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(54) **Title**: ANTISENSE MODULATION OF GCCR EXPRESSION

(57) **Abstract**: Provided herein are methods, compounds, and compositions for reducing expression of GCCR mRNA and protein in an animal. Such methods, compounds, and compositions are useful to treat, prevent, delay, or ameliorate metabolic disease, for example, diabetes, or a symptom thereof.

ANTISENSE MODULATION OF GCCR 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 BIOL0159WOSEQ.txt created September 19, 2012, which is 393 Kb in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

FIELD

Provided herein are methods, compounds, and compositions for reducing expression of GCCR mRNA and protein in an animal. Such methods, compounds, and compositions are useful, for example, to treat, prevent, delay or ameliorate diseases associated with metabolic disorders, particularly disorders associated with diabetes.

BACKGROUND

Diabetes is a chronic metabolic disorder characterized by impaired insulin secretion and/or action. In type 2 diabetes (T2DM), insulin resistance leads to an inability of insulin to control the activity of gluconeogenic enzymes, and many subjects also exhibit inappropriate levels of circulating glucagon (GC) in the fasting and postprandial state. Glucagon is secreted from the α-cells of the pancreatic islets and regulates glucose homeostasis through modulation of hepatic glucose production (Quesada et al., J. Endocrinol. 2008. 199: 5-19). .

Glucagon exerts its action on target tissues via the activation of glucocorticoid receptor (GCCR). The glucocorticoid receptor is a 62 kDa protein that is a member of the class B G-protein coupled family of receptors (Brubaker et al., Recept. Channels. 2002. 8: 179-88). GCCR activation leads to signal transduction by G proteins ($G_s\alpha$ and G_q), whereby $G_s\alpha$ activates adenylate cyclase, which causes cAMP production, resulting in an increase in levels of protein kinase A. GCCR signaling in the liver results in increased hepatic glucose production by induction of glycogenolysis and gluconeogenesis along with inhibition of glycogenesis (Jiang and Zhang. Am. J. Physiol. Endocrinol. Metab. 2003. 284: E671-E678). GCCR is also expressed in extrahepatic tissues, which includes heart, intestinal smooth muscle, kidney, brain, and adipose tissue (Hansen et al., Peptides. 1995. 16: 1163-1166).

Development of GCCR inhibitors have been hampered by the unfavorable side effects associated with systemic GCCR inhibition, including activation of the hypothalamic-pituitary adrenal (HPA) axis.

Inhibition of GCCR activity in the brain can lead to an increase in circulating adrenocorticotropic hormone due to feedback regulation and a consequent increase in secretion of adrenal steroids (Philibert et al., Front. Horm. Res. 1991. 19: 1-17). This, in turn, can produce a myriad of negative chronic steroid-related side-effects. Other studies have demonstrated that specific inactivation of GCCR resulted in hypoglycemia upon prolonged fasting (Opherk et al., Mol. Endocronol. 2004. 18: 1346-1353).

It has previously been demonstrated in pre-clinical models that administration of GCCR antisense oligonucleotides results in tissue-specific accumulation and reduced GCCR expression in liver and adipose tissue (PCT Pub. No. WO2005/071080; PCT Pub. No. WO2007/035759) without affecting GCCR mRNA levels in the CNS or adrenal glands. Thus, antisense inhibition of GCCR mRNA expression has be shown to improve hyperglycemia and hyperlipidemia without activating the HPA axis. The present invention provides compositions and methods for modulating GCCR expression. Antisense compounds for modulating expression of GCCR are disclosed in the aforementioned published patent applications. However, there remains a need for additional improved compounds. The compounds and treatment methods described herein provide significant advantages over the treatments options currently available for GCCR related disorders.

All documents, or portions of documents, cited in this application, including, but not limited to, patents, patent applications, articles, books, and treatises, are hereby expressly incorporated-by-reference for the portions of the document discussed herein, as well as in their entirety.

SUMMARY

Provided herein are methods, compounds, and compositions for modulating expression of GCCR and treating, preventing, delaying or ameliorating diseases associated with metabolic disorders, particularly disorders associated with diabetes and/or a symptom thereof.

DETAILED DESCRIPTION

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive described herein, as claimed. Herein, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the use of "or" means "and/or" unless stated otherwise. 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, and treatises, are hereby expressly incorporated-by-reference for the portions of the document discussed herein, as well as in their entirety.

Definitions

Unless specific definitions are provided, the nomenclature utilized in connection with, and the procedures and techniques of analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques can be used for chemical synthesis, and chemical analysis. Where permitted, all documents, or portions of documents, cited in this application, including, but not limited to, all patents, applications, published applications and other journal publications, GENBANK Accession Numbers and associated sequence information obtainable through databases such as National Center for Biotechnology Information (NCBI) and other data referred to throughout in the disclosure herein are incorporated by reference for the portions of the document discussed herein, as well as in their entirety.

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

"2'-O-methoxyethyl" (also 2'-MOE and 2'-$O(CH_2)_2$-OCH_3) refers to an O-methoxy-ethyl modification of the 2' position of a furosyl ring. A 2'-O-methoxyethyl modified sugar is a modified sugar.

"2'-O-methoxyethyl nucleotide" means a nucleotide comprising a 2'-O-methoxyethyl modified sugar moiety.

"3' target site" refers to the nucleotide of a target nucleic acid which is complementary to the 3'-most nucleotide of a particular antisense compound.

"5' target site" refers to the nucleotide of a target nucleic acid which is complementary to the 5'-most nucleotide of a particular antisense compound.

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

"About" means within ±10% of a value. For example, if it is stated, "a marker may be increased by about 50%", it is implied that the marker may be increased between 45%-55%.

"Active pharmaceutical agent" means the substance or substances in a pharmaceutical composition that provide a therapeutic benefit when administered to an individual. For example, in certain embodiments an antisense oligonucleotide targeted to GCCR is an active pharmaceutical agent.

"Active target region" or "target region" means a region to which one or more active antisense compounds is targeted. "Active antisense compounds" means antisense compounds that reduce target nucleic acid levels or protein levels.

"Adiposity" or "Obesity" refers to the state of being obese or an excessively high amount of body fat or adipose tissue in relation to lean body mass. The amount of body fat includes concern for both the distribution of fat throughout the body and the size and mass of the adipose tissue deposits. Body fat distribution can be estimated by skin-fold measures, waist-to-hip circumference ratios, or techniques such as ultrasound, computed tomography, or magnetic resonance imaging. According to the Center for Disease

Control and Prevention, individuals with a body mass index (BMI) of 30 or more are considered obese. The term "Obesity" as used herein includes conditions where there is an increase in body fat beyond the physical requirement as a result of excess accumulation of adipose tissue in the body. The term "obesity" includes, but is not limited to, the following conditions: adult-onset obesity; alimentary obesity; endogenous or inflammatory obesity; endocrine obesity; familial obesity; hyperinsulinar obesity; hyperplastic-hypertrophic obesity; hypogonadal obesity; hypothyroid obesity; lifelong obesity; morbid obesity and exogenous obesity.

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

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

"Agent" means an active substance that can provide a therapeutic benefit when administered to an animal. "First Agent" means a therapeutic compound provided herein. For example, a first agent can be an antisense oligonucleotide targeting GCCR. "Second agent" means a second therapeutic compound described herein (e.g. a second antisense oligonucleotide targeting GCCR) and/or a non- GCCR therapeutic compound.

"Amelioration" refers to a lessening of at least one indicator, sign, or symptom of an associated disease, disorder, or condition. The severity of indicators can be determined by subjective or objective measures, which are known to those skilled in the art.

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

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

"Antisense compound" means an oligomeric compound that is capable of undergoing hybridization to a target nucleic acid through hydrogen bonding.

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

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

"Bicyclic sugar" means a furosyl ring modified by the bridging of two non-geminal ring atoms. A bicyclic sugar is a modified sugar.

"Bicyclic nucleic acid" or "BNA" refers to a nucleoside or nucleotide wherein the furanose portion of the nucleoside or nucleotide includes a bridge connecting two carbon atoms on the furanose ring, thereby forming a bicyclic ring system.

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

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

"Chimeric antisense compound" means an antisense compound that has at least two chemically distinct regions.

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

"Cholesterol" is a sterol molecule found in the cell membranes of all animal tissues. Cholesterol must be transported in an animal's blood plasma by lipoproteins including very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), and high density lipoprotein (HDL). "Plasma cholesterol" refers to the sum of all lipoproteins (VDL, IDL, LDL, HDL) esterified and/or non-esterified cholesterol present in the plasma or serum.

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

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

"Deoxyribonucleotide" means a nucleotide having a hydrogen at the 2' position of the sugar portion of the nucleotide. Deoxyribonucleotides may be modified with any of a variety of substituents.

"Diabetes mellitus" or "diabetes" is a syndrome characterized by disordered metabolism and abnormally high blood sugar (hyperglycemia) resulting from insufficient levels of insulin or reduced insulin sensitivity. The characteristic symptoms are excessive urine production (polyuria) due to high blood glucose levels, excessive thirst and increased fluid intake (polydipsia) attempting to compensate for increased urination, blurred vision due to high blood glucose effects on the eye's optics, unexplained weight loss, and lethargy.

"Diabetic dyslipidemia" or "type 2 diabetes with dyslipidemia" means a condition characterized by Type 2 diabetes and, typically, elevated lipids such as cholesterol and triglycerides as well as lipoproteins such as low-density lipoprotein (LDL) and elevated small, dense LDL particles. Such condition may also be characterized by reduced HDL-C,.

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

"Dyslipidemia" refers to a disorder of lipid and/or lipoprotein metabolism, including lipid and/or lipoprotein overproduction or deficiency. Dyslipidemias may be manifested by elevation of lipids such as cholesterol and triglycerides as well as lipoproteins such as low-density lipoprotein (LDL) cholesterol.

"Dosage unit" means a form in which a pharmaceutical agent is provided, e.g. pill, tablet, or other dosage unit known in the art. In certain embodiments, a dosage unit is a vial containing lyophilized antisense oligonucleotide. In certain embodiments, a dosage unit is a vial containing reconstituted antisense oligonucleotide.

"Dose" means a specified quantity of a pharmaceutical agent provided in a single administration, or in a specified time period. In certain embodiments, a dose can be administered in one, two, or more boluses, tablets, or injections. For example, in certain embodiments where subcutaneous administration is desired, the desired dose requires a volume not easily accommodated by a single injection, therefore, two or more injections can be used to achieve the desired dose. In certain embodiments, the pharmaceutical agent is administered by infusion over an extended period of time or continuously. Doses can be stated as the amount of pharmaceutical agent per hour, day, week, or month.

"Effective amount" or "therapeutically effective amount" means the amount of active pharmaceutical agent sufficient to effectuate a desired physiological outcome in an individual in need of the agent. The effective amount can vary among individuals depending on the health and physical condition of the individual to be treated, the taxonomic group of the individuals to be treated, the formulation of the composition, assessment of the individual's medical condition, and other relevant factors.

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

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

"Gap-widened" means a chimeric antisense compound having a gap segment of 12 or more contiguous 2'-deoxyribonucleosides positioned between and immediately adjacent to 5' and 3' wing segments having from one to six nucleosides.

"Glucocorticoid receptor" or "GCCR" means any nucleic acid or protein of GCCR.

"GCCR expression" means the level of mRNA transcribed from the gene encoding GCCR or the level of protein translated from the mRNA. GCCR expression can be determined by art known methods such as a Northern or Western blot.

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

"Glucose" is a monosaccharide used by cells as a source of energy and inflammatory intermediate. "Plasma glucose" refers to glucose present in the plasma.

"Hybridization" means the annealing of complementary nucleic acid molecules. In certain embodiments, complementary nucleic acid molecules include an antisense compound and a target nucleic acid.

"Hyperlipidemia" or "hyperlipemia" is a condition characterized by elevated serum lipids or circulating (plasma) lipids. This condition manifests an abnormally high concentration of fats. The lipid fractions in the circulating blood are cholesterol, low density lipoproteins, very low density lipoproteins and triglycerides.

"Hypertriglyceridemia" means a condition characterized by elevated triglyceride levels.

"Identifying" or "selecting an animal with metabolic" means identifying or selecting a subject having been diagnosed with a metabolic disease, or a metabolic disorder; or, identifying or selecting a subject having any symptom of a metabolic disease, including, but not limited to, metabolic syndrome, hyperglycemia, hypertriglyceridemia, hypertension increased insulin resistance, decreased insulin sensitivity, above normal body weight, and/or above normal body fat or any combination thereof. Such identification may be accomplished by any method, including but not limited to, standard clinical tests or assessments, such as measuring serum or circulating (plasma) blood-glucose, measuring serum or circulating (plasma) triglycerides, measuring blood-pressure, measuring body fat, measuring body weight, and the like.

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

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

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

"Insulin resistance" is defined as the condition in which normal amounts of insulin are inadequate to produce a normal insulin response from fat, muscle and liver cells. Insulin resistance in fat cells results in hydrolysis of stored triglycerides, which elevates free fatty acids in the blood plasma. Insulin resistance in muscle reduces glucose uptake whereas insulin resistance in liver reduces glucose storage, with both effects

serving to elevate blood glucose. High plasma levels of insulin and glucose due to insulin resistance often leads to metabolic syndrome and type 2 diabetes.

"Insulin sensitivity" is a measure of how effectively an individual processes glucose. An individual having high insulin sensitivity effectively processes glucose whereas an individual with low insulin sensitivity does not effectively process glucose.

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

"Intravenous administration" means administration into a vein.

"Linked nucleosides" means adjacent nucleosides which are bonded together.

"Lipid-lowering therapy" or "lipid lowering agent" means a therapeutic regimen provided to a subject to reduce one or more lipids in a subject. In certain embodiments, a lipid-lowering therapy is provided to reduce one or more of ApoB, total cholesterol, LDL-C, VLDL-C, IDL-C, non-HDL-C, triglycerides, small dense LDL particles, and Lp(a) in a subject. Examples of lipid-lowering therapy include statins, fibrates, and MTP inhibitors.

"Major risk factors" refers to factors that contribute to a high risk for a particular disease or condition. In certain embodiments, major risk factors for coronary heart disease include, without limitation, cigarette smoking, hypertension, low HDL-C, family history of coronary heart disease, age, and other factors disclosed herein.

"Metabolic disease" or "metabolic disorder" refers to a condition characterized by an alteration or disturbance in metabolic function. "Metabolic" and "metabolism" are terms well known in the art and generally include the whole range of biochemical processes that occur within a living organism. Metabolic diseases or disorders include, but are not limited to, obesity, diabetes, hyperglycemia, prediabetes, non-alcoholic fatty liver disease (NAFLD), metabolic syndrome, insulin resistance, diabetic dyslipidemia, or hypertriglyceridemia or a combination thereof.

"Metabolic syndrome" means a condition characterized by a clustering of lipid and non-lipid cardiovascular risk factors of metabolic origin. In certain embodiments, metabolic syndrome is identified by the presence of any 3 of the following factors: waist circumference of greater than 102 cm in men or greater than 88 cm in women; serum triglyceride of at least 150 mg/dL; HDL-C less than 40 mg/dL in men or less than 50 mg/dL in women; blood pressure of at least 130/85 mmHg; and fasting glucose of at least 110 mg/dL. These determinants can be readily measured in clinical practice (JAMA, 2001, 285: 2486-2497).

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

"Mixed dyslipidemia" means a condition characterized by elevated cholesterol and elevated triglycerides.

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

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

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

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

"Modified oligonucleotide" means an oligonucleotide comprising at least one modified nucleotide.

"Modified sugar" refers to a substitution or change from a natural sugar.

"Motif" means the pattern of chemically distinct regions in an antisense compound.

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

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

"Non-alcoholic fatty liver disease" or "NAFLD" means a condition characterized by fatty inflammation of the liver that is not due to excessive alcohol use (for example, alcohol consumption of over 20 g/day). In certain embodiments, NAFLD is related to insulin resistance and the metabolic syndrome. NAFLD encompasses a disease spectrum ranging from simple triglyceride accumulation in hepatocytes (hepatic steatosis) to hepatic steatosis with inflammation (steatohepatitis), fibrosis, and cirrhosis.

"Nonalcoholic steatohepatitis" (NASH) occurs from progression of NAFLD beyond deposition of triglycerides. A "second hit" capable of inducing necrosis, inflammation, and fibrosis is required for development of NASH. Candidates for the second-hit can be grouped into broad categories: factors causing an increase in oxidative stress and factors promoting expression of proinflammatory cytokines

"Nucleic acid" refers to molecules composed of monomeric nucleotides. A nucleic acid includes ribonucleic acids (RNA), deoxyribonucleic acids (DNA), single-stranded nucleic acids, double-stranded nucleic acids, small interfering ribonucleic acids (siRNA), and microRNAs (miRNA). A nucleic acid can also comprise a combination of these elements in a single molecule.

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

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

"Nucleoside" means a nucleobase linked to a sugar.

"Nucleoside mimetic" includes those structures used to replace the sugar or the sugar and the base and not necessarily the linkage at one or more positions of an oligomeric compound such as for example nucleoside mimetics having morpholino, cyclohexenyl, cyclohexyl, tetrahydropyranyl, bicyclo or tricyclo sugar mimetics e.g. non furanose sugar units.

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

"Nucleotide mimetic" includes those structures used to replace the nucleoside and the linkage at one or more positions of an oligomeric compound such as for example peptide nucleic acids or morpholinos (morpholinos linked by -N(H)-C(=O)-O- or other non-phosphodiester linkage).

"Oligomeric compound" or "oligomer" refers to a polymeric structure comprising two or more sub-structures and capable of hybridizing to a region of a nucleic acid molecule. In certain embodiments, oligomeric compounds are oligonucleosides. In certain embodiments, oligomeric compounds are oligonucleotides. In certain embodiments, oligomeric compounds are antisense compounds. In certain embodiments, oligomeric compounds are antisense oligonucleotides. In certain embodiments, oligomeric compounds are chimeric oligonucleotides.

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

"Parenteral administration" means administration through injection or infusion. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration, e.g. intrathecal or intracerebroventricular administration. Administration can be continuous, or chronic, or short or intermittent.

"Peptide" means a molecule formed by linking at least two amino acids by amide bonds. Peptide refers to polypeptides and proteins.

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

"Pharmaceutical composition" means a mixture of substances suitable for administering to an individual. For example, a pharmaceutical composition can comprise one or more active agents and a sterile aqueous solution.

"Pharmaceutically acceptable carrier" means a medium or diluent that does not interfere with the structure of the oligonucleotide. Certain, of such carries 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. For example, a pharmaceutically acceptable carrier can be a sterile aqueous solution.

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

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

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

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

"Prevent" refers to delaying or forestalling the onset or development of a disease, disorder, or condition for a period of time from minutes to indefinitely. Prevent also means reducing risk of developing a disease, disorder, or condition.

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

"Side effects" means physiological responses attributable to a treatment other than the desired effects. In certain embodiments, side effects include injection site reactions, liver function test abnormalities, renal function abnormalities, liver toxicity, renal toxicity, central nervous system abnormalities, myopathies, and malaise. For example, increased aminotransferase levels in serum can indicate liver toxicity or liver function abnormality. For example, increased bilirubin can indicate liver toxicity or liver function abnormality.

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

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

"Statin" means an agent that inhibits the activity of HMG-CoA reductase.

"Subcutaneous administration" means administration just below the skin.

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

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

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

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

"Therapeutic lifestyle change" means dietary and lifestyle changes intended to lower fat /adipose tissue mass and/or cholesterol. Such change can reduce the risk of developing heart disease, and may includes recommendations for dietary intake of total daily calories, total fat, saturated fat, polyunsaturated fat, monounsaturated fat, carbohydrate, protein, cholesterol, insoluble fiber, as well as recommendations for physical activity.

"Triglyceride" or "TG" means a lipid or neutral fat consisting of glycerol combined with three fatty acid molecules.

"Type 2 diabetes," (also known as "type 2 diabetes mellitus" or "diabetes mellitus, type 2", and formerly called "diabetes mellitus type 2" , "non-insulin-dependent diabetes (NIDDM)", "obesity related diabetes", or "adult-onset diabetes") is a metabolic disorder that is primarily characterized by insulin resistance, relative insulin deficiency, and hyperglycemia.

"Treat" refers to administering a pharmaceutical composition to an animal to effect an alteration or improvement of a disease, disorder, or condition.

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

Certain embodiments

Certain embodiments provide methods, compounds, and compositions for inhibiting GCCR expression.

Certain embodiments provide antisense compounds targeted to a GCCR nucleic acid. In certain embodiments, the GCCR nucleic acid sequence is a human sequence. In certain embodiments, the GCCR nucleic acid is the complement of GENBANK Accession No. NT_029289.10 truncated from nucleotides 3818000 to 3980000 (incorporated herein as SEQ ID NO: 1). In certain embodiments, the GCCR nucleic acid is a rhesus monkey sequence. In certain embodiment, the GCCR nucleic acid sequence is the complement of GENBANK Accession No. NW_001120987.1 truncated from nucleotides 1334000 to 1491000 (incorporated herein as SEQ ID NO: 2).

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 12 to 30 nucleosides having a nucleobase sequence complementary to an equal length portion of SEQ ID NO: 1 or 2 or both.

In certain embodiments, the compounds or compositions provided herein consist of 12 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 4-56.

In certain embodiments, the compounds or compositions provided herein consist of 12 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 6, 7, 10, 11, 33, 35, 36, 39, 42, and 43.

In certain embodiments, the compound or composition provided herein is or comprises ISIS NOs: 420470, 420476, 426130, 426183, 426261, 426262, 426115, 426168, 426246, 426172, 426325, and 426267.

In certain embodiments, the compounds or compositions provided herein consist of 12 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NO: 36.

In certain embodiments, the compound or composition is or comprises ISIS NO: 426115.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 15 to 30 nucleosides having a nucleobase sequence complementary to an equal length portion of SEQ ID NO: 1 or 2 or both.

In certain embodiments, the compounds or compositions provided herein consist of 15 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 4-56.

In certain embodiments, the compounds or compositions provided herein consist of 15 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 6, 7, 10, 11, 33, 35, 36, 39, 42, and 43.

In certain embodiments, the compound or composition provided herein is or comprises ISIS NOs: 420470, 420476, 426130, 426183, 426261, 426262, 426115, 426168, 426246, 426172, 426325, and 426267.

In certain embodiments, the compounds or compositions provided herein consist of 15 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NO: 36.

In certain embodiments, the compound or composition provided herein is or comprise ISIS NO: 426115.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 16 to 21 nucleosides having a nucleobase sequence complementary to an equal length portion of SEQ ID NO: 1 or 2 or both

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 16 to 21 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 4-56.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 16 to 21 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of any of SEQ ID NOs: 6, 7, 10, 11, 33, 35, 36, 39, 42, and 43.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 16 to 21 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NO: 36.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 35 nucleosides having a nucleobase sequence complementary to an equal length portion of any of SEQ ID NOs: 1 and 2.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 35 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NOs: 4-56.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 35 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NOs: 6, 7, 10, 11, 33, 35, 36, 39, 42, and 43.

In certain embodiments, the compounds or compositions provided herein can consist of 17 to 35 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NO: 36.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 30 nucleosides having a nucleobase sequence complementary to an equal length portion of SEQ ID NO: 1 or 2 or both.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NOs: 4-56.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NOs: 6, 7, 10, 11, 33, 35, 36, 39, 42, and 43.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 30 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NO: 36.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 25 nucleosides having a nucleobase sequence complementary to an equal length portion of SEQ ID NO: 1 or 2 or both.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 25 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NOs: 4-56.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 25 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NOs: 6, 7, 10, 11, 33, 35, 36, 39, 42, and 43.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 25 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NO: 36.

In certain embodiments, the compounds or compositions described herein comprise a modified oligonucleotide consisting of 17 to 24 nucleosides having a nucleobase sequence complementary to an equal length portion of SEQ ID NO: 1 or 2 or both.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 24 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NOs: 4-56.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 24 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NOs: 6, 7, 10, 11, 33, 35, 36, 39, 42, and 43.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 24 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NO: 36.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 23 nucleosides having a nucleobase sequence complementary to an equal length portion of SEQ ID NO: 1 or 2 or both.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 23 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NOs: 4-56.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 23 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NOs: 6, 7, 10, 11, 33, 35, 36, 39, 42, and 43.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 23 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NO: 36.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 22 nucleosides having a nucleobase sequence complementary to an equal length portion of SEQ ID NO: 1 or 2 or both.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 22 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NOs: 4-56.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 22 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NOs: 6, 7, 10, 11, 33, 35, 36, 39, 42, and 43.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 22 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NO: 36.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 21 nucleosides having a nucleobase sequence complementary to an equal length portion of SEQ ID NO: 1 or 2 or both.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 21 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NOs: 4-56.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17 to 21 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NOs: 6, 7, 10, 11, 33, 35, 36, 39, 42, and 43.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 17to 21 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NO: 36.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 nucleosides having a nucleobase sequence complementary to an equal length portion of SEQ ID NO: 1 or 2 or both.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NOs: 4-56.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides and have a nucleobase sequence comprising at least 8, 9,

10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NOs: 6, 7, 10, 11, 33, 35, 36, 39, 42, and 43.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NO: 36.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 nucleosides having a nucleobase sequence complementary to an equal length portion of SEQ ID NO: 1 or 2 or both.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NOs: 4-56.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NOs: 6, 7, 10, 11, 33, 35, 36, 39, 42, and 43.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides and have a nucleobase sequence comprising at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 contiguous nucleobases of SEQ ID NO: 36.

In certain embodiments, the compounds or compositions provided herein comprise a salt of the modified oligonucleotide.

In certain embodiments, the compounds or compositions provided herein further comprise a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% complementary to any one of SEQ ID NOs: 1 and 2 as measured over the entirety of the modified oligonucleotide.

In certain embodiments, the nucleobase sequence of the modified oligonucleotide has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to any one of SEQ ID NOs: 4-56 as measured over the entirety of the modified oligonucleotide.

In certain embodiments, the nucleobase sequence of the modified oligonucleotide has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to any one of SEQ ID NOs: 6, 7, 10, 11, 33, 35, 36, 39, 42, and 43 as measured over the entirety of the modified oligonucleotide.

In certain embodiments, the nucleobase sequence of the modified oligonucleotide has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 36 as measured over the entirety of the modified oligonucleotide.

In certain embodiments, antisense compounds or modified oligonucleotides target a region of a GCCR nucleic acid. In certain embodiments, such compounds or oligonucleotides targeted to a region of a GCCR nucleic acid have a contiguous nucleobase portion that is complementary to an equal length nucleobase portion of the region. For example, the portion can be at least an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 contiguous nucleobases. Such portion is complementary to an equal length portion of a region recited herein. In certain embodiments, such compounds or oligonucleotide target the following nucleotide regions of SEQ ID NO: 1: 33116-33135, 33296-33315, 33673-33692, 33716-33755, 33716-33751, 33716-33735, 33732-33755, 33732-33755, 33736-33755, 37217-37236, 51878-51898, 51878-51897, 51879-51898, 57825-57846, 57825-57844, 57827-57846, 59951-59978, 59951-59975, 59951-59974, 59951-59971, 59951-59970, 59952-59978, 59952-59975, 59952-59974, 59952-59971, 59955-59978, 59955-59975, 59955-59974, 59956-59978, 59956-59975, 59959-59978, 60935-60958, 60935-60956, 60935-60955, 60935-60954, 60936-60958, 60936-60956, 60936-60955, 60937-60958, 60937-60956, 60939-60958, 63677-63698, 63677-63697, 63677-63696, 63678-63698, 63678-63697, 63679-63698, 65938-65961, 65938-65960, 65938-65959, 65938-65958, 65938-65957, 65939-65961, 65939-65960, 65939-65959, 65939-65958, 65940-65961, 65940-65960, 65940-65959, 65941-65961, 65941-65960, 65942-65961, 76224-76248, 76224-76247, 76224-76246, 76224-76244, 76224-76243, 76225-76248, 76225-76247, 76225-76246, 76225-76244, 76227-76248, 76227-76247, 76227-76246, 76228-76248, 76228-76247, 76229-76248, 95513-95538, 95513-95537, 95513-95532, 95518-95538, 95518-95537, 95519-95538, 104247-104266, 109346-109368, 109346-109366, 109346-109365, 109347-109368, 109347-109366, 109349-109368, 109473-109492, 112218-112242, 112218-112241, 112218-122240, 112218-112239, 112218-112238, 112218-112237, 112219-112242, 112219-112241, 112219-112240, 112219-112239, 112219-112238, 112220-112242, 112220-112241, 112220-112240, 112220-112239, 112221-112242, 112221-112241, 112221-112240, 112222-112242, 112222-112241, 112223-112242, 114154-114178, 114154-114177, 114154-114176, 114154-114175, 114154-114174, 114154-114173, 114155-114178, 114155-114177, 114155-114176, 114155-114175, 114155-114174, 114156-114178, 114156-114177, 114156-114176, 114156-114175, 114157-114178, 114157-114177, 114157-114176, 114158-114178, 114158-114177, 114159-114178, 114587-114610, 114587-114609, 114587-114608, 114587-114606, 114589-114610, 114589-114609, 114589-114608, 114590-114610, 114590-114609, 114591-114610, 139287-139306, 143259-143280, 143259-143279, 143259-143278, 143260-143280, 143260-143279, 143261-143280, 143737-143757, 143737-143756, and 143738-143757.

In certain embodiments, antisense compounds or modified oligonucleotides target a region of a GCCR nucleic acid. In certain embodiments, such compounds or oligonucleotides targeted to a region of a GCCR nucleic acid have a contiguous nucleobase portion that is complementary to an equal length nucleobase portion of the region. For example, the portion can be at least an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 contiguous nucleobases portion complementary to an equal length portion of a region recited

herein. In certain embodiments, such compounds or oligonucleotide target the following nucleotide regions of SEQ ID NO: 1: 57825-57844, 59956-59975, 63677-63696, 65938-65959, 65938-65958, 65938-65957, 65939-65959, 65939-65958, 65940-65959, 76224-76243, 76225-76244, 76229-76248, and 95513-95532.

In certain embodiments, antisense compounds or modified oligonucleotides target a region of a GCCR nucleic acid. In certain embodiments, such compounds or oligonucleotides targeted to a region of a GCCR nucleic acid have a contiguous nucleobase portion that is complementary to an equal length nucleobase portion of the region. For example, the portion can be at least an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 contiguous nucleobases portion complementary to an equal length portion of a region recited herein. In certain embodiments, such compounds or oligonucleotide target the following nucleotide region of SEQ ID NO: 1: 65940-65959.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate at least 55% inhibition of GCCR expression: ISIS NOs: 361137, 361141, 361151, 361155, 361156, 377131, 414641, 414648, 414681, 420450, 420470, 420476, 420479, 420488, 420493, 420522, 420599, 420634, 420644, 420764, 426110, 426115, 426116, 426117, 426128, 426136, 426142, 426143, 426161, 426172, 426177, 426183, 426187, 426189, 426246, 426255, 426261, 426262, 426263, 426264, 426325, and 426345.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate at least 60% inhibition of GCCR expression: ISIS NOs: 361137, 361141, 361151, 361155, 361156, 377131, 414641, 414648, 414681, 420450, 420470, 420476, 420479, 420488, 420493, 420522, 420599, 420634, 420644, 420764, 426110, 426115, 426116, 426117, 426128, 426143, 426177, 426183, 426187, 426246, 426255, 426261, and 426262.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate at least 65% inhibition of GCCR expression: ISIS NOs: 361137, 361141, 361151, 361155, 361156, 377131, 414641, 414648, 414681, 420450, 420470, 420476, 420479, 420488, 420493, 420522, 420599, 420634, 420644, 420764, 426110, 426115, 426117, 426128, 426143, 426177, 426183, 426187, 426246, 426255, and 426261.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate at least 70% inhibition of GCCR expression: ISIS NOs: 361137, 361155, 361156, 377131, 414641, 414648, 414681, 420450, 420470, 420476, 420479, 420488, 420493, 420522, 420599, 420634, 420644, 420764, 426115, 426117, 426128, 426183, and 426261.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate at least 75% inhibition of GCCR expression: ISIS NOs:

361137, 361155, 377131, 414641, 414681, 420450, 420470, 420476, 420488, 420493, 420522, 420599, 420634, 420644, and 420764.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate at least 80% inhibition of GCCR expression: ISIS NOs: 377131, 414641, 414681, 420450, 420476, and 420634.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate at least 85% inhibition of GCCR expression: ISIS NOs: 414681, 420450, 420476, and 420634.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate an IC_{50} value of less than 3 μM using electroporation for transfection: ISIS NOs: 377131, 414641, 414681, 420450, 420470, 420476, 420493, 420522, 420599, 420644, 426110, 426115, 426116, 426117, 426119, 426124, 426128, 426130, 426131, 426136, 426137, 426142, 426143, 426144, 426150, 426157, 426161, 426168, 426171, 426172, 426177, 426183, 426185, 426187, 426189, 426199, 426203, 426229, 426246, 426255, 426261, 426262, 426263, 426264, 426267, 426281, 426301, 426302, 426306, 426323, 426324, 426325, 426343, 426345, 426346, 426347, 426401, 426403, 426404, and 426405.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate an IC_{50} value of less than 2 μM using electroporation for transfection: ISIS NOs: 377131, 414641, 414681, 420450, 420470, 420476, 420493, 420522, 420599, 420644, 426110, 426115, 426116, 426117, 426119, 426128, 426130, 426136, 426137, 426142, 426143, 426144, 426150, 426157, 426168, 426171, 426172, 426183, 426185, 426189, 426203, 426246, 426261, 426262, 426263, 426264, 426267, 426281, 426301, 426324, 426325, 426345, and 426347.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate an IC_{50} value of less than 1 μM using electroporation for transfection: ISIS NOs: 426115, 426128, 426172, 426261, and 426325.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate an IC_{50} value of less than 50 nM using lipofectin as a transfection agent: ISIS NOs: 377131, 414641, 414648, 414681, 420450, 420470, 420488, 420493, 420522, 420599, 420644, 426110, 426115, 426116, 426117, 426119, 426124, 426128, 426130, 426131, 426136, 426137, 426142, 426143, 426144, 426150, 426157, 426161, 426168, 426171, 426172, 426177, 426183, 426185, 426187, 426189, 426199, 426203, 426216, 426229, 426246, 426255, 426261, 426262, 426263, 426264, 426267, 426276, 426281, 426293, 426301, 426302, 426306, 426323, 426324, 426325, 426331, 426334, 426336, 426337, 426343, 426344, 426345, 426347, 426390, 426401, 426402, 426403, 426404, and 426405.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate an IC_{50} value of less than 40 nM using lipofectin as a transfection agent: ISIS NOs: 377131, 414641, 414681, 420450, 420493, 420522, 420599, 420644, 426110, 426115, 426116, 426117, 426119, 426124, 426128, 426130, 426131, 426142, 426143, 426157, 426168, 426171, 426172, 426177, 426183, 426185, 426187, 426189, 426199, 426203, 426216, 426246, 426255, 426261, 426262, 426263, 426264, 426267, 426276, 426281, 426293, 426301, 426302, 426306, 426324, 426331, 426336, 426337, 426343, 426344, 426345, 426347, 426401, 426402, 426403, 426404, and 426405.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate an IC_{50} value of less than 30 nM using lipofectin as a transfection agent: ISIS NOs: 414641, 420493, 420599, 426110, 426115, 426116, 426117, 426130, 426131, 426168, 426171, 426172, 426177, 426183, 426185, 426187, 426189, 426246, 426255, 426261, 426262, 426263, 426264, 426324, 426344, 426345, and 426402.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate an IC_{50} value of less than 20 nM using lipofectin as a transfection agent: ISIS NOs: 414641, 426110, 426115, 426116, 426117, 426172, 426177, 426183, 426187, 426255, 426262, and 426263.

Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 20 linked nucleosides wherein the linked nucleosides comprise at least an 8 contiguous nucleobase portion that is complementary to an equal length nucleobase portion within the region selected from nucleotides 57825-57844, 59956-59975, 63677-63696, 65938-65959, 65938-65958, 65938-65957, 65939-65959, 65939-65958, 65940-65959, 76224-76243, 76225-76244, 76229-76248, and 95513-95532 of SEQ ID NO: 1. In certain embodiments, the modified oligonucleotide has at least a 9, at least a 10, at least an 11, at least a 12, at least a 13, at least a 14, at least a 15, at least a 16, at least a 17, at an 18, or at least a 19 contiguous nucleobase portion of which is complementary to an equal length portion within the region selected from nucleotides 57825-57844, 59956-59975, 63677-63696, 65938-65959, 65938-65958, 65938-65957, 65939-65959, 65939-65958, 65940-65959, 76224-76243, 76225-76244, 76229-76248, and 95513-95532 of SEQ ID NO: 1. In certain embodiments, the modified oligonucleotide is 90%, 95%, 99%, or 100% complementary to a nucleic acid encoding human GCCR e.g. SEQ ID NO: 1

Certain embodiments provide compounds comprising a modified oligonucleotide consisting of at least 20 linked nucleosides 60% complementary within the region selected from nucleotides 57825-57844, 59956-59975, 63677-63696, 65938-65959, 65938-65958, 65938-65957, 65939-65959, 65939-65958, 65940-65959, 76224-76243, 76225-76244, 76229-76248, and 95513-95532 of SEQ ID NO: 1.

Certain embodiments provide compounds comprising a modified oligonucleotide consisting of at least 20 linked nucleosides 70% complementary within the region selected from nucleotides 57825-57844,

59956-59975, 63677-63696, 65938-65959, 65938-65958, 65938-65957, 65939-65959, 65939-65958, 65940-65959, 76224-76243, 76225-76244, 76229-76248, and 95513-95532 of SEQ ID NO: 1.

Certain embodiments provide compounds comprising a modified oligonucleotide consisting of at least 20 linked nucleosides 80% complementary within the region selected from 57825-57844, 59956-59975, 63677-63696, 65938-65959, 65938-65958, 65938-65957, 65939-65959, 65939-65958, 65940-65959, 76224-76243, 76225-76244, 76229-76248, and 95513-95532 of SEQ ID NO: 1.

Certain embodiments provide compounds comprising a modified oligonucleotide consisting of at least 20 linked nucleosides 90% complementary within the region selected from nucleotides 57825-57844, 59956-59975, 63677-63696, 65938-65959, 65938-65958, 65938-65957, 65939-65959, 65939-65958, 65940-65959, 76224-76243, 76225-76244, 76229-76248, and 95513-95532 of SEQ ID NO: 1.

Certain embodiments provide compounds comprising a modified oligonucleotide consisting of at least 20 linked nucleosides 95% complementary within the region selected from nucleotides 57825-57844, 59956-59975, 63677-63696, 65938-65959, 65938-65958, 65938-65957, 65939-65959, 65939-65958, 65940-65959, 76224-76243, 76225-76244, 76229-76248, and 95513-95532 of SEQ ID NO: 1.

Certain embodiments provide compounds comprising a modified oligonucleotide consisting of at least 20 linked nucleosides 99% complementary within the region selected from nucleotides 57825-57844, 59956-59975, 63677-63696, 65938-65959, 65938-65958, 65938-65957, 65939-65959, 65939-65958, 65940-65959, 76224-76243, 76225-76244, 76229-76248, and 95513-95532 of SEQ ID NO: 1.

Certain embodiments provide compounds comprising a modified oligonucleotide consisting of at least 20 linked nucleosides 100% complementary within the region selected from nucleotides 57825-57844, 59956-59975, 63677-63696, 65938-65959, 65938-65958, 65938-65957, 65939-65959, 65939-65958, 65940-65959, 76224-76243, 76225-76244, 76229-76248, and 95513-95532 of SEQ ID NO: 1.

Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 20 linked nucleosides 60% complementary within nucleotides 65940-65959 of SEQ ID NO: 1.

Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 20 linked nucleosides 70% complementary within nucleotides 65940-65959 of SEQ ID NO: 1.

Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 20 linked nucleosides 80% complementary within nucleotides 65940-65959 of SEQ ID NO: 1.

Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 20 linked nucleosides 90% complementary within nucleotides 65940-65959 of SEQ ID NO: 1.

Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 20 linked nucleosides 95% complementary within nucleotides 65940-65959 of SEQ ID NO: 1

Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 20 linked nucleosides 99% complementary within nucleotides 65940-65959 of SEQ ID NO: 1.

Certain embodiments provide compounds comprising a modified oligonucleotide consisting of 20 linked nucleosides 100% complementary within nucleotides 65940-65959 of SEQ ID NO: 1.

In certain embodiments, such compounds or oligonucleotides targeted to a region of a GCCR nucleic acid have a contiguous nucleobase portion that is complementary to an equal length nucleobase portion of the region 65940-65959 of SEQ ID NO: 1

In certain embodiments, the following nucleotide regions of SEQ ID NO: 1, when targeted by antisense compounds or oligonucleotides, displays at least 65% inhibition: 57825-57844, 59956-59975, 63677-63696, 65938-65959, 65938-65958, 65938-65957, 65939-65959, 65939-65958, 65940-65959, 76224-76243, 76225-76244, 76229-76248, and 95513-95532.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate at least 55% inhibition of GCCR expression: ISIS NOs: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 30, 31, 35, 36, 37, 38, 42, 43, 45, 48, 54, and 56.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate at least 60% inhibition of GCCR expression: ISIS NOs: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 31, 35, 36, 37, 38, and 45.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate at least 65% inhibition of GCCR expression: ISIS NOs: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 31, 36, 38, and 45.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate at least 70% inhibition of GCCR expression: ISIS NOs: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 20, 21, 22, 23, 24, 36, and 38.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate at least 75% inhibition of GCCR expression: ISIS NOs: 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17, 20, and 22.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate at least 80 % inhibition of GCCR expression: ISIS NOs: 4, 5, 7, 13, 16, and 22.

In certain embodiments, the following antisense compounds target a region of SEQ ID NO: 1, a nucleic acid encoding human GCCR and demonstrate at least 85 % inhibition of a GCCR expression: ISIS NOs: 5, 7, 13, and 16.

In certain embodiments, the compounds provided herein have a greater therapeutic potential than ISIS NOs: 361137, 361141, 361151, 361156, 377131, 361143, and 361155 (Disclosed in PCT Pub No. WO 2007/035759 incorporated herein by reference). In certain embodiments, the compounds provided herein have better *in vitro* inhibition over ISIS NOs: 361137, 361141, 361151, 361156, 377131, 361143, and 361155. In certain embodiments, the compounds provided herein have better *in vivo* inhibition over ISIS NOs: 361137, 361141, 361151, 361156, 377131, 361143, and 361155. In certain embodiments, the compounds provided herein have a better tolerability profile than ISIS NOs: 361137, 361141, 361151, 361156, 377131, 361143, and 361155.

In certain embodiments, the compound provided herein consists of a single-stranded modified oligonucleotide.

In certain embodiments, the modified oligonucleotide consists of 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 or 35 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 21 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 19 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 18 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides. In certain embodiments, the modified oligonucleotide consists of 16 linked nucleosides.

In certain embodiments, at least one internucleoside linkage of the modified oligonucleotide is a modified internucleoside linkage. In certain embodiments, each internucleoside linkage is a phosphorothioate internucleoside linkage.

In certain embodiments, at least one nucleoside of said modified oligonucleotide comprises a modified nucleobase. In certain embodiments, the modified nucleobase is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide comprises: a) a gap segment consisting of linked deoxynucleosides; b) a 5' wing segment consisting of linked nucleosides; and c) a 3' wing segment consisting of linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment and each nucleoside of each wing segment comprises a modified sugar.

In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides, the gap segment consisting of ten linked deoxynucleosides, the 5' wing segment consisting of five linked nucleosides, the 3' wing segment consisting of five linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides, the gap segment consisting of fourteen linked deoxynucleosides, the 5' wing segment consisting of three linked nucleosides, the 3' wing segment consisting of three linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide consists of 20 linked nucleosides, the gap segment consisting of thirteen linked deoxynucleosides, the 5' wing segment consisting of two linked nucleosides, the 3' wing segment consisting of five linked nucleosides, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine is a 5-methylcytosine.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1 and 2, wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NO: 1, wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising at least 19 contiguous nucleobases of SEQ ID NOs: 6, 7, 10, 11, 33, 35, 36, 39, 42, and 43,wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising at least 19contiguous nucleobases of SEQ ID NO: 36 wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising at least 19 contiguous nucleobases of SEQ ID NO: 36, wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of four linked nucleosides; and c) a 3' wing segment consisting of four linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NOs: 1 and 2, wherein the modified oligonucleotide comprises: a) a gap segment consisting of fourteen linked deoxynucleosides; b) a 5' wing segment consisting of three linked nucleosides; and c) a 3' wing segment consisting of three linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to an equal length portion of any of SEQ ID NO: 1, wherein the modified oligonucleotide comprises: a) a gap segment consisting of thirteen linked deoxynucleosides; b) a 5' wing segment consisting of two linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising at least 19 contiguous nucleobases of SEQ ID NOs: 6, 7, 10, 11, 33, 35, 36, 39, 42, and 43, wherein the modified

oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence comprising at least 19 contiguous nucleobases of SEQ ID NO: 36, wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of five linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

In certain embodiments, the compounds or compositions provided herein comprise a modified oligonucleotide consisting of 20 linked nucleosides having a nucleobase sequence of SEQ ID NO: 36 wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten linked deoxynucleosides; b) a 5' wing segment consisting of five linked nucleosides; and c) a 3' wing segment consisting of six linked nucleosides. The gap segment is positioned between the 5' wing segment and the 3' wing segment, each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, each internucleoside linkage is a phosphorothioate linkage and each cytosine residue is a 5-methylcytosine.

Certain embodiments provide methods, compounds, and compositions for inhibiting GCCR expression.

Certain embodiments provide a method of reducing GCCR expression in an animal comprising administering to the animal a compound as described herein. In certain embodiments, the compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length targeted to GCCR. In certain embodiments, the compound comprises a modified oligonucleotide 15 to 30 linked nucleosides in length targeted to GCCR. In certain embodiments, the compound comprises a modified oligonucleotide 18 to 21 linked nucleosides in length targeted to GCCR. In certain embodiments, the compound comprises a modified oligonucleotide 17 to 35 linked nucleosides in length targeted to GCCR. In certain embodiments, the compound comprises a modified oligonucleotide 17 to 25 linked nucleosides in length targeted to GCCR. In certain embodiments, the compound comprises a modified oligonucleotide 17 to 24 linked nucleosides in length targeted to GCCR. In certain embodiments, the compound comprises a modified oligonucleotide 17 to 23 linked nucleosides in length targeted to GCCR. In certain embodiments, the compound comprises a modified oligonucleotide 17 to 22 linked nucleosides in length targeted to GCCR. In certain embodiments, the compound comprises a modified oligonucleotide 17 to 21 linked nucleosides in length targeted to GCCR.

In certain embodiments, the compound comprises a modified oligonucleotide 20 linked nucleosides in length targeted to GCCR. In certain embodiments, the compound comprises a modified oligonucleotide 20 linked nucleosides in length targeted to GCCR.

Certain embodiments provide a method of preventing, ameliorating or treating a metabolic disease in an animal comprising administering to the animal a compound as described herein. In certain embodiments, the compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length targeted to GCCR. In certain embodiments, the compound comprises a modified oligonucleotide 20 linked nucleosides in length targeted to GCCR. Examples of metabolic diseases or disorders include, but are not limited to diabetes, hyperglycemia, prediabetes, obesity, non-alcoholic fatty liver disease (NAFLD), metabolic syndrome, insulin resistance, diabetic dyslipidemia, or hypertriglyceridemia or a combination thereof.

Certain embodiments provide a method of preventing, ameliorating or treating obesity in an animal comprising administering to the animal a compound as described herein. In certain embodiments, the compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length targeted to GCCR. In certain embodiments, the compound comprises a modified oligonucleotide 20 linked nucleosides in length targeted to GCCR. In certain embodiments, the compound comprises a modified oligonucleotide 20 linked nucleosides in length targeted to GCCR. In certain embodiments, the compound or composition comprises the compound of ISIS NOs: 420470, 420476, 426130, 426183, 426261, 426262, 426115, 426168, 426246, 426172, 426325, and 426267. In certain embodiments, the compound or composition comprises the compound of ISIS NO: 426115.

Certain embodiments provide a method of reducing body weight in an animal comprising administering to the animal a compound as described herein. In certain embodiments, the compound comprises a modified oligonucleotide 12 to 30 linked nucleosides in length targeted to GCCR. In certain embodiments, the compound comprises a modified oligonucleotide 20 linked nucleosides in length targeted to GCCR. In certain embodiments, reduction of body weight in an animal prevents, ameliorates or treats a metabolic disease. In certain embodiments, reduction of body weight in an animal prevents, ameliorates or treats diabetes. In certain embodiments, reduction of body weight in an animal prevents, ameliorates or treats obesity. In certain embodiments, reduction of body weight in an animal prevents, ameliorates or treats metabolic syndrome. In certain embodiments, reduction of body weight in an animal prevents, ameliorates or treats insulin resistance. In certain embodiments, reduction of body weight in an animal prevents, ameliorates or treats hyperglycemia. In certain embodiments, reduction of body weight in an animal prevents, ameliorates or treats NAFLD. In certain embodiments, reduction of body weight in an animal prevents, ameliorates or treats diabetic dyslipidemia. In certain embodiments, the glucose levels are reduced by at least 5%, 10%, 20%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100%.

Certain embodiments provide a method of reducing glucose levels in an animal comprising administering to the animal a compound as described herein. In certain embodiments, the compound

comprises a modified oligonucleotide 12 to 30 linked nucleosides in length targeted to GCCR. In certain embodiments, the compound comprises a modified oligonucleotide 20 linked nucleosides in length targeted to GCCR. In certain embodiments, reduction of glucose levels in an animal prevents, ameliorates or treats a metabolic disease. In certain embodiments, reduction of glucose levels in an animal prevents, ameliorates or treats diabetes. In certain embodiments, reduction of glucose levels in an animal prevents, ameliorates or treats obesity. In certain embodiments, reduction of glucose levels in an animal prevents, ameliorates or treats metabolic syndrome. In certain embodiments, reduction of glucose levels in an animal prevents, ameliorates or treats insulin resistance. In certain embodiments, reduction of glucose levels in an animal prevents, ameliorates or treats hyperglycemia. In certain embodiments, reduction of glucose levels in an animal prevents, ameliorates or treats NAFLD. In certain embodiments, reduction of glucose levels in an animal prevents, ameliorates or treats diabetic dyslipidemia. In certain embodiments, the glucose level is reduced by at least 5%, 10%, 20%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100%.

In certain embodiments, GCCR has the human sequence as set forth in any of the GENBANK Accession Numbers: the complement of GENBANK Accession No. NT_029289.10 truncated from nucleotides 3818000 to 3980000 (incorporated herein as SEQ ID NO: 1). In certain embodiments, GCCR has the rhesus monkey sequence as set forth in the complement of GENBANK Accession No. NW_001120987.1 truncated from nucleotides 1334000 to 1491000 (incorporated herein as SEQ ID NO: 2).

In certain embodiments, the compounds or compositions provided herein comprise a salt thereof, and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition comprises a modified oligonucleotide consisting of 17 to 35 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence recited in SEQ ID NOs: 6, 7, 10, 11, 33, 35, 36, 39, 42, and 43 or a salt thereof and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition comprises a modified oligonucleotide consisting of 20 to 25 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence recited in SEQ ID NOs: 6, 7, 10, 11, 33, 35, 36, 39, 42, and 43 or a salt thereof and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition comprises a modified oligonucleotide consisting of 20 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence recited in SEQ ID NOs: 6, 7, 10, 11, 33, 35, 36, 39, 42, and 43 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the compounds or compositions provided herein comprise a salt thereof, and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition comprises a modified oligonucleotide consisting of 17 to 35 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence recited in SEQ ID NO: 36 or a salt thereof and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition

comprises a modified oligonucleotide consisting of 17 to 25 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence recited in SEQ ID NO: 36 or a salt thereof and a pharmaceutically acceptable carrier or diluent. In certain embodiments, the composition comprises a modified oligonucleotide consisting of 20 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence recited in SEQ ID NO: 36 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

Certain embodiments provide a method for treating an animal with a GCCR related disease or condition comprising: a) identifying said animal with the GCCR related disease or condition, and b) administering to said animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence at least 90% complementary to any of SEQ ID NOs: 1 and 2 as measured over the entirety of said modified oligonucleotide. In certain embodiments, the therapeutically effective amount of the compound administered to the animal treats or reduces the GCCR related disease or condition, or a symptom thereof, in the animal. In certain embodiments, the GCCR related disease or condition is obesity. In certain embodiments, the GCCR related disease or condition is diabetes.

Certain embodiments provide a method for treating an animal with a GCCR related disease or condition comprising: a) identifying said animal with the GCCR related disease or condition, and b) administering to said animal a therapeutically effective amount of a compound comprising a modified oligonucleotide consisting of 20 linked nucleosides and having a nucleobase sequence at least 100% complementary to any of SEQ ID NOs: 1 and 2 as measured over the entirety of said modified oligonucleotide. In certain embodiments, the therapeutically effective amount of the compound administered to the animal treats or reduces the GCCR related disease or condition, or a symptom thereof, in the animal. In certain embodiments, the GCCR related disease or condition is obesity. In certain embodiments, the GCCR related disease or condition is diabetes.

Certain embodiments provide methods of treating, preventing, or ameliorating a metabolic disease. In certain embodiments the metabolic disease is obesity, diabetes, hyperglycemia, prediabetes, non-alcoholic fatty liver disease (NAFLD), metabolic syndrome, insulin resistance, diabetic dyslipidemia, or hypertriglyceridemia or a combination thereof.

Certain embodiments provide methods comprising administering to an animal a compound as described herein to an animal. In certain embodiments, the method comprises administering to an animal a modified oligonucleotide consisting of 17 to 35 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence recited in SEQ ID NOs: 6, 7, 10, 11, 33, 35, 36, 39, 42, and 43.

Certain embodiments provide methods comprising administering to an animal a compound as described herein to an animal. In certain embodiments, the method comprises administering to an animal a

modified oligonucleotide consisting of 17 to 35 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence recited in SEQ ID NO: 6, 7, 10, 11, 33, 35, 36, 39, 42, and 43.

Certain embodiments provide methods comprising administering to an animal a compound as described herein to an animal. In certain embodiments, the method comprises administering to an animal a modified oligonucleotide consisting of 17 to 35 linked nucleosides and having a nucleobase sequence comprising at least 20 contiguous nucleobases of a nucleobase sequence selected from among the nucleobase sequences recited in SEQ ID NO: 36.

In certain embodiments, the animal is a human.

In certain embodiments, the administering prevents, treats, ameliorates, or slows progression of a metabolic disease as described herein.

In certain embodiments, the administering prevents, treats, ameliorates, or slows progression of obesity as described herein.

In certain embodiments, the administering prevents, treats, ameliorates, or slows progression of diabetes as described herein.

In certain embodiments, the compound is co-administered with a second agent.

In certain embodiments, the compound and the second agent are administered concomitantly.

In certain embodiments, the administering is parenteral administration.

Certain embodiments further provide a method to reduce GCCR mRNA or protein expression in an animal comprising administering to the animal a compound or composition as described herein to reduce GCCR mRNA or protein expression in the animal. In certain embodiments, the animal is a human. In certain embodiments, reducing GCCR mRNA or protein expression prevents, treats, ameliorates, or slows progression of metabolic disease. In certain embodiments, the metabolic disease or condition is diabetes. In certain embodiments, the metabolic disease or condition is obesity.

Certain embodiments provide a method for treating a human with a metabolic disease comprising identifying the human with the disease and administering to the human a therapeutically effective amount of a compound or composition as described herein. In certain embodiments, the treatment reduces a symptom selected from the group consisting of metabolic syndrome, hyperglycemia, hypertriglyceridemia, hypertension, increased glucose levels, increased insulin resistance, decreased insulin sensitivity, above normal body weight, and/or above normal body fat or any combination thereof.

Certain embodiments provide a method for treating a human with obesity comprising identifying the human with the disease and administering to the human a therapeutically effective amount of a compound or composition as described herein. In certain embodiments, the treatment reduces a symptom selected from the group consisting of metabolic syndrome, hyperglycemia, hypertriglyceridemia, hypertension, increased

glucose levels, increased insulin resistance, decreased insulin sensitivity, above normal body weight, and/or above normal body fat or any combination thereof

Certain embodiments provide a method for treating a human with diabetes comprising identifying the human with the disease and administering to the human a therapeutically effective amount of a compound or composition as described herein. In certain embodiments, the treatment reduces a symptom selected from the group consisting of metabolic syndrome, hyperglycemia, hypertriglyceridemia, hypertension, increased glucose levels, increased insulin resistance, decreased insulin sensitivity, above normal body weight, and/or above normal body fat or any combination thereof

Further provided is a method for reducing or preventing metabolic disease comprising administering to a human a therapeutically effective amount compound or composition as described herein, thereby reducing or preventing metabolic disease.

Further provided is a method for reducing or preventing obesity comprising administering to a human a therapeutically effective amount compound or composition as described herein, thereby reducing or preventing diabetes.

Further provided is a method for reducing or preventing diabetes comprising administering to a human a therapeutically effective amount compound or composition as described herein, thereby reducing or preventing diabetes.

Further provided is a method for ameliorating a symptom of metabolic disease, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 17 to 35 linked nucleosides, wherein said modified oligonucleotide specifically hybridizes to SEQ ID NO: 1 or 2, thereby ameliorating a symptom of metabolic disease in the human.

Further provided is a method for ameliorating a symptom of diabetes, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 17 to 35 linked nucleosides, wherein said modified oligonucleotide specifically hybridizes to SEQ ID NO: 1 or 2, thereby ameliorating a symptom of diabetes in the human.

Further provided is a method for ameliorating a symptom of metabolic disease, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein said modified oligonucleotide specifically hybridizes to SEQ ID NO: 1 or 2, thereby ameliorating a symptom of metabolic disease in the human.

Further provided is a method for ameliorating a symptom of diabetes, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein said modified oligonucleotide specifically hybridizes to SEQ ID NO: 1 or 2, thereby ameliorating a symptom of diabetes in the human.

Further provided is a method for ameliorating a symptom of metabolic disease, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 20

linked nucleosides, wherein said modified oligonucleotide specifically hybridizes to SEQ ID NO: 1 or 2, thereby ameliorating a symptom of metabolic disease in the human.

Further provided is a method for ameliorating a symptom of diabetes, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 20 linked nucleosides, wherein said modified oligonucleotide specifically hybridizes to SEQ ID NO: 1 or 2, thereby ameliorating a symptom of diabetes in the human.

Further provided is a method for reducing the rate of progression of a symptom associated with metabolic disease, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 20 to 35 linked nucleosides, wherein said modified oligonucleotide specifically hybridizes to SEQ ID NO: 1 or 2, thereby reducing the rate of progression a symptom of metabolic disease in the human.

Further provided is a method for reducing the rate of progression of a symptom associated with diabetes, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 17 to 35 linked nucleosides, wherein said modified oligonucleotide specifically hybridizes to SEQ ID NO: 1 or 2, thereby reducing the rate of progression a symptom of diabetes in the human.

Further provided is a method for reducing the rate of progression of a symptom associated with metabolic disease, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein said modified oligonucleotide specifically hybridizes to SEQ ID NO: 1 or 2, thereby reducing the rate of progression a symptom of metabolic disease in the human.

Further provided is a method for reducing the rate of progression of a symptom associated with diabetes, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides, wherein said modified oligonucleotide specifically hybridizes to SEQ ID NO: 1 or 2, thereby reducing the rate of progression a symptom of diabetes in the human.

Further provided is a method for reducing the rate of progression of a symptom associated with metabolic disease, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 20 linked nucleosides, wherein said modified oligonucleotide specifically hybridizes to SEQ ID NO: 1 or 2, thereby reducing the rate of progression a symptom of metabolic disease in the human.

Further provided is a method for reducing the rate of progression of a symptom associated with diabetes, comprising administering to a human in need thereof a compound comprising a modified oligonucleotide consisting of 20 linked nucleosides, wherein said modified oligonucleotide specifically

hybridizes to SEQ ID NO: 1 or 2, thereby reducing the rate of progression a symptom of diabetes in the human.

Also provided are methods and compounds for the preparation of a medicament for the treatment, prevention, or amelioration of metabolic disease.

Also provided are methods and compounds for the preparation of a medicament for the treatment, prevention, or amelioration of obesity.

Also provided are methods and compounds for the preparation of a medicament for the treatment, prevention, or amelioration of diabetes.

Also provided are methods and compounds for the preparation of a medicament for the treatment, prevention, or amelioration of metabolic syndrome.

Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, ameliorating, or preventing metabolic disease.

Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, ameliorating, or preventing obesity.

Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, ameliorating, or preventing diabetes.

Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, ameliorating, or preventing metabolic syndrome.

Certain embodiments provide a compound as described herein for use in treating, preventing, or ameliorating metabolic disease as described herein by combination therapy with an additional agent or therapy as described herein. Agents or therapies can be co-administered or administered concomitantly.

Certain embodiments provide a compound as described herein for use in treating, preventing, or ameliorating diabetes as described herein by combination therapy with an additional agent or therapy as described herein. Agents or therapies can be co-administered or administered concomitantly.

Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, preventing, or ameliorating metabolic disease as described herein by combination therapy with an additional agent or therapy as described herein. Agents or therapies can be co-administered or administered concomitantly.

Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, preventing, or ameliorating obesity as described herein by combination therapy with an additional agent or therapy as described herein. Agents or therapies can be co-administered or administered concomitantly.

Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, preventing, or ameliorating diabetes as described herein by combination therapy

with an additional agent or therapy as described herein. Agents or therapies can be co-administered or administered concomitantly.

Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, preventing, or ameliorating diabetes as described herein by combination therapy with an additional agent or therapy as described herein. Agents or therapies can be co-administered or administered concomitantly.

Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, preventing, or ameliorating metabolic disease as described herein in a patient who is subsequently administered an additional agent or therapy as described herein.

Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, preventing, or ameliorating obesity as described herein in a patient who is subsequently administered an additional agent or therapy as described herein.

Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, preventing, or ameliorating diabetes as described herein in a patient who is subsequently administered an additional agent or therapy as described herein.

Certain embodiments provide the use of a compound as described herein in the manufacture of a medicament for treating, preventing, or ameliorating metabolic syndrome as described herein in a patient who is subsequently administered an additional agent or therapy as described herein.

Certain embodiments provide a kit for treating, preventing, or ameliorating metabolic disease as described herein wherein the kit comprises:

(i) a compound as described herein; and alternatively

(ii) an additional agent or therapy as described herein.

Certain embodiments provide a kit for treating, preventing, or ameliorating obesity as described herein wherein the kit comprises:

(i) a compound as described herein; and alternatively

(ii) an additional agent or therapy as described herein.

Certain embodiments provide a kit for treating, preventing, or ameliorating diabetes as described herein wherein the kit comprises:

(i) a compound as described herein; and alternatively

(ii) an additional agent or therapy as described herein.

Certain embodiments provide a kit for treating, preventing, or ameliorating metabolic syndrome as described herein wherein the kit comprises:

(i) a compound as described herein; and alternatively

(ii) an additional agent or therapy as described herein.

A kit as described herein may further include instructions for using the kit to treat, prevent, or ameliorate metabolic disease as described herein by combination therapy as described herein. In certain embodiments, the metabolic disease is obesity. In certain embodiments, the metabolic disease is diabetes.

Antisense Compounds

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

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

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

In certain embodiments, the antisense compound comprises a shortened or truncated modified oligonucleotide. The shortened or truncated modified oligonucleotide can have a single nucleoside deleted from the 5' end (5' truncation), or alternatively from the 3' end (3' truncation). A shortened or truncated oligonucleotide may have two nucleosides deleted from the 5' end, or alternatively may have two subunits deleted from the 3' end. Alternatively, the deleted nucleosides may be dispersed throughout the modified oligonucleotide, for example, in an antisense compound having one nucleoside deleted from the 5' end and one nucleoside deleted from the 3' end.

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

antisense compound, for example, in an oligonucleotide having one nucleoside added to the 5' end and one subunit added to the 3' end.

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

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

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

Antisense Compound Motifs

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

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

Antisense compounds having a gapmer motif are considered chimeric antisense compounds. In a gapmer an internal region having a plurality of nucleotides that supports RNaseH cleavage is positioned between external regions having a plurality of nucleotides that are chemically distinct from the nucleosides of the internal region. In the case of an antisense oligonucleotide having a gapmer motif, the gap segment

generally serves as the substrate for endonuclease cleavage, while the wing segments comprise modified nucleosides. In certain embodiments, the regions of a gapmer are differentiated by the types of sugar moieties comprising each distinct region. The types of sugar moieties that are used to differentiate the regions of a gapmer may in some embodiments include β-D-ribonucleosides, β-D-deoxyribonucleosides, 2'-modified nucleosides (such 2'-modified nucleosides may include 2'-MOE and 2'-O-CH_3, among others), and bicyclic sugar modified nucleosides (such bicyclic sugar modified nucleosides may include those having a constrained ethyl). In certain embodiments, wings may include several modified sugar moieties, including, for example 2'-MOE and constrained ethyl. In certain embodiments, wings may include several modified and unmodified sugar moieties. In certain embodiments, wings may include various combinations of 2'-MOE nucleosides, constrained ethyl nucleosides, and 2'-deoxynucleosides.

Each distinct region may comprise uniform sugar moieties, variant, or alternating sugar moieties. The wing-gap-wing motif is frequently described as "X-Y-Z", where "X" represents the length of the 5'-wing, "Y" represents the length of the gap, and "Z" represents the length of the 3'-wing. "X" and "Z" may comprise uniform, variant, or alternating sugar moieties. In certain embodiments, "X" and "Y" may include one or more 2'-deoxynucleosides."Y" may comprise 2'-deoxynucleosides. As used herein, a gapmer described as "X-Y-Z" has a configuration such that the gap is positioned immediately adjacent to each of the 5'-wing and the 3' wing. Thus, no intervening nucleotides exist between the 5'-wing and gap, or the gap and the 3'-wing. Any of the antisense compounds described herein can have a gapmer motif. In certain embodiments, "X" and "Z" are the same, in other embodiments they are different. In certain embodiments, "Y" is between 8 and 15 nucleosides. X, Y, or Z can be any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more nucleosides.

In certain embodiments, antisense compounds targeted to a GCCR nucleic acid possess a 3-14-3 gapmer motif.

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

In certain embodiments, antisense compounds targeted to a GCCR nucleic acid possess a 5-10-6 gapmer motif.

In certain embodiments, antisense compounds targeted to a GCCR nucleic acid possess a 3-10-3 gapmer motif.

In certain embodiments, antisense compounds targeted to a GCCR nucleic acid possess a 4-12-4 gapmer motif.

In certain embodiments, antisense compounds targeted to a GCCR nucleic acid possess a 2-13-5 gapmer motif.

Target Nucleic Acids, Target Regions and Nucleotide Sequences

In certain embodiments, GCCR has the human sequence as set forth in any of the GENBANK Accession Numbers: the complement of GENBANK Accession No. NT_029289.10 truncated from nucleotides 3818000 to 3980000 (incorporated herein as SEQ ID NO: 1). In certain embodiments, GCCR has the rhesus monkey sequence as set forth in the complement of GENBANK Accession No. NW_001120987.1 truncated from nucleotides 1334000 to 1491000 (incorporated herein as SEQ ID NO: 2)

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

In certain embodiments, a target region is a structurally defined region of the target nucleic acid. For example, a target region may encompass a 3' UTR, a 5' UTR, an exon, an intron, an exon/intron junction, a coding region, a translation initiation region, translation termination region, or other defined nucleic acid region. The structurally defined regions for GCCR can be obtained by accession number from sequence databases such as NCBI and such information is incorporated herein by reference. In certain embodiments, a target region may encompass the sequence from a 5' target site of one target segment within the target region to a 3' target site of another target segment within the same target region.

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

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

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

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

There may be variation in activity (e.g., as defined by percent reduction of target nucleic acid levels) of the antisense compounds within an active target region. In certain embodiments, reductions in GCCR mRNA levels are indicative of inhibition of GCCR expression. Reductions in levels of a GCCR protein are also indicative of inhibition of target mRNA expression. Further, phenotypic changes are indicative of inhibition of GCCR expression. In certain embodiments, reduced glucose levels, reduced lipid levels, and reduced body weight can be indicative of inhibition of GCCR expression. In certain embodiments, amelioration of symptoms associated with metabolic disease can be indicative of inhibition of GCCR expression. In certain embodiments, amelioration of symptoms associated with diabetes can be indicative of inhibition of GCCR expression. In certain embodiments, reduction of insulin resistance is indicative of inhibition of GCCR expression. In certain embodiments, reduction of diabetes biomarkers can be indicative of inhibition of GCCR expression.

Hybridization

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

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

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

Complementarity

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

An antisense compound may hybridize over one or more segments of a GCCR nucleic acid such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure, mismatch or hairpin structure).

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

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

In certain embodiments, the antisense compounds provided herein, or specified portions thereof, are fully complementary (i.e. 100% complementary) to a target nucleic acid, or specified portion thereof. For example, antisense compound may be fully complementary to a GCCR nucleic acid, or a target region, or a target segment or target sequence thereof. As used herein, "fully complementary" means each nucleobase of an antisense compound is capable of precise base pairing with the corresponding nucleobases of a target nucleic acid. For example, a 20 nucleobase antisense compound is fully complementary to a target sequence that is 400 nucleobases long, so long as there is a corresponding 20 nucleobase portion of the target nucleic acid that is fully complementary to the antisense compound. Fully complementary can also be used in reference to a specified portion of the first and /or the second nucleic acid. For example, a 20 nucleobase portion of a 30 nucleobase antisense compound can be "fully complementary" to a target sequence that is 400 nucleobases long. The 20 nucleobase portion of the 30 nucleobase oligonucleotide is fully complementary to the target sequence if the target sequence has a corresponding 20 nucleobase portion wherein each nucleobase is complementary to the 20 nucleobase portion of the antisense compound. At the same time, the entire 30

nucleobase antisense compound may or may not be fully complementary to the target sequence, depending on whether the remaining 10 nucleobases of the antisense compound are also complementary to the target sequence.

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

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

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

The antisense compounds provided herein also include those which are complementary to a portion of a target nucleic acid. As used herein, "portion" refers to a defined number of contiguous (i.e. linked) nucleobases within a region or segment of a target nucleic acid. A "portion" can also refer to a defined number of contiguous nucleobases of an antisense compound. In certain embodiments, the antisense compounds, are complementary to at least an 8 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 12 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 13 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 14 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 15 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 16 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 17 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 18 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 19 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 20 nucleobase portion of a target segment. Also contemplated are antisense compounds that are complementary to at least a 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more nucleobase portion of a target segment, or a range defined by any two of these values.

Identity

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

In certain embodiments, the antisense compounds, or portions thereof, are at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to one or more of the antisense compounds or SEQ ID NOs, or a portion thereof, disclosed herein.

Modifications

A nucleoside is a base-sugar combination. The nucleobase (also known as base) portion of the nucleoside is normally a heterocyclic base moiety. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to the 2', 3' or 5' hydroxyl moiety of the sugar. Oligonucleotides are formed through the covalent linkage of adjacent nucleosides to one another, to form a linear polymeric oligonucleotide. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside linkages of the oligonucleotide.

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

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

Modified Internucleoside Linkages

The naturally occurring internucleoside linkage of RNA and DNA is a 3' to 5' phosphodiester linkage. Antisense compounds having one or more modified, i.e. non-naturally occurring, internucleoside linkages are often selected over antisense compounds having naturally occurring internucleoside linkages

because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for target nucleic acids, and increased stability in the presence of nucleases.

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

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

Modified Sugar Moieties

Antisense compounds provided herein can optionally contain one or more nucleosides wherein the sugar group has been modified. Such sugar modified nucleosides may impart enhanced nuclease stability, increased binding affinity, or some other beneficial biological property to the antisense compounds. In certain embodiments, nucleosides comprise a chemically modified ribofuranose ring moiety. Examples of chemically modified ribofuranose rings include, without limitation, addition of substitutent groups (including 5' and 2' substituent groups); bridging of non-geminal ring atoms to form bicyclic nucleic acids (BNA); replacement of the ribosyl ring oxygen atom with S, N(R), or C(R1)(R)2 (R = H, C_1-C_{12} alkyl or a protecting group); and combinations thereof. Examples of chemically modified sugars include, 2'-F-5'-methyl substituted nucleoside (*see*, PCT International Application WO 2008/101157, published on 8/21/08 for other disclosed 5', 2'-bis substituted nucleosides), replacement of the ribosyl ring oxygen atom with S with further substitution at the 2'-position (*see*, published U.S. Patent Application US2005/0130923, published on June 16, 2005), or, alternatively, 5'-substitution of a BNA (*see*, PCT International Application WO 2007/134181, published on 11/22/07, wherein LNA is substituted with, for example, a 5'-methyl or a 5'-vinyl group).

Examples of nucleosides having modified sugar moieties include, without limitation, nucleosides comprising 5'-vinyl, 5'-methyl (R or S), 4'-S, 2'-F, 2'-OCH_3, and 2'-$O(CH_2)2OCH_3$ substituent groups. The substituent at the 2' position can also be selected from allyl, amino, azido, thio, O-allyl, O-C_1-C_{10} alkyl, OCF_3, $O(CH_2)2SCH_3$, $O(CH_2)2$-O-N(Rm)(Rn), and O-CH_2-C(=O)-N(Rm)(Rn), where each Rm and Rn is, independently, H or substituted or unsubstituted C_1-C_{10} alkyl.

As used herein, "bicyclic nucleosides" refer to modified nucleosides comprising a bicyclic sugar moiety. Examples of bicyclic nucleosides include, without limitation, nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, antisense compounds provided herein include one or more bicyclic nucleosides wherein the bridge comprises a 4' to 2' bicyclic nucleoside.

Examples of such 4' to 2' bicyclic nucleosides, include, but are not limited to, one of the formulae: 4'-(CH_2)-O-2' (LNA); 4'-(CH_2)-S-2'; 4'-$(CH_2)_2$-O-2' (ENA); 4'-$CH(CH_3)$-O-2' and 4'-$CH(CH_2OCH_3)$-O-2', and analogs thereof (*see*, U.S. Patent 7,399,845, issued on July 15, 2008); 4'-$C(CH_3)(CH_3)$-O-2', and analogs thereof (*see*, published PCT International Application WO2009/006478, published January 8, 2009); 4'-CH_2-$N(OCH_3)$-2', and analogs thereof (*see*, published PCT International Application WO2008/150729, published December 11, 2008); 4'-CH_2-O-$N(CH_3)$-2' (*see*, published U.S. Patent Application US2004/0171570, published September 2, 2004); 4'-CH_2-N(R)-O-2', wherein R is H, C_1-C_{12} alkyl, or a protecting group (*see*, U.S. Patent 7,427,672, issued on September 23, 2008); 4'-CH_2-$C(H)(CH_3)$-2' (*see*, Chattopadhyaya, *et al., J. Org. Chem.*,2009, *74*, 118-134); and 4'-CH_2-$C(=CH_2)$-2', and analogs thereof (*see*, published PCT International Application WO 2008/154401, published on December 8, 2008). Also see, for example: Singh et al., *Chem. Commun.*, 1998, *4*, 455-456; Koshkin et al., *Tetrahedron*, 1998, *54*, 3607-3630; Wahlestedt et al., *Proc. Natl. Acad. Sci. U. S. A.*, 2000, *97*, 5633-5638; 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.*, *129(26)* 8362-8379 (Jul. 4, 2007); Elayadi *et al., Curr. Opinion Invens. Drugs,* 2001, *2*, 558-561; Braasch *et al., Chem. Biol.,* 2001, *8*, 1-7; Orum *et al., Curr. Opinion Mol. Ther.,* 2001, *3*, 239-243; U.S. Patent Nos U.S. 6,670,461, 7,053,207, 6,268,490, 6,770,748, 6,794,499, 7,034,133, 6,525,191, 7,399,845; published PCT International applications WO 2004/106356, WO 94/14226, WO 2005/021570, and WO 2007/134181; U.S. Patent Publication Nos. US2004/0171570, US2007/0287831, and US2008/0039618; and U.S. Patent Serial Nos. 12/129,154, 60/989,574, 61/026,995, 61/026,998, 61/056,564, 61/086,231, 61/097,787, and 61/099,844; and PCT International Application Nos. PCT/US2008/064591, PCT/US2008/066154, and PCT/US2008/068922. Each of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α-L-ribofuranose and β-D-ribofuranose (see PCT international application PCT/DK98/00393, published on March 25, 1999 as WO 99/14226).

In certain embodiments, bicyclic sugar moieties of BNA nucleosides include, but are not limited to, compounds having at least one bridge between the 4' and the 2' position of the pentofuranosyl sugar moiety wherein such bridges independently comprises 1 or from 2 to 4 linked groups independently selected from -$[C(R_a)(R_b)]_n$-, -$C(R_a)$=$C(R_b)$-, -$C(R_a)$=N-, -$C(=NR_a)$-, -C(=O)-, -C(=S)-, -O-, -$Si(R_a)_2$-, -$S(=O)_x$-, and -$N(R_a)$-;

wherein:

x is 0, 1, or 2;

n is 1, 2, 3, or 4;

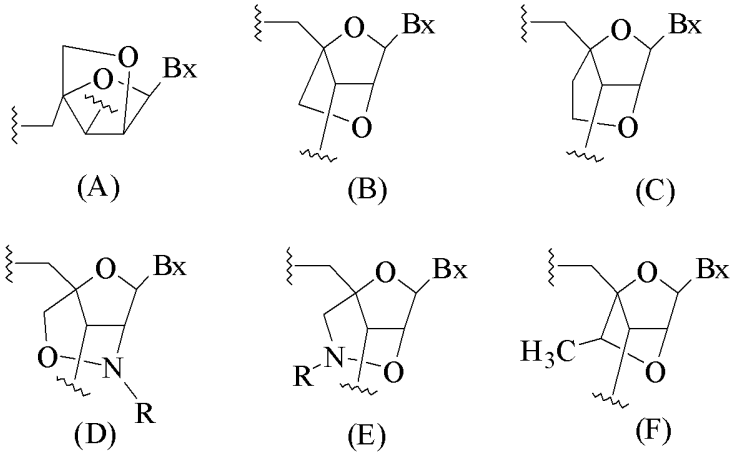
each R_a and R_b is, independently, H, a protecting group, hydroxyl, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, heterocycle radical, substituted heterocycle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

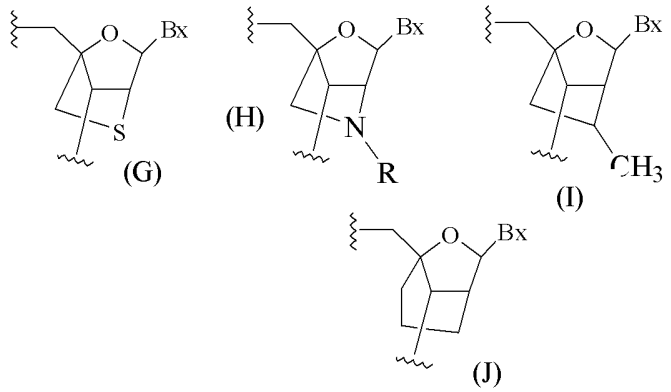
each J_1 and J_2 is, independently, H, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, acyl (C(=O)-H), substituted acyl, a heterocycle radical, a substituted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

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

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

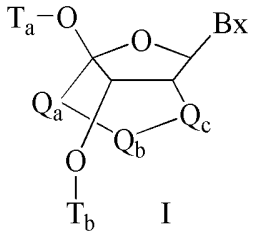
In certain embodiments, bicyclic nucleosides include, but are not limited to, (A) α-L-Methyleneoxy (4'-CH_2-O-2') BNA , (B) β-D-Methyleneoxy (4'-CH_2-O-2') BNA , (C) Ethyleneoxy (4'-$(CH_2)_2$-O-2') BNA , (D) Aminooxy (4'-CH_2-O-N(R)-2') BNA, (E) Oxyamino (4'-CH_2-N(R)-O-2') BNA, (F) Methyl(methyleneoxy) (4'-$CH(CH_3)$-O-2') BNA, (G) methylene-thio (4'-CH_2-S-2') BNA, (H) methylene-amino (4'-CH2-N(R)-2') BNA, (I) methyl carbocyclic (4'-CH_2-$CH(CH_3)$-2') BNA, and (J) propylene carbocyclic (4'-$(CH_2)_3$-2') BNA as depicted below.





wherein Bx is the base moiety and R is, independently, H, a protecting group or C_1-C_{12} alkyl.

In certain embodiments, bicyclic nucleoside having Formula I:



I

wherein:

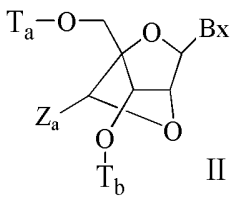
Bx is a heterocyclic base moiety;

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

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

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

In certain embodiments, bicyclic nucleoside having Formula II:



II

wherein:

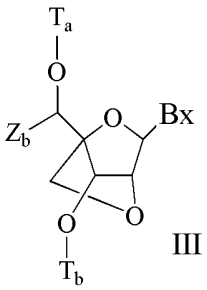
Bx is a heterocyclic base moiety;

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

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

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

In certain embodiments, bicyclic nucleoside having Formula III:



III

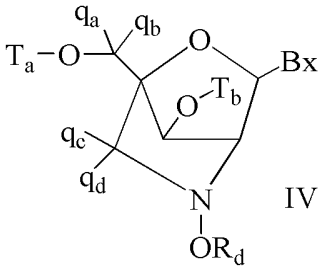
wherein:

Bx is a heterocyclic base moiety;

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

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

In certain embodiments, bicyclic nucleoside having Formula IV:



IV

wherein:

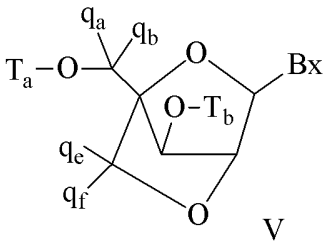
Bx is a heterocyclic base moiety;

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

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

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

In certain embodiments, bicyclic nucleoside having Formula V:



V

wherein:

Bx is a heterocyclic base moiety;

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

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

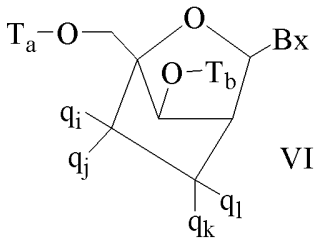
or q_e and q_f together are $=C(q_g)(q_h)$;

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

The synthesis and preparation of the methyleneoxy (4'-CH_2-O-2') BNA monomers adenine, cytosine, guanine, 5-methyl-cytosine, thymine, and uracil, along with their oligomerization, and nucleic acid recognition properties have been described (*see, e.g.*, Koshkin et al., *Tetrahedron*, 1998, *54*, 3607-3630). BNAs and preparation thereof are also described in WO 98/39352 and WO 99/14226.

Analogs of methyleneoxy (4'-CH_2-O-2') BNA, methyleneoxy (4'-CH_2-O-2') BNA, and 2'-thio-BNAs, have also been prepared (*see, e.g.*, Kumar et al., *Bioorg. Med. Chem. Lett.*, 1998, *8*, 2219-2222). Preparation of locked nucleoside analogs comprising oligodeoxyribonucleotide duplexes as substrates for nucleic acid polymerases has also been described (*see, e.g.*, Wengel et al., WO 99/14226). Furthermore, synthesis of 2'-amino-BNA, a novel comformationally restricted high-affinity oligonucleotide analog, has been described in the art (*see, e.g.*, Singh et al., *J. Org. Chem.*, 1998, *63*, 10035-10039). In addition, 2'-amino- and 2'-methylamino-BNA's have been prepared and the thermal stability of their duplexes with complementary RNA and DNA strands has been previously reported.

In certain embodiments, bicyclic nucleoside having Formula VI:



VI

wherein:

Bx is a heterocyclic base moiety;

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

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

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

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

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

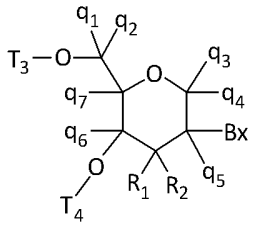
As used herein, "monocylic nucleosides" refer to nucleosides comprising modified sugar moieties that are not bicyclic sugar moieties. In certain embodiments, the sugar moiety, or sugar moiety analogue, of a nucleoside may be modified or substituted at any position.

As used herein, "2'-modified sugar" means a furanosyl sugar modified at the 2' position. In certain embodiments, such modifications include substituents selected from: a halide, including, but not limited to substituted and unsubstituted alkoxy, substituted and unsubstituted thioalkyl, substituted and unsubstituted amino alkyl, substituted and unsubstituted alkyl, substituted and unsubstituted allyl, and substituted and unsubstituted alkynyl. In certain embodiments, 2' modifications are selected from substituents including, but not limited to: $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, $OCH_2C(=O)N(H)CH_3$, and $O(CH_2)_nON[(CH_2)_nCH_3]_2$, where n and m are from 1 to about 10. Other 2'- substituent groups can also be selected from: C_1-C_{12} alkyl; substituted alkyl; alkenyl; alkynyl; alkaryl; aralkyl; O-alkaryl or O-aralkyl; SH;

SCH_3; OCN; Cl; Br; CN; CF_3; OCF_3; $SOCH_3$; SO_2CH_3; ONO_2; NO_2; N_3; NH_2; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a reporter group; an intercalator; a group for improving pharmacokinetic properties; and a group for improving the pharmacodynamic properties of an antisense compound, and other substituents having similar properties. In certain embodiments, modifed nucleosides comprise a 2'-MOE side chain (*see, e.g.*, Baker et al., J. Biol. Chem., 1997, 272, 11944-12000). Such 2'-MOE substitution have been described as having improved binding affinity compared to unmodified nucleosides and to other modified nucleosides, such as 2'- *O*-methyl, *O*-propyl, and *O*-aminopropyl. Oligonucleotides having the 2'-MOE substituent also have been shown to be antisense inhibitors of gene expression with promising features for *in vivo* use (*see, e.g.*, Martin, P., *Helv. Chim. Acta*, 1995, *78*, 486-504; Altmann et al., *Chimia*, 1996, *50*, 168-176; Altmann et al., *Biochem. Soc. Trans.*, 1996, *24*, 630-637; and Altmann et al., *Nucleosides Nucleotides*, 1997, *16*, 917-926).

As used herein, a "modified tetrahydropyran nucleoside" or "modified THP nucleoside" means a nucleoside having a six-membered tetrahydropyran "sugar" substituted in for the pentofuranosyl residue in normal nucleosides (a sugar surrogate). Modified THP nucleosides include, but are not limited to, what is referred to in the art as hexitol nucleic acid (HNA), anitol nucleic acid (ANA), manitol nucleic acid (MNA) (*see* Leumann, CJ. *Bioorg. & Med. Chem.* (2002) **10**:841-854), fluoro HNA (F-HNA), or those compounds having Formula X:

Formula X:



X

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

Bx is a heterocyclic base moiety;

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

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

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

In certain embodiments, the modified THP nucleosides of Formula X are provided wherein q_m, q_n, q_p, q_r, q_s, q_t, and q_u are each H. In certain embodiments, at least one of q_m, q_n, q_p, q_r, q_s, q_t, and q_u is other than H. In certain embodiments, at least one of q_m, q_n, q_p, q_r, q_s, q_t and q_u is methyl. In certain embodiments, THP nucleosides of Formula X are provided wherein one of R_1 and R_2 is F. In certain embodiments, R_1 is fluoro and R_2 is H, R_1 is methoxy and R_2 is H, and R_1 is methoxyethoxy and R_2 is H.

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

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

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

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

Many other bicyclo and tricyclo sugar surrogate ring systems are also known in the art that can be used to modify nucleosides for incorporation into antisense compounds (*see, e.g.*, review article: Leumann, J.C., *Bioorganic & Medicinal Chemistry*, **2002**, *10*, 841-854).

Such ring systems can undergo various additional substitutions to enhance activity.

Methods for the preparations of modified sugars are well known to those skilled in the art.

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

In certain embodiments, antisense compounds comprise one or more nucleotides having modified sugar moieties. In certain embodiments, the modified sugar moiety is 2'-MOE. In certain embodiments, the 2'-MOE modified nucleotides are arranged in a gapmer motif. In certain embodiments, the modified sugar moiety is a cEt. In certain embodiments, the cEt modified nucleotides are arranged throughout the wings of a gapmer motif.

Modified Nucleobases

Nucleobase (or base) modifications or substitutions are structurally distinguishable from, yet functionally interchangeable with, naturally occurring or synthetic unmodified nucleobases. Both natural and

modified nucleobases are capable of participating in hydrogen bonding. Such nucleobase modifications may impart nuclease stability, binding affinity or some other beneficial biological property to antisense compounds. Modified nucleobases include synthetic and natural nucleobases such as, for example, 5-methylcytosine (5-me-C). Certain nucleobase substitutions, including 5-methylcytosine substitutions, are particularly useful for increasing the binding affinity of an antisense compound for a target nucleic acid. For example, 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y.S., Crooke, S.T. and Lebleu, B., eds., *Antisense Research and Applications*, CRC Press, Boca Raton, **1993**, pp. 276-278).

Additional unmodified nucleobases include 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl (-C≡C-CH_3) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

Heterocyclic base moieties 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. Nucleobases that are particularly useful for increasing the binding affinity of antisense compounds include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2 aminopropyladenine, 5-propynyluracil and 5-propynylcytosine.

In certain embodiments, antisense compounds targeted to a GCCR nucleic acid comprise one or more modified nucleobases. In certain embodiments, gap-widened antisense oligonucleotides targeted to a GCCR nucleic acid comprise one or more modified nucleobases. In certain embodiments, the modified nucleobase is 5-methylcytosine. In certain embodiments, each cytosine is a 5-methylcytosine.

Compositions and Methods for Formulating Pharmaceutical Compositions

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

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

described herein, is a pharmaceutical composition comprising an antisense compound targeted to a GCCR nucleic acid and a pharmaceutically acceptable diluent. In certain embodiments, the pharmaceutically acceptable diluent is PBS. In certain embodiments, the antisense compound is an antisense oligonucleotide.

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

Pharmaceutically acceptable salts of the compounds described herein may be prepared by methods well-known in the art. For a review of pharmaceutically acceptable salts, see Stahl and Wermuth, Handbook of Pharmaceutical Salts: Properties, Selection and Use (Wiley-VCH, Weinheim, Germany, 2002). Sodium salts of antisense oligonucleotides are useful and are well accepted for therapeutic administration to humans. Accordingly, in one embodiment the compounds described herein are in the form of a sodium salt.

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

Conjugated Antisense Compounds

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

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

Cell culture and antisense compound treatment

The effects of antisense compounds on the level, activity or expression of GCCR nucleic acids can be tested *in vitro* in a variety of cell types. Cell types used for such analyses are available from commercial vendors (*e.g.* American Type Culture Collection, Manassus, VA; Zen-Bio, Inc., Research Triangle Park, NC; Clonetics Corporation, Walkersville, MD) and cells are cultured according to the vendor's instructions using commercially available reagents (e.g. Invitrogen Life Technologies, Carlsbad, CA). Illustrative cell types include, but are not limited to, HepG2 cells and primary hepatocytes.

In vitro testing of antisense oligonucleotides

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

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

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

Another reagent used to introduce antisense oligonucleotides into cultured cells includes LIPOFECTAMINE 2000® (Invitrogen, Carlsbad, CA). Antisense oligonucleotide is mixed with LIPOFECTAMINE 2000® in OPTI-MEM® 1 reduced serum medium (Invitrogen, Carlsbad, CA) to achieve the desired concentration of antisense oligonucleotide and a LIPOFECTAMINE® concentration that typically ranges 2 to 12 ug/mL per 100 nM antisense oligonucleotide.

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

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

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

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

transfected with LIPOFECTAMINE2000®, Lipofectin or Cytofectin. Antisense oligonucleotides are used at higher concentrations ranging from 625 to 20,000 nM when transfected using electroporation.

RNA Isolation

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

Analysis of inhibition of target levels or expression

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

Quantitative Real-Time PCR Analysis of Target RNA Levels

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

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

Gene (or RNA) target quantities obtained by real time PCR are normalized using either the expression level of a gene whose expression is constant, such as cyclophilin A, or by quantifying total RNA using RIBOGREEN® (Invitrogen, Inc. Carlsbad, CA). Cyclophilin A expression is quantified by real time PCR, by being run simultaneously with the target, multiplexing, or separately. Total RNA is quantified using RIBOGREEN® RNA quantification reagent (Invitrogen, Inc. Eugene, OR). Methods of RNA quantification by RIBOGREEN® are taught in Jones, L.J., et al, (Analytical Biochemistry, 1998, 265, 368-374). A CYTOFLUOR® 4000 instrument (PE Applied Biosystems) is used to measure RIBOGREEN® fluorescence.

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

Analysis of Protein Levels

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

In vivo testing of antisense compounds

Antisense compounds, for example, antisense oligonucleotides, are tested in animals to assess their ability to inhibit expression of GCCR and produce phenotypic changes. Testing may be performed in normal animals, or in experimental disease models. For administration to animals, antisense oligonucleotides are formulated in a pharmaceutically acceptable diluent, such as phosphate-buffered saline. Administration includes parenteral routes of administration. Following a period of treatment with antisense oligonucleotides, RNA is isolated from tissue and changes in GCCR nucleic acid expression are measured. Changes in GCCR protein levels are also measured.

Certain Indications

In certain embodiments, provided herein are methods of treating an individual comprising administering one or more pharmaceutical compositions as described herein. In certain embodiments, the individual has metabolic related disease.

As shown in the examples below, compounds targeted to GCCR, as described herein, have been shown to reduce the severity of physiological symptoms of metabolic related diseases, including metabolic syndrome, diabetes mellitus, insulin resistance, diabetic dyslipidemia, hypertriglyceridemia, obesity and weight gain *e.g.*, the animals continued to experience symptoms, but the symptoms were less severe compared to untreated animals. In certain experiments, the compounds reduced blood glucose levels,. In other experiments, the compounds reduce the symptoms of diabetes. In other experiments, the compounds inhibit weight gain. In other experiments, the compounds inhibit hypertriglyceridemia. In certain embodiements, the compounds restore function therefore demonstratingreversal of disease by treatment with a compound as described herein. In certain embodiments, animals treated for a longer period of time experience less severe symptoms than those administered the compounds for a shorter period of time.

Diabetes mellitus is characterized by numerous physical and physiological signs and/or symptoms. Any symptom known to one of skill in the art to be associated with Type 2 diabetes can be ameliorated or otherwise modulated as set forth above in the methods described above. In certain embodiments, the sign or symptom is a physical symptom such as increased glucose levels, increased weight gain, frequent urination, unusual thirst, extreme hunger, extreme fatigue, blurred vision, frequent infections, tingling or numbness at the extremities, dry and itchy skin, weight loss, slow-healing sores, and swollen gums. In certain embodiments, the sign or symptom is a physiological symptom such as increased insulin resistance, increased glucose levels, increased fat mass, decreased metabolic rate, decreased glucose clearance, decreased glucose tolerance, decreased insulin sensitivity, decreased hepatic insulin sensitivity, increased adipose tissue size and weight, increased body fat, and increased body weight.

In certain embodiments, the physical sign or symptom is increased glucose levels. In certain embodiments, the sign or symptom is weight gain. In certain embodiments, the sign or symptom is frequent urination. In certain embodiments, the sign or symptom is unusual thirst. In certain embodiments, the sign or symptom is extreme hunger. In certain embodiments, the sign or symptom is extreme fatigue. In certain embodiments, the sign or symptom is blurred vision. In certain embodiments, the sign or symptom is frequent infections. In certain embodiments, the sign or symptom is tingling or numbness at the extremities. In certain embodiments, the sign or symptom is dry and itchy skin. In certain embodiments, the sign or symptom is weight loss. In certain embodiments, the sign or symptom is slow-healing sores. In certain embodiments, the sign or symptom is swollen gums. In certain embodiments, the sign or symptom is increased insulin resistance. In certain embodiments, the sign or symptom is increased glucose levels. In certain embodiments, the sign or symptom is increased fat mass. In certain embodiments, the sign or symptom is decreased metabolic rate. In certain embodiments, the sign or symptom is decreased glucose clearance. In certain embodiments, the sign or symptom is decreased glucose tolerance. In certain embodiments, the sign or symptom is decreased insulin sensitivity. In certain embodiments, the sign or symptom is decreased hepatic insulin sensitivity. In certain embodiments, the sign or symptom is increased adipose tissue size and weight. In certain embodiments, the sign or symptom is increased body fat. In certain embodiments, the sign or symptom is increased body weight.

In certain embodiments, provided are methods of treating an individual comprising administering one or more pharmaceutical compositions as described herein. In certain embodiments, the individual has metabolic related disease.

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

In certain embodiments, pharmaceutical compositions comprising an antisense compound targeted to GCCR are used for the preparation of a medicament for treating a patient suffering or susceptible to metabolic related disease.

In certain embodiments, the methods described herein include administering a compound comprising a modified oligonucleotide having a contiguous nucleobases portion as described herein of a sequence recited in SEQ ID NO: 36. In certain embodiments, the compound is ISIS 426115.

Certain Combination Therapies

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

In certain embodiments, a first agent and one or more second agents are administered at the same time. In certain embodiments, the first agent and one or more second agents are administered at different times. In certain embodiments, the first agent and one or more second agents are prepared together in a single pharmaceutical formulation. In certain embodiments, the first agent and one or more second agents are prepared separately.

In certain embodiments, the second compound is administered prior to administration of a pharmaceutical composition described herein. In certain embodiments, the second compound is administered following administration of a pharmaceutical composition described herein. In certain embodiments, the second compound is administered at the same time as a pharmaceutical composition described herein. In certain embodiments, the dose of a co-administered second compound is the same as the dose that would be administered if the second compound was administered alone. In certain embodiments, the dose of a co-administered second compound is lower than the dose that would be administered if the second compound

was administered alone. In certain embodiments, the dose of a co-administered second compound is greater than the dose that would be administered if the second compound was administered alone.

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

In certain embodiments, second agents include, but are not limited to, a glucose-lowering agent. The glucose lowering agent can include, but is not limited to, a therapeutic lifestyle change, PPAR agonist, a dipeptidyl peptidase (IV) inhibitor, a GLP-1 analog, insulin or an insulin analog, an insulin secretagogue, a SGLT2 inhibitor, a human amylin analog, a biguanide, an alpha-glucosidase inhibitor, or a combination thereof. The glucose-lowering agent can include, but is not limited to metformin, sulfonylurea, rosiglitazone, meglitinide, thiazolidinedione, alpha-glucosidase inhibitor or a combination thereof. The sulfonylurea can be acetohexamide, chlorpropamide, tolbutamide, tolazamide, glimepiride, a glipizide, a glyburide, or a gliclazide. The meglitinide can be nateglinide or repaglinide. The thiazolidinedione can be pioglitazone or rosiglitazone. The alpha-glucosidase can be acarbose or miglitol.

In some embodiments, the glucose-lowering therapeutic is a GLP-1 analog. In some embodiments, the GLP-1 analog is exendin-4 or liraglutide.

In other embodiments, the glucose-lowering therapeutic is a sulfonylurea. In some embodiments, the sulfonylurea is acetohexamide, chlorpropamide, tolbutamide, tolazamide, glimepiride, a glipizide, a glyburide, or a gliclazide.

In some embodiments, the glucose-lowering drug is a biguanide. In some embodiments, the biguanide is metformin, and in some embodiments, blood glucose levels are decreased without increased lactic acidosis as compared to the lactic acidosis observed after treatment with metformin alone.

In some embodiments, the glucose-lowering drug is a meglitinide. In some embodiments, the meglitinide is nateglinide or repaglinide.

In some embodiments, the glucose-lowering drug is a thiazolidinedione. In some embodiments, the thiazolidinedione is pioglitazone, rosiglitazone, or troglitazone. In some embodiments, blood glucose levels are decreased without greater weight gain than observed with rosiglitazone treatment alone.

In some embodiments, the glucose-lowering drug is an alpha-glucosidase inhibitor. In some embodiments, the alpha-glucosidase inhibitor is acarbose or miglitol.

In a certain embodiment, a co-administered glucose-lowering agent is ISIS 113715.

In a certain embodiment, glucose-lowering therapy is therapeutic lifestyle change.

In certain embodiments, second agents include, but are not limited to, lipid-lowering agents. The lipid-lowering agent can include, but is not limited to atorvastatin, simvastatin, rosuvastatin, and ezetimibe. In certain such embodiments, the lipid-lowering agent is administered prior to administration of a pharmaceutical composition described herein. In certain such embodiments, the lipid-lowering agent is administered following administration of a pharmaceutical composition described herein. In certain such embodiments the lipid-lowering agent is administered at the same time as a pharmaceutical composition described herein. In certain such embodiments the dose of a co-administered lipid-lowering agent is the same as the dose that would be administered if the lipid-lowering agent was administered alone. In certain such embodiments the dose of a co-administered lipid-lowering agent is lower than the dose that would be administered if the lipid-lowering agent was administered alone. In certain such embodiments the dose of a co-administered lipid-lowering agent is greater than the dose that would be administered if the lipid-lowering agent was administered alone.

In certain embodiments, a co-administered lipid-lowering agent is a HMG-CoA reductase inhibitor. In certain such embodiments the HMG-CoA reductase inhibitor is a statin. In certain such embodiments the statin is selected from atorvastatin, simvastatin, pravastatin, fluvastatin, and rosuvastatin.

In certain embodiments, a co-administered lipid-lowering agent is a cholesterol absorption inhibitor. In certain such embodiments, cholesterol absorption inhibitor is ezetimibe.

In certain embodiments, a co-administered lipid-lowering agent is a co-formulated HMG-CoA reductase inhibitor and cholesterol absorption inhibitor. In certain such embodiments the co-formulated lipid-lowering agent is ezetimibe/simvastatin.

In certain embodiments, a co-administered lipid-lowering agent is a microsomal triglyceride transfer protein inhibitor (MTP inhibitor).

In certain embodiments, a co-administered lipid-lowering agent is an oligonucleotide targeted to ApoB.

In certain embodiments, second agents include, but are not limited to an anti-obesity drug or agent. Such anti-obesity agents include but are not limited to Orlistat or Rimonabant, and may be administered as described above as adipose or body weight lowering agents. In certain embodiments, the antisense compound may be co-administered with appetite suppressants. Such appetite suppressants include but are not

limited to diethylpropion tenuate, mazindol, orlistat, phendimetrazine, and phentermine, and may be administered as described herein. In certain embodiment, the anti-obesity agents are CNS based or GLP-1 based such as, but not limited to, liraglutide.

Formulations

The compounds provided herein may also be admixed, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as for example, liposomes, receptor-targeted molecules, or other formulations, for assisting in uptake, distribution and/or absorption. Representative United States patents that teach the preparation of such uptake, distribution and/or absorption-assisting formulations include, but are not limited to, U.S.: 5,108,921; 5,354,844; 5,416,016; 5,459,127; 5,521,291; 5,543,158; 5,547,932; 5,583,020; 5,591,721; 4,426,330; 4,534,899; 5,013,556; 5,108,921; 5,213,804; 5,227,170; 5,264,221; 5,356,633; 5,395,619; 5,416,016; 5,417,978; 5,462,854; 5,469,854; 5,512,295; 5,527,528; 5,534,259; 5,543,152; 5,556,948; 5,580,575; and 5,595,756, each of which is herein incorporated by reference.

The antisense compounds provided herein can be included in a pharmaceutical composition or formulation. The pharmaceutical composition can include any pharmaceutically acceptable salts, esters, or salts of such esters, or any other compound which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof.

The term "pharmaceutically acceptable salts" refers to physiologically and pharmaceutically acceptable salts of the compounds provided herein: i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto. The term “pharmaceutically acceptable salt” includes a salt prepared from pharmaceutically acceptable non-toxic acids or bases, including inorganic or organic acids and bases. For oligonucleotides, preferred examples of pharmaceutically acceptable salts and their uses are further described in U.S. Patent 6,287,860, which is incorporated herein in its entirety. Sodium salts have been shown to be suitable forms of oligonucleotide drugs.

The term “pharmaceutically acceptable derivative” encompasses , but is not limited to, pharmaceutically acceptable salts, solvates, hydrates, esters, prodrugs, polymorphs, isomers, isotopically labeled variants of the compounds described herein.

The pharmaceutical compositions described herein may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be parenteral. Parenteral administration includes but is not limited to subcutaneous, intravenous or intramuscular injection or infusion.

Parenteral administration, is preferred to target GCCR expression in the liver and plasma. Oligonucleotides with at least one 2'-O-methoxyethyl modification are believed to be particularly useful for parenteral administration.

The pharmaceutical formulations described herein, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both.

The compositions described herein may also be formulated as suspensions in aqueous, non-aqueous or mixed media. The suspension may also contain stabilizers.

Pharmaceutical compositions described herein include, but are not limited to, solutions, emulsions, and liposome-containing formulations. The pharmaceutical compositions and formulations described herein may comprise one or more penetration enhancers, carriers, excipients or other active or inactive ingredients.

Formulations include liposomal formulations. As used in the present invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers. Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior that contains the composition to be delivered. Cationic liposomes are positively charged liposomes which are believed to interact with negatively charged DNA molecules to form a stable complex. Liposomes that are pH-sensitive or negatively-charged are believed to entrap DNA rather than complex with it. Both cationic and noncationic liposomes have been used to deliver DNA to cells.

Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Liposomes and their uses are further described in U.S. Patent 6,287,860, which is incorporated herein in its entirety.

In another embodiment, formulations include saline formulations. In certain embodiments, a formulation consists of the compounds described herein and saline. In certain embodiments, a formulation consists essentially of the compounds described herein and saline. In certain embodiments, the saline is pharmaceutically acceptable grade saline. In certain embodiments, the saline is buffered saline. In certain embodiments, the saline is phosphate buffered saline (PBS).

In certain embodiments, a formulation excludes liposomes. In certain embodiments, the formulation excludes sterically stabilized liposomes. In certain embodiments, a formulation excludes phospholipids. In certain embodiments, the formulation consists essentially of the compounds described herein and saline and excludes liposomes.

The pharmaceutical formulations and compositions may also include surfactants. Surfactants and their uses are further described in U.S. Patent 6,287,860, which is incorporated herein in its entirety.

In one embodiment, the present invention employs various penetration enhancers to affect the efficient delivery of nucleic acids, particularly oligonucleotides. Penetration enhancers and their uses are further described in U.S. Patent 6,287,860, which is incorporated herein in its entirety.

Compositions and formulations for parenteral administration, including intravenous, subcutaneous and intramuscular injection or infusion may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

In another related embodiment, compositions provided herein may contain one or more antisense compounds, particularly oligonucleotides, targeted to a first nucleic acid and one or more additional antisense compounds targeted to a second nucleic acid target. Alternatively, compositions provided herein may contain two or more antisense compounds targeted to different regions of the same nucleic acid target. Numerous examples of antisense compounds are known in the art. Two or more combined compounds may be used together or sequentially.

Dosing

Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Optimum dosages may vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC_{50}s found to be effective in *in vitro* and *in vivo* animal models. In general, dosage is from 0.01 μg to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or at desired intervals. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 μg to 100 g per kg of body weight, once or more daily.

While the present invention has been described with specificity in accordance with certain of its preferred embodiments, the following examples serve only to illustrate the invention 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.

Certain Compounds

About seven hundred and sixty newly designed and previously disclosed antisense compounds of various lengths, motifs and backbone composition were tested for their effect on human GCCR mRNA *in vitro* in several cell types (Examples 1 and 2). The new compounds were compared with previously designed compounds, including ISIS 377131, ISIS 361137, ISIS 361141, ISIS 361151, ISIS 361155, and ISIS 361156

which have previously been determined to be some of the most potent antisense compounds *in vitro* (see e.g., PCT Pub. No. WO 2007/035759). Of the about seven hundred and sixty newly designed and previously designed antisense compounds, only those compounds which were selected for further study based on *in vitro* activity are presented.

Fifteen of these compounds were selected and additional new compounds were designed based on these compounds. The 317 new compounds were designed by creating compounds shifted slightly upstream and downstream (i.e. microwalk) of the original compounds. The new and original compounds were tested using electroporation for transfection and separately using lipofectin as the transfection reagent (Example 3). Of the 332 compounds tested, only those compounds which were selected for further study based on *in vitro* activity are presented. The 72 compounds selected for dose response assay were tested by individually using electroporation and lipofectin as the transfection reagents (Example 4). In the dose response assays described in Examples 4 and 5, several exemplary compounds were found to be more potent than the benchmark compound, ISIS 377131. Twenty nine compounds were selected for a dose response assay with electroporation as the transfection reagent (Example 5), from which twelve oligonucleotides were selected for *in vivo* rodent tolerability studies.

Twelve compounds, ISIS 420470 (SEQ ID NO: 6), ISIS 420476 (SEQ ID NO: 7), ISIS 426115 (SEQ ID NO: 36), ISIS 426130 (SEQ ID NO: 33), ISIS 426168 (SEQ ID NO: 39), ISIS 426172 (SEQ ID NO: 42), ISIS 426183 (SEQ ID NO: 10), ISIS 426246 (SEQ ID NO: 11), ISIS 426261 (SEQ ID NO: 10), ISIS 426262 (SEQ ID NO: 35), ISIS 426267 (SEQ ID NO: 43), and ISIS 426325 (SEQ ID NO: 42), were tested for tolerability in a CD1 mouse model, as well as a Sprague-Dawley rat model. The compound ISIS 377131 (SEQ ID NO: 4) was carried forward as a benchmark. The compounds are complementary to the regions 57825-57844, 59956-59975, 63677-63696, 65938-65957, 65939-65958, 65940-65959, 76224-76243, 76229-76248, 76255-76274, and 95513-95532 of SEQ ID NO: 1.

Liver function markers, such as alanine transaminase, aspartate transaminase and bilirubin, and kidney function markers, such as BUN and creatinine, as well as markers of inflammation were measured (Examples 6 and 7). The 13 compounds were also assayed for long-term effects on tolerability in a CD/1GS rat model for 12 weeks (Example 8). Liver function markers, such as alanine transaminase and aspartate transaminase, and kidney function markers, such as urine protein to creatinine were measured.

Final evaluation of these rodent tolerability studies (Examples 6-8) led to the selection of all twelve of the compounds for additional studies.

Due to having advantageous properties including *in vitro* potency and *in vivo* tolerability, in certain embodiments the compounds provided herein have a nucleobase sequence containing a portion of at least an 8, at least a 9, at least a 10, at least an 11, at least a 12, at least a 14, at least a 15, at least a 16, at least a 17, at least a 18, at least a 19 or at least a 20 contiguous nucleobases of one of SEQ ID NOs: 6, 7, 36, 33, 39, 42, 10, 11, 35 and 43. In certain embodiments, the compounds have a nucleobase sequence containing a portion of at

least an 8, at least a 9, at least a 10, at least an 11, at least a 12, at least a 14, at least a 15, at least a 16, at least a 17, at least a 18, at least a 19 or at least a 20 contiguous nucleobases complementary to an equal length portion of one of the regions 57825-57844, 59956-59975, 63677-63696, 65938-65957, 65939-65958, 65940-65959, 76224-76243, 76225-76244, 76229-76248, and 95513-95532 of SEQ ID NO: 1. In certain embodiments, the compounds targeting the listed regions or having a nucleobase portion of a sequence recited in the listed SEQ ID NOs can be of various length, as further described herein, and can have one of various motifs, as further described herein. In certain embodiments, a compound has the specific length and motif, as indicated by the ISIS NOs: 420470, 426476, 426115, 426130, 426168, 426172, 426183, 426246, 426261, 426262, 426267, and 426325.

These twelve compounds were tested for activity, pharmacokinetic profile and tolerability in cynomolgus monkeys (Example 9). Treatment with some of the compounds caused reduction of GCCR mRNA expression in liver tissue. Specifically, treatment with ISIS 420476, ISIS 426115, and ISIS 426325 caused significant reduction of GCCR mRNA expression in liver tissue, compared to the PBS control.

Tolerability studies in cynomolgus monkeys (Example 9) were also performed, with measurements of body and organ weights; measurements of ALT, AST, alkaline phosphatase, and bilirubin levels to assess liver function; measurements of BUN and creatinine levels to assess renal function; measurements of CRP and immune cell counts to assess inflammation status; and measurement of oligonucleotide concentrations in the liver and kidney to assess pharmacokinetics of the compounds. Treatment with ISIS 426115 was well tolerated, as indicated by baseline values of all the parameters listed above.

Viscosity of ISIS 420476, ISIS 426115 and ISIS 426325 was also measured (Example 10) and found to be optimal in all three cases.

Accordingly, provided herein are antisense compounds with any one or more of the improved characteristics. In a certain embodiments, the compounds as described herein are efficacious by virtue of having at least one of an *in vitro* IC_{50} in a human cell of less than 3 μM, less than 2.5 μM, less than 2 μM, less than 1.5 μM, less than 1 μM, when delivered to a HepG2 cell line using electroporation, as described in Examples 4 and 5. In certain embodiments, the compounds as described herein are efficacious by virtue of having at least one of an *in vitro* IC_{50} in a human cell of less than 50 nM, less than 45 nM less than 40 nM, less than 35 nM less than 30 nM, less than 25 nM less than 20 nM, when delivered to a HepG2 cell line using lipofectin reagent, as described in Example 4. In certain embodiments, the compounds as described herein are highly tolerable, as demonstrated by having at least one of an increase of ALT or AST value of no more than 50 fold, no more than 40 fold, no more than 30 fold, no more than 20 fold, no more than 10 fold, no more than 5 fold, no more than, no more than 4 fold, no more than 3 fold, or no more than 2 fold over saline treated animals; or an increase in liver, spleen or kidney weight of no more than 30%, no more than 20%, no more than 15%, no more than 12%, no more than 10%, 5 no more than % or no more than 2%.

EXAMPLES

Non-limiting disclosure and incorporation by reference

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

Example 1: Antisense inhibition of human glucocorticoid receptor (GCCR) in HepG2 cells

Antisense oligonucleotides were designed to target a human GCCR nucleic acid and were tested for their effects on GCCR mRNA *in vitro*. ISIS 377131, previously described in PCT Pub No. WO2005/071080, was also included in the assay. Cultured HepG2 cells at a density of 10,000 cells per well were transfected using lipofectin reagent with 120 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and GCCR mRNA levels were measured by quantitative real-time PCR using human primer probe set RTS1408 (forward sequence GGAGATCATATAGACAATCAAGTGCAA, designated herein as SEQ ID NO: 58; reverse sequence GGGTAGAGTCATTCTCTGCTCATTAA, designated herein as SEQ ID NO: 29; probe sequence CTGTGTTTTGCTCCTGATCTGAT, designated herein as SEQ ID NO: 60). GCCR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of GCCR, relative to untreated control cells. Of the 460 oligonucleotides tested, only those selected for further studies are presented.

The newly designed chimeric antisense oligonucleotides in Table 1 were designed as 5-10-5 MOE gapmers. The gapmers are 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Human Target start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. The gapmers were targeted to intronic sequences or intron-exon junctions of the human GCCR genomic sequence, designated herein as SEQ ID NO: 1 (the complement of GENBANK Accession No. NT_029289.10 truncated from nucleotides 3818000 to 3980000). The data indicates that antisense oligonucleotides targeted to the intronic regions of SEQ ID NO: 1 significantly reduce GCCR mRNA levels.

Table 1

Inhibition of human GCCR mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 1

ISIS NO	Human Start Site	Region	Sequence	% inhibition	SEQ ID NO
377131	37217	exon 2	GTCAAAGGTGCTTTGGTCTG	81	4
420450	51879	intron 2	TCCACAGATCTCTAGGGCAG	87	5
420470	57825	intron 2	GGTAGAAATATAGTTGTTCC	77	6
420476	59956	intron 2	TTCATGTGTCTGCATCATGT	86	7
420479	60939	intron 2	ATTTGGCTATTGTGGGATTC	71	8
420488	63678	intron 2	GGCATCCAGCGAGCACCAAA	79	9
420493	65938	intron 2	AGCCATGGTGATCAGGAGGC	78	10
420522	76225	intron 2	GGTCTGGATTACAGCATAAA	78	11
420599	95518	intron 2	TACTGGTGCTTGTCCAGGAT	79	12
420634	109349	intron 2	TCTGCGCACCTGCAGGCCCA	91	13
420644	112219 114155	intron 2	ACTTCTTACATGGTGGTGGC	76	14
420764	143259	intron 7	GCAACTATGAAACCACAGTT	76	15
414681	143737	intron 7	GGTATATATTTCCATCCTTA	83	16

Example 2: Antisense inhibition of human GCCR in HepG2 cells

Additional antisense oligonucleotides were designed targeting a GCCR nucleic acid and were tested for their effects on GCCR mRNA *in vitro*. ISIS 361137, ISIS 361141, ISIS 361151, ISIS 361156, ISIS 377131, ISIS 361143, and ISIS 361155, previously described in PCT Pub No. WO2005/071080, were also included in the assay. Cultured HepG2 cells at a density of 10,000 cells per well were transfected using lipofectin reagent with 120 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and GCCR mRNA levels were measured by quantitative real-time PCR using human primer probe set RTS1408. GCCR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of GCCR, relative to untreated control cells. Of the 298 new oligonucleotides tested, only those selected for further studies are presented.

The newly designed chimeric antisense oligonucleotides in Table 2 were designed as 5-10-5 MOE gapmers. The gapmers are 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Human Target start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. The gapmers were targeted to exonic sequences, intronic sequences or intron-exon junctions of SEQ ID NO: 1.

Table 2
Inhibition of human GCCR mRNA levels by chimeric antisense oligonucleotides having 5-10-5 MOE wings and deoxy gap targeted to SEQ ID NO: 1

ISIS NO	Human Start Site	Region	Sequence	% inhibition	SEQ ID NO
361137	33116	exon 2	CGACCTATTGAGGTTTGCAA	77	17
361141	33673	exon 2	GCAGACATTTTATTACCAAT	65	18
361151	33716	exon 2	GTACATCTGTCCTCCAGAGG	66	19
361155	33732	exon 2	TATTCATGTCATAGTGGTAC	75	20
361156	33736	exon 2	GCTGTATTCATGTCATAGTG	73	21
377131	33296	exon 2	GTCAAAGGTGCTTTGGTCTG	82	4
414641	104247	intron 2	GCGCACCTGCAGGCCCAACA	80	22
414648	109473	intron 2	CCCTCAGGTTTTGATGCTGC	74	23
414681	139287	intron 7	GGTATATATTTCCATCCTTA	87	16

Example 3: Antisense inhibition of human GCCR in HepG2 cells by oligonucleotides designed by microwalk

Additional gapmers were designed based on the gapmers presented in Tables 1 and 2. These gapmers were designed by creating gapmers shifted slightly upstream and downstream (i.e. "microwalk") of the original gapmers from Tables 1 and 2. Gapmers were also created with various motifs, e.g. 5-10-5 MOE, 3-14-3 MOE, and 2-13-5 MOE motifs. These gapmers were tested *in vitro*. Cultured HepG2 cells at a density of 20,000 cells per well were transfected using electroporation with 2,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and GCCR mRNA levels were measured by quantitative real-time PCR. The human primer probe set RTS1408 was used to measure GCCR mRNA levels. GCCR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of GCCR, relative to untreated control cells. The results are presented in Table 3.

The gapmers were also tested for their activity using lipofectin as the transfection reagent. Cultured HepG2 cells at a density of 10,000 cells per well were transfected using lipofectin with 50 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and GCCR mRNA levels were measured by quantitative real-time PCR. The human primer probe set RTS1408 was used to measure GCCR mRNA levels. GCCR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of GCCR, relative to untreated control cells. The results are also presented in Table 3.

The chimeric antisense oligonucleotides in Table 3 were designed as 5-10-5 MOE, 3-14-3 MOE, or 2-13-5 MOE gapmers. The gapmers designated with an asterisk (*) in Table 3 are the original gapmers from

which gapmers, ISIS 426106-426405, were designed via microwalk. ISIS 377131 was included in this assay and the activity of the newly designed gapmers was compared to the activity of ISIS 377131. The 5-10-5 gapmers are 20 nucleosides in length, wherein the central gap segment is comprised of ten 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising five nucleosides each. The 3-14-3 gapmers are 20 nucleosides in length, wherein the central gap segment is comprised of fourteen 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising three nucleosides each. The 2-13-5 gapmers are 20 nucleosides in length, wherein the central gap segment is comprised of thirteen 2'-deoxynucleosides and is flanked on the 5' and the 3' directions with wings comprising two and five nucleosides respectively. For each of the motifs (5-10-5, 3-14-3, and 2-113-5), each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Target start site" indicates the 5'-most nucleoside to which the gapmer is targeted. Each gapmer listed in Table 3 is targeted to SEQ ID NO: 1 (the complement of GENBANK Accession No. NT_029289.10 truncated from nucleotides 3818000 to 3980000). Only those gapmers which were selected for further studies are presented.

Table 3

Inhibition of human GCCR mRNA levels by chimeric antisense oligonucleotides

ISIS No	Start Site	Sequence	Motif	% inhibition using electroporation	% inhibition using lipofectin	SEQ ID NO
377131	37217	GTCAAAGGTGCTTTGGTCTG	4-12-4	67	37	4
426128	51878	CCACAGATCTCTAGGGCAGG	5-10-5	73	45	24
426199	51878	CCACAGATCTCTAGGGCAGG	3-14-3	54	28	24
426276	51878	CCACAGATCTCTAGGGCAGG	2-13-5	47	47	24
420450*	51879	TCCACAGATCTCTAGGGCAG	5-10-5	73	21	5
420470*	57825	GGTAGAAATATAGTTGTTCC	5-10-5	54	26	6
426331	57827	GTGGTAGAAATATAGTTGTT	5-10-5	33	29	25
426150	59951	GTGTCTGCATCATGTCTCTC	5-10-5	50	20	26
426301	59951	GTGTCTGCATCATGTCTCTC	2-13-5	50	22	26
426302	59952	TGTGTCTGCATCATGTCTCT	2-13-5	17	48	27
426229	59955	TCATGTGTCTGCATCATGTC	3-14-3	23	34	28
420476*	59956	TTCATGTGTCTGCATCATGT	5-10-5	53	59	7
426306	59956	TTCATGTGTCTGCATCATGT	2-13-5	24	46	7
426157	59959	TATTTCATGTGTCTGCATCA	5-10-5	45	20	29
426142	60935	GGCTATTGTGGGATTCTCCT	5-10-5	59	52	30
426216	60935	GGCTATTGTGGGATTCTCCT	3-14-3	50	46	30
426143	60936	TGGCTATTGTGGGATTCTCC	5-10-5	60	50	31

426293	60936	TGGCTATTGTGGGATTCTCC	2-13-5	51	7	31
426144	60937	TTGGCTATTGTGGGATTCTC	5-10-5	48	25	32
420479*	60939	ATTTGGCTATTGTGGGATTC	5-10-5	30	26	8
426130	63677	GCATCCAGCGAGCACCAAAG	5-10-5	49	46	33
420488*	63678	GGCATCCAGCGAGCACCAAA	5-10-5	55	50	9
426203	63678	GGCATCCAGCGAGCACCAAA	3-14-3	31	38	9
426131	63679	GGGCATCCAGCGAGCACCAA	5-10-5	52	32	34
426281	63679	GGGCATCCAGCGAGCACCAA	2-13-5	38	53	34
420493*	65938	AGCCATGGTGATCAGGAGGC	5-10-5	53	49	10
426183	65938	AGCCATGGTGATCAGGAGGC	3-14-3	68	70	10
426261	65938	AGCCATGGTGATCAGGAGGC	2-13-5	72	65	10
426262	65939	CAGCCATGGTGATCAGGAGG	2-13-5	34	61	35
426115	65940	GCAGCCATGGTGATCAGGAG	5-10-5	56	71	36
426185	65940	GCAGCCATGGTGATCAGGAG	3-14-3	41	51	36
426263	65940	GCAGCCATGGTGATCAGGAG	2-13-5	46	57	36
426116	65941	TGCAGCCATGGTGATCAGGA	5-10-5	45	61	37
426264	65941	TGCAGCCATGGTGATCAGGA	2-13-5	42	58	37
426117	65942	CTGCAGCCATGGTGATCAGG	5-10-5	58	70	38
426187	65942	CTGCAGCCATGGTGATCAGG	3-14-3	42	69	38
426168	76224	GTCTGGATTACAGCATAAAC	5-10-5	43	31	39
420522*	76225	GGTCTGGATTACAGCATAAA	5-10-5	44	33	11
426246	76225	GGTCTGGATTACAGCATAAA	3-14-3	60	39	11
426323	76227	TTGGTCTGGATTACAGCATA	2-13-5	32	50	40
426171	76228	CTTGGTCTGGATTACAGCAT	5-10-5	53	47	41
426324	76228	CTTGGTCTGGATTACAGCAT	2-13-5	51	33	41
426172	76229	CCTTGGTCTGGATTACAGCA	5-10-5	53	56	42
426325	76229	CCTTGGTCTGGATTACAGCA	2-13-5	43	57	42
426119	95513	GTGCTTGTCCAGGATGATGC	5-10-5	44	45	43
426189	95513	GTGCTTGTCCAGGATGATGC	3-14-3	44	59	43
426267	95513	GTGCTTGTCCAGGATGATGC	2-13-5	41	45	43
420599*	95518	TACTGGTGCTTGTCCAGGAT	5-10-5	63	51	12
426124	95519	CTACTGGTGCTTGTCCAGGA	5-10-5	41	54	44
414641*	109346	GCGCACCTGCAGGCCCAACA	5-10-5	43	76	22
426177	109346	GCGCACCTGCAGGCCCAACA	3-14-3	29	68	22
426255	109346	GCGCACCTGCAGGCCCAACA	2-13-5	13	68	22
426110	109347	TGCGCACCTGCAGGCCCAAC	5-10-5	45	69	45
420634*	109349	TCTGCGCACCTGCAGGCCCA	5-10-5	37	62	13
426343	112218 114154	CTTCTTACATGGTGGTGGCA	5-10-5	42	21	46
420644*	112219 114155	ACTTCTTACATGGTGGTGGC	5-10-5	44	40	14

426401	112219 114155	ACTTCTTACATGGTGGTGGC	2-13-5	31	50	14
426344	112220 114156	TACTTCTTACATGGTGGTGG	5-10-5	32	44	47
426402	112220 114156	TACTTCTTACATGGTGGTGG	2-13-5	33	40	47
426345	112221 114157	GTACTTCTTACATGGTGGTG	5-10-5	49	55	48
426403	112221 114157	GTACTTCTTACATGGTGGTG	2-13-5	31	37	48
426346	112222 114158	GGTACTTCTTACATGGTGGT	5-10-5	38	37	49
426404	112222 114158	GGTACTTCTTACATGGTGGT	2-13-5	40	34	49
426347	112223 114159	AGGTACTTCTTACATGGTGG	5-10-5	42	41	50
426405	112223 114159	AGGTACTTCTTACATGGTGG	2-13-5	30	31	50
426334	114587	CAGGTTTTGATGCTGCTGCT	5-10-5	15	37	51
426390	114587	CAGGTTTTGATGCTGCTGCT	2-13-5	15	42	51
426336	114589	CTCAGGTTTTGATGCTGCTG	5-10-5	15	36	52
426337	114590	CCTCAGGTTTTGATGCTGCT	5-10-5	20	44	53
414648*	114591	CCCTCAGGTTTTGATGCTGC	5-10-5	23	37	23
420764*	143259	GCAACTATGAAACCACAGTT	5-10-5	41	14	15
426136	143260	GGCAACTATGAAACCACAGT	5-10-5	56	33	54
426137	143261	TGGCAACTATGAAACCACAG	5-10-5	47	28	55
414681*	143737	GGTATATATTTCCATCCTTA	5-10-5	36	57	16
426161	143738	AGGTATATATTTCCATCCTT	5-10-5	13	55	56

Example 4: Dose-dependent antisense inhibition of human GCCR in HepG2 cells

Gapmers from Example 3 exhibiting significant *in vitro* inhibition of human GCCR were tested under various conditions in HepG2 cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.8 μM, 1.5 μM, 3.0 μM, or 6.0 μM concentrations of antisense oligonucleotide, as specified in Table 4. After a treatment period of approximately 16 hours, RNA was isolated from the cells and GCCR mRNA levels were measured by quantitative real-time PCR. Human GCCR primer probe set RTS1408 was used to measure mRNA levels. GCCR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of GCCR, relative to untreated control cells.

The gapmers were also tested at various doses in HepG2 cells using the transfection reagent, lipofectin. Cells were plated at a density of 10,000 cells per well and transfected using lipofectin reagent with 17.5 nM, 35 nM, 70 nM or 140 nM concentrations of antisense oligonucleotide, as specified in Table 5. After a treatment period of approximately 16 hours, RNA was isolated from the cells and GCCR mRNA levels were measured by quantitative real-time PCR. Human GCCR primer probe set RTS1408 was used to measure mRNA levels. GCCR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of GCCR, relative to untreated control cells.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented in Tables 4 and 5, and was calculated by plotting the concentrations of oligonucleotides used versus the percent inhibition of GCCR mRNA expression achieved at each concentration, and noting the concentration of oligonucleotide at which 50% inhibition of GCCR mRNA expression was achieved compared to the control. As illustrated in Tables 4 and 5, GCCR mRNA levels were significantly reduced in a dose-dependent manner in antisense oligonucleotide treated cells. Certain exemplary compounds demonstrated greater potency than the benchmark, ISIS 377131.

Table 4

Dose-dependent antisense inhibition of human GCCR expression in HepG2 cells using electroporation

ISIS No	0.8 μM	1.5 μM	3.0 μM	6.0 μM	IC_{50} (μM)
377131	28	43	66	83	2.0
414641	30	50	77	93	1.4
414648	8	32	50	61	3.4
414681	28	43	61	84	1.8
420450	36	57	68	90	1.3
420470	34	58	70	89	1.3
420476	36	51	81	93	1.3
420488	12	28	54	58	3.5
420493	32	42	66	82	1.7
420522	32	52	73	90	1.4
420599	28	52	73	80	1.5
420644	30	48	58	72	1.9
426110	20	40	57	78	2.2
426115	35	51	76	82	1.3
426116	32	48	74	80	1.5
426117	20	41	72	88	1.8
426119	33	52	72	80	1.4
426124	18	30	64	78	2.3
426128	40	51	82	91	1.2
426130	5	32	47	74	3.0
426131	26	23	41	60	4.3

426136	19	42	71	81	1.9
426137	5	25	48	73	3.1
426142	28	36	69	85	1.8
426143	14	38	59	80	2.3
426144	8	29	50	69	3.1
426150	26	42	69	81	1.8
426157	23	48	71	88	1.7
426161	17	34	52	68	2.8
426168	36	56	75	94	1.2
426171	34	49	78	90	1.4
426172	46	63	83	92	0.8
426177	19	35	55	83	2.3
426183	36	71	77	93	1.0
426185	36	43	65	78	1.6
426187	22	42	57	81	2.1
426189	31	45	68	84	1.6
426199	13	37	40	76	2.9
426203	0	6	16	33	1.8
426216	3	28	32	60	4.5
426229	5	23	55	83	2.6
426246	38	59	86	94	1.1
426255	19	29	62	77	2.4
426261	62	76	92	97	<0.8
426262	23	26	57	71	2.7
426263	25	40	70	90	1.7
426264	18	46	67	88	1.8
426267	45	54	78	90	1.0
426276	0	14	33	68	4.1
426281	0	8	15	44	1.0
426293	5	11	48	55	4.5
426301	26	47	76	92	1.6
426302	18	36	64	75	2.3
426306	12	17	60	85	2.5
426323	16	28	58	76	2.5
426324	27	54	81	94	1.4
426325	75	61	86	97	<0.8
426331	13	33	45	72	3.0
426334	1	16	41	63	4.1
426336	5	31	38	63	3.9
426337	16	29	35	64	4.1
426343	19	34	45	74	2.8
426344	11	26	42	70	3.4
426345	23	42	74	83	1.8

426346	23	41	60	82	2.0
426347	29	43	65	83	1.8
426390	13	19	30	60	5.2
426401	21	39	60	76	2.2
426402	14	16	37	67	4.0
426403	24	33	52	77	2.4
426404	27	39	54	86	2.0
426405	19	31	51	73	2.7

Table 5

Dose-dependent antisense inhibition of human GCCR expression in HepG2 cells using lipofectin reagent

ISIS No	17.5 nM	35.0 nM	70.0 nM	140.0 nM	IC_{50} (nM)
377131	27	55	78	87	33.0
414641	54	74	89	96	<17.5
414648	28	41	66	83	42.0
414681	30	48	68	85	37.0
420450	27	47	74	77	39.1
420470	22	45	59	75	49.4
420476	38	58	74	88	27.3
420488	28	48	72	71	40.3
420493	41	62	75	85	23.2
420522	32	57	72	78	31.8
420599	37	55	73	82	28.9
420644	32	53	75	84	32.0
426110	55	69	89	95	<17.5
426115	45	62	76	69	17.8
426116	47	67	81	92	18.1
426117	49	68	83	92	16.7
426119	36	53	68	70	33.4
426124	22	53	73	89	37.0
426128	34	48	73	83	33.7
426130	32	55	81	93	29.7
426131	41	52	71	79	28.4
426136	12	41	63	80	50.8
426137	14	41	62	87	47.8
426142	32	51	74	81	33.6
426143	34	54	76	82	30.7
426144	21	48	71	86	40.2
426150	27	49	66	76	40.8
426157	31	55	68	79	34.6
426161	23	43	70	86	41.6
426168	37	56	75	86	27.9

426171	42	56	73	83	25.3
426172	52	67	83	90	<17.5
426177	42	72	88	97	19.1
426183	54	70	86	92	<17.5
426185	36	61	82	87	25.9
426187	50	64	83	95	17.9
426189	40	62	79	86	23.5
426199	33	58	74	84	30.4
426203	29	46	74	90	36.0
426216	26	51	67	80	39.6
426229	23	44	70	90	40.2
426246	41	54	74	84	26.6
426255	43	69	88	96	19.8
426261	43	67	86	96	20.3
426262	44	65	82	90	19.7
426263	45	65	80	87	19.0
426264	36	57	83	95	27.0
426267	22	51	73	85	38.2
426276	28	56	77	92	32.4
426281	25	48	72	89	37.4
426293	30	46	72	79	37.5
426301	29	60	70	85	32.2
426302	22	48	72	89	39.2
426306	37	45	76	91	31.8
426323	19	44	71	88	41.9
426324	34	57	76	84	29.1
426325	2	48	70	89	46.9
426331	29	54	67	78	36.8
426334	20	39	65	81	47.3
426336	30	47	67	84	37.9
426337	31	55	71	89	32.7
426343	33	52	70	76	34.3
426344	38	53	72	85	29.5
426345	43	59	78	83	22.7
426346	34	56	62	35	>140.0
426347	36	53	71	79	31.3
426390	24	38	62	84	46.6
426401	35	49	69	82	34.0
426402	39	52	71	83	29.7
426403	29	54	72	86	33.9
426404	36	56	70	78	30.1
426405	33	53	73	86	32.1

Example 5: Dose-dependent antisense inhibition of human GCCR in HepG2 cells

Gapmers selected from Example 4 were tested at various doses in HepG2 cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.5 μM, 1.0 μM, 2.0 μM, 4.0 μM or 8.0 μM concentrations of antisense oligonucleotide, as specified in Table 6. After a treatment period of approximately 16 hours, RNA was isolated from the cells and GCCR mRNA levels were measured by quantitative real-time PCR. Human GCCR primer probe set RTS1408 was used to measure mRNA levels. GCCR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of GCCR, relative to untreated control cells. As illustrated in Table 6, GCCR mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells. Certain exemplary compounds demonstrated greater potency than the benchmark ISIS 377131.

Table 6

Dose-dependent antisense inhibition of human GCCR expression in HepG2 cells using electroporation

ISIS No	0.5 μM	1.0 μM	2.0 μM	4.0 μM	8.0 μM	IC_{50} (μM)
377131	19	42	65	83	90	1.4
414641	23	48	67	88	95	1.2
420450	29	49	65	81	94	1.1
420470	15	25	47	72	91	2.0
420476	14	36	67	86	94	1.5
420644	22	33	51	69	87	1.8
426110	13	33	52	77	93	1.8
426115	32	53	70	84	90	0.9
426116	27	44	71	87	90	1.1
426119	30	41	66	78	84	1.2
426128	37	54	77	82	94	0.8
426130	21	38	55	80	92	1.5
426131	1	33	39	74	86	2.2
426142	33	45	72	89	93	1.0
426143	29	44	69	85	93	1.1
426168	15	47	59	77	91	1.5
426171	15	23	45	72	88	2.1
426172	31	48	68	81	91	1.1
426183	23	51	79	91	97	1.0
426246	0	5	0	5	0	>8.0
426261	36	60	81	88	95	0.7
426262	15	26	55	76	92	1.8
426267	18	44	57	80	90	1.5
426325	25	46	74	89	97	1.1
426344	11	3	37	60	78	3.1

426345	7	20	43	65	82	2.5
426347	16	26	41	72	85	2.1
426402	3	9	35	54	80	3.2
426404	15	26	40	70	89	2.1

Example 6: Tolerability of antisense oligonucleotides targeting human GCCR in CD1 mice

CD1® mice (Charles River, MA) are a multipurpose model of mice frequently utilized for safety and efficacy testing. The mice were treated with ISIS antisense oligonucleotides selected from the study described in Example 5, and evaluated for changes in the levels of various markers.

Treatment

Eight-week old male CD1 mice were maintained at a 12-hour light/dark cycle and fed Purina mouse chow 5001 *ad libitum*. The mice were acclimated for at least 7 days in the research facility before initiation of the experiment. Groups of four CD1 mice each were injected subcutaneously twice a week for 4 weeks with 50 mg/kg of ISIS 377131, ISIS 420470, ISIS 420476, ISIS 426115, ISIS 426130, ISIS 426168, ISIS 426172, ISIS 426183, ISIS 426246, ISIS 426261, ISIS 426262, ISIS 426267, or ISIS 426325. Blood samples were collected via tail snip prior to dosing and at weeks 2, 3, and 4 after dosing. Three days after the last dose at each time point, mice were euthanized and organs and plasma were harvested for further analysis. Mice treated with ISIS 426267 died before the end of the study. Therefore, samples from mice treated with ISIS 426267 was not included in any assay

Plasma chemistry

To evaluate the effect of ISIS oligonucleotides on liver and kidney function, plasma levels of transaminases, cholesterol, glucose, and triglycerides were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma levels of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in Tables 7 and 8 expressed in IU/L. Plasma levels of cholesterol, glucose and triglycerides were also measured using the same clinical chemistry analyzer and the results are also presented in Tables 9, 10, and 11.

Table 7
ALT levels (IU/L) of CD1 mice at week 4

	Week 0	Week 2	Week 3	Week 4
PBS	25	23	31	25
ISIS 377131	24	41	32	50
ISIS 420470	31	53	62	97
ISIS 420476	24	46	56	83
ISIS 426115	23	29	39	47

ISIS 426130	21	29	41	37
ISIS 426168	22	31	64	65
ISIS 426172	24	32	35	39
ISIS 426183	22	29	43	50
ISIS 426261	23	39	77	93
ISIS 426262	28	34	43	81
ISIS 426246	25	291	535	1061
ISIS 426325	26	32	52	145

Table 8
AST levels (IU/L) of CD1 mice at week 4

	Week 0	Week 2	Week 3	Week 4
PBS	46	40	45	38
ISIS 377131	42	43	38	62
ISIS 420470	38	64	62	152
ISIS 420476	41	47	77	112
ISIS 426115	42	34	43	66
ISIS 426130	41	33	42	43
ISIS 426168	50	37	63	81
ISIS 426172	45	41	44	48
ISIS 426183	55	35	46	62
ISIS 426261	52	47	64	75
ISIS 426262	45	43	47	88
ISIS 426246	43	236	245	525
ISIS 426325	45	48	53	88

Table 9
Cholesterol levels (mg/dL) of CD1 mice at week 4

	Week 0	Week 2	Week 3	Week 4
PBS	152	166	176	161
ISIS 377131	141	162	149	175
ISIS 420470	159	181	193	201
ISIS 420476	132	161	165	179
ISIS 426115	115	131	143	140
ISIS 426130	120	148	160	157
ISIS 426168	123	138	161	159
ISIS 426172	134	163	161	161
ISIS 426183	135	166	154	164
ISIS 426261	128	146	158	172
ISIS 426262	149	208	197	248
ISIS 426246	156	283	225	183
ISIS 426325	128	140	117	81

Table 10
Glucose levels (mg/dL) of CD1 mice at week 4

	Week 0	Week 2	Week 3	Week 4
PBS	205	196	223	185
ISIS 377131	188	211	203	175
ISIS 420470	200	194	206	186
ISIS 420476	192	222	216	175
ISIS 426115	184	180	185	167
ISIS 426130	166	225	205	218
ISIS 426168	170	209	190	181
ISIS 426172	200	220	232	190
ISIS 426183	176	229	217	203
ISIS 426261	174	212	219	192
ISIS 426262	203	232	200	197
ISIS 426246	209	220	202	142
ISIS 426325	172	204	204	154

Table 11
Triglyceride levels (mg/dL) of CD1 mice at week 4

	Week 2	Week 3	Week 4
PBS	165	212	143
ISIS 377131	187	137	158
ISIS 420470	170	138	104
ISIS 420476	172	130	109
ISIS 426115	176	142	127
ISIS 426130	125	133	173
ISIS 426168	167	123	124
ISIS 426172	175	166	177
ISIS 426183	162	92	108
ISIS 426261	139	70	91
ISIS 426262	126	88	98
ISIS 426246	67	58	63
ISIS 426325	136	132	102

Example 7: Tolerability of antisense oligonucleotides targeting human GCCR in Sprague-Dawley rats

Sprague-Dawley rats were treated with ISIS antisense oligonucleotides from the study described in Example 6 and evaluated for changes in the levels of various markers.

Treatment

Eight week-old male rats were maintained on a 12-hour light/dark cycle and fed Purina normal rat chow *ad libitum*. Animals were acclimated at least 7 days in the research facility before the initiation of the experiment. Groups of four Sprague-Dawley rats each were injected subcutaneously twice a week with 50 mg/kg of ISIS 377131, ISIS 420470, ISIS 420476, ISIS 426115, ISIS 426130, ISIS 426168, ISIS 426172, ISIS 426183, ISIS 426246, ISIS 426261, ISIS 426262, ISIS 426267, or ISIS 426325. Blood samples were collected via tail snip prior to dosing and at weeks 2, 3, and 4 after dosing. Three days after the last dose at each time point, rats were euthanized and organs and plasma were harvested for further analysis.

Plasma chemistry

To evaluate the effect of ISIS oligonucleotides on liver and kidney function, plasma levels of transaminases, cholesterol, glucose, and triglycerides were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma levels of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in Tables 12 and 13, expressed in IU/L. Plasma levels of cholesterol, glucose and triglycerides were also measured using the same clinical chemistry analyzer and the results are also presented in Tables 14-16, expressed in mg/dL. 'n/a' indicates that the plasma chemistry marker for that particular time point had not been measured.

Table 12
ALT levels (IU/L) of Sprague-Dawley rats

	Week 0	Week 2	Week 3	Week 4
PBS	47	49	52	71
ISIS 377131	46	59	51	103
ISIS 420470	55	59	64	105
ISIS 420476	47	59	41	63
ISIS 426115	53	79	151	198
ISIS 426130	50	56	50	74
ISIS 426168	44	54	53	106
ISIS 426172	46	60	46	123
ISIS 426183	54	61	140	288
ISIS 426261	46	63	116	132
ISIS 426262	41	66	56	78
ISIS 426246	58	56	74	362
ISIS 426267	50	487	242	227
ISIS 426325	51	63	71	108

Table 13
AST levels (IU/L) of Sprague-Dawley rats

	Week 0	Week 2	Week 3	Week 4
PBS	73	87	83	85
ISIS 377131	71	76	72	127

ISIS 420470	95	83	109	141
ISIS 420476	72	80	78	104
ISIS 426115	82	92	226	192
ISIS 426130	74	75	75	86
ISIS 426168	72	78	112	155
ISIS 426172	76	77	87	188
ISIS 426183	75	90	207	361
ISIS 426261	72	87	144	140
ISIS 426262	72	94	97	119
ISIS 426246	92	82	108	269
ISIS 426267	86	400	264	206
ISIS 426325	83	75	90	126

Table 14
Cholesterol levels (mg/dL) of Sprague-Dawley rats

	Week 0	Week 2	Week 3	Week 4
PBS	93	72	71	65
ISIS 377131	111	41	36	40
ISIS 420470	103	37	42	42
ISIS 420476	85	59	59	59
ISIS 426115	116	81	95	110
ISIS 426130	89	59	49	54
ISIS 426168	68	43	46	72
ISIS 426172	81	49	53	118
ISIS 426183	87	89	111	245
ISIS 426261	84	67	54	70
ISIS 426262	80	60	49	60
ISIS 426246	78	59	62	91
ISIS 426267	89	58	70	72
ISIS 426325	83	44	49	71

Table 15
Glucose levels (mg/dL) of Sprague-Dawley rats

	Week 0	Week 2	Week 3	Week 4
PBS	184	172	159	157
ISIS 377131	191	175	146	138
ISIS 420470	191	134	162	161
ISIS 420476	185	151	159	188
ISIS 426115	191	151	124	142
ISIS 426130	191	161	161	154
ISIS 426168	189	158	142	233

ISIS 426172	189	150	143	288
ISIS 426183	183	154	146	268
ISIS 426261	176	150	134	142
ISIS 426262	163	169	143	141
ISIS 426246	200	152	148	156
ISIS 426267	193	121	137	142
ISIS 426325	174	146	154	147

Table 16
Triglyceride levels (mg/dL) of Sprague-Dawley rats

	Week 0	Week 2	Week 3	Week 4
PBS	73	66	124	96
ISIS 377131	81	32	33	32
ISIS 420470	71	42	35	31
ISIS 420476	79	41	59	43
ISIS 426115	48	43	35	26
ISIS 426130	84	37	52	40
ISIS 426168	62	44	56	37
ISIS 426172	65	46	51	n/a
ISIS 426183	74	26	44	n/a
ISIS 426261	71	55	37	40
ISIS 426262	91	36	34	27
ISIS 426246	136	56	43	36
ISIS 426267	120	42	34	29
ISIS 426325	75	82	86	67

Example 8: Long-term tolerability of antisense oligonucleotides targeting human GCCR in CD/1GS **rats**

CD/1GS rats were treated for 12 weeks with ISIS antisense oligonucleotides selected from the studies described in Examples 6 and 7, and evaluated for changes in the levels of various markers.

Treatment

Eight week-old male rats were placed in metabolic cages, maintained on a 12-hour light/dark cycle and fed Purina normal rat chow *ad libitum*. Animals were acclimated at least 7 days in the research facility before the initiation of the experiment. Groups of four rats each were injected subcutaneously twice a week for 12 weeks with 30 mg/kg of ISIS 377131, ISIS 420470, ISIS 420476, ISIS 426115, ISIS 426130, ISIS 426168, ISIS 426172, ISIS 426183, ISIS 426246, ISIS 426261, ISIS 426262, ISIS 426267, or ISIS 426325. Blood samples were collected via tail snip prior to dosing and at weeks 2, 4, 6, 8, 10 and 12 after dosing.

Three days after the last dose at each time point, rats were euthanized and organs and plasma were harvested for further analysis. Rats treated with ISIS 426267 died before the end of the study. Therefore, ISIS 426267 was not included in any further studies.

Liver function

To evaluate the effect of ISIS oligonucleotides on liver function, plasma levels of transaminases, cholesterol, glucose, and triglycerides were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The plasma levels of ALT and AST taken at week 12 are presented in Table 17, expressed as fold increase over the values of the PBS control. None of the antisense oligonucleotides caused any changes in any plasma chemistry markers outside the expected range for antisense oligonucleotides.

Table 17

Fold-increase in plasma chemistry markers of Sprague-Dawley rats compared to the PBS control

	ALT	AST
ISIS 377131	1.0	1.5
ISIS 420470	0.7	0.6
ISIS 420476	1.7	2.6
ISIS 426115	4.2	2.5
ISIS 426130	1.1	1.4
ISIS 426168	1.6	1.7
ISIS 426172	1.8	2.1
ISIS 426183	1.3	0.8
ISIS 426261	1.2	0.9
ISIS 426262	2.0	1.4
ISIS 426246	1.1	0.8
ISIS 426325	3.8	3.4

Renal function

To evaluate the effect of ISIS oligonucleotides on renal function, urine concentrations of total urine protein and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The results are presented in Table 18 as a ratio, as well as the fold increase in the ratio taken at week 12. Those antisense oligonucleotides that did not cause any changes in any renal metabolic marker outside the expected range for antisense oligonucleotides were selected for further study.

Table 18

Effect of antisense oligonucleotide treatment on renal metabolic markers of Sprague-Dawley rats

	Fold Increase
PBS	1

ISIS 377131	7
ISIS 420470	63
ISIS 420476	6
ISIS 426115	12
ISIS 426130	5
ISIS 426168	16
ISIS 426172	7
ISIS 426183	61
ISIS 426261	60
ISIS 426262	54
ISIS 426246	68
ISIS 426325	11

The results of both the mouse and rat studies indicated that ISIS 426115 was the most well tolerated antisense oligonucleotide targeting GCCR.

Example 9: Tolerability of ISIS antisense oligonucleotides targeting human GCCR in cynomolgus monkeys

Cynomolgus monkeys were treated with ISIS antisense oligonucleotides from studies described in Examples 6, 7, and 8. Antisense oligonucleotide activity and tolerability was evaluated.

The human oligonucleotides selected are fully cross-reactive with rhesus monkey gene sequences. The greater the complementarity between the human oligonucleotide and the rhesus monkey sequence, the more likely the human oligonucleotide can cross-react with the rhesus monkey sequence. The human oligonucleotides were compared to the rhesus monkey genomic sequence (SEQ ID NO: 2; the complement of GENBANK Accession No. NW_001120987.1 truncated from nucleotides 1334000 to 1491000), based on similarity to human exons, and the results are displayed in Table 19. "Rhesus start site" indicates the 5'-most nucleotide to which the gapmer is targeted in the rhesus monkey gene sequence.

Table 19

Complementarity of antisense oligonucleotides targeting human GCCR to SEQ ID NO: 2

ISIS No	Motif	Rhesus Start Site	Sequence	SEQ ID NO
420470	5-10-5	53479	GGTAGAAATATAGTTGTTCC	6
420476	5-10-5	55628	TTCATGTGTCTGCATCATGT	7
426130	5-10-5	59602	GCATCCAGCGAGCACCAAAG	33
426183	3-14-3	61848	AGCCATGGTGATCAGGAGGC	10
426261	2-13-5	61848	AGCCATGGTGATCAGGAGGC	10
426262	2-13-5	61849	CAGCCATGGTGATCAGGAGG	35
426115	5-10-5	61850	GCAGCCATGGTGATCAGGAG	36

426168	5-10-5	72083	GTCTGGATTACAGCATAAAC	39
426246	3-14-3	72084	GGTCTGGATTACAGCATAAA	11
426172	5-10-5	72088	CCTTGGTCTGGATTACAGCA	42
426325	2-13-5	72088	CCTTGGTCTGGATTACAGCA	42
426267	2-13-5	91877	GTGCTTGTCCAGGATGATGC	43

Treatment

The study was conducted at WuXi PharmaTech testing facility, People's Republic of China. Male 2-5 year-old cynomolgous monkeys were tested twice for tuberculosis before being used for the study and were quarantined for at least 14 days prior to the initiation of dosing. Twenty four groups of five randomly assigned male cynomolgus monkeys each were injected subcutaneously thrice per week for the first week, and subsequently once a week for the next 11 weeks, with either 8 mg/kg or 20 mg/kg of ISIS 420470, ISIS 420476, ISIS 426115, ISIS 426130, ISIS 426168, ISIS 426172, ISIS 426183, ISIS 426246, ISIS 426261, ISIS 426262, ISIS 426267, or ISIS 426325. A control group of 16 cynomolgus monkeys was injected with PBS subcutaneously thrice per week for the first week, and subsequently once a week for the next 11 weeks.

During the study period, the monkeys were observed daily for signs of illness or distress. Any animal showing adverse effects to the treatment was removed and referred to the veterinarian and Study Director. Clinical observations and mortality checks were performed pre-dose and at least once a day during the dosing regimen. Body weights were measured once a week. Blood samples were collected 5 days before the treatment as well as on various days of the study period and analyzed. The animals were fasted for at least 13 hours (overnight) prior to blood collection. Blood was collected by venipuncture from a peripheral vein from restrained, conscious animals. Terminal sacrifices of all groups were conducted on day 86, which was 48 hours after the last dose.

Inhibition studies

RNA analysis

At the end of the study, RNA was extracted from liver tissue for real-time PCR analysis of GCCR using primer probe set mkGCCR_1 (forward sequence TTAGGAGGGCGGCAAGTG, designated herein as SEQ ID NO: 61; reverse sequence AGGTGTAAGTTCCTGAAACCTGGTA, designated herein as SEQ ID NO: 62; probe sequence TGCAGCAGTGAAATGGGCAAAGGC; designated herein as SEQ ID NO: 63).The data was also analyzed using prime probe set mkGCCR_5 (forward sequence GGAGATCATATAGACAATCAAGTGCAA, designated herein as SEQ ID NO: 64; reverse sequence GGGTAGAGTCATTCTCTGCTCATTAA, designated herein as SEQ ID NO: 65; probe sequence CTGTGTTTTGCTCCTGATCTGAT; designated herein as SEQ ID NO: 66). Results are presented as percent inhibition of GCCR, relative to PBS control, normalized to the house-keeping gene, cyclophilin. As shown in

Table 20, treatment with ISIS 426325, ISIS 420476, and ISIS 426115 significantly reduced GCCR mRNA levels.

Table 20

Inhibition of GCCR mRNA in the cynomolgus monkey liver relative to the PBS control

ISIS No	8 mg/kg dose		20 mg/kg dose	
	primer probe set mkGCCR_1	primer probe set mkGCCR_5	primer probe set mkGCCR_1	primer probe set mkGCCR_5
420470	34	0	51	57
420476	53	67	76	87
426115	52	66	6	49
426130	27	38	34	48
426168	31	53	42	54
426172	28	37	41	51
426183	43	55	49	59
426246	31	61	50	68
426261	41	55	36	73
426262	41	8	49	59
426267	45	64	43	64
426325	68	72	73	79

Protein analysis

Approximately 1 mL of blood was collected from all available animals at week 11 and placed in tubes containing the potassium salt of EDTA. The tubes were centrifuged (3000 rpm for 10 min at room temperature) to obtain plasma. GCCR protein levels were measured in the plasma by western analysis using Santa Cruz sc-1003 rabbit polyclonal antibody. The results are presented in Table 21, expressed as percentage inhibition compared to the PBS control levels. The results indicate that ISIS 426325, ISIS 420476, and ISIS 426115 significantly reduced GCCR protein levels.

Table 21

GCCR protein level reduction in the cynomolgus monkey plasma relative to control levels

	Dose (mg/kg)	% reduction
ISIS 426325	8	70
	20	61
ISIS 420476	8	63
	20	62
ISIS 426115	8	57
	20	52
ISIS 426261	8	21
	20	28
ISIS 426183	8	0

	20	0

Tolerability studies

Body and organ weight measurements

To evaluate the effect of ISIS oligonucleotides on the overall health of the animals, body and organ weights were measured at week 12. The data is presented in Table 22. Treatment with ISIS 420476 caused increase in spleen weight. Treatment with the remaining ISIS oligonucleotides caused no significant change outside the expected range for antisense oligonucleotides.

Table 22

Final body and organ weight in the cynomolgus monkey at week 12

	Dose (mg/kg)	Body weight	Kidney	Spleen	Liver
PBS	-	2744	5	3	52
ISIS 426325	8	3000	6	6	63
	20	2882	7	6	72
ISIS 426172	8	2786	6	4	63
	20	2750	6	5	63
ISIS 426183	8	3026	6	4	58
	20	2822	6	5	58
ISIS 426168	8	2724	6	4	60
	20	2868	7	5	72
ISIS 420476	8	2980	7	4	71
	20	2798	7	9	77
ISIS 426267	8	2788	7	6	73
	20	2826	6	5	78
ISIS 426261	8	2590	6	4	57
	20	2596	5	6	59
ISIS 426246	8	2612	6	4	57
	20	2470	6	6	67
ISIS 426115	8	2572	5	5	56
	20	2642	6	7	62
ISIS 426262	8	2952	6	6	60
	20	2980	6	6	67
ISIS 420470	8	2588	8	9	70
	20	2782	7	6	80
ISIS 426130	8	2958	6	3	62
	20	2870	6	4	61

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, blood samples were collected from all the study groups on week 11. Approximately 3 mL of blood was collected from fasted animals and placed in tubes for serum separation. Serum was obtained by stabilization of the tubes at room temperature for 30-80 min followed by centrifugation (2,000 g x 15 minutes at room temperature). Levels of transaminases were measured using a Hitachi-917/911 chemistry analyzer. Plasma levels of ALT and AST were measured and the results are presented in Table 23, expressed in IU/L. Alkaline phosphatase (ALP), which is synthesized in increased amounts by damaged liver cells and is also a marker of liver disease, was similarly measured. The data is also presented in Table 23. Bilirubin is also a liver metabolic marker and was similarly measured. and the data is also presented in Table 23, expressed in mg/dL. None of the ISIS oligonucleotides caused any change in these liver function markers outside the expected range for antisense oligonucleotides.

Table 23

Levels of liver function markers in cynomolgus monkey plasma

	Dose (mg/kg)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Bilirubin (mg/dL)
PBS	-	52	71	1310	4
ISIS 426325	8	51	62	1408	4
	20	38	46	1464	5
ISIS 426172	8	55	61	1643	4
	20	56	55	1442	4
ISIS 426183	8	47	57	1633	3
	20	65	61	1603	6
ISIS 426168	8	37	41	1907	2
	20	55	46	1990	2
ISIS 420476	8	65	41	2088	2
	20	53	46	1698	2
ISIS 426267	8	42	40	1738	2
	20	68	47	1982	1
ISIS 426261	8	59	93	1193	4
	20	41	47	1158	4
ISIS 426246	8	57	64	1108	3
	20	35	60	1376	3
ISIS 426115	8	42	61	1369	3
	20	87	68	1418	3
ISIS 426262	8	41	45	1973	3
	20	49	48	1637	3
ISIS 420470	8	51	57	2137	2
	20	65	59	2568	2

ISIS 426130	8	33	42	1884	2
	20	44	54	2279	3

Kidney function

To evaluate the effect of ISIS oligonucleotides on renal function, blood samples were collected from all the study groups on week 11. Approximately 3 mL of blood was collected from fasted animals and placed in tubes for serum separation. Serum was obtained by stabilization of the tubes at room temperature for 30-80 min followed by centrifugation (2,000 g x 15 minutes at room temperature). Concentrations of BUN and creatinine were measured at week 11 using a Hitachi-917/911 chemistry analyzer. Results are presented in Table 24, expressed in mg/dL. None of the ISIS oligonucleotides caused any change in these renal function markers outside the expected range for antisense oligonucleotides.

Table 24

Plasma BUN and creatinine levels (mg/dL) in cynomolgus monkeys

	Dose (mg/kg)	BUN	Creatinine
PBS	-	7	56
ISIS 426325	8	8	58
	20	7	57
ISIS 426172	8	8	54
	20	7	53
ISIS 426183	8	6	62
	20	8	66
ISIS 426168	8	7	46
	20	6	46
ISIS 420476	8	7	51
	20	8	55
ISIS 426267	8	7	43
	20	6	50
ISIS 426261	8	7	56
	20	7	54
ISIS 426246	8	7	53
	20	6	54
ISIS 426115	8	8	57
	20	7	52
ISIS 426262	8	7	54
	20	6	58
ISIS 420470	8	7	61
	20	6	61
ISIS 426130	8	7	56
	20	6	57

Markers of inflammation

To evaluate any inflammatory effect of ISIS oligonucleotides in cynomolgus monkeys, blood samples were taken at week 11. C-reactive protein (CRP), which is synthesized in the liver and which serves as a marker of inflammation, was also similarly measured on week 11 using a Hitachi-917/911 chemistry analyzer. The results are presented in Table 25. Treatment with ISIS 426172 and ISIS 420470 caused increase in CRP levels. Treatment with the remaining ISIS oligonucleotides did not cause any change outside the expected range for antisense oligonucleotides.

Approximately 1.3 mL blood was collected in a tube treated with EDTA and used for the measurement of hematology parameter. Samples were analyzed for red blood cell (RBC) count, white blood cells (WBC) count, individual white blood cell percentages, such as that of monocytes, neutrophils, lymphocytes, as well as for platelet count and hematocrit (%), using an ADVIA120 hematology analyzer (Bayer, USA). The data is presented in Table 26. Treatment with ISIS 426168 and ISIS 420476 caused increase in lymphocyte counts. Treatment with ISSI 426325, ISIS 426172, ISIS 426262, and ISIS 420470 caused increase in neutrophil counts. Treatment with the remaining ISIS oligonucleotides did not cause any significant pro-inflammatory response beyond that expected for antisense oligonucleotides.

Table 25
CRP levels in cynomolgus monkeys

	Dose (mg/kg)	CRP (mg/L)
PBS	-	4
ISIS 426325	8	6
	20	5
ISIS 426172	8	17
	20	11
ISIS 426183	8	5
	20	3
ISIS 426168	8	3
	20	3
ISIS 420476	8	4
	20	6
ISIS 426267	8	4
	20	4
ISIS 426261	8	4
	20	3
ISIS 426246	8	6
	20	3
ISIS 426115	8	4
	20	3
ISIS 426262	8	4

	20	6
ISIS 420470	8	20
	20	12
ISIS 426130	8	3
	20	4

Table 26
Blood cells counts in cynomolgus monkeys

	Dose (mg/kg)	WBC (x 10^3/μL)	RBC (x 10^6/μL)	Platelet (x 1000/μL)	Hematocrit (%)	Lymphocytes (%)	Neutrophil (%)	Monocytes (%)
PBS	-	13	6	500	47	56	39	2
ISIS 426325	8	11	6	471	43	52	44	2
	20	13	6	454	45	45	52	2
ISIS 426172	8	12	6	496	48	44	51	2
	20	14	6	437	45	42	54	2
ISIS 426183	8	15	6	494	42	44	52	2
	20	12	6	466	45	61	34	2
ISIS 426168	8	15	6	334	43	76	18	3
	20	18	6	401	44	73	22	3
ISIS 420476	8	15	6	484	44	71	22	4
	20	15	6	455	42	70	24	3
ISIS 426267	8	16	5	377	41	52	43	2
	20	14	5	488	41	42	52	3
ISIS 426261	8	13	6	414	43	48	48	2
	20	12	5	414	40	47	48	3
ISIS 426246	8	10	6	403	44	47	49	3
	20	17	6	421	45	43	54	2
ISIS 426115	8	12	6	408	42	49	45	3
	20	15	6	457	44	47	50	2
ISIS 426262	8	13	5	443	41	46	50	3
	20	15	6	402	44	46	50	2
ISIS 420470	8	14	6	461	43	39	56	3
	20	12	5	445	43	45	50	2
ISIS 426130	8	15	6	466	44	41	54	3
	20	16	6	425	42	48	45	2

Pharmacokinetic studies

Measurement of oligonucleotide concentration

The concentration of the full-length oligonucleotide, as well as the total oligonucleotide concentration (including the degraded form), was measured at week 12. The method used is a modification of previously published methods (Leeds et al., 1996; Geary et al., 1999) which consist of a phenol-chloroform (liquid-liquid) extraction followed by a solid phase extraction. An internal standard (ISIS 355868, a 27-mer

2'-O-methoxyethyl modified phosphorothioate oligonucleotide, GCGTTTGCTCTTCTTCTTGCGTTTTTT, designated herein as SEQ ID NO: 57) was added prior to extraction. Tissue sample concentrations were calculated using calibration curves, with a lower limit of quantitation (LLOQ) of approximately 1.14 μg/g. The ratio of the concentrations in the kidney versus the liver was calculated. The results are presented in Tables 27 and 28, expressed as μg/g tissue.

Table 27

Full-length oligonucleotide concentration (μg/g) in the liver of cynomolgus monkey

ISIS No	Dose (mg/kg)	Kidney	Liver	Kidney/ Liver Ratio
426325	8	685	390	1.8
	20	1558	654	2.4
426172	8	643	483	1.3
	20	1159	1042	1.1
426183	8	655	537	1.2
	20	1245	820	1.5
426168	8	751	388	1.9
	20	1906	765	2.5
420476	8	939	463	2.0
	20	1318	689	1.9
426267	8	709	401	1.8
	20	1507	893	1.7
426261	8	453	382	1.2
	20	930	720	1.3
426246	8	595	248	2.4
	20	1479	425	3.5
426115	8	1035	511	2.0
	20	1403	1067	1.3
426262	8	558	410	1.4
	20	1506	921	1.6
420470	8	811	275	2.9
	20	2938	609	4.8
426130	8	718	425	1.7
	20	1715	769	2.2

Table 28

Total oligonucleotide concentration (μg/g) in the liver of cynomolgus monkey

ISIS No	Dose (mg/kg)	Kidney	Liver	Kidney/ Liver Ratio
426325	8	870	523	1.7
	20	2139	875	2.4

426172	8	922	688	1.3
	20	1681	1313	1.3
426183	8	905	809	1.1
	20	1791	1232	1.5
426168	8	909	507	1.8
	20	2477	951	2.6
420476	8	1367	636	2.1
	20	2057	948	2.2
426267	8	858	505	1.7
	20	1816	1103	1.6
426261	8	607	580	1.0
	20	1770	1098	1.6
426246	8	898	404	2.2
	20	2897	653	4.4
426115	8	1478	773	1.9
	20	2102	1542	1.4
426262	8	815	786	1.0
	20	2340	1438	1.6
420470	8	1051	401	2.6
	20	4012	815	4.9
426130	8	987	677	1.5
	20	2496	1144	2.2

Example 10: Measurement of viscosity of ISIS antisense oligonucleotides targeting human GCCR

The viscosity of three of the antisense oligonucleotides tested in the monkey study described in Example 9 was measured with the aim of screening out antisense oligonucleotides which have a viscosity more than 40 cP. Oligonucleotides having a viscosity greater than 40 cP would be too viscous to be administered to any subject.

ISIS oligonucleotides (32-35 mg) were weighed into a glass vial, 120 µL of water was added and the antisense oligonucleotide was dissolved into solution by heating the vial at 50^0C. Part of (75 µL) the pre-heated sample was pipetted to a micro-viscometer (Cambridge). The temperature of the micro-viscometer was set to 25^0C and the viscosity of the sample was measured. Another part (20 µL) of the pre-heated sample was pipetted into 10 mL of water for UV reading at 260 nM at 85^0C (Cary UV instrument). The results are presented in Table 29 and indicate that all the antisense oligonucleotides solutions are optimal in their viscosity under the criterion stated above.

Table 29
Viscosity and concentration of ISIS antisense oligonucleotides targeting human GCCR

ISIS No.	Viscosity (cP)	Concentration (mg/mL)
420476	4.18	179
426115	17.6	178
426325	4.17	164

Example 11: Dose response confirmation of antisense oligonucleotides targeting human GCCR in rhesus monkey LLC-MK2 cells

Select gapmers from the monkey study described in Example 9 were tested at various doses in LLC-MK2 cells. The ISIS oligonucleotides tested are cross-reactive with rhesus monkey GCCR gene (SEQ ID NO: 2; the complement of GENBANK Accession No. NW_001120987.1 truncated from nucleotides 1334000 to 1491000).

Cells were plated at a density of 25,000 cells per well and transfected using electroporation with 0.09 μM, 0.19 nM, 0.38 μM, 0.75 μM 1.50 μM, 3.00 μM, 6.00 μM or 12.00 μM concentrations of antisense oligonucleotide, as specified in Table 32. After a treatment period of approximately 16 hours, RNA was isolated from the cells and GCCR mRNA levels were measured by quantitative real-time PCR. Human GCCR primer probe set RTS1408 was used to measure mRNA levels. GCCR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of GCCR, relative to untreated control cells. As illustrated in Table 30, GCCR mRNA levels were reduced in a dose-dependent manner in LLC-MK2 cells treated with the ISIS oligonucleotides tested.

Table 30
Dose-dependent antisense inhibition of human GCCR in LLC-MK2 using electroporation

ISIS No	0.09 μM	0.19 μM	0.38 μM	0.75 μM	1.50 μM	3.00 μM	6.00 μM	12.00 μM	IC_{50} (μM)
377131	10	21	31	63	82	94	98	97	0.6
420476	0	2	4	30	45	71	93	97	1.7
426115	3	6	20	46	67	87	94	95	0.9
426261	6	24	31	52	77	94	97	97	0.6
426325	3	12	22	28	51	77	95	99	1.2

Example 12: Effect of ISIS 426115 targeting human/rhesus GCCR in cynomolgus monkeys

Since ISIS 426115 was demonstrated by the study above to be both a highly potent and tolerable antisense oligonucleotide, it was selected for a second cynomolgus monkey study.

Treatment

Prior to the study, the monkeys were kept in quarantine for a 5-week period, during which the animals were observed daily for general health. The monkeys were 2-3 years old and weighed between 2 and 5 kg. One group of five randomly assigned male cynomolgus monkeys was injected subcutaneously with ISIS 426115 using a stainless steel dosing needle and syringe of appropriate size into the intracapsular region and outer thigh of the monkeys. The monkeys were dosed four times a week for the first week (days 1, 3, 5, and 7) as loading doses, and subsequently once a week for weeks 2-13, with 40 mg/kg of ISIS 426115. A control group of 8 cynomolgus monkeys was injected with PBS subcutaneously thrice four times a week for the first week (days 1, 3, 5, and 7), and subsequently once a week for weeks 2-13.

During the study period, the monkeys were observed twice daily for signs of illness or distress. Any animal experiencing more than momentary or slight pain or distress due to the treatment, injury or illness was treated by the veterinary staff with approved analgesics or agents to relieve the pain after consultation with the Study Director. Any animal in poor health or in a possible moribund condition was identified for further monitoring and possible euthanasia. Scheduled euthanasia of the animals was conducted on day 93 by exsanguination after ketamine/xylazine-induced anesthesia and administration of sodium pentobarbital. The protocols described in the Example were approved by the Institutional Animal Care and Use Committee (IACUC).

Tolerability studies

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, blood samples were collected from all groups. The blood samples were collected via femoral venipuncture on day 95, 48 hrs post-dosing. The monkeys were fasted overnight prior to blood collection. Blood was collected in tubes containing K_2-EDTA anticoagulant, which were centrifuged to obtain plasma. Levels of various liver function markers were measured using a Toshiba 200FR NEO chemistry analyzer (Toshiba Co., Japan). Plasma levels of ALT and AST were measured and the results are presented in Table 31, expressed in IU/L. Bilirubin, a liver function marker, was similarly measured and is presented in Table 31, expressed in mg/dL. Treatment with ISIS 426115 was well tolerated in terms of the liver function in monkeys.

Table 31
Levels of liver metabolic markers in cynomolgus monkey plasma

	PBS	ISIS 426115
ALT (IU/L)	42	46
AST (IU/L)	42	46
Bilirubin (mg/dL)	0.18	0.26

Kidney function

To evaluate the effect of ISIS 426115 on kidney function, blood samples were collected from all groups. The blood samples were collected via femoral venipuncture on day 95, 48 hrs post-dosing. The monkeys were fasted overnight prior to blood collection. Blood was collected in tubes containing K_2-EDTA anticoagulant, which were centrifuged to obtain plasma. Concentrations of BUN and creatinine were measured using a Toshiba 200FR NEO chemistry analyzer (Toshiba Co., Japan). Results are presented in Table 32, expressed in mg/dL.

The plasma data indicate that treatment with ISIS 426115 was well tolerated in terms of the kidney function in monkeys.

Table 32

Effect Plasma BUN and creatinine levels (mg/dL) in cynomolgus monkeys

	PBS	ISIS 426115
BUN	17	19
Creatinine	0.60	0.58

This study taken into account with the study described in Example 9 further corroborates that ISIS 426115 is a well-tolerated antisense oligonucleotide targeting GCCR.

What is claimed is:

1. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases of any one of SEQ ID NOs: 4-56, wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

2. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases of any one of SEQ ID NOs: 36, 6, 7, 10, 11, 33, 35, 39, 42, or 43, wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

3. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 57825-57844, 59956-59975, 63677-63696, 65938-65957, 65939-65958, 65940-65959, 76224-76243, 76229-76248, 76255-76274 or 95513-95532 of SEQ ID NO: 1, wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

4. The compound of claim 1, 2 or 3, consisting of a single-stranded modified oligonucleotide.

5. The compound of claim 1 or 2, wherein said modified oligonucleotide is at least 95% complementary to SEQ ID NO: 1.

6. The compound of claim 1, 2 or 3, wherein said modified oligonucleotide is at least 96% complementary to SEQ ID NO: 1.

7. The compound of claim 1, 2 or 3, wherein said modified oligonucleotide is at least 97% complementary to SEQ ID NO: 1.

8. The compound of claim 1, 2 or 3, wherein said modified oligonucleotide is at least 98% complementary to SEQ ID NO: 1.

9. The compound of claim 1, 2 or 3, wherein said modified oligonucleotide is at least 99% complementary to SEQ ID NO: 1.

10. The compound of claim 1, 2 or 3, wherein said modified oligonucleotide is 100% complementary to SEQ ID NO: 1.

11. The compound of any one of the preceding claims, wherein at least one internucleoside linkage is a modified internucleoside linkage.

12. The compound of claim 11, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage.

13. The compound of any one of the preceding claims, wherein at least one nucleoside of the modified oligonucleotide comprises a modified sugar.

14. The compound of claim 13, wherein the at least one modified sugar is a bicyclic sugar.

15. The compound of claim 14, wherein each of the at least one bicyclic sugar comprises a 4'-(CH_2)-O-2', 4'-$(CH_2)_2$-O-2', or 4'-$CH(CH_3)$-O-2' group.

16. The compound of claim 14, wherein each of the at least one bicyclic sugar comprises a 4'-$CH(CH_3)$-O-2' bridge.

17. The compound of claim 13, wherein at least one modified sugar comprises a 2'-O-methoxyethyl group.

18. The compound of any one of the preceding claims, wherein at least one nucleoside comprises a modified nucleobase.

19. The compound of claim 18, wherein the modified nucleobase is a 5-methylcytosine.

20. The compound of claim of any one of the preceding claims, wherein the modified oligonucleotide comprises:

a gap segment consisting of linked deoxynucleosides;

a 5' wing segment consisting of linked nucleosides; and

a 3' wing segment consisting of linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

21. The compound of claim 20, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of five linked nucleosides; and

a 3' wing segment consisting of five linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

22. The compound of claim 20, wherein the modified oligonucleotide comprises:

a gap segment consisting of thirteen linked deoxynucleosides;

a 5' wing segment consisting of two linked nucleosides; and

a 3' wing segment consisting of five linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

23. The compound of claim 20, wherein the modified oligonucleotide comprises:

a gap segment consisting of fourteen linked deoxynucleosides;

a 5' wing segment consisting of three linked nucleosides; and

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.

24. The compound of any of claims 20-23, wherein each wing segment comprises a 2'-O-methoxyethyl modified sugar; and wherein each internucleoside linkage is a phosphorothioate linkage.

25. The compound of any one of the preceding claims, wherein said modified oligonucleotide consists of 14 to 25 linked nucleosides.

26. The compound of any one of the preceding claims, wherein said modified oligonucleotide consists of 17 to 21 linked nucleosides.

27. The compound of any one of the preceding claims, wherein said modified oligonucleotide consists of 18 to 21 linked nucleosides.

28. The compound of any one of the preceding claims, wherein said modified oligonucleotide consists of 20 linked nucleosides.

29. The compound of any one of the preceding claims, wherein said modified oligonucleotide consists of the nucleobase sequences of any one of SEQ ID NOs: 6, 7, 10, 11, 33, 35, 36, 39, 42 or 43.

30. A compound comprising a modified oligonucleotide consisting of 20 linked nucleosides having the nucleobase sequences of SEQ ID NO: 36, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

a 5' wing segment consisting of five linked nucleosides;

a 3' wing segment consisting of five linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment, wherein each nucleoside of each wing segment comprises a 2'-O-methoxyethyl modified sugar, wherein each internucleoside linkage of said modified oligonucleotide is a phosphorothioate linkage, and wherein each cytosine of said modified oligonucleotide is a 5-methylcytosine.

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

32. A method comprising administering to an animal any one of the preceding compounds or compositions.

33. The method of claim 32, wherein the animal is a human.

34. The method of claim 32, wherein administering the compound prevents, treats, ameliorates, or slows progression of a metabolic disease or condition.

35. The method of claim 34, wherein the disease or condition is diabetes.

36. The method of claim 34, wherein the disease or condition is Type 2 diabetes.

37. A method of decreasing blood glucose levels in an animal comprising administering to said animal the compound of claim 1.

38. The method of claim 37, wherein the animal is human.

39. The method of claim 37, wherein the blood glucose levels are plasma glucose levels or serum glucose levels.

40. The method of claim 37, wherein the animal is a diabetic animal.

41. A method of preventing, ameliorating or delaying the onset of a disease or condition associated with GCCR in an animal comprising administering to the animal a therapeutically or prophylactically effective amount of any one of the preceding compounds or compositions.

42. The method of claim 41, wherein the animal is human.

43. The method of claim 41, wherein the disease is a metabolic disease.

44. The method of claim 41, wherein the disease or condition is diabetes.

45. The method of claim 41, wherein the disease or condition is Type 2 diabetes.

46. A method of lowering blood glucose levels in an animal comprising administering to the animal a therapeutically or prophylactically effective amount of any one of the preceding compounds or compositions.

47. The method of claim 46, wherein the animal is human.

48. The method of claim 46, wherein the blood glucose levels are plasma glucose levels or serum glucose levels.

49. The method of claim 46, wherein the animal is a diabetic animal.

50. The method of claim 46, comprising co-administering the compound or composition and a second agent.

51. The method of claim 50, wherein the second agent is a glucose lowering agent.

52. The method of claim 51, wherein the compound or composition and the second agent are administered concomitantly.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/61984

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 15/11; C07H 21/04; C12N 5/00 (2013.01)
USPC - 514/44A
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C12N 15/11; C07H 21/04; C12N 5/00 (2013.01)
USPC - 514/44A

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 536/24.5; 435/375
Search not restricted by classification

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar; (oligonucleotide, linked nucleoside, glucocorticoid, gccl, isis 37217, modulat*, gtcaaaggtgctttggtctg, isis 37217)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/0222412 A1 (MONIA et al.) 02 September 2010 (02.09.2010). Paras [0005], [0006], [0008], [0009], [0022], [0024], [0025], [0050], [0051], [0053], [0065] and [0124].	1, 4-10 and 37-40
A	US 2007/0066557 A1 (MONIA et al.) 22 March 2007 (23.03.2007). Paras [0005]-[0010], [0021], [0022], [0024], [0025], [0028]-[0034], [0050], [0051], [0065] and [0123].	1, 4-10 and 37-40
A	US 2006/0160760 A1 (BHANOT et al.) 20 July 2006 (20.07.2006). Paras [0035], [0042], [0043], [0048], [0077], [0078], [0080], [0083], [0249].	1, 4-10 and 37-40

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
18 February 2012 (18.02.2013)	18 MAR 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/61984

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

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

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

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

3. ☒ Claims Nos.: 11-29, 31-36, 41-52
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:
- Please see extra sheet for continuation -

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

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

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

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Claims 1, 4-10, and 37-40 restricted to SEQ ID NO: 4

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/61984

Continuation of:
Box No III. Observations where unity of invention is lacking

Group I+: claims 1-10, 30 and 37-40, drawn to a compound comprising a modified oligonucleotide of 12 to 30 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases of any one of SEQ ID NOs: 4-56, wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementarity to SEQ ID NO: 1. The first invention is restricted to SEQ ID NO: 4 (Claims 1, 4-10, and 37-40). Should an additional fee(s) be paid, Applicant is invited to elect an additional sequence(s) to be searched. The exact claims searched will depend on Applicant's election.

The inventions of Group I+ share the technical feature of a a compound comprising a modified oligonucleotide of 12 to 30 linked nucleosides, wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementarity to SEQ ID NO: 1. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2010/0222412 A1 to Monia et al. (hereinafter 'Monia'). Monia discloses a compound comprising a modified oligonucleotide (para [0005]-[0006]) of 12 to 30 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases of SEQ ID NO: 4, wherein the nucleobase sequence of the modified oligonucleotide is at least 90% complementarity to SEQ ID NO: 1 (para [0125], SEQ ID NO: 37 is 100% identical to claimed SEQ ID NO: 4). As said composition was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Another special technical feature of the inventions listed as Group I+ is the specific sequence(s) recited therein. The inventions do not share a special technical feature, because no significant structural similarities can readily be ascertained among sequence(s). Without a shared special technical feature, the inventions lack unity with one another.

Group I+ therefore lacks unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

摘要

本文提供了用于减少动物中 GCCR mRNA 和蛋白表达的方法、化合物和组合物。该方法、化合物和组合物用于治疗、预防、延缓或改进代谢疾病，例如糖尿病，或其症状。