COMPOUNDS AND METHODS FOR MODULATING ANGIOTENSINOGEN EXPRESSION

Sequence Listing

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled BIOL0393WOSEQ_ST25.txt, created on November 3, 2021, which is 32 KB in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

Field

Provided are compounds, methods, and pharmaceutical compositions for reducing the amount or activity of Angiotensinogen RNA in a cell or subject, and in certain instances reducing the amount of Angiotensinogen (AGT) in a cell or subject. Such compounds and pharmaceutical compositions are useful to ameliorate at least one symptom or hallmark of a RAAS pathway-related disease or disorder. Such diseases and disorders include hypertension, hypertensive emergency (i.e. malignant hypertension), resistant hypertension, kidney disease (e.g., chronic kidney disease, polycystic kidney disease), pre-eclampsia, Marfan Syndrome, stroke, cardiac disease (e.g., myocardial infarction, heart failure, congestive heart failure, valvular heart disease), aneurysms of the blood vessels, abdominal aneurysm, peripheral artery disease, organ damage, pulmonary arterial hypertension, obesity, metabolic syndrome, NASH, NAFLD and other RAAS related diseases, disorders and/or conditions or symptoms thereof.

Background

Angiotensinogen (AGT), also known as SERPINA8 or ANHU, is a member of the serpin family and is a component of the renin-angiotensin-aldosterone system (RAAS). It is primarily produced in the liver and is released into the circulation where renin converts it into angiotensin I. Angiotensin I is subsequently converted into angiotensin II by angiotension converting enzyme (ACE). Angiotensin II is a peptide hormone which causes vasoconstriction which, in turn, can increase blood pressure. Angiotensin II also stimulates secretion of the hormone aldosterone from the adrenal cortex. Aldosterone causes the kidneys to increase reabsorption of sodium and water leading to an increase of the fluid volume in a body which, in turn, can increase blood pressure. Over stimulation or activity of the RAAS pathway can lead to high blood pressure. Chronic high blood pressure is known as hypertension. The high blood pressure in a hypertensive subject requires the heart to work harder to circulate blood through the blood vessels.

Hypertension remains a leading cause of global death and disability from cardiovascular disease and stroke. Despite extensive research and the existence of multiple effective therapeutic interventions, hypertension remains an important public health challenge in the United States (Sigmund et al., Hypertension 2020, 75: 902-917). There are limitations to the therapies currently approved for treating hypertension as a significant subset of all hypertensive patients do not achieve adequate blood pressure control. For example,

drugs such as ACE inhibitors and angiotensin receptor blockers (ARBs) that target parts of the renin-angiotensin system (RAS) pathway are limited in their ability to inhibit the RAAS pathway (Nobakht et al., Nat Rev Nephrol, 2011, 7:356-359). Additionally, certain anti-hypertensive drugs such as ACE inhibitors are contra-indicated in hypertensive patients with renal disease due to their potential to compromise renal function in patients.

Accordingly, there is a need to find alternative treatments to inhibit the RAAS pathway and treat hypertension. It is therefore an object herein to provide compounds, methods, and pharmaceutical compositions for the treatment of such diseases.

Summary of the Invention

Provided herein are compounds, methods and pharmaceutical compositions for reducing the amount or activity of AGT RNA and, in certain embodiments, reducing the expression of AGT protein in a cell or subject. In certain embodiments, the subject has a cardiovascular disease. In certain embodiments, the subject has hypertension. In certain embodiments, the subject has resistant hypertension. In certain embodiments, the subject has Marfan syndrome. In certain embodiments, the subject has kidney disease. In certain embodiments, compounds useful for reducing the amount or activity of AGT RNA are oligomeric compounds. In certain embodiments, compounds useful for reducing the amount or activity of AGT RNA are modified oligonucleotides. In certain embodiments, compounds useful for decreasing expression of AGT protein are oligomeric compounds. In certain embodiments, compounds useful for decreasing expression of AGT protein are modified oligonucleotides.

Also provided are methods useful for ameliorating at least one symptom or hallmark of an RAAS pathway-related disease or indication. In certain embodiments, the disease is hypertension. In certain embodiments, the disease is resistant hypertension. In certain embodiments, the disease is Marfan syndrome. In certain embodiments, the indication is heart failure. In certain embodiments, the symptom or hallmark includes hypertension, hypertensive emergency (i.e. malignant hypertension), pre-eclampsia, stroke, cardiac disease (e.g., myocardial infarction, heart failure, congestive heart failure, valvular heart disease), aneurysms of the blood vessels, abdominal aneurysm, organ damage, pulmonary arterial hypertension, obesity, and other RAAS pathway-related diseases, disorders and/or conditions or symptoms thereof.

Detailed Description of the Invention

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

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, treatises, and GenBank, ENSEMBL, 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.

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 herein in their entirety.

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

DEFINITIONS

As used herein, "2'-deoxynucleoside" means a nucleoside comprising a 2'-H(H) deoxyfuranosyl sugar moiety. In certain embodiments, a 2'-deoxynucleoside is a 2'-β-D-deoxynucleoside 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'-deoxynucleoside may comprise a modified nucleobase or may comprise an RNA nucleobase (uracil).

As used herein, "2'-MOE" means a 2'-$OCH_2CH_2OCH_3$ group in place of the 2'-OH group of a furanosyl sugar moiety. A "2'-MOE sugar moiety" means a sugar moiety with a 2'-$OCH_2CH_2OCH_3$ group in place of the 2'-OH group of a furanosyl sugar moiety. Unless otherwise indicated, a 2'-MOE sugar moiety is in the β-D-ribosyl configuration. "MOE" means O-methoxyethyl.

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

As used herein, "2'-OMe" means a 2'-OCH_3 group in place of the 2'-OH group of a furanosyl sugar moiety. As used herein, "2'-O-methyl sugar moiety" or "2'-OMe sugar moiety" means a sugar moiety with a 2'-OCH_3 group in place of the 2'-OH group of a furanosyl sugar moiety. Unless otherwise indicated, a 2'-OMe sugar moiety is in the β-D-ribosyl configuration.

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, "About" means within ±10% of a value. For example, if it is stated, "the compounds affected about 70% inhibition of AGT", it is implied that AGT levels are inhibited within a range of 63% and 77%.

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

As used herein, "Angiotensinogen" and "AGT" is used interchangeably herein. Angiotensinogen is also known as SERPINA8 and ANHU.

As used herein, "Anti-hypertensive drug" refers to a drug capable of lowering blood pressure. Examples of such drugs include, but are not limited to, RAAS inhibitors, diuretics, calcium channel blockers, adrenergic receptor antagonists, adrenergic agonists and vasodilators. In one example, the anti-hypertensive drug captopril can be used in combination with the AGT compound described herein to treat an animal having or at risk of having a RAAS pathway related disease, disorder and/or condition.

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. In certain embodiments, antisense activity is a decrease in the amount or expression of a target nucleic acid or protein encoded by such target nucleic acid compared to target nucleic acid levels or target protein levels in the absence of the antisense compound.

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

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. The progression or severity of indicators may be determined by subjective or objective measures, which are known to those skilled in the art.

As used herein, "Blood pressure" refers to the pressure of the blood in the circulatory system against the walls of the blood vessel. The blood pressure is due mainly to the beating of the heart in an animal. During each heartbeat, the blood pressure varies between a maximum (systolic) blood pressure (SBP) and minimum (diastolic) blood pressure (DBP). The mean arterial pressure (MAP) is the average arterial pressure during a heartbeat cycle. Blood pressure can be measure by a blood pressure meter (i.e., a sphygmomanometer). Normal blood pressure at rest is less than 120 mmHg systolic and less than 80 mmHg diastolic and is commonly expressed as the systolic pressure (top reading) / diastolic pressure (bottom reading) mmHg.

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 sugar moiety is a ribosyl moiety. In certain embodiments, the bicyclic sugar moiety does not comprise a furanosyl moiety.

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(CH_3)-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(CH_3)-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, “cleavable moiety” means a bond or group of atoms that is cleaved under physiological conditions, for example, inside a cell, a subject, an animal, or a human.

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. As used herein, “complementary nucleobases” means nucleobases that are capable of forming hydrogen bonds with one another. Complementary nucleobase pairs include adenine (A) and thymine (T), adenine (A) and uracil (U), cytosine (C) and guanine (G), and 5-methyl cytosine (^{m}C) and 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, “conjugate group” means a group of atoms that is directly or indirectly attached to an oligonucleotide. Conjugate groups include a conjugate moiety and a conjugate linker that attaches the conjugate moiety to the oligonucleotide.

As used herein, “conjugate linker” means a single bond or a group of atoms comprising at least one bond that connects a conjugate moiety to an oligonucleotide.

As used herein, “conjugate moiety” means a group of atoms that is attached to an oligonucleotide via a conjugate linker.

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, "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, "chirally controlled" in reference to an internucleoside linkage means chirality at that linkage is enriched for a particular stereochemical configuration.

As used herein, "deoxy region" means a region of 5-12 contiguous nucleotides, wherein at least 70% of the nucleosides are 2'-β-D-deoxynucleosides. In certain embodiments, each nucleoside is selected from a 2'-β-D-deoxynucleoside, a bicyclic nucleoside, and a 2'-susbstituted nucleoside. In certain embodiments, a deoxy region supports RNase H activity. In certain embodiments, a deoxy region is the gap or internal region of a gapmer.

As used herein, "gapmer" means a modified oligonucleotide comprising an internal region having a plurality of nucleosides that support RNase H cleavage positioned between external regions having one or more nucleosides, wherein the nucleosides comprising the internal region are chemically distinct from the nucleoside or nucleosides comprising the external regions. The internal region may be referred to as the "gap" and the external regions may be referred to as the "wings." The internal region is a deoxy region. The positions of the internal region or gap refer to the order of the nucleosides of the internal region and are counted starting from the 5'-end of the internal region. Unless otherwise indicated, "gapmer" refers to a sugar motif. In certain embodiments, each nucleoside of the gap is a 2'-β-D-deoxynucleoside. In certain embodiments, the gap comprises one 2'-substituted nucleoside at position 1, 2, 3, 4, or 5 of the gap, and the remainder of the nucleosides of the gap are 2'-β-D-deoxynucleosides. As used herein, the term "MOE gapmer" indicates a gapmer having a gap comprising 2'-β-D-deoxynucleosides and wings comprising 2'-MOE nucleosides. As used herein, the term "mixed wing gapmer" indicates a gapmer having wings comprising modified nucleosides comprising at least two different sugar modifications. Unless otherwise indicated, a gapmer may comprise one or more modified internucleoside linkages and/or modified nucleobases and such modifications do not necessarily follow the gapmer pattern of the sugar modifications.

As used herein, "hotspot region" is a range of nucleobases on a target nucleic acid that is amenable to oligomeric compound-mediated reduction of the amount or activity of the target nucleic acid.

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, "Hypertension" or "HTN" refers to a chronic medical condition where the blood pressure in an animal is elevated. The elevated blood pressure requires the heart to work harder to circulate blood through the blood vessels. High blood pressure is said to be present if it is persistently at or above 130/80 mmHg (Stage 1) or 140/90 mmHg (Stage 2). Hypertension is classified as primary (essential) or secondary. Primary hypertension has no clear cause and is thought to be linked to genetics, diet, lack of exercise and obesity. Secondary hypertension is caused by another medical condition. Hypertension is a major risk factor for shortened life expectancy, chronic kidney disease, stroke, myocardial infarction, heart failure, aneurysms of the blood vessels (e.g. aortic aneurysm), peripheral artery disease, organ damage (e.g., heart enlargement or hypertrophy) and other cardiovascular diseases, disorders and/or conditions or symptoms thereof. Anti-hypertensive drugs, diet changes and lifestyle changes may reduce hypertension and reduce the diseases, disorders and/or conditions associated with hypertension. Hypertension can be nonresistant to drug intervention (i.e., controllable by commercially available drug therapies) or resistant to drug intervention.

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, "linker-nucleoside" means a nucleoside that links, either directly or indirectly, an oligonucleotide to a conjugate moiety. Linker-nucleosides are located within the conjugate linker of an oligomeric compound. Linker-nucleosides are not considered part of the oligonucleotide portion of an oligomeric compound even if they are contiguous with the 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, "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, "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, or a fragment of 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. Modified nucleosides include abasic nucleosides, which lack a nucleobase. “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, “Organ damage” or “end organ damage” refers to damage occurring in major organs fed by the circulatory system such as the heart (e.g., heart muscle hypertrophy, reduced heart function and/or heart failure), kidney (e.g., albuminurea, proteinurea, reduced renal function and/or renal failure), eyes (e.g., hypertensive retinopathy), brain (e.g., stroke) and the like. The organs can be damaged by hypertension in an animal. In certain embodiments, the heart damage is fibrosis, heart cell and/or muscle hypertrophy leading to heart enlargement.

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.

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. In certain embodiments, a pharmaceutical composition shows activity in free uptake assay in certain cell lines.

As used herein, "prodrug" means a therapeutic agent in a form outside the body that is converted to a different form within a subject or cells thereof. Typically, conversion of a prodrug within the subject is facilitated by the action of an enzymes (e.g., endogenous or viral enzyme) or chemicals present in cells or tissues and/or by physiologic conditions.

As used herein, "reducing the amount or activity" refers to a reduction or blockade of the transcriptional expression or activity relative to the transcriptional expression or activity in an untreated or control sample and does not necessarily indicate a total elimination of transcriptional expression or activity.

As used herein, "Renin-angiotensin-aldosterone system", "Renin-angiotensin-aldosterone system pathway", "RAAS pathway" or "RAAS" refer to a multi-component enzymatic pathway where a precursor component (angiotensinogen) is converted by various enzymes such as renin and enzyme angiotensin-converting-enzyme (ACE) into downstream components such as angiotensin I and angiotensin II. Angiotensin I stimulates secretion of the steroid aldosterone in the pathway. The RAAS pathway regulates blood pressure and fluid balance.

As used herein, "Renin-angiotensin System", or "RAS" or "RAS pathway" refer to a portion of the RAAS pathway. Various components of this pathway have been targeted by agonists or antagonists to block the production of the components. For example renin inhibitors, ACE inhibitors, angiotensin-receptor blockers (ARBs) and the like have been developed to inhibit or block the RAS pathway. However, commercially available therapies targeting various RAS pathway components have been ineffective in completely inhibiting or blocking the RAS pathway due to various mechanisms or adverse effects (Nobakht et al., Nat Rev Nephrol, 2011, 7:356-359).

As used herein, "RAAS related disease, disorder and/or condition" or "RAAS pathway related disease, disorder and/or condition" refers to any disease, disorder or condition related to RAAS in an animal. Examples of RAAS related diseases, disorders and/or conditions include shortened life expectancy, hypertension (e.g. nonresistant hypertension, resistant hypertension), kidney disease (e.g., chronic kidney disease, polycystic kidney disease), stroke, heart disease (e.g., myocardial infarction, heart failure, valvular heart disease), aneurysms of the blood vessels (e.g. aortic aneurysm), peripheral artery disease, organ damage (e.g., heart damage or hypertrophy), tissue fibrosis and other cardiovascular diseases, disorders and/or

conditions or symptoms thereof. In certain embodiments, RAAS related disease, disorder and/or condition does not include hypertension.

As used herein, "Resistant hypertension" or "RHTN" is defined as either a) blood pressure above the therapeutic goal (typically ≥130/80 mmHg) despite concurrent use of 3 or more anti-hypertensive agents from different drug classes administered at maximal tolerated doses; or b) blood pressure that is controlled at or below the therapeutic goal only after administration of at least 4 anti-hypertensive agents of different classes to achieve control.

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

As used herein, "RNAi compound" means an antisense compound that acts, at least in part, through RISC or Ago2 to modulate a target nucleic acid and/or protein encoded by a target nucleic acid. RNAi compounds include, but are not limited to double-stranded siRNA, single-stranded RNA (ssRNA), and microRNA, including microRNA mimics. In certain embodiments, an RNAi compound modulates the amount, activity, and/or splicing of a target nucleic acid. The term RNAi compound excludes antisense compounds that act through RNase H.

As used herein, "self-complementary" in reference to an oligonucleotide means an oligonucleotide that at least partially hybridizes to itself.

As used herein, "standard *in vitro* assay" means the assays described in Examples and reasonable variations thereof.

As used herein, "standard *in vivo* assay" means the assays described in Examples and reasonable variations thereof.

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 sugar 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.

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.

As used herein, "symptom" or "hallmark" 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. In certain embodiments, a hallmark is apparent upon invasive diagnostic testing, including, but not limited to, post-mortem tests.

As used herein, "target nucleic acid" and "target RNA" mean 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.

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 14 to 30 linked nucleosides and having a nucleobase sequence comprising at least 14, at least 15, or 16 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 12-15, wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

Embodiment 2: An oligomeric compound comprising a modified oligonucleotide consisting of 14 to 30 linked nucleosides and having a nucleobase sequence comprising at least 14, at least 15, or at least 16 contiguous nucleobases complementary to:

an equal length portion of nucleobases 2046-2061 of SEQ ID NO: 1;
an equal length portion of nucleobases 2271-2286 of SEQ ID NO: 1;
an equal length portion of nucleobases 2272-2287 of SEQ ID NO: 1;

wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

Embodiment 3: An oligomeric compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of SEQ ID NO: 12, wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

Embodiment 4: An oligomeric compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of SEQ ID NO: 13, wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

Embodiment 5: An oligomeric compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of SEQ ID NO: 14, wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

Embodiment 6: An oligomeric compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of SEQ ID NO: 15, wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

Embodiment 7: The oligomeric compound of any of embodiments 1-6, wherein the modified oligonucleotide has a nucleobase sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% complementary to the nucleobase sequence of any SEQ ID NO: 1 or SEQ ID NO: 2, when measured across the entire nucleobase sequence of the modified oligonucleotide.

Embodiment 8: The oligomeric compound of any of embodiments 1-7, wherein the modified oligonucleotide comprises at least one bicyclic sugar moiety.

Embodiment 9: The oligomeric compound of embodiment 8, wherein the bicyclic sugar moiety has a 4'-2' bridge, wherein the 4'-2' bridge is selected from $-CH_2-O-$; and $-CH(CH_3)-O-$.

Embodiment 10: The oligomeric compound of any of embodiments 1-9, wherein the modified oligonucleotide comprises at least one non-bicyclic modified sugar moiety.

Embodiment 11: The oligomeric compound of embodiment 10, wherein the non-bicyclic modified sugar moiety is a 2'-MOE sugar moiety or a 2'-OMe sugar moiety.

Embodiment 12: The oligomeric compound of any of embodiments 1-11, wherein the modified oligonucleotide comprises at least one sugar surrogate.

Embodiment 13: The oligomeric compound of embodiment 12, wherein the sugar surrogate is any of morpholino, modified morpholino, PNA, THP, and F-HNA.

Embodiment 14: The oligomeric compound of any of embodiments 1-13, wherein the modified oligonucleotide is a gapmer.

Embodiment 15: The oligomeric compound of embodiment 14, wherein the modified oligonucleotide has a sugar motif comprising:

a 5'-region consisting of 1-6 linked 5'-region nucleosides;

a central region consisting of 6-10 linked central region nucleosides; and

a 3'-region consisting of 1-6 linked 3'-region nucleosides; wherein

each of the 5'-region nucleosides and each of the 3'-region nucleosides comprises a modified sugar moiety and at least 6 of the central region nucleosides comprises a 2'-β-D-deoxyribosyl sugar moiety.

Embodiment 16: The oligomeric compound of embodiment 14, wherein the modified oligonucleotide has a sugar motif comprising:

a 5'-region consisting of 1-6 linked 5'-region nucleosides;

a central region consisting of 6-10 linked central region nucleosides; and

a 3'-region consisting of 1-6 linked 3'-region nucleosides; wherein

each of the 5'-region nucleosides and each of the 3'-region nucleosides comprises a modified sugar moiety and each of the central region nucleosides comprises a 2'-deoxyribosyl sugar moiety

Embodiment 17: The oligomeric compound of embodiment 14, wherein the modified oligonucleotide has a sugar motif comprising:

a 5'-region consisting of 3 linked 5'-region nucleosides;

a central region consisting of 10 linked central region nucleosides; and

a 3'-region consisting of 3 linked 3'-region nucleosides; wherein

each of the 5'-region nucleosides and each of the 3'-region nucleosides comprises a 2'-MOE sugar moiety or a cEt modified sugar moiety, and each of the central region nucleosides comprises a 2'-β-D- deoxyribosyl sugar moiety.

Embodiment 18: The oligomeric compound of embodiment 14, wherein the modified oligonucleotide has a sugar motif comprising:

a 5'-region consisting of 3 linked 5'-region nucleosides;

a central region consisting of 10 linked central region nucleosides; and

a 3'-region consisting of 3 linked 3'-region nucleosides; wherein

each of the 5'-region nucleosides and each of the 3'-region nucleosides comprises a 2'-MOE modified sugar moiety or a cEt modified sugar moiety, and at least 6 of the central region nucleosides comprises a 2'-β-D-deoxyribosyl sugar moiety.

Embodiment 19: The oligomeric compound of any of embodiments 1-18, wherein the modified oligonucleotide has a sugar motif (5' to 3') selected from eekddddddddddkke, ekkddddddddddkke, kkkdyddddddddkkk, kkkddydddddddkkk, kkkdddydddddddkkk, kkkddddddddddkkk, or

eeeeeddddddddddeeeee; wherein 'e' represents a 2'-MOE sugar moiety, 'k' represents a cEt sugar moiety, 'd' represents a 2'-β-D-deoxyribosyl sugar moiety, and 'y' represents a 2'-OMe sugar moiety.

Embodiment 20: The oligomeric compound of any of embodiments 1-19, 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.

Embodiment 22: The oligomeric compound of embodiment 20 or embodiment 21, wherein at least one internucleoside linkage is a phosphorothioate internucleoside linkage.

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

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

Embodiment 25: The oligomeric compound of embodiment 21, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage.

Embodiment 26: The oligomeric compound of any of embodiments 1-20 or 22-24, wherein the modified oligonucleotide has an internucleoside linkage motif of soosssssssssos; wherein,

s = a phosphorothioate internucleoside linkage and o = a phosphodiester internucleoside linkage.

Embodiment 27: The oligomeric compound of any of embodiments 1-26, wherein the modified oligonucleotide comprises at least one modified nucleobase.

Embodiment 28: The oligomeric compound of embodiment 27, wherein the modified nucleobase is a 5-methylcytosine.

Embodiment 29: The oligomeric compound of any of embodiments 1-28, wherein the modified oligonucleotide consists of 12-30, 12-22, 12-20,14-18, 14-20, 15-17, 15-25, or 16-20 linked nucleosides.

Embodiment 30: The oligomeric compound of any of embodiments 1-28, wherein the modified oligonucleotide consists of 16 linked nucleosides.

Embodiment 31: The oligomeric compound of any of embodiments 1-30, comprising a conjugate group.

Embodiment 32: The oligomeric compound of embodiment 31, wherein the conjugate group comprises a GalNAc cluster comprising 1-3 GalNAc ligands.

Embodiment 33: The oligomeric compound of any one of embodiments 31 and 32, wherein the conjugate group comprises a conjugate linker consisting of a single bond.

Embodiment 34: The oligomeric compound of any one of embodiments 31-33, wherein the conjugate group comprises a cleavable linker.

Embodiment 35: The oligomeric compound of any one of embodiments 31-34, wherein the conjugate group comprises a conjugate linker comprising 1-3 linker-nucleosides.

Embodiment 36: The oligomeric compound of any one of embodiments 31-35, wherein the conjugate group is attached to the modified oligonucleotide at the 5'-end of the modified oligonucleotide.

Embodiment 37: The oligomeric compound of any one of embodiments 31-35, wherein the conjugate group is attached to the modified oligonucleotide at the 3'-end of the modified oligonucleotide.

Embodiment 38: The oligomeric compound of any of embodiments 1-37, wherein the oligomeric compound is a singled-stranded oligomeric compound.

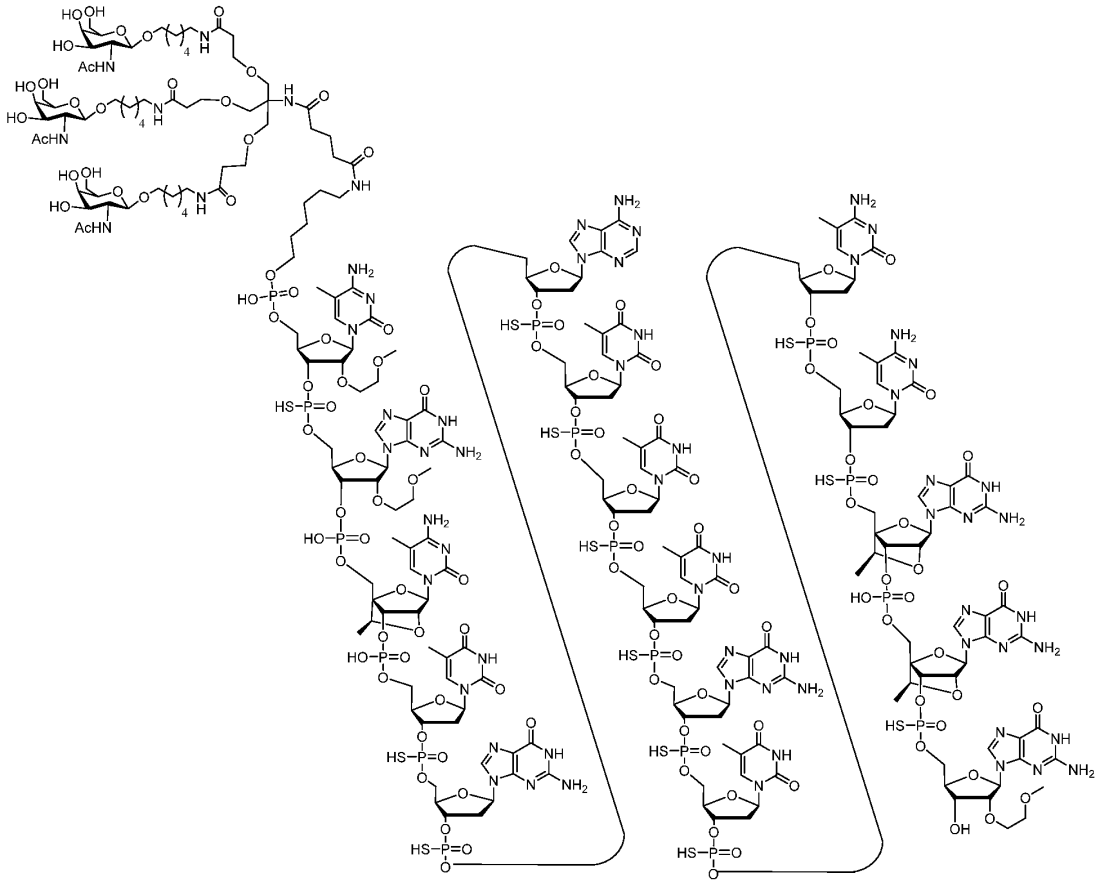
Embodiment 39: The oligomeric compound of any of embodiments 1-30 or 38, consisting of the modified oligonucleotide.

Embodiment 40: An oligomeric duplex comprising an oligomeric compound of any of embodiments 1-37.

Embodiment 41: An antisense compound comprising or consisting of an oligomeric compound of any of embodiments 1-39 or an oligomeric duplex of embodiment 40.

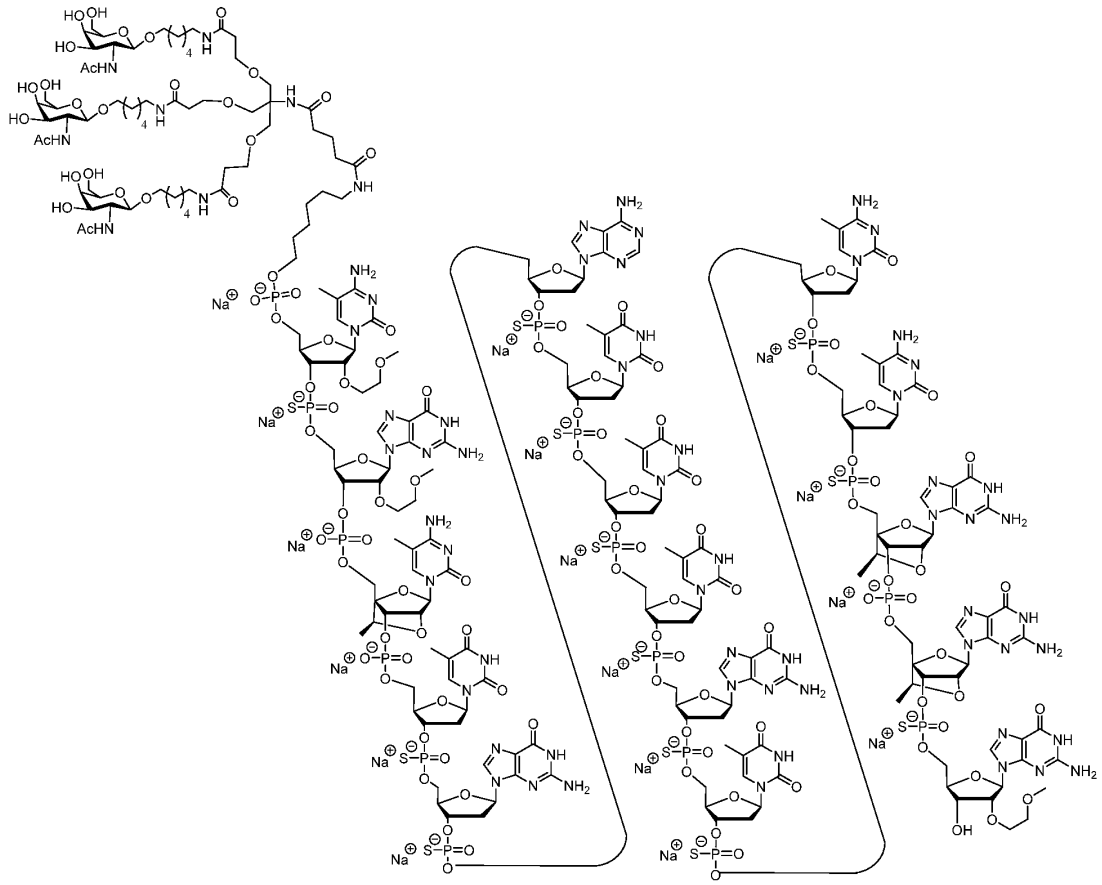
Embodiment 42: A pharmaceutical composition comprising an oligomeric compound of any of embodiments 1-39 or an oligomeric duplex of embodiment 40 and a pharmaceutically acceptable carrier or diluent.

Embodiment 43: A compound according to the following chemical structure:



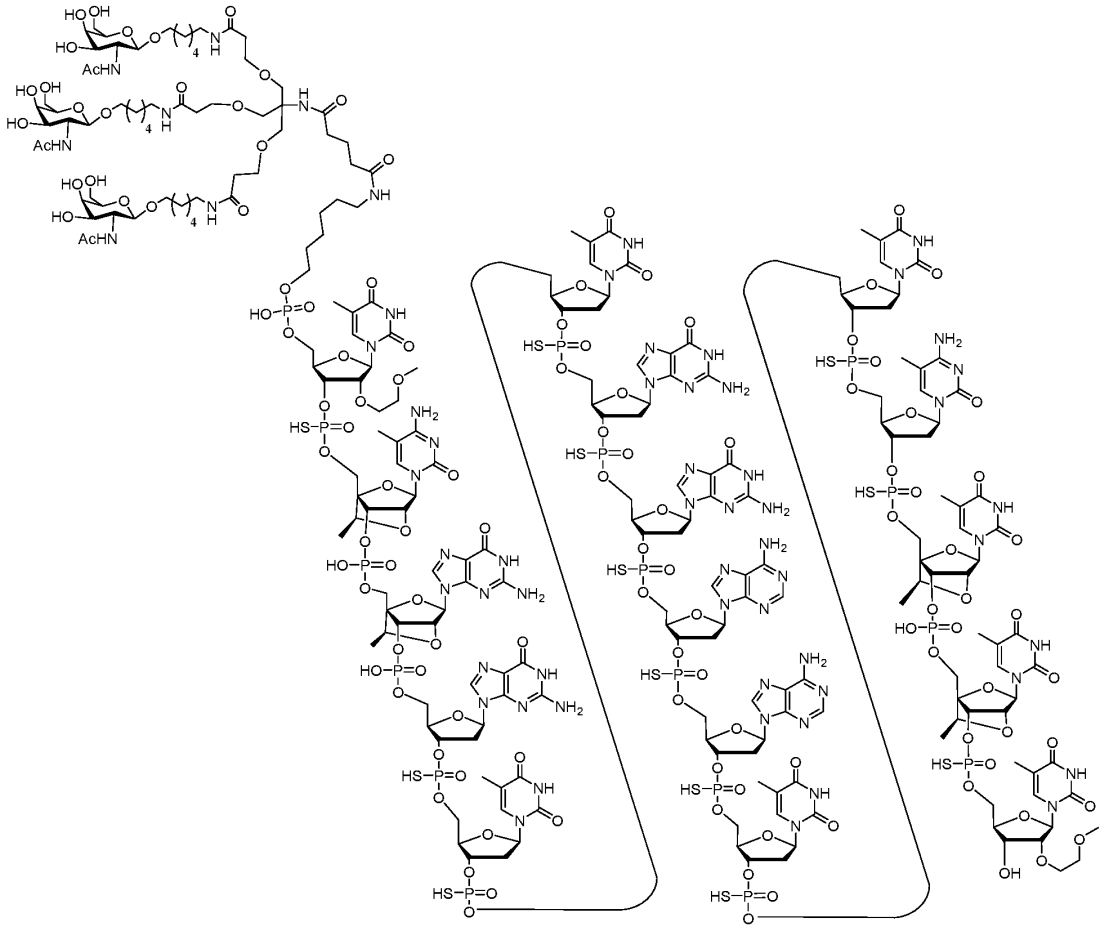
(SEQ ID NO: 12), or a salt thereof.

Embodiment 44: A compound according to the following chemical structure:



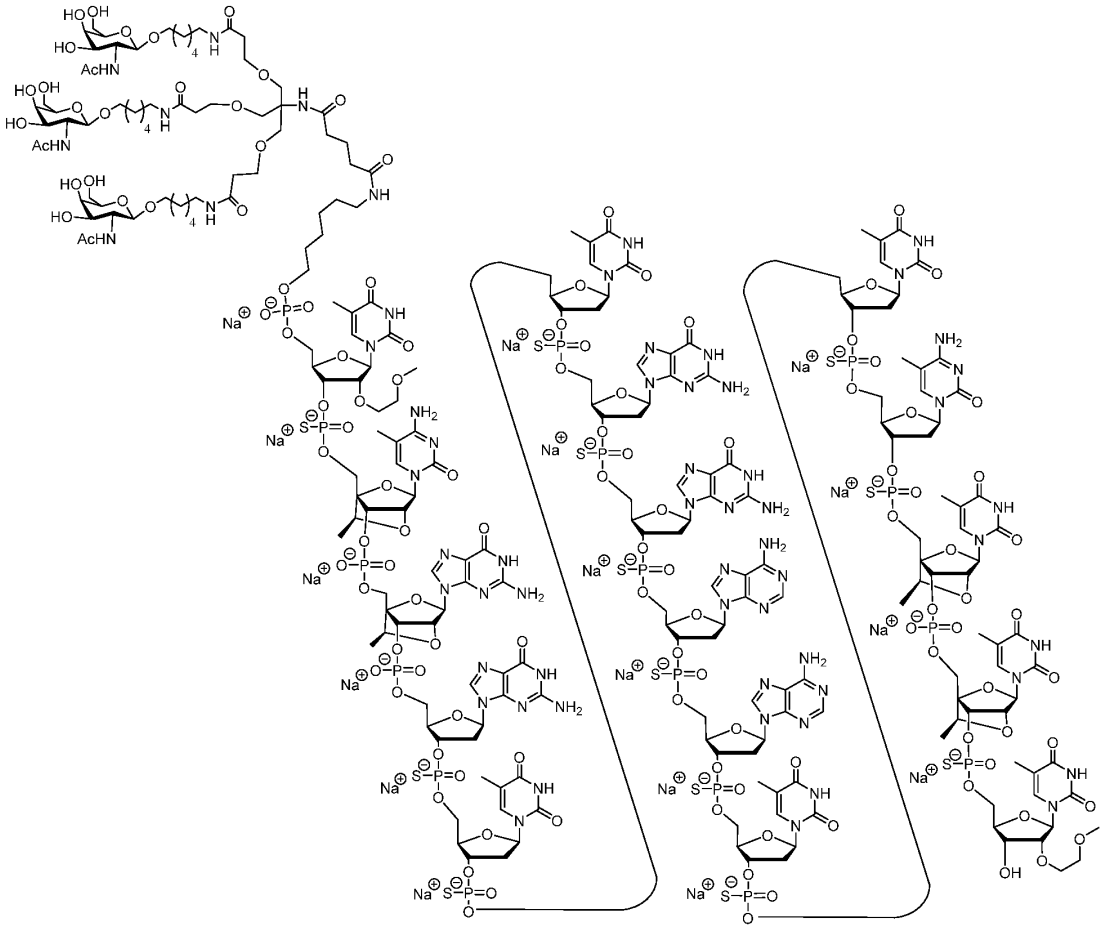
(SEQ ID NO: 12).

Embodiment 45: A compound according to the following chemical structure:



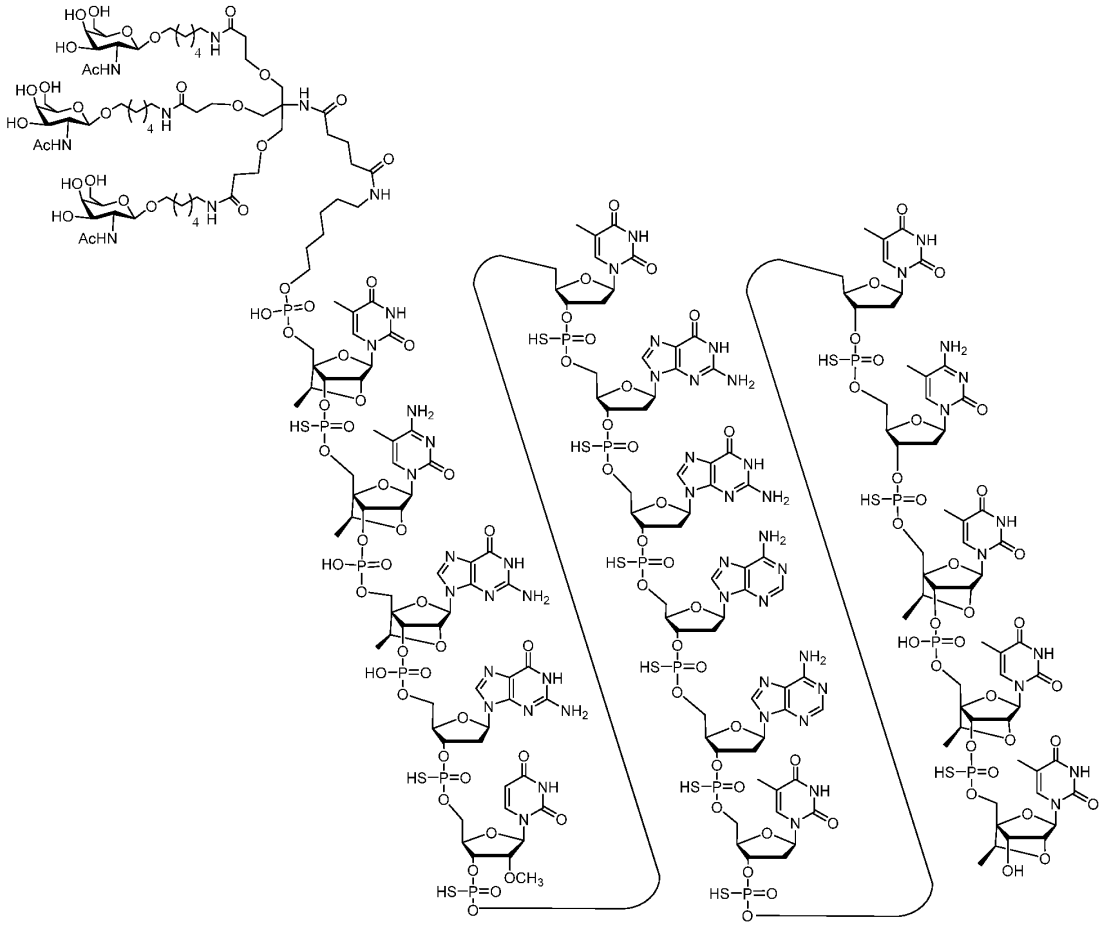
(SEQ ID NO: 13), or a salt thereof.

Embodiment 46: A compound according to the following chemical structure:



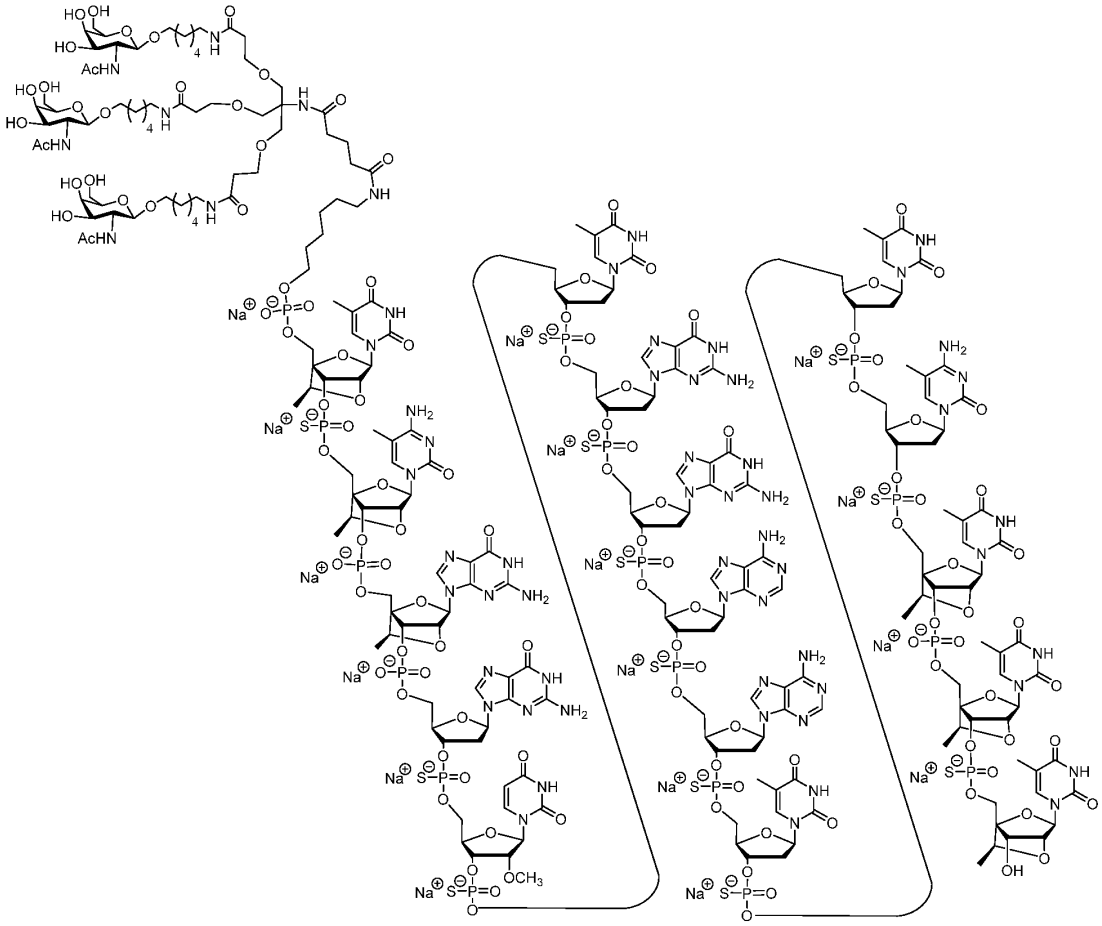
(SEQ ID NO: 13).

Embodiment 47: A compound according to the following chemical structure:



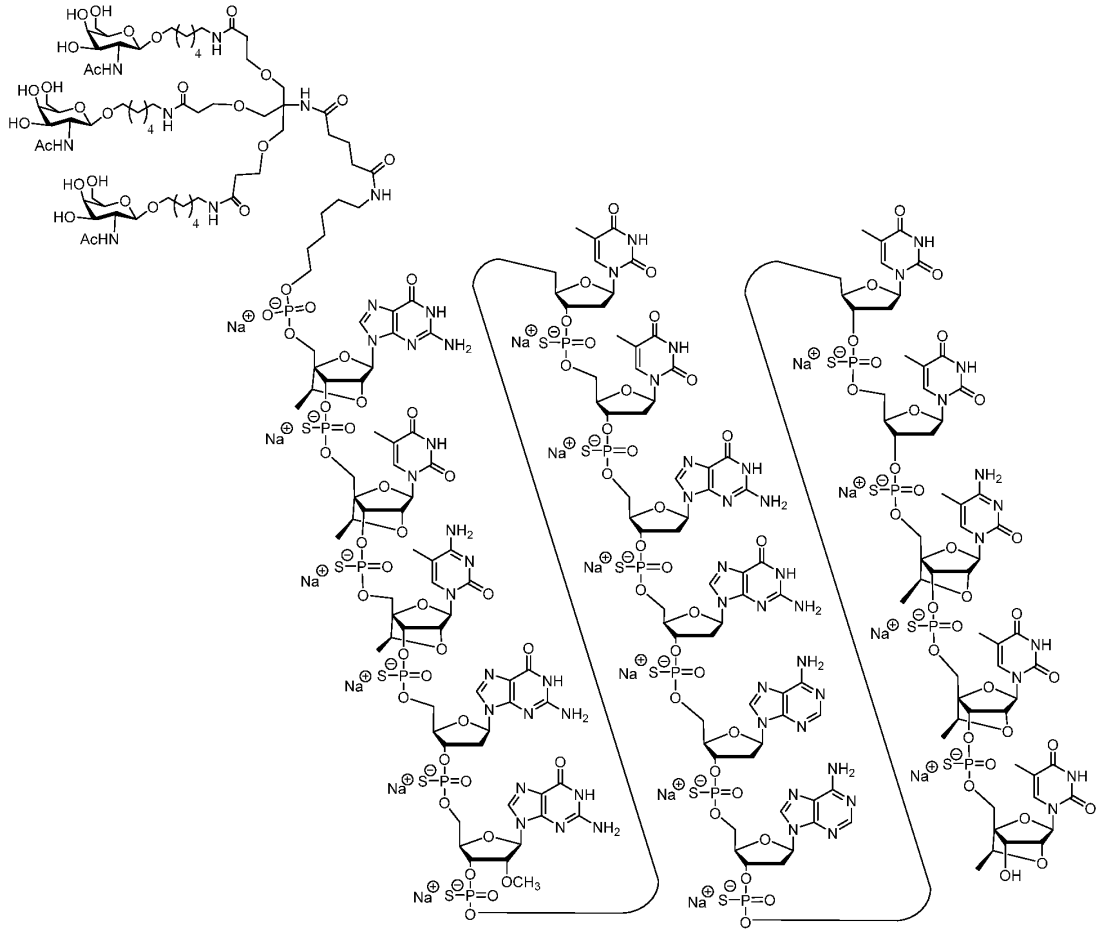
(SEQ ID NO: 14), or a salt thereof.

Embodiment 48: A compound according to the following chemical structure:



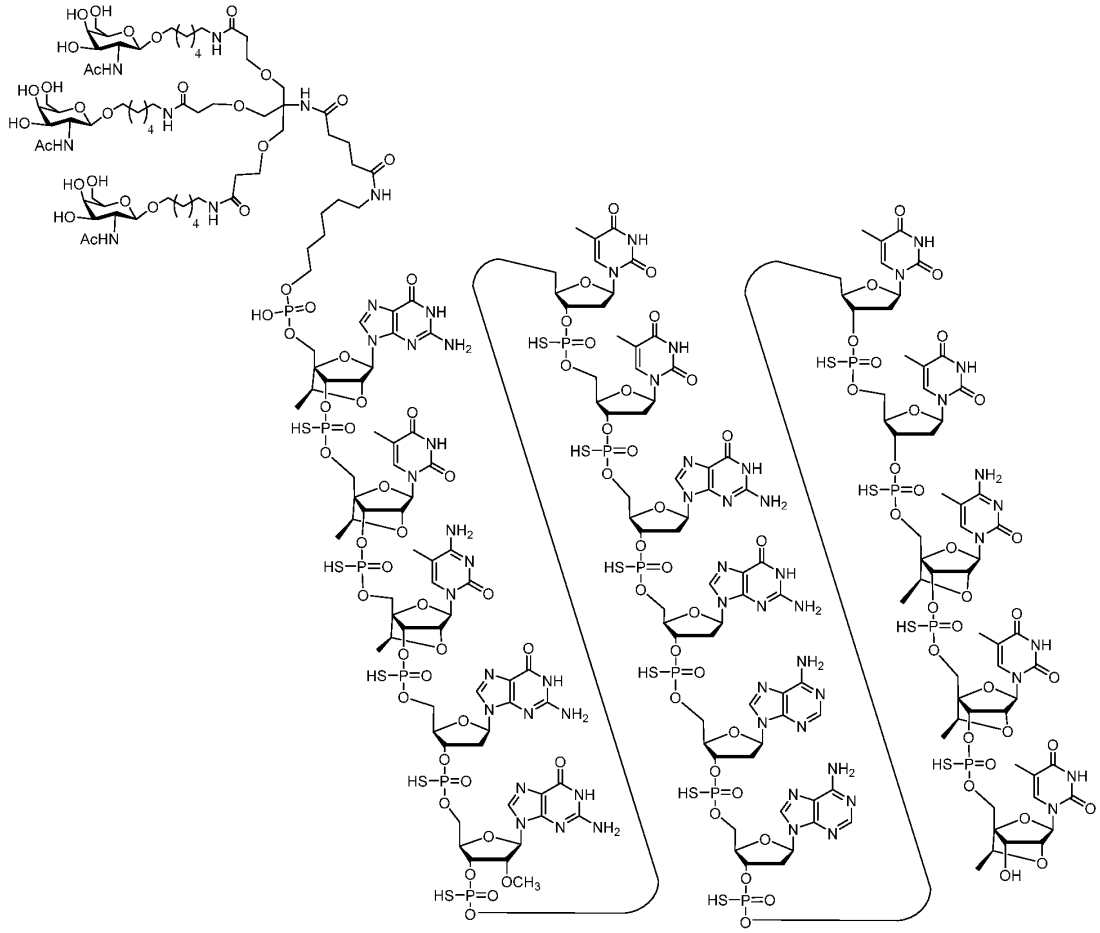
(SEQ ID NO: 14).

Embodiment 49: A compound according to the following chemical structure:



(SEQ ID NO: 15), or salt thereof.

Embodiment 50: A compound according to the following chemical structure:



(SEQ ID NO: 15).

Embodiment 51: The modified oligonucleotide of any of embodiments 43, 45, 47, and 49, which is the sodium salt or potassium salt of the chemical structure.

Embodiment 52: A pharmaceutical composition comprising the modified oligonucleotide of any of embodiments 43-51 and a pharmaceutically acceptable carrier or diluent.

Embodiment 53: A compound comprising a modified oligonucleotide according to the following chemical notation: $^{m}C_{es}G_{eo}{}^{m}C_{ko}T_{ds}G_{ds}A_{ds}T_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}G_{ko}G_{ks}G_{e}$ (SEQ ID NO: 12), wherein:

A = an adenine nucleobase,

^{m}C = a 5-methyl cytosine nucleobase,

G = a guanine nucleobase,

T = a thymine nucleobase,

e = a 2'-β-D-MOE sugar moiety,

k = a cEt sugar moiety,

d = a 2'-β-D-deoxyribosyl sugar moiety,

s = a phosphorothioate internucleoside linkage, and

o = a phosphodiester internucleoside linkage.

Embodiment 54: A compound comprising a modified oligonucleotide according to the following chemical notation: $T_{es}{}^{m}C_{ko}G_{ko}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}A_{ds}A_{ds}T_{ds}T_{ds}{}^{m}C_{ds}T_{ko}T_{ks}T_{e}$ (SEQ ID NO: 13), wherein:

A = an adenine nucleobase,

^{m}C = a 5-methyl cytosine nucleobase,

G = a guanine nucleobase,

T = a thymine nucleobase,

e = a 2'-β-D-MOE sugar moiety,

k = a cEt sugar moiety,

d = a 2'-β-D-deoxyribosyl sugar moiety,

s = a phosphorothioate internucleoside linkage, and

o = a phosphodiester internucleoside linkage.

Embodiment 55: A compound comprising a modified oligonucleotide according to the following chemical notation: $G_{ks}T_{ks}{}^{m}C_{ks}G_{ds}G_{ys}T_{ds}T_{ds}G_{ds}G_{ds}A_{ds}A_{ds}T_{ds}T_{ds}{}^{m}C_{ks}T_{ks}T_{k}$ (SEQ ID NO: 15), wherein:

A = an adenine nucleobase,

^{m}C = a 5-methyl cytosine nucleobase,

G = a guanine nucleobase,

T = a thymine nucleobase,

k = a cEt sugar moiety,

d = a 2'-β-D-deoxyribosyl sugar moiety,

y = a 2'-OMe ribose sugar moiety, and

s = a phosphorothioate internucleoside linkage.

Embodiment 56: A compound comprising a modified oligonucleotide according to the following chemical notation: $T_{ks}{}^{m}C_{ko}G_{ko}G_{ds}U_{ys}T_{ds}G_{ds}G_{ds}A_{ds}A_{ds}T_{ds}T_{ds}{}^{m}C_{ds}T_{ko}T_{ks}T_{k}$ (SEQ ID NO: 14), wherein:

A = an adenine nucleobase,

^{m}C = a 5-methyl cytosine nucleobase,

G = a guanine nucleobase,

T = a thymine nucleobase,

U = a uracil nucleobase,

k = a cEt sugar moiety,

d = a 2'-β-D-deoxyribosyl sugar moiety,

y = a 2'-OMe ribose sugar moiety,

s = a phosphorothioate internucleoside linkage, and

o = a phosphodiester internucleoside linkage.

Embodiment 57: The compound of any of embodiments 53-56, comprising the modified oligonucleotide covalently linked to a conjugate group.

Embodiment 58: A pharmaceutical composition of any of embodiments 53-56, and a pharmaceutically acceptable diluent or carrier.

Embodiment 59: A chirally enriched population of modified oligonucleotides of any of embodiments 53-56, wherein the population is enriched for modified oligonucleotides comprising at least one particular phosphorothioate internucleoside linkage having a particular stereochemical configuration.

Embodiment 60: The chirally enriched population of embodiment 59, wherein the population is enriched for modified oligonucleotides comprising at least one particular phosphorothioate internucleoside linkage having the (*S*p) configuration.

Embodiment 61: The chirally enriched population of embodiment 59, wherein the population is enriched for modified oligonucleotides comprising at least one particular phosphorothioate internucleoside linkage having the (*R*p) configuration.

Embodiment 62: The chirally enriched population of embodiment 59, wherein the population is enriched for modified oligonucleotides having a particular, independently selected stereochemical configuration at each phosphorothioate internucleoside linkage.

Embodiment 63: The chirally enriched population of embodiment 59, wherein the population is enriched for modified oligonucleotides having the (*S*p) configuration at each phosphorothioate internucleoside linkage or for modified oligonucleotides having the (*R*p) configuration at each phosphorothioate internucleoside linkage.

Embodiment 64: The chirally enriched population of embodiment 59, 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 65: The chirally enriched population of embodiment 59, 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 66: A population of modified oligonucleotides of any of embodiments 59-65, wherein all the phosphorothioate internucleoside linkages of the modified oligonucleotide are stereorandom.

Embodiment 67: A method comprising administering to an individual the pharmaceutical composition of any preceding embodiment.

Embodiment 68: A method of treating a disease associated with the RAAS pathway, comprising administering to an individual having or at risk of having a disease associated with the RAAS pathway a

therapeutically effective amount of a pharmaceutical composition according to any preceding embodiment, thereby treating the disease associated with the RAAS pathway.

Embodiment 69: The method of embodiment 68, wherein the disease is a cardiovascular disease.

Embodiment 70: The method of any of embodiments 68 and 69, wherein the disease is selected from hypertension, resistant hypertension, Marfan syndrome, heart failure, kidney disease, obesity, metabolic syndrome, NASH, and NAFLD.

Embodiment 71: The method of any of embodiments 68-70, wherein at least one symptom or hallmark of the disease is ameliorated.

Embodiment 72: The method of embodiment 71, wherein the symptom or hallmark is any of hypertension, hypertensive emergency (i.e. malignant hypertension), stroke, pre-eclampsia, aneurysms of the blood vessels, abdominal aneurysm, peripheral artery disease, organ damage, or pulmonary arterial hypertension.

Embodiment 73: The method of any of embodiments 67-72, wherein the pharmaceutical composition is administered systemically.

Embodiment 74: The method of any of embodiments 73, wherein the pharmaceutical composition is administered any of subcutaneously, or intramuscularly.

Embodiment 75: Use of an oligomeric compound of any of embodiments 1-37 or an oligomeric duplex of embodiment 40 for reducing AGT expression in a cell.

Embodiment 76: The use of embodiment 75, wherein the level of AGT RNA is reduced.

Embodiment 77: The use of embodiment 75, wherein the level of AGT protein is reduced.

Embodiment 78. An oligomeric compound comprising a modified oligonucleotide consisting of 14 to 30 linked nucleosides and having a nucleobase sequence comprising at least 14, at least 15, or at least 16 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 12-15, wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

Embodiment 79. An oligomeric compound comprising a modified oligonucleotide consisting of 14 to 30 linked nucleosides and having a nucleobase sequence comprising at least 14, at least 15, or at least 16 contiguous nucleobases complementary to:

a. an equal length portion of nucleobases 2046-2061 of SEQ ID NO: 1;
b. an equal length portion of nucleobases 2271-2286 of SEQ ID NO: 1;
c. an equal length portion of nucleobases 2272-2287 of SEQ ID NO: 1;

wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

Embodiment 80. An oligomeric compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of SEQ ID

NO: 12, wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

Embodiment 81. An oligomeric compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of SEQ ID NO: 13, wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

Embodiment 82. An oligomeric compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of SEQ ID NO: 14, wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

Embodiment 83. An oligomeric compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of SEQ ID NO: 15, wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

Embodiment 84. The oligomeric compound of any of embodiments 78-83, wherein the modified oligonucleotide has a nucleobase sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% complementary to the nucleobase sequence of any SEQ ID NO: 1 or SEQ ID NO: 2, when measured across the entire nucleobase sequence of the modified oligonucleotide.

Embodiment 85. The oligomeric compound of any of embodiments 78-84, wherein the modified oligonucleotide comprises at least one bicyclic sugar moiety.

Embodiment 86. The oligomeric compound of embodiment 85, wherein the bicyclic sugar moiety has a 4'-2' bridge, wherein the 4'-2' bridge is selected from $-CH_2-O-$; and $-CH(CH_3)-O-$.

Embodiment 87. The oligomeric compound of any of embodiments 78-86, wherein the modified oligonucleotide comprises at least one non-bicyclic modified sugar moiety.

Embodiment 88. The oligomeric compound of embodiment 87, wherein the non-bicyclic modified sugar moiety is a 2'-MOE sugar moiety or a 2'-OMe sugar moiety.

Embodiment 89. The oligomeric compound of any of embodiments 78-88, wherein the modified oligonucleotide comprises at least one sugar surrogate.

Embodiment 90. The oligomeric compound of embodiment 89, wherein the sugar surrogate is any of morpholino, modified morpholino, PNA, THP, and F-HNA.

Embodiment 91. The oligomeric compound of any of embodiments 78-90, wherein the modified oligonucleotide is a gapmer.

Embodiment 92. The oligomeric compound of any of embodiments 78-91, wherein the modified oligonucleotide has a sugar motif comprising:

a 5'-region consisting of 1-6 linked 5'-region nucleosides;

a central region consisting of 6-10 linked central region nucleosides; and

a 3'-region consisting of 1-6 linked 3'-region nucleosides; wherein

each of the 5'-region nucleosides and each of the 3'-region nucleosides comprises a modified sugar moiety and at least 6 of the central region nucleosides comprises a 2'-β-D-deoxyribosyl sugar moiety.

Embodiment 93. The oligomeric compound of any of embodiments 78-91, wherein the modified oligonucleotide has a sugar motif comprising:

a 5'-region consisting of 1-6 linked 5'-region nucleosides;

a central region consisting of 6-10 linked central region nucleosides; and

a 3'-region consisting of 1-6 linked 3'-region nucleosides; wherein

each of the 5'-region nucleosides and each of the 3'-region nucleosides comprises a modified sugar moiety and each of the central region nucleosides comprises a 2'-deoxyribosyl sugar moiety

Embodiment 94. The oligomeric compound of any of embodiments 78-91, wherein the modified oligonucleotide has a sugar motif comprising:

a 5'-region consisting of 3 linked 5'-region nucleosides;

a central region consisting of 10 linked central region nucleosides; and

a 3'-region consisting of 3 linked 3'-region nucleosides; wherein

each of the 5'-region nucleosides and each of the 3'-region nucleosides comprises a 2'-MOE sugar moiety or a cEt sugar moiety, and each of the central region nucleosides comprises a 2'-β-D-deoxyribosyl sugar moiety.

Embodiment 95. The oligomeric compound of any of embodiments 78-91, wherein the modified oligonucleotide has a sugar motif comprising:

a 5'-region consisting of 3 linked 5'-region nucleosides;

a central region consisting of 10 linked central region nucleosides; and

a 3'-region consisting of 43 linked 3'-region nucleosides; wherein

each of the 5'-region nucleosides and each of the 3'-region nucleosides comprises a 2'-MOE sugar moiety or a cEt sugar moiety, and at least 6 of the central region nucleosides comprises a 2'-β-D-deoxyribosyl sugar moiety.

Embodiment 96. The oligomeric compound of any of embodiments 78-95, wherein the modified oligonucleotide has a sugar motif (5' to 3') selected from eekddddddddddkke, ekkddddddddddkke, kkkdyddddddddkkk, kkkddyddddddddkkk, kkkdddyddddddkkk, kkkddddddddddkkk, or eeeeeddddddddddeeeee; wherein 'e' represents a 2'-MOE sugar moiety, 'k' represents a cEt sugar moiety, 'd' represents a 2'-β-D-deoxyribosyl sugar moiety, and 'y' represents a 2'-OMe sugar moiety.

Embodiment 97. The oligomeric compound of any of embodiments 78-96, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage.

Embodiment 98. The oligomeric compound of embodiment 97, wherein each internucleoside linkage of the modified oligonucleotide is a modified internucleoside linkage.

Embodiment 99. The oligomeric compound of embodiment 97 or embodiment 98, wherein at least one internucleoside linkage is a phosphorothioate internucleoside linkage.

Embodiment 100. The oligomeric compound of any of embodiments 97 and embodiment 99, wherein the modified oligonucleotide comprises at least one phosphodiester internucleoside linkage.

Embodiment 101. The oligomeric compound of any of embodiments 97, 99, and 100, wherein each internucleoside linkage is either a phosphodiester internucleoside linkage or a phosphorothioate internucleoside linkage.

Embodiment 102. The oligomeric compound of embodiment 98, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage.

Embodiment 103. The oligomeric compound of any of embodiments 78-97 or 99-101, wherein the modified oligonucleotide has an internucleoside linkage motif of soosssssssssos; wherein,

s = a phosphorothioate internucleoside linkage, and

o = a phosphodiester internucleoside linkage.

Embodiment 104. The oligomeric compound of any of embodiments 78-103, wherein the modified oligonucleotide comprises at least one modified nucleobase.

Embodiment 105. The oligomeric compound of embodiment 104, wherein the modified nucleobase is a 5-methylcytosine.

Embodiment 106. The oligomeric compound of any of embodiments 78-105, wherein the modified oligonucleotide consists of 14-30, 14-22, 14-20, 14-18, 14-20, 15-17, 15-25, or 16-20 linked nucleosides.

Embodiment 107. The oligomeric compound of any of embodiments 78-106, wherein the modified oligonucleotide consists of 16 linked nucleosides.

Embodiment 108. The oligomeric compound of any of embodiments 78-107, comprising a conjugate group.

Embodiment 109. The oligomeric compound of embodiment 108, wherein the conjugate group comprises a GalNAc cluster comprising 1-3 GalNAc ligands.

Embodiment 110. The oligomeric compound of any one of embodiments 108 and 109, wherein the conjugate group comprises a conjugate linker consisting of a single bond.

Embodiment 111. The oligomeric compound of any one of embodiments 108-110, wherein the conjugate group comprises a cleavable linker.

Embodiment 112. The oligomeric compound of any one of embodiments 108-111, wherein the conjugate group comprises a conjugate linker comprising 1-3 linker-nucleosides.

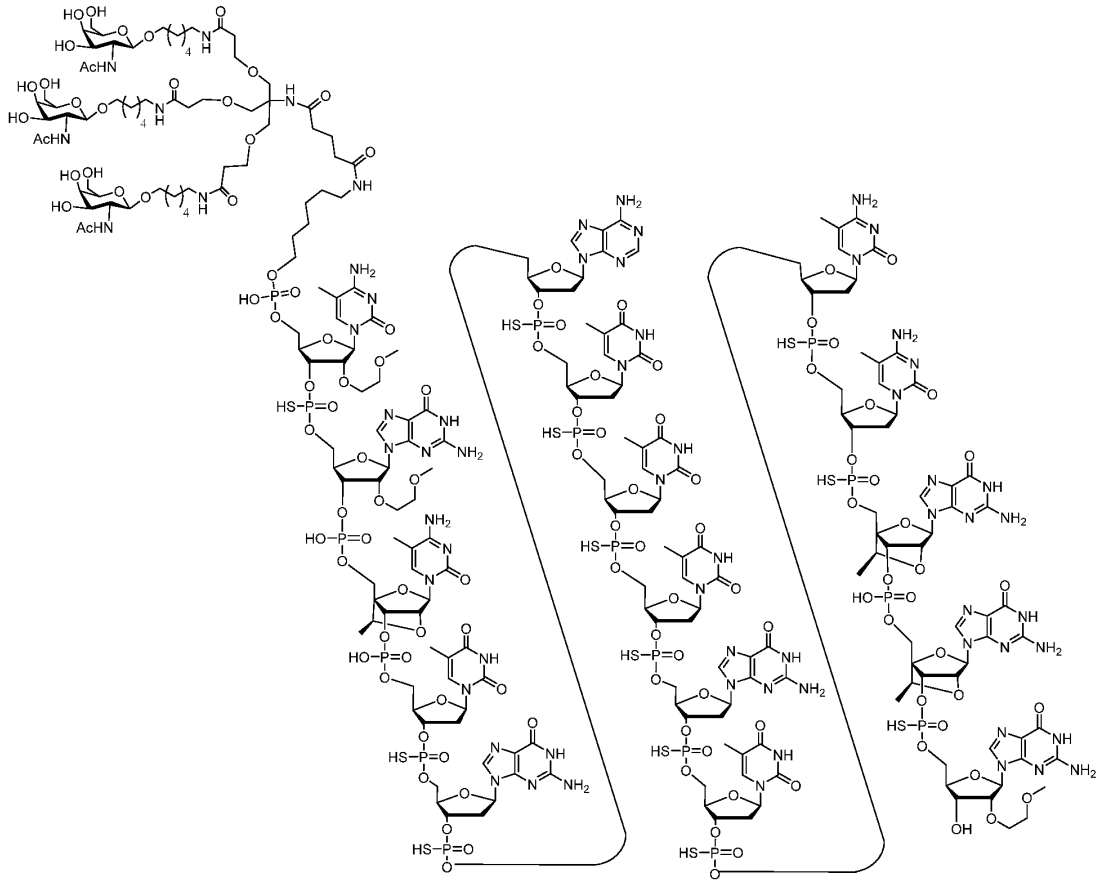
Embodiment 113. The oligomeric compound of any one of embodiments 108-112, wherein the conjugate group is attached to the modified oligonucleotide at the 5'-nucleoside of the modified oligonucleotide.

Embodiment 114. The oligomeric compound of any one of embodiments 108-113, wherein the conjugate group is attached to the modified oligonucleotide at the 3'-nucleoside of the modified oligonucleotide.

Embodiment 115. The oligomeric compound of any of embodiments 78-114, wherein the oligomeric compound is a singled-stranded oligomeric compound.

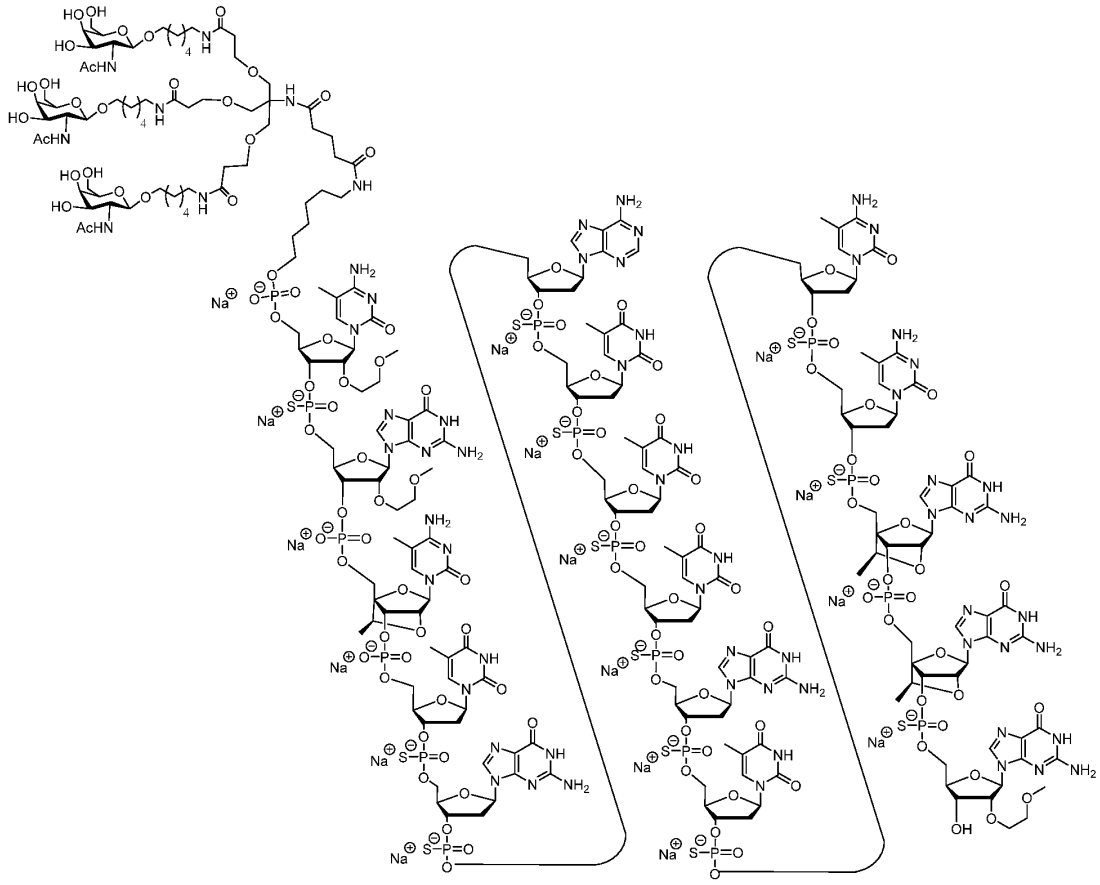
Embodiment 116. The oligomeric compound of any of embodiments 78-107 or 115, consisting of the modified oligonucleotide.

Embodiment 117. An oligomeric compound according to the following chemical structure:



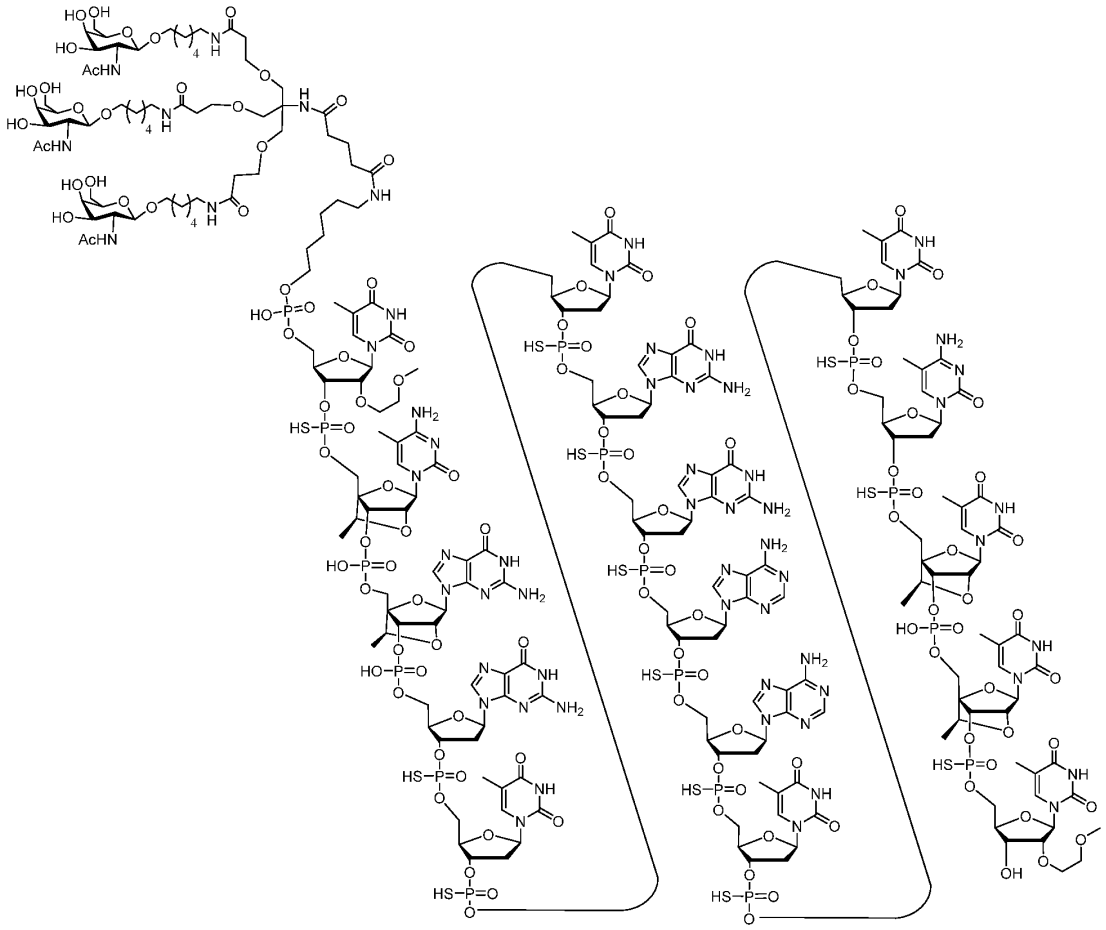
(SEQ ID NO: 12), or a salt thereof.

Embodiment 118. An oligomeric compound according to the following chemical structure:



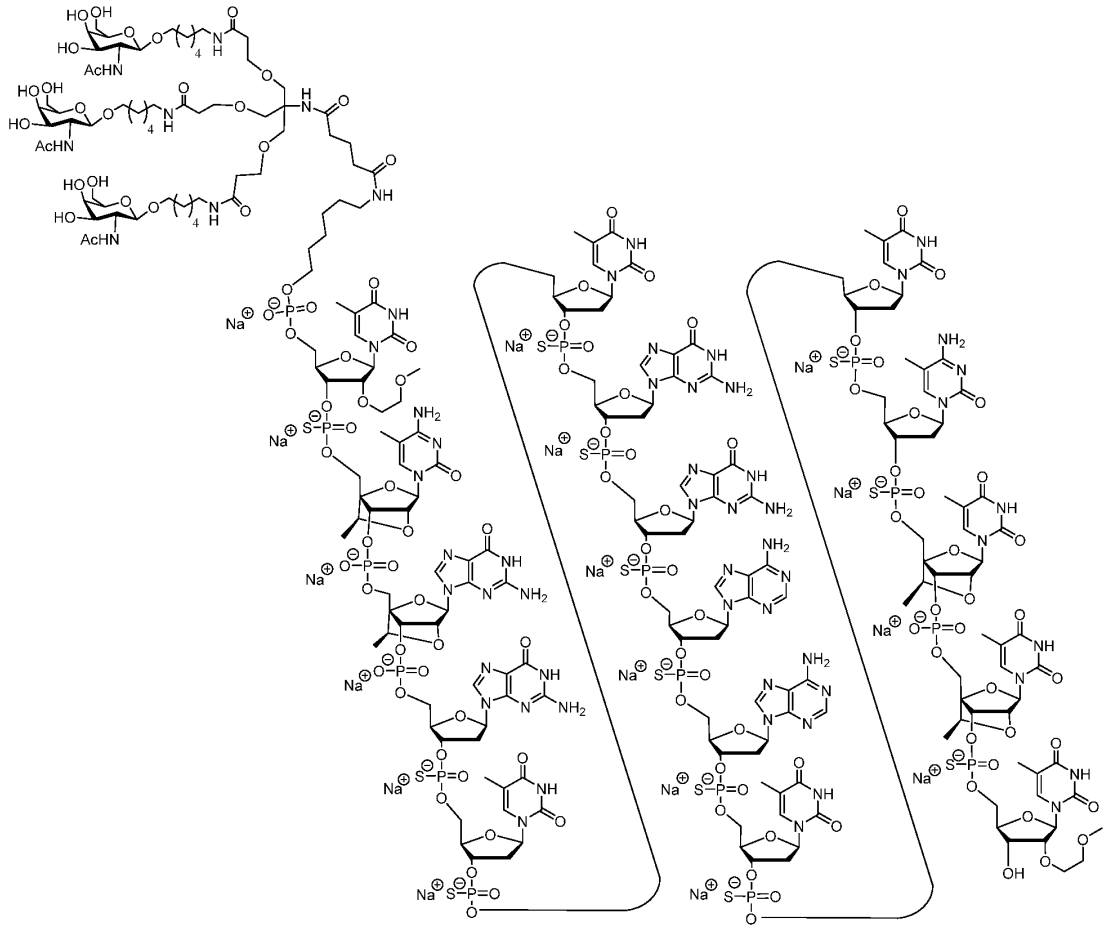
(SEQ ID NO: 12).

Embodiment 119. An oligomeric compound according to the following chemical structure:



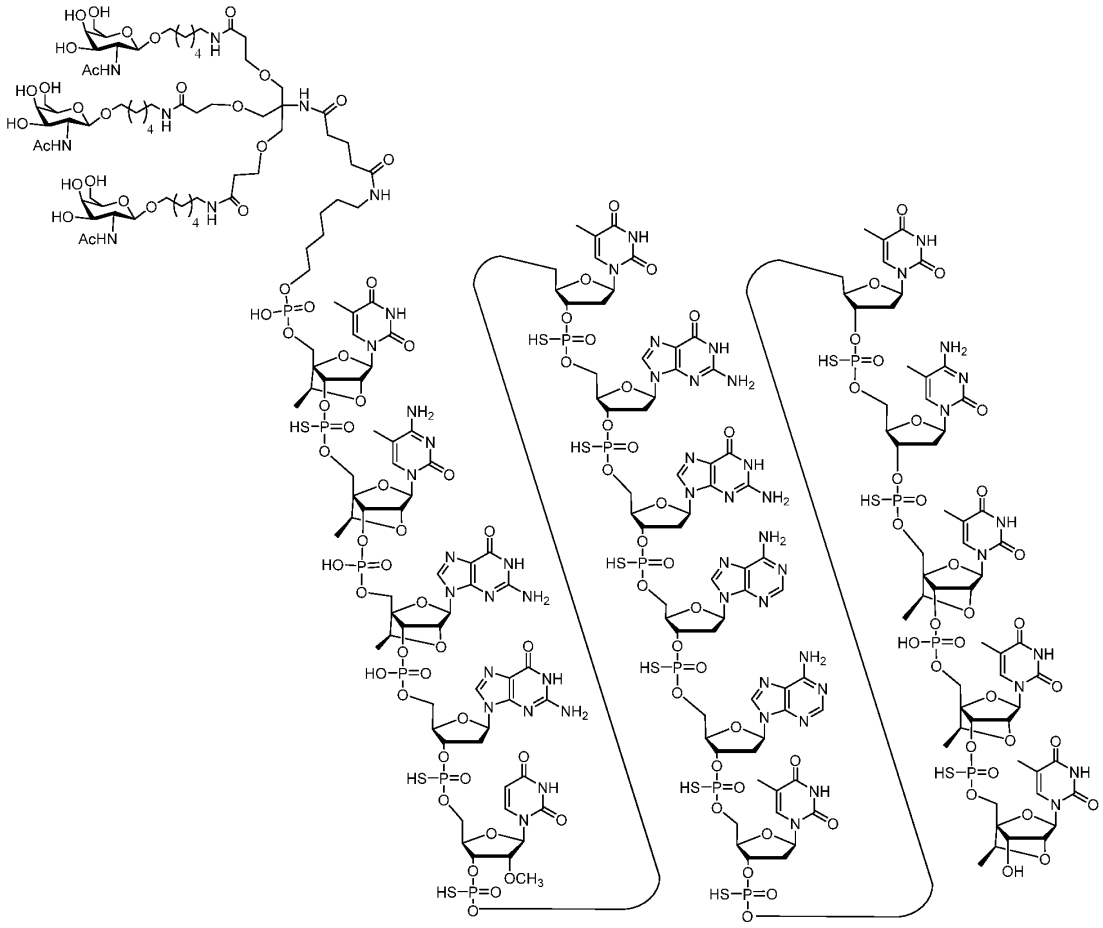
(SEQ ID NO: 13), or a salt thereof.

Embodiment 120. An oligomeric compound according to the following chemical structure:



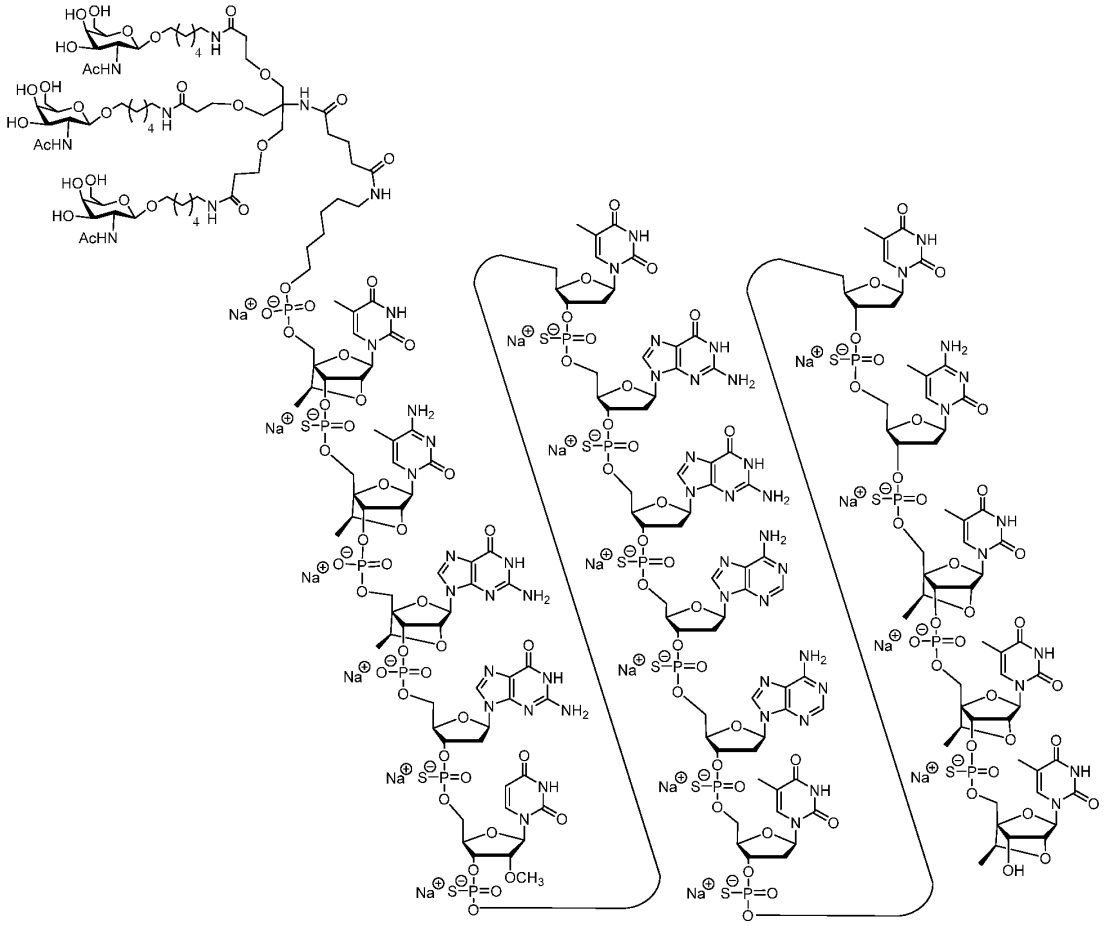
(SEQ ID NO: 13).

Embodiment 121. An oligomeric compound according to the following chemical structure:



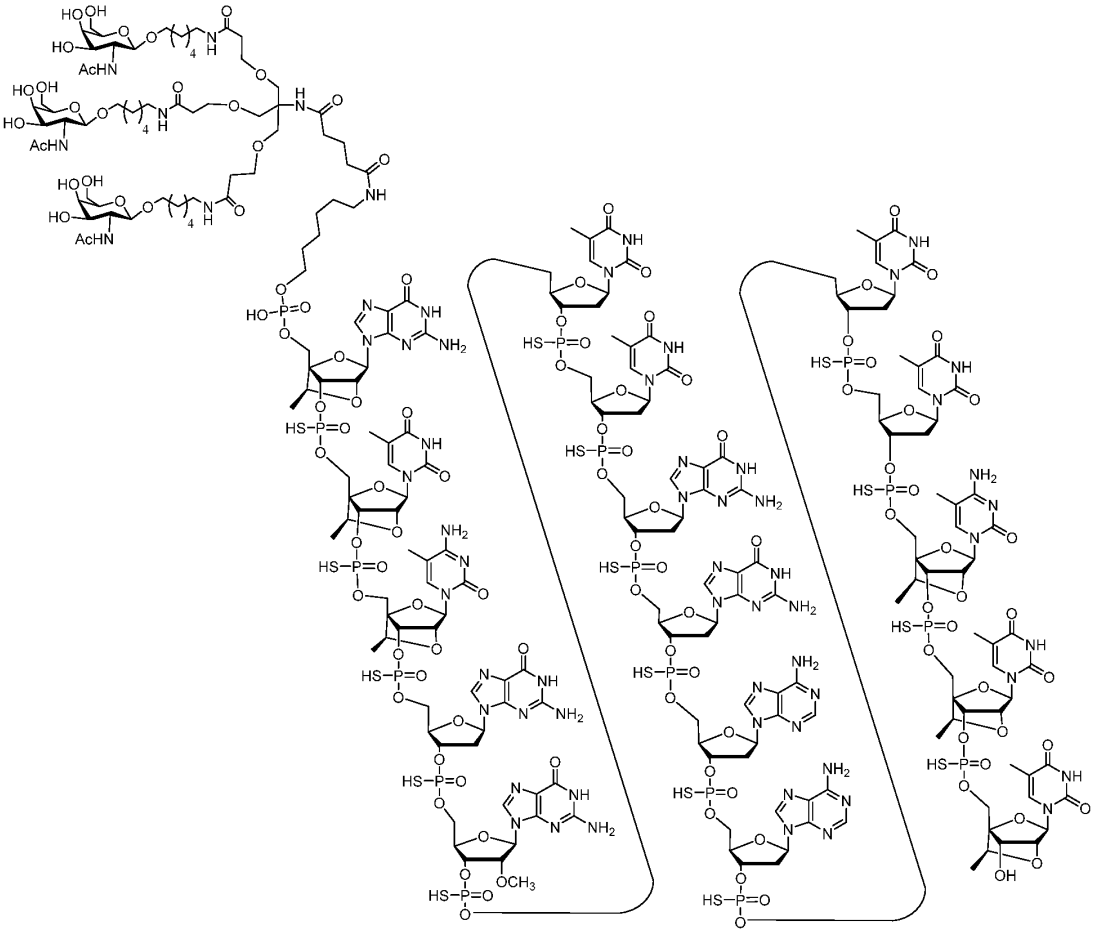
(SEQ ID NO: 14), or a salt thereof.

Embodiment 122. An oligomeric compound according to the following chemical structure:



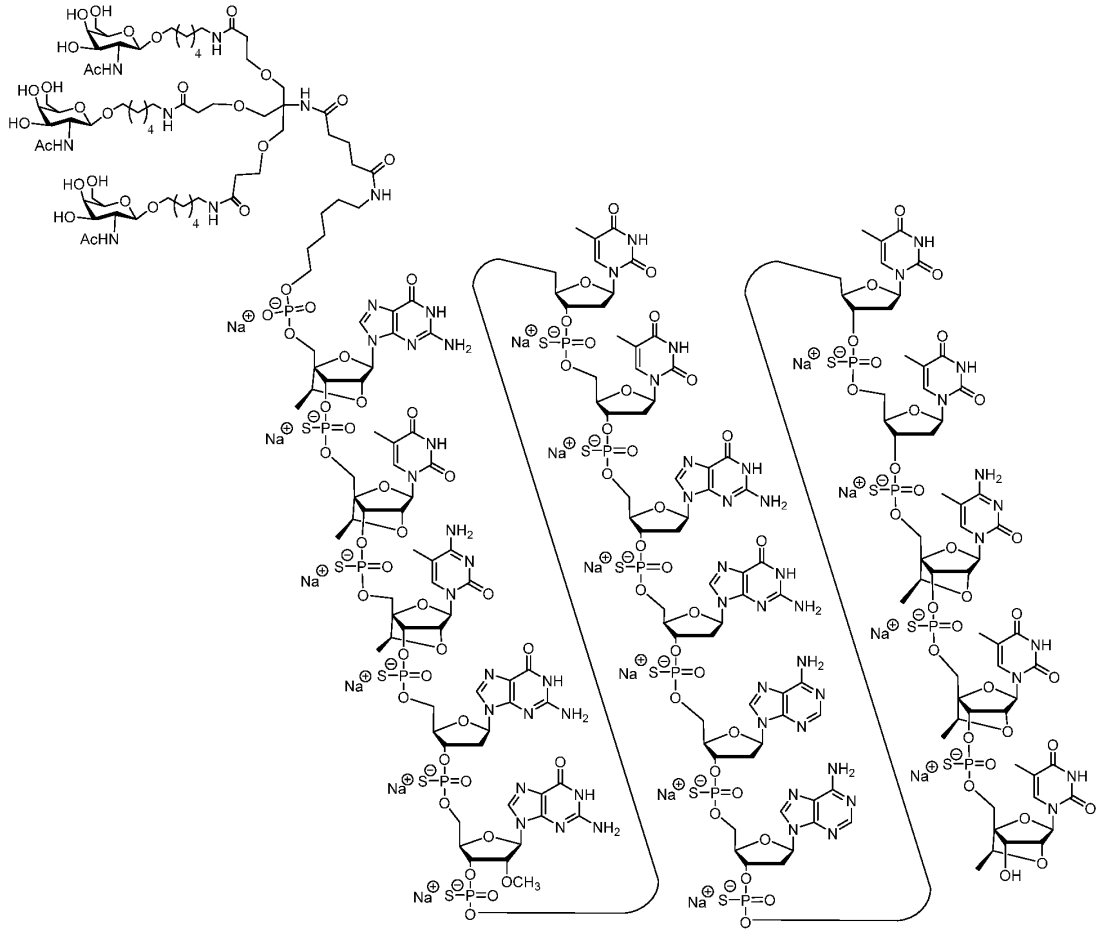
(SEQ ID NO: 14).

Embodiment 123. An oligomeric compound according to the following chemical structure:



(SEQ ID NO: 15), or salt thereof.

Embodiment 124. An oligomeric compound according to the following chemical structure:



(SEQ ID NO: 15).

Embodiment 125. The oligomeric compound of any of embodiments 117, 119,121, or 123, which is the sodium salt or potassium salt.

Embodiment 126. An oligomeric compound comprising a modified oligonucleotide according to the following chemical notation: $^{m}C_{es}G_{eo}{}^{m}C_{ko}T_{ds}G_{ds}A_{ds}T_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}G_{ko}G_{ks}G_{e}$ (SEQ ID NO: 12), wherein:

A = an adenine nucleobase,

^{m}C = a 5-methyl cytosine nucleobase,

G = a guanine nucleobase,

T = a thymine nucleobase,

e = a 2'-β-D-MOE sugar moiety,

k = a cEt sugar moiety,

d = a 2'-β-D-deoxyribosyl sugar moiety,

s = a phosphorothioate internucleoside linkage, and

o = a phosphodiester internucleoside linkage.

Embodiment 127. An oligomeric compound comprising a modified oligonucleotide according to the following chemical notation: $T_{es}{}^{m}C_{ko}G_{ko}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}A_{ds}A_{ds}T_{ds}T_{ds}{}^{m}C_{ds}T_{ko}T_{ks}T_{e}$ (SEQ ID NO: 13), wherein:

A = an adenine nucleobase,

^{m}C = a 5-methyl cytosine nucleobase,

G = a guanine nucleobase,

T = a thymine nucleobase,

e = a 2'-β-D-MOE sugar moiety,

k = a cEt sugar moiety,

d = a 2'-β-D-deoxyribosyl sugar moiety,

s = a phosphorothioate internucleoside linkage, and

o = a phosphodiester internucleoside linkage.

Embodiment 128. An oligomeric compound comprising a modified oligonucleotide according to the following chemical notation: $G_{ks}T_{ks}{}^{m}C_{ks}G_{ds}G_{ys}T_{ds}T_{ds}G_{ds}G_{ds}A_{ds}A_{ds}T_{ds}T_{ds}{}^{m}C_{ks}T_{ks}T_{k}$ (SEQ ID NO: 15), wherein:

A = an adenine nucleobase,

^{m}C = a 5-methyl cytosine nucleobase,

G = a guanine nucleobase,

T = a thymine nucleobase,

k = a cEt sugar moiety,

d = a 2'-β-D-deoxyribosyl sugar moiety,

y = a 2'-OMe ribose sugar moiety, and

s = a phosphorothioate internucleoside linkage.

Embodiment 129. An oligomeric compound comprising a modified oligonucleotide according to the following chemical notation: $T_{ks}{}^{m}C_{ko}G_{ko}G_{ds}U_{ys}T_{ds}G_{ds}G_{ds}A_{ds}A_{ds}T_{ds}T_{ds}{}^{m}C_{ds}T_{ko}T_{ks}T_{k}$ (SEQ ID NO: 14), wherein:

A = an adenine nucleobase,

^{m}C = a 5-methyl cytosine nucleobase,

G = a guanine nucleobase,

T = a thymine nucleobase,

U = a uracil nucleobase,

k = a cEt sugar moiety,

d = a 2'-β-D-deoxyribosyl sugar moiety,

y = a 2'-OMe ribose sugar moiety,

s = a phosphorothioate internucleoside linkage, and

o = a phosphodiester internucleoside linkage.

Embodiment 130. The oligomeric compound of any of embodiments 126-129, comprising the modified oligonucleotide covalently linked to a conjugate group.

Embodiment 131. An oligomeric duplex comprising an oligomeric compound of any of embodiments 78-130.

Embodiment 132. An antisense compound comprising or consisting of an oligomeric compound of any of embodiments 78-130 or an oligomeric duplex of embodiment 131.

Embodiment 133. A chirally enriched population of oligomeric compounds of any of embodiments 117-130, wherein the population is enriched for compounds comprising at least one particular phosphorothioate internucleoside linkage having a particular stereochemical configuration.

Embodiment 134. The chirally enriched population of embodiment 133, wherein the population is enriched for compounds comprising at least one particular phosphorothioate internucleoside linkage having the (*S*p) configuration.

Embodiment 135. The chirally enriched population of embodiment 133, wherein the population is enriched for compounds comprising at least one particular phosphorothioate internucleoside linkage having the (*R*p) configuration.

Embodiment 136. The chirally enriched population of embodiment 133, wherein the population is enriched for compounds having a particular, independently selected stereochemical configuration at each phosphorothioate internucleoside linkage.

Embodiment 137. The chirally enriched population of embodiment 133, wherein the population is enriched for compounds having the (*S*p) configuration at each phosphorothioate internucleoside linkage or for modified oligonucleotides having the (*R*p) configuration at each phosphorothioate internucleoside linkage.

Embodiment 138. The chirally enriched population of embodiment 133, wherein the population is enriched for compounds having the (Rp) configuration at one particular phosphorothioate internucleoside linkage and the (Sp) configuration at each of the remaining phosphorothioate internucleoside linkages.

Embodiment 139. The chirally enriched population of embodiment 133, wherein the population is enriched for compounds having at least 3 contiguous phosphorothioate internucleoside linkages in the Sp, Sp, and Rp configurations, in the 5' to 3' direction.

Embodiment 140. A population of oligomeric compounds of any of embodiments 117-130, in which all phosphorothioate internucleoside linkages of the oligomeric compound are stereorandom.

Embodiment 141. A pharmaceutical composition comprising the oligomeric compound of any of embodiments 78-130, the oligomeric duplex of embodiment 131, the antisense compound of embodiment 132, or the population of any of embodiments 133-140 and a pharmaceutically acceptable carrier or diluent.

Embodiment 142. A method comprising administering to an individual the pharmaceutical composition of embodiment 141.

Embodiment 143. A method of treating a disease associated with the RAAS pathway, comprising administering to an individual having or at risk of having a disease associated with the RAAS pathway a therapeutically effective amount of the pharmaceutical composition according to embodiment 142, thereby treating the disease associated with the RAAS pathway.

Embodiment 144. The method of embodiment 143, wherein the disease is a cardiovascular disease.

Embodiment 145. The method of any of embodiments 143 and 144, wherein the disease is selected from hypertension, resistant hypertension, Marfan syndrome, heart failure, kidney disease, obesity, metabolic syndrome, NASH, and NAFLD.

Embodiment 146. The method of any of embodiments 143-145, wherein at least one symptom or hallmark of the disease is ameliorated.

Embodiment 147. The method of embodiment 146, wherein the symptom or hallmark is any of hypertension, hypertensive emergency (i.e. malignant hypertension), stroke, pre-eclampsia, aneurysms of the blood vessels, abdominal aneurysm, peripheral artery disease, organ damage, or pulmonary arterial hypertension.

Embodiment 148. The method of any of embodiments 142-147, wherein the pharmaceutical composition is administered systemically.

Embodiment 149. The method of any of embodiments 142-148, wherein the pharmaceutical composition is administered subcutaneously or intramuscularly.

Embodiment 150. Use of the oligomeric compound of any of embodiments 78-130, the oligomeric duplex of embodiment 131, the antisense compound of embodiment 132, or the population of any of embodiments 133-140 for reducing AGT expression in a cell.

Embodiment 151. The use of embodiment 150, wherein the level of AGT RNA is reduced.

Embodiment 152. The use of embodiment 150, wherein the level of AGT protein is reduced.

I. 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.

A. Certain Modified Nucleosides

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

1. 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'-OCH_3 ("OMe" or "O-methyl"), and 2'-$O(CH_2)_2OCH_3$ ("MOE" or "O-methoxyethyl"). In certain embodiments, 2'-substituent groups are selected from among: halo, allyl, amino, azido, SH, CN, OCN, CF_3, OCF_3, O-C_1-C_{10} alkoxy, O-C_1-C_{10} substituted alkoxy, O-C_1-C_{10} alkyl, O-C_1-C_{10} 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, $O(CH_2)_2SCH_3$, $O(CH_2)_2ON(R_m)(R_n)$ or $OCH_2C(=O)$-$N(R_m)(R_n)$, where each R_m and R_n is, independently, H, an amino protecting group, or substituted or unsubstituted C_1-C_{10} 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 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 (NO_2), 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, NH_2, N_3, OCF_3, OCH_3, $O(CH_2)_3NH_2$, $CH_2CH{=}CH_2$, $OCH_2CH{=}CH_2$, $OCH_2CH_2OCH_3$, $O(CH_2)_2SCH_3$, $O(CH_2)_2ON(R_m)(R_n)$, $O(CH_2)_2O(CH_2)_2N(CH_3)_2$, and N-substituted acetamide ($OCH_2C(=O)$-$N(R_m)(R_n)$), where each R_m and R_n is, independently, H, an amino protecting group, or substituted or unsubstituted C_1-C_{10} alkyl.

In certain embodiments, a 2'-substituted non-bicyclic modified nucleoside comprises a sugar moiety comprising a non-bridging 2'-substituent group selected from: F, OCF_3, OCH_3, $OCH_2CH_2OCH_3$, $O(CH_2)_2SCH_3$, $O(CH_2)_2ON(CH_3)_2$, $O(CH_2)_2O(CH_2)_2N(CH_3)_2$, and $OCH_2C(=O)$-$N(H)CH_3$ ("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, OCH_3, and $OCH_2CH_2OCH_3$.

In certain embodiments, modified furanosyl sugar moieties and nucleosides incorporating such modified furanosyl sugar moieties are further defined by isomeric configuration. For example, a 2'-deoxyfuranosyl sugar moiety may be in seven isomeric configurations other than the naturally occurring β-D-deoxyribosyl configuration. Such modified sugar moieties are described in, e.g., WO 2019/157531, incorporated by reference herein. A 2'-modified sugar moiety has an additional stereocenter at the 2'-position relative to a 2'-deoxyfuranosyl sugar moiety; therefore, such sugar moieties have a total of sixteen possible isomeric configurations. 2'-modified sugar moieties described herein are in the β-D-ribosyl isomeric configuration unless otherwise specified.

Certain modifed 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'-CH_2-2', 4'-$(CH_2)_2$-2', 4'-$(CH_2)_3$-2', 4'-CH_2-O-2' ("LNA"), 4'-CH_2-S-2', 4'-$(CH_2)_2$-O-2' ("ENA"), 4'-$CH(CH_3)$-O-2' (referred to as "constrained ethyl" or "cEt"), 4'-CH_2-O-CH_2-2', 4'-CH_2-N(R)-2', 4'-$CH(CH_2OCH_3)$-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'-$C(CH_3)(CH_3)$-O-2' and analogs thereof (*see, e.g.,*Seth et al., U.S. 8,278,283), 4'-CH_2-$N(OCH_3)$-2' and analogs thereof (*see, e.g.,* Prakash et al., U.S. 8,278,425), 4'-CH_2-O-$N(CH_3)$-2' (*see, e.g.,* Allerson et al., U.S. 7,696,345 and Allerson et al., U.S. 8,124,745), 4'-CH_2-$C(H)(CH_3)$-2' (*see, e.g.,* Zhou, *et al., J. Org. Chem.,*2009, *74*, 118-134), 4'-CH_2-$C(=CH_2)$-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'-CH_2-O-N(R)-2', and 4'-CH_2-N(R)-O-2', wherein each R, R_a, and R_b is, independently, H, a protecting group, or C_1-C_{12} 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 $-N(R_a)-$;

wherein:

x is 0, 1, or 2;

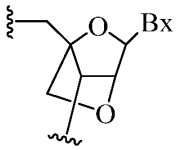
n is 1, 2, 3, or 4;

each R_a and R_b is, independently, H, a protecting group, hydroxyl, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, heterocycle radical, substituted heterocycle radical, heteroaryl, substituted heteroaryl, C_5-C_7 alicyclic radical, substituted C_5-C_7 alicyclic radical, halogen, OJ_1, NJ_1J_2, SJ_1, N_3, $COOJ_1$, acyl (C(=O)-H), substituted acyl, CN, sulfonyl ($S(=O)_2-J_1$), or sulfoxyl ($S(=O)-J_1$); and

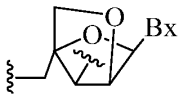
each J_1 and J_2 is, independently, H, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, acyl (C(=O)-H), substituted acyl, a heterocycle radical, a substituted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

Additional bicyclic sugar moieties are known in the art, see, for example: Freier *et al., Nucleic Acids Research*, 1997, *25*(*22*), 4429-4443, Albaek *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 a., 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; 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 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'-CH_2-O-2'



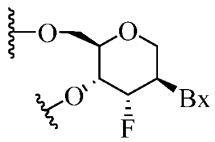
α-*L*-LNA (α-*L*-configuration)
bridge = 4'-CH_2-O-2'

α-L-methyleneoxy (4'-CH_2-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).

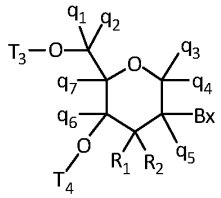
In certain embodiments, modified sugar moieties are sugar surrogates. In certain such embodiments, the oxygen atom of the sugar moiety is replaced, *e.g.*, with a sulfur, carbon or nitrogen atom. In certain such embodiments, such modified sugar moieties also comprise bridging and/or non-bridging substituents 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 the modified THP nucleosides:

Bx is a nucleobase moiety;

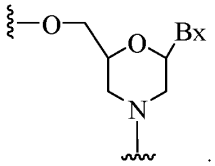
T_3 and T_4 are each, independently, an internucleoside linking group linking the modified THP nucleoside to the remainder of an oligonucleotide or one of T_3 and T_4 is an internucleoside linking group linking the modified THP nucleoside to the remainder of an oligonucleotide and the other of T_3 and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

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

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

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

In certain embodiments, sugar surrogates comprise rings having more than 5 atoms and more than one heteroatom. For example, nucleosides comprising morpholino sugar moieties and their use in 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:



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

In certain embodiments, sugar surrogates comprise acyclic moieites. 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.

2. Certain Modified Nucleobases

In certain embodiments, modified oligonucleotides comprise one or more nucleosides comprising an unmodified nucleobase. In certain embodiments, modified oligonucleotides comprise one or more nucleoside comprising a modified nucleobase. In certain embodiments, modified oligonucleotides comprise one or more nucleoside 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-propyladenine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-propynyl (-C≡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-isobutyrylguanine, 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-deaza-adenine, 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.

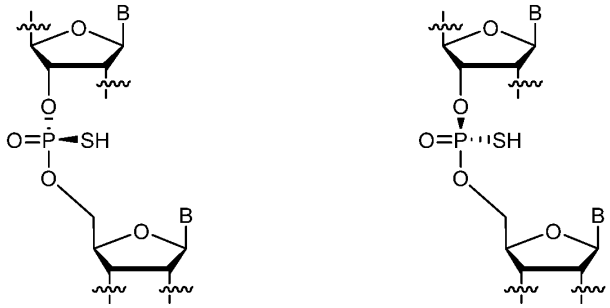
5,134,066; Bischofberger et al., U.S. 5,175,273; Urdea et al., U.S. 5,367,066; Benner et al., U.S. 5,432,272; Matteucci et al., U.S. 5,434,257; Gmeiner et al., U.S. 5,457,187; Cook et al., U.S. 5,459,255; Froehler et al., U.S. 5,484,908; Matteucci et al., U.S. 5,502,177; Hawkins et al., U.S. 5,525,711; 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.

3. 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 phosphodiesters, which contain a phosphodiester bond ("$P(O_2)=O$") (also referred to as unmodified or naturally occurring linkages), phosphotriesters, methylphosphonates, 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 phosphodiester internucleoside 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 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 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 linkage in a particular, independently

selected stereochemical configuration. In certain embodiments, the particular configuration of the 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 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* 125, 8307 (2003), Wan et al. *Nuc. Acid. Res.* 42, 13456 (2014), and WO 2017/015555. In certain embodiments, a population of modified oligonucleotides is enriched for modified oligonucleotides having at least one indicated phosphorothioate in the *(S*p*)* configuration. In certain embodiments, a population of modified oligonucleotides is enriched for modified oligonucleotides having at least one phosphorothioate in the *(R*p*)* configuration. In certain embodiments, modified oligonucleotides comprising *(R*p*)* and/or *(S*p*)* phosphorothioates comprise one or more of the following formulas, respectively, wherein "B" indicates a nucleobase:



(R_p) (S_p)

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

Neutral internucleoside linkages include, without limitation, phosphotriesters, methylphosphonates, MMI (3'-CH_2-N(CH_3)-O-5'), amide-3 (3'-CH_2-C(=O)-N(H)-5'), amide-4 (3'-CH_2-N(H)-C(=O)-5'), formacetal (3'-O-CH_2-O-5'), methoxypropyl (MOP), and thioformacetal (3'-S-CH_2-O-5'). Further neutral internucleoside linkages include nonionic linkages 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 CH_2 component parts.

B. 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 comprising a modified nucleobase. In certain embodiments, modified oligonucleotides comprise one or more modified internucleoside linkage. 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 linkage motif (as used herein, nucleobase motif describes the modifications to the nucleobases independent of the sequence of nucleobases).

1. **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 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. 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 nucleoside of each wing of a gapmer comprises a modified sugar moiety. In certain embodiments, at least two nucleosides of each wing of a gapmer comprises a modified sugar moiety. In certain embodiments, at least three nucleosides of each wing of a gapmer comprises a modified sugar moiety. In certain embodiments, at least four nucleosides of each wing of a gapmer comprises a modified sugar moiety. In certain embodiments, at least five 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, at least six nucleosides of the gap of a gapmer comprise a 2'-β-D-deoxyribosyl sugar moiety. In certain embodiments, each nucleoside of the gap of a gapmer comprises a 2'-deoxyribosyl sugar moietyIn certain embodiments, each nucleoside of the gap of a gapmer comprises a 2'-β-D-deoxyribosyl sugar moiety. In certain embodiments, at least one nucleoside of the gap of a gapmer comprises a modified sugar moiety. In certain embodiments, at least one nucleoside of the gap of a gapmer comprises a 2'-OMe sugar moiety.

In certain embodiments, the gapmer is a deoxy gapmer. In certain embodiments, the nucleosides on the gap side of each wing/gap junction comprise 2'- deoxyribosyl sugar moieties and the nucleosides on the wing sides of each wing/gap junction comprise modified sugar moieties. In certain embodiments, at least six nucleosides of the gap of a gapmer comprise a 2'-β-D-deoxyribosyl sugar moiety. In certain embodiments, each nucleoside of the gap comprises a 2'-deoxyribosyl sugar moiety. In certain embodiments, each nucleoside of each wing of a gapmer comprises a modified sugar moiety. In certain embodiments, one nucleoside of the gap comprises a modified sugar moiety and each remaining nucleoside of the gap comprises a 2'-deoxyribosyl sugar moiety.

In certain embodiments, modified oligonucleotides comprise or consist of a portion having a fully modified sugar motif. In such embodiments, each nucleoside of the fully modified portion of the modified oligonucleotide comprises a modified sugar moiety. In certain embodiments, each nucleoside of the entire modified oligonucleotide comprises a modified sugar moiety. In certain embodiments, modified oligonucleotides comprise or consist of a portion having a fully modified sugar motif, wherein each nucleoside within the fully modified portion comprises the same modified sugar moiety, referred to herein as a uniformly modified sugar motif. In certain embodiments, a fully modified oligonucleotide is a uniformly modified oligonucleotide. In certain embodiments, each nucleoside of a uniformly modified oligonucleotide comprises the same 2'-modification.

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 comprises a 2'- β-D-deoxyribosyl sugar moiety. Thus, a 5-10-5 MOE gapmer consists of 5 linked 2'-MOE nucleosides in the 5'-wing, 10 linked 2'- β-D-deoxynucleosides in the gap, and 5 linked 2'-MOE nucleosides in the 3'-wing. A 3-10-3 cEt gapmer consists of 3 linked cEt nucleosides in the 5'-wing, 10 linked 2'- β-D-deoxynucleosides in the gap, and 3 linked cEt nucleosides in the 3'-wing. A 5-8-5 gapmer consists of 5 linked nucleosides comprising a modified sugar moiety in the 5'-wing, 8 linked 2'-deoxynucleosides in the gap, and 5 linked nucleosides comprising a modified sugar moiety in the 3'-wing. A mixed wing gapmer has at least two different modified sugars in the

5' and/or 3' wing. A 5-8-5 or 5-8-4 mixed wing gapmer has at least two different modified sugar moieties in the 5'- and/or the 3'-wing.

In certain embodiments, modified oligonucleotides are 5-10-5 MOE gapmers. In certain embodiments, modified oligonucleotides are 4-10-6 MOE gapmers. In certain embodiments, modified oligonucleotides are 6-10-4 MOE gapmers. In certain embodiments, modified oligonucleotides are 5-8-5 MOE gapmers. In certain embodiments, modified oligonucleotides are X-Y-Z MOE gapmers, wherein X and Z are independently selected from 1, 2, 3, 4, 5, or 6 linked 2'-MOE nucleosides and Y is 7, 8, 9, 10, or 11 linked deoxynucleosides.

In certain embodiments, modified oligonucleotides have a sugar motif selected from the following (5' to 3'): meeemddddddddddmmmmm, wherein 'd' represents a 2'-deoxyribosyl sugar moiety, 'e' represents a 2'-MOE sugar moiety, and 'm' represents a 2'-OMe sugar moiety.

2. 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'-deoxyribosyl sugar moiety. In certain embodiments, the modified nucleobase is selected from: a 2-thiopyrimidine and a 5-propynepyrimidine.

3. 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 ($P(O_2)=O$). In

certain embodiments, each internucleoside linking group of a modified oligonucleotide is a phosphorothioate internucleoside linkage ($P(O_2)=S$). 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 *(Sp)* phosphorothioate, and a *(Rp)* 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 *S*p, *S*p, *R*p motif. In certain embodiments, populations of modified oligonucleotides are enriched for modified oligonucleotides comprising such internucleoside linkage motifs.

In certain embodiments, all of the internucleoside linkages are either phosphodiester internucleoside linkages or phosphorothioate internucleoside linkages, and the chiral motif is (5' to 3'): *S*p-o-o-o-*S*p-*S*p-*S*p-*R*p-*S*p-*S*p-*R*p-*S*p-*S*p-*S*p-*S*p-*S*p-*S*p-*S*p-*S*p or *S*p-o-o-o-*S*p-*S*p-*S*p-*R*p-*S*p-*S*p-*S*p-*S*p-*S*p-*S*p-*S*p-*S*p-*S*p-*S*p-*S*p, wherein each '*S*p' represents a (*S*p) phosphorothioate internucleoside linkage, each '*R*p' is a *R*p internucleoside linkage, and each 'o' represents a phosphodiester internucleoside linkage. In certain embodiments, populations of modified oligonucleotides are enriched for modified oligonucleotides comprising such internucleoside linkage motifs.

In certain embodiments, modified oligonucleotides have an internucleoside linkage motif of sooossssssssssssooss, wherein each "s" represents a phosphorothioate internucleoside linkage and each "o" represents a phosphodiester internucleoside linkage. In certain embodiments, modified oligonucleotides have an internucleoside linkage motif of (5' to 3'): sooooossssssssssoss, wherein each "s" represents a phosphorothioate internucleoside linkage and each "o" represents a phosphodiester internucleoside linkage. In certain embodiments, modified oligonucleotides have an internucleoside linkage motif of (5' to 3'): soooossssssssssooss, wherein each "s" represents a phosphorothioate internucleoside linkage and each "o" represents a phosphodiester internucleoside linkage. In certain embodiments, modified oligonucleotides have an internucleoside linkage motif of (5' to 3'):sooossssssssssooss, wherein each "s" represents a phosphorothioate internucleoside linkage and each "o" represents a phosphodiester internucleoside linkage. In certain embodiments, modified oligonucleotides have an internucleoside linkage motif of (5' to 3'):

sooossssssssssoooss, wherein each "s" represents a phosphorothioate internucleoside linkage and each "o" represents a phosphodiester internucleoside linkage. In certain embodiments, modified oligonucleotides have an internucleoside linkage motif of (5' to 3'): sooossssssssssssss, wherein each "s" represents a phosphorothioate internucleoside linkage and each "o" represents a phosphodiester internucleoside linkage.

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C. 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 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
10 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
15 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,
20 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
25 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,
30 19 to 29, 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
35 30, or 29 to 30 linked nucleosides.

D. 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.

E. 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.

F. 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.

II. 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. 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 embodiments, conjugate groups are attached near the 3'-end of oligonucleotides. In certain embodiments, conjugate groups (or terminal groups) are attached at the 5'-end of oligonucleotides. In certain embodiments, conjugate 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.

A. 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 palmityl 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).

1. **Conjugate Moieties**

Conjugate moieties include, without limitation, intercalators, reporter molecules, polyamines,

polyamides, peptides, carbohydrates, vitamin moieties, polyethylene glycols, thioethers, polyethers, cholesterols, thiocholesterols, 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, folinic acid, a benzothiadiazide, chlorothiazide, a diazepine, indo-methicin, a barbiturate, a cephalosporin, a sulfa drug, an antidiabetic, an antibacterial or an antibiotic.

2. **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 (AHEX or AHA). Other conjugate linkers include but are not limited to substituted or unsubstituted C_1-C_{10} alkyl, substituted or unsubstituted C_2-C_{10} alkenyl or substituted or unsubstituted C_2-C_{10} 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.

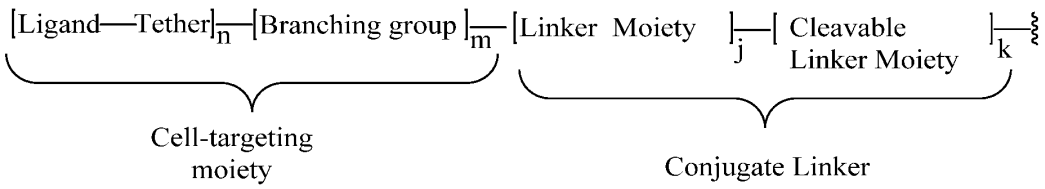
For example, in certain circumstances oligomeric compounds comprising a particular conjugate moiety are better taken up by a particular cell type, but once the oligomeric compound has been taken up, it is desirable that the conjugate group be cleaved to release the unconjugated or parent oligonucleotide. 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 or phosphodiester 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'-deoxynucleoside that is attached to either the 3' or 5'-terminal nucleoside of an oligonucleotide by a phosphodiester 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.

3. **Cell-Targeting Moieties**

In certain embodiments, a conjugate group comprises a cell-targeting moiety. In certain embodiments, a conjugate group has the general formula:



wherein n is from 1 to about 3, m is 0 when n is 1, m is 1 when n is 2 or greater, j is 1 or 0, and k is 1 or 0.

In certain embodiments, n is 1, j is 1 and k is 0. In certain embodiments, n is 1, j is 0 and k is 1. In certain embodiments, n is 1, j is 1 and k is 1. In certain embodiments, n is 2, j is 1 and k is 0. In certain

embodiments, n is 2, j is 0 and k is 1. In certain embodiments, n is 2, j is 1 and k is 1. In certain embodiments, n is 3, j is 1 and k is 0. In certain embodiments, n is 3, j is 0 and k is 1. In certain embodiments, n is 3, j is 1 and k is 1.

In certain embodiments, conjugate groups comprise cell-targeting moieties that have at least one tethered ligand. In certain embodiments, cell-targeting moieties comprise two tethered ligands covalently attached to a branching group. In certain embodiments, cell-targeting moieties comprise three tethered ligands covalently attached to a branching group.

B. 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'-phosphonates, 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.

III. 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.

IV. 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 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, described 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.

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.

V. 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.

A. 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, 20, 21, 22, 23, or 24 nucleobases in length.

In certain embodiments, oligonucleotides comprise one or more mismatched nucleobases relative to the target nucleic acid. In certain embodiments, antisense activity against the target is reduced by such mismatch, but activity against a non-target is reduced by a greater amount. Thus, in certain embodiments selectivity of the oligonucleotide is improved. In certain embodiments, the mismatch is specifically positioned within an oligonucleotide having a gapmer motif. In certain embodiments, the mismatch is at position 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 from the 5'-end of the gap region. In certain embodiments, the mismatch is at position 1, 2, 3, 4, 5, or 6 from the 5'-end of the 5' wing region or the 3' wing region.

B. AGT

In certain embodiments, oligomeric compounds comprise or consist of an oligonucleotide that is complementary to a target nucleic acid, wherein the target nucleic acid is an AGT nucleic acid. In certain embodiments, AGT nucleic acid has the sequence set forth in SEQ ID NO: 1 (GENBANK Accession No. NM_000029.3) or SEQ ID NO: 2 (the complement of GENBANK Accession No. NC_000001.11 truncated from nucleotides 230700001 to 230718000).

In certain embodiments, contacting a cell with an oligomeric compound complementary to any of SEQ ID NO: 1 and 2 reduces the amount of AGT RNA and in certain embodiments reduces the amount of AGT protein. In certain embodiments, the oligomeric compound consists of a modified oligonucleotide. In certain embodiments, contacting a cell with an oligomeric compound complementary to any of SEQ ID NO: 1 and 2 reduces the amount of AGT RNA in a cell, and in certain embodiments reduces the amount of AGT protein in a cell. In certain embodiments, the cell is *in vitro*. In certain embodiments, the cell is in a subject. In certain embodiments, the oligomeric compound consists of a modified oligonucleotide. In certain embodiments, contacting a cell in a subject with an oligomeric compound complementary to any of SEQ ID

NO: 1 and 2 ameliorates one or more symptom or hallmark of a cardiovascular disease. In certain embodiments, the disease is hypertension. In certain embodiments, the disease is resistant hypertension. In certain embodiments, the disease is Marfan syndrome. In certain embodiments, the disease is heart failure. In certain embodiments, the symptom or hallmark is selected from hypertension, chronic kidney disease, stroke, myocardial infarction, heart failure, valvular heart disease, aneurysms of the blood vessels, peripheral artery disease, and organ damage.

In certain embodiments, an oligomeric compound complementary to any of SEQ ID NO: 1 and 2 is capable of reducing the detectable amount of AGT RNA *in vitro* by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% when administered according to the standard cell assay. In certain embodiments, an oligomeric compound complementary to SEQ ID NO: 1 or 2 is capable of decreasing the amount of AGT *in vitro* by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% when administered according to the standard *in vitro* assay. In certain embodiments, an oligomeric compound complementary to SEQ ID NO: 1 or SEQ ID NO: 2 is capable of reducing the detectable amount of AGT RNA in a subject by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%.

VI. Certain Conjugated Compounds

In certain embodiments, the oligomeric compounds described herein comprise or 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. 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 embodiments, conjugate groups are attached near the 3'-end of oligonucleotides. In certain embodiments, conjugate groups (or terminal groups) are attached at the 5'-end of oligonucleotides. In certain embodiments, conjugate groups are attached near the 5'-end of oligonucleotides.

In certain embodiments, the oligonucleotide is modified. In certain embodiments, the oligonucleotide of a compound has a nucleobase sequence that is complementary to a target nucleic acid. In certain embodiments, oligonucleotides are complementary to a messenger RNA (mRNA). In certain embodiments, oligonucleotides are complementary to a pre-mRNA. In certain embodiments, oligonucleotides are complementary to a sense transcript.

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

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 palmityl 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).

1. Conjugate Moieties

Conjugate moieties include, without limitation, intercalators, reporter molecules, polyamines, polyamides, peptides, carbohydrates (e.g., GalNAc), vitamin moieties, polyethylene glycols, thioethers, polyethers, cholesterols, thiocholesterols, cholic acid moieties, folate, lipids, 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, folinic acid, a benzothiadiazide, chlorothiazide, a diazepine, indo-methicin, a barbiturate, a cephalosporin, a sulfa drug, an antidiabetic, an antibacterial or an antibiotic.

2. Conjugate linkers

Conjugate moieties are attached to oligonucleotides through conjugate linkers. In certain 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 compounds, a conjugate moiety is attached to an oligonucleotide via a more complex conjugate linker comprising one or more conjugate linker moieities, 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 (AHEX or AHA). Other conjugate linkers include but are not limited to substituted or unsubstituted C_1-C_{10} alkyl, substituted or unsubstituted C_2-C_{10} alkenyl or substituted or unsubstituted C_2-C_{10} 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, 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-methylcytosine, 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 compound after it reaches a target tissue. Accordingly, linker-nucleosides are typically linked to one another and to the remainder of the compound through cleavable bonds. In certain embodimements, such cleavable bonds are phosphodiester bonds.

Herein, linker-nucleosides are not considered to be part of the oligonucleotide. Accordingly, in embodiments in which an 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 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, a 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 a compound is more than 30. Alternatively, a compound may comprise a modified oligonucleotide consisting of 8-30 nucleosides and no conjugate group. The total number of contiguous linked nucleosides in such a 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.

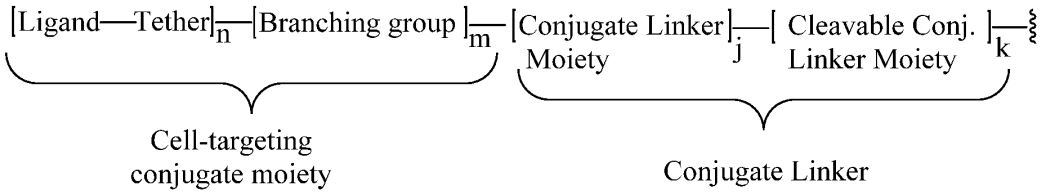
In certain embodiments, it is desirable for a conjugate group to be cleaved from the oligonucleotide. For example, in certain circumstances compounds comprising a particular conjugate moiety are better taken up by a particular cell type, but once the compound has been taken up, it is desirable that the 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 compound through cleavable bonds. In certain embodiments, such cleavable bonds are unmodified phosphodiester bonds. In certain embodiments, a cleavable moiety is 2'-deoxy nucleoside 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 linkage. In certain such embodiments, the cleavable moiety is 2'-deoxyadenosine.

3. Certain Cell-Targeting Conjugate Moietiess

In certain embodiments, a conjugate group comprises a cell-targeting conjugate moiety. In certain embodiments, a conjugate group has the general formula:



wherein n is from 1 to about 3, m is 0 when n is 1, m is 1 when n is 2 or greater, j is 1 or 0, and k is 1 or 0.

In certain embodiments, n is 1, j is 1 and k is 0. In certain embodiments, n is 1, j is 0 and k is 1. In certain embodiments, n is 1, j is 1 and k is 1. In certain embodiments, n is 2, j is 1 and k is 0. In certain embodiments, n is 2, j is 0 and k is 1. In certain embodiments, n is 2, j is 1 and k is 1. In certain embodiments, n is 3, j is 1 and k is 0. In certain embodiments, n is 3, j is 0 and k is 1. In certain embodiments, n is 3, j is 1 and k is 1.

In certain embodiments, conjugate groups comprise cell-targeting moieties that have at least one tethered ligand. In certain embodiments, cell-targeting moieties comprise two tethered ligands covalently attached to a branching group. In certain embodiments, cell-targeting moieties comprise three tethered ligands covalently attached to a branching group.

In certain embodiments, the cell-targeting moiety comprises a branching group comprising one or more groups selected from alkyl, amino, oxo, amide, disulfide, polyethylene glycol, ether, thioether and hydroxylamino groups. In certain embodiments, the branching group comprises a branched aliphatic group comprising groups selected from alkyl, amino, oxo, amide, disulfide, polyethylene glycol, ether, thioether and hydroxylamino groups. In certain such embodiments, the branched aliphatic group comprises groups selected from alkyl, amino, oxo, amide and ether groups. In certain such embodiments, the branched aliphatic group comprises groups selected from alkyl, amino and ether groups. In certain such embodiments, the branched

aliphatic group comprises groups selected from alkyl and ether groups. In certain embodiments, the branching group comprises a mono or polycyclic ring system.

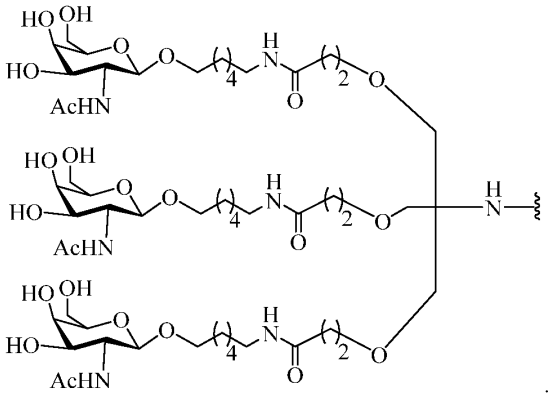
In certain embodiments, each tether of a cell-targeting moiety comprises one or more groups selected from alkyl, substituted alkyl, ether, thioether, disulfide, amino, oxo, amide, phosphodiester, and polyethylene glycol, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, ether, thioether, disulfide, amino, oxo, amide, and polyethylene glycol, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, phosphodiester, ether, amino, oxo, and amide, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, ether, amino, oxo, and amid, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, amino, and oxo, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl and oxo, in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl and phosphodiester, in any combination. In certain embodiments, each tether comprises at least one phosphorus linking group or neutral linking group. In certain embodiments, each tether comprises a chain from about 6 to about 20 atoms in length. In certain embodiments, each tether comprises a chain from about 10 to about 18 atoms in length. In certain embodiments, each tether comprises about 10 atoms in chain length.

In certain embodiments, each ligand of a cell-targeting moiety has an affinity for at least one type of receptor on a target cell. In certain embodiments, each ligand has an affinity for at least one type of receptor on the surface of a mammalian liver cell. In certain embodiments, each ligand has an affinity for the hepatic asialoglycoprotein receptor (ASGP-R). In certain embodiments, each ligand is a carbohydrate. In certain embodiments, each ligand is, independently selected from galactose, N-acetyl galactoseamine (GalNAc), mannose, glucose, glucoseamine and fucose. In certain embodiments, each ligand is N-acetyl galactoseamine (GalNAc). In certain embodiments, the cell-targeting moiety comprises 3 GalNAc ligands. In certain embodiments, the cell-targeting moiety comprises 2 GalNAc ligands. In certain embodiments, the cell-targeting moiety comprises 1 GalNAc ligand.

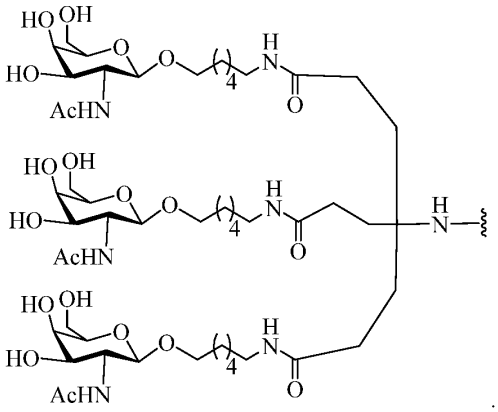
In certain embodiments, each ligand of a cell-targeting moiety is a carbohydrate, carbohydrate derivative, modified carbohydrate, polysaccharide, modified polysaccharide, or polysaccharide derivative. In certain such embodiments, the conjugate group comprises a carbohydrate cluster (*see*, e.g., Maier et al., "Synthesis of Antisense Oligonucleotides Conjugated to a Multivalent Carbohydrate Cluster for Cellular Targeting," *Bioconjugate Chemistry*, 2003, *14*, 18-29 or Rensen et al., "Design and Synthesis of Novel *N*-Acetylgalactosamine-Terminated Glycolipids for Targeting of Lipoproteins to the Hepatic Asiaglycoprotein Receptor," *J. Med. Chem.* 2004, *47*, 5798-5808). In certain such embodiments, each ligand is an amino sugar or a thio sugar. For example, amino sugars may be selected from any number of compounds known in the art, such as sialic acid, α-D-galactosamine, β-muramic acid, 2-deoxy-2-methylamino-L-glucopyranose, 4,6-

dideoxy-4-formamido-2,3-di-*O*-methyl-D-mannopyranose, 2-deoxy-2-sulfoamino-D-glucopyranose and *N*-sulfo-D-glucosamine, and *N*-glycoloyl-α-neuraminic acid. For example, thio sugars may be selected from 5-Thio-β-D-glucopyranose, methyl 2,3,4-tri-*O*-acetyl-1-thio-6-*O*-trityl-α-D-glucopyranoside, 4-thio-β-D-galactopyranose, and ethyl 3,4,6,7-tetra-*O*-acetyl-2-deoxy-1,5-dithio-α-D-*gluco*-heptopyranoside.

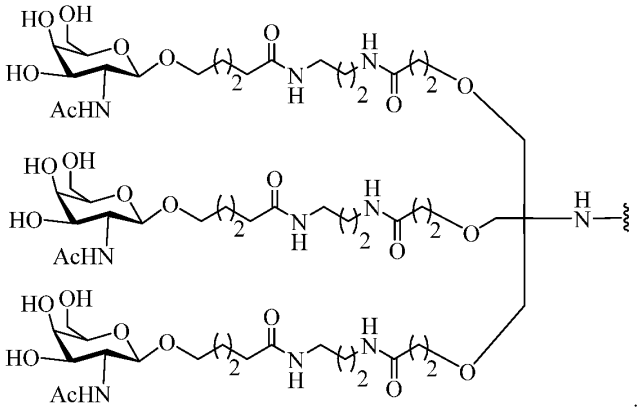
In certain embodiments, conjugate groups comprise a cell-targeting moiety having the formula:



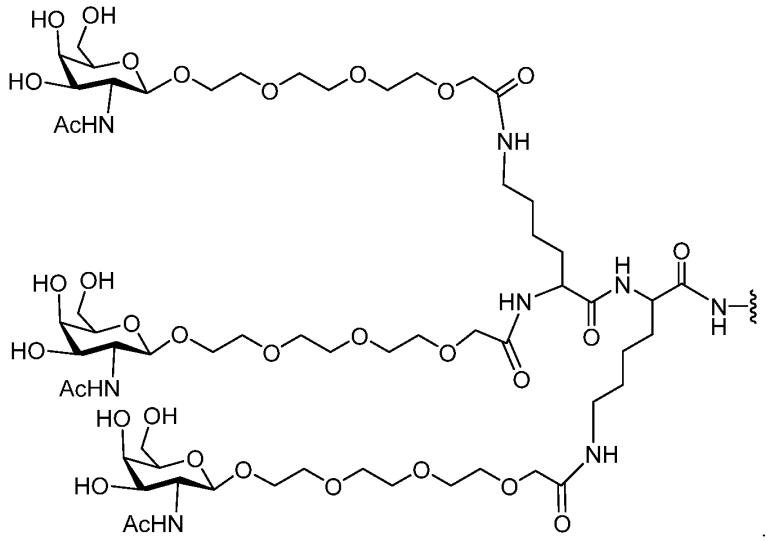
In certain embodiments, conjugate groups comprise a cell-targeting moiety having the formula:



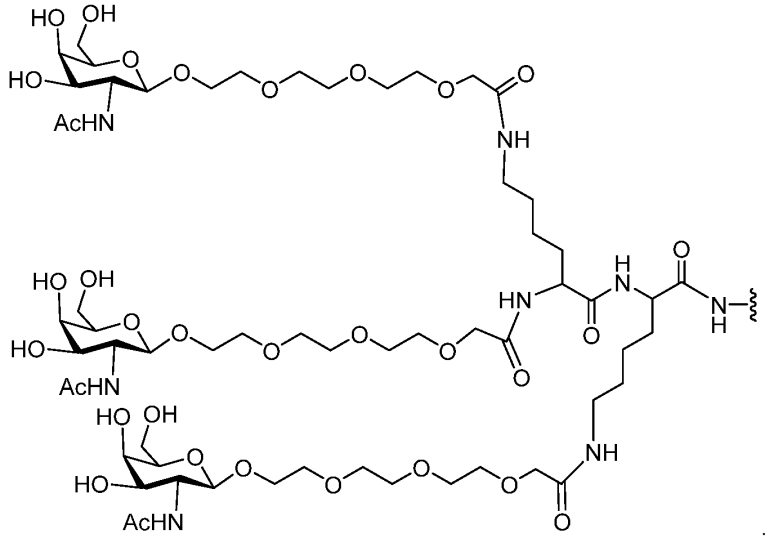
In certain embodiments, conjugate groups comprise a cell-targeting moiety having the formula:



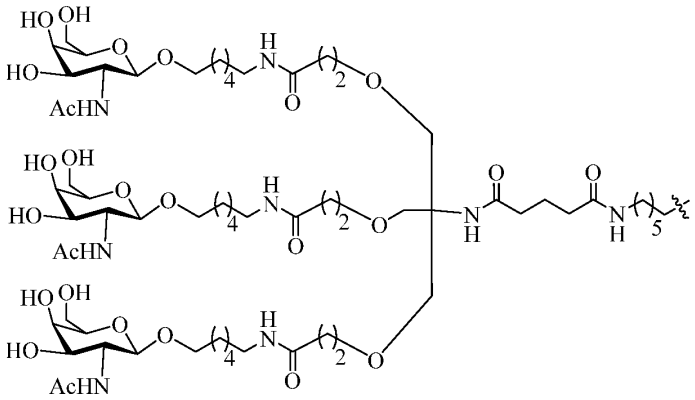
In certain embodiments, conjugate groups comprise a cell-targeting moiety having the formula:



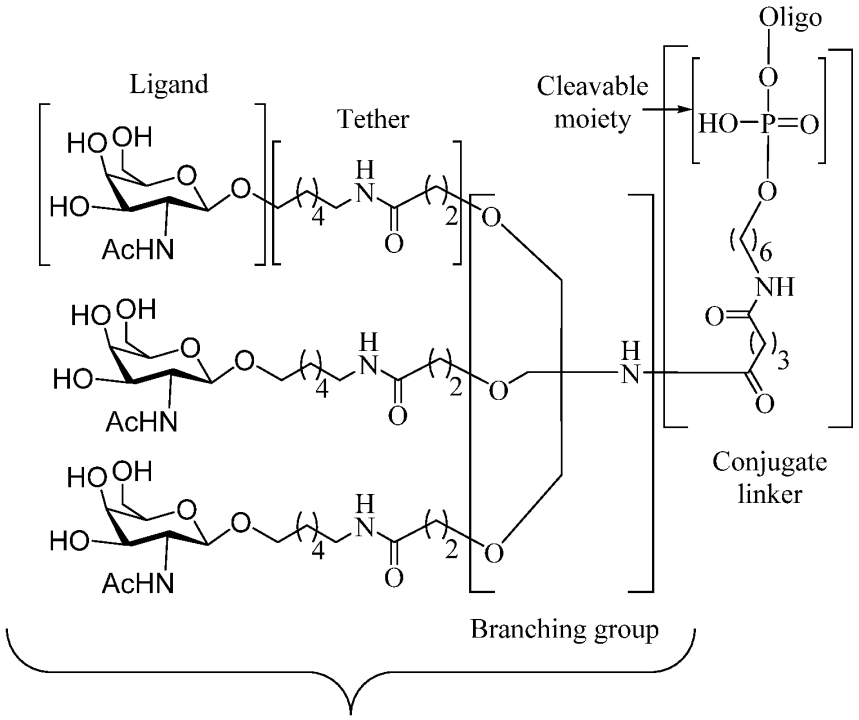
In certain embodiments, conjugate groups comprise a cell-targeting moiety having the formula:



In certain embodiments, compounds comprise a conjugate group described herein as "LICA-1". LICA-1 has the formula:



In certain embodiments, compounds described herein comprise LICA-1 and a cleavable moiety within the conjugate linker have the formula:



wherein oligo is an oligonucleotide.

Representative United States patents, United States patent application publications, international patent application publications, and other publications that teach the preparation of certain of the above noted conjugate groups, compounds comprising conjugate groups, tethers, conjugate linkers, branching groups, ligands, cleavable moieties as well as other modifications include without limitation, US 5,994,517, US 6,300,319, US 6,660,720, US 6,906,182, US 7,262,177, US 7,491,805, US 8,106,022, US 7,723,509, US 2006/0148740, US 2011/0123520, WO 2013/033230 and WO 2012/037254, Biessen et al., *J. Med. Chem.* 1995, *38*, 1846-1852, Lee et al., *Bioorganic & Medicinal Chemistry* 2011,*19*, 2494-2500, Rensen et al., *J. Biol. Chem.* 2001, *276*, 37577-37584, Rensen et al., *J. Med. Chem.* 2004, *47*, 5798-5808, Sliedregt et al., *J. Med. Chem.* 1999, *42*, 609-618, and Valentijn et al., *Tetrahedron,* 1997, *53,* 759-770.

In certain embodiments, modified oligonucleotides comprise a gapmer or fully modified sugar motif and a conjugate group comprising at least one, two, or three GalNAc ligands. In certain embodiments, compounds comprise a conjugate group found in any of the following references: Lee, *Carbohydr Res*, 1978, 67, 509-514; Connolly et al., *J Biol Chem*, 1982, 257, 939-945; Pavia et al., *Int J Pep Protein Res*, 1983, 22,

539-548; Lee et al., *Biochem*, 1984, 23, 4255-4261; Lee et al., *Glycoconjugate J*, 1987, 4, 317-328; Toyokuni et al., *Tetrahedron Lett*, 1990, 31, 2673-2676; Biessen et al., *J Med Chem*, 1995, 38, 1538-1546; Valentijn et al., *Tetrahedron*, 1997, 53, 759-770; Kim et al., *Tetrahedron Lett*, 1997, 38, 3487-3490; Lee et al., *Bioconjug Chem*, 1997, 8, 762-765; Kato et al., *Glycobiol*, 2001, 11, 821-829; Rensen et al., *J Biol Chem*, 2001, 276, 37577-37584; Lee et al., *Methods Enzymol*, 2003, 362, 38-43; Westerlind et al., *Glycoconj J*, 2004, 21, 227-241; Lee et al., *Bioorg Med Chem Lett*, 2006, 16(19), 5132-5135; Maierhofer et al., *Bioorg Med Chem*, 2007, 15, 7661-7676; Khorev et al., *Bioorg Med Chem*, 2008, 16, 5216-5231; Lee et al., *Bioorg Med Chem*, 2011, 19, 2494-2500; Kornilova et al., Analyt Biochem, 2012, 425, 43-46; Pujol et al., *Angew Chemie Int Ed Engl*, 2012, 51, 7445-7448; Biessen et al., *J Med Chem*, 1995, 38, 1846-1852; Sliedregt et al., *J Med Chem*, 1999, 42, 609-618; Rensen et al., *J Med Chem*, 2004, 47, 5798-5808; Rensen et al., *Arterioscler Thromb Vasc Biol*, 2006, 26, 169-175; van Rossenberg et al., *Gene Ther*, 2004, 11, 457-464; Sato et al., *J Am Chem Soc*, 2004, 126, 14013-14022; Lee et al., *J Org Chem*, 2012, 77, 7564-7571; Biessen et al., *FASEB J*, 2000, 14, 1784-1792; Rajur et al., *Bioconjug Chem*, 1997, 8, 935-940; Duff et al., *Methods Enzymol*, 2000, 313, 297-321; Maier et al., *Bioconjug Chem*, 2003, 14, 18-29; Jayaprakash et al., *Org Lett*, 2010, 12, 5410-5413; Manoharan, *Antisense Nucleic Acid Drug Dev*, 2002, 12, 103-128; Merwin et al., *Bioconjug Chem*, 1994, 5, 612-620; Tomiya et al., Bioorg Med Chem, 2013, 21, 5275-5281; International applications WO1998/013381; WO2011/038356; WO1997/046098; WO2008/098788; WO2004/101619; WO2012/037254; WO2011/120053; WO2011/100131; WO2011/163121; WO2012/177947; WO2013/033230; WO2013/075035; WO2012/083185; WO2012/083046; WO2009/082607; WO2009/134487; WO2010/144740; WO2010/148013; WO1997/020563; WO2010/088537; WO2002/043771; WO2010/129709; WO2012/068187; WO2009/126933; WO2004/024757; WO2010/054406; WO2012/089352; WO2012/089602; WO2013/166121; WO2013/165816; U.S. Patents 4,751,219; 8,552,163; 6,908,903; 7,262,177; 5,994,517; 6,300,319; 8,106,022; 7,491,805; 7,491,805; 7,582,744; 8,137,695; 6,383,812; 6,525,031; 6,660,720; 7,723,509; 8,541,548; 8,344,125; 8,313,772; 8,349,308; 8,450,467; 8,501,930; 8,158,601; 7,262,177; 6,906,182; 6,620,916; 8,435,491; 8,404,862; 7,851,615; Published U.S. Patent Application Publications US2011/0097264; US2011/0097265; US2013/0004427; US2005/0164235; US2006/0148740; US2008/0281044; US2010/0240730; US2003/0119724; US2006/0183886; US2008/0206869; US2011/0269814; US2009/0286973; US2011/0207799; US2012/0136042; US2012/0165393; US2008/0281041; US2009/0203135; US2012/0035115; US2012/0095075; US2012/0101148; US2012/0128760; US2012/0157509; US2012/0230938; US2013/0109817; US2013/0121954; US2013/0178512; US2013/0236968; US2011/0123520; US2003/0077829; US2008/0108801; and US2009/0203132.

VII. 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 saline solution and one or more oligomeric compound. 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 water. 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 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, 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.

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.

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 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.

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, 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 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 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 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), intraneural, perineural, etc.). In certain of such embodiments, a pharmaceutical composition 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 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 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 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.

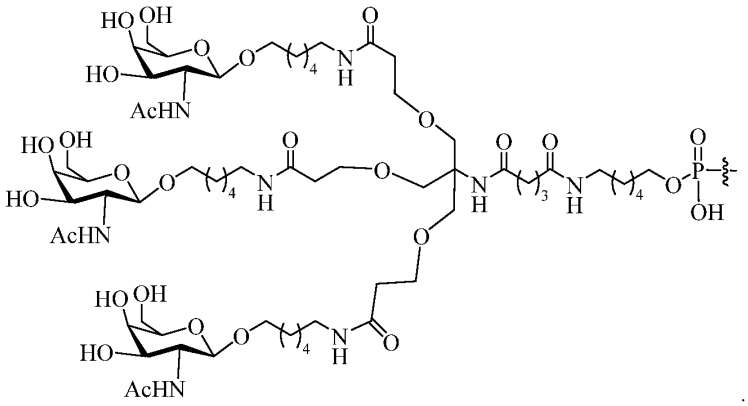
In certain embodiments, modified oligonucleotides or oligomeric compounds are in aqueous solution with sodium. In certain embodiments, modified oligonucleotides or oligomeric compounds are in aqueous solution with potassium. In certain embodiments, modified oligonucleotides or oligomeric compounds are 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. 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.

VIII. Certain Compositions

1. Compound No. 1205407

In certain embodiments, Compound No. 1205407 is characterized as a 3-10-3 MOE/cEt mixed wing gapmer conjugated at the 5'-end to a conjugate group. Compound 1205407 has a sequence (from 5' to 3') of CGCTGATTTGTCCGGG (SEQ ID NO: 12), wherein nucleosides 1-3 have sugar modifications of e-e-k (from 5' to 3'), wherein nucleosides 14-16 have sugar modifications of k-k-e, wherein each 'e' represents a 2'- MOE sugar moiety, and each 'k' refers to a cEt sugar moiety; and each of nucleosides 4-13 are 2'-β-D-deoxynucleosides; wherein the internucleoside linkages between nucleosides 2 to 3, 3 to 4, and 14 to 15 are phosphodiester internucleoside linkages and the internucleoside linkages between nucleosides 1 to 2, 4 to 5, 5 to 6, 6 to 7, 7 to 8, 8 to 9, 9 to 10, 10 to 11, 11 to 12, 12 to 13, 13 to 14, and 15 to 16 are phosphorothioate internucleoside linkages, and wherein each cytosine is a 5-methylcytosine. Compound No. 1205407 has a 5'-trishexylamino-(THA)-C_6GalNAc$_3$ endcap, represented by the structure below, wherein the phosphate group is attached to the 5'-oxygen atom of the 5'-nucleoside:



In certain embodiments, Compound No. 1205407 is represented by the following chemical notation: THA-C6-GalNAc$_3$-$^{m}C_{es}G_{eo}{}^{m}C_{ko}T_{ds}G_{ds}A_{ds}T_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}G_{ko}G_{ks}G_{e}$ (SEQ ID NO: 12), wherein:

A = an adenine nucleobase,

^{m}C = a 5-methyl cytosine nucleobase,

G = a guanine nucleobase,

T = a thymine nucleobase,

e = a 2'-β-D-MOE sugar moiety,

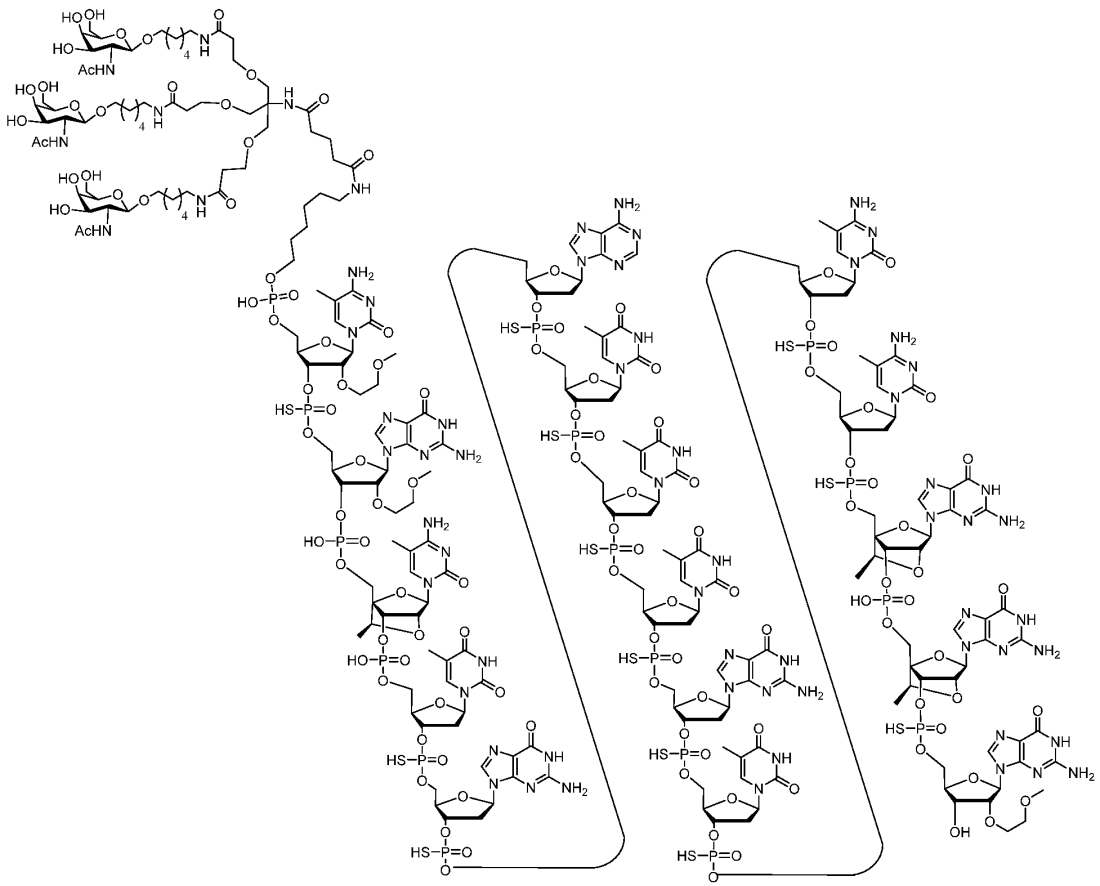
k = a cEt sugar moiety,

d = a 2'-β-D-deoxyribosyl sugar moiety,

s = a phosphorothioate internucleoside linkage, and

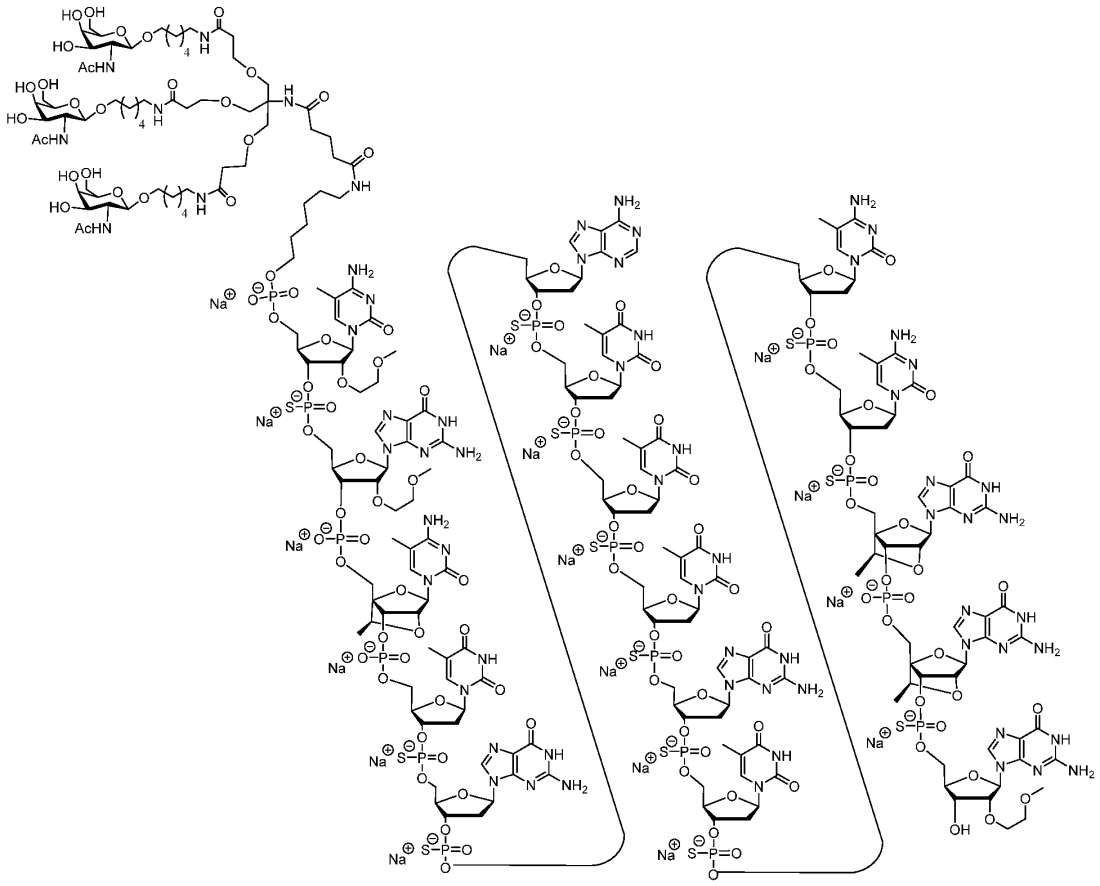
o = a phosphodiester internucleoside linkage.

In certain embodiments, Compound No. 1205407 is represented by the following chemical structure:



(SEQ ID NO: 12) or a salt thereof.

In certain embodiments, the sodium salt of Compound No. 1205407 is represented by the following chemical structure:



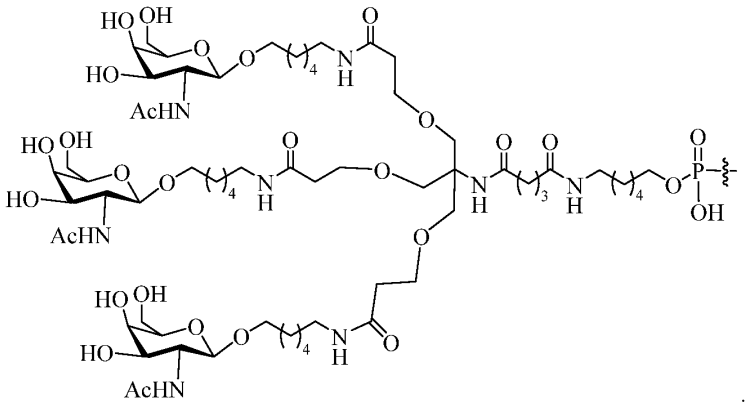
(SEQ ID NO: 12).

In certain embodiments, Compound No. 1205407 is in anionic form.

2. Compound No. 1205408

In certain embodiments, Compound No. 1205408 is characterized as a 3-10-3 MOE/cEt mixed wing gapmer conjugated at the 5'-end to a conjugate group. Compound 1205408 has a sequence (from 5' to 3') of TCGGTTGGAATTCTTT (SEQ ID NO: 13), wherein nucleosides 1-3 have sugar modifications of e-k-k (from 5' to 3') and wherein nucleosides 14-16 have sugar modifications of k-k-e; wherein each 'e' represents a 2'- MOE sugar moiety, and each 'k' refers to a cEt sugar moiety; and each of nucleosides 4-13 are 2'-β-D-deoxynucleosides; wherein the internucleoside linkages between nucleosides 2 to 3, 3 to 4, and 14 to 15 are phosphodiester internucleoside linkages and the internucleoside linkages between nucleosides 1 to 2, 4 to 5, 5 to 6, 6 to 7, 7 to 8, 8 to 9, 9 to 10, 10 to 11, 11 to 12, 12 to 13, 13 to 14, and 15 to 16 are phosphorothioate internucleoside linkages, and wherein each cytosine is a 5-methyl cytosine. Compound No. 1205408 has a

5'-trishexylamino-(THA)-C_6GalNAc$_3$ endcap, represented by the structure below, wherein the phosphate group is attached to the 5'-oxygen atom of the 5'-nucleoside:



In certain embodiments, Compound No. 1205408 is represented by the following chemical notation: THA-C6-GalNAc$_3$- $T_{es}{}^{m}C_{ko}G_{ko}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}A_{ds}A_{ds}T_{ds}T_{ds}{}^{m}C_{ds}T_{ko}T_{ks}T_{e}$ (SEQ ID NO: 13), wherein:

A = an adenine nucleobase,

^{m}C = a 5-methyl cytosine nucleobase,

G = a guanine nucleobase,

T = a thymine nucleobase,

e = a 2'- β-D-MOE sugar moiety,

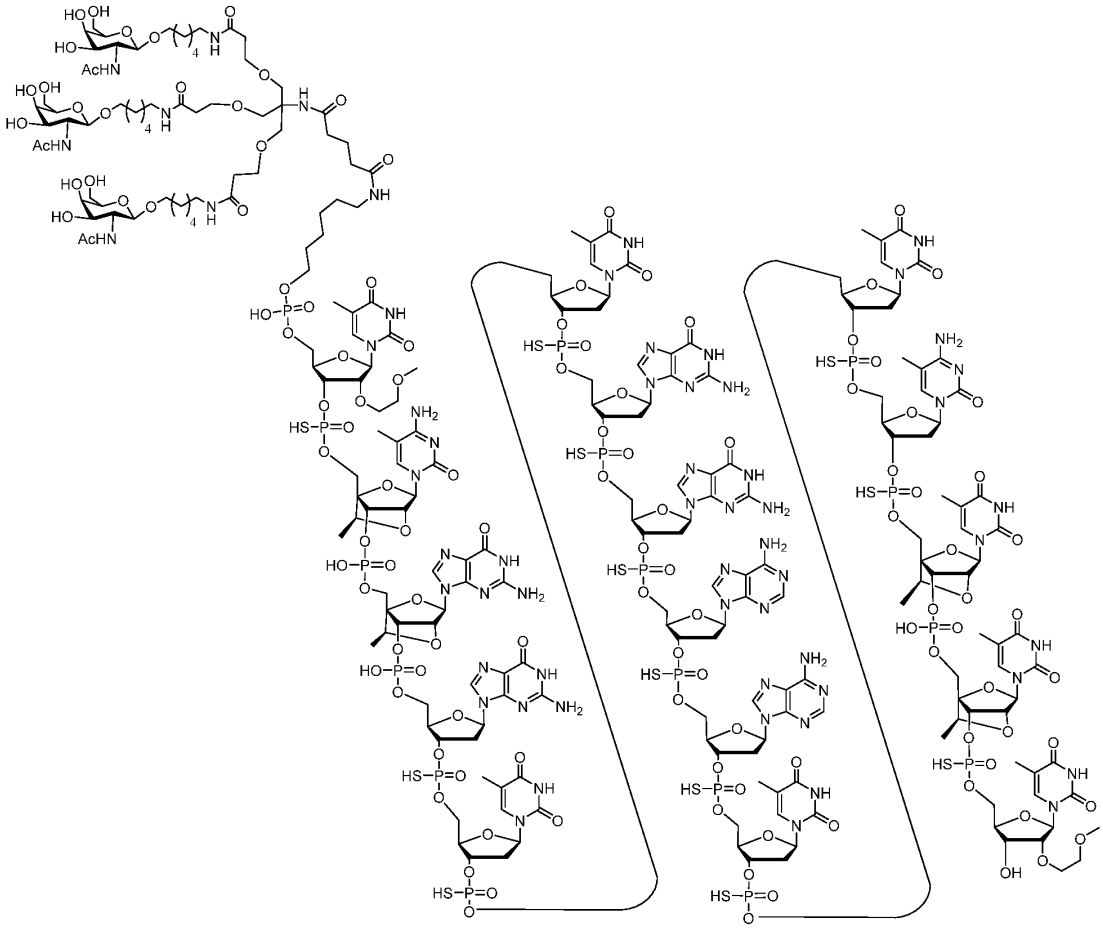
k = a cEt sugar moiety,

d = a 2'-β-D-deoxyribosyl sugar moiety,

s = a phosphorothioate internucleoside linkage, and

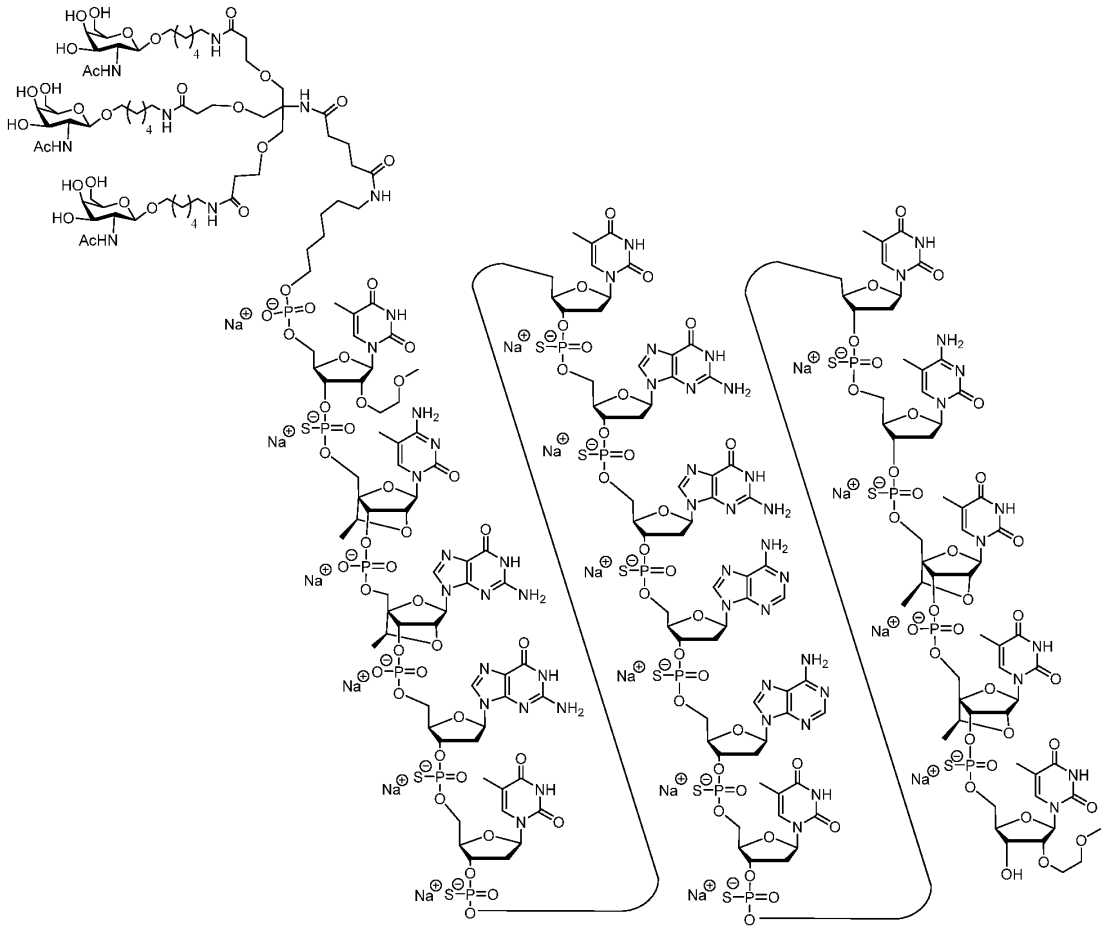
o = a phosphodiester internucleoside linkage.

In certain embodiments, Compound No. 1205408 is represented by the following chemical structure:



(SEQ ID NO: 13) or salt thereof.

In certain embodiments, the sodium salt of Compound No. 1205408 is represented by the following chemical structure:

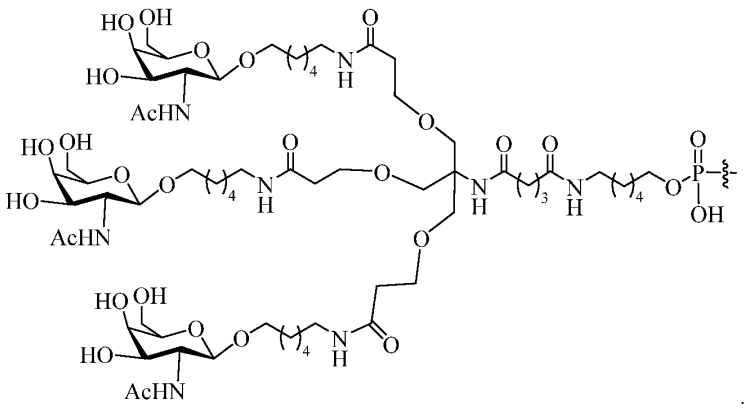


(SEQ ID NO: 13).

In certain embodiments, Compound No. 1205408 is in anionic form.

3. Compound No. 1250837

In certain embodiments, Compound No. 1250837 is characterized as a 3-10-3 gapmer conjugated at the 5'-end to a conjugate group. Compound 1250837 has a sequence (from 5' to 3') of GTCGGTTGGAATTCTT (SEQ ID NO: 15), wherein nucleosides 1-3 and 14-16 have cEt sugar modifications, wherein nucleoside 5 has a 2'-OMe ribose sugar, and wherein each of nucleosides 4 and 6-13 are 2'-β-D-deoxynucleosides; wherein each internucleoside linkage between the nucleosides is a phosphorothioate internucleoside linkage, and wherein each cytosine is a 5-methyl cytosine. Compound No. 1250837 has a 5'-trishexylamino-(THA)-C_6GalNAc$_3$ endcap, represented by the structure below, wherein the phosphate group is attached to the 5'-oxygen atom of the 5'-nucleoside:



In certain embodiments, Compound No. 1250837 is represented by the following chemical notation: THA-C6-GalNAc$_3$- $G_{ks}T_{ks}{}^{m}C_{ks}G_{ds}G_{ys}T_{ds}T_{ds}G_{ds}G_{ds}A_{ds}A_{ds}T_{ds}T_{ds}{}^{m}C_{ks}T_{ks}T_{k}$ (SEQ ID NO: 15), wherein:

A = an adenine nucleobase,

^{m}C = a 5-methyl cytosine nucleobase,

G = a guanine nucleobase,

T = a thymine nucleobase,

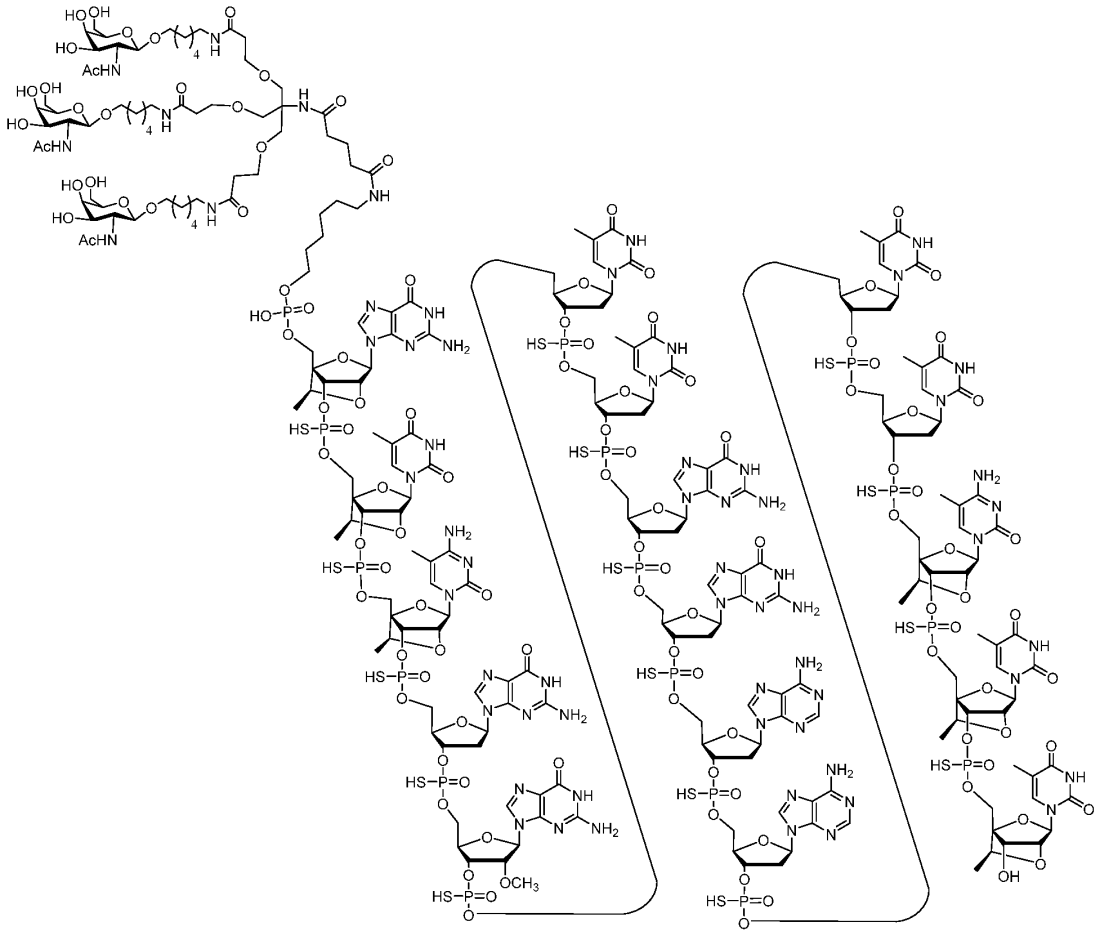
k = a cEt sugar moiety,

d = a 2'-β-D-deoxyribosyl sugar moiety,

y = a 2'-OMe ribose sugar moiety, and

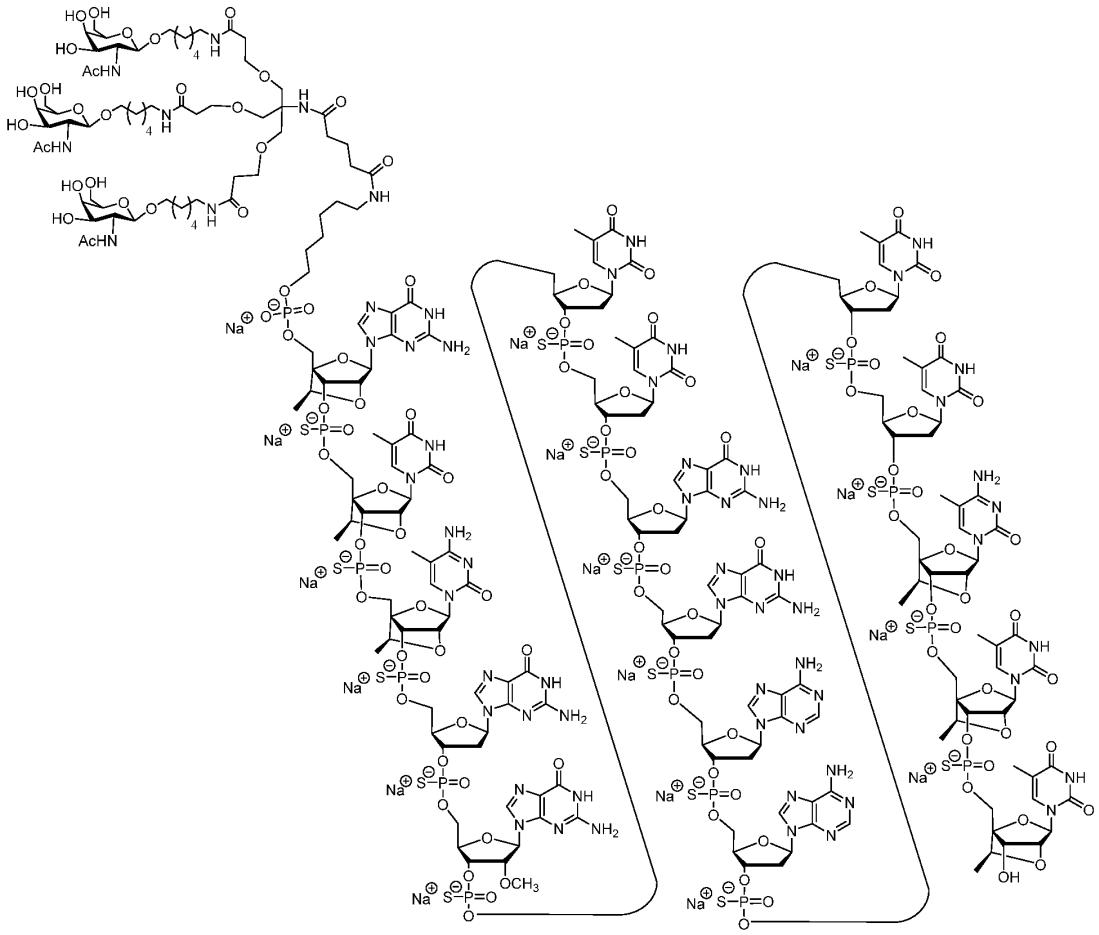
s = a phosphorothioate internucleoside linkage.

In certain embodiments, Compound No. 1250837 is represented by the following chemical structure:



(SEQ ID NO: 15) or a salt thereof.

In certain embodiments, the sodium salt of Compound No. 1250837 is represented by the following chemical structure:



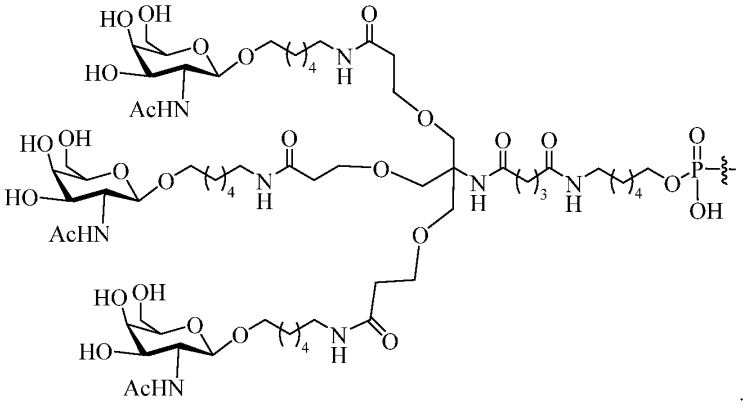
(SEQ ID NO: 15).

In certain embodiments, Compound No. 1250837 is in anionic form.

4. Compound No. 1250851

In certain embodiments, Compound No. 1250851 is characterized as a 3-10-3 gapmer conjugated at the 5'-end to a conjugate group. Compound 1250851 has a sequence (from 5' to 3') of TCGGUTGGAATTCTTT (SEQ ID NO: 14), wherein nucleosides 1-3 and 14-16 have cEt sugar modifications, wherein nucleoside 5 has a 2'-OMe ribose sugar, and wherein each of nucleosides 4 and 6-13 are 2'-β-D-deoxynucleosides; wherein the internucleoside linkages between nucleosides 2 to 3, 3 to 4, and 14 to 15 are phosphodiester internucleoside linkages and the internucleoside linkages between nucleosides 1 to 2, 4 to 5, 5 to 6, 6 to 7, 7 to 8, 8 to 9, 9 to 10, 10 to 11, 11 to 12, 12 to 13, 13 to 14, and 15 to 16 are

phosphorothioate internucleoside linkages, and wherein each cytosine is a 5-methyl cytosine. Compound No. 1250851 has a 5'-trishexylamino-(THA)-$C_6GalNAc_3$ endcap, represented by the structure below, wherein the phosphate group is attached to the 5'-oxygen atom of the 5'-nucleoside:



In certain embodiments, Compound No. 1250851 is represented by the following chemical notation: THA-C6-$GalNAc_3$- $T_{ks}{}^{m}C_{ko}G_{ko}G_{ds}U_{ys}T_{ds}G_{ds}G_{ds}A_{ds}A_{ds}T_{ds}T_{ds}{}^{m}C_{ds}T_{ko}T_{ks}T_{k}$ (SEQ ID NO: 14), wherein:

A = an adenine nucleobase,

^{m}C = a 5-methyl cytosine nucleobase,

G = a guanine nucleobase,

T = a thymine nucleobase,

U = a uracil nucleobase,

k = a cEt sugar moiety,

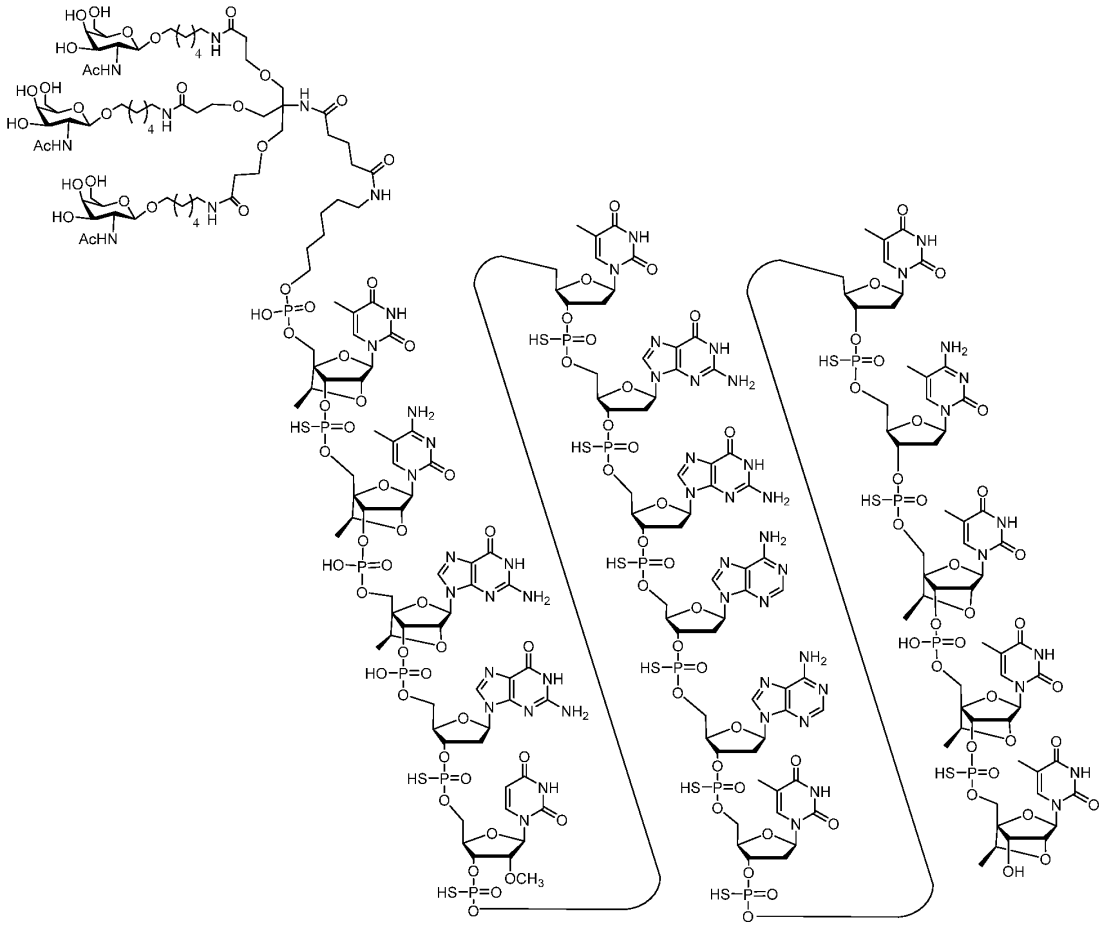
d = a 2'-β-D-deoxyribosyl sugar moiety,

y = a 2'-OMe ribose sugar moiety,

s = a phosphorothioate internucleoside linkage, and

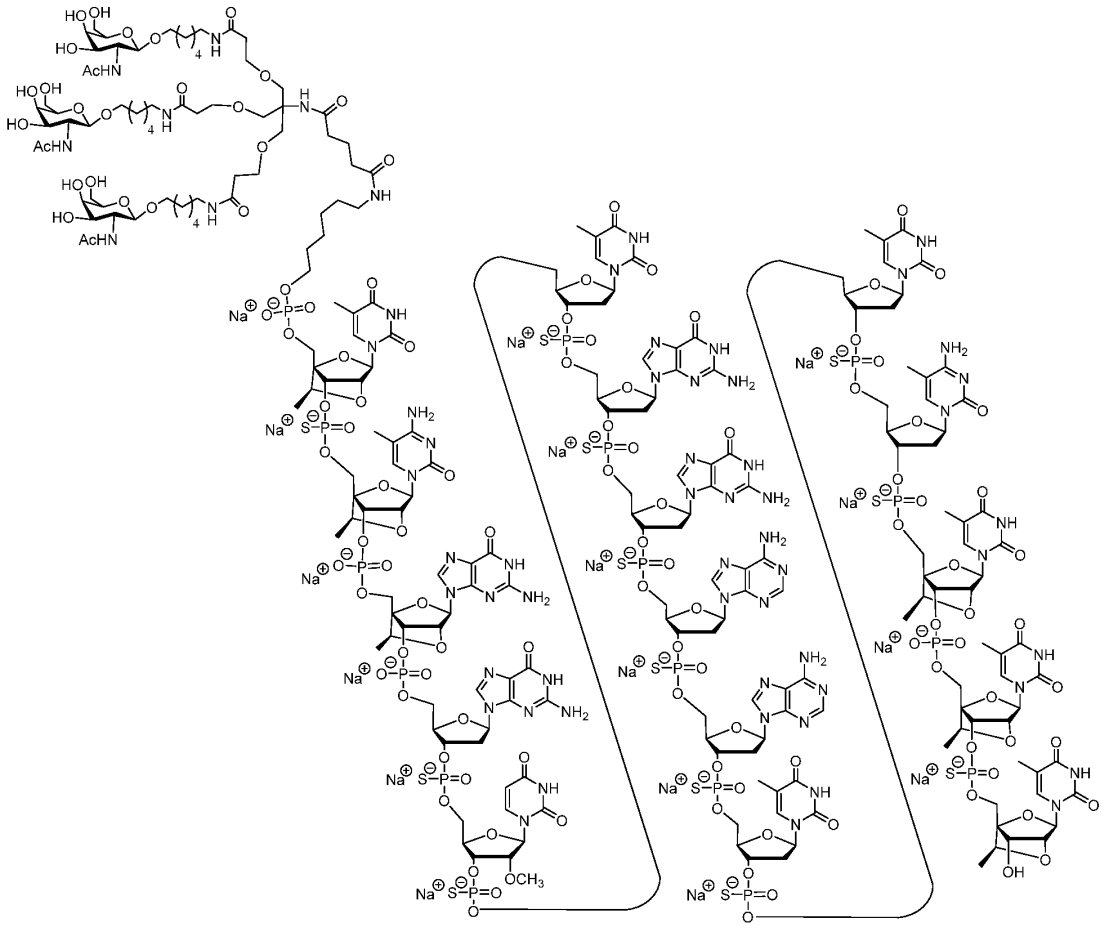
o = a phosphodiester internucleoside linkage.

In certain embodiments, Compound No. 1250851 is represented by the following chemical structure:



(SEQ ID NO: 14) or a salt thereof.

In certain embodiments, the sodium salt of Compound No. 1250851 is represented by the following chemical structure:



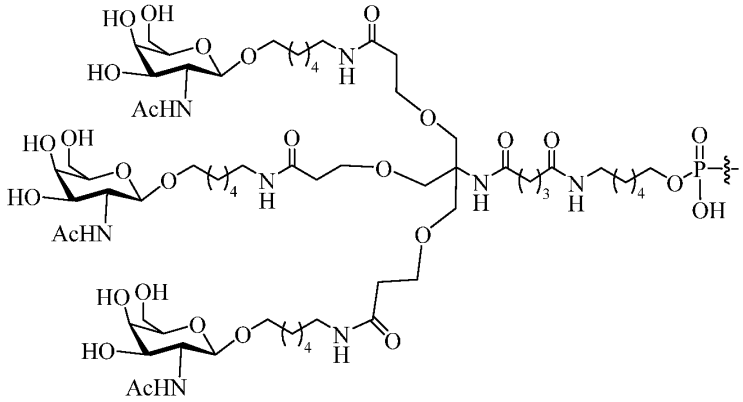
(SEQ ID NO: 14).

In certain embodiments, Compound No. 1250851 is in anionic form.

VI. Certain Comparator Compositions

In certain embodiments, Compound No. 757456 is a comparator compound. Compound No. 757456 was previously described in WO2017062816, incorporated herein by reference, and has a sequence (from 5' to 3') of CACAAACAAGCTGGTCGGTT (SEQ ID NO: 28), wherein the compound comprises a conjugate group and a modified oligonucleotide; wherein the modified oligonucleotide is a 5-10-5 MOE gapmer, wherein the central gap segment consists of ten 2'-β-D-deoxynucleosides and the 5' and 3' wing segments each consists of five 2'-MOE modified nucleosides. Each internucleoside linkage is a phosphorothioate internucleoside linkage. All cytosine residues are 5-methylcytosines. Compound No.

757456 has a 5'-trishexylamino-(THA)-C_6GalNAc$_3$ endcap, represented by the structure below, wherein the phosphate group is attached to the 5'-oxygen atom of the 5'-nucleoside:



In certain embodiments, Compound No. 568637 is a comparator compound. Compound No. 568637 was previously described in WO2017062816, incorporated herein by reference, and has a sequence (from 5' to 3') of CGCTGATTTGTCCGGG (SEQ ID NO: 12), wherein the compound consists a modified oligonucleotide; wherein the modified oligonucleotide is 16 nucleosides in length with mixed sugar moieties, as described by the sugar motif eekddddddddddkke; wherein each 'd' represents a 2'-β-D-deoxyribosyl sugar moiety, each 'e' represents a 2'- MOE sugar moiety, and each 'k' represents a cEt sugar moiety. Each internucleoside linkage is a phosphorothioate internucleoside linkage. All cytosine residues are 5-methylcytosines.

In certain embodiments, Compound No. 1176644 is a comparator compound. Compound No. 1176644 is Compound No. 568637 that has a 5'-trishexylamino-(THA)-C_6GalNAc$_3$ endcap. Compound No. 1176644, similar to Compound No. 568637, has a sequence (from 5' to 3') of CGCTGATTTGTCCGGG (SEQ ID NO: 12), wherein the compound comprises a modified oligonucleotide; wherein the modified oligonucleotide is 16 nucleosides in length with mixed sugar moieties, as described by the sugar motif eekddddddddddkke; wherein each 'd' represents a 2'-β-D-deoxyribosyl sugar moiety, each 'e' represents a 2'-MOE sugar moiety, and each 'k' represents a cEt sugar moiety. Each internucleoside linkage is a phosphorothioate internucleoside linkage. All cytosine residues are 5-methylcytosines.

In certain embodiments, compounds described herein are superior relative to compounds described in WO2017062816 because they demonstrate one or more improved properties, such as potency.

For example, Compound No. 1205407 demonstrated improved potency *in vivo* as compared to Compound No. 757456. As shown in Example 5, Compound No. 1205407 achieved 93% and 90% inhibition of AGT RNA and protein respectively at a dose of 2.7 mg/kg. In comparison, Compound No. 757456 achieved 65% and 60% inhibition of AGT RNA and protein respectively at a dose of 3.3 mg/kg. Therefore, Compound No. 1205407 is more potent than Compound No. 757456 in this assay. For example, as shown in Study 1 of Example 6, Compound No. 1205407 achieved an ED_{50} of 0.1 in the liver and plasma. In

comparison, Compound No. 757456 achieved an ED_{50} of 1.3 in the liver and plasma. Therefore, Compound No. 1205407 is more potent than Compound No. 757456 in this assay.

For example, Compound No. 1205407 demonstrated improved potency *ex-vivo* as compared to Compound No. 757456. As shown in Example 8, Compound No. 1205407 achieved an IC_{50} of 0.04 nM *ex vivo* using the Hepatopac system. In comparison, Compound No. 757456 had an IC_{50} of >20 μM *ex vivo*. Therefore, Compound No. 1205407 is more potent than Compound No. 757456 in this assay.

For example, Compound No. 1205407 demonstrated improved potency *in vitro* as compared to Compound No. 757456 or Compound No. 1176644. As shown in Example 7, Compound No. 1205407 achieved an IC_{50} of 8 nM and 12 nM when tested with two different primer probe sets *in vitro*. In comparison, Compound No. 757456 achieved an IC_{50} of 868 nM and 709 nM under the same culture conditions *in vitro*. In comparison, Compound No. 1176644 achieved an IC_{50} of 35 nM and 43 nM under the same culture conditions *in vitro*. Therefore, Compound No. 1205407 is more potent than Compound No. 757456 or Compound No. 1176644 in this assay.

For example, Compound No. 1205407 demonstrated improved potency *in vivo* as compared to Compound No. 757456 or Compound No. 1176644. As shown in Study 2 of Example 6, Compound No. 1205407 achieved an ED_{50} of 0.11 and an ED_{75} of 0.38 in a transgenic mouse study. In comparison, Compound No. 757456 achieved an ED_{50} of 2.1 and an ED_{75} of 2.68. In comparison, Compound No. 1176644 achieved an ED_{50} of 0.38 and an ED_{75} of 0.61. Therefore, Compound No. 1205407 is more potent than Compound No. 757456 or Compound No. 1176644 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^{m}CGAUCG," wherein ^{m}C indicates a cytosine base comprising a methyl group at the 5-position.

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 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 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 ^{1}H hydrogen atoms. Isotopic substitutions encompassed by the compounds herein include but are not limited to: ^{2}H or ^{3}H in place of ^{1}H, ^{13}C or ^{14}C in place of ^{12}C, ^{15}N in place of ^{14}N, ^{17}O or ^{18}O in place of ^{16}O, and ^{33}S, ^{34}S, ^{35}S, or ^{36}S in place of ^{32}S. 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.

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.

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

Modified oligonucleotides complementary to a human AGT nucleic acid were designed, as described in the tables below. "Start site" in all the tables below 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. Each modified oligonucleotide listed in the tables below is 100% complementary to SEQ ID NO: 1 (GENBANK Accession No. NM_000029.3), or to SEQ ID NO: 2 (the complement of GENBANK Accession No. NC_000001.11 truncated from nucleotides 230700001 to 230718000), or to both.

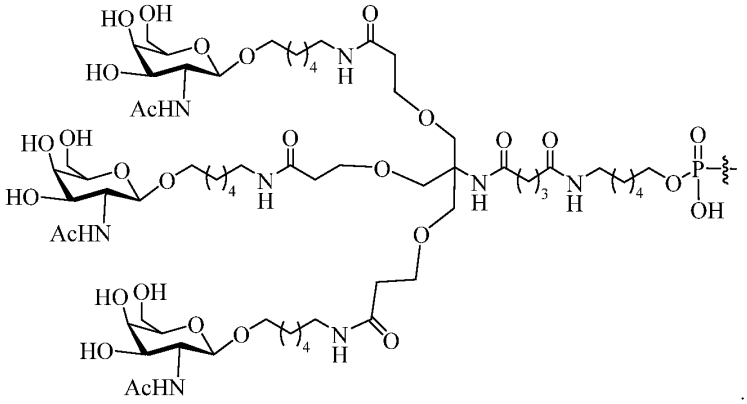
The modified oligonucleotide in Table 1 is 16 nucleosides in length with mixed sugar moieties as indicated in the table below, wherein each 'd' represents a 2'-β-D-deoxyribosyl sugar moiety, each 'e' represents a 2'- MOE sugar moiety, and each 'k' represents a cEt sugar moiety. Each internucleoside linkage is a phosphorothioate internucleoside linkage. All cytosine residues are 5-methylcytosines. 568637 is a comparator compound previously described in WO 2017/062816.

Table 1

3-10-3 MOE/cEt mixed wing gapmers with uniform PS internucleoside linkages complementary to human AGT RNA

Compound ID	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	Sugar Motif (5' to 3')	SEQ ID NO
568637	2046	2061	14940	14955	CGCTGATTTGTCCGGG	eekddddddddddkke	12

The modified oligonucleotides in Tables 2-6 all have a 5'-trishexylamino-(THA)-C_6GalNAc$_3$ endcap, represented by the structure below, wherein the phosphate group is attached to the 5'-oxygen atom of the 5'-nucleoside:



The modified oligonucleotides in Table 2 are 16 nucleosides in length with mixed sugar moieties as indicated, wherein each 'd' represents a 2'-β-D-deoxyribosyl sugar moiety, each 'e' represents a 2'- MOE sugar moiety, and each 'k' refers to a cEt sugar moiety. The internucleoside motif for the gapmers is (from 5' to 3'): soosssssssssssos; wherein each 'o' represents a phosphodiester internucleoside linkage and each 's' represents a phosphorothioate internucleoside linkage. All cytosine residues are 5-methylcytosines.

Table 2

GalNAc-conjugated 3-10-3 MOE/cEt mixed wing gapmers with mixed PO/PS internucleoside linkages complementary to human AGT RNA

Compound ID	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	Sugar Motif (5' to 3')	SEQ ID NO
1205407	2046	2061	14940	14955	THA-GalNAc-CGCTGATTTGTCCGGG	eekddddddddddkke	12
1205408	2271	2286	15165	15180	THA-GalNAc-TCGGTTGGAATTCTTT	ekkddddddddddkke	13
1205410	2046	2061	14940	14955	THA-GalNAc-CGCTGATTTGTCCGGG	ekkddddddddddkke	12

The modified oligonucleotides in Table 3 are 16 nucleosides in length with mixed sugar moieties as indicated, wherein each 'd' represents a 2'-β-D-deoxyribosyl sugar moiety, each 'e' represents a 2'- MOE sugar moiety, each 'k' refers to a cEt sugar moiety, and each 'y' refers to a 2'-OMe ribose sugar. The internucleoside motif for the gapmers is (from 5' to 3'): soosssssssssssos; wherein each 'o' represents a phosphodiester internucleoside linkage and each 's' represents a phosphorothioate internucleoside linkage. All cytosine residues are 5-methylcytosines, unless indicated by a bold underlined '**C**', in which case, the cytosine is not methylated.

Table 3

GalNAc-conjugated 3-10-3 cEt gapmers having a 2'-OMe in the gap with mixed PO/PS internucleoside linkages complementary to human AGT RNA

Compound ID	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	Sugar Motif (5' to 3')	SEQ ID NO
1299239	637	652	7279	7294	THA-GalNAc-CTCATUGTGGATGACG	kkkddyddddddkkk	16

1299240	637	652	7279	7294	THA-GalNAc-CTCATTGTGGATGACG	kkkdddydddddkkk	17
1299247	711	726	7353	7368	THA-GalNAc-TGAATUGGAGCAGGTA	kkkddydddddkkk	18
1299248	711	726	7353	7368	THA-GalNAc-TGAATTGGAGCAGGTA	kkkdddydddddkkk	19
1251199	785	800	7427	7442	THA-GalNAc-CGGTGTCAAGTTTTGC	kkkdyddddddddkkk	20
1251204	1826	1841	14720	14735	THA-GalNAc-GTTGGGTAGACTCTGT	kkkdyddddddddkkk	21
1250850	2046	2061	14940	14955	THA-GalNAc-CGCTGATTTGTCCGGG	kkkdyddddddddkkk	12
1251213	2268	2283	15162	15177	THA-GalNAc-GTTGGAATTCTTTTTG	kkkdyddddddddkkk	22
1250851	2271	2286	15165	15180	THA-GalNAc-TCGGUTGGAATTCTTT	kkkdyddddddddkkk	14

The modified oligonucleotides in Table 4 are 16 nucleosides in length with mixed sugar motifs as indicated, wherein 'd' represents a 2'-β-D-deoxyribosyl sugar moiety, 'e' represents a 2'-MOE sugar moiety, and 'k' refers to a cEt sugar moiety. Each internucleoside linkage is a phosphorothioate internucleoside linkage. All cytosine residues are 5-methylcytosines.

Table 4

GalNAc-conjugated 3-10-3 MOE/cEt mixed wing gapmers with uniform PS internucleoside linkages complementary to human AGT RNA

Compound ID	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	Sugar Motif (5' to 3')	SEQ ID NO
1176644	2046	2061	14940	14955	THA-GalNAc-CGCTGATTTGTCCGGG	eekddddddddddkke	12
1176648	2271	2286	15165	15180	THA-GalNAc-TCGGTTGGAATTCTTT	ekkddddddddddkke	13
1176649	2272	2287	15166	15181	THA-GalNAc-GTCGGTTGGAATTCTT	ekkddddddddddkke	15
1176653	2046	2061	14940	14955	THA-GalNAc-CGCTGATTTGTCCGGG	ekkddddddddddkke	12
1231463	1834	1849	14728	14743	THA-GalNAc-GTTAAGCTGTTGGGTA	kkkddddddddddkkk	23

The modified oligonucleotides in Table 5 are 16 nucleosides in length with mixed sugar motifs as indicated, wherein 'd' represents a 2'-β-D-deoxyribosyl sugar moiety, 'e' represents a 2'-MOE sugar moiety, 'k' refers to a cEt sugar moiety, and 'y' refers to a 2'-OMe ribose sugar. Each internucleoside linkage is a

phosphorothioate internucleoside linkage. All cytosine residues are 5-methylcytosines, unless indicated by a bold underlined '**C**', in which case, the cytosine is not methylated.

Table 5

GalNAc-conjugated 3-10-3 cEt gapmers having a 2'-OMe in the gap with uniform PS internucleoside linkages complementary to human AGT RNA

Compound ID	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	Sugar Motif (5' to 3')	SEQ ID NO
1250835	2046	2061	14940	14955	THA-GalNAc-CGCTGATTTGTCCGGG	kkkdyddddddddkkk	12
1250836	2271	2286	15165	15180	THA-GalNAc-TCGGUTGGAATTCTTT	kkkdyddddddddkkk	14
1250837	2272	2287	15166	15181	THA-GalNAc-GTCGGTTGGAATTCTT	kkkdyddddddddkkk	15
1250840	711	726	7353	7368	THA-GalNAc-TGAAUTGGAGCAGGTA	kkkdyddddddddkkk	24
1250842	1729	1744	13760	13775	THA-GalNAc-TTGCAGGTTCAGCTCG	kkkdyddddddddkkk	25
1251216	1822	1837	14716	14731	THA-GalNAc-GGTAGACTCTGTGGGC	kkkdyddddddddkkk	26
1251228	2268	2283	15162	15177	THA-GalNAc-GTTGGAATTCTTTTTG	kkkdyddddddddkkk	27

The modified oligonucleotide in Table 6 is a 5-10-5 MOE gapmer with uniform phosphorothioate internucleoside linkages. The compound is 20 nucleosides in length, wherein the central gap segment consists of ten 2'-β-D-deoxynucleosides and the 5' and 3' wing segments each consists of five 2'-MOE modified nucleosides. Each internucleoside linkage is a phosphorothioate internucleoside linkage. All cytosine residues are 5-methylcytosines. 757456 is comparator compound previously described in WO 2017/062816.

Table 6

GalNAc-conjugated 5-10-5 MOE gapmer with uniform PS internucleoside linkages complementary to human AGT RNA

Compound ID	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	Sequence (5' to 3')	Sugar Motif (5' to 3')	SEQ ID NO
757456	2281	2300	15175	15194	THA-GalNAc-CACAAACAAGCTGGTCGGTT	eeeeddddddddddeeeee	28

Example 2: Dose-dependent *in vitro* inhibition of human AGT in HepG2 cells

Cultured HepG2 cells at a density of 10,000 cells per well treated by electroporation with modified oligonucleotides diluted to different concentrations as specified in the tables below. After a treatment period of approximately 24 hours, AGT RNA levels were measured as previously described using the Human AGT primer-probe set RTS3721 (described herein above). AGT RNA levels were normalized to Human GAPDH expression level using the primer probe set RTS104 (forward sequence GAAGGTGAAGGTCGGAGTC, designated herein as SEQ ID NO: 9; reverse sequence GAAGATGGTGATGGGATTTC, designated herein as SEQ ID NO: 10; probe sequence CAAGCTTCCCGTTCTCAGCC, designated herein as SEQ ID NO: 11). Results are presented in the tables below as percent inhibition of AGT, relative to untreated control cells. As used herein, a value of '0' indicates that treatment with the modified oligonucleotide did not inhibit AGT mRNA levels.

Table 7

Multi-dose assay of modified oligonucleotides in HepG2 cells

Compound No.	%Inhibition					IC_{50} (μM)
	23nM	94nM	375nM	1500nM	6000nM	
1250840	0	0	5	25	51	5.3
1231463	0	8	14	47	77	1.8
1205407	0	19	52	81	91	0.4
1250850	12	25	49	74	93	0.4
1251213	0	6	3	33	71	2.8
1251228	0	0	4	34	58	3.9
1205408	3	5	15	47	77	1.8
1250836	0	9	22	48	75	1.7
1250851	11	7	24	61	80	1.1
1250837	11	4	29	61	83	1.0
1299239	13	37	65	92	97	0.8
1299240	23	56	85	97	96	1.9
1205410	6	16	31	68	98	0.2
1250835	10	20	50	87	86	0.4

Example 3: Tolerability of modified oligonucleotides targeting human AGT in CD-1 mice

CD1 mice are a multipurpose mouse model frequently utilized for safety and efficacy testing. The mice were treated with modified oligonucleotides selected from studies described above and evaluated for changes in the levels of various plasma chemistry markers.

Treatment

Groups of 6- to 8-week-old male CD-1 mice were injected subcutaneously once a week for six weeks (for a total of 7 treatments) with 15 mg/kg of modified oligonucleotides. One group of male CD-1 mice was injected with saline. Mice were euthanized 72 hours following the final administration.

Plasma chemistry markers

To evaluate the effect of modified oligonucleotides on liver function, plasma levels of blood urea nitrogen (BUN), albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CREA) and total bilirubin (TBIL) were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400c, Melville, NY). The results are presented in the table below.

Table 8

Plasma chemistry markers in male CD-1 mice

Compound No.	BUN (mg/dL)	Albumin (g/dL)	ALT (IU/L)	AST (IU/L)	TBIL (mg/dL)	CREA (mg/dL)
Saline	23	3	31	49	0.14	0.08
1176644	24	3	52	56	0.13	0.11
1176648	25	3	70	51	0.12	0.11
1176653	22	3	309	330	0.12	0.09
1205407	21	3	123	83	0.12	0.09
1205408	24	3	64	78	0.12	0.13
1205410	26	3	161	121	0.13	0.11
1231463	27	3	132	188	0.19	0.13

Blood obtained from mouse groups at week 6 were sent to IDEXX BioResearch for measurement of blood cell counts. Counts taken include red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (HGB), hematocrit (HCT), Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and individual white blood cell counts, such as that of monocytes (MON), neutrophils (NEU), lymphocytes (LYM), and platelets (PLT). The results are presented in the tables below.

Table 9

Blood Cell Count in male CD-1 mice

Compound No.	WBC (K/uL)	RBC (M/uL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	NEU (/uL)	LYM (/uL)	MON (/uL)	PLT (K/uL)
Saline	8	9	14	47	52	16	31	1133	6142	390	1290
1176644	6	9	14	46	50	16	31	919	4533	231	1182

1176648	4	9	14	44	50	16	31	703	3370	227	1186
1176653	8	10	15	48	48	15	32	989	6505	361	829
1205407	9	10	15	47	47	15	33	901	7171	355	1228
1205408	9	9	15	46	48	15	32	1633	6745	501	1446
1205410	6	9	14	45	50	16	32	714	5112	227	994
1231463	8	10	15	46	48	16	32	872	6365	481	1260

Body weights of mice were measured at days 1 and 35, and the average body weight for each group is presented in the table below. Liver, spleen and kidney weights were measured at the end of the study and are presented in the table below. Modified oligonucleotides that caused any changes in organ weights outside the expected range for modified oligonucleotides were excluded from further studies

Table 10

Body and organ weights (g)

Compound No.	Body Weight (g)		Liver Weight (g)	Kidney Weight (g)	Spleen Weight (g)
	Day 1	Day 35			
PBS	31.7	37.7	2.1	0.7	0.2
1176644	31.0	37.7	2.5	0.5	0.1
1176648	32.4	40.0	2.5	0.6	0.1
1176653	32.0	40.7	2.8	0.7	0.1
1205407	31.7	39.2	2.7	0.6	0.1
1205408	30.5	38.3	2.2	0.5	0.1
1205410	29.8	35.2	2.4	0.5	0.1
1231463	32.5	39.6	2.5	0.5	0.1

Example 4: Tolerability of modified oligonucleotides targeting human AGT in Sprague-Dawley rats

Sprague-Dawley rats are a multipurpose model used for safety and efficacy evaluations. The rats were treated with Ionis modified oligonucleotides from the studies described in the Examples above and evaluated for changes in the levels of various plasma chemistry markers.

STUDY 1

Treatment

Male Sprague-Dawley rats were maintained on a 12-hour light/dark cycle and fed *ad libitum* with Purina normal rat chow. Groups of 4 Sprague-Dawley rats each were weekly injected subcutaneously with 15 mg/kg of Ionis oligonucleotide for 6 weeks (total 6 doses). 72 hours after the last dose, rats were euthanized; and organs, urine and plasma were harvested for further analysis.

Plasma chemistry markers

To evaluate the effect of Ionis oligonucleotides on hepatic function, plasma levels of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400c, Melville, NY). Plasma levels of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in the Table below expressed in IU/L. Plasma levels of total bilirubin (TBIL), creatinine, albumin, and Blood Urea Nitrogen (BUN) were also measured using the same clinical chemistry analyzer and the results are also presented in the table below.

Table 11

Plasma chemistry markers in Sprague-Dawley rats

Compound No .	BUN (mg/dL)	Albumin (g/dL)	ALT (IU/L)	AST (IU/L)	TBIL (mg/dL)	CREA (mg/dL)
Saline	17	3	27	78	0.13	0.23
1205407	15	3	69	182	0.15	0.25
1205408	20	3	34	151	0.36	0.25

Organ weights

Liver, heart, spleen and kidney weights were measured at the end of the study and are presented in the Table below.

Table 12

Organ weights (g)

Compound No.	Liver (g)	Kidney (g)	Spleen (g)
Saline	13.7	3.4	0.8
1205407	15.2	3.6	1.2
1205408	18.3	4.0	1.2

Kidney function

To evaluate the effect of Ionis modified oligonucleotides on kidney function, urinary levels of total protein and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400c, Melville, NY). The ratios of total protein to creatinine (P/C ratio) are presented in the Table below.

Table 13

Total protein to creatinine ratio in Sprague-Dawley rats

Compound No.	P/C Ratio
Saline	1.1
1205407	2.1
1205408	0.7

STUDY 2

Treatment

Male Sprague-Dawley rats were maintained on a 12-hour light/dark cycle and fed *ad libitum* with Purina normal rat chow. Groups of 4 Sprague-Dawley rats each were weekly injected subcutaneously with 15 mg/kg of Ionis oligonucleotide for 6 weeks (total 6 doses). 72 hours after the last dose, rats were euthanized; and organs, urine and plasma were harvested for further analysis.

Plasma chemistry markers

To evaluate the effect of Ionis oligonucleotides on hepatic function, plasma levels of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400c, Melville, NY). Plasma levels of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in the Table below expressed in IU/L. Plasma levels of total bilirubin (TBIL), creatinine, albumin, and Blood Urea Nitrogen (BUN) were also measured using the same clinical chemistry analyzer and the results are also presented in the Table below.

Table 14

Plasma chemistry markers in Sprague-Dawley rats

Compound No .	BUN (mg/dL)	Albumin (g/dL)	ALT (IU/L)	AST (IU/L)	TBIL (mg/dL)	CREA (mg/dL)
Saline	16	3	28	112	0.11	0.27
1250837	18	4	32	129	0.14	0.23
1250851	18	3	119	641	0.41	0.25

Organ weights

Liver, heart, spleen and kidney weights were measured at the end of the study and are presented in the Table below.

Table 15

Organ weights (g)

Compound No.	Liver (g)	Kidney (g)	Spleen (g)
Saline	12.242	3.388	1.051
1250837	13.771	3.580	1.184
1250851	14.850	3.756	1.166

Kidney function

To evaluate the effect of Ionis modified oligonucleotides on kidney function, urinary levels of total protein and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400c, Melville, NY). The ratios of total protein to creatinine (P/C ratio) are presented in the Table below.

Table 16

Total protein to creatinine ratio in Sprague-Dawley rats

Compound No.	P/C Ratio
Saline	0.9
1250837	1.1
1250851	0.8

Example 5: Activity of modified oligonucleotides complementary to human AGT in transgenic mice

A transgenic AGT mouse model was developed in Dr. Curt Sigmund's lab by insertion of a 14 kb transgenic construct containing the entire human angiotensinogen gene (approximately 11.5 kb) and 5' (1.2 kb) and 3' (1.4 kb) flanking sequence (Yang G; et al. 1994. J Biol Chem 269(51):32497-502) and used for further testing of the modified oligonucleotides described above.

Treatment

The AGT transgenic mice were divided into groups of 2 mice each. Each mouse was injected subcutaneously weekly with 2.7 mg/kg of modified oligonucleotides (total of 2 doses on Days 0 and 7). A group of 2 mice received saline as a negative control. In addition, a group of 2 mice received 3.3 mg/kg of comparator modified oligonucleotide 757456 (total of 2 doses on Days 0 and 7). The mice were sacrificed three days after the last dose (Day 10). Liver and plasma were collected for analysis.

RNA and protein analysis

RNA was extracted from liver tissue for real-time PCR analysis to measure the amount of AGT RNA using Human primer probe set RTS3721 (described herein above). Results are presented as percent inhibition of AGT RNA relative to saline control, normalized to total RNA content, as measured by RIBOGREEN®. In addition, plasma was extracted to measure human AGT protein levels in plasma using an ELISA kit (Human Total Angiotensinogen Assay Kit, IBL, Cat#27412). Results are presented as percent inhibition of AGT protein relative to saline control.

Table 17

Reduction of human AGT RNA and protein in transgenic mice

Compound No.	Dose (mg/kg)	%inhibition (AGT RNA) in liver	%inhibition (AGT protein) in plasma
757456	3.3	65	60

1231463	2.7	93	87
1176644	2.7	90	91
1176648	2.7	93	90
1176653	2.7	96	93
1205407	2.7	93	90
1205408	2.7	94	91
1205410	2.7	96	92
1250842	2.7	88	65
1251204	2.7	44	47

Example 6: Potency of modified oligonucleotides complementary to human AGT RNA in transgenic mice, multiple dose

Modified oligonucleotides were further tested in a dose-dependent manner in the transgenic mouse model described above.

Treatment

The AGT transgenic mice were divided into groups of 2 mice each. Each mouse was injected subcutaneously with two doses (on Days 0 and 7) of modified oligonucleotide at the concentrations indicated in the table below. A group of 4 mice received PBS as a negative control. Seventy-two hours after the last dose (Day 10), the mice were euthanized. Liver and plasma were collected for analysis. Compound No. 757456 was added as a comparator compound in some studies.

STUDY 1

RNA and protein analysis

RNA was extracted from liver tissue for real-time PCR analysis to measure the amount of AGT RNA using Human primer probe set RTS3721 (described herein above). Results are presented as percent inhibition of AGT RNA relative to saline control, normalized to total RNA content, as measured by RIBOGREEN®. In addition, plasma was extracted to measure human AGT protein levels in plasma using an ELISA kit (Human Total Angiotensinogen Assay Kit, IBL, Cat#27412). Results are presented as percent inhibition of AGT protein relative to saline control.

Table 18

Reduction (%) of human AGT RNA and protein in transgenic mice

Compound No.	**Dose (mg/kg)**	**%inhibition (AGT RNA) in liver**	**ED_{50} (mg/kg)**	**%inhibition (AGT protein) in plasma**
757456	4.5	81	1.3	81

	1.5	41		49
	0.5	39		31
1205407	4.5	97	0.1	97
	1.5	95		94
	0.5	75		79
1205408	4.5	97	0.2	95
	1.5	91		90
	0.5	75		74
1205410	4.5	98	0.1	98
	1.5	97		96
	0.5	83		85
1231463	4.5	97	0.3	94
	1.5	90		87
	0.5	70		65
1250835	4.5	88	0.2	91
	1.5	86		84
	0.5	72		73
1250836	4.5	95	0.1	96
	1.5	93		93
	0.5	82		77
1250837	4.5	96	0.2	94
	1.5	89		88
	0.5	62		69
1250840	4.5	96	0.3	95
	1.5	81		85
	0.5	47		64
1250850	4.5	91	0.2	93
	1.5	78		84
	0.5	74		78
1250851	4.5	96	0.1	96
	1.5	94		95
	0.5	77		81
1251199	4.5	92	0.2	91
	1.5	84		83
	0.5	69		69
1251213	4.5	91	0.2	93
	1.5	84		86
	0.5	74		75
1251216	4.5	90	0.3	91
	1.5	79		80
	0.5	46		68
1251228	4.5	94	0.2	95
	1.5	83		88
	0.5	58		67

1299247	4.5	99	0.1	98
	1.5	98		97
	0.5	83		84
1299248	4.5	89	0.3	91
	1.5	74		84
	0.5	49		61

STUDY 2

RNA and protein analysis

RNA was extracted from liver tissue for real-time PCR analysis to measure the amount of AGT RNA using Human primer probe set RTS3721 (described herein above). Results are presented as percent inhibition of AGT RNA relative to saline control, normalized to total RNA content, as measured by RIBOGREEN®. In addition, plasma was extracted to measure human AGT protein levels in plasma using an ELISA kit (Human Total Angiotensinogen Assay Kit, IBL, Cat#27412). Results are presented as percent inhibition of AGT protein relative to saline control.

Table 19

Reduction of human AGT RNA and protein in transgenic mice

Compound No.	**Dose (mg/kg)**	**%inhibition (AGT RNA) in liver**	**ED50 (mg/kg)**	**%inhibition (AGT protein) in plasma**
1205407	5.0	98	0.14	96
	1.7	93		90
	0.6	85		80
	0.2	56		37
	0.1	30		29
1205408	5.0	98	0.27	95
	1.7	93		88
	0.6	73		76
	0.2	37		23
	0.1	9		0
1250837	5.0	85	0.53	83
	1.7	84		84
	0.6	57		64
	0.2	26		40
	0.1	0		46
1250851	5.0	94	0.23	95
	1.7	89		91
	0.6	76		76
	0.2	32		49
	0.1	32		37

STUDY 3

RNA and protein analysis

RNA was extracted from liver tissue for real-time PCR analysis to measure the amount of AGT RNA using Human primer probe set RTS3721 (described herein above). Results are presented as percent inhibition of AGT RNA relative to saline control, normalized to total RNA content, as measured by RIBOGREEN®. In addition, plasma was extracted to measure human AGT protein levels in plasma using an ELISA kit (Human Total Angiotensinogen Assay Kit, IBL, Cat#27412). Results are presented as percent inhibition of AGT protein relative to saline control. As used herein, a value of '0' indicates that treatment with the modified oligonucleotide did not inhibit AGT levels.

Table 20

Reduction of human AGT RNA and protein in transgenic mice

Compound No.	Dose (mg/kg)	%inhibition (AGT RNA) in liver	ED50 (mg/kg)	ED75 (mg/kg)	%inhibition (AGT protein) in plasma
757456	10	88	2.1	2.68	88
	3.3	74			72
	0.1	0			0
	0.04	0			0
1205407	1.5	86	0.11	0.38	87
	0.5	68			62
	0.17	38			29
	0.06	35			11
1176644	1.5	76	0.38	0.61	71
	0.5	62			51
	0.17	0			5
	0.06	0			0
1250837	1.5	74	0.22	0.67	67
	0.5	60			52
	0.17	38			28
	0.06	0			0
1176649	1.5	83	0.20	0.59	82
	0.5	50			56
	0.17	17			17
	0.06	0			0

Example 7: Dose-dependent *in vitro* inhibition of human AGT in transgenic mouse hepatocytes

The transgenic AGT mouse model described above was used in this study. Modified oligonucleotides described in the studies above were tested for inhibition of AGT RNA at various doses in primary mouse hepatocytes extracted from these transgenic mice.

Primary mouse transgenic hepatocytes were plated at a density of 20,000 cells per well and were treated by free uptake with modified oligonucleotides diluted to different concentrations as specified in the tables below. After an overnight incubation, AGT RNA levels were measured using the Human AGT primer-probe set RTS3721(forward sequence CCCTGATGGGAGCCAGTGT, designated herein as SEQ ID NO: 3; reverse sequence AGCAGGGAGAAGCCCTTCA, designated herein as SEQ ID NO: 4; probe sequence CCCTGGCTTTCAACACCTACGTCCACT, designated herein as SEQ ID NO: 5). In addition, data was confirmed a second human AGT primer probe set RTS4039 (forward sequence GGACAAGGTGGAGGGTCTCA, designated herein as SEQ ID NO: 6; reverse sequence AGATCCTTGCAGCACCAGTTG, designated herein as SEQ ID NO: 7; probe sequence ATGAAGAAACTATCTCCCCGGACCATCCA, designated herein as SEQ ID NO: 8) to measure human AGT RNA levels. AGT RNA levels were normalized to total RNA content, as measured by RIBOGREEN®. Results are presented in the tables below as percent inhibition of AGT, relative to untreated control cells. As used herein, a value of '0' indicates that treatment with the modified oligonucleotide did not inhibit AGT mRNA levels. The half maximal inhibitory concentration (IC_{50}) of each modified oligonucleotide is also presented. IC_{50} was calculated using a nonlinear regression using 4 parameter variable slope method of log(inhibitor) vs. response with the bottom and top fixed to 0 and 100, respectively (Prism).

Table 21

Multi-dose assay of modified oligonucleotides in primary mouse hepatocytes

Compound No.	%Inhibition (RTS3721)								IC_{50} (μM)
	0.61nM	2.44nM	9.77nM	39.06nM	156.25nM	625nM	2500nM	10000nM	
757456	0	0	0	0	39	52	32	20	868
1205407	36	19	51	74	86	94	88	77	8
1176644	0	8	25	51	79	88	86	88	35
1176649	0	0	25	33	60	74	72	53	95
1250837	0	0	12	27	61	67	72	73	145

Table 22

Multi-dose assay of modified oligonucleotides in primary mouse hepatocytes

Compound No.	% Inhibition (RTS4039)								IC_{50} (μM)
	0.61nM	2.44nM	9.77nM	39.06nM	156.25nM	625nM	2500nM	10000nM	
757456	0	0	0	3	43	50	37	7	709

1205407	27	21	44	69	84	89	87	83	12
1176644	0	16	16	48	74	85	82	82	43
1176649	0	0	13	35	55	74	73	65	116
1250837	2	0	14	28	57	66	70	67	169

Example 8: Dose-dependent *ex-vivo* inhibition of human AGT in HepatoPac®

The HepatoPac® kit is a commercially available liver model system available from BIOIVT that consists of micropatterned hepatocyte "islands" co-cultured with supportive stromal cells. A 96-well HepatoPac plate was equilibrated for 48hrs at 37°C and 10% CO_2 in fresh Maintenance medium prior to treatment. Modified oligonucleotides were diluted into maintenance medium at the concentrations described in the table below for 48 hours. After 48 hours, medium was replaced with fresh maintenance medium without additional oligonucleotide. Cell lysates were collected at 96 hours post oligonucleotide addition and analyzed by RT-PCR using primer probe set RTS3721 (described herein above). Results are presented in the tables below as percent inhibition of AGT, relative to untreated control cells. As used herein, a value of '0' indicates that treatment with the modified oligonucleotide did not inhibit AGT mRNA levels. IC_{50}s were calculated using variable slope 4 parameter logistic regression in Prism with the bottom and top of the curves set to 5 and 100, respectively.

Table 23

Multi-dose assay of modified oligonucleotides in Hepatopac® cells

Compound No.	%Inhibition						IC_{50} (μM)
	6.4nM	32nM	160nM	800nM	4000nM	20000nM	
757456	31	31	21	16	29	16	>20
1250850	0	3	18	61	78	86	0.85
1205407	27	52	70	84	93	92	0.04
1205410	9	46	63	89	95	99	0.06

Example 9: Effect of modified oligonucleotides targeting human AGT in cynomolgus monkeys

Cynomolgus monkeys were treated with modified oligonucleotides selected from studies described in the Examples above.

Treatment

Prior to the study, the monkeys were kept in quarantine, followed by an acclimation period during which the animals were observed daily for general health. The monkeys were 2-4 years old and weighed 2-4 kg. Nine groups of 4 randomly assigned male cynomolgus monkeys each were injected subcutaneously with Ionis oligonucleotide or saline in a clockwise rotation between four different sites on the back. Following

loading doses on days 1, 4 and 8, the monkeys were dosed once per week (on days 15, 22, 29, 36, 43, 50, 57, 64, 71, 78 and 85) with 20 mg/kg of Ionis oligonucleotide. A control group of 4 cynomolgus monkeys was injected with 0.9% saline in a similar manner and served as the control group.

During the study period, the monkeys were observed at least once daily for signs of illness or distress. Any animal showing signs of severe debility or toxicity, particularly if death appeared imminent, was euthanized for humane reasons as soon as possible with attending veterinarian consultation. Scheduled euthanasia of the animals was conducted on day 87 approximately 48 hours after the last dose by exsanguination while under deep anesthesia. The protocols described in the Example were approved by the Institutional Animal Care and Use Committee (IACUC).

Body and organ weight measurements

To evaluate the effect of Ionis oligonucleotides on the overall health of the animals, body and organ weights were measured. Terminal body weight was measured prior to necropsy. Organ weights were measured as well, and all weight measurements are presented in the table below.

Table 24
Body and Organ weights (g)

Compound No.	Terminal Body Weight	Liver with gallbladder	Kidneys	Spleen
saline	2967	60	13	3
1205407	2956	96	15	5
1205408	2971	72	13	3
1205410	2868	101	14	4
1231463	2923	69	13	5
1250835	2949	93	16	6
1250836	2973	71	14	6
1250837	2712	63	15	3
1250850	3044	97	17	5
1250851	2806	63	15	3

Kidney and Liver function

To evaluate the effect of Ionis oligonucleotides on hepatic and kidney function, blood samples were collected from all the study groups on day 87. The monkeys were fasted overnight prior to blood collection. Blood was collected in tubes without anticoagulant for serum separation. The tubes were kept at room temperature for a minimum of 90 minutes and then centrifuged at 3000 rpm for 10 minutes to obtain serum. Levels of various liver function markers were measured using a Toshiba 200FR NEO chemistry analyzer (Toshiba Co., Japan). Plasma levels of blood urea nitrogen (BUN), creatinine (CREA), total protein (TP),

albumin (ALB), globulin (GLO), albumin/globulin (A/G) ratio calculated, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL) were measured and the results are presented in the table below.

Table 25

Liver function markers in cynomolgus monkey plasma

Compound No.	BUN (mg/dL)	CREA (mg/dL)	TP (g/dL)	ALB (g/dL)	GLO (g/dL)	A/G ratio	ALT (IU/L)	AST (IU/L)	TBIL (mg/dL)
saline	28	0.8	6.9	4.1	2.8	1.5	50	57	0.25
1205407	23	0.8	6.9	4.0	2.9	1.4	67	55	0.20
1205408	26	0.8	6.7	4.0	2.7	1.5	53	50	0.27
1205410	21	0.9	6.9	3.9	3.0	1.3	145	72	0.26
1231463	22	0.8	7.3	4.0	3.4	1.2	112	75	0.26
1250835	19	0.9	7.4	3.8	3.6	1.1	129	82	0.25
1250836	23	1.1	7.7	3.9	3.8	1.1	150	95	0.34
1250837	21	0.8	6.9	4.2	2.7	1.6	91	68	0.28
1250850	21	0.8	7.4	4.0	3.4	1.2	85	86	0.25
1250851	20	0.8	6.8	4.1	2.7	1.6	63	64	0.27

Pro-inflammatory proteins analysis

To evaluate any inflammatory effect of Ionis modified oligonucleotides in cynomolgus monkeys, blood samples were taken for analysis. The monkeys were fasted overnight prior to blood collection. On day 85 (pre-dose and 24hours post-dose), approximately 0.8 mL of blood was collected from each animal and put into tubes without anticoagulant for serum separation. The tubes were kept at room temperature for a minimum of 90 min and then centrifuged at 3,000 rpm for 10 min at room temperature to obtain serum. Complement C3 were measured using a Toshiba 120 FR NEO chemistry analyzer (Toshiba Co., Japan). Another marker of inflammation, C-Reactive Protein (CRP) was tested together with the clinical chemistry parameters tested for liver function above.

Table 26

Pro-inflammatory protein analysis in cynomolgus monkeys

Compound No.	Complement C3 (mg/dL)		CRP (mg/L)
	Day 85 (pre-dose)	Day86 (24hr post-dose)	Day 87
saline	110	110	2
1205407	94	91	10
1205408	93	93	4
1205410	117	111	14

1231463	92	102	6
1250835	84	75	15
1250836	78	82	9
1250837	82	87	1
1250850	86	83	12
1250851	86	92	3

Hematology

To evaluate any effect of Ionis modified oligonucleotides in cynomolgus monkeys on hematologic parameters, blood samples of approximately 0.5 mL of blood was collected from each of the available study animals on day 87. The samples were collected in tubes containing K_2-EDTA. Samples were analyzed for red blood cell (RBC) count, Hemoglobin (HGB), Hematocrit (HCT), Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), white blood cells (WBC) count, individual white blood cell counts, such as that of monocytes (MON), neutrophils (NEU), and lymphocytes (LYM) using an ADVIA2120i hematology analyzer (Siemens, USA).

Table 27

Blood cell counts in cynomolgus monkeys

Compound No.	WBC (^3/μL)	RBC (^6/μL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	NEU (%)	LYM (%)	MON (%)	PLT (^3/μL)
saline	13	6	14	45	78	24	30	39	55	4	403
1205407	11	6	13	43	77	23	30	40	56	3	377
1205408	12	6	13	44	78	23	30	43	51	3	375
1205410	8	6	13	45	81	24	30	32	63	2	312
1231463	10	6	13	45	78	24	30	28	66	3	338
1250835	10	6	14	47	79	24	30	28	65	4	370
1250836	12	6	14	45	75	23	31	29	66	3	354
1250837	7	6	13	43	77	24	31	37	59	3	288
1250850	8	5	13	42	78	23	30	29	66	3	376
1250851	9	6	14	45	77	24	31	45	51	2	356

Urine Analysis

Food was removed overnight the day before fresh urine collection, but water was supplied. Fresh urine samples for urinalysis and urine chemistry were collected from all animals using a clean cage pan on wet ice (first in the morning) on day 87. Urinalysis/Urine Chemistry parameters include creatinine (UCRE), protein/creatinine (P/C) ratio, microprotein (UTP) and urine microalbumin (UALB), which were measured using a Toshiba 120FR automated chemistry analyzer (Toshiba Co., Japan).

Table 28

Urinalysis and Urine Chemistry Markers in cynomolgus monkeys

Compound No.	UTP (mg/dL)	UALB (mg/dL)	P/C ratio	UCRE (mg/dL)
saline	7	0.57	0.15	55
1205407	7	0.29	0.19	37
1205408	7	0.47	0.14	52
1205410	7	0.34	0.19	52
1231463	7	0.37	0.13	63
1250835	12	0.58	0.14	95
1250836	10	0.54	0.11	114
1250837	7	0.41	0.16	49
1250850	7	0.55	0.08	105
1250851	9	0.74	0.07	140

CLAIMS:

1. An oligomeric compound comprising a modified oligonucleotide consisting of 14 to 30 linked nucleosides and having a nucleobase sequence comprising at least 14, at least 15, or at least 16 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 12-15, wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

2. An oligomeric compound comprising a modified oligonucleotide consisting of 14 to 30 linked nucleosides and having a nucleobase sequence comprising at least 14, at least 15, or at least 16 contiguous nucleobases complementary to:

an equal length portion of nucleobases 2046-2061 of SEQ ID NO: 1;
an equal length portion of nucleobases 2271-2286 of SEQ ID NO: 1;
an equal length portion of nucleobases 2272-2287 of SEQ ID NO: 1;

wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

3. An oligomeric compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of SEQ ID NO: 12, wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

4. An oligomeric compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of SEQ ID NO: 13, wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

5. An oligomeric compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of SEQ ID NO: 14, wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

6. An oligomeric compound comprising a modified oligonucleotide consisting of 16 linked nucleosides and having a nucleobase sequence comprising the nucleobase sequence of SEQ ID NO: 15, wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

7. The oligomeric compound of any of claims 1-6, wherein the modified oligonucleotide has a nucleobase sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% complementary to

the nucleobase sequence of any SEQ ID NO: 1 or SEQ ID NO: 2, when measured across the entire nucleobase sequence of the modified oligonucleotide.

8. The oligomeric compound of any of claims 1-7, wherein the modified oligonucleotide comprises at least one bicyclic sugar moiety.

9. The oligomeric compound of claim 8, wherein the bicyclic sugar moiety has a 4'-2' bridge, wherein the 4'-2' bridge is selected from $-CH_2-O-$; and $-CH(CH_3)-O-$.

10. The oligomeric compound of any of claims 1-9, wherein the modified oligonucleotide comprises at least one non-bicyclic modified sugar moiety.

11. The oligomeric compound of claim 10, wherein the non-bicyclic modified sugar moiety is a 2'-MOE sugar moiety or a 2'-OMe sugar moiety.

12. The oligomeric compound of any of claims 1-11, wherein the modified oligonucleotide comprises at least one sugar surrogate.

13. The oligomeric compound of claim 12, wherein the sugar surrogate is any of morpholino, modified morpholino, PNA, THP, and F-HNA.

14. The oligomeric compound of any of claims 1-13, wherein the modified oligonucleotide is a gapmer.

15. The oligomeric compound of claim 14, wherein the modified oligonucleotide has a sugar motif comprising:

a 5'-region consisting of 1-6 linked 5'-region nucleosides;

a central region consisting of 6-10 linked central region nucleosides; and

a 3'-region consisting of 1-6 linked 3'-region nucleosides; wherein

each of the 5'-region nucleosides and each of the 3'-region nucleosides comprises a modified sugar moiety and at least 6 of the central region nucleosides comprises a 2'-β-D-deoxyribosyl sugar moiety.

16. The oligomeric compound of claim 14, wherein the modified oligonucleotide has a sugar motif comprising:

a 5'-region consisting of 1-6 linked 5'-region nucleosides;

a central region consisting of 6-10 linked central region nucleosides; and

a 3'-region consisting of 1-6 linked 3'-region nucleosides; wherein

each of the 5'-region nucleosides and each of the 3'-region nucleosides comprises a modified sugar moiety and each of the central region nucleosides comprises a 2'-deoxyribosyl sugar moiety

17. The oligomeric compound of claim 14, wherein the modified oligonucleotide has a sugar motif comprising:

a 5'-region consisting of 3 linked 5'-region nucleosides;

a central region consisting of 10 linked central region nucleosides; and

a 3'-region consisting of 3 linked 3'-region nucleosides; wherein

each of the 5'-region nucleosides and each of the 3'-region nucleosides comprises a 2'-MOE modified sugar moiety or a cEt modified sugar moiety, and each of the central region nucleosides comprises a 2'-β-D-deoxyribosyl sugar moiety.

18. The oligomeric compound of claim 14, wherein the modified oligonucleotide has a sugar motif comprising:

a 5'-region consisting of 3 linked 5'-region nucleosides;

a central region consisting of 10 linked central region nucleosides; and

a 3'-region consisting of 43 linked 3'-region nucleosides; wherein

each of the 5'-region nucleosides and each of the 3'-region nucleosides comprises a 2'-MOE modified sugar moiety or a cEt modified sugar moiety, and at least 6 of the central region nucleosides comprises a 2'-β-D-deoxyribosyl sugar moiety.

19. The oligomeric compound of any of claims 1-18, wherein the modified oligonucleotide has a sugar motif (5' to 3') selected from eekddddddddddkke, ekkddddddddddkke, kkkdyddddddddkkk, kkkddyddddddddkkk, kkkdddyddddddkkk, kkkddddddddddkkk, or eeeeddddddddddeeeee; wherein 'e' represents a 2'-MOE sugar moiety, 'k' represents a cEt sugar moiety, 'd' represents a 2'-β-D-deoxyribosyl sugar moiety, and 'y' represents a 2'-OMe sugar moiety.

20. The oligomeric compound of any of claims 1-19, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage.

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

22. The oligomeric compound of claim 20 or claim 21, wherein at least one internucleoside linkage is a phosphorothioate internucleoside linkage.

23. The oligomeric compound of any of claims 20 and claim 22, wherein the modified oligonucleotide comprises at least one phosphodiester internucleoside linkage.

24. The oligomeric compound of any of claims 20, 22 and 23, wherein each internucleoside linkage is either a phosphodiester internucleoside linkage or a phosphorothioate internucleoside linkage.

25. The oligomeric compound of claim 21, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage.

26. The oligomeric compound of any of claims 1-20 or 22-24, wherein the modified oligonucleotide has an internucleoside linkage motif of soosssssssssos; wherein,

s = a phosphorothioate internucleoside linkage, and

o = a phosphodiester internucleoside linkage.

27. The oligomeric compound of any of claims 1-26, wherein the modified oligonucleotide comprises at least one modified nucleobase.

28. The oligomeric compound of claim 27, wherein the modified nucleobase is a 5-methylcytosine.

29. The oligomeric compound of any of claims 1-28, wherein the modified oligonucleotide consists of 14-30, 14-22, 14-20, 14-18, 14-20, 15-17, 15-25, or 16-20 linked nucleosides.

30. The oligomeric compound of any of claims 1-28, wherein the modified oligonucleotide consists of 16 linked nucleosides.

31. The oligomeric compound of any of claims 1-30, comprising a conjugate group.

32. The oligomeric compound of claim 31, wherein the conjugate group comprises a GalNAc cluster comprising 1-3 GalNAc ligands.

33. The oligomeric compound of any one of claims 31 and 32, wherein the conjugate group comprises a conjugate linker consisting of a single bond.

34. The oligomeric compound of any one of claims 31-33, wherein the conjugate group comprises a cleavable linker.

35. The oligomeric compound of any one of claims 31-34, wherein the conjugate group comprises a conjugate linker comprising 1-3 linker-nucleosides.

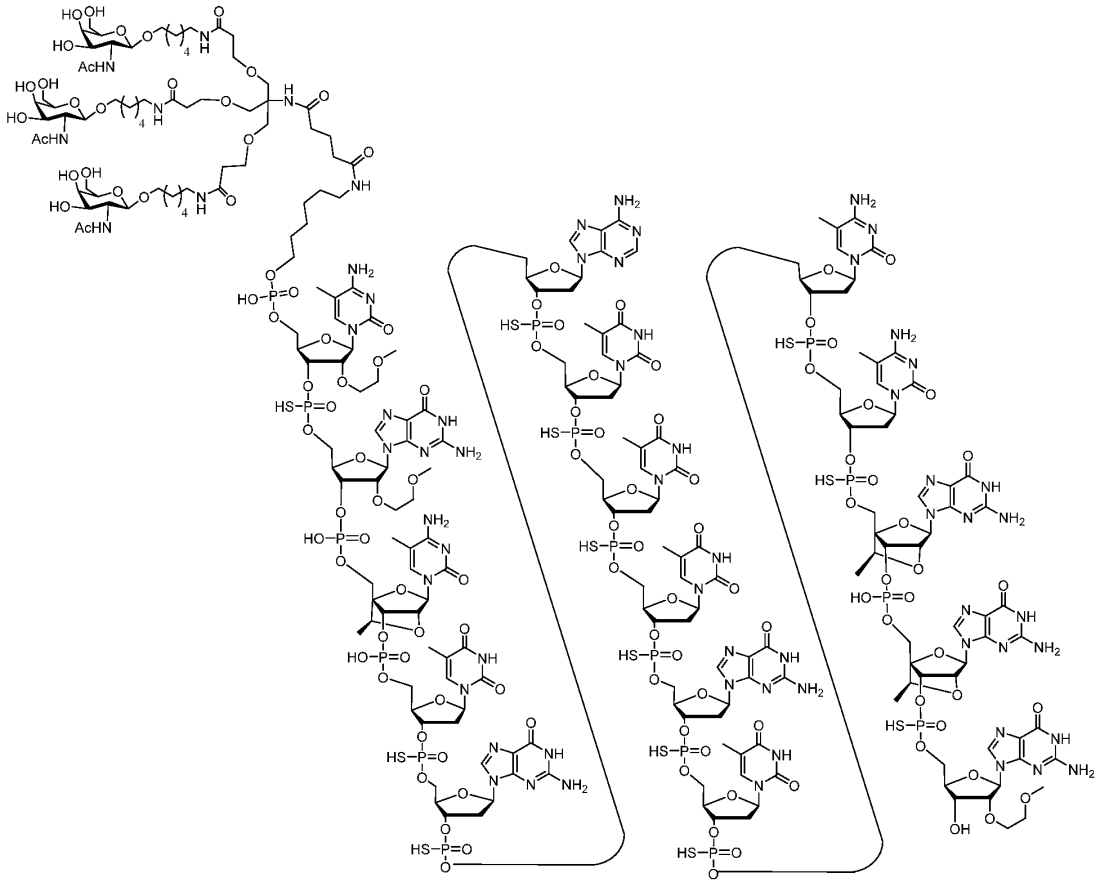
36. The oligomeric compound of any one of claims 31-35, wherein the conjugate group is attached to the modified oligonucleotide at the 5'-nucleoside of the modified oligonucleotide.

37. The oligomeric compound of any one of claims 31-35, wherein the conjugate group is attached to the modified oligonucleotide at the 3'-nucleoside of the modified oligonucleotide.

38. The oligomeric compound of any of claims 1-37, wherein the oligomeric compound is a singled-stranded oligomeric compound.

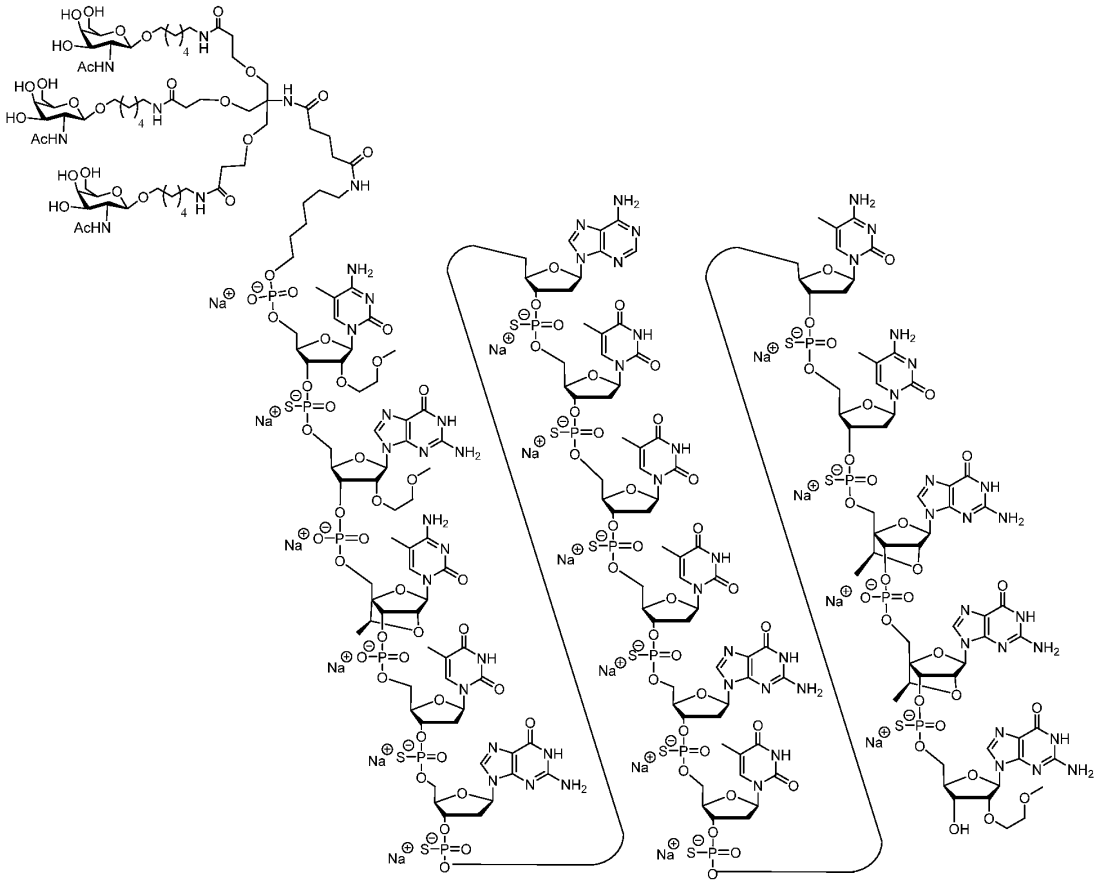
39. The oligomeric compound of any of claims 1-30, consisting of the modified oligonucleotide.

40. An oligomeric compound according to the following chemical structure:



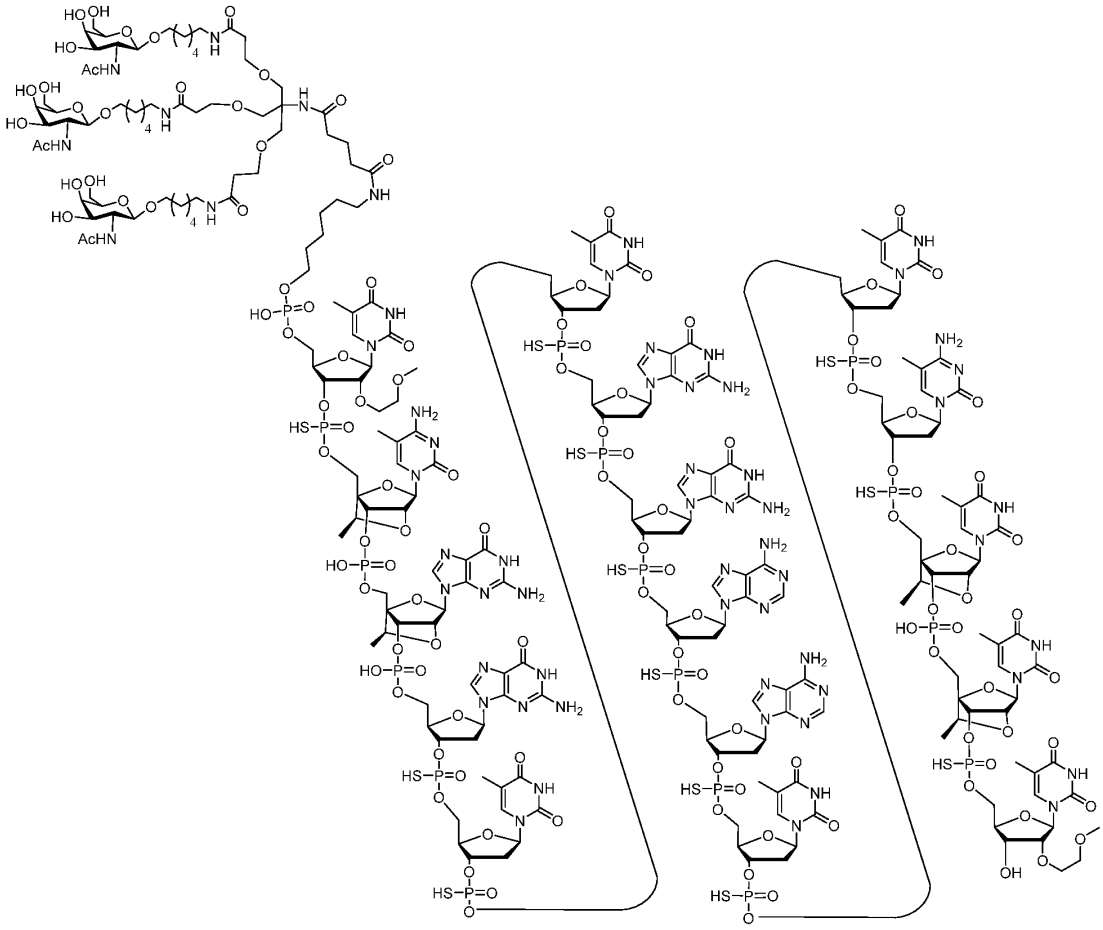
(SEQ ID NO: 12), or a salt thereof.

41. An oligomeric compound according to the following chemical structure:



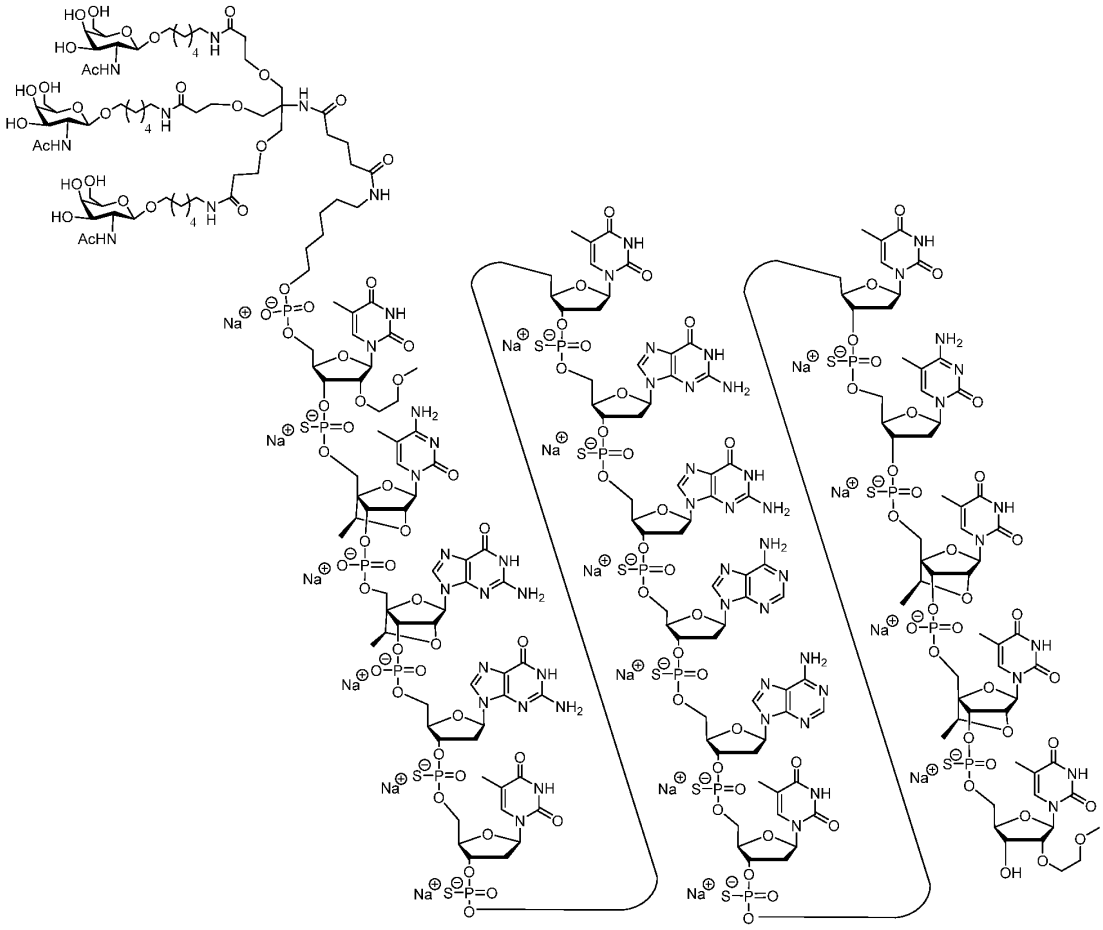
(SEQ ID NO: 12).

42. An oligomeric compound according to the following chemical structure:



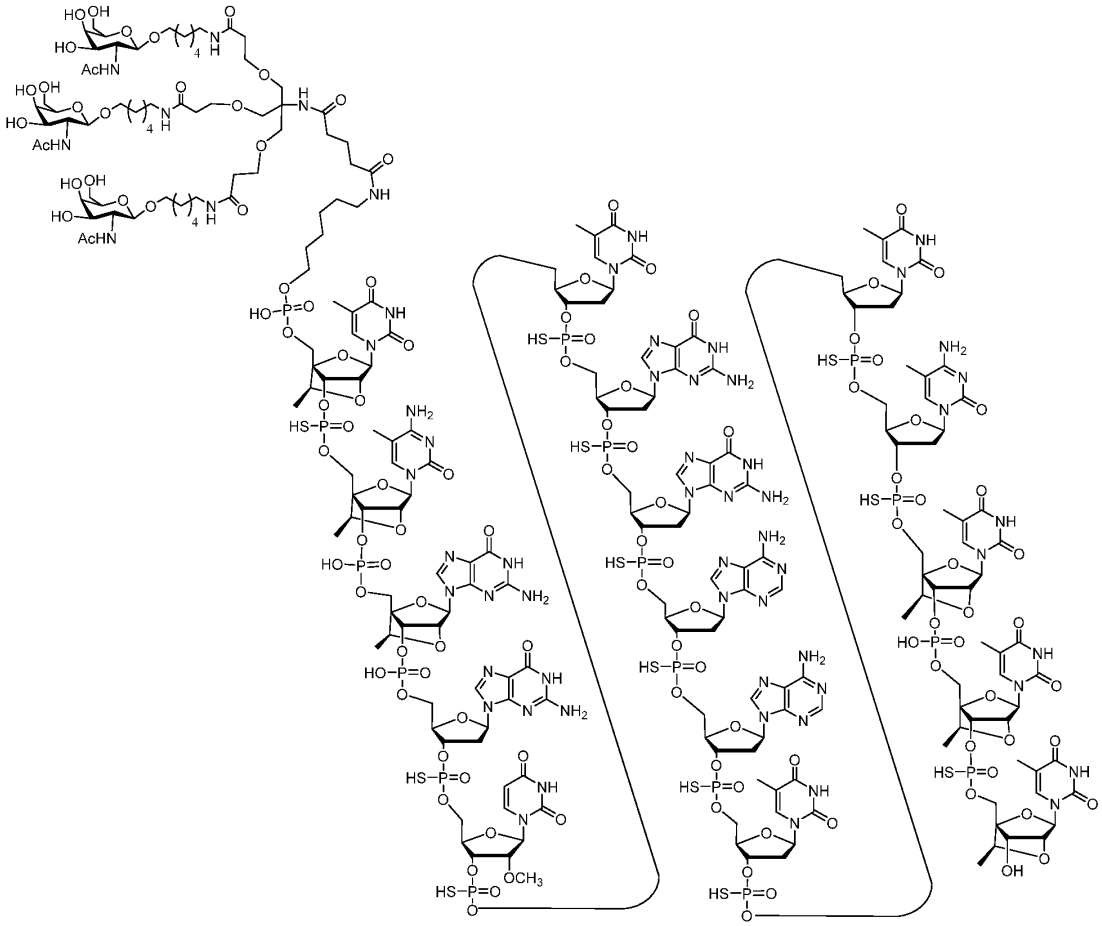
(SEQ ID NO: 13), or a salt thereof.

43. An oligomeric compound according to the following chemical structure:



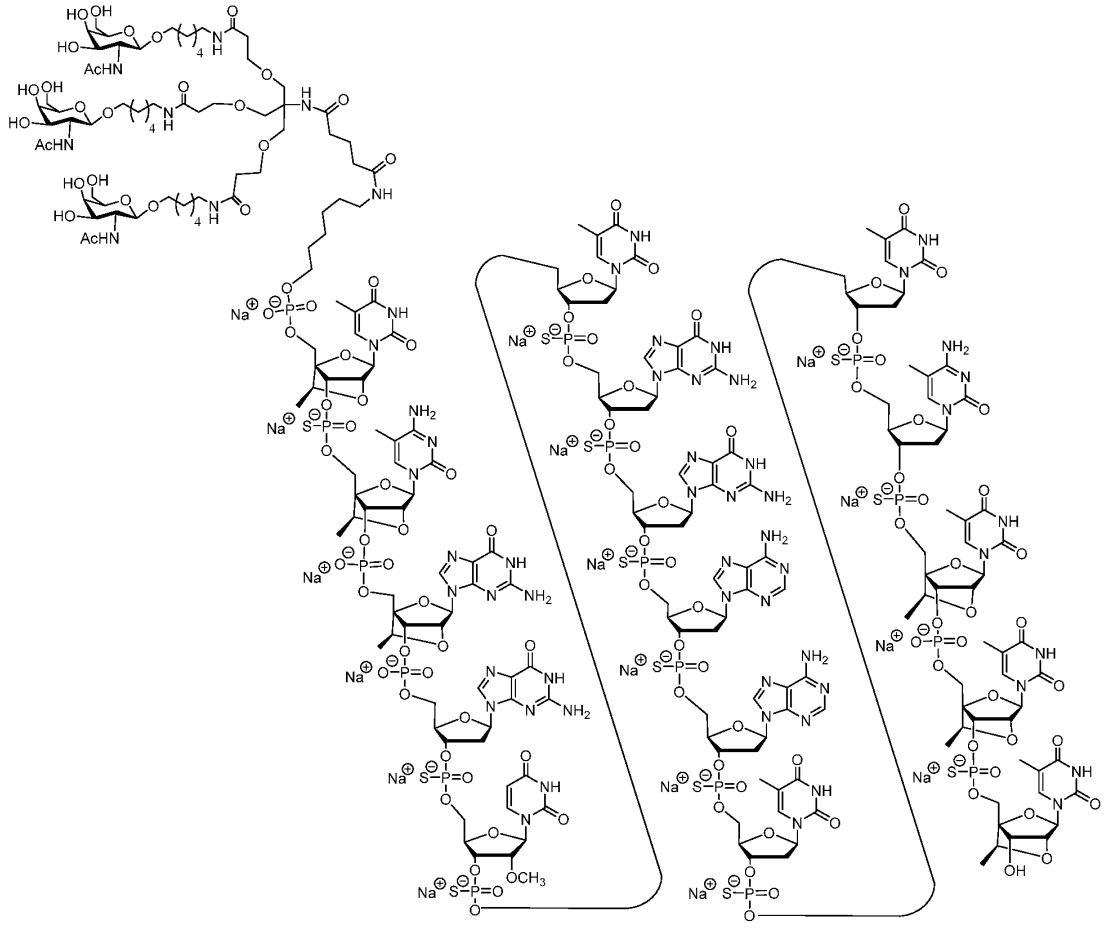
(SEQ ID NO: 13).

44. An oligomeric compound according to the following chemical structure:



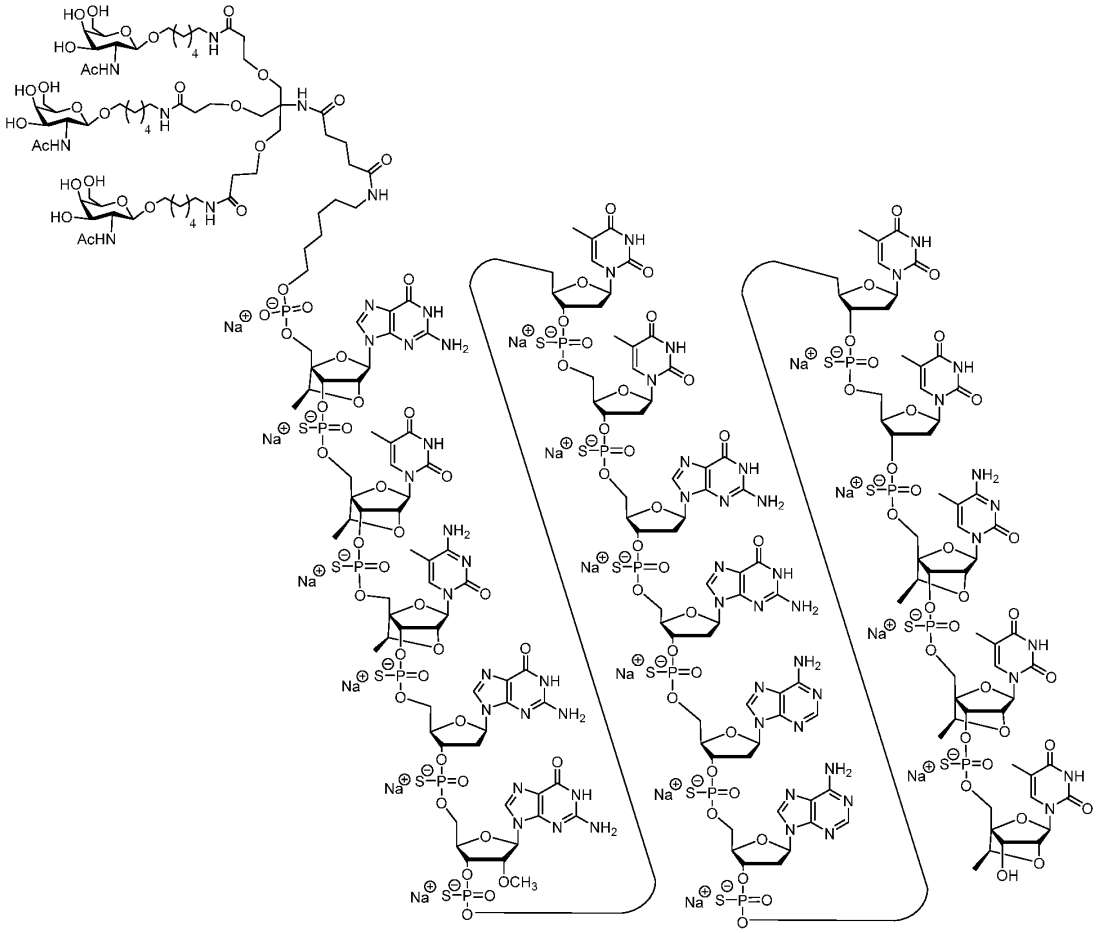
(SEQ ID NO: 14), or a salt thereof.

45. An oligomeric compound according to the following chemical structure:



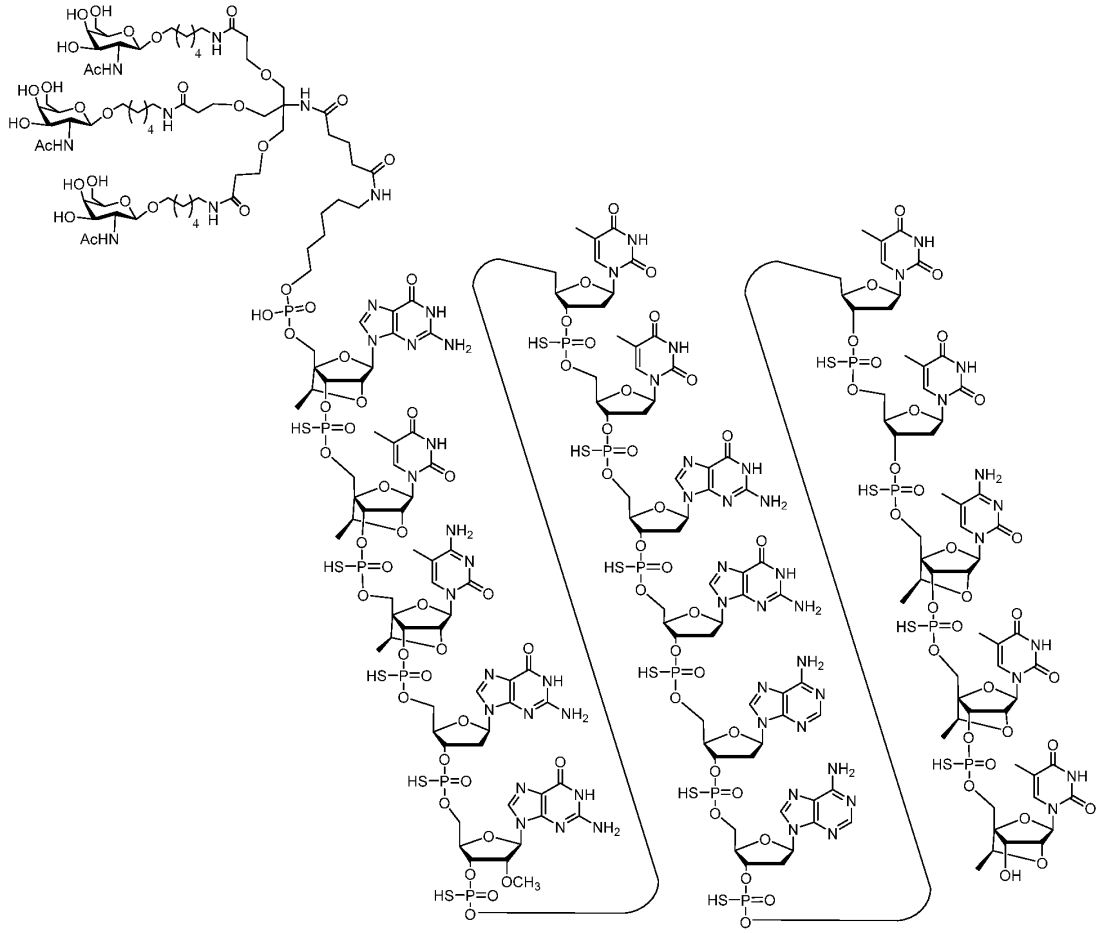
(SEQ ID NO: 14).

46. An oligomeric compound according to the following chemical structure:



(SEQ ID NO: 15), or salt thereof.

47. An oligomeric compound according to the following chemical structure:



(SEQ ID NO: 15).

48. The oligomeric compound of any of claims 40, 42, 44, and 47, which is the sodium salt or potassium salt.

49. An oligomeric compound comprising a modified oligonucleotide according to the following chemical notation: $^{m}C_{es}G_{eo}{}^{m}C_{ko}T_{ds}G_{ds}A_{ds}T_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}G_{ko}G_{ks}G_{e}$ (SEQ ID NO: 12), wherein:

A = an adenine nucleobase,

^{m}C = a 5-methyl cytosine nucleobase,

G = a guanine nucleobase,

T = a thymine nucleobase,

e = a 2'-β-D-MOE sugar moiety,

k = a cEt sugar moiety,

d = a 2'-β-D-deoxyribosyl sugar moiety,

s = a phosphorothioate internucleoside linkage, and

o = a phosphodiester internucleoside linkage.

50. An oligomeric compound comprising a modified oligonucleotide according to the following chemical notation: $T_{es}{}^{m}C_{ko}G_{ko}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}A_{ds}A_{ds}T_{ds}T_{ds}{}^{m}C_{ds}T_{ko}T_{ks}T_{e}$ (SEQ ID NO: 13), wherein:

A = an adenine nucleobase,

^{m}C = a 5-methyl cytosine nucleobase,

G = a guanine nucleobase,

T = a thymine nucleobase,

e = a 2'-β-D-MOE sugar moiety,

k = a cEt sugar moiety,

d = a 2'-β-D-deoxyribosyl sugar moiety,

s = a phosphorothioate internucleoside linkage, and

o = a phosphodiester internucleoside linkage.

51. An oligomeric compound comprising a modified oligonucleotide according to the following chemical notation: $G_{ks}T_{ks}{}^{m}C_{ks}G_{ds}G_{ys}T_{ds}T_{ds}G_{ds}G_{ds}A_{ds}A_{ds}T_{ds}T_{ds}{}^{m}C_{ks}T_{ks}T_{k}$ (SEQ ID NO: 15), wherein:

A = an adenine nucleobase,

^{m}C = a 5-methyl cytosine nucleobase,

G = a guanine nucleobase,

T = a thymine nucleobase,

k = a cEt sugar moiety,

d = a 2'-β-D-deoxyribosyl sugar moiety,

y = a 2'-OMe ribose sugar moiety, and

s = a phosphorothioate internucleoside linkage.

52. An oligomeric compound comprising a modified oligonucleotide according to the following chemical notation: $T_{ks}{}^{m}C_{ko}G_{ko}G_{ds}U_{ys}T_{ds}G_{ds}G_{ds}A_{ds}A_{ds}T_{ds}T_{ds}{}^{m}C_{ds}T_{ko}T_{ks}T_{k}$ (SEQ ID NO: 14), wherein:

A = an adenine nucleobase,

^{m}C = a 5-methyl cytosine nucleobase,

G = a guanine nucleobase,

T = a thymine nucleobase,

U = a uracil nucleobase,

k = a cEt sugar moiety,

d = a 2'-β-D-deoxyribosyl sugar moiety,

y = a 2'-OMe ribose sugar moiety,

s = a phosphorothioate internucleoside linkage, and

o = a phosphodiester internucleoside linkage.

53. The oligomeric compound of any of claims 49-52, comprising the modified oligonucleotide covalently linked to a conjugate group.

54. An oligomeric duplex comprising an oligomeric compound of any of claims 1-53.

55. An antisense compound comprising or consisting of an oligomeric compound of any of claims 1-53 or an oligomeric duplex of claim 54.

56. A chirally enriched population of oligomeric compounds of any of claims 40-53, wherein the population is enriched for compounds comprising at least one particular phosphorothioate internucleoside linkage having a particular stereochemical configuration.

57. The chirally enriched population of claim 56, wherein the population is enriched for compounds comprising at least one particular phosphorothioate internucleoside linkage having the (*S*p) configuration.

58. The chirally enriched population of claim 56, wherein the population is enriched for compounds comprising at least one particular phosphorothioate internucleoside linkage having the (*R*p) configuration.

59. The chirally enriched population of claim 56, wherein the population is enriched for compounds having a particular, independently selected stereochemical configuration at each phosphorothioate internucleoside linkage.

60. The chirally enriched population of claim 56, wherein the population is enriched for compounds having the (*S*p) configuration at each phosphorothioate internucleoside linkage or for modified oligonucleotides having the (*R*p) configuration at each phosphorothioate internucleoside linkage.

61. The chirally enriched population of claim 56, wherein the population is enriched for compounds having the (Rp) configuration at one particular phosphorothioate internucleoside linkage and the (Sp) configuration at each of the remaining phosphorothioate internucleoside linkages.

62. The chirally enriched population of claim 56, wherein the population is enriched for compounds having at least 3 contiguous phosphorothioate internucleoside linkages in the Sp, Sp, and Rp configurations, in the 5' to 3' direction.

63. A population of oligomeric compounds of any of claims 40-53, in which all phosphorothioate internucleoside linkages of the oligomeric compound are stereorandom.

64. A pharmaceutical composition comprising the oligomeric compound of any of claims 1-53, the oligomeric duplex of claim 54, the antisense compound of claim 55, or the population of any of claims 56-63 and a pharmaceutically acceptable carrier or diluent.

65. A method comprising administering to an individual the pharmaceutical composition of claim 64.

66. A method of treating a disease associated with the RAAS pathway, comprising administering to an individual having or at risk of having a disease associated with the RAAS pathway a therapeutically effective amount of the pharmaceutical composition according to claim 64, thereby treating the disease associated with

the RAAS pathway.

67. The method of claim 66, wherein the disease is a cardiovascular disease.

68. The method of any of claims 66 and 67, wherein the disease is selected from hypertension, resistant hypertension, Marfan syndrome, heart failure, kidney disease, obesity, metabolic syndrome, NASH, and NAFLD.

69. The method of any of claims 66-68, wherein at least one symptom or hallmark of the disease is ameliorated.

70. The method of claim 69, wherein the symptom or hallmark is any of hypertension, hypertensive emergency (i.e. malignant hypertension), stroke, pre-eclampsia, aneurysms of the blood vessels, abdominal aneurysm, peripheral artery disease, organ damage, or pulmonary arterial hypertension.

71. The method of any of claims 65 to 70, wherein the pharmaceutical composition is administered systemically.

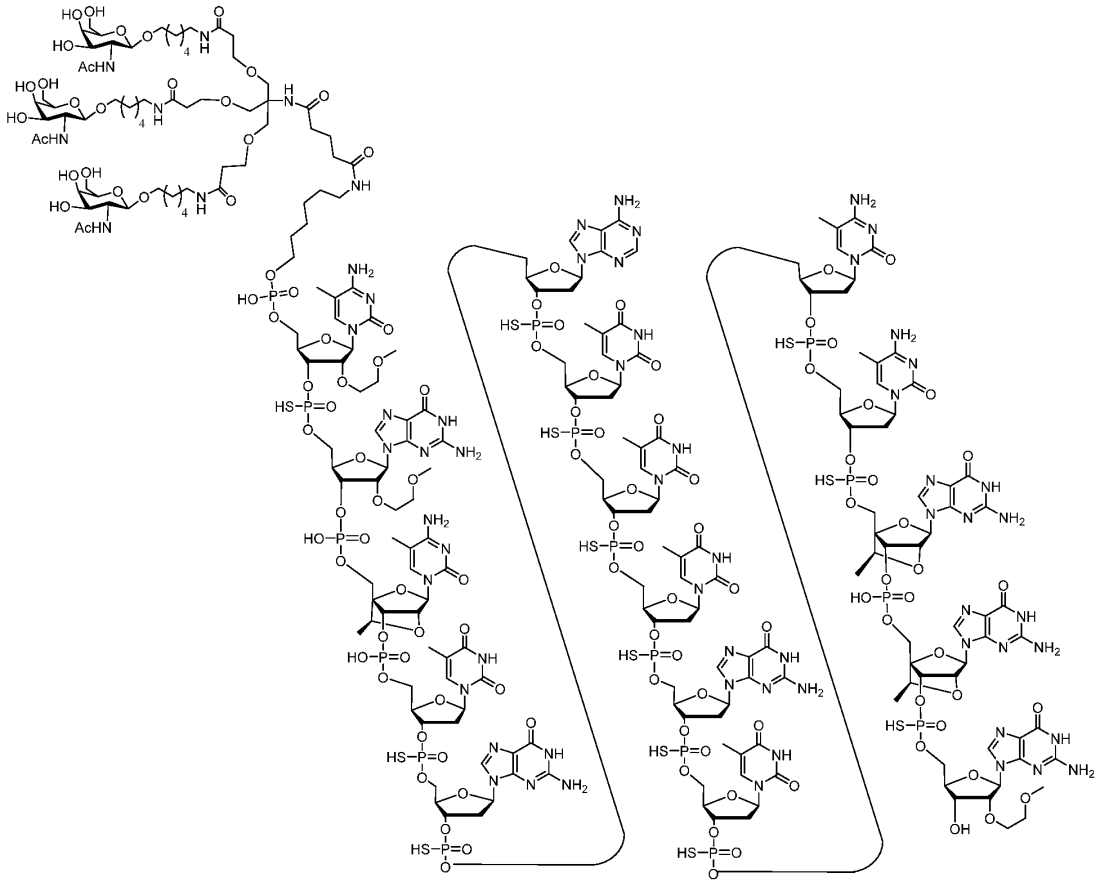
72. The method of any of claims 65 to 71, wherein the pharmaceutical composition is administered subcutaneously or intramuscularly.

73. Use of the oligomeric compound of any of claims 1-53, the oligomeric duplex of claim 54, the antisense compound of claim 55, or the population of any of claims 56-63 for reducing AGT expression in a cell.

74. The use of claim 73, wherein the level of AGT RNA is reduced.

75. The use of claim 73, wherein the level of AGT protein is reduced.

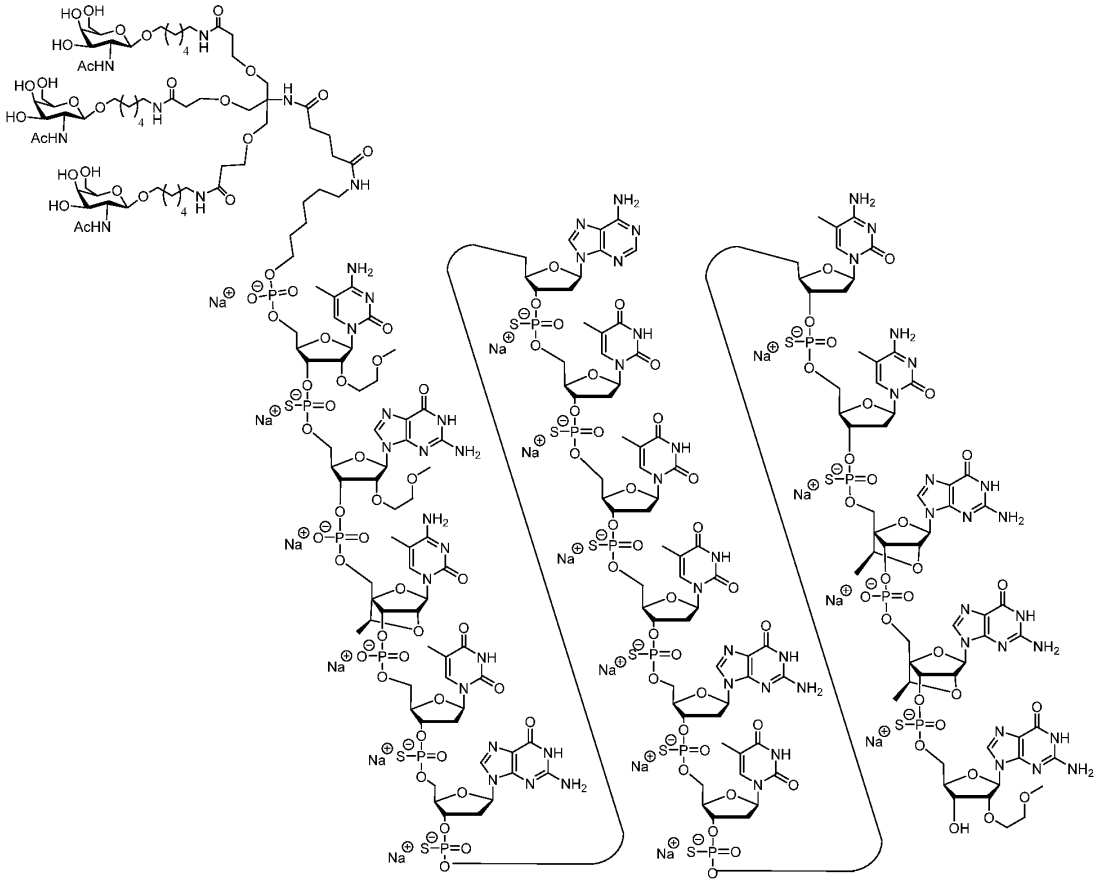
76. An oligomeric compound according to the following chemical structure:



(SEQ ID NO: 12), or a salt thereof.

77. The oligomeric compound of claim 76, which is the sodium salt or potassium salt.

78. An oligomeric compound according to the following chemical structure:



(SEQ ID NO: 12).

79. An oligomeric compound comprising a modified oligonucleotide according to the following chemical notation: $^{m}C_{es}G_{eo}{}^{m}C_{ko}T_{ds}G_{ds}A_{ds}T_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}G_{ko}G_{ks}G_{e}$ (SEQ ID NO: 12), wherein:

A = an adenine nucleobase,

^{m}C = a 5-methyl cytosine nucleobase,

G = a guanine nucleobase,

T = a thymine nucleobase,

e = a 2'-β-D-MOE sugar moiety,

k = a cEt sugar moiety,

d = a 2'-β-D-deoxyribosyl sugar moiety,

s = a phosphorothioate internucleoside linkage, and

o = a phosphodiester internucleoside linkage.

80. The oligomeric compound of claim 79, comprising the modified oligonucleotide covalently linked to a conjugate group.

81. A population of oligomeric compounds of claim 76, in which all phosphorothioate internucleoside linkages of the oligomeric compound are stereorandom.

82. A pharmaceutical composition comprising the oligomeric compound of claim 76 and a pharmaceutically acceptable carrier or diluent.

83. The pharmaceutical composition of claim 82, wherein the pharmaceutically acceptable diluent is water or PBS.

84. A pharmaceutical composition comprising the population of claim 81 and a pharmaceutically acceptable carrier or diluent.

85. A population of oligomeric compounds of claim 77, in which all phosphorothioate internucleoside linkages of the oligomeric compound are stereorandom.

86. A pharmaceutical composition comprising the oligomeric compound of claim 77 and a pharmaceutically acceptable carrier or diluent.

87. The pharmaceutical composition of claim 86, wherein the pharmaceutically acceptable diluent is water or PBS.

88. A pharmaceutical composition comprising the population of claim 85 and a pharmaceutically acceptable carrier or diluent.

89. A population of oligomeric compounds of claim 78, in which all phosphorothioate internucleoside linkages of the oligomeric compound are stereorandom.

90. A pharmaceutical composition comprising the oligomeric compound of claim 78 and a pharmaceutically acceptable carrier or diluent.

91. The pharmaceutical composition of claim 90, wherein the pharmaceutically acceptable diluent is water or PBS.

92. A pharmaceutical composition comprising the population of claim 89 and a pharmaceutically acceptable carrier or diluent.

93. A population of oligomeric compounds of claim 79 in which all phosphorothioate internucleoside linkages of the oligomeric compound are stereorandom.

94. A pharmaceutical composition comprising the oligomeric compound of claim 79 and a pharmaceutically acceptable carrier or diluent.

95. The pharmaceutical composition of claim 94, wherein the pharmaceutically acceptable diluent is water or PBS.

96. A pharmaceutical composition comprising the population of claim 93 and a pharmaceutically acceptable carrier or diluent.

97. A population of oligomeric compounds of claim 80, in which all phosphorothioate internucleoside linkages of the oligomeric compound are stereorandom.

98. A pharmaceutical composition comprising the oligomeric compound of claim 80 and a pharmaceutically acceptable carrier or diluent.

99. The pharmaceutical composition of claim 98, wherein the pharmaceutically acceptable diluent is water or PBS.

100. A pharmaceutical composition comprising the population of claim 97 and a pharmaceutically acceptable carrier or diluent.