(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 May 2016 (19.05.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/077704 A1

(51) International Patent Classification:
*C12N 9/10* (2006.01) *A61P 3/00* (2006.01)
*C12N 15/63* (2006.01) *A61P 3/06* (2006.01)
*C12N 15/113* (2010.01) *A61P 3/10* (2006.01)

(21) International Application Number:
PCT/US2015/060591

(22) International Filing Date:
13 November 2015 (13.11.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/079,983 14 November 2014 (14.11.2014) US

(71) Applicants: **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 1111 Franklin Street, Twelfth Floor, Oakland, CA 94607-5200 (US). **IONIS PHARMACEUTICALS, INC.** [US/US]; 2855 Gazelle Ct., Carlsbad, CA 92010 (US).

(72) Inventors: **LUSIS, Aldons, J.**; 5321 Amigo Avenue, Tarzana, CA 91356 (US). **PARKS, Brian**; 1605 Armacost Avenue #203, Los Angeles, CA 90025 (US). **LEE, Richard**; 2855 Gazelle Ct., Carlsbad, CA 92010 (US).

(74) Agents: **LIANG, Teresa, Y.** et al.; Ionis Pharmaceuticals, Inc., 2855 Gazelle Ct., Carlsbad, CA 92010 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

**Published**:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2016/077704 A1

(54) **Title**: MODULATION OF AGPAT5 EXPRESSION

(57) **Abstract**: Provided herein are methods, compounds, and compositions for reducing expression of an AGPAT5 mRNA and protein in an animal. Also provided herein are methods, compounds, and compositions for reducing lipids, insulin resistance and/or glucose in an animal. Such methods, compounds, and compositions are useful to treat, prevent, delay, or ameliorate a cardiometabolic disease, disorder or condition, or a physiological marker thereof, in an individual in need.

# MODULATION OF AGPAT5 EXPRESSION

## Statement of Government Support

This invention was made with government support under HL028481 awarded by National Institute of Health. The government has certain rights in the invention.

## 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 BIOL0241WOSEQ_ST25, created on November 12, 2015 which is 144 MB in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

## Field of the Invention

Provided herein are methods, compounds, and compositions for reducing expression of 1-acylglycerol-3-phosphate O-acyltransferase 5 (also known as AGPAT5, LPAATE or 1AGPAT5) mRNA and protein in an animal. Also, provided herein are methods, compounds, and compositions comprising an AGPAT5 inhibitor for reducing AGPAT5 related diseases or conditions in an animal. Such methods, compounds, and compositions are useful, for example, to treat, prevent, delay or ameliorate a cardiometabolic disease, disorder or condition, or a physiological marker thereof, in an animal.

## Background

Diabetes and obesity (sometimes collectively referred to as "diabesity") are interrelated in that obesity is known to exacerbate the pathology of diabetes and greater than 60% of diabetics are obese. Most human obesity is associated with insulin resistance and leptin resistance. In fact, it has been suggested that obesity may have an even greater impact on insulin action than diabetes itself (Sindelka et al., *Physiol Res.*, 2002, *51*, 85-91). Additionally, several compounds on the market for the treatment of diabetes are known to induce weight gain, a very undesirable side effect to the treatment of this disease.

Cardiovascular disease is also interrelated to obesity and diabetes. Cardiovascular disease encompasses a wide variety of etiologies and has an equally wide variety of causative agents and interrelated players. Many causative agents contribute to symptoms such as elevated plasma levels of cholesterol, including non-high density lipoprotein cholesterol (non-HDL-C), as well as other lipid-related disorders. Such lipid-related disorders, generally referred to as dyslipidemia, include hyperlipidemia, hypercholesterolemia and hypertriglyceridemia among other indications. Elevated non-HDL cholesterol is associated with atherogenesis and its sequelae, including cardiovascular diseases such as arteriosclerosis, coronary artery disease, myocardial infarction, ischemic stroke, and other forms of heart disease. These rank

as the most prevalent types of illnesses in industrialized countries. Indeed, an estimated 12 million people in the United States suffer with coronary artery disease and about 36 million require treatment for elevated cholesterol levels.

Epidemiological and experimental evidence has shown that high levels of circulating triglyceride (TG) can contribute to cardiovascular disease and a myriad of metabolic disorders (Valdivielso et al., 2009, *Atherosclerosis* Zhang et al., 2008, *Circ Res.* 1;102(2):250-6). TG derived from either exogenous or endogenous sources is incorporated and secreted in chylomicrons from the intestine or in very low density lipoproteins (VLDL) from the liver. Once in circulation, TG is hydrolyzed by lipoprotein lipase (LpL) and the resulting free fatty acids can then be taken up by local tissues and used as an energy source. Due to the profound effect LpL has on plasma TG and metabolism in general, discovering and developing compounds that affect LpL activity are of great interest.

Metabolic syndrome is a combination of medical disorders that increase one's risk for cardiovascular disease and diabetes. The symptoms or physiological markers of metabolic syndrome, include high blood pressure, high triglycerides, decreased HDL and obesity, tend to appear together in some individuals. It affects a large number of people in a clustered fashion. In some studies, the prevalence in the USA is calculated as being up to 25% of the population. Metabolic syndrome is known under various other names, such as (metabolic) syndrome X, insulin resistance syndrome, Reaven's syndrome or CHAOS.

Despite several drugs for treating cardiometabolic diseases commercially available, the high prevalence of cardiovascular disorders and metabolic disorders shows that there remains a need for improved approaches to treat these conditions. It is therefore an object herein to provide compounds and methods for the treatment of such diseases and disorder.

AGPAT5 is a lipid acyltransferase gene that is important in the conversion of lysophosphatidic acid to phosphatidic acid and biochemical studies indicate that *Agpat5* is localized to the mitochondria where its function is unknown (Prasad *et al.*, 2011, J Lipid Res *52*, 451-462). A genome-wide association scan (GWAS) surveying the murine genome for common variants associated with insulin resistance (IR) found an association between AGPAT5 and IR (Parks *et al.*, unpublished).

The potential role of AGPAT5 in IR makes it an attractive target for investigation. Antisense technology is emerging as an effective means for reducing the expression of certain gene products and may therefore prove to be uniquely useful in a number of therapeutic, diagnostic, and research applications for the modulation of AGPAT5.

**Summary of the Invention**

Provided herein are compositions, compounds and methods for modulating expression of AGPAT5 mRNA and protein. In certain embodiments, AGPAT5 modulators include nucleic acids, proteins and small molecules. In certain embodiments, the AGPAT5 modulator is an AGPAT5 specific inhibitor. In certain

embodiments, the AGPAT5 specific inhibitor decreases expression of AGPAT5 mRNA and protein. In certain embodiments, AGPAT5 specific inhibitors include nucleic acids, proteins and small molecules. In certain embodiments, the AGPAT5 specific inhibitor is a nucleic acid. In certain embodiments, the nucleic acid is an antisense compound. In certain embodiments, the antisense compound is a modified oligonucleotide. In certain embodiments, the modified oligonucleotide can be single stranded or double stranded.

Certain embodiments disclosed herein provide a method of reducing AGPAT5 expression in an animal comprising administering to the animal a compound or composition comprising an AGPAT5 specific inhibitor.

Certain embodiments disclosed herein provide a method of reducing insulin resistance in an animal comprising administering to the animal a compound or composition comprising an AGPAT5 specific inhibitor.

Certain embodiments disclosed herein provide a method of increasing insulin sensitivity in an animal comprising administering to the animal a compound or composition comprising an AGPAT5 specific inhibitor.

Certain embodiments disclosed herein provide a method of reducing liver triglyceride levels in an animal comprising administering to the animal a compound or composition comprising an AGPAT5 specific inhibitor.

Certain embodiments disclosed herein provide a method of reducing insulin levels in an animal comprising administering to the animal a compound or composition comprising an AGPAT5 specific inhibitor.

Certain embodiments disclosed herein provide a method of increasing glucose tolerance in an animal comprising administering to the animal a compound or composition comprising an AGPAT5 specific inhibitor.

Certain embodiments disclosed herein provide a method of increasing glucose clearance in an animal comprising administering to the animal a compound or composition comprising an AGPAT5 specific inhibitor.

Certain embodiments disclosed herein provide a method of reducing the ratio of fat to body weight in an animal comprising administering to the animal a compound or composition comprising an AGPAT5 specific inhibitor.

Certain embodiments disclosed herein provide a method of ameliorating a metabolic and/or cardiovascular disease, disorder or condition, or a symptom or physiological marker thereof, in an animal comprising administering to the animal a therapeutically effective amount of a compound or composition comprising an AGPAT5 specific inhibitor. In certain embodiments, the therapeutically effective amount of the compound or composition administered to the animal reduces insulin resistance in a diabetic or non-diabetic animal.

Certain embodiments disclosed herein provide a method for treating an animal at risk for a metabolic and/or cardiovascular disease, disorder or condition, or a symptom or physiological marker thereof, comprising administering to said animal a therapeutically effective amount of a compound or composition comprising an AGPAT5 specific inhibitor. In certain embodiments, the therapeutically effective amount of the compound or composition administered to the animal reduces insulin resistance in a pre-diabetic or non-diabetic animal.

Certain embodiments disclosed herein provide compounds or compositions comprising an AGPAT5 modulator. In certain embodiments, the AGPAT5 modulator is an AGPAT5 specific inhibitor. In certain embodiments, AGPAT5 specific inhibitor is a nucleic acid, polypeptide, antibody, small molecules, or other agent capable of inhibiting the expression of AGPAT5. In certain embodiments, the nucleic acid is an antisense compound or composition targeting AGPAT5. In certain embodiments, the antisense compound or composition is single stranded. In certain embodiments, the antisense compound or composition is double stranded. In certain embodiments, the antisense compound or composition targeting AGPAT5 is an oligonucleotide. In certain embodiments, the oligonucleotide is single stranded. In certain embodiments, the oligonucleotide is double stranded. In certain embodiments, the oligonucleotide is a modified oligonucleotide. In certain embodiments, the modified oligonucleotide is single stranded. In certain embodiments, the modified oligonucleotide is double stranded.

Certain embodiments disclosed herein provide an antisense compound or composition comprising a modified oligonucleotide that is 10 to 30 linked nucleosides in length targeted to AGPAT5. The AGPAT5 target can have a nucleobase sequence selected from any one of SEQ ID NOs: 1-2, 24-26. In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 70%, 75%, 80%, 85%, 90%, 95% or 100% complementary to the nucleobase sequences recited in any one of SEQ ID NOs: 1-2, 24-26 as measured over the entirety of the modified oligonucleotide. In certain embodiments, the modified oligonucleotide comprises a nucleobase sequence comprising a portion of at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, or 16 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 1, 2, 24, 25 or 26.

Certain embodiments disclosed herein provide an antisense compound or composition comprising a modified oligonucleotide that is 10 to 30 linked nucleosides in length wherein the nucleobase sequence of the modified oligonucleotide comprises at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, or 16 contiguous nucleobases of a sequence recited in any one of SEQ ID NOs: 6-23.

Certain embodiments provide compositions and methods for use in therapy. Certain embodiments provide compositions and uses for preventing, treating, delaying, slowing the progression and/or ameliorating AGPAT5 related diseases, disorders, and conditions, or symptoms or physiological markers thereof. In certain embodiments, such diseases, disorders, and conditions are cardiovascular and/or metabolic diseases, disorders, and conditions, or symptoms or physiological markers thereof. In certain embodiments, the compositions and uses for therapy include administering therapeutically effective amount of an AGPAT5

specific inhibitor to an individual in need thereof. In certain embodiments, the AGPAT5 specific inhibitor is a nucleic acid, protein or small molecule. In certain embodiments, the nucleic acid is an antisense compound. In certain embodiments, the antisense compound is a modified oligonucleotide.

**Detailed Description of the Invention**

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed. Herein, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the use of "or" means "and/or" unless stated otherwise. 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 patents, applications, published applications and other 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" or "3' stop 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" or "5' start 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 AGPAT5 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.

"Adipogenesis" means the development of fat cells from preadipocytes. "Lipogenesis" means the production or formation of fat, either fatty degeneration or fatty infiltration.

"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 metabolic 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 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 of the invention. For example, a first agent can be an antisense oligonucleotide targeting AGPAT5. "Second agent" means a second therapeutic compound of the invention (e.g. a second antisense oligonucleotide targeting AGPAT5) and/or a non-AGPAT5 therapeutic compound.

"Amelioration" refers to a lessening of at least one indicator, sign, physiological marker 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.

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

"AGPAT5" means any nucleic acid or protein of AGPAT5.

"AGPAT5 expression" means the level of mRNA transcribed from the gene encoding AGPAT5 or the level of protein translated from the mRNA. AGPAT5 expression can be determined by art known methods such as a Northern or Western blot.

"AGPAT5 nucleic acid" means any nucleic acid encoding AGPAT5. For example, in certain embodiments, an AGPAT5 nucleic acid includes a DNA sequence encoding AGPAT5, a RNA sequence transcribed from DNA encoding AGPAT5 (including genomic DNA comprising introns and exons), and a mRNA sequence encoding AGPAT5. "AGPAT5 mRNA" means a mRNA encoding an AGPAT5 protein.

"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.

"Atherosclerosis" means a hardening of the arteries affecting large and medium-sized arteries and is characterized by the presence of fatty deposits. The fatty deposits are called "atheromas" or "plaques," which consist mainly of cholesterol and other fats, calcium and scar tissue, and damage the lining of arteries.

"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.

"Cardiovascular disease" or "cardiovascular disorder" refers to a group of conditions related to the heart, blood vessels, or the circulation. Examples of cardiovascular diseases or disorders include, but are not limited to, aneurysm, angina, arrhythmia, atherosclerosis, cerebrovascular disease (stroke), coronary heart disease, hypertension, dyslipidemia, hyperlipidemia, and hypercholesterolemia.

"Cardiometabolic disease, disorder or condition" are diseases, disorders and conditions concerning both the cardiovascular system and the metabolic system. Examples of cardiometabolic diseases or disorders include, but are not limited to, diabetes, obesity, insulin resistance and dyslipidemias.

"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.

"Cholesterol absorption inhibitor" means an agent that inhibits the absorption of exogenous cholesterol obtained from diet.

"Complementarity" means the capacity for pairing between nucleobases of a first nucleic acid and a second nucleic acid. In certain embodiments, complementarity between the first and second nucleic acid may be between two DNA strands, between two RNA strands, or between a DNA and an RNA strand. In certain embodiments, some of the nucleobases on one strand are matched to a complementary hydrogen bonding base on the other strand. In certain embodiments, all of the nucleobases on one strand are matched to a complementary hydrogen bonding base on the other strand. In certain embodiments, a first nucleic acid is an antisense compound and a second nucleic acid is a target nucleic acid. In certain such embodiments, an antisense oligonucleotide is a first nucleic acid and a target nucleic acid is a second nucleic acid.

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

"Coronary heart disease (CHD)" means a narrowing of the small blood vessels that supply blood and oxygen to the heart, which is often a result of atherosclerosis.

"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, reduced HDL-C, elevated triglycerides, and elevated small, dense LDL particles.

"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. Doses can be expressed as mg/kg or g/kg.

"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."

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

"High density lipoprotein-C (HDL-C)" means cholesterol associated with high density lipoprotein particles. Concentration of HDL-C in serum (or plasma) is typically quantified in mg/dL or nmol/L. "serum HDL-C" and "plasma HDL-C" mean HDL-C in serum and plasma, respectively.

"HMG-CoA reductase inhibitor" means an agent that acts through the inhibition of the enzyme HMG-CoA reductase, such as atorvastatin, rosuvastatin, fluvastatin, lovastatin, pravastatin, and simvastatin.

"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.

"Hypercholesterolemia" means a condition characterized by elevated cholesterol or circulating (plasma) cholesterol, LDL-cholesterol and VLDL-cholesterol, as per the guidelines of the Expert Panel Report of the National Cholesterol Educational Program (NCEP) of Detection, Evaluation of Treatment of high cholesterol in adults (see, Arch. Int. Med. (1988) 148, 36-39).

"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 a subject having a metabolic or cardiovascular disease" means identifying or selecting a subject having been diagnosed with a metabolic disease, a cardiovascular disease, or a metabolic syndrome; or, identifying or selecting a subject having any symptom of a metabolic disease, cardiovascular disease, or metabolic syndrome including, but not limited to, hypercholesterolemia, hyperglycemia, hyperlipidemia, hypertriglyceridemia, hypertension, increased insulin resistance, decreased insulin sensitivity, above normal body weight, and/or above normal body fat content 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) cholesterol, measuring serum or circulating (plasma) blood-glucose, measuring serum or circulating (plasma) triglycerides, measuring blood-pressure, measuring body fat content, measuring body weight, and the like.

"Identifying" or "selecting a diabetic subject" means identifying or selecting a subject having been identified as diabetic or identifying or selecting a subject having any symptom or physiological marker of diabetes (type 1 or type 2) such as, but not limited to, having a fasting glucose of at least 110 mg/dL, glycosuria, polyuria, polydipsia, increased insulin resistance, and/or decreased insulin sensitivity.

"Identifying" or "selecting an obese subject" means identifying or selecting a subject having been diagnosed as obese or identifying or selecting a subject with a BMI over 30 and/or a waist circumference of greater than 102 cm in men or greater than 88 cm in women.

"Identifying" or "selecting a subject having dyslipidemia" means identifying or selecting a subject diagnosed with 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.

"Identifying" or "selecting" a subject having increased adiposity" means identifying or selecting a subject having an increased amount of body fat (or adiposity) that includes concern for one or 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, computer 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.

"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 and does not necessarily indicate a total elimination of expression or activity.

"Insulin resistance" or "IR" is defined as the condition in which normal amounts of insulin are inadequate to produce a normal insulin response from cells, e.g., fat, muscle and/or 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" means a reduction in one or more lipids in a subject. Lipid-lowering can occur with one or more doses over time.

"Lipid-lowering agent" means an agent, for example, an AGPAT5-specific modulator, provided to a subject to achieve a lowering of lipids in the subject. For example, in certain embodiments, a lipid-lowering agent is provided to a subject to reduce one or more of apoB, apoC-III, total cholesterol, LDL-C, VLDL-C, IDL-C, non-HDL-C, triglycerides, small dense LDL particles, and Lp(a) in a subject.

"Lipid-lowering therapy" 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, apoC-III, total cholesterol, LDL-C, VLDL-C, IDL-C, non-HDL-C, triglycerides, small dense LDL particles, and Lp(a) in a subject.

"Lipoprotein", such as VLDL, LDL and HDL, refers to a group of proteins found in the serum, plasma and lymph and are important for lipid transport. The chemical composition of each lipoprotein differs in that the HDL has a higher proportion of protein versus lipid, whereas the VLDL has a lower proportion of protein versus lipid.

"Low density lipoprotein-cholesterol (LDL-C)" means cholesterol carried in low density lipoprotein particles. Concentration of LDL-C in serum (or plasma) is typically quantified in mg/dL or nmol/L. "Serum LDL-C" and "plasma LDL-C" mean LDL-C in the serum and plasma, respectively.

"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, family history of coronary heart disease, age, and other factors disclosed herein. In certain embodiments, major risk factors for Type 2 diabetes include, without limitation, obesity, sedentary lifestyle, hypertension, family history of diabetes, age, low HDL, high TG and other factors.

"Metabolic disorder" or "metabolic disease" 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 disorders include, but are not limited to, hyperglycemia, prediabetes, diabetes (type I and type 2), obesity, insulin resistance, metabolic syndrome, non-alcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), and dyslipidemia due to type 2 diabetes.

"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, one or more modified sugar moiety or modified nucleobase.

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

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

"Modified sugar" refers to a substitution or change from a natural sugar. Representative modified sugars include 2'-O-alkyl sugars (such as 2'-O-methoxyethyl sugars and 2'-O-methyl sugars), 2'-amino sugars, 2'-fluoro sugars, 4'-thio sugars, cyclopentyl or cyclohexyl sugar analogs, and bicyclic sugars, such as a locked nucleic acid ("LNA"), as well as sugars modified with a constrained ethyl, a 3'-fluoro-HNA, a 4'-$CH(CH_3)$-O-2' bridge, or a 4'- $(CH_2)_n$-O-2' bridge, wherein n is 1 or 2, but the term "modified sugar" should be understood to include any suitable replacement for a natural sugar in a nucleic acid sequence.

"Modulating" refers to changing or adjusting a feature in a cell, tissue, organ or organism. For example, modulating AGPAT5 mRNA can mean to increase or decrease the level of AGPAT5 mRNA and/or AGPAT5 protein in a cell, tissue, organ or organism. Modulating AGPAT5 mRNA and/or protein can lead to an increase or decrease in insulin resistance or insulin sensitivity in a cell, tissue, organ or organism. A "modulator" effects the change in the cell, tissue, organ or organism. For example, an AGPAT5 antisense oligonucleotide can be a modulator that increases or decreases the amount of AGPAT5 mRNA and/or AGPAT5 protein in a cell, tissue, organ or organism.

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

"MTP inhibitor" means an agent that inhibits the enzyme microsomal triglyceride transfer protein.

"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 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" or "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. It has been suggested that increased liver triglycerides lead to increased oxidative stress in hepatocytes of animals and humans, indicating a potential cause-and-effect relationship between hepatic triglyceride accumulation, oxidative stress, and the progression of hepatic steatosis to NASH (Browning and Horton, *J Clin Invest,* 2004, *114*, 147-152). Hypertriglyceridemia and hyperfattyacidemia can cause triglyceride accumulation in peripheral tissues (Shimamura et al., *Biochem Biophys Res Commun,* 2004, *322*, 1080-1085).

"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 by a manner other than through the digestive tract. Parenteral administration includes topical administration, 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 AGPAT5 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 or function 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. Certain of such carriers enable pharmaceutical compositions to be formulated for injection or infusion. For example, a pharmaceutically acceptable carrier can be a sterile aqueous solution.

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

"Target segment" means the sequence of nucleotides of a target nucleic acid to which one or more antisense compound is targeted. "5' target site" or "5' start site" refers to the 5'-most nucleotide of a target segment. "3' target site" or "3' stop 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 cardiometabolic disease, and may include 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 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 a 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 modulating a cardiometabolic disease, disorder or condition, or a symptom thereof, in an animal by administering a therapeutically effective amount of the compound or composition to the animal, wherein the compound or composition comprises an AGPAT5 modulator. Modulation of AGPAT5 can lead to a decrease of AGPAT5 mRNA and protein expression in order to treat, prevent, ameliorate or delay the cardiometabolic disease, disorder or condition, or a symptom thereof. In certain embodiments, the AGPAT5 modulator is an AGPAT5 specific inhibitor. In certain embodiments, AGPAT5 specific inhibitors are nucleic acids (including antisense compounds), peptides, antibodies, small molecules, and other agents capable of inhibiting the expression of AGPAT5 mRNA and/or AGPAT5 protein.

In certain embodiments disclosed herein, AGPAT5 has the murine sequence as set forth in GENBANK Accession No. NM_026792.3 (incorporated herein as SEQ ID NO: 1) and/or GENBANK Accession No. NT_039455.7 truncated from nucleotides 15844020 to 15885425 (incorporated herein as SEQ ID NO: 2). In certain embodiments disclosed herein, AGPAT5 has the human sequence as set forth in GENBANK Accession No. NM_018361.3 (incorporated herein as SEQ ID NO: 24), GENBANK Accession No. AK310545.1 (incorporated herein as SEQ ID NO: 25) and/or GENBANK Accession No. NT_023736.17 truncated from nucleotides 6554870 to 6610015 (incorporated herein as SEQ ID NO: 26).

Certain embodiments disclosed herein provide compounds or compositions comprising an AGPAT5 modulator. In certain embodiments, the AGPAT5 modulator is an AGPAT5 specific inhibitor. In certain embodiments, AGPAT5 specific inhibitor is a nucleic acid, polypeptide, antibody, small molecules, or other agent capable of inhibiting the expression of AGPAT5. In certain embodiments, the nucleic acid is an antisense compound or composition targeting AGPAT5. In certain embodiments, the antisense compound or composition is single stranded. In certain embodiments, the antisense compound or composition is double stranded. In certain embodiments, the antisense compound or composition targeting AGPAT5 is an oligonucleotide. In certain embodiments, the oligonucleotide is single stranded. In certain embodiments, the oligonucleotide is double stranded. In certain embodiments, the oligonucleotide is a modified oligonucleotide. In certain embodiments, the modified oligonucleotide is single stranded. In certain embodiments, the modified oligonucleotide is double stranded.

Certain embodiments disclosed herein provide an antisense compound or composition comprising a modified oligonucleotide that is 10 to 30 linked nucleosides in length targeted to AGPAT5. The AGPAT5 target can have a nucleobase sequence selected from any one of SEQ ID NOs: 1-2, 24-26. In certain embodiments, the nucleobase sequence of the modified oligonucleotide is at least 70%, 75%, 80%, 85%, 90%, 95% or 100% complementary to the nucleobase sequences recited in any one of SEQ ID NOs: 1-2, 24-26 as measured over the entirety of the modified oligonucleotide. In certain embodiments, the modified oligonucleotide comprises a nucleobase sequence comprising a portion of at least 8, at least 9, at least 10, at

least 11, at least 12, at least 13, at least 14, at least 15, or 16 contiguous nucleobases complementary to an equal length portion of SEQ ID NO: 1, 2, 24, 25 or 26.

Certain embodiments disclosed herein provide an antisense compound or composition comprising a modified oligonucleotide that is 10 to 30 linked nucleosides in length wherein the nucleobase sequence of the modified oligonucleotide comprises at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, or 16 contiguous nucleobases of a sequence recited in any one of SEQ ID NOs: 6-23.

Certain embodiments disclosed herein provide an antisense compound or composition comprising a modified oligonucleotide that is 10 to 30 linked nucleosides in length wherein the nucleobase sequence of the modified oligonucleotide consists of a sequence recited in any one of SEQ ID NOs: 6-23.

In certain embodiments, the modified oligonucleotide consists of 10 to 50, 10 to 30, 12 to 30, 13 to 24, 14 to 24, 15 to 30, 15 to 24, 15 to 20, 15 to 18, 16 to 30, 16 to 24, 16 to 20, 16 to 18, 18 to 24 or 19 to 22 linked nucleosides. 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 or 30 linked nucleosides or a range defined by any two of these values. In certain embodiments, the modified oligonucleotide is 16 linked nucleosides in length. In certain embodiments, the modified oligonucleotide is 20 linked nucleosides in length.

In certain embodiments, at least one internucleoside linkage of said 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 the modified oligonucleotide comprises a modified sugar. In certain embodiments, at least one modified sugar is a bicyclic sugar. In certain embodiments, at least one modified sugar comprises a 2'-O-methoxyethyl, a constrained ethyl, a 3'-fluoro-HNA, a 4'-$CH(CH_3)$-O-2' bridge or a 4'- $(CH_2)_n$-O-2' bridge, wherein n is 1 or 2.

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.

Certain embodiments disclosed herein provide an antisense compound or composition comprising a modified oligonucleotide with: 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, at least one internucleoside linkage is a phosphorothioate linkage. In certain embodiments, and at least one cytosine is a 5-methylcytosine. In certain embodiments, the modified oligonucleotide is 16 linked nucleosides in length. In certain embodiments, the modified oligonucleotide is 20 linked nucleosides in length.

In certain embodiments, the antisense compound or composition comprises a modified oligonucleotide consisting of 10 to 30 linked nucleosides and 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 and each nucleoside of each wing segment comprises a modified sugar. In certain embodiments, at least one internucleoside linkage is a phosphorothioate linkage. In certain embodiments, and at least one cytosine is a 5-methylcytosine. In certain embodiments, the modified oligonucleotide is 16 linked nucleosides in length. In certain embodiments, the modified oligonucleotide is 20 linked nucleosides in length.

Certain embodiments disclosed herein provide antisense compounds or compositions comprising a modified oligonucleotide consisting of 16 linked nucleosides, the modified oligonucleotide having a nucleobase sequence comprising at least 8 contiguous nucleobases of a nucleobase sequence selected from any of SEQ ID NOs: 6-23 and comprising: a) a gap segment consisting of ten 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 with each nucleoside of each wing segment comprising a 4'-$CH(CH_3)$-O-2' sugar. In certain embodiments, at least one internucleoside linkage is a phosphorothioate linkage. In certain embodiments, at least one cytosine is a 5-methylcytosine.

Certain embodiments disclosed herein provide antisense compounds or compositions comprising a modified oligonucleotide consisting of 16 linked nucleosides, the modified oligonucleotide having a nucleobase sequence selected from any of SEQ ID NOs: 6-23 and comprising: a) a gap segment consisting of ten 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 with each nucleoside of each wing segment comprising a 4'-$CH(CH_3)$-O-2' sugar. In certain embodiments, at least one internucleoside linkage is a phosphorothioate linkage. In certain embodiments, at least one cytosine is a 5-methylcytosine.

In certain embodiments, the antisense compounds or compositions disclosed herein comprise a modified oligonucleotide consisting of 16 linked nucleosides having a nucleobase sequence comprising at least 8 contiguous nucleobases complementary to an equal length portion of a sequence selected from any of SEQ ID NOs: 1-2, 24-26 wherein the modified oligonucleotide comprises: a) a gap segment consisting of ten 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 and each nucleoside of each wing segment comprises a modified sugar. In certain embodiments, at least one internucleoside linkage is a phosphorothioate linkage. In certain embodiments, at least one cytosine is a 5-methylcytosine.

In certain embodiments, the antisense compounds or compositions disclosed 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 a sequence selected from any of SEQ ID NOs: 1-2, 24-26 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 and each nucleoside of each wing segment comprises a modified sugar. In certain embodiments, at least one internucleoside linkage is a phosphorothioate linkage. In certain embodiments, at least one cytosine is a 5-methylcytosine.

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

Certain embodiments disclosed herein provide a method of reducing AGPAT5 expression in an animal comprising administering to the animal a compound or composition comprising an AGPAT5 specific inhibitor. In certain embodiments, the AGPAT5 specific inhibitor is a nucleic acid, peptide, antibody, small molecule or other agent capable of inhibiting the expression of AGPAT5. In certain embodiments, the AGPAT5 specific inhibitor comprises an antisense compound. In certain embodiments, the AGPAT5 specific inhibitor comprises a modified oligonucleotide.

Certain embodiments disclosed herein provide a method of reducing insulin resistance in an animal comprising administering to the animal a compound or composition comprising an AGPAT5 specific inhibitor. In certain embodiments, the AGPAT5 specific inhibitor is a nucleic acid, peptide, antibody, small molecule or other agent capable of inhibiting the expression of AGPAT5. In certain embodiments, the AGPAT5 specific inhibitor comprises an antisense compound. In certain embodiments, the AGPAT5 specific inhibitor comprises a modified oligonucleotide.

Certain embodiments disclosed herein provide a method of increasing insulin sensitivity in an animal comprising administering to the animal a compound or composition comprising an AGPAT5 specific inhibitor. In certain embodiments, the AGPAT5 specific inhibitor is a nucleic acid, peptide, antibody, small molecule or other agent capable of inhibiting the expression of AGPAT5. In certain embodiments, the AGPAT5 specific inhibitor comprises an antisense compound. In certain embodiments, the AGPAT5 specific inhibitor comprises a modified oligonucleotide.

Certain embodiments disclosed herein provide a method of reducing liver triglyceride levels in an animal comprising administering to the animal a compound or composition comprising an AGPAT5 specific inhibitor. In certain embodiments, the AGPAT5 specific inhibitor is a nucleic acid, peptide, antibody, small molecule or other agent capable of inhibiting the expression of AGPAT5. In certain embodiments, the AGPAT5 specific inhibitor comprises an antisense compound. In certain embodiments, the AGPAT5 specific inhibitor comprises a modified oligonucleotide.

Certain embodiments disclosed herein provide a method of reducing insulin levels in an animal comprising administering to the animal a compound or composition comprising an AGPAT5 specific inhibitor. In certain embodiments, the AGPAT5 specific inhibitor is a nucleic acid, peptide, antibody, small molecule or other agent capable of inhibiting the expression of AGPAT5. In certain embodiments, the AGPAT5 specific inhibitor comprises an antisense compound. In certain embodiments, the AGPAT5 specific inhibitor comprises a modified oligonucleotide.

Certain embodiments disclosed herein provide a method of increasing glucose tolerance in an animal comprising administering to the animal a compound or composition comprising an AGPAT5 specific inhibitor. In certain embodiments, the AGPAT5 specific inhibitor is a nucleic acid, peptide, antibody, small molecule or other agent capable of inhibiting the expression of AGPAT5. In certain embodiments, the AGPAT5 specific inhibitor comprises an antisense compound. In certain embodiments, the AGPAT5 specific inhibitor comprises a modified oligonucleotide.

Certain embodiments disclosed herein provide a method of increasing glucose clearance in an animal comprising administering to the animal a compound or composition comprising an AGPAT5 specific inhibitor. In certain embodiments, the AGPAT5 specific inhibitor is a nucleic acid, peptide, antibody, small molecule or other agent capable of inhibiting the expression of AGPAT5. In certain embodiments, the AGPAT5 specific inhibitor comprises an antisense compound. In certain embodiments, the AGPAT5 specific inhibitor comprises a modified oligonucleotide.

Certain embodiments disclosed herein provide a method of reducing the ratio of fat to body weight in an animal comprising administering to the animal a compound or composition comprising an AGPAT5 specific inhibitor. In certain embodiments, the AGPAT5 specific inhibitor is a nucleic acid, peptide, antibody, small molecule or other agent capable of inhibiting the expression of AGPAT5. In certain embodiments, the AGPAT5 specific inhibitor comprises an antisense compound. In certain embodiments, the AGPAT5 specific inhibitor comprises a modified oligonucleotide.

Certain embodiments disclosed herein provide a method of ameliorating metabolic and/or cardiovascular disease in an animal comprising administering to the animal a therapeutically effective amount of a compound or composition comprising an AGPAT5 specific inhibitor. In certain embodiments, the AGPAT5 specific inhibitor is a nucleic acid, peptide, antibody, small molecule or other agent capable of inhibiting the expression of AGPAT5. In certain embodiments, the AGPAT5 specific inhibitor comprises an antisense compound. In certain embodiments, the AGPAT5 specific inhibitor comprises a modified oligonucleotide. In certain embodiments, the therapeutically effective amount of the compound or composition administered to the animal reduces insulin resistance in a diabetic or non-diabetic animal.

Certain embodiments disclosed herein provide a method for treating an animal at risk for a metabolic and/or cardiovascular disease comprising administering to said animal a therapeutically effective amount of a compound or composition comprising an AGPAT5 specific inhibitor. In certain embodiments, the AGPAT5 specific inhibitor is a nucleic acid, peptide, antibody, small molecule or other agent capable of inhibiting the

expression of AGPAT5. In certain embodiments, the AGPAT5 specific inhibitor comprises an antisense compound. In certain embodiments, the AGPAT5 specific inhibitor comprises a modified oligonucleotide. In certain embodiments, the therapeutically effective amount of the compound or composition administered to the animal reduces insulin resistance in a pre-diabetic or non-diabetic animal.

In certain embodiments, the metabolic and/or cardiovascular disease is obesity, diabetes, insulin resistance, dyslipidemia, non-alcoholic fatty liver disease (NAFLD), hypertension, hyperglycemia, or metabolic syndrome, or a combination thereof. In certain embodiments, the NAFLD is hepatic steatosis or steatohepatitis. In certain embodiments, the diabetes is type 2 diabetes or type 2 diabetes with dyslipidemia.

In certain embodiments, administering the compound or composition disclosed herein reduces triglyceride levels, insulin levels, insulin resistance levels, glucose levels or a combination thereof. In certain embodiments, the levels are independently reduced by at least 5%, at least 10%, at least 20%, at least 30%, at least 35%, or at least 40%.

In certain embodiments, administering the compound or composition disclosed herein increases glucose tolerance, glucose clearance and/or insulin sensitivity. In certain embodiments, glucose tolerance, glucose clearance and/or insulin sensitivity is increased by at least 5%, at least 10%, at least 20%, at least 30%, at least 35%, or at least 40%.

Certain embodiments provide uses of the compounds and compositions described herein for inhibiting AGPAT5 expression. In certain embodiments, the compounds or compositions inhibit AGPAT5 by at least 5%, at least 10%, at least 20%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. In a preferred embodiment, an antisense compound comprising a modified oligonucleotide decreases AGPAT5 by at least 50%. In a preferred embodiment, an antisense compound comprising a modified oligonucleotide decreases AGPAT5 by at least 55%. In a preferred embodiment an antisense compound comprising a modified oligonucleotide decreases AGPAT5 by at least 60%. In a preferred embodiment, an antisense compound comprising a modified oligonucleotide decreases AGPAT5 by at least 65%. In a preferred embodiment, an antisense compound comprising a modified oligonucleotide decreases AGPAT5 by at least 70%. In a preferred embodiment, an antisense compound comprising a modified oligonucleotide decreases AGPAT5 by at least 75%. In a preferred embodiment, an antisense compound comprising a modified oligonucleotide decreases AGPAT5 by at least 80%. In a preferred embodiment, an antisense compound comprising a modified oligonucleotide decreases AGPAT5 by at least 85%. In a preferred embodiment, an antisense compound comprising a modified oligonucleotide decreases AGPAT5 by at least 90%. In a preferred embodiment, an antisense compound comprising a modified oligonucleotide decreases AGPAT5 by at least 95%.

Certain embodiments provide uses of the compounds and compositions described herein for use in therapy. In certain embodiments, the therapy is used in treating, preventing, delaying the onset or slowing progression of a disease related to elevated AGPAT5. In certain embodiments, the disease is a cardiovascular

and/or metabolic disease, disorder or condition, or symptom or marker thereof. In certain embodiments, the metabolic and/or cardiovascular disease includes, but is not limited to, obesity, diabetes, insulin resistance, dyslipidemia, hyperglycemia, non-alcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), metabolic syndrome, high blood pressure, or a combination thereof. The dyslipidemia can be hypertriglyceridemia. The diabetes can be type 2 diabetes or type 2 diabetes with dyslipidemia.

In certain embodiments, the compounds or compositions disclosed herein further comprise a conjugate group. In certain embodiments, the conjugate group is a carbohydrate group. In certain embodiments, the conjugate group is a GalNAc group.

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

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

In certain embodiments, the animal is a human.

In certain embodiments, administration comprises parenteral administration. In certain embodiments, parenteral administration comprises subcutaneous administration.

In certain embodiments, the compounds or compositions disclosed herein are designated as a first agent and the methods or uses disclosed herein further comprise administering a second agent. In certain embodiments, the first agent and the second agent are co-administered. In certain embodiments the first agent and the second agent are co-administered sequentially or concomitantly.

In certain embodiments, the second agent is 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 certain embodiments, the second agent is a lipid-lowering therapy. In certain embodiments the lipid lowering therapy can include, but is not limited to, a therapeutic lifestyle change, HMG-CoA reductase inhibitor, cholesterol absorption inhibitor, MTP inhibitor (e.g., a small molecule, polypeptide, antibody or antisense compound targeted to MTP), ApoB inhibitor (e.g., a small molecule, polypeptide, antibody or antisense compound targeted to ApoB), ApoC3 inhibitor (e.g., a small molecule, polypeptide, antibody or antisense compound targeted to ApoC3), PCSK9 inhibitor (e.g., a small molecule, polypeptide, antibody or antisense compound targeted to PCSK9), CETP inhibitor (e.g., a small molecule, polypeptide, antibody or

antisense compound targeted to CETP), fibrate, beneficial oil (e.g., krill or fish oils (e.g., Vascepa$^{R}$), flaxseed oil, or other oils rich in omega-3 fatty acids such as α-linolenic acid (ALA), docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA)), or any combination thereof. The HMG-CoA reductase inhibitor can be atorvastatin, rosuvastatin, fluvastatin, lovastatin, pravastatin, or simvastatin. The cholesterol absorption inhibitor can be ezetimibe. The fibrate can be fenofibrate, bezafibrate, ciprofibrate, clofibrate, gemfibrozil and the like.

In certain embodiments, use of a compound or composition disclosed herein results in a reduction of lipid levels (e.g., triglyceride levels), insulin resistance levels, glucose levels or a combination thereof. One or more of the levels can be independently reduced by at least 5%, at least 10%, at least 20%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. Administering the compound can result in improved insulin sensitivity (e.g., hepatic insulin sensitivity), glucose clearance and/or glucose tolerance by at least 5%, at least 10%, at least 20%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95%. Administering the compound disclosed herein can result in a reduction in insulin, insulin resistance, obesity, glucose, lipids, glucose resistance, cholesterol, or improvement in insulin sensitivity or any combination thereof.

Certain embodiments provide the use of a compound or composition as described herein in the manufacture of a medicament for treating, ameliorating, delaying or preventing one or more diseases, disorders, conditions, symptoms or physiological markers associated with AGPAT5. In certain embodiments, the compound or composition as described herein is used in the manufacture of a medicament for treating, ameliorating, delaying or preventing one or more of a metabolic disease or a cardiovascular disease, or a symptom or physiological marker thereof.

Certain embodiments provide a kit for treating, preventing, delaying, or ameliorating one or more of a metabolic disease or a cardiovascular disease as described herein wherein the kit comprises: a) a compound or composition as described herein; and optionally b) an additional agent or therapy as described herein. The kit can further include instructions or a label for using the kit to treat, prevent, or ameliorate one or more of a metabolic disease or a cardiovascular disease.

*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 can 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 AGPAT5 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 80, 12 to 50, 12 to 30, 15 to 30, 18 to 24, 19 to 22, 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 can have two or more nucleosides deleted from the 5' end, or alternatively can have two or more nucleosides deleted from the 3' end. Alternatively, the deleted nucleosides can be dispersed throughout the modified oligonucleotide, for example, in an antisense compound having one or more nucleosides deleted from the 5' end and one or more nucleoside deleted from the 3' end.

When a single additional nucleoside is present in a lengthened oligonucleotide, the additional nucleoside can be located at the 5', 3' end or central portion of the oligonucleotide. When two or more additional nucleosides are present, the added nucleosides can 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) or the central portion, of the oligonucleotide. Alternatively, the added nucleosides can be dispersed throughout the antisense compound, for example, in an oligonucleotide having one or more nucleosides added to the 5' end, one or more nucleosides added to the 3' end, and/or one or more nucleosides added to the central portion.

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.

*Certain Antisense Compound Motifs and Mechanisms*

In certain embodiments, antisense compounds 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 confer another desired property e.g., serve as a substrate for the cellular endonuclease RNase H, which cleaves the RNA strand of an RNA:DNA duplex.

Antisense activity may result from any mechanism involving the hybridization of the antisense compound (e.g., oligonucleotide) with a target nucleic acid, wherein the hybridization ultimately results in a biological effect. In certain embodiments, the amount and/or activity of the target nucleic acid is modulated. In certain embodiments, the amount and/or activity of the target nucleic acid is reduced. In certain embodiments, hybridization of the antisense compound to the target nucleic acid ultimately results in target nucleic acid degradation. In certain embodiments, hybridization of the antisense compound to the target nucleic acid does not result in target nucleic acid degradation. In certain such embodiments, the presence of the antisense compound hybridized with the target nucleic acid (occupancy) results in a modulation of antisense activity. In certain embodiments, antisense compounds having a particular chemical motif or pattern of chemical modifications are particularly suited to exploit one or more mechanisms. In certain embodiments, antisense compounds function through more than one mechanism and/or through mechanisms that have not been elucidated. Accordingly, the antisense compounds described herein are not limited by particular mechanism.

Antisense mechanisms include, without limitation, RNase H mediated antisense; RNAi mechanisms, which utilize the RISC pathway and include, without limitation, siRNA, ssRNA and microRNA mechanisms;

and occupancy based mechanisms. Certain antisense compounds may act through more than one such mechanism and/or through additional mechanisms.

*RNase H-Mediated Antisense*

In certain embodiments, antisense activity results at least in part from degradation of target RNA by RNase H. RNase H is a cellular endonuclease that cleaves the RNA strand of an RNA:DNA duplex. It is known in the art that single-stranded antisense compounds which are "DNA-like" elicit RNase H activity in mammalian cells. Accordingly, antisense compounds comprising at least a portion of DNA or DNA-like nucleosides may activate RNase H, resulting in cleavage of the target nucleic acid. In certain embodiments, antisense compounds that utilize RNase H comprise one or more modified nucleosides. In certain embodiments, such antisense compounds comprise at least one block of 1-8 modified nucleosides. In certain such embodiments, the modified nucleosides do not support RNase H activity. In certain embodiments, such antisense compounds are gapmers, as described herein. In certain such embodiments, the gap of the gapmer comprises DNA nucleosides. In certain such embodiments, the gap of the gapmer comprises DNA-like nucleosides. In certain such embodiments, the gap of the gapmer comprises DNA nucleosides and DNA-like nucleosides.

Certain 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, nucleosides in the wings may include several modified sugar moieties, including, for example 2'-MOE and bicyclic sugar moieties such as constrained ethyl or LNA. In certain embodiments, wings may include several modified and unmodified sugar moieties. In certain embodiments, wings may include various combinations of 2'-MOE nucleosides, bicyclic sugar moieties such as constrained ethyl nucleosides or LNA 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, the antisense compound targeted to an AGPAT5 nucleic acid has a gapmer motif in which the gap consists of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 linked nucleosides.

In certain embodiments, the antisense oligonucleotide has a sugar motif described by Formula A as follows: $(J)_m$-$(B)_n$-$(J)_p$-$(B)_r$-$(A)_t$-$(D)_g$-$(A)_v$-$(B)_w$-$(J)_x$-$(B)_y$-$(J)_z$

wherein:

each A is independently a 2'-substituted nucleoside;

each B is independently a bicyclic nucleoside;

each J is independently either a 2'-substituted nucleoside or a 2'-deoxynucleoside;

each D is a 2'-deoxynucleoside;

m is 0-4; n is 0-2; p is 0-2; r is 0-2; t is 0-2; v is 0-2; w is 0-4; x is 0-2; y is 0-2; z is 0-4; g is 6-14; provided that:

at least one of m, n, and r is other than 0;

at least one of w and y is other than 0;

the sum of m, n, p, r, and t is from 2 to 5; and

the sum of v, w, x, y, and z is from 2 to 5.

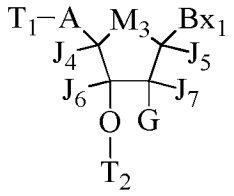
*RNAi Compounds*

In certain embodiments, antisense compounds are interfering RNA compounds (RNAi), which include double-stranded RNA compounds (also referred to as short-interfering RNA or siRNA) and single-stranded RNAi compounds (or ssRNA). Such compounds work at least in part through the RISC pathway to degrade and/or sequester a target nucleic acid (thus, include microRNA/microRNA-mimic compounds). In certain embodiments, antisense compounds comprise modifications that make them particularly suited for such mechanisms.

*i. ssRNA compounds*

In certain embodiments, antisense compounds including those particularly suited for use as single-stranded RNAi compounds (ssRNA) comprise a modified 5'-terminal end. In certain such embodiments, the 5'-terminal end comprises a modified phosphate moiety. In certain embodiments, such modified phosphate is stabilized (e.g., resistant to degradation/cleavage compared to unmodified 5'-phosphate). In certain embodiments, such 5'-terminal nucleosides stabilize the 5'-phosphorous moiety. Certain modified 5'-terminal nucleosides may be found in the art, for example in WO/2011/139702.

In certain embodiments, the 5'-nucleoside of an ssRNA compound has Formula IIc:



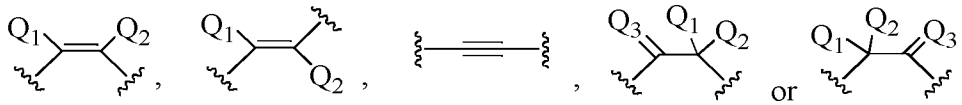
IIc

wherein:

$T_1$ is an optionally protected phosphorus moiety;

$T_2$ is an internucleoside linking group linking the compound of Formula IIc to the oligomeric compound;

A has one of the formulas:



$Q_1$ and $Q_2$ are each, independently, H, halogen, $C_1$-$C_6$ alkyl, substituted $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkoxy, substituted $C_1$-$C_6$ alkoxy, $C_2$-$C_6$ alkenyl, substituted $C_2$-$C_6$ alkenyl, $C_2$-$C_6$ alkynyl, substituted $C_2$-$C_6$ alkynyl or $N(R_3)(R_4)$;

$Q_3$ is O, S, $N(R_5)$ or $C(R_6)(R_7)$;

each $R_3$, $R_4$ $R_5$, $R_6$ and $R_7$ is, independently, H, $C_1$-$C_6$ alkyl, substituted $C_1$-$C_6$ alkyl or $C_1$-$C_6$ alkoxy;

$M_3$ is O, S, $NR_{14}$, $C(R_{15})(R_{16})$, $C(R_{15})(R_{16})C(R_{17})(R_{18})$, $C(R_{15})=C(R_{17})$, $OC(R_{15})(R_{16})$ or $OC(R_{15})(Bx_2)$;

$R_{14}$ is H, $C_1$-$C_6$ alkyl, substituted $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkoxy, substituted $C_1$-$C_6$ alkoxy, $C_2$-$C_6$ alkenyl, substituted $C_2$-$C_6$ alkenyl, $C_2$-$C_6$ alkynyl or substituted $C_2$-$C_6$ alkynyl;

$R_{15}$, $R_{16}$, $R_{17}$ and $R_{18}$ are each, independently, H, halogen, $C_1$-$C_6$ alkyl, substituted $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkoxy, substituted $C_1$-$C_6$ alkoxy, $C_2$-$C_6$ alkenyl, substituted $C_2$-$C_6$ alkenyl, $C_2$-$C_6$ alkynyl or substituted $C_2$-$C_6$ alkynyl;

$Bx_1$ is a heterocyclic base moiety;

or if $Bx_2$ is present then $Bx_2$ is a heterocyclic base moiety and $Bx_1$ is H, halogen, $C_1$-$C_6$ alkyl, substituted $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkoxy, substituted $C_1$-$C_6$ alkoxy, $C_2$-$C_6$ alkenyl, substituted $C_2$-$C_6$ alkenyl, $C_2$-$C_6$ alkynyl or substituted $C_2$-$C_6$ alkynyl;

$J_4$, $J_5$, $J_6$ and $J_7$ are each, independently, H, halogen, $C_1$-$C_6$ alkyl, substituted $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkoxy, substituted $C_1$-$C_6$ alkoxy, $C_2$-$C_6$ alkenyl, substituted $C_2$-$C_6$ alkenyl, $C_2$-$C_6$ alkynyl or substituted $C_2$-$C_6$ alkynyl;

or $J_4$ forms a bridge with one of $J_5$ or $J_7$ wherein said bridge comprises from 1 to 3 linked biradical groups selected from O, S, $NR_{19}$, $C(R_{20})(R_{21})$, $C(R_{20})=C(R_{21})$, $C[=C(R_{20})(R_{21})]$ and C(=O) and the other two of $J_5$, $J_6$ and $J_7$ are each, independently, H, halogen, $C_1$-$C_6$ alkyl, substituted $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkoxy, substituted $C_1$-$C_6$ alkoxy, $C_2$-$C_6$ alkenyl, substituted $C_2$-$C_6$ alkenyl, $C_2$-$C_6$ alkynyl or substituted $C_2$-$C_6$ alkynyl;

each $R_{19}$, $R_{20}$ and $R_{21}$ is, independently, H, $C_1$-$C_6$ alkyl, substituted $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkoxy, substituted $C_1$-$C_6$ alkoxy, $C_2$-$C_6$ alkenyl, substituted $C_2$-$C_6$ alkenyl, $C_2$-$C_6$ alkynyl or substituted $C_2$-$C_6$ alkynyl;

G is H, OH, halogen or $O\text{-}[C(R_8)(R_9)]_n\text{-}[(C=O)_m\text{-}X_1]_j\text{-}Z$;

each $R_8$ and $R_9$ is, independently, H, halogen, $C_1$-$C_6$ alkyl or substituted $C_1$-$C_6$ alkyl;

$X_1$ is O, S or $N(E_1)$;

Z is 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, substituted $C_2$-$C_6$ alkynyl or $N(E_2)(E_3)$;

$E_1$, $E_2$ and $E_3$ are each, independently, H, $C_1$-$C_6$ alkyl or substituted $C_1$-$C_6$ alkyl;

n is from 1 to about 6;

m is 0 or 1;

j is 0 or 1;

each substituted group comprises one or more optionally protected substituent groups independently selected from halogen, $OJ_1$, $N(J_1)(J_2)$, $=NJ_1$, $SJ_1$, $N_3$, CN, $OC(=X_2)J_1$, $OC(=X_2)N(J_1)(J_2)$ and $C(=X_2)N(J_1)(J_2)$;

$X_2$ is O, S or $NJ_3$;

each $J_1$, $J_2$ and $J_3$ is, independently, H or $C_1$-$C_6$ alkyl;

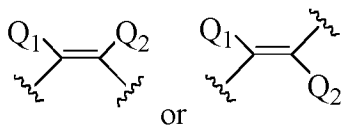
when j is 1 then Z is other than halogen or $N(E_2)(E_3)$; and

wherein said oligomeric compound comprises from 8 to 40 monomeric subunits and is hybridizable to at least a portion of a target nucleic acid.

In certain embodiments, $M_3$ is O, CH=CH, $OCH_2$ or $OC(H)(Bx_2)$. In certain embodiments, $M_3$ is O.

In certain embodiments, $J_4$, $J_5$, $J_6$ and $J_7$ are each H. In certain embodiments, $J_4$ forms a bridge with one of $J_5$ or $J_7$.

In certain embodiments, A has one of the formulas:



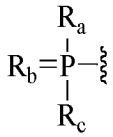

or

wherein:

$Q_1$ and $Q_2$ are each, independently, H, halogen, $C_1$-$C_6$ alkyl, substituted $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkoxy or substituted $C_1$-$C_6$ alkoxy. In certain embodiments, $Q_1$ and $Q_2$ are each H. In certain embodiments, $Q_1$ and $Q_2$

are each, independently, H or halogen. In certain embodiments, $Q_1$ and $Q_2$ is H and the other of $Q_1$ and $Q_2$ is F, $CH_3$ or $OCH_3$.

In certain embodiments, $T_1$ has the formula:



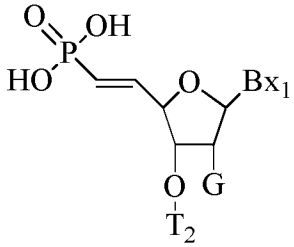
wherein:

$R_a$ and $R_c$ are each, independently, protected hydroxyl, protected thiol, $C_1$-$C_6$ alkyl, substituted $C_1$-$C_6$ alkyl, $C_1$-$C_6$ alkoxy, substituted $C_1$-$C_6$ alkoxy, protected amino or substituted amino; and

$R_b$ is O or S. In certain embodiments, $R_b$ is O and $R_a$ and $R_c$ are each, independently, $OCH_3$, $OCH_2CH_3$ or $CH(CH_3)_2$.

In certain embodiments, G is halogen, $OCH_3$, $OCH_2F$, $OCHF_2$, $OCF_3$, $OCH_2CH_3$, $O(CH_2)_2F$, $OCH_2CHF_2$, $OCH_2CF_3$, $OCH_2$-$CH=CH_2$, $O(CH_2)_2$-$OCH_3$, $O(CH_2)_2$-$SCH_3$, $O(CH_2)_2$-$OCF_3$, $O(CH_2)_3$-$N(R_{10})(R_{11})$, $O(CH_2)_2$-$ON(R_{10})(R_{11})$, $O(CH_2)_2$-$O(CH_2)_2$-$N(R_{10})(R_{11})$, $OCH_2C(=O)$-$N(R_{10})(R_{11})$, $OCH_2C(=O)$-$N(R_{12})$-$(CH_2)_2$-$N(R_{10})(R_{11})$ or $O(CH_2)_2$-$N(R_{12})$-$C(=NR_{13})[N(R_{10})(R_{11})]$ wherein $R_{10}$, $R_{11}$, $R_{12}$ and $R_{13}$ are each, independently, H or $C_1$-$C_6$ alkyl. In certain embodiments, G is halogen, $OCH_3$, $OCF_3$, $OCH_2CH_3$, $OCH_2CF_3$, $OCH_2$-$CH=CH_2$, $O(CH_2)_2$-$OCH_3$, $O(CH_2)_2$-$O(CH_2)_2$-$N(CH_3)_2$, $OCH_2C(=O)$-$N(H)CH_3$, $OCH_2C(=O)$-$N(H)$-$(CH_2)_2$-$N(CH_3)_2$ or $OCH_2$-$N(H)$-$C(=NH)NH_2$. In certain embodiments, G is F, $OCH_3$ or $O(CH_2)_2$-$OCH_3$. In certain embodiments, G is $O(CH_2)_2$-$OCH_3$.

In certain embodiments, the 5'-terminal nucleoside has Formula IIe:



IIe

In certain embodiments, antisense compounds, including those particularly suitable for ssRNA comprise one or more type of modified sugar moieties and/or naturally occurring sugar moieties arranged along an oligonucleotide or region thereof in a defined pattern or sugar modification motif. Such motifs may include any of the sugar modifications discussed herein and/or other known sugar modifications.

In certain embodiments, the oligonucleotides comprise or consist of a region having uniform sugar modifications. In certain such embodiments, each nucleoside of the region comprises the same RNA-like sugar modification. In certain embodiments, each nucleoside of the region is a 2'-F nucleoside. In certain embodiments, each nucleoside of the region is a 2'-OMe nucleoside. In certain embodiments, each

nucleoside of the region is a 2'-MOE nucleoside. In certain embodiments, each nucleoside of the region is a cEt nucleoside. In certain embodiments, each nucleoside of the region is an LNA nucleoside. In certain embodiments, the uniform region constitutes all or essentially all of the oligonucleotide. In certain embodiments, the region constitutes the entire oligonucleotide except for 1-4 terminal nucleosides.

In certain embodiments, oligonucleotides comprise one or more regions of alternating sugar modifications, wherein the nucleosides alternate between nucleotides having a sugar modification of a first type and nucleotides having a sugar modification of a second type. In certain embodiments, nucleosides of both types are RNA-like nucleosides. In certain embodiments the alternating nucleosides are selected from: 2'-OMe, 2'-F, 2'-MOE, LNA, and cEt. In certain embodiments, the alternating modifications are 2'-F and 2'-OMe. Such regions may be contiguous or may be interupted by differently modified nucleosides or conjugated nucleosides.

In certain embodiments, the alternating region of alternating modifications each consist of a single nucleoside (i.e., the patern is $(AB)_xA_y$ wheren A is a nucleoside having a sugar modification of a first type and B is a nucleoside having a sugar modification of a second type; x is 1-20 and y is 0 or 1). In certain embodiments, one or more alternating regions in an alternating motif includes more than a single nucleoside of a type. For example, oligonucleotides may include one or more regions of any of the following nucleoside motifs:

AABBAA;

ABBABB;

AABAAB;

ABBABAABB;

ABABAA;

AABABAB;

ABABAA;

ABBAABBABABAA;

BABBAABBABABAA; or

ABABBAABBABABAA;

wherein A is a nucleoside of a first type and B is a nucleoside of a second type. In certain embodiments, A and B are each selected from 2'-F, 2'-OMe, BNA, and MOE.

In certain embodiments, oligonucleotides having such an alternating motif also comprise a modified 5' terminal nucleoside, such as those of formula IIc or IIe.

In certain embodiments, oligonucleotides comprise a region having a 2-2-3 motif. Such regions comprises the following motif:

$-(A)_2-(B)_x-(A)_2-(C)_y-(A)_3-$

wherein: A is a first type of modifed nucleoside;

B and C, are nucleosides that are differently modified than A, however, B and C may have the same or different modifications as one another;

x and y are from 1 to 15.

In certain embodiments, A is a 2'-OMe modified nucleoside. In certain embodiments, B and C are both 2'-F modified nucleosides. In certain embodiments, A is a 2'-OMe modified nucleoside and B and C are both 2'-F modified nucleosides.

In certain embodiments, oligonucleosides have the following sugar motif:

5'- (Q)- $(AB)_xA_y$-$(D)_z$

wherein:

Q is a nucleoside comprising a stabilized phosphate moiety. In certain embodiments, Q is a nucleoside having Formula IIc or IIe;

A is a first type of modifed nucleoside;

B is a second type of modified nucleoside;

D is a modified nucleoside comprising a modification different from the nucleoside adjacent to it. Thus, if y is 0, then D must be differently modified than B and if y is 1, then D must be differently modified than A. In certain embodiments, D differs from both A and B.

X is 5-15;

Y is 0 or 1;

Z is 0-4.

In certain embodiments, oligonucleosides have the following sugar motif:

5'- (Q)- $(A)_x$-$(D)_z$

wherein:

Q is a nucleoside comprising a stabilized phosphate moiety. In certain embodiments, Q is a nucleoside having Formula IIc or IIe;

A is a first type of modifed nucleoside;

D is a modified nucleoside comprising a modification different from A.

X is 11-30;

Z is 0-4.

In certain embodiments A, B, C, and D in the above motifs are selected from: 2'-OMe, 2'-F, 2'-MOE, LNA, and cEt. In certain embodiments, D represents terminal nucleosides. In certain embodiments, such terminal nucleosides are not designed to hybridize to the target nucleic acid (though one or more might hybridize by chance). In certain embodiments, the nucleobase of each D nucleoside is adenine, regardless of the identity of the nucleobase at the corresponding position of the target nucleic acid. In certain embodiments the nucleobase of each D nucleoside is thymine.

In certain embodiments, antisense compounds, including those particularly suited for use as ssRNA comprise modified internucleoside linkages arranged along the oligonucleotide or region thereof in a defined pattern or modified internucleoside linkage motif. In certain embodiments, oligonucleotides comprise a region having an alternating internucleoside linkage motif. In certain embodiments, oligonucleotides comprise a region of uniformly modified internucleoside linkages. In certain such embodiments, the oligonucleotide comprises a region that is uniformly linked by phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide is uniformly linked by phosphorothioate internucleoside linkages. In certain embodiments, each internucleoside linkage of the oligonucleotide is selected from phosphodiester and phosphorothioate. In certain embodiments, each internucleoside linkage of the oligonucleotide is selected from phosphodiester and phosphorothioate and at least one internucleoside linkage is phosphorothioate.

In certain embodiments, the oligonucleotide comprises at least 6 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least 8 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least 10 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 6 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 8 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 10 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least one 12 consecutive phosphorothioate internucleoside linkages. In certain such embodiments, at least one such block is located at the 3' end of the oligonucleotide. In certain such embodiments, at least one such block is located within 3 nucleosides of the 3' end of the oligonucleotide.

Oligonucleotides having any of the various sugar motifs described herein, may have any linkage motif. For example, the oligonucleotides, including but not limited to those described above, may have a linkage motif selected from non-limiting the table below:

| 5' most linkage | Central region | 3'-region |
|---|---|---|
| PS | Alternating PO/PS | 6 PS |
| PS | Alternating PO/PS | 7 PS |
| PS | Alternating PO/PS | 8 PS |

*ii. siRNA compounds*

In certain embodiments, antisense compounds are double-stranded RNAi compounds (siRNA). In such embodiments, one or both strands may comprise any modification motif described above for ssRNA. In

certain embodiments, ssRNA compounds may be unmodified RNA. In certain embodiments, siRNA compounds may comprise unmodified RNA nucleosides, but modified internucleoside linkages.

Several embodiments relate to double-stranded compositions wherein each strand comprises a motif defined by the location of one or more modified or unmodified nucleosides. In certain embodiments, compositions are provided comprising a first and a second oligomeric compound that are fully or at least partially hybridized to form a duplex region and further comprising a region that is complementary to and hybridizes to a nucleic acid target. It is suitable that such a composition comprise a first oligomeric compound that is an antisense strand having full or partial complementarity to a nucleic acid target and a second oligomeric compound that is a sense strand having one or more regions of complementarity to and forming at least one duplex region with the first oligomeric compound.

The compositions of several embodiments modulate gene expression by hybridizing to a nucleic acid target resulting in loss of its normal function. In some embodiments, the target nucleic acid is AGPAT5. In certain embodiment, the degradation of the targeted AGPAT5 is facilitated by an activated RISC complex that is formed with compositions disclosed herein.

Several embodiments are directed to double-stranded compositions wherein one of the strands is useful in, for example, influencing the preferential loading of the opposite strand into the RISC (or cleavage) complex. The compositions are useful for targeting selected nucleic acid molecules and modulating the expression of one or more genes. In some embodiments, the compositions of the present invention hybridize to a portion of a target RNA resulting in loss of normal function of the target RNA.

Certain embodiments are drawn to double-stranded compositions wherein both the strands comprises a hemimer motif, a fully modified motif, a positionally modified motif or an alternating motif. Each strand of the compositions of the present invention can be modified to fulfil a particular role in for example the siRNA pathway. Using a different motif in each strand or the same motif with different chemical modifications in each strand permits targeting the antisense strand for the RISC complex while inhibiting the incorporation of the sense strand. Within this model, each strand can be independently modified such that it is enhanced for its particular role. The antisense strand can be modified at the 5'-end to enhance its role in one region of the RISC while the 3'-end can be modified differentially to enhance its role in a different region of the RISC.

The double-stranded oligonucleotide molecules can be a double-stranded polynucleotide molecule comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The double-stranded oligonucleotide molecules can be assembled from two separate oligonucleotides, where one strand is the sense strand and the other is the antisense strand, wherein the antisense and sense strands are self-complementary (i.e. each strand comprises nucleotide sequence that is complementary to nucleotide sequence in the other strand; such as where the antisense strand and sense strand form a duplex or double-stranded structure, for example wherein the double-stranded region is about

15 to about 30, e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 base pairs; the antisense strand comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof (e.g., about 15 to about 25 or more nucleotides of the double-stranded oligonucleotide molecule are complementary to the target nucleic acid or a portion thereof). Alternatively, the double-stranded oligonucleotide is assembled from a single oligonucleotide, where the self-complementary sense and antisense regions of the siRNA are linked by means of a nucleic acid based or non-nucleic acid-based linker(s).

The double-stranded oligonucleotide can be a polynucleotide with a duplex, asymmetric duplex, hairpin or asymmetric hairpin secondary structure, having self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a separate target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The double-stranded oligonucleotide can be a circular single-stranded polynucleotide having two or more loop structures and a stem comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof, and wherein the circular polynucleotide can be processed either in vivo or in vitro to generate an active siRNA molecule capable of mediating RNAi.

In certain embodiments, the double-stranded oligonucleotide comprises separate sense and antisense sequences or regions, wherein the sense and antisense regions are covalently linked by nucleotide or non-nucleotide linkers molecules as is known in the art, or are alternately non-covalently linked by ionic interactions, hydrogen bonding, van der waals interactions, hydrophobic interactions, and/or stacking interactions. In certain embodiments, the double-stranded oligonucleotide comprises nucleotide sequence that is complementary to nucleotide sequence of a target gene. In another embodiment, the double-stranded oligonucleotide interacts with nucleotide sequence of a target gene in a manner that causes inhibition of expression of the target gene.

As used herein, double-stranded oligonucleotides need not be limited to those molecules containing only RNA, but further encompasses chemically modified nucleotides and non-nucleotides. In certain embodiments, the short interfering nucleic acid molecules lack 2'-hydroxy (2'-OH) containing nucleotides. In certain embodiments short interfering nucleic acids optionally do not include any ribonucleotides (e.g., nucleotides having a 2'-OH group). Such double-stranded oligonucleotides that do not require the presence of ribonucleotides within the molecule to support RNAi can however have an attached linker or linkers or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2'-OH groups. Optionally, double-stranded oligonucleotides can comprise ribonucleotides at about 5, 10, 20, 30, 40, or 50% of the nucleotide positions. As used herein, the term siRNA is meant to be equivalent to other terms used to

describe nucleic acid molecules that are capable of mediating sequence specific RNAi, for example short interfering RNA (siRNA), double-stranded RNA (dsRNA), micro-RNA (miRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically modified siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as used herein, the term RNAi is meant to be equivalent to other terms used to describe sequence specific RNA interference, such as post transcriptional gene silencing, translational inhibition, or epigenetics. For example, double-stranded oligonucleotides can be used to epigenetically silence genes at both the post-transcriptional level and the pre-transcriptional level. In a non-limiting example, epigenetic regulation of gene expression by siRNA molecules of the invention can result from siRNA mediated modification of chromatin structure or methylation pattern to alter gene expression (see, for example, Verdel et al., 2004, Science, 303, 672-676; Pal-Bhadra et al., 2004, Science, 303, 669-672; Allshire, 2002, Science, 297, 1818-1819; Volpe et al., 2002, Science, 297, 1833-1837; Jenuwein, 2002, Science, 297, 2215-2218; and Hall et al., 2002, Science, 297, 2232-2237).

It is contemplated that compounds and compositions of several embodiments provided herein can target AGPAT5 by a dsRNA-mediated gene silencing or RNAi mechanism, including, e.g., "hairpin" or stem-loop double-stranded RNA effector molecules in which a single RNA strand with self-complementary sequences is capable of assuming a double-stranded conformation, or duplex dsRNA effector molecules comprising two separate strands of RNA. In various embodiments, the dsRNA consists entirely of ribonucleotides or consists of a mixture of ribonucleotides and deoxynucleotides, such as the RNA/DNA hybrids disclosed, for example, by WO 00/63364, filed Apr. 19, 2000, or U.S. Ser. No. 60/130,377, filed Apr. 21, 1999. The dsRNA or dsRNA effector molecule may be a single molecule with a region of self-complementarity such that nucleotides in one segment of the molecule base pair with nucleotides in another segment of the molecule. In various embodiments, a dsRNA that consists of a single molecule consists entirely of ribonucleotides or includes a region of ribonucleotides that is complementary to a region of deoxyribonucleotides. Alternatively, the dsRNA may include two different strands that have a region of complementarity to each other.

In various embodiments, both strands consist entirely of ribonucleotides, one strand consists entirely of ribonucleotides and one strand consists entirely of deoxyribonucleotides, or one or both strands contain a mixture of ribonucleotides and deoxyribonucleotides. In certain embodiments, the regions of complementarity are at least 70%, 80%, 90%, 95%, 98%, or 100% complementary to each other and to a target nucleic acid sequence. In certain embodiments, the region of the dsRNA that is present in a double-stranded conformation includes at least 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 50, 75,100, 200, 500, 1000, 2000 or 5000 nucleotides or includes all of the nucleotides in a cDNA or other target nucleic acid sequence being represented in the dsRNA. In some embodiments, the dsRNA does not contain any single stranded regions, such as single stranded ends, or the dsRNA is a hairpin. In other embodiments, the dsRNA has one or more single stranded regions or overhangs. In certain embodiments, RNA/DNA hybrids include a

DNA strand or region that is an antisense strand or region (e.g, has at least 70%, 80%, 90%, 95%, 98%, or 100% complementarity to a target nucleic acid) and an RNA strand or region that is a sense strand or region (e.g, has at least 70%, 80%, 90%, 95%, 98%, or 100% identity to a target nucleic acid), and vice versa.

In various embodiments, the RNA/DNA hybrid is made in vitro using enzymatic or chemical synthetic methods such as those described herein or those described in WO 00/63364, filed Apr. 19, 2000, or U.S. Ser. No. 60/130,377, filed Apr. 21, 1999. In other embodiments, a DNA strand synthesized in vitro is complexed with an RNA strand made in vivo or in vitro before, after, or concurrent with the transformation of the DNA strand into the cell. In yet other embodiments, the dsRNA is a single circular nucleic acid containing a sense and an antisense region, or the dsRNA includes a circular nucleic acid and either a second circular nucleic acid or a linear nucleic acid (see, for example, WO 00/63364, filed Apr. 19, 2000, or U.S. Ser. No. 60/130,377, filed Apr. 21, 1999.) Exemplary circular nucleic acids include lariat structures in which the free 5' phosphoryl group of a nucleotide becomes linked to the 2' hydroxyl group of another nucleotide in a loop back fashion.

In other embodiments, the dsRNA includes one or more modified nucleotides in which the 2' position in the sugar contains a halogen (such as fluorine group) or contains an alkoxy group (such as a methoxy group) which increases the half-life of the dsRNA in vitro or in vivo compared to the corresponding dsRNA in which the corresponding 2' position contains a hydrogen or an hydroxyl group. In yet other embodiments, the dsRNA includes one or more linkages between adjacent nucleotides other than a naturally-occurring phosphodiester linkage. Examples of such linkages include phosphoramide, phosphorothioate, and phosphorodithioate linkages. The dsRNAs may also be chemically modified nucleic acid molecules as taught in U.S. Pat. No. 6,673,661. In other embodiments, the dsRNA contains one or two capped strands, as disclosed, for example, by WO 00/63364, filed Apr. 19, 2000, or U.S. Ser. No. 60/130,377, filed Apr. 21, 1999.

In other embodiments, the dsRNA can be any of the at least partially dsRNA molecules disclosed in WO 00/63364, as well as any of the dsRNA molecules described in U.S. Provisional Application 60/399,998; and U.S. Provisional Application 60/419,532, and PCT/US2003/033466, the teaching of which is hereby incorporated by reference. Any of the dsRNAs may be expressed in vitro or in vivo using the methods described herein or standard methods, such as those described in WO 00/63364.

*Occupancy*

In certain embodiments, antisense compounds are not expected to result in cleavage or the target nucleic acid via RNase H or to result in cleavage or sequestration through the RISC pathway. In certain such embodiments, antisense activity may result from occupancy, wherein the presence of the hybridized antisense compound disrupts the activity of the target nucleic acid. In certain such embodiments, the antisense

compound may be uniformly modified or may comprise a mix of modifications and/or modified and unmodified nucleosides.

*Target Nucleic Acids, Target Regions and Nucleotide Sequences*

Nucleotide sequences that encode AGPAT5 include, without limitation, the following: the murine sequence as set forth in GENBANK Accession No. NM_026792.3 (incorporated herein as SEQ ID NO: 1) or GENBANK Accession No. NT_039455.7 truncated from nucleotides 15844020 to 15885425 (incorporated herein as SEQ ID NO: 2); the human sequence as set forth in GENBANK Accession No. NM_018361.3 (incorporated herein as SEQ ID NO: 24), GENBANK Accession No. AK310545.1 (incorporated herein as SEQ ID NO: 25) or GENBANK Accession No. NT_023736.17 truncated from nucleotides 6554870 to 6610015 (incorporated herein as SEQ ID NO: 26).

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 can 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 can 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 AGPAT5 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 can 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 target region.

In certain embodiments, a "target segment" is a smaller, sub-portion of a target region within a nucleic acid. For example, a target segment can be the sequence of nucleotides of a target nucleic acid to which one or more antisense compound is targeted. "5' target site" or "5' start site" refers to the 5'-most nucleotide of a target segment. "3' target site" or "3' stop site" refers to the 3'-most nucleotide of a target segment.

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 can contain one or more target segments. Multiple target segments within a target region can be overlapping. Alternatively, they can be non-overlapping. In certain embodiments, target segments within a target region are separated by no more than about 300 nucleotides. In certain

embodiments, 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 preceding 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 can 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 can specifically exclude a certain structurally defined region such as the start codon or stop codon.

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

There can 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 AGPAT5 mRNA levels are indicative of inhibition of AGPAT5 protein expression. Reductions in levels of an AGPAT5 protein are also indicative of inhibition of target mRNA expression. Further, phenotypic changes, such as a reduction of the level of triglyceride, insulin resistance or glucose levels, can be indicative of inhibition of AGPAT5 mRNA and/or protein expression.

*Hybridization*

In some embodiments, hybridization occurs between an antisense compound disclosed herein and an AGPAT5 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 (Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 3rd Ed., 2001). In certain embodiments, the antisense compounds provided herein are specifically hybridizable with an AGPAT5 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 an AGPAT5 nucleic acid).

An antisense compound can hybridize over one or more segments of an AGPAT5 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%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementary to an AGPAT5 nucleic acid, a target region, target segment, or specified portion thereof. In certain embodiments, the antisense compounds provided herein, or a specified portion thereof, are, or are at least, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementary to the sequence of one or more of SEQ ID NOs: 1-2,24-26. Percent complementarity of an antisense compound with a target nucleic acid can be determined using routine methods.

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

In certain embodiments, the antisense compounds provided herein, or specified portions thereof, are fully complementary (i.e. 100% complementary) to a target nucleic acid, or specified portion thereof. For example, an antisense compound can be fully complementary to an AGPAT5 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 can 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 can be at the 5' end or 3' end of the antisense compound. Alternatively, the non-complementary nucleobase or nucleobases can be at an internal position of the antisense compound. When two or more non-complementary nucleobases are present, they can be either 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 10, 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 an AGPAT5 nucleic acid, or specified portion thereof.

In certain embodiments, antisense compounds that are, or are up to 10, 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 an AGPAT5 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 10 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 15 nucleobase portion of a target segment. Also contemplated are antisense compounds that are complementary to at least an 8, 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 can also have a defined percent identity to a particular nucleotide sequence, SEQ ID NO, or the sequence of a 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 can be adjacent to each other or dispersed throughout the antisense compound. Percent identity of an antisense compound is calculated according to the number of bases that have identical base pairing relative to the sequence to which it is being compared.

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

*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 can 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 an AGPAT5 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.

In certain embodiments, oligonucleotides comprise modified internucleoside linkages arranged along the oligonucleotide or region thereof in a defined pattern or modified internucleoside linkage motif. In certain embodiments, internucleoside linkages are arranged in a gapped motif. In such embodiments, the internucleoside linkages in each of two wing regions are different from the internucleoside linkages in the gap region. In certain embodiments the internucleoside linkages in the wings are phosphodiester and the internucleoside linkages in the gap are phosphorothioate. The nucleoside motif is independently selected, so such oligonucleotides having a gapped internucleoside linkage motif may or may not have a gapped nucleoside motif and if it does have a gapped nucleoside motif, the wing and gap lengths may or may not be the same.

In certain embodiments, oligonucleotides comprise a region having an alternating internucleoside linkage motif. In certain embodiments, oligonucleotides of the present invention comprise a region of uniformly modified internucleoside linkages. In certain such embodiments, the oligonucleotide comprises a region that is uniformly linked by phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide is uniformly linked by phosphorothioate. In certain embodiments, each internucleoside linkage of the oligonucleotide is selected from phosphodiester and phosphorothioate. In certain embodiments, each internucleoside linkage of the oligonucleotide is selected from phosphodiester and phosphorothioate and at least one internucleoside linkage is phosphorothioate.

In certain embodiments, the oligonucleotide comprises at least 6 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least 8 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least 10 phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 6 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 8 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least one block of at least 10 consecutive phosphorothioate internucleoside linkages. In certain embodiments, the oligonucleotide comprises at least block of at least one 12 consecutive

phosphorothioate internucleoside linkages. In certain such embodiments, at least one such block is located at the 3' end of the oligonucleotide. In certain such embodiments, at least one such block is located within 3 nucleosides of the 3' end of the oligonucleotide.

In certain embodiments, oligonucleotides comprise one or more methylphosponate linkages. In certain embodiments, oligonucleotides having a gapmer nucleoside motif comprise a linkage motif comprising all phosphorothioate linkages except for one or two methylphosphonate linkages. In certain embodiments, one methylphosponate linkage is in the central gap of an oligonucleotide having a gapmer nucleoside motif.

In certain embodiments, it is desirable to arrange the number of phosphorothioate internucleoside linkages and phosphodiester internucleoside linkages to maintain nuclease resistance. In certain embodiments, it is desirable to arrange the number and position of phosphorothioate internucleoside linkages and the number and position of phosphodiester internucleoside linkages to maintain nuclease resistance. In certain embodiments, the number of phosphorothioate internucleoside linkages may be decreased and the number of phosphodiester internucleoside linkages may be increased. In certain embodiments, the number of phosphorothioate internucleoside linkages may be decreased and the number of phosphodiester internucleoside linkages may be increased while still maintaining nuclease resistance. In certain embodiments it is desirable to decrease the number of phosphorothioate internucleoside linkages while retaining nuclease resistance. In certain embodiments it is desirable to increase the number of phosphodiester internucleoside linkages while retaining nuclease resistance.

*Modified Sugar Moieties*

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

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

As used herein, "bicyclic nucleosides" refer to modified nucleosides comprising a bicyclic sugar moiety. Examples of bicyclic nucleic acids (BNAs) include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, antisense compounds provided herein include one or more BNA nucleosides wherein the bridge comprises one of the formulas: 4'-$(CH_2)$-O-2' (LNA); 4'-$(CH_2)$-S-2'; 4'-$(CH_2)_2$-O-2' (ENA); 4'-$CH(CH_3)$-O-2' and 4'-$CH(CH_2OCH_3)$-O-2' (and analogs thereof see U.S. Patent 7,399,845, issued on July 15, 2008); 4'-$C(CH_3)(CH_3)$-O-2' (and analogs thereof see PCT/US2008/068922 published as WO/2009/006478, published January 8, 2009); 4'-$CH_2$-$N(OCH_3)$-2' (and analogs thereof see PCT/US2008/064591 published as WO/2008/150729, published December 11, 2008); 4'-$CH_2$-O-$N(CH_3)$-2' (see published U.S. Patent Application US2004-0171570, published September 2, 2004 ); 4'-$CH_2$-N(R)-O-2', wherein R is H, $C_1$-$C_{12}$ alkyl, or a protecting group (see U.S. Patent 7,427,672, issued on September 23, 2008); 4'-$CH_2$-$C(H)(CH_3)$-2' (see Chattopadhyaya *et al., J. Org. Chem.,* 2009, *74*, 118-134); and 4'-$CH_2$-$C(=CH_2)$-2' (and analogs thereof see PCT/US2008/066154 published as WO 2008/154401, published on December 8, 2008).

Further bicyclic nucleosides have been reported in published literature (see for example: Srivastava et al., *J. Am. Chem. Soc.*, 2007, *129(26)* 8362-8379; Frieden *et al., Nucleic Acids Research,* 2003, *21*, 6365-6372; Elayadi *et al., Curr. Opinion Invens. Drugs,* 2001, *2*, 558-561; Braasch *et al., Chem. Biol.,* 2001, *8*, 1-7; Orum *et al., Curr. Opinion Mol. Ther.,* 2001, *3*, 239-243; Wahlestedt et al., *Proc. Natl. Acad. Sci. U. S. A.*, 2000, *97*, 5633-5638; Singh et al., *Chem. Commun.*, 1998, *4*, 455-456; Koshkin et al., *Tetrahedron*, 1998, *54*, 3607-3630; Kumar et al., *Bioorg. Med. Chem. Lett.*, 1998, *8*, 2219-2222; Singh et al., *J. Org. Chem.*, 1998, *63*, 10035-10039; U.S. Patents Nos.: 7,399,845; 7,053,207; 7,034,133; 6,794,499; 6,770,748; 6,670,461; 6,525,191; 6,268,490; U.S. Patent Publication Nos.: US2008-0039618; US2007-0287831; US2004-0171570; U.S. Patent Applications, Serial Nos.: 12/129,154; 61/099,844; 61/097,787; 61/086,231; 61/056,564; 61/026,998; 61/026,995; 60/989,574; International applications WO 2007/134181; WO 2005/021570; WO 2004/106356; WO 94/14226; and PCT International Applications Nos.: PCT/US2008/068922; PCT/US-2008/066154; and PCT/US2008/064591). Each of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α-L-ribofuranose and β-D-ribofuranose (see PCT international application PCT/DK98/00393, published on March 25, 1999 as WO 99/14226).

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

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

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

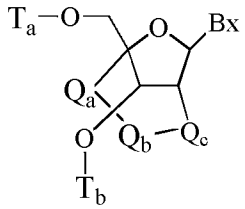
each $J_1$ and $J_2$ is, independently, H, $C_1$-$C_{12}$ alkyl, substituted $C_1$-$C_{12}$ alkyl, $C_2$-$C_{12}$ alkenyl, substituted $C_2$-$C_{12}$ alkenyl, $C_2$-$C_{12}$ alkynyl, substituted $C_2$-$C_{12}$ alkynyl, $C_5$-$C_{20}$ aryl, substituted $C_5$-$C_{20}$ aryl, acyl (C(=O)-H), substituted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

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

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

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

In certain embodiment, bicyclic nucleosides have the formula:



wherein:

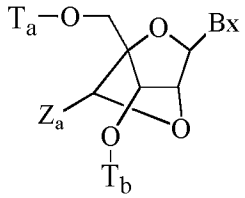
Bx is a heterocyclic base moiety;

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

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

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

In certain embodiments, bicyclic nucleosides have the formula:



wherein:

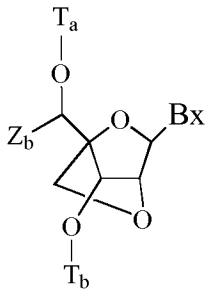
Bx is a heterocyclic base moiety;

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

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

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

In certain embodiments, bicyclic nucleosides have the formula:



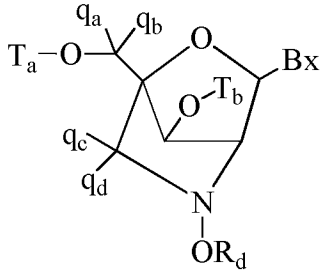
wherein:

Bx is a heterocyclic base moiety;

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

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

In certain embodiments, bicyclic nucleosides have the formula:




wherein:

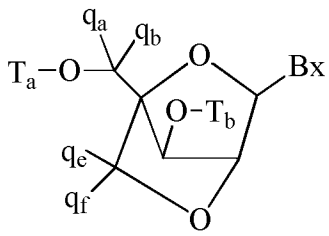
Bx is a heterocyclic base moiety;

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

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

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

In certain embodiments, bicyclic nucleosides have the formula:




wherein:

Bx is a heterocyclic base moiety;

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

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

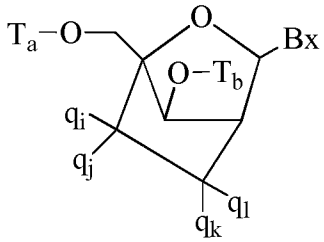
or $q_e$ and $q_f$ together are $=C(q_g)(q_h)$;

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

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

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

In certain embodiments, bicyclic nucleosides have the formula:




wherein:

Bx is a heterocyclic base moiety;

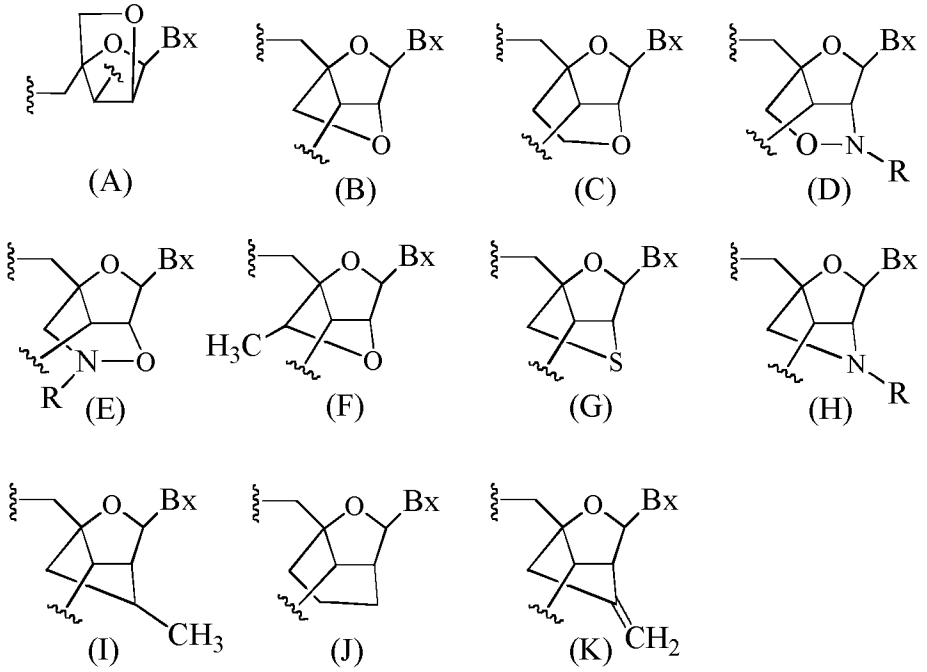
$T_a$ and $T_b$ are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;

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

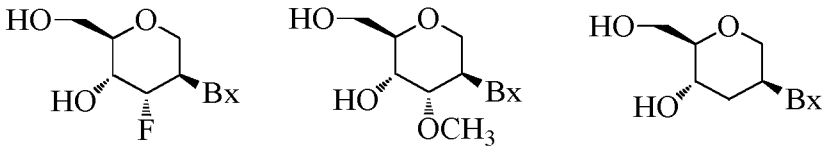
One carbocyclic bicyclic nucleoside having a 4'-$(CH_2)_3$-2' bridge and the alkenyl analog bridge 4'-CH=CH-$CH_2$-2' have been described (Frier *et al., Nucleic Acids Research*, 1997, *25*(*22*), 4429-4443 and Albaek *et al., J. Org. Chem.*, 2006, *71*, 7731-7740). The synthesis and preparation of carbocyclic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

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

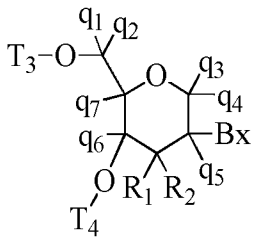



wherein Bx is the base moiety and R is, independently, H, a protecting group, $C_1$-$C_6$ alkyl or $C_1$-$C_6$ alkoxy.

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



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



wherein:

Bx is a heterocyclic base moiety;

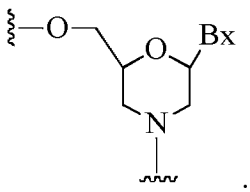
$T_3$ and $T_4$ are each, independently, an internucleoside linking group linking the tetrahydropyran nucleoside analog to the oligomeric compound or one of $T_3$ and $T_4$ is an internucleoside linking group linking the tetrahydropyran nucleoside analog to an oligomeric compound or oligonucleotide and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

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

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

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

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

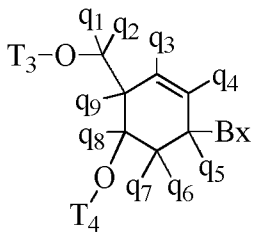


In certain embodiments, morpholinos may be modified, for example by adding or altering various substituent groups from the above morpholino structure. Such sugar surrogates are referred to herein as "modifed morpholinos."

Combinations of modifications are also provided without limitation, such as 2'-F-5'-methyl substituted nucleosides (see PCT International Application WO 2008/101157 published on 8/21/08 for other disclosed 5', 2'-bis substituted nucleosides) and replacement of the ribosyl ring oxygen atom with S and

further substitution at the 2'-position (see published U.S. Patent Application US2005-0130923, published on June 16, 2005) or alternatively 5'-substitution of a bicyclic nucleic acid (see PCT International Application WO 2007/134181, published on 11/22/07 wherein a 4'-$CH_2$-O-2' bicyclic nucleoside is further substituted at the 5' position with a 5'-methyl or a 5'-vinyl group). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (*see, e.g.,* Srivastava *et al., J. Am. Chem. Soc.* 2007, *129(26)*, 8362-8379).

In certain embodiments, antisense compounds comprise one or more modified cyclohexenyl nucleosides, which is a nucleoside having a six-membered cyclohexenyl in place of the pentofuranosyl residue in naturally occurring nucleosides. Modified cyclohexenyl nucleosides include, but are not limited to those described in the art (see for example commonly owned, published PCT Application WO 2010/036696, published on April 10, 2010, Robeyns *et al., J. Am. Chem. Soc.,* 2008, *130(6)*, 1979-1984; Horváth *et al., Tetrahedron Letters,* 2007, *48*, 3621-3623; Nauwelaerts *et al., J. Am. Chem. Soc.*, 2007, *129(30)*, 9340-9348; Gu *et al.,, Nucleosides, Nucleotides & Nucleic Acids*, 2005, *24(5-7)*, 993-998; Nauwelaerts *et al., Nucleic Acids Research,* 2005, *33(8)*, 2452-2463; Robeyns et al., *Acta Crystallographica, Section F: Structural Biology and Crystallization Communications,* 2005, *F61(6)*, 585-586; Gu *et al., Tetrahedron*, 2004, *60(9)*, 2111-2123; Gu *et al., Oligonucleotides*, 2003, *13(6)*, 479-489; Wang *et al., J. Org. Chem.,* 2003, *68*, 4499-4505; Verbeure *et al., Nucleic Acids Research*, 2001, *29(24)*, 4941-4947; Wang *et al., J. Org. Chem.,* 2001, *66*, 8478-82; Wang *et al., Nucleosides, Nucleotides & Nucleic Acids*, 2001, *20(4-7)*, 785-788; Wang *et al., J. Am. Chem.,* 2000, *122*, 8595-8602; Published PCT application, WO 06/047842; and Published PCT Application WO 01/049687; the text of each is incorporated by reference herein, in their entirety). Certain modified cyclohexenyl nucleosides have Formula X.




X

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

Bx is a heterocyclic base moiety;

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

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

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

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

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

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

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

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

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

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

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

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

*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 can 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 modified 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-CH3) 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 can 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 an AGPAT5 nucleic acid comprise one or more modified nucleobases. In certain embodiments, shortened or gap-widened antisense oligonucleotides targeted to an AGPAT5 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 can 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.

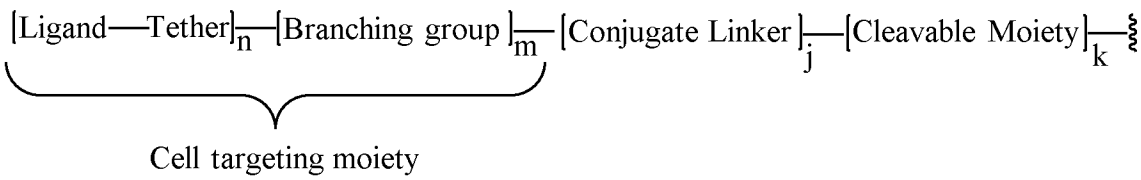
Antisense compound targeted to an AGPAT5 nucleic acid can be utilized in pharmaceutical compositions by combining the antisense compound with a suitable pharmaceutically acceptable diluent or carrier. A pharmaceutically acceptable diluent includes phosphate-buffered saline (PBS). PBS is a diluent suitable 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 an AGPAT5 nucleic acid and a pharmaceutically acceptable diluent. In certain embodiments, the pharmaceutically acceptable diluent is PBS. In certain embodiments, the antisense compound is an antisense oligonucleotide.

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

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

*Conjugated Antisense Compounds*

In certain embodiments, the oligomeric compounds as provided herein are modified by covalent attachment of one or more conjugate groups. As used herein, "conjugate group" means a radical group comprising a group of atoms that are attached to an oligomeric compound. In general, conjugate groups modify one or more properties of the compound to which they are attached, including, but not limited to pharmacodynamic, pharmacokinetic, binding, absorption, cellular distribution, cellular uptake, charge and/or clearance properties. Conjugate groups are routinely used in the chemical arts and can include a conjugate linker that covalently links the conjugate group to an oligomeric compound. In certain embodiments, conjugate groups include a cleavable moiety that covalently links the conjugate group to an oligomeric compound. In certain embodiments, conjugate groups include a conjugate linker and a cleavable moiety to covalently link the conjugate group to an oligomeric compound. In certain embodiments, a conjugate group has the general formula:




wherein n is from 1 to about 3, m is 0 when n is 1 or m is 1 when n is 2 or 3, j is 1 or 0, k is 1 or 0 and the sum of j and k is at least one.

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

Conjugate groups are shown herein as radicals, providing a bond for forming covalent attachment to an oligomeric compound such as an antisense oligonucleotide. In certain embodiments, the point of attachment on the oligomeric compound is at the 3'-terminal nucleoside or modified nucleoside. In certain embodiments, the point of attachment on the oligomeric compound is the 3'-oxygen atom of the 3'-hydroxyl group of the 3' terminal nucleoside or modified nucleoside. In certain embodiments, the point of attachment on the oligomeric compound is at the 5'-terminal nucleoside or modified nucleoside. In certain embodiments

the point of attachment on the oligomeric compound is the 5'-oxygen atom of the 5'-hydroxyl group of the 5'-terminal nucleoside or modified nucleoside. In certain embodiments, the point of attachment on the oligomeric compound is at any reactive site on a nucleoside, a modified nucleoside or an internucleoside linkage.

As used herein, "cleavable moiety" and "cleavable bond" mean a cleavable bond or group of atoms that is capable of being split or cleaved under certain physiological conditions. In certain embodiments, a cleavable moiety is a cleavable bond. In certain embodiments, a cleavable moiety comprises a cleavable bond. In certain embodiments, a cleavable moiety is a group of atoms. In certain embodiments, a cleavable moiety is selectively cleaved inside a cell or sub-cellular compartment, such as a lysosome. In certain embodiments, a cleavable moiety is selectively cleaved by endogenous enzymes, such as nucleases. In certain embodiments, a cleavable moiety comprises a group of atoms having one, two, three, four, or more than four cleavable bonds.

In certain embodiments, conjugate groups comprise a cleavable moiety. In certain such embodiments, the cleavable moiety covalently attaches the oligomeric compound to the conjugate linker. In certain such embodiments, the cleavable moiety covalently attaches the oligomeric compound to the cell-targeting moiety.

In certain embodiments, a cleavable bond is selected from among: an amide, a polyamide, an ester, an ether, one or both esters of a phosphodiester, a phosphate ester, a carbamate, a di-sulfide, or a peptide. In certain embodiments, a cleavable bond is one of the esters of a phosphodiester. In certain embodiments, a cleavable bond is one or both esters of a phosphodiester. In certain embodiments, the cleavable moiety is a phosphodiester linkage between an oligomeric compound and the remainder of the conjugate group. In certain embodiments, the cleavable moiety comprises a phosphodiester linkage that is located between an oligomeric compound and the remainder of the conjugate group. In certain embodiments, the cleavable moiety comprises a phosphate or phosphodiester. In certain embodiments, the cleavable moiety is attached to the conjugate linker by either a phosphodiester or a phosphorothioate linkage. In certain embodiments, the cleavable moiety is attached to the conjugate linker by a phosphodiester linkage. In certain embodiments, the conjugate group does not include a cleavable moiety.

In certain embodiments, the cleavable moiety is a cleavable nucleoside or a modified nucleoside. In certain embodiments, the nucleoside or modified nucleoside comprises an optionally protected heterocyclic base selected from a purine, substituted purine, pyrimidine or substituted pyrimidine. In certain embodiments, the cleavable moiety is a nucleoside selected from uracil, thymine, cytosine, 4-N-benzoylcytosine, 5-methylcytosine, 4-N-benzoyl-5-methylcytosine, adenine, 6-N-benzoyladenine, guanine and 2-N-isobutyrylguanine.

In certain embodiments, the cleavable moiety is 2'-deoxy nucleoside that is attached to either the 3' or 5'-terminal nucleoside of an oligomeric compound by a phosphodiester linkage and covalently attached to the remainder of the conjugate group by a phosphodiester or phosphorothioate linkage. In certain embodiments,

the cleavable moiety is 2'-deoxy adenosine that is attached to either the 3' or 5'-terminal nucleoside of an oligomeric compound by a phosphodiester linkage and covalently attached to the remainder of the conjugate group by a phosphodiester or phosphorothioate linkage. In certain embodiments, the cleavable moiety is 2'-deoxy adenosine that is attached to the 3'-oxygen atom of the 3'-hydroxyl group of the 3'-terminal nucleoside or modified nucleoside by a phosphodiester linkage. In certain embodiments, the cleavable moiety is 2'-deoxy adenosine that is attached to the 5'-oxygen atom of the 5'-hydroxyl group of the 5'-terminal nucleoside or modified nucleoside by a phosphodiester linkage. In certain embodiments, the cleavable moiety is attached to a 2'-position of a nucleoside or modified nucleoside of an oligomeric compound.

As used herein, "conjugate linker" in the context of a conjugate group means a portion of a conjugate group comprising any atom or group of atoms that covalently link the cell-targeting moiety to the oligomeric compound either directly or through the cleavable moiety. In certain embodiments, the conjugate linker comprises groups selected from alkyl, amino, oxo, amide, disulfide, polyethylene glycol, ether, thioether (-S-) and hydroxylamino (-O-N(H)-). In certain embodiments, the conjugate linker comprises groups selected from alkyl, amino, oxo, amide and ether groups. In certain embodiments, the conjugate linker comprises groups selected from alkyl and amide groups. In certain embodiments, the conjugate linker comprises groups selected from alkyl and ether groups. In certain embodiments, the conjugate linker comprises at least one phosphorus linking group. In certain embodiments, the conjugate linker comprises at least one phosphodiester group. In certain embodiments, the conjugate linker includes at least one neutral linking group.

In certain embodiments, the conjugate linker is covalently attached to the oligomeric compound. In certain embodiments, the conjugate linker is covalently attached to the oligomeric compound and the branching group. In certain embodiments, the conjugate linker is covalently attached to the oligomeric compound and a tethered ligand. In certain embodiments, the conjugate linker is covalently attached to the cleavable moiety. In certain embodiments, the conjugate linker is covalently attached to the cleavable moiety and the branching group. In certain embodiments, the conjugate linker is covalently attached to the cleavable moiety and a tethered ligand. In certain embodiments, the conjugate linker includes one or more cleavable bonds. In certain embodiments, the conjugate group does not include a conjugate linker.

As used herein, "branching group" means a group of atoms having at least 3 positions that are capable of forming covalent linkages to two or more tether-ligands and the remainder of the conjugate group. In general a branching group provides a plurality of reactive sites for connecting tethered ligands to the oligomeric compound through the conjugate linker and/or the cleavable moiety. In certain embodiments, the branching group comprises groups selected from alkyl, amino, oxo, amide, disulfide, polyethylene glycol, ether, thioether and hydroxylamino groups. In certain embodiments, the branching group comprises a branched aliphatic group comprising groups selected from alkyl, amino, oxo, amide, disulfide, polyethylene glycol, ether, thioether and hydroxylamino groups. In certain such embodiments, the branched aliphatic group comprises groups selected from alkyl, amino, oxo, amide and ether groups. In certain such

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

In certain embodiments, the branching group is covalently attached to the conjugate linker. In certain embodiments, the branching group is covalently attached to the cleavable moiety. In certain embodiments, the branching group is covalently attached to the conjugate linker and each of the tethered ligands. In certain embodiments, the branching group comprises one or more cleavable bond. In certain embodiments, the conjugate group does not include a branching group.

In certain embodiments, conjugate groups as provided herein include a cell-targeting moiety that has at least one tethered ligand. In certain embodiments, the cell-targeting moiety comprises two tethered ligands covalently attached to a branching group. In certain embodiments, the cell-targeting moiety comprises three tethered ligands covalently attached to a branching group.

As used herein, "tether" means a group of atoms that connect a ligand to the remainder of the conjugate group. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, substituted alkyl, ether, thioether, disulfide, amino, oxo, amide, phosphodiester and polyethylene glycol groups in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, ether, thioether, disulfide, amino, oxo, amide and polyethylene glycol groups in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, substituted alkyl, phosphodiester, ether and amino, oxo, amide groups in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, ether and amino, oxo, amide groups in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, amino and oxo groups in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl and oxo groups in any combination. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl and phosphodiester in any combination. In certain embodiments, each tether comprises at least one phosphorus linking group or neutral linking group.

In certain embodiments, tethers include one or more cleavable bond. In certain embodiments, each tethered ligand is attached to a branching group. In certain embodiments, each tethered ligand is attached to a branching group through an amide group. In certain embodiments, each tethered ligand is attached to a branching group through an ether group. In certain embodiments, each tethered ligand is attached to a branching group through a phosphorus linking group or neutral linking group. In certain embodiments, each tethered ligand is attached to a branching group through a phosphodiester group. In certain embodiments, each tether is attached to a ligand through either an amide or an ether group. In certain embodiments, each tether is attached to a ligand through an ether group.

In certain embodiments, each tether comprises from about 8 to about 20 atoms in chain length between the ligand and the branching group. In certain embodiments, each tether comprises from about 10 to about 18 atoms in chain length between the ligand and the branching group. In certain embodiments, each tether comprises about 13 atoms in chain length.

In certain embodiments, the present disclosure provides ligands wherein each ligand is covalently attached to the remainder of the conjugate group through a tether. In certain embodiments, each ligand is selected to have an affinity for at least one type of receptor on a target cell. In certain embodiments, ligands are selected that have an affinity for at least one type of receptor on the surface of a mammalian liver cell. In certain embodiments, ligands are selected that have an affinity for the hepatic asialoglycoprotein receptor (ASGP-R). In certain embodiments, each ligand is a carbohydrate. In certain embodiments, each ligand is, independently selected from galactose, N-acetyl galactoseamine, mannose, glucose, glucosamine and fucose. In certain embodiments, each ligand is N-acetyl galactoseamine (GalNAc). In certain embodiments, the targeting moiety comprises 1 to 3 ligands. In certain embodiments, the targeting moiety comprises 3 ligands. In certain embodiments, the targeting moiety comprises 2 ligands. In certain embodiments, the targeting moiety comprises 1 ligand. In certain embodiments, the targeting moiety comprises 3 N-acetyl galactoseamine ligands. In certain embodiments, the targeting moiety comprises 2 N-acetyl galactoseamine ligands. In certain embodiments, the targeting moiety comprises 1 N-acetyl galactoseamine ligand.

In certain embodiments, each ligand is a carbohydrate, carbohydrate derivative, modified carbohydrate, multivalent carbohydrate cluster, polysaccharide, modified polysaccharide, or polysaccharide derivative. In certain embodiments, each ligand is an amino sugar or a thio sugar. For example, amino sugars may be selected from any number of compounds known in the art, for example glucosamine, sialic acid, α-D-galactosamine, N-Acetylgalactosamine, 2-acetamido-2-deoxy-D-galactopyranose (GalNAc), 2-Amino-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-β-D-glucopyranose (β-muramic acid), 2-Deoxy-2-methylamino-L-glucopyranose, 4,6-Dideoxy-4-formamido-2,3-di-*O*-methyl-D-mannopyranose, 2-Deoxy-2-sulfoamino-D-glucopyranose and *N*-sulfo-D-glucosamine, and *N*-Glycoloyl-α-neuraminic acid. For example, thio sugars may be selected from the group consisting of 5-Thio-β-D-glucopyranose, Methyl 2,3,4-tri-*O*-acetyl-1-thio-6-*O*-trityl-α-D-glucopyranoside, 4-Thio-β-D-galactopyranose, and ethyl 3,4,6,7-tetra-*O*-acetyl-2-deoxy-1,5-dithio-α-D-*gluco*-heptopyranoside.

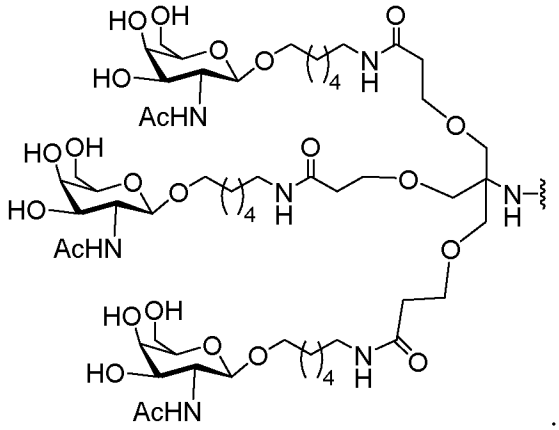
In certain embodiments, conjugate groups as provided herein comprise a carbohydrate cluster. As used herein, "carbohydrate cluster" means a portion of a conjugate group wherein two or more carbohydrate residues are attached to a branching group through tether groups. (*see*, e.g., Maier et al., "Synthesis of Antisense Oligonucleotides Conjugated to a Multivalent Carbohydrate Cluster for Cellular Targeting," *Bioconjugate Chemistry*, 2003, (14): 18-29, which is incorporated herein by reference in its entirety, or Rensen et al., "Design and Synthesis of Novel *N*-Acetylgalactosamine-Terminated Glycolipids for Targeting of Lipoproteins to the Hepatic Asiaglycoprotein Receptor," *J. Med. Chem.* 2004, (47): 5798-5808, for examples of carbohydrate conjugate clusters).

As used herein, “modified carbohydrate” means any carbohydrate having one or more chemical modifications relative to naturally occurring carbohydrates.

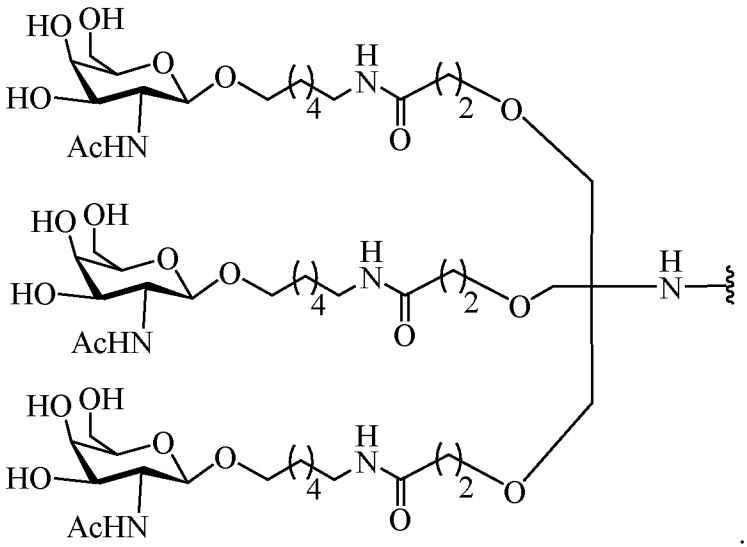
As used herein, “carbohydrate derivative” means any compound which may be synthesized using a carbohydrate as a starting material or intermediate.

As used herein, "carbohydrate” means a naturally occurring carbohydrate, a modified carbohydrate, or a carbohydrate derivative.

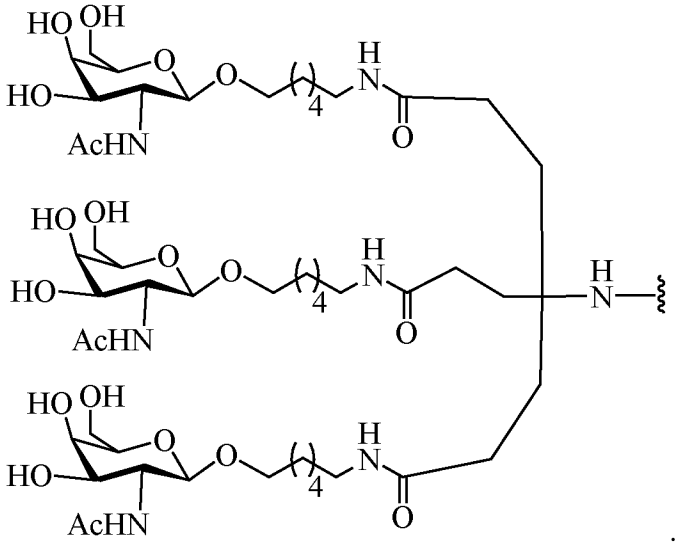
In certain embodiments, conjugate groups are provided wherein the cell-targeting moiety has the formula:



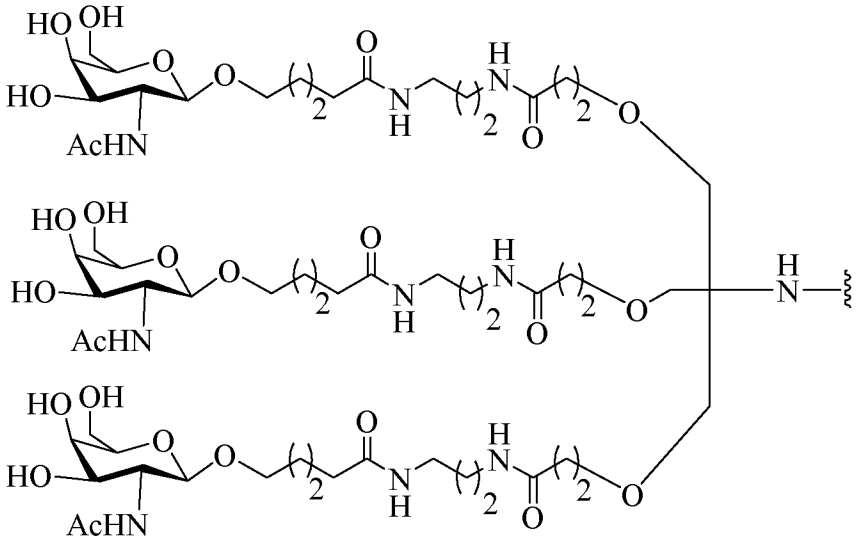
In certain embodiments, conjugate groups are provided wherein the cell-targeting moiety has the formula:



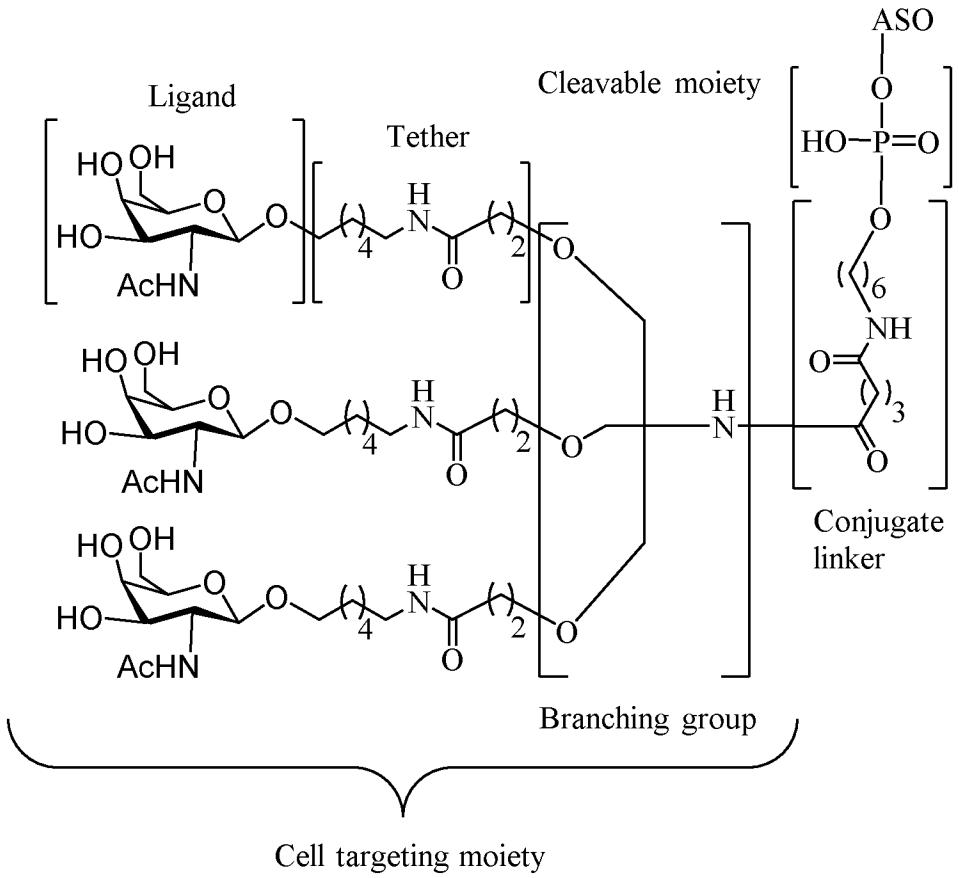
In certain embodiments, conjugate groups are provided wherein the cell-targeting moiety has the formula:



In certain embodiments, conjugate groups are provided wherein the cell-targeting moiety has the formula:



In certain embodiments, conjugate groups have the formula:




Representative United States patents, United States patent application publications, and international patent application publications that teach the preparation of certain of the above noted conjugates, conjugated oligomeric compounds such as antisense compounds, tethers, conjugate linkers, branching groups, ligands, cleavable moieties as well as other modifications include without limitation, US 5,994,517, US 6,300,319, US 6,660,720, US 6,906,182, US 7,262,177, US 7,491,805, US 8,106,022, US 7,723,509, US 2006/0148740, US 2011/0123520, WO 2013/033230 and WO 2012/037254, each of which is incorporated by reference herein in its entirety.

Representative publications that teach the preparation of certain of the above noted conjugates, conjugated oligomeric compounds such as antisense compounds, tethers, conjugate linkers, branching groups, ligands, cleavable moieties as well as other modifications include without limitation, BIESSEN et al., "The Cholesterol Derivative of a Triantennary Galactoside with High Affinity for the Hepatic Asialoglycoprotein Receptor: a Potent Cholesterol Lowering Agent" J. Med. Chem. (1995) 38:1846-1852, BIESSEN et al., "Synthesis of Cluster Galactosides with High Affinity for the Hepatic Asialoglycoprotein Receptor" J. Med. Chem. (1995) 38:1538-1546, LEE et al., "New and more efficient multivalent glyco-ligands for asialoglycoprotein receptor of mammalian hepatocytes" Bioorganic & Medicinal Chemistry (2011) 19:2494-

2500, RENSEN et al., "Determination of the Upper Size Limit for Uptake and Processing of Ligands by the Asialoglycoprotein Receptor on Hepatocytes in Vitro and in Vivo" J. Biol. Chem. (2001) 276(40):37577-37584, RENSEN et al., "Design and Synthesis of Novel N-Acetylgalactosamine-Terminated Glycolipids for Targeting of Lipoproteins to the Hepatic Asialoglycoprotein Receptor" J. Med. Chem. (2004) 47:5798-5808, SLIEDREGT et al., "Design and Synthesis of Novel Amphiphilic Dendritic Galactosides for Selective Targeting of Liposomes to the Hepatic Asialoglycoprotein Receptor" J. Med. Chem. (1999) 42:609-618, and Valentijn *et al.,* "Solid-phase synthesis of lysine-based cluster galactosides with high affinity for the Asialoglycoprotein Receptor" *Tetrahedron,* 1997, *53(2),* 759-770, each of which is incorporated by reference herein in its entirety.

Representative United States patents, United States patent application publications, and international patent application publications that teach the preparation of certain of the above noted conjugates, conjugated antisense compounds, tethers, linkers, branching groups, ligands, cleavable moieties as well as other modifications include without limitation, US 5,994,517, US 6,300,319, US 6,660,720, US 6,906,182, US 7,262,177, US 7,491,805, US 8,106,022, US 7,723,509, US 2006/0148740, US 2011/0123520, PCT/US2014/036452, WO 2013/033230 and WO 2012/037254, each of which is incorporated by reference herein in its entirety.

Representative publications that teach the preparation of certain of the above noted conjugates, conjugated antisense compounds, tethers, linkers, branching groups, ligands, cleavable moieties as well as other modifications include without limitation, BIESSEN et al., "The Cholesterol Derivative of a Triantennary Galactoside with High Affinity for the Hepatic Asialoglycoprotein Receptor: a Potent Cholesterol Lowering Agent" J. Med. Chem. (1995) 38:1846-1852, BIESSEN et al., "Synthesis of Cluster Galactosides with High Affinity for the Hepatic Asialoglycoprotein Receptor" J. Med. Chem. (1995) 38:1538-1546, LEE et al., "New and more efficient multivalent glyco-ligands for asialoglycoprotein receptor of mammalian hepatocytes" Bioorganic & Medicinal Chemistry (2011) 19:2494-2500, RENSEN et al., "Determination of the Upper Size Limit for Uptake and Processing of Ligands by the Asialoglycoprotein Receptor on Hepatocytes in Vitro and in Vivo" J. Biol. Chem. (2001) 276(40):37577-37584, RENSEN et al., "Design and Synthesis of Novel N-Acetylgalactosamine-Terminated Glycolipids for Targeting of Lipoproteins to the Hepatic Asialoglycoprotein Receptor" J. Med. Chem. (2004) 47:5798-5808, SLIEDREGT et al., "Design and Synthesis of Novel Amphiphilic Dendritic Galactosides for Selective Targeting of Liposomes to the Hepatic Asialoglycoprotein Receptor" J. Med. Chem. (1999) 42:609-618, and Valentijn *et al.,* "Solid-phase synthesis of lysine-based cluster galactosides with high affinity for the Asialoglycoprotein Receptor" *Tetrahedron,* 1997, *53(2),* 759-770, each of which is incorporated by reference herein in its entirety.

In certain embodiments, conjugated antisense compounds comprise an RNase H based oligonucleotide (such as a gapmer) or a splice modulating oligonucleotide (such as a fully modified oligonucleotide) and any conjugate group comprising at least one, two, or three GalNAc groups. In certain

embodiments a conjugated antisense compound comprises any conjugate group found in any of the following references: Lee, *Carbohydr Res*, 1978, 67, 509-514; Connolly et al., *J Biol Chem*, 1982, 257, 939-945; Pavia et al., *Int J Pep Protein Res*, 1983, 22, 539-548; Lee et al., *Biochem*, 1984, 23, 4255-4261; Lee et al., *Glycoconjugate J*, 1987, 4, 317-328; Toyokuni et al., *Tetrahedron Lett*, 1990, 31, 2673-2676; Biessen et al., *J Med Chem*, 1995, 38, 1538-1546; Valentijn et al., *Tetrahedron*, 1997, 53, 759-770; Kim et al., *Tetrahedron Lett*, 1997, 38, 3487-3490; Lee et al., *Bioconjug Chem*, 1997, 8, 762-765; Kato et al., *Glycobiol*, 2001, 11, 821-829; Rensen et al., *J Biol Chem*, 2001, 276, 37577-37584; Lee et al., *Methods Enzymol*, 2003, 362, 38-43; Westerlind et al., *Glycoconj J*, 2004, 21, 227-241; Lee et al., *Bioorg Med Chem Lett*, 2006, 16(19), 5132-5135; Maierhofer et al., *Bioorg Med Chem*, 2007, 15, 7661-7676; Khorev et al., *Bioorg Med Chem*, 2008, 16, 5216-5231; Lee et al., *Bioorg Med Chem*, 2011, 19, 2494-2500; Kornilova et al., Analyt Biochem, 2012, 425, 43-46; Pujol et al., *Angew Chemie Int Ed Engl*, 2012, 51, 7445-7448; Biessen et al., *J Med Chem*, 1995, 38, 1846-1852; Sliedregt et al., *J Med Chem*, 1999, 42, 609-618; Rensen et al., *J Med Chem*, 2004, 47, 5798-5808; Rensen et al., *Arterioscler Thromb Vasc Biol*, 2006, 26, 169-175; van Rossenberg et al., *Gene Ther*, 2004, 11, 457-464; Sato et al., *J Am Chem Soc*, 2004, 126, 14013-14022; Lee et al., *J Org Chem*, 2012, 77, 7564-7571; Biessen et al., *FASEB J*, 2000, 14, 1784-1792; Rajur et al., *Bioconjug Chem*, 1997, 8, 935-940; Duff et al., *Methods Enzymol*, 2000, 313, 297-321; Maier et al., *Bioconjug Chem*, 2003, 14, 18-29; Jayaprakash et al., *Org Lett*, 2010, 12, 5410-5413; Manoharan, *Antisense Nucleic Acid Drug Dev*, 2002, 12, 103-128; Merwin et al., *Bioconjug Chem*, 1994, 5, 612-620; Tomiya et al., Bioorg Med Chem, 2013, 21, 5275-5281; International applications WO1998/013381; WO2011/038356; WO1997/046098; WO2008/098788; WO2004/101619; WO2012/037254; WO2011/120053; WO2011/100131; WO2011/163121; WO2012/177947; WO2013/033230; WO2013/075035; WO2012/083185; WO2012/083046; WO2009/082607; WO2009/134487; WO2010/144740; WO2010/148013; WO1997/020563; WO2010/088537; WO2002/043771; WO2010/129709; WO2012/068187; WO2009/126933; WO2004/024757; WO2010/054406; WO2012/089352; WO2012/089602; WO2013/166121; WO2013/165816; U.S. Patents 4,751,219; 8,552,163; 6,908,903; 7,262,177; 5,994,517; 6,300,319; 8,106,022; 7,491,805; 7,491,805; 7,582,744; 8,137,695; 6,383,812; 6,525,031; 6,660,720; 7,723,509; 8,541,548; 8,344,125; 8,313,772; 8,349,308; 8,450,467; 8,501,930; 8,158,601; 7,262,177; 6,906,182; 6,620,916; 8,435,491; 8,404,862; 7,851,615; Published U.S. Patent Application Publications US2011/0097264; US2011/0097265; US2013/0004427; US2005/0164235; US2006/0148740; US2008/0281044; US2010/0240730; US2003/0119724; US2006/0183886; US2008/0206869; US2011/0269814; US2009/0286973; US2011/0207799; US2012/0136042; US2012/0165393; US2008/0281041; US2009/0203135; US2012/0035115; US2012/0095075; US2012/0101148; US2012/0128760; US2012/0157509; US2012/0230938; US2013/0109817; US2013/0121954; US2013/0178512; US2013/0236968; US2011/0123520; US2003/0077829; US2008/0108801; and US2009/0203132; each of which is incorporated by reference in its entirety.

*Cell culture and antisense compounds treatment*

The effects of antisense compounds on the level, activity or expression of AGPAT5 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, Hep3B cells, Huh7 (hepatocellular carcinoma) cells, primary hepatocytes, A549 cells, GM04281 fibroblasts and LLC-MK2 cells.

*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 reagent used to introduce antisense oligonucleotides into cultured cells includes Oligofectamine™ (Invitrogen Life Technologies, Carlsbad, CA). Antisense oligonucleotide is mixed with Oligofectamine™ in Opti-MEM™-1 reduced serum medium (Invitrogen Life Technologies, Carlsbad, CA) to achieve the desired concentration of oligonucleotide with an Oligofectamine™ to oligonucleotide ratio of approximately 0.2 to 0.8 µL per 100 nM.

Another reagent used to introduce antisense oligonucleotides into cultured cells includes FuGENE 6 (Roche Diagnostics Corp., Indianapolis, IN). Antisense oligomeric compound was mixed with FuGENE 6 in

1 mL of serum-free RPMI to achieve the desired concentration of oligonucleotide with a FuGENE 6 to oligomeric compound ratio of 1 to 4 μL of FuGENE 6 per 100 nM.

Another technique used to introduce antisense oligonucleotides into cultured cells includes electroporation (Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 3rd Ed., 2001).

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 (Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 3rd Ed., 2001). 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 an AGPAT5 nucleic acid can be assayed in a variety of ways known in the art (Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 3rd Ed., 2001). 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 can 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 and 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 can be normalized using either the expression level of a gene whose expression is constant, such as cyclophilin A or GADPH or by quantifying total RNA using RIBOGREEN® (Life Technologies™, Inc. Carlsbad, CA). Cyclophilin A or GADPH expression can be quantified by real time PCR, by being run simultaneously with the target, multiplexing, or separately. Total RNA can be quantified using RIBOGREEN® RNA quantification reagent. 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) can be used to measure RIBOGREEN® fluorescence.

Methods for designing real-time PCR probes and primers are well known in the art, and can include the use of software such as PRIMER EXPRESS® Software (Applied Biosystems, Foster City, CA). Probes and primers used in real-time PCR were designed to hybridize to AGPAT5 specific sequences and are disclosed in the Examples below. The target specific PCR probes can have FAM covalently linked to the 5' end and TAMRA or MGB covalently linked to the 3' end, where FAM is the fluorescent dye and TAMRA or MGB is the quencher dye.

*Analysis of Protein Levels*

Antisense inhibition of AGPAT5 nucleic acids can be assessed by measuring AGPAT5 protein levels. Protein levels of AGPAT5 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) (Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 3rd Ed., 2001). 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.

*In vivo testing of antisense compounds*

Antisense compounds, for example, antisense oligonucleotides, are tested in animals to assess their ability to inhibit expression of AGPAT5 and produce phenotypic changes. Testing can 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 AGPAT5 nucleic acid expression are measured. Changes in AGPAT5 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 a cardiometabolic disease, disorder or condition, or physiological marker thereof. In certain embodiments, the individual has one or more of diabetes (Type I or Type II), metabolic syndrome, insulin resistance, dyslipidemia, hypertriglyceridemia, hyperglycemia, non-alcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), diabetes (e.g., Type 2 diabetes) and high blood pressure.

In certain embodiments, the compounds targeted to AGPAT5 described herein modulate lipid and/or energy metabolism in an animal. In certain embodiments, the compounds targeted to AGPAT5 described herein modulate physiological markers or phenotypes of dyslipidemia, hypertriglyceridemia, metabolic syndrome, insulin resistance, NAFLD, NASH and/or diabetes. For example, administration of the compounds to animals can modulate one or more of triglyceride levels, glucose levels, insulin levels, insulin sensitivity, insulin resistance or AGPAT5 levels. In certain embodiments, the modulation of the physiological markers or phenotypes can be associated with inhibition of AGPAT5 by the compounds.

In certain embodiments, the compounds targeted to AGPAT5 described herein reduce and/or prevent one or more of hepatic TG accumulation (i.e. hepatic steatosis), dyslipidemia, metabolic syndrome, non-alcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), diabetes (e.g., Type 1 or Type 2 diabetes), insulin resistance and high blood pressure. In certain embodiments, the compounds targeted to AGPAT5 described herein improve insulin sensitivity.

In certain embodiments, administration of an antisense compound targeted to an AGPAT5 nucleic acid results in reduction of AGPAT5 expression by about at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99%, or a range defined by any two of these values.

In certain embodiments, pharmaceutical compositions comprising an antisense compound targeted to AGPAT5 are used for the preparation of a medicament for treating a patient suffering from, or susceptible to, a cardiometabolic disease, disorder or condition, or a symptom or physiological marker thereof. In certain embodiments, pharmaceutical compositions comprising an antisense compound targeted to AGPAT5 are used in the preparation of a medicament for treating a patient suffering from, or susceptible to, one or more of diabetes (Type I or Type II), metabolic syndrome, insulin resistance, dyslipidemia, hypertriglyceridemia, non-alcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), diabetes (e.g., Type 2 diabetes) and high blood pressure.

*Administration*

In certain embodiments, the compounds and compositions as described herein are administered parenterally.

In certain embodiments, parenteral administration is by infusion. Infusion can be chronic or continuous or short or intermittent. In certain embodiments, infused pharmaceutical agents are delivered with a pump.

In certain embodiments, parenteral administration is by injection. The injection can be delivered with a syringe or a pump. In certain embodiments, the injection is a bolus injection. In certain embodiments, the injection is administered directly to a tissue or organ. In certain embodiments, the injection is subcutaneous.

*Certain Combination Therapies*

In certain embodiments, a first agent comprising the modified oligonucleotide disclosed herein is co-administered with one or more secondary agents. In certain embodiments, such second agents are designed to treat the same disease, disorder or condition as the first agent described herein. In certain embodiments, such second agents are designed to treat a different disease, disorder, or condition as the first agent described herein. In certain embodiments, such second agents are designed to treat an undesired side effect of one or more pharmaceutical compositions as described herein. In certain embodiments, second agents are co-administered with the first agent to treat an undesired effect of the first agent. In certain embodiments, second agents are co-administered with the first agent to produce a combinational effect. In certain embodiments, second agents are co-administered with the first 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, second agents include, but are not limited to a glucose-lowering agent or a lipid-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 certain embodiments the lipid lowering therapy can include, but is not limited to, a therapeutic lifestyle

change, niacin, HMG-CoA reductase inhibitor, cholesterol absorption inhibitor, MTP inhibitor (e.g., a small molecule, polypeptide, antibody or antisense compound targeted to MTP), fibrate, PCSK9 inhibitor (e.g., PCSK9 antibodies, polypeptides, small molecules nucleic acid compounds targeting PCSK9), CETP inhibitor (e.g., small molecules such as torcetrapib and anacetrapib, polypeptides, antibodies or nucleic acid compounds targeted to CETP), apoC-III inhibitor (e.g., a small molecule, polypeptide, antibody or nucleic acid compounds targeted to apoC-III), apoB inhibitor (e.g., a small molecule, polypeptide, antibody or nucleic acid compounds targeted to apoB), beneficial oils rich in omega-3 fatty acids, omega-3 fatty acids or any combination thereof. The HMG-CoA reductase inhibitor can be atorvastatin, rosuvastatin, fluvastatin, lovastatin, pravastatin, simvastatin and the like. The cholesterol absorption inhibitor can be ezetimibe. The fibrate can be fenofibrate, bezafibrate, ciprofibrate, clofibrate, gemfibrozil and the like. The beneficial oil rich in omega-3 fatty acids can be krill, fish (e.g., Vascepa$^{R}$), flaxseed oil and the like. The omega-3 fatty acid can be ALA, DHA, EPA and the like.

## ADVANTAGES OF THE INVENTION

Provided herein, for the first time, are methods and compositions for the modulation of AGPAT5 that can treat, delay, prevent and/or ameliorate a cardiometabolic disease, disorder or condition, or a physiological marker thereof. In a particular embodiment, for the first time AGPAT5 inhibitors (e.g., antisense oligonucleotides targeting a nucleic acid encoding AGPAT5) are provided for reducing of insulin, insulin resistance, glucose and triglyceride levels in an animal. In a particular embodiment, for the first time AGPAT5 inhibitors (e.g., antisense oligonucleotides targeting a nucleic acid encoding AGPAT5) are provided for increasing insulin sensitivity, glucose clearance and glucose tolerance in an animal

## 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 mouse AGPAT5 by oligomeric compounds

A series of oligomeric compounds was designed to target different regions of mouse 1-acylglycerol-3-phosphate-O-acyltransferase 5 (AGPAT5). The oligomeric compounds are targeted to the mouse mRNA

SEQ ID NO: 1 (GENBANK Accession No. NM_026792.3) and/or the mouse genomic SEQ ID NO: 2 (GENBANK Accession No. NT_039455.7 truncated from nucleotides 15844020 to 15885425).

The compounds were analyzed for their effects on gene target mRNA levels. Mouse bEND cells were plated at a density of 20,000 cells per well in 96 well plates and were transfected using electroporation with 3,000 nM compound or with no compound for untreated controls. After approximately 24 hours, RNA was isolated from the cells and AGPAT5 transcript levels were measured by quantitative real-time PCR using primer probe set RTS3749 (forward: 5'-GAACAAGGTATAATGCAACATACACAAA-3', SEQ ID NO: 3; reverse: 5'-TGGCCTTTATTCTTGGTGTCAGT-3', SEQ ID NO: 4; probe: 5'-CCTTTCAGCCAGTCAGGCATTTGCTG-3', SEQ ID NO: 5). AGPAT5 RNA transcript levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results for the compounds that exhibited greatest inhibition of AGPAT5 transcript expression are presented as average percent inhibition of AGPAT5, relative to untreated control cells, in Table 1.

All compounds in Table 1 are chimeric oligonucleotides ("gapmers") 16 nucleotides in length, composed of a central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3') by three-nucleotide "wings". The "gap" region elicits RNase H activity, and the "wing" regions increase binding affinity to the target. The wings are composed of 4'-$CH(CH_3)$-O-2' nucleotides, also known as constrained ethyl or cEt nucleotides. The internucleoside (backbone) linkages are phosphorothioate throughout the oligonucleotides. All cytosine residues are 5-methylcytosines. The start and stop sites for the compounds are listed in Table 1.

**Table 1**

**Oligomeric compounds targeting mouse AGPAT5**

| ISIS No. | Sequence 5' to 3' | Start site on SEQ ID NO: 1 | Stop Site on SEQ ID NO:1 | Start site on SEQ ID NO:2 | Stop Site on SEQ ID NO:2 | % Inhibition | SEQ ID NO. |
|---|---|---|---|---|---|---|---|
| 574936 | GAGTAGAGGTCCCTGA | n/a | n/a | 3882 | 3897 | 63.3 | 6 |
| 574938 | GGAATAGTGTTTACAC | n/a | n/a | 9144 | 9159 | 61.5 | 7 |
| 574979 | GCATAAGTTAGTGAAC | n/a | n/a | 16656 | 16671 | 60.8 | 8 |
| 574842 | CTTGTCTTTCAGTACG | 632 | 647 | 24935 | 24950 | 66.5 | 9 |
| 574981 | AGTGTACTTACAGTGG | n/a | n/a | 28183 | 28198 | 59.8 | 10 |
| 574905 | GTATTTTCCTGAACCT | 992 | 1007 | 34112 | 34127 | 54.3 | 11 |
| 574948 | AAGTGTTCGATTTTGC | n/a | n/a | 34887 | 34902 | 63.9 | 12 |
| 574919 | CCATGTACAGTTTCCT | 1308 | 1323 | 37873 | 37888 | 48.8 | 13 |
| 574933 | GATAACCAATATAGCG | 1743 | 1758 | 38308 | 38323 | 68.6 | 14 |
| 574868 | AGCTGCCAAGTAGTCA | 1766 | 1781 | 38331 | 38346 | 58.6 | 15 |
| 574935 | AGTATTAAGAGTCTGA | 1781 | 1796 | 38346 | 38361 | 61.8 | 16 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 574874 | CCTTTTTCTTGGAACA | 1897 | 1912 | 38462 | 38477 | 68.1 | 17 |
| 574880 | GACACTTTACACATTA | 2063 | 2078 | 38628 | 38643 | 64.2 | 18 |
| 574890 | AGCACCACTGGAGGAC | 2379 | 2394 | 38944 | 38959 | 60.7 | 19 |
| 574959 | CACGGCAAATCACACG | 2663 | 2678 | 39228 | 39243 | 52.2 | 20 |
| 574962 | GATTCAATTGGTCTAT | 2790 | 2805 | 39355 | 39370 | 53.1 | 21 |
| 574968 | CCAATTCCATGAAGCT | 3276 | 3291 | 39841 | 39856 | 50 | 22 |
| 574972 | TACCACATTTGATAGG | 3542 | 3557 | 40107 | 40122 | 48.7 | 23 |

**Example 2: Antisense inhibition of mouse AGPAT5 *in vivo***

Select compounds listed in Table 1 were further evaluated for antisense inhibition of mouse AGPAT5 *in vivo*. Male C57BL/6 mice were injected intraperitoneally (i.p.) once per week for 6 weeks (a total of 6 doses) with 10 mg/kg or 25 mg/kg of a compound listed in Table 2 or with PBS. Each treatment group consisted of 4 animals. Two days following the final dose, the mice were sacrificed, and hepatic AGPAT5 mRNA expression was quantitated using qRT-PCR, as described in Example 1. The results presented in Table 2 are the average values for each treatment group relative to the average values for the PBS control group.

**Table 2**

**Antisense inhibition of mouse AGPAT5 *in vivo***

| ISIS No. | Dose (mg/kg) | % Inhibition |
|---|---|---|
| 574979 | 10 | 19.4 |
| | 25 | 61.5 |
| 574948 | 10 | 81.5 |
| | 25 | 76.0 |
| 574933 | 10 | 43.0 |
| | 25 | 88.1 |
| 574935 | 10 | 27.7 |
| | 25 | 72.5 |
| 574880 | 10 | 68.7 |
| | 25 | 86.3 |

**Example 3: Effect of an oligomeric compound targeting mouse AGPAT5 in a diet-induced obesity mouse model**

ISIS 574933 (see Tables 1-2) was selected for evaluation in a diet-induced obesity (DIO) mouse model. Male C57BL/6 mice were fed a high fat diet comprising 60% of total calories as fat beginning at 3

weeks of age and continuing until they were sacrificed. After consuming the high fat diet for 3 months, baseline levels of plasma transaminases, cholesterol, glucose, HDL, LDL, triglycerides (TG), nonesterified fatty acids (NEFA), and 3-hydroxybutyrate (3HB) were measured, and body weights of the mice were assessed. Following the baseline assessments, the mice were i.p. injected once per week for 9 weeks (a total of 10 doses) with one of the three doses for ISIS 574933 listed in Table 3, with 25 mg/kg of control cEt gapmer ISIS 549144 (control ASO) that is not targeted to any mouse gene, or with PBS. Each treatment group consisted of 8 animals.

Five days before the final oligonucleotide or PBS dose was administered, an i.p. glucose tolerance test was performed. Following an overnight fast, baseline blood glucose (time= 0 minutes) was measured using a glucometer (Abbott Laboratories, Bedford, MA). Animals were subsequently i.p. injected with 1.5 mg/kg glucose, and additional blood glucose measurements were performed at the time points listed in Table 3. The average results for each treatment group are presented in Table 3, and show that after about 8 weeks of treatment with ISIS 574933, treated mice were able to clear glucose at a higher rate than control treated mice, indicating an improvement in glucose tolerance with ISIS 574933 treatment.

Two days following the final oligonucleotide or PBS dose, the mice were sacrificed. Liver and adipose mRNA levels of AGPAT5 were evaluated using qRT-PCR, as described in Example 1, except that the AGPAT5 mRNA levels were normalized to Cyclophilin A instead of RIBOGREEN®. Results are presented in Table 4 as average % inhibition of AGPAT5 mRNA expression relative to the PBS control group. ISIS 574933 was able to knockdown AGPAT5 expression by more than 70% in both the liver and adipose tissue. Insulin levels were also measured at this time using an ELISA kit from ALPCO according to the manufacturer's instructions. The average results for each treatment group are presented in Table 4 and show that the mice had significantly reduced plasma insulin levels. Taken together, the data in Tables 3-4 show that ISIS 574933 treatment decreased insulin resistance and increased insulin sensitivity, indicated by the decreased levels of insulin needed to affect glucose levels.

Body weights, organ weights, and epididymal white adipose tissue weights (epiWAT) were assessed (Table 5), and plasma transaminases, cholesterol, glucose, HDL, LDL, TG, NEFA, and 3HB levels were measured (Table 6). Body weights and plasma marker results are presented in Tables 5 and 6, respectively, as average percent change relative to baseline levels for each treatment group. The data show that ISIS 574933 ameliorated the body weight gain in the treated mice compared to the control ASO treated mice.

**Table 3**

**Effect of ISIS 574933 on glucose tolerance in a DIO mouse model**

| ISIS No. | Dose (mg/kg) | Glucose (mg/dL) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 minutes | 15 minutes | 30 minutes | 60 minutes | 90 minutes | 120 minutes |
| PBS | n/a | 147 | 347 | 459 | 460 | 418 | 351 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 549144 | 25 | 141 | 439 | 476 | 448 | 397 | 310 |
| 574933 | 5 | 139 | 384 | 464 | 434 | 375 | 326 |
| | 10 | 152 | 356 | 390 | 425 | 382 | 321 |
| | 25 | 128 | 336 | 370 | 358 | 313 | 243 |

**Table 4**

**Effect of ISIS 574933 on AGPAT5 mRNA levels and insulin levels in a DIO mouse model**

| ISIS No. | Dose (mg/kg) | % mRNA Inhibition in liver | % mRNA inhibition in adipose | Insulin (ng/mL) |
|---|---|---|---|---|
| PBS | n/a | 0.0 | 0.0 | 46.2 |
| 549144 | 25 | 4.3 | 1.8 | 22.1 |
| 574933 | 5 | 78.4 | 52.4 | 25.4 |
| | 10 | 88.8 | 72.2 | 11.9 |
| | 25 | 92.8 | 79.8 | 3.9 |

**Table 5**

**Effect of ISIS 574933 on body and organ weights in a DIO mouse model**

| ISIS No. | Dose (mg/kg) | % Body weight change | Kidney weight (g) | Liver weight (g) | Spleen weight (g) | epiWAT mass (g) |
|---|---|---|---|---|---|---|
| PBS | n/a | 22.8 | 0.38 | 2.6 | 0.12 | 1.5 |
| 549144 | 25 | 24.6 | 0.38 | 2.5 | 0.13 | 1.9 |
| 574933 | 5 | 20.8 | 0.37 | 2.1 | 0.14 | 1.8 |
| | 10 | 23.7 | 0.35 | 2.6 | 0.15 | 1.6 |
| | 25 | 13.4 | 0.33 | 2.6 | 0.48 | 1.6 |

**Table 6**

**% change of plasma markers relative to baseline values in a DIO mouse model**

| ISIS No. | Dose | ALT | AST | Cholesterol | Glucose | HDL | LDL | TG | NEFA | 3HB |
|---|---|---|---|---|---|---|---|---|---|---|
| PBS | n/a | 425 | 132 | 14.9 | -2.8 | 26.2 | 40.0 | 25.6 | -61.9 | 51.2 |
| 549144 | 25 | 291 | 109 | 26.7 | -2.5 | 36.4 | 52.5 | 43.4 | -57.3 | 45.1 |
| 574933 | 5 | 234 | 123 | 10.2 | 1.4 | 30.0 | 35.6 | 39.1 | -29.7 | 3.4 |
| | 10 | 485 | 207 | 36.8 | 0.6 | 57.6 | 61.2 | 12.2 | -31.4 | 52.1 |
| | 25 | 1400 | 727 | 17.3 | -4.2 | 37.3 | 37.7 | 27.7 | -45.3 | 23.9 |

**Example 4: Effect of an oligomeric compound targeting mouse AGPAT5 in a diet-induced obesity mouse model**

ISIS 574933 (see Table 1) was further evaluated in the diet-induced obesity (DIO) mouse model described in Example 3 fed the high fat diet for a longer time period. After feeding the male C57BL/6 mice the high fat diet for nearly 5 months, baseline levels of plasma transaminases, cholesterol, glucose, HDL, triglycerides (TG), nonesterified fatty acids (NEFA), and 3-hydroxybutyrate (3HB) were measured, and body weights of the mice were assessed. MRI scans were also performed to analyze body fat, lean, and water masses of the mice using an EchoMRI whole body composition analyzer (Echo Medical Systems, Houston, TX). Following the baseline assessments, the mice were i.p. injected once per week for 8 weeks (a total of 9 doses) with 25 mg/kg of ISIS 574933 or with PBS. Each treatment group consisted of 6 animals.

Two days before the final oligonucleotide or PBS dose was administered, an i.p. glucose tolerance test (IPGTT) was performed as described in Example 3. The results are presented in Table 7, and show that after 8 weeks of treatment with ISIS 574933, treated mice were able to clear plasma glucose at a higher rate than PBS treated mice indicating ISIS 574933 improves glucose tolerance.

Insulin levels were measured using the ALPCO ELISA kit 4 weeks following the first dose (midpoint of the oligonucleotide treatment) and 8 weeks following the first dose (end of the oligonucleotide treatment). The average results for each treatment group are presented in Table 8 and show that the ISIS 574933 treated mice had significantly reduced plasma insulin levels at both the 4 and 8 week timepoints. Taken together, the data in Tables 7-8 indicate that ISIS 574933 treatment decreased insulin resistance and and increased insulin sensitivity as measured by the decreased levels of insulin needed to affect glucose levels.

MRI scans were performed to analyze body fat, lean mass, and water mass 7 weeks following the first oligonucleotide dose. The results are presented in Table 9 as average percent change for each treatment group relative to baseline levels and show that ISIS 574933 ameliorated the fat gain in the treated mice compared to the PBS treated mice.

Five days following the final oligonucleotide or PBS dose, the mice were sacrificed. Liver mRNA levels of AGPAT5 were evaluated using qRT-PCR as described in Example 1, except that the AGPAT5 mRNA levels were normalized to Cyclophilin A instead of RIBOGREEN®. Results are presented in Table 8 as average percent inhibition of AGPAT5 mRNA expression relative to the PBS control group and show that ISIS 574933 was able to significantly knockdown AGPAT5 expression in the liver.

Body weights, organ weights, and epididymal white adipose tissue weights (epiWAT) were assessed, and plasma transaminases, cholesterol, creatinine, glucose, HDL, TG, BUN, NEFA, and 3HB levels were measured. Body weights and plasma marker results are presented in Tables 10 and 11, respectively, as average percent change relative to baseline levels, with the exceptions of creatinine and BUN, which are presented as average absolute levels at time of sacrifice for each treatment group. Organ weights and epiWAT ratios are presented in Table 10 as average absolute levels at time of sacrifice for each treatment

group. The data show that ISIS 574933 administration ameliorated the body weight gain in the treated mice compared to the PBS treated mice.

Liver triglycerides were also measured, and the average results for each treatment group are presented in Table 12. The data show that ISIS 574933 significantly reduced the level of liver triglycerides (hepatic steatosis) in the treated mice.

**Table 7**

**Effect of ISIS 574933 on glucose tolerance in a DIO mouse model**

| ISIS No. | Glucose (mg/dL) | | | | | |
|---|---|---|---|---|---|---|
| | 0 minutes | 15 minutes | 30 minutes | 60 minutes | 90 minutes | 120 minutes |
| PBS | 100 | 315 | 306 | 214 | 159 | 137 |
| 574933 | 105 | 257 | 250 | 202 | 163 | 138 |

**Table 8**

**Effect of ISIS 574933 on AGPAT5 mRNA levels and insulin levels in a DIO mouse model**

| ISIS No. | % mRNA Inhibition in liver | Insulin at 4 weeks (ng/mL) | Insulin at 8 weeks (ng/mL) |
|---|---|---|---|
| PBS | 0.0 | 55.3 | 33.3 |
| 574933 | 86.6 | 6.0 | 4.0 |

**Table 9**

**% change of body fat, lean mass, and water mass in a DIO mouse model**

| ISIS No. | Body fat | Lean mass | Total fluids | Free water | Fat/Body weight |
|---|---|---|---|---|---|
| PBS | 21.4 | 10.3 | 49.5 | 11.3 | 8.4 |
| 574933 | -9.4 | 5.3 | 11.1 | 5.4 | -8. |

**Table 10**

**Effect of ISIS 574933 on body and organ weights in a DIO mouse model**

| ISIS No. | % Body weight change | Liver weight (g) | Kidney weight (g) | Spleen weight (g) | epiWAT mass (g) |
|---|---|---|---|---|---|
| PBS | 9.2 | 2.5 | 0.42 | 0.17 | 1.1 |
| 574933 | -0.8 | 1.7 | 0.43 | 0.26 | 1.2 |

**Table 11**

**% change of plasma markers relative to baseline values in a DIO mouse model**

| ISIS No. | ALT | AST | Cholesterol | Glucose | HDL | TG | NEFA | 3HB | Creatinine (mg/dL) | BUN (mg/dL) |
|---|---|---|---|---|---|---|---|---|---|---|
| PBS | 12.5 | -3.1 | -21.8 | -6.7 | -22.4 | 9.7 | 7.1 | 156 | 0.13 | 25.1 |
| 574933 | 169 | -16.1 | 12.3 | 23.4 | 8.7 | 18.0 | -34.2 | 57.8 | 0.10 | 22.0 |

**Table 12**

**Liver triglycerides in a DIO mouse model**

| ISIS No. | Liver triglycerides (mg TG/g liver wet weight) |
|---|---|
| PBS | 226 |
| 574933 | 51 |

**Example 5: *In vivo* antisense inhibition of murine 1-acylglycerol-3-phosphate O-acyltransferase 5 (AGPAT5)**

Several antisense oligonucleotides were designed that were targeted to murine AGPAT5 (GENBANK Accession No. NM_026792.3, incorporated herein as SEQ ID NO: 1) mRNA.

ISIS 574933 (GATAACCAATATAGCG; SEQ ID NO: 14), which was one of the designed antisense oligonucleotides to target both rat and mouse AGPAT5, is a 3-10-3 cEt gapmer, and is 16 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 3 nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt modification. The internucleoside linkages throughout the gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout the gapmer are 5-methylcytosines. ISIS 574933 is targeted to nucleobases 1743 to 1758 of SEQ ID NO: 1.

ISIS 549144 (GGCCAATACGCCGTCA; SEQ ID NO: 27) is a control oligonucleotide with no known gene target. It was designed as a 3-10-3 cEt gapmer, and is 16 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 3 nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a cEt modification. The internucleoside linkages throughout the gapmer are phosphorothioate (P=S) linkages.

*Treatment*

Groups of Sprague-Dawley rats were placed on a fructose-fed diet for 12 weeks and then randomized, based on fed insulin and fed glucose levels, into one of three groups. Each group contained 10 rats. Two groups of rats were injected subcutaneously with 25 mg/kg of rat AGPAT5 ASO (ISIS 574933) or control oligonucleotide (ISIS 549144), administered weekly for 8 weeks. A control group of rats was injected with phosphate buffered saline (PBS) administered weekly for 8 weeks. Rats underwent IPGTT after 7 weeks of dosing. The animals were sacrificed one day after the last oligonucleotide injection.

*AGPAT5 RNA Analysis*

RNA was extracted from liver and fat tissues for real-time PCR analysis of AGPAT5, using rat AGPAT5 primer probe set (Life Technologies, Assay ID Rn01482872_ml).

The mRNA levels were normalized using Cyclophilin. As shown in Table 13, ISIS 574933 achieved significant reduction of murine AGPAT5 over the PBS control in both liver and fat tissues. Results are presented as percent inhibition of AGPAT5, relative to control.

**Table 13**

**Percent inhibition of murine AGPAT5 mRNA by ISIS 574933 in Sprague-Dawley rats**

| | % |
|---|---|
| Liver | 88 |
| Fat | 53 |

*Effect on glucose tolerance*

Glucose tolerance was measured via the intraperitoneal glucose tolerance test (IPGTT) at week 7. The rats were fasted overnight and then an intraperitoneal administration of 40% glucose at 2 g/kg was given. Blood glucose levels were measured before the glucose challenge and at different time points after challenge up to 120 min.

As presented in Table 14, in antisense oligonucleotide-treated rats, the increase in glucose levels during the IPGTT assay was substantially less than in the control. Therefore, antisense oligonucleotide treated rats had enhanced glucose clearance as compared to the control animals.

**Table 14**

**Glucose tolerance as measured by IPGTT in Sprague-Dawley rats**

| | Time | ISIS 549144 (control) | ISIS 574933 (AGPAT5 ASO) |
|---|---|---|---|
| Body weight | n/a | 558 | 500 |
| Glucose levels mg/kg) | 0 min | 83 | 86 |
| | 15 min | n/a | 419 |
| | 30 min | 496 | 416 |
| | 60 min | 506 | 262 |
| | 90 min | 426 | 194 |
| | 120 min | 355 | 154 |

*Triglyceride levels*

Triglyceride levels were measured with the use of an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY) (Nyblom, H. et al., Alcohol & Alcoholism 39: 336-339, 2004; Tietz NW (Ed): Clinical Guide to Laboratory Tests, 3rd ed. W. B. Saunders, Philadelphia, PA, 1995). The results are presented in Table 15 and are expressed in mg/dL. Antisense inhibition of AGPAT5 resulted in reduction of plasma triglyceride levels compared to the PBS control.

**Table 15**

**Plasma triglyceride levels (mg/dL) in Sprague-Dawley rats**

| | |
|---|---|
| PBS | 465 |
| ISIS 549144 | 210 |
| ISIS 574933 | 112 |

*Liver function*

To evaluate the effect of representative oligonucleotides on hepatic function, plasma concentrations of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY) (Nyblom, H. et al., Alcohol & Alcoholism 39: 336-339, 2004; Tietz NW (Ed): Clinical Guide to Laboratory Tests, 3rd ed. W. B. Saunders, Philadelphia, PA, 1995). Plasma concentrations of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in Table 16

expressed in IU/L. Antisense inhibition of AGPAT5 was considered tolerable in the rats, as demonstrated by their liver transaminase profile.

**Table 16**

**ALT and AST levels (IU/L) of Sprague-Dawley rats**

| | ALT | AST |
|---|---|---|
| PBS | 31 | 52 |
| ISIS 549144 | 46 | 65 |
| ISIS 574933 | 41 | 59 |

**Example 6: Effect of an oligomeric compound targeting mouse AGPAT5 in DBA/2J mice fed a high fat diet**

ISIS 574933 (see Table 1) was further evaluated in DBA/2J mice (a strain prone to obesity and diabetes) fed a high fat diet. Mice received weekly i.p. injection of 20mg/kg or 40mg/kg of ISIS 574933 or 40 mg/kg of control antisense oligonucleotide ISIS 549144 while being fed a high fat diet. After 6 weeks of high fat diet feeding and 6 total doses of oligonucleotide, baseline levels of glucose were measured. Each treatment group consisted of 10 animals.

Three days after the final oligonucleotide dose was administered, insulin sensitivity was assessed by an insulin tolerance test (ITT). The mice were fasted for 5 hours before the ITT. Baseline blood glucose levels were measured before the insulin challenge. The mice were injected intraperitoneally (ip) with insulin (1U/kg) and blood glucose measured at specific time points. The ability of the ip injected insulin to reduce glucose is an indicator of insulin sensitivity.

The results are presented in Table 17 as an average percent reduction in glucose from baseline for each treatment group.

**Table 17**

**Insulin sensitivity as measured by ITT in DBA/2J mice**

| | Time | ISIS 574933 (40 mg/kg) | ISIS 574933 (20 mg/kg) | ISIS 549144 (40 mg/kg) |
|---|---|---|---|---|
| Body weight (grams) | n/a | 36.4 | 38.4 | 40.9 |
| Glucose levels (% basal) | 0 min | 0 | 0 | 0 |
| | 15 min | -19.6 | -16.3 | -6.6 |
| | 30 min | -32.4 | -25.0 | -15.0 |
| | 60 min | -28.3 | -20.8 | -9.4 |
| | 120 min | -16.5 | -12.0 | -7.2 |

As presented in Table 17, in ISIS 574933 treated mice, the decrease in glucose levels during the ITT was substantially more than in control oligonucleotide ISIS 549144 treated mice after 6 weeks of treatment. Additionally, there was a dose-dependent reduction of glucose with the higher dose of ISIS 574933 (40 mg/kg) reducing glucose levels more than the lower dose of ISIS 574933 (20 mg/kg). Taken together, the data in Table 17 indicate that AGPAT5 antisense oligonucleotide treatment increased insulin sensitivity in a statistically significant and dose-dependent manner.

**What is claimed is:**

1. A method of reducing AGPAT5 expression in an animal comprising administering to the animal a composition comprising an AGPAT5 specific inhibitor, wherein expression of AGPAT5 is reduced in the animal.

2. A method of reducing insulin resistance in an animal comprising administering to the animal a composition comprising an AGPAT5 specific inhibitor, wherein the level of insulin resistance is reduced in the animal.

3. A method of increasing insulin sensitivity in an animal comprising administering to the animal a composition comprising an AGPAT5 specific inhibitor, wherein the level of insulin sensitivity is increased in the animal.

4. A method of reducing liver triglyceride levels in an animal comprising administering to the animal a composition comprising an AGPAT5 specific inhibitor, wherein the level of liver triglyceride is reduced in the animal.

5. A method of reducing insulin levels in an animal comprising administering to the animal a composition comprising an AGPAT5 specific inhibitor, wherein the level of insulin is reduced in the animal.

6. A method of increasing glucose tolerance and/or glucose clearance in an animal comprising administering to the animal a composition comprising an AGPAT5 specific inhibitor, wherein the glucose tolerance and/or glucose clearance of the animal is increased.

7. A method of reducing the ratio of fat to body weight in an animal comprising administering to the animal a composition comprising an AGPAT5 specific inhibitor, wherein the ratio of fat to body weight is reduced in the animal.

8. A method of ameliorating metabolic and/or cardiovascular disease in an animal comprising administering to the animal a therapeutically effective amount of a composition comprising an AGPAT5 specific inhibitor, wherein the metabolic and/or cardiovascular disease is ameliorated in the animal.

9. A method for treating an animal at risk for a metabolic and/or cardiovascular disease comprising administering to said animal a therapeutically effective amount of a composition comprising an AGPAT5 specific inhibitor, wherein said animal at risk for the metabolic and/or cardiovascular disease is treated.

10. The method of any of claims 1-9, wherein the AGPAT5 specific inhibitor is selected from any of a nucleic acid compound, a polypeptide, an antibody and a small molecule.

11. The method of claim 10, wherein the nucleic acid compound is an antisense compound.

12. The method of claim 11, wherein the antisense compound comprises a modified oligonucleotide that is 10 to 30 linked nucleosides in length and has a nucleobase sequence at least 90% complementary to any of SEQ ID NOs: 1-2, 24-26 as measured over the entirety of said modified oligonucleotide.

13. The method of claim 12, wherein the nucleobase sequence comprises at least 8 contiguous nucleobases of a sequence recited in any one of SEQ ID NOs: 6-23.

14. The method of any one of claims 1-13, wherein the animal is a human.

15. The method of claim 12, wherein the modified oligonucleotide is single-stranded.

16. The method of claim 12, wherein the nucleobase sequence of the modified oligonucleotide is at least 95% complementary to any one of SEQ ID NOs: 1-2, 24-26 as measured over the entirety of said modified oligonucleotide.

17. The method of claim 12, wherein the nucleobase sequence of the modified oligonucleotide is 100% complementary to any one of SEQ ID NOs: 1-2, 24-26 as measured over the entirety of said modified oligonucleotide.

18. The method of claim 12, wherein at least one internucleoside linkage of said modified oligonucleotide is a modified internucleoside linkage.

19. The method of claim 18, wherein each internucleoside linkage is a phosphorothioate internucleoside linkage.

20. The method of claim 12, wherein at least one nucleoside of said modified oligonucleotide comprises a modified sugar.

21. The method of claim 20, wherein at least one modified sugar is a bicyclic sugar.

22. The method of claim 20, wherein at least one modified sugar comprises a 2'-O- methoxyethyl.

23. The method of claim 21, wherein at least one modified sugar comprises a 4'- $CH(CH_3)$-O-2' bridge or a 4'- $(CH_2)_n$-O-2' bridge, wherein n is 1 or 2.

24. The method of claim 12, wherein at least one nucleoside of said modified oligonucleotide comprises a modified nucleobase.

25. The method of claim 24, wherein the modified nucleobase is a 5-methylcytosine.

26. The method of claim 12, wherein the modified oligonucleotide consists of 16-20 linked nucleosides.

27. The method of claim 12, wherein the modified oligonucleotide comprises:

a). a gap segment consisting of linked deoxynucleosides;

b). a 5' wing segment consisting of linked nucleosides;

c). 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.

28. The method of claim 12, wherein the modified oligonucleotide consists of 16 linked nucleosides, has a nucleobase sequence comprising at least 8 contiguous nucleobases of a nucleobase sequence selected from any of SEQ ID NO: 6-23 and comprises:

a) a gap segment consisting of ten linked deoxynucleosides;

b) a 5' wing segment consisting of three linked nucleosides;

c) 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, wherein each nucleoside of each wing segment comprises a 4'-$CH(CH_3)$-O-2' sugar, wherein each internucleoside linkage is a phosphorothioate linkage, and wherein each cytosine is a 5-methylcytosine.

29. The method of claim 8 or 9, wherein the therapeutically effective amount of the composition administered to the animal reduces insulin resistance in a diabetic or non-diabetic animal.

30. The method of claim 8 or 9, wherein the metabolic and/or cardiovascular disease is obesity, diabetes, insulin resistance, atherosclerosis, dyslipidemia, coronary heart disease, non-alcoholic fatty liver disease (NAFLD), hyperfattyacidemia or metabolic syndrome, or a combination thereof.

31. The method of claim 30, wherein the NAFLD is hepatic steatosis or steatohepatitis.

32. The method of claim 30, wherein the diabetes is type 2 diabetes or type 2 diabetes with dyslipidemia.

33. The method of claim 1, 8 or 9, wherein administering the composition reduces triglyceride levels, insulin levels, insulin resistance, glucose levels or a combination thereof.

34. The method of claim 33, wherein the levels are independently reduced by at least 5%, 10%, 20%, 30%, 35%, or 40%.

35. The method of claim 1, 8 or 9, wherein administering the composition increases glucose tolerance, glucose clearance and/or insulin sensitivity.

36. The method of claim 35, wherein glucose tolerance, glucose clearance and/or insulin sensitivity is increased by at least 5%, 10%, 20%, 30%, 35%, or 40%.

37. The method of any one of claims 1-9, wherein the composition is a first agent and further comprising administering a second agent.

38. The method of claim 37, wherein the first agent and the second agent are co-administered.

39. The method of any of claim 37, wherein the second agent is a glucose-lowering agent.

40. The method of claim 39, wherein the glucose-lowering agent is 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.

41. The method of claim 39, wherein the glucose-lowering agent is metformin, sulfonylurea, meglitinide, thiazolidinedione, rosiglitazone, or a combination thereof.

42. The method of claim 39, wherein the glucose-lowering agent is selected from acetohexamide, chlorpropamide, tolbutamide, tolazamide, glimepiride, a glipizide, a glyburide, or a gliclazide.

43. The method of claim 39, wherein the glucose-lowering agent is selected from nateglinide or repaglinide.

44. The method of claim 39, wherein the glucose-lowering agent is selected from pioglitazone or rosiglitazone.

45. The method of claim 39, wherein the glucose-lowering agent is selected from acarbose or miglitol.

46. The method of claim 37, wherein the second agent is a lipid-lowering therapy.

47. The method of claim 46, wherein the lipid-lowering therapy is a therapeutic lifestyle change, HMG CoA reductase inhibitor, cholesterol absorption inhibitor, MTP inhibitor, antisense compound targeted to ApoB or any combination thereof

48. The method of claim 46, wherein the lipid-lowering therapy is selected from atorvastatin, rosuvastatin, fluvastatin, lovastatin, pravastatin, or simvastatin.

49. The method of claim 46, wherein the lipid-lowering therapy is ezetimibe.

50. The method of any one of claims 1-9, wherein administration comprises parenteral administration.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/60591

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 9/10, C12N 15/63, C12N 15/113, A61P 3/00, A61P 3/06, A61P 3/10 (2016.01)
CPC - C12N 9/1025, A61K 38/00, A61K 48/00, C12N 15/113, C12N 15/1137
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 9/10, C12N 15/63, C12N 15/113, A61P 3/00, A61P 3/06, A61P 3/10 (2016.01)
CPC - C12N 9/1025, A61K 38/00, A61K 48/00, C12N 15/113, C12N 15/1137

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched (keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar
Search terms: AGPAT5 or LPAATE or 1AGPAT5 or 1-AGPAT5 or AGPAT-5 or AGPAT 5, acylglycerolphosphate acyltransferase, inhibit, antagonize, reduce, block, lower, slience, antisense, RNAi, siRNA, miRNA,1-acylglycerol-3-phosphate O-acyltransferase 5, 1-acyl-sn-gly

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | US 2003/0064439 A1 (BANDARU et al.) 03 April 2003 (03.04.2003) para [0176], [0385], [0728], [0732], [0744], [0745], [0746], [0750], [1065], [1074],[1144], [1465] | 1-13, 15-50 |
| Y | TAKEUCHI et al., Biochemistry, physiology, and genetics of GPAT, AGPAT, and lipin enzymes in triglyceride synthesis. Am J Physiol Endocrinol Metab, June 2009, Vol 296, No 6, pp E1195-209. Especially abstract; p E1202, col 1, para 3 | 1-13, 15-50 |
| Y | US 2010/0048545 A1 (JETTE et al.) 25 February 2010 (25.02.2010) para [0329] | 31-32, 39-45 |
| Y | US 2004/0198733 A1 (SCHWINK et al.) 07 October 2004 (07.10.2004) para [0004], [0118] | 46-49 |
| Y | NM_026792.3, Mus musculus 1-acylglycerol-3-phosphate O-acyltransferase 5 (lysophosphatidic acid acyltransferase, epsilon) (Agpat5), mRNA. 19 April 2014 [online]. [Retrieved on 4 February 2016]. Retrieved from the internet: <URL: http://www.ncbi.nlm.nih.gov/nuccore/127139102?sat=21&satkey=2754421> entire document | 12-13, 15-28 |
| Y | JN959516, GenBank Accession No. JN959516, Mus musculus targeted non-conditional, lacZ-tagged mutant allele Agpat5:tm1e(EUCOMM)Hmgu; transgenic, 5 November 2011 [online]. [Retrieved on 4 April 2016]. Retrieved from the internet: <URL: http://www.ncbi.nlm.nih.gov/nuccore/JN959516> entire document | 13, 28 |

☒ 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 |
|---|---|
| 04 April 2016 | 14 APR 2016 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-8300 | Authorized officer:<br>Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

## INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/60591

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | WO 2004/063329 A2 (ISIS PHARMACEUTICALS INC. et al.) 29 July 2004 (29.07.2004) p 7, ln 26-27; p 9, ln 3-6; p 17, ln 2-3; p 17, ln 9-10; p 19, ln 1-14; p 20, ln 1-11; p 43, ln 21-26; p 60, ln 17-23 | 18-25, 27-28 |
| A | US 2009/0036355 A1 (BHANOT et al.) 05 February 2009 (05.02.2009) para [0008] | 1-11, 29-30, 33-38, 50 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/60591

**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.: 14
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:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-13, 15-50, drawn to a method of reducing AGPAT5 expression in an animal. The method of reducing AGPAT5 expression will be searched to the extent that the nucleic acid compound is complementary to SEQ ID NO: 1 (claims 12, 16, 17) and comprises the nucleobase sequence of SEQ ID NO: 6 (claims 13, 28). It is believed that claims 1-13, 15-50, limited to SEQ ID NOs: 1 and 6, encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass a method of reducing AGPAT5 expression wherein the nucleic acid compound is complementary to SEQ ID NO: 1 and comprises the nucleobase sequence of SEQ ID NO: 6. Additional sequences to which the nucleic acid compound is complementary and nucleobase sequences of the nucleic acid compound will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected complementary sequence(s) and nucleobase sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to ----
----please seee extra sheet-------

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.:
1-13, 15-50, limited to SEQ ID NOs: 1 and 6

**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.

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

clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a method of reducing AGPAT5 expression wherein the nucleic acid compound is complementary to SEQ ID NO: 2 and comprises the nucleobase sequence of SEQ ID NO: 7, i.e. claims 1-13, 15-50, limited to SEQ ID NOs: 2 and 7.

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

The technical feature of each of the inventions listed as Group I+ is the specific complementary sequence and nucleobase sequence recited therein. Each invention requires a complementary sequence and nucleobase sequence, not required by any of the other inventions.

Common Technical Features

The feature shared by Group I+ is an AGPAT5 specific inhibitor and methods of administering to an animal a composition comprising an AGPAT5 specific inhibitor,
Another feature shared by Group I+ is wherein the inhibitor is an antisense compound of claim 11.
Another feature shared by Group I+ is wherein the antisense compound comprises a modified oligonucleotide that is 10 to 30 linked nucleosides in length of claim 12
Another feature shared by Group I+ is the method of reducing AGPAT5 expression in an animal of claim 1.
Another feature shared by Group I+ is the methods of reducing insulin resistance, increasing insulin sensitivity, and reducing insulin levels of claims 2, 3, and 5.
Another feature shared by Group I+ is the method of reducing liver triglyceride levels of claim 4.
Another feature shared by Group I+ is the method of increasing glucose tolerance and/or glucose clearance of claim 6.
Another feature shared by Group I+ is the method of of reducing the ratio of fat to body weight in an animal of claim 7
Another feature shared by Group I+ is the methods of ameliorating metabolic and/or cardiovascular disease in an animal and for treating an animal at risk for a metabolic and/or cardiovascular disease of claims 8 and 9.

However, these shared technical features do not represent a contribution over prior art, because the shared technical features are taught by US 2003/0064439 A1 to Bandaru et al. (hereinafter 'Bandaru') in view of the article entitled "Biochemistry, physiology, and genetics of GPAT, AGPAT, and lipin enzymes in triglyceride synthesis" by Takeuchi et al. (Am J Physiol Endocrinol Metab, June 2009, Vol 296, No 6, pp E1195-209) (hereinafter 'Takeuchi').

Bandaru discloses [claim 1] a method of reducing acyltransferase expression in an animal comprising administering to the animal a composition comprising an acyltransferase specific inhibitor, wherein expression of acyltransferase is reduced in the animal (para [0732] "antisense and ribozyme molecules that inhibit expression of the target gene can also be used in accordance with the invention to reduce the level of target gene expression, thus effectively reducing the level of target gene activity"; para [0744] "In another embodiment, the agent inhibits one or more acyltransferase activities. Examples of such inhibitory agents include antisense acyltransferase nucleic acid molecules"; para [0745] "inhibition of acyltransferase activity is desirable in situations in which acyltransferase is abnormally upregulated and/or in which decreased acyltransferase activity is likely to have a beneficial effect").

Bandaru does not specifically teach that the acyltransferase is AGPAT5. However, Takeuchi teaches that AGPAT5, an acylglycerolphosphate acyltransferase, is overexpressed in a mouse model exhibiting elevated plasma glucose and insulin levels characteristic of insulin resistance (abstract "acylglycerolphosphate acyltransferase (AGPAT)"; p E1202, col 1, para 3 "As observed in CGL patients, Agpat2-/-mice have elevated plasma glucose and insulin levels, characteristic of insulin resistance (26). Evidence suggests that impaired insulin signaling in the liver may also occur, contributing to insulin resistance. ... Expression levels for several other AGPAT genes were increased in the liver of Agpat2-/- male mice, including ... Agpat5 (1.6-fold)").

Given that Bandaru teaches that an acyltransferase inhibitor can be used to treat a subject having a disorder associated with aberrant acyltransferase expression, including metabolic disorders such as diabetes that is marked by insulin resistance (para [0728] "The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a, disorder associated with aberrant or unwanted acyltransferase expression or activity"; para [0746] "The acyltransferase molecules can act as novel diagnostic targets and therapeutic agents for controlling ... cardiovascular disorders, as described above, as well as disorders associated with lipid metabolism, ... or metabolic disorders"; para [0750] "acyltransferase may play an important role in the regulation of metabolism or pain disorders. Diseases of metabolic imbalance include, but are not limited to, obesity, ... lipid disorders, and diabetes", note that diabetes is marked by insulin resistance see for example US 2009/0036355 A1 to Bhanot et al. para [0008]), one of ordinary skill in the art would have found it obvious to target the acyltransferase inhibitor of Bandaru to AGPAT5, as taught by Takeuchi, in order to treat insulin resistance and other hallmarks of metabolic disorders.

Bandaru further teaches [claim 11] that the inhibitory compound is an antisense compound (para [0732] "antisense and ribozyme molecules that inhibit expression of the target gene can also be used in accordance with the invention to reduce the level of target gene expression, thus effectively reducing the level of target gene activity").

Bandaru further teaches [claim 12] that the antisense compound comprises a modified oligonucleotide that is 10 to 30 linked nucleosides in length (para [0175] "An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids"; para [0174] "An antisense oligonucleotide can be, for example, about 7, 10, 15, 20, 25, 30, ... nucleotides in length").

------- please see continuation on next extra sheet -------

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

Bandaru discloses [claim 2] a method of reducing acyltransferase expression in an animal comprising administering to the animal a composition comprising an acyltransferase specific inhibitor, wherein expression of acyltransferase is reduced in the animal (para [0732] "antisense and ribozyme molecules that inhibit expression of the target gene can also be used in accordance with the invention to reduce the level of target gene expression, thus effectively reducing the level of target gene activity"; para [0744] "In another embodiment, the agent inhibits one or more acyltransferase activities. Examples of such inhibitory agents include antisense acyltransferase nucleic acid molecules"; para [0745] "inhibition of acyltransferase activity is desirable in situations in which *acyltransferase is abnormally upregulated and/or in which decreased acyltransferase activity is likely to have a beneficial effect*").

Bandaru does not specifically teach that the acyltransferase is AGPAT5, or that the AGPAT5 specific inhibitor reduces insulin resistance in an animal. However, Takeuchi teaches that AGPAT5, an acylglycerolphosphate acyltransferase, is overexpressed in a mouse model exhibiting elevated plasma glucose and insulin levels characteristic of insulin resistance (abstract "acylglycerolphosphate acyltransferase (AGPAT)"; p E1202, col 1, para 3 "As observed in CGL patients, Agpat2-/-mice have elevated plasma glucose and insulin levels, characteristic of insulin resistance (26). Evidence suggests that impaired insulin signaling in the liver may also occur, contributing to insulin resistance. ... Expression levels for several other AGPAT genes were increased in the liver of Agpat2-/- male mice, including ... Agpat5 (1.6-fold)"). Given that Bandaru teaches that an acyltransferase inhibitor can be used to treat a subject having a disorder associated with aberrant acyltransferase expression, including metabolic disorders such as diabetes that is marked by insulin resistance (para [0728] "The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a, disorder associated with aberrant or unwanted acyltransferase expression or activity"; para [0746] "The acyltransferase molecules can act as novel diagnostic targets and therapeutic agents for controlling ... cardiovascular disorders, as described above, as well as disorders associated with lipid metabolism, ... or metabolic disorders"; para [0750] "acyltransferase may play an important role in the regulation of metabolism or pain disorders. Diseases of metabolic imbalance include, but are not limited to, obesity, ... lipid disorders, and diabetes", note that diabetes is marked by insulin resistance see for example US 2009/0036355 A1 to Bhanot et al. para [0008]), one of ordinary skill in the art would have found it obvious to target the acyltransferase inhibitor of Bandaru to AGPAT5, as taught by Takeuchi, in order to treat insulin resistance in subjects with diabetes.

Bandaru discloses [claim 3] a method of reducing acyltransferase expression in an animal comprising administering to the animal a composition comprising an acyltransferase specific inhibitor, wherein expression of acyltransferase is reduced in the animal (para [0732] "antisense and ribozyme molecules that inhibit expression of the target gene can also be used in accordance with the invention to reduce the level of target gene expression, thus effectively reducing the level of target gene activity"; para [0744] "In another embodiment, the agent inhibits one or more acyltransferase activities. Examples of such inhibitory agents include antisense acyltransferase nucleic acid molecules"; para [0745] "inhibition of acyltransferase activity is desirable in situations in which acyltransferase is abnormally upregulated and/or in which decreased acyltransferase activity is likely to have a beneficial effect").

Bandaru does not specifically teach that the acyltransferase is AGPAT5, or that the AGPAT5 specific inhibitor increases insulin sensitivity in an animal. However, Takeuchi teaches that AGPAT5, an acylglycerolphosphate acyltransferase, is overexpressed in a mouse model exhibiting elevated plasma glucose and insulin levels characteristic of insulin resistance (abstract "acylglycerolphosphate acyltransferase (AGPAT)"; p E1202, col 1, para 3 "As observed in CGL patients, Agpat2-/-mice have elevated plasma glucose and insulin levels, characteristic of insulin resistance (26). Evidence suggests that impaired insulin signaling in the liver may also occur, contributing to insulin resistance. ... Expression levels for several other AGPAT genes were increased in the liver of Agpat2-/- male mice, including ... Agpat5 (1.6-fold)"). Given that Bandaru teaches that an acyltransferase inhibitor can be used to treat a subject having a disorder associated with aberrant acyltransferase expression, including metabolic disorders such as diabetes that is marked by insulin resistance (para [0728] "The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a, disorder associated with aberrant or unwanted acyltransferase expression or activity"; para [0746] "The acyltransferase molecules can act as novel diagnostic targets and therapeutic agents for controlling ... cardiovascular disorders, as described above, as well as disorders associated with lipid metabolism, ... or metabolic disorders"; para [0750] "acyltransferase may play an important role in the regulation of metabolism or pain disorders. Diseases of metabolic imbalance include, but are not limited to, obesity, ... lipid disorders, and diabetes", note that diabetes is marked by insulin resistance see for example US 2009/0036355 A1 to Bhanot et al. para [0008])), one of ordinary skill in the art would have found it obvious to target the acyltransferase inhibitor of Bandaru to AGPAT5, as taught by Takeuchi, in order to reverse insulin resistance in subjects with diabetes by increasing insulin sensitivity.

Bandaru discloses [claim 4] a method of reducing acyltransferase expression in an animal comprising administering to the animal a composition comprising an acyltransferase specific inhibitor, wherein expression of acyltransferase is reduced in the animal (para [0732] "antisense and ribozyme molecules that inhibit expression of the target gene can also be used in accordance with the invention to reduce the level of target gene expression, thus effectively reducing the level of target gene activity"; para [0744] "In another embodiment, the agent inhibits one or more acyltransferase activities. Examples of such inhibitory agents include antisense acyltransferase nucleic acid molecules"; para [0745] "inhibition of acyltransferase activity is desirable in situations in which acyltransferase is abnormally upregulated and/or in which decreased acyltransferase activity is likely to have a beneficial effect").

Bandaru does not specifically teach that the acyltransferase is AGPAT5, or that the AGPAT5 specific inhibitor reduces liver triglyceride levels in an animal. However, Takeuchi teaches that acylglycerolphosphate acyltransferase (AGPAT) is involved in triglyceride biosynthesis (p col 2, para 2) and that AGPAT5 is overexpressed in a mouse model exhibiting elevated liver TAG (abstract "acylglycerolphosphate acyltransferase (AGPAT)"; p E1202, col 1, para 3 "As observed in CGL patients, Agpat2-/-mice have elevated plasma glucose and insulin levels, characteristic of insulin resistance (26). Evidence suggests that impaired insulin signaling in the liver may also occur, contributing to insulin resistance. Male mice exhibited more profound hepatic steatosis than female mice, characterized by a sixfold increase in liver TAG content. Expression levels for several other AGPAT genes were increased in the liver of Agpat2-/- male mice, including ... Agpat5 (1.6-fold)"). Given that Bandaru teaches that an acyltransferase inhibitor can be used to treat a subject having a disorder associated with aberrant acyltransferase expression, including metabolic disorders such as obesity that is marked by high triglycerides (para [0728] "The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a, disorder associated with aberrant or unwanted acyltransferase expression or activity"; para [0746] "The acyltransferase molecules can act as novel diagnostic targets and therapeutic agents for controlling ... cardiovascular disorders, as described above, as well as disorders associated with lipid metabolism, ... or metabolic disorders"; para [0750] "acyltransferase may play an important role in the regulation of metabolism or pain disorders. Diseases of metabolic imbalance include, but are not limited to, obesity, ... lipid disorders, and diabetes", note that metabolic disorders such as obesity exhibit high triglycerides see for example US 2009/0036355 A1 to Bhanot et al. para [0008])), one of ordinary skill in the art would have found it obvious to target

------ please see continuation on next extra sheet -------

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

the acyltransferase inhibitor of Bandaru to AGPAT5, as taught by Takeuchi, in order to reduce liver triglyceride levels in an animal with a metabolic disorder such as obesity.

Bandaru discloses [claim 5] a method of reducing acyltransferase expression in an animal comprising administering to the animal a composition comprising an acyltransferase specific inhibitor, wherein expression of acyltransferase is reduced in the animal (para [0732] "antisense and ribozyme molecules that inhibit expression of the target gene can also be used in accordance with the invention to reduce the level of target gene expression, thus effectively reducing the level of target gene activity"; para [0744] "In another embodiment, the agent inhibits one or more acyltransferase activities. Examples of such inhibitory agents include antisense acyltransferase nucleic acid molecules"; para [0745] "inhibition of acyltransferase activity is desirable in situations in which acyltransferase is abnormally upregulated and/or in which decreased acyltransferase activity is likely to have a beneficial effect").
Bandaru does not specifically teach that the acyltransferase is AGPAT5, or that the AGPAT5 specific inhibitor reduces insulin levels in an animal. However, Takeuchi teaches that AGPAT5, an acylglycerolphosphate acyltransferase, is overexpressed in a mouse model exhibiting elevated plasma glucose and insulin levels characteristic of insulin resistance (abstract "acylglycerolphosphate acyltransferase (AGPAT)"; p E1202, col 1, para 3 "As observed in CGL patients, Agpat2-/-mice have elevated plasma glucose and insulin levels, characteristic of insulin resistance (26). Evidence suggests that impaired insulin signaling in the liver may also occur, contributing to insulin resistance. ... Expression levels for several other AGPAT genes were increased in the liver of Agpat2-/- male mice, including ... Agpat5 (1.6-fold)"). Given that Bandaru teaches that an acyltransferase inhibitor can be used to treat a subject having a disorder associated with aberrant acyltransferase expression, including metabolic disorders such as diabetes that is marked by insulin resistance (para [0728] "The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a, disorder associated with aberrant or unwanted acyltransferase expression or activity"; para [0746] "The acyltransferase molecules can act as novel diagnostic targets and therapeutic agents for controlling ... cardiovascular disorders, as described above, as well as disorders associated with lipid metabolism, ... or metabolic disorders"; para [0750] "acyltransferase may play an important role in the regulation of metabolism or pain disorders. Diseases of metabolic imbalance include, but are not limited to, obesity, ... lipid disorders, and diabetes", note that diabetes is marked by insulin resistance see for example US 2009/0036355 A1 to Bhanot et al. para [0008])), one of ordinary skill in the art would have found it obvious to target the acyltransferase inhibitor of Bandaru to AGPAT5, as taught by Takeuchi, in order to reduce insulin levels in subjects with diabetes.

Bandaru discloses [claim 6] a method of reducing acyltransferase expression in an animal comprising administering to the animal a composition comprising an acyltransferase specific inhibitor, wherein expression of acyltransferase is reduced in the animal (para [0732] "antisense and ribozyme molecules that inhibit expression of the target gene can also be used in accordance with the invention to reduce the level of target gene expression, thus effectively reducing the level of target gene activity"; para [0744] "In another embodiment, the agent inhibits one or more acyltransferase activities. Examples of such inhibitory agents include antisense acyltransferase nucleic acid molecules"; para [0745] "inhibition of acyltransferase activity is desirable in situations in which acyltransferase is abnormally upregulated and/or in which decreased acyltransferase activity is likely to have a beneficial effect").
Bandaru does not specifically teach that the acyltransferase is AGPAT5, or that the AGPAT5 specific inhibitor increases glucose tolerance and/or glucose clearance in an animal. However, Takeuchi teaches that AGPAT5, an acylglycerolphosphate acyltransferase, is overexpressed in a mouse model exhibiting elevated plasma glucose and insulin levels characteristic of insulin resistance (abstract "acylglycerolphosphate acyltransferase (AGPAT)"; p E1202, col 1, para 3 "As observed in CGL patients, Agpat2-/-mice have elevated plasma glucose and insulin levels, characteristic of insulin resistance (26). Evidence suggests that impaired insulin signaling in the liver may also occur, contributing to insulin resistance. ... Expression levels for several other AGPAT genes were increased in the liver of Agpat2-/- male mice, including ... Agpat5 (1.6-fold)"). Given that Bandaru teaches that an acyltransferase inhibitor can be used to treat a subject having a disorder associated with aberrant acyltransferase expression, including metabolic disorders such as diabetes that is marked by insulin resistance (para [0728] "The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a, disorder associated with aberrant or unwanted acyltransferase expression or activity"; para [0746] "The acyltransferase molecules can act as novel diagnostic targets and therapeutic agents for controlling ... cardiovascular disorders, as described above, as well as disorders associated with lipid metabolism, ... or metabolic disorders"; para [0750] "acyltransferase may play an important role in the regulation of metabolism or pain disorders. Diseases of metabolic imbalance include, but are not limited to, obesity, ... lipid disorders, and diabetes", note that diabetes is marked by insulin resistance see for example US 2009/0036355 A1 to Bhanot et al. para [0008])), one of ordinary skill in the art would have found it obvious to target the acyltransferase inhibitor of Bandaru to AGPAT5, as taught by Takeuchi, in order to increase glucose tolerance and/or glucose clearance in in subjects with diabetes.

Bandaru discloses [claim 7] a method of reducing acyltransferase expression in an animal comprising administering to the animal a composition comprising an acyltransferase specific inhibitor, wherein expression of acyltransferase is reduced in the animal (para [0732] "antisense and ribozyme molecules that inhibit expression of the target gene can also be used in accordance with the invention to reduce the level of target gene expression, thus effectively reducing the level of target gene activity"; para [0744] "In another embodiment, the agent inhibits one or more acyltransferase activities. Examples of such inhibitory agents include antisense acyltransferase nucleic acid molecules"; para [0745] "inhibition of acyltransferase activity is desirable in situations in which acyltransferase is abnormally upregulated and/or in which decreased acyltransferase activity is likely to have a beneficial effect").
Bandaru does not specifically teach that the acyltransferase is AGPAT5, or that the AGPAT5 specific inhibitor reduces the ratio of fat to body weight in an animal. However, Takeuchi teaches that acylglycerolphosphate acyltransferase (AGPAT) is involved in triglyceride biosynthesis (p col 2, para 2) and that AGPAT5 is overexpressed in a mouse model exhibiting elevated liver TAG (abstract "acylglycerolphosphate acyltransferase (AGPAT)"; p E1202, col 1, para 3 "As observed in CGL patients, Agpat2-/-mice have elevated plasma glucose and insulin levels, characteristic of insulin resistance (26). Evidence suggests that impaired insulin signaling in the liver may also occur, contributing to insulin resistance. Male mice exhibited more profound hepatic steatosis than female mice, characterized by a sixfold increase in liver TAG content. Expression levels for several other AGPAT genes were increased in the liver of Agpat2-/- male mice, including ... Agpat5 (1.6-fold)"). Given that Bandaru teaches that an acyltransferase inhibitor can be used to treat a subject having a disorder associated with aberrant acyltransferase expression, including metabolic disorders such as obesity that is marked by high triglycerides (para [0728] "The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a, disorder associated with aberrant or unwanted acyltransferase expression or activity"; para [0746] "The acyltransferase molecules can act as novel diagnostic targets and therapeutic agents for controlling ... cardiovascular disorders, as described above, as well as disorders associated with lipid metabolism, ... or metabolic disorders"; para [0750] "acyltransfe

------- please see continuation on next extra sheet--------

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

may play an important role in the regulation of metabolism or pain disorders. Diseases of metabolic imbalance include, but are not limited to, obesity, ... lipid disorders, and diabetes", note that metabolic disorders such as obesity exhibit high triglycerides see for example US 2009/0036355 A1 to Bhanot et al. para [0008])), one of ordinary skill in the art would have found it obvious to target the acyltransferase inhibitor of Bandaru to AGPAT5, as taught by Takeuchi, in order to reduce the ratio of fat to body weight in an animal with a metabolic disorder such as obesity.

Bandaru discloses [claim 8] a method of ameliorating metabolic and/or cardiovascular disease in an animal (para [0728] "The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a, disorder associated with aberrant or unwanted acyltransferase expression or activity"; para [0746] "The acyltransferase molecules can act as novel diagnostic targets and therapeutic agents for controlling ... cardiovascular disorders, as described above, as well as disorders associated with lipid metabolism, ... or metabolic disorders") comprising administering to the animal a therapeutically effective amount of a composition comprising an acyltransferase specific inhibitor (para [0732] "antisense and ribozyme molecules that inhibit expression of the target gene can also be used in accordance with the invention to reduce the level of target gene expression, thus effectively reducing the level of target gene activity"; para [0744] "In another embodiment, the agent inhibits one or more acyltransferase activities. Examples of such inhibitory agents include antisense acyltransferase nucleic acid molecules"; para [0745] "inhibition of acyltransferase activity is desirable in situations in which acyltransferase is abnormally upregulated and/or in which decreased acyltransferase activity is likely to have a beneficial effect"), wherein the metabolic and/or cardiovascular disease is ameliorated in the animal (para [0728], [0746]).
Bandaru does not specifically teach that the acyltransferase is AGPAT5. However, Takeuchi teaches that AGPAT5, an acylglycerolphosphate acyltransferase, is overexpressed in a mouse model exhibiting elevated plasma glucose and insulin levels characteristic of insulin resistance found in metabolic disorders (abstract "acylglycerolphosphate acyltransferase (AGPAT)"; p E1202, col 1, para 3 "As observed in CGL patients, Agpat2-/-mice have elevated plasma glucose and insulin levels, characteristic of insulin resistance (26). Evidence suggests that impaired insulin signaling in the liver may also occur, contributing to insulin resistance. ... Expression levels for several other AGPAT genes were increased in the liver of Agpat2-/- male mice, including ... Agpat5 (1.6-fold)", note that metabolic syndrome is marked by insulin resistance see for example US 2009/0036355 A1 to Bhanot et al. para [0008]). Given that Bandaru teaches that an acyltransferase inhibitor can be used to treat a subject having a disorder associated with aberrant acyltransferase expression, including metabolic disorders and cardiovascular disease (para [0728], [0746]). one of ordinary skill in the art would have found it obvious to target the acyltransferase inhibitor of Bandaru to AGPAT5, as taught by Takeuchi, in order to ameliorate metabolic and/or cardiovascular disease.

Bandaru discloses [claim 9] a method for treating an animal at risk for a metabolic and/or cardiovascular disease (para [0728] "The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a, disorder associated with aberrant or unwanted acyltransferase expression or activity"; para [0746] "The acyltransferase molecules can act as novel diagnostic targets and therapeutic agents for controlling ... cardiovascular disorders, as described above, as well as disorders associated with lipid metabolism, ... or metabolic disorders") comprising administering to said animal a therapeutically effective amount of a composition comprising an acyltransferase specific inhibitor (para [0732] "antisense and ribozyme molecules that inhibit expression of the target gene can also be used in accordance with the invention to reduce the level of target gene expression, thus effectively reducing the level of target gene activity"; para [0744] "In another embodiment, the agent inhibits one or more acyltransferase activities. Examples of such inhibitory agents include antisense acyltransferase nucleic acid molecules"; para [0745] "inhibition of acyltransferase activity is desirable in situations in which acyltransferase is abnormally upregulated and/or in which decreased acyltransferase activity is likely to have a beneficial effect"), wherein said animal at risk for the metabolic and/or cardiovascular disease is treated (para [0728], [0746]).
Bandaru does not specifically teach that the acyltransferase is AGPAT5. However, Takeuchi teaches that AGPAT5, an acylglycerolphosphate acyltransferase, is overexpressed in a mouse model exhibiting elevated plasma glucose and insulin levels characteristic of insulin resistance found in metabolic disorders (abstract "acylglycerolphosphate acyltransferase (AGPAT)"; p E1202, col 1, para 3 "As observed in CGL patients, Agpat2-/-mice have elevated plasma glucose and insulin levels, characteristic of insulin resistance (26). Evidence suggests that impaired insulin signaling in the liver may also occur, contributing to insulin resistance. ... Expression levels for several other AGPAT genes were increased in the liver of Agpat2-/- male mice, including ... Agpat5 (1.6-fold)", note that metabolic syndrome is marked by insulin resistance see for example US 2009/0036355 A1 to Bhanot et al. para [0008]). Given that Bandaru teaches that an acyltransferase inhibitor can be used to treat a subject having a disorder associated with aberrant acyltransferase expression, including metabolic disorders and cardiovascular disease (para [0728], [0746]). one of ordinary skill in the art would have found it obvious to target the acyltransferase inhibitor of Bandaru to AGPAT5, as taught by Takeuchi, in order to treat an animal at risk for a metabolic and/or cardiovascular disease.

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups. The inventions listed as Group I+ therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Item 4 (continued)

Claim 14 is a dependent claim and is not drafted in accordance with the second and third sentences of Rule 6.4(a).