COMPOSITIONS AND METHODS FOR MODULATING COMPLEMENT FACTOR B EXPRESSION

Sequence Listing

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled BIOL0251WOSEQ_ST25.txt created April 28, 2015, which is 204 kb in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

Field

The present embodiments provide methods, compounds, and compositions for treating, preventing, or ameliorating a disease associated with dysregulation of the complement alternative pathway by administering a Complement Factor B (CFB) specific inhibitor to a subject.

Background

The complement system is part of the host innate immune system involved in lysing foreign cells, enhancing phagocytosis of antigens, clumping antigen-bearing agents, and attracting macrophages and neutrophils. The complement system is divided into three initiation pathways—the classical, lectin, and alternative pathways—that converge at component C3 to generate an enzyme complex known as C3 convertase, which cleaves C3 into C3a and C3b. C3b associates with C3 convertase mediated by CFB and results in generation of C5 convertase, which cleaves C5 into C5a and C5b, which initiates the membrane attack pathway resulting in the formation of the membrane attack complex (MAC) comprising components C5b, C6, C7, C8, and C9. The membrane-attack complex (MAC) forms transmembrane channels and disrupts the phospholipid bilayer of target cells, leading to cell lysis.

In the homeostatic state, the alternative pathway is continuously activated at a low "tickover" level as a result of activation of the alternative pathway by spontaneous hydrolysis of C3 and the production of C3b, which generates C5 convertase.

Summary

The complement system mediates innate immunity and plays an important role in normal inflammatory response to injury, but its dysregulation may cause severe injury. Activation of the alternative complement pathway beyond its constitutive "tickover" level can lead to unrestrained hyperactivity and manifest as diseases of complement dysregulation.

Certain embodiments provided herein relate to methods of treating, preventing, or ameliorating a disease associated with dysregulation of the complement alternative pathway in a subject by administration of a Complement Factor B (CFB) specific inhibitor. Several embodiments provided herein are drawn to a method of inhibiting expression of CFB in a subject having, or at risk of having, a disease associated with dysregulation of the complement alternative pathway by administering a CFB specific inhibitor to the subject. In certain embodiments, a method of reducing or inhibiting accumulation of C3 deposits in the eye of a subject having, or at risk of having, a disease associated with dysregulation of the complement alternative pathway comprises administering a CFB specific inhibitor to the subject. In several embodiments, a method of reducing or inhibiting accumulation of C3 deposits in the kidney of a subject having, or at risk of having, a disease associated with dysregulation of the complement alternative pathway comprises administering a CFB specific inhibitor to the subject.

Detailed Description

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

Unless specific definitions are provided, the nomenclature used in connection with, and the procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques may be used for chemical synthesis, and chemical analysis. Certain such techniques and procedures may be found for example in "Carbohydrate Modifications in Antisense Research" Edited by Sangvi and Cook, American Chemical Society , Washington D.C., 1994; "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., 21st edition, 2005; and "Antisense Drug Technology, Principles, Strategies, and Applications" Edited by Stanley T. Crooke, CRC Press, Boca Raton, Florida; and Sambrook et al., "Molecular Cloning, A laboratory Manual," 2nd Edition, Cold Spring Harbor Laboratory Press, 1989, which are hereby incorporated by reference for any purpose. Where permitted, all patents, applications, published applications and other publications and other data referred to throughout in the disclosure are

incorporated by reference herein in their entirety.

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

"2'-F nucleoside" refers to a nucleoside comprising a sugar comprising fluorine at the 2' position. Unless otherwise indicated, the fluorine in a 2'-F nucleoside is in the ribo position (replacing the OH of a natural ribose).

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

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

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

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

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

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

"About" means within ±10% of a value. For example, if it is stated, "the compounds affected at least about 70% inhibition of CFB", it is implied that CFB levels are inhibited within a range of 60% and 80%.

"Administration" or "administering" refers to routes of introducing an antisense compound provided herein to a subject to perform its intended function. An example of a route of administration that can be used includes, but is not limited to parenteral administration, such as subcutaneous, intravenous, or intramuscular injection or infusion.

"Alkyl," as used herein, means a saturated straight or branched hydrocarbon radical containing up to twenty four carbon atoms. Examples of alkyl groups include without limitation, methyl, ethyl, propyl, butyl, isopropyl, n-hexyl, octyl, decyl, dodecyl and the like. Alkyl groups typically include from 1 to about 24 carbon atoms, more typically from 1 to about 12 carbon atoms (C1-C12 alkyl) with from 1 to about 6 carbon atoms being more preferred.

As used herein, "alkenyl," means a straight or branched hydrocarbon chain radical containing up to twenty four carbon atoms and having at least one carbon-carbon double bond. Examples of alkenyl groups

include without limitation, ethenyl, propenyl, butenyl, 1-methyl-2-buten-1-yl, dienes such as 1,3-butadiene and the like. Alkenyl groups typically include from 2 to about 24 carbon atoms, more typically from 2 to about 12 carbon atoms with from 2 to about 6 carbon atoms being more preferred. Alkenyl groups as used herein may optionally include one or more further substituent groups.

As used herein, "alkynyl," means a straight or branched hydrocarbon radical containing up to twenty four carbon atoms and having at least one carbon-carbon triple bond. Examples of alkynyl groups include, without limitation, ethynyl, 1-propynyl, 1-butynyl, and the like. Alkynyl groups typically include from 2 to about 24 carbon atoms, more typically from 2 to about 12 carbon atoms with from 2 to about 6 carbon atoms being more preferred. Alkynyl groups as used herein may optionally include one or more further substituent groups.

As used herein, "acyl," means a radical formed by removal of a hydroxyl group from an organic acid and has the general Formula -C(O)-X where X is typically aliphatic, alicyclic or aromatic. Examples include aliphatic carbonyls, aromatic carbonyls, aliphatic sulfonyls, aromatic sulfinyls, aliphatic sulfinyls, aromatic phosphates, aliphatic phosphates and the like. Acyl groups as used herein may optionally include further substituent groups.

As used herein, "alicyclic" means a cyclic ring system wherein the ring is aliphatic. The ring system can comprise one or more rings wherein at least one ring is aliphatic. Preferred alicyclics include rings having from about 5 to about 9 carbon atoms in the ring. Alicyclic as used herein may optionally include further substituent groups.

As used herein, "aliphatic" means a straight or branched hydrocarbon radical containing up to twenty four carbon atoms wherein the saturation between any two carbon atoms is a single, double or triple bond. An aliphatic group preferably contains from 1 to about 24 carbon atoms, more typically from 1 to about 12 carbon atoms with from 1 to about 6 carbon atoms being more preferred. The straight or branched chain of an aliphatic group may be interrupted with one or more heteroatoms that include nitrogen, oxygen, sulfur and phosphorus. Such aliphatic groups interrupted by heteroatoms include without limitation, polyalkoxys, such as polyalkylene glycols, polyamines, and polyimines. Aliphatic groups as used herein may optionally include further substituent groups.

As used herein, "alkoxy" means a radical formed between an alkyl group and an oxygen atom wherein the oxygen atom is used to attach the alkoxy group to a parent molecule. Examples of alkoxy groups include without limitation, methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, sec-butoxy, tert-butoxy, n-pentoxy, neopentoxy, n-hexoxy and the like. Alkoxy groups as used herein may optionally include further substituent groups.

As used herein, "aminoalkyl" means an amino substituted C1-C12 alkyl radical. The alkyl portion of the radical forms a covalent bond with a parent molecule. The amino group can be located at any position and the aminoalkyl group can be substituted with a further substituent group at the alkyl and/or amino portions.

As used herein, "aralkyl" and "arylalkyl" mean an aromatic group that is covalently linked to a C1-C12 alkyl radical. The alkyl radical portion of the resulting aralkyl (or arylalkyl) group forms a covalent bond with a parent molecule. Examples include without limitation, benzyl, phenethyl and the like. Aralkyl groups as used herein may optionally include further substituent groups attached to the alkyl, the aryl or both groups that form the radical group.

As used herein, "aryl" and "aromatic" mean a mono- or polycyclic carbocyclic ring system radicals having one or more aromatic rings. Examples of aryl groups include without limitation, phenyl, naphthyl, tetrahydronaphthyl, indanyl, idenyl and the like. Preferred aryl ring systems have from about 5 to about 20 carbon atoms in one or more rings. Aryl groups as used herein may optionally include further substituent groups.

“Amelioration” refers to a lessening of at least one indicator, sign, or symptom of an associated disease, disorder, or condition. In certain embodiments, amelioration includes a delay or slowing in the progression of one or more indicators of a condition or disease. The severity of indicators may be determined by subjective or objective measures, which are known to those skilled in the art.

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

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

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

“Antisense mechanisms” are all those mechanisms involving hybridization of a compound with target nucleic acid, wherein the outcome or effect of the hybridization is either target degradation or target

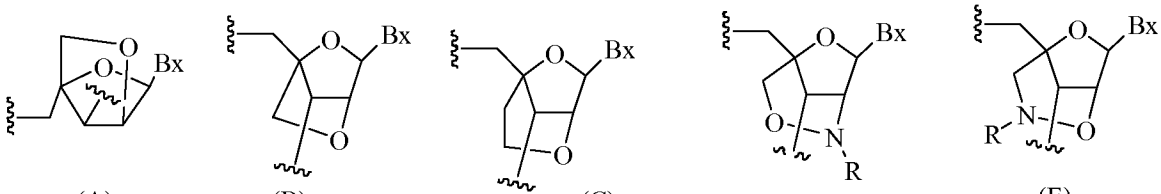
occupancy with concomitant stalling of the cellular machinery involving, for example, transcription or splicing.

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

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

"Bicyclic sugar moiety" means a modified sugar moiety comprising a 4 to 7 membered ring (including but not limited to a furanosyl) comprising a bridge connecting two atoms of the 4 to 7 membered ring to form a second ring, resulting in a bicyclic structure. In certain embodiments, the 4 to 7 membered ring is a sugar ring. In certain embodiments the 4 to 7 membered ring is a furanosyl. In certain such embodiments, the bridge connects the 2'-carbon and the 4'-carbon of the furanosyl.

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



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

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

alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, acyl (C(=O)-H), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C_1-C_{12} aminoalkyl, substituted C_1-C_{12} aminoalkyl or a protecting group.

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

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

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

"Carbohydrate" means a naturally occurring carbohydrate, a modified carbohydrate, or a carbohydrate derivative.

"Carbohydrate cluster" means a compound having one or more carbohydrate residues attached to a scaffold or linker group. (*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).

"Carbohydrate derivative" means any compound which may be synthesized using a carbohydrate as a starting material or intermediate.

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

"Chemical modification" means a chemical difference in a compound when compared to a naturally occurring counterpart. Chemical modifications of oligonucleotides include nucleoside modifications (including sugar moiety modifications and nucleobase modifications) and internucleoside linkage

modifications. In reference to an oligonucleotide, chemical modification does not include differences only in nucleobase sequence.

"Cleavable bond" means any chemical bond capable of being split. 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.

"Cleavable moiety" means a bond or group that is capable of being split under physiological conditions. In certain embodiments, a cleavable moiety is cleaved inside a cell or sub-cellular compartments, such as a lysosome. In certain embodiments, a cleavable moiety is 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.

"Conjugate" or "conjugate group" means an atom or group of atoms bound to an oligonucleotide or 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 linker" or "linker" in the context of a conjugate group means a portion of a conjugate group comprising any atom or group of atoms and which covalently link (1) an oligonucleotide to another portion of the conjugate group or (2) two or more portions of the conjugate group.

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 the 3'-oxygen atom of the 3'-hydroxyl group of the 3' terminal nucleoside of the oligomeric compound. 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 of the oligomeric compound. In certain embodiments, the bond for forming attachment to the oligomeric compound is a cleavable bond. In certain such embodiments, such cleavable bond constitutes all or part of a cleavable moiety.

In certain embodiments, conjugate groups comprise a cleavable moiety (e.g., a cleavable bond or cleavable nucleoside) and a carbohydrate cluster portion, such as a GalNAc cluster portion. Such carbohydrate cluster portion comprises: a targeting moiety and, optionally, a conjugate linker. In certain embodiments, the carbohydrate cluster portion is identified by the number and identity of the ligand. For example, in certain embodiments, the carbohydrate cluster portion comprises 3 GalNAc groups and is designated "GalNAc3". In certain embodiments, the carbohydrate cluster portion comprises 4 GalNAc groups and is designated "GalNAc4". Specific carbohydrate cluster portions (having specific tether, branching and conjugate linker groups) are described herein and designated by Roman numeral followed by

subscript "a". Accordingly "GalNac3-1a" refers to a specific carbohydrate cluster portion of a conjugate group having 3 GalNac groups and specifically identified tether, branching and linking groups. Such carbohydrate cluster fragment is attached to an oligomeric compound via a cleavable moiety, such as a cleavable bond or cleavable nucleoside.

"Conjugate compound" means any atoms, group of atoms, or group of linked atoms suitable for use as a conjugate group. In certain embodiments, conjugate compounds may possess or impart one or more properties, including, but not limited to pharmacodynamic, pharmacokinetic, binding, absorption, cellular distribution, cellular uptake, charge and/or clearance properties.

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

"Complement Factor B (CFB)" means any nucleic acid or protein of CFB. "CFB nucleic acid" means any nucleic acid encoding CFB. For example, in certain embodiments, a CFB nucleic acid includes a DNA sequence encoding CFB, an RNA sequence transcribed from DNA encoding CFB (including genomic DNA comprising introns and exons), including a non-protein encoding (*i.e.* non-coding) RNA sequence, and an mRNA sequence encoding CFB. "CFB mRNA" means an mRNA encoding a CFB protein.

"CFB specific inhibitor" refers to any agent capable of specifically inhibiting CFB RNA and/or CFB protein expression or activity at the molecular level. For example, CFB specific inhibitors include nucleic acids (including antisense compounds), peptides, antibodies, small molecules, and other agents capable of inhibiting the expression of CFB RNA and/or CFB protein.

"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 compounds" means antisense compounds that have at least 2 chemically distinct regions, each position having a plurality of subunits.

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

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

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

"Deoxynucleoside" means a nucleoside comprising 2'-H furanosyl sugar moiety, as found in naturally occurring deoxyribonucleosides (DNA). In certain embodiments, a 2'-deoxynucleoside may comprise a modified nucleobase or may comprise an RNA nucleobase (e.g., uracil).

"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.

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

"Differently modified" mean chemical modifications or chemical substituents that are different from one another, including absence of modifications. Thus, for example, a MOE nucleoside and an unmodified DNA nucleoside are "differently modified," even though the DNA nucleoside is unmodified. Likewise, DNA and RNA are "differently modified," even though both are naturally-occurring unmodified nucleosides. Nucleosides that are the same but for comprising different nucleobases are not differently modified. For example, a nucleoside comprising a 2'-OMe modified sugar and an unmodified adenine nucleobase and a nucleoside comprising a 2'-OMe modified sugar and an unmodified thymine nucleobase are not differently modified.

"Double-stranded" refers to two separate oligomeric compounds that are hybridized to one another. Such double stranded compounds may have one or more or non-hybridizing nucleosides at one or both ends of one or both strands (overhangs) and/or one or more internal non-hybridizing nucleosides (mismatches) provided there is sufficient complementarity to maintain hybridization under physiologically relevant conditions.

"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 may vary among individuals depending on the health and physical condition of the individual to be treated, the taxonomic group of the individuals to be treated, the formulation of the composition, assessment of the individual's medical condition, and other relevant factors.

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

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

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

"Furanosyl" means a structure comprising a 5-membered ring comprising four carbon atoms and one oxygen atom.

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

"Halo" and "halogen," mean an atom selected from fluorine, chlorine, bromine and iodine.

"Heteroaryl," and "heteroaromatic," mean a radical comprising a mono- or poly-cyclic aromatic ring, ring system or fused ring system wherein at least one of the rings is aromatic and includes one or more heteroatoms. Heteroaryl is also meant to include fused ring systems including systems where one or more of the fused rings contain no heteroatoms. Heteroaryl groups typically include one ring atom selected from sulfur, nitrogen or oxygen. Examples of heteroaryl groups include without limitation, pyridinyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl, oxadiazolyl, thiophenyl, furanyl, quinolinyl, isoquinolinyl, benzimidazolyl, benzooxazolyl, quinoxalinyl and the like. Heteroaryl radicals can be attached to a parent molecule directly or through a linking moiety such as an aliphatic group or hetero atom. Heteroaryl groups as used herein may optionally include further substituent groups.

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

"Identifying an animal having, or at risk for having, a disease, disorder and/or condition" means identifying an animal having been diagnosed with the disease, disorder and/or condition or identifying an animal predisposed to develop the disease, disorder and/or condition. Such identification may be accomplished by any method including evaluating an individual's medical history and standard clinical tests or assessments.

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

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

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

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

"Internucleoside neutral linking group" means a neutral linking group that directly links two nucleosides.

"Internucleoside phosphorus linking group" means a phosphorus linking group that directly links two nucleosides.

"Lengthened" antisense oligonucleotides are those that have one or more additional nucleosides relative to an antisense oligonucleotide disclosed herein.

"Linkage motif" means a pattern of linkage modifications in an oligonucleotide or region thereof. The nucleosides of such an oligonucleotide may be modified or unmodified. Unless otherwise indicated, motifs herein describing only linkages are intended to be linkage motifs. Thus, in such instances, the nucleosides are not limited.

"Linked deoxynucleoside" means a nucleic acid base (A, G, C, T, U) substituted by deoxyribose linked by a phosphate ester to form a nucleotide.

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

"Locked nucleic acid nucleoside" or "LNA" means a nucleoside comprising a bicyclic sugar moiety comprising a 4'-CH2-O-2'bridge.

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

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

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

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

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

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

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

"Modulating" refers to changing or adjusting a feature in a cell, tissue, organ or organism. For example, modulating CFB mRNA can mean to increase or decrease the level of CFB mRNA and/or CFB protein in a cell, tissue, organ or organism. A "modulator" effects the change in the cell, tissue, organ or organism. For example, a CFB antisense compound can be a modulator that decreases the amount of CFB mRNA and/or CFB protein in a cell, tissue, organ or organism.

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

"Mono or polycyclic ring system" is meant to include all ring systems selected from single or polycyclic radical ring systems wherein the rings are fused or linked and is meant to be inclusive of single and mixed ring systems individually selected from aliphatic, alicyclic, aryl, heteroaryl, aralkyl, arylalkyl, heterocyclic, heteroaryl, hetero¬aromatic and heteroarylalkyl. Such mono and poly cyclic structures can contain rings that each have the same level of saturation or each, independently, have varying degrees of saturation including fully saturated, partially saturated or fully unsaturated. Each ring can comprise ring atoms selected from C, N, O and S to give rise to hetero¬cyclic rings as well as rings comprising only C ring atoms which can be present in a mixed motif such as for example benzimidazole wherein one ring has only carbon ring atoms and the fused ring has two nitrogen atoms. The mono or polycyclic ring system can be further substituted with substituent groups such as for example phthalimide which has two =O groups attached to one of the rings. Mono or polycyclic ring systems can be attached to parent molecules using various strategies such as directly through a ring atom, fused through multiple ring atoms, through a substituent group or through a bifunctional linking moiety.

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

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

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

"Neutral linking group" means a linking group that is not charged. Neutral linking groups include without limitation phospho¬triesters, methylphosphonates, MMI (-CH2-N(CH3)-O-), amide-3 (-CH2-C(=O)-N(H)-), amide-4 (-CH2-N(H)-C(=O)-), formacetal (-O-CH2-O-), and thioformacetal (-S-CH2-O-). Further neutral linking groups include nonionic linkages comprising siloxane (dialkylsiloxane), carboxylate ester, carboxamide, sulfide, sulfonate ester and amides (See for example: Carbohydrate Modifications in Antisense Research; Y.S. Sanghvi and P.D. Cook Eds. ACS Symposium Series 580; Chapters 3 and 4, (pp. 40-65)). Further neutral linking groups include nonionic linkages comprising mixed N, O, S and CH2 component parts.

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

"Non-internucleoside neutral linking group" means a neutral linking group that does not directly link two nucleosides. In certain embodiments, a non-internucleoside neutral linking group links a nucleoside to a group other than a nucleoside. In certain embodiments, a non-internucleoside neutral linking group links two groups, neither of which is a nucleoside.

"Non-internucleoside phosphorus linking group" means a phosphorus linking group that does not directly link two nucleosides. In certain embodiments, a non-internucleoside phosphorus linking group links a nucleoside to a group other than a nucleoside. In certain embodiments, a non-internucleoside phosphorus linking group links two groups, neither of which is a nucleoside.

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

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

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

"Nucleobase modification motif" means a pattern of modifications to nucleobases along an oligonucleotide. Unless otherwise indicated, a nucleobase modification motif is independent of the nucleobase sequence.

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

"Nucleoside" means a nucleobase linked to a sugar.

"Nucleoside mimetic" includes those structures used to replace the sugar or the sugar and the base and not necessarily the linkage at one or more positions of an oligomeric compound such as for example nucleoside mimetics having morpholino, cyclohexenyl, cyclohexyl, tetrahydropyranyl, bicyclo or tricyclo sugar mimetics, *e.g.*, non furanose sugar units. Nucleotide mimetic includes those structures used to replace the nucleoside and the linkage at one or more positions of an oligomeric compound such as for example peptide nucleic acids or morpholinos (morpholinos linked by -N(H)-C(=O)-O- or other non-phosphodiester linkage). Sugar surrogate overlaps with the slightly broader term nucleoside mimetic but is intended to

indicate replacement of the sugar unit (furanose ring) only. The tetrahydropyranyl rings provided herein are illustrative of an example of a sugar surrogate wherein the furanose sugar group has been replaced with a tetrahydropyranyl ring system. "Mimetic" refers to groups that are substituted for a sugar, a nucleobase, and/or internucleoside linkage. Generally, a mimetic is used in place of the sugar or sugar-internucleoside linkage combination, and the nucleobase is maintained for hybridization to a selected target.

"Nucleoside motif" means a pattern of nucleoside modifications in an oligonucleotide or a region thereof. The linkages of such an oligonucleotide may be modified or unmodified. Unless otherwise indicated, motifs herein describing only nucleosides are intended to be nucleoside motifs. Thus, in such instances, the linkages are not limited.

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

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

"Oligonucleoside" means an oligonucleotide in which the internucleoside linkages do not contain a phosphorus atom.

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

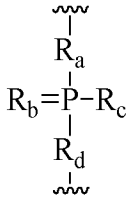
"Parenteral administration" means administration through injection or infusion. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration, e.g.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

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

"Phosphorus linking group" means a linking group comprising a phosphorus atom. Phosphorus linking groups include without limitation groups having the formula:



wherein:

R_a and R_d are each, independently, O, S, CH_2, NH, or NJ_1 wherein J_1 is C_1-C_6 alkyl or substituted C_1-C_6 alkyl;

R_b is O or S;

R_c is OH, SH, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_1-C_6 alkoxy, substituted C_1-C_6 alkoxy, amino or substituted amino; and

J_1 is R_b is O or S.

Phosphorus linking groups include without limitation, phosphodiester, phosphorothioate, phosphorodithioate, phosphonate, phosphoramidate, phosphorothioamidate, thionoalkylphosphonate, phosphotriesters, thionoalkylphosphotriester and boranophosphate.

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

"Prodrug" means an inactive or less active form of a compound which, when administered to a subject, is metabolized to form the active, or more active, compound (e.g., drug).

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

"Protecting group" means any compound or protecting group known to those having skill in the art. Non-limiting examples of protecting groups may be found in "Protective Groups in Organic Chemistry", T. W. Greene, P. G. M. Wuts, ISBN 0-471-62301-6, John Wiley & Sons, Inc, New York, which is incorporated herein by reference in its entirety.

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

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

"RISC based antisense compound" means an antisense compound wherein at least some of the antisense activity of the antisense compound is attributable to the RNA Induced Silencing Complex (RISC).

"RNase H based antisense compound" means an antisense compound wherein at least some of the antisense activity of the antisense compound is attributable to hybridization of the antisense compound to a target nucleic acid and subsequent cleavage of the target nucleic acid by RNase H.

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

"Separate regions" means portions of an oligonucleotide wherein the chemical modifications or the motif of chemical modifications of any neighboring portions include at least one difference to allow the separate regions to be distinguished from one another.

"Sequence motif" means a pattern of nucleobases arranged along an oligonucleotide or portion thereof. Unless otherwise indicated, a sequence motif is independent of chemical modifications and thus may have any combination of chemical modifications, including no chemical modifications.

"Side effects" means physiological disease and/or conditions 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 may indicate liver toxicity or liver function abnormality. For example, increased bilirubin may indicate liver toxicity or liver function abnormality.

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

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

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

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

"Substituent" and "substituent group," means an atom or group that replaces the atom or group of a named parent compound. For example a substituent of a modified nucleoside is any atom or group that differs from the atom or group found in a naturally occurring nucleoside (e.g., a modified 2'-substuent is any atom or group at the 2'-position of a nucleoside other than H or OH). Substituent groups can be protected or unpro¬tected. In certain embodiments, compounds of the present disclosure have substituents at one or at

more than one position of the parent compound. Substituents may also be further substituted with other substituent groups and may be attached directly or via a linking group such as an alkyl or hydro¬carbyl group to a parent compound.

Likewise, as used herein, "substituent" in reference to a chemical functional group means an atom or group of atoms that differs from the atom or a group of atoms normally present in the named functional group. In certain embodiments, a substituent replaces a hydrogen atom of the functional group (e.g., in certain embodiments, the substituent of a substituted methyl group is an atom or group other than hydrogen which replaces one of the hydrogen atoms of an unsubstituted methyl group). Unless otherwise indicated, groups amenable for use as substituents include without limitation, halogen, hydroxyl, alkyl, alkenyl, alkynyl, acyl (-C¬(O)¬Raa), carboxyl (-C(O)O-Raa), aliphatic groups, ali-cyclic groups, alkoxy, substituted oxy (-O-Raa), aryl, aralkyl, heterocyclic radical, hetero¬aryl, hetero-arylalkyl, amino (N(Rbb)¬(Rcc)), imino(=NRbb), amido (C(O)N¬(Rbb)(Rcc) or N(Rbb)C(O)Raa), azido (-N3), nitro (NO2), cyano (-CN), carbamido (OC(O)N(Rbb)(Rcc) or N(Rbb)¬C(O)¬ORaa), ureido (N(Rbb)C(O)¬N(Rbb)(Rcc)), thioureido (N(Rbb)C¬¬¬(S)N(Rbb)¬(Rcc)), guanidinyl (N(Rbb)¬C(=NRbb)-N(Rbb)(Rcc)), amidinyl (C(=NRbb)¬¬¬N(Rbb)(Rcc) or N(Rbb)C(=NRbb)(Raa)), thiol (-SRbb), sulfinyl (S(O)Rbb), sulfonyl (-S(O)2Rbb) and sulfonamidyl (-S(O)2N(Rbb)(Rcc) or N(Rbb)¬S¬¬(O)2Rbb). Wherein each Raa, Rbb and Rcc is, independently, H, an optionally linked chemical functional group or a further substituent group with a preferred list including without limitation, alkyl, alkenyl, alkynyl, aliphatic, alkoxy, acyl, aryl, aralkyl, heteroaryl, alicyclic, heterocyclic and hetero¬aryl¬alkyl. Selected substituents within the compounds described herein are present to a recursive degree.

"Substituted sugar moiety" means a furanosyl that is not a naturally occurring sugar moiety. Substituted sugar moieties include, but are not limited to furanosyls comprising substituents at the 2'-position, the 3'-position, the 5'-position and/or the 4'-position. Certain substituted sugar moieties are bicyclic sugar moieties.

"Sugar moiety" means a naturally occurring sugar moiety or a modified sugar moiety of a nucleoside.

"Sugar motif" means a pattern of sugar modifications in an oligonucleotide or a region thereof.

"Sugar surrogate" means a structure that does not comprise a furanosyl and that is capable of replacing the naturally occurring sugar moiety of a nucleoside, such that the resulting nucleoside sub-units are capable of linking together and/or linking to other nucleosides to form an oligomeric compound which is capable of hybridizing to a complementary oligomeric compound. Such structures include rings comprising a different number of atoms than furanosyl (e.g., 4, 6, or 7-membered rings); replacement of the oxygen of a furanosyl with a non-oxygen atom (e.g., carbon, sulfur, or nitrogen); or both a change in the number of atoms and a replacement of the oxygen. Such structures may also comprise substitutions corresponding to those described for substituted sugar moieties (e.g., 6-membered carbocyclic bicyclic sugar surrogates optionally

comprising additional substituents). Sugar surrogates also include more complex sugar replacements (e.g., the non-ring systems of peptide nucleic acid). Sugar surrogates include without limitation morpholinos, cyclohexenyls and cyclohexitols.

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

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

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

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

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

"Terminal group" means one or more atom attached to either, or both, the 3' end or the 5' end of an oligonucleotide. In certain embodiments a terminal group is a conjugate group. In certain embodiments, a terminal group comprises one or more terminal group nucleosides.

"Terminal internucleoside linkage" means the linkage between the last two nucleosides of an oligonucleotide or defined region thereof.

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

"Treat" refers to administering a pharmaceutical composition to an animal in order to effect an alteration or improvement of a disease, disorder, or condition in the animal. In certain embodiments, one or more pharmaceutical compositions can be administered to the animal.

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

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

Certain Embodiments

Certain embodiments provide methods, compounds and compositions for inhibiting Complement Factor B (CFB) expression.

Certain embodiments provide antisense compounds targeted to a CFB nucleic acid. In certain embodiments, the CFB nucleic acid has the sequence set forth in GENBANK Accession No. NM_001710.5 (incorporated herein as SEQ ID NO: 1), GENBANK Accession No. NT_007592.15 truncated from nucleotides 31852000 to 31861000 (incorporated herein as SEQ ID NO: 2), GENBANK Accession No NW_001116486.1 truncated from nucleotides 536000 to 545000 (incorporated herein as SEQ ID NO: 3), GENBANK Accession No. XM_001113553.2 (incorporated herein as SEQ ID NO: 4), or GENBANK Accession No. NM_008198.2 (incorporated herein as SEQ ID NO: 5).

Certain embodiments provide a compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides and has a nucleobase sequence comprising at least 8 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 6-808.

Certain embodiments provide a compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides and has a nucleobase sequence comprising at least 9 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 6-808.

Certain embodiments provide a compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides and has a nucleobase sequence comprising at least 10 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 6-808.

Certain embodiments provide a compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides and has a nucleobase sequence comprising at least 11 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 6-808.

Certain embodiments provide a compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides and has a nucleobase sequence comprising at least 12 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 6-808.

Certain embodiments provide a compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides and has a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 6-808.

Certain embodiments provide a compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of the nucleobase sequence of any one of SEQ ID NOs: 6-808.

Certain embodiments provide a compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides complementary within nucleobases 30-49, 48-63, 150-169, 151-170, 152-171, 154-169, 154-173, 156-171, 156-175, 157-176, 158-173, 158-177, 480-499, 600-619, 638-657, 644-663, 738-757, 1089-1108, 1135-1154, 1141-1160, 1147-1166, 1150-1169, 1153-1172, 1159-1178, 1162-1181, 1165-1184, 1171-1186, 1171-1190, 1173-1188, 1173-1192, 1175-1190, 1175-1194, 1177-1196, 1183-1202, 1208-1227, 1235-1254, 1298-1317, 1304-1323, 1310-1329, 1316-1335, 1319-1338, 1322-1341, 1328-1347, 1349-1368, 1355-1374, 1393-1412, 1396-1415, 1399-1418, 1405-1424, 1421-1440, 1621-1640, 1646-1665, 1646-1665, 1647-1666, 1689-1708, 1749-1768, 1763-1782, 1912-1931, 2073-2092, 2085-2104, 2166-2185, 2172-2191, 2189-2208, 2191-2210, 2193-2212, 2195-2210, 2195-2214, 2196-2215, 2197-2212, 2197-2216, 2202-2221, 2223-2238, 2223-2242, 2225-2240, 2226-2245, 2227-2242, 2227-2246, 2238-2257, 2241-2260, 2267-2286, 2361-2380, 2388-2407, 2397-2416, 2448-2467, 2453-2472, 2455-2474, 2457-2472, 2457-2476, 2459-2474, 2459-2478, 2461-2476, 2461-2480, 2532-2551, 2550-2569, 2551-2566, 2551-2570, 2552-2568, 2552-2570, 2552-2571, 2553-2568, 2553-2570, 2553-2571, 2553-2572, 2554-2571, 2554-2572, 2554-2573, 2555-2570, 2555-2572, 2555-2574, 2556-2573, 2556-2574, 2556-2575, 2557-2573, 2557-2574, 2557-2575, 2557-2576, 2558-2575, 2558-2576, 2558-2577, 2559-2576, 2559-2577, 2559-2578, 2560-2577, 2560-2578, 2560-2579, 2561-2576, 2561-2578, 2561-2579, 2561-2580, 2562-2577, 2562-2579, 2562-2581, 2563-2578, 2563-2580, 2563-2582, 2564-2581, 2564-2583, 2565-2584, 2566-2583, 2566-2585, 2567-2582, 2567-2584, 2567-2586, 2568-2583, 2568-2585, 2568-2587, 2569-2586, 2569-2588, 2570-2585, 2570-2587, 2570-2589, 2571-2586, 2571-2588, 2571-2590, 2572-2589, 2572-2590, 2572-2591, 2573-2590, 2573-2592, 2574-2590, 2574-2591, 2574-2593, 2575-2590, 2575-2591, 2575-2592, 2575-2594, 2576-2593, 2576-2595, 2577-2594, 2577-2595, 2577-2596, 2578-2594, 2578-2596, 2578-2597, 2579-2598, 2580-2596, 2580-2597, 2580-2598, 2580-2599, 2581-2597, 2581-2598, 2581-2599, 2581-2600, 2582-2598, 2582-2599, 2582-2600, 2582-2601, 2583-2599, 2583-2600, 2583-2601, 2583-2602, 2584-2600, 2584-2601, 2584-2602, 2584-2603, 2585-2601, 2585-2603, 2585-2604, 2586-2601, 2586-2602, 2586-2604, 2586-2605, 2587-2602, 2587-2603, 2587-2605, 2587-2606, 2588-2603, 2588-2604, 2588-2605, 2588-2606, 2588-2607, 2589-2604, 2589-2605, 2589-2606, 2589-2607, 2589-2608, 2590-2605, 2590-2606, 2590-2607, 2590-2608, 2590-2609, 2590-2609, 2591-2607, 2591-2608, 2591-2609, 2591-2610, 2592-2607, 2592-2608, 2592-2609, 2592-2610, 2592-2611, 2593-2608, 2593-2609, 2593-2610, 2593-2612, 2594-2609, 2594-2610, 2594-2611, 2594-2612, 2594-2613, 2595-2610, 2595-2611, 2595-2612, 2595-2613, 2595-2614, 2596-2611, 2596-2612, 2596-2613, 2596-2614, 2596-2615, 2597-2612, 2597-2612, 2597-2613, 2597-2614, 2597-2615, 2597-2616, 2598-2613, 2598-2614, 2598-2615, 2598-2616, 2598-2617, 2599-2614, 2599-2615, 2599-2616, 2599-2617, 2599-2618, 2600-2615, 2600-2616, 2600-2617, 2600-2618, 2600-2619, 2601-2616, 2601-

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Certain embodiments provide a compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 30-49, 48-63, 150-169, 151-170, 152-171, 154-169, 154-173, 156-171, 156-175, 157-176, 158-173, 158-177, 480-499, 600-619, 638-657, 644-663, 738-757, 1089-1108, 1135-1154, 1141-1160, 1147-1166, 1150-1169, 1153-1172, 1159-1178, 1162-1181, 1165-1184, 1171-1186, 1171-1190, 1173-1188, 1173-1192, 1175-1190, 1175-1194, 1177-1196, 1183-1202, 1208-1227, 1235-1254, 1298-1317, 1304-1323, 1310-1329, 1316-1335, 1319-1338, 1322-1341, 1328-1347, 1349-1368, 1355-1374, 1393-1412, 1396-1415, 1399-1418, 1405-1424, 1421-1440, 1621-1640, 1646-1665, 1646-1665, 1647-1666, 1689-1708, 1749-1768, 1763-1782, 1912-1931, 2073-2092, 2085-2104, 2166-2185, 2172-2191, 2189-2208, 2191-2210, 2193-2212, 2195-2210, 2195-2214, 2196-2215, 2197-2212, 2197-2216, 2202-2221, 2223-2238, 2223-2242, 2225-2240, 2226-2245, 2227-2242, 2227-2246, 2238-2257, 2241-2260, 2267-2286, 2361-2380, 2388-2407, 2397-2416, 2448-2467, 2453-2472, 2455-2474, 2457-2472, 2457-2476, 2459-2474, 2459-2478, 2461-2476, 2461-2480, 2532-2551, 2550-2569, 2551-2566, 2551-2570, 2552-2568, 2552-2570, 2552-2571, 2553-2568, 2553-2570, 2553-2571, 2553-2572, 2554-2571, 2554-2572, 2554-2573, 2555-2570, 2555-2572, 2555-2574, 2556-2573, 2556-2574, 2556-2575, 2557-2573, 2557-2574, 2557-2575, 2557-2576, 2558-2575, 2558-2576, 2558-2577, 2559-2576, 2559-2577, 2559-2578, 2560-2577, 2560-2578, 2560-2579, 2561-2576, 2561-2578, 2561-2579, 2561-2580, 2562-2577, 2562-2579, 2562-2581, 2563-2578, 2563-2580, 2563-2582, 2564-2581, 2564-2583, 2565-2584, 2566-2583, 2566-2585, 2567-2582, 2567-2584, 2567-2586, 2568-2583, 2568-2585, 2568-2587, 2569-2586, 2569-2588, 2570-2585, 2570-2587, 2570-2589, 2571-2586, 2571-2588, 2571-2590, 2572-2589, 2572-2590, 2572-2591, 2573-2590, 2573-2592, 2574-2590, 2574-2591, 2574-2593, 2575-2590, 2575-2591, 2575-2592, 2575-2594, 2576-2593, 2576-2595, 2577-2594, 2577-2595, 2577-2596, 2578-2594, 2578-2596, 2578-2597, 2579-2598, 2580-2596, 2580-2597, 2580-2598, 2580-2599, 2581-2597, 2581-2598, 2581-2599, 2581-2600, 2582-2598, 2582-2599, 2582-2600, 2582-2601, 2583-2599, 2583-2600, 2583-2601, 2583-2602, 2584-2600, 2584-2601, 2584-2602, 2584-2603, 2585-2601, 2585-2603, 2585-2604, 2586-2601, 2586-2602, 2586-

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Certain embodiments provide a compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides complementary within nucleobases 1608-1627, 1685-1704, 1686-1705, 1751-1770, 1769-1784, 1871-1890, 1872-1891, 1873-1892, 1875-1890, 1875-1894, 1877-1892, 1877-1896, 1878-1897, 1879-1894, 1879-1898, 2288-2307, 2808-2827, 2846-2865, 2852-2871, 2946-2965, 3773-3792, 3819-3838, 3825-3844, 3831-3850, 3834-3853, 3837-3856, 3843-3862, 4151-4166, 4151-4170, 4153-4172, 4159-4178, 4184-4203, 4211-4230, 4609-4628, 4612-4631, 4615-4634, 4621-4640, 4642-4661, 4648-4667, 4686-4705, 4689-4708, 4692-4711, 4698-4717, 4714-4733, 5270-5289, 5295-5314, 5296-5315, 5830-5849, 5890-5909, 5904-5923, 6406-6425, 6662-6681, 6674-6693, 6954-6973, 6960-6979, 6977-6996, 6979-6998, 6981-7000, 6983-6998, 6983-7002, 6984-7003, 6985-7000, 6985-7004, 6990-7009, 7122-7141, 7125-7144, 7151-7170, 7353-7372, 7362-7381, 7683-7702, 7688-7707, 7690-7709, 7692-7707, 7692-7711, 7694-7709, 7694-7713, 7696-7711, 7696-7715, 7767-7786, 7785-7804, 7786-7801, 7787-7803, 7787-7805, 7787-7806, 7788-7803, 7788-7805, 7788-7806, 7788-7807, 7789-7806, 7789-7807, 7789-7808, 7790-7805, 7790-7807, 7790-7809, 7791-7808, 7791-7809, 7791-7810, 7792-7808, 7792-7809, 7792-7810, 7792-7811, 7793-7810, 7793-7811, 7793-7812, 7794-7811, 7794-7812, 7794-7813, 7795-7812, 7795-7813, 7795-7814, 7796-7811, 7796-7813, 7796-7814, 7796-7815, 7797-7812, 7797-7814, 7797-7816, 7798-7813, 7798-7815, 7798-7817, 7799-7816, 7799-7818, 7800-7819, 7801-7818, 7801-7820, 7802-7817, 7802-7819, 7802-7821, 7803-7818, 7803-7820, 7803-7822, 7804-7821, 7804-7823, 7805-7820,

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Certain embodiments provide a compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases complementary to an equal length portion of nucleobases 1608-1627, 1685-1704, 1686-1705, 1751-1770, 1769-1784, 1871-1890, 1872-1891, 1873-1892, 1875-1890, 1875-1894, 1877-1892, 1877-1896, 1878-1897, 1879-1894, 1879-1898, 2288-2307, 2808-2827, 2846-2865, 2852-2871, 2946-2965, 3773-3792, 3819-3838, 3825-3844, 3831-3850, 3834-3853, 3837-3856, 3843-3862, 4151-4166, 4151-4170, 4153-4172, 4159-4178, 4184-4203, 4211-4230, 4609-4628, 4612-4631, 4615-4634, 4621-4640, 4642-4661, 4648-4667, 4686-4705, 4689-4708, 4692-4711, 4698-4717, 4714-4733, 5270-5289, 5295-5314, 5296-5315, 5830-5849, 5890-5909, 5904-5923, 6406-6425, 6662-6681, 6674-6693, 6954-6973, 6960-6979, 6977-6996, 6979-6998, 6981-7000, 6983-6998, 6983-7002, 6984-7003, 6985-7000, 6985-7004, 6990-7009, 7122-7141, 7125-7144, 7151-7170, 7353-7372, 7362-7381, 7683-7702, 7688-7707, 7690-7709, 7692-7707, 7692-7711, 7694-7709, 7694-7713, 7696-7711, 7696-7715, 7767-7786, 7785-7804, 7786-7801, 7787-7803, 7787-7805, 7787-7806, 7788-7803, 7788-7805, 7788-7806, 7788-7807, 7789-7806, 7789-7807, 7789-7808, 7790-7805, 7790-7807, 7790-7809, 7791-7808, 7791-7809, 7791-7810, 7792-

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In certain embodiments, antisense compounds or oligonucleotides target a region of a CFB nucleic acid. In certain embodiments, such compounds or oligonucleotides targeted to a region of a CFB nucleic acid have a contiguous nucleobase portion that is complementary to an equal length nucleobase portion of the region. For example, the portion can be at least an 8, 9, 10, 11, 12, 13, 14, 15, or 16 contiguous nucleobase portion complementary to an equal length portion of a region recited herein. In certain embodiments, a compound comprises or consists of a conjugate and a modified oligonucleotide targeting any of the following nucleotide regions of SEQ ID NO: 1: 30-49, 48-63, 150-169, 151-170, 152-171, 154-169, 154-173, 156-171, 156-175, 157-176, 158-173, 158-177, 480-499, 600-619, 638-657, 644-663, 738-757, 1089-1108, 1135-1154, 1141-1160, 1147-1166, 1150-1169, 1153-1172, 1159-1178, 1162-1181, 1165-1184, 1171-1186, 1171-1190, 1173-1188, 1173-1192, 1175-1190, 1175-1194, 1177-1196, 1183-1202, 1208-1227, 1235-1254, 1298-1317,

1304-1323, 1310-1329, 1316-1335, 1319-1338, 1322-1341, 1328-1347, 1349-1368, 1355-1374, 1393-1412, 1396-1415, 1399-1418, 1405-1424, 1421-1440, 1621-1640, 1646-1665, 1646-1665, 1647-1666, 1689-1708, 1749-1768, 1763-1782, 1912-1931, 2073-2092, 2085-2104, 2166-2185, 2172-2191, 2189-2208, 2191-2210, 2193-2212, 2195-2210, 2195-2214, 2196-2215, 2197-2212, 2197-2216, 2202-2221, 2223-2238, 2223-2242, 2225-2240, 2226-2245, 2227-2242, 2227-2246, 2238-2257, 2241-2260, 2267-2286, 2361-2380, 2388-2407, 2397-2416, 2448-2467, 2453-2472, 2455-2474, 2457-2472, 2457-2476, 2459-2474, 2459-2478, 2461-2476, 2461-2480, 2532-2551, 2550-2569, 2551-2566, 2551-2570, 2552-2568, 2552-2570, 2552-2571, 2553-2568, 2553-2570, 2553-2571, 2553-2572, 2554-2571, 2554-2572, 2554-2573, 2555-2570, 2555-2572, 2555-2574, 2556-2573, 2556-2574, 2556-2575, 2557-2573, 2557-2574, 2557-2575, 2557-2576, 2558-2575, 2558-2576, 2558-2577, 2559-2576, 2559-2577, 2559-2578, 2560-2577, 2560-2578, 2560-2579, 2561-2576, 2561-2578, 2561-2579, 2561-2580, 2562-2577, 2562-2579, 2562-2581, 2563-2578, 2563-2580, 2563-2582, 2564-2581, 2564-2583, 2565-2584, 2566-2583, 2566-2585, 2567-2582, 2567-2584, 2567-2586, 2568-2583, 2568-2585, 2568-2587, 2569-2586, 2569-2588, 2570-2585, 2570-2587, 2570-2589, 2571-2586, 2571-2588, 2571-2590, 2572-2589, 2572-2590, 2572-2591, 2573-2590, 2573-2592, 2574-2590, 2574-2591, 2574-2593, 2575-2590, 2575-2591, 2575-2592, 2575-2594, 2576-2593, 2576-2595, 2577-2594, 2577-2595, 2577-2596, 2578-2594, 2578-2596, 2578-2597, 2579-2598, 2580-2596, 2580-2597, 2580-2598, 2580-2599, 2581-2597, 2581-2598, 2581-2599, 2581-2600, 2582-2598, 2582-2599, 2582-2600, 2582-2601, 2583-2599, 2583-2600, 2583-2601, 2583-2602, 2584-2600, 2584-2601, 2584-2602, 2584-2603, 2585-2601, 2585-2603, 2585-2604, 2586-2601, 2586-2602, 2586-2604, 2586-2605, 2587-2602, 2587-2603, 2587-2605, 2587-2606, 2588-2603, 2588-2604, 2588-2605, 2588-2606, 2588-2607, 2589-2604, 2589-2605, 2589-2606, 2589-2607, 2589-2608, 2590-2605, 2590-2606, 2590-2607, 2590-2608, 2590-2609, 2590-2609, 2591-2607, 2591-2608, 2591-2609, 2591-2610, 2592-2607, 2592-2608, 2592-2609, 2592-2610, 2592-2611, 2593-2608, 2593-2609, 2593-2610, 2593-2612, 2594-2609, 2594-2610, 2594-2611, 2594-2612, 2594-2613, 2595-2610, 2595-2611, 2595-2612, 2595-2613, 2595-2614, 2596-2611, 2596-2612, 2596-2613, 2596-2614, 2596-2615, 2597-2612, 2597-2612, 2597-2613, 2597-2614, 2597-2615, 2597-2616, 2598-2613, 2598-2614, 2598-2615, 2598-2616, 2598-2617, 2599-2614, 2599-2615, 2599-2616, 2599-2617, 2599-2618, 2600-2615, 2600-2616, 2600-2617, 2600-2618, 2600-2619, 2601-2616, 2601-2617, 2601-2618, 2601-2619, 2601-2620, 2602-2617, 2602-2618, 2602-2619, 2602-2620, 2602-2621, 2603-2618, 2603-2619, 2603-2620, 2603-2621, 2603-2622, 2604-2619, 2604-2620, 2604-2621, 2604-2622, 2604-2623, 2605-2620, 2605-2621, 2605-2622, 2605-2623, 2605-2624, 2606-2621, 2606-2622, 2606-2623, 2606-2624, 2606-2625, 2607-2622, 2607-2623, 2607-2624, 2607-2625, 2607-2626, 2608-2623, 2608-2624, 2608-2625, 2608-2626, 2608-2627, 2609-2624, 2609-2625, 2609-2626, 2609-2627, 2609-2628, 2610-2625, 2610-2626, 2610-2627, 2610-2628, 2610-2629, 2611-2626, 2611-2627, 2611-2628, 2611-2629, 2611-2630, 2612-2627, 2612-2628, 2612-2629, 2612-2630, 2612-2631, 2613-2628, 2613-2629, 2613-2630, 2613-2631, 2614-2629, 2614-2630, 2614-2631, 2615-2630, 2615-2631, and 2616-2631.

In certain embodiments, antisense compounds or oligonucleotides target a region of a CFB nucleic acid. In certain embodiments, such compounds or oligonucleotides targeted to a region of a CFB nucleic acid have a contiguous nucleobase portion that is complementary to an equal length nucleobase portion of the region. For example, the portion can be at least an 8, 9, 10, 11, 12, 13, 14, 15, or 16 contiguous nucleobase portion complementary to an equal length portion of a region recited herein. In certain embodiments, a compound comprises or consists of a conjugate and a modified oligonucleotide targeting the following nucleotide regions of SEQ ID NO: 2: 1608-1627, 1685-1704, 1686-1705, 1751-1770, 1769-1784, 1871-1890, 1872-1891, 1873-1892, 1875-1890, 1875-1894, 1877-1892, 1877-1896, 1878-1897, 1879-1894, 1879-1898, 2288-2307, 2808-2827, 2846-2865, 2852-2871, 2946-2965, 3773-3792, 3819-3838, 3825-3844, 3831-3850, 3834-3853, 3837-3856, 3843-3862, 4151-4166, 4151-4170, 4153-4172, 4159-4178, 4184-4203, 4211-4230, 4609-4628, 4612-4631, 4615-4634, 4621-4640, 4642-4661, 4648-4667, 4686-4705, 4689-4708, 4692-4711, 4698-4717, 4714-4733, 5270-5289, 5295-5314, 5296-5315, 5830-5849, 5890-5909, 5904-5923, 6406-6425, 6662-6681, 6674-6693, 6954-6973, 6960-6979, 6977-6996, 6979-6998, 6981-7000, 6983-6998, 6983-7002, 6984-7003, 6985-7000, 6985-7004, 6990-7009, 7122-7141, 7125-7144, 7151-7170, 7353-7372, 7362-7381, 7683-7702, 7688-7707, 7690-7709, 7692-7707, 7692-7711, 7694-7709, 7694-7713, 7696-7711, 7696-7715, 7767-7786, 7785-7804, 7786-7801, 7787-7803, 7787-7805, 7787-7806, 7788-7803, 7788-7805, 7788-7806, 7788-7807, 7789-7806, 7789-7807, 7789-7808, 7790-7805, 7790-7807, 7790-7809, 7791-7808, 7791-7809, 7791-7810, 7792-7808, 7792-7809, 7792-7810, 7792-7811, 7793-7810, 7793-7811, 7793-7812, 7794-7811, 7794-7812, 7794-7813, 7795-7812, 7795-7813, 7795-7814, 7796-7811, 7796-7813, 7796-7814, 7796-7815, 7797-7812, 7797-7814, 7797-7816, 7798-7813, 7798-7815, 7798-7817, 7799-7816, 7799-7818, 7800-7819, 7801-7818, 7801-7820, 7802-7817, 7802-7819, 7802-7821, 7803-7818, 7803-7820, 7803-7822, 7804-7821, 7804-7823, 7805-7820, 7805-7822, 7805-7824, 7806-7821, 7806-7823, 7806-7825, 7807-7824, 7807-7825, 7807-7826, 7808-7825, 7808-7827, 7809-7825, 7809-7826, 7809-7828, 7810-7825, 7810-7826, 7810-7827, 7810-7829, 7811-7828, 7811-7830, 7812-7829, 7812-7830, 7812-7831, 7813-7829, 7813-7831, 7813-7832, 7814-7833, 7815-7831, 7815-7832, 7815-7833, 7815-7834, 7816-7832, 7816-7833, 7816-7834, 7816-7835, 7817-7833, 7817-7834, 7817-7835, 7817-7836, 7818-7834, 7818-7835, 7818-7836, 7818-7837, 7819-7835, 7819-7836, 7819-7837, 7819-7838, 7820-7836, 7820-7838, 7820-7839, 7821-7836, 7821-7837, 7821-7839, 7821-7840, 7822-7837, 7822-7838, 7822-7840, 7822-7841, 7823-7838, 7823-7839, 7823-7839, 7823-7840, 7823-7841, 7823-7842, 7824-7839, 7824-7840, 7824-7840, 7824-7841, 7824-7842, 7824-7843, 7825-7840, 7825-7841, 7825-7842, 7825-7843, 7825-7844, 7826-7842, 7826-7843, 7826-7844, 7826-7845, 7827-7842, 7827-7843, 7827-7844, 7827-7845, 7827-7846, 7828-7843, 7828-7844, 7828-7845, 7828-7847, 7829-7844, 7829-7845, 7829-7846, 7829-7847, 7829-7848, 7830-7845, 7830-7846, 7830-7847, 7830-7848, 7830-7849, 7831-7846, 7831-7847, 7831-7848, 7831-7849, 7831-7850, 7832-7847, 7832-7848, 7832-7849, 7832-7850, 7832-7851, 7833-7848, 7833-7849, 7833-7850, 7833-7851, 7833-7852, 7834-7849, 7834-7850, 7834-7851, 7834-7852, 7834-7853, 7835-7850, 7835-7851, 7835-7852, 7835-7853, 7835-7854, 7836-7851, 7836-7852,

7836-7853, 7836-7854, 7836-7855, 7837-7852, 7837-7853, 7837-7854, 7837-7855, 7837-7856, 7838-7853, 7838-7854, 7838-7855, 7838-7856, 7838-7857, 7839-7854, 7839-7855, 7839-7856, 7839-7857, 7839-7858, 7840-7855, 7840-7856, 7840-7857, 7840-7858, 7840-7859, 7841-7856, 7841-7857, 7841-7858, 7841-7859, 7841-7860, 7842-7857, 7842-7858, 7842-7859, 7842-7860, 7842-7861, 7843-7858, 7843-7859, 7843-7860, 7843-7861, 7843-7862, 7844-7859, 7844-7860, 7844-7861, 7844-7862, 7845-7860, 7845-7861, 7845-7862, 7846-7861, and 7846-7862.

In certain embodiments, a compound comprises or consists of a conjugate and a modified oligonucleotide targeting the 3'UTR of a CFB nucleic acid. In certain aspects, the modified oligonucleotide targets within nucleotides 2574-2626 of a CFB nucleic acid having the nucleobase sequence of SEQ ID NO: 1. In certain aspects, the modified oligonucleotide has at least an 8, 9, 10, 11, 12, 13, 14, 15, or 16 contiguous nucleobase portion complementary to an equal length portion within nucleotides 2574-2626 of a CFB nucleic acid having the nucleobase sequence of SEQ ID NO: 1.

In certain embodiments, a compound comprises or consists of a conjugate and a modified oligonucleotide targeting a region of a CFB nucleic acid having the nucleobase sequence of SEQ ID NO: 1 within nucleobases 2457-2631, 2457-2472, 2457-2474, 2457-2476, 2457-2566, 2457-2570, 2457-2571, 2457-2572, 2457-2573, 2457-2574, 2457-2575, 2457-2576, 2457-2577, 2457-2578, 2457-2579, 2457-2580, 2457-2581, 2457-2582, 2457-2583, 2457-2584, 2457-2585, 2457-2586, 2457-2587, 2457-2588, 2457-2589, 2457-2590, 2457-2591, 2457-2592, 2457-2593, 2457-2594, 2457-2595, 2457-2596, 2457-2597, 2457-2598, 2457-2599, 2457-2600, 2457-2601, 2457-2602, 2457-2603, 2457-2604, 2457-2605, 2457-2606, 2457-2607, 2457-2608, 2457-2609, 2457-2610, 2457-2611, 2457-2612, 2457-2613, 2457-2614, 2457-2615, 2457-2616, 2457-2617, 2457-2618, 2457-2619, 2457-2620, 2457-2621, 2457-2622, 2457-2623, 2457-2624, 2457-2625, 2457-2626, 2457-2627, 2457-2628, 2457-2629, 2457-2630, 2457-2631, 2459-2474, 2459-2476, 2459-2566, 2459-2570, 2459-2571, 2459-2572, 2459-2573, 2459-2574, 2459-2575, 2459-2576, 2459-2577, 2459-2578, 2459-2579, 2459-2580, 2459-2581, 2459-2582, 2459-2583, 2459-2584, 2459-2585, 2459-2586, 2459-2587, 2459-2588, 2459-2589, 2459-2590, 2459-2591, 2459-2592, 2459-2593, 2459-2594, 2459-2595, 2459-2596, 2459-2597, 2459-2598, 2459-2599, 2459-2600, 2459-2601, 2459-2602, 2459-2603, 2459-2604, 2459-2605, 2459-2606, 2459-2607, 2459-2608, 2459-2609, 2459-2610, 2459-2611, 2459-2612, 2459-2613, 2459-2614, 2459-2615, 2459-2616, 2459-2617, 2459-2618, 2459-2619, 2459-2620, 2459-2621, 2459-2622, 2459-2623, 2459-2624, 2459-2625, 2459-2626, 2459-2627, 2459-2628, 2459-2629, 2459-2630, 2459-2631, 2461-2476, 2461-2566, 2461-2570, 2461-2571, 2461-2572, 2461-2573, 2461-2574, 2461-2575, 2461-2576, 2461-2577, 2461-2578, 2461-2579, 2461-2580, 2461-2581, 2461-2582, 2461-2583, 2461-2584, 2461-2585, 2461-2586, 2461-2587, 2461-2588, 2461-2589, 2461-2590, 2461-2591, 2461-2592, 2461-2593, 2461-2594, 2461-2595, 2461-2596, 2461-2597, 2461-2598, 2461-2599, 2461-2600, 2461-2601, 2461-2602, 2461-2603, 2461-2604, 2461-2605, 2461-2606, 2461-2607, 2461-2608, 2461-2609, 2461-2610, 2461-2611, 2461-2612, 2461-2613, 2461-2614, 2461-2615, 2461-2616, 2461-2617, 2461-2618, 2461-2619, 2461-2620, 2461-2621, 2461-2622,

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In certain embodiments, the following nucleotide regions of SEQ ID NO: 1, when targeted by antisense compounds or oligonucleotides, display at least 50% inhibition: 30-49, 48-63, 150-169, 151-170, 152-171, 154-169, 154-173, 156-171, 156-175, 157-176, 158-173, 158-177, 480-499, 600-619, 638-657, 644-663, 738-757, 1089-1108, 1135-1154, 1141-1160, 1147-1166, 1150-1169, 1153-1172, 1159-1178, 1162-1181, 1165-1184, 1171-1186, 1171-1190, 1173-1188, 1173-1192, 1175-1190, 1175-1194, 1177-1196, 1183-1202, 1208-1227, 1235-1254, 1298-1317, 1304-1323, 1310-1329, 1316-1335, 1319-1338, 1322-1341, 1328-1347, 1349-1368, 1355-1374, 1393-1412, 1396-1415, 1399-1418, 1405-1424, 1421-1440, 1621-1640, 1646-1665, 1646-1665, 1647-1666, 1689-1708, 1749-1768, 1763-1782, 1912-1931, 2073-2092, 2085-2104, 2166-2185, 2172-2191, 2189-2208, 2191-2210, 2193-2212, 2195-2210, 2195-2214, 2196-2215, 2197-2212, 2197-2216, 2202-2221, 2223-2238, 2223-2242, 2225-2240, 2226-2245, 2227-2242, 2227-2246, 2238-2257, 2241-2260, 2267-2286, 2361-2380, 2388-2407, 2397-2416, 2448-2467, 2453-2472, 2455-2474, 2457-2472, 2457-2476, 2459-2474, 2459-2478, 2461-2476, 2461-2480, 2532-2551, 2550-2569, 2551-2566, 2551-2570, 2552-2568, 2552-2570, 2552-2571, 2553-2568, 2553-2570, 2553-2571, 2553-2572, 2554-2571, 2554-2572, 2554-2573, 2555-2570, 2555-2572, 2555-2574, 2556-2573, 2556-2574, 2556-2575, 2557-2573, 2557-2574, 2557-2575, 2557-2576, 2558-2575, 2558-2576, 2558-2577, 2559-2576, 2559-2577, 2559-2578, 2560-2577, 2560-2578, 2560-2579, 2561-2576, 2561-2578, 2561-2579, 2561-2580, 2562-2577, 2562-2579, 2562-2581, 2563-2578, 2563-2580, 2563-2582, 2564-2581, 2564-2583, 2565-2584, 2566-2583, 2566-2585, 2567-2582, 2567-2584, 2567-2586, 2568-2583, 2568-2585, 2568-2587, 2569-2586, 2569-2588, 2570-2585, 2570-2587, 2570-2589, 2571-2586, 2571-2588, 2571-2590, 2572-2589, 2572-2590, 2572-2591, 2573-2590, 2573-2592, 2574-

2590, 2574-2591, 2574-2593, 2575-2590, 2575-2591, 2575-2592, 2575-2594, 2576-2593, 2576-2595, 2577-2594, 2577-2595, 2577-2596, 2578-2594, 2578-2596, 2578-2597, 2579-2598, 2580-2596, 2580-2597, 2580-2598, 2580-2599, 2581-2597, 2581-2598, 2581-2599, 2581-2600, 2582-2598, 2582-2599, 2582-2600, 2582-2601, 2583-2599, 2583-2600, 2583-2601, 2583-2602, 2584-2600, 2584-2601, 2584-2602, 2584-2603, 2585-2601, 2585-2603, 2585-2604, 2586-2601, 2586-2602, 2586-2604, 2586-2605, 2587-2602, 2587-2603, 2587-2605, 2587-2606, 2588-2603, 2588-2604, 2588-2605, 2588-2606, 2588-2607, 2589-2604, 2589-2605, 2589-2606, 2589-2607, 2589-2608, 2590-2605, 2590-2606, 2590-2607, 2590-2608, 2590-2609, 2590-2609, 2591-2607, 2591-2608, 2591-2609, 2591-2610, 2592-2607, 2592-2608, 2592-2609, 2592-2610, 2592-2611, 2593-2608, 2593-2609, 2593-2610, 2593-2612, 2594-2609, 2594-2610, 2594-2611, 2594-2612, 2594-2613, 2595-2610, 2595-2611, 2595-2612, 2595-2613, 2595-2614, 2596-2611, 2596-2612, 2596-2613, 2596-2614, 2596-2615, 2597-2612, 2597-2612, 2597-2613, 2597-2614, 2597-2615, 2597-2616, 2598-2613, 2598-2614, 2598-2615, 2598-2616, 2598-2617, 2599-2614, 2599-2615, 2599-2616, 2599-2617, 2599-2618, 2600-2615, 2600-2616, 2600-2617, 2600-2618, 2600-2619, 2601-2616, 2601-2617, 2601-2618, 2601-2619, 2601-2620, 2602-2617, 2602-2618, 2602-2619, 2602-2620, 2602-2621, 2603-2618, 2603-2619, 2603-2620, 2603-2621, 2603-2622, 2604-2619, 2604-2620, 2604-2621, 2604-2622, 2604-2623, 2605-2620, 2605-2621, 2605-2622, 2605-2623, 2605-2624, 2606-2621, 2606-2622, 2606-2623, 2606-2624, 2606-2625, 2607-2622, 2607-2623, 2607-2624, 2607-2625, 2607-2626, 2608-2623, 2608-2624, 2608-2625, 2608-2626, 2608-2627, 2609-2624, 2609-2625, 2609-2626, 2609-2627, 2609-2628, 2610-2625, 2610-2626, 2610-2627, 2610-2628, 2610-2629, 2611-2626, 2611-2627, 2611-2628, 2611-2629, 2611-2630, 2612-2627, 2612-2628, 2612-2629, 2612-2630, 2612-2631, 2613-2628, 2613-2629, 2613-2630, 2613-2631, 2614-2629, 2614-2630, 2614-2631, 2615-2630, 2615-2631, and 2616-2631.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 2, when targeted by antisense compounds or oligonucleotides, display at least 50% inhibition: 1608-1627, 1685-1704, 1686-1705, 1751-1770, 1769-1784, 1871-1890, 1872-1891, 1873-1892, 1875-1890, 1875-1894, 1877-1892, 1877-1896, 1878-1897, 1879-1894, 1879-1898, 2288-2307, 2808-2827, 2846-2865, 2852-2871, 2946-2965, 3773-3792, 3819-3838, 3825-3844, 3831-3850, 3834-3853, 3837-3856, 3843-3862, 4151-4166, 4151-4170, 4153-4172, 4159-4178, 4184-4203, 4211-4230, 4609-4628, 4612-4631, 4615-4634, 4621-4640, 4642-4661, 4648-4667, 4686-4705, 4689-4708, 4692-4711, 4698-4717, 4714-4733, 5270-5289, 5295-5314, 5296-5315, 5830-5849, 5890-5909, 5904-5923, 6406-6425, 6662-6681, 6674-6693, 6954-6973, 6960-6979, 6977-6996, 6979-6998, 6981-7000, 6983-6998, 6983-7002, 6984-7003, 6985-7000, 6985-7004, 6990-7009, 7122-7141, 7125-7144, 7151-7170, 7353-7372, 7362-7381, 7683-7702, 7688-7707, 7690-7709, 7692-7707, 7692-7711, 7694-7709, 7694-7713, 7696-7711, 7696-7715, 7767-7786, 7785-7804, 7786-7801, 7787-7803, 7787-7805, 7787-7806, 7788-7803, 7788-7805, 7788-7806, 7788-7807, 7789-7806, 7789-7807, 7789-7808, 7790-7805, 7790-7807, 7790-7809, 7791-7808, 7791-7809, 7791-7810, 7792-7808, 7792-7809, 7792-7810, 7792-7811, 7793-7810, 7793-7811, 7793-7812, 7794-7811, 7794-7812, 7794-7813, 7795-7812, 7795-7813, 7795-7814, 7796-7811,

7796-7813, 7796-7814, 7796-7815, 7797-7812, 7797-7814, 7797-7816, 7798-7813, 7798-7815, 7798-7817, 7799-7816, 7799-7818, 7800-7819, 7801-7818, 7801-7820, 7802-7817, 7802-7819, 7802-7821, 7803-7818, 7803-7820, 7803-7822, 7804-7821, 7804-7823, 7805-7820, 7805-7822, 7805-7824, 7806-7821, 7806-7823, 7806-7825, 7807-7824, 7807-7825, 7807-7826, 7808-7825, 7808-7827, 7809-7825, 7809-7826, 7809-7828, 7810-7825, 7810-7826, 7810-7827, 7810-7829, 7811-7828, 7811-7830, 7812-7829, 7812-7830, 7812-7831, 7813-7829, 7813-7831, 7813-7832, 7814-7833, 7815-7831, 7815-7832, 7815-7833, 7815-7834, 7816-7832, 7816-7833, 7816-7834, 7816-7835, 7817-7833, 7817-7834, 7817-7835, 7817-7836, 7818-7834, 7818-7835, 7818-7836, 7818-7837, 7819-7835, 7819-7836, 7819-7837, 7819-7838, 7820-7836, 7820-7838, 7820-7839, 7821-7836, 7821-7837, 7821-7839, 7821-7840, 7822-7837, 7822-7838, 7822-7840, 7822-7841, 7823-7838, 7823-7839, 7823-7839, 7823-7840, 7823-7841, 7823-7842, 7824-7839, 7824-7840, 7824-7840, 7824-7841, 7824-7842, 7824-7843, 7825-7840, 7825-7841, 7825-7842, 7825-7843, 7825-7844, 7826-7842, 7826-7843, 7826-7844, 7826-7845, 7827-7842, 7827-7843, 7827-7844, 7827-7845, 7827-7846, 7828-7843, 7828-7844, 7828-7845, 7828-7847, 7829-7844, 7829-7845, 7829-7846, 7829-7847, 7829-7848, 7830-7845, 7830-7846, 7830-7847, 7830-7848, 7830-7849, 7831-7846, 7831-7847, 7831-7848, 7831-7849, 7831-7850, 7832-7847, 7832-7848, 7832-7849, 7832-7850, 7832-7851, 7833-7848, 7833-7849, 7833-7850, 7833-7851, 7833-7852, 7834-7849, 7834-7850, 7834-7851, 7834-7852, 7834-7853, 7835-7850, 7835-7851, 7835-7852, 7835-7853, 7835-7854, 7836-7851, 7836-7852, 7836-7853, 7836-7854, 7836-7855, 7837-7852, 7837-7853, 7837-7854, 7837-7855, 7837-7856, 7838-7853, 7838-7854, 7838-7855, 7838-7856, 7838-7857, 7839-7854, 7839-7855, 7839-7856, 7839-7857, 7839-7858, 7840-7855, 7840-7856, 7840-7857, 7840-7858, 7840-7859, 7841-7856, 7841-7857, 7841-7858, 7841-7859, 7841-7860, 7842-7857, 7842-7858, 7842-7859, 7842-7860, 7842-7861, 7843-7858, 7843-7859, 7843-7860, 7843-7861, 7843-7862, 7844-7859, 7844-7860, 7844-7861, 7844-7862, 7845-7860, 7845-7861, 7845-7862, 7846-7861, and 7846-7862.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 1, when targeted by antisense compounds or oligonucleotides, display at least 60% inhibition: 48-63, 150-169, 152-171, 154-169, 154-173, 156-171, 156-175, 158-173, 158-177, 600-619, 1135-1154, 1141-1160, 1147-1166, 1153-1172, 1171-1186, 1173-1188, 1175-1190, 1749-1768, 1763-1782, 1763-1782, 1912-1931, 2189-2208, 2191-2210, 2193-2212, 2195-2210, 2195-2214, 2197-2212, 2197-2216, 2223-2238, 2225-2240, 2227-2242, 2238-2257, 2448-2467, 2453-2472, 2455-2474, 2457-2472, 2457-2476, 2459-2474, 2459-2478, 2461-2476, 2461-2480, 2550-2569, 2551-2566, 2552-2571, 2553-2568, 2553-2570, 2553-2571, 2553-2572, 2554-2571, 2554-2572, 2554-2573, 2555-2572, 2555-2574, 2556-2573, 2556-2574, 2556-2575, 2557-2574, 2557-2575, 2557-2576, 2558-2575, 2558-2576, 2558-2577, 2559-2576, 2559-2577, 2559-2578, 2560-2577, 2560-2578, 2560-2579, 2561-2578, 2561-2579, 2561-2580, 2562-2577, 2562-2579, 2562-2581, 2563-2578, 2563-2580, 2563-2582, 2564-2581, 2564-2583, 2565-2584, 2566-2583, 2566-2585, 2567-2582, 2567-2584, 2567-2586, 2568-2583, 2568-2585, 2568-2587, 2569-2586, 2569-2588, 2570-2587, 2570-2589, 2571-2588, 2572-2590, 2572-2591, 2573-2590, 2573-2592, 2574-2591, 2574-2593, 2575-2590, 2575-2592, 2575-2594, 2576-2593, 2576-2595,

2577-2594, 2577-2595, 2577-2596, 2578-2594, 2578-2597, 2579-2598, 2580-2596, 2580-2597, 2580-2598, 2580-2599, 2581-2597, 2581-2598, 2581-2599, 2581-2600, 2582-2598, 2582-2599, 2582-2600, 2582-2601, 2583-2599, 2583-2600, 2583-2601, 2583-2602, 2584-2600, 2584-2602, 2584-2603, 2585-2601, 2585-2603, 2585-2604, 2586-2602, 2586-2604, 2586-2605, 2587-2603, 2587-2605, 2587-2606, 2588-2603, 2588-2604, 2588-2606, 2588-2607, 2589-2605, 2589-2606, 2589-2607, 2589-2608, 2590-2605, 2590-2606, 2590-2607, 2590-2608, 2590-2609, 2591-2607, 2591-2609, 2591-2610, 2592-2608, 2592-2609, 2592-2611, 2593-2608, 2593-2609, 2593-2612, 2594-2609, 2594-2610, 2594-2611, 2594-2612, 2594-2613, 2595-2610, 2595-2611, 2595-2612, 2595-2613, 2595-2614, 2596-2611, 2596-2612, 2596-2613, 2596-2614, 2596-2615, 2597-2612, 2597-2613, 2597-2614, 2597-2615, 2597-2616, 2598-2613, 2598-2614, 2598-2615, 2598-2616, 2598-2617, 2599-2614, 2599-2615, 2599-2616, 2599-2617, 2599-2618, 2600-2615, 2600-2616, 2600-2617, 2600-2618, 2600-2619, 2601-2616, 2601-2617, 2601-2618, 2601-2619, 2601-2620, 2602-2617, 2602-2618, 2602-2619, 2602-2620, 2602-2621, 2603-2618, 2603-2619, 2603-2620, 2603-2621, 2603-2622, 2604-2619, 2604-2620, 2604-2621, 2604-2622, 2604-2623, 2605-2620, 2605-2621, 2605-2622, 2605-2623, 2605-2624, 2606-2621, 2606-2622, 2606-2623, 2606-2624, 2606-2625, 2607-2622, 2607-2623, 2607-2624, 2607-2625, 2607-2626, 2608-2623, 2608-2624, 2608-2625, 2608-2625, 2608-2626, 2608-2627, 2609-2624, 2609-2625, 2609-2626, 2609-2627, 2609-2628, 2610-2625, 2610-2626, 2610-2627, 2610-2628, 2610-2629, 2611-2626, 2611-2626, 2611-2627, 2611-2628, 2611-2629, 2611-2630, 2612-2627, 2612-2628, 2612-2629, 2612-2630, 2612-2631, 2613-2628, 2613-2629, 2613-2630, 2613-2631, 2614-2629, 2614-2630, 2614-2631, 2615-2630, 2615-2630, 2615-2631, 2615-2631, and 2616-2631.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 2, when targeted by antisense compounds or oligonucleotides, display at least 60% inhibition: 1685-1704, 1686-1705, 1769-1784, 1871-1890, 1873-1892, 1875-1890, 1875-1894, 1877-1892, 1877-1896, 1879-1894, 1879-1898, 2808-2827, 3819-3838, 3825-3844, 3831-3850, 3837-3856, 4151-4166, 5890-5909, 5904-5923, 5904-5923, 6406-6425, 6977-6996, 6979-6998, 6981-7000, 6983-6998, 6983-7002, 6985-7000, 6985-7004, 7122-7141, 7683-7702, 7688-7707, 7690-7709, 7692-7707, 7692-7711, 7694-7709, 7696-7711, 7696-7715, 7786-7801, 7787-7806, 7788-7803, 7788-7805, 7788-7806, 7788-7807, 7789-7806, 7789-7807, 7789-7808, 7790-7807, 7790-7809, 7791-7808, 7791-7809, 7791-7810, 7792-7809, 7792-7810, 7792-7811, 7793-7810, 7793-7811, 7793-7812, 7794-7811, 7794-7812, 7794-7813, 7795-7812, 7795-7813, 7795-7814, 7796-7813, 7796-7814, 7796-7815, 7797-7812, 7797-7814, 7797-7816, 7798-7813, 7798-7815, 7798-7817, 7799-7816, 7799-7818, 7800-7819, 7801-7818, 7801-7820, 7802-7817, 7802-7819, 7802-7821, 7803-7818, 7803-7820, 7803-7822, 7804-7821, 7804-7823, 7805-7822, 7805-7824, 7806-7823, 7806-7825, 7807-7824, 7807-7825, 7807-7826, 7808-7825, 7808-7827, 7809-7826, 7809-7828, 7810-7825, 7810-7827, 7810-7829, 7811-7828, 7811-7830, 7812-7829, 7812-7830, 7812-7831, 7813-7829, 7813-7832, 7814-7833, 7815-7831, 7815-7832, 7815-7833, 7815-7834, 7816-7832, 7816-7833, 7816-7834, 7816-7835, 7817-7833, 7817-7834, 7817-7835, 7817-7836, 7818-7834, 7818-7835, 7818-7836, 7818-7837, 7819-7835, 7819-7837, 7819-7838, 7820-7836, 7820-7838, 7820-7839,

7821-7837, 7821-7839, 7821-7840, 7822-7838, 7822-7840, 7822-7841, 7823-7838, 7823-7839, 7823-7841, 7823-7842, 7824-7840, 7824-7841, 7824-7842, 7824-7843, 7825-7840, 7825-7841, 7825-7842, 7825-7843, 7825-7844, 7826-7842, 7826-7844, 7826-7845, 7827-7843, 7827-7844, 7827-7846, 7828-7843, 7828-7844, 7828-7847, 7829-7844, 7829-7845, 7829-7846, 7829-7847, 7829-7848, 7830-7845, 7830-7846, 7830-7847, 7830-7848, 7830-7849, 7831-7846, 7831-7847, 7831-7848, 7831-7849, 7831-7850, 7832-7847, 7832-7848, 7832-7849, 7832-7850, 7832-7851, 7833-7848, 7833-7849, 7833-7850, 7833-7851, 7833-7852, 7834-7849, 7834-7850, 7834-7851, 7834-7852, 7834-7853, 7835-7850, 7835-7851, 7835-7852, 7835-7853, 7835-7854, 7836-7851, 7836-7852, 7836-7853, 7836-7854, 7836-7855, 7837-7852, 7837-7853, 7837-7854, 7837-7855, 7837-7856, 7838-7853, 7838-7854, 7838-7855, 7838-7856, 7838-7857, 7839-7854, 7839-7855, 7839-7856, 7839-7857, 7839-7858, 7840-7855, 7840-7856, 7840-7857, 7840-7858, 7840-7859, 7841-7856, 7841-7857, 7841-7858, 7841-7859, 7841-7860, 7842-7857, 7842-7858, 7842-7859, 7842-7860, 7842-7861, 7843-7858, 7843-7859, 7843-7860, 7843-7861, 7843-7862, 7844-7859, 7844-7860, 7844-7861, 7844-7862, 7845-7860, 7845-7861, 7845-7862, 7846-7861, 7846-7862, and 7847-7862.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 1, when targeted by antisense compounds or oligonucleotides, display at least 70% inhibition: 48-63, 150-169, 152-171, 154-169, 154-173, 156-171, 156-175, 158-173, 158-177, 1135-1154, 1141-1160, 1147-1166, 1171-1186, 1173-1188, 1175-1190, 1749-1768, 1763-1782, 1912-1931, 2193-2212, 2195-2210, 2195-2214, 2197-2212, 2197-2216, 2223-2238, 2225-2240, 2227-2242, 2453-2472, 2455-2474, 2457-2472, 2457-2476, 2459-2474, 2461-2476, 2461-2480, 2550-2569, 2551-2566, 2552-2571, 2553-2570, 2553-2571, 2553-2572, 2554-2571, 2554-2572, 2554-2573, 2554-2573, 2555-2572, 2555-2574, 2555-2574, 2556-2573, 2556-2574, 2556-2575, 2557-2574, 2557-2576, 2558-2575, 2558-2576, 2558-2577, 2559-2576, 2559-2577, 2559-2578, 2560-2577, 2560-2578, 2560-2579, 2561-2578, 2561-2579, 2561-2580, 2562-2577, 2562-2579, 2562-2581, 2563-2578, 2563-2580, 2563-2582, 2564-2581, 2564-2583, 2565-2584, 2566-2583, 2566-2585, 2567-2582, 2567-2584, 2567-2586, 2568-2585, 2568-2587, 2569-2586, 2569-2588, 2570-2587, 2570-2589, 2571-2588, 2571-2590, 2572-2589, 2572-2591, 2573-2590, 2573-2592, 2574-2591, 2574-2593, 2575-2592, 2575-2594, 2576-2593, 2576-2595, 2577-2594, 2577-2596, 2578-2597, 2579-2598, 2580-2596, 2580-2598, 2580-2599, 2581-2597, 2581-2600, 2582-2598, 2582-2600, 2582-2601, 2583-2599, 2583-2601, 2583-2602, 2584-2600, 2584-2602, 2584-2603, 2585-2601, 2585-2603, 2585-2604, 2586-2605, 2587-2606, 2588-2604, 2588-2606, 2588-2607, 2589-2605, 2589-2606, 2589-2607, 2589-2608, 2590-2605, 2590-2606, 2590-2607, 2590-2609, 2591-2607, 2591-2610, 2592-2611, 2593-2608, 2593-2612, 2594-2609, 2594-2610, 2594-2612, 2594-2613, 2595-2610, 2595-2611, 2595-2612, 2595-2613, 2595-2614, 2596-2611, 2596-2614, 2596-2615, 2597-2612, 2597-2613, 2597-2614, 2597-2615, 2597-2616, 2598-2613, 2598-2614, 2598-2615, 2598-2616, 2598-2617, 2599-2614, 2599-2615, 2599-2616, 2599-2617, 2599-2618, 2600-2615, 2600-2616, 2600-2617, 2600-2618, 2600-2619, 2601-2616, 2601-2617, 2601-2618, 2601-2619, 2601-2620, 2602-2617, 2602-2618, 2602-2619, 2602-2620, 2602-2621, 2603-2619, 2603-2620, 2603-2621, 2603-2622, 2604-2619, 2604-2620, 2604-2621, 2604-2622, 2604-2623,

2605-2620, 2605-2621, 2605-2622, 2605-2623, 2605-2624, 2606-2621, 2606-2622, 2606-2623, 2606-2624, 2606-2625, 2607-2622, 2607-2623, 2607-2624, 2607-2625, 2607-2626, 2608-2623, 2608-2624, 2608-2625, 2608-2626, 2608-2627, 2609-2624, 2609-2625, 2609-2626, 2609-2627, 2609-2628, 2610-2625, 2610-2626, 2610-2627, 2610-2628, 2610-2629, 2611-2626, 2611-2627, 2611-2629, 2611-2630, 2612-2627, 2612-2628, 2612-2629, 2612-2630, 2612-2631, 2613-2628, 2613-2629, 2613-2630, 2613-2631, 2614-2629, 2614-2630, 2614-2631, 2615-2630, 2615-2630, 2615-2631, and 2616-2631.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 2, when targeted by antisense compounds or oligonucleotides, display at least 70% inhibition: 1685-1704, 1686-1705, 1769-1784, 1871-1890, 1873-1892, 1875-1890, 1875-1894, 1877-1892, 1877-1896, 1879-1894, 1879-1898, 3819-3838, 3825-3844, 3831-3850, 4151-4166, 5890-5909, 5904-5923, 5904-5923, 6406-6425, 6983-6998, 6983-7002, 6985-7000, 6985-7004, 7688-7707, 7690-7709, 7692-7707, 7692-7711, 7694-7709, 7696-7711, 7696-7715, 7786-7801, 7787-7806, 7788-7805, 7788-7806, 7788-7807, 7789-7806, 7789-7807, 7789-7808, 7790-7807, 7790-7809, 7791-7808, 7791-7809, 7791-7810, 7792-7809, 7792-7811, 7793-7810, 7793-7811, 7793-7812, 7794-7811, 7794-7812, 7794-7813, 7795-7812, 7795-7813, 7795-7814, 7796-7813, 7796-7814, 7796-7815, 7797-7812, 7797-7814, 7797-7816, 7798-7813, 7798-7815, 7798-7817, 7799-7816, 7799-7818, 7800-7819, 7801-7818, 7801-7820, 7802-7817, 7802-7819, 7802-7821, 7803-7820, 7803-7822, 7804-7821, 7804-7823, 7805-7822, 7805-7824, 7806-7823, 7806-7825, 7807-7824, 7807-7826, 7808-7825, 7808-7827, 7809-7826, 7809-7828, 7810-7827, 7811-7828, 7811-7830, 7812-7829, 7812-7831, 7813-7832, 7814-7833, 7815-7831, 7815-7833, 7815-7834, 7816-7832, 7816-7835, 7817-7833, 7817-7835, 7817-7836, 7818-7834, 7818-7836, 7818-7837, 7819-7835, 7819-7837, 7819-7838, 7820-7836, 7820-7838, 7820-7839, 7821-7840, 7822-7841, 7823-7839, 7823-7841, 7823-7842, 7824-7840, 7824-7841, 7824-7842, 7824-7843, 7825-7840, 7825-7841, 7825-7842, 7825-7844, 7826-7842, 7826-7845, 7827-7846, 7828-7843, 7828-7847, 7829-7844, 7829-7845, 7829-7847, 7829-7848, 7830-7845, 7830-7846, 7830-7847, 7830-7848, 7830-7849, 7831-7846, 7831-7849, 7831-7850, 7832-7847, 7832-7848, 7832-7849, 7832-7850, 7832-7851, 7833-7848, 7833-7849, 7833-7850, 7833-7851, 7833-7852, 7834-7849, 7834-7850, 7834-7851, 7834-7852, 7834-7853, 7835-7850, 7835-7851, 7835-7852, 7835-7853, 7835-7854, 7836-7851, 7836-7852, 7836-7853, 7836-7854, 7836-7855, 7837-7852, 7837-7853, 7837-7854, 7837-7855, 7837-7856, 7838-7854, 7838-7855, 7838-7856, 7838-7857, 7839-7854, 7839-7855, 7839-7856, 7839-7857, 7839-7858, 7840-7855, 7840-7856, 7840-7857, 7840-7858, 7840-7859, 7841-7856, 7841-7857, 7841-7858, 7841-7859, 7841-7860, 7842-7857, 7842-7858, 7842-7859, 7842-7860, 7842-7861, 7843-7858, 7843-7859, 7843-7860, 7843-7861, 7843-7862, 7844-7859, 7844-7860, 7844-7861, 7844-7862, 7845-7860, 7845-7861, 7845-7862, 7846-7861, 7846-7862, and 7847-7862.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 1, when targeted by antisense compounds or oligonucleotides, display at least 80% inhibition: 152-171, 154-169, 156-171, 158-173, 1135-1154, 1171-1186, 1173-1188, 1175-1190, 1763-1782, 1912-1931, 2197-2212, 2223-2238, 2225-2240, 2227-2242, 2457-2472, 2459-2474, 2461-2476, 2551-2566, 2553-2570, 2553-2571, 2553-2572, 2554-

2573, 2555-2572, 2555-2574, 2556-2573, 2556-2574, 2556-2575, 2557-2574, 2557-2576, 2558-2575, 2558-2576, 2559-2577, 2559-2578, 2560-2577, 2560-2578, 2560-2579, 2561-2578, 2561-2579, 2561-2580, 2562-2577, 2562-2579, 2562-2581, 2563-2580, 2563-2582, 2564-2581, 2564-2583, 2565-2584, 2566-2583, 2567-2584, 2567-2586, 2568-2585, 2568-2587, 2569-2586, 2569-2588, 2570-2587, 2571-2588, 2571-2590, 2572-2589, 2572-2591, 2573-2590, 2573-2592, 2574-2591, 2574-2593, 2575-2592, 2576-2593, 2576-2595, 2577-2594, 2577-2596, 2578-2597, 2580-2598, 2580-2599, 2581-2597, 2581-2600, 2582-2601, 2583-2602, 2584-2603, 2585-2604, 2586-2605, 2587-2606, 2588-2607, 2589-2608, 2590-2606, 2590-2607, 2590-2609, 2591-2610, 2592-2611, 2593-2608, 2593-2612, 2594-2613, 2595-2611, 2595-2614, 2596-2615, 2597-2612, 2597-2613, 2597-2614, 2597-2615, 2597-2616, 2598-2613, 2598-2613, 2598-2614, 2598-2615, 2598-2616, 2598-2617, 2599-2614, 2599-2617, 2599-2618, 2600-2615, 2600-2617, 2600-2618, 2600-2619, 2601-2616, 2601-2617, 2601-2619, 2601-2620, 2602-2618, 2602-2621, 2603-2620, 2603-2621, 2603-2622, 2604-2619, 2604-2620, 2604-2621, 2604-2622, 2604-2623, 2605-2620, 2605-2621, 2605-2622, 2605-2623, 2605-2624, 2606-2621, 2606-2622, 2606-2623, 2606-2624, 2606-2625, 2607-2622, 2607-2623, 2607-2624, 2607-2625, 2607-2626, 2608-2623, 2608-2624, 2608-2625, 2608-2627, 2609-2624, 2609-2626, 2609-2627, 2609-2628, 2610-2625, 2610-2626, 2610-2628, 2610-2629, 2611-2626, 2611-2627, 2611-2629, 2611-2630, 2612-2627, 2612-2628, 2612-2630, 2612-2631, 2613-2628, 2613-2629, 2613-2631, 2614-2629, 2614-2630, 2614-2631, 2615-2630, and 2616-2631.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 2, when targeted by antisense compounds or oligonucleotides, display at least 80% inhibition: 1685-1704, 1686-1705, 1873-1892, 1875-1890, 1877-1892, 1879-1894, 3819-3838, 4151-4166, 5904-5923, 6406-6425, 6985-7000, 7692-7707, 7694-7709, 7696-7711, 7786-7801, 7788-7805, 7788-7806, 7788-7807, 7789-7808, 7790-7807, 7790-7809, 7791-7808, 7791-7809, 7791-7810, 7792-7809, 7792-7811, 7793-7810, 7793-7811, 7794-7812, 7794-7813, 7795-7812, 7795-7813, 7795-7814, 7796-7813, 7796-7814, 7796-7815, 7797-7812, 7797-7814, 7797-7816, 7798-7815, 7798-7817, 7799-7816, 7799-7818, 7800-7819, 7801-7818, 7802-7819, 7802-7821, 7803-7820, 7803-7822, 7804-7821, 7804-7823, 7805-7822, 7806-7823, 7806-7825, 7807-7824, 7807-7826, 7808-7825, 7808-7827, 7809-7826, 7809-7828, 7810-7827, 7811-7828, 7812-7829, 7812-7831, 7813-7832, 7814-7833, 7815-7834, 7816-7832, 7816-7835, 7817-7836, 7818-7837, 7819-7838, 7820-7839, 7821-7840, 7822-7841, 7823-7842, 7824-7843, 7825-7841, 7825-7842, 7825-7844, 7826-7845, 7827-7846, 7828-7843, 7828-7847, 7829-7848, 7830-7846, 7830-7849, 7831-7850, 7832-7847, 7832-7848, 7832-7849, 7832-7850, 7832-7851, 7833-7848, 7833-7849, 7833-7850, 7833-7851, 7833-7852, 7834-7849, 7834-7852, 7834-7853, 7835-7850, 7835-7852, 7835-7853, 7835-7854, 7836-7851, 7836-7852, 7836-7854, 7836-7855, 7837-7853, 7837-7856, 7838-7855, 7838-7856, 7838-7857, 7839-7854, 7839-7855, 7839-7856, 7839-7857, 7839-7858, 7840-7855, 7840-7856, 7840-7857, 7840-7858, 7840-7859, 7841-7856, 7841-7857, 7841-7858, 7841-7859, 7841-7860, 7842-7857, 7842-7858, 7842-7859, 7842-7860, 7842-7861, 7843-7858, 7843-7859, 7843-7860, 7843-7862, 7844-7859, 7844-7861, 7844-7862, 7845-7860, 7845-7861, 7846-7862, and 7847-7862.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 1, when targeted by antisense compounds or oligonucleotides, display at least 90% inhibition: 154-169, 156-171, 158-173, 1135-1154, 1171-1186, 1173-1188, 1763-1782, 1912-1931, 2223-2238, 2227-2242, 2459-2474, 2461-2476, 2554-2573, 2555-2574, 2560-2577, 2561-2578, 2561-2579, 2562-2581, 2563-2580, 2563-2582, 2564-2581, 2566-2583, 2567-2584, 2568-2585, 2568-2587, 2569-2586, 2570-2587, 2576-2593, 2577-2594, 2577-2596, 2578-2597, 2580-2599, 2581-2600, 2582-2601, 2583-2602, 2584-2603, 2586-2605, 2587-2605, 2587-2606, 2588-2607, 2589-2608, 2590-2607, 2590-2609, 2592-2611, 2595-2614, 2596-2615, 2597-2612, 2597-2613, 2597-2615, 2597-2616, 2598-2613, 2598-2613, 2598-2617, 2599-2614, 2599-2618, 2600-2615, 2600-2619, 2601-2617, 2601-2620, 2602-2621, 2603-2622, 2604-2623, 2605-2621, 2605-2622, 2605-2624, 2606-2625, 2607-2626, 2608-2623, 2608-2625, 2609-2628, 2611-2627, 2611-2630, 2612-2628, 2612-2631, 2613-2629, 2614-2629, 2615-2630, and 2616-2631.

In certain embodiments, the following nucleotide regions of SEQ ID NO: 2, when targeted by antisense compounds or oligonucleotides, display at least 90% inhibition: 1685-1704, 1686-1705, 1875-1890, 1877-1892, 1879-1894, 3819-3838, 5904-5923, 6406-6425, 7694-7709, 7696-7711, 7789-7808, 7790-7809, 7795-7812, 7795-7813, 7796-7813, 7796-7814, 7797-7814, 7797-7816, 7798-7815, 7798-7817, 7799-7816, 7801-7818, 7802-7819, 7803-7820, 7803-7822, 7804-7821, 7805-7822, 7811-7828, 7812-7829, 7812-7831, 7813-7832, 7815-7834, 7818-7837, 7819-7838, 7821-7840, 7822-7840, 7822-7841, 7825-7842, 7832-7847, 7832-7848, 7832-7850, 7833-7848, 7833-7852, 7834-7849, 7834-7853, 7835-7850, 7836-7852, 7836-7855, 7837-7856, 7838-7856, 7839-7857, 7839-7858, 7840-7856, 7840-7857, 7840-7859, 7843-7858, 7843-7860, and 7846-7862.

In certain embodiments, the following antisense compounds or oligonucleotides target a region of a CFB nucleic acid and effect at least a 50% inhibition of a CFB mRNA, ISIS NOs: 516350, 532614, 532632, 532635, 532638, 532639, 532686, 532687, 532688, 532689, 532690, 532691, 532692, 532692, 532693, 532694, 532695, 532696, 532697, 532698, 532699, 532700, 532701, 532702, 532703, 532704, 532705, 532706, 532707, 532770, 532775, 532778, 532780, 532791, 532800, 532809, 532810, 532811, 532917, 532952, 588509, 588510, 588511, 588512, 588513, 588514, 588515, 588516, 588517, 588518, 588519, 588520, 588522, 588523, 588524, 588525, 588527, 588528, 588529, 588530, 588531, 588532, 588533, 588534, 588535, 588536, 588537, 588538, 588539, 588540, 588541, 588542, 588543, 588544, 588545, 588546, 588547, 588548, 588549, 588550, 588551, 588552, 588553, 588554, 588555, 588556, 588557, 588558, 588559, 588560, 588561, 588562, 588563, 588564, 588565, 588566, 588567, 588568, 588569, 588570, 588571, 588572, 588573, 588574, 588575, 588576, 588577, 588580, 588581, 588585, 588586, 588589, 588590, 588599, 588603, 588606, 588608, 588610, 588614, 588616, 588628, 588631, 588632, 588634, 588636, 588638, 588640, 588645, 588646, 588654, 588656, 588658, 588660, 588662, 588664, 588670, 588672, 588676, 588682, 588688, 588696, 588698, 588807, 588808, 588809, 588813, 588814, 588815, 588819, 588820, 588822, 588823, 588838, 588839, 588840, 588841, 588842, 588846, 588847,

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In certain embodiments, the following antisense compounds or oligonucleotides target a region of a CFB nucleic acid and effect at least a 50% inhibition of a CFB mRNA, SEQ ID NOs: 12, 30, 33, 36, 37, 84, 85, 86, 87, 88, 89, 90, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 198, 203, 206, 208, 219, 228, 237, 238, 239, 317, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 468, 472, 473, 475, 478, 479, 488, 492, 494, 495, 498, 499, 500, 502, 503, 509, 510, 511, 512, 513, 514, 515, 517, 518, 522, 523, 524, 525, 529, 530, 531, 534, 535, 537, 540, 541, 542, 543, 544, 545, 546, 547, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 563, 564, 565, 569, 570, 572, 573, 577, 588, 589, 590, 591, 592, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 623, 640, 641, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 700, 704, 705, 706, 707, 708, 709, 711, 712, 713, 714, 715, 716, 717, 718, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 758, 759, 760, 761, 762, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 813, 833, 834, 841, 846, 849, 850, 867, and 873.

In certain embodiments, the following antisense compounds or oligonucleotides target a region of a CFB nucleic acid and effect at least a 60% inhibition of a CFB mRNA, ISIS NOs: 516350, 532614, 532635, 532686, 532687, 532688, 532689, 532770, 532800, 532809, 532810, 532811, 532917, 532952, 588512, 588513, 588514, 588515, 588516, 588517, 588518, 588519, 588522, 588523, 588524, 588525, 588527, 588528, 588529, 588530, 588531, 588532, 588533, 588534, 588535, 588536, 588537, 588538, 588539, 588540, 588541, 588542, 588543, 588544, 588545, 588546, 588547, 588548, 588549, 588550, 588551, 588552, 588553, 588554, 588555, 588556, 588557, 588558, 588559, 588560, 588561, 588562, 588563, 588564, 588565, 588566, 588567, 588568, 588569, 588570, 588571, 588572, 588573, 588574, 588575, 588576, 588577, 588636, 588638, 588640, 588664, 588676, 588696, 588698, 588807, 588808, 588814, 588815, 588819, 588820, 588840, 588842, 588846, 588847, 588848, 588849, 588850, 588851, 588852, 588853, 588854, 588855, 588856, 588857, 588858, 588859, 588860, 588861, 588862, 588863, 588864, 588866, 588867, 588868, 588870, 588871, 588872, 588873, 588874, 588875, 588876, 588877, 588878, 588879, 588880, 588881, 588882, 588883, 588884, 598999, 599000, 599001, 599002, 599003, 599004,

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In certain embodiments, the following antisense compounds or oligonucleotides target a region of a CFB nucleic acid and effect at least a 60% inhibition of a CFB mRNA, SEQ ID NOs: 12, 33, 84, 85, 86, 87, 198, 228, 237, 238, 239, 317, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455,

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In certain embodiments, the following antisense compounds or oligonucleotides target a region of a CFB nucleic acid and effect at least a 70% inhibition of a CFB mRNA, ISIS NOs: 516350, 532614, 532686, 532687, 532688, 532770, 532800, 532809, 532810, 532811, 532917, 532952, 588512, 588513, 588514, 588515, 588516, 588517, 588518, 588524, 588529, 588530, 588531, 588532, 588533, 588534, 588535, 588536, 588537, 588538, 588539, 588540, 588541, 588542, 588543, 588544, 588545, 588546, 588547, 588548, 588549, 588550, 588551, 588552, 588553, 588554, 588555, 588556, 588557, 588558, 588559, 588560, 588561, 588562, 588563, 588564, 588565, 588568, 588569, 588570, 588571, 588572, 588573, 588574, 588575, 588577, 588636, 588638, 588640, 588696, 588698, 588807, 588814, 588815, 588819, 588842, 588847, 588848, 588849, 588850, 588851, 588852, 588853, 588856, 588857, 588858, 588859, 588860, 588861, 588862, 588863, 588866, 588867, 588870, 588871, 588872, 588873, 588874, 588875, 588876, 588877, 588878, 588879, 588880, 588881, 588882, 588883, 588884, 599000, 599001, 599003, 599004, 599005, 599008, 599009, 599010, 599011, 599014, 599015, 599024, 599025, 599027, 599028, 599029, 599030, 599031, 599032, 599033, 599034, 599072, 599077, 599080, 599085, 599086, 599087, 599088, 599089, 599090, 599091, 599093, 599094, 599095, 599096, 599097, 599125, 599126, 599134, 599138, 599139, 599148, 599149, 599150, 599151, 599152, 599154, 599155, 599156, 599157, 599158, 599187, 599188, 599193, 599195, 599196, 599197, 599198, 599199, 599200, 599201, 599202, 599203, 599204, 599205, 599206, 599207, 599208, 599210, 599211, 599212, 599213, 599214, 599215, 599216, 599217, 599218, 599219, 599220, 599221, 599222, 599223, 599224, 599225, 599226, 599227, 599228, 599229, 599230, 599231, 599232, 599233, 599234, 599235, 599236, 599266, 599272, 599272, 599273, 599274, 599275, 599277, 599278, 599279, 599280, 599280, 599306, 599311, 599312, 599313, 599314, 599315, 599316, 599317, 599318, 599319, 599320, 599321, 599322, 599323, 599325, 599327, 599328, 599329, 599330, 599355, 599357, 599358, 599359, 599360, 599361, 599362, 599363, 599364, 599369, 599371, 599372, 599373, 599378, 599379, 599382, 599384, 599386, 599387, 599388, 599389, 599390, 599391, 599392, 599393, 599394, 599395, 599396, 599397, 599398, 599399, 599400, 599401, 599402, 599403, 599404, 599405, 599406, 599407, 599408, 599409, 599410, 599413, 599414, 599415, 599416,

599417, 599418, 599419, 599420, 599421, 599422, 599423, 599424, 599433, 599434, 599435, 599436, 599437, 599438, 599439, 599440, 599441, 599442, 599443, 599445, 599446, 599447, 599448, 599472, 599473, 599474, 599475, 599476, 599477, 599478, 599479, 599480, 599481, 599482, 599483, 599484, 599485, 599486, 599487, 599488, 599489, 599490, 599491, 599492, 599493, 599494, 599495, 599496, 599497, 599498, 599499, 599500, 599501, 599502, 599503, 599504, 599505, 599506, 599507, 599508, 599512, 599547, 599548, 599552, 599553, 599554, 599555, 599558, 599562, 599563, 599564, 599566, 599567, 599568, 599569, 599570, 599577, 599578, 599579, 599580, 599581, 599582, 599585, 599586, 599587, 599588, 599589, 599590, 599591, 599592, 599593, 599594, 599595, 601332, 601335, 601341, 601343, 601344, 601345, 601346, 601347, 601348, 601349, 601371, 601372, 601380, 601382, 601383, 601384, 601385, 601386, and 601387.

In certain embodiments, the following antisense compounds or oligonucleotides target a region of a CFB nucleic acid and effect at least a 70% inhibition of a CFB mRNA, SEQ ID NOs: 12, 84, 85, 86, 198, 228, 237, 238, 239, 317, 395, 396, 397, 398, 399, 402, 403, 404, 405, 407, 408, 410, 411, 412, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 464, 465, 472, 473, 513, 514, 515, 541, 542, 543, 544, 545, 546, 547, 549, 550, 551, 552, 553, 554, 555, 556, 557, 564, 565, 569, 592, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 645, 646, 647, 648, 649, 650, 653, 654, 655, 656, 659, 660, 662, 663, 664, 665, 666, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 677, 678, 679, 680, 682, 683, 684, 686, 687, 688, 689, 706, 708, 709, 711, 712, 713, 714, 715, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 767, 768, 773, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 793, 794, 795, 797, 798, 799, 813, 833, 834, 841, 846, 849, 867, and 873.

In certain embodiments, the following antisense compounds or oligonucleotides target a region of a CFB nucleic acid and effect at least an 80% inhibition of a CFB mRNA, ISIS NOs: 532686, 532809, 532810, 532811, 532917, 532952, 588512, 588517, 588518, 588533, 588534, 588535, 588536, 588537, 588538, 588539, 588540, 588542, 588543, 588544, 588545, 588546, 588547, 588548, 588549, 588550, 588551, 588552, 588553, 588554, 588555, 588556, 588557, 588558, 588559, 588560, 588561, 588562, 588563, 588564, 588565, 588571, 588638, 588640, 588696, 588698, 588807, 588814, 588849, 588850, 588851, 588853, 588857, 588858, 588859, 588860, 588861, 588862, 588863, 588866, 588867, 588871, 588872, 588873, 588874, 588875, 588876, 588877, 588878, 588879, 588880, 588881, 588882, 588883, 599001, 599024, 599025, 599033, 599086, 599087, 599088, 599089, 599093, 599094, 599095, 599096, 599134, 599139, 599148, 599149, 599151, 599154, 599155, 599156, 599158, 599188, 599195, 599196, 599198, 599201, 599202, 599203, 599204, 599205, 599206, 599207, 599212, 599213, 599215, 599216, 599217, 599218, 599219, 599220, 599221, 599222, 599223, 599224, 599225, 599226, 599227, 599228, 599229,

599230, 599231, 599232, 599233, 599234, 599235, 599236, 599272, 599273, 599275, 599277, 599278, 599279, 599280, 599311, 599313, 599314, 599316, 599317, 599318, 599320, 599321, 599322, 599323, 599327, 599328, 599329, 599330, 599355, 599357, 599358, 599359, 599360, 599361, 599362, 599363, 599364, 599371, 599372, 599373, 599378, 599379, 599382, 599384, 599386, 599387, 599388, 599389, 599390, 599391, 599392, 599393, 599397, 599398, 599399, 599400, 599401, 599403, 599404, 599405, 599407, 599408, 599409, 599410, 599413, 599414, 599415, 599416, 599417, 599418, 599419, 599420, 599421, 599422, 599423, 599424, 599433, 599434, 599435, 599436, 599437, 599438, 599439, 599440, 599441, 599445, 599446, 599447, 599448, 599474, 599476, 599477, 599479, 599481, 599482, 599483, 599485, 599486, 599487, 599488, 599489, 599490, 599491, 599492, 599494, 599495, 599496, 599497, 599498, 599499, 599500, 599502, 599503, 599504, 599505, 599506, 599507, 599508, 599547, 599552, 599553, 599554, 599558, 599563, 599567, 599568, 599569, 599570, 599577, 599578, 599581, 599582, 599585, 599587, 599588, 599590, 599591, 599592, 599593, 599594, 601332, 601344, 601345, 601382, 601383, and 601385.

In certain embodiments, the following antisense compounds or oligonucleotides target a region of a CFB nucleic acid and effect at least a 80% inhibition of a CFB mRNA, SEQ ID NOs: 84, 237, 238, 239, 317, 395, 397, 411, 412, 413, 414, 415, 417, 418, 419, 420, 421, 422, 423, 425, 426, 427, 429, 430, 431, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 472, 473, 514, 515, 542, 543, 544, 545, 546, 547, 550, 551, 552, 553, 554, 555, 556, 557, 564, 595, 599, 600, 601, 602, 603, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 646, 655, 660, 662, 663, 666, 669, 670, 671, 672, 673, 675, 676, 677, 678, 679, 682, 684, 686, 687, 688, 689, 706, 708, 709, 711, 712, 713, 714, 715, 720, 722, 723, 724, 725, 726, 727, 729, 730, 731, 732, 733, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 768, 775, 776, 778, 781, 782, 783, 784, 785, 787, 788, 789, 790, 791, 792, 793, 794, 799, 813, 833, 834, 841, 849, 867, and 873.

In certain embodiments, the following antisense compounds or oligonucleotides target a region of a CFB nucleic acid and effect at least a 90% inhibition of a CFB mRNA, ISIS NOs: 532686, 532811, 532917, 588536, 588537, 588538, 588539, 588544, 588545, 588546, 588548, 588551, 588552, 588553, 588554, 588555, 588556, 588557, 588558, 588559, 588560, 588561, 588562, 588564, 588638, 588640, 588696, 588698, 588849, 588850, 588851, 588860, 588866, 588867, 588872, 588873, 588874, 588876, 588877, 588878, 588879, 588881, 588883, 599149, 599188, 599203, 599206, 599220, 599221, 599222, 599223, 599224, 599225, 599226, 599227, 599228, 599229, 599235, 599236, 599279, 599280, 599314, 599321, 599362, 599378, 599390, 599391, 599398, 599399, 599404, 599413, 599414, 599416, 599419, 599420, 599422, 599435, 599437, 599438, 599441, 599483, 599494, 599508, 599552, 599553, 599554, 599568, 599570, 599577, 599581, 599591, 599592, and 599593.

In certain embodiments, the following antisense compounds or oligonucleotides target a region of a CFB nucleic acid and effect at least a 90% inhibition of a CFB mRNA, SEQ ID NOs: 84, 238, 239, 317, 412, 413, 420, 421, 426, 434, 436, 437, 438, 439, 440, 442, 443, 444, 445, 446, 448, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 464, 465, 472, 473, 514, 515, 542, 543, 544, 545, 546, 551, 553, 555, 556, 599, 600, 601, 602, 610, 616, 617, 618, 662, 666, 670, 676, 677, 678, 688, 689, 713, 723, 729, 730, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 755, 756, 768, 783, 793, 833, and 867.

In certain embodiments, a compound can comprise or consist of any oligonucleotide targeted to CFB described herein and a conjugate group.

In certain embodiments, a compound comprises a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides complementary within nucleotides 2193-2212, 2195-2210, 2457-2476, 2571-2590, 2584-2603, 2588-2607, 2592-2611, 2594-2613, 2597-2616, 2600-2619, or 2596-2611 of SEQ ID NO: 1.

In certain embodiments, a compound comprises a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 198, 228, 237, 440, 444, 448, 450, 453, 455, 549, and 598.

In certain embodiments, a compound comprises a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide has a nucleobase sequence consisting of any one of SEQ ID NOs: 198, 228, 237, 440, 444, 448, 450, 453, 455, 549, and 598.

In certain embodiments, any of the foregoing compounds or oligonucleotides can comprise at least one modified internucleoside linkage, at least one modified sugar, and/or at least one modified nucleobase.

In certain aspects, any of the foregoing compounds or oligonucleotides can comprise at least one modified sugar. In certain aspects, at least one modified sugar comprises a 2'-O-methoxyethyl group. In certain aspects, at least one modified sugar is a bicyclic sugar, such as a 4'-$CH(CH_3)$-O-2' group, a 4'-CH_2-O-2' group, or a 4'-$(CH_2)_2$-O-2'group.

In certain aspects, the modified oligonucleotide comprises at least one modified internucleoside linkage, such as a phosphorothioate internucleoside linkage.

In certain embodiments, the modified oligonucleotide comprises at least 1, 2, 3, 4, 5, 6, or 7 phosphodiester internucleoside linkages.

In certain embodiments, each internucleoside linkage of the modified oligonucleotide is selected from a phosphodiester internucleoside linkage and a phosphorothioate internucleoside linkage.

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

In certain embodiments, any of the foregoing compounds or oligonucleotides comprises at least one modified nucleobase, such as 5-methylcytosine.

In certain embodiments, a compound comprises a conjugate group and a modified oligonucleotide comprising:

a gap segment consisting of linked deoxynucleosides;

a 5' wing segment consisting of linked nucleosides; and

a 3' wing segment consisting of linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar. In certain embodiments, the oligonucleotide consists of 10 to 30 linked nucleosides having a nucleobase sequence comprising the sequence recited in SEQ ID NO: 198, 228, 237, 440, 444, 448, 450, 453, 455, 549, or 598.

In certain embodiments, the modified oligonucleotide has a nucleobase sequence comprising or consisting of the sequence recited in SEQ ID NO: 198, 228, 237, 440, 444, 448, 450, 453, or 455, wherein the modified oligonucleotide comprises:

a gap segment consisting of ten linked deoxynucleosides;

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

a 3' wing segment consisting of five linked nucleosides;

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

In certain embodiments, a compound comprises or consists of a single-stranded modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 20 linked nucleosides having a nucleobase sequence consisting of the sequence recited in SEQ ID NO: 198, 228, 237, 440, 444, 448, 450, 453, or 455, wherein the oligonucleotide comprises:

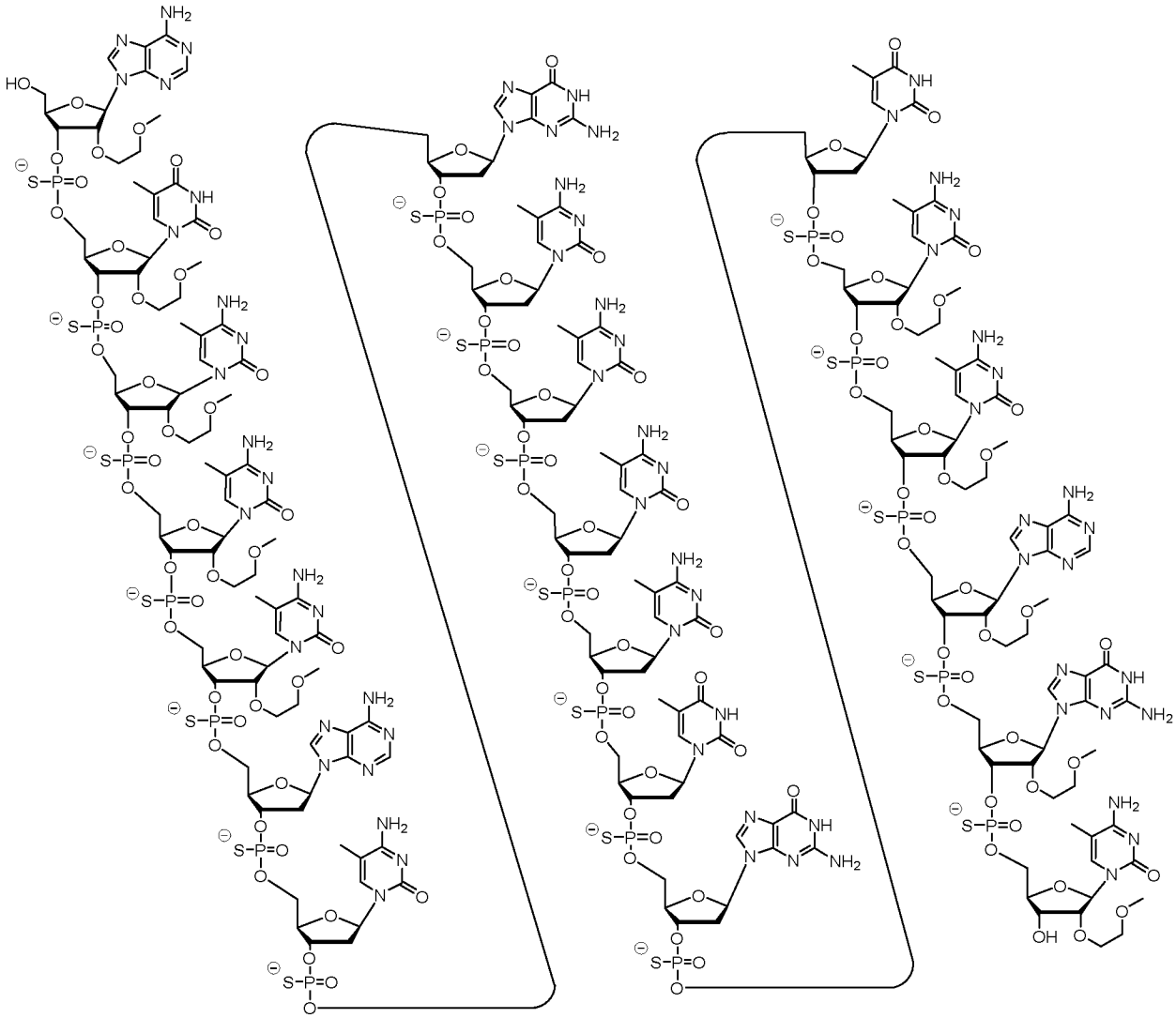
a gap segment consisting of ten linked deoxynucleosides;

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

a 3' wing segment consisting of five linked nucleosides;

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

In certain embodiments, a compound comprises or consists of ISIS 588540 and a conjugate group. In certain embodiments, ISIS 588540 has the following chemical structure:



In certain embodiments, the modified oligonucleotide has a nucleobase sequence comprising or consisting of the sequence recited in SEQ ID NO: 549, wherein the modified oligonucleotide comprises

a gap segment consisting of ten linked deoxynucleosides;

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

a 3' wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein each nucleoside of each wing segment comprises a cEt sugar; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In certain aspects, the modified oligonucleotide has a nucleobase sequence comprising or consisting of the sequence recited in SEQ ID NO: 598, wherein the modified oligonucleotide comprises

a gap segment consisting of ten linked deoxynucleosides;

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

a 3' wing segment consisting of three linked nucleosides;

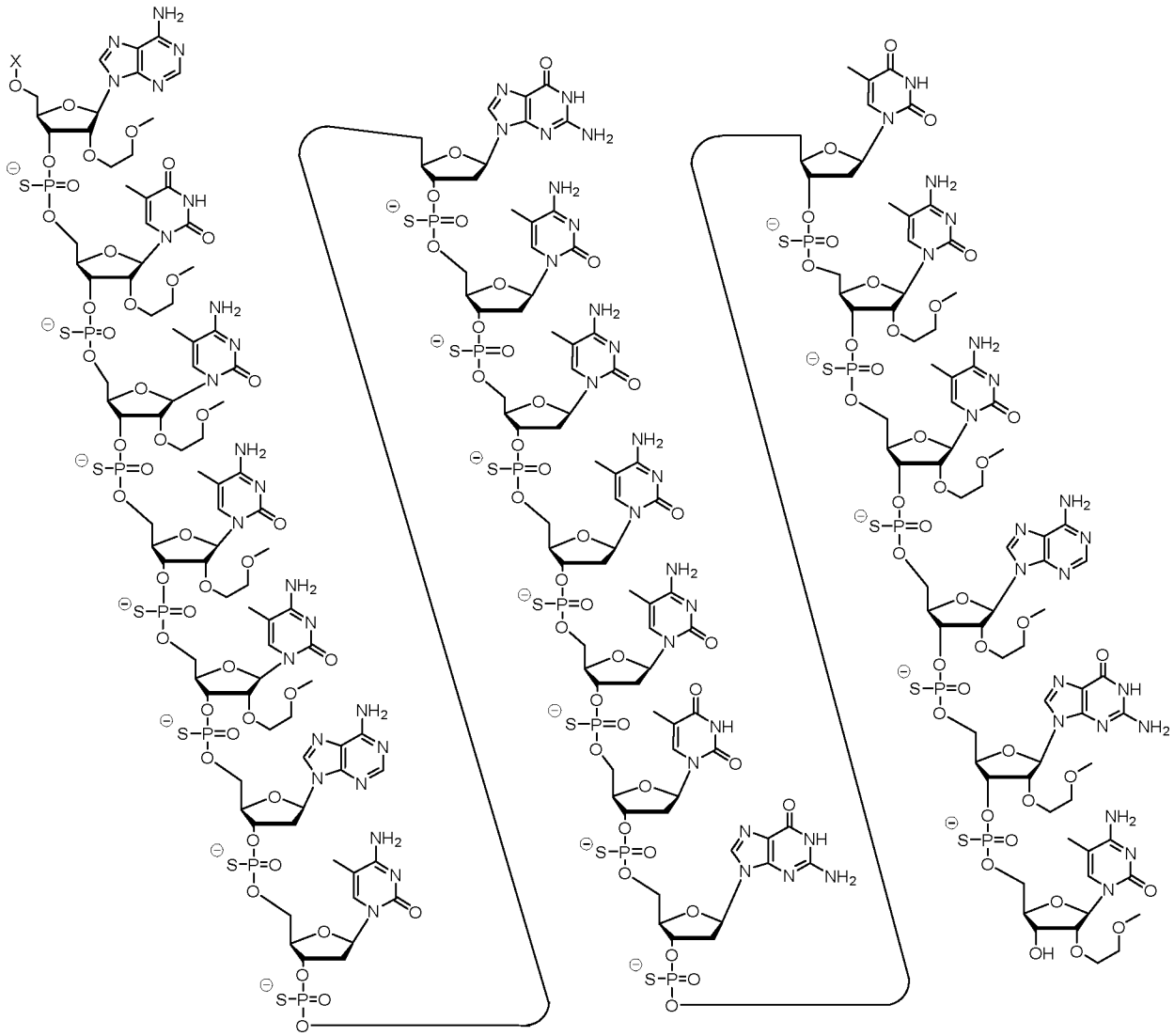
wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment; wherein the 5' wing segment comprises a 2'-O-methoxyethyl sugar, 2'-O-methoxyethyl sugar, and cEt sugar in the 5' to 3' direction; wherein the 3' wing segment comprises a cEt sugar, cEt sugar, and 2'-O-methoxyethyl sugar in the 5' to 3' direction; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In any of the foregoing embodiments, the compound or oligonucleotide can be at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% complementary to a nucleic acid encoding CFB.

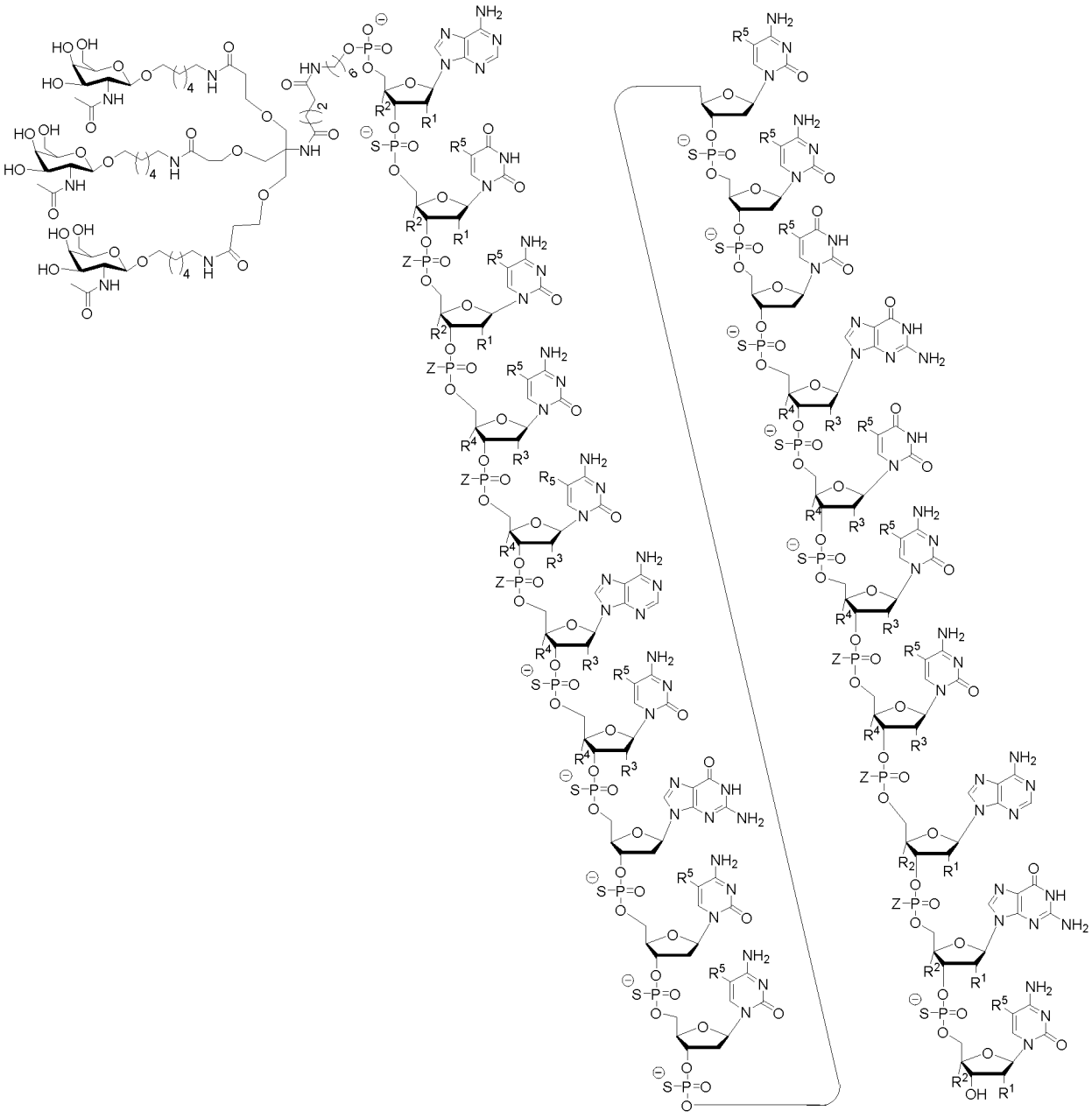
In any of the foregoing embodiments, the compound or oligonucleotide can be single-stranded.

In certain embodiments, the conjugate group is linked to the modified oligonucleotide at the 5' end of the modified oligonucleotide. In certain embodiments, the conjugate group is linked to the modified oligonucleotide at the 3' end of the modified oligonucleotide. In certain embodiments, the conjugate group comprises at least one *N*- Acetylgalactosamine (GalNAc), at least two *N*- Acetylgalactosamines (GalNAcs), or at least three *N*- Acetylgalactosamines (GalNAcs).

In certain embodiments, a compound having the following chemical structure comprises or consists of ISIS 588540 with a 5'-X, wherein X is a conjugate group comprising GalNAc as described herein:



In certain embodiments, a compound comprises or consists of SEQ ID NO: 440, 5'-GalNAc, and chemical modifications as represented by the following chemical structure:



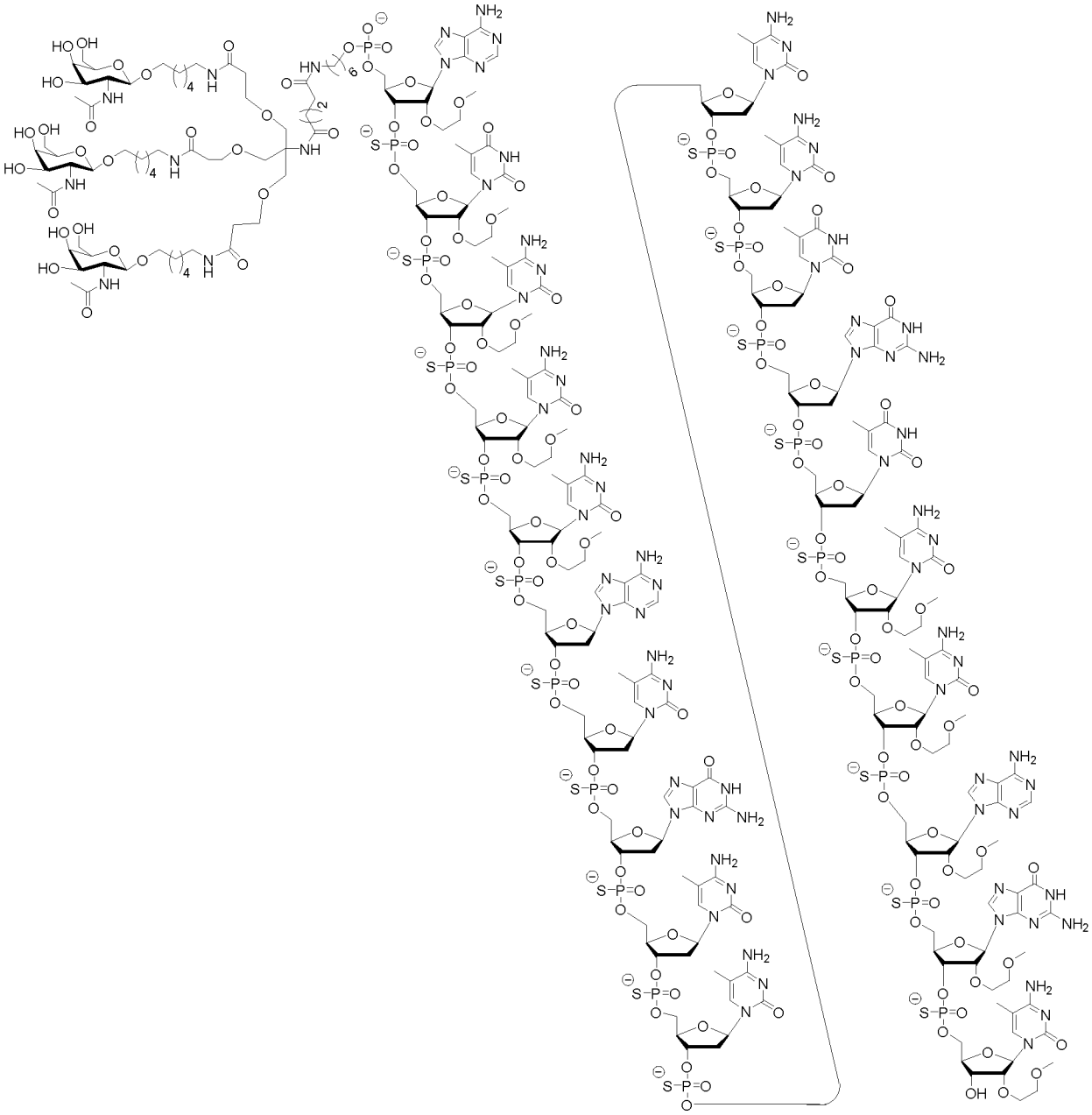
wherein either R^1 is $-OCH_2CH_2OCH_3$ (MOE)and R^2 is H; or R^1 and R^2 together form a bridge, wherein R^1 is –O- and R^2 is $-CH_2-$, $-CH(CH_3)-$, or $-CH_2CH_2-$, and R^1 and R^2 are directly connected such that the resulting bridge is selected from: $-O-CH_2-$, $-O-CH(CH_3)-$, and $-O-CH_2CH_2-$;

And for each pair of R^3 and R^4 on the same ring, independently for each ring: either R^3 is selected from H and $-OCH_2CH_2OCH_3$ and R^4 is H; or R^3 and R^4 together form a bridge, wherein R^3 is –O-, and R^4 is $-CH_2-$, $-CH(CH_3)-$, or $-CH_2CH_2-$and R^3 and R^4 are directly connected such that the resulting bridge is selected from: $-O-CH_2-$, $-O-CH(CH_3)-$, and $-O-CH_2CH_2-$;

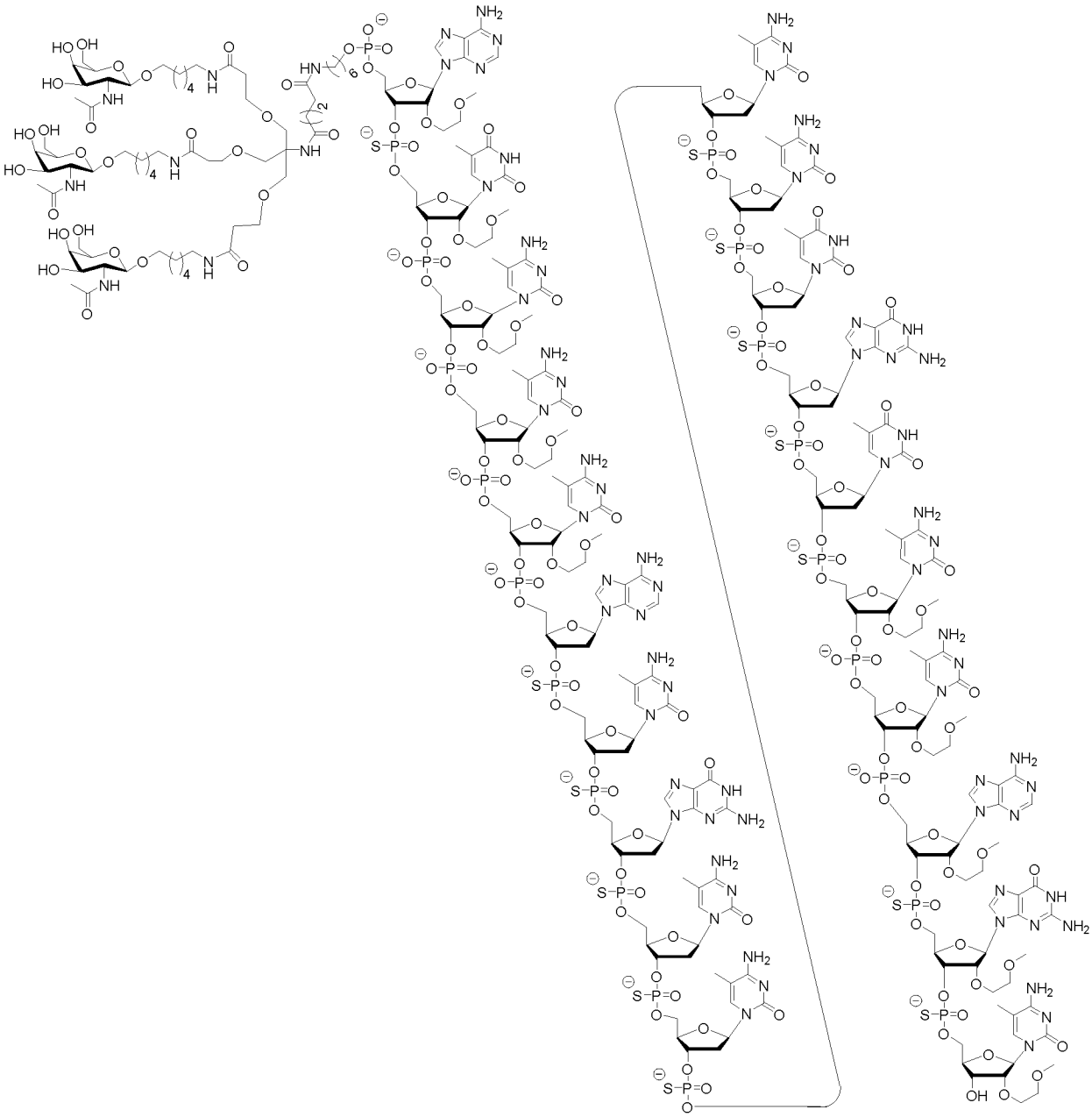
And R^5 is selected from H and $-CH_3$;

And Z is selected from S^- and O^-.

In certain embodiments, a compound comprises ISIS 696844. In certain embodiments, a compound consists of ISIS 696844. In certain embodiments, ISIS 696844 has the following chemical structure:

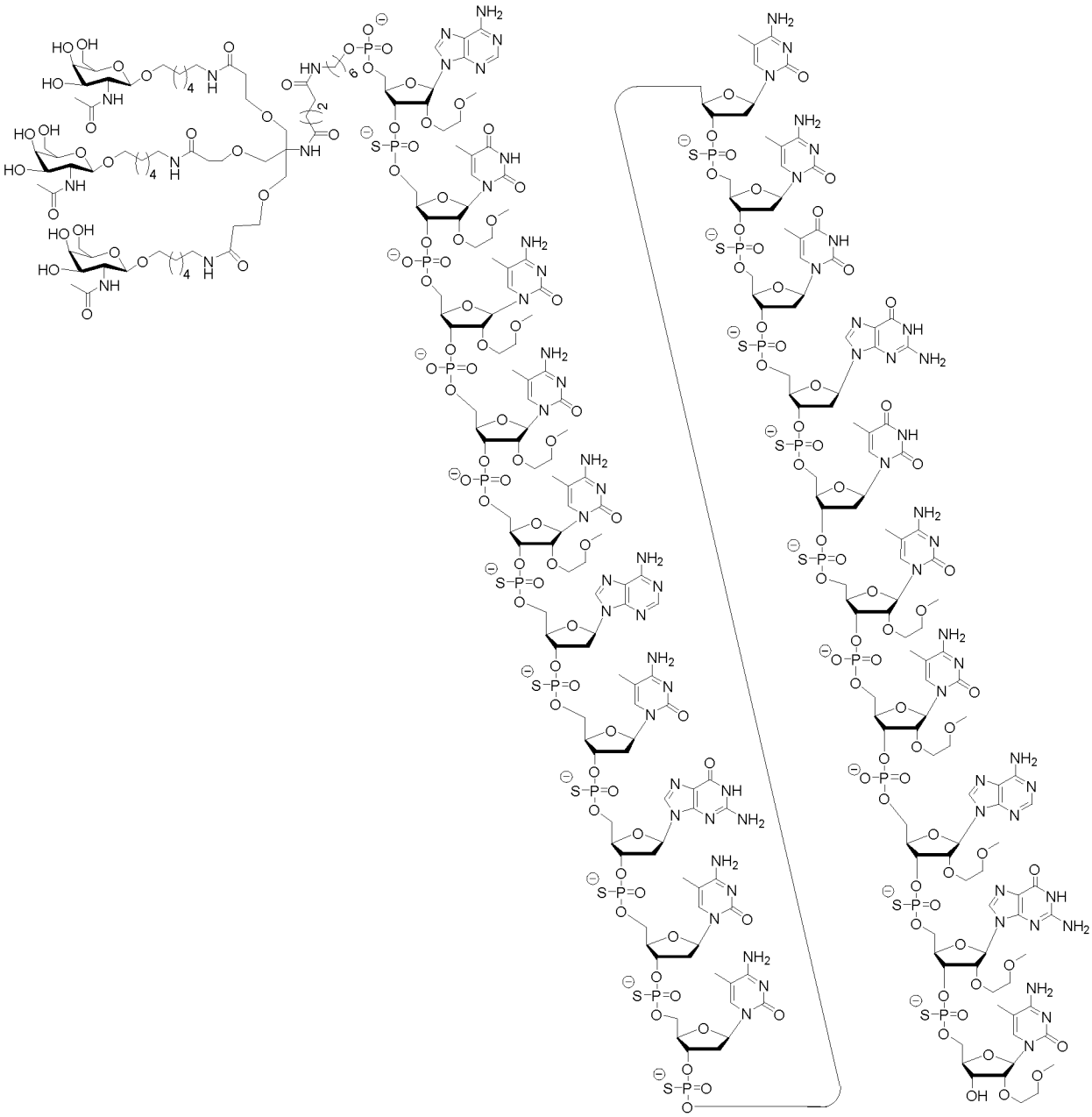


In certain embodiments, a compound comprises ISIS 696845. In certain embodiments, a compound consists of ISIS 696845. In certain embodiments, ISIS 696845 has the following chemical structure:

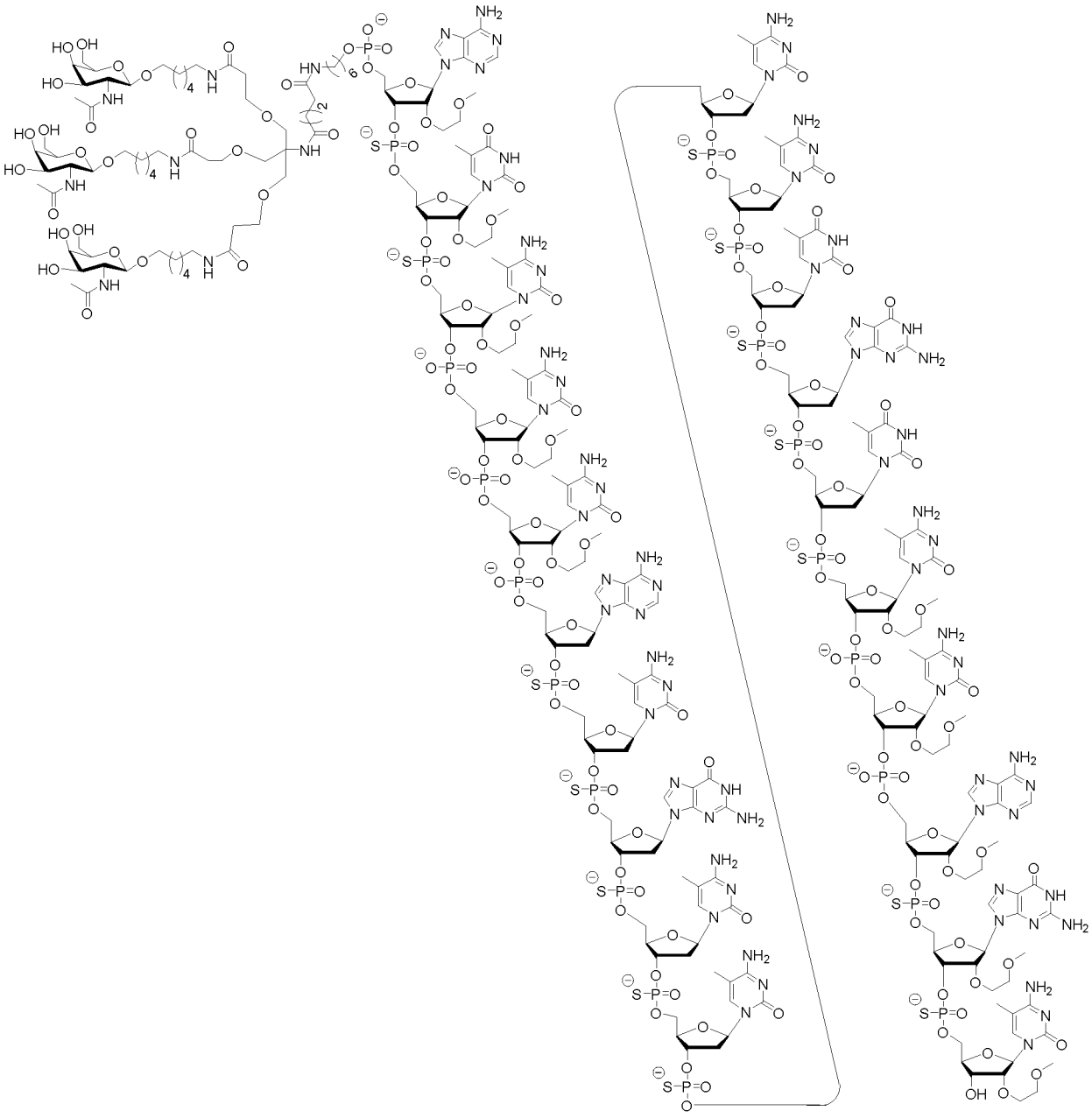


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In certain embodiments, a compound comprises ISIS 698969. In certain embodiments, a compound consists of ISIS 698969. In certain embodiments, ISIS 698969 has the following chemical structure:



In certain embodiments, a compound comprises ISIS 698970. In certain embodiments, a compound consists of ISIS 698970. In certain embodiments, ISIS 698970 has the following chemical structure:



Certain embodiments provide compositions comprising any of the compounds comprising or consisting of a modified oligonucleotide targeted to CFB or salt thereof and a conjugate group, and at least one of a pharmaceutically acceptable carrier or diluent.

In certain embodiments, the compounds or compositions as described herein are efficacious by virtue of having at least one of an *in vitro* IC_{50} of less than 250 nM, less than 200 nM, less than 150 nM, less than 100 nM, less than 90 nM, less than 80 nM, less than 70 nM, less than 65 nM, less than 60 nM, less than 55

nM, less than 50 nM, less than 45 nM, less than 40 nM, less than 35 nM, less than 30 nM, less than 25 nM, or less than 20 nM.

In certain embodiments, the compounds or compositions as described herein are highly tolerable as demonstrated by having at least one of an increase an ALT or AST value of no more than 4 fold, 3 fold, or 2 fold over saline treated animals or an increase in liver, spleen, or kidney weight of no more than 30%, 20%, 15%, 12%, 10%, 5%, or 2%. In certain embodiments, the compounds or compositions as described herein are highly tolerable as demonstrated by having no increase of ALT or AST over saline treated animals. In certain embodiments, the compounds or compositions as described herein are highly tolerable as demonstrated by having no increase in liver, spleen, or kidney weight over saline treated animals.

Certain embodiments provide a composition comprising the compound of any of the aforementioned embodiments or salt thereof and at least one of a pharmaceutically acceptable carrier or diluent. In certain aspects, the composition has a viscosity less than about 40 centipoise (cP), less than about 30 centipose (cP), less than about 20 centipose (cP), less than about 15 centipose (cP), or less than about 10 centipose (cP). In certain aspects, the composition having any of the aforementioned viscosities comprises a compound provided herein at a concentration of about 100 mg/mL, about 125 mg/mL, about 150 mg/mL, about 175 mg/mL, about 200 mg/mL, about 225 mg/mL, about 250 mg/mL, about 275 mg/mL, or about 300 mg/mL. In certain aspects, the composition having any of the aforementioned viscosities and/or compound concentrations has a temperature of room temperature or about 20°C, about 21°C, about 22°C, about 23°C, about 24°C, about 25°C, about 26°C, about 27°C, about 28°C, about 29°C, or about 30°C.

In certain embodiments, a method of treating, preventing, or ameliorating a disease associated with dysregulation of the complement alternative pathway in a subject comprises administering to the subject a compound or composition described herein, thereby treating, preventing, or ameliorating the disease. In certain aspects, the complement alternative pathway is activated greater than normal. In certain embodiments, a method of treating, preventing, or ameliorating a disease associated with dysregulation of the complement alternative pathway in a subject comprises administering to the subject a compound comprising or consisting of a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides and has a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 6-808. In certain embodiments, a method of treating, preventing, or ameliorating a disease associated with dysregulation of the complement alternative pathway in a subject comprises administering to the subject a compound comprising or consisting of a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 198, 228, 237, 440, 444, 448, 450, 453, 455, 549, and 598. In certain embodiments, a method of treating, preventing, or ameliorating a disease associated with

dysregulation of the complement alternative pathway in a subject comprises administering to the subject a compound comprising or consisting of ISIS 696844, ISIS 696845, ISIS 698969, or ISIS 698970.

In certain embodiments, a method of treating, preventing, or ameliorating macular degeneration, such as age-related macular degeneration (AMD) in a subject comprises administering to the subject a compound or composition described herein, thereby treating, preventing, or ameliorating AMD. In certain aspects, the complement alternative pathway is activated greater than normal. In certain aspects, the AMD is wet AMD. In certain aspects, the AMD is dry AMD, such as Geographic Atrophy. In certain embodiments, a method of treating, preventing, or ameliorating macular degeneration in a subject, such as age-related macular degeneration (AMD), wet AMD, dry AMD, or Geographic Atrophy comprises administering to the subject a a compound comprising or consisting of a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides and has a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 6-808. In certain embodiments, a method of treating, preventing, or ameliorating macular degeneration, such as age-related macular degeneration (AMD), wet AMD, dry AMD, or Geographic Atrophy in a subject comprises administering to the subject a comprises administering to the subject a compound comprising or consisting of a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 198, 228, 237, 440, 444, 448, 450, 453, 455, 549, and 598. In certain embodiments, a method of treating, preventing, or ameliorating macular degeneration, such as age-related macular degeneration (AMD), wet AMD, dry AMD, or Geographic Atrophy in a subject comprises administering to the subject a compound comprising or consisting of ISIS 696844, ISIS 696845, ISIS 698969, or ISIS 698970. In certain aspects, the compound or composition is administered to the subject parenterally.

In certain embodiments, a method of treating, preventing, or ameliorating a kidney disease associated with dysregulation of the complement alternative pathway in a subject comprises administering to the subject a compound or composition described herein, thereby treating, preventing, or ameliorating the kidney disease. In certain embodiments, a method of treating, preventing, or ameliorating a kidney disease associated with dysregulation of the complement alternative pathway in a subject comprises administering to the subject a compound comprising or consisting of a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides and has a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 6-808. In certain embodiments, a method of treating, preventing, or ameliorating a kidney disease associated with dysregulation of the complement alternative pathway in a subject comprises administering to the subject a compound comprising or consisting of a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 198, 228, 237, 440,

444, 448, 450, 453, 455, 549, and 598. In certain embodiments, a method of treating, preventing, or ameliorating a kidney disease associated with dysregulation of the complement alternative pathway in a subject comprises administering to the subject a compound comprising or consisting of ISIS 696844, ISIS 696845, ISIS 698969, or ISIS 698970. In certain aspects, the complement alternative pathway is activated greater than normal. In certain aspects, the kidney disease is lupus nephritis, systemic lupus erythematosus (SLE), dense deposit disease (DDD), C3 glomerulonephritis (C3GN), CFHR5 nephropathy, or atypical hemolytic uremic syndrome (aHUS), or any combination thereof. In certain aspects, the kidney disease is associated with C3 deposits, such as C3 deposits in the glomerulus. In certain aspects, the kidney disease is associated with lower than normal circulating C3 levels, such as serum or plasma C3 levels. In certain aspects, administering the compound or composition reduces or inhibits accumulation of ocular C3 levels, such as C3 protein levels. In certain aspects, administering the compound or composition reduces the level of ocular C3 deposits or inhibits accumulation of ocular C3 deposits. In certain aspects, the compound or composition is administered to the subject parenterally. In certain aspects, administering the compound or composition reduces or inhibits accumulation of C3 levels in the kidney, such as C3 protein levels. In certain aspects, administering the compound or composition reduces the level of kidney C3 deposits or inhibits accumulation of kidney C3 deposits, such as C3 levels in the glomerulus. In certain aspects, the subject is identified as having or at risk of having a disease associated with dysregulation of the complement alternative pathway, for example by detecting complement levels or membrane-attack complex levels in the subject's blood and/or performing a genetic test for gene mutations of complement factors associated with the disease.

In certain embodiments, a method of inhibiting expression of Complement Factor B (CFB) in a subject having, or at risk of having, a disease associated with dysregulation of the complement alternative pathway comprises administering a compound or composition described herein to the subject, thereby inhibiting expression of CFB in the subject. In certain embodiemnts, a method of inhibiting expression of Complement Factor B (CFB) in a subject having, or at risk of having, a disease associated with dysregulation of the complement alternative pathway comprises administering to the subject a compound comprising or consisting of a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides and has a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 6-808. In certain embodiemnts, a method of inhibiting expression of Complement Factor B (CFB) in a subject having, or at risk of having, a disease associated with dysregulation of the complement alternative pathway comprises administering to the subject a compound comprising or consisting of a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 198, 228, 237, 440, 444, 448, 450, 453, 455, 549, and 598. In certain embodiments, a method of inhibiting expression of Complement Factor B (CFB) in a subject having, or at risk of having, a disease associated with dysregulation of the complement alternative pathway comprises administering to the subject a compound comprising or

consisting of ISIS 696844, ISIS 696845, ISIS 698969, or ISIS 698970. In certain aspects, administering the compound or composition inhibits expression of CFB in the eye. In certain aspects, the subject has, or is at risk of having, age related macular degeneration (AMD), such as wet AMD and dry AMD. In certain aspects, dry AMD can be Geographic Atrophy. Geographic Atrophy is considered an advanced form of dry AMD involving degeneration of the retina. In certain aspects, administering the compound or composition inhibits expression of CFB in the kidney, such as in the glomerulus. In certain aspects, the subject has, or is at risk of having, lupus nephritis, systemic lupus erythematosus (SLE), dense deposit disease (DDD), C3 glomerulonephritis (C3GN), CFHR5 nephropathy, or atypical hemolytic uremic syndrome (aHUS), or any combination thereof.

In certain embodiments, a method of reducing or inhibiting accumulation of C3 deposits in the eye of a subject having, or at risk of having, a disease associated with dysregulation of the complement alternative pathway comprises administering a compound or composition described herein to the subject, thereby reducing or inhibiting accumulation of C3 deposits in the eye of the subject. In certain embodiemnts, a method of reducing or inhibiting accumulation of C3 deposits in the eye of a subject having, or at risk of having, a disease associated with dysregulation of the complement alternative pathway comprises administering to the subject a compound comprising or consisting of a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides and has a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 6-808. In certain embodiemnts, a method of reducing or inhibiting accumulation of C3 deposits in the eye of a subject having, or at risk of having, a disease associated with dysregulation of the complement alternative pathway comprises administering to the subject a compound comprising or consisting of a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 198, 228, 237, 440, 444, 448, 450, 453, 455, 549, and 598. In certain embodiemnts, a method of reducing or inhibiting accumulation of C3 deposits in the eye of a subject having, or at risk of having, a disease associated with dysregulation of the complement alternative pathway comprises administering to the subject a compound comprising or consisting of ISIS 696844, ISIS 696845, ISIS 698969, or ISIS 698970. In certain aspects, the subject has, or is at risk of having, age related macular degeneration (AMD), such as wet AMD and dry AMD. In certain aspects, dry AMD can be Geographic Atrophy. In certain aspects, the compound or composition is administered to the subject parenterally.

In certain embodiments, a method of reducing or inhibiting accumulation of C3 deposits in the kidney of a subject having, or at risk of having, a disease associated with dysregulation of the complement alternative pathway comprises administering a compound or composition described herein to the subject, thereby reducing or inhibiting accumulation of C3 deposits in the kidney of the subject. In certain embodiments, a method of reducing or inhibiting accumulation of C3 deposits in the kidney of a subject

having, or at risk of having, a disease associated with dysregulation of the complement alternative pathway comprises administering to the subject a compound comprising or consisting of a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides and has a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 6-808. In certain embodiments, a method of reducing or inhibiting accumulation of C3 deposits in the kidney of a subject having, or at risk of having, a disease associated with dysregulation of the complement alternative pathway comprises administering to the subject a compound comprising or consisting of a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 198, 228, 237, 440, 444, 448, 450, 453, 455, 549, and 598. In certain embodiments, a method of reducing or inhibiting accumulation of C3 deposits in the kidney of a subject having, or at risk of having, a disease associated with dysregulation of the complement alternative pathway comprises administering to the subject a compound comprising or consisting of ISIS 696844, ISIS 696845, ISIS 698969, or ISIS 698970. In certain aspects, the subject has, or is at risk of having, lupus nephritis, systemic lupus erythematosus (SLE), dense deposit disease (DDD), C3 glomerulonephritis (C3GN), CFHR5 nephropathy, or atypical hemolytic uremic syndrome (aHUS), or any combination thereof. In certain aspects, the compound or composition is administered to the subject parenterally.

Certain embodiments are drawn to use of a compound or composition described herein for treating a disease associated with dysregulation of the complement alternative pathway. Certain embodiments are drawn to use of a compound comprising or consisting of a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides and has a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 6-808, for treating a disease associated with dysregulation of the complement alternative pathway. Certain embodiments are drawn to use of a compound comprising or consisting of a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 198, 228, 237, 440, 444, 448, 450, 453, 455, 549, and 598, for treating a disease associated with dysregulation of the complement alternative pathway. Certain embodiments are drawn to use of a compound comprising or consisting of ISIS 696844, ISIS 696845, ISIS 698969, or ISIS 698970 for treating a disease associated with dysregulation of the complement alternative pathway. In certain aspects, the complement alternative pathway is activated greater than normal. In certain aspects, the disease is macular degeneration, such as age related macular degeneration (AMD), which can be wet AMD or dry AMD. In certain aspects, dry AMD can be Geographic Atrophy. In certain aspects, the disease is a kidney disease such as lupus nephritis, systemic lupus erythematosus (SLE), dense deposit disease (DDD), C3 glomerulonephritis (C3GN), CFHR5 nephropathy, or atypical hemolytic uremic syndrome (aHUS), or any combination thereof. In certain aspects, the compound or composition is administered to the subject parenterally.

In certain embodiments, a compound or composition described herein is administered parenterally. For example, in certain embodiments the compound or composition can be administered through injection or infusion. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration, e.g. intrathecal or intracerebroventricular administration.

Antisense compounds

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

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

In certain embodiments, an antisense compound is 10 to 30 subunits in length. In certain embodiments, an antisense compound is 12 to 30 subunits in length. In certain embodiments, an antisense compound is 12 to 22 subunits in length. In certain embodiments, an antisense compound is 14 to 30 subunits in length. In certain embodiments, an antisense compound is 14 to 20 subunits in length. In certain embodiments, an antisense compoun is 15 to 30 subunits in length. In certain embodiments, an antisense compound is 15 to 20 subunits in length. In certain embodiments, an antisense compound is 16 to 30 subunits in length. In certain embodiments, an antisense compound is 16 to 20 subunits in length. In certain embodiments, an antisense compound is 17 to 30 subunits in length. In certain embodiments, an antisense compound is 17 to 20 subunits in length. In certain embodiments, an antisense compound is 18 to 30 subunits in length. In certain embodiments, an antisense compound is 18 to 21 subunits in length. In certain embodiments, an antisense compound is 18 to 20 subunits in length. In certain embodiments, an antisense compound is 20 to 30 subunits in length. In other words, such antisense compounds are from 12 to 30 linked subunits, 14 to 30 linked subunits, 14 to 20 subunits, 15 to 30 subunits, 15 to 20 subunits, 16 to 30 subunits, 16 to 20 subunits, 17 to 30 subunits, 17 to 20 subunits, 18 to 30 subunits, 18 to 20 subunits, 18 to 21 subunits, 20 to 30 subunits, or 12 to 22 linked subunits, respectively. In certain embodiments, an antisense compound is 14 subunits in length. In certain embodiments, an antisense compound is 16 subunits in length. In certain embodiments, an antisense compound is 17 subunits in length. In certain embodiments, an antisense compound is 18 subunits in length. In certain embodiments, an antisense compound is 19 subunits in length. In certain embodiments, an antisense compound is 20 subunits in length. In other embodiments, the antisense compound is 8 to 80, 12 to 50, 13 to 30, 13 to 50, 14 to 30, 14 to 50, 15 to 30, 15 to 50, 16 to 30, 16 to 50, 17 to 30, 17 to 50, 18 to 22, 18 to 24, 18 to 30, 18 to 50, 19 to 22, 19 to 30, 19 to 50, or 20 to 30 linked subunits. In certain such embodiments, the antisense compounds are 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21,

22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 linked subunits in length, or a range defined by any two of the above values. In some embodiments the antisense compound is an antisense oligonucleotide, and the linked subunits are nucleotides.

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

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

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

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

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

was able to inhibit translation, albeit at a more modest level than the 28 or 42 nucleobase antisense oligonucleotides.

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 a CFB 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.

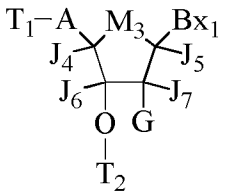
RNAi Compounds

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:



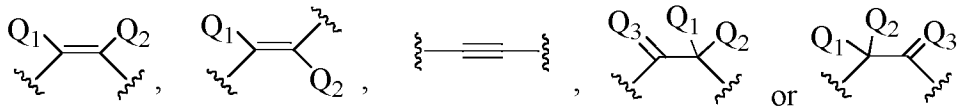
IIc

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-[$C(R_8)(R_9)]_n$-[$(C=O)_m$-$X_1]_j$-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;

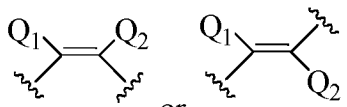
when j is 1 then Z is other than halogen or $N(E_2)(E_3)$; and

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.

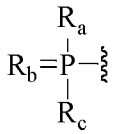
In certain embodiments, A has one of the formulas:



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:



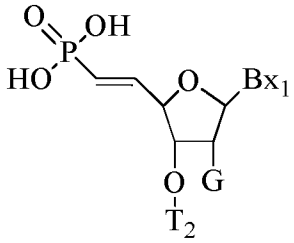
wherein:

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 modificatios 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 certan 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 nucleosde;

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 certiain 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 CFB. In certain embodiment, the degradation of the targeted CFB is facilitated by an activated RISC complex that is formed with compositions of the invention.

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 CFB 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 Complement Factor B (CFB) include, without limitation, the following: GENBANK Accession No. NM_001710.5 (incorporated herein as SEQ ID NO: 1), GENBANK Accession No. NT_007592.15 truncated from nucleotides 31852000 to 31861000 (incorporated herein as SEQ ID NO: 2), GENBANK Accession No NW_001116486.1 truncated from nucleotides 536000 to 545000 (incorporated herein as SEQ ID NO: 3), GENBANK Accession No. XM_001113553.2 (incorporated herein as SEQ ID NO: 4), or GENBANK Accession No. NM_008198.2 (incorporated herein as SEQ ID NO: 5).

Hybridization

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

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

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

Complementarity

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

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

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

For example, an antisense compound in which 18 of 20 nucleobases of the antisense compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining noncomplementary nucleobases may be clustered or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. As such, an antisense compound which is 18 nucleobases in length having four noncomplementary nucleobases which are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid and would thus fall within the scope of the present invention. Percent complementarity of an antisense compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul *et al.*, *J. Mol. Biol.*, 1990, 215, 403

410; Zhang and Madden, Genome Res., 1997, 7, 649 656). Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482 489).

In certain embodiments, the antisense compounds provided herein, or specified portions thereof, are fully complementary (i.e. 100% complementary) to a target nucleic acid, or specified portion thereof. For example, an antisense compound may be fully complementary to a CFB nucleic acid, or a target region, or a target segment or target sequence thereof. As used herein, "fully complementary" means each nucleobase of an antisense compound is capable of precise base pairing with the corresponding nucleobases of a target nucleic acid. For example, a 20 nucleobase antisense compound is fully complementary to a target sequence that is 400 nucleobases long, so long as there is a corresponding 20 nucleobase portion of the target nucleic acid that is fully complementary to the antisense compound. Fully complementary can also be used in reference to a specified portion of the first and /or the second nucleic acid. For example, a 20 nucleobase portion of a 30 nucleobase antisense compound can be "fully complementary" to a target sequence that is 400 nucleobases long. The 20 nucleobase portion of the 30 nucleobase oligonucleotide is fully complementary to the target sequence if the target sequence has a corresponding 20 nucleobase portion wherein each nucleobase is complementary to the 20 nucleobase portion of the antisense compound. At the same time, the entire 30 nucleobase antisense compound may or may not be fully complementary to the target sequence, depending on whether the remaining 10 nucleobases of the antisense compound are also complementary to the target sequence.

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

In certain embodiments, antisense compounds that are, or are up to 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleobases in length comprise no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as a CFB nucleic acid, or specified portion thereof.

In certain embodiments, antisense compounds that are, or are up to 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleobases in length comprise no more than 6, no more than 5, no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as a CFB nucleic acid, or specified portion thereof.

The antisense compounds provided also include those which are complementary to a portion of a target nucleic acid. As used herein, "portion" refers to a defined number of contiguous (i.e. linked)

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

Identity

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

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

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

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

Modifications

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

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

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

Modified Internucleoside Linkages

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

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

In certain embodiments, antisense compounds targeted to a CFB 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 methylphosponate 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 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 nucleosides include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, antisense compounds provided herein include one or more bicyclic nucleosides comprising a 4' to 2' bridge. Examples of such 4' to 2' bridged bicyclic nucleosides, include but are not limited to one of the formulae: 4'-(CH_2)-O-2' (LNA); 4'-(CH_2)-S-2'; 4'-$(CH_2)_2$-O-2' (ENA); 4'-$CH(CH_3)$-O-2' (also referred to as constrained ethyl or cEt) and 4'-$CH(CH_2OCH_3)$-O-2' (and analogs thereof see U.S. Patent 7,399,845, issued on July 15, 2008); 4'-$C(CH_3)(CH_3)$-O-2' (and analogs thereof see published International Application WO 2009/006478, published January 8, 2009); 4'-CH_2-$N(OCH_3)$-2' (and analogs thereof see published International Application 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 Zhou *et al., J. Org. Chem.,* 2009, *74*, 118-134); and 4'-CH_2-C(=CH_2)-2' (and analogs thereof see published International Application WO 2008/154401, published on December 8, 2008).

Further reports related to bicyclic nucleosides can also be found in published literature (see for example: Singh *et al., Chem. Commun.*, 1998, *4*, 455-456; Koshkin *et al., Tetrahedron*, 1998, *54*, 3607-3630; Wahlestedt *et al., Proc. Natl. Acad. Sci. U. S. A.*, 2000, *97*, 5633-5638; Kumar *et al., Bioorg. Med. Chem. Lett.*, 1998, *8*, 2219-2222; Singh *et al., J. Org. Chem.*, 1998, *63*, 10035-10039; Srivastava *et al., J. Am. Chem. Soc.*, 2007, *129(26)* 8362-8379; Elayadi *et al., Curr. Opinion Invest. Drugs,* 2001, *2*, 558-561; Braasch *et al., Chem. Biol.,* 2001, *8*, 1-7; and Orum *et al., Curr. Opinion Mol. Ther.,* 2001, *3*, 239-243; U.S. Patent Nos. 6,268,490; 6,525,191; 6,670,461; 6,770,748; 6,794,499; 7,034,133; 7,053,207; 7,399,845; 7,547,684; 8,530,640; and 7,696,345; U.S. Patent Publication No. US2008-0039618; US2009-0012281; U.S. Patent Serial Nos. 61/026,995 and 61/097,787; Published PCT International applications; WO 2009/067647; WO 2011/017521; WO 2010/036698 WO 1999/014226; WO 2004/106356; WO 2005/021570; WO 2007/134181; WO 2008/150729; WO 2008/154401; and WO 2009/006478. Each of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α-L-ribofuranose and β-D-ribofuranose (see PCT international application PCT/DK98/00393, published on March 25, 1999 as WO 99/14226).

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

wherein:

x is 0, 1, or 2;

n is 1, 2, 3, or 4;

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

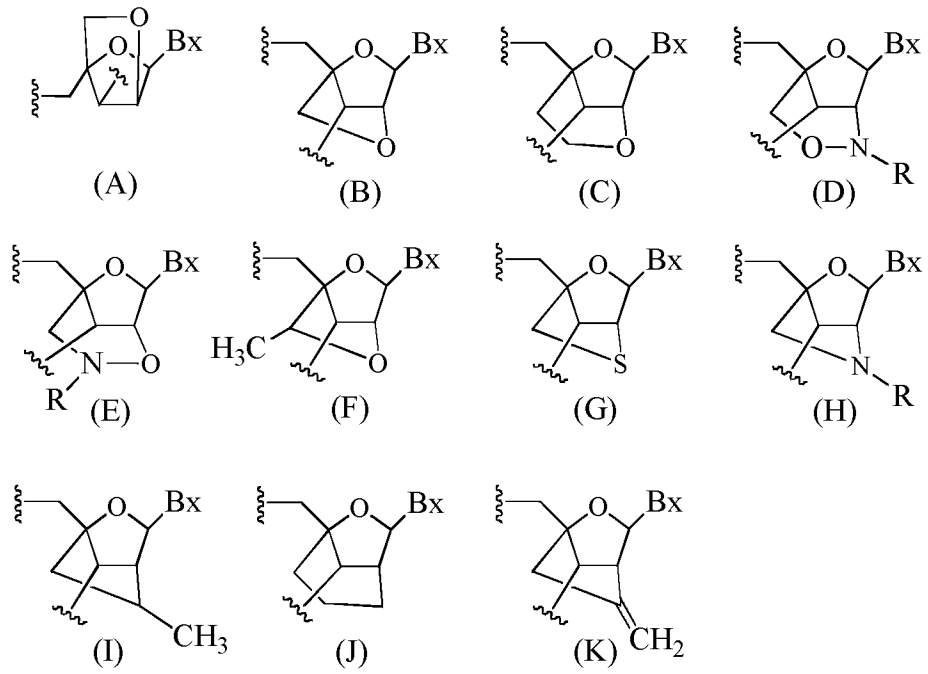
each J_1 and J_2 is, independently, H, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, acyl (C(=O)-H), substituted acyl, a heterocycle radical, a substituted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

In certain embodiments, the bridge of a bicyclic sugar moiety is -$[C(R_a)(R_b)]_n$-, -$[C(R_a)(R_b)]_n$-O-, -$C(R_aR_b)$-N(R)-O- or –$C(R_aR_b)$-O-N(R)-. In certain embodiments, the bridge is 4'-CH_2-2', 4'-$(CH_2)_2$-2', 4'-

$(CH_2)_3$-2', 4'-CH_2-O-2', 4'-$(CH_2)_2$-O-2', 4'-CH_2-O-N(R)-2' and 4'-CH_2-N(R)-O-2'- wherein each R is, independently, H, a protecting group or C_1-C_{12} alkyl.

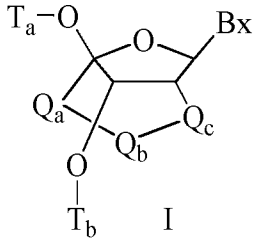
In certain embodiments, bicyclic nucleosides are further defined by isomeric configuration. For example, a nucleoside comprising a 4'-2' methylene-oxy bridge, may be in the α-L configuration or in the β-D configuration. Previously, α-L-methyleneoxy (4'-CH_2-O-2') BNA's have been incorporated into antisense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

In certain embodiments, bicyclic nucleosides include, but are not limited to, (A) α-L-methyleneoxy (4'-CH_2-O-2') BNA , (B) β-D-methyleneoxy (4'-CH_2-O-2') BNA , (C) ethyleneoxy (4'-$(CH_2)_2$-O-2') BNA , (D) aminooxy (4'-CH_2-O-N(R)-2') BNA, (E) oxyamino (4'-CH_2-N(R)-O-2') BNA, and (F) methyl(methyleneoxy) (4'-$CH(CH_3)$-O-2') BNA, (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_{12} alkyl or C_1-C_{12} alkoxy.

In certain embodiments, bicyclic nucleosides are provided having Formula I:



I

wherein:

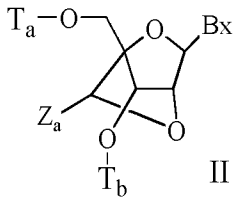
Bx is a heterocyclic base moiety;

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

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

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

In certain embodiments, bicyclic nucleosides are provided having Formula II:



II

wherein:

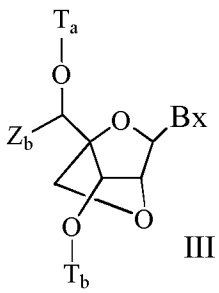
Bx is a heterocyclic base moiety;

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

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

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

In certain embodiments, bicyclic nucleosides are provided having Formula III:



III

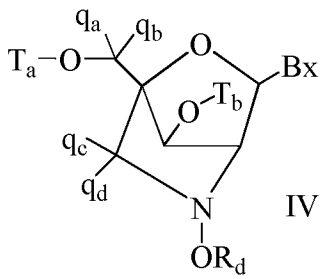
wherein:

Bx is a heterocyclic base moiety;

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

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

In certain embodiments, bicyclic nucleosides are provided having Formula IV:



IV

wherein:

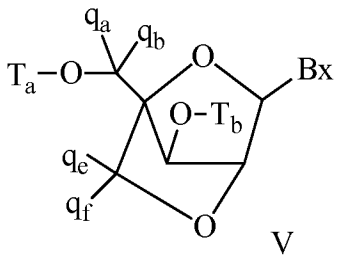
Bx is a heterocyclic base moiety;

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

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

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

In certain embodiments, bicyclic nucleosides are provided having Formula V:



V

wherein:

Bx is a heterocyclic base moiety;

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

q_a, q_b, q_e and q_f are each, independently, hydrogen, halogen, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_1-C_{12} alkoxy, substituted

C_1-C_{12} alkoxy, OJ_j, SJ_j, SOJ_j, SO_2J_j, NJ_jJ_k, N_3, CN, $C(=O)OJ_j$, $C(=O)NJ_jJ_k$, $C(=O)J_j$, O-$C(=O)NJ_jJ_k$, $N(H)C(=NH)NJ_jJ_k$, $N(H)C(=O)NJ_jJ_k$ or $N(H)C(=S)NJ_jJ_k$;

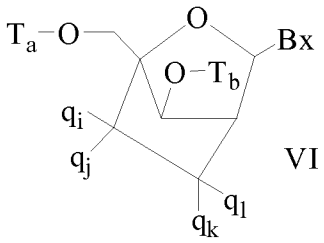
or q_e and q_f together are $=C(q_g)(q_h)$;

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

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

Analogs of methyleneoxy (4'-CH_2-O-2') BNA and 2'-thio-BNAs, have also been prepared (Kumar *et al.*, *Bioorg. Med. Chem. Lett.*, 1998, *8*, 2219-2222). Preparation of locked nucleoside analogs comprising oligodeoxyribonucleotide duplexes as substrates for nucleic acid polymerases has also been described (Wengel *et al.*, WO 99/14226). Furthermore, synthesis of 2'-amino-BNA, a novel comformationally restricted high-affinity oligonucleotide analog has been described in the art (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 are provided having Formula VI:



VI

wherein:

Bx is a heterocyclic base moiety;

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

each q_i, q_j, q_k and q_l is, independently, H, halogen, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_1-C_{12} alkoxyl, substituted C_1-C_{12} alkoxyl, OJ_j, SJ_j, SOJ_j, SO_2J_j, NJ_jJ_k, N_3, CN, $C(=O)OJ_j$, $C(=O)NJ_jJ_k$, $C(=O)J_j$, O-$C(=O)NJ_jJ_k$, $N(H)C(=NH)NJ_jJ_k$, $N(H)C(=O)NJ_jJ_k$ 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 (Freier *et al., Nucleic Acids Research*, 1997, *25*(*22*), 4429-4443 and Albaek *et al., J. Org. Chem.*, 2006, *71*, 7731-7740). The synthesis and preparation of carbocyclic bicyclic

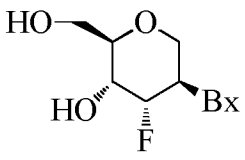
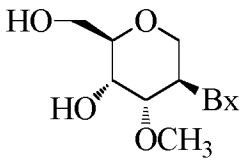
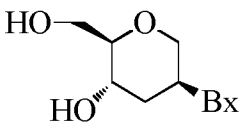
nucleosides along with their oligomerization and biochemical studies have also been described (Srivastava *et al., J. Am. Chem. Soc.,* 2007, *129(26)*, 8362-8379).

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

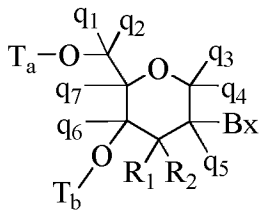
As used herein, "monocylic nucleosides" refer to nucleosides comprising modified sugar moieties that are not bicyclic sugar moieties. In certain embodiments, the sugar moiety, or sugar moiety analogue, of a nucleoside may be modified or substituted at any position.

As used herein, "2'-modified sugar" means a furanosyl sugar modified at the 2' position. In certain embodiments, such modifications include substituents selected from: a halide, including, but not limited to substituted and unsubstituted alkoxy, substituted and unsubstituted thioalkyl, substituted and unsubstituted amino alkyl, substituted and unsubstituted alkyl, substituted and unsubstituted allyl, and substituted and unsubstituted alkynyl. In certain embodiments, 2' modifications are selected from substituents including, but not limited to: $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nF$, $O(CH_2)_nONH_2$, $OCH_2C(=O)N(H)CH_3$, 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, a "modified tetrahydropyran nucleoside" or "modified THP nucleoside" means a nucleoside having a six-membered tetrahydropyran "sugar" substituted in for the pentofuranosyl residue in normal nucleosides (a sugar surrogate). Modified THP nucleosides include, but are not limited to, what is referred to in the art as hexitol nucleic acid (HNA), anitol nucleic acid (ANA), manitol nucleic acid (MNA) (see Leumann, *Bioorg. Med. Chem.,* 2002, *10*, 841-854) or fluoro HNA (F-HNA) having a tetrahydropyran ring system as illustrated below:

In certain embodiments, sugar surrogates are selected having Formula VII:



VII

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

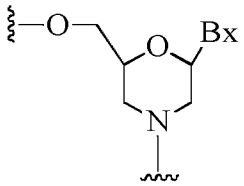
Bx is a heterocyclic base moiety;

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

q_1, q_2, q_3, q_4, q_5, q_6 and q_7 are each independently, H, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl; and each of R_1 and R_2 is selected from hydrogen, hydroxyl, halogen, subsitituted or unsubstituted alkoxy, NJ_1J_2, SJ_1, N_3, $OC(=X)J_1$, $OC(=X)NJ_1J_2$, $NJ_3C(=X)NJ_1J_2$ and CN, wherein X is O, S or NJ_1 and each J_1, J_2 and J_3 is, independently, H or C_1-C_6 alkyl.

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

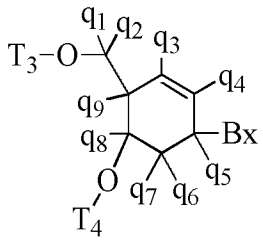
In certain embodiments, sugar surrogates comprise rings having more than 5 atoms and more than one heteroatom. For example nucleosides comprising morpholino sugar moieties and their use in oligomeric compounds has been reported (see for example: Braasch *et al.*, *Biochemistry*, 2002, *41*, 4503-4510; 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.

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" or "2'-O-methyl" 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.

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

Many other bicyclo and tricyclo sugar surrogate ring systems are also known in the art that can be used to modify nucleosides for incorporation into antisense compounds (see for example review article: Leumann, *Bioorg. Med. Chem.*, 2002, *10*, 841-854). Such ring systems can undergo various additional substitutions to enhance activity.

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

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 a CFB nucleic acid comprise one or more modified nucleobases. In certain embodiments, shortened or gap-widened antisense oligonucleotides targeted to a CFB 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.

Conjugated Antisense compounds

In certain embodiments, the present disclosure provides conjugated antisense compounds. In certain embodiments, the present disclosure provides conjugated antisense compounds comprising an antisense oligonucleotide complementary to a nucleic acid transcript. In certain embodiments, the present disclosure provides methods comprising contacting a cell with a conjugated antisense compound comprising an antisense oligonucleotide complementary to a nucleic acid transcript. In certain embodiments, the present disclosure provides methods comprising contacting a cell with a conjugated antisense compound comprising an antisense oligonucleotide and reducing the amount or activity of a nucleic acid transcript in a cell.

The asialoglycoprotein receptor (ASGP-R) has been described previously. See e.g., Park et al., PNAS vol. 102, No. 47, pp 17125-17129 (2005). Such receptors are expressed on liver cells, particularly hepatocytes. Further, it has been shown that compounds comprising clusters of three N-acetylgalactosamine (GalNAc) ligands are capable of binding to the ASGP-R, resulting in uptake of the compound into the cell. See e.g., Khorev et al., Bioorganic and Medicinal Chemistry, 16, 9, pp 5216-5231 (May 2008). Accordingly, conjugates comprising such GalNAc clusters have been used to facilitate uptake of certain compounds into liver cells, specifically hepatocytes. For example it has been shown that certain GalNAc-containing conjugates increase activity of duplex siRNA compounds in liver cells in vivo. In such instances, the GalNAc-containing conjugate is typically attached to the sense strand of the siRNA duplex. Since the sense strand is discarded before the antisense strand ultimately hybridizes with the target nucleic acid, there is little concern that the conjugate will interfere with activity. Typically, the conjugate is attached to the 3' end of the sense strand of the siRNA. See e.g., U.S. Patent 8,106,022. Certain conjugate groups described herein are more active and/or easier to synthesize than conjugate groups previously described.

In certain embodiments of the present invention, conjugates are attached to single-stranded antisense compounds, including, but not limited to RNase H based antisense compounds and antisense compounds that alter splicing of a pre-mRNA target nucleic acid. In such embodiments, the conjugate should remain attached to the antisense compound long enough to provide benefit (improved uptake into cells) but then should either be cleaved, or otherwise not interfere with the subsequent steps necessary for activity, such as hybridization to a target nucleic acid and interaction with RNase H or enzymes associated with splicing or splice modulation. This balance of properties is more important in the setting of single-stranded antisense compounds than in siRNA compounds, where the conjugate may simply be attached to the sense strand. Disclosed herein are conjugated single-stranded antisense compounds having improved potency in liver cells in vivo compared with the same antisense compound lacking the conjugate. Given the required balance of properties for these compounds such improved potency is surprising.

In certain embodiments, conjugate groups herein comprise a cleavable moiety. As noted, without

wishing to be bound by mechanism, it is logical that the conjugate should remain on the compound long enough to provide enhancement in uptake, but after that, it is desirable for some portion or, ideally, all of the conjugate to be cleaved, releasing the parent compound (e.g., antisense compound) in its most active form. In certain embodiments, the cleavable moiety is a cleavable nucleoside. Such embodiments take advantage of endogenous nucleases in the cell by attaching the rest of the conjugate (the cluster) to the antisense oligonucleotide through a nucleoside via one or more cleavable bonds, such as those of a phosphodiester linkage. In certain embodiments, the cluster is bound to the cleavable nucleoside through a phosphodiester linkage. In certain embodiments, the cleavable nucleoside is attached to the antisense oligonucleotide (antisense compound) by a phosphodiester linkage. In certain embodiments, the conjugate group may comprise two or three cleavable nucleosides. In such embodiments, such cleavable nucleosides are linked to one another, to the antisense compound and/or to the cluster via cleavable bonds (such as those of a phosphodiester linkage). Certain conjugates herein do not comprise a cleavable nucleoside and instead comprise a cleavable bond. It is shown that that sufficient cleavage of the conjugate from the oligonucleotide is provided by at least one bond that is vulnerable to cleavage in the cell (a cleavable bond).

In certain embodiments, conjugated antisense compounds are prodrugs. Such prodrugs are administered to an animal and are ultimately metabolized to a more active form. For example, conjugated antisense compounds are cleaved to remove all or part of the conjugate resulting in the active (or more active) form of the antisense compound lacking all or some of the conjugate.

In certain embodiments, conjugates are attached at the 5' end of an oligonucleotide. Certain such 5'-conjugates are cleaved more efficiently than counterparts having a similar conjugate group attached at the 3' end. In certain embodiments, improved activity may correlate with improved cleavage. In certain embodiments, oligonucleotides comprising a conjugate at the 5' end have greater efficacy than oligonucleotides comprising a conjugate at the 3' end (see, for example, Examples 56, 81, 83, and 84). Further, 5'-attachment allows simpler oligonucleotide synthesis. Typically, oligonucleotides are synthesized on a solid support in the 3' to 5' direction. To make a 3'-conjugated oligonucleotide, typically one attaches a pre-conjugated 3' nucleoside to the solid support and then builds the oligonucleotide as usual. However, attaching that conjugated nucleoside to the solid support adds complication to the synthesis. Further, using that approach, the conjugate is then present throughout the synthesis of the oligonucleotide and can become degraded during subsequent steps or may limit the sorts of reactions and reagents that can be used. Using the structures and techniques described herein for 5'-conjugated oligonucleotides, one can synthesize the oligonucleotide using standard automated techniques and introduce the conjugate with the final (5'-most) nucleoside or after the oligonucleotide has been cleaved from the solid support.

In view of the art and the present disclosure, one of ordinary skill can easily make any of the conjugates and conjugated oligonucleotides herein. Moreover, synthesis of certain such conjugates and conjugated oligonucleotides disclosed herein is easier and/or requires few steps, and is therefore less

expensive than that of conjugates previously disclosed, providing advantages in manufacturing. For example, the synthesis of certain conjugate groups consists of fewer synthetic steps, resulting in increased yield, relative to conjugate groups previously described. Conjugate groups such as GalNAc3-10 in Example 46 and GalNAc3-7 in Example 48 are much simpler than previously described conjugates such as those described in U.S. 8,106,022 or U.S. 7,262,177 that require assembly of more chemical intermediates . Accordingly, these and other conjugates described herein have advantages over previously described compounds for use with any oligonucleotide, including single-stranded oligonucleotides and either strand of double-stranded oligonucleotides (e.g., siRNA).

Similarly, disclosed herein are conjugate groups having only one or two GalNAc ligands. As shown, such conjugates groups improve activity of antisense compounds. Such compounds are much easier to prepare than conjugates comprising three GalNAc ligands. Conjugate groups comprising one or two GalNAc ligands may be attached to any antisense compounds, including single-stranded oligonucleotides and either strand of double-stranded oligonucleotides (e.g., siRNA).

In certain embodiments, the conjugates herein do not substantially alter certain measures of tolerability. For example, it is shown herein that conjugated antisense compounds are not more immunogenic than unconjugated parent compounds. Since potency is improved, embodiments in which tolerability remains the same (or indeed even if tolerability worsens only slightly compared to the gains in potency) have improved properties for therapy.

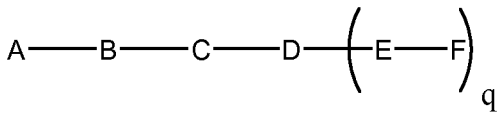
In certain embodiments, conjugation allows one to alter antisense compounds in ways that have less attractive consequences in the absence of conjugation. For example, in certain embodiments, replacing one or more phosphorothioate linkages of a fully phosphorothioate antisense compound with phosphodiester linkages results in improvement in some measures of tolerability. For example, in certain instances, such antisense compounds having one or more phosphodiester are less immunogenic than the same compound in which each linkage is a phosphorothioate. However, in certain instances, as shown in Example 26, that same replacement of one or more phosphorothioate linkages with phosphodiester linkages also results in reduced cellular uptake and/or loss in potency. In certain embodiments, conjugated antisense compounds described herein tolerate such change in linkages with little or no loss in uptake and potency when compared to the conjugated full-phosphorothioate counterpart. In fact, in certain embodiments, for example, in Examples 44, 57, 59, and 86, oligonucleotides comprising a conjugate and at least one phosphodiester internucleoside linkage actually exhibit increased potency in vivo even relative to a full phosphorothioate counterpart also comprising the same conjugate. Moreover, since conjugation results in substantial increases in uptake/potency a small loss in that substantial gain may be acceptable to achieve improved tolerability. Accordingly, in certain embodiments, conjugated antisense compounds comprise at least one phosphodiester linkage.

In certain embodiments, conjugation of antisense compounds herein results in increased delivery,

uptake and activity in hepatocytes. Thus, more compound is delivered to liver tissue. However, in certain embodiments, that increased delivery alone does not explain the entire increase in activity. In certain such embodiments, more compound enters hepatocytes. In certain embodiments, even that increased hepatocyte uptake does not explain the entire increase in activity. In such embodiments, productive uptake of the conjugated compound is increased. For example, as shown in Example 102, certain embodiments of GalNAc-containing conjugates increase enrichment of antisense oligonucleotides in hepatocytes versus non-parenchymal cells. This enrichment is beneficial for oligonucleotides that target genes that are expressed in hepatocytes.

In certain embodiments, conjugated antisense compounds herein result in reduced kidney exposure. For example, as shown in Example 20, the concentrations of antisense oligonucleotides comprising certain embodiments of GalNAc-containing conjugates are lower in the kidney than that of antisense oligonucleotides lacking a GalNAc-containing conjugate. This has several beneficial therapeutic implications. For therapeutic indications where activity in the kidney is not sought, exposure to kidney risks kidney toxicity without corresponding benefit. Moreover, high concentration in kidney typically results in loss of compound to the urine resulting in faster clearance. Accordingly for non-kidney targets, kidney accumulation is undesired.

In certain embodiments, the present disclosure provides conjugated antisense compounds represented by the formula:



wherein

A is the antisense oligonucleotide;

B is the cleavable moiety

C is the conjugate linker

D is the branching group

each E is a tether;

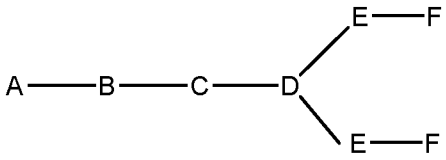
each F is a ligand; and

q is an integer between 1 and 5.

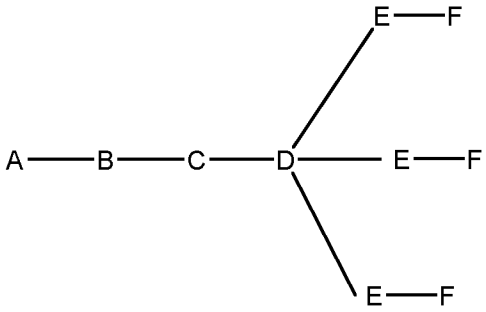
In the above diagram and in similar diagrams herein, the branching group "D" branches as many times as is necessary to accommodate the number of (E-F) groups as indicated by "q". Thus, where q = 1, the formula is:



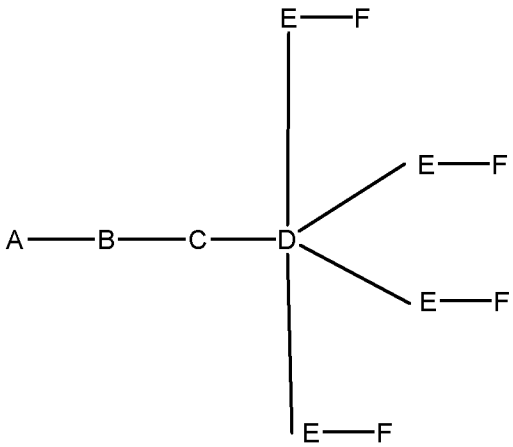
where q = 2, the formula is:



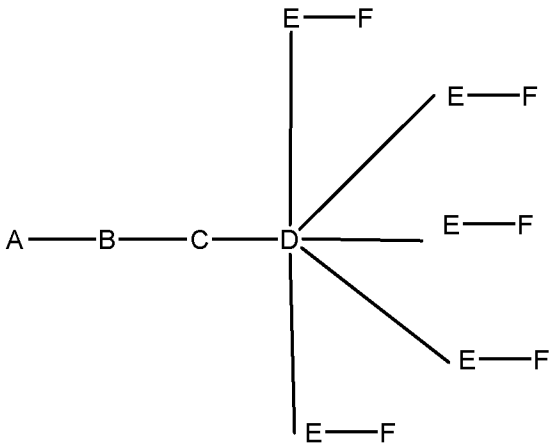
where q = 3, the formula is:



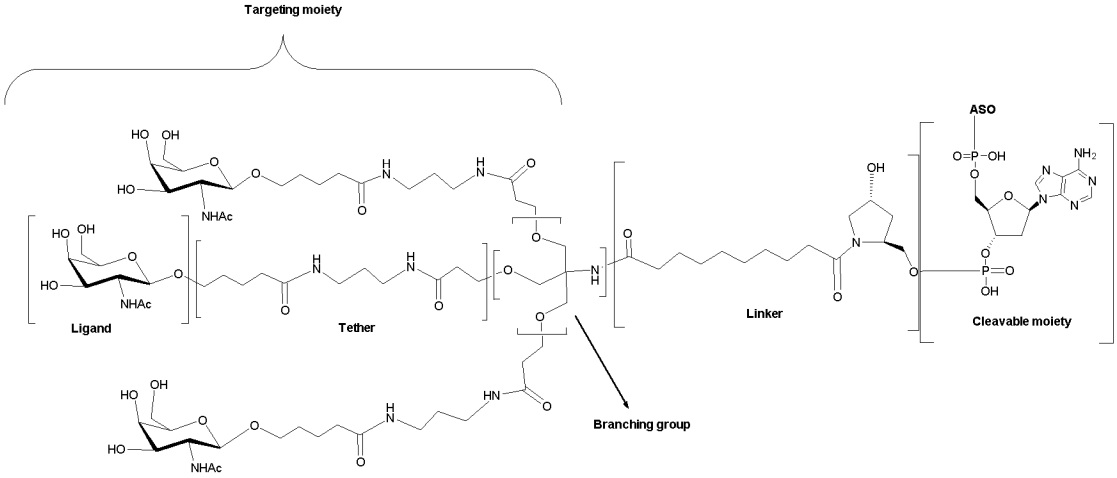
where q = 4, the formula is:



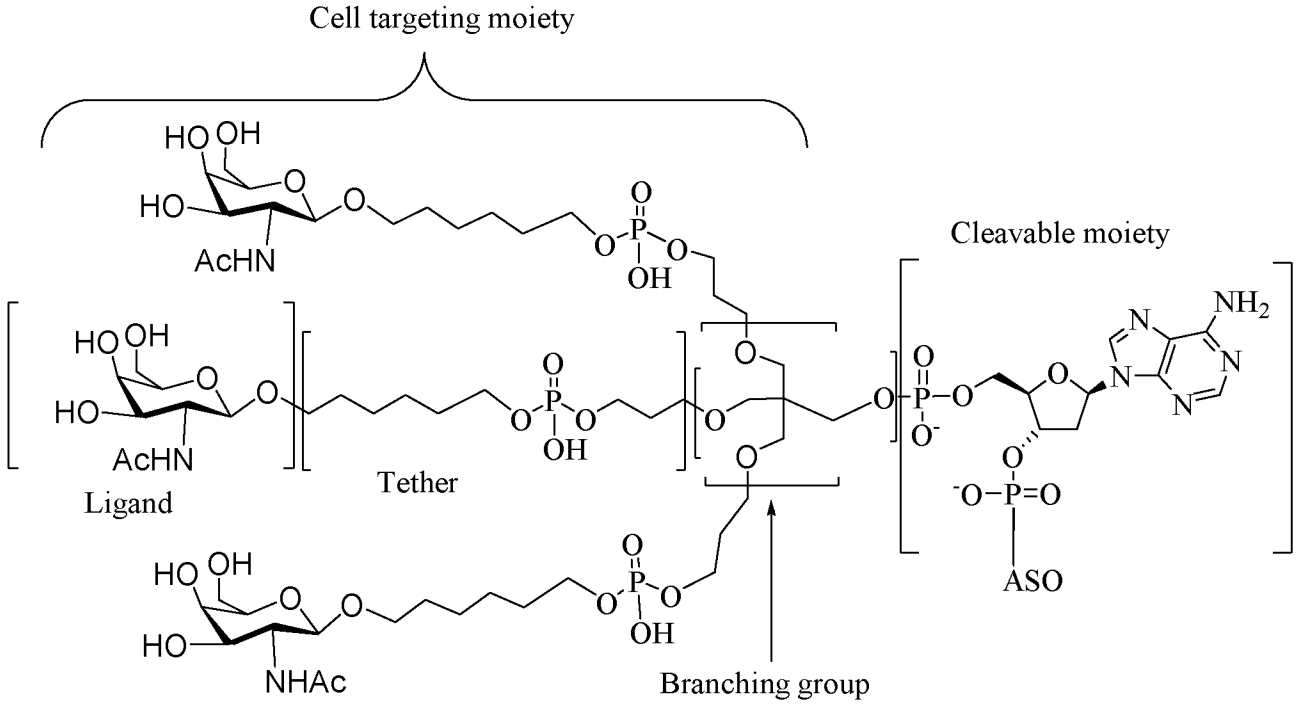
where q = 5, the formula is:



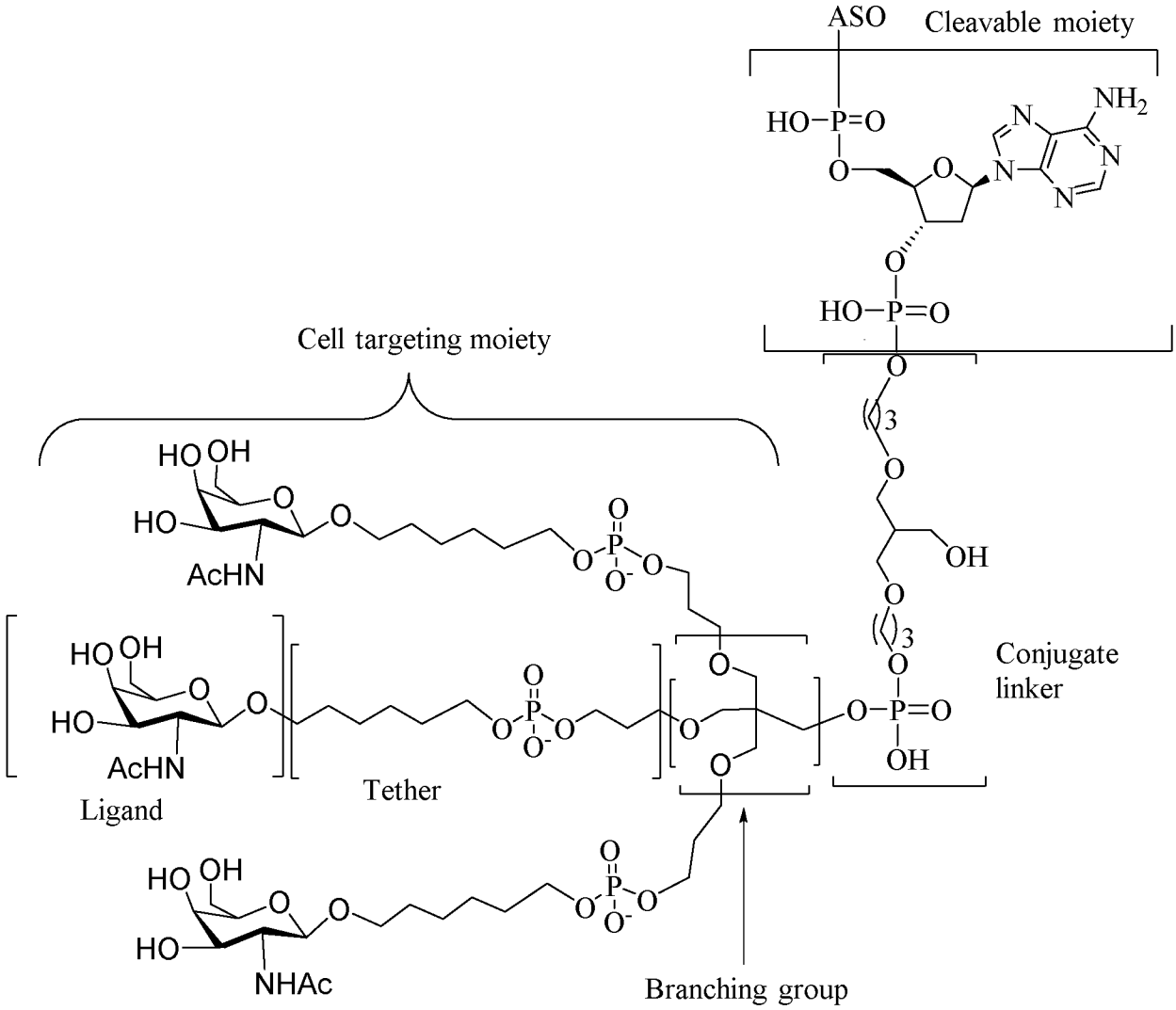
In certain embodiments, conjugated antisense compounds are provided having the structure:



In certain embodiments, conjugated antisense compounds are provided having the structure:

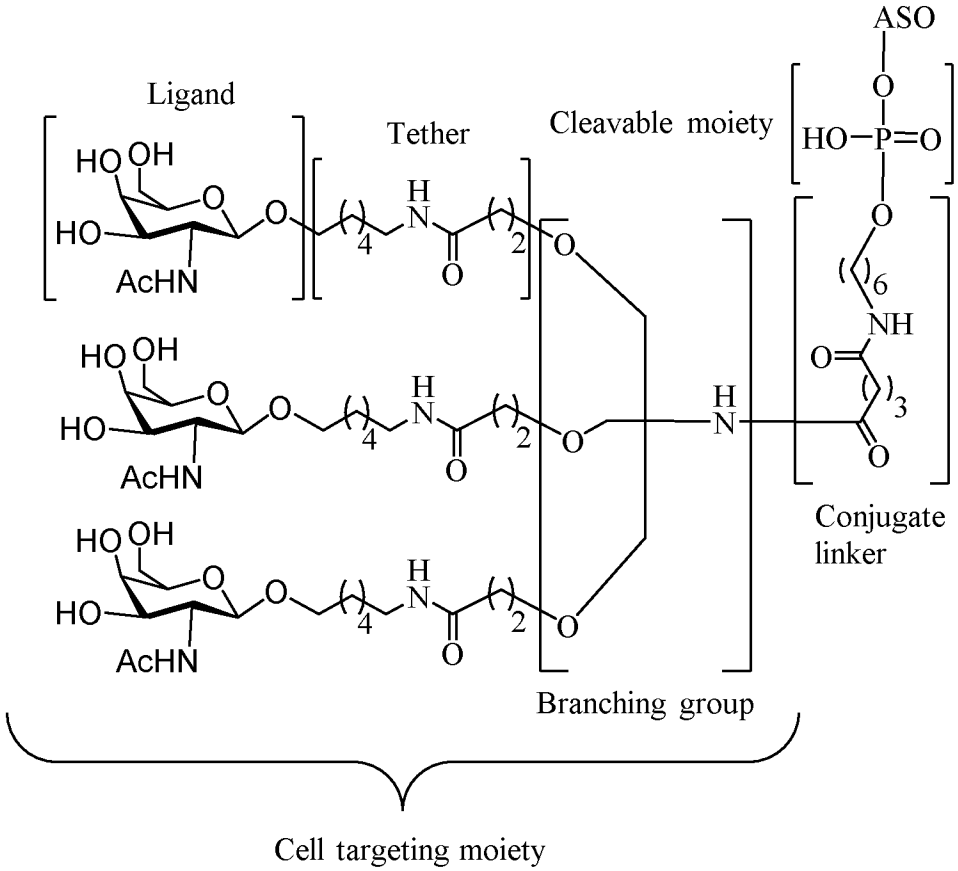


In certain embodiments, conjugated antisense compounds are provided having the structure:



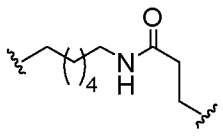
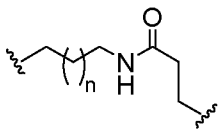
.

In certain embodiments, conjugated antisense compounds are provided having the structure:

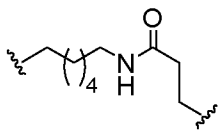


The present disclosure provides the following non-limiting numbered embodiments:

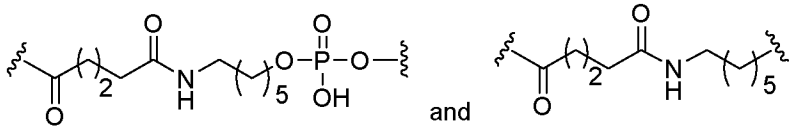
Embodiment 1. The conjugated antisense compound of any of embodiments 1179 to 1182, wherein the tether has a structure selected from among:

 , or  ; wherein each n is independently, 0, 1, 2, 3, 4, 5, 6, or 7.

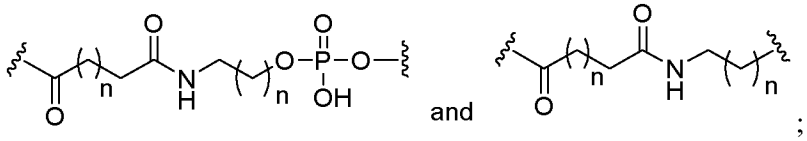
Embodiment 2. The conjugated antisense compound of any of embodiments 1179 to 1182, wherein the tether has the structure:

 .

Embodiment 3. The conjugated antisense compound of any of embodiments 1179 to 1182 or 1688 to 1689, wherein the linker has a structure selected from among:

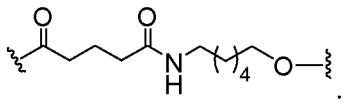


Embodiment 4. The conjugated antisense compound of any of embodiments 1179 to 1182 or 1688 to 1689, wherein the linker has a structure selected from among:



wherein each n is independently, 0, 1, 2, 3, 4, 5, 6, or 7.

Embodiment 5. The conjugated antisense compound of any of embodiments 1179 to 1182 or 1688 to 1689, wherein the linker has the structure:



In embodiments having more than one of a particular variable (e.g., more than one "m" or "n"), unless otherwise indicated, each such particular variable is selected independently. Thus, for a structure having more than one n, each n is selected independently, so they may or may not be the same as one another.

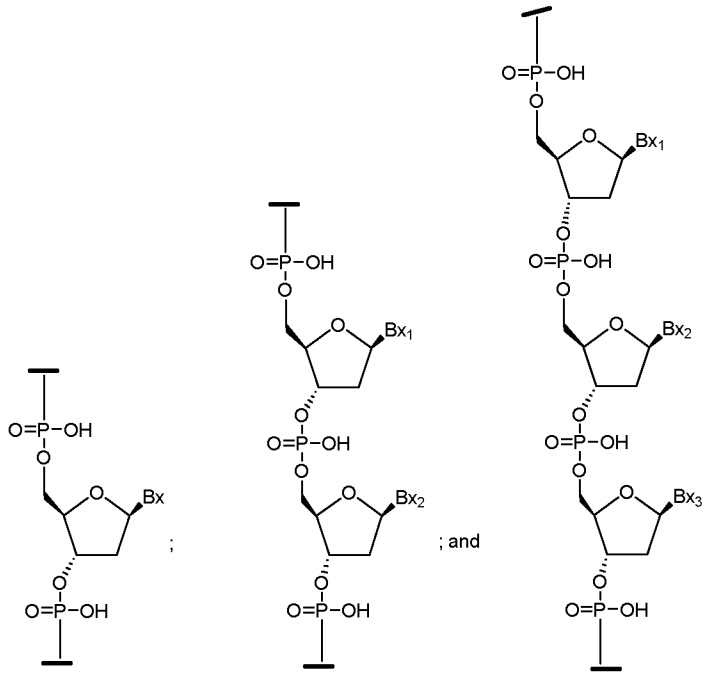
i. **Certain Cleavable Moieties**

In certain embodiments, a cleavable moiety is a cleavable bond. In certain embodiments, a cleavable moiety comprises a cleavable bond. In certain embodiments, the conjugate group comprises a cleavable moiety. In certain such embodiments, the cleavable moiety attaches to the antisense oligonucleotide. In certain such embodiments, the cleavable moiety attaches directly to the cell-targeting moiety. In certain such embodiments, the cleavable moiety attaches to the conjugate linker. In certain embodiments, the cleavable moiety comprises a phosphate or phosphodiester. In certain embodiments, the cleavable moiety is a cleavable nucleoside or nucleoside analog. In certain embodiments, the nucleoside or nucleoside analog 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 comprising an optionally protected heterocyclic base selected from uracil, thymine, cytosine, 4-N-benzoylcytosine, 5-methylcytosine, 4-N-benzoyl-5-methylcytosine, adenine, 6-N-benzoyladenine, guanine

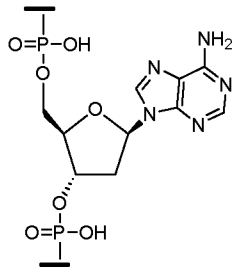
and 2-N-isobutyrylguanine. In certain embodiments, the cleavable moiety is 2'-deoxy nucleoside that is attached to the 3' position of the antisense oligonucleotide by a phosphodiester linkage and is attached to the linker by a phosphodiester or phosphorothioate linkage. In certain embodiments, the cleavable moiety is 2'-deoxy adenosine that is attached to the 3' position of the antisense oligonucleotide by a phosphodiester linkage and is attached to the linker by a phosphodiester or phosphorothioate linkage. In certain embodiments, the cleavable moiety is 2'-deoxy adenosine that is attached to the 3' position of the antisense oligonucleotide by a phosphodiester linkage and is attached to the linker by a phosphodiester linkage.

In certain embodiments, the cleavable moiety is attached to the 3' position of the antisense oligonucleotide. In certain embodiments, the cleavable moiety is attached to the 5' position of the antisense oligonucleotide. In certain embodiments, the cleavable moiety is attached to a 2' position of the antisense oligonucleotide. In certain embodiments, the cleavable moiety is attached to the antisense oligonucleotide by a phosphodiester linkage. In certain embodiments, the cleavable moiety is attached to the linker by either a phosphodiester or a phosphorothioate linkage. In certain embodiments, the cleavable moiety is attached to the linker by a phosphodiester linkage. In certain embodiments, the conjugate group does not include a cleavable moiety.

In certain embodiments, the cleavable moiety is cleaved after the complex has been administered to an animal only after being internalized by a targeted cell. Inside the cell the cleavable moiety is cleaved thereby releasing the active antisense oligonucleotide. While not wanting to be bound by theory it is believed that the cleavable moiety is cleaved by one or more nucleases within the cell. In certain embodiments, the one or more nucleases cleave the phosphodiester linkage between the cleavable moiety and the linker. In certain embodiments, the cleavable moiety has a structure selected from among the following:



wherein each of Bx, Bx_1, Bx_2, and Bx_3 is independently a heterocyclic base moiety. In certain embodiments, the cleavable moiety has a structure selected from among the following:



ii. **Certain Linkers**

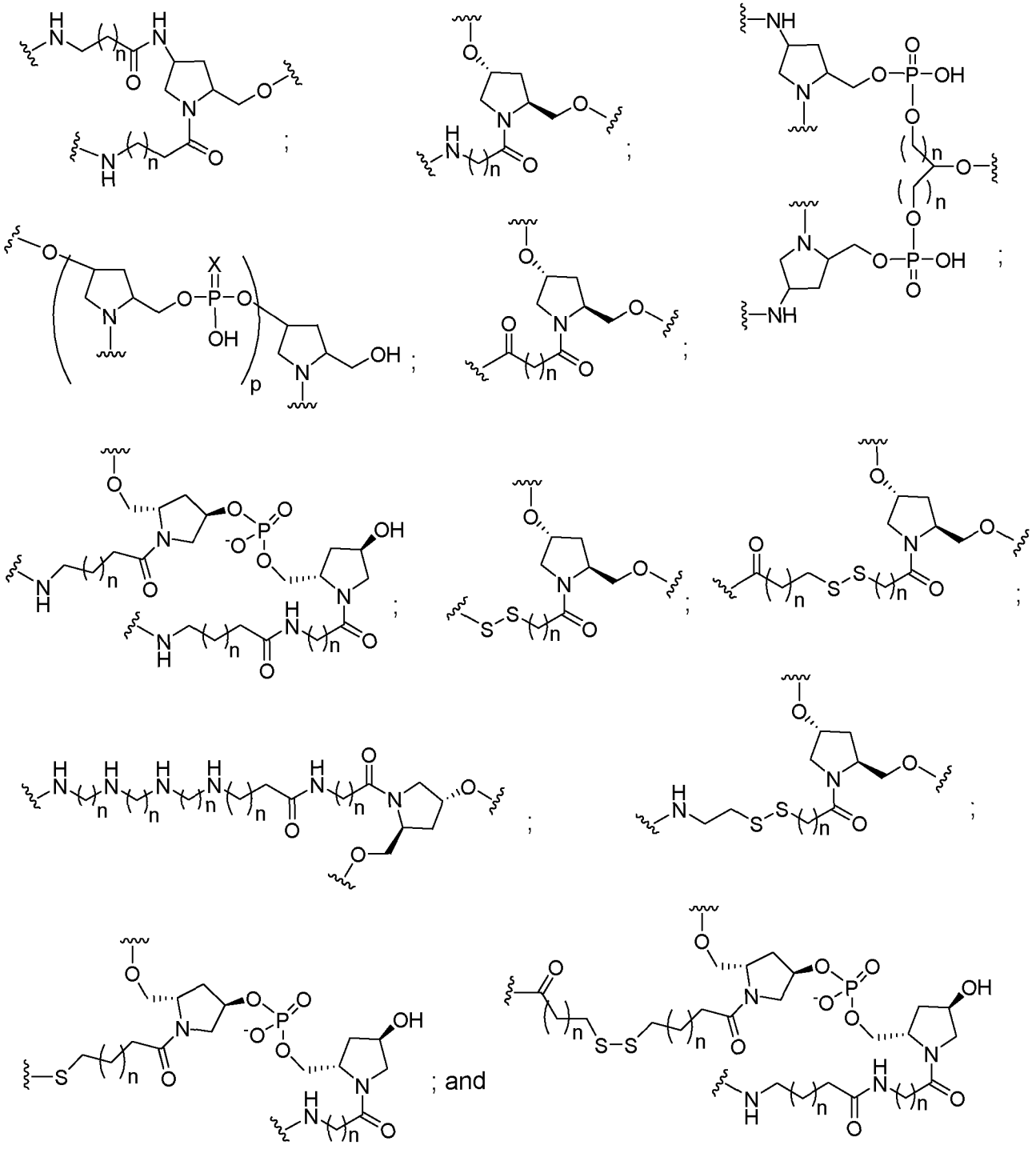
In certain embodiments, the conjugate groups comprise a linker. In certain such embodiments, the linker is covalently bound to the cleavable moiety. In certain such embodiments, the linker is covalently bound to the antisense oligonucleotide. In certain embodiments, the linker is covalently bound to a cell-targeting moiety. In certain embodiments, the linker further comprises a covalent attachment to a solid support. In certain embodiments, the linker further comprises a covalent attachment to a protein binding moiety. In certain embodiments, the linker further comprises a covalent attachment to a solid support and further comprises a covalent attachment to a protein binding moiety. In certain embodiments, the linker includes multiple positions for attachment of tethered ligands. In certain embodiments, the linker includes multiple positions for attachment of tethered ligands and is not attached to a branching group. In certain embodiments, the linker further comprises one or more cleavable bond. In certain embodiments, the conjugate group does not include a linker.

In certain embodiments, the linker includes at least a linear group comprising groups selected from alkyl, amide, disulfide, polyethylene glycol, ether, thioether (-S-) and hydroxylamino (-O-N(H)-) groups. In certain embodiments, the linear group comprises groups selected from alkyl, amide and ether groups. In certain embodiments, the linear group comprises groups selected from alkyl and ether groups. In certain embodiments, the linear group comprises at least one phosphorus linking group. In certain embodiments, the linear group comprises at least one phosphodiester group. In certain embodiments, the linear group includes at least one neutral linking group. In certain embodiments, the linear group is covalently attached to the cell-targeting moiety and the cleavable moiety. In certain embodiments, the linear group is covalently attached to the cell-targeting moiety and the antisense oligonucleotide. In certain embodiments, the linear group is covalently attached to the cell-targeting moiety, the cleavable moiety and a solid support. In certain embodiments, the linear group is covalently attached to the cell-targeting moiety, the cleavable moiety, a solid support and a protein binding moiety. In certain embodiments, the linear group includes one or more cleavable bond.

In certain embodiments, the linker includes the linear group covalently attached to a scaffold group. In certain embodiments, the scaffold includes a branched aliphatic group comprising groups selected from alkyl, amide, disulfide, polyethylene glycol, ether, thioether and hydroxylamino groups. In certain embodiments, the scaffold includes a branched aliphatic group comprising groups selected from alkyl, amide and ether groups. In certain embodiments, the scaffold includes at least one mono or polycyclic ring system. In certain embodiments, the scaffold includes at least two mono or polycyclic ring systems. In certain embodiments, the linear group is covalently attached to the scaffold group and the scaffold group is covalently attached to the cleavable moiety and the linker. In certain embodiments, the linear group is covalently attached to the scaffold group and the scaffold group is covalently attached to the cleavable moiety, the linker and a solid support. In certain embodiments, the linear group is covalently attached to the scaffold group and the scaffold group is covalently attached to the cleavable moiety, the linker and a protein binding moiety. In certain embodiments, the linear group is covalently attached to the scaffold group and the scaffold group is covalently attached to the cleavable moiety, the linker, a protein binding moiety and a solid support. In certain embodiments, the scaffold group includes one or more cleavable bond.

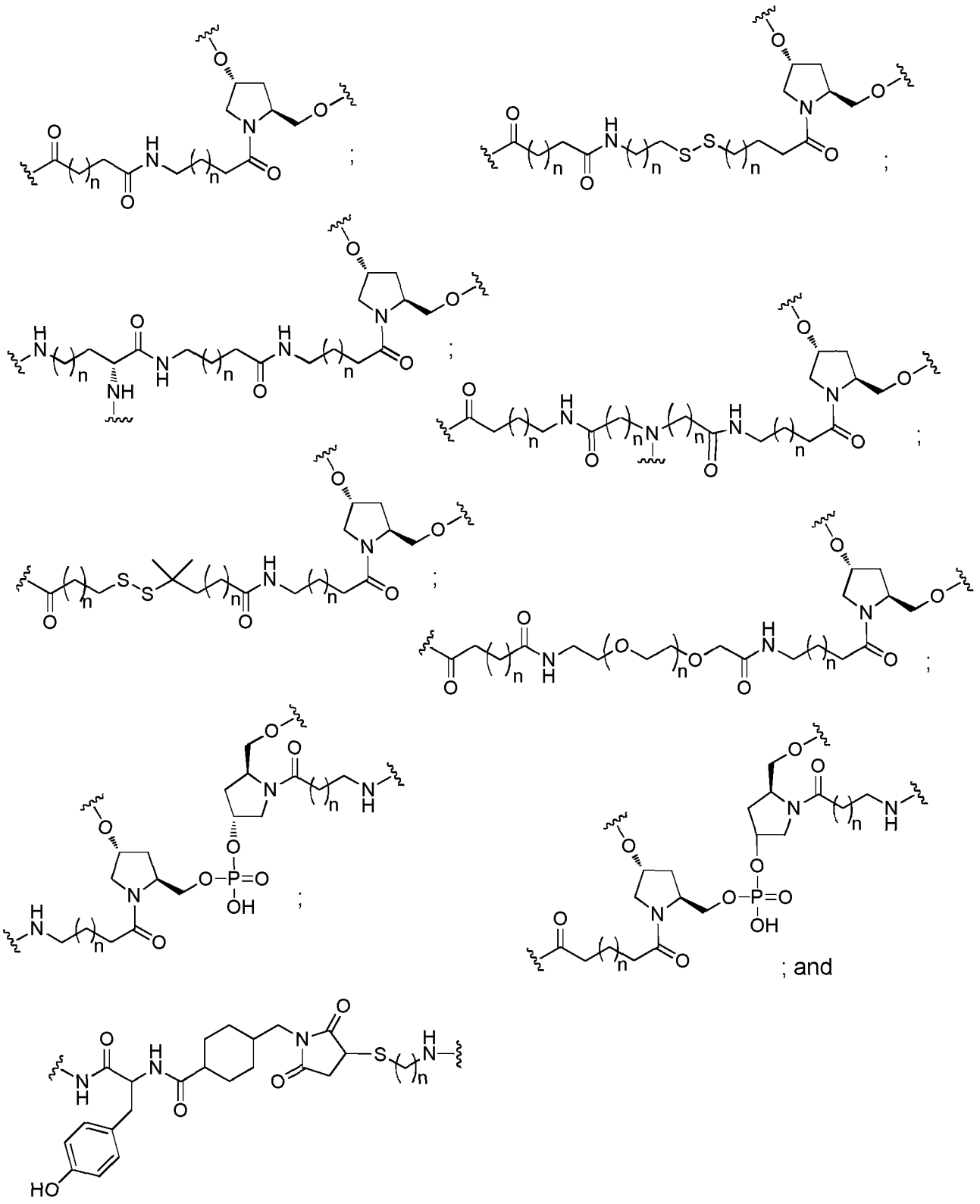
In certain embodiments, the linker includes a protein binding moiety. In certain embodiments, the protein binding moiety is a lipid such as for example including but not limited to cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine), a vitamin (e.g., folate, vitamin A, vitamin E, biotin, pyridoxal), a peptide, a carbohydrate (e.g., monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, polysaccharide), an endosomolytic component, a steroid (e.g., uvaol, hecigenin, diosgenin), a terpene (e.g., triterpene, e.g., sarsasapogenin, friedelin, epifriedelanol derivatized lithocholic acid), or a cationic lipid. In certain embodiments, the protein binding moiety is a C16 to C22 long chain saturated or unsaturated fatty acid, cholesterol, cholic acid, vitamin E, adamantane or 1-pentafluoropropyl.

In certain embodiments, a linker has a structure selected from among:



wherein each n is, independently, from 1 to 20; and p is from 1 to 6.

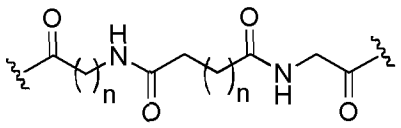
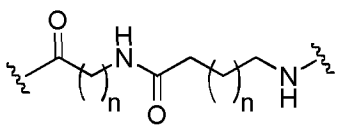
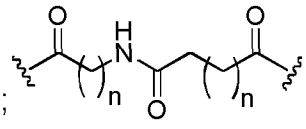
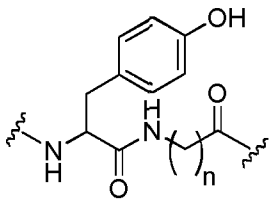
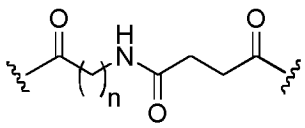
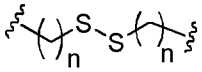
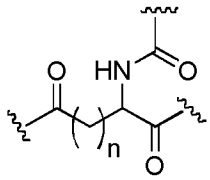
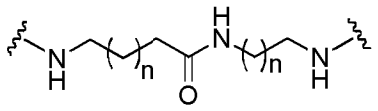
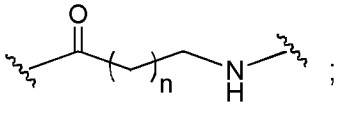
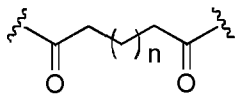
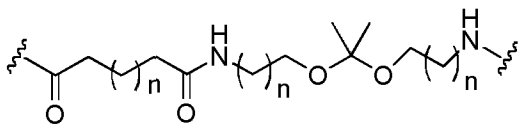
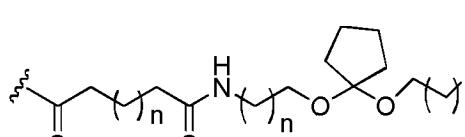
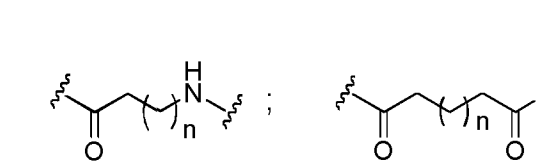
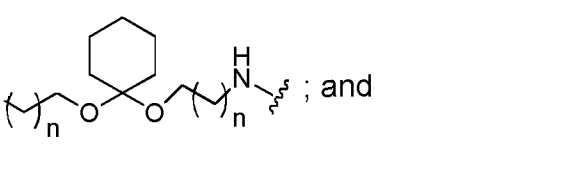
In certain embodiments, a linker has a structure selected from among:



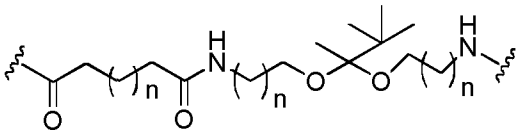
; and

wherein each n is, independently, from 1 to 20.

In certain embodiments, a linker has a structure selected from among:

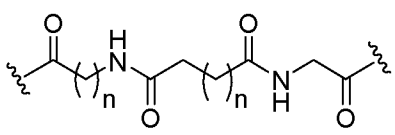
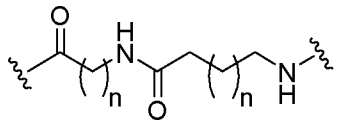
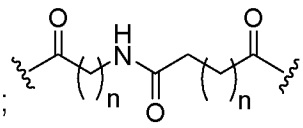
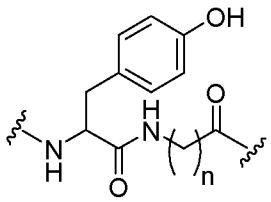
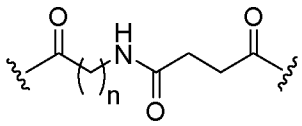
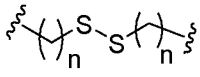
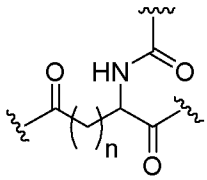
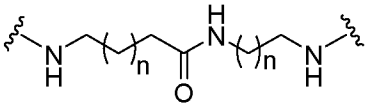
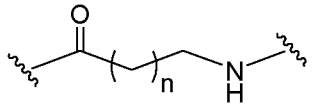
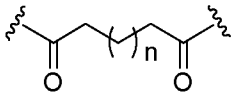
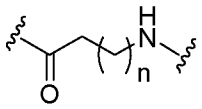
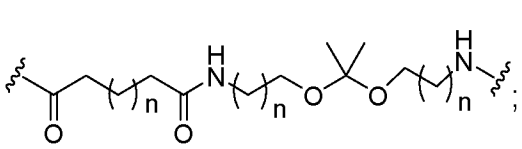
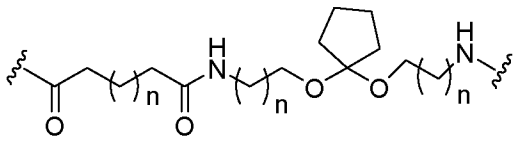
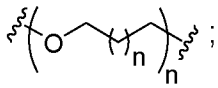
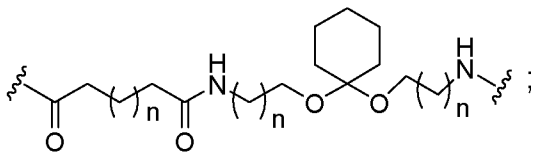
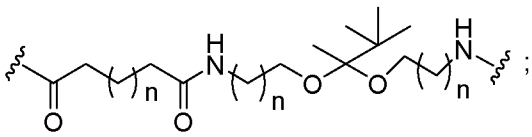
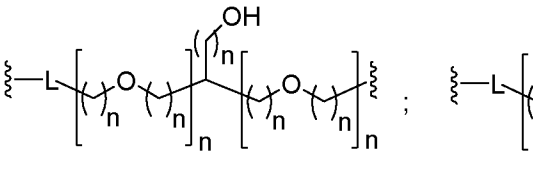
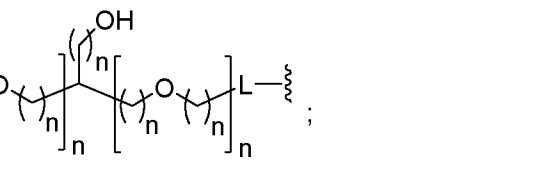
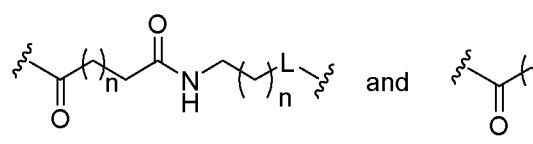
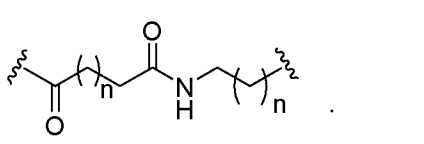
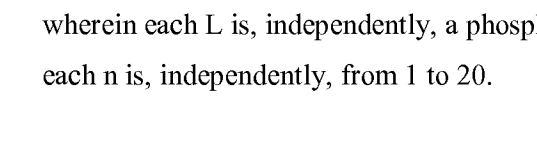
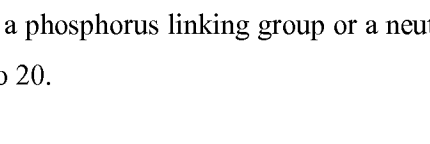
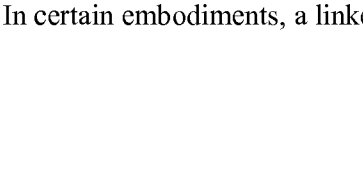
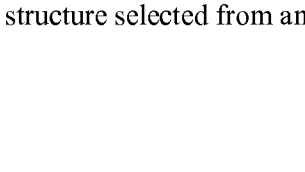

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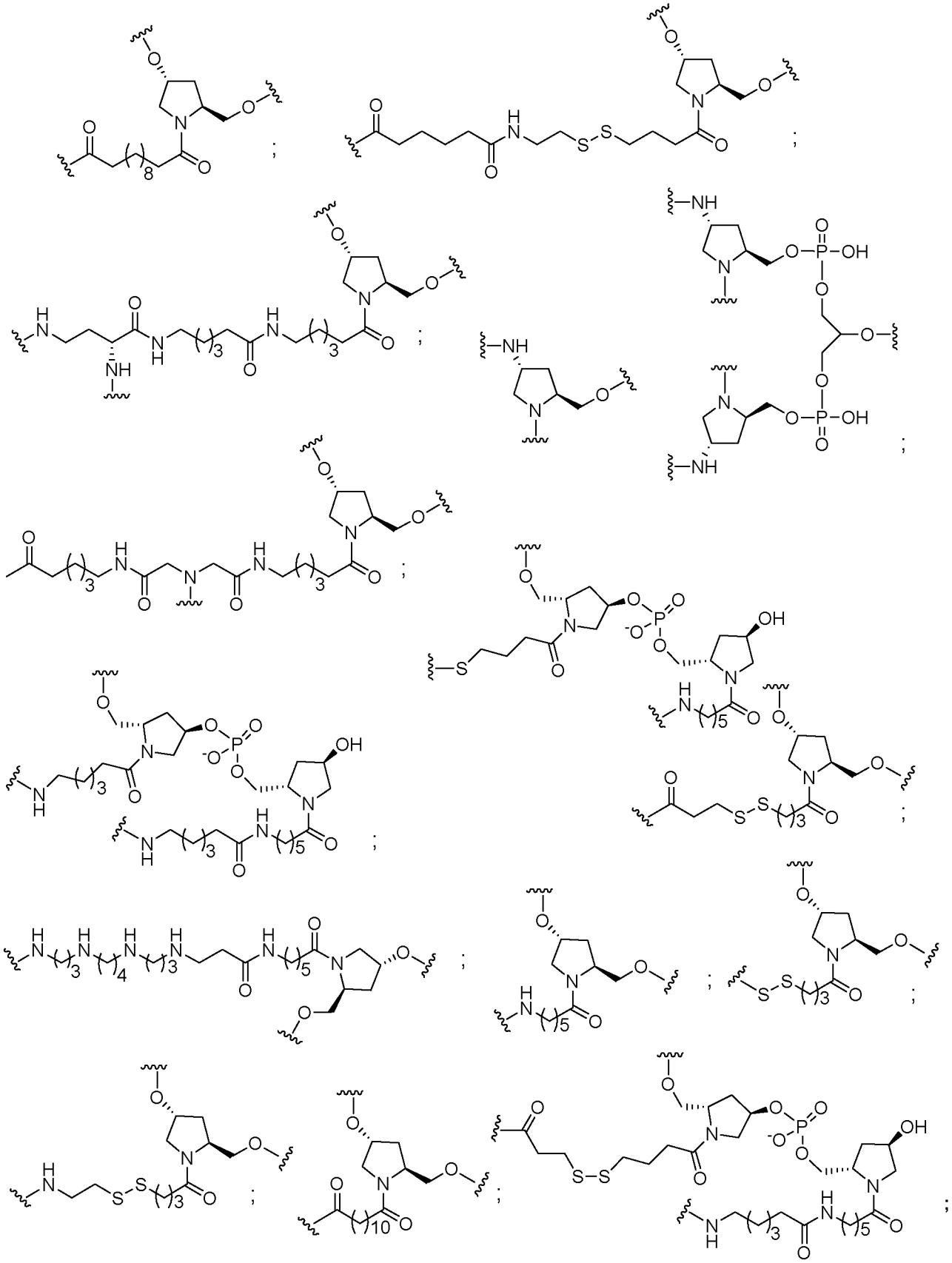
wherein n is from 1 to 20.

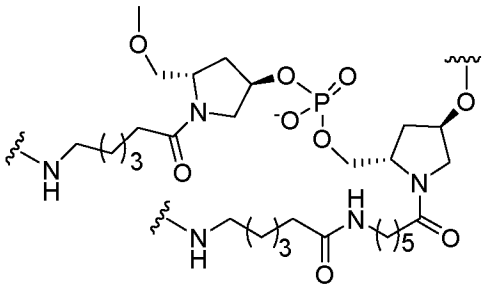
In certain embodiments, a linker has a structure selected from among:

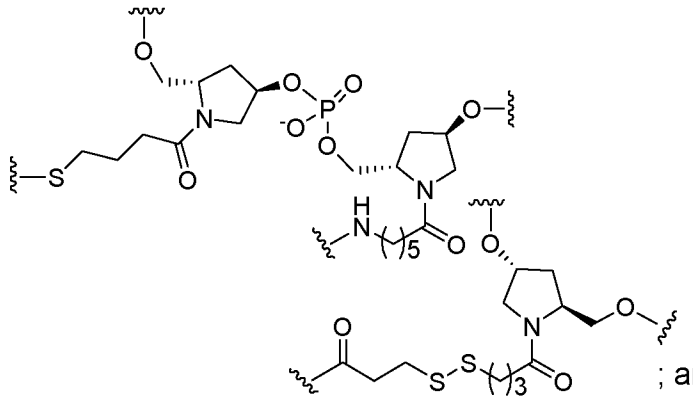

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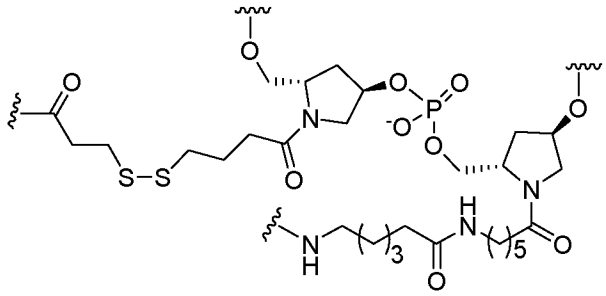
wherein each L is, independently, a phosphorus linking group or a neutral linking group; and each n is, independently, from 1 to 20.

In certain embodiments, a linker has a structure selected from among:

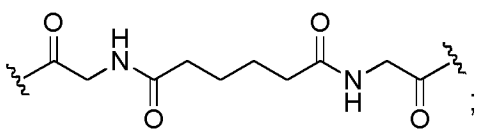


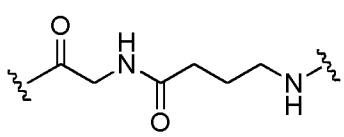

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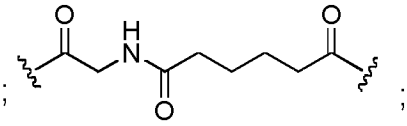

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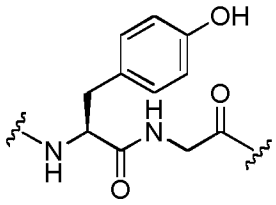

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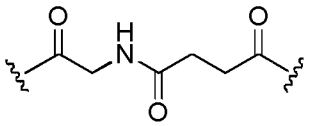
In certain embodiments, a linker has a structure selected from among:

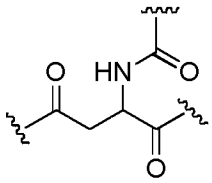

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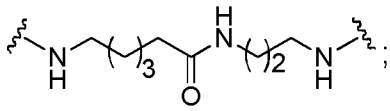

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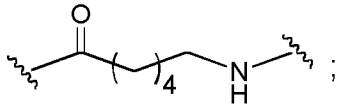

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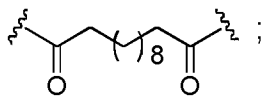

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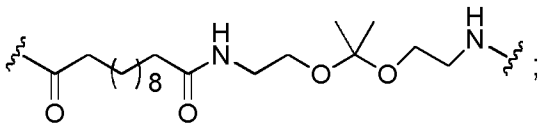

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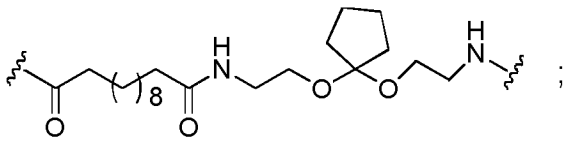

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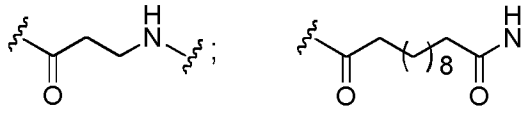

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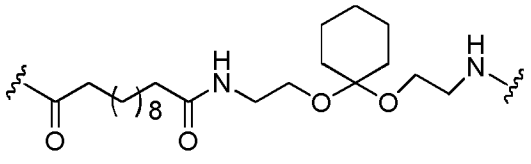

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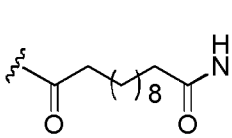

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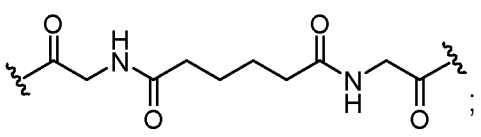
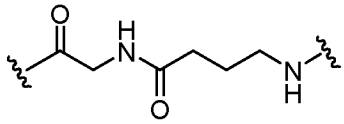
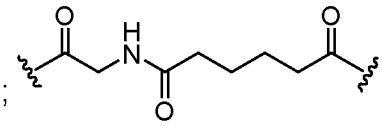

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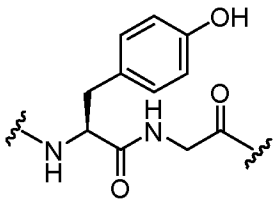
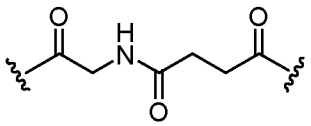
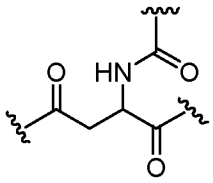

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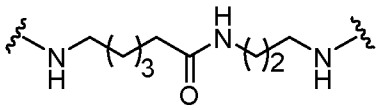
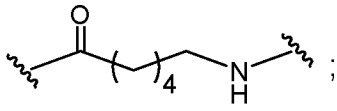
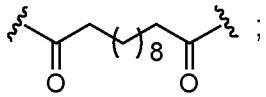

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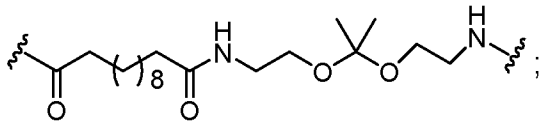
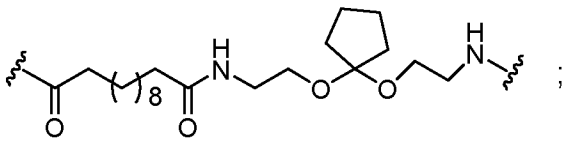
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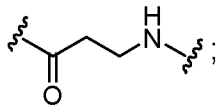
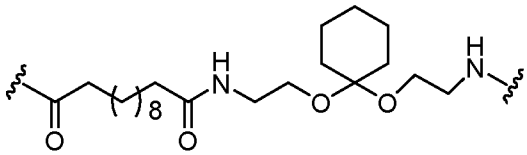
In certain embodiments, a linker has a structure selected from among:

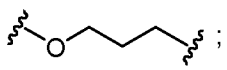
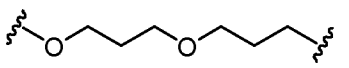
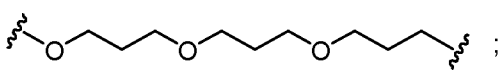

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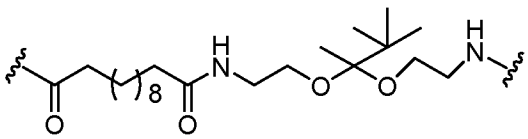
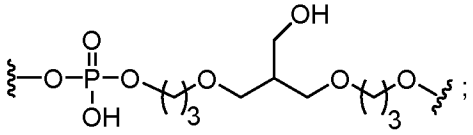

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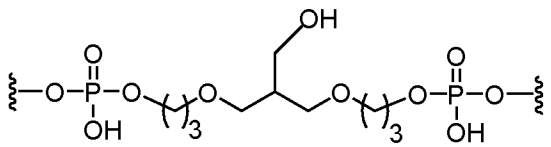
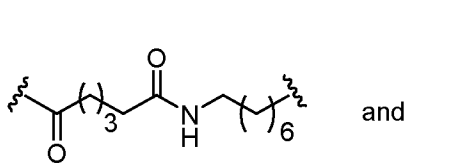

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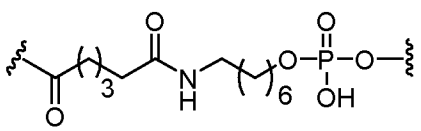

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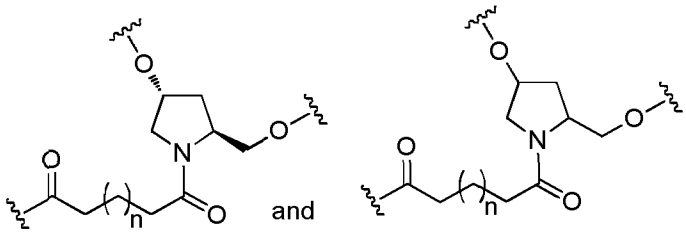

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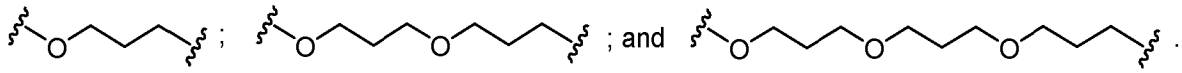

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In certain embodiments, a linker has a structure selected from among:

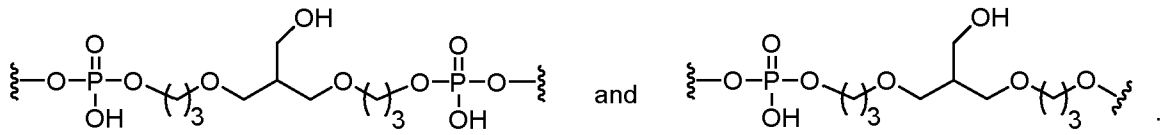


wherein n is from 1 to 20.

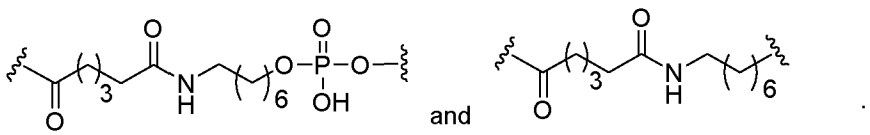
In certain embodiments, a linker has a structure selected from among:



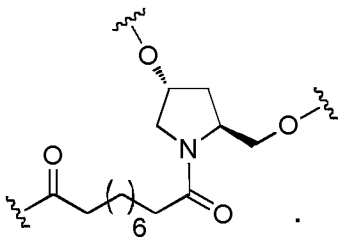
In certain embodiments, a linker has a structure selected from among:



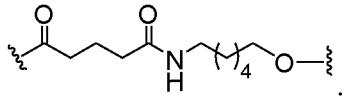
In certain embodiments, a linker has a structure selected from among:



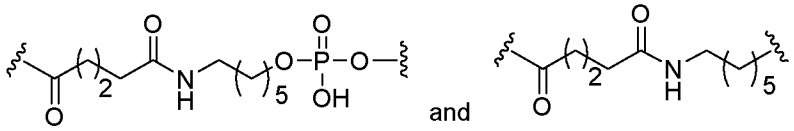
In certain embodiments, the conjugate linker has the structure:



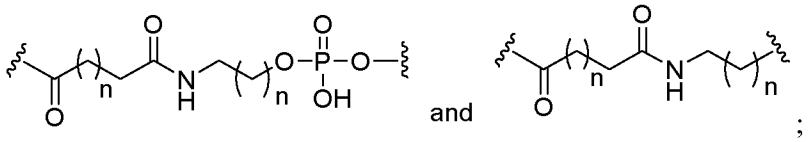
In certain embodiments, the conjugate linker has the structure:



In certain embodiments, a linker has a structure selected from among:



In certain embodiments, a linker has a structure selected from among:



wherein each n is independently, 0, 1, 2, 3, 4, 5, 6, or 7.

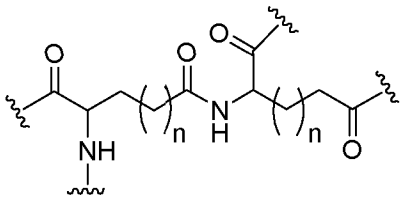
iii. **Certain Cell-Targeting Moieties**

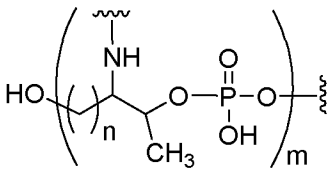
In certain embodiments, conjugate groups comprise cell-targeting moieties. Certain such cell-targeting moieties increase cellular uptake of antisense compounds. In certain embodiments, cell-targeting moieties comprise a branching group, one or more tether, and one or more ligand. In certain embodiments, cell-targeting moieties comprise a branching group, one or more tether, one or more ligand and one or more cleavable bond.

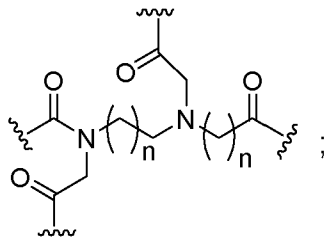
1. **Certain Branching Groups**

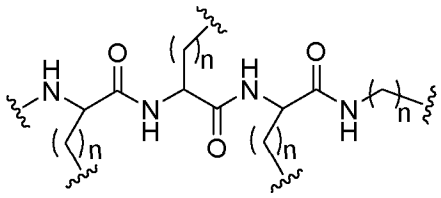
In certain embodiments, the conjugate groups comprise a targeting moiety comprising a branching group and at least two tethered ligands. In certain embodiments, the branching group attaches the conjugate linker. In certain embodiments, the branching group attaches the cleavable moiety. In certain embodiments, the branching group attaches the antisense oligonucleotide. In certain embodiments, the branching group is covalently attached to the linker and each of the tethered ligands. In certain embodiments, the branching group comprises a branched aliphatic group comprising groups selected from alkyl, amide, disulfide, polyethylene glycol, ether, thioether and hydroxylamino groups. In certain embodiments, the branching group comprises groups selected from alkyl, amide and ether groups. In certain embodiments, the branching 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 comprises one or more cleavable bond. In certain embodiments, the conjugate group does not include a branching group.

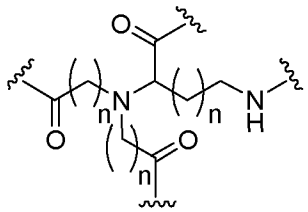
In certain embodiments, a branching group has a structure selected from among:

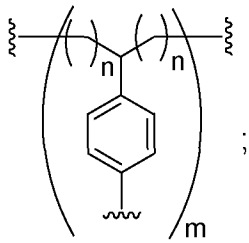

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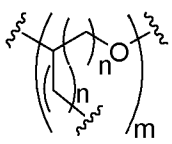

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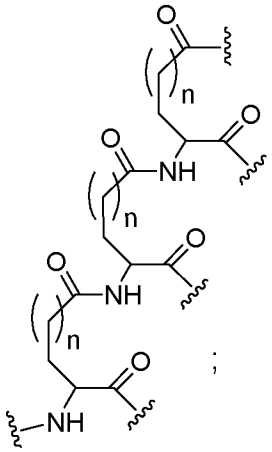

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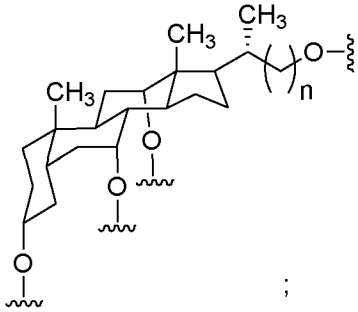

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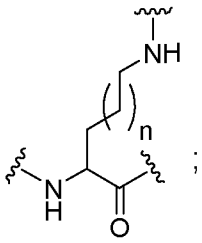

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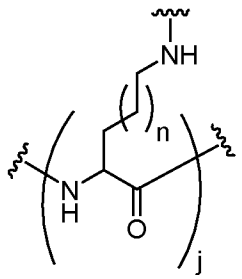
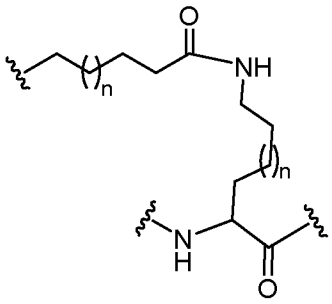
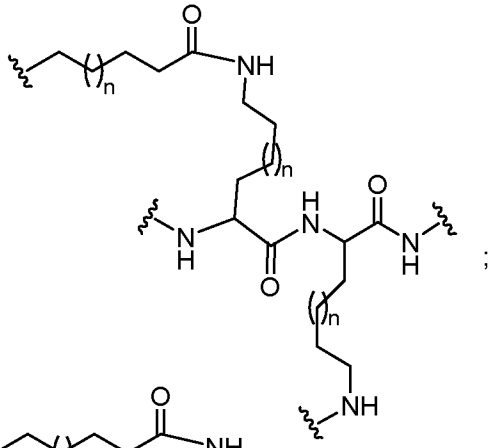

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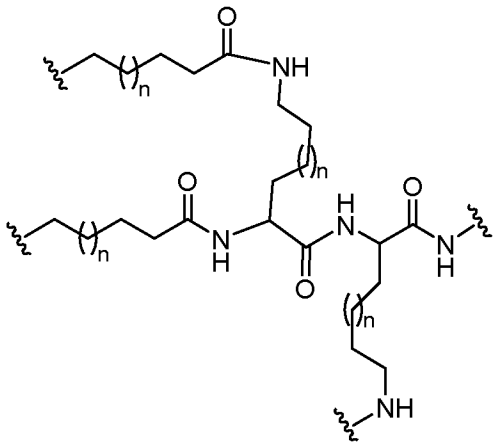
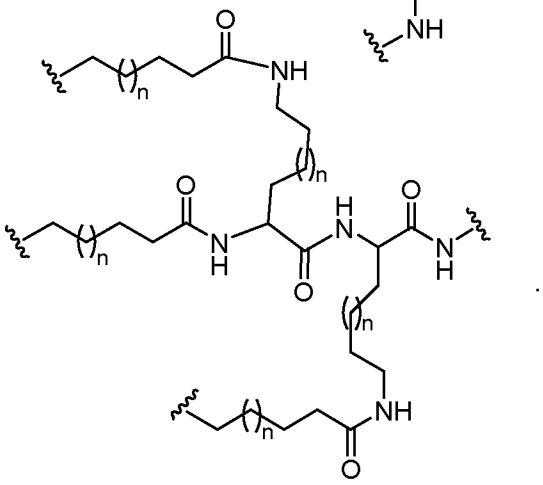

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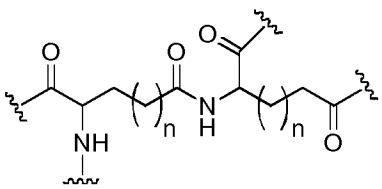
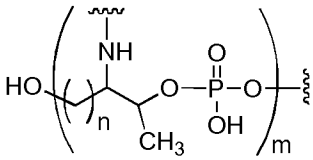
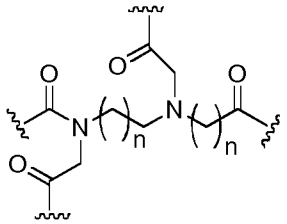
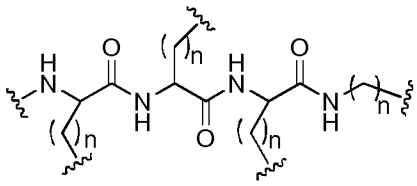
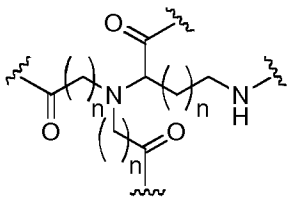
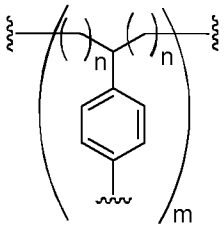
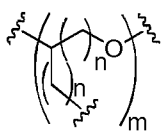
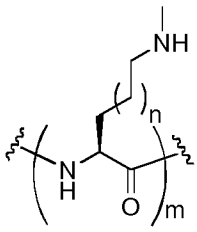
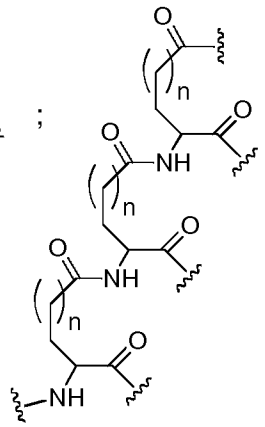
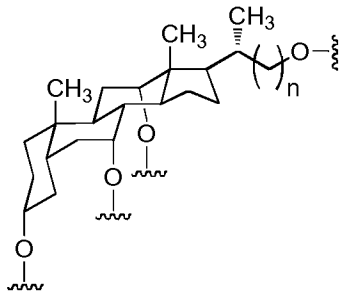
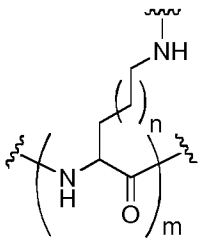

; and 
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wherein each n is, independently, from 1 to 20;

j is from 1 to 3; and

m is from 2 to 6.

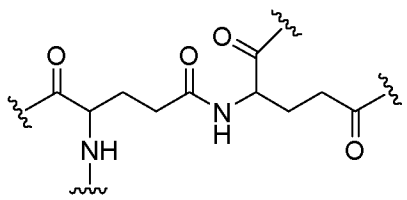
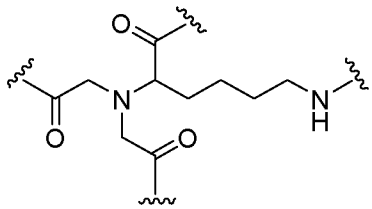
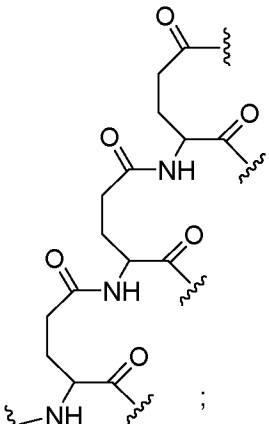
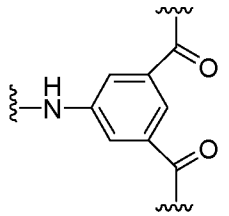
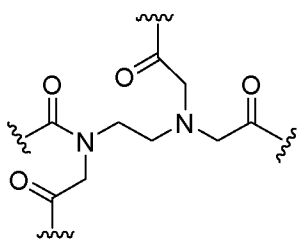
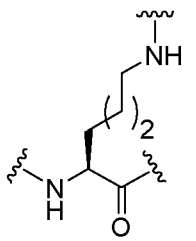
In certain embodiments, a branching group has a structure selected from among:

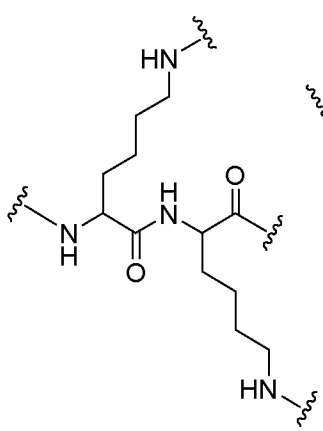
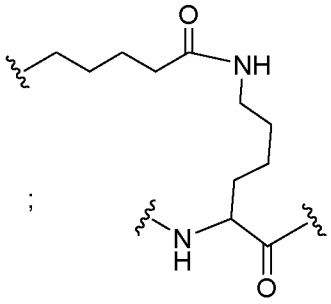
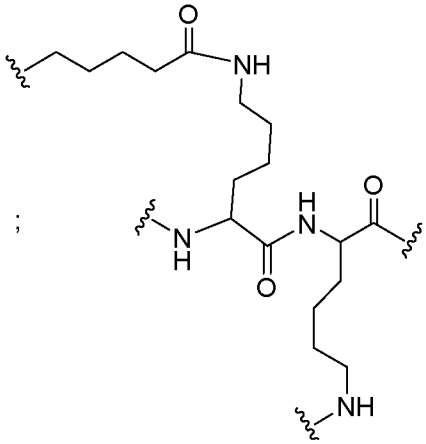
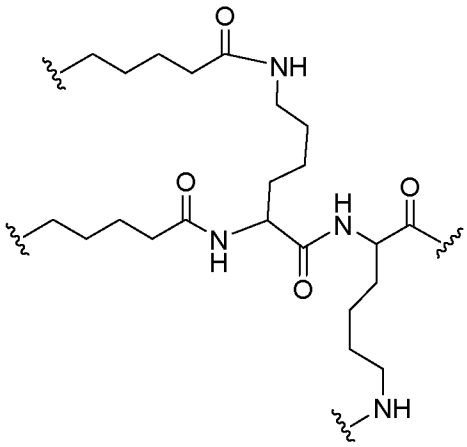
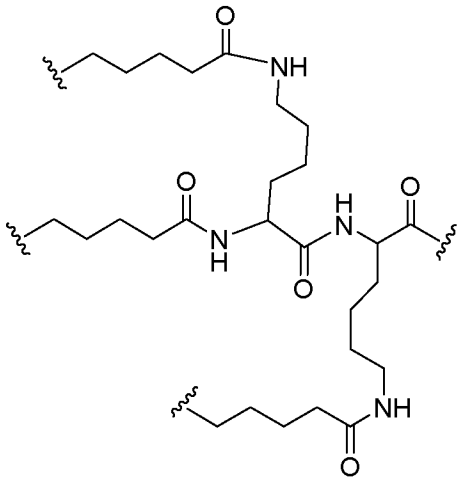

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; and


wherein each n is, independently, from 1 to 20; and

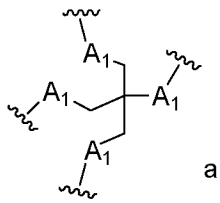
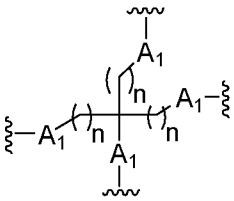
m is from 2 to 6.

In certain embodiments, a branching group has a structure selected from among:


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; and


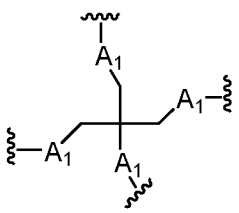
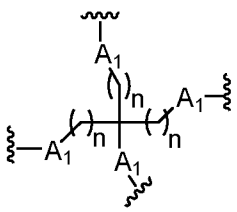
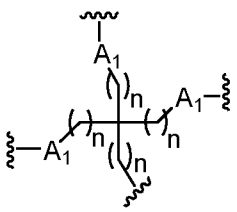
In certain embodiments, a branching group has a structure selected from among:


and


wherein each A_1 is independently, O, S, C=O or NH; and

each n is, independently, from 1 to 20.

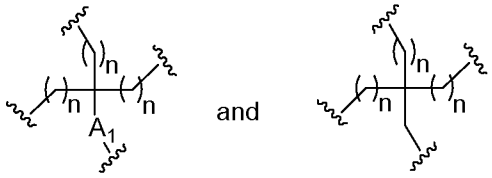
In certain embodiments, a branching group has a structure selected from among:


;

and


wherein each A_1 is independently, O, S, C=O or NH; and

each n is, independently, from 1 to 20.

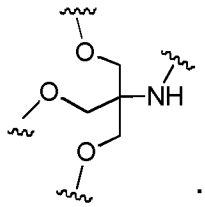
In certain embodiments, a branching group has a structure selected from among:



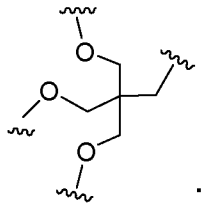
wherein A_1 is O, S, C=O or NH; and

each n is, independently, from 1 to 20.

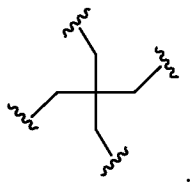
In certain embodiments, a branching group has a structure selected from among:



In certain embodiments, a branching group has a structure selected from among:



In certain embodiments, a branching group has a structure selected from among:



2. **Certain Tethers**

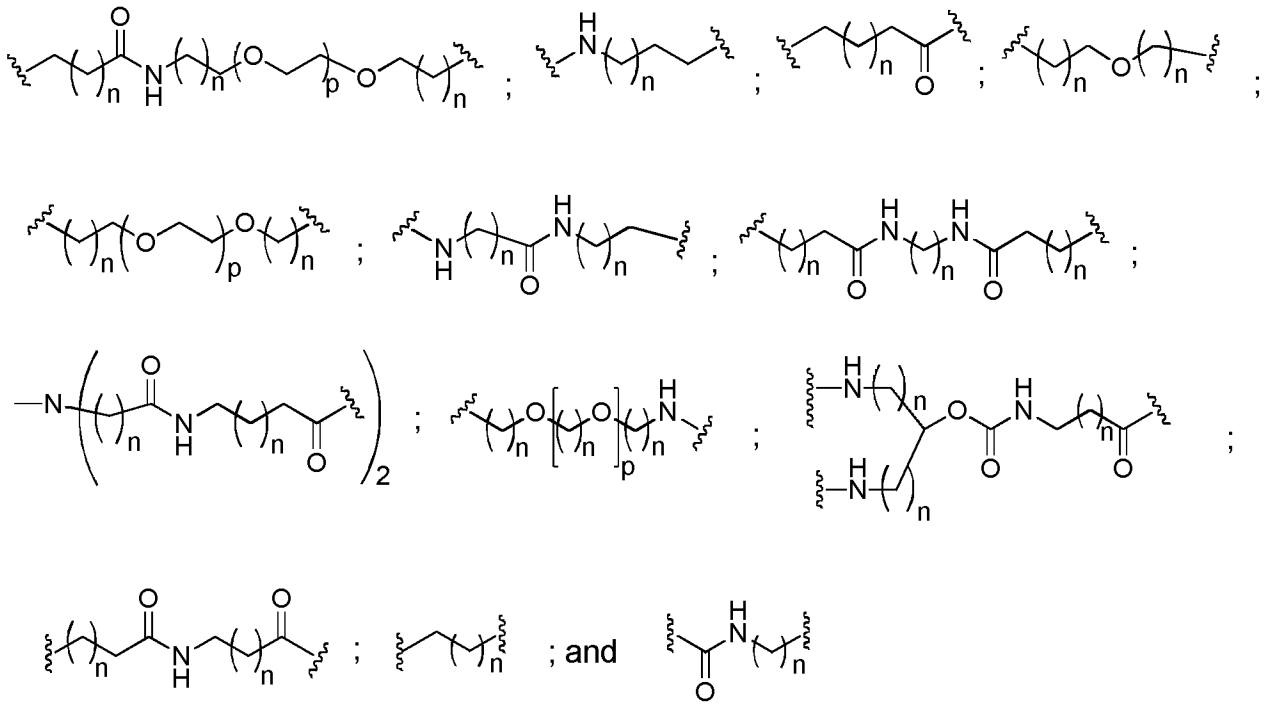
In certain embodiments, conjugate groups comprise one or more tethers covalently attached to the branching group. In certain embodiments, conjugate groups comprise one or more tethers covalently attached to the linking group. In certain embodiments, each tether is a linear aliphatic group comprising one or more groups selected from alkyl, ether, thioether, disulfide, 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, ether, thioether, disulfide, 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 and amide 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 amide 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, the tether includes one or more cleavable bond. In certain embodiments, the tether is attached to the branching group through either an amide or an ether group. In certain embodiments, the tether is attached to the branching group through a phosphodiester group. In certain embodiments, the tether is attached to the branching group through a phosphorus linking group or neutral linking group. In certain embodiments, the tether is attached to the branching group through an ether group. In certain embodiments, the tether is attached to the ligand through either an amide or an ether group. In certain embodiments, the tether is attached to the ligand through an ether group. In certain embodiments, the tether is attached to the ligand through either an amide or an ether group. In certain embodiments, the tether is attached to the 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 group comprises from about 10 to about 18 atoms in chain length between the ligand and the branching group. In certain embodiments, each tether group comprises about 13 atoms in chain length.

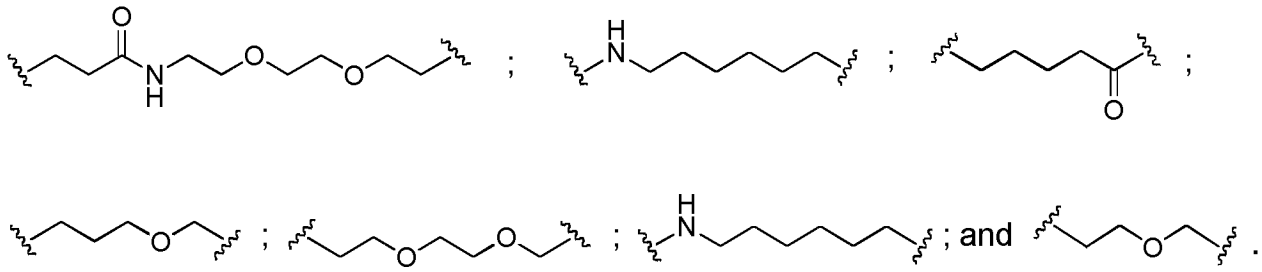
In certain embodiments, a tether has a structure selected from among:



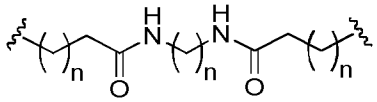
wherein each n is, independently, from 1 to 20; and

each p is from 1 to about 6.

In certain embodiments, a tether has a structure selected from among:

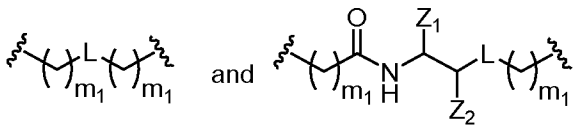


In certain embodiments, a tether has a structure selected from among:



wherein each n is, independently, from 1 to 20.

In certain embodiments, a tether has a structure selected from among:



wherein L is either a phosphorus linking group or a neutral linking group;

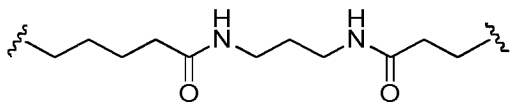
Z_1 is C(=O)O-R_2;

Z_2 is H, C_1-C_6 alkyl or substituted C_1-C_6 alky;

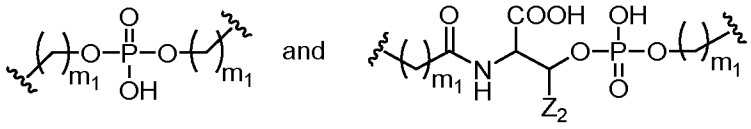
R_2 is H, C_1-C_6 alkyl or substituted C_1-C_6 alky; and

each m_1 is, independently, from 0 to 20 wherein at least one m_1 is greater than 0 for each tether.

In certain embodiments, a tether has a structure selected from among:



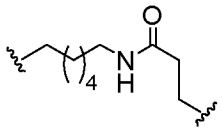
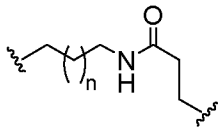
In certain embodiments, a tether has a structure selected from among:



wherein Z_2 is H or CH_3; and

each m_1 is, independently, from 0 to 20 wherein at least one m_1 is greater than 0 for each tether.

In certain embodiments, a tether has a structure selected from among:

 , or  ; wherein each n is independently, 0, 1, 2, 3, 4, 5, 6, or 7.

In certain embodiments, a tether comprises a phosphorus linking group. In certain embodiments, a tether does not comprise any amide bonds. In certain embodiments, a tether comprises a phosphorus linking group and does not comprise any amide bonds.

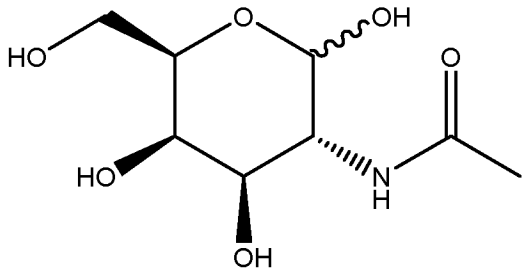
3. Certain Ligands

In certain embodiments, the present disclosure provides ligands wherein each ligand is covalently attached to 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, glucosamone and fucose. In certain embodiments, each ligand is N-acetyl galactoseamine (GalNAc). In certain embodiments, the targeting moiety comprises 2 to 6 ligands. In certain embodiments, the targeting moiety comprises 3 ligands. In certain embodiments, the targeting moiety comprises 3 N-acetyl galactoseamine ligands.

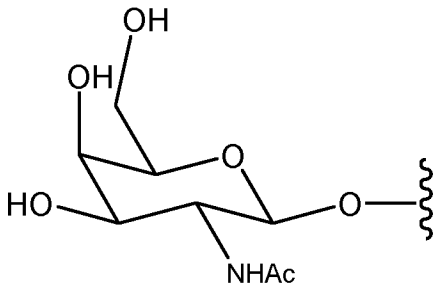
In certain embodiments, the ligand is a carbohydrate, carbohydrate derivative, modified carbohydrate, multivalent carbohydrate cluster, polysaccharide, modified polysaccharide, or polysaccharide derivative. In certain embodiments, the ligand is an amino sugar or a thio sugar. For example, amino sugars 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, "GalNac" or "Gal-NAc" refers to 2-(Acetylamino)-2-deoxy-D-galactopyranose, commonly referred to in the literature as N-acetyl galactosamine. In certain embodiments, "N-acetyl galactosamine" refers to 2-(Acetylamino)-2-deoxy-D-galactopyranose. In certain embodiments, "GalNac" or "Gal-NAc" refers to 2-(Acetylamino)-2-deoxy-D-galactopyranose. In certain embodiments,

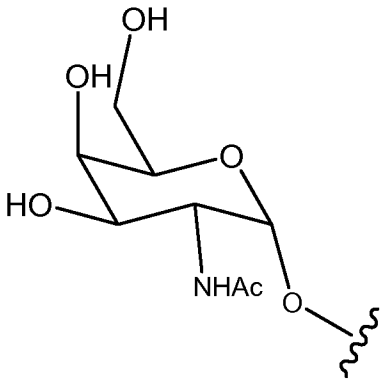
"GalNac" or "Gal-NAc" refers to 2-(Acetylamino)-2-deoxy-D-galactopyranose, which includes both the β-form: 2-(Acetylamino)-2-deoxy-β-D-galactopyranose and α-form: 2-(Acetylamino)-2-deoxy-D-galactopyranose. In certain embodiments, both the β-form: 2-(Acetylamino)-2-deoxy-β-D-galactopyranose and α-form: 2-(Acetylamino)-2-deoxy-D-galactopyranose may be used interchangeably. Accordingly, in structures in which one form is depicted, these structures are intended to include the other form as well. For example, where the structure for an α-form: 2-(Acetylamino)-2-deoxy-D-galactopyranose is shown, this structure is intended to include the other form as well. In certain embodiments, In certain preferred embodiments, the β-form 2-(Acetylamino)-2-deoxy-D-galactopyranose is the preferred embodiment.



2-(Acetylamino)-2-deoxy-D-galactopyranose

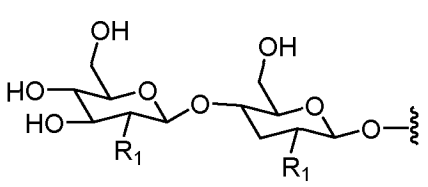
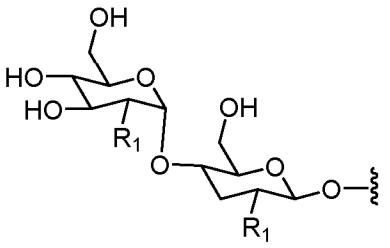


2-(Acetylamino)-2-deoxy-β-D-galactopyranose



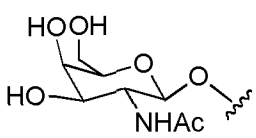
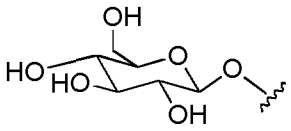
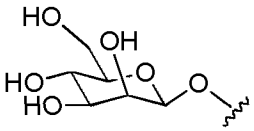
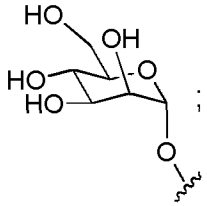
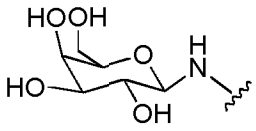
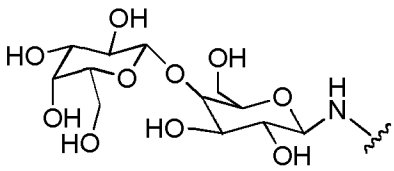
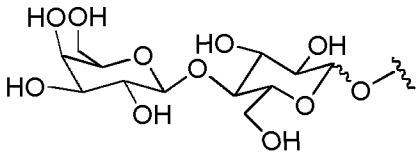
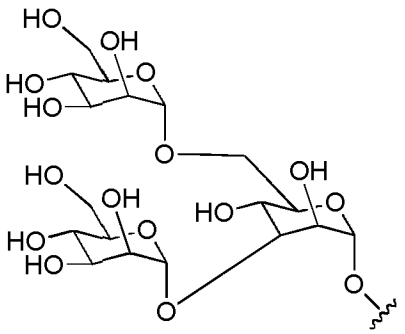
2-(Acetylamino)-2-deoxy-α-D-galactopyranose

In certain embodiments one or more ligand has a structure selected from among:

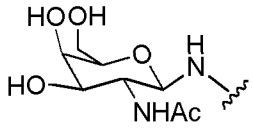



wherein each R_1 is selected from OH and NHCOOH.

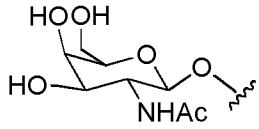
In certain embodiments one or more ligand has a structure selected from among:








; and

.

In certain embodiments one or more ligand has a structure selected from among:


.

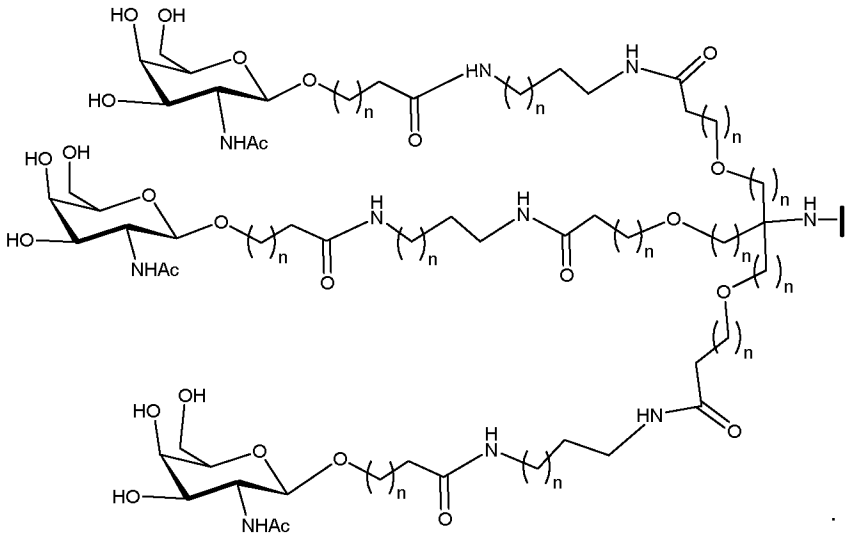
In certain embodiments one or more ligand has a structure selected from among:



.

i. **Certain Conjugates**

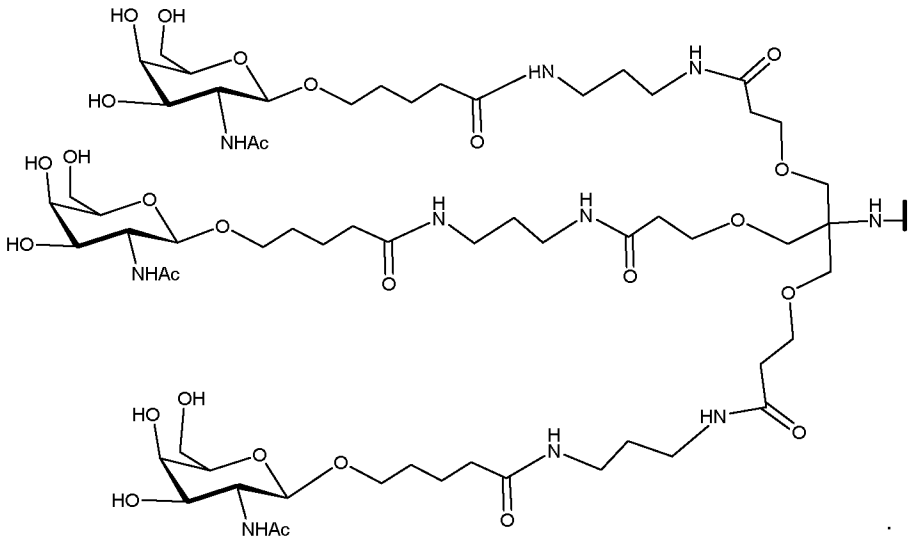
In certain embodiments, conjugate groups comprise the structural features above. In certain such embodiments, conjugate groups have the following structure:



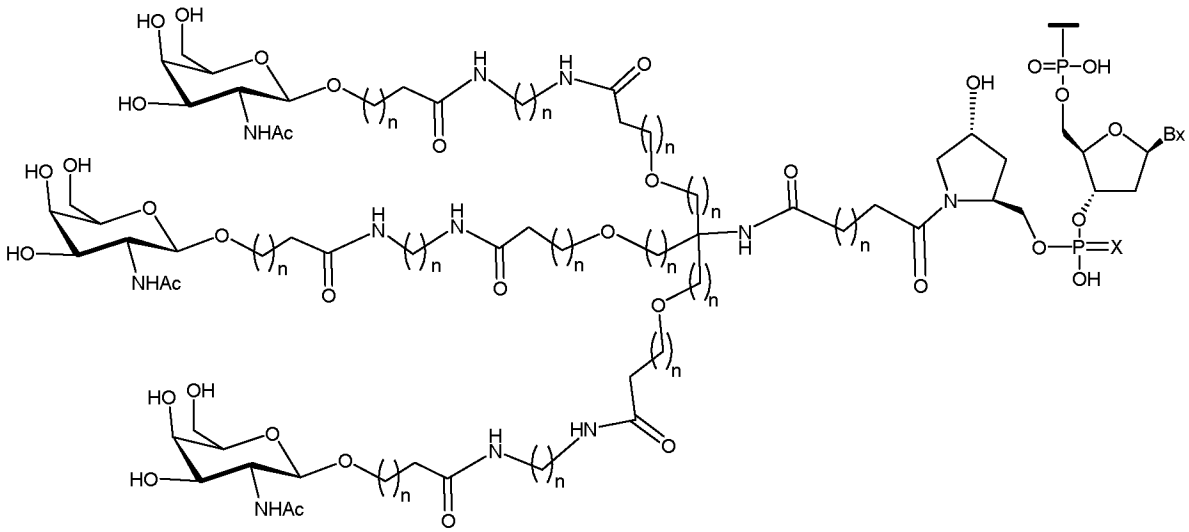
.

wherein each n is, independently, from 1 to 20.

In certain such embodiments, conjugate groups have the following structure:



In certain such embodiments, conjugate groups have the following structure:



wherein each n is, independently, from 1 to 20;

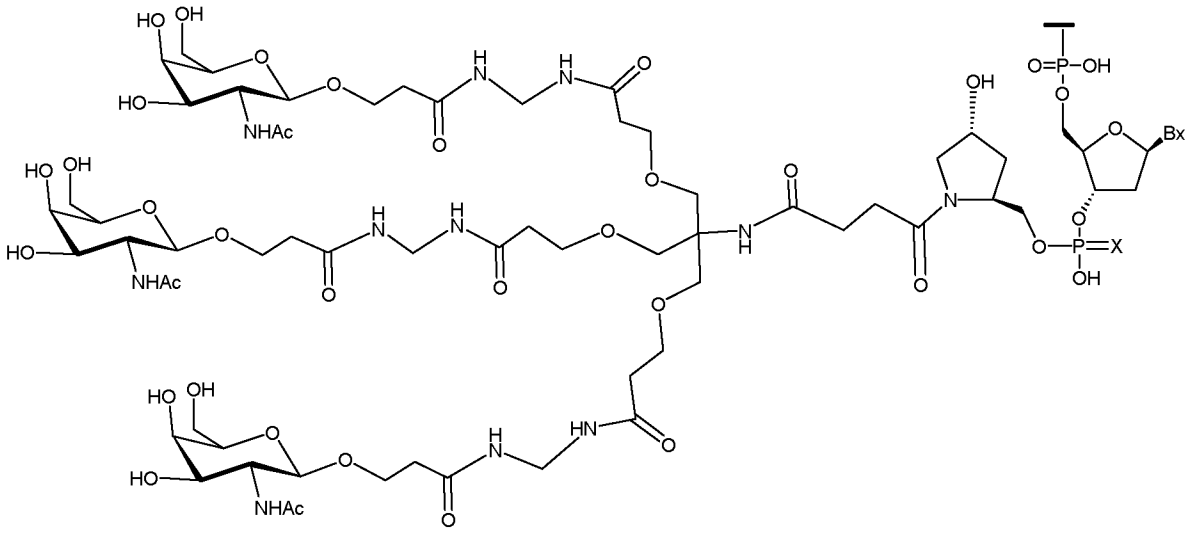
Z is H or a linked solid support;

Q is an antisense compound;

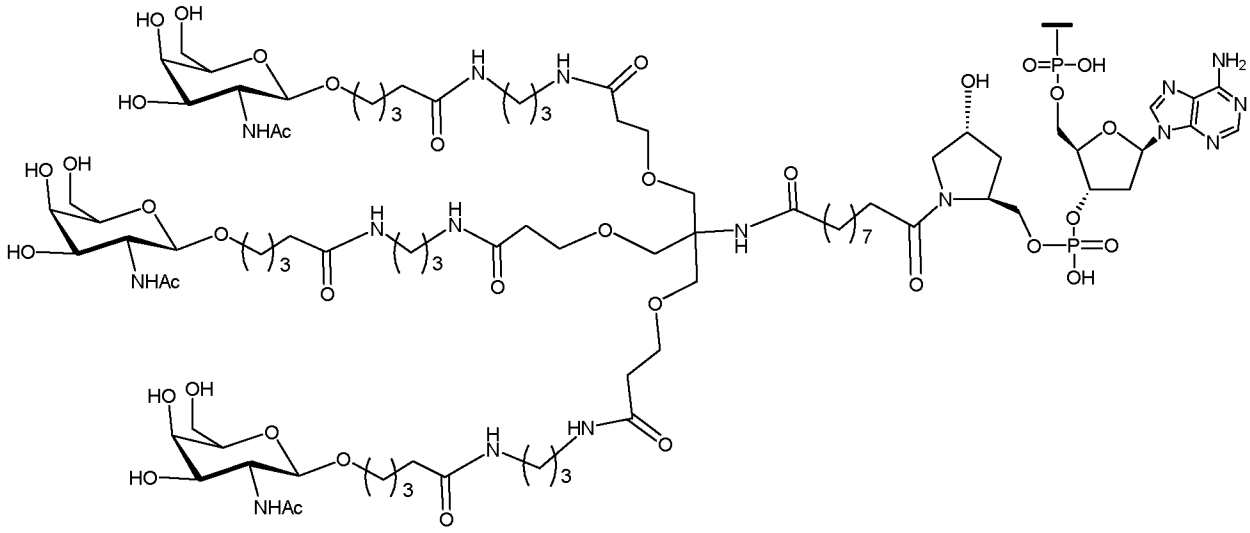
X is O or S; and

Bx is a heterocyclic base moiety.

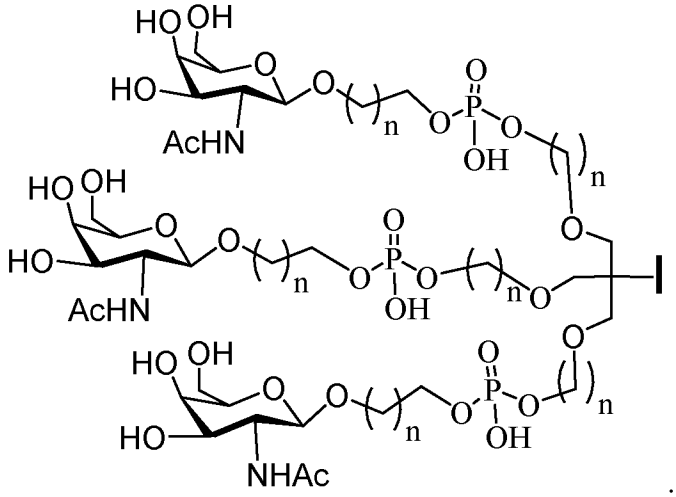
In certain such embodiments, conjugate groups have the following structure:



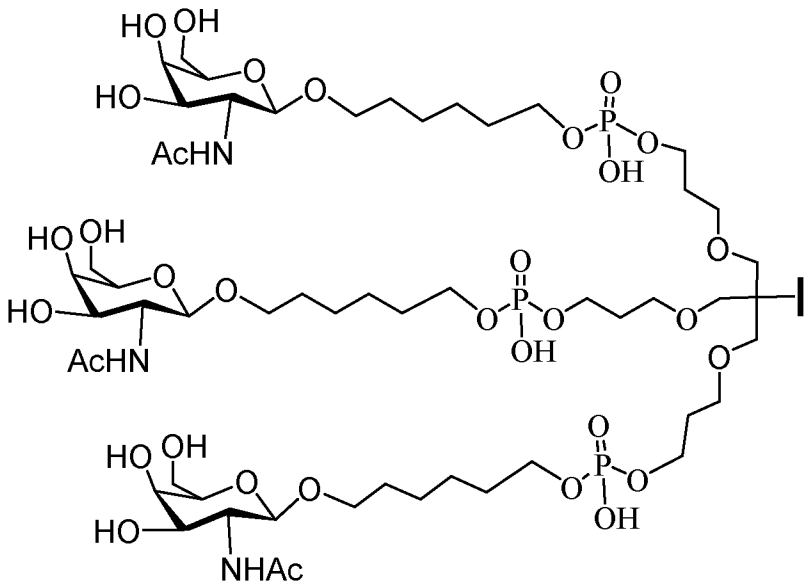
In certain such embodiments, conjugate groups have the following structure:



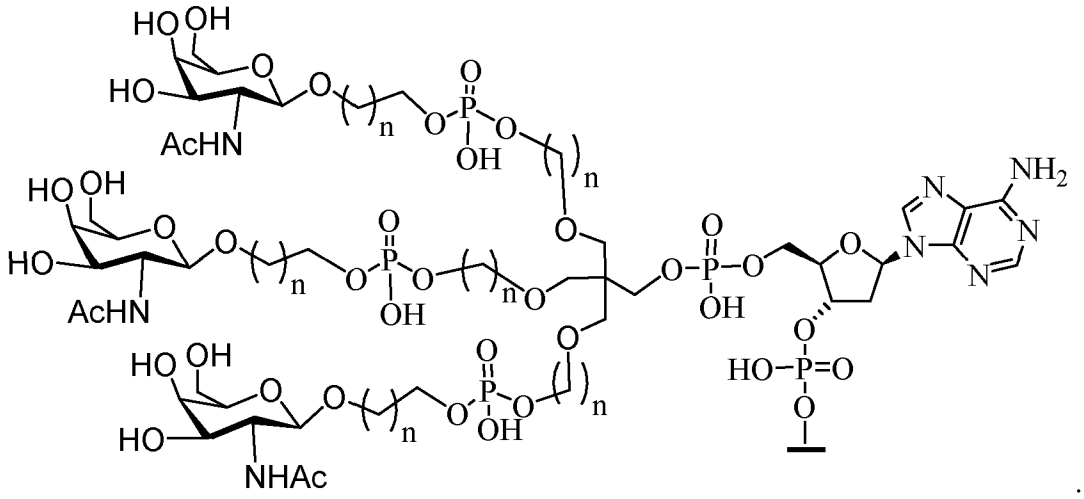
In certain such embodiments, conjugate groups have the following structure:



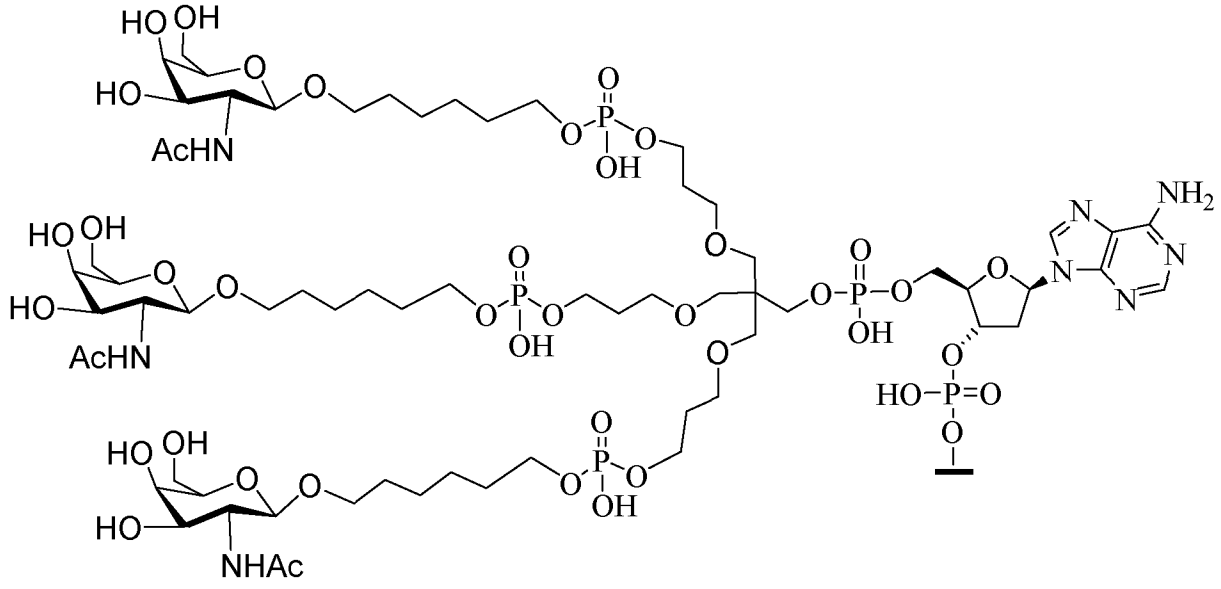
In certain such embodiments, conjugate groups have the following structure:



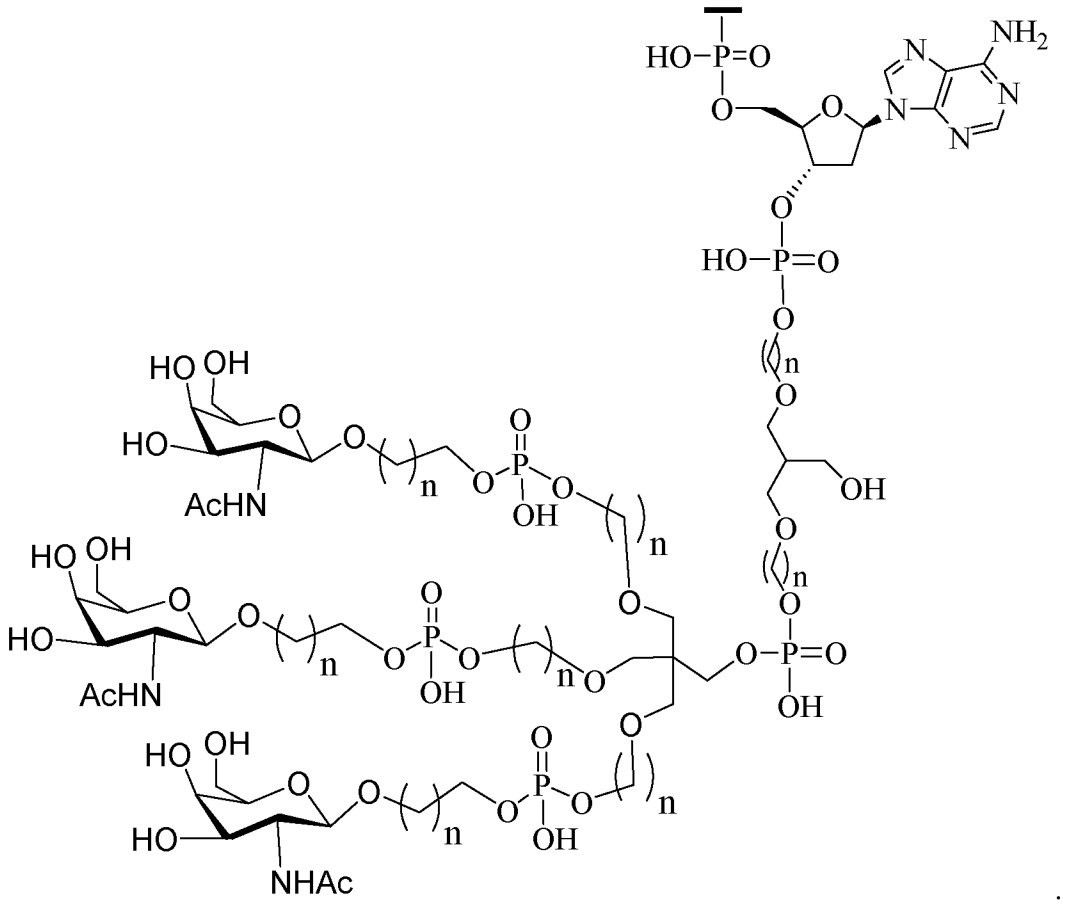
In certain such embodiments, conjugate groups have the following structure:



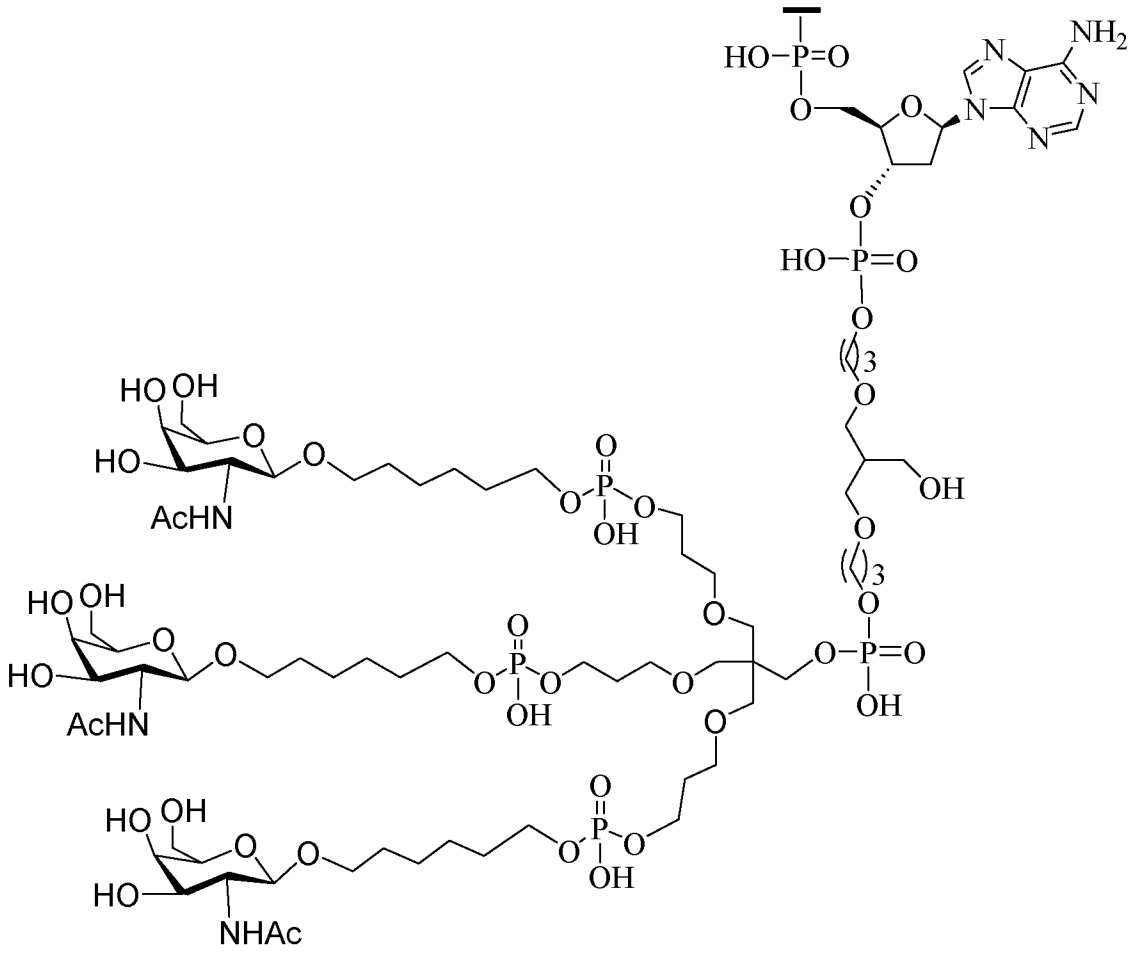
In certain such embodiments, conjugate groups have the following structure:



In certain such embodiments, conjugate groups have the following structure:

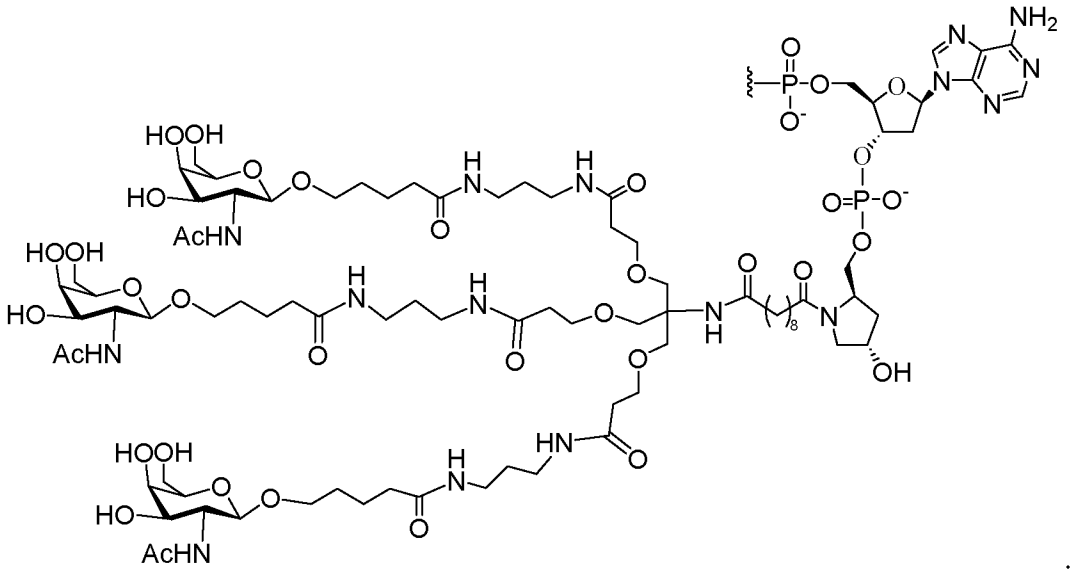


In certain such embodiments, conjugate groups have the following structure:

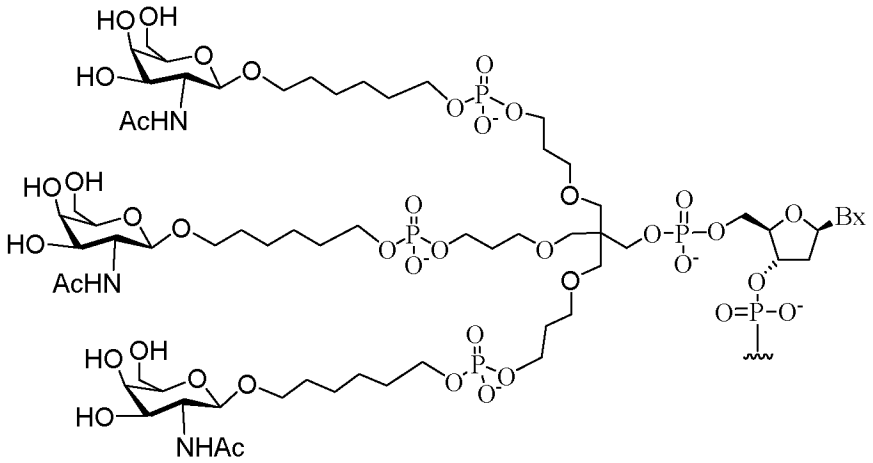


In certain embodiments, conjugates do not comprise a pyrrolidine.

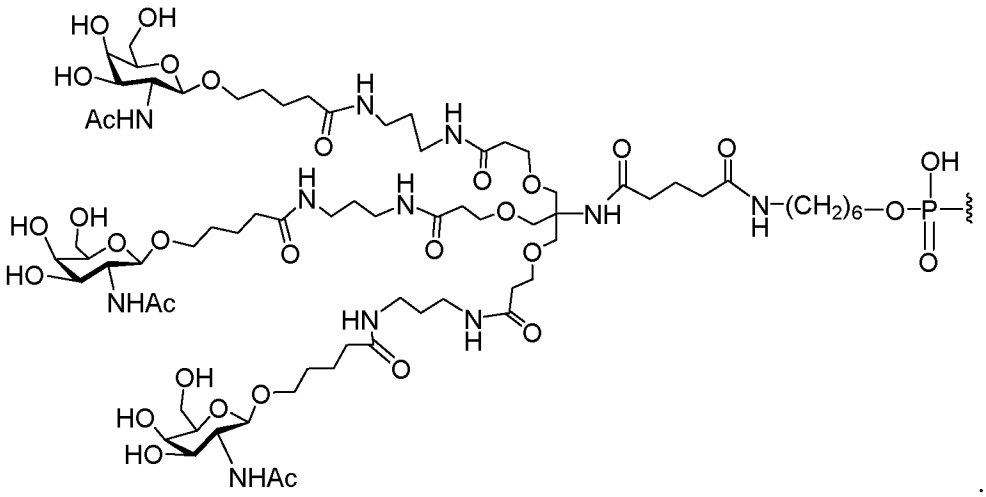
In certain such embodiments, conjugate groups have the following structure:



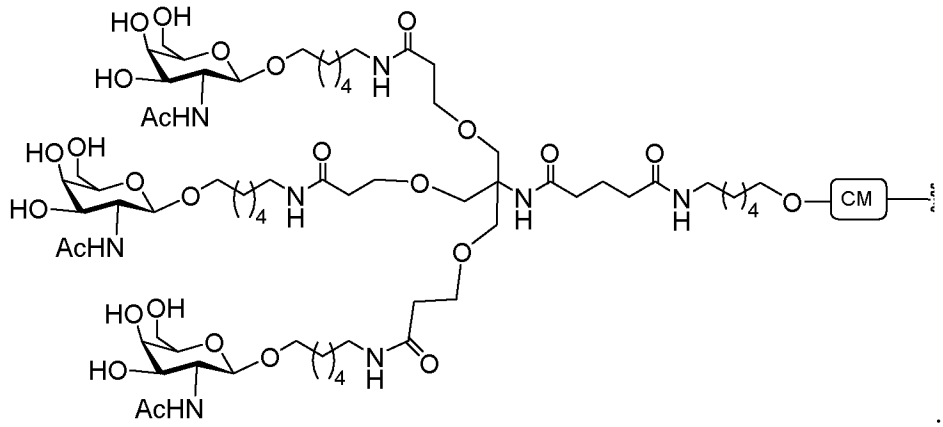
In certain such embodiments, conjugate groups have the following structure:



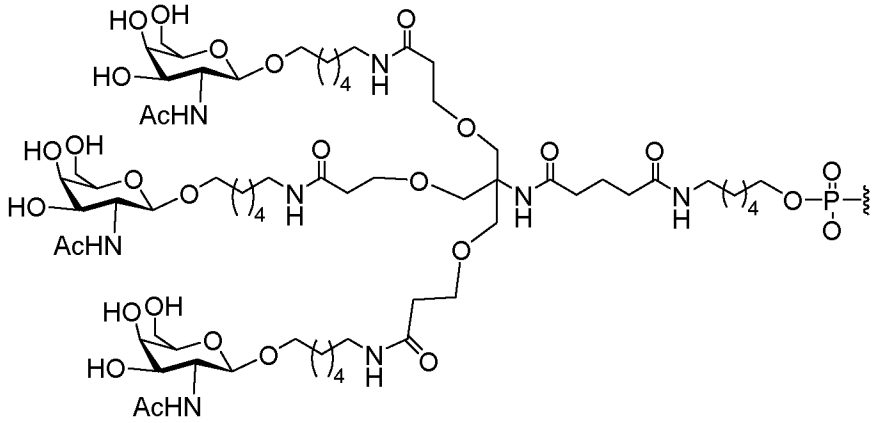
In certain such embodiments, conjugate groups have the following structure:



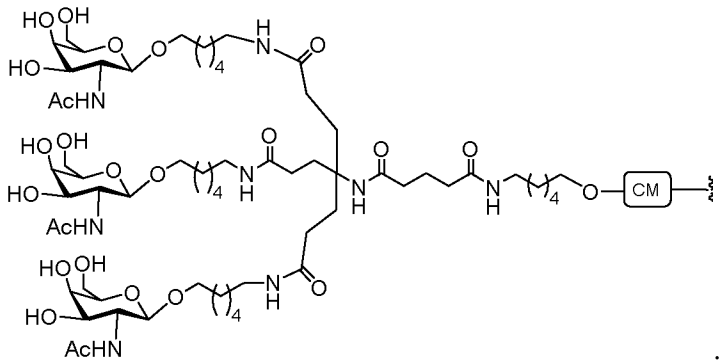
In certain such embodiments, conjugate groups have the following structure:



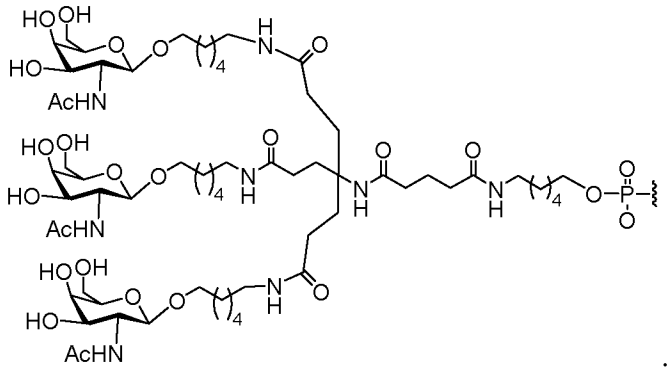
In certain such embodiments, conjugate groups have the following structure:



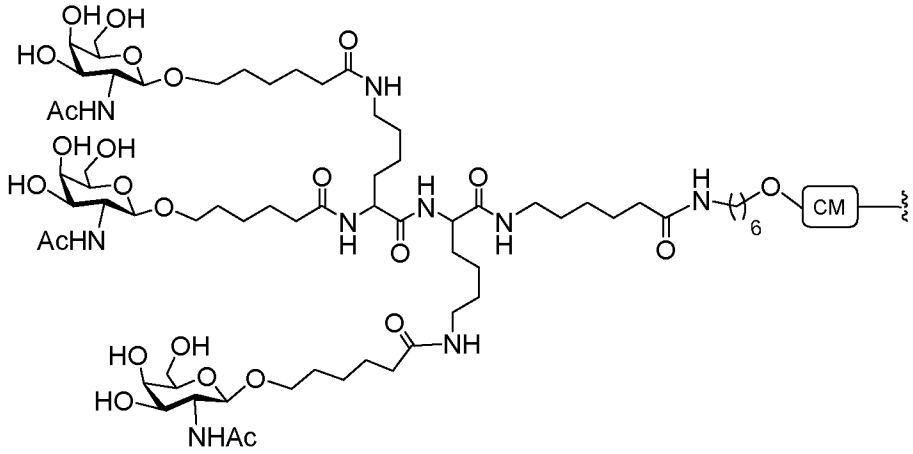
In certain such embodiments, conjugate groups have the following structure:



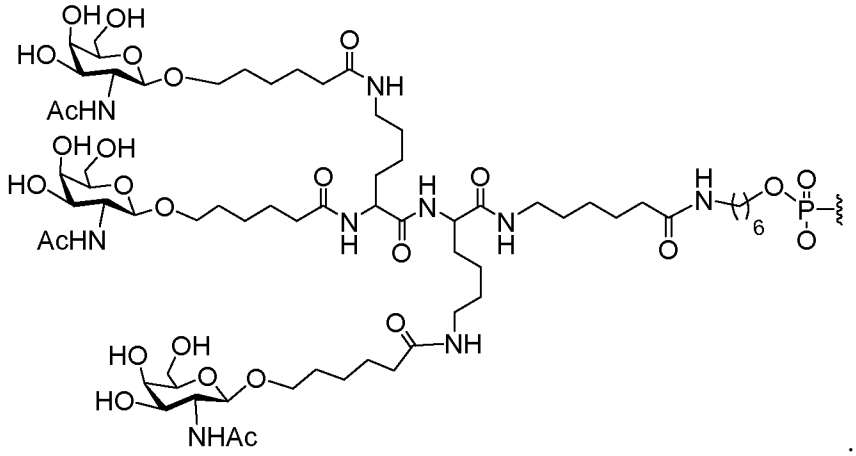
In certain such embodiments, conjugate groups have the following structure:



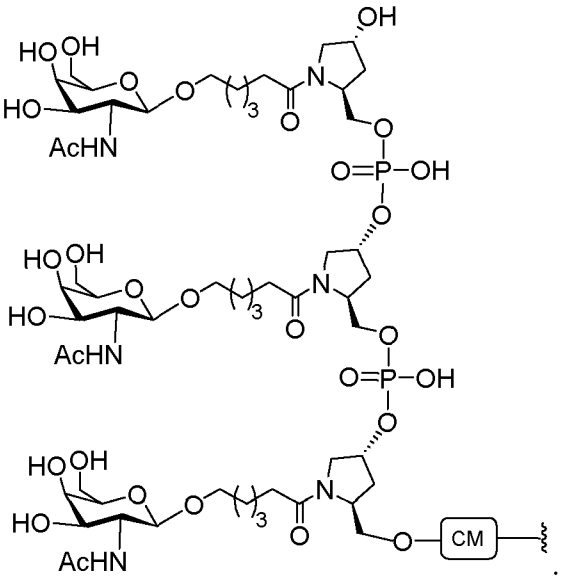
In certain such embodiments, conjugate groups have the following structure:



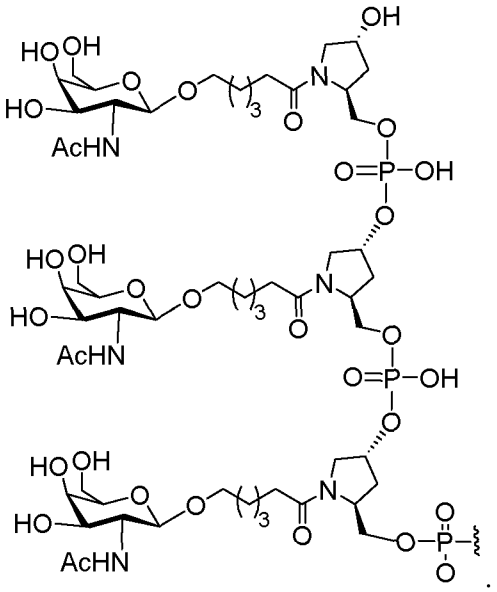
In certain such embodiments, conjugate groups have the following structure:



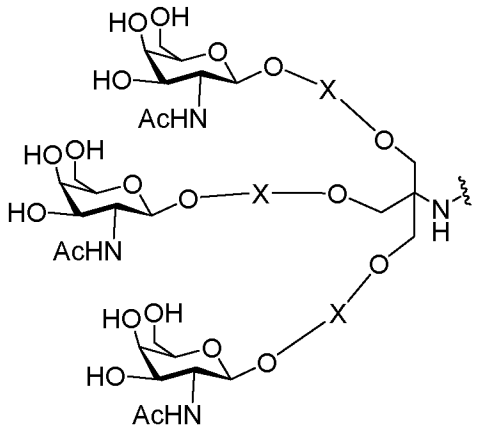
In certain such embodiments, conjugate groups have the following structure:



In certain such embodiments, conjugate groups have the following structure:

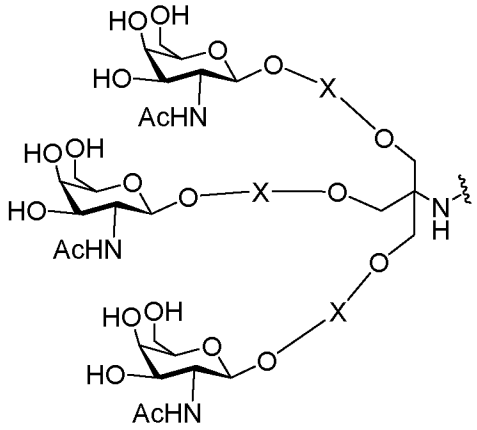


In certain embodiments, the cell-targeting moiety of the conjugate group has the following structure:



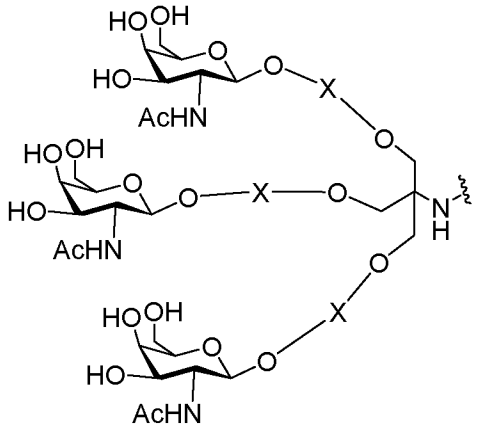
wherein X is a substituted or unsubstituted tether of six to eleven consecutively bonded atoms.

In certain embodiments, the cell-targeting moiety of the conjugate group has the following structure:



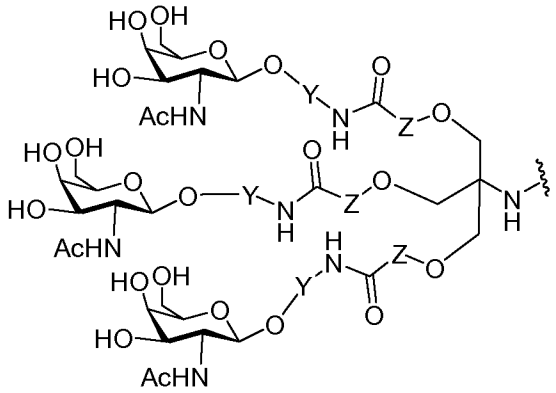
wherein X is a substituted or unsubstituted tether of ten consecutively bonded atoms.

In certain embodiments, the cell-targeting moiety of the conjugate group has the following structure:



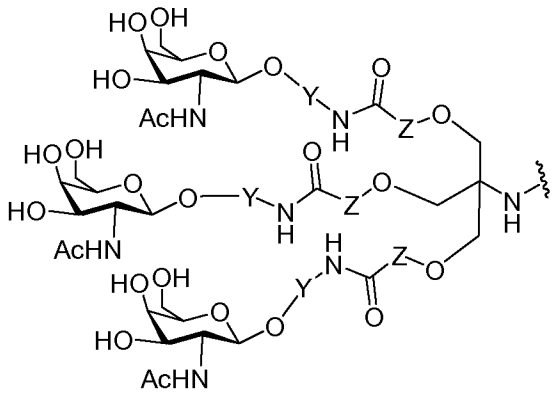
wherein X is a substituted or unsubstituted tether of four to eleven consecutively bonded atoms and wherein the tether comprises exactly one amide bond.

In certain embodiments, the cell-targeting moiety of the conjugate group has the following structure:



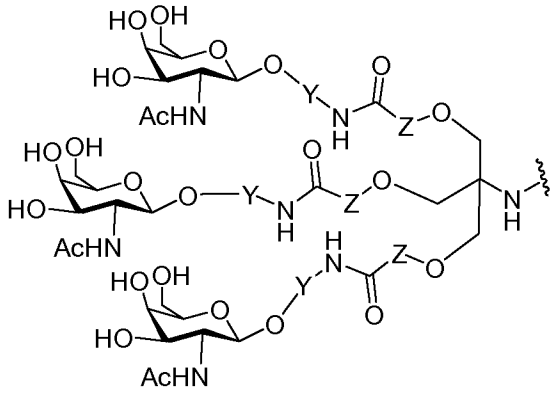
wherein Y and Z are independently selected from a C_1-C_{12} substituted or unsubstituted alkyl, alkenyl, or alkynyl group, or a group comprising an ether, a ketone, an amide, an ester, a carbamate, an amine, a piperidine, a phosphate, a phosphodiester, a phosphorothioate, a triazole, a pyrrolidine, a disulfide, or a thioether.

In certain such embodiments, the cell-targeting moiety of the conjugate group has the following structure:



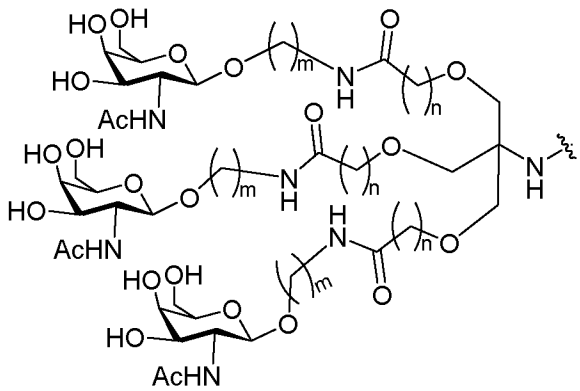
wherein Y and Z are independently selected from a C_1-C_{12} substituted or unsubstituted alkyl group, or a group comprising exactly one ether or exactly two ethers, an amide, an amine, a piperidine, a phosphate, a phosphodiester, or a phosphorothioate.

In certain such embodiments, the cell-targeting moiety of the conjugate group has the following structure:



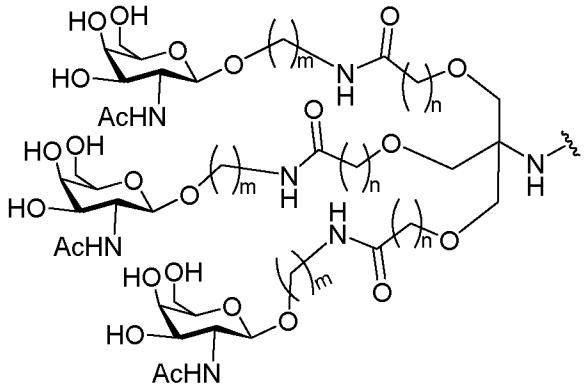
wherein Y and Z are independently selected from a C_1-C_{12} substituted or unsubstituted alkyl group.

In certain such embodiments, the cell-targeting moiety of the conjugate group has the following structure:



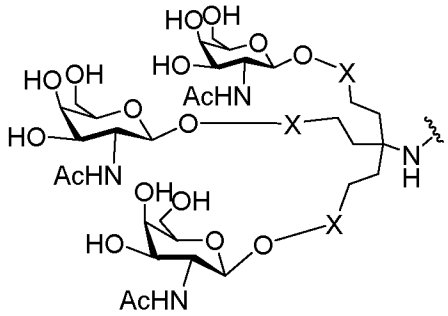
wherein m and n are independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12.

In certain such embodiments, the cell-targeting moiety of the conjugate group has the following structure:



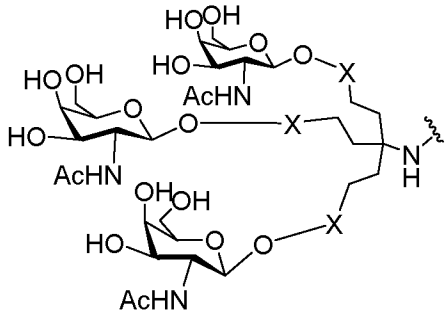
wherein m is 4, 5, 6, 7, or 8, and n is 1, 2, 3, or 4.

In certain embodiments, the cell-targeting moiety of the conjugate group has the following structure:



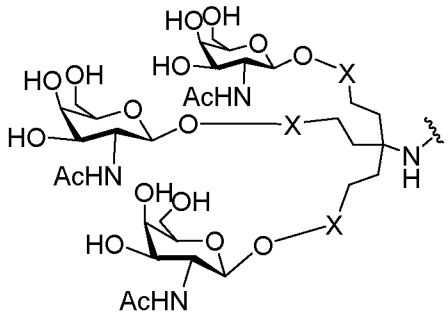
wherein X is a substituted or unsubstituted tether of four to thirteen consecutively bonded atoms, and wherein X does not comprise an ether group.

In certain embodiments, the cell-targeting moiety of the conjugate group has the following structure:



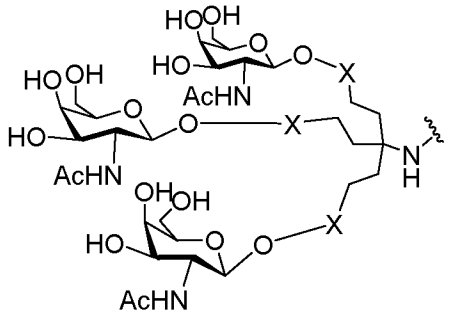
wherein X is a substituted or unsubstituted tether of eight consecutively bonded atoms, and wherein X does not comprise an ether group.

In certain embodiments, the cell-targeting moiety of the conjugate group has the following structure:



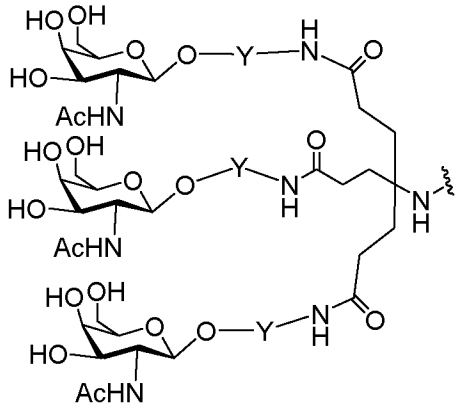
wherein X is a substituted or unsubstituted tether of four to thirteen consecutively bonded atoms, and wherein the tether comprises exactly one amide bond, and wherein X does not comprise an ether group.

In certain embodiments, the cell-targeting moiety of the conjugate group has the following structure:



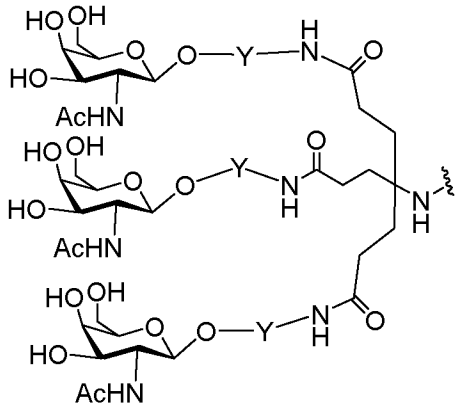
wherein X is a substituted or unsubstituted tether of four to thirteen consecutively bonded atoms and wherein the tether consists of an amide bond and a substituted or unsubstituted C_2-C_{11} alkyl group.

In certain embodiments, the cell-targeting moiety of the conjugate group has the following structure:



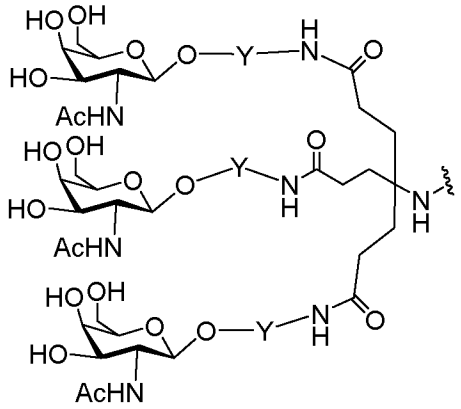
wherein Y is selected from a C_1-C_{12} substituted or unsubstituted alkyl, alkenyl, or alkynyl group, or a group comprising an ether, a ketone, an amide, an ester, a carbamate, an amine, a piperidine, a phosphate, a phosphodiester, a phosphorothioate, a triazole, a pyrrolidine, a disulfide, or a thioether.

In certain such embodiments, the cell-targeting moiety of the conjugate group has the following structure:



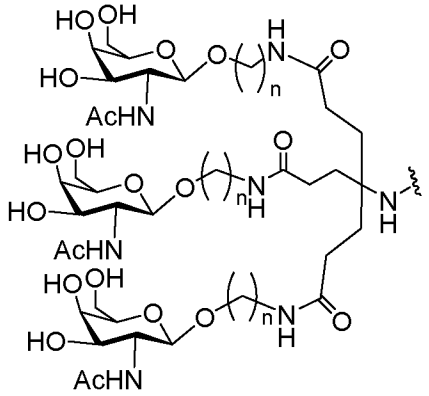
wherein Y is selected from a C_1-C_{12} substituted or unsubstituted alkyl group, or a group comprising an ether, an amine, a piperidine, a phosphate, a phosphodiester, or a phosphorothioate.

In certain such embodiments, the cell-targeting moiety of the conjugate group has the following structure:



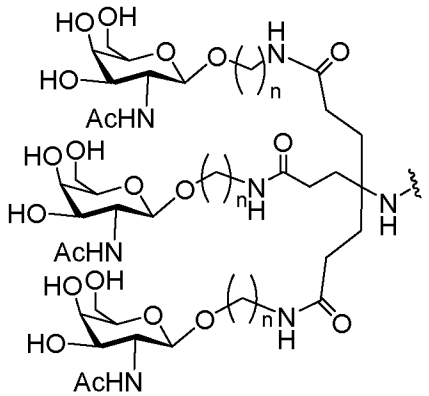
wherein Y is selected from a C_1-C_{12} substituted or unsubstituted alkyl group.

In certain such embodiments, the cell-targeting moiety of the conjugate group has the following structure:



Wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12.

In certain such embodiments, the cell-targeting moiety of the conjugate group has the following structure:

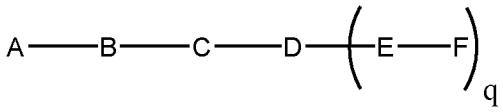


wherein n is 4, 5, 6, 7, or 8.

In certain embodiments, conjugates do not comprise a pyrrolidine.

a Certain conjugated antisense compounds

In certain embodiments, the conjugates are bound to a nucleoside of the antisense oligonucleotide at the 2', 3', of 5' position of the nucleoside. In certain embodiments, a conjugated antisense compound has the following structure:



wherein

A is the antisense oligonucleotide;

B is the cleavable moiety

C is the conjugate linker

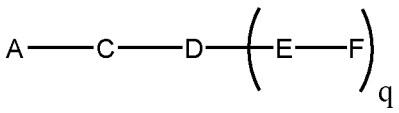
D is the branching group

each E is a tether;

each F is a ligand; and

q is an integer between 1 and 5.

In certain embodiments, a conjugated antisense compound has the following structure:



wherein

A is the antisense oligonucleotide;

C is the conjugate linker

D is the branching group

each E is a tether;

each F is a ligand; and

q is an integer between 1 and 5.

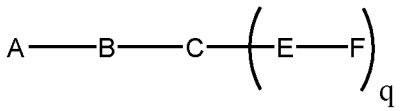
In certain such embodiments, the conjugate linker comprises at least one cleavable bond.

In certain such embodiments, the branching group comprises at least one cleavable bond.

In certain embodiments each tether comprises at least one cleavable bond.

In certain embodiments, the conjugates are bound to a nucleoside of the antisense oligonucleotide at the 2', 3', of 5' position of the nucleoside.

In certain embodiments, a conjugated antisense compound has the following structure:



wherein

A is the antisense oligonucleotide;

B is the cleavable moiety

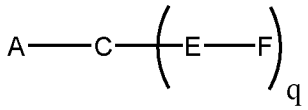
C is the conjugate linker

each E is a tether;

each F is a ligand; and

q is an integer between 1 and 5.

In certain embodiments, the conjugates are bound to a nucleoside of the antisense oligonucleotide at the 2', 3', of 5' position of the nucleoside. In certain embodiments, a conjugated antisense compound has the following structure:



wherein

A is the antisense oligonucleotide;

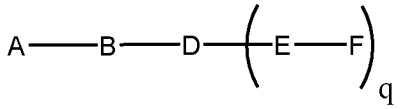
C is the conjugate linker

each E is a tether;

each F is a ligand; and

q is an integer between 1 and 5.

In certain embodiments, a conjugated antisense compound has the following structure:



wherein

A is the antisense oligonucleotide;

B is the cleavable moiety

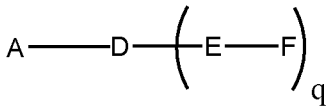
D is the branching group

each E is a tether;

each F is a ligand; and

q is an integer between 1 and 5.

In certain embodiments, a conjugated antisense compound has the following structure:



wherein

A is the antisense oligonucleotide;

D is the branching group

each E is a tether;

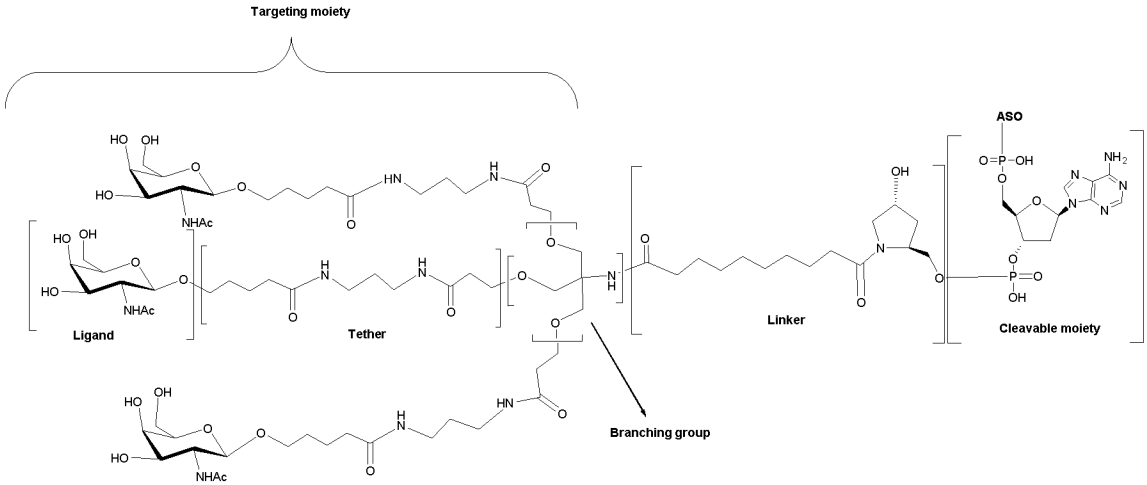
each F is a ligand; and

q is an integer between 1 and 5.

In certain such embodiments, the conjugate linker comprises at least one cleavable bond.

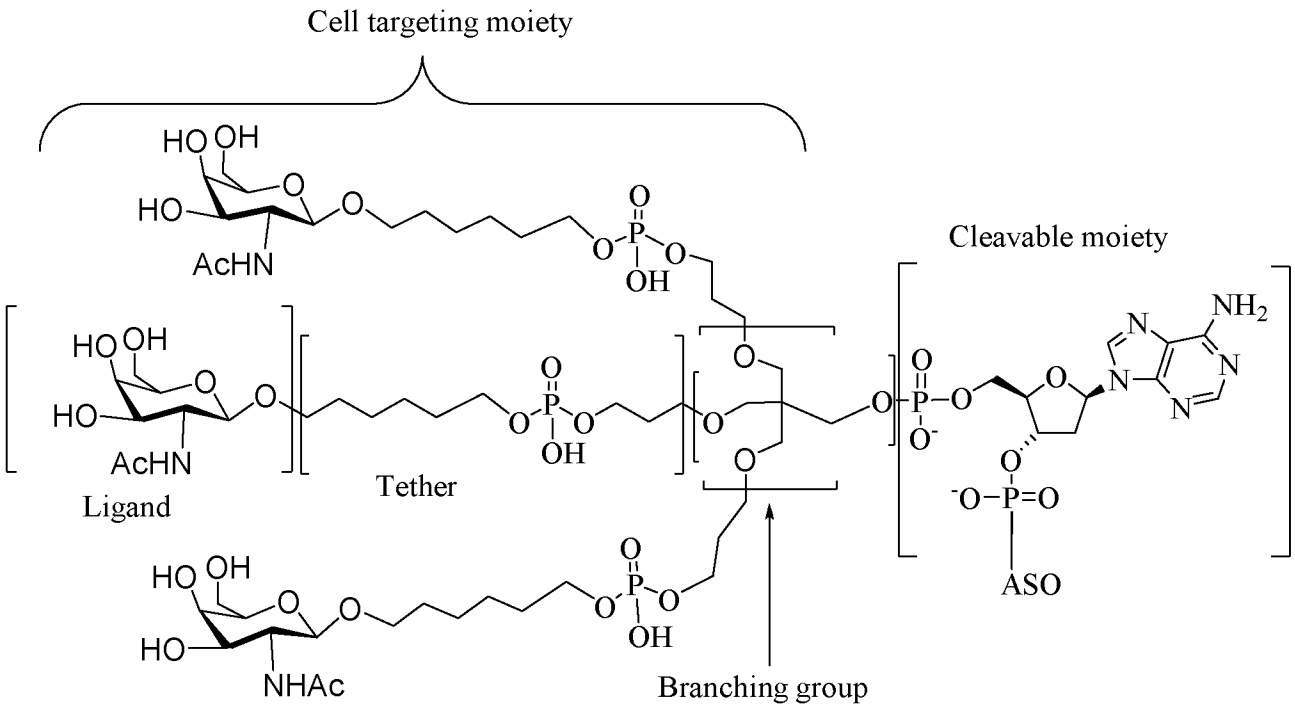
In certain embodiments each tether comprises at least one cleavable bond.

In certain embodiments, a conjugated antisense compound has a structure selected from among the following:

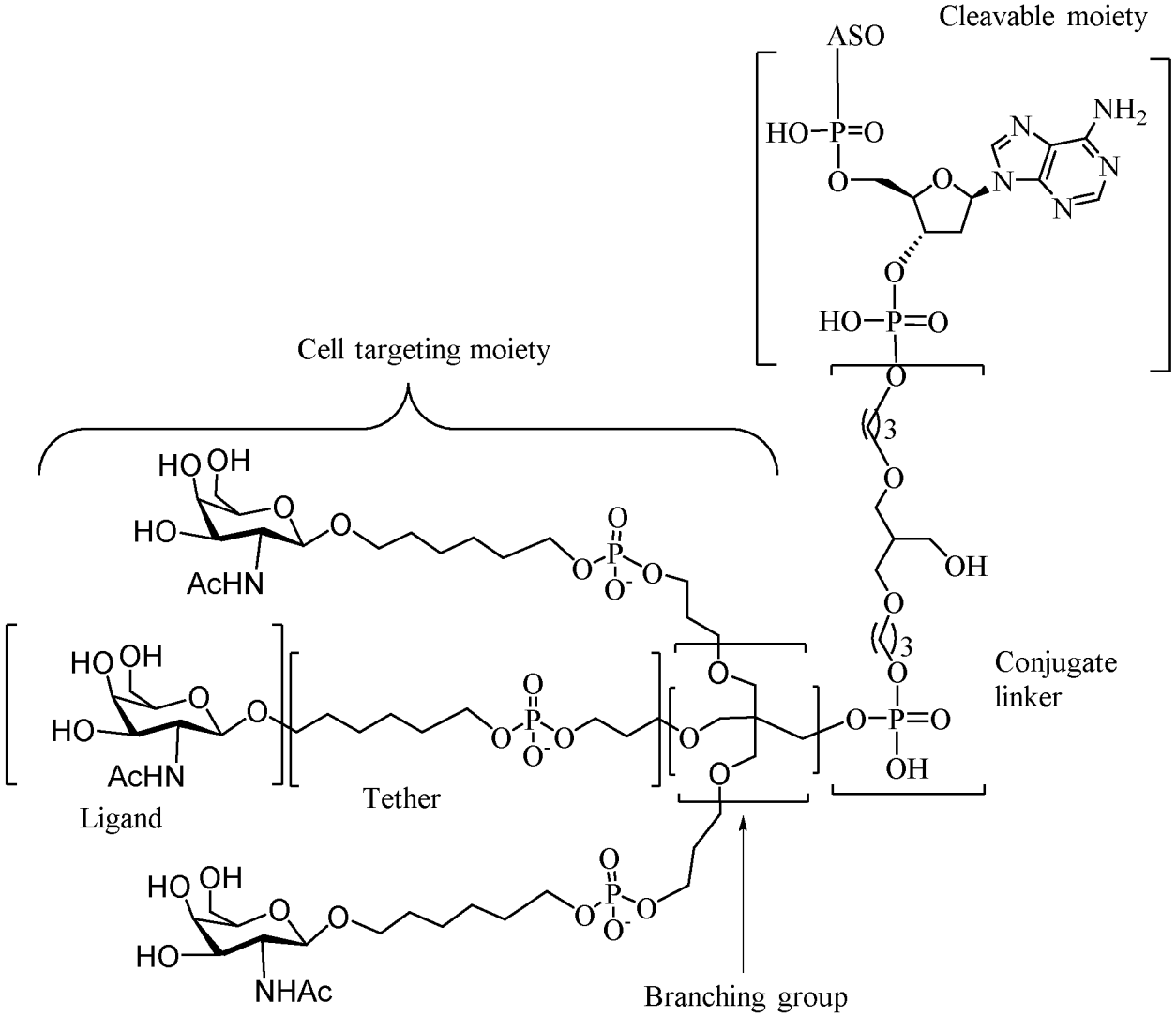


.

In certain embodiments, a conjugated antisense compound has a structure selected from among the following:


Cell targeting moiety
Cleavable moiety
Ligand
Tether
Branching group
ASO

In certain embodiments, a conjugated antisense compound has a structure selected from among the following:



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, 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.

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.

Cells may be treated with antisense oligonucleotides when the cells reach approximately 60-80% confluency in culture.

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

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

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

Yet another technique used to introduce antisense oligonucleotides into cultured cells includes free uptake of the oligonucleotides by the cells.

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

The concentration of antisense oligonucleotide used varies from cell line to cell line. Methods to determine the optimal antisense oligonucleotide concentration for a particular cell line are well known in the art. Antisense oligonucleotides are typically used at concentrations ranging from 1 nM to 300 nM when transfected with LIPOFECTAMINE. Antisense oligonucleotides are used at higher concentrations ranging from 625 to 20,000 nM when transfected using electroporation.

RNA Isolation

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

Certain Indications

Certain embodiments provided herein relate to methods of treating, preventing, or ameliorating a disease associated with dysregulation of the complement alternative pathway in a subject by administration of a CFB specific inhibitor, such as an antisense compound targeted to CFB.

Examples of renal diseases associated with dysregulation of the complement alternative pathway treatable, preventable, and/or ameliorable with the methods provided herein include C3 glomerulopathy, atypical hemolytic uremic syndrome (aHUS), dense deposit disease (DDD; also known as MPGN Type II or C3Neph), and CFHR5 nephropathy.

Additional renal diseases associated with dysregulation of the complement alternative pathway treatable, preventable, and/or ameliorable with the methods provided herein include IgA nephropathy; mesangiocapillary (membranoproliferative) glomerulonephritis (MPGN); autoimmune disorders including lupus nephritis and systemic lupus erythematosus (SLE); infection-induced glomerulonephritis (also known as Postinfectious glomerulonephritis); and renal ischemia-reperfusion injury, for example post-transplant renal ischemia-reperfusion injury.

Examples of non-renal disorders associated with dysregulation of the complement alternative pathway treatable and/or preventable with the methods provided herein include ocular diseases such as macular degeneration, for example age-related macular degeneration (AMD), including wet AMD and dry AMD, such as Geographic Atrophy; neuromyelitis optica; corneal disease, such as corneal inflammation; autoimmune uveitis; and diabetic retinopathy. It has been reported that complement system is involved in ocular diseases. Jha P, et al., *Mol Immunol* (2007) 44(16): 3901-3908. Additional examples of non-renal disorders associated with dysregulation of the complement alternative pathway treatable and/or preventable with the methods provided herein include ANCA-assocaited vasculitis, antiphospholipid syndrome (also known as antiphospholipid antibody syndrome (APS)), asthma, rheumatoid arthritis, Myasthenia Gravis, and multiple sclerosis.

Certain embodiments provided herein relate to methods of treating, preventing, or ameliorating a renal disease associated with dysregulation of the complement alternative pathway in a subject by administration of a CFB specific inhibitor, such as an antisense compound targeted to CFB. In certain aspects, the renal disease is lupus nephritis, systemic lupus erythematosus (SLE), dense deposit disease (DDD), C3 glomerulonephritis (C3GN), CFHR5 nephropathy, or atypical hemolytic uremic syndrome (aHUS), or any combination thereof.

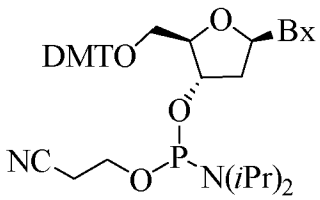
Certain embodiments provided herein relate to methods of treating, preventing, or ameliorating macular degeneration, such as age-related macular degeneration (AMD), in a subject by administration of a CFB specific inhibitor, such as an antisense compound targeted to CFB. In certain aspects, the AMD is wet AMD or dry AMD. In certain aspects, dry AMD can be Geographic Atrophy. Studies have demonstrated the association of complement alternative pathway dysregulation and AMD. Complement components are common constituents of ocular drusen, the extracellular material that accumulates in the macula of AMD patients. Furthermore, it has been reported that CFH and CFB variants account for nearly 75% of AMD cases in northern Europe and North America. It has also been found that a specific CFB polymorphism confers protection against AMD. Patel, N. et al., *Eye* (2008) 22(6):768-76. Additionally, CFB homozygous null mice have lower complement pathway activity, exhibit smaller ocular lesions, and choroidal neovascularization (CNV) after laser photocoagulation. Rohrer, B. et al., *Invest Ophthalmol Vis Sci.* (2009) 50(7):3056-64. Furthermore, CFB siRNA treatment protects mice from laser induced CNV. Bora, NS et al., *J Immunol.* (2006) 177(3):1872-8. Studies have also shown that the kidney and eye share developmental pathways and structural features including basement membrane collagen IV protomer composition and vascularity. Savige et al., *J Am Soc Nephrol.* (2011) 22(8):1403-15. There is evidence that the complement pathway is involved in renal and ocular diseases. For instance, inherited complement regulatory protein deficiency causes predisposition to atypical hemolytic uremic syndrome and AMD. Richards A et al., *Adv Immunol.* (2007) 96:141-77. Additionally, chronic kidney disease has been associated with AMD. Nitsch, D.

et al., *Ophthalmic Epidemiol.* (2009) 16(3):181-6; Choi, J. et al, *Ophthalmic Epidemiol.* (2011) 18(6):259-63. Dense deposit disease (DDD), a kidney disease associated with dysregulated complement alternative pathway, is characterized by acute nephritic syndrome and ocular drusen. Cruz and Smith, *GeneReviews* (2007) Jul 20. Moreover, mice harboring genetic deletion of a component of the complement alternative pathway have coexisting renal and ocular disease phenotypes. It has been reported that CFH homozygous null mice develop DDD and present retinal abnormalities and visual dysfunction. Pickering et al., *Nat Genet.* (2002) 31(4):424-8. Mouse models of renal diseases associated with dysregulation of the complement alternative pathway are also accepted as models of AMD. Pennesi ME et al., *Mol Aspects Med* (2012) 33:487-509. CFH null mice, for example, are an accepted model for renal diseases, such as DDD, and AMD. Furthermore, it has been reported that AMD is associated with the systemic source of complement factors, which accumulate locally in the eye to drive alternative pathway complement activation. Loyet et al., *Invest Ophthalmol Vis Sci.* (2012) 53(10):6628-37.

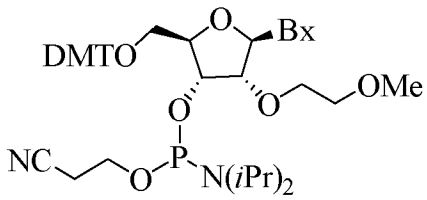
EXAMPLES

The following examples illustrate certain embodiments of the present disclosure and are not limiting. Moreover, where specific embodiments are provided, the inventors have contemplated generic application of those specific embodiments. For example, disclosure of an oligonucleotide having a particular motif provides reasonable support for additional oligonucleotides having the same or similar motif. And, for example, where a particular high-affinity modification appears at a particular position, other high-affinity modifications at the same position are considered suitable, unless otherwise indicated.

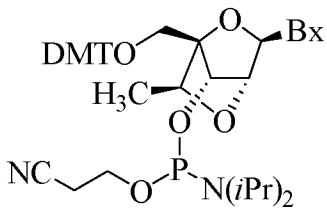
Example 1: General Method for the Preparation of Phosphoramidites, Compounds 1, 1a and 2



1



1a

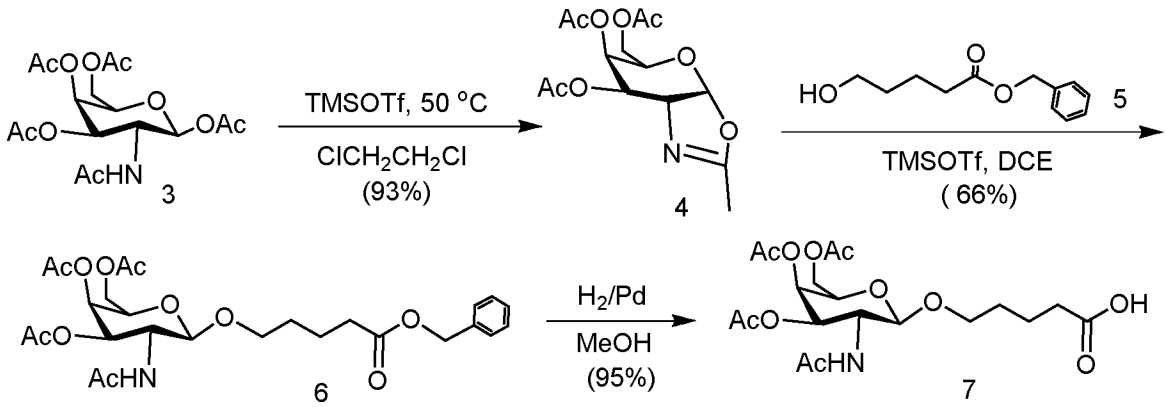


2

Bx is a heterocyclic base;

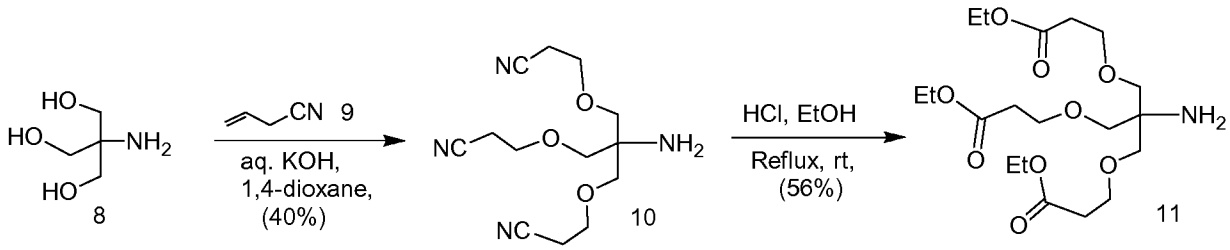
Compounds 1, 1a and 2 were prepared as per the procedures well known in the art as described in the specification herein (see Seth et al., Bioorg. Med. Chem., 2011, 21(4), 1122-1125, J. Org. Chem., 2010, 75(5), 1569-1581, Nucleic Acids Symposium Series, 2008, 52(1), 553-554); and also see published PCT International Applications (WO 2011/115818, WO 2010/077578, WO2010/036698, WO2009/143369, WO 2009/006478, and WO 2007/090071), and US patent 7,569,686).

Example 2: Preparation of Compound 7



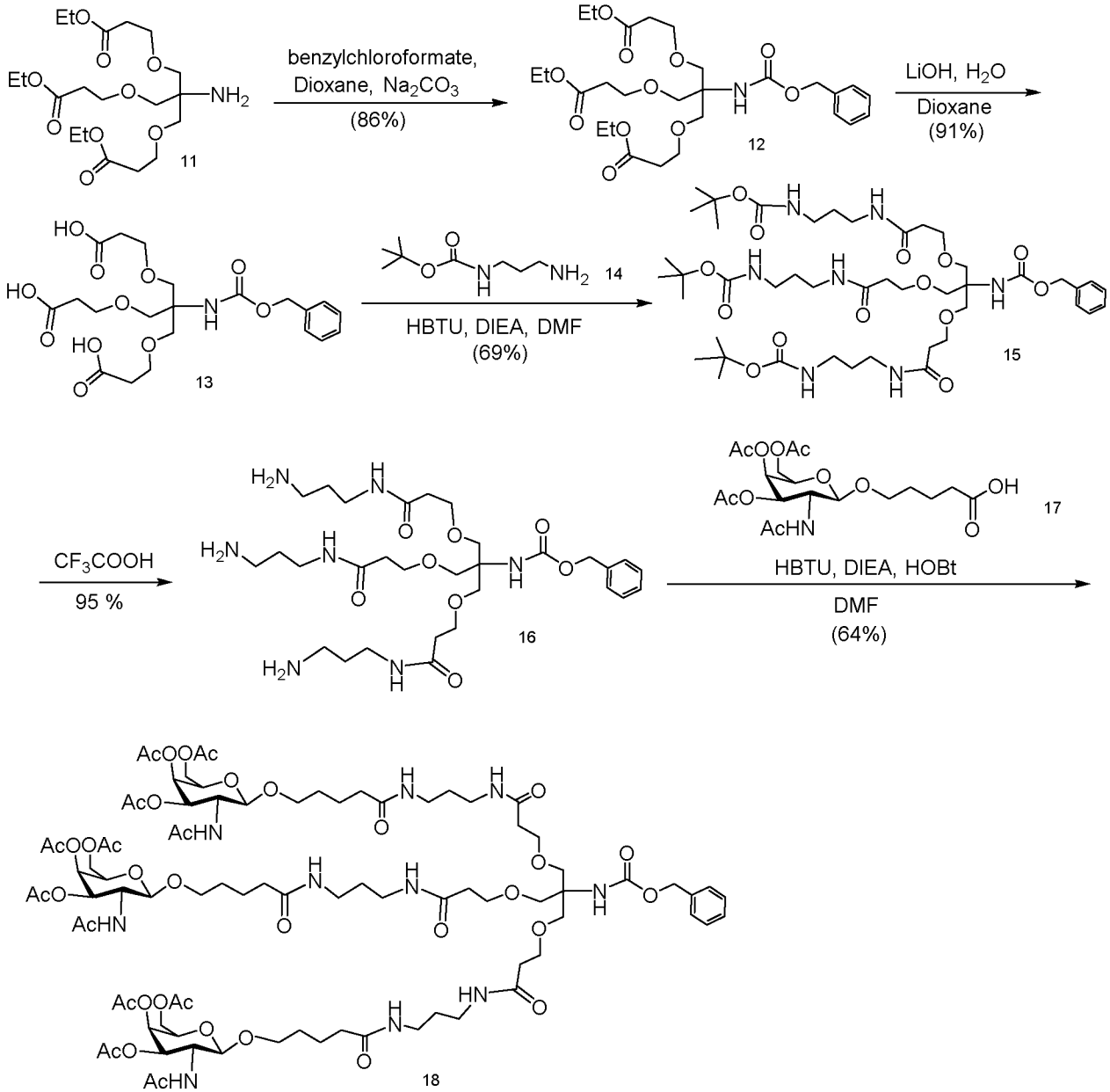
Compounds 3 (2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-Dgalactopyranose or galactosamine pentaacetate) is commercially available. Compound 5 was prepared according to published procedures (Weber *et al., J. Med. Chem.*, 1991, *34*, 2692).

Example 3: Preparation of Compound 11



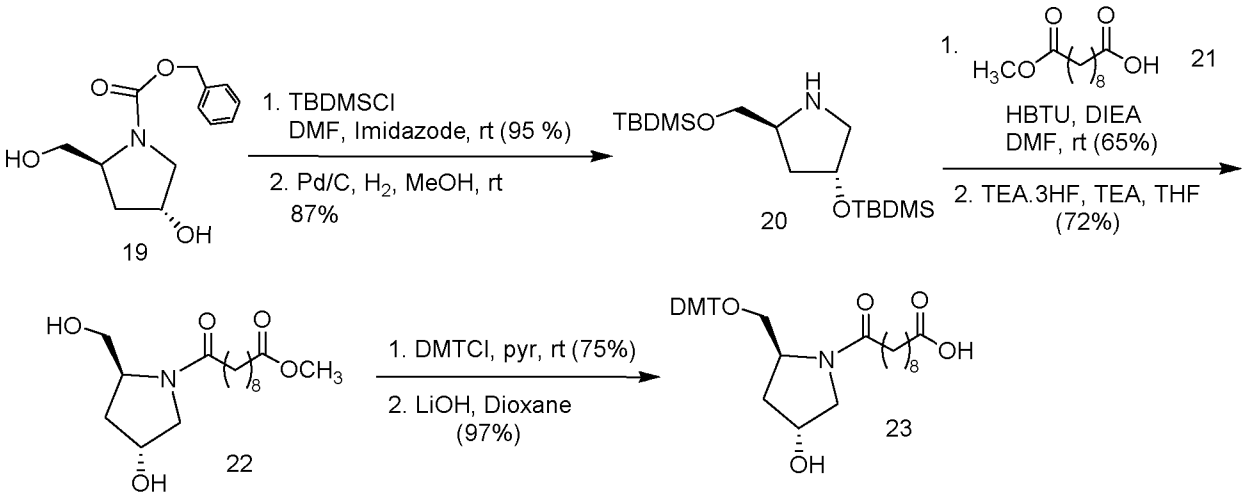
Compounds 8 and 9 are commercially available.

Example 4: Preparation of Compound 18



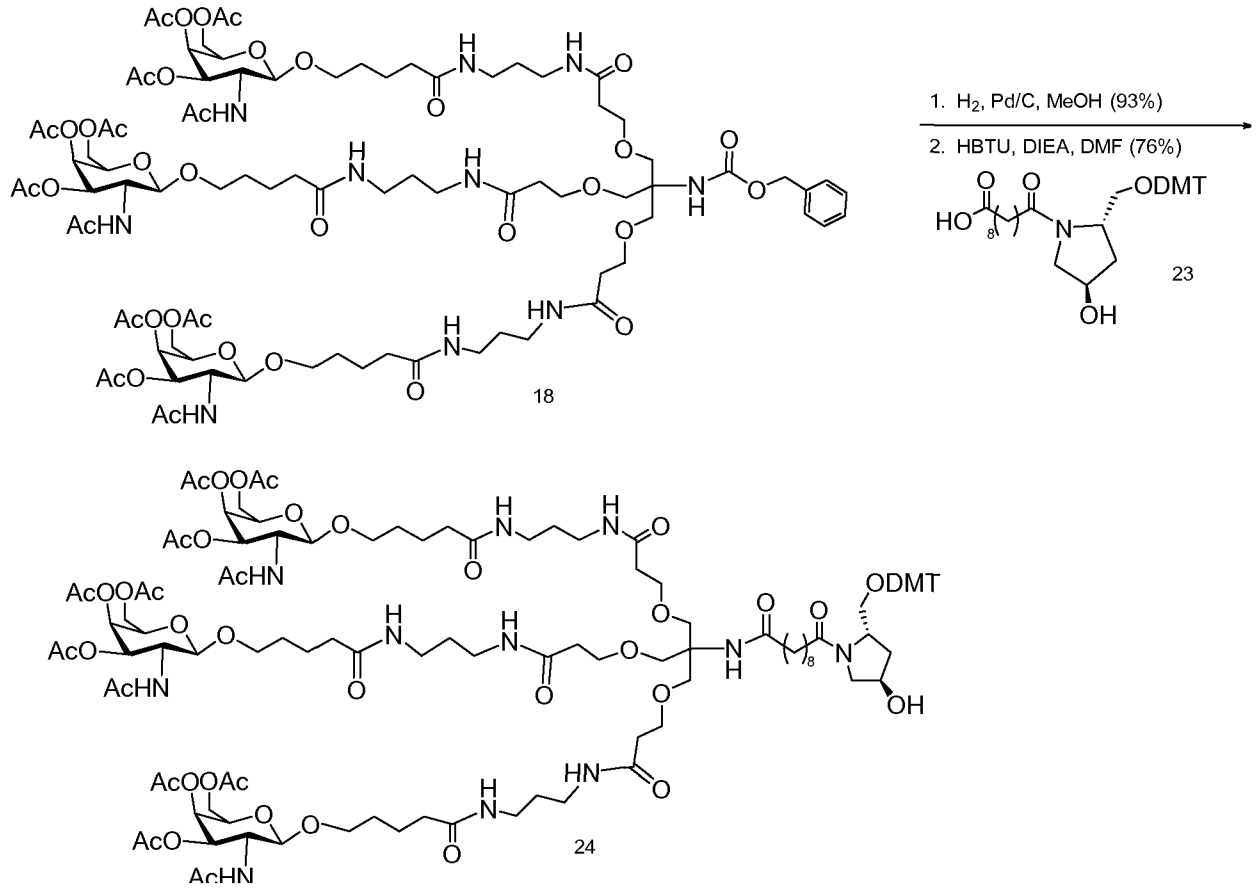
Compound 11 was prepared as per the procedures illustrated in Example 3. Compound 14 is commercially available. Compound 17 was prepared using similar procedures reported by Rensen *et al., J. Med. Chem.,* 2004, *47,* 5798-5808.

Example 5: Preparation of Compound 23



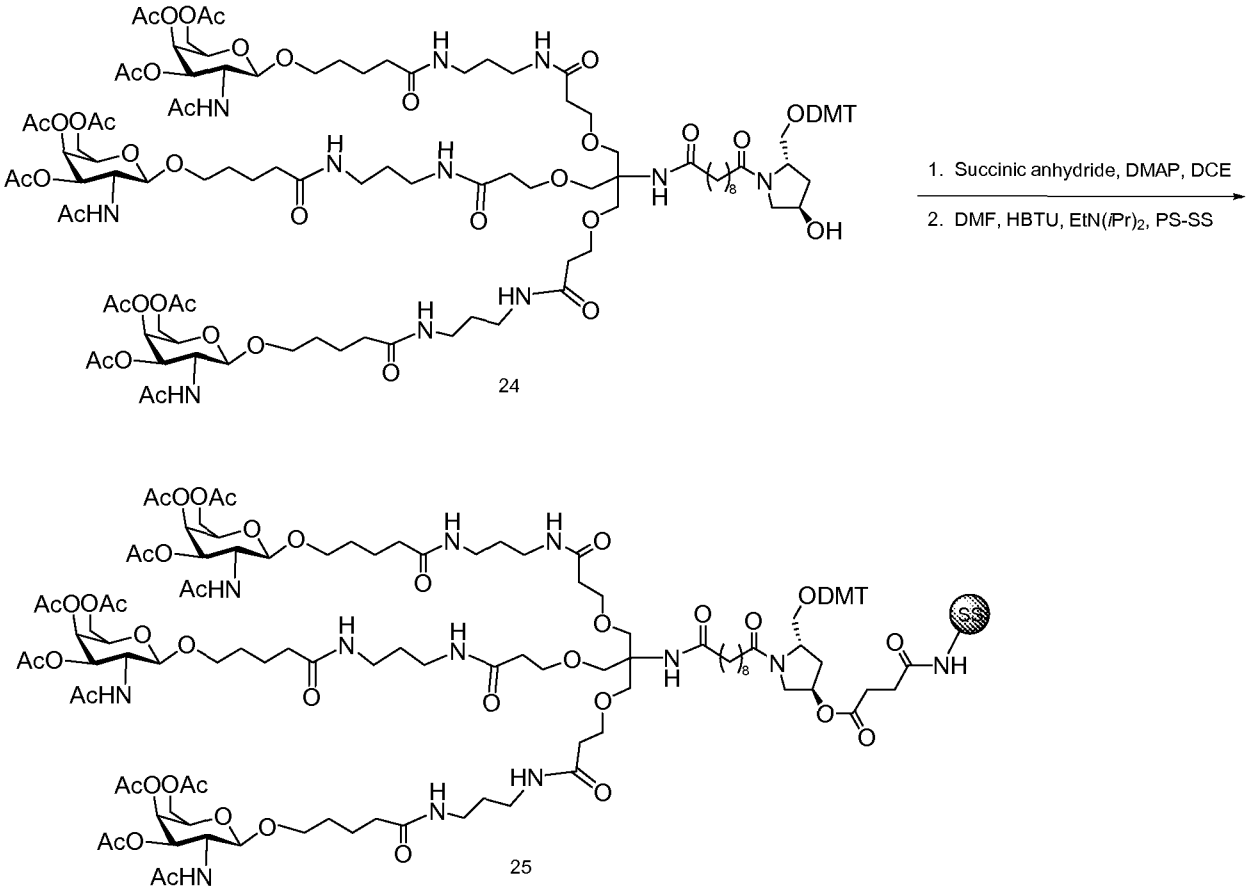
Compounds 19 and 21 are commercially available.

Example 6: Preparation of Compound 24



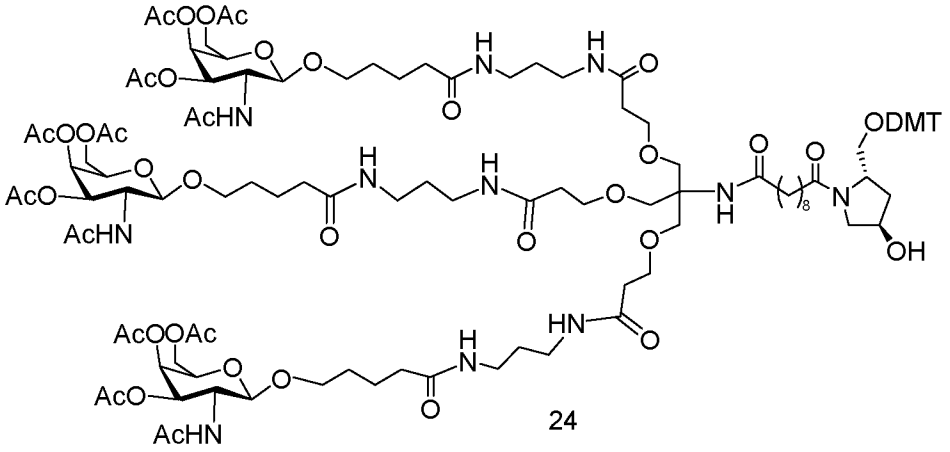
Compounds 18 and 23 were prepared as per the procedures illustrated in Examples 4 and 5.

Example 7: Preparation of Compound 25

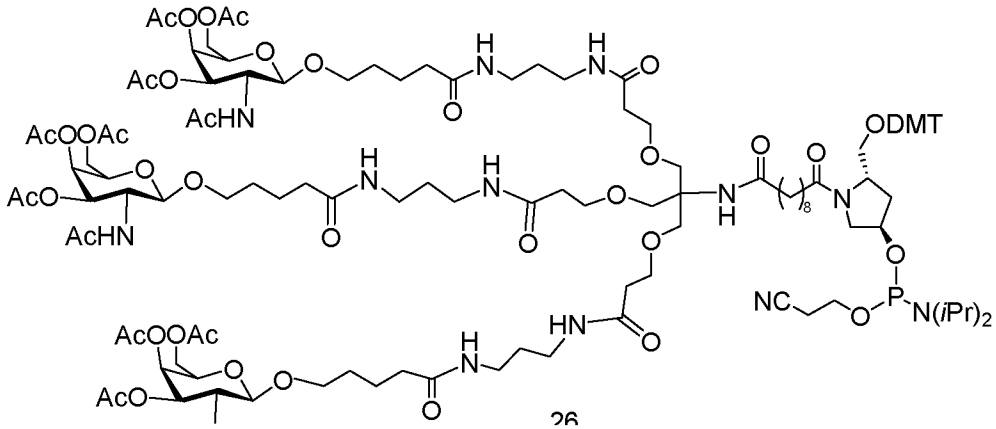


Compound 24 was prepared as per the procedures illustrated in Example 6.

Example 8: Preparation of Compound 26

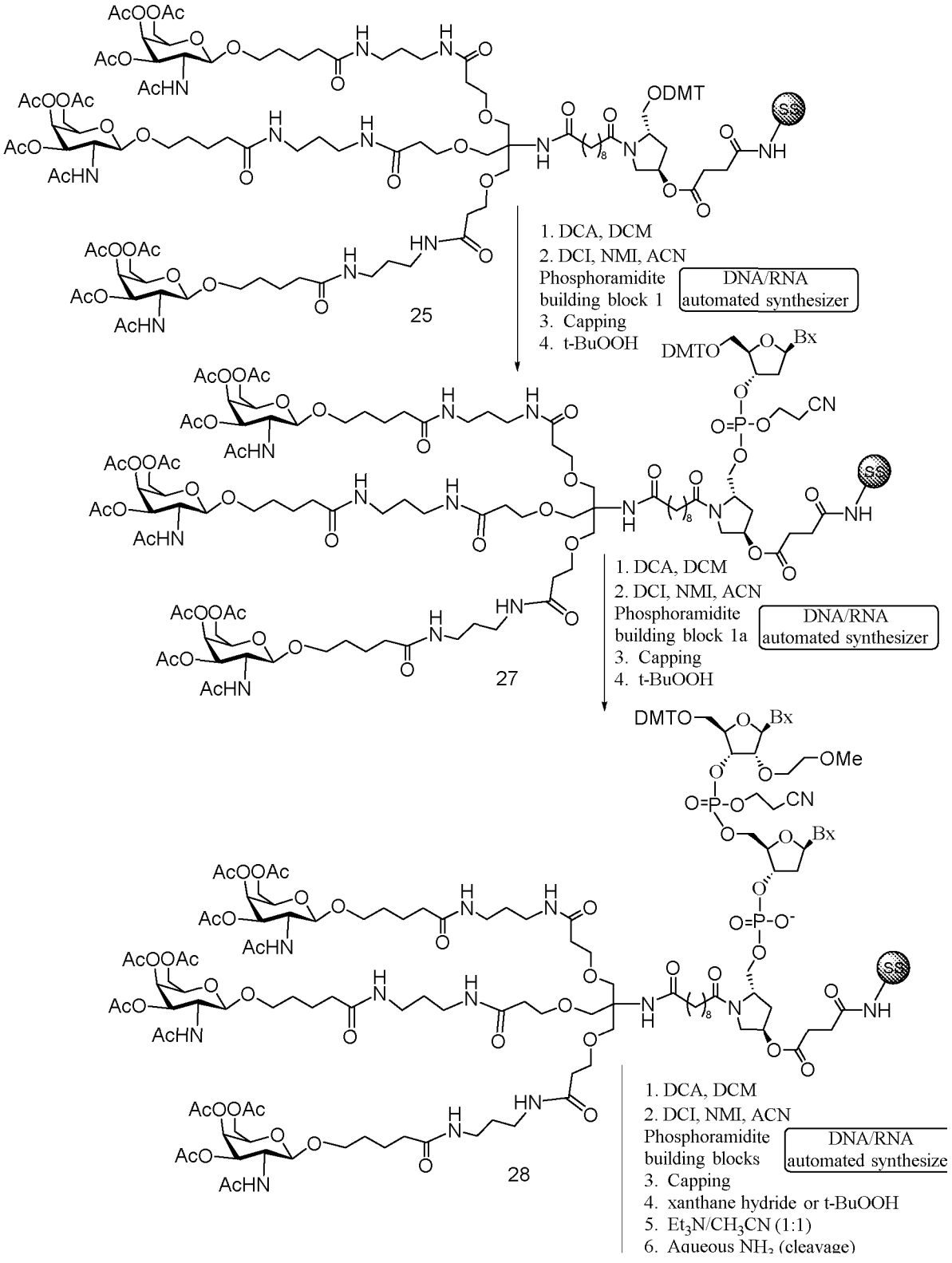


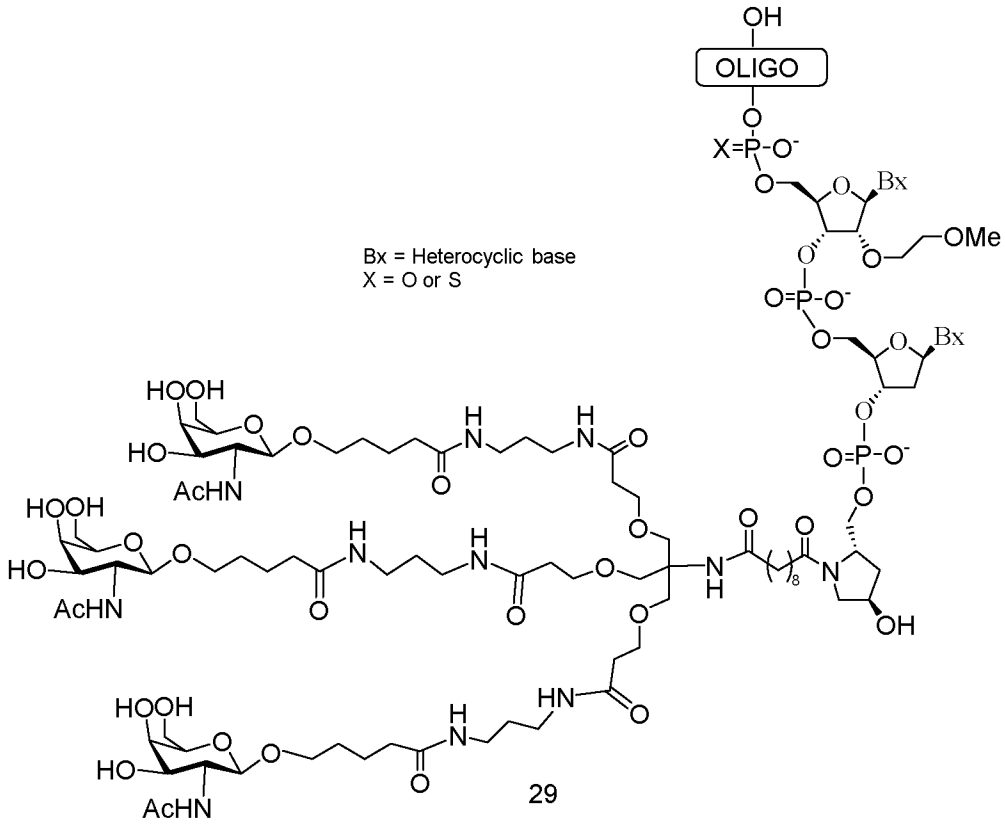
Phosphitylation



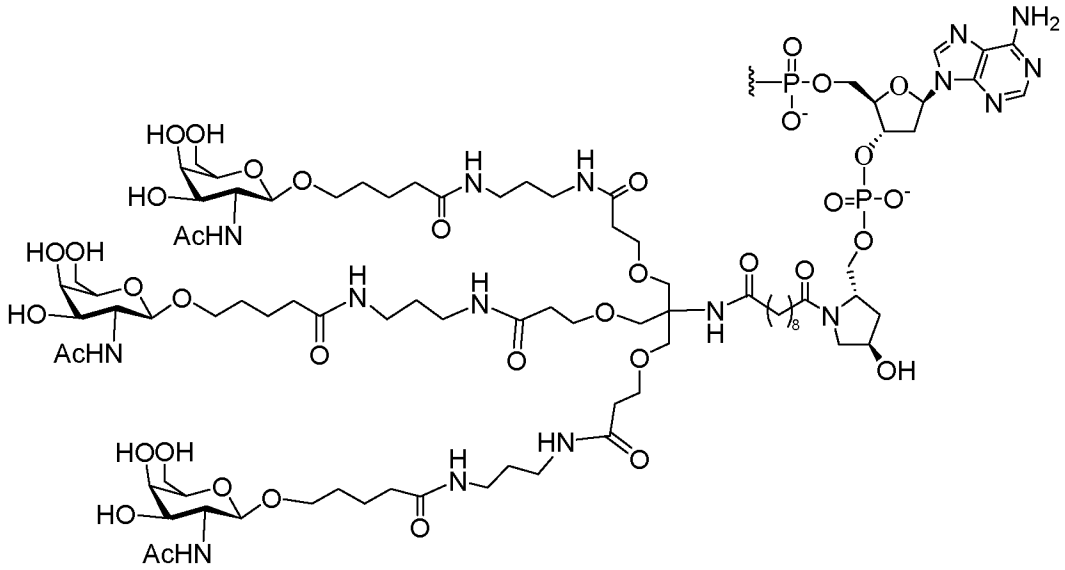
Compound 24 is prepared as per the procedures illustrated in Example 6.

Example 9: General preparation of conjugated ASOs comprising GalNAc$_3$-1 at the 3' terminus, Compound 29

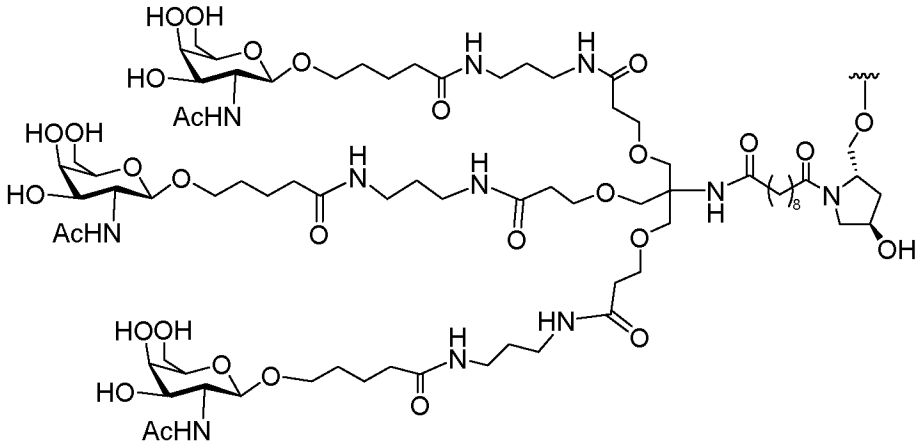




Wherein the protected **GalNAc$_3$-1** has the structure:

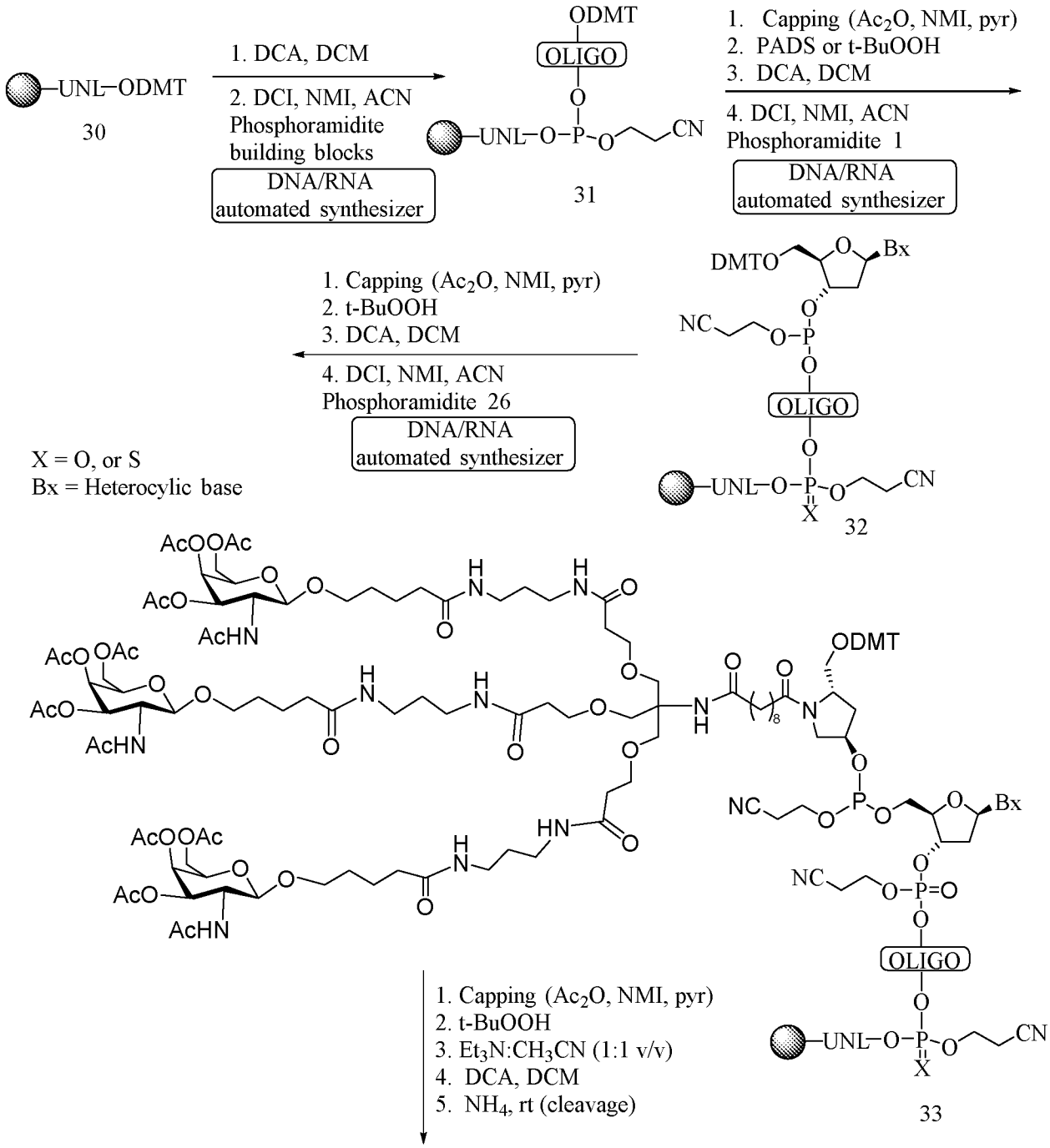


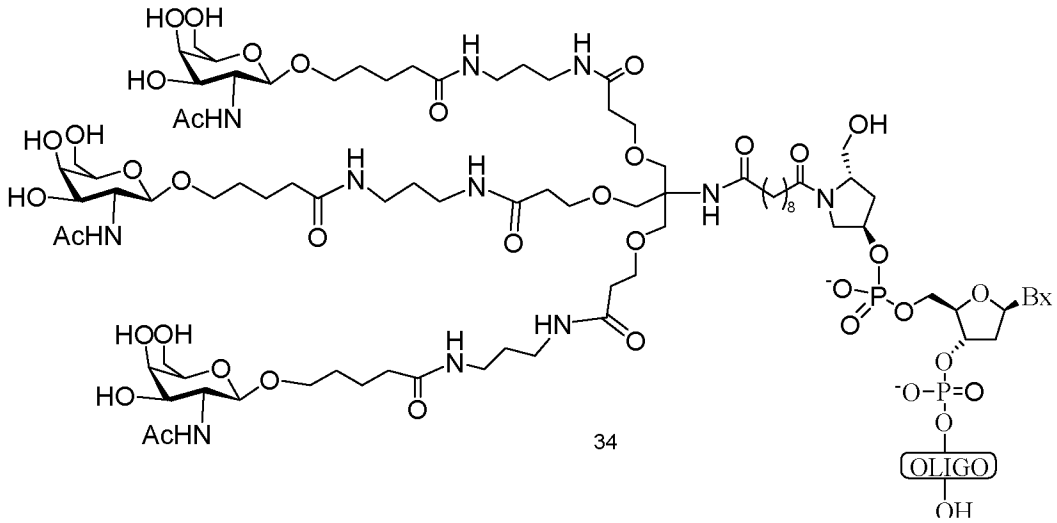
The GalNAc$_3$ cluster portion of the conjugate group GalNAc$_3$-1 (GalNAc$_3$-1$_a$) can be combined with any cleavable moiety to provide a variety of conjugate groups. Wherein GalNAc$_3$-1$_a$ has the formula:



The solid support bound protected **GalNAc$_3$-1**, Compound 25, was prepared as per the procedures illustrated in Example 7. Oligomeric Compound 29 comprising **GalNAc$_3$-1** at the 3' terminus was prepared using standard procedures in automated DNA/RNA synthesis (see Dupouy *et al., Angew. Chem. Int. Ed.,* 2006, *45,* 3623-3627). Phosphoramidite building blocks, Compounds 1 and 1a were prepared as per the procedures illustrated in Example 1. The phosphoramidites illustrated are meant to be representative and not intended to be limiting as other phosphoramidite building blocks can be used to prepare oligomeric compounds having a predetermined sequence and composition. The order and quantity of phosphoramidites added to the solid support can be adjusted to prepare gapped oligomeric compounds as described herein. Such gapped oligomeric compounds can have predetermined composition and base sequence as dictated by any given target.

Example 10: General preparation conjugated ASOs comprising GalNAc$_3$-1 at the 5' terminus, Compound 34

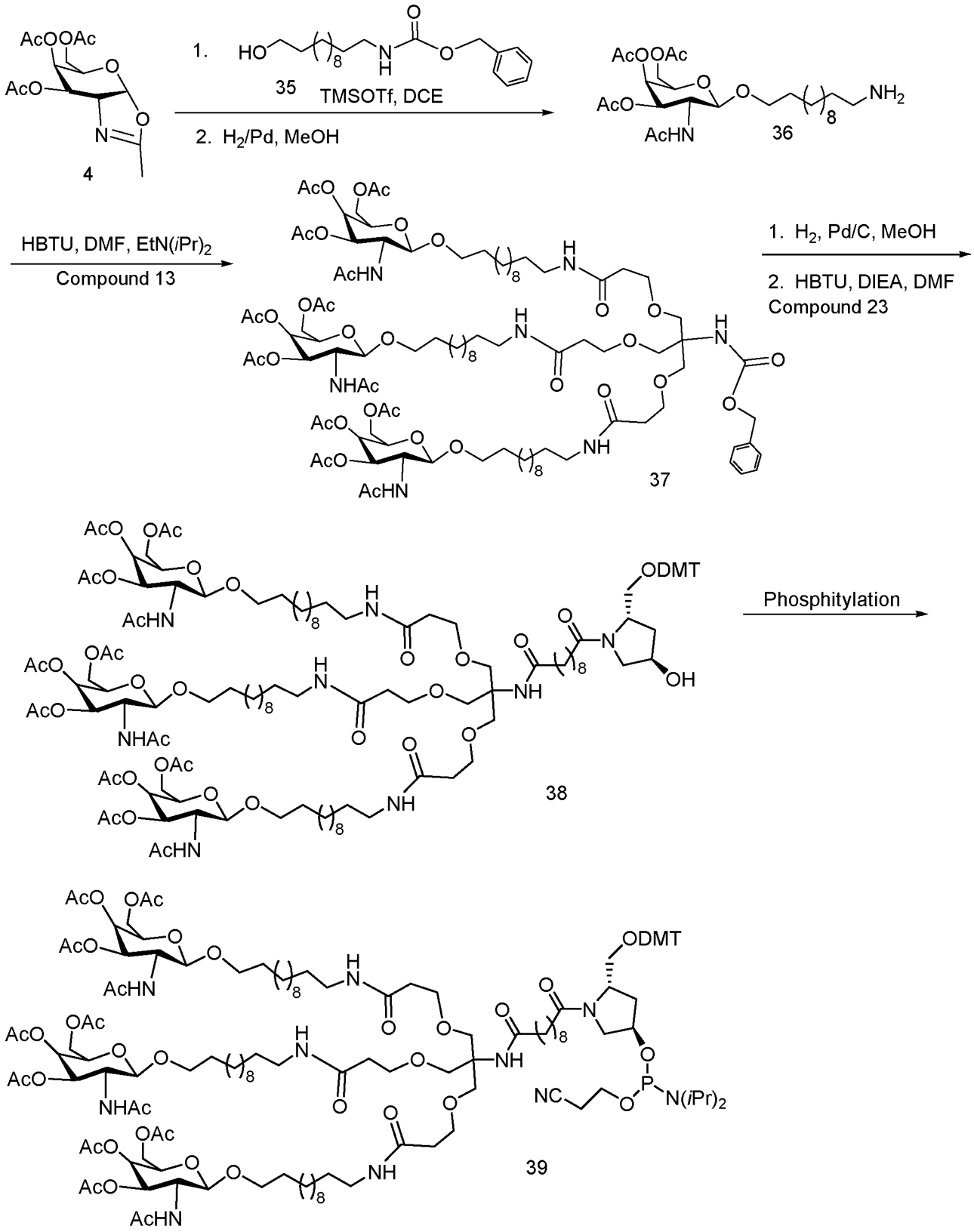




34

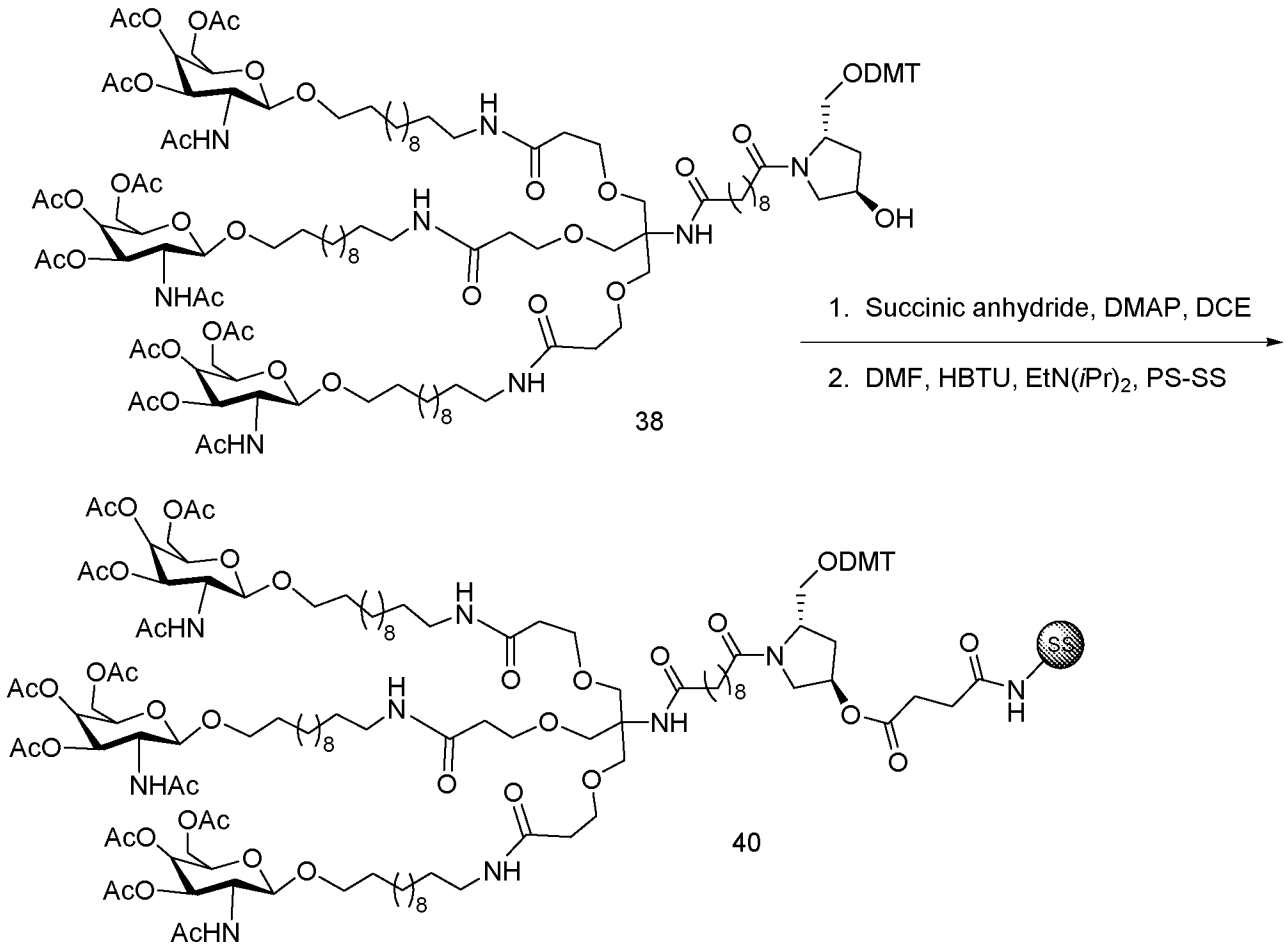
The Unylinker™ 30 is commercially available. Oligomeric Compound 34 comprising a **GalNAc$_3$-1** cluster at the 5' terminus is prepared using standard procedures in automated DNA/RNA synthesis (see Dupouy *et al., Angew. Chem. Int. Ed.,* 2006, *45,* 3623-3627). Phosphoramidite building blocks, Compounds 1 and 1a were prepared as per the procedures illustrated in Example 1. The phosphoramidites illustrated are meant to be representative and not intended to be limiting as other phosphoramidite building blocks can be used to prepare an oligomeric compound having a predetermined sequence and composition. The order and quantity of phosphoramidites added to the solid support can be adjusted to prepare gapped oligomeric compounds as described herein. Such gapped oligomeric compounds can have predetermined composition and base sequence as dictated by any given target.

Example 11: Preparation of Compound 39


1.
35
TMSOTf, DCE
2. H2/Pd, MeOH
4
36
HBTU, DMF, EtN(iPr)2
Compound 13
37
1. H2, Pd/C, MeOH
2. HBTU, DIEA, DMF
Compound 23
38
Phosphitylation
39

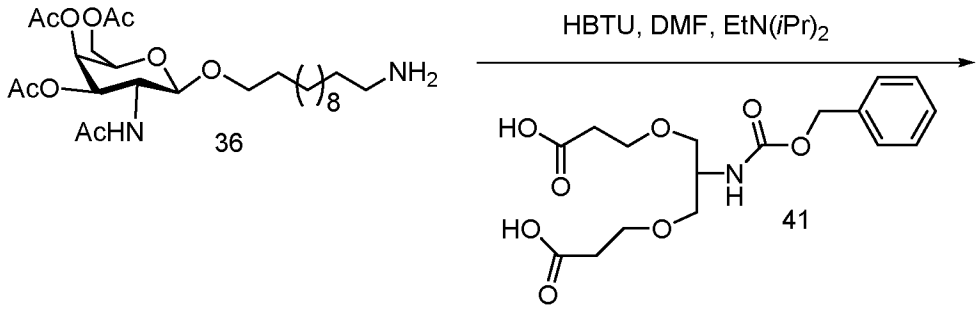
Compounds 4, 13 and 23 were prepared as per the procedures illustrated in Examples 2, 4, and 5. Compound 35 is prepared using similar procedures published in Rouchaud *et al., Eur. J. Org. Chem.,* 2011, *12*, 2346-2353.

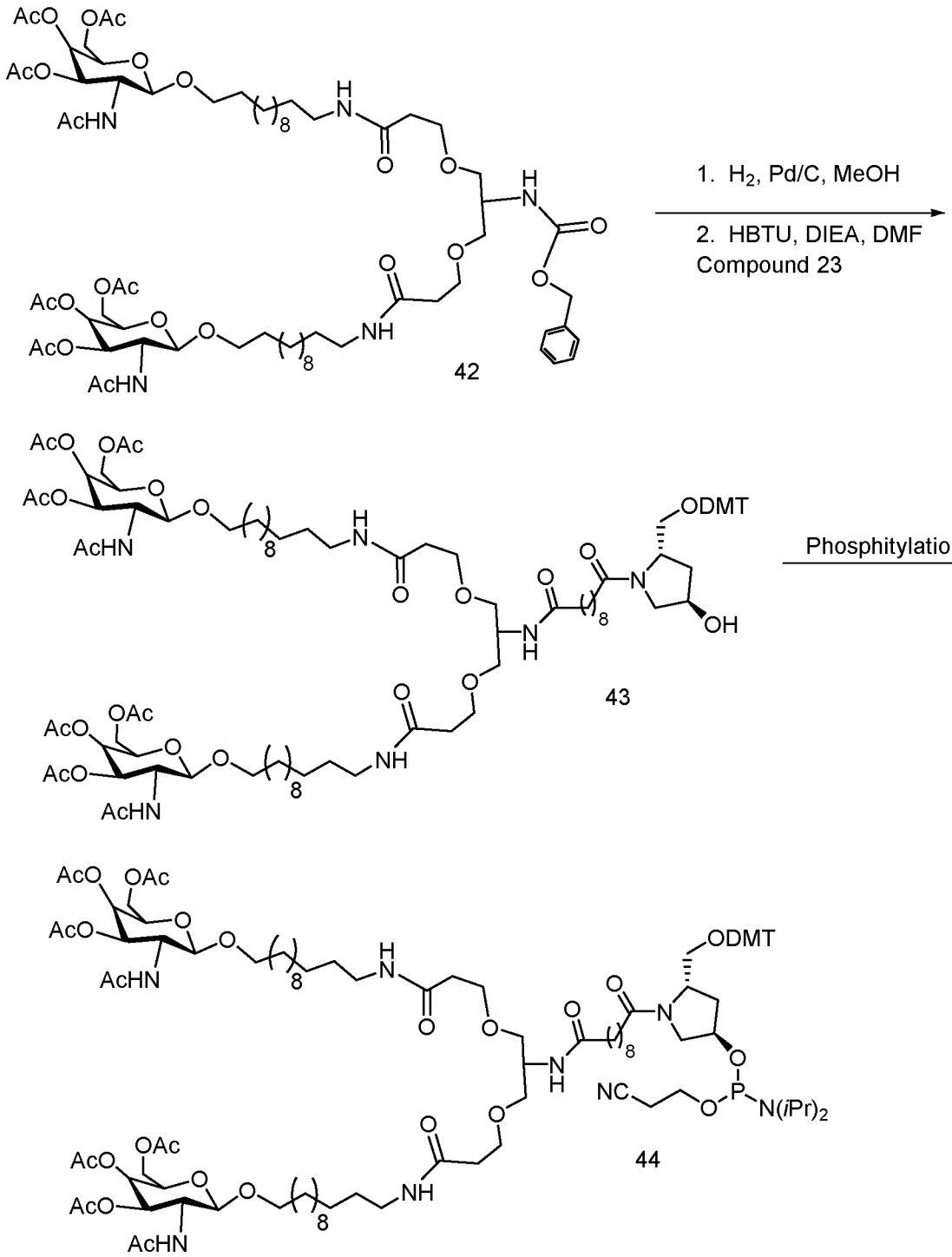
Example 12: Preparation of Compound 40



Compound 38 is prepared as per the procedures illustrated in Example 11.

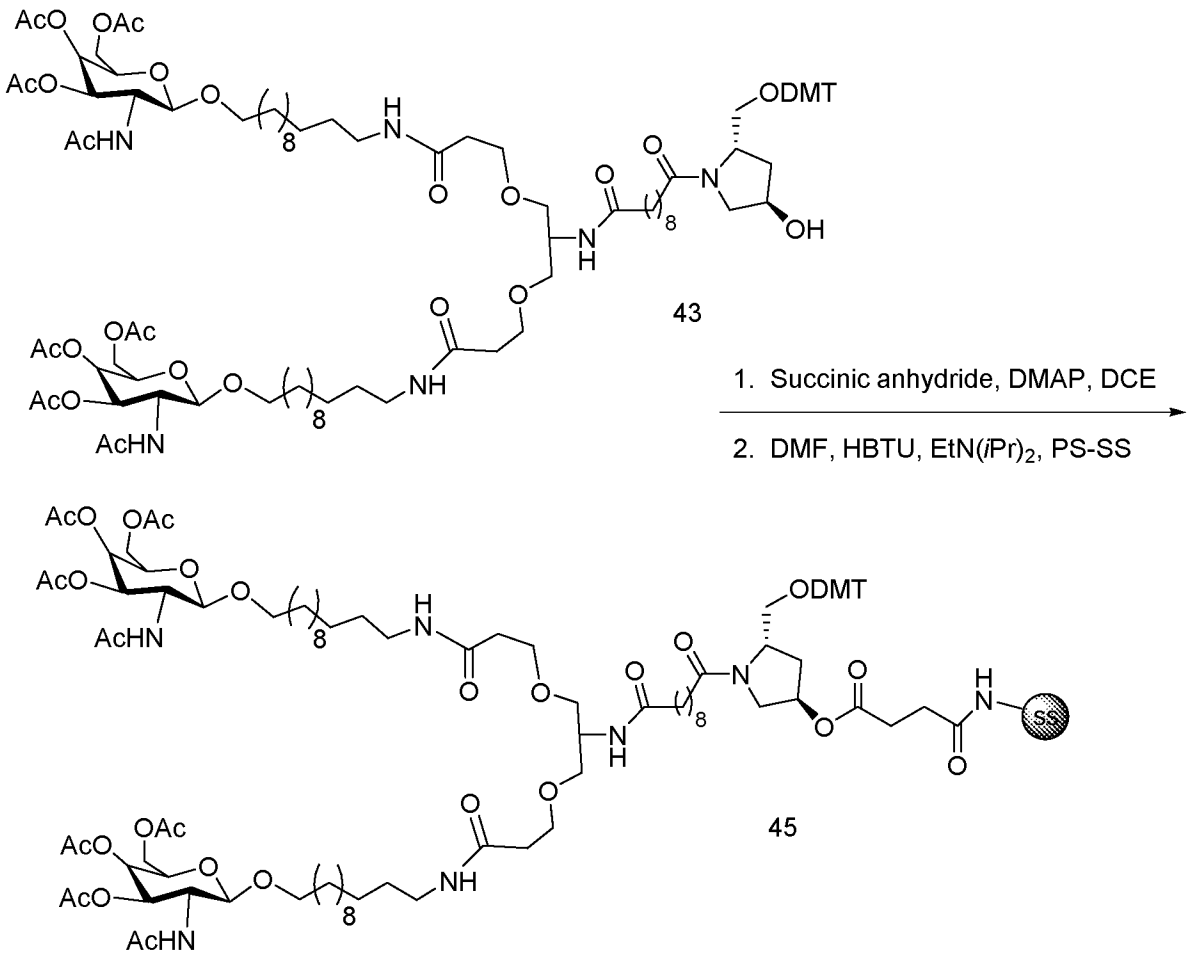
Example 13: Preparation of Compound 44





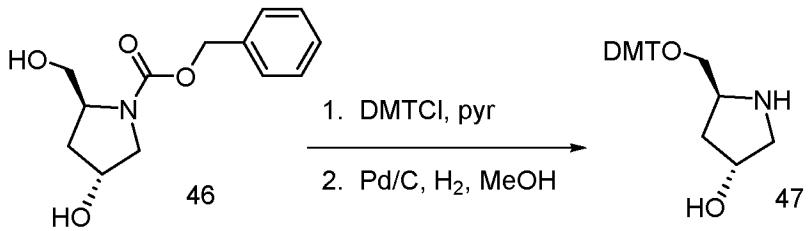
Compounds 23 and 36 are prepared as per the procedures illustrated in Examples 5 and 11. Compound 41 is prepared using similar procedures published in WO 2009082607.

Example 14: Preparation of Compound 45



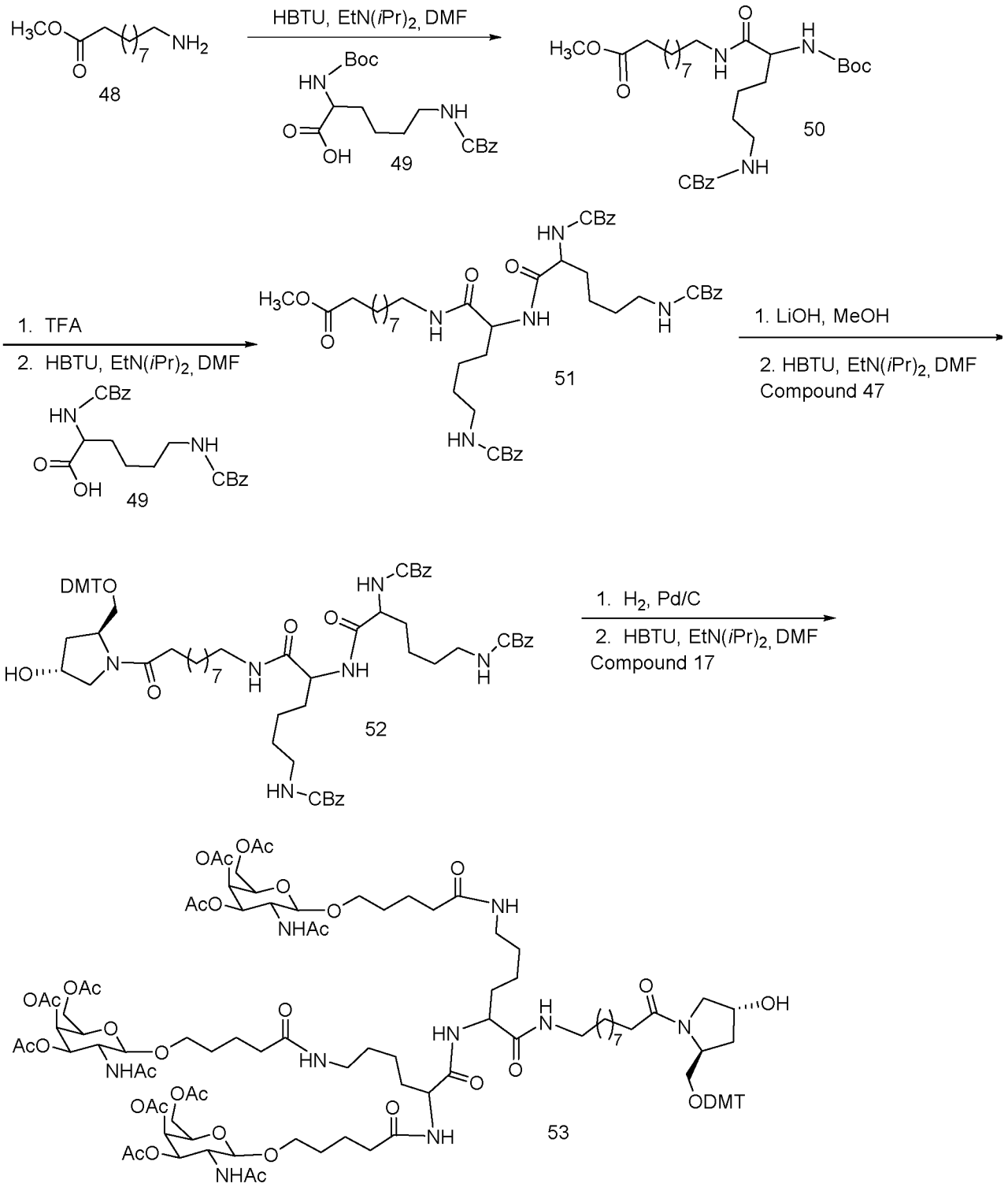
Compound 43 is prepared as per the procedures illustrated in Example 13.

Example 15: Preparation of Compound 47



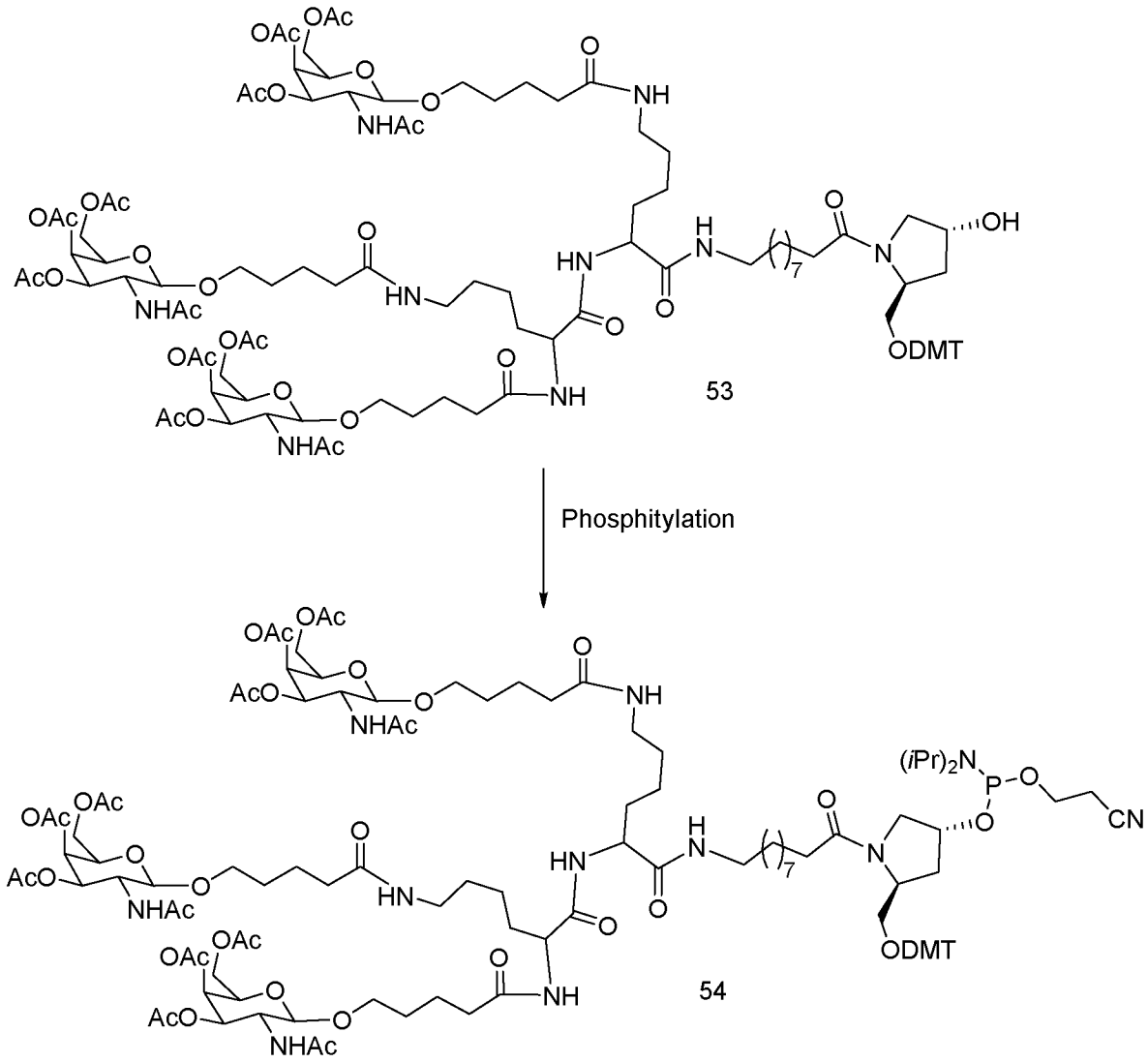
Compound 46 is commercially available.

Example 16: Preparation of Compound 53



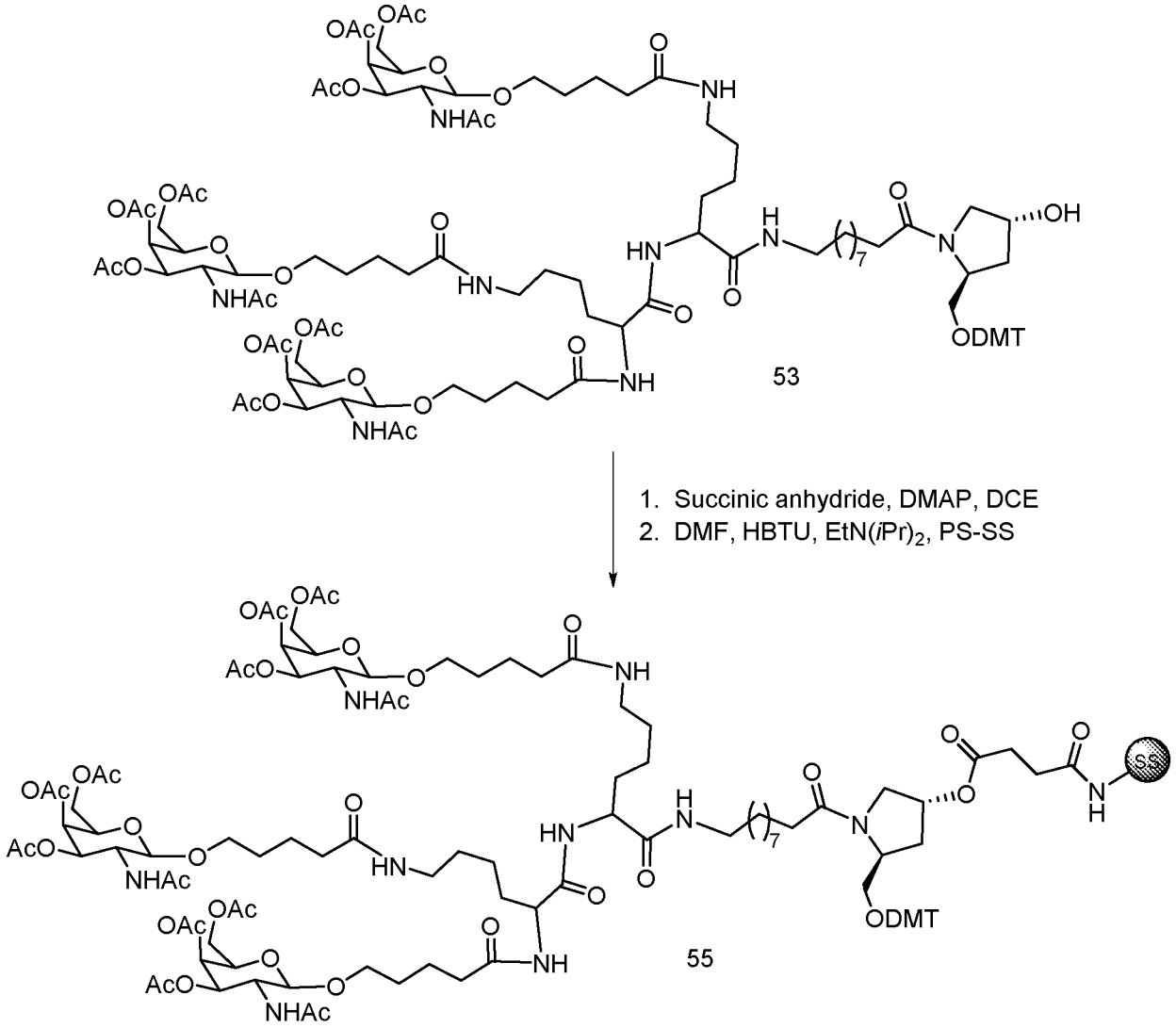
Compounds 48 and 49 are commercially available. Compounds 17 and 47 are prepared as per the procedures illustrated in Examples 4 and 15.

Example 17: Preparation of Compound 54



Compound 53 is prepared as per the procedures illustrated in Example 16.

Example 18: Preparation of Compound 55



Compound 53 is prepared as per the procedures illustrated in Example 16.

Example 19: General method for the preparation of conjugated ASOs comprising GalNAc$_3$-1 at the 3' position via solid phase techniques (preparation of ISIS 647535, 647536 and 651900)

Unless otherwise stated, all reagents and solutions used for the synthesis of oligomeric compounds are purchased from commercial sources. Standard phosphoramidite building blocks and solid support are used for incorporation nucleoside residues which include for example T, A, G, and ^{m}C residues. A 0.1 M solution of phosphoramidite in anhydrous acetonitrile was used for β-D-2'-deoxyribonucleoside and 2'-MOE.

The ASO syntheses were performed on ABI 394 synthesizer (1-2 μmol scale) or on GE Healthcare Bioscience ÄKTA oligopilot synthesizer (40-200 μmol scale) by the phosphoramidite coupling method on an **GalNAc$_3$-1** loaded VIMAD solid support (110 μmol/g, Guzaev *et al.*, 2003)

packed in the column. For the coupling step, the phosphoramidites were delivered 4 fold excess over the loading on the solid support and phosphoramidite condensation was carried out for 10 min. All other steps followed standard protocols supplied by the manufacturer. A solution of 6% dichloroacetic acid in toluene was used for removing dimethoxytrityl (DMT) group from 5'-hydroxyl group of the nucleotide. 4,5-Dicyanoimidazole (0.7 M) in anhydrous CH_3CN was used as activator during coupling step. Phosphorothioate linkages were introduced by sulfurization with 0.1 M solution of xanthane hydride in 1:1 pyridine/CH_3CN for a contact time of 3 minutes. A solution of 20% *tert*-butylhydroperoxide in CH_3CN containing 6% water was used as an oxidizing agent to provide phosphodiester internucleoside linkages with a contact time of 12 minutes.

After the desired sequence was assembled, the cyanoethyl phosphate protecting groups were deprotected using a 1:1 (v/v) mixture of triethylamine and acetonitrile with a contact time of 45 minutes. The solid-support bound ASOs were suspended in aqueous ammonia (28-30 wt %) and heated at 55 °C for 6 h.

The unbound ASOs were then filtered and the ammonia was boiled off. The residue was purified by high pressure liquid chromatography on a strong anion exchange column (GE Healthcare Bioscience, Source 30Q, 30 μm, 2.54 x 8 cm, A = 100 mM ammonium acetate in 30% aqueous CH_3CN, B = 1.5 M NaBr in A, 0-40% of B in 60 min, flow 14 mL min-1, λ = 260 nm). The residue was desalted by HPLC on a reverse phase column to yield the desired ASOs in an isolated yield of 15-30% based on the initial loading on the solid support. The ASOs were characterized by ion-pair-HPLC coupled MS analysis with Agilent 1100 MSD system.

Antisense oligonucleotides not comprising a conjugate were synthesized using standard oligonucleotide synthesis procedures well known in the art.

Using these methods, three separate antisense compounds targeting ApoC III were prepared. As summarized in Table 17, below, each of the three antisense compounds targeting ApoC III had the same nucleobase sequence; ISIS 304801 is a 5-10-5 MOE gapmer having all phosphorothioate linkages; ISIS 647535 is the same as ISIS 304801, except that it had a **GalNAc$_3$-1** conjugated at its 3'end; and ISIS 647536 is the same as ISIS 647535 except that certain internucleoside linkages of that compound are phosphodiester linkages. As further summarized in Table 17, two separate antisense compounds targeting SRB-1 were synthesized. ISIS 440762 was a 2-10-2 cEt gapmer with all phosphorothioate internucleoside linkages; ISIS 651900 is the same as ISIS 440762, except that it included a **GalNAc$_3$-1** at its 3'-end.

Table 17

Modified ASO targeting ApoC III and SRB-1

ASO	Sequence (5' to 3')	Target	CalCd Mass	Observed Mass	SEQ ID No.
ISIS 304801	$A_{es}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{es}T_{es}T_{es}A_{es}T_{e}$	ApoC III	7165.4	7164.4	821
ISIS 647535	$A_{es}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{es}T_{es}T_{es}A_{es}T_{eo}\mathbf{A_{do}}$'-**GalNAc$_3-1_a$**	ApoC III	9239.5	9237.8	822
ISIS 647536	$A_{es}G_{eo}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{eo}T_{eo}T_{es}A_{es}T_{eo}\mathbf{A_{do}}$'-**GalNAc$_3-1_a$**	ApoC III	9142.9	9140.8	822
ISIS 440762	$T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{k}$	SRB-1	4647.0	4646.4	823
ISIS 651900	$T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{ko}\mathbf{A_{do}}$'-**GalNAc$_3-1_a$**	SRB-1	6721.1	6719.4	824

Subscripts: "e" indicates 2'-MOE modified nucleoside; "d" indicates β-D-2'-deoxyribonucleoside; "k" indicates 6'-*(S)*-CH_3 bicyclic nucleoside (e.g. cEt); "s" indicates phosphorothioate internucleoside linkages (PS); "o" indicates phosphodiester internucleoside linkages (PO); and "o'" indicates -O-P(=O)(OH)-. Superscript "m" indicates 5-methylcytosines. "**GalNAc$_3$-1**" indicates a conjugate group having the structure shown previously in Example 9. Note that **GalNAc$_3$-1** comprises a cleavable adenosine which links the ASO to remainder of the conjugate, which is designated "**GalNAc$_3$-1$_a$.**" This nomenclature is used in the above table to show the full nucleobase sequence, including the adenosine, which is part of the conjugate. Thus, in the above table, the sequences could also be listed as ending with "**GalNAc$_3$-1**" with the "**A$_{do}$**" omitted. This convention of using the subscript "a" to indicate the portion of a conjugate group lacking a cleavable nucleoside or cleavable moiety is used throughout these Examples. This portion of a conjugate group lacking the cleavable moiety is referred to herein as a "cluster" or "conjugate cluster" or "GalNAc$_3$ cluster." In certain instances it is convenient to describe a conjugate group by separately providing its cluster and its cleavable moiety.

Example 20: Dose-dependent antisense inhibition of human ApoC III in huApoC III transgenic mice

ISIS 304801 and ISIS 647535, each targeting human ApoC III and described above, were separately tested and evaluated in a dose-dependent study for their ability to inhibit human ApoC III in human ApoC III transgenic mice.

Treatment

Human ApoCIII transgenic mice were maintained on a 12-hour light/dark cycle and fed *ad libitum* Teklad lab chow. Animals were acclimated for at least 7 days in the research facility before

initiation of the experiment. ASOs were prepared in PBS and sterilized by filtering through a 0.2 micron filter. ASOs were dissolved in 0.9% PBS for injection.

Human ApoC III transgenic mice were injected intraperitoneally once a week for two weeks with ISIS 304801 or 647535 at 0.08, 0.25. 0.75, 2.25 or 6.75 μmol/kg or with PBS as a control. Each treatment group consisted of 4 animals. Forty-eight hours after the administration of the last dose, blood was drawn from each mouse and the mice were sacrificed and tissues were collected.

ApoC III mRNA Analysis

ApoC III mRNA levels in the mice's livers were determined using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. ApoC III mRNA levels were determined relative to total RNA (using Ribogreen), prior to normalization to PBS-treated control. The results below are presented as the average percent of ApoC III mRNA levels for each treatment group, normalized to PBS-treated control and are denoted as "% PBS". The half maximal effective dosage (ED_{50}) of each ASO is also presented in Table 18, below.

As illustrated, both antisense compounds reduced ApoC III RNA relative to the PBS control. Further, the antisense compound conjugated to **$GalNAc_3$-1** (ISIS 647535) was substantially more potent than the antisense compound lacking the **$GalNAc_3$-1** conjugate (ISIS 304801).

Table 18

Effect of ASO treatment on ApoC III mRNA levels in human ApoC III transgenic mice

ASO	Dose (μmol/kg)	% PBS	ED_{50} (μmol/kg)	3' Conjugate	Internucleoside linkage/Length	SEQ ID No.
PBS	0	100	--	-	--	
ISIS 304801	0.08	95	0.77	None	PS/20	821
	0.75	42				
	2.25	32				
	6.75	19				
ISIS 647535	0.08	50	0.074	**$GalNAc_3$-1**	PS/20	822
	0.75	15				
	2.25	17				
	6.75	8				

ApoC III Protein Analysis (Turbidometric Assay)

Plasma ApoC III protein analysis was determined using procedures reported by Graham *et al, Circulation Research,* published online before print March 29, 2013.

Approximately 100 µl of plasma isolated from mice was analyzed without dilution using an Olympus Clinical Analyzer and a commercially available turbidometric ApoC III assay (Kamiya, Cat# KAI-006, Kamiya Biomedical, Seattle, WA). The assay protocol was performed as described by the vendor.

As shown in the Table 19 below, both antisense compounds reduced ApoC III protein relative to the PBS control. Further, the antisense compound conjugated to **GalNAc$_3$-1** (ISIS 647535) was substantially more potent than the antisense compound lacking the **GalNAc$_3$-1** conjugate (ISIS 304801).

Table 19

Effect of ASO treatment on ApoC III plasma protein levels in human ApoC III transgenic mice

ASO	Dose (µmol/kg)	% PBS	ED_{50} (µmol/kg)	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
PBS	0	100	--	--	--	
ISIS 304801	0.08	86	0.73	None	PS/20	821
	0.75	51				
	2.25	23				
	6.75	13				
ISIS 647535	0.08	72	0.19	**GalNAc$_3$-1**	PS/20	822
	0.75	14				
	2.25	12				
	6.75	11				

Plasma triglycerides and cholesterol were extracted by the method of Bligh and Dyer (Bligh, E.G. and Dyer, W.J. Can. J. Biochem. Physiol. 37: 911-917, 1959)(Bligh, E and Dyer, W, *Can J Biochem Physiol*, 37, 911-917, 1959)(Bligh, E and Dyer, W, *Can J Biochem Physiol*, 37, 911-917, 1959) and measured by using a Beckmann Coulter clinical analyzer and commercially available reagents.

The triglyceride levels were measured relative to PBS injected mice and are denoted as "% PBS". Results are presented in Table 20. As illustrated, both antisense compounds lowered triglyceride levels. Further, the antisense compound conjugated to **GalNAc$_3$-1** (ISIS 647535) was

substantially more potent than the antisense compound lacking the **GalNAc$_3$-1** conjugate (ISIS 304801).

Table 20

Effect of ASO treatment on triglyceride levels in transgenic mice

ASO	Dose (μmol/kg)	% PBS	ED_{50} (μmol/kg)	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
PBS	0	100	--	--	--	
ISIS 304801	0.08	87	0.63	None	PS/20	821
	0.75	46				
	2.25	21				
	6.75	12				
ISIS 647535	0.08	65	0.13	**GalNAc$_3$-1**	PS/20	822
	0.75	9				
	2.25	8				
	6.75	9				

Plasma samples were analyzed by HPLC to determine the amount of total cholesterol and of different fractions of cholesterol (HDL and LDL). Results are presented in Tables 21 and 22. As illustrated, both antisense compounds lowered total cholesterol levels; both lowered LDL; and both raised HDL. Further, the antisense compound conjugated to **GalNAc$_3$-1** (ISIS 647535) was substantially more potent than the antisense compound lacking the **GalNAc$_3$-1** conjugate (ISIS 304801). An increase in HDL and a decrease in LDL levels is a cardiovascular beneficial effect of antisense inhibition of ApoC III.

Table 21

Effect of ASO treatment on total cholesterol levels in transgenic mice

ASO	Dose (μmol/kg)	Total Cholesterol (mg/dL)	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
PBS	0	257	--	--	
ISIS 304801	0.08	226	None	PS/20	821
	0.75	164			
	2.25	110			
	6.75	82			
ISIS 647535	0.08	230	**GalNAc$_3$-1**	PS/20	822
	0.75	82			

	2.25	86			
	6.75	99			

Table 22

Effect of ASO treatment on HDL and LDL cholesterol levels in transgenic mice

ASO	Dose (μmol/kg)	HDL (mg/dL)	LDL (mg/dL)	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
PBS	0	17	28	--	--	
ISIS 304801	0.08	17	23	None	PS/20	821
	0.75	27	12			
	2.25	50	4			
	6.75	45	2			
ISIS 647535	0.08	21	21	**GalNAc$_3$-1**	PS/20	822
	0.75	44	2			
	2.25	50	2			
	6.75	58	2			

Pharmacokinetics Analysis (PK)

The PK of the ASOs was also evaluated. Liver and kidney samples were minced and extracted using standard protocols. Samples were analyzed on MSD1 utilizing IP-HPLC-MS. The tissue level (μg/g) of full-length ISIS 304801 and 647535 was measured and the results are provided in Table 23. As illustrated, liver concentrations of total full-length antisense compounds were similar for the two antisense compounds. Thus, even though the **GalNAc$_3$-1** -conjugated antisense compound is more active in the liver (as demonstrated by the RNA and protein data above), it is not present at substantially higher concentration in the liver. Indeed, the calculated EC_{50} (provided in Table 23) confirms that the observed increase in potency of the conjugated compound cannot be entirely attributed to increased accumulation. This result suggests that the conjugate improved potency by a mechanism other than liver accumulation alone, possibly by improving the productive uptake of the antisense compound into cells.

The results also show that the concentration of **GalNAc$_3$-1** conjugated antisense compound in the kidney is lower than that of antisense compound lacking the GalNAc conjugate. This has several beneficial therapeutic implications. For therapeutic indications where activity in the kidney is not sought, exposure to kidney risks kidney toxicity without corresponding benefit. Moreover, high concentration in kidney typically results in loss of compound to the urine resulting in faster

clearance. Accordingly, for non-kidney targets, kidney accumulation is undesired. These data suggest that **GalNAc$_3$-1** conjugation reduces kidney accumulation.

Table 23

PK analysis of ASO treatment in transgenic mice

ASO	Dose (μmol/kg)	Liver (μg/g)	Kidney (μg/g)	Liver EC_{50} (μg/g)	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
ISIS 304801	0.1	5.2	2.1	53	None	PS/20	821
	0.8	62.8	119.6				
	2.3	142.3	191.5				
	6.8	202.3	337.7				
ISIS 647535	0.1	3.8	0.7	3.8	**GalNAc$_3$-1**	PS/20	822
	0.8	72.7	34.3				
	2.3	106.8	111.4				
	6.8	237.2	179.3				

Metabolites of ISIS 647535 were also identified and their masses were confirmed by high resolution mass spectrometry analysis. The cleavage sites and structures of the observed metabolites are shown below. The relative % of full length ASO was calculated using standard procedures and the results are presented in Table 23a. The major metabolite of ISIS 647535 was full-length ASO lacking the entire conjugate (i.e. ISIS 304801), which results from cleavage at cleavage site A, shown below. Further, additional metabolites resulting from other cleavage sites were also observed. These results suggest that introducing other cleabable bonds such as esters, peptides, disulfides, phosphoramidates or acyl-hydrazones between the **GalNAc$_3$-1** sugar and the ASO, which can be cleaved by enzymes inside the cell, or which may cleave in the reductive environment of the cytosol, or which are labile to the acidic pH inside endosomes and lyzosomes, can also be useful.

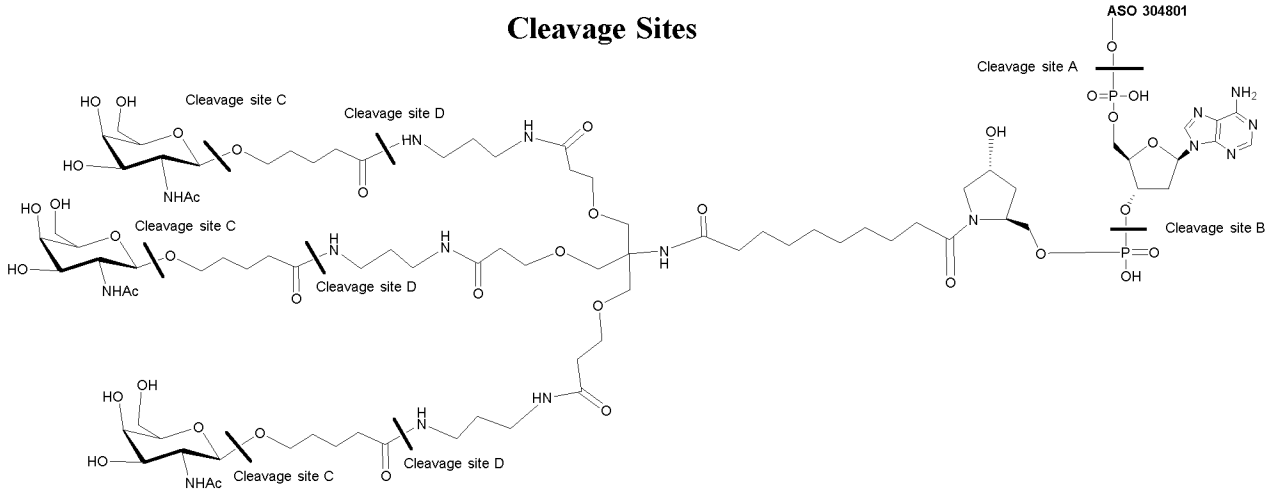
Table 23a

Observed full length metabolites of ISIS 647535

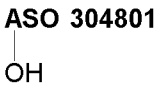
Metabolite	ASO	Cleavage site	Relative %
1	ISIS 304801	A	36.1
2	ISIS 304801 + dA	B	10.5
3	ISIS 647535 minus [3 GalNAc]	C	16.1
4	ISIS 647535 minus [3 GalNAc + 1 5-hydroxy-pentanoic acid tether]	D	17.6

5	ISIS 647535 minus [2 GalNAc + 2 5-hydroxy-pentanoic acid tether]	D	9.9
6	ISIS 647535 minus [3 GalNAc + 3 5-hydroxy-pentanoic acid tether]	D	9.8

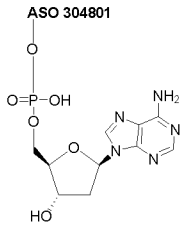
Cleavage Sites



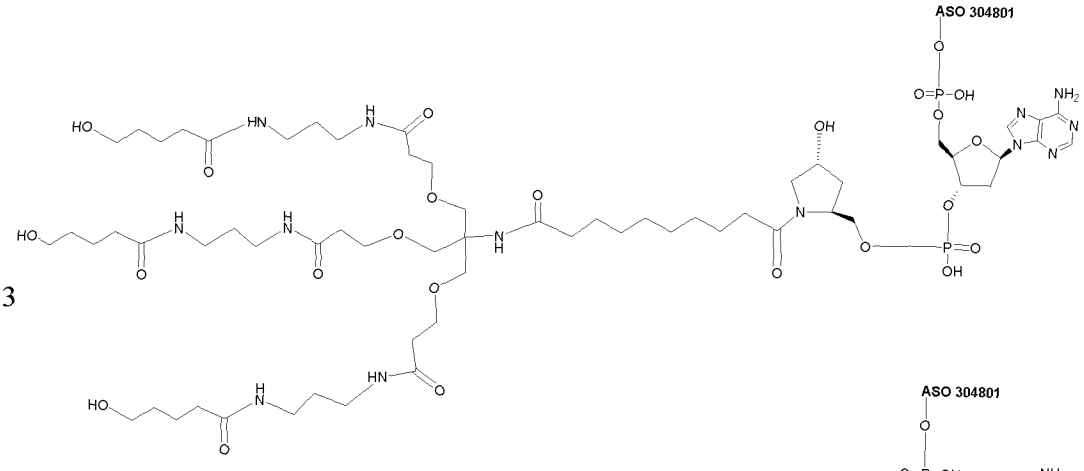
Metabolite 1



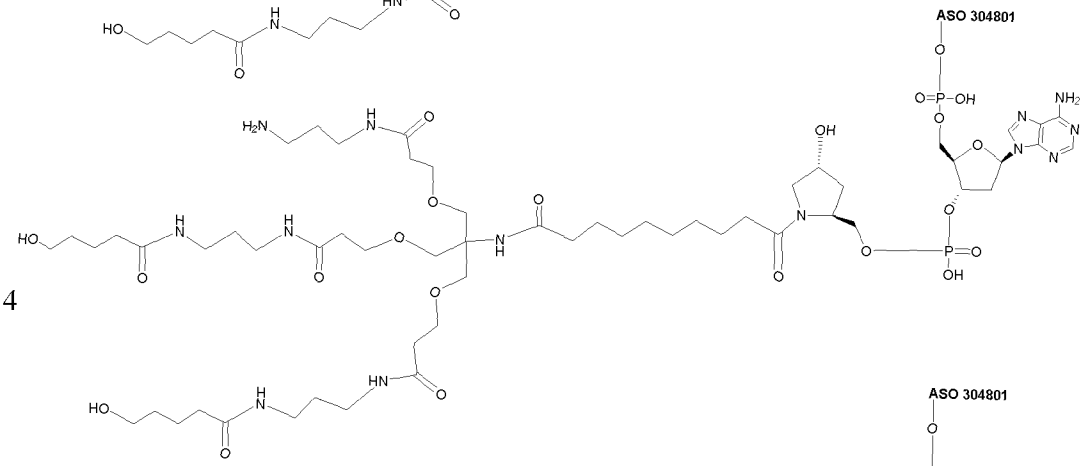
Metabolite 2



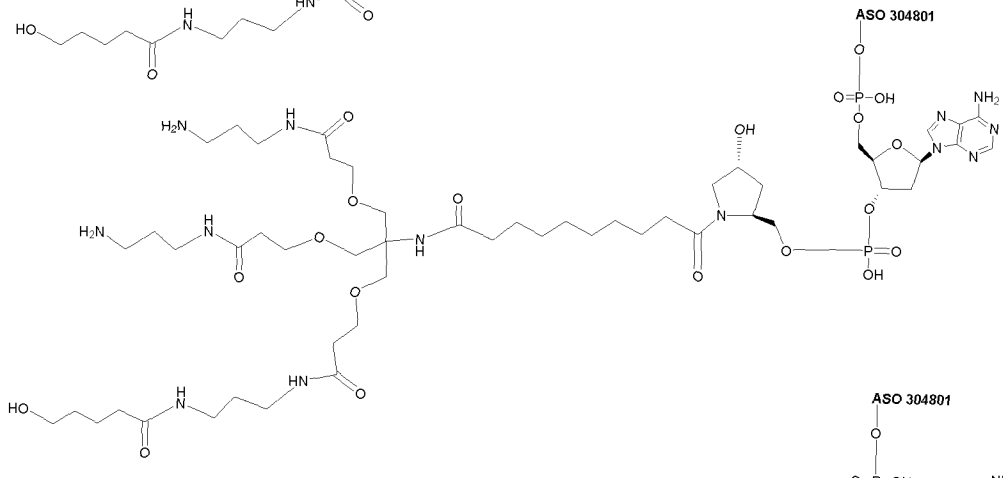
Metabolite 3



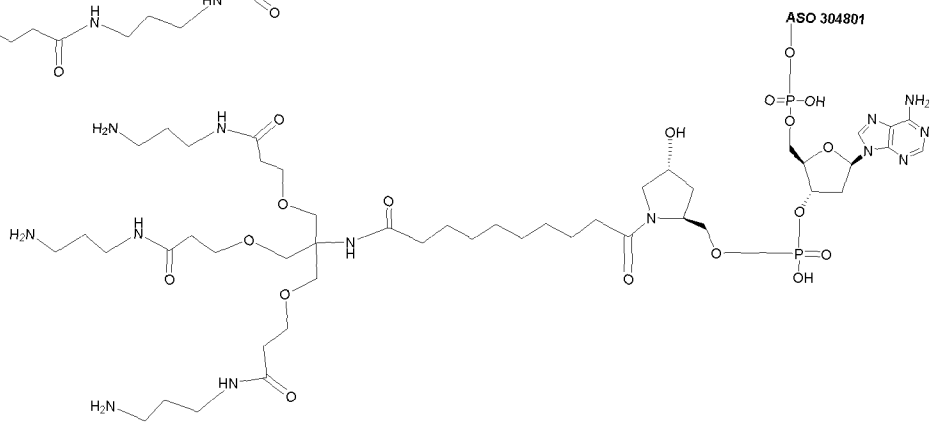
Metabolite 4



Metabolite 5



Metabolite 6



Example 21: Antisense inhibition of human ApoC III in human ApoC III transgenic mice in single administration study

ISIS 304801, 647535 and 647536 each targeting human ApoC III and described in Table 17, were further evaluated in a single administration study for their ability to inhibit human ApoC III in human ApoC III transgenic mice.

Treatment

Human ApoCIII transgenic mice were maintained on a 12-hour light/dark cycle and fed *ad libitum* Teklad lab chow. Animals were acclimated for at least 7 days in the research facility before initiation of the experiment. ASOs were prepared in PBS and sterilized by filtering through a 0.2 micron filter. ASOs were dissolved in 0.9% PBS for injection.

Human ApoC III transgenic mice were injected intraperitoneally once at the dosage shown below with ISIS 304801, 647535 or 647536 (described above) or with PBS treated control. The treatment group consisted of 3 animals and the control group consisted of 4 animals. Prior to the treatment as well as after the last dose, blood was drawn from each mouse and plasma samples were analyzed. The mice were sacrificed 72 hours following the last administration.

Samples were collected and analyzed to determine the ApoC III mRNA and protein levels in the liver; plasma triglycerides; and cholesterol, including HDL and LDL fractions were assessed as described above (Example 20). Data from those analyses are presented in Tables 24-28, below. Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum were measured relative to saline injected mice using standard protocols. The ALT and AST levels showed that the antisense compounds were well tolerated at all administered doses.

These results show improvement in potency for antisense compounds comprising a **GalNAc$_3$-1** conjugate at the 3' terminus (ISIS 647535 and 647536) compared to the antisense compound lacking a **GalNAc$_3$-1** conjugate (ISIS 304801). Further, ISIS 647536, which comprises a **GalNAc$_3$-1** conjugate and some phosphodiester linkages was as potent as ISIS 647535, which comprises the same conjugate and all internucleoside linkages within the ASO are phosphorothioate.

Table 24

Effect of ASO treatment on ApoC III mRNA levels in human ApoC III transgenic mice

ASO	Dose (mg/kg)	% PBS	ED_{50} (mg/kg)	3' Conjugate	Internucleoside linkage/Length	SEQ ID No.
PBS	0	99	--	-	--	
ISIS 304801	1	104	13.2	None	PS/20	821
	3	92				

	10	71				
	30	40				
ISIS 647535	0.3	98	1.9	**GalNAc$_3$-1**	PS/20	822
	1	70				
	3	33				
	10	20				
ISIS 647536	0.3	103	1.7	**GalNAc$_3$-1**	PS/PO/20	822
	1	60				
	3	31				
	10	21				

Table 25

Effect of ASO treatment on ApoC III plasma protein levels in human ApoC III transgenic mice

ASO	Dose (mg/kg)	% PBS	ED$_{50}$ (mg/kg)	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
PBS	0	99	--	--	--	
ISIS 304801	1	104	23.2	None	PS/20	821
	3	92				
	10	71				
	30	40				
ISIS 647535	0.3	98	2.1	**GalNAc$_3$-1**	PS/20	822
	1	70				
	3	33				
	10	20				
ISIS 647536	0.3	103	1.8	**GalNAc$_3$-1**	PS/PO/20	822
	1	60				
	3	31				
	10	21				

Table 26

Effect of ASO treatment on triglyceride levels in transgenic mice

ASO	Dose (mg/kg)	% PBS	ED$_{50}$ (mg/kg)	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
PBS	0	98	--	--	--	
ISIS 304801	1	80	29.1	None	PS/20	821
	3	92				
	10	70				

	30	47				
ISIS 647535	0.3	100	2.2	**GalNAc$_3$-1**	PS/20	822
	1	70				
	3	34				
	10	23				
ISIS 647536	0.3	95	1.9	**GalNAc$_3$-1**	PS/PO/20	822
	1	66				
	3	31				
	10	23				

Table 27

Effect of ASO treatment on total cholesterol levels in transgenic mice

ASO	Dose (mg/kg)	% PBS	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
PBS	0	96	--	--	
ISIS 304801	1	104	None	PS/20	821
	3	96			
	10	86			
	30	72			
ISIS 647535	0.3	93	**GalNAc$_3$-1**	PS/20	822
	1	85			
	3	61			
	10	53			
ISIS 647536	0.3	115	**GalNAc$_3$-1**	PS/PO/20	822
	1	79			
	3	51			
	10	54			

Table 28

Effect of ASO treatment on HDL and LDL cholesterol levels in transgenic mice

ASO	Dose (mg/kg)	HDL % PBS	LDL % PBS	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
PBS	0	131	90	--	--	
ISIS 304801	1	130	72	None	PS/20	821
	3	186	79			
	10	226	63			
	30	240	46			

ISIS 647535	0.3	98	86	**GalNAc$_3$-1**	PS/20	822
	1	214	67			
	3	212	39			
	10	218	35			
ISIS 647536	0.3	143	89	**GalNAc$_3$-1**	PS/PO/20	822
	1	187	56			
	3	213	33			
	10	221	34			

These results confirm that the **GalNAc$_3$-1** conjugate improves potency of an antisense compound. The results also show equal potency of a **GalNAc$_3$-1** conjugated antisense compounds where the antisense oligonucleotides have mixed linkages (ISIS 647536 which has six phosphodiester linkages) and a full phosphorothioate version of the same antisense compound (ISIS 647535).

Phosphorothioate linkages provide several properties to antisense compounds. For example, they resist nuclease digestion and they bind proteins resulting in accumulation of compound in the liver, rather than in the kidney/urine. These are desirable properties, particularly when treating an indication in the liver. However, phosphorothioate linkages have also been associated with an inflammatory response. Accordingly, reducing the number of phosphorothioate linkages in a compound is expected to reduce the risk of inflammation, but also lower concentration of the compound in liver, increase concentration in the kidney and urine, decrease stability in the presence of nucleases, and lower overall potency. The present results show that a **GalNAc$_3$-1** conjugated antisense compound where certain phosphorothioate linkages have been replaced with phosphodiester linkages is as potent against a target in the liver as a counterpart having full phosphorothioate linkages. Such compounds are expected to be less proinflammatory (See Example 24 describing an experiment showing reduction of PS results in reduced inflammatory effect).

Example 22: Effect of GalNAc$_3$-1 conjugated modified ASO targeting SRB-1 *in vivo*

ISIS 440762 and 651900, each targeting SRB-1 and described in Table 17, were evaluated in a dose-dependent study for their ability to inhibit SRB-1 in Balb/c mice.

Treatment

Six week old male Balb/c mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously once at the dosage shown below with ISIS 440762, 651900 or with PBS treated

control. Each treatment group consisted of 4 animals. The mice were sacrificed 48 hours following the final administration to determine the SRB-1 mRNA levels in liver using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. SRB-1 mRNA levels were determined relative to total RNA (using Ribogreen), prior to normalization to PBS-treated control. The results below are presented as the average percent of SRB-1 mRNA levels for each treatment group, normalized to PBS-treated control and is denoted as "% PBS".

As illustrated in Table 29, both antisense compounds lowered SRB-1 mRNA levels. Further, the antisense compound comprising the **GalNAc$_3$-1** conjugate (ISIS 651900) was substantially more potent than the antisense compound lacking the **GalNAc$_3$-1** conjugate (ISIS 440762). These results demonstrate that the potency benefit of **GalNAc$_3$-1** conjugates are observed using antisense oligonucleotides complementary to a different target and having different chemically modified nucleosides, in this instance modified nucleosides comprise constrained ethyl sugar moieties (a bicyclic sugar moiety).

Table 29

Effect of ASO treatment on SRB-1 mRNA levels in Balb/c mice

ASO	Dose (mg/kg)	Liver % PBS	ED_{50} (mg/kg)	3' Conjugate		Internucleoside linkage/Length	SEQ ID No.
PBS	0	100		-		--	
ISIS 440762	0.7	85	2.2	None		PS/14	823
	2	55					
	7	12					
	20	3					
ISIS 651900	0.07	98	0.3	**GalNAc$_3$-1**		PS/14	824
	0.2	63					
	0.7	20					
	2	6					
	7	5					

Example 23: Human Peripheral Blood Mononuclear Cells (hPBMC) Assay Protocol

The hPBMC assay was performed using BD Vautainer CPT tube method. A sample of whole blood from volunteered donors with informed consent at US HealthWorks clinic (Faraday & El Camino Real, Carlsbad) was obtained and collected in 4-15 BD Vacutainer CPT 8 ml tubes (VWR Cat.# BD362753). The approximate starting total whole blood volume in the CPT tubes for

each donor was recorded using the PBMC assay data sheet.

The blood sample was remixed immediately prior to centrifugation by gently inverting tubes 8-10 times. CPT tubes were centrifuged at rt (18-25 °C) in a horizontal (swing-out) rotor for 30 min. at 1500-1800 RCF with brake off (2700 RPM Beckman Allegra 6R). The cells were retrieved from the buffy coat interface (between Ficoll and polymer gel layers); transferred to a sterile 50 ml conical tube and pooled up to 5 CPT tubes/50 ml conical tube/donor. The cells were then washed twice with PBS (Ca^{++}, Mg^{++} free; GIBCO). The tubes were topped up to 50 ml and mixed by inverting several times. The sample was then centrifuged at 330 x g for 15 minutes at rt (1215 RPM in Beckman Allegra 6R) and aspirated as much supernatant as possible without disturbing pellet. The cell pellet was dislodged by gently swirling tube and resuspended cells in RPMI+10% FBS+pen/strep (~1 ml / 10 ml starting whole blood volume). A 60 µl sample was pipette into a sample vial (Beckman Coulter) with 600 µl VersaLyse reagent (Beckman Coulter Cat# A09777) and was gently vortexed for 10-15 sec. The sample was allowed to incubate for 10 min. at rt and being mixed again before counting. The cell suspension was counted on Vicell XR cell viability analyzer (Beckman Coulter) using PBMC cell type (dilution factor of 1:11 was stored with other parameters). The live cell/ml and viability were recorded. The cell suspension was diluted to 1 x 10^7 live PBMC/ml in RPMI+ 10% FBS+pen/strep.

The cells were plated at 5 x 10^5 in 50 µl/well of 96-well tissue culture plate (Falcon Microtest). 50 µl/well of 2x concentration oligos/controls diluted in RPMI+10% FBS+pen/strep. was added according to experiment template (100 µl/well total). Plates were placed on the shaker and allowed to mix for approx. 1 min. After being incubated for 24 hrs at 37 °C; 5% CO_2, the plates were centrifuged at 400 x g for 10 minutes before removing the supernatant for MSD cytokine assay (i.e. human IL-6, IL-10, IL-8 and MCP-1).

Example 24: Evaluation of Proinflammatory Effects in hPBMC Assay for $GalNAc_3$-1 conjugated ASOs

The antisense oligonucleotides (ASOs) listed in Table 30 were evaluated for proinflammatory effect in hPBMC assay using the protocol described in Example 23. ISIS 353512 is an internal standard known to be a high responder for IL-6 release in the assay. The hPBMCs were isolated from fresh, volunteered donors and were treated with ASOs at 0, 0.0128, 0.064, 0.32, 1.6, 8, 40 and 200 µM concentrations. After a 24 hr treatment, the cytokine levels were measured.

The levels of IL-6 were used as the primary readout. The EC_{50} and E_{max} was calculated using standard procedures. Results are expressed as the average ratio of E_{max}/EC_{50} from two donors and is denoted as "E_{max}/EC_{50}." The lower ratio indicates a relative decrease in the proinflammatory response and the higher ratio indicates a relative increase in the proinflammatory response.

With regard to the test compounds, the least proinflammatory compound was the PS/PO linked ASO (ISIS 616468). The **GalNAc$_3$-1** conjugated ASO, ISIS 647535 was slightly less proinflammatory than its non-conjugated counterpart ISIS 304801. These results indicate that incorporation of some PO linkages reduces proinflammatory reaction and addition of a **GalNAc$_3$-1** conjugate does not make a compound more proinflammatory and may reduce proinflammatory response. Accordingly, one would expect that an antisense compound comprising both mixed PS/PO linkages and a **GalNAc$_3$-1** conjugate would produce lower proinflammatory responses relative to full PS linked antisense compound with or without a **GalNAc$_3$-1** conjugate. These results show that **GalNAc$_3$-1** conjugated antisense compounds, particularly those having reduced PS content are less proinflammatory.

Together, these results suggest that a **GalNAc$_3$-1** conjugated compound, particularly one with reduced PS content, can be administered at a higher dose than a counterpart full PS antisense compound lacking a **GalNAc$_3$-1** conjugate. Since half-life is not expected to be substantially different for these compounds, such higher administration would result in less frequent dosing. Indeed such administration could be even less frequent, because the **GalNAc$_3$-1** conjugated compounds are more potent (See Examples 20-22) and re-dosing is necessary once the concentration of a compound has dropped below a desired level, where such desired level is based on potency.

Table 30
Modified ASOs

ASO	Sequence (5' to 3')	Target	SEQ ID No.
ISIS 104838	$G_{es}{}^{m}C_{es}T_{es}G_{es}A_{es}T_{ds}T_{ds}A_{ds}G_{ds}A_{ds}G_{ds}$ $A_{ds}G_{ds}A_{ds}G_{ds}G_{es}T_{es}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$	TNFα	825
ISIS 353512	$T_{es}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{ds}A_{ds}T_{ds}T_{ds}T_{ds}{}^{m}C_{ds}A_{ds}G_{ds}$ $G_{ds}A_{ds}G_{ds}A_{ds}{}^{m}C_{ds}{}^{m}C_{ds}T_{es}G_{es}G_{e}$	CRP	826
ISIS 304801	$A_{es}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}$ $^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{es}T_{es}T_{es}A_{es}T_{e}$	ApoC III	821
ISIS 647535	$A_{es}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}$ $^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{es}T_{es}T_{es}A_{es}T_{eo}$**A$_{do}$'-GalNAc$_3-1_a$**	ApoC III	822
ISIS 616468	$A_{es}G_{eo}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}$ $^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{eo}T_{eo}T_{es}A_{es}T_{e}$	ApoC III	821

Subscripts: "e" indicates 2'-MOE modified nucleoside; "d" indicates β-D-2'-deoxyribonucleoside; "k" indicates 6'-*(S)*-CH_3 bicyclic nucleoside (e.g. cEt); "s" indicates phosphorothioate internucleoside linkages (PS); "o" indicates phosphodiester internucleoside linkages (PO); and "o'" indicates -O-P(=O)(OH)-. Superscript "m" indicates 5-methylcytosines. "**A_{do}-GalNAc$_3$-1$_a$**" indicates a conjugate having the structure **GalNAc$_3$-1** shown in Example 9 attached to the 3'-end of the antisense oligonucleotide, as indicated.

Table 31

Proinflammatory Effect of ASOs targeting ApoC III in hPBMC assay

ASO	EC_{50} (μM)	E_{max} (μM)	E_{max}/EC_{50}	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
ISIS 353512 (high responder)	0.01	265.9	26,590	None	PS/20	826
ISIS 304801	0.07	106.55	1,522	None	PS/20	821
ISIS 647535	0.12	138	1,150	**GalNAc$_3$-1**	PS/20	822
ISIS 616468	0.32	71.52	224	None	PS/PO/20	821

Example 25: Effect of GalNAc$_3$-1 conjugated modified ASO targeting human ApoC III *in vitro*

ISIS 304801 and 647535 described above were tested *in vitro*. Primary hepatocyte cells from transgenic mice at a density of 25,000 cells per well were treated with 0.03,0.08, 0.24, 0.74, 2.22, 6.67 and 20 μM concentrations of modified oligonucleotides. After a treatment period of approximately 16 hours, RNA was isolated from the cells and mRNA levels were measured by quantitative real-time PCR and the hApoC III mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN.

The IC_{50} was calculated using the standard methods and the results are presented in Table 32. As illustrated, comparable potency was observed in cells treated with ISIS 647535 as compared to the control, ISIS 304801.

Table 32

Modified ASO targeting human ApoC III in primary hepatocytes

ASO	IC_{50} (μM)	3' Conjugate	Internucleoside linkage/Length	SEQ ID No.
ISIS 304801	0.44	None	PS/20	821
ISIS 647535	0.31	**GalNAc$_3$-1**	PS/20	822

In this experiment, the large potency benefits of **GalNAc$_3$-1** conjugation that are observed *in vivo* were not observed *in vitro*. Subsequent free uptake experiments in primary hepatocytes *in vitro* did show increased potency of oligonucleotides comprising various GalNAc conjugates relative to oligonucleotides that lacking the GalNAc conjugate.(see Examples 60, 82, and 92)

Example 26: Effect of PO/PS linkages on ApoC III ASO Activity

Human ApoC III transgenic mice were injected intraperitoneally once at 25 mg/kg of ISIS 304801, or ISIS 616468 (both described above) or with PBS treated control once per week for two weeks. The treatment group consisted of 3 animals and the control group consisted of 4 animals. Prior to the treatment as well as after the last dose, blood was drawn from each mouse and plasma samples were analyzed. The mice were sacrificed 72 hours following the last administration.

Samples were collected and analyzed to determine the ApoC III protein levels in the liver as described above (Example 20). Data from those analyses are presented in Table 33, below.

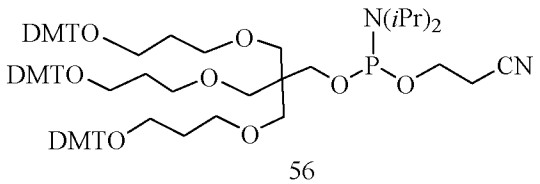
These results show reduction in potency for antisense compounds with PO/PS (ISIS 616468) in the wings relative to full PS (ISIS 304801).

Table 33

Effect of ASO treatment on ApoC III protein levels in human ApoC III transgenic mice

ASO	Dose (mg/kg)	% PBS	3' Conjugate	Internucleoside linkage/Length	SEQ ID No.
PBS	0	99	-	--	
ISIS 304801	25 mg/kg/wk for 2 wks	24	None	Full PS	821
ISIS 616468	25 mg/kg/wk for 2 wks	40	None	14 PS/6 PO	821

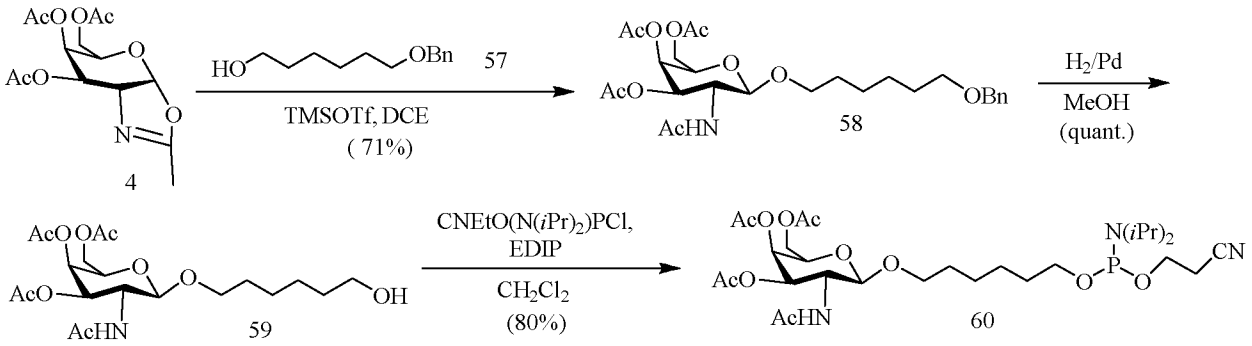
Example 27: Compound 56



56

Compound 56 is commercially available from Glen Research or may be prepared according to published procedures reported by Shchepinov *et al., Nucleic Acids Research,* 1997, *25(22)*, 4447-4454.

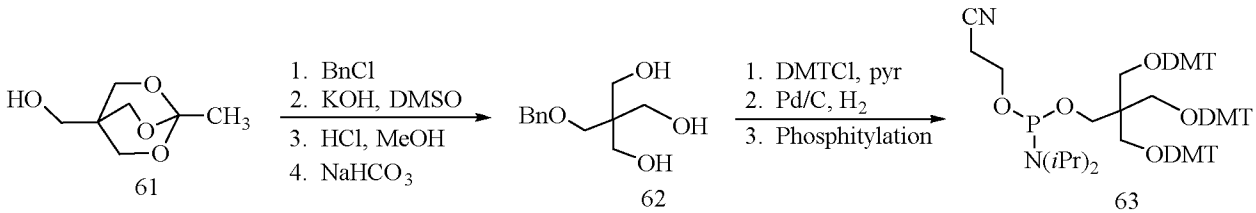
Example 28: Preparation of Compound 60



Compound 4 was prepared as per the procedures illustrated in Example 2. Compound 57 is commercially available. Compound 60 was confirmed by structural analysis.

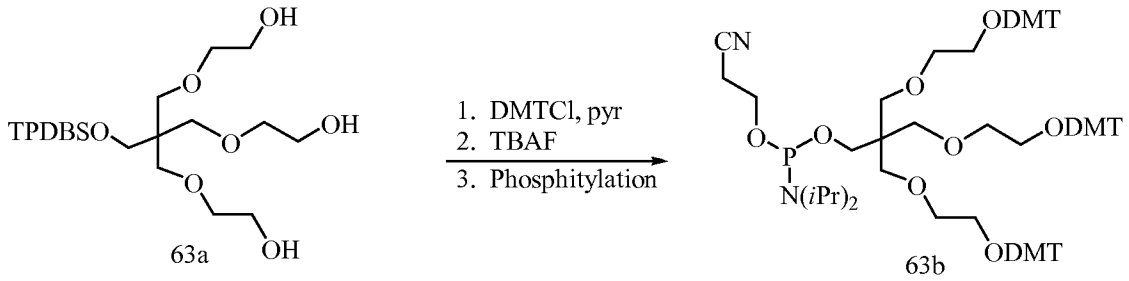
Compound 57 is meant to be representative and not intended to be limiting as other mono-protected substituted or unsubstituted alkyl diols including but not limited to those presented in the specification herein can be used to prepare phosphoramidites having a predetermined composition.

Example 29: Preparation of Compound 63



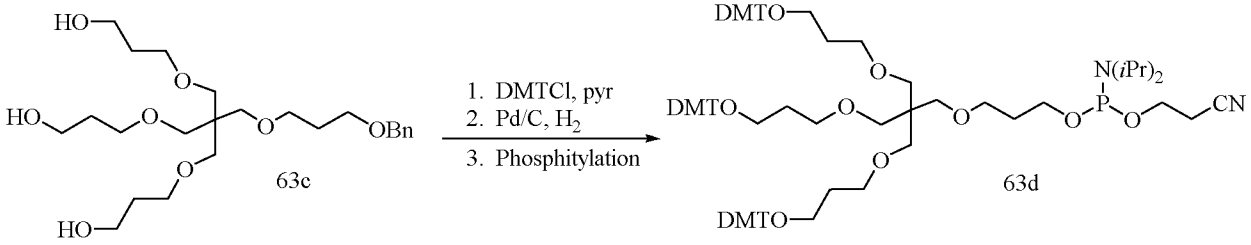
Compounds 61 and 62 are prepared using procedures similar to those reported by Tober *et al., Eur. J. Org. Chem.*, 2013, *3,* 566-577; and Jiang *et al., Tetrahedron,* 2007, *63(19),* 3982-3988.

Alternatively, Compound 63 is prepared using procedures similar to those reported in scientific and patent literature by Kim *et al., Synlett,* 2003, *12,* 1838-1840; and Kim *et al.,* published PCT International Application, WO 2004063208.**Example 30: Preparation of Compound 63b**



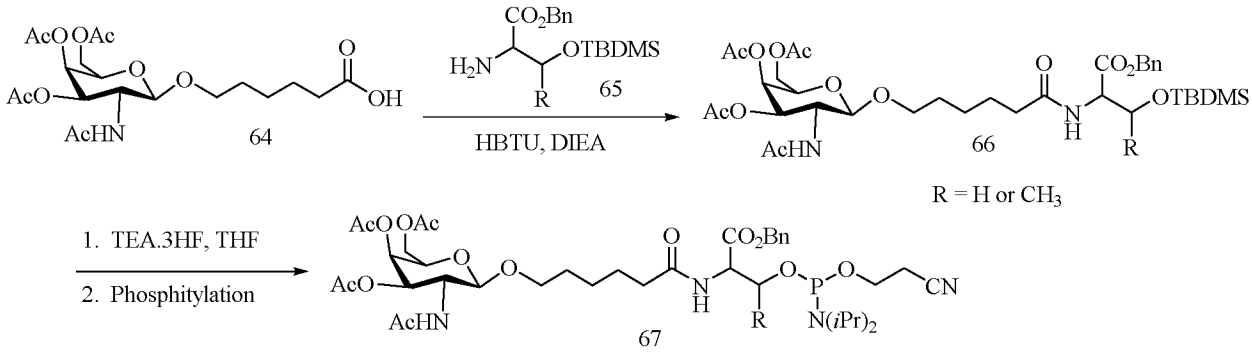
Compound 63a is prepared using procedures similar to those reported by Hanessian *et al., Canadian Journal of Chemistry,* 1996, *74(9),* 1731-1737.

Example 31: Preparation of Compound 63d



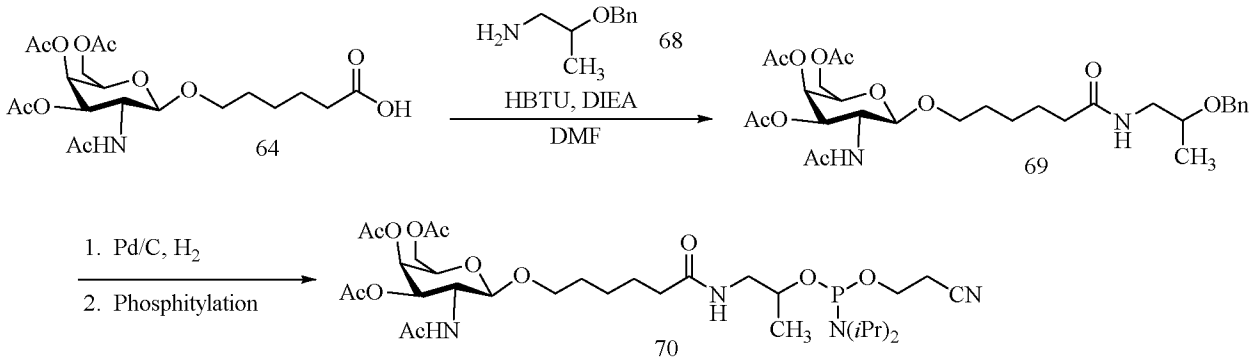
Compound 63c is prepared using procedures similar to those reported by Chen *et al., Chinese Chemical Letters,* 1998, *9(5),* 451-453.

Example 32: Preparation of Compound 67



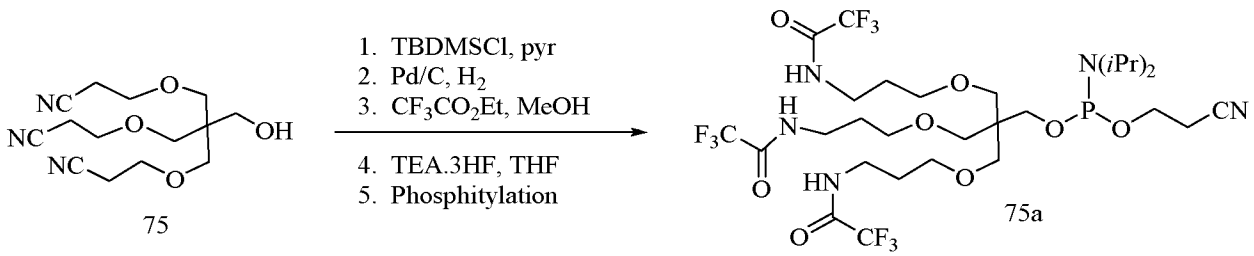
Compound 64 was prepared as per the procedures illustrated in Example 2. Compound 65 is prepared using procedures similar to those reported by Or *et al.,* published PCT International Application, WO 2009003009. The protecting groups used for Compound 65 are meant to be representative and not intended to be limiting as other protecting groups including but not limited to those presented in the specification herein can be used.

Example 33: Preparation of Compound 70



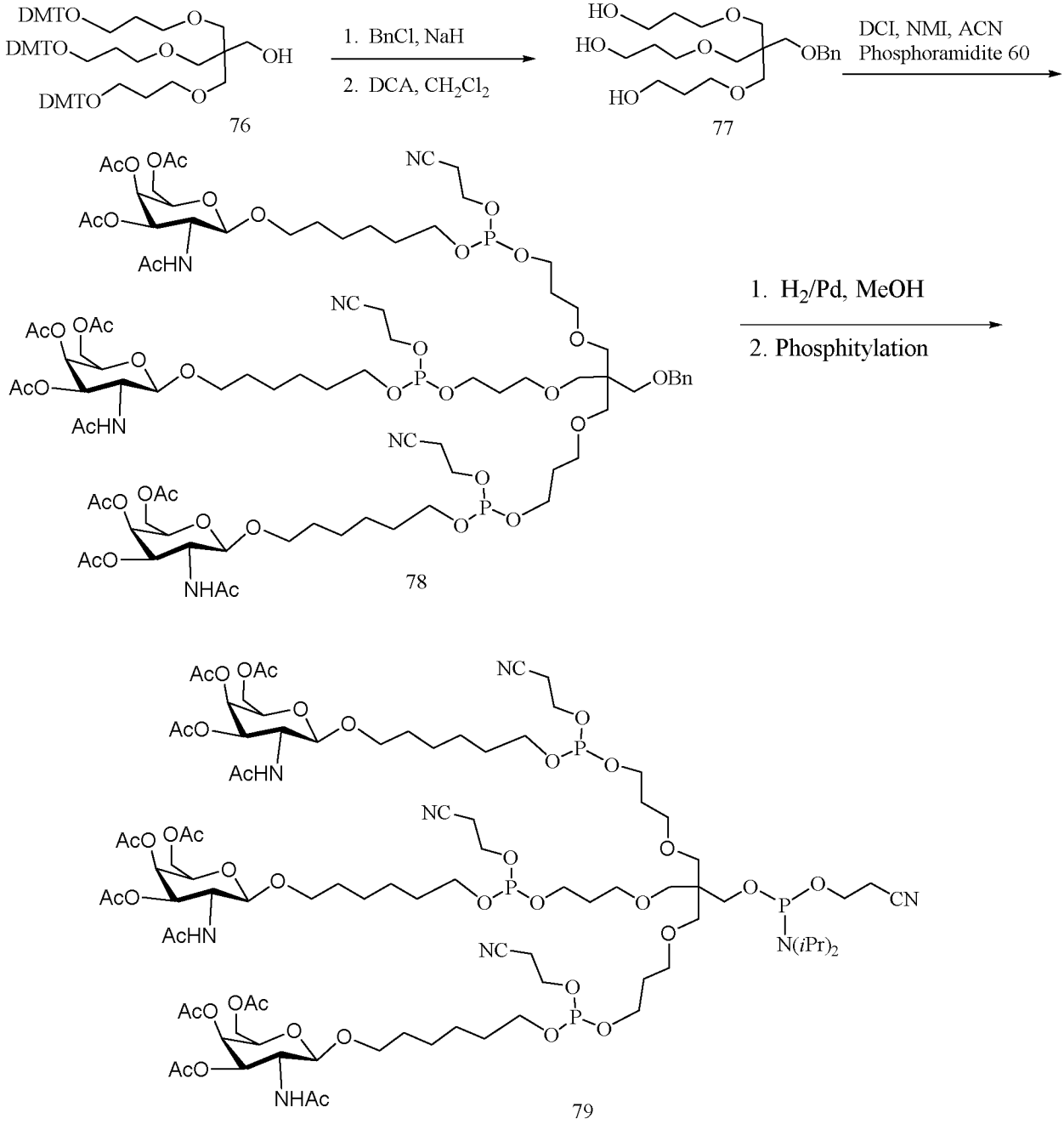
Compound 64 was prepared as per the procedures illustrated in Example 2. Compound 68 is commercially available. The protecting group used for Compound 68 is meant to be representative and not intended to be limiting as other protecting groups including but not limited to those presented in the specification herein can be used.

Example 34: Preparation of Compound 75a



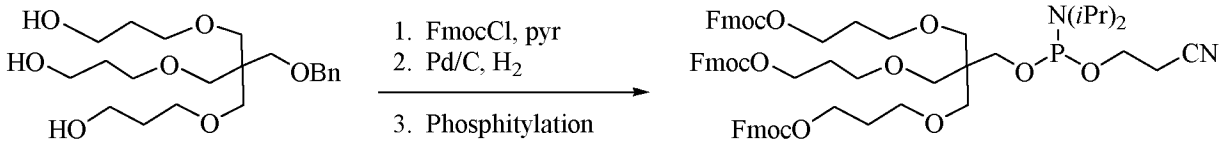
Compound 75 is prepared according to published procedures reported by Shchepinov *et al., Nucleic Acids Research,* 1997, *25(22)*, 4447-4454.

Example 35: Preparation of Compound 79



Compound 76 was prepared according to published procedures reported by Shchepinov *et al., Nucleic Acids Research,* 1997, *25(22)*, 4447-4454.

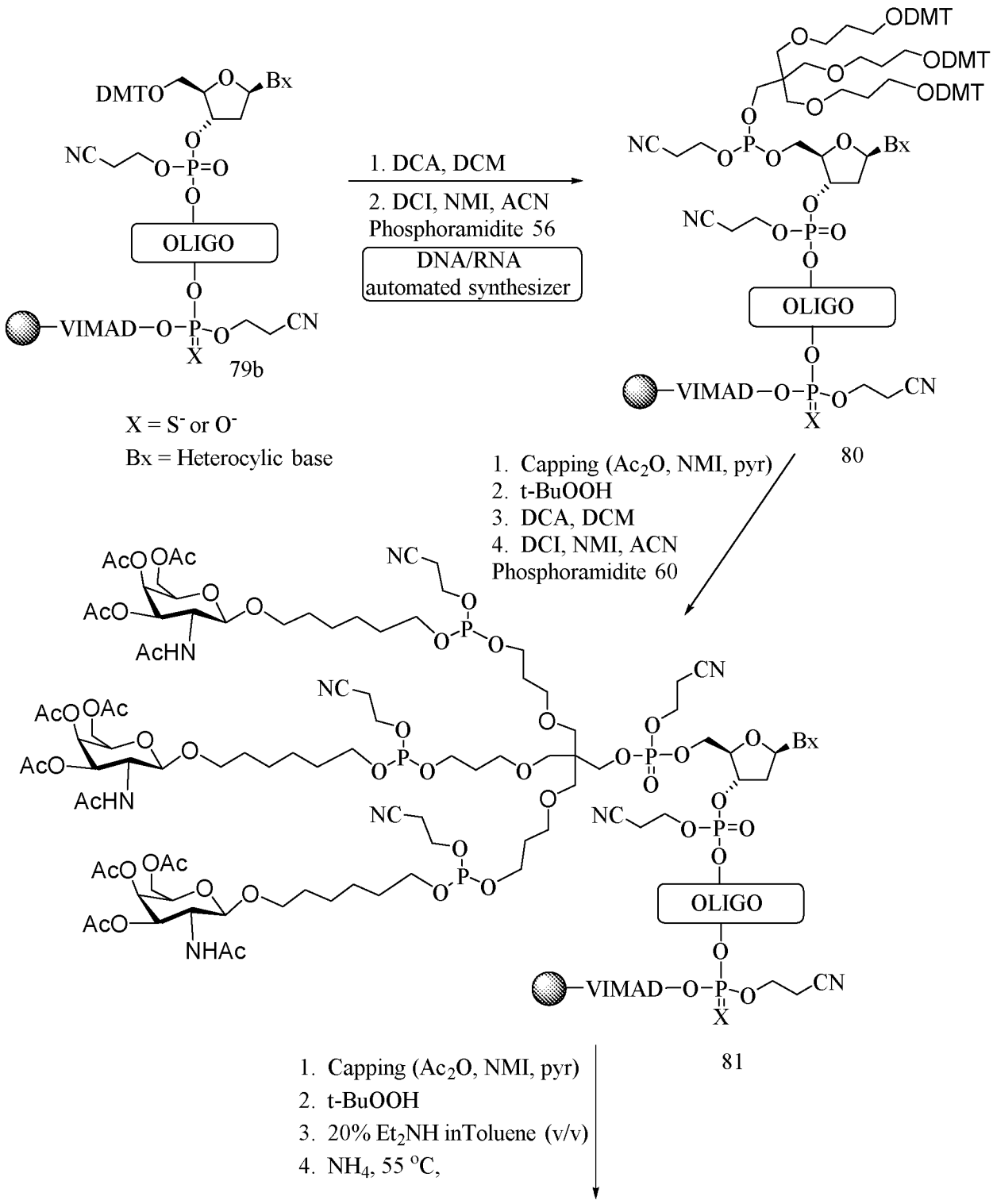
Example 36: Preparation of Compound 79a

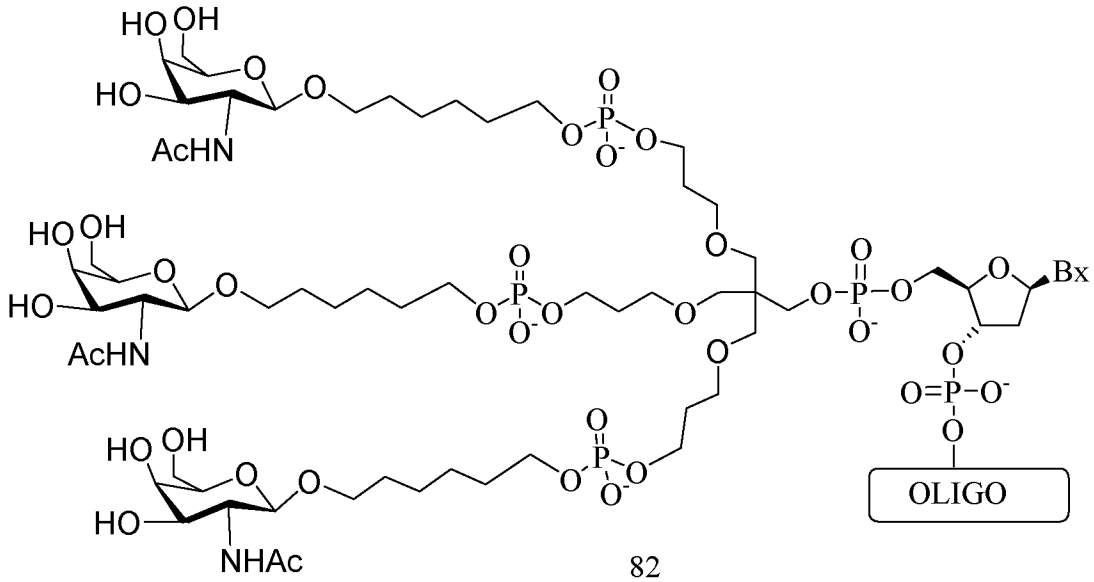


77 79a

Compound 77 is prepared as per the procedures illustrated in Example 35.

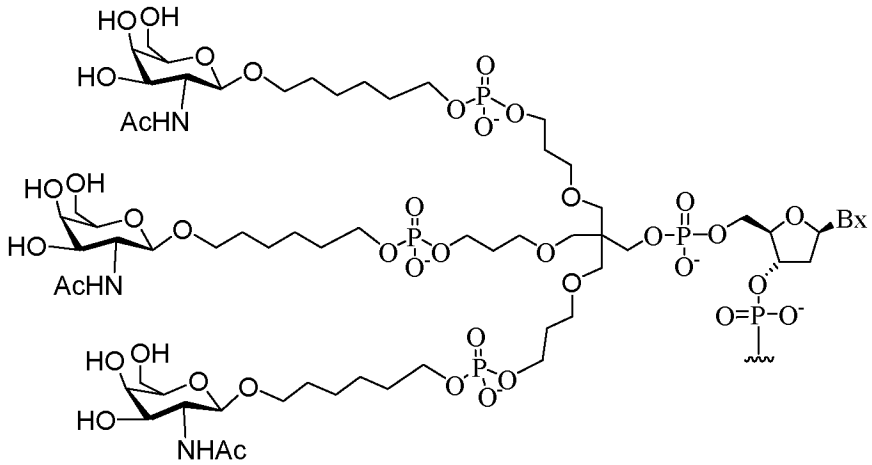
Example 37: General method for the preparation of conjugated oligomeric compound 82 comprising a phosphodiester linked GalNAc$_3$-2 conjugate at 5' terminus *via* solid support (Method I)



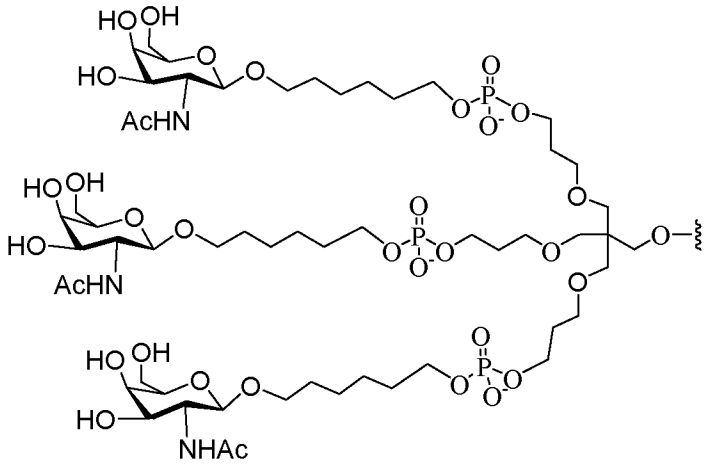


82

wherein $GalNAc_3$-2 has the structure:

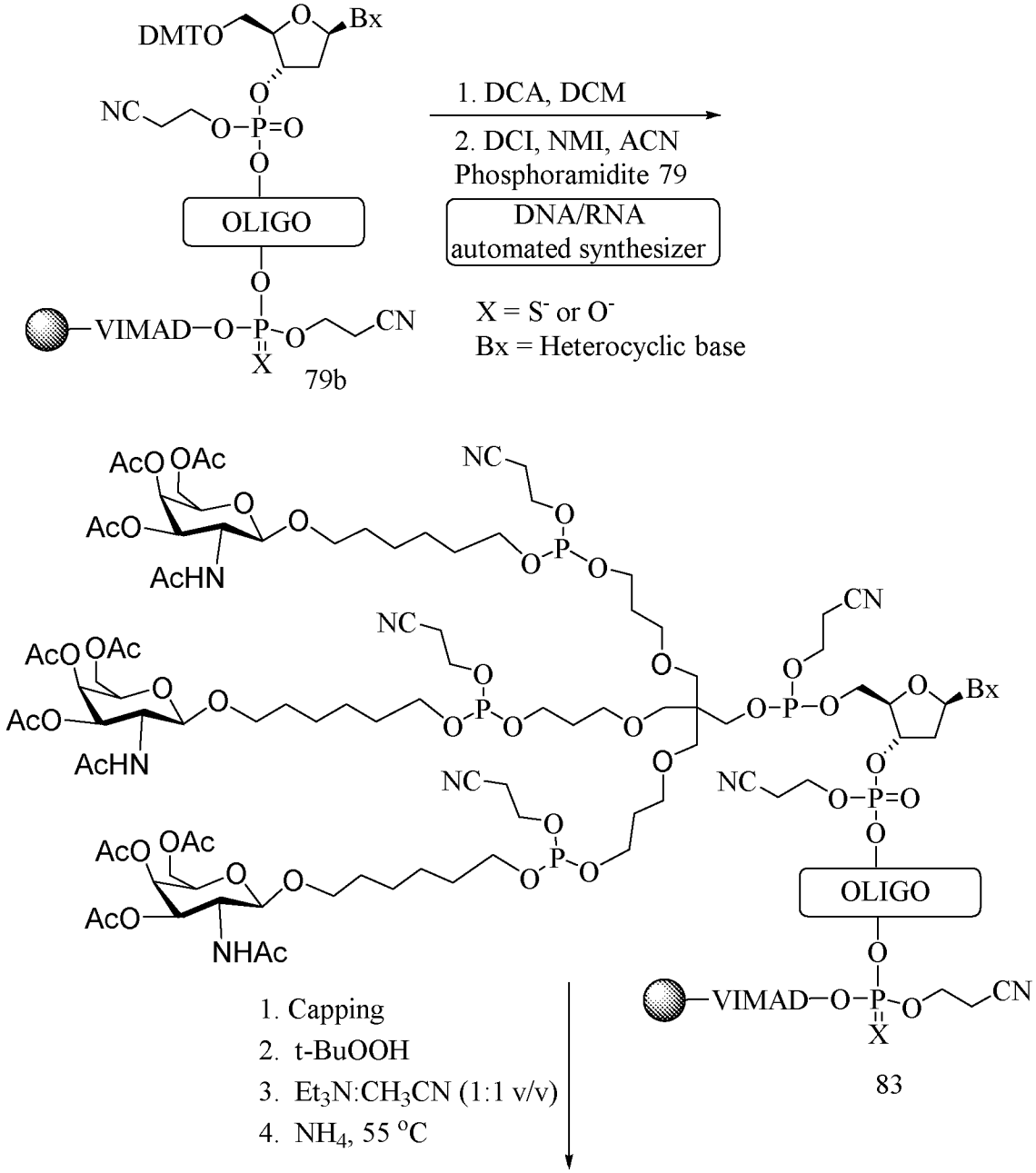


The $GalNAc_3$ cluster portion of the conjugate group $GalNAc_3$-2 ($GalNAc_3$-2_a) can be combined with any cleavable moiety to provide a variety of conjugate groups. Wherein $GalNAc_3$-2_a has the formula:



The VIMAD-bound oligomeric compound 79b was prepared using standard procedures for automated DNA/RNA synthesis (see Dupouy *et al., Angew. Chem. Int. Ed.,* 2006, *45,* 3623-3627). The phosphoramidite Compounds 56 and 60 were prepared as per the procedures illustrated in Examples 27 and 28, respectively. The phosphoramidites illustrated are meant to be representative and not intended to be limiting as other phosphoramidite building blocks including but not limited those presented in the specification herein can be used to prepare an oligomeric compound having a phosphodiester linked conjugate group at the 5' terminus. The order and quantity of phosphoramidites added to the solid support can be adjusted to prepare the oligomeric compounds as described herein having any predetermined sequence and composition.

Example 38: Alternative method for the preparation of oligomeric compound 82 comprising a phosphodiester linked GalNAc$_3$-2 conjugate at 5' terminus (Method II)

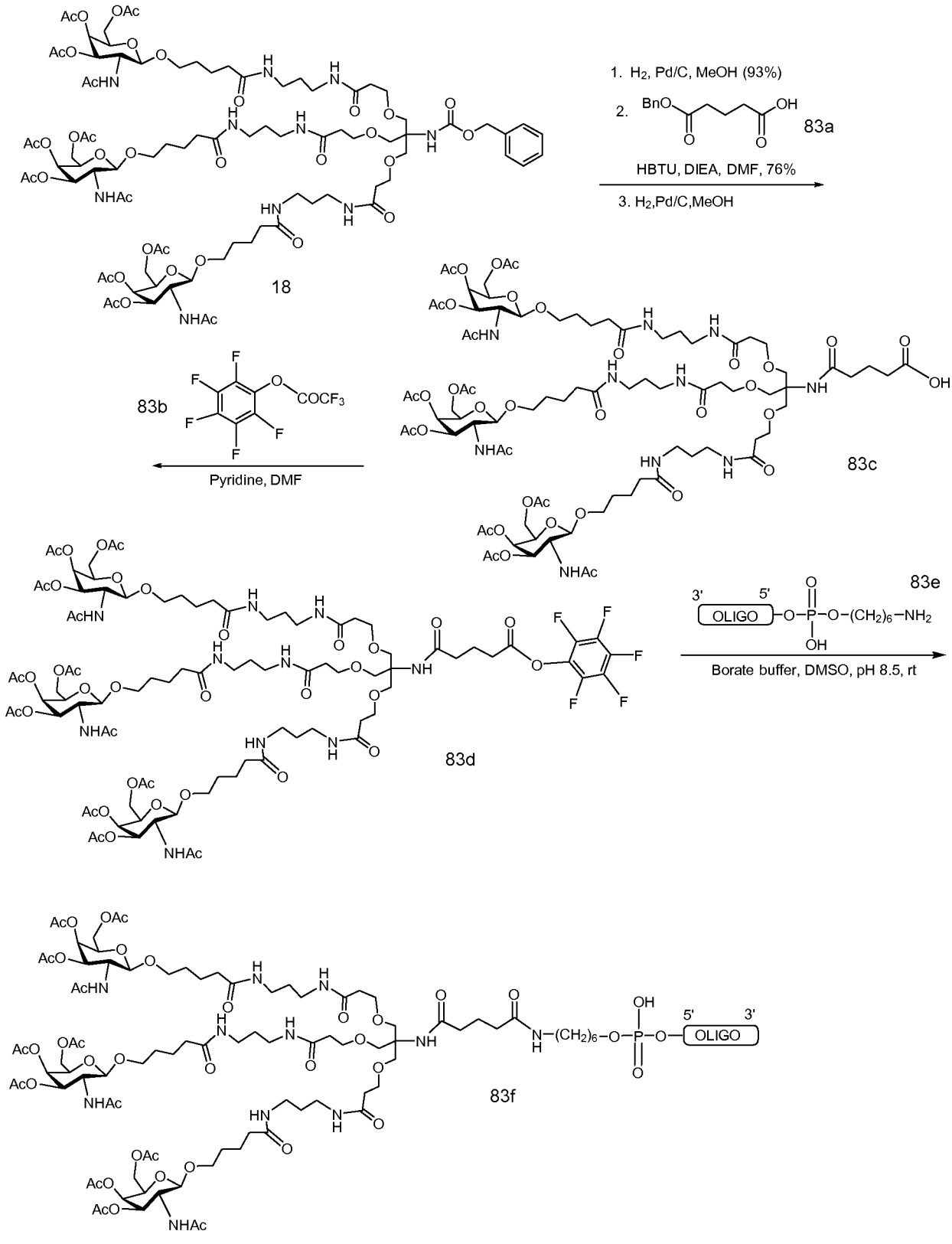


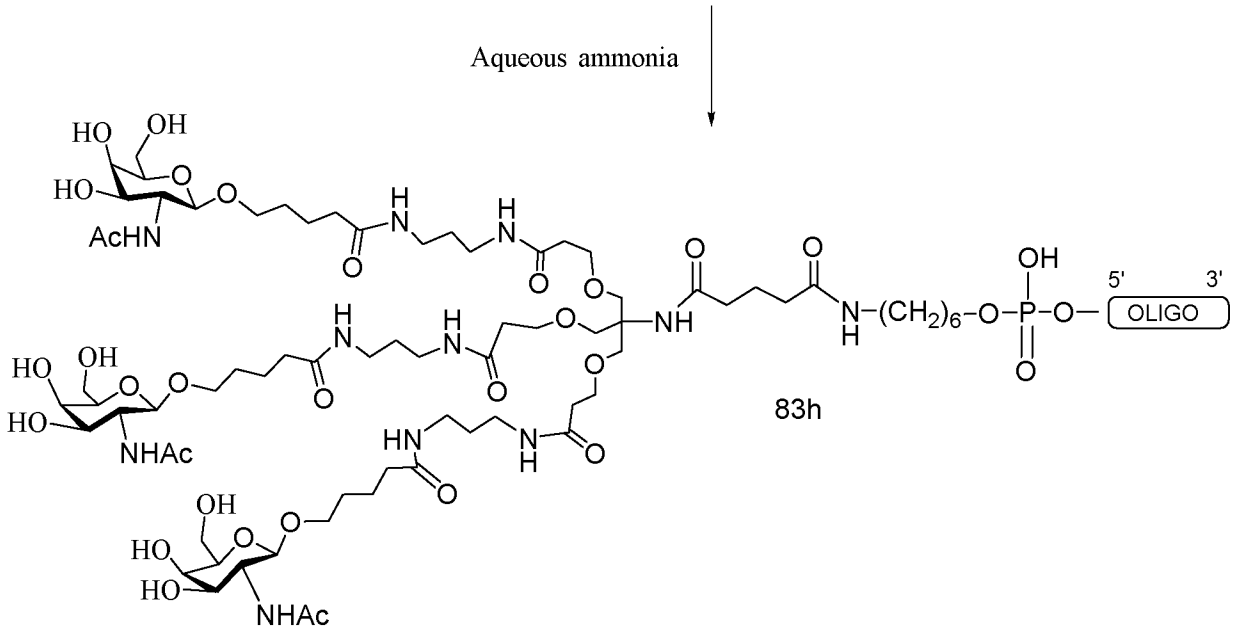
Oligomeric Compound 82

The VIMAD-bound oligomeric compound 79b was prepared using standard procedures for automated DNA/RNA synthesis (see Dupouy *et al., Angew. Chem. Int. Ed.,* 2006, *45,* 3623-3627). The GalNAc$_3$-2 cluster phosphoramidite, Compound 79 was prepared as per the procedures illustrated in Example 35. This alternative method allows a one-step installation of the

phosphodiester linked $GalNAc_3$-2 conjugate to the oligomeric compound at the final step of the synthesis. The phosphoramidites illustrated are meant to be representative and not intended to be limiting, as other phosphoramidite building blocks including but not limited to those presented in the specification herein can be used to prepare oligomeric compounds having a phosphodiester conjugate at the 5' terminus. The order and quantity of phosphoramidites added to the solid support can be adjusted to prepare the oligomeric compounds as described herein having any predetermined sequence and composition.

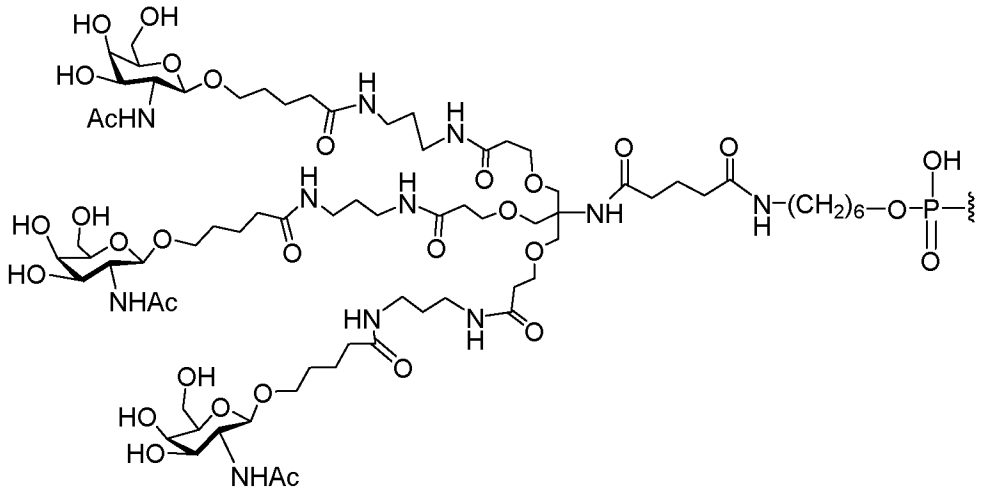
Example 39: General method for the preparation of oligomeric compound 83h comprising a GalNAc$_3$-3 Conjugate at the 5' Terminus (GalNAc$_3$-1 modified for 5' end attachment) *via* Solid Support





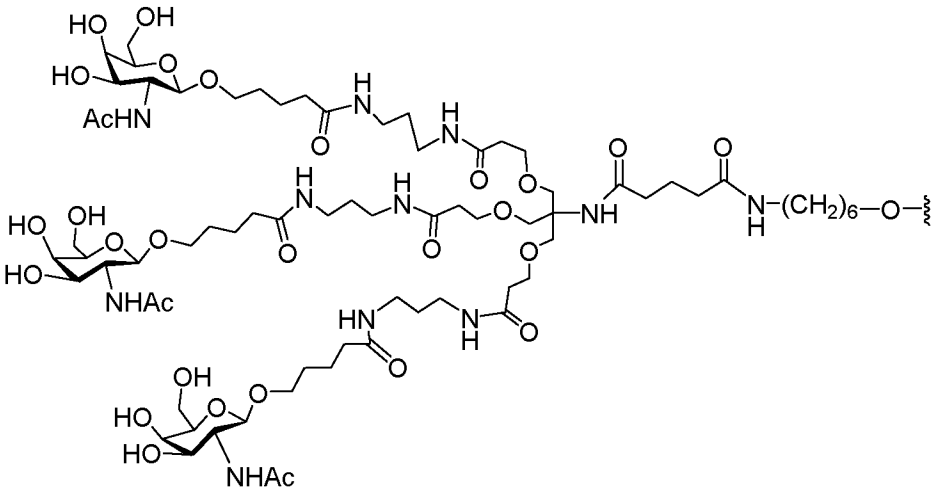
Compound 18 was prepared as per the procedures illustrated in Example 4. Compounds 83a and 83b are commercially available. Oligomeric Compound 83e comprising a phosphodiester linked hexylamine was prepared using standard oligonucleotide synthesis procedures. Treatment of the protected oligomeric compound with aqueous ammonia provided the 5'-$GalNAc_3$-3 conjugated oligomeric compound (83h).

Wherein $GalNAc_3$-3 has the structure:

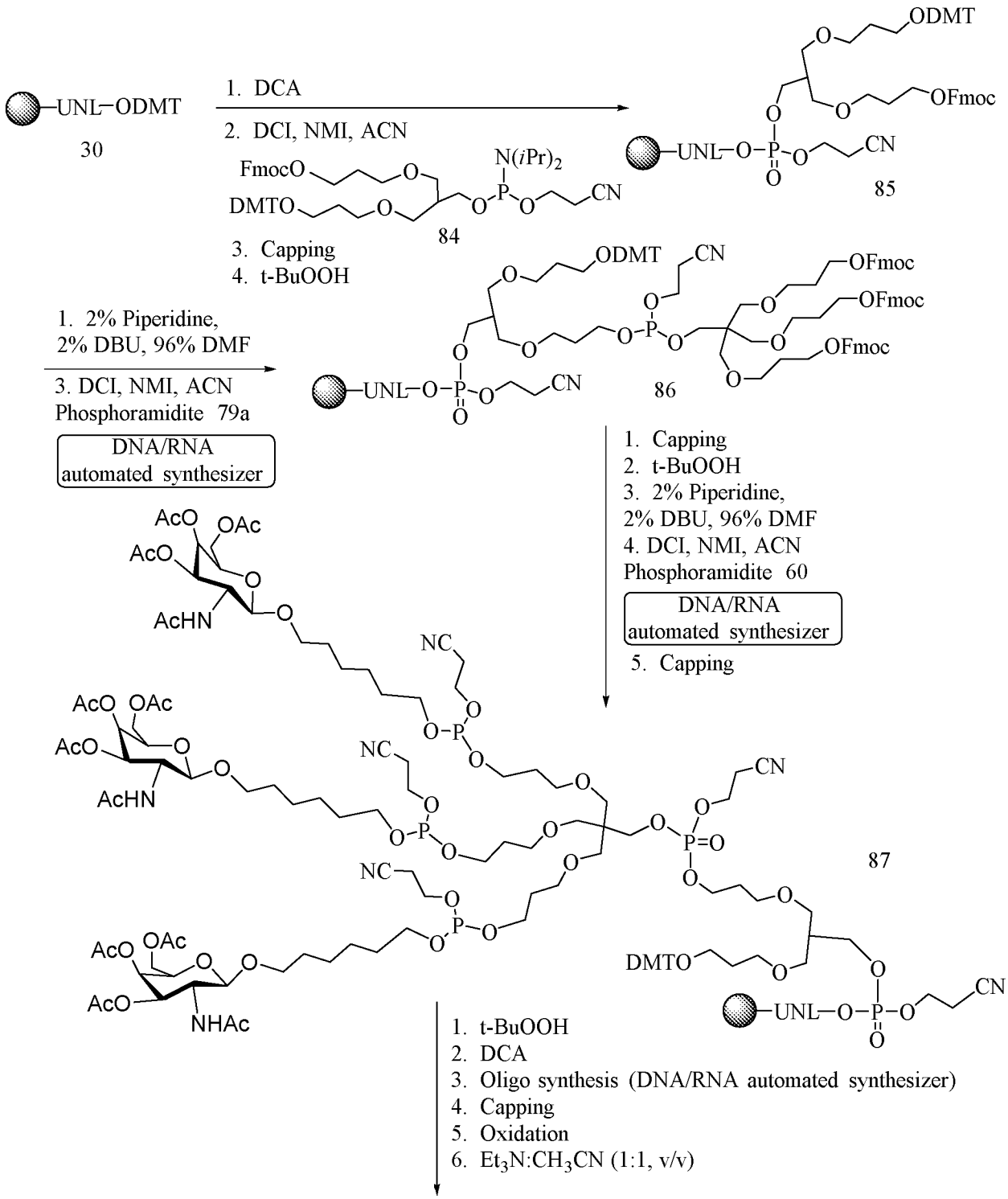


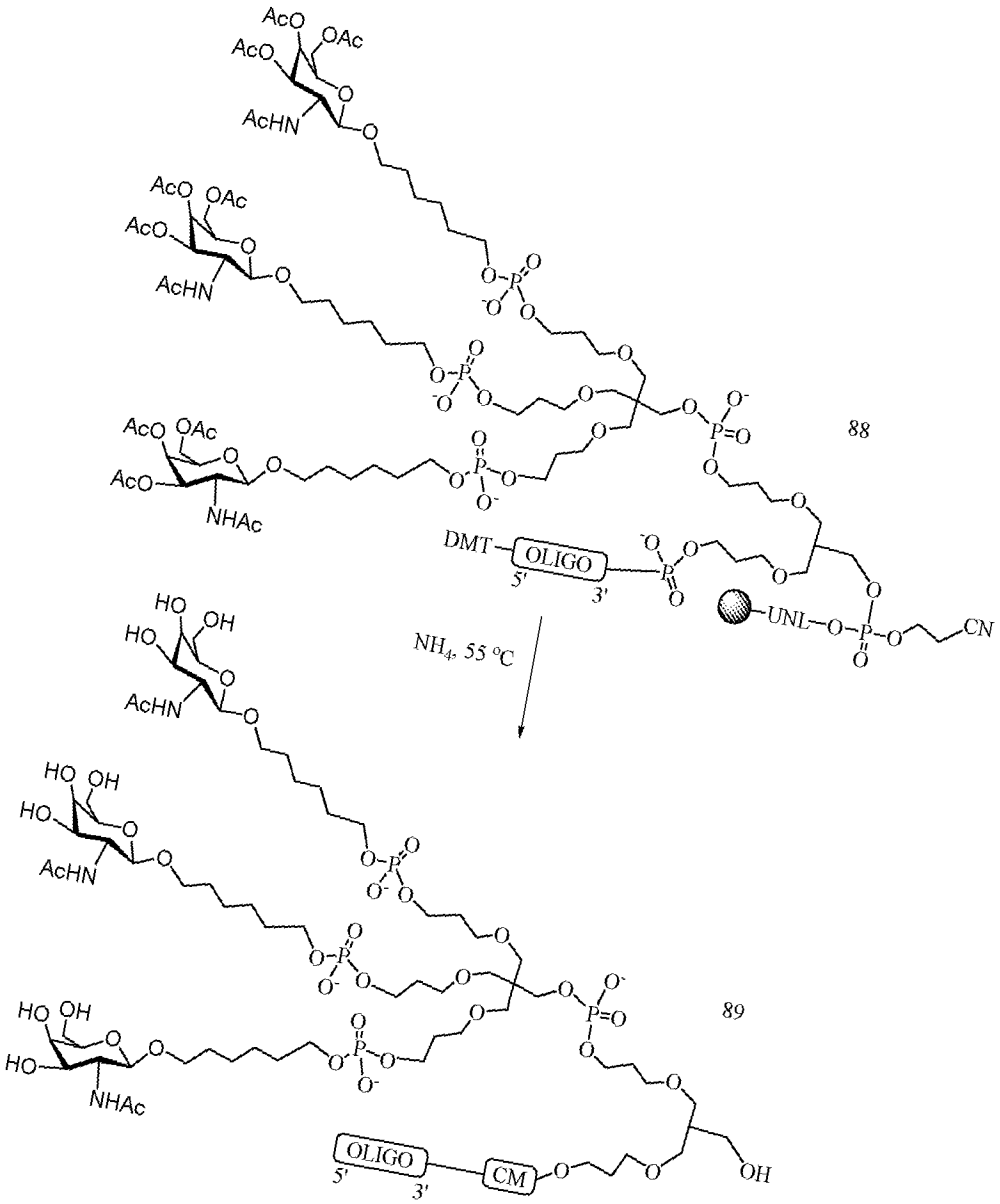
.

The $GalNAc_3$ cluster portion of the conjugate group $GalNAc_3$-3 ($GalNAc_3$-3_a) can be combined with any cleavable moiety to provide a variety of conjugate groups. Wherein $GalNAc_3$-3_a has the formula:

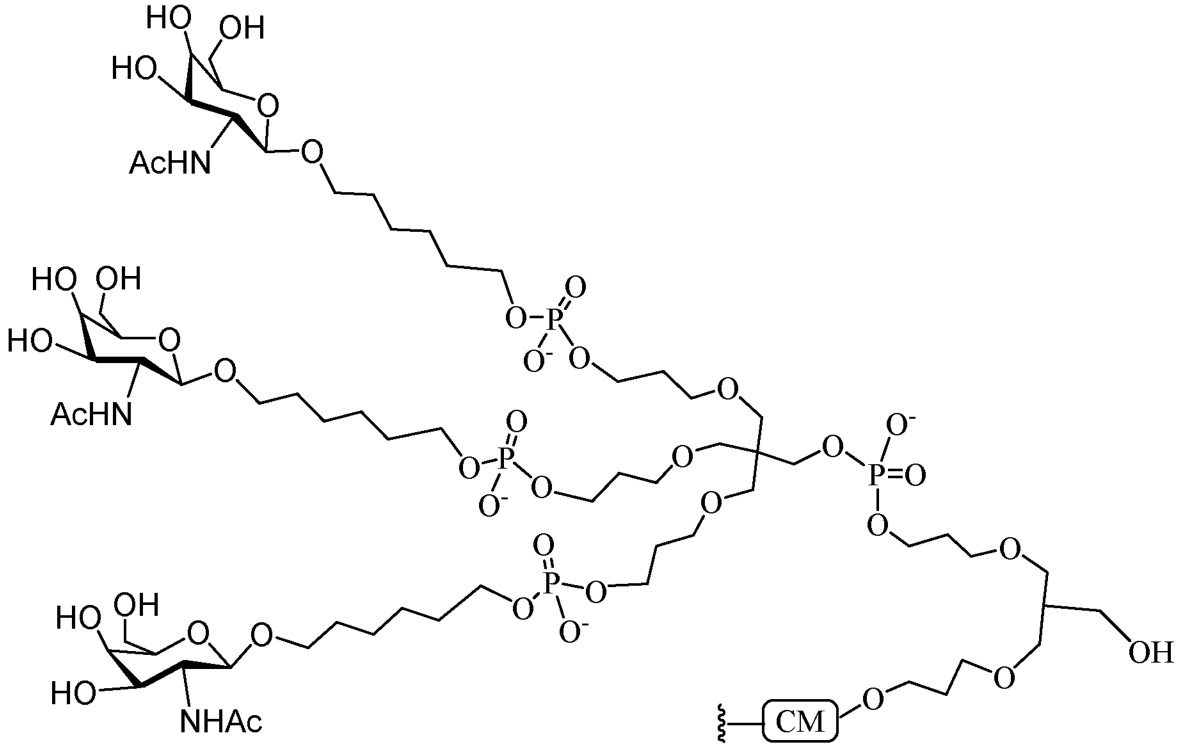


Example 40: General method for the preparation of oligomeric compound 89 comprising a phosphodiester linked GalNAc$_3$-4 conjugate at the 3' terminus *via* solid support

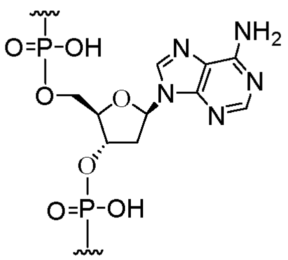



AcO
OAc
AcHN
NHAc
88
DMT
OLIGO
5'
3'
UNL
CN
NH4, 55 °C
HO
OH
89
CM

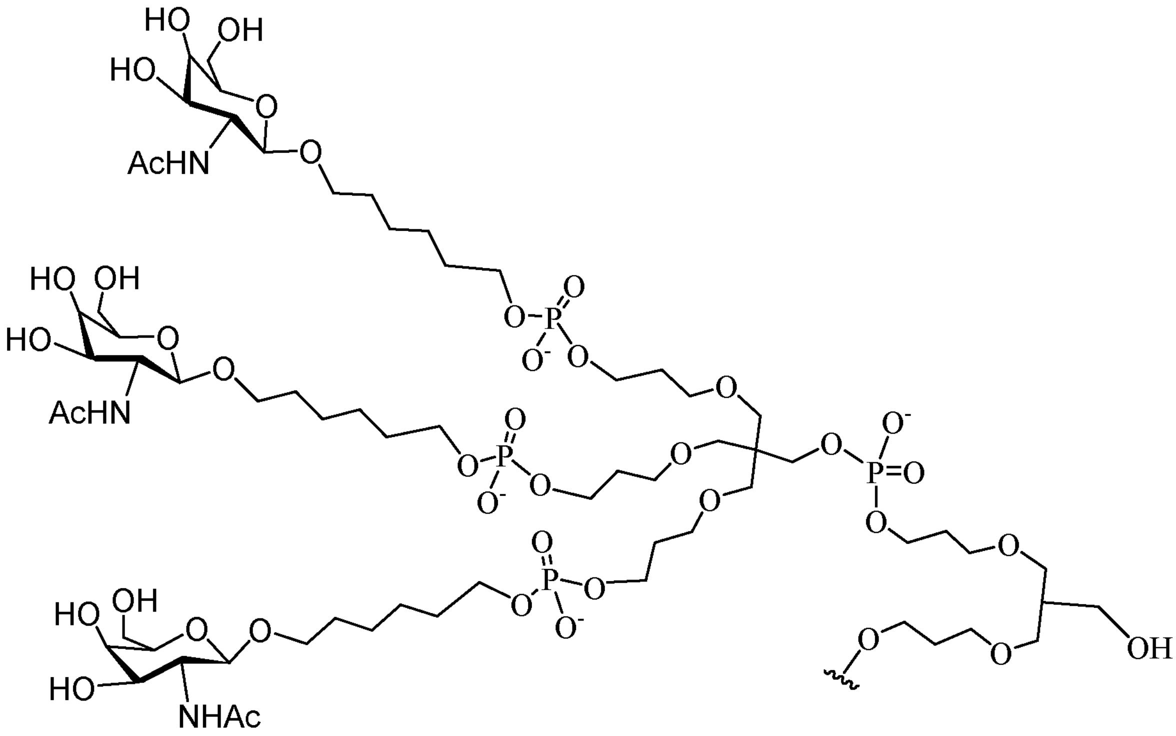
Wherein GalNAc$_3$-4 has the structure:



Wherein CM is a cleavable moiety. In certain embodiments, cleavable moiety is:



The GalNAc$_3$ cluster portion of the conjugate group GalNAc$_3$-4 (GalNAc$_3$-4$_a$) can be combined with any cleavable moiety to provide a variety of conjugate groups. Wherein GalNAc$_3$-4$_a$ has the formula:


HO
OH
HO
O
AcHN
O
O
P
O
O
O-
O
O
HO
OH
HO
O
AcHN
O
O
P
O
O
O-
O
O
O
O
P
O
O-
O
O
HO
OH
HO
O
NHAc
O
O
P
O
O
O-
O
O
O
OH

The protected Unylinker functionalized solid support Compound 30 is commercially available. Compound 84 is prepared using procedures similar to those reported in the literature (*see* Shchepinov *et al., Nucleic Acids Research,* 1997, *25(22)*, 4447-4454; Shchepinov *et al., Nucleic Acids Research,* 1999, *27*, 3035-3041; and Hornet *et al., Nucleic Acids Research,* 1997, *25*, 4842-4849).

The phosphoramidite building blocks, Compounds 60 and 79a are prepared as per the procedures illustrated in Examples 28 and 36. The phosphoramidites illustrated are meant to be representative and not intended to be limiting as other phosphoramidite building blocks can be used to prepare an oligomeric compound having a phosphodiester linked conjugate at the 3' terminus with a predetermined sequence and composition. The order and quantity of phosphoramidites added to the solid support can be adjusted to prepare the oligomeric compounds as described herein having any predetermined sequence and composition.

Example 41: General method for the preparation of ASOs comprising a phosphodiester linked GalNAc$_3$-2 (see Example 37, Bx is adenine) conjugate at the 5' position *via* solid phase techniques (preparation of ISIS 661134)

Unless otherwise stated, all reagents and solutions used for the synthesis of oligomeric compounds are purchased from commercial sources. Standard phosphoramidite building blocks and solid support are used for incorporation nucleoside residues which include for example T, A, G, and ^{m}C residues. Phosphoramidite compounds 56 and 60 were used to synthesize the phosphodiester linked GalNAc$_3$-2 conjugate at the 5' terminus. A 0.1 M solution of phosphoramidite in anhydrous acetonitrile was used for β-D-2'-deoxyribonucleoside and 2'-MOE.

The ASO syntheses were performed on ABI 394 synthesizer (1-2 μmol scale) or on GE Healthcare Bioscience ÄKTA oligopilot synthesizer (40-200 μmol scale) by the phosphoramidite coupling method on VIMAD solid support (110 μmol/g, Guzaev *et al.*, 2003) packed in the column. For the coupling step, the phosphoramidites were delivered at a 4 fold excess over the initial loading of the solid support and phosphoramidite coupling was carried out for 10 min. All other steps followed standard protocols supplied by the manufacturer. A solution of 6% dichloroacetic acid in toluene was used for removing the dimethoxytrityl (DMT) groups from 5'-hydroxyl groups of the nucleotide. 4,5-Dicyanoimidazole (0.7 M) in anhydrous CH_3CN was used as activator during the

coupling step. Phosphorothioate linkages were introduced by sulfurization with 0.1 M solution of xanthane hydride in 1:1 pyridine/CH_3CN for a contact time of 3 minutes. A solution of 20% *tert*-butylhydroperoxide in CH_3CN containing 6% water was used as an oxidizing agent to provide phosphodiester internucleoside linkages with a contact time of 12 minutes.

After the desired sequence was assembled, the cyanoethyl phosphate protecting groups were deprotected using a 20% diethylamine in toluene (v/v) with a contact time of 45 minutes. The solid-support bound ASOs were suspended in aqueous ammonia (28-30 wt %) and heated at 55 °C for 6 h. The unbound ASOs were then filtered and the ammonia was boiled off. The residue was purified by high pressure liquid chromatography on a strong anion exchange column (GE Healthcare Bioscience, Source 30Q, 30 μm, 2.54 x 8 cm, A = 100 mM ammonium acetate in 30% aqueous CH_3CN, B = 1.5 M NaBr in A, 0-40% of B in 60 min, flow 14 mL min-1, λ = 260 nm). The residue was desalted by HPLC on a reverse phase column to yield the desired ASOs in an isolated yield of 15-30% based on the initial loading on the solid support. The ASOs were characterized by ion-pair-HPLC coupled MS analysis with Agilent 1100 MSD system.

Table 34

ASO comprising a phosphodiester linked $GalNAc_3$-2 conjugate at the 5' position targeting SRB-1

ISIS No.	Sequence (5' to 3')	CalCd Mass	Observed Mass	SEQ ID No.
661134	**$GalNAc_3$-2_a-$_{o'}$$A_{do}$**$T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{k}$	6482.2	6481.6	827

Subscripts: "e" indicates 2'-MOE modified nucleoside; "d" indicates β-D-2'-deoxyribonucleoside; "k" indicates 6'-*(S)*-CH_3 bicyclic nucleoside (e.g. cEt); "s" indicates phosphorothioate internucleoside linkages (PS); "o" indicates phosphodiester internucleoside linkages (PO); and "o'" indicates -O-P(=O)(OH)-. Superscript "m" indicates 5-methylcytosines. The structure of $GalNAc_3$-2_a is shown in Example 37.

Example 42: General method for the preparation of ASOs comprising a $GalNAc_3$-3 conjugate at the 5' position *via* solid phase techniques (preparation of ISIS 661166)

The synthesis for ISIS 661166 was performed using similar procedures as illustrated in Examples 39 and 41.

ISIS 661166 is a 5-10-5 MOE gapmer, wherein the 5' position comprises a $GalNAc_3$-3 conjugate. The ASO was characterized by ion-pair-HPLC coupled MS analysis with Agilent 1100 MSD system.

Table 34a
ASO comprising a $GalNAc_3$-3 conjugate at the 5' position via a hexylamino phosphodiester linkage targeting Malat-1

ISIS No.	Sequence (5' to 3')	Conjugate	Calcd Mass	Observed Mass	SEQ ID No.
661166	5'-**$GalNAc_3$-3_{a-o}**-$^{m}C_{es}G_{es}G_{es}T_{es}G_{es}$ $^{m}C_{ds}A_{ds}A_{ds}G_{ds}G_{ds}$ $^{m}C_{ds}T_{ds}T_{ds}A_{ds}G_{ds}$ $G_{es}A_{es}A_{es}T_{es}T_{e}$	**5'-$GalNAc_3$-3**	8992.16	8990.51	828

Subscripts: "e" indicates 2'-MOE modified nucleoside; "d" indicates β-D-2'-deoxyribonucleoside; "s" indicates phosphorothioate internucleoside linkages (PS); "o" indicates phosphodiester internucleoside linkages (PO); and "o'" indicates -O-P(=O)(OH)-. Superscript "m" indicates 5-methylcytosines. The structure of "5'-$GalNAc_3$-3a" is shown in Example 39.

Example 43: Dose-dependent study of phosphodiester linked $GalNAc_3$-2 (see examples 37 and 41, Bx is adenine) at the 5' terminus targeting SRB-1 *in vivo*

ISIS 661134 (see Example 41) comprising a phosphodiester linked $GalNAc_3$-2 conjugate at the 5' terminus was tested in a dose-dependent study for antisense inhibition of SRB-1 in mice. Unconjugated ISIS 440762 and 651900 ($GalNAc_3$-1 conjugate at 3' terminus, see Example 9) were included in the study for comparison and are described previously in Table 17.

Treatment

Six week old male Balb/c mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously once at the dosage shown below with ISIS 440762, 651900, 661134 or with PBS treated control. Each treatment group consisted of 4 animals. The mice were sacrificed 72 hours following the final administration to determine the liver SRB-1 mRNA levels using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. SRB-1 mRNA levels were determined relative to total RNA (using Ribogreen), prior to normalization to PBS-treated control. The results below are presented as the average percent of SRB-1 mRNA levels for each treatment group, normalized to PBS-treated control and is

denoted as "% PBS". The ED_{50}s were measured using similar methods as described previously and are presented below.

As illustrated in Table 35, treatment with antisense oligonucleotides lowered SRB-1 mRNA levels in a dose-dependent manner. Indeed, the antisense oligonucleotides comprising the phosphodiester linked $GalNAc_3$-2 conjugate at the 5' terminus (ISIS 661134) or the $GalNAc_3$-1 conjugate linked at the 3' terminus (ISIS 651900) showed substantial improvement in potency compared to the unconjugated antisense oligonucleotide (ISIS 440762). Further, ISIS 661134, which comprises the phosphodiester linked $GalNAc_3$-2 conjugate at the 5' terminus was equipotent compared to ISIS 651900, which comprises the $GalNAc_3$-1 conjugate at the 3' terminus.

Table 35
ASOs containing $GalNAc_3$-1 or $GalNAc_3$-2 targeting SRB-1

ISIS No.	Dosage (mg/kg)	SRB-1 mRNA levels (% PBS)	ED_{50} (mg/kg)	Conjugate	SEQ ID No.
PBS	0	100	--	--	
440762	0.2	116	2.58	No conjugate	823
	0.7	91			
	2	69			
	7	22			
	20	5			
651900	0.07	95	0.26	**3' $GalNAc_3$-1**	824
	0.2	77			
	0.7	28			
	2	11			
	7	8			
661134	0.07	107	0.25	**5' $GalNAc_3$-2**	827
	0.2	86			
	0.7	28			
	2	10			
	7	6			

Structures for 3' $GalNAc_3$-1 and 5' $GalNAc_3$-2 were described previously in Examples 9 and 37.

Pharmacokinetics Analysis (PK)

The PK of the ASOs from the high dose group (7 mg/kg) was examined and evaluated in the same manner as illustrated in Example 20. Liver sample was minced and extracted using standard protocols. The full length metabolites of 661134 (5' $GalNAc_3$-2) and ISIS 651900 (3' $GalNAc_3$-1) were identified and their masses were confirmed by high resolution mass spectrometry analysis.

The results showed that the major metabolite detected for the ASO comprising a phosphodiester linked $GalNAc_3$-2 conjugate at the 5' terminus (ISIS 661134) was ISIS 440762 (data not shown). No additional metabolites, at a detectable level, were observed. Unlike its counterpart, additional metabolites similar to those reported previously in Table 23a were observed for the ASO having the $GalNAc_3$-1 conjugate at the 3' terminus (ISIS 651900). These results suggest that having the phosphodiester linked $GalNAc_3$-1 or $GalNAc_3$-2 conjugate may improve the PK profile of ASOs without compromising their potency.

Example 44: Effect of PO/PS linkages on antisense inhibition of ASOs comprising $GalNAc_3$-1 conjugate (see Example 9) at the 3' terminus targeting SRB-1

ISIS 655861 and 655862 comprising a $GalNAc_3$-1 conjugate at the 3' terminus each targeting SRB-1 were tested in a single administration study for their ability to inhibit SRB-1 in mice. The parent unconjugated compound, ISIS 353382 was included in the study for comparison.

The ASOs are 5-10-5 MOE gapmers, wherein the gap region comprises ten 2'-deoxyribonucleosides and each wing region comprises five 2'-MOE modified nucleosides. The ASOs were prepared using similar methods as illustrated previously in Example 19 and are described Table 36, below.

Table 36

Modified ASOs comprising $GalNAc_3$-1 conjugate at the 3' terminus targeting SRB-1

ISIS No.	Sequence (5' to 3')	Chemistry	SEQ ID No.
353382 (parent)	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	Full PS no conjugate	829
655861	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{eo}\mathbf{A_{do'}\text{-}GalNAc_3\text{-}1_a}$	Full PS with **$GalNAc_3$-1** conjugate	830
655862	$G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T_{eo}\mathbf{A_{do'}\text{-}GalNAc_3\text{-}1_a}$	Mixed PS/PO with **$GalNAc_3$-1** conjugate	830

Subscripts: "e" indicates 2'-MOE modified nucleoside; "d" indicates β-D-2'-deoxyribonucleoside; "s" indicates phosphorothioate internucleoside linkages (PS); "o" indicates phosphodiester internucleoside linkages (PO); and "o'" indicates -O-P(=O)(OH)-. Superscript "m" indicates 5-methylcytosines. The structure of "$GalNAc_3$-1" is shown in Example 9.

Treatment

Six week old male Balb/c mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously once at the dosage shown below with ISIS 353382, 655861, 655862 or with PBS treated control. Each treatment group consisted of 4 animals. Prior to the treatment as well as after the last dose, blood was drawn from each mouse and plasma samples were analyzed. The mice were sacrificed 72 hours following the final administration to determine the liver SRB-1 mRNA levels using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. SRB-1 mRNA levels were determined relative to total RNA (using Ribogreen), prior to normalization to PBS-treated control. The results below are presented as the average percent of SRB-1 mRNA levels for each treatment group, normalized to PBS-treated control and is denoted as "% PBS". The ED_{50}s were measured using similar methods as described previously and are reported below.

As illustrated in Table 37, treatment with antisense oligonucleotides lowered SRB-1 mRNA levels in a dose-dependent manner compared to PBS treated control. Indeed, the antisense oligonucleotides comprising the $GalNAc_3$-1 conjugate at the 3' terminus (ISIS 655861 and 655862) showed substantial improvement in potency comparing to the unconjugated antisense oligonucleotide (ISIS 353382). Further, ISIS 655862 with mixed PS/PO linkages showed an improvement in potency relative to full PS (ISIS 655861).

Table 37
Effect of PO/PS linkages on antisense inhibition of ASOs comprising $GalNAc_3$-1 conjugate at 3' terminus targeting SRB-1

ISIS No.	Dosage (mg/kg)	SRB-1 mRNA levels (% PBS)	ED_{50} (mg/kg)	Chemistry	SEQ ID No.
PBS	0	100	--	--	
353382 (parent)	3	76.65	10.4	Full PS without conjugate	829
	10	52.40			
	30	24.95			
655861	0.5	81.22	2.2	Full PS with $GalNAc_3$-1 conjugate	830
	1.5	63.51			
	5	24.61			
	15	14.80			
655862	0.5	69.57	1.3	Mixed PS/PO with $GalNAc_3$-1 conjugate	830
	1.5	45.78			
	5	19.70			
	15	12.90			

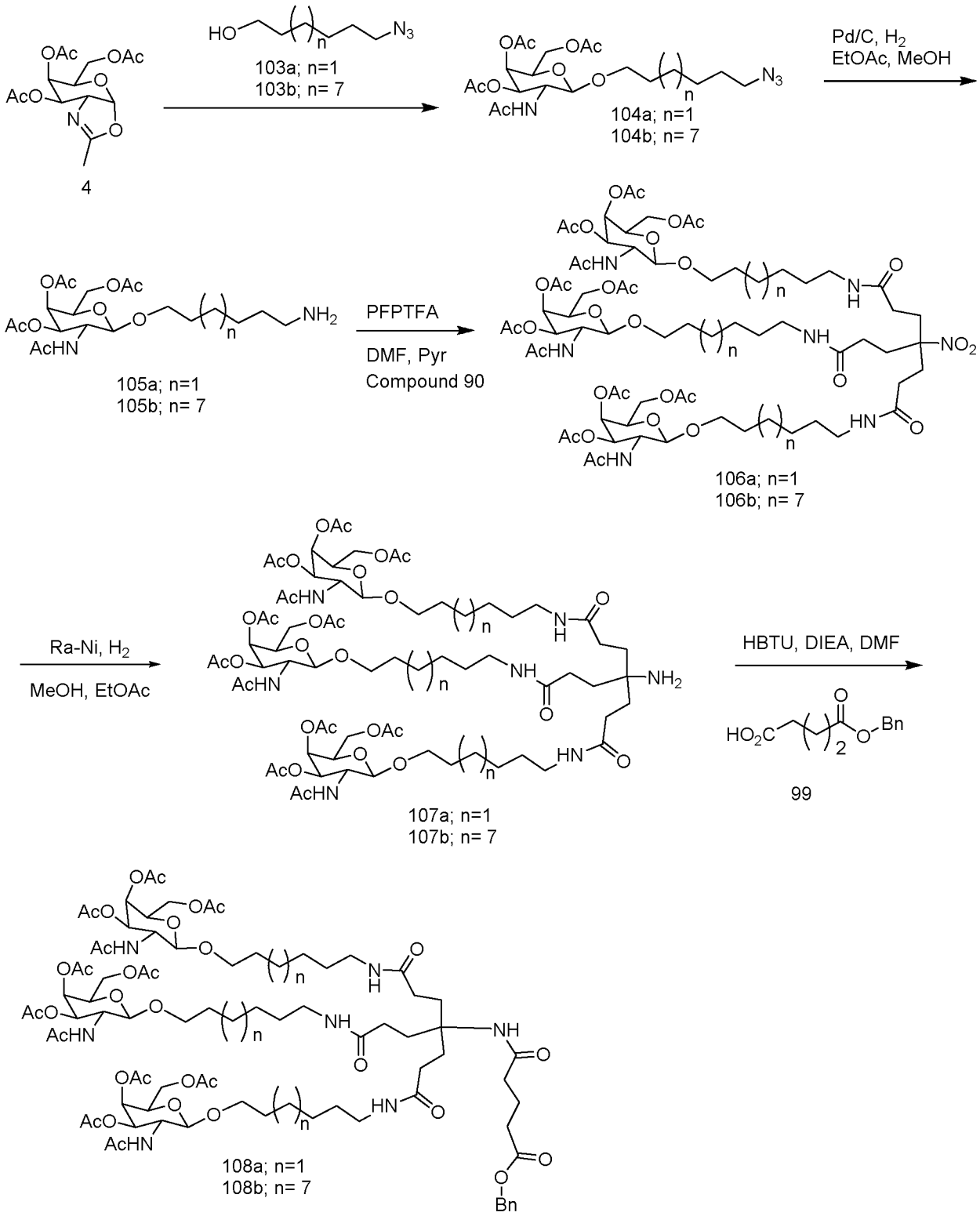
Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum were measured relative to saline injected mice using standard protocols. Organ

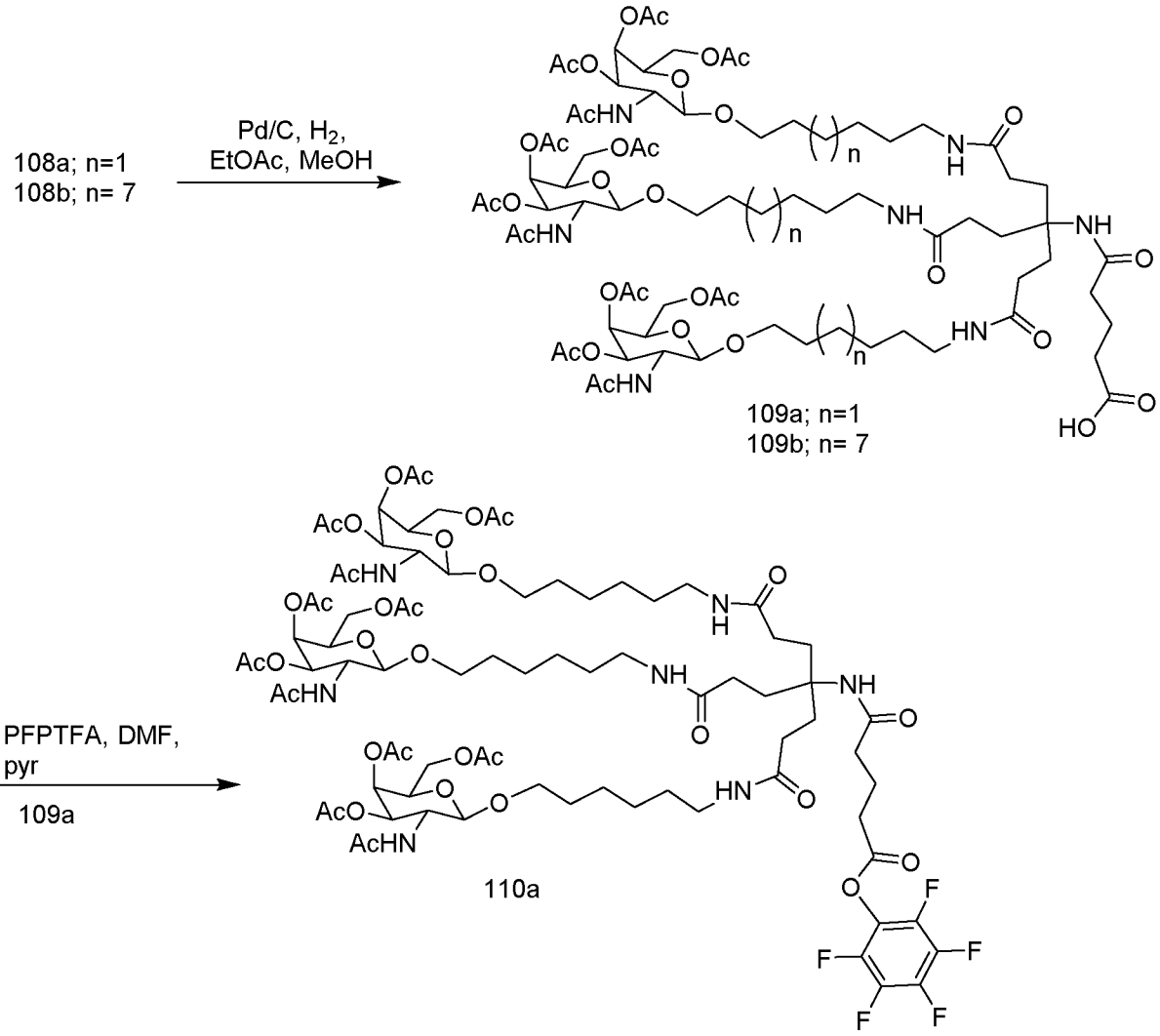
weights were also evaluated. The results demonstrated that no elevation in transaminase levels (Table 38) or organ weights (data not shown) were observed in mice treated with ASOs compared to PBS control. Further, the ASO with mixed PS/PO linkages (ISIS 655862) showed similar transaminase levels compared to full PS (ISIS 655861).

Table 38
Effect of PO/PS linkages on transaminase levels of ASOs comprising $GalNAc_3$-1 conjugate at 3' terminus targeting SRB-1

ISIS No.	Dosage (mg/kg)	ALT (U/L)	AST (U/L)	Chemistry	SEQ ID No.
PBS	0	28.5	65	--	
353382 (parent)	3	50.25	89	Full PS without conjugate	829
	10	27.5	79.3		
	30	27.3	97		
655861	0.5	28	55.7	Full PS with **$GalNAc_3$-1**	830
	1.5	30	78		
	5	29	63.5		
	15	28.8	67.8		
655862	0.5	50	75.5	Mixed PS/PO with **$GalNAc_3$-1**	830
	1.5	21.7	58.5		
	5	29.3	69		
	15	22	61		

Example 45: Preparation of PFP Ester, Compound 110a





Compound 4 (9.5g, 28.8 mmoles) was treated with compound 103a or 103b (38 mmoles), individually, and TMSOTf (0.5 eq.) and molecular sieves in dichloromethane (200 mL), and stirred for 16 hours at room temperature. At that time, the organic layer was filtered thru celite, then washed with sodium bicarbonate, water and brine. The organic layer was then separated and dried over sodium sulfate, filtered and reduced under reduced pressure. The resultant oil was purified by silica gel chromatography (2%-->10% methanol/dichloromethane) to give compounds 104a and 104b in >80% yield. LCMS and proton NMR was consistent with the structure.

Compounds 104a and 104b were treated to the same conditions as for compounds 100a-d (Example 47), to give compounds 105a and 105b in >90% yield. LCMS and proton NMR was consistent with the structure.

Compounds 105a and 105b were treated, individually, with compound 90 under the same conditions as for compounds 901a-d, to give compounds 106a (80%) and 106b (20%). LCMS and proton NMR was consistent with the structure.

Compounds 106a and 106b were treated to the same conditions as for compounds 96a-d (Example 47), to give 107a (60%) and 107b (20%). LCMS and proton NMR was consistent with the structure.

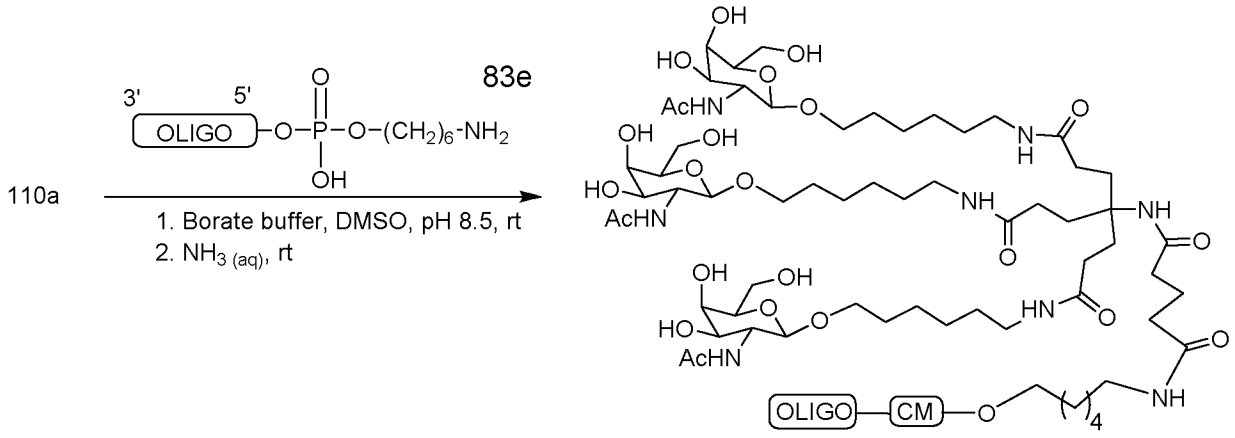
Compounds 107a and 107b were treated to the same conditions as for compounds 97a-d (Example 47), to give compounds 108a and 108b in 40-60% yield. LCMS and proton NMR was consistent with the structure.

Compounds 108a (60%) and 108b (40%) were treated to the same conditions as for compounds 100a-d (Example 47), to give compounds 109a and 109b in >80% yields. LCMS and proton NMR was consistent with the structure.

Compound 109a was treated to the same conditions as for compounds 101a-d (Example 47), to give Compound 110a in 30-60% yield. LCMS and proton NMR was consistent with the structure. Alternatively, Compound 110b can be prepared in a similar manner starting with Compound 109b.

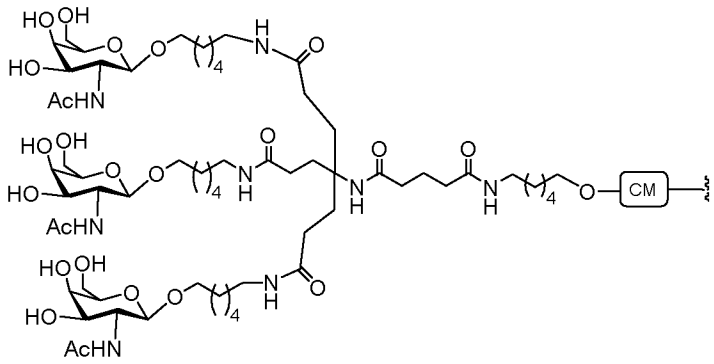
Example 46: General Procedure for Conjugation with PFP Esters (Oligonucleotide 111); Preparation of ISIS 666881 ($GalNAc_3$-10)

A 5'-hexylamino modified oligonucleotide was synthesized and purified using standard solid-phase oligonucleotide procedures. The 5'-hexylamino modified oligonucleotide was dissolved in 0.1 M sodium tetraborate, pH 8.5 (200 μL) and 3 equivalents of a selected PFP esterified $GalNAc_3$ cluster dissolved in DMSO (50 μL) was added. If the PFP ester precipitated upon addition to the ASO solution DMSO was added until all PFP ester was in solution. The reaction was complete after about 16 h of mixing at room temperature. The resulting solution was diluted with water to 12 mL and then spun down at 3000 rpm in a spin filter with a mass cut off of 3000 Da. This process was repeated twice to remove small molecule impurities. The solution was then lyophilized to dryness and redissolved in concentrated aqueous ammonia and mixed at room temperature for 2.5 h followed by concentration *in vacuo* to remove most of the ammonia. The conjugated oligonucleotide was purified and desalted by RP-HPLC and lyophilized to provide the $GalNAc_3$ conjugated oligonucleotide.



111

Oligonucleotide 111 is conjugated with $GalNAc_3$-10. The $GalNAc_3$ cluster portion of the conjugate group $GalNAc_3$-10 ($GalNAc_3$-10_a) can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the cleavable moiety is -P(=O)(OH)-A_d-P(=O)(OH)- as shown in the oligonucleotide (ISIS 666881) synthesized with $GalNAc_3$-10 below. The structure of $GalNAc_3$-10 ($GalNAc_3$-10_a-CM-) is shown below:



Following this general procedure ISIS 666881 was prepared. 5'-hexylamino modified oligonucleotide, ISIS 660254, was synthesized and purified using standard solid-phase oligonucleotide procedures. ISIS 660254 (40 mg, 5.2 μmol) was dissolved in 0.1 M sodium tetraborate, pH 8.5 (200 μL) and 3 equivalents PFP ester (Compound 110a) dissolved in DMSO (50 μL) was added. The PFP ester precipitated upon addition to the ASO solution requiring additional DMSO (600 μL) to fully dissolve the PFP ester. The reaction was complete after 16 h of mixing at room temperature. The solution was diluted with water to 12 mL total volume and spun down at 3000 rpm in a spin filter with a mass cut off of 3000 Da. This process was repeated twice to remove small molecule impurities. The solution was lyophilized to dryness and redissolved in concentrated aqueous ammonia with mixing at room temperature for 2.5 h followed by concentration *in vacuo* to

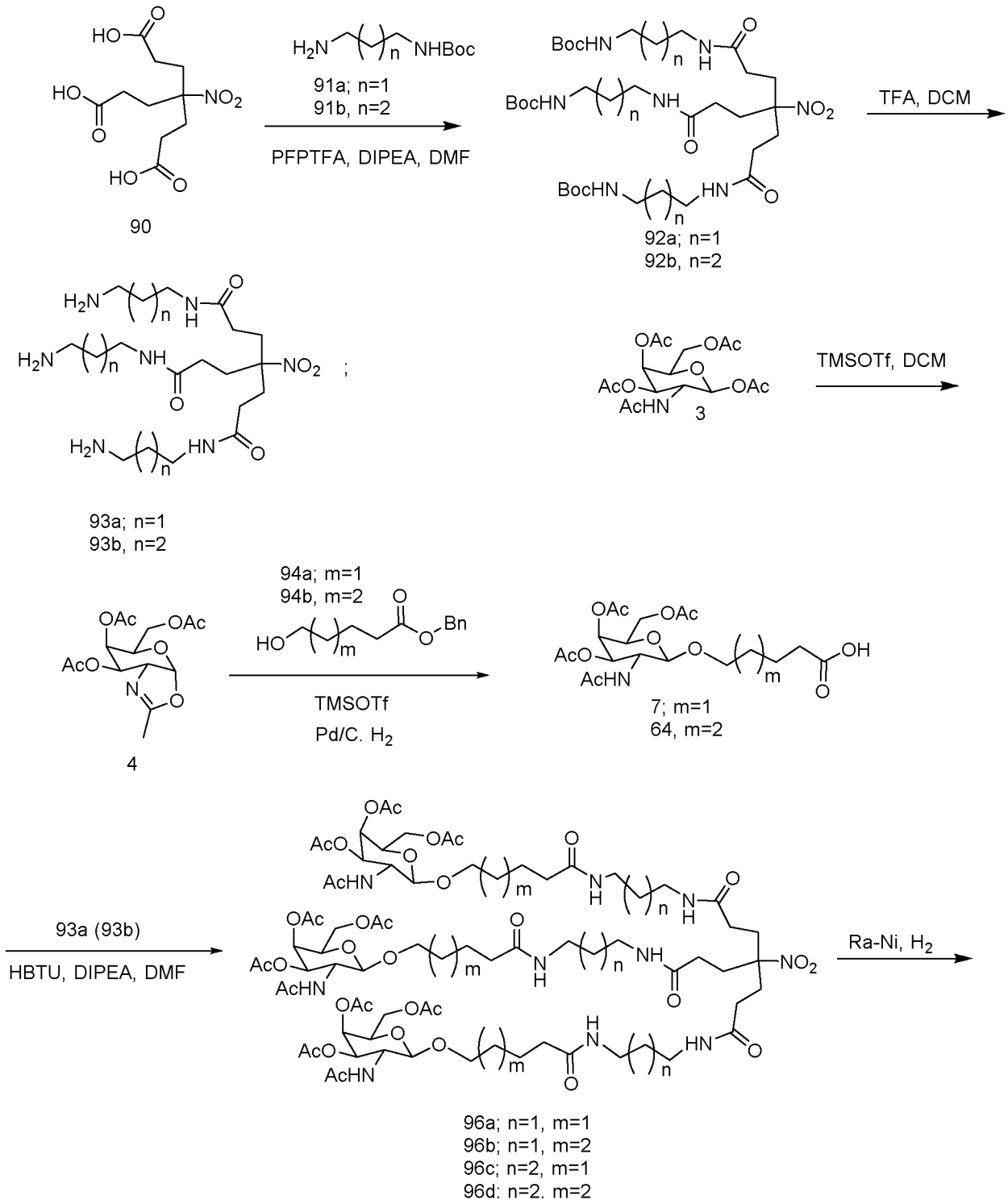
remove most of the ammonia. The conjugated oligonucleotide was purified and desalted by RP-HPLC and lyophilized to give ISIS 666881 in 90% yield by weight (42 mg, 4.7 μmol).

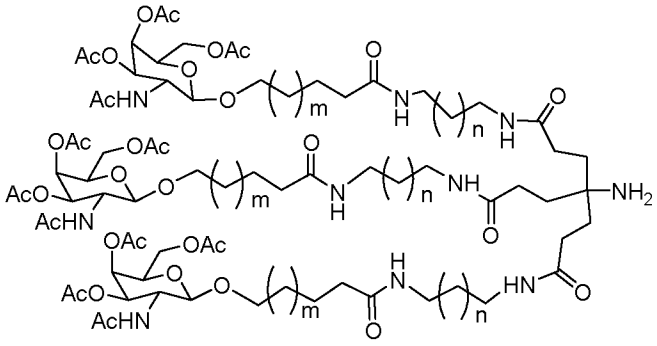
$GalNAc_3$-10 conjugated oligonucleotide

ASO	Sequence (5' to 3')	5' group	SEQ ID No.
ISIS 660254	$NH_2(CH_2)_6$- $_oA_{do}G_{es}{}^mC_{es}T_{es}T_{es}{}^mC_{es}A_{ds}G_{ds}T_{ds}$ $^mC_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^mC_{ds}T_{ds}T_{es}{}^mC_{es}{}^mC_{es}T_{es}T_e$	Hexylamine	831
ISIS 666881	**$GalNAc_3$-10_a-** **$_o$'A_{do}**$G_{es}{}^mC_{es}T_{es}T_{es}{}^mC_{es}A_{ds}G_{ds}T_{ds}$ $^mC_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^mC_{ds}T_{ds}T_{es}{}^mC_{es}{}^mC_{es}T_{es}T_e$	**$GalNAc_3$-10**	831

Capital letters indicate the nucleobase for each nucleoside and ^mC indicates a 5-methyl cytosine. Subscripts: "e" indicates a 2'-MOE modified nucleoside; "d" indicates a β-D-2'-deoxyribonucleoside; "s" indicates a phosphorothioate internucleoside linkage (PS); "o" indicates a phosphodiester internucleoside linkage (PO); and "o'" indicates -O-P(=O)(OH)-. Conjugate groups are in bold.

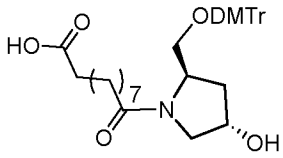
Example 47: Preparation of Oligonucleotide 102 Comprising GalNAc$_3$-8



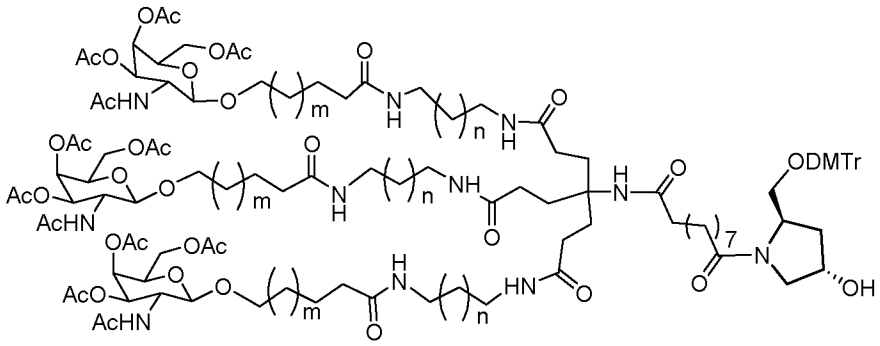


97a; n=1, m=1
97b; n=1, m=2
97c; n=2, m=1
97d; n=2, m=2

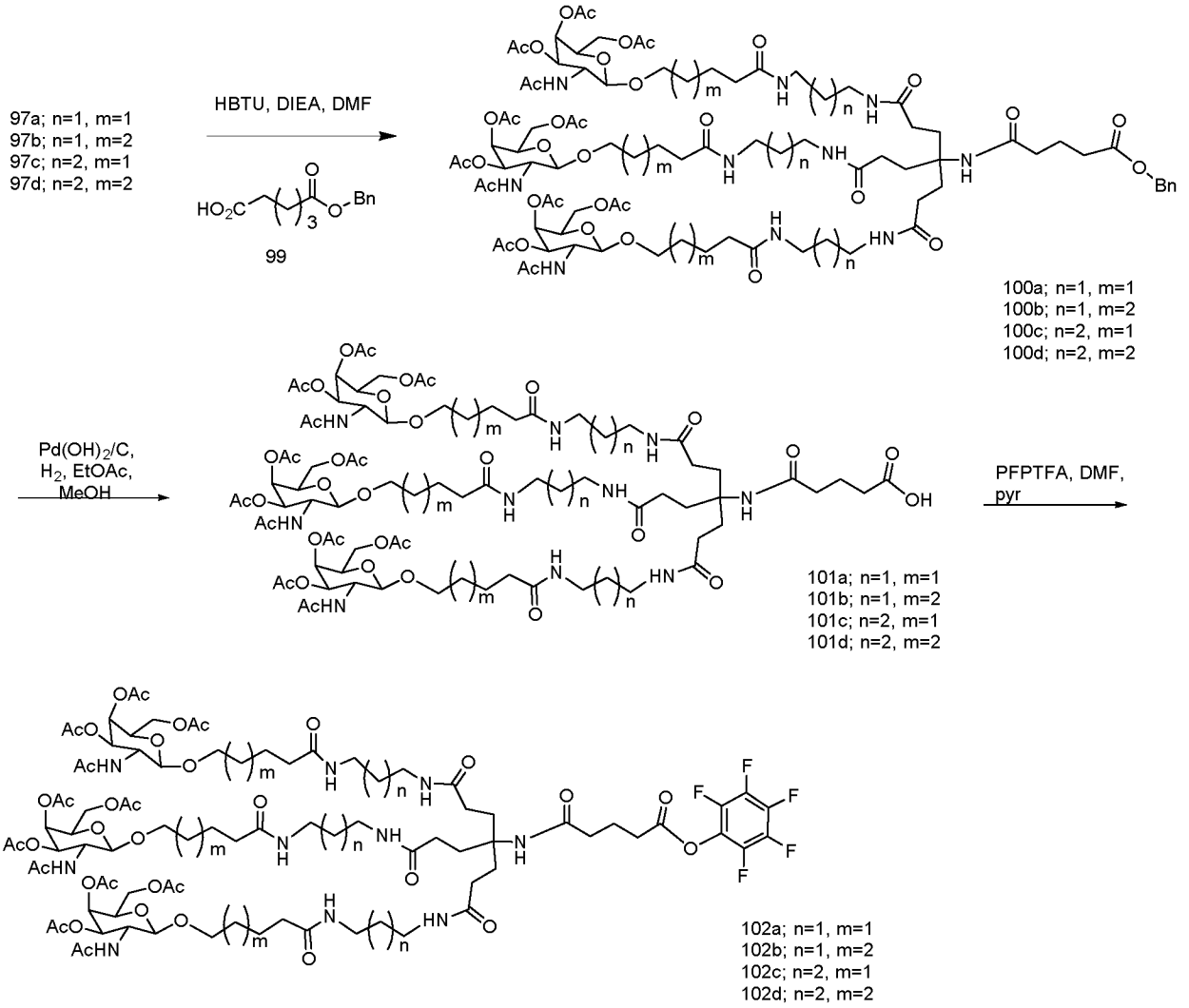
HBTU, DIEA, DMF



23



98a; n=1, m=1
98b; n=1, m=2
98c; n=2, m=1
98d; n=2, m=2



The triacid 90 (4 g, 14.43 mmol) was dissolved in DMF (120 mL) and *N*,*N*-Diisopropylethylamine (12.35 mL, 72 mmoles). Pentafluorophenyl trifluoroacetate (8.9 mL, 52 mmoles) was added dropwise, under argon, and the reaction was allowed to stir at room temperature for 30 minutes. Boc-diamine 91a or 91b (68.87 mmol) was added, along with *N*,*N*-Diisopropylethylamine (12.35 mL, 72 mmoles), and the reaction was allowed to stir at room temperature for 16 hours. At that time, the DMF was reduced by >75% under reduced pressure, and then the mixture was dissolved in dichloromethane. The organic layer was washed with sodium bicarbonate, water and brine. The organic layer was then separated and dried over sodium sulfate, filtered and reduced to an oil under reduced pressure. The resultant oil was purified by silica gel chromatography (2%-->10% methanol/dichloromethane) to give compounds 92a and 92b in an approximate 80% yield. LCMS and proton NMR were consistent with the structure.

Compound 92a or 92b (6.7 mmoles) was treated with 20 mL of dichloromethane and 20 mL of trifluoroacetic acid at room temperature for 16 hours. The resultant solution was evaporated and then dissolved in methanol and treated with DOWEX-OH resin for 30 minutes. The resultant solution was filtered and reduced to an oil under reduced pressure to give 85-90% yield of compounds 93a and 93b.

Compounds 7 or 64 (9.6 mmoles) were treated with HBTU (3.7g, 9.6 mmoles) and *N*,*N*-Diisopropylethylamine (5 mL) in DMF (20 mL) for 15 minutes. To this was added either compounds 93a or 93b (3 mmoles), and allowed to stir at room temperature for 16 hours. At that time, the DMF was reduced by >75% under reduced pressure, and then the mixture was dissolved in dichloromethane. The organic layer was washed with sodium bicarbonate, water and brine. The organic layer was then separated and dried over sodium sulfate, filtered and reduced to an oil under reduced pressure. The resultant oil was purified by silica gel chromatography (5%-->20% methanol/dichloromethane) to give compounds 96a-d in 20-40% yield. LCMS and proton NMR was consistent with the structure.

Compounds 96a-d (0.75 mmoles), individually, were hydrogenated over Raney Nickel for 3 hours in Ethanol (75 mL). At that time, the catalyst was removed by filtration thru celite, and the ethanol removed under reduced pressure to give compounds 97a-d in 80-90% yield. LCMS and proton NMR were consistent with the structure.

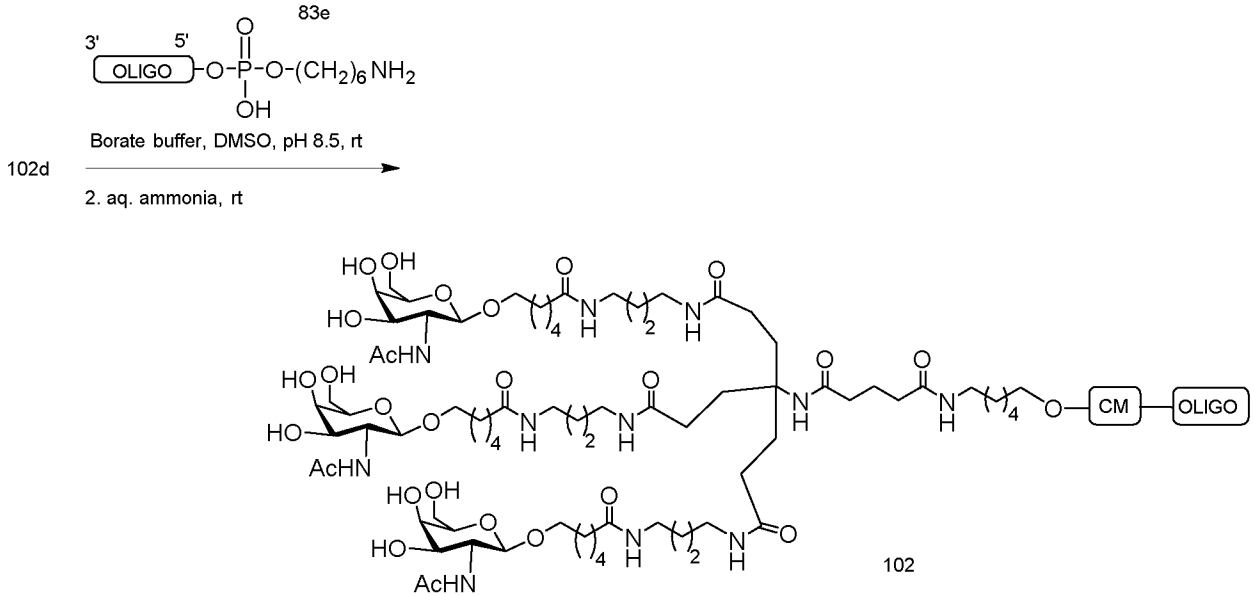
Compound 23 (0.32g, 0.53 mmoles) was treated with HBTU (0.2g, 0.53 mmoles) and *N*,*N*-Diisopropylethylamine (0.19 mL, 1.14 mmoles) in DMF (30mL) for 15 minutes. To this was added compounds 97a-d (0.38 mmoles), individually, and allowed to stir at room temperature for 16 hours. At that time, the DMF was reduced by >75% under reduced pressure, and then the mixture was dissolved in dichloromethane. The organic layer was washed with sodium bicarbonate, water and brine. The organic layer was then separated and dried over sodium sulfate, filtered and reduced to an oil under reduced pressure. The resultant oil was purified by silica gel chromatography (2%-->20% methanol/dichloromethane) to give compounds 98a-d in 30-40% yield. LCMS and proton NMR was consistent with the structure.

Compound 99 (0.17g, 0.76 mmoles) was treated with HBTU (0.29 g, 0.76 mmoles) and *N*,*N*-Diisopropylethylamine (0.35 mL, 2.0 mmoles) in DMF (50mL) for 15 minutes. To this was added compounds 97a-d (0.51 mmoles), individually, and allowed to stir at room temperature for 16 hours. At that time, the DMF was reduced by >75% under reduced pressure, and then the mixture was dissolved in dichloromethane. The organic layer was washed with sodium bicarbonate, water and

brine. The organic layer was then separated and dried over sodium sulfate, filtered and reduced to an oil under reduced pressure. The resultant oil was purified by silica gel chromatography (5%-->20% methanol/ dichloromethane) to give compounds 100a-d in 40-60% yield. LCMS and proton NMR was consistent with the structure.

Compounds 100a-d (0.16 mmoles), individually, were hydrogenated over 10% $Pd(OH)_2/C$ for 3 hours in methanol/ethyl acetate (1:1, 50 mL). At that time, the catalyst was removed by filtration thru celite, and the organics removed under reduced pressure to give compounds 101a-d in 80-90% yield. LCMS and proton NMR was consistent with the structure.

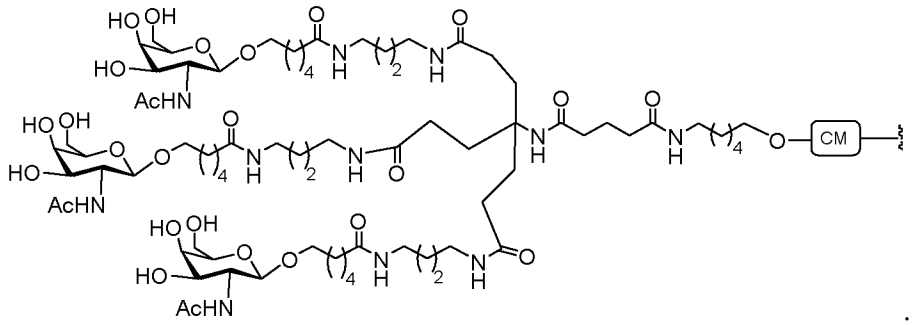
Compounds 101a-d (0.15 mmoles), individually, were dissolved in DMF (15 mL) and pyridine (0.016 mL, 0.2 mmoles). Pentafluorophenyl trifluoroacetate (0.034 mL, 0.2 mmoles) was added dropwise, under argon, and the reaction was allowed to stir at room temperature for 30 minutes. At that time, the DMF was reduced by >75% under reduced pressure, and then the mixture was dissolved in dichloromethane. The organic layer was washed with sodium bicarbonate, water and brine. The organic layer was then separated and dried over sodium sulfate, filtered and reduced to an oil under reduced pressure. The resultant oil was purified by silica gel chromatography (2%-->5% methanol/dichloromethane) to give compounds 102a-d in an approximate 80% yield. LCMS and proton NMR were consistent with the structure.



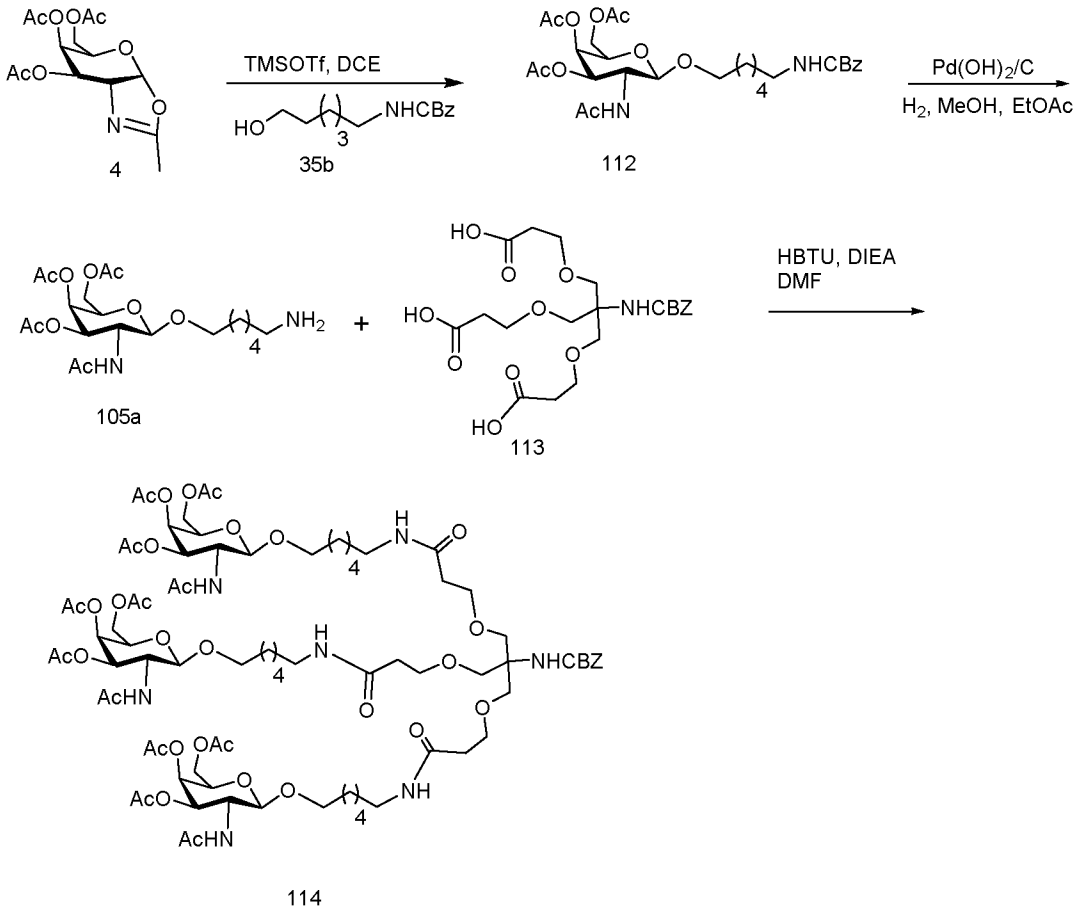
Oligomeric Compound 102, comprising a $GalNAc_3$-8 conjugate group, was prepared using the general procedures illustrated in Example 46. The $GalNAc_3$ cluster portion of the conjugate

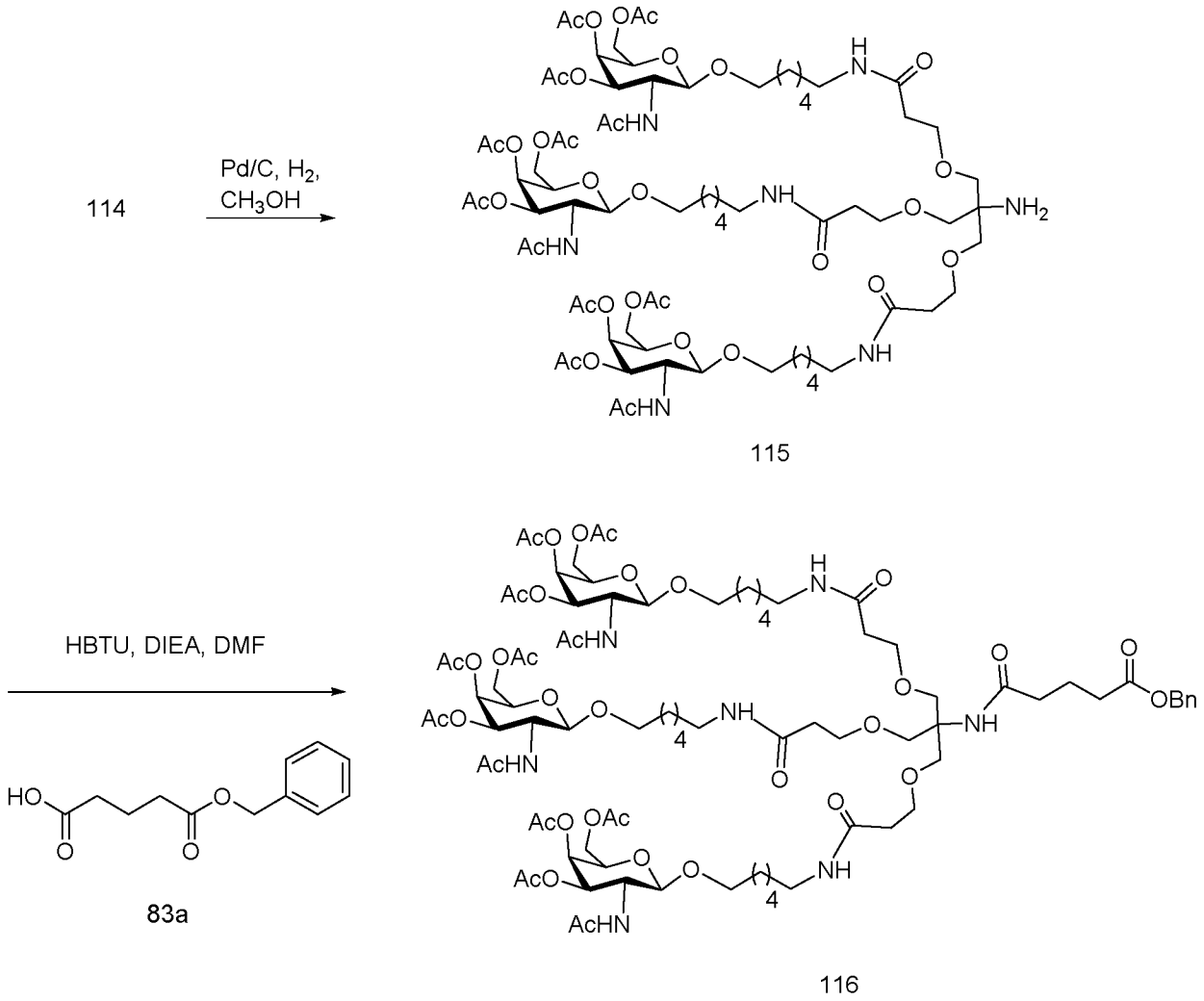
group GalNAc$_3$-8 (GalNAc$_3$-8$_a$) can be combined with any cleavable moiety to provide a variety of conjugate groups. In a preferred embodiment, the cleavable moiety is -P(=O)(OH)-A$_d$-P(=O)(OH)-.

The structure of GalNAc$_3$-8 (GalNAc$_3$-8$_a$-CM-) is shown below:



Example 48: Preparation of Oligonucleotide 119 Comprising GalNAc$_3$-7





Compound 112 was synthesized following the procedure described in the literature (*J. Med. Chem.* 2004, *47*, 5798-5808).

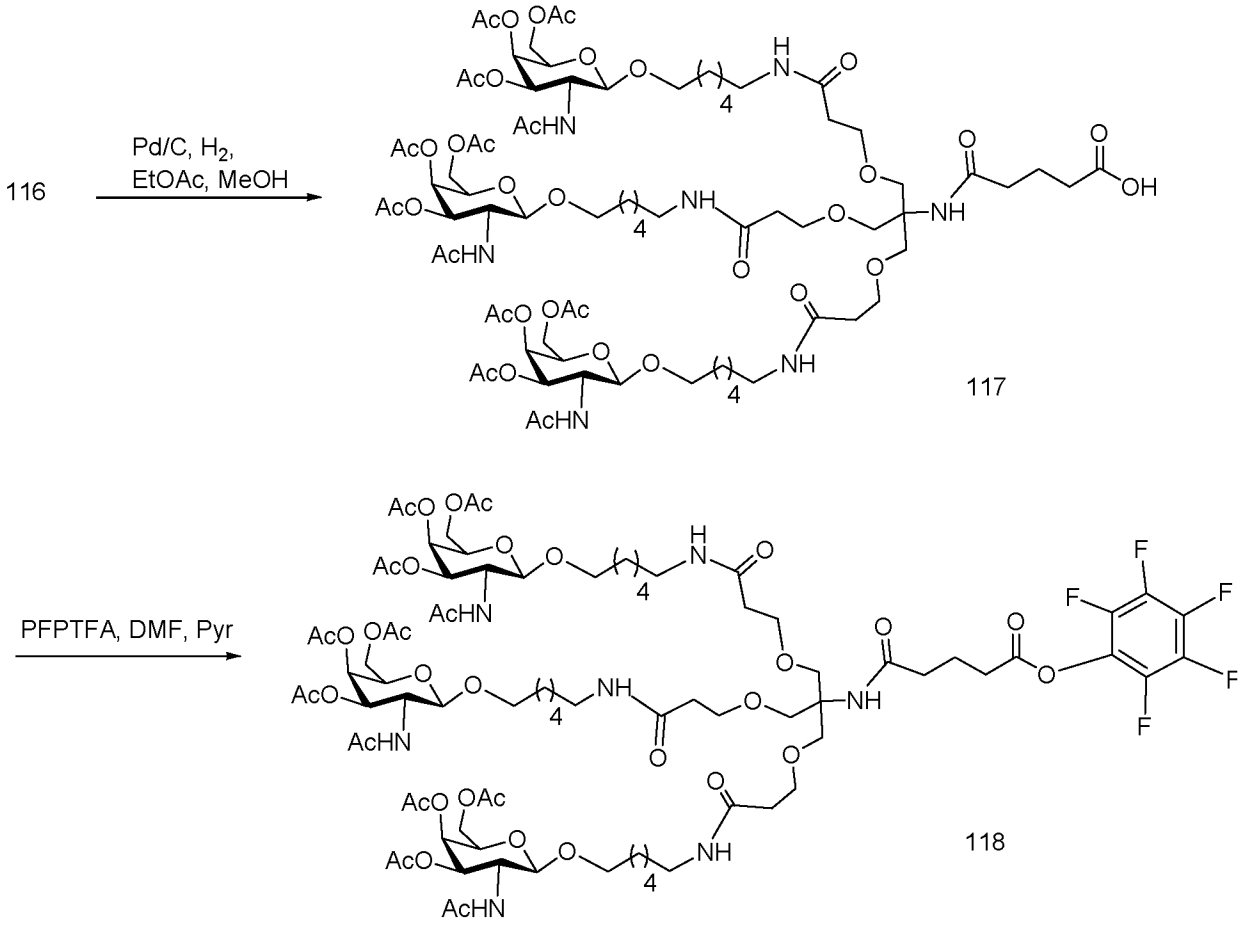
Compound 112 (5 g, 8.6 mmol) was dissolved in 1:1 methanol/ethyl acetate (22 mL/22 mL). Palladium hydroxide on carbon (0.5 g) was added. The reaction mixture was stirred at room temperature under hydrogen for 12 h. The reaction mixture was filtered through a pad of celite and washed the pad with 1:1 methanol/ethyl acetate. The filtrate and the washings were combined and concentrated to dryness to yield Compound 105a (quantitative). The structure was confirmed by LCMS.

Compound 113 (1.25 g, 2.7 mmol), HBTU (3.2 g, 8.4 mmol) and DIEA (2.8 mL, 16.2 mmol) were dissolved in anhydrous DMF (17 mL) and the reaction mixture was stirred at room temperature for 5 min. To this a solution of Compound 105a (3.77 g, 8.4 mmol) in anhydrous DMF (20 mL) was added. The reaction was stirred at room temperature for 6 h. Solvent was removed

under reduced pressure to get an oil. The residue was dissolved in CH_2Cl_2 (100 mL) and washed with aqueous saturated $NaHCO_3$ solution (100 mL) and brine (100 mL). The organic phase was separated, dried (Na_2SO_4), filtered and evaporated. The residue was purified by silica gel column chromatography and eluted with 10 to 20 % MeOH in dichloromethane to yield Compound 114 (1.45 g, 30%). The structure was confirmed by LCMS and ^1H NMR analysis.

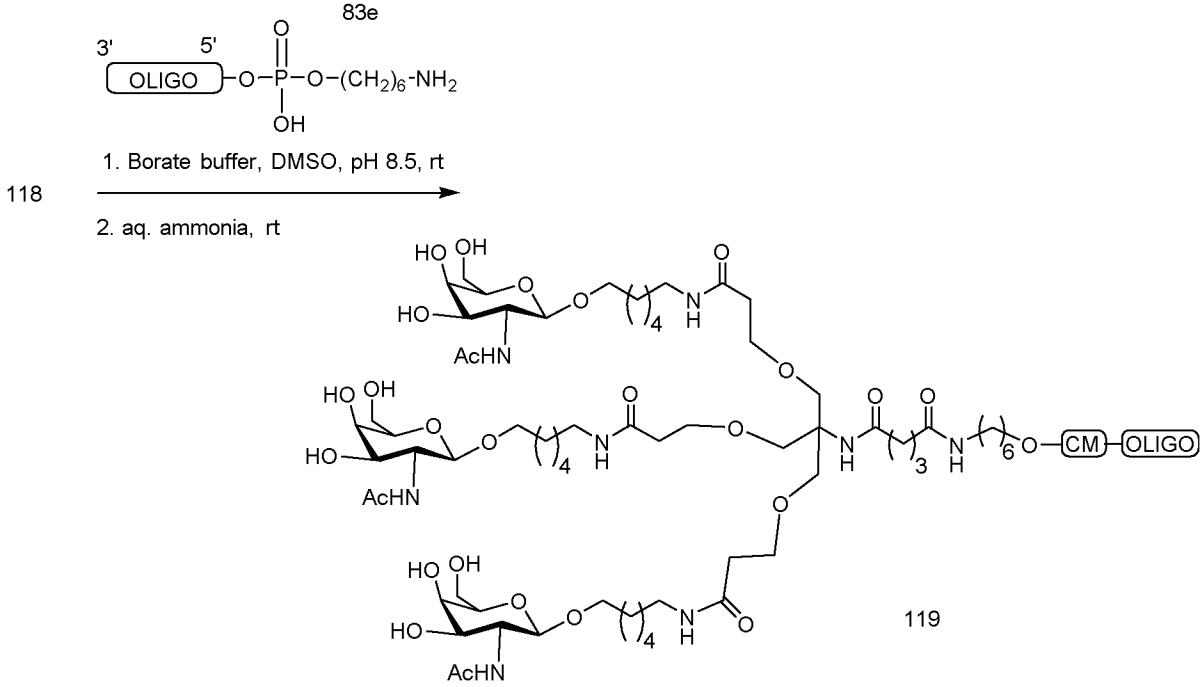
Compound 114 (1.43 g, 0.8 mmol) was dissolved in 1:1 methanol/ethyl acetate (4 mL/4 mL). Palladium on carbon (wet, 0.14 g) was added. The reaction mixture was flushed with hydrogen and stirred at room temperature under hydrogen for 12 h. The reaction mixture was filtered through a pad of celite. The celite pad was washed with methanol/ethyl acetate (1:1). The filtrate and the washings were combined together and evaporated under reduced pressure to yield Compound 115 (quantitative). The structure was confirmed by LCMS and ^1H NMR analysis.

Compound 83a (0.17 g, 0.75 mmol), HBTU (0.31 g, 0.83 mmol) and DIEA (0.26 mL, 1.5 mmol) were dissolved in anhydrous DMF (5 mL) and the reaction mixture was stirred at room temperature for 5 min. To this a solution of Compound 115 (1.22 g, 0.75 mmol) in anhydrous DMF was added and the reaction was stirred at room temperature for 6 h. The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2. The organic layer was washed aqueous saturated $NaHCO_3$ solution and brine and dried over anhydrous Na_2SO_4 and filtered. The organic layer was concentrated to dryness and the residue obtained was purified by silica gel column chromatography and eluted with 3 to 15 % MeOH in dichloromethane to yield Compound 116 (0.84 g, 61%). The structure was confirmed by LC MS and ^1H NMR analysis.



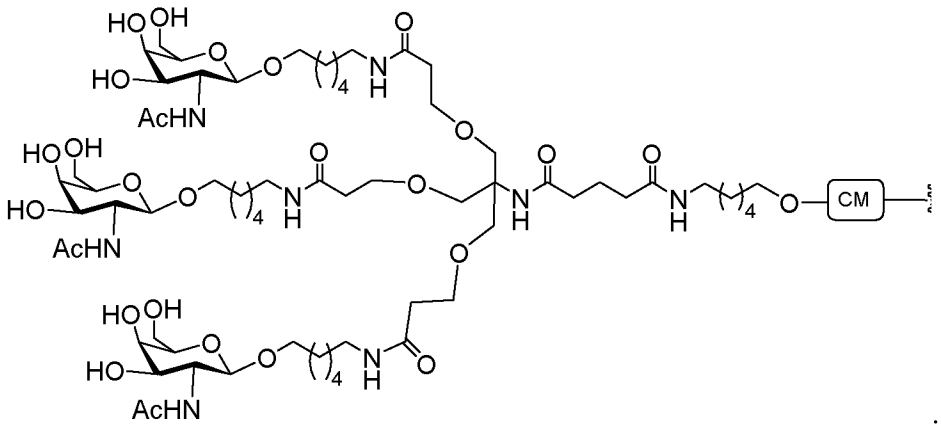
Compound 116 (0.74 g, 0.4 mmol) was dissolved in 1:1 methanol/ethyl acetate (5 mL/5 mL). Palladium on carbon (wet, 0.074 g) was added. The reaction mixture was flushed with hydrogen and stirred at room temperature under hydrogen for 12 h. The reaction mixture was filtered through a pad of celite. The celite pad was washed with methanol/ethyl acetate (1:1). The filtrate and the washings were combined together and evaporated under reduced pressure to yield compound 117 (0.73 g, 98%). The structure was confirmed by LCMS and ^{1}H NMR analysis.

Compound 117 (0.63 g, 0.36 mmol) was dissolved in anhydrous DMF (3 mL). To this solution *N*,*N*-Diisopropylethylamine (70 µL, 0.4 mmol) and pentafluorophenyl trifluoroacetate (72 µL, 0.42 mmol) were added. The reaction mixture was stirred at room temperature for 12 h and poured into a aqueous saturated $NaHCO_3$ solution. The mixture was extracted with dichloromethane, washed with brine and dried over anhydrous Na_2SO_4. The dichloromethane solution was concentrated to dryness and purified with silica gel column chromatography and eluted with 5 to 10 % MeOH in dichloromethane to yield compound 118 (0.51 g, 79%). The structure was confirmed by LCMS and ^{1}H and ^{1}H and ^{19}F NMR.

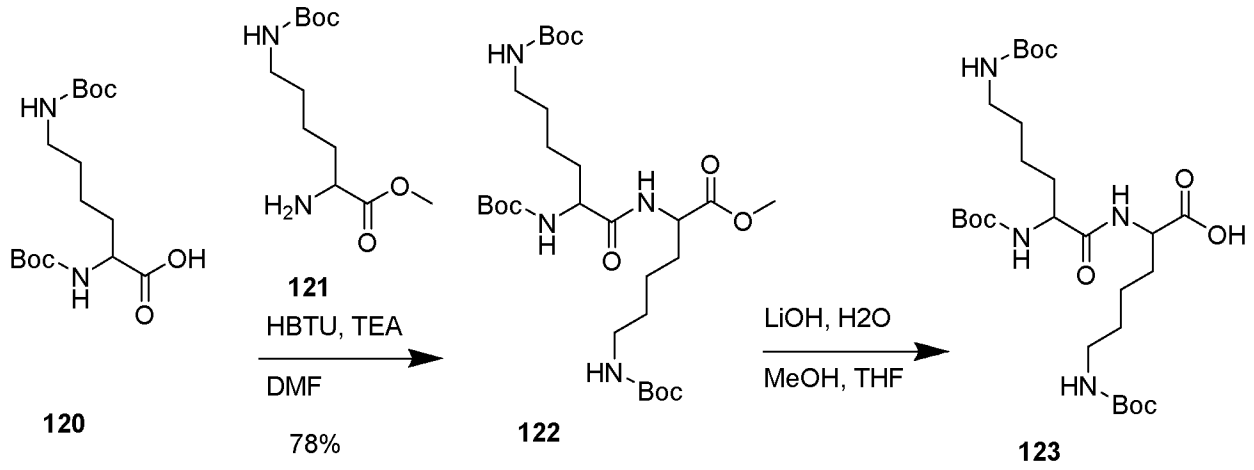


Oligomeric Compound 119, comprising a $GalNAc_3$-7 conjugate group, was prepared using the general procedures illustrated in Example 46. The $GalNAc_3$ cluster portion of the conjugate group $GalNAc_3$-7 ($GalNAc_3$-7_a) can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the cleavable moiety is -P(=O)(OH)-A_d-P(=O)(OH)-.

The structure of $GalNAc_3$-7 ($GalNAc_3$-7_a-CM-) is shown below:



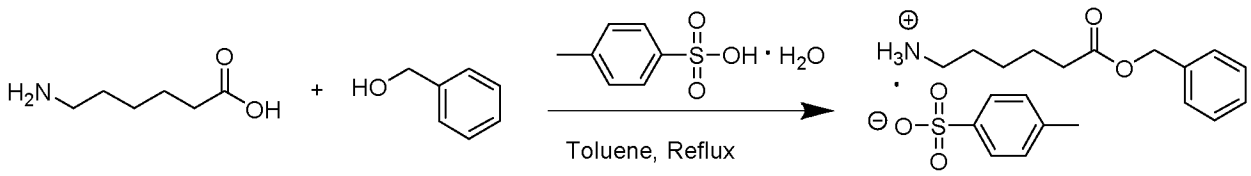
Example 49: Preparation of Oligonucleotide 132 Comprising GalNAc$_3$-5



Compound 120 (14.01 g, 40 mmol) and HBTU (14.06 g, 37 mmol) were dissolved in anhydrous DMF (80 mL). Triethylamine (11.2 mL, 80.35 mmol) was added and stirred for 5 min. The reaction mixture was cooled in an ice bath and a solution of compound 121 (10 g, mmol) in anhydrous DMF (20 mL) was added. Additional triethylamine (4.5 mL, 32.28 mmol) was added and the reaction mixture was stirred for 18 h under an argon atmosphere. The reaction was monitored by TLC (ethyl acetate:hexane; 1:1; Rf = 0.47). The solvent was removed under reduced pressure. The residue was taken up in EtOAc (300 mL) and washed with 1M $NaHSO_4$ (3 x 150 mL), aqueous saturated $NaHCO_3$ solution (3 x 150 mL) and brine (2 x 100 mL). Organic layer was dried with Na_2SO_4. Drying agent was removed by filtration and organic layer was concentrated by rotary evaporation. Crude mixture was purified by silica gel column chromatography and eluted by using 35 – 50% EtOAc in hexane to yield a compound 122 (15.50 g, 78.13%). The structure was confirmed by LCMS and ^{1}H NMR analysis. Mass *m/z* 589.3 $[M + H]^+$.

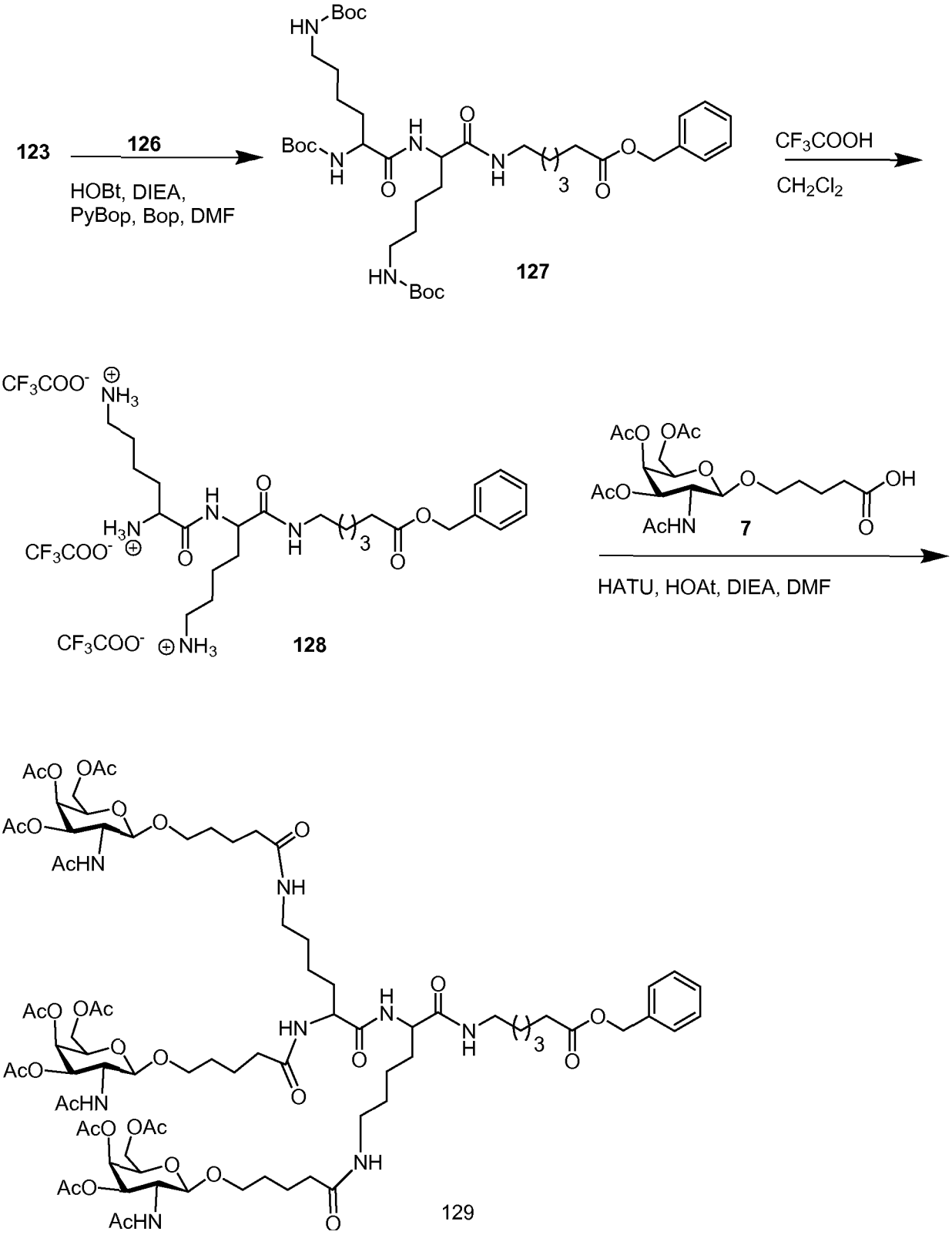
A solution of LiOH (92.15 mmol) in water (20 mL) and THF (10 mL) was added to a cooled solution of Compound 122 (7.75 g,13.16 mmol) dissolved in methanol (15 mL). The reaction mixture was stirred at room temperature for 45 min. and monitored by TLC (EtOAc:hexane; 1:1). The reaction mixture was concentrated to half the volume under reduced pressure. The remaining solution was cooled an ice bath and neutralized by adding concentrated HCl. The reaction mixture was diluted, extracted with EtOAc (120 mL) and washed with brine (100 mL). An emulsion formed and cleared upon standing overnight. The organic layer was separated dried (Na_2SO_4), filtered and evaporated to yield Compound 123 (8.42 g). Residual salt is the likely cause of excess mass. LCMS

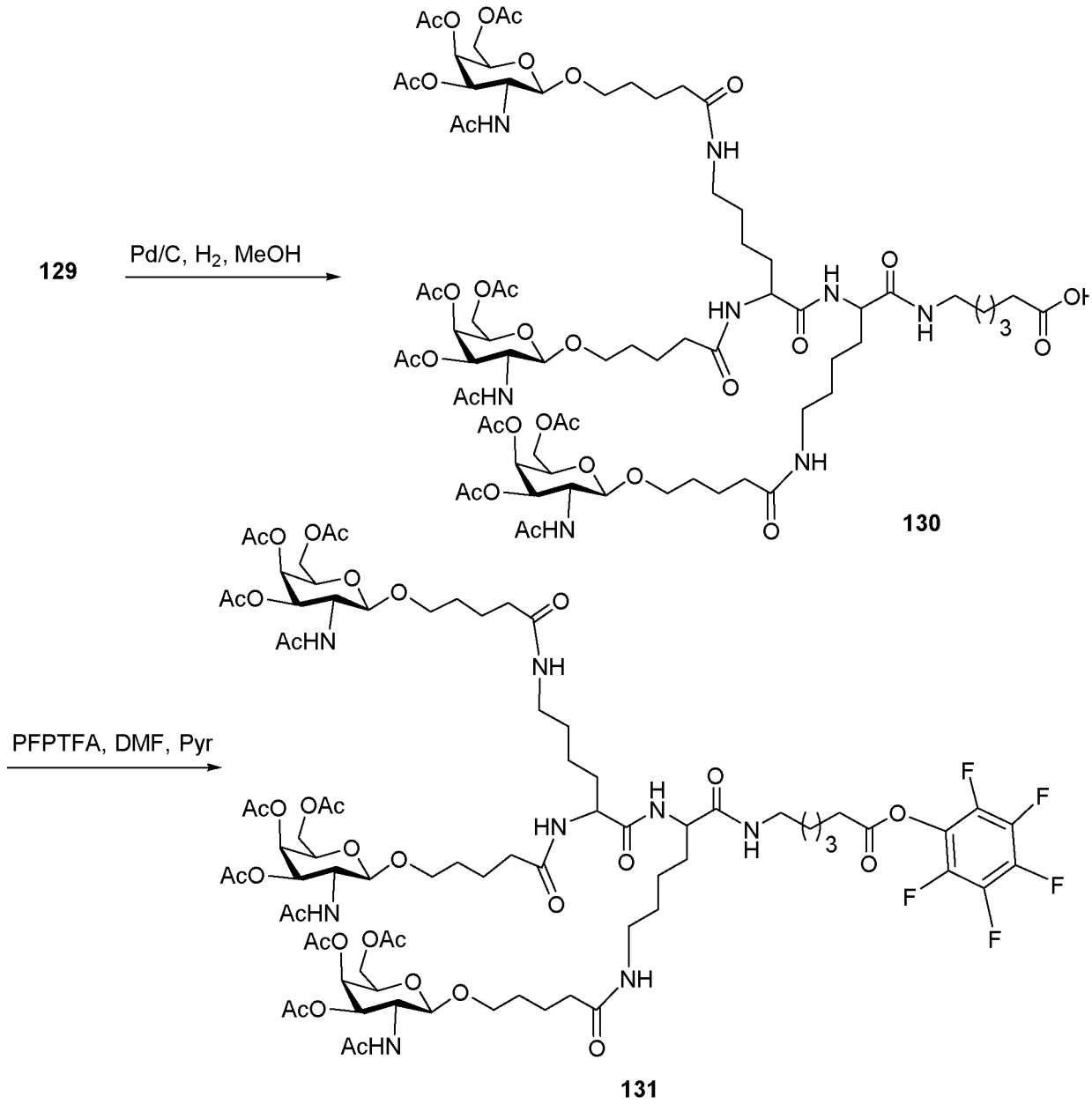
is consistent with structure. Product was used without any further purification. M.W.cal:574.36; M.W.fd:575.3 $[M + H]^+$.



124 125 126

Compound 126 was synthesized following the procedure described in the literature (*J. Am. Chem. Soc.* 2011, *133*, 958-963).


123
126
HOBt, DIEA,
PyBop, Bop, DMF
127
CF3COOH
CH2Cl2
128
7
HATU, HOAt, DIEA, DMF
129



Compound 123 (7.419 g, 12.91 mmol), HOBt (3.49 g, 25.82 mmol) and compound 126 (6.33 g, 16.14 mmol) were dissolved in and DMF (40 mL) and the resulting reaction mixture was cooled in an ice bath. To this *N*,*N*-Diisopropylethylamine (4.42 mL, 25.82 mmol), PyBop (8.7 g, 16.7 mmol) followed by Bop coupling reagent (1.17 g, 2.66 mmol) were added under an argon atmosphere. The ice bath was removed and the solution was allowed to warm to room temperature. The reaction was completed after 1 h as determined by TLC (DCM:MeOH:AA; 89:10:1). The reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (200 mL) and washed with 1 M $NaHSO_4$ (3x100 mL), aqueous saturated $NaHCO_3$ (3x100 mL) and brine (2x100 mL). The organic phase separated dried (Na_2SO_4), filtered and concentrated. The residue was purified by silica gel column chromatography with a gradient of 50% hexanes/EtOAC to 100% EtOAc to yield Compound 127 (9.4 g) as a white foam. LCMS and ^1H NMR

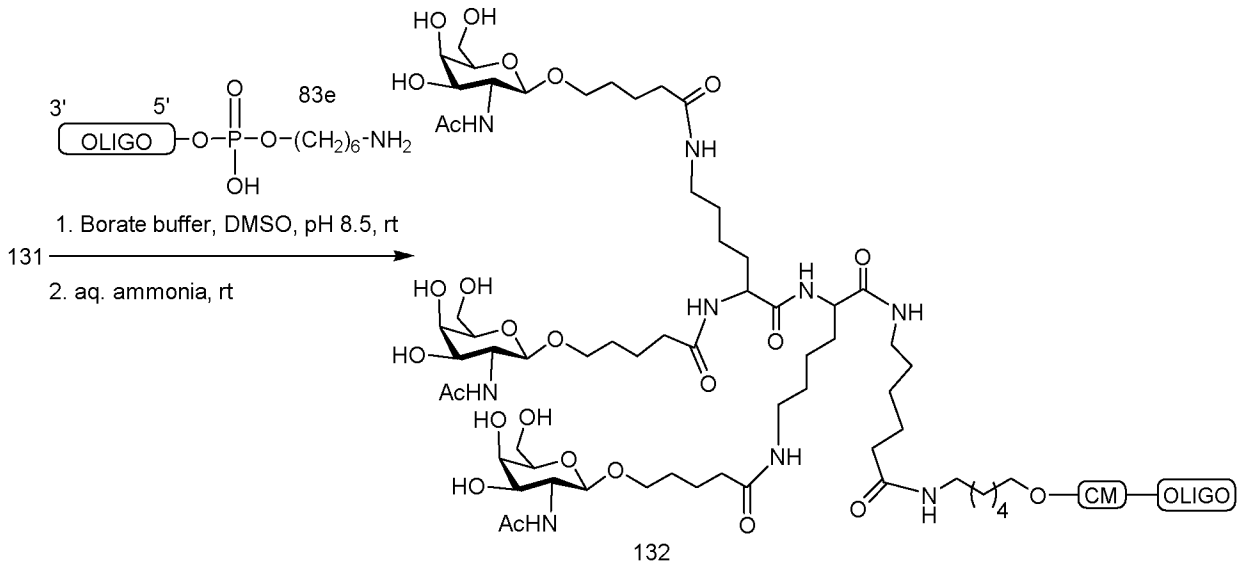
were consistent with structure. Mass *m/z* 778.4 $[M + H]^+$.

Trifluoroacetic acid (12 mL) was added to a solution of compound 127 (1.57 g, 2.02 mmol) in dichloromethane (12 mL) and stirred at room temperature for 1 h. The reaction mixture was co-evaporated with toluene (30 mL) under reduced pressure to dryness. The residue obtained was co-evaporated twice with acetonitrile (30 mL) and toluene (40 mL) to yield Compound 128 (1.67 g) as trifluoro acetate salt and used for next step without further purification. LCMS and ^{1}H NMR were consistent with structure. Mass *m/z* 478.2 $[M + H]^+$.

Compound 7 (0.43 g, 0.963 mmol), HATU (0.35 g, 0.91 mmol), and HOAt (0.035 g, 0.26 mmol) were combined together and dried for 4 h over P_2O_5 under reduced pressure in a round bottom flask and then dissolved in anhydrous DMF (1 mL) and stirred for 5 min. To this a solution of compound 128 (0.20 g, 0.26 mmol) in anhydrous DMF (0.2 mL) and *N*,*N*-Diisopropylethylamine (0.2 mL) was added. The reaction mixture was stirred at room temperature under an argon atmosphere. The reaction was complete after 30 min as determined by LCMS and TLC (7% MeOH/DCM). The reaction mixture was concentrated under reduced pressure. The residue was dissolved in DCM (30 mL) and washed with 1 M $NaHSO_4$ (3x20 mL), aqueous saturated $NaHCO_3$ (3 x 20 mL) and brine (3x20 mL). The organic phase was separated, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography using 5-15% MeOH in dichloromethane to yield Compound 129 (96.6 mg). LC MS and ^{1}H NMR are consistent with structure. Mass *m/z* 883.4 $[M + 2H]^+$.

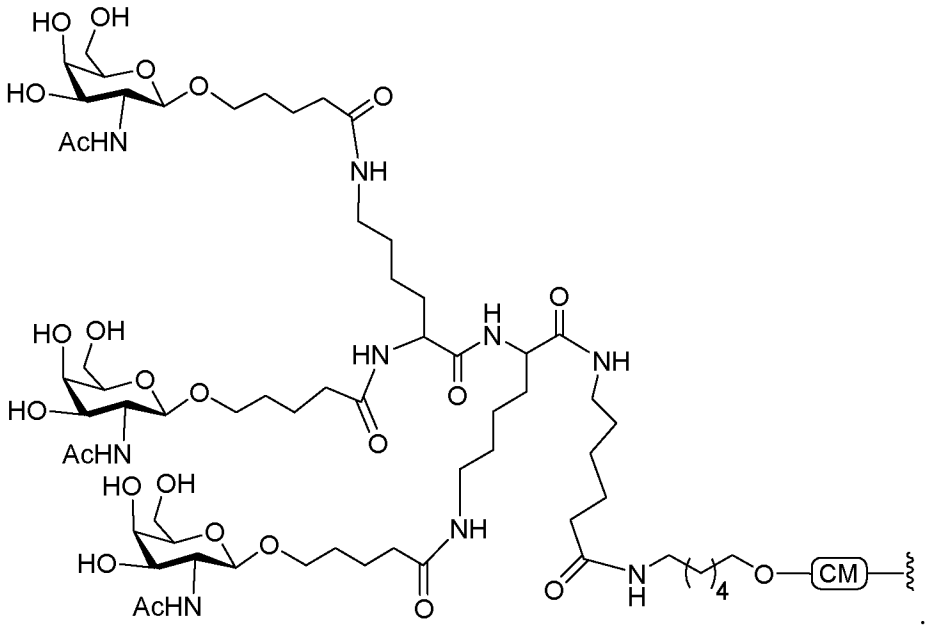
Compound 129 (0.09 g, 0.051 mmol) was dissolved in methanol (5 mL) in 20 mL scintillation vial. To this was added a small amount of 10% Pd/C (0.015 mg) and the reaction vessel was flushed with H_2 gas. The reaction mixture was stirred at room temperature under H_2 atmosphere for 18 h. The reaction mixture was filtered through a pad of Celite and the Celite pad was washed with methanol. The filtrate washings were pooled together and concentrated under reduced pressure to yield Compound 130 (0.08 g). LCMS and ^{1}H NMR were consistent with structure. The product was used without further purification. Mass *m/z* 838.3 $[M + 2H]^+$.

To a 10 mL pointed round bottom flask were added compound 130 (75.8 mg, 0.046 mmol), 0.37 M pyridine/DMF (200 µL) and a stir bar. To this solution was added 0.7 M pentafluorophenyl trifluoroacetate/DMF (100 µL) drop wise with stirring. The reaction was completed after 1 h as determined by LC MS. The solvent was removed under reduced pressure and the residue was dissolved in $CHCl_3$ (~ 10 mL). The organic layer was partitioned against $NaHSO_4$ (1 M, 10 mL) , aqueous saturated $NaHCO_3$ (10 mL) and brine (10 mL) three times each. The organic phase separated and dried over Na_2SO_4, filtered and concentrated to yield Compound 131 (77.7 mg). LCMS is consistent with structure. Used without further purification. Mass *m/z* 921.3 $[M + 2H]^+$.

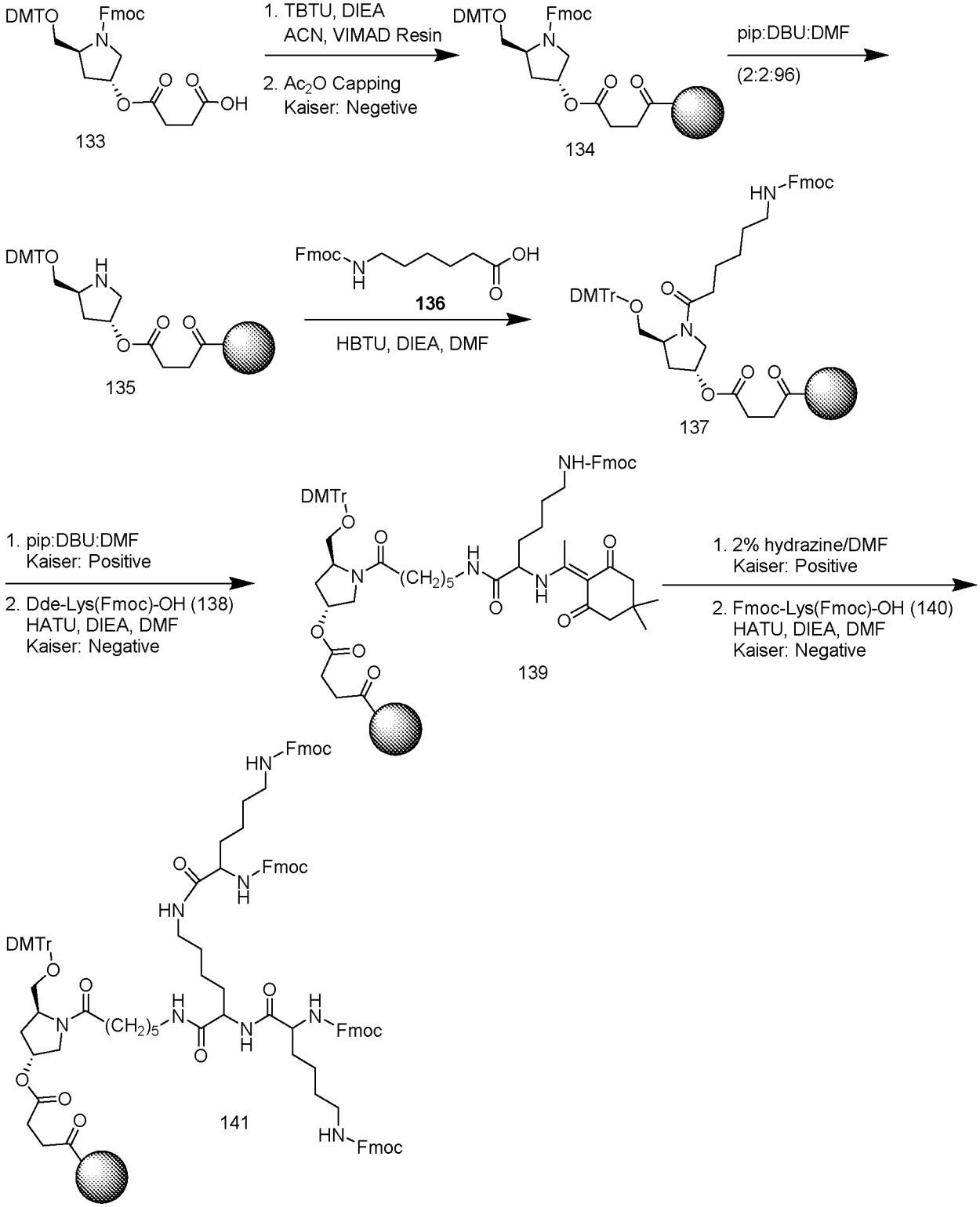


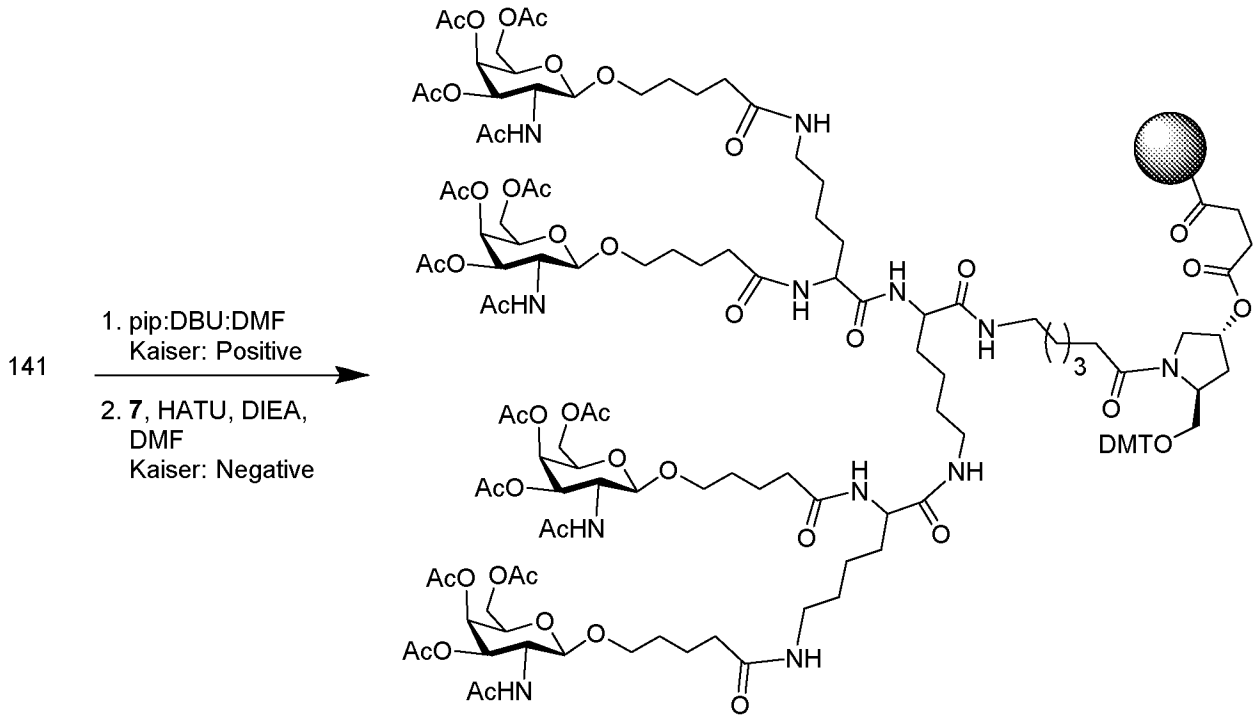
Oligomeric Compound 132, comprising a $GalNAc_3$-5 conjugate group, was prepared using the general procedures illustrated in Example 46. The $GalNAc_3$ cluster portion of the conjugate group $GalNAc_3$-5 ($GalNAc_3$-5_a) can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the cleavable moiety is -P(=O)(OH)-A_d-P(=O)(OH)-.

The structure of $GalNAc_3$-5 ($GalNAc_3$-5_a-CM-) is shown below:



Example 50: Preparation of Oligonucleotide 144 Comprising GalNAc$_4$-11



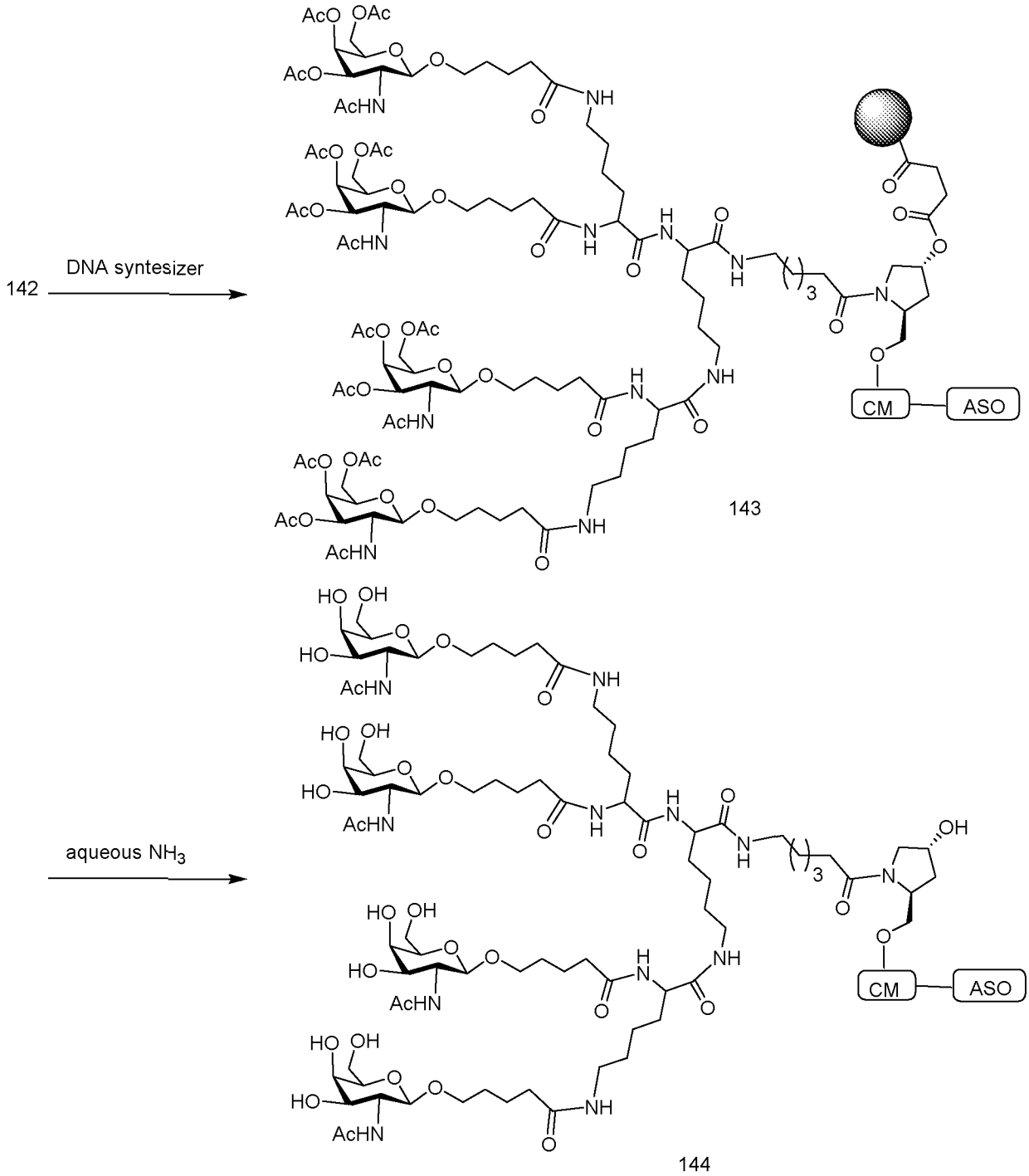


142

Synthesis of Compound 134. To a Merrifield flask was added aminomethyl VIMAD resin (2.5 g, 450 μmol/g) that was washed with acetonitrile, dimethylformamide, dichloromethane and acetonitrile. The resin was swelled in acetonitrile (4 mL). Compound 133 was pre-activated in a 100 mL round bottom flask by adding 20 (1.0 mmol, 0.747 g), TBTU (1.0 mmol, 0.321 g), acetonitrile (5 mL) and DIEA (3.0 mmol, 0.5 mL). This solution was allowed to stir for 5 min and was then added to the Merrifield flask with shaking. The suspension was allowed to shake for 3 h. The reaction mixture was drained and the resin was washed with acetonitrile, DMF and DCM. New resin loading was quantitated by measuring the absorbance of the DMT cation at 500 nm (extinction coefficient = 76000) in DCM and determined to be 238 μmol/g. The resin was capped by suspending in an acetic anhydride solution for ten minutes three times.

The solid support bound compound 141 was synthesized using iterative Fmoc-based solid phase peptide synthesis methods. A small amount of solid support was withdrawn and suspended in aqueous ammonia (28-30 wt%) for 6 h. The cleaved compound was analyzed by LC-MS and the observed mass was consistent with structure. Mass *m/z* 1063.8 $[M + 2H]^+$.

The solid support bound compound 142 was synthesized using solid phase peptide synthesis methods.



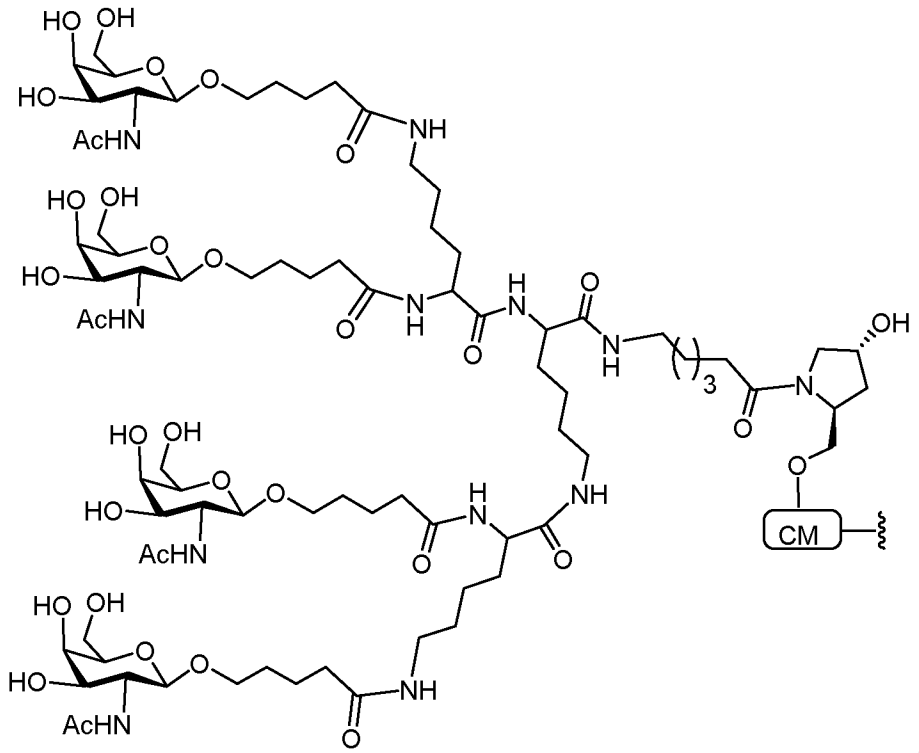
The solid support bound compound 143 was synthesized using standard solid phase synthesis on a DNA synthesizer.

The solid support bound compound 143 was suspended in aqueous ammonia (28-30 wt%) and heated at 55 °C for 16 h. The solution was cooled and the solid support was filtered. The filtrate was concentrated and the residue dissolved in water and purified by HPLC on a strong anion exchange column. The fractions containing full length compound 144 were pooled together and

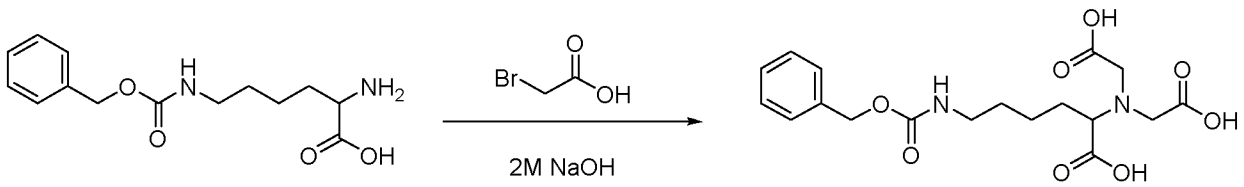
desalted. The resulting GalNAc$_4$-11 conjugated oligomeric compound was analyzed by LC-MS and the observed mass was consistent with structure.

The GalNAc$_4$ cluster portion of the conjugate group GalNAc$_4$-11 (GalNAc$_4$-11$_a$) can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the cleavable moiety is -P(=O)(OH)-A$_d$-P(=O)(OH)-.

The structure of GalNAc$_4$-11 (GalNAc$_4$-11$_a$-CM) is shown below:

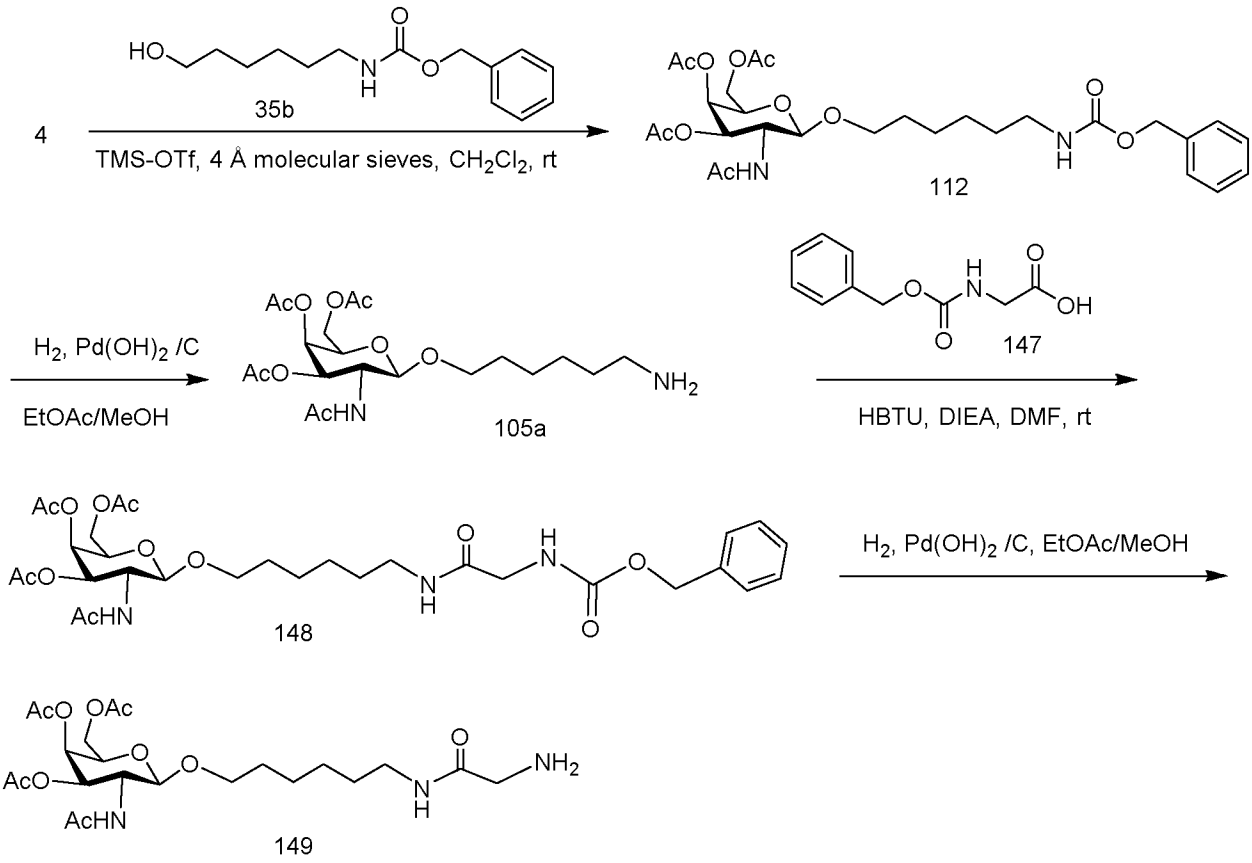


Example 51: Preparation of Oligonucleotide 155 Comprising GalNAc$_3$-6



145 146

Compound 146 was synthesized as described in the literature (*Analytical Biochemistry* 1995, *229*, 54-60).

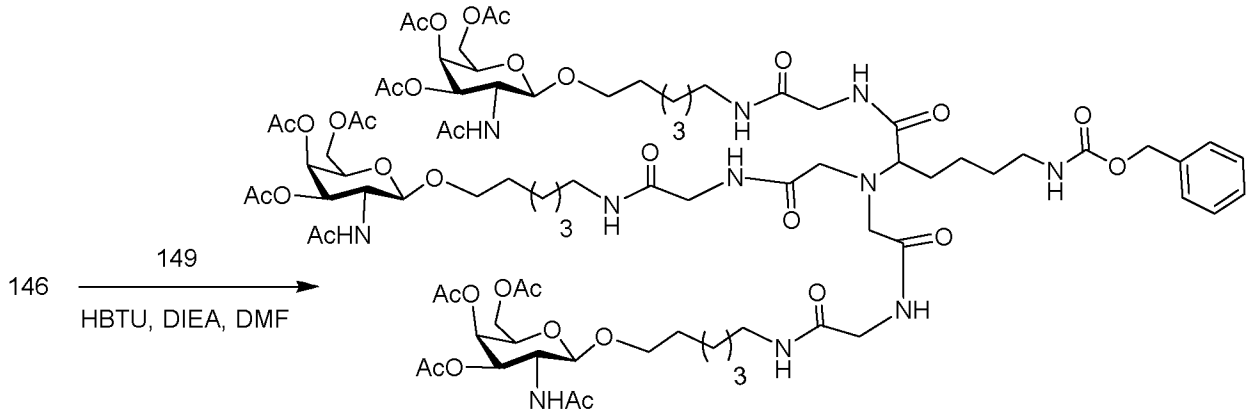


Compound 4 (15 g, 45.55 mmol) and compound 35b (14.3 grams, 57 mmol) were dissolved in CH_2Cl_2 (200 ml). Activated molecular sieves (4 Å. 2 g, powdered) were added, and the reaction was allowed to stir for 30 minutes under nitrogen atmosphere. TMS-OTf was added (4.1 ml, 22.77 mmol) and the reaction was allowed to stir at room temp overnight. Upon completion, the reaction was quenched by pouring into solution of saturated aqueous $NaHCO_3$ (500 ml) and crushed ice (~ 150 g). The organic layer was separated, washed with brine, dried over $MgSO_4$, filtered, and was concentrated to an orange oil under reduced pressure. The crude material was purified by silica gel column chromatography and eluted with 2-10 % MeOH in CH_2Cl_2 to yield Compound 112 (16.53 g, 63 %). LCMS and ^1H NMR were consistent with the expected compound.

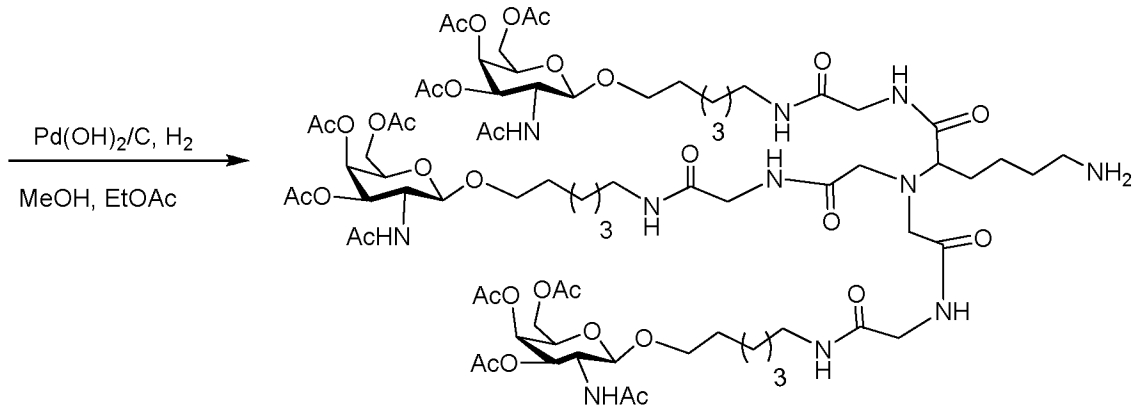
Compound 112 (4.27 g, 7.35 mmol) was dissolved in 1:1 MeOH/EtOAc (40 ml). The reaction mixture was purged by bubbling a stream of argon through the solution for 15 minutes. Pearlman's catalyst (palladium hydroxide on carbon, 400 mg) was added, and hydrogen gas was bubbled through the solution for 30 minutes. Upon completion (TLC 10% MeOH in CH_2Cl_2, and LCMS), the catalyst was removed by filtration through a pad of celite. The filtrate was concentrated by rotary evaporation, and was dried briefly under high vacuum to yield Compound 105a (3.28 g). LCMS and 1H NMR were consistent with desired product.

Compound 147 (2.31 g, 11 mmol) was dissolved in anhydrous DMF (100 mL). *N,N*-Diisopropylethylamine (DIEA, 3.9 mL, 22 mmol) was added, followed by HBTU (4 g, 10.5 mmol). The reaction mixture was allowed to stir for ~ 15 minutes under nitrogen. To this a solution of compound 105a (3.3 g, 7.4 mmol) in dry DMF was added and stirred for 2 h under nitrogen atmosphere. The reaction was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$ and brine. The organics phase was separated, dried ($MgSO_4$), filtered, and concentrated to an orange syrup. The crude material was purified by column chromatography 2-5 % MeOH in CH_2Cl_2 to yield Compound 148 (3.44 g, 73 %). LCMS and ^1H NMR were consistent with the expected product.

Compound 148 (3.3 g, 5.2 mmol) was dissolved in 1:1 MeOH/EtOAc (75 ml). The reaction mixture was purged by bubbling a stream of argon through the solution for 15 minutes. Pearlman's catalyst (palladium hydroxide on carbon) was added (350 mg). Hydrogen gas was bubbled through the solution for 30 minutes. Upon completion (TLC 10% MeOH in DCM, and LCMS), the catalyst was removed by filtration through a pad of celite. The filtrate was concentrated by rotary evaporation, and was dried briefly under high vacuum to yield Compound 149 (2.6 g). LCMS was consistent with desired product. The residue was dissolved in dry DMF (10 ml) was used immediately in the next step.



150

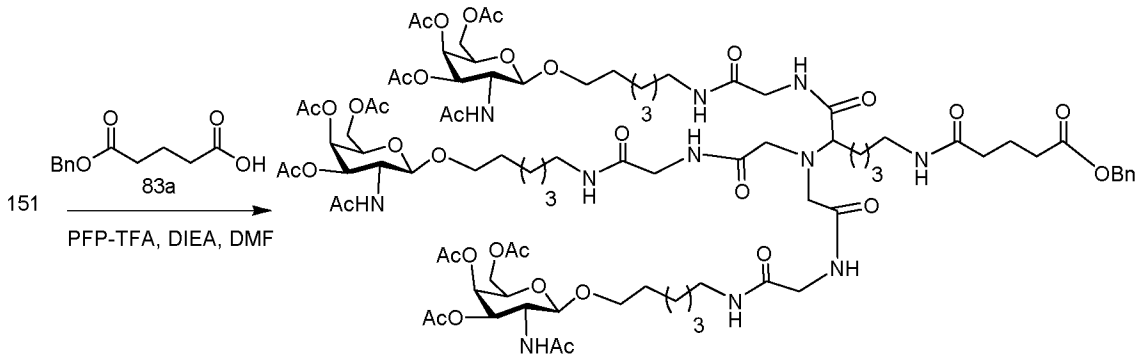


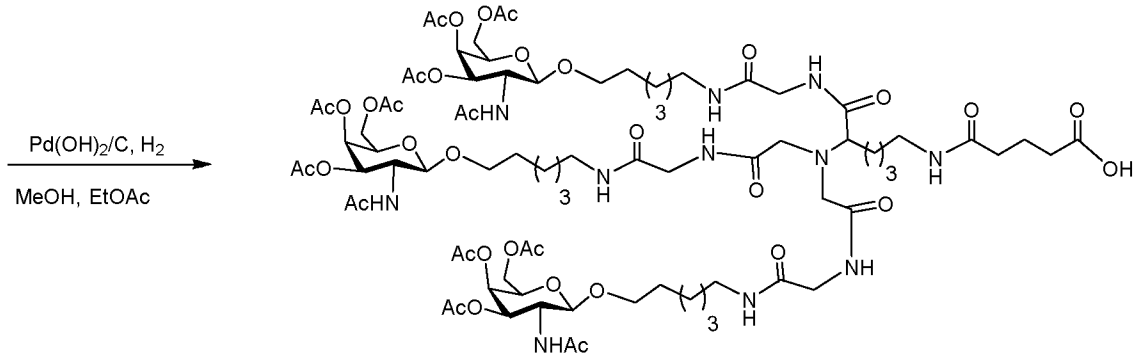
151

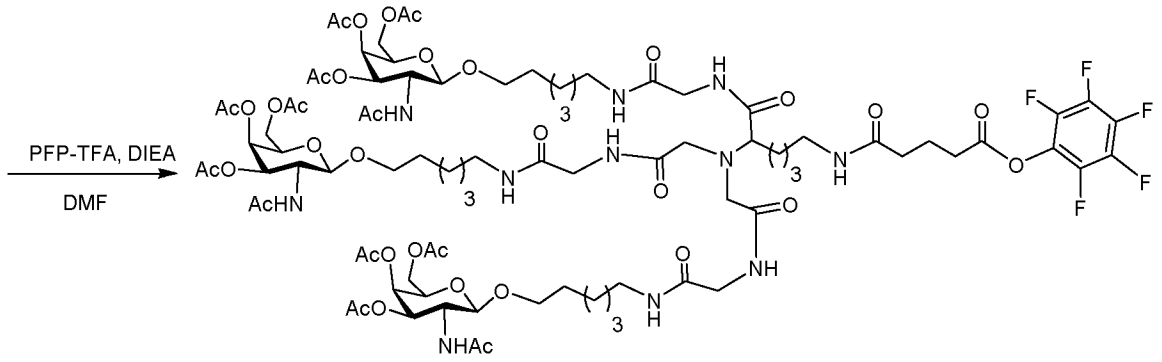
Compound 146 (0.68 g, 1.73 mmol) was dissolved in dry DMF (20 ml). To this DIEA (450 µL, 2.6 mmol, 1.5 eq.) and HBTU (1.96 g, 0.5.2 mmol) were added. The reaction mixture was allowed to stir for 15 minutes at room temperature under nitrogen. A solution of compound 149 (2.6 g) in anhydrous DMF (10 mL) was added. The pH of the reaction was adjusted to pH = 9-10 by addition of DIEA (if necessary). The reaction was allowed to stir at room temperature under nitrogen for 2 h. Upon completion the reaction was diluted with EtOAc (100 mL), and washed with aqueous saturated aqueous $NaHCO_3$, followed by brine. The organic phase was separated, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography and eluted with 2-10 % MeOH in CH_2Cl_2 to yield Compound 150 (0.62 g, 20 %). LCMS and ^1H NMR were consistent with the desired product.

Compound 150 (0.62 g) was dissolved in 1:1 MeOH/ EtOAc (5 L). The reaction mixture was purged by bubbling a stream of argon through the solution for 15 minutes. Pearlman's catalyst (palladium hydroxide on carbon) was added (60 mg). Hydrogen gas was bubbled through the solution for 30 minutes. Upon completion (TLC 10% MeOH in DCM, and LCMS), the catalyst was

removed by filtration (syringe-tip Teflon filter, 0.45 μm). The filtrate was concentrated by rotary evaporation, and was dried briefly under high vacuum to yield Compound 151 (0.57 g). The LCMS was consistent with the desired product. The product was dissolved in 4 mL dry DMF and was used immediately in the next step.





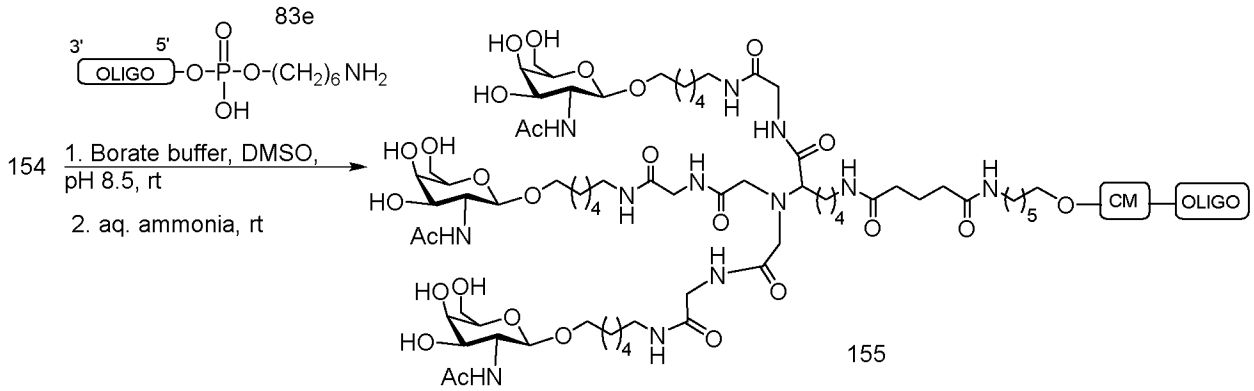


Compound 83a (0.11 g, 0.33 mmol) was dissolved in anhydrous DMF (5 mL) and *N*,*N*-Diisopropylethylamine (75 μL, 1 mmol) and PFP-TFA (90 μL, 0.76 mmol) were added. The

reaction mixture turned magenta upon contact, and gradually turned orange over the next 30 minutes. Progress of reaction was monitored by TLC and LCMS. Upon completion (formation of the PFP ester), a solution of compound 151 (0.57 g, 0.33 mmol) in DMF was added. The pH of the reaction was adjusted to pH = 9-10 by addition of *N*,*N*-Diisopropylethylamine (if necessary). The reaction mixture was stirred under nitrogen for ~ 30 min. Upon completion, the majority of the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2 and washed with aqueous saturated $NaHCO_3$, followed by brine. The organic phase separated, dried over $MgSO_4$, filtered, and concentrated to an orange syrup. The residue was purified by silica gel column chromatography (2-10 % MeOH in CH_2Cl_2) to yield Compound 152 (0.35 g, 55 %). LCMS and ^1H NMR were consistent with the desired product.

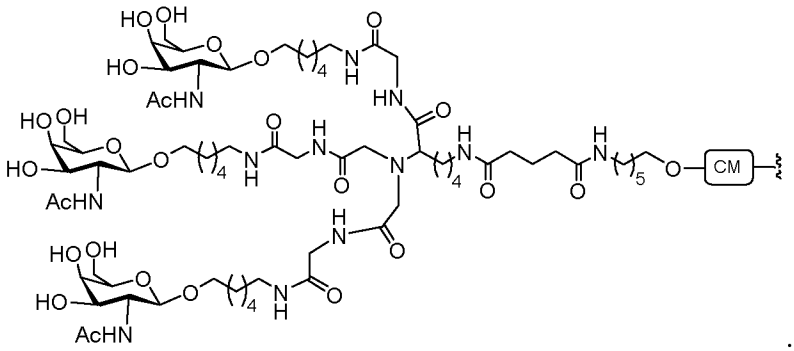
Compound 152 (0.35 g, 0.182 mmol) was dissolved in 1:1 MeOH/EtOAc (10 mL). The reaction mixture was purged by bubbling a stream of argon thru the solution for 15 minutes. Pearlman's catalyst (palladium hydroxide on carbon) was added (35 mg). Hydrogen gas was bubbled thru the solution for 30 minutes. Upon completion (TLC 10% MeOH in DCM, and LCMS), the catalyst was removed by filtration (syringe-tip Teflon filter, 0.45 μm). The filtrate was concentrated by rotary evaporation, and was dried briefly under high vacuum to yield Compound 153 (0.33 g, quantitative). The LCMS was consistent with desired product.

Compound 153 (0.33 g, 0.18 mmol) was dissolved in anhydrous DMF (5 mL) with stirring under nitrogen. To this *N*,*N*-Diisopropylethylamine (65 μL, 0.37 mmol) and PFP-TFA (35 μL, 0.28 mmol) were added. The reaction mixture was stirred under nitrogen for ~ 30 min. The reaction mixture turned magenta upon contact, and gradually turned orange. The pH of the reaction mixture was maintained at pH = 9-10 by adding more *N*,-Diisopropylethylamine. The progress of the reaction was monitored by TLC and LCMS. Upon completion, the majority of the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2 (50 mL), and washed with saturated aqueous $NaHCO_3$, followed by brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated to an orange syrup. The residue was purified by column chromatography and eluted with 2-10 % MeOH in CH_2Cl_2 to yield Compound 154 (0.29 g, 79 %). LCMS and ^1H NMR were consistent with the desired product.

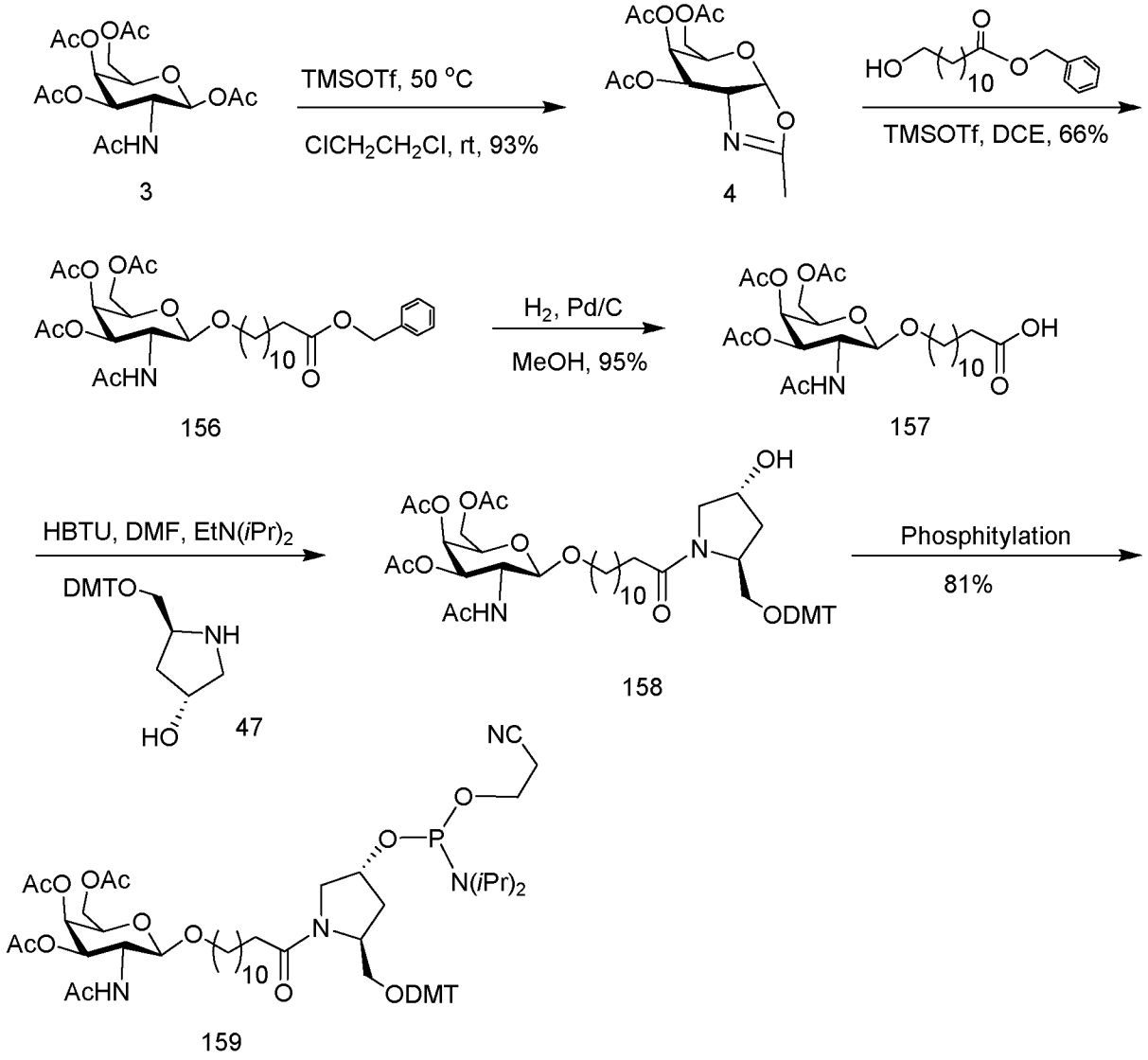


Oligomeric Compound 155, comprising a $GalNAc_3$-6 conjugate group, was prepared using the general procedures illustrated in Example 46. The $GalNAc_3$ cluster portion of the conjugate group $GalNAc_3$-6 ($GalNAc_3$-6_a) can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the cleavable moiety is -P(=O)(OH)-A_d-P(=O)(OH)-.

The structure of $GalNAc_3$-6 ($GalNAc_3$-6_a-CM-) is shown below:



Example 52: Preparation of Oligonucleotide 160 Comprising GalNAc$_3$-9



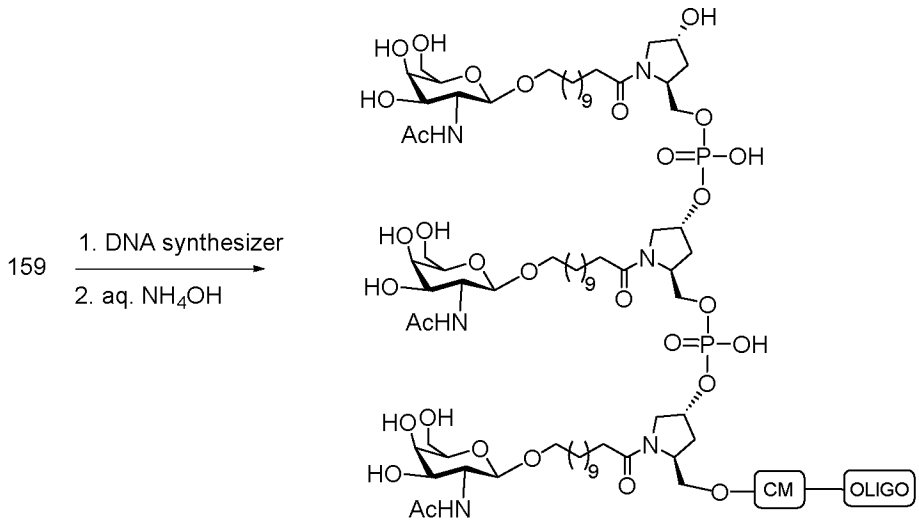
Compound 156 was synthesized following the procedure described in the literature (*J. Med. Chem.* 2004, *47*, 5798-5808).

Compound 156, (18.60 g, 29.28 mmol) was dissolved in methanol (200 mL). Palladium on carbon (6.15 g, 10 wt%, loading (dry basis), matrix carbon powder, wet) was added. The reaction mixture was stirred at room temperature under hydrogen for 18 h. The reaction mixture was filtered through a pad of celite and the celite pad was washed thoroughly with methanol. The combined filtrate was washed and concentrated to dryness. The residue was purified by silica gel column chromatography and eluted with 5-10 % methanol in dichloromethane to yield Compound 157 (14.26 g, 89%). Mass *m/z* 544.1 $[M-H]^-$.

Compound 157 (5 g, 9.17 mmol) was dissolved in anhydrous DMF (30 mL). HBTU (3.65 g, 9.61 mmol) and *N*,*N*-Diisopropylethylamine (13.73 mL, 78.81 mmol) were added and the reaction

mixture was stirred at room temperature for 5 minutes. To this a solution of compound 47 (2.96 g, 7.04 mmol) was added. The reaction was stirred at room temperature for 8 h. The reaction mixture was poured into a saturated $NaHCO_3$ aqueous solution. The mixture was extracted with ethyl acetate and the organic layer was washed with brine and dried (Na_2SO_4), filtered and evaporated. The residue obtained was purified by silica gel column chromatography and eluted with 50% ethyl acetate in hexane to yield compound 158 (8.25g, 73.3%). The structure was confirmed by MS and ^{1}H NMR analysis.

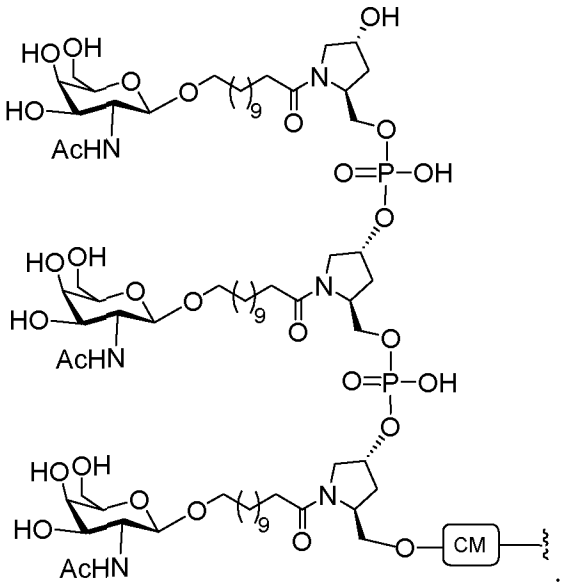
Compound 158 (7.2 g, 7.61 mmol) was dried over P_2O_5 under reduced pressure. The dried compound was dissolved in anhydrous DMF (50 mL). To this 1H-tetrazole (0.43 g, 6.09 mmol) and *N*-methylimidazole (0.3 mL, 3.81 mmol) and 2-cyanoethyl-*N*,*N*,*N*',*N*'-tetraisopropyl phosphorodiamidite (3.65 mL, 11.50 mmol) were added. The reaction mixture was stirred t under an argon atmosphere for 4 h. The reaction mixture was diluted with ethyl acetate (200 mL). The reaction mixture was washed with saturated $NaHCO_3$ and brine. The organic phase was separated, dried (Na_2SO_4), filtered and evaporated. The residue was purified by silica gel column chromatography and eluted with 50-90 % ethyl acetate in hexane to yield Compound 159 (7.82 g, 80.5%). The structure was confirmed by LCMS and ^{31}P NMR analysis.



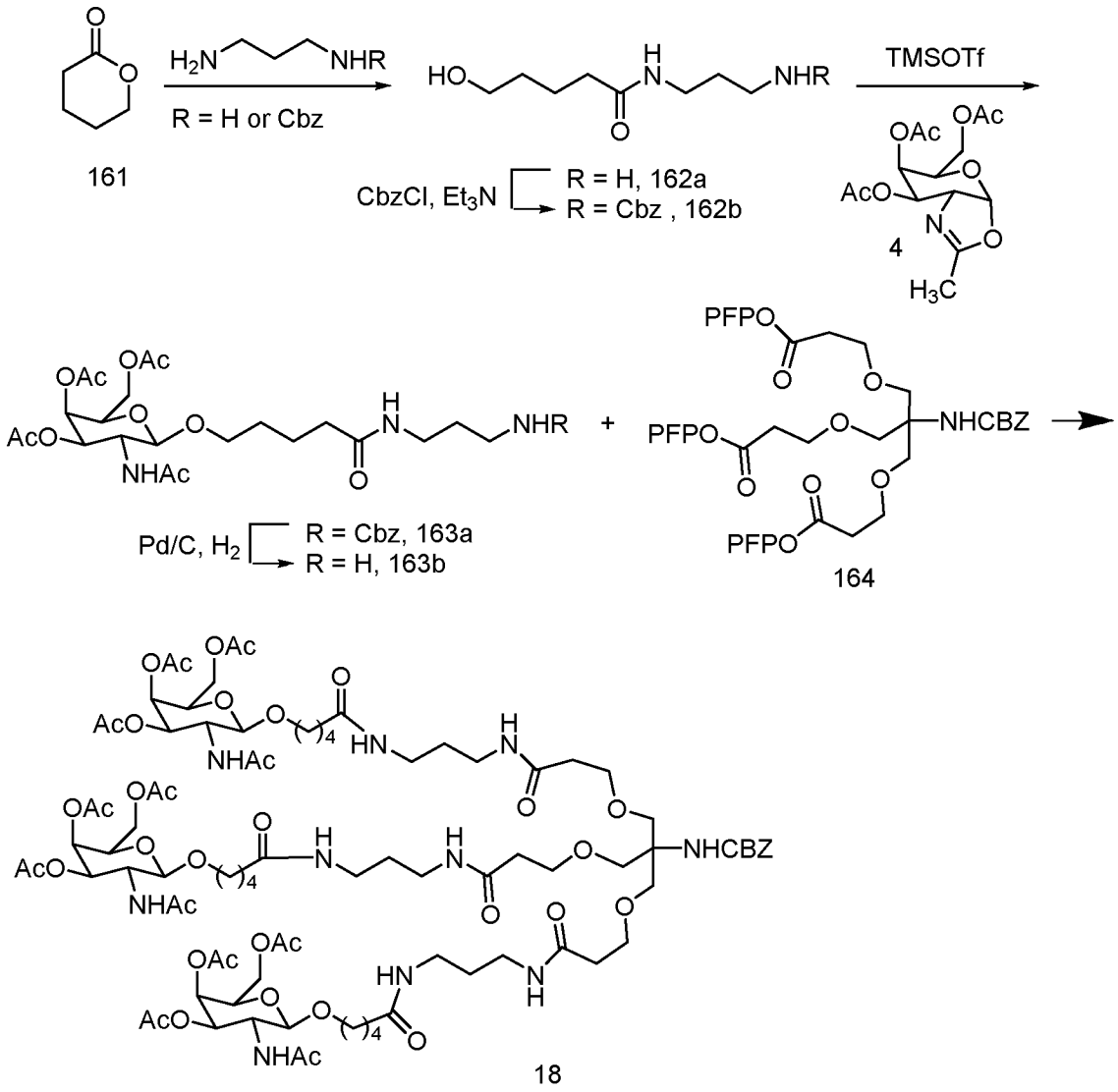
160

Oligomeric Compound 160, comprising a $GalNAc_3$-9 conjugate group, was prepared using standard oligonucleotide synthesis procedures. Three units of compound 159 were coupled to the solid support, followed by nucleotide phosphoramidites. Treatment of the protected oligomeric compound with aqueous ammonia yielded compound 160. The $GalNAc_3$ cluster portion of the conjugate group $GalNAc_3$-9 ($GalNAc_3$-9_a) can be combined with any cleavable moiety to provide a

variety of conjugate groups. In certain embodiments, the cleavable moiety is -P(=O)(OH)-A_d-P(=O)(OH)-. The structure of $GalNAc_3$-9 ($GalNAc_3$-9_a-CM) is shown below:



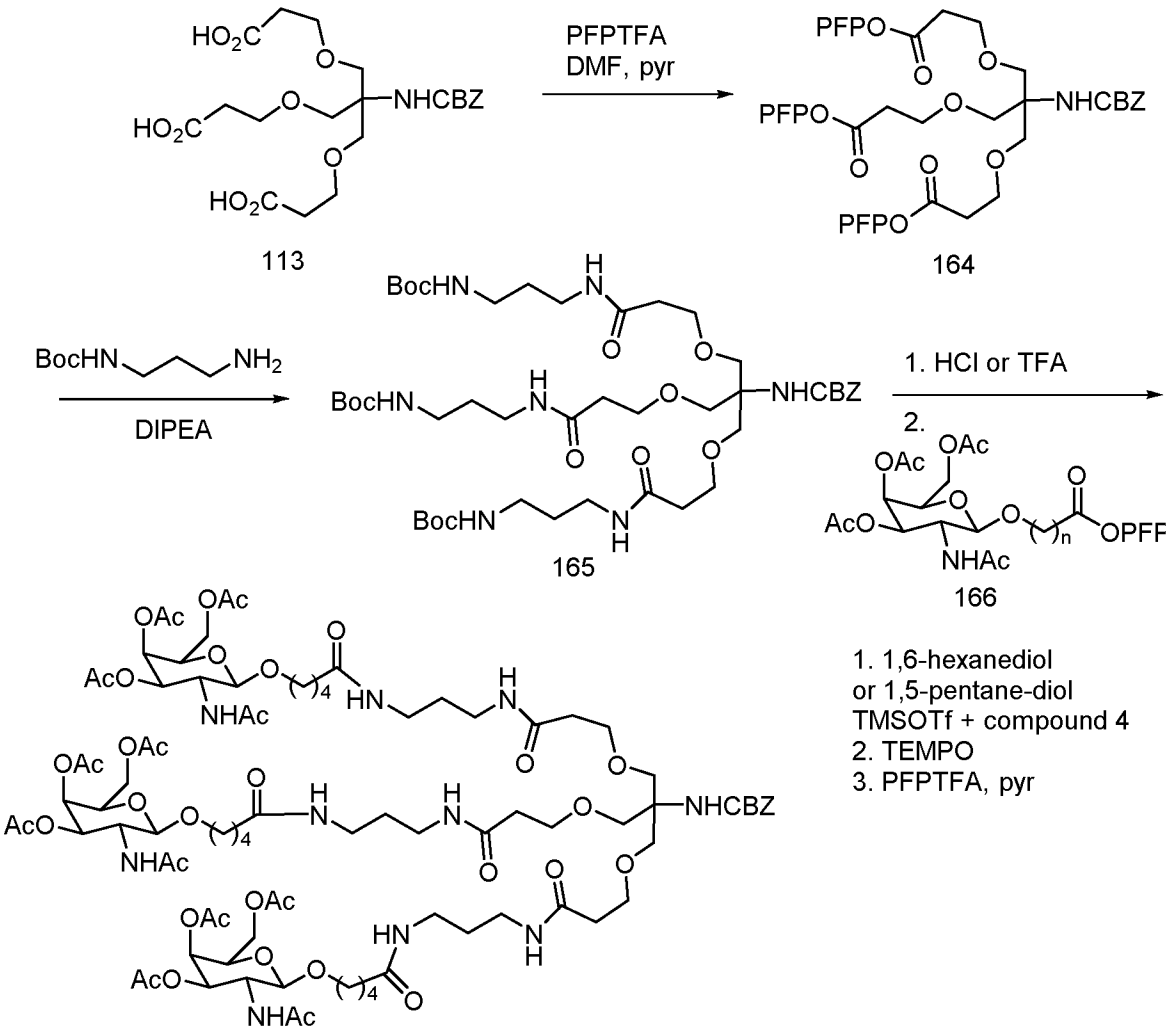
Example 53: Alternate procedure for preparation of Compound 18 (GalNAc$_3$-1a and GalNAc$_3$-3a)



Lactone 161 was reacted with diamino propane (3-5 eq) or Mono-Boc protected diamino propane (1 eq) to provide alcohol 162a or 162b. When unprotected propanediamine was used for the above reaction, the excess diamine was removed by evaporation under high vacuum and the free amino group in 162a was protected using CbzCl to provide 162b as a white solid after purification by column chromatography. Alcohol 162b was further reacted with compound 4 in the presence of TMSOTf to provide 163a which was converted to 163b by removal of the Cbz group using catalytic hydrogenation. The pentafluorophenyl (PFP) ester 164 was prepared by reacting triacid 113 (see Example 48) with PFPTFA (3.5 eq) and pyridine (3.5 eq) in DMF (0.1 to 0.5 M). The triester 164 was directly reacted with the amine 163b (3–4 eq) and DIPEA (3–4 eq) to provide Compound 18.

The above method greatly facilitates purification of intermediates and minimizes the formation of byproducts which are formed using the procedure described in Example 4.

Example 54: Alternate procedure for preparation of Compound 18 (GalNAc$_3$-1a and GalNAc$_3$-3a)



18

The triPFP ester 164 was prepared from acid 113 using the procedure outlined in example 53 above and reacted with mono-Boc protected diamine to provide 165 in essentially quantitative yield. The Boc groups were removed with hydrochloric acid or trifluoroacetic acid to provide the triamine which was reacted with the PFP activated acid 166 in the presence of a suitable base such as DIPEA to provide Compound 18.

The PFP protected Gal-NAc acid 166 was prepared from the corresponding acid by treatment with PFPTFA (1-1.2 eq) and pyridine (1-1.2 eq) in DMF. The precursor acid in turn was prepared from the corresponding alcohol by oxidation using TEMPO (0.2 eq) and BAIB in acetonitrile and water. The precursor alcohol was prepared from sugar intermediate 4 by reaction with 1,6-hexanediol (or 1,5-pentanediol or other diol for other n values) (2-4 eq) and TMSOTf using conditions described previously in example 47.

Example 55: Dose-dependent study of oligonucleotides comprising either a 3' or 5'-conjugate group (comparison of GalNAc$_3$-1, 3, 8 and 9) targeting SRB-1 *in vivo*

The oligonucleotides listed below were tested in a dose-dependent study for antisense inhibition of SRB-1 in mice. Unconjugated ISIS 353382 was included as a standard. Each of the various GalNAc$_3$ conjugate groups was attached at either the 3' or 5' terminus of the respective oligonucleotide by a phosphodiester linked 2'-deoxyadenosine nucleoside (cleavable moiety).

Table 39

Modified ASO targeting SRB-1

ASO	Sequence (5' to 3')	Motif	Conjugate	SEQ ID No.
ISIS 353382 (parent)	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	none	829
ISIS 655861	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{eo}$**$A_{do}$'-GalNAc$_3-1_a$**	5/10/5	**GalNAc$_3$-1**	830
ISIS 664078	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{eo}$**$A_{do}$'-GalNAc$_3-9_a$**	5/10/5	**GalNAc$_3$-9**	830
ISIS 661161	**GalNAc$_3$-3$_{a\text{-}o}$'A_{do}** $G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-3**	831
ISIS 665001	**GalNAc$_3$-8$_{a\text{-}o}$'A_{do}** $G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-8**	831

Capital letters indicate the nucleobase for each nucleoside and ^{m}C indicates a 5-methyl cytosine. Subscripts: "e" indicates a 2'-MOE modified nucleoside; "d" indicates a β-D-2'-deoxyribonucleoside; "s" indicates a phosphorothioate internucleoside linkage (PS); "o" indicates a phosphodiester internucleoside linkage (PO); and "o'" indicates -O-P(=O)(OH)-. Conjugate groups are in bold.

The structure of $GalNAc_3$-1_a was shown previously in Example 9. The structure of $GalNAc_3$-9 was shown previously in Example 52. The structure of $GalNAc_3$-3 was shown previously in Example 39. The structure of $GalNAc_3$-8 was shown previously in Example 47.

Treatment

Six week old male Balb/c mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously once at the dosage shown below with ISIS 353382, 655861, 664078, 661161, 665001 or with saline. Each treatment group consisted of 4 animals. The mice were sacrificed 72 hours following the final administration to determine the liver SRB-1 mRNA levels using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. The results below are presented as the average percent of SRB-1 mRNA levels for each treatment group, normalized to the saline control.

As illustrated in Table 40, treatment with antisense oligonucleotides lowered SRB-1 mRNA levels in a dose-dependent manner. Indeed, the antisense oligonucleotides comprising the phosphodiester linked $GalNAc_3$-1 and $GalNAc_3$-9 conjugates at the 3' terminus (ISIS 655861 and ISIS 664078) and the $GalNAc_3$-3 and $GalNAc_3$-8 conjugates linked at the 5' terminus (ISIS 661161 and ISIS 665001) showed substantial improvement in potency compared to the unconjugated antisense oligonucleotide (ISIS 353382). Furthermore, ISIS 664078, comprising a $GalNAc_3$-9 conjugate at the 3' terminus was essentially equipotent compared to ISIS 655861, which comprises a $GalNAc_3$-1 conjugate at the 3' terminus. The 5' conjugated antisense oligonucleotides, ISIS 661161 and ISIS 665001, comprising a $GalNAc_3$-3 or $GalNAc_3$-9, respectively, had increased potency compared to the 3' conjugated antisense oligonucleotides (ISIS 655861 and ISIS 664078).

Table 40

ASOs containing $GalNAc_3$-1, 3, 8 or 9 targeting SRB-1

ISIS No.	Dosage (mg/kg)	SRB-1 mRNA (% Saline)	Conjugate
Saline	n/a	100	
353382	3	88	none
	10	68	
	30	36	
655861	0.5	98	**$GalNac_3$ -1** (3')
	1.5	76	
	5	31	
	15	20	

664078	0.5	88	**GalNac$_3$-9** (3')
	1.5	85	
	5	46	
	15	20	
661161	0.5	92	**GalNac$_3$-3** (5')
	1.5	59	
	5	19	
	15	11	
665001	0.5	100	**GalNac$_3$-8** (5')
	1.5	73	
	5	29	
	15	13	

Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum were measured relative to saline injected mice using standard protocols. Total bilirubin and BUN were also evaluated. The change in body weights was evaluated with no significant change from the saline group. ALTs, ASTs, total bilirubin and BUN values are shown in the table below.

Table 41

ISIS No.	**Dosage mg/kg**	**ALT**	**AST**	**Total Bilirubin**	**BUN**	**Conjugate**
Saline		24	59	0.1	37.52	
353382	3	21	66	0.2	34.65	none
	10	22	54	0.2	34.2	
	30	22	49	0.2	33.72	
655861	0.5	25	62	0.2	30.65	**GalNac$_3$-1** (3')
	1.5	23	48	0.2	30.97	
	5	28	49	0.1	32.92	
	15	40	97	0.1	31.62	
664078	0.5	40	74	0.1	35.3	**GalNac$_3$-9** (3')
	1.5	47	104	0.1	32.75	
	5	20	43	0.1	30.62	
	15	38	92	0.1	26.2	
661161	0.5	101	162	0.1	34.17	**GalNac$_3$-3** (5')
	1.5 g	42	100	0.1	33.37	
	5 g	23	99	0.1	34.97	
	15	53	83	0.1	34.8	
665001	0.5	28	54	0.1	31.32	**GalNac$_3$-8** (5')
	1.5	42	75	0.1	32.32	
	5	24	42	0.1	31.85	
	15	32	67	0.1	31.	

Example 56: Dose-dependent study of oligonucleotides comprising either a 3' or 5'-conjugate group (comparison of GalNAc$_3$-1, 2, 3, 5, 6, 7 and 10) targeting SRB-1 *in vivo*

The oligonucleotides listed below were tested in a dose-dependent study for antisense inhibition of SRB-1 in mice. Unconjugated ISIS 353382 was included as a standard. Each of the various GalNAc$_3$ conjugate groups was attached at the 5' terminus of the respective oligonucleotide by a phosphodiester linked 2'-deoxyadenosine nucleoside (cleavable moiety) except for ISIS 655861 which had the GalNAc$_3$ conjugate group attached at the 3' terminus.

Table 42

Modified ASO targeting SRB-1

ASO	Sequence (5' to 3')	Motif	**Conjugate**	SEQ ID No.
ISIS 353382 (parent)	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	no conjugate	829
ISIS 655861	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{eo}\mathbf{A_{do'}\text{-}GalNAc_3\text{-}1_a}$	5/10/5	**GalNAc$_3$-1**	830
ISIS 664507	$\mathbf{GalNAc_3\text{-}2_a\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-2**	831
ISIS 661161	$\mathbf{GalNAc_3\text{-}3_{a\text{-}o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-3**	831
ISIS 666224	$\mathbf{GalNAc_3\text{-}5_a\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-5**	831
ISIS 666961	$\mathbf{GalNAc_3\text{-}6_a\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-6**	831
ISIS 666981	$\mathbf{GalNAc_3\text{-}7_a\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-7**	831
ISIS 666881	$\mathbf{GalNAc_3\text{-}10_a\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-10**	831

Capital letters indicate the nucleobase for each nucleoside and ^{m}C indicates a 5-methyl cytosine. Subscripts: "e" indicates a 2'-MOE modified nucleoside; "d" indicates a β-D-2'-deoxyribonucleoside; "s" indicates a phosphorothioate internucleoside linkage (PS); "o" indicates a phosphodiester internucleoside linkage (PO); and "o'" indicates -O-P(=O)(OH)-. Conjugate groups are in bold.

The structure of $GalNAc_3$-1_a was shown previously in Example 9. The structure of $GalNAc_3$-2_a was shown previously in Example 37. The structure of $GalNAc_3$-3_a was shown previously in Example 39. The structure of $GalNAc_3$-5_a was shown previously in Example 49. The structure of $GalNAc_3$-6_a was shown previously in Example 51. The structure of $GalNAc_3$-7_a was shown previously in Example 48. The structure of $GalNAc_3$-10_a was shown previously in Example 46.

Treatment

Six week old male Balb/c mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously once at the dosage shown below with ISIS 353382, 655861, 664507, 661161, 666224, 666961, 666981, 666881 or with saline. Each treatment group consisted of 4 animals. The mice were sacrificed 72 hours following the final administration to determine the liver SRB-1 mRNA levels using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. The results below are presented as the average percent of SRB-1 mRNA levels for each treatment group, normalized to the saline control.

As illustrated in Table 43, treatment with antisense oligonucleotides lowered SRB-1 mRNA levels in a dose-dependent manner. Indeed, the conjugated antisense oligonucleotides showed substantial improvement in potency compared to the unconjugated antisense oligonucleotide (ISIS 353382). The 5' conjugated antisense oligonucleotides showed a slight increase in potency compared to the 3' conjugated antisense oligonucleotide.

Table 43

ISIS No.	Dosage (mg/kg)	SRB-1 mRNA (% Saline)	Conjugate
Saline	n/a	100.0	
353382	3	96.0	none
	10	73.1	
	30	36.1	
655861	0.5	99.4	**$GalNac_3$-1** (3')
	1.5	81.2	
	5	33.9	
	15	15.2	
664507	0.5	102.0	**$GalNac_3$-2** (5')
	1.5	73.2	
	5	31.3	
	15	10.8	

661161	0.5	90.7	**GalNac$_3$-3** (5')
	1.5	67.6	
	5	24.3	
	15	11.5	
666224	0.5	96.1	**GalNac$_3$-5** (5')
	1.5	61.6	
	5	25.6	
	15	11.7	
666961	0.5	85.5	**GalNAc$_3$-6** (5')
	1.5	56.3	
	5	34.2	
	15	13.1	
666981	0.5	84.7	**GalNAc$_3$-7** (5')
	1.5	59.9	
	5	24.9	
	15	8.5	
666881	0.5	100.0	**GalNAc$_3$-10** (5')
	1.5	65.8	
	5	26.0	
	15	13.0	

Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum were measured relative to saline injected mice using standard protocols. Total bilirubin and BUN were also evaluated. The change in body weights was evaluated with no significant change from the saline group. ALTs, ASTs, total bilirubin and BUN values are shown in Table 44 below.

Table 44

ISIS No.	Dosage mg/kg	ALT	AST	Total Bilirubin	BUN	Conjugate
Saline		26	57	0.2	27	
353382	3	25	92	0.2	27	none
	10	23	40	0.2	25	
	30	29	54	0.1	28	
655861	0.5	25	71	0.2	34	**GalNac$_3$-1** (3')
	1.5	28	60	0.2	26	
	5	26	63	0.2	28	
	15	25	61	0.2	28	
664507	0.5	25	62	0.2	25	**GalNac$_3$-2** (5')
	1.5	24	49	0.2	26	
	5	21	50	0.2	26	
	15	59	84	0.1	22	
661161	0.5	20	42	0.2	29	**GalNac$_3$-3** (5')
	1.5 g	37	74	0.2	25	

	5 g	28	61	0.2	29	
	15	21	41	0.2	25	
666224	0.5	34	48	0.2	21	**GalNac$_3$-5** (5')
	1.5	23	46	0.2	26	
	5	24	47	0.2	23	
	15	32	49	0.1	26	
666961	0.5	17	63	0.2	26	**GalNAc$_3$-6** (5')
	1.5	23	68	0.2	26	
	5	25	66	0.2	26	
	15	29	107	0.2	28	
666981	0.5	24	48	0.2	26	**GalNAc$_3$-7** (5')
	1.5	30	55	0.2	24	
	5	46	74	0.1	24	
	15	29	58	0.1	26	
666881	0.5	20	65	0.2	27	**GalNAc$_3$-10** (5')
	1.5	23	59	0.2	24	
	5	45	70	0.2	26	
	15	21	57	0.2	24	

Example 57: Duration of action study of oligonucleotides comprising a 3'-conjugate group targeting ApoC III *in vivo*

Mice were injected once with the doses indicated below and monitored over the course of 42 days for ApoC-III and plasma triglycerides (Plasma TG) levels. The study was performed using 3 transgenic mice that express human APOC-III in each group.

Table 45

Modified ASO targeting ApoC III

ASO	Sequence (5' to 3')	Linkages	SEQ ID No.
ISIS 304801	$A_{es}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}$ $^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{es}T_{es}T_{es}A_{es}T_{e}$	PS	821
ISIS 647535	$A_{es}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}$ $A_{ds}G_{ds}{}^{m}C_{ds}T_{es}T_{es}T_{es}A_{es}T_{eo}\mathbf{A_{do'}}$-**GalNAc$_3-1_a$**	PS	822
ISIS 647536	$A_{es}G_{eo}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}$ $A_{ds}G_{ds}{}^{m}C_{ds}T_{eo}T_{eo}T_{es}A_{es}T_{eo}\mathbf{A_{do'}}$-**GalNAc$_3-1_a$**	PO/PS	822

Capital letters indicate the nucleobase for each nucleoside and ^{m}C indicates a 5-methyl cytosine. Subscripts: "e" indicates a 2'-MOE modified nucleoside; "d" indicates a β-D-2'-deoxyribonucleoside; "s" indicates a phosphorothioate internucleoside linkage (PS); "o" indicates a

phosphodiester internucleoside linkage (PO); and "o'" indicates -O-P(=O)(OH)-. Conjugate groups are in bold.

The structure of $GalNAc_3$-1_a was shown previously in Example 9.

Table 46

ApoC III mRNA (% Saline on Day 1) and Plasma TG Levels (% Saline on Day 1)

ASO	Dose	Target	Day 3	Day 7	Day 14	Day 35	Day 42
Saline	0 mg/kg	ApoC-III	98	100	100	95	116
ISIS 304801	30 mg/kg	ApoC-III	28	30	41	65	74
ISIS 647535	10 mg/kg	ApoC-III	16	19	25	74	94
ISIS 647536	10 mg/kg	ApoC-III	18	16	17	35	51
Saline	0 mg/kg	Plasma TG	121	130	123	105	109
ISIS 304801	30 mg/kg	Plasma TG	34	37	50	69	69
ISIS 647535	10 mg/kg	Plasma TG	18	14	24	18	71
ISIS 647536	10 mg/kg	Plasma TG	21	19	15	32	35

As can be seen in the table above the duration of action increased with addition of the 3'-conjugate group compared to the unconjugated oligonucleotide. There was a further increase in the duration of action for the conjugated mixed PO/PS oligonucleotide 647536 as compared to the conjugated full PS oligonucleotide 647535.

Example 58: Dose-dependent study of oligonucleotides comprising a 3'-conjugate group (comparison of $GalNAc_3$-1 and $GalNAc_4$-11) targeting SRB-1 *in vivo*

The oligonucleotides listed below were tested in a dose-dependent study for antisense inhibition of SRB-1 in mice. Unconjugated ISIS 440762 was included as an unconjugated standard. Each of the conjugate groups were attached at the 3' terminus of the respective oligonucleotide by a phosphodiester linked 2'-deoxyadenosine nucleoside cleavable moiety.

The structure of $GalNAc_3$-1_a was shown previously in Example 9. The structure of $GalNAc_3$-11_a was shown previously in Example 50.

Treatment

Six week old male Balb/c mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously once at the dosage shown below with ISIS 440762, 651900, 663748 or with saline. Each treatment group consisted of 4 animals. The mice were sacrificed 72 hours following the final administration to determine the liver SRB-1 mRNA levels using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. The results below are presented as the average percent of SRB-1 mRNA levels for each treatment group, normalized to the saline control.

As illustrated in Table 47, treatment with antisense oligonucleotides lowered SRB-1 mRNA levels in a dose-dependent manner. The antisense oligonucleotides comprising the phosphodiester linked $GalNAc_3$-1 and $GalNAc_4$-11 conjugates at the 3' terminus (ISIS 651900 and ISIS 663748) showed substantial improvement in potency compared to the unconjugated antisense oligonucleotide (ISIS 440762). The two conjugated oligonucleotides, $GalNAc_3$-1 and $GalNAc_4$-11, were equipotent.

Table 47

Modified ASO targeting SRB-1

ASO	Sequence (5' to 3')	Dose mg/kg	% Saline control	SEQ ID No.
Saline			100	
ISIS 440762	$T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{k}$	0.6	73.45	823
		2	59.66	
		6	23.50	
ISIS 651900	$T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{k}$**$_{o}A_{do'}$-GalNAc$_3-1_a$**	0.2	62.75	824
		0.6	29.14	
		2	8.61	
		6	5.62	
ISIS 663748	$T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{k}$**$_{o}A_{do'}$-GalNAc$_4-11_a$**	0.2	63.99	824
		0.6	33.53	
		2	7.58	
		6	5.52	

Capital letters indicate the nucleobase for each nucleoside and ^{m}C indicates a 5-methyl cytosine. Subscripts: "e" indicates a 2'-MOE modified nucleoside; "k" indicates 6'-*(S)*-CH_3 bicyclic nucleoside; "d" indicates a β-D-2'-deoxyribonucleoside; "s" indicates a phosphorothioate internucleoside linkage (PS); "o" indicates a phosphodiester internucleoside linkage (PO); and "o'" indicates -O-P(=O)(OH)-. Conjugate groups are in bold.

Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum were measured relative to saline injected mice using standard protocols. Total bilirubin and BUN were also evaluated. The change in body weights was evaluated with no significant change from the saline group. ALTs, ASTs, total bilirubin and BUN values are shown in Table 48 below.

Table 48

ISIS No.	Dosage mg/kg	ALT	AST	Total Bilirubin	BUN	Conjugate
Saline		30	76	0.2	40	
440762	0.60	32	70	0.1	35	none
	2	26	57	0.1	35	
	6	31	48	0.1	39	
651900	0.2	32	115	0.2	39	**GalNac$_3$-1** (3')
	0.6	33	61	0.1	35	
	2	30	50	0.1	37	
	6	34	52	0.1	36	
663748	0.2	28	56	0.2	36	**GalNac$_4$-11** (3')
	0.6	34	60	0.1	35	
	2	44	62	0.1	36	
	6	38	71	0.1	33	

Example 59: Effects of GalNAc$_3$-1 conjugated ASOs targeting FXI *in vivo*

The oligonucleotides listed below were tested in a multiple dose study for antisense inhibition of FXI in mice. ISIS 404071 was included as an unconjugated standard. Each of the conjugate groups was attached at the 3' terminus of the respective oligonucleotide by a phosphodiester linked 2'-deoxyadenosine nucleoside cleavable moiety.

Table 49

Modified ASOs targeting FXI

ASO	Sequence (5' to 3')	Linkages	SEQ ID No.
ISIS 404071	$T_{es}G_{es}G_{es}T_{es}A_{es}A_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}{}^{m}C_{ds}$ $T_{ds}T_{ds}T_{ds}{}^{m}C_{ds}A_{es}G_{es}A_{es}G_{es}G_{e}$	PS	832
ISIS 656172	$T_{es}G_{es}G_{es}T_{es}A_{es}A_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}{}^{m}C_{ds}$ $T_{ds}T_{ds}T_{ds}{}^{m}C_{ds}A_{es}G_{es}A_{es}G_{es}G_{eo}\mathbf{A_{do}}$'-**GalNAc$_3-1_a$**	PS	833
ISIS 656173	$T_{es}G_{eo}G_{eo}T_{eo}A_{eo}A_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}{}^{m}C_{ds}$ $T_{ds}T_{ds}T_{ds}{}^{m}C_{ds}A_{eo}G_{eo}A_{es}G_{es}G_{eo}\mathbf{A_{do}}$'-**GalNAc$_3-1_a$**	PO/PS	833

Capital letters indicate the nucleobase for each nucleoside and ^{m}C indicates a 5-methyl cytosine. Subscripts: "e" indicates a 2'-MOE modified nucleoside; "d" indicates a β-D-2'-deoxyribonucleoside; "s" indicates a phosphorothioate internucleoside linkage (PS); "o" indicates a phosphodiester internucleoside linkage (PO); and "o'" indicates -O-P(=O)(OH)-. Conjugate groups are in bold.

The structure of GalNAc$_3$-1$_a$ was shown previously in Example 9.

Treatment

Six week old male Balb/c mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously twice a week for 3 weeks at the dosage shown below with ISIS 404071, 656172, 656173 or with PBS treated control. Each treatment group consisted of 4 animals. The mice were sacrificed 72 hours following the final administration to determine the liver FXI mRNA levels using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. Plasma FXI protein levels were also measured using ELISA. FXI mRNA levels were determined relative to total RNA (using RIBOGREEN®), prior to normalization to PBS-treated control. The results below are presented as the average percent of FXI mRNA levels for each treatment group. The data was normalized to PBS-treated control and is denoted as "% PBS". The ED$_{50}$s were measured using similar methods as described previously and are presented below.

Table 50

Factor XI mRNA (% Saline)

ASO	Dose mg/kg	% Control	Conjugate	Linkages
Saline		100	none	
ISIS 404071	3	92	none	PS
	10	40		
	30	15		
ISIS 656172	0.7	74	**GalNAc$_3$-1**	PS
	2	33		
	6	9		
ISIS 656173	0.7	49	**GalNAc$_3$-1**	PO/PS
	2	22		
	6	1		

As illustrated in Table 50, treatment with antisense oligonucleotides lowered FXI mRNA levels in a dose-dependent manner. The oligonucleotides comprising a 3'-GalNAc$_3$-1 conjugate

group showed substantial improvement in potency compared to the unconjugated antisense oligonucleotide (ISIS 404071). Between the two conjugated oligonucleotides an improvement in potency was further provided by substituting some of the PS linkages with PO (ISIS 656173).

As illustrated in Table 50a, treatment with antisense oligonucleotides lowered FXI protein levels in a dose-dependent manner. The oligonucleotides comprising a 3'-$GalNAc_3$-1 conjugate group showed substantial improvement in potency compared to the unconjugated antisense oligonucleotide (ISIS 404071). Between the two conjugated oligonucleotides an improvement in potency was further provided by substituting some of the PS linkages with PO (ISIS 656173).

Table 50a

Factor XI protein (% Saline)

ASO	Dose mg/kg	Protein (% Control)	Conjugate	Linkages
Saline		100	none	
ISIS 404071	3	127	none	PS
	10	32		
	30	3		
ISIS 656172	0.7	70	**$GalNAc_3$-1**	PS
	2	23		
	6	1		
ISIS 656173	0.7	45	**$GalNAc_3$-1**	PO/PS
	2	6		
	6	0		

Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum were measured relative to saline injected mice using standard protocols. Total bilirubin, total albumin, CRE and BUN were also evaluated. The change in body weights was evaluated with no significant change from the saline group. ALTs, ASTs, total bilirubin and BUN values are shown in the table below.

Table 51

ISIS No.	Dosage mg/kg	ALT	AST	Total Albumin	Total Bilirubin	CRE	BUN	Conjugate
Saline		71.8	84.0	3.1	0.2	0.2	22.9	
404071	3	152.8	176.0	3.1	0.3	0.2	23.0	none
	10	73.3	121.5	3.0	0.2	0.2	21.4	
	30	82.5	92.3	3.0	0.2	0.2	23.0	
656172	0.7	62.5	111.5	3.1	0.2	0.2	23.8	**$GalNac_3$-1**

	2	33.0	51.8	2.9	0.2	0.2	22.0	(3')
	6	65.0	71.5	3.2	0.2	0.2	23.9	
656173	0.7	54.8	90.5	3.0	0.2	0.2	24.9	**GalNac$_3$-1** (3')
	2	85.8	71.5	3.2	0.2	0.2	21.0	
	6	114.0	101.8	3.3	0.2	0.2	22.7	

Example 60: Effects of conjugated ASOs targeting SRB-1 *in vitro*

The oligonucleotides listed below were tested in a multiple dose study for antisense inhibition of SRB-1 in primary mouse hepatocytes. ISIS 353382 was included as an unconjugated standard. Each of the conjugate groups were attached at the 3' or 5' terminus of the respective oligonucleotide by a phosphodiester linked 2'-deoxyadenosine nucleoside cleavable moiety.

Table 52

Modified ASO targeting SRB-1

ASO	Sequence (5' to 3')	Motif	Conjugate	SEQ ID No.
ISIS 353382	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	none	829
ISIS 655861	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{eo}$**$A_{do'}$-GalNAc$_3-1_a$**	5/10/5	**GalNAc$_3$-1**	830
ISIS 655862	$G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T_{eo}$**$A_{do'}$-GalNAc$_3-1_a$**	5/10/5	**GalNAc$_3$-1**	830
ISIS 661161	**GalNAc$_3$-3$_{a\text{-}o}$,A_{do}**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-3**	831
ISIS 665001	**GalNAc$_3$-8$_{a\text{-}o}$,A_{do}**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-8**	831
ISIS 664078	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{eo}$**$A_{do'}$-GalNAc$_3-9_a$**	5/10/5	**GalNAc$_3$-9**	830
ISIS 666961	**GalNAc$_3$-6$_{a\text{-}o}$,A_{do}**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-6**	831
ISIS 664507	**GalNAc$_3$-2$_{a\text{-}o}$,A_{do}**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-2**	831
ISIS 666881	**GalNAc$_3$-10$_{a\text{-}o}$,A_{do}**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-10**	831
ISIS 666224	**GalNAc$_3$-5$_{a\text{-}o}$,A_{do}**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-5**	831
ISIS 666981	**GalNAc$_3$-7$_{a\text{-}o}$,A_{do}**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-7**	831

Capital letters indicate the nucleobase for each nucleoside and ^{m}C indicates a 5-methyl cytosine. Subscripts: "e" indicates a 2'-MOE modified nucleoside; "d" indicates a β-D-2'-deoxyribonucleoside; "s" indicates a phosphorothioate internucleoside linkage (PS); "o" indicates a phosphodiester internucleoside linkage (PO); and "o'" indicates -O-P(=O)(OH)-. Conjugate groups are in bold.

The structure of GalNAc$_3$-1$_a$ was shown previously in Example 9. The structure of GalNAc$_3$-3a was shown previously in Example 39. The structure of GalNAc$_3$-8a was shown previously in Example 47. The structure of GalNAc$_3$-9a was shown previously in Example 52. The structure of GalNAc$_3$-6a was shown previously in Example 51. The structure of GalNAc$_3$-2a was shown previously in Example 37. The structure of GalNAc$_3$-10a was shown previously in Example 46. The structure of GalNAc$_3$-5a was shown previously in Example 49. The structure of GalNAc$_3$-7a was shown previously in Example 48.

Treatment

The oligonucleotides listed above were tested *in vitro* in primary mouse hepatocyte cells plated at a density of 25,000 cells per well and treated with 0.03, 0.08, 0.24, 0.74, 2.22, 6.67 or 20 nM modified oligonucleotide. After a treatment period of approximately 16 hours, RNA was isolated from the cells and mRNA levels were measured by quantitative real-time PCR and the SRB-1 mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®.

The IC$_{50}$ was calculated using standard methods and the results are presented in Table 53. The results show that, under free uptake conditions in which no reagents or electroporation techniques are used to artificially promote entry of the oligonucleotides into cells, the oligonucleotides comprising a GalNAc conjugate were significantly more potent in hepatocytes than the parent oligonucleotide (ISIS 353382) that does not comprise a GalNAc conjugate.

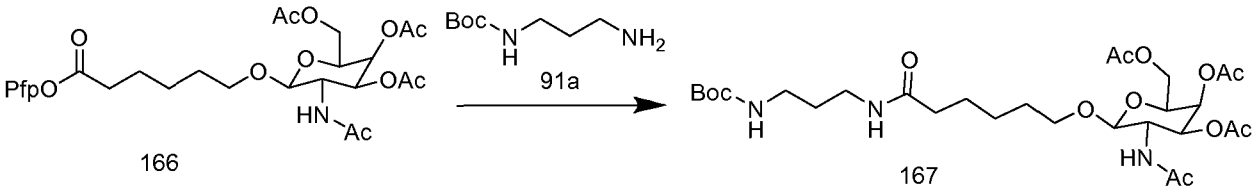
Table 53

ASO	IC$_{50}$ (nM)	Internucleoside linkages	Conjugate	SEQ ID No.
ISIS 353382	190[a]	PS	none	829
ISIS 655861	11[a]	PS	**GalNAc$_3$-1**	830
ISIS 655862	3	PO/PS	**GalNAc$_3$-1**	830

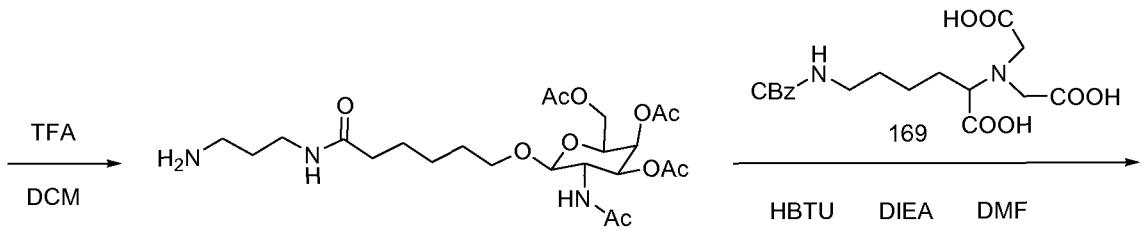
ISIS 661161	15[a]	PS	**GalNAc$_3$-3**	831
ISIS 665001	20	PS	**GalNAc$_3$-8**	831
ISIS 664078	55	PS	**GalNAc$_3$-9**	830
ISIS 666961	22[a]	PS	**GalNAc$_3$-6**	831
ISIS 664507	30	PS	**GalNAc$_3$-2**	831
ISIS 666881	30	PS	**GalNAc$_3$-10**	831
ISIS 666224	30[a]	PS	**GalNAc$_3$-5**	831
ISIS 666981	40	PS	**GalNAc$_3$-7**	831

[a]Average of multiple runs.

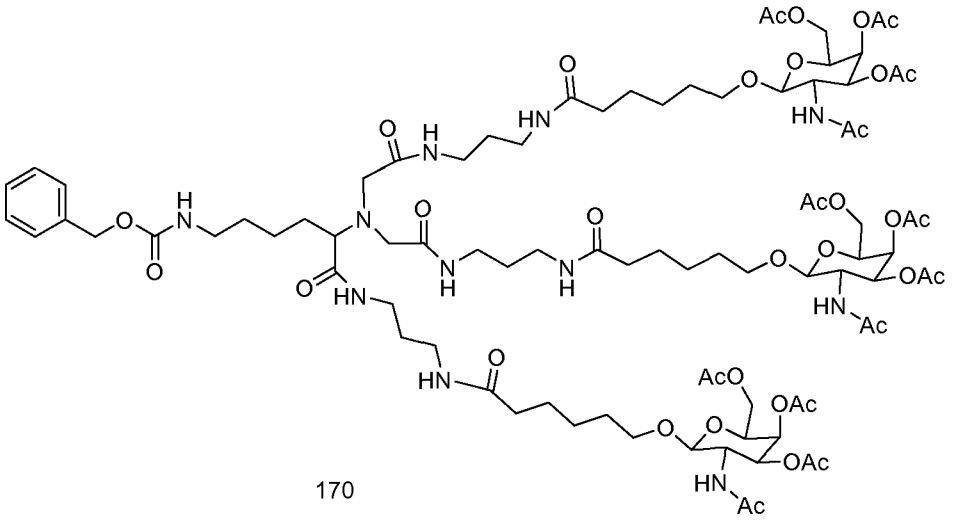
Example 61: Preparation of oligomeric compound 175 comprising GalNAc$_3$-12



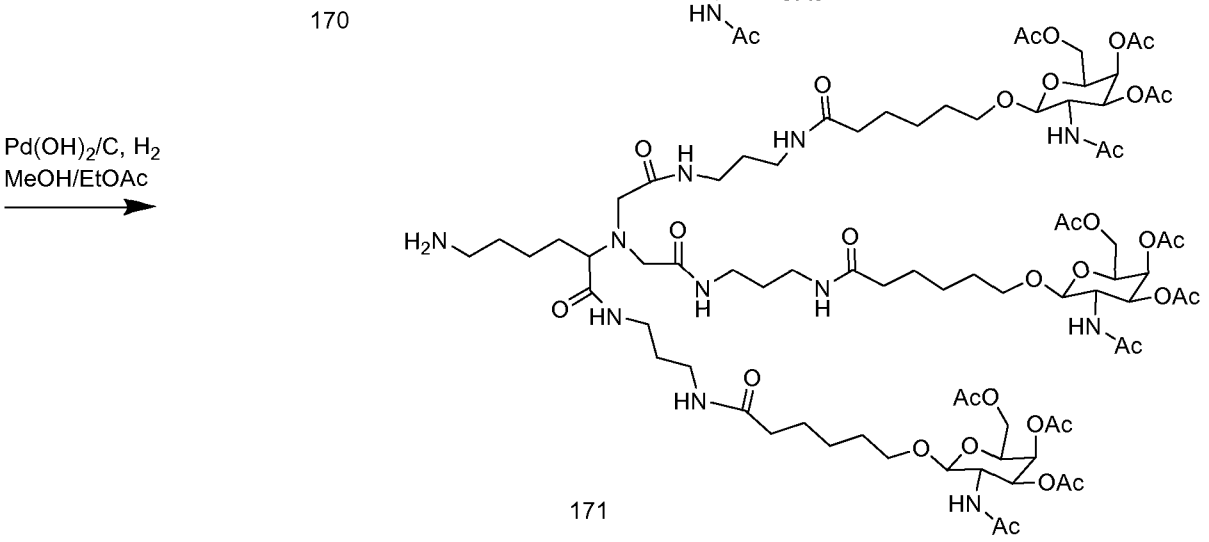
166 167



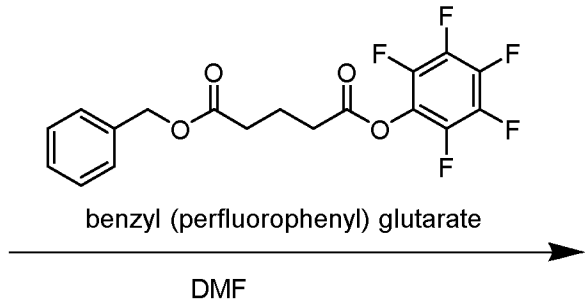
168 169

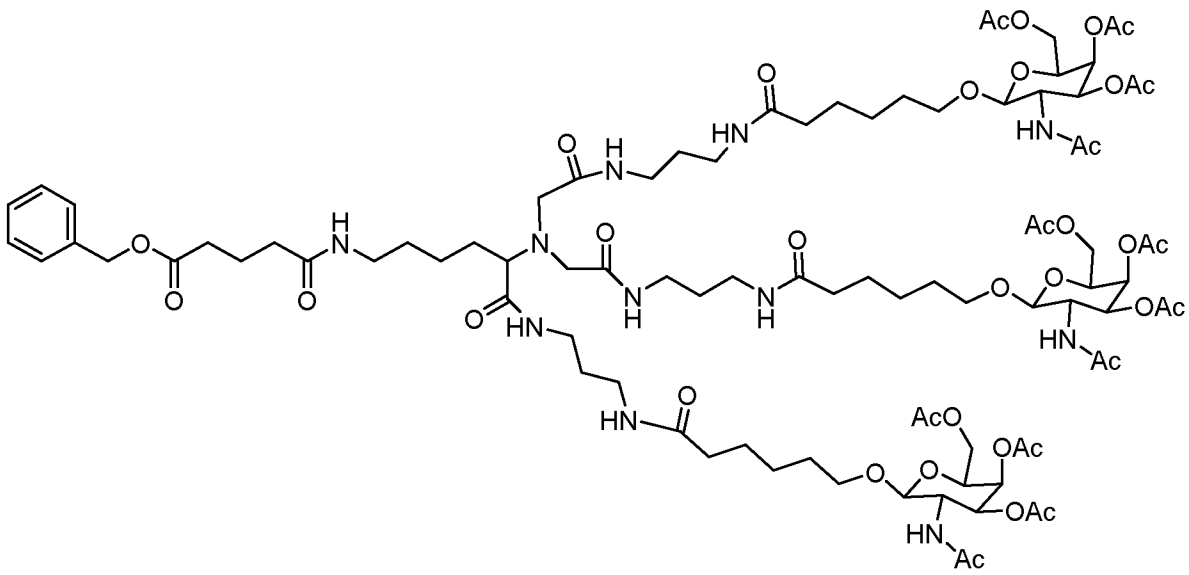


170

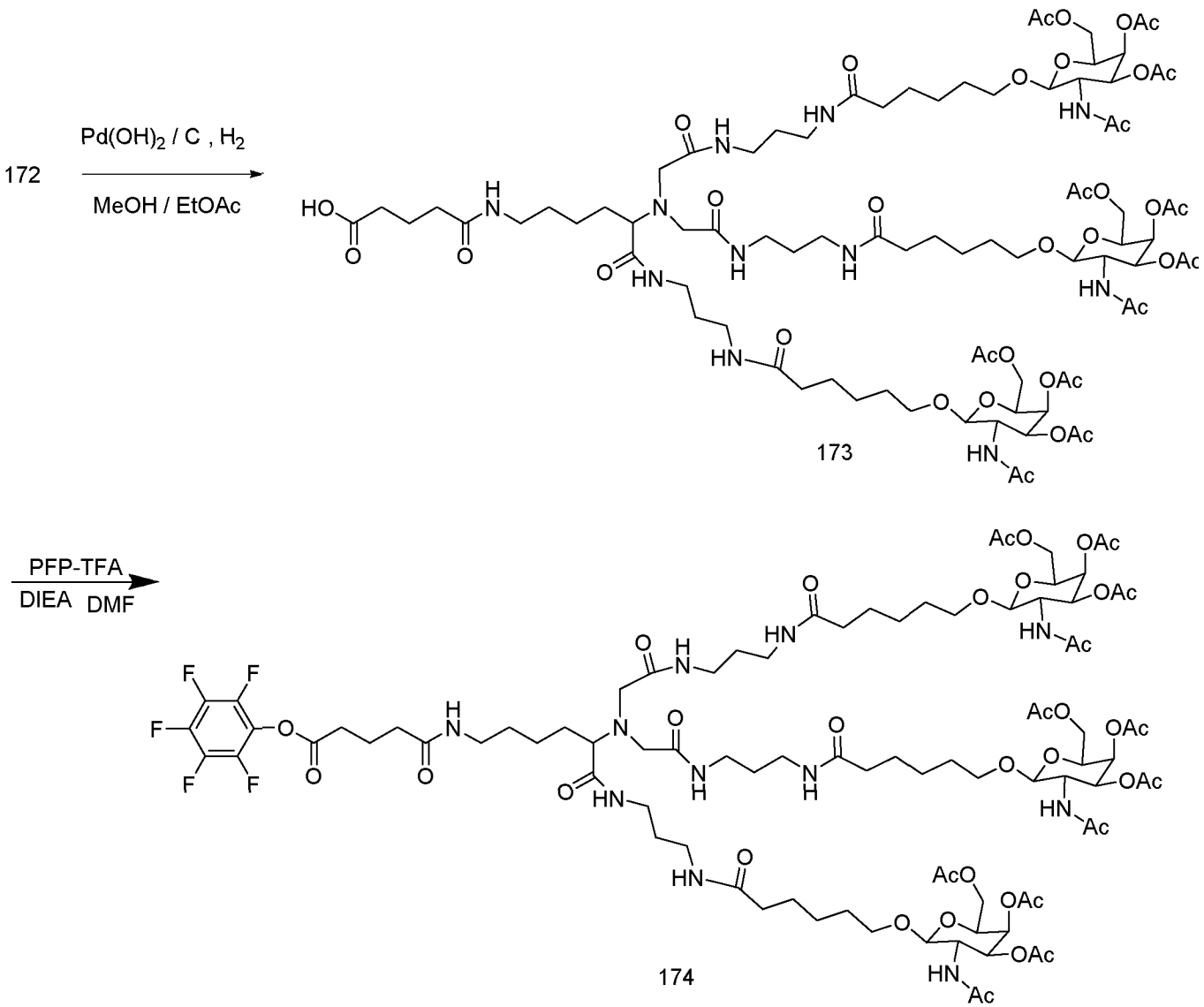


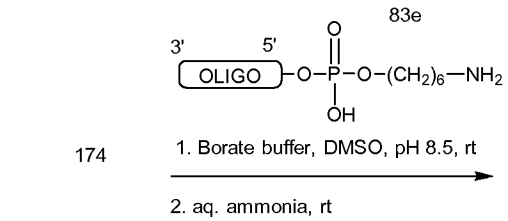
171

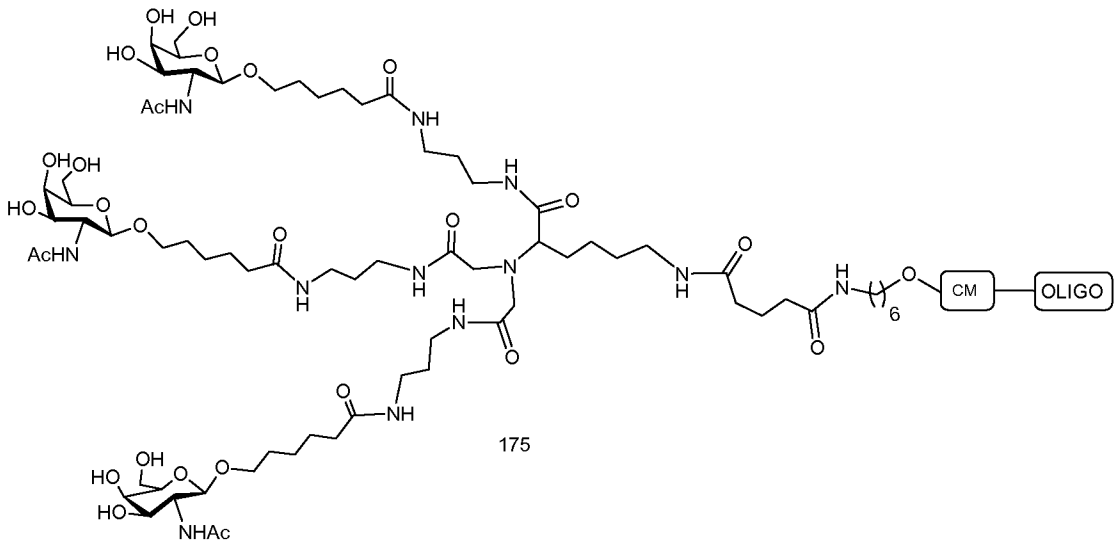




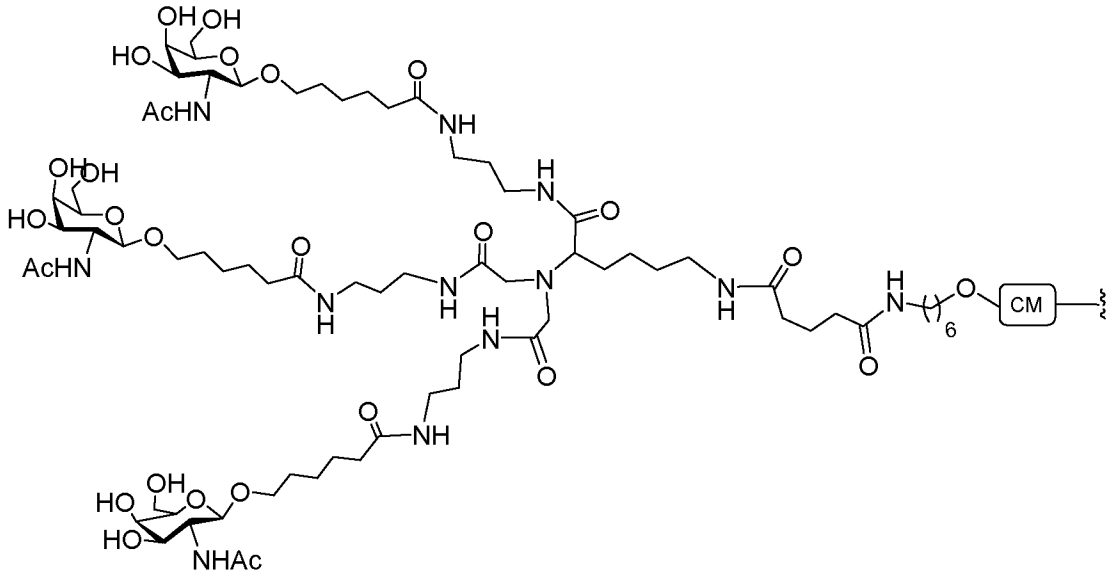
172


172
Pd(OH)2 / C , H2
MeOH / EtOAc
173
PFP-TFA
DIEA DMF
174

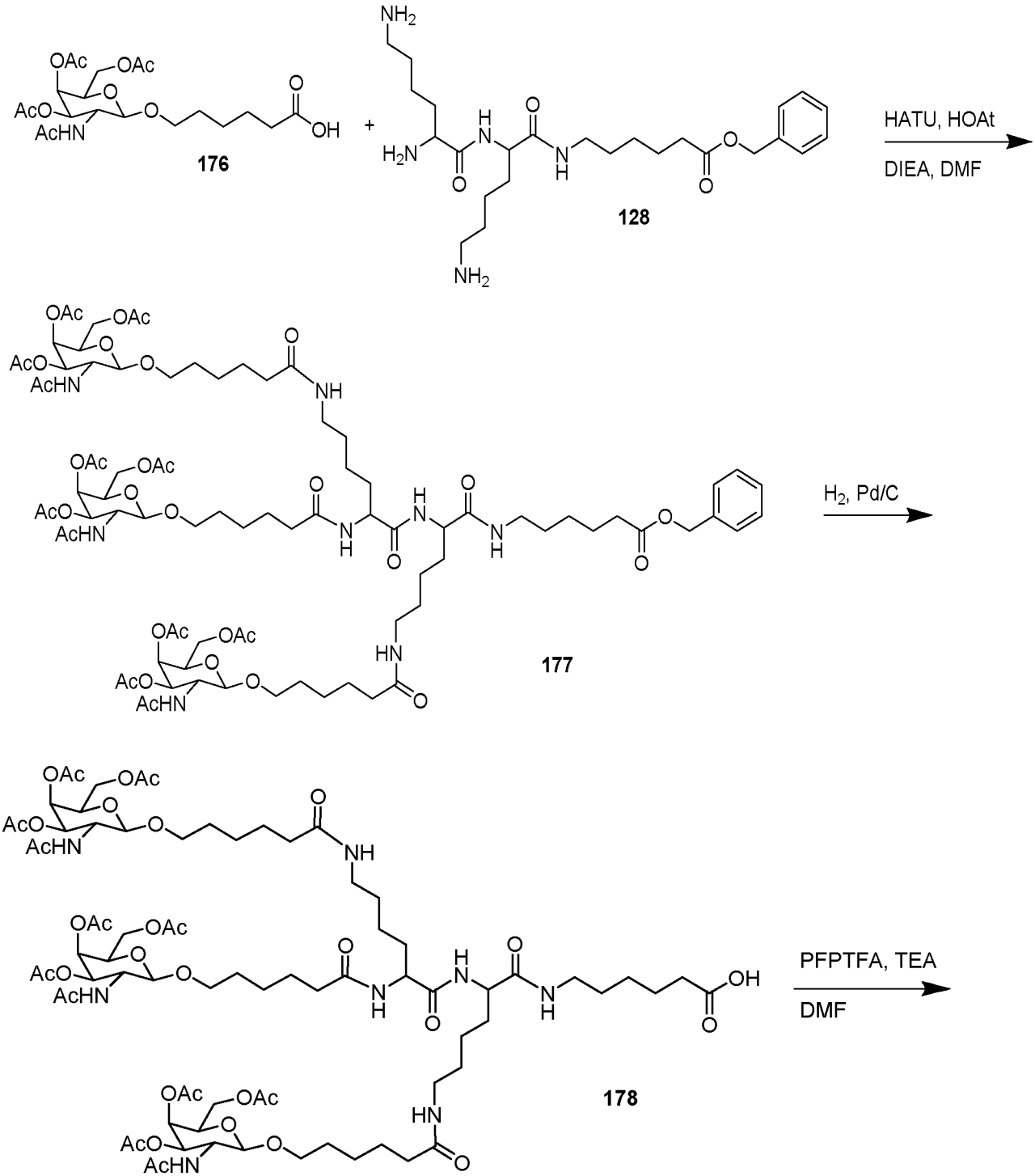


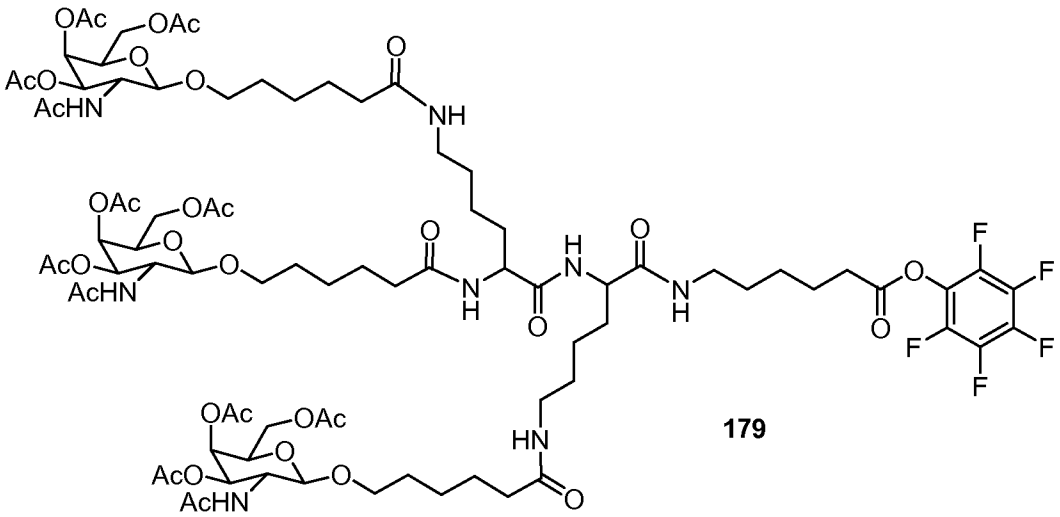


Compound 169 is commercially available. Compound 172 was prepared by addition of benzyl (perfluorophenyl) glutarate to compound 171. The benzyl (perfluorophenyl) glutarate was prepared by adding PFP-TFA and DIEA to 5-(benzyloxy)-5-oxopentanoic acid in DMF. Oligomeric compound 175, comprising a $GalNAc_3$-12 conjugate group, was prepared from compound 174 using the general procedures illustrated in Example 46. The $GalNAc_3$ cluster portion of the conjugate group $GalNAc_3$-12 ($GalNAc_3$-12_a) can be combined with any cleavable moiety to provide a variety of conjugate groups. In a certain embodiments, the cleavable moiety is -P(=O)(OH)-A_d-P(=O)(OH)-. The structure of $GalNAc_3$-12 ($GalNAc_3$-12_a-CM-) is shown below:

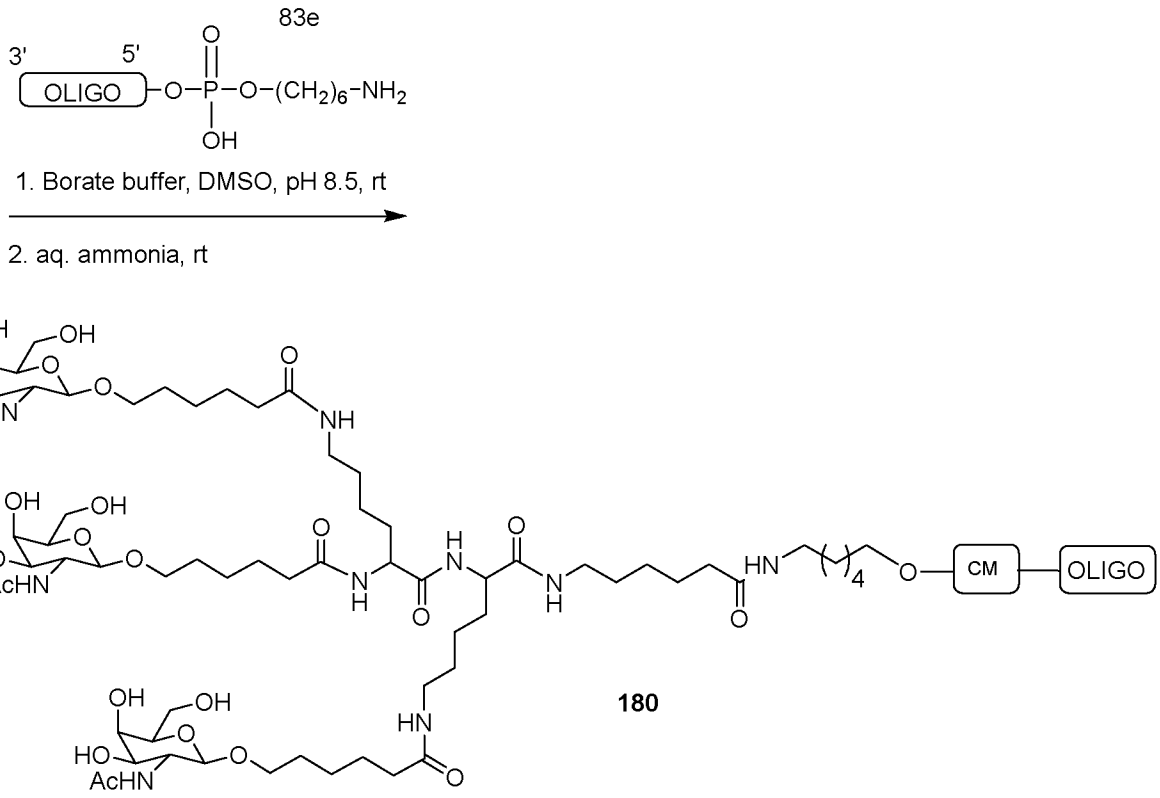


Example 62: Preparation of oligomeric compound 180 comprising $GalNAc_3$-13


176
128
HATU, HOAt
DIEA, DMF
177
H2, Pd/C
178
PFPTFA, TEA
DMF



179

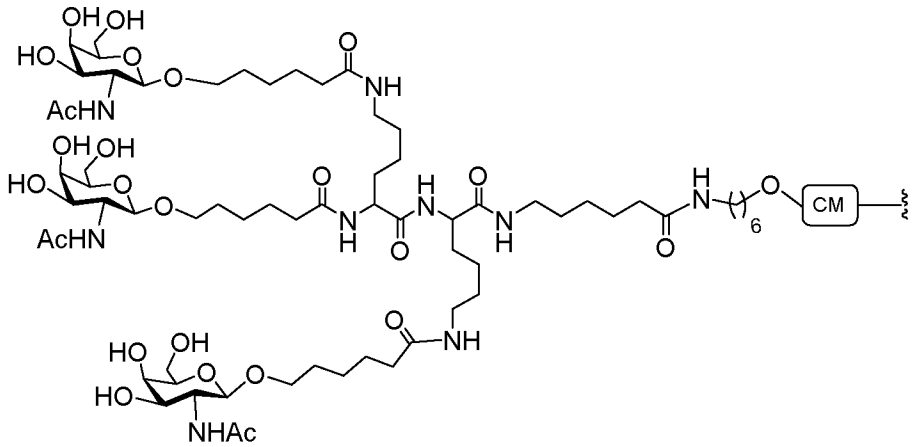


180

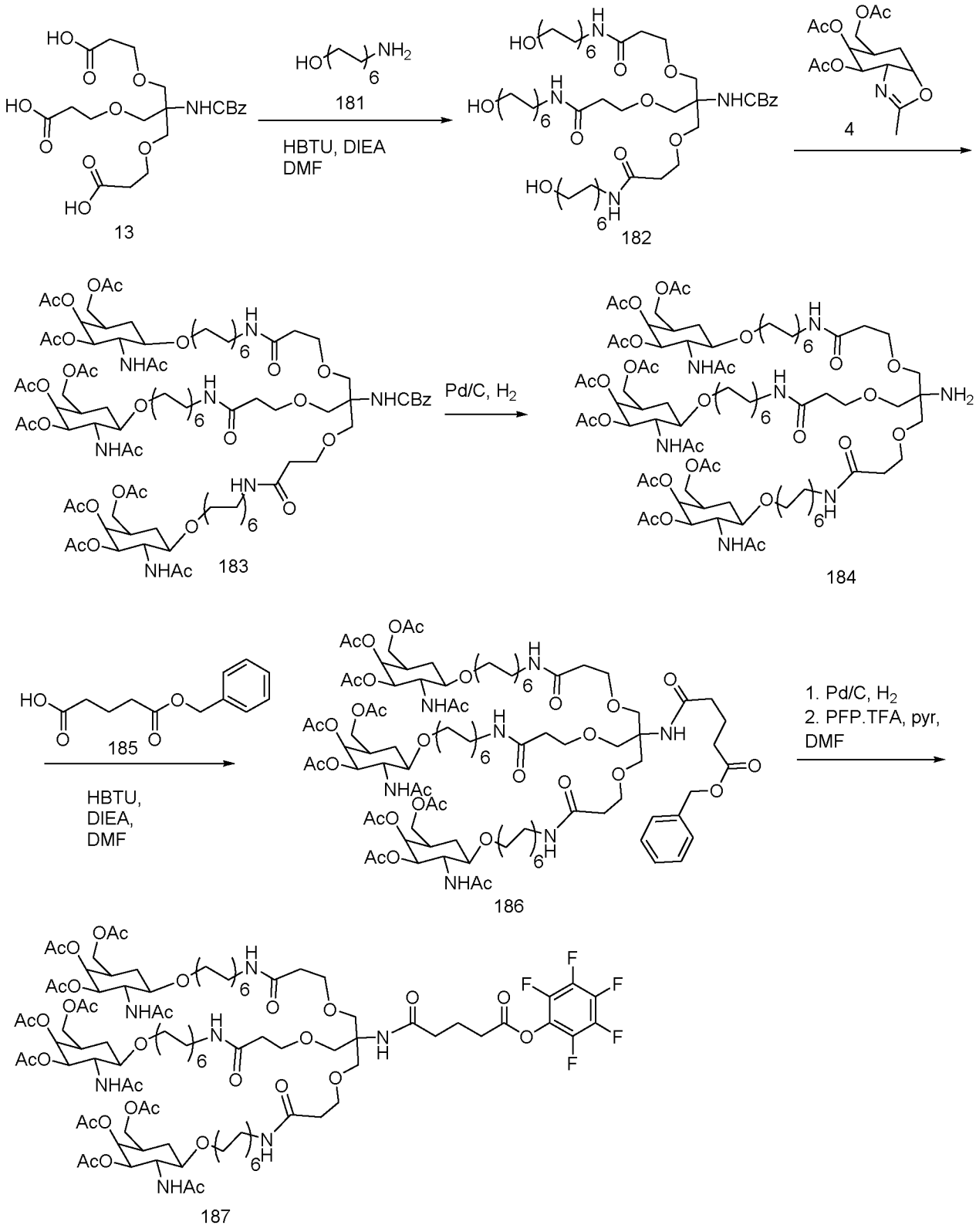
5

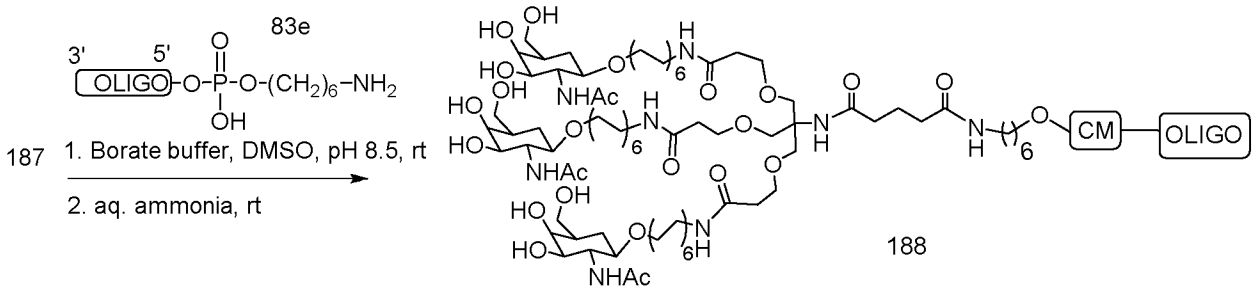
Compound 176 was prepared using the general procedure shown in Example 2. Oligomeric compound 180, comprising a $GalNAc_3$-13 conjugate group, was prepared from compound 177 using the general procedures illustrated in Example 49. The $GalNAc_3$ cluster portion of the conjugate group $GalNAc_3$-13 ($GalNAc_3$-13_a) can be combined with any cleavable moiety to provide a variety

of conjugate groups. In a certainembodiments, the cleavable moiety is -P(=O)(OH)-A_d-P(=O)(OH)-. The structure of $GalNAc_3$-13 ($GalNAc_3$-13_a-CM-) is shown below:

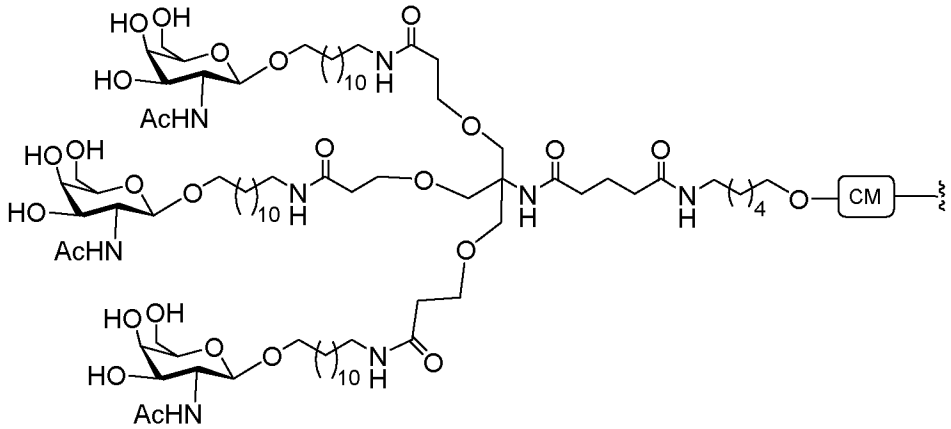


Example 63: Preparation of oligomeric compound 188 comprising $GalNAc_3$-14

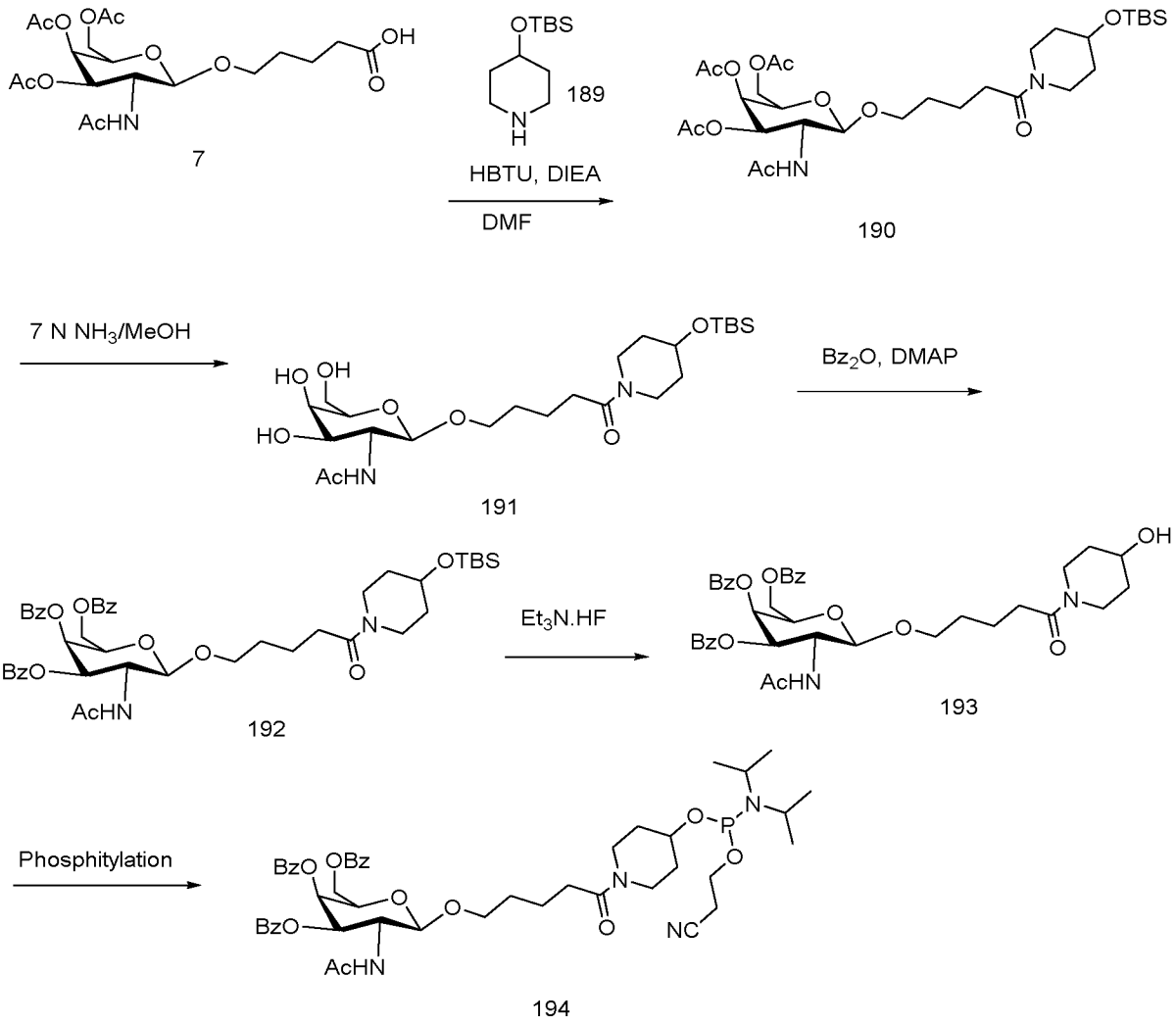

181
HBTU, DIEA
DMF
13
182
4
183
Pd/C, H2
184
185
HBTU,
DIEA,
DMF
186
1. Pd/C, H2
2. PFP.TFA, pyr,
DMF
187

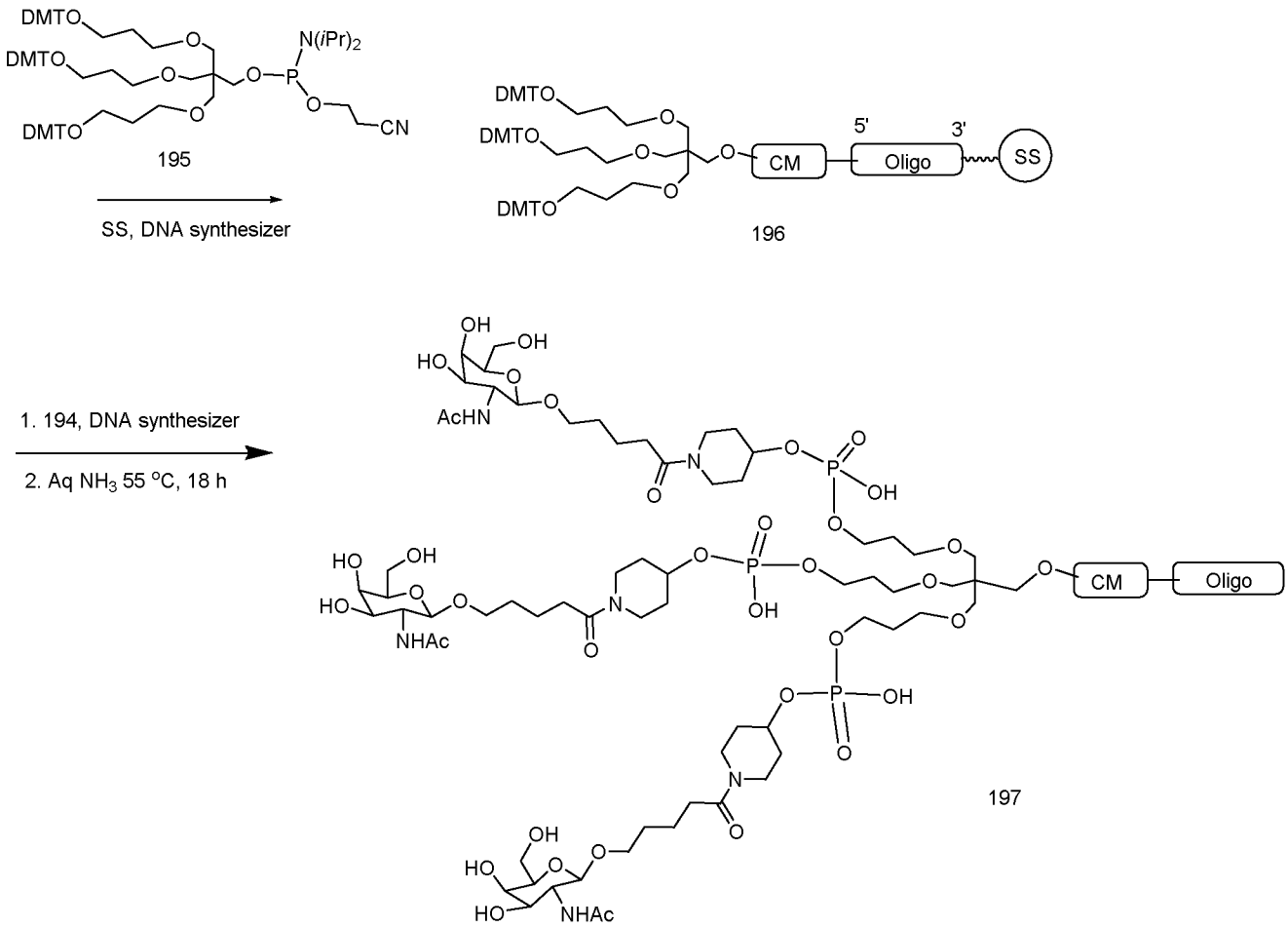


Compounds 181 and 185 are commercially available. Oligomeric compound 188, comprising a $GalNAc_3$-14 conjugate group, was prepared from compound 187 using the general procedures illustrated in Example 46. The $GalNAc_3$ cluster portion of the conjugate group $GalNAc_3$-14 ($GalNAc_3$-14_a) can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the cleavable moiety is -P(=O)(OH)-A_d-P(=O)(OH)-. The structure of $GalNAc_3$-14 ($GalNAc_3$-14_a-CM-) is shown below:

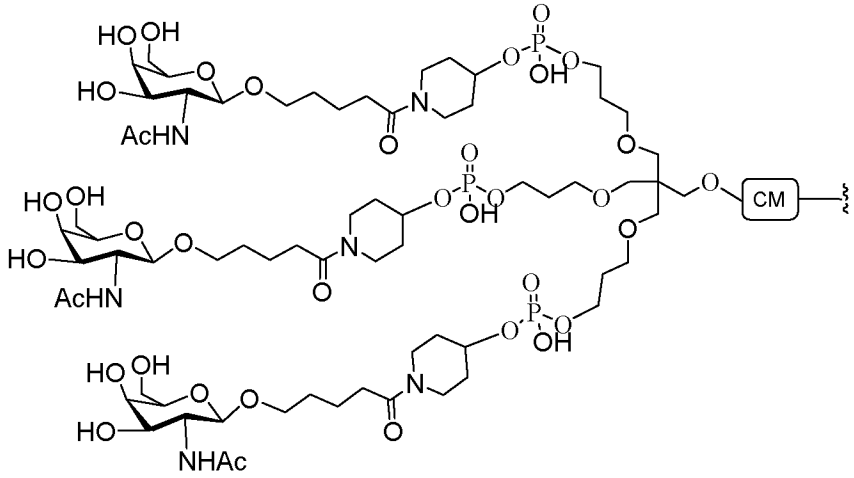


Example 64: Preparation of oligomeric compound 197 comprising $GalNAc_3$-15


AcO
OAc
AcO
AcHN
OH
O
7
OTBS
189
N
H
HBTU, DIEA
DMF
190
7 N NH3/MeOH
HO
OH
HO
AcHN
191
Bz2O, DMAP
BzO
OBz
BzO
AcHN
192
Et3N.HF
193
Phosphitylation
P
NC
194



Compound 189 is commercially available. Compound 195 was prepared using the general procedure shown in Example 31. Oligomeric compound 197, comprising a $GalNAc_3$-15 conjugate group, was prepared from compounds 194 and 195 using standard oligonucleotide synthesis procedures. The $GalNAc_3$ cluster portion of the conjugate group $GalNAc_3$-15 ($GalNAc_3$-15_a) can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the cleavable moiety is -P(=O)(OH)-A_d-P(=O)(OH)-. The structure of $GalNAc_3$-15 ($GalNAc_3$-15_a-CM-) is shown below:



Example 65: Dose-dependent study of oligonucleotides comprising a 5'-conjugate group (comparison of GalNAc$_3$-3, 12, 13, 14, and 15) targeting SRB-1 *in vivo*

The oligonucleotides listed below were tested in a dose-dependent study for antisense inhibition of SRB-1 in mice. Unconjugated ISIS 353382 was included as a standard. Each of the GalNAc$_3$ conjugate groups was attached at the 5' terminus of the respective oligonucleotide by a phosphodiester linked 2'-deoxyadenosine nucleoside (cleavable moiety).

Table 54

Modified ASOs targeting SRB-1

ISIS No.	Sequences (5' to 3')	Conjugate	SEQ ID No.
353382	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	none	829
661161	**GalNAc$_3$-3$_a$-$_o$'A$_{do}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	GalNAc$_3$-3	831
671144	**GalNAc$_3$-12$_a$-$_o$'A$_{do}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	GalNAc$_3$-12	831
670061	**GalNAc$_3$-13$_a$-$_o$'A$_{do}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	GalNAc$_3$-13	831
671261	**GalNAc$_3$-14$_a$-$_o$'A$_{do}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	GalNAc$_3$-14	831
671262	**GalNAc$_3$-15$_a$-$_o$'A$_{do}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	GalNAc$_3$-15	831

Capital letters indicate the nucleobase for each nucleoside and ^{m}C indicates a 5-methyl cytosine. Subscripts: "e" indicates a 2'-MOE modified nucleoside; "d" indicates a β-D-2'-deoxyribonucleoside; "s" indicates a phosphorothioate internucleoside linkage (PS); "o" indicates a phosphodiester internucleoside linkage (PO); and "o'" indicates -O-P(=O)(OH)-. Conjugate groups are in bold.

The structure of GalNAc$_3$-3$_a$ was shown previously in Example 39. The structure of GalNAc$_3$-12a was shown previously in Example 61. The structure of GalNAc$_3$-13a was shown previously in Example 62. The structure of GalNAc$_3$-14a was shown previously in Example 63. The structure of GalNAc$_3$-15a was shown previously in Example 64.

Treatment

Six to eight week old C57bl6 mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously once or twice at the dosage shown below with ISIS 353382, 661161, 671144, 670061, 671261, 671262, or with saline. Mice that were dosed twice received the second dose three days after the first dose. Each treatment group consisted of 4 animals. The mice were sacrificed 72 hours following the final administration to determine the liver SRB-1 mRNA levels using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. The results below are presented as the average percent of SRB-1 mRNA levels for each treatment group, normalized to the saline control.

As illustrated in Table 55, treatment with antisense oligonucleotides lowered SRB-1 mRNA levels in a dose-dependent manner. No significant differences in target knockdown were observed between animals that received a single dose and animals that received two doses (see ISIS 353382 dosages 30 and 2 x 15 mg/kg; and ISIS 661161 dosages 5 and 2 x 2.5 mg/kg). The antisense oligonucleotides comprising the phosphodiester linked GalNAc$_3$-3, 12, 13, 14, and 15 conjugates showed substantial improvement in potency compared to the unconjugated antisense oligonucleotide (ISIS 335382).

Table 55

SRB-1 mRNA (% Saline)

ISIS No.	Dosage (mg/kg)	SRB-1 mRNA (% Saline)	ED_{50} (mg/kg)	Conjugate
Saline	n/a	100.0	n/a	n/a
353382	3	85.0	22.4	none
	10	69.2		

	30	34.2		
	2 x 15	36.0		
661161	0.5	87.4	2.2	$GalNAc_3$-3
	1.5	59.0		
	5	25.6		
	2 x 2.5	27.5		
	15	17.4		
671144	0.5	101.2	3.4	$GalNAc_3$-12
	1.5	76.1		
	5	32.0		
	15	17.6		
670061	0.5	94.8	2.1	$GalNAc_3$-13
	1.5	57.8		
	5	20.7		
	15	13.3		
671261	0.5	110.7	4.1	$GalNAc_3$-14
	1.5	81.9		
	5	39.8		
	15	14.1		
671262	0.5	109.4	9.8	$GalNAc_3$-15
	1.5	99.5		
	5	69.2		
	15	36.1		

Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum were measured relative to saline injected mice using standard protocols. Total bilirubin and BUN were also evaluated. The changes in body weights were evaluated with no significant differences from the saline group (data not shown). ALTs, ASTs, total bilirubin and BUN values are shown in Table 56 below.

Table 56

ISIS No.	Dosage (mg/kg)	ALT (U/L)	AST (U/L)	Total Bilirubin (mg/dL)	BUN (mg/dL)	Conjugate
Saline	n/a	28	60	0.1	39	n/a
353382	3	30	77	0.2	36	none
	10	25	78	0.2	36	
	30	28	62	0.2	35	
	2 x 15	22	59	0.2	33	
661161	0.5	39	72	0.2	34	$GalNAc_3$-3
	1.5	26	50	0.2	33	
	5	41	80	0.2	32	
	2 x 2.5	24	72	0.2	28	
	15	32	69	0.2	36	

671144	0.5	25	39	0.2	34	GalNAc$_3$-12
	1.5	26	55	0.2	28	
	5	48	82	0.2	34	
	15	23	46	0.2	32	
670061	0.5	27	53	0.2	33	GalNAc$_3$-13
	1.5	24	45	0.2	35	
	5	23	58	0.1	34	
	15	24	72	0.1	31	
671261	0.5	69	99	0.1	33	GalNAc$_3$-14
	1.5	34	62	0.1	33	
	5	43	73	0.1	32	
	15	32	53	0.2	30	
671262	0.5	24	51	0.2	29	GalNAc$_3$-15
	1.5	32	62	0.1	31	
	5	30	76	0.2	32	
	15	31	64	0.1	32	

Example 66: Effect of various cleavable moieties on antisense inhibition *in vivo* by oligonucleotides targeting SRB-1 comprising a 5'-GalNAc$_3$ cluster

The oligonucleotides listed below were tested in a dose-dependent study for antisense inhibition of SRB-1 in mice. Each of the GalNAc$_3$ conjugate groups was attached at the 5' terminus of the respective oligonucleotide by a phosphodiester linked nucleoside (cleavable moiety (CM)).

Table 57

Modified ASOs targeting SRB-1

ISIS No.	Sequences (5' to 3')	GalNAc$_3$ Cluster	CM	SEQ ID No.
661161	**GalNAc$_3$-3$_{a\text{-}o}$,A$_{do}$**G$_{es}$^{m}C$_{es}$T$_{es}$T$_{es}$^{m}C$_{es}$A$_{ds}$G$_{ds}$T$_{ds}$^{m}C$_{ds}$A$_{ds}$T$_{ds}$ G$_{ds}$A$_{ds}$^{m}C$_{ds}$T$_{ds}$T$_{es}$^{m}C$_{es}$^{m}C$_{es}$T$_{es}$T$_{e}$	GalNAc$_3$-3a	A$_d$	831
670699	**GalNAc$_3$-3$_{a\text{-}o}$,T$_{do}$**G$_{es}$^{m}C$_{eo}$T$_{eo}$T$_{eo}$^{m}C$_{eo}$A$_{ds}$G$_{ds}$T$_{ds}$^{m}C$_{ds}$A$_{ds}$T$_{ds}$ G$_{ds}$A$_{ds}$^{m}C$_{ds}$T$_{ds}$T$_{eo}$^{m}C$_{eo}$^{m}C$_{es}$T$_{es}$T$_{e}$	GalNAc$_3$-3a	T$_d$	834
670700	**GalNAc$_3$-3$_{a\text{-}o}$,A$_{eo}$**G$_{es}$^{m}C$_{eo}$T$_{eo}$T$_{eo}$^{m}C$_{eo}$A$_{ds}$G$_{ds}$T$_{ds}$^{m}C$_{ds}$A$_{ds}$T$_{ds}$ G$_{ds}$A$_{ds}$^{m}C$_{ds}$T$_{ds}$T$_{eo}$^{m}C$_{eo}$^{m}C$_{es}$T$_{es}$T$_{e}$	GalNAc$_3$-3a	A$_e$	831
670701	**GalNAc$_3$-3$_{a\text{-}o}$,T$_{eo}$**G$_{es}$^{m}C$_{eo}$T$_{eo}$T$_{eo}$^{m}C$_{eo}$A$_{ds}$G$_{ds}$T$_{ds}$^{m}C$_{ds}$A$_{ds}$T$_{ds}$ G$_{ds}$A$_{ds}$^{m}C$_{ds}$T$_{ds}$T$_{eo}$^{m}C$_{eo}$^{m}C$_{es}$T$_{es}$T$_{e}$	GalNAc$_3$-3a	T$_e$	834
671165	**GalNAc$_3$-13$_{a\text{-}o}$,A$_{do}$**G$_{es}$^{m}C$_{eo}$T$_{eo}$T$_{eo}$^{m}C$_{eo}$A$_{ds}$G$_{ds}$T$_{ds}$^{m}C$_{ds}$A$_{ds}$T$_{ds}$ G$_{ds}$A$_{ds}$^{m}C$_{ds}$T$_{ds}$T$_{eo}$^{m}C$_{eo}$^{m}C$_{es}$T$_{es}$T$_{e}$	GalNAc$_3$-13a	A$_d$	831

Capital letters indicate the nucleobase for each nucleoside and ^{m}C indicates a 5-methyl cytosine. Subscripts: "e" indicates a 2'-MOE modified nucleoside; "d" indicates a β-D-2'-deoxyribonucleoside; "s" indicates a phosphorothioate internucleoside linkage (PS); "o" indicates a

phosphodiester internucleoside linkage (PO); and "o'" indicates -O-P(=O)(OH)-. Conjugate groups are in bold.

The structure of $GalNAc_3$-3_a was shown previously in Example 39. The structure of $GalNAc_3$-13a was shown previously in Example 62.

Treatment

Six to eight week old C57bl6 mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously once at the dosage shown below with ISIS 661161, 670699, 670700, 670701, 671165, or with saline. Each treatment group consisted of 4 animals. The mice were sacrificed 72 hours following the final administration to determine the liver SRB-1 mRNA levels using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. The results below are presented as the average percent of SRB-1 mRNA levels for each treatment group, normalized to the saline control.

As illustrated in Table 58, treatment with antisense oligonucleotides lowered SRB-1 mRNA levels in a dose-dependent manner. The antisense oligonucleotides comprising various cleavable moieties all showed similar potencies.

Table 58

SRB-1 mRNA (% Saline)

ISIS No.	Dosage (mg/kg)	SRB-1 mRNA (% Saline)	$GalNAc_3$ Cluster	CM
Saline	n/a	100.0	n/a	n/a
661161	0.5	87.8	$GalNAc_3$-3a	A_d
	1.5	61.3		
	5	33.8		
	15	14.0		
670699	0.5	89.4	$GalNAc_3$-3a	T_d
	1.5	59.4		
	5	31.3		
	15	17.1		
670700	0.5	79.0	$GalNAc_3$-3a	A_e
	1.5	63.3		
	5	32.8		
	15	17.9		
670701	0.5	79.1	$GalNAc_3$-3a	T_e
	1.5	59.2		
	5	35.8		
	15	17.7		

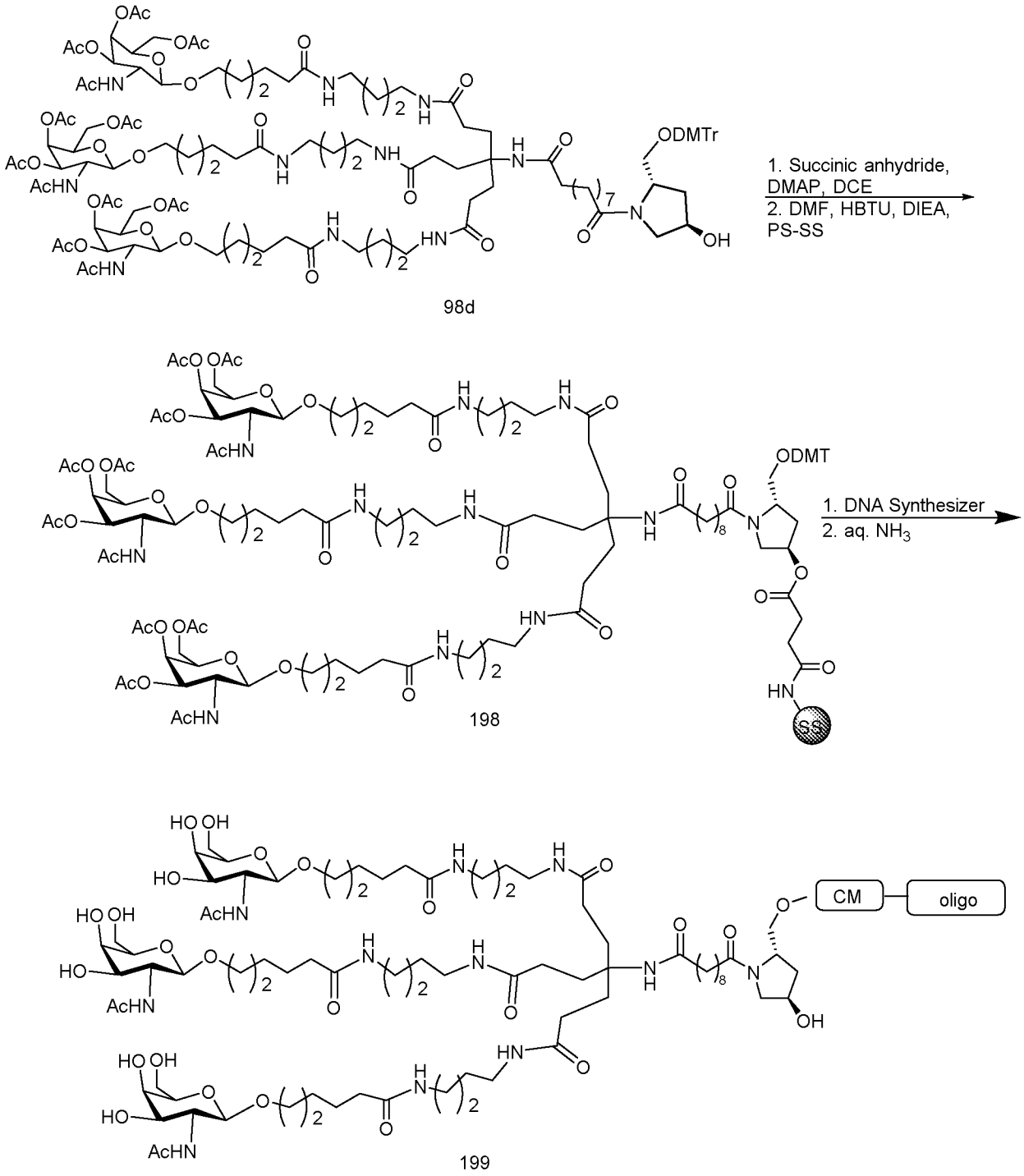
671165	0.5	76.4	$GalNAc_3$-13a	A_d
	1.5	43.2		
	5	22.6		
	15	10.0		

Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum were measured relative to saline injected mice using standard protocols. Total bilirubin and BUN were also evaluated. The changes in body weights were evaluated with no significant differences from the saline group (data not shown). ALTs, ASTs, total bilirubin and BUN values are shown in Table 56 below.

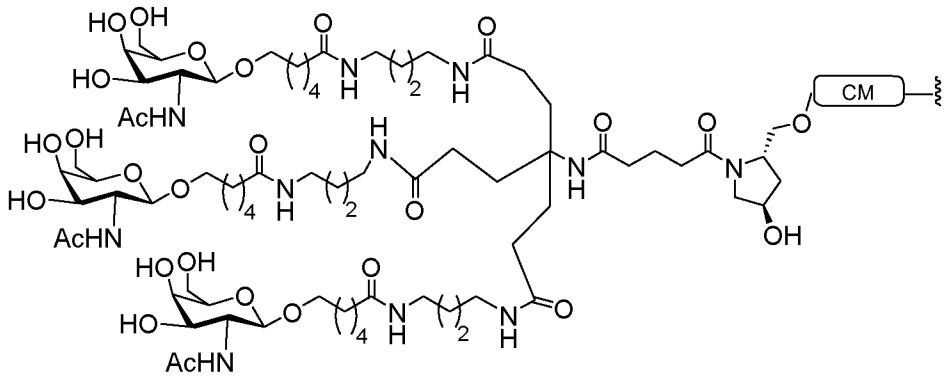
Table 59

ISIS No.	Dosage (mg/kg)	ALT (U/L)	AST (U/L)	Total Bilirubin (mg/dL)	BUN (mg/dL)	$GalNAc_3$ Cluster	CM
Saline	n/a	24	64	0.2	31	n/a	n/a
661161	0.5	25	64	0.2	31	$GalNAc_3$-3a	A_d
	1.5	24	50	0.2	32		
	5	26	55	0.2	28		
	15	27	52	0.2	31		
670699	0.5	42	83	0.2	31	$GalNAc_3$-3a	T_d
	1.5	33	58	0.2	32		
	5	26	70	0.2	29		
	15	25	67	0.2	29		
670700	0.5	40	74	0.2	27	$GalNAc_3$-3a	A_e
	1.5	23	62	0.2	27		
	5	24	49	0.2	29		
	15	25	87	0.1	25		
670701	0.5	30	77	0.2	27	$GalNAc_3$-3a	T_e
	1.5	22	55	0.2	30		
	5	81	101	0.2	25		
	15	31	82	0.2	24		
671165	0.5	44	84	0.2	26	$GalNAc_3$-13a	A_d
	1.5	47	71	0.1	24		
	5	33	91	0.2	26		
	15	33	56	0.2	29		

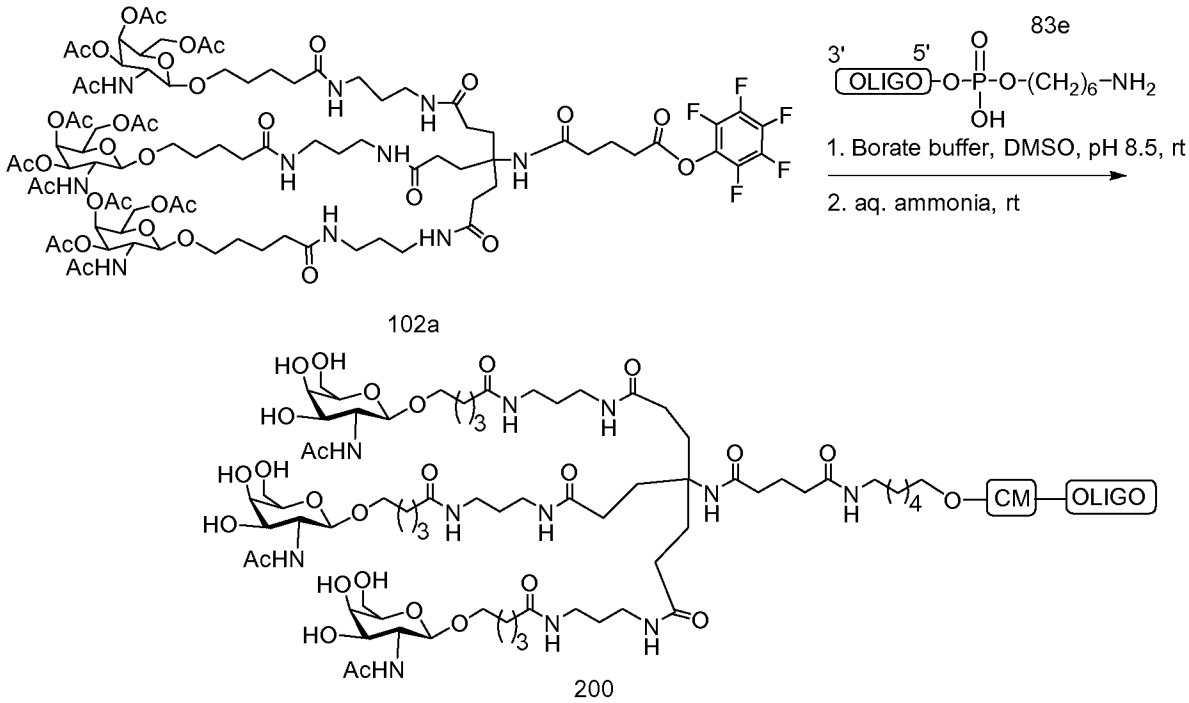
Example 67: Preparation of oligomeric compound 199 comprising GalNAc$_3$-16



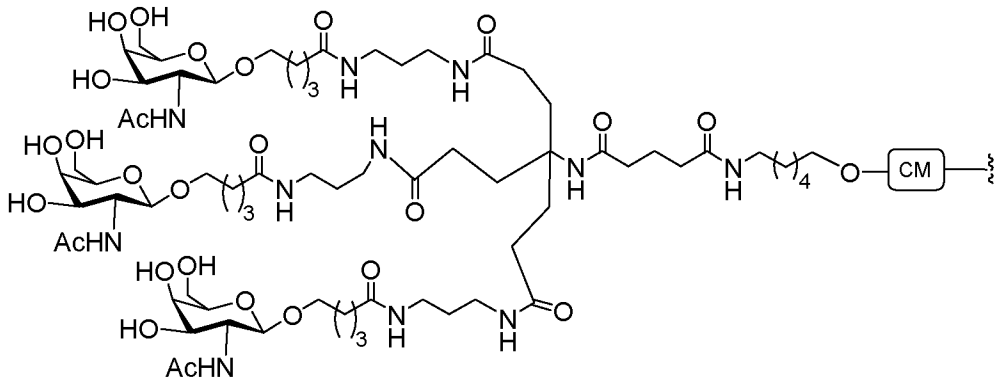
Oligomeric compound 199, comprising a GalNAc$_3$-16 conjugate group, is prepared using the general procedures illustrated in Examples 7 and 9. The GalNAc$_3$ cluster portion of the conjugate group GalNAc$_3$-16 (GalNAc$_3$-16$_a$) can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the cleavable moiety is -P(=O)(OH)-A$_d$-P(=O)(OH)-. The structure of GalNAc$_3$-16 (GalNAc$_3$-16$_a$-CM-) is shown below:



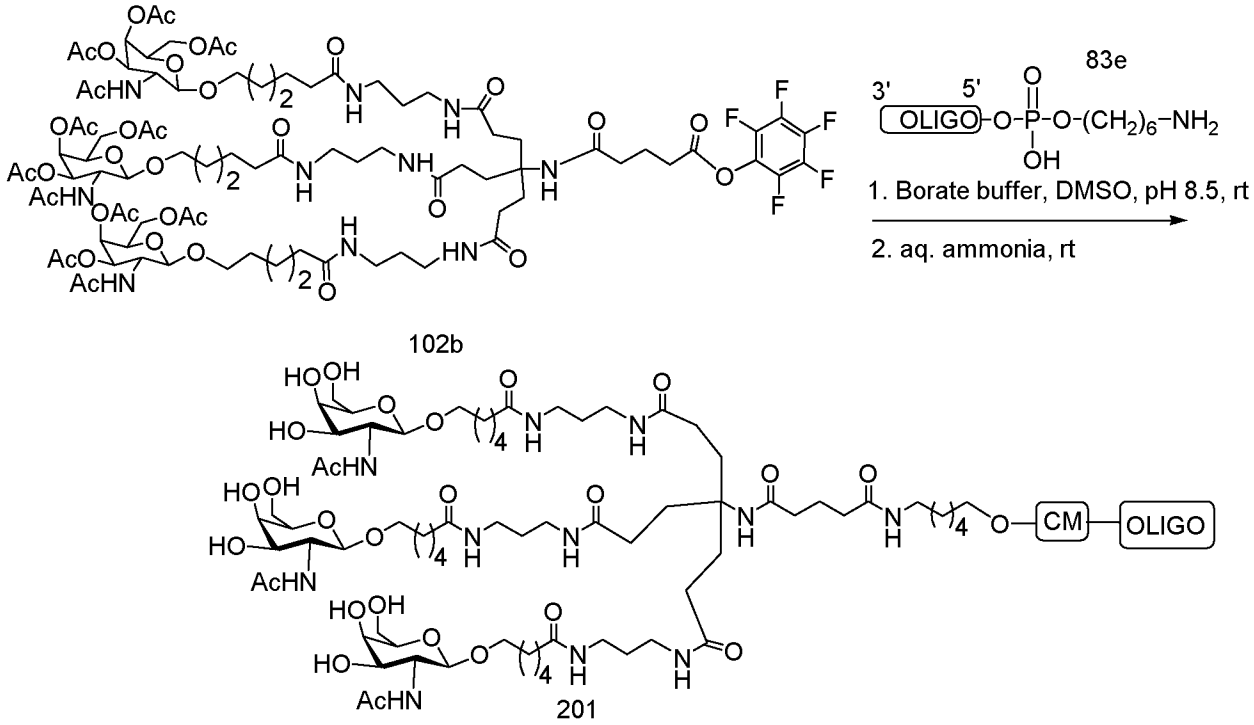
Example 68: Preparation of oligomeric compound 200 comprising GalNAc$_3$-17



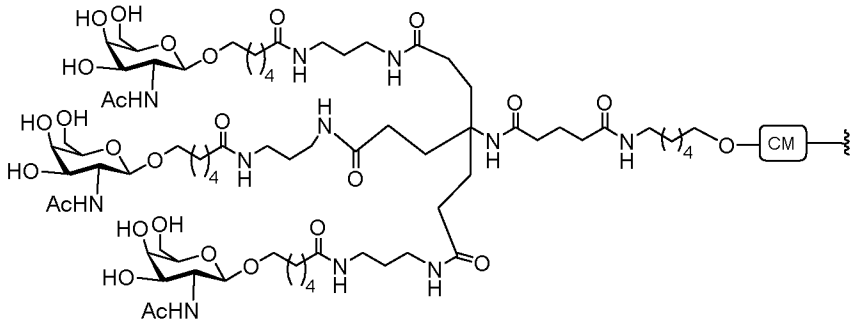
Oligomeric compound 200, comprising a GalNAc$_3$-17 conjugate group, was prepared using the general procedures illustrated in Example 46. The GalNAc$_3$ cluster portion of the conjugate group GalNAc$_3$-17 (GalNAc$_3$-17$_a$) can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the cleavable moiety is -P(=O)(OH)-A$_d$-P(=O)(OH)-. The structure of GalNAc$_3$-17 (GalNAc$_3$-17$_a$-CM-) is shown below:



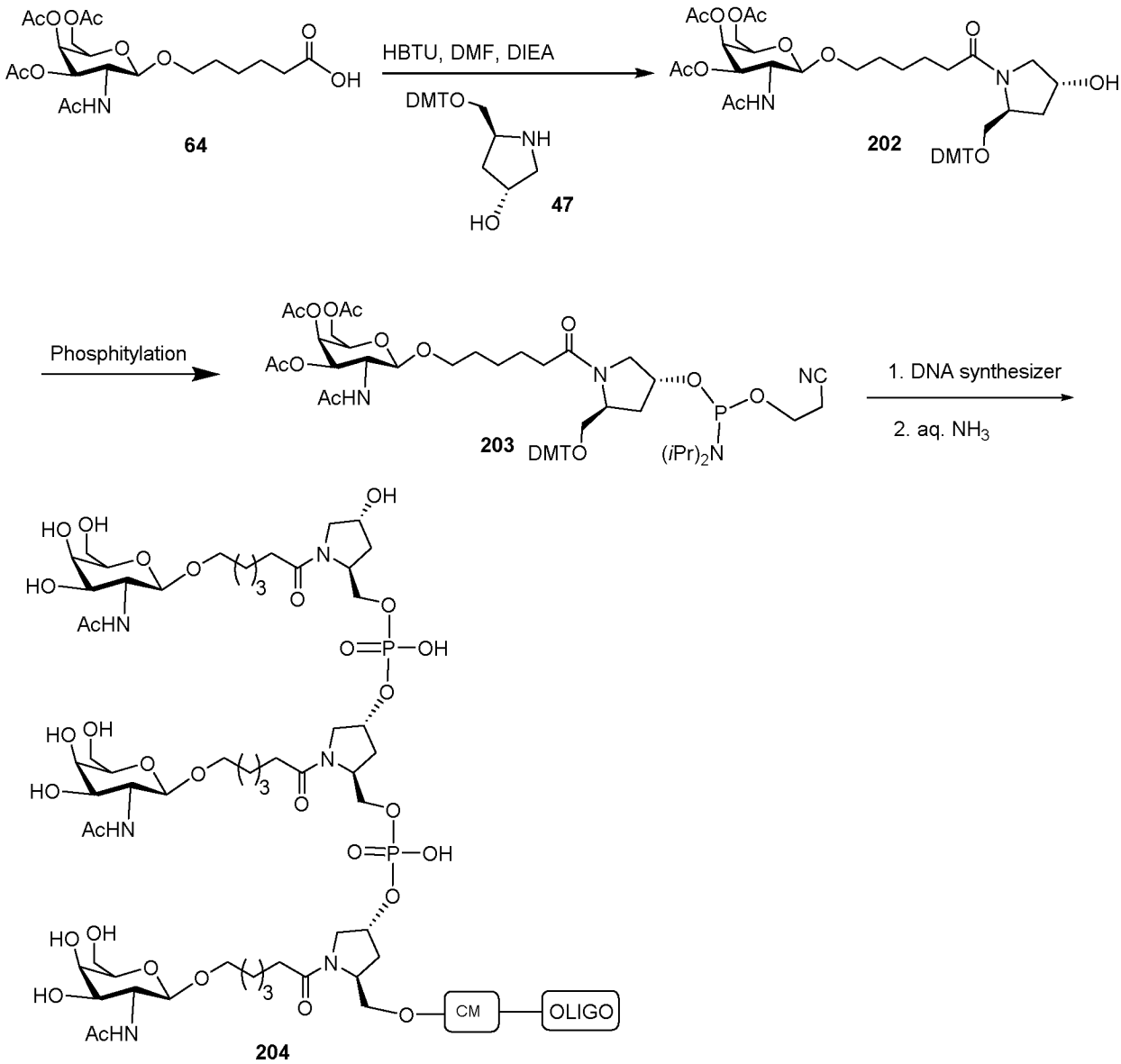
Example 69: Preparation of oligomeric compound 201 comprising GalNAc$_3$-18



Oligomeric compound 201, comprising a GalNAc$_3$-18 conjugate group, was prepared using the general procedures illustrated in Example 46. The GalNAc$_3$ cluster portion of the conjugate group GalNAc$_3$-18 (GalNAc$_3$-18$_a$) can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the cleavable moiety is -P(=O)(OH)-A$_d$-P(=O)(OH)-. The structure of GalNAc$_3$-18 (GalNAc$_3$-18$_a$-CM-) is shown below:

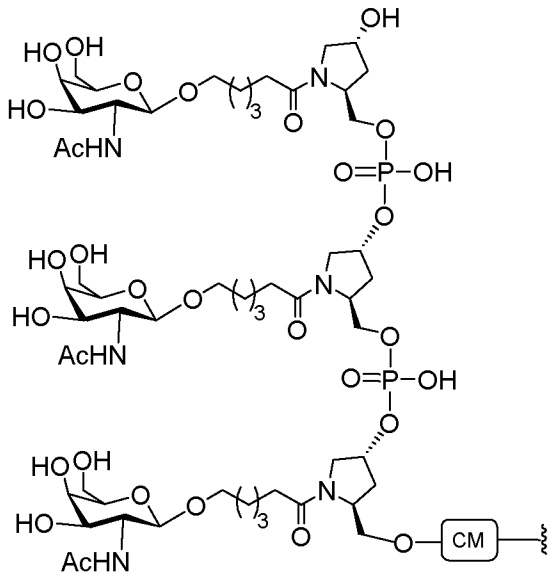


Example 70: Preparation of oligomeric compound 204 comprising GalNAc$_3$-19

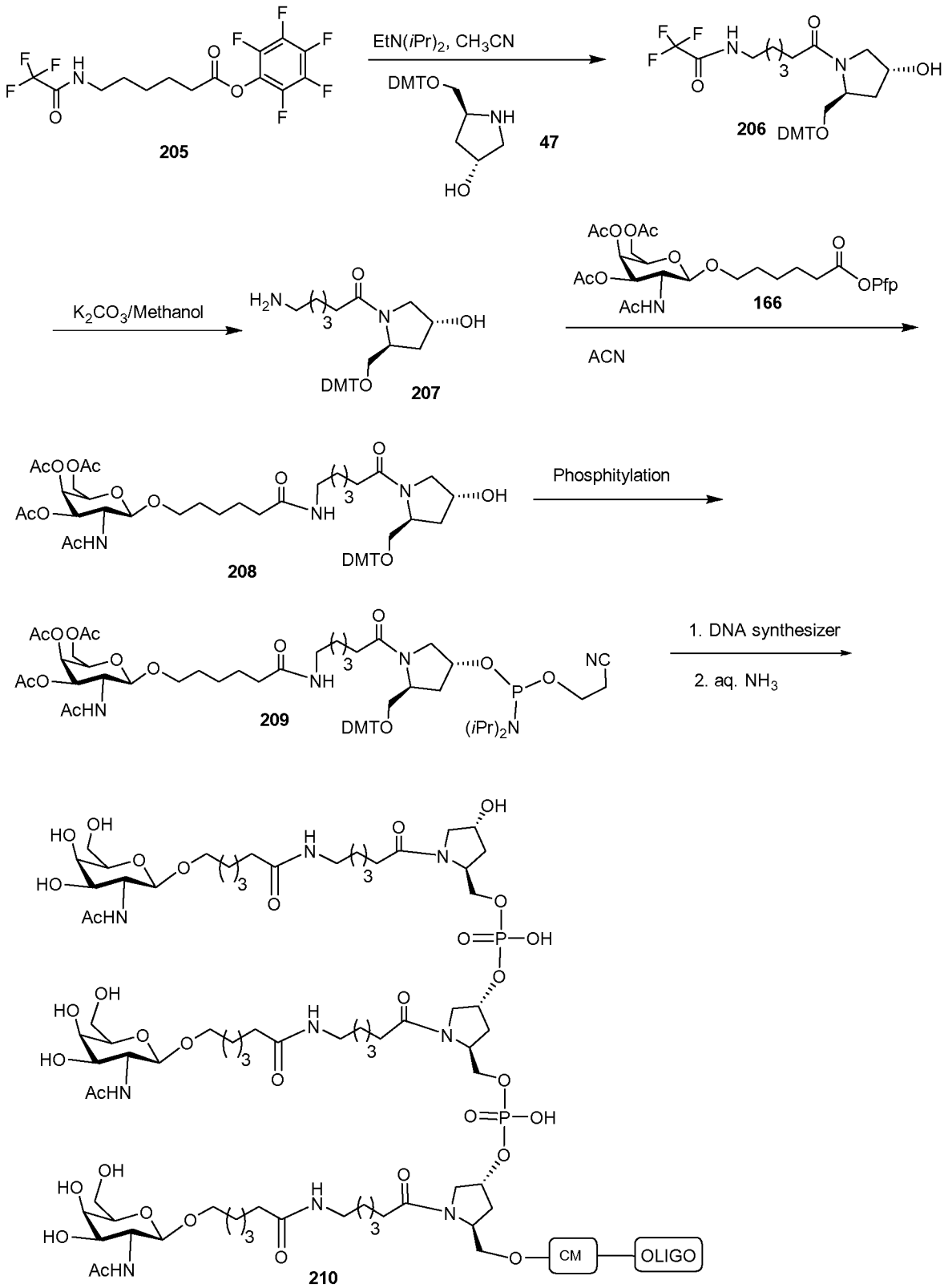


Oligomeric compound 204, comprising a GalNAc$_3$-19 conjugate group, was prepared from compound 64 using the general procedures illustrated in Example 52. The GalNAc$_3$ cluster portion

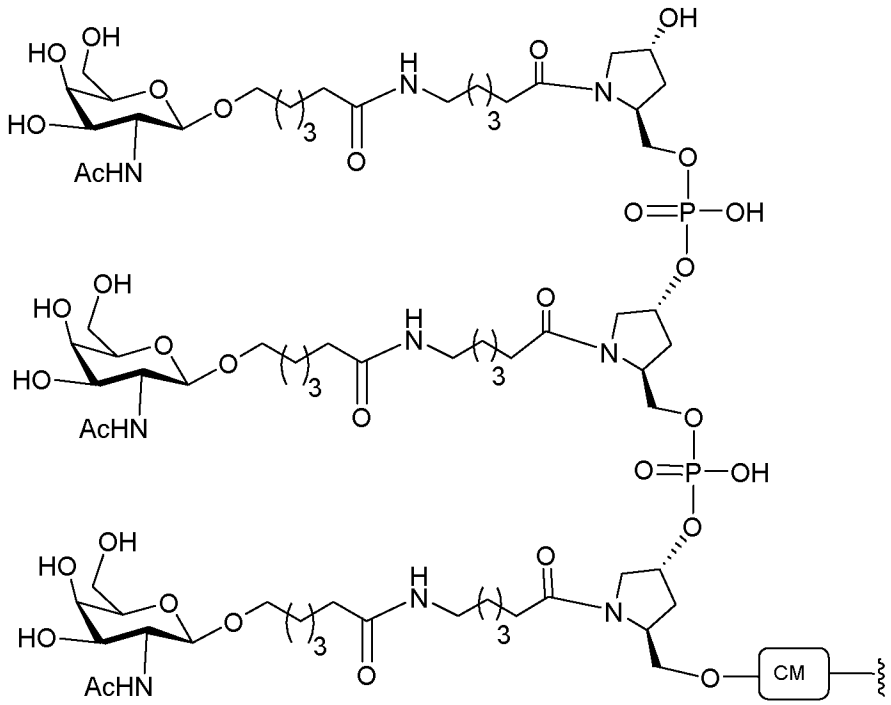
of the conjugate group GalNAc$_3$-19 (GalNAc$_3$-19$_a$) can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the cleavable moiety is -P(=O)(OH)-A$_d$-P(=O)(OH)-. The structure of GalNAc$_3$-19 (GalNAc$_3$-19$_a$-CM-) is shown below:



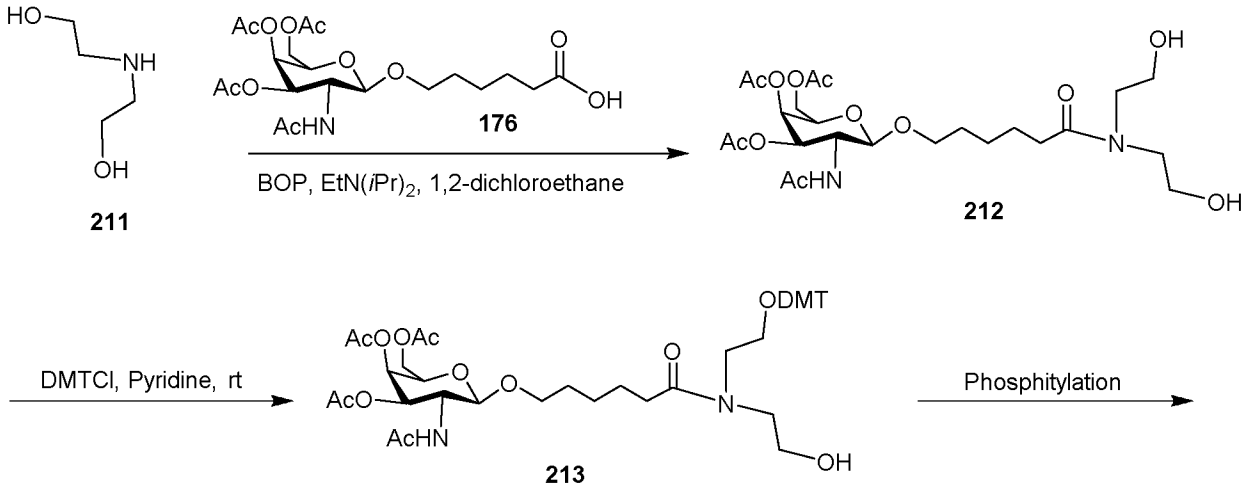
Example 71: Preparation of oligomeric compound 210 comprising $GalNAc_3$-20

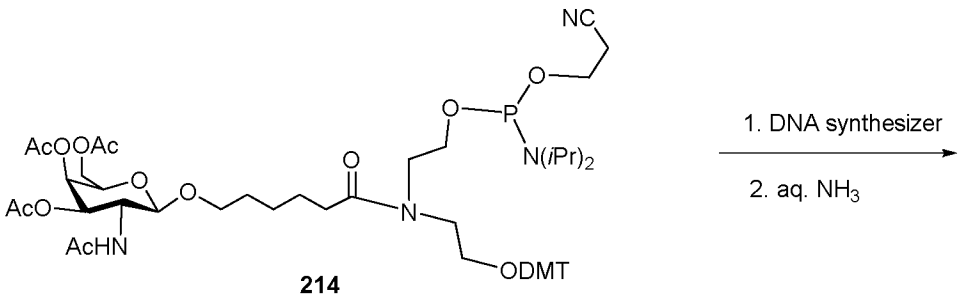


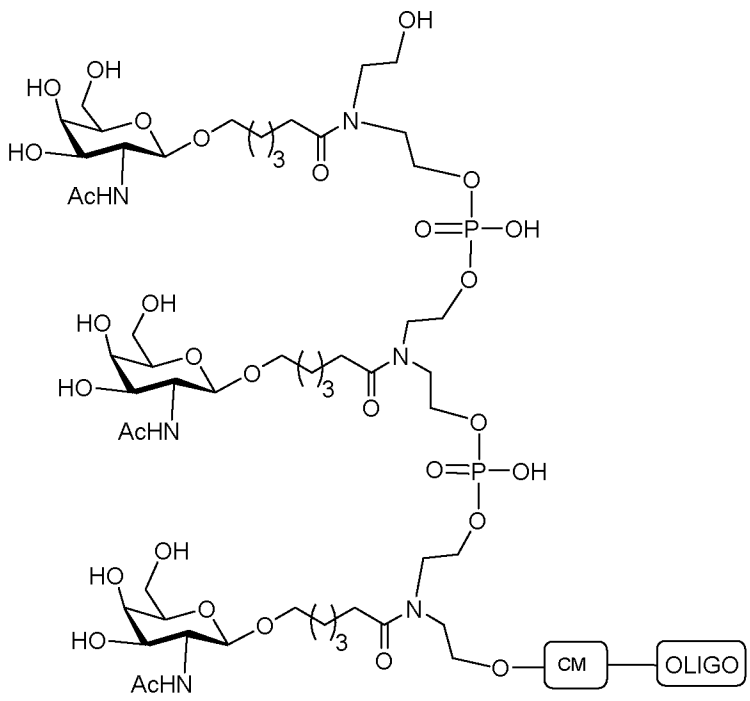
Compound 205 was prepared by adding PFP-TFA and DIEA to 6-(2,2,2-trifluoroacetamido)hexanoic acid in acetonitrile ,which was prepared by adding triflic anhydride to 6-aminohexanoic acid. The reaction mixture was heated to 80 °C, then lowered to rt. Oligomeric compound 210, comprising a $GalNAc_3$-20 conjugate group, was prepared from compound 208 using the general procedures illustrated in Example 52. The $GalNAc_3$ cluster portion of the conjugate group $GalNAc_3$-20 ($GalNAc_3$-20_a) can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the cleavable moiety is -P(=O)(OH)-A_d-P(=O)(OH)-. The structure of $GalNAc_3$-20 ($GalNAc_3$-20_a-CM-) is shown below:



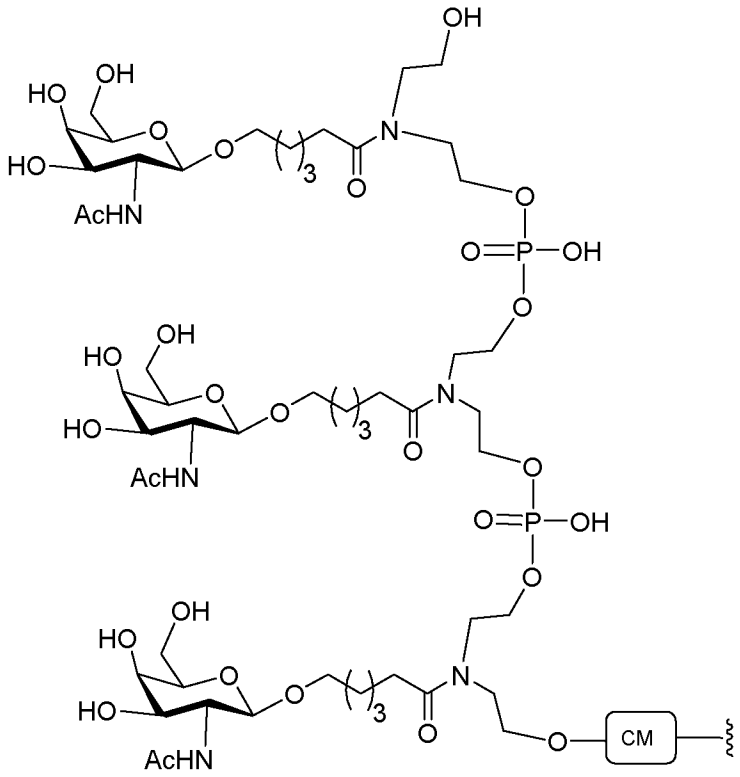
Example 72: Preparation of oligomeric compound 215 comprising GalNAc$_3$-21



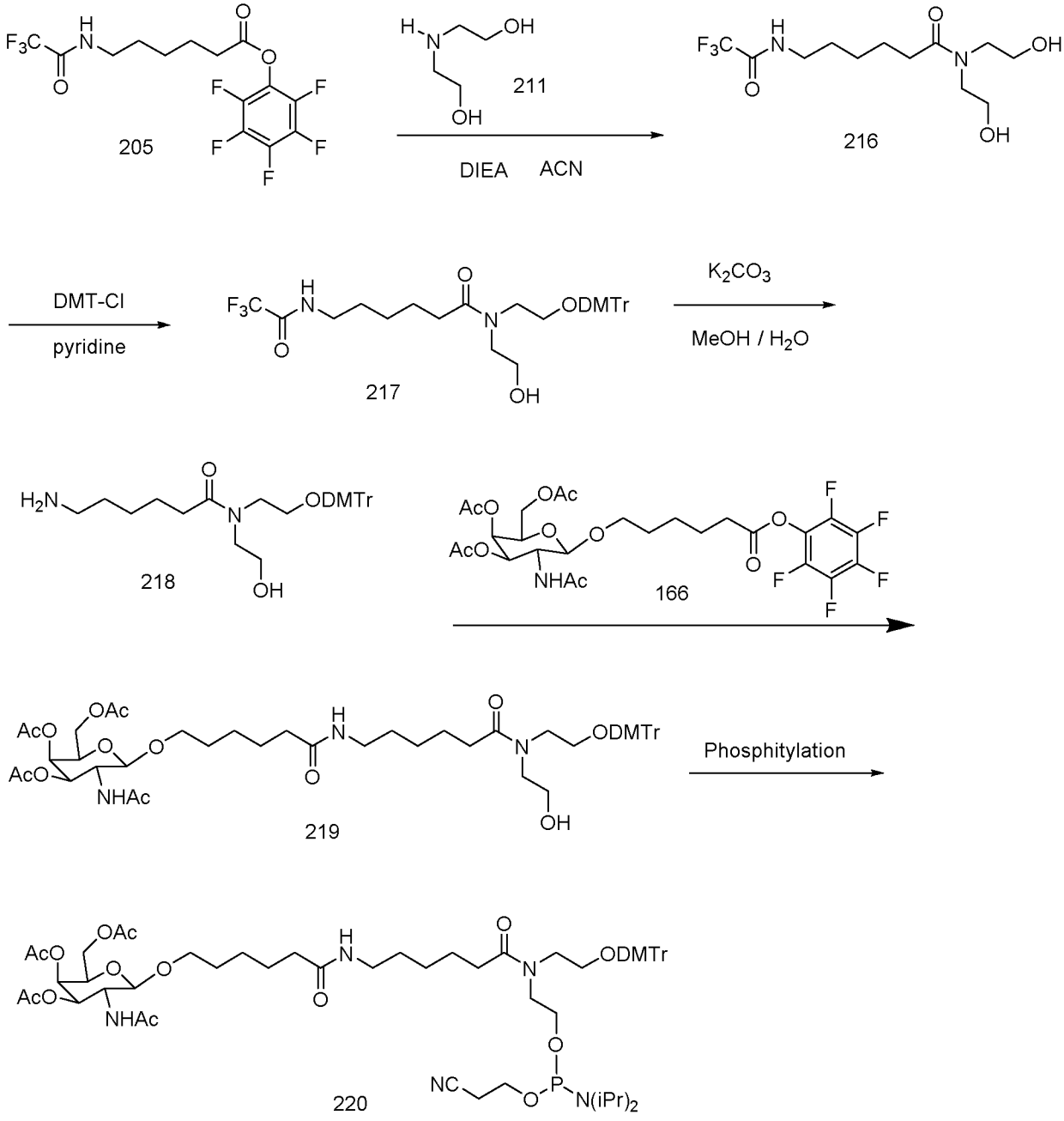


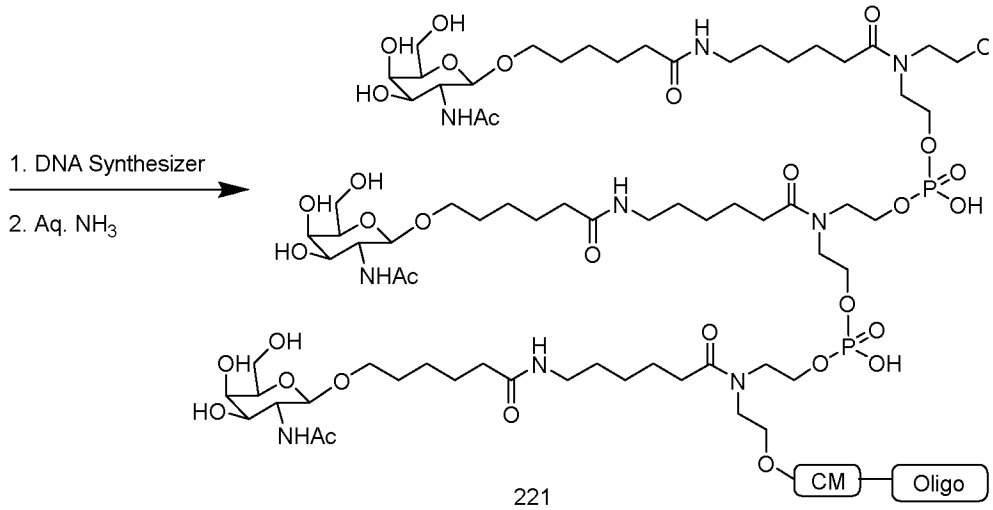


Compound 211 is commercially available. Oligomeric compound 215, comprising a $GalNAc_3$-21 conjugate group, was prepared from compound 213 using the general procedures illustrated in Example 52. The $GalNAc_3$ cluster portion of the conjugate group $GalNAc_3$-21 ($GalNAc_3$-21_a) can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the cleavable moiety is -P(=O)(OH)-A_d-P(=O)(OH)-. The structure of $GalNAc_3$-21 ($GalNAc_3$-21_a-CM-) is shown below:



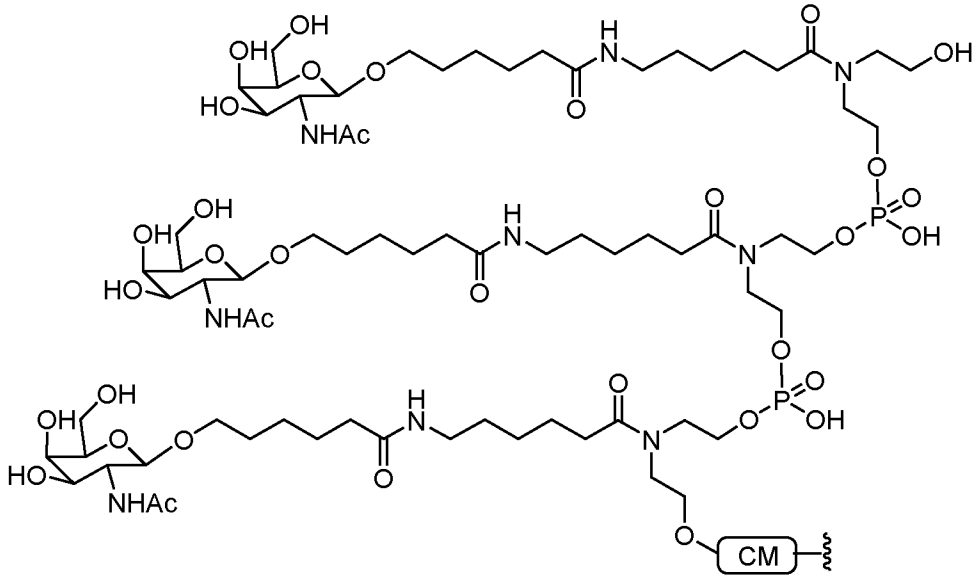
Example 73: Preparation of oligomeric compound 221 comprising GalNAc$_3$-22





221

Compound 220 was prepared from compound 219 using diisopropylammonium tetrazolide. Oligomeric compound 221, comprising a $GalNAc_3$-21 conjugate group, is prepared from compound 220 using the general procedure illustrated in Example 52. The $GalNAc_3$ cluster portion of the conjugate group $GalNAc_3$-22 ($GalNAc_3$-22_a) can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the cleavable moiety is -P(=O)(OH)-A_d-P(=O)(OH)-. The structure of $GalNAc_3$-22 ($GalNAc_3$-22_a-CM-) is shown below:



Example 74: Effect of various cleavable moieties on antisense inhibition *in vivo* by oligonucleotides targeting SRB-1 comprising a 5'-$GalNAc_3$ conjugate

The oligonucleotides listed below were tested in a dose-dependent study for antisense inhibition of SRB-1 in mice. Each of the $GalNAc_3$ conjugate groups was attached at the 5' terminus of the respective oligonucleotide.

Table 60

Modified ASOs targeting SRB-1

ISIS No.	Sequences (5' to 3')	$GalNAc_3$ Cluster	CM	SEQ ID No.
353382	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	n/a	n/a	829
661161	**$GalNAc_3$-$3_{a\text{-}o}$,A_{do}**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	$GalNAc_3$-3a	A_d	831
666904	**$GalNAc_3$-$3_{a\text{-}o}$,**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	$GalNAc_3$-3a	PO	829
675441	**$GalNAc_3$-$17_{a\text{-}o}$,A_{do}**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	$GalNAc_3$-17a	A_d	831
675442	**$GalNAc_3$-$18_{a\text{-}o}$,A_{do}**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	$GalNAc_3$-18a	A_d	831

In all tables, capital letters indicate the nucleobase for each nucleoside and ^{m}C indicates a 5-methyl cytosine. Subscripts: "e" indicates a 2'-MOE modified nucleoside; "d" indicates a β-D-2'-deoxyribonucleoside; "s" indicates a phosphorothioate internucleoside linkage (PS); "o" indicates a phosphodiester internucleoside linkage (PO); and "o'" indicates -O-P(=O)(OH)-. Conjugate groups are in bold.

The structure of $GalNAc_3$-3_a was shown previously in Example 39. The structure of $GalNAc_3$-17a was shown previously in Example 68, and the structure of $GalNAc_3$-18a was shown in Example 69.

Treatment

Six to eight week old C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously once at the dosage shown below with an oligonucleotide listed in Table 60 or with saline. Each treatment group consisted of 4 animals. The mice were sacrificed 72 hours following the final administration to determine the SRB-1 mRNA levels using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. The results below are presented as the average percent of SRB-1 mRNA levels for each treatment group, normalized to the saline control.

As illustrated in Table 61, treatment with antisense oligonucleotides lowered SRB-1 mRNA levels in a dose-dependent manner. The antisense oligonucleotides comprising a GalNAc conjugate showed similar potencies and were significantly more potent than the parent oligonucleotide lacking a GalNAc conjugate.

Table 61

SRB-1 mRNA (% Saline)

ISIS No.	Dosage (mg/kg)	SRB-1 mRNA (% Saline)	$GalNAc_3$ Cluster	CM
Saline	n/a	100.0	n/a	n/a
353382	3	79.38	n/a	n/a
	10	68.67		
	30	40.70		
661161	0.5	79.18	$GalNAc_3$-3a	A_d
	1.5	75.96		
	5	30.53		
	15	12.52		
666904	0.5	91.30	$GalNAc_3$-3a	PO
	1.5	57.88		
	5	21.22		
	15	16.49		
675441	0.5	76.71	$GalNAc_3$-17a	A_d
	1.5	63.63		
	5	29.57		
	15	13.49		
675442	0.5	95.03	$GalNAc_3$-18a	A_d
	1.5	60.06		
	5	31.04		
	15	19.40		

Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum were measured relative to saline injected mice using standard protocols. Total bilirubin and BUN were also evaluated. The change in body weights was evaluated with no significant change from the saline group (data not shown). ALTs, ASTs, total bilirubin and BUN values are shown in Table 62 below.

Table 62

ISIS No.	Dosage (mg/kg)	ALT (U/L)	AST (U/L)	Total Bilirubin (mg/dL)	BUN (mg/dL)	$GalNAc_3$ Cluster	CM
Saline	n/a	26	59	0.16	42	n/a	n/a
353382	3	23	58	0.18	39	n/a	n/a
	10	28	58	0.16	43		

	30	20	48	0.12	34		
661161	0.5	30	47	0.13	35	$GalNAc_3$-3a	A_d
	1.5	23	53	0.14	37		
	5	26	48	0.15	39		
	15	32	57	0.15	42		
666904	0.5	24	73	0.13	36	$GalNAc_3$-3a	PO
	1.5	21	48	0.12	32		
	5	19	49	0.14	33		
	15	20	52	0.15	26		
675441	0.5	42	148	0.21	36	$GalNAc_3$-17a	A_d
	1.5	60	95	0.16	34		
	5	27	75	0.14	37		
	15	24	61	0.14	36		
675442	0.5	26	65	0.15	37	$GalNAc_3$-18a	A_d
	1.5	25	64	0.15	43		
	5	27	69	0.15	37		
	15	30	84	0.14	37		

Example 75: Pharmacokinetic analysis of oligonucleotides comprising a 5'-conjugate group

The PK of the ASOs in Tables 54, 57 and 60 above was evaluated using liver samples that were obtained following the treatment procedures described in Examples 65, 66, and 74. The liver samples were minced and extracted using standard protocols and analyzed by IP-HPLC-MS alongside an internal standard. The combined tissue level (μg/g) of all metabolites was measured by integrating the appropriate UV peaks, and the tissue level of the full-length ASO missing the conjugate ("parent," which is Isis No. 353382 in this case) was measured using the appropriate extracted ion chromatograms (EIC).

Table 63

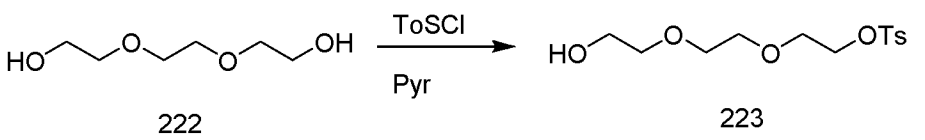
PK Analysis in Liver

ISIS No.	Dosage (mg/kg)	Total Tissue Level by UV (μg/g)	Parent ASO Tissue Level by EIC (μg/g)	$GalNAc_3$ Cluster	CM
353382	3	8.9	8.6	n/a	n/a
	10	22.4	21.0		
	30	54.2	44.2		
661161	5	32.4	20.7	$GalNAc_3$-3a	A_d
	15	63.2	44.1		
671144	5	20.5	19.2	$GalNAc_3$-12a	A_d
	15	48.6	41.5		
670061	5	31.6	28.0	$GalNAc_3$-13a	A_d
	15	67.6	55.5		
671261	5	19.8	16.8	$GalNAc_3$-14a	A_d

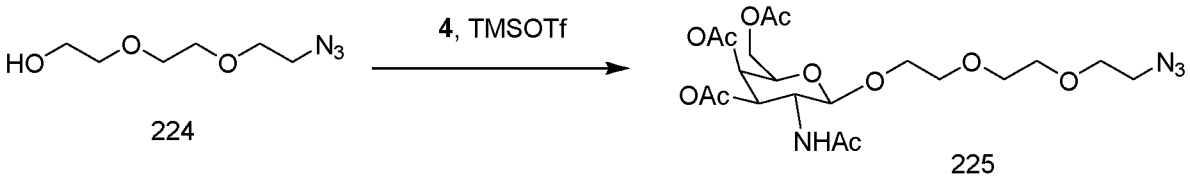
	15	64.7	49.1		
671262	5	18.5	7.4	GalNAc$_3$-15a	A_d
	15	52.3	24.2		
670699	5	16.4	10.4	GalNAc$_3$-3a	T_d
	15	31.5	22.5		
670700	5	19.3	10.9	GalNAc$_3$-3a	A_e
	15	38.1	20.0		
670701	5	21.8	8.8	GalNAc$_3$-3a	T_e
	15	35.2	16.1		
671165	5	27.1	26.5	GalNAc$_3$-13a	A_d
	15	48.3	44.3		
666904	5	30.8	24.0	GalNAc$_3$-3a	PO
	15	52.6	37.6		
675441	5	25.4	19.0	GalNAc$_3$-17a	A_d
	15	54.2	42.1		
675442	5	22.2	20.7	GalNAc$_3$-18a	A_d
	15	39.6	29.0		

The results in Table 63 above show that there were greater liver tissue levels of the oligonucleotides comprising a GalNAc$_3$ conjugate group than of the parent oligonucleotide that does not comprise a GalNAc$_3$ conjugate group (ISIS 353382) 72 hours following oligonucleotide administration, particularly when taking into consideration the differences in dosing between the oligonucleotides with and without a GalNAc$_3$ conjugate group. Furthermore, by 72 hours, 40-98% of each oligonucleotide comprising a GalNAc$_3$ conjugate group was metabolized to the parent compound, indicating that the GalNAc$_3$ conjugate groups were cleaved from the oligonucleotides.

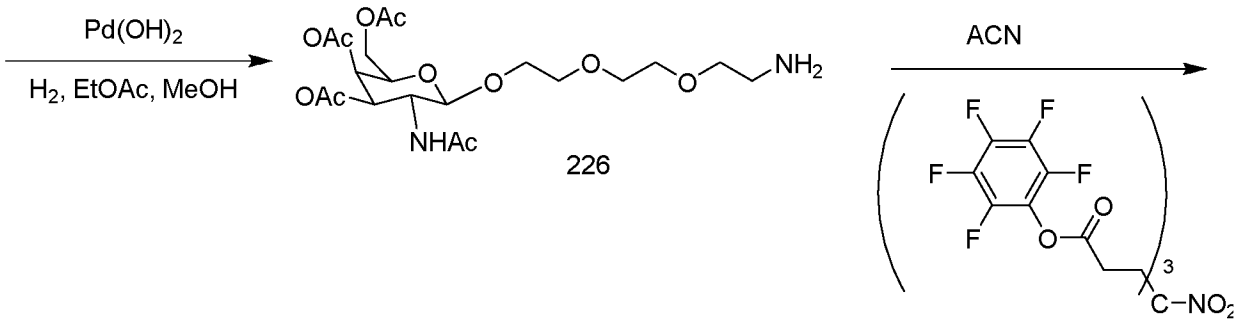
Example 76: Preparation of oligomeric compound 230 comprising GalNAc$_3$-23



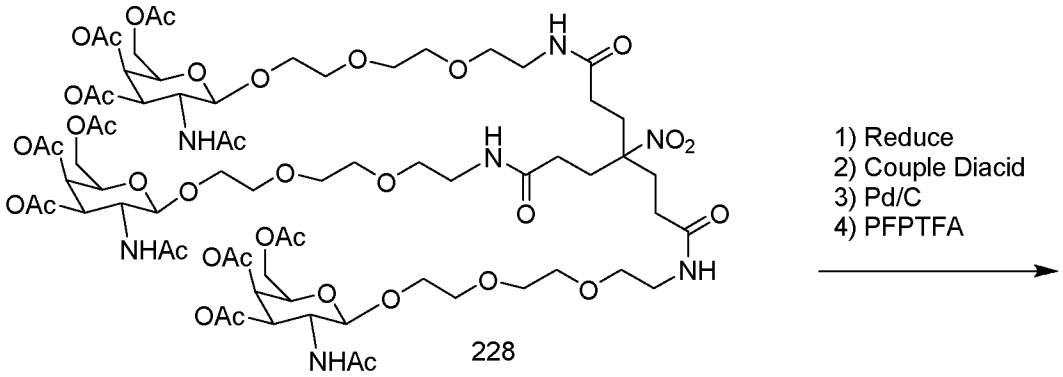
222 223



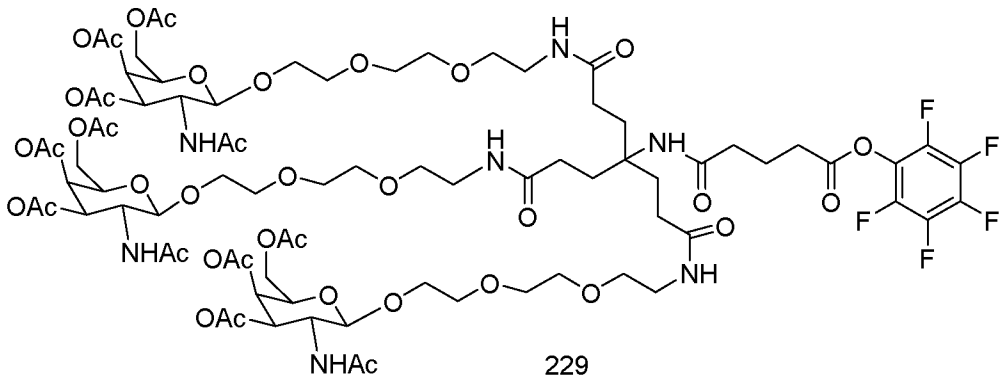
224 225



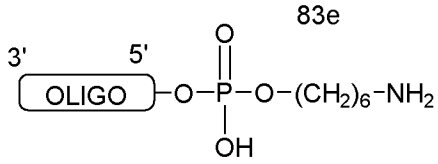
226 227



228

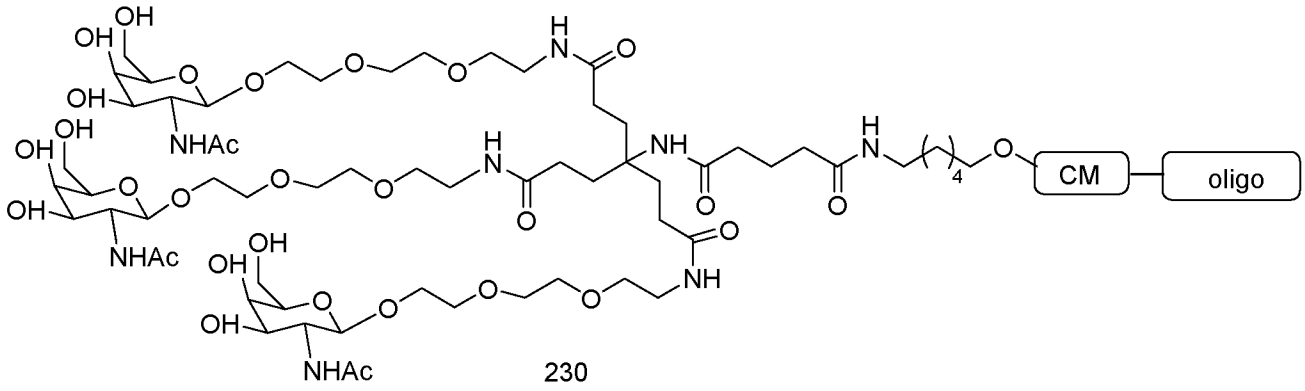


229



1. Borate buffer, DMSO, pH 8.5, rt

2. aq. ammonia, rt



230

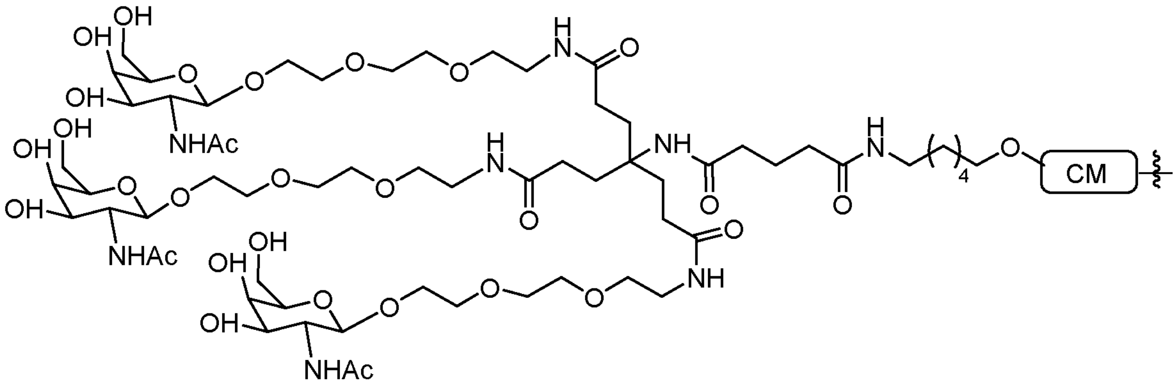
Compound 222 is commercially available. 44.48 ml (0.33 mol) of compound 222 was treated with tosyl chloride (25.39 g, 0.13 mol) in pyridine (500mL) for 16 hours. The reaction was then evaporated to an oil, dissolved in EtOAc and washed with water, sat. $NaHCO_3$, brine, and dried over Na_2SO_4. The ethyl acetate was concentrated to dryness and purified by column chromatography, eluted with EtOAc/hexanes (1:1) followed by 10% methanol in CH_2Cl_2 to give compound 223 as a colorless oil. LCMS and NMR were consistent with the structure. 10 g (32.86 mmol) of 1-Tosyltriethylene glycol (compound 223) was treated with sodium azide (10.68 g, 164.28 mmol) in DMSO (100mL) at room temperature for 17 hours. The reaction mixture was then poured onto water, and extracted with EtOAc. The organic layer was washed with water three times and dried over Na_2SO_4. The organic layer was concentrated to dryness to give 5.3g of compound 224 (92%). LCMS and NMR were consistent with the structure. 1-Azidotriethylene glycol (compound 224, 5.53 g, 23.69 mmol) and compound 4 (6 g, 18.22 mmol) were treated with 4A molecular sieves (5g), and TMSOTf (1.65 ml, 9.11 mmol) in dichloromethane (100mL) under an inert atmosphere. After 14 hours, the reaction was filtered to remove the sieves, and the organic layer was washed with sat. $NaHCO_3$, water, brine, and dried over Na_2SO_4. The organic layer was concentrated to dryness and purified by column chromatography, eluted with a gradient of 2 to 4% methanol in dichloromethane

to give compound 225. LCMS and NMR were consistent with the structure. Compound 225 (11.9 g, 23.59 mmol) was hydrogenated in EtOAc/Methanol (4:1, 250mL) over Pearlman's catalyst. After 8 hours, the catalyst was removed by filtration and the solvents removed to dryness to give compound 226. LCMS and NMR were consistent with the structure.

In order to generate compound 227, a solution of nitromethanetrispropionic acid (4.17 g, 15.04 mmol) and Hunig's base (10.3 ml, 60.17 mmol) in DMF (100mL) were treated dropwise with pentaflourotrifluoro acetate (9.05 ml, 52.65 mmol). After 30 minutes, the reaction was poured onto ice water and extracted with EtOAc. The organic layer was washed with water, brine, and dried over Na_2SO_4. The organic layer was concentrated to dryness and then recrystallized from heptane to give compound 227 as a white solid. LCMS and NMR were consistent with the structure. Compound 227 (1.5 g, 1.93 mmol) and compound 226 (3.7 g, 7.74 mmol) were stirred at room temperature in acetonitrile (15 mL) for 2 hours. The reaction was then evaporated to dryness and purified by column chromatography, eluting with a gradient of 2 to10% methanol in dichloromethane to give compound 228. LCMS and NMR were consistent with the structure. Compound 228 (1.7 g, 1.02 mmol) was treated with Raney Nickel (about 2g wet) in ethanol (100mL) in an atmosphere of hydrogen. After 12 hours, the catalyst was removed by filtration and the organic layer was evaporated to a solid that was used directly in the next step. LCMS and NMR were consistent with the structure. This solid (0.87 g, 0.53 mmol) was treated with benzylglutaric acid (0.18 g, 0.8 mmol), HBTU (0.3 g, 0.8 mmol) and DIEA (273.7 µl, 1.6 mmol) in DMF (5mL). After 16 hours, the DMF was removed under reduced pressure at 65°C to an oil, and the oil was dissolved in dichloromethane. The organic layer was washed with sat. $NaHCO_3$, brine, and dried over Na_2SO_4. After evaporation of the organic layer, the compound was purified by column chromatography and eluted with a gradient of 2 to 20% methanol in dichloromethane to give the coupled product. LCMS and NMR were consistent with the structure. The benzyl ester was deprotected with Pearlman's catalyst under a hydrogen atmosphere for 1 hour. The catalyst was them removed by filtration and the solvents removed to dryness to give the acid. LCMS and NMR were consistent with the structure. The acid (486 mg, 0.27 mmol) was dissolved in dry DMF (3 mL). Pyridine (53.61 µl, 0.66 mmol) was added and the reaction was purged with argon. Pentaflourotriflouro acetate (46.39 µl, 0.4 mmol) was slowly added to the reaction mixture. The color of the reaction changed from pale yellow to burgundy, and gave off a light smoke which was blown away with a stream of argon. The reaction was allowed to stir at room temperature for one hour (completion of reaction was confirmed by LCMS). The solvent was removed under reduced

pressure (rotovap) at 70 °C. The residue was diluted with DCM and washed with 1N $NaHSO_4$, brine, saturated sodium bicarbonate and brine again. The organics were dried over Na_2SO_4, filtered, and were concentrated to dryness to give 225 mg of compound 229 as a brittle yellow foam. LCMS and NMR were consistent with the structure.

Oligomeric compound 230, comprising a GalNAc$_3$-23 conjugate group, was prepared from compound 229 using the general procedure illustrated in Example 46. The GalNAc$_3$ cluster portion of the GalNAc$_3$-23 conjugate group (GalNAc$_3$-23$_a$) can be combined with any cleavable moiety to provide a variety of conjugate groups. The structure of GalNAc$_3$-23 (GalNAc$_3$-23$_a$-CM) is shown below:



Example 77: Antisense inhibition *in vivo* by oligonucleotides targeting SRB-1 comprising a GalNAc$_3$ conjugate

The oligonucleotides listed below were tested in a dose-dependent study for antisense inhibition of SRB-1 in mice.

Table 64

Modified ASOs targeting SRB-1

ISIS No.	Sequences (5' to 3')	GalNAc$_3$ Cluster	CM	SEQ ID No.
661161	**GalNAc$_3$-3$_{a}$-$_{o'}$A$_{do}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	GalNAc$_3$-3a	A$_d$	831
666904	**GalNAc$_3$-3$_{a}$-$_{o'}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	GalNAc$_3$-3a	PO	829
673502	**GalNAc$_3$-10$_{a}$-$_{o'}$A$_{do}$**$G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T_{e}$	GalNAc$_3$-10a	A$_d$	831
677844	**GalNAc$_3$-9$_{a}$-$_{o'}$A$_{do}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	GalNAc$_3$-9a	A$_d$	831
677843	**GalNAc$_3$-23$_{a}$-$_{o'}$A$_{do}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}$	GalNAc$_3$-23a	A$_d$	831

	$G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$			
655861	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}$ $^{m}C_{es}T_{es}T_{eo}\mathbf{A_{do'}\text{-}GalNAc_3\text{-}1_a}$	$GalNAc_3$-1a	A_d	830
677841	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}$ $^{m}C_{es}T_{es}T_{eo}\mathbf{A_{do'}\text{-}GalNAc_3\text{-}19_a}$	$GalNAc_3$-19a	A_d	830
677842	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}$ $^{m}C_{es}T_{es}T_{eo}\mathbf{A_{do'}\text{-}GalNAc_3\text{-}20_a}$	$GalNAc_3$-20a	A_d	830

The structure of $GalNAc_3$-1_a was shown previously in Example 9, $GalNAc_3$-3_a was shown in Example 39, $GalNAc_3$-9a was shown in Example 52, $GalNAc_3$-10a was shown in Example 46, $GalNAc_3$-19_a was shown in Example 70, $GalNAc_3$-20_a was shown in Example 71, and $GalNAc_3$-23_a was shown in Example 76.

Treatment

Six to eight week old C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) were each injected subcutaneously once at a dosage shown below with an oligonucleotide listed in Table 64 or with saline. Each treatment group consisted of 4 animals. The mice were sacrificed 72 hours following the final administration to determine the SRB-1 mRNA levels using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. The results below are presented as the average percent of SRB-1 mRNA levels for each treatment group, normalized to the saline control.

As illustrated in Table 65, treatment with antisense oligonucleotides lowered SRB-1 mRNA levels in a dose-dependent manner.

Table 65

SRB-1 mRNA (% Saline)

ISIS No.	Dosage (mg/kg)	SRB-1 mRNA (% Saline)	$GalNAc_3$ Cluster	CM
Saline	n/a	100.0	n/a	n/a
661161	0.5	89.18	$GalNAc_3$-3a	A_d
	1.5	77.02		
	5	29.10		
	15	12.64		
666904	0.5	93.11	$GalNAc_3$-3a	PO
	1.5	55.85		
	5	21.29		
	15	13.43		
673502	0.5	77.75	$GalNAc_3$-10a	A_d
	1.5	41.05		

	5	19.27		
	15	14.41		
677844	0.5	87.65	$GalNAc_3$-9a	A_d
	1.5	93.04		
	5	40.77		
	15	16.95		
677843	0.5	102.28	$GalNAc_3$-23a	A_d
	1.5	70.51		
	5	30.68		
	15	13.26		
655861	0.5	79.72	$GalNAc_3$-1a	A_d
	1.5	55.48		
	5	26.99		
	15	17.58		
677841	0.5	67.43	$GalNAc_3$-19a	A_d
	1.5	45.13		
	5	27.02		
	15	12.41		
677842	0.5	64.13	$GalNAc_3$-20a	A_d
	1.5	53.56		
	5	20.47		
	15	10.23		

Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum were also measured using standard protocols. Total bilirubin and BUN were also evaluated. Changes in body weights were evaluated, with no significant change from the saline group (data not shown). ALTs, ASTs, total bilirubin and BUN values are shown in Table 66 below.

Table 66

ISIS No.	Dosage (mg/kg)	ALT (U/L)	AST (U/L)	Total Bilirubin (mg/dL)	BUN (mg/dL)	$GalNAc_3$ Cluster	CM
Saline	n/a	21	45	0.13	34	n/a	n/a
661161	0.5	28	51	0.14	39	$GalNAc_3$-3a	A_d
	1.5	23	42	0.13	39		
	5	22	59	0.13	37		
	15	21	56	0.15	35		
666904	0.5	24	56	0.14	37	$GalNAc_3$-3a	PO
	1.5	26	68	0.15	35		
	5	23	77	0.14	34		
	15	24	60	0.13	35		
673502	0.5	24	59	0.16	34	$GalNAc_3$-10a	A_d
	1.5	20	46	0.17	32		
	5	24	45	0.12	31		
	15	24	47	0.13	34		

677844	0.5	25	61	0.14	37	GalNAc$_3$-9a	A$_d$
	1.5	23	64	0.17	33		
	5	25	58	0.13	35		
	15	22	65	0.14	34		
677843	0.5	53	53	0.13	35	GalNAc$_3$-23a	A$_d$
	1.5	25	54	0.13	34		
	5	21	60	0.15	34		
	15	22	43	0.12	38		
655861	0.5	21	48	0.15	33	GalNAc$_3$-1a	A$_d$
	1.5	28	54	0.12	35		
	5	22	60	0.13	36		
	15	21	55	0.17	30		
677841	0.5	32	54	0.13	34	GalNAc$_3$-19a	A$_d$
	1.5	24	56	0.14	34		
	5	23	92	0.18	31		
	15	24	58	0.15	31		
677842	0.5	23	61	0.15	35	GalNAc$_3$-20a	A$_d$
	1.5	24	57	0.14	34		
	5	41	62	0.15	35		
	15	24	37	0.14	32		

Example 78: Antisense inhibition *in vivo* by oligonucleotides targeting Angiotensinogen comprising a GalNAc$_3$ conjugate

The oligonucleotides listed below were tested in a dose-dependent study for antisense inhibition of Angiotensinogen (AGT) in normotensive Sprague Dawley rats.

Table 67

Modified ASOs targeting AGT

ISIS No.	Sequences (5' to 3')	GalNAc$_3$ Cluster	CM	SEQ ID No.
552668	$^{m}C_{es}A_{es}{}^{m}C_{es}T_{es}G_{es}A_{ds}T_{ds}T_{ds}T_{ds}T_{ds}T_{ds}G_{ds}{}^{m}C_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{es}G_{es}G_{es}A_{es}T_{e}$	n/a	n/a	835
669509	$^{m}C_{es}A_{es}{}^{m}C_{es}T_{es}G_{es}A_{ds}T_{ds}T_{ds}T_{ds}T_{ds}T_{ds}G_{ds}{}^{m}C_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{es}G_{es}G_{es}A_{es}T_{eo}$**$A_{do'}$-GalNAc$_3-1_a$**	GalNAc$_3$-1$_a$	A$_d$	836

The structure of GalNAc$_3$-1$_a$ was shown previously in Example 9.

Treatment

Six week old, male Sprague Dawley rats were each injected subcutaneously once per week at a dosage shown below, for a total of three doses, with an oligonucleotide listed in Table 67 or with PBS. Each treatment group consisted of 4 animals. The rats were sacrificed 72 hours following the

final dose. AGT liver mRNA levels were measured using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. AGT plasma protein levels were measured using the Total Angiotensinogen ELISA (Catalog # JP27412, IBL International, Toronto, ON) with plasma diluted 1:20,000. The results below are presented as the average percent of AGT mRNA levels in liver or AGT protein levels in plasma for each treatment group, normalized to the PBS control.

As illustrated in Table 68, treatment with antisense oligonucleotides lowered AGT liver mRNA and plasma protein levels in a dose-dependent manner, and the oligonucleotide comprising a GalNAc conjugate was significantly more potent than the parent oligonucleotide lacking a GalNAc conjugate.

Table 68

AGT liver mRNA and plasma protein levels

ISIS No.	Dosage (mg/kg)	AGT liver mRNA (% PBS)	AGT plasma protein (% PBS)	$GalNAc_3$ Cluster	CM
PBS	n/a	100	100	n/a	n/a
552668	3	95	122	n/a	n/a
	10	85	97		
	30	46	79		
	90	8	11		
669509	0.3	95	70	$GalNAc_3$-1a	A_d
	1	95	129		
	3	62	97		
	10	9	23		

Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in plasma and body weights were also measured at time of sacrifice using standard protocols. The results are shown in Table 69 below.

Table 69

Liver transaminase levels and rat body weights

ISIS No.	Dosage (mg/kg)	ALT (U/L)	AST (U/L)	Body Weight (% of baseline)	$GalNAc_3$ Cluster	CM
PBS	n/a	51	81	186	n/a	n/a
552668	3	54	93	183	n/a	n/a
	10	51	93	194		
	30	59	99	182		

	90	56	78	170		
669509	0.3	53	90	190	GalNAc$_3$-1a	A_d
	1	51	93	192		
	3	48	85	189		
	10	56	95	189		

Example 79: Duration of action *in vivo* of oligonucleotides targeting APOC-III comprising a GalNAc$_3$ conjugate

The oligonucleotides listed in Table 70 below were tested in a single dose study for duration of action in mice.

Table 70

Modified ASOs targeting APOC-III

ISIS No.	Sequences (5' to 3')	GalNAc$_3$ Cluster	CM	SEQ ID No.
304801	$A_{es}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{es}T_{es}T_{es}A_{es}T_e$	n/a	n/a	821
647535	$A_{es}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{es}T_{es}T_{es}A_{es}T_{eo}$**$A_{do'}$-GalNAc$_3-1_a$**	GalNAc$_3$-1a	A_d	822
663083	**GalNAc$_3$-3$_a$-$_o$,A_{do}**$A_{es}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{es}T_{es}T_{es}A_{es}T_e$	GalNAc$_3$-3a	A_d	837
674449	**GalNAc$_3$-7$_a$-$_o$,A_{do}**$A_{es}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{es}T_{es}T_{es}A_{es}T_e$	GalNAc$_3$-7a	A_d	837
674450	**GalNAc$_3$-10$_a$-$_o$,A_{do}**$A_{es}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{es}T_{es}T_{es}A_{es}T_e$	GalNAc$_3$-10a	A_d	837
674451	**GalNAc$_3$-13$_a$-$_o$,A_{do}**$A_{es}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{es}T_{es}T_{es}A_{es}T_e$	GalNAc$_3$-13a	A_d	837

The structure of GalNAc$_3$-1$_a$ was shown previously in Example 9, GalNAc$_3$-3$_a$ was shown in Example 39, GalNAc$_3$-7$_a$ was shown in Example 48, GalNAc$_3$-10$_a$ was shown in Example 46, and GalNAc$_3$-13$_a$ was shown in Example 62.

Treatment

Six to eight week old transgenic mice that express human APOC-III were each injected subcutaneously once with an oligonucleotide listed in Table 70 or with PBS. Each treatment group consisted of 3 animals. Blood was drawn before dosing to determine baseline and at 72 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, and 6 weeks following the dose. Plasma triglyceride and APOC-III protein levels were measured as described in Example 20. The results below are presented as the average percent of plasma triglyceride and APOC-III levels for each treatment group,

normalized to baseline levels, showing that the oligonucleotides comprising a GalNAc conjugate group exhibited a longer duration of action than the parent oligonucleotide without a conjugate group (ISIS 304801) even though the dosage of the parent was three times the dosage of the oligonucleotides comprising a GalNAc conjugate group.

Table 71
Plasma triglyceride and APOC-III protein levels in transgenic mice

ISIS No.	Dosage (mg/kg)	Time point (days post-dose)	Triglycerides (% baseline)	APOC-III protein (% baseline)	$GalNAc_3$ Cluster	CM
PBS	n/a	3	97	102	n/a	n/a
		7	101	98		
		14	108	98		
		21	107	107		
		28	94	91		
		35	88	90		
		42	91	105		
304801	30	3	40	34	n/a	n/a
		7	41	37		
		14	50	57		
		21	50	50		
		28	57	73		
		35	68	70		
		42	75	93		
647535	10	3	36	37	$GalNAc_3$-1a	A_d
		7	39	47		
		14	40	45		
		21	41	41		
		28	42	62		
		35	69	69		
		42	85	102		
663083	10	3	24	18	$GalNAc_3$-3a	A_d
		7	28	23		
		14	25	27		
		21	28	28		
		28	37	44		
		35	55	57		
		42	60	78		
674449	10	3	29	26	$GalNAc_3$-7a	A_d
		7	32	31		
		14	38	41		
		21	44	44		
		28	53	63		

		35	69	77		
		42	78	99		
674450	10	3	33	30	GalNAc$_3$-10a	A_d
		7	35	34		
		14	31	34		
		21	44	44		
		28	56	61		
		35	68	70		
		42	83	95		
674451	10	3	35	33	GalNAc$_3$-13a	A_d
		7	24	32		
		14	40	34		
		21	48	48		
		28	54	67		
		35	65	75		
		42	74	97		

Example 80: Antisense inhibition *in vivo* by oligonucleotides targeting Alpha-1 Antitrypsin (A1AT) comprising a GalNAc$_3$ Conjugate

The oligonucleotides listed in Table 72 below were tested in a study for dose-dependent inhibition of A1AT in mice.

Table 72

Modified ASOs targeting A1AT

ISIS No.	Sequences (5' to 3')	GalNAc$_3$ Cluster	CM	SEQ ID No.
476366	$A_{es}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{es}A_{es}A_{ds}T_{ds}T_{ds}{}^{m}C_{ds}A_{ds}G_{ds}A_{ds}A_{ds}G_{ds}G_{ds}A_{es}A_{es}G_{es}G_{es}A_e$	n/a	n/a	838
656326	$A_{es}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{es}A_{es}A_{ds}T_{ds}T_{ds}{}^{m}C_{ds}A_{ds}G_{ds}A_{ds}A_{ds}G_{ds}G_{ds}A_{es}A_{es}G_{es}G_{es}A_{eo}\mathbf{A_{do'}\text{-}GalNAc_3\text{-}1_a}$	GalNAc$_3$-1a	A_d	839
678381	$\mathbf{GalNAc_3\text{-}3_{a\text{-}o'}A_{do}}A_{es}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{es}A_{es}A_{ds}T_{ds}T_{ds}{}^{m}C_{ds}A_{ds}G_{ds}A_{ds}A_{ds}G_{ds}G_{ds}A_{es}A_{es}G_{es}G_{es}A_e$	GalNAc$_3$-3a	A_d	840
678382	$\mathbf{GalNAc_3\text{-}7_{a\text{-}o'}A_{do}}A_{es}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{es}A_{es}A_{ds}T_{ds}T_{ds}{}^{m}C_{ds}A_{ds}G_{ds}A_{ds}A_{ds}G_{ds}G_{ds}A_{es}A_{es}G_{es}G_{es}A_e$	GalNAc$_3$-7a	A_d	840
678383	$\mathbf{GalNAc_3\text{-}10_{a\text{-}o'}A_{do}}A_{es}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{es}A_{es}A_{ds}T_{ds}T_{ds}{}^{m}C_{ds}A_{ds}G_{ds}A_{ds}A_{ds}G_{ds}G_{ds}A_{es}A_{es}G_{es}G_{es}A_e$	GalNAc$_3$-10a	A_d	840
678384	$\mathbf{GalNAc_3\text{-}13_{a\text{-}o'}A_{do}}A_{es}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{es}A_{es}A_{ds}T_{ds}T_{ds}{}^{m}C_{ds}A_{ds}G_{ds}A_{ds}A_{ds}G_{ds}G_{ds}A_{es}A_{es}G_{es}G_{es}A_e$	GalNAc$_3$-13a	A_d	840

The structure of GalNAc$_3$-1$_a$ was shown previously in Example 9, GalNAc$_3$-3$_a$ was shown in Example 39, GalNAc$_3$-7$_a$ was shown in Example 48, GalNAc$_3$-10$_a$ was shown in Example 46, and GalNAc$_3$-13$_a$ was shown in Example 62.

Treatment

Six week old, male C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) were each injected subcutaneously once per week at a dosage shown below, for a total of three doses, with an oligonucleotide listed in Table 72 or with PBS. Each treatment group consisted of 4 animals. The mice were sacrificed 72 hours following the final administration. A1AT liver mRNA levels were determined using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. A1AT plasma protein levels were determined using the Mouse Alpha 1-Antitrypsin ELISA (catalog # 41-A1AMS-E01, Alpco, Salem, NH). The results below are presented as the average percent of A1AT liver mRNA and plasma protein levels for each treatment group, normalized to the PBS control.

As illustrated in Table 73, treatment with antisense oligonucleotides lowered A1AT liver mRNA and A1AT plasma protein levels in a dose-dependent manner. The oligonucleotides comprising a GalNAc conjugate were significantly more potent than the parent (ISIS 476366).

Table 73

A1AT liver mRNA and plasma protein levels

ISIS No.	Dosage (mg/kg)	A1AT liver mRNA (% PBS)	A1AT plasma protein (% PBS)	$GalNAc_3$ Cluster	CM
PBS	n/a	100	100	n/a	n/a
476366	5	86	78	n/a	n/a
	15	73	61		
	45	30	38		
656326	0.6	99	90	$GalNAc_3$-1a	A_d
	2	61	70		
	6	15	30		
	18	6	10		
678381	0.6	105	90	$GalNAc_3$-3a	A_d
	2	53	60		
	6	16	20		
	18	7	13		
678382	0.6	90	79	$GalNAc_3$-7a	A_d
	2	49	57		
	6	21	27		
	18	8	11		
678383	0.6	94	84	$GalNAc_3$-10a	A_d
	2	44	53		
	6	13	24		
	18	6	10		

678384	0.6	106	91	$GalNAc_3$-13a	A_d
	2	65	59		
	6	26	31		
	18	11	15		

Liver transaminase and BUN levels in plasma were measured at time of sacrifice using standard protocols. Body weights and organ weights were also measured. The results are shown in Table 74 below. Body weight is shown as % relative to baseline. Organ weights are shown as % of body weight relative to the PBS control group.

Table 74

ISIS No.	Dosage (mg/kg)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	Body weight (% baseline)	Liver weight (Rel % BW)	Kidney weight (Rel % BW)	Spleen weight (Rel % BW)
PBS	n/a	25	51	37	119	100	100	100
476366	5	34	68	35	116	91	98	106
	15	37	74	30	122	92	101	128
	45	30	47	31	118	99	108	123
656326	0.6	29	57	40	123	100	103	119
	2	36	75	39	114	98	111	106
	6	32	67	39	125	99	97	122
	18	46	77	36	116	102	109	101
678381	0.6	26	57	32	117	93	109	110
	2	26	52	33	121	96	106	125
	6	40	78	32	124	92	106	126
	18	31	54	28	118	94	103	120
678382	0.6	26	42	35	114	100	103	103
	2	25	50	31	117	91	104	117
	6	30	79	29	117	89	102	107
	18	65	112	31	120	89	104	113
678383	0.6	30	67	38	121	91	100	123
	2	33	53	33	118	98	102	121
	6	32	63	32	117	97	105	105
	18	36	68	31	118	99	103	108
678384	0.6	36	63	31	118	98	103	98
	2	32	61	32	119	93	102	114
	6	34	69	34	122	100	100	96
	18	28	54	30	117	98	101	104

Example 81: Duration of action *in vivo* of oligonucleotides targeting A1AT comprising a $GalNAc_3$ cluster

The oligonucleotides listed in Table 72 were tested in a single dose study for duration of action in mice.

Treatment

Six week old, male C57BL/6 mice were each injected subcutaneously once with an oligonucleotide listed in Table 72 or with PBS. Each treatment group consisted of 4 animals. Blood was drawn the day before dosing to determine baseline and at 5, 12, 19, and 25 days following the dose. Plasma A1AT protein levels were measured via ELISA (see Example 80). The results below are presented as the average percent of plasma A1AT protein levels for each treatment group, normalized to baseline levels. The results show that the oligonucleotides comprising a GalNAc conjugate were more potent and had longer duration of action than the parent lacking a GalNAc conjugate (ISIS 476366). Furthermore, the oligonucleotides comprising a 5'-GalNAc conjugate (ISIS 678381, 678382, 678383, and 678384) were generally even more potent with even longer duration of action than the oligonucleotide comprising a 3'-GalNAc conjugate (ISIS 656326).

Table 75

Plasma A1AT protein levels in mice

ISIS No.	Dosage (mg/kg)	Time point (days post-dose)	A1AT (% baseline)	$GalNAc_3$ Cluster	CM
PBS	n/a	5	93	n/a	n/a
		12	93		
		19	90		
		25	97		
476366	100	5	38	n/a	n/a
		12	46		
		19	62		
		25	77		
656326	18	5	33	$GalNAc_3$-1a	A_d
		12	36		
		19	51		
		25	72		
678381	18	5	21	$GalNAc_3$-3a	A_d
		12	21		
		19	35		
		25	48		
678382	18	5	21	$GalNAc_3$-7a	A_d
		12	21		

		19	39		
		25	60		
678383	18	5	24	$GalNAc_3$-10a	A_d
		12	21		
		19	45		
		25	73		
678384	18	5	29	$GalNAc_3$-13a	A_d
		12	34		
		19	57		
		25	76		

Example 82: Antisense inhibition *in vitro* by oligonucleotides targeting SRB-1 comprising a $GalNAc_3$ conjugate

Primary mouse liver hepatocytes were seeded in 96 well plates at 15,000 cells/well 2 hours prior to treatment. The oligonucleotides listed in Table 76 were added at 2, 10, 50, or 250 nM in Williams E medium and cells were incubated overnight at 37 °C in 5% CO_2. Cells were lysed 16 hours following oligonucleotide addition, and total RNA was purified using RNease 3000 BioRobot (Qiagen). SRB-1 mRNA levels were determined using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. IC_{50} values were determined using Prism 4 software (GraphPad). The results show that oligonucleotides comprising a variety of different GalNAc conjugate groups and a variety of different cleavable moieties are significantly more potent in an *in vitro* free uptake experiment than the parent oligonucleotides lacking a GalNAc conjugate group (ISIS 353382 and 666841).

Table 76

Inhibition of SRB-1 expression *in vitro*

ISIS No.	Sequence (5' to 3')	Linkages	GalNAc cluster	CM	IC_{50} (nM)	SEQ ID No.
353382	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	n/a	n/a	250	829
655861	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{eo}\mathbf{A_{do}}$-**$GalNAc_3$-$1_a$**	PS	$GalNAc_3$-1_a	A_d	40	830
661161	**$GalNAc_3$-3_a-$_o$'A_{do}**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	$GalNAc_3$-3_a	A_d	40	831
661162	**$GalNAc_3$-3_a-$_o$'A_{do}**$G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{ds}G_{ds}T_{ds}$	PO/PS	$GalNAc_3$-3_a	A_d	8	831

	$^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T_{e}$					
664078	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{eo}\mathbf{A_{do}}$-**GalNAc$_3-9_a$**	PS	GalNAc$_3$-9$_a$	A_d	20	830
665001	**GalNAc$_3$-8$_a$-$_o$'$\mathbf{A_{do}}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}$-$A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	GalNAc$_3$-8$_a$	A_d	70	831
666224	**GalNAc$_3$-5$_a$-$_o$'$\mathbf{A_{do}}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	GalNAc$_3$-5$_a$	A_d	80	831
666841	$G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T_{e}$	PO/PS	n/a	n/a	>250	829
666881	**GalNAc$_3$-10$_a$-$_o$'$\mathbf{A_{do}}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	GalNAc$_3$-10$_a$	A_d	30	831
666904	**GalNAc$_3$-3$_a$-$_o$'**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	GalNAc$_3$-3$_a$	PO	9	829
666924	**GalNAc$_3$-3$_a$-$_o$'$\mathbf{T_{do}}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	GalNAc$_3$-3$_a$	T_d	15	834
666961	**GalNAc$_3$-6$_a$-$_o$'$\mathbf{A_{do}}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	GalNAc$_3$-6$_a$	A_d	150	831
666981	**GalNAc$_3$-7$_a$-$_o$'$\mathbf{A_{do}}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	GalNAc$_3$-7$_a$	A_d	20	831
670061	**GalNAc$_3$-13$_a$-$_o$'$\mathbf{A_{do}}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	GalNAc$_3$-13$_a$	A_d	30	831
670699	**GalNAc$_3$-3$_a$-$_o$'$\mathbf{T_{do}}$**$G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T_{e}$	PO/PS	GalNAc$_3$-3$_a$	T_d	15	834
670700	**GalNAc$_3$-3$_a$-$_o$'$\mathbf{A_{eo}}$**$G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T$	PO/PS	GalNAc$_3$-3$_a$	A_e	30	831
670701	**GalNAc$_3$-3$_a$-$_o$'$\mathbf{T_{eo}}$**$G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T_{e}$	PO/PS	GalNAc$_3$-3$_a$	T_e	25	834
671144	**GalNAc$_3$-12$_a$-$_o$'$\mathbf{A_{do}}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	GalNAc$_3$-12$_a$	A_d	40	831
671165	**GalNAc$_3$-13$_a$-**	PO/PS	GalNAc	A_d	8	831

	$_{o}$'$\mathbf{A_{do}}$G$_{es}$ ^{m}C$_{eo}$T$_{eo}$T$_{eo}$ ^{m}C$_{eo}$A$_{ds}$G$_{ds}$T$_{ds}$ ^{m}C$_{ds}$A$_{ds}$T$_{ds}$G$_{ds}$A$_{ds}$ ^{m}C$_{ds}$T$_{ds}$T$_{eo}$ ^{m}C$_{eo}$ ^{m}C$_{es}$T$_{es}$T		GalNAc$_3$-13$_a$			
671261	**GalNAc$_3$-14$_a$-** $_{o}$'$\mathbf{A_{do}}$G$_{es}$ ^{m}C$_{es}$T$_{es}$T$_{es}$ ^{m}C$_{es}$A$_{ds}$G$_{ds}$T$_{ds}$ ^{m}C$_{ds}$A$_{ds}$T$_{ds}$G$_{ds}$A$_{ds}$ ^{m}C$_{ds}$T$_{ds}$ T$_{es}$ ^{m}C$_{es}$ ^{m}C$_{es}$T$_{es}$T$_{e}$	PS	GalNAc$_3$-14$_a$	A$_d$	>250	831
671262	**GalNAc$_3$-15$_a$-** $_{o}$'$\mathbf{A_{do}}$G$_{es}$ ^{m}C$_{es}$T$_{es}$T$_{es}$ ^{m}C$_{es}$A$_{ds}$G$_{ds}$T$_{ds}$ ^{m}C$_{ds}$A$_{ds}$T$_{ds}$G$_{ds}$A$_{ds}$ ^{m}C$_{ds}$T$_{ds}$ T$_{es}$ ^{m}C$_{es}$ ^{m}C$_{es}$T$_{es}$T$_{e}$	PS	GalNAc$_3$-15$_a$	A$_d$	>250	831
673501	**GalNAc$_3$-7$_a$-** $_{o}$'$\mathbf{A_{do}}$G$_{es}$ ^{m}C$_{eo}$T$_{eo}$T$_{eo}$ ^{m}C$_{eo}$A$_{ds}$G$_{ds}$T$_{ds}$ ^{m}C$_{ds}$A$_{ds}$T$_{ds}$G$_{ds}$A$_{ds}$ ^{m}C$_{ds}$T$_{ds}$ T$_{eo}$ ^{m}C$_{eo}$ ^{m}C$_{es}$T$_{es}$T$_{e}$	PO/PS	GalNAc$_3$-7$_a$	A$_d$	30	831
673502	**GalNAc$_3$-10$_a$-** $_{o}$'$\mathbf{A_{do}}$G$_{es}$ ^{m}C$_{eo}$T$_{eo}$T$_{eo}$ ^{m}C$_{eo}$A$_{ds}$G$_{ds}$T$_{ds}$ ^{m}C$_{ds}$A$_{ds}$T$_{ds}$G$_{ds}$A$_{ds}$ ^{m}C$_{ds}$T$_{ds}$ T$_{eo}$ ^{m}C$_{eo}$ ^{m}C$_{es}$T$_{es}$T$_{e}$	PO/PS	GalNAc$_3$-10$_a$	A$_d$	8	831
675441	**GalNAc$_3$-17$_a$-** $_{o}$'$\mathbf{A_{do}}$G$_{es}$ ^{m}C$_{es}$T$_{es}$T$_{es}$ ^{m}C$_{es}$A$_{ds}$G$_{ds}$T$_{ds}$ ^{m}C$_{ds}$A$_{ds}$T$_{ds}$G$_{ds}$A$_{ds}$ ^{m}C$_{ds}$T$_{ds}$ T$_{es}$ ^{m}C$_{es}$ ^{m}C$_{es}$T$_{es}$T$_{e}$	PS	GalNAc$_3$-17$_a$	A$_d$	30	831
675442	**GalNAc$_3$-18$_a$-** $_{o}$'$\mathbf{A_{do}}$G$_{es}$ ^{m}C$_{es}$T$_{es}$T$_{es}$ ^{m}C$_{es}$A$_{ds}$G$_{ds}$T$_{ds}$ ^{m}C$_{ds}$A$_{ds}$T$_{ds}$G$_{ds}$A$_{ds}$ ^{m}C$_{ds}$T$_{ds}$ T$_{es}$ ^{m}C$_{es}$ ^{m}C$_{es}$T$_{es}$T$_{e}$	PS	GalNAc$_3$-18$_a$	A$_d$	20	831
677841	G$_{es}$ ^{m}C$_{es}$T$_{es}$T$_{es}$ ^{m}C$_{es}$A$_{ds}$G$_{ds}$T$_{ds}$ ^{m}C$_{ds}$A$_{ds}$T$_{ds}$G$_{ds}$ A$_{ds}$ ^{m}C$_{ds}$T$_{ds}$T$_{es}$ ^{m}C$_{es}$ ^{m}C$_{es}$T$_{es}$T$_{eo}$**A$_{do}$**'- **GalNAc$_3$-19$_a$**	PS	GalNAc$_3$-19$_a$	A$_d$	40	830
677842	G$_{es}$ ^{m}C$_{es}$T$_{es}$T$_{es}$ ^{m}C$_{es}$A$_{ds}$G$_{ds}$T$_{ds}$ ^{m}C$_{ds}$A$_{ds}$T$_{ds}$G$_{ds}$ A$_{ds}$ ^{m}C$_{ds}$T$_{ds}$T$_{es}$ ^{m}C$_{es}$ ^{m}C$_{es}$T$_{es}$T$_{eo}$**A$_{do}$**'- **GalNAc$_3$-20$_a$**	PS	GalNAc$_3$-20$_a$	A$_d$	30	830
677843	**GalNAc$_3$-23$_a$-** $_{o}$'$\mathbf{A_{do}}$G$_{es}$ ^{m}C$_{es}$T$_{es}$T$_{es}$ ^{m}C$_{es}$A$_{ds}$G$_{ds}$T$_{ds}$ ^{m}C$_{ds}$A$_{ds}$T$_{ds}$G$_{ds}$A$_{ds}$ ^{m}C$_{ds}$T$_{ds}$ T$_{es}$ ^{m}C$_{es}$ ^{m}C$_{es}$T$_{es}$T$_{e}$	PS	GalNAc$_3$-23$_a$	A$_d$	40	831

The structure of GalNAc$_3$-1$_a$ was shown previously in Example 9, GalNAc$_3$-3$_a$ was shown in Example 39, GalNAc$_3$-5$_a$ was shown in Example 49, GalNAc$_3$-6$_a$ was shown in Example 51, GalNAc$_3$-7$_a$ was shown in Example 48, GalNAc$_3$-8$_a$ was shown in Example 47, GalNAc$_3$-9$_a$ was shown in Example 52, GalNAc$_3$-10$_a$ was shown in Example 46, GalNAc$_3$-12$_a$ was shown in Example 61, GalNAc$_3$-13$_a$ was shown in Example 62, GalNAc$_3$-14$_a$ was shown in Example 63, GalNAc$_3$-15$_a$ was shown in Example 64, GalNAc$_3$-17$_a$ was shown in Example 68, GalNAc$_3$-18$_a$ was shown in Example 69, GalNAc$_3$-19$_a$ was shown in Example 70, GalNAc$_3$-20$_a$ was shown in Example 71, and GalNAc$_3$-23$_a$ was shown in Example 76.

Example 83: Antisense inhibition *in vivo* by oligonucleotides targeting Factor XI comprising a $GalNAc_3$ cluster

The oligonucleotides listed in Table 77 below were tested in a study for dose-dependent inhibition of Factor XI in mice.

Table 77

Modified oligonucleotides targeting Factor XI

ISIS No.	Sequence (5' to 3')	GalNAc cluster	CM	SEQ ID No.
404071	$T_{es}G_{es}G_{es}T_{es}A_{es}A_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ds}T_{ds}{}^{m}C_{ds}A_{es}G_{es}A_{es}G_{es}G_{e}$	n/a	n/a	832
656173	$T_{es}G_{eo}G_{eo}T_{eo}A_{eo}A_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ds}T_{ds}{}^{m}C_{ds}A_{eo}G_{eo}A_{es}G_{es}G_{eo}\mathbf{A_{do}}$-**$GalNAc_3$-$1_a$**	$GalNAc_3$-1_a	A_d	833
663086	**$GalNAc_3$-3_a-**$_o$'$\mathbf{A_{do}}T_{es}G_{eo}G_{eo}T_{eo}A_{eo}A_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ds}T_{ds}{}^{m}C_{ds}A_{eo}G_{eo}A_{es}G_{es}G_{e}$	$GalNAc_3$-3_a	A_d	841
678347	**$GalNAc_3$-7_a-**$_o$'$\mathbf{A_{do}}T_{es}G_{eo}G_{eo}T_{eo}A_{eo}A_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ds}T_{ds}{}^{m}C_{ds}A_{eo}G_{eo}A_{es}G_{es}G_{e}$	$GalNAc_3$-7_a	A_d	841
678348	**$GalNAc_3$-10_a-**$_o$'$\mathbf{A_{do}}T_{es}G_{eo}G_{eo}T_{eo}A_{eo}A_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ds}T_{ds}{}^{m}C_{ds}A_{eo}G_{eo}A_{es}G_{es}G_{e}$	$GalNAc_3$-10_a	A_d	841
678349	**$GalNAc_3$-13_a-**$_o$'$\mathbf{A_{do}}T_{es}G_{eo}G_{eo}T_{eo}A_{eo}A_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ds}T_{ds}{}^{m}C_{ds}A_{eo}G_{eo}A_{es}G_{es}G_{e}$	$GalNAc_3$-13_a	A_d	841

The structure of $GalNAc_3$-1_a was shown previously in Example 9, $GalNAc_3$-3_a was shown in Example 39, $GalNAc_3$-7_a was shown in Example 48, $GalNAc_3$-10_a was shown in Example 46, and $GalNAc_3$-13_a was shown in Example 62.

Treatment

Six to eight week old mice were each injected subcutaneously once per week at a dosage shown below, for a total of three doses, with an oligonucleotide listed below or with PBS. Each treatment group consisted of 4 animals. The mice were sacrificed 72 hours following the final dose. Factor XI liver mRNA levels were measured using real-time PCR and normalized to cyclophilin according to standard protocols. Liver transaminases, BUN, and bilirubin were also measured. The results below are presented as the average percent for each treatment group, normalized to the PBS control.

As illustrated in Table 78, treatment with antisense oligonucleotides lowered Factor XI liver mRNA in a dose-dependent manner. The results show that the oligonucleotides comprising a

GalNAc conjugate were more potent than the parent lacking a GalNAc conjugate (ISIS 404071). Furthermore, the oligonucleotides comprising a 5'-GalNAc conjugate (ISIS 663086, 678347, 678348, and 678349) were even more potent than the oligonucleotide comprising a 3'-GalNAc conjugate (ISIS 656173).

Table 78

Factor XI liver mRNA, liver transaminase, BUN, and bilirubin levels

ISIS No.	Dosage (mg/kg)	Factor XI mRNA (% PBS)	ALT (U/L)	AST (U/L)	BUN (mg/dL)	Bilirubin (mg/dL)	$GalNAc_3$ Cluster	SEQ ID No.
PBS	n/a	100	63	70	21	0.18	n/a	n/a
404071	3	65	41	58	21	0.15	n/a	832
	10	33	49	53	23	0.15		
	30	17	43	57	22	0.14		
656173	0.7	43	90	89	21	0.16	$GalNAc_3$-1a	833
	2	9	36	58	26	0.17		
	6	3	50	63	25	0.15		
663086	0.7	33	91	169	25	0.16	$GalNAc_3$-3a	841
	2	7	38	55	21	0.16		
	6	1	34	40	23	0.14		
678347	0.7	35	28	49	20	0.14	$GalNAc_3$-7a	841
	2	10	180	149	21	0.18		
	6	1	44	76	19	0.15		
678348	0.7	39	43	54	21	0.16	$GalNAc_3$-10a	841
	2	5	38	55	22	0.17		
	6	2	25	38	20	0.14		
678349	0.7	34	39	46	20	0.16	$GalNAc_3$-13a	841
	2	8	43	63	21	0.14		
	6	2	28	41	20	0.14		

Example 84: Duration of action *in vivo* of oligonucleotides targeting Factor XI comprising a $GalNAc_3$ Conjugate

The oligonucleotides listed in Table 77 were tested in a single dose study for duration of action in mice.

Treatment

Six to eight week old mice were each injected subcutaneously once with an oligonucleotide listed in Table 77 or with PBS. Each treatment group consisted of 4 animals. Blood was drawn by tail bleeds the day before dosing to determine baseline and at 3, 10, and 17 days following the dose.

Plasma Factor XI protein levels were measured by ELISA using Factor XI capture and biotinylated detection antibodies from R & D Systems, Minneapolis, MN (catalog # AF2460 and # BAF2460, respectively) and the OptEIA Reagent Set B (Catalog # 550534, BD Biosciences, San Jose, CA). The results below are presented as the average percent of plasma Factor XI protein levels for each treatment group, normalized to baseline levels. The results show that the oligonucleotides comprising a GalNAc conjugate were more potent with longer duration of action than the parent lacking a GalNAc conjugate (ISIS 404071). Furthermore, the oligonucleotides comprising a 5'-GalNAc conjugate (ISIS 663086, 678347, 678348, and 678349) were even more potent with an even longer duration of action than the oligonucleotide comprising a 3'-GalNAc conjugate (ISIS 656173).

Table 79

Plasma Factor XI protein levels in mice

ISIS No.	Dosage (mg/kg)	Time point (days post-dose)	Factor XI (% baseline)	$GalNAc_3$ Cluster	CM	SEQ ID No.
PBS	n/a	3	123	n/a	n/a	n/a
		10	56			
		17	100			
404071	30	3	11	n/a	n/a	832
		10	47			
		17	52			
656173	6	3	1	$GalNAc_3$-1a	A_d	833
		10	3			
		17	21			
663086	6	3	1	$GalNAc_3$-3a	A_d	841
		10	2			
		17	9			
678347	6	3	1	$GalNAc_3$-7a	A_d	841
		10	1			
		17	8			
678348	6	3	1	$GalNAc_3$-10a	A_d	841
		10	1			
		17	6			
678349	6	3	1	$GalNAc_3$-13a	A_d	841
		10	1			
		17	5			

Example 85: Antisense inhibition *in vivo* by oligonucleotides targeting SRB-1 comprising a $GalNAc_3$ Conjugate

Oligonucleotides listed in Table 76 were tested in a dose-dependent study for antisense inhibition of SRB-1 in mice.

Treatment

Six to eight week old C57BL/6 mice were each injected subcutaneously once per week at a dosage shown below, for a total of three doses, with an oligonucleotide listed in Table 76 or with saline. Each treatment group consisted of 4 animals. The mice were sacrificed 48 hours following the final administration to determine the SRB-1 mRNA levels using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. The results below are presented as the average percent of liver SRB-1 mRNA levels for each treatment group, normalized to the saline control.

As illustrated in Tables 80 and 81, treatment with antisense oligonucleotides lowered SRB-1 mRNA levels in a dose-dependent manner.

Table 80

SRB-1 mRNA in liver

ISIS No.	Dosage (mg/kg)	SRB-1 mRNA (% Saline)	$GalNAc_3$ Cluster	CM
Saline	n/a	100	n/a	n/a
655861	0.1	94	$GalNAc_3$-1a	A_d
	0.3	119		
	1	68		
	3	32		
661161	0.1	120	$GalNAc_3$-3a	A_d
	0.3	107		
	1	68		
	3	26		
666881	0.1	107	$GalNAc_3$-10a	A_d
	0.3	107		
	1	69		
	3	27		
666981	0.1	120	$GalNAc_3$-7a	A_d
	0.3	103		
	1	54		
	3	21		
670061	0.1	118	$GalNAc_3$-13a	A_d
	0.3	89		
	1	52		
	3	18		
677842	0.1	119	$GalNAc_3$-20a	A_d
	0.3	96		
	1	65		
	3	23		

Table 81

SRB-1 mRNA in liver

ISIS No.	Dosage (mg/kg)	SRB-1 mRNA (% Saline)	$GalNAc_3$ Cluster	CM
661161	0.1	107	$GalNAc_3$-3a	A_d
	0.3	95		
	1	53		
	3	18		
677841	0.1	110	$GalNAc_3$-19a	A_d
	0.3	88		
	1	52		
	3	25		

Liver transaminase levels, total bilirubin, BUN, and body weights were also measured using standard protocols. Average values for each treatment group are shown in Table 82 below.

Table 82

ISIS No.	Dosage (mg/kg)	ALT (U/L)	AST (U/L)	Bilirubin (mg/dL)	BUN (mg/dL)	Body Weight (% baseline)	$GalNAc_3$ Cluster	CM
Saline	n/a	19	39	0.17	26	118	n/a	n/a
655861	0.1	25	47	0.17	27	114	$GalNAc_3$-1a	A_d
	0.3	29	56	0.15	27	118		
	1	20	32	0.14	24	112		
	3	27	54	0.14	24	115		
661161	0.1	35	83	0.13	24	113	$GalNAc_3$-3a	A_d
	0.3	42	61	0.15	23	117		
	1	34	60	0.18	22	116		
	3	29	52	0.13	25	117		
666881	0.1	30	51	0.15	23	118	$GalNAc_3$-10a	A_d
	0.3	49	82	0.16	25	119		
	1	23	45	0.14	24	117		
	3	20	38	0.15	21	112		
666981	0.1	21	41	0.14	22	113	$GalNAc_3$-7a	A_d
	0.3	29	49	0.16	24	112		
	1	19	34	0.15	22	111		
	3	77	78	0.18	25	115		
670061	0.1	20	63	0.18	24	111	$GalNAc_3$-13a	A_d
	0.3	20	57	0.15	21	115		
	1	20	35	0.14	20	115		
	3	27	42	0.12	20	116		
677842	0.1	20	38	0.17	24	114	$GalNAc_3$-20a	A_d
	0.3	31	46	0.17	21	117		
	1	22	34	0.15	21	119		
	3	41	57	0.14	23	118		

Example 86: Antisense inhibition *in vivo* by oligonucleotides targeting TTR comprising a $GalNAc_3$ cluster

Oligonucleotides listed in Table 83 below were tested in a dose-dependent study for antisense inhibition of human transthyretin (TTR) in transgenic mice that express the human TTR gene.

Treatment

Eight week old TTR transgenic mice were each injected subcutaneously once per week for three weeks, for a total of three doses, with an oligonucleotide and dosage listed in the tables below or with PBS. Each treatment group consisted of 4 animals. The mice were sacrificed 72 hours following the final administration. Tail bleeds were performed at various time points throughout the experiment, and plasma TTR protein, ALT, and AST levels were measured and reported in Tables 85-87. After the animals were sacrificed, plasma ALT, AST, and human TTR levels were measured, as were body weights, organ weights, and liver human TTR mRNA levels. TTR protein levels were measured using a clinical analyzer (AU480, Beckman Coulter, CA). Real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) were used according to standard protocols to determine liver human TTR mRNA levels. The results presented in Tables 84-87 are the average values for each treatment group. The mRNA levels are the average values relative to the average for the PBS group. Plasma protein levels are the average values relative to the average value for the PBS group at baseline. Body weights are the average percent weight change from baseline until sacrifice for each individual treatment group. Organ weights shown are normalized to the animal's body weight, and the average normalized organ weight for each treatment group is then presented relative to the average normalized organ weight for the PBS group.

In Tables 84-87, "BL" indicates baseline, measurements that were taken just prior to the first dose. As illustrated in Tables 84 and 85, treatment with antisense oligonucleotides lowered TTR expression levels in a dose-dependent manner. The oligonucleotides comprising a GalNAc conjugate were more potent than the parent lacking a GalNAc conjugate (ISIS 420915). Furthermore, the oligonucleotides comprising a GalNAc conjugate and mixed PS/PO internucleoside linkages were even more potent than the oligonucleotide comprising a GalNAc conjugate and full PS linkages.

Table 83

Oligonucleotides targeting human TTR

Isis No.	Sequence 5' to 3'	Linkages	GalNAc cluster	CM	SEQ ID No.
420915	$T_{es}{}^{m}C_{es}T_{es}T_{es}G_{es}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{es}T_{es}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$	PS	n/a	n/a	842
660261	$T_{es}{}^{m}C_{es}T_{es}T_{es}G_{es}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{es}T_{es}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{eo}\mathbf{A_{do}}$-**GalNAc$_3-1_a$**	PS	GalNAc$_3$-1a	A_d	843
682883	**GalNAc$_3$-3$_a$**-$_o$,$T_{es}{}^{m}C_{eo}T_{eo}T_{eo}G_{eo}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{eo}T_{eo}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$	PS/PO	GalNAc$_3$-3a	PO	842
682884	**GalNAc$_3$-7$_a$**-$_o$,$T_{es}{}^{m}C_{eo}T_{eo}T_{eo}G_{eo}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{eo}T_{eo}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$	PS/PO	GalNAc$_3$-7a	PO	842
682885	**GalNAc$_3$-10$_a$**-$_o$,$T_{es}{}^{m}C_{eo}T_{eo}T_{eo}G_{eo}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{eo}T_{eo}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$	PS/PO	GalNAc$_3$-10a	PO	842
682886	**GalNAc$_3$-13$_a$**-$_o$,$T_{es}{}^{m}C_{eo}T_{eo}T_{eo}G_{eo}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{eo}T_{eo}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$	PS/PO	GalNAc$_3$-13a	PO	842
684057	$T_{es}{}^{m}C_{eo}T_{eo}T_{eo}G_{eo}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{eo}T_{eo}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{eo}\mathbf{A_{do}}$-**GalNAc$_3-19_a$**	PS/PO	GalNAc$_3$-19a	A_d	843

The legend for Table 85 can be found in Example 74. The structure of GalNAc$_3$-1 was shown in Example 9. The structure of GalNAc$_3$-3$_a$ was shown in Example 39. The structure of GalNAc$_3$-7$_a$ was shown in Example 48. The structure of GalNAc$_3$-10$_a$ was shown in Example 46. The structure of GalNAc$_3$-13$_a$ was shown in Example 62. The structure of GalNAc$_3$-19$_a$ was shown in Example 70.

Table 84

Antisense inhibition of human TTR *in vivo*

Isis No.	Dosage (mg/kg)	TTR mRNA (% PBS)	Plasma TTR protein (% PBS)	GalNAc cluster	CM	SEQ ID No.
PBS	n/a	100	100	n/a	n/a	
420915	6	99	95	n/a	n/a	842
	20	48	65			
	60	18	28			
660261	0.6	113	87	GalNAc$_3$-1a	A_d	843
	2	40	56			
	6	20	27			
	20	9	11			

Table 85

Antisense inhibition of human TTR *in vivo*

Isis No.	Dosage (mg/kg)	TTR mRNA (% PBS)	Plasma TTR protein (% PBS at BL)				GalNAc cluster	CM	SEQ ID No.
			BL	Day 3	Day 10	Day 17 (After sac)			
PBS	n/a	100	100	96	90	114	n/a	n/a	
420915	6	74	106	86	76	83	n/a	n/a	842
	20	43	102	66	61	58			
	60	24	92	43	29	32			
682883	0.6	60	88	73	63	68	$GalNAc_3$-3a	PO	842
	2	18	75	38	23	23			
	6	10	80	35	11	9			
682884	0.6	56	88	78	63	67	$GalNAc_3$-7a	PO	842
	2	19	76	44	25	23			
	6	15	82	35	21	24			
682885	0.6	60	92	77	68	76	$GalNAc_3$-10a	PO	842
	2	22	93	58	32	32			
	6	17	85	37	25	20			
682886	0.6	57	91	70	64	69	$GalNAc_3$-13a	PO	842
	2	21	89	50	31	30			
	6	18	102	41	24	27			
684057	0.6	53	80	69	56	62	$GalNAc_3$-19a	A_d	843
	2	21	92	55	34	30			
	6	11	82	50	18	13			

Table 86

Transaminase levels, body weight changes, and relative organ weights

Isis No.	Dosage (mg/kg)	ALT (U/L)				AST (U/L)				Body (% BL)	Liver (% PBS)	Spleen (% PBS)	Kidney (% PBS)	SEQ ID No.
		BL	Day 3	Day 10	Day 17	BL	Day 3	Day 10	Day 17					
PBS	n/a	33	34	33	24	58	62	67	52	105	100	100	100	n/a
420915	6	34	33	27	21	64	59	73	47	115	99	89	91	842
	20	34	30	28	19	64	54	56	42	111	97	83	89	
	60	34	35	31	24	61	58	71	58	113	102	98	95	
660261	0.6	33	38	28	26	70	71	63	59	111	96	99	92	843
	2	29	32	31	34	61	60	68	61	118	100	92	90	
	6	29	29	28	34	58	59	70	90	114	99	97	95	
	20	33	32	28	33	64	54	68	95	114	101	106	92	

Table 87

Transaminase levels, body weight changes, and relative organ weights

Isis No.	Dosage (mg/kg)	ALT (U/L)				AST (U/L)				Body (% BL)	Liver (% PBS)	Spleen (% PBS)	Kidney (% PBS)	SEQ ID No.
		BL	Day 3	Day 10	Day 17	BL	Day 3	Day 10	Day 17					
PBS	n/a	32	34	37	41	62	78	76	77	104	100	100	100	n/a
420915	6	32	30	34	34	61	71	72	66	102	103	102	105	842
	20	41	34	37	33	80	76	63	54	106	107	135	101	
	60	36	30	32	34	58	81	57	60	106	105	104	99	
682883	0.6	32	35	38	40	53	81	74	76	104	101	112	95	842
	2	38	39	42	43	71	84	70	77	107	98	116	99	
	6	35	35	41	38	62	79	103	65	105	103	143	97	
682884	0.6	33	32	35	34	70	74	75	67	101	100	130	99	842
	2	31	32	38	38	63	77	66	55	104	103	122	100	
	6	38	32	36	34	65	85	80	62	99	105	129	95	
682885	0.6	39	26	37	35	63	63	77	59	100	109	109	112	842
	2	30	26	38	40	54	56	71	72	102	98	111	102	
	6	27	27	34	35	46	52	56	64	102	98	113	96	
682886	0.6	30	40	34	36	58	87	54	61	104	99	120	101	842
	2	27	26	34	36	51	55	55	69	103	91	105	92	
	6	40	28	34	37	107	54	61	69	109	100	102	99	
684057	0.6	35	26	33	39	56	51	51	69	104	99	110	102	843
	2	33	32	31	40	54	57	56	87	103	100	112	97	
	6	39	33	35	40	67	52	55	92	98	104	121	108	

Example 87: Duration of action *in vivo* by single doses of oligonucleotides targeting TTR comprising a $GalNAc_3$ cluster

ISIS numbers 420915 and 660261 (see Table 83) were tested in a single dose study for duration of action in mice. ISIS numbers 420915, 682883, and 682885 (see Table 83) were also tested in a single dose study for duration of action in mice.

Treatment

Eight week old, male transgenic mice that express human TTR were each injected subcutaneously once with 100 mg/kg ISIS No. 420915 or 13.5 mg/kg ISIS No. 660261. Each treatment group consisted of 4 animals. Tail bleeds were performed before dosing to determine baseline and at days 3, 7, 10, 17, 24, and 39 following the dose. Plasma TTR protein levels were measured as described in Example 86. The results below are presented as the average percent of plasma TTR levels for each treatment group, normalized to baseline levels.

Table 88

Plasma TTR protein levels

ISIS No.	Dosage (mg/kg)	Time point (days post-dose)	TTR (% baseline)	$GalNAc_3$ Cluster	CM	SEQ ID No.
420915	100	3	30	n/a	n/a	842
		7	23			
		10	35			
		17	53			
		24	75			
		39	100			
660261	13.5	3	27	$GalNAc_3$-1a	A_d	843
		7	21			
		10	22			
		17	36			
		24	48			
		39	69			

Treatment

Female transgenic mice that express human TTR were each injected subcutaneously once with 100 mg/kg ISIS No. 420915, 10.0 mg/kg ISIS No. 682883, or 10.0 mg/kg 682885. Each treatment group consisted of 4 animals. Tail bleeds were performed before dosing to determine baseline and at days 3, 7, 10, 17, 24, and 39 following the dose. Plasma TTR protein levels were measured as described in Example 86. The results below are presented as the average percent of plasma TTR levels for each treatment group, normalized to baseline levels.

Table 89

Plasma TTR protein levels

ISIS No.	Dosage (mg/kg)	Time point (days post-dose)	TTR (% baseline)	$GalNAc_3$ Cluster	CM	SEQ ID No.
420915	100	3	48	n/a	n/a	842
		7	48			
		10	48			
		17	66			
		31	80			
682883	10.0	3	45	$GalNAc_3$-3a	PO	842
		7	37			
		10	38			
		17	42			
		31	65			
682885	10.0	3	40	$GalNAc_3$-	PO	842

		7	33	10a		
		10	34			
		17	40			
		31	64			

The results in Tables 88 and 89 show that the oligonucleotides comprising a GalNAc conjugate are more potent with a longer duration of action than the parent oligonucleotide lacking a conjugate (ISIS 420915).

Example 88: Splicing modulation *in vivo* by oligonucleotides targeting SMN comprising a GalNAc$_3$ conjugate

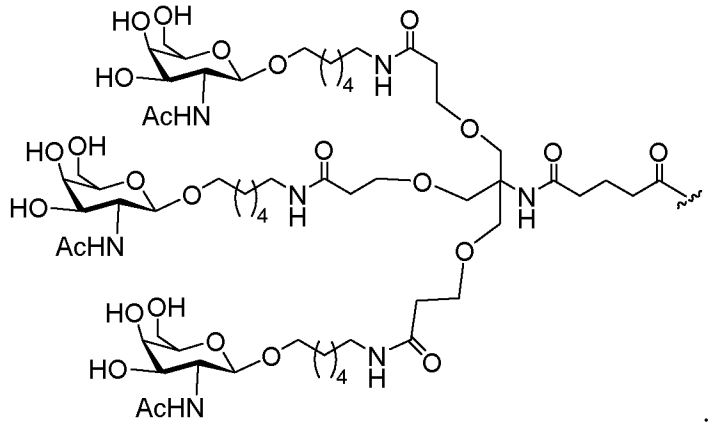
The oligonucleotides listed in Table 90 were tested for splicing modulation of human survival of motor neuron (SMN) in mice.

Table 90

Modified ASOs targeting SMN

ISIS No.	Sequences (5' to 3')	GalNAc$_3$ Cluster	CM	SEQ ID No.
387954	$A_{es}T_{es}T_{es}{}^{m}C_{es}A_{es}{}^{m}C_{es}T_{es}T_{es}T_{es}{}^{m}C_{es}A_{es}T_{es}A_{es}A_{es}T_{es}G_{es}{}^{m}C_{es}T_{es}G_{es}G_{e}$	n/a	n/a	844
699819	**GalNAc$_3$-7$_{a}$-$_{o}$**,$A_{es}T_{es}T_{es}{}^{m}C_{es}A_{es}{}^{m}C_{es}T_{es}T_{es}T_{es}{}^{m}C_{es}A_{es}T_{es}A_{es}A_{es}T_{es}G_{es}{}^{m}C_{es}T_{es}G_{es}G_{e}$	GalNAc$_3$-7a	PO	844
699821	**GalNAc$_3$-7$_{a}$-$_{o}$**,$A_{es}T_{eo}T_{eo}{}^{m}C_{eo}A_{eo}{}^{m}C_{eo}T_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{eo}T_{eo}A_{eo}A_{eo}T_{eo}G_{eo}{}^{m}C_{eo}T_{es}G_{es}G_{e}$	GalNAc$_3$-7a	PO	844
700000	$A_{es}T_{es}T_{es}{}^{m}C_{es}A_{es}{}^{m}C_{es}T_{es}T_{es}T_{es}{}^{m}C_{es}A_{es}T_{es}A_{es}A_{es}T_{es}G_{es}{}^{m}C_{es}T_{es}G_{es}G_{eo}$**A$_{do}$-GalNAc$_3-1_a$**	GalNAc$_3$-1a	A$_d$	845
703421	X-ATT^{m}CA^{m}CTTT^{m}CATAATG^{m}CTGG	n/a	n/a	844
703422	**GalNAc$_3$-7$_b$**-X-ATT^{m}CA^{m}CTTT^{m}CATAATG^{m}CTGG	GalNAc$_3$-7b	n/a	844

The structure of GalNAc$_3$-7$_a$ was shown previously in Example 48. "X" indicates a 5' primary amine generated by Gene Tools (Philomath, OR), and GalNAc$_3$-7$_b$ indicates the structure of GalNAc$_3$-7$_a$ lacking the –NH-C$_6$-O portion of the linker as shown below:



ISIS numbers 703421 and 703422 are morphlino oligonucleotides, wherein each nucleotide of the two oligonucleotides is a morpholino nucleotide.

Treatment

Six week old transgenic mice that express human SMN were injected subcutaneously once with an oligonucleotide listed in Table 91 or with saline. Each treatment group consisted of 2 males and 2 females. The mice were sacrificed 3 days following the dose to determine the liver human SMN mRNA levels both with and without exon 7 using real-time PCR according to standard protocols. Total RNA was measured using Ribogreen reagent. The SMN mRNA levels were normalized to total mRNA, and further normalized to the averages for the saline treatment group. The resulting average ratios of SMN mRNA including exon 7 to SMN mRNA missing exon 7 are shown in Table 91. The results show that fully modified oligonucleotides that modulate splicing and comprise a GalNAc conjugate are significantly more potent in altering splicing in the liver than the parent oligonucleotides lacking a GlaNAc conjugate. Furthermore, this trend is maintained for multiple modification chemistries, including 2'-MOE and morpholino modified oligonucleotides.

Table 91

Effect of oligonucleotides targeting human SMN *in vivo*

ISIS No.	Dose (mg/kg)	+Exon 7 / -Exon 7	$GalNAc_3$ Cluster	CM	SEQ ID No.
Saline	n/a	1.00	n/a	n/a	n/a
387954	32	1.65	n/a	n/a	844
387954	288	5.00	n/a	n/a	844
699819	32	7.84	$GalNAc_3$-7a	PO	844
699821	32	7.22	$GalNAc_3$-7a	PO	844
700000	32	6.91	$GalNAc_3$-1a	A_d	845
703421	32	1.27	n/a	n/a	844
703422	32	4.12	$GalNAc_3$-7b	n/a	844

Example 89: Antisense inhibition *in vivo* by oligonucleotides targeting Apolipoprotein A (Apo(a)) comprising a GalNAc$_3$ conjugate

The oligonucleotides listed in Table 92 below were tested in a study for dose-dependent inhibition of Apo(a) in transgenic mice.

Table 92

Modified ASOs targeting Apo(a)

ISIS No.	Sequences (5' to 3')	GalNAc$_3$ Cluster	CM	SEQ ID No.
494372	$T_{es}G_{es}{}^{m}C_{es}T_{es}{}^{m}C_{es}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}T_{ds}G_{ds}{}^{m}C_{ds}$ $T_{ds}T_{es}G_{es}T_{es}T_{es}{}^{m}C_{e}$	n/a	n/a	847
681257	**GalNAc$_3$-7$_{a}$-$_{o}$**$T_{es}G_{eo}{}^{m}C_{eo}T_{eo}{}^{m}C_{eo}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}$ $T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{eo}G_{eo}T_{es}T_{es}{}^{m}C_{e}$	GalNAc$_3$-7a	PO	847

The structure of GalNAc$_3$-7$_a$ was shown in Example 48.

Treatment

Eight week old, female C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) were each injected subcutaneously once per week at a dosage shown below, for a total of six doses, with an oligonucleotide listed in Table 92 or with PBS. Each treatment group consisted of 3-4 animals. Tail bleeds were performed the day before the first dose and weekly following each dose to determine plasma Apo(a) protein levels. The mice were sacrificed two days following the final administration. Apo(a) liver mRNA levels were determined using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc. Eugene, OR) according to standard protocols. Apo(a) plasma protein levels were determined using ELISA, and liver transaminase levels were determined. The mRNA and plasma protein results in Table 93 are presented as the treatment group average percent relative to the PBS treated group. Plasma protein levels were further normalized to the baseline (BL) value for the PBS group. Average absolute transaminase levels and body weights (% relative to baseline averages) are reported in Table 94.

As illustrated in Table 93, treatment with the oligonucleotides lowered Apo(a) liver mRNA and plasma protein levels in a dose-dependent manner. Furthermore, the oligonucleotide comprising the GalNAc conjugate was significantly more potent with a longer duration of action than the parent oligonucleotide lacking a GalNAc conjugate. As illustrated in Table 94, transaminase levels and body weights were unaffected by the oligonucleotides, indicating that the oligonucleotides were well tolerated.

Table 93

Apo(a) liver mRNA and plasma protein levels

ISIS No.	Dosage (mg/kg)	Apo(a) mRNA (% PBS)	Apo(a) plasma protein (% PBS)						
			BL	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
PBS	n/a	100	100	120	119	113	88	121	97
494372	3	80	84	89	91	98	87	87	79
	10	30	87	72	76	71	57	59	46
	30	5	92	54	28	10	7	9	7
681257	0.3	75	79	76	89	98	71	94	78
	1	19	79	88	66	60	54	32	24
	3	2	82	52	17	7	4	6	5
	10	2	79	17	6	3	2	4	5

Table 94

ISIS No.	Dosage (mg/kg)	ALT (U/L)	AST (U/L)	Body weight (% baseline)
PBS	n/a	37	54	103
494372	3	28	68	106
	10	22	55	102
	30	19	48	103
681257	0.3	30	80	104
	1	26	47	105
	3	29	62	102
	10	21	52	107

Example 90: Antisense inhibition *in vivo* by oligonucleotides targeting TTR comprising a $GalNAc_3$ cluster

Oligonucleotides listed in Table 95 below were tested in a dose-dependent study for antisense inhibition of human transthyretin (TTR) in transgenic mice that express the human TTR gene.

Treatment

TTR transgenic mice were each injected subcutaneously once per week for three weeks, for a total of three doses, with an oligonucleotide and dosage listed in Table 96 or with PBS. Each treatment group consisted of 4 animals. Prior to the first dose, a tail bleed was performed to determine plasma TTR protein levels at baseline (BL). The mice were sacrificed 72 hours following the final administration. TTR protein levels were measured using a clinical analyzer (AU480, Beckman Coulter, CA). Real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular

Probes, Inc. Eugene, OR) were used according to standard protocols to determine liver human TTR mRNA levels. The results presented in Table 96 are the average values for each treatment group. The mRNA levels are the average values relative to the average for the PBS group. Plasma protein levels are the average values relative to the average value for the PBS group at baseline. "BL" indicates baseline, measurements that were taken just prior to the first dose. As illustrated in Table 96, treatment with antisense oligonucleotides lowered TTR expression levels in a dose-dependent manner. The oligonucleotides comprising a GalNAc conjugate were more potent than the parent lacking a GalNAc conjugate (ISIS 420915), and oligonucleotides comprising a phosphodiester or deoxyadenosine cleavable moiety showed significant improvements in potency compared to the parent lacking a conjugate (see ISIS numbers 682883 and 666943 vs 420915 and see Examples 86 and 87).

Table 95

Oligonucleotides targeting human TTR

Isis No.	Sequence 5' to 3'	Linkages	GalNAc cluster	CM	SEQ ID No.
420915	$T_{es}{}^{m}C_{es}T_{es}T_{es}G_{es}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{es}T_{es}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$	PS	n/a	n/a	842
682883	**$GalNAc_3$-3_a-$_o$'**$T_{es}{}^{m}C_{eo}T_{eo}T_{eo}G_{eo}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{eo}T_{eo}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$	PS/PO	$GalNAc_3$-3a	PO	842
666943	**$GalNAc_3$-3_a-$_o$'A_{do}**$T_{es}{}^{m}C_{eo}T_{eo}T_{eo}G_{eo}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{eo}T_{eo}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$	PS/PO	$GalNAc_3$-3a	A_d	846
682887	**$GalNAc_3$-7_a-$_o$'A_{do}**$T_{es}{}^{m}C_{eo}T_{eo}T_{eo}G_{eo}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{eo}T_{eo}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$	PS/PO	$GalNAc_3$-7a	A_d	846
682888	**$GalNAc_3$-10_a-$_o$'A_{do}**$T_{es}{}^{m}C_{eo}T_{eo}T_{eo}G_{eo}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{eo}T_{eo}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$	PS/PO	$GalNAc_3$-10a	A_d	846
682889	**$GalNAc_3$-13_a-$_o$'A_{do}**$T_{es}{}^{m}C_{eo}T_{eo}T_{eo}G_{eo}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{eo}T_{eo}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$	PS/PO	$GalNAc_3$-13a	A_d	846

The legend for Table 95 can be found in Example 74. The structure of $GalNAc_3$-3_a was shown in Example 39. The structure of $GalNAc_3$-7_a was shown in Example 48. The structure of $GalNAc_3$-10_a was shown in Example 46. The structure of $GalNAc_3$-13_a was shown in Example 62.

Table 96

Antisense inhibition of human TTR *in vivo*

Isis No.	Dosage (mg/kg)	TTR mRNA (% PBS)	TTR protein (% BL)	GalNAc cluster	CM
PBS	n/a	100	124	n/a	n/a
420915	6	69	114	n/a	n/a
	20	71	86		
	60	21	36		
682883	0.6	61	73	$GalNAc_3$-3a	PO
	2	23	36		
	6	18	23		
666943	0.6	74	93	$GalNAc_3$-3a	A_d
	2	33	57		
	6	17	22		
682887	0.6	60	97	$GalNAc_3$-7a	A_d
	2	36	49		
	6	12	19		
682888	0.6	65	92	$GalNAc_3$-10a	A_d
	2	32	46		
	6	17	22		
682889	0.6	72	74	$GalNAc_3$-13a	A_d
	2	38	45		
	6	16	18		

Example 91: Antisense inhibition *in vivo* by oligonucleotides targeting Factor VII comprising a $GalNAc_3$ conjugate in non-human primates

Oligonucleotides listed in Table 97 below were tested in a non-terminal, dose escalation study for antisense inhibition of Factor VII in monkeys.

Treatment

Non-naïve monkeys were each injected subcutaneously on days 0, 15, and 29 with escalating doses of an oligonucleotide listed in Table 97 or with PBS. Each treatment group consisted of 4 males and 1 female. Prior to the first dose and at various time points thereafter, blood draws were performed to determine plasma Factor VII protein levels. Factor VII protein levels were measured by ELISA. The results presented in Table 98 are the average values for each treatment group relative to the average value for the PBS group at baseline (BL), the measurements taken just prior to the first dose. As illustrated in Table 98, treatment with antisense oligonucleotides lowered Factor VII expression levels in a dose-dependent manner, and the oligonucleotide comprising the GalNAc

conjugate was significantly more potent in monkeys compared to the oligonucleotide lacking a GalNAc conjugate.

Table 97

Oligonucleotides targeting Factor VII

Isis No.	Sequence 5' to 3'	Linkages	GalNAc cluster	CM	SEQ ID No.
407935	$A_{es}T_{es}G_{es}{}^{m}C_{es}A_{es}T_{ds}G_{ds}G_{ds}T_{ds}G_{ds}A_{ds}T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}T_{es}G_{es}A_{e}$	PS	n/a	n/a	848
686892	**$GalNAc_3$-10_{a}-$_{o}$**$A_{es}T_{es}G_{es}{}^{m}C_{es}A_{es}T_{ds}G_{ds}G_{ds}T_{ds}G_{ds}A_{ds}T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}T_{es}G_{es}A_{e}$	PS	$GalNAc_3$-10a	PO	848

The legend for Table 97 can be found in Example 74. The structure of $GalNAc_3$-10_a was shown in Example 46.

Table 98

Factor VII plasma protein levels

ISIS No.	Day	Dose (mg/kg)	Factor VII (% BL)
407935	0	n/a	100
	15	10	87
	22	n/a	92
	29	30	77
	36	n/a	46
	43	n/a	43
686892	0	3	100
	15	10	56
	22	n/a	29
	29	30	19
	36	n/a	15
	43	n/a	11

Example 92: Antisense inhibition in primary hepatocytes by antisense oligonucleotides targeting Apo-CIII comprising a $GalNAc_3$ conjugate

Primary mouse hepatocytes were seeded in 96-well plates at 15,000 cells per well, and the oligonucleotides listed in Table 99, targeting mouse ApoC-III, were added at 0.46, 1.37, 4.12, or 12.35, 37.04, 111.11, or 333.33 nM or 1.00 μM. After incubation with the oligonucleotides for 24 hours, the cells were lysed and total RNA was purified using RNeasy (Qiagen). ApoC-III mRNA levels were determined using real-time PCR and RIBOGREEN® RNA quantification reagent (Molecular Probes, Inc.) according to standard protocols. IC_{50} values were determined using Prism 4

software (GraphPad). The results show that regardless of whether the cleavable moiety was a phosphodiester or a phosphodiester-linked deoxyadensoine, the oligonucleotides comprising a GalNAc conjugate were significantly more potent than the parent oligonucleotide lacking a conjugate.

Table 99

Inhibition of mouse APOC-III expression in mouse primary hepatocytes

ISIS No.	Sequence (5' to 3')	CM	IC_{50} (nM)	SEQ ID No.
440670	$^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$	n/a	13.20	849
661180	$^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}A_{eo}$ **A_{do}'-GalNAc$_3$-1$_a$**	A_d	1.40	850
680771	**GalNAc$_3$-3$_a$-$_o$'**$^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$	PO	0.70	849
680772	**GalNAc$_3$-7$_a$-$_o$'**$^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$	PO	1.70	849
680773	**GalNAc$_3$-10$_a$-$_o$'**$^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$	PO	2.00	849
680774	**GalNAc$_3$-13$_a$-$_o$'**$^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$	PO	1.50	849
681272	**GalNAc$_3$-3$_a$-$_o$'**$^{m}C_{es}A_{eo}G_{eo}{}^{m}C_{eo}T_{eo}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{eo}A_{eo}G_{es}{}^{m}C_{es}A_{e}$	PO	< 0.46	849
681273	**GalNAc$_3$-3$_a$-$_o$'-A_{do}**$^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$	A_d	1.10	851
683733	$^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}A_{eo}$ **A_{do}'-GalNAc$_3$-19$_a$**	A_d	2.50	850

The structure of GalNAc$_3$-1$_a$ was shown previously in Example 9, GalNAc$_3$-3$_a$ was shown in Example 39, GalNAc$_3$-7$_a$ was shown in Example 48, GalNAc$_3$-10$_a$ was shown in Example 46, GalNAc$_3$-13$_a$ was shown in Example 62, and GalNAc$_3$-19$_a$ was shown in Example 70.

Example 93: Antisense inhibition *in vivo* by oligonucleotides targeting SRB-1 comprising mixed wings and a 5'-GalNAc$_3$ conjugate

The oligonucleotides listed in Table 100 were tested in a dose-dependent study for antisense inhibition of SRB-1 in mice.

Table 100

Modified ASOs targeting SRB-1

ISIS No.	Sequences (5' to 3')	$GalNAc_3$ Cluster	CM	SEQ ID No.
449093	$T_{ks}T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}\ A_{ds}T_{ds}\ G_{ds}\ A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{ks}{}^{m}C_{k}$	n/a	n/a	852
699806	**$GalNAc_3$-3_{a}-$_{o}$**,$T_{ks}T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}\ A_{ds}T_{ds}\ G_{ds}A_{ds}{}^{m}C_{ds}\ T_{ds}T_{ks}{}^{m}C_{ks}{}^{m}C_{k}$	$GalNAc_3$-3a	PO	852
699807	**$GalNAc_3$-7_{a}-$_{o}$**,$T_{ks}T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}\ A_{ds}T_{ds}\ G_{ds}A_{ds}{}^{m}C_{ds}\ T_{ds}T_{ks}{}^{m}C_{ks}{}^{m}C_{k}$	$GalNAc_3$-7a	PO	852
699809	**$GalNAc_3$-7_{a}-$_{o}$**, $T_{ks}T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}\ A_{ds}T_{ds}\ G_{ds}\ A_{ds}{}^{m}C_{ds}\ T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{e}$	$GalNAc_3$-7a	PO	852
699811	**$GalNAc_3$-7_{a}-$_{o}$**,$T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}\ A_{ds}T_{ds}\ G_{ds}A_{ds}{}^{m}C_{ds}\ T_{ds}T_{ks}{}^{m}C_{ks}{}^{m}C_{k}$	$GalNAc_3$-7a	PO	852
699813	**$GalNAc_3$-7_{a}-$_{o}$**,$T_{ks}T_{ds}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}\ A_{ds}T_{ds}\ G_{ds}A_{ds}{}^{m}C_{ds}\ T_{ds}T_{ks}{}^{m}C_{ds}{}^{m}C_{k}$	$GalNAc_3$-7a	PO	852
699815	**$GalNAc_3$-7_{a}-$_{o}$**,$T_{es}T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}\ A_{ds}T_{ds}\ G_{ds}A_{ds}{}^{m}C_{ds}\ T_{ds}T_{ks}{}^{m}C_{ks}{}^{m}C_{e}$	$GalNAc_3$-7a	PO	852

The structure of $GalNAc_3$-3_a was shown previously in Example 39, and the structure of $GalNAc_3$-7a was shown previously in Example 48. Subscripts: "e" indicates 2'-MOE modified nucleoside; "d" indicates β-D-2'-deoxyribonucleoside; "k" indicates 6'-*(S)*-CH_3 bicyclic nucleoside (cEt); "s" indicates phosphorothioate internucleoside linkages (PS); "o" indicates phosphodiester internucleoside linkages (PO). Supersript "m" indicates 5-methylcytosines.

Treatment

Six to eight week old C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously once at the dosage shown below with an oligonucleotide listed in Table 100 or with saline. Each treatment group consisted of 4 animals. The mice were sacrificed 72 hours following the final administration. Liver SRB-1 mRNA levels were measured using real-time PCR. SRB-1 mRNA levels were normalized to cyclophilin mRNA levels according to standard protocols. The results are presented as the average percent of SRB-1 mRNA levels for each treatment group relative to the saline control group. As illustrated in Table 101, treatment with antisense oligonucleotides lowered SRB-1 mRNA levels in a dose-dependent manner, and the gapmer oligonucleotides comprising a GalNAc conjugate and having wings that were either full cEt or mixed sugar modifications were significantly more potent than the parent oligonucleotide lacking a conjugate and comprising full cEt modified wings.

Body weights, liver transaminases, total bilirubin, and BUN were also measured, and the average values for each treatment group are shown in Table 101. Body weight is shown as the

average percent body weight relative to the baseline body weight (% BL) measured just prior to the oligonucleotide dose.

Table 101

SRB-1 mRNA, ALT, AST, BUN, and total bilirubin levels and body weights

ISIS No.	Dosage (mg/kg)	SRB-1 mRNA (% PBS)	ALT (U/L)	AST (U/L)	Bil	BUN	Body weight (% BL)
PBS	n/a	100	31	84	0.15	28	102
449093	1	111	18	48	0.17	31	104
	3	94	20	43	0.15	26	103
	10	36	19	50	0.12	29	104
699806	0.1	114	23	58	0.13	26	107
	0.3	59	21	45	0.12	27	108
	1	25	30	61	0.12	30	104
699807	0.1	121	19	41	0.14	25	100
	0.3	73	23	56	0.13	26	105
	1	24	22	69	0.14	25	102
699809	0.1	125	23	57	0.14	26	104
	0.3	70	20	49	0.10	25	105
	1	33	34	62	0.17	25	107
699811	0.1	123	48	77	0.14	24	106
	0.3	94	20	45	0.13	25	101
	1	66	57	104	0.14	24	107
699813	0.1	95	20	58	0.13	28	104
	0.3	98	22	61	0.17	28	105
	1	49	19	47	0.11	27	106
699815	0.1	93	30	79	0.17	25	105
	0.3	64	30	61	0.12	26	105
	1	24	18	41	0.14	25	106

Example 94: Antisense inhibition *in vivo* by oligonucleotides targeting SRB-1 comprising 2'-sugar modifications and a 5'-GalNAc$_3$ conjugate

The oligonucleotides listed in Table 102 were tested in a dose-dependent study for antisense inhibition of SRB-1 in mice.

Table 102

Modified ASOs targeting SRB-1

ISIS No.	Sequences (5' to 3')	GalNAc$_3$ Cluster	CM	SEQ ID No.
353382	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	n/a	n/a	829
700989	$G_{ms}C_{ms}U_{ms}U_{ms}C_{ms}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}U_{ms}C_{ms}C_{ms}U_{ms}U_{m}$	n/a	n/a	853

666904	**GalNAc$_3$-3$_{a-o}$**,$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	GalNAc$_3$-3a	PO	829
700991	**GalNAc$_3$-7$_{a-o}$**,$G_{ms}C_{ms}U_{ms}U_{ms}C_{ms}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}U_{ms}C_{ms}C_{ms}U_{ms}U_{m}$	GalNAc$_3$-7a	PO	853

Subscript "m" indicates a 2'-O-methyl modified nucleoside. See Example 74 for complete table legend. The structure of GalNAc$_3$-3$_a$ was shown previously in Example 39, and the structure of GalNAc$_3$-7a was shown previously in Example 48.

Treatment

The study was completed using the protocol described in Example 93. Results are shown in Table 103 below and show that both the 2'-MOE and 2'-OMe modified oligonucleotides comprising a GalNAc conjugate were significantly more potent than the respective parent oligonucleotides lacking a conjugate. The results of the body weights, liver transaminases, total bilirubin, and BUN measurements indicated that the compounds were all well tolerated.

Table 103

SRB-1 mRNA

ISIS No.	Dosage (mg/kg)	SRB-1 mRNA (% PBS)
PBS	n/a	100
353382	5	116
	15	58
	45	27
700989	5	120
	15	92
	45	46
666904	1	98
	3	45
	10	17
700991	1	118
	3	63
	10	14

Example 95: Antisense inhibition *in vivo* by oligonucleotides targeting SRB-1 comprising bicyclic nucleosides and a 5'-GalNAc$_3$ conjugate

The oligonucleotides listed in Table 104 were tested in a dose-dependent study for antisense inhibition of SRB-1 in mice.

Table 104

Modified ASOs targeting SRB-1

ISIS No.	Sequences (5' to 3')	$GalNAc_3$ Cluster	CM	SEQ ID No
440762	$T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{k}$	n/a	n/a	823
666905	**$GalNAc_3$-3_a-$_o$**,$T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{k}$	$GalNAc_3$-3_a	PO	823
699782	**$GalNAc_3$-7_a-$_o$**,$T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{k}$	$GalNAc_3$-7_a	PO	823
699783	**$GalNAc_3$-3_a-$_o$**,$T_{ls}{}^{m}C_{ls}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ls}{}^{m}C_{l}$	$GalNAc_3$-3_a	PO	823
653621	$T_{ls}{}^{m}C_{ls}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ls}{}^{m}C_{lo}$**$A_{do}$**,-**$GalNAc_3$-$1_a$**	$GalNAc_3$-1_a	A_d	824
439879	$T_{gs}{}^{m}C_{gs}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{d}\ G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{gs}{}^{m}C_{g}$	n/a	n/a	823
699789	**$GalNAc_3$-3_a-$_o$**,$T_{gs}{}^{m}C_{gs}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{d}\ G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{gs}{}^{m}C_{g}$	$GalNAc_3$-3_a	PO	823

Subscript "g" indicates a fluoro-HNA nucleoside, subscript "l" indicates a locked nucleoside comprising a 2'-O-CH_2-4' bridge. See the Example 74 table legend for other abbreviations. The structure of $GalNAc_3$-1_a was shown previously in Example 9, the structure of $GalNAc_3$-3_a was shown previously in Example 39, and the structure of $GalNAc_3$-7a was shown previously in Example 48.

Treatment

The study was completed using the protocol described in Example 93. Results are shown in Table 105 below and show that oligonucleotides comprising a GalNAc conjugate and various bicyclic nucleoside modifications were significantly more potent than the parent oligonucleotide lacking a conjugate and comprising bicyclic nucleoside modifications. Furthermore, the oligonucleotide comprising a GalNAc conjugate and fluoro-HNA modifications was significantly more potent than the parent lacking a conjugate and comprising fluoro-HNA modifications. The results of the body weights, liver transaminases, total bilirubin, and BUN measurements indicated that the compounds were all well tolerated.

Table 105

SRB-1 mRNA, ALT, AST, BUN, and total bilirubin levels and body weights

ISIS No.	Dosage (mg/kg)	SRB-1 mRNA (% PBS)
PBS	n/a	100
440762	1	104
	3	65
	10	35
666905	0.1	105
	0.3	56
	1	18
699782	0.1	93
	0.3	63

	1	15
699783	0.1	105
	0.3	53
	1	12
653621	0.1	109
	0.3	82
	1	27
439879	1	96
	3	77
	10	37
699789	0.1	82
	0.3	69
	1	26

Example 96: Plasma protein binding of antisense oligonucleotides comprising a GalNAc$_3$ conjugate group

Oligonucleotides listed in Table 70 targeting ApoC-III and oligonucleotides in Table 106 targeting Apo(a) were tested in an ultra-filtration assay in order to assess plasma protein binding.

Table 106

Modified oligonucleotides targeting Apo(a)

ISIS No.	Sequences (5' to 3')	GalNAc$_3$ Cluster	CM	SEQ ID No
494372	$T_{es}G_{es}{}^{m}C_{es}T_{es}{}^{m}C_{es}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{es}G_{es}T_{es}T_{es}{}^{m}C_{e}$	n/a	n/a	847
693401	$T_{es}G_{eo}{}^{m}C_{eo}T_{eo}{}^{m}C_{eo}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{eo}G_{eo}T_{es}T_{es}{}^{m}C_{e}$	n/a	n/a	847
681251	**GalNAc$_3$-7$_{a}$-$_{o}$**$T_{es}G_{es}{}^{m}C_{es}T_{es}{}^{m}C_{es}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{es}G_{es}T_{es}T_{es}{}^{m}C_{e}$	GalNAc$_3$-7$_a$	PO	847
681257	**GalNAc$_3$-7$_{a}$-$_{o}$**$T_{es}G_{eo}{}^{m}C_{eo}T_{eo}{}^{m}C_{eo}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{eo}G_{eo}T_{es}T_{es}{}^{m}C_{e}$	GalNAc$_3$-7$_a$	PO	847

See the Example 74 for table legend. The structure of GalNAc$_3$-7a was shown previously in Example 48.

Ultrafree-MC ultrafiltration units (30,000 NMWL, low-binding regenerated cellulose membrane, Millipore, Bedford, MA) were pre-conditioned with 300 µL of 0.5% Tween 80 and centrifuged at 2000 g for 10 minutes, then with 300µL of a 300 µg/mL solution of a control oligonucleotide in H_2O and centrifuged at 2000 g for 16 minutes. In order to assess non-specific binding to the filters of each test oligonucleotide from Tables 70 and 106 to be used in the studies, 300 µL of a 250 ng/mL solution of oligonucleotide in H_2O at pH 7.4 was placed in the pre-conditioned filters and centrifuged at 2000 g for 16 minutes. The unfiltered and filtered samples

were analyzed by an ELISA assay to determine the oligonucleotide concentrations. Three replicates were used to obtain an average concentration for each sample. The average concentration of the filtered sample relative to the unfiltered sample is used to determine the percent of oligonucleotide that is recovered through the filter in the absence of plasma (% recovery).

Frozen whole plasma samples collected in K3-EDTA from normal, drug-free human volunteers, cynomolgus monkeys, and CD-1 mice, were purchased from Bioreclamation LLC (Westbury, NY). The test oligonucleotides were added to 1.2 mL aliquots of plasma at two concentrations (5 and 150 μg/mL). An aliquot (300 μL) of each spiked plasma sample was placed in a pre-conditioned filter unit and incubated at 37°C for 30 minutes, immediately followed by centrifugation at 2000 g for 16 minutes. Aliquots of filtered and unfiltered spiked plasma samples were analyzed by an ELISA to determine the oligonucleotide concentration in each sample. Three replicates per concentration were used to determine the average percentage of bound and unbound oligonucleotide in each sample. The average concentration of the filtered sample relative to the concentration of the unfiltered sample is used to determine the percent of oligonucleotide in the plasma that is not bound to plasma proteins (% unbound). The final unbound oligonucleotide values are corrected for non-specific binding by dividing the % unbound by the % recovery for each oligonucleotide. The final % bound oligonucleotide values are determined by subtracting the final % unbound values from 100. The results are shown in Table 107 for the two concentrations of oligonucleotide tested (5 and 150 μg/mL) in each species of plasma. The results show that GalNAc conjugate groups do not have a significant impact on plasma protein binding. Furthermore, oligonucleotides with full PS internucleoside linkages and mixed PO/PS linkages both bind plasma proteins, and those with full PS linkages bind plasma proteins to a somewhat greater extent than those with mixed PO/PS linkages.

Table 107

Percent of modified oligonucleotide bound to plasma proteins

ISIS No.	Human plasma		Monkey plasma		Mouse plasma	
	5 μg/mL	150 μg/mL	5 μg/mL	150 μg/mL	5 μg/mL	150 μg/mL
304801	99.2	98.0	99.8	99.5	98.1	97.2
663083	97.8	90.9	99.3	99.3	96.5	93.0
674450	96.2	97.0	98.6	94.4	94.6	89.3
494372	94.1	89.3	98.9	97.5	97.2	93.6
693401	93.6	89.9	96.7	92.0	94.6	90.2
681251	95.4	93.9	99.1	98.2	97.8	96.1
681257	93.4	90.5	97.6	93.7	95.6	92.7

Example 97: Modified oligonucleotides targeting TTR comprising a GalNAc$_3$ conjugate group

The oligonucleotides shown in Table 108 comprising a GalNAc conjugate were designed to target TTR.

Table 108

Modified oligonucleotides targeting TTR

ISIS No.	Sequences (5' to 3')	GalNAc$_3$ Cluster	CM	SEQ ID No
666941	**GalNAc$_3$-3$_{a\text{-}o}$,A$_{do}$** T_{es} $^{m}C_{es}$ T_{es} T_{es} G_{es} G_{ds} T_{ds} T_{ds} A_{ds} $^{m}C_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} A_{ds} A_{es} T_{es} $^{m}C_{es}$ $^{m}C_{es}$ $^{m}C_{e}$	GalNAc$_3$-3	A$_d$	846
666942	T_{es} $^{m}C_{eo}$ T_{eo} T_{eo} G_{eo} G_{ds} T_{ds} T_{ds} A_{ds} $^{m}C_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} A_{ds} A_{eo} T_{eo} $^{m}C_{es}$ $^{m}C_{es}$ $^{m}C_{eo}$ **A$_{do}$-GalNAc$_3$-3$_a$**	GalNAc$_3$-1	A$_d$	843
682876	**GalNAc$_3$-3$_{a\text{-}o}$**,T_{es} $^{m}C_{es}$ T_{es} T_{es} G_{es} G_{ds} T_{ds} T_{ds} A_{ds} $^{m}C_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} A_{ds} A_{es} T_{es} $^{m}C_{es}$ $^{m}C_{es}$ $^{m}C_{e}$	GalNAc$_3$-3	PO	842
682877	**GalNAc$_3$-7$_{a\text{-}o}$**,T_{es} $^{m}C_{es}$ T_{es} T_{es} G_{es} G_{ds} T_{ds} T_{ds} A_{ds} $^{m}C_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} A_{ds} A_{es} T_{es} $^{m}C_{es}$ $^{m}C_{es}$ $^{m}C_{e}$	GalNAc$_3$-7	PO	842
682878	**GalNAc$_3$-10$_{a\text{-}o}$**,T_{es} $^{m}C_{es}$ T_{es} T_{es} G_{es} G_{ds} T_{ds} T_{ds} A_{ds} $^{m}C_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} A_{ds} A_{es} T_{es} $^{m}C_{es}$ $^{m}C_{es}$ $^{m}C_{e}$	GalNAc$_3$-10	PO	842
682879	**GalNAc$_3$-13$_{a\text{-}o}$**,T_{es} $^{m}C_{es}$ T_{es} T_{es} G_{es} G_{ds} T_{ds} T_{ds} A_{ds} $^{m}C_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} A_{ds} A_{es} T_{es} $^{m}C_{es}$ $^{m}C_{es}$ $^{m}C_{e}$	GalNAc$_3$-13	PO	842
682880	**GalNAc$_3$-7$_{a\text{-}o}$,A$_{do}$** T_{es} $^{m}C_{es}$ T_{es} T_{es} G_{es} G_{ds} T_{ds} T_{ds} A_{ds} $^{m}C_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} A_{ds} A_{es} T_{es} $^{m}C_{es}$ $^{m}C_{es}$ $^{m}C_{e}$	GalNAc$_3$-7	A$_d$	846
682881	**GalNAc$_3$-10$_{a\text{-}o}$,A$_{do}$** T_{es} $^{m}C_{es}$ T_{es} T_{es} G_{es} G_{ds} T_{ds} T_{ds} A_{ds} $^{m}C_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} A_{ds} A_{es} T_{es} $^{m}C_{es}$ $^{m}C_{es}$ $^{m}C_{e}$	GalNAc$_3$-10	A$_d$	846
682882	**GalNAc$_3$-13$_{a\text{-}o}$,A$_{do}$** T_{es} $^{m}C_{es}$ T_{es} T_{es} G_{es} G_{ds} T_{ds} T_{ds} A_{ds} $^{m}C_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} A_{ds} A_{es} T_{es} $^{m}C_{es}$ $^{m}C_{es}$ $^{m}C_{e}$	GalNAc$_3$-13	A$_d$	846
684056	T_{es} $^{m}C_{es}$ T_{es} T_{es} G_{es} G_{ds} T_{ds} T_{ds} A_{ds} $^{m}C_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} A_{ds} A_{es} T_{es} $^{m}C_{es}$ $^{m}C_{es}$ $^{m}C_{eo}$ **A$_{do}$-GalNAc$_3$-19$_a$**	GalNAc$_3$-19	A$_d$	846

The legend for Table 108 can be found in Example 74. The structure of GalNAc$_3$-1 was shown in Example 9. The structure of GalNAc$_3$-3$_a$ was shown in Example 39. The structure of GalNAc$_3$-7$_a$ was shown in Example 48. The structure of GalNAc$_3$-10$_a$ was shown in Example 46. The structure of GalNAc$_3$-13$_a$ was shown in Example 62. The structure of GalNAc$_3$-19$_a$ was shown in Example 70.

Example 98: Evaluation of pro-inflammatory effects of oligonucleotides comprising a GalNAc conjugate in hPMBC assay

The oligonucleotides listed in Table 109 and were tested for pro-inflammatory effects in an hPMBC assay as described in Examples 23 and 24. (See Tables 30, 83, 95, and 108 for descriptions of the oligonucleotides.) ISIS 353512 is a high responder used as a positive control, and the other

oligonucleotides are described in Tables 83, 95, and 108. The results shown in Table 109 were obtained using blood from one volunteer donor. The results show that the oligonucleotides comprising mixed PO/PS internucleoside linkages produced significantly lower pro-inflammatory responses compared to the same oligonucleotides having full PS linkages. Furthermore, the GalNAc conjugate group did not have a significant effect in this assay.

Table 109

ISIS No.	E_{max}/EC_{50}	$GalNAc_3$ cluster	Linkages	CM
353512	3630	n/a	PS	n/a
420915	802	n/a	PS	n/a
682881	1311	$GalNAc_3$-10	PS	A_d
682888	0.26	$GalNAc_3$-10	PO/PS	A_d
684057	1.03	$GalNAc_3$-19	PO/PS	A_d

Example 99: Binding affinities of oligonucleotides comprising a GalNAc conjugate for the asialoglycoprotein receptor

The binding affinities of the oligonucleotides listed in Table 110 (see Table 76 for descriptions of the oligonucleotides) for the asialoglycoprotein receptor were tested in a competitive receptor binding assay. The competitor ligand, α1-acid glycoprotein (AGP), was incubated in 50 mM sodium acetate buffer (pH 5) with 1 U neuraminidase-agarose for 16 hours at 37°C, and > 90% desialylation was confirmed by either sialic acid assay or size exclusion chromatography (SEC). Iodine monochloride was used to iodinate the AGP according to the procedure by Atsma et al. (*see* J Lipid Res. 1991 Jan; 32(1):173-81.) In this method, desialylated α1-acid glycoprotein (de-AGP) was added to 10 mM iodine chloride, $Na^{125}I$, and 1 M glycine in 0.25 M NaOH. After incubation for 10 minutes at room temperature, ^{125}I -labeled de-AGP was separated from free ^{125}I by concentrating the mixture twice utilizing a 3 KDMWCO spin column. The protein was tested for labeling efficiency and purity on a HPLC system equipped with an Agilent SEC-3 column (7.8x300mm) and a ß-RAM counter. Competition experiments utilizing ^{125}I -labeled de-AGP and various GalNAc-cluster containing ASOs were performed as follows. Human HepG2 cells (10^6 cells/ml) were plated on 6-well plates in 2 ml of appropriate growth media. MEM media supplemented with 10% fetal bovine serum (FBS), 2 mM L-Glutamine and 10mM HEPES was used. Cells were incubated 16-20 hours @ 37°C with 5% and 10% CO_2 respectively. Cells were washed with media without FBS prior to the experiment. Cells were incubated for 30 min @37°C with 1ml competition mix containing appropriate growth media with 2% FBS, 10^{-8} M ^{125}I -labeled de-AGP and GalNAc-cluster containing ASOs at concentrations ranging from 10^{-11} to 10^{-5} M. Non-specific binding was determined in the presence of 10^{-2} M GalNAc sugar. Cells were washed twice with media without FBS to remove unbound ^{125}I -labeled de-AGP and competitor GalNAc ASO. Cells were lysed using Qiagen's RLT buffer containing 1% ß-mercaptoethanol. Lysates were transferred to round bottom

assay tubes after a brief 10 min freeze/thaw cycle and assayed on a ɣ-counter. Non-specific binding was subtracted before dividing ^{125}I protein counts by the value of the lowest GalNAc-ASO concentration counts. The inhibition curves were fitted according to a single site competition binding equation using a nonlinear regression algorithm to calculate the binding affinities (K_D's).

The results in Table 110 were obtained from experiments performed on five different days. Results for oligonucleotides marked with superscript "a" are the average of experiments run on two different days. The results show that the oligonucleotides comprising a GalNAc conjugate group on the 5'-end bound the asialoglycoprotein receptor on human HepG2 cells with 1.5 to 16-fold greater affinity than the oligonucleotides comprising a GalNAc conjugate group on the 3'-end.

Table 110
Asialoglycoprotein receptor binding assay results

ISIS No.	GalNAc conjugate	Oligonucleotide end to which GalNAc conjugate is attached	K_D (nM)
661161[a]	$GalNAc_3$-3	5'	3.7
666881[a]	$GalNAc_3$-10	5'	7.6
666981	$GalNAc_3$-7	5'	6.0
670061	$GalNAc_3$-13	5'	7.4
655861[a]	$GalNAc_3$-1	3'	11.6
677841[a]	$GalNAc_3$-19	3'	60.8

Example 100: Antisense inhibition *in vivo* by oligonucleotides comprising a GalNAc conjugate group targeting Apo(a) *in vivo*

The oligonucleotides listed in Table 111a below were tested in a single dose study for duration of action in mice.

Table 111a
Modified ASOs targeting APO(a)

ISIS No.	Sequences (5' to 3')	$GalNAc_3$ Cluster	CM	SEQ ID No.
681251	**$GalNAc_3$-7_{a}-$_{o}$'**$T_{es}G_{es}{}^{m}C_{es}T_{es}{}^{m}C_{es}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}$ $T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{es}G_{es}$ $T_{es}T_{es}{}^{m}C_{e}$	$GalNAc_3$-7a	PO	847
681257	**$GalNAc_3$-7_{a}-$_{o}$'**$T_{es}G_{eo}{}^{m}C_{eo}T_{eo}{}^{m}C_{eo}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}$ $T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{eo}G_{eo}$ $T_{es}T_{es}{}^{m}C_{e}$	$GalNAc_3$-7a	PO	847

The structure of $GalNAc_3$-7_a was shown in Example 48.

Treatment

Female transgenic mice that express human Apo(a) were each injected subcutaneously once per week, for a total of 6 doses, with an oligonucleotide and dosage listed in Table 111b or with PBS. Each treatment group consisted of 3 animals. Blood was drawn the day before dosing to determine baseline levels of Apo(a) protein in plasma and at 72 hours, 1 week, and 2 weeks following the first dose. Additional blood draws will occur at 3 weeks, 4 weeks, 5 weeks, and 6 weeks following the first dose. Plasma Apo(a) protein levels were measured using an ELISA. The results in Table 111b are presented as the average percent of plasma Apo(a) protein levels for each treatment group, normalized to baseline levels (% BL), The results show that the oligonucleotides comprising a GalNAc conjugate group exhibited potent reduction in Apo(a) expression. This potent effect was observed for the oligonucleotide that comprises full PS internucleoside linkages and the oligonucleotide that comprises mixed PO and PS linkages.

Table 111b
Apo(a) plasma protein levels

ISIS No.	Dosage (mg/kg)	Apo(a) at 72 hours (% BL)	Apo(a) at 1 week (% BL)	Apo(a) at 3 weeks (% BL)
PBS	n/a	116	104	107
681251	0.3	97	108	93
	1.0	85	77	57
	3.0	54	49	11
	10.0	23	15	4
681257	0.3	114	138	104
	1.0	91	98	54
	3.0	69	40	6
	10.0	30	21	4

Example 101: Antisense inhibition by oligonucleotides comprising a GalNAc cluster linked via a stable moiety

The oligonucleotides listed in Table 112 were tested for inhibition of mouse APOC-III expression *in vivo*. C57Bl/6 mice were each injected subcutaneously once with an oligonucleotide listed in Table 112 or with PBS. Each treatment group consisted of 4 animals. Each mouse treated with ISIS 440670 received a dose of 2, 6, 20, or 60 mg/kg. Each mouse treated with ISIS 680772 or 696847 received 0.6, 2, 6, or 20 mg/kg. The GalNAc conjugate group of ISIS 696847 is linked via a stable moiety, a phosphorothioate linkage instead of a readily cleavable phosphodiester containing linkage. The animals were sacrificed 72 hours after the dose. Liver APOC-III mRNA levels were

measured using real-time PCR. APOC-III mRNA levels were normalized to cyclophilin mRNA levels according to standard protocols. The results are presented in Table 112 as the average percent of APOC-III mRNA levels for each treatment group relative to the saline control group. The results show that the oligonucleotides comprising a GalNAc conjugate group were significantly more potent than the oligonucleotide lacking a conjugate group. Furthermore, the oligonucleotide comprising a GalNAc conjugate group linked to the oligonucleotide via a cleavable moiety (ISIS 680772) was even more potent than the oligonucleotide comprising a GalNAc conjugate group linked to the oligonucleotide via a stable moiety (ISIS 696847).

Table 112

Modified oligonucleotides targeting mouse APOC-III

ISIS No.	Sequences (5' to 3')	CM	Dosage (mg/kg)	APOC-III mRNA (% PBS)	SEQ ID No.
440670	$^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$	n/a	2	92	849
			6	86	
			20	59	
			60	37	
680772	**GalNAc$_3$-7$_{a-o}$**,$^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$	PO	0.6	79	849
			2	58	
			6	31	
			20	13	
696847	**GalNAc$_3$-7$_{a-s}$**,$^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$	n/a (PS)	0.6	83	849
			2	73	
			6	40	
			20	28	

The structure of GalNAc$_3$-7$_a$ was shown in Example 48.

Example 102: Distribution in liver of antisense oligonucleotides comprising a GalNAc conjugate

The liver distribution of ISIS 353382 (see Table 36) that does not comprise a GalNAc conjugate and ISIS 655861 (see Table 36) that does comprise a GalNAc conjugate was evaluated. Male balb/c mice were subcutaneously injected once with ISIS 353382 or 655861 at a dosage listed in Table 113. Each treatment group consisted of 3 animals except for the 18 mg/kg group for ISIS 655861, which consisted of 2 animals. The animals were sacrificed 48 hours following the dose to determine the liver distribution of the oligonucleotides. In order to measure the number of antisense oligonucleotide molecules per cell, a Ruthenium (II) tris-bipyridine tag (MSD TAG, Meso Scale Discovery) was conjugated to an oligonucleotide probe used to detect the antisense oligonucleotides.

The results presented in Table 113 are the average concentrations of oligonucleotide for each treatment group in units of millions of oligonucleotide molecules per cell. The results show that at equivalent doses, the oligonucleotide comprising a GalNAc conjugate was present at higher concentrations in the total liver and in hepatocytes than the oligonucleotide that does not comprise a GalNAc conjugate. Furthermore, the oligonucleotide comprising a GalNAc conjugate was present at lower concentrations in non-parenchymal liver cells than the oligonucleotide that does not comprise a GalNAc conjugate. And while the concentrations of ISIS 655861 in hepatocytes and non-parenchymal liver cells were similar per cell, the liver is approximately 80% hepatocytes by volume. Thus, the majority of the ISIS 655861 oligonucleotide that was present in the liver was found in hepatocytes, whereas the majority of the ISIS 353382 oligonucleotide that was present in the liver was found in non-parenchymal liver cells.

Table 113

ISIS No.	Dosage (mg/kg)	Concentration in whole liver (molecules*10^6 per cell)	Concentration in hepatocytes (molecules*10^6 per cell)	Concentration in non-parenchymal liver cells (molecules*10^6 per cell)
353382	3	9.7	1.2	37.2
	10	17.3	4.5	34.0
	20	23.6	6.6	65.6
	30	29.1	11.7	80.0
	60	73.4	14.8	98.0
	90	89.6	18.5	119.9
655861	0.5	2.6	2.9	3.2
	1	6.2	7.0	8.8
	3	19.1	25.1	28.5
	6	44.1	48.7	55.0
	18	76.6	82.3	77.1

Example 103: Duration of action *in vivo* of oligonucleotides targeting APOC-III comprising a $GalNAc_3$ conjugate

The oligonucleotides listed in Table 114 below were tested in a single dose study for duration of action in mice.

Table 114

Modified ASOs targeting APOC-III

ISIS No.	Sequences (5' to 3')	$GalNAc_3$ Cluster	CM	SEQ ID No.
304801	$A_{es}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{es}T_{es}$ $T_{es}A_{es}T_{e}$	n/a	n/a	821
663084	**$GalNAc_3$-3_a-$_o$'A_{do}**$A_{es}G_{eo}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}$ $^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{eo}T_{eo}T_{es}A_{es}T_{e}$	$GalNAc_3$-3a	A_d	837
679241	$A_{es}G_{eo}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{eo}T_{eo}$ $T_{es}A_{es}T_{eo}$**A_{do}'-$GalNAc_3$-19_a**	$GalNAc_3$-19a	A_d	822

The structure of $GalNAc_3$-3_a was shown in Example 39, and $GalNAc_3$-19_a was shown in Example 70.

Treatment

Female transgenic mice that express human APOC-III were each injected subcutaneously once with an oligonucleotide listed in Table 114 or with PBS. Each treatment group consisted of 3 animals. Blood was drawn before dosing to determine baseline and at 3, 7, 14, 21, 28, 35, and 42 days following the dose. Plasma triglyceride and APOC-III protein levels were measured as described in Example 20. The results in Table 115 are presented as the average percent of plasma triglyceride and APOC-III levels for each treatment group, normalized to baseline levels. A comparison of the results in Table 71 of example 79 with the results in Table 115 below show that oligonucleotides comprising a mixture of phosphodiester and phosphorothioate internucleoside linkages exhibited increased duration of action than equivalent oligonucleotides comprising only phosphorothioate internucleoside linkages.

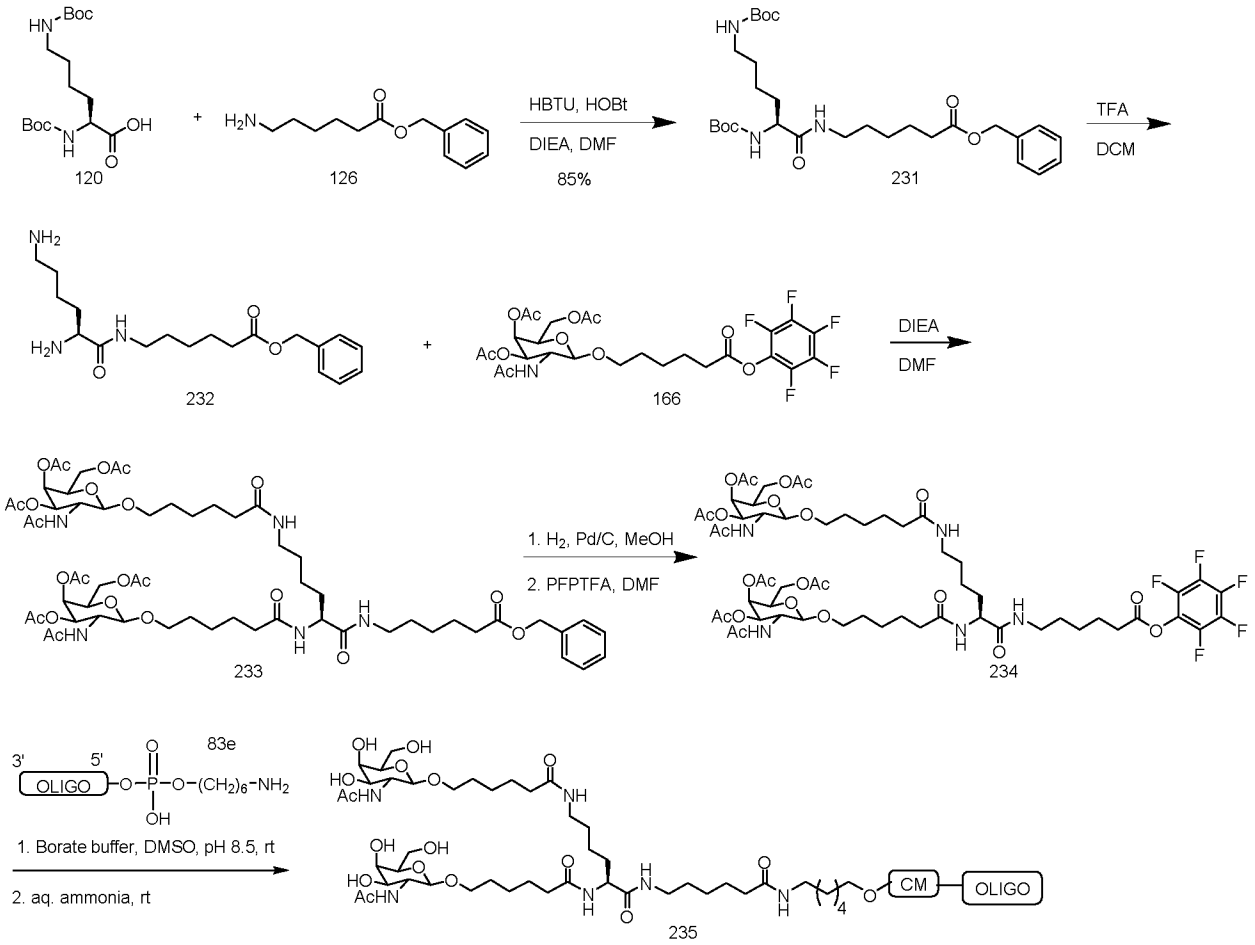
Table 115

Plasma triglyceride and APOC-III protein levels in transgenic mice

ISIS No.	Dosage (mg/kg)	Time point (days post-dose)	Triglycerides (% baseline)	APOC-III protein (% baseline)	$GalNAc_3$ Cluster	CM
PBS	n/a	3	96	101	n/a	n/a
		7	88	98		
		14	91	103		
		21	69	92		
		28	83	81		
		35	65	86		
		42	72	88		

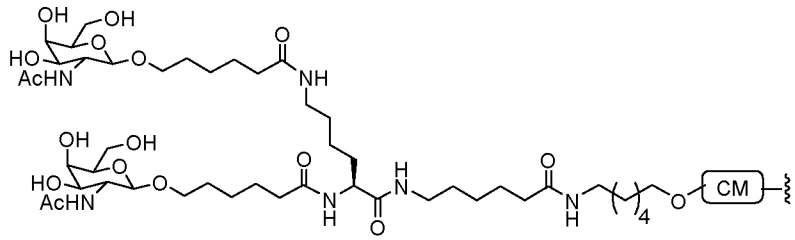
304801	30	3	42	46	n/a	n/a
		7	42	51		
		14	59	69		
		21	67	81		
		28	79	76		
		35	72	95		
		42	82	92		
663084	10	3	35	28	$GalNAc_3$-3a	A_d
		7	23	24		
		14	23	26		
		21	23	29		
		28	30	22		
		35	32	36		
		42	37	47		
679241	10	3	38	30	$GalNAc_3$-19a	A_d
		7	31	28		
		14	30	22		
		21	36	34		
		28	48	34		
		35	50	45		
		42	72	64		

Example 104: Synthesis of oligonucleotides comprising a 5'-GalNAc$_2$ conjugate

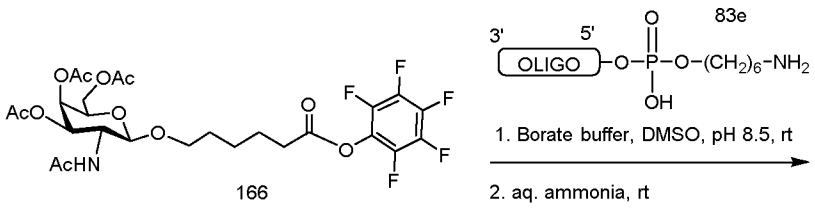


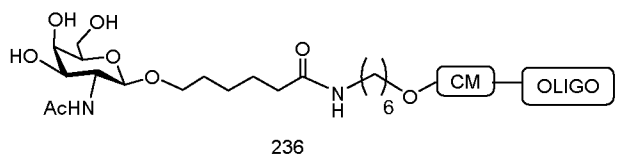
Compound 120 is commercially available, and the synthesis of compound 126 is described in Example 49. Compound 120 (1 g, 2.89 mmol), HBTU (0.39 g, 2.89 mmol), and HOBt (1.64 g, 4.33 mmol) were dissolved in DMF (10 mL. and *N*,*N*-diisopropylethylamine (1.75 mL, 10.1 mmol) were added. After about 5 min, aminohexanoic acid benzyl ester (1.36 g, 3.46 mmol) was added to the reaction. After 3h, the reaction mixture was poured into 100 mL of 1 M NaHSO4 and extracted with 2 x 50 mL ethyl acetate. Organic layers were combined and washed with 3 x 40 mL sat $NaHCO_3$ and 2 x brine, dried with Na_2SO_4, filtered and concentrated. The product was purified by silica gel column chromatography (DCM:EA:Hex , 1:1:1) to yield compound 231. LCMS and NMR were consistent with the structure. Compounds 231 (1.34 g, 2.438 mmol) was dissolved in dichloromethane (10 mL) and trifluoracetic acid (10 mL) was added. After stirring at room temperature for 2h, the reaction mixture was concentrated under reduced pressure and co-evaporated with toluene (3 x 10 mL). The residue was dried under reduced pressure to yield compound 232 as the trifuloracetate salt. The synthesis of compound 166 is described in Example 54. Compound 166 (3.39 g, 5.40 mmol) was dissolved in DMF (3 mL). A solution of compound 232 (1.3 g, 2.25 mmol)

was dissolved in DMF (3 mL) and *N*,*N*-diisopropylethylamine (1.55 mL) was added. The reaction was stirred at room temperature for 30 minutes, then poured into water (80 mL) and the aqueous layer was extracted with EtOAc (2x100 mL). The organic phase was separated and washed with sat. aqueous $NaHCO_3$ (3 x 80 mL), 1 M $NaHSO_4$ (3 x 80 mL) and brine (2 x 80 mL), then dried (Na_2SO_4), filtered, and concentrated. The residue was purified by silica gel column chromatography to yield compound 233. LCMS and NMR were consistent with the structure. Compound 233 (0.59 g, 0.48 mmol) was dissolved in methanol (2.2 mL) and ethyl acetate (2.2 mL). Palladium on carbon (10 wt% Pd/C, wet , 0.07 g) was added, and the reaction mixture was stirred under hydrogen atmosphere for 3 h. The reaction mixture was filtered through a pad of Celite and concentrated to yield the carboxylic acid. The carboxylic acid (1.32 g, 1.15 mmol, cluster free acid) was dissolved in DMF (3.2 mL). To this *N*,*N*-diisopropylehtylamine (0.3 mL, 1.73 mmol) and PFPTFA (0.30 mL, 1.73 mmol) were added. After 30 min stirring at room temperature the reaction mixture was poured into water (40 mL) and extracted with EtOAc (2 x 50 mL). A standard work-up was completed as described above to yield compound 234. LCMS and NMR were consistent with the structure. Oligonucleotide 235 was prepared using the general procedure described in Example 46. The $GalNAc_2$ cluster portion ($GalNAc_2$-24_a) of the conjugate group $GalNAc_2$-24 can be combined with any cleavable moiety present on the oligonucleotide to provide a variety of conjugate groups. The structure of $GalNAc_2$-24 ($GalNAc_2$-24_a-CM) is shown below:



Example 105: Synthesis of oligonucleotides comprising a $GalNAc_1$-25 conjugate

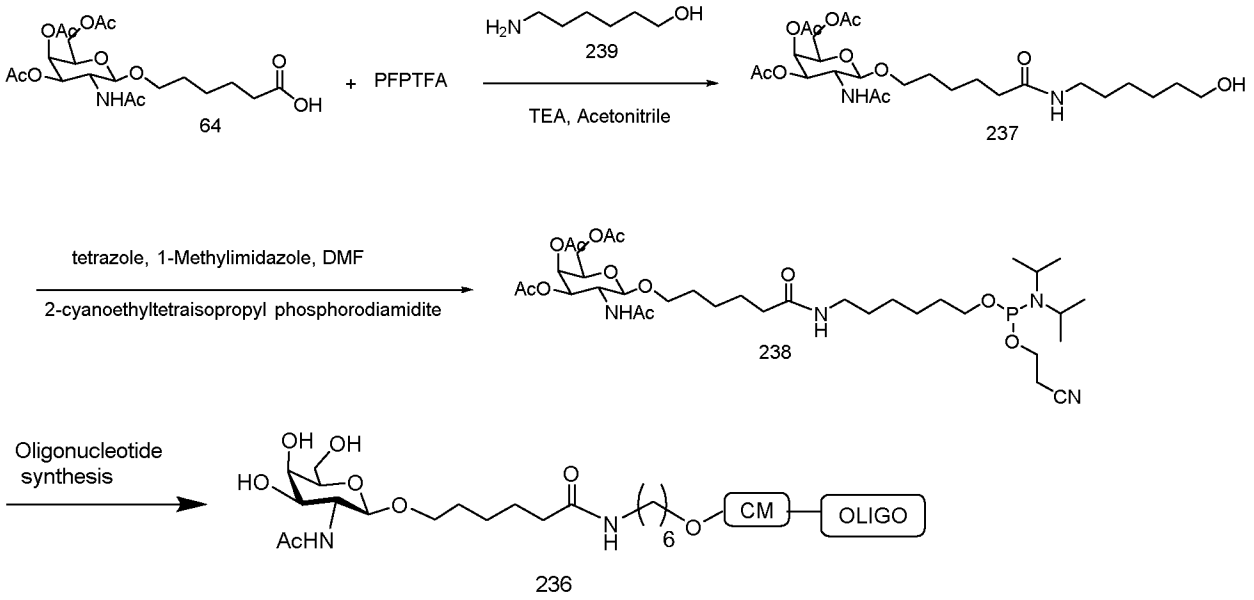




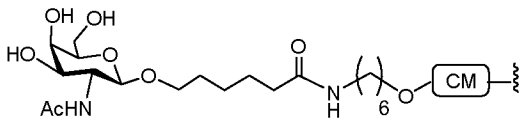
236

The synthesis of compound 166 is described in Example 54. Oligonucleotide 236 was prepared using the general procedure described in Example 46.

Alternatively, oligonucleotide 236 was synthesized using the scheme shown below, and compound 238 was used to form the oligonucleotide 236 using procedures described in Example 10.



The $GalNAc_1$ cluster portion ($GalNAc_1$-25_a) of the conjugate group $GalNAc_1$-25 can be combined with any cleavable moiety present on the oligonucleotide to provide a variety of conjugate groups. The structure of $GalNAc_1$-25 ($GalNAc_1$-25_a-CM) is shown below:



Example 106: Antisense inhibition *in vivo* by oligonucleotides targeting SRB-1 comprising a 5'-$GalNAc_2$ or a 5'-$GalNAc_3$ conjugate

Oligonucleotides listed in Tables 116 and 117 were tested in dose-dependent studies for antisense inhibition of SRB-1 in mice.

Treatment

Six to week old, male C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously once with 2, 7, or 20 mg/kg of ISIS No. 440762; or with 0.2, 0.6, 2, 6, or 20 mg/kg of ISIS No. 686221, 686222, or 708561; or with saline. Each treatment group consisted of 4 animals. The mice were sacrificed 72 hours following the final administration. Liver SRB-1 mRNA

levels were measured using real-time PCR. SRB-1 mRNA levels were normalized to cyclophilin mRNA levels according to standard protocols. The antisense oligonucleotides lowered SRB-1 mRNA levels in a dose-dependent manner, and the ED_{50} results are presented in Tables 116 and 117. Although previous studies showed that trivalent GalNAc-conjugated oligonucleotides were significantly more potent than divalent GalNAc-conjugated oligonucleotides, which were in turn significantly more potent than monovalent GalNAc conjugated oligonucleotides (*see*, e.g., Khorev et al., *Bioorg. & Med. Chem.*, Vol. 16, 5216-5231 (2008)), treatment with antisense oligonucleotides comprising monovalent, divalent, and trivalent GalNAc clusters lowered SRB-1 mRNA levels with similar potencies as shown in Tables 116 and 117.

Table 116

Modified oligonucleotides targeting SRB-1

ISIS No.	Sequences (5' to 3')	GalNAc Cluster	ED_{50} (mg/kg)	SEQ ID No
440762	$T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{k}$	n/a	4.7	823
686221	**$GalNAc_2$-24_a-$_o$,A_{do}**$T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{k}$	$GalNAc_2$-24_a	0.39	827
686222	**$GalNAc_3$-13_a-$_o$,A_{do}**$T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{k}$	$GalNAc_3$-13_a	0.41	827

See Example 93 for table legend. The structure of $GalNAc_3$-13a was shown in Example 62, and the structure of $GalNAc_2$-24a was shown in Example 104.

Table 117

Modified oligonucleotides targeting SRB-1

ISIS No.	Sequences (5' to 3')	GalNAc Cluster	ED_{50} (mg/kg)	SEQ ID No
440762	$T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{k}$	n/a	5	823
708561	**$GalNAc_1$-25_a-$_o$,**$T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{k}$	$GalNAc_1$-25_a	0.4	823

See Example 93 for table legend. The structure of $GalNAc_1$-25a was shown in Example 105.

The concentrations of the oligonucleotides in Tables 116 and 117 in liver were also assessed, using procedures described in Example 75. The results shown in Tables 117a and 117b below are the average total antisense oligonucleotide tissues levels for each treatment group, as measured by UV in units of μg oligonucleotide per gram of liver tissue. The results show that the oligonucleotides comprising a GalNAc conjugate group accumulated in the liver at significantly

higher levels than the same dose of the oligonucleotide lacking a GalNAc conjugate group. Furthermore, the antisense oligonucleotides comprising one, two, or three GalNAc ligands in their respective conjugate groups all accumulated in the liver at similar levels. This result is surprising in view of the Khorev et al. literature reference cited above and is consistent with the activity data shown in Tables 116 and 117 above.

Table 117a

Liver concentrations of oligonucleotides comprising a $GalNAc_2$ or $GalNAc_3$ conjugate group

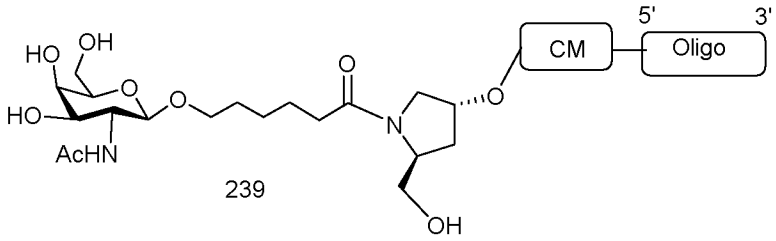
ISIS No.	Dosage (mg/kg)	[Antisense oligonucleotide] (μg/g)	GalNAc cluster	CM
440762	2	2.1	n/a	n/a
	7	13.1		
	20	31.1		
686221	0.2	0.9	$GalNAc_2$-24_a	A_d
	0.6	2.7		
	2	12.0		
	6	26.5		
686222	0.2	0.5	$GalNAc_3$-13_a	A_d
	0.6	1.6		
	2	11.6		
	6	19.8		

Table 117b

Liver concentrations of oligonucleotides comprising a $GalNAc_1$ conjugate group

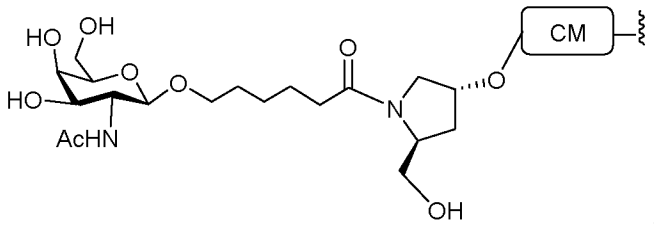
ISIS No.	Dosage (mg/kg)	[Antisense oligonucleotide] (μg/g)	GalNAc cluster	CM
440762	2	2.3	n/a	n/a
	7	8.9		
	20	23.7		
708561	0.2	0.4	$GalNAc_1$-25_a	PO
	0.6	1.1		
	2	5.9		
	6	23.7		
	20	53.9		

Example 107: Synthesis of oligonucleotides comprising a GalNAc$_1$-26 or GalNAc$_1$-27 conjugate



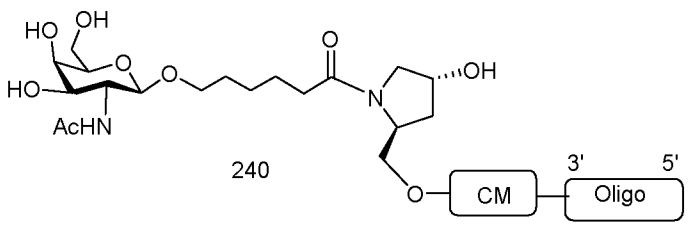
239

Oligonucleotide 239 is synthesized via coupling of compound 47 (see Example 15) to acid 64 (see Example 32) using HBTU and DIEA in DMF. The resulting amide containing compound is phosphitylated, then added to the 5'-end of an oligonucleotide using procedures described in Example 10. The GalNAc$_1$ cluster portion (GalNAc$_1$-26$_a$) of the conjugate group GalNAc$_1$-26 can be combined with any cleavable moiety present on the oligonucleotide to provide a variety of conjugate groups. The structure of GalNAc$_1$-26 (GalNAc$_1$-26$_a$-CM) is shown below:



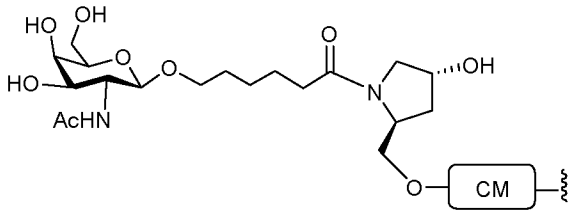
.

In order to add the GalNAc$_1$ conjugate group to the 3'-end of an oligonucleotide, the amide formed from the reaction of compounds 47 and 64 is added to a solid support using procedures described in Example 7. The oligonucleotide synthesis is then completed using procedures described in Example 9 in order to form oligonucleotide 240.



240

The GalNAc$_1$ cluster portion (GalNAc$_1$-27$_a$) of the conjugate group GalNAc$_1$-27 can be combined with any cleavable moiety present on the oligonucleotide to provide a variety of conjugate groups. The structure of GalNAc$_1$-27 (GalNAc$_1$-27$_a$-CM) is shown below:



Example 108: Antisense inhibition *in vivo* by oligonucleotides comprising a GalNAc conjugate group targeting Apo(a) *in vivo*

The oligonucleotides listed in Table 118 below were tested in a single dose study in mice.

Table 118

Modified ASOs targeting APO(a)

ISIS No.	Sequences (5' to 3')	$GalNAc_3$ Cluster	CM	SEQ ID No.
494372	$T_{es}G_{es}{}^{m}C_{es}T_{es}{}^{m}C_{es}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}T_{ds}G_{ds}{}^{m}C_{ds}$ $T_{ds}T_{es}G_{es}T_{es}T_{es}{}^{m}C_{e}$	n/a	n/a	847
681251	**$GalNAc_3$-7_{a}-$_{o}$**,$T_{es}G_{es}{}^{m}C_{es}T_{es}{}^{m}C_{es}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}$ $T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{es}G_{es}T_{es}T_{es}{}^{m}C_{e}$	$GalNAc_3$-7a	PO	847
681255	**$GalNAc_3$-3_{a}-$_{o}$**,$T_{es}G_{eo}{}^{m}C_{eo}T_{eo}{}^{m}C_{eo}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}$ $T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{eo}G_{eo}T_{es}T_{es}{}^{m}C_{e}$	$GalNAc_3$-3a	PO	847
681256	**$GalNAc_3$-10_{a}-$_{o}$**,$T_{es}G_{eo}{}^{m}C_{eo}T_{eo}{}^{m}C_{eo}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}$ $T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{eo}G_{eo}T_{es}T_{es}{}^{m}C_{e}$	$GalNAc_3$-10a	PO	847
681257	**$GalNAc_3$-7_{a}-$_{o}$**,$T_{es}G_{eo}{}^{m}C_{eo}T_{eo}{}^{m}C_{eo}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}$ $T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{eo}G_{eo}T_{es}T_{es}{}^{m}C_{e}$	$GalNAc_3$-7a	PO	847
681258	**$GalNAc_3$-13_{a}-$_{o}$**,$T_{es}G_{eo}{}^{m}C_{eo}T_{eo}{}^{m}C_{eo}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}$ $T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{eo}G_{eo}T_{es}T_{es}{}^{m}C_{e}$	$GalNAc_3$-13a	PO	847
681260	$T_{es}G_{eo}{}^{m}C_{eo}T_{eo}{}^{m}C_{eo}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{eo}G_{eo}$ $T_{es}T_{es}{}^{m}C_{eo}$**A_{do}-$GalNAc_3$-19**	$GalNAc_3$-19a	A_d	854

The structure of $GalNAc_3$-7_a was shown in Example 48.

Treatment

Male transgenic mice that express human Apo(a) were each injected subcutaneously once with an oligonucleotide and dosage listed in Table 119 or with PBS. Each treatment group consisted of 4 animals. Blood was drawn the day before dosing to determine baseline levels of Apo(a) protein in plasma and at 1 week following the first dose. Additional blood draws will occur weekly for approximately 8 weeks. Plasma Apo(a) protein levels were measured using an ELISA. The results in Table 119 are presented as the average percent of plasma Apo(a) protein levels for each treatment group, normalized to baseline levels (% BL), The results show that the antisense oligonucleotides reduced Apo(a) protein expression. Furthermore, the oligonucleotides comprising a GalNAc

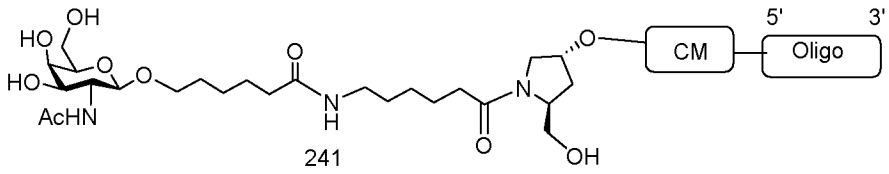
conjugate group exhibited even more potent reduction in Apo(a) expression than the oligonucleotide that does not comprise a conjugate group.

Table 119

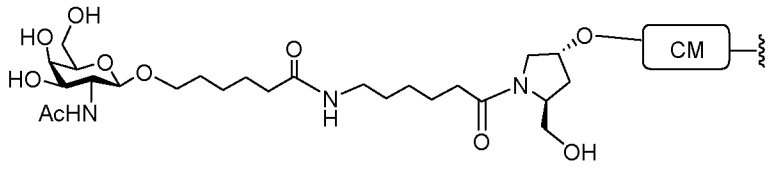
Apo(a) plasma protein levels

ISIS No.	Dosage (mg/kg)	Apo(a) at 1 week (% BL)
PBS	n/a	143
494372	50	58
681251	10	15
681255	10	14
681256	10	17
681257	10	24
681258	10	22
681260	10	26

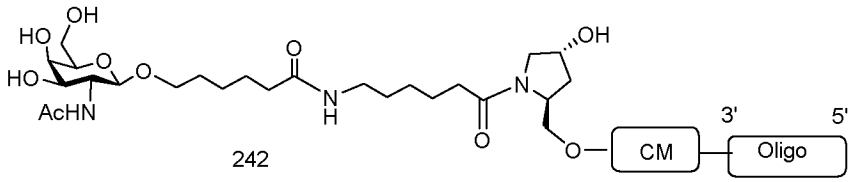
Example 109: Synthesis of oligonucleotides comprising a GalNAc$_1$-28 or GalNAc$_1$-29 conjugate



Oligonucleotide 241 is synthesized using procedures similar to those described in Example 71 to form the phosphoramidite intermediate, followed by procedures described in Example 10 to synthesize the oligonucleotide. The GalNAc$_1$ cluster portion (GalNAc$_1$-28$_a$) of the conjugate group GalNAc$_1$-28 can be combined with any cleavable moiety present on the oligonucleotide to provide a variety of conjugate groups. The structure of GalNAc$_1$-28 (GalNAc$_1$-28$_a$-CM) is shown below:

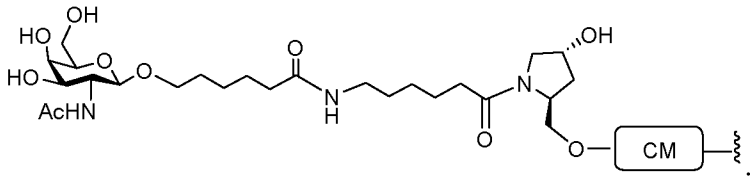


In order to add the $GalNAc_1$ conjugate group to the 3'-end of an oligonucleotide, procedures similar to those described in Example 71 are used to form the hydroxyl intermediate, which is then added to the solid support using procedures described in Example 7. The oligonucleotide synthesis is then completed using procedures described in Example 9 in order to form oligonucleotide 242.

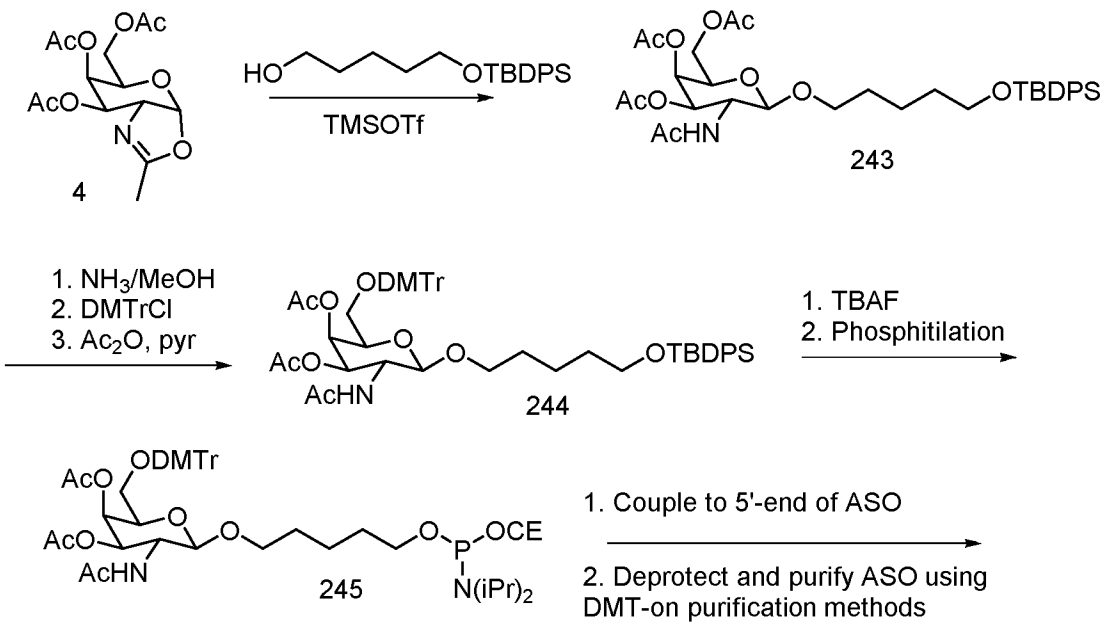


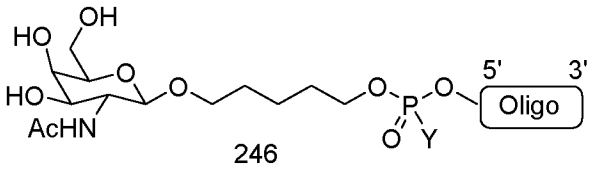
242

The $GalNAc_1$ cluster portion ($GalNAc_1$-29_a) of the conjugate group $GalNAc_1$-29 can be combined with any cleavable moiety present on the oligonucleotide to provide a variety of conjugate groups. The structure of $GalNAc_1$-29 ($GalNAc_1$-29_a-CM) is shown below:

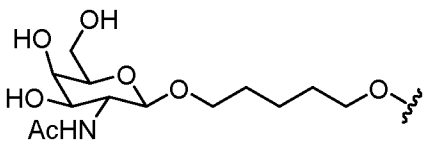


Example 110: Synthesis of oligonucleotides comprising a GalNAc$_1$-30 conjugate

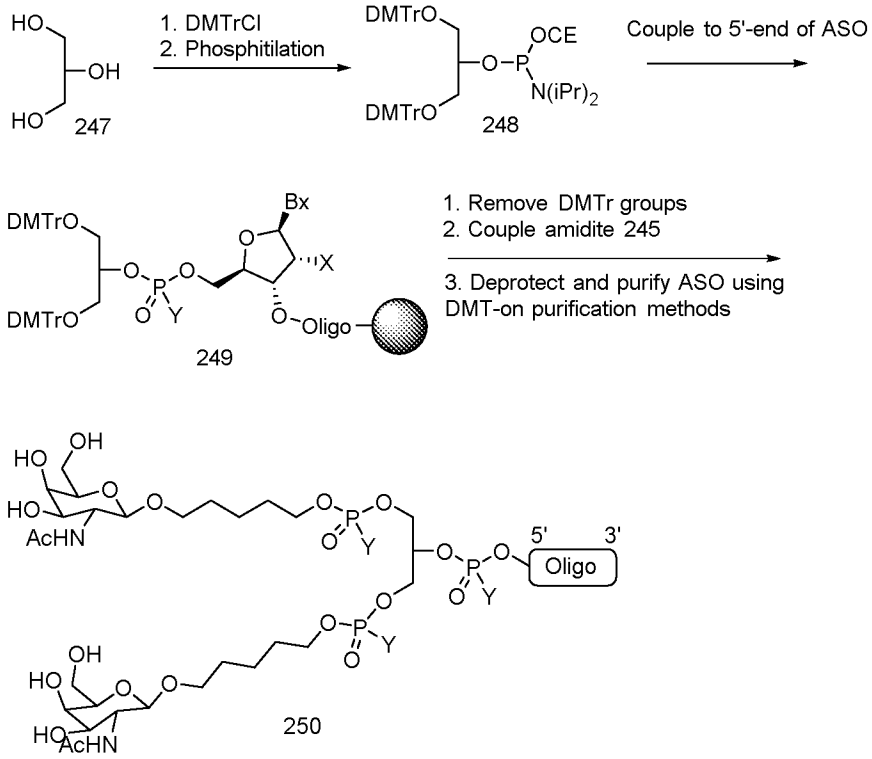




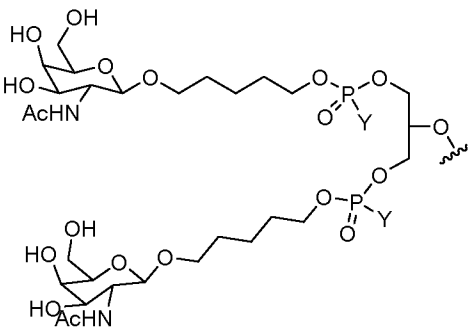
Oligonucleotide 246 comprising a GalNAc$_1$-30 conjugate group, wherein Y is selected from O, S, a substituted or unsubstituted C_1-C_{10} alkyl, amino, substituted amino, azido, alkenyl or alkynyl, is synthesized as shown above. The GalNAc$_1$ cluster portion (GalNAc$_1$-30$_a$) of the conjugate group GalNAc$_1$-30 can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, Y is part of the cleavable moiety. In certain embodiments, Y is part of a stable moiety, and the cleavable moiety is present on the oligonucleotide. The structure of GalNAc$_1$-30$_a$ is shown below:



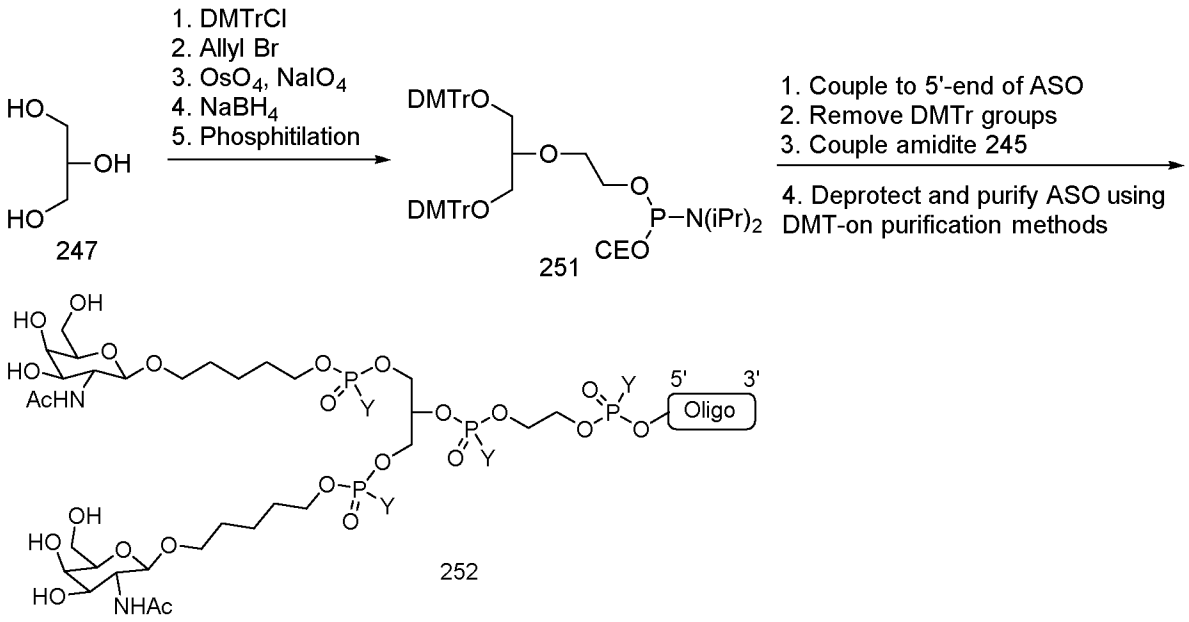
Example 111: Synthesis of oligonucleotides comprising a GalNAc$_2$-31 or GalNAc$_2$-32 conjugate



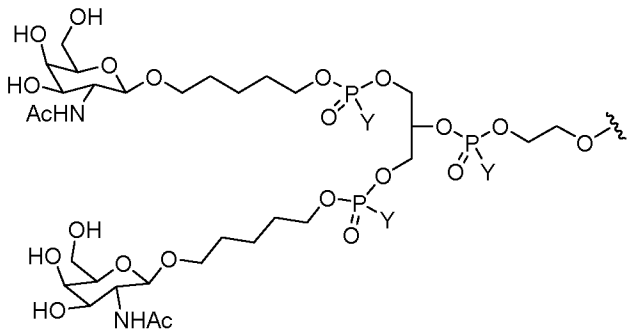
Oligonucleotide 250 comprising a $GalNAc_2$-31 conjugate group, wherein Y is selected from O, S, a substituted or unsubstituted C_1-C_{10} alkyl, amino, substituted amino, azido, alkenyl or alkynyl, is synthesized as shown above. The $GalNAc_2$ cluster portion ($GalNAc_2$-31_a) of the conjugate group $GalNAc_2$-31 can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the Y-containing group directly adjacent to the 5'-end of the oligonucleotide is part of the cleavable moiety. In certain embodiments, the Y-containing group directly adjacent to the 5'-end of the oligonucleotide is part of a stable moiety, and the cleavable moiety is present on the oligonucleotide. The structure of $GalNAc_2$-31_a is shown below:



The synthesis of an oligonucleotide comprising a GalNAc$_2$-32 conjugate is shown below.



Oligonucleotide 252 comprising a GalNAc$_2$-32 conjugate group, wherein Y is selected from O, S, a substituted or unsubstituted C_1-C_{10} alkyl, amino, substituted amino, azido, alkenyl or alkynyl, is synthesized as shown above. The GalNAc$_2$ cluster portion (GalNAc$_2$-32$_a$) of the conjugate group GalNAc$_2$-32 can be combined with any cleavable moiety to provide a variety of conjugate groups. In certain embodiments, the Y-containing group directly adjacent to the 5'-end of the oligonucleotide is part of the cleavable moiety. In certain embodiments, the Y-containing group directly adjacent to the 5'-end of the oligonucleotide is part of a stable moiety, and the cleavable moiety is present on the oligonucleotide. The structure of GalNAc$_2$-32$_a$ is shown below:



Example 112: Modified oligonucleotides comprising a GalNAc$_1$ conjugate

The oligonucleotides in Table 120 targeting SRB-1 were synthesized with a GalNAc$_1$ conjugate group in order to further test the potency of oligonucleotides comprising conjugate groups that contain one GalNAc ligand.

Table 120

ISIS No.	Sequence (5' to 3')	GalNAc cluster	CM	SEQ ID NO.
711461	**$GalNAc_1$-$25_{a\text{-}o}$,A_{do}** G_{es} $^mC_{es}$ T_{es} T_{es} $^mC_{es}$ A_{ds} G_{ds} T_{ds} $^mC_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} $^mC_{ds}$ T_{ds} T_{es} $^mC_{es}$ $^mC_{es}$ T_{es} T_e	$GalNAc_1$-25_a	A_d	831
711462	**$GalNAc_1$-$25_{a\text{-}o}$,**G_{es} $^mC_{es}$ T_{es} T_{es} $^mC_{es}$ A_{ds} G_{ds} T_{ds} $^mC_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} $^mC_{ds}$ T_{ds} T_{es} $^mC_{es}$ $^mC_{es}$ T_{es} T_e	$GalNAc_1$-25_a	PO	829
711463	**$GalNAc_1$-$25_{a\text{-}o}$,**G_{es} $^mC_{eo}$ T_{eo} T_{eo} $^mC_{eo}$ A_{ds} G_{ds} T_{ds} $^mC_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} $^mC_{ds}$ T_{ds} T_{eo} $^mC_{eo}$ $^mC_{es}$ T_{es} T_e	$GalNAc_1$-25_a	PO	829
711465	**$GalNAc_1$-$26_{a\text{-}o}$,A_{do}** G_{es} $^mC_{es}$ T_{es} T_{es} $^mC_{es}$ A_{ds} G_{ds} T_{ds} $^mC_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} $^mC_{ds}$ T_{ds} T_{es} $^mC_{es}$ $^mC_{es}$ T_{es} T_e	$GalNAc_1$-26_a	A_d	831
711466	**$GalNAc_1$-$26_{a\text{-}o}$,**G_{es} $^mC_{es}$ T_{es} T_{es} $^mC_{es}$ A_{ds} G_{ds} T_{ds} $^mC_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} $^mC_{ds}$ T_{ds} T_{es} $^mC_{es}$ $^mC_{es}$ T_{es} T_e	$GalNAc_1$-26_a	PO	829
711467	**$GalNAc_1$-$26_{a\text{-}o}$,**G_{es} $^mC_{eo}$ T_{eo} T_{eo} $^mC_{eo}$ A_{ds} G_{ds} T_{ds} $^mC_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} $^mC_{ds}$ T_{ds} T_{eo} $^mC_{eo}$ $^mC_{es}$ T_{es} T_e	$GalNAc_1$-26_a	PO	829
711468	**$GalNAc_1$-$28_{a\text{-}o}$,A_{do}** G_{es} $^mC_{es}$ T_{es} T_{es} $^mC_{es}$ A_{ds} G_{ds} T_{ds} $^mC_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} $^mC_{ds}$ T_{ds} T_{es} $^mC_{es}$ $^mC_{es}$ T_{es} T_e	$GalNAc_1$-28_a	A_d	831
711469	**$GalNAc_1$-$28_{a\text{-}o}$,**G_{es} $^mC_{es}$ T_{es} T_{es} $^mC_{es}$ A_{ds} G_{ds} T_{ds} $^mC_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} $^mC_{ds}$ T_{ds} T_{es} $^mC_{es}$ $^mC_{es}$ T_{es} T_e	$GalNAc_1$-28_a	PO	829
711470	**$GalNAc_1$-$28_{a\text{-}o}$,**G_{es} $^mC_{eo}$ T_{eo} T_{eo} $^mC_{eo}$ A_{ds} G_{ds} T_{ds} $^mC_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} $^mC_{ds}$ T_{ds} T_{eo} $^mC_{eo}$ $^mC_{es}$ T_{es} T_e	$GalNAc_1$-28_a	PO	829
713844	G_{es} $^mC_{es}$ T_{es} T_{es} $^mC_{es}$ A_{ds} G_{ds} T_{ds} $^mC_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} $^mC_{ds}$ T_{ds} T_{es} $^mC_{es}$ $^mC_{es}$ T_{es} T_{eo}'-**$GalNAc_1$-27_a**	$GalNAc_1$-27_a	PO	829
713845	G_{es} $^mC_{eo}$ T_{eo} T_{eo} $^mC_{eo}$ A_{ds} G_{ds} T_{ds} $^mC_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} $^mC_{ds}$ T_{ds} T_{eo} $^mC_{eo}$ $^mC_{es}$ T_{es} T_{eo}'-**$GalNAc_1$-27_a**	$GalNAc_1$-27_a	PO	829
713846	G_{es} $^mC_{eo}$ T_{eo} T_{eo} $^mC_{eo}$ A_{ds} G_{ds} T_{ds} $^mC_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} $^mC_{ds}$ T_{ds} T_{eo} $^mC_{eo}$ $^mC_{es}$ T_{es} T_{eo} **A_{do}'-$GalNAc_1$-27_a**	$GalNAc_1$-27_a	A_d	830
713847	G_{es} $^mC_{es}$ T_{es} T_{es} $^mC_{es}$ A_{ds} G_{ds} T_{ds} $^mC_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} $^mC_{ds}$ T_{ds} T_{es} $^mC_{es}$ $^mC_{es}$ T_{es} T_{eo}'-**$GalNAc_1$-29_a**	$GalNAc_1$-29_a	PO	829
713848	G_{es} $^mC_{eo}$ T_{eo} T_{eo} $^mC_{eo}$ A_{ds} G_{ds} T_{ds} $^mC_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} $^mC_{ds}$ T_{ds} T_{eo} $^mC_{eo}$ $^mC_{es}$ T_{es} T_{eo}'-**$GalNAc_1$-29_a**	$GalNAc_1$-29_a	PO	829
713849	G_{es} $^mC_{es}$ T_{es} T_{es} $^mC_{es}$ A_{ds} G_{ds} T_{ds} $^mC_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} $^mC_{ds}$ T_{ds} T_{es} $^mC_{es}$ $^mC_{es}$ T_{es} T_{eo} **A_{do}'-$GalNAc_1$-29_a**	$GalNAc_1$-29_a	A_d	830
713850	G_{es} $^mC_{eo}$ T_{eo} T_{eo} $^mC_{eo}$ A_{ds} G_{ds} T_{ds} $^mC_{ds}$ A_{ds} T_{ds} G_{ds} A_{ds} $^mC_{ds}$ T_{ds} T_{eo} $^mC_{eo}$ $^mC_{es}$ T_{es} T_{eo} **A_{do}'-$GalNAc_1$-29_a**	$GalNAc_1$-29_a	A_d	830

Example 113: Antisense inhibition *in vivo* by oligonucleotides targeting CFB

The oligonucleotides listed in Table 121 were tested in a dose-dependent study for antisense inhibition of human Complement Factor B (CFB) in mice.

Table 121
Modified ASOs targeting CFB

ISIS No.	Sequences (5' to 3')	$GalNAc_3$ Cluster	CM	SEQ ID No.
588540	$A_{es}T_{es}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{es}A_{ds}{}^{m}C_{ds}G_{ds}{}^{m}C_{ds}{}^{m}C_{ds}{}^{m}C_{ds}$ $^{m}C_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{es}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{e}$	n/a	n/a	440
687301	**$GalNAc_3$-3_{a-o}**,$A_{es}T_{es}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{es}A_{ds}{}^{m}C_{ds}G_{ds}$ $^{m}C_{ds}{}^{m}C_{ds}{}^{m}C_{ds}{}^{m}C_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{es}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{e}$	$GalNAc_3$-3a	PO	440

The structure of $GalNAc_3$-3_a was shown previously in Example 39.

Treatment

Transgenic mice that express human CFB (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously once per week for 3 weeks (a total of 4 doses) with an oligonucleotide listed in Table 122 or with saline. The four treatment groups that received ISIS No. 588540 were given 6, 12, 25, or 50 mg/kg per dose. The four treatment groups that received ISIS No. 687301 were given 0.25, 0.5, 2, or 6 mg/kg per dose. Each treatment group consisted of 4 animals. The mice were sacrificed 2 days following the final administration to determine the liver and kidney human CFB and cyclophilin mRNA levels using real-time PCR according to standard protocols. The CFB mRNA levels were normalized to the cyclophilin levels, and the averages for each treatment group were used to determine the dose that achieved 50% inhibition of the human CFB transcript expression (ED_{50}). The results are the averages of four experiments completed with two different primer probe sets and are shown in Table 122.

Table 122
Potencies of oligonucleotides targeting human CFB *in vivo*

ISIS No.	ED_{50} in liver (mg/kg)	ED_{50} in kidney (mg/kg)	$GalNAc_3$ Cluster	CM
588540	7.9	11.7	n/a	n/a
687301	0.49	0.35	$GalNAc_3$-3a	PO

Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum were measured relative to saline injected mice using standard protocols. Total bilirubin, BUN, and body weights were also evaluated. The results show that there were no significant changes in any of the treatment groups relative to the saline treated group (data not shown), indicating that both oligonucleotides were very well tolerated.

Example 114: Antisense inhibition *in vivo* by oligonucleotides targeting CFB

The oligonucleotides listed in Table 123 were tested in a dose-dependent study for antisense inhibition of human CFB in mice.

Treatment

Transgenic mice that express human CFB (Jackson Laboratory, Bar Harbor, ME) were injected subcutaneously once with 0.6, 1, 6, or 18 mg/kg of an oligonucleotide listed in Table 123 or with saline. Each treatment group consisted of 4 or 5 animals. The mice were sacrificed 72 hours following the dose to determine the liver human CFB and cyclophilin mRNA levels using real-time PCR according to standard protocols. The CFB mRNA levels were normalized to the cyclophilin levels, and the averages for each treatment group were used to determine the dose that achieved 50% inhibition of the human CFB transcript expression (ED_{50}). The results are shown in Table 123.

Table 123
Modified ASOs targeting CFB

ISIS No.	Sequences (5' to 3')	$GalNAc_3$ Cluster	CM	ED_{50} in liver (mg/kg)	SEQ ID No.
696844	**$GalNAc_3$-7_{a}-$_{o}$**,$A_{es}T_{es}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{es}A_{ds}{}^{m}C_{ds}G_{ds}{}^{m}C_{ds}{}^{m}C_{ds}{}^{m}C_{ds}{}^{m}C_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{es}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{e}$	$GalNAc_3$-7a	PO	0.86	440
696845	**$GalNAc_3$-7_{a}-$_{o}$**,$A_{es}T_{eo}{}^{m}C_{eo}{}^{m}C_{eo}{}^{m}C_{eo}A_{ds}{}^{m}C_{ds}G_{ds}{}^{m}C_{ds}{}^{m}C_{ds}{}^{m}C_{ds}{}^{m}C_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{eo}{}^{m}C_{eo}A_{es}G_{es}{}^{m}C_{e}$	$GalNAc_3$-7a	PO	0.71	440
698969	**$GalNAc_3$-7_{a}-$_{o}$**,$A_{es}T_{eo}{}^{m}C_{eo}{}^{m}C_{eo}{}^{m}C_{es}A_{ds}{}^{m}C_{ds}G_{ds}{}^{m}C_{ds}{}^{m}C_{ds}{}^{m}C_{ds}{}^{m}C_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{eo}{}^{m}C_{eo}A_{es}G_{es}{}^{m}C_{e}$	$GalNAc_3$-7a	PO	0.51	440
698970	**$GalNAc_3$-7_{a}-$_{o}$**,$A_{es}T_{es}{}^{m}C_{eo}{}^{m}C_{eo}{}^{m}C_{eo}A_{ds}{}^{m}C_{ds}G_{ds}{}^{m}C_{ds}{}^{m}C_{ds}{}^{m}C_{ds}{}^{m}C_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{eo}{}^{m}C_{eo}A_{es}G_{es}{}^{m}C_{e}$	$GalNAc_3$-7a	PO	0.55	440

The structure of $GalNAc_3$-7_a was shown previously in Example 48.

Example 115: Antisense inhibition of human Complement Factor B (CFB) in HepG2 cells by MOE gapmers

Antisense oligonucleotides were designed targeting human Complement Factor B (CFB) nucleic acid and were tested for their effects on CFB mRNA in vitro. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. Cultured HepG2 cells at a density of 20,000 cells per well were transfected using electroporation with 4,500 nM antisense oligonucleotide. After a treatment period of approximately 24

hours, RNA was isolated from the cells and CFB mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3459 (forward sequence AGTCTCTGTGGCATGGTTTGG, designated herein as SEQ ID NO: 810; reverse sequence GGGCGAATGACTGAGATCTTG, designated herein as SEQ ID NO: 811; probe sequence TACCGATTACCACAAGCAACCATGGCA, designated herein as SEQ ID NO: 812) was used to measure mRNA levels. CFB mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of CFB, relative to untreated control cells.

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 5-10-5 MOE gapmers. The 5-10-5 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising five nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in the Tables below is targeted to either the human CFB mRNA, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NM_001710.5) or the human CFB genomic sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NT_007592.15 truncated from nucleotides 31852000 to 31861000), or both. 'n/a' indicates that the antisense oligonucleotide does not target that particular gene sequence with 100% complementarity.

Table 124

Inhibition of CFB mRNA by 5-10-5 MOE gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target Region	Sequence	% inhibition	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	SEQ ID NO:
532608	20	39	Exon 1	GCTGAGCTGCCAGTCAAGGA	36	1741	1760	6
532609	26	45	Exon 1	GGCCCCGCTGAGCTGCCAGT	16	1747	1766	7
532610	45	64	Exon 1	CGGAACATCCAAGCGGGAGG	11	1766	1785	8
532611	51	70	Exon 1	CTTTCCCGGAACATCCAAGC	26	1772	1791	9
532612	100	119	Exon 1	ATCTGTGTTCTGGCACCTGC	25	1821	1840	10
532613	148	167	Exon 1	GTCACATTCCCTTCCCCTGC	39	1869	1888	11
532614	154	173	Exon 1	GACCTGGTCACATTCCCTTC	71	1875	1894	12
532615	160	179	Exon 1	GACCTAGACCTGGTCACATT	35	1881	1900	13
532616	166	185	Exon 1	ACTCCAGACCTAGACCTGGT	39	1887	1906	14

532617	172	191	Exon 1	GCTGAAACTCCAGACCTAGA	27	1893	1912	15
532618	178	197	Exon 1	GTCCAAGCTGAAACTCCAGA	29	1899	1918	16
532619	184	203	Exon 1	CTCAGTGTCCAAGCTGAAAC	21	1905	1924	17
532620	246	265	Exon 1	AGGAGAGAAGCTGGGCCTGG	31	1967	1986	18
532621	252	271	Exon 1	GAAGGCAGGAGAGAAGCTGG	25	1973	1992	19
532622	336	355	Exon 1-2 Junction	GTGGTGGTCACACCTCCAGA	28	n/a	n/a	20
532623	381	400	Exon 2	CCCTCCAGAGAGCAGGATCC	22	2189	2208	21
532624	387	406	Exon 2	TCTACCCCCTCCAGAGAGCA	37	2195	2214	22
532625	393	412	Exon 2	TTGATCTCTACCCCCTCCAG	30	2201	2220	23
532626	417	436	Exon 2	TGGAGAAGTCGGAAGGAGCC	35	2225	2244	24
532627	423	442	Exon 2	CCCTCTTGGAGAAGTCGGAA	37	2231	2250	25
532628	429	448	Exon 2	GCCTGGCCCTCTTGGAGAAG	0	2237	2256	26
532629	435	454	Exon 2	TCCAGTGCCTGGCCCTCTTG	26	2243	2262	27
532630	458	477	Exon 2	AGAAGCCAGAAGGACACACG	30	2266	2285	28
532631	464	483	Exon 2	ACGGGTAGAAGCCAGAAGGA	43	2272	2291	29
532632	480	499	Exon 2	CGTGTCTGCACAGGGTACGG	57	2288	2307	30
532633	513	532	Exon 2	AGGGTGCTCCAGGACCCCGT	27	2321	2340	31
532634	560	579	Exon 2-3 Junction	TTGCTCTGCACTCTGCCTTC	41	n/a	n/a	32
532635	600	619	Exon 3	TATTCCCCGTTCTCGAAGTC	67	2808	2827	33
532636	626	645	Exon 3	CATTGTAGTAGGGAGACCGG	24	2834	2853	34
532637	632	651	Exon 3	CACTCACATTGTAGTAGGGA	49	2840	2859	35
532638	638	657	Exon 3	TCTCATCACTCACATTGTAG	50	2846	2865	36
532639	644	663	Exon 3	AAGAGATCTCATCACTCACA	52	2852	2871	37
532640	650	669	Exon 3	AGTGGAAAGAGATCTCATCA	34	2858	2877	38
532641	656	675	Exon 3	CATAGCAGTGGAAAGAGATC	32	2864	2883	39
532642	662	681	Exon 3	AACCGTCATAGCAGTGGAAA	45	2870	2889	40
532643	668	687	Exon 3	GAGTGTAACCGTCATAGCAG	36	2876	2895	41
532644	674	693	Exon 3	CCCGGAGAGTGTAACCGTCA	30	2882	2901	42
532645	680	699	Exon 3	CAGAGCCCCGGAGAGTGTAA	27	2888	2907	43
532646	686	705	Exon 3	GATTGGCAGAGCCCCGGAGA	20	2894	2913	44
532647	692	711	Exon 3	AGGTGCGATTGGCAGAGCCC	28	2900	2919	45
532648	698	717	Exon 3	CTTGGCAGGTGCGATTGGCA	24	2906	2925	46
532649	704	723	Exon 3	CATTCACTTGGCAGGTGCGA	28	2912	2931	47
532650	729	748	Exon 3	ATCGCTGTCTGCCCACTCCA	44	2937	2956	48
532651	735	754	Exon 3	TCACAGATCGCTGTCTGCCC	44	2943	2962	49
532652	741	760	Exon 3	CCGTTGTCACAGATCGCTGT	27	2949	2968	50
532653	747	766	Exon 3-4 Junction	CCCGCTCCGTTGTCACAGAT	28	n/a	n/a	51

532654	753	772	Exon 3-4 Junction	CAGTACCCCGCTCCGTTGTC	13	n/a	n/a	52
532655	759	778	Exon 3-4 Junction	TTGGAGCAGTACCCCGCTCC	8	n/a	n/a	53
532656	789	808	Exon 4	ACCTTCCTTGTGCCAATGGG	40	3152	3171	54
532657	795	814	Exon 4	CTGCCCACCTTCCTTGTGCC	41	3158	3177	55
532658	818	837	Exon 4	CGCTGTCTTCAAGGCGGTAC	33	3181	3200	56
532659	835	854	Exon 4	GCTGCAGTGGTAGGTGACGC	32	3198	3217	57
532660	841	860	Exon 4	CCCCCGGCTGCAGTGGTAGG	17	3204	3223	58
532661	847	866	Exon 4	GGTAAGCCCCCGGCTGCAGT	28	3210	3229	59
532662	853	872	Exon 4	ACGCAGGGTAAGCCCCCGGC	13	3216	3235	60
532663	859	878	Exon 4	GGAGCCACGCAGGGTAAGCC	33	3222	3241	61
532664	866	885	Exon 4	GCCGCTGGGAGCCACGCAGG	10	3229	3248	62
532665	891	910	Exon 4	CAAGAGCCACCTTCCTGACA	17	3254	3273	63
532666	897	916	Exon 4	CCGCTCCAAGAGCCACCTTC	25	3260	3279	64
532667	903	922	Exon 4	TCCGTCCCGCTCCAAGAGCC	29	3266	3285	65
532668	909	928	Exon 4	GAAGGCTCCGTCCCGCTCCA	14	3272	3291	66
532669	915	934	Exon 4	TGGCAGGAAGGCTCCGTCCC	18	3278	3297	67
532670	921	940	Exon 4-5 Junction	GAGTCTTGGCAGGAAGGCTC	20	n/a	n/a	68
532671	927	946	Exon 4-5 Junction	ATGAAGGAGTCTTGGCAGGA	14	n/a	n/a	69
532672	956	975	Exon 5	CTTCGGCCACCTCTTGAGGG	45	3539	3558	70
532673	962	981	Exon 5	GGAAAGCTTCGGCCACCTCT	37	3545	3564	71
532674	968	987	Exon 5	AAGACAGGAAAGCTTCGGCC	28	3551	3570	72
532675	974	993	Exon 5	TCAGGGAAGACAGGAAAGCT	16	3557	3576	73
532676	996	1015	Exon 5	TCGACTCCTTCTATGGTCTC	31	3579	3598	74
532677	1033	1052	Exon 5-6 Junction	CTTCTGTTGTTCCCCTGGGC	36	n/a	n/a	75
532678	1068	1087	Exon 6	TTCATGGAGCCTGAAGGGTC	19	3752	3771	76
532679	1074	1093	Exon 6	TAGATGTTCATGGAGCCTGA	24	3758	3777	77
532680	1080	1099	Exon 6	ACCAGGTAGATGTTCATGGA	13	3764	3783	78
532681	1086	1105	Exon 6	TCTAGCACCAGGTAGATGTT	20	3770	3789	79
532682	1092	1111	Exon 6	GATCCATCTAGCACCAGGTA	33	3776	3795	80
532683	1098	1117	Exon 6	CTGTCTGATCCATCTAGCAC	44	3782	3801	81
532684	1104	1123	Exon 6	CCAATGCTGTCTGATCCATC	29	3788	3807	82
532685	1129	1148	Exon 6	TTTGGCTCCTGTGAAGTTGC	40	3813	3832	83

Table 125

Inhibition of CFB mRNA by 5-10-5 MOE gapmers targeting SEQ ID NO: 1 and 2

ISIS No	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target region	Sequence	% inhibition	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	SEQ ID NO:
532686	1135	1154	Exon 6	ACACTTTTTGGCTCCTGTGA	91	3819	3838	84
532687	1141	1160	Exon 6	GACTAGACACTTTTTGGCTC	77	3825	3844	85
532688	1147	1166	Exon 6	TAAGTTGACTAGACACTTTT	70	3831	3850	86
532689	1153	1172	Exon 6	CTCAATTAAGTTGACTAGAC	61	3837	3856	87
532690	1159	1178	Exon 6-7 Junction	CACCTTCTCAATTAAGTTGA	57	3843	3862	88
532691	1165	1184	Exon 6-7 Junction	ACTTGCCACCTTCTCAATTA	56	n/a	n/a	89
532692	1171	1190	Exon 6-7 Junction	ACCATAACTTGCCACCTTCT	56	n/a	n/a	90
532693	1177	1196	Exon 7	CTTCACACCATAACTTGCCA	56	4153	4172	91
532694	1183	1202	Exon 7	TCTTGGCTTCACACCATAAC	55	4159	4178	92
532695	1208	1227	Exon 7	ATGTGGCATATGTCACTAGA	55	4184	4203	93
532696	1235	1254	Exon 7	CAGACACTTTGACCCAAATT	55	4211	4230	94
532697	1298	1317	Exon 7-8 Junction	GGTCTTCATAATTGATTTCA	53	n/a	n/a	95
532698	1304	1323	Exon 7-8 Junction	ACTTGTGGTCTTCATAATTG	53	n/a	n/a	96
532699	1310	1329	Exon 7-8 Junction	ACTTCAACTTGTGGTCTTCA	52	n/a	n/a	97
532700	1316	1335	Exon 8	TCCCTGACTTCAACTTGTGG	52	4609	4628	98
532701	1322	1341	Exon 8	TGTTAGTCCCTGACTTCAAC	52	4615	4634	99
532702	1328	1347	Exon 8	TCTTGGTGTTAGTCCCTGAC	51	4621	4640	100
532703	1349	1368	Exon 8	TGTACACTGCCTGGAGGGCC	51	4642	4661	101
532704	1355	1374	Exon 8	TCATGCTGTACACTGCCTGG	51	4648	4667	102
532705	1393	1412	Exon 8	GTTCCAGCCTTCAGGAGGGA	50	4686	4705	103
532706	1399	1418	Exon 8	GGTGCGGTTCCAGCCTTCAG	50	4692	4711	104
532707	1405	1424	Exon 8	ATGGCGGGTGCGGTTCCAGC	50	4698	4717	105
532708	1411	1430	Exon 8	GATGACATGGCGGGTGCGGT	49	4704	4723	106
532709	1417	1436	Exon 8	GAGGATGATGACATGGCGGG	49	4710	4729	107
532710	1443	1462	Exon 8-9 Junction	CCCATGTTGTGCAATCCATC	48	n/a	n/a	108
532711	1449	1468	Exon 9	TCCCCGCCCATGTTGTGCAA	48	5023	5042	109
532712	1455	1474	Exon 9	ATTGGGTCCCCGCCCATGTT	48	5029	5048	110
532713	1461	1480	Exon 9	ACAGTAATTGGGTCCCCGCC	48	5035	5054	111

532714	1467	1486	Exon 9	TCAATGACAGTAATTGGGTC	47	5041	5060	112
532715	1473	1492	Exon 9	ATCTCATCAATGACAGTAAT	47	5047	5066	113
532716	1479	1498	Exon 9	TCCCGGATCTCATCAATGAC	46	5053	5072	114
532717	1533	1552	Exon 9-10 Junction	ACATCCAGATAATCCTCCCT	46	n/a	n/a	115
532718	1539	1558	Exon 9-10 Junction	ACATAGACATCCAGATAATC	46	n/a	n/a	116
532719	1545	1564	Exon 9-10 Junction	CCAAACACATAGACATCCAG	46	n/a	n/a	117
532720	1582	1601	Exon 10	AGCATTGATGTTCACTTGGT	46	5231	5250	118
532721	1588	1607	Exon 10	AGCCAAAGCATTGATGTTCA	45	5237	5256	119
532722	1594	1613	Exon 10	CTTGGAAGCCAAAGCATTGA	45	5243	5262	120
532723	1600	1619	Exon 10	GTCTTTCTTGGAAGCCAAAG	45	5249	5268	121
532724	1606	1625	Exon 10	CTCATTGTCTTTCTTGGAAG	44	5255	5274	122
532725	1612	1631	Exon 10	ATGTTGCTCATTGTCTTTCT	44	5261	5280	123
532726	1618	1637	Exon 10	GAACACATGTTGCTCATTGT	44	5267	5286	124
532727	1624	1643	Exon 10	GACTTTGAACACATGTTGCT	43	5273	5292	125
532728	1630	1649	Exon 10	ATCCTTGACTTTGAACACAT	43	5279	5298	126
532729	1636	1655	Exon 10	TTCCATATCCTTGACTTTGA	43	5285	5304	127
532730	1642	1661	Exon 10	CAGGTTTTCCATATCCTTGA	42	5291	5310	128
532731	1686	1705	Exon 11	CTCAGAGACTGGCTTTCATC	42	5827	5846	129
532732	1692	1711	Exon 11	CAGAGACTCAGAGACTGGCT	42	5833	5852	130
516252	1698	1717	Exon 11	ATGCCACAGAGACTCAGAGA	42	5839	5858	131
532733	1704	1723	Exon 11	CAAACCATGCCACAGAGACT	41	5845	5864	132
532734	1710	1729	Exon 11	TGTTCCCAAACCATGCCACA	41	5851	5870	133
532735	1734	1753	Exon 11	TTGTGGTAATCGGTACCCTT	41	5875	5894	134
532736	1740	1759	Exon 11	GGTTGCTTGTGGTAATCGGT	40	5881	5900	135
532737	1746	1765	Exon 11	TGCCATGGTTGCTTGTGGTA	40	5887	5906	136
532738	1752	1771	Exon 11	TTGGCCTGCCATGGTTGCTT	40	5893	5912	137
532739	1758	1777	Exon 11	GAGATCTTGGCCTGCCATGG	38	5899	5918	138
532740	1803	1822	Exon 12	ACAGCCCCCATACAGCTCTC	38	6082	6101	139
532741	1809	1828	Exon 12	GACACCACAGCCCCCATACA	38	6088	6107	140
532742	1815	1834	Exon 12	TACTCAGACACCACAGCCCC	38	6094	6113	141
532743	1821	1840	Exon 12	ACAAAGTACTCAGACACCAC	37	6100	6119	142
532744	1827	1846	Exon 12	GTCAGCACAAAGTACTCAGA	37	6106	6125	143
532745	1872	1891	Exon 12	TTGATTGAGTGTTCCTTGTC	36	6151	6170	144
532746	1878	1897	Exon 12	CTGACCTTGATTGAGTGTTC	35	6157	6176	145
532747	1909	1928	Exon 13	TATCTCCAGGTCCCGCTTCT	35	6403	6422	146
532748	1967	1986	Exon 13	GAATTCCTGCTTCTTTTTTC	32	6461	6480	147
532749	1973	1992	Exon 13	ATTCAGGAATTCCTGCTTCT	32	6467	6486	148
532750	1979	1998	Exon 13	CATAAAATTCAGGAATTCCT	32	6473	6492	149
532751	1985	2004	Exon 13	CATAGTCATAAAATTCAGGA	31	6479	6498	150
532752	2006	2025	Exon 13	TGAGCTTGATCAGGGCAACG	30	6500	6519	151

532753	2012	2031	Exon 13	TATTCTTGAGCTTGATCAGG	30	6506	6525	152
532754	2048	2067	Exon 13-14 Junction	GACAAATGGGCCTGATAGTC	30	n/a	n/a	153
532755	2070	2089	Exon 14	GTTGTTCCCTCGGTGCAGGG	29	6659	6678	154
532756	2076	2095	Exon 14	GCTCGAGTTGTTCCCTCGGT	28	6665	6684	155
532757	2082	2101	Exon 14	CTCAAAGCTCGAGTTGTTCC	28	6671	6690	156
532758	2088	2107	Exon 14	GGAAGCCTCAAAGCTCGAGT	25	6677	6696	157
532759	2094	2113	Exon 14	GTTGGAGGAAGCCTCAAAGC	23	6683	6702	158
532760	2100	2119	Exon 14	GTGGTAGTTGGAGGAAGCCT	23	6689	6708	159
532761	2106	2125	Exon 14	TGGCAAGTGGTAGTTGGAGG	18	6695	6714	160
532762	2112	2131	Exon 14	TGTTGCTGGCAAGTGGTAGT	14	6701	6720	161

Table 126

Inhibition of CFB mRNA by 5-10-5 MOE gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target Region	Sequence	% inhibition	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	SEQ ID NO:
532812	n/a	n/a	Exon 1	TCCAGCTCACTCCCCTGTTG	19	1593	1612	162
532813	n/a	n/a	Exon 1	TAAGGATCCAGCTCACTCCC	40	1599	1618	163
532814	n/a	n/a	Exon 1	CAGAAATAAGGATCCAGCTC	39	1605	1624	164
532815	n/a	n/a	Exon 1	AGGGACCAGAAATAAGGATC	0	1611	1630	165
532816	n/a	n/a	Exon 1	CCACTTAGGGACCAGAAATA	27	1617	1636	166
532817	n/a	n/a	Exon 1	TCCAGGACTCTCCCCTTCAG	39	1682	1701	167
532818	n/a	n/a	Exon 1	AAGTCCCACCCTTTGCTGCC	15	1707	1726	168
532819	n/a	n/a	Exon 1	CTGCAGAAGTCCCACCCTTT	26	1713	1732	169
532820	n/a	n/a	Exon 1	CAGAAACTGCAGAAGTCCCA	8	1719	1738	170
532821	n/a	n/a	Exon 2 - Intron 2	AACCTCTGCACTCTGCCTTC	39	2368	2387	171
532822	n/a	n/a	Exon 2 - Intron 2	CCCTCAAACCTCTGCACTCT	3	2374	2393	172
532823	n/a	n/a	Exon 2 - Intron 2	TCATTGCCCTCAAACCTCTG	19	2380	2399	173
532824	n/a	n/a	Intron 2	CCACACTCATTGCCCTCAAA	37	2386	2405	174
532825	n/a	n/a	Intron 2	CACTGCCCACACTCATTGCC	23	2392	2411	175
532826	n/a	n/a	Intron 2	TTAGGCCACTGCCCACACTC	15	2398	2417	176
532827	n/a	n/a	Intron 2	CTAGTCCTGACCTTGCTGCC	28	2436	2455	177

532828	n/a	n/a	Intron 2	CTCATCCTAGTCCTGACCTT	25	2442	2461	178
532829	n/a	n/a	Intron 2	CCTAGTCTCATCCTAGTCCT	23	2448	2467	179
532830	n/a	n/a	Intron 2	ACCCTGCCTAGTCTCATCCT	30	2454	2473	180
532831	n/a	n/a	Intron 2	CTTGTCACCCTGCCTAGTCT	34	2460	2479	181
532832	n/a	n/a	Intron 2	GCCCACCTTGTCACCCTGCC	36	2466	2485	182
532833	n/a	n/a	Intron 2	CCTAAAACTGCTCCTACTCC	9	2492	2511	183
532834	n/a	n/a	Intron 4	GAGTCAGAAATGAGGTCAAA	19	3494	3513	184
532835	n/a	n/a	Intron 11	CCCTACTCCCATTTCACCTT	16	5971	5990	185
532836	n/a	n/a	Intron 8 - Exon 9	TGTTGTGCAATCCTGCAGAA	25	5013	5032	186
532837	n/a	n/a	Intron 1	AAAGGCTGATGAAGCCTGGC	18	2123	2142	187
532838	n/a	n/a	Intron 7	CCTTTGACCACAAAGTGGCC	21	4461	4480	188
532839	n/a	n/a	Intron 12	AGGTACCACCTCTTTGTGGG	29	6362	6381	189
532840	n/a	n/a	Intron 1 - Exon 2	TGGTGGTCACACCTGAAGAG	34	2143	2162	190
532763	2133	2152	Exon 14-15 Junction	GCAGGGAGCAGCTCTTCCTT	40	n/a	n/a	191
532764	2139	2158	Exon 15	TCCTGTGCAGGGAGCAGCTC	28	6927	6946	192
532765	2145	2164	Exon 15	TTGATATCCTGTGCAGGGAG	41	6933	6952	193
532766	2151	2170	Exon 15	AGAGCTTTGATATCCTGTGC	36	6939	6958	194
532767	2157	2176	Exon 15	ACAAACAGAGCTTTGATATC	33	6945	6964	195
532768	2163	2182	Exon 15	TCAGACACAAACAGAGCTTT	41	6951	6970	196
532769	2169	2188	Exon 15	TCCTCCTCAGACACAAACAG	49	6957	6976	197
532770	2193	2212	Exon 15	ACCTCCTTCCGAGTCAGCTT	61	6981	7000	198
532771	2199	2218	Exon 15	ATGTAGACCTCCTTCCGAGT	39	6987	7006	199
532772	2205	2224	Exon 15	TTCTTGATGTAGACCTCCTT	30	6993	7012	200
532773	2211	2230	Exon 15	TCCCCATTCTTGATGTAGAC	31	6999	7018	201
532774	2217	2236	Exon 15-16 Junction	TTCTTATCCCCATTCTTGAT	36	n/a	n/a	202
532775	2223	2242	Exon 15-16 Junction	CTGCCTTTCTTATCCCCATT	56	n/a	n/a	203
532776	2229	2248	Exon 15-16 Junction	TCACAGCTGCCTTTCTTATC	33	n/a	n/a	204
532777	2235	2254	Exon 16	TCTCTCTCACAGCTGCCTTT	38	7119	7138	205
532778	2241	2260	Exon 16	TGAGCATCTCTCTCACAGCT	51	7125	7144	206
532779	2247	2266	Exon 16	GCATATTGAGCATCTCTCTC	39	7131	7150	207

532780	2267	2286	Exon 16	TGACTTTGTCATAGCCTGGG	56	7151	7170	208
532781	2273	2292	Exon 16	TGTCCTTGACTTTGTCATAG	36	7157	7176	209
532782	2309	2328	Exon 16	CAGTACAAAGGAACCGAGGG	30	7193	7212	210
532783	2315	2334	Exon 16	CTCCTCCAGTACAAAGGAAC	21	7199	7218	211
532784	2321	2340	Exon 16	GACTCACTCCTCCAGTACAA	31	7205	7224	212
532785	2327	2346	Exon 16	CATAGGGACTCACTCCTCCA	30	7211	7230	213
532786	2333	2352	Exon 16	GGTCAGCATAGGGACTCACT	31	7217	7236	214
532787	2352	2371	Exon 16-17 Junction	TCACCTCTGCAAGTATTGGG	42	7236	7255	215
532788	2358	2377	Exon 16-17 Junction	CCAGAATCACCTCTGCAAGT	32	n/a	n/a	216
532789	2364	2383	Exon 16-17 Junction	GGGCCGCCAGAATCACCTCT	35	n/a	n/a	217
532790	2382	2401	Exon 17	CTCTTGTGAACTATCAAGGG	33	7347	7366	218
532791	2388	2407	Exon 17	CGACTTCTCTTGTGAACTAT	52	7353	7372	219
532792	2394	2413	Exon 17	ATGAAACGACTTCTCTTGTG	16	7359	7378	220
532793	2400	2419	Exon 17-18 Junction	ACTTGAATGAAACGACTTCT	45	7365	7384	221
532794	2406	2425	Exon 17-18 Junction	ACACCAACTTGAATGAAACG	18	n/a	n/a	222
532795	2427	2446	Exon 18	TCCACTACTCCCCAGCTGAT	30	7662	7681	223
532796	2433	2452	Exon 18	CAGACATCCACTACTCCCCA	38	7668	7687	224
532797	2439	2458	Exon 18	TTTTTGCAGACATCCACTAC	35	7674	7693	225
532798	2445	2464	Exon 18	TTCTGGTTTTTGCAGACATC	45	7680	7699	226
532799	2451	2470	Exon 18	TGCCGCTTCTGGTTTTTGCA	47	7686	7705	227
532800	2457	2476	Exon 18	TGCTTTTGCCGCTTCTGGTT	61	7692	7711	228
532801	2463	2482	Exon 18	GGTACCTGCTTTTGCCGCTT	47	7698	7717	229
532802	2469	2488	Exon 18	TGAGCAGGTACCTGCTTTTG	31	7704	7723	230
532803	2517	2536	Exon 18	TTCAGCCAGGGCAGCACTTG	41	7752	7771	231
532804	2523	2542	Exon 18	TTCTCCTTCAGCCAGGGCAG	44	7758	7777	232
532805	2529	2548	Exon 18	TGGAGTTTCTCCTTCAGCCA	46	7764	7783	233
532806	2535	2554	Exon 18	TCATCTTGGAGTTTCTCCTT	49	7770	7789	234
532807	2541	2560	Exon 18	AAATCCTCATCTTGGAGTTT	30	7776	7795	235
532808	2547	2566	Exon 18	AAACCCAAATCCTCATCTTG	20	7782	7801	236
532809	2571	2590	Exon 18	GTCCAGCAGGAAACCCCTTA	65	7806	7825	237
532810	2577	2596	Exon 18	GCCCCTGTCCAGCAGGAAAC	74	7812	7831	238
532811	2599	2618	Exon 18	AGCTGTTTTAATTCAATCCC	96	7834	7853	239

Table 127

Inhibition of CFB mRNA by 5-10-5 MOE gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target region	Sequence	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	% inhibition	SEQ ID NO:
532841	n/a	n/a	Intron 6-Exon 7	AACTTGCCACCTGTGGGTGA	4142	4161	11	240
532842	n/a	n/a	Exon 15 - Intron 15	TCACCTTATCCCCATTCTTG	7007	7026	16	241
532843	n/a	n/a	Intron 11	TCAACTTTCACAAACCACCA	6015	6034	19	242
532844	n/a	n/a	Intron 16 - Exon 17	CCGCCAGAATCACCTGCAAG	7326	7345	33	243
532845	n/a	n/a	Intron 10	AGGAGGAATGAAGAAGGCTT	5431	5450	29	244
532846	n/a	n/a	Intron 13	GCCTTTCCTCAGGGATCTGG	6561	6580	26	245
532847	n/a	n/a	Intron 4	AAATGTCTGGGAGTGTCAGG	3477	3496	18	246
532848	n/a	n/a	Intron 15	GCCTAGAGTGCCTCCTTAGG	7038	7057	20	247
532849	n/a	n/a	Intron 17	GGCATCTCCCCAGATAGGAA	7396	7415	16	248
532850	n/a	n/a	Intron 6	AGGGAGCTAGTCCTGGAAGA	3906	3925	14	249
532851	n/a	n/a	Intron 1 - Exon 2	ACACCTGAAGAGAAAGGCTG	2135	2154	6	250
532852	n/a	n/a	Intron 7	CCCTTTGACCACAAAGTGGC	4462	4481	25	251
532853	n/a	n/a	Intron 7	GCCCTCAAGGTAGTCTCATG	4354	4373	26	252
532854	n/a	n/a	Intron 6	AAGGGAAGGAGGACAGAATA	3977	3996	18	253
532855	n/a	n/a	Intron 1	AAAGGCCAAGGAGGGATGCT	2099	2118	9	254
532856	n/a	n/a	Exon 8 - Intron 8	AGAGGTCCCTTCTGACCATC	4736	4755	4	255
532857	n/a	n/a	Intron 8	GCTGGGACAGGAGAGAGGTC	4749	4768	0	256
532858	n/a	n/a	Intron 4	TCAAATGTCTGGGAGTGTCA	3479	3498	13	257
532859	n/a	n/a	Intron 10	AGAAGGAGAATGTGCTGAAA	5801	5820	20	258
532860	n/a	n/a	Intron 17	TGCTGACCACTTGGCATCTC	7408	7427	20	259
532861	n/a	n/a	Intron 11	CAACTTTCACAAACCACCAT	6014	6033	18	260
532862	n/a	n/a	Intron 10	AGCTCTGTGATTCTAAGGTT	5497	5516	15	261
532863	n/a	n/a	Intron 6-Exon 7	CCACCTGTGGGTGAGGAGAA	4136	4155	16	262
532864	n/a	n/a	Exon 17 - Intron 17	GAGGACTCACTTGAATGAAA	7373	7392	21	263
532865	n/a	n/a	Intron 6	TGGAATGATCAGGGAGCTAG	3916	3935	30	264
532866	n/a	n/a	Intron 5	GTCCCTTCTCCATTTTCCCC	3659	3678	26	265
532867	n/a	n/a	Intron 7	TCAACTTTTTAAGTTAATCA	4497	4516	14	266
532868	n/a	n/a	Intron 6	GGGTGAGGAGAACAAGGCGC	4128	4147	21	267
532869	n/a	n/a	Intron 7	CTTCCAAGCCATCTTTTAAC	4553	4572	5	268

532870	n/a	n/a	Exon 17 - Intron 17	AGGACTCACTTGAATGAAAC	7372	7391	18	269
532871	n/a	n/a	Intron 10	TTCCAGGCAACTAGAGCTTC	5412	5431	15	270
532872	n/a	n/a	Exon 1	CAGAGTCCAGCCACTGTTTG	1557	1576	13	271
532873	n/a	n/a	Intron 17 - Exon 18	CCAACCTGCAGAGGCAGTGG	7638	7657	23	272
532874	n/a	n/a	Intron 16	TGCAAGGAGAGGAGAAGCTG	7312	7331	10	273
532875	n/a	n/a	Exon 9 - Intron 9	CTAGGCAGGTTACTCACCCA	5120	5139	21	274
532876	n/a	n/a	Intron 6- Exon 7	CACCATAACTTGCCACCTGT	4148	4167	41	275
532877	n/a	n/a	Intron 12	TAGGTACCACCTCTTTGTGG	6363	6382	27	276
532878	n/a	n/a	Intron 11	CTTGACCTCACCTCCCCCAA	5954	5973	13	277
532879	n/a	n/a	Intron 12	CCACCTCTTTGTGGGCAGCT	6357	6376	33	278
532880	n/a	n/a	Intron 11	TTCACAAACCACCATCTCTT	6009	6028	8	279
532881	n/a	n/a	Exon 3 - Intron 3	TTCTCACCTCCGTTGTCACA	2958	2977	17	280
532882	n/a	n/a	Intron 12	GAAAGTGGGAGGTGTTGCCT	6225	6244	19	281
532883	n/a	n/a	Intron 1	ACAGCAGGAAGGGAAGGTTA	2075	2094	34	282
532884	n/a	n/a	Intron 17	CATGCTGACCACTTGGCATC	7410	7429	18	283
532885	n/a	n/a	Exon 4 - Intron 4	GGTCACCTTGGCAGGAAGGC	3286	3305	0	284
532886	n/a	n/a	Intron 8	GTATAGTGTTACAAGTGGAC	4804	4823	13	285
532887	n/a	n/a	Intron 7	GGACTTCCCTTTGACCACAA	4468	4487	18	286
532888	n/a	n/a	Intron 11	TCACCTTGACCTCACCTCCC	5958	5977	20	287
532889	n/a	n/a	Intron 15	TAGAGTGCCTCCTTAGGATG	7035	7054	27	288
532890	n/a	n/a	Intron 7	TGACTTCAACTTGTGGTCTG	4605	4624	16	289
532891	n/a	n/a	Intron 10	CAGAGAAGGAGAATGTGCTG	5804	5823	25	290
532892	n/a	n/a	Intron 14 - Exon 15	AGGGAGCAGCTCTTCCTCTG	6919	6938	47	291
532893	n/a	n/a	Intron 5 - Exon 6	TGTTCCCCTGGGTGCCAGGA	3710	3729	24	292
532894	n/a	n/a	Intron 10	GGCCTGGCTGTTTTCAAGCC	5612	5631	15	293
532895	n/a	n/a	Intron 10 - Exon 11	GACTGGCTTTCATCTGGCAG	5821	5840	25	294
532896	n/a	n/a	Intron 10	GAAGGCTTTCCAGGCAACTA	5419	5438	19	295
532897	n/a	n/a	Exon 17 - Intron 17	TCACTTGAATGAAACGACTT	7367	7386	11	296
532898	n/a	n/a	Intron 1	GGCCCCAAAAGGCCAAGGAG	2106	2125	5	297
532899	n/a	n/a	Intron 16 - Exon 17	AATCACCTGCAAGGAGAGGA	7319	7338	19	298
532900	n/a	n/a	Intron 12	GACCTTCAGTTGCATCCTTA	6183	6202	25	299
532901	n/a	n/a	Intron 1	TGATGAAGCCTGGCCCCAAA	2117	2136	0	300
532902	n/a	n/a	Intron 12	TAGAAAGTGGGAGGTGTTGC	6227	6246	0	301

532903	n/a	n/a	Intron 12	CCCATCCCTGACTGGTCTGG	6295	6314	14	302
532904	n/a	n/a	Intron 8	CCATGGGTATAGTGTTACAA	4810	4829	13	303
532905	n/a	n/a	Intron 2	GTGTTCTCTTGACTTCCAGG	2586	2605	23	304
532906	n/a	n/a	Intron 13	GGCCTGCTCCTCACCCCAGT	6597	6616	27	305
532907	n/a	n/a	Intron 10	GAGGCCTGGCTGTTTTCAAG	5614	5633	32	306
532908	n/a	n/a	Exon 1	GACTCTCCCCTTCAGTACCT	1677	1696	16	307
532909	n/a	n/a	Intron 8	CATGGGTATAGTGTTACAAG	4809	4828	10	308
532910	n/a	n/a	Intron 10	GAAGGAGAATGTGCTGAAAA	5800	5819	0	309
532911	n/a	n/a	Intron 7	TCACCTGGTCTTCCAAGCCA	4562	4581	0	310
532912	n/a	n/a	Intron 17	CTCCCCAGATAGGAAAGGGA	7391	7410	0	311
532913	n/a	n/a	Exon 17 - Intron 17	GGACTCACTTGAATGAAACG	7371	7390	0	312
532914	n/a	n/a	Intron 16 - Exon 17	GGCCGCCAGAATCACCTGCA	7328	7347	30	313
532915	n/a	n/a	Exon 17 - Intron 17	CTCACTTGAATGAAACGACT	7368	7387	22	314
532916	n/a	n/a	Intron 13	CTTTCCCAGCCTTTCCTCAG	6569	6588	28	315
532918	n/a	n/a	Intron 12	AGAAAGTGGGAGGTGTTGCC	6226	6245	3	316
532917	2604	2623	Exon 18	GTCGCAGCTGTTTTAATTCA	7839	7858	90	317

Table 128

Inhibition of CFB mRNA by 5-10-5 MOE gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target region	Sequence	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	% inhibition	SEQ ID NO:
532919	n/a	n/a	Exon 1	CCAGGACTCTCCCCTTCAGT	1681	1700	4	318
532920	n/a	n/a	Intron 6	AGGGAAGGAGGACAGAATAG	3976	3995	25	319
532921	n/a	n/a	Intron 4	GAAATGAGGTCAAATGTCTG	3488	3507	30	320
532922	n/a	n/a	Intron 4	GGAGAGTCAGAAATGAGGTC	3497	3516	25	321
532923	n/a	n/a	Intron 12	GTAGAAAGTGGGAGGTGTTG	6228	6247	26	322
532924	n/a	n/a	Intron 10	TAGAAAGATCTCTGAAGTGC	5521	5540	24	323
532925	n/a	n/a	Intron 13	CTGCTCCTCACCCCAGTCCT	6594	6613	26	324
532926	n/a	n/a	Intron 11	CTACTGGGATTCTGTGCTTA	5927	5946	30	325
532927	n/a	n/a	Intron 1	CCCAAAAGGCCAAGGAGGGA	2103	2122	13	326
532928	n/a	n/a	Intron 17	TGACCACTTGGCATCTCCCC	7405	7424	27	327
532929	n/a	n/a	Intron 16 -	CCTGCAAGGAGAGGAGAAGC	7314	7333	29	328

			Exon 17					
532930	n/a	n/a	Exon 16 - Intron 16	CTCTCACCTCTGCAAGTATT	7239	7258	44	329
532931	n/a	n/a	Intron 1	CCCCAAAAGGCCAAGGAGGG	2104	2123	21	330
532932	n/a	n/a	Intron 7	GTCTTCCAAGCCATCTTTTA	4555	4574	20	331
532933	n/a	n/a	Intron 8	GTTACAAGTGGACTTAAGGG	4797	4816	30	332
532934	n/a	n/a	Intron 8 - Exon 9	CCCATGTTGTGCAATCCTGC	5017	5036	30	333
532935	n/a	n/a	Intron 15	GAGGTGGGAAGCATGGAGAA	7091	7110	17	334
532936	n/a	n/a	Intron 14	TGCTCCCACCACTGTCATCT	6874	6893	21	335
532937	n/a	n/a	Exon 9 - Intron 9	AGGCAGGTTACTCACCCAGA	5118	5137	18	336
532938	n/a	n/a	Intron 11	TACTGGGATTCTGTGCTTAC	5926	5945	15	337
532939	n/a	n/a	Intron 13	GCCTTTCCCAGCCTTTCCTC	6571	6590	27	338
532940	n/a	n/a	Intron 8 - Exon 9	GTGCAATCCTGCAGAAGAGA	5009	5028	21	339
532941	n/a	n/a	Intron 8	ACAGGAGAGAGGTCCCTTCT	4743	4762	20	340
532942	n/a	n/a	Intron 10	CCCAAAAGGAGAAAGGGAAA	5717	5736	14	341
532943	n/a	n/a	Intron 2	AAGCCCAGGGTAAATGCTTA	2557	2576	32	342
532944	n/a	n/a	Intron 1	GATGAAGCCTGGCCCCAAAA	2116	2135	22	343
532945	n/a	n/a	Intron 10	TGGCAGAGAAGGAGAATGTG	5807	5826	22	344
532946	n/a	n/a	Intron 13	TTCCCAGCCTTTCCTCAGGG	6567	6586	35	345
532947	n/a	n/a	Intron 10	GGCAGAGAAGGAGAATGTGC	5806	5825	30	346
532948	n/a	n/a	Intron 10	ACAGTGCCAGGAAACAAGAA	5471	5490	25	347
532949	n/a	n/a	Exon 9 - Intron 9	TAGGCAGGTTACTCACCCAG	5119	5138	22	348
532950	n/a	n/a	Intron 2	TTCTCTTGACTTCCAGGGCT	2583	2602	22	349
532951	n/a	n/a	Intron 13	CCTGCTCCTCACCCCAGTCC	6595	6614	16	350
532953	n/a	n/a	Intron 7	TCCCACTAACCTCCATTGCC	4422	4441	14	351
532954	n/a	n/a	Intron 7	TTCCCTTTGACCACAAAGTG	4464	4483	16	352
532955	n/a	n/a	Intron 9	CTGGGTCCTAGGCAGGTTAC	5127	5146	30	353
532956	n/a	n/a	Intron 10	TCCAGGCAACTAGAGCTTCA	5411	5430	20	354
532957	n/a	n/a	Intron 8 - Exon 9	GCCCATGTTGTGCAATCCTG	5018	5037	45	355

532958	n/a	n/a	Intron 7	GGTTCCCACTAACCTCCATT	4425	4444	18	356
532959	n/a	n/a	Intron 3	AGGTAGAGAGCAAGAGTTAC	3052	3071	26	357
532960	n/a	n/a	Intron 7	CCACTAACCTCCATTGCCCA	4420	4439	10	358
532961	n/a	n/a	Intron 11	TCACAAACCACCATCTCTTA	6008	6027	40	359
532962	n/a	n/a	Exon 9 - Intron 9	TACTCACCCAGATAATCCTC	5110	5129	27	360
532963	n/a	n/a	Intron 13	TGCTCCTCACCCCAGTCCTC	6593	6612	24	361
532964	n/a	n/a	Intron 15 - Exon 16	TCTCACAGCTGCCTTTCTGT	7115	7134	25	362
532965	n/a	n/a	Exon 17 - Intron 17	GAAAGGGAGGACTCACTTGA	7379	7398	11	363
532966	n/a	n/a	Intron 7	CCATCTTTTAACCCCAGAGA	4545	4564	18	364
532967	n/a	n/a	Intron 13	TCCTCACCCCAGTCCTCCAG	6590	6609	27	365
532968	n/a	n/a	Intron 10	CTGGCAGAGAAGGAGAATGT	5808	5827	15	366
532969	n/a	n/a	Intron 17	TCTCCCCAGATAGGAAAGGG	7392	7411	23	367
532970	n/a	n/a	Intron 14	ACTTCAGCTGCTCCCACCAC	6882	6901	18	368
532971	n/a	n/a	Intron 1	GACAGCAGGAAGGGAAGGTT	2076	2095	13	369
532972	n/a	n/a	Intron 13 - Exon 14	GGAGACAAATGGGCCTATAA	6640	6659	33	370
532973	n/a	n/a	Intron 14	CTGCTCCCACCACTGTCATC	6875	6894	11	371
532974	n/a	n/a	Intron 10	AGGAATGAAGAAGGCTTTCC	5428	5447	21	372
532975	n/a	n/a	Intron 14	GGGATCTCATCCTTATCCTC	6741	6760	31	373
532976	n/a	n/a	Intron 9	GTGCTGGGTCCTAGGCAGGT	5130	5149	16	374
532977	n/a	n/a	Intron 1	CAAAAGGCCAAGGAGGGATG	2101	2120	14	375
532978	n/a	n/a	Intron 17	CCATGCTGACCACTTGGCAT	7411	7430	20	376
532979	n/a	n/a	Intron 8	GGAGGCTGGGACAGGAGAGA	4753	4772	25	377
532980	n/a	n/a	Intron 14 - Exon 15	GGAGCAGCTCTTCCTCTGGA	6917	6936	36	378
532981	n/a	n/a	Exon 3 - Intron 3	TCTCACCTCCGTTGTCACAG	2957	2976	20	379
532982	n/a	n/a	Intron 13	CAGTCCTCCAGCCTTTCCCA	6581	6600	21	380
532983	n/a	n/a	Intron 13	AGTCCTCCAGCCTTTCCCAG	6580	6599	22	381
532984	n/a	n/a	Intron 4 - Exon 5	TGAAGGAGTCTGGGAGAGTC	3509	3528	12	382

532985	n/a	n/a	Intron 16 - Exon 17	CAGAATCACCTGCAAGGAGA	7322	7341	20	383
532986	n/a	n/a	Exon 17 - Intron 17	TAGGAAAGGGAGGACTCACT	7382	7401	3	384
532987	n/a	n/a	Exon 4 - Intron 4	ACCTTGGCAGGAAGGCTCCG	3282	3301	12	385
532988	n/a	n/a	Intron 13 - Exon 14	GAGACAAATGGGCCTATAAA	6639	6658	15	386
532989	n/a	n/a	Intron 1	CTGAAGAGAAAGGCTGATGA	2131	2150	17	387
532990	n/a	n/a	Intron 6	AATGATCAGGGAGCTAGTCC	3913	3932	30	388
532991	n/a	n/a	Intron 17	CTTAGCTGACCTAAAGGAAT	7557	7576	22	389
532992	n/a	n/a	Intron 8	TGGGTATAGTGTTACAAGTG	4807	4826	17	390
532993	n/a	n/a	Intron 1	TGAAGAGAAAGGCTGATGAA	2130	2149	19	391
532994	n/a	n/a	Intron 8	GTGTTACAAGTGGACTTAAG	4799	4818	25	392
532995	n/a	n/a	Intron 6	ACCTGTGGGTGAGGAGAACA	4134	4153	24	393
532996	n/a	n/a	Exon 9 - Intron 9	TCACCCAGATAATCCTCCCT	5107	5126	36	394
532952	2608	2627	Exon 18	TGTTGTCGCAGCTGTTTTAA	7843	7862	90	395

Example 116: Antisense inhibition of human Complement Factor B (CFB) in HepG2 cells by MOE gapmers

Additional antisense oligonucleotides were designed targeting human Complement Factor B (CFB) nucleic acid and were tested for their effects on CFB mRNA in vitro. Cultured HepG2 cells at a density of 20,000 cells per well were transfected using electroporation with 4,500 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and CFB mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3460_MGB (forward sequence CGAAGCAGCTCAATGAAATCAA, designated herein as SEQ ID NO: 813; reverse sequence TGCCTGGAGGGCCTTCTT, designated herein as SEQ ID NO: 814; probe sequence AGACCACAAGTTGAAGTC, designated herein as SEQ ID NO: 815) was used to measure mRNA levels. CFB mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of CFB, relative to untreated control cells.

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 5-10-5 MOE gapmers. The 5-10-5 MOE gapmers are 20 nucleosides in length, wherein the central gap segment

comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising five nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in the Tables below is targeted to either the human CFB mRNA, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NM_001710.5) or the human CFB genomic sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NT_007592.15 truncated from nucleotides 31852000 to 31861000) , or both. 'n/a' indicates that the antisense oligonucleotide does not target that particular gene sequence with 100% complementarity.

Table 129

Inhibition of CFB mRNA by 5-10-5 MOE gapmers targeting SEQ ID NO: 1 and 2

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO:1 stop site	Target region	Sequence	% inhibition	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	SEQ ID NO:
532686	1135	1154	Exon 6	ACACTTTTTGGCTCCTGTGA	48	3819	3838	84
532687	1141	1160	Exon 6	GACTAGACACTTTTTGGCTC	63	3825	3844	85
532688	1147	1166	Exon 6	TAAGTTGACTAGACACTTTT	47	3831	3850	86
532689	1153	1172	Exon 6	CTCAATTAAGTTGACTAGAC	57	3837	3856	87
532690	1159	1178	Exon 6-7 Junction	CACCTTCTCAATTAAGTTGA	49	3843	3862	88
532691	1165	1184	Exon 6-7 Junction	ACTTGCCACCTTCTCAATTA	33	n/a	n/a	89
532692	1171	1190	Exon 6-7 Junction	ACCATAACTTGCCACCTTCT	67	n/a	n/a	90
532693	1177	1196	Exon 7	CTTCACACCATAACTTGCCA	56	4153	4172	91
532694	1183	1202	Exon 7	TCTTGGCTTCACACCATAAC	50	4159	4178	92
532695	1208	1227	Exon 7	ATGTGGCATATGTCACTAGA	53	4184	4203	93
532696	1235	1254	Exon 7	CAGACACTTTGACCCAAATT	52	4211	4230	94
532697	1298	1317	Exon 7-8 Juncion	GGTCTTCATAATTGATTTCA	59	n/a	n/a	95
532698	1304	1323	Exon 7-8 Juncion	ACTTGTGGTCTTCATAATTG	52	n/a	n/a	96
532699	1310	1329	Exon 7-8 Juncion	ACTTCAACTTGTGGTCTTCA	85	n/a	n/a	97
532700	1316	1335	Exon 8	TCCCTGACTTCAACTTGTGG	96	4609	4628	98

532701	1322	1341	Exon 8	TGTTAGTCCCTGACTTCAAC	56	4615	4634	99
532702	1328	1347	Exon 8	TCTTGGTGTTAGTCCCTGAC	86	4621	4640	100
532703	1349	1368	Exon 8	TGTACACTGCCTGGAGGGCC	35	4642	4661	101
532704	1355	1374	Exon 8	TCATGCTGTACACTGCCTGG	12	4648	4667	102
532705	1393	1412	Exon 8	GTTCCAGCCTTCAGGAGGGA	27	4686	4705	103
532706	1399	1418	Exon 8	GGTGCGGTTCCAGCCTTCAG	67	4692	4711	104
532707	1405	1424	Exon 8	ATGGCGGGTGCGGTTCCAGC	26	4698	4717	105
532708	1411	1430	Exon 8	GATGACATGGCGGGTGCGGT	28	4704	4723	106
532709	1417	1436	Exon 8	GAGGATGATGACATGGCGGG	6	4710	4729	107
532710	1443	1462	Exon 8-9 Junction	CCCATGTTGTGCAATCCATC	35	n/a	n/a	108
532711	1449	1468	Exon 9	TCCCCGCCCATGTTGTGCAA	28	5023	5042	109
532712	1455	1474	Exon 9	ATTGGGTCCCCGCCCATGTT	19	5029	5048	110
532713	1461	1480	Exon 9	ACAGTAATTGGGTCCCCGCC	29	5035	5054	111
532714	1467	1486	Exon 9	TCAATGACAGTAATTGGGTC	49	5041	5060	112
532715	1473	1492	Exon 9	ATCTCATCAATGACAGTAAT	45	5047	5066	113
532716	1479	1498	Exon 9	TCCCGGATCTCATCAATGAC	54	5053	5072	114
532717	1533	1552	Exon 9-10 Junction	ACATCCAGATAATCCTCCCT	22	n/a	n/a	115
532718	1539	1558	Exon 9-10 Junction	ACATAGACATCCAGATAATC	8	n/a	n/a	116
532719	1545	1564	Exon 9-10 Junction	CCAAACACATAGACATCCAG	30	n/a	n/a	117
532720	1582	1601	Exon 10	AGCATTGATGTTCACTTGGT	62	5231	5250	118
532721	1588	1607	Exon 10	AGCCAAAGCATTGATGTTCA	46	5237	5256	119
532722	1594	1613	Exon 10	CTTGGAAGCCAAAGCATTGA	35	5243	5262	120
532723	1600	1619	Exon 10	GTCTTTCTTGGAAGCCAAAG	43	5249	5268	121
532724	1606	1625	Exon 10	CTCATTGTCTTTCTTGGAAG	40	5255	5274	122
532725	1612	1631	Exon 10	ATGTTGCTCATTGTCTTTCT	49	5261	5280	123
532726	1618	1637	Exon 10	GAACACATGTTGCTCATTGT	68	5267	5286	124
532727	1624	1643	Exon 10	GACTTTGAACACATGTTGCT	54	5273	5292	125
532728	1630	1649	Exon 10	ATCCTTGACTTTGAACACAT	61	5279	5298	126
532729	1636	1655	Exon 10	TTCCATATCCTTGACTTTGA	55	5285	5304	127
532730	1642	1661	Exon 10	CAGGTTTTCCATATCCTTGA	51	5291	5310	440
532731	1686	1705	Exon 10-11 Junction	CTCAGAGACTGGCTTTCATC	41	5827	5846	129
532732	1692	1711	Exon 11	CAGAGACTCAGAGACTGGCT	59	5833	5852	130
516252	1698	1717	Exon 11	ATGCCACAGAGACTCAGAGA	57	5839	5858	131
532733	1704	1723	Exon 11	CAAACCATGCCACAGAGACT	34	5845	5864	132
532734	1710	1729	Exon 11	TGTTCCCAAACCATGCCACA	51	5851	5870	133

532735	1734	1753	Exon 11	TTGTGGTAATCGGTACCCTT	50	5875	5894	134
532736	1740	1759	Exon 11	GGTTGCTTGTGGTAATCGGT	64	5881	5900	135
532737	1746	1765	Exon 11	TGCCATGGTTGCTTGTGGTA	40	5887	5906	136
532738	1752	1771	Exon 11	TTGGCCTGCCATGGTTGCTT	49	5893	5912	137
532739	1758	1777	Exon 11	GAGATCTTGGCCTGCCATGG	47	5899	5918	138
532740	1803	1822	Exon 12	ACAGCCCCCATACAGCTCTC	48	6082	6101	139
532741	1809	1828	Exon 12	GACACCACAGCCCCCATACA	40	6088	6107	140
532742	1815	1834	Exon 12	TACTCAGACACCACAGCCCC	33	6094	6113	141
532743	1821	1840	Exon 12	ACAAAGTACTCAGACACCAC	39	6100	6119	142
532744	1827	1846	Exon 12	GTCAGCACAAAGTACTCAGA	45	6106	6125	143
532745	1872	1891	Exon 12	TTGATTGAGTGTTCCTTGTC	42	6151	6170	144
532746	1878	1897	Exon 12	CTGACCTTGATTGAGTGTTC	53	6157	6176	145
532747	1909	1928	Exon 13	TATCTCCAGGTCCCGCTTCT	31	6403	6422	146
532748	1967	1986	Exon 13	GAATTCCTGCTTCTTTTTTC	30	6461	6480	147
532749	1973	1992	Exon 13	ATTCAGGAATTCCTGCTTCT	40	6467	6486	148
532750	1979	1998	Exon 13	CATAAAATTCAGGAATTCCT	45	6473	6492	149
532751	1985	2004	Exon 13	CATAGTCATAAAATTCAGGA	43	6479	6498	150
532752	2006	2025	Exon 13	TGAGCTTGATCAGGGCAACG	61	6500	6519	151
532753	2012	2031	Exon 13	TATTCTTGAGCTTGATCAGG	47	6506	6525	152
532754	2048	2067	Exon 13-14 Junction	GACAAATGGGCCTGATAGTC	35	n/a	n/a	153
532755	2070	2089	Exon 14	GTTGTTCCCTCGGTGCAGGG	43	6659	6678	154
532756	2076	2095	Exon 14	GCTCGAGTTGTTCCCTCGGT	51	6665	6684	155
532757	2082	2101	Exon 14	CTCAAAGCTCGAGTTGTTCC	36	6671	6690	156
532758	2088	2107	Exon 14	GGAAGCCTCAAAGCTCGAGT	54	6677	6696	157
532759	2094	2113	Exon 14	GTTGGAGGAAGCCTCAAAGC	52	6683	6702	158
532760	2100	2119	Exon 14	GTGGTAGTTGGAGGAAGCCT	22	6689	6708	159
532761	2106	2125	Exon 14	TGGCAAGTGGTAGTTGGAGG	34	6695	6714	160
532762	2112	2131	Exon 14	TGTTGCTGGCAAGTGGTAGT	52	6701	6720	161

Example 117: Antisense inhibition of human Complement Factor B (CFB) in HepG2 cells by MOE gapmers

Additional antisense oligonucleotides were designed targeting human Complement Factor B (CFB) nucleic acid and were tested for their effects on CFB mRNA in vitro. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. Cultured HepG2 cells at a density of 20,000 cells per well were transfected using electroporation with 5,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and CFB mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3459 was used to measure mRNA levels. CFB

mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of CFB, relative to untreated control cells.

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 5-10-5 MOE gapmers. The gapmers are 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising five nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines. "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in the Tables below is targeted to either the human CFB mRNA, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NM_001710.5) or the human CFB genomic sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NT_007592.15 truncated from nucleotides 31852000 to 31861000) , or both. 'n/a' indicates that the antisense oligonucleotide does not target that particular gene sequence with 100% complementarity. In case the sequence alignment for a target gene in a particular table is not shown, it is understood that none of the oligonucleotides presented in that table align with 100% complementarity with that target gene.

Table 130

Inhibition of CFB mRNA by 5-10-5 MOE gapmers targeting SEQ ID NO: 1

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target region	Sequence	% inhibition	SEQ ID NO:
588570	150	169	Exon 1	TGGTCACATTCCCTTCCCCT	54	396
588571	152	171	Exon 1	CCTGGTCACATTCCCTTCCC	63	397
532614	154	173	Exon 1	GACCTGGTCACATTCCCTTC	64	12
588572	156	175	Exon 1	TAGACCTGGTCACATTCCCT	62	398
588573	158	177	Exon 1	CCTAGACCTGGTCACATTCC	53	399
588566	2189	2208	Exon 15	CCTTCCGAGTCAGCTTTTTC	60	400
588567	2191	2210	Exon 15	CTCCTTCCGAGTCAGCTTTT	61	401
532770	2193	2212	Exon 15	ACCTCCTTCCGAGTCAGCTT	77	198
588568	2195	2214	Exon 15	AGACCTCCTTCCGAGTCAGC	72	402
588569	2197	2216	Exon 15	GTAGACCTCCTTCCGAGTCA	46	403
588574	2453	2472	Exon 18	TTTGCCGCTTCTGGTTTTTG	46	404
588575	2455	2474	Exon 18	CTTTTGCCGCTTCTGGTTTT	41	405
532800	2457	2476	Exon 18	TGCTTTTGCCGCTTCTGGTT	69	228
588576	2459	2478	Exon 18	CCTGCTTTTGCCGCTTCTGG	61	406
588577	2461	2480	Exon 18	TACCTGCTTTTGCCGCTTCT	51	407

516350	2550	2569	Exon 18	AGAAAACCCAAATCCTCATC	71	408
588509	2551	2570	Exon 18	TAGAAAACCCAAATCCTCAT	58	409
588510	2552	2571	Exon 18	ATAGAAAACCCAAATCCTCA	57	410
588511	2553	2572	Exon 18	TATAGAAAACCCAAATCCTC	57	411
588512	2554	2573	Exon 18	TTATAGAAAACCCAAATCCT	44	412
588513	2555	2574	Exon 18	CTTATAGAAAACCCAAATCC	37	413
588514	2556	2575	Exon 18	CCTTATAGAAAACCCAAATC	50	414
588515	2557	2576	Exon 18	CCCTTATAGAAAACCCAAAT	45	415
588516	2558	2577	Exon 18	CCCCTTATAGAAAACCCAAA	60	416
588517	2559	2578	Exon 18	ACCCCTTATAGAAAACCCAA	67	417
588518	2560	2579	Exon 18	AACCCCTTATAGAAAACCCA	57	418
588519	2561	2580	Exon 18	AAACCCCTTATAGAAAACCC	61	419
588520	2562	2581	Exon 18	GAAACCCCTTATAGAAAACC	27	420
588521	2563	2582	Exon 18	GGAAACCCCTTATAGAAAAC	25	421
588522	2564	2583	Exon 18	AGGAAACCCCTTATAGAAAA	36	422
588523	2565	2584	Exon 18	CAGGAAACCCCTTATAGAAA	36	423
588524	2566	2585	Exon 18	GCAGGAAACCCCTTATAGAA	46	424
588525	2567	2586	Exon 18	AGCAGGAAACCCCTTATAGA	38	425
588526	2568	2587	Exon 18	CAGCAGGAAACCCCTTATAG	47	426
588527	2569	2588	Exon 18	CCAGCAGGAAACCCCTTATA	68	427
588528	2570	2589	Exon 18	TCCAGCAGGAAACCCCTTAT	63	428
532809	2571	2590	Exon 18	GTCCAGCAGGAAACCCCTTA	85	237
588529	2572	2591	Exon 18	TGTCCAGCAGGAAACCCCTT	76	429
588530	2573	2592	Exon 18	CTGTCCAGCAGGAAACCCCT	74	430
588531	2574	2593	Exon 18	CCTGTCCAGCAGGAAACCCC	75	431
588532	2575	2594	Exon 18	CCCTGTCCAGCAGGAAACCC	73	432
588533	2576	2595	Exon 18	CCCCTGTCCAGCAGGAAACC	82	433
532810	2577	2596	Exon 18	GCCCCTGTCCAGCAGGAAAC	88	238
588534	2578	2597	Exon 18	CGCCCCTGTCCAGCAGGAAA	86	434
588535	2579	2598	Exon 18	ACGCCCCTGTCCAGCAGGAA	86	435
588536	2580	2599	Exon 18	CACGCCCCTGTCCAGCAGGA	93	436
588537	2581	2600	Exon 18	CCACGCCCCTGTCCAGCAGG	92	437
588538	2582	2601	Exon 18	CCCACGCCCCTGTCCAGCAG	94	438
588539	2583	2602	Exon 18	TCCCACGCCCCTGTCCAGCA	96	439
588540	2584	2603	Exon 18	ATCCCACGCCCCTGTCCAGC	88	440
588541	2585	2604	Exon 18	AATCCCACGCCCCTGTCCAG	79	441
588542	2586	2605	Exon 18	CAATCCCACGCCCCTGTCCA	83	442
588543	2587	2606	Exon 18	TCAATCCCACGCCCCTGTCC	86	443
588544	2588	2607	Exon 18	TTCAATCCCACGCCCCTGTC	90	444
588545	2589	2608	Exon 18	ATTCAATCCCACGCCCCTGT	92	445
588546	2590	2609	Exon 18	AATTCAATCCCACGCCCCTG	92	446
588547	2591	2610	Exon 18	TAATTCAATCCCACGCCCCT	88	447
588548	2592	2611	Exon 18	TTAATTCAATCCCACGCCCC	93	448

588549	2593	2612	Exon 18	TTTAATTCAATCCCACGCCC	88	449
588550	2594	2613	Exon 18	TTTTAATTCAATCCCACGCC	89	450
588551	2595	2614	Exon 18	GTTTTAATTCAATCCCACGC	94	451
588552	2596	2615	Exon 18	TGTTTTAATTCAATCCCACG	93	452
588553	2597	2616	Exon 18	CTGTTTTAATTCAATCCCAC	96	453
588554	2598	2617	Exon 18	GCTGTTTTAATTCAATCCCA	98	454
532811	2599	2618	Exon 18	AGCTGTTTTAATTCAATCCC	97	239
532811	2599	2618	Exon 18	AGCTGTTTTAATTCAATCCC	95	239
588555	2600	2619	Exon 18	CAGCTGTTTTAATTCAATCC	93	455
588556	2601	2620	Exon 18	GCAGCTGTTTTAATTCAATC	96	456
588557	2602	2621	Exon 18	CGCAGCTGTTTTAATTCAAT	98	457
588558	2603	2622	Exon 18	TCGCAGCTGTTTTAATTCAA	95	458
532917	2604	2623	Exon 18	GTCGCAGCTGTTTTAATTCA	97	317
588559	2605	2624	Exon 18	TGTCGCAGCTGTTTTAATTC	95	459
588560	2606	2625	Exon 18	TTGTCGCAGCTGTTTTAATT	92	460
588561	2607	2626	Exon 18	GTTGTCGCAGCTGTTTTAAT	93	461
532952	2608	2627	Exon 18	TGTTGTCGCAGCTGTTTTAA	88	395
588562	2609	2628	Exon 18 / Repeat	TTGTTGTCGCAGCTGTTTTA	90	462
588563	2610	2629	Exon 18 / Repeat	TTTGTTGTCGCAGCTGTTTT	89	463
588564	2611	2630	Exon 18 / Repeat	TTTTGTTGTCGCAGCTGTTT	92	464
588565	2612	2631	Exon 18 / Repeat	TTTTTGTTGTCGCAGCTGTT	88	465

Table 131

Inhibition of CFB mRNA by 5-10-5 MOE gapmers targeting SEQ ID NO: 1 or SEQ ID NO: 2

ISIS NO	SEQ ID NO:1 start site	SEQ ID NO: 1 stop site	Target region	Sequence	% inhibition	SEQ ID NO 2: start site	SEQ ID NO: 2 stop site	SEQ ID NO:
588685	n/a	n/a	Exon 1	GGATCCAGCTCACTCCCCTG	48	1596	1615	466
588686	n/a	n/a	Exon 1	AAATAAGGATCCAGCTCACT	29	1602	n/a	467
588688	n/a	n/a	Exon 1	GACCAGAAATAAGGATCCAG	58	1608	1627	468
588690	n/a	n/a	Exon 1	CTTAGGGACCAGAAATAAGG	45	1614	1633	469
588692	n/a	n/a	Exon 1	CACCCACTTAGGGACCAGAA	36	1620	1639	470
588694	n/a	n/a	Exon 1	ACCACCCACTTAGGGACCAG	47	1622	1641	471
588696	n/a	n/a	Exon 1	AGGTCCAGGACTCTCCCCTT	96	1685	1704	472
588698	n/a	n/a	Exon 1	AAGGTCCAGGACTCTCCCCT	96	1686	1705	473
588700	n/a	n/a	Exon 1	AAACTGCAGAAGTCCCACCC	2	1716	1735	474
588586	30	49	Exon 1	GGAGGGCCCCGCTGAGCTGC	59	1751	1770	475

588587	48	67	Exon 1	TCCCGGAACATCCAAGCGGG	45	1769	1788	476
588588	56	75	Exon 1	CATCACTTTCCCGGAACATC	39	1777	n/a	477
588589	151	170	Exon 1	CTGGTCACATTCCCTTCCCC	29	1872	1891	478
588590	157	176	Exon 1	CTAGACCTGGTCACATTCCC	47	1878	1897	479
588591	339	358	Exon 1-2 Junction	GGAGTGGTGGTCACACCTCC	44	n/a	n/a	480
588592	384	403	Exon 2	ACCCCCTCCAGAGAGCAGGA	43	2192	2211	481
588593	390	409	Exon 2	ATCTCTACCCCCTCCAGAGA	34	2198	2217	482
588594	467	486	Exon 2	GGTACGGGTAGAAGCCAGAA	17	2275	2294	483
588595	671	690	Exon 3	GGAGAGTGTAACCGTCATAG	37	2879	2898	484
588596	689	708	Exon 3	TGCGATTGGCAGAGCCCCGG	18	2897	2916	485
588597	695	714	Exon 3	GGCAGGTGCGATTGGCAGAG	32	2903	2922	486
588598	707	726	Exon 3	GGCCATTCACTTGGCAGGTG	45	2915	2934	487
588599	738	757	Exon 3	TTGTCACAGATCGCTGTCTG	52	2946	2965	488
588600	924	943	Exon 4-5 Junction	AAGGAGTCTTGGCAGGAAGG	39	n/a	n/a	489
588601	931	950	Exon 4-5 Junction	GTACATGAAGGAGTCTTGGC	37	n/a	n/a	490
588602	959	978	Exon 5	AAGCTTCGGCCACCTCTTGA	21	3542	3561	491
588603	1089	1108	Exon 6	CCATCTAGCACCAGGTAGAT	22	3773	3792	492
588604	1108	1127	Exon 6	GGCCCCAATGCTGTCTGATC	21	3792	3811	493
588606	1150	1169	Exon 6	AATTAAGTTGACTAGACACT	56	3834	3853	494
588608	1162	1181	Exon 6-7 Junction	TGCCACCTTCTCAATTAAGT	50		19	495
588578	1167	1186	Exon 6-7 Junction	TAACTTGCCACCTTCTCAAT	23	n/a	n/a	496
588579	1169	1188	Exon 6-7 Junction	CATAACTTGCCACCTTCTCA	23	n/a	n/a	497
532692	1171	1190	Exon 6-7 Junction	ACCATAACTTGCCACCTTCT	15	n/a	n/a	90
588580	1173	1192	Exon 6-7 Junction	ACACCATAACTTGCCACCTT	16	n/a	n/a	498
588581	1175	1194	Exon 6-7 Junction	TCACACCATAACTTGCCACC	14	4151	4170	499
588610	1319	1338	Exon 8	TAGTCCCTGACTTCAACTTG	50	4612	4631	500
588612	1325	1344	Exon 8	TGGTGTTAGTCCCTGACTTC	47	4618	4637	501
588614	1396	1415	Exon 8	GCGGTTCCAGCCTTCAGGAG	47	4689	4708	502
588616	1421	1440	Exon 8	TCATGAGGATGATGACATGG	51	4714	4733	503
588618	1446	1465	Exon 9	CCGCCCATGTTGTGCAATCC	18	5020	5039	504
588620	1458	1477	Exon 9	GTAATTGGGTCCCCGCCCAT	40	5032	5051	505
588623	1482	1501	Exon 9	AAGTCCCGGATCTCATCAAT	40	5056	5075	506
588624	1542	1561	Exon 9-10 Junction	AACACATAGACATCCAGATA	45	n/a	n/a	507

588626	1585	1604	Exon 10	CAAAGCATTGATGTTCACTT	43	5234	5253	508
588628	1621	1640	Exon 10	TTTGAACACATGTTGCTCAT	45	5270	5289	509
588631	1646	1665	Exon 10	CTTCCAGGTTTTCCATATCC	53	5295	5314	510
588632	1647	1666	Exon 10	TCTTCCAGGTTTTCCATATC	56	5296	5315	511
588634	1689	1708	Exon 11	AGACTCAGAGACTGGCTTTC	35	5830	5849	512
588636	1749	1768	Exon 11	GCCTGCCATGGTTGCTTGTG	55	5890	5909	513
588638	1763	1782	Exon 11	TGACTGAGATCTTGGCCTGC	78	5904	5923	514
588640	1912	1931	Exon 13	TTCTATCTCCAGGTCCCGCT	95	6406	6425	515
588642	1982	2001	Exon 13	AGTCATAAAATTCAGGAATT	44	6476	6495	516
588645	2073	2092	Exon 14	CGAGTTGTTCCCTCGGTGCA	40	6662	6681	517
588646	2085	2104	Exon 14	AGCCTCAAAGCTCGAGTTGT	57	6674	6693	518
588648	2091	2110	Exon 14	GGAGGAAGCCTCAAAGCTCG	48	6680	6699	519
588651	2097	2116	Exon 14	GTAGTTGGAGGAAGCCTCAA	40	6686	6705	520
588652	2103	2122	Exon 14	CAAGTGGTAGTTGGAGGAAG	43	6692	6711	521
588654	2166	2185	Exon 15	TCCTCAGACACAAACAGAGC	13	6954	6973	522
588656	2172	2191	Exon 15	TTCTCCTCCTCAGACACAAA	55	6960	6979	523
588658	2196	2215	Exon 15	TAGACCTCCTTCCGAGTCAG	44	6984	7003	524
588660	2202	2221	Exon 15	TTGATGTAGACCTCCTTCCG	50	6990	7009	525
588582	2219	2238	Exon 15-16 Junction	CTTTCTTATCCCCATTCTTG	19	n/a	n/a	526
588583	2221	2240	Exon 15-16 Junction	GCCTTTCTTATCCCCATTCT	14	n/a	n/a	527
532775	2223	2242	Exon 15-16 Junction	CTGCCTTTCTTATCCCCATT	3	n/a	n/a	203
588584	2225	2244	Exon 15-16 Junction	AGCTGCCTTTCTTATCCCCA	18	n/a	n/a	528
588662	2226	2245	Exon 15-16 Junction	CAGCTGCCTTTCTTATCCCC	27	n/a	n/a	529
588585	2227	2246	Exon 15-16 Junction	ACAGCTGCCTTTCTTATCCC	59	n/a	n/a	530
588664	2238	2257	Exon 16	GCATCTCTCTCACAGCTGCC	49	7122	7141	531
588666	2276	2295	Exon 16	AGATGTCCTTGACTTTGTCA	41	7160	7179	532
588668	2330	2349	Exon 16	CAGCATAGGGACTCACTCCT	41	7214	7233	533
588670	2361	2380	Exon 16-17 Junction	CCGCCAGAATCACCTCTGCA	43	n/a	n/a	534
588672	2397	2416	Exon 17	TGAATGAAACGACTTCTCTT	52	7362	7381	535
588674	2430	2449	Exon 18	ACATCCACTACTCCCCAGCT	39	7665	7684	536

588676	2448	2467	Exon 18	CGCTTCTGGTTTTTGCAGAC	69	7683	7702	537
588678	2454	2473	Exon 18	TTTTGCCGCTTCTGGTTTTT	46	7689	7708	538
588680	2466	2485	Exon 18	GCAGGTACCTGCTTTTGCCG	47	7701	7720	539
588682	2532	2551	Exon 18	TCTTGGAGTTTCTCCTTCAG	58	7767	7786	540
532811	2599	2618	Exon 18	AGCTGTTTTAATTCAATCCC	10	7834	7853	239
532917	2604	2623	Exon 18	GTCGCAGCTGTTTTAATTCA	11	7839	7858	317

Example 118: Antisense inhibition of human Complement Factor B (CFB) in HepG2 cells by MOE gapmers

Antisense oligonucleotides were designed targeting human Complement Factor B (CFB) nucleic acid and were tested for their effects on CFB mRNA in vitro. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. Cultured HepG2 cells at a density of 20,000 cells per well were transfected using electroporation with 3,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and CFB mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3459 was used to measure mRNA levels. CFB mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of CFB, relative to untreated control cells.

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 4-8-5 MOE, 5-9-5 MOE, 5-10-5 MOE, 3-10-4 MOE, 3-10-7 MOE, 6-7-6- MOE, 6-8-6 MOE, or 5-7-5 MOE gapmers, or as deoxy, MOE, and (S)-cEt oligonucleotides.

The 4-8-5 MOE gapmers are 17 nucleosides in length, wherein the central gap segment comprises of eight 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising four and five nucleosides respectively. The 5-9-5 MOE gapmers are 19 nucleosides in length, wherein the central gap segment comprises of nine 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising five nucleosides each. The 5-10-5 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising five nucleosides each. The 5-7-5 MOE gapmers are 17 nucleosides in length, wherein the central gap segment comprises of seven 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising five nucleosides each. The 3-10-4 MOE gapmers are 17 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three and four nucleosides respectively. The 3-10-7 MOE gapmers are 20 nucleosides in

length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three and seven nucleosides respectively. The 6-7-6 MOE gapmers are 19 nucleosides in length, wherein the central gap segment comprises of seven 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising six nucleosides each. The 6-8-6 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises of eight 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising six nucleosides each. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines.

The deoxy, MOE and (S)-cEt oligonucleotides are 16 nucleosides in length wherein the nucleoside have either a MOE sugar modification, an (S)-cEt sugar modification, or a deoxy modification. The 'Chemistry' column describes the sugar modifications of each oligonucleotide. 'k' indicates an (S)-cEt sugar modification; 'd' indicates deoxyribose; and 'e' indicates a MOE modification.

"Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in the Tables below is targeted to either the human CFB mRNA, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NM_001710.5) or the human CFB genomic sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NT_007592.15 truncated from nucleotides 31852000 to 31861000), or both. 'n/a' indicates that the antisense oligonucleotide does not target that particular gene sequence with 100% complementarity.

Table 132

Inhibition of CFB mRNA by deoxy, MOE and (S)-cEt oligonucleotides targeting SEQ ID NO: 1 or SEQ ID NO: 2

ISIS NO	SEQ ID NO: 1 Start site	SEQ ID NO: 1 stop site	Target region	Sequence	% inhibition	SEQ ID NO: 2 Start site	SEQ ID NO: 2 Stop site	Motif	SEQ ID NO:
532811	2599	2618	Exon 18	AGCTGTTTTAATTCAATCCC	10	7834	7853	eeeeddddddddddeeeee	239
588884	48	63	Exon 1	GGAACATCCAAGCGGG	79	1769	1784	eekddddddddddkke	541
588872	154	169	Exon 1	TGGTCACATTCCCTTC	91	1875	1890	eekddddddddddkke	542
588873	156	171	Exon 1	CCTGGTCACATTCCCT	91	1877	1892	eekddddddddddkke	543
588874	158	173	Exon 1	GACCTGGTCACATTCC	91	1879	1894	eekddddddddddkke	544
588878	1171	1186	Exon 6-7 Junction	TAACTTGCCACCTTCT	92	n/a	n/a	eekddddddddddkke	545
588879	1173	1188	Exon 6-7 Junction	CATAACTTGCCACCTT	94	n/a	n/a	eekddddddddddkke	546
588880	1175	1190	Exon 6-7 Junction	ACCATAACTTGCCACC	89	4151	4166	eekddddddddddkke	547

588869	2193	2208	Exon 15	CCTTCCGAGTCAGCTT	17	6981	6996	eekddddddddddkke	548
588870	2195	2210	Exon 15	CTCCTTCCGAGTCAGC	78	6983	6998	eekddddddddddkke	549
588871	2197	2212	Exon 15	ACCTCCTTCCGAGTCA	80	6985	7000	eekddddddddddkke	550
588881	2223	2238	Exon 15-16 Junction	CTTTCTTATCCCCATT	93	n/a	n/a	eekddddddddddkke	551
588882	2225	2240	Exon 15-16 Junction	GCCTTTCTTATCCCCA	88	n/a	n/a	eekddddddddddkke	552
588883	2227	2242	Exon 15-16 Junction	CTGCCTTTCTTATCCC	90	n/a	n/a	eekddddddddddkke	553
588875	2457	2472	Exon 18	TTTGCCGCTTCTGGTT	81	7692	7707	eekddddddddddkke	554
588876	2459	2474	Exon 18	CTTTTGCCGCTTCTGG	95	7694	7709	eekddddddddddkke	555
588877	2461	2476	Exon 18	TGCTTTTGCCGCTTCT	91	7696	7711	eekddddddddddkke	556
588807	2551	2566	Exon 18	AAACCCAAATCCTCAT	82	7786	7801	eekddddddddddkke	557
588808	2553	2568	Exon 18	GAAAACCCAAATCCTC	69	7788	7803	eekddddddddddkke	558
588809	2555	2570	Exon 18	TAGAAAACCCAAATCC	51	7790	7805	eekddddddddddkke	559
588810	2556	2571	Exon 18	ATAGAAAACCCAAATC	23	7791	7806	eekddddddddddkke	560
588811	2559	2574	Exon 18	CTTATAGAAAACCCAA	13	7794	7809	eekddddddddddkke	561
588812	2560	2575	Exon 18	CCTTATAGAAAACCCA	29	7795	7810	eekddddddddddkke	562
588813	2561	2576	Exon 18	CCCTTATAGAAAACCC	53	7796	7811	eekddddddddddkke	563
588814	2562	2577	Exon 18	CCCCTTATAGAAAACC	86	7797	7812	eekddddddddddkke	564
588815	2563	2578	Exon 18	ACCCCTTATAGAAAAC	76	7798	7813	eekddddddddddkke	565
588816	2564	2579	Exon 18	AACCCCTTATAGAAAA	33	7799	7814	eekddddddddddkke	566
588817	2565	2580	Exon 18	AAACCCCTTATAGAAA	48	7800	7815	eekddddddddddkke	567
588818	2566	2581	Exon 18	GAAACCCCTTATAGAA	44	7801	7816	eekddddddddddkke	568
588819	2567	2582	Exon 18	GGAAACCCCTTATAGA	74	7802	7817	eekddddddddddkke	569
588820	2568	2583	Exon 18	AGGAAACCCCTTATAG	68	7803	7818	eekddddddddddkke	570
588821	2569	2584	Exon 18	CAGGAAACCCCTTATA	45	7804	7819	eekddddddddddkke	571
588822	2570	2585	Exon 18	GCAGGAAACCCCTTAT	50	7805	7820	eekddddddddddkke	572
588823	2571	2586	Exon 18	AGCAGGAAACCCCTTA	54	7806	7821	eekddddddddddkke	573
588824	2572	2587	Exon 18	CAGCAGGAAACCCCTT	35	7807	7822	eekddddddddddkke	574
588825	2573	2588	Exon 18	CCAGCAGGAAACCCCT	11	7808	7823	eekddddddddddkke	575
588826	2574	2589	Exon 18	TCCAGCAGGAAACCCC	19	7809	7824	eekddddddddddkke	576
588827	2575	2590	Exon 18	GTCCAGCAGGAAACCC	42	7810	7825	eekddddddddddkke	577
588828	2576	2591	Exon 18	TGTCCAGCAGGAAACC	0	7811	7826	eekddddddddddkke	578
588829	2577	2592	Exon 18	CTGTCCAGCAGGAAAC	49	7812	7827	eekddddddddddkke	579
588830	2578	2593	Exon 18	CCTGTCCAGCAGGAAA	11	7813	7828	eekddddddddddkke	580
588831	2579	2594	Exon 18	CCCTGTCCAGCAGGAA	20	7814	7829	eekddddddddddkke	581
588832	2580	2595	Exon 18	CCCCTGTCCAGCAGGA	19	7815	7830	eekddddddddddkke	582
588833	2581	2596	Exon 18	GCCCCTGTCCAGCAGG	12	7816	7831	eekddddddddddkke	583
588834	2582	2597	Exon 18	CGCCCCTGTCCAGCAG	10	7817	7832	eekddddddddddkke	584
588835	2583	2598	Exon 18	ACGCCCCTGTCCAGCA	13	7818	7833	eekddddddddddkke	585

588836	2584	2599	Exon 18	CACGCCCCTGTCCAGC	13	7819	7834	eekddddddddddkke	586
588837	2585	2600	Exon 18	CCACGCCCCTGTCCAG	39	7820	7835	eekddddddddddkke	587
588838	2586	2601	Exon 18	CCCACGCCCCTGTCCA	54	7821	7836	eekddddddddddkke	588
588839	2587	2602	Exon 18	TCCCACGCCCCTGTCC	51	7822	7837	eekddddddddddkke	589
588840	2588	2603	Exon 18	ATCCCACGCCCCTGTC	65	7823	7838	eekddddddddddkke	590
588841	2589	2604	Exon 18	AATCCCACGCCCCTGT	59	7824	7839	eekddddddddddkke	591
588842	2590	2605	Exon 18	CAATCCCACGCCCCTG	70	7825	7840	eekddddddddddkke	592
588843	2591	2606	Exon 18	TCAATCCCACGCCCCT	0	7826	7841	eekddddddddddkke	593
588844	2592	2607	Exon 18	TTCAATCCCACGCCCC	48	7827	7842	eekddddddddddkke	594
588845	2593	2608	Exon 18	ATTCAATCCCACGCCC	46	7828	7843	eekddddddddddkke	595
588846	2594	2609	Exon 18	AATTCAATCCCACGCC	67	7829	7844	eekddddddddddkke	596
588847	2595	2610	Exon 18	TAATTCAATCCCACGC	75	7830	7845	eekddddddddddkke	597
588848	2596	2611	Exon 18	TTAATTCAATCCCACG	76	7831	7846	eekddddddddddkke	598
588849	2597	2612	Exon 18	TTTAATTCAATCCCAC	94	7832	7847	eekddddddddddkke	599
588850	2598	2613	Exon 18	TTTTAATTCAATCCCA	91	7833	7848	eekddddddddddkke	600
588851	2599	2614	Exon 18	GTTTTAATTCAATCCC	91	7834	7849	eekddddddddddkke	601
588852	2600	2615	Exon 18	TGTTTTAATTCAATCC	78	7835	7850	eekddddddddddkke	602
588853	2601	2616	Exon 18	CTGTTTTAATTCAATC	81	7836	7851	eekddddddddddkke	603
588854	2602	2617	Exon 18	GCTGTTTTAATTCAAT	63	7837	7852	eekddddddddddkke	604
588855	2603	2618	Exon 18	AGCTGTTTTAATTCAA	65	7838	7853	eekddddddddddkke	605
588856	2604	2619	Exon 18	CAGCTGTTTTAATTCA	76	7839	7854	eekddddddddddkke	606
588857	2605	2620	Exon 18	GCAGCTGTTTTAATTC	89	7840	7855	eekddddddddddkke	607
588858	2606	2621	Exon 18	CGCAGCTGTTTTAATT	89	7841	7856	eekddddddddddkke	608
588859	2607	2622	Exon 18	TCGCAGCTGTTTTAAT	89	7842	7857	eekddddddddddkke	609
588860	2608	2623	Exon 18	GTCGCAGCTGTTTTAA	76	7843	7858	eekddddddddddkke	610
588861	2609	2624	Exon 18	TGTCGCAGCTGTTTTA	87	7844	7859	eekddddddddddkke	611
588862	2610	2625	Exon 18	TTGTCGCAGCTGTTTT	85	7845	7860	eekddddddddddkke	612
588863	2611	2626	Exon 18	GTTGTCGCAGCTGTTT	87	7846	7861	eekddddddddddkke	613
588864	2612	2627	Exon 18	TGTTGTCGCAGCTGTT	67	7847	7862	eekddddddddddkke	614
588865	2613	2628	Exon 18	TTGTTGTCGCAGCTGT	51	n/a	n/a	eekddddddddddkke	615
588866	2614	2629	Exon 18	TTTGTTGTCGCAGCTG	95	n/a	n/a	eekddddddddddkke	616
588867	2615	2630	Exon 18	TTTTGTTGTCGCAGCT	92	n/a	n/a	eekddddddddddkke	617
588868	2616	2631	Exon 18	TTTTTGTTGTCGCAGC	66	n/a	n/a	eekddddddddddkke	618

Table 133

Inhibition of CFB mRNA by 5-10-5 MOE gapmers targeting SEQ ID NO: 1 or SEQ ID NO: 2

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target region	Sequence	% inhibition	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	SEQ ID NO:

588685	n/a	n/a	Exon 1	GGATCCAGCTCACTCCCCTG	14	1596	1615	466
588686	n/a	n/a	Exon 1	AAATAAGGATCCAGCTCACT	2	1602	1621	467
588688	n/a	n/a	Exon 1	GACCAGAAATAAGGATCCAG	3	1608	1627	468
588690	n/a	n/a	Exon 1	CTTAGGGACCAGAAATAAGG	10	1614	1633	469
588692	n/a	n/a	Exon 1	CACCCACTTAGGGACCAGAA	23	1620	1639	470
588694	n/a	n/a	Exon 1	ACCACCCACTTAGGGACCAG	23	1622	1641	471
588696	n/a	n/a	Exon 1	AGGTCCAGGACTCTCCCCTT	15	1685	1704	472
588698	n/a	n/a	Exon 1	AAGGTCCAGGACTCTCCCCT	19	1686	1705	473
588700	n/a	n/a	Exon 1	AAACTGCAGAAGTCCCACCC	16	1716	1735	474
588586	30	49	Exon 1	GGAGGGCCCCGCTGAGCTGC	11	1751	1770	475
588587	48	67	Exon 1	TCCCGGAACATCCAAGCGGG	14	1769	1788	476
588588	56	75	Exon 1	CATCACTTTCCCGGAACATC	18	1777	1796	477
588589	151	170	Exon 1	CTGGTCACATTCCCTTCCCC	59	1872	1891	478
588590	157	176	Exon 1	CTAGACCTGGTCACATTCCC	59	1878	1897	479
588591	339	358	Exon 1-2 Junction	GGAGTGGTGGTCACACCTCC	45	n/a	n/a	480
588592	384	403	Exon 2	ACCCCCTCCAGAGAGCAGGA	39	2192	2211	481
588593	390	409	Exon 2	ATCTCTACCCCCTCCAGAGA	29	2198	2217	482
588594	467	486	Exon 2	GGTACGGGTAGAAGCCAGAA	47	2275	2294	483
588595	671	690	Exon 3	GGAGAGTGTAACCGTCATAG	44	2879	2898	484
588596	689	708	Exon 3	TGCGATTGGCAGAGCCCCGG	43	2897	2916	638
588597	695	714	Exon 3	GGCAGGTGCGATTGGCAGAG	34	2903	2922	486
588598	707	726	Exon 3	GGCCATTCACTTGGCAGGTG	17	2915	2934	487
588599	738	757	Exon 3	TTGTCACAGATCGCTGTCTG	37	2946	2965	488
588600	924	943	Exon 3-4 Junction	AAGGAGTCTTGGCAGGAAGG	18	n/a	n/a	489
588601	931	950	Exon 3-4 Junction	GTACATGAAGGAGTCTTGGC	32	n/a	n/a	490
588602	959	978	Exon 5	AAGCTTCGGCCACCTCTTGA	45	3542	3561	491
588603	1089	1108	Exon 6	CCATCTAGCACCAGGTAGAT	52	3773	3792	492
588604	1108	1127	Exon 6	GGCCCCAATGCTGTCTGATC	39	3792	3811	493
588606	1150	1169	Exon 6	AATTAAGTTGACTAGACACT	37	3834	3853	494
588608	1162	1181	Exon 6-7 Junction	TGCCACCTTCTCAATTAAGT	21	n/a	n/a	648
588578	1167	1186	Exon 6-7 Junction	TAACTTGCCACCTTCTCAAT	22	n/a	n/a	496
588579	1169	1188	Exon 6-7 Junction	CATAACTTGCCACCTTCTCA	21	n/a	n/a	497
532692	1171	1190	Exon 6-7 Junction	ACCATAACTTGCCACCTTCT	56	n/a	n/a	90
588580	1173	1192	Exon 6-7 Junction	ACACCATAACTTGCCACCTT	50	n/a	n/a	498
588581	1175	1194	Exon 7	TCACACCATAACTTGCCACC	50	4151	4170	499
588610	1319	1338	Exon 8	TAGTCCCTGACTTCAACTTG	47	4612	4631	500

588612	1325	1344	Exon 8	TGGTGTTAGTCCCTGACTTC	47	4618	4637	501
588614	1396	1415	Exon 8	GCGGTTCCAGCCTTCAGGAG	51	4689	4708	502
588616	1421	1440	Exon 8	TCATGAGGATGATGACATGG	18	4714	4733	503
588618	1446	1465	Exon 9	CCGCCCATGTTGTGCAATCC	40	5020	5039	504
588620	1458	1477	Exon 9	GTAATTGGGTCCCCGCCCAT	40	5032	5051	505
588623	1482	1501	Exon 9	AAGTCCCGGATCTCATCAAT	45	5056	5075	506
588624	1542	1561	Exon 9-10 Junction	AACACATAGACATCCAGATA	43	n/a	n/a	507
588626	1585	1604	Exon 10	CAAAGCATTGATGTTCACTT	45	5234	5253	508
588628	1621	1640	Exon 10	TTTGAACACATGTTGCTCAT	53	5270	5289	509
588631	1646	1665	Exon 10	CTTCCAGGTTTTCCATATCC	56	5295	5314	510
588632	1647	1666	Exon 10	TCTTCCAGGTTTTCCATATC	35	5296	5315	511
588634	1689	1708	Exon 11	AGACTCAGAGACTGGCTTTC	55	5830	5849	512
588636	1749	1768	Exon 11	GCCTGCCATGGTTGCTTGTG	78	5890	5909	513
588638	1763	1782	Exon 11	TGACTGAGATCTTGGCCTGC	95	5904	5923	514
588640	1912	1931	Exon 13	TTCTATCTCCAGGTCCCGCT	44	6406	6425	515
588642	1982	2001	Exon 13	AGTCATAAAATTCAGGAATT	40	6476	6495	516
588645	2073	2092	Exon 14	CGAGTTGTTCCCTCGGTGCA	57	6662	6681	517
588646	2085	2104	Exon 14	AGCCTCAAAGCTCGAGTTGT	48	6674	6693	518
588648	2091	2110	Exon 14	GGAGGAAGCCTCAAAGCTCG	40	6680	6699	519
588651	2097	2116	Exon 14	GTAGTTGGAGGAAGCCTCAA	43	6686	6705	520
588652	2103	2122	Exon 14	CAAGTGGTAGTTGGAGGAAG	13	6692	6711	521
588654	2166	2185	Exon 15	TCCTCAGACACAAACAGAGC	55	6954	6973	522
588656	2172	2191	Exon 15	TTCTCCTCCTCAGACACAAA	44	6960	6979	523
588658	2196	2215	Exon 15	TAGACCTCCTTCCGAGTCAG	50	6984	7003	524
588660	2202	2221	Exon 15	TTGATGTAGACCTCCTTCCG	27	6990	7009	525
588582	2219	2238	Exon 15-16 Junction	CTTTCTTATCCCCATTCTTG	49	n/a	n/a	526
588583	2221	2240	Exon 15-16 Junction	GCCTTTCTTATCCCCATTCT	41	n/a	n/a	527
532775	2223	2242	Exon 15-16 Junction	CTGCCTTTCTTATCCCCATT	41	n/a	n/a	203
588584	2225	2244	Exon 15-16 Junction	AGCTGCCTTTCTTATCCCCA	43	n/a	n/a	528
588662	2226	2245	Exon 15-16 Junction	CAGCTGCCTTTCTTATCCCC	52	n/a	n/a	529
588585	2227	2246	Exon 15-16 Junction	ACAGCTGCCTTTCTTATCCC	39	n/a	n/a	530

588664	2238	2257	Exon 16	GCATCTCTCTCACAGCTGCC	69	7122	7141	531
588666	2276	2295	Exon 16	AGATGTCCTTGACTTTGTCA	46	7160	7179	532
588668	2330	2349	Exon 16	CAGCATAGGGACTCACTCCT	47	7214	7233	533
588670	2361	2380	Exon 16-17 Junction	CCGCCAGAATCACCTCTGCA	58	n/a	n/a	534
588672	2397	2416	Exon 17	TGAATGAAACGACTTCTCTT	48	7362	7381	535
588674	2430	2449	Exon 18	ACATCCACTACTCCCCAGCT	29	7665	7684	536
588676	2448	2467	Exon 18	CGCTTCTGGTTTTTGCAGAC	58	7683	7702	537
588678	2454	2473	Exon 18	TTTTGCCGCTTCTGGTTTTT	45	7689	7708	538
588680	2466	2485	Exon 18	GCAGGTACCTGCTTTTGCCG	36	7701	7720	539
588682	2532	2551	Exon 18	TCTTGGAGTTTCTCCTTCAG	47	7767	7786	540
532811	2599	2618	Exon 18	AGCTGTTTTAATTCAATCCC	96	7834	7853	239
532917	2604	2623	Exon 18	GTCGCAGCTGTTTTAATTCA	96	7839	7858	317

Table 134

Inhibition of CFB mRNA by MOE gapmers targeting SEQ ID NO: 1 or SEQ ID NO: 2

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target region	Sequence	% inhibition	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	Motif	SEQ ID NO:
598973	2552	2568	Exon 18	GAAAACCCAAATCCTCA	40	7787	7803	3-10-4	619
599036	2552	2568	Exon 18	GAAAACCCAAATCCTCA	18	7787	7803	5-7-5	619
598974	2553	2569	Exon 18	AGAAAACCCAAATCCTC	28	7788	7804	3-10-4	620
599037	2553	2569	Exon 18	AGAAAACCCAAATCCTC	19	7788	7804	5-7-5	620
598975	2554	2570	Exon 18	TAGAAAACCCAAATCCT	15	7789	7805	3-10-4	621
599038	2554	2570	Exon 18	TAGAAAACCCAAATCCT	32	7789	7805	5-7-5	621
598976	2555	2571	Exon 18	ATAGAAAACCCAAATCC	12	7790	7806	3-10-4	622
599039	2555	2571	Exon 18	ATAGAAAACCCAAATCC	7	7790	7806	5-7-5	622
598977	2557	2573	Exon 18	TTATAGAAAACCCAAAT	13	7792	7808	3-10-4	623
599040	2557	2573	Exon 18	TTATAGAAAACCCAAAT	13	7792	7808	5-7-5	623
598978	2558	2574	Exon 18	CTTATAGAAAACCCAAA	0	7793	7809	3-10-4	624
599041	2558	2574	Exon 18	CTTATAGAAAACCCAAA	0	7793	7809	5-7-5	624
598979	2559	2575	Exon 18	CCTTATAGAAAACCCAA	8	7794	7810	3-10-4	625
599042	2559	2575	Exon 18	CCTTATAGAAAACCCAA	19	7794	7810	5-7-5	625
598980	2560	2576	Exon 18	CCCTTATAGAAAACCCA	42	7795	7811	3-10-4	626
599043	2560	2576	Exon 18	CCCTTATAGAAAACCCA	10	7795	7811	5-7-5	626
598981	2561	2577	Exon 18	CCCCTTATAGAAAACCC	20	7796	7812	3-10-4	627
599044	2561	2577	Exon 18	CCCCTTATAGAAAACCC	12	7796	7812	5-7-5	627
598982	2562	2578	Exon 18	ACCCCTTATAGAAAACC	10	7797	7813	3-10-4	628
599045	2562	2578	Exon 18	ACCCCTTATAGAAAACC	3	7797	7813	5-7-5	628

598983	2563	2579	Exon 18	AACCCCTTATAGAAAAC	0	7798	7814	3-10-4	629
599046	2563	2579	Exon 18	AACCCCTTATAGAAAAC	18	7798	7814	5-7-5	629
598984	2564	2580	Exon 18	AAACCCCTTATAGAAAA	0	7799	7815	3-10-4	630
599047	2564	2580	Exon 18	AAACCCCTTATAGAAAA	7	7799	7815	5-7-5	630
598985	2565	2581	Exon 18	GAAACCCCTTATAGAAA	0	7800	7816	3-10-4	631
599048	2565	2581	Exon 18	GAAACCCCTTATAGAAA	9	7800	7816	5-7-5	631
598986	2566	2582	Exon 18	GGAAACCCCTTATAGAA	0	7801	7817	3-10-4	632
599049	2566	2582	Exon 18	GGAAACCCCTTATAGAA	18	7801	7817	5-7-5	632
598988	2567	2583	Exon 18	AGGAAACCCCTTATAGA	0	7802	7818	3-10-4	633
599050	2567	2583	Exon 18	AGGAAACCCCTTATAGA	8	7802	7818	5-7-5	633
598989	2568	2584	Exon 18	CAGGAAACCCCTTATAG	0	7803	7819	3-10-4	634
598990	2569	2585	Exon 18	GCAGGAAACCCCTTATA	8	7804	7820	3-10-4	635
598991	2570	2586	Exon 18	AGCAGGAAACCCCTTAT	25	7805	7821	3-10-4	636
598992	2571	2587	Exon 18	CAGCAGGAAACCCCTTA	12	7806	7822	3-10-4	637
598993	2572	2588	Exon 18	CCAGCAGGAAACCCCTT	37	7807	7823	3-10-4	638
598994	2573	2589	Exon 18	TCCAGCAGGAAACCCCT	29	7808	7824	3-10-4	639
598995	2574	2590	Exon 18	GTCCAGCAGGAAACCCC	42	7809	7825	3-10-4	640
598996	2575	2591	Exon 18	TGTCCAGCAGGAAACCC	36	7810	7826	3-10-4	641
598997	2576	2592	Exon 18	CTGTCCAGCAGGAAACC	18	7811	7827	3-10-4	642
598998	2577	2593	Exon 18	CCTGTCCAGCAGGAAAC	27	7812	7828	3-10-4	643
598999	2578	2594	Exon 18	CCCTGTCCAGCAGGAAA	61	7813	7829	3-10-4	644
599000	2580	2596	Exon 18	GCCCCTGTCCAGCAGGA	71	7815	7831	3-10-4	645
599001	2581	2597	Exon 18	CGCCCCTGTCCAGCAGG	80	7816	7832	3-10-4	646
599002	2582	2598	Exon 18	ACGCCCCTGTCCAGCAG	68	7817	7833	3-10-4	647
599003	2583	2599	Exon 18	CACGCCCCTGTCCAGCA	71	7818	7834	3-10-4	648
599004	2584	2600	Exon 18	CCACGCCCCTGTCCAGC	76	7819	7835	3-10-4	649
599005	2585	2601	Exon 18	CCCACGCCCCTGTCCAG	70	7820	7836	3-10-4	650
599006	2586	2602	Exon 18	TCCCACGCCCCTGTCCA	65	7821	7837	3-10-4	651
599007	2587	2603	Exon 18	ATCCCACGCCCCTGTCC	60	7822	7838	3-10-4	652
599008	2588	2604	Exon 18	AATCCCACGCCCCTGTC	72	7823	7839	3-10-4	653
599009	2589	2605	Exon 18	CAATCCCACGCCCCTGT	79	7824	7840	3-10-4	654
599010	2590	2606	Exon 18	TCAATCCCACGCCCCTG	73	7825	7841	3-10-4	655
599011	2591	2607	Exon 18	TTCAATCCCACGCCCCT	79	7826	7842	3-10-4	656
599012	2592	2608	Exon 18	ATTCAATCCCACGCCCC	67	7827	7843	3-10-4	657
599013	2593	2609	Exon 18	AATTCAATCCCACGCCC	65	7828	7844	3-10-4	658
599014	2594	2610	Exon 18	TAATTCAATCCCACGCC	74	7829	7845	3-10-4	659
599015	2595	2611	Exon 18	TTAATTCAATCCCACGC	71	7830	7846	3-10-4	660
599016	2596	2612	Exon 18	TTTAATTCAATCCCACG	48	7831	7847	3-10-4	661
599017	2597	2613	Exon 18	TTTTAATTCAATCCCAC	34	7832	7848	3-10-4	662
599018	2598	2614	Exon 18	GTTTTAATTCAATCCCA	56	7833	7849	3-10-4	663
599019	2599	2615	Exon 18	TGTTTTAATTCAATCCC	60	7834	7850	3-10-4	664
599020	2600	2616	Exon 18	CTGTTTTAATTCAATCC	0	7835	7851	3-10-4	665
599021	2601	2617	Exon 18	GCTGTTTTAATTCAATC	33	7836	7852	3-10-4	666

599022	2602	2618	Exon 18	AGCTGTTTTAATTCAAT	17	7837	7853	3-10-4	667
599023	2603	2619	Exon 18	CAGCTGTTTTAATTCAA	52	7838	7854	3-10-4	668
532917	2604	2623	Exon 18	GTCGCAGCTGTTTTAATTCA	86	7839	7858	5-10-5	317
599024	2604	2620	Exon 18	GCAGCTGTTTTAATTCA	88	7839	7855	3-10-4	669
599025	2605	2621	Exon 18	CGCAGCTGTTTTAATTC	85	7840	7856	3-10-4	670
599026	2606	2622	Exon 18	TCGCAGCTGTTTTAATT	69	7841	7857	3-10-4	671
599027	2607	2623	Exon 18	GTCGCAGCTGTTTTAAT	77	7842	7858	3-10-4	672
599028	2608	2624	Exon 18	TGTCGCAGCTGTTTTAA	73	7843	7859	3-10-4	673
599029	2609	2625	Exon 18	TTGTCGCAGCTGTTTTA	78	7844	7860	3-10-4	674
599030	2610	2626	Exon 18	GTTGTCGCAGCTGTTTT	75	7845	7861	3-10-4	675
599031	2611	2627	Exon 18	TGTTGTCGCAGCTGTTT	77	7846	7862	3-10-4	676
599032	2612	2628	Exon 18 / Repeat	TTGTTGTCGCAGCTGTT	79	n/a	n/a	3-10-4	677
599033	2613	2629	Exon 18 / Repeat	TTTGTTGTCGCAGCTGT	80	n/a	n/a	3-10-4	678
599034	2614	2630	Exon 18 / Repeat	TTTTGTTGTCGCAGCTG	78	n/a	n/a	3-10-4	679
599035	2615	2631	Exon 18 / Repeat	TTTTTGTTGTCGCAGCT	63	n/a	n/a	3-10-4	680

Table 135

Inhibition of CFB mRNA by MOE gapmers targeting SEQ ID NO: 1 or SEQ ID NO: 2

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target region	Sequence	% inhibition	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	Motif	SEQ ID NO:
599098	2552	2568	Exon 18	GAAAACCCAAATCCTCA	57	7787	7803	4-8-5	619
599099	2553	2569	Exon 18	AGAAAACCCAAATCCTC	33	7788	7804	4-8-5	620
599100	2554	2570	Exon 18	TAGAAAACCCAAATCCT	32	7789	7805	4-8-5	621
599101	2555	2571	Exon 18	ATAGAAAACCCAAATCC	47	7790	7806	4-8-5	622
599102	2557	2573	Exon 18	TTATAGAAAACCCAAAT	59	7792	7808	4-8-5	623
599103	2558	2574	Exon 18	CTTATAGAAAACCCAAA	10	7793	7809	4-8-5	624
599104	2559	2575	Exon 18	CCTTATAGAAAACCCAA	3	7794	7810	4-8-5	625
599105	2560	2576	Exon 18	CCCTTATAGAAAACCCA	45	7795	7811	4-8-5	626
599106	2561	2577	Exon 18	CCCCTTATAGAAAACCC	49	7796	7812	4-8-5	627
599107	2562	2578	Exon 18	ACCCCTTATAGAAAACC	35	7797	7813	4-8-5	628
599108	2563	2579	Exon 18	AACCCCTTATAGAAAAC	17	7798	7814	4-8-5	629
599109	2564	2580	Exon 18	AAACCCCTTATAGAAAA	36	7799	7815	4-8-5	630
599110	2565	2581	Exon 18	GAAACCCCTTATAGAAA	20	7800	7816	4-8-5	631
599111	2566	2582	Exon 18	GGAAACCCCTTATAGAA	20	7801	7817	4-8-5	632

599112	2567	2583	Exon 18	AGGAAACCCCTTATAGA	15	7802	7818	4-8-5	633
599113	2568	2584	Exon 18	CAGGAAACCCCTTATAG	19	7803	7819	4-8-5	634
599051	2568	2584	Exon 18	CAGGAAACCCCTTATAG	26	7803	7819	5-7-5	634
599114	2569	2585	Exon 18	GCAGGAAACCCCTTATA	18	7804	7820	4-8-5	635
599052	2569	2585	Exon 18	GCAGGAAACCCCTTATA	21	7804	7820	5-7-5	635
599115	2570	2586	Exon 18	AGCAGGAAACCCCTTAT	31	7805	7821	4-8-5	636
599053	2570	2586	Exon 18	AGCAGGAAACCCCTTAT	25	7805	7821	5-7-5	636
599116	2571	2587	Exon 18	CAGCAGGAAACCCCTTA	39	7806	7822	4-8-5	637
599054	2571	2587	Exon 18	CAGCAGGAAACCCCTTA	36	7806	7822	5-7-5	637
599117	2572	2588	Exon 18	CCAGCAGGAAACCCCTT	46	7807	7823	4-8-5	638
599055	2572	2588	Exon 18	CCAGCAGGAAACCCCTT	22	7807	7823	5-7-5	638
599118	2573	2589	Exon 18	TCCAGCAGGAAACCCCT	40	7808	7824	4-8-5	639
599056	2573	2589	Exon 18	TCCAGCAGGAAACCCCT	32	7808	7824	5-7-5	639
599119	2574	2590	Exon 18	GTCCAGCAGGAAACCCC	50	7809	7825	4-8-5	640
599057	2574	2590	Exon 18	GTCCAGCAGGAAACCCC	46	7809	7825	5-7-5	640
599120	2575	2591	Exon 18	TGTCCAGCAGGAAACCC	30	7810	7826	4-8-5	641
599058	2575	2591	Exon 18	TGTCCAGCAGGAAACCC	52	7810	7826	5-7-5	641
599121	2576	2592	Exon 18	CTGTCCAGCAGGAAACC	31	7811	7827	4-8-5	642
599059	2576	2592	Exon 18	CTGTCCAGCAGGAAACC	24	7811	7827	5-7-5	642
599122	2577	2593	Exon 18	CCTGTCCAGCAGGAAAC	23	7812	7828	4-8-5	643
599060	2577	2593	Exon 18	CCTGTCCAGCAGGAAAC	37	7812	7828	5-7-5	643
599123	2578	2594	Exon 18	CCCTGTCCAGCAGGAAA	51	7813	7829	4-8-5	644
599061	2578	2594	Exon 18	CCCTGTCCAGCAGGAAA	34	7813	7829	5-7-5	644
599124	2580	2596	Exon 18	GCCCCTGTCCAGCAGGA	56	7815	7831	4-8-5	645
599062	2580	2596	Exon 18	GCCCCTGTCCAGCAGGA	51	7815	7831	5-7-5	645
599125	2581	2597	Exon 18	CGCCCCTGTCCAGCAGG	70	7816	7832	4-8-5	646
599063	2581	2597	Exon 18	CGCCCCTGTCCAGCAGG	56	7816	7832	5-7-5	646
599126	2582	2598	Exon 18	ACGCCCCTGTCCAGCAG	76	7817	7833	4-8-5	647
599064	2582	2598	Exon 18	ACGCCCCTGTCCAGCAG	61	7817	7833	5-7-5	647
599127	2583	2599	Exon 18	CACGCCCCTGTCCAGCA	67	7818	7834	4-8-5	648
599065	2583	2599	Exon 18	CACGCCCCTGTCCAGCA	64	7818	7834	5-7-5	648
599066	2584	2600	Exon 18	CCACGCCCCTGTCCAGC	40	7819	7835	5-7-5	649
599067	2585	2601	Exon 18	CCCACGCCCCTGTCCAG	37	7820	7836	5-7-5	650
599068	2586	2602	Exon 18	TCCCACGCCCCTGTCCA	31	7821	7837	5-7-5	651
599069	2587	2603	Exon 18	ATCCCACGCCCCTGTCC	39	7822	7838	5-7-5	652
599070	2588	2604	Exon 18	AATCCCACGCCCCTGTC	59	7823	7839	5-7-5	653
599071	2589	2605	Exon 18	CAATCCCACGCCCCTGT	63	7824	7840	5-7-5	657
599072	2590	2606	Exon 18	TCAATCCCACGCCCCTG	74	7825	7841	5-7-5	655
599073	2591	2607	Exon 18	TTCAATCCCACGCCCCT	53	7826	7842	5-7-5	656
599074	2592	2608	Exon 18	ATTCAATCCCACGCCCC	56	7827	7843	5-7-5	657
599075	2593	2609	Exon 18	AATTCAATCCCACGCCC	49	7828	7844	5-7-5	658
599076	2594	2610	Exon 18	TAATTCAATCCCACGCC	54	7829	7845	5-7-5	659
599077	2595	2611	Exon 18	TTAATTCAATCCCACGC	79	7830	7846	5-7-5	660
599078	2596	2612	Exon 18	TTTAATTCAATCCCACG	67	7831	7847	5-7-5	661

599079	2597	2613	Exon 18	TTTTAATTCAATCCCAC	69	7832	7848	5-7-5	662
599080	2598	2614	Exon 18	GTTTTAATTCAATCCCA	79	7833	7849	5-7-5	663
599081	2599	2615	Exon 18	TGTTTTAATTCAATCCC	57	7834	7850	5-7-5	664
599082	2600	2616	Exon 18	CTGTTTTAATTCAATCC	50	7835	7851	5-7-5	665
599083	2601	2617	Exon 18	GCTGTTTTAATTCAATC	67	7836	7852	5-7-5	666
599084	2602	2618	Exon 18	AGCTGTTTTAATTCAAT	60	7837	7853	5-7-5	667
599085	2603	2619	Exon 18	CAGCTGTTTTAATTCAA	71	7838	7854	5-7-5	668
532917	2604	2623	Exon 18	GTCGCAGCTGTTTTAATTCA	82	7839	7858	5-10-5	317
599086	2604	2620	Exon 18	GCAGCTGTTTTAATTCA	81	7839	7855	5-7-5	669
599087	2605	2621	Exon 18	CGCAGCTGTTTTAATTC	88	7840	7856	5-7-5	670
599088	2606	2622	Exon 18	TCGCAGCTGTTTTAATT	84	7841	7857	5-7-5	671
599089	2607	2623	Exon 18	GTCGCAGCTGTTTTAAT	81	7842	7858	5-7-5	672
599090	2608	2624	Exon 18	TGTCGCAGCTGTTTTAA	77	7843	7859	5-7-5	673
599091	2609	2625	Exon 18	TTGTCGCAGCTGTTTTA	74	7844	7860	5-7-5	674
599092	2610	2626	Exon 18	GTTGTCGCAGCTGTTTT	66	7845	7861	5-7-5	675
599093	2611	2627	Exon 18	TGTTGTCGCAGCTGTTT	89	7846	7862	5-7-5	676
599094	2612	2628	Exon 18 / Repeat	TTGTTGTCGCAGCTGTT	82	n/a	n/a	5-7-5	677
599095	2613	2629	Exon 18 / Repeat	TTTGTTGTCGCAGCTGT	87	n/a	n/a	5-7-5	678
599096	2614	2630	Exon 18 / Repeat	TTTTGTTGTCGCAGCTG	85	n/a	n/a	5-7-5	679
599097	2615	2631	Exon 18 / Repeat	TTTTTGTTGTCGCAGCT	78	n/a	n/a	5-7-5	680

Table 136

Inhibition of CFB mRNA by MOE gapmers targeting SEQ ID NO: 1 or SEQ ID NO: 2

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target region	Sequence	% inhibition	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	Motif	SEQ ID NO:
599510	2552	2570	Exon 18	TAGAAAACCCAAATCCTCA	45	7787	7805	5-9-5	681
599331	2553	2571	Exon 18	ATAGAAAACCCAAATCCTC	46	7788	7806	5-9-5	682
599332	2554	2572	Exon 18	TATAGAAAACCCAAATCCT	38	7789	7807	5-9-5	683
599333	2556	2574	Exon 18	CTTATAGAAAACCCAAATC	1	7791	7809	5-9-5	684
599334	2557	2575	Exon 18	CCTTATAGAAAACCCAAAT	5	7792	7810	5-9-5	685
599335	2558	2576	Exon 18	CCCTTATAGAAAACCCAAA	34	7793	7811	5-9-5	686
599336	2559	2577	Exon 18	CCCCTTATAGAAAACCCAA	40	7794	7812	5-9-5	687
599337	2560	2578	Exon 18	ACCCCTTATAGAAAACCCA	39	7795	7813	5-9-5	688
599338	2561	2579	Exon 18	AACCCCTTATAGAAAACCC	57	7796	7814	5-9-5	689
599339	2562	2580	Exon 18	AAACCCCTTATAGAAAACC	26	7797	7815	5-9-5	690

599281	2562	2580	Exon 18	AAACCCCTTATAGAAAACC	15	7797	7815	6-7-6	690
599340	2563	2581	Exon 18	GAAACCCCTTATAGAAAAC	17	7798	7816	5-9-5	691
599282	2563	2581	Exon 18	GAAACCCCTTATAGAAAAC	12	7798	7816	6-7-6	691
599341	2564	2582	Exon 18	GGAAACCCCTTATAGAAAA	23	7799	7817	5-9-5	692
599283	2564	2582	Exon 18	GGAAACCCCTTATAGAAAA	18	7799	7817	6-7-6	692
599342	2565	2583	Exon 18	AGGAAACCCCTTATAGAAA	10	7800	7818	5-9-5	693
599284	2565	2583	Exon 18	AGGAAACCCCTTATAGAAA	14	7800	7818	6-7-6	693
599343	2566	2584	Exon 18	CAGGAAACCCCTTATAGAA	10	7801	7819	5-9-5	694
599285	2566	2584	Exon 18	CAGGAAACCCCTTATAGAA	13	7801	7819	6-7-6	694
599344	2567	2585	Exon 18	GCAGGAAACCCCTTATAGA	22	7802	7820	5-9-5	695
599286	2567	2585	Exon 18	GCAGGAAACCCCTTATAGA	31	7802	7820	6-7-6	695
599345	2568	2586	Exon 18	AGCAGGAAACCCCTTATAG	19	7803	7821	5-9-5	696
599287	2568	2586	Exon 18	AGCAGGAAACCCCTTATAG	12	7803	7821	6-7-6	696
599346	2569	2587	Exon 18	CAGCAGGAAACCCCTTATA	30	7804	7822	5-9-5	697
599288	2569	2587	Exon 18	CAGCAGGAAACCCCTTATA	28	7804	7822	6-7-6	697
599347	2570	2588	Exon 18	CCAGCAGGAAACCCCTTAT	46	7805	7823	5-9-5	698
599289	2570	2588	Exon 18	CCAGCAGGAAACCCCTTAT	32	7805	7823	6-7-6	698
599348	2571	2589	Exon 18	TCCAGCAGGAAACCCCTTA	44	7806	7824	5-9-5	699
599290	2571	2589	Exon 18	TCCAGCAGGAAACCCCTTA	24	7806	7824	6-7-6	699
599349	2572	2590	Exon 18	GTCCAGCAGGAAACCCCTT	60	7807	7825	5-9-5	700
599291	2572	2590	Exon 18	GTCCAGCAGGAAACCCCTT	38	7807	7825	6-7-6	700
599350	2573	2591	Exon 18	TGTCCAGCAGGAAACCCCT	49	7808	7826	5-9-5	701
599292	2573	2591	Exon 18	TGTCCAGCAGGAAACCCCT	35	7808	7826	6-7-6	701
599351	2575	2593	Exon 18	CCTGTCCAGCAGGAAACCC	46	7810	7828	5-9-5	702
599293	2575	2593	Exon 18	CCTGTCCAGCAGGAAACCC	12	7810	7828	6-7-6	702
599352	2576	2594	Exon 18	CCCTGTCCAGCAGGAAACC	49	7811	7829	5-9-5	703
599294	2576	2594	Exon 18	CCCTGTCCAGCAGGAAACC	38	7811	7829	6-7-6	703
599353	2577	2595	Exon 18	CCCCTGTCCAGCAGGAAAC	64	7812	7830	5-9-5	704
599295	2577	2595	Exon 18	CCCCTGTCCAGCAGGAAAC	33	7812	7830	6-7-6	704
599354	2578	2596	Exon 18	GCCCCTGTCCAGCAGGAAA	56	7813	7831	5-9-5	705
599296	2578	2596	Exon 18	GCCCCTGTCCAGCAGGAAA	13	7813	7831	6-7-6	705
599355	2580	2598	Exon 18	ACGCCCCTGTCCAGCAGGA	81	7815	7833	5-9-5	706
599297	2580	2598	Exon 18	ACGCCCCTGTCCAGCAGGA	57	7815	7833	6-7-6	706
599356	2581	2599	Exon 18	CACGCCCCTGTCCAGCAGG	64	7816	7834	5-9-5	707
599298	2581	2599	Exon 18	CACGCCCCTGTCCAGCAGG	39	7816	7834	6-7-6	707
599299	2582	2600	Exon 18	CCACGCCCCTGTCCAGCAG	55	7817	7835	6-7-6	708
599300	2583	2601	Exon 18	CCCACGCCCCTGTCCAGCA	45	7818	7836	6-7-6	709
599301	2584	2602	Exon 18	TCCCACGCCCCTGTCCAGC	39	7819	7837	6-7-6	710
599302	2585	2603	Exon 18	ATCCCACGCCCCTGTCCAG	27	7820	7838	6-7-6	711
599303	2586	2604	Exon 18	AATCCCACGCCCCTGTCCA	35	7821	7839	6-7-6	712
599304	2587	2605	Exon 18	CAATCCCACGCCCCTGTCC	16	7822	7840	6-7-6	713
599305	2588	2606	Exon 18	TCAATCCCACGCCCCTGTC	41	7823	7841	6-7-6	714
599306	2589	2607	Exon 18	TTCAATCCCACGCCCCTGT	70	7824	7842	6-7-6	715

599307	2590	2608	Exon 18	ATTCAATCCCACGCCCCTG	66	7825	7843	6-7-6	716
599308	2591	2609	Exon 18	AATTCAATCCCACGCCCCT	68	7826	7844	6-7-6	717
599309	2592	2610	Exon 18	TAATTCAATCCCACGCCCC	52	7827	7845	6-7-6	718
599310	2593	2611	Exon 18	TTAATTCAATCCCACGCCC	39	7828	7846	6-7-6	719
599311	2594	2612	Exon 18	TTTAATTCAATCCCACGCC	83	7829	7847	6-7-6	720
599312	2595	2613	Exon 18	TTTTAATTCAATCCCACGC	72	7830	7848	6-7-6	721
599313	2596	2614	Exon 18	GTTTTAATTCAATCCCACG	86	7831	7849	6-7-6	722
599314	2597	2615	Exon 18	TGTTTTAATTCAATCCCAC	91	7832	7850	6-7-6	723
599315	2598	2616	Exon 18	CTGTTTTAATTCAATCCCA	71	7833	7851	6-7-6	724
599316	2599	2617	Exon 18	GCTGTTTTAATTCAATCCC	89	7834	7852	6-7-6	725
599317	2600	2618	Exon 18	AGCTGTTTTAATTCAATCC	87	7835	7853	6-7-6	726
599318	2601	2619	Exon 18	CAGCTGTTTTAATTCAATC	81	7836	7854	6-7-6	727
599319	2602	2620	Exon 18	GCAGCTGTTTTAATTCAAT	75	7837	7855	6-7-6	728
599320	2603	2621	Exon 18	CGCAGCTGTTTTAATTCAA	84	7838	7856	6-7-6	729
532917	2604	2623	Exon 18	GTCGCAGCTGTTTTAATTCA	92	7839	7858	5-10-5	317
599321	2604	2622	Exon 18	TCGCAGCTGTTTTAATTCA	90	7839	7857	6-7-6	730
599322	2605	2623	Exon 18	GTCGCAGCTGTTTTAATTC	89	7840	7858	6-7-6	731
599323	2606	2624	Exon 18	TGTCGCAGCTGTTTTAATT	81	7841	7859	6-7-6	732
599324	2607	2625	Exon 18	TTGTCGCAGCTGTTTTAAT	68	7842	7860	6-7-6	733
599325	2608	2626	Exon 18	GTTGTCGCAGCTGTTTTAA	71	7843	7861	6-7-6	734
599326	2609	2627	Exon 18	TGTTGTCGCAGCTGTTTTA	52	7844	7862	6-7-6	735
599327	2610	2628	Exon 18 / Repeat	TTGTTGTCGCAGCTGTTTT	88	n/a	n/a	6-7-6	736
599328	2611	2629	Exon 18 / Repeat	TTTGTTGTCGCAGCTGTTT	87	n/a	n/a	6-7-6	737
599329	2612	2630	Exon 18 / Repeat	TTTTGTTGTCGCAGCTGTT	84	n/a	n/a	6-7-6	738
599330	2613	2631	Exon 18 / Repeat	TTTTTGTTGTCGCAGCTGT	87	n/a	n/a	6-7-6	739

Table 137

Inhibition of CFB mRNA by MOE gapmers targeting SEQ ID NO: 1 or SEQ ID NO: 2

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target region	Sequence	% inhibition	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	Motif	SEQ ID NO:
599512	2552	2571	Exon 18	ATAGAAAACCCAAATCCTCA	74	7787	7806	3-10-7	410
599449	2553	2572	Exon 18	TATAGAAAACCCAAATCCTC	43	7788	7807	3-10-7	411
599450	2554	2573	Exon 18	TTATAGAAAACCCAAATCCT	51	7789	7808	3-10-7	412
599451	2555	2574	Exon 18	CTTATAGAAAACCCAAATCC	35	7790	7809	3-10-7	413

599452	2556	2575	Exon 18	CCTTATAGAAAACCCAAATC	34	7791	7810	3-10-7	414
599453	2557	2576	Exon 18	CCCTTATAGAAAACCCAAAT	44	7792	7811	3-10-7	415
599454	2558	2577	Exon 18	CCCCTTATAGAAAACCCAAA	54	7793	7812	3-10-7	416
599455	2559	2578	Exon 18	ACCCCTTATAGAAAACCCAA	53	7794	7813	3-10-7	417
599456	2560	2579	Exon 18	AACCCCTTATAGAAAACCCA	69	7795	7814	3-10-7	418
599457	2561	2580	Exon 18	AAACCCCTTATAGAAAACCC	46	7796	7815	3-10-7	419
599458	2562	2581	Exon 18	GAAACCCCTTATAGAAAACC	0	7797	7816	3-10-7	420
599459	2563	2582	Exon 18	GGAAACCCCTTATAGAAAAC	12	7798	7817	3-10-7	421
599460	2564	2583	Exon 18	AGGAAACCCCTTATAGAAAA	17	7799	7818	3-10-7	422
599461	2565	2584	Exon 18	CAGGAAACCCCTTATAGAAA	24	7800	7819	3-10-7	423
599462	2566	2585	Exon 18	GCAGGAAACCCCTTATAGAA	33	7801	7820	3-10-7	424
599463	2567	2586	Exon 18	AGCAGGAAACCCCTTATAGA	38	7802	7821	3-10-7	425
599464	2568	2587	Exon 18	CAGCAGGAAACCCCTTATAG	33	7803	7822	3-10-7	426
599465	2569	2588	Exon 18	CCAGCAGGAAACCCCTTATA	49	7804	7823	3-10-7	427
599466	2570	2589	Exon 18	TCCAGCAGGAAACCCCTTAT	45	7805	7824	3-10-7	428
599467	2571	2590	Exon 18	GTCCAGCAGGAAACCCCTTA	60	7806	7825	3-10-7	237
599468	2572	2591	Exon 18	TGTCCAGCAGGAAACCCCTT	61	7807	7826	3-10-7	429
599469	2573	2592	Exon 18	CTGTCCAGCAGGAAACCCCT	52	7808	7827	3-10-7	430
599470	2574	2593	Exon 18	CCTGTCCAGCAGGAAACCCC	45	7809	7828	3-10-7	431
599471	2575	2594	Exon 18	CCCTGTCCAGCAGGAAACCC	67	7810	7829	3-10-7	432
599472	2576	2595	Exon 18	CCCCTGTCCAGCAGGAAACC	79	7811	7830	3-10-7	433
599473	2577	2596	Exon 18	GCCCCTGTCCAGCAGGAAAC	72	7812	7831	3-10-7	238
599474	2578	2597	Exon 18	CGCCCCTGTCCAGCAGGAAA	87	7813	7832	3-10-7	434
599475	2579	2598	Exon 18	ACGCCCCTGTCCAGCAGGAA	76	7814	7833	3-10-7	435
599476	2580	2599	Exon 18	CACGCCCCTGTCCAGCAGGA	81	7815	7834	3-10-7	436
599477	2581	2600	Exon 18	CCACGCCCCTGTCCAGCAGG	83	7816	7835	3-10-7	437
599478	2582	2601	Exon 18	CCCACGCCCCTGTCCAGCAG	72	7817	7836	3-10-7	438
599479	2583	2602	Exon 18	TCCCACGCCCCTGTCCAGCA	81	7818	7837	3-10-7	439
599480	2584	2603	Exon 18	ATCCCACGCCCCTGTCCAGC	77	7819	7838	3-10-7	440
599481	2585	2604	Exon 18	AATCCCACGCCCCTGTCCAG	83	7820	7839	3-10-7	441
599482	2586	2605	Exon 18	CAATCCCACGCCCCTGTCCA	87	7821	7840	3-10-7	442
599483	2587	2606	Exon 18	TCAATCCCACGCCCCTGTCC	90	7822	7841	3-10-7	443
599484	2588	2607	Exon 18	TTCAATCCCACGCCCCTGTC	72	7823	7842	3-10-7	444
599485	2589	2608	Exon 18	ATTCAATCCCACGCCCCTGT	82	7824	7843	3-10-7	445
599486	2590	2609	Exon 18	AATTCAATCCCACGCCCCTG	84	7825	7844	3-10-7	446
599487	2591	2610	Exon 18	TAATTCAATCCCACGCCCCT	84	7826	7845	3-10-7	447
599488	2592	2611	Exon 18	TTAATTCAATCCCACGCCCC	87	7827	7846	3-10-7	448
599489	2593	2612	Exon 18	TTTAATTCAATCCCACGCCC	87	7828	7847	3-10-7	449
599490	2594	2613	Exon 18	TTTTAATTCAATCCCACGCC	86	7829	7848	3-10-7	450
599491	2595	2614	Exon 18	GTTTTAATTCAATCCCACGC	87	7830	7849	3-10-7	451
599492	2596	2615	Exon 18	TGTTTTAATTCAATCCCACG	88	7831	7850	3-10-7	452
599493	2597	2616	Exon 18	CTGTTTTAATTCAATCCCAC	75	7832	7851	3-10-7	453
599433	2597	2616	Exon 18	CTGTTTTAATTCAATCCCAC	89	7832	7851	6-8-6	453

599494	2598	2617	Exon 18	GCTGTTTTAATTCAATCCCA	90	7833	7852	3-10-7	454
599434	2598	2617	Exon 18	GCTGTTTTAATTCAATCCCA	89	7833	7852	6-8-6	454
599495	2599	2618	Exon 18	AGCTGTTTTAATTCAATCCC	88	7834	7853	3-10-7	239
599435	2599	2618	Exon 18	AGCTGTTTTAATTCAATCCC	91	7834	7853	6-8-6	239
599496	2600	2619	Exon 18	CAGCTGTTTTAATTCAATCC	89	7835	7854	3-10-7	455
599436	2600	2619	Exon 18	CAGCTGTTTTAATTCAATCC	89	7835	7854	6-8-6	455
599497	2601	2620	Exon 18	GCAGCTGTTTTAATTCAATC	89	7836	7855	3-10-7	456
599437	2601	2620	Exon 18	GCAGCTGTTTTAATTCAATC	91	7836	7855	6-8-6	456
599498	2602	2621	Exon 18	CGCAGCTGTTTTAATTCAAT	88	7837	7856	3-10-7	457
599438	2602	2621	Exon 18	CGCAGCTGTTTTAATTCAAT	90	7837	7856	6-8-6	457
599499	2603	2622	Exon 18	TCGCAGCTGTTTTAATTCAA	81	7838	7857	3-10-7	458
599439	2603	2622	Exon 18	TCGCAGCTGTTTTAATTCAA	88	7838	7857	6-8-6	458
532917	2604	2623	Exon 18	GTCGCAGCTGTTTTAATTCA	90	7839	7858	5-10-5	317
599500	2604	2623	Exon 18	GTCGCAGCTGTTTTAATTCA	88	7839	7858	3-10-7	317
599440	2604	2623	Exon 18	GTCGCAGCTGTTTTAATTCA	88	7839	7858	6-8-6	317
599501	2605	2624	Exon 18	TGTCGCAGCTGTTTTAATTC	78	7840	7859	3-10-7	459
599441	2605	2624	Exon 18	TGTCGCAGCTGTTTTAATTC	90	7840	7859	6-8-6	459
599502	2606	2625	Exon 18	TTGTCGCAGCTGTTTTAATT	87	7841	7860	3-10-7	460
599442	2606	2625	Exon 18	TTGTCGCAGCTGTTTTAATT	76	7841	7860	6-8-6	460
599503	2607	2626	Exon 18	GTTGTCGCAGCTGTTTTAAT	83	7842	7861	3-10-7	461
599443	2607	2626	Exon 18	GTTGTCGCAGCTGTTTTAAT	77	7842	7861	6-8-6	461
599504	2608	2627	Exon 18	TGTTGTCGCAGCTGTTTTAA	89	7843	7862	3-10-7	395
599444	2608	2627	Exon 18	TGTTGTCGCAGCTGTTTTAA	69	7843	7862	6-8-6	395
599505	2609	2628	Exon 19 / Repeat	TTGTTGTCGCAGCTGTTTTA	83	n/a	n/a	3-10-7	462
599445	2609	2628	Exon 19 / Repeat	TTGTTGTCGCAGCTGTTTTA	85	n/a	n/a	6-8-6	462
599506	2610	2629	Exon 19 / Repeat	TTTGTTGTCGCAGCTGTTTT	89	n/a	n/a	3-10-7	463
599446	2610	2629	Exon 19 / Repeat	TTTGTTGTCGCAGCTGTTTT	85	n/a	n/a	6-8-6	463
599507	2611	2630	Exon 19 / Repeat	TTTTGTTGTCGCAGCTGTTT	82	n/a	n/a	3-10-7	464
599447	2611	2630	Exon 19 / Repeat	TTTTGTTGTCGCAGCTGTTT	83	n/a	n/a	6-8-6	464
599508	2612	2631	Exon 19 / Repeat	TTTTTGTTGTCGCAGCTGTT	90	n/a	n/a	3-10-7	465
599448	2612	2631	Exon 19 / Repeat	TTTTTGTTGTCGCAGCTGTT	87	n/a	n/a	6-8-6	465

Example 119: Antisense inhibition of human Complement Factor B (CFB) in HepG2 cells by MOE gapmers

Additional antisense oligonucleotides were designed targeting human Complement Factor B (CFB) nucleic acid and were tested for their effects on CFB mRNA in vitro. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. Cultured HepG2 cells at a density of 20,000 cells per well were transfected using electroporation with 2,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and CFB mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3459 was used to measure mRNA levels. CFB mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of CFB, relative to untreated control cells.

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 4-8-5 MOE, 5-8-5 MOE, 5-9-5 MOE, 5-10-5 MOE, 6-7-6- MOE, 3-10-5 MOE, or 6-8-6 MOE gapmers.

The 4-8-5 MOE gapmers are 17 nucleosides in length, wherein the central gap segment comprises of eight 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising four and five nucleosides respectively. The 5-8-5 MOE gapmers are 18 nucleosides in length, wherein the central gap segment comprises of eight 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising five nucleosides each. The 5-9-5 MOE gapmers are 19 nucleosides in length, wherein the central gap segment comprises of nine 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising five nucleosides each. The 5-10-5 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising five nucleosides each. The 3-10-5 MOE gapmers are 18 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three and five nucleosides respectively. The 6-7-6 MOE gapmers are 19 nucleosides in length, wherein the central gap segment comprises of seven 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising six nucleosides each. The 6-8-6 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises of eight 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising six nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines.

"Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence.

Each gapmer listed in the Tables below is targeted to either the human CFB mRNA, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NM_001710.5) or the human CFB genomic sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NT_007592.15 truncated from nucleotides 31852000 to 31861000), or both. 'n/a' indicates that the antisense oligonucleotide does not target that particular gene sequence with 100% complementarity.

Table 138

Inhibition of CFB mRNA by MOE gapmers targeting SEQ ID NO: 1 or SEQ ID NO: 2

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target region	Sequence	% inhibition	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	Motif	SEQ ID NO:
599160	2560	2577	Exon 18	CCCCTTATAGAAAACCCA	26	7795	7812	5-8-5	740
599161	2561	2578	Exon 18	ACCCCTTATAGAAAACCC	20	7796	7813	5-8-5	741
599162	2562	2579	Exon 18	AACCCCTTATAGAAAACC	12	7797	7814	5-8-5	742
599163	2563	2580	Exon 18	AAACCCCTTATAGAAAAC	11	7798	7815	5-8-5	743
599164	2564	2581	Exon 18	GAAACCCCTTATAGAAAA	11	7799	7816	5-8-5	744
599165	2566	2583	Exon 18	AGGAAACCCCTTATAGAA	0	7801	7818	5-8-5	745
599166	2567	2584	Exon 18	CAGGAAACCCCTTATAGA	12	7802	7819	5-8-5	746
599167	2568	2585	Exon 18	GCAGGAAACCCCTTATAG	14	7803	7820	5-8-5	747
599168	2569	2586	Exon 18	AGCAGGAAACCCCTTATA	16	7804	7821	5-8-5	748
599169	2570	2587	Exon 18	CAGCAGGAAACCCCTTAT	24	7805	7822	5-8-5	749
599170	2571	2588	Exon 18	CCAGCAGGAAACCCCTTA	37	7806	7823	5-8-5	750
599171	2572	2589	Exon 18	TCCAGCAGGAAACCCCTT	30	7807	7824	5-8-5	751
599172	2573	2590	Exon 18	GTCCAGCAGGAAACCCCT	43	7808	7825	5-8-5	752
599173	2574	2591	Exon 18	TGTCCAGCAGGAAACCCC	47	7809	7826	5-8-5	753
599174	2575	2592	Exon 18	CTGTCCAGCAGGAAACCC	27	7810	7827	5-8-5	754
599175	2576	2593	Exon 18	CCTGTCCAGCAGGAAACC	30	7811	7828	5-8-5	755
599176	2577	2594	Exon 18	CCCTGTCCAGCAGGAAAC	34	7812	7829	5-8-5	756
599177	2578	2595	Exon 18	CCCCTGTCCAGCAGGAAA	41	7813	7830	5-8-5	757
599178	2580	2597	Exon 18	CGCCCCTGTCCAGCAGGA	67	7815	7832	5-8-5	758
599179	2581	2598	Exon 18	ACGCCCCTGTCCAGCAGG	61	7816	7833	5-8-5	759
599180	2582	2599	Exon 18	CACGCCCCTGTCCAGCAG	62	7817	7834	5-8-5	760
599181	2583	2600	Exon 18	CCACGCCCCTGTCCAGCA	63	7818	7835	5-8-5	761
599128	2584	2600	Exon 18	CCACGCCCCTGTCCAGC	55	7819	7835	4-8-5	649
599182	2584	2601	Exon 18	CCCACGCCCCTGTCCAGC	58	7819	7836	5-8-5	762
599129	2585	2601	Exon 18	CCCACGCCCCTGTCCAG	41	7820	7836	4-8-5	650
599183	2585	2602	Exon 18	TCCCACGCCCCTGTCCAG	43	7820	7837	5-8-5	763
599130	2586	2602	Exon 18	TCCCACGCCCCTGTCCA	46	7821	7837	4-8-5	651
599184	2586	2603	Exon 18	ATCCCACGCCCCTGTCCA	32	7821	7838	5-8-5	764

599131	2587	2603	Exon 18	ATCCCACGCCCCTGTCC	30	7822	7838	4-8-5	652
599185	2587	2604	Exon 18	AATCCCACGCCCCTGTCC	35	7822	7839	5-8-5	765
599132	2588	2604	Exon 18	AATCCCACGCCCCTGTC	52	7823	7839	4-8-5	653
599186	2588	2605	Exon 18	CAATCCCACGCCCCTGTC	55	7823	7840	5-8-5	766
599133	2589	2605	Exon 18	CAATCCCACGCCCCTGT	66	7824	7840	4-8-5	654
599187	2589	2606	Exon 18	TCAATCCCACGCCCCTGT	72	7824	7841	5-8-5	767
599134	2590	2606	Exon 18	TCAATCCCACGCCCCTG	80	7825	7841	4-8-5	655
599188	2590	2607	Exon 18	TTCAATCCCACGCCCCTG	92	7825	7842	5-8-5	768
599135	2591	2607	Exon 18	TTCAATCCCACGCCCCT	61	7826	7842	4-8-5	656
599189	2591	2608	Exon 18	ATTCAATCCCACGCCCCT	52	7826	7843	5-8-5	769
599136	2592	2608	Exon 18	ATTCAATCCCACGCCCC	68	7827	7843	4-8-5	657
599190	2592	2609	Exon 18	AATTCAATCCCACGCCCC	62	7827	7844	5-8-5	770
599137	2593	2609	Exon 18	AATTCAATCCCACGCCC	51	7828	7844	4-8-5	658
599191	2593	2610	Exon 18	TAATTCAATCCCACGCCC	54	7828	7845	5-8-5	771
599138	2594	2610	Exon 18	TAATTCAATCCCACGCC	71	7829	7845	4-8-5	659
599192	2594	2611	Exon 18	TTAATTCAATCCCACGCC	66	7829	7846	5-8-5	772
599139	2595	2611	Exon 18	TTAATTCAATCCCACGC	80	7830	7846	4-8-5	660
599193	2595	2612	Exon 18	TTTAATTCAATCCCACGC	74	7830	7847	5-8-5	773
599140	2596	2612	Exon 18	TTTAATTCAATCCCACG	66	7831	7847	4-8-5	786
599194	2596	2613	Exon 18	TTTTAATTCAATCCCACG	66	7831	7848	5-8-5	774
599141	2597	2613	Exon 18	TTTTAATTCAATCCCAC	63	7832	7848	4-8-5	662
599195	2597	2614	Exon 18	GTTTTAATTCAATCCCAC	86	7832	7849	5-8-5	775
599142	2598	2614	Exon 18	GTTTTAATTCAATCCCA	69	7833	7849	4-8-5	663
599196	2598	2615	Exon 18	TGTTTTAATTCAATCCCA	82	7833	7850	5-8-5	776
599143	2599	2615	Exon 18	TGTTTTAATTCAATCCC	59	7834	7850	4-8-5	664
599197	2599	2616	Exon 18	CTGTTTTAATTCAATCCC	79	7834	7851	5-8-5	777
599144	2600	2616	Exon 18	CTGTTTTAATTCAATCC	52	7835	7851	4-8-5	665
599198	2600	2617	Exon 18	GCTGTTTTAATTCAATCC	86	7835	7852	5-8-5	778
599145	2601	2617	Exon 18	GCTGTTTTAATTCAATC	53	7836	7852	4-8-5	666
599199	2601	2618	Exon 18	AGCTGTTTTAATTCAATC	72	7836	7853	5-8-5	779
599146	2602	2618	Exon 18	AGCTGTTTTAATTCAAT	42	7837	7853	4-8-5	667
599200	2602	2619	Exon 18	CAGCTGTTTTAATTCAAT	76	7837	7854	5-8-5	780
599147	2603	2619	Exon 18	CAGCTGTTTTAATTCAA	55	7838	7854	4-8-5	668
599201	2603	2620	Exon 18	GCAGCTGTTTTAATTCAA	87	7838	7855	5-8-5	781
532917	2604	2623	Exon 18	GTCGCAGCTGTTTTAATTCA	93	7839	7858	5-10-5	317
599148	2604	2620	Exon 18	GCAGCTGTTTTAATTCA	84	7839	7855	4-8-5	669
599202	2604	2621	Exon 18	CGCAGCTGTTTTAATTCA	89	7839	7856	5-8-5	782
599149	2605	2621	Exon 18	CGCAGCTGTTTTAATTC	92	7840	7856	4-8-5	670
599203	2605	2622	Exon 18	TCGCAGCTGTTTTAATTC	90	7840	7857	5-8-5	783
599150	2606	2622	Exon 18	TCGCAGCTGTTTTAATT	75	7841	7857	4-8-5	671
599151	2607	2623	Exon 18	GTCGCAGCTGTTTTAAT	80	7842	7858	4-8-5	672
599152	2608	2624	Exon 18	TGTCGCAGCTGTTTTAA	76	7843	7859	4-8-5	673

599153	2609	2625	Exon 18	TTGTCGCAGCTGTTTTA	56	7844	7860	4-8-5	674
599154	2610	2626	Exon 18	GTTGTCGCAGCTGTTTT	85	7845	7861	4-8-5	675
599155	2611	2627	Exon 18	TGTTGTCGCAGCTGTTT	89	7846	7862	4-8-5	676
599156	2612	2628	Exon 18 / Repeat	TTGTTGTCGCAGCTGTT	83	n/a	n/a	4-8-5	813
599157	2613	2629	Exon 18 / Repeat	TTTGTTGTCGCAGCTGT	78	n/a	n/a	4-8-5	678
599158	2614	2630	Exon 18 / Repeat	TTTTGTTGTCGCAGCTG	83	n/a	n/a	4-8-5	679
599159	2615	2631	Exon 18 / Repeat	TTTTTGTTGTCGCAGCT	65	n/a	n/a	4-8-5	680
599204	2606	2623	Exon 18	GTCGCAGCTGTTTTAATT	83	7841	7858	5-8-5	784

Table 139

Inhibition of CFB mRNA by MOE gapmers targeting SEQ ID NO: 1 or SEQ ID NO: 2

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target region	Sequence	% inhibition	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	Motif	SEQ ID NO:
599509	2552	2570	Exon 18	TAGAAAACCCAAATCCTCA	45	7787	7805	6-7-6	681
599213	2553	2570	Exon 18	TAGAAAACCCAAATCCTC	89	7788	7805	3-10-5	785
599273	2553	2571	Exon 18	ATAGAAAACCCAAATCCTC	85	7788	7806	6-7-6	682
599214	2554	2571	Exon 18	ATAGAAAACCCAAATCCT	79	7789	7806	3-10-5	786
599274	2554	2572	Exon 18	TATAGAAAACCCAAATCCT	75	7789	7807	6-7-6	683
599215	2555	2572	Exon 18	TATAGAAAACCCAAATCC	81	7790	7807	3-10-5	787
599216	2556	2573	Exon 18	TTATAGAAAACCCAAATC	87	7791	7808	3-10-5	788
599275	2556	2574	Exon 18	CTTATAGAAAACCCAAATC	84	7791	7809	6-7-6	684
599217	2557	2574	Exon 18	CTTATAGAAAACCCAAAT	84	7792	7809	3-10-5	789
599276	2557	2575	Exon 18	CCTTATAGAAAACCCAAAT	68	7792	7810	6-7-6	685
599218	2558	2575	Exon 18	CCTTATAGAAAACCCAAA	82	7793	7810	3-10-5	790
599277	2558	2576	Exon 18	CCCTTATAGAAAACCCAAA	82	7793	7811	6-7-6	686
599219	2559	2576	Exon 18	CCCTTATAGAAAACCCAA	81	7794	7811	3-10-5	791
599278	2559	2577	Exon 18	CCCCTTATAGAAAACCCAA	84	7794	7812	6-7-6	687
599220	2560	2577	Exon 18	CCCCTTATAGAAAACCCA	92	7795	7812	3-10-5	740
599279	2560	2578	Exon 18	ACCCCTTATAGAAAACCCA	92	7795	7813	6-7-6	688
599221	2561	2578	Exon 18	ACCCCTTATAGAAAACCC	93	7796	7813	3-10-5	741
599280	2561	2579	Exon 18	AACCCCTTATAGAAAACCC	90	7796	7814	6-7-6	689
599222	2562	2579	Exon 18	AACCCCTTATAGAAAACC	95	7797	7814	3-10-5	742
599223	2563	2580	Exon 18	AAACCCCTTATAGAAAAC	93	7798	7815	3-10-5	743
599224	2564	2581	Exon 18	GAAACCCCTTATAGAAAA	90	7799	7816	3-10-5	744
599225	2566	2583	Exon 18	AGGAAACCCCTTATAGAA	93	7801	7818	3-10-5	745
599226	2567	2584	Exon 18	CAGGAAACCCCTTATAGA	95	7802	7819	3-10-5	746

599227	2568	2585	Exon 18	GCAGGAAACCCCTTATAG	94	7803	7820	3-10-5	747
599228	2569	2586	Exon 18	AGCAGGAAACCCCTTATA	96	7804	7821	3-10-5	748
599229	2570	2587	Exon 18	CAGCAGGAAACCCCTTAT	92	7805	7822	3-10-5	749
599230	2571	2588	Exon 18	CCAGCAGGAAACCCCTTA	88	7806	7823	3-10-5	750
599231	2572	2589	Exon 18	TCCAGCAGGAAACCCCTT	83	7807	7824	3-10-5	751
599232	2573	2590	Exon 18	GTCCAGCAGGAAACCCCT	89	7808	7825	3-10-5	752
599233	2574	2591	Exon 18	TGTCCAGCAGGAAACCCC	83	7809	7826	3-10-5	753
599234	2575	2592	Exon 18	CTGTCCAGCAGGAAACCC	88	7810	7827	3-10-5	754
599235	2576	2593	Exon 18	CCTGTCCAGCAGGAAACC	91	7811	7828	3-10-5	755
599236	2577	2594	Exon 18	CCCTGTCCAGCAGGAAAC	90	7812	7829	3-10-5	756
599237	2578	2595	Exon 18	CCCCTGTCCAGCAGGAAA	34	7813	7830	3-10-5	757
599238	2580	2597	Exon 18	CGCCCCTGTCCAGCAGGA	14	7815	7832	3-10-5	758
599239	2581	2598	Exon 18	ACGCCCCTGTCCAGCAGG	10	7816	7833	3-10-5	759
599240	2582	2599	Exon 18	CACGCCCCTGTCCAGCAG	26	7817	7834	3-10-5	760
599241	2583	2600	Exon 18	CCACGCCCCTGTCCAGCA	11	7818	7835	3-10-5	761
599242	2584	2601	Exon 18	CCCACGCCCCTGTCCAGC	24	7819	7836	3-10-5	762
599243	2585	2602	Exon 18	TCCCACGCCCCTGTCCAG	23	7820	7837	3-10-5	763
599244	2586	2603	Exon 18	ATCCCACGCCCCTGTCCA	29	7821	7838	3-10-5	764
599245	2587	2604	Exon 18	AATCCCACGCCCCTGTCC	11	7822	7839	3-10-5	765
599246	2588	2605	Exon 18	CAATCCCACGCCCCTGTC	0	7823	7840	3-10-5	766
599247	2589	2606	Exon 18	TCAATCCCACGCCCCTGT	21	7824	7841	3-10-5	767
599248	2590	2607	Exon 18	TTCAATCCCACGCCCCTG	0	7825	7842	3-10-5	768
599249	2591	2608	Exon 18	ATTCAATCCCACGCCCCT	9	7826	7843	3-10-5	769
599250	2592	2609	Exon 18	AATTCAATCCCACGCCCC	4	7827	7844	3-10-5	770
599251	2593	2610	Exon 18	TAATTCAATCCCACGCCC	12	7828	7845	3-10-5	771
599252	2594	2611	Exon 18	TTAATTCAATCCCACGCC	2	7829	7846	3-10-5	772
599253	2595	2612	Exon 18	TTTAATTCAATCCCACGC	28	7830	7847	3-10-5	773
599254	2596	2613	Exon 18	TTTTAATTCAATCCCACG	27	7831	7848	3-10-5	774
599255	2597	2614	Exon 18	GTTTTAATTCAATCCCAC	38	7832	7849	3-10-5	775
599256	2598	2615	Exon 18	TGTTTTAATTCAATCCCA	36	7833	7850	3-10-5	776
599257	2599	2616	Exon 18	CTGTTTTAATTCAATCCC	48	7834	7851	3-10-5	777
599258	2600	2617	Exon 18	GCTGTTTTAATTCAATCC	19	7835	7852	3-10-5	778
599259	2601	2618	Exon 18	AGCTGTTTTAATTCAATC	36	7836	7853	3-10-5	779
599260	2602	2619	Exon 18	CAGCTGTTTTAATTCAAT	58	7837	7854	3-10-5	780
599261	2603	2620	Exon 18	GCAGCTGTTTTAATTCAA	35	7838	7855	3-10-5	781
532917	2604	2623	Exon 18	GTCGCAGCTGTTTTAATTCA	96	7839	7858	5-10-5	317
599262	2604	2621	Exon 18	CGCAGCTGTTTTAATTCA	52	7839	7856	3-10-5	782
599263	2605	2622	Exon 18	TCGCAGCTGTTTTAATTC	66	7840	7857	3-10-5	783
599264	2606	2623	Exon 18	GTCGCAGCTGTTTTAATT	48	7841	7858	3-10-5	784
599265	2607	2624	Exon 18	TGTCGCAGCTGTTTTAAT	46	7842	7859	3-10-5	792
599205	2607	2624	Exon 18	TGTCGCAGCTGTTTTAAT	83	7842	7859	5-8-5	792
599266	2608	2625	Exon 18	TTGTCGCAGCTGTTTTAA	76	7843	7860	3-10-5	793

599206	2608	2625	Exon 18	TTGTCGCAGCTGTTTTAA	90	7843	7860	5-8-5	793
599267	2609	2626	Exon 18	GTTGTCGCAGCTGTTTTA	53	7844	7861	3-10-5	794
599207	2609	2626	Exon 18	GTTGTCGCAGCTGTTTTA	82	7844	7861	5-8-5	794
599268	2610	2627	Exon 18	TGTTGTCGCAGCTGTTTT	58	7845	7862	3-10-5	795
599208	2610	2627	Exon 18	TGTTGTCGCAGCTGTTTT	70	7845	7862	5-8-5	795
599269	2611	2628	Exon 18 / Repeat	TTGTTGTCGCAGCTGTTT	38	n/a	n/a	3-10-5	796
599209	2611	2628	Exon 18 / Repeat	TTGTTGTCGCAGCTGTTT	50	n/a	n/a	5-8-5	796
599270	2612	2629	Exon 18 / Repeat	TTTGTTGTCGCAGCTGTT	46	n/a	n/a	3-10-5	797
599210	2612	2629	Exon 18 / Repeat	TTTGTTGTCGCAGCTGTT	76	n/a	n/a	5-8-5	797
599271	2613	2630	Exon 18 / Repeat	TTTTGTTGTCGCAGCTGT	64	n/a	n/a	3-10-5	798
599211	2613	2630	Exon 18 / Repeat	TTTTGTTGTCGCAGCTGT	78	n/a	n/a	5-8-5	798
599272	2614	2631	Exon 18 / Repeat	TTTTTGTTGTCGCAGCTG	89	n/a	n/a	3-10-5	799
599212	2614	2631	Exon 18 / Repeat	TTTTTGTTGTCGCAGCTG	84	n/a	n/a	5-8-5	799

Table 140

Inhibition of CFB mRNA by MOE gapmers targeting SEQ ID NO: 1 or SEQ ID NO: 2

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target region	Sequence	% inhibition	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	Motif	SEQ ID NO:
599511	2552	2571	Exon 18	ATAGAAAACCCAAATCCTCA	38	7787	7806	6-8-6	410
599389	2553	2572	Exon 18	TATAGAAAACCCAAATCCTC	80	7788	7807	6-8-6	411
599390	2554	2573	Exon 18	TTATAGAAAACCCAAATCCT	92	7789	7808	6-8-6	412
599391	2555	2574	Exon 18	CTTATAGAAAACCCAAATCC	90	7790	7809	6-8-6	413
599392	2556	2575	Exon 18	CCTTATAGAAAACCCAAATC	87	7791	7810	6-8-6	414
599393	2557	2576	Exon 18	CCCTTATAGAAAACCCAAAT	87	7792	7811	6-8-6	415
599394	2558	2577	Exon 18	CCCCTTATAGAAAACCCAAA	74	7793	7812	6-8-6	416
599395	2559	2578	Exon 18	ACCCCTTATAGAAAACCCAA	78	7794	7813	6-8-6	417
599396	2560	2579	Exon 18	AACCCCTTATAGAAAACCCA	77	7795	7814	6-8-6	418
599397	2561	2580	Exon 18	AAACCCCTTATAGAAAACCC	89	7796	7815	6-8-6	419
599398	2562	2581	Exon 18	GAAACCCCTTATAGAAAACC	90	7797	7816	6-8-6	420
599399	2563	2582	Exon 18	GGAAACCCCTTATAGAAAAC	91	7798	7817	6-8-6	421
599400	2564	2583	Exon 18	AGGAAACCCCTTATAGAAAA	88	7799	7818	6-8-6	422
599401	2565	2584	Exon 18	CAGGAAACCCCTTATAGAAA	85	7800	7819	6-8-6	423
599402	2566	2585	Exon 18	GCAGGAAACCCCTTATAGAA	77	7801	7820	6-8-6	424

599403	2567	2586	Exon 18	AGCAGGAAACCCCTTATAGA	85	7802	7821	6-8-6	425
599404	2568	2587	Exon 18	CAGCAGGAAACCCCTTATAG	90	7803	7822	6-8-6	426
599405	2569	2588	Exon 18	CCAGCAGGAAACCCCTTATA	89	7804	7823	6-8-6	427
599406	2570	2589	Exon 18	TCCAGCAGGAAACCCCTTAT	72	7805	7824	6-8-6	428
599407	2571	2590	Exon 18	GTCCAGCAGGAAACCCCTTA	87	7806	7825	6-8-6	237
599408	2572	2591	Exon 18	TGTCCAGCAGGAAACCCCTT	87	7807	7826	6-8-6	429
599409	2573	2592	Exon 18	CTGTCCAGCAGGAAACCCCT	83	7808	7827	6-8-6	430
599410	2574	2593	Exon 18	CCTGTCCAGCAGGAAACCCC	88	7809	7828	6-8-6	431
599411	2575	2594	Exon 18	CCCTGTCCAGCAGGAAACCC	45	7810	7829	6-8-6	432
599412	2576	2595	Exon 18	CCCCTGTCCAGCAGGAAACC	66	7811	7830	6-8-6	433
599413	2577	2596	Exon 18	GCCCCTGTCCAGCAGGAAAC	92	7812	7831	6-8-6	238
599414	2578	2597	Exon 18	CGCCCCTGTCCAGCAGGAAA	92	7813	7832	6-8-6	434
599415	2579	2598	Exon 18	ACGCCCCTGTCCAGCAGGAA	87	7814	7833	6-8-6	435
599416	2580	2599	Exon 18	CACGCCCCTGTCCAGCAGGA	91	7815	7834	6-8-6	436
599417	2581	2600	Exon 18	CCACGCCCCTGTCCAGCAGG	84	7816	7835	6-8-6	437
599357	2582	2600	Exon 18	CCACGCCCCTGTCCAGCAG	88	7817	7835	5-9-5	708
599418	2582	2601	Exon 18	CCCACGCCCCTGTCCAGCAG	85	7817	7836	6-8-6	438
599358	2583	2601	Exon 18	CCCACGCCCCTGTCCAGCA	86	7818	7836	5-9-5	709
599419	2583	2602	Exon 18	TCCCACGCCCCTGTCCAGCA	91	7818	7837	6-8-6	833
599359	2584	2602	Exon 18	TCCCACGCCCCTGTCCAGC	85	7819	7837	5-9-5	834
599420	2584	2603	Exon 18	ATCCCACGCCCCTGTCCAGC	91	7819	7838	6-8-6	440
599360	2585	2603	Exon 18	ATCCCACGCCCCTGTCCAG	89	7820	7838	5-9-5	711
599421	2585	2604	Exon 18	AATCCCACGCCCCTGTCCAG	87	7820	7839	6-8-6	441
599361	2586	2604	Exon 18	AATCCCACGCCCCTGTCCA	89	7821	7839	5-9-5	712
599422	2586	2605	Exon 18	CAATCCCACGCCCCTGTCCA	90	7821	7840	6-8-6	442
599362	2587	2605	Exon 18	CAATCCCACGCCCCTGTCC	94	7822	7840	5-9-5	713
599423	2587	2606	Exon 18	TCAATCCCACGCCCCTGTCC	85	7822	7841	6-8-6	841
599363	2588	2606	Exon 18	TCAATCCCACGCCCCTGTC	88	7823	7841	5-9-5	714
599424	2588	2607	Exon 18	TTCAATCCCACGCCCCTGTC	88	7823	7842	6-8-6	444
599364	2589	2607	Exon 18	TTCAATCCCACGCCCCTGT	88	7824	7842	5-9-5	715
599425	2589	2608	Exon 18	ATTCAATCCCACGCCCCTGT	68	7824	7843	6-8-6	445
599365	2590	2608	Exon 18	ATTCAATCCCACGCCCCTG	48	7825	7843	5-9-5	716
599426	2590	2609	Exon 18	AATTCAATCCCACGCCCCTG	55	7825	7844	6-8-6	446
599366	2591	2609	Exon 18	AATTCAATCCCACGCCCCT	28	7826	7844	5-9-5	717
599427	2591	2610	Exon 18	TAATTCAATCCCACGCCCCT	13	7826	7845	6-8-6	849
599367	2592	2610	Exon 18	TAATTCAATCCCACGCCCC	21	7827	7845	5-9-5	718
599428	2592	2611	Exon 18	TTAATTCAATCCCACGCCCC	39	7827	7846	6-8-6	448
599368	2593	2611	Exon 18	TTAATTCAATCCCACGCCC	20	7828	7846	5-9-5	719
599429	2593	2612	Exon 18	TTTAATTCAATCCCACGCCC	18	7828	7847	6-8-6	449
599369	2594	2612	Exon 18	TTTAATTCAATCCCACGCC	78	7829	7847	5-9-5	720
599430	2594	2613	Exon 18	TTTTAATTCAATCCCACGCC	24	7829	7848	6-8-6	450
599370	2595	2613	Exon 18	TTTTAATTCAATCCCACGC	25	7830	7848	5-9-5	721
599431	2595	2614	Exon 18	GTTTTAATTCAATCCCACGC	30	7830	7849	6-8-6	451

599371	2596	2614	Exon 18	GTTTTAATTCAATCCCACG	84	7831	7849	5-9-5	722
599432	2596	2615	Exon 18	TGTTTTAATTCAATCCCACG	29	7831	7850	6-8-6	452
599372	2597	2615	Exon 18	TGTTTTAATTCAATCCCAC	83	7832	7850	5-9-5	723
599373	2598	2616	Exon 18	CTGTTTTAATTCAATCCCA	81	7833	7851	5-9-5	724
599374	2599	2617	Exon 18	GCTGTTTTAATTCAATCCC	26	7834	7852	5-9-5	725
599375	2600	2618	Exon 18	AGCTGTTTTAATTCAATCC	26	7835	7853	5-9-5	726
599376	2601	2619	Exon 18	CAGCTGTTTTAATTCAATC	62	7836	7854	5-9-5	727
599377	2602	2620	Exon 18	GCAGCTGTTTTAATTCAAT	21	7837	7855	5-9-5	728
599378	2603	2621	Exon 18	CGCAGCTGTTTTAATTCAA	90	7838	7856	5-9-5	729
532917	2604	2623	Exon 18	GTCGCAGCTGTTTTAATTCA	95	7839	7858	5-10-5	867
599379	2604	2622	Exon 18	TCGCAGCTGTTTTAATTCA	88	7839	7857	5-9-5	730
599380	2605	2623	Exon 18	GTCGCAGCTGTTTTAATTC	37	7840	7858	5-9-5	869
599381	2606	2624	Exon 18	TGTCGCAGCTGTTTTAATT	33	7841	7859	5-9-5	732
599382	2607	2625	Exon 18	TTGTCGCAGCTGTTTTAAT	81	7842	7860	5-9-5	733
599383	2608	2626	Exon 18	GTTGTCGCAGCTGTTTTAA	54	7843	7861	5-9-5	734
599384	2609	2627	Exon 18	TGTTGTCGCAGCTGTTTTA	85	7844	7862	5-9-5	873
599385	2610	2628	Exon 18 / Repeat	TTGTTGTCGCAGCTGTTTT	59	n/a	n/a	5-9-5	736
599386	2611	2629	Exon 18 / Repeat	TTTGTTGTCGCAGCTGTTT	81	n/a	n/a	5-9-5	737
599387	2612	2630	Exon 18 / Repeat	TTTTGTTGTCGCAGCTGTT	80	n/a	n/a	5-9-5	738
599388	2613	2631	Exon 18 / Repeat	TTTTTGTTGTCGCAGCTGT	84	n/a	n/a	5-9-5	739

Example 120: Antisense inhibition of human Complement Factor B (CFB) in HepG2 cells by MOE gapmers

Additional antisense oligonucleotides were designed targeting human Complement Factor B (CFB) nucleic acid and were tested for their effects on CFB mRNA in vitro. Cultured HepG2 cells at a density of 20,000 cells per well were transfected using electroporation with 1,000 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and CFB mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3459 was used to measure mRNA levels. CFB mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of CFB, relative to untreated control cells.

The newly designed chimeric antisense oligonucleotides in the Tables below were designed deoxy, MOE and (S)-cEt oligonucleotides. The deoxy, MOE and (S)-cEt oligonucleotides are 16 nucleosides in length wherein the nucleoside have either a MOE sugar modification, an (S)-cEt sugar modification, or a deoxy modification. The 'Chemistry' column describes the sugar modifications of each oligonucleotide. 'k' indicates an (S)-cEt sugar modification; 'd' indicates deoxyribose; and 'e' indicates a MOE modification.

"Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in the Tables below is targeted to either the human CFB mRNA, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NM_001710.5) or the human CFB genomic sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NT_007592.15 truncated from nucleotides 31852000 to 31861000), or both. 'n/a' indicates that the antisense oligonucleotide does not target that particular gene sequence with 100% complementarity.

Table 141

Inhibition of CFB mRNA by deoxy, MOE and (S)-cEt oligonucleotides targeting SEQ ID NO: 1 or SEQ ID NO: 2

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target region	Sequence	% inhibition	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	Motif	SEQ ID NO:
599513	2551	2566	Exon 18	AAACCCAAATCCTCAT	11	7786	7801	ekkeekkddddddddkk	557
599514	2553	2568	Exon 18	GAAAACCCAAATCCTC	13	7788	7803	ekkeekkddddddddkk	801
599515	2555	2570	Exon 18	TAGAAAACCCAAATCC	54	7790	7805	ekkeekkddddddddkk	559
599516	2559	2574	Exon 18	CTTATAGAAAACCCAA	16	7794	7809	ekkeekkddddddddkk	561
599517	2560	2575	Exon 18	CCTTATAGAAAACCCA	29	7795	7810	ekkeekkddddddddkk	562
599518	2561	2576	Exon 18	CCCTTATAGAAAACCC	55	7796	7811	ekkeekkddddddddkk	563
599519	2562	2577	Exon 18	CCCCTTATAGAAAACC	31	7797	7812	ekkeekkddddddddkk	564
599520	2563	2578	Exon 18	ACCCCTTATAGAAAAC	14	7798	7813	ekkeekkddddddddkk	565
599521	2564	2579	Exon 18	AACCCCTTATAGAAAA	9	7799	7814	ekkeekkddddddddkk	566
599522	2565	2580	Exon 18	AAACCCCTTATAGAAA	8	7800	7815	ekkeekkddddddddkk	567
599523	2566	2581	Exon 18	GAAACCCCTTATAGAA	6	7801	7816	ekkeekkddddddddkk	568
599524	2567	2582	Exon 18	GGAAACCCCTTATAGA	14	7802	7817	ekkeekkddddddddkk	569
599525	2568	2583	Exon 18	AGGAAACCCCTTATAG	6	7803	7818	ekkeekkddddddddkk	570
599526	2569	2584	Exon 18	CAGGAAACCCCTTATA	16	7804	7819	ekkeekkddddddddkk	571
599527	2570	2585	Exon 18	GCAGGAAACCCCTTAT	0	7805	7820	ekkeekkddddddddkk	572
599528	2571	2586	Exon 18	AGCAGGAAACCCCTTA	6	7806	7821	ekkeekkddddddddkk	573
599529	2572	2587	Exon 18	CAGCAGGAAACCCCTT	6	7807	7822	ekkeekkddddddddkk	574
599530	2574	2589	Exon 18	TCCAGCAGGAAACCCC	29	7809	7824	ekkeekkddddddddkk	576
599531	2575	2590	Exon 18	GTCCAGCAGGAAACCC	64	7810	7825	ekkeekkddddddddkk	577
599532	2576	2591	Exon 18	TGTCCAGCAGGAAACC	43	7811	7826	ekkeekkddddddddkk	578
599533	2577	2592	Exon 18	CTGTCCAGCAGGAAAC	25	7812	7827	ekkeekkddddddddkk	820
599534	2578	2593	Exon 18	CCTGTCCAGCAGGAAA	12	7813	7828	ekkeekkddddddddkk	580
599535	2580	2595	Exon 18	CCCCTGTCCAGCAGGA	16	7815	7830	ekkeekkddddddddkk	582
599536	2582	2597	Exon 18	CGCCCCTGTCCAGCAG	27	7817	7832	ekkeekkddddddddkk	584

599537	2583	2598	Exon 18	ACGCCCCTGTCCAGCA	35	7818	7833	ekkeekkddddddddkk	585
599538	2584	2599	Exon 18	CACGCCCCTGTCCAGC	26	7819	7834	ekkeekkddddddddkk	586
599539	2585	2600	Exon 18	CCACGCCCCTGTCCAG	33	7820	7835	ekkeekkddddddddkk	587
599540	2586	2601	Exon 18	CCCACGCCCCTGTCCA	27	7821	7836	ekkeekkddddddddkk	588
599541	2587	2602	Exon 18	TCCCACGCCCCTGTCC	52	7822	7837	ekkeekkddddddddkk	589
599542	2588	2603	Exon 18	ATCCCACGCCCCTGTC	16	7823	7838	ekkeekkddddddddkk	590
599543	2589	2604	Exon 18	AATCCCACGCCCCTGT	19	7824	7839	ekkeekkddddddddkk	591
599544	2590	2605	Exon 18	CAATCCCACGCCCCTG	33	7825	7840	ekkeekkddddddddkk	831
599545	2591	2606	Exon 18	TCAATCCCACGCCCCT	24	7826	7841	ekkeekkddddddddkk	593
599546	2592	2607	Exon 18	TTCAATCCCACGCCCC	54	7827	7842	ekkeekkddddddddkk	594
599547	2593	2608	Exon 18	ATTCAATCCCACGCCC	87	7828	7843	ekkeekkddddddddkk	595
599548	2594	2609	Exon 18	AATTCAATCCCACGCC	79	7829	7844	ekkeekkddddddddkk	596
599549	2595	2610	Exon 18	TAATTCAATCCCACGC	62	7830	7845	ekkeekkddddddddkk	597
599550	2596	2611	Exon 18	TTAATTCAATCCCACG	52	7831	7846	ekkeekkddddddddkk	598
599551	2597	2612	Exon 18	TTTAATTCAATCCCAC	27	7832	7847	ekkeekkddddddddkk	599
599577	2597	2613	Exon 18	TTTTAATTCAATCCCAC	90	7832	7848	eeekkddddddddkkeee	662
599552	2598	2613	Exon 18	TTTTAATTCAATCCCA	92	7833	7848	ekkeekkddddddddkk	600
599578	2598	2614	Exon 18	GTTTTAATTCAATCCCA	88	7833	7849	eeekkddddddddkkeee	663
599553	2599	2614	Exon 18	GTTTTAATTCAATCCC	91	7834	7849	ekkeekkddddddddkk	601
599579	2599	2615	Exon 18	TGTTTTAATTCAATCCC	79	7834	7850	eeekkddddddddkkeee	664
599554	2600	2615	Exon 18	TGTTTTAATTCAATCC	90	7835	7850	ekkeekkddddddddkk	602
599580	2600	2616	Exon 18	CTGTTTTAATTCAATCC	79	7835	7851	eeekkddddddddkkeee	665
599555	2601	2616	Exon 18	CTGTTTTAATTCAATC	79	7836	7851	ekkeekkddddddddkk	846
599581	2601	2617	Exon 18	GCTGTTTTAATTCAATC	90	7836	7852	eeekkddddddddkkeee	666
599556	2602	2617	Exon 18	GCTGTTTTAATTCAAT	47	7837	7852	ekkeekkddddddddkk	604
599582	2602	2618	Exon 18	AGCTGTTTTAATTCAAT	89	7837	7853	eeekkddddddddkkeee	849
599557	2603	2618	Exon 18	AGCTGTTTTAATTCAA	67	7838	7853	ekkeekkddddddddkk	850
599583	2603	2619	Exon 18	CAGCTGTTTTAATTCAA	49	7838	7854	eeekkddddddddkkeee	668
532917	2604	2623	Exon 18	GTCGCAGCTGTTTTAATTCA	78	7839	7858	eeeeeddddddddddeeeee	317
599558	2604	2619	Exon 18	CAGCTGTTTTAATTCA	80	7839	7854	ekkeekkddddddddkk	606
599584	2604	2620	Exon 18	GCAGCTGTTTTAATTCA	66	7839	7855	eeekkddddddddkkeee	669
599559	2605	2620	Exon 18	GCAGCTGTTTTAATTC	38	7840	7855	ekkeekkddddddddkk	607
599585	2605	2621	Exon 18	CGCAGCTGTTTTAATTC	80	7840	7856	eeekkddddddddkkeee	670
599560	2606	2621	Exon 18	CGCAGCTGTTTTAATT	16	7841	7856	ekkeekkddddddddkk	608
599586	2606	2622	Exon 18	TCGCAGCTGTTTTAATT	78	7841	7857	eeekkddddddddkkeee	671
599561	2607	2622	Exon 18	TCGCAGCTGTTTTAAT	58	7842	7857	ekkeekkddddddddkk	609
599587	2607	2623	Exon 18	GTCGCAGCTGTTTTAAT	81	7842	7858	eeekkddddddddkkeee	672
588860	2608	2623	Exon 18	GTCGCAGCTGTTTTAA	92	7843	7858	eekddddddddddkke	610
599562	2608	2623	Exon 18	GTCGCAGCTGTTTTAA	78	7843	7858	ekkeekkddddddddkk	610
599588	2608	2624	Exon 18	TGTCGCAGCTGTTTTAA	81	7843	7859	eeekkddddddddkkeee	673
599563	2609	2624	Exon 18	TGTCGCAGCTGTTTTA	86	7844	7859	ekkeekkddddddddkk	611
599589	2609	2625	Exon 18	TTGTCGCAGCTGTTTTA	75	7844	7860	eeekkddddddddkkeee	674

599564	2610	2625	Exon 18	TTGTCGCAGCTGTTTT	75	7845	7860	ekkeekkddddddkk	612
599590	2610	2626	Exon 18	GTTGTCGCAGCTGTTTT	88	7845	7861	eeekkddddddkkeee	675
599565	2611	2626	Exon 18	GTTGTCGCAGCTGTTT	65	7846	7861	ekkeekkddddddkk	613
599591	2611	2627	Exon 18	TGTTGTCGCAGCTGTTT	94	7846	7862	eeekkddddddkkeee	676
599566	2612	2627	Exon 18	TGTTGTCGCAGCTGTT	72	7847	7862	ekkeekkddddddkk	614
599592	2612	2628	Exon 18 / Repeat	TTGTTGTCGCAGCTGTT	90	n/a	n/a	eeekkddddddkkeee	677
599567	2613	2628	Exon 18 / Repeat	TTGTTGTCGCAGCTGT	82	n/a	n/a	ekkeekkddddddkk	615
599593	2613	2629	Exon 18 / Repeat	TTTGTTGTCGCAGCTGT	95	n/a	n/a	eeekkddddddkkeee	678
599568	2614	2629	Exon 18 / Repeat	TTTGTTGTCGCAGCTG	92	n/a	n/a	ekkeekkddddddkk	616
599594	2614	2630	Exon 18 / Repeat	TTTTGTTGTCGCAGCTG	86	n/a	n/a	eeekkddddddkkeee	679
599569	2615	2630	Exon 18 / Repeat	TTTTGTTGTCGCAGCT	89	n/a	n/a	ekkeekkddddddkk	617
599595	2615	2631	Exon 18 / Repeat	TTTTTGTTGTCGCAGCT	76	n/a	n/a	eeekkddddddkkeee	680
599570	2616	2631	Exon 18 / Repeat	TTTTTGTTGTCGCAGC	95	n/a	n/a	ekkeekkddddddkk	618

Example 121: Antisense inhibition of human Complement Factor B (CFB) in HepG2 cells by MOE gapmers

Additional antisense oligonucleotides were designed targeting human Complement Factor B (CFB) nucleic acid and were tested for their effects on CFB mRNA in vitro. The antisense oligonucleotides were tested in a series of experiments that had similar culture conditions. The results for each experiment are presented in separate tables shown below. Cultured HepG2 cells at a density of 20,000 cells per well were transfected using electroporation with 500 nM antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA was isolated from the cells and CFB mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3459 was used to measure mRNA levels. CFB mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of CFB, relative to untreated control cells.

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as deoxy, MOE and (S)-cEt oligonucleotides, or as 5-8-5 MOE, 5-9-5 MOE, 5-10-5 MOE, 6-7-6- MOE, 3-10-5 MOE, or 6-8-6 MOE gapmers.

The deoxy, MOE and (S)-cEt oligonucleotides are 16 nucleosides in length wherein the nucleoside have either a MOE sugar modification, an (S)-cEt sugar modification, or a deoxy modification. The 'Chemistry' column describes the sugar modifications of each oligonucleotide. 'k' indicates an (S)-cEt sugar modification; 'd' indicates deoxyribose; and 'e' indicates a MOE modification.

The 5-8-5 MOE gapmers are 18 nucleosides in length, wherein the central gap segment comprises of eight 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising five nucleosides each. The 5-9-5 MOE gapmers are 19 nucleosides in length, wherein the central gap segment comprises of nine 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising five nucleosides each. The 5-10-5 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising five nucleosides each. The 3-10-5 MOE gapmers are 18 nucleosides in length, wherein the central gap segment comprises of ten 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising three and five nucleosides respectively. The 6-7-6 MOE gapmers are 19 nucleosides in length, wherein the central gap segment comprises of seven 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising six nucleosides each. The 6-8-6 MOE gapmers are 20 nucleosides in length, wherein the central gap segment comprises of eight 2'-deoxynucleosides and is flanked by wing segments on the 5' direction and the 3' direction comprising six nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines.

"Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human gene sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human gene sequence. Each gapmer listed in the Tables below is targeted to either the human CFB mRNA, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NM_001710.5) or the human CFB genomic sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NT_007592.15 truncated from nucleotides 31852000 to 31861000), or both. 'n/a' indicates that the antisense oligonucleotide does not target that particular gene sequence with 100% complementarity.

Table 142

Inhibition of CFB mRNA by deoxy, MOE and (S)-cEt oligonucleotides targeting SEQ ID NO: 1 or SEQ ID NO: 2

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target region	Sequence	% inhibition	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	Motif	SEQ ID NO:
601152	2551	2566	Exon 18	AAACCCAAATCCTCAT	22	7786	7801	eekkddddddddkkee	557
601218	2551	2566	Exon 18	AAACCCAAATCCTCAT	21	7786	7801	ekkkddddddddkeee	557
601153	2552	2567	Exon 18	AAAACCCAAATCCTCA	27	7787	7802	eekkddddddddkkee	800
601219	2552	2567	Exon 18	AAAACCCAAATCCTCA	19	7787	7802	ekkkddddddddkeee	800

601154	2553	2568	Exon 18	GAAAACCCAAATCCTC	23	7788	7803	eekkddddddddkkee	558
601220	2553	2568	Exon 18	GAAAACCCAAATCCTC	24	7788	7803	ekkkddddddddkeee	558
601155	2554	2569	Exon 18	AGAAAACCCAAATCCT	20	7789	7804	eekkddddddddkkee	801
601221	2554	2569	Exon 18	AGAAAACCCAAATCCT	0	7789	7804	ekkkddddddddkeee	801
601156	2555	2570	Exon 18	TAGAAAACCCAAATCC	11	7790	7805	eekkddddddddkkee	559
601222	2555	2570	Exon 18	TAGAAAACCCAAATCC	23	7790	7805	ekkkddddddddkeee	559
601157	2556	2571	Exon 18	ATAGAAAACCCAAATC	9	7791	7806	eekkddddddddkkee	560
601223	2556	2571	Exon 18	ATAGAAAACCCAAATC	0	7791	7806	ekkkddddddddkeee	560
601158	2557	2572	Exon 18	TATAGAAAACCCAAAT	0	7792	7807	eekkddddddddkkee	802
601224	2557	2572	Exon 18	TATAGAAAACCCAAAT	0	7792	7807	ekkkddddddddkeee	802
601159	2558	2573	Exon 18	TTATAGAAAACCCAAA	2	7793	7808	eekkddddddddkkee	803
601225	2558	2573	Exon 18	TTATAGAAAACCCAAA	0	7793	7808	ekkkddddddddkeee	803
601160	2559	2574	Exon 18	CTTATAGAAAACCCAA	0	7794	7809	eekkddddddddkkee	561
601226	2559	2574	Exon 18	CTTATAGAAAACCCAA	0	7794	7809	ekkkddddddddkeee	561
601161	2560	2575	Exon 18	CCTTATAGAAAACCCA	1	7795	7810	eekkddddddddkkee	562
601227	2560	2575	Exon 18	CCTTATAGAAAACCCA	14	7795	7810	ekkkddddddddkeee	562
601162	2561	2576	Exon 18	CCCTTATAGAAAACCC	9	7796	7811	eekkddddddddkkee	563
601228	2561	2576	Exon 18	CCCTTATAGAAAACCC	9	7796	7811	ekkkddddddddkeee	563
601163	2562	2577	Exon 18	CCCCTTATAGAAAACC	0	7797	7812	eekkddddddddkkee	564
601164	2563	2578	Exon 18	ACCCCTTATAGAAAAC	3	7798	7813	eekkddddddddkkee	565
601165	2564	2579	Exon 18	AACCCCTTATAGAAAA	0	7799	7814	eekkddddddddkkee	566
601166	2565	2580	Exon 18	AAACCCCTTATAGAAA	0	7800	7815	eekkddddddddkkee	567
601167	2566	2581	Exon 18	GAAACCCCTTATAGAA	0	7801	7816	eekkddddddddkkee	568
601168	2567	2582	Exon 18	GGAAACCCCTTATAGA	0	7802	7817	eekkddddddddkkee	569
601169	2568	2583	Exon 18	AGGAAACCCCTTATAG	0	7803	7818	eekkddddddddkkee	570
601170	2569	2584	Exon 18	CAGGAAACCCCTTATA	10	7804	7819	eekkddddddddkkee	571
601171	2570	2585	Exon 18	GCAGGAAACCCCTTAT	9	7805	7820	eekkddddddddkkee	572
601172	2571	2586	Exon 18	AGCAGGAAACCCCTTA	15	7806	7821	eekkddddddddkkee	573
601173	2572	2587	Exon 18	CAGCAGGAAACCCCTT	29	7807	7822	eekkddddddddkkee	574
601174	2573	2588	Exon 18	CCAGCAGGAAACCCCT	25	7808	7823	eekkddddddddkkee	575
601175	2574	2589	Exon 18	TCCAGCAGGAAACCCC	15	7809	7824	eekkddddddddkkee	576
601176	2575	2590	Exon 18	GTCCAGCAGGAAACCC	18	7810	7825	eekkddddddddkkee	577
601177	2576	2591	Exon 18	TGTCCAGCAGGAAACC	10	7811	7826	eekkddddddddkkee	578
601178	2577	2592	Exon 18	CTGTCCAGCAGGAAAC	11	7812	7827	eekkddddddddkkee	579
601179	2578	2593	Exon 18	CCTGTCCAGCAGGAAA	19	7813	7828	eekkddddddddkkee	580
601180	2579	2594	Exon 18	CCCTGTCCAGCAGGAA	7	7814	7829	eekkddddddddkkee	581
601181	2580	2595	Exon 18	CCCCTGTCCAGCAGGA	3	7815	7830	eekkddddddddkkee	582
601182	2581	2596	Exon 18	GCCCCTGTCCAGCAGG	0	7816	7831	eekkddddddddkkee	583
601183	2582	2597	Exon 18	CGCCCCTGTCCAGCAG	4	7817	7832	eekkddddddddkkee	584
601184	2583	2598	Exon 18	ACGCCCCTGTCCAGCA	14	7818	7833	eekkddddddddkkee	585
601185	2584	2599	Exon 18	CACGCCCCTGTCCAGC	26	7819	7834	eekkddddddddkkee	586
601186	2585	2600	Exon 18	CCACGCCCCTGTCCAG	8	7820	7835	eekkddddddddkkee	587
601187	2586	2601	Exon 18	CCCACGCCCCTGTCCA	18	7821	7836	eekkddddddddkkee	588

601188	2587	2602	Exon 18	TCCCACGCCCCTGTCC	20	7822	7837	eekkddddddddkkee	589
601189	2588	2603	Exon 18	ATCCCACGCCCCTGTC	12	7823	7838	eekkddddddddkkee	590
601190	2589	2604	Exon 18	AATCCCACGCCCCTGT	33	7824	7839	eekkddddddddkkee	591
601191	2590	2605	Exon 18	CAATCCCACGCCCCTG	52	7825	7840	eekkddddddddkkee	592
601192	2591	2606	Exon 18	TCAATCCCACGCCCCT	46	7826	7841	eekkddddddddkkee	593
601193	2592	2607	Exon 18	TTCAATCCCACGCCCC	30	7827	7842	eekkddddddddkkee	594
601194	2593	2608	Exon 18	ATTCAATCCCACGCCC	41	7828	7843	eekkddddddddkkee	595
601195	2594	2609	Exon 18	AATTCAATCCCACGCC	40	7829	7844	eekkddddddddkkee	596
601196	2595	2610	Exon 18	TAATTCAATCCCACGC	71	7830	7845	eekkddddddddkkee	597
601197	2596	2611	Exon 18	TTAATTCAATCCCACG	42	7831	7846	eekkddddddddkkee	598
601198	2597	2612	Exon 18	TTTAATTCAATCCCAC	63	7832	7847	eekkddddddddkkee	599
601199	2598	2613	Exon 18	TTTTAATTCAATCCCA	51	7833	7848	eekkddddddddkkee	600
601200	2599	2614	Exon 18	GTTTTAATTCAATCCC	65	7834	7849	eekkddddddddkkee	601
601201	2600	2615	Exon 18	TGTTTTAATTCAATCC	49	7835	7850	eekkddddddddkkee	602
601202	2601	2616	Exon 18	CTGTTTTAATTCAATC	33	7836	7851	eekkddddddddkkee	603
601203	2602	2617	Exon 18	GCTGTTTTAATTCAAT	63	7837	7852	eekkddddddddkkee	604
601204	2603	2618	Exon 18	AGCTGTTTTAATTCAA	69	7838	7853	eekkddddddddkkee	605
532917	2604	2623	Exon 18	GTCGCAGCTGTTTTAATTCA	73	7839	7858	eeeeeddddddddddeeeee	317
601205	2604	2619	Exon 18	CAGCTGTTTTAATTCA	51	7839	7854	eekkddddddddkkee	606
601206	2605	2620	Exon 18	GCAGCTGTTTTAATTC	43	7840	7855	eekkddddddddkkee	607
601207	2606	2621	Exon 18	CGCAGCTGTTTTAATT	52	7841	7856	eekkddddddddkkee	608
601208	2607	2622	Exon 18	TCGCAGCTGTTTTAAT	61	7842	7857	eekkddddddddkkee	609
588860	2608	2623	Exon 18	GTCGCAGCTGTTTTAA	75	7843	7858	eekddddddddddkke	610
601209	2608	2623	Exon 18	GTCGCAGCTGTTTTAA	73	7843	7858	eekkddddddddkkee	610
601210	2609	2624	Exon 18	TGTCGCAGCTGTTTTA	80	7844	7859	eekkddddddddkkee	611
601211	2610	2625	Exon 18	TTGTCGCAGCTGTTTT	64	7845	7860	eekkddddddddkkee	612
601212	2611	2626	Exon 18	GTTGTCGCAGCTGTTT	86	7846	7861	eekkddddddddkkee	613
601213	2612	2627	Exon 18	TGTTGTCGCAGCTGTT	87	7847	7862	eekkddddddddkkee	614
601214	2613	2628	Exon 18 / Repeat	TTGTTGTCGCAGCTGT	84	n/a	n/a	eekkddddddddkkee	615
601215	2614	2629	Exon 18 / Repeat	TTTGTTGTCGCAGCTG	78	n/a	n/a	eekkddddddddkkee	616
601216	2615	2630	Exon 18 / Repeat	TTTTGTTGTCGCAGCT	73	n/a	n/a	eekkddddddddkkee	617
601217	2616	2631	Exon 18 / Repeat	TTTTTGTTGTCGCAGC	66	n/a	n/a	eekkddddddddkkee	618

Table 143

Inhibition of CFB mRNA by deoxy, MOE and (S)-cEt oligonucleotides targeting SEQ ID NO: 1 or SEQ ID NO: 2

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target region	Sequence	% inhibition	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	Motif	SEQ ID NO:
601284	2551	2566	Exon 18	AAACCCAAATCCTCAT	8	7786	7801	ekkddddddddkkeee	557
601285	2552	2567	Exon 18	AAAACCCAAATCCTCA	15	7787	7802	ekkddddddddkkeee	800
601286	2553	2568	Exon 18	GAAAACCCAAATCCTC	21	7788	7803	ekkddddddddkkeee	558
601287	2554	2569	Exon 18	AGAAAACCCAAATCCT	9	7789	7804	ekkddddddddkkeee	801
601288	2555	2570	Exon 18	TAGAAAACCCAAATCC	0	7790	7805	ekkddddddddkkeee	559
601289	2556	2571	Exon 18	ATAGAAAACCCAAATC	40	7791	7806	ekkddddddddkkeee	560
601290	2557	2572	Exon 18	TATAGAAAACCCAAAT	16	7792	7807	ekkddddddddkkeee	802
601291	2558	2573	Exon 18	TTATAGAAAACCCAAA	15	7793	7808	ekkddddddddkkeee	803
601292	2559	2574	Exon 18	CTTATAGAAAACCCAA	5	7794	7809	ekkddddddddkkeee	561
601293	2560	2575	Exon 18	CCTTATAGAAAACCCA	15	7795	7810	ekkddddddddkkeee	562
601294	2561	2576	Exon 18	CCCTTATAGAAAACCC	3	7796	7811	ekkddddddddkkeee	563
601229	2562	2577	Exon 18	CCCCTTATAGAAAACC	15	7797	7812	ekkkddddddddkeee	564
601295	2562	2577	Exon 18	CCCCTTATAGAAAACC	5	7797	7812	ekkddddddddkkeee	564
601230	2563	2578	Exon 18	ACCCCTTATAGAAAAC	14	7798	7813	ekkkddddddddkeee	565
601296	2563	2578	Exon 18	ACCCCTTATAGAAAAC	0	7798	7813	ekkddddddddkkeee	565
601231	2564	2579	Exon 18	AACCCCTTATAGAAAA	14	7799	7814	ekkkddddddddkeee	566
601297	2564	2579	Exon 18	AACCCCTTATAGAAAA	14	7799	7814	ekkddddddddkkeee	566
601232	2565	2580	Exon 18	AAACCCCTTATAGAAA	15	7800	7815	ekkkddddddddkeee	567
601298	2565	2580	Exon 18	AAACCCCTTATAGAAA	7	7800	7815	ekkddddddddkkeee	567
601233	2566	2581	Exon 18	GAAACCCCTTATAGAA	0	7801	7816	ekkkddddddddkeee	568
601299	2566	2581	Exon 18	GAAACCCCTTATAGAA	0	7801	7816	ekkddddddddkkeee	568
601234	2567	2582	Exon 18	GGAAACCCCTTATAGA	0	7802	7817	ekkkddddddddkeee	569
601300	2567	2582	Exon 18	GGAAACCCCTTATAGA	9	7802	7817	ekkddddddddkkeee	569
601235	2568	2583	Exon 18	AGGAAACCCCTTATAG	3	7803	7818	ekkkddddddddkeee	570
601301	2568	2583	Exon 18	AGGAAACCCCTTATAG	14	7803	7818	ekkddddddddkkeee	570
601236	2569	2584	Exon 18	CAGGAAACCCCTTATA	0	7804	7819	ekkkddddddddkeee	571
601302	2569	2584	Exon 18	CAGGAAACCCCTTATA	0	7804	7819	ekkddddddddkkeee	571
601237	2570	2585	Exon 18	GCAGGAAACCCCTTAT	16	7805	7820	ekkkddddddddkeee	572
601303	2570	2585	Exon 18	GCAGGAAACCCCTTAT	16	7805	7820	ekkddddddddkkeee	572
601238	2571	2586	Exon 18	AGCAGGAAACCCCTTA	11	7806	7821	ekkkddddddddkeee	573
601304	2571	2586	Exon 18	AGCAGGAAACCCCTTA	10	7806	7821	ekkddddddddkkeee	573
601239	2572	2587	Exon 18	CAGCAGGAAACCCCTT	21	7807	7822	ekkkddddddddkeee	574
601305	2572	2587	Exon 18	CAGCAGGAAACCCCTT	7	7807	7822	ekkddddddddkkeee	574
601240	2573	2588	Exon 18	CCAGCAGGAAACCCCT	6	7808	7823	ekkkddddddddkeee	575

601241	2574	2589	Exon 18	TCCAGCAGGAAACCCC	10	7809	7824	ekkkddddddddkeee	576
601242	2575	2590	Exon 18	GTCCAGCAGGAAACCC	19	7810	7825	ekkkddddddddkeee	577
601243	2576	2591	Exon 18	TGTCCAGCAGGAAACC	10	7811	7826	ekkkddddddddkeee	578
601244	2577	2592	Exon 18	CTGTCCAGCAGGAAAC	28	7812	7827	ekkkddddddddkeee	579
601245	2578	2593	Exon 18	CCTGTCCAGCAGGAAA	5	7813	7828	ekkkddddddddkeee	580
601246	2579	2594	Exon 18	CCCTGTCCAGCAGGAA	18	7814	7829	ekkkddddddddkeee	581
601247	2580	2595	Exon 18	CCCCTGTCCAGCAGGA	4	7815	7830	ekkkddddddddkeee	582
601248	2581	2596	Exon 18	GCCCCTGTCCAGCAGG	6	7816	7831	ekkkddddddddkeee	583
601249	2582	2597	Exon 18	CGCCCCTGTCCAGCAG	18	7817	7832	ekkkddddddddkeee	584
601250	2583	2598	Exon 18	ACGCCCCTGTCCAGCA	26	7818	7833	ekkkddddddddkeee	585
601251	2584	2599	Exon 18	CACGCCCCTGTCCAGC	27	7819	7834	ekkkddddddddkeee	586
601252	2585	2600	Exon 18	CCACGCCCCTGTCCAG	21	7820	7835	ekkkddddddddkeee	587
601253	2586	2601	Exon 18	CCCACGCCCCTGTCCA	0	7821	7836	ekkkddddddddkeee	588
601254	2587	2602	Exon 18	TCCCACGCCCCTGTCC	31	7822	7837	ekkkddddddddkeee	589
601255	2588	2603	Exon 18	ATCCCACGCCCCTGTC	3	7823	7838	ekkkddddddddkeee	590
601256	2589	2604	Exon 18	AATCCCACGCCCCTGT	21	7824	7839	ekkkddddddddkeee	591
601257	2590	2605	Exon 18	CAATCCCACGCCCCTG	47	7825	7840	ekkkddddddddkeee	592
601258	2591	2606	Exon 18	TCAATCCCACGCCCCT	48	7826	7841	ekkkddddddddkeee	593
601259	2592	2607	Exon 18	TTCAATCCCACGCCCC	38	7827	7842	ekkkddddddddkeee	594
601260	2593	2608	Exon 18	ATTCAATCCCACGCCC	33	7828	7843	ekkkddddddddkeee	595
601261	2594	2609	Exon 18	AATTCAATCCCACGCC	17	7829	7844	ekkkddddddddkeee	596
601262	2595	2610	Exon 18	TAATTCAATCCCACGC	40	7830	7845	ekkkddddddddkeee	597
601263	2596	2611	Exon 18	TTAATTCAATCCCACG	31	7831	7846	ekkkddddddddkeee	598
601264	2597	2612	Exon 18	TTTAATTCAATCCCAC	72	7832	7847	ekkkddddddddkeee	599
601265	2598	2613	Exon 18	TTTTAATTCAATCCCA	48	7833	7848	ekkkddddddddkeee	600
601266	2599	2614	Exon 18	GTTTTAATTCAATCCC	64	7834	7849	ekkkddddddddkeee	601
601267	2600	2615	Exon 18	TGTTTTAATTCAATCC	43	7835	7850	ekkkddddddddkeee	602
601268	2601	2616	Exon 18	CTGTTTTAATTCAATC	44	7836	7851	ekkkddddddddkeee	603
601269	2602	2617	Exon 18	GCTGTTTTAATTCAAT	66	7837	7852	ekkkddddddddkeee	604
601270	2603	2618	Exon 18	AGCTGTTTTAATTCAA	47	7838	7853	ekkkddddddddkeee	605
532917	2604	2623	Exon 18	GTCGCAGCTGTTTTAATTCA	3	7839	7858	eeeeeddddddddddeeeee	317
601271	2604	2619	Exon 18	CAGCTGTTTTAATTCA	26	7839	7854	ekkkddddddddkeee	606
601272	2605	2620	Exon 18	GCAGCTGTTTTAATTC	33	7840	7855	ekkkddddddddkeee	607
601273	2606	2621	Exon 18	CGCAGCTGTTTTAATT	34	7841	7856	ekkkddddddddkeee	608
601274	2607	2622	Exon 18	TCGCAGCTGTTTTAAT	39	7842	7857	ekkkddddddddkeee	609
588860	2608	2623	Exon 18	GTCGCAGCTGTTTTAA	72	7843	7858	eekddddddddddkke	610
601275	2608	2623	Exon 18	GTCGCAGCTGTTTTAA	65	7843	7858	ekkkddddddddkeee	610
601276	2609	2624	Exon 18	TGTCGCAGCTGTTTTA	65	7844	7859	ekkkddddddddkeee	611
601277	2610	2625	Exon 18	TTGTCGCAGCTGTTTT	51	7845	7860	ekkkddddddddkeee	612
601278	2611	2626	Exon 18	GTTGTCGCAGCTGTTT	78	7846	7861	ekkkddddddddkeee	613
601279	2612	2627	Exon 18	TGTTGTCGCAGCTGTT	79	7847	7862	ekkkddddddddkeee	614
601280	2613	2628	Exon 18 /	TTGTTGTCGCAGCTGT	70	n/a	n/a	ekkkddddddddkeee	615

			Repeat						
601281	2614	2629	Exon 18 / Repeat	TTTGTTGTCGCAGCTG	78	n/a	n/a	ekkkddddddddkeee	616
601282	2615	2630	Exon 18 / Repeat	TTTTGTTGTCGCAGCT	68	n/a	n/a	ekkkddddddddkeee	617
601283	2616	2631	Exon 18 / Repeat	TTTTTGTTGTCGCAGC	61	n/a	n/a	ekkkddddddddkeee	618

Table 144

Inhibition of CFB mRNA by deoxy, MOE and (S)-cEt oligonucleotides targeting SEQ ID NO: 1 or SEQ ID NO: 2

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target region	Sequence	% inhibition	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	Motif	SEQ ID NO:
601306	2573	2588	Exon 18	CCAGCAGGAAACCCCT	22	7808	7823	ekkddddddddkkeee	575
601307	2574	2589	Exon 18	TCCAGCAGGAAACCCC	22	7809	7824	ekkddddddddkkeee	576
601308	2575	2590	Exon 18	GTCCAGCAGGAAACCC	33	7810	7825	ekkddddddddkkeee	577
601309	2576	2591	Exon 18	TGTCCAGCAGGAAACC	33	7811	7826	ekkddddddddkkeee	578
601310	2577	2592	Exon 18	CTGTCCAGCAGGAAAC	28	7812	7827	ekkddddddddkkeee	579
601311	2578	2593	Exon 18	CCTGTCCAGCAGGAAA	33	7813	7828	ekkddddddddkkeee	580
601312	2579	2594	Exon 18	CCCTGTCCAGCAGGAA	13	7814	7829	ekkddddddddkkeee	581
601313	2580	2595	Exon 18	CCCCTGTCCAGCAGGA	32	7815	7830	ekkddddddddkkeee	582
601314	2581	2596	Exon 18	GCCCCTGTCCAGCAGG	0	7816	7831	ekkddddddddkkeee	583
601315	2582	2597	Exon 18	CGCCCCTGTCCAGCAG	36	7817	7832	ekkddddddddkkeee	584
601316	2583	2598	Exon 18	ACGCCCCTGTCCAGCA	39	7818	7833	ekkddddddddkkeee	585
601317	2584	2599	Exon 18	CACGCCCCTGTCCAGC	33	7819	7834	ekkddddddddkkeee	586
601356	2584	2599	Exon 18	CACGCCCCTGTCCAGC	27	7819	7834	kkkddddddddkeeee	586
601318	2585	2600	Exon 18	CCACGCCCCTGTCCAG	35	7820	7835	ekkddddddddkkeee	587
601357	2585	2600	Exon 18	CCACGCCCCTGTCCAG	26	7820	7835	kkkddddddddkeeee	587
601319	2586	2601	Exon 18	CCCACGCCCCTGTCCA	33	7821	7836	ekkddddddddkkeee	588
601358	2586	2601	Exon 18	CCCACGCCCCTGTCCA	26	7821	7836	kkkddddddddkeeee	588
601320	2587	2602	Exon 18	TCCCACGCCCCTGTCC	25	7822	7837	ekkddddddddkkeee	589
601359	2587	2602	Exon 18	TCCCACGCCCCTGTCC	23	7822	7837	kkkddddddddkeeee	589
601321	2588	2603	Exon 18	ATCCCACGCCCCTGTC	50	7823	7838	ekkddddddddkkeee	590
601360	2588	2603	Exon 18	ATCCCACGCCCCTGTC	33	7823	7838	kkkddddddddkeeee	590
601322	2589	2604	Exon 18	AATCCCACGCCCCTGT	52	7824	7839	ekkddddddddkkeee	591
601361	2589	2604	Exon 18	AATCCCACGCCCCTGT	48	7824	7839	kkkddddddddkeeee	591
601323	2590	2605	Exon 18	CAATCCCACGCCCCTG	67	7825	7840	ekkddddddddkkeee	592
601362	2590	2605	Exon 18	CAATCCCACGCCCCTG	51	7825	7840	kkkddddddddkeeee	592
601324	2591	2606	Exon 18	TCAATCCCACGCCCCT	42	7826	7841	ekkddddddddkkeee	593
601363	2591	2606	Exon 18	TCAATCCCACGCCCCT	42	7826	7841	kkkddddddddkeeee	593

601325	2592	2607	Exon 18	TTCAATCCCACGCCCC	52	7827	7842	ekkddddddddkkeee	594
601364	2592	2607	Exon 18	TTCAATCCCACGCCCC	48	7827	7842	kkkddddddddkeeee	594
601326	2593	2608	Exon 18	ATTCAATCCCACGCCC	27	7828	7843	ekkddddddddkkeee	595
601365	2593	2608	Exon 18	ATTCAATCCCACGCCC	36	7828	7843	kkkddddddddkeeee	595
601327	2594	2609	Exon 18	AATTCAATCCCACGCC	66	7829	7844	ekkddddddddkkeee	596
601366	2594	2609	Exon 18	AATTCAATCCCACGCC	49	7829	7844	kkkddddddddkeeee	596
601328	2595	2610	Exon 18	TAATTCAATCCCACGC	55	7830	7845	ekkddddddddkkeee	597
601367	2595	2610	Exon 18	TAATTCAATCCCACGC	57	7830	7845	kkkddddddddkeeee	597
601329	2596	2611	Exon 18	TTAATTCAATCCCACG	69	7831	7846	ekkddddddddkkeee	598
601368	2596	2611	Exon 18	TTAATTCAATCCCACG	68	7831	7846	kkkddddddddkeeee	598
601330	2597	2612	Exon 18	TTTAATTCAATCCCAC	58	7832	7847	ekkddddddddkkeee	599
601369	2597	2612	Exon 18	TTTAATTCAATCCCAC	65	7832	7847	kkkddddddddkeeee	599
601331	2598	2613	Exon 18	TTTTAATTCAATCCCA	45	7833	7848	ekkddddddddkkeee	600
601370	2598	2613	Exon 18	TTTTAATTCAATCCCA	42	7833	7848	kkkddddddddkeeee	600
601332	2599	2614	Exon 18	GTTTTAATTCAATCCC	84	7834	7849	ekkddddddddkkeee	601
601371	2599	2614	Exon 18	GTTTTAATTCAATCCC	79	7834	7849	kkkddddddddkeeee	601
601333	2600	2615	Exon 18	TGTTTTAATTCAATCC	61	7835	7850	ekkddddddddkkeee	602
601372	2600	2615	Exon 18	TGTTTTAATTCAATCC	71	7835	7850	kkkddddddddkeeee	602
601334	2601	2616	Exon 18	CTGTTTTAATTCAATC	61	7836	7851	ekkddddddddkkeee	603
601373	2601	2616	Exon 18	CTGTTTTAATTCAATC	57	7836	7851	kkkddddddddkeeee	603
601335	2602	2617	Exon 18	GCTGTTTTAATTCAAT	73	7837	7852	ekkddddddddkkeee	604
601374	2602	2617	Exon 18	GCTGTTTTAATTCAAT	66	7837	7852	kkkddddddddkeeee	604
601336	2603	2618	Exon 18	AGCTGTTTTAATTCAA	64	7838	7853	ekkddddddddkkeee	605
601375	2603	2618	Exon 18	AGCTGTTTTAATTCAA	61	7838	7853	kkkddddddddkeeee	605
532917	2604	2623	Exon 18	GTCGCAGCTGTTTTAATTCA	66	7839	7858	eeeeeddddddddddeeee	317
601337	2604	2619	Exon 18	CAGCTGTTTTAATTCA	53	7839	7854	ekkddddddddkkeee	606
601376	2604	2619	Exon 18	CAGCTGTTTTAATTCA	39	7839	7854	kkkddddddddkeeee	606
601338	2605	2620	Exon 18	GCAGCTGTTTTAATTC	67	7840	7855	ekkddddddddkkeee	607
601377	2605	2620	Exon 18	GCAGCTGTTTTAATTC	67	7840	7855	kkkddddddddkeeee	607
601339	2606	2621	Exon 18	CGCAGCTGTTTTAATT	63	7841	7856	ekkddddddddkkeee	608
601378	2606	2621	Exon 18	CGCAGCTGTTTTAATT	60	7841	7856	kkkddddddddkeeee	608
601340	2607	2622	Exon 18	TCGCAGCTGTTTTAAT	40	7842	7857	ekkddddddddkkeee	609
601379	2607	2622	Exon 18	TCGCAGCTGTTTTAAT	36	7842	7857	kkkddddddddkeeee	609
588860	2608	2623	Exon 18	GTCGCAGCTGTTTTAA	84	7843	7858	eekddddddddddkke	610
601341	2608	2623	Exon 18	GTCGCAGCTGTTTTAA	74	7843	7858	ekkddddddddkkeee	610
601380	2608	2623	Exon 18	GTCGCAGCTGTTTTAA	78	7843	7858	kkkddddddddkeeee	610
601342	2609	2624	Exon 18	TGTCGCAGCTGTTTTA	68	7844	7859	ekkddddddddkkeee	611
601381	2609	2624	Exon 18	TGTCGCAGCTGTTTTA	66	7844	7859	kkkddddddddkeeee	611
601343	2610	2625	Exon 18	TTGTCGCAGCTGTTTT	71	7845	7860	ekkddddddddkkeee	612
601382	2610	2625	Exon 18	TTGTCGCAGCTGTTTT	84	7845	7860	kkkddddddddkeeee	612
601344	2611	2626	Exon 18	GTTGTCGCAGCTGTTT	87	7846	7861	ekkddddddddkkeee	613
601383	2611	2626	Exon 18	GTTGTCGCAGCTGTTT	85	7846	7861	kkkddddddddkeeee	613

601345	2612	2627	Exon 18	TGTTGTCGCAGCTGTT	82	7847	7862	ekkddddddddkkeee	614
601384	2612	2627	Exon 18	TGTTGTCGCAGCTGTT	79	7847	7862	kkkddddddddkeeee	614
601346	2613	2628	Exon 18 / Repeat	TTGTTGTCGCAGCTGT	73	n/a	n/a	ekkddddddddkkeee	615
601385	2613	2628	Exon 18 / Repeat	TTGTTGTCGCAGCTGT	84	n/a	n/a	kkkddddddddkeeee	615
601347	2614	2629	Exon 18 / Repeat	TTTGTTGTCGCAGCTG	70	n/a	n/a	ekkddddddddkkeee	616
601386	2614	2629	Exon 18 / Repeat	TTTGTTGTCGCAGCTG	71	n/a	n/a	kkkddddddddkeeee	616
601348	2615	2630	Exon 18 / Repeat	TTTTGTTGTCGCAGCT	71	n/a	n/a	ekkddddddddkkeee	617
601387	2615	2630	Exon 18 / Repeat	TTTTGTTGTCGCAGCT	76	n/a	n/a	kkkddddddddkeeee	617
601349	2616	2631	Exon 18 / Repeat	TTTTTGTTGTCGCAGC	71	n/a	n/a	ekkddddddddkkeee	618
601388	2616	2631	Exon 18 / Repeat	TTTTTGTTGTCGCAGC	67	n/a	n/a	kkkddddddddkeeee	618

Table 145

Inhibition of CFB mRNA by MOE gapmers targeting SEQ ID NO: 1 or SEQ ID NO: 2

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target region	Sequence	% inhibition	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	Motif	SEQ ID NO:
599357	2582	2600	Exon 18	CCACGCCCCTGTCCAGCAG	26	7817	7835	5-9-5	708
599358	2583	2601	Exon 18	CCCACGCCCCTGTCCAGCA	22	7818	7836	5-9-5	709
599359	2584	2602	Exon 18	TCCCACGCCCCTGTCCAGC	13	7819	7837	5-9-5	710
599360	2585	2603	Exon 18	ATCCCACGCCCCTGTCCAG	7	7820	7838	5-9-5	711
599361	2586	2604	Exon 18	AATCCCACGCCCCTGTCCA	11	7821	7839	5-9-5	712
599362	2587	2605	Exon 18	CAATCCCACGCCCCTGTCC	14	7822	7840	5-9-5	713
599363	2588	2606	Exon 18	TCAATCCCACGCCCCTGTC	17	7823	7841	5-9-5	714
599364	2589	2607	Exon 18	TTCAATCCCACGCCCCTGT	20	7824	7842	5-9-5	715
599365	2590	2608	Exon 18	ATTCAATCCCACGCCCCTG	22	7825	7843	5-9-5	716
599366	2591	2609	Exon 18	AATTCAATCCCACGCCCCT	13	7826	7844	5-9-5	717
599367	2592	2610	Exon 18	TAATTCAATCCCACGCCCC	11	7827	7845	5-9-5	718
599368	2593	2611	Exon 18	TTAATTCAATCCCACGCCC	10	7828	7846	5-9-5	719
599369	2594	2612	Exon 18	TTTAATTCAATCCCACGCC	19	7829	7847	5-9-5	720
599370	2595	2613	Exon 18	TTTTAATTCAATCCCACGC	23	7830	7848	5-9-5	721
599371	2596	2614	Exon 18	GTTTTAATTCAATCCCACG	4	7831	7849	5-9-5	722
599372	2597	2615	Exon 18	TGTTTTAATTCAATCCCAC	16	7832	7850	5-9-5	723
599373	2598	2616	Exon 18	CTGTTTTAATTCAATCCCA	3	7833	7851	5-9-5	724

599374	2599	2617	Exon 18	GCTGTTTTAATTCAATCCC	10	7834	7852	5-9-5	725
599375	2600	2618	Exon 18	AGCTGTTTTAATTCAATCC	17	7835	7853	5-9-5	726
599376	2601	2619	Exon 18	CAGCTGTTTTAATTCAATC	18	7836	7854	5-9-5	727
599377	2602	2620	Exon 18	GCAGCTGTTTTAATTCAAT	22	7837	7855	5-9-5	728
599378	2603	2621	Exon 18	CGCAGCTGTTTTAATTCAA	11	7838	7856	5-9-5	729
599511	2552	2571	Exon 18	ATAGAAAACCCAAATCCTCA	7	7787	7806	6-8-6	410
599389	2553	2572	Exon 18	TATAGAAAACCCAAATCCTC	22	7788	7807	6-8-6	411
599390	2554	2573	Exon 18	TTATAGAAAACCCAAATCCT	21	7789	7808	6-8-6	412
599391	2555	2574	Exon 18	CTTATAGAAAACCCAAATCC	27	7790	7809	6-8-6	413
599392	2556	2575	Exon 18	CCTTATAGAAAACCCAAATC	30	7791	7810	6-8-6	414
599393	2557	2576	Exon 18	CCCTTATAGAAAACCCAAAT	30	7792	7811	6-8-6	415
599394	2558	2577	Exon 18	CCCCTTATAGAAAACCCAAA	28	7793	7812	6-8-6	416
599395	2559	2578	Exon 18	ACCCCTTATAGAAAACCCAA	23	7794	7813	6-8-6	417
599396	2560	2579	Exon 18	AACCCCTTATAGAAAACCCA	53	7795	7814	6-8-6	418
599397	2561	2580	Exon 18	AAACCCCTTATAGAAAACCC	33	7796	7815	6-8-6	419
599398	2562	2581	Exon 18	GAAACCCCTTATAGAAAACC	58	7797	7816	6-8-6	420
599399	2563	2582	Exon 18	GGAAACCCCTTATAGAAAAC	23	7798	7817	6-8-6	421
599400	2564	2583	Exon 18	AGGAAACCCCTTATAGAAAA	54	7799	7818	6-8-6	422
599401	2565	2584	Exon 18	CAGGAAACCCCTTATAGAAA	30	7800	7819	6-8-6	423
599402	2566	2585	Exon 18	GCAGGAAACCCCTTATAGAA	25	7801	7820	6-8-6	424
599403	2567	2586	Exon 18	AGCAGGAAACCCCTTATAGA	17	7802	7821	6-8-6	425
599404	2568	2587	Exon 18	CAGCAGGAAACCCCTTATAG	20	7803	7822	6-8-6	426
599405	2569	2588	Exon 18	CCAGCAGGAAACCCCTTATA	12	7804	7823	6-8-6	427
599406	2570	2589	Exon 18	TCCAGCAGGAAACCCCTTAT	51	7805	7824	6-8-6	428
599407	2571	2590	Exon 18	GTCCAGCAGGAAACCCCTTA	39	7806	7825	6-8-6	237
599408	2572	2591	Exon 18	TGTCCAGCAGGAAACCCCTT	53	7807	7826	6-8-6	429
599409	2573	2592	Exon 18	CTGTCCAGCAGGAAACCCCT	65	7808	7827	6-8-6	430
599410	2574	2593	Exon 18	CCTGTCCAGCAGGAAACCCC	56	7809	7828	6-8-6	431
599411	2575	2594	Exon 18	CCCTGTCCAGCAGGAAACCC	60	7810	7829	6-8-6	432
599412	2576	2595	Exon 18	CCCCTGTCCAGCAGGAAACC	61	7811	7830	6-8-6	433
599413	2577	2596	Exon 18	GCCCCTGTCCAGCAGGAAAC	40	7812	7831	6-8-6	238
599414	2578	2597	Exon 18	CGCCCCTGTCCAGCAGGAAA	41	7813	7832	6-8-6	434
599415	2579	2598	Exon 18	ACGCCCCTGTCCAGCAGGAA	37	7814	7833	6-8-6	435
599416	2580	2599	Exon 18	CACGCCCCTGTCCAGCAGGA	54	7815	7834	6-8-6	436
599417	2581	2600	Exon 18	CCACGCCCCTGTCCAGCAGG	36	7816	7835	6-8-6	437
599418	2582	2601	Exon 18	CCCACGCCCCTGTCCAGCAG	53	7817	7836	6-8-6	438
599419	2583	2602	Exon 18	TCCCACGCCCCTGTCCAGCA	54	7818	7837	6-8-6	439
599420	2584	2603	Exon 18	ATCCCACGCCCCTGTCCAGC	50	7819	7838	6-8-6	440
599421	2585	2604	Exon 18	AATCCCACGCCCCTGTCCAG	48	7820	7839	6-8-6	441
599422	2586	2605	Exon 18	CAATCCCACGCCCCTGTCCA	55	7821	7840	6-8-6	442
599423	2587	2606	Exon 18	TCAATCCCACGCCCCTGTCC	75	7822	7841	6-8-6	443
599424	2588	2607	Exon 18	TTCAATCCCACGCCCCTGTC	69	7823	7842	6-8-6	444
599425	2589	2608	Exon 18	ATTCAATCCCACGCCCCTGT	77	7824	7843	6-8-6	445

599426	2590	2609	Exon 18	AATTCAATCCCACGCCCCTG	60	7825	7844	6-8-6	446
599427	2591	2610	Exon 18	TAATTCAATCCCACGCCCCT	72	7826	7845	6-8-6	447
599428	2592	2611	Exon 18	TTAATTCAATCCCACGCCCC	81	7827	7846	6-8-6	448
599429	2593	2612	Exon 18	TTTAATTCAATCCCACGCCC	68	7828	7847	6-8-6	449
599430	2594	2613	Exon 18	TTTTAATTCAATCCCACGCC	58	7829	7848	6-8-6	450
599431	2595	2614	Exon 18	GTTTTAATTCAATCCCACGC	70	7830	7849	6-8-6	451
599432	2596	2615	Exon 18	TGTTTTAATTCAATCCCACG	85	7831	7850	6-8-6	452
532917	2604	2623	Exon 18	GTCGCAGCTGTTTTAATTCA	85	7839	7858	5-10-5	317
599379	2604	2622	Exon 18	TCGCAGCTGTTTTAATTCA	73	7839	7857	5-9-5	730
599380	2605	2623	Exon 18	GTCGCAGCTGTTTTAATTC	77	7840	7858	5-9-5	731
599381	2606	2624	Exon 18	TGTCGCAGCTGTTTTAATT	69	7841	7859	5-9-5	732
599382	2607	2625	Exon 18	TTGTCGCAGCTGTTTTAAT	58	7842	7860	5-9-5	733
599383	2608	2626	Exon 18	GTTGTCGCAGCTGTTTTAA	52	7843	7861	5-9-5	734
599384	2609	2627	Exon 18	TGTTGTCGCAGCTGTTTTA	63	7844	7862	5-9-5	735
599385	2610	2628	Exon 18 / Repeat	TTGTTGTCGCAGCTGTTTT	53	n/a	n/a	5-9-5	736
599386	2611	2629	Exon 18 / Repeat	TTTGTTGTCGCAGCTGTTT	63	n/a	n/a	5-9-5	737
599387	2612	2630	Exon 18 / Repeat	TTTTGTTGTCGCAGCTGTT	64	n/a	n/a	5-9-5	438
599388	2613	2631	Exon 18 / Repeat	TTTTTGTTGTCGCAGCTGT	66	n/a	n/a	5-9-5	739

Table 146

Inhibition of CFB mRNA by MOE gapmers targeting SEQ ID NO: 1 or SEQ ID NO: 2

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target region	Sequence	% inhibition	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	Motif	SEQ ID NO:
599213	2553	2570	Exon 18	TAGAAAACCCAAATCCTC	0	7788	7805	3-10-5	785
599214	2554	2571	Exon 18	ATAGAAAACCCAAATCCT	0	7789	7806	3-10-5	786
599215	2555	2572	Exon 18	TATAGAAAACCCAAATCC	36	7790	7807	3-10-5	787
599216	2556	2573	Exon 18	TTATAGAAAACCCAAATC	8	7791	7808	3-10-5	788
599217	2557	2574	Exon 18	CTTATAGAAAACCCAAAT	5	7792	7809	3-10-5	789
599218	2558	2575	Exon 18	CCTTATAGAAAACCCAAA	0	7793	7810	3-10-5	790
599219	2559	2576	Exon 18	CCCTTATAGAAAACCCAA	8	7794	7811	3-10-5	791
599220	2560	2577	Exon 18	CCCCTTATAGAAAACCCA	0	7795	7812	3-10-5	740
599221	2561	2578	Exon 18	ACCCCTTATAGAAAACCC	54	7796	7813	3-10-5	741
599222	2562	2579	Exon 18	AACCCCTTATAGAAAACC	3	7797	7814	3-10-5	742
599223	2563	2580	Exon 18	AAACCCCTTATAGAAAAC	0	7798	7815	3-10-5	743
599224	2564	2581	Exon 18	GAAACCCCTTATAGAAAA	0	7799	7816	3-10-5	744
599225	2566	2583	Exon 18	AGGAAACCCCTTATAGAA	60	7801	7818	3-10-5	745

599226	2567	2584	Exon 18	CAGGAAACCCCTTATAGA	0	7802	7819	3-10-5	746
599227	2568	2585	Exon 18	GCAGGAAACCCCTTATAG	37	7803	7820	3-10-5	747
599228	2569	2586	Exon 18	AGCAGGAAACCCCTTATA	0	7804	7821	3-10-5	748
599229	2570	2587	Exon 18	CAGCAGGAAACCCCTTAT	39	7805	7822	3-10-5	749
599230	2571	2588	Exon 18	CCAGCAGGAAACCCCTTA	10	7806	7823	3-10-5	750
599231	2572	2589	Exon 18	TCCAGCAGGAAACCCCTT	16	7807	7824	3-10-5	751
599232	2573	2590	Exon 18	GTCCAGCAGGAAACCCCT	9	7808	7825	3-10-5	752
599233	2574	2591	Exon 18	TGTCCAGCAGGAAACCCC	44	7809	7826	3-10-5	753
599234	2575	2592	Exon 18	CTGTCCAGCAGGAAACCC	14	7810	7827	3-10-5	754
599235	2576	2593	Exon 18	CCTGTCCAGCAGGAAACC	0	7811	7828	3-10-5	755
599236	2577	2594	Exon 18	CCCTGTCCAGCAGGAAAC	43	7812	7829	3-10-5	756
599237	2578	2595	Exon 18	CCCCTGTCCAGCAGGAAA	0	7813	7830	3-10-5	757
599238	2580	2597	Exon 18	CGCCCCTGTCCAGCAGGA	9	7815	7832	3-10-5	758
599239	2581	2598	Exon 18	ACGCCCCTGTCCAGCAGG	36	7816	7833	3-10-5	759
599240	2582	2599	Exon 18	CACGCCCCTGTCCAGCAG	11	7817	7834	3-10-5	760
599241	2583	2600	Exon 18	CCACGCCCCTGTCCAGCA	51	7818	7835	3-10-5	761
599242	2584	2601	Exon 18	CCCACGCCCCTGTCCAGC	7	7819	7836	3-10-5	762
599243	2585	2602	Exon 18	TCCCACGCCCCTGTCCAG	47	7820	7837	3-10-5	763
599244	2586	2603	Exon 18	ATCCCACGCCCCTGTCCA	37	7821	7838	3-10-5	764
599245	2587	2604	Exon 18	AATCCCACGCCCCTGTCC	35	7822	7839	3-10-5	765
599246	2588	2605	Exon 18	CAATCCCACGCCCCTGTC	21	7823	7840	3-10-5	766
599247	2589	2606	Exon 18	TCAATCCCACGCCCCTGT	61	7824	7841	3-10-5	767
599248	2590	2607	Exon 18	TTCAATCCCACGCCCCTG	51	7825	7842	3-10-5	768
599249	2591	2608	Exon 18	ATTCAATCCCACGCCCCT	58	7826	7843	3-10-5	769
599250	2592	2609	Exon 18	AATTCAATCCCACGCCCC	49	7827	7844	3-10-5	770
599251	2593	2610	Exon 18	TAATTCAATCCCACGCCC	46	7828	7845	3-10-5	771
599252	2594	2611	Exon 18	TTAATTCAATCCCACGCC	32	7829	7846	3-10-5	772
599253	2595	2612	Exon 18	TTTAATTCAATCCCACGC	23	7830	7847	3-10-5	773
599254	2596	2613	Exon 18	TTTTAATTCAATCCCACG	0	7831	7848	3-10-5	774
599255	2597	2614	Exon 18	GTTTTAATTCAATCCCAC	61	7832	7849	3-10-5	775
599256	2598	2615	Exon 18	TGTTTTAATTCAATCCCA	64	7833	7850	3-10-5	776
599257	2599	2616	Exon 18	CTGTTTTAATTCAATCCC	66	7834	7851	3-10-5	777
599258	2600	2617	Exon 18	GCTGTTTTAATTCAATCC	59	7835	7852	3-10-5	778
599259	2601	2618	Exon 18	AGCTGTTTTAATTCAATC	40	7836	7853	3-10-5	779
599260	2602	2619	Exon 18	CAGCTGTTTTAATTCAAT	38	7837	7854	3-10-5	780
599261	2603	2620	Exon 18	GCAGCTGTTTTAATTCAA	54	7838	7855	3-10-5	781
599509	2552	2570	Exon 18	TAGAAAACCCAAATCCTCA	54	7787	7805	6-7-6	681
599273	2553	2571	Exon 18	ATAGAAAACCCAAATCCTC	0	7788	7806	6-7-6	682
599274	2554	2572	Exon 18	TATAGAAAACCCAAATCCT	57	7789	7807	6-7-6	683
599275	2556	2574	Exon 18	CTTATAGAAAACCCAAATC	0	7791	7809	6-7-6	684
599276	2557	2575	Exon 18	CCTTATAGAAAACCCAAAT	44	7792	7810	6-7-6	685
599277	2558	2576	Exon 18	CCCTTATAGAAAACCCAAA	0	7793	7811	6-7-6	686
599278	2559	2577	Exon 18	CCCCTTATAGAAAACCCAA	0	7794	7812	6-7-6	687

599279	2560	2578	Exon 18	ACCCCTTATAGAAAACCCA	20	7795	7813	6-7-6	688
599280	2561	2579	Exon 18	AACCCCTTATAGAAAACCC	70	7796	7814	6-7-6	689
532917	2604	2623	Exon 18	GTCGCAGCTGTTTTAATTCA	85	7839	7858	5-10-5	317
599262	2604	2621	Exon 18	CGCAGCTGTTTTAATTCA	49	7839	7856	3-10-5	782
599263	2605	2622	Exon 18	TCGCAGCTGTTTTAATTC	49	7840	7857	3-10-5	783
599264	2606	2623	Exon 18	GTCGCAGCTGTTTTAATT	62	7841	7858	3-10-5	784
599265	2607	2624	Exon 18	TGTCGCAGCTGTTTTAAT	63	7842	7859	3-10-5	792
599266	2608	2625	Exon 18	TTGTCGCAGCTGTTTTAA	41	7843	7860	3-10-5	793
599267	2609	2626	Exon 18	GTTGTCGCAGCTGTTTTA	52	7844	7861	3-10-5	794
599268	2610	2627	Exon 18	TGTTGTCGCAGCTGTTTT	51	7845	7862	3-10-5	795
599269	2611	2628	Exon 18 / Repeat	TTGTTGTCGCAGCTGTTT	58	n/a	n/a	3-10-5	796
599270	2612	2629	Exon 18 / Repeat	TTTGTTGTCGCAGCTGTT	69	n/a	n/a	3-10-5	797
599271	2613	2630	Exon 18 / Repeat	TTTTGTTGTCGCAGCTGT	69	n/a	n/a	3-10-5	798
599272	2614	2631	Exon 18 / Repeat	TTTTTGTTGTCGCAGCTG	72	n/a	n/a	3-10-5	799
599205	2607	2624	Exon 18	TGTCGCAGCTGTTTTAAT	54	7842	7859	5-8-5	792
599206	2608	2625	Exon 18	TTGTCGCAGCTGTTTTAA	62	7843	7860	5-8-5	793
599207	2609	2626	Exon 18	GTTGTCGCAGCTGTTTTA	62	7844	7861	5-8-5	794
599208	2610	2627	Exon 18	TGTTGTCGCAGCTGTTTT	66	7845	7862	5-8-5	795
599209	2611	2628	Exon 18 / Repeat	TTGTTGTCGCAGCTGTTT	60	n/a	n/a	5-8-5	796
599210	2612	2629	Exon 18 / Repeat	TTTGTTGTCGCAGCTGTT	62	n/a	n/a	5-8-5	797
599211	2613	2630	Exon 18 / Repeat	TTTTGTTGTCGCAGCTGT	65	n/a	n/a	5-8-5	798
599212	2614	2631	Exon 18 / Repeat	TTTTTGTTGTCGCAGCTG	67	n/a	n/a	5-8-5	799

Table 147

Inhibition of CFB mRNA by 5-10-5 MOE gapmers targeting SEQ ID NO: 1 or SEQ ID NO: 2

ISIS NO	SEQ ID NO: 1 start site	SEQ ID NO: 1 stop site	Target region	Sequence	% inhibition	SEQ ID NO: 2 start site	SEQ ID NO: 2 stop site	SEQ ID NO:
588570	150	169	Exon 1	TGGTCACATTCCCTTCCCCT	72	1871	1890	396
588571	152	171	Exon 1	CCTGGTCACATTCCCTTCCC	80	1873	1892	397
532614	154	173	Exon 1	GACCTGGTCACATTCCCTTC	65	1875	1894	12
588572	156	175	Exon 1	TAGACCTGGTCACATTCCCT	74	1877	1896	398
588573	158	177	Exon 1	CCTAGACCTGGTCACATTCC	72	1879	1898	399

588566	2189	2208	Exon 15	CCTTCCGAGTCAGCTTTTTC	66	6977	6996	400
588567	2191	2210	Exon 15	CTCCTTCCGAGTCAGCTTTT	66	6979	6998	401
532770	2193	2212	Exon 15	ACCTCCTTCCGAGTCAGCTT	64	6981	7000	198
588568	2195	2214	Exon 15	AGACCTCCTTCCGAGTCAGC	78	6983	7002	402
588569	2197	2216	Exon 15	GTAGACCTCCTTCCGAGTCA	74	6985	7004	403
588574	2453	2472	Exon 18	TTTGCCGCTTCTGGTTTTTG	71	7688	7707	404
588575	2455	2474	Exon 18	CTTTTGCCGCTTCTGGTTTT	72	7690	7709	405
532800	2457	2476	Exon 18	TGCTTTTGCCGCTTCTGGTT	71	7692	7711	228
588576	2459	2478	Exon 18	CCTGCTTTTGCCGCTTCTGG	59	7694	7713	406
588577	2461	2480	Exon 18	TACCTGCTTTTGCCGCTTCT	76	7696	7715	407
516350	2550	2569	Exon 18	AGAAAACCCAAATCCTCATC	58	7785	7804	408
588509	2551	2570	Exon 18	TAGAAAACCCAAATCCTCAT	6	7786	7805	409
588510	2552	2571	Exon 18	ATAGAAAACCCAAATCCTCA	10	7787	7806	410
588511	2553	2572	Exon 18	TATAGAAAACCCAAATCCTC	9	7788	7807	411
588512	2554	2573	Exon 18	TTATAGAAAACCCAAATCCT	80	7789	7808	412
588513	2555	2574	Exon 18	CTTATAGAAAACCCAAATCC	70	7790	7809	413
588514	2556	2575	Exon 18	CCTTATAGAAAACCCAAATC	71	7791	7810	414
588515	2557	2576	Exon 18	CCCTTATAGAAAACCCAAAT	78	7792	7811	415
588516	2558	2577	Exon 18	CCCCTTATAGAAAACCCAAA	72	7793	7812	416
588517	2559	2578	Exon 18	ACCCCTTATAGAAAACCCAA	80	7794	7813	417
588518	2560	2579	Exon 18	AACCCCTTATAGAAAACCCA	80	7795	7814	418
588519	2561	2580	Exon 18	AAACCCCTTATAGAAAACCC	62	7796	7815	419
588520	2562	2581	Exon 18	GAAACCCCTTATAGAAAACC	59	7797	7816	420
588521	2563	2582	Exon 18	GGAAACCCCTTATAGAAAAC	40	7798	7817	421
588522	2564	2583	Exon 18	AGGAAACCCCTTATAGAAAA	66	7799	7818	422
588523	2565	2584	Exon 18	CAGGAAACCCCTTATAGAAA	63	7800	7819	423
588524	2566	2585	Exon 18	GCAGGAAACCCCTTATAGAA	70	7801	7820	424
588525	2567	2586	Exon 18	AGCAGGAAACCCCTTATAGA	67	7802	7821	425
588526	2568	2587	Exon 18	CAGCAGGAAACCCCTTATAG	0	7803	7822	426
588527	2569	2588	Exon 18	CCAGCAGGAAACCCCTTATA	11	7804	7823	427
588528	2570	2589	Exon 18	TCCAGCAGGAAACCCCTTAT	15	7805	7824	428
532809	2571	2590	Exon 18	GTCCAGCAGGAAACCCCTTA	75	7806	7825	237
588529	2572	2591	Exon 18	TGTCCAGCAGGAAACCCCTT	16	7807	7826	429
588530	2573	2592	Exon 18	CTGTCCAGCAGGAAACCCCT	16	7808	7827	430
588531	2574	2593	Exon 18	CCTGTCCAGCAGGAAACCCC	19	7809	7828	431
588532	2575	2594	Exon 18	CCCTGTCCAGCAGGAAACCC	15	7810	7829	432
588533	2576	2595	Exon 18	CCCCTGTCCAGCAGGAAACC	29	7811	7830	433
532810	2577	2596	Exon 18	GCCCCTGTCCAGCAGGAAAC	74	7812	7831	238
588534	2578	2597	Exon 18	CGCCCCTGTCCAGCAGGAAA	21	7813	7832	434
588535	2579	2598	Exon 18	ACGCCCCTGTCCAGCAGGAA	16	7814	7833	435
588536	2580	2599	Exon 18	CACGCCCCTGTCCAGCAGGA	0	7815	7834	436
588537	2581	2600	Exon 18	CCACGCCCCTGTCCAGCAGG	8	7816	7835	437
588538	2582	2601	Exon 18	CCCACGCCCCTGTCCAGCAG	10	7817	7836	438

588539	2583	2602	Exon 18	TCCCACGCCCCTGTCCAGCA	23	7818	7837	439
588540	2584	2603	Exon 18	ATCCCACGCCCCTGTCCAGC	16	7819	7838	440
588541	2585	2604	Exon 18	AATCCCACGCCCCTGTCCAG	16	7820	7839	441
588542	2586	2605	Exon 18	CAATCCCACGCCCCTGTCCA	12	7821	7840	442
588543	2587	2606	Exon 18	TCAATCCCACGCCCCTGTCC	26	7822	7841	443
588544	2588	2607	Exon 18	TTCAATCCCACGCCCCTGTC	26	7823	7842	444
588545	2589	2608	Exon 18	ATTCAATCCCACGCCCCTGT	31	7824	7843	445
588546	2590	2609	Exon 18	AATTCAATCCCACGCCCCTG	22	7825	7844	446
588547	2591	2610	Exon 18	TAATTCAATCCCACGCCCCT	12	7826	7845	447
588548	2592	2611	Exon 18	TTAATTCAATCCCACGCCCC	20	7827	7846	448
588549	2593	2612	Exon 18	TTTAATTCAATCCCACGCCC	26	7828	7847	449
588550	2594	2613	Exon 18	TTTTAATTCAATCCCACGCC	32	7829	7848	450
588551	2595	2614	Exon 18	GTTTTAATTCAATCCCACGC	48	7830	7849	451
588552	2596	2615	Exon 18	TGTTTTAATTCAATCCCACG	57	7831	7850	452
588553	2597	2616	Exon 18	CTGTTTTAATTCAATCCCAC	49	7832	7851	453
588554	2598	2617	Exon 18	GCTGTTTTAATTCAATCCCA	64	7833	7852	454
532811	2599	2618	Exon 18	AGCTGTTTTAATTCAATCCC	78	7834	7853	239
588555	2600	2619	Exon 18	CAGCTGTTTTAATTCAATCC	48	7835	7854	455
588556	2601	2620	Exon 18	GCAGCTGTTTTAATTCAATC	55	7836	7855	456
588557	2602	2621	Exon 18	CGCAGCTGTTTTAATTCAAT	51	7837	7856	457
588558	2603	2622	Exon 18	TCGCAGCTGTTTTAATTCAA	51	7838	7857	458
532917	2604	2623	Exon 18	GTCGCAGCTGTTTTAATTCA	82	7839	7858	317
588559	2605	2624	Exon 18	TGTCGCAGCTGTTTTAATTC	58	7840	7859	459
588560	2606	2625	Exon 18	TTGTCGCAGCTGTTTTAATT	72	7841	7860	460
588561	2607	2626	Exon 18	GTTGTCGCAGCTGTTTTAAT	75	7842	7861	461
532952	2608	2627	Exon 18	TGTTGTCGCAGCTGTTTTAA	39	7843	7862	395
588562	2609	2628	Exon 18 / Repeat	TTGTTGTCGCAGCTGTTTTA	53	n/a	n/a	462
588563	2610	2629	Exon 18 / Repeat	TTTGTTGTCGCAGCTGTTTT	62	n/a	n/a	463
588564	2611	2630	Exon 18 / Repeat	TTTTGTTGTCGCAGCTGTTT	63	n/a	n/a	464
588565	2612	2631	Exon 18 / Repeat	TTTTTGTTGTCGCAGCTGTT	64	n/a	n/a	465

Example 122: Dose-dependent antisense inhibition of human CFB in HepG2 cells by 5-10-5 MOE gapmers

Gapmers from studies described above exhibiting *in vitro* inhibition of CFB mRNA were selected and tested at various doses in HepG2 cells. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.313 μM, 0.625 μM, 1.25 μM, 2.50 μM, 5.00 μM, or 10.00 μM

concentrations of antisense oligonucleotide, as specified in the Table below. After a treatment period of approximately 16 hours, RNA was isolated from the cells and CFB mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3459 was used to measure mRNA levels. CFB mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of CFB, relative to untreated control cells.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented. CFB mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 148

ISIS No	0.313 μM	0.625 μM	1.25 μM	2.50 μM	5.00 μM	10.00 μM	IC_{50} (μM)
532614	7	13	43	72	65	71	2.2
532635	12	0	3	28	0	0	>10
532692	26	0	12	52	55	74	3.7
532770	21	18	32	73	64	88	1.8
532775	8	0	26	35	47	59	6.2
532800	0	5	30	65	50	75	3.1
532809	12	30	28	40	46	66	4.6
532810	28	44	32	69	84	95	1.2
532811	66	83	90	94	97	99	<0.3
532917	64	85	88	96	97	99	<0.3
532952	50	53	68	80	91	94	0.4

Example 123: Dose-dependent antisense inhibition of human CFB in HepG2 cells

Gapmers from studies described above exhibiting *in vitro* inhibition of CFB mRNA were selected and tested at various doses in HepG2 cells. The antisense oligonucleotides were tested in a number of experiments with similar culture conditions. The results for each experiment are presented in separate tables shown below. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.08 μM, 0.25 μM, 0.74 μM, 2.22 μM, 6.67 μM, and 20.00 μM concentrations of antisense oligonucleotide, as specified in the Table below. After a treatment period of approximately 16 hours, RNA was isolated from the cells and CFB mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3459 was used to measure mRNA levels. CFB mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of CFB, relative to untreated control cells.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented. CFB mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 149

ISIS No	0.08 μM	0.25 μM	0.74 μM	2.22 μM	6.67 μM	20.00 μM	IC_{50} (μM)
532811	19	53	81	87	96	97	0.2
588834	7	42	64	92	98	98	0.5
588835	11	30	66	89	97	97	0.5
588836	14	40	61	91	97	97	0.5
588837	6	39	67	89	96	97	0.5
588838	0	27	41	81	87	97	1.0
588842	17	51	68	86	93	95	0.3
588843	21	38	72	90	95	96	0.4
588870	9	31	56	88	95	97	0.6
588871	14	25	47	79	93	97	0.7
588872	18	28	59	84	92	97	0.6

Table 150

ISIS No	0.08 μM	0.25 μM	0.74 μM	2.22 μM	6.67 μM	20.00 μM	IC_{50} (μM)
532811	31	70	89	94	97	97	0.1
588844	31	60	77	91	95	96	0.1
588846	32	52	78	89	95	97	0.2
588847	22	52	77	91	95	97	0.2
588848	20	40	73	91	96	98	0.3
588851	40	52	82	94	97	97	0.1
588854	17	55	59	84	94	96	0.4
588855	10	32	56	82	93	96	0.6
588856	13	46	75	90	96	97	0.3
588857	11	52	73	94	96	97	0.3
588858	19	48	75	94	97	98	0.3

Table 151

ISIS No	0.08 μM	0.25 μM	0.74 μM	2.22 μM	6.67 μM	20.00 μM	IC_{50} (μM)
532811	42	66	88	96	97	98	0.1
588859	18	46	66	90	96	97	0.4
588860	55	80	94	97	97	97	<0.1
588861	24	61	86	93	96	97	0.2
588862	25	64	85	94	96	98	0.1
588863	50	73	89	96	96	98	<0.1
588864	52	80	92	96	98	98	<0.1

588865	46	72	91	96	96	99	<0.1
588866	47	76	88	96	97	98	<0.1
588867	43	69	83	92	96	99	0.1
588868	43	56	65	84	93	97	0.1

Table 152

ISIS No	0.08 μM	0.25 μM	0.74 μM	2.22 μM	6.67 μM	20.00 μM	IC_{50} (μM)
532810	0	14	38	72	89	96	1.2
532811	18	54	79	93	96	97	0.3
532952	19	34	73	87	94	96	0.4
588534	17	13	44	77	93	97	0.9
588544	12	43	69	86	89	93	0.4
588545	17	55	67	86	91	93	0.3
588546	10	32	67	85	91	93	0.6
588552	27	54	76	90	94	97	0.2
588553	32	68	87	93	95	97	0.1
588560	16	54	76	90	94	96	0.3
588561	18	45	68	85	93	96	0.4

Table 153

ISIS No	0.08 μM	0.25 μM	0.74 μM	2.22 μM	6.67 μM	20.00 μM	IC_{50} (μM)
532811	22	60	82	94	97	98	0.2
588536	2	38	65	90	96	97	0.6
588537	12	38	63	87	94	97	0.5
588547	19	35	61	86	93	97	0.5
588548	19	36	75	88	95	96	0.4
588554	0	76	92	95	97	97	<0.1
588555	31	61	89	96	97	98	0.1
588556	33	56	82	95	94	97	0.1
588562	12	39	71	87	94	97	0.4
588563	25	48	72	86	94	96	0.3
588564	15	33	63	89	91	97	0.5

Table 154

ISIS No	0.08 μM	0.25 μM	0.74 μM	2.22 μM	6.67 μM	20.00 μM	IC_{50} (μM)
532811	39	68	86	96	98	98	0.1
588538	0	40	82	94	97	98	0.3

588539	34	65	88	95	98	98	0.1
588540	30	51	81	91	97	98	0.2
588549	31	57	82	95	96	98	0.1
588550	34	65	88	96	98	98	0.1
588551	47	66	87	96	98	99	<0.1
588557	40	84	95	98	98	98	<0.1
588558	45	73	93	97	98	99	<0.1
588559	51	69	83	96	98	99	<0.1
588565	19	56	81	92	96	98	0.2

Example 124: Dose-dependent antisense inhibition of human CFB in HepG2 cells

Gapmers from studies described above exhibiting *in vitro* inhibition of CFB mRNA were selected and tested at various doses in HepG2 cells. The antisense oligonucleotides were tested in a number of experiments with similar culture conditions. The results for each experiment are presented in separate tables shown below. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.06 μM, 0.25 μM, 1.00 μM, and 4.00 μM concentrations of antisense oligonucleotide, as specified in the Table below. After a treatment period of approximately 16 hours, RNA was isolated from the cells and CFB mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3459 was used to measure mRNA levels. CFB mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of CFB, relative to untreated control cells.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented. CFB mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 155

ISIS No	0.06 μM	0.25 μM	1.00 μM	4.00 μM	IC_{50} (μM)
532917	31	58	87	92	0.2
588860	18	50	79	93	0.3
599001	16	28	69	90	0.5
599024	14	32	74	90	0.4
599025	0	31	56	92	0.7
599032	28	44	62	88	0.3
599033	28	46	80	92	0.2
599077	8	20	59	80	0.8
599080	9	33	48	76	0.9
599086	7	22	53	83	0.8
599087	21	31	74	87	0.4

599088	13	37	69	82	0.5
599089	3	36	55	79	0.7
599093	25	59	79	88	0.2
599094	19	29	75	89	0.4
599095	29	43	67	87	0.3
599096	23	51	70	88	0.3
599149	20	53	82	92	0.3
599188	0	21	62	85	0.8

Table 156

ISIS No	0.06 μM	0.25 μM	1.00 μM	4.00 μM	IC_{50} (μM)
532917	0	42	81	91	0.4
588860	17	49	74	92	0.3
599155	29	52	67	87	0.3
599198	3	25	64	89	0.6
599201	13	26	67	91	0.5
599202	0	44	72	87	0.5
599203	22	41	75	88	0.3
599314	12	34	71	84	0.5
599316	7	37	66	88	0.5
599317	8	1	54	83	1.0
599321	8	33	70	85	0.5
599322	24	38	66	87	0.4
599327	22	32	66	89	0.4
599328	0	31	59	88	0.7
599330	5	43	67	84	0.5
599374	23	42	80	91	0.3
599378	21	57	80	93	0.2
599380	23	56	82	93	0.2
599432	17	37	73	93	0.4

Table 157

ISIS No	0.06 μM	0.25 μM	1.00 μM	4.00 μM	IC_{50} (μM)
532917	23	65	76	93	0.2
588860	17	60	76	90	0.3
601282	48	68	81	88	0.1
601269	18	59	80	94	0.2
601276	34	64	81	91	0.1

601275	14	39	78	90	0.4
601344	52	84	92	94	<0.06
601383	53	81	86	94	<0.06
601382	41	76	88	94	0.1
601385	52	74	89	91	<0.06
601332	41	69	86	94	0.1
601345	36	75	86	95	0.1
601371	34	72	91	93	0.1
601384	50	78	91	95	<0.06
601380	28	57	83	92	0.2
601387	48	61	82	88	0.1
601341	28	65	83	91	0.2
601346	31	69	82	93	0.1
601335	24	56	85	92	0.2

Table 158

ISIS No	0.06 µM	0.25 µM	1.00 µM	4.00 µM	IC_{50} (µM)
532917	31	66	86	93	0.1
588860	28	62	85	94	0.2
599208	24	50	71	89	0.3
599261	31	49	81	94	0.2
599267	41	48	80	88	0.2
599268	28	56	75	92	0.2
599313	14	24	71	92	0.5
599441	24	57	80	87	0.2
599494	13	55	86	94	0.3
599552	30	69	93	95	0.1
599553	34	71	93	96	0.1
599554	30	74	93	96	0.1
599568	40	77	90	97	0.1
599570	61	82	93	96	<0.06
599577	18	62	81	93	0.2
599581	27	60	80	94	0.2
599591	49	74	93	96	<0.06
599592	46	76	90	94	0.1
599593	44	72	91	95	0.1

Table 159

ISIS No	0.06 μM	0.25 μM	1.00 μM	4.00 μM	IC_{50} (μM)
532917	25	56	84	92	0.2
588860	11	51	80	92	0.3
599547	23	60	82	90	0.2
599569	42	73	85	88	0.1
599578	29	49	82	89	0.2
599582	21	56	78	91	0.2
599590	24	62	80	90	0.2
601209	21	49	85	88	0.3
601210	34	64	86	92	0.1
601212	46	68	88	90	0.1
601213	54	80	90	92	<0.06
601214	38	77	88	95	0.1
601215	42	64	85	92	0.1
601216	45	57	76	89	0.1
601264	29	58	86	95	0.2
601278	51	82	83	93	<0.06
601279	44	80	92	96	0.1
601280	44	73	87	94	0.1
601281	51	80	91	94	<0.06

Example 125: Dose-dependent antisense inhibition of human CFB in HepG2 cells

Gapmers from studies described above exhibiting *in vitro* inhibition of CFB mRNA were selected and tested at various doses in HepG2 cells. Additionally, a deoxy, MOE and (S)-cEt oligonucleotide, ISIS 594430, was designed with the same sequence (CTCCTTCCGAGTCAGC, SEQ ID NO: 549) and target region (target start site 2195 of SEQ ID NO: 1 and target start site 6983 of SED ID NO: 2) as ISIS 588870, another deoxy, MOE, and (S)-cEt oligonucleotide. ISIS 594430 is a 3-10-3 (S)-cEt gapmer.

Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.01 μM, 0.04 μM,0.12 μM, 0.37 μM, 1.11 μM, 3.33 μM, and 10.00 μM concentrations of antisense oligonucleotide, as specified in the Table below. After a treatment period of approximately 16 hours, RNA was isolated from the cells and CFB mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3459 was used to measure mRNA levels. CFB mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of CFB, relative to untreated control cells.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented. CFB mRNA levels were reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 160

ISIS No	0.01 μM	0.04 μM	0.12 μM	0.37 μM	1.11 μM	3.33 μM	10.00 μM	IC_{50} (μM)
588536	0	0	0	5	45	73	94	1.4
588548	0	0	0	19	52	78	90	1.2
588553	0	0	9	42	76	85	94	0.6
588555	0	52	23	58	78	83	95	0.3
588847	4	1	18	45	67	84	96	0.5
588848	0	3	13	38	67	83	95	0.6
594430	0	0	10	34	50	55	84	1.4

Example 126: Tolerability of MOE gapmers targeting human CFB in CD1 mice

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

Study 1 (with 5-10-5 MOE gapmers)

Groups of seven-week old male CD1 mice were injected subcutaneously once a week for 6 weeks with 100 mg/kg of ISIS oligonucleotide. A group of male CD1 mice was injected subcutaneously once a week for 6 weeks with PBS. One group of mice was injected with subcutaneously once a week for 6 weeks with 100 mg/kg of control oligonucleotide ISIS 141923 (CCTTCCCTGAAGGTTCCTCC, designated herein as SEQ ID NO: 809, 5-10-5 MOE gapmer with no known murine target). Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

Plasma chemistry markers

To evaluate the effect of ISIS oligonucleotides on liver and kidney function, plasma levels of transaminases, and BUN were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The results are presented in the Table below. ISIS oligonucleotides that caused changes in the levels of any of the liver or kidney function markers outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 161

Plasma chemistry markers in CD1 mice plasma on day 40

	ALT (IU/L)	AST (IU/L)	BUN (mg/dL)
PBS	25	46	20
ISIS 532614	513	407	22
ISIS 532692	131	130	24
ISIS 532770	36	53	25
ISIS 532775	193	158	23
ISIS 532800	127	110	25
ISIS 532809	36	42	22
ISIS 532810	229	286	26
ISIS 532811	197	183	21
ISIS 532917	207	204	27
ISIS 532952	246	207	25
ISIS 141923	39	67	23

Weights

Body weights of the mice were measured on day 40 before sacrificing the mice. Weights of organs, liver, kidney, and spleen were also measured after the mice were sacrificed. The results are presented in the Table below. ISIS oligonucleotides that caused changes in the weights outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 162

Weights (g) of CD1 mice on day 40

	Body	Kidney	Liver	Spleen
PBS	44	0.8	2.0	0.1
ISIS 532614	43	0.7	4.3	0.2
ISIS 532692	42	0.7	2.6	0.2
ISIS 532770	42	0.6	2.3	0.2
ISIS 532775	42	0.7	2.5	0.2
ISIS 532800	43	0.6	2.8	0.3
ISIS 532809	42	0.6	2.2	0.1
ISIS 532810	43	0.6	2.3	0.2
ISIS 532811	41	0.7	2.4	0.2
ISIS 532917	42	0.7	3.0	0.2
ISIS 532952	44	0.8	2.5	0.3
ISIS 141923	41	0.6	2.0	0.1

Study 2 (with 5-10-5 MOE gapmers)

Groups of six- to eight-week old male CD1 mice were injected subcutaneously once a week for 6 weeks with 100 mg/kg of ISIS oligonucleotide. Two groups of male CD1 mice were injected subcutaneously once a week for 6 weeks with PBS. One group of mice was injected with subcutaneously once a week for 6 weeks with 100 mg/kg of control oligonucleotide ISIS 141923. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

Plasma chemistry markers

To evaluate the effect of ISIS oligonucleotides on liver and kidney function, plasma levels of transaminases, albumin, and BUN were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The results are presented in the Table below. ISIS oligonucleotides that caused changes in the levels of any of the liver or kidney function markers outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 163

Plasma chemistry markers in CD1 mice plasma on day 45

	ALT (IU/L)	AST (IU/L)	Albumin (g/dL)	BUN (mg/dL)
PBS	39	53	2.9	29
PBS	50	97	2.9	30
ISIS 141923	163	174	4.1	25
ISIS 532810	321	297	2.5	26
ISIS 532952	182	199	2.7	27
ISIS 588534	276	248	2.6	29
ISIS 588536	48	60	2.9	31
ISIS 588537	72	79	4.0	25
ISIS 588538	63	67	4.5	29
ISIS 588539	238	177	3.9	28
ISIS 588545	496	256	4.4	24
ISIS 588547	323	210	4.4	25
ISIS 588548	61	63	4.2	27
ISIS 588549	127	132	4.1	23
ISIS 588551	302	282	4.2	22
ISIS 588552	76	98	4.0	30
ISIS 588558	1066	521	3.9	27
ISIS 588559	76	94	4.1	26
ISIS 588561	502	500	4.4	26
ISIS 588563	50	99	4.4	28

Weights

Body weights of the mice were measured on day 42. Weights of organs, liver, kidney, and spleen were also measured after the mice were sacrificed on day 45. The results are presented in the Table below.

ISIS oligonucleotides that caused changes in the weights outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 164

Weights (g) of CD1 mice on day 40

	Body	Kidney	Liver	Spleen
PBS	44	0.7	2.4	0.1
PBS	43	0.7	2.4	0.2
ISIS 141923	43	0.6	2.4	0.2
ISIS 532810	41	0.6	1.9	0.1
ISIS 532952	43	0.6	2.4	0.2
ISIS 588534	44	0.7	2.8	0.2
ISIS 588536	43	0.7	2.7	0.2
ISIS 588537	43	0.7	2.4	0.2
ISIS 588538	44	0.7	2.8	0.2
ISIS 588539	44	0.6	2.7	0.2
ISIS 588545	44	0.8	3.3	0.3
ISIS 588547	42	0.6	3.3	0.3
ISIS 588548	43	0.6	2.8	0.2
ISIS 588549	42	0.6	2.8	0.3
ISIS 588551	39	0.6	2.2	0.2
ISIS 588552	41	0.6	2.2	0.2
ISIS 588558	44	0.7	3.3	0.3
ISIS 588559	43	0.6	2.7	0.3
ISIS 588561	40	0.7	2.4	0.3
ISIS 588563	41	0.7	2.4	0.2

Study 3 (with 5-10-5 MOE gapmers)

Groups of six- to eight-week old male CD1 mice were injected subcutaneously once a week for 6 weeks with 100 mg/kg of ISIS oligonucleotide. Two groups of male CD1 mice were injected subcutaneously once a week for 6 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

Plasma chemistry markers

To evaluate the effect of ISIS oligonucleotides on liver and kidney function, plasma levels of transaminases, albumin, and BUN were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The results are presented in the Table below. ISIS oligonucleotides that caused changes in the levels of any of the liver or kidney function markers outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 165

Plasma chemistry markers in CD1 mice plasma on day 42

	ALT (IU/L)	AST (IU/L)	Albumin (g/dL)	BUN (mg/dL)
PBS	37	108	3.1	30
PBS	45	51	3.0	27
ISIS 588544	209	168	2.9	26
ISIS 588546	526	279	3.0	22
ISIS 588550	82	136	2.7	25
ISIS 588553	79	105	3.0	24
ISIS 588554	112	220	3.2	19
ISIS 588555	95	162	2.8	25
ISIS 588556	345	236	3.0	26
ISIS 588557	393	420	2.8	24
ISIS 588560	109	148	2.7	27
ISIS 588562	279	284	2.8	22
ISIS 588564	152	188	3.0	23
ISIS 588565	247	271	2.8	28

Weights

Body weights of the mice were measured on day 42. Weights of organs, liver, kidney, and spleen were also measured after the mice were sacrificed on day 42. The results are presented in the Table below. ISIS oligonucleotides that caused changes in the weights outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 166

Weights (g) of CD1 mice on day 40

	Body	Kidney	Liver	Spleen
PBS	42	0.7	2.4	0.1
PBS	41	0.7	2.4	0.2
ISIS 588544	44	0.6	1.9	0.1
ISIS 588546	43	0.6	2.4	0.2
ISIS 588550	41	0.7	2.8	0.2
ISIS 588553	44	0.7	2.7	0.2
ISIS 588554	40	0.7	2.4	0.2
ISIS 588555	44	0.7	2.8	0.2
ISIS 588556	39	0.6	2.7	0.2
ISIS 588557	41	0.8	3.3	0.3
ISIS 588560	38	0.6	3.2	0.3
ISIS 588562	41	0.6	2.8	0.2
ISIS 588564	40	0.6	2.8	0.3
ISIS 588565	39	0.6	2.2	0.2

Study 4 (with (S) cEt gapmers and deoxy, MOE and (S)-cEt oligonucleotides)

Groups of ten-week old male CD1 mice were injected subcutaneously once a week for 6 weeks with 50 mg/kg of ISIS oligonucleotide from the studies described above. In addition, two oligonucleotides, ISIS 594431 and ISIS 594432, were designed as 3-10-3 (S)-cEt gapmers and were also tested in this study. ISIS 594431(ACCTCCTTCCGAGTCA, SEQ ID NO: 550) targets the same region as ISIS 588871, a deoxy, MOE and (S)-cEt gapmer (target start site 2197 of SEQ ID NO: 1 and target start site 6985 of SEQ ID NO: 2). ISIS 594432 (TGGTCACATTCCCTTC, SEQ ID NO: 542) targets the same region as ISIS 588872 a deoxy, MOE and (S)-cEt gapmer (target start site 154 of SEQ ID NO: 1 and target start site 1875 of SEQ ID NO: 2).

Two groups of male CD1 mice were injected subcutaneously once a week for 6 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

Plasma chemistry markers

To evaluate the effect of ISIS oligonucleotides on liver and kidney function, plasma levels of transaminases, albumin, creatinine, and BUN were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The results are presented in the Table below. ISIS oligonucleotides that caused changes in the levels of any of the liver or kidney function markers outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 167

Plasma chemistry markers in CD1 mice plasma on day 42

	Chemistry	ALT (IU/L)	AST (IU/L)	Albumin (g/dL)	Creatinine (mg/dL)	BUN (mg/dL)
PBS	-	71	77	2.7	0.2	29
PBS	-	30	36	2.7	0.2	26
ISIS 588834	Deoxy, MOE and (S)-cEt	436	510	2.8	0.2	25
ISIS 588835	Deoxy, MOE and (S)-cEt	70	98	3.0	0.2	27
ISIS 588836	Deoxy, MOE and (S)-cEt	442	312	2.7	0.2	27
ISIS 588846	Deoxy, MOE and (S)-cEt	50	75	2.5	0.1	28
ISIS 588847	Deoxy, MOE and (S)-cEt	44	71	2.6	0.1	24
ISIS 588848	Deoxy, MOE and (S)-cEt	47	70	2.4	0.1	27
ISIS 588857	Deoxy, MOE and (S)-cEt	1287	655	2.7	0.2	26
ISIS 588858	Deoxy, MOE and (S)-cEt	1169	676	2.5	0.2	26
ISIS 588859	Deoxy, MOE and (S)-cEt	1036	1300	3.2	0.2	25
ISIS 588861	Deoxy, MOE and (S)-cEt	749	466	3.1	0.1	24
ISIS 588862	Deoxy, MOE and (S)-cEt	1564	1283	2.9	0.2	22
ISIS 588863	Deoxy, MOE and (S)-cEt	477	362	2.8	0.1	23

ISIS 588864	Deoxy, MOE and (S)-cEt	118	165	2.9	0.2	27
ISIS 588866	Deoxy, MOE and (S)-cEt	843	784	3.2	0.2	25
ISIS 594430	3-10-3 (S)-cEt	89	99	2.4	0.1	28
ISIS 594431	3-10-3 (S)-cEt	590	433	3.0	0.2	24
ISIS 594432	3-10-3 (S)-cEt	2595	2865	2.4	0.1	25

Weights

Body weights of the mice were measured on day 39. Weights of organs, liver, kidney, and spleen were also measured after the mice were sacrificed on day 42. The results are presented in the Table below. ISIS oligonucleotides that caused changes in the weights outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 168

Weights (g) of CD1 mice

	Chemistry	Body	Kidney	Liver	Spleen
PBS	-	37	0.6	2.1	0.1
PBS	-	45	0.7	2.5	0.2
ISIS 588834	Deoxy, MOE and (S)-cEt	40	0.6	3.2	0.2
ISIS 588835	Deoxy, MOE and (S)-cEt	38	0.7	2.8	0.3
ISIS 588836	Deoxy, MOE and (S)-cEt	41	0.7	2.3	0.2
ISIS 588837	Deoxy, MOE and (S)-cEt	38	0.6	2.4	0.3
ISIS 588846	Deoxy, MOE and (S)-cEt	39	0.6	2.3	0.2
ISIS 588847	Deoxy, MOE and (S)-cEt	40	0.7	2.5	0.2
ISIS 588848	Deoxy, MOE and (S)-cEt	43	0.7	2.6	0.3
ISIS 588857	Deoxy, MOE and (S)-cEt	39	0.6	3.3	0.2
ISIS 588858	Deoxy, MOE and (S)-cEt	37	0.6	3.4	0.2
ISIS 588859	Deoxy, MOE and (S)-cEt	41	0.7	2.5	0.3
ISIS 588861	Deoxy, MOE and (S)-cEt	39	0.6	2.6	0.4
ISIS 588862	Deoxy, MOE and (S)-cEt	34	0.6	2.5	0.4
ISIS 588863	Deoxy, MOE and (S)-cEt	40	0.6	2.7	0.3
ISIS 588864	Deoxy, MOE and (S)-cEt	40	0.7	2.3	0.2
ISIS 588866	Deoxy, MOE and (S)-cEt	45	0.7	3.0	0.2
ISIS 594430	3-10-3 (S)-cEt	39	0.6	2.2	0.2
ISIS 594431	3-10-3 (S)-cEt	36	0.6	3.2	0.2
ISIS 594432	3-10-3 (S)-cEt	31	0.4	1.9	0.1

Study 5 (with MOE gapmers, (S) cEt gapmers and deoxy, MOE and (S)-cEt oligonucleotides)

Groups of eight- to nine-week old male CD1 mice were injected subcutaneously once a week for 6 weeks with 50 mg/kg of ISIS oligonucleotide. Two groups of male CD1 mice were injected subcutaneously

once a week for 6 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

Plasma chemistry markers

To evaluate the effect of ISIS oligonucleotides on liver and kidney function, plasma levels of transaminases, albumin, creatinine, and BUN were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The results are presented in the Table below. ISIS oligonucleotides that caused changes in the levels of any of the liver or kidney function markers outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 169

Plasma chemistry markers in CD1 mice plasma on day 42

	Chemistry	ALT (IU/L)	AST (IU/L)	Albumin (g/dL)	Creatinine (mg/dL)	BUN (mg/dL)
PBS	-	33	84	2.9	0.2	28
PBS	-	32	65	2.5	0.1	27
ISIS 532692	5-10-5 MOE	363	281	3.0	0.2	30
ISIS 532770	5-10-5 MOE	69	100	2.9	0.1	28
ISIS 532775	5-10-5 MOE	371	333	2.6	0.1	29
ISIS 532800	5-10-5 MOE	104	106	2.7	0.1	31
ISIS 532809	5-10-5 MOE	69	127	2.8	0.1	26
ISIS 588540	5-10-5 MOE	66	110	2.8	0.1	26
ISIS 588838	3-10-3 (S)-cEt	391	330	2.9	0.1	25
ISIS 588842	Deoxy, MOE and (S)-cEt	224	264	2.6	0.1	26
ISIS 588843	3-10-3 (S)-cEt	185	160	2.8	0.1	24
ISIS 588844	Deoxy, MOE and (S)-cEt	304	204	2.7	0.1	25
ISIS 588851	Deoxy, MOE and (S)-cEt	186	123	2.7	0.1	31
ISIS 588854	Deoxy, MOE and (S)-cEt	1232	925	2.7	0.1	25
ISIS 588855	Deoxy, MOE and (S)-cEt	425	321	2.7	0.1	28
ISIS 588856	Deoxy, MOE and (S)-cEt	78	101	2.4	0.1	31
ISIS 588865	Deoxy, MOE and (S)-cEt	126	145	2.5	0.1	23
ISIS 588867	Deoxy, MOE and (S)-cEt	108	112	2.5	0.1	32
ISIS 588868	Deoxy, MOE and (S)-cEt	61	124	2.5	0.1	28
ISIS 588870	Deoxy, MOE and (S)-cEt	48	69	2.4	0.1	31
ISIS 588871	Deoxy, MOE and (S)-cEt	723	881	2.5	0.1	24
ISIS 588872	Deoxy, MOE and (S)-cEt	649	654	2.7	0.1	26

Weights

Body weights of the mice were measured on day 40. Weights of organs, liver, kidney, and spleen were also measured after the mice were sacrificed on day 42. The results are presented in the Table below.

ISIS oligonucleotides that caused changes in the weights outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 170

Weights (g) of CD1 mice

	Chemistry	Body	Kidney	Liver	Spleen
PBS	-	46	0.7	2.3	0.2
PBS	-	44	0.7	2.3	0.2
ISIS 532692	5-10-5 MOE	44	0.6	2.8	0.2
ISIS 532770	5-10-5 MOE	43	0.6	2.2	0.2
ISIS 532775	5-10-5 MOE	43	0.6	2.8	0.2
ISIS 532800	5-10-5 MOE	47	0.7	2.9	0.2
ISIS 532809	5-10-5 MOE	44	0.7	2.6	0.2
ISIS 588540	5-10-5 MOE	44	0.7	2.5	0.2
ISIS 588838	3-10-3 (S)-cEt	45	0.7	3.1	0.2
ISIS 588842	Deoxy, MOE and (S)-cEt	41	0.6	2.6	0.2
ISIS 588843	3-10-3 (S)-cEt	43	0.7	2.9	0.2
ISIS 588844	Deoxy, MOE and (S)-cEt	43	0.7	2.8	0.2
ISIS 588851	Deoxy, MOE and (S)-cEt	46	0.6	2.6	0.2
ISIS 588854	Deoxy, MOE and (S)-cEt	45	0.7	4.1	0.2
ISIS 588855	Deoxy, MOE and (S)-cEt	44	0.7	2.9	0.3
ISIS 588856	Deoxy, MOE and (S)-cEt	44	0.7	3.2	0.2
ISIS 588865	Deoxy, MOE and (S)-cEt	45	0.7	2.6	0.3
ISIS 588867	Deoxy, MOE and (S)-cEt	46	0.7	3.2	0.3
ISIS 588868	Deoxy, MOE and (S)-cEt	42	0.7	2.9	0.3
ISIS 588870	Deoxy, MOE and (S)-cEt	43	0.6	2.2	0.2
ISIS 588871	Deoxy, MOE and (S)-cEt	41	0.7	3.1	0.2
ISIS 588872	Deoxy, MOE and (S)-cEt	39	0.6	3.2	0.3

Study 6 (with deoxy, MOE and (S)-cEt oligonucleotides)

Groups of eight- to nine-week old male CD1 mice were injected subcutaneously once a week for 6 weeks with 50 mg/kg of deoxy, MOE, and (S)-cEt oligonucleotides. Two groups of male CD1 mice were injected subcutaneously once a week for 6 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

Plasma chemistry markers

To evaluate the effect of ISIS oligonucleotides on liver and kidney function, plasma levels of transaminases, albumin, creatinine, bilirubin, and BUN were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The results are presented in the Table below. ISIS

oligonucleotides that caused changes in the levels of any of the liver or kidney function markers outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 171

Plasma chemistry markers in CD1 mice plasma on day 45

	ALT (IU/L)	AST (IU/L)	Albumin (g/dL)	Creatinine (mg/dL)	Bilirubin (mg/dL)	BUN (mg/dL)
PBS	39	78	3.4	0.2	0.2	31
PBS	37	59	2.9	0.1	0.2	27
ISIS 599552	167	208	3.0	0.1	0.2	32
ISIS 599553	43	86	2.9	0.1	0.2	28
ISIS 599554	57	101	2.2	0.2	0.2	31
ISIS 599569	469	530	3.5	0.2	0.3	27
ISIS 599577	37	84	2.9	0.1	0.1	31
ISIS 599578	45	104	2.8	0.1	0.2	30
ISIS 599581	54	88	3.1	0.1	0.2	31
ISIS 599590	1741	1466	3.1	0.1	0.3	25
ISIS 599591	2230	1183	3.2	0.1	0.3	27
ISIS 601209	68	104	2.9	0.1	0.2	30
ISIS 601212	1795	968	3.2	0.1	0.3	22
ISIS 601215	424	385	3.1	0.1	0.4	25
ISIS 601216	90	125	2.9	0.1	0.2	29
ISIS 601276	946	366	2.9	0.1	0.5	31
ISIS 601282	831	540	3.3	0.2	0.2	32

Weights

Body weights of the mice were measured on day 40. Weights of organs, liver, kidney, and spleen were also measured after the mice were sacrificed on day 45. The results are presented in the Table below. ISIS oligonucleotides that caused changes in the weights outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 172

Weights (g) of CD1 mice

	Body	Kidney	Liver	Spleen
PBS	40	0.7	2.1	0.2
PBS	42	0.8	2.3	0.2
ISIS 599552	38	0.6	2.3	0.2
ISIS 599553	39	0.7	2.2	0.2
ISIS 599554	39	0.7	2.4	0.2
ISIS 599569	39	0.7	2.2	0.2
ISIS 599577	41	0.7	2.5	0.2
ISIS 599578	37	0.6	2.0	0.2

ISIS 599581	40	0.6	2.5	0.2
ISIS 599590	34	0.6	3.5	0.2
ISIS 599591	38	0.8	2.7	0.2
ISIS 601209	42	0.7	2.6	0.3
ISIS 601212	38	0.6	2.9	0.2
ISIS 601215	36	0.7	2.6	0.2
ISIS 601216	42	0.6	2.7	0.2
ISIS 601276	42	0.7	3.2	0.2
ISIS 601282	38	0.7	3.2	0.2

Study 7 (with MOE gapmers and deoxy, MOE and (S)-cEt oligonucleotides)

Groups of eight- to nine-week old male CD1 mice were injected subcutaneously once a week for 6 weeks with 100 mg/kg of ISIS oligonucleotides. One group of male CD1 mice was injected subcutaneously once a week for 6 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

Plasma chemistry markers

To evaluate the effect of ISIS oligonucleotides on liver and kidney function, plasma levels of transaminases, albumin, creatinine, and BUN were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The results are presented in the Table below. ISIS oligonucleotides that caused changes in the levels of any of the liver or kidney function markers outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 173

Plasma chemistry markers in CD1 mice plasma on day 45

	Chemistry	ALT (IU/L)	AST (IU/L)	Albumin (g/dL)	Creatinine (mg/dL)	BUN (mg/dL)
PBS	-	120	102	2.7	0.2	26
ISIS 588842	Deoxy, MOE and (S)-cEt	177	164	2.7	0.1	23
ISIS 588843	Deoxy, MOE and (S)-cEt	98	194	2.7	0.1	24
ISIS 588851	Deoxy, MOE and (S)-cEt	91	142	2.6	0.1	23
ISIS 588856	Deoxy, MOE and (S)-cEt	78	110	2.7	0.1	23
ISIS 599024	3-10-4 MOE	91	108	2.7	0.1	23
ISIS 599087	5-7-5 MOE	198	183	2.6	0.2	28
ISIS 599093	5-7-5 MOE	3285	2518	2.6	0.2	24
ISIS 599149	4-8-5 MOE	30	64	2.9	0.2	25
ISIS 599155	4-8-5 MOE	145	189	2.6	0.2	25
ISIS 599202	5-8-5 MOE	150	128	2.8	0.2	23
ISIS 599203	5-8-5 MOE	111	127	2.8	0.2	24
ISIS 599208	5-8-5 MOE	146	178	2.9	0.2	22

ISIS 599261	3-10-5 MOE	144	165	2.8	0.2	26
ISIS 599267	3-10-5 MOE	96	132	2.6	0.2	27
ISIS 599268	3-10-5 MOE	87	115	2.6	0.1	23
ISIS 599322	6-7-6 MOE	115	138	2.7	0.1	22
ISIS 599374	5-9-5 MOE	375	271	2.6	0.1	21
ISIS 599378	5-9-5 MOE	77	99	2.7	0.1	23
ISIS 599441	6-8-6 MOE	150	250	2.9	0.1	23

Weights

Body weights of the mice were measured on day 44. Weights of organs, liver, kidney, and spleen were also measured after the mice were sacrificed on day 49. The results are presented in the Table below. ISIS oligonucleotides that caused changes in the weights outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 174

Weights (g) of CD1 mice

	Chemistry	Body	Kidney	Liver	Spleen
PBS	-	39	0.6	1.9	0.1
ISIS 588842	Deoxy, MOE and (S)-cEt	38	0.5	2.1	0.1
ISIS 588843	Deoxy, MOE and (S)-cEt	41	0.6	2.4	0.2
ISIS 588851	Deoxy, MOE and (S)-cEt	42	0.6	2.2	0.2
ISIS 588856	Deoxy, MOE and (S)-cEt	42	0.7	2.6	0.2
ISIS 599024	3-10-4 MOE	41	0.6	4.0	0.2
ISIS 599087	5-7-5 MOE	44	0.8	2.6	0.3
ISIS 599093	5-7-5 MOE	39	0.6	2.3	0.2
ISIS 599149	4-8-5 MOE	42	0.7	2.8	0.2
ISIS 599155	4-8-5 MOE	41	0.7	2.1	0.2
ISIS 599202	5-8-5 MOE	43	0.6	2.6	0.2
ISIS 599203	5-8-5 MOE	42	0.6	2.6	0.2
ISIS 599208	5-8-5 MOE	40	0.6	2.1	0.2
ISIS 599261	3-10-5 MOE	39	0.7	3.4	0.3
ISIS 599267	3-10-5 MOE	42	0.8	2.5	0.3
ISIS 599268	3-10-5 MOE	41	0.7	2.1	0.2
ISIS 599322	6-7-6 MOE	43	0.6	2.2	0.2
ISIS 599374	5-9-5 MOE	37	0.6	2.2	0.2
ISIS 599378	5-9-5 MOE	43	0.7	2.7	0.2
ISIS 599441	6-8-6 MOE	42	0.6	2.5	0.3

Study 8 (with MOE gapmers, deoxy, MOE and (S)-cEt oligonucleotides, and (S)-cEt gapmers)

Groups of eight- to nine-week old male CD1 mice were injected subcutaneously once a week for 6 weeks with 100 mg/kg of MOE gapmers, or 50 mg/kg of deoxy, MOE and (S)-cEt oligonucleotides or (S)-cEt gapmers. One group of male CD1 mice was injected subcutaneously once a week for 6 weeks with PBS. Mice were euthanized 48 hours after the last dose, and organs and plasma were harvested for further analysis.

Plasma chemistry markers

To evaluate the effect of ISIS oligonucleotides on liver and kidney function, plasma levels of transaminases, albumin, creatinine, and BUN were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). The results are presented in the Table below.

Table 175

Plasma chemistry markers in CD1 mice plasma on day 43

	Chemistry	Dose (mg/kg/wk)	ALT (IU/L)	AST (IU/L)	Albumin (g/dL)	Creatinine (mg/dL)	BUN (mg/dL)
PBS	-	-	37	57	2.5	0.08	26
ISIS 532770	5-10-5 MOE	100	57	73	2.5	0.07	24
ISIS 532800	5-10-5 MOE	100	74	126	2.8	0.10	26
ISIS 532809	5-10-5 MOE	100	83	73	2.5	0.07	23
ISIS 588540	5-10-5 MOE	100	106	102	2.7	0.09	27
ISIS 588544	5-10-5 MOE	100	66	62	2.6	0.10	24
ISIS 588548	5-10-5 MOE	100	48	67	2.6	0.08	23
ISIS 588550	5-10-5 MOE	100	65	106	2.5	0.10	25
ISIS 588553	5-10-5 MOE	100	78	90	2.6	0.09	25
ISIS 588555	5-10-5 MOE	100	94	89	2.5	0.08	23
ISIS 588848	Deoxy, MOE and (S)-cEt	50	38	54	2.3	0.07	25
ISIS 594430	3-10-3 (S)-cEt	50	63	72	2.5	0.10	27

Weights

Body weights of the mice were measured on day 36. Weights of organs, liver, kidney, and spleen were also measured after the mice were sacrificed on day 43. The results for the organ weights were expressed as a ratio to the body weights and normalized to the PBS control ratio.

Table 176

Organ Weights/Body weight (BW) of CD1 mice

	Chemistry	Dose (mg/kg/wk)	Kidney/BW	Liver/BW	Spleen/BW
PBS	-	-	1.0	1.0	1.0
ISIS 532770	5-10-5 MOE	100	1.4	1.1	1.0

ISIS 532800	5-10-5 MOE	100	1.5	1.1	0.9
ISIS 532809	5-10-5 MOE	100	1.3	1.2	0.9
ISIS 588540	5-10-5 MOE	100	1.3	1.2	1.0
ISIS 588544	5-10-5 MOE	100	1.6	1.1	1.0
ISIS 588548	5-10-5 MOE	100	1.7	1.2	1.0
ISIS 588550	5-10-5 MOE	100	1.5	1.2	1.0
ISIS 588553	5-10-5 MOE	100	1.5	1.0	0.8
ISIS 588555	5-10-5 MOE	100	1.8	1.2	1.0
ISIS 588848	Deoxy, MOE and (S)-cEt	50	1.3	1.0	0.9
ISIS 594430	3-10-3 (S)-cEt	50	1.4	1.1	0.9

Cytokine assays

Blood obtained from all mice groups were sent to Antech Diagnostics for measurements of the various cytokine levels, such as IL-6, MDC, MIP1β, IP-10, MCP1, MIP-1α, and RANTES. The results are presented in Table 54.

Table 177

Cytokine levels (pg/mL) in CD1 mice plasma

	Chemistry	IL-6	MDC	MIP1β	IP-10	MCP1	MIP-1α	RANTES
PBS	-	70	16	23	20	17	6	2
ISIS 532770	5-10-5 MOE	101	18	146	116	101	24	6
ISIS 532800	5-10-5 MOE	78	17	83	53	105	1	3
ISIS 532809	5-10-5 MOE	66	19	60	32	55	20	4
ISIS 588540	5-10-5 MOE	51	18	126	70	75	4	3
ISIS 588544	5-10-5 MOE	157	14	94	34	102	1	3
ISIS 588548	5-10-5 MOE	164	12	90	66	84	10	4
ISIS 588550	5-10-5 MOE	58	21	222	124	157	3	5
ISIS 588553	5-10-5 MOE	62	14	183	60	103	9	4
ISIS 588555	5-10-5 MOE	70	19	172	171	178	16	9
ISIS 588848	Deoxy, MOE and (S)-cEt	59	13	61	27	63	12	4
ISIS 594430	3-10-3 (S)-cEt	48	14	56	38	85	10	3

Hematology assays

Blood obtained from all mice groups were sent to Antech Diagnostics for measurements of hematocrit (HCT), as well as of the various blood cells, such as WBC, RBC, and platelets, and total hemoglobin (Hb) content. The results are presented in Table 55.

Table 178

Hematology markers in CD1 mice plasma

	Chemistry	HCT (%)	Hb (g/dL)	WBC (10^3/μL)	RBC (10^6/μL)	Platelets (10^3/μL)
PBS	-	46	15	7	9	960
ISIS 532770	5-10-5 MOE	45	14	5	9	879
ISIS 532800	5-10-5 MOE	45	14	5	9	690
ISIS 532809	5-10-5 MOE	46	14	6	9	1005
ISIS 588540	5-10-5 MOE	49	15	6	10	790
ISIS 588544	5-10-5 MOE	36	11	7	7	899
ISIS 588548	5-10-5 MOE	46	14	6	9	883
ISIS 588550	5-10-5 MOE	42	13	8	8	721
ISIS 588553	5-10-5 MOE	45	14	6	9	719
ISIS 588555	5-10-5 MOE	43	13	8	9	838
ISIS 588848	Deoxy, MOE and (S)-cEt	40	15	8	10	840
ISIS 594430	3-10-3 (S)-cEt	45	14	8	9	993

Example 127: Tolerability of antisense oligonucleotides targeting human CFB in Sprague-Dawley rats

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

Study 1 (with 5-10-5 MOE gapmers)

Male Sprague-Dawley rats, seven- to eight-week old, were maintained on a 12-hour light/dark cycle and fed ad libitum with Purina normal rat chow, diet 5001. Groups of 4 Sprague-Dawley rats each were injected subcutaneously once a week for 6 weeks with 100 mg/kg of 5-10-5 MOE gapmers. One control group of 6 rats was injected subcutaneously once a week for 6 weeks with PBS. Forty eight hours after the last dose, rats were euthanized and organs and plasma were harvested for further analysis.

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma levels of transaminases were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma levels of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in the Table below expressed in IU/L. ISIS oligonucleotides that caused changes in the

levels of any markers of liver function outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 179

Liver function markers in Sprague-Dawley rats

	ALT (IU/L)	AST (IU/L)
PBS	66	134
ISIS 588544	101	329
ISIS 588550	69	157
ISIS 588553	88	304
ISIS 588554	202	243
ISIS 588555	94	113
ISIS 588556	102	117
ISIS 588560	206	317
ISIS 588564	292	594

Kidney function

To evaluate the effect of ISIS oligonucleotides on kidney function, plasma levels of blood urea nitrogen (BUN) and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in the Table below, expressed in mg/dL. ISIS oligonucleotides that caused changes in the levels of any of the kidney function markers outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 180

Kidney function markers (mg/dL) in Sprague-Dawley rats

	BUN	Creatinine
PBS	18	3.5
ISIS 588544	21	3.1
ISIS 588550	21	3.0
ISIS 588553	22	2.8
ISIS 588554	23	3.0
ISIS 588555	22	3.5
ISIS 588556	21	3.2
ISIS 588560	26	2.4
ISIS 588564	24	2.7

Weights

Body weight measurements were taken on day 39. Liver, heart, spleen and kidney weights were measured at the end of the study on day 42, and are presented in the Table below. ISIS oligonucleotides that

caused any changes in organ weights outside the expected range for antisense oligonucleotides were excluded from further studies.

Table 181

Weights (g)

	Body	Liver	Spleen	Kidney
PBS	422	16	1.2	3.9
ISIS 588544	353	15	1.7	2.9
ISIS 588550	321	14	2.1	3.2
ISIS 588553	313	15	2.3	3.2
ISIS 588554	265	11	1.6	2.7
ISIS 588555	345	14	1.4	3.3
ISIS 588556	328	13	1.7	3.1
ISIS 588560	270	13	2.4	3.0
ISIS 588564	253	12	2.9	3.0

Study 2 (with deoxy, MOE and (S)-cEt oligonucleotides)

Male Sprague-Dawley rats, nine- to ten-week old, were maintained on a 12-hour light/dark cycle and fed ad libitum with Purina normal rat chow, diet 5001. Groups of 4 Sprague-Dawley rats each were injected subcutaneously once a week for 6 weeks with 100 mg/kg of deoxy, MOE, and (S)-cEt oligonucleotides. Two control groups of 3 rats each were injected subcutaneously once a week for 6 weeks with PBS. Forty eight hours after the last dose, rats were euthanized and organs and plasma were harvested for further analysis.

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma levels of transaminases were measured on day 42 using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma levels of ALT (alanine transaminase) and AST (aspartate transaminase), and albumin were measured and the results are presented in the Table below. ISIS oligonucleotides that caused changes in the levels of any markers of liver function outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 182

Liver function markers in Sprague-Dawley rats

	ALT (IU/L)	AST (IU/L)	Albumin (g/dL)
PBS	55	150	3.4
PBS	64	91	3.5
ISIS 588554	52	92	3.2
ISIS 588835	971	844	4.1
ISIS 588842	317	359	3.8

ISIS 588843	327	753	2.9
ISIS 588846	70	111	3.2
ISIS 588847	65	100	3.0
ISIS 588864	91	109	3.0
ISIS 594430	85	106	3.7

Kidney function

To evaluate the effect of ISIS oligonucleotides on kidney function, plasma levels of blood urea nitrogen (BUN) and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in the Table below, expressed in mg/dL. ISIS oligonucleotides that caused changes in the levels of any of the kidney function markers outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 183

Kidney function markers (mg/dL) in Sprague-Dawley rats

	BUN	Creatinine
PBS	17	0.4
PBS	21	0.4
ISIS 588554	20	0.4
ISIS 588835	23	0.5
ISIS 588842	22	0.4
ISIS 588843	51	0.4
ISIS 588846	25	0.5
ISIS 588847	23	0.5
ISIS 588864	23	0.4
ISIS 594430	22	0.5

Weights

Body weight measurements were taken on day 39. Liver, heart, spleen and kidney weights were measured at the end of the study on day 42, and are presented in the Table below. ISIS oligonucleotides that caused any changes in organ weights outside the expected range for antisense oligonucleotides were excluded from further studies.

Table 184

Weights (g)

	Body	Liver	Spleen	Kidney
PBS	466	16	0.9	3.8
PBS	485	16	0.9	3.6
ISIS 588554	393	15	2.3	2.6
ISIS 588835	387	16	1.0	3.3

ISIS 588842	414	22	1.5	3.7
ISIS 588843	427	20	2.5	4.2
ISIS 588846	366	16	2.1	3.3
ISIS 588847	402	15	1.6	3.1
ISIS 588864	364	15	2.1	3.8
ISIS 594430	420	16	1.2	3.6

Study 3 (with MOE gapmers)

Male Sprague-Dawley rats, nine- to ten-week old, were maintained on a 12-hour light/dark cycle and fed ad libitum with Purina normal rat chow, diet 5001. Groups of 4 Sprague-Dawley rats each were injected subcutaneously once a week for 6 weeks with 100 mg/kg of MOE gapmers. One control group of 6 rats was injected subcutaneously once a week for 6 weeks with PBS. Forty eight hours after the last dose, rats were euthanized and organs and plasma were harvested for further analysis.

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma levels of transaminases were measured on day 43 using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma levels of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in the Table below expressed in IU/L. ISIS oligonucleotides that caused changes in the levels of any markers of liver function outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 185

Liver function markers in Sprague-Dawley rats

	Chemistry	ALT (IU/L)	AST (IU/L)	Albumin (g/dL)
PBS	-	52	110	3.7
ISIS 588563	5-10-5 MOE	175	291	2.9
ISIS 599024	3-10-4 MOE	139	173	1.4
ISIS 599093	5-7-5 MOE	116	238	2.6
ISIS 599149	4-8-5 MOE	232	190	3.4
ISIS 599155	4-8-5 MOE	108	215	2.5
ISIS 599202	5-8-5 MOE	65	86	3.5
ISIS 599203	5-8-5 MOE	71	97	3.1
ISIS 599208	5-8-5 MOE	257	467	1.9
ISIS 599261	3-10-5 MOE	387	475	1.5
ISIS 599267	3-10-5 MOE	201	337	2.7

Kidney function

To evaluate the effect of ISIS oligonucleotides on kidney function, plasma levels of blood urea nitrogen (BUN) and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in the Table below, expressed in mg/dL. ISIS oligonucleotides that caused changes in the levels of any of the kidney function markers outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 186

Kidney function markers (mg/dL) in Sprague-Dawley rats

	Chemistry	BUN	Creatinine
PBS	-	16	0.3
ISIS 588563	5-10-5 MOE	26	0.4
ISIS 599024	3-10-4 MOE	135	1.2
ISIS 599093	5-7-5 MOE	29	0.4
ISIS 599149	4-8-5 MOE	23	0.4
ISIS 599155	4-8-5 MOE	29	0.4
ISIS 599202	5-8-5 MOE	19	0.4
ISIS 599203	5-8-5 MOE	22	0.4
ISIS 599208	5-8-5 MOE	26	0.3
ISIS 599261	3-10-5 MOE	228	1.6
ISIS 599267	3-10-5 MOE	24	0.4

Weights

Body weight measurements were taken on day 39. Liver, heart, spleen and kidney weights were measured at the end of the study on day 42, and are presented in the Table below. ISIS oligonucleotides that caused any changes in organ weights outside the expected range for antisense oligonucleotides were excluded from further studies.

Table 187

Weights (g)

	Chemistry	Body	Liver	Spleen	Kidney
PBS	-	471	16	1.0	4.1
ISIS 588563	5-10-5 MOE	311	16	3.4	4.1
ISIS 599024	3-10-4 MOE	297	11	1.0	3.5
ISIS 599093	5-7-5 MOE	332	18	4.1	5.0
ISIS 599149	4-8-5 MOE	388	16	2.3	3.7
ISIS 599155	4-8-5 MOE	290	15	2.9	4.5
ISIS 599202	5-8-5 MOE	359	13	1.3	3.2
ISIS 599203	5-8-5 MOE	334	14	1.8	3.3
ISIS 599208	5-8-5 MOE	353	29	4.7	4.6
ISIS 599261	3-10-5 MOE	277	10	0.9	3.2

ISIS 599267	3-10-5 MOE	344	21	3.9	4.7

Study 4 (with MOE gapmers)

Male Sprague-Dawley rats, nine- to ten-week old, were maintained on a 12-hour light/dark cycle and fed ad libitum with Purina normal rat chow, diet 5001. Groups of 4 Sprague-Dawley rats each were injected subcutaneously once a week for 6 weeks with 100 mg/kg of MOE gapmers. One control group of 6 rats was injected subcutaneously once a week for 6 weeks with PBS. Forty eight hours after the last dose, rats were euthanized and organs and plasma were harvested for further analysis.

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma levels of transaminases were measured on day 42 using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma levels of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in the Table below expressed in IU/L. ISIS oligonucleotides that caused changes in the levels of any markers of liver function outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 188

Liver function markers in Sprague-Dawley rats

	Chemistry	ALT (IU/L)	AST (IU/L)	Albumin (g/dL)
PBS	-	48	77	3.9
ISIS 532800	5-10-5 MOE	72	111	3.4
ISIS 532809	5-10-5 MOE	59	89	3.8
ISIS 588540	5-10-5 MOE	146	259	3.8
ISIS 599268	3-10-5 MOE	175	206	2.7
ISIS 599322	6-7-6 MOE	523	567	3.3
ISIS 599374	5-9-5 MOE	114	176	3.0
ISIS 599378	5-9-5 MOE	124	116	3.2
ISIS 599380	5-9-5 MOE	148	210	3.4
ISIS 599441	6-8-6 MOE	51	91	2.6

Kidney function

To evaluate the effect of ISIS oligonucleotides on kidney function, plasma levels of blood urea nitrogen (BUN) and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in the Table below, expressed in mg/dL. ISIS oligonucleotides that caused changes in the levels of any of the kidney function markers outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 189

Kidney function markers (mg/dL) in Sprague-Dawley rats

	Chemistry	BUN	Creatinine
PBS	-	15	0.4
ISIS 532800	5-10-5 MOE	26	0.5
ISIS 532809	5-10-5 MOE	18	0.5
ISIS 588540	5-10-5 MOE	22	0.5
ISIS 599268	3-10-5 MOE	28	0.5
ISIS 599322	6-7-6 MOE	24	0.5
ISIS 599374	5-9-5 MOE	29	0.5
ISIS 599378	5-9-5 MOE	22	0.4
ISIS 599380	5-9-5 MOE	26	0.5
ISIS 599441	6-8-6 MOE	24	0.4

Weights

Body weight measurements were taken on day 39. Liver, heart, spleen and kidney weights were measured at the end of the study on day 42, and are presented in the Table below. ISIS oligonucleotides that caused any changes in organ weights outside the expected range for antisense oligonucleotides were excluded from further studies.

Table 190

Weights (g)

	Chemistry	Body	Liver	Spleen	Kidney
PBS	-	502	16	0.9	3.7
ISIS 532800	5-10-5 MOE	376	16	2.0	3.4
ISIS 532809	5-10-5 MOE	430	16	1.4	3.4
ISIS 588540	5-10-5 MOE	391	16	1.8	3.5
ISIS 599268	3-10-5 MOE	332	16	3.6	3.6
ISIS 599322	6-7-6 MOE	348	13	2.1	3.4
ISIS 599374	5-9-5 MOE	302	12	2.0	3.3
ISIS 599378	5-9-5 MOE	332	11	1.1	2.8
ISIS 599380	5-9-5 MOE	350	11	1.5	3.3
ISIS 599441	6-8-6 MOE	368	16	2.5	3.3

Study 5 (with MOE gapmers and deoxy, MOE and (S)-cEt oligonucleotides)

Male Sprague-Dawley rats, nine- to ten-week old, were maintained on a 12-hour light/dark cycle and fed ad libitum with Purina normal rat chow, diet 5001. Groups of 4 Sprague-Dawley rats each were injected subcutaneously once a week for 6 weeks with 100 mg/kg of MOE gapmer or with 50 mg/kg of deoxy, MOE and (S)-cEt oligonucleotides. One control group of 4 rats was injected subcutaneously once a week for 6

weeks with PBS. Forty eight hours after the last dose, rats were euthanized and organs and plasma were harvested for further analysis.

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma levels of transaminases were measured on day 42 using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma levels of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in the Table below expressed in IU/L. ISIS oligonucleotides that caused changes in the levels of any markers of liver function outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 191

Liver function markers in Sprague-Dawley rats

	Chemistry	ALT (IU/L)	AST (IU/L)	Albumin (g/dL)
PBS	-	49	74	3.3
ISIS 532770	5-10-5 MOE	95	132	3.3
ISIS 588851	Deoxy, MOE, and (S)-cEt	47	72	3.1
ISIS 588856	Deoxy, MOE, and (S)-cEt	56	75	3.0
ISIS 588865	Deoxy, MOE, and (S)-cEt	62	84	2.9
ISIS 588867	Deoxy, MOE, and (S)-cEt	73	214	2.9
ISIS 588868	Deoxy, MOE, and (S)-cEt	59	83	3.1
ISIS 588870	Deoxy, MOE, and (S)-cEt	144	144	3.4

Kidney function

To evaluate the effect of ISIS oligonucleotides on kidney function, plasma and urine levels of blood urea nitrogen (BUN) and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in the Tables below, expressed in mg/dL. ISIS oligonucleotides that caused changes in the levels of any of the kidney function markers outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 192

Kidney function markers (mg/dL) in the plasma of Sprague-Dawley rats

	Chemistry	BUN	Creatinine
PBS	-	18	0.3
ISIS 532770	5-10-5 MOE	20	0.4
ISIS 588851	Deoxy, MOE, and (S)-cEt	20	0.4
ISIS 588856	Deoxy, MOE, and (S)-cEt	22	0.4

ISIS 588865	Deoxy, MOE, and (S)-cEt	24	0.5
ISIS 588867	Deoxy, MOE, and (S)-cEt	22	0.4
ISIS 588868	Deoxy, MOE, and (S)-cEt	19	0.4
ISIS 588870	Deoxy, MOE, and (S)-cEt	20	0.5

Table 193

Kidney function markers (mg/dL) in the urine of Sprague-Dawley rats

	Chemistry	Total protein	Creatinine
PBS	-	80	92
ISIS 532770	5-10-5 MOE	466	69
ISIS 588851	Deoxy, MOE, and (S)-cEt	273	64
ISIS 588856	Deoxy, MOE, and (S)-cEt	259	68
ISIS 588865	Deoxy, MOE, and (S)-cEt	277	67
ISIS 588867	Deoxy, MOE, and (S)-cEt	337	68
ISIS 588868	Deoxy, MOE, and (S)-cEt	326	75
ISIS 588870	Deoxy, MOE, and (S)-cEt	388	82

Weights

Body weight measurements were taken on day 39. Liver, heart, spleen and kidney weights were measured at the end of the study on day 42, and are presented in the Table below. ISIS oligonucleotides that caused any changes in organ weights outside the expected range for antisense oligonucleotides were excluded from further studies.

Table 194

Weights (g)

	Chemistry	Body	Liver	Spleen	Kidney
PBS	-	489	16	0.9	3.5
ISIS 532770	5-10-5 MOE	372	15	1.7	3.1
ISIS 588851	Deoxy, MOE, and (S)-cEt	285	14	1.4	3.2
ISIS 588856	Deoxy, MOE, and (S)-cEt	415	15	1.1	3.3
ISIS 588865	Deoxy, MOE, and (S)-cEt	362	14	2.0	3.3
ISIS 588867	Deoxy, MOE, and (S)-cEt	406	15	2.4	3.4
ISIS 588868	Deoxy, MOE, and (S)-cEt	399	15	1.5	3.4
ISIS 588870	Deoxy, MOE, and (S)-cEt	446	14	1.4	3.3

Study 6 (with MOE gapmers, deoxy, MOE and (S)-cEt oligonucleotides, and (S)-cEt gapmers)

Male rats were maintained on a 12-hour light/dark cycle and fed ad libitum with Purina normal rat chow, diet 5001. Groups of 4 rats each were injected subcutaneously once a week for 6 weeks with 100

mg/kg of MOE gapmers or with 50 mg/kg of deoxy, MOE and (S)-cEt oligonucleotide or (S)-cEt gapmer. One control group of 4 rats was injected subcutaneously once a week for 6 weeks with PBS. Forty eight hours after the last dose, rats were euthanized and organs and plasma were harvested for further analysis.

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, plasma levels of transaminases were measured on day 42 using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Plasma levels of ALT (alanine transaminase) and AST (aspartate transaminase) were measured and the results are presented in the Table below expressed in IU/L.

Table 195

Liver function markers

	Chemistry	Dose (mg/kg/wk)	ALT (IU/L)	AST (IU/L)	Albumin (g/dL)
PBS	-	-	54	73	4.3
ISIS 532770	5-10-5 MOE	100	57	114	4.4
ISIS 532800	5-10-5 MOE	100	176	180	4.3
ISIS 532809	5-10-5 MOE	100	71	132	4.1
ISIS 588540	5-10-5 MOE	100	89	202	4.4
ISIS 588544	5-10-5 MOE	100	75	152	3.9
ISIS 588548	5-10-5 MOE	100	50	71	4.1
ISIS 588550	5-10-5 MOE	100	80	133	3.6
ISIS 588553	5-10-5 MOE	100	59	112	3.9
ISIS 588555	5-10-5 MOE	100	97	142	3.8
ISIS 588848	Deoxy, MOE and (S)-cEt	50	53	82	3.9
ISIS 594430	3-10-3 (S)-cEt	50	198	172	4.4

Kidney function

To evaluate the effect of ISIS oligonucleotides on kidney function, urine levels of total protein and creatinine were measured using an automated clinical chemistry analyzer (Hitachi Olympus AU400e, Melville, NY). Results are presented in the Table below. ISIS oligonucleotides that caused changes in the levels of any of the kidney function markers outside the expected range for antisense oligonucleotides were excluded in further studies.

Table 196

Total protein/creatinine ratio in the urine of rats

	Chemistry	Dose (mg/kg/wk)	P/C ratio
PBS	-	-	1.1
ISIS 532770	5-10-5 MOE	100	8.3
ISIS 532800	5-10-5 MOE	100	6.5

ISIS 532809	5-10-5 MOE	100	6.1
ISIS 588540	5-10-5 MOE	100	10.1
ISIS 588544	5-10-5 MOE	100	7.9
ISIS 588548	5-10-5 MOE	100	6.6
ISIS 588550	5-10-5 MOE	100	7.6
ISIS 588553	5-10-5 MOE	100	7.0
ISIS 588555	5-10-5 MOE	100	6.2
ISIS 588848	Deoxy, MOE and (S)-cEt	50	5.2
ISIS 594430	3-10-3 (S)-cEt	50	5.3

Weights

Body weight measurements were taken on day 39. Liver, heart, spleen and kidney weights were measured at the end of the study on day 42, and are presented in the Table below. The results for the organ weights were expressed as a ratio to the body weights and normalized to the PBS control ratio.

Table 197

Organ weights/Body weight (BW) ratios

	Chemistry	Dose (mg/kg/wk)	Spleen/BW	Liver/BW	Kidney/BW
PBS	-	-	1.0	1.0	1.0
ISIS 532770	5-10-5 MOE	100	2.0	1.2	1.0
ISIS 532800	5-10-5 MOE	100	2.8	1.3	1.0
ISIS 532809	5-10-5 MOE	100	2.2	1.1	1.0
ISIS 588540	5-10-5 MOE	100	2.2	1.4	1.0
ISIS 588544	5-10-5 MOE	100	2.5	1.3	1.1
ISIS 588548	5-10-5 MOE	100	2.1	1.3	1.1
ISIS 588550	5-10-5 MOE	100	3.9	1.4	1.1
ISIS 588553	5-10-5 MOE	100	4.1	1.4	1.4
ISIS 588555	5-10-5 MOE	100	1.8	1.3	1.0
ISIS 588848	Deoxy, MOE and (S)-cEt	50	3.1	1.3	1.1
ISIS 594430	3-10-3 (S)-cEt	50	1.7	1.0	1.1

Example 128: Efficacy of antisense oligonucleotides against CFB mRNA in hCFB mice

Selected compounds were tested for efficacy in human CFB transgenic mice, founder line #6 The human CFB gene is located on chromosome 6: position 31913721- 31919861. A Fosmid (ABC14-50933200C23) containing the CFB sequence was selected to make transgenic mice expressing the human CFB gene. Cla I (31926612) and Age I (31926815) restriction enzymes were used to generate a 22,127 bp fragment containing the CFB gene for pronuclear injection. DNA was confirmed by restriction enzyme analysis using Pvu I. The 22,127 bp DNA fragment was injected into C57BL/6NTac embryos. 6 positive

founders were bred. Founder #6 expressed the liver human CFB mRNA and was crossbreed to the 3rd generation. Progeny from 3rd generation mice were used to evaluate human CFB ASOs for human CFB mRNA reduction.

Treatment

Groups of 3 mice each were injected subcutaneously twice a week for the first week with 50 mg/kg of ISIS oligonucleotides, followed by once a week dosing with 50 mg/kg of ISIS oligonucleotides for an additional three weeks. One control group of 4 mice was injected subcutaneously twice a week for 2 weeks for the first week with PBS for the first week for an additional three weeks. Forty eight hours after the last dose, mice were euthanized and organs and plasma were harvested for further analysis.

RNA Analysis

At the end of the dosing period, RNA was extracted from the liver and kidney for real-time PCR analysis of CFB mRNA levels. Human CFB mRNA levels were measured using the human primer probe set RTS3459. CFB mRNA levels were normalized to RIBOGREEN®, and also to the housekeeping gene, Cyclophilin. Results were calculated as percent inhibition of CFB mRNA expression compared to the control. All the antisense oligonucleotides effected inhibition of human CFB mRNA levels in the liver.

Table 198

Percent reduction of CFB mRNA levels in hCFB mice

ISIS No	Normalized to RIBOGREEN	Normalized to Cyclophilin
532770	86	87
532800	88	87
532809	69	69
588540	95	94
588544	91	91
588548	78	77
588550	89	88
588553	94	94
588555	94	94
588848	83	82
594430	78	76

Example 129: *In vivo* antisense inhibition of murine CFB

Several antisense oligonucleotides were designed that were targeted to murine CFB mRNA (GENBANK Accession No. NM_008198.2, incorporated herein as SEQ ID NO: 5). The target start sites and sequences of each oligonucleotide are described in the table below. The chimeric antisense oligonucleotides in the table below were designed as 5-10-5 MOE gapmers. The gapmers are 20 nucleosides in length, wherein the central gap segment is comprised of 10 2'-deoxynucleosides and is flanked on both sides (in the 5' and 3' directions) by wings comprising 5 nucleosides each. Each nucleoside in the 5' wing segment and each nucleoside in the 3' wing segment has a 2'-MOE modification. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. All cytosine residues throughout each gapmer are 5-methylcytosines.

Table 199

Gapmers targeting murine CFB

ISIS No	Sequence	Target Start Site on SEQ ID NO: 5	SEQ ID NO
516269	GCATAAGAGGGTACCAGCTG	2593	804
516272	GTCCTTTAGCCAGGGCAGCA	2642	805
516323	TCCACCCATGTTGTGCAAGC	1568	806
516330	CCACACCATGCCACAGAGAC	1826	807
516341	TTCCGAGTCAGGCTCTTCCC	2308	808

Treatment

Groups of four C57BL/6 mice each were injected with 50 mg/kg of ISIS 516269, ISIS 516272, ISIS 516323, ISIS 516330, or ISIS 516341 administered weekly for 3 weeks. A control group of mice was injected with phosphate buffered saline (PBS) administered weekly for 3 weeks.

CFB RNA Analysis

At the end of the study, RNA was extracted from liver tissue for real-time PCR analysis of CFB, using primer probe set RTS3430 (forward sequence GGGCAAACAGCAATTTGTGA, designated herein as SEQ ID NO: 816; reverse sequence TGGCTACCCACCTTCCTTGT, designated herein as SEQ ID NO: 817; probe sequence CTGGATACTGTCCCAATCCCGGTATTCCX, designated herein as SEQ ID NO: 818). The mRNA levels were normalized using RIBOGREEN®. As shown in the Table below, some of the antisense oligonucleotides achieved reduction of murine CFB over the PBS control. Results are presented as percent inhibition of CFB, relative to control.

Table 200

Percent inhibition of murine CFB mRNA in C57BL/6 mice

ISIS No	%
516269	29
516272	72
516323	77
516330	62
516341	72

Protein Analysis

CFB protein levels were measured in the kidney, liver, plasma, and in the eye by western Blot using goat anti-CFB antibody (Sigma Aldrich). Results are presented as percent inhibition of CFB, relative to PBS control. 'n/a' indicates that measurements were not taken for that sample. As shown in the Table below, antisense inhibition of CFB by ISIS oligonucleotides resulted in a reduction of CFB protein in various tissues. As shown in the Table below, systemic administration of ISIS oligonucleotides was effective in reducing CFB levels in the eye.

Table 201

Percent inhibition of murine CFB protein in C57BL/6 mice

ISIS No	Kidney	Liver	Plasma	Eye
516269	20	58	n/a	70
516272	48	74	n/a	99
516323	73	85	90	93
516330	77	80	n/a	n/a
516341	80	88	68	n/a

Example 130: Dose-dependent antisense inhibition of murine CFB

Groups of four C57BL/6 mice each were injected with 25 mg/kg, 50 mg/kg, or 100 mg/kg of ISIS 516272, and ISIS 516323 administered weekly for 6 weeks. Another two groups of mice were injected with 100 mg/kg of ISIS 516330 or ISIS 516341 administered weekly for 6 weeks. Two control groups of mice were injected with phosphate buffered saline (PBS) administered weekly for 6 weeks.

CFB RNA Analysis

RNA was extracted from liver and kidney tissues for real-time PCR analysis of CFB, using primer probe set RTS3430. The mRNA levels were normalized using RIBOGREEN®. As shown in the Table below,

the antisense oligonucleotides achieved dose-dependent reduction of murine CFB over the PBS control. Results are presented as percent inhibition of CFB, relative to control.

Table 202

Percent inhibition of murine CFB mRNA in C57BL/6 mice

ISIS No	Dose (mg/kg/wk)	Liver	Kidney
516272	25	39	32
	50	73	36
	100	87	42
516323	25	36	41
	50	65	47
	100	79	71
516330	100	85	45
516341	200	89	65

Protein Analysis

CFB protein levels were measured in the plasma by western Blot using goat anti-CFB antibody (Sigma Aldrich). As shown in the table below, antisense inhibition of CFB by the ISIS oligonucleotides resulted in a reduction of CFB protein. Results are presented as percent inhibition of CFB, relative to PBS control. 'n/a' indicates that measurements were not taken for that sample.

CFB protein levels were also measured in the eye by Western Blot. All treatment groups demonstrated an inhibition of CFB by 95%, with some sample measurements being below detection levels of the assay.

Table 203

Percent inhibition of murine CFB protein in C57BL/6 mice

ISIS No	Dose (mg/kg/wk)	Liver
516272	25	32
	50	70
	100	83
516323	25	43
	50	80
	100	90
516330	100	n/a
516341	200	n/a

Example 131: Effect of antisense inhibition of CFB in the NZB/W F1 mouse model

The NZB/W F1 is the oldest classical model of lupus, where the mice develop severe lupus-like phenotypes comparable to that of lupus patients (Theofilopoulos, A.N. and Dixon, F.J. Advances in Immunology, vol. 37, pp. 269–390, 1985). These lupus-like phenotypes include lymphadenopathy, splenomegaly, elevated serum antinuclear autoantibodies (ANA) including anti-dsDNA IgG, a majority of which are IgG2a and IgG3, and immune complex-mediated glomerulonephritis (GN) that becomes apparent at 5-6 months of age, leading to kidney failure and death at 10–12 months of age.

Study 1

A study was conducted to demonstrate that treatment with antisense oligonucleotides targeting CFB would improve renal pathology in the mouse model. Female NZB/W F1 mice, 17 weeks old, were purchased from Jackson Laboratories. Groups of 16 mice each received doses of 100 µg/kg/week of ISIS 516272 or ISIS 516323 for 20 weeks. Another group of 16 mice received doses of 100 µg/kg/week of control oligonucleotide ISIS 141923 for 20 weeks. Another group of 10 mice received doses of PBS for 20 weeks and served as the control group to which all the other groups were compared. Terminal endpoints were collected 48 hours after the last dose was injected.

CFB RNA Analysis

RNA was extracted from liver and kidney tissue for real-time PCR analysis of CFB, using primer probe set RTS3430. The mRNA levels were normalized using RIBOGREEN®. As shown in the Table below, some of the antisense oligonucleotides achieved reduction of murine CFB over the PBS control. Results are presented as percent inhibition of CFB, relative to control.

Table 204

Percent inhibition of murine CFB mRNA in NZB/W F1 mice

ISIS No	Liver	Kidney
516272	55	25
516323	63	43
141923	0	0

Proteinuria

Proteinuria is expected in 60% of animals in this mouse model. The cumulative incidence of severe proteinuria was measured by calculating the total protein to creatinine ratio using a clinical analyzer. The results are presented in the table below and demonstrate that treatment with antisense oligonucleotides targeting CFB achieved reduction of proteinuria in the mice compared to the PBS control and the control oligonucleotide treated mice.

Table 205

Percent cumulative incidence of severe proteinuria in NZB/W F1 mice

	%
PBS	40
ISIS 516272	6
ISIS 516323	0
ISIS 141923	25

Survival

Survival of the mice was monitored by keeping count of the mice at the start of treatment and then again at week 20. The results are presented in the table below and demonstrate that treatment with antisense oligonucleotides targeting CFB increased survival in the mice compared to the PBS control and the control oligonucleotide treated mice.

Table 206

Number of surviving mice and % survival

	Week 1	Week 20	% survival at week 20
PBS	10	6	60
ISIS 516272	16	15	94
ISIS 516323	16	16	100
ISIS 141923	16	12	75

Glomerular deposition

The amount of C3 deposition, as well as IgG deposition, in the glomeruli of the kidneys was measured by immunohistochemistry with an anti-C3 antibody. The results are presented in the table below and demonstrate that treatment with antisense oligonucleotides targeting CFB achieved reduction of both C3 and IgG depositions in the kidney glomeruli compared to the PBS control and the control oligonucleotide treated mice.

Table 207

Percent inhibition of glomerula deposition in NZB/W F1 mice

ISIS No	C3	IgG
516272	45	20
516323	48	2
141923	0	0

Study 2

Female NZB/W F1 mice, 16 weeks old, were purchased from Jackson Laboratories. A group of 10 mice received doses of 100 µg/kg/week of ISIS 516323 for 12 weeks. Another group of 10 mice received doses of 100 µg/kg/week of control oligonucleotide ISIS 141923 for 12 weeks. Another group of 10 mice received doses of PBS for 12 weeks and served as the control group to which all the other groups were compared. Terminal endpoints were collected 48 hours after the last dose was injected.

CFB RNA Analysis

RNA was extracted from liver and kidney tissue for real-time PCR analysis of CFB, using primer probe set RTS3430. As shown in the table below, treatment with ISIS 516323 achieved reduction of murine CFB over the PBS control. Results are presented as percent inhibition of CFB, relative to control.

Table 208

Percent inhibition of murine CFB mRNA in NZB/W F1 mice

ISIS No	Liver	Kidney
516323	75	46
141923	0	6

Proteinuria

The cumulative incidence of severe proteinuria was assessed by measuring urine total protein to creatinine ratio, as well as by measuring total microalbumin levels. The results are presented in the tables below and demonstrate that treatment with antisense oligonucleotides targeting CFB reduced proteinuria in the mice compared to the PBS control and the control oligonucleotide treated mice.

Table 209

Proteinuria in NZB/W F1 mice measured as urine microalbumin levels (mg/dl)

ISIS No	Week 0	Week 6	Week 8	Week 10
516323	0	0	5.4	0.4
141923	0	8.28	8.6	5.6

Table 210

Proteinuria in NZB/W F1 mice measured as total protein to creatinine ratio

ISIS No	Week 0	Week 6	Week 8	Week 10
516323	5.5	7.8	8.6	7.2
141923	6.9	10.0	13.5	7.2

Survival

Survival of the mice was monitored by keeping count of the mice at the start of treatment and then again at week 12. The results are presented in the table below and demonstrate that treatment with antisense oligonucleotides targeting CFB increased survival in the mice compared to the PBS control and the control oligonucleotide treated mice.

Table 211

Number of surviving mice

	Week 1	Week 12
PBS	10	9
ISIS 516323	10	10
ISIS 141923	10	9

Example 132: Effect of antisense inhibition of CFB in the MRL mouse model

The MRL/lpr lupus nephritis mouse model develops an SLE-like phenotype characterized by lymphadenopathy due to an accumulation of double negative ($CD4^-$ $CD8^-$) and $B220^+$ T-cells. These mice display an accelerated mortality rate. In addition, the mice have high concentrations of circulating immunoglobulins, which included elevated levels of autoantibodies such as ANA, anti-ssDNA, anti-dsDNA, anti-Sm, and rheumatoid factors, resulting in large amounts of immune complexes (Andrews, B. et al., J. Exp. Med. 148: 1198-1215, 1978).

Treatment

A study was conducted to investigate whether treatment with antisense oligonucleotides targeting CFB would reverse renal pathology in the mouse model. Female MRL/lpr mice, 14 weeks old, were purchased from Jackson Laboratories. A group of 10 mice received doses of 50 µg/kg/week of ISIS 516323 for 7 weeks. Another group of 10 mice received doses of 50 µg/kg/week of control oligonucleotide ISIS 141923 for 7 weeks. Another group of 10 mice received doses of PBS for 7 weeks and served as the control group to which all the other groups were compared. Terminal endpoints were collected 48 hours after the last dose was injected.

CFB RNA Analysis

RNA was extracted from liver tissue for real-time PCR analysis of CFB, using primer probe set RTS3430. As shown in the Table below, ISIS 516323 reduced CFB over the PBS control. Results are presented as percent inhibition of CFB, relative to control.

Table 212

Percent inhibition of murine CFB mRNA in MRL/lpr mice

ISIS No	%
516323	68
141923	4

Renal pathology

Renal pathology was evaluated by two methods. Histological sections of the kidney were stained with Haematoxylin &Eosin. The PBS control demonstrated presence of multiglomerular crescents tubular casts, which is a symptom of glomerulosclerosis. In contrast, the sections from mice treated with ISIS 516323 showed absent crescents tubular casts with minimal bowman capsule fibrotic changes, moderate to severe segmental mesangial cell expansion and glomerular basement membrane thickening.

Accumulation of C3 in the kidney was also assessed by immunohistochemistry with anti-C3 antibodies. The whole kidney C3 immunohistochemistry intensity score was calculated by intensity scoring system, which was computed by capturing 10 glomeruli per kidney and calculation the intensity of positive C3 staining. The results are presented in the table below and demonstrate that treatment with ISIS 516323 reduced renal C3 accumulation compared to the control groups.

Table 213

Renal C3 accumulation in MRL/lpr mice

	Whole kidney C3 intensity score	C3 quantification (area/total area) % of average PBS
PBS	2.5	100
ISIS 516323	1.6	68
ISIS 141923	2.2	99

Plasma C3 levels

Reduction of CFB inhibits activation of the alternative complement pathway, preventing C3 consumption and leading to an apparent elevation of plasma C3 levels. Plasma C3 levels from terminal bleed were measured by clinical analyzer. The results are presented in the table below and demonstrate that treatment with ISIS 516323 increased C3 levels ($p < 0.001$) in the plasma compared to the control groups.

Table 214

Plasma C3 levels (mg/dL) in MRL/lpr mice

ISIS No	C3
516323	28
141923	16

The results indicate that treatment with antisense oligonucleotides targeting CFB reverses renal pathology in the lupus mouse model.

Example 133: Effect of antisense inhibition of CFB in the CFH Het mouse model

CFH heterozygous (CFH Het, $CFH^{+/-}$) mouse model express a mutant Factor H protein in combination with the full-length mouse protein (Pickering, M.C. et al., J. Exp. Med. 2007. 204: 1249-56). Renal histology remains normal in these mice up to six months old.

Study 1

Groups of 8 $CFH^{+/-}$ mice, 6 weeks old, each received doses of 75 mg/kg/week of ISIS 516323 or ISIS 516341 for 6 weeks. Another group of 8 mice received doses of 75 mg/kg/week of control oligonucleotide ISIS 141923 for 6 weeks. Another group of 8 mice received doses of PBS for 6 weeks and served as the control group to which all the other groups were compared. Terminal endpoints were collected 48 hours after the last dose was injected.

CFB RNA Analysis

RNA was extracted from liver and kidney tissue for real-time PCR analysis of CFB, using primer probe set RTS3430. As shown in the Table below, the antisense oligonucleotides reduced CFB over the PBS control. Results are presented as percent inhibition of CFB, relative to control.

Table 215

Percent inhibition of murine CFB mRNA in $CFH^{+/-}$ mice

ISIS No	Liver	Kidney
516323	80	38
516341	90	44
141923	0	17

Plasma C3 levels

Reduction of CFB inhibits activation of the alternative complement pathway, preventing C3 consumption and leading to an apparent elevation of plasma C3 levels. Plasma C3 levels from terminal plasma collection were measured by clinical analyzer. The results are presented in the table below and demonstrate that treatment with ISIS 516323 increased C3 to normal levels in the plasma.

Table 216

Plasma C3 levels (mg/dL) in $CFH^{+/-}$ mice

ISIS No	C3
516323	15
516341	17
141923	8

Study 2

Groups of 5 $CFH^{+/-}$ mice each received doses of 12.5 mg/kg/week, 25 mg/kg/week, 50 mg/kg/week, 75 mg/kg/week, or 100 mg/kg/week of ISIS 516323 or ISIS 516341 for 6 weeks. Another group of 5 mice received doses of 75 μg/kg/week of control oligonucleotide ISIS 141923 for 6 weeks. Another group of 5 mice received doses of PBS for 6 weeks and served as the control group to which all the other groups were compared. Terminal endpoints were collected 48 hours after the last dose was injected.

CFB RNA Analysis

RNA was extracted from liver and kidney tissue for real-time PCR analysis of CFB, using primer probe set RTS3430. As shown in the Table below, the antisense oligonucleotides reduced CFB over the PBS control in a dose dependent manner. Results are presented as percent inhibition of CFB, relative to control.

Table 217

Percent inhibition of murine CFB mRNA in the liver of $CFH^{+/-}$ mice

ISIS No	Dose (mg/kg/week)	%
516323	12.5	34
	25	51
	50	72
	75	79
	100	92
516341	12.5	38
	25	57
	50	89
	75	92
	100	90
141923	75	13

Plasma C3 levels

Reduction of CFB inhibits activation of the alternative complement pathway, preventing C3 consumption and leading to an apparent elevation of plasma C3 levels. Plasma C3 levels from terminal plasma collection were measured by clinical analyzer. The results are presented in the table below and demonstrate that treatment with ISIS oligonucleotides targeting CFB increased C3 levels in the plasma.

Table 218

Plasma C3 levels (mg/dL) in $CFH^{+/-}$ mice

	Dose (mg/kg/week)	C3
PBS	-	10.1
516323	12.5	11.4

	25	15.5
	50	17.0
	75	18.3
	100	18.8
516341	12.5	12.1
	25	16.3
	50	18.6
	75	22.1
	100	19.1
141923	75	8.9

Example 134: Effect of ISIS antisense oligonucleotides targeting human CFB in cynomolgus monkeys

Cynomolgus monkeys were treated with ISIS antisense oligonucleotides selected from studies described in the Examples above. Antisense oligonucleotide efficacy and tolerability, as well as their pharmacokinetic profile in the liver and kidney, were evaluated.

At the time this study was undertaken, the cynomolgus monkey genomic sequence was not available in the National Center for Biotechnology Information (NCBI) database; therefore cross-reactivity with the cynomolgus monkey gene sequence could not be confirmed. Instead, the sequences of the ISIS antisense oligonucleotides used in the cynomolgus monkeys was compared to a rhesus monkey sequence for homology. It is expected that ISIS oligonucleotides with homology to the rhesus monkey sequence are fully cross-reactive with the cynomolgus monkey sequence as well. The human antisense oligonucleotides tested are cross-reactive with the rhesus genomic sequence (GENBANK Accession No. NW_001116486.1 truncated from nucleotides 536000 to 545000, designated herein as SEQ ID NO: 3). The greater the complementarity between the human oligonucleotide and the rhesus monkey sequence, the more likely the human oligonucleotide can cross-react with the rhesus monkey sequence. The start and stop sites of each oligonucleotide targeted to SEQ ID NO: 3 is presented in the Table below. “Start site” indicates the 5’-most nucleotide to which the gapmer is targeted in the rhesus monkey gene sequence. ‘Mismatches’ indicates the number of nucleobases in the human oligonucleotide that are mismatched with the rhesus genomic sequence.

Table 219

Antisense oligonucleotides complementary to the rhesus CFB genomic sequence (SEQ ID NO: 3)

ISIS No	Target Start Site	Mismatches	Chemistry	SEQ ID NO
532770	6788	0	5-10-5 MOE	198
532800	7500	0	5-10-5 MOE	228
532809	7614	0	5-10-5 MOE	237

588540	7627	1	5-10-5 MOE	440
588544	7631	1	5-10-5 MOE	444
588548	7635	1	5-10-5 MOE	448
588550	7637	1	5-10-5 MOE	450
588553	7640	1	5-10-5 MOE	453
588555	7643	0	5-10-5 MOE	455
588848	7639	1	Deoxy, MOE and cEt	598
594430	6790	0	3-10-3 cEt	549

Treatment

Prior to the study, the monkeys were kept in quarantine for at least a 30 day period, during which the animals were observed daily for general health. The monkeys were 2-4 years old and weighed between 2 and 4 kg. Eleven groups of 4-6 randomly assigned male cynomolgus monkeys each were injected subcutaneously with ISIS oligonucleotide or PBS at four sites on the back in a clockwise rotation (i.e. left, top, right, and bottom), one site per dose. The monkeys were given four loading doses of PBS or 40 mg/kg of ISIS 532800, ISIS 532809, ISIS 588540, ISIS 588544, ISIS 588548, ISIS 588550, ISIS 588553, ISIS 588555, ISIS 588848, or ISIS 594430 for the first week (days 1, 3, 5, and 7), and were subsequently dosed once a week for 12 weeks (days 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, and 84) with PBS or 40 mg/kg of ISIS oligonucleotide. ISIS 532770 was tested in a separate study with similar conditions with two male and two female cynomolgus monkeys in the group.

Hepatic Target Reduction

RNA analysis

On day 86, liver and kidney samples were collected in duplicate (approximately 250 mg each) for CFB mRNA analysis. The samples were flash frozen in liquid nitrogen at necropsy within approximately 10 minutes of sacrifice.

RNA was extracted from liver and kidney for real-time PCR analysis of measurement of mRNA expression of CFB. Results are presented as percent change of mRNA, relative to PBS control, normalized with RIBOGREEN®. RNA levels were also normalized with the house-keeping gene, Cyclophilin A. RNA levels were measured with the primer probe sets RTS3459, described above, or RTS4445_MGB (forward sequence CGAAGAAGCTCAGTGAAATCAA, designated herein as SEQ ID NO: 819; reverse sequence TGCCTGGAGGGCCCTCTT, designated herein as SEQ ID NO: 820; probe sequence AGACCACAAGTTGAAGTC, designated herein as SEQ ID NO: 815).

As shown in the Tables below, treatment with ISIS antisense oligonucleotides resulted in reduction of CFB mRNA in comparison to the PBS control. Analysis of CFB mRNA levels revealed that several of the ISIS oligonucleotides reduced CFB levels in liver and/or kidney. Here '0' indicates that the expression levels were not inhibited. '*' indicates that the oligonucleotide was tested in a separate study with similar conditions.

Table 220

Percent inhibition of CFB mRNA in the cynomolgus monkey liver relative to the PBS control

ISIS No	RTS3459/ Cyclophilin A	RTS3459/ RIBOGREEN	RTS445_MGB/ Cyclophilin A	RTS445_MGB / RIBOGREEN
532770*	12	37	24	45
532800	54	45	56	46
588540	31	27	28	24
588548	68	67	68	67
588550	53	39	51	37
588553	74	59	74	59
588555	73	71	71	69
588848	9	0	6	0
594430	24	26	23	25

Table 221

Percent inhibition of CFB mRNA in the cynomolgus monkey kidney relative to the PBS control

ISIS No	RTS3459/ Cyclophilin A	RTS3459/ RIBOGREEN	RTS445_MGB/ Cyclophilin A	RTS445_MGB / RIBOGREEN
532770*	34	56	2	31
532800	36	30	43	37
588540	70	71	67	69
588548	83	84	82	83
588550	81	77	78	74
588553	86	84	86	85
588555	32	34	48	50
588848	89	91	87	90
594430	33	37	19	23

Protein analysis

Approximately 1 mL of blood was collected from all available animals at day 85 and placed in tubes containing the potassium salt of EDTA. The blood samples were placed in wet-ice or Kryorack immediately, and centrifuged (3000 rpm for 10 min at 4°C) to obtain plasma (approximately 0.4 mL) within 60 minutes of collection. Plasma levels of CFB were measured in the plasma by radial immunodiffusion (RID), using a polyclonal anti-Factor B antibody. The results are presented in the Table below. ISIS 532770 was tested in a separate study and plasma protein levels were measured on day 91 or 92 in that group.

Analysis of plasma CFB revealed that several ISIS oligonucleotides reduced protein levels in a sustained manner. ISIS 532770, which was tested in a separate study, reduced CFB protein levels on day 91/92 by 50% compared to baseline values. The reduction in plasma CFB protein levels correlates well with liver CFB mRNA level reduction in the corresponding groups of animals.

Table 222

Plasma protein levels (% baseline values) in the cynomolgus monkey

	Day 1	Day 30	Day 58	Day 72	Day 86
PBS	113	115	95	83	86
ISIS 532800	117	68	52	39	34
ISIS 532809	104	121	100	80	71
ISIS 588540	108	72	61	40	38
ISIS 588544	118	74	53	33	29
ISIS 588548	110	41	28	20	16
ISIS 588550	104	64	54	38	37
ISIS 588553	97	42	35	18	16
ISIS 588555	107	35	37	18	18
ISIS 588848	116	95	92	69	71
ISIS 594430	104	64	59	45	46

Tolerability studies

Body weight measurements

To evaluate the effect of ISIS oligonucleotides on the overall health of the animals, body and organ weights were measured and are presented in the Table below. '*' indicates that the oligonucleotide was tested in a separate study with similar conditions and is the average of the measurements from male and female monkeys. The results indicate that effect of treatment with antisense oligonucleotides on body and organ weights was within the expected range for antisense oligonucleotides.

Table 223

Final body weights (g) in cynomolgus monkey

	Day 1	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84
PBS	2887	2953	3028	3094	3125	3143	3193
ISIS 532770*	2963	2947	2966	3050	3097	3138	3160
ISIS 532800	2886	2976	3072	3149	3220	3269	3265
ISIS 532809	2755	2836	2927	2983	3019	3071	3098
ISIS 588540	2779	2834	2907	2934	2981	3034	3057
ISIS 588544	2837	2896	3009	3064	3132	3163	3199

ISIS 588548	2694	2816	2882	2990	3073	3149	3161
ISIS 588550	2855	2988	3062	3188	3219	3282	3323
ISIS 588553	3033	3156	3256	3335	3379	3372	3442
ISIS 588555	2757	2863	2965	3022	3075	3088	3158
ISIS 588848	2850	3018	3032	3187	3230	3212	3291
ISIS 594430	2884	2963	2953	3149	3187	3204	3256

Table 224

Final organ weights (g) in cynomolgus monkey

	Spleen	Heart	Kidney	Liver
PBS	2.8	11.6	11.9	55.8
ISIS 532770*	5.0	11.3	20.6	77.9
ISIS 532800	6.2	11.9	18.6	94.4
ISIS 588540	4.0	11.4	13.5	67.1
ISIS 588548	4.1	11.7	17.3	72.0
ISIS 588550	5.8	10.9	18.5	81.8
ISIS 588553	5.0	12.7	17.2	85.9
ISIS 588555	4.7	11.8	15.9	88.3
ISIS 588848	5.0	12.7	14.4	75.7
ISIS 594430	3.9	11.9	14.8	69.9

Liver function

To evaluate the effect of ISIS oligonucleotides on hepatic function, blood samples were collected from all the study groups. The blood samples were collected from the cephalic, saphenous, or femoral veins, 48 hours post-dosing. The monkeys were fasted overnight prior to blood collection. Blood (1.5 mL) was collected in tubes without anticoagulant for serum separation. The tubes were kept at room temperature for a minimum of 90 minutes and then centrifuged (approximately 3,000 rpm for 10 min) to obtain serum. Levels of various liver function markers were measured using a Toshiba 200FR NEO chemistry analyzer (Toshiba Co., Japan).

Plasma levels of ALT and AST were measured and the results are presented in the Table below, expressed in IU/L. Bilirubin, a liver function marker, was similarly measured and is presented in the Table below expressed in mg/dL. '*' indicates that the oligonucleotide was tested in a separate study with similar conditions and is the average of the measurements from male and female monkeys. The results indicate that most of the antisense oligonucleotides had no effect on liver function outside the expected range for antisense oligonucleotides.

Table 225

Liver chemistry marker levels in cynomolgus monkey plasma on day 86

	ALT (IU/L)	AST (IU/L)	Bilirubin (mg/dL)
PBS	71	57	0.3
ISIS 532770*	59	58	0.1
ISIS 532800	65	86	0.1
ISIS 532809	35	58	0.1
ISIS 588540	70	88	0.2
ISIS 588544	55	97	0.2
ISIS 588548	61	85	0.2
ISIS 588550	94	84	0.2
ISIS 588553	44	65	0.2
ISIS 588555	63	84	0.2
ISIS 588848	69	65	0.2
ISIS 594430	86	53	0.2

Kidney function

To evaluate the effect of ISIS oligonucleotides on kidney function, blood samples were collected from all the study groups. The blood samples were collected from the cephalic, saphenous, or femoral veins, 48 hours post-dosing. The monkeys were fasted overnight prior to blood collection. Blood was collected in tubes without anticoagulant for serum separation. The tubes were kept at room temperature for a minimum of 90 minutes and then centrifuged (approximately 3,000 rpm for 10 min) to obtain serum. Levels of BUN and creatinine were measured using a Toshiba 200FR NEO chemistry analyzer (Toshiba Co., Japan). Results are presented in the Table below, expressed in mg/dL. '*' indicates that the oligonucleotide was tested in a separate study with similar conditions and is the average of the measurements from male and female monkeys.

For urinalysis, fresh urine from all the animals was collected in the morning using a clean cage pan on wet ice. Food was removed overnight the day before urine collection but water was supplied. Urine samples (approximately 1 mL) were analyzed for protein to creatinine (P/C) ratio using a Toshiba 200FR NEO automated chemistry analyzer (Toshiba Co., Japan). 'n.d.' indicates that the urine protein level was under the detection limit of the analyzer.

The plasma and urine chemistry data indicate that most of the ISIS oligonucleotides did not have any effect on the kidney function outside the expected range for antisense oligonucleotides.

Table 226

Renal chemistry marker levels (mg/dL) in cynomolgus monkey plasma on day 86

	BUN	Creatinine	Total protein
PBS	28	0.9	8.0
ISIS 532770*	20	0.9	6.9
ISIS 532800	25	0.9	7.5
ISIS 532809	23	0.8	7.4
ISIS 588540	30	0.8	7.5
ISIS 588544	26	0.9	7.4
ISIS 588548	25	0.9	7.6
ISIS 588550	24	0.9	7.2
ISIS 588553	25	0.8	7.2
ISIS 588555	25	0.8	7.6
ISIS 588848	24	0.9	7.5
ISIS 594430	25	0.8	7.2

Table 227

Renal chemistry marker levels in cynomolgus monkey urine on day 44 and day 86

	Day 44	Day 86
PBS	0.03	n.d.
ISIS 532800	0.01	n.d.
ISIS 532809	0.01	n.d.
ISIS 588540	0.03	n.d.
ISIS 588544	0.01	0.09
ISIS 588548	0.01	0.01
ISIS 588550	0.04	0.01
ISIS 588553	0.05	n.d.
ISIS 588555	0.03	0.03
ISIS 588848	0.09	n.d.
ISIS 594430	0.03	n.d.

Hematology

To evaluate any effect of ISIS oligonucleotides in cynomolgus monkeys on hematologic parameters, blood samples of approximately 0.5 mL of blood was collected from each of the available study animals in tubes containing K_2-EDTA. Samples were analyzed for red blood cell (RBC) count, white blood cells (WBC) count, individual white blood cell counts, such as that of monocytes, neutrophils, lymphocytes, as well as for platelet count, hemoglobin content and hematocrit, using an ADVIA120 hematology analyzer (Bayer, USA).

The data is presented in the Tables below. '*' indicates that the oligonucleotide was tested in a separate study with similar conditions and is the average of the measurements from male and female monkeys.

The data indicate the oligonucleotides did not cause any changes in hematologic parameters outside the expected range for antisense oligonucleotides at this dose.

Table 228

Blood cell counts in cynomolgus monkeys

	RBC (x 10^6/μL)	Platelets (x 10^3/μL)	WBC (x 10^3/μL)	Neutrophils (% WBC)	Lymphocytes (% total)	Monocytes (% total)
PBS	5.8	347	9.4	42.7	53.1	3.0
ISIS 532770*	5.4	386	10.8	22.3	71.7	3.3
ISIS 532800	5.6	360	13.1	29.5	61.1	6.5
ISIS 532809	5.2	400	11.5	56.6	38.2	2.5
ISIS 588540	5.5	367	11.7	50.9	42.7	2.1
ISIS 588544	5.2	373	14.3	56.6	37.6	4.3
ISIS 588548	5.1	373	9.7	40.4	54.3	3.9
ISIS 588550	6.1	343	9.9	32.1	61.7	4.6
ISIS 588553	5.2	424	9.3	41.7	53.2	3.6
ISIS 588555	5.1	411	9.6	45.1	49.7	3.5
ISIS 588848	5.7	370	10.0	39.8	55.8	3.1
ISIS 594430	5.7	477	10.6	47.3	47.8	3.6

Table 229

Hematologic parameters in cynomolgus monkeys

	Hemoglobin (g/dL)	HCT (%)
PBS	14.1	46.6
ISIS 532770*	12.4	40.9
ISIS 532800	12.3	40.5
ISIS 532809	12.2	40.4
ISIS 588540	12.5	41.5
ISIS 588544	11.9	38.1
ISIS 588548	12.3	39.6
ISIS 588550	13.4	45.0
ISIS 588553	12.6	39.8
ISIS 588555	11.6	38.1
ISIS 588848	13.2	42.7
ISIS 594430	13.4	43.1

Measurement of oligonucleotide concentration

The concentration of the full-length oligonucleotide was measured in the kidney and liver tissues. The method used is a modification of previously published methods (Leeds et al., 1996; Geary et al., 1999) which consist of a phenol-chloroform (liquid-liquid) extraction followed by a solid phase extraction. Tissue sample concentrations were calculated using calibration curves, with a lower limit of quantitation (LLOQ) of approximately 1.14 µg/g. The results are presented in the Table below, expressed as µg/g liver or kidney tissue.

Table 230

Antisense oligonucleotide distribution

	Kidney (µg/g)	Liver (µg/g)	Kidney/Liver ratio
ISIS 532800	3881	1633	2.4
ISIS 588540	3074	1410	2.2
ISIS 588548	3703	1233	3.0
ISIS 588550	4242	860	4.9
ISIS 588553	3096	736	4.2
ISIS 588555	4147	1860	2.2
ISIS 588848	2235	738	3.0
ISIS 594430	1548	752	2.1

Example 135: 6 week efficacy study of unconjugated and 5'-THA-GalNAc3 conjugated antisense oligonucleotides targeted to human CFB in transgenic mice

Two antisense oligonucleotides having the same nucleobase sequence: uncongugated antisense oligonucleotide ISIS 588540 and 5'-THA-GalNAc3-conjugated antisense oligonucleotide ISIS 696844, were tested in human CFB transgenic mice (hCFB-Tg mice).

The mice were administered subcutaneously with ISIS 696844 at doses of 0.1, 1.25, 0.5, 2.0, 6.0, or 12.0 mg/kg/week or with ISIS 588540 at doses of 2, 6, 12, 25, or 50 mg/kg/week for 6 weeks. A control group of mice were administered subcutaneously with PBS for 6 weeks. Mice were sacrificed 48 hours after the last dose. Hepatic mRNA levels were analyzed by qRT-PCR.

Study 1

The results are presented in the Table below and demonstrate that the 5'-THA-GalNAc3-conjugated antisense oligonucleotide targeting CFB is more potent than the unconjugated antisense oligonucleotide with the same sequence.

Table 231

Efficacy of antisense oligonucleotides targeting CFB

	ED_{50} (mg/kg)	ED_{75} (mg/kg)
ISIS 588540	4.52	9.26
ISIS 696844	0.52	1.12

Study 2

Liver mRNA levels were measured with two different primer probe sets targeting different regions of the mRNA and normalized to either RIBOGREEN® (RGB) or Cyclophilin. The primer probe sets were RTS3459, described above, and RTS3460 (forward sequence CGAAGCAGCTCAATGAAATCAA, designated herein as SEQ ID NO: 813; reverse sequence TGCCTGGAGGGCCTTCTT, designated herein as SEQ ID NO: 814; probe sequence AGACCACAAGTTGAAGTC, designated herein as SEQ ID NO: 815). The results are presented in the Table below and demonstrate that the 5'-THA-GalNAc3-conjugated antisense oligonucleotide targeting CFB is more potent than the unconjugated antisense oligonucleotide with the same sequence, irrespective of the primer probe set used.

Table 231

Efficacy of antisense oligonucleotides targeting CFB

	ED_{50} RTS3459 (RGB)	ED_{50} RTS3460 (RGB)	ED_{50} RTS3459 (Cyclophilin)	ED_{50} RTS3460 (Cyclophilin)	ED_{75} RTS3459 (RGB)	ED_{75} RTS3460 (RGB)	ED_{75} RTS3459 (Cyclophilin)	ED_{75} RTS3460 (Cyclophilin)
ISIS 588540	4.5	4.1	5.2	5.4	9.3	10.0	10.0	9.3
ISIS 696844	0.5	0.5	0.6	0.5	1.1	1.3	1.2	0.9

What is Claimed:

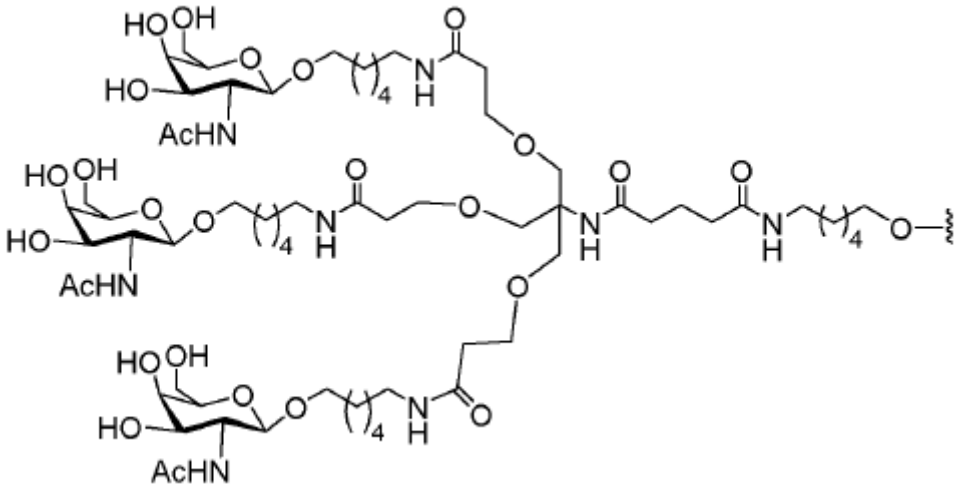
1. A compound comprising a single stranded modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 16 to 30 linked nucleosides and has a nucleobase sequence comprising any one of SEQ ID NOs: 455, 237, 448, 453, 444, 450, 598, 198, 549 and 228 wherein the modified oligonucleotide has:

a gap segment consisting of linked deoxynucleosides;

a 5' wing segment consisting of linked nuclesides;

and a 3' wing segment consisting of linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar; wherein the conjugate group comprises:



and wherein the compound is capable of inhibiting Complement Factor B (CFB) expression.

2. The compound of claim 1, wherein the modified oligonucleotide consists of 20 linked nucleosides and has a nucleobase sequence consisting of the sequence recited in SEQ ID NO: 455, 237, 453, 444, 448, 450, 198 or 228 wherein the modified oligonucleotide has:

a gap segment consisting of ten linked deoxynucleosides;

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

a 3' wing segment consisting of five linked nucleosides;

wherein the gap segment is positioned between the 5' wing segment and the 3' wing

segment, wherein each nucleoside of each wing segment comprises a 2’-O-methoxyethyl sugar; wherein each internucleoside linkage of the modified oligonucleotide is a phosphorothioate linkage and wherein each cytosine is a 5-methylcytosine.

3. The compound of claim 1, wherein the modified oligonucleotide consists of 16 linked nucleosides and has a nucleobase sequence consisting of the sequence recited in SEQ ID NO: 598, wherein the modified oligonucleotide has:

a gap segment consisting of ten linked deoxynucleosides;

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

a 3’ wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5’ wing segment and the 3’ wing segment; wherein the 5’ wing segment comprises a 2’-O-methoxyethyl sugar, 2’-O-methoxyethyl sugar and cEt sugar in the 5’ to 3’ direction; wherein the 3’ wing segment comprises a cEt sugar, cEt sugar, and 2’-O-methoxyethyl sugar in the 5’ to 3’ direction; wherein each internucleoside linkage of the modified oligonucleotide is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

4. The compound of claim 1, wherein the modified oligonucleotide consists of 16 linked nucleosides and has a nucleobase sequence consisting of the sequence recited in SEQ ID NO: 549, wherein the modified oligonucleotide has:

a gap segment consisting of ten linked deoxynucleosides;

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

a 3’ wing segment consisting of three linked nucleosides;

wherein the gap segment is positioned between the 5’ wing segment and the 3’ wing segment; wherein each nucleoside of each wing segment comprises a cEt sugar; wherein each internucleoside linkage of the modified oligonucleotide is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

5. The compound of claim 1, wherein the oligonucleotide is at least 80%, 85%, 90%, 95% or 100% complementary to SEQ ID NO: 1 or 2.

6. The compound of claim 1 or claim 5, wherein the modified sugar is a bicyclic sugar, optionally wherein the bicyclic sugar is selected from the group consisting of: 4'-(CH2)-O-2' (LNA); 4'-(CH2) 2-O-2' (ENA); and 4'-CH(CH3)-O-2' (cEt).

7. The compound of any one of claims 1, 5 or 6, wherein the modified sugar is a 2’-O-

methoxyethyl.

8. The compound of claim 1, or any one of claims 5 – 7, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage or at least one modified nucleobase.

9. The compound of claim 8, wherein the modified internucleoside linkage is a phosphorothioate internucleoside linkage or, wherein each internucleoside linkage of the modified oligonucleotide comprises a phosphorothioate internucleoside linkage.

10. The compound of claim 8 or claim 9, wherein the modified nucleobase is a 5-methylcytosine.

11. The compound of any one of claims 1 – 10, wherein the conjugate group is linked to the modified oligonucleotide:

(i) at the 5’ end of the modified oligonucleotide; or

(ii) at the 3’ end of the modified oligonucleotide.

12. A composition comprising the compound of any one of claims 1-11 or a salt thereof and at least one of a pharmaceutically acceptable carrier or diluent.

13. A compound according to any one of claims 1-11 or a composition according to claim 12 for use as a medicament.

14. Use of a compound according to any one of claims 1-11 or a composition according to claim 12 in the manufacture of a medicament for the treatment of a disease associated with dysregulation of the complement alternative pathway in a subject.

15. The use of claim 14 wherein the disease is macular degeneration, age related macular degeneration (AMD), wet AMD, dry AMD or Geographic Atrophy.

16. The use of claim 14 wherein the disease is a kidney disease.

17. The use of claim 16 wherein the kidney disease is lupus nephritis, systemic lupus erythematosus (SLE), dense deposit disease (DDD), C3 glomerulonephritis (C3GN), CFHR5 nephropathy or atypical hemolytic uremic syndrome (aHUS).

ABSTRACT

COMPOSITIONS AND METHODS FOR MODULATING COMPLEMENT FACTOR B EXPRESSION

The present embodiments provide methods, compounds, and compositions for treating, preventing, or ameliorating a disease associated with dysregulation of the complement alternative pathway by administering a Complement Factor B (CFB) specific inhibitor to a subject.

(no figure)