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(54) Title: COMPOSITIONS AND METHODS FOR MODULATING GROWTH HORMONE RECEPTOR EXPRESSION

(57) Abrégé/Abstract:
The present embodiments provide methods, compounds, and compositions for treating, preventing, ameliorating a disease associated with excess growth hormone using antisense compounds oligonucleotides targeted to growth hormone receptor (GHR).

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COMPOSITIONS AND METHODS FOR MODULATING GROWTH HORMONE RECEPTOR 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 BIOL0253WOSEQ_ST25.txt created April 27, 2015, which is 1.29 MB 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 excess growth hormone using antisense compounds or oligonucleotides targeted to growth hormone receptor (GHR).

Background

Growth hormone is produced in the pituitary and secreted into the bloodstream where it binds to growth hormone receptor (GHR) on many cell types, causing production of insulin-like growth factor-1 (IGF-1). IGF-1 is produced mainly in the liver, but also in adipose tissue and the kidney, and secreted into the bloodstream. Several disorders, such as acromegaly and gigantism, are associated with elevated growth hormone levels and/or elevated IGF-I levels in plasma and/or tissues.

Excessive production of growth hormone can lead to diseases such as acromegaly or gigantism. Acromegaly and gigantism are associated with excess growth hormone, often caused by a pituitary tumor, and affects 40-50 per million people worldwide with about 15,000 patients in each of the US and Europe and an annual incidence of about 4-5 per million people. Acromegaly and gigantism are initially characterized by abnormal growth of the hands and feet and bony changes in the facial features. Many of the growth related outcomes are mediated by elevated levels of serum IGF-1.

Summary

Embodiments provided herein relate to methods, compounds, and compositions for treating, preventing, or ameliorating a disease associated with excess growth hormone. Several embodiments provided herein are drawn to antisense compounds or oligonucleotides targeted to growth hormone receptor (GHR). Several embodiments are directed to treatment, prevention, or amelioration of acromegaly with antisense compounds or oligonucleotides targeted to growth hormone receptor (GHR).

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 other than H or OH. Unless otherwise indicated, a 2'-substituted nucleoside is not a bicyclic nucleoside..

“2’-substituted sugar moiety” means a furanosyl comprising a substituent at the 2’-position other than H or OH. Unless otherwise indicated, a 2’-substituted sugar moiety is not a bicyclic sugar moiety (i.e., the 2’-substituent of a 2’-substituted sugar moiety does not form a bridge to another atom of the furanosyl ring.

“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 GHR”, it is implied that GHR 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 (C_1-C_{12} 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 C_1-C_{12} 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 C_1-C_{12} 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 a nucleoside having a sugar moiety comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4'-carbon and the 2'-carbon of the sugar ring.

"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'.

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

"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.

"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.

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

"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.

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

"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.

"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 "GalNAc$_3$". In certain embodiments, the carbohydrate cluster portion comprises 4 GalNAc groups and is designated "GalNAc$_4$". 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 "GalNAc3-1$_a$" 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.

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

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

"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).

"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.

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

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

"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.

"Downstream" refers to the relative direction towards the 3' end or C-terminal end of a nucleic acid.

"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.

"Effective amount" in the context of modulating an activity or of treating or preventing a condition means the administration of that amount of pharmaceutical agent to a subject in need of such modulation, treatment, or prophylaxis, either in a single dose or as part of a series, that is effective for modulation of that effect, or for treatment or prophylaxis or improvement of that condition. 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.

"Essentially unchanged" means little or no change in a particular parameter, particularly relative to another parameter which changes much more. In certain embodiments, a parameter is essentially unchanged when it changes less than 5%. In certain embodiments, a parameter is essentially unchanged if it changes less than two-fold while another parameter changes at least ten-fold. For example, in certain embodiments, an antisense activity is a change in the amount of a target nucleic acid. In certain such embodiments, the amount of a non-target nucleic acid is essentially unchanged if it changes much less than the target nucleic acid does, but the change need not be zero.

"Expression" means the process by which a gene ultimately results in a protein. Expression includes, but is not limited to, transcription, post-transcriptional modification (e.g., splicing, polyadenlyation, addition of 5'-cap), 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."

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

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

"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.

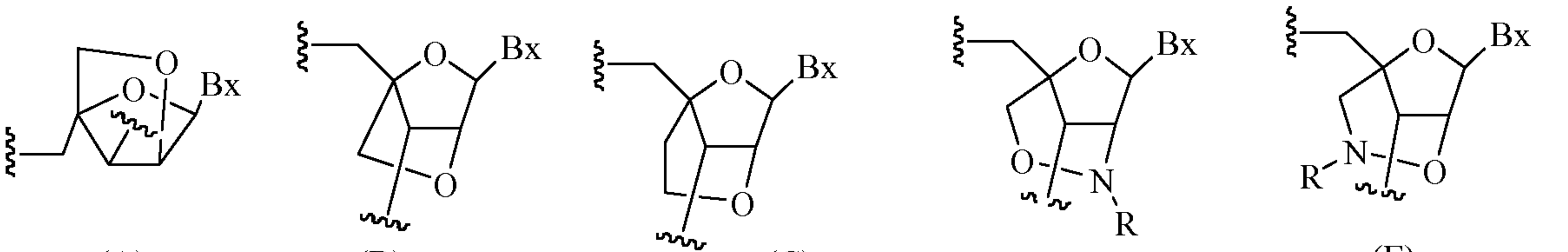
"Lengthened" antisense oligonucleotides are those that have one or more additional nucleosides relative to an antisense oligonucleotide disclosed herein.

"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" "Locked nucleic acid" or " LNA" or "LNA 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.

"Metabolic disorder" means a disease or condition principally characterized by dysregulation of metabolism – the complex set of chemical reactions associated with breakdown of food to produce energy.

"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 carbohydrate" means any carbohydrate having one or more chemical modifications relative to naturally occurring carbohydrates.

"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. "Modified sugar moiety" means a substituted sugar moiety or a sugar surrogate.

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

"MOE" means $-OCH_2CH_2OCH_3$.

"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, heteroaromatic 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 heterocyclic 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 sugar moiety" means a ribofuranosyl as found in naturally occurring RNA or a deoxyribofuranosyl as found in naturally occurring DNA.

"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 phosphotriesters, methylphosphonates, MMI ($-CH_2-N(CH_3)-O-$), amide-3 ($-CH_2-C(=O)-N(H)-$), amide-4 ($-CH_2-N(H)-C(=O)-$), formacetal ($-O-CH_2-O-$), and thioformacetal ($-S-CH_2-O-$). 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 CH_2 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" or "complementarity" when in reference to nucleobases means 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 means 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. Nucleobases comprising certain modifications may maintain the ability to pair with a counterpart nucleobase and thus, are still capable of nucleobase complementarity.

"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 compound comprising a nucleobase moiety and a sugar moiety. Nucleosides include, but are not limited to, naturally occurring nucleosides (as found in DNA and RNA) and modified nucleosides. Nucleosides may be linked to a phosphate moiety.

"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.

"Off-target effect" refers to an unwanted or deleterious biological effect associated with modulation of RNA or protein expression of a gene other than the intended target nucleic acid.

"Oligomeric compound" means a polymeric structure comprising two or more sub-structures. In certain embodiments, an oligomeric compound comprises an oligonucleotide. In certain embodiments, an oligomeric compound comprises one or more conjugate groups and/or terminal groups. In certain embodiments, an oligomeric compound consists of an oligonucleotide. Oligomeric compounds also include naturally occurring nucleic acids. In certain embodiments, an oligomeric compound comprises a backbone of one or more linked monomeric subunits where each linked monomeric subunit is directly or indirectly attached to a heterocyclic base moiety. In certain embodiments, oligomeric compounds may also include monomeric subunits that are not linked to a heterocyclic base moiety, thereby providing abasic sites. In certain embodiments, the linkages joining the monomeric subunits, the sugar moieties or surrogates and the heterocyclic base moieties can be independently modified. In certain embodiments, the linkage-sugar unit, which may or may not include a heterocyclic base, may be substituted with a mimetic such as the monomers in peptide nucleic acids.

"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.

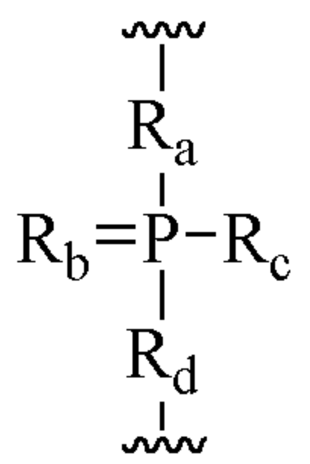
"Peptide" means a molecular formed by linking at least two amino acids by amide bonds. Without limitation, as used herein, peptide refers to polypeptides and proteins.

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

"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.

"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.

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

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

"Prevent" refers to delaying or forestalling the onset, 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.

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

"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.

"Single-stranded" means an oligomeric compound that is not hybridized to its complement and which lacks sufficient self-complementarity to form a stable self-duplex.

"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 unprotected. 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 hydrocarbyl 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 ($-C(O)R_{aa}$), carboxyl ($-C(O)O-R_{aa}$), aliphatic groups, alicyclic groups, alkoxy, substituted oxy ($-O-R_{aa}$), aryl, aralkyl, heterocyclic radical, heteroaryl, heteroarylalkyl, amino ($-N(R_{bb})(R_{cc})$), imino($=NR_{bb}$), amido ($-C(O)N(R_{bb})(R_{cc})$ or $-N(R_{bb})C(O)R_{aa}$), azido ($-N_3$), nitro ($-NO_2$), cyano ($-CN$), carbamido ($-OC(O)N(R_{bb})(R_{cc})$ or $-N(R_{bb})C(O)OR_{aa}$), ureido ($-N(R_{bb})C(O)N(R_{bb})(R_{cc})$), thioureido ($-N(R_{bb})C(S)N(R_{bb})-(R_{cc})$), guanidinyl ($-N(R_{bb})C(=NR_{bb})N(R_{bb})(R_{cc})$), amidinyl ($-C(=NR_{bb})N(R_{bb})(R_{cc})$ or $-N(R_{bb})C(=NR_{bb})(R_{aa})$), thiol ($-SR_{bb}$), sulfinyl ($-S(O)R_{bb}$), sulfonyl ($-S(O)_2R_{bb}$) and sulfonamidyl ($-S(O)_2N(R_{bb})(R_{cc})$ or $-N(R_{bb})S-(O)_2R_{bb}$). Wherein each R_{aa}, R_{bb} and R_{cc} 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 heteroarylalkyl. 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" or "targeted" means the process of design and selection of an antisense compound that will specifically hybridize to a target nucleic acid and induce a desired effect.

"Target nucleic acid," "target RNA," "target RNA transcript" and "nucleic acid target" all mean a nucleic acid capable of being targeted by antisense compounds. "Target nucleic acid" means a nucleic acid molecule to which an antisense compound is intended to hybridize to result in a desired antisense activity. Antisense oligonucleotides have sufficient complementarity to their target nucleic acids to allow hybridization under physiological conditions.

"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.

“The same type of modifications” refers to modifications that are the same as one another, including absence of modifications. Thus, for example, two unmodified DNA nucleosides have “the same type of modification,” even though the DNA nucleoside is unmodified. Such nucleosides having the same type modification may comprise different nucleobases.

“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.

“Type of modification” in reference to a nucleoside or a nucleoside of a “type” means the chemical modification of a nucleoside and includes modified and unmodified nucleosides. Accordingly, unless otherwise indicated, a “nucleoside having a modification of a first type” may be an unmodified nucleoside.

"Unmodified" nucleobases or "naturally occurring nucleobase" means the naturally occurring heterocyclic nucleobases of RNA or DNA: the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) (including 5-methyl 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).

“Upstream” refers to the relative direction towards the 5’ end or N-terminal end of a nucleic acid.

“Wing segment” means a plurality of nucleosides modified to impart to an oligonucleotide properties such as enhanced inhibitory activity, increased binding affinity for a target nucleic acid, or resistance to degradation by in vivo nucleases.

Certain Embodiments

Certain embodiments provide methods, compounds and compositions for inhibiting growth hormone receptor (GHR) expression.

Certain embodiments provide antisense compounds targeted to a GHR nucleic acid. In certain embodiments, the GHR nucleic acid has the sequence set forth in GENBANK Accession No. NM_000163.4 (incorporated herein as SEQ ID NO: 1), GENBANK Accession No. NT_006576.16 truncated from nucleotides 42411001 to 42714000 (incorporated herein as SEQ ID NO: 2), GENBANK Accession No

X06562.1 (incorporated herein as SEQ ID NO: 3), GENBANK Accession No. DR006395.1 (incorporated herein as SEQ ID NO: 4), GENBANK Accession No. DB052048.1 (incorporated herein as SEQ ID NO: 5), GENBANK Accession No. AF230800.1 (incorporated herein as SEQ ID NO: 6), the complement of GENBANK Accession No. AA398260.1 (incorporated herein as SEQ ID NO: 7), GENBANK Accession No. BC136496.1 (incorporated herein as SEQ ID NO: 8), GENBANK Accession No. NM_001242399.2 (incorporated herein as SEQ ID NO: 9), GENBANK Accession No. NM_001242400.2 (incorporated herein as SEQ ID NO: 10), GENBANK Accession No. NM_001242401.3 (incorporated herein as SEQ ID NO: 11), GENBANK Accession No. NM_001242402.2 (incorporated herein as SEQ ID NO: 12), GENBANK Accession No. NM_001242403.2 (incorporated herein as SEQ ID NO: 13), GENBANK Accession No. NM_001242404.2 (incorporated herein as SEQ ID NO: 14), GENBANK Accession No. NM_001242405.2 (incorporated herein as SEQ ID NO: 15), GENBANK Accession No. NM_001242406.2 (incorporated herein as SEQ ID NO: 16), GENBANK Accession No. NM_001242460.1 (incorporated herein as SEQ ID NO: 17), GENBANK Accession NM_001242461.1 (incorporated herein as SEQ ID NO: 18), or GENBANK Accession No. NM_001242462.1 (incorporated herein as SEQ ID NO: 19).

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: 20-2295.

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: 20-2295.

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 the nucleobase sequences of any of SEQ ID NOs: 20-2295.

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 the nucleobase sequences of any of SEQ ID NOs: 20-2295.

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 the nucleobase sequences of any of SEQ ID NOs: 20-2295.

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 sequences of any of SEQ ID NOs: 20-2295.

Certain embodiments provide a compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of the nucleobase sequences of any one of SEQ ID NOs: 20-2295.

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 nucleotides 30-51, 63-82, 103-118, 143-159, 164-197, 206-259, 361-388, 554-585, 625-700, 736-776, 862-887, 923-973, 978-996, 1127-1142, 1170-1195, 1317-1347, 1360-1383, 1418-1449, 1492-1507, 1524-1548, 1597-1634, 1641-1660, 1683-1698, 1744-1768, 1827-1860, 1949-2002, 2072-2092, 2095-2110, 2306-2321, 2665-2683, 2685-2719, 2739-2770, 2859-2880, 2941-2960, 2963-2978, 3037-3052, 3205-3252, 3306-3332, 3371-3386, 3518-3542, 3975-3990, 4041-4087, 4418-4446, 4528-4546, 7231-7246, 7570-7585, 8395-8410, 9153-9168, 9554-9569, 9931-9946, 10549-10564, 11020-11035, 11793-11808, 12214-12229, 12474-12489, 12905-12920, 13400-13415, 13717-13732, 14149-14164, 14540-14555, 15264-15279, 15849-15864, 16530-16545, 17377-17392, 17581-17596, 17943-17958, 18353-18368, 18636-18651, 19256-19271, 19814-19829, 20365-20380, 20979-20994, 21566-21581, 22150-22165, 22803-22818, 29049-29064, 29554-29569, 30245-30260, 30550-30565, 30915-30930, 31468-31483, 32366-32381, 32897-32912, 33187-33202, 33780-33795, 34407-34422, 34846-34861, 35669-35684, 36312-36327, 36812-36827, 37504-37519, 38841-38856, 40250-40265, 40706-40721, 40922-40937, 41424-41439, 41999-42014, 42481-42496, 42700-42715, 43291-43306, 43500-43515, 43947-43962, 44448-44463, 45162-45177, 46010-46025, 46476-46491, 47447-47462, 47752-47767, 48001-48016, 48423-48438, 50195-50210, 50470-50485, 51104-51119, 51756-51771, 52015-52030, 52230-52245, 52588-52603, 53532-53547, or 54645-54660 of SEQ ID NO: 1, wherein said modified oligonucleotide is at least 90% complementary to SEQ ID NO: 1.

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 100% complementary to an equal length portion of nucleobases 30-51, 63-82, 103-118, 143-159, 164-197, 206-259, 361-388, 554-585, 625-700, 736-776, 862-887, 923-973, 978-996, 1127-1142, 1170-1195, 1317-1347, 1360-1383, 1418-1449, 1492-1507, 1524-1548, 1597-1634, 1641-1660, 1683-1698, 1744-1768, 1827-1860, 1949-2002, 2072-2092, 2095-2110, 2306-2321, 2665-2683, 2685-2719, 2739-2770, 2859-2880, 2941-2960, 2963-2978, 3037-3052, 3205-3252, 3306-3332, 3371-3386, 3518-3542, 3975-3990, 4041-4087, 4418-4446, 4528-4546, 7231-7246, 7570-7585, 8395-8410, 9153-9168, 9554-9569, 9931-9946, 10549-10564, 11020-11035, 11793-11808, 12214-12229, 12474-12489, 12905-12920, 13400-13415, 13717-13732, 14149-14164, 14540-14555, 15264-15279, 15849-

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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 nucleotides 2571-2586, 2867-3059, 3097-3116, 3341-3695, 4024-4039, 4446-4894, 5392-5817, 6128-6265, 6499-6890, 7231-7246, 8395-8410, 9153-9168, 9554-9569, 9931-9946, 10549-10564, 10660-10679, 11020-11035, 11793-12229, 12469-12920, 13351-13415, 13717-13732, 14149-14164, 14361-14555, 14965-15279, 15849-16001, 16253-16272, 16447-16545, 17130-17149, 17377-17669, 17927-17958, 18353-18368, 18636-18773, 19661-19918, 20288-20470, 20979-20994, 21215-21606, 21820-21837, 22150-22165, 22518-22536, 22803-22818, 26494-26522, 29049-29069, 29323-29489, 30550-30565, 30915-31191, 31468-31483, 32363-32382, 32827-33202, 33635-33795, 34138-34157, 34407-34422, 34845-34864, 35466-35485, 35669-35684, 36023-36042, 36266-36327, 36721-36827, 37032-37130, 37276-37295, 37504-37675, 38094-38118, 38841-38856, 39716-40538, 40706-40937, 41164-41183, 41342-41439, 42141-42164, 42700-42760, 43173-43537, 43765-46025, 46476-46532, 48423-48438, 50072-50210, 50470-50485, 50719-51234, 51747-51797, 52015-52143, 52230-52245, 52573-52652, 53466-54660, 54886-54901, 63751-64662, 64882-65099, 65363-65378, 65600-65615, 65988-66183, 66566-66581, 66978-67080, 67251-67270, 67662-67929, 68727-68742, 69203-69242, 69565-69620, 69889-70145, 70352-70584, 70925-71071, 71314-71329, 71617-71769, 72107-72241, 72584-72670, 73061-73076, 73350-73369, 73689-73723, 74107-74131, 74317-74557, 74947-75009, 75192-75207, 75979-76066, 76410-77095, 77292-77307, 77638-77869, 78122-78326, 79006-79021, 79478-79505, 80277-80292, 80575-80939, 81207-81222, 81524-81543, 81761-81776, 82233-82248, 82738-83198, 83330-83416, 83884-84063, 84381-85964, 86220-86392, 86554-86655, 86901-86920, 87181-87262, 88063-88082, 88293-88308, 88605-88967, 89160-89175, 89940-90255, 90473-90528, 91073-91088, 91273-91292, 91647-91662, 91930-92126, 92356-92371, 93190-93443, 93762-94111, 94374-94389, 94581-94653, 94839-94858, 95292-95583, 95829-95844, 96137-96503, 96793-97013, 97539-97554, 97800-97889, 98132-98151, 98624-98672, 98810-99115, 99258-99273, 99478-99503, 99791-99858, 100281-100300, 100406-100421, 100742-100828, 101080-101103, 101242-101320, 101788-101906, 102549-102568, 103566-103625, 104067-104086, 104277-104858, 105255-105274, 106147-106364, 106632-106647, 106964-107735, 108514-108788, 109336-109505, 109849-109864, 110403-110442, 110701-110974, 111203-111322, 112030-

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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 100% complementary to an equal length portion of nucleobases 2571-2586, 2867-3059, 3097-3116, 3341-3695, 4024-4039, 4446-4894, 5392-5817, 6128-6265, 6499-6890, 7231-7246, 8395-8410, 9153-9168, 9554-9569, 9931-9946, 10549-10564, 10660-10679, 11020-11035, 11793-12229, 12469-12920, 13351-13415, 13717-13732, 14149-14164, 14361-14555, 14965-15279, 15849-16001, 16253-16272, 16447-16545, 17130-17149, 17377-17669, 17927-17958, 18353-18368, 18636-18773, 19661-19918, 20288-20470, 20979-20994, 21215-21606, 21820-21837, 22150-22165, 22518-22536, 22803-22818, 26494-26522, 29049-29069, 29323-29489, 30550-30565, 30915-31191, 31468-31483, 32363-32382, 32827-33202, 33635-33795, 34138-34157, 34407-34422, 34845-34864, 35466-35485, 35669-35684, 36023-36042, 36266-36327, 36721-36827, 37032-37130, 37276-37295, 37504-37675, 38094-38118, 38841-38856, 39716-40538, 40706-40937, 41164-41183, 41342-41439, 42141-42164, 42700-42760, 43173-43537, 43765-46025, 46476-46532, 48423-48438, 50072-50210, 50470-50485, 50719-51234, 51747-51797, 52015-52143, 52230-52245, 52573-52652, 53466-54660, 54886-54901, 63751-64662, 64882-65099, 65363-65378, 65600-65615, 65988-66183, 66566-66581, 66978-67080, 67251-67270, 67662-67929, 68727-68742, 69203-69242, 69565-69620, 69889-70145, 70352-70584, 70925-71071, 71314-71329, 71617-71769, 72107-72241, 72584-72670, 73061-73076, 73350-73369, 73689-73723, 74107-74131, 74317-74557, 74947-75009, 75192-75207, 75979-76066, 76410-77095, 77292-77307, 77638-77869, 78122-78326, 79006-79021, 79478-79505, 80277-80292, 80575-80939, 81207-81222, 81524-81543, 81761-81776, 82233-82248, 82738-83198, 83330-83416, 83884-84063, 84381-85964, 86220-86392, 86554-86655, 86901-86920, 87181-87262, 88063-88082, 88293-88308, 88605-88967, 89160-89175, 89940-90255, 90473-90528, 91073-91088, 91273-91292, 91647-91662, 91930-92126, 92356-92371, 93190-93443, 93762-94111, 94374-94389, 94581-94653, 94839-94858, 95292-95583, 95829-95844, 96137-96503, 96793-97013, 97539-97554, 97800-97889, 98132-98151, 98624-98672, 98810-99115, 99258-99273, 99478-99503, 99791-99858, 100281-100300, 100406-100421, 100742-100828, 101080-101103, 101242-101320, 101788-101906, 102549-102568, 103566-

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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 and having a nucleobase sequence comprising the nucleobase sequence of any one of SEQ ID NOs: 20-2295.

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: 20-2295.

In certain embodiments, a compound comprising an antisense compound or oligonucleotide and a conjugate group, wherein the antisense compound or oligonucleotide is targeted to a growth hormone receptor nucleic acid and is complementary within the following nucleotide regions of SEQ ID NO: 1: 30-51, 63-82, 103-118, 143-159, 164-197, 206-259, 361-388, 554-585, 625-700, 736-776, 862-887, 923-973, 978-996, 1127-1142, 1170-1195, 1317-1347, 1360-1383, 1418-1449, 1492-1507, 1524-1548, 1597-1634, 1641-1660, 1683-1698, 1744-1768, 1827-1860, 1949-2002, 2072-2092, 2095-2110, 2306-2321, 2665-2683, 2685-2719, 2739-2770, 2859-2880, 2941-2960, 2963-2978, 3037-3052, 3205-3252, 3306-3332, 3371-3386, 3518-3542, 3975-3990, 4041-4087, 4418-4446, 4528-4546, 7231-7246, 7570-7585, 8395-8410, 9153-9168, 9554-9569, 9931-9946, 10549-10564, 11020-11035, 11793-11808, 12214-12229, 12474-12489, 12905-12920, 13400-13415, 13717-13732, 14149-14164, 14540-14555, 15264-15279, 15849-15864, 16530-16545, 17377-17392, 17581-17596, 17943-17958, 18353-18368, 18636-18651, 19256-19271, 19814-19829, 20365-20380, 20979-20994, 21566-21581, 22150-22165, 22803-22818, 29049-29064, 29554-29569, 30245-30260, 30550-30565, 30915-30930, 31468-31483, 32366-32381, 32897-32912, 33187-33202, 33780-33795, 34407-34422, 34846-34861, 35669-35684, 36312-36327, 36812-36827, 37504-37519, 38841-38856, 40250-40265, 40706-40721, 40922-40937, 41424-41439, 41999-42014, 42481-42496, 42700-42715, 43291-43306, 43500-43515, 43947-43962, 44448-44463, 45162-45177, 46010-46025, 46476-46491, 47447-47462, 47752-47767, 48001-48016, 48423-48438, 50195-50210, 50470-50485, 51104-51119, 51756-51771, 52015-52030, 52230-52245, 52588-52603, 53532-53547, or 54645-54660.

In certain embodiments, a compound comprising an antisense compound or oligonucleotide and a conjugate group, wherein the antisense compound or oligonucleotide is targeted to a growth hormone receptor nucleic acid and targets the following nucleotide regions of SEQ ID NO: 1: 30-51, 63-82, 103-118, 143-159, 164-197, 206-259, 361-388, 554-585, 625-700, 736-776, 862-887, 923-973, 978-996, 1127-1142, 1170-1195, 1317-1347, 1360-1383, 1418-1449, 1492-1507, 1524-1548, 1597-1634, 1641-1660, 1683-1698, 1744-1768, 1827-1860, 1949-2002, 2072-2092, 2095-2110, 2306-2321, 2665-2683, 2685-2719, 2739-2770, 2859-2880, 2941-2960, 2963-2978, 3037-3052, 3205-3252, 3306-3332, 3371-3386, 3518-3542, 3975-3990, 4041-4087, 4418-4446, 4528-4546, 7231-7246, 7570-7585, 8395-8410, 9153-9168, 9554-9569, 9931-9946, 10549-10564, 11020-11035, 11793-11808, 12214-12229, 12474-12489, 12905-12920, 13400-13415, 13717-13732, 14149-14164, 14540-14555, 15264-15279, 15849-15864, 16530-16545, 17377-17392, 17581-17596, 17943-17958, 18353-18368, 18636-18651, 19256-19271, 19814-19829, 20365-20380, 20979-20994, 21566-21581, 22150-22165, 22803-22818, 29049-29064, 29554-29569, 30245-30260, 30550-30565, 30915-30930, 31468-31483, 32366-32381, 32897-32912, 33187-33202, 33780-33795, 34407-34422, 34846-34861, 35669-35684, 36312-36327, 36812-36827, 37504-37519, 38841-38856, 40250-40265, 40706-40721, 40922-40937, 41424-41439, 41999-42014, 42481-42496, 42700-42715, 43291-43306, 43500-43515, 43947-43962, 44448-44463, 45162-45177, 46010-46025, 46476-46491, 47447-47462, 47752-47767, 48001-48016, 48423-48438, 50195-50210, 50470-50485, 51104-51119, 51756-51771, 52015-52030, 52230-52245, 52588-52603, 53532-53547, or 54645-54660.

In certain embodiments, a compound comprises an antisense compound or oligonucleotide and a conjugate group, wherein the antisense compound or oligonucleotide is targeted to a region of a growth hormone receptor nucleic acid. In certain embodiments, such compounds or oligonucleotides targeted to a region of a GHR 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 nucleobases portion complementary to an equal length portion of a region recited herein. In certain embodiments, such compounds or oligonucleotide target the following nucleotide regions of SEQ ID NO: 1: 30-51, 63-82, 103-118, 143-159, 164-197, 206-259, 361-388, 554-585, 625-700, 736-776, 862-887, 923-973, 978-996, 1127-1142, 1170-1195, 1317-1347, 1360-1383, 1418-1449, 1492-1507, 1524-1548, 1597-1634, 1641-1660, 1683-1698, 1744-1768, 1827-1860, 1949-2002, 2072-2092, 2095-2110, 2306-2321, 2665-2683, 2685-2719, 2739-2770, 2859-2880, 2941-2960, 2963-2978, 3037-3052, 3205-3252, 3306-3332, 3371-3386, 3518-3542, 3975-3990, 4041-4087, 4418-4446, 4528-4546, 7231-7246, 7570-7585, 8395-8410, 9153-

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In certain embodiments, a compound comprising an antisense compound or oligonucleotide and a conjugate group, wherein the antisense compound or oligonucleotide is targeted to a growth hormone receptor nucleic acid is complementary within the following nucleotide regions of SEQ ID NO: 2: 2571-2586, 2867-3059, 3097-3116, 3341-3695, 4024-4039, 4446-4894, 5392-5817, 6128-6265, 6499-6890, 7231-7246, 8395-8410, 9153-9168, 9554-9569, 9931-9946, 10549-10564, 10660-10679, 11020-11035, 11793-12229, 12469-12920, 13351-13415, 13717-13732, 14149-14164, 14361-14555, 14965-15279, 15849-16001, 16253-16272, 16447-16545, 17130-17149, 17377-17669, 17927-17958, 18353-18368, 18636-18773, 19661-19918, 20288-20470, 20979-20994, 21215-21606, 21820-21837, 22150-22165, 22518-22536, 22803-22818, 26494-26522, 29049-29069, 29323-29489, 30550-30565, 30915-31191, 31468-31483, 32363-32382, 32827-33202, 33635-33795, 34138-34157, 34407-34422, 34845-34864, 35466-35485, 35669-35684, 36023-36042, 36266-36327, 36721-36827, 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In certain embodiments, a compound comprising an antisense compound or oligonucleotide and a conjugate group, wherein the antisense compound or oligonucleotide is targeted to a growth hormone receptor nucleic acid targets the following nucleotide regions of SEQ ID NO: 2: : 2571-2586, 2867-3059, 3097-3116, 3341-3695, 4024-4039, 4446-4894, 5392-5817, 6128-6265, 6499-6890, 7231-7246, 8395-8410, 9153-9168, 9554-9569, 9931-9946, 10549-10564, 10660-10679, 11020-11035, 11793-12229, 12469-12920, 13351-13415, 13717-13732, 14149-14164, 14361-14555, 14965-15279, 15849-16001, 16253-16272, 16447-16545, 17130-17149, 17377-17669, 17927-17958, 18353-18368, 18636-18773, 19661-19918, 20288-20470, 20979-20994, 21215-21606, 21820-21837, 22150-22165, 22518-22536, 22803-22818, 26494-26522, 29049-29069, 29323-29489, 30550-30565, 30915-31191, 31468-31483, 32363-32382, 32827-33202, 33635-33795, 34138-34157, 34407-34422, 34845-34864, 35466-35485, 35669-35684, 36023-36042, 36266-36327, 36721-36827, 37032-37130, 37276-37295, 37504-37675, 38094-38118, 38841-38856, 39716-40538, 40706-40937, 41164-41183, 41342-41439, 42141-42164, 42700-42760, 43173-43537, 43765-46025, 46476-46532, 48423-48438, 50072-50210, 50470-50485, 50719-51234, 51747-51797, 52015-52143, 52230-52245, 52573-52652, 53466-54660, 54886-54901, 63751-64662, 64882-65099, 65363-65378, 65600-65615, 65988-66183, 66566-66581, 66978-67080, 67251-67270, 67662-67929, 68727-68742, 69203-69242, 69565-69620, 69889-70145, 70352-70584, 70925-71071, 71314-71329, 71617-71769, 72107-72241, 72584-72670, 73061-73076, 73350-73369, 73689-73723, 74107-74131, 74317-74557, 74947-75009, 75192-75207, 75979-76066, 76410-77095, 77292-77307, 77638-77869, 78122-78326, 79006-79021, 79478-79505, 80277-80292, 80575-80939, 81207-81222, 81524-81543, 81761-81776, 82233-82248, 82738-83198, 83330-83416, 83884-84063, 84381-85964, 86220-86392, 86554-86655, 86901-86920, 87181-87262, 88063-88082, 88293-88308, 88605-88967, 89160-89175, 89940-90255, 90473-90528, 91073-91088, 91273-91292, 91647-91662, 91930-92126, 92356-92371, 93190-93443, 93762-94111, 94374-94389, 94581-94653, 94839-94858, 95292-95583, 95829-95844, 96137-96503, 96793-97013, 97539-97554, 97800-97889, 98132-98151, 98624-98672, 98810-99115, 99258-99273, 99478-99503, 99791-99858, 100281-100300, 100406-100421, 100742-100828, 101080-101103, 101242-

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In certain embodiments, a compound comprises an antisense compound or oligonucleotide and a conjugate group, wherein the antisense compound or oligonucleotide is targeted to a region of a growth hormone receptor nucleic acid. In certain embodiments, such compounds or oligonucleotides targeted to a region of a GHR 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 nucleobases portion complementary to an equal length portion of a region recited herein. In certain embodiments, such compounds or oligonucleotide target the following nucleotide regions of SEQ ID NO: 2: : 2571-2586, 2867-3059, 3097-3116, 3341-3695, 4024-4039, 4446-4894, 5392-5817, 6128-6265, 6499-6890, 7231-7246, 8395-8410, 9153-9168, 9554-9569, 9931-9946, 10549-10564, 10660-10679, 11020-11035, 11793-12229, 12469-12920, 13351-13415, 13717-13732, 14149-14164, 14361-14555, 14965-15279, 15849-16001, 16253-16272, 16447-16545, 17130-17149, 17377-17669, 17927-17958, 18353-18368, 18636-18773, 19661-19918, 20288-20470, 20979-20994, 21215-21606, 21820-21837, 22150-22165, 22518-22536, 22803-22818, 26494-26522, 29049-29069, 29323-29489, 30550-30565, 30915-31191, 31468-31483, 32363-32382, 32827-33202, 33635-33795, 34138-34157, 34407-34422, 34845-34864, 35466-35485, 35669-35684, 36023-36042, 36266-36327, 36721-36827, 37032-37130, 37276-37295, 37504-37675, 38094-38118, 38841-38856, 39716-40538, 40706-40937, 41164-41183, 41342-41439, 42141-42164, 42700-42760, 43173-43537, 43765-46025, 46476-46532, 48423-48438, 50072-50210, 50470-50485, 50719-51234, 51747-51797, 52015-52143, 52230-52245, 52573-52652, 53466-54660, 54886-54901, 63751-64662, 64882-65099, 65363-65378, 65600-65615, 65988-66183, 66566-66581, 66978-67080, 67251-67270, 67662-67929, 68727-68742, 69203-69242, 69565-69620, 69889-70145, 70352-70584, 70925-71071, 71314-71329, 71617-71769, 72107-72241, 72584-72670, 73061-73076, 73350-73369, 73689-73723, 74107-74131, 74317-74557, 74947-75009, 75192-75207, 75979-76066, 76410-77095, 77292-77307, 77638-77869, 78122-78326, 79006-79021, 79478-79505, 80277-80292, 80575-80939, 81207-81222, 81524-81543, 81761-81776, 82233-82248, 82738-83198, 83330-

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In certain embodiments, a compound comprises an antisense compound or oligonucleotide and a conjugate group, wherein the antisense compound or oligonucleotide is targeted to target intron 1 of a growth hormone receptor nucleic acid. In certain aspects, antisense compounds or oligonucleotides target within nucleotides 3058-144965 (intron 1) of a growth hormone receptor nucleic acid having the nucleobase sequence of SEQ ID NO: 2 (GENBANK Accession No. NT_006576.16 truncated from nucleotides 42411001 to 42714000).

In certain embodiments, a compound comprises an antisense compound or oligonucleotide and a conjugate group, wherein the antisense compound or oligonucleotide is targeted to intron 2 of a growth hormone receptor nucleic acid. In certain aspects, antisense compounds or oligonucleotides target within nucleotides 145047-208139 (intron 2) of a growth hormone receptor nucleic acid having the nucleobase sequence of SEQ ID NO: 2 (GENBANK Accession No. NT_006576.16 truncated from nucleotides 42411001 to 42714000).

In certain embodiments, a compound comprises an antisense compound or oligonucleotide and a conjugate group, wherein the antisense compound or oligonucleotide is targeted to intron 3 of a growth hormone receptor nucleic acid. In certain aspects, antisense compounds or oligonucleotides target within nucleotides 208206-267991 (intron 3) of a growth hormone receptor nucleic acid having the nucleobase sequence of SEQ ID NO: 2 (GENBANK Accession No. NT_006576.16 truncated from nucleotides 42411001 to 42714000).

In certain embodiments, a compound comprises an antisense compound or oligonucleotide and a conjugate group, wherein the antisense compound or oligonucleotide is targeted to intron 4 of a growth hormone receptor nucleic acid. In certain aspects, antisense compounds or oligonucleotides target within nucleotides 268122-274018 (intron 4) of a growth hormone receptor nucleic acid having the nucleobase sequence of SEQ ID NO: 2 (GENBANK Accession No. NT_006576.16 truncated from nucleotides 42411001 to 42714000).

In certain embodiments, a compound comprises an antisense compound or oligonucleotide and a conjugate group, wherein the antisense compound or oligonucleotide is targeted to intron 5 of a growth hormone receptor nucleic acid. In certain aspects, antisense compounds or oligonucleotides target within nucleotides 274192-278925 (intron 5) of a growth hormone receptor nucleic acid having the nucleobase sequence of SEQ ID NO: 2 (GENBANK Accession No. NT_006576.16 truncated from nucleotides 42411001 to 42714000).

In certain embodiments, a compound comprises an antisense compound or oligonucleotide and a conjugate group, wherein the antisense compound or oligonucleotide is targeted to intron 6 of a growth hormone receptor nucleic acid. In certain aspects, antisense compounds or oligonucleotides target within nucleotides 279105-290308 (intron 6) of a growth hormone receptor nucleic acid having the nucleobase sequence of SEQ ID NO: 2 (GENBANK Accession No. NT_006576.16 truncated from nucleotides 42411001 to 42714000).

In certain embodiments, a compound comprises an antisense compound or oligonucleotide and a conjugate group, wherein the antisense compound or oligonucleotide is targeted to intron 7 of a growth hormone receptor nucleic acid. In certain aspects, antisense compounds or oligonucleotides target within nucleotides 290475-292530 (intron 7) of a growth hormone receptor nucleic acid having the nucleobase sequence of SEQ ID NO: 2 (GENBANK Accession No. NT_006576.16 truncated from nucleotides 42411001 to 42714000).

In certain embodiments, a compound comprises an antisense compound or oligonucleotide and a conjugate group, wherein the antisense compound or oligonucleotide is targeted to intron 8 of a growth hormone receptor nucleic acid. In certain aspects, antisense compounds or oligonucleotides target within nucleotides 292622-297153 (intron 8) of a growth hormone receptor nucleic acid having the nucleobase sequence of SEQ ID NO: 2 (GENBANK Accession No. NT_006576.16 truncated from nucleotides 42411001 to 42714000).

In certain embodiments, a compound comprises an antisense compound or oligonucleotide and a conjugate group, wherein the antisense compound or oligonucleotide is targeted to intron 9 of a growth hormone receptor nucleic acid. In certain aspects, antisense compounds or oligonucleotides target within nucleotides 297224-297554 (intron 9) of a growth hormone receptor nucleic acid having the nucleobase sequence of SEQ ID NO: 2 (GENBANK Accession No. NT_006576.16 truncated from nucleotides 42411001 to 42714000).

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

In certain embodiments, any of the foregoing compounds or oligonucleotides comprises 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(CH3)-O-2' group, a 4'-CH2-O-2' group, or a 4'-(CH2)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, any of the foregoing compounds or oligonucleotides comprises at least one modified nucleobase, such as 5-methylcytosine.

In certain embodiments, any of the foregoing compounds or oligonucleotides comprises:

a gap segment consisting of linked deoxynucleosides;

a 5' wing segment consisting of linked nucleosides; and

a 3' wing segment consisting of linked nucleosides;

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

Certain embodiments provide a compound comprising a modified oligonucleotide consisting of 10 to 30 linked nucleosides having a nucleobase sequence comprising the sequence recited in SEQ ID NO: 918, 479, 703, 1800, 1904, 2122, 2127, or 2194.

In certain aspects, the modified oligonucleotide has a nucleobase sequence comprising the sequence recited in SEQ ID NOs: 918, 479 or 703, 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 aspects, the modified oligonucleotide has a nucleobase sequence comprising the sequence recited in SEQ ID NOs: 1800, 1904, 2122, 2127, or 2194, wherein the modified oligonucleotide comprises of nucleosides that have either a MOE sugar modification, an (S)-cEt sugar modification, or a deoxy modification; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In certain embodiments, a compound comprises a single-stranded modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 20 linked nucleosides and has a nucleobase sequence comprising the sequence recited in SEQ ID NOs: 918, 479 or 703, 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 a single-stranded modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 16 linked nucleosides and has a nucleobase sequence comprising the sequence recited in SEQ ID NOs: 1800, 1904, 2122, 2127, or 2194, wherein the modified oligonucleotide comprises of nucleosides that have either a MOE sugar modification, an (S)-cEt sugar modification, or a deoxy modification; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

In certain embodiments, a compound comprises an ISIS oligonucleotide targeting GHR and a conjugate group. For instance, in certain embodiments, a compound comprises ISIS 532401and a conjugate group.

In any of the foregoing embodiments, the compound or oligonucleotide can be at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% complementary to a nucleic acid encoding growth hormone receptor.

In any of the foregoing embodiments, the nucleic acid encoding growth hormone receptor can comprise the nucleotide sequence of any one of SEQ ID NOs: 1-19.

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

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

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

In certain embodiments, at least one modified internucleoside linkage of the modified oligonucleotide is 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, at least one nucleoside of the modified oligonucleotide comprises a modified nucleobase.

In certain embodiments, the modified nucleobase is a 5-methylcytosine.

In certain embodiments, the modified oligonucleotide comprises at least one modified sugar.

In certain embodiments, the modified sugar is a 2' modified sugar, a BNA, or a THP.

In certain embodiments, the modified sugar is any of a 2'-O-methoxyethyl, 2'-O-methyl, a constrained ethyl, a LNA, or a 3'-fluoro-HNA.

In certain embodiments, the compound comprises at least one 2'-O-methoxyethyl nucleoside, 2'-O-methyl nucleoside, constrained ethyl nucleoside, LNA nucleoside, or 3'-fluoro-HNA nucleoside.

In certain embodiments, the modified oligonucleotide comprises:

a gap segment consisting of 10 linked deoxynucleosides;

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

a 3' wing segment consisting of 5 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 modified oligonucleotide consists of 20 linked nucleosides.

In certain embodiments, the modified oligonucleotide consists of 19 linked nucleosides.

In certain embodiments, the modified oligonucleotide consists of 18 linked nucleosides.

Certain embodiments provide compounds consisting of a conjugate group and a modified oligonucleotide according to the following formula: mCes mCes Aes mCes mCes Tds Tds Tds Gds Gds Gds Tds Gds Ads Ads Tes Aes Ges mCes Ae; wherein,

A = an adenine,

mC = a 5'-methylcytosine

G = a guanine,

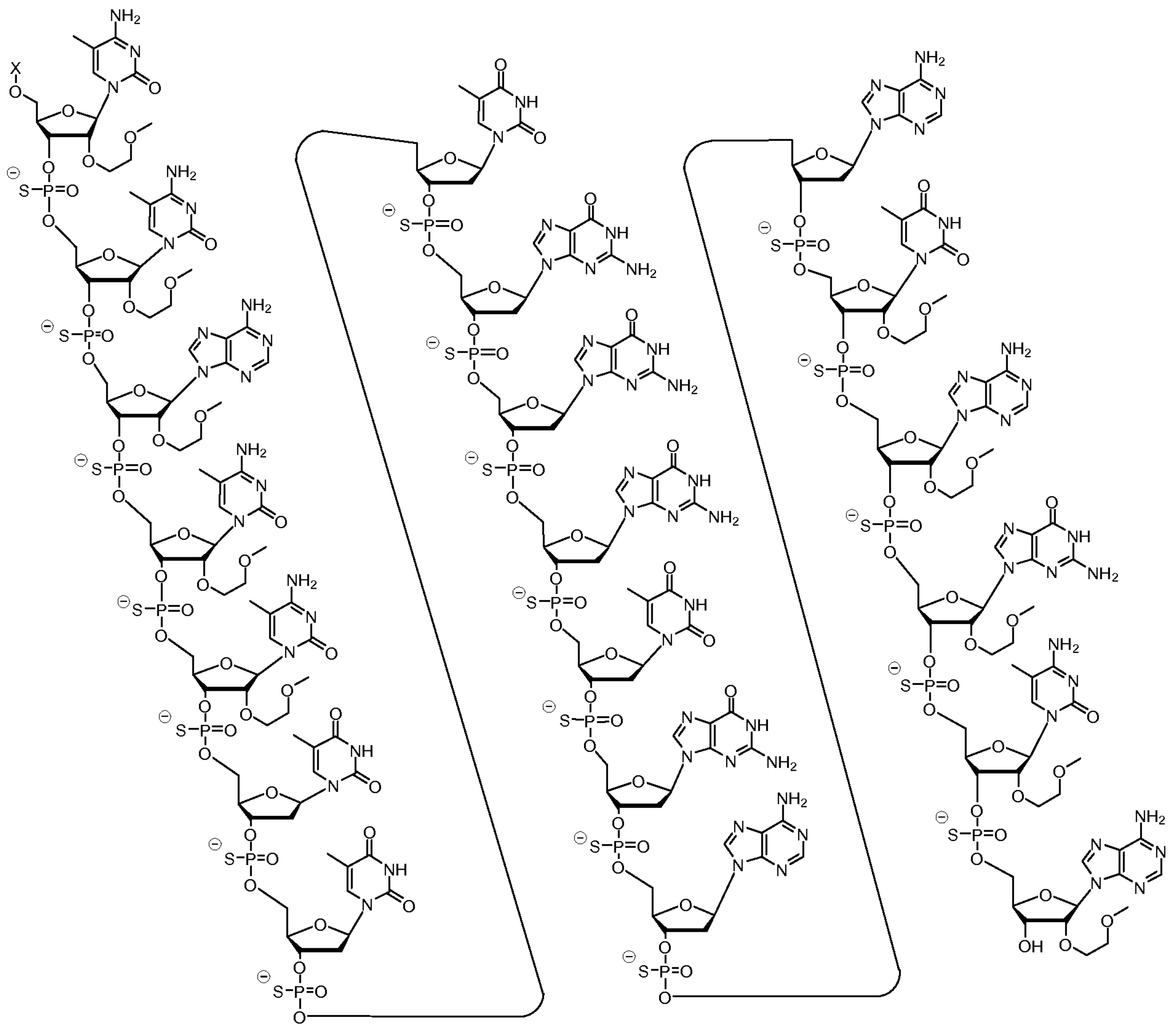
T = a thymine,

e = a 2'-O-methoxyethyl modified nucleoside,

d = a 2'-deoxynucleoside, and

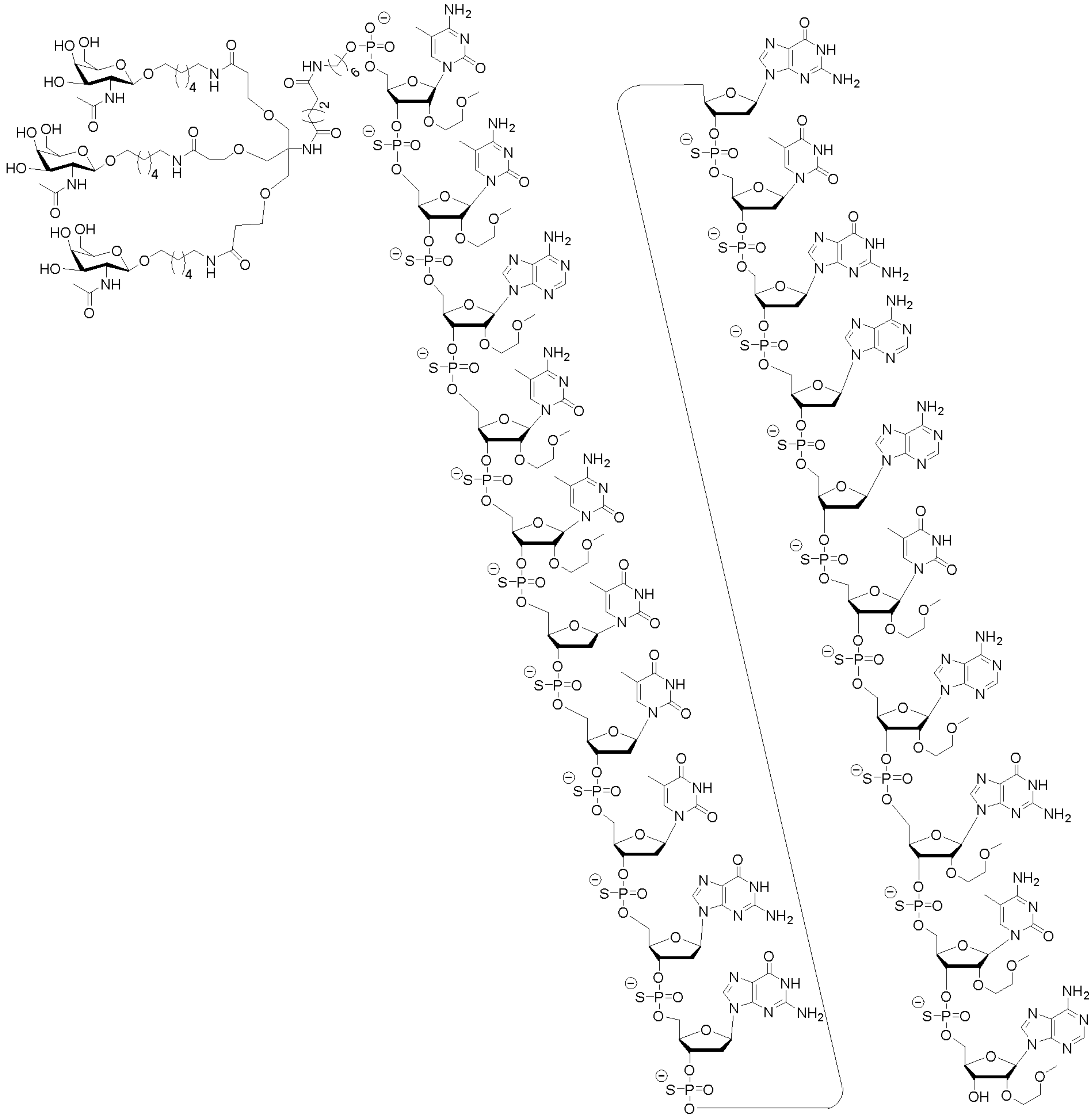
s = a phosphorothioate internucleoside linkage.

In certain embodiments, a compound comprises an ISIS oligonucleotide targeting GHR conjugated to GalNAc on the 5' end. For instance, in certain embodiments, a compound comprises ISIS 532401 conjugated to GalNAc on the 5' end. . In further embodiments, the compound has the following chemical structure comprising or consisting of ISIS 532401 with 5'-X, wherein X is a conjugate group comprising GalNAc as described herein:

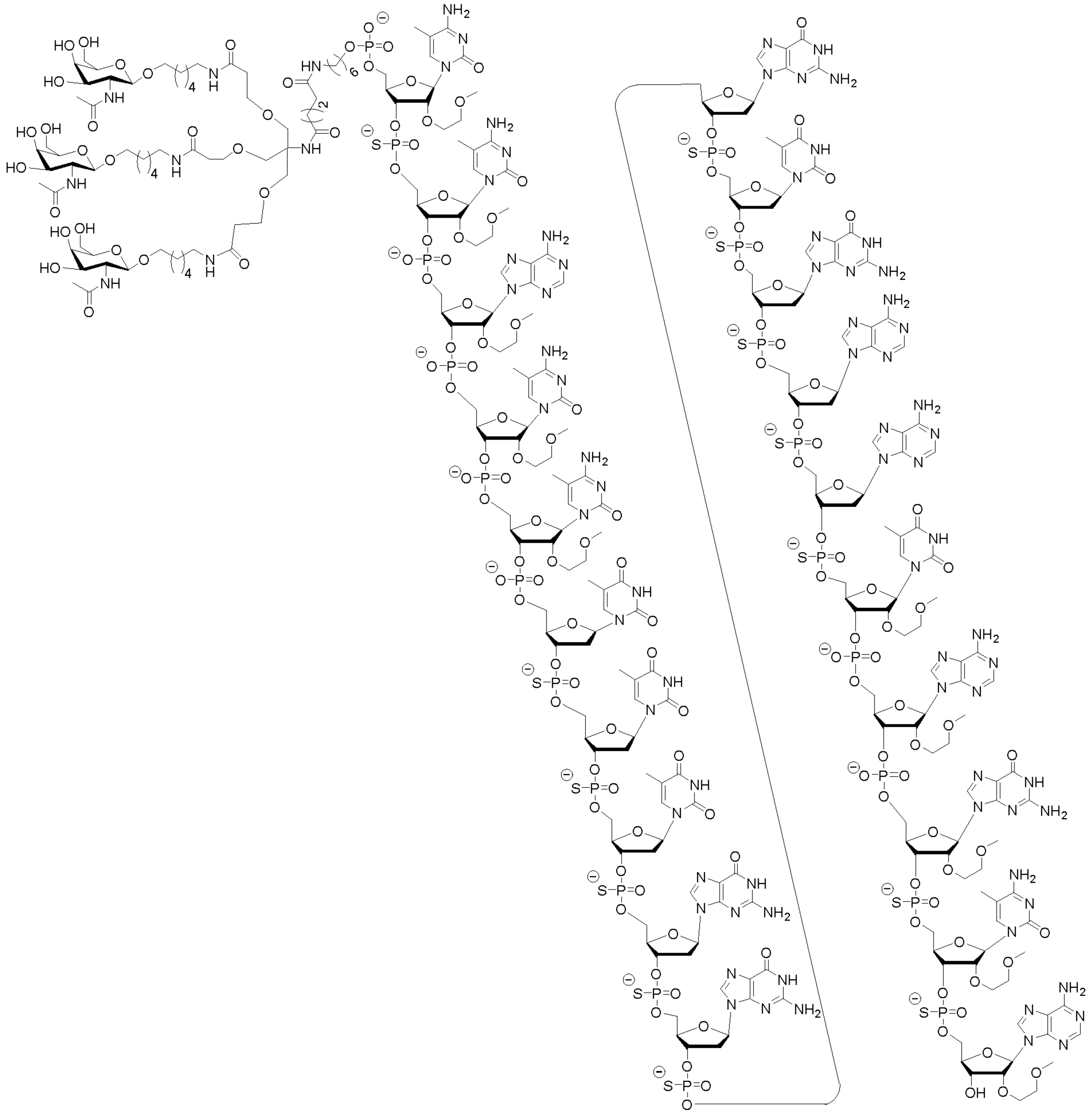


wherein X is a conjugate group comprising GalNAc.

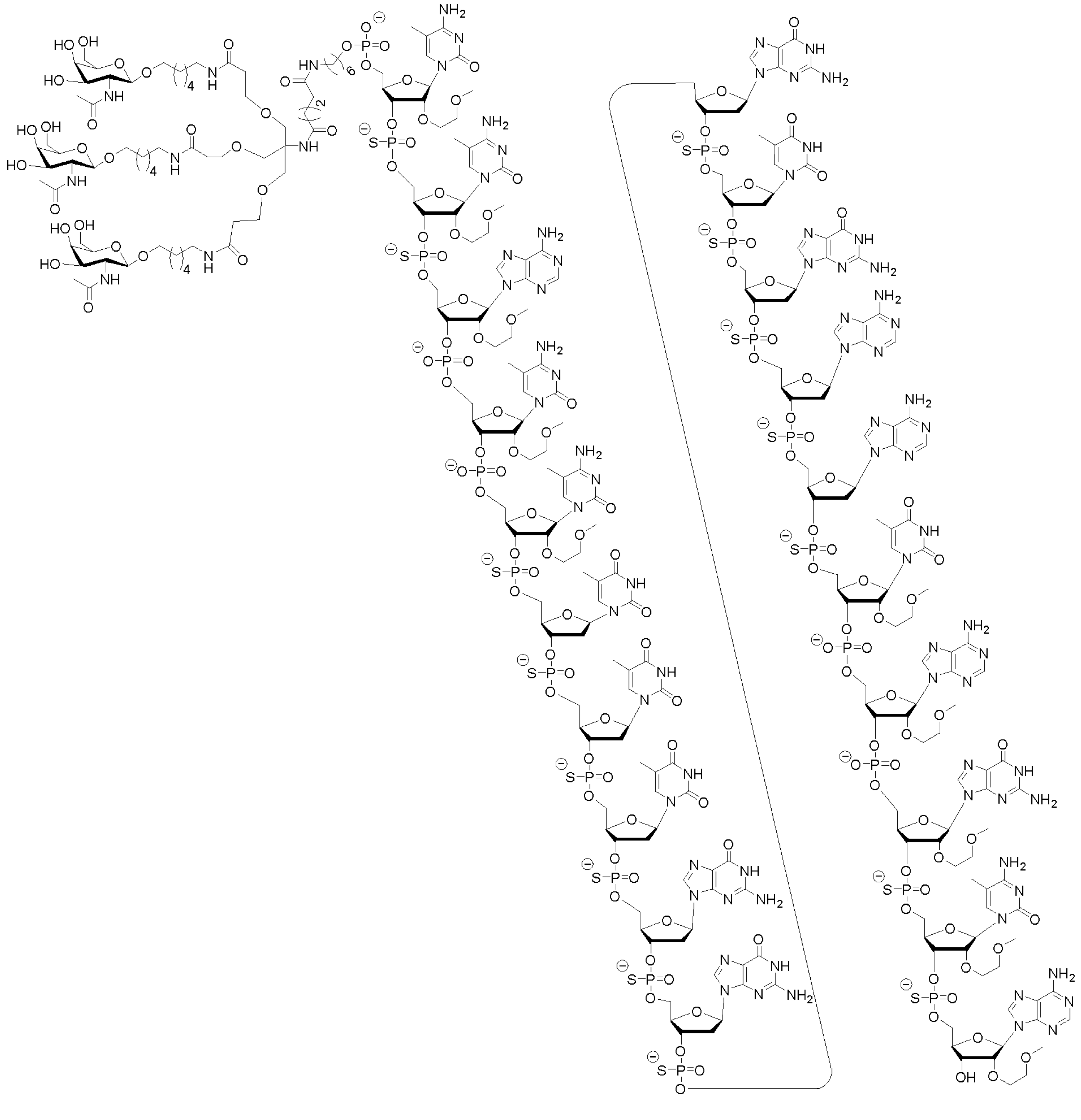
In certain embodiments, a compound comprises an ISIS oligonucleotide targeting GHR conjugated to GalNAc, and wherein each internucleoside linkage of the oligonucleotide is a phosphorothioate linkage. In further embodiments, a compound having the following chemical structure comprises or consists of ISIS 719223 with a 5'-X, wherein X is a conjugate group comprising GalNAc as described herein:



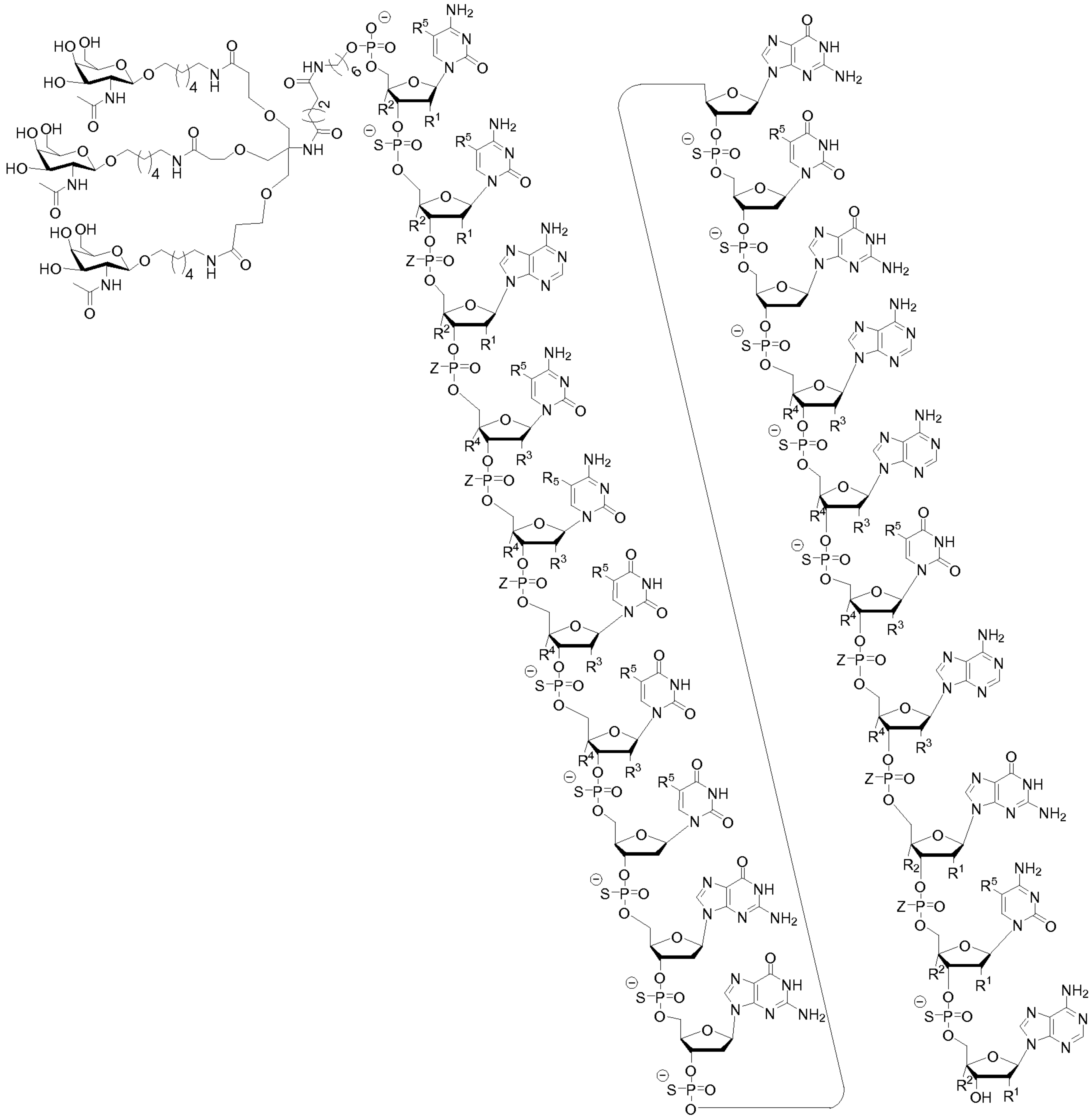
In certain embodiments, a compound comprises an ISIS oligonucleotide targeting GHR conjugated to GalNAc, and wherein each internucleoside linkage of the oligonucleotide is a phosphorothioate linkage or a phosphodiester linkage. In further embodiments, a compound having the following chemical structure comprises or consists of ISIS 719224 with a 5'-X, wherein X is a conjugate group comprising GalNAc as described herein:



In certain embodiments, a compound comprises an ISIS oligonucleotide targeting GHR conjugated to GalNAc, and wherein each internucleoside linkage of the oligonucleotide is a phosphorothioate linkage or a phosphodiester linkage. In further embodiments, a compound having the following chemical structure comprises or consists of ISIS 766720 with a 5'-X, wherein X is a conjugate group comprising GalNAc as described herein:

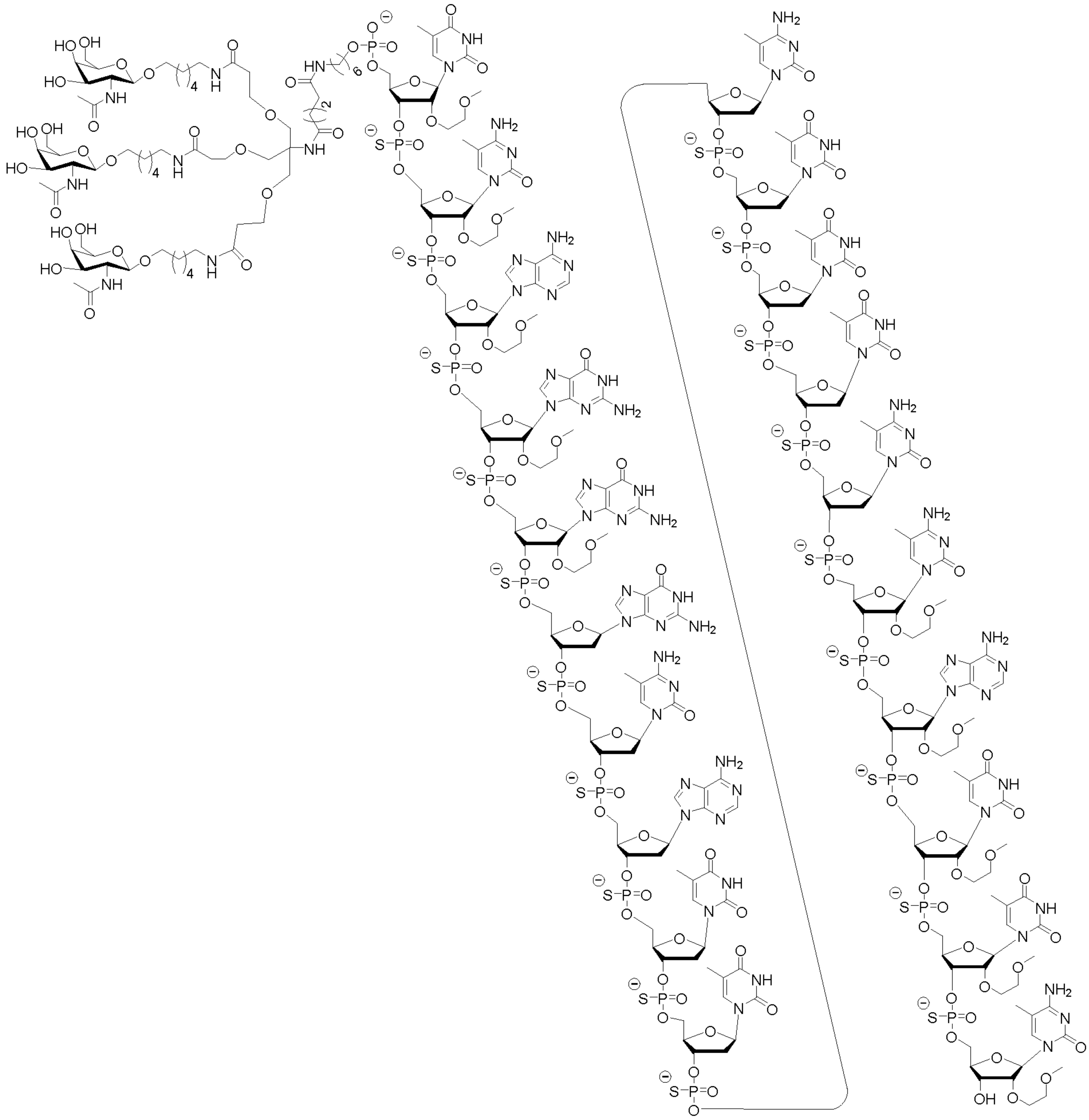


In certain embodiments, a compound comprises an ISIS oligonucleotide targeting GHR conjugated to GalNAc. In further such embodiments, the compound comprises the sequence of ISIS 532401 conjugated to GalNAc, and is represented by the following chemical structure:

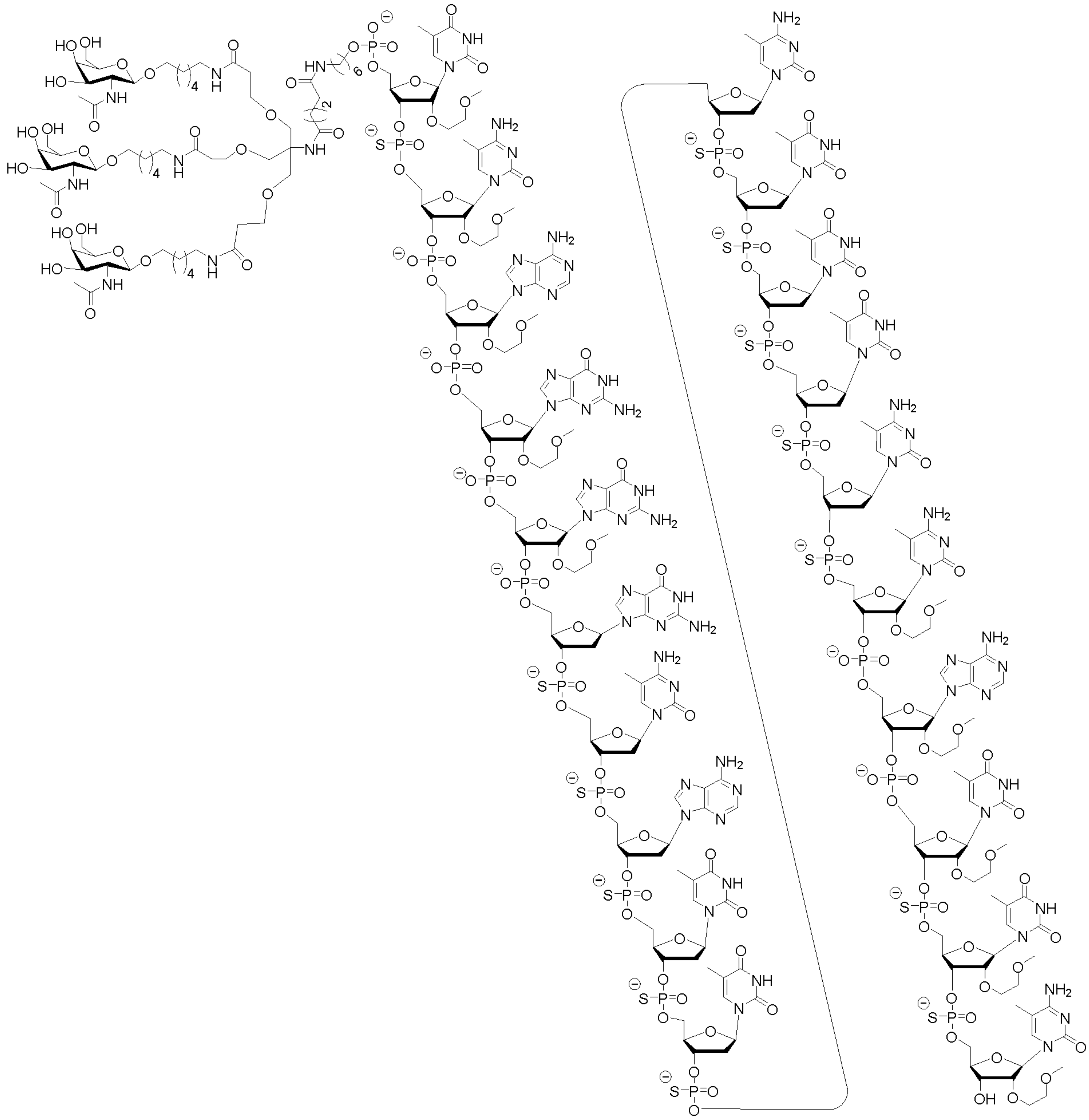


wherein either R^1 is $-OCH_2CH_2OCH_3$ (MOE)and R^2 is H; or R^1 and R^2 together form a bridge, wherein R^1 is $-O-$ and R^2 is $-CH_2-$, $-CH(CH_3)-$, or $-CH_2CH_2-$, and R^1 and R^2 are directly connected such that the resulting bridge is selected from: $-O-CH_2-$, $-O-CH(CH_3)-$, and $-O-CH_2CH_2-$; 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 an antisense oligonucleotide having a nucleobase sequence of any of SEQ ID NOs disclosed in WO 2004/078922 and a conjugate group described herein. The nucleobase sequences of all of the aforementioned referenced SEQ ID NOs are incorporated by reference herein. For example, a compound comprises an oligonucleotide disclosed in WO 2004/078922 conjugated to GalNAc, and wherein each internucleoside linkage of the oligonucleotide is a phosphorothioate linkage and has the following chemical structure:



For example, a compound comprises an oligonucleotide disclosed in WO 2004/078922 conjugated to GalNAc, and wherein each internucleoside linkage of the oligonucleotide compound is a phosphorothioate linkage or a phosphodiester linkage, and has the following chemical structure:



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.

Certain embodiments provide a method of treating a disease associated with excess growth hormone in a human comprising administering to the human a therapeutically effective amount of the compound or composition of any of the aforementioned embodiments, thereby treating the disease associated with excess growth hormone. In certain aspects, the disease associated with excess growth hormone is acromegaly. In certain aspects, the treatment reduces IGF-1 levels.

Certain embodiments provide a method of preventing a disease associated with excess growth hormone in a human comprising administering to the human a therapeutically effective amount of a compound or composition of any of the aforementioned embodiments, thereby preventing the disease associated with excess growth hormone. In certain embodiments, the disease associated with excess growth hormone is acromegaly.

Certain embodiments provide a method of reducing growth hormone receptor (GHR) levels in a human comprising administering to the human a therapeutically effective amount of the compound or composition of any of the aforementioned embodiments, thereby reducing GHR levels in the human. In certain aspects, the human has a disease associated with excess growth hormone. In certain aspects, the disease associated with excess growth hormone is acromegaly.

In certain aspects, the foregoing methods comprise co-administering the compound or composition and a second agent. In certain aspects, the compound or composition and the second agent are administered concomitantly.

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

In certain embodiments, an antisense compound 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 a GHR 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 GHR 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.

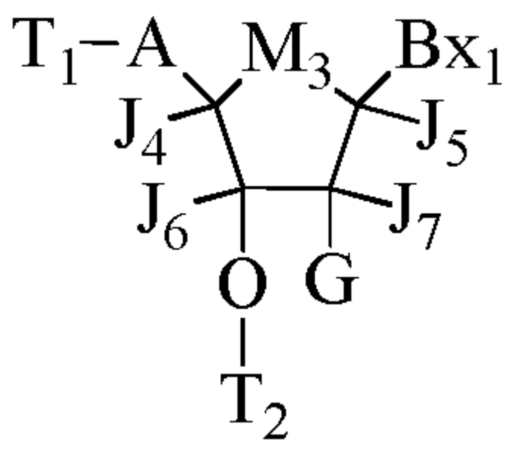
RNAi Compounds

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:



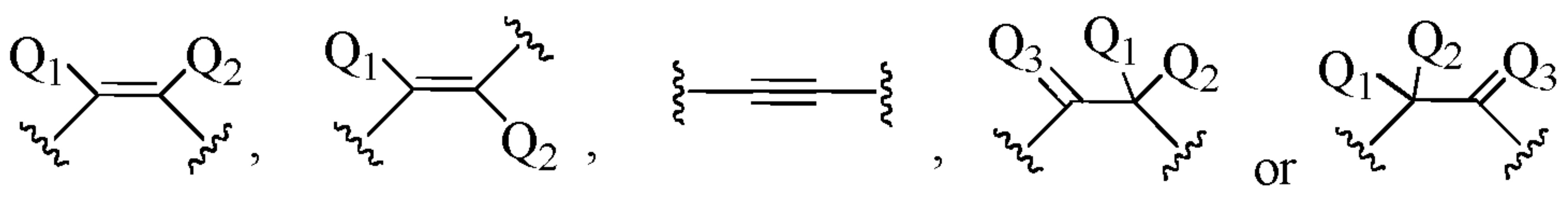
IIc

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;

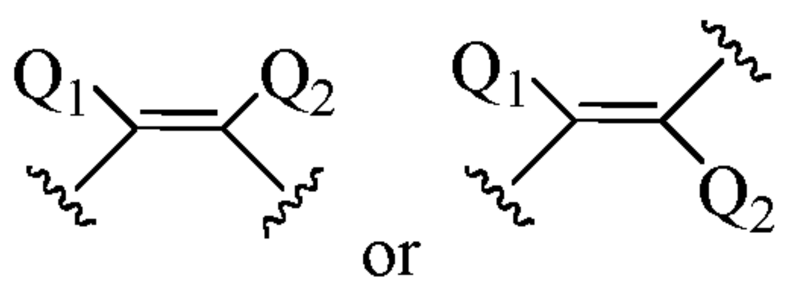
when j is 1 then Z is other than halogen or $N(E_2)(E_3)$; and

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.

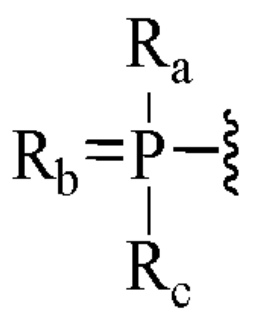
In certain embodiments, A has one of the formulas:



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:



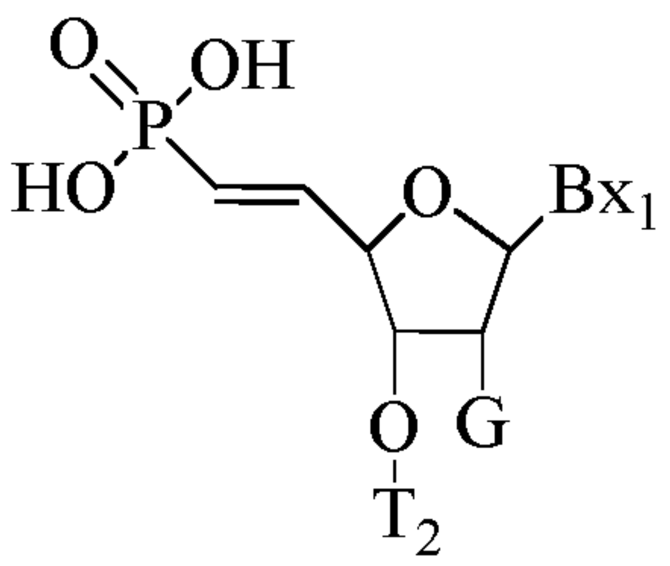
wherein:

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 GHR. In certain embodiment, the degradation of the targeted GHR 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 GHR 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, published on April 29, 2004 as WO 2004/035765, 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 growth hormone receptor (GHR) targetable with the compounds provided herein include, without limitation, the following: GENBANK Accession No. NM_000163.4 (incorporated herein as SEQ ID NO: 1), GENBANK Accession No. NT_006576.16 truncated from nucleotides 42411001 to 42714000 (incorporated herein as SEQ ID NO: 2), GENBANK Accession No X06562.1 (incorporated herein as SEQ ID NO: 3), GENBANK Accession No. DR006395.1 (incorporated herein as SEQ ID NO: 4), GENBANK Accession No. DB052048.1 (incorporated herein as SEQ ID NO: 5), GENBANK Accession No. AF230800.1 (incorporated herein as SEQ ID NO: 6), the complement of GENBANK Accession No. AA398260.1 (incorporated herein as SEQ ID NO: 7), GENBANK Accession No. BC136496.1 (incorporated herein as SEQ ID NO: 8), GENBANK Accession No. NM_001242399.2 (incorporated herein as SEQ ID NO: 9), GENBANK Accession No. NM_001242400.2 (incorporated herein as SEQ ID NO: 10), GENBANK Accession No. NM_001242401.3 (incorporated herein as SEQ ID NO: 11), GENBANK Accession No. NM_001242402.2 (incorporated herein as SEQ ID NO: 12), GENBANK Accession No. NM_001242403.2 (incorporated herein as SEQ ID NO: 13), GENBANK Accession No.

NM_001242404.2 (incorporated herein as SEQ ID NO: 14), GENBANK Accession No. NM_001242405.2 (incorporated herein as SEQ ID NO: 15), GENBANK Accession No. NM_001242406.2 (incorporated herein as SEQ ID NO: 16), GENBANK Accession No. NM_001242460.1 (incorporated herein as SEQ ID NO: 17), GENBANK Accession NM_001242461.1 (incorporated herein as SEQ ID NO: 18), GENBANK Accession No. NM_001242462.1 (incorporated herein as SEQ ID NO: 19), or GENBANK Accession No NW_001120958.1 truncated from nucleotides 4410000 to 4720000 (incorporated herein as SEQ ID NO: 2332).

Hybridization

In some embodiments, hybridization occurs between an antisense compound disclosed herein and a GHR 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 GHR 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 GHR nucleic acid).

Non-complementary nucleobases between an antisense compound and a GHR 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 GHR 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 GHR 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 GHR 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 GHR 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 GHR 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 GHR 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; 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 1999/014226; WO 2004/106356; WO 2005/021570; WO 2007/134181; WO 2008/150729; WO 2008/154401; WO 2009/006478; WO 2010/036698; WO 2011/017521; WO 2009/067647; WO 20009/100320. 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;

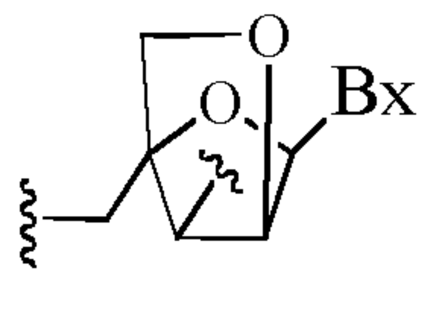
each R_a and R_b is, independently, H, a protecting group, hydroxyl, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, heterocycle radical, substituted heterocycle 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: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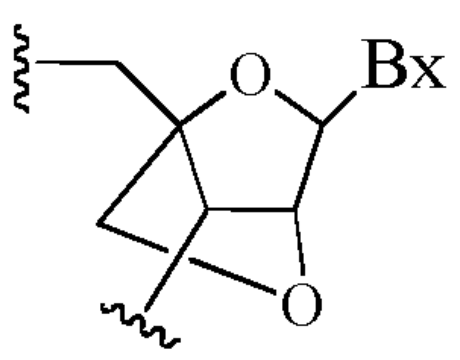
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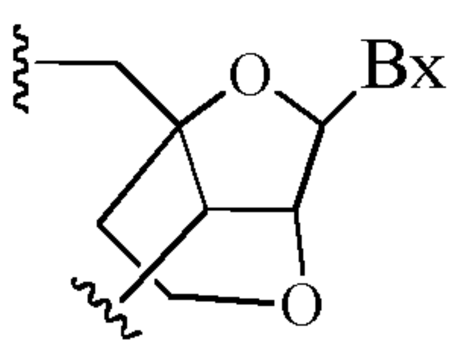
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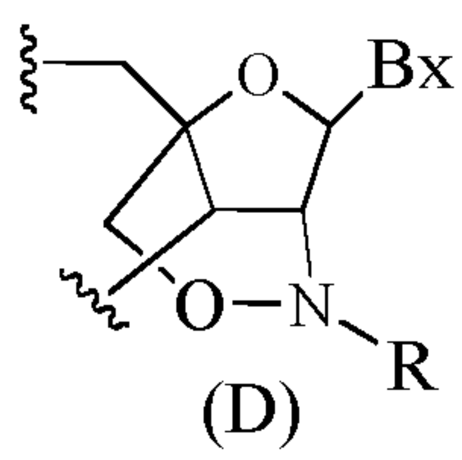
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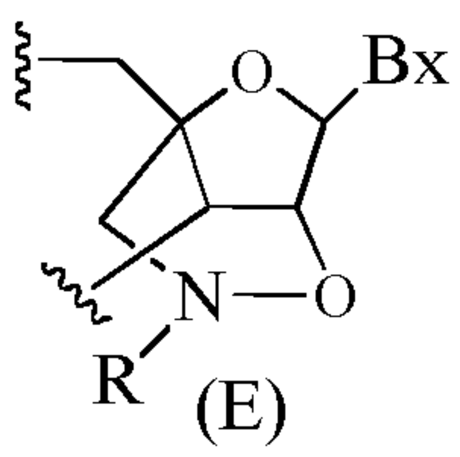
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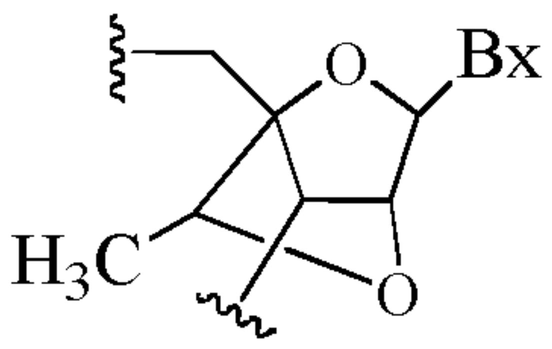
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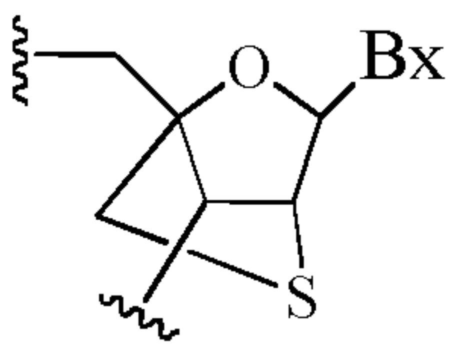
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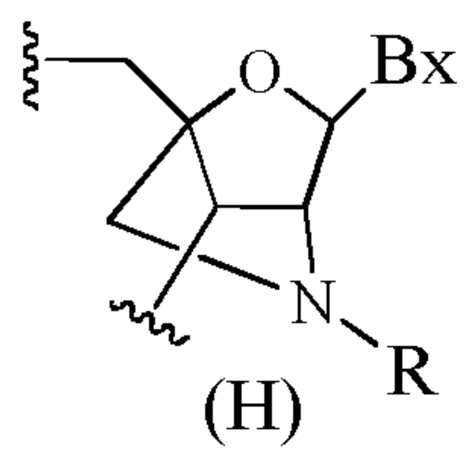
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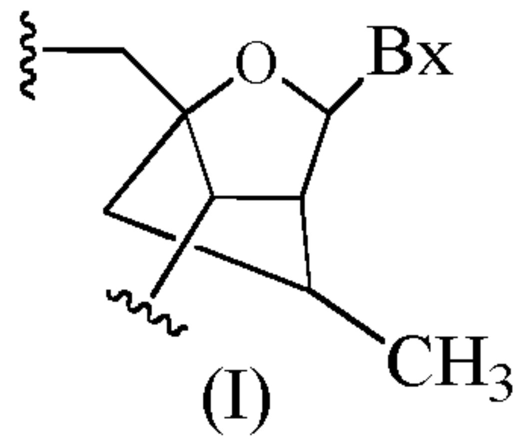
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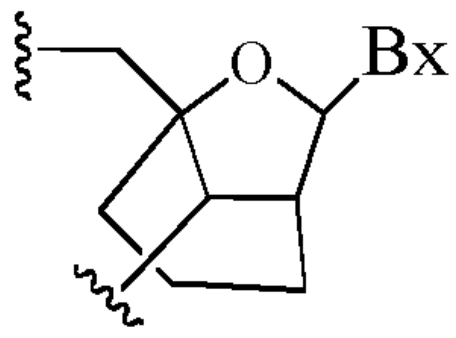
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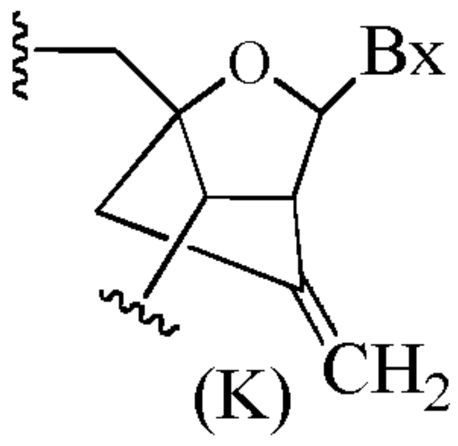
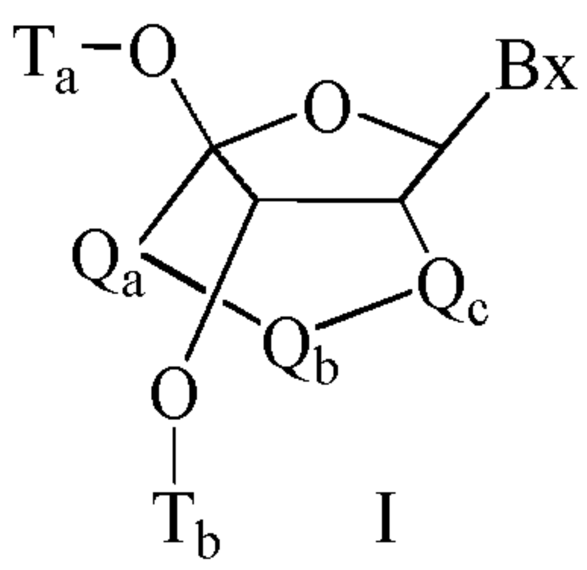

(K)

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:

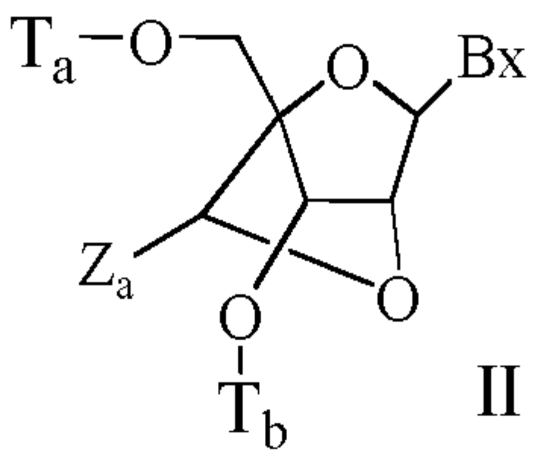
Bx is a heterocyclic base moiety;

-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:

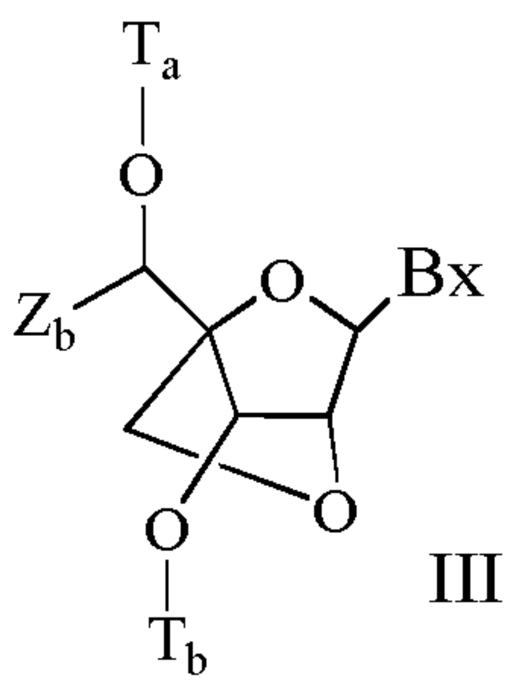
Bx is a heterocyclic base moiety;

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

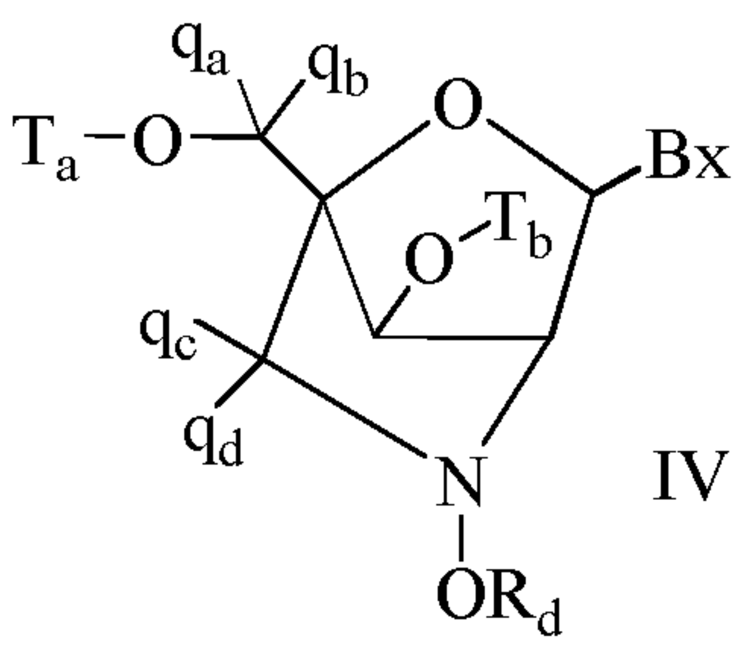
wherein:

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:

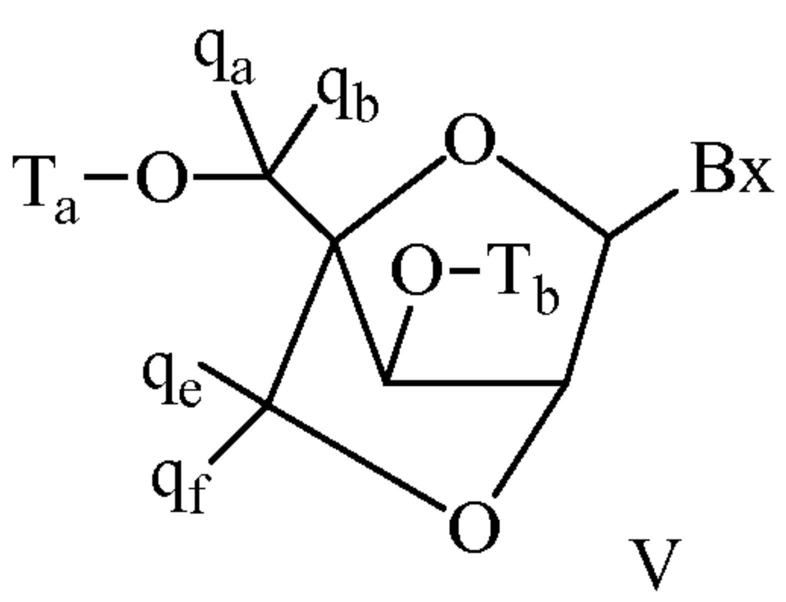
Bx is a heterocyclic base moiety;

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, $C(=O)OJ_j$, $C(=O)NJ_jJ_k$, $C(=O)J_j$, $O\text{-}C(=O)NJ_jJ_k$, $N(H)C(=NH)NJ_jJ_k$, $N(H)C(=O)NJ_jJ_k$ or $N(H)C(=S)NJ_jJ_k$;

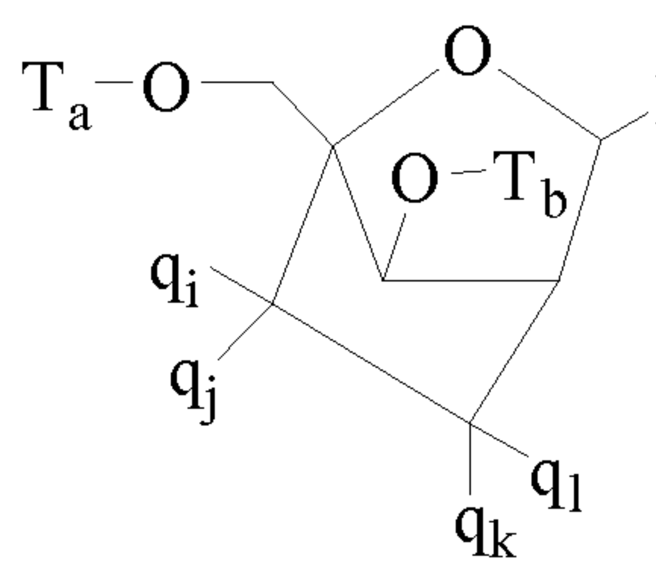
or q_e and q_f together are $=C(q_g)(q_h)$;

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

The synthesis and preparation of the methyleneoxy (4'-CH_2-O-2') BNA monomers adenine, cytosine, guanine, 5-methyl-cytosine, thymine and uracil, along with their oligomerization, and nucleic acid recognition properties have been described (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.

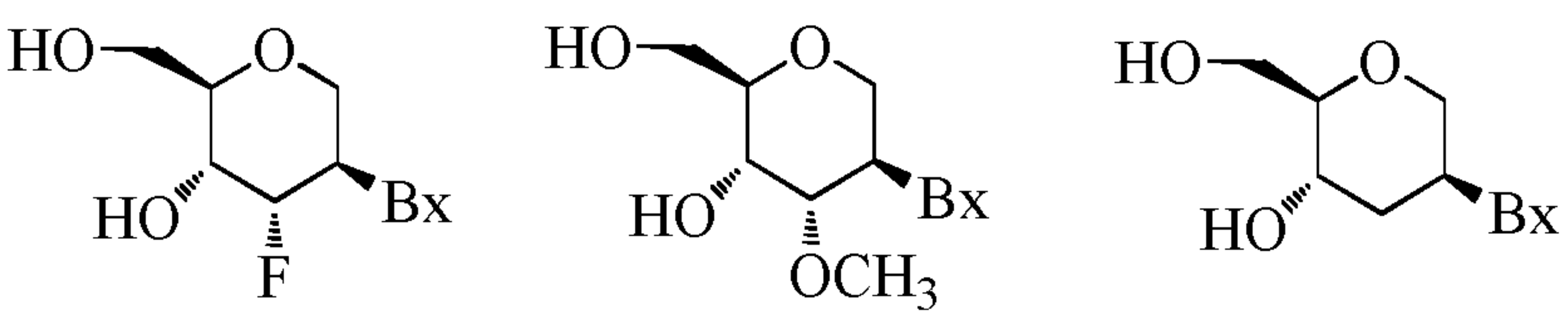
One carbocyclic bicyclic nucleoside having a 4'-$(CH_2)_3$-2' bridge and the alkenyl analog bridge 4'-CH=CH-CH_2-2' have been described (Freier *et al., Nucleic Acids Research*, 1997, *25*(*22*), 4429-4443 and Albaek *et al., J. Org. Chem.*, 2006, *71*, 7731-7740). The synthesis and preparation of carbocyclic 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.

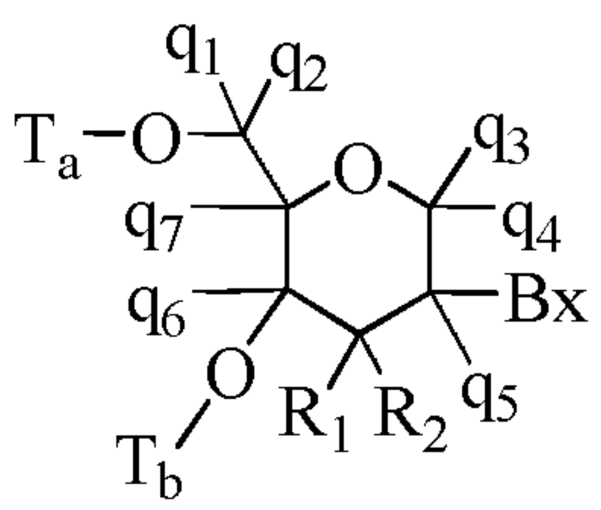
As used herein, "monocylic nucleosides" refer to nucleosides comprising modified sugar moieties that are not bicyclic sugar moieties. In certain embodiments, the sugar moiety, or sugar moiety analogue, of a nucleoside may be modified or substituted at any position.

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:

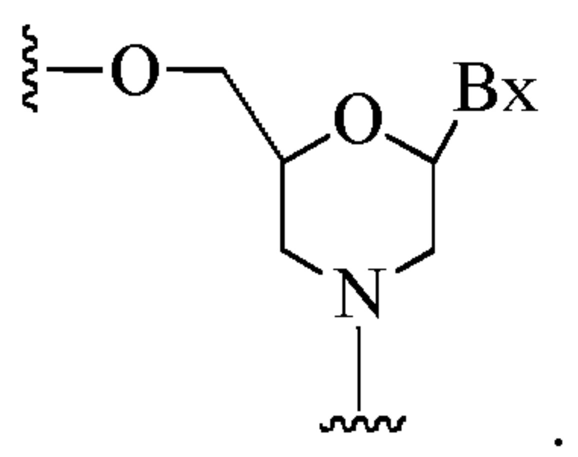
Bx is a heterocyclic base moiety;

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.

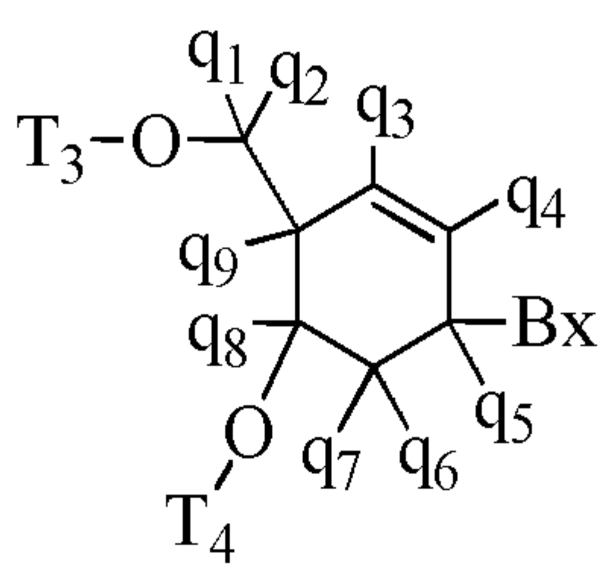
In certain embodiments, sugar surrogates comprise rings having more than 5 atoms and more than one heteroatom. For example nucleosides comprising morpholino sugar moieties and their use in oligomeric compounds has been reported (see for example: Braasch *et al.*, *Biochemistry*, 2002, *41*, 4503-4510; 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 GHR nucleic acid comprise one or more modified nucleobases. In certain embodiments, shortened or gap-widened antisense oligonucleotides targeted to a GHR 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:

$$A - B - C - D \left(E - F \right)_q$$

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;

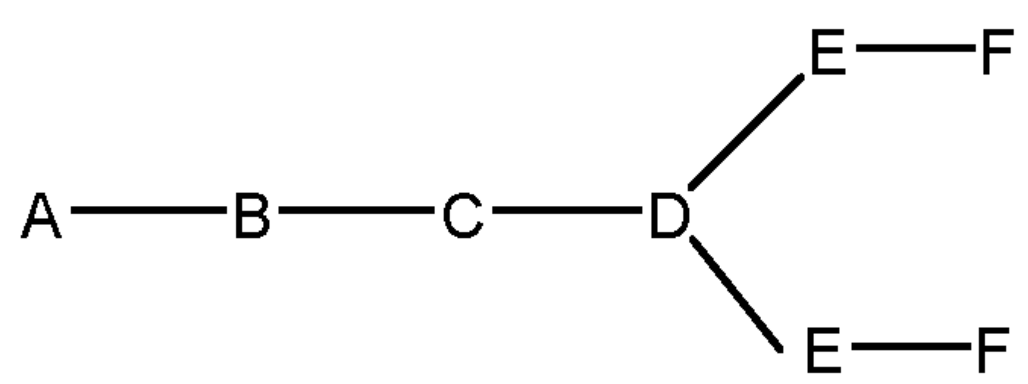
each F is a ligand; and

q is an integer between 1 and 5.

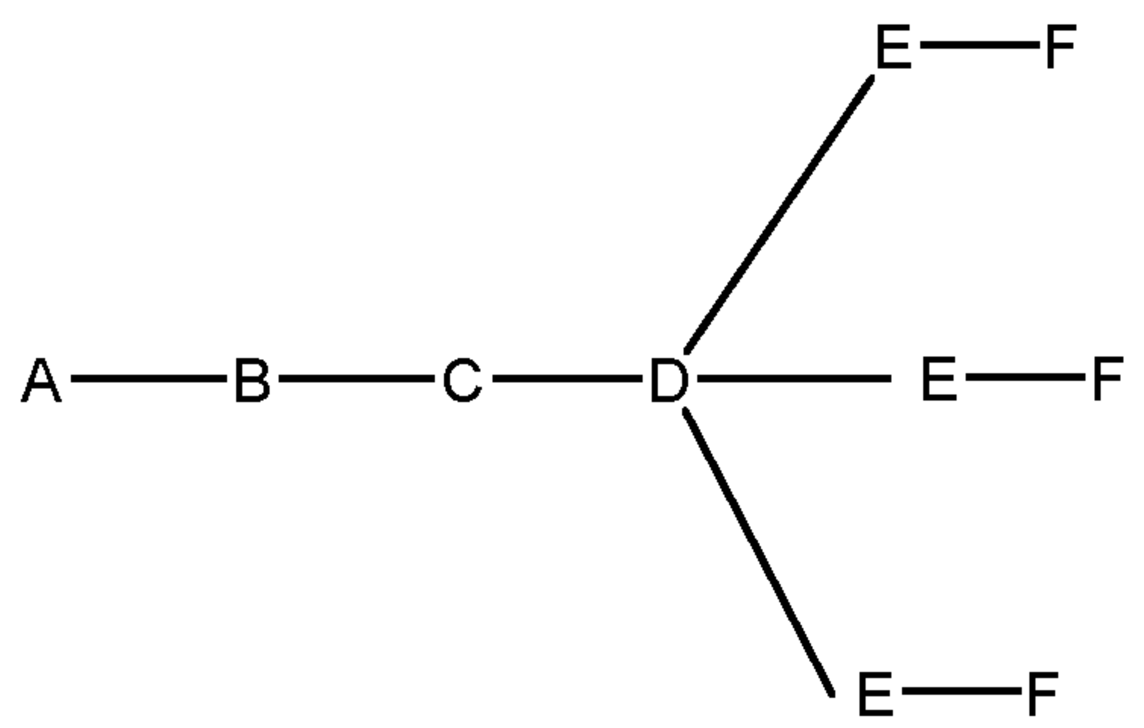
In the above diagram and in similar diagrams herein, the branching group "D" branches as many times as is necessary to accommodate the number of (E-F) groups as indicated by "q". Thus, where q = 1, the formula is:

$$A - B - C - D - E - F$$

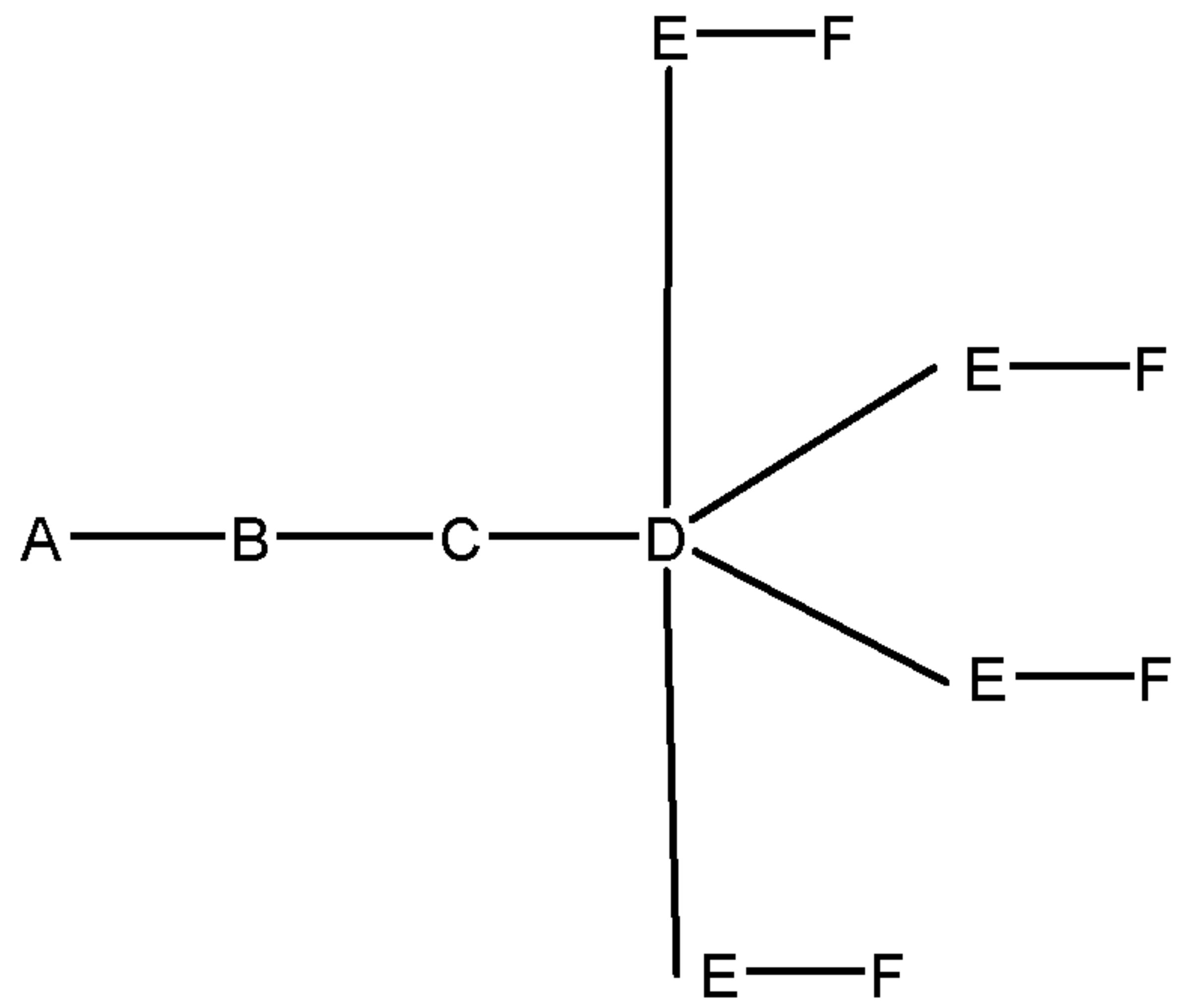
where q = 2, the formula is:



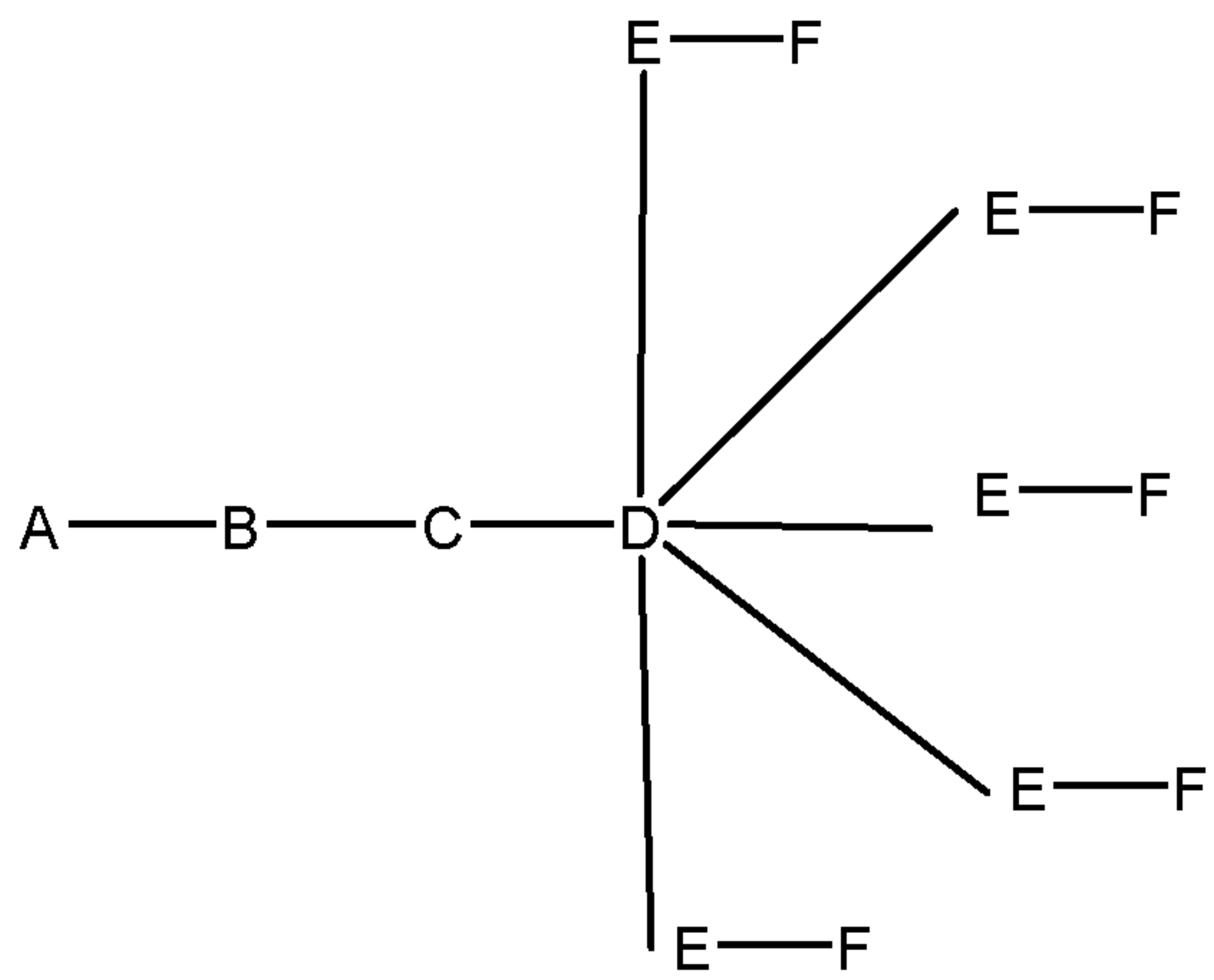
where q = 3, the formula is:



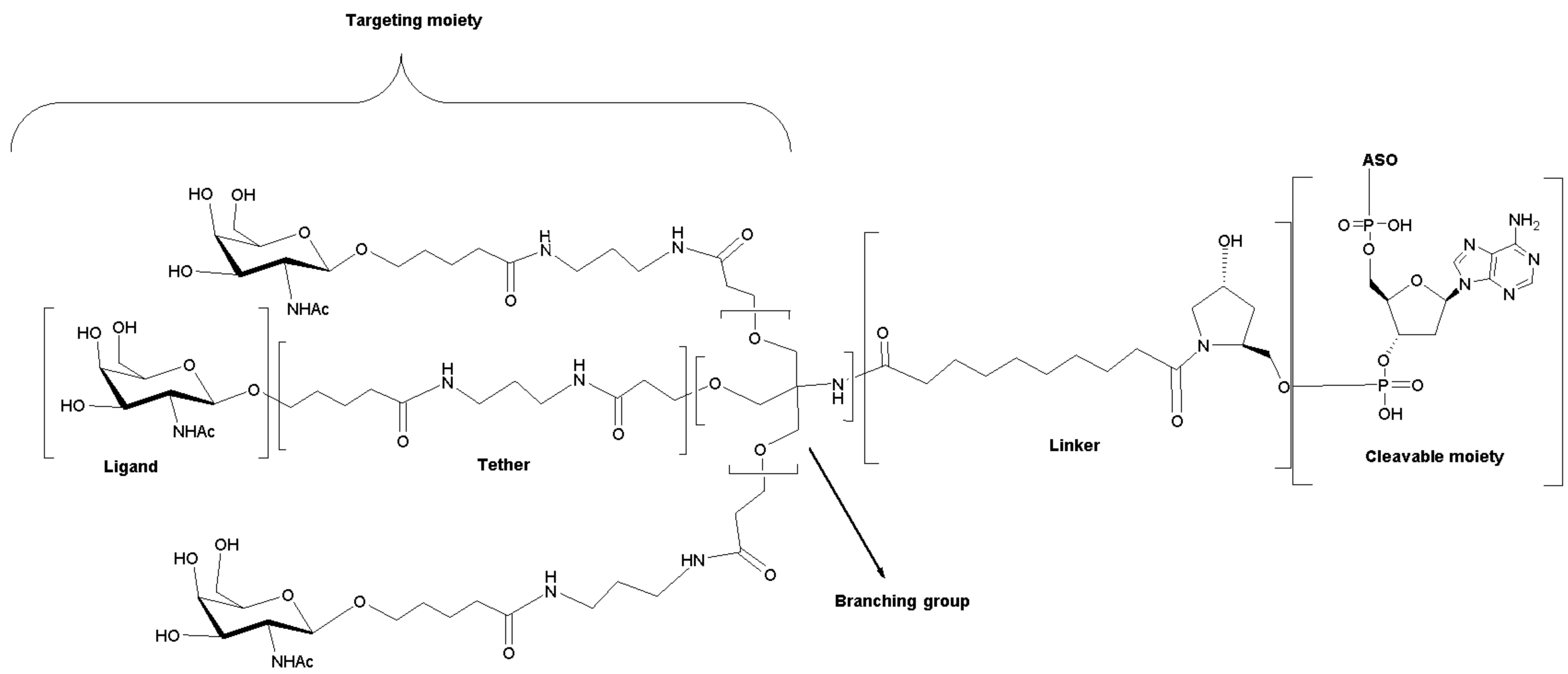
where q = 4, the formula is:



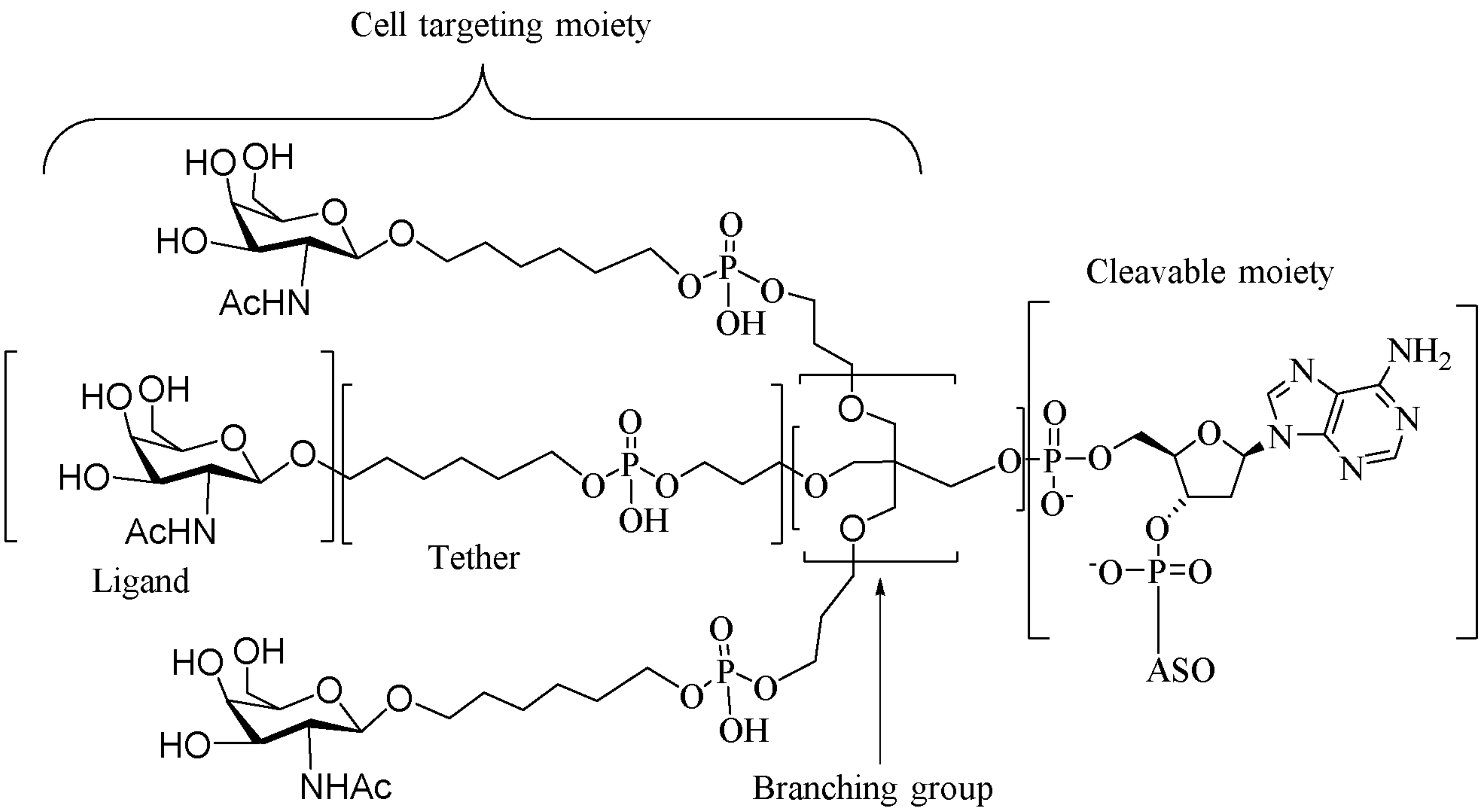
where q = 5, the formula is:



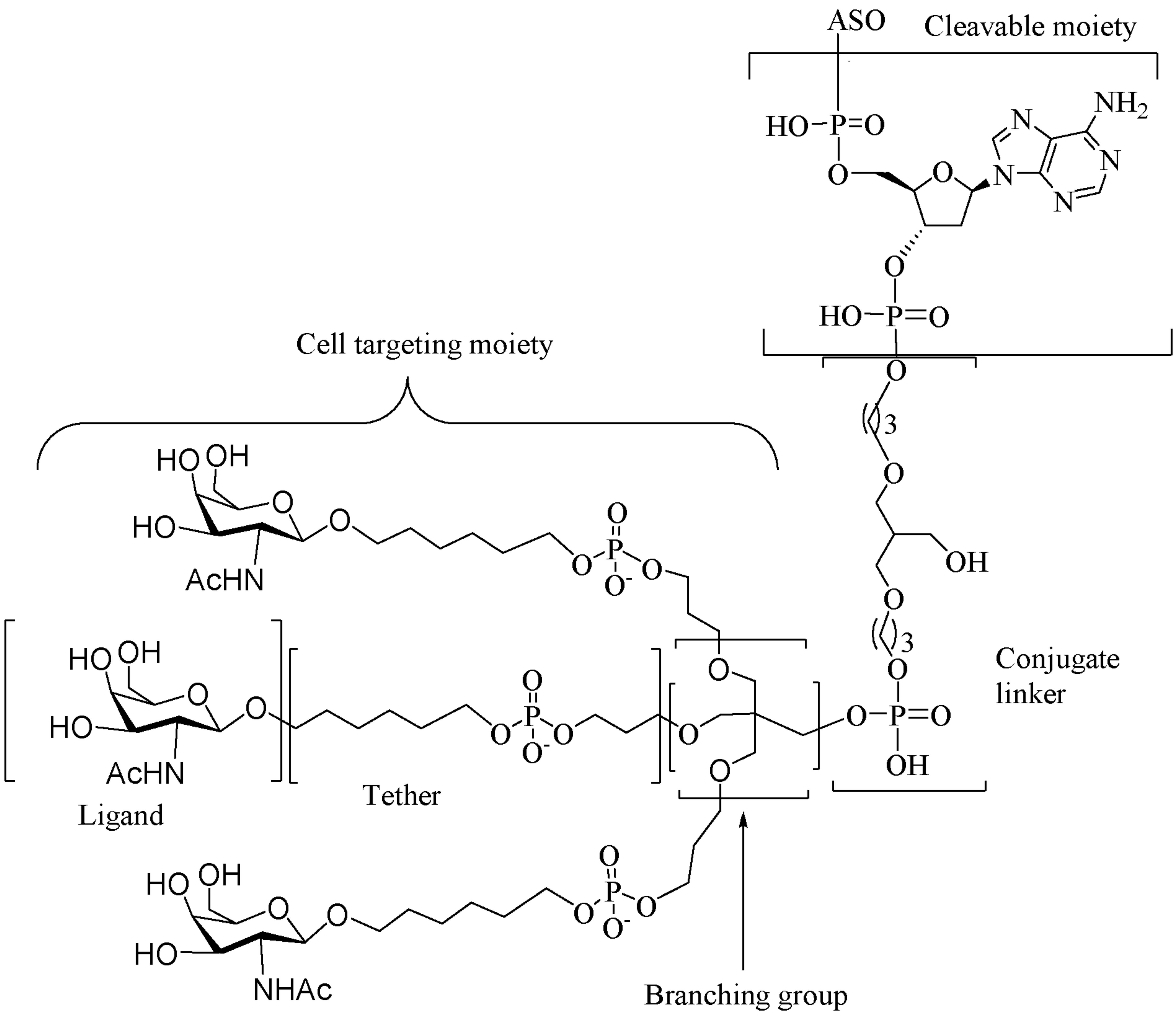
In certain embodiments, conjugated antisense compounds are provided having the structure:



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

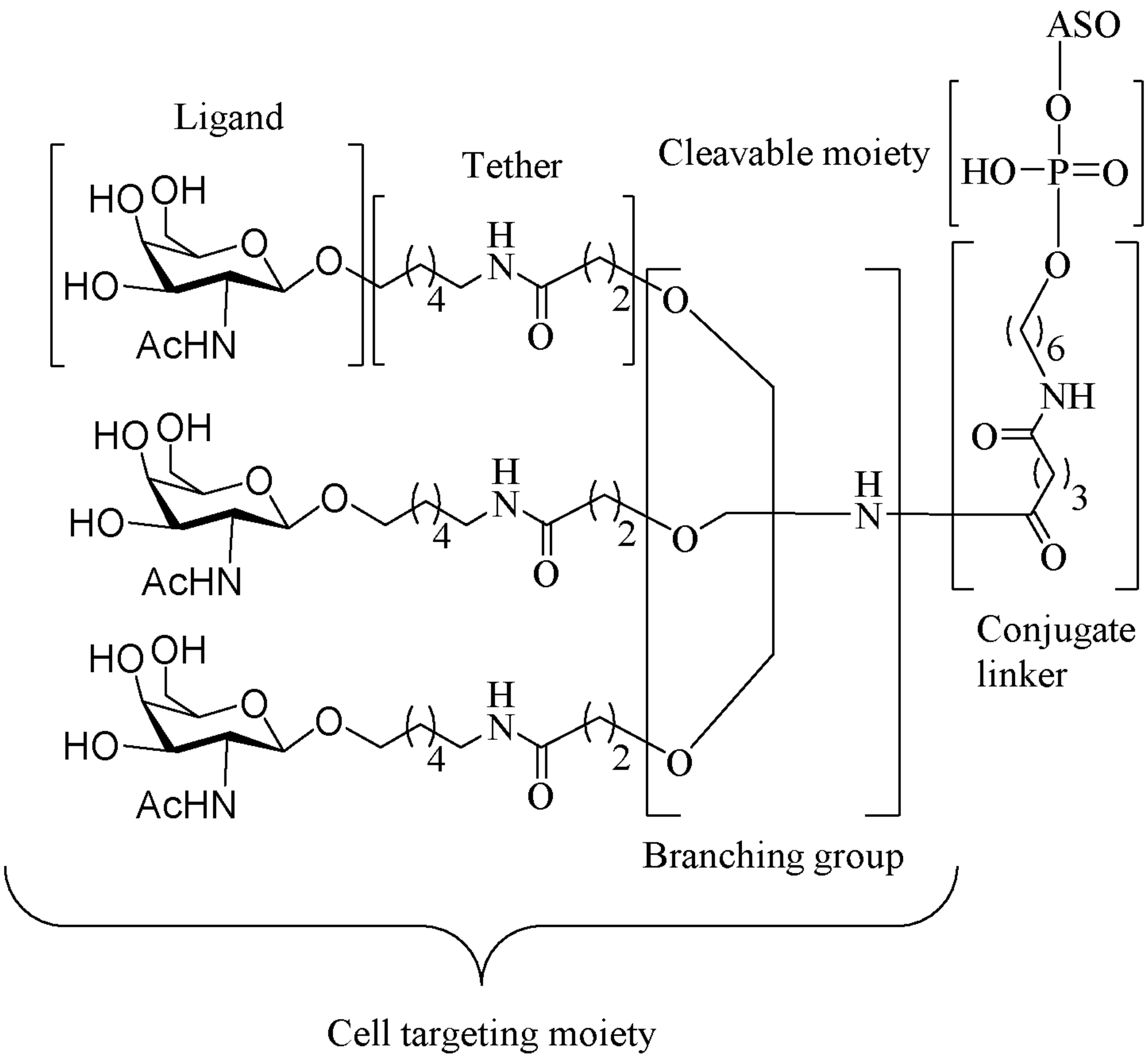


In certain embodiments, conjugated antisense compounds are provided having the structure:



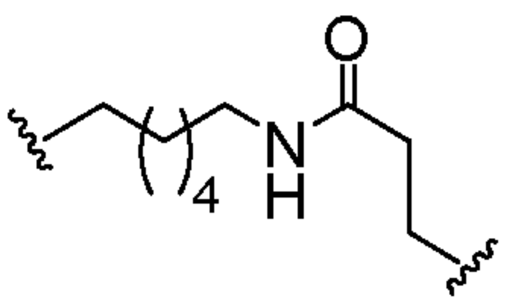
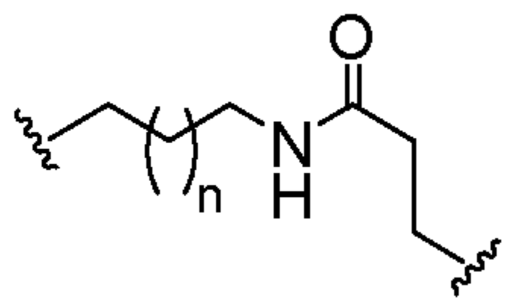
5 .

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

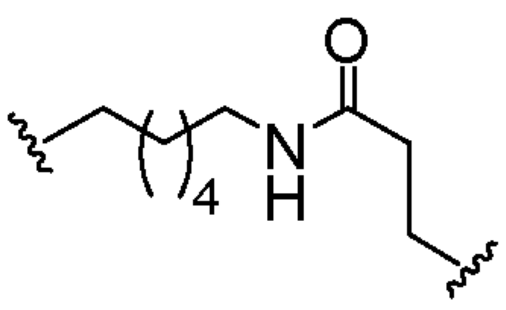


The present disclosure provides the following non-limiting numbered embodiments:

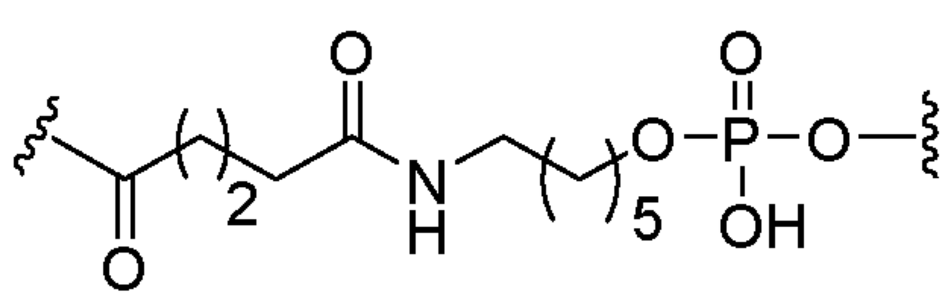
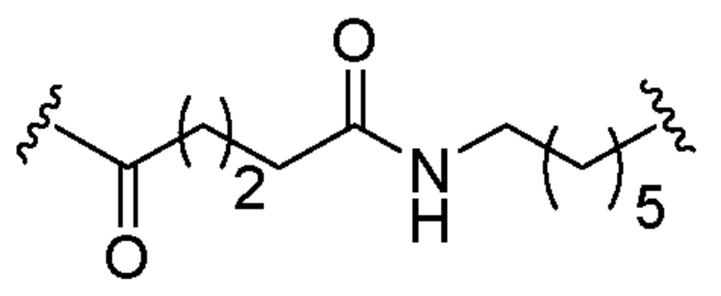
Embodiment 1. The conjugated antisense compound of any of embodiments 1179 to 1182, wherein the tether has a structure selected from among:

, 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:


and

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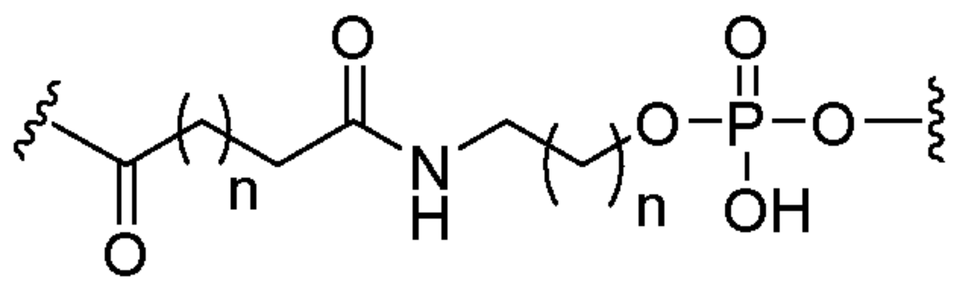
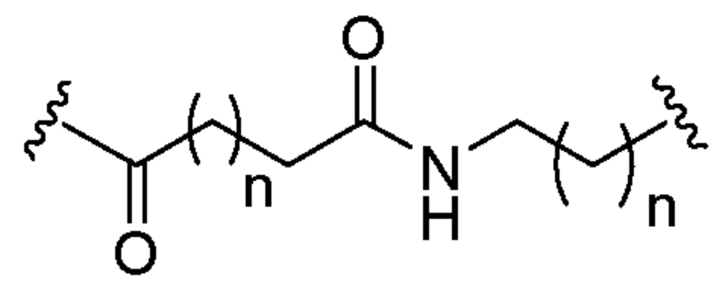
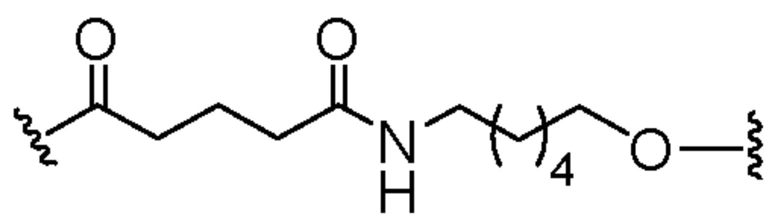
Embodiment 4. The conjugated antisense compound of any of embodiments 1179 to 1182 or 1688 to 1689, wherein the linker has a structure selected from among:


and

;

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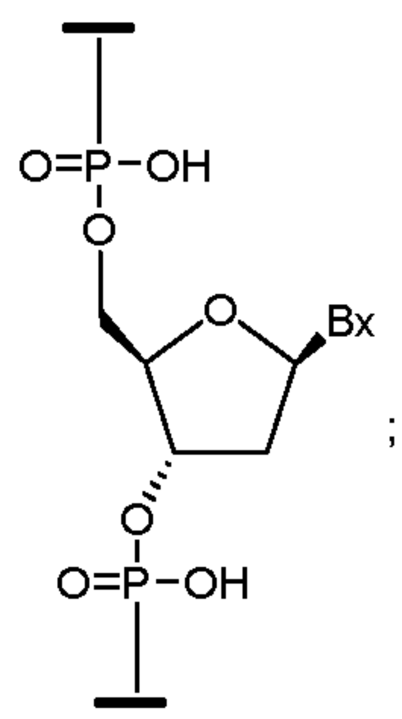
In embodiments having more than one of a particular variable (e.g., more than one “m” or “n”), unless otherwise indicated, each such particular variable is selected independently. Thus, for a structure having more than one n, each n is selected independently, so they may or may not be the same as one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

i. <u>Certain Cleavable Moieties</u>

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: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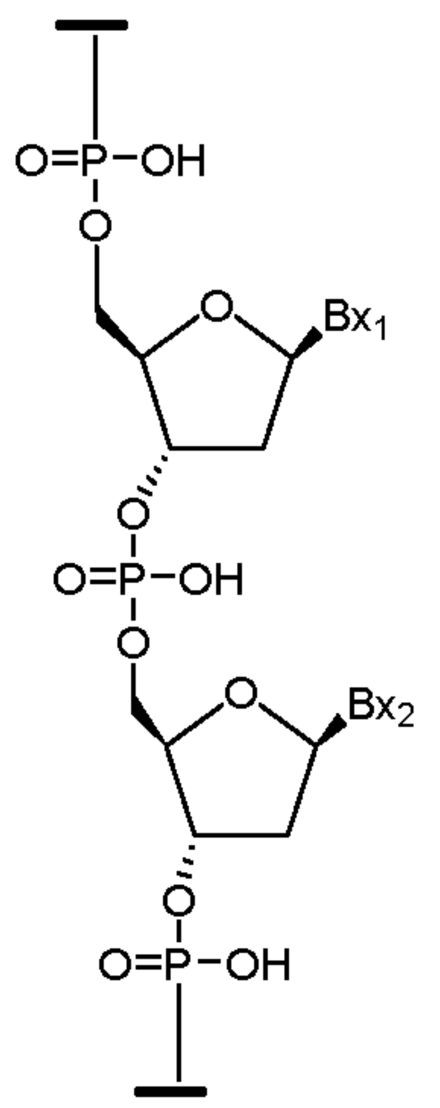
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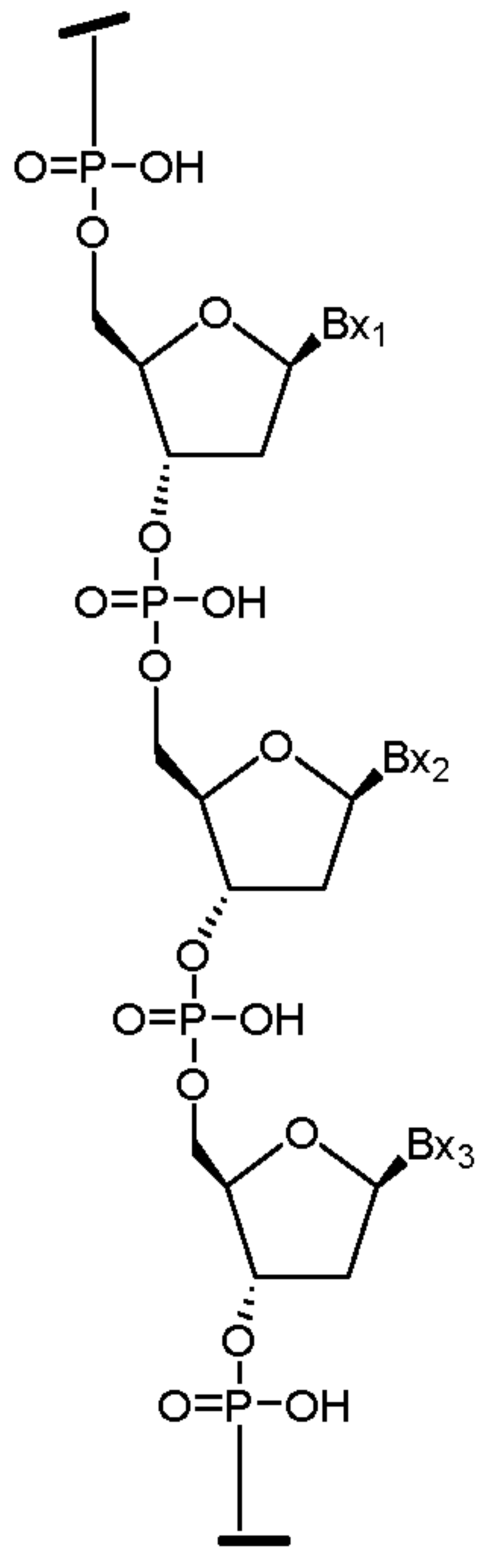
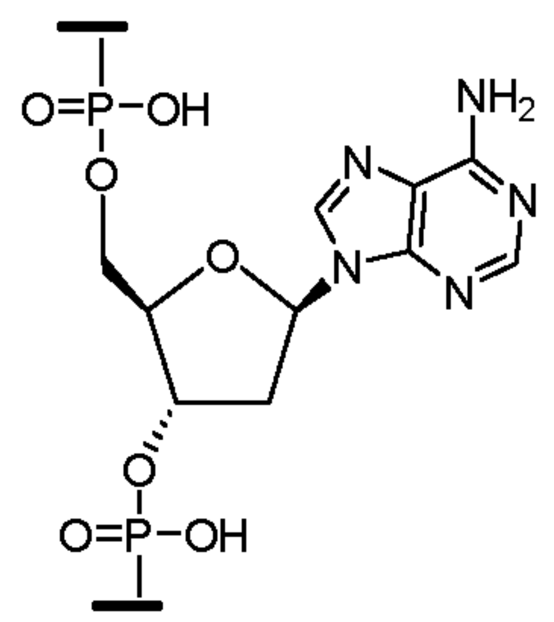


wherein each of Bx, Bx_1, Bx_2, and Bx_3 is independently a heterocyclic base moiety. In certain embodiments, the cleavable moiety has a structure selected from among the following:



ii. Certain Linkers

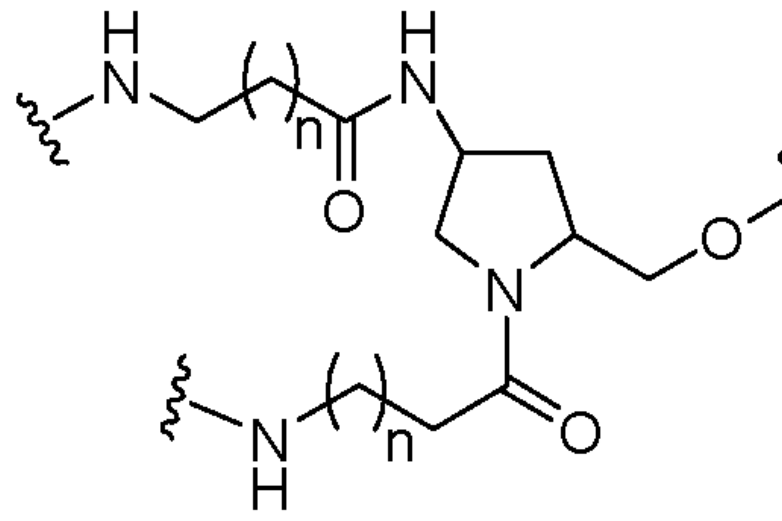
In certain embodiments, the conjugate groups comprise a linker. In certain such embodiments, the linker is covalently bound to the cleavable moiety. In certain such embodiments, the linker is covalently bound to the antisense oligonucleotide. In certain embodiments, the linker is covalently bound to 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: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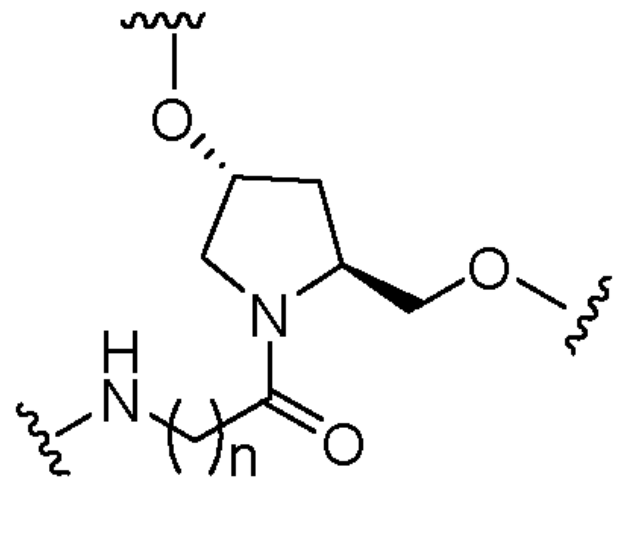
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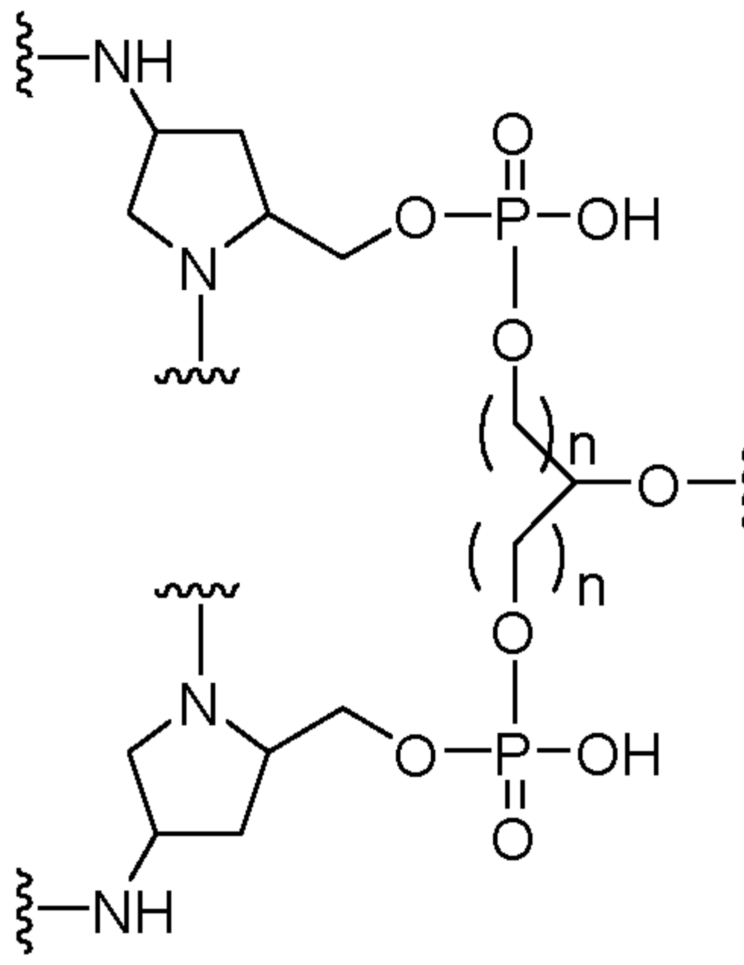
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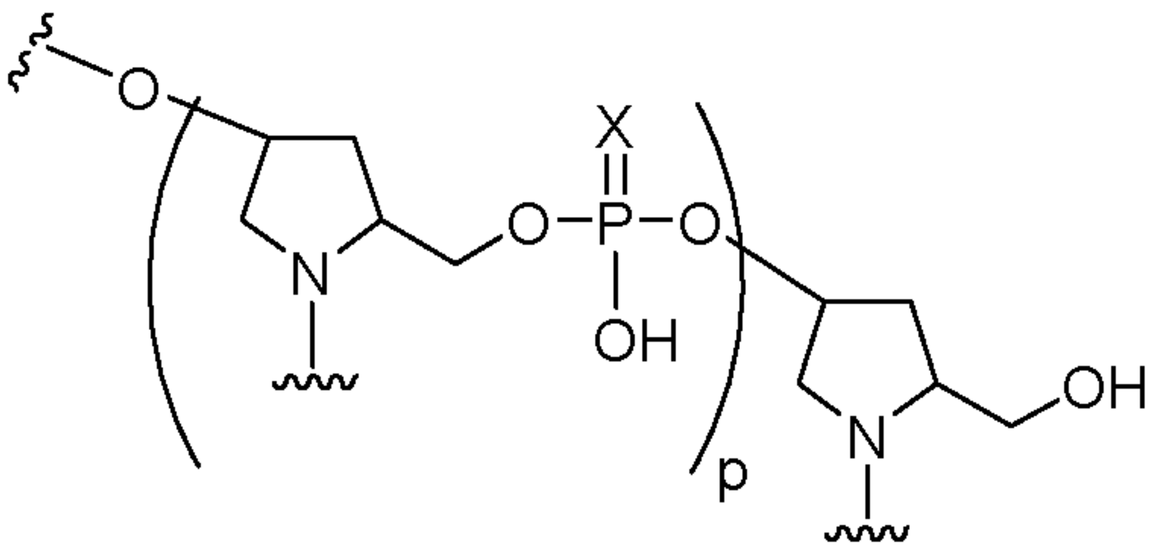
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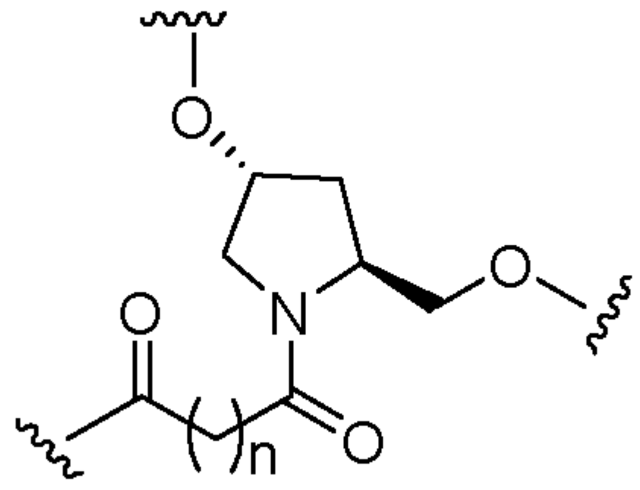
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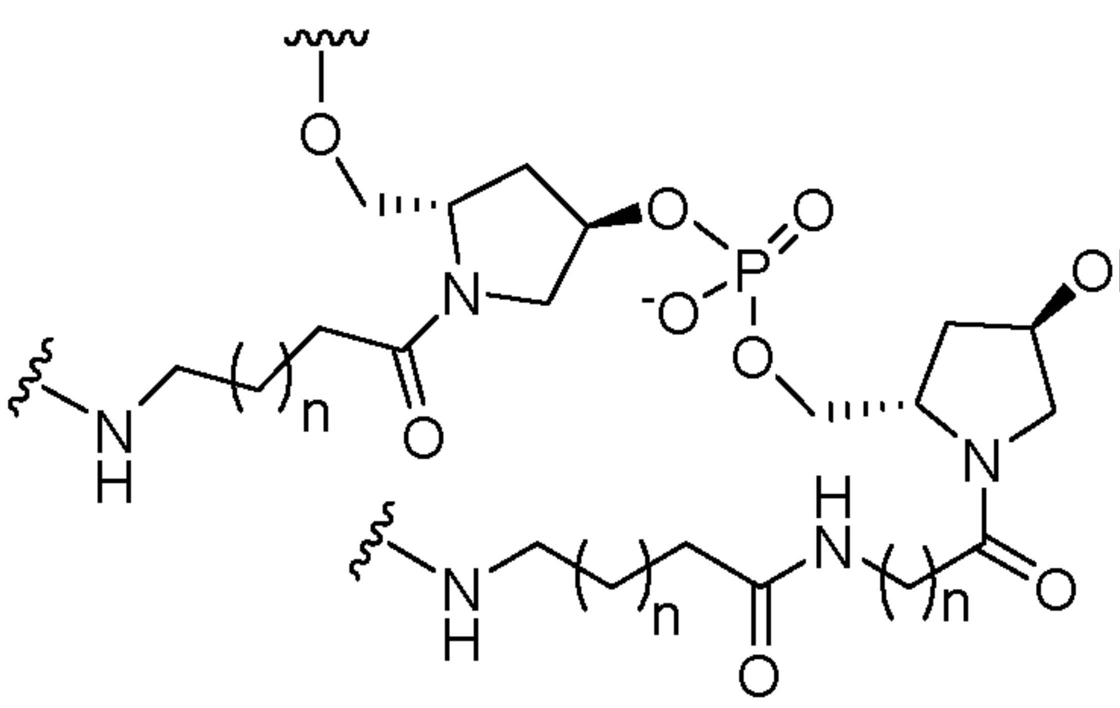
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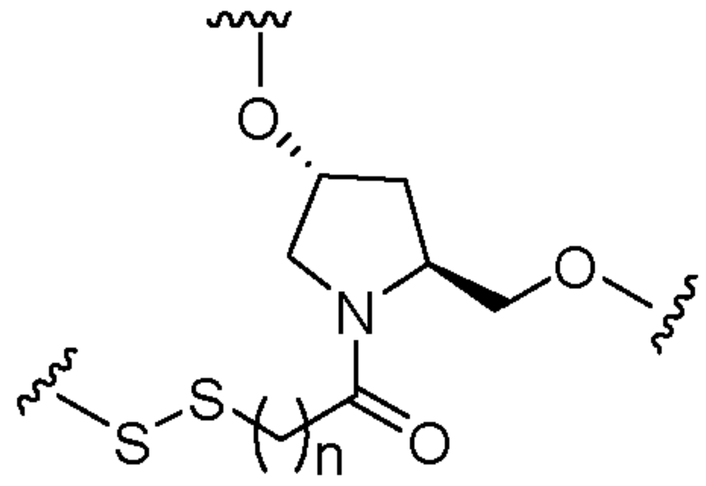
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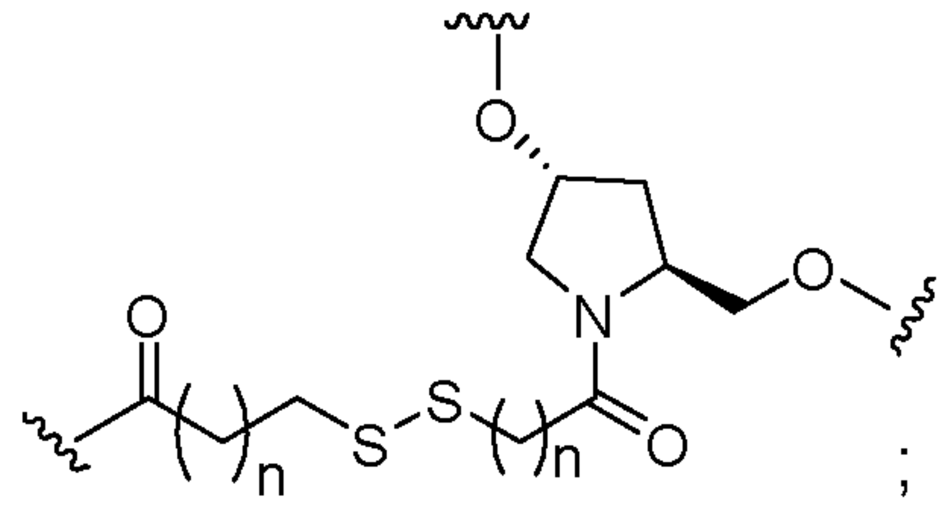
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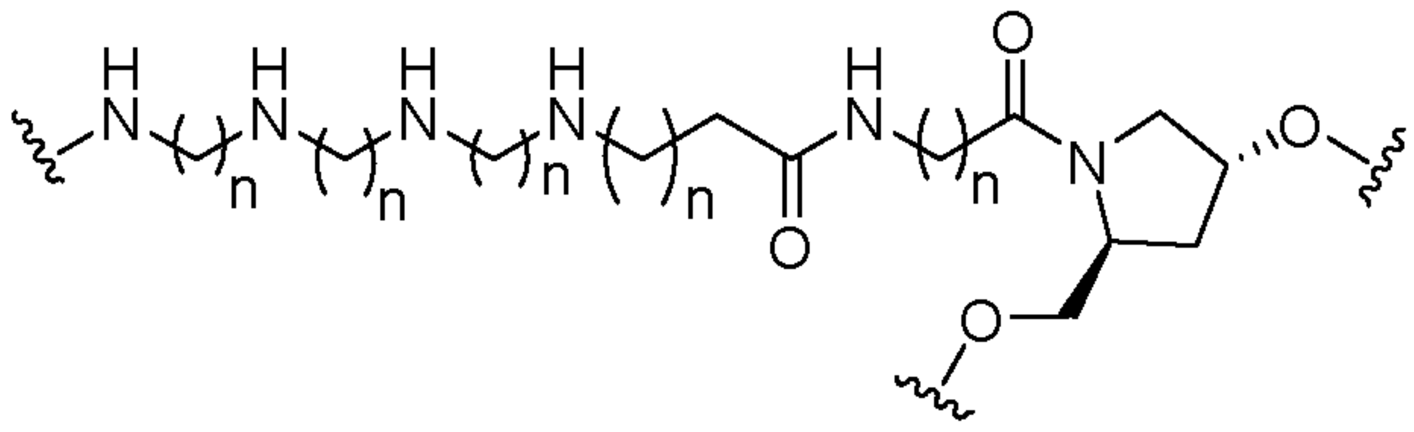
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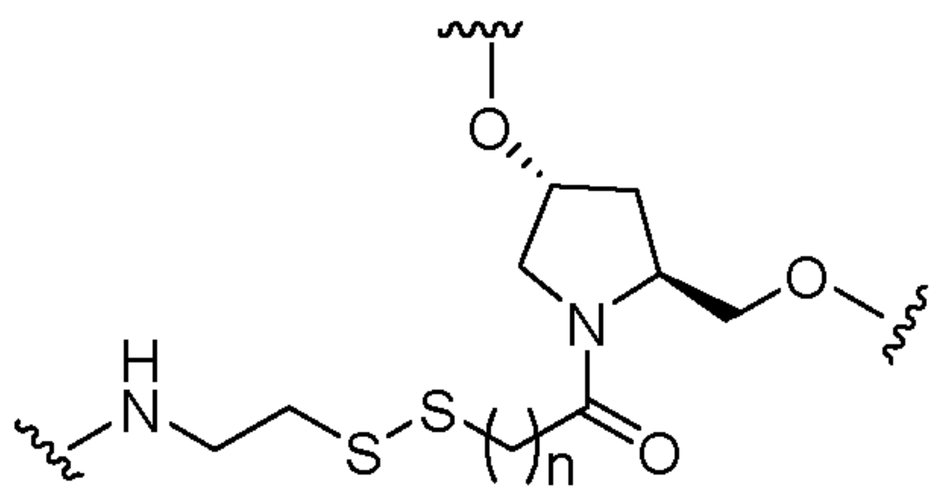
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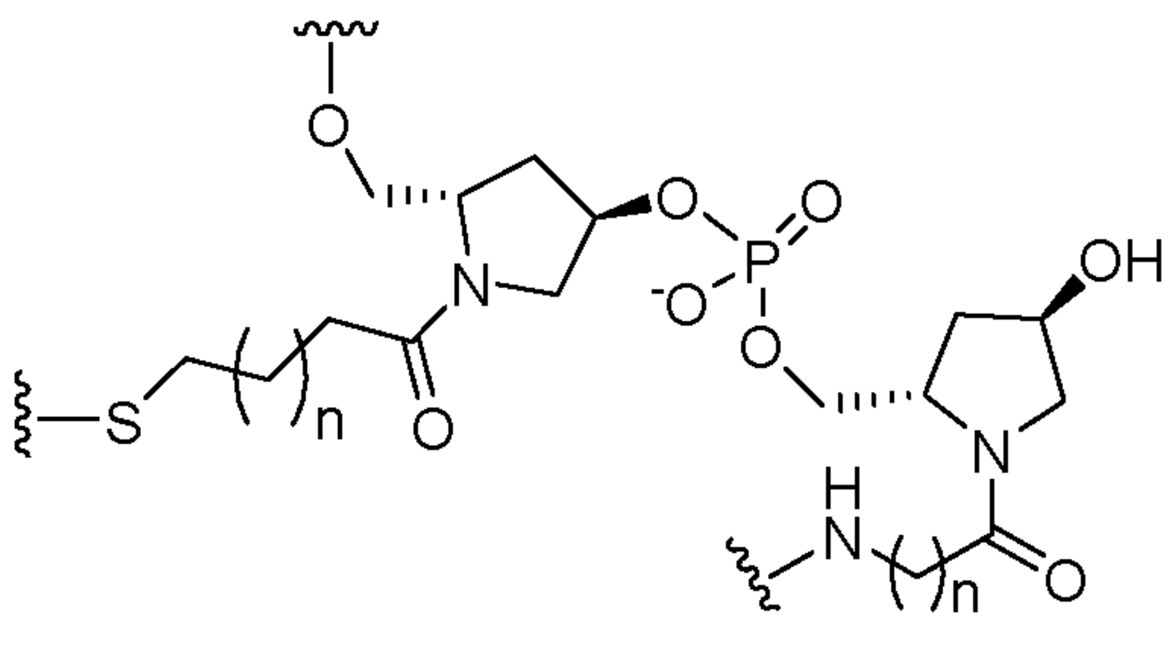
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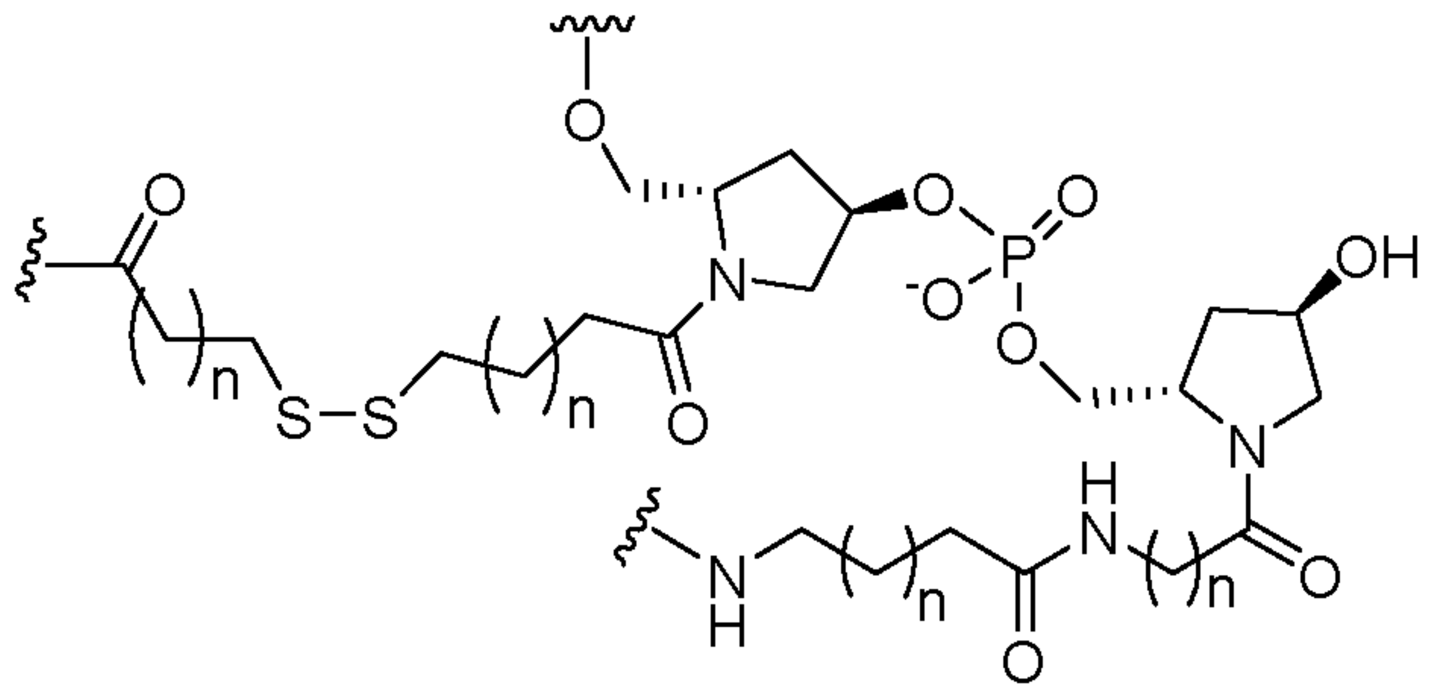
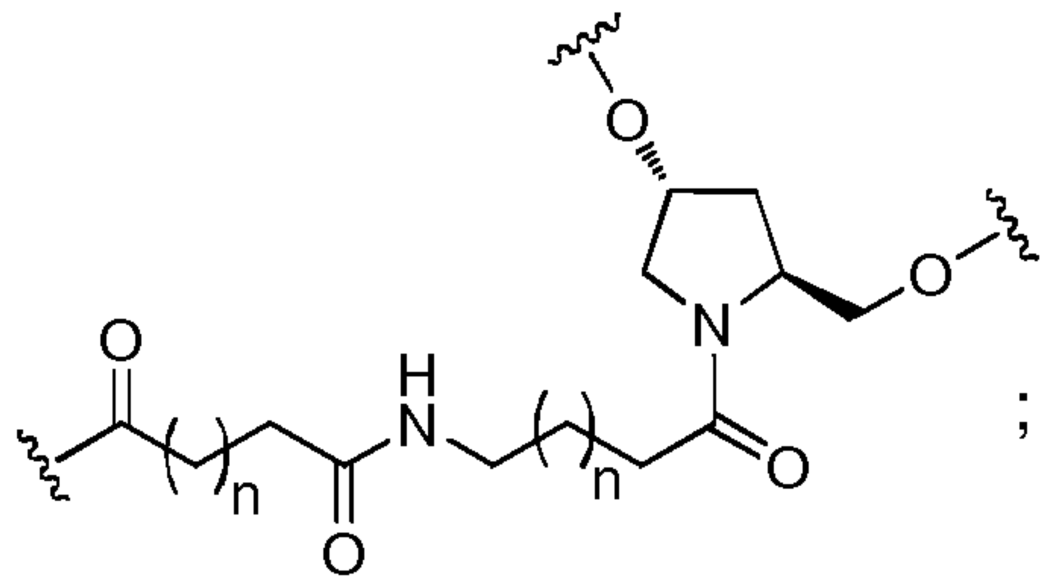


wherein each n is, independently, from 1 to 20; and p is from 1 to 6.

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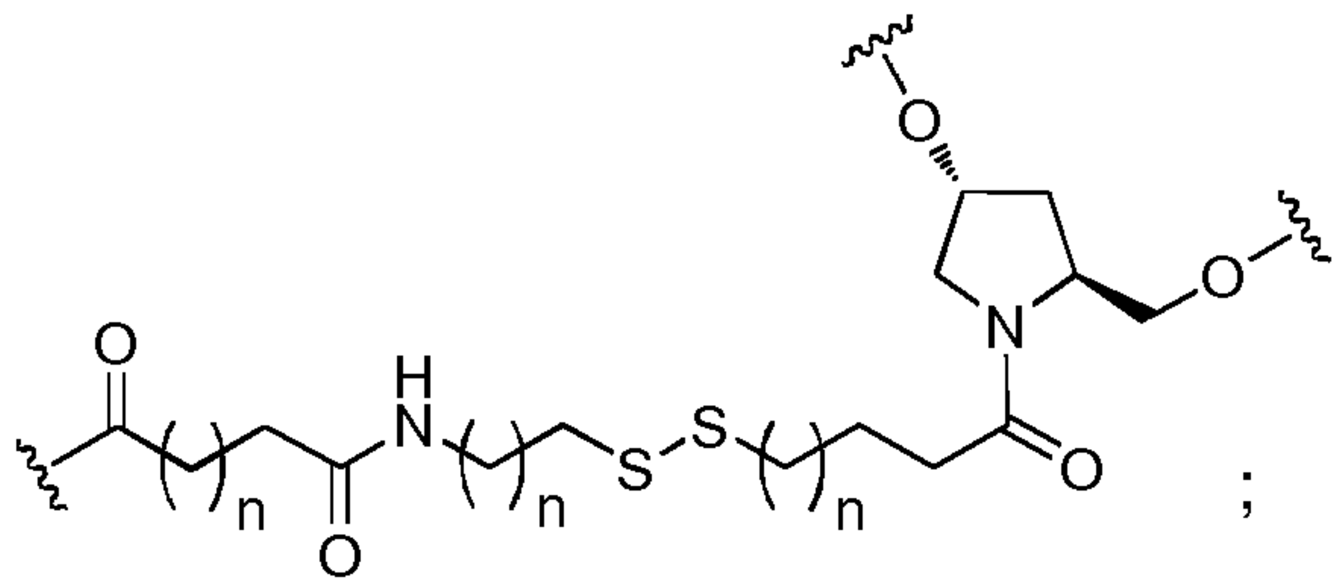
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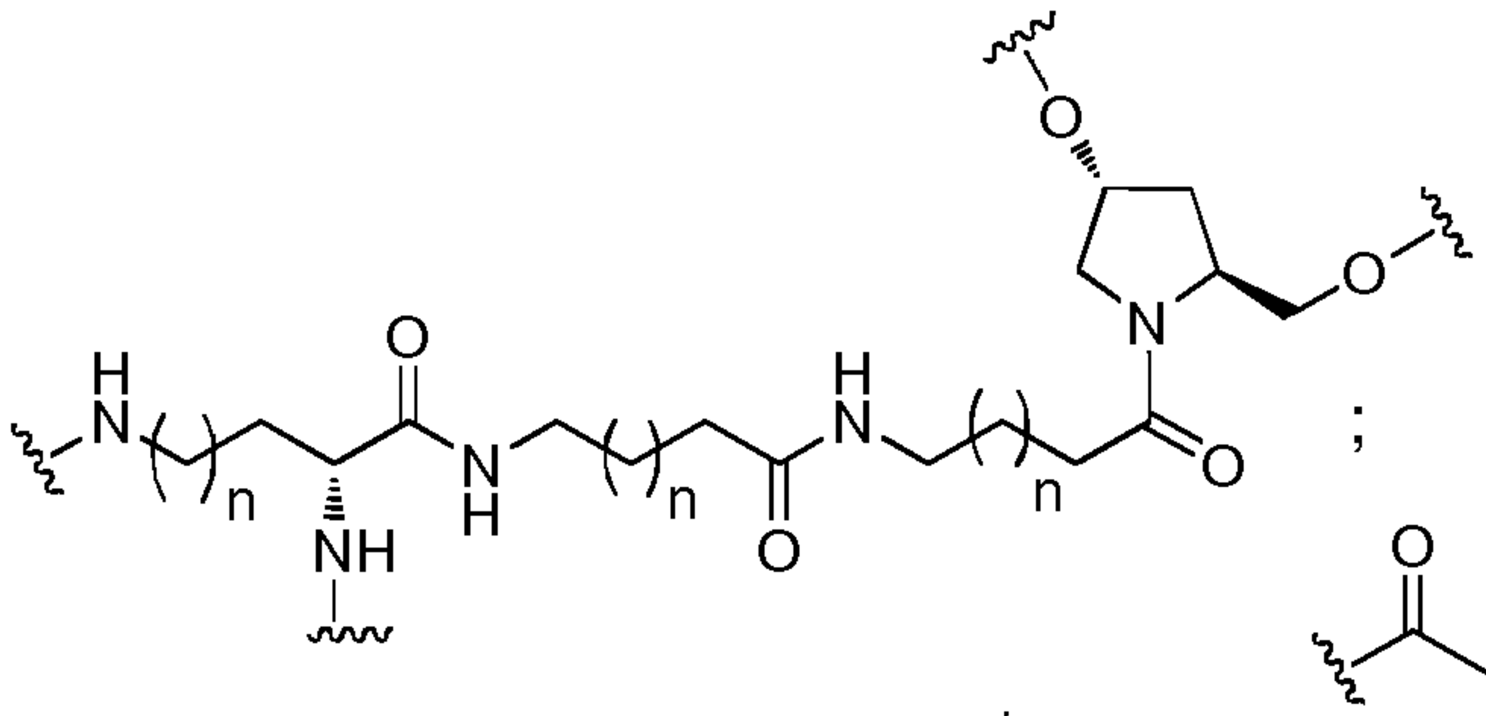
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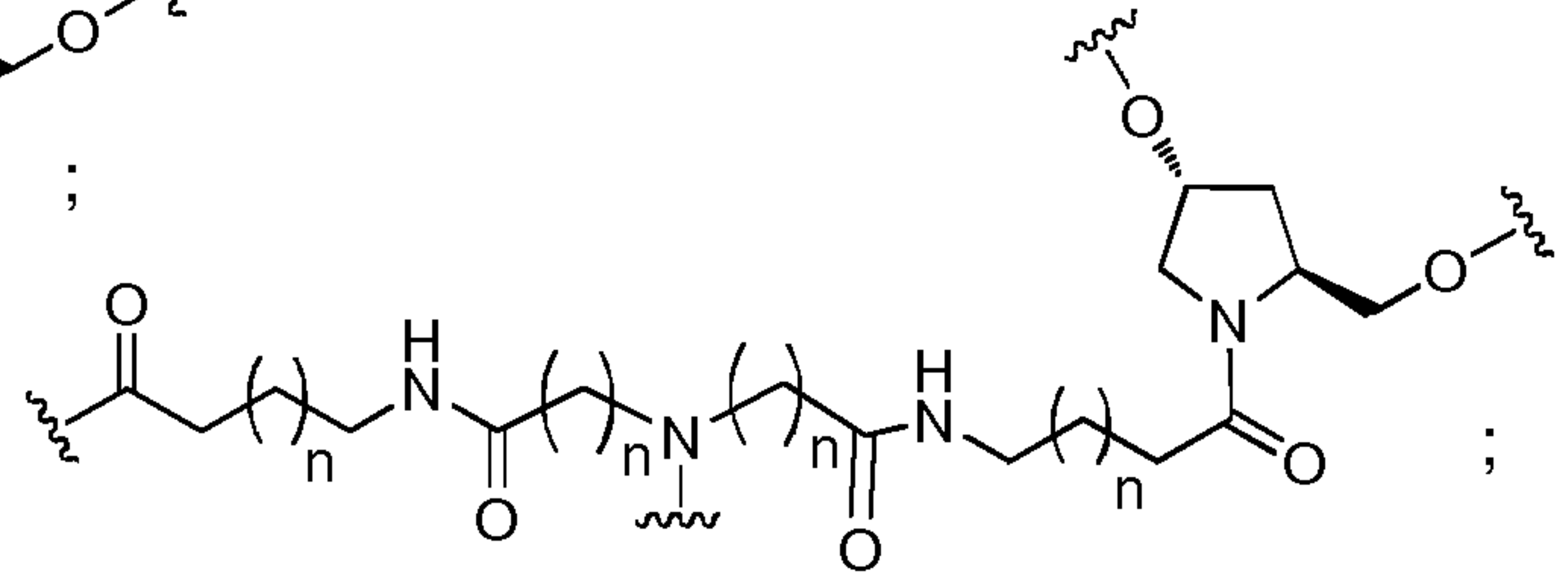
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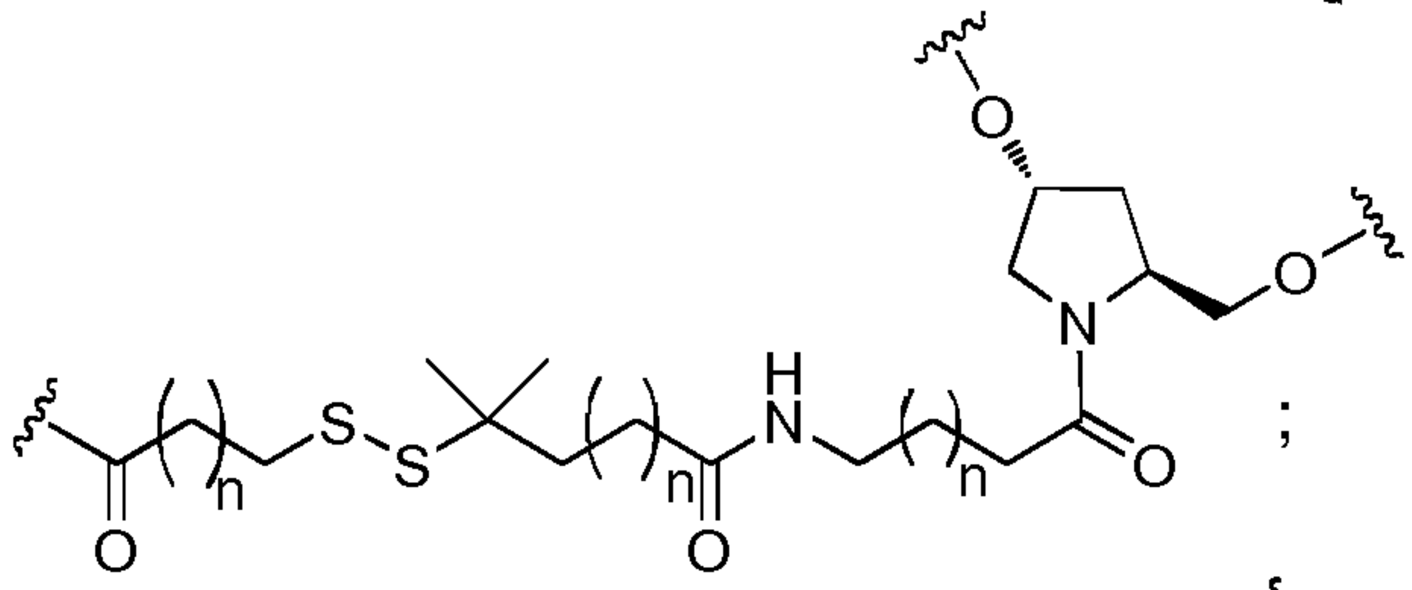
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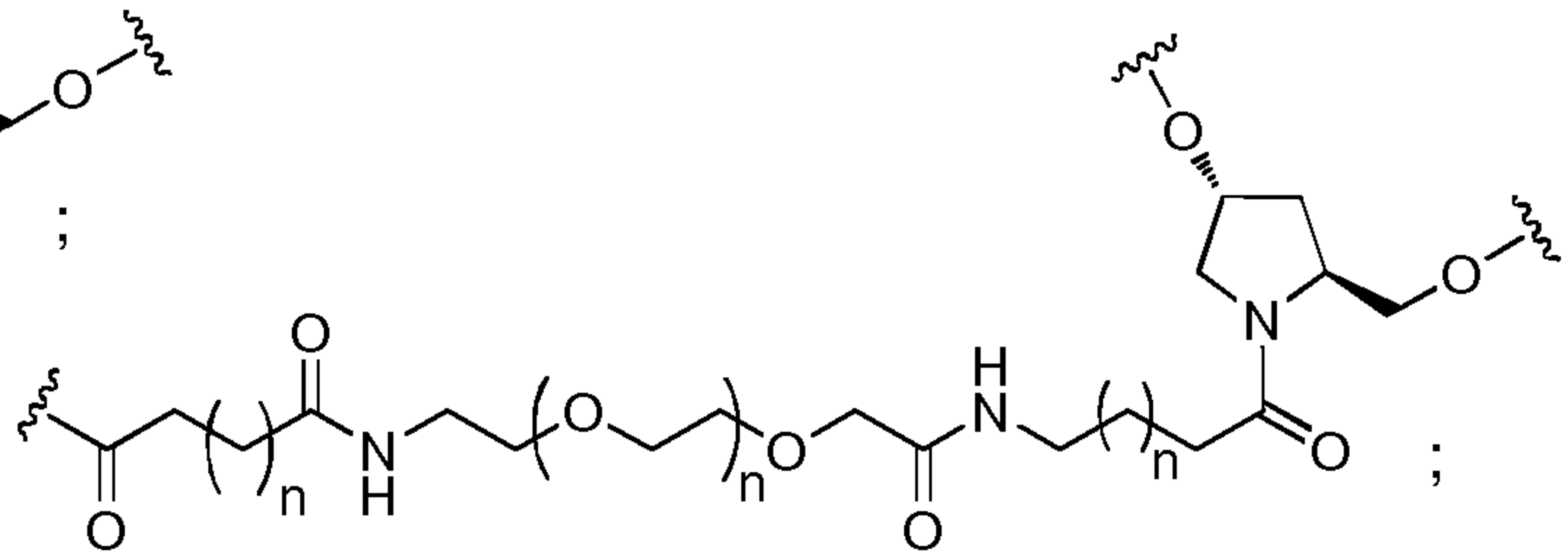
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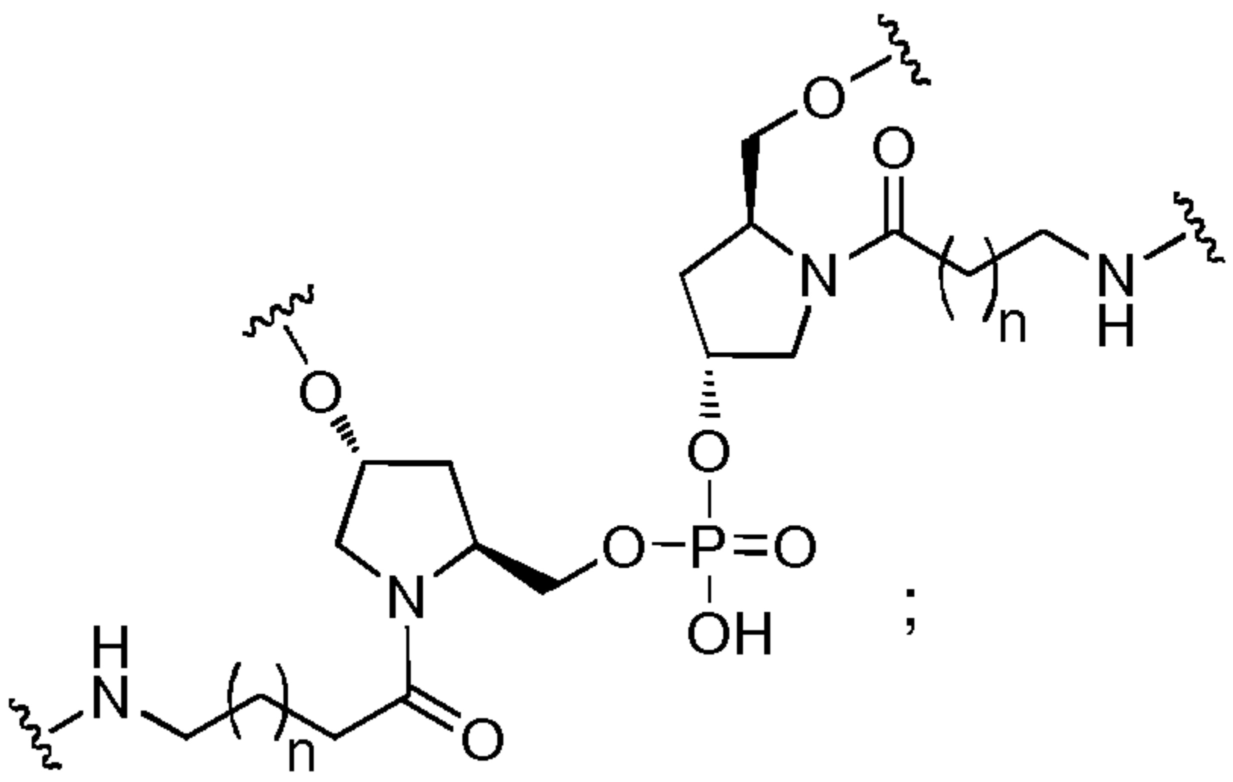
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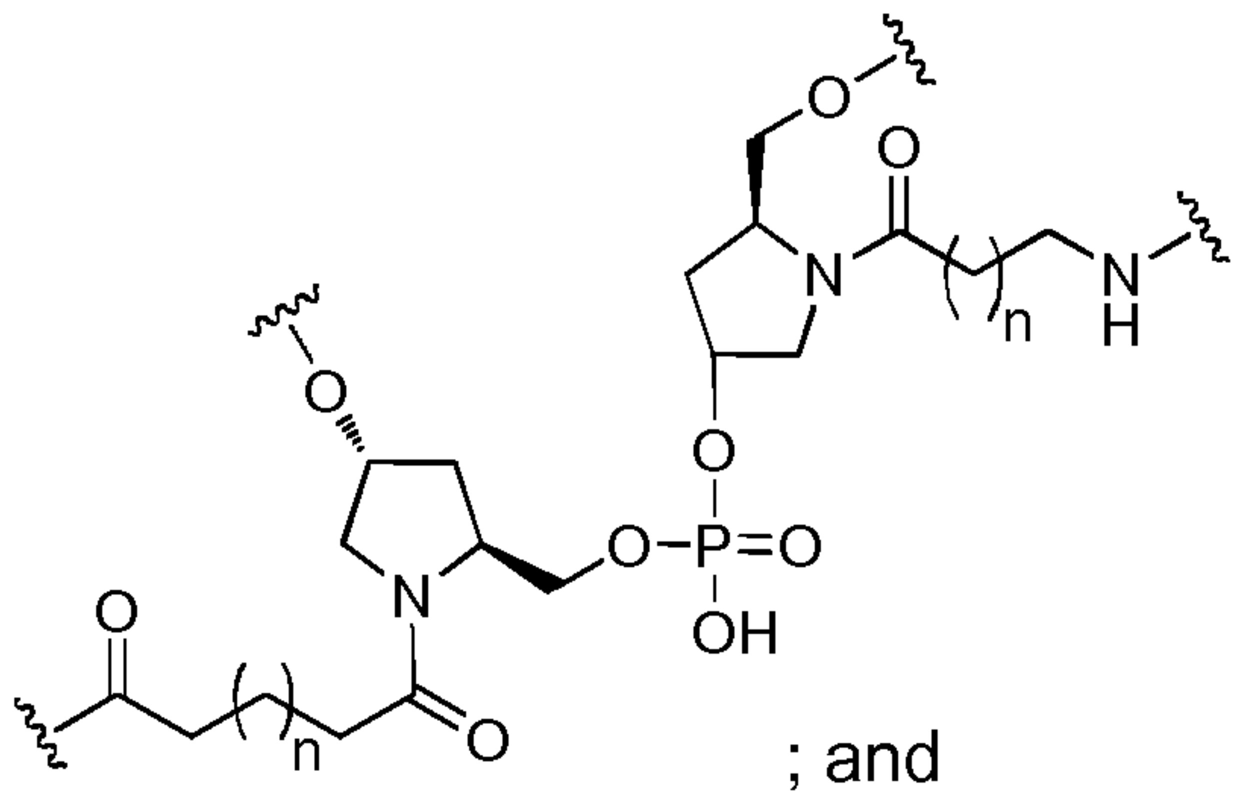
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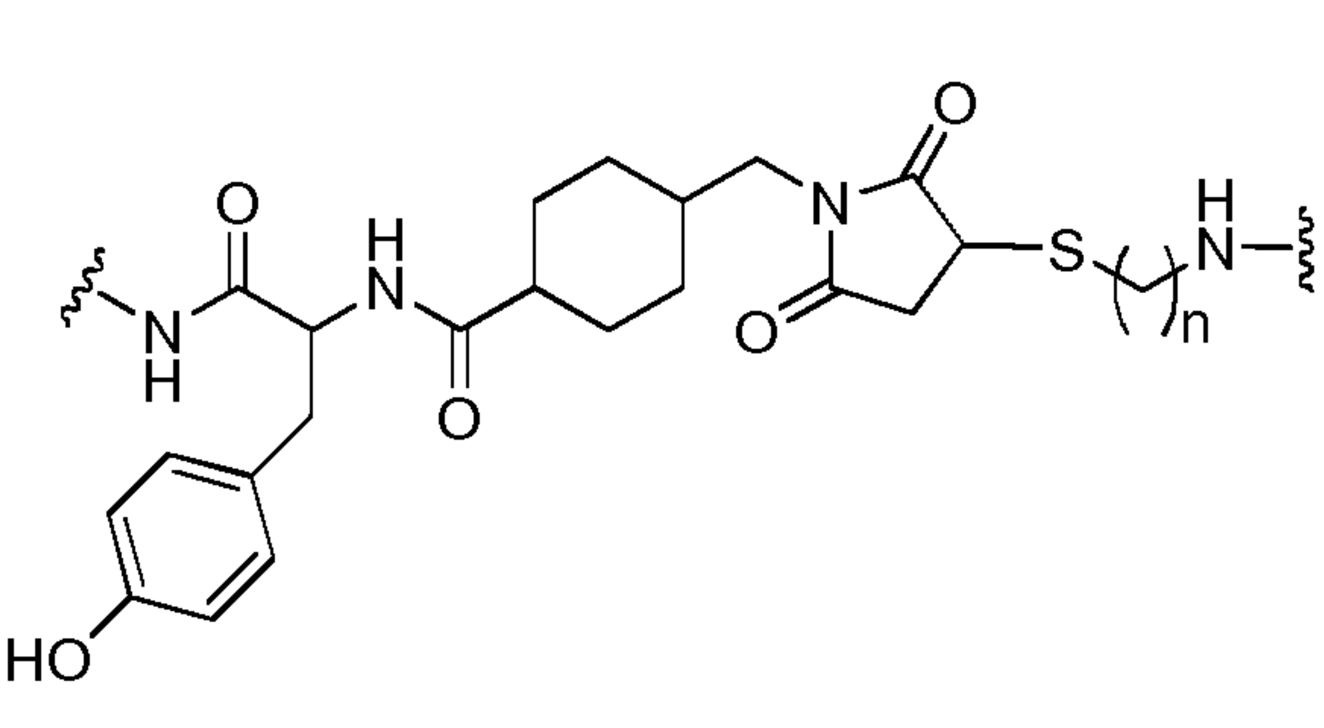
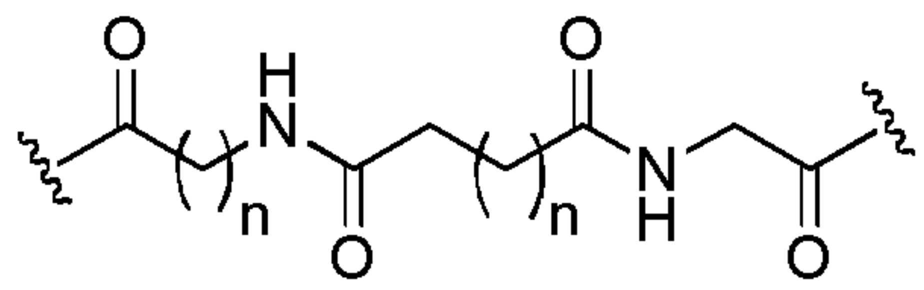
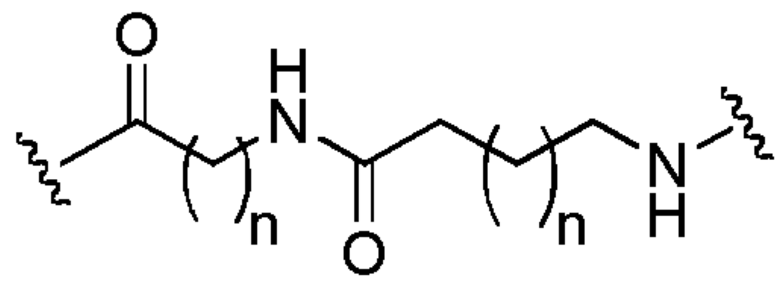
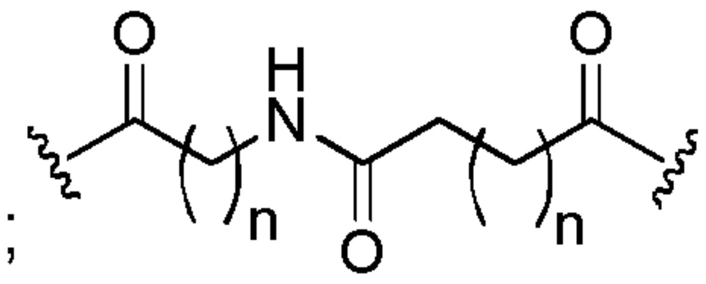
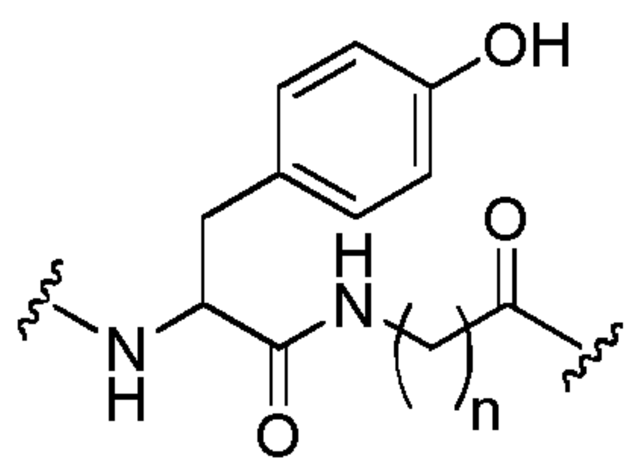
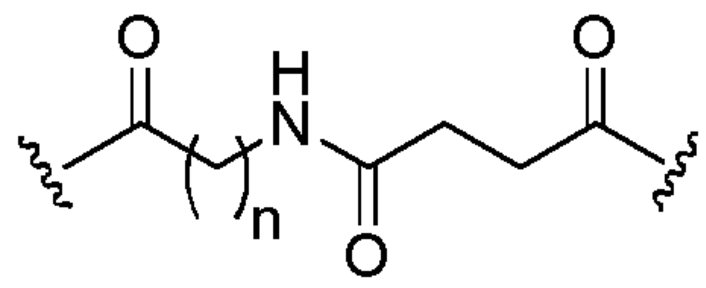
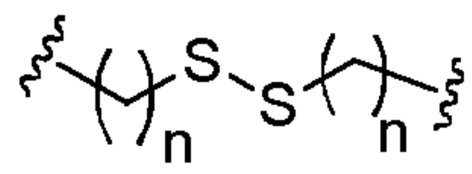
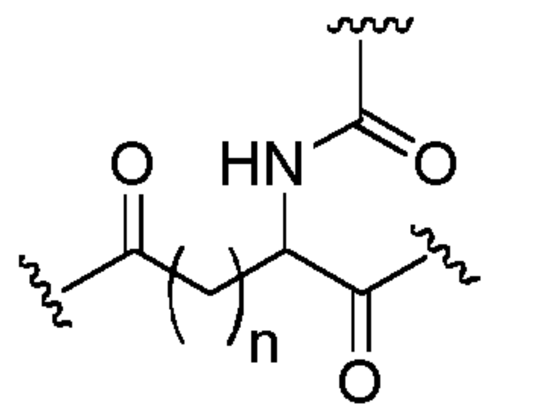
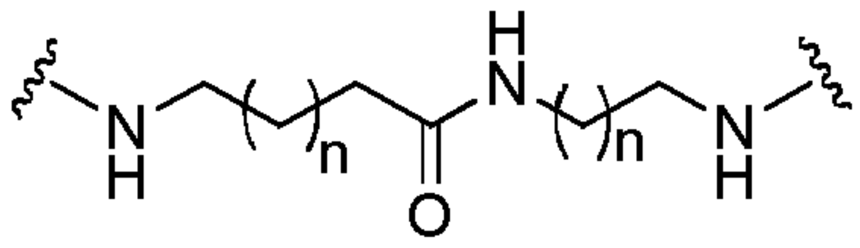
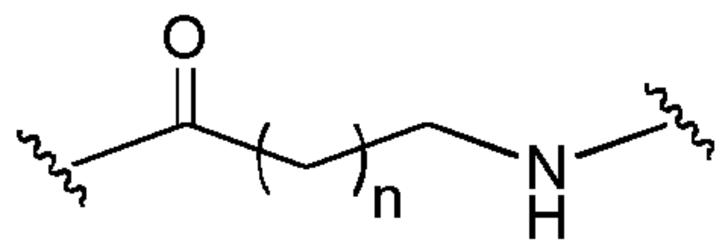
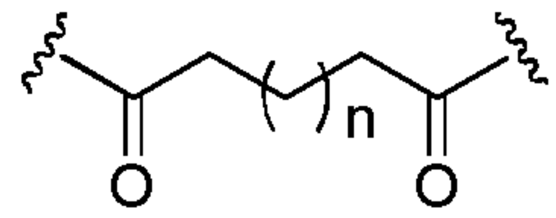


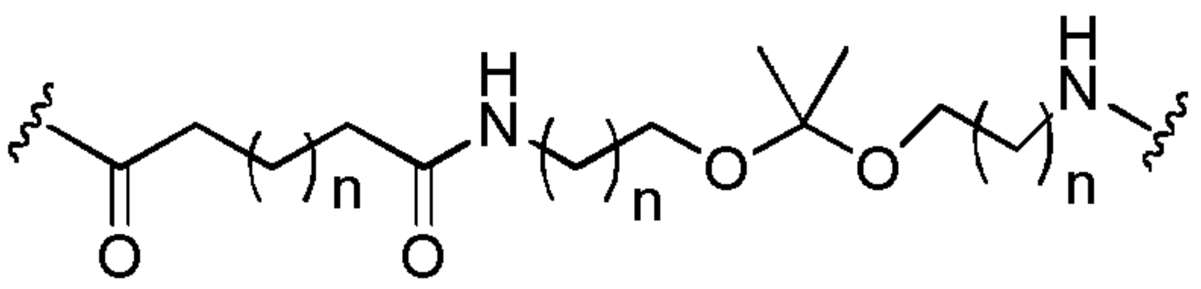
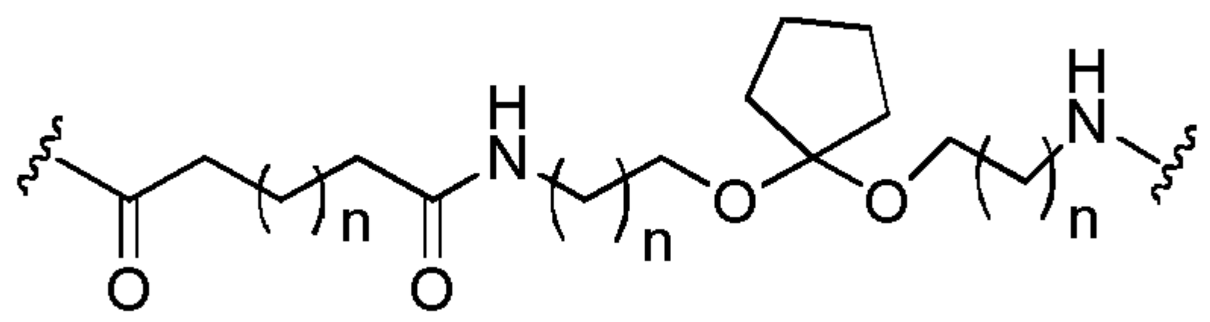
wherein each n is, independently, from 1 to 20.

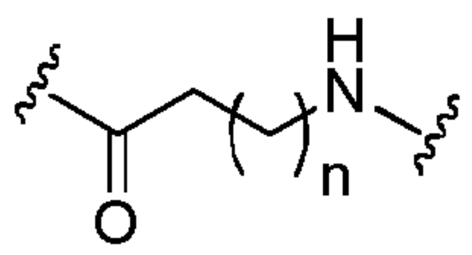
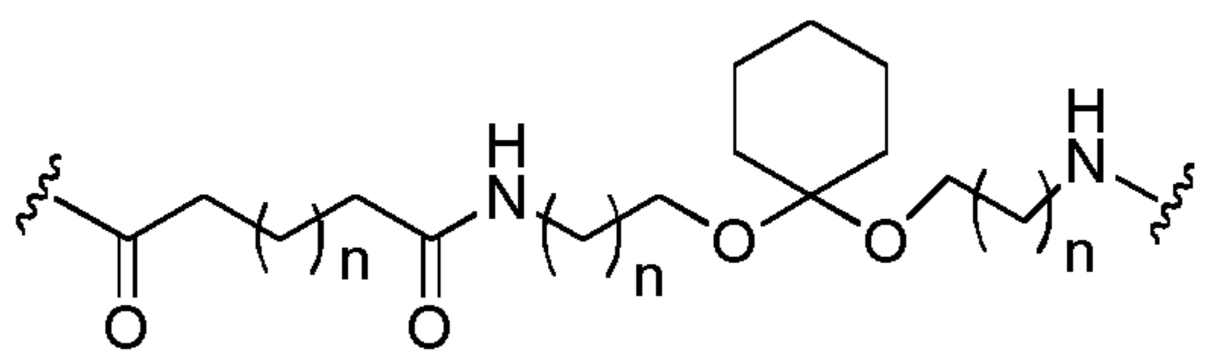
In certain embodiments, a linker has a structure selected from among:

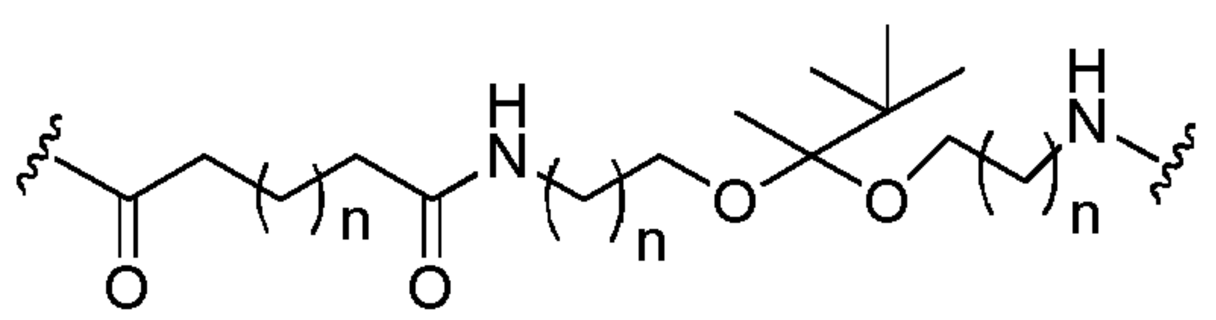
 ;  ;  ;

 ;  ;  ;  ;

 ;  ;  ;

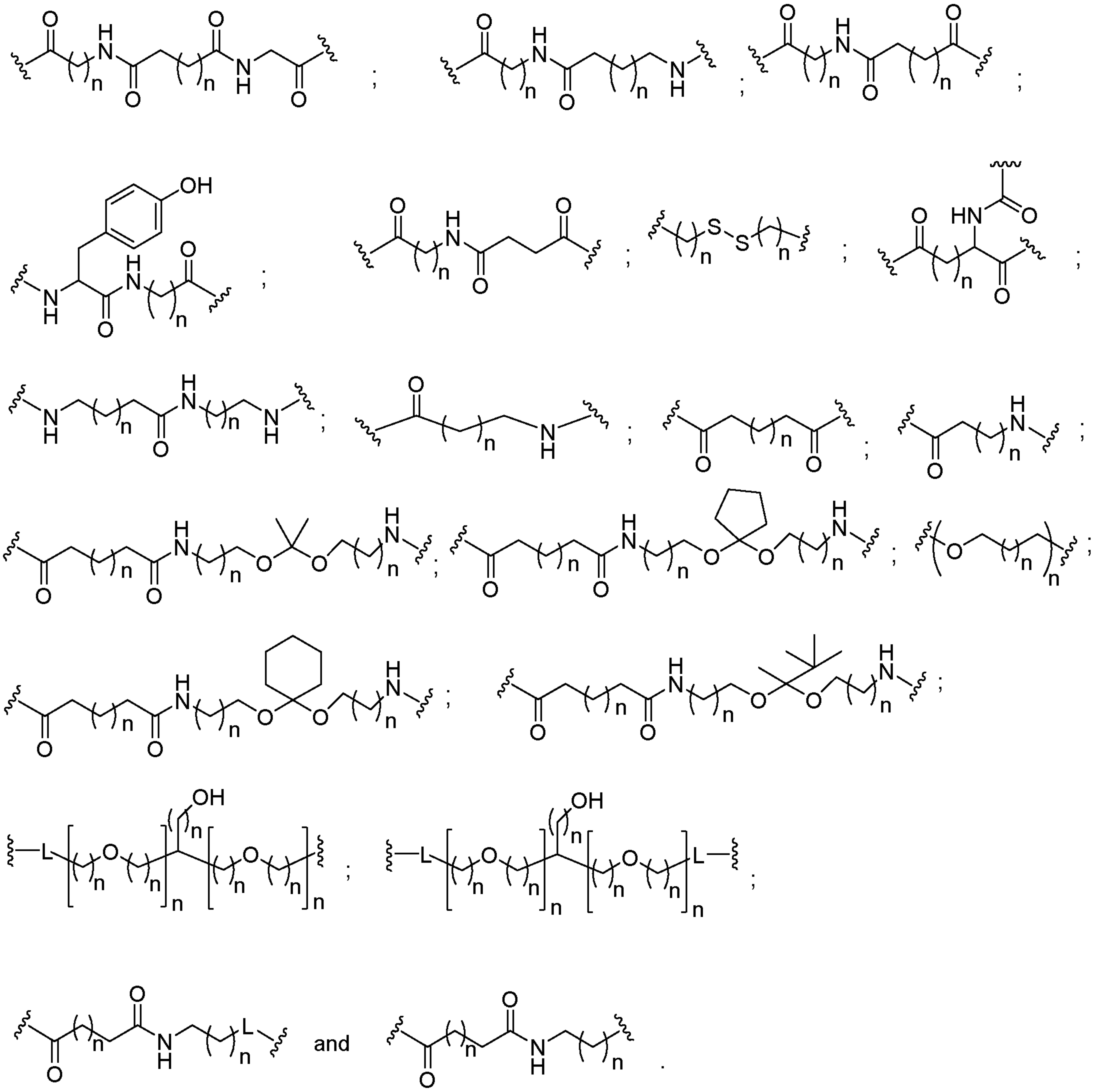
 ;  ;

 ;  ; and



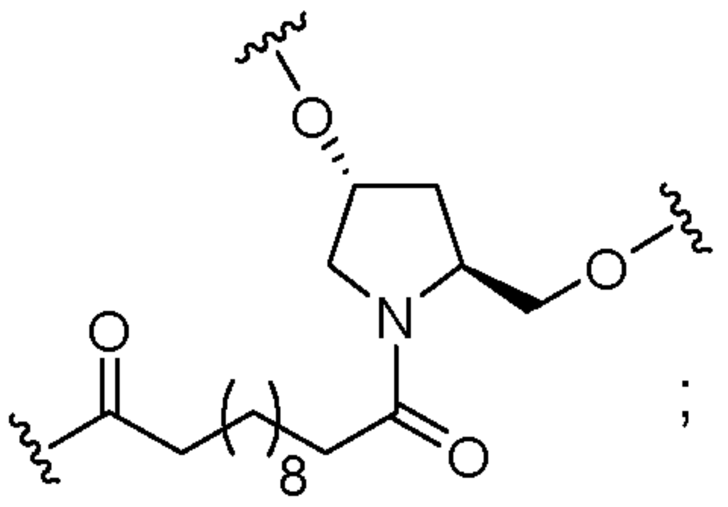
wherein n is from 1 to 20.

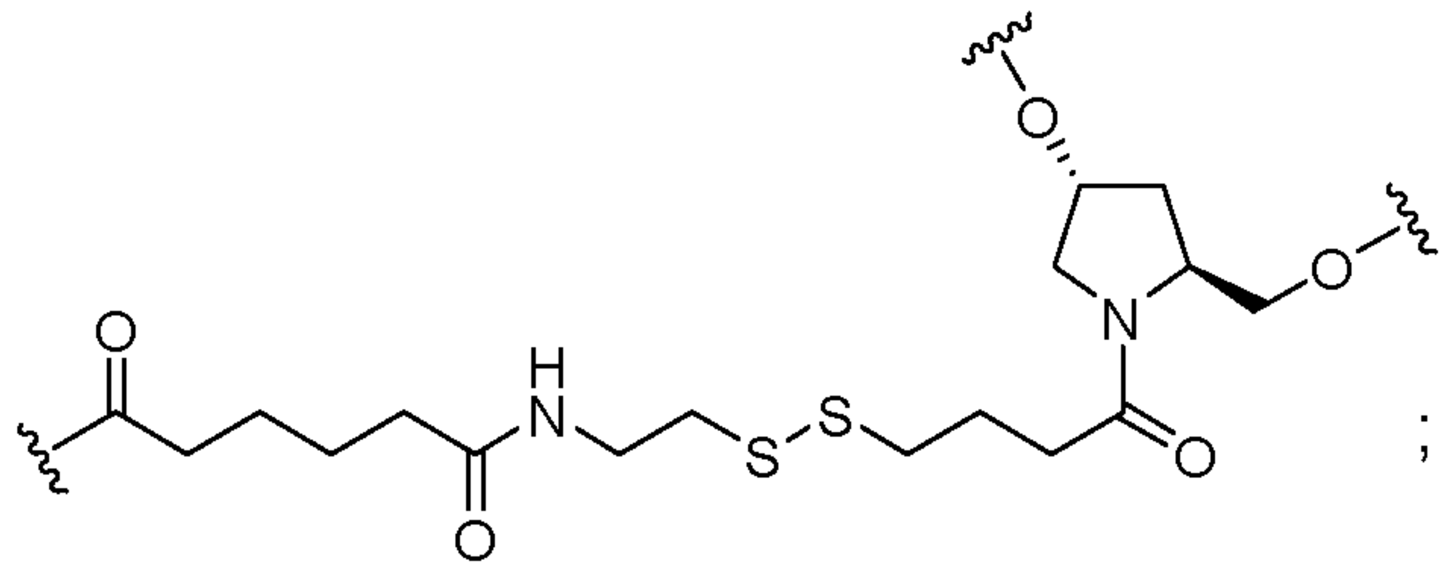
In certain embodiments, a linker has a structure selected from among:

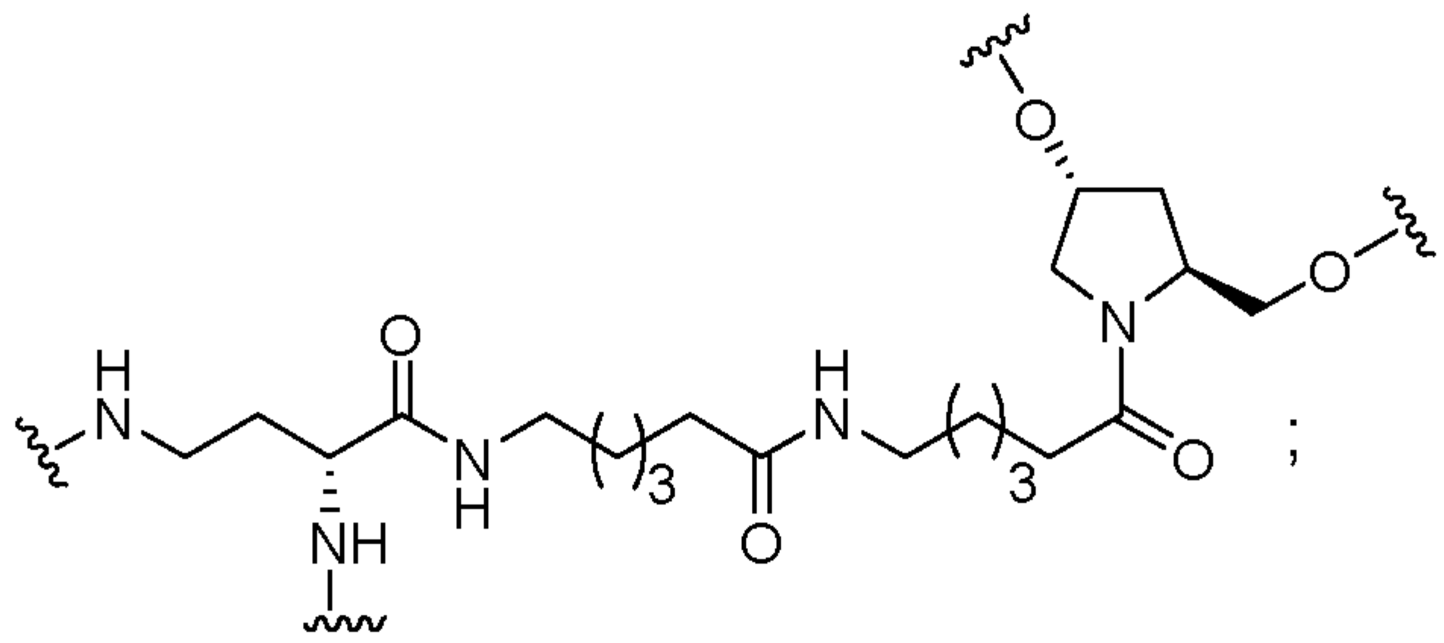


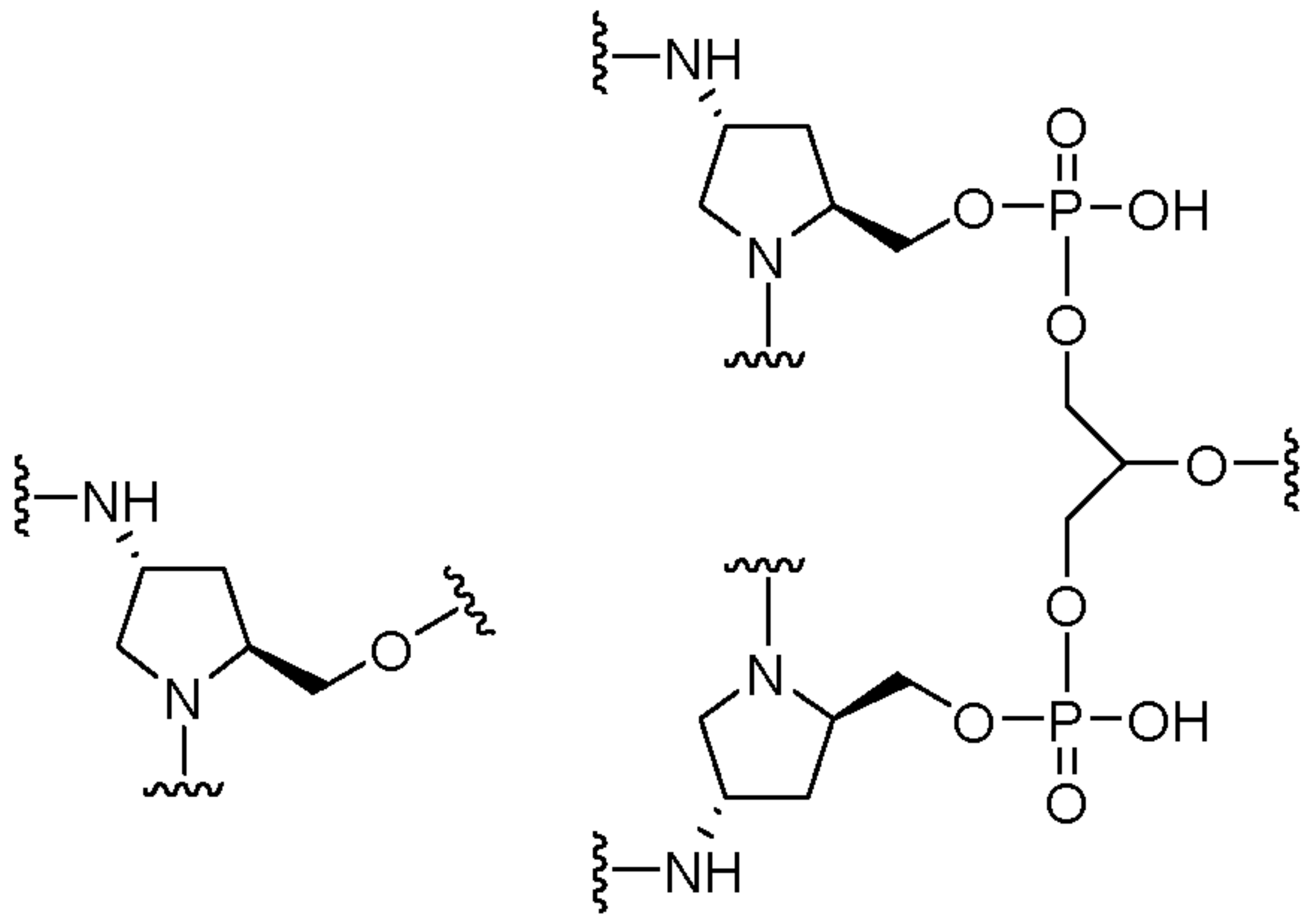
wherein each L is, independently, a phosphorus linking group or a neutral linking group; and each n is, independently, from 1 to 20.

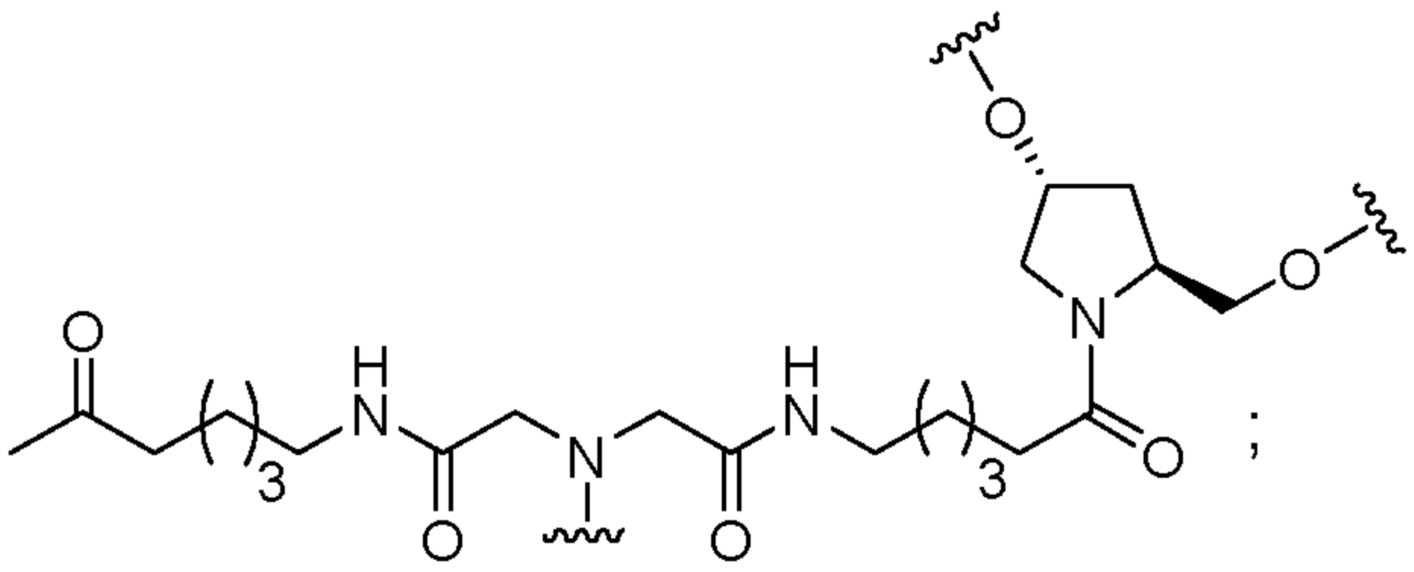
In certain embodiments, a linker has a structure selected from among:

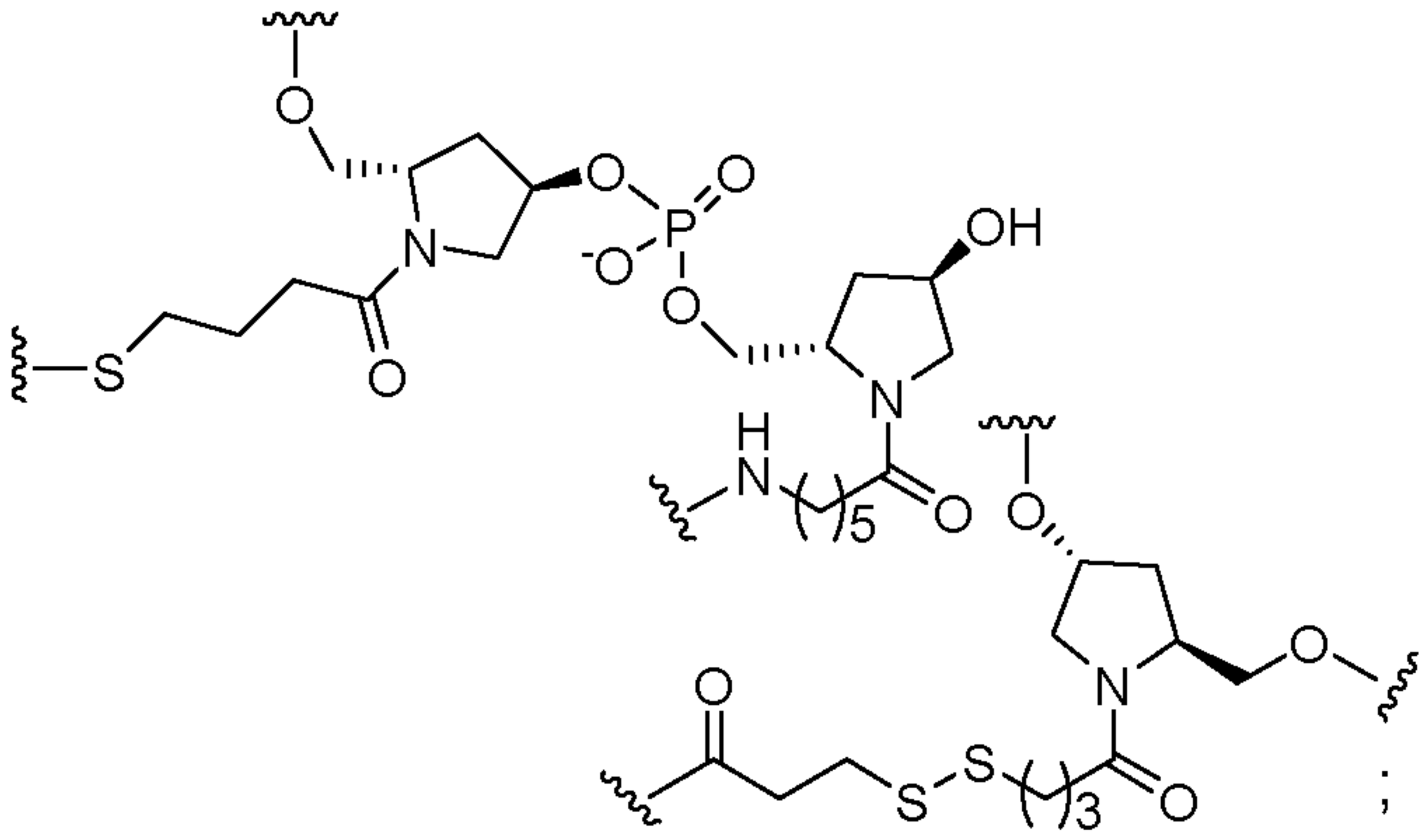
 ;

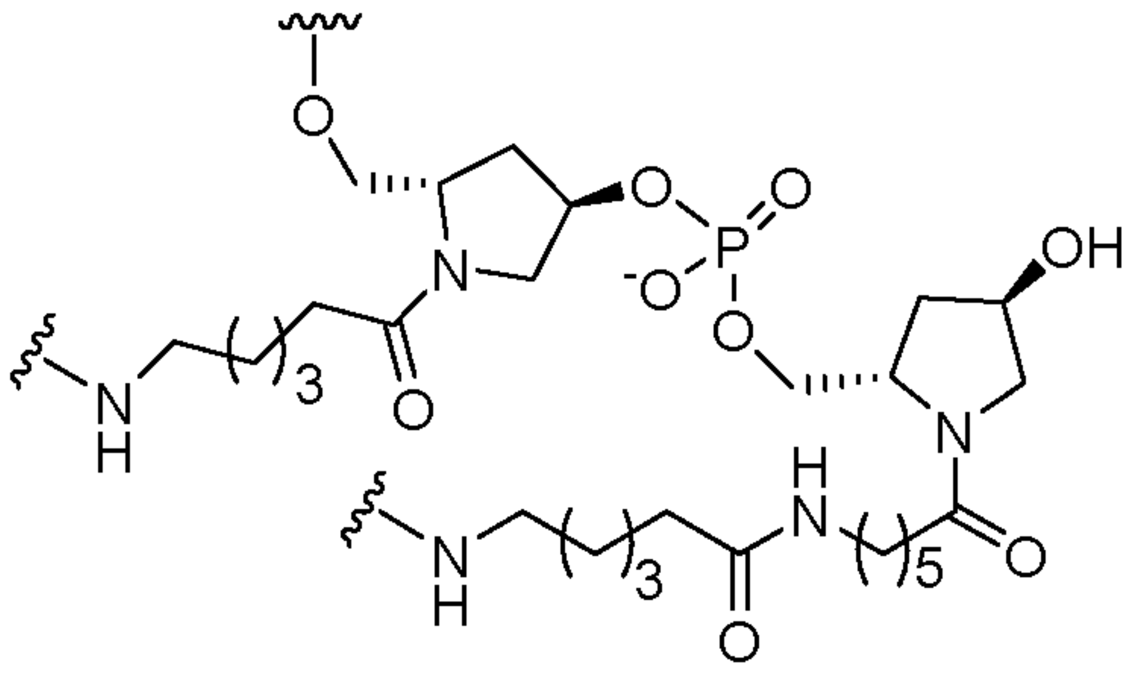
 ;

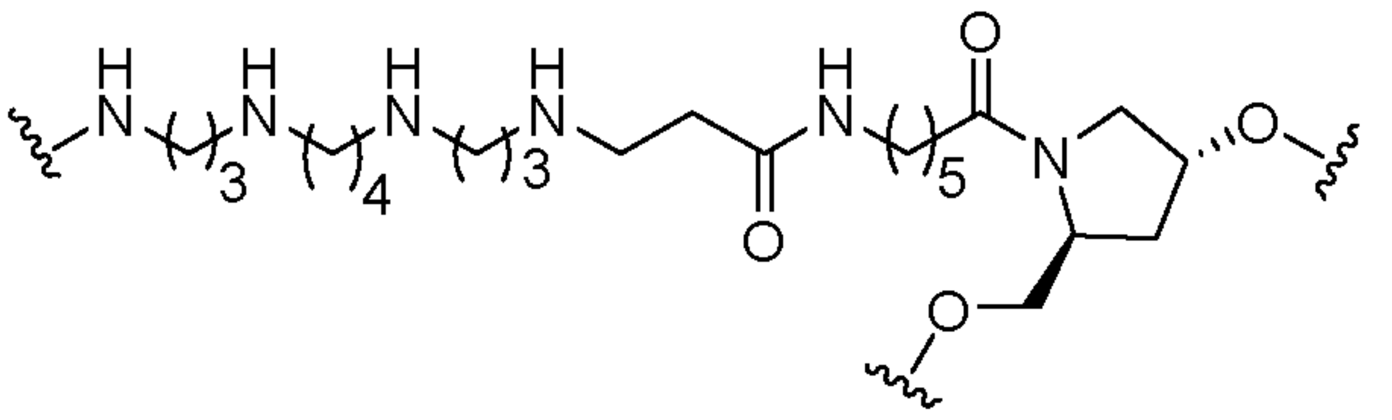
 ;

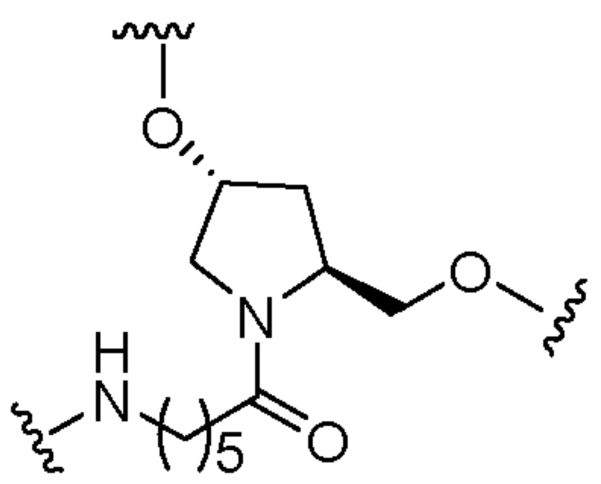
 ;

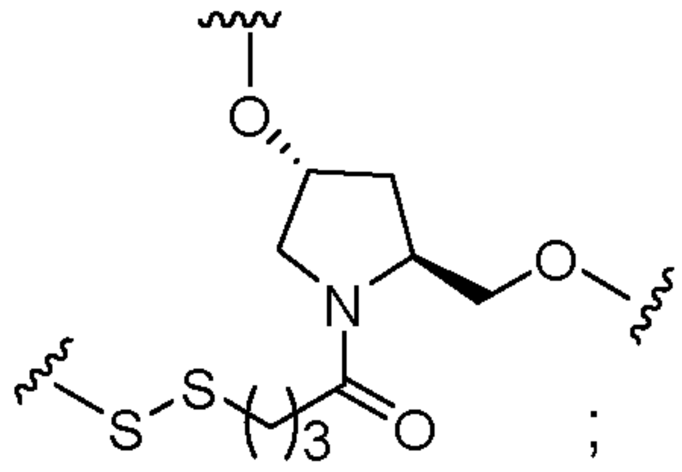
 ;

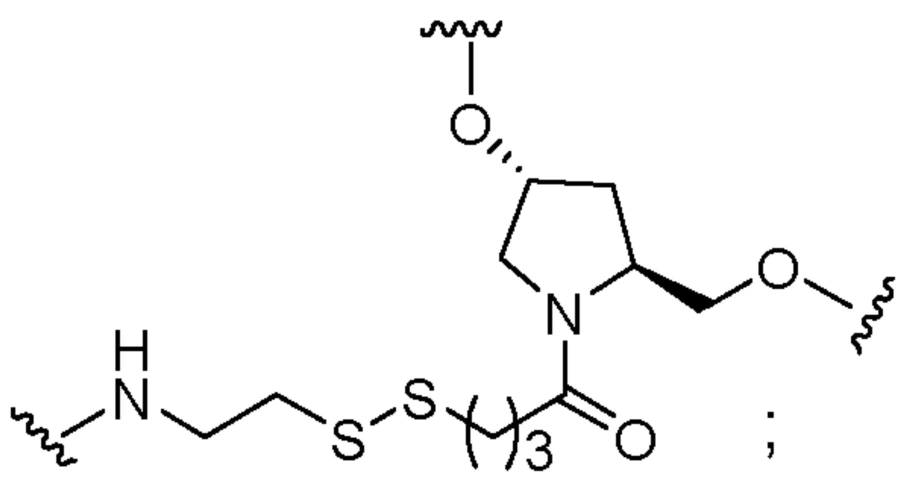
 ;

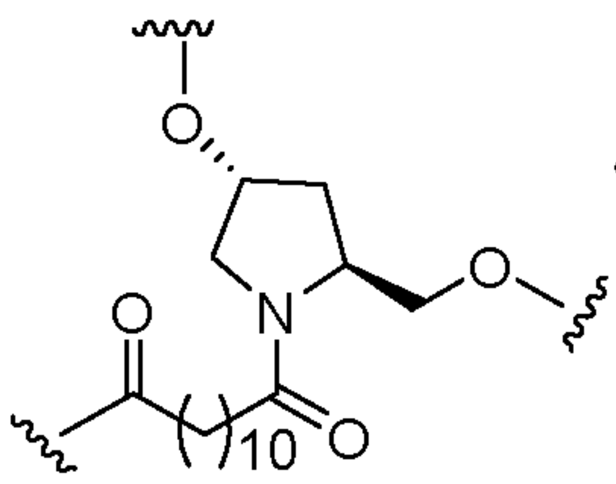
 ;

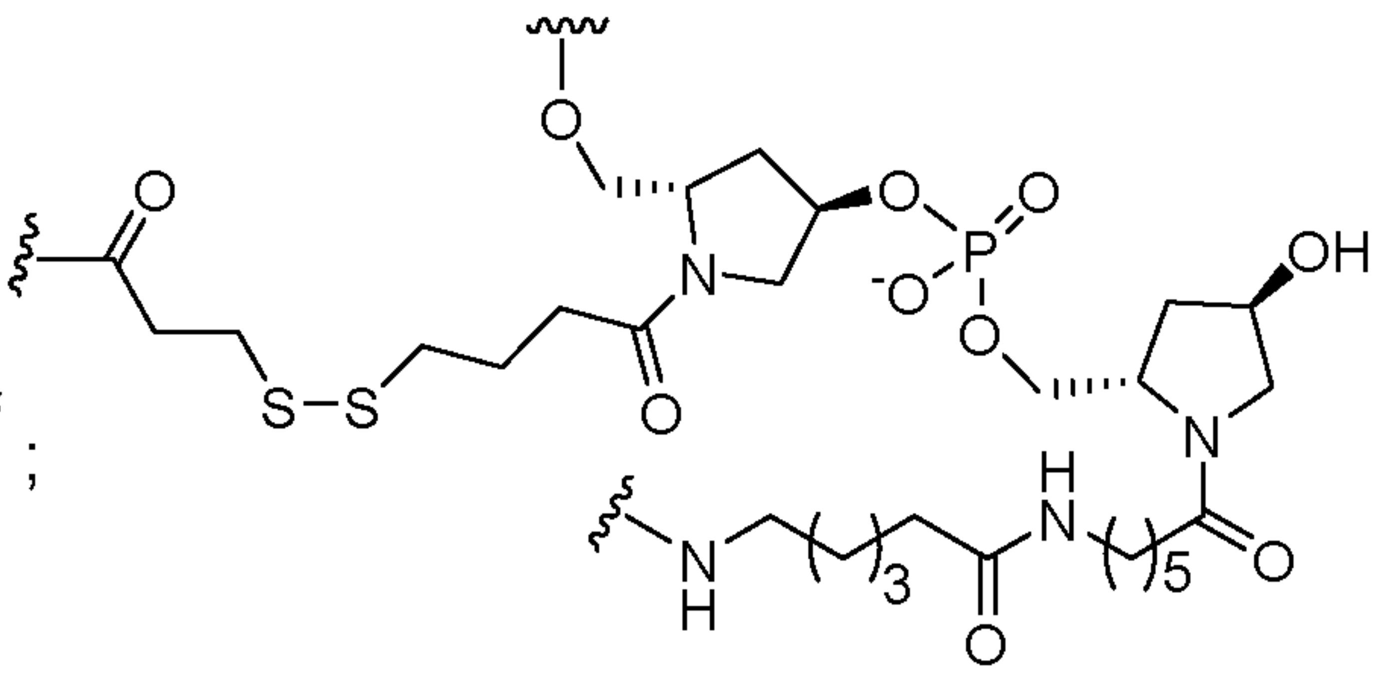
 ;

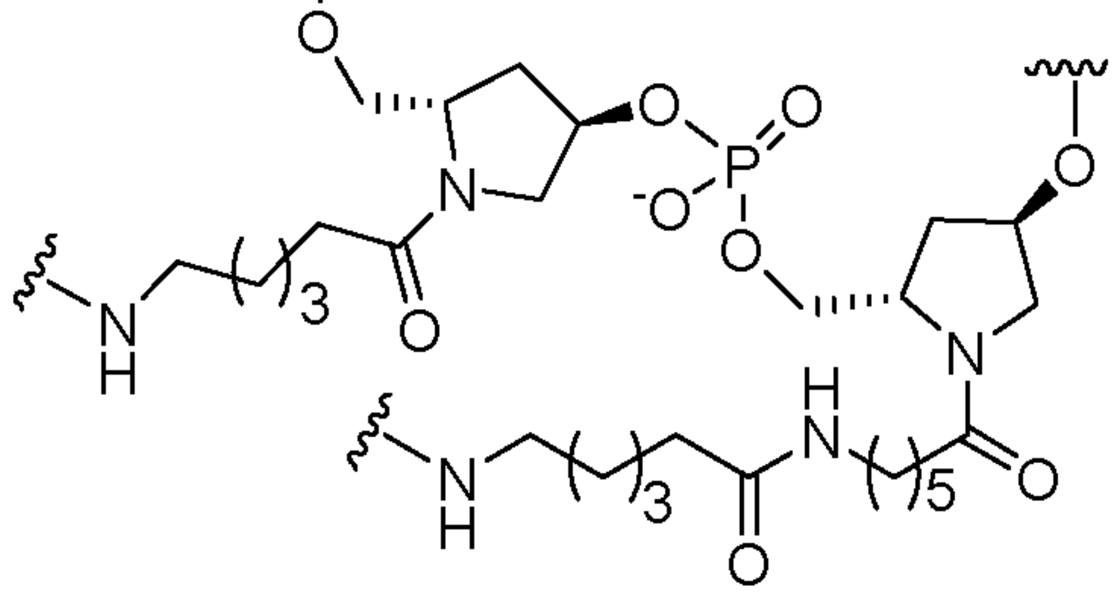
 ;

 ;

 ;

 ;

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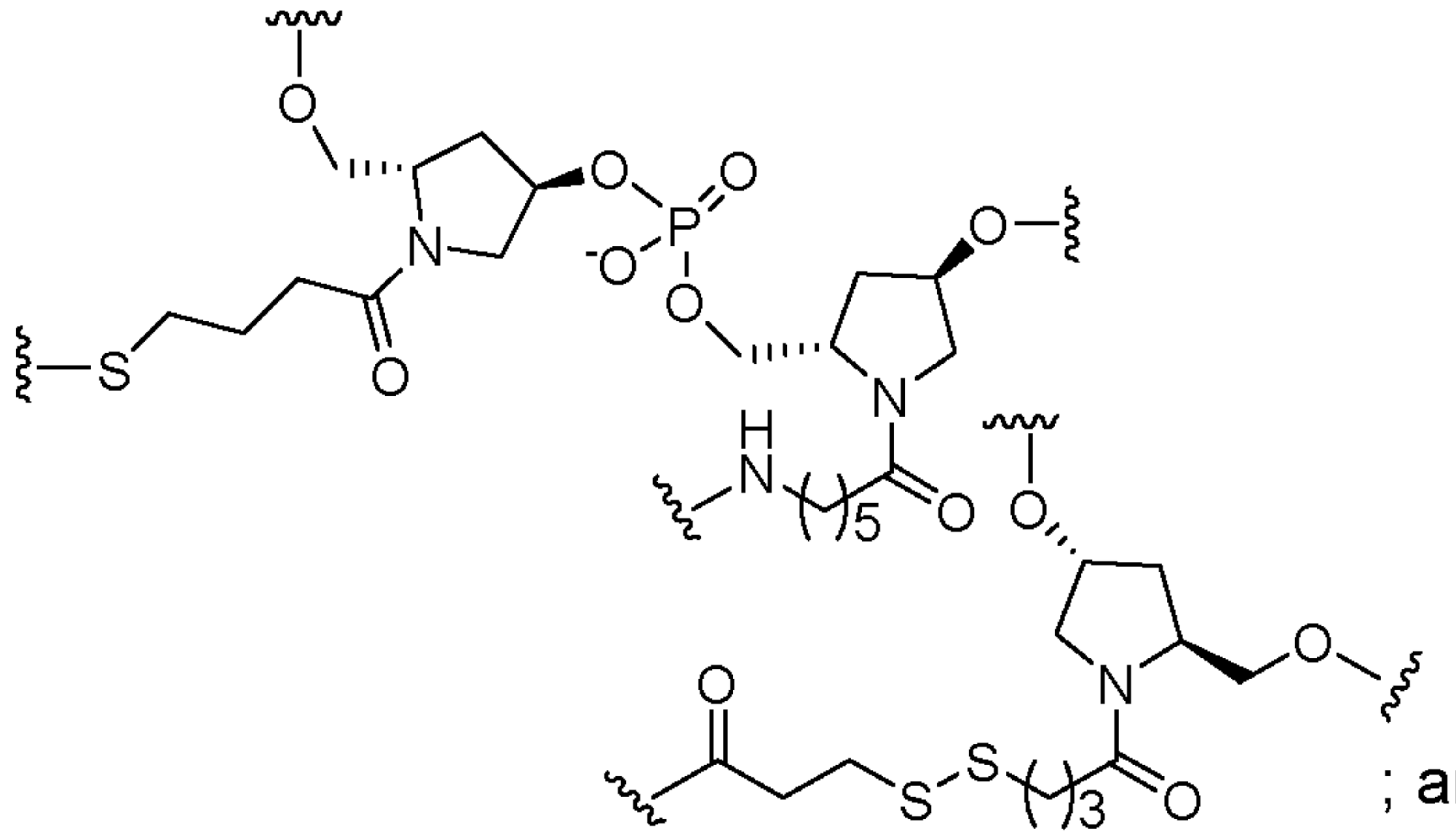
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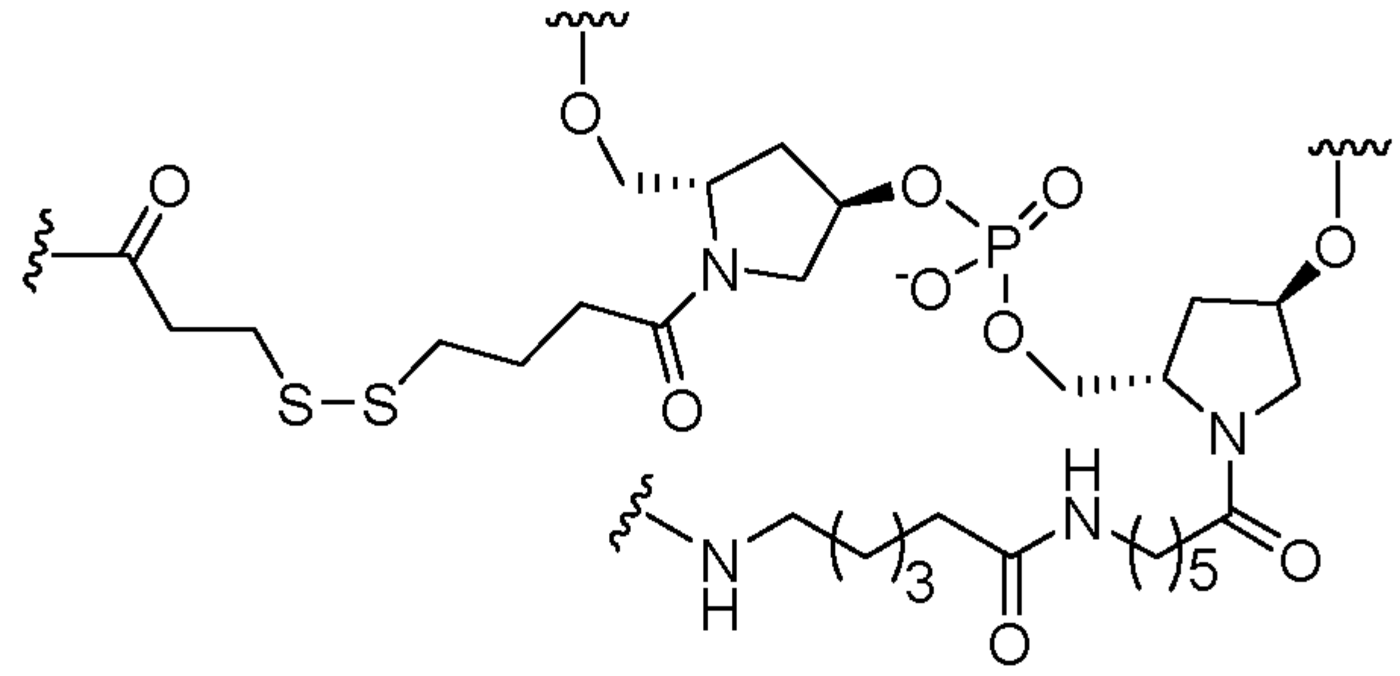
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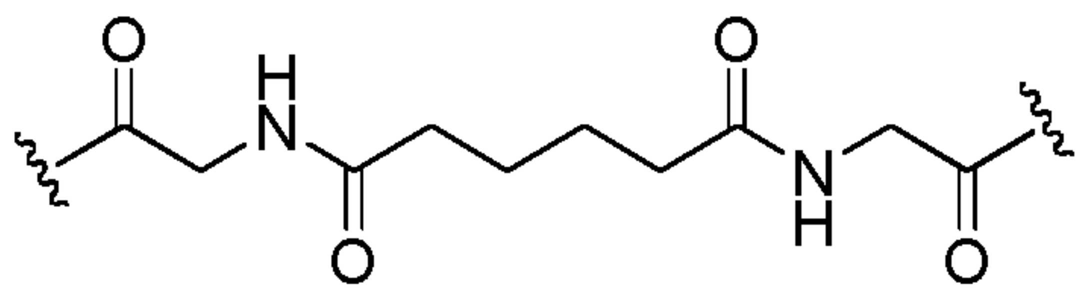
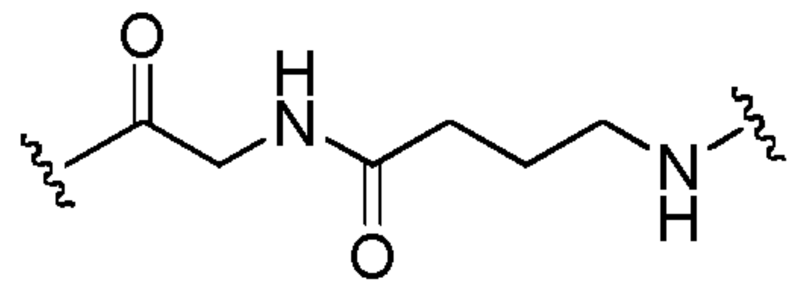
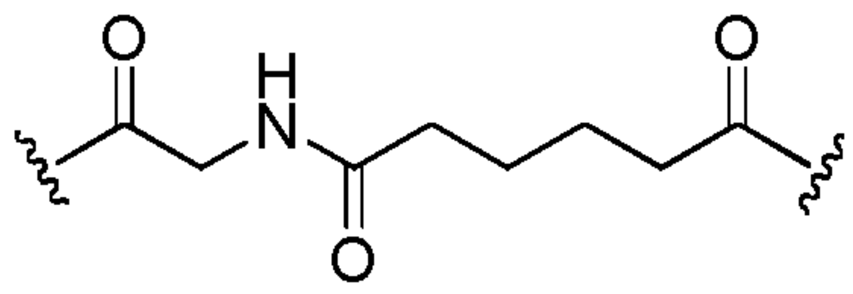
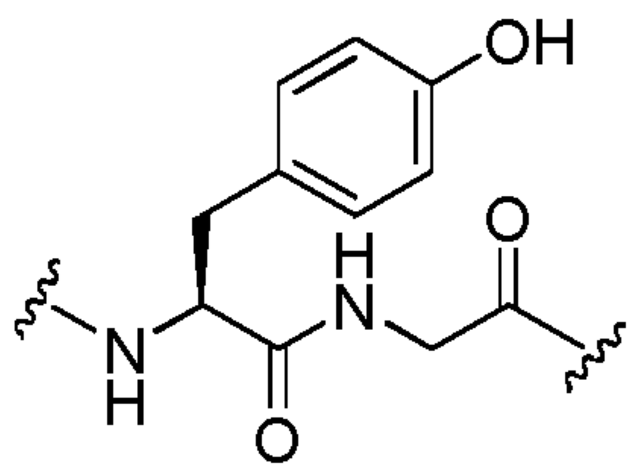
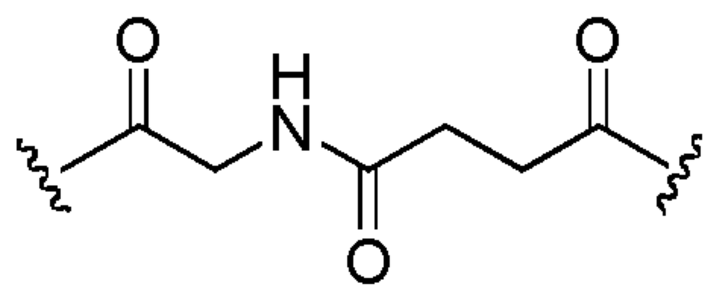
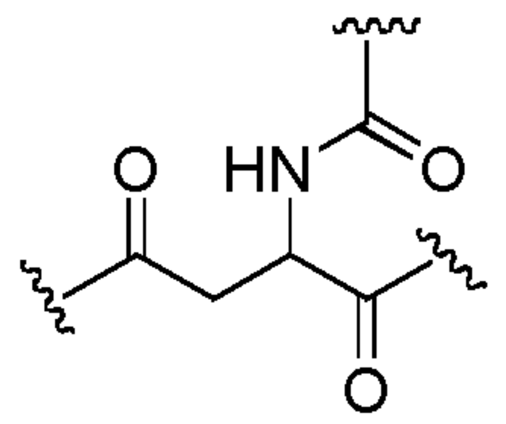
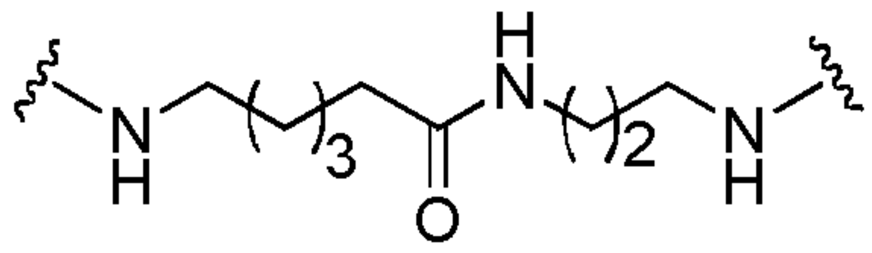
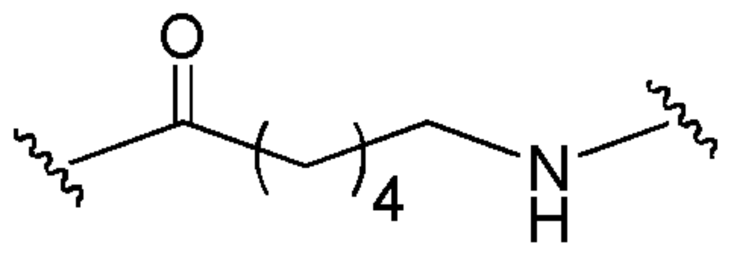
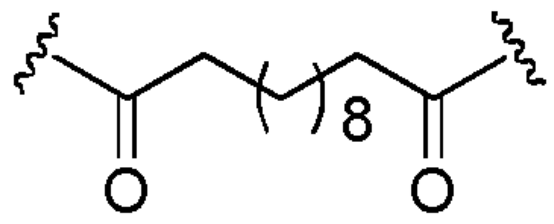
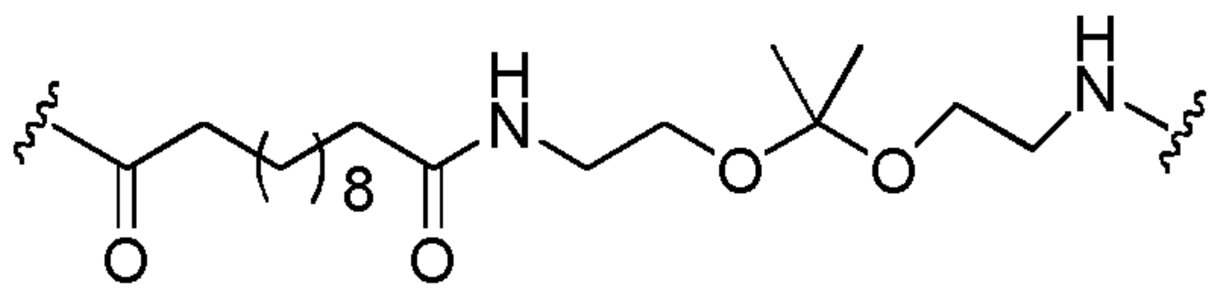
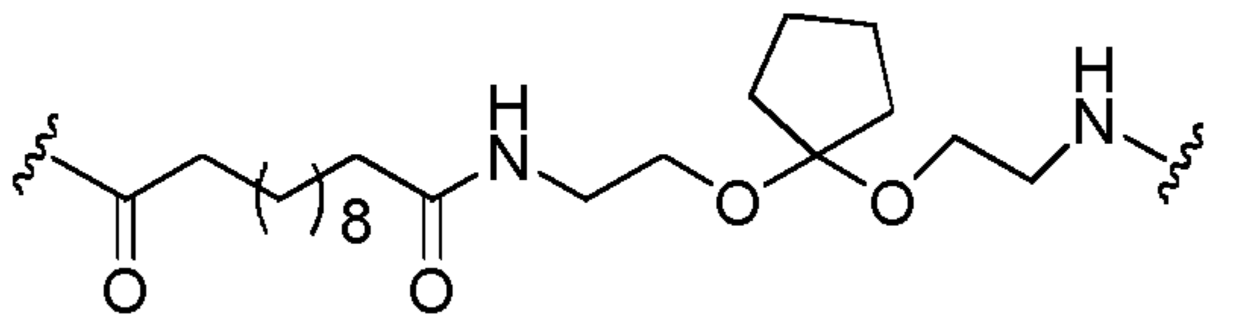
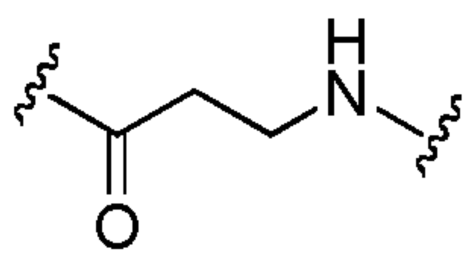
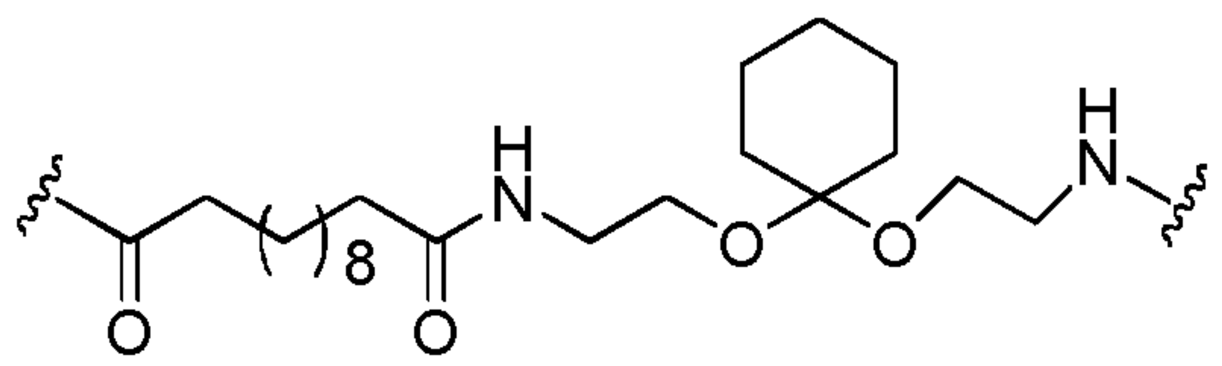
In certain embodiments, a linker has a structure selected from among:

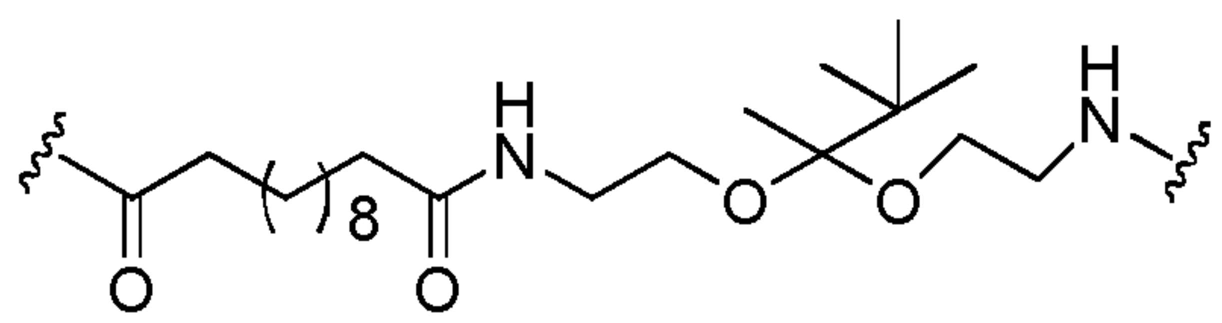
 ;  ;  ;

 ;  ;  ;

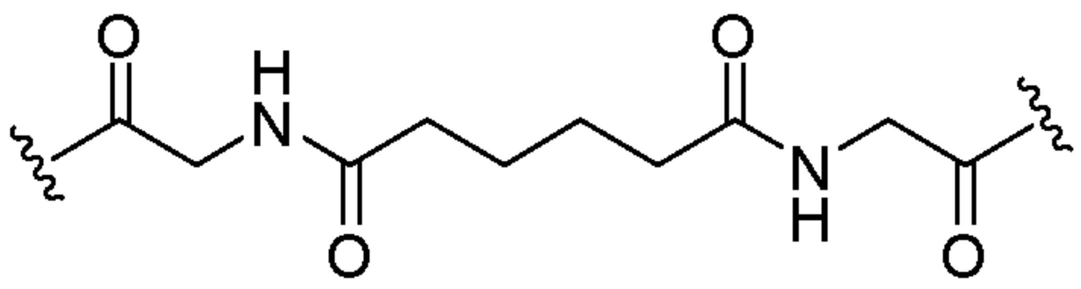
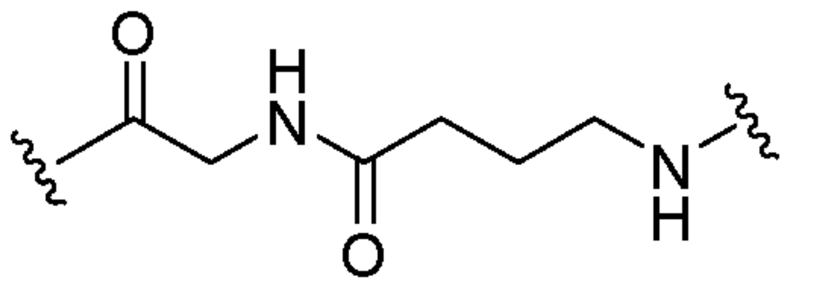
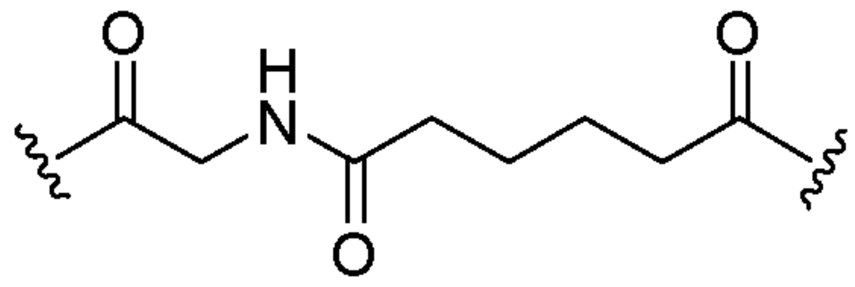
 ;  ;  ;

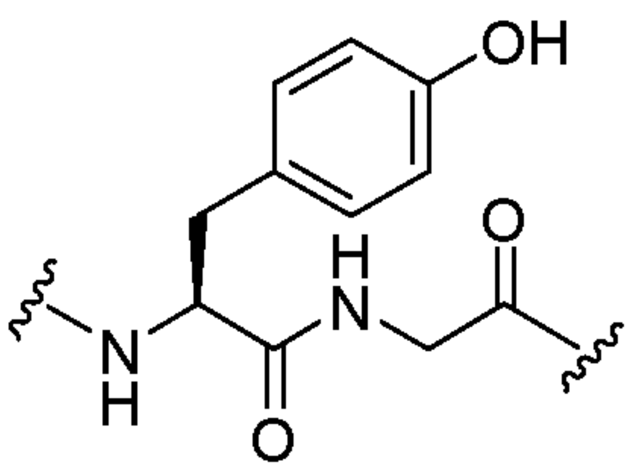
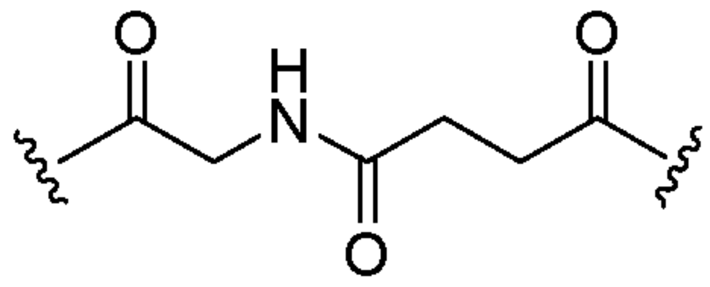
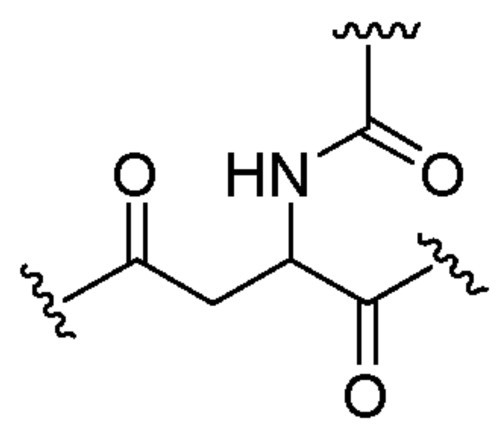
 ;  ;

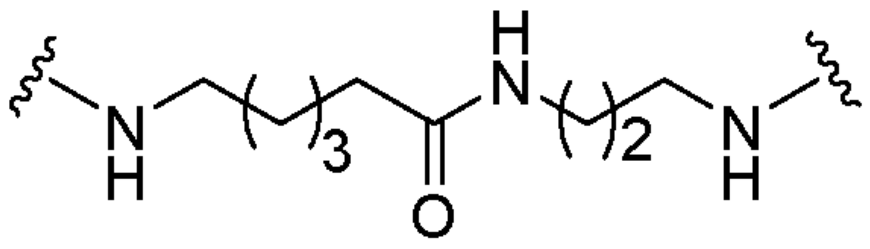
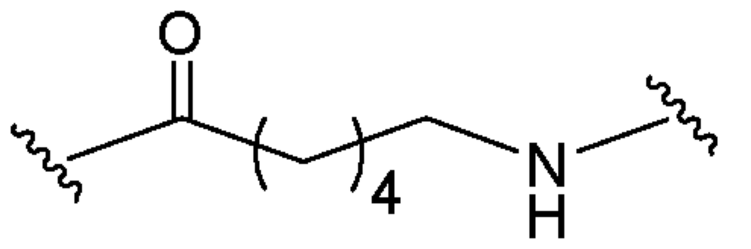
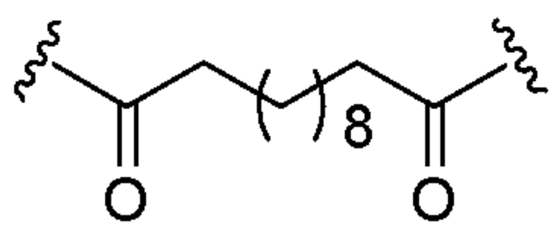
 ;  ; and

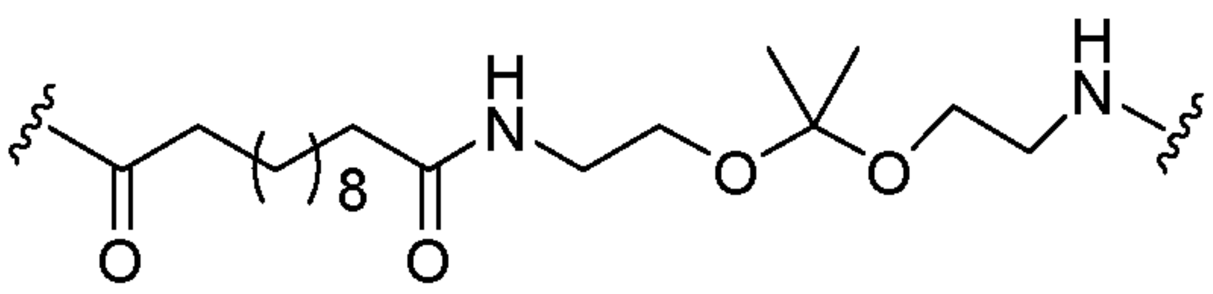
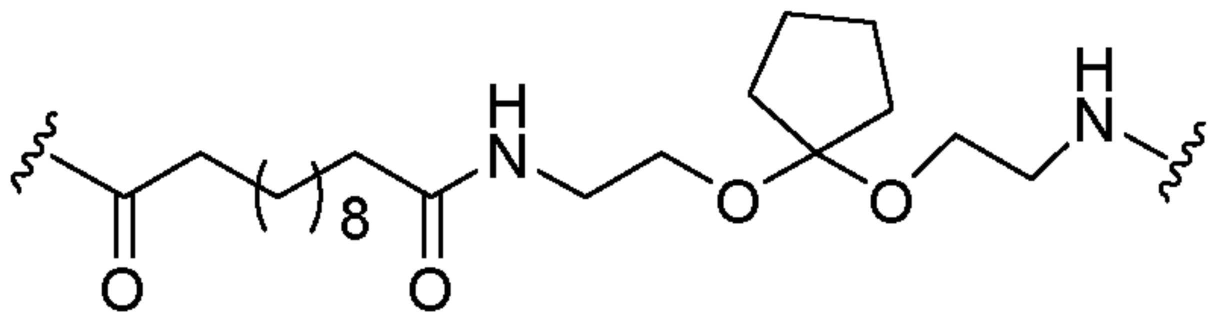
 .

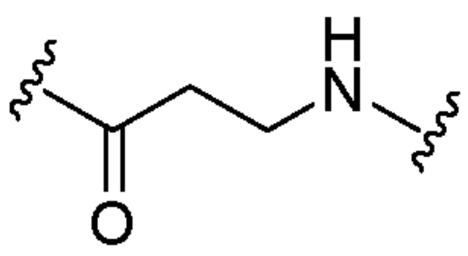
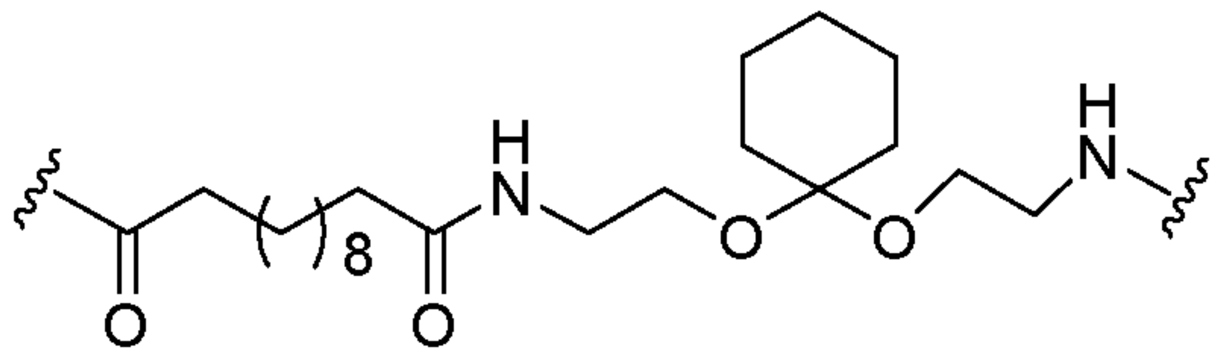
In certain embodiments, a linker has a structure selected from among:

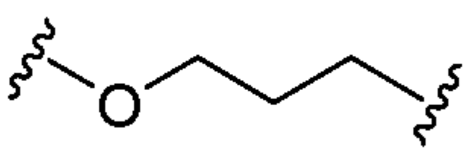
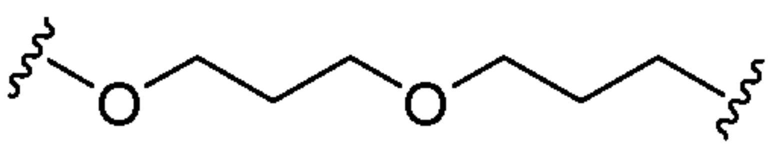
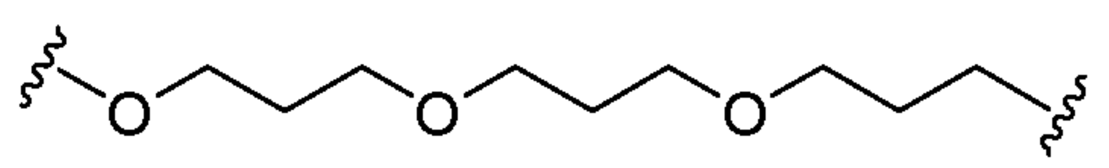
 ;  ;  ;

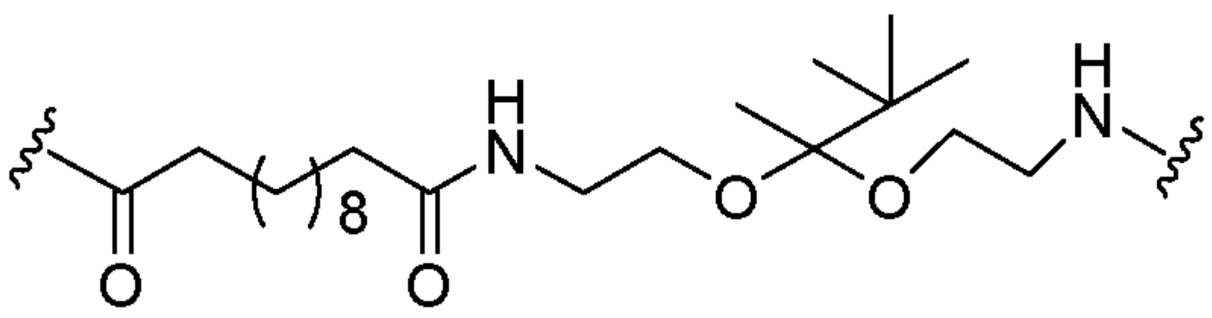
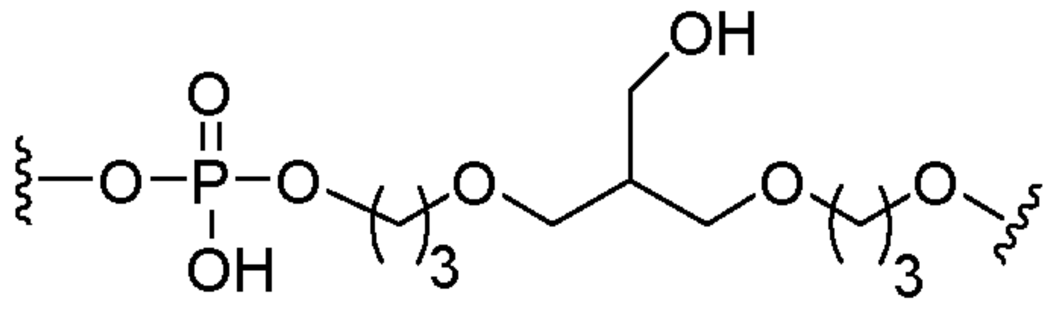
 ;  ;  ;

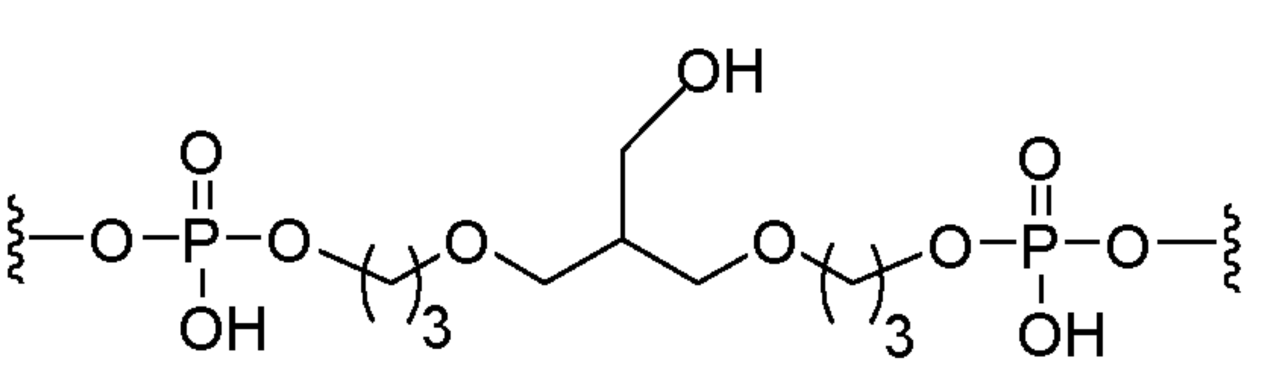
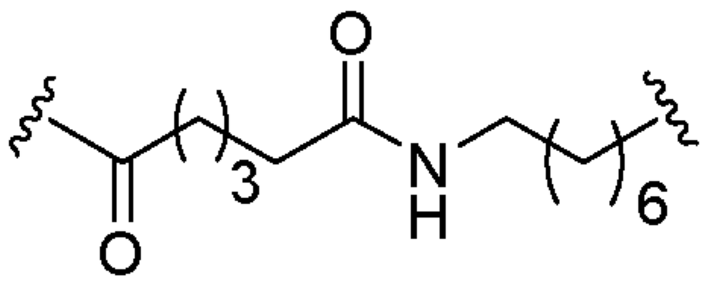
 ;  ;  ;

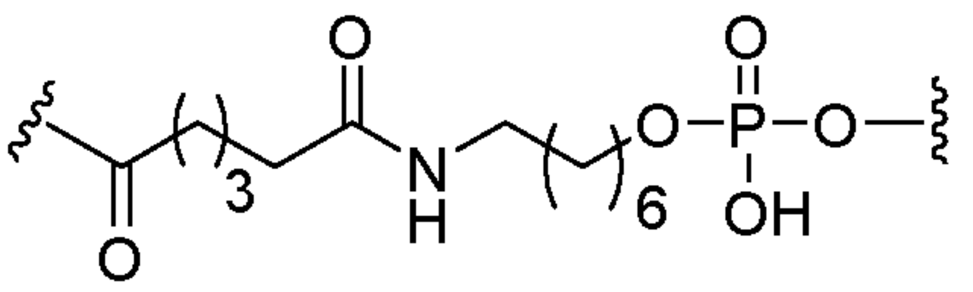
 ;  ;

 ; 

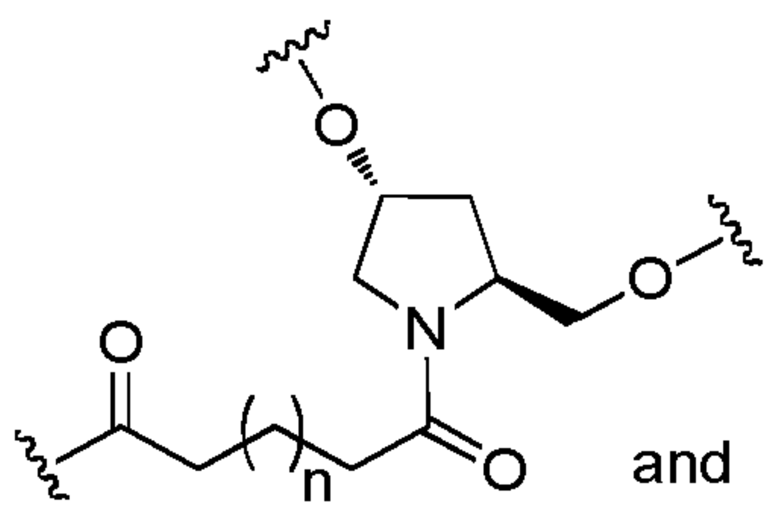
 ;  ;  ;

 ;  ;

 ;  and

 .

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



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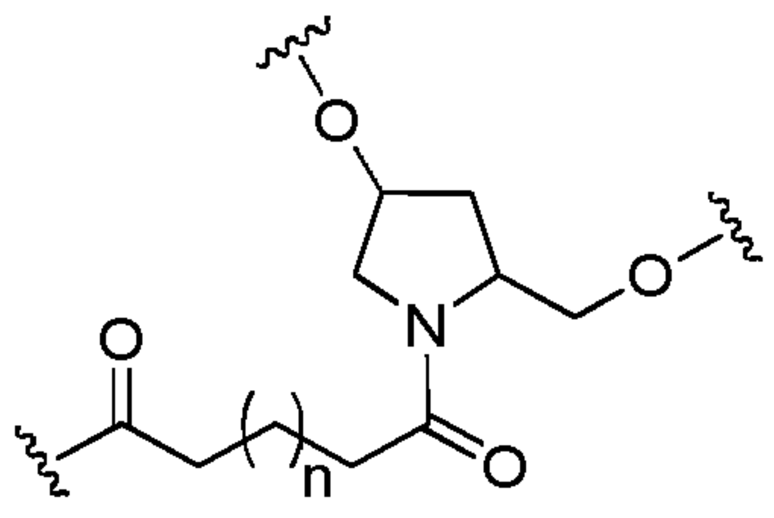
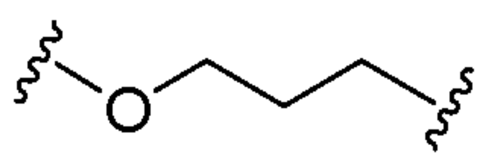
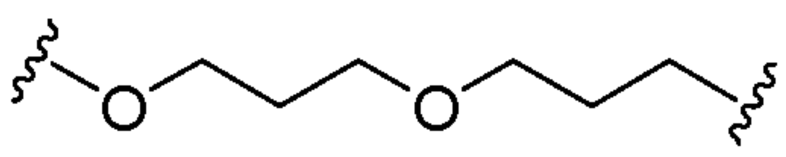
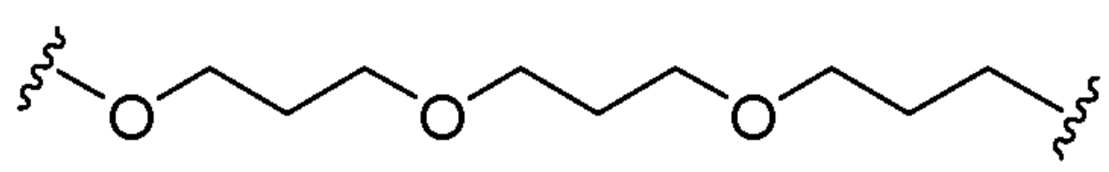
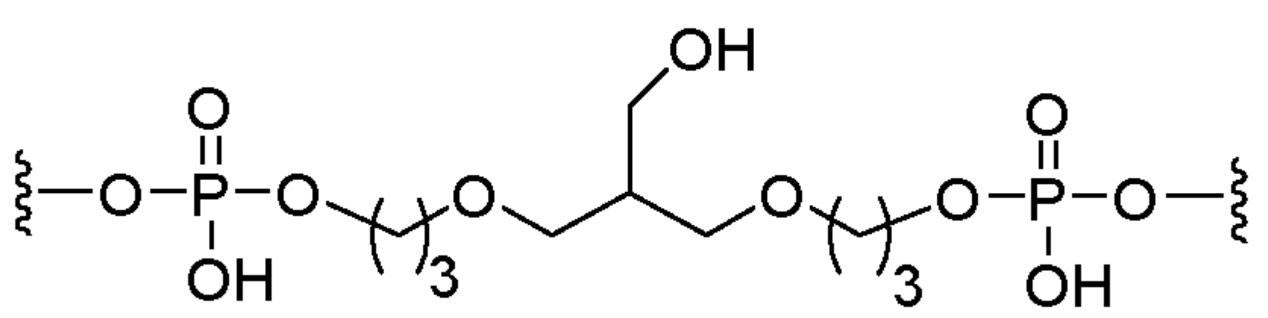
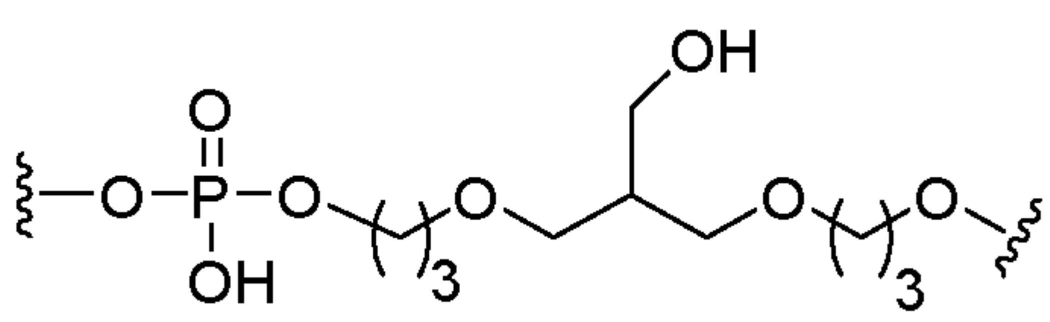


wherein n is from 1 to 20.

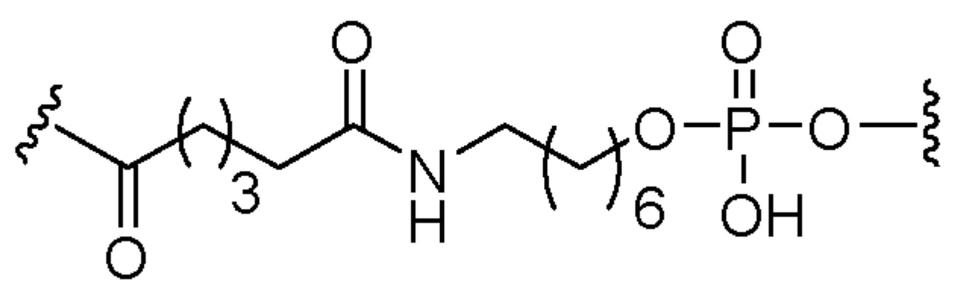
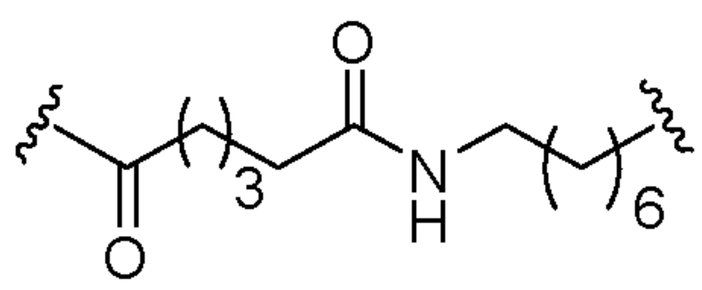
In certain embodiments, a linker has a structure selected from among:

 ;  ; and  .

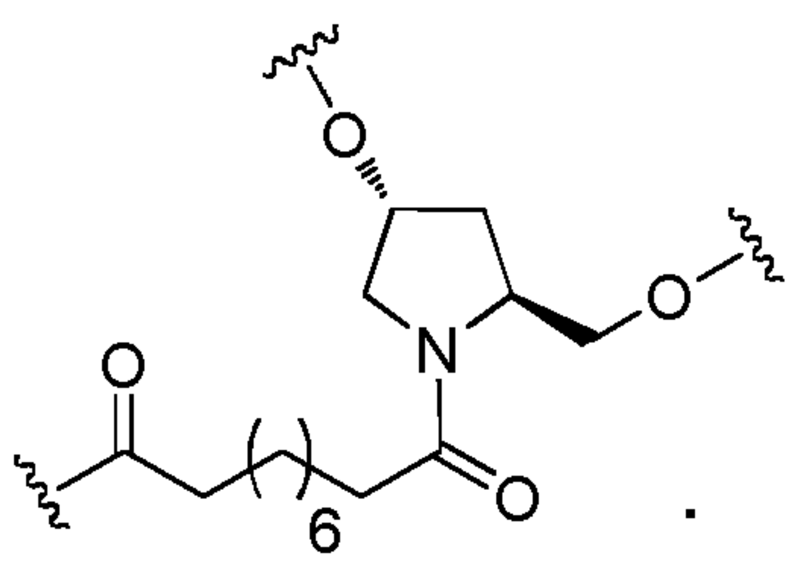
In certain embodiments, a linker has a structure selected from among:

 and  .

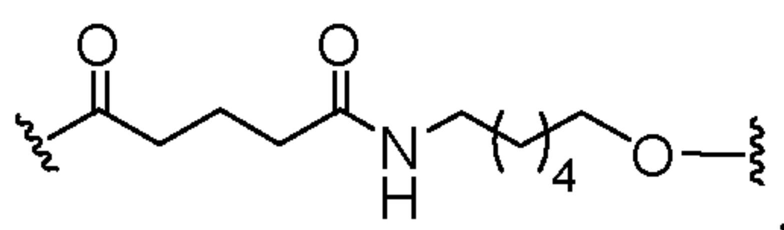
In certain embodiments, a linker has a structure selected from among:

 and  .

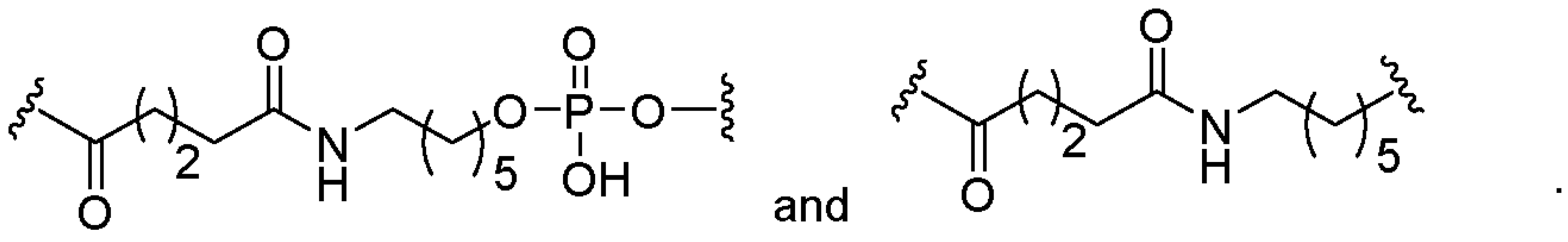
In certain embodiments, the conjugate linker has the structure:

 .

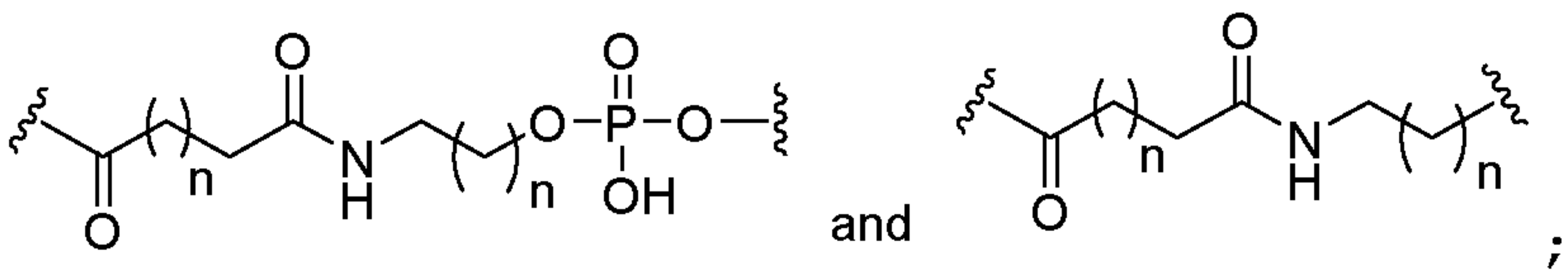
In certain embodiments, the conjugate linker has the structure:

 .

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.

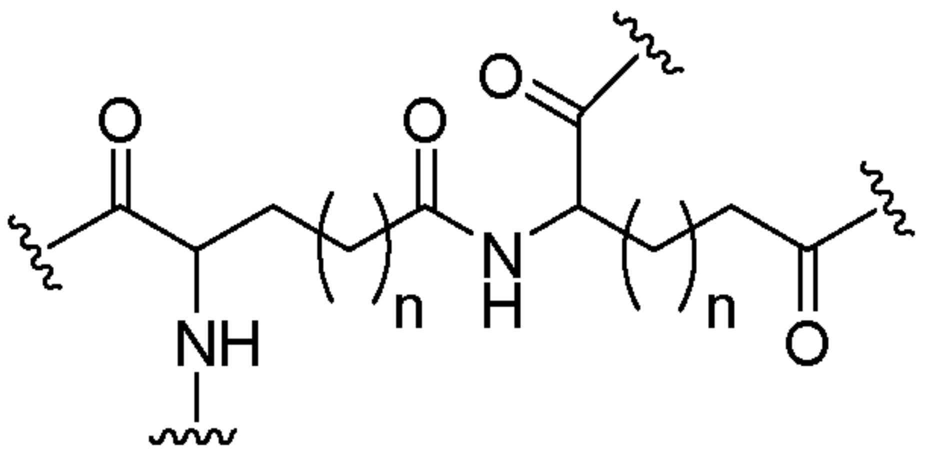
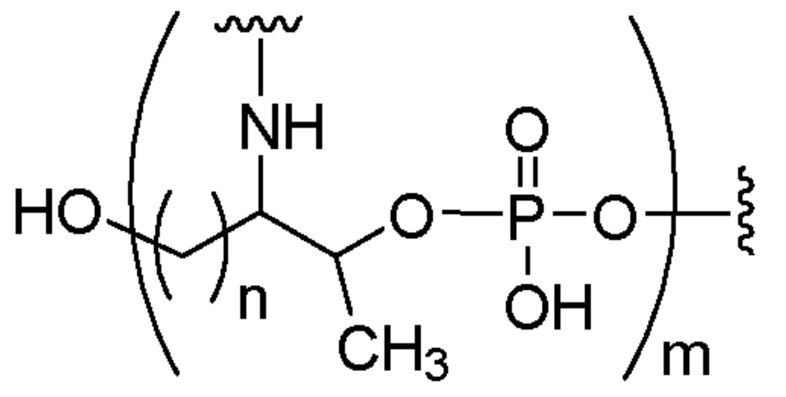
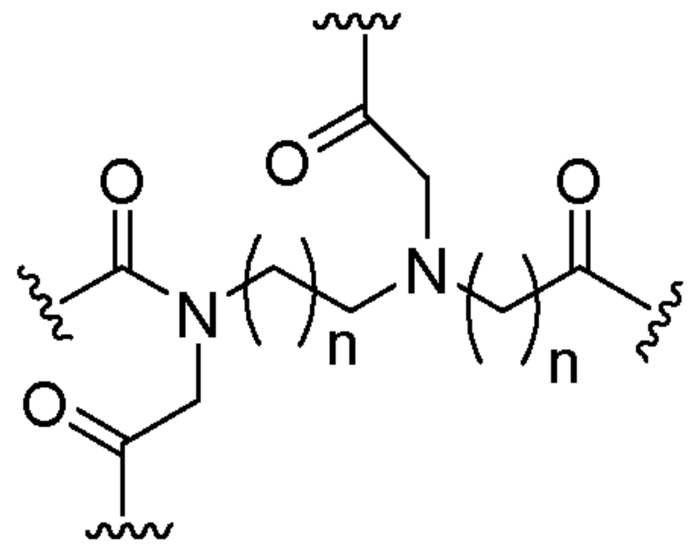
iii. Certain Cell-Targeting Moieties

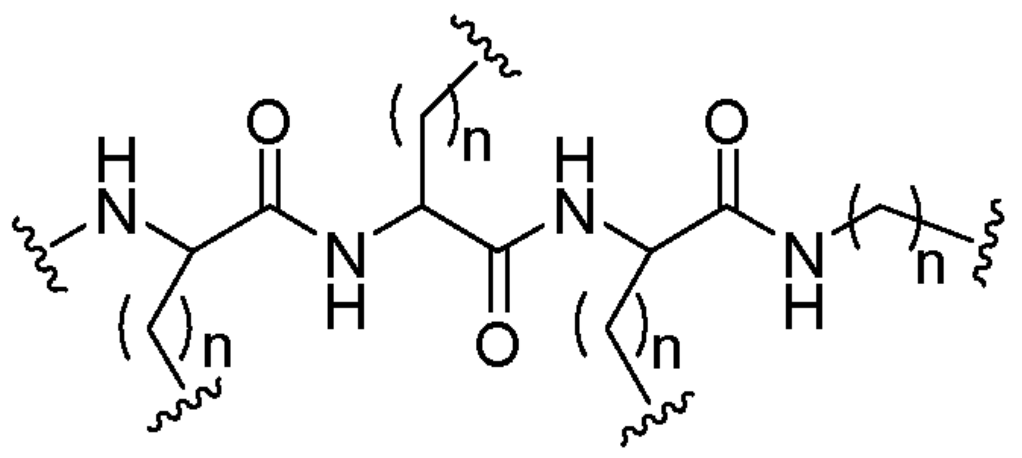
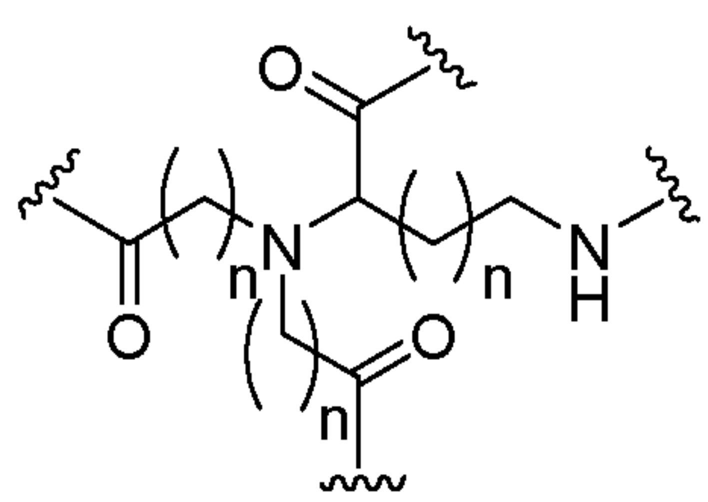
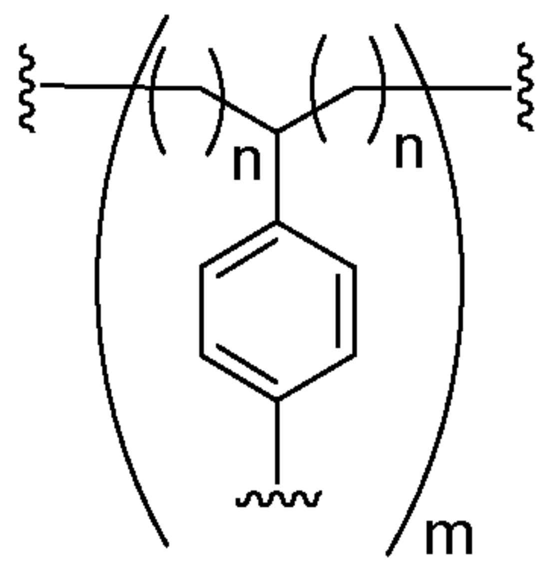
In certain embodiments, conjugate groups comprise cell-targeting moieties. Certain such cell-targeting moieties increase cellular uptake of antisense compounds. In certain embodiments, cell-targeting moieties comprise a branching group, one or more tether, and one or more ligand. In certain embodiments,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

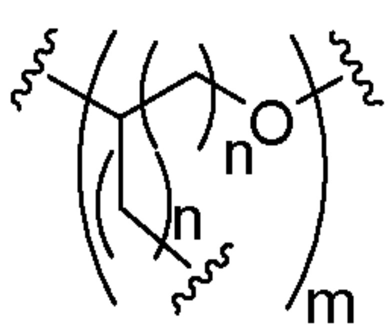
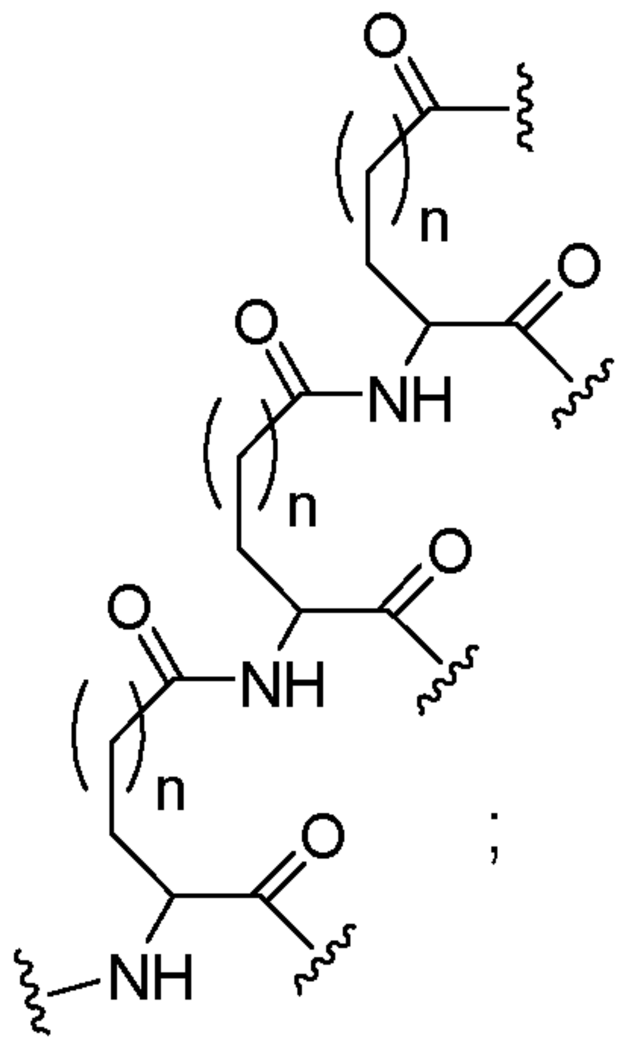
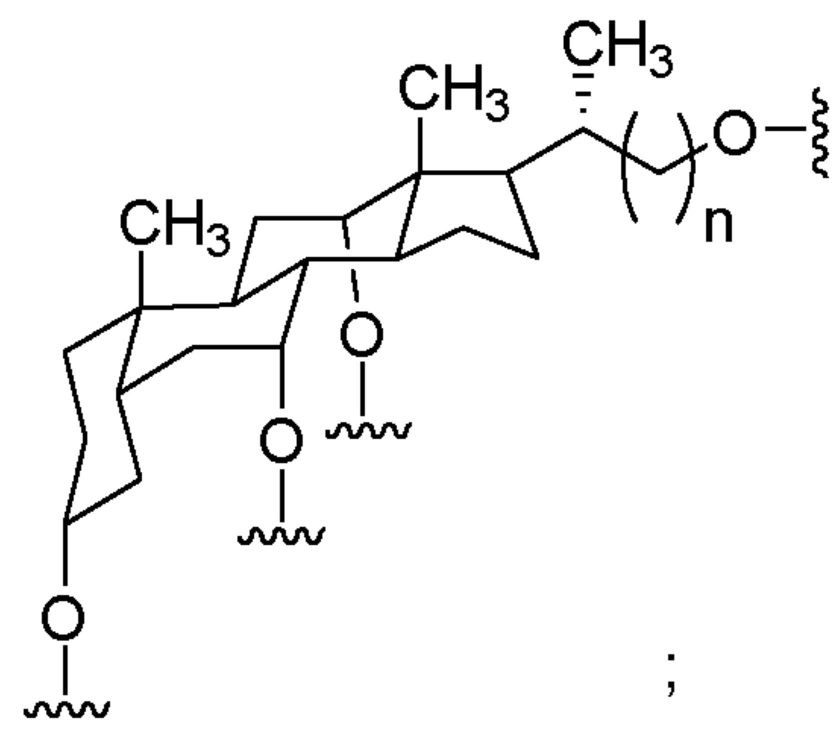
1. Certain Branching Groups

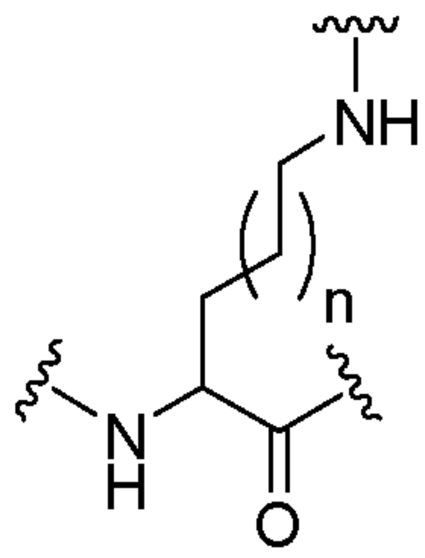
In certain embodiments, the conjugate groups comprise a targeting moiety comprising a branching group and at least two tethered ligands. In certain embodiments, the branching group attaches the conjugate linker. In certain embodiments, the branching group attaches the cleavable moiety. In certain embodiments, 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.

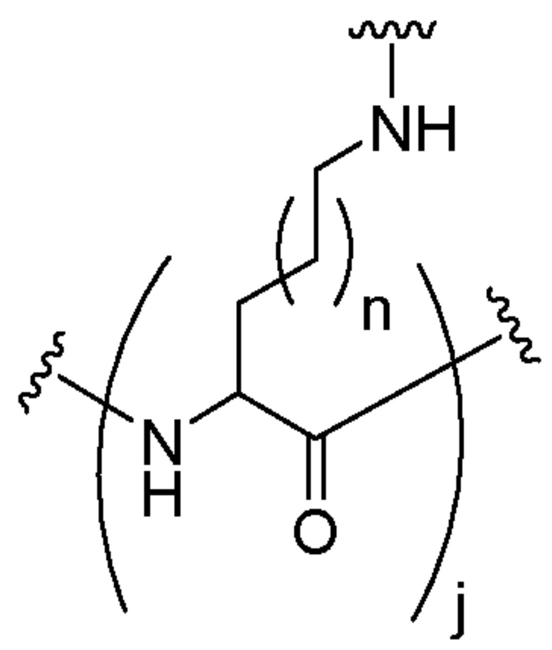
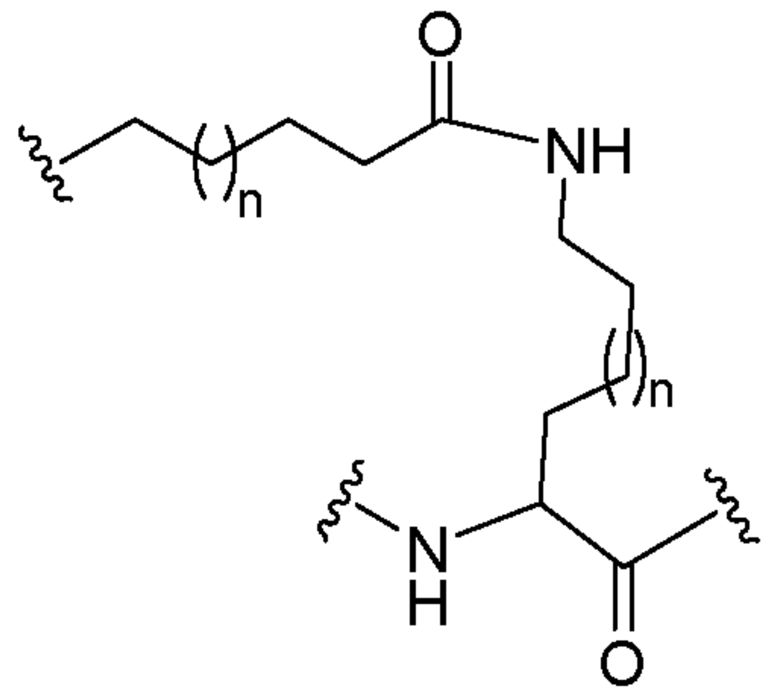
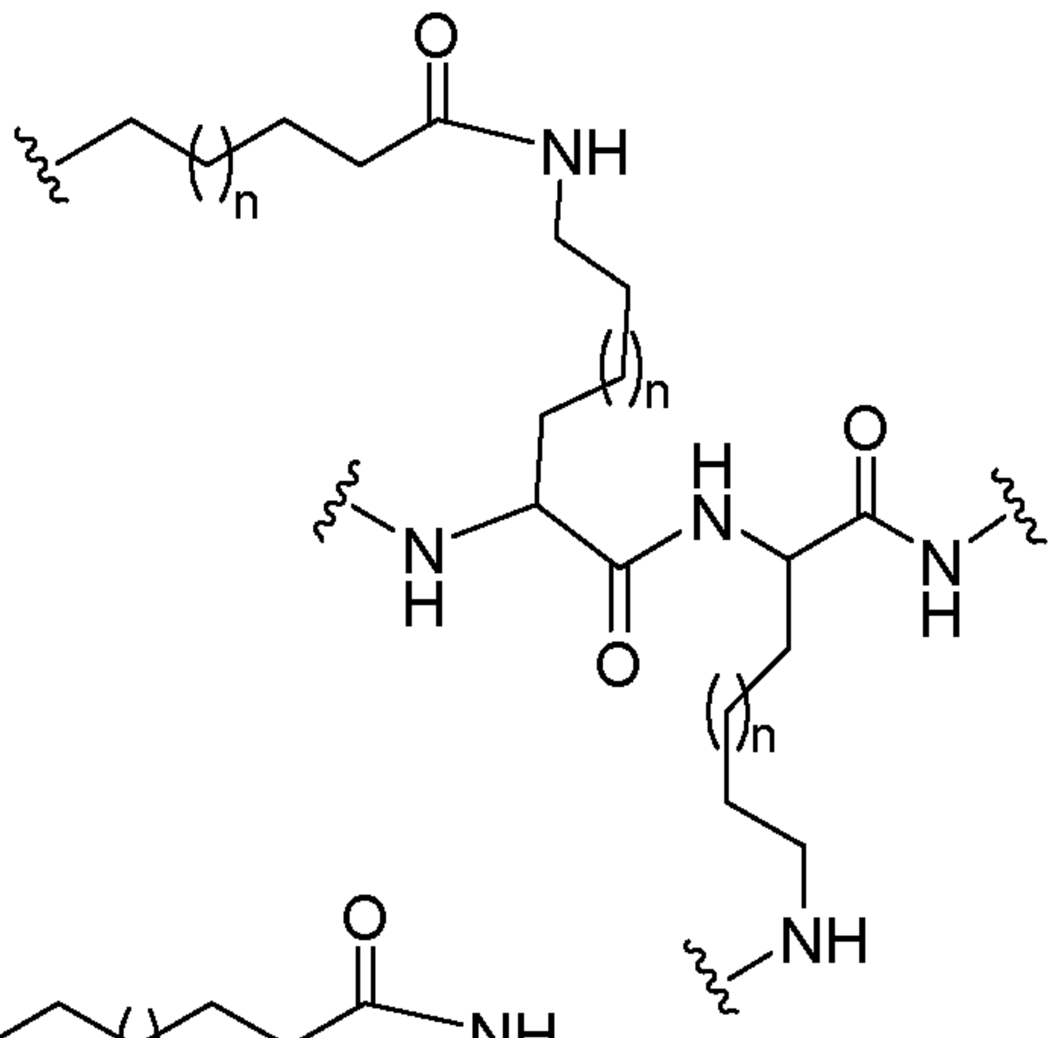
In certain embodiments, a branching group has a structure selected from among:

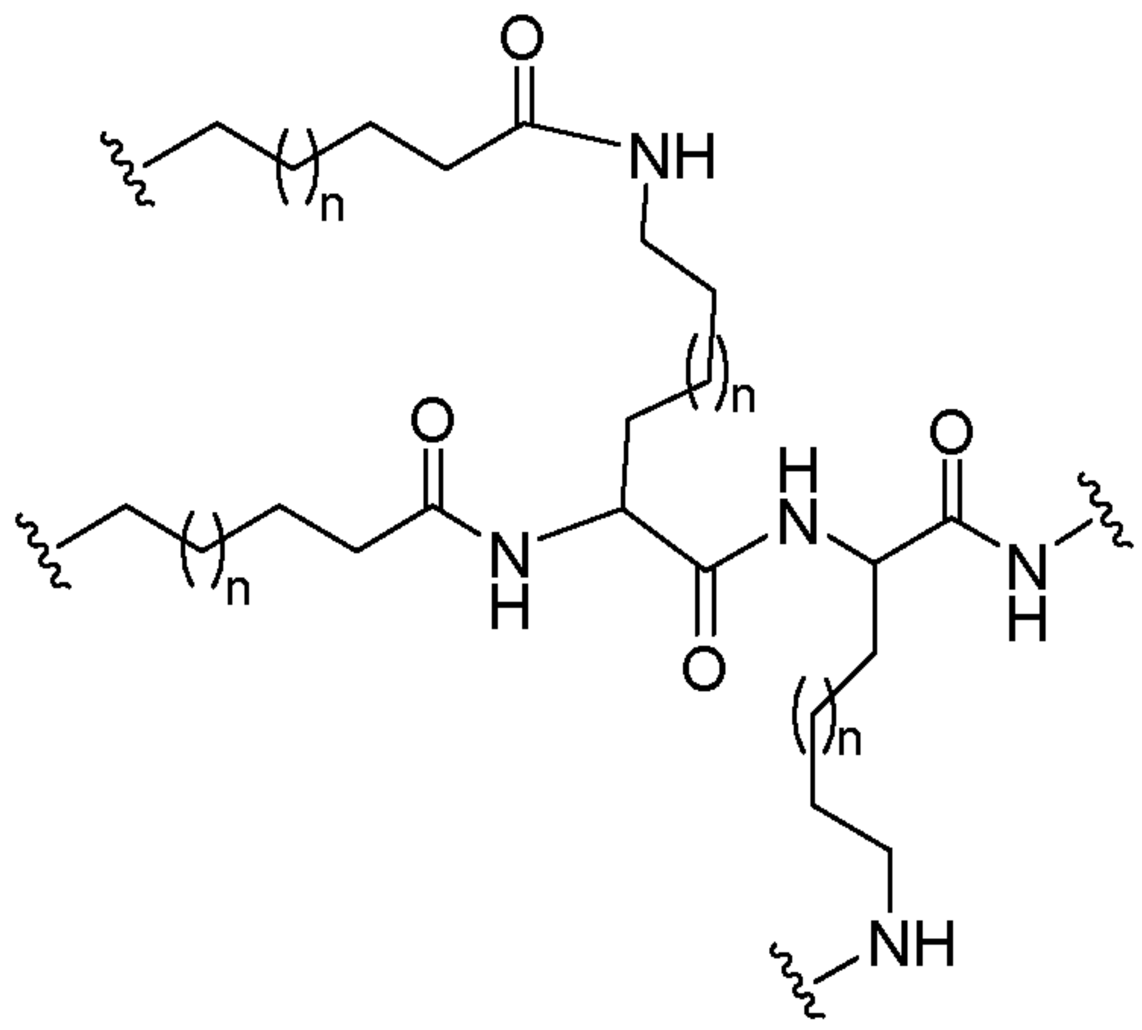
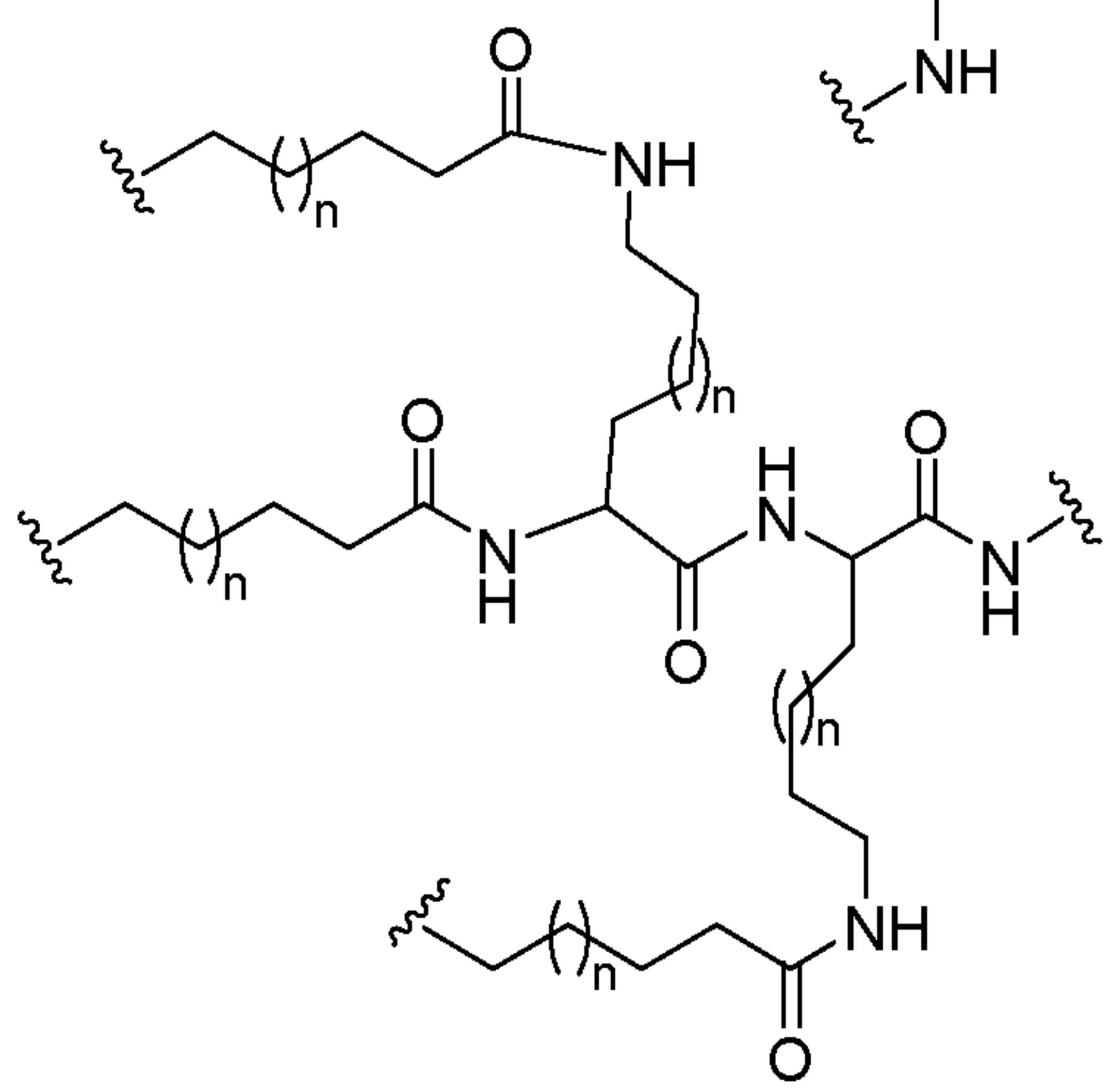
 ;  ;  ;

 ;  ;  ;

 ;  ;  ;

 ;

 ;  ;  ;

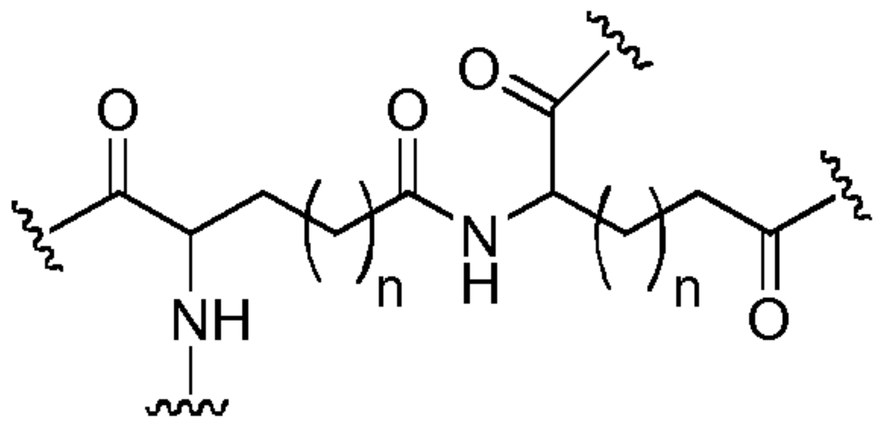
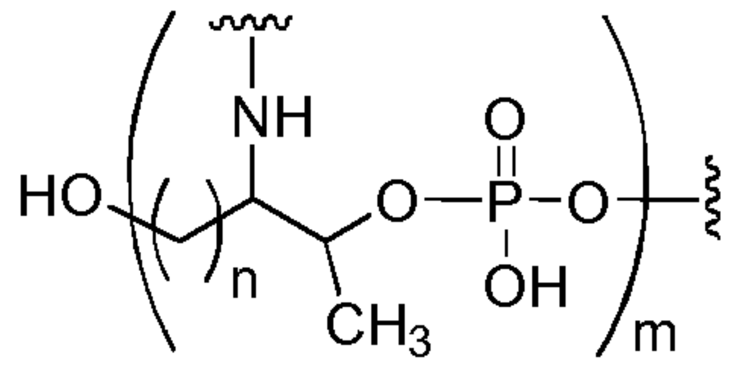
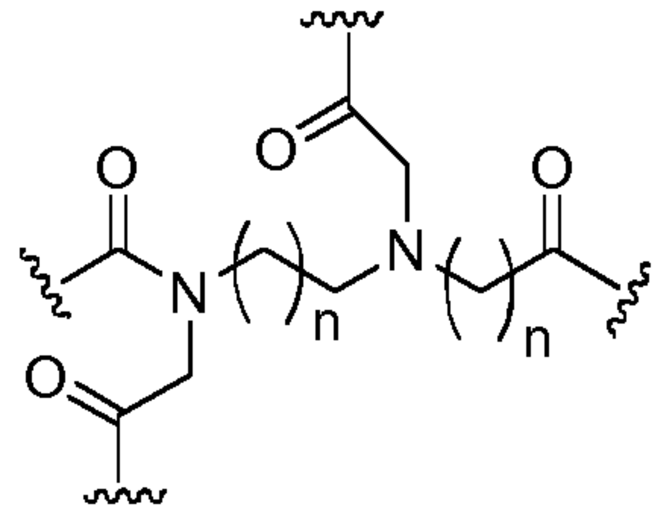
 ; and  .

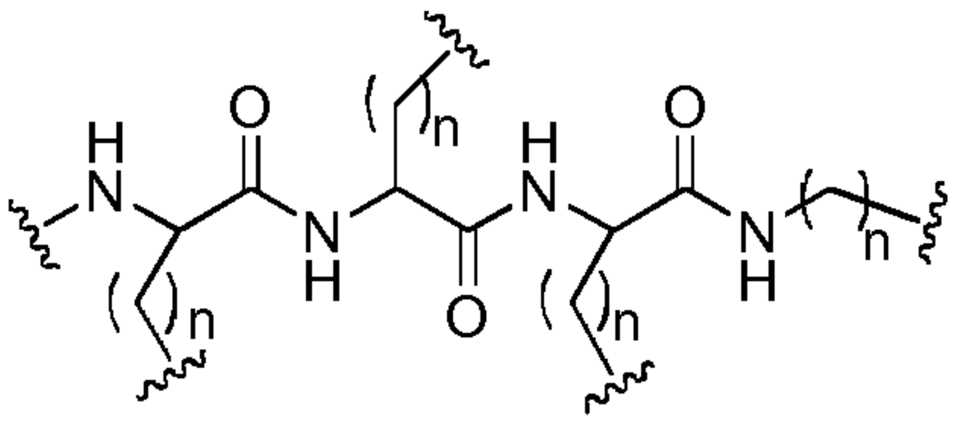
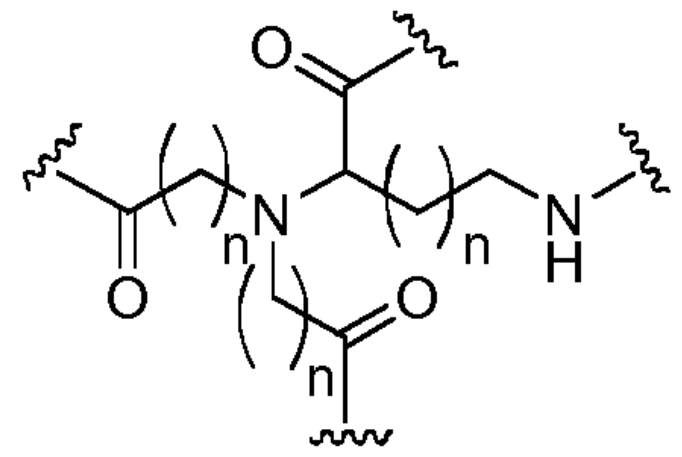
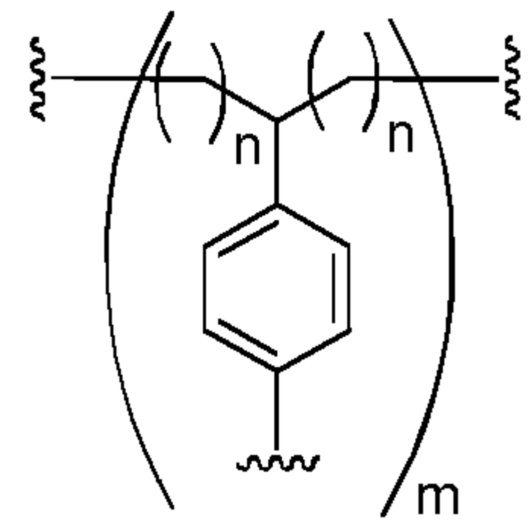
wherein each n is, independently, from 1 to 20;

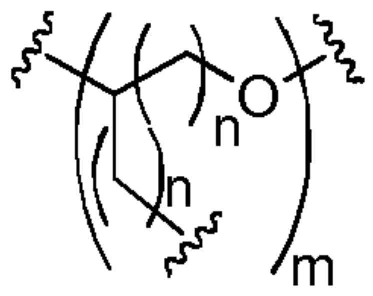
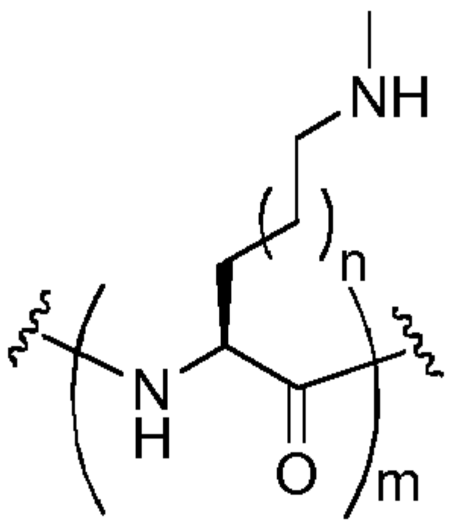
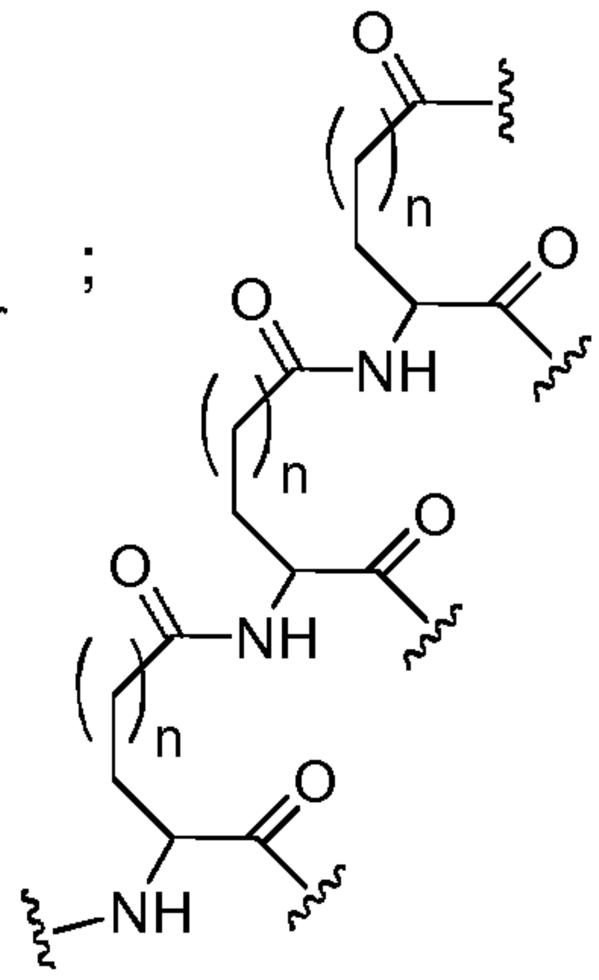
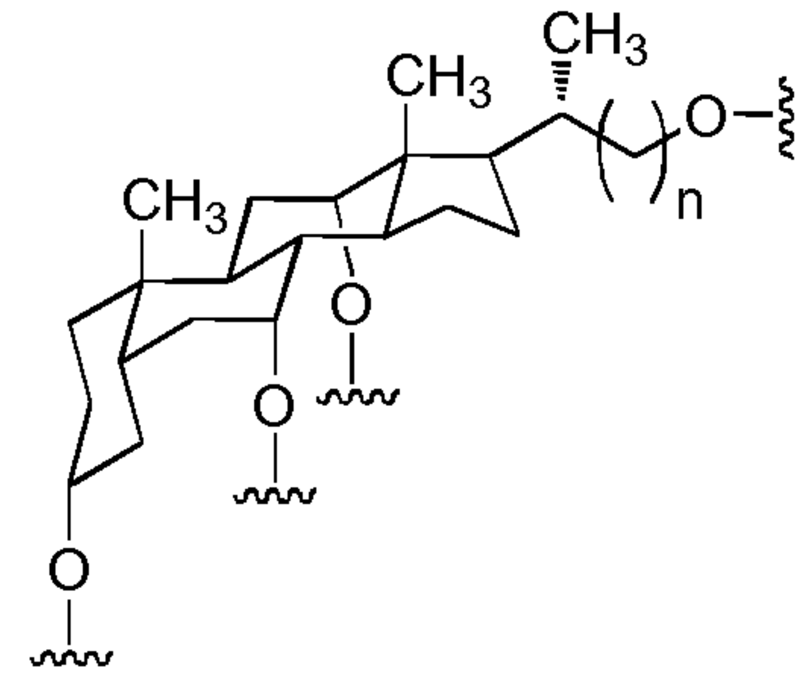
j is from 1 to 3; and

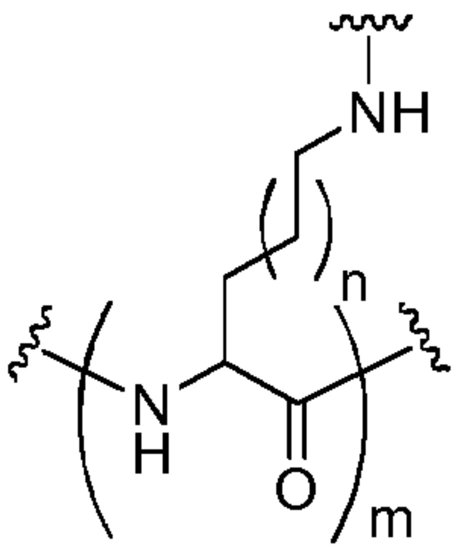
m is from 2 to 6.

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

 ;  ;  ;

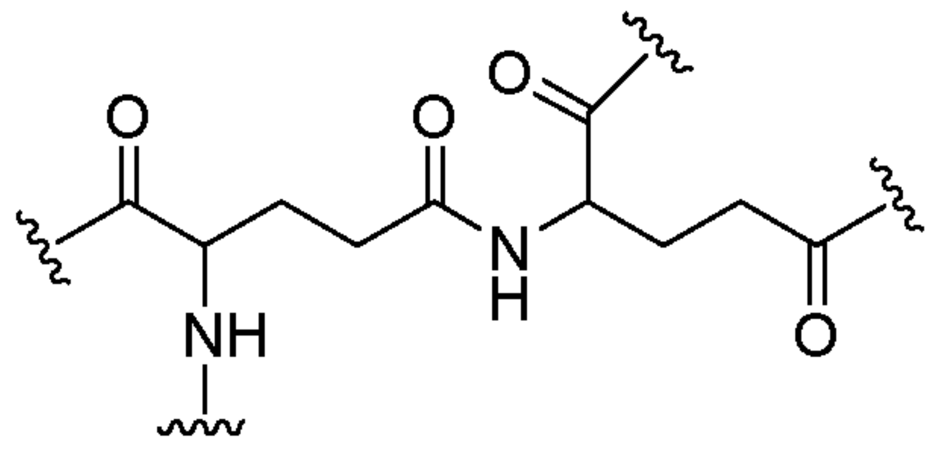
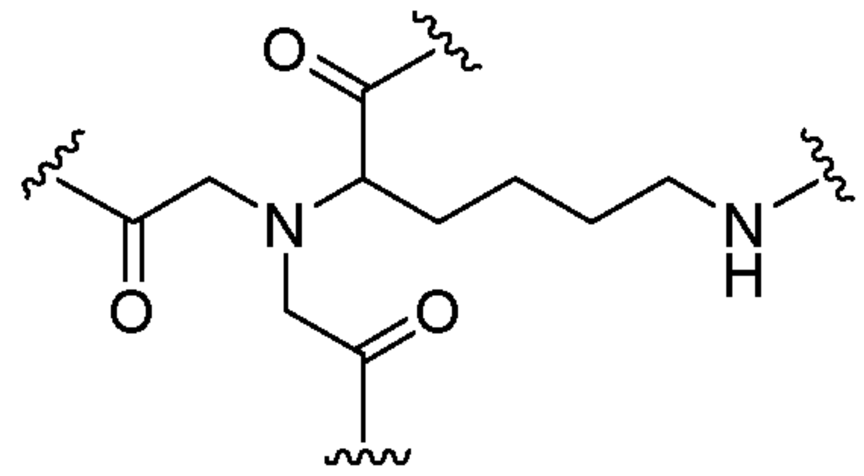
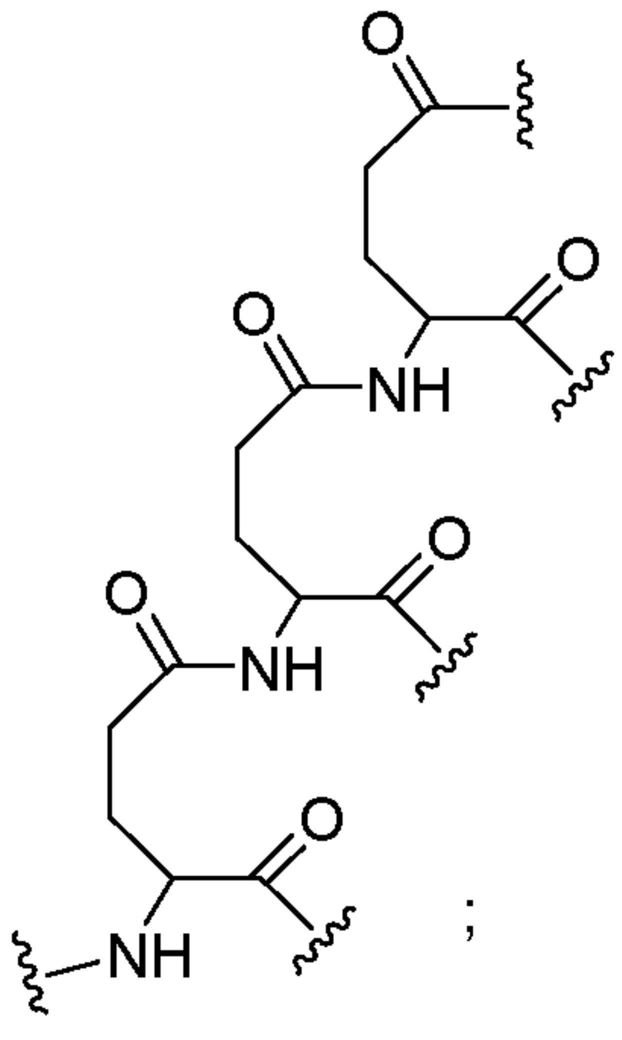
 ;  ;  ;

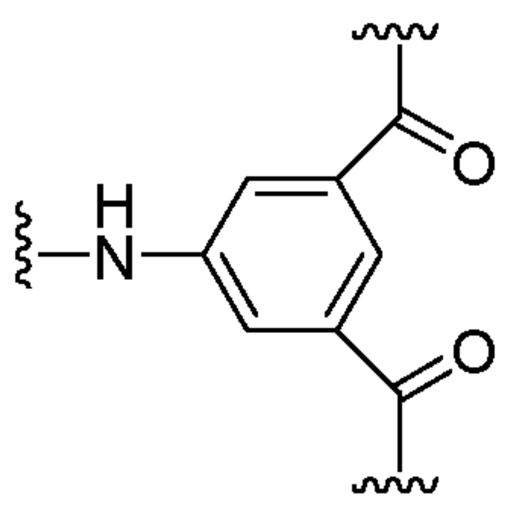
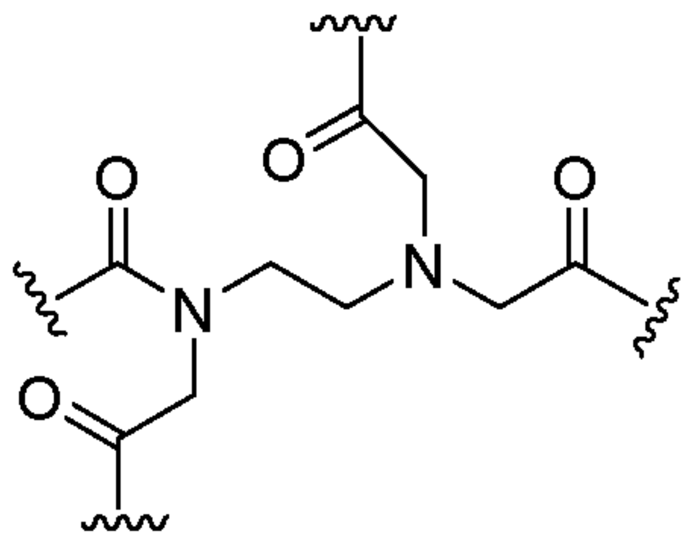
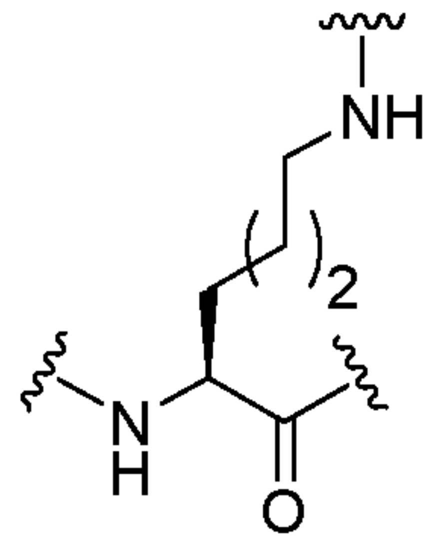
 ;  ;  ;  ; and

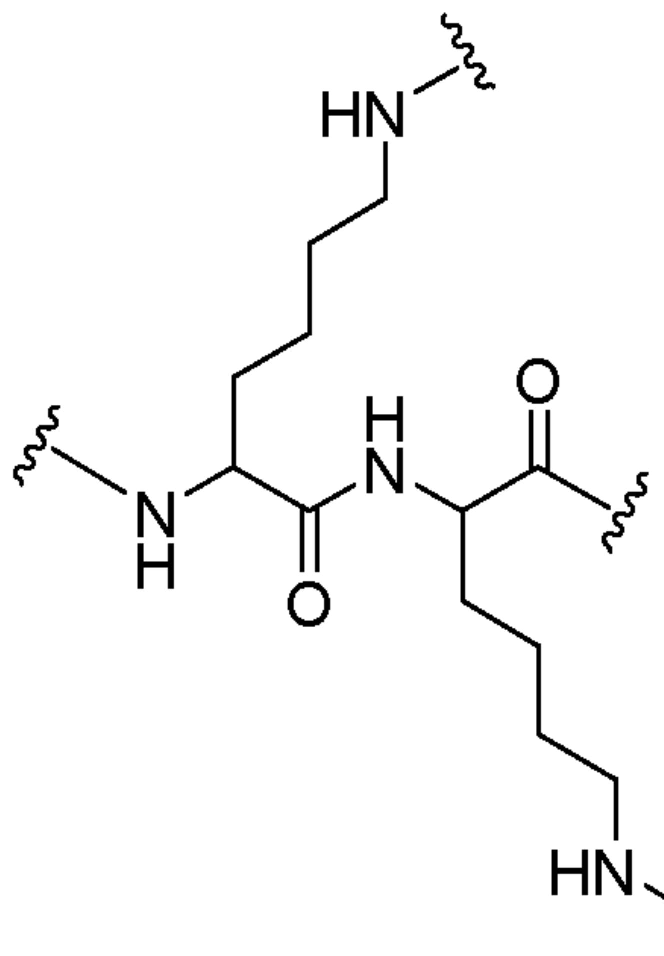
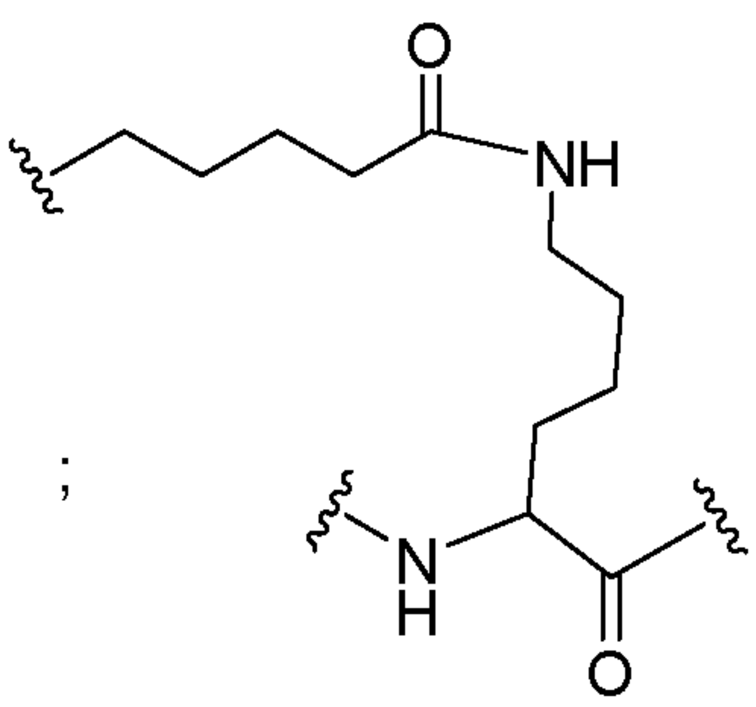
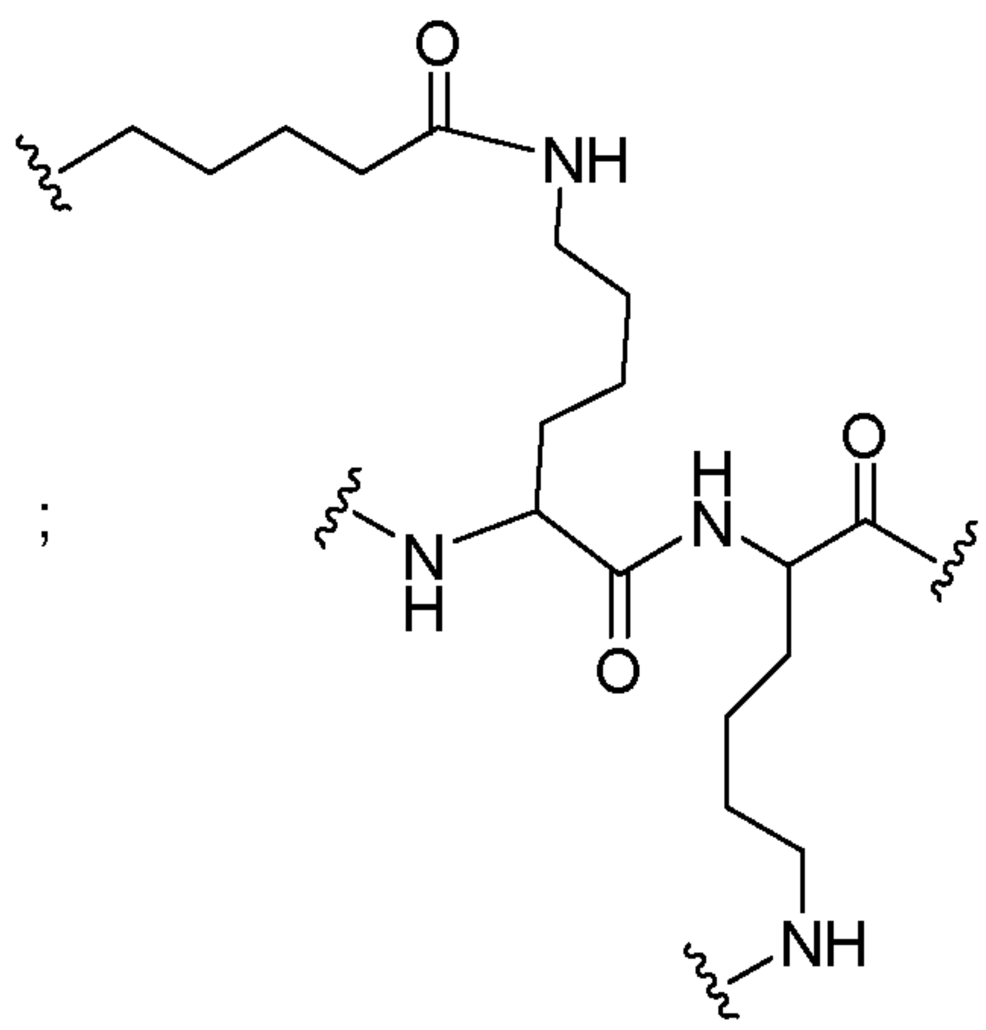


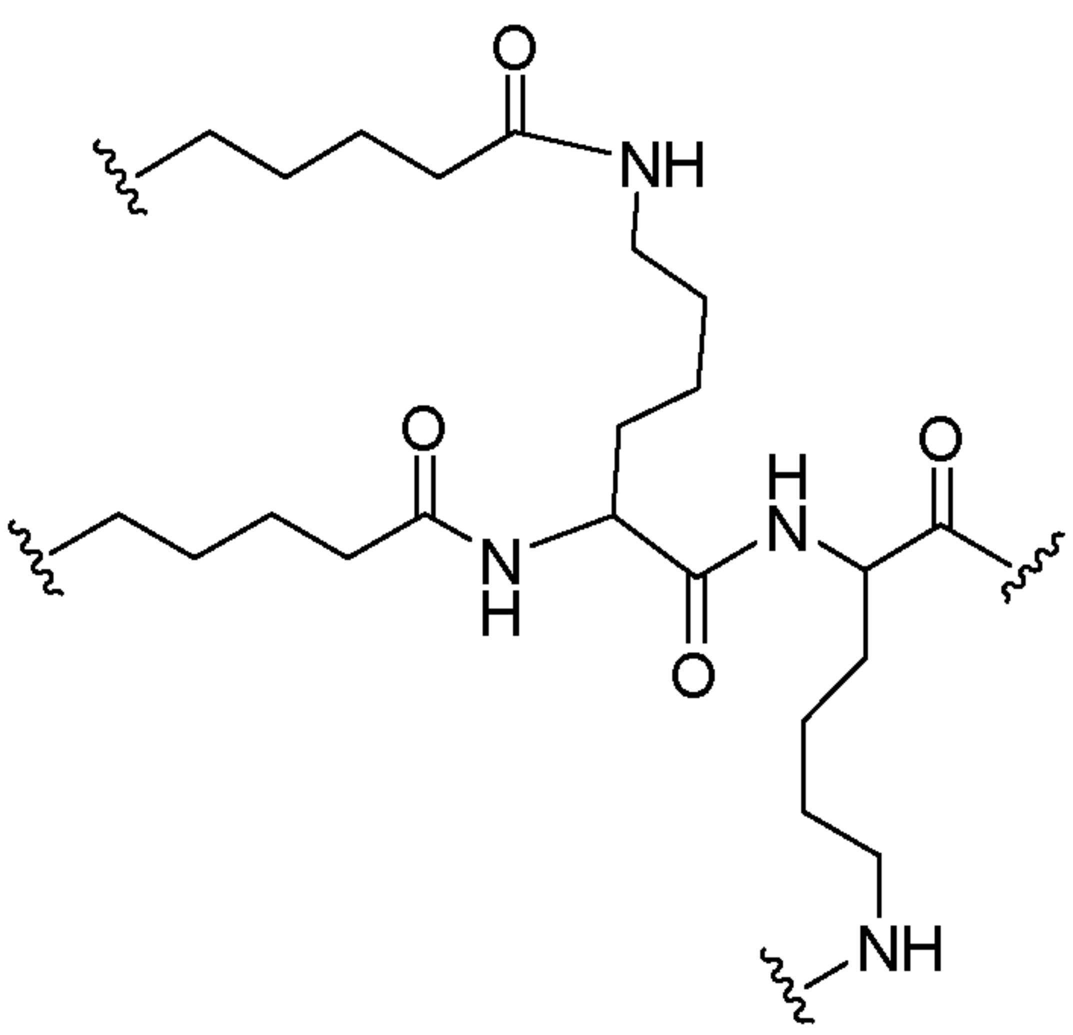
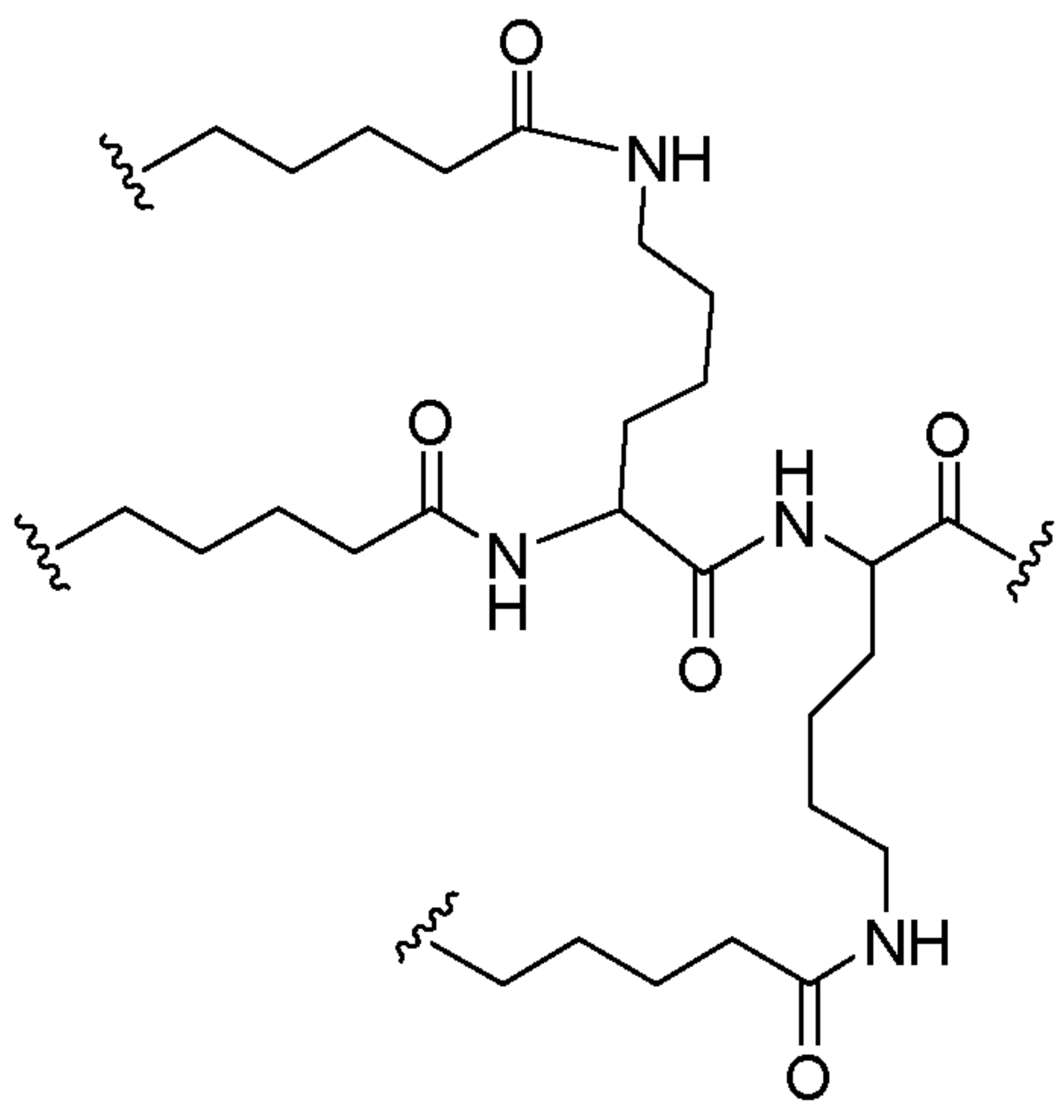
wherein each n is, independently, from 1 to 20; and
m is from 2 to 6.

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

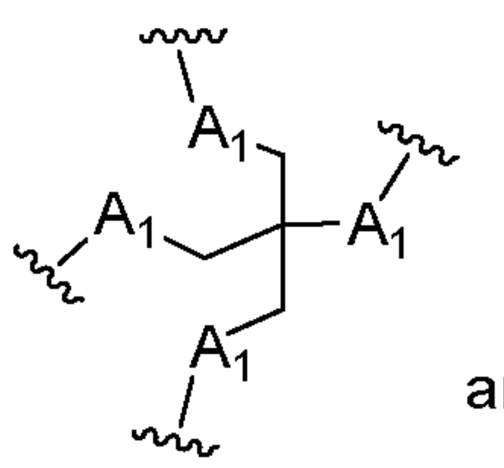
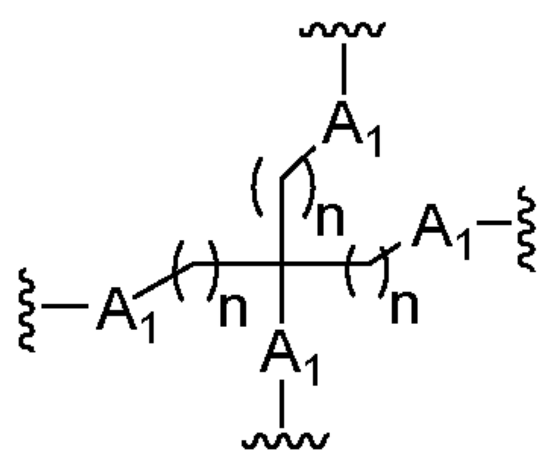
 ;  ;  ;

 ;  ;  ;

 ;  ;  ;

 ; and  .

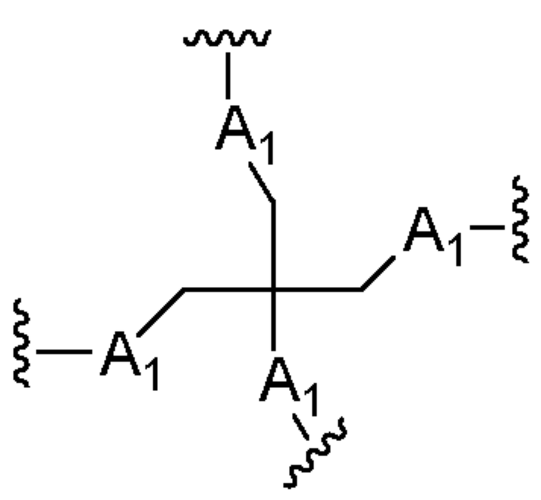
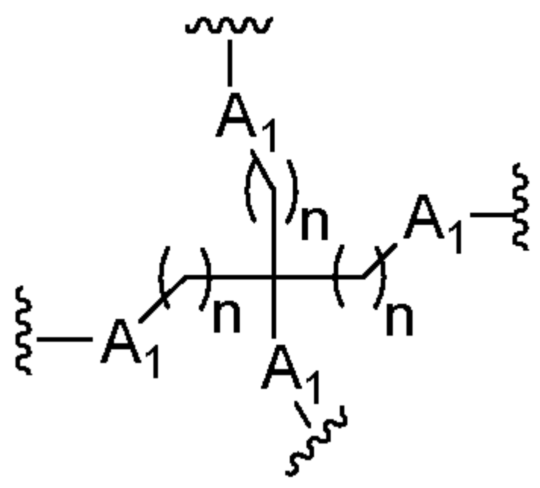
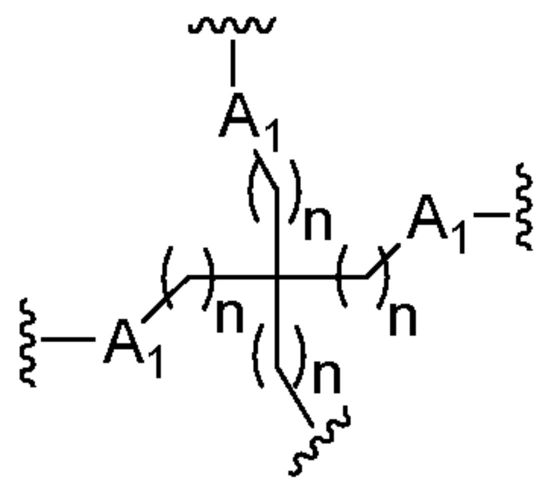
In certain embodiments, a branching group has a structure selected from among:

 and 

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

each n is, independently, from 1 to 20.

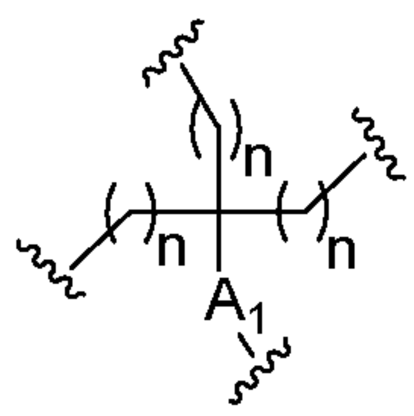
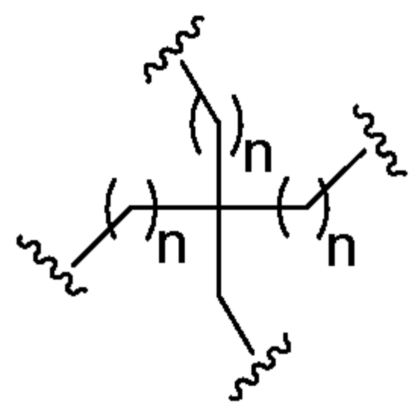
In certain embodiments, a branching group has a structure selected from among:

 ;  and 

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

each n is, independently, from 1 to 20.

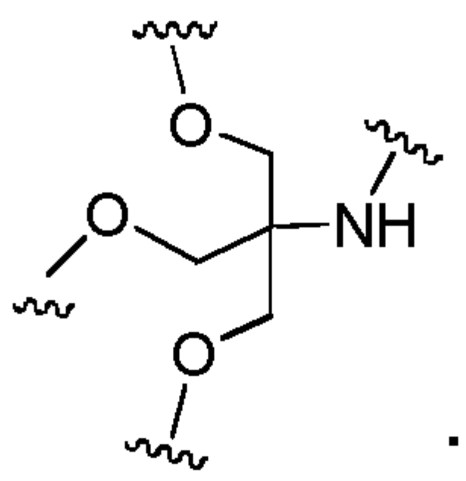
In certain embodiments, a branching group has a structure selected from among:

 and 

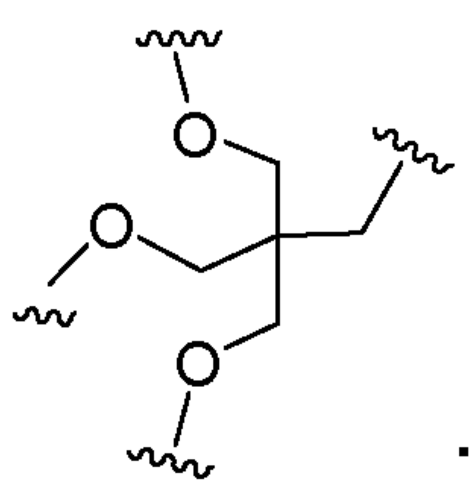
wherein A_1 is O, S, C=O or NH; and

each n is, independently, from 1 to 20.

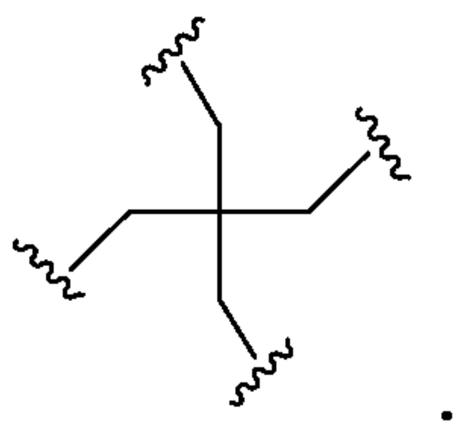
In certain embodiments, a branching group has a structure selected from among:

 .

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

 .

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



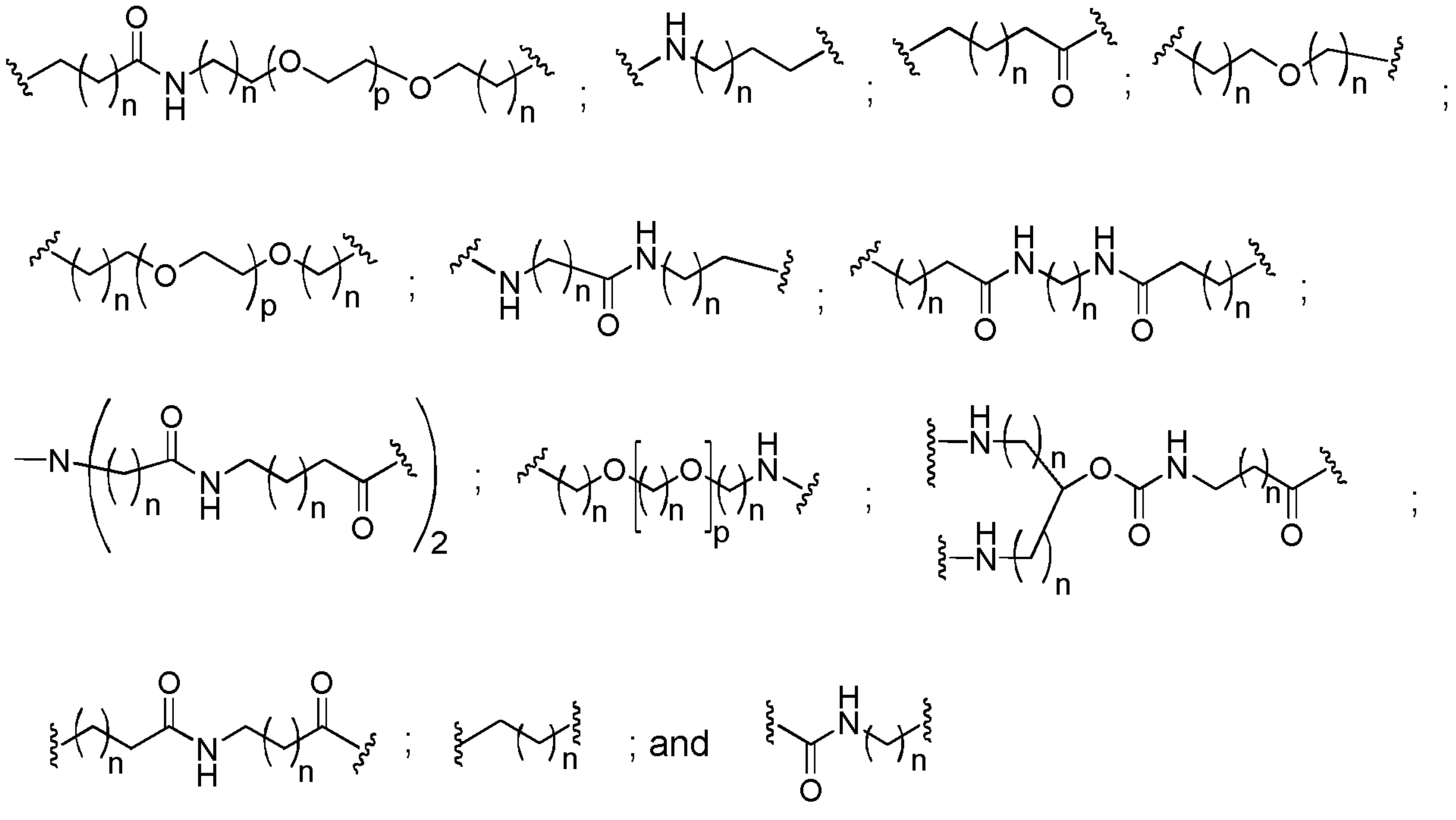
2. Certain Tethers

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.

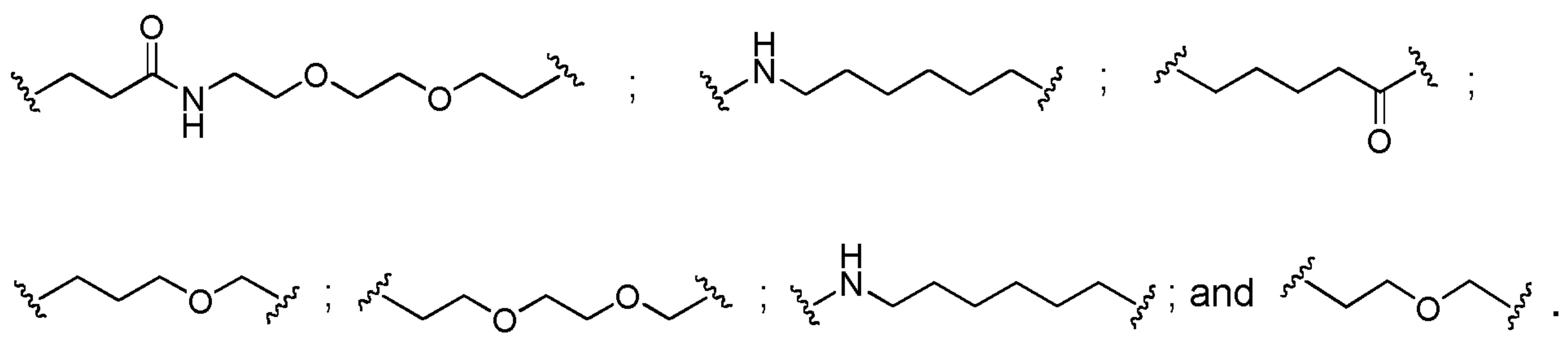
In certain embodiments, a tether has a structure selected from among:



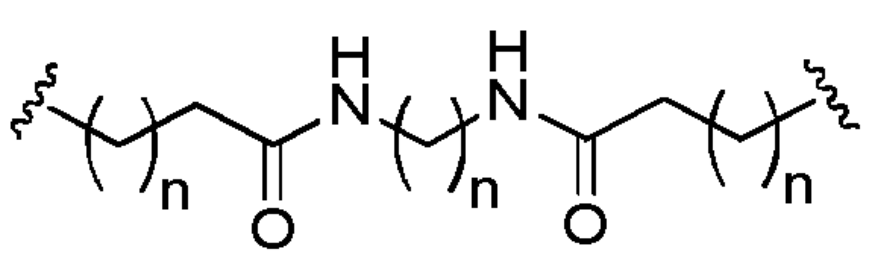
wherein each n is, independently, from 1 to 20; and

each p is from 1 to about 6.

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

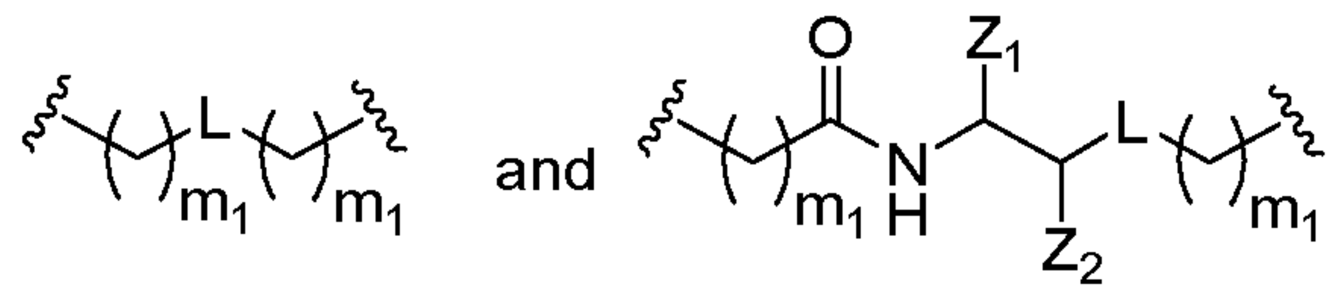


In certain embodiments, a tether has a structure selected from among:



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;

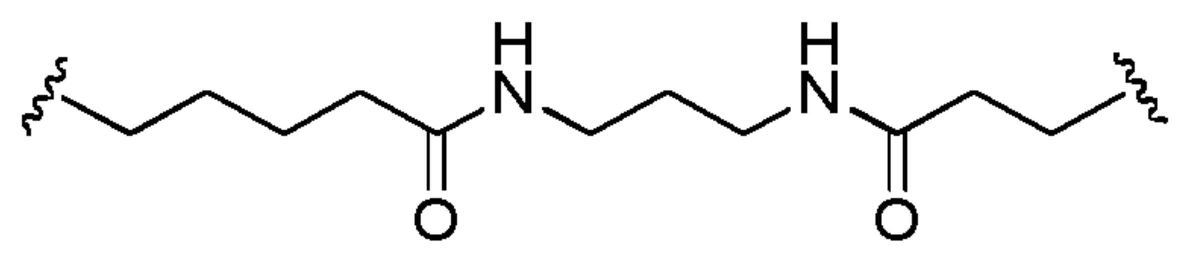
Z_1 is C(=O)O-R_2;

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

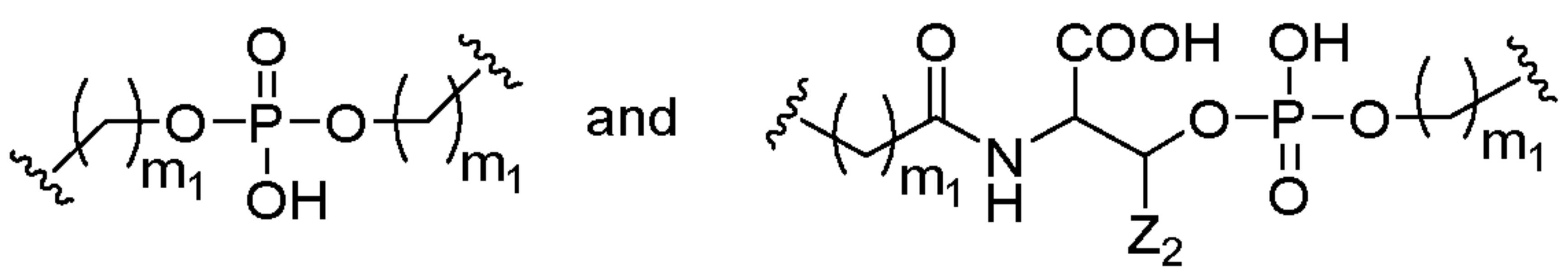
R_2 is H, C_1-C_6 alkyl or substituted C_1-C_6 alky; and

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:



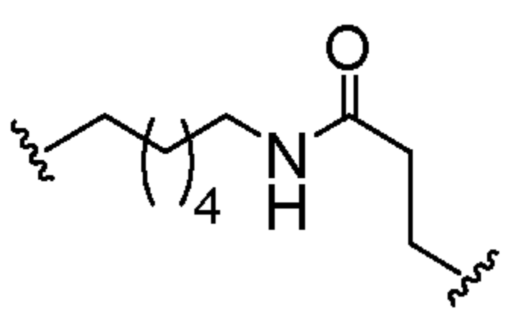
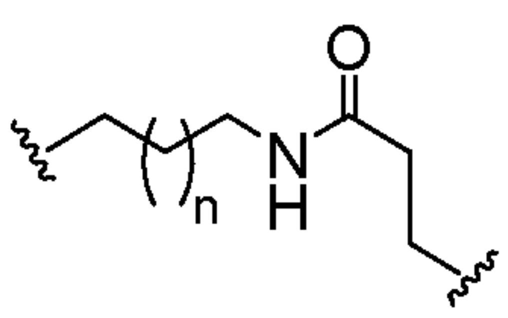
In certain embodiments, a tether has a structure selected from among:



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.

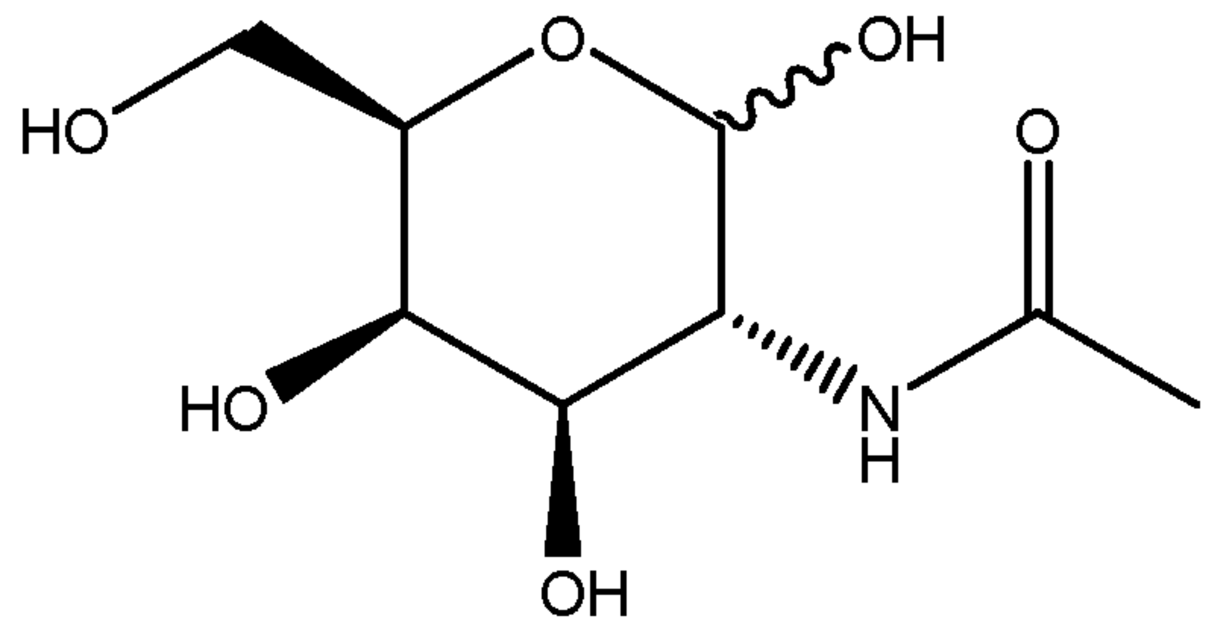
In certain embodiments, a tether comprises a phosphorus linking group. In certain embodiments, a tether does not comprise any amide bonds. In certain embodiments, a tether comprises a phosphorus linking group and does not comprise any amide bonds.

3. Certain Ligands

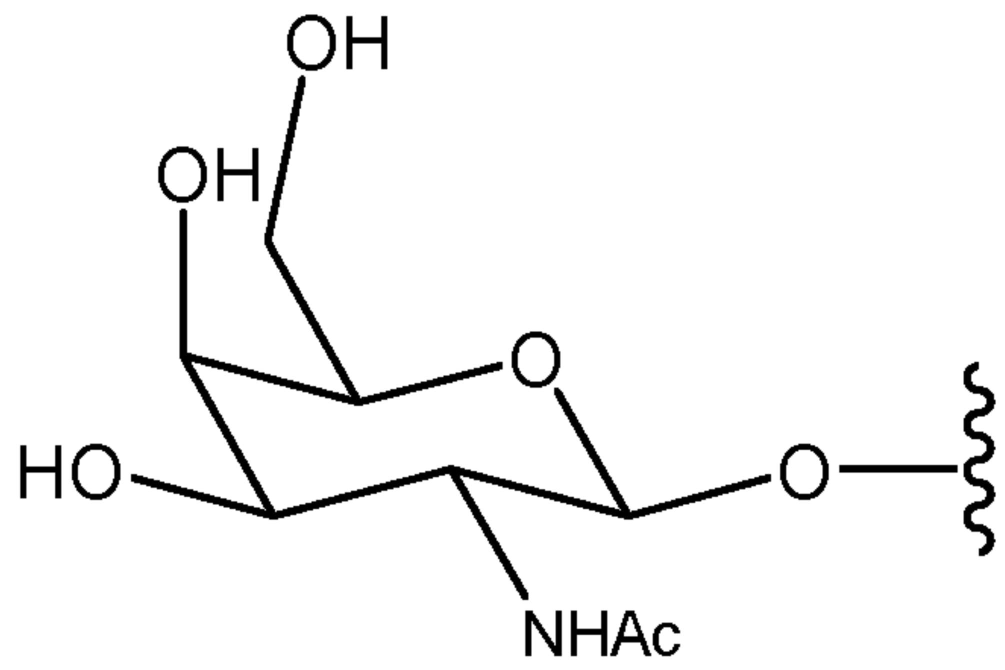
In certain embodiments, the present disclosure provides ligands wherein each ligand is covalently attached to a tether. In certain embodiments, each ligand is selected to have an affinity for at least one type of receptor on a target cell. In certain embodiments, ligands are selected that have an affinity 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.

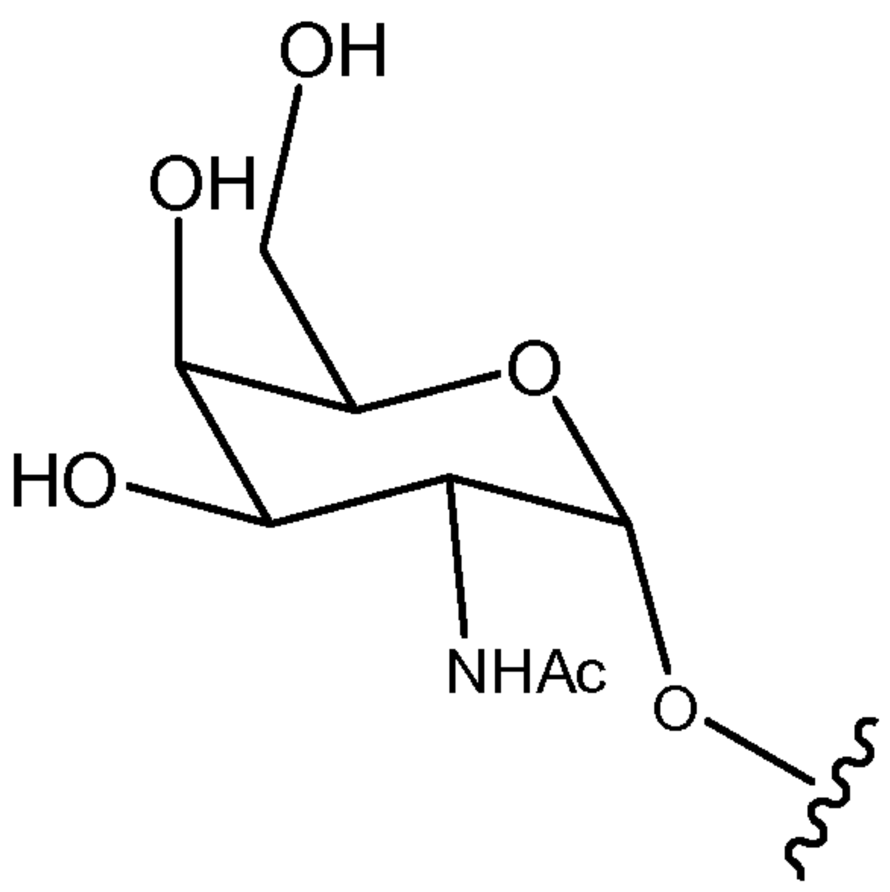
In certain embodiments, “GalNAc” or “Gal-NAc” refers to 2-(Acetylamino)-2-deoxy-D-galactopyranose, commonly referred to in the literature as N-acetyl galactosamine. In certain embodiments, “N-acetyl galactosamine” refers to 2-(Acetylamino)-2-deoxy-D-galactopyranose. In certain embodiments, “GalNAc” or “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

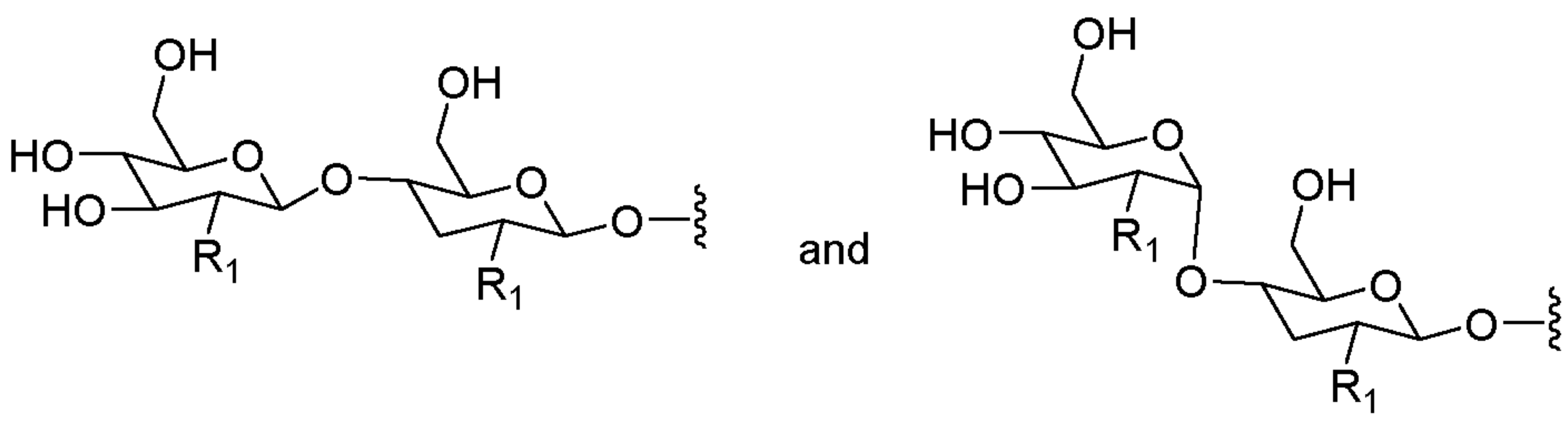


2-(Acetylamino)-2-deoxy-β-D-galactopyranose



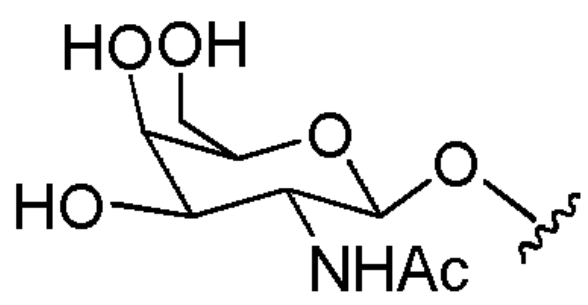
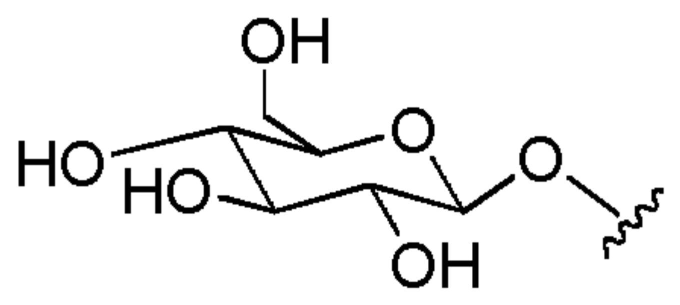
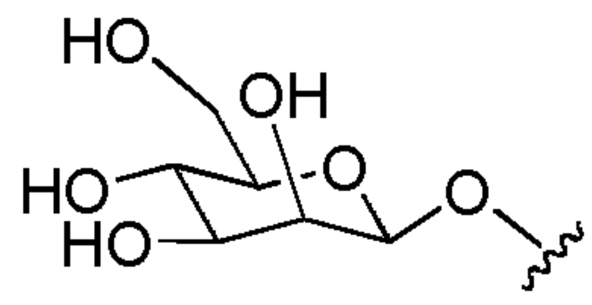
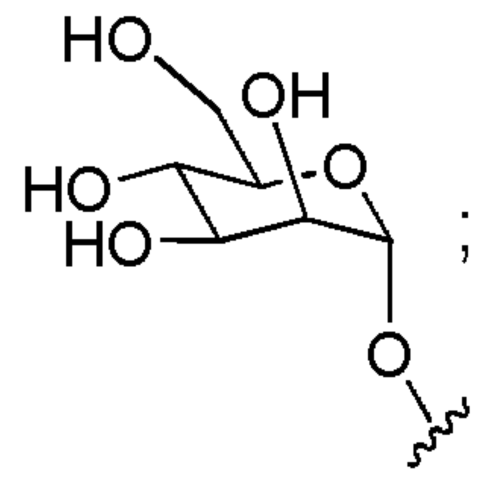
2-(Acetylamino)-2-deoxy-α-D-galactopyranose

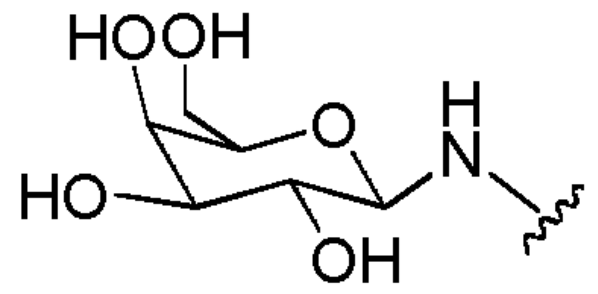
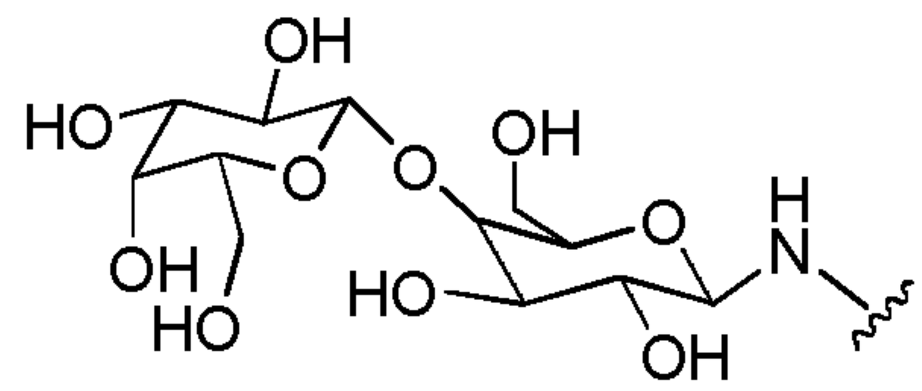
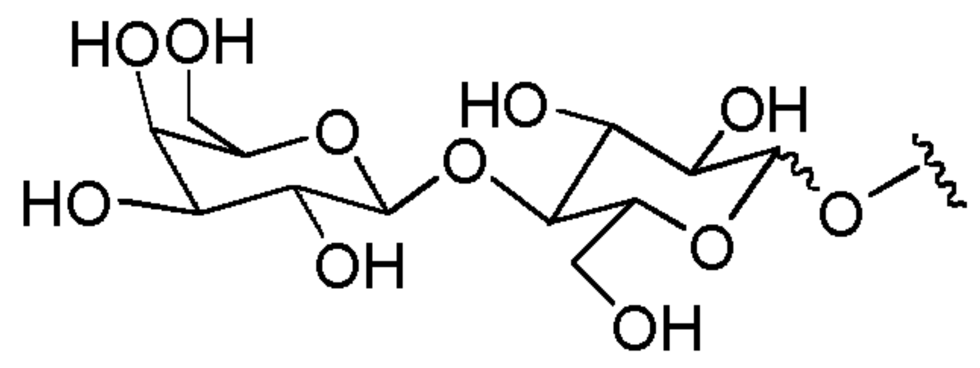
In certain embodiments one or more ligand has a structure selected from among:

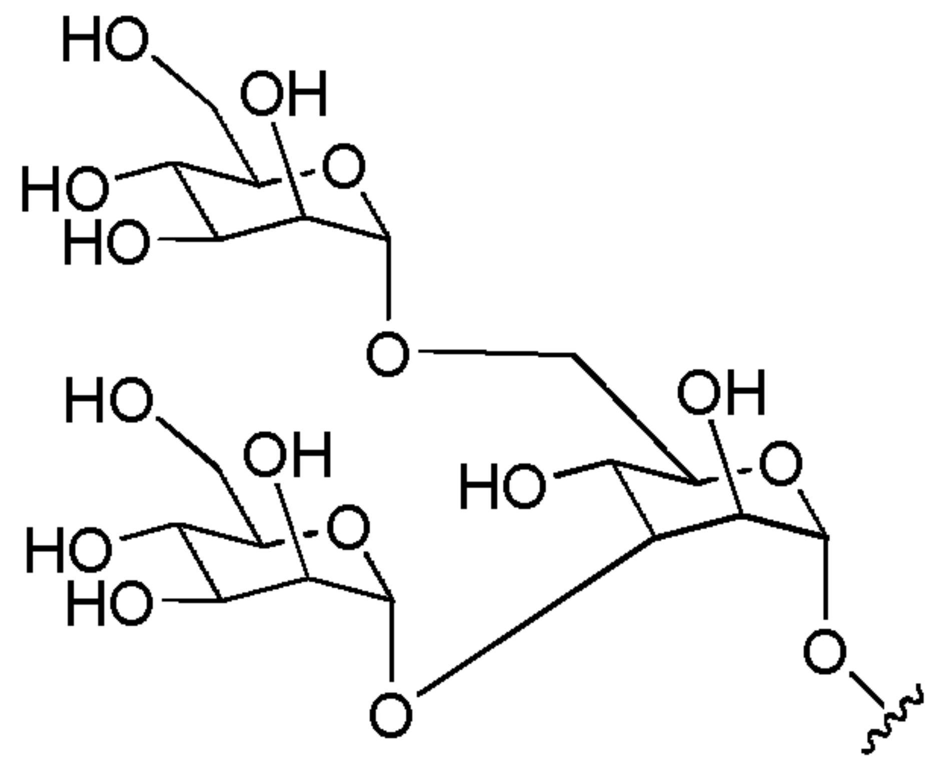


wherein each R_1 is selected from OH and NHCOOH.

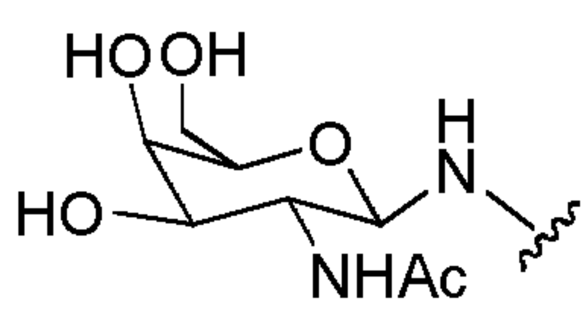
In certain embodiments one or more ligand has a structure selected from among:

 ;  ;  ;  ;

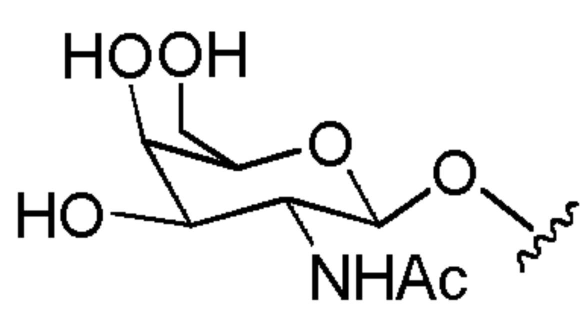
 ;  ;  ; and

 .

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

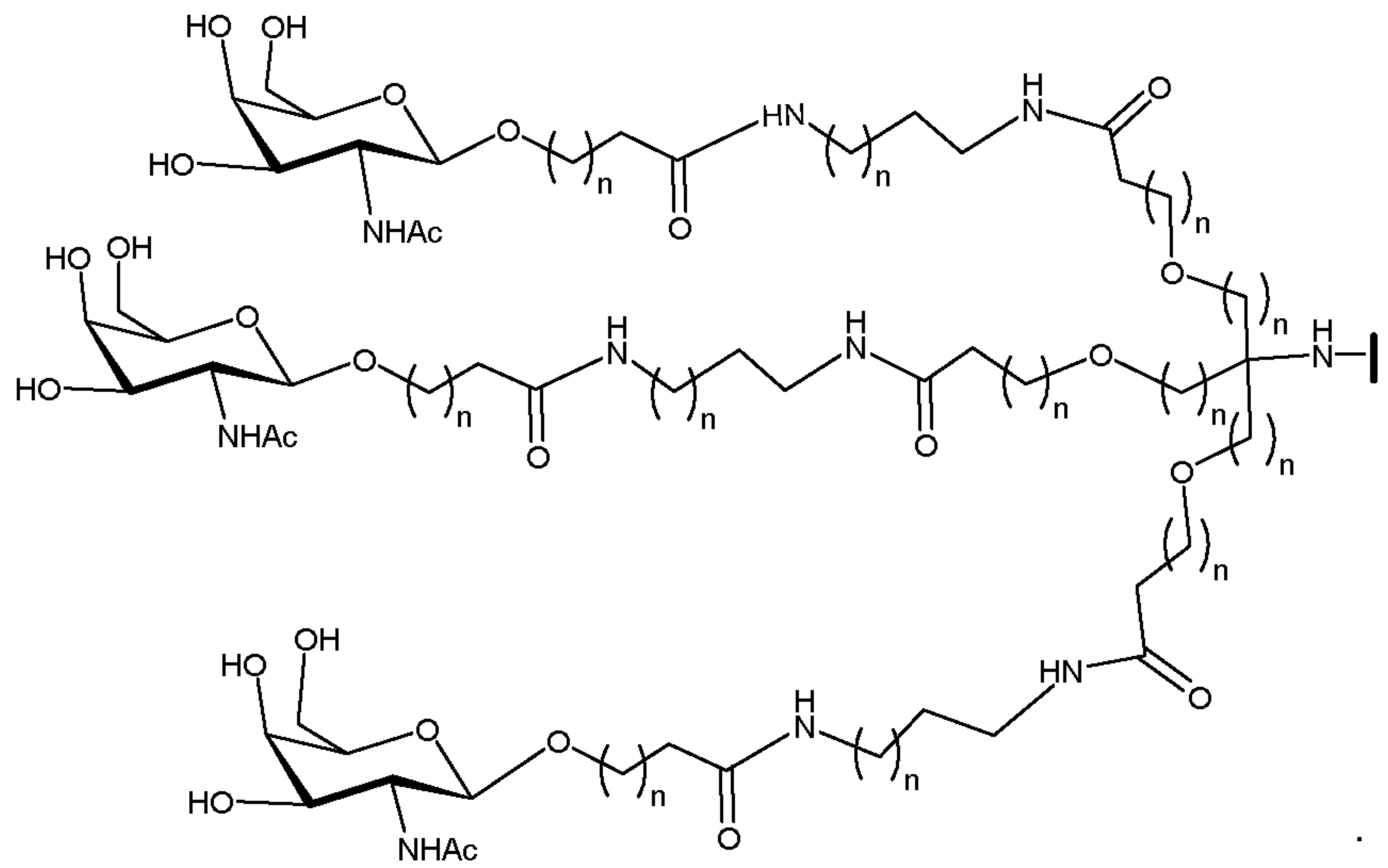
 .

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

 .

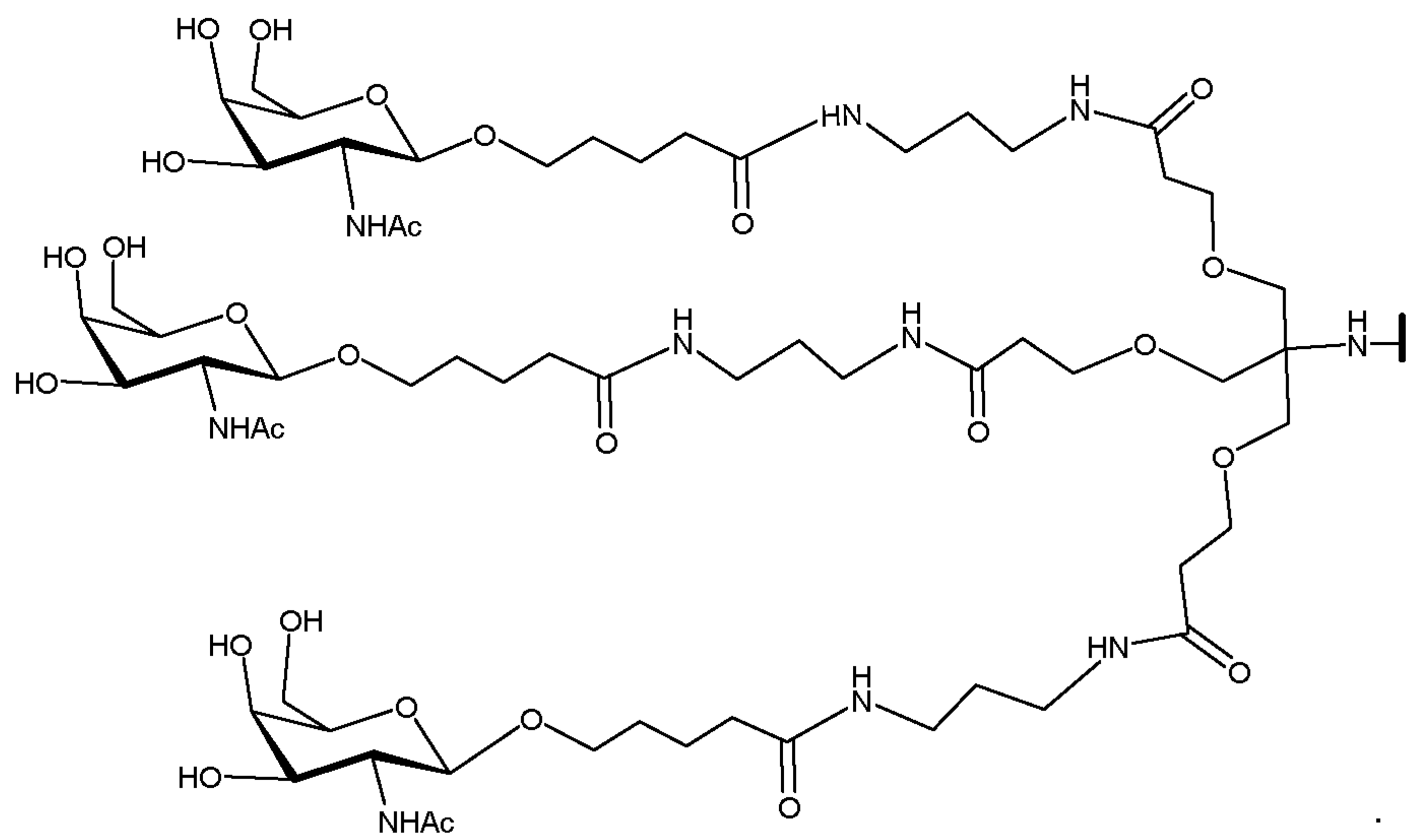
i. Certain Conjugates

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

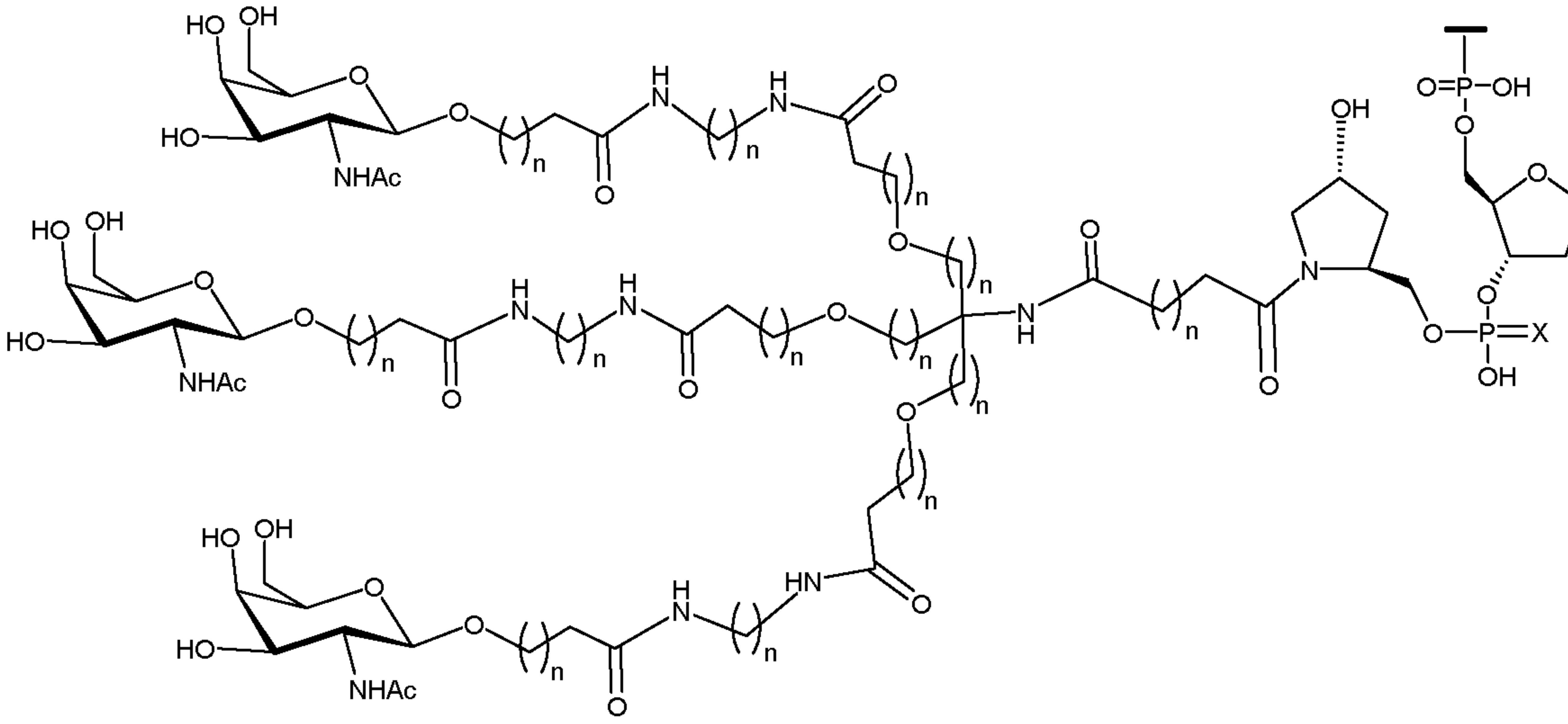


wherein each n is, independently, from 1 to 20.

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;

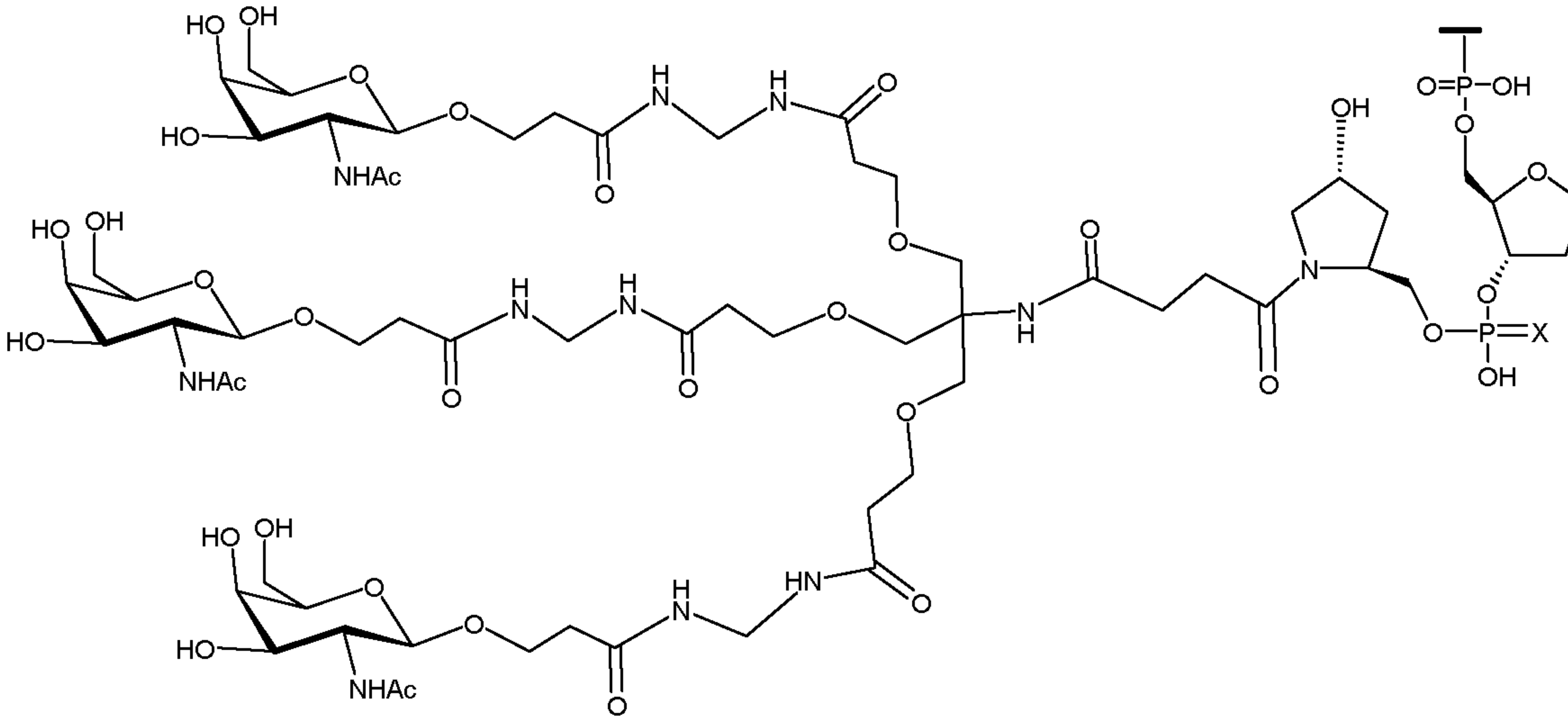
Z is H or a linked solid support;

Q is an antisense compound;

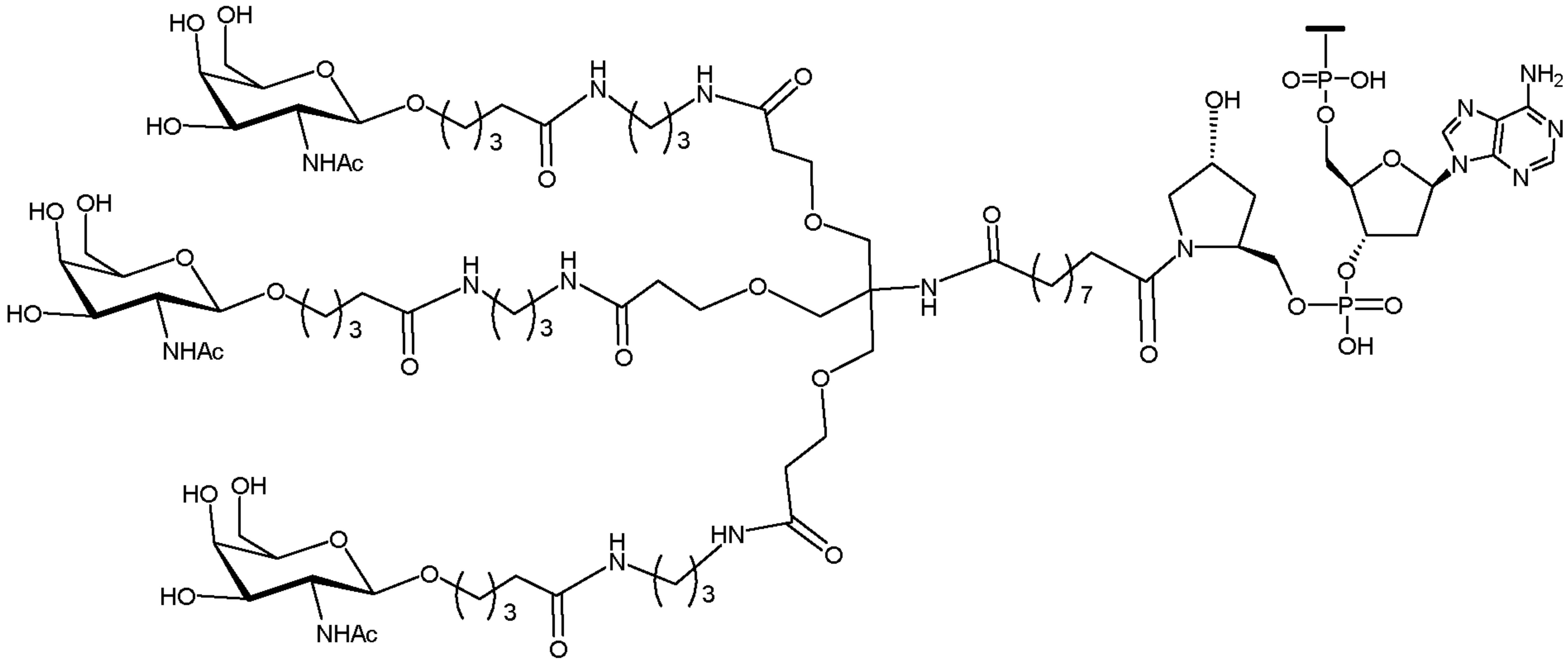
X is O or S; and

Bx is a heterocyclic base moiety.

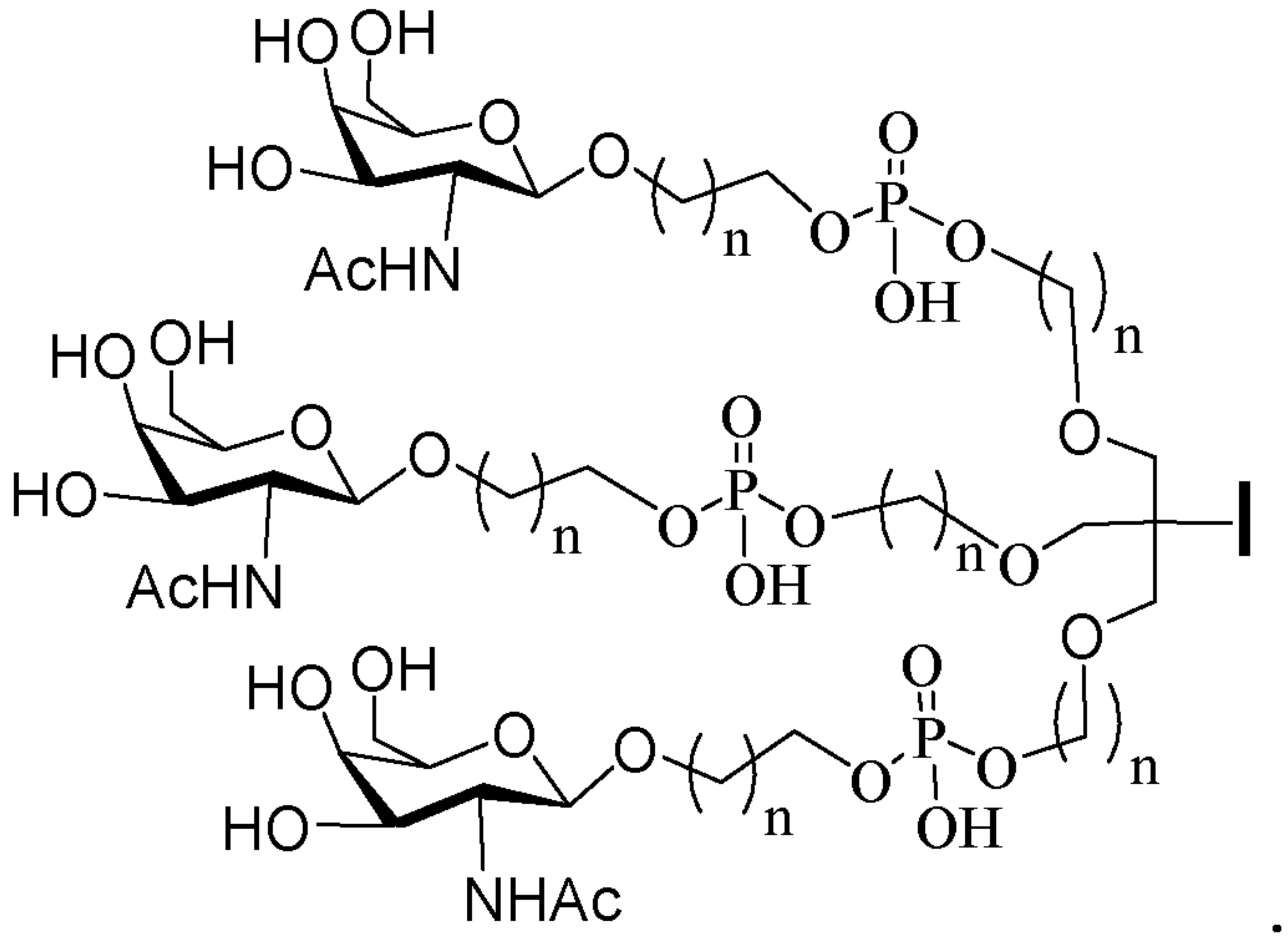
In certain such embodiments, conjugate groups have the following structure:



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

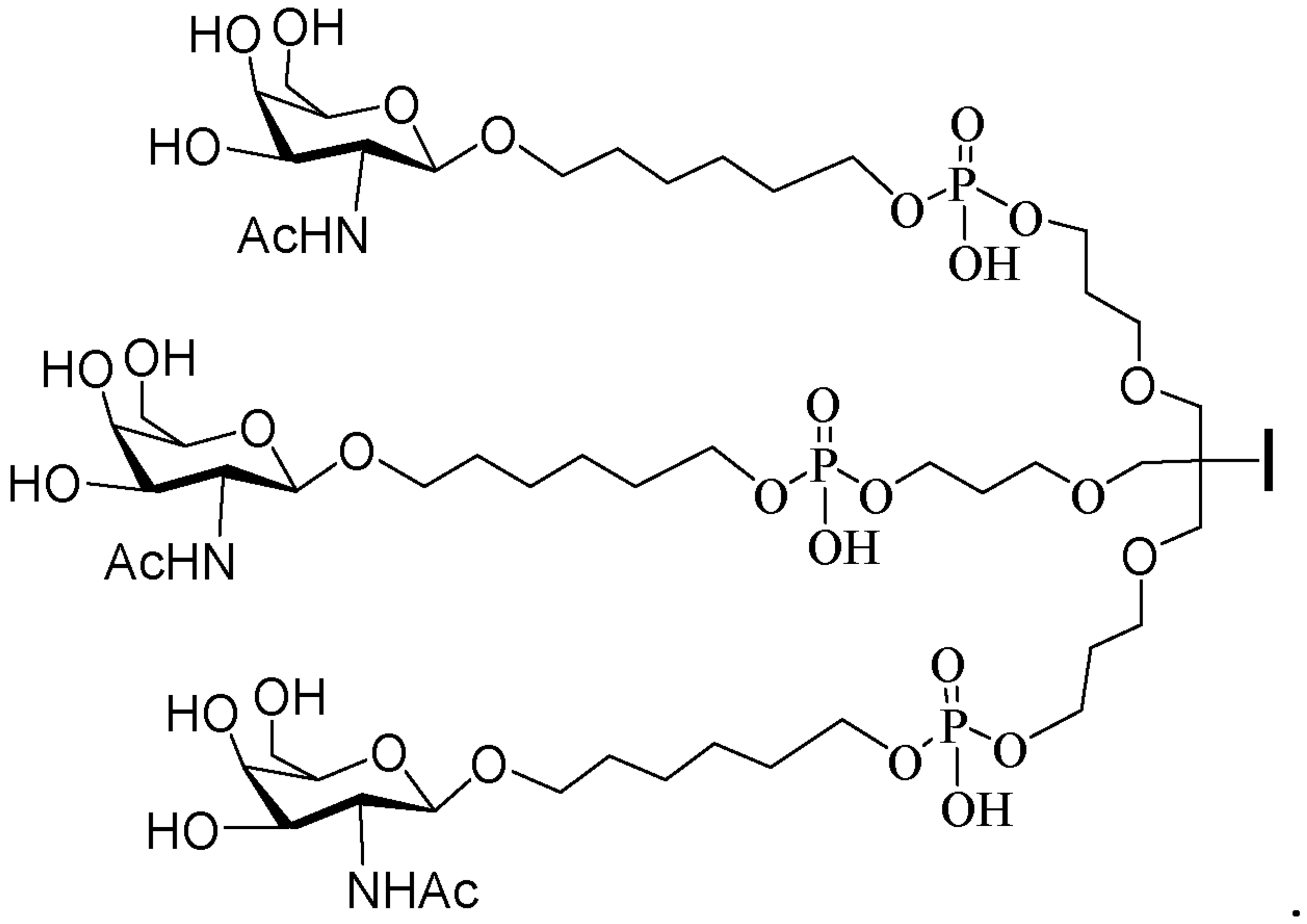


In certain such embodiments, conjugate groups have the following structure:

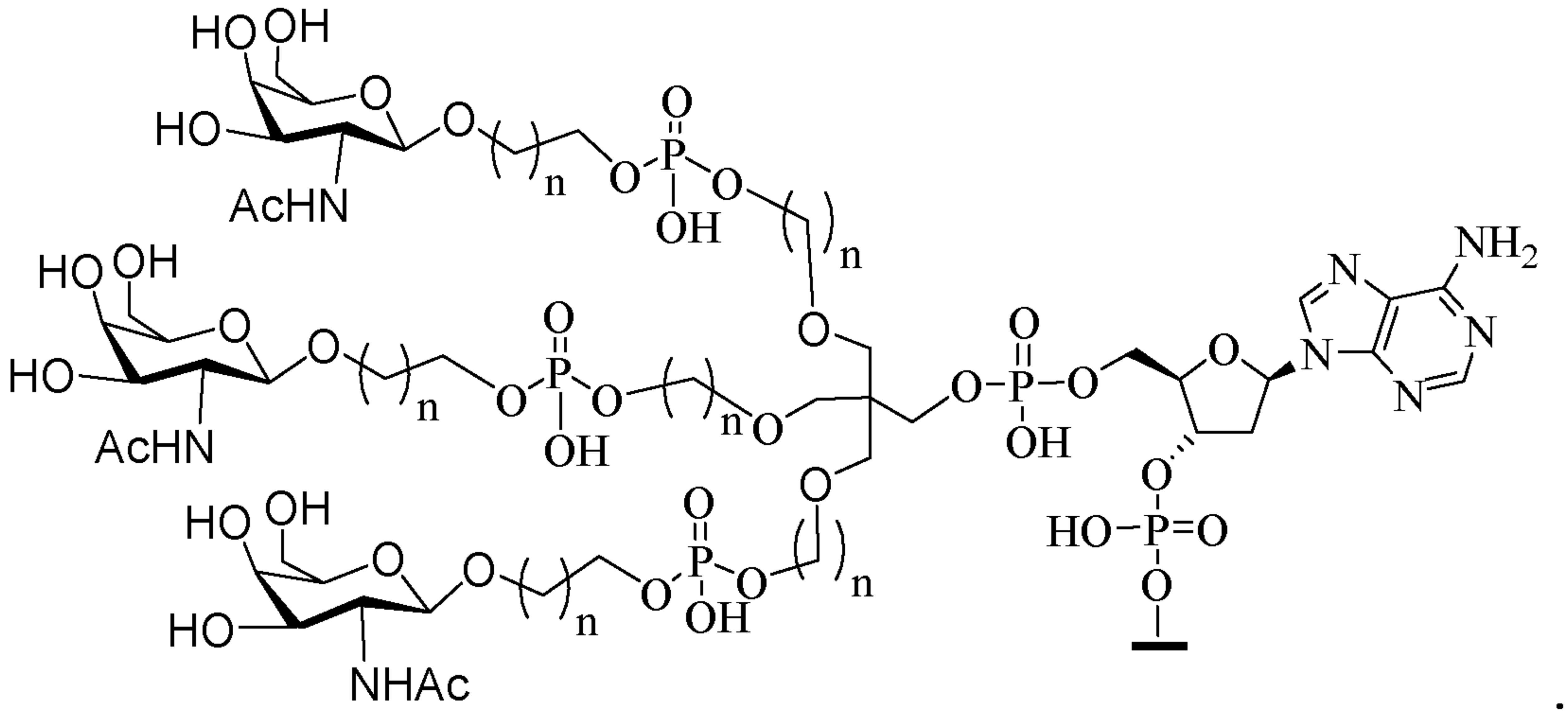


.

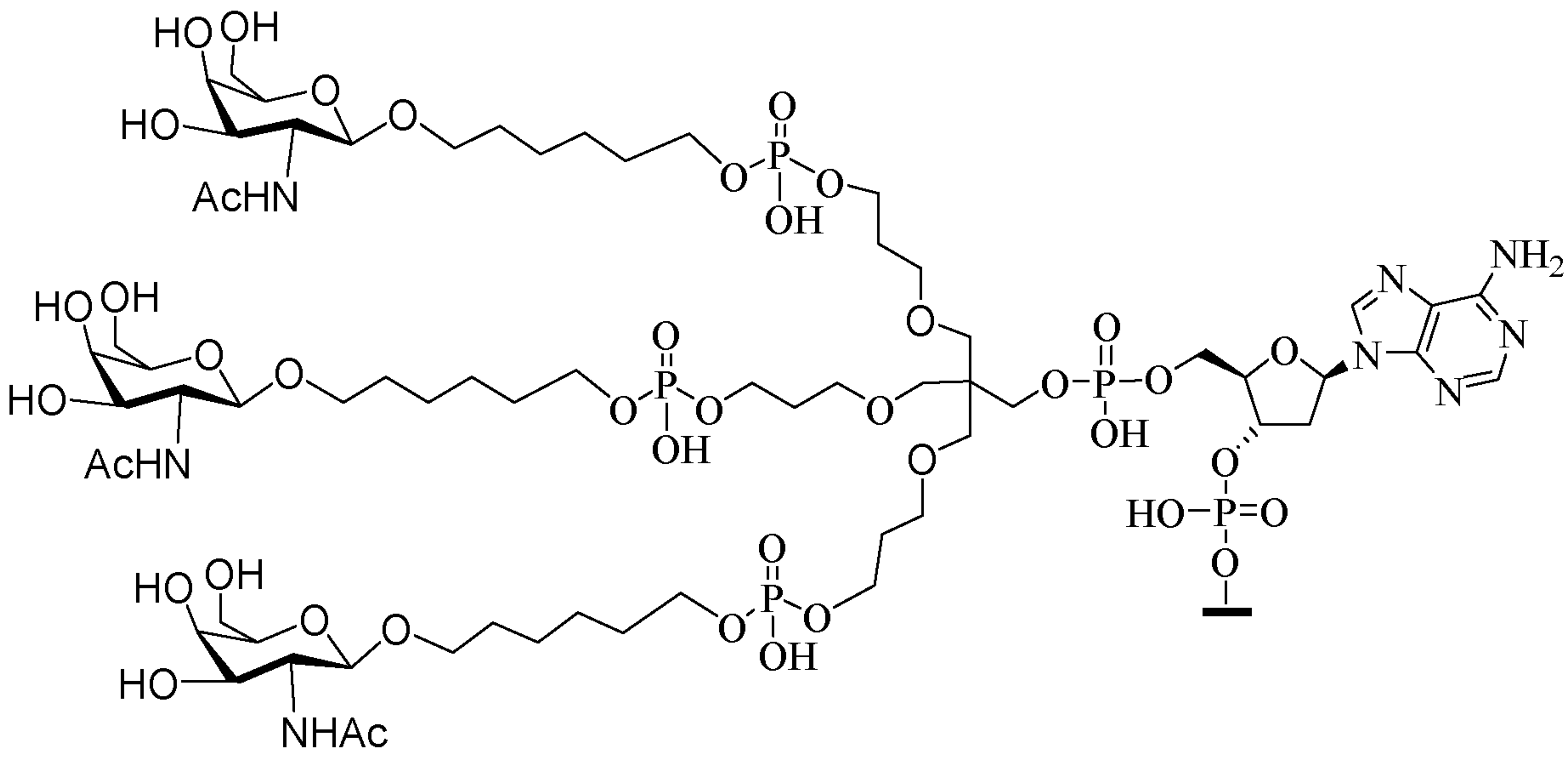
In certain such embodiments, conjugate groups have the following structure:



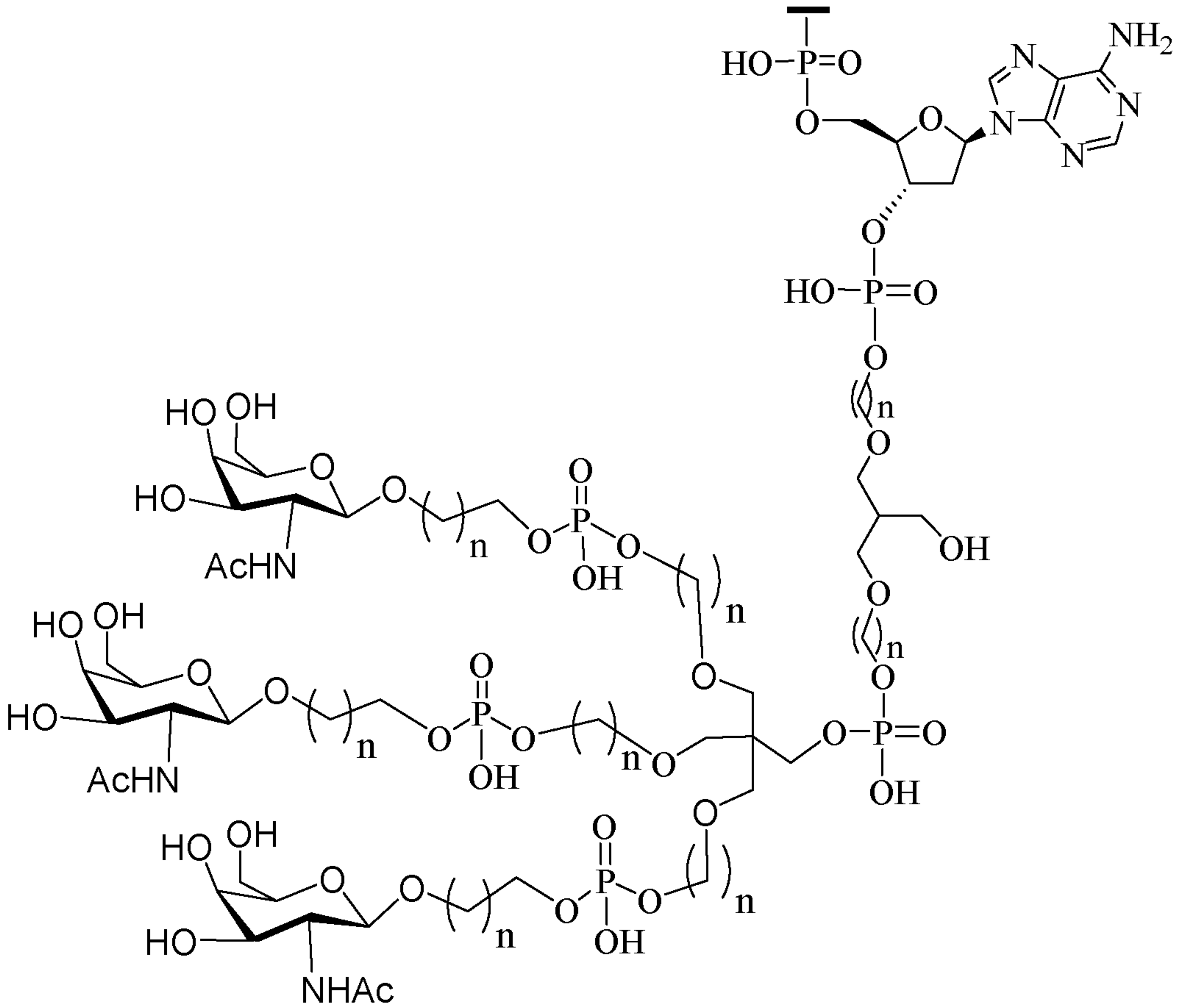
In certain such embodiments, conjugate groups have the following structure:



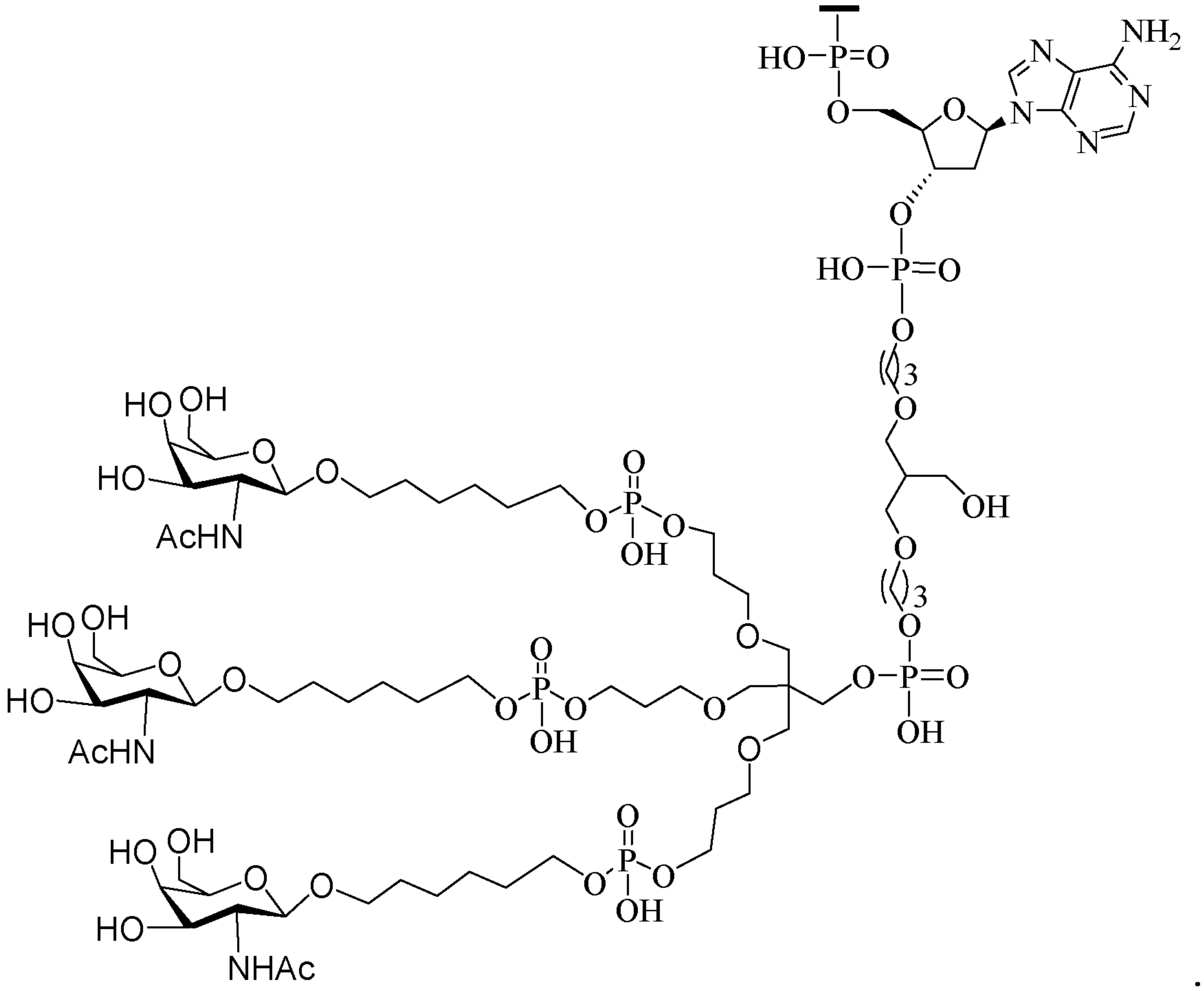
In certain such embodiments, conjugate groups have the following structure:



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

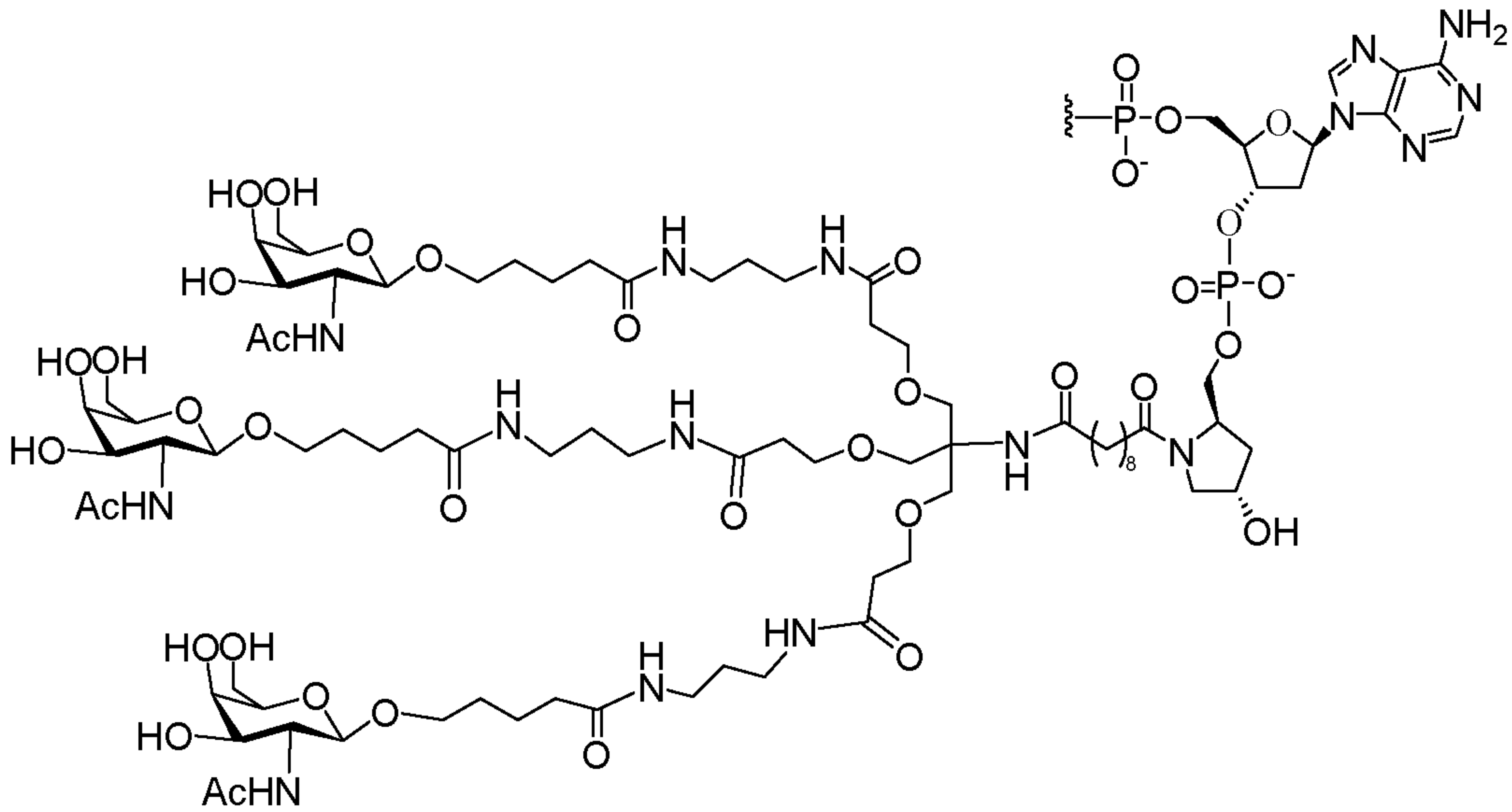


In certain such embodiments, conjugate groups have the following structure:



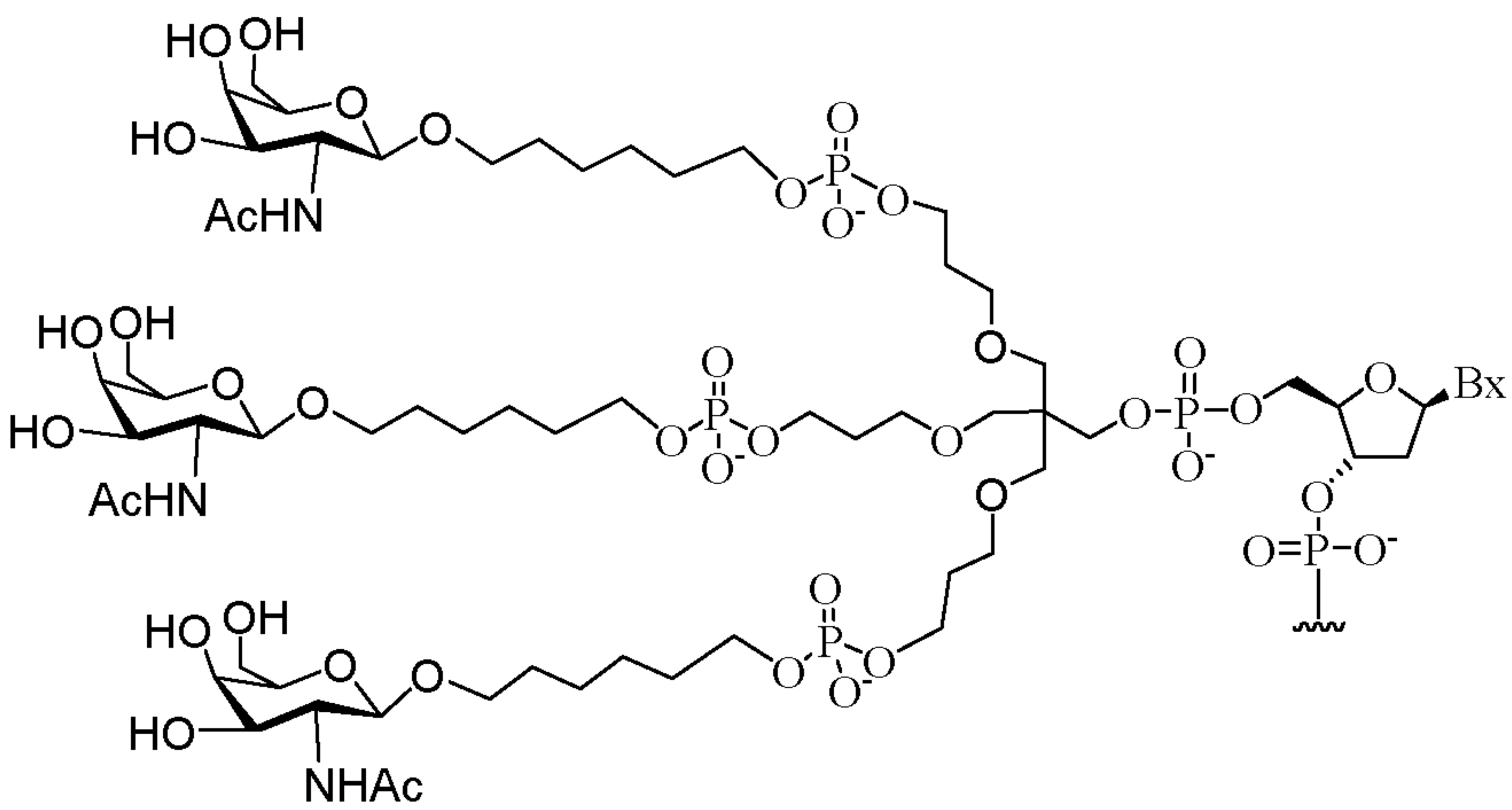
In certain embodiments, conjugates do not comprise a pyrrolidine.

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



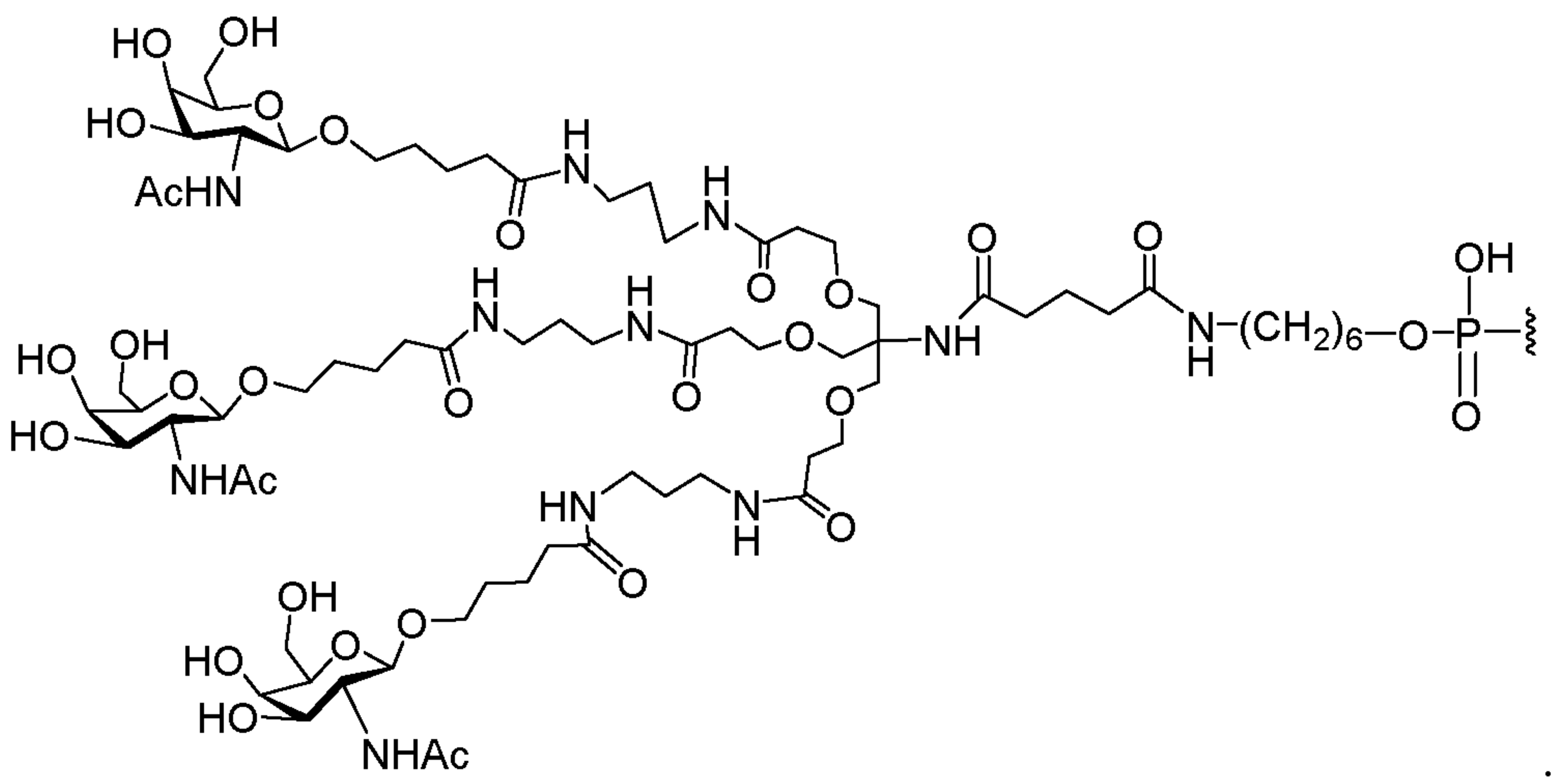
.

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



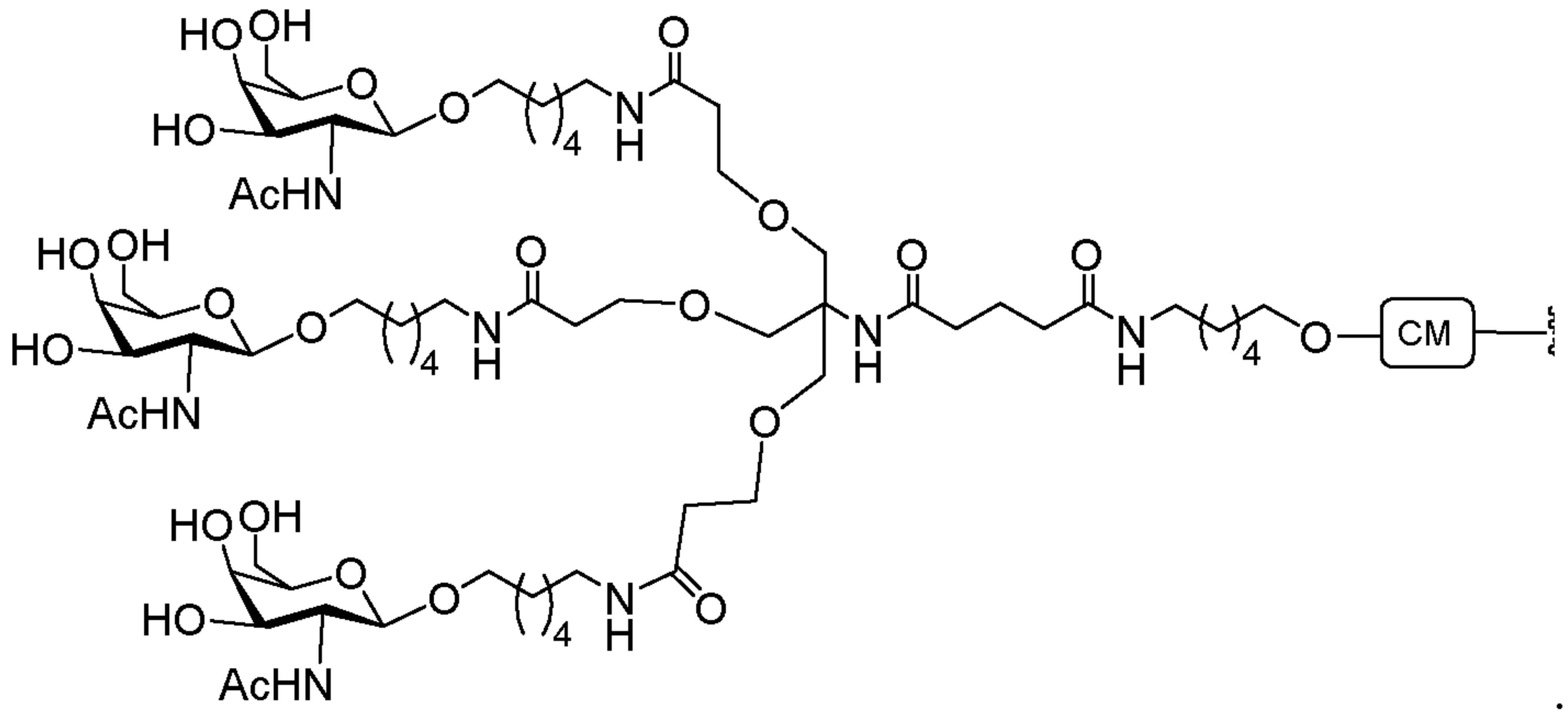
.

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

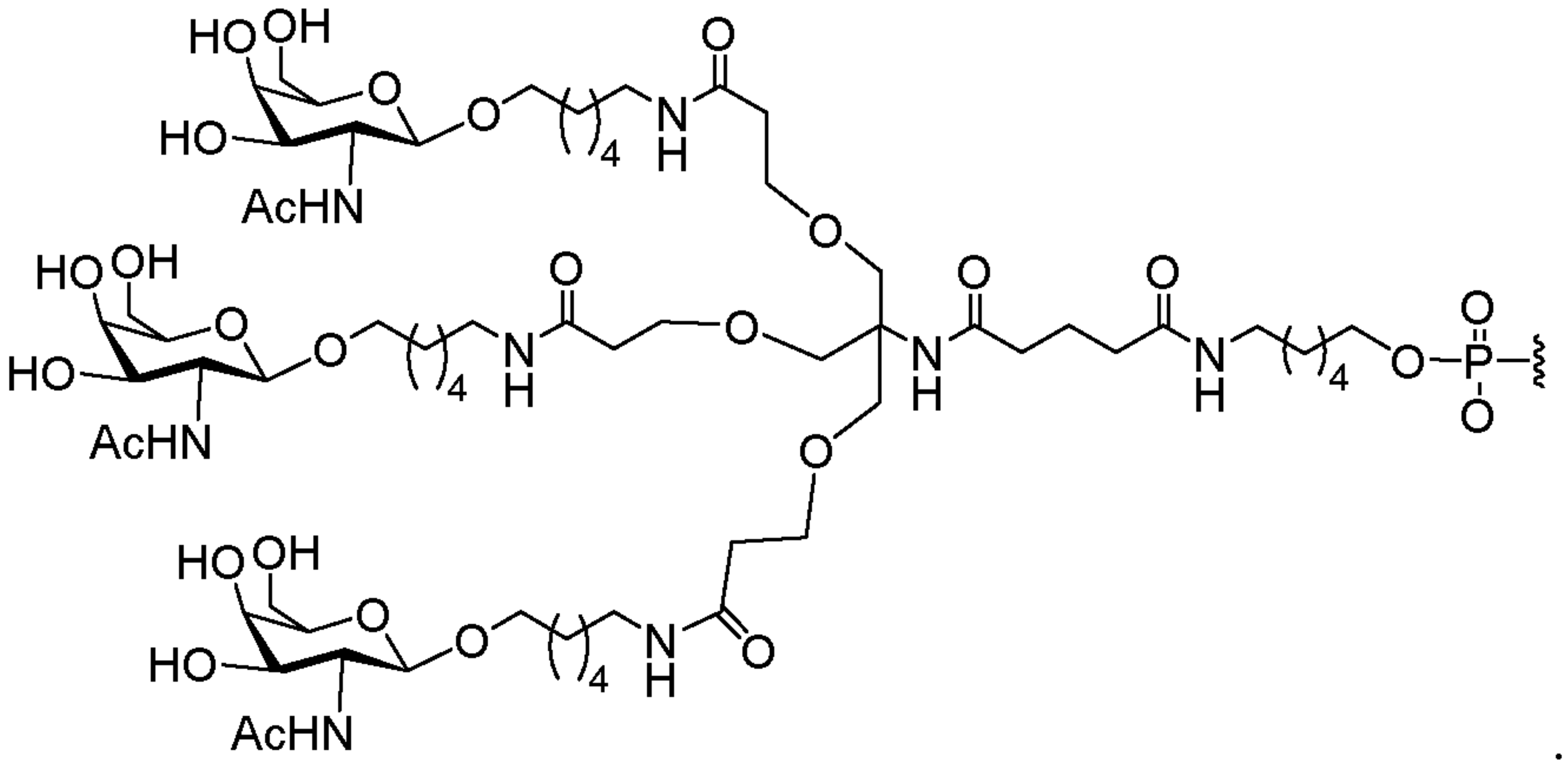


.

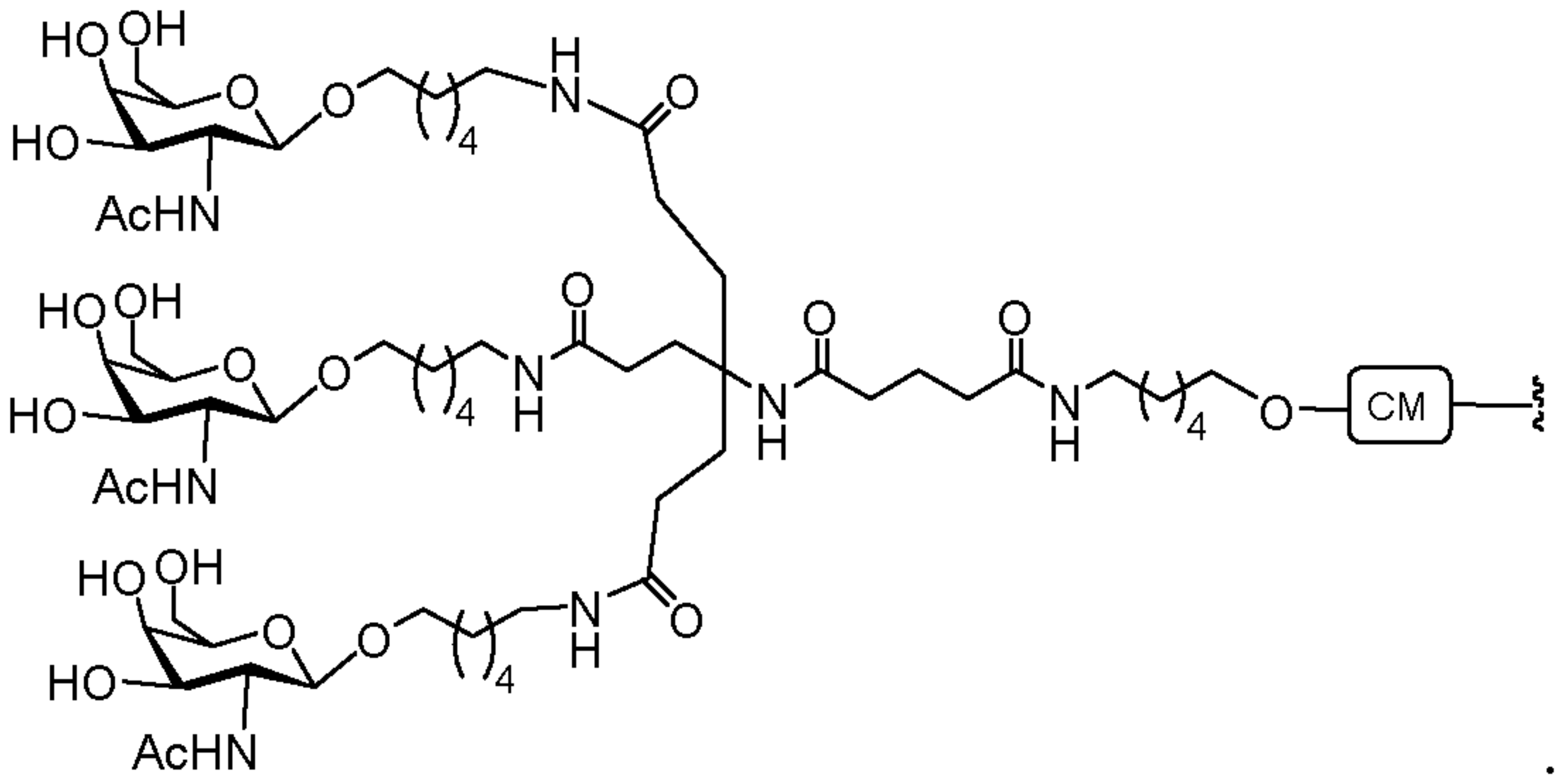
In certain such embodiments, conjugate groups have the following structure:



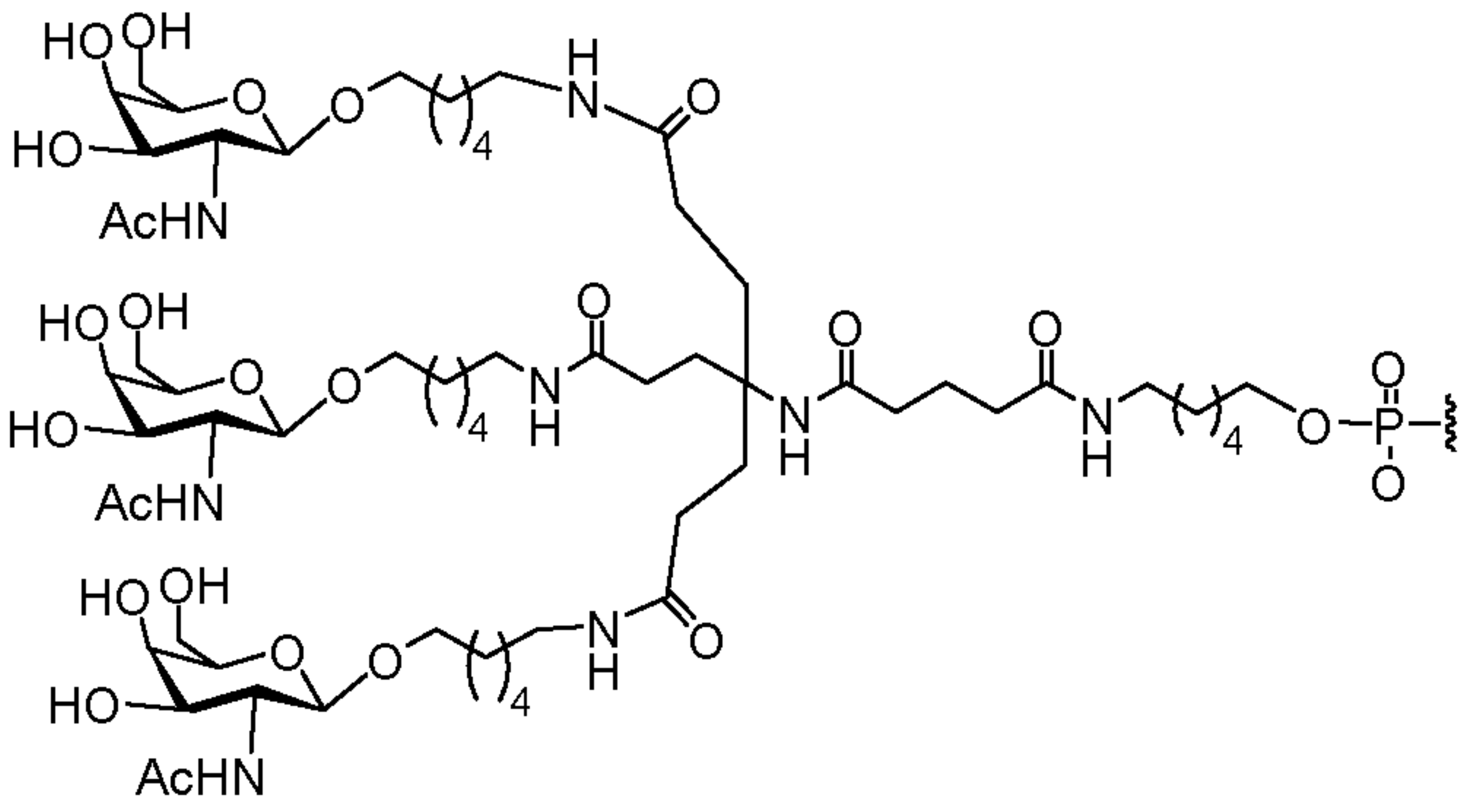
In certain such embodiments, conjugate groups have the following structure:



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

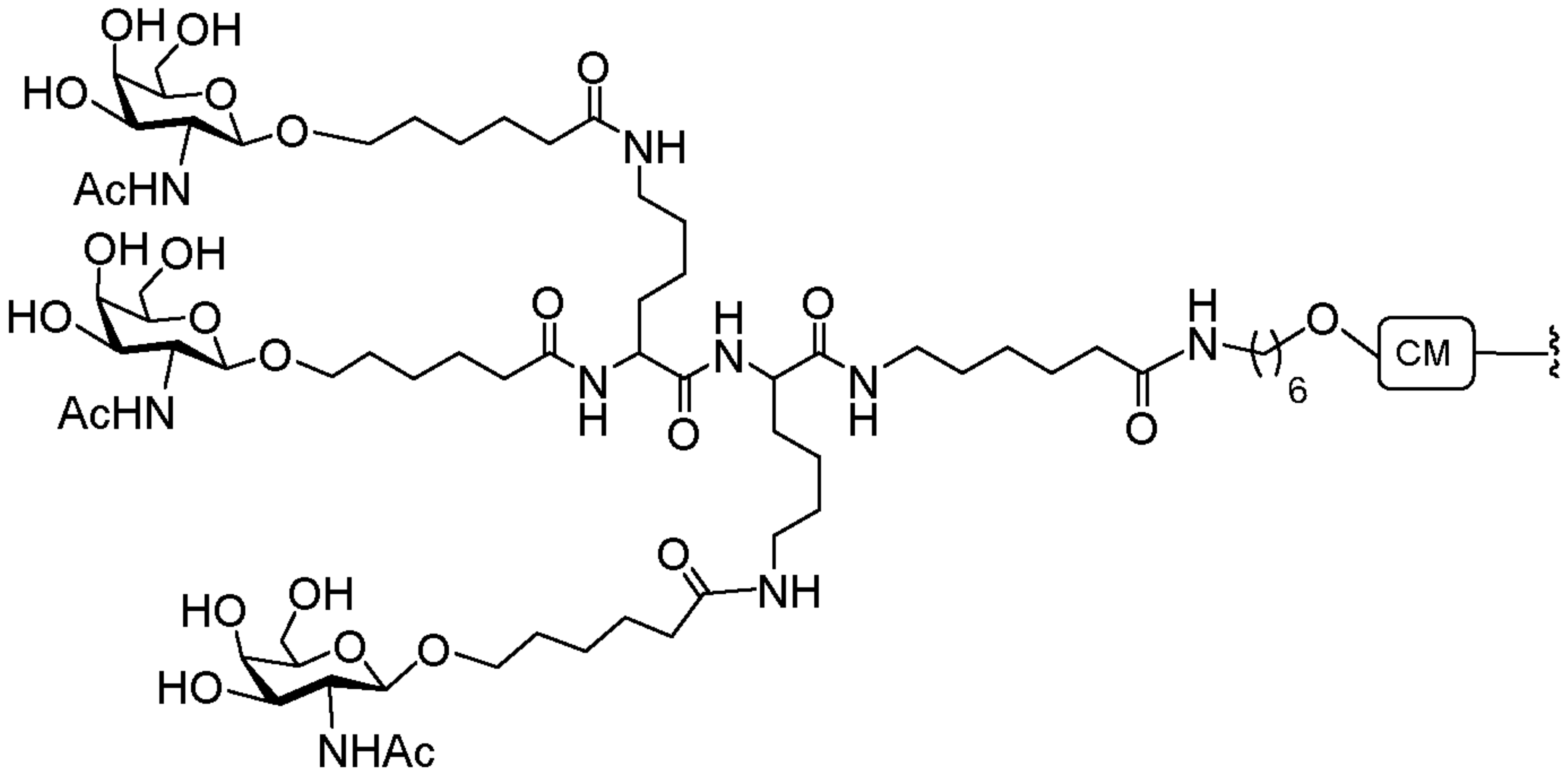


In certain such embodiments, conjugate groups have the following structure:



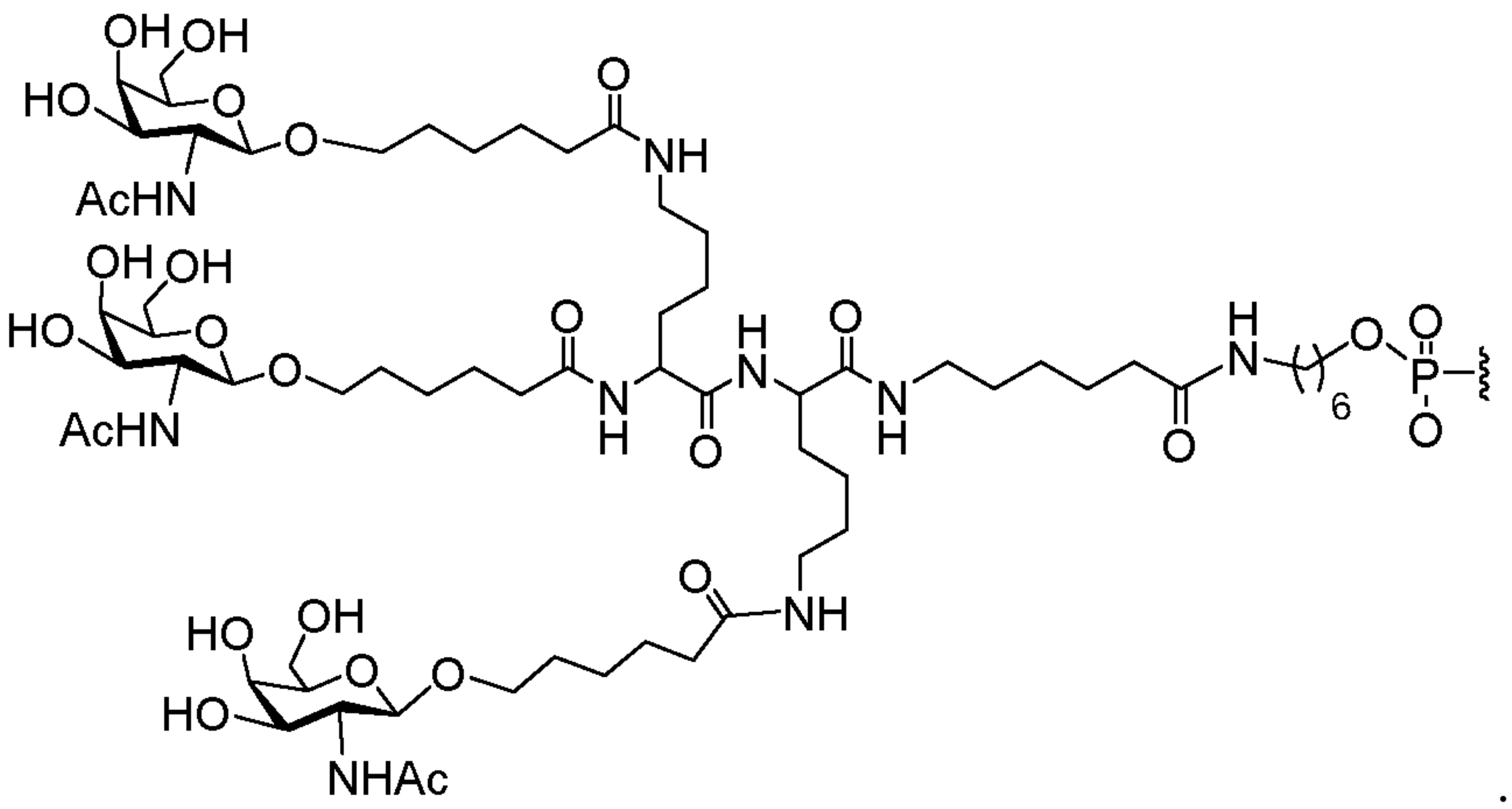
.

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



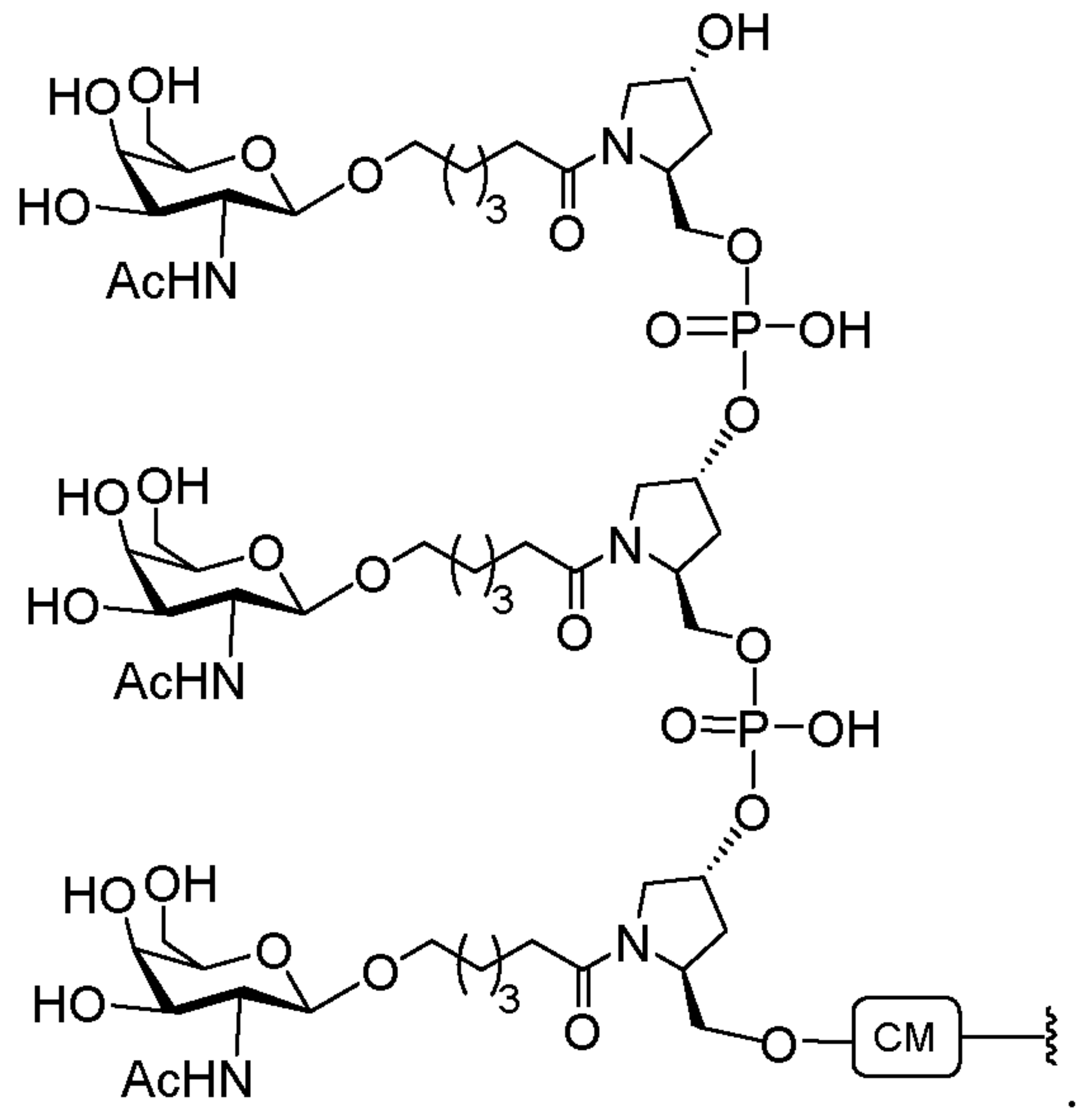
.

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

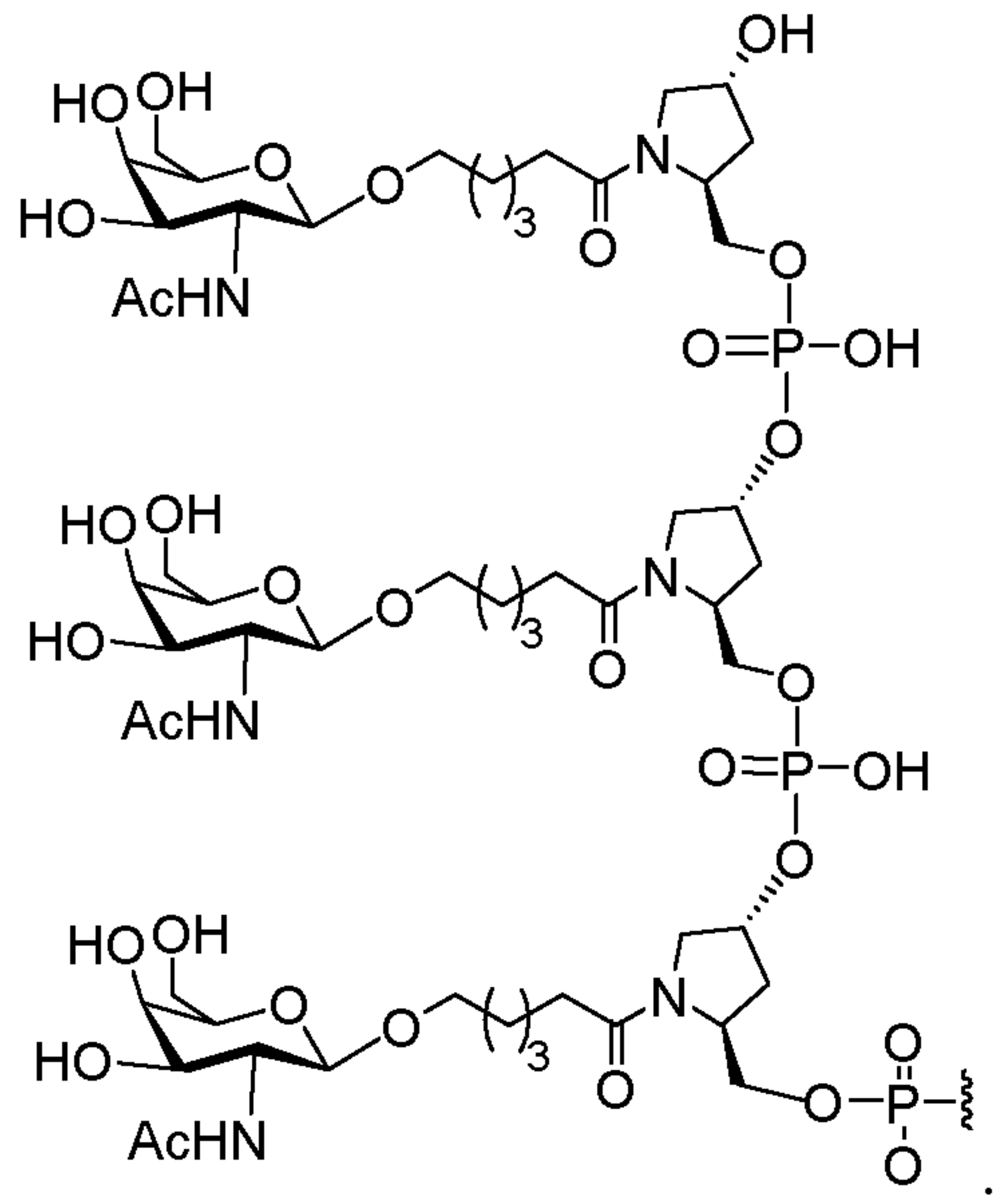


.

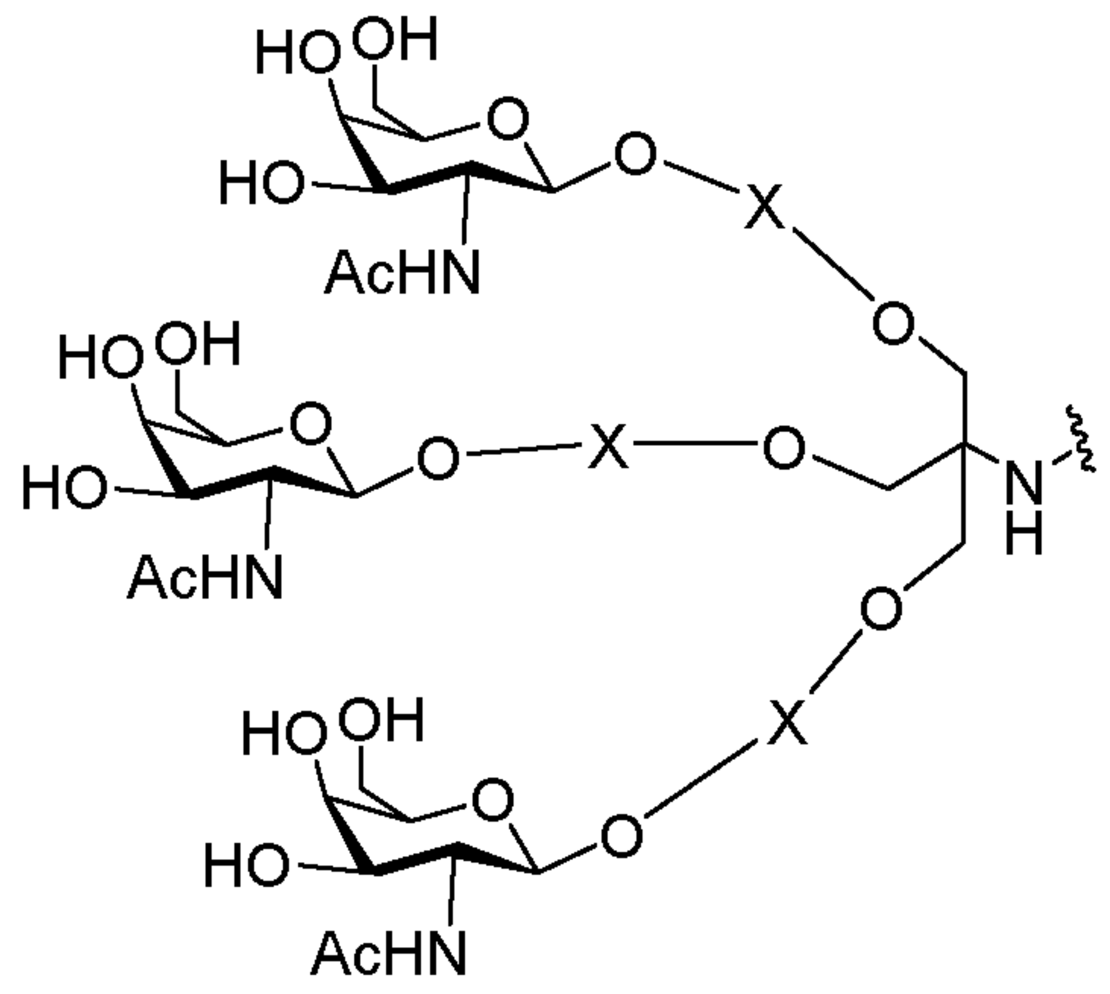
In certain such embodiments, conjugate groups have the following structure:



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

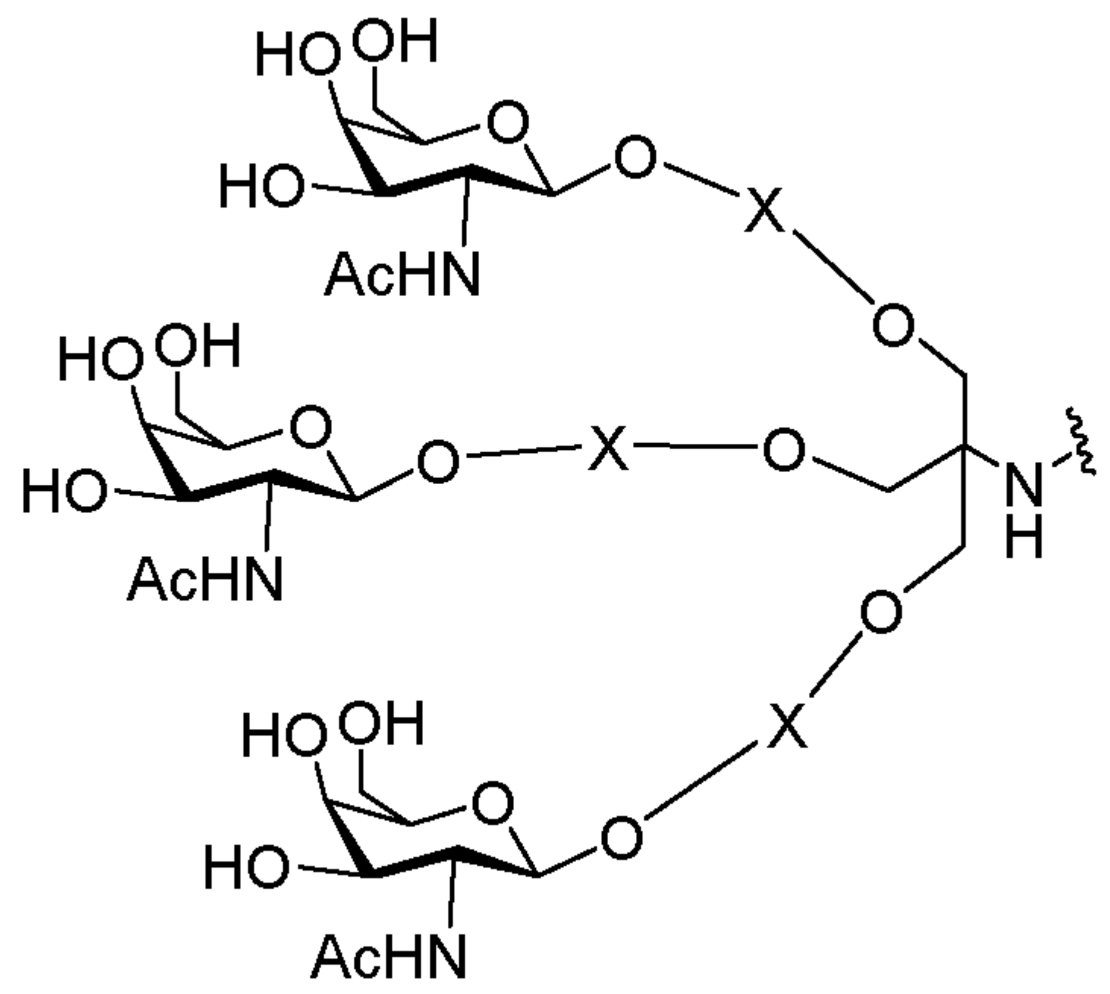


In certain embodiments, the cell-targeting moiety of the conjugate group has the following structure:



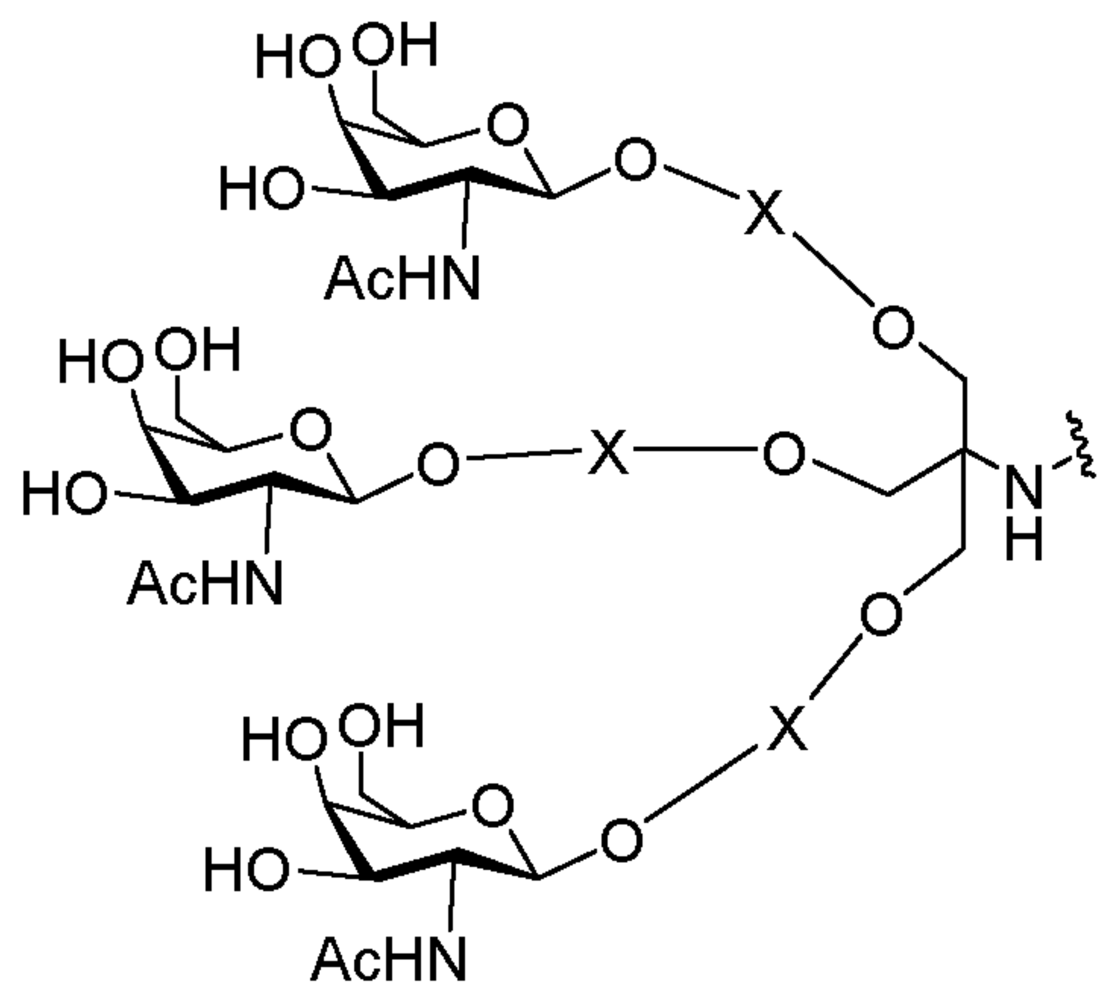
wherein X is a substituted or unsubstituted tether of six to eleven consecutively bonded atoms.

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



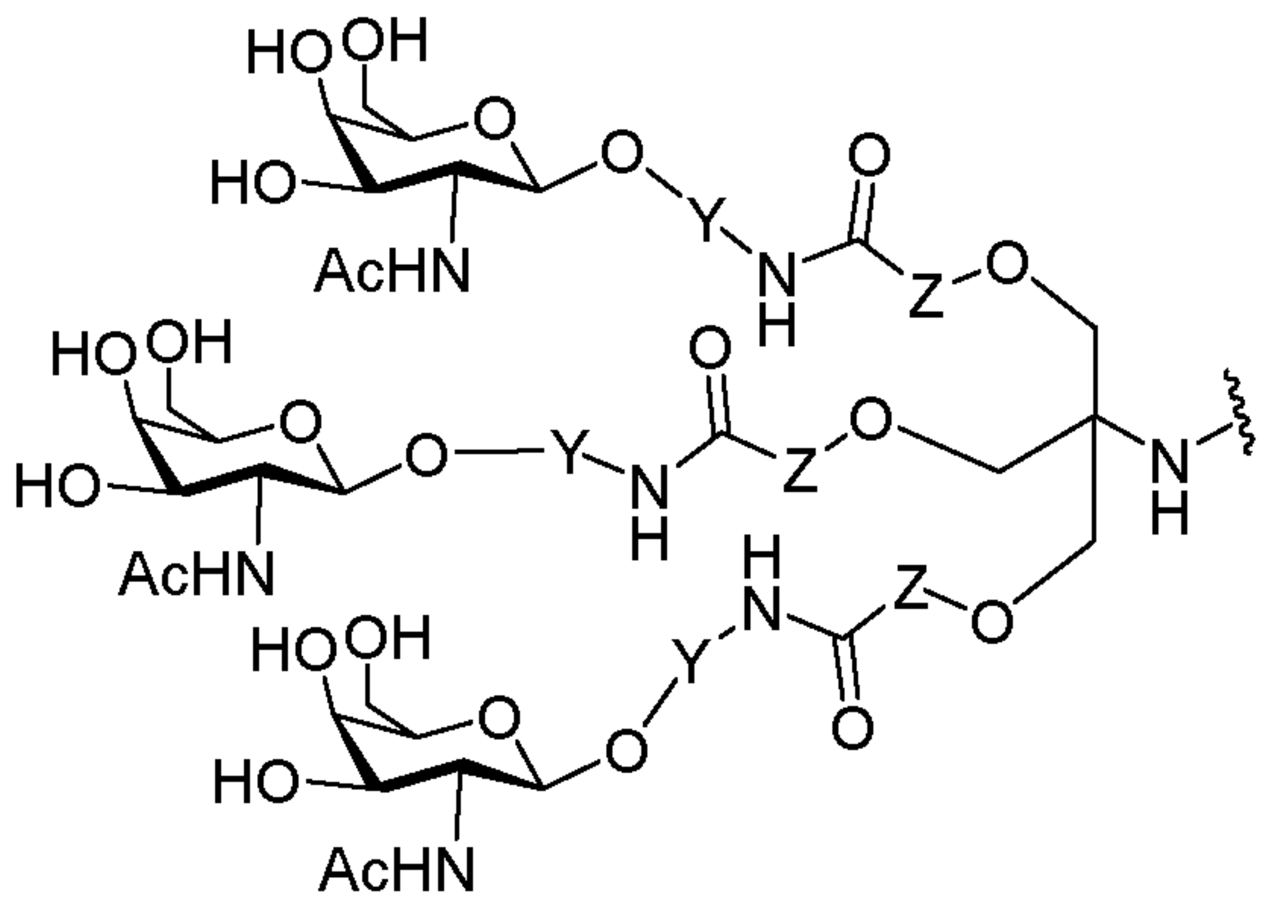
wherein X is a substituted or unsubstituted tether of ten consecutively bonded atoms.

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



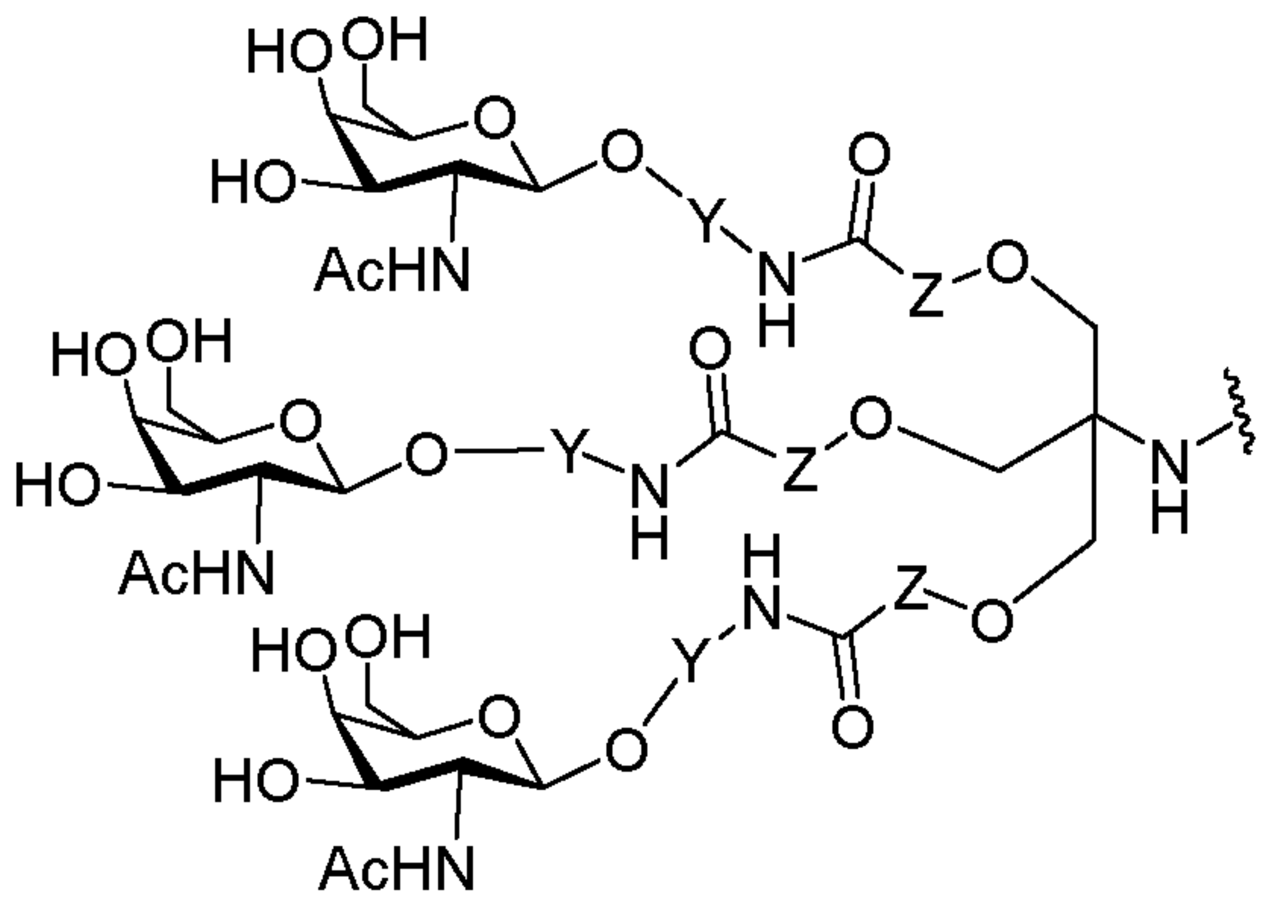
wherein X is a substituted or unsubstituted tether of four to eleven consecutively bonded atoms and wherein the tether comprises exactly one amide bond.

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



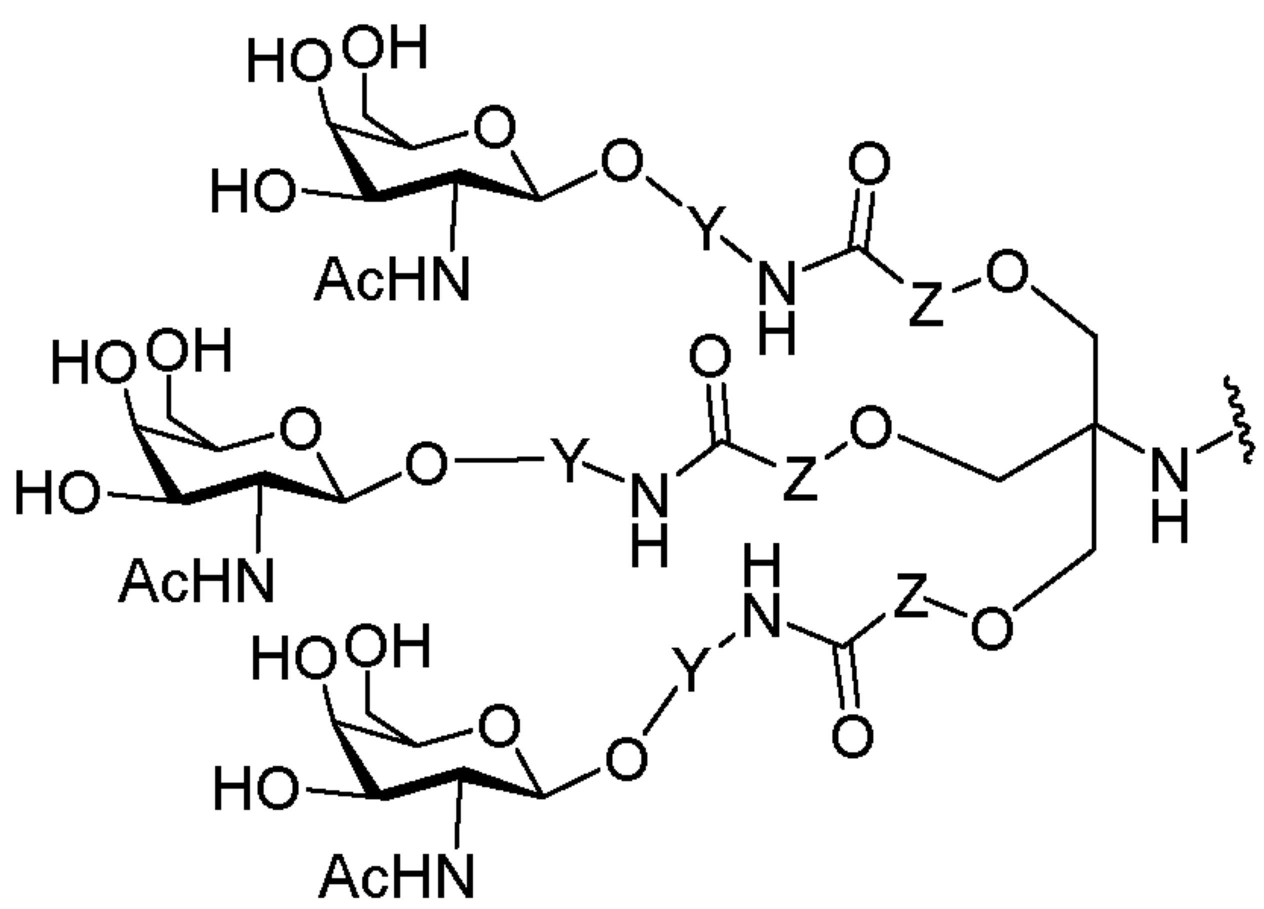
wherein Y and Z are independently selected from a C_1-C_{12} substituted or unsubstituted alkyl, alkenyl, or alkynyl group, or a group comprising an ether, a ketone, an amide, an ester, a carbamate, an amine, a piperidine, a phosphate, a phosphodiester, a phosphorothioate, a triazole, a pyrrolidine,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

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



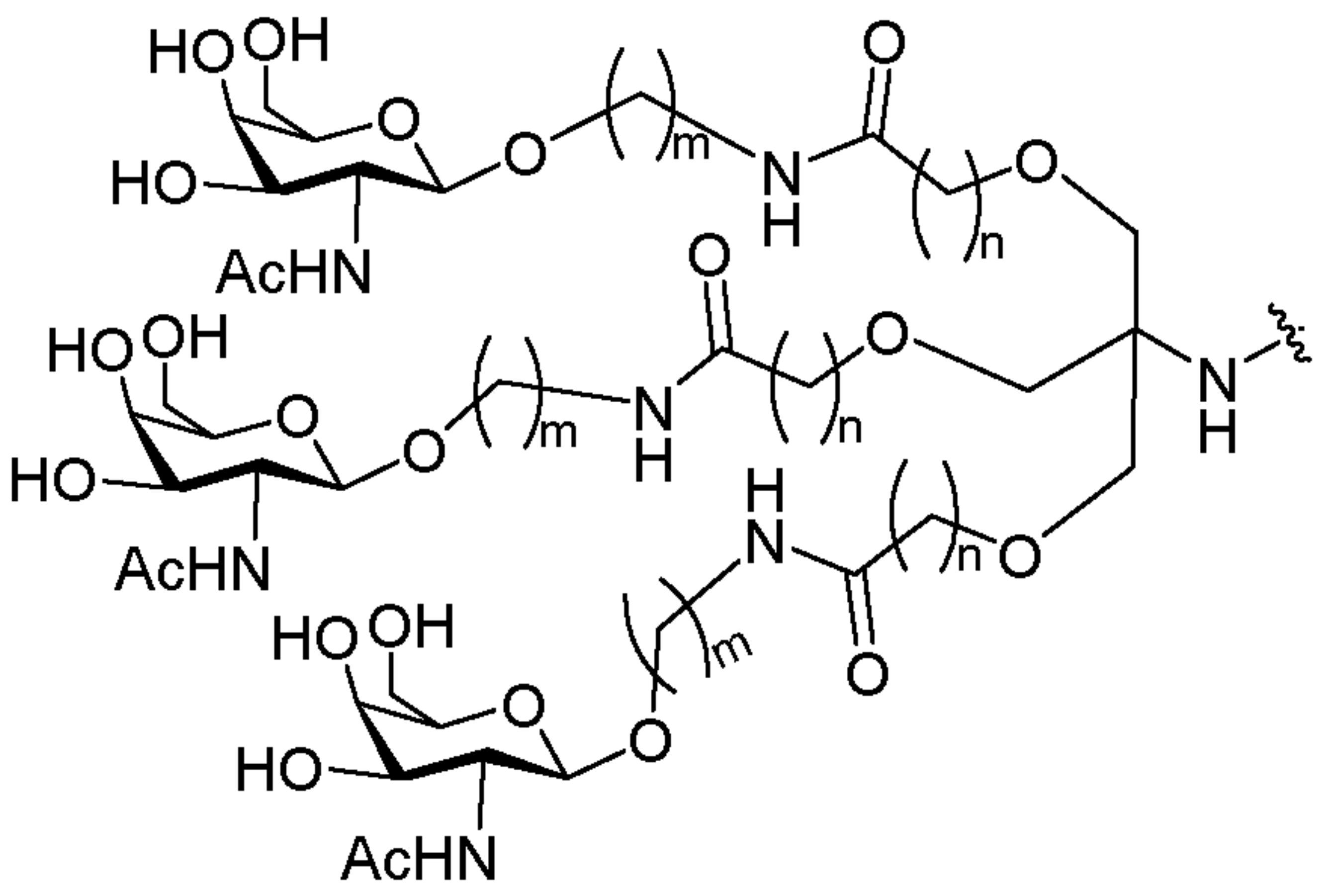
wherein Y and Z are independently selected from a C_1-C_{12} substituted or unsubstituted alkyl group, or a group comprising exactly one ether or exactly two ethers, an amide, an amine, a piperidine, a phosphate, a phosphodiester, or a phosphorothioate.

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



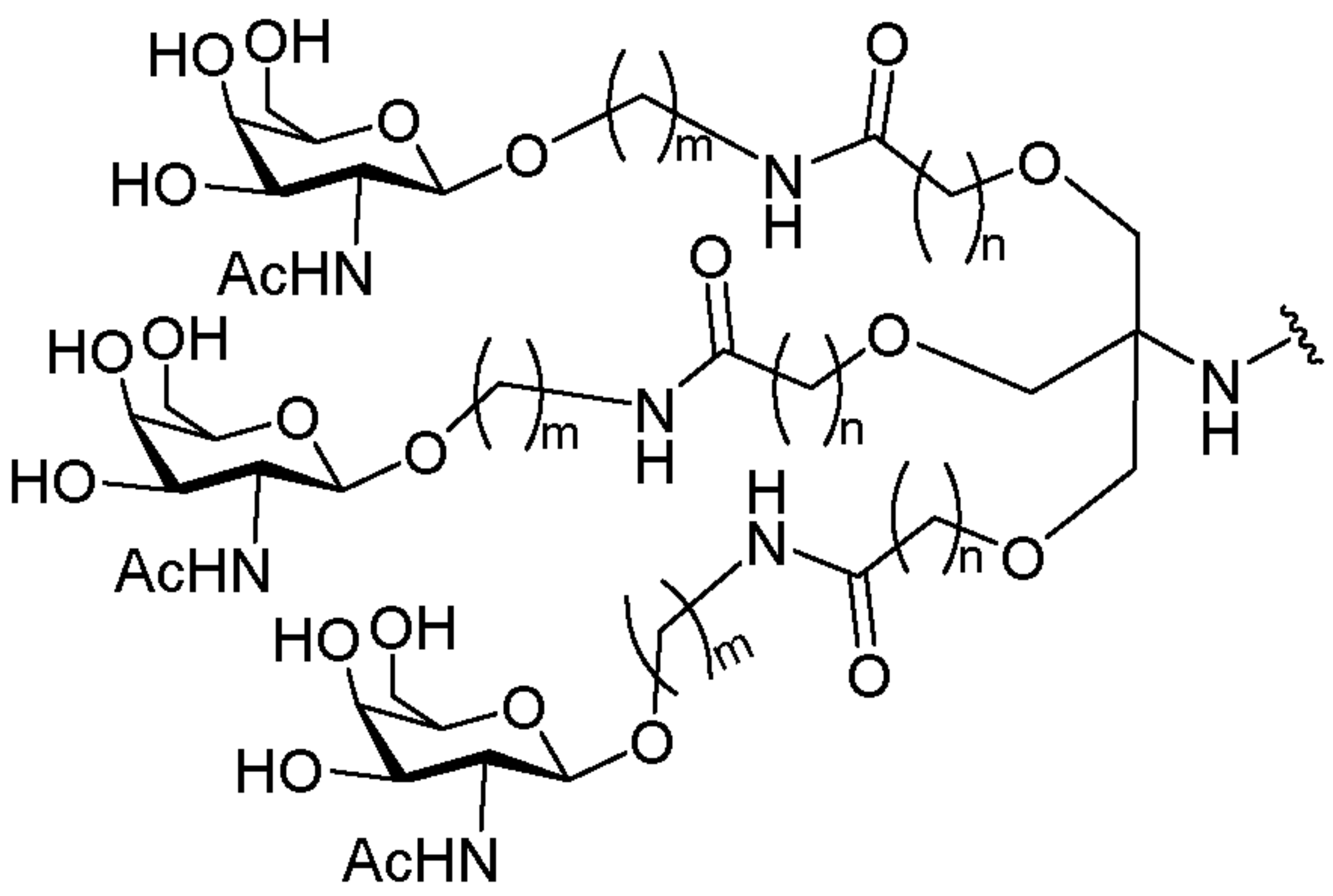
wherein Y and Z are independently selected from a C_1-C_{12} substituted or unsubstituted alkyl group.

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



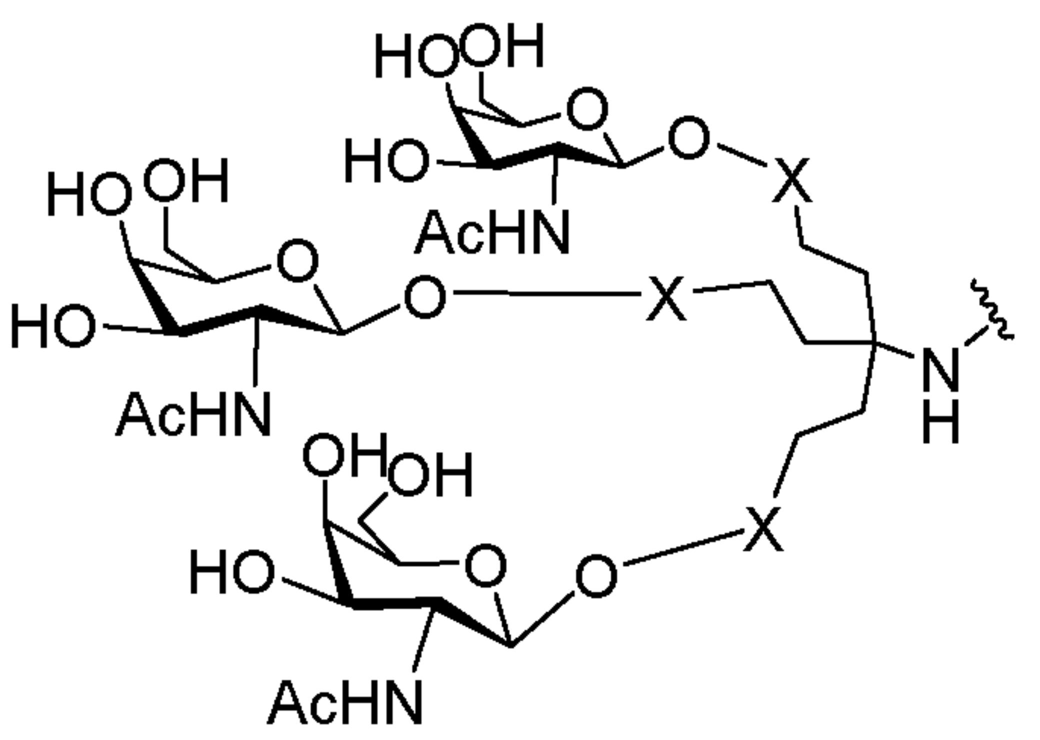
wherein m and n are independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12.

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



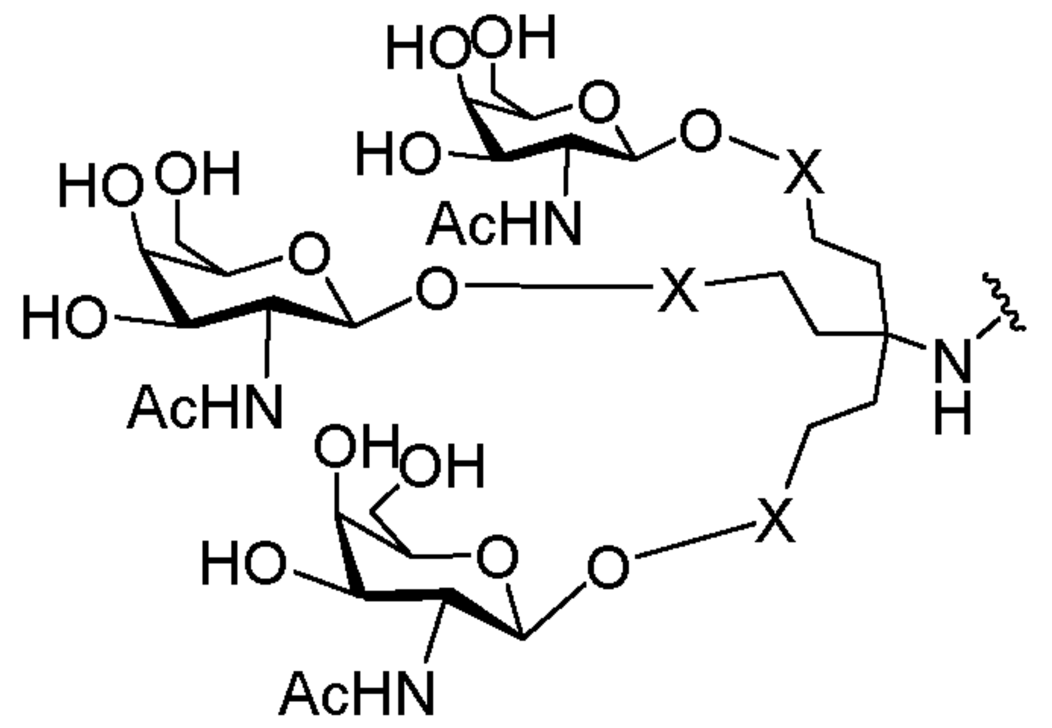
wherein m is 4, 5, 6, 7, or 8, and n is 1, 2, 3, or 4.

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



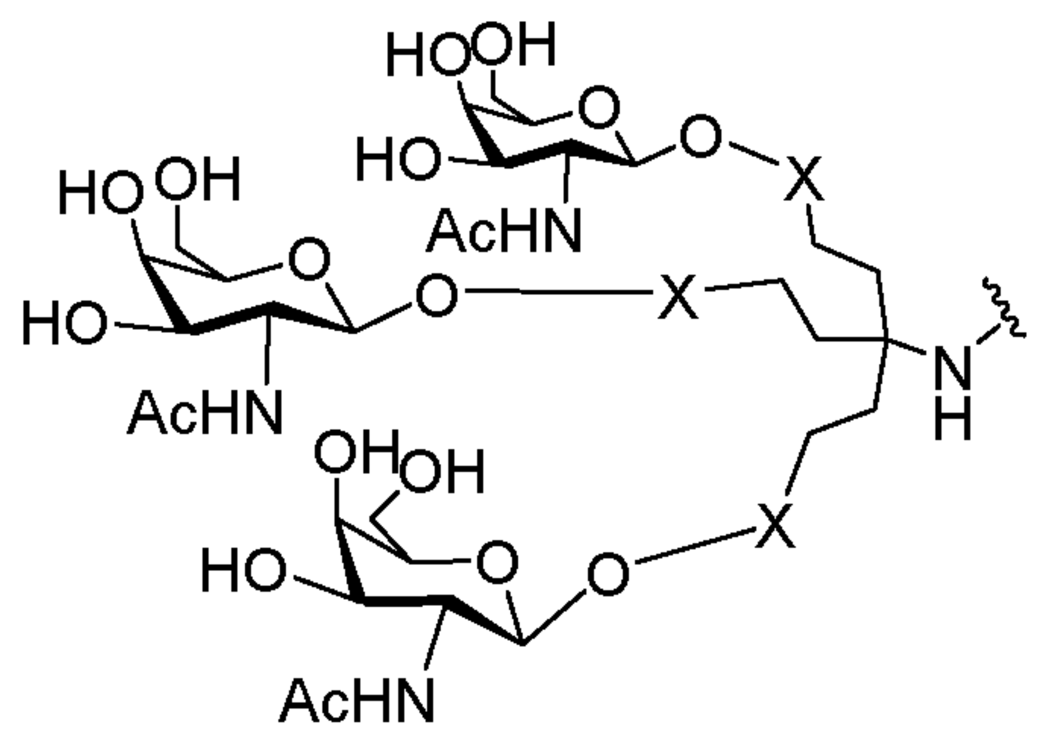
wherein X is a substituted or unsubstituted tether of four to thirteen consecutively bonded atoms, and wherein X does not comprise an ether group.

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



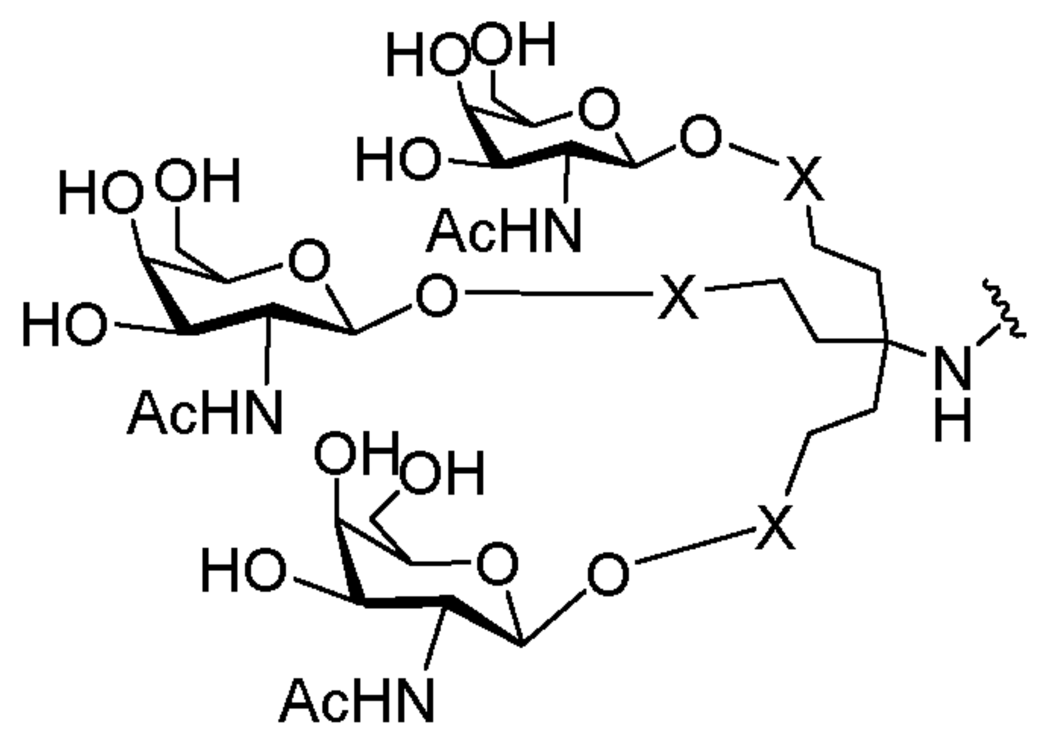
wherein X is a substituted or unsubstituted tether of eight consecutively bonded atoms, and wherein X does not comprise an ether group.

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



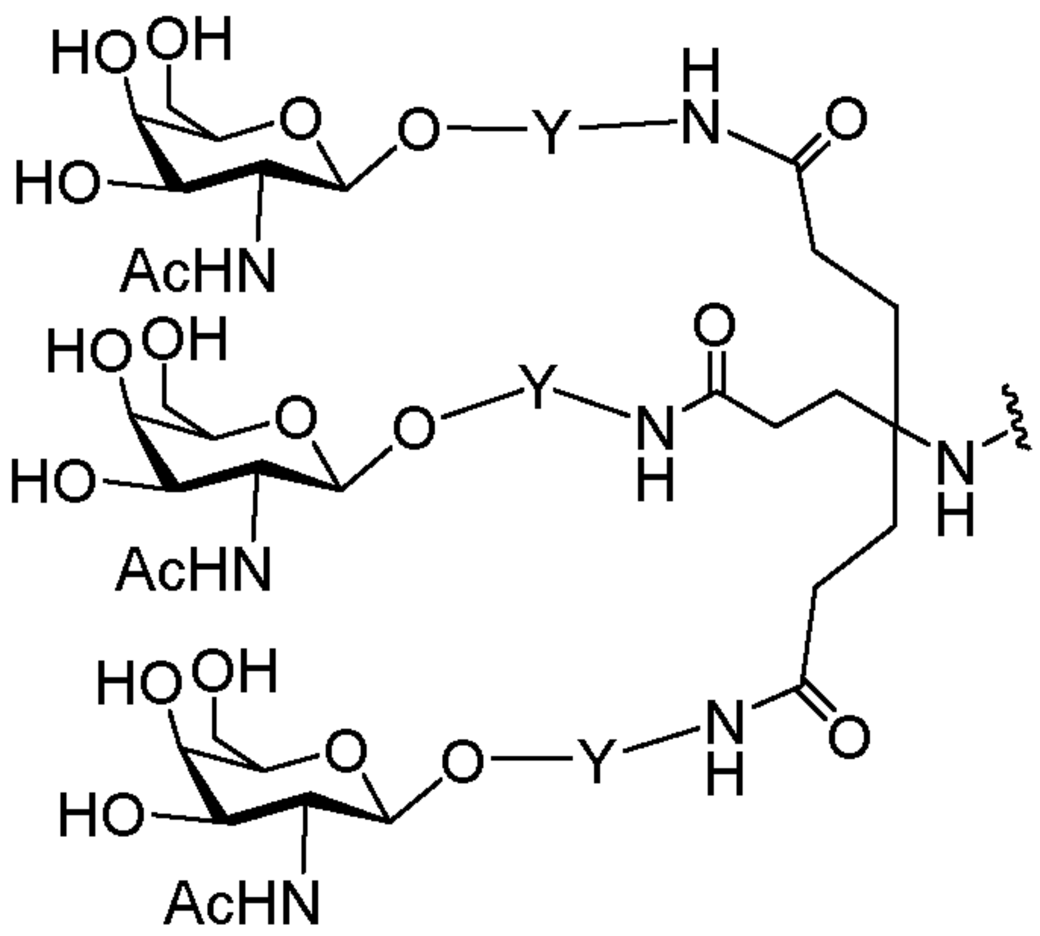
wherein X is a substituted or unsubstituted tether of four to thirteen consecutively bonded atoms, and wherein the tether comprises exactly one amide bond, and wherein X does not comprise an ether group.

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



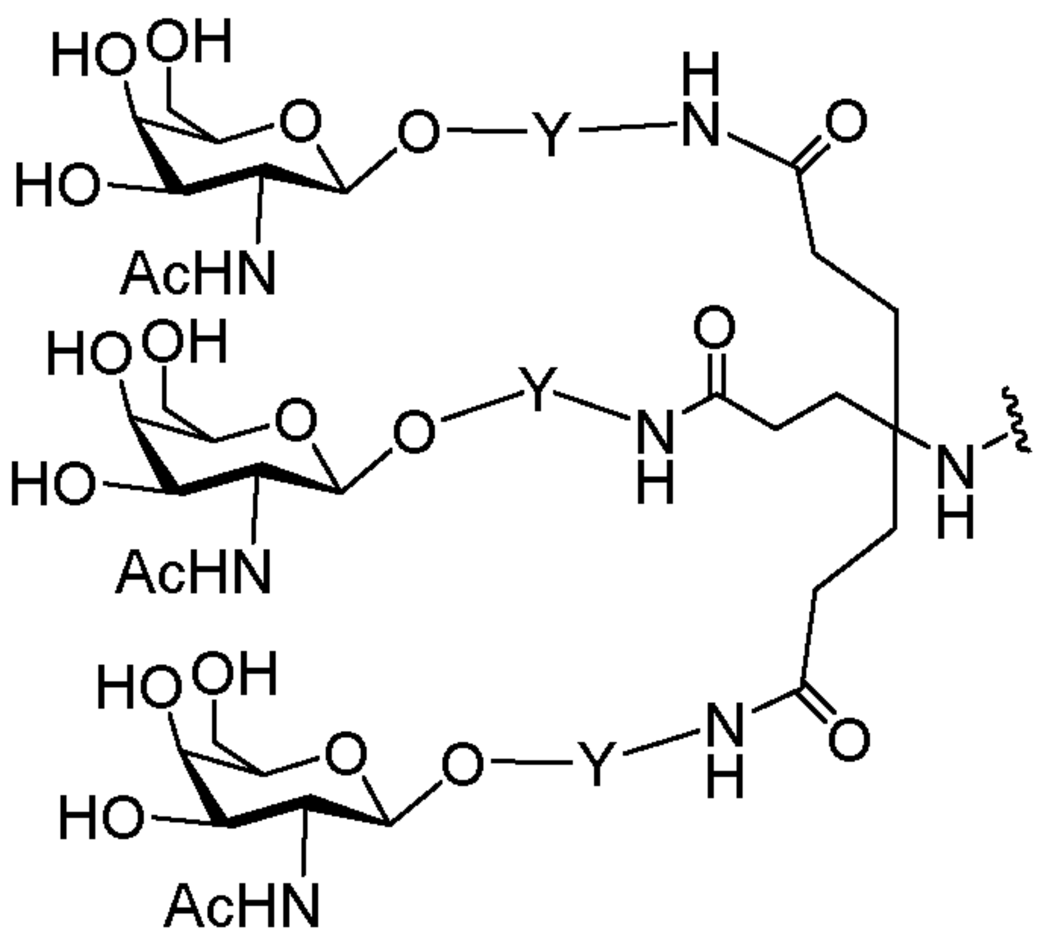
wherein X is a substituted or unsubstituted tether of four to thirteen consecutively bonded atoms and wherein the tether consists of an amide bond and a substituted or unsubstituted C_2-C_{11} alkyl group.

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



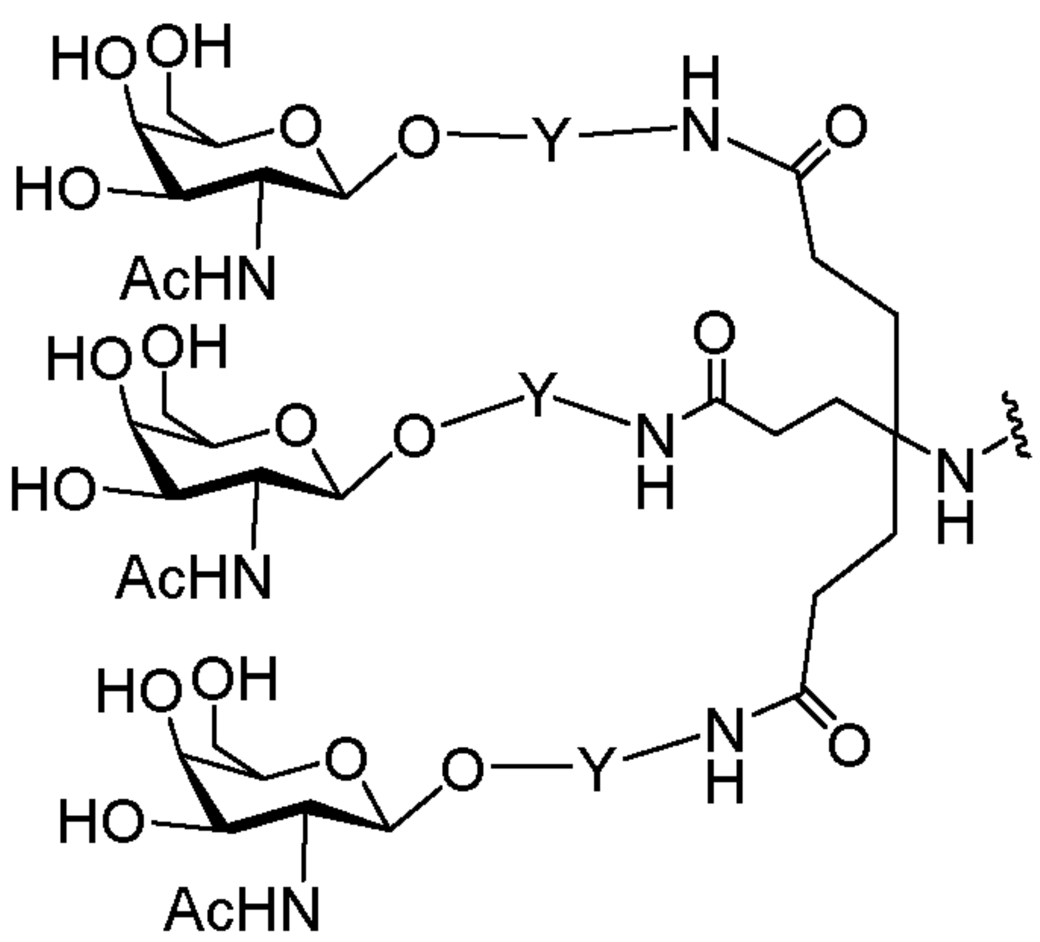
wherein Y is selected from a C_1-C_{12} substituted or unsubstituted alkyl, alkenyl, or alkynyl group, or a group comprising an ether, a ketone, an amide, an ester, a carbamate, an amine, a piperidine, a phosphate, a phosphodiester, a phosphorothioate, a triazole, a pyrrolidine, a disulfide, or a thioether.

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



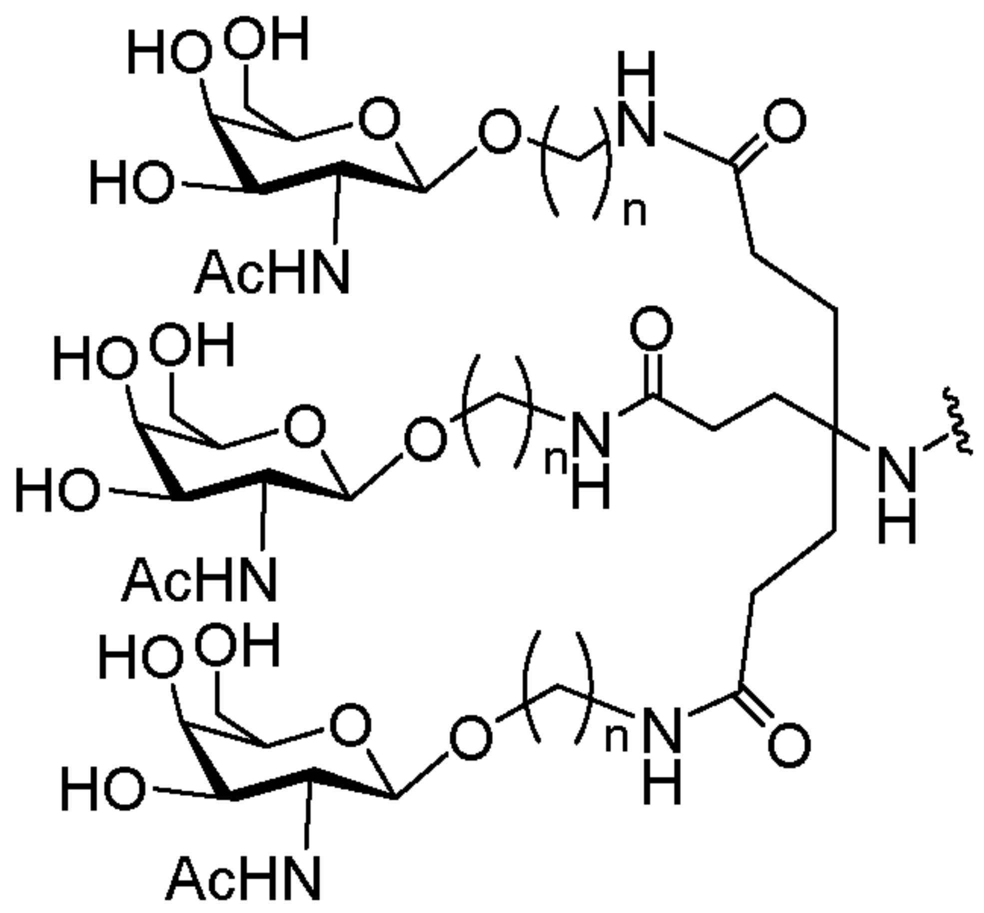
wherein Y is selected from a C_1-C_{12} substituted or unsubstituted alkyl group, or a group comprising an ether, an amine, a piperidine, a phosphate, a phosphodiester, or a phosphorothioate.

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



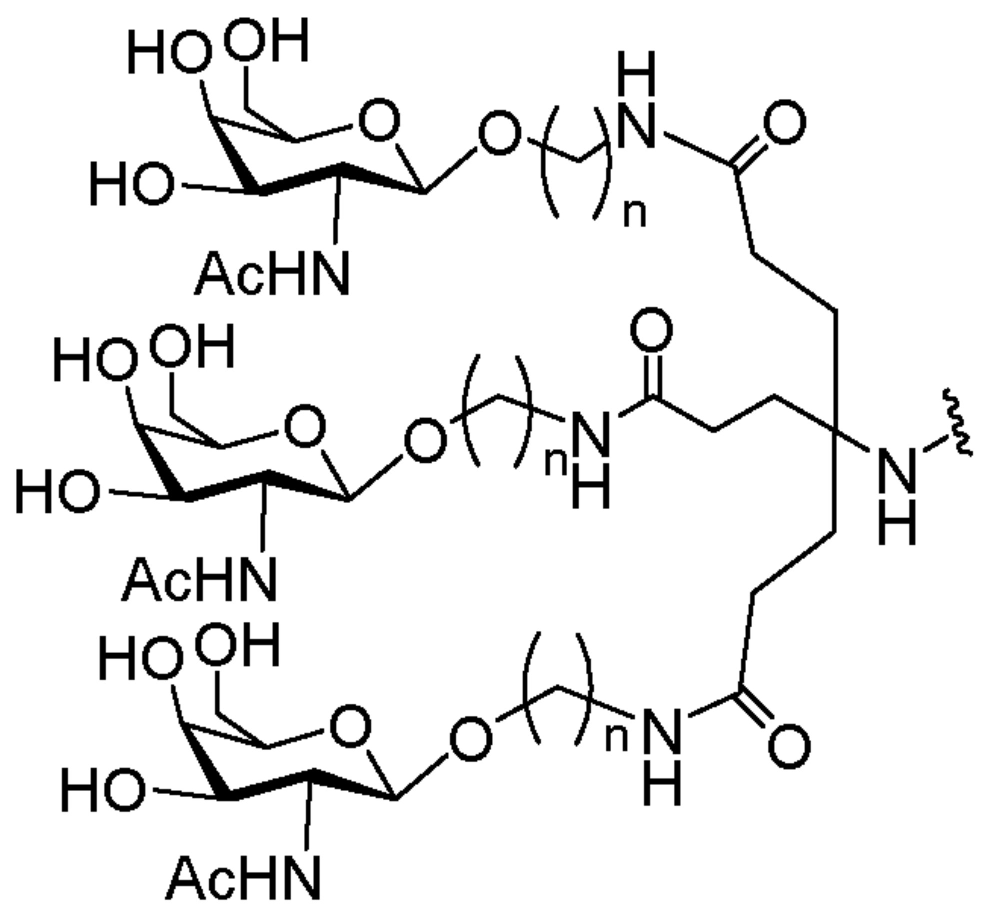
wherein Y is selected from a C_1-C_{12} substituted or unsubstituted alkyl group.

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:

$$A - B - C - D \left(- E - F \right)_q$$

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:

$$A-C-D\left(E-F\right)_q$$

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:

$$A-B-C\left(E-F\right)_q$$

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:

$$A-C\left(-E-F\right)_q$$

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:

$$A-B-D\left(-E-F\right)_q$$

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:

$$A-D\left(-E-F\right)_q$$

wherein
A is the antisense oligonucleotide;

D is the branching group

each E is a tether;

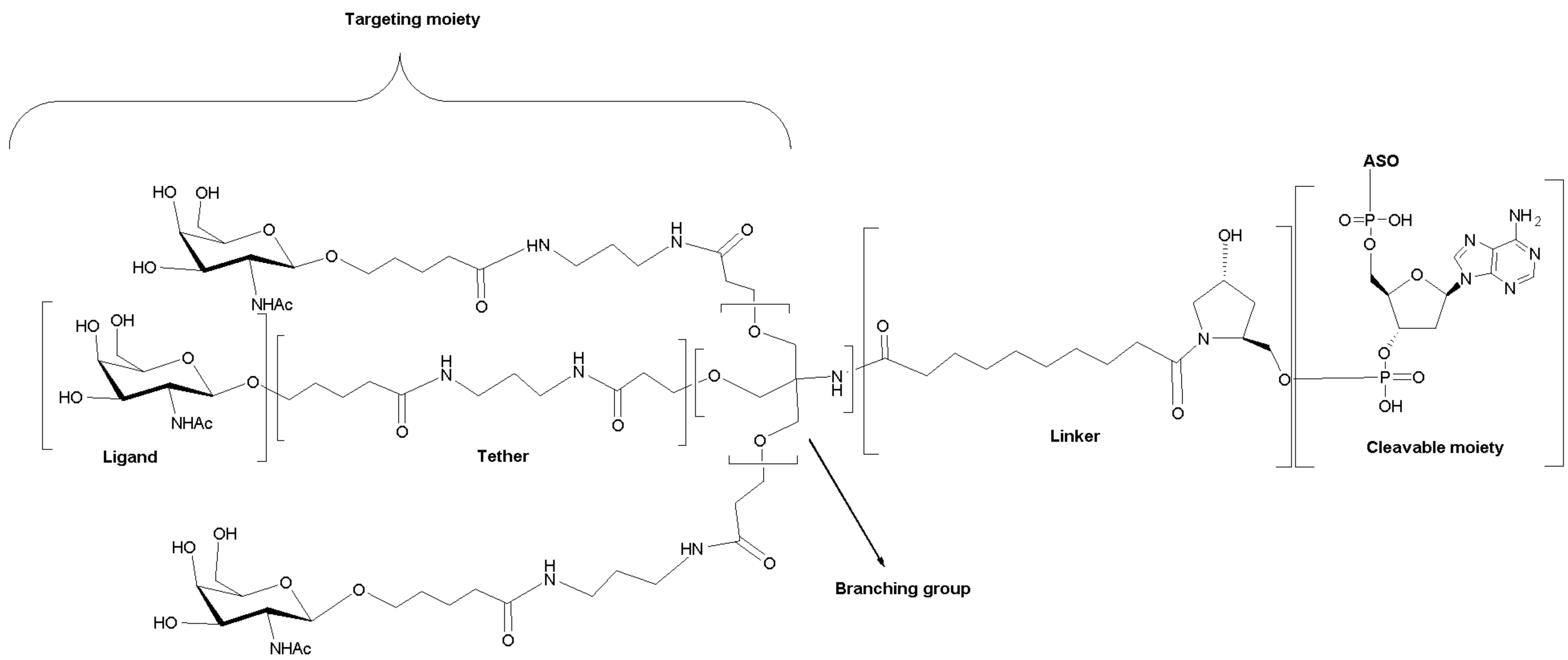
each F is a ligand; and

q is an integer between 1 and 5.

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

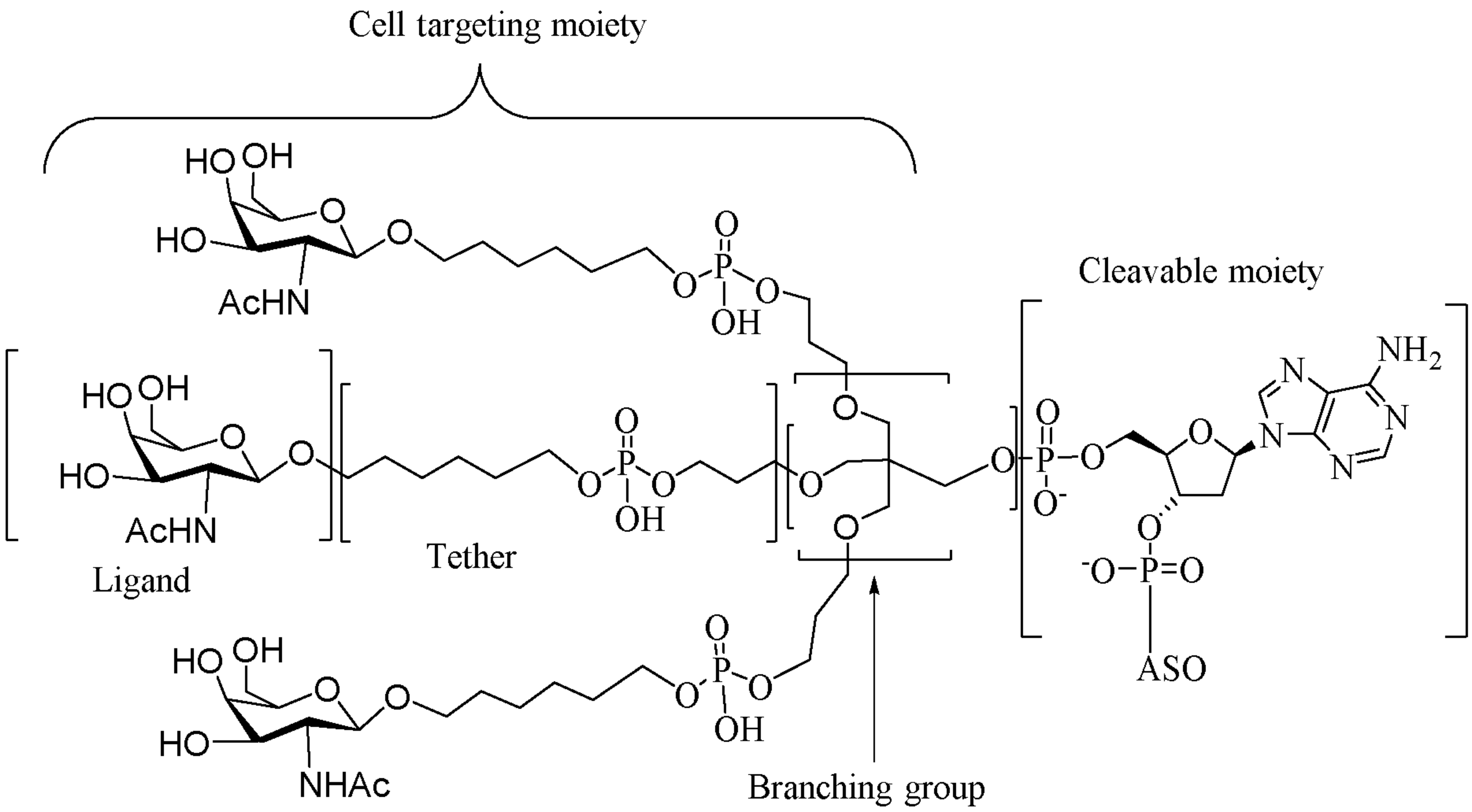
In certain embodiments each tether comprises at least one cleavable bond.

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



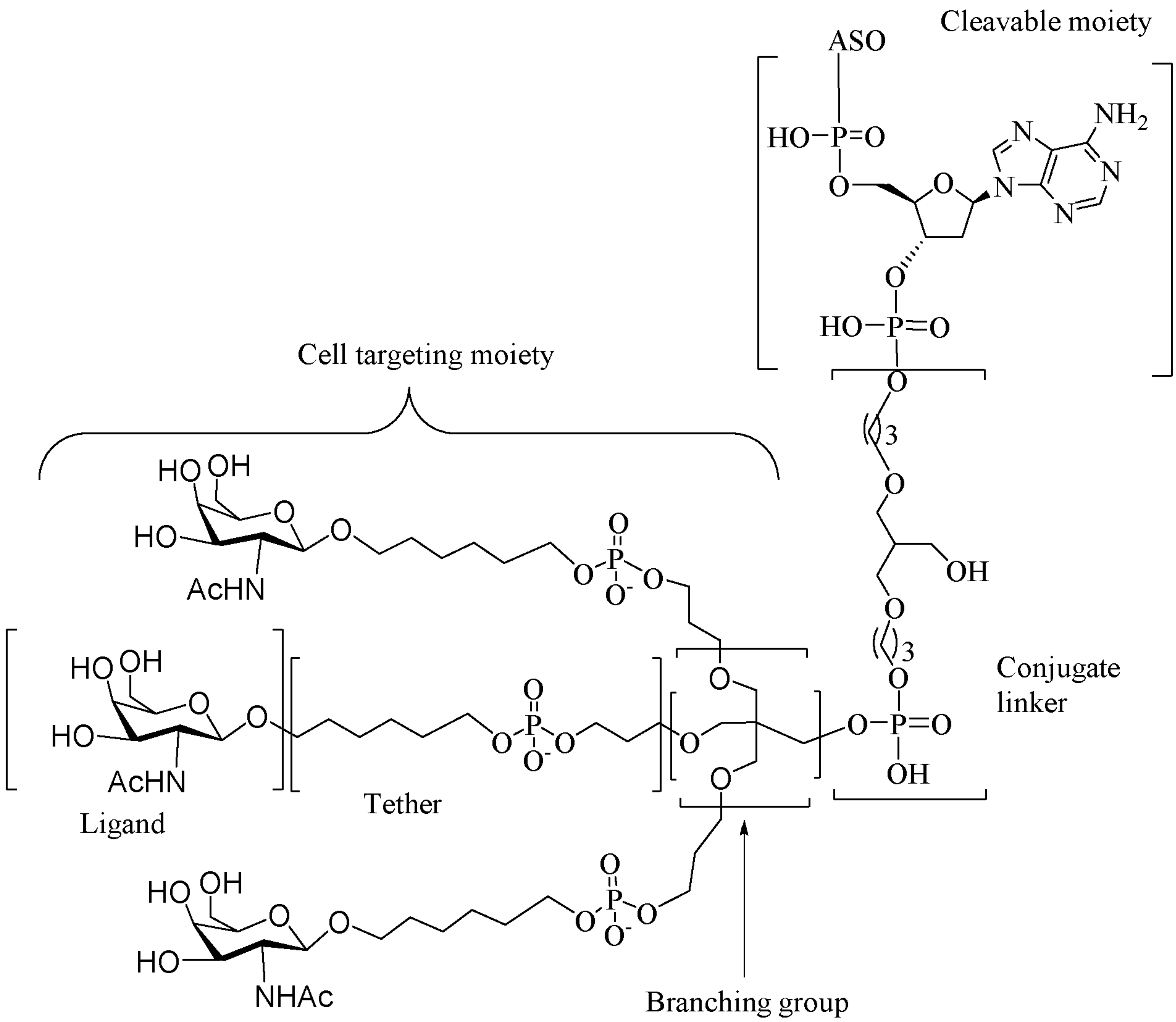
.

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

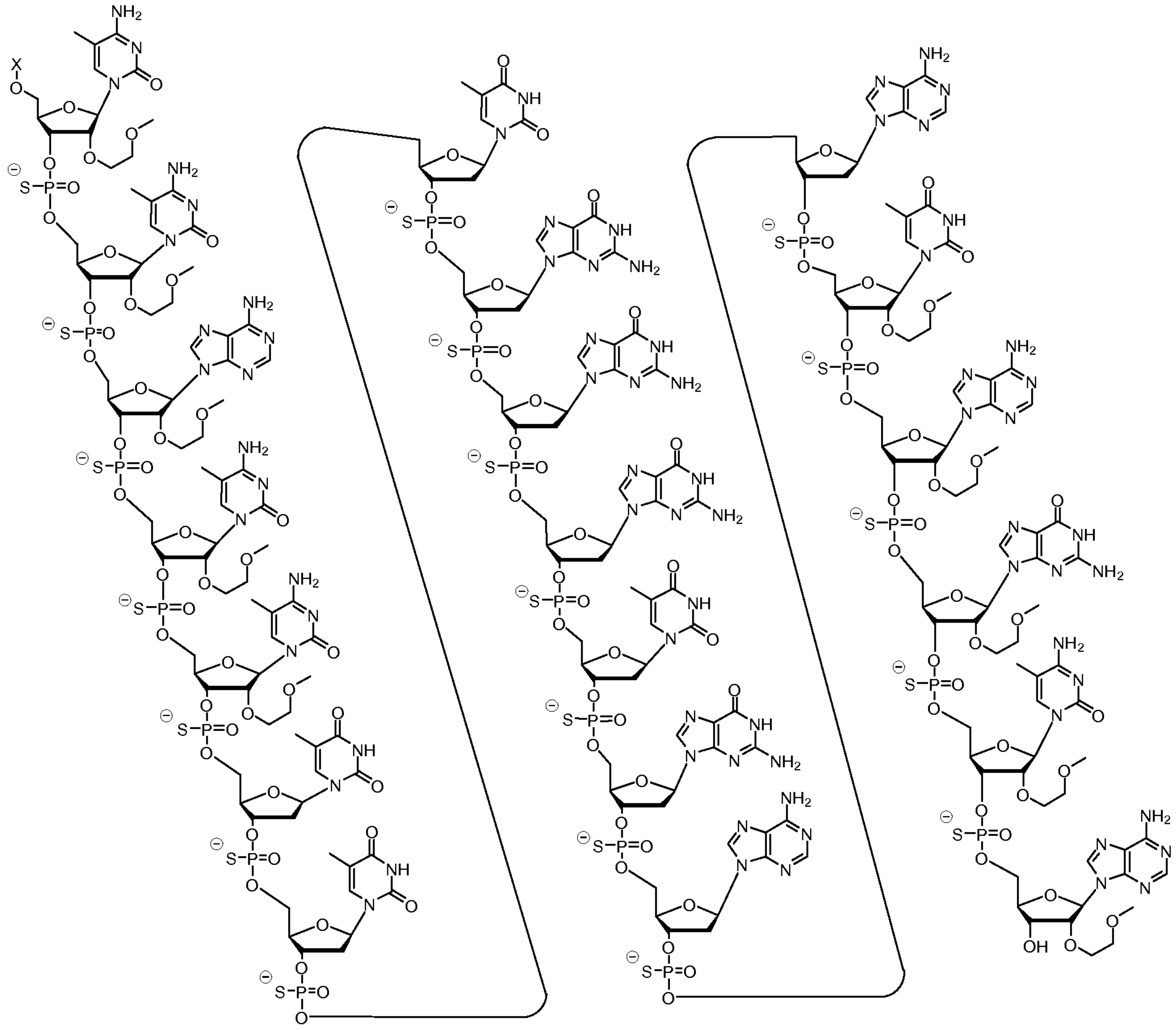


.

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

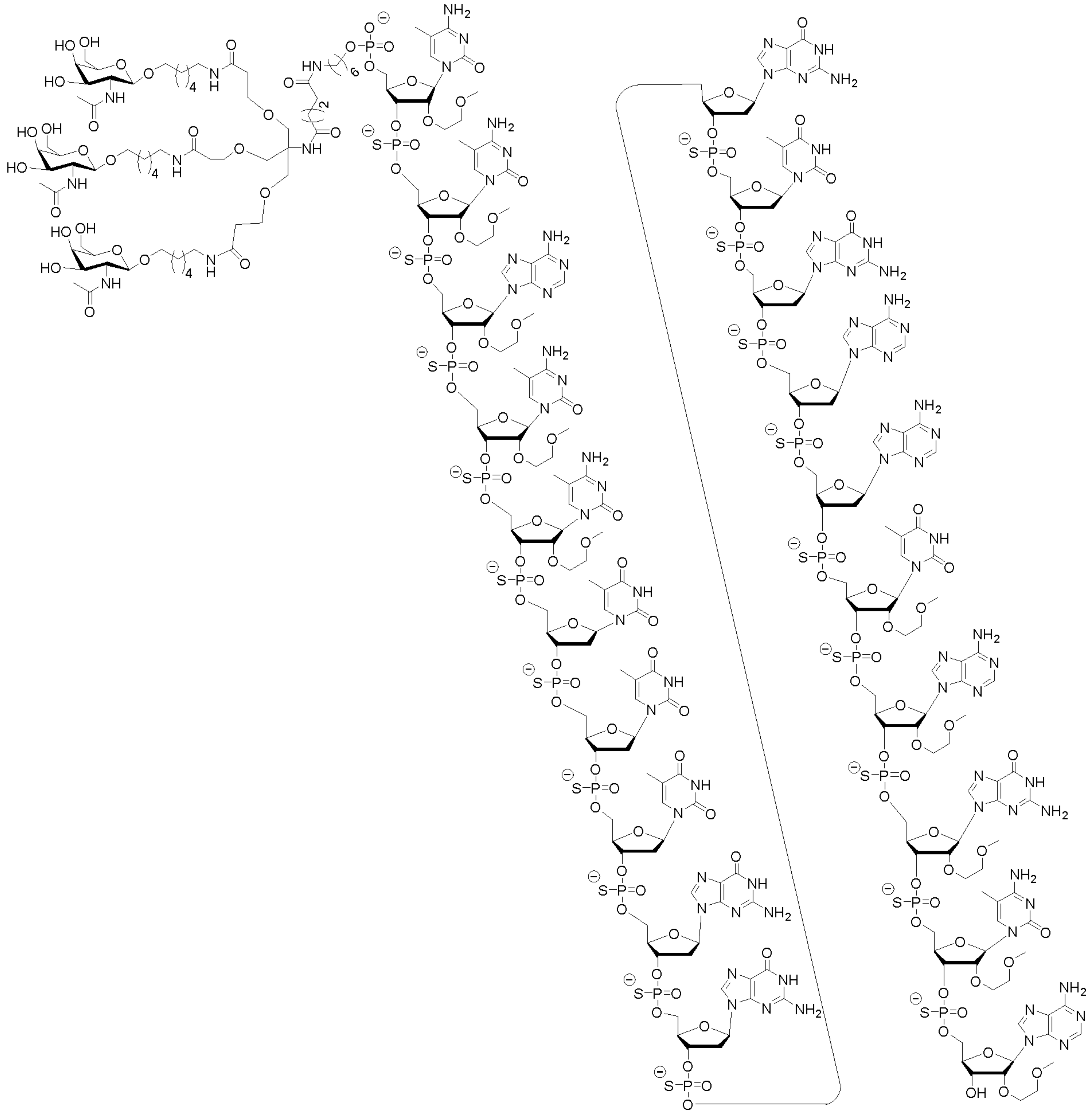


In certain embodiments, the conjugated antisense compound has the following structure: In certain embodiments, a compound comprises an ISIS oligonucleotide targeting GHR conjugated to GalNAc on the 5' end. For instance, in certain embodiments, a compound comprises ISIS 532401 conjugated to GalNAc on the 5' end. . In further embodiments, the compound has the following chemical structure comprising or consisting of ISIS 532401 with 5'-X, wherein X is a conjugate group comprising GalNAc as described herein:

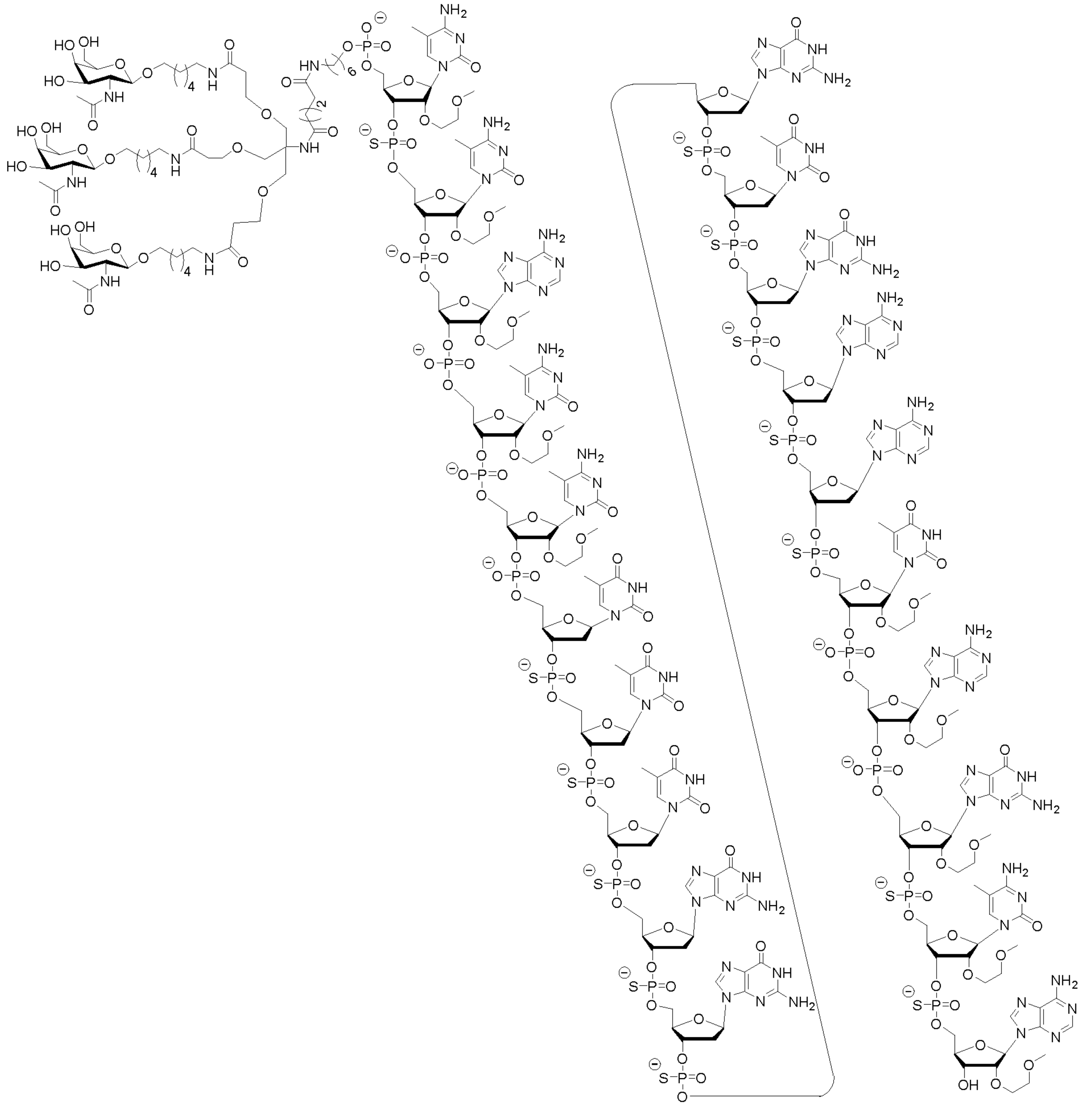


wherein X is a conjugate group comprising GalNAc.

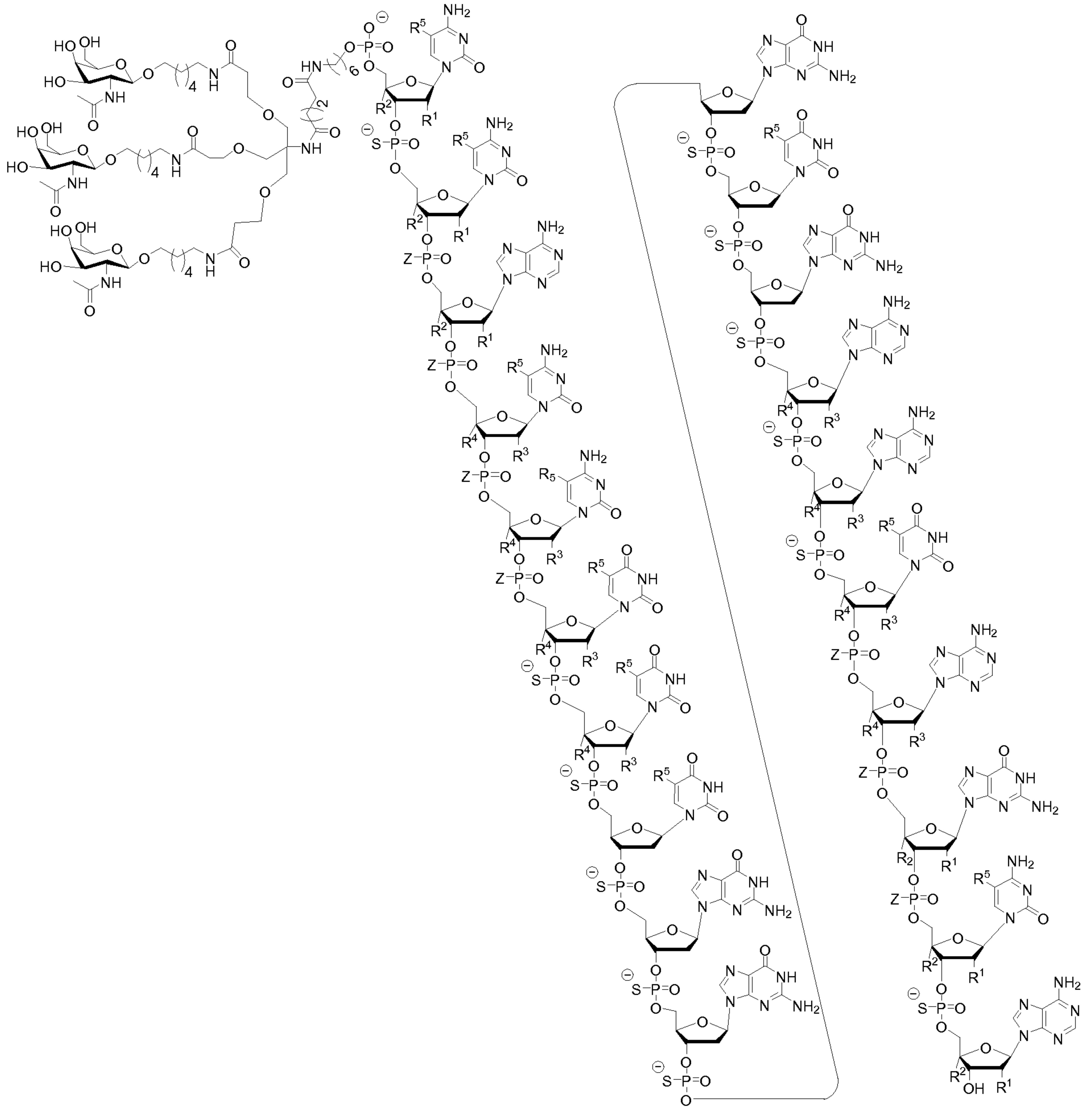
In certain embodiments, a compound comprises an ISIS oligonucleotide targeting GHR conjugated to GalNAc, and wherein each internucleoside linkage of the oligonucleotide com is a phosphorothioate linkage. In further embodiments, the compound comprises the sequence of ISIS 532401 conjugated to GalNAc, and wherein each internucleoside linkage of the oligonucleotide com is a phosphorothioate linkage. In such embodiments, the chemical structure is as follows:



In certain embodiments, a compound comprises an ISIS oligonucleotide targeting GHR conjugated to GalNAc, and wherein each internucleoside linkage of the oligonucleotide com is a phosphorothioate linkage or a phosphodiester linkage. In further embodiments, the compound comprises the sequence of ISIS 532401 conjugated to GalNAc, and wherein each internucleoside linkage of the oligonucleotide com is a phosphorothioate linkage or a phosphodiester linkage. In such embodiments, the chemical structure is as follows:



In certain embodiments, a compound comprises an ISIS oligonucleotide targeting GHR conjugated to GalNAc. In further such embodiments, the compound comprises the sequence of ISIS 532401 conjugated to GalNAc, and is 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^-.

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 excess growth hormone in a subject by administering a GHR specific inhibitor, such as an antisense compound or oligonucleotide targeted to GHR. In certain aspects, the disease associated with excess growth hormone is acromegaly. In certain aspects, the disease associated with excess growth hormone is gigantism.

Certain embodiments provide a method of treating, preventing, or ameliorating acromegaly in a subject by administering a GHR specific inhibitor, such as an antisense compound or oligonucleotide targeted to GHR. Acromegaly is a disease associated with excess growth hormone (GH). In over 90 percent of acromegaly patients, the overproduction of growth hormones is caused by a benign tumor of the pituitary gland, called an adenoma, which produces excess growth hormone and compresses surrounding brain tissues. Expansion of the adenoma can cause headaches and visual impairment that often accompany acromegaly. In some instances, acromegaly is caused by tumors of the pancreas, lungs, or adrenal glands that lead to an excess of GH, either by producing GH or by producing Growth Hormone Releasing Hormone (GHRH), the hormone that stimulates the pituitary to make GH.

Acromegaly most commonly affects adults in middle age and can result in severe disfigurement, complicating conditions, and premature death. Because of its pathogenesis and slow progression, acromegaly often goes undiagnosed until changes in external features become noticeable, such as changes in the face. Acromegaly is often associated with gigantism.

Features of acromegaly include soft tissue swelling resulting in enlargement of the hands, feet, nose, lips and ears, and a general thickening of the skin; soft tissue swelling of internal organs, such as the heart and kidney; vocal cord swelling resulting in a low voice and slow speech; expansion of the skull; pronounced eyebrow protrusion, often with ocular distension; pronounced lower jaw protrusion and enlargement of the tongue; teeth gapping; and carpal tunnel syndrome. In certain embodiments, any one or combination of these features of acromegaly can be treated, prevented, or ameliorated by administering a compound or composition targeted to GHR provided herein.

EXAMPLES

Non-limiting disclosure and incorporation by reference

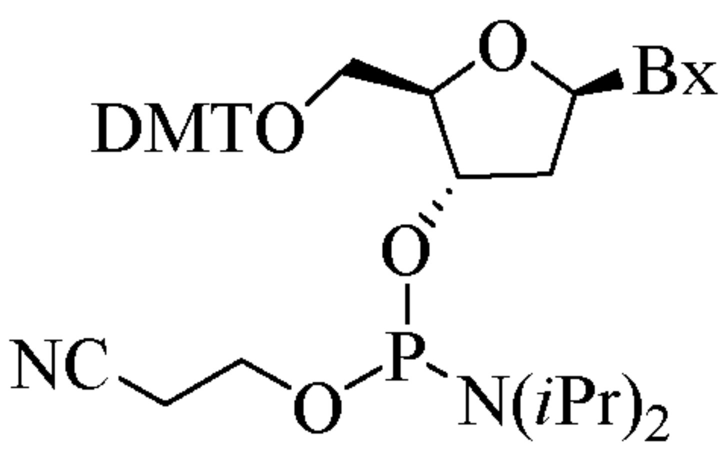
While certain compounds, compositions and methods described herein have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the compounds described herein and are not intended to limit the same. Each of the references recited in the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

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

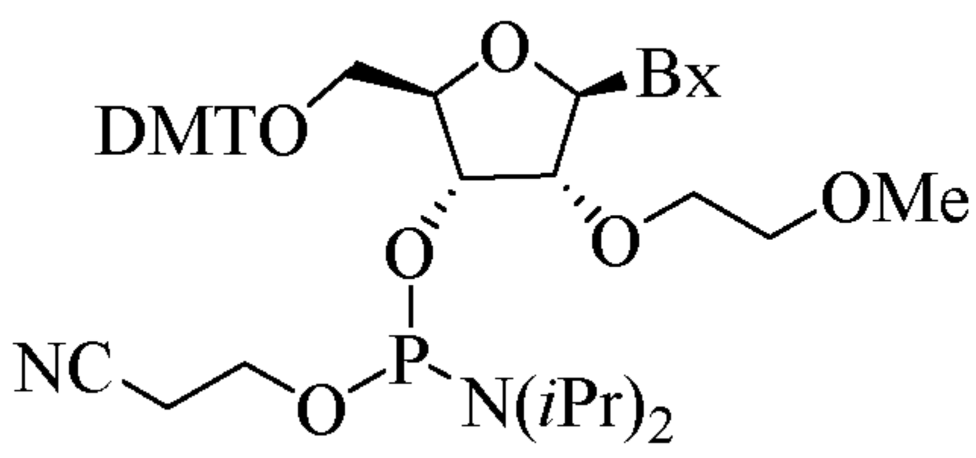
The following examples illustrate certain embodiments of the present disclosure and are not limiting. Moreover, where specific embodiments are provided, the inventors have contemplated generic application of those specific embodiments. For example, disclosure of an oligonucleotide having a particular 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

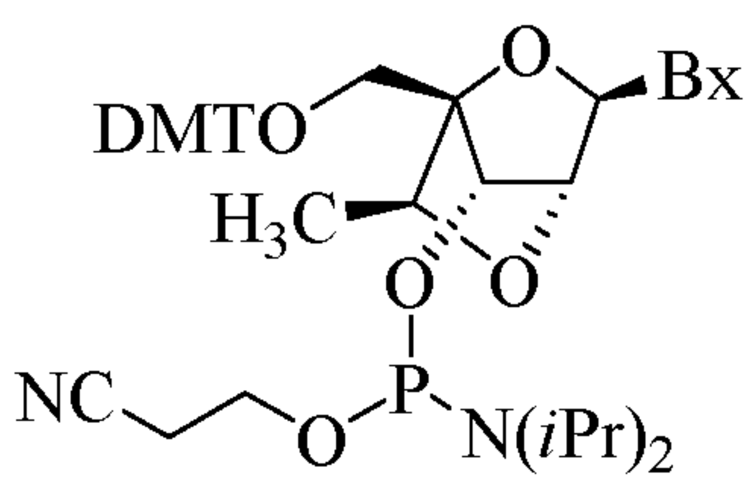
Compounds 1, 1a and 2 were prepared as per the procedures well known in the art as described in the specification herein (see Seth et al., Bioorg. Med. Chem., 2011, 21(4), 1122-1125, J. Org. Chem., 2010, 75(5), 1569-1581, Nucleic Acids Symposium Series, 2008, 52(1), 553-554); and also see published PCT 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).



1



1a

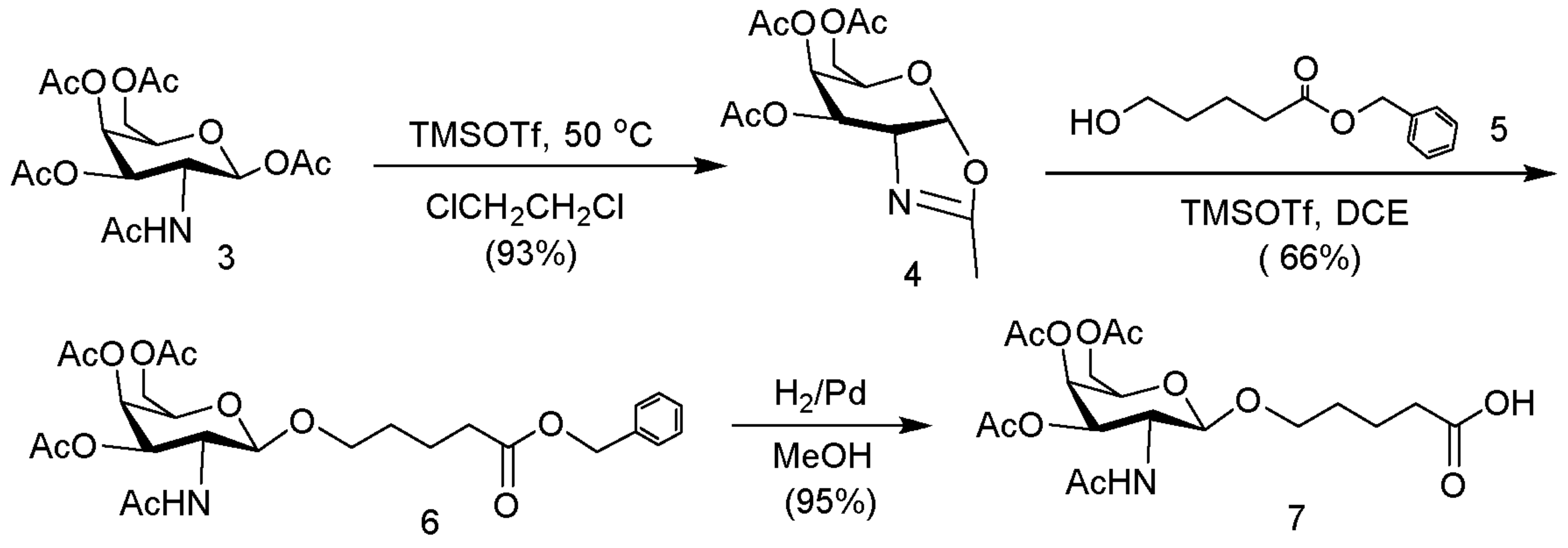


2

Bx is a heterocyclic base;

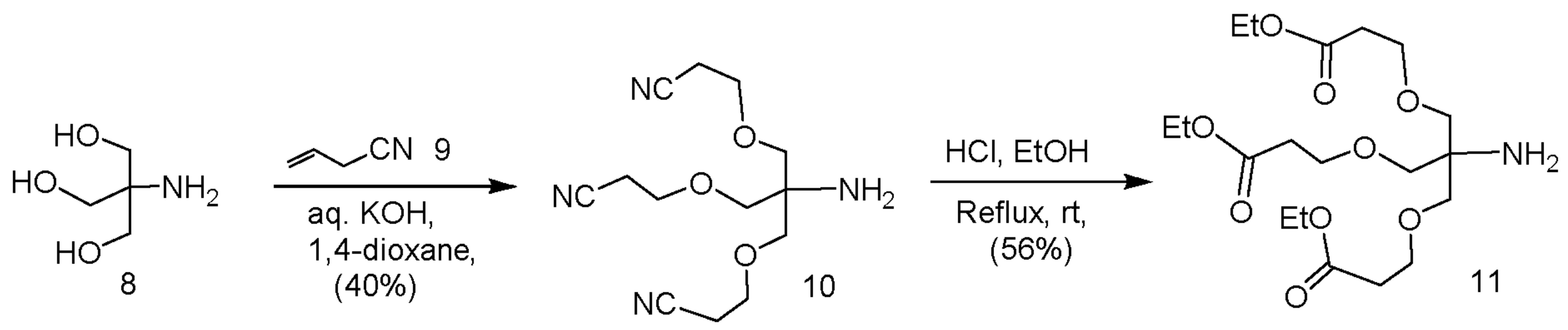
Example 2: Preparation of Compound 7

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).



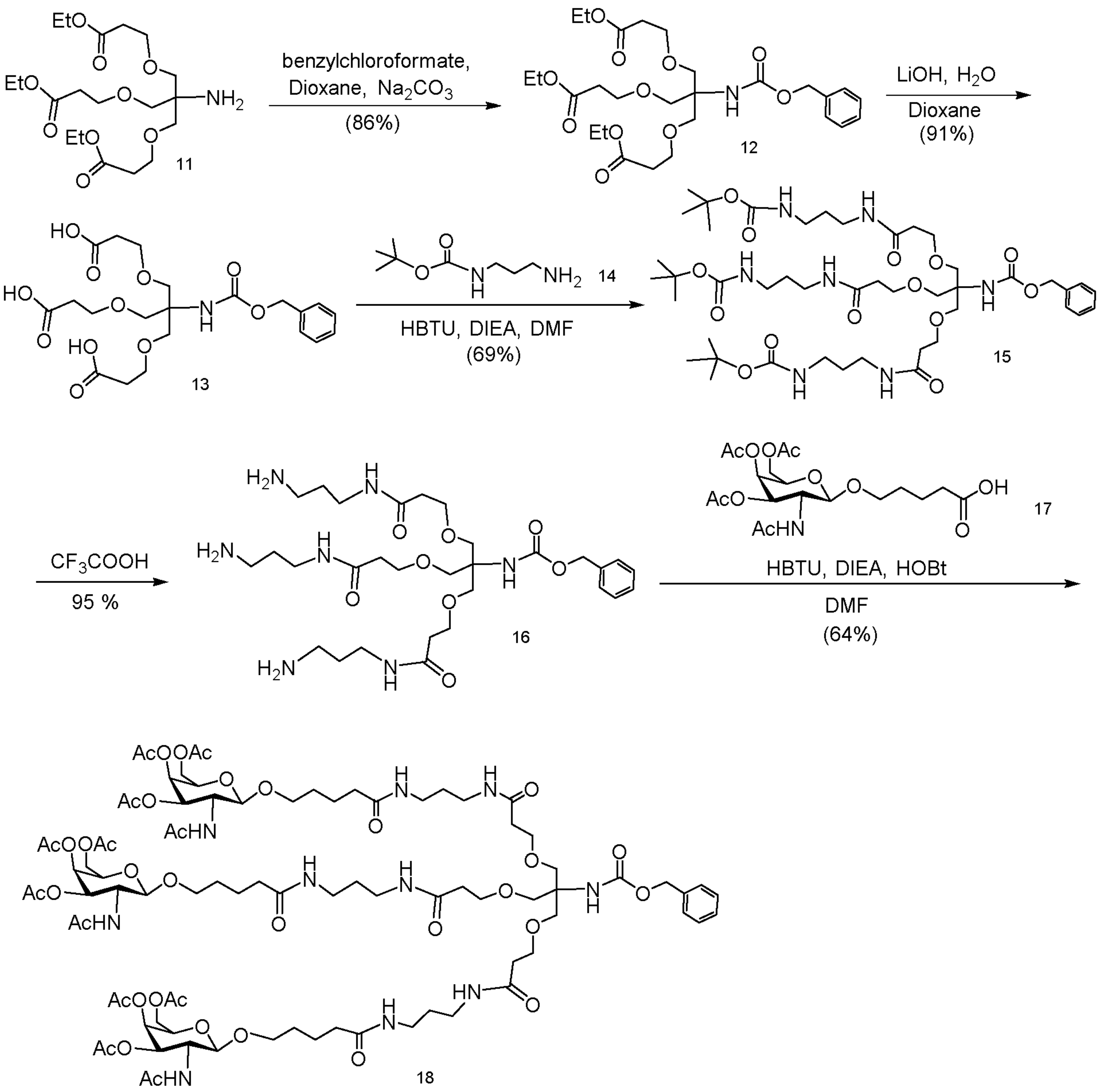
Example 3: Preparation of Compound 11

Compounds 8 and 9 are commercially available.



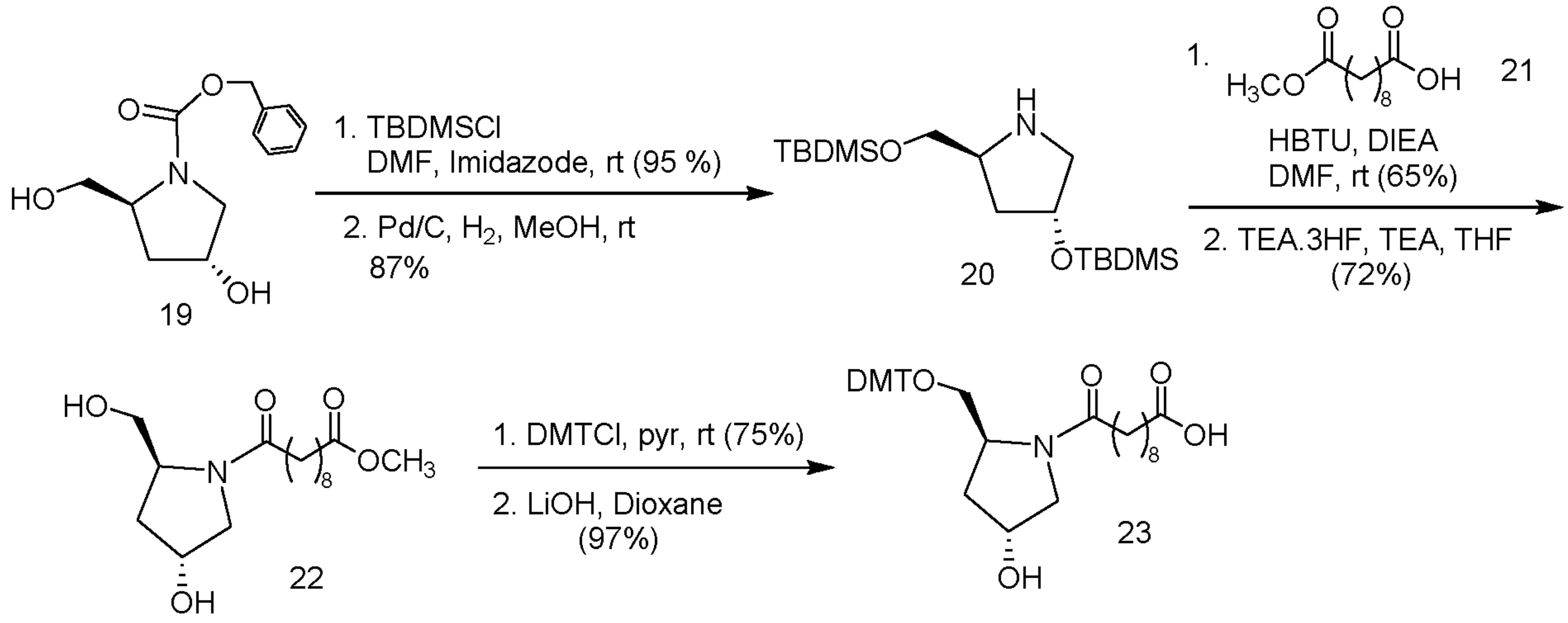
Example 4: Preparation of Compound 18

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.


benzylchloroformate,
Dioxane, Na_2CO_3
(86%)
LiOH, H_2O
Dioxane
(91%)
11
12
13
14
15
16
17
18
HBTU, DIEA, DMF
(69%)
CF_3COOH
95 %
HBTU, DIEA, HOBt
DMF
(64%)

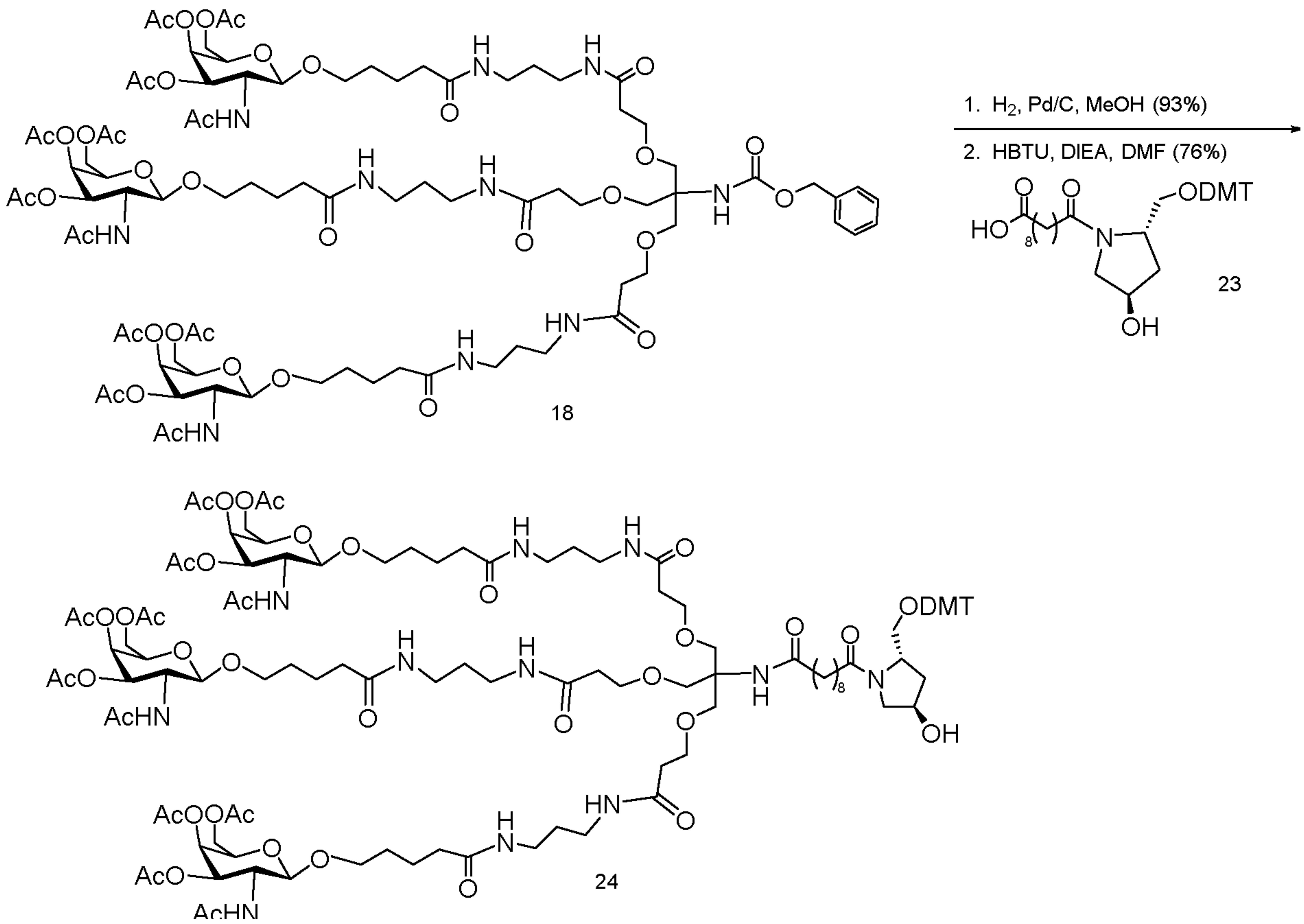
Example 5: Preparation of Compound 23

Compounds 19 and 21 are commercially available.



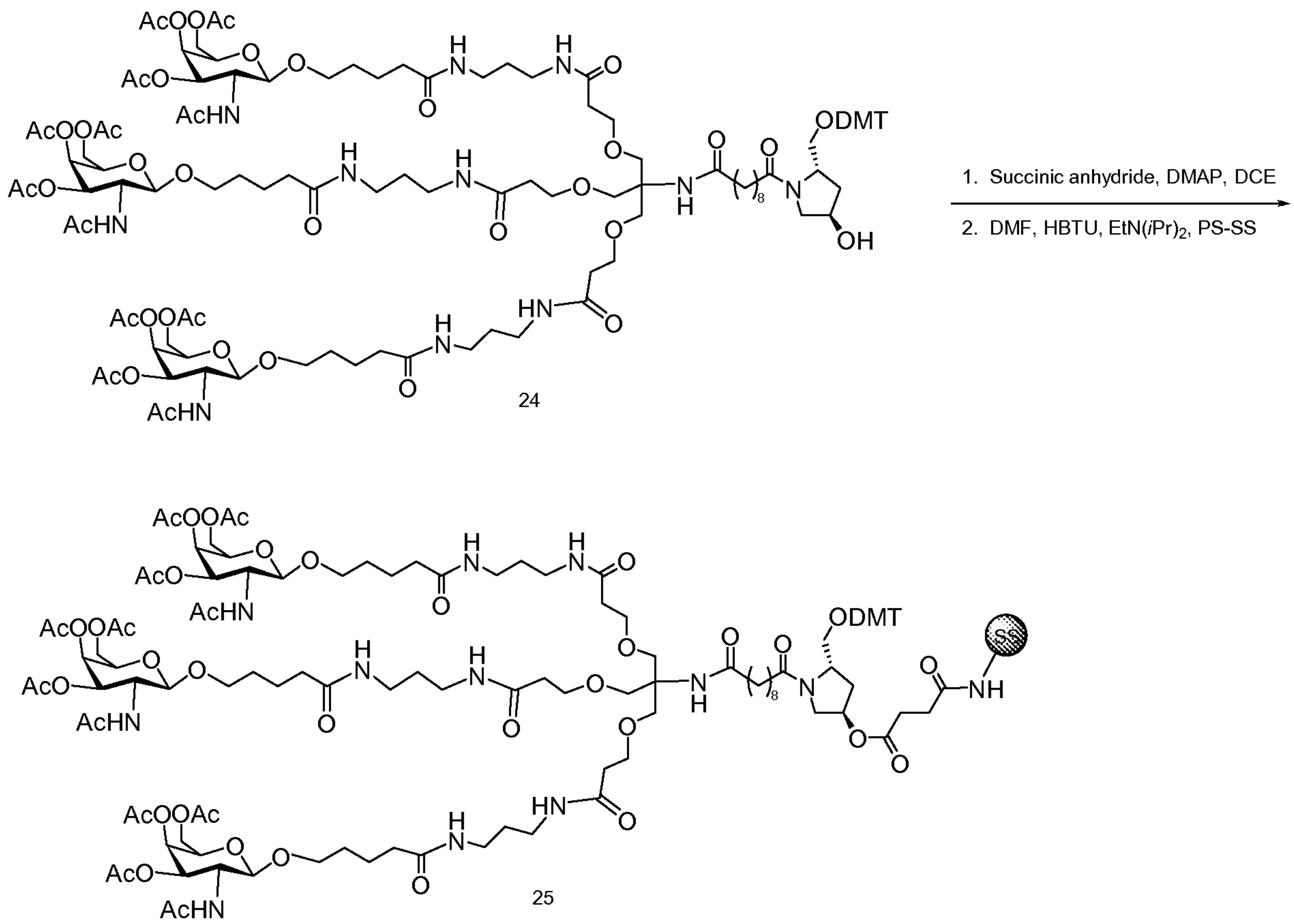
Example 6: Preparation of Compound 24

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.

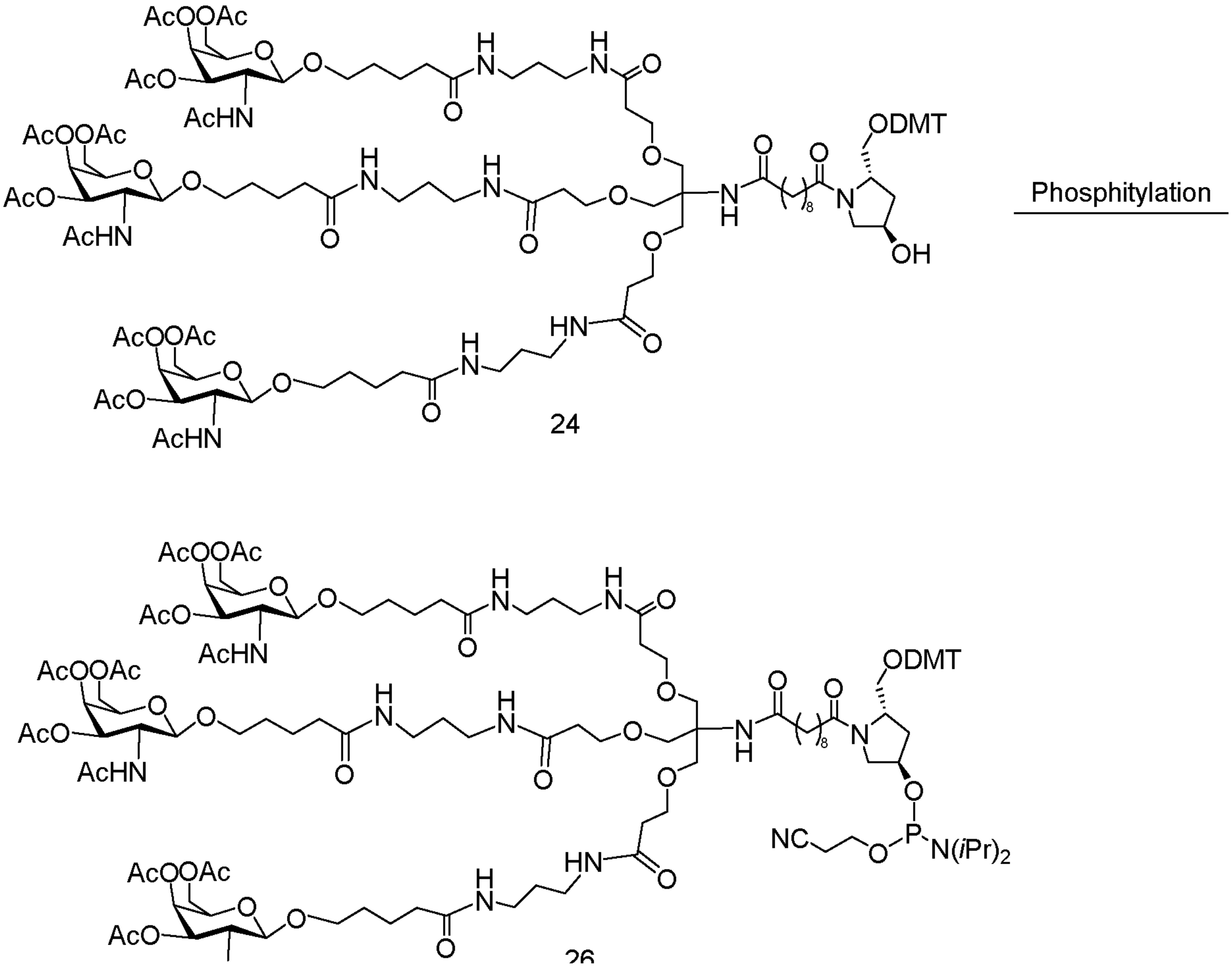


24

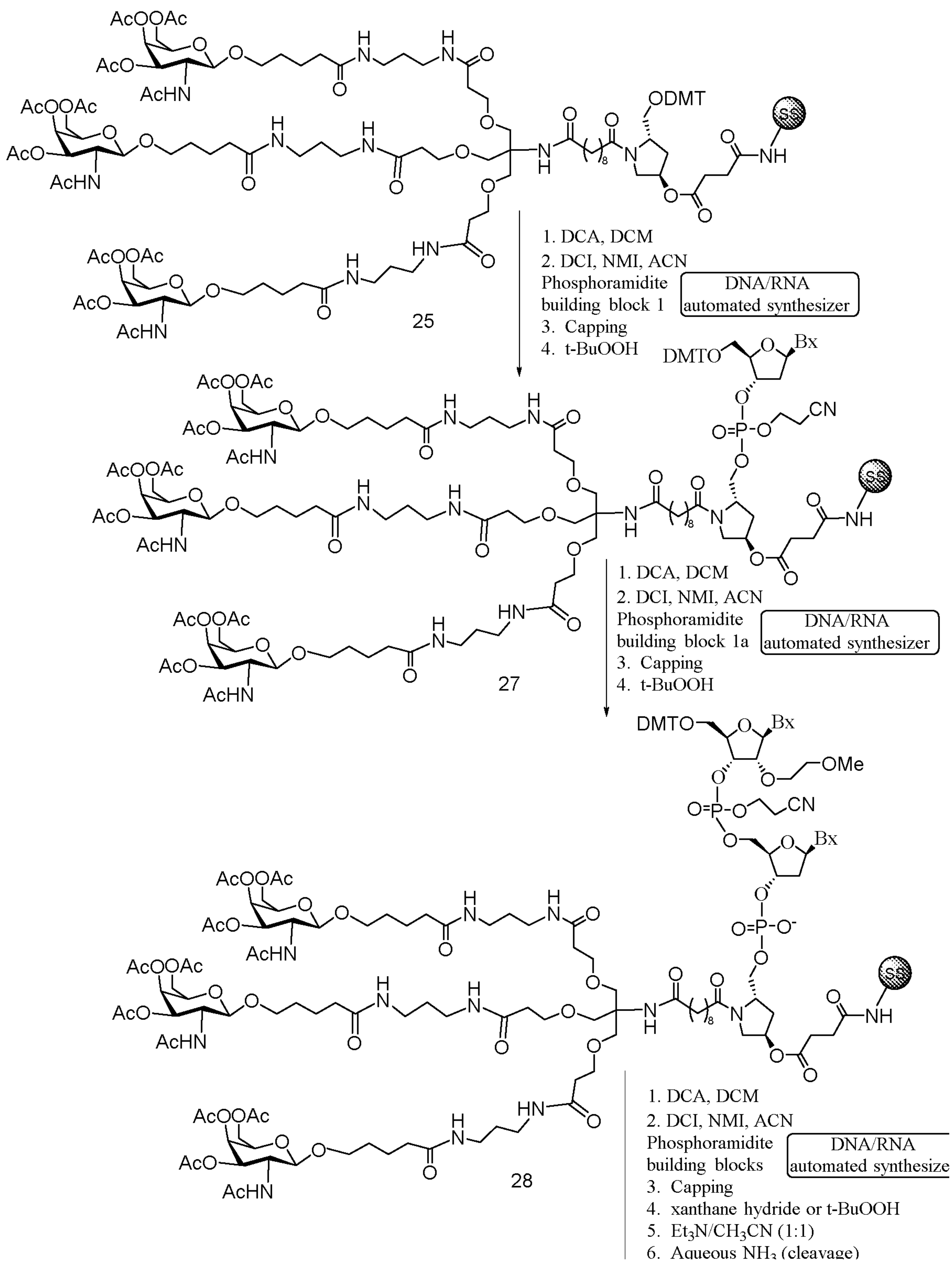
25

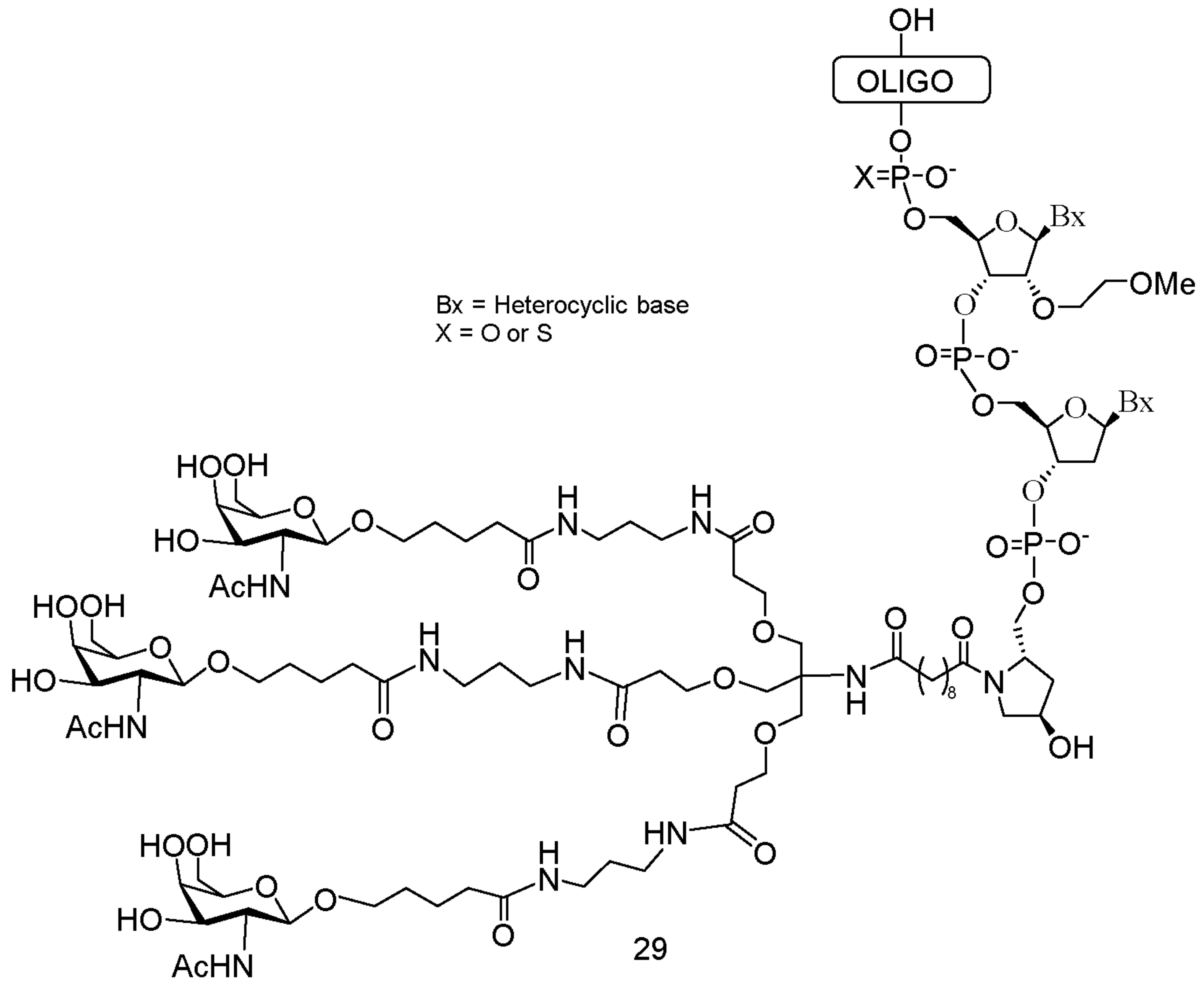
Example 8: Preparation of Compound 26

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

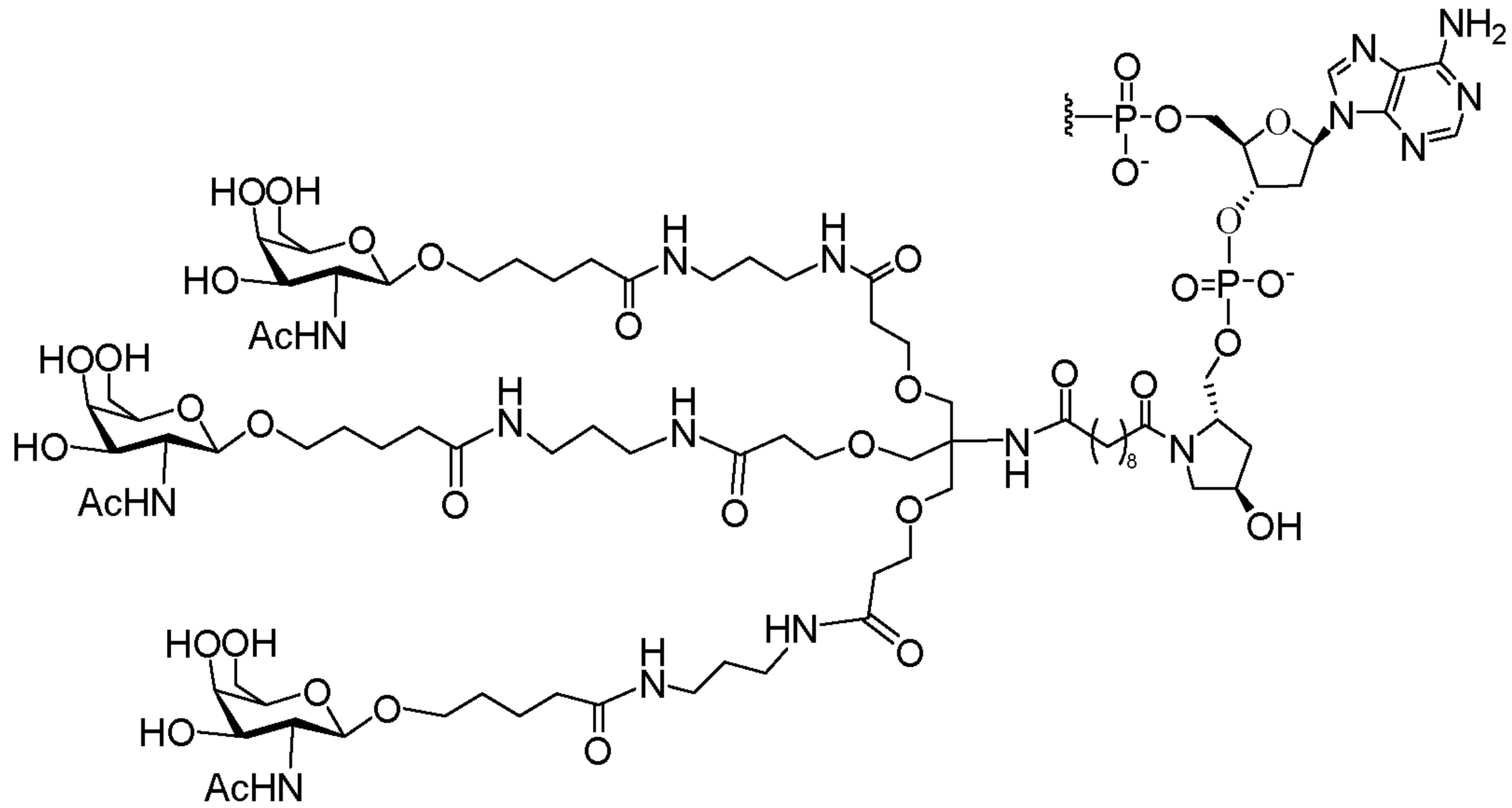




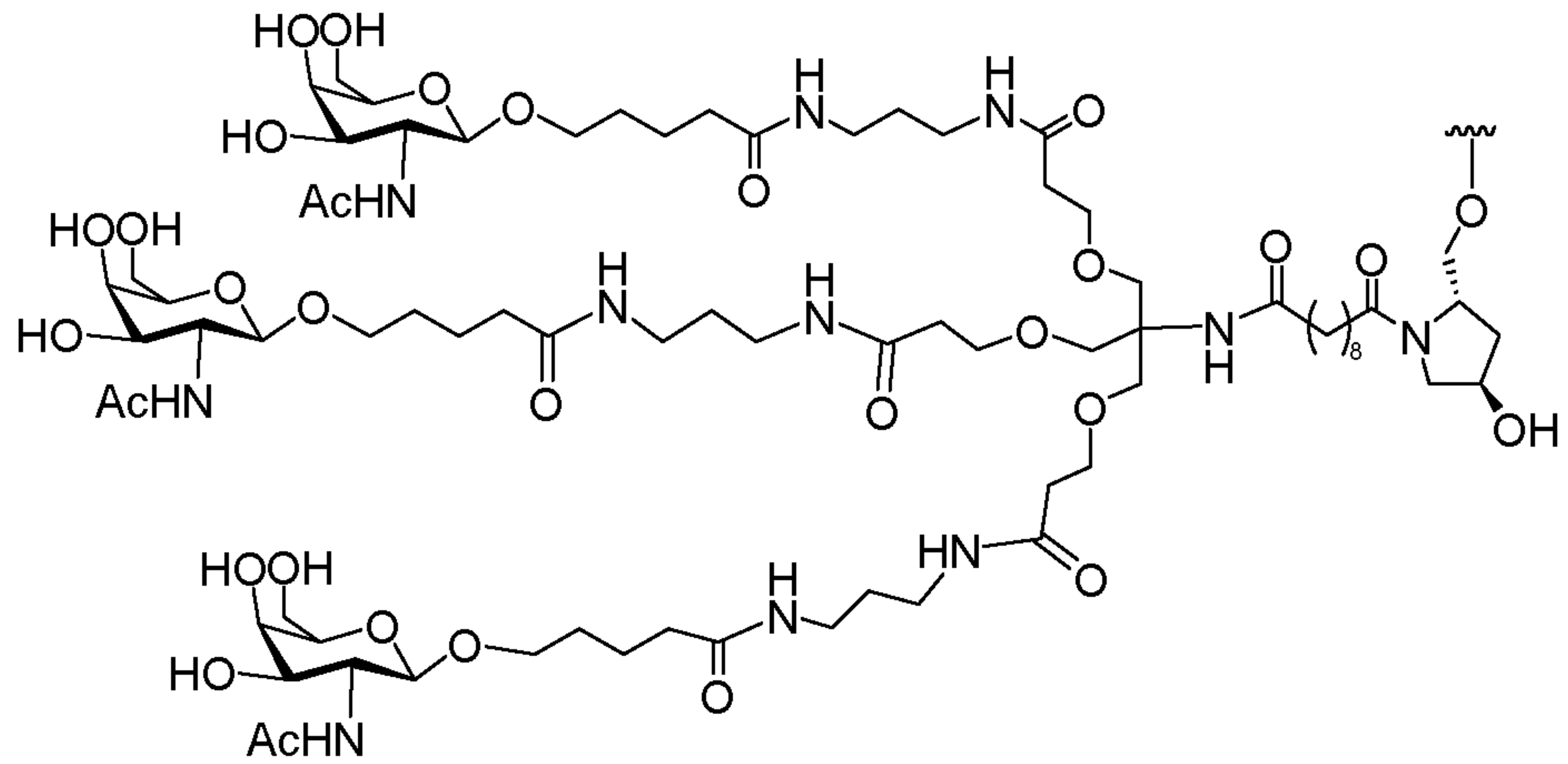
Bx = Heterocyclic base
X = O or S

29

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

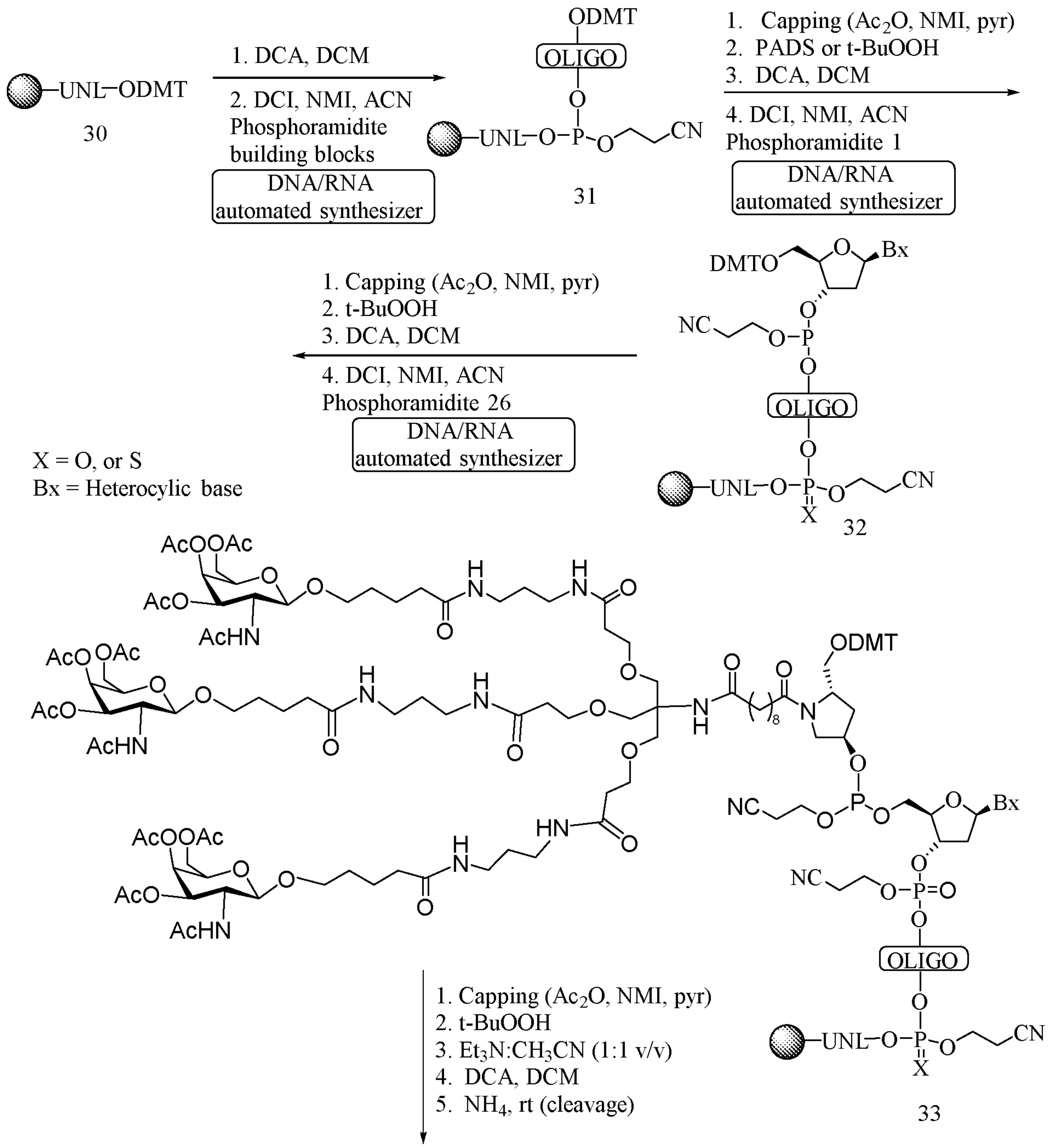


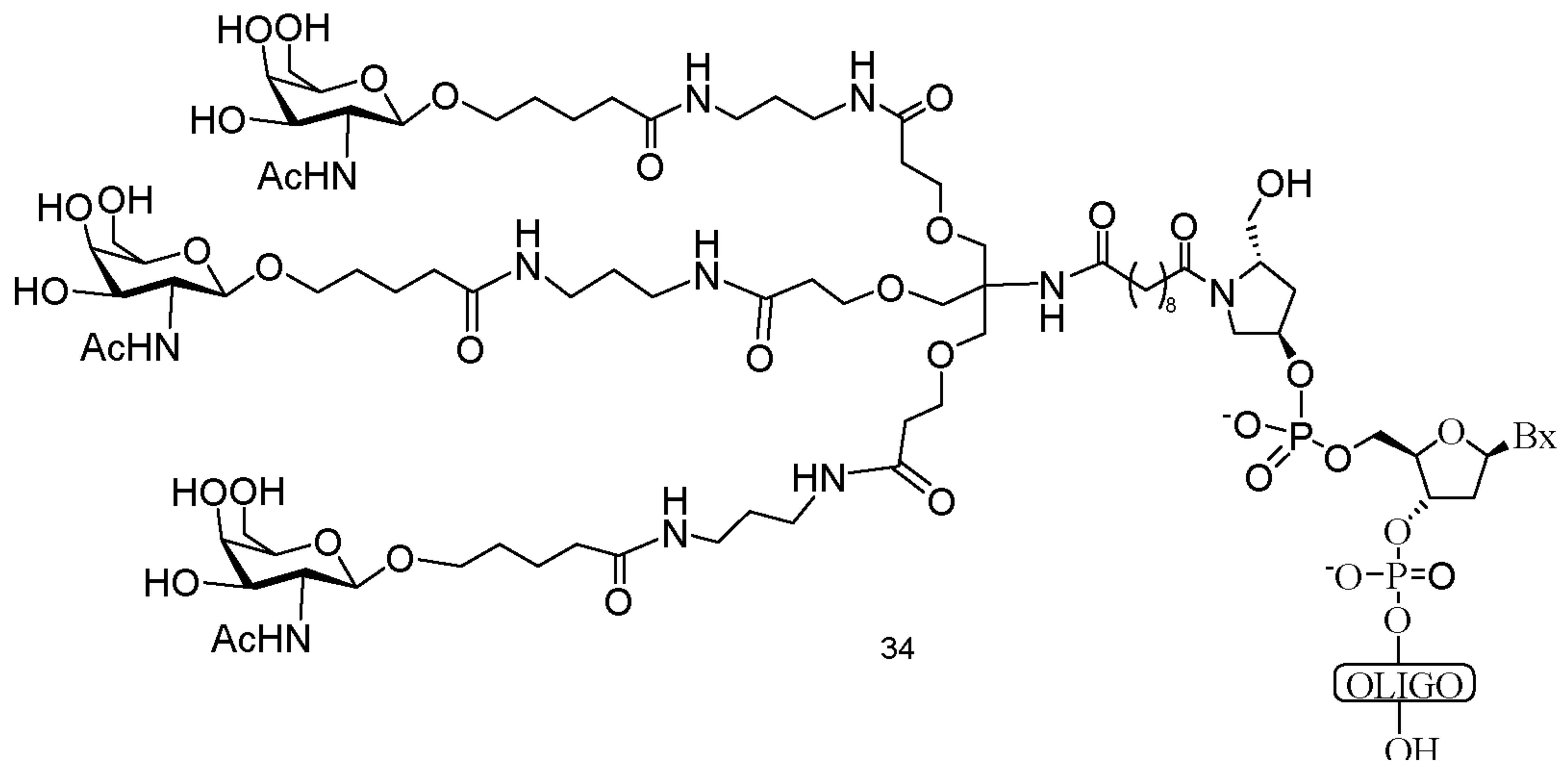
The GalNAc$_3$ cluster portion of the conjugate group GalNAc$_3$-1 (GalNAc$_3$-1$_a$) can be combined with any cleavable moiety to provide a variety of conjugate groups. Wherein GalNAc$_3$-1$_a$ has the formula:



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

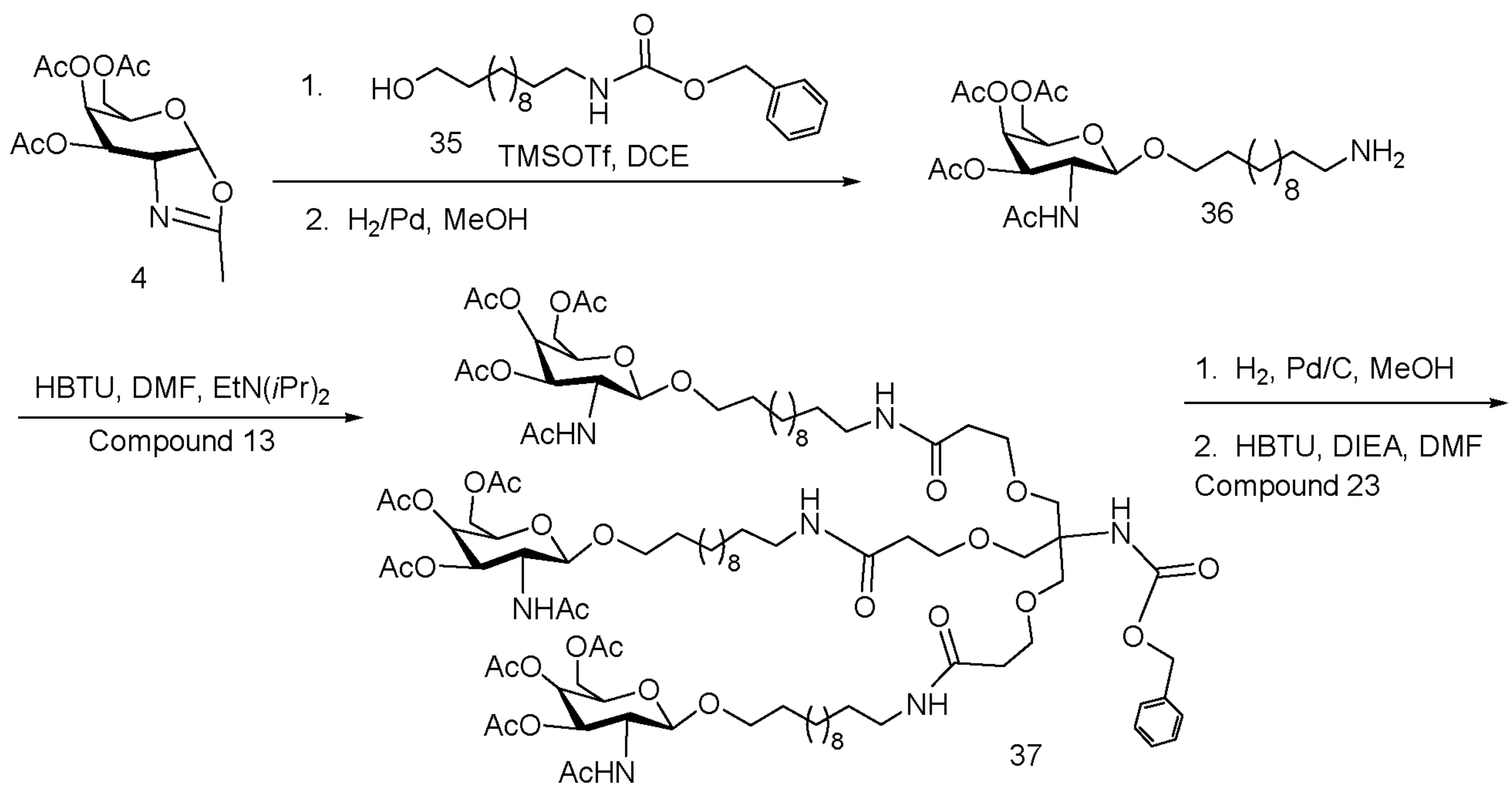


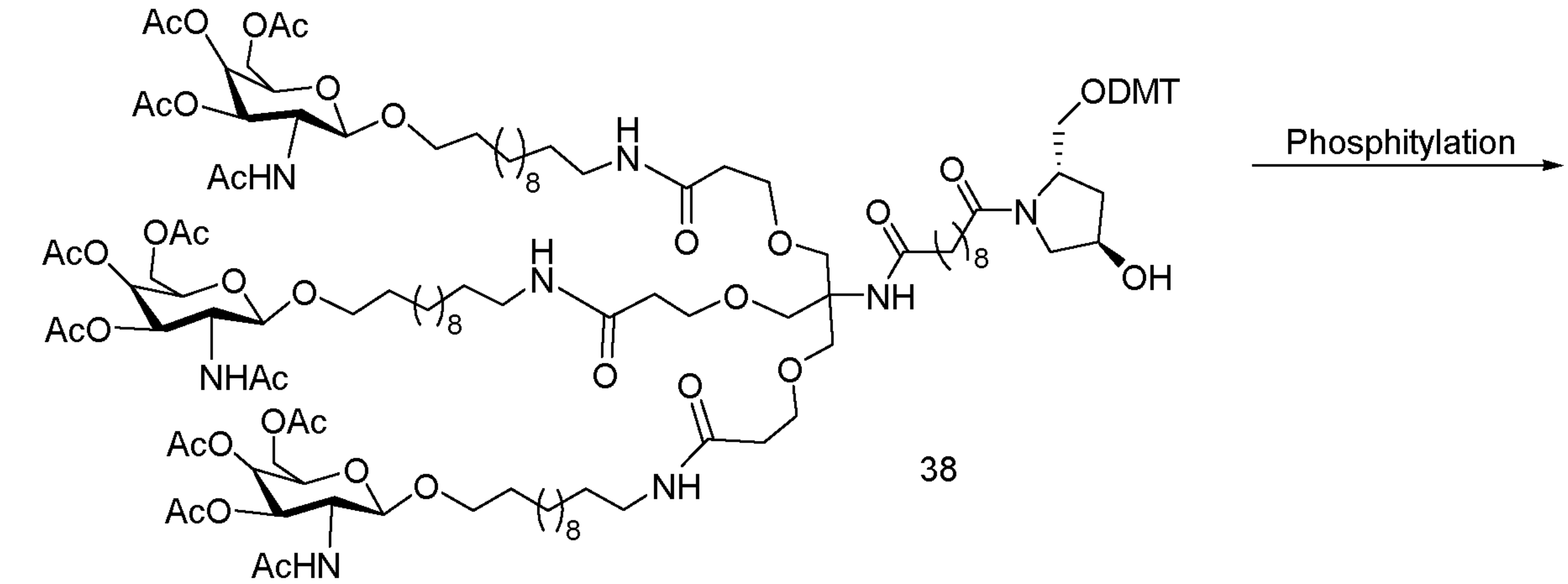


34

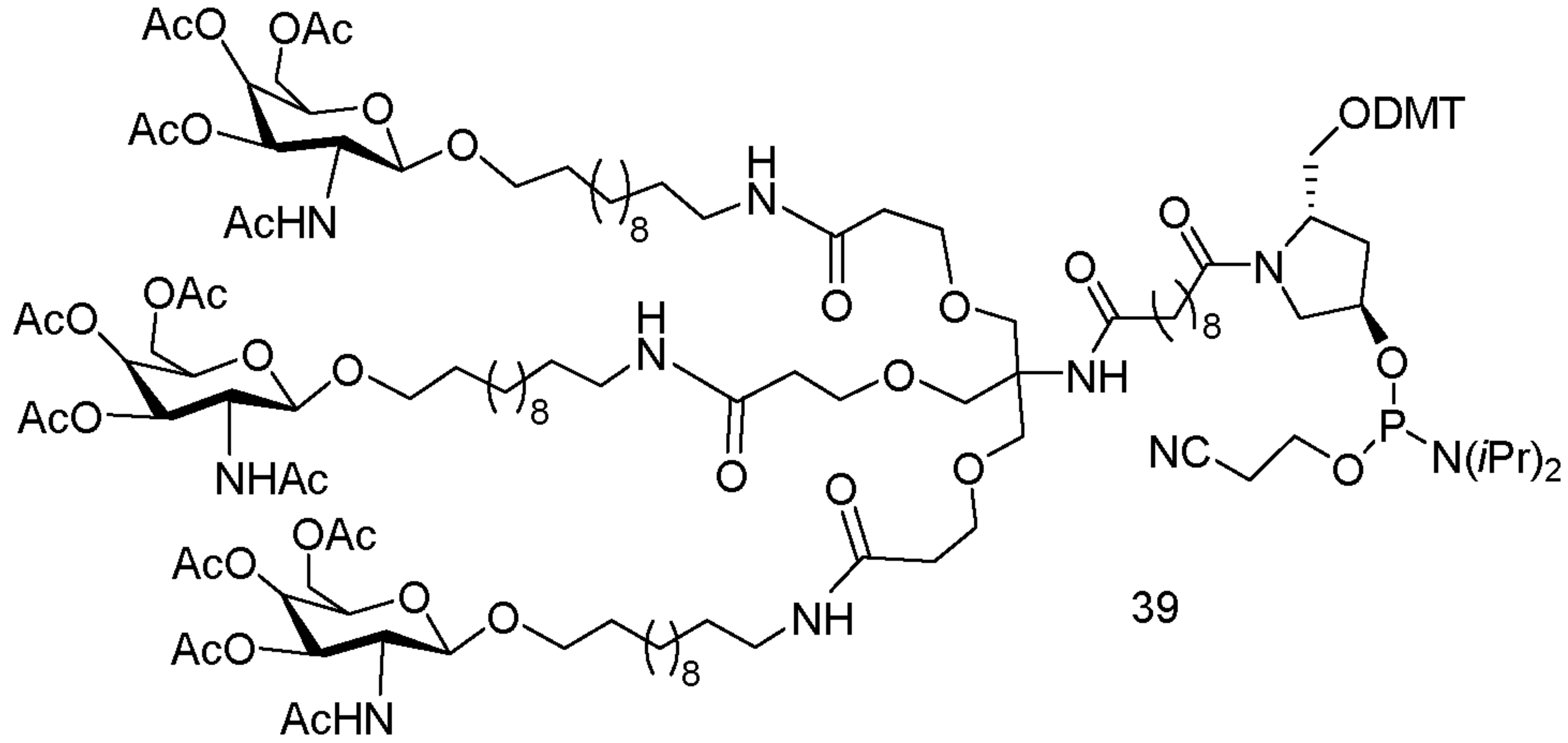
The Unylinker™ 30 is commercially available. Oligomeric Compound 34 comprising a **GalNAc$_3$-1** cluster at the 5' terminus is prepared using standard procedures in automated DNA/RNA synthesis (see Dupouy *et al., Angew. Chem. Int. Ed.,* 2006, *45,* 3623-3627). Phosphoramidite building blocks, Compounds 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





38

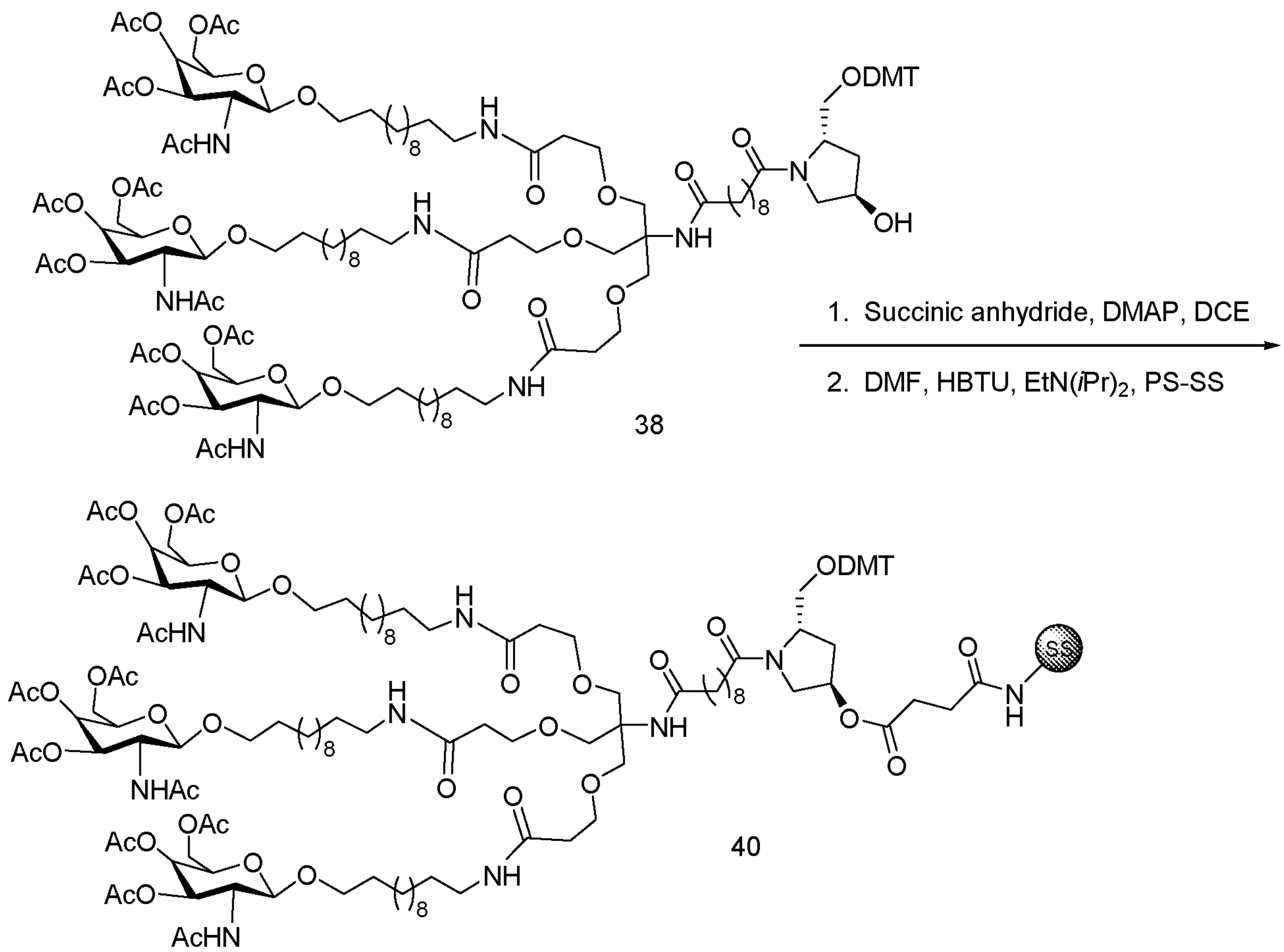


39

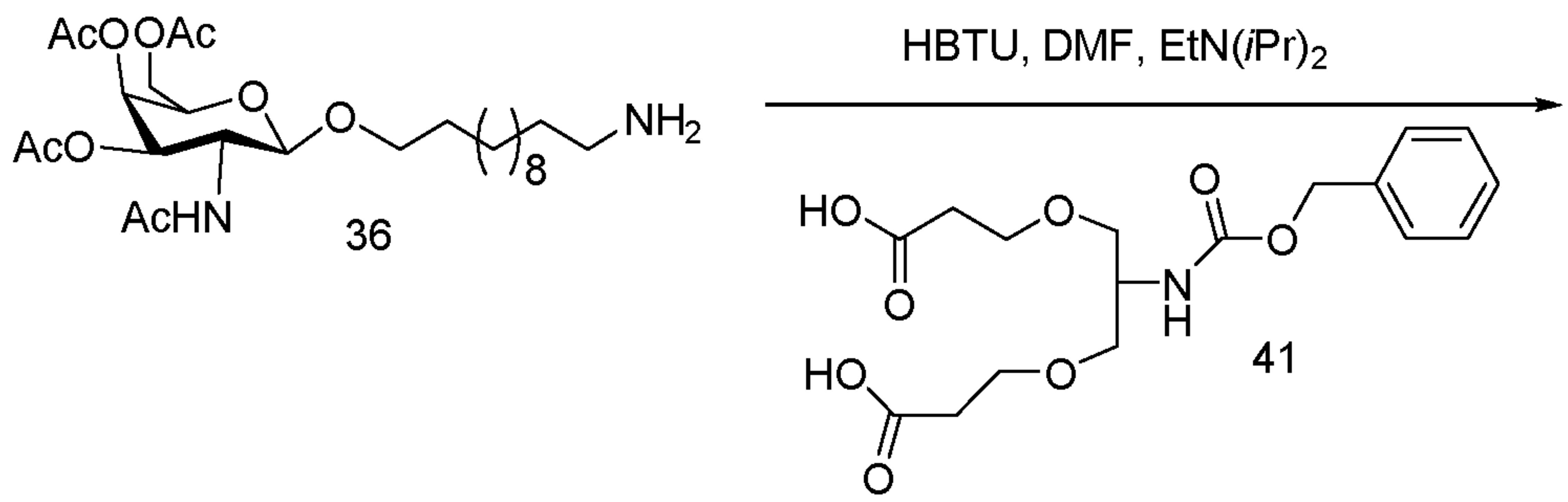
Compounds 4, 13 and 23 were prepared as per the procedures illustrated in Examples 2, 4, and 5. Compound 35 is prepared using similar procedures published in Rouchaud *et al., Eur. J. Org. Chem.,* 2011, *12*, 2346-2353.

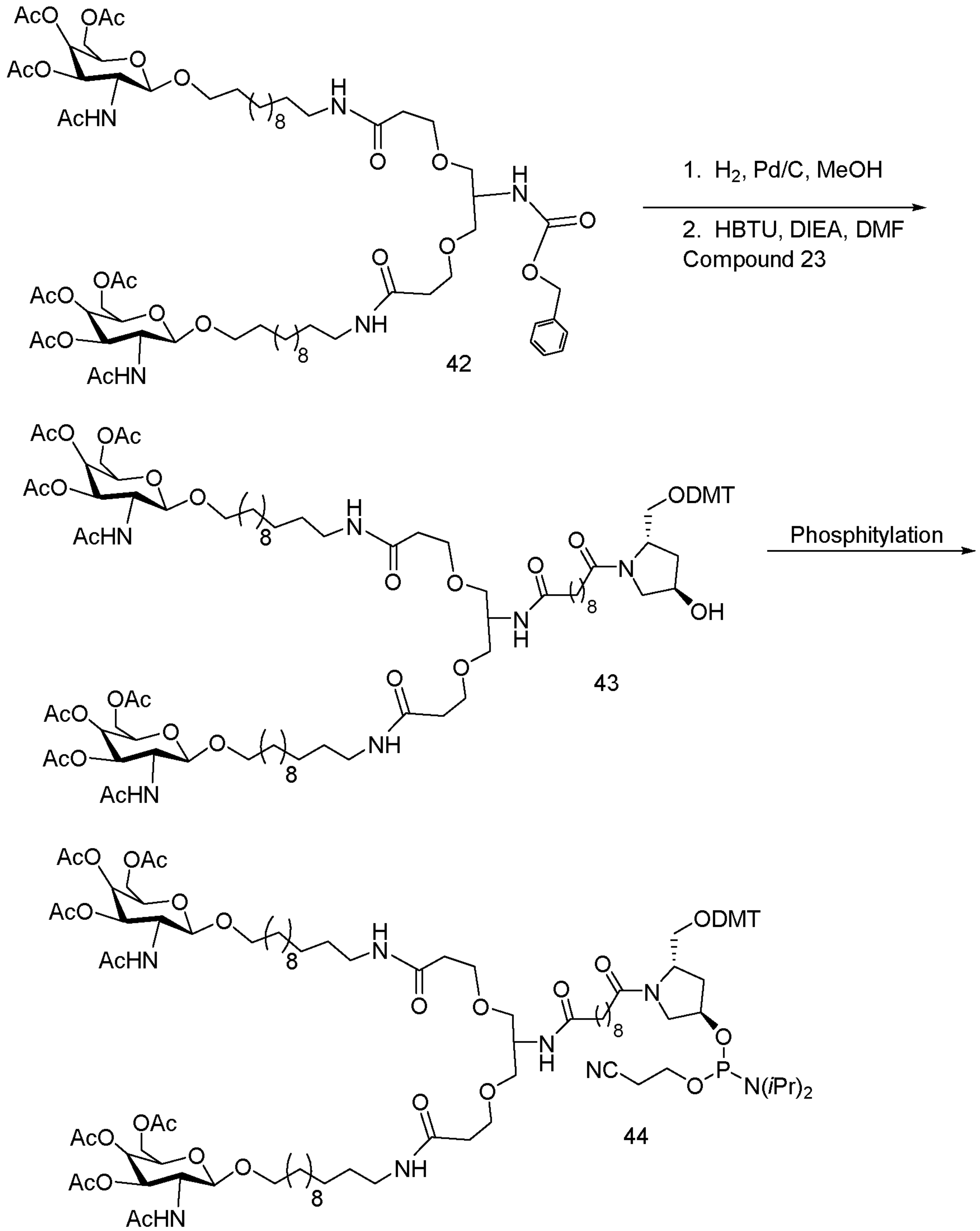
Example 12: Preparation of Compound 40

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



Example 13: Preparation of Compound 44





42

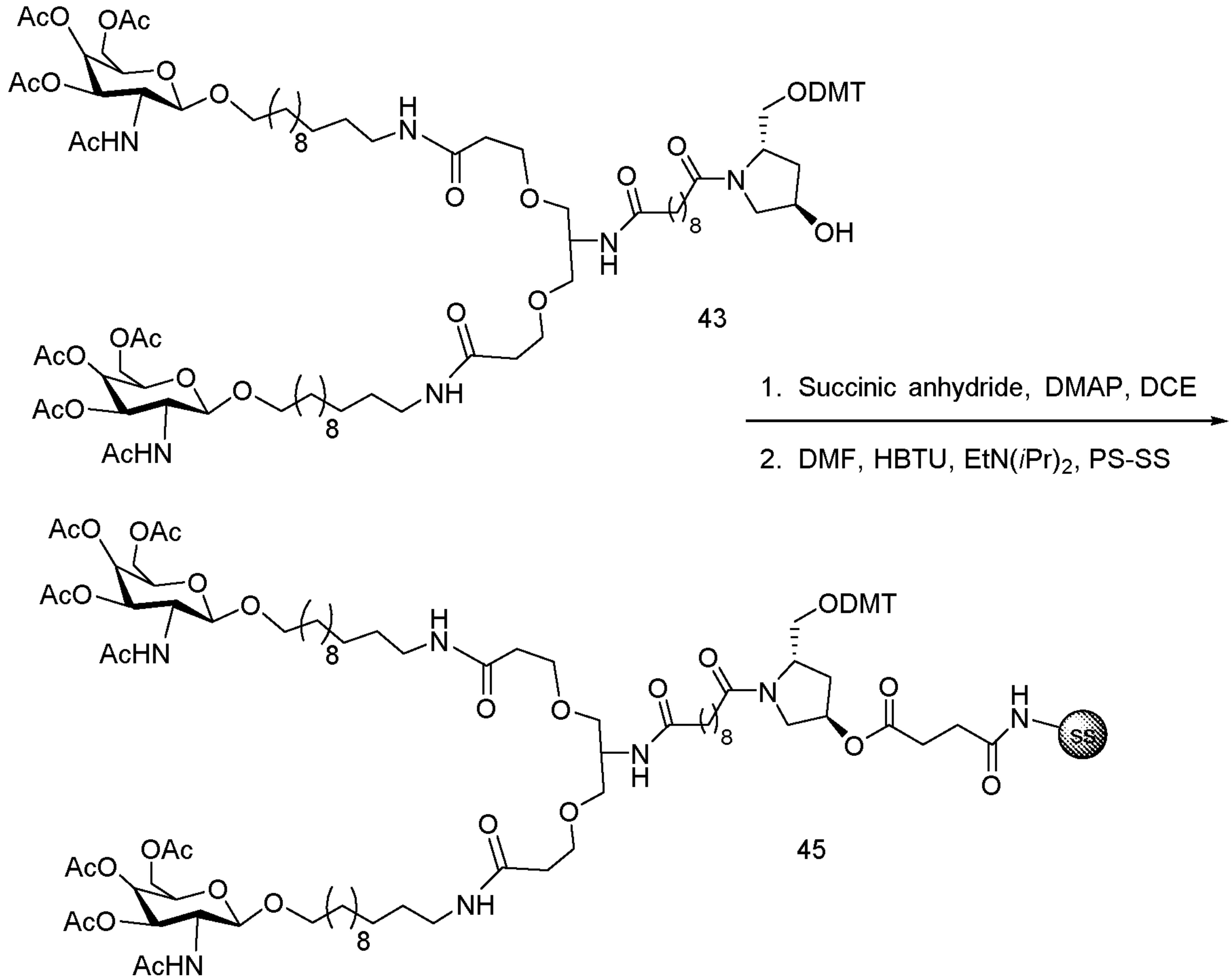
43

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.

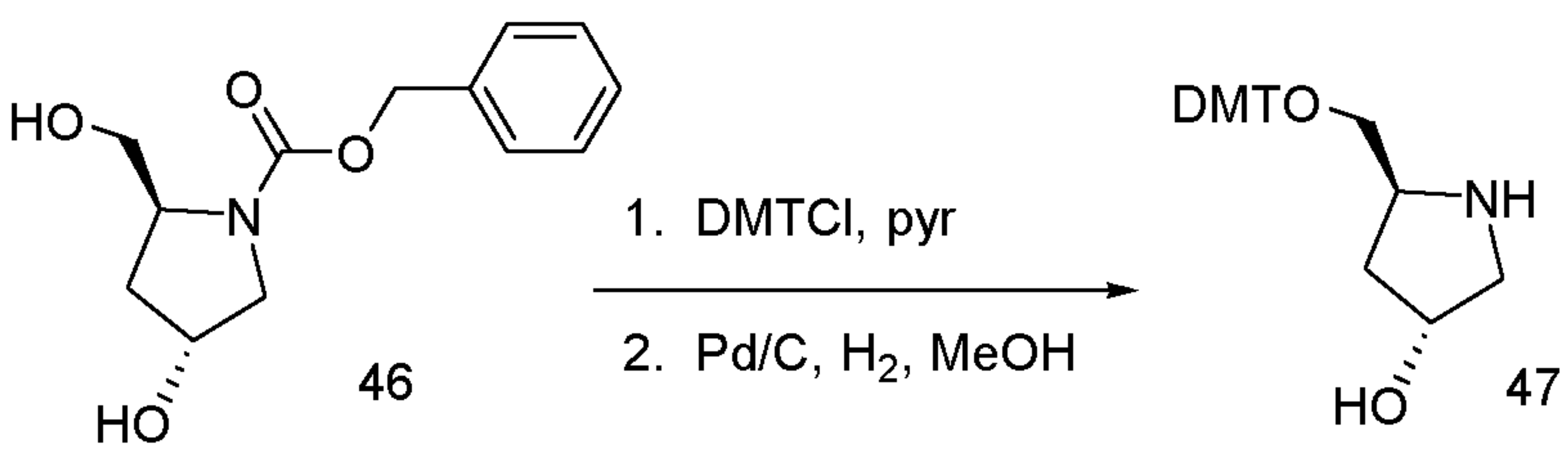
Example 14: Preparation of Compound 45

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

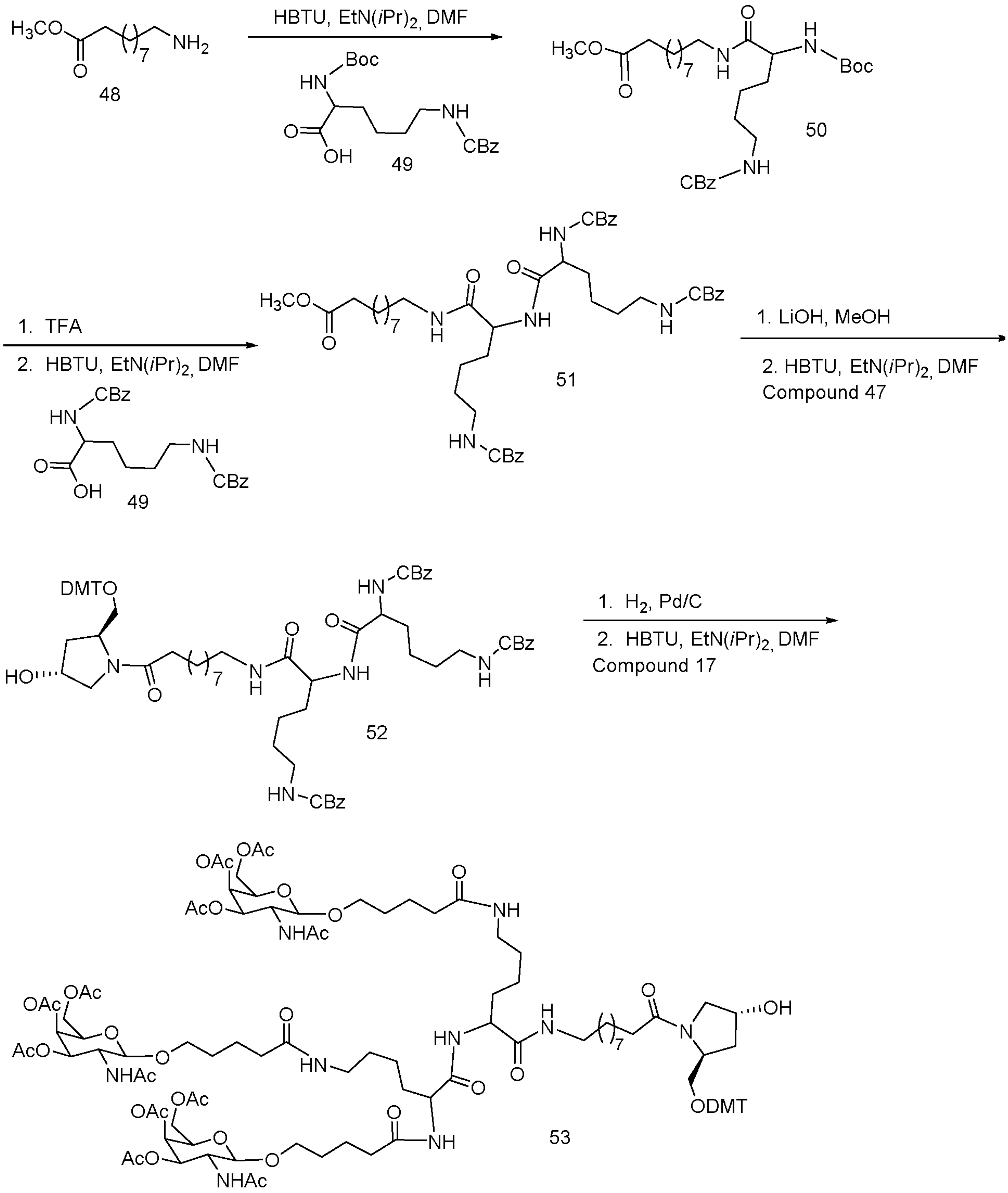


Example 15: Preparation of Compound 47

Compound 46 is commercially available.

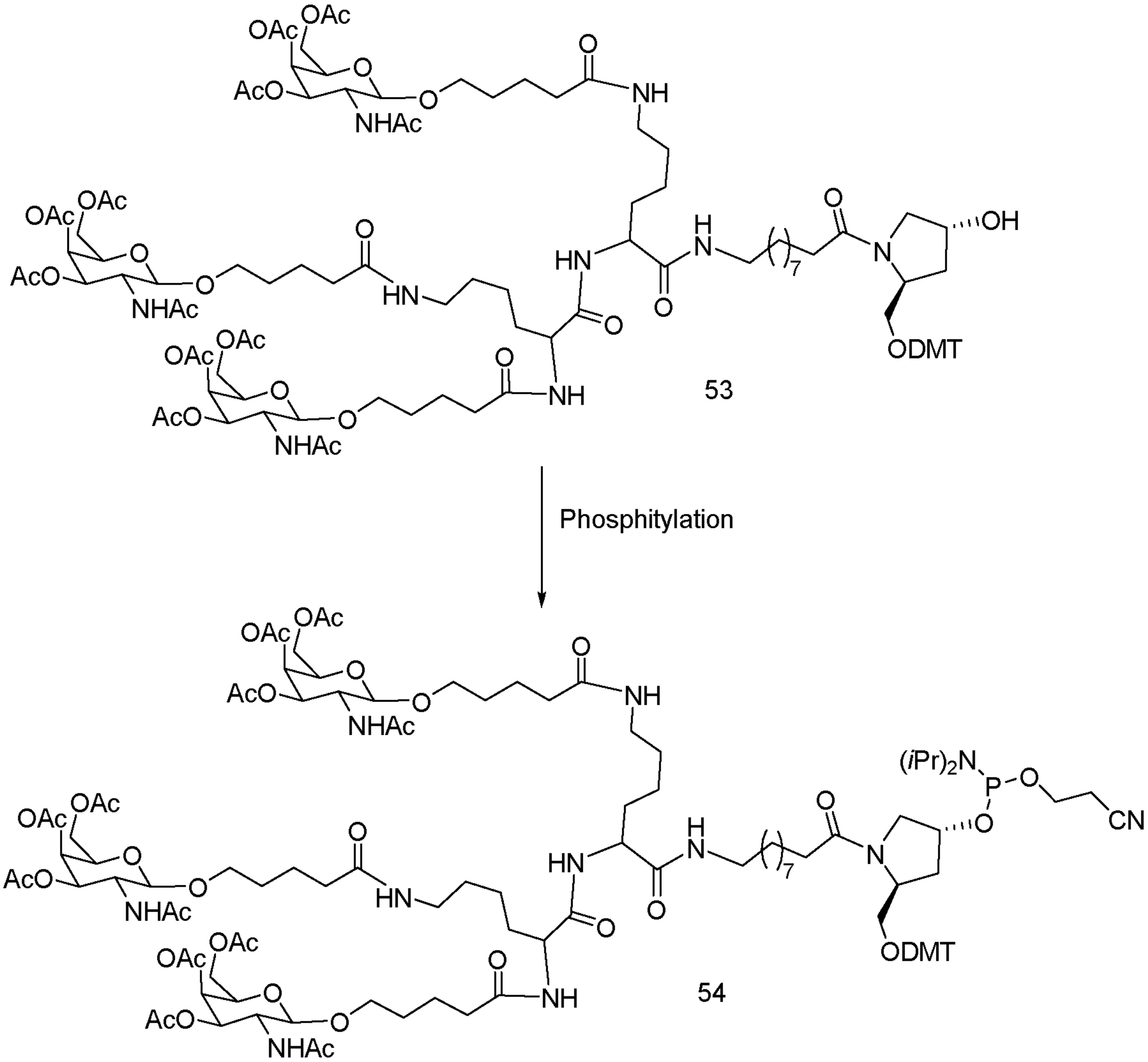


Example 16: Preparation of Compound 53



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

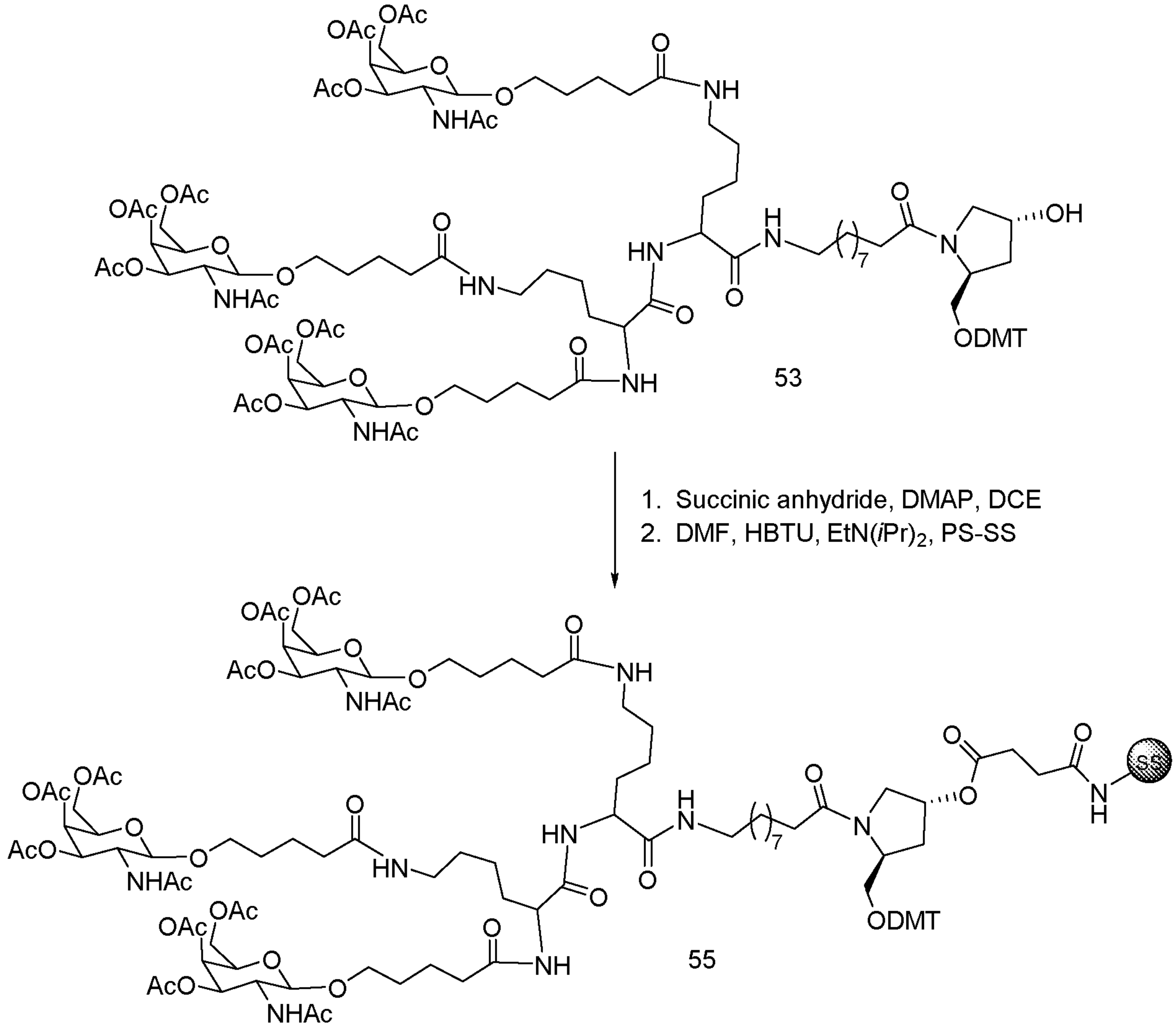
Example 17: Preparation of Compound 54



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 ^{o}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	2296
ISIS 647535	$A_{es}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{es}T_{es}T_{es}A_{es}T_{\mathbf{eo}}\mathbf{A_{do'}}$-**GalNAc$_3-1_a$**	ApoC III	9239.5	9237.8	2297

ISIS 647536	$A_{es}G_{eo}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{eo}T_{eo}T_{es}A_{es}T_{\mathbf{eo}}\mathbf{A_{do'}}$-$\mathbf{GalNAc_3\text{-}1_a}$	ApoC III	9142.9	9140.8	2297
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	2298
ISIS 651900	$T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{k\mathbf{o}}\mathbf{A_{do'}}$-$\mathbf{GalNAc_3\text{-}1_a}$	SRB-1	6721.1	6719.4	2299

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. “$\mathbf{GalNAc_3\text{-}1}$” indicates a conjugate group having the structure shown previously in Example 9. Note that $\mathbf{GalNAc_3\text{-}1}$ comprises a cleavable adenosine which links the ASO to remainder of the conjugate, which is designated “$\mathbf{GalNAc_3\text{-}1_a}$.” 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 “$\mathbf{GalNAc_3\text{-}1}$” with the “$\mathbf{A_{do}}$” 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	2296
	0.75	42				
	2.25	32				
	6.75	19				
ISIS 647535	0.08	50	0.074	**GalNAc$_3$-1**	PS/20	2297
	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	2296
	0.75	51				
	2.25	23				
	6.75	13				

ISIS 647535	0.08	72	0.19	**GalNAc$_3$-1**	PS/20	2297
	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	2296
	0.75	46				
	2.25	21				
	6.75	12				
ISIS 647535	0.08	65	0.13	**GalNAc$_3$-1**	PS/20	2297
	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	2296
	0.75	164			
	2.25	110			
	6.75	82			
ISIS 647535	0.08	230	**GalNAc$_3$-1**	PS/20	2297
	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	2296
	0.75	27	12			
	2.25	50	4			
	6.75	45	2			
ISIS 647535	0.08	21	21	**GalNAc$_3$-1**	PS/20	2297
	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

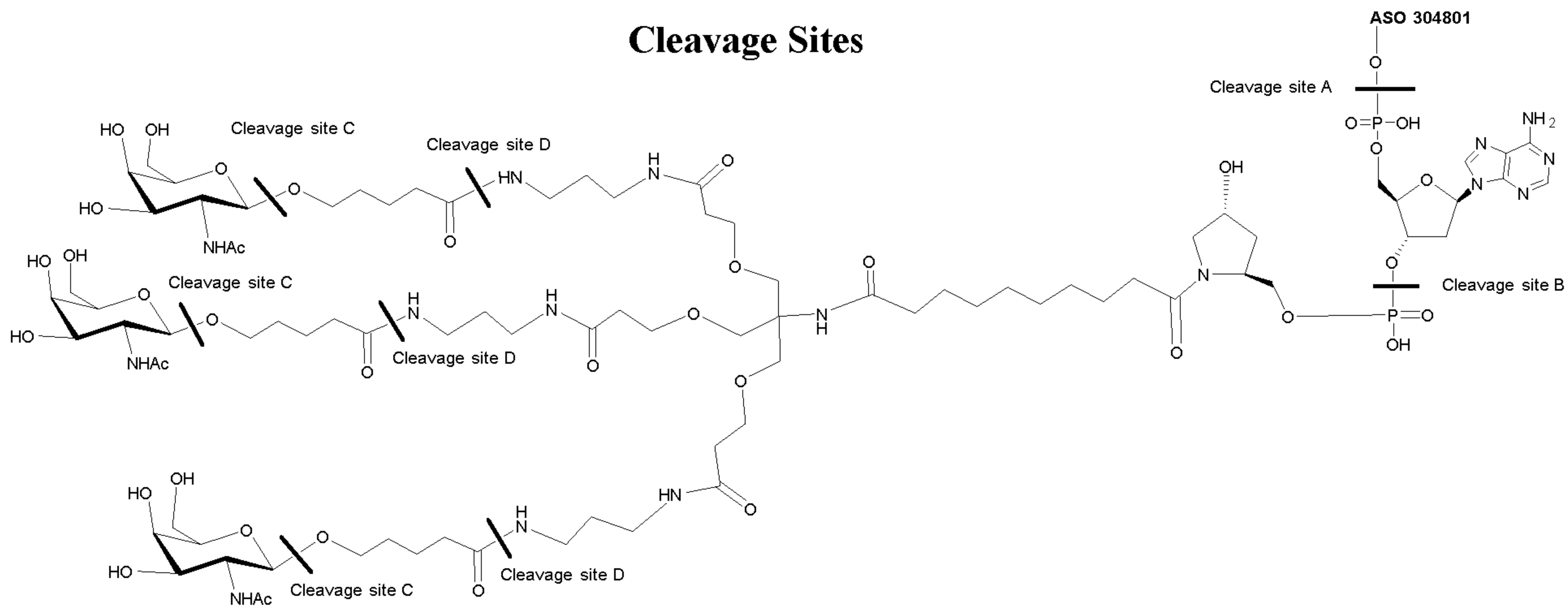
ASO	Dose (μmol/kg)	Liver (μg/g)	Kidney (μg/g)	Liver EC_{50} (μg/g)	3' Conjugate	Internucleoside Linkage/Length	SEQ ID No.
ISIS 304801	0.1	5.2	2.1	53	None	PS/20	2296
	0.8	62.8	119.6				
	2.3	142.3	191.5				
	6.8	202.3	337.7				
ISIS 647535	0.1	3.8	0.7	3.8	**GalNAc$_3$-1**	PS/20	2297
	0.8	72.7	34.3				
	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 cleavable 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.

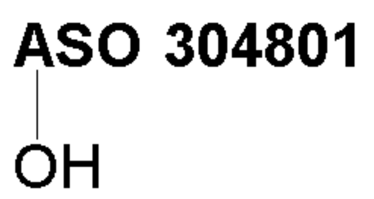
Table 23a
Observed full length metabolites of ISIS 647535

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

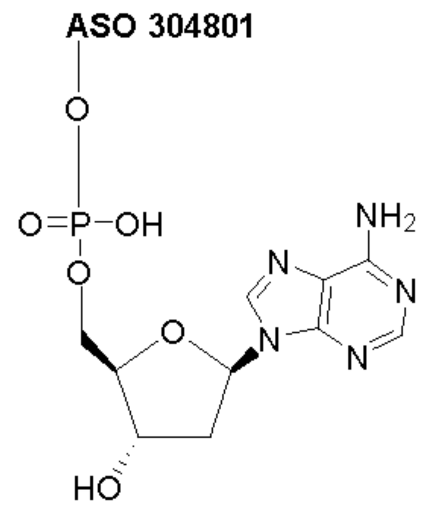
Cleavage Sites

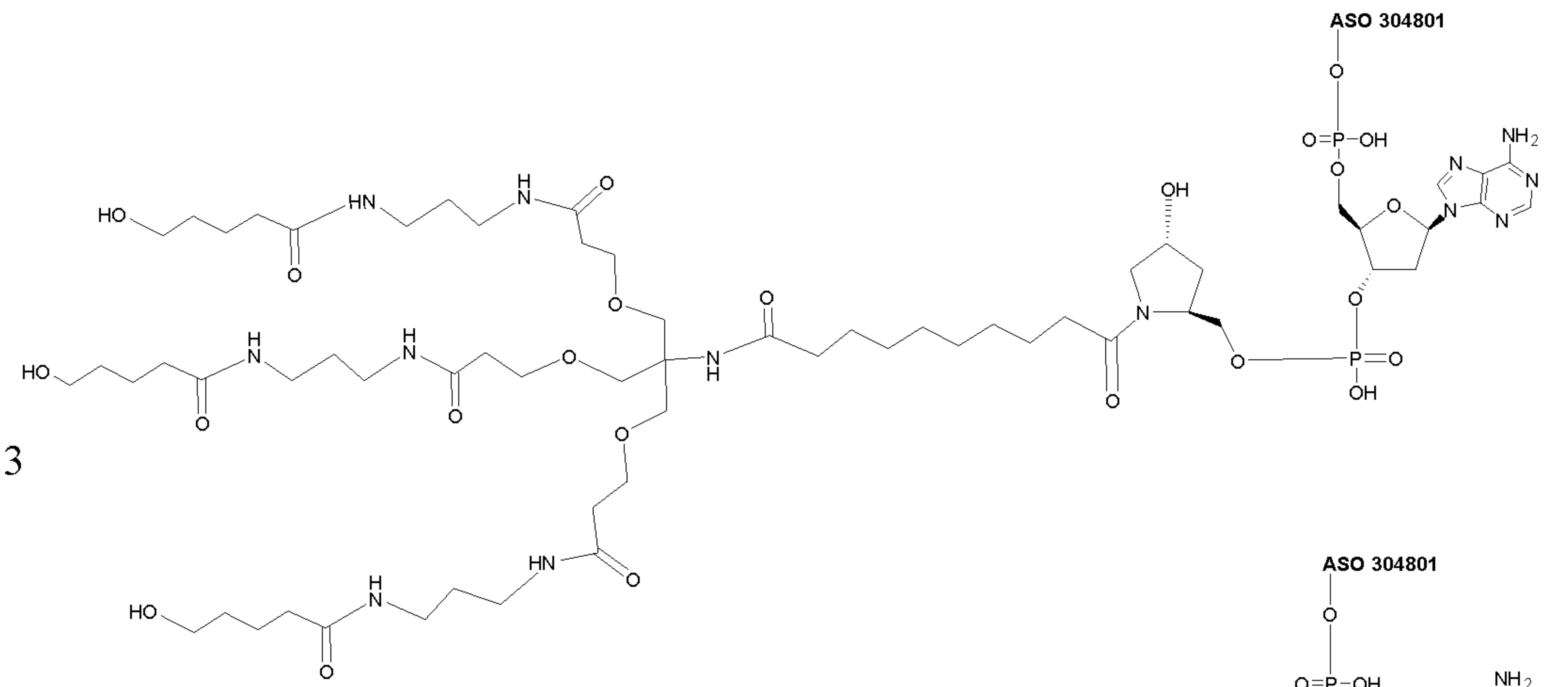


Metabolite 1

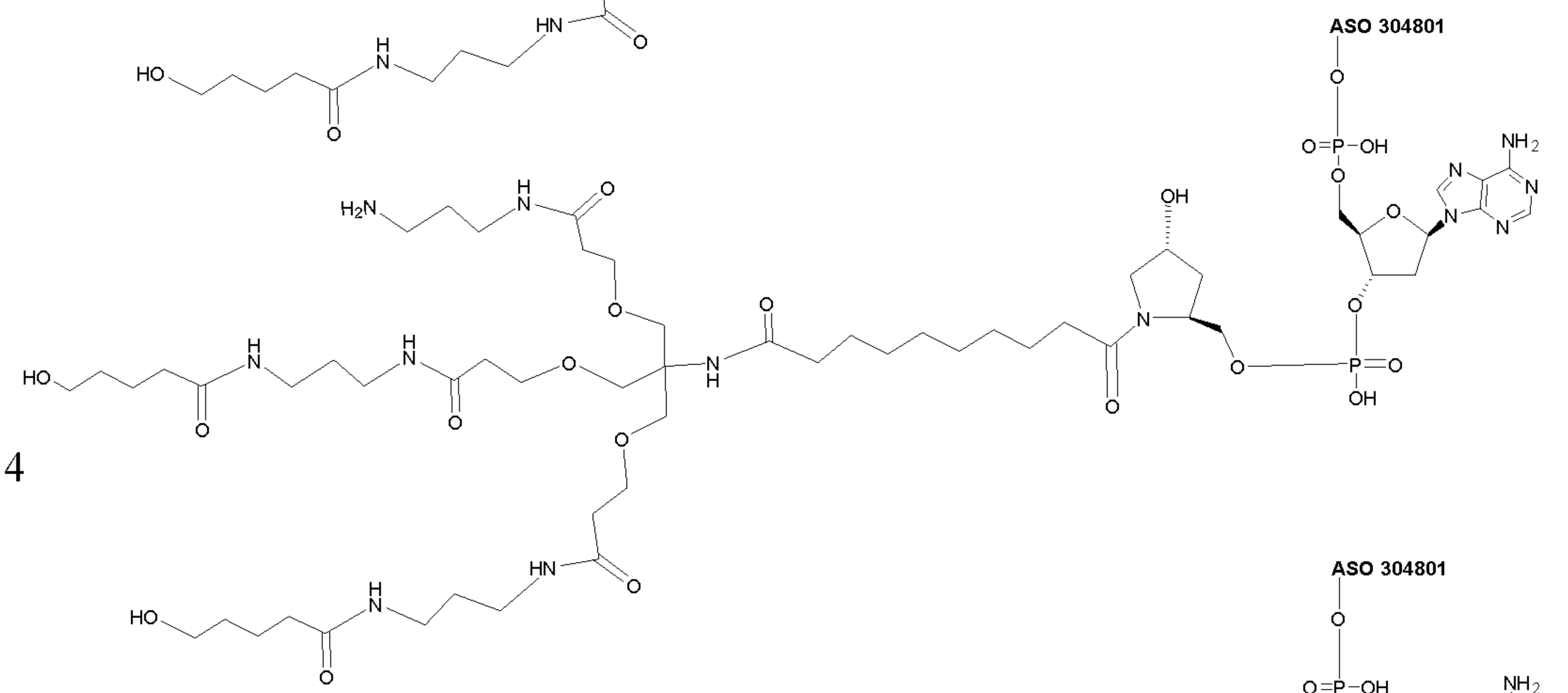


Metabolite 2

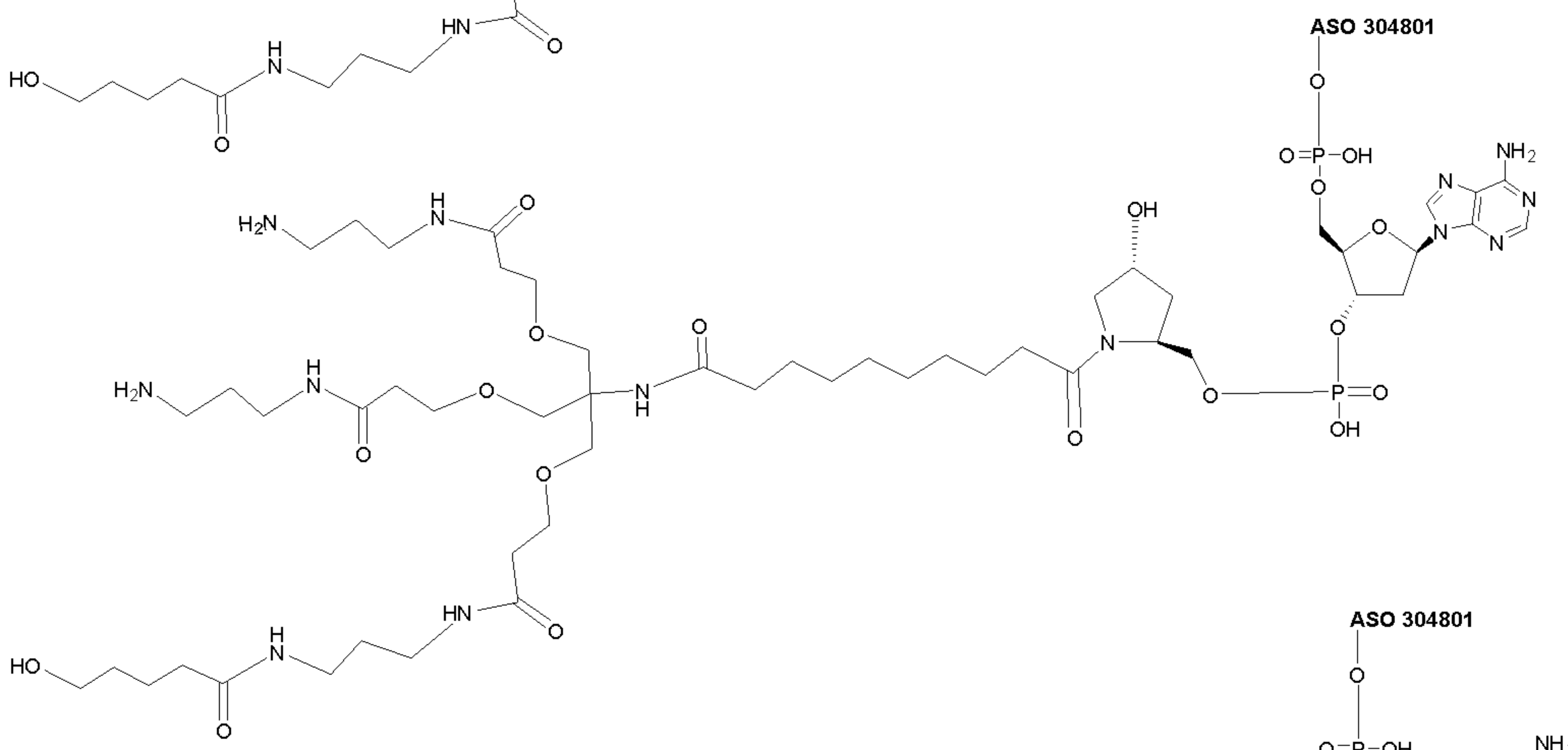




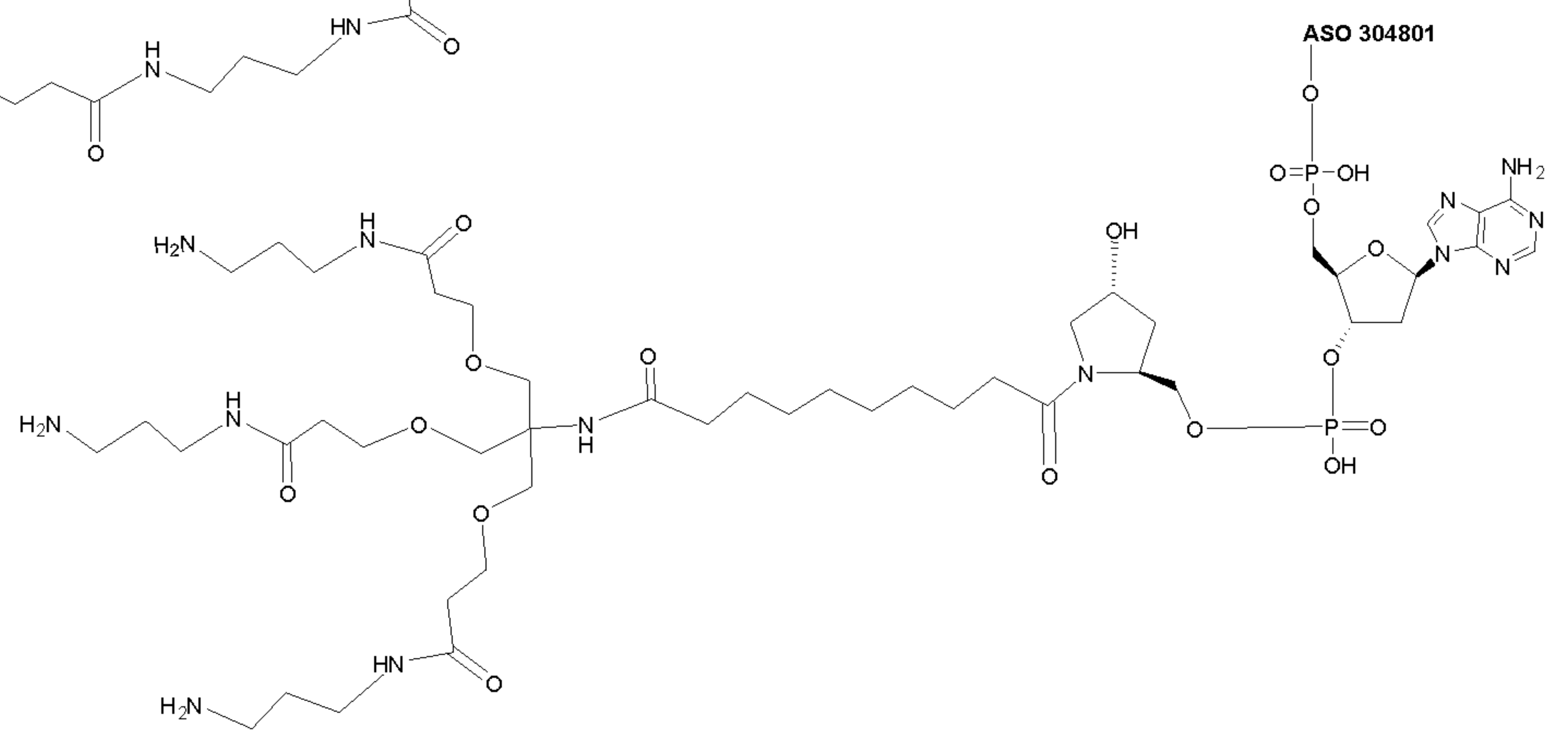
Metabolite 3



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 the 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	2296
	3	92				
	10	71				
	30	40				
ISIS 647535	0.3	98	1.9	**GalNAc$_3$-1**	PS/20	2297
	1	70				

	3	33				
	10	20				
ISIS 647536	0.3	103	1.7	**GalNAc$_3$-1**	PS/PO/20	2297
	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	2296
	3	92				
	10	71				
	30	40				
ISIS 647535	0.3	98	2.1	**GalNAc$_3$-1**	PS/20	2297
	1	70				
	3	33				
	10	20				
ISIS 647536	0.3	103	1.8	**GalNAc$_3$-1**	PS/PO/20	2297
	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	2296
	3	92				
	10	70				
	30	47				
ISIS 647535	0.3	100	2.2	**GalNAc$_3$-1**	PS/20	2297
	1	70				
	3	34				
	10	23				
ISIS 647536	0.3	95	1.9	**GalNAc$_3$-1**	PS/PO/20	2297
	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	1	104	None	PS/20	2296

304801	3	96			
	10	86			
	30	72			
ISIS 647535	0.3	93	**GalNAc$_3$-1**	PS/20	2297
	1	85			
	3	61			
	10	53			
ISIS 647536	0.3	115	**GalNAc$_3$-1**	PS/PO/20	2297
	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	2296
	3	186	79			
	10	226	63			
	30	240	46			
ISIS 647535	0.3	98	86	**GalNAc$_3$-1**	PS/20	2297
	1	214	67			
	3	212	39			
	10	218	35			
ISIS 647536	0.3	143	89	**GalNAc$_3$-1**	PS/PO/20	2297
	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	2298
	2	55					
	7	12					
	20	3					
ISIS 651900	0.07	98	0.3	**GalNAc$_3$-1**		PS/14	2299
	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	2300
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	2301
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	2296
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	2297
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	2296

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	2301
ISIS 304801	0.07	106.55	1,522	None	PS/20	2296
ISIS 647535	0.12	138	1,150	**GalNAc$_3$-1**	PS/20	2297
ISIS 616468	0.32	71.52	224	None	PS/PO/20	2296

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	2296
ISIS 647535	0.31	**GalNAc$_3$-1**	PS/20	2297

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 lack 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.

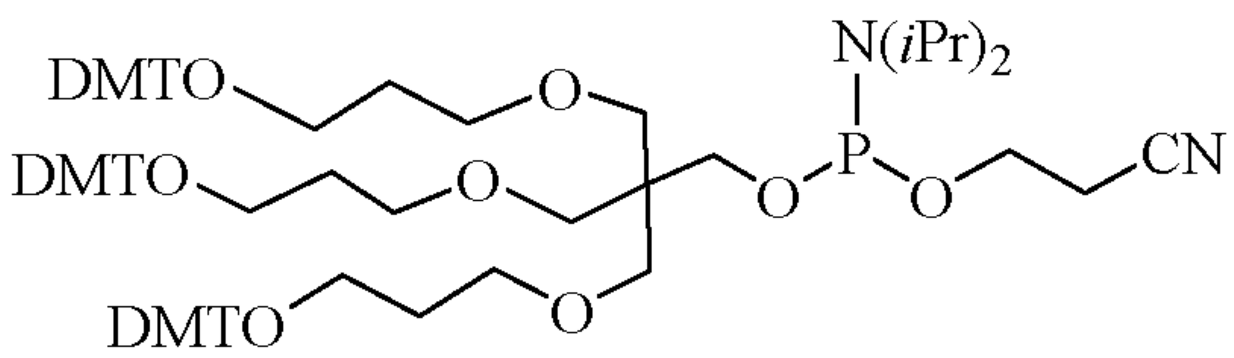
These results show reduction in potency for antisense compounds with PO/PS (ISIS 616468) in the wings relative to full PS (ISIS 304801).

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	2296
ISIS 616468	25 mg/kg/wk for 2 wks	40	None	14 PS/6 PO	2296

Example 27: Compound 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.

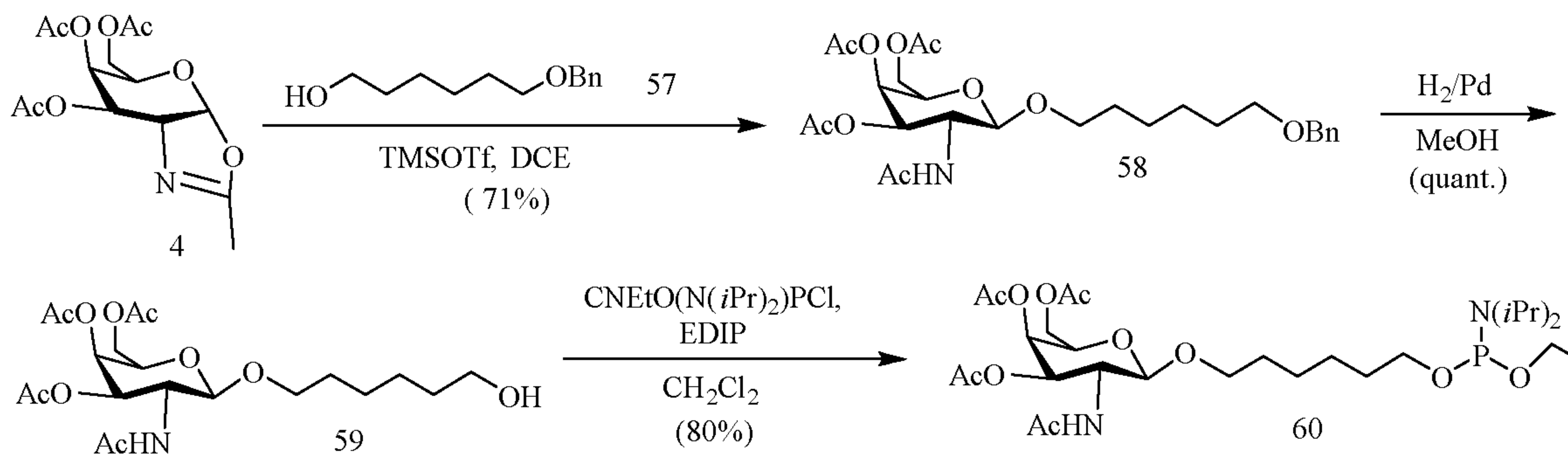


56

Example 28: Preparation of Compound 60

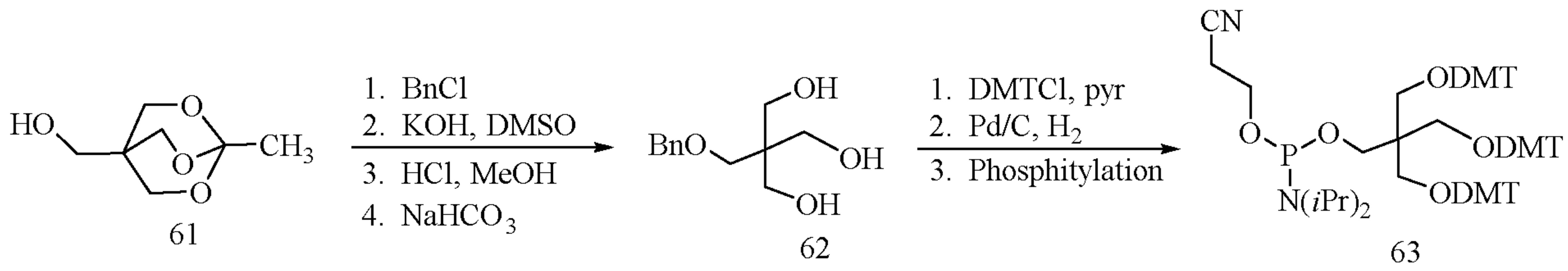
Compound 4 was prepared as per the procedures illustrated in Example 2. Compound 57 is commercially available. Compound 60 was confirmed by structural analysis.

Compound 57 is meant to be representative and not intended to be limiting as other monoprotected 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

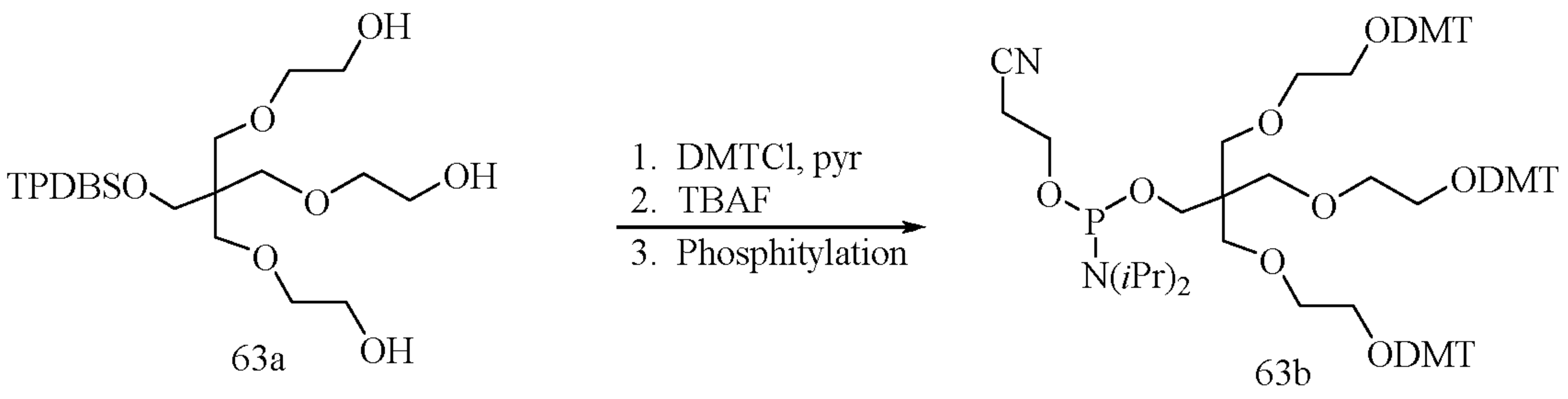
Compounds 61 and 62 are prepared using procedures similar to those reported by Tober *et al., Eur. J. Org. Chem.*, 2013, *3,* 566-577; and Jiang *et al., Tetrahedron,* 2007, *63(19),* 3982-3988.



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.

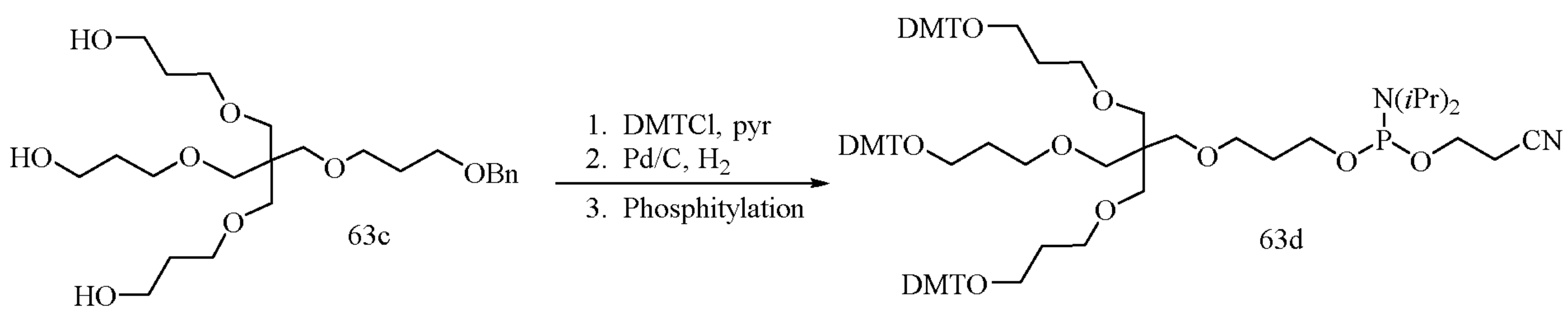
Example 30: Preparation of Compound 63b

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



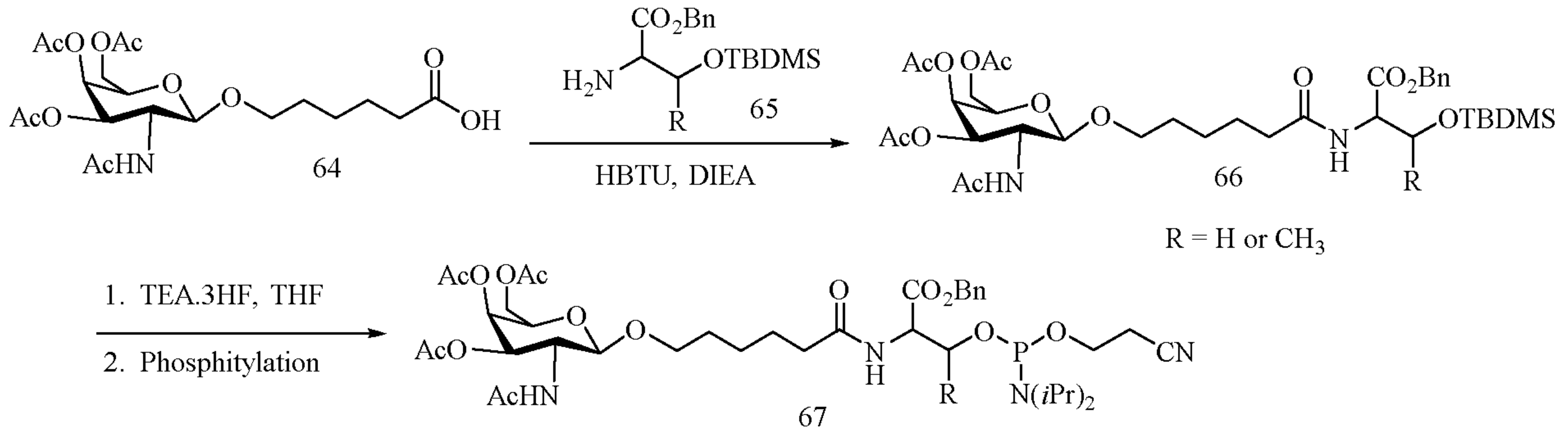
Example 31: Preparation of Compound 63d

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



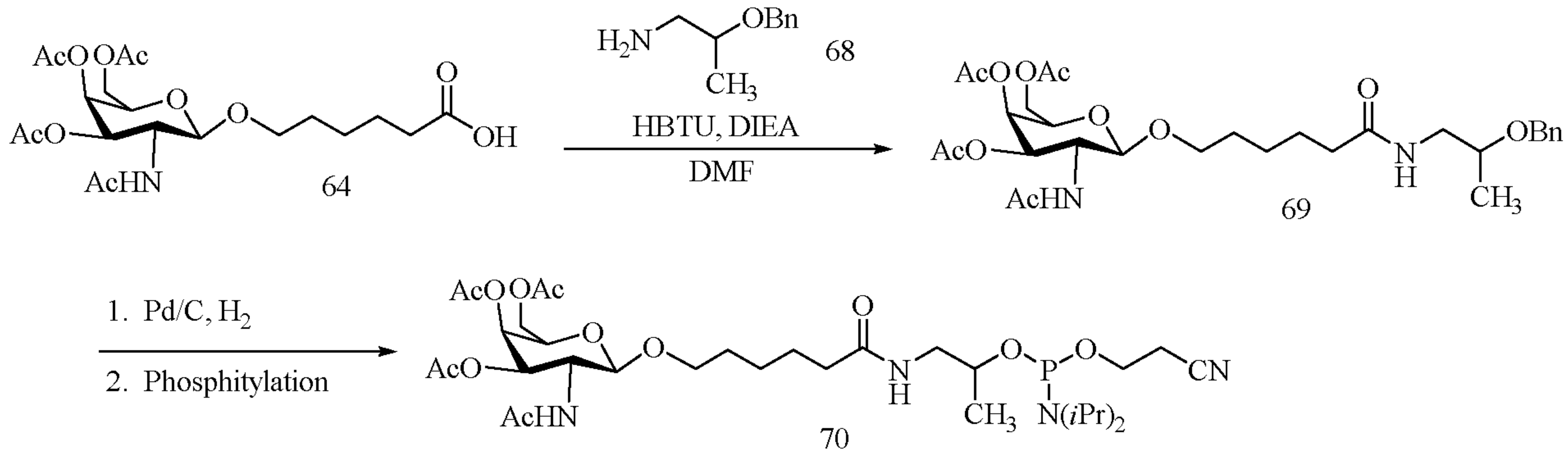
Example 32: Preparation of Compound 67

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.



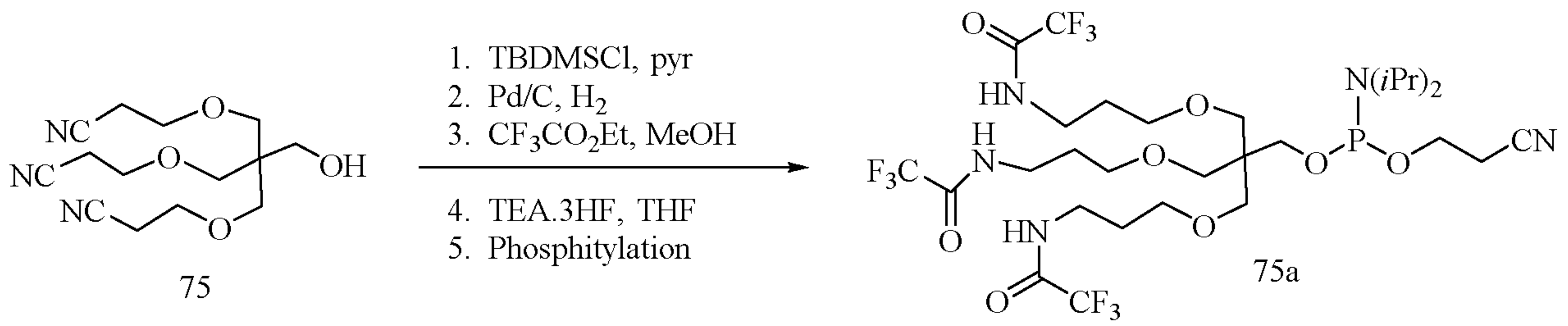
Example 33: Preparation of Compound 70

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.



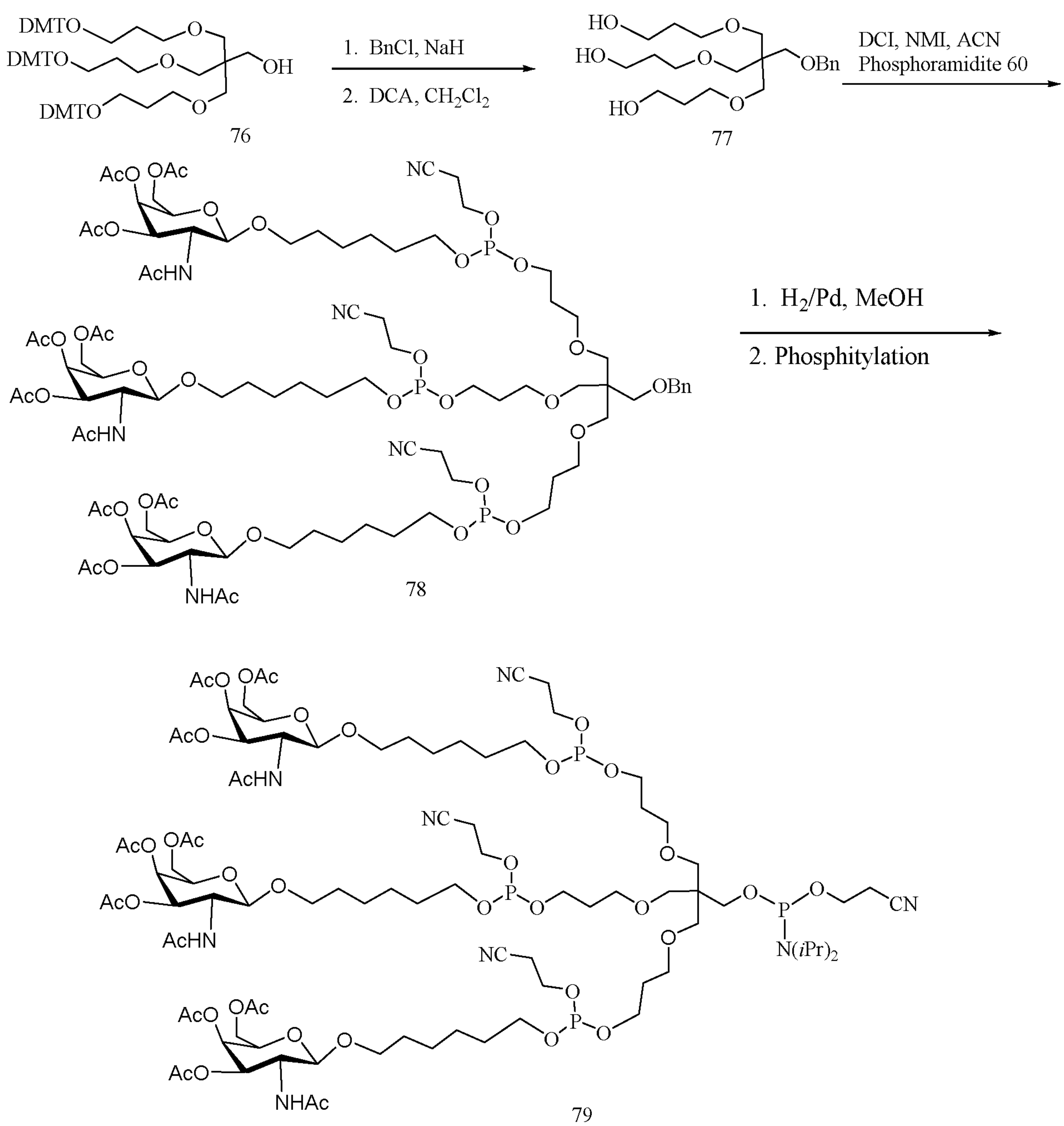
Example 34: Preparation of Compound 75a

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

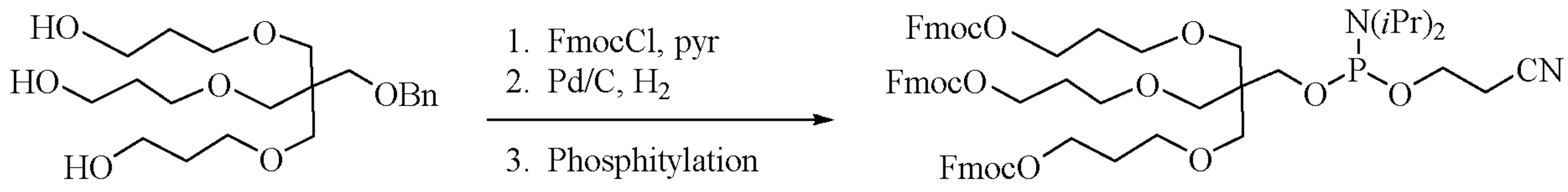
Compound 76 was prepared according to published procedures reported by Shchepinov *et al., Nucleic Acids Research,* 1997, *25(22)*, 4447-4454.



79

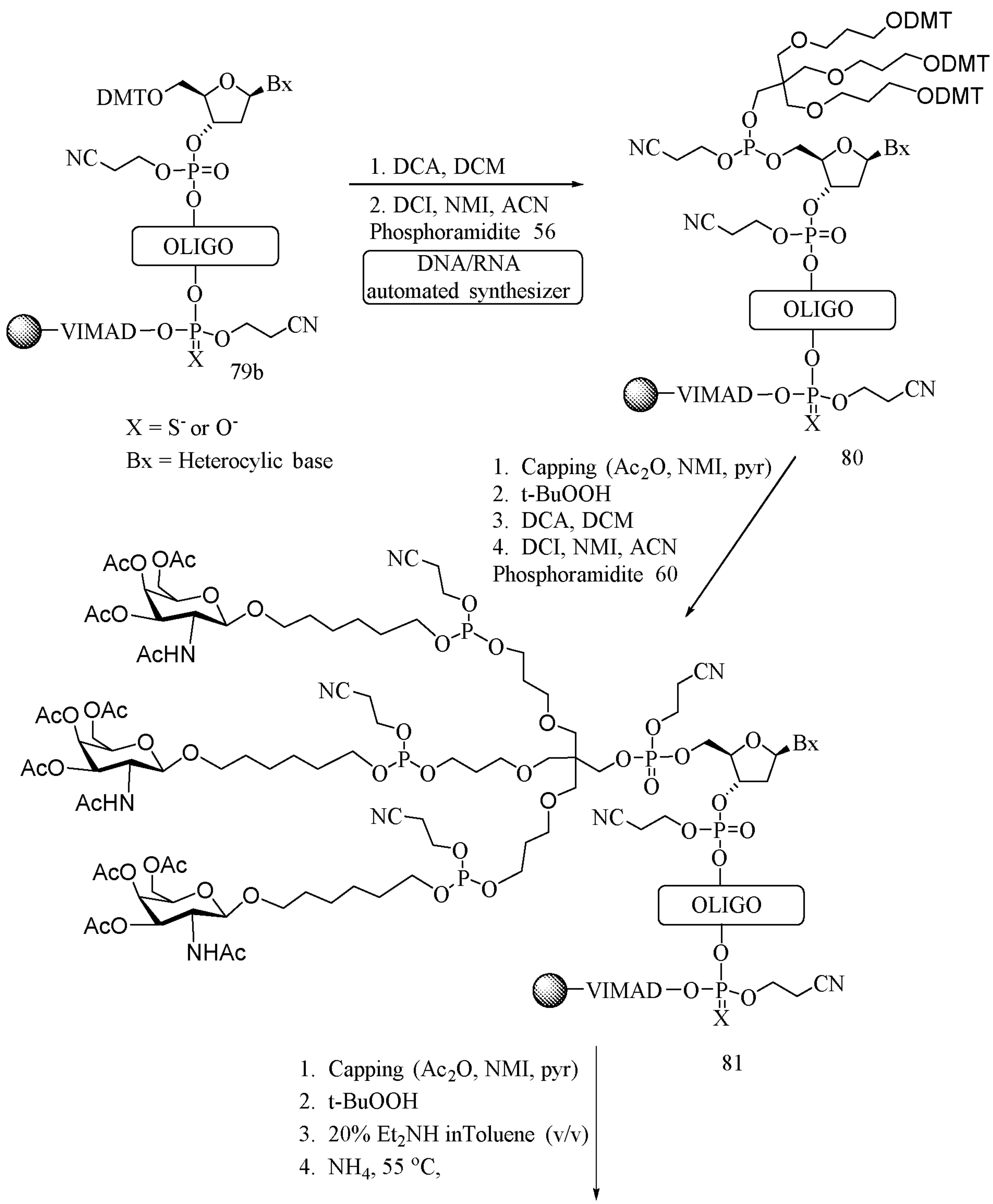
Example 36: Preparation of Compound 79a

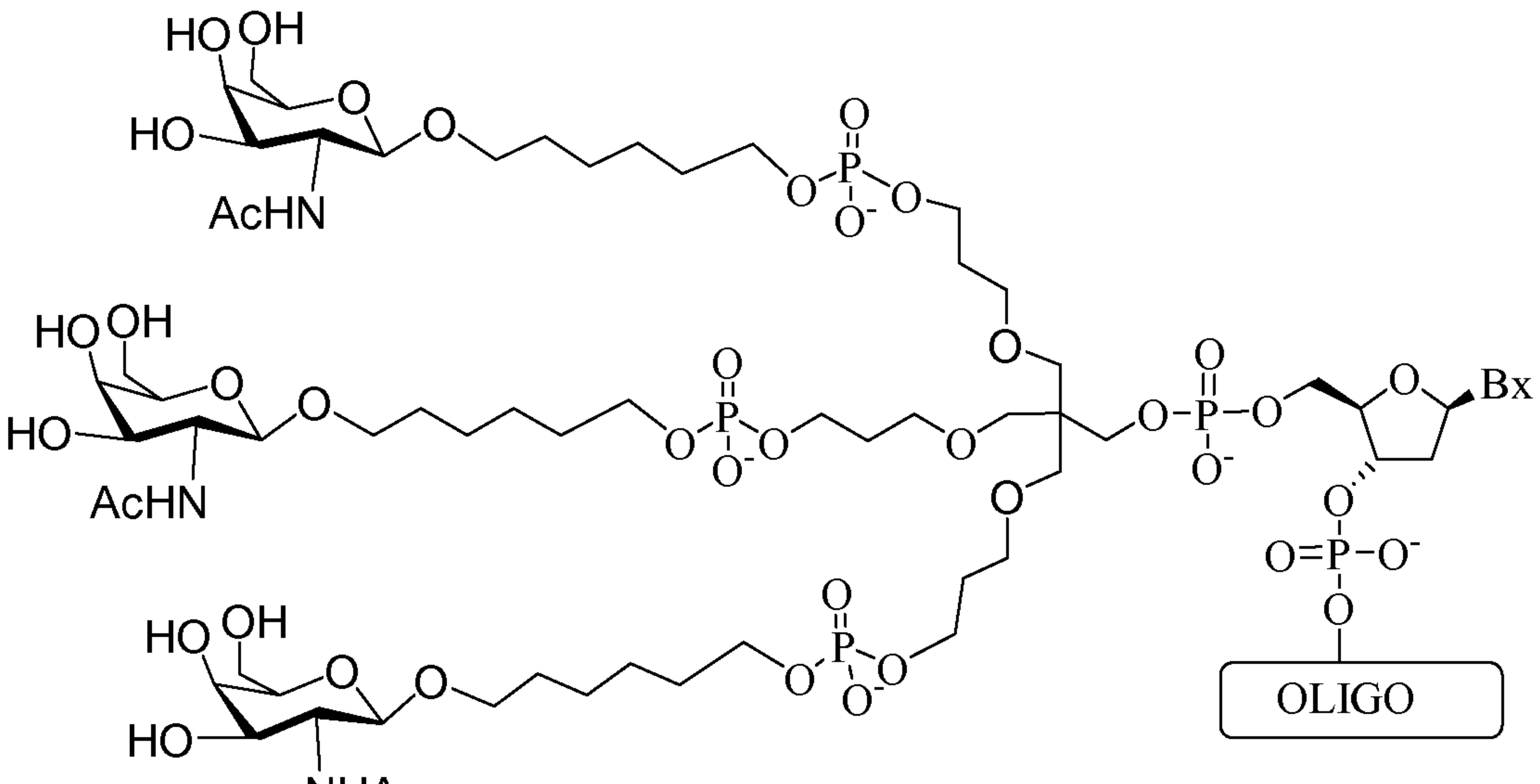
Compound 77 is prepared as per the procedures illustrated in Example 35.



77 79a

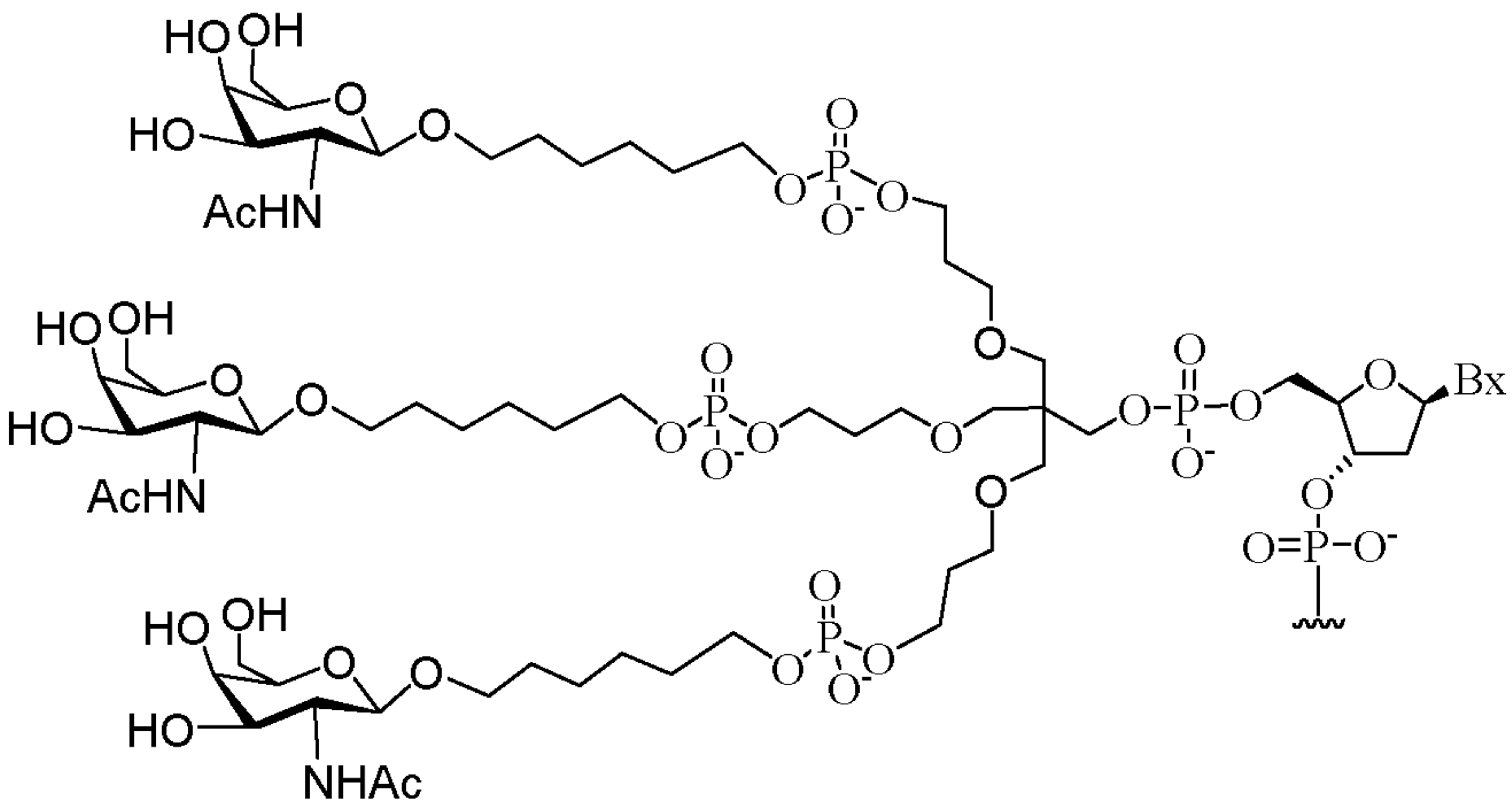
Example 37: General method for the preparation of conjugated oligomeric compound 82 comprising a phosphodiester linked GalNAc$_3$-2 conjugate at 5' terminus *via* solid support (Method I)



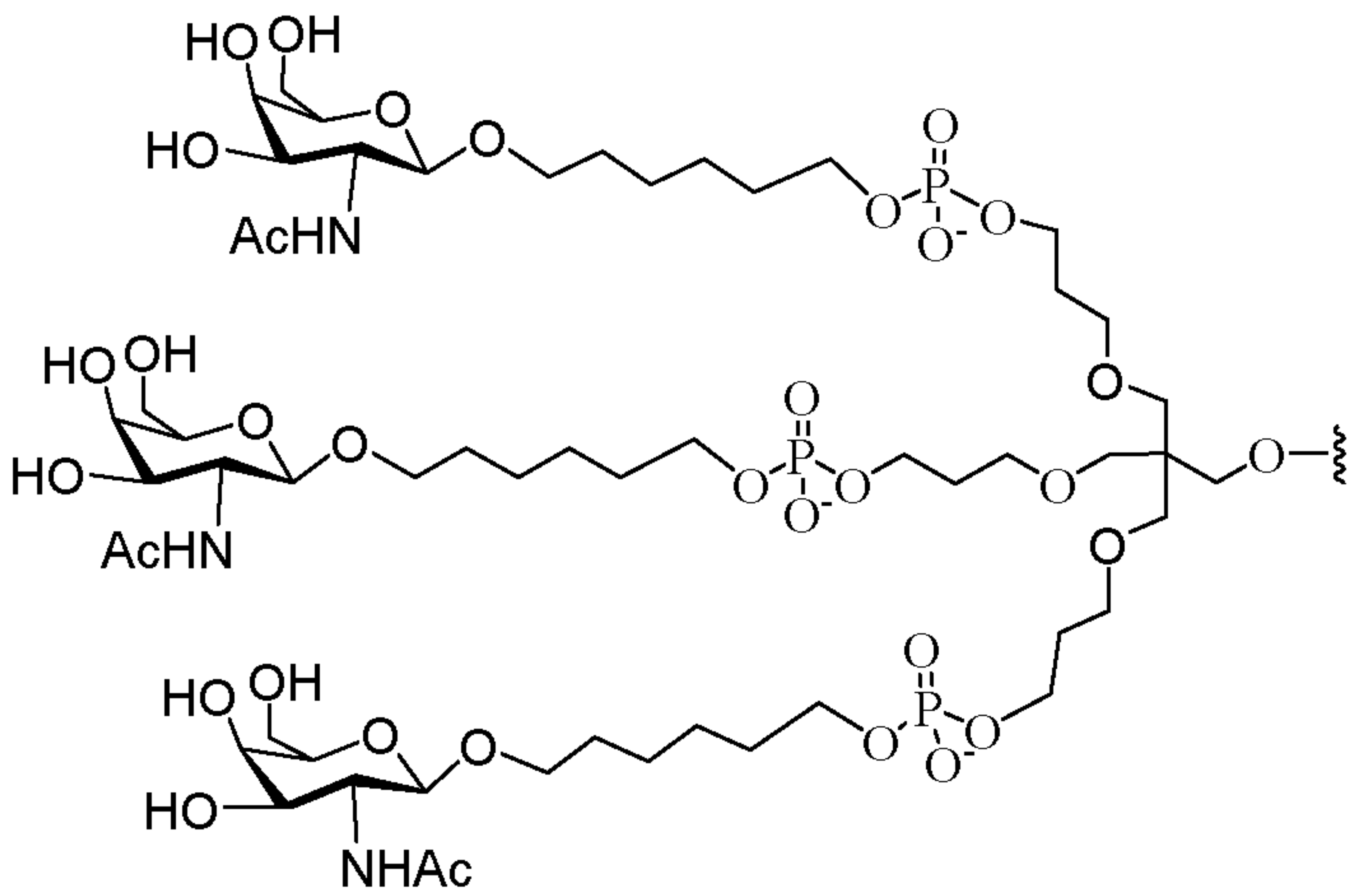


82

wherein GalNAc$_3$-2 has the structure:

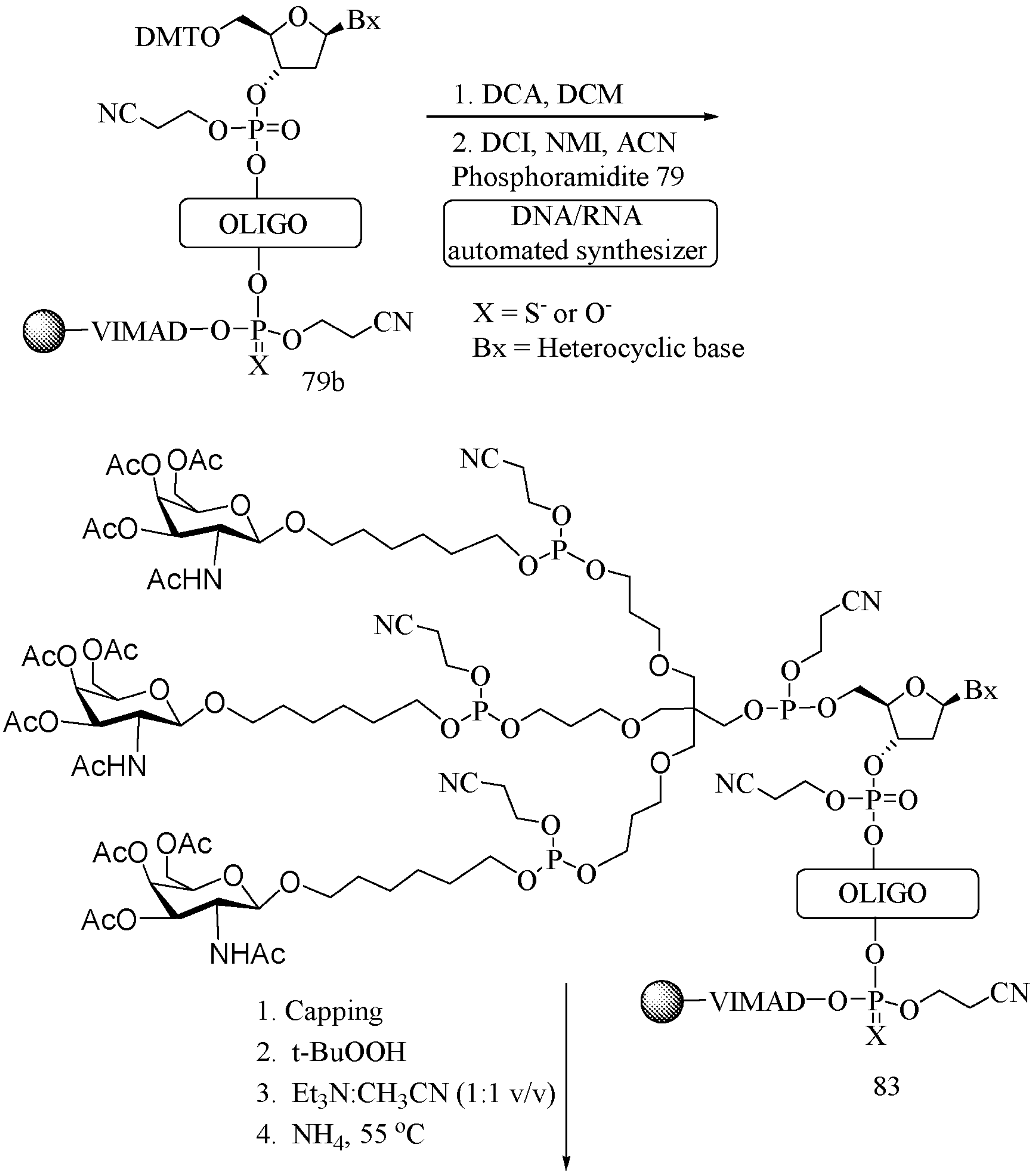


The GalNAc$_3$ cluster portion of the conjugate group GalNAc$_3$-2 (GalNAc$_3$-2$_a$) can be combined with any cleavable moiety to provide a variety of conjugate groups. Wherein GalNAc$_3$-2$_a$ has the formula:



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.

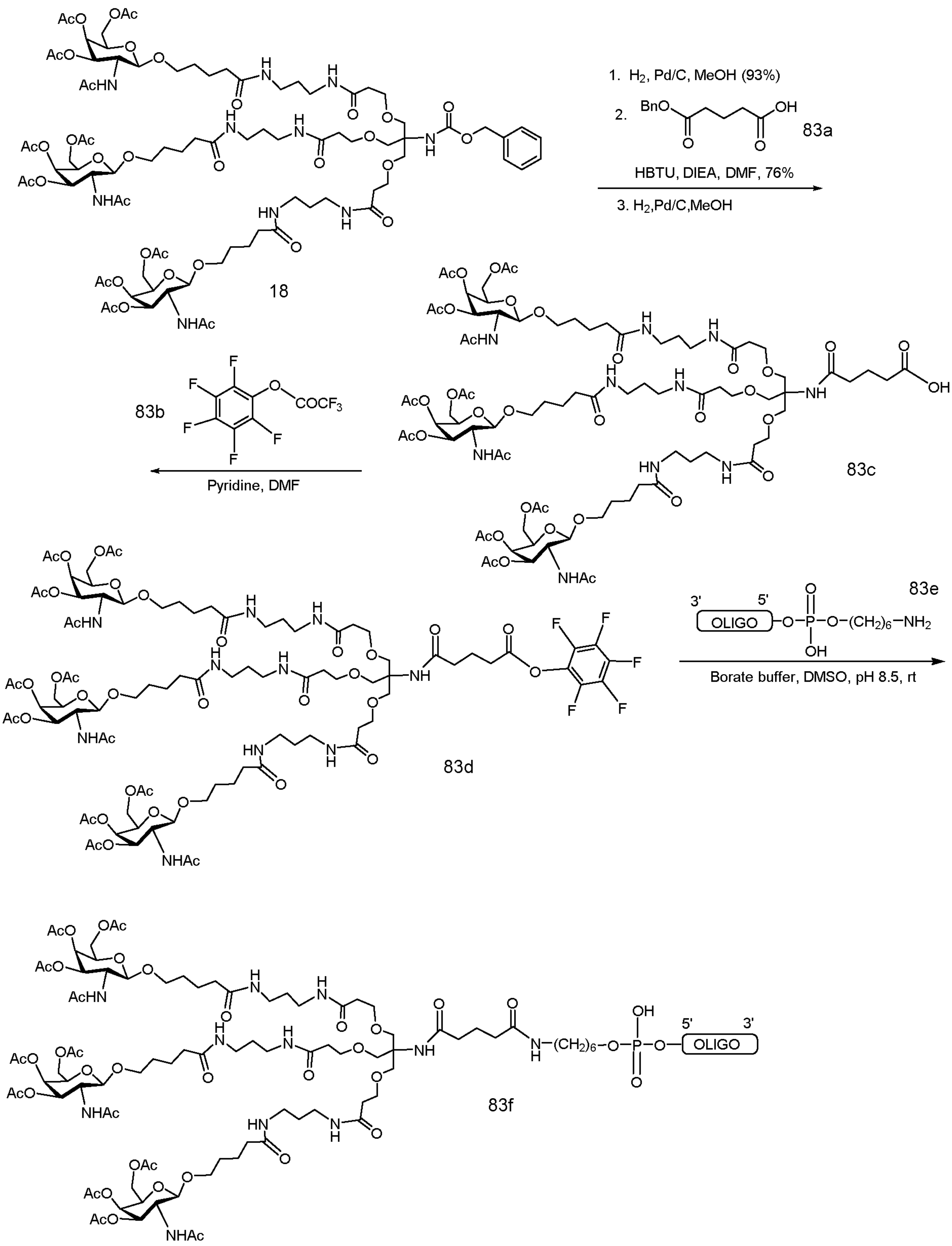
Example 38: Alternative method for the preparation of oligomeric compound 82 comprising a phosphodiester linked GalNAc$_3$-2 conjugate at 5' terminus (Method II)

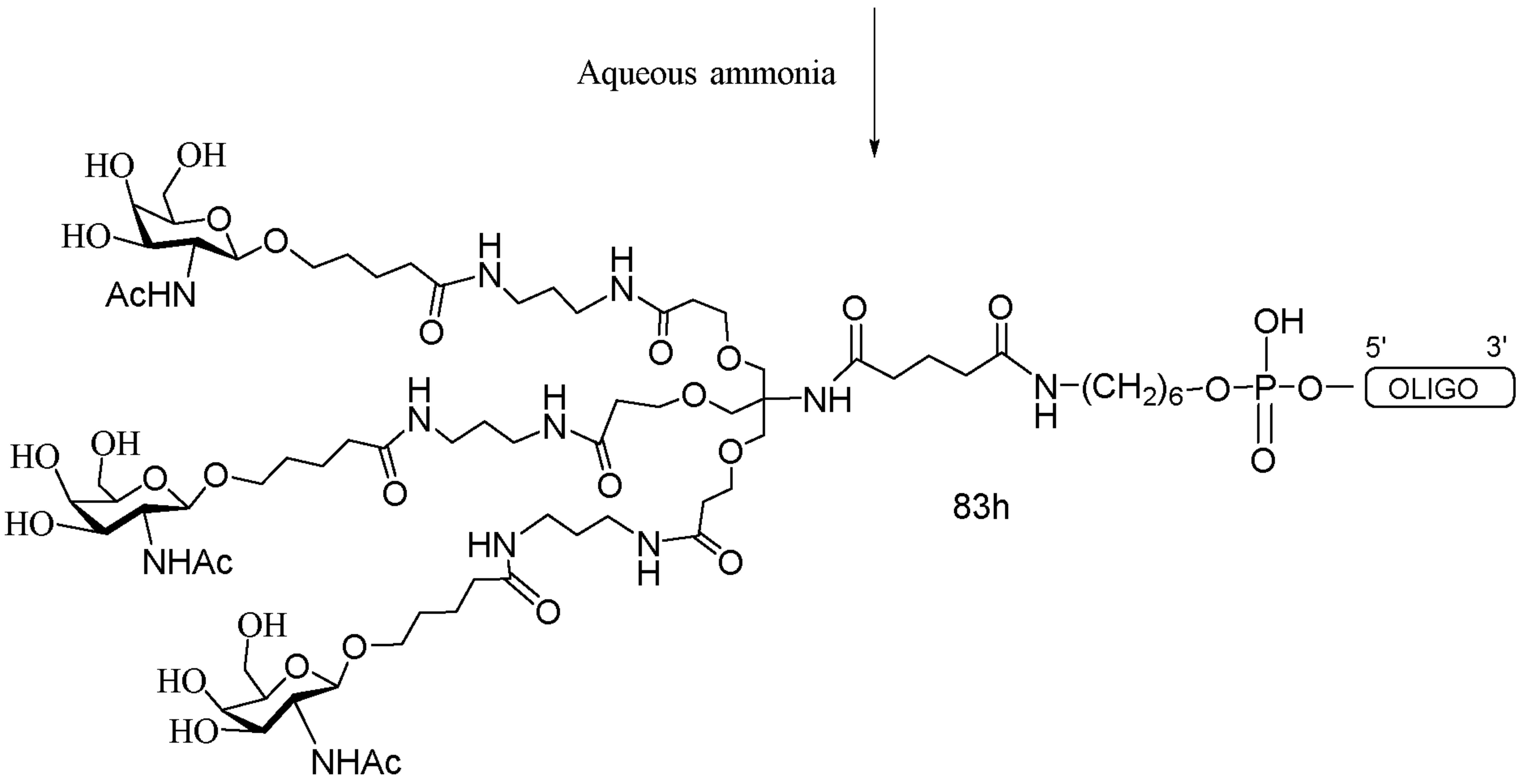


Oligomeric Compound 82

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

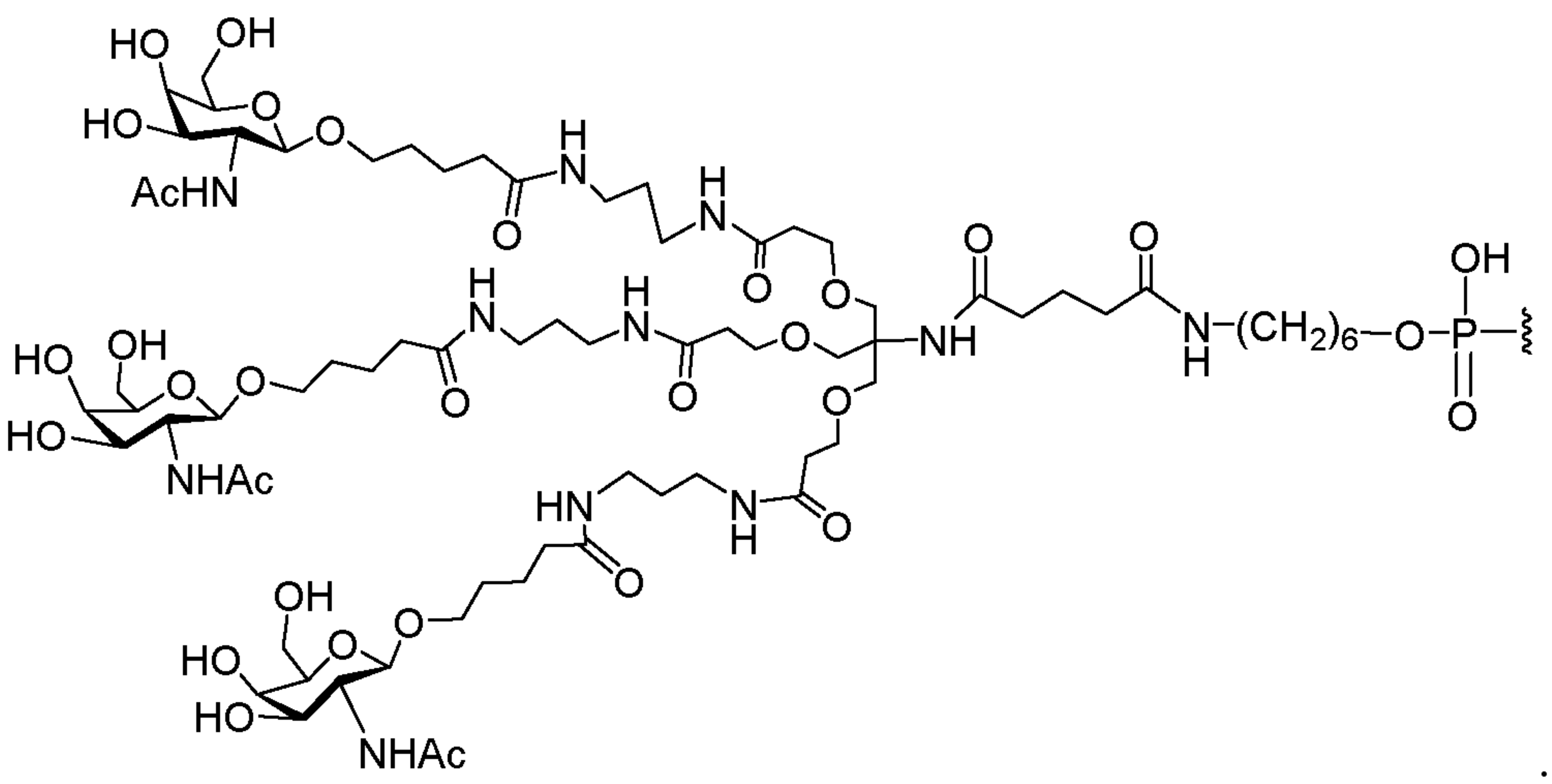

18
1. H_2, Pd/C, MeOH (93%)
2. 83a
HBTU, DIEA, DMF, 76%
3. H_2,Pd/C,MeOH
83c
83b
Pyridine, DMF
83d
83e
Borate buffer, DMSO, pH 8.5, rt
83f



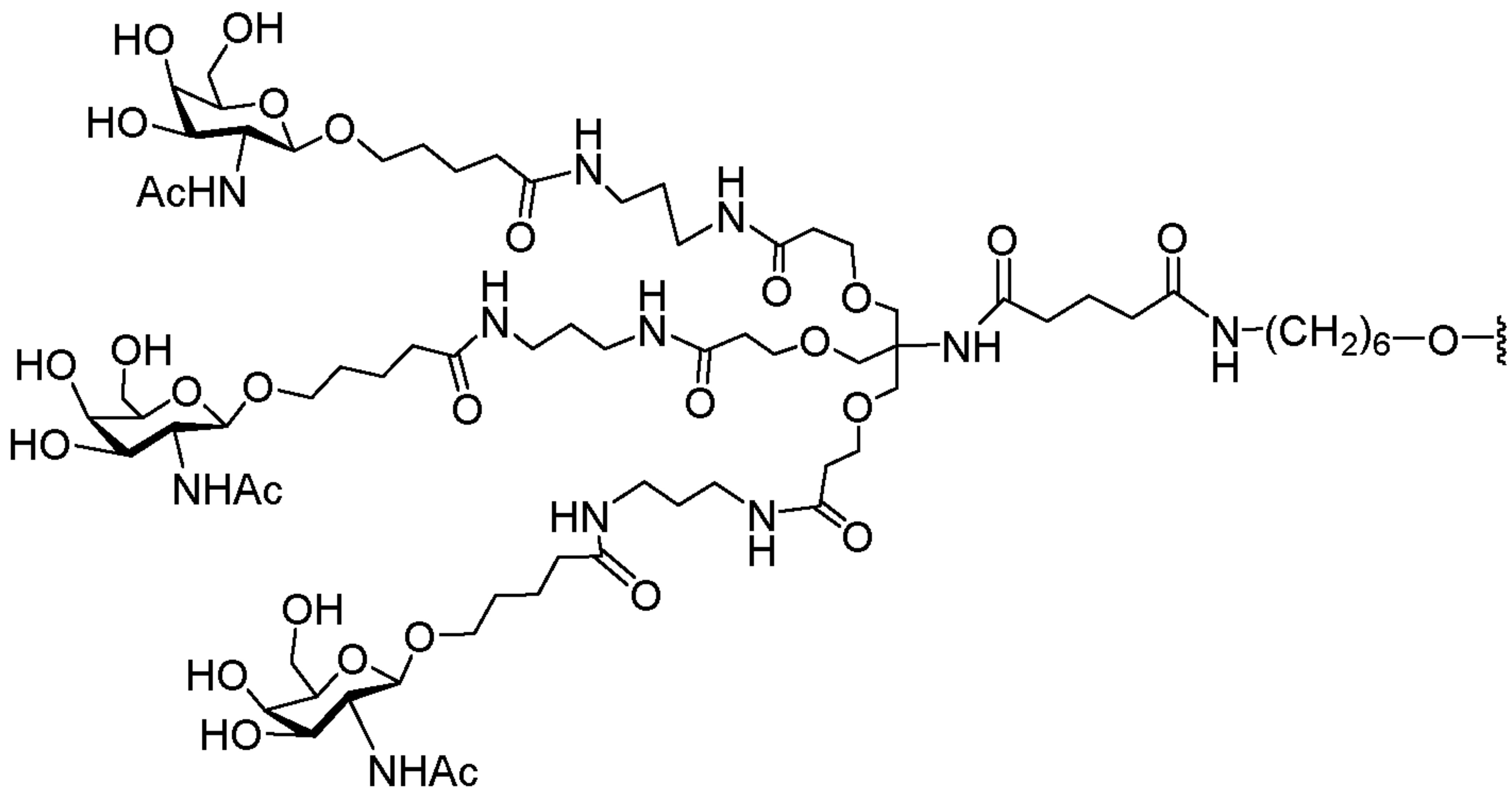
83h

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).

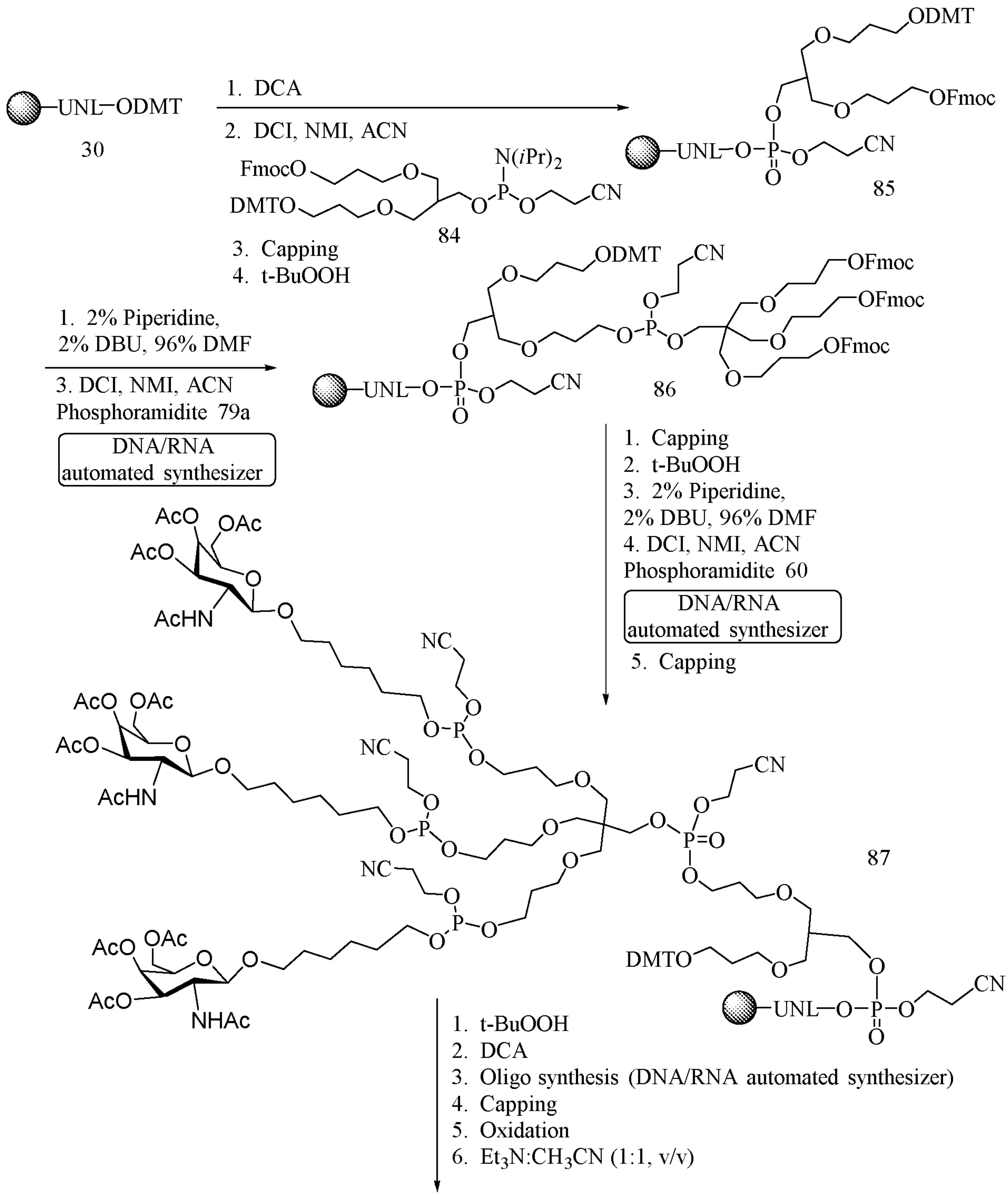
Wherein GalNAc$_3$-3 has the structure:

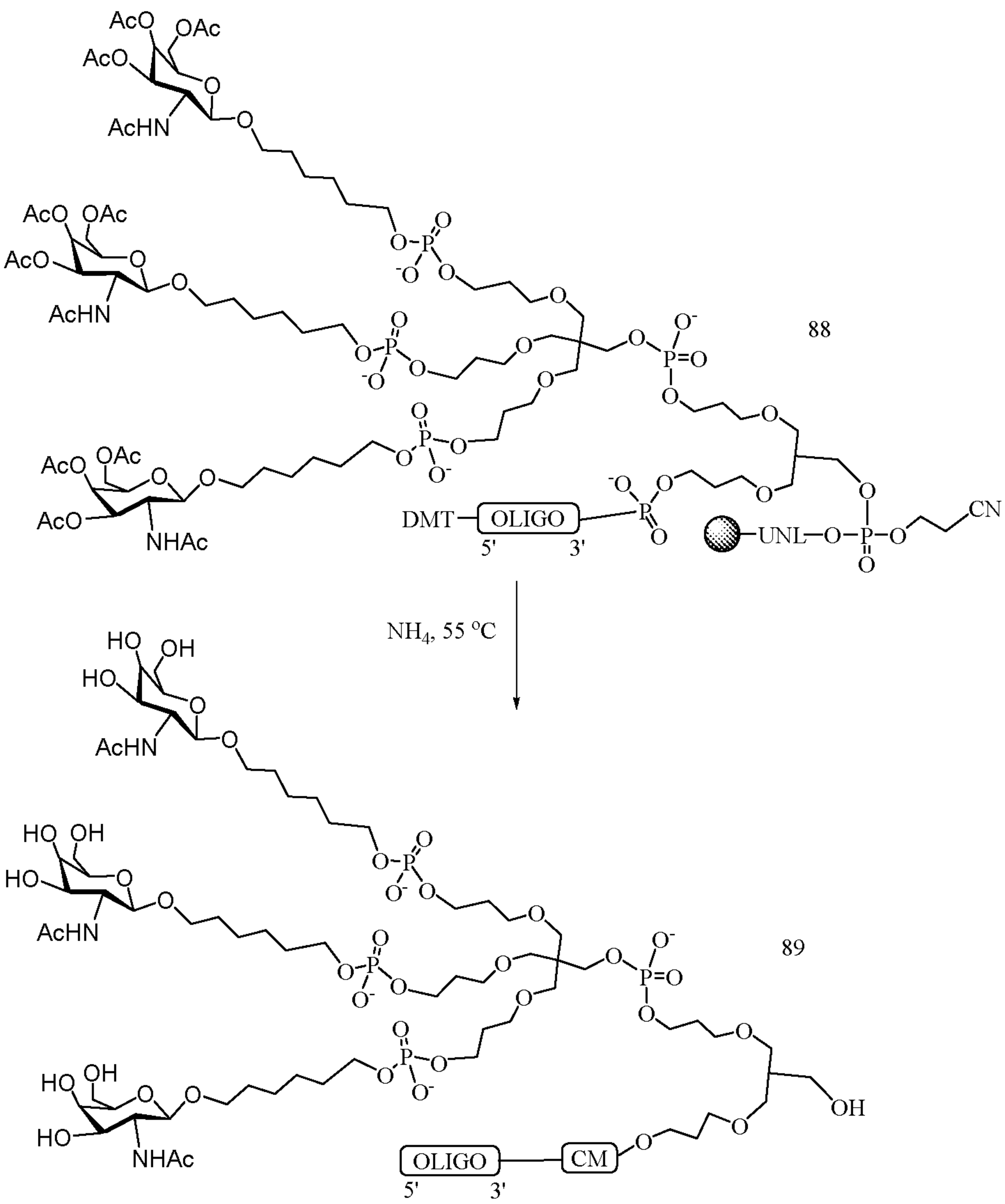


The GalNAc$_3$ cluster portion of the conjugate group GalNAc$_3$-3 (GalNAc$_3$-3$_a$) can be combined with any cleavable moiety to provide a variety of conjugate groups. Wherein GalNAc$_3$-3$_a$ has the formula:


HO
OH
HO
O
O
AcHN
H
N
O
H
N
O
O
O
O
NH
N
H
(CH2)6
O
HO
OH
HO
NHAc
H
N
H
N
O
O
O
HN
N
H
O
OH
HO
HO
NHAc
.

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

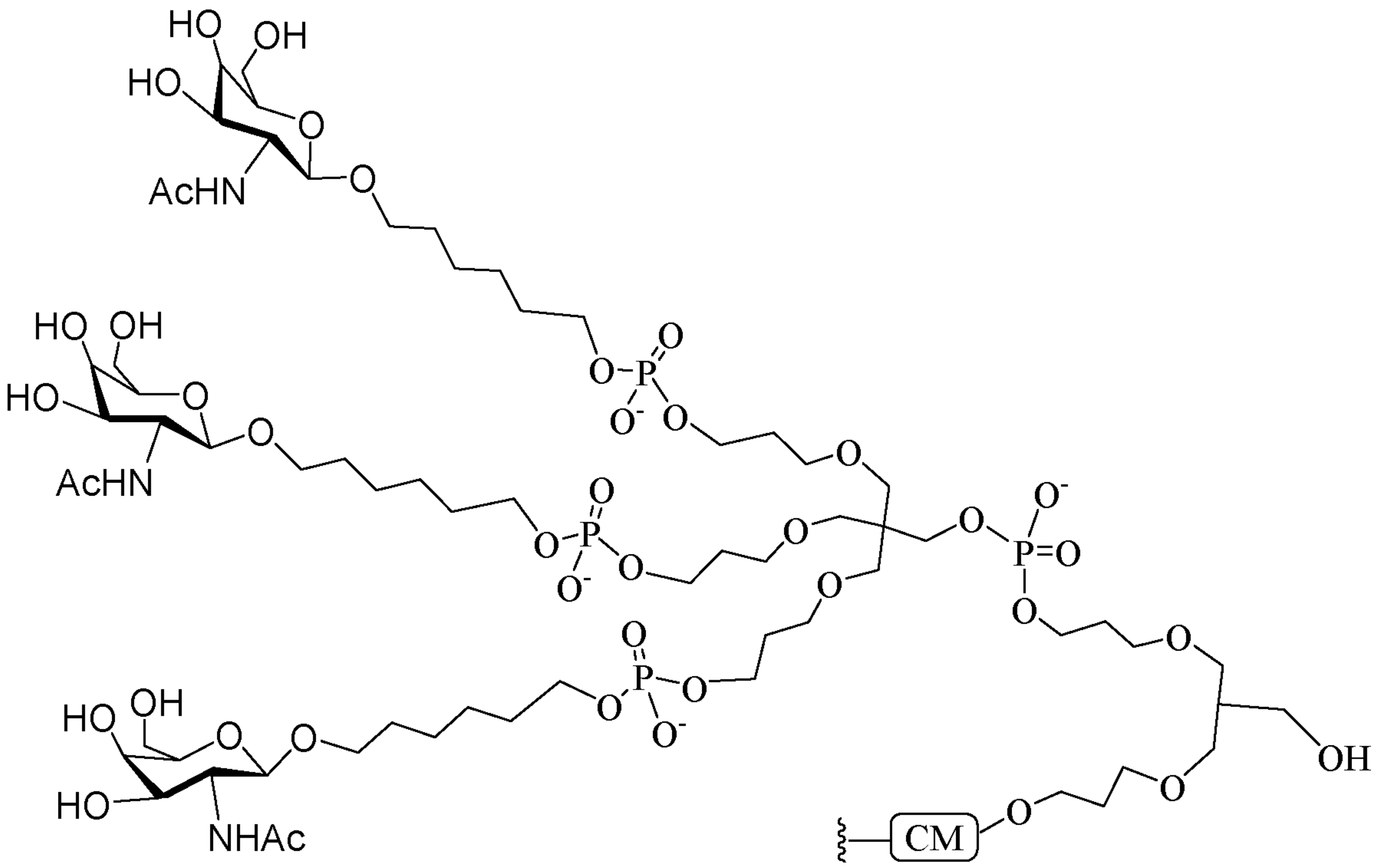




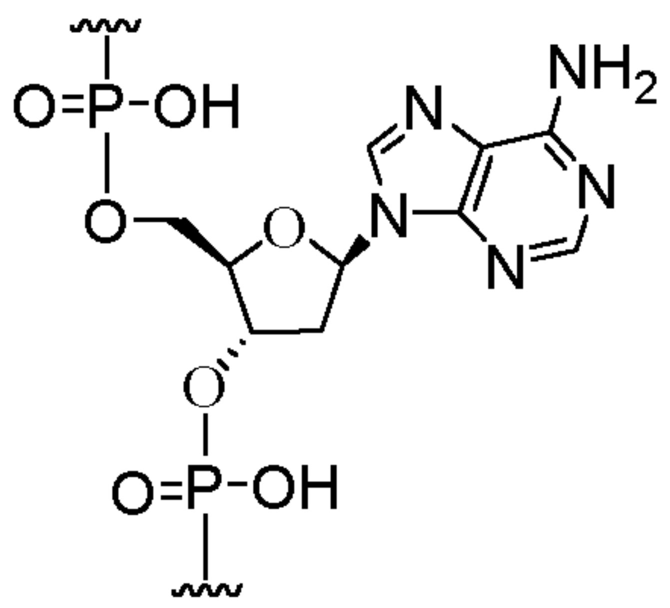
88

89

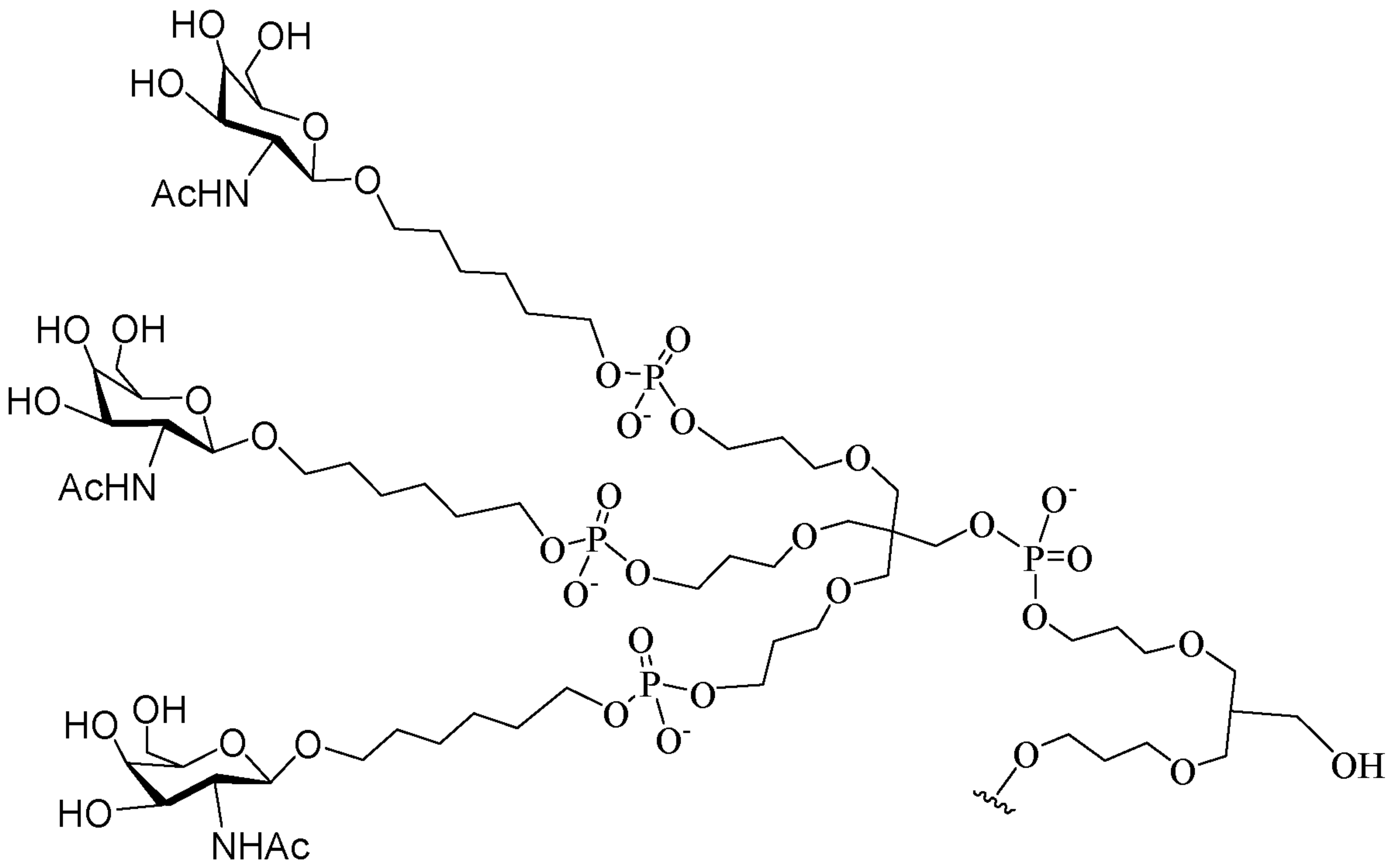
Wherein GalNAc3-4 has the structure:



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:



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 GalNAc3-2 conjugate at the 5' position targeting SRB-1

ISIS No.	Sequence (5' to 3')	CalCd Mass	Observed Mass	SEQ ID No.
661134	$\mathbf{GalNAc_3\text{-}2_{a}\text{-}_{o'}A_{do}}T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{k}$	6482.2	6481.6	2302

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 $GalNAc_3\text{-}2_a$ 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	2303

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	2298
	0.7	91			
	2	69			
	7	22			
	20	5			
651900	0.07	95	0.26	**3' GalNAc$_3$-1**	2299
	0.2	77			
	0.7	28			
	2	11			
	7	8			
661134	0.07	107	0.25	**5' GalNAc$_3$-2**	2302
	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	2304
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	2305
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	2305

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	2304
	10	52.40			
	30	24.95			
655861	0.5	81.22	2.2	Full PS with GalNAc$_3$-1 conjugate	2305
	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	2305
	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

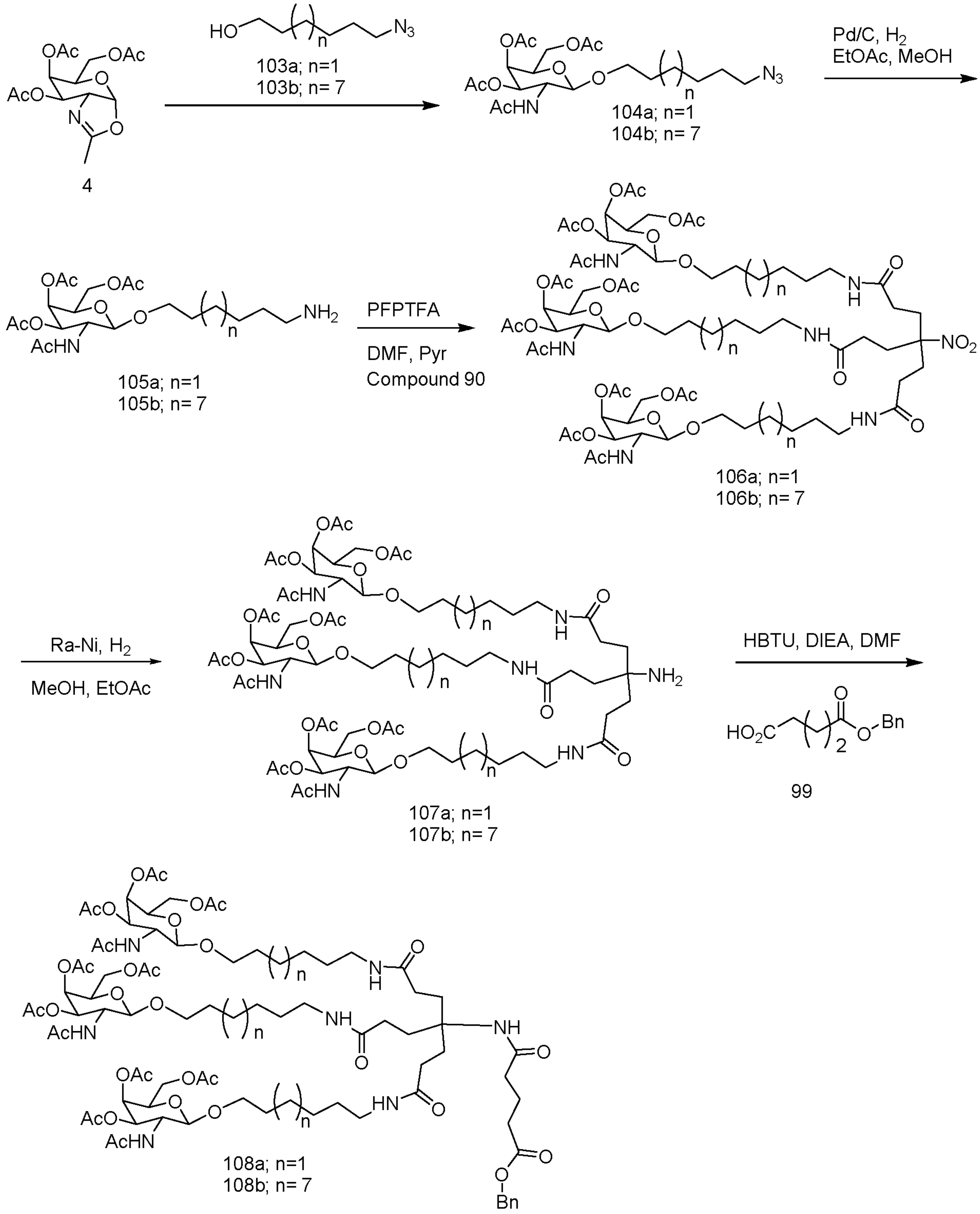
Effect of PO/PS linkages on transaminase levels of ASOs comprising GalNAc$_3$-1 conjugate at 3' terminus targeting SRB-1

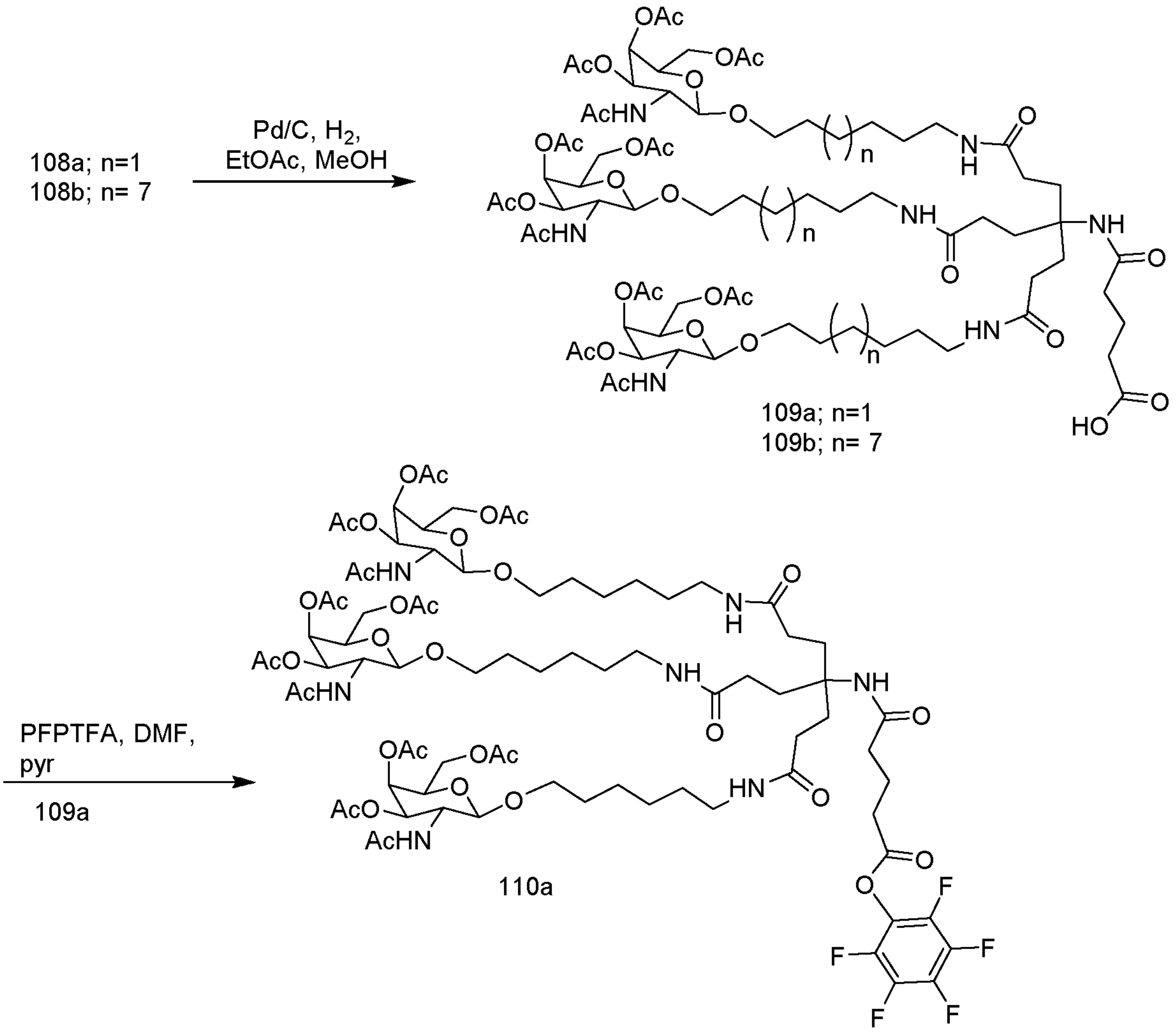
ISIS No.	Dosage (mg/kg)	ALT (U/L)	AST (U/L)	Chemistry	SEQ ID No.
PBS	0	28.5	65	--	
353382 (parent)	3	50.25	89	Full PS without conjugate	2304
	10	27.5	79.3		
	30	27.3	97		
655861	0.5	28	55.7	Full PS with **GalNAc$_3$-1**	2305
	1.5	30	78		
	5	29	63.5		
	15	28.8	67.8		
655862	0.5	50	75.5	Mixed PS/PO with	2305

	1.5	21.7	58.5	**GalNAc$_3$-1**	
	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.

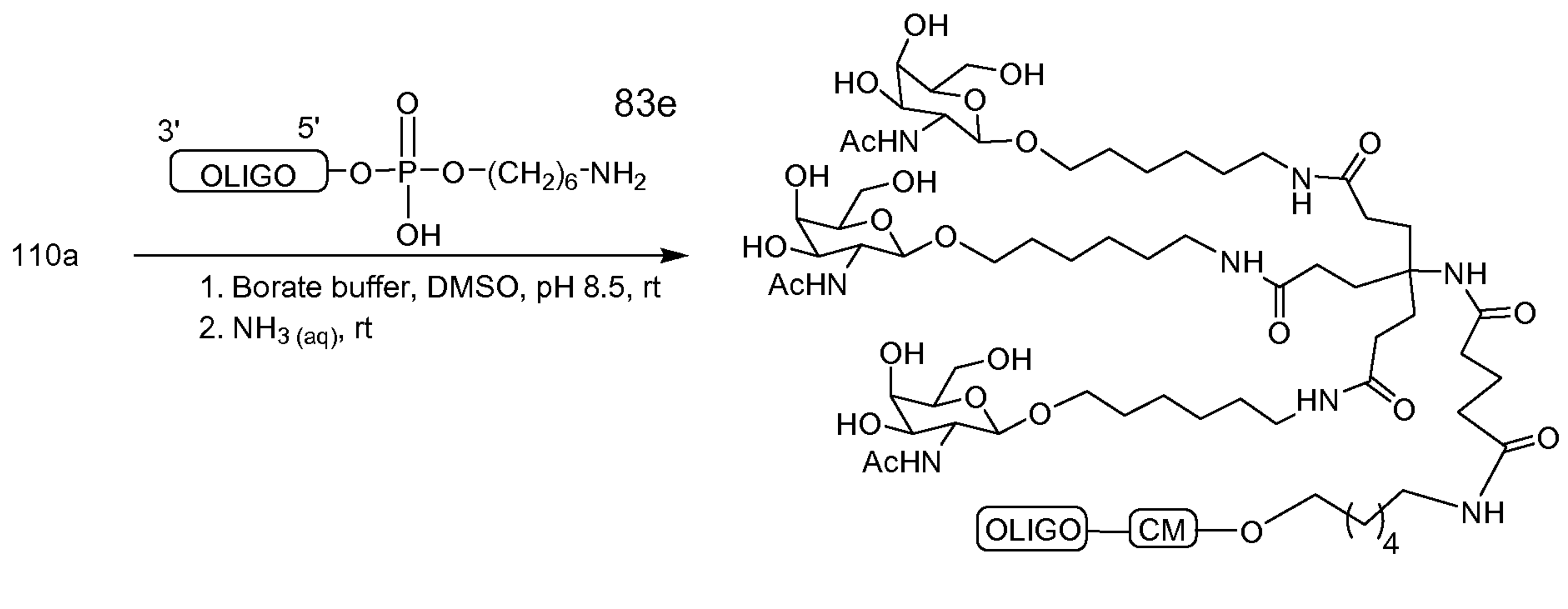
Compounds 107a and 107b were treated to the same conditions as for compounds 97a-d (Example 47), to give compounds 108a and 108b in 40-60% yield. LCMS and proton NMR was consistent with the structure.

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.

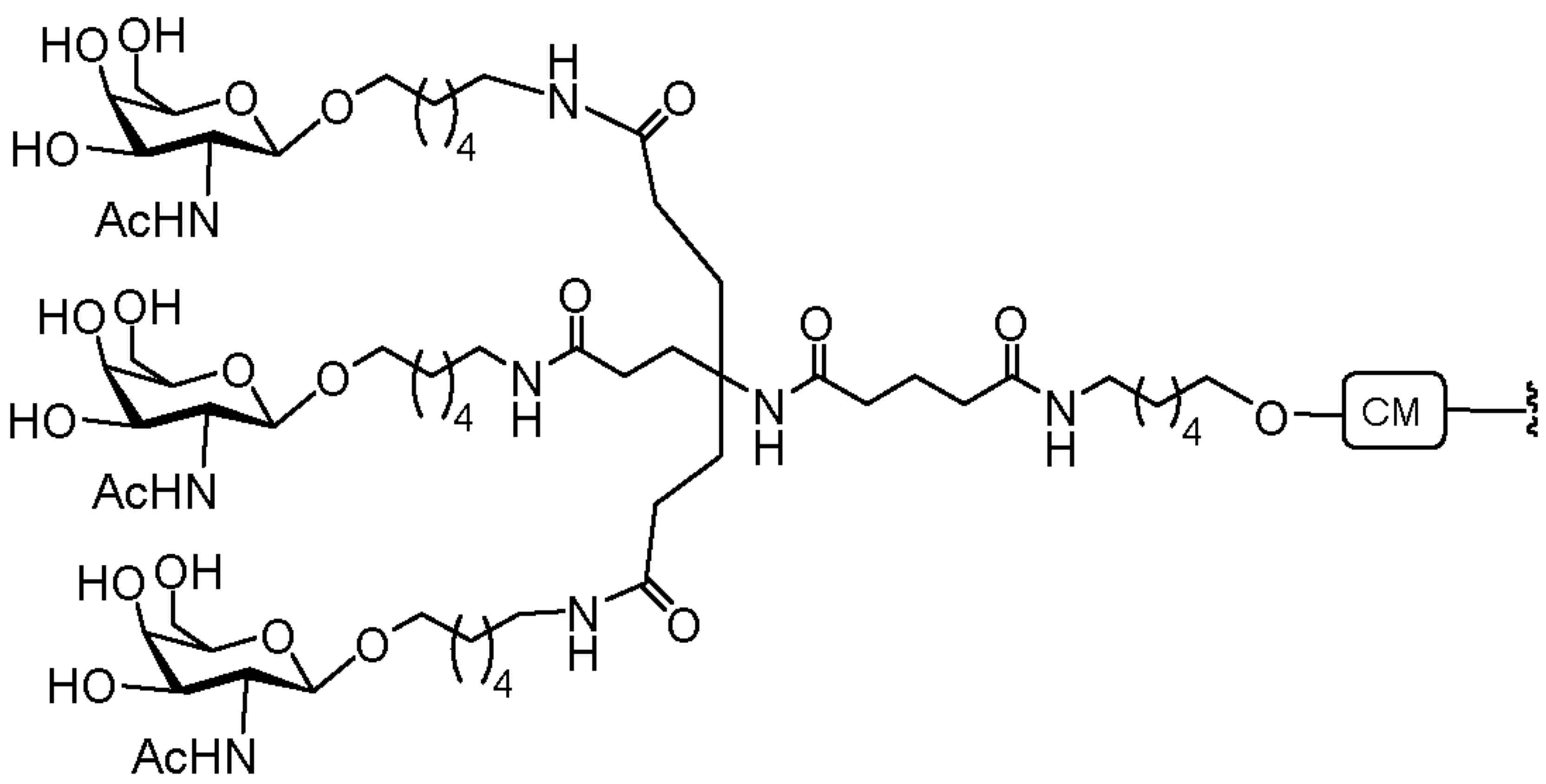
Example 46: General Procedure for Conjugation with PFP Esters (Oligonucleotide 111); Preparation of ISIS 666881 (GalNAc$_3$-10)

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).

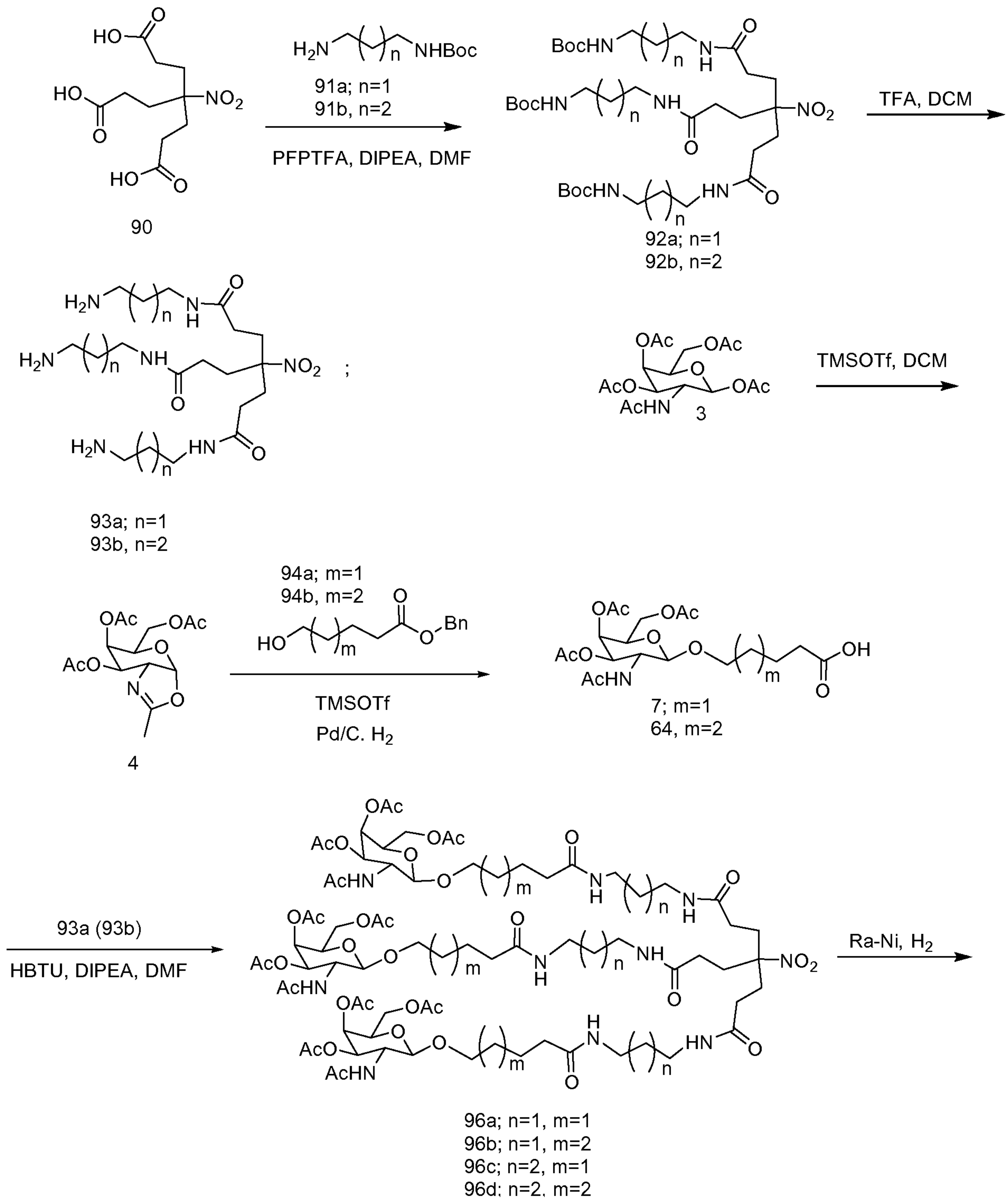
Table 38a

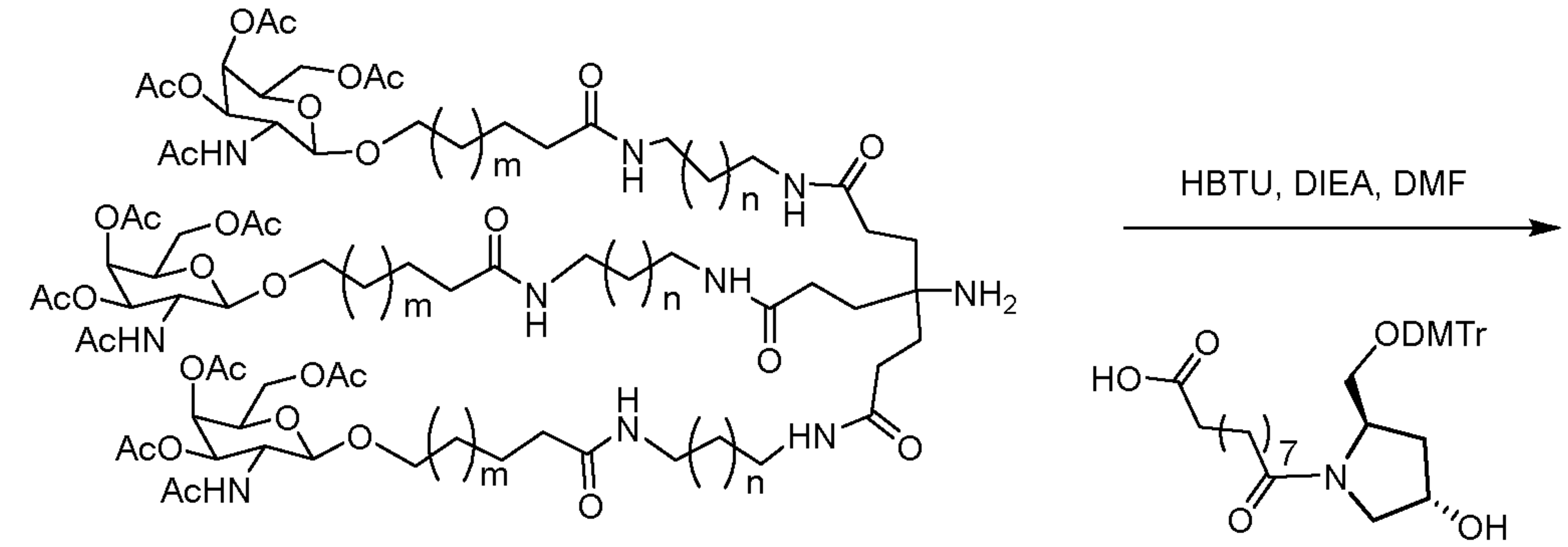
GalNAc$_3$-10 conjugated oligonucleotide

ASO	Sequence (5' to 3')	5' group	SEQ ID No.
ISIS 660254	$NH_2(CH_2)_6$-$_o$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$_e$	Hexylamine	2306
ISIS 666881	**GalNAc$_3$-10$_a$-$_o$'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$_3$-10**	2306

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.

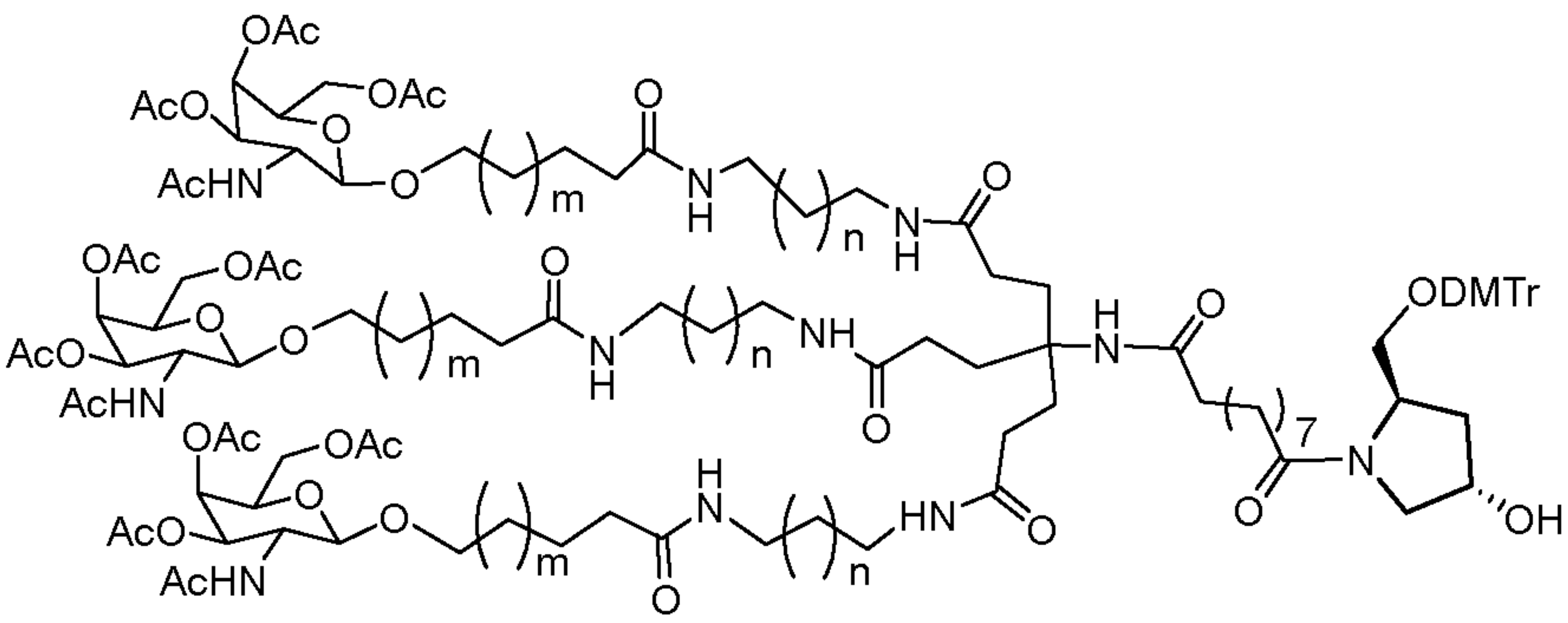
Example 47: Preparation of Oligonucleotide 102 Comprising GalNAc$_3$-8



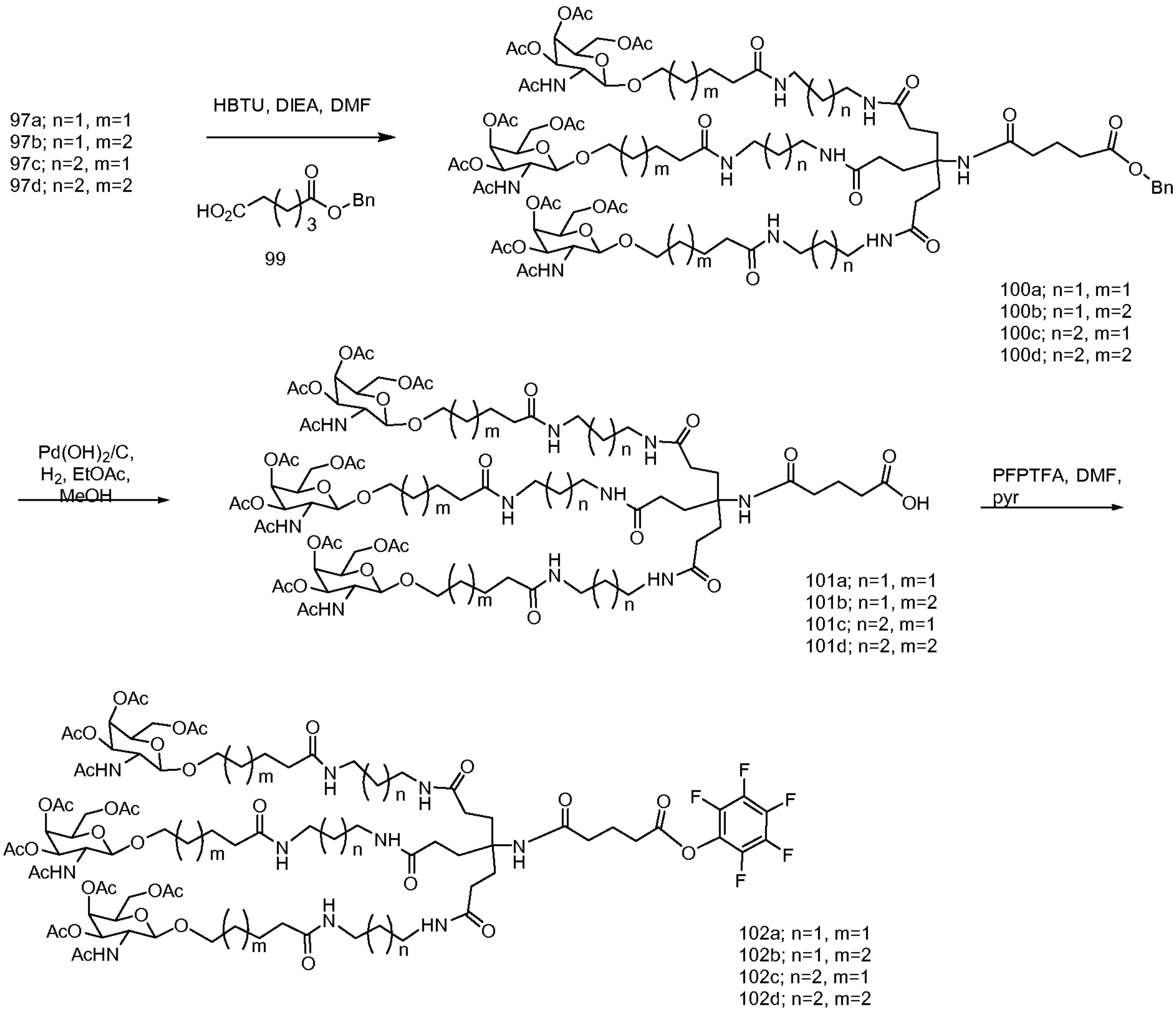


97a; n=1, m=1
97b; n=1, m=2
97c; n=2, m=1
97d; n=2, m=2

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.

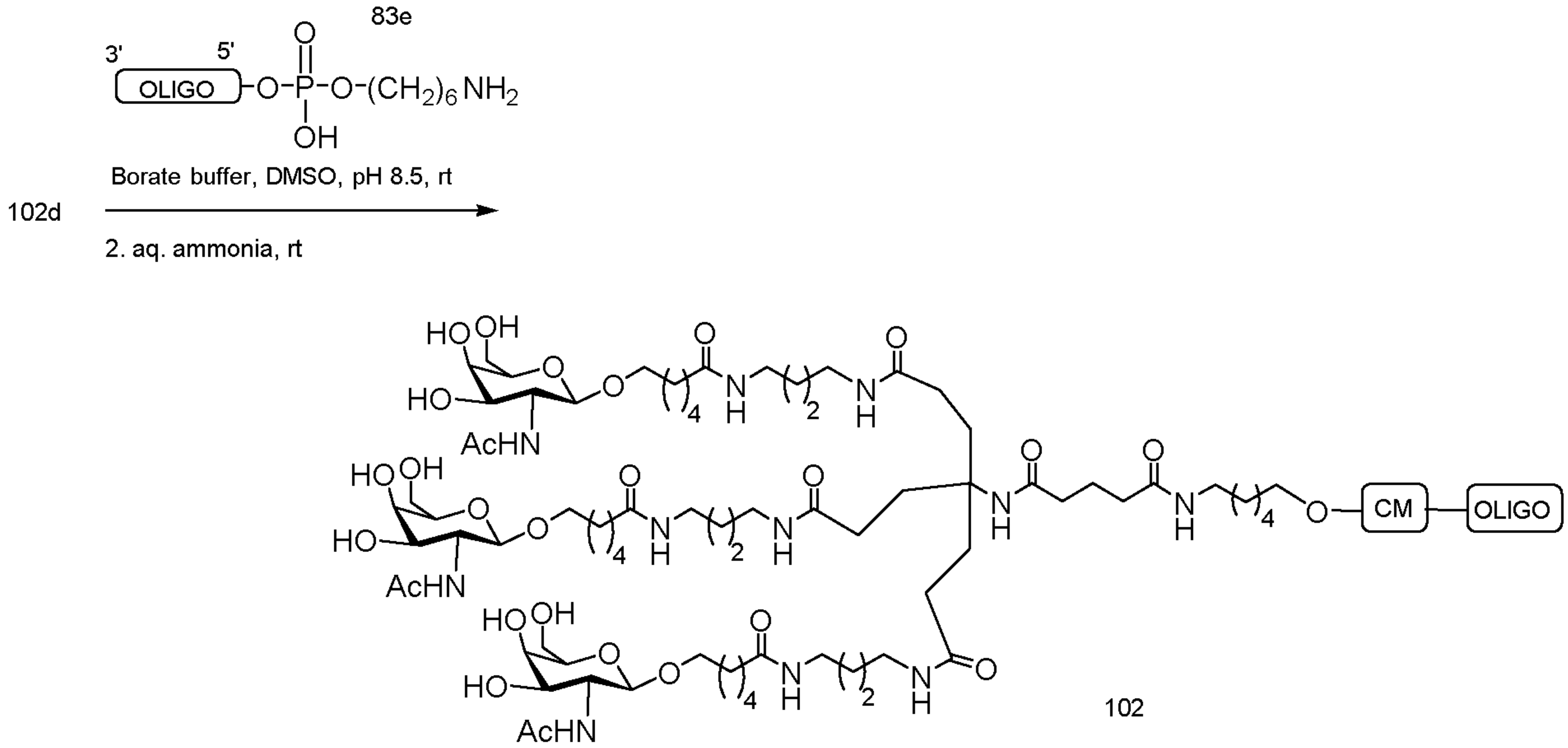
Compounds 96a-d (0.75 mmoles), individually, were hydrogenated over Raney Nickel for 3 hours in Ethanol (75 mL). At that time, the catalyst was removed by filtration thru celite, and the ethanol removed under reduced pressure to give compounds 97a-d in 80-90% yield. LCMS and proton NMR were consistent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

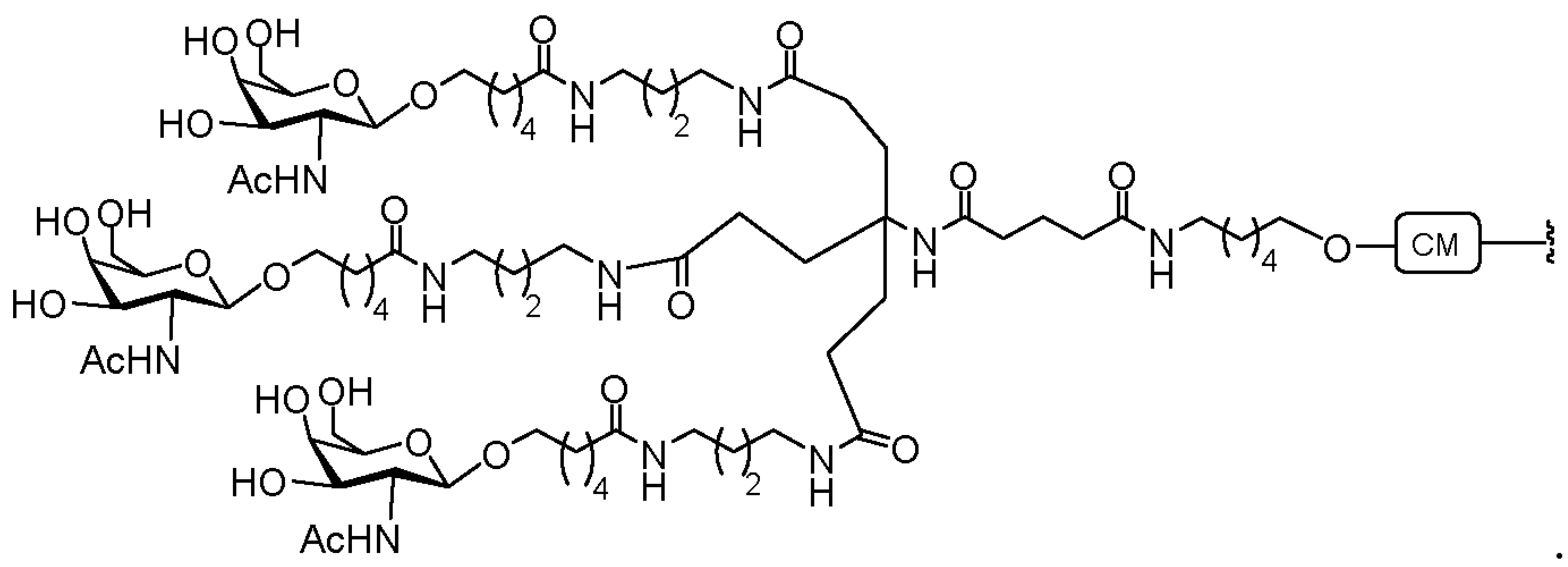
Compounds 101a-d (0.15 mmoles), individually, were dissolved in DMF (15 mL) and pyridine (0.016 mL, 0.2 mmoles). Pentafluorophenyl trifluoroacetate (0.034 mL, 0.2 mmoles) was added dropwise, under argon, and the reaction was allowed to stir at room temperature for 30 minutes. At that time, the DMF was 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.



102

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:



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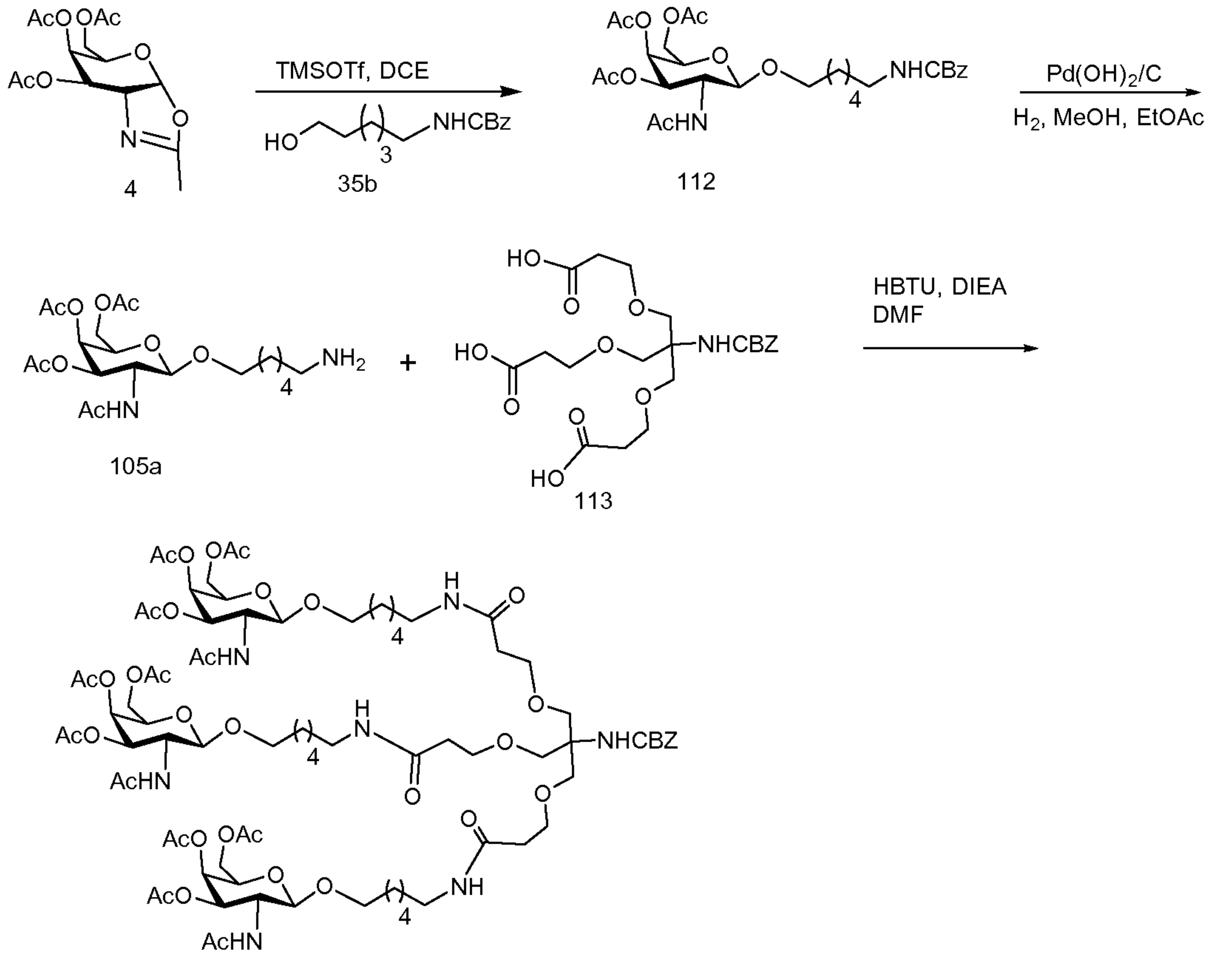
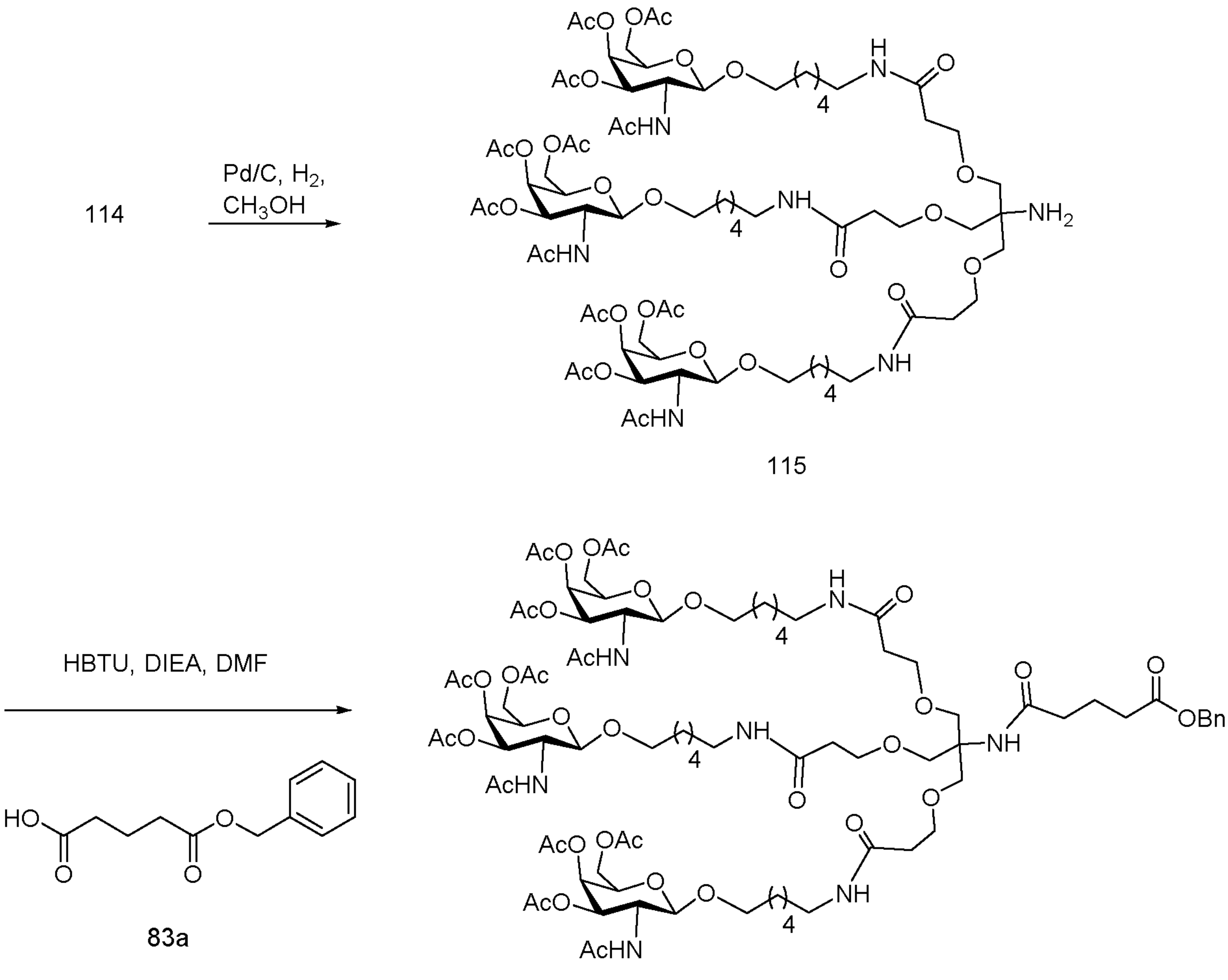
Example 48: Preparation of Oligonucleotide 119 Comprising GalNAc$_3$-7



114



116

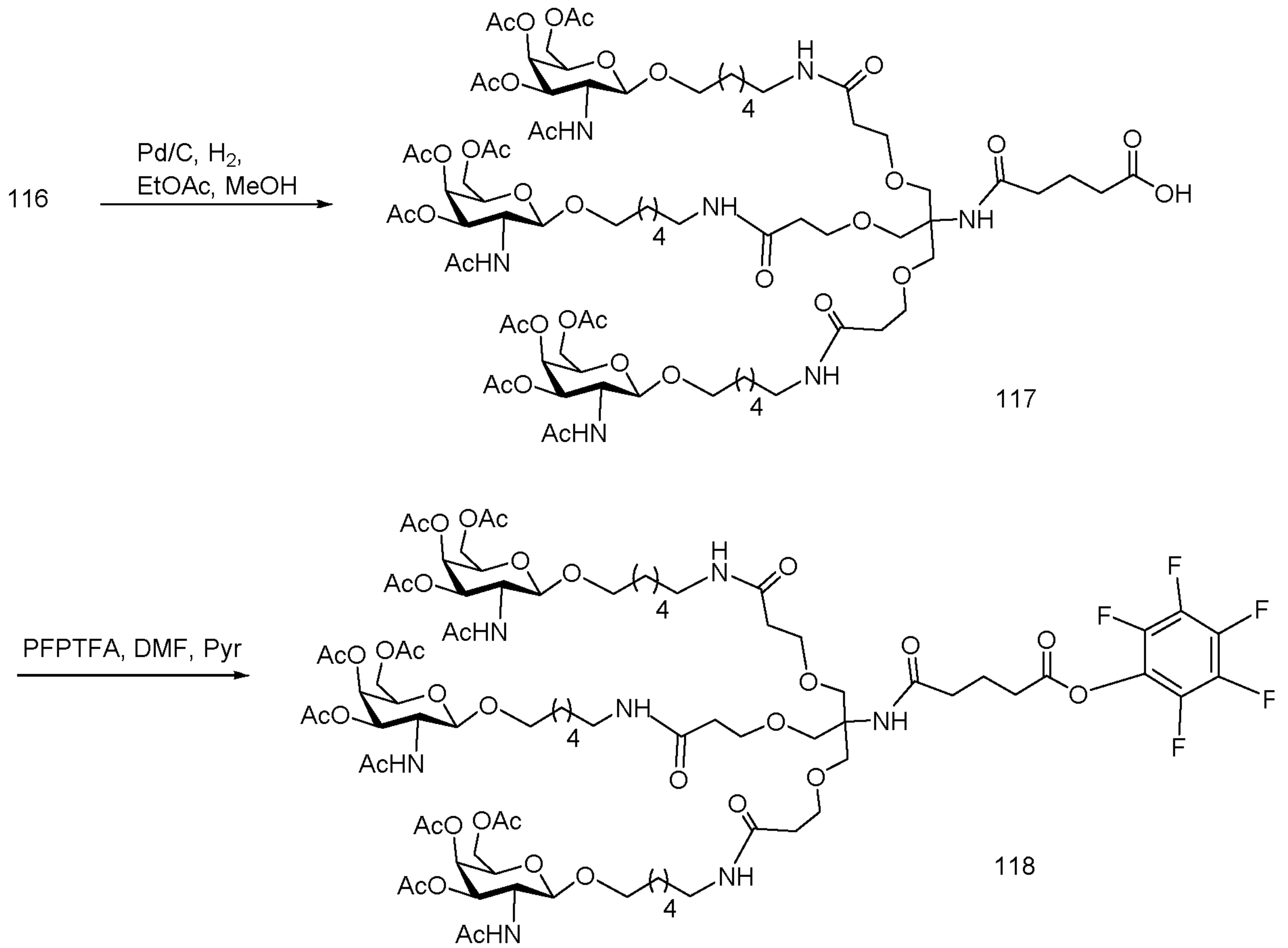
Compound 112 was synthesized following the procedure described in the literature (*J. Med. Chem.* 2004, *47*, 5798-5808).

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 ^{1}H NMR analysis.

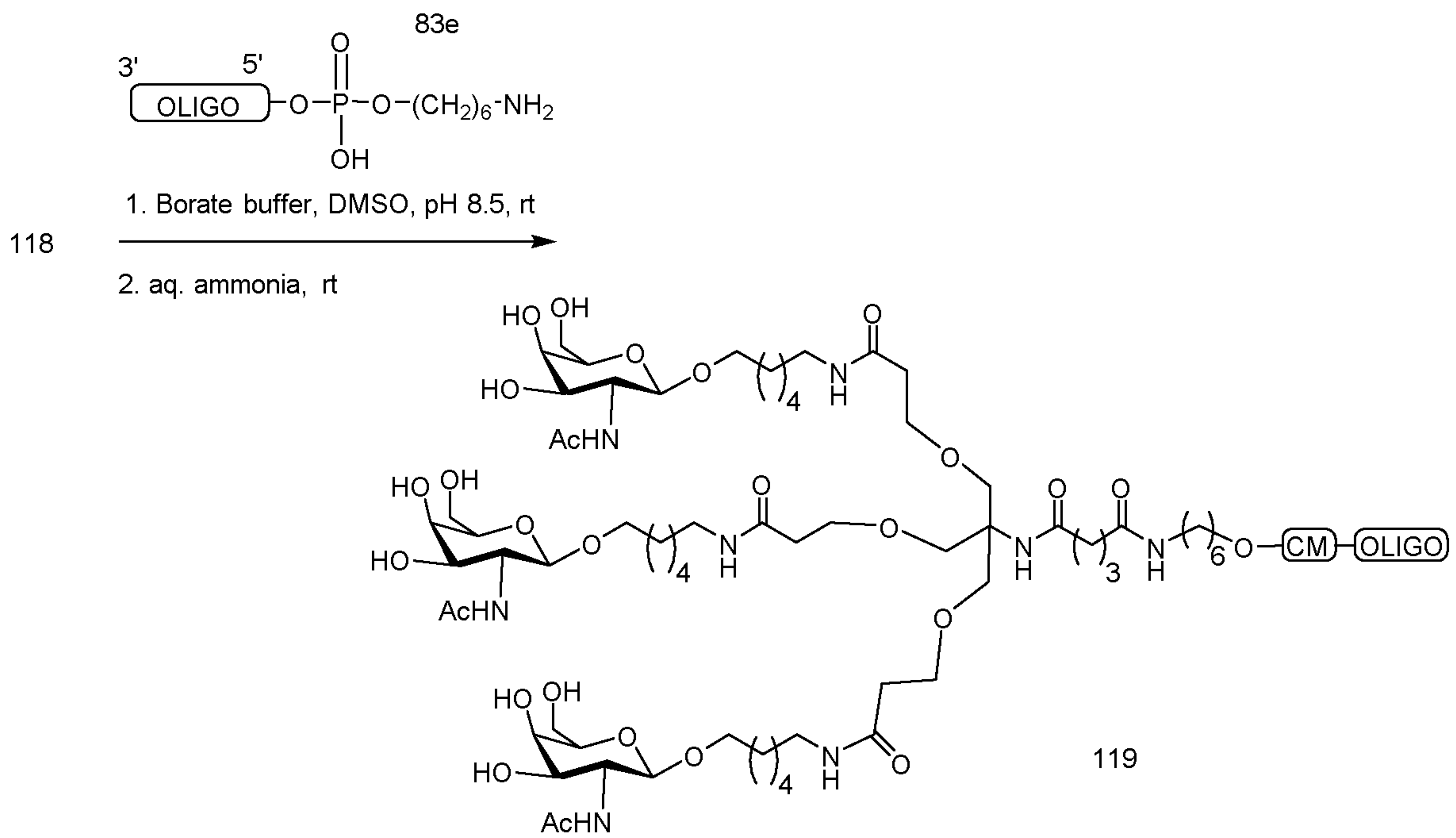
Compound 114 (1.43 g, 0.8 mmol) was dissolved in 1:1 methanol/ethyl acetate (4 mL/4 mL). Palladium on carbon (wet, 0.14 g) was added. The reaction mixture was flushed with hydrogen and stirred at room temperature under hydrogen for 12 h. The reaction mixture was filtered through a pad of celite. The 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 ^{1}H 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 ^{1}H NMR analysis.



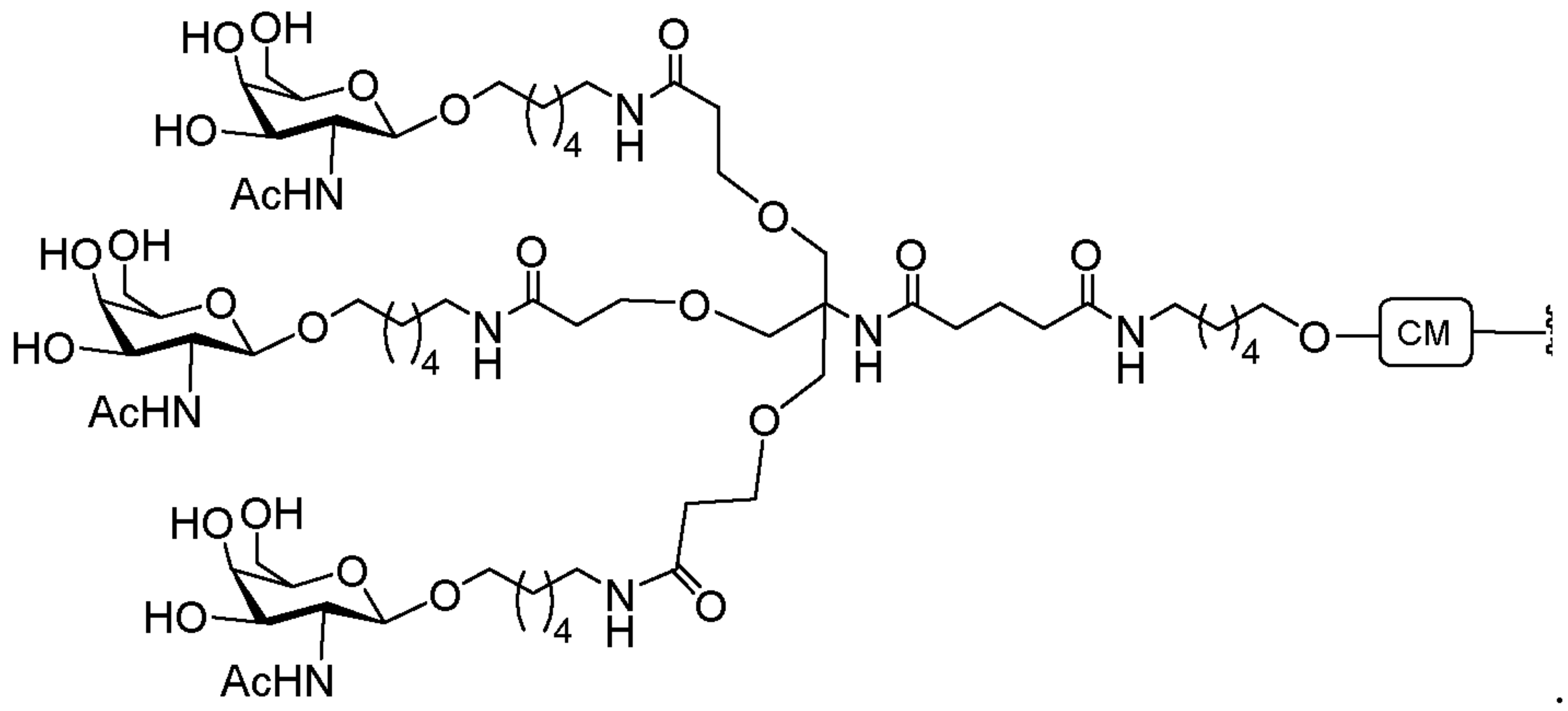
Compound 116 (0.74 g, 0.4 mmol) was dissolved in 1:1 methanol/ethyl acetate (5 mL/5 mL). Palladium on carbon (wet, 0.074 g) was added. The reaction mixture was flushed with hydrogen and stirred at room temperature under hydrogen for 12 h. The reaction mixture was filtered through a pad of celite. The 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:



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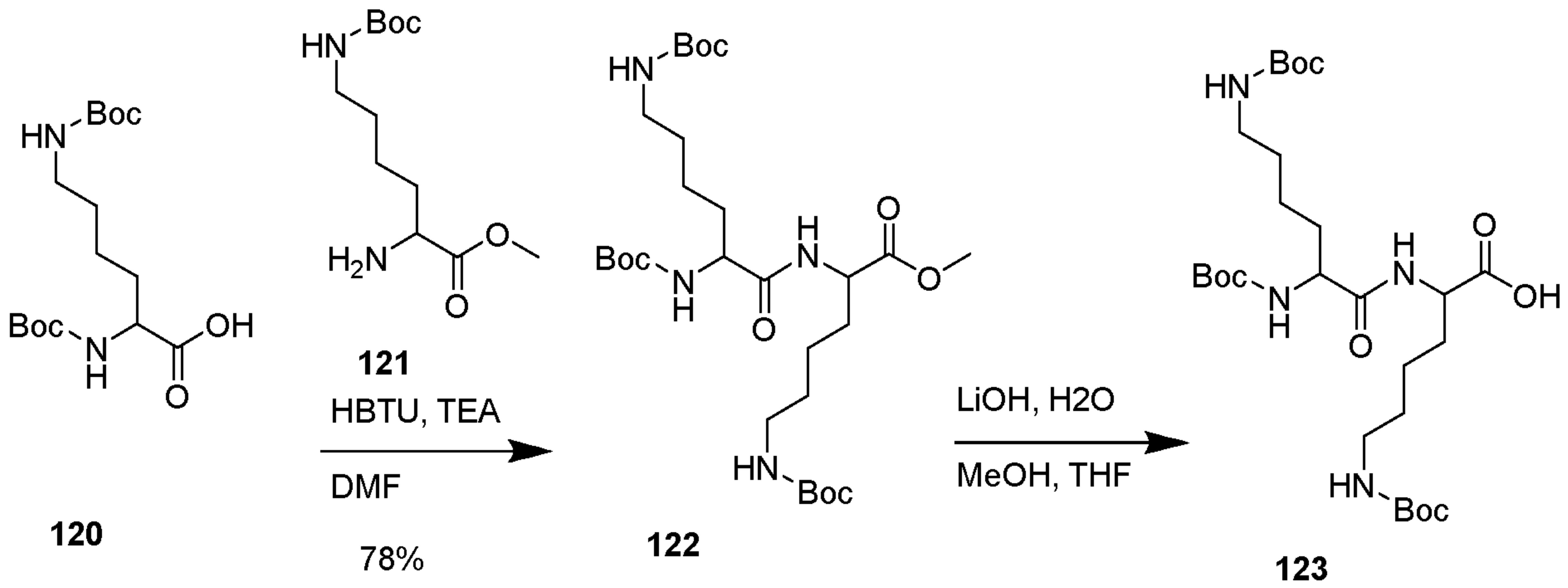
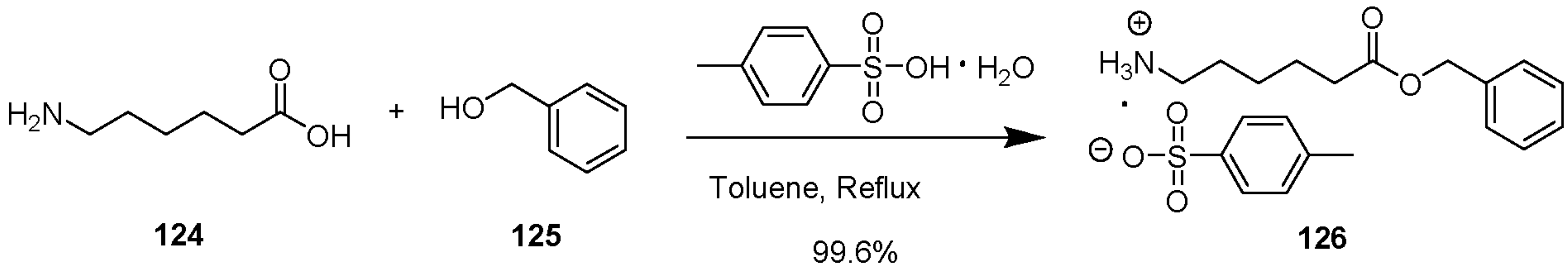
Example 49: Preparation of Oligonucleotide 132 Comprising GalNAc$_3$-5



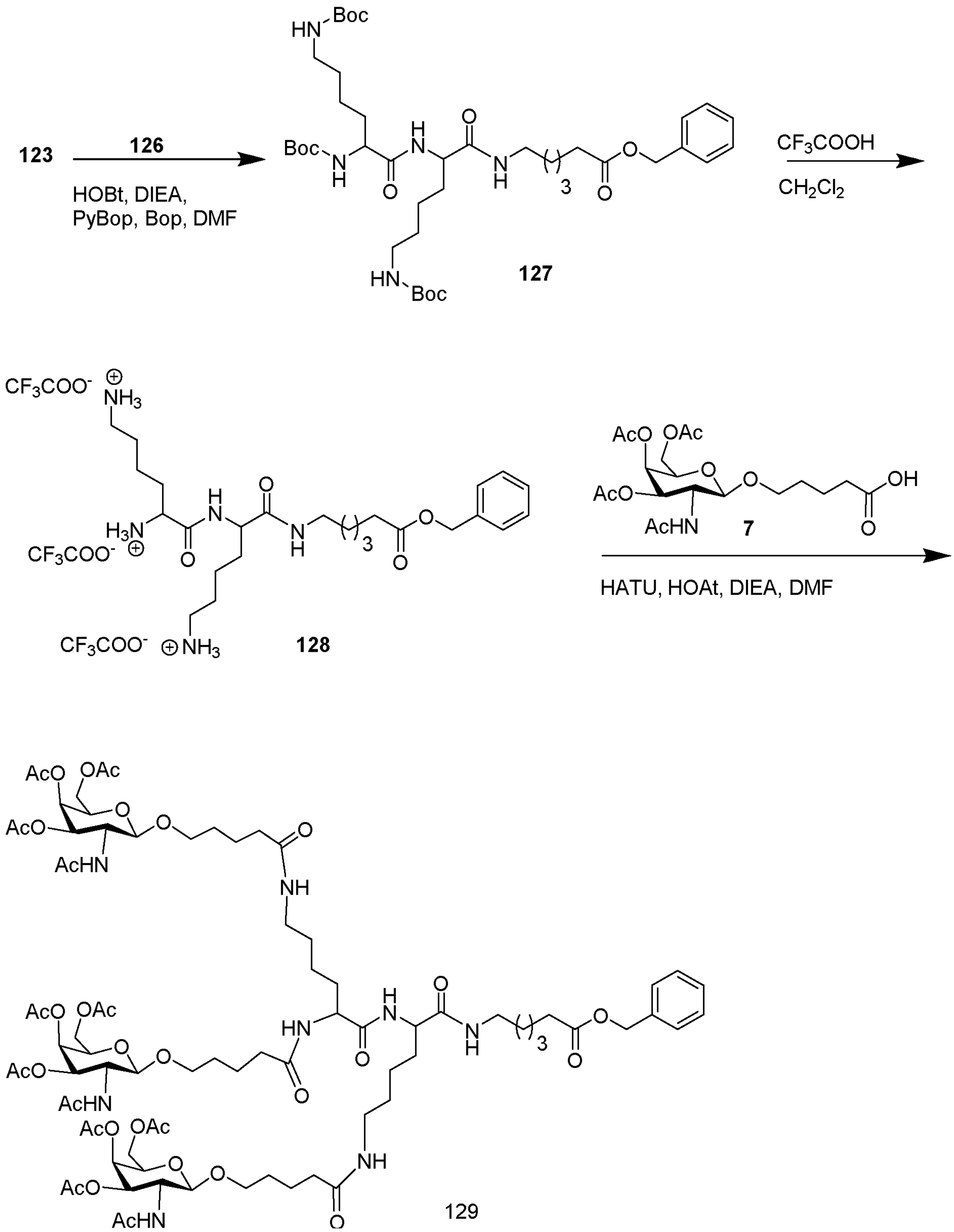
Compound 120 (14.01 g, 40 mmol) and HBTU (14.06 g, 37 mmol) were dissolved in anhydrous DMF (80 mL). Triethylamine (11.2 mL, 80.35 mmol) was added and stirred for 5 min. The reaction mixture was cooled in an ice bath and a solution of compound 121 (10 g, mmol) in anhydrous DMF (20 mL) was added. Additional 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 ^1H NMR analysis. Mass m/z 589.3 $[M + H]^+$.

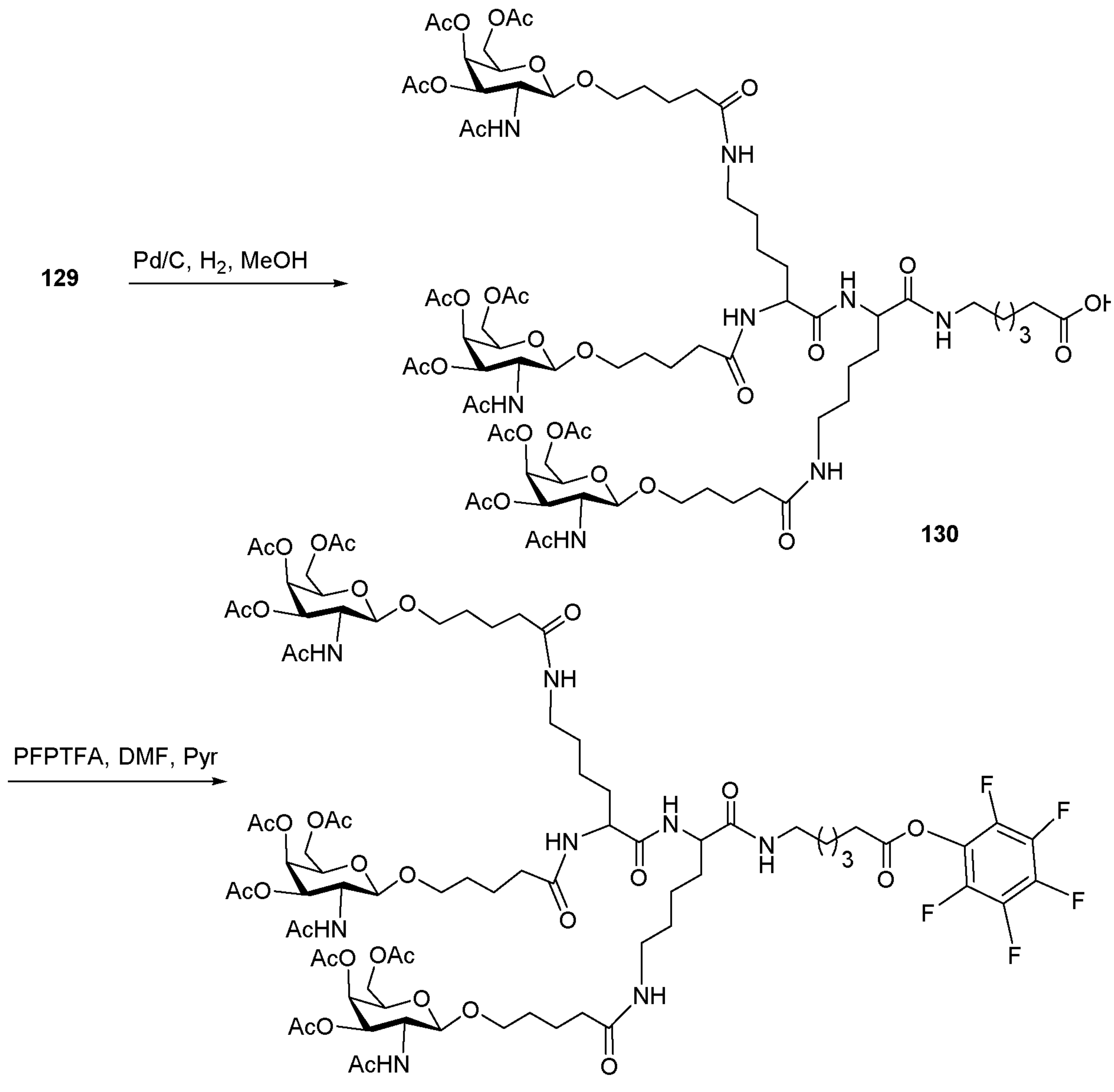
A solution of LiOH (92.15 mmol) in water (20 mL) and THF (10 mL) was added to a cooled solution of Compound 122 (7.75 g,13.16 mmol) dissolved in methanol (15 mL). The reaction mixture was stirred at room temperature for 45 min. and monitored by TLC (EtOAc:hexane; 1:1). The reaction mixture was concentrated 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).





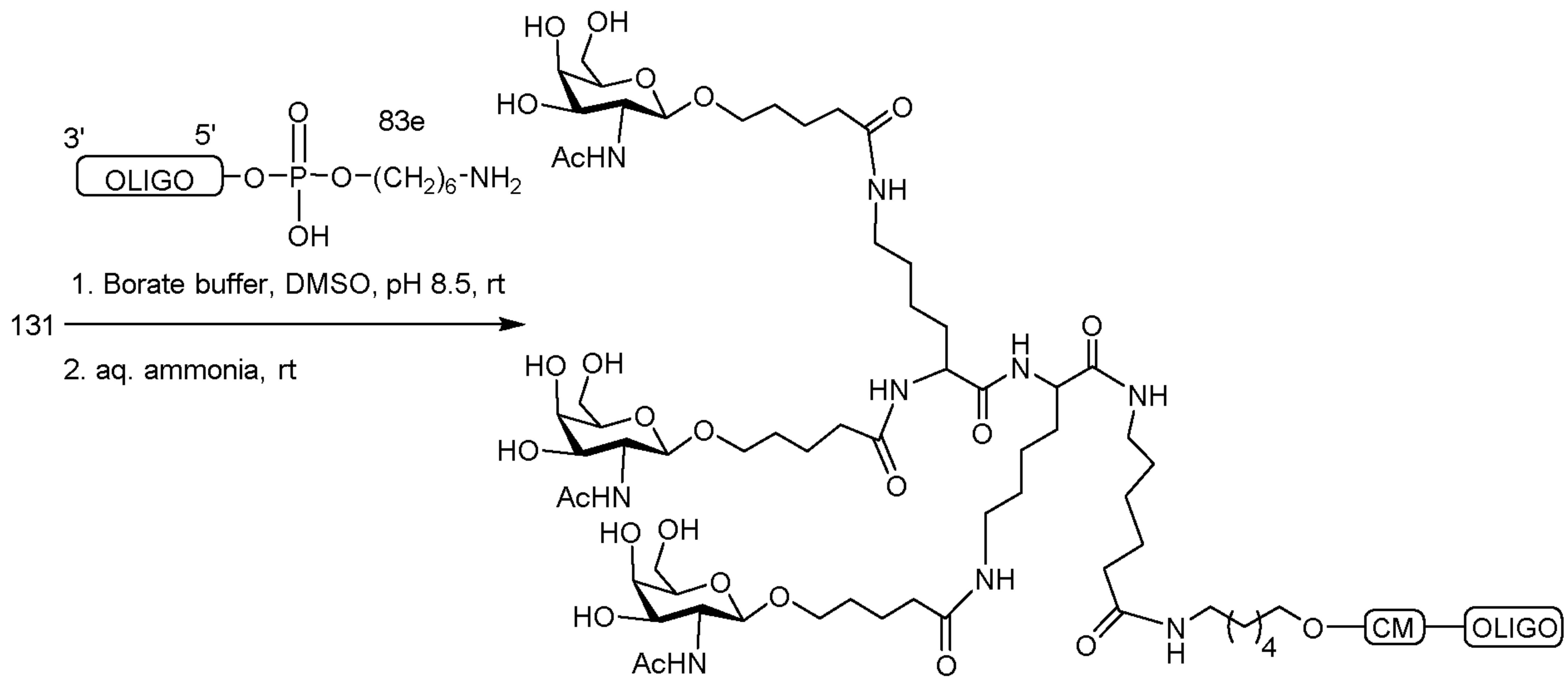
Compound 123 (7.419 g, 12.91 mmol), HOBt (3.49 g, 25.82 mmol) and compound 126 (6.33 g, 16.14 mmol) were dissolved in and DMF (40 mL) and the resulting reaction mixture was cooled in an ice bath. To this *N,N*-Diisopropylethylamine (4.42 mL, 25.82 mmol), PyBop (8.7 g, 16.7 mmol) followed by Bop coupling 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 ^{1}H 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 $[M + 2H]^+$.

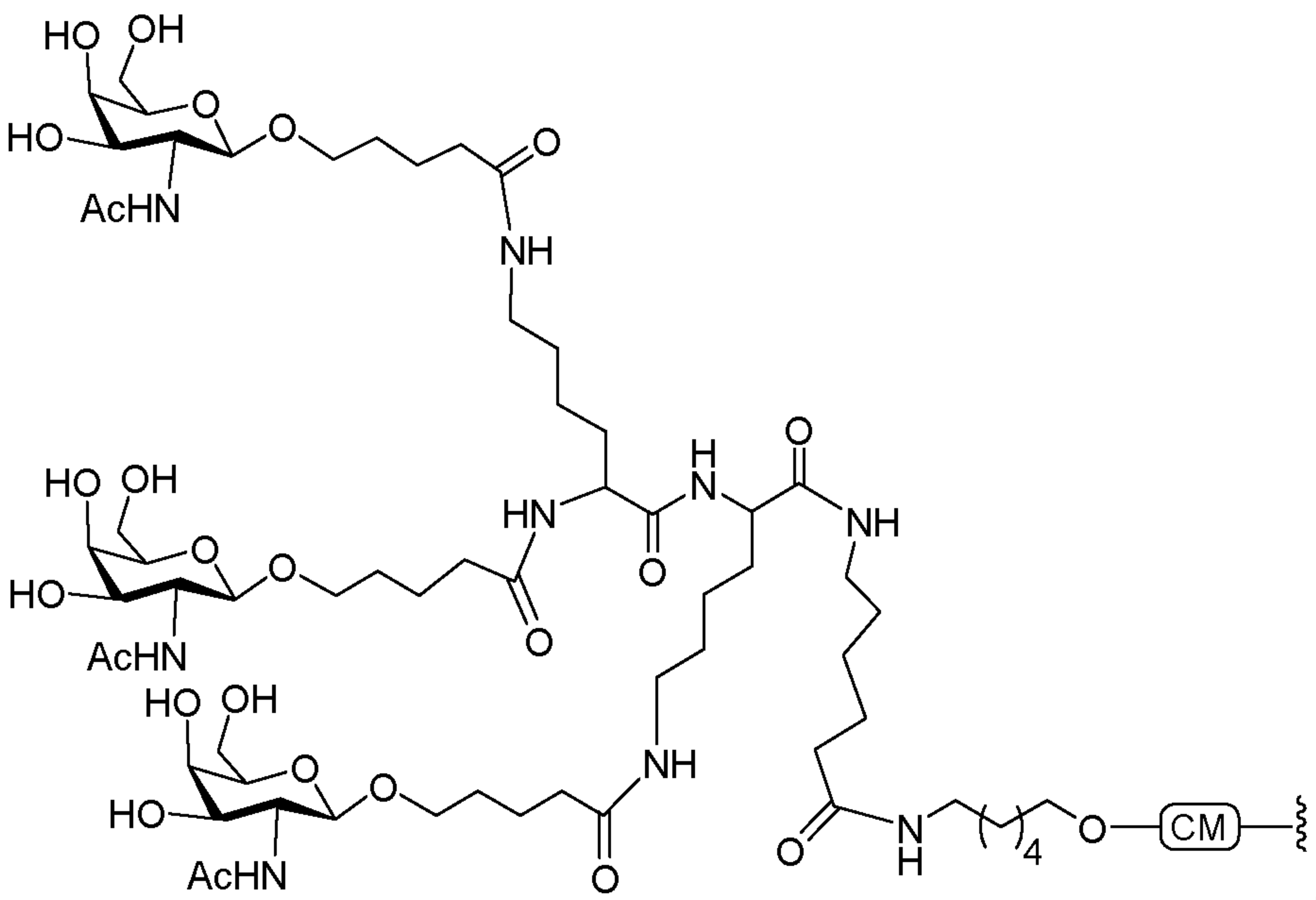
To a 10 mL pointed round bottom flask were added compound 130 (75.8 mg, 0.046 mmol), 0.37 M pyridine/DMF (200 µL) and a stir bar. To this solution was added 0.7 M pentafluorophenyl trifluoroacetate/DMF (100 µL) drop wise with stirring. The reaction was completed after 1 h as determined by LC MS. The 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 $[M + 2H]^+$.



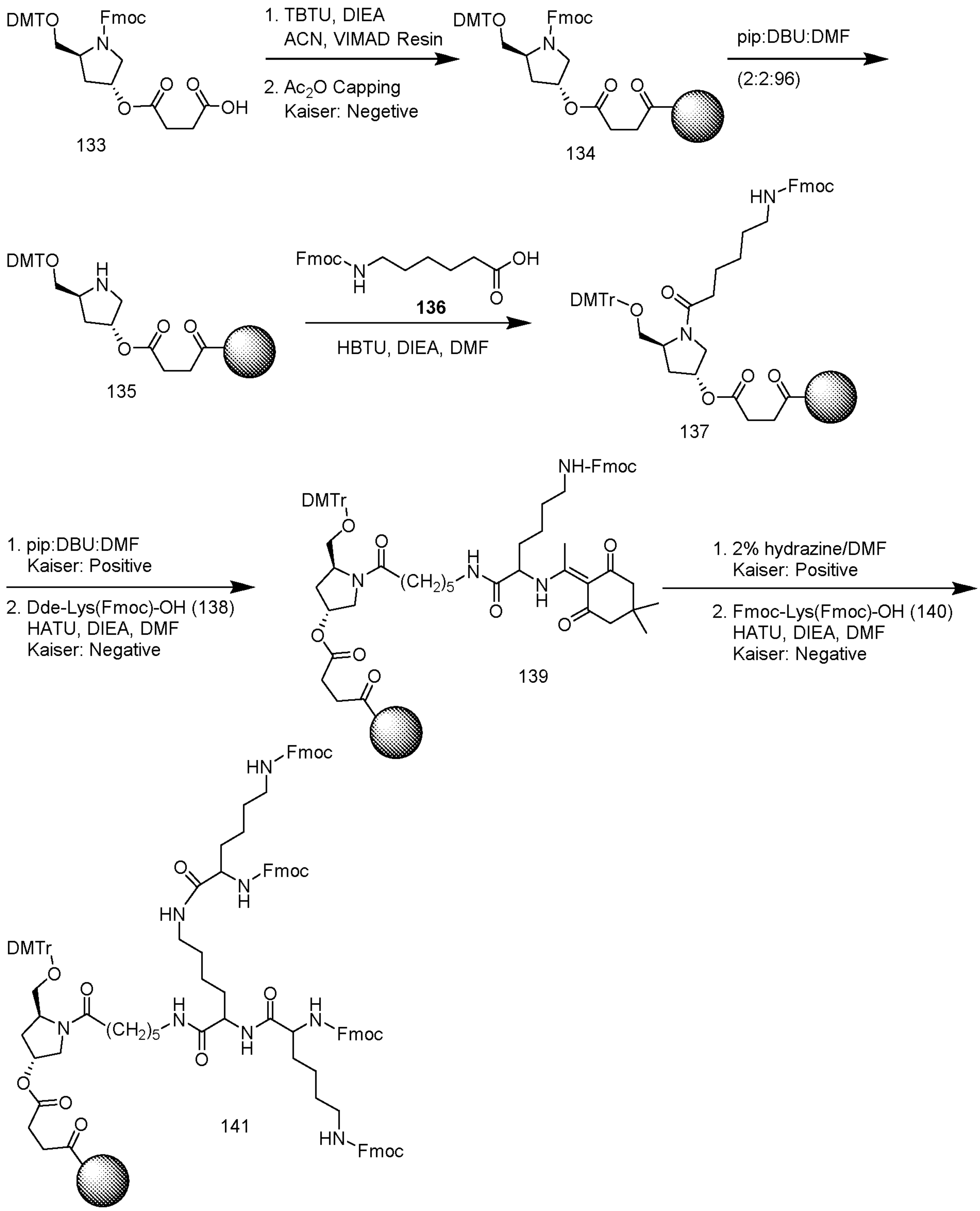
132

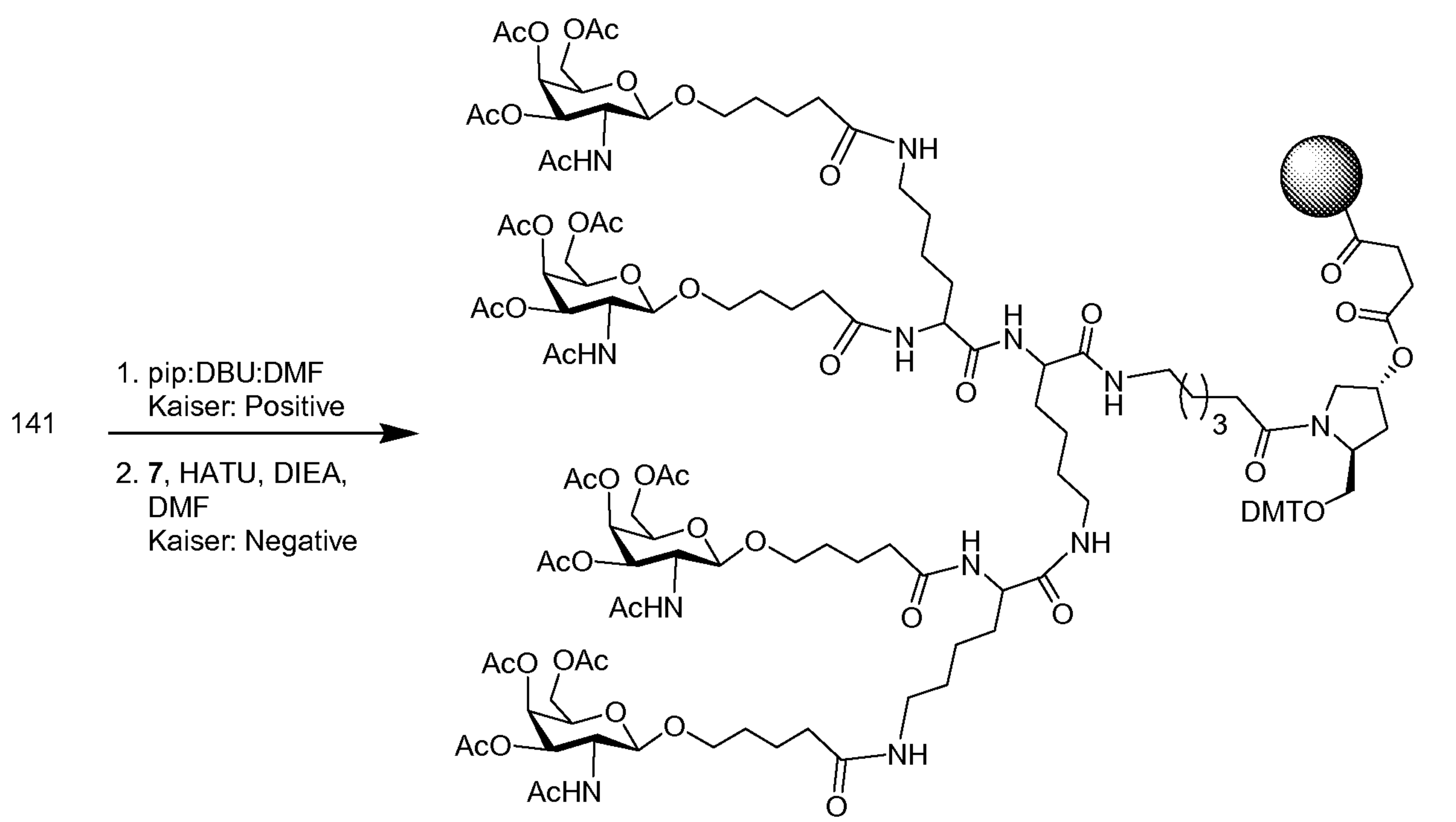
Oligomeric Compound 132, comprising a GalNAc$_3$-5 conjugate group, was prepared using the general procedures illustrated in Example 46. The GalNAc$_3$ cluster portion of the conjugate group GalNAc$_3$-5 (GalNAc$_3$-5$_a$) can be combined with any cleavable moiety to provide a variety of conjugate groups. 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



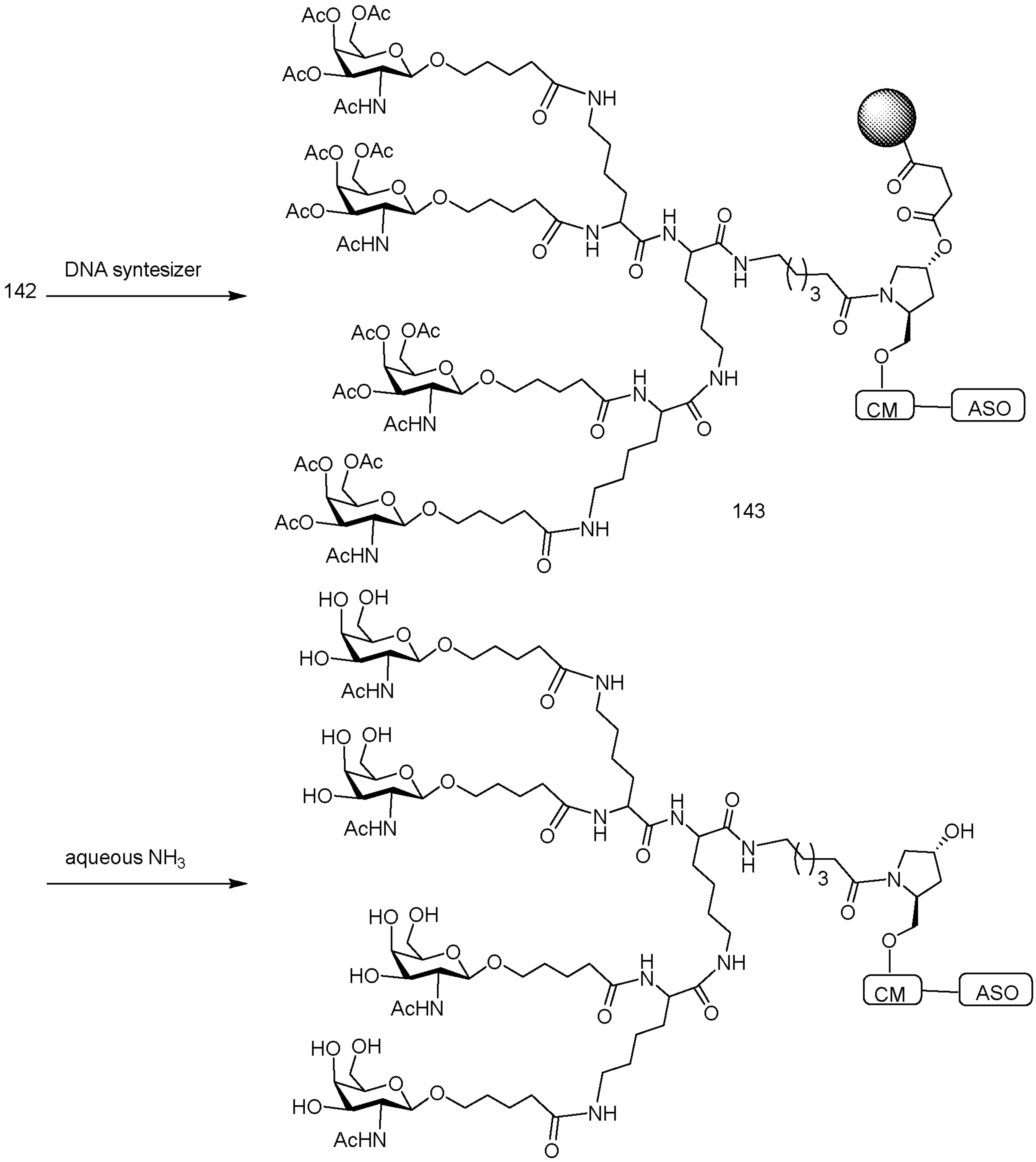


142

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.



143

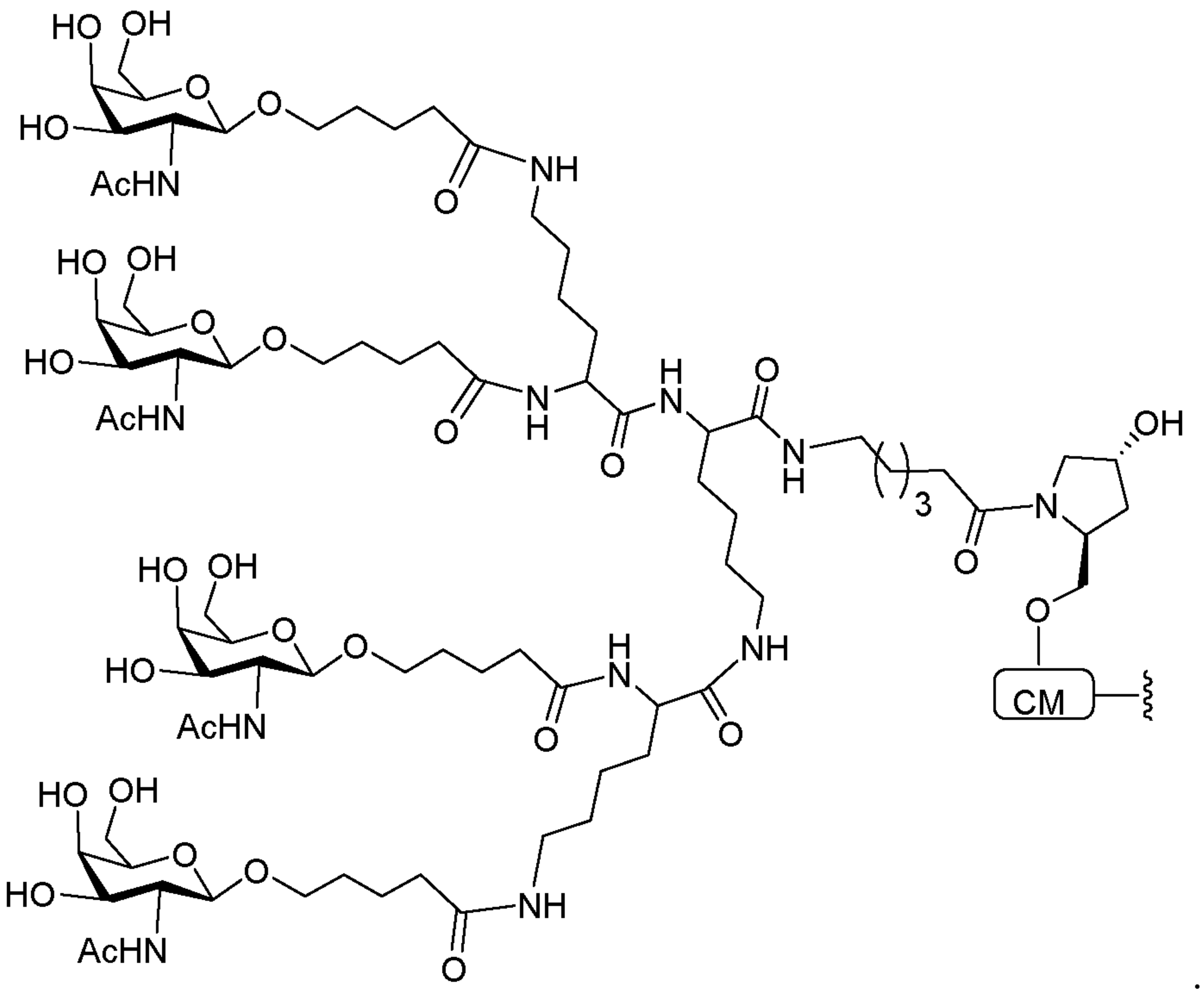
144

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

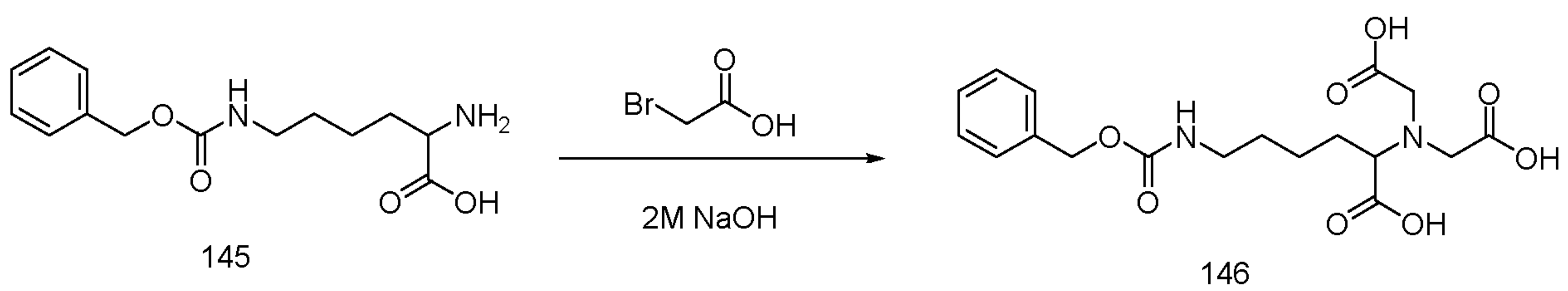
The solid support bound compound 143 was suspended in aqueous ammonia (28-30 wt%) and heated at 55 °C for 16 h. The solution was cooled and the solid support was filtered. The filtrate was concentrated and the residue dissolved in water and purified by HPLC on a strong anion exchange column. The fractions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

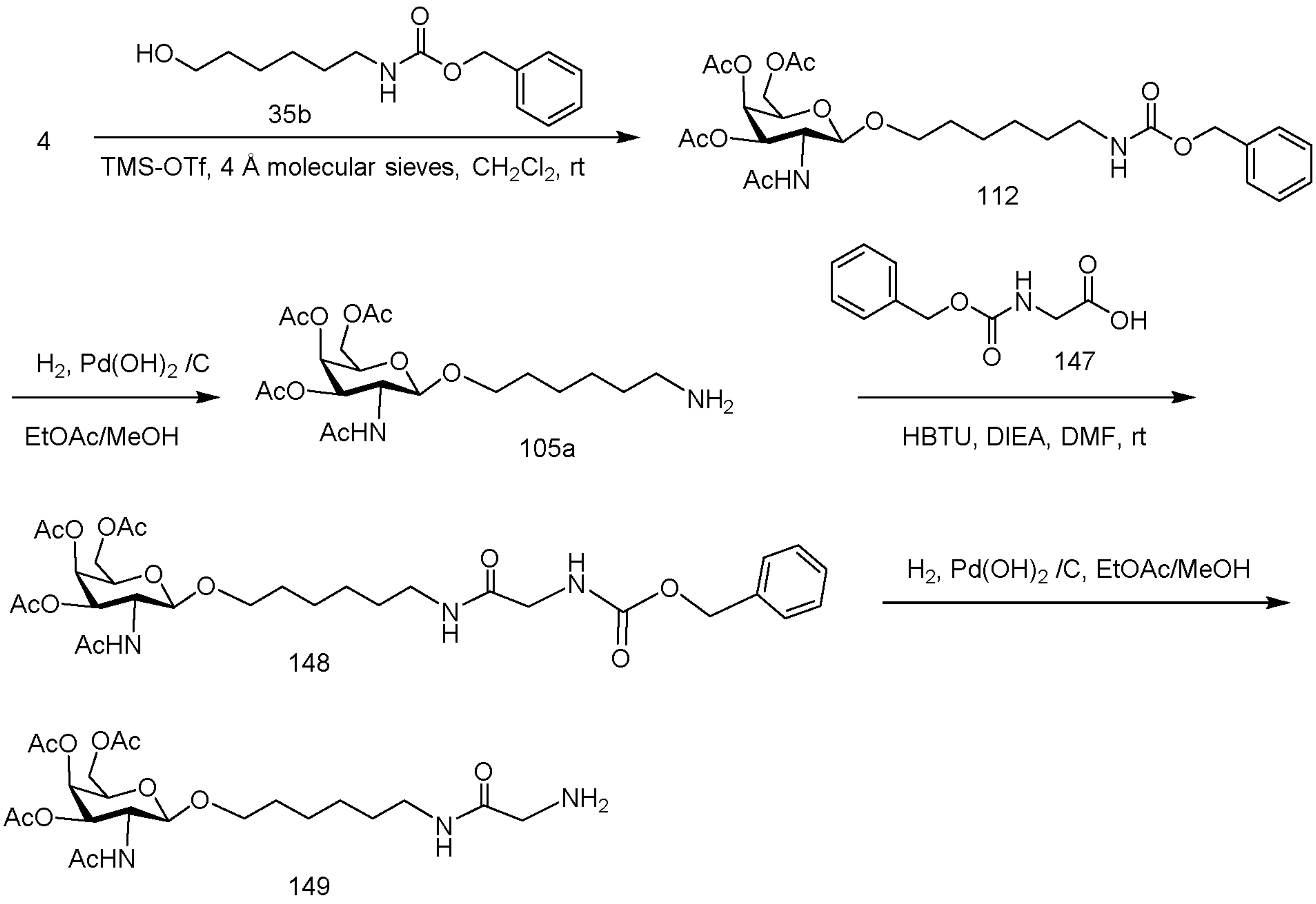
The structure of $GalNAc_4$-11 ($GalNAc_4$-11_a-CM) is shown below:



Example 51: Preparation of Oligonucleotide 155 Comprising $GalNAc_3$-6



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

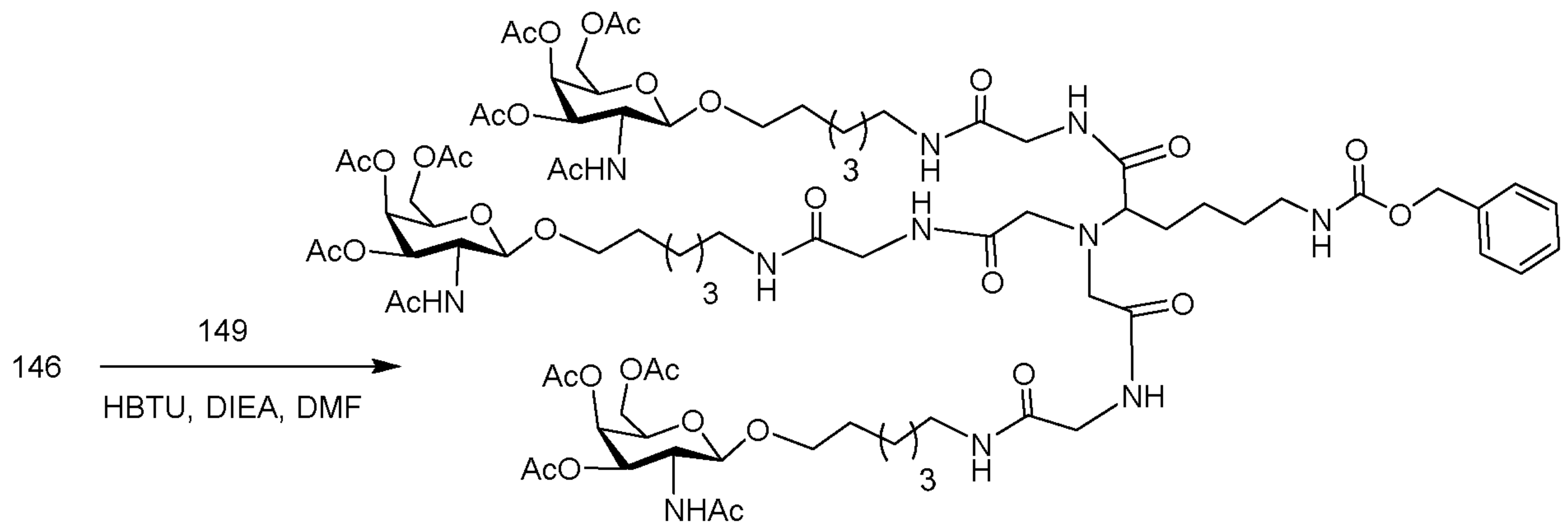


Compound 4 (15 g, 45.55 mmol) and compound 35b (14.3 grams, 57 mmol) were dissolved in CH_2Cl_2 (200 ml). Activated molecular sieves (4 Å. 2 g, powdered) were added, and the reaction was allowed to stir for 30 minutes under nitrogen atmosphere. TMS-OTf was added (4.1 ml, 22.77 mmol) and the reaction 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.

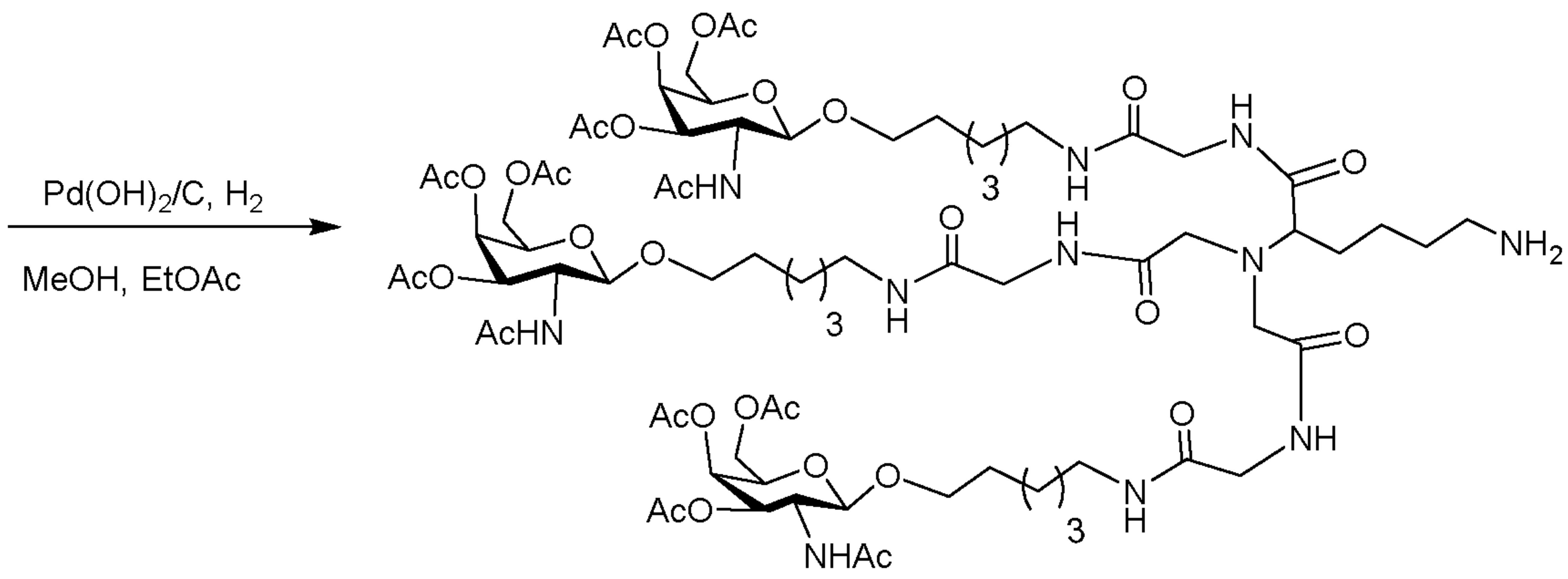
Compound 112 (4.27 g, 7.35 mmol) was dissolved in 1:1 MeOH/EtOAc (40 ml). The reaction mixture was purged by bubbling a stream of argon through the solution for 15 minutes. Pearlman's catalyst (palladium hydroxide on carbon, 400 mg) was added, and hydrogen gas was bubbled through the solution for 30 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 ^{1}H 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.



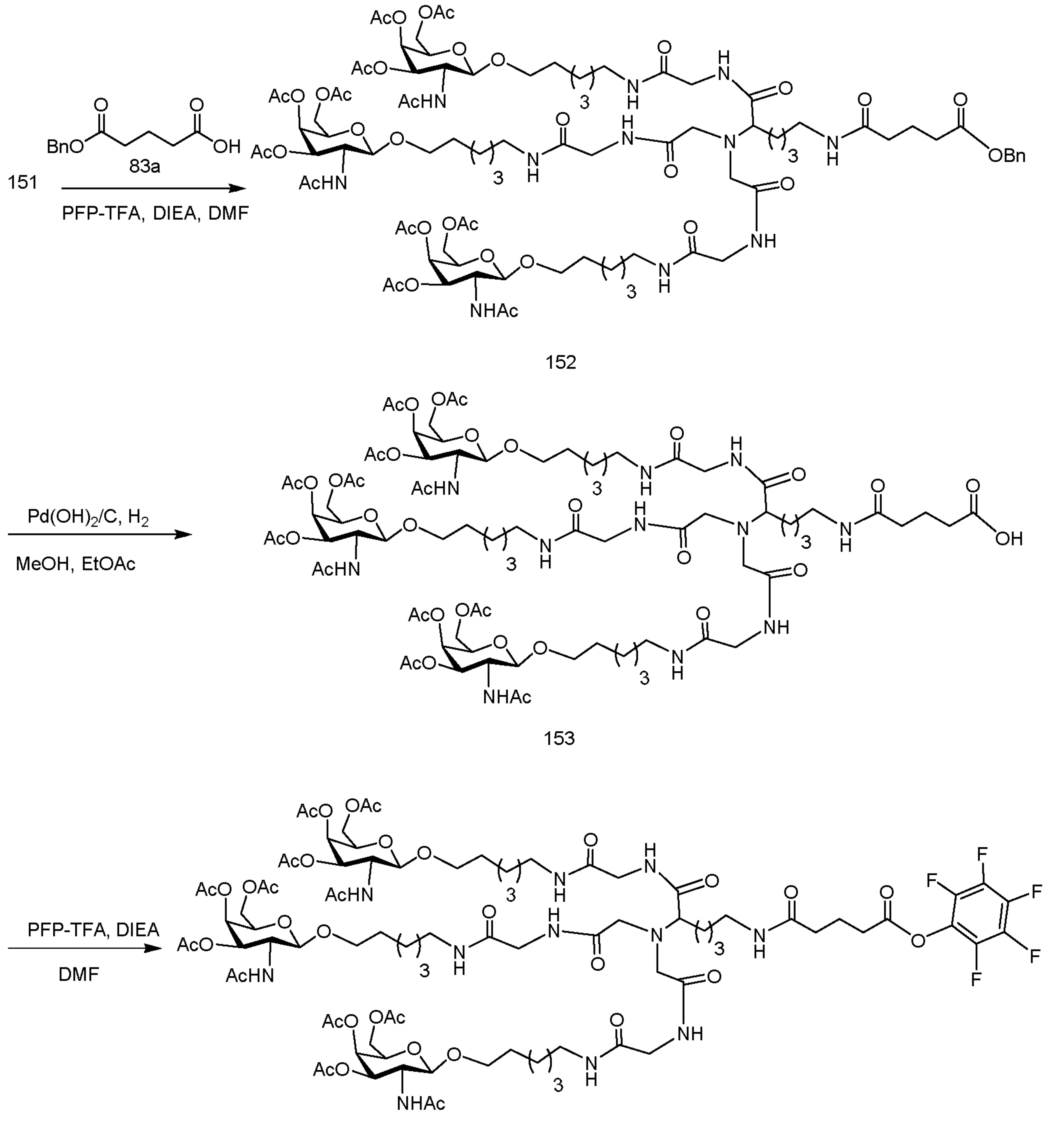
150



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.



152

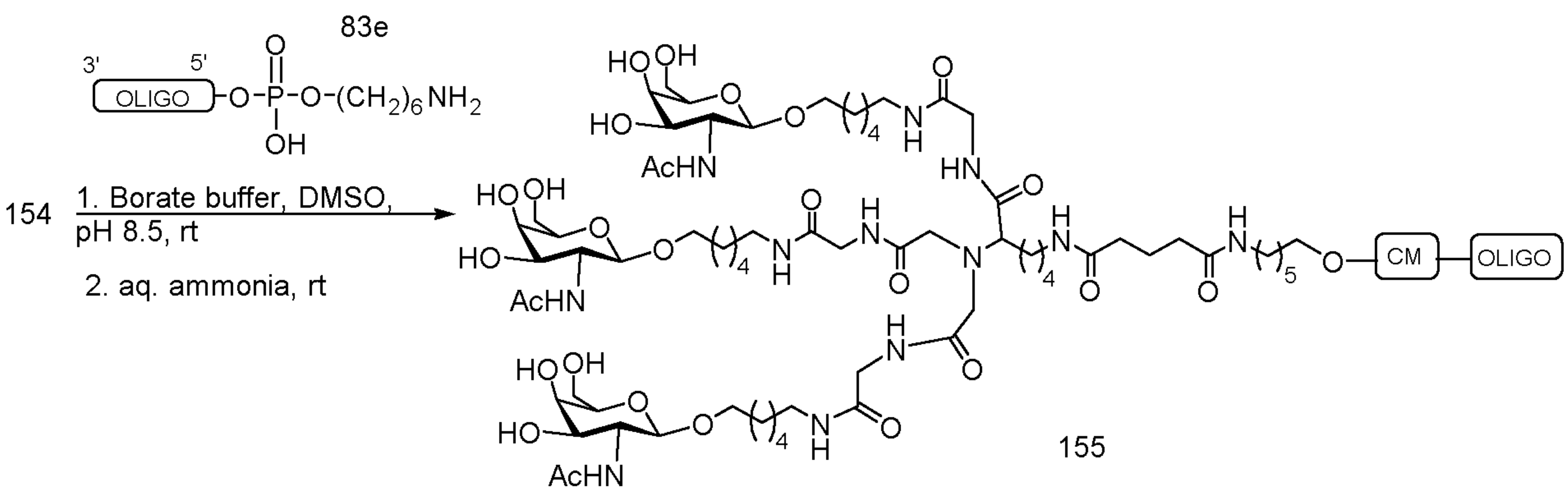
153

154

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 [1]H NMR were consistent with the desired product.

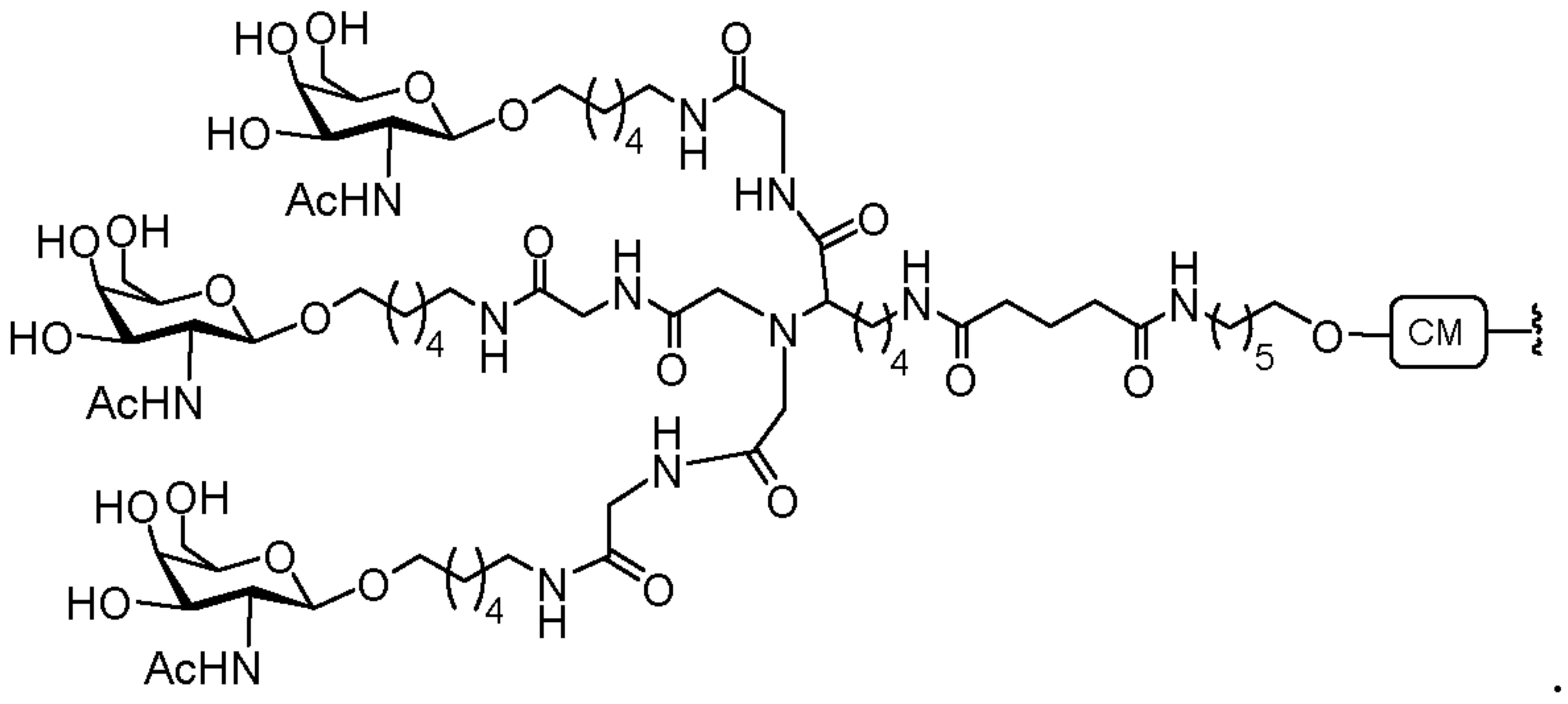
Compound 152 (0.35 g, 0.182 mmol) was dissolved in 1:1 MeOH/EtOAc (10 mL). The reaction mixture was purged by bubbling a stream of argon thru the solution for 15 minutes. Pearlman's catalyst (palladium hydroxide on carbon) was added (35 mg). Hydrogen gas was bubbled thru the solution for 30 minutes. 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 [1]H NMR were consistent with the desired product.

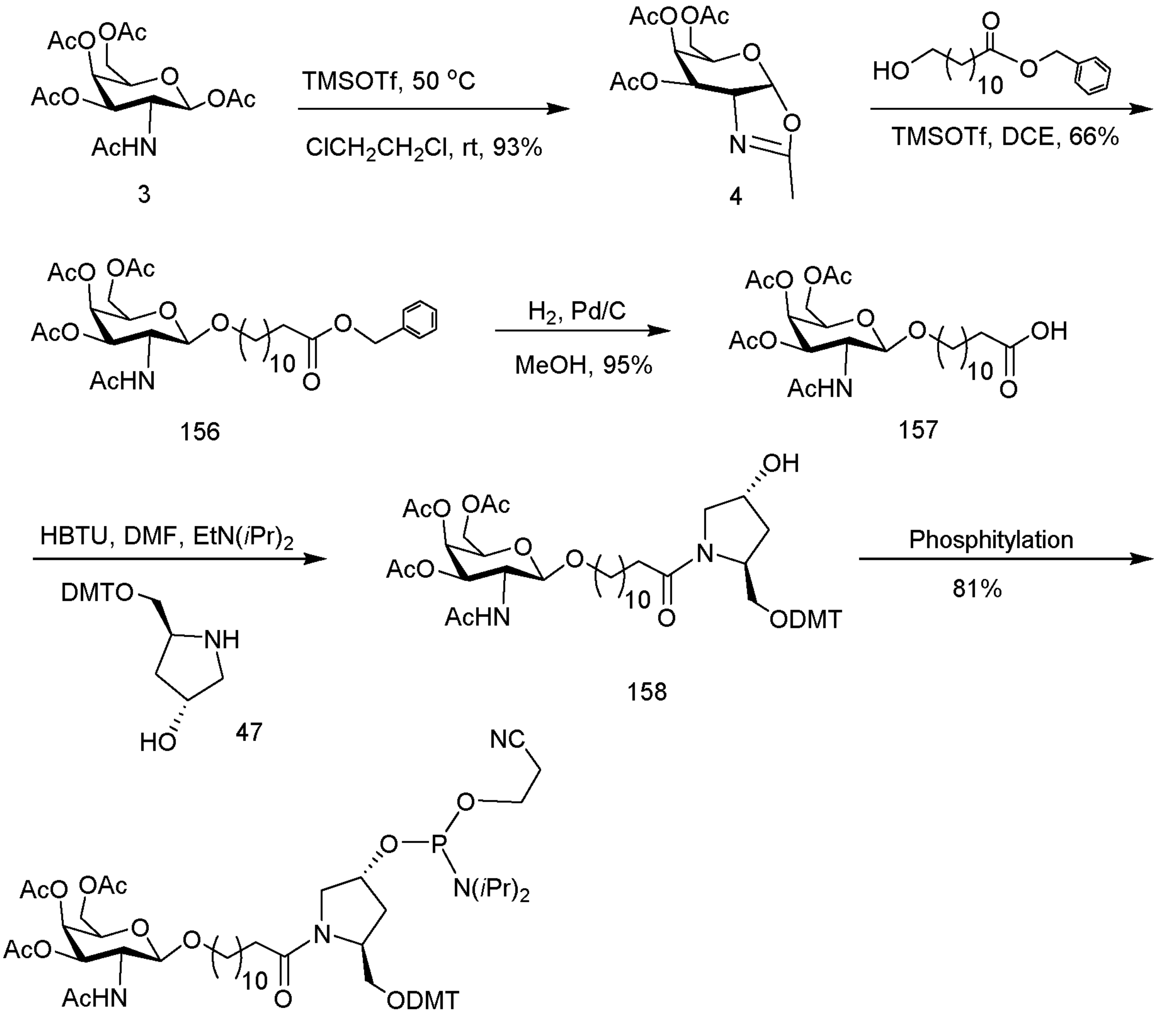


Oligomeric Compound 155, comprising a GalNAc$_3$-6 conjugate group, was prepared using the general procedures illustrated in Example 46. The GalNAc$_3$ cluster portion of the conjugate group GalNAc$_3$-6 (GalNAc$_3$-6$_a$) can be combined with any cleavable moiety to provide a variety of conjugate groups. 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



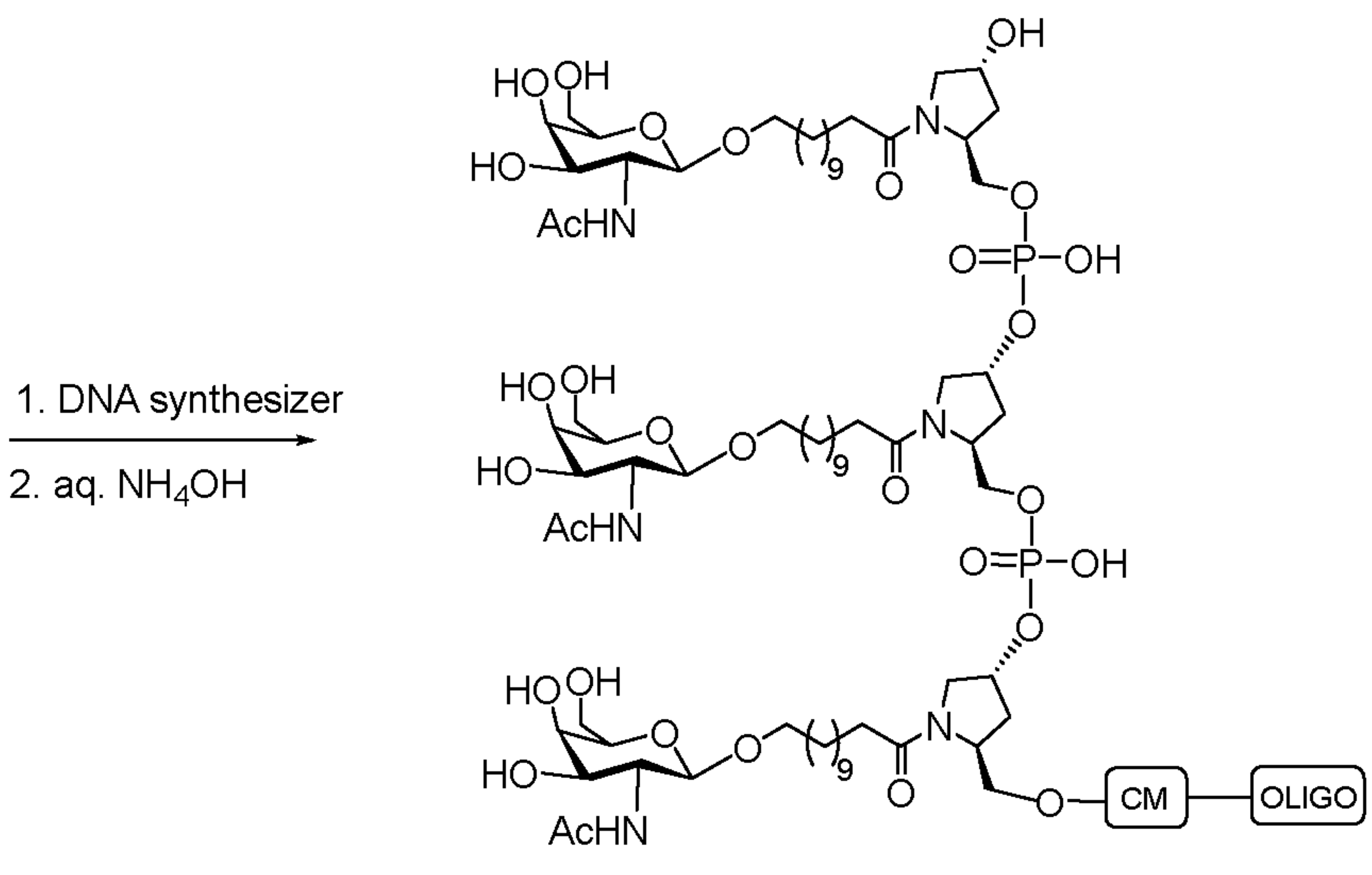
Compound 156 was synthesized following the procedure described in the literature (*J. Med. Chem.* 2004, *47*, 5798-5808).

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 ^1H NMR analysis.

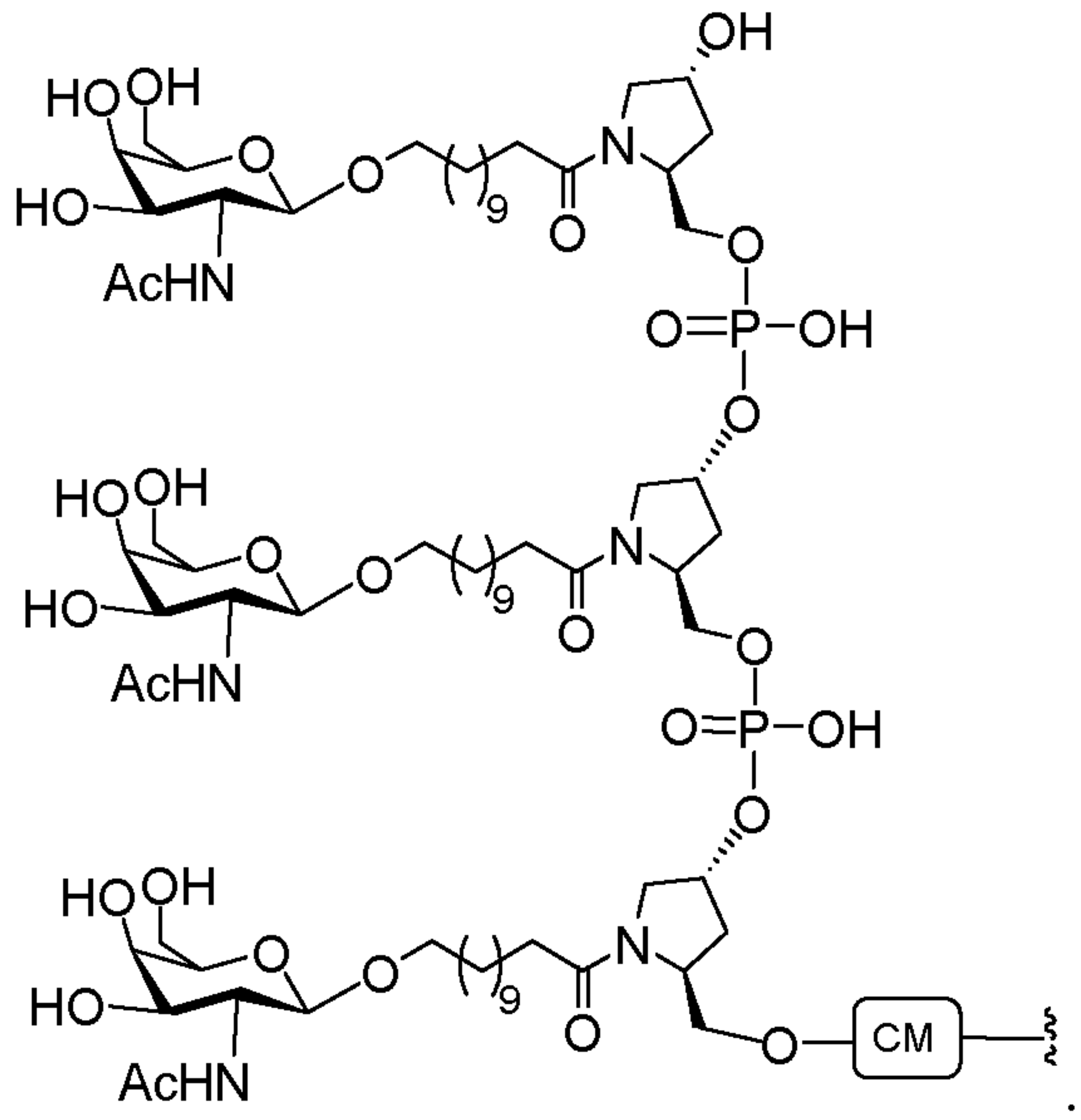
Compound 158 (7.2 g, 7.61 mmol) was dried over P_2O_5 under reduced pressure. The dried compound was dissolved in anhydrous DMF (50 mL). To this 1H-tetrazole (0.43 g, 6.09 mmol) and *N*-methylimidazole (0.3 mL, 3.81 mmol) and 2-cyanoethyl-*N,N,N',N'*-tetraisopropyl phosphorodiamidite (3.65 mL, 11.50 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.

159



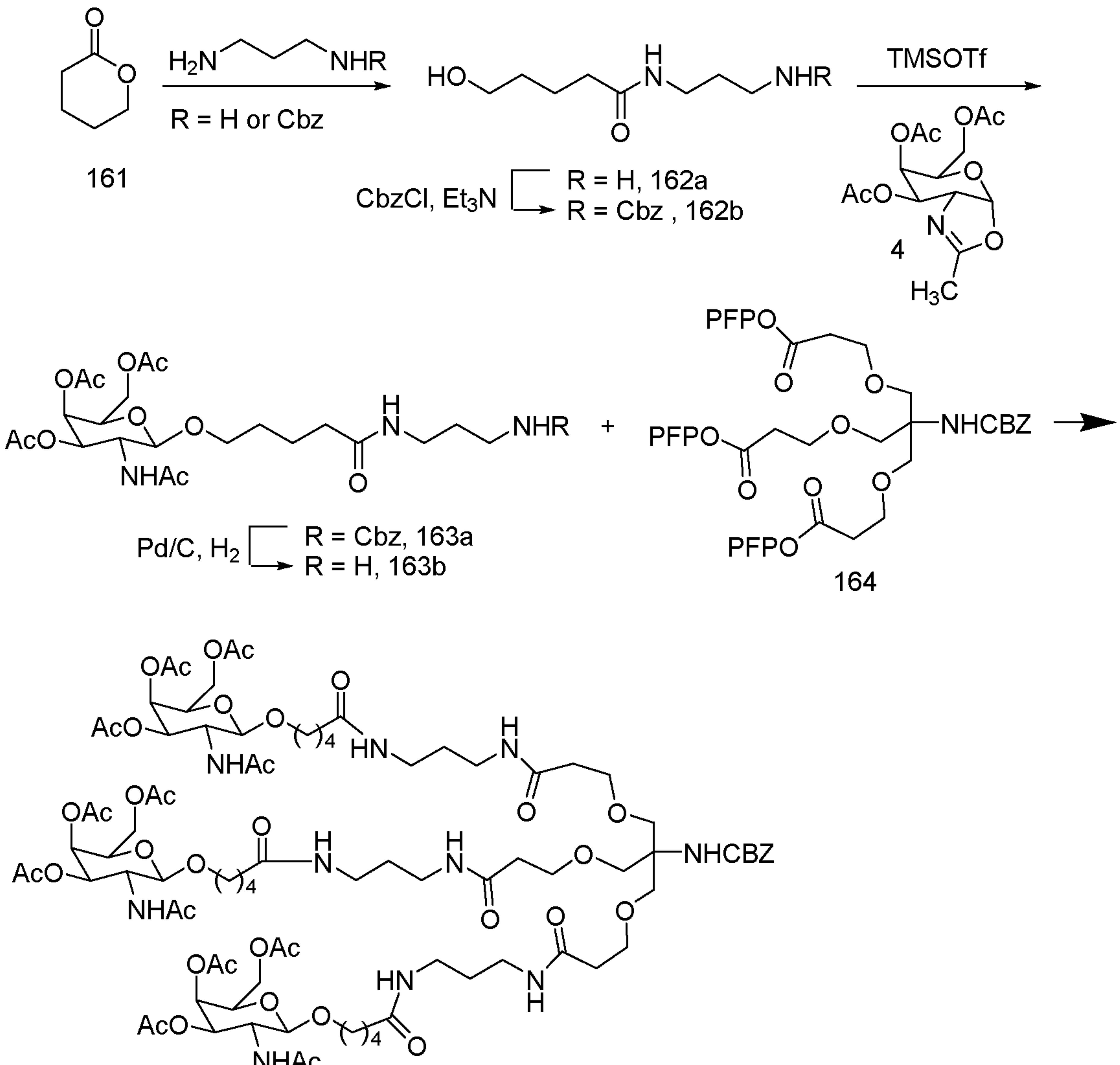
160

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.



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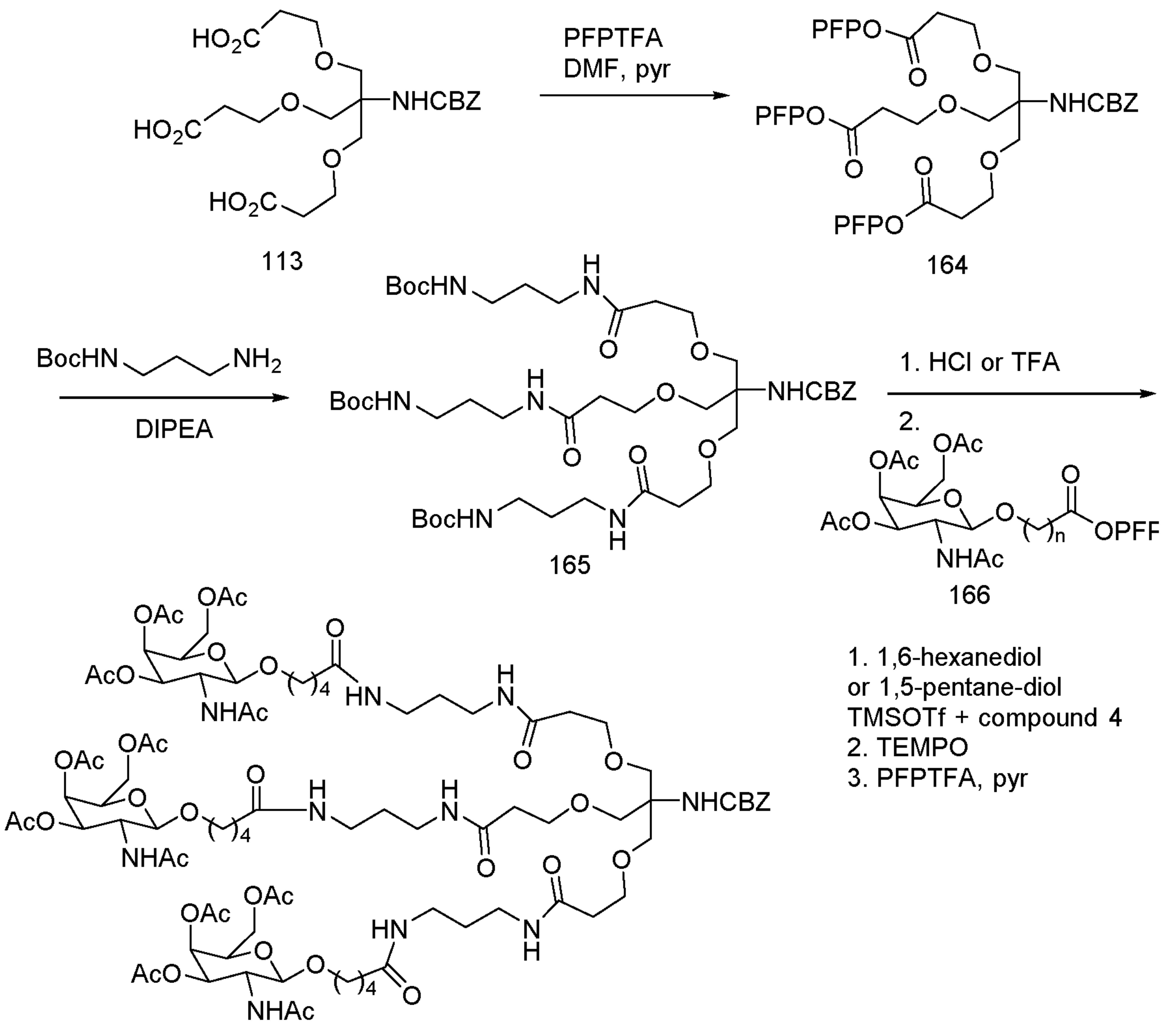
Example 54: Alternate procedure for preparation of Compound 18 (GalNAc$_3$-1a and GalNAc$_3$-3a)

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.



18

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	2304

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**	2305
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**	2305
ISIS 661161	**GalNAc$_3$-3$_a$-$_{o'}$A$_{do}$** $G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}$ $^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-3**	2304
ISIS 665001	**GalNAc$_3$-8$_a$-$_{o'}$A$_{do}$** $G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}$ $^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-8**	2304

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	2304
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**	2305
ISIS 664507	**GalNAc$_3$-2$_a$-$_{o'}$A$_{do}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-2**	2306
ISIS 661161	**GalNAc$_3$-3$_a$-$_{o'}$A$_{do}$** $G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-3**	2304
ISIS 666224	**GalNAc$_3$-5$_a$-$_{o'}$A$_{do}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-5**	2306
ISIS 666961	**GalNAc$_3$-6$_a$-$_{o'}$A$_{do}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-6**	2306
ISIS 666981	**GalNAc$_3$-7$_a$-$_{o'}$A$_{do}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-7**	2306
ISIS 666881	**GalNAc$_3$-10$_a$-$_{o'}$A$_{do}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-10**	2306

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	2296
ISIS 647535	$A_{es}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{es}T_{es}T_{es}A_{es}T_{eo}\mathbf{A_{do'}\text{-}GalNAc_3\text{-}1_a}$	PS	2297
ISIS 647536	$A_{es}G_{eo}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{eo}T_{eo}T_{es}A_{es}T_{eo}\mathbf{A_{do'}\text{-}GalNAc_3\text{-}1_a}$	PO/PS	2297

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 $GalNAc_3\text{-}1_a$ 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	2298
		2	59.66	
		6	23.50	
ISIS 651900	$T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{ko}\mathbf{A_{do'}\text{-}GalNAc_3\text{-}1_a}$	0.2	62.75	2299
		0.6	29.14	
		2	8.61	
		6	5.62	

ISIS 663748	$T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{ko}\mathbf{A_{do'}\text{-}GalNAc_4\text{-}11_a}$	0.2	63.99	2299
		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	2307

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	2308
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	2308

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** (3')
	2	33.0	51.8	2.9	0.2	0.2	22.0	
	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	2304
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**	2305
ISIS 655862	$G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T_{eo}\mathbf{A_{do'}\text{-}GalNAc_{3}\text{-}1_{a}}$	5/10/5	**GalNAc$_3$-1**	2305
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**	2306
ISIS 665001	$\mathbf{GalNAc_{3}\text{-}8_{a\text{-}o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	5/10/5	**GalNAc$_3$-8**	2306
ISIS 664078	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{eo}\mathbf{A_{do'}\text{-}GalNAc_{3}\text{-}9_{a}}$	5/10/5	**GalNAc$_3$-9**	2305
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**	2306
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**	2306
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**	2306
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**	2306
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**	2306

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®.

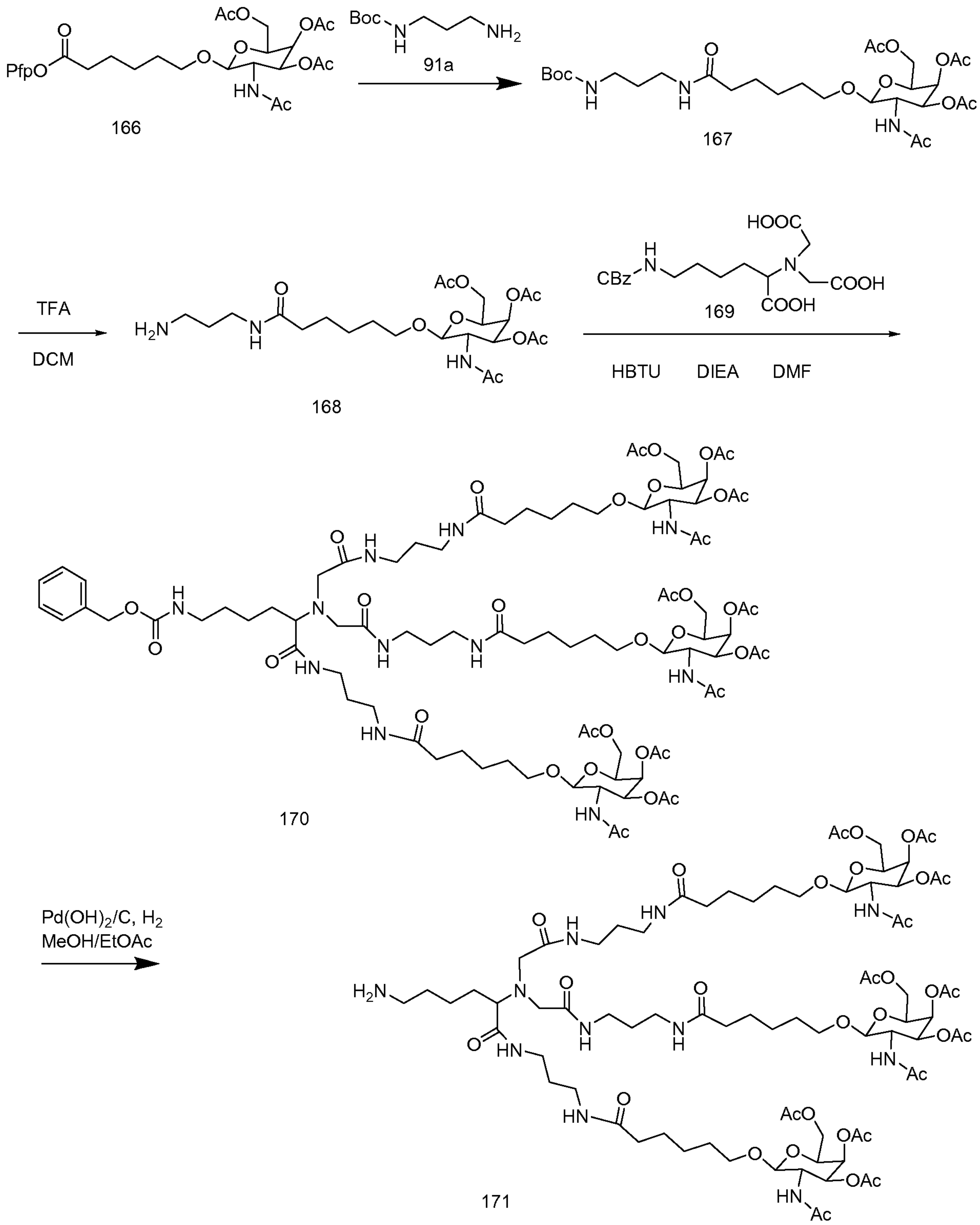
The IC_{50} was calculated using standard methods and the results are presented in Table 53. The results show that, under free uptake conditions in which no reagents or electroporation techniques are used to artificially promote entry of the oligonucleotides into cells, the oligonucleotides comprising a GalNAc conjugate were significantly more potent in hepatocytes than the parent oligonucleotide (ISIS 353382) that does not comprise a GalNAc conjugate.

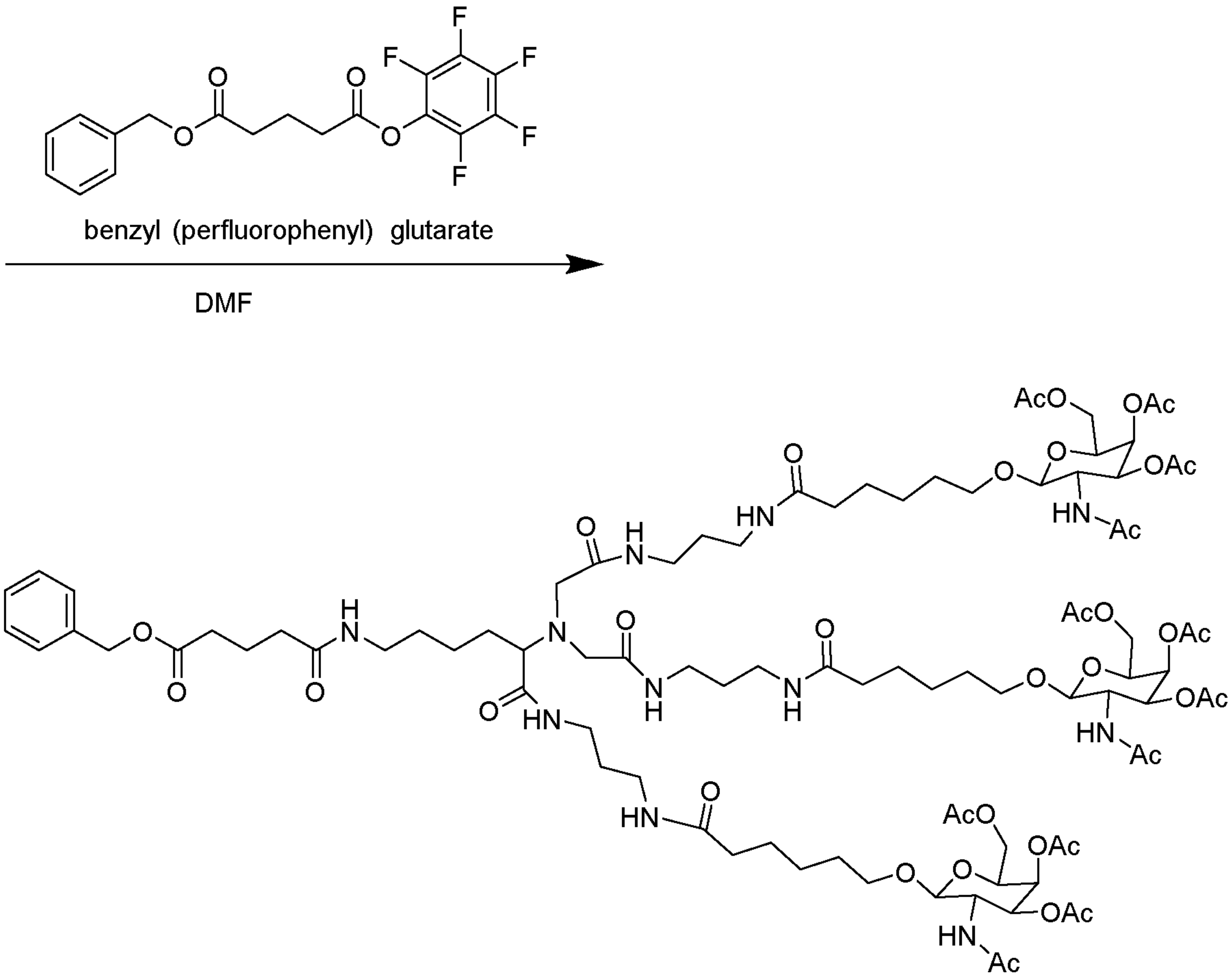
Table 53

ASO	IC_{50} (nM)	Internucleoside linkages	Conjugate	SEQ ID No.
ISIS 353382	190[a]	PS	none	2304
ISIS 655861	11[a]	PS	**GalNAc$_3$-1**	2305
ISIS 655862	3	PO/PS	**GalNAc$_3$-1**	2305
ISIS 661161	15[a]	PS	**GalNAc$_3$-3**	2306
ISIS 665001	20	PS	**GalNAc$_3$-8**	2306
ISIS 664078	55	PS	**GalNAc$_3$-9**	2305
ISIS 666961	22[a]	PS	**GalNAc$_3$-6**	2306
ISIS 664507	30	PS	**GalNAc$_3$-2**	2306
ISIS 666881	30	PS	**GalNAc$_3$-10**	2306
ISIS 666224	30[a]	PS	**GalNAc$_3$-5**	2306
ISIS 666981	40	PS	**GalNAc$_3$-7**	2306

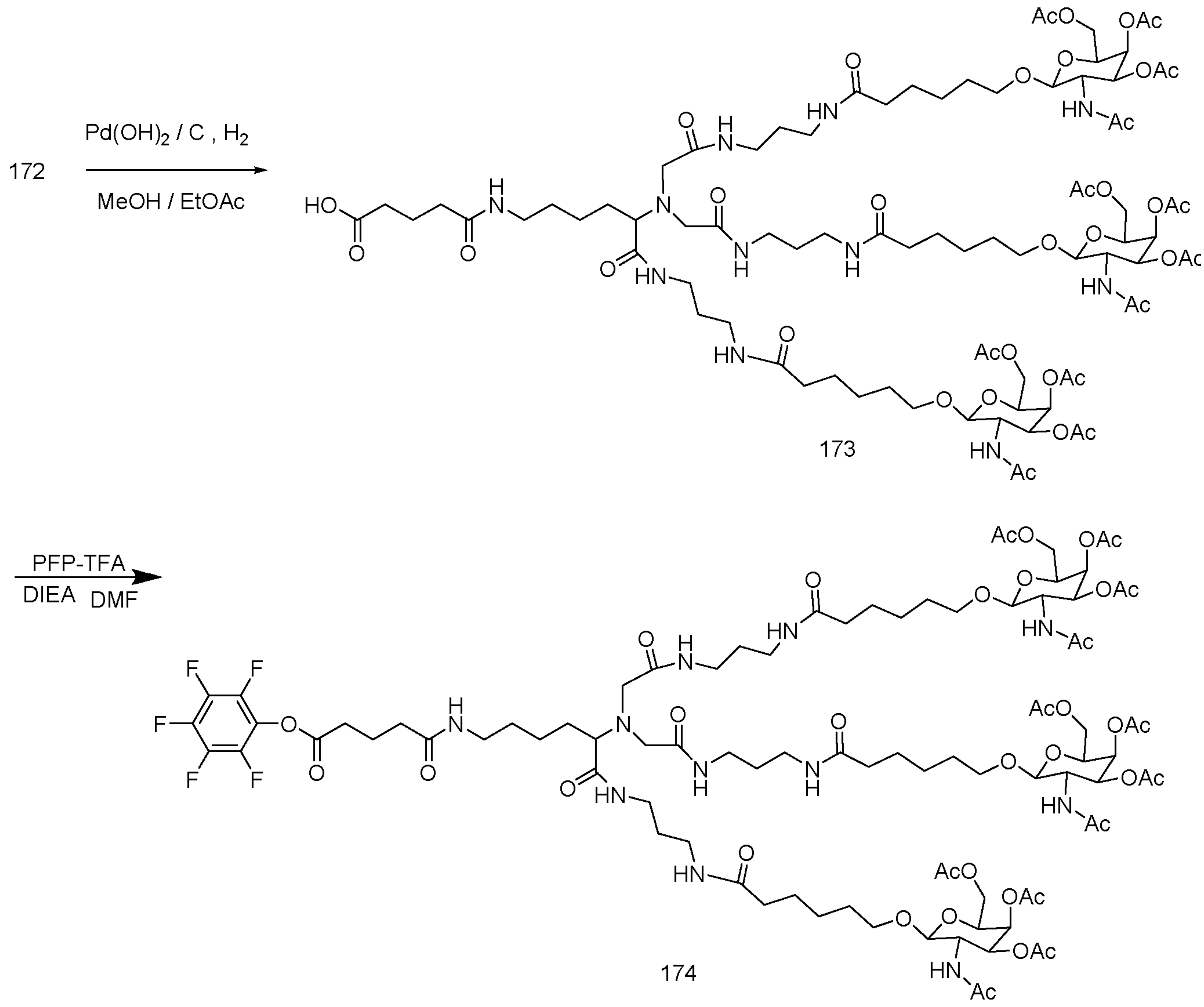
[a]Average of multiple runs.

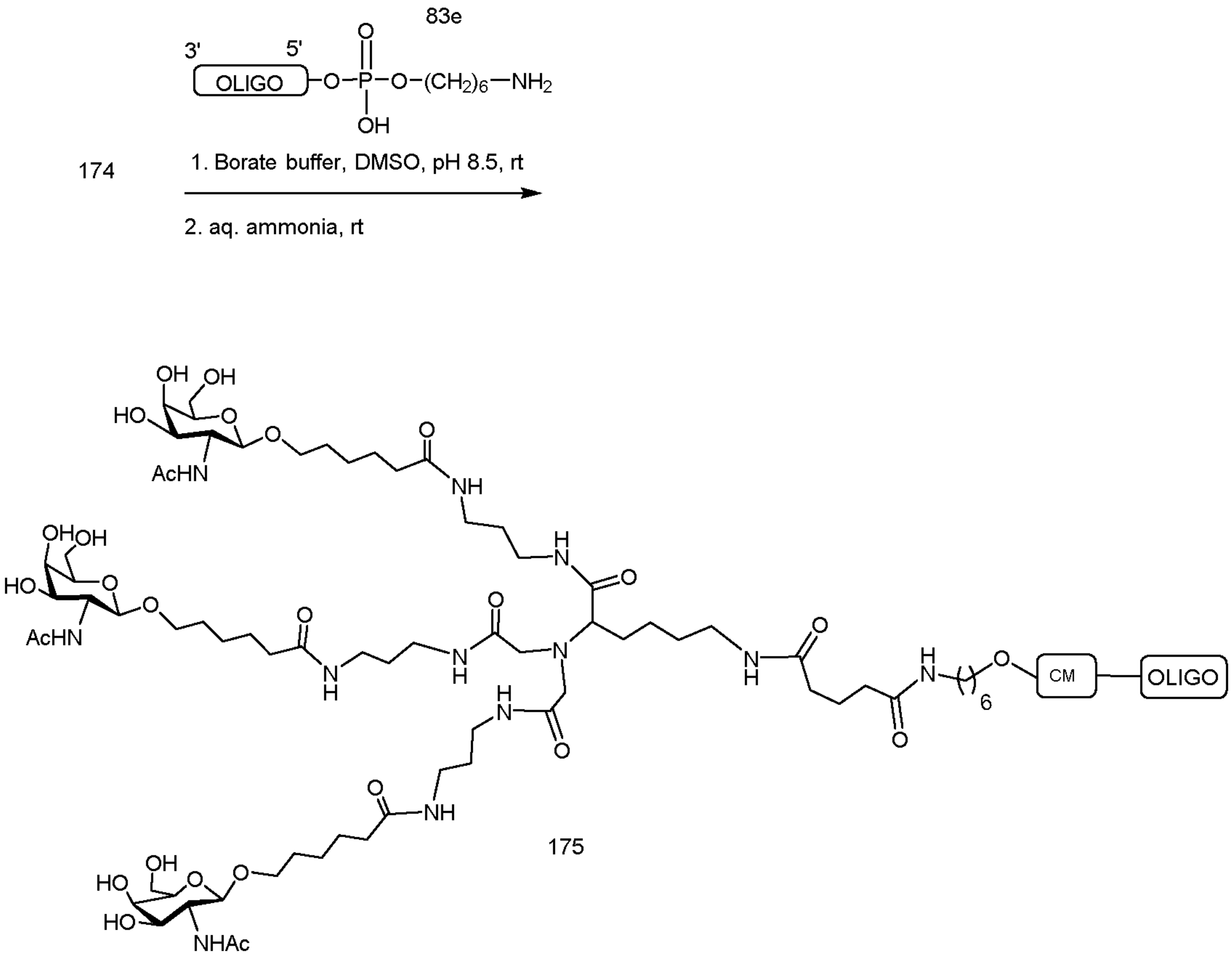
Example 61: Preparation of oligomeric compound 175 comprising GalNAc$_3$-12


166
91a
167
TFA
DCM
168
169
HBTU DIEA DMF
170
$Pd(OH)_2/C$, H_2
MeOH/EtOAc
171



172

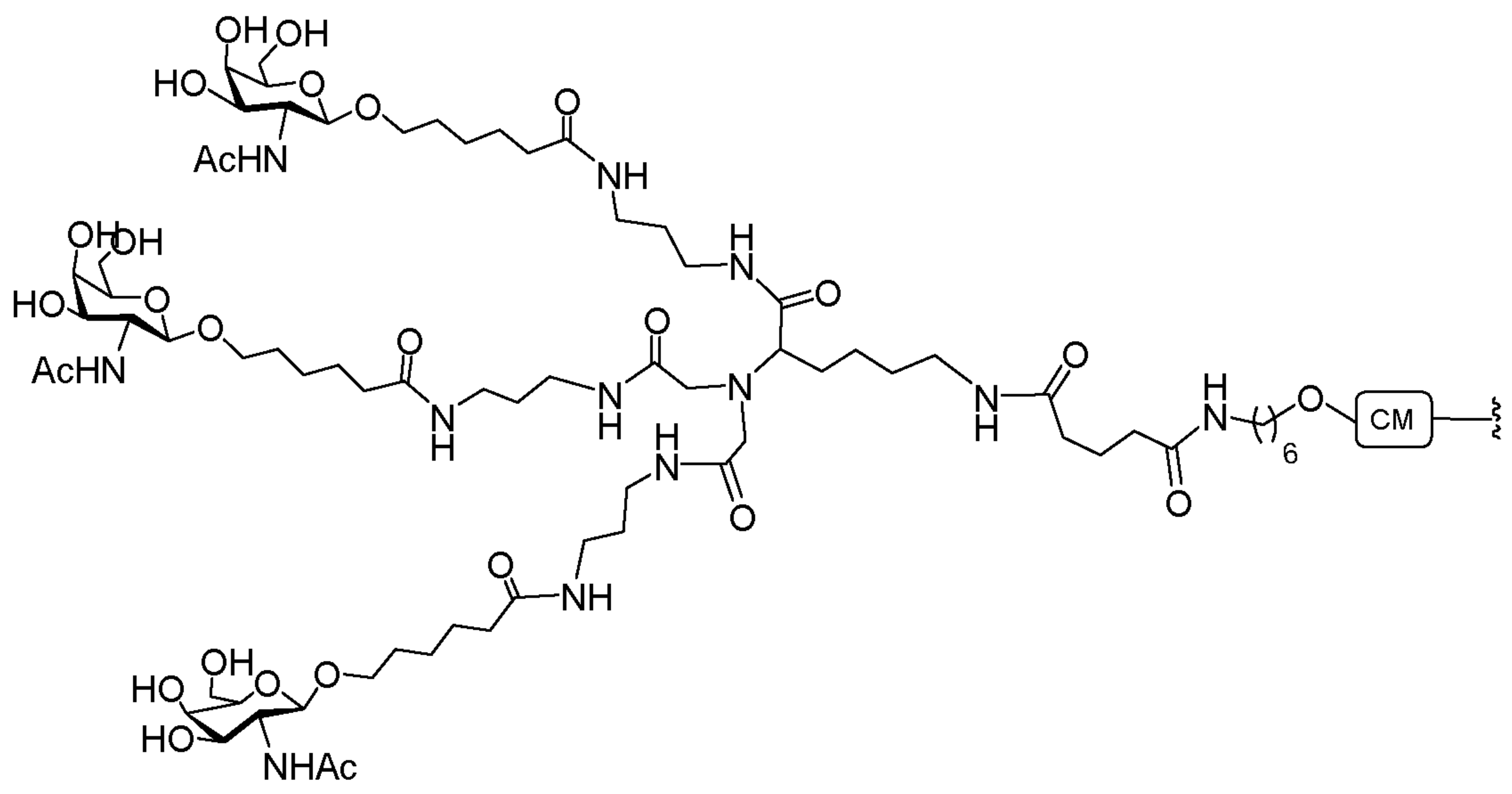

172
Pd(OH)2 / C , H2
MeOH / EtOAc
173
PFP-TFA
DIEA DMF
174



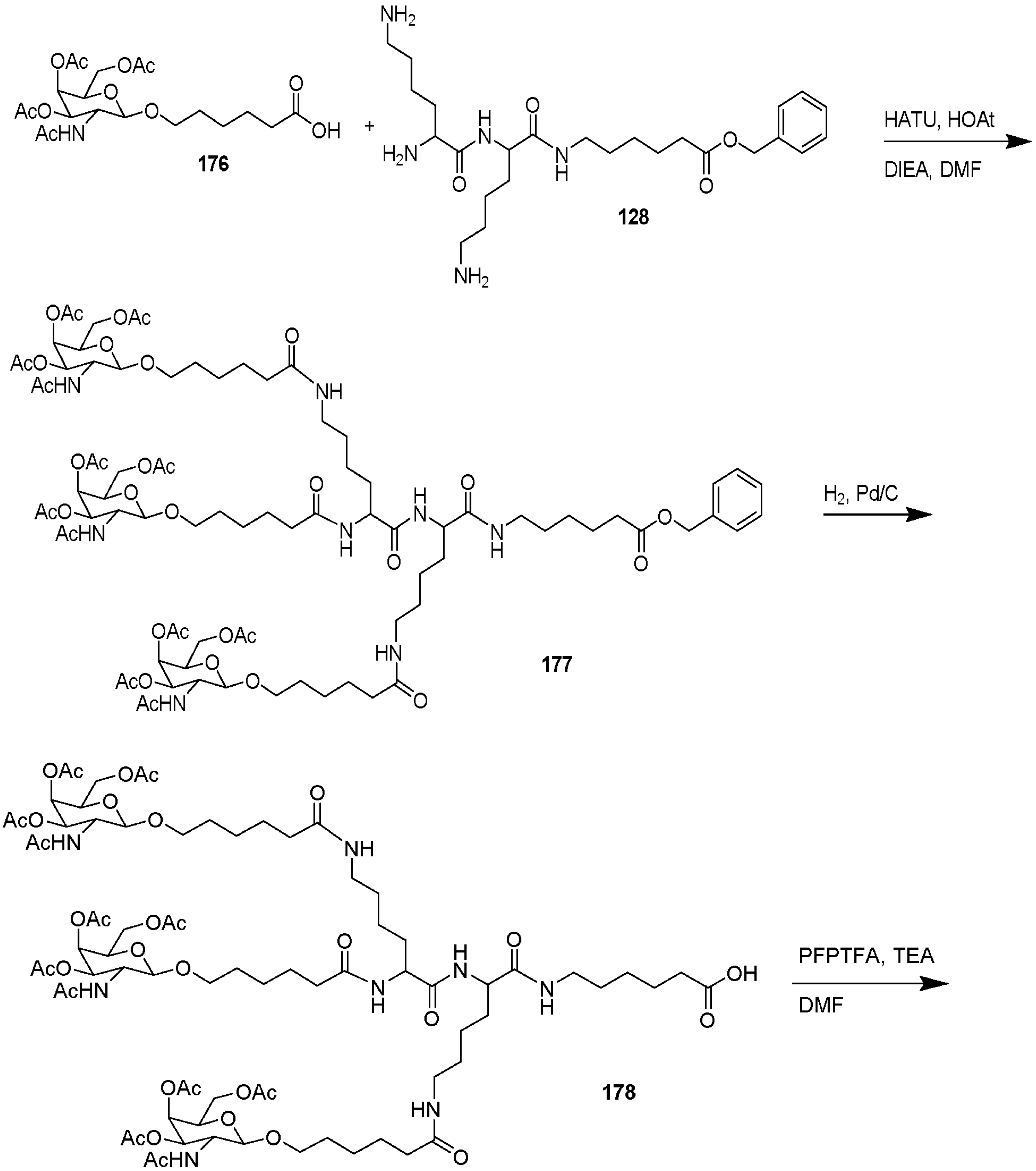
174

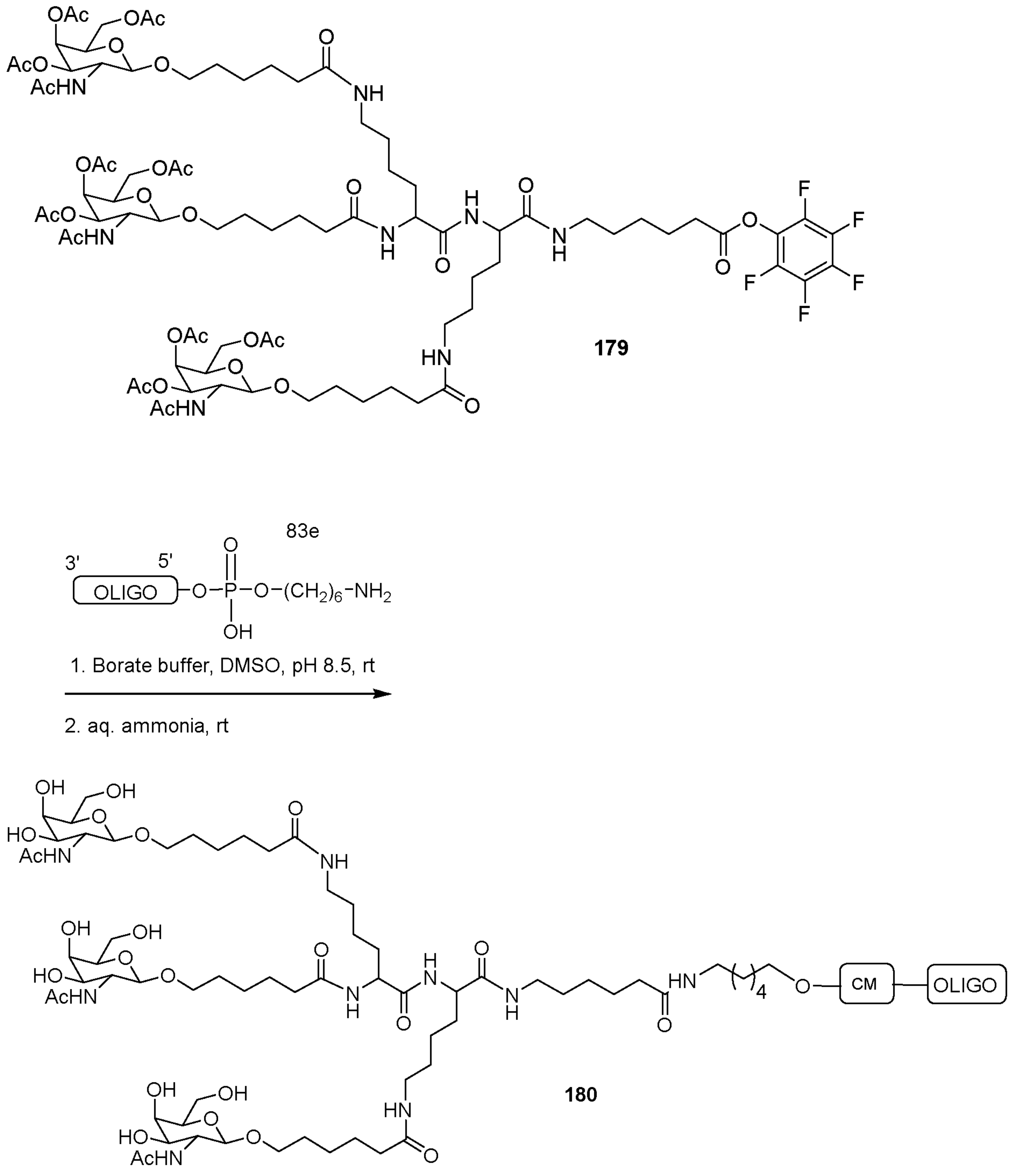
175

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

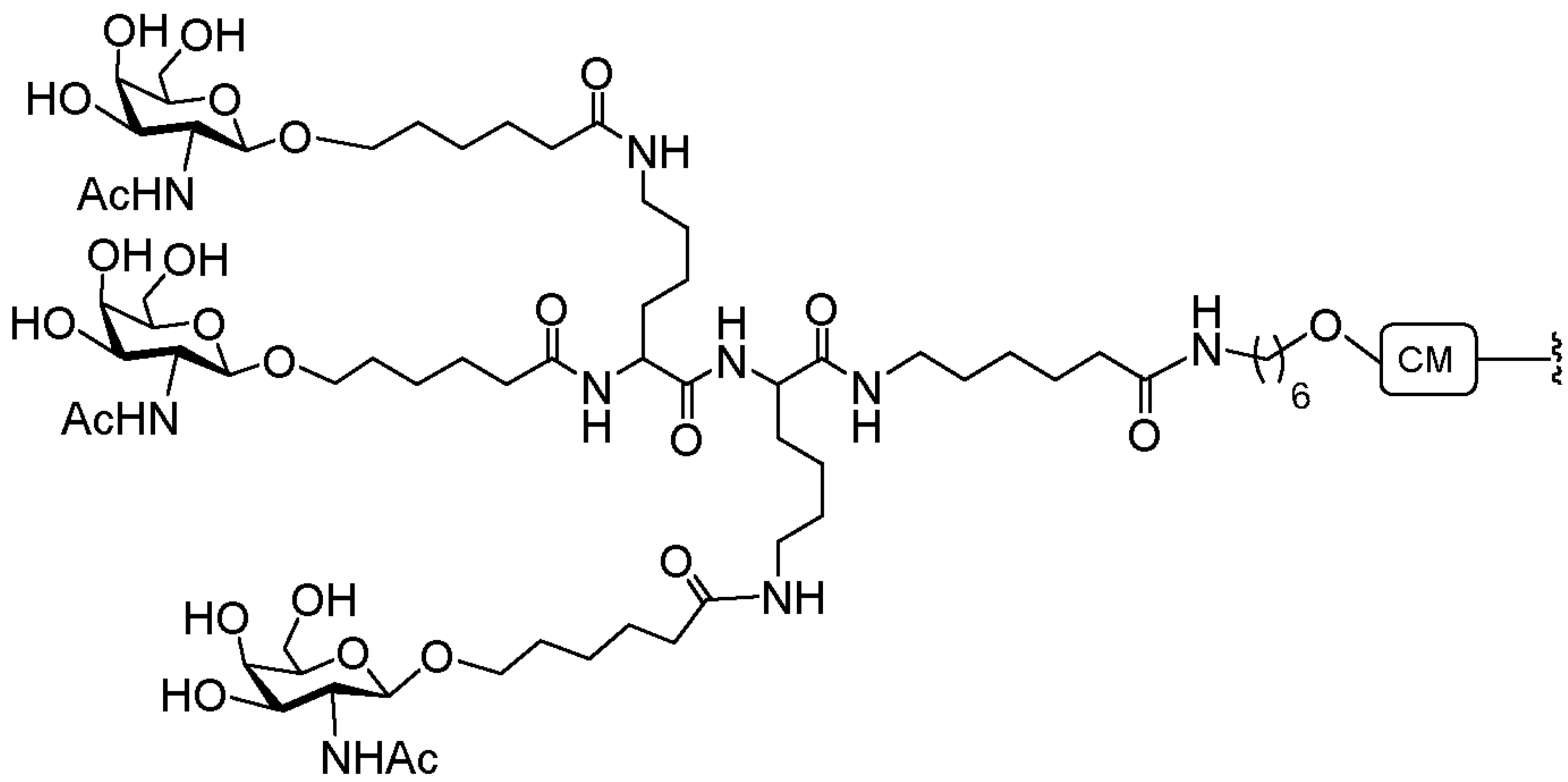

176
128
HATU, HOAt
DIEA, DMF
177
H_2, Pd/C
178
PFPTFA, TEA
DMF



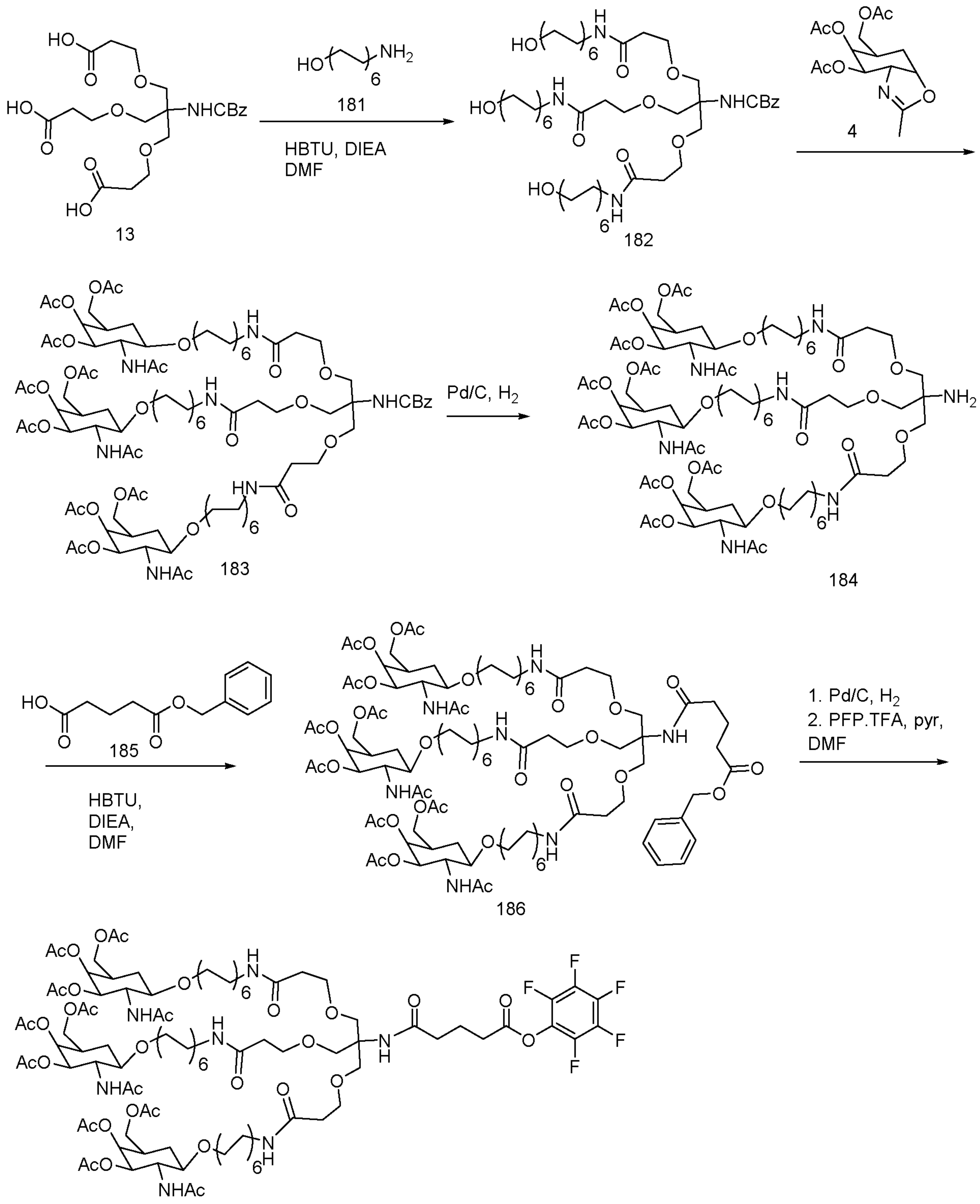
179

180

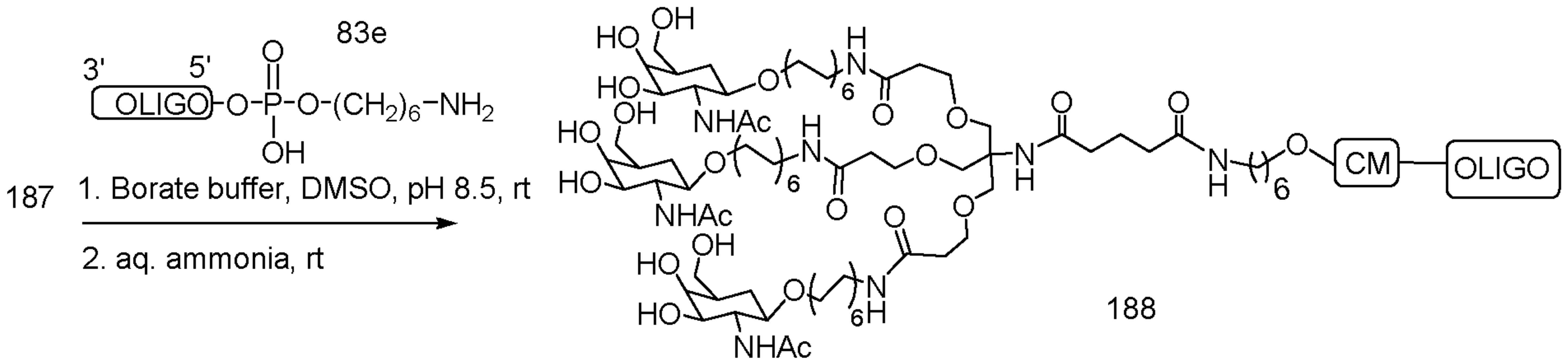
Compound 176 was prepared using the general procedure shown in Example 2. Oligomeric compound 180, comprising a GalNAc$_3$-13 conjugate group, was prepared from compound 177 using the general procedures illustrated in Example 49. The GalNAc$_3$ cluster portion of the conjugate group GalNAc$_3$-13 (GalNAc$_3$-13$_a$) 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:



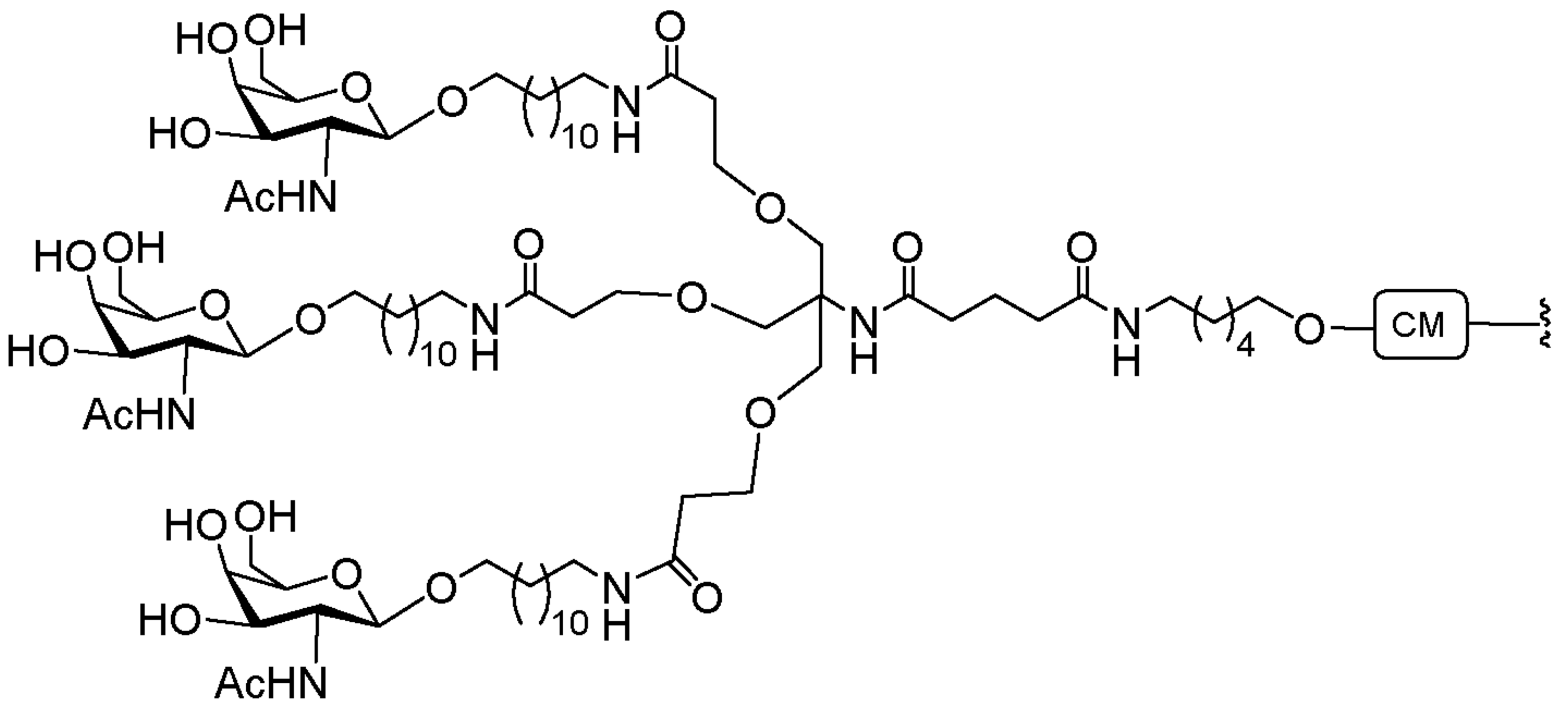
Example 63: Preparation of oligomeric compound 188 comprising GalNAc$_3$-14


13
181
HBTU, DIEA
DMF
182
4
183
Pd/C, H_2
184
185
HBTU,
DIEA,
DMF
186
1. Pd/C, H_2
2. PFP.TFA, pyr,
DMF

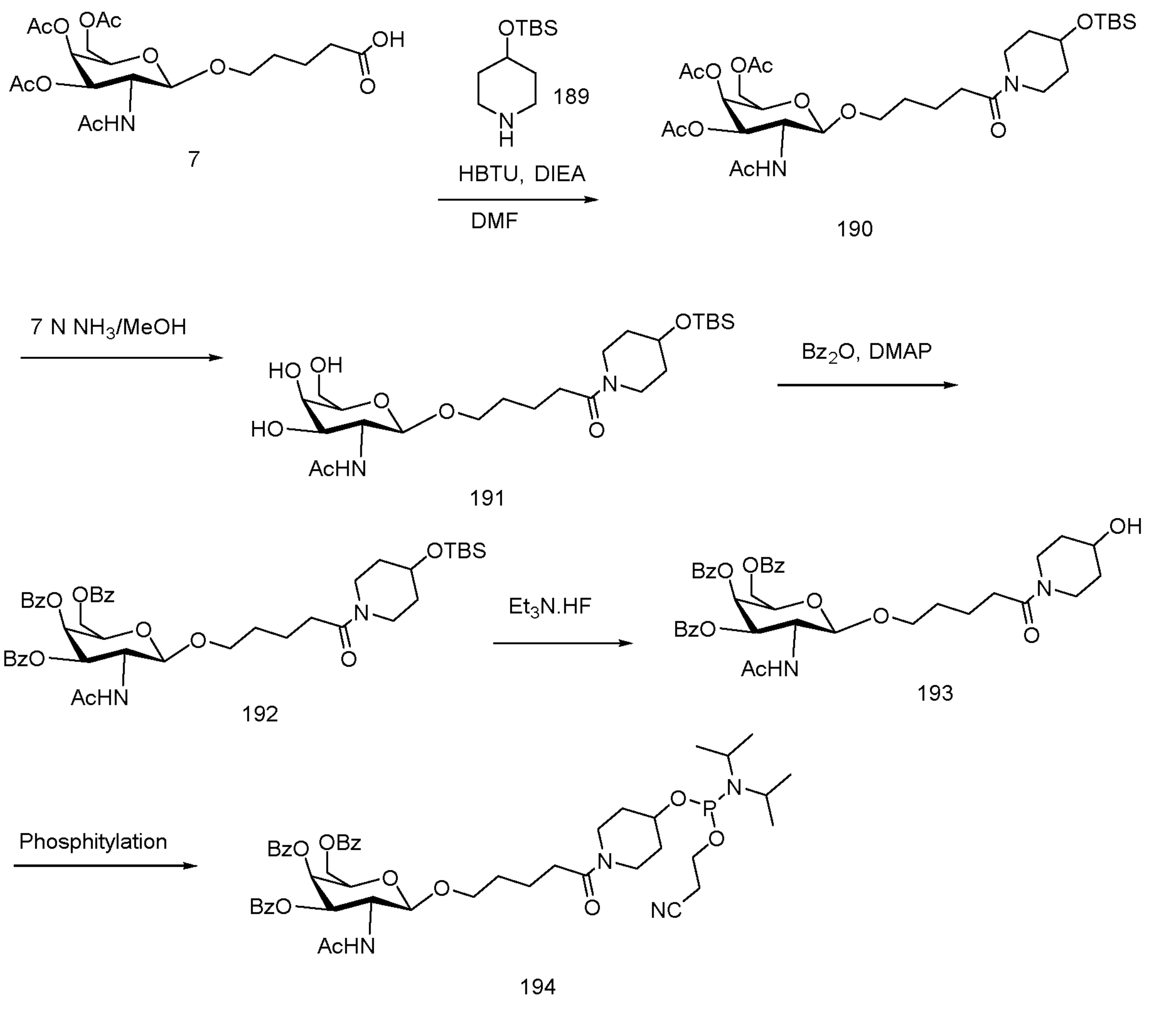
187

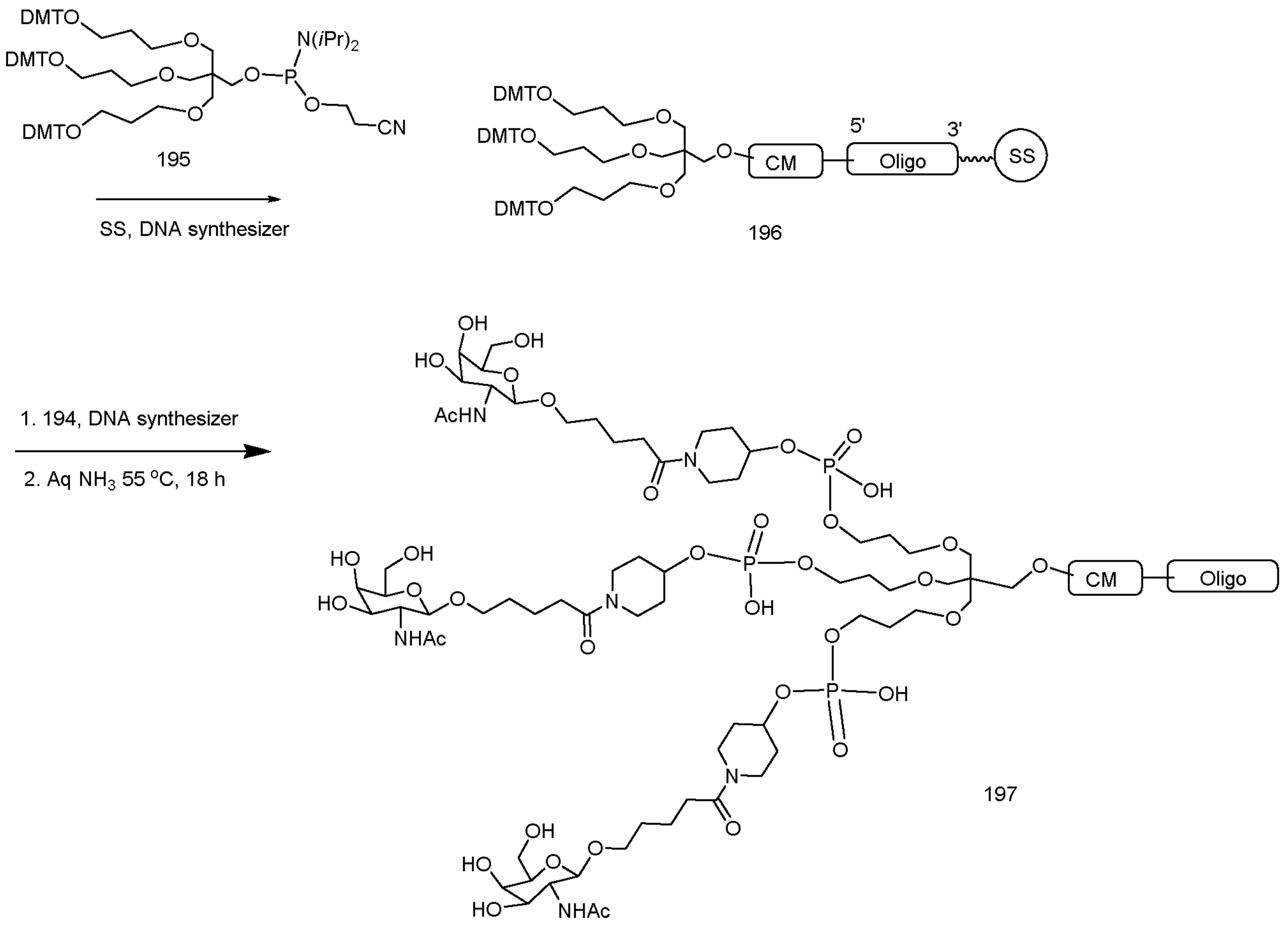


Compounds 181 and 185 are commercially available. Oligomeric compound 188, comprising a GalNAc$_3$-14 conjugate group, was prepared from compound 187 using the general procedures illustrated in Example 46. The GalNAc$_3$ cluster portion of the conjugate group GalNAc$_3$-14 (GalNAc$_3$-14$_a$) can be 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:

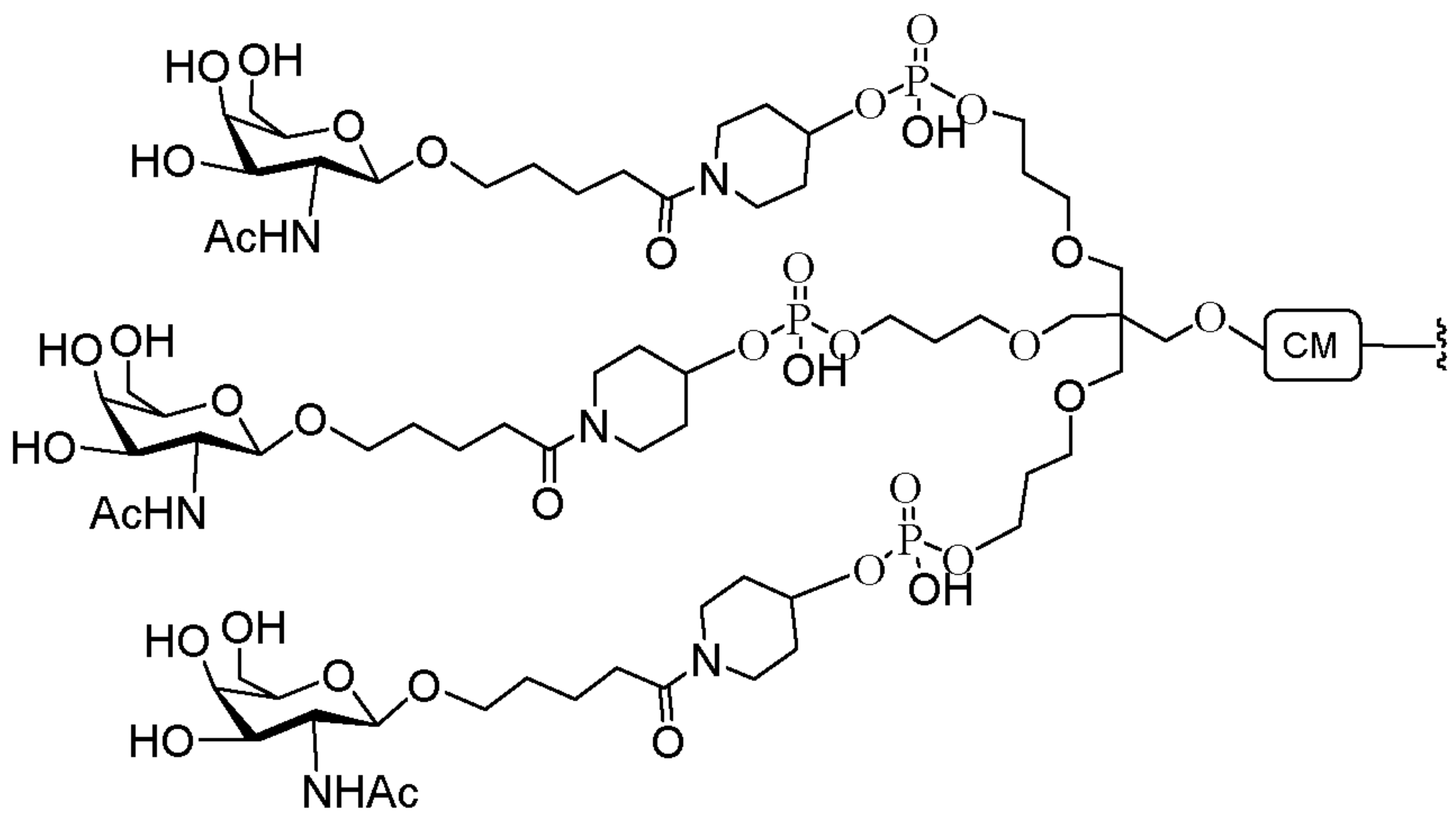


Example 64: Preparation of oligomeric compound 197 comprising GalNAc$_3$-15


AcO
OAc
OH
AcO
O
O
AcHN
7
OTBS
189
N
H
HBTU, DIEA
DMF
190
7 N NH3/MeOH
HO
OH
HO
AcHN
191
Bz2O, DMAP
BzO
OBz
192
Et3N.HF
193
Phosphitylation
P
N
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	2304
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	2306
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	2306
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	2306
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	2306
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	2306

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	$\mathbf{GalNAc_3\text{-}3_{a}\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	GalNAc$_3$-3a	A_d	2306
670699	$\mathbf{GalNAc_3\text{-}3_{a}\text{-}_{o'}T_{do}}G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T_{e}$	GalNAc$_3$-3a	T_d	2309
670700	$\mathbf{GalNAc_3\text{-}3_{a}\text{-}_{o'}A_{eo}}G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T_{e}$	GalNAc$_3$-3a	A_e	2306
670701	$\mathbf{GalNAc_3\text{-}3_{a}\text{-}_{o'}T_{eo}}G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T_{e}$	GalNAc$_3$-3a	T_e	2309
671165	$\mathbf{GalNAc_3\text{-}13_{a}\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T_{e}$	GalNAc$_3$-13a	A_d	2306

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 C57BL/6 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 demonstrated 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		

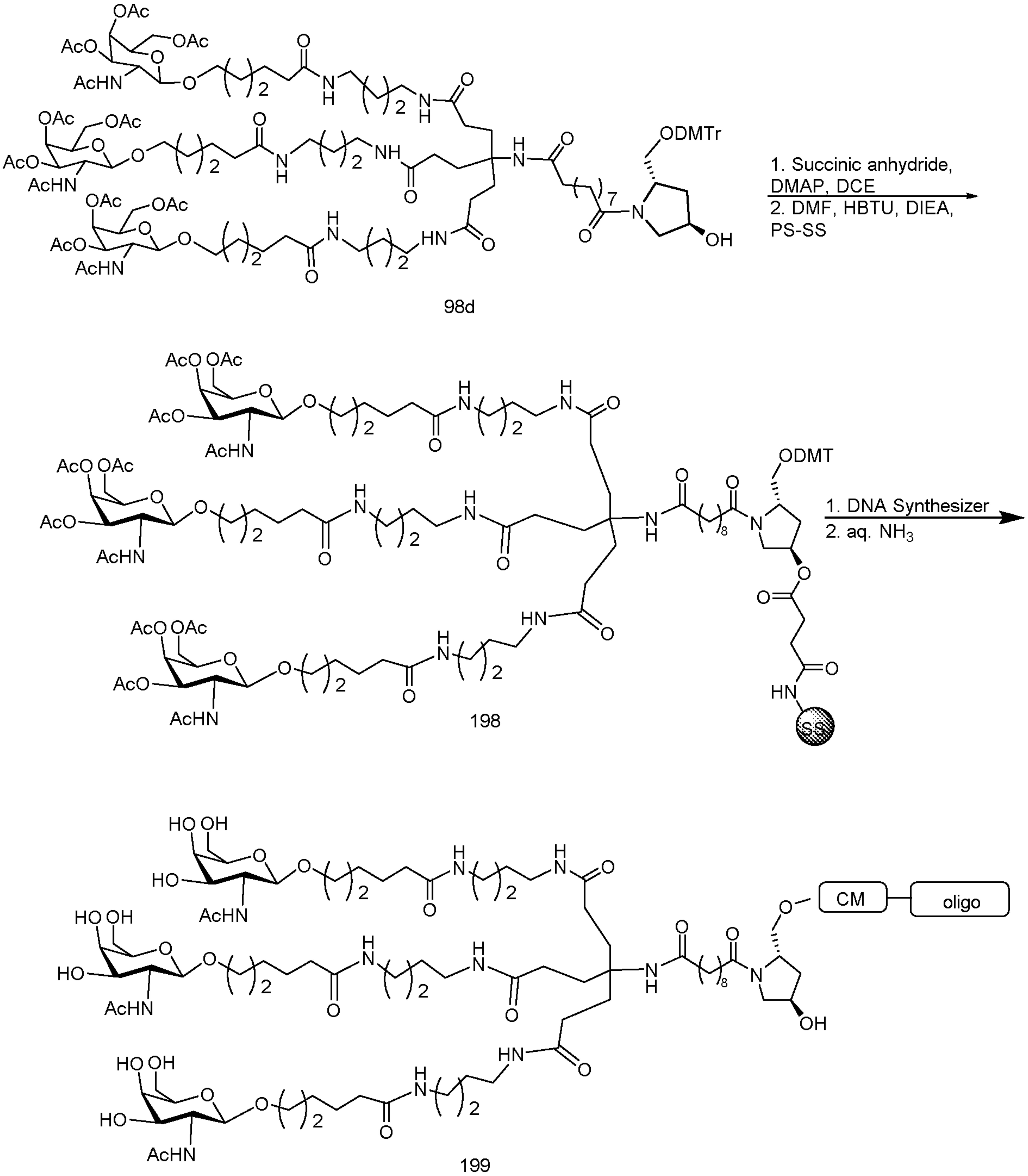
Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum were measured relative to saline injected mice using standard protocols. Total bilirubin and BUN were also evaluated. The changes in body weights were evaluated with no significant differences from the saline group (data not shown). ALTs, ASTs, total bilirubin and BUN values are shown in Table 59 below.

Table 59

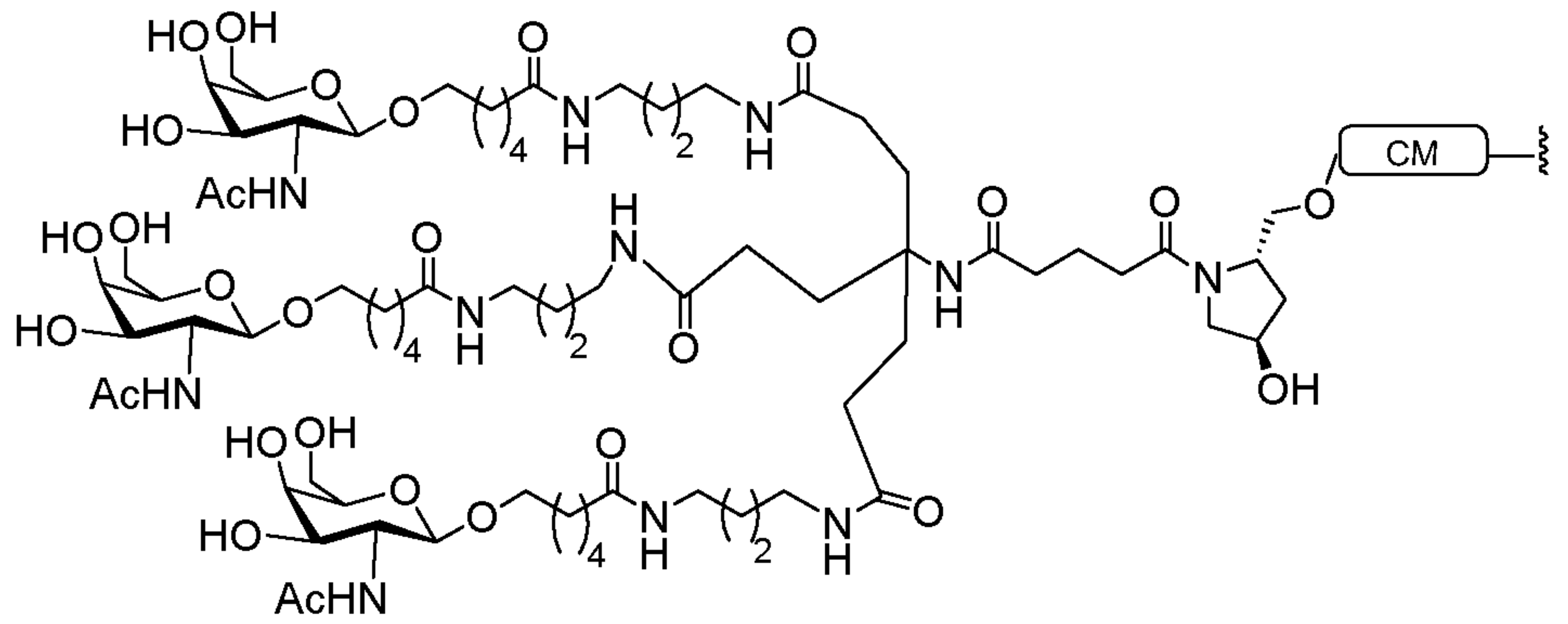
ISIS No.	Dosage (mg/kg)	ALT (U/L)	AST (U/L)	Total Bilirubin (mg/dL)	BUN (mg/dL)	$GalNAc_3$ Cluster	CM
Saline	n/a	24	64	0.2	31	n/a	n/a
661161	0.5	25	64	0.2	31	$GalNAc_3$-3a	A_d
	1.5	24	50	0.2	32		
	5	26	55	0.2	28		
	15	27	52	0.2	31		
670699	0.5	42	83	0.2	31	$GalNAc_3$-3a	T_d
	1.5	33	58	0.2	32		
	5	26	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		

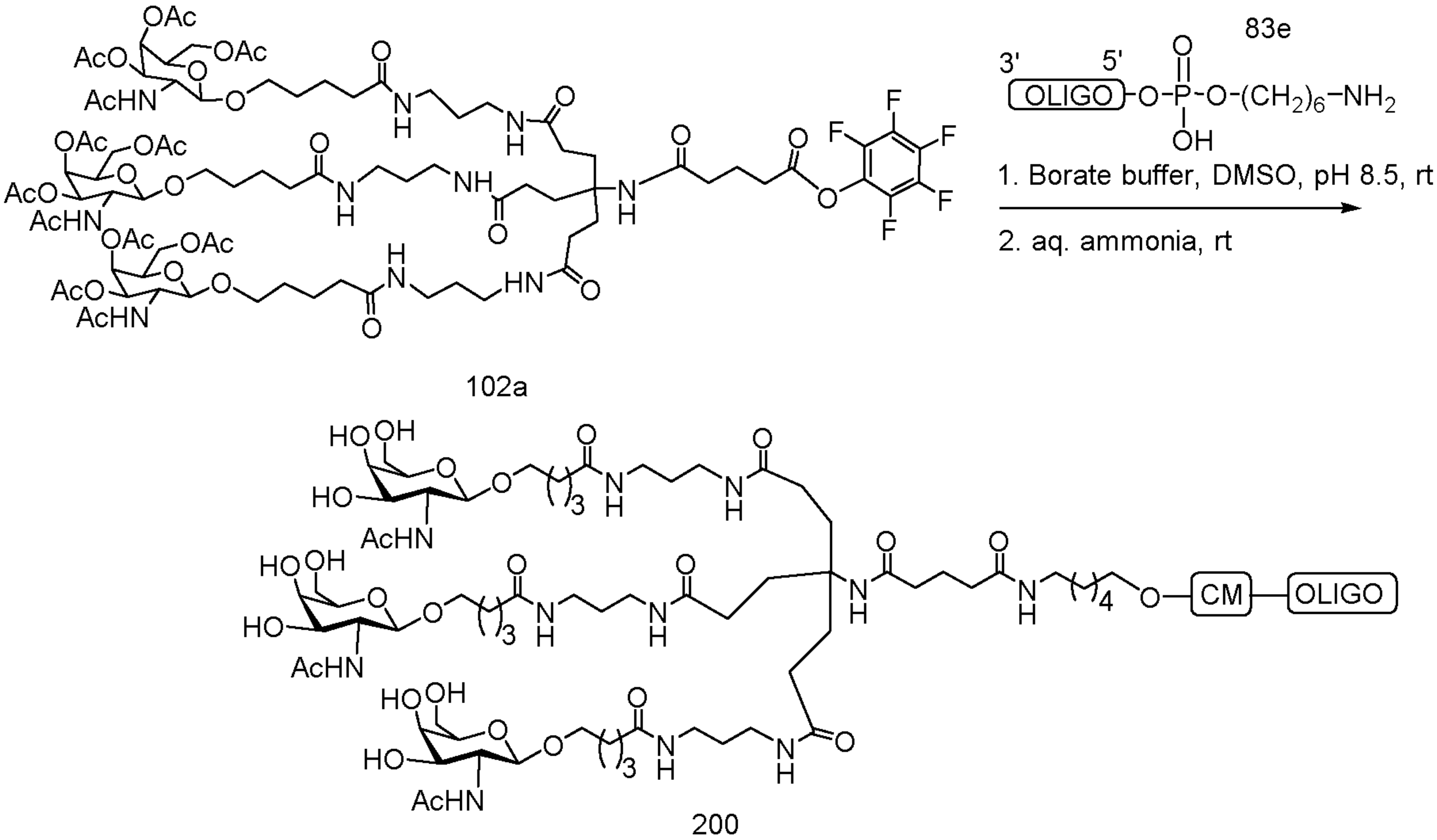
Example 67: Preparation of oligomeric compound 199 comprising GalNAc$_3$-16



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:



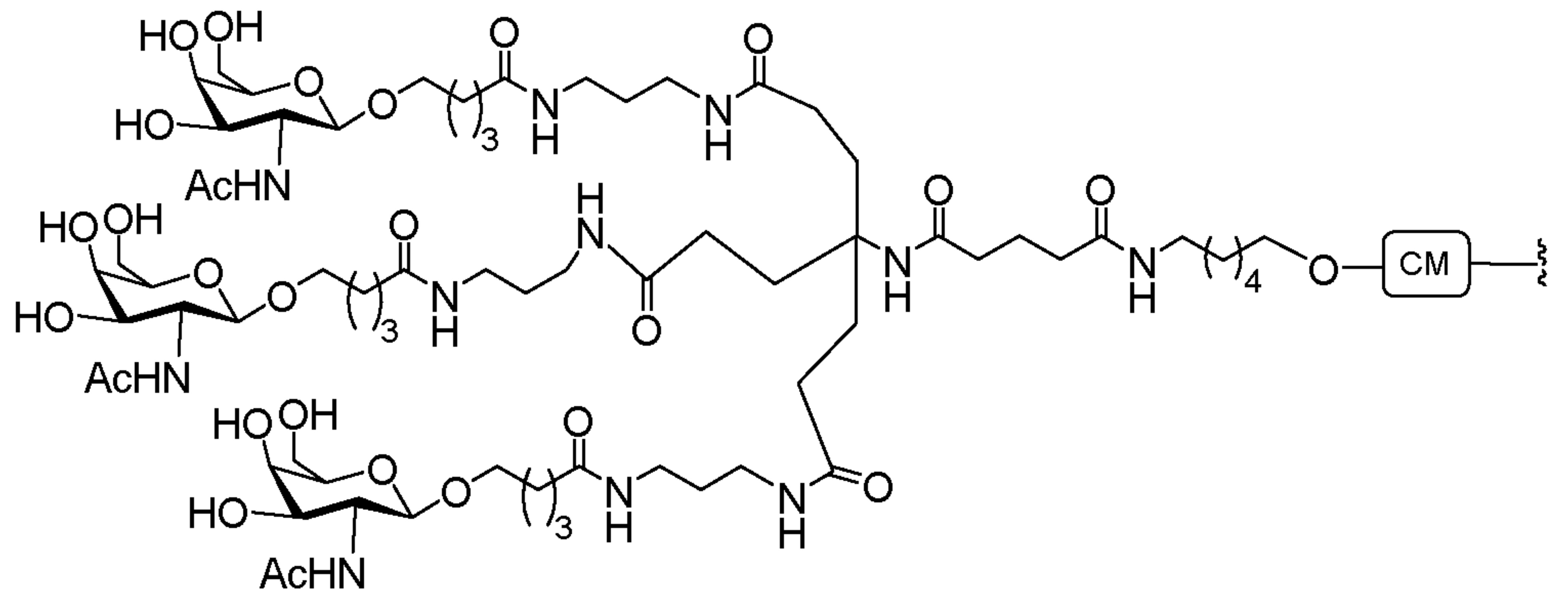
Example 68: Preparation of oligomeric compound 200 comprising GalNAc$_3$-17



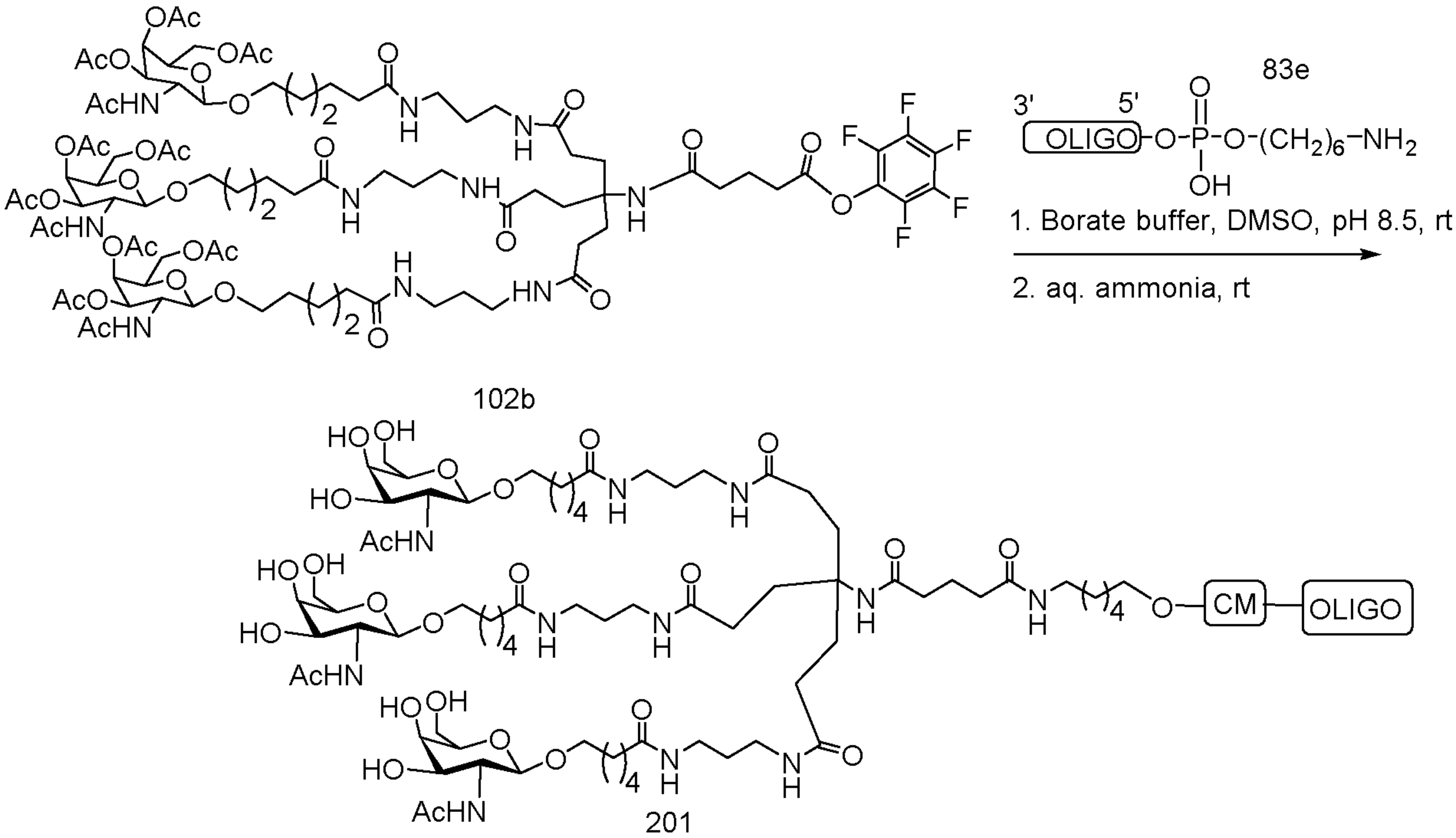
102a

200

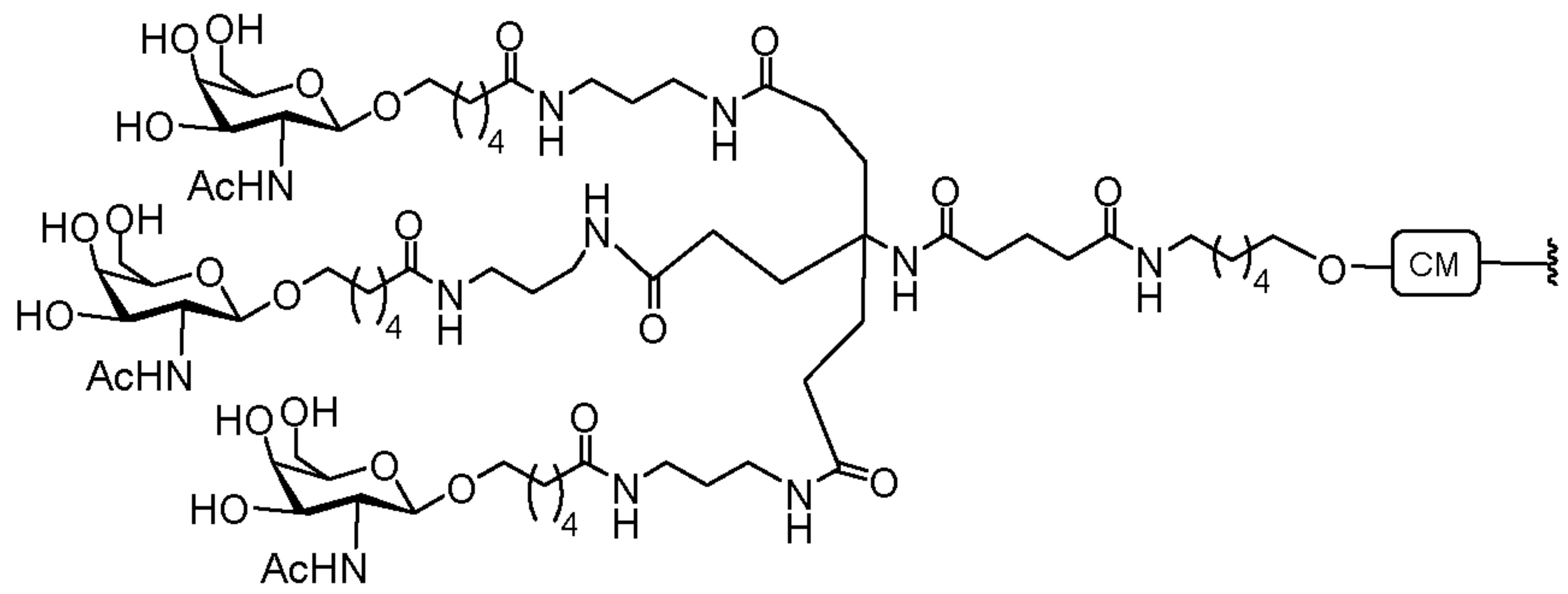
Oligomeric compound 200, comprising a GalNAc$_3$-17 conjugate group, was prepared using the general procedures illustrated in Example 46. The GalNAc$_3$ cluster portion of the conjugate group GalNAc$_3$-17 (GalNAc$_3$-17$_a$) can be combined with any cleavable moiety to provide a variety of conjugate 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:



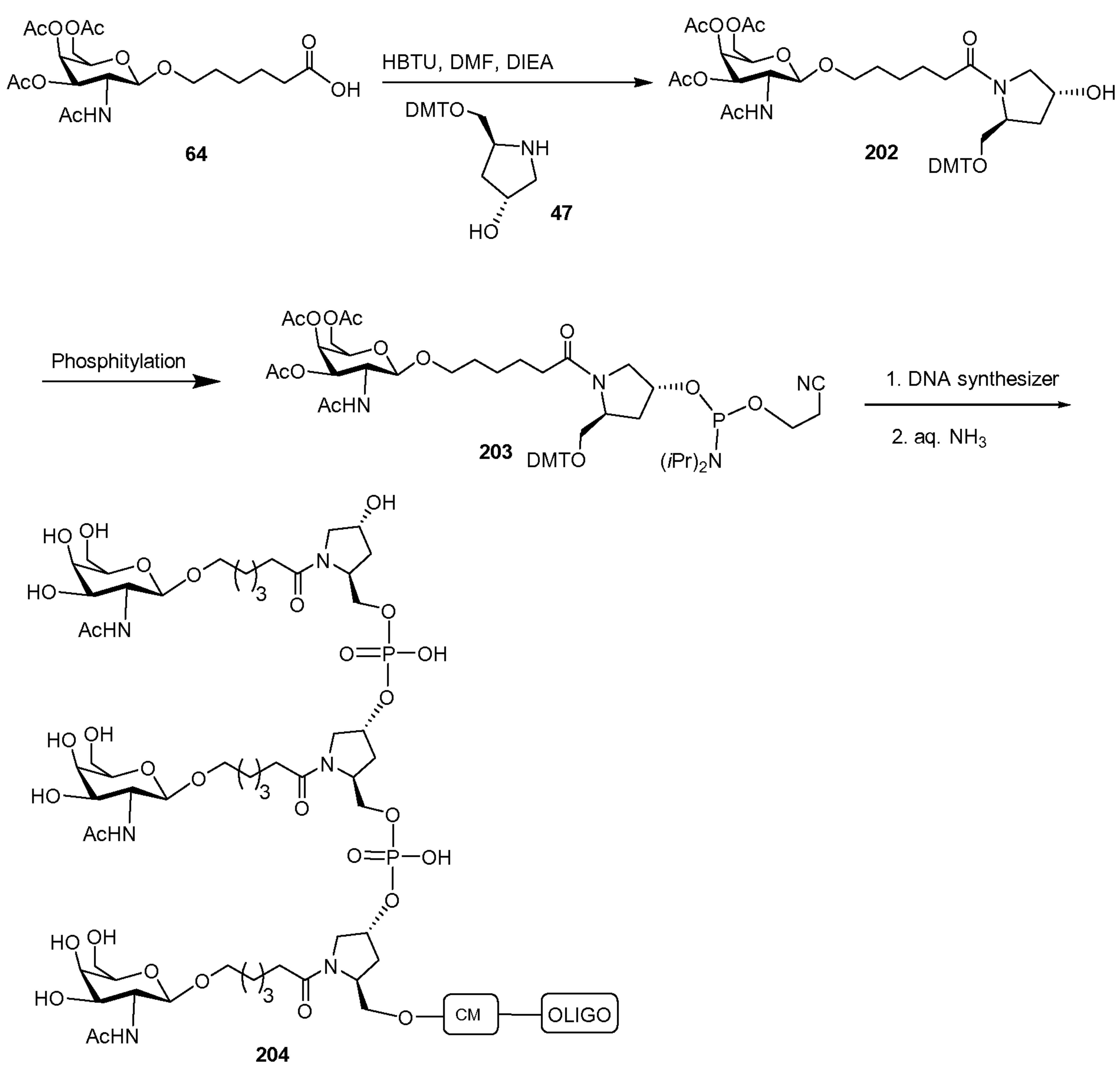
Example 69: Preparation of oligomeric compound 201 comprising GalNAc$_3$-18



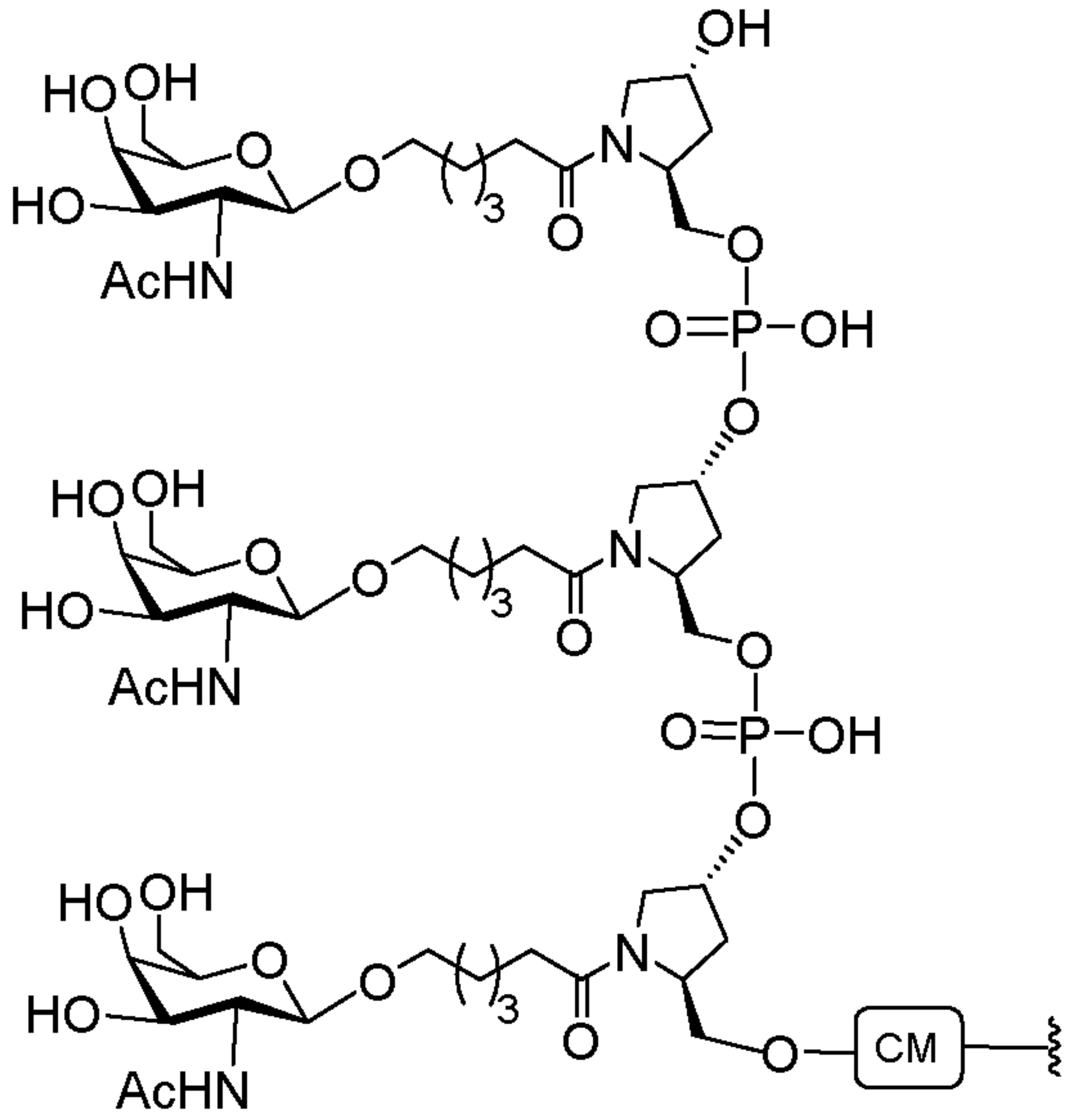
Oligomeric compound 201, comprising a GalNAc$_3$-18 conjugate group, was prepared using the general procedures illustrated in Example 46. The GalNAc$_3$ cluster portion of the conjugate group GalNAc$_3$-18 (GalNAc$_3$-18$_a$) can be combined with any cleavable moiety to provide a variety of conjugate 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:



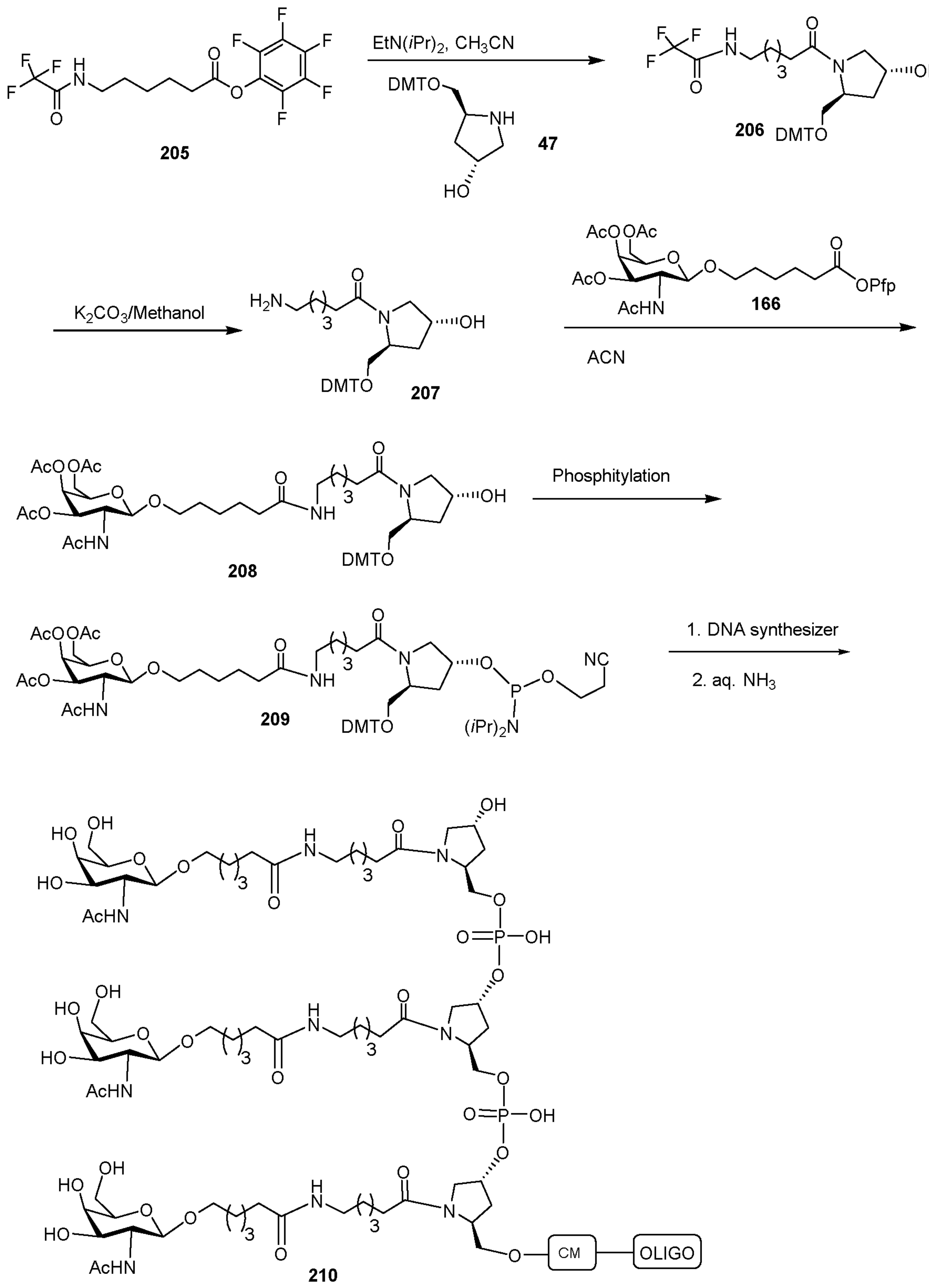
Example 70: Preparation of oligomeric compound 204 comprising GalNAc$_3$-19



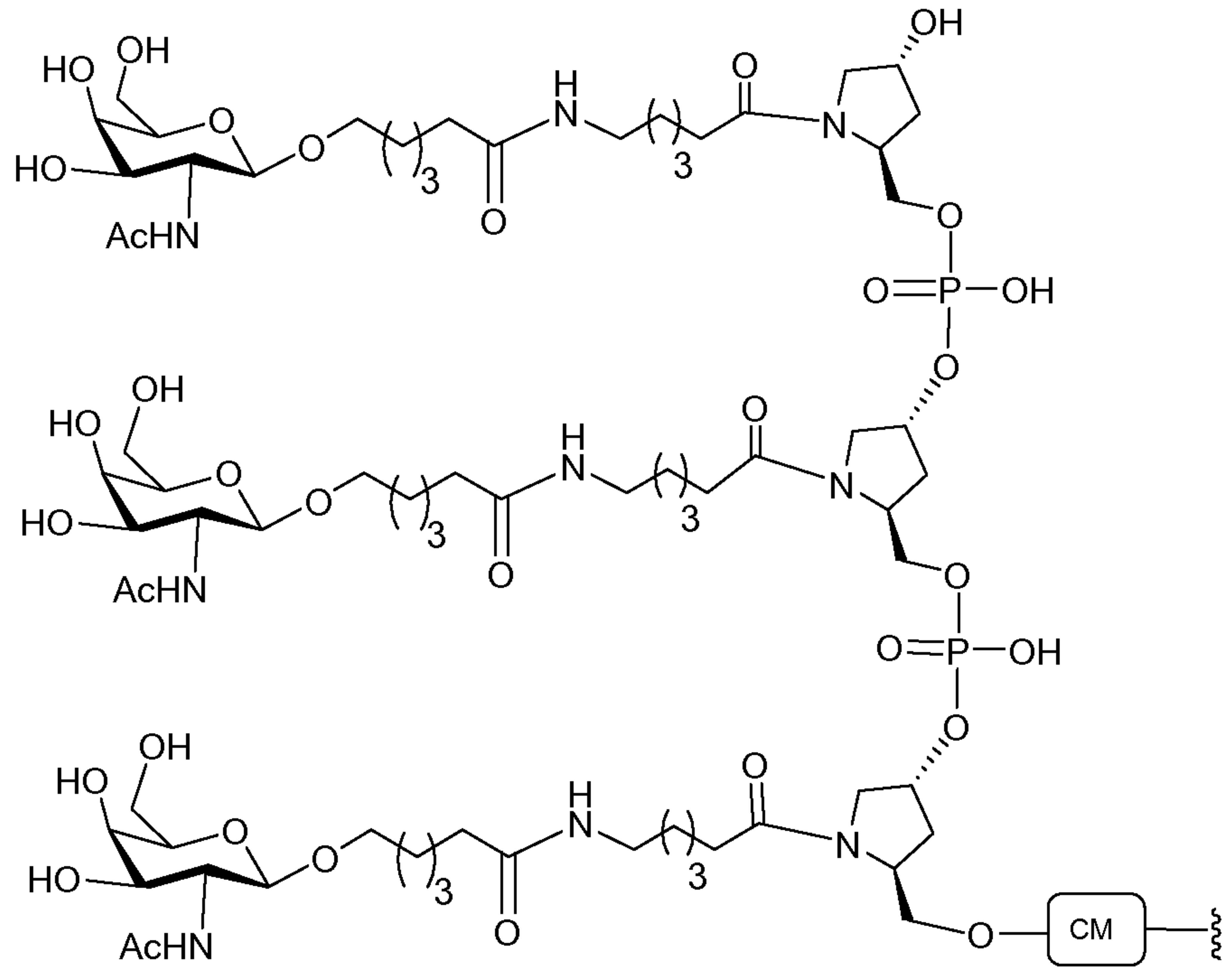
Oligomeric compound 204, comprising a GalNAc$_3$-19 conjugate group, was prepared from compound 64 using the general procedures illustrated in Example 52. The GalNAc$_3$ cluster portion of the conjugate group GalNAc$_3$-19 (GalNAc$_3$-19$_a$) can be combined with any cleavable moiety to provide a variety 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:



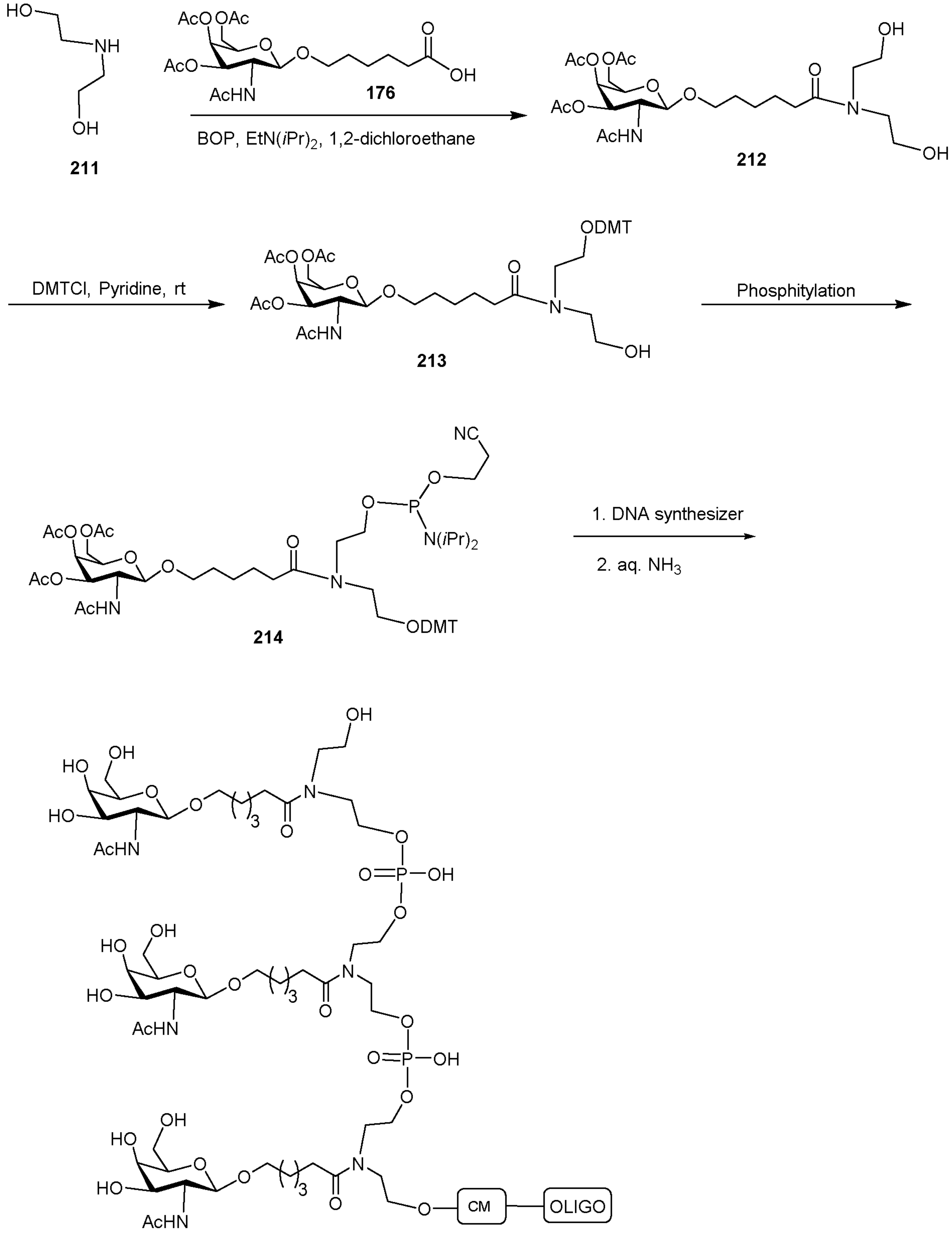
Example 71: Preparation of oligomeric compound 210 comprising GalNAc$_3$-20



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:

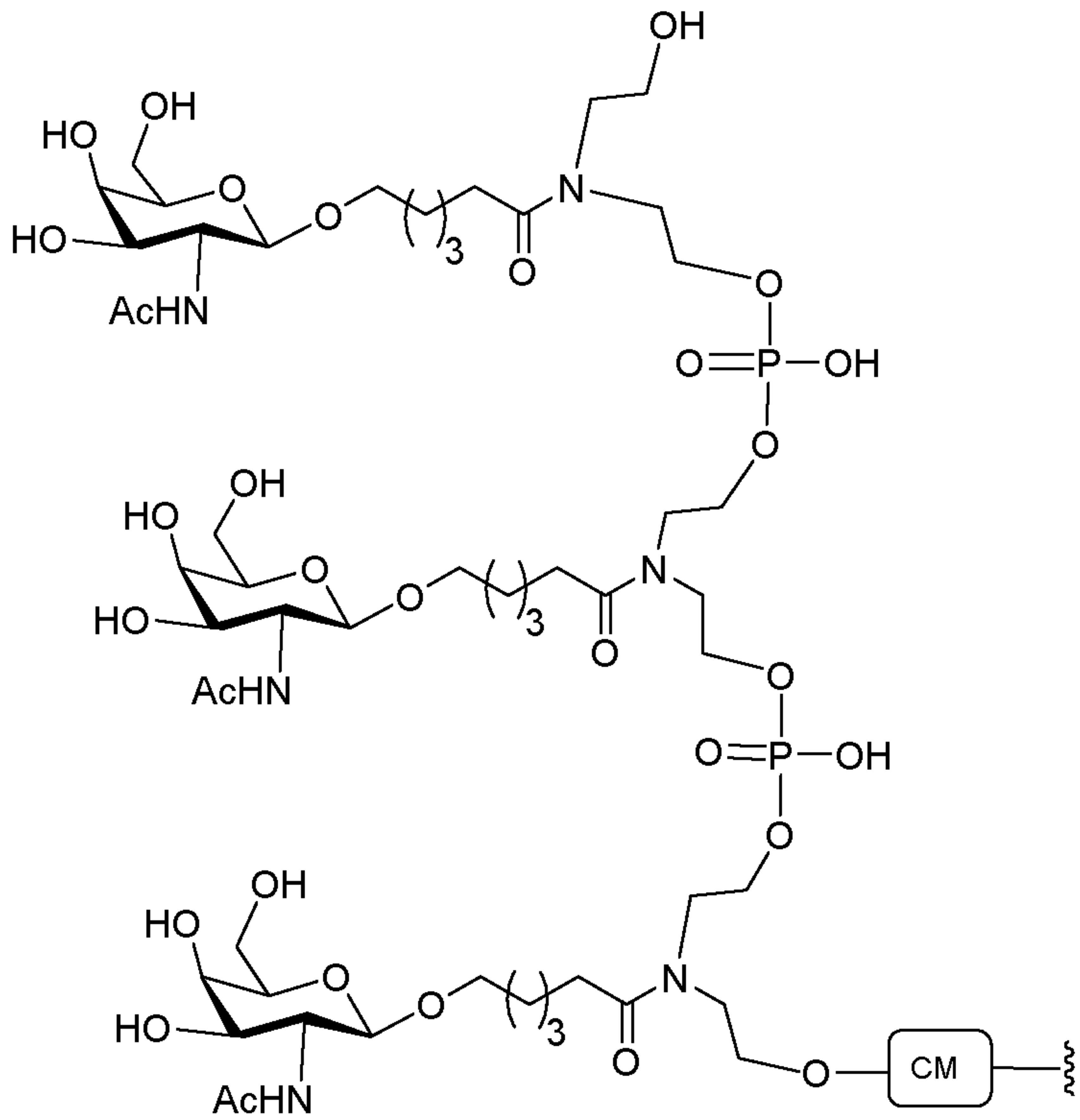


Example 72: Preparation of oligomeric compound 215 comprising GalNAc$_3$-21

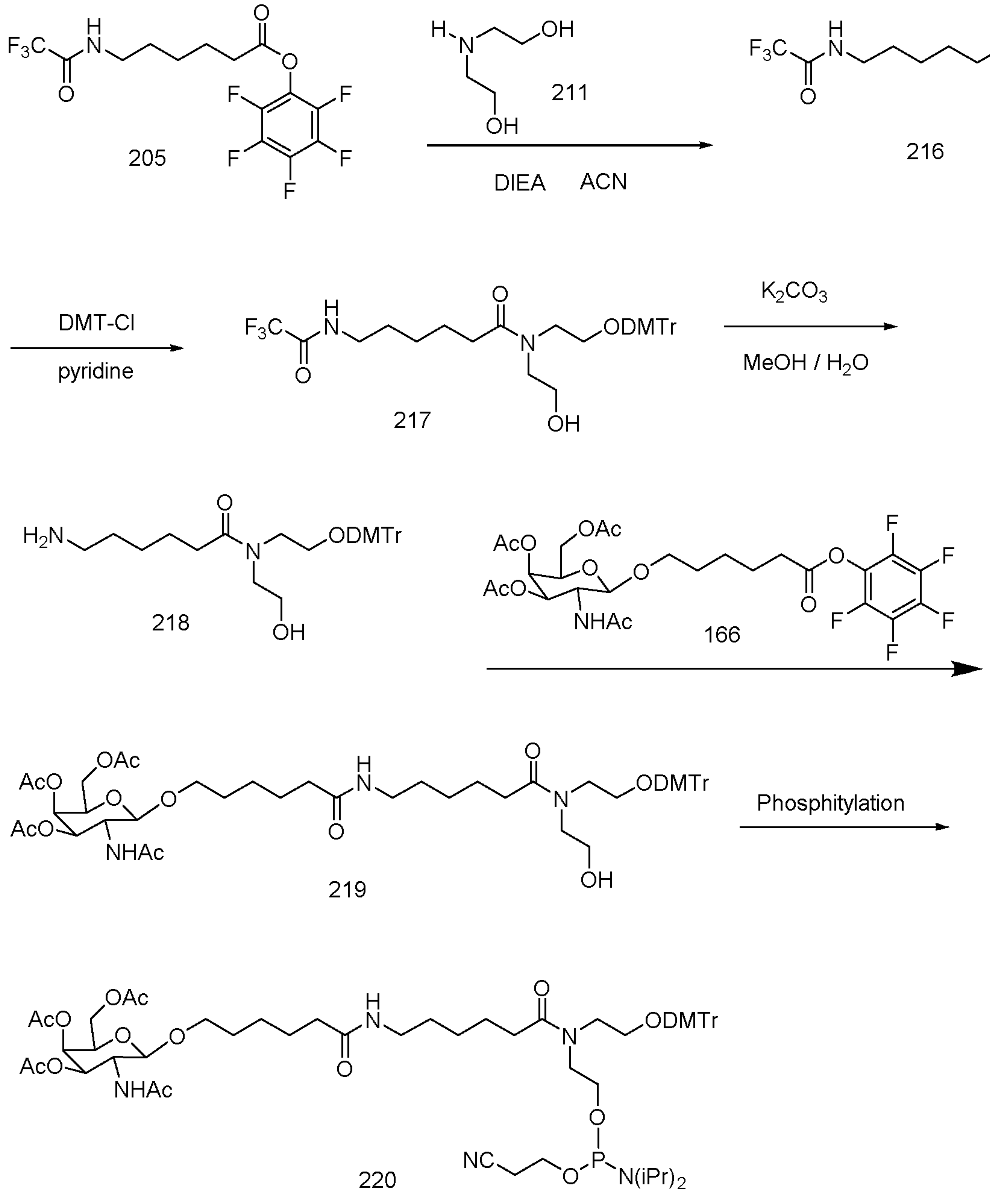


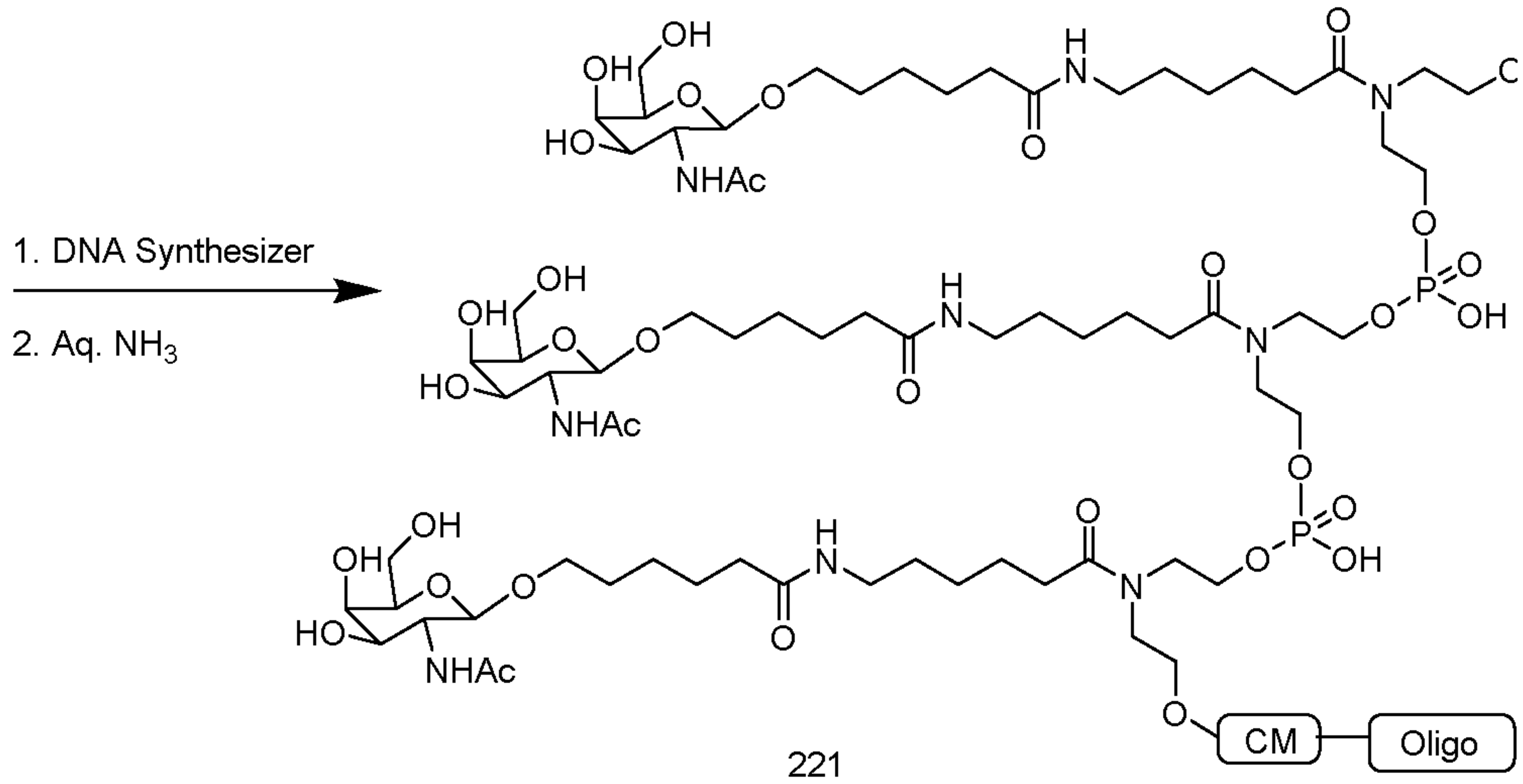
215

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:



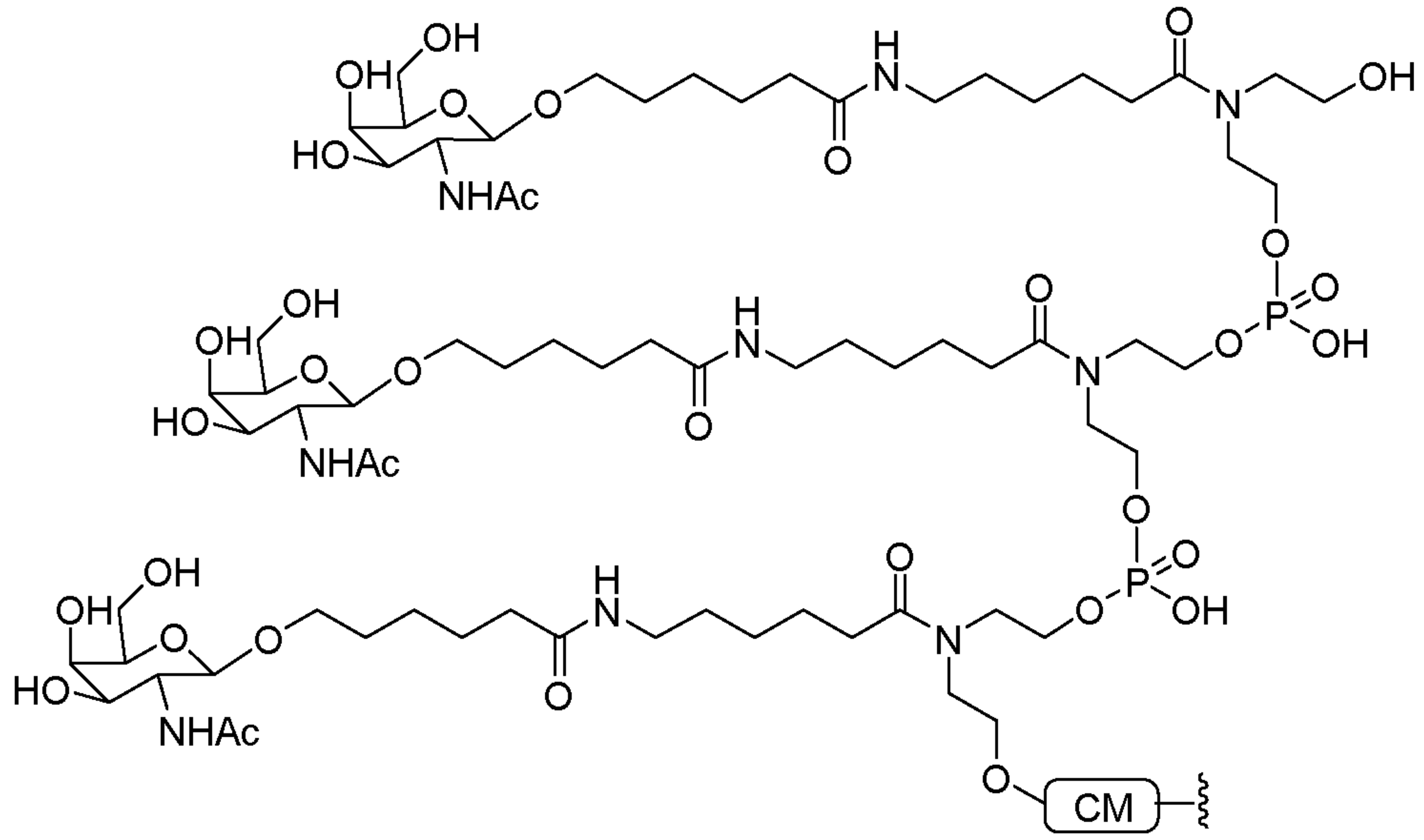
Example 73: Preparation of oligomeric compound 221 comprising GalNAc$_3$-22





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	2304
661161	$\mathbf{GalNAc_3\text{-}3_{a}\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	$GalNAc_3$-3a	A_d	2306
666904	$\mathbf{GalNAc_3\text{-}3_{a}\text{-}_{o'}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	$GalNAc_3$-3a	PO	2304
675441	$\mathbf{GalNAc_3\text{-}17_{a}\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	$GalNAc_3$-17a	A_d	2306
675442	$\mathbf{GalNAc_3\text{-}18_{a}\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	$GalNAc_3$-18a	A_d	2306

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).

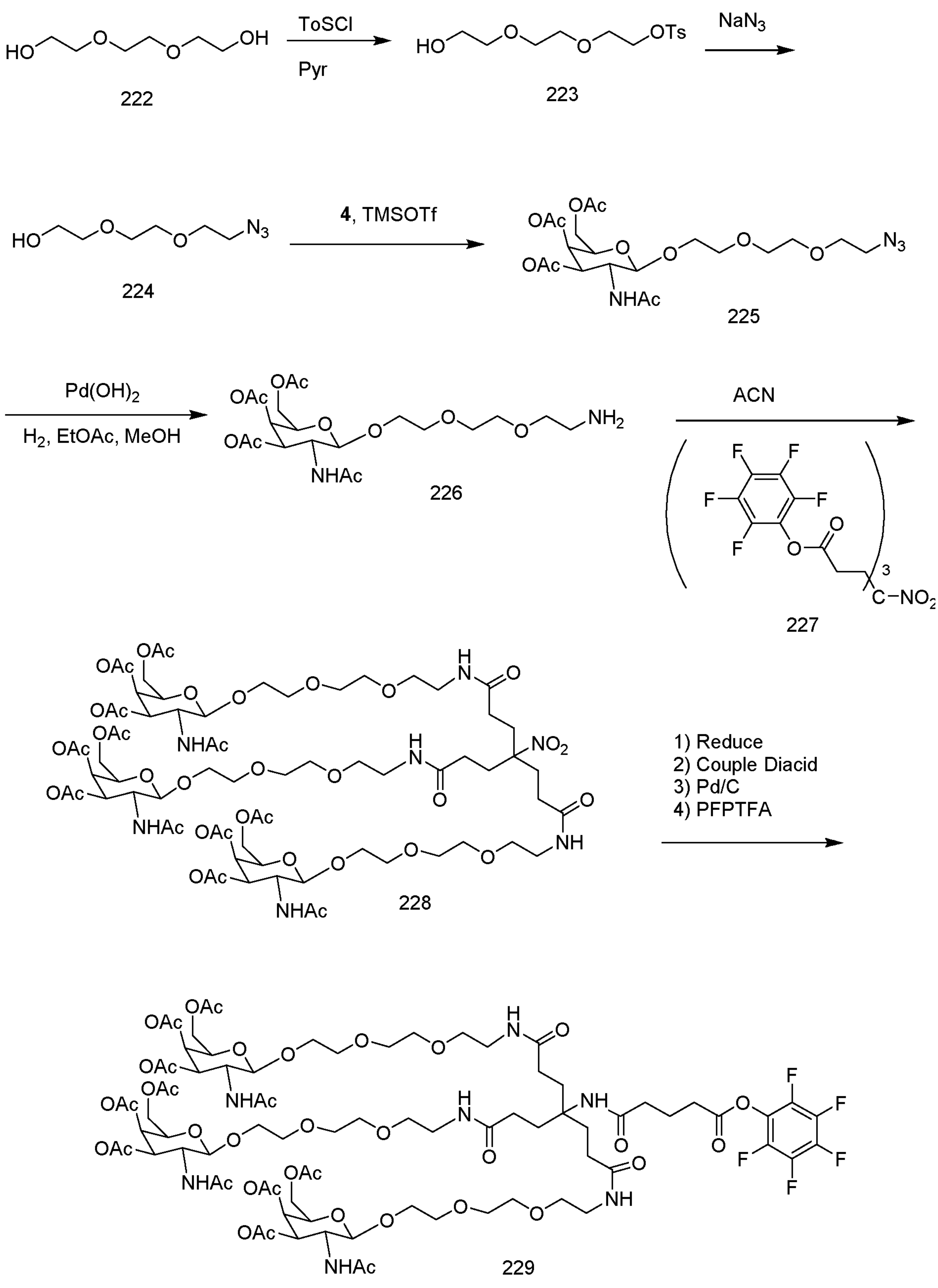
Table 63

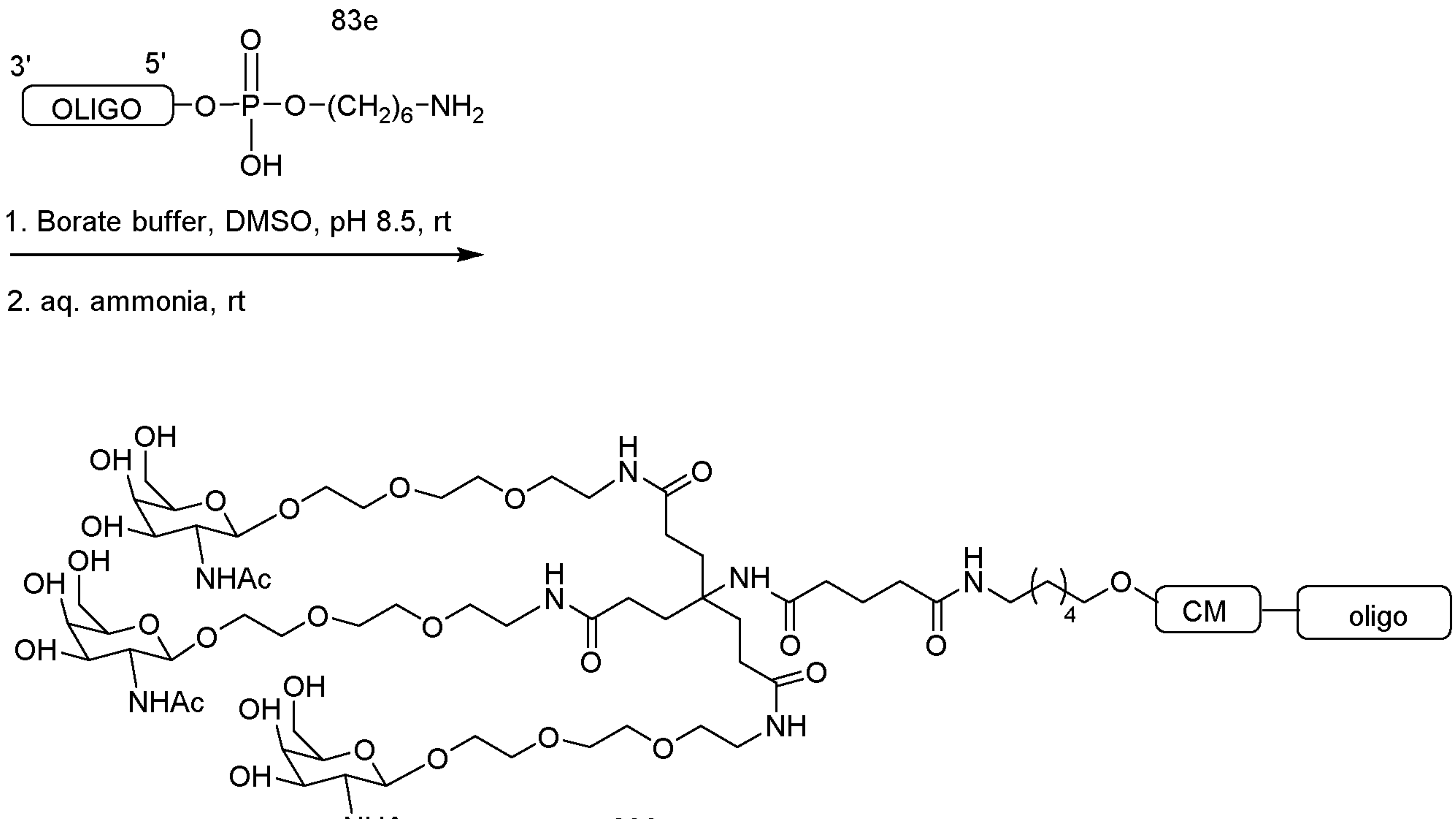
PK Analysis in Liver

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


ToSCl
Pyr
222
223
NaN3
224
4, TMSOTf
225
Pd(OH)2
H2, EtOAc, MeOH
226
ACN
227
228
1) Reduce
2) Couple Diacid
3) Pd/C
4) PFPTFA
229



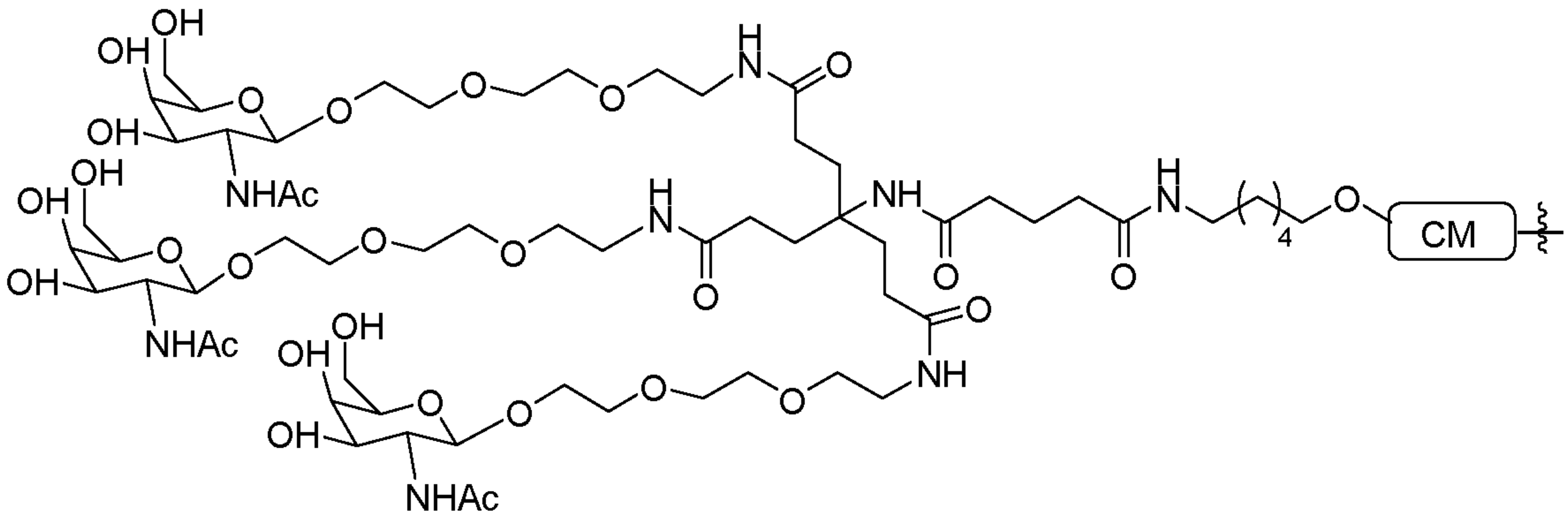
230

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	$\mathbf{GalNAc_3\text{-}3_a\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	GalNAc$_3$-3a	A$_d$	2306
666904	$\mathbf{GalNAc_3\text{-}3_a\text{-}_{o'}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	GalNAc$_3$-3a	PO	2304
673502	$\mathbf{GalNAc_3\text{-}10_a\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T_{e}$	GalNAc$_3$-10a	A$_d$	2306
677844	$\mathbf{GalNAc_3\text{-}9_a\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	GalNAc$_3$-9a	A$_d$	2306
677843	$\mathbf{GalNAc_3\text{-}23_a\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	GalNAc$_3$-23a	A$_d$	2306
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$_d$	2305
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$_d$	2305
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$_d$	2305

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	2310
669509	$^{m}C_{es}A_{es}{}^{m}C_{es}T_{es}G_{es}A_{ds}T_{ds}T_{ds}T_{ds}T_{ds}T_{ds}G_{ds}{}^{m}C_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{es}G_{es}G_{es}A_{es}T_{eo}\mathbf{A_{do'}\text{-}GalNAc_3\text{-}1_a}$	$GalNAc_3$-1_a	A_d	2311

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	2296
647535	$A_{es}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{es}T_{es}T_{es}A_{es}T_{eo}\mathbf{A_{do'}\text{-}GalNAc_3\text{-}1_a}$	$GalNAc_3$-1a	A_d	2297
663083	$\mathbf{GalNAc_3\text{-}3_a\text{-}_{o'}A_{do}}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{es}T_{es}T_{es}A_{es}T_{e}$	$GalNAc_3$-3a	A_d	2312
674449	$\mathbf{GalNAc_3\text{-}7_a\text{-}_{o'}A_{do}}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{es}T_{es}T_{es}A_{es}T_{e}$	$GalNAc_3$-7a	A_d	2312
674450	$\mathbf{GalNAc_3\text{-}10_a\text{-}_{o'}A_{do}}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{es}T_{es}T_{es}A_{es}T_{e}$	$GalNAc_3$-10a	A_d	2312
674451	$\mathbf{GalNAc_3\text{-}13_a\text{-}_{o'}A_{do}}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{es}T_{es}T_{es}A_{es}T_{e}$	$GalNAc_3$-13a	A_d	2312

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	2313
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	2314
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	2315
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	2315
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	2315
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	2315

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$_3$ conjugate

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	2304
655861	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{eo}\mathbf{A_{do'}\text{-}GalNAc_{3}\text{-}1_{a}}$	PS	$GalNAc_3$-1_a	A_d	40	2305
661161	$\mathbf{GalNAc_{3}\text{-}3_{a}\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	$GalNAc_3$-3_a	A_d	40	2306
661162	$\mathbf{GalNAc_{3}\text{-}3_{a}\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T_{e}$	PO/PS	$GalNAc_3$-3_a	A_d	8	2306
664078	$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{eo}\mathbf{A_{do'}\text{-}GalNAc_{3}\text{-}9_{a}}$	PS	$GalNAc_3$-9_a	A_d	20	2305
665001	$\mathbf{GalNAc_{3}\text{-}8_{a}\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	$GalNAc_3$-8_a	A_d	70	2306
666224	$\mathbf{GalNAc_{3}\text{-}5_{a}\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	$GalNAc_3$-5_a	A_d	80	2306
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	2304
666881	$\mathbf{GalNAc_{3}\text{-}10_{a}\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	$GalNAc_3$-10_a	A_d	30	2306
666904	$\mathbf{GalNAc_{3}\text{-}3_{a}\text{-}_{o'}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	$GalNAc_3$-3_a	PO	9	2304
666924	$\mathbf{GalNAc_{3}\text{-}3_{a}\text{-}_{o'}T_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	$GalNAc_3$-3_a	T_d	15	2309
666961	$\mathbf{GalNAc_{3}\text{-}6_{a}\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	$GalNAc_3$-6_a	A_d	150	2306
666981	$\mathbf{GalNAc_{3}\text{-}7_{a}\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	$GalNAc_3$-7_a	A_d	20	2306
670061	$\mathbf{GalNAc_{3}\text{-}13_{a}\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	$GalNAc_3$-13_a	A_d	30	2306
670699	$\mathbf{GalNAc_{3}\text{-}3_{a}\text{-}_{o'}T_{do}}G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T_{e}$	PO/PS	$GalNAc_3$-3_a	T_d	15	2309
670700	$\mathbf{GalNAc_{3}\text{-}3_{a}\text{-}_{o'}A_{eo}}G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T$	PO/PS	$GalNAc_3$-3_a	A_e	30	2306
670701	$\mathbf{GalNAc_{3}\text{-}3_{a}\text{-}_{o'}T_{eo}}G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T_{e}$	PO/PS	$GalNAc_3$-3_a	T_e	25	2306
671144	$\mathbf{GalNAc_{3}\text{-}12_{a}\text{-}_{o'}A_{do}}G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	$GalNAc_3$-12_a	A_d	40	2306

671165	**GalNAc$_3$-13$_a$-$_{o'}$A$_{do}$**$G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T$	PO/PS	GalNAc$_3$ -13$_a$	A_d	8	2306
671261	**GalNAc$_3$-14$_a$-$_{o'}$A$_{do}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	GalNAc$_3$ -14$_a$	A_d	>250	2306
671262	**GalNAc$_3$-15$_a$-$_{o'}$A$_{do}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	GalNAc$_3$ -15$_a$	A_d	>250	2306
673501	**GalNAc$_3$-7$_a$-$_{o'}$A$_{do}$**$G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T_{e}$	PO/PS	GalNAc$_3$ -7$_a$	A_d	30	2306
673502	**GalNAc$_3$-10$_a$-$_{o'}$A$_{do}$**$G_{es}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{eo}{}^{m}C_{eo}{}^{m}C_{es}T_{es}T_{e}$	PO/PS	GalNAc$_3$ -10$_a$	A_d	8	2306
675441	**GalNAc$_3$-17$_a$-$_{o'}$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$_3$ -17$_a$	A_d	30	2306
675442	**GalNAc$_3$-18$_a$-$_{o'}$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$_3$ -18$_a$	A_d	20	2306
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	2305
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	2305
677843	**GalNAc$_3$-23$_a$-$_{o'}$A$_{do}$**$G_{es}{}^{m}C_{es}T_{es}T_{es}{}^{m}C_{es}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}{}^{m}C_{es}T_{es}T_{e}$	PS	GalNAc$_3$ -23$_a$	A_d	40	2306

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$_3$ conjugate

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	2307
656173	$T_{es}G_{eo}G_{eo}T_{eo}A_{eo}A_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ds}T_{ds}{}^{m}C_{ds}A_{eo}G_{eo}A_{es}G_{es}G_{eo}$**A$_{do'}$-GalNAc$_3-1_a$**	GalNAc$_3$-1$_a$	A_d	2308
663086	**GalNAc$_3$-3$_a$-$_{o'}$A$_{do}$**$T_{es}G_{eo}G_{eo}T_{eo}A_{eo}A_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}{}^{m}C_{ds}T_{ds}$	GalNAc$_3$-3$_a$	A_d	2316

	$T_{ds}T_{ds}{}^{m}C_{ds}A_{eo}G_{eo}A_{es}G_{es}G_{e}$			
678347	$\mathbf{GalNAc_3\text{-}7_{a}\text{-}_{o'}A_{do}}T_{es}G_{eo}G_{eo}T_{eo}A_{eo}A_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ds}T_{ds}{}^{m}C_{ds}A_{eo}G_{eo}A_{es}G_{es}G_{e}$	$GalNAc_3\text{-}7_a$	A_d	2316
678348	$\mathbf{GalNAc_3\text{-}10_{a}\text{-}_{o'}A_{do}}T_{es}G_{eo}G_{eo}T_{eo}A_{eo}A_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ds}T_{ds}{}^{m}C_{ds}A_{eo}G_{eo}A_{es}G_{es}G_{e}$	$GalNAc_3\text{-}10_a$	A_d	2316
678349	$\mathbf{GalNAc_3\text{-}13_{a}\text{-}_{o'}A_{do}}T_{es}G_{eo}G_{eo}T_{eo}A_{eo}A_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ds}T_{ds}{}^{m}C_{ds}A_{eo}G_{eo}A_{es}G_{es}G_{e}$	$GalNAc_3\text{-}13_a$	A_d	2316

The structure of $GalNAc_3\text{-}1_a$ was shown previously in Example 9, $GalNAc_3\text{-}3_a$ was shown in Example 39, $GalNAc_3\text{-}7_a$ was shown in Example 48, $GalNAc_3\text{-}10_a$ was shown in Example 46, and $GalNAc_3\text{-}13_a$ 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	2307
	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	2308
	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	2316
	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	2316
	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	2316
	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	2316
	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	2307
		10	47			
		17	52			
656173	6	3	1	GalNAc$_3$-1a	A_d	2308
		10	3			
		17	21			
663086	6	3	1	GalNAc$_3$-3a	A_d	2316
		10	2			
		17	9			
678347	6	3	1	GalNAc$_3$-7a	A_d	2316
		10	1			
		17	8			
678348	6	3	1	GalNAc$_3$-10a	A_d	2316
		10	1			

		17	6			
678349	6	3	1	GalNAc$_3$-13a	A$_d$	2316
		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 $GalNAc_3$ conjugate

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 84-87. 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	2317
660261	$T_{es}{}^{m}C_{es}T_{es}T_{es}G_{es}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{es}T_{es}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{eo}\mathbf{A_{do'}\text{-}GalNAc_{3}\text{-}1_{a}}$	PS	$GalNAc_3$-1a	A_d	2318
682883	$\mathbf{GalNAc_{3}\text{-}3_{a\text{-}o'}}T_{es}{}^{m}C_{eo}T_{eo}T_{eo}G_{eo}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{eo}T_{eo}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$	PS/PO	$GalNAc_3$-3a	PO	2317
682884	$\mathbf{GalNAc_{3}\text{-}7_{a\text{-}o'}}T_{es}{}^{m}C_{eo}T_{eo}T_{eo}G_{eo}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{eo}T_{eo}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$	PS/PO	$GalNAc_3$-7a	PO	2317

682885	$\mathbf{GalNAc_3\text{-}10_{a\text{-}o'}}T_{es}{}^{m}C_{eo}T_{eo}T_{eo}G_{eo}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{eo}T_{eo}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$	PS/PO	$GalNAc_3$-10a	PO	2317
682886	$\mathbf{GalNAc_3\text{-}13_{a\text{-}o'}}T_{es}{}^{m}C_{eo}T_{eo}T_{eo}G_{eo}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{eo}T_{eo}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$	PS/PO	$GalNAc_3$-13a	PO	2317
684057	$T_{es}{}^{m}C_{eo}T_{eo}T_{eo}G_{eo}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{eo}T_{eo}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{eo}\mathbf{A_{do'}\text{-}GalNAc_3\text{-}19_{a}}$	PS/PO	$GalNAc_3$-19a	A_d	2318

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	2317
	20	48	65			
	60	18	28			
660261	0.6	113	87	$GalNAc_3$-1a	A_d	2318
	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	2317
	20	43	102	66	61	58			
	60	24	92	43	29	32			
682883	0.6	60	88	73	63	68	$GalNAc_3$-3a	PO	2317
	2	18	75	38	23	23			
	6	10	80	35	11	9			
682884	0.6	56	88	78	63	67	$GalNAc_3$-7a	PO	2317
	2	19	76	44	25	23			
	6	15	82	35	21	24			
682885	0.6	60	92	77	68	76	$GalNAc_3$-10a	PO	2317
	2	22	93	58	32	32			
	6	17	85	37	25	20			
682886	0.6	57	91	70	64	69	$GalNAc_3$-13a	PO	2317
	2	21	89	50	31	30			

	6	18	102	41	24	27			
684057	0.6	53	80	69	56	62	GalNAc$_3$-19a	A$_d$	2318
	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	2317
	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	2318
	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	2317
	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	2317
	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	2317
	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	2317
	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	2317
	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	2318
	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$_3$ conjugate

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	2317
		7	23			
		10	35			
		17	53			
		24	75			
		39	100			
660261	13.5	3	27	$GalNAc_3$-1a	A_d	2318
		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	2317
		7	48			
		10	48			
		17	66			

		31	80			
682883	10.0	3	45	$GalNAc_3$-3a	PO	2317
		7	37			
		10	38			
		17	42			
		31	65			
682885	10.0	3	40	$GalNAc_3$-10a	PO	2317
		7	33			
		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

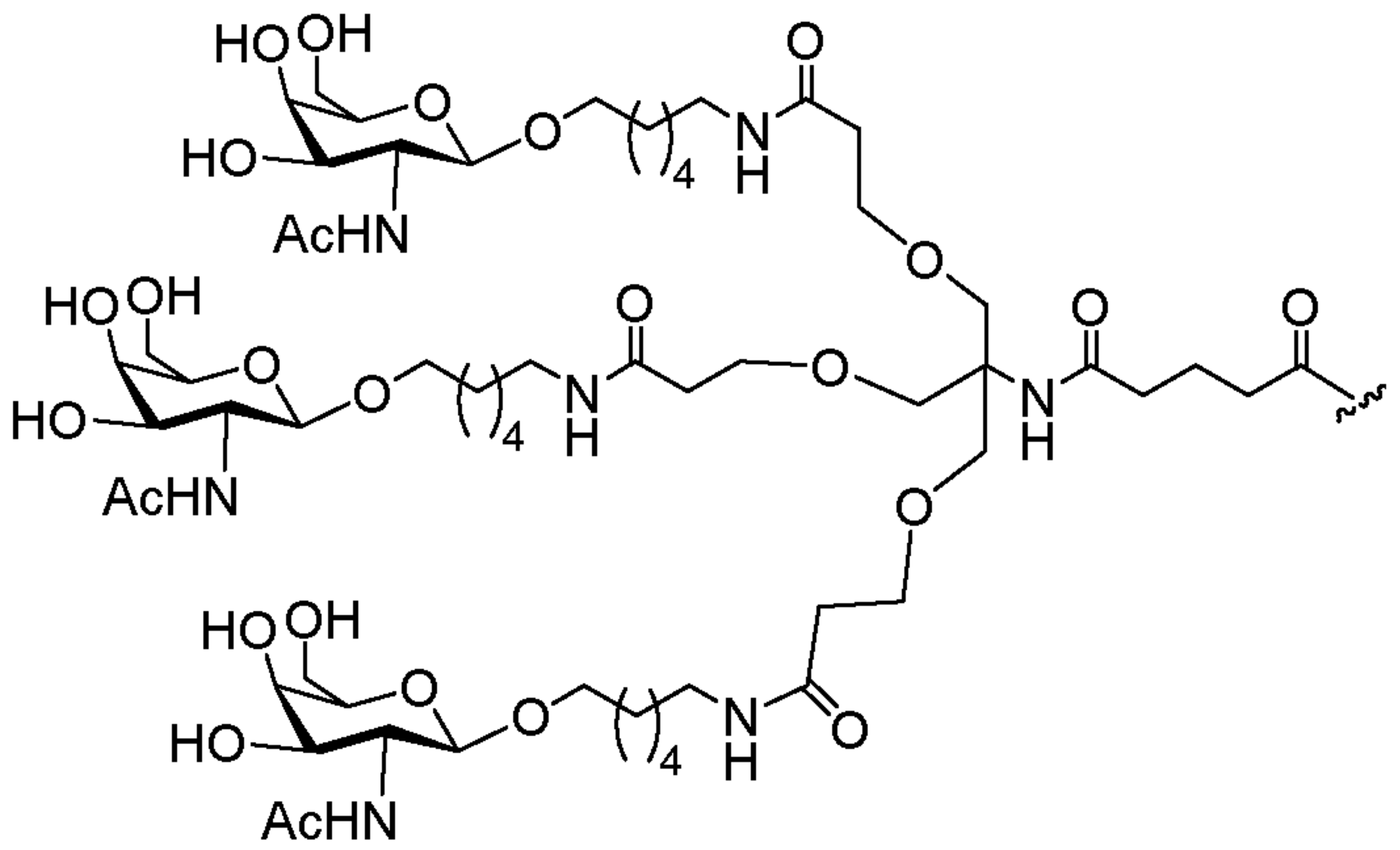
The oligonucleotides listed in Table 90 were tested for splicing modulation of human survival of motor neuron (SMN) in mice.

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	2319
699819	$\mathbf{GalNAc_3\text{-}7_{a}\text{-}_{o'}}A_{es}T_{es}T_{es}{}^{m}C_{es}A_{es}{}^{m}C_{es}T_{es}T_{es}T_{es}{}^{m}C_{es}A_{es}T_{es}A_{es}A_{es}T_{es}G_{es}{}^{m}C_{es}T_{es}G_{es}G_{e}$	$GalNAc_3$-7a	PO	2319
699821	$\mathbf{GalNAc_3\text{-}7_{a}\text{-}_{o'}}A_{es}T_{eo}T_{eo}{}^{m}C_{eo}A_{eo}{}^{m}C_{eo}T_{eo}T_{eo}T_{eo}{}^{m}C_{eo}A_{eo}T_{eo}A_{eo}A_{eo}T_{eo}G_{eo}{}^{m}C_{eo}T_{es}G_{es}G_{e}$	$GalNAc_3$-7a	PO	2319
700000	$A_{es}T_{es}T_{es}{}^{m}C_{es}A_{es}{}^{m}C_{es}T_{es}T_{es}T_{es}{}^{m}C_{es}A_{es}T_{es}A_{es}A_{es}T_{es}G_{es}{}^{m}C_{es}T_{es}G_{es}G_{eo}\mathbf{A_{do'}\text{-}GalNAc_3\text{-}1_{a}}$	$GalNAc_3$-1a	A_d	2320
703421	X-ATT^{m}CA^{m}CTTT^{m}CATAATG^{m}CTGG	n/a	n/a	2319
703422	**$GalNAc_3$-7$_b$-X-**ATT^{m}CA^{m}CTTT^{m}CATAATG^{m}CTGG	$GalNAc_3$-7b	n/a	2319

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	2319
387954	288	5.00	n/a	n/a	2319
699819	32	7.84	$GalNAc_3$-7a	PO	2319
699821	32	7.22	$GalNAc_3$-7a	PO	2319
700000	32	6.91	$GalNAc_3$-1a	A_d	2320
703421	32	1.27	n/a	n/a	2319
703422	32	4.12	$GalNAc_3$-7b	n/a	2319

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	2321
681257	$\mathbf{GalNAc_3\text{-}7_a\text{-}_{o'}}T_{es}G_{eo}{}^{m}C_{eo}T_{eo}{}^{m}C_{eo}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{eo}G_{eo}T_{es}T_{es}{}^{m}C_{e}$	$GalNAc_3$-7a	PO	2321

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$_3$ conjugate

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	2317
682883	$\mathbf{GalNAc_3\text{-}3_{a\text{-}o'}}T_{es}{}^{m}C_{eo}T_{eo}T_{eo}G_{eo}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}$	PS/PO	GalNAc$_3$-3a	PO	2317

	$T_{ds}G_{ds}A_{ds}A_{ds}A_{eo}T_{eo}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$				
666943	$\mathbf{GalNAc_3\text{-}3_{a\text{-}o'}A_{do}}T_{es}{}^{m}C_{eo}T_{eo}T_{eo}G_{eo}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{eo}T_{eo}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$	PS/PO	$GalNAc_3$-3a	A_d	2322
682887	$\mathbf{GalNAc_3\text{-}7_{a\text{-}o'}A_{do}}T_{es}{}^{m}C_{eo}T_{eo}T_{eo}G_{eo}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{eo}T_{eo}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$	PS/PO	$GalNAc_3$-7a	A_d	2322
682888	$\mathbf{GalNAc_3\text{-}10_{a\text{-}o'}A_{do}}T_{es}{}^{m}C_{eo}T_{eo}T_{eo}G_{eo}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{eo}T_{eo}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$	PS/PO	$GalNAc_3$-10a	A_d	2322
682889	$\mathbf{GalNAc_3\text{-}13_{a\text{-}o'}A_{do}}T_{es}{}^{m}C_{eo}T_{eo}T_{eo}G_{eo}G_{ds}T_{ds}T_{ds}A_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}A_{ds}A_{eo}T_{eo}{}^{m}C_{es}{}^{m}C_{es}{}^{m}C_{e}$	PS/PO	$GalNAc_3$-13a	A_d	2322

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	2323
686892	$\mathbf{GalNAc_3\text{-}10_{a\text{-}o'}}A_{es}T_{es}G_{es}{}^{m}C_{es}A_{es}T_{ds}G_{ds}G_{ds}T_{ds}G_{ds}A_{ds}T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{es}{}^{m}C_{es}T_{es}G_{es}A_{e}$	PS	$GalNAc_3$-10a	PO	2323

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 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	2324
661180	${}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}A_{eo}\mathbf{A_{do'}\text{-}GalNAc_3\text{-}1_a}$	A_d	1.40	2325
680771	$\mathbf{GalNAc_3\text{-}3_{a\text{-}o'}}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$	PO	0.70	2324
680772	$\mathbf{GalNAc_3\text{-}7_{a\text{-}o'}}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$	PO	1.70	2324
680773	$\mathbf{GalNAc_3\text{-}10_{a\text{-}o'}}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$	PO	2.00	2324
680774	$\mathbf{GalNAc_3\text{-}13_{a\text{-}o'}}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$	PO	1.50	2324
681272	$\mathbf{GalNAc_3\text{-}3_{a\text{-}o'}}{}^{m}C_{es}A_{eo}G_{eo}{}^{m}C_{eo}T_{eo}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{eo}A_{eo}G_{es}{}^{m}C_{es}A_{e}$	PO	< 0.46	2324
681273	$\mathbf{GalNAc_3\text{-}3_{a\text{-}o'}A_{do}}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$	A_d	1.10	2324
683733	${}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}A_{eo}\mathbf{A_{do'}\text{-}GalNAc_3\text{-}19_a}$	A_d	2.50	2325

The structure of $GalNAc_3\text{-}1_a$ was shown previously in Example 9, $GalNAc_3\text{-}3_a$ was shown in Example 39, $GalNAc_3\text{-}7_a$ was shown in Example 48, $GalNAc_3\text{-}10_a$ was shown in Example 46, $GalNAc_3\text{-}13_a$ was shown in Example 62, and $GalNAc_3\text{-}19_a$ 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	2326
699806	$\mathbf{GalNAc_3\text{-}3_{a\text{-}o'}}T_{ks}T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{ks}{}^{m}C_{k}$	$GalNAc_3$-3a	PO	2326
699807	$\mathbf{GalNAc_3\text{-}7_{a\text{-}o'}}T_{ks}T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{ks}{}^{m}C_{k}$	$GalNAc_3$-7a	PO	2326

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	2326
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	2326
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	2326
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	2326

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 are 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	2304
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	2327
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	2304
700991	**GalNAc$_3$-7$_{a\text{-}o'}$**$G_{ms}C_{ms}U_{ms}U_{ms}C_{ms}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}U_{ms}C_{ms}C_{ms}U_{ms}U_{m}$	GalNAc$_3$-7a	PO	2327

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	2298
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	2298
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	2298
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	2298
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	2299
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	2298
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	2298

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	2321
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	2321
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	2321
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	2321

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	$\mathbf{GalNAc_3\text{-}3_{a\text{-}o'}A_{do}}\ T_{es}\ {}^{m}C_{es}\ T_{es}\ T_{es}\ G_{es}\ G_{ds}\ T_{ds}\ T_{ds}\ A_{ds}\ {}^{m}C_{ds}\ A_{ds}\ T_{ds}\ G_{ds}\ A_{ds}\ A_{ds}\ A_{es}\ T_{es}\ {}^{m}C_{es}\ {}^{m}C_{es}\ {}^{m}C_{e}$	GalNAc$_3$-3	A$_d$	2322
666942	$T_{es}\ {}^{m}C_{eo}\ T_{eo}\ T_{eo}\ G_{eo}\ G_{ds}\ T_{ds}\ T_{ds}\ A_{ds}\ {}^{m}C_{ds}\ A_{ds}\ T_{ds}\ G_{ds}\ A_{ds}\ A_{ds}\ A_{eo}\ T_{eo}\ {}^{m}C_{es}\ {}^{m}C_{es}\ {}^{m}C_{eo}\ \mathbf{A_{do'}\text{-}GalNAc_3\text{-}3_a}$	GalNAc$_3$-1	A$_d$	2318
682876	$\mathbf{GalNAc_3\text{-}3_{a\text{-}o'}}T_{es}\ {}^{m}C_{es}\ T_{es}\ T_{es}\ G_{es}\ G_{ds}\ T_{ds}\ T_{ds}\ A_{ds}\ {}^{m}C_{ds}\ A_{ds}\ T_{ds}\ G_{ds}\ A_{ds}\ A_{ds}\ A_{es}\ T_{es}\ {}^{m}C_{es}\ {}^{m}C_{es}\ {}^{m}C_{e}$	GalNAc$_3$-3	PO	2317
682877	$\mathbf{GalNAc_3\text{-}7_{a\text{-}o'}}T_{es}\ {}^{m}C_{es}\ T_{es}\ T_{es}\ G_{es}\ G_{ds}\ T_{ds}\ T_{ds}\ A_{ds}\ {}^{m}C_{ds}\ A_{ds}\ T_{ds}\ G_{ds}\ A_{ds}\ A_{ds}\ A_{es}\ T_{es}\ {}^{m}C_{es}\ {}^{m}C_{es}\ {}^{m}C_{e}$	GalNAc$_3$-7	PO	2317
682878	$\mathbf{GalNAc_3\text{-}10_{a\text{-}o'}}T_{es}\ {}^{m}C_{es}\ T_{es}\ T_{es}\ G_{es}\ G_{ds}\ T_{ds}\ T_{ds}\ A_{ds}\ {}^{m}C_{ds}\ A_{ds}\ T_{ds}\ G_{ds}\ A_{ds}\ A_{ds}\ A_{es}\ T_{es}\ {}^{m}C_{es}\ {}^{m}C_{es}\ {}^{m}C_{e}$	GalNAc$_3$-10	PO	2317
682879	$\mathbf{GalNAc_3\text{-}13_{a\text{-}o'}}T_{es}\ {}^{m}C_{es}\ T_{es}\ T_{es}\ G_{es}\ G_{ds}\ T_{ds}\ T_{ds}\ A_{ds}\ {}^{m}C_{ds}\ A_{ds}\ T_{ds}\ G_{ds}\ A_{ds}\ A_{ds}\ A_{es}\ T_{es}\ {}^{m}C_{es}\ {}^{m}C_{es}\ {}^{m}C_{e}$	GalNAc$_3$-13	PO	2317
682880	$\mathbf{GalNAc_3\text{-}7_{a\text{-}o'}A_{do}}\ T_{es}\ {}^{m}C_{es}\ T_{es}\ T_{es}\ G_{es}\ G_{ds}\ T_{ds}\ T_{ds}\ A_{ds}\ {}^{m}C_{ds}\ A_{ds}\ T_{ds}\ G_{ds}\ A_{ds}\ A_{ds}\ A_{es}\ T_{es}\ {}^{m}C_{es}\ {}^{m}C_{es}\ {}^{m}C_{e}$	GalNAc$_3$-7	A$_d$	2322
682881	$\mathbf{GalNAc_3\text{-}10_{a\text{-}o'}A_{do}}\ T_{es}\ {}^{m}C_{es}\ T_{es}\ T_{es}\ G_{es}\ G_{ds}\ T_{ds}\ T_{ds}\ A_{ds}\ {}^{m}C_{ds}\ A_{ds}\ T_{ds}\ G_{ds}\ A_{ds}\ A_{ds}\ A_{es}\ T_{es}\ {}^{m}C_{es}\ {}^{m}C_{es}\ {}^{m}C_{e}$	GalNAc$_3$-10	A$_d$	2322
682882	$\mathbf{GalNAc_3\text{-}13_{a\text{-}o'}A_{do}}\ T_{es}\ {}^{m}C_{es}\ T_{es}\ T_{es}\ G_{es}\ G_{ds}\ T_{ds}\ T_{ds}\ A_{ds}\ {}^{m}C_{ds}\ A_{ds}\ T_{ds}\ G_{ds}\ A_{ds}\ A_{ds}\ A_{es}\ T_{es}\ {}^{m}C_{es}\ {}^{m}C_{es}\ {}^{m}C_{e}$	GalNAc$_3$-13	A$_d$	2322
684056	$T_{es}\ {}^{m}C_{es}\ T_{es}\ T_{es}\ G_{es}\ G_{ds}\ T_{ds}\ T_{ds}\ A_{ds}\ {}^{m}C_{ds}\ A_{ds}\ T_{ds}\ G_{ds}\ A_{ds}\ A_{ds}\ A_{es}\ T_{es}\ {}^{m}C_{es}\ {}^{m}C_{es}\ {}^{m}C_{eo}\ \mathbf{A_{do'}\text{-}GalNAc_3\text{-}19_a}$	GalNAc$_3$-19	A$_d$	2318

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 γ-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	$\mathbf{GalNAc_3\text{-}7_{a}\text{-}_{o'}}T_{es}G_{es}{}^{m}C_{es}T_{es}{}^{m}C_{es}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{es}G_{es}T_{es}T_{es}{}^{m}C_{e}$	$GalNAc_3$-7a	PO	2321
681257	$\mathbf{GalNAc_3\text{-}7_{a}\text{-}_{o'}}T_{es}G_{eo}{}^{m}C_{eo}T_{eo}{}^{m}C_{eo}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{eo}G_{eo}T_{es}T_{es}{}^{m}C_{e}$	$GalNAc_3$-7a	PO	2321

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	2324
			6	86	
			20	59	
			60	37	
680772	**GalNAc$_3$-7$_{a\text{-}o'}$**$^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$	PO	0.6	79	2324
			2	58	
			6	31	
			20	13	
696847	**GalNAc$_3$-7$_{a\text{-}s'}$**$^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}T_{es}T_{ds}T_{ds}A_{ds}T_{ds}T_{ds}A_{ds}G_{ds}G_{ds}G_{ds}A_{ds}{}^{m}C_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$	n/a (PS)	0.6	83	2324
			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	2296
663084	$\mathbf{GalNAc_3\text{-}3_a\text{-}_{o'}A_{do}}A_{es}G_{eo}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{eo}T_{eo}T_{es}A_{es}T_{e}$	GalNAc$_3$-3a	A_d	2312
679241	$A_{es}G_{eo}{}^{m}C_{eo}T_{eo}T_{eo}{}^{m}C_{ds}T_{ds}T_{ds}G_{ds}T_{ds}{}^{m}C_{ds}{}^{m}C_{ds}A_{ds}G_{ds}{}^{m}C_{ds}T_{eo}T_{eo}T_{es}A_{es}T_{eo}\mathbf{A_{do'}\text{-}GalNAc_3\text{-}19_a}$	GalNAc$_3$-19a	A_d	2297

The structure of GalNAc$_3$-3$_a$ was shown in Example 39, and GalNAc$_3$-19$_a$ was shown in Example 70.

Treatment

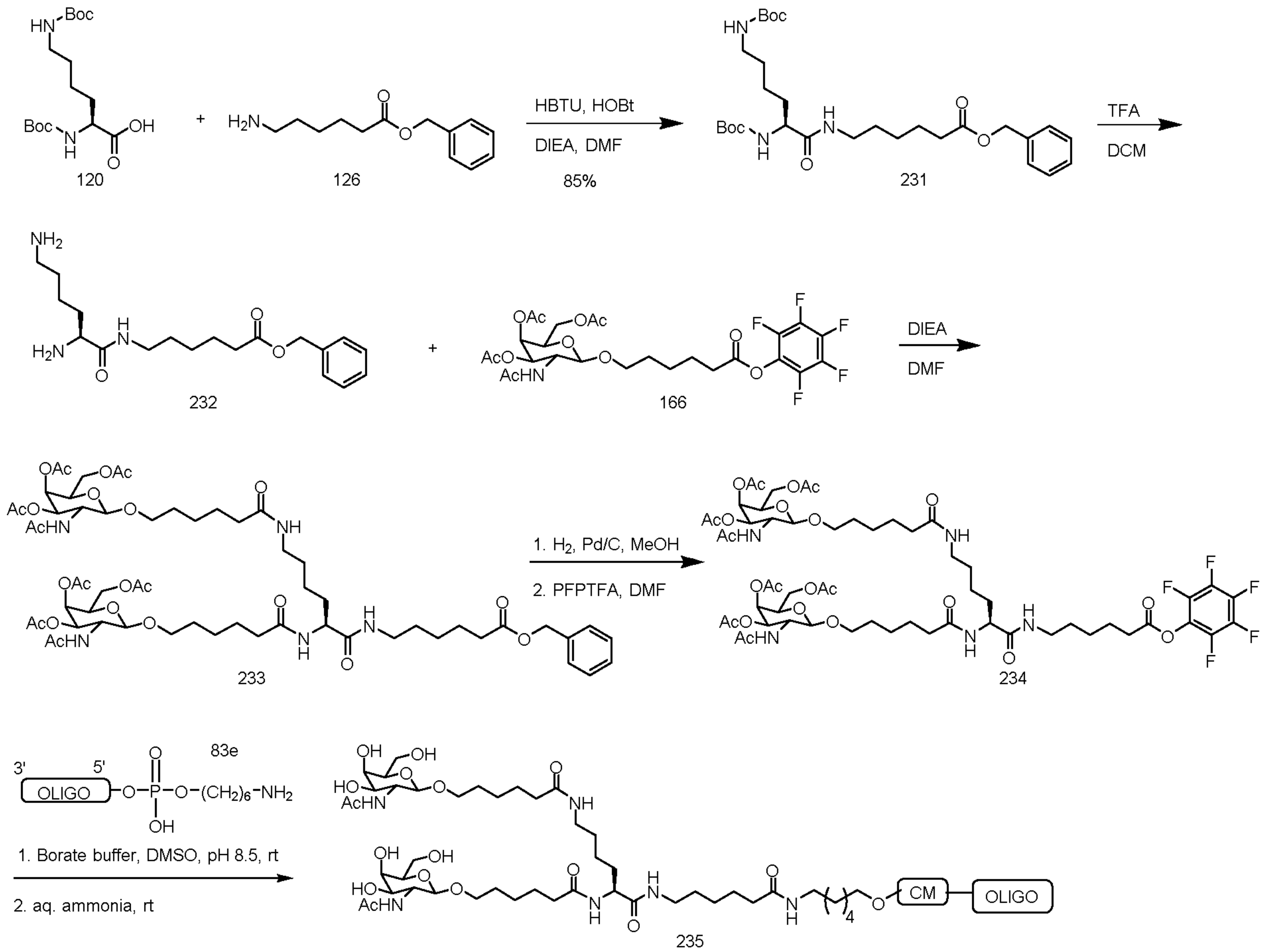
Female transgenic mice that express human APOC-III were each injected subcutaneously once with an oligonucleotide listed in Table 114 or with PBS. Each treatment group consisted of 3 animals. Blood was drawn before dosing to determine baseline and at 3, 7, 14, 21, 28, 35, and 42 days following the dose. 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

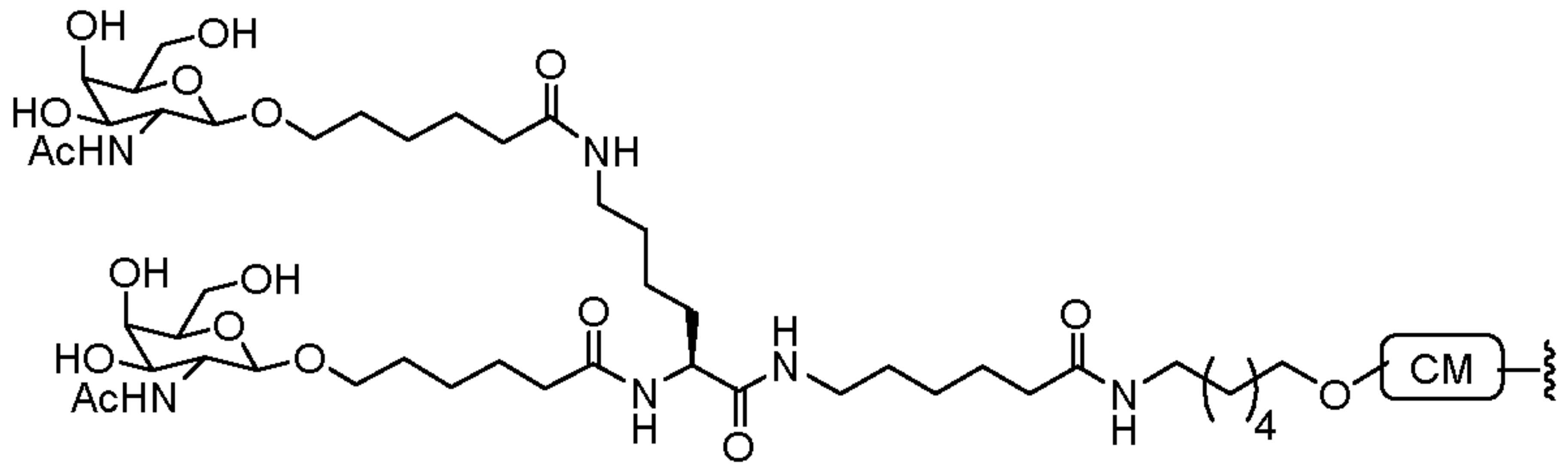
ISIS No.	Dosage (mg/kg)	Time point (days post-dose)	Triglycerides (% baseline)	APOC-III protein (% baseline)	$GalNAc_3$ Cluster	CM
PBS	n/a	3	96	101	n/a	n/a
		7	88	98		
		14	91	103		
		21	69	92		
		28	83	81		
		35	65	86		
		42	72	88		
304801	30	3	42	46	n/a	n/a
		7	42	51		
		14	59	69		
		21	67	81		
		28	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

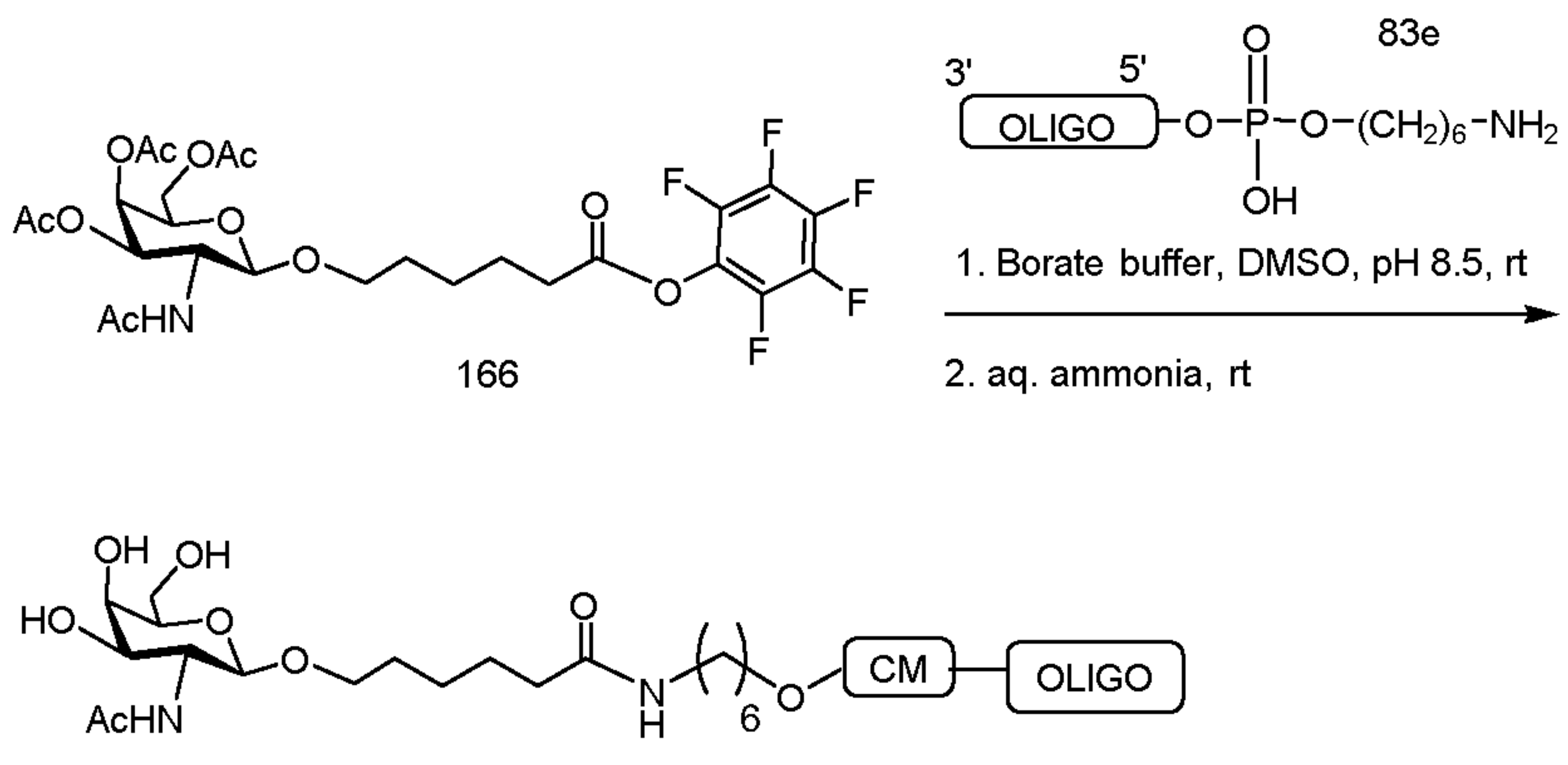


Compound 120 is commercially available, and the synthesis of compound 126 is described in Example 49. Compound 120 (1 g, 2.89 mmol), HBTU (0.39 g, 2.89 mmol), and HOBt (1.64 g, 4.33 mmol) were dissolved in DMF (10 mL. and *N,N*-diisopropylethylamine (1.75 mL, 10.1 mmol) were added. After about 5 min, 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:

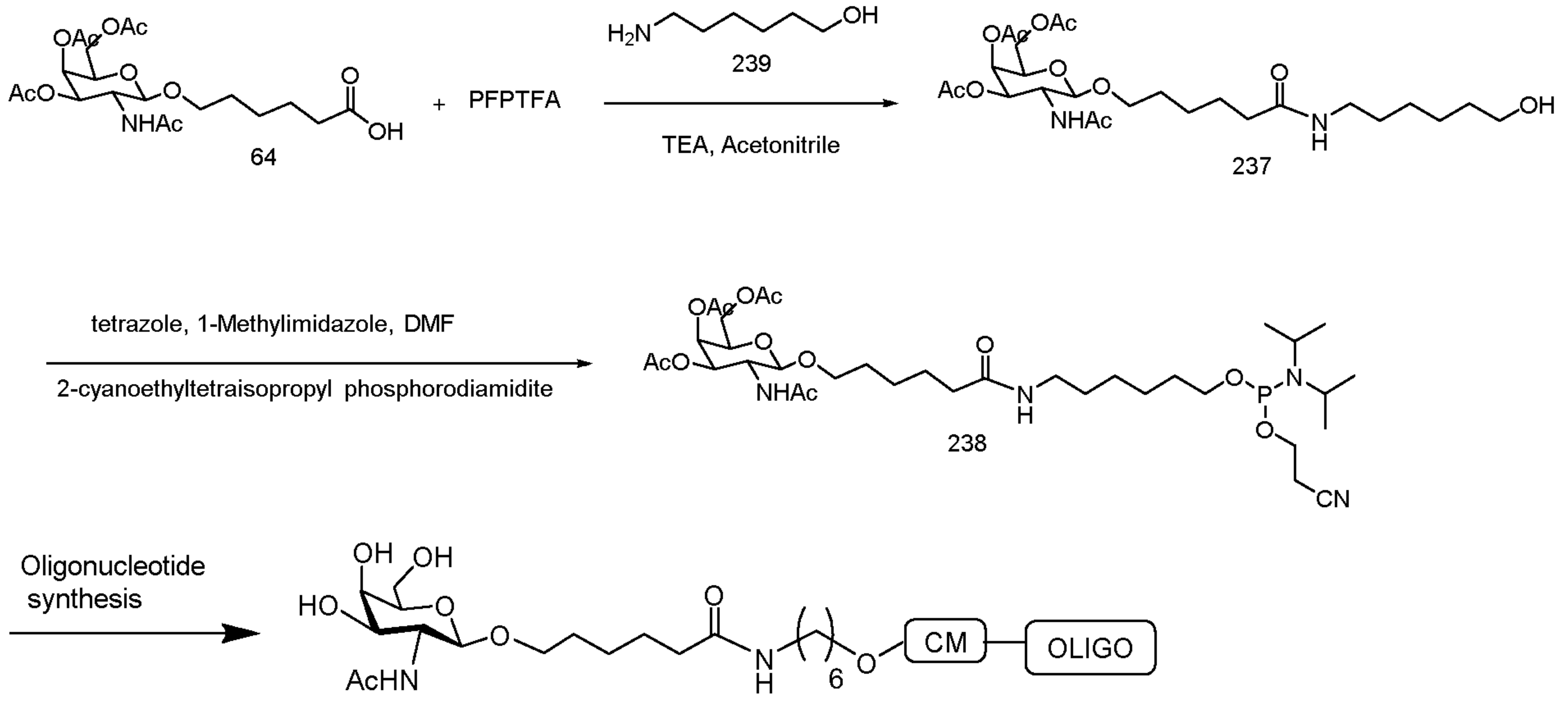


Example 105: Synthesis of oligonucleotides comprising a $GalNAc_1$-25 conjugate



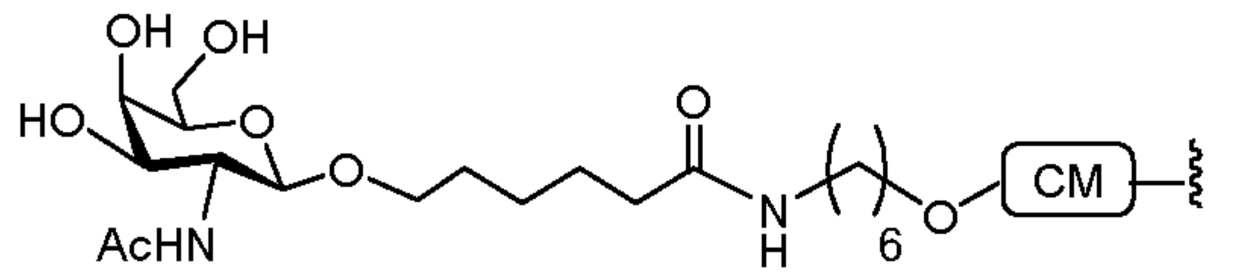
236

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.



236

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	2298
686221	$\mathbf{GalNAc_2\text{-}24_{a}\text{-}_{o'}A_{do}}T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{k}$	$GalNAc_2$-24_a	0.39	2302
686222	$\mathbf{GalNAc_3\text{-}13_{a}\text{-}_{o'}A_{do}}T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{k}$	$GalNAc_3$-13_a	0.41	2302

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	2298
708561	$\mathbf{GalNAc_1\text{-}25_{a}\text{-}_{o'}}T_{ks}{}^{m}C_{ks}A_{ds}G_{ds}T_{ds}{}^{m}C_{ds}A_{ds}T_{ds}G_{ds}A_{ds}{}^{m}C_{ds}T_{ds}T_{ks}{}^{m}C_{k}$	$GalNAc_1$-25_a	0.4	2298

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		

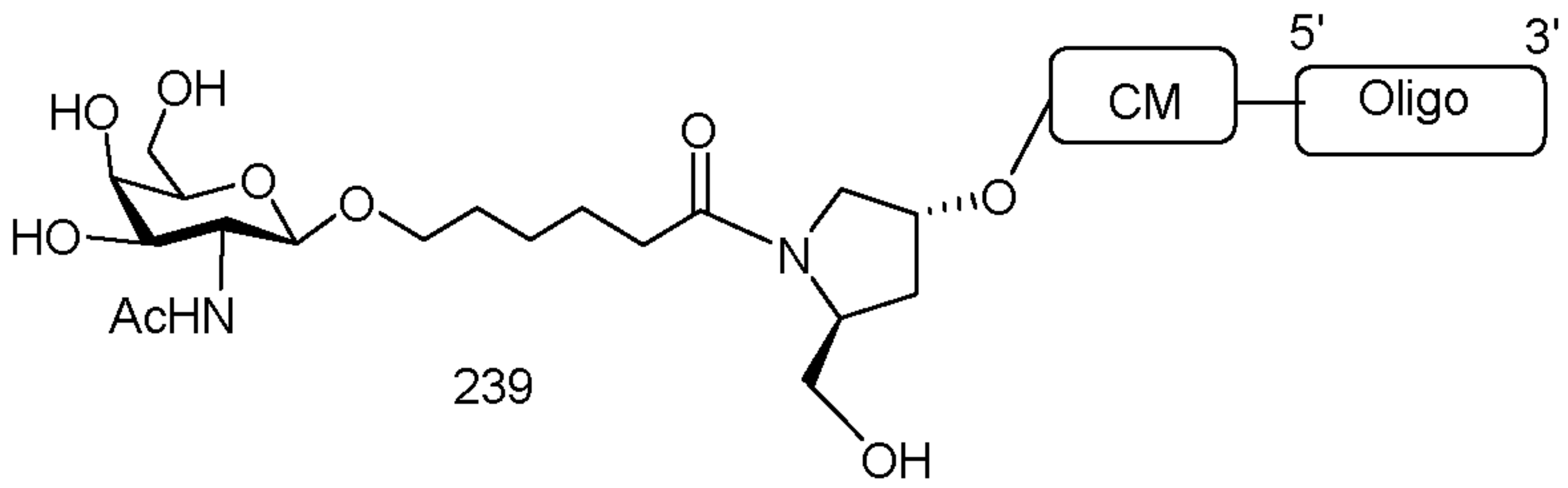
	6	26.5		
686222	0.2	0.5	GalNAc$_3$-13$_a$	A$_d$
	0.6	1.6		
	2	11.6		
	6	19.8		

Table 117b

Liver concentrations of oligonucleotides comprising a GalNAc$_1$ conjugate group

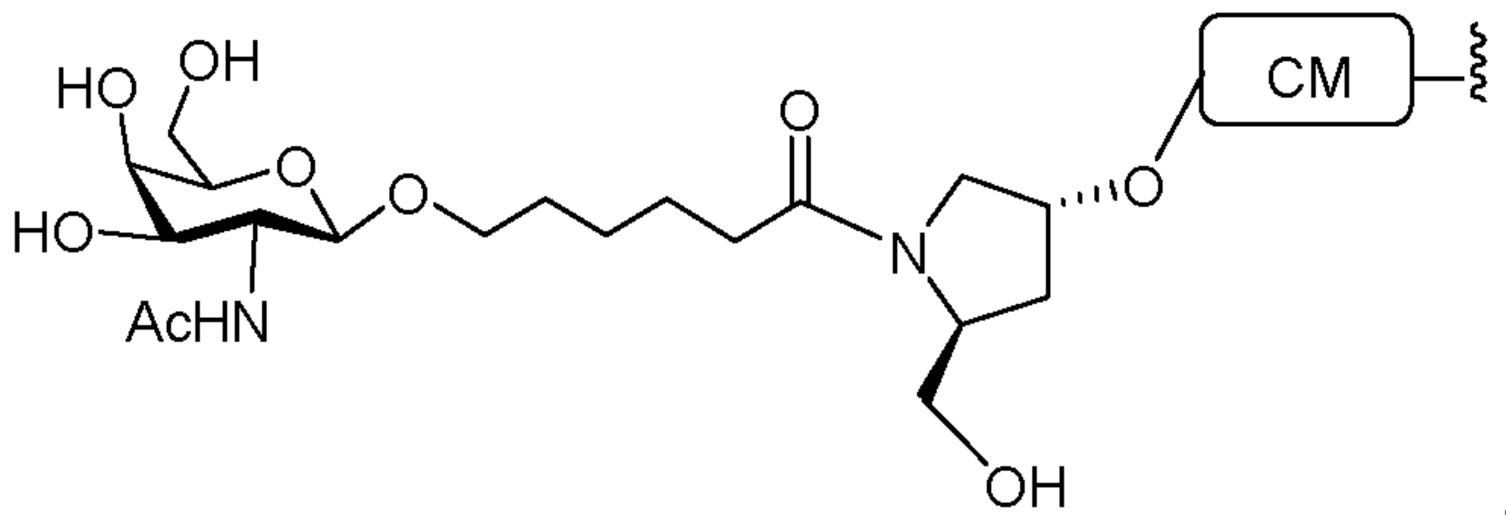
ISIS No.	Dosage (mg/kg)	Antisense oligonucleotide (μg/g)	GalNAc cluster	CM
440762	2	2.3	n/a	n/a
	7	8.9		
	20	23.7		
708561	0.2	0.4	GalNAc$_1$-25$_a$	PO
	0.6	1.1		
	2	5.9		
	6	23.7		
	20	53.9		

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:



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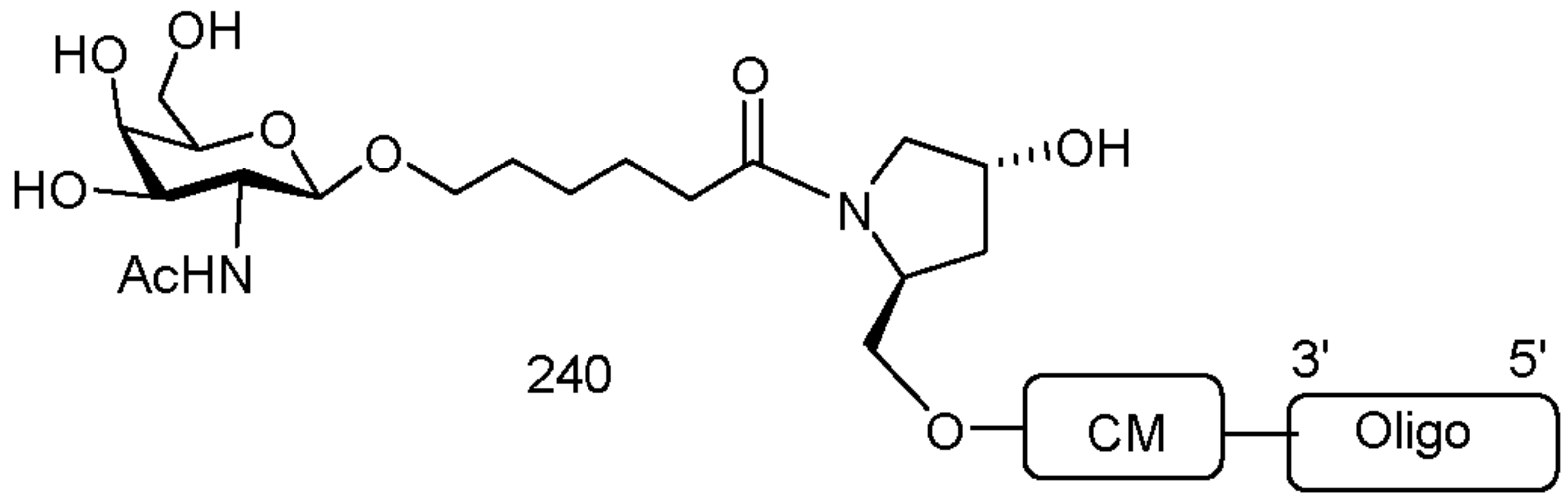
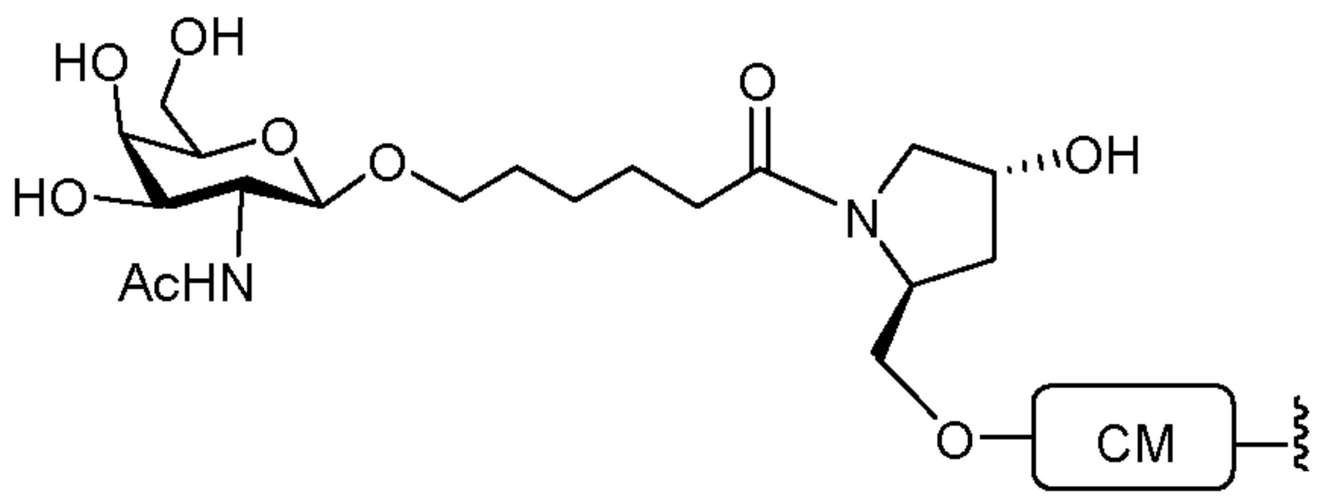
In order to add the $GalNAc_1$ conjugate group to the 3'-end of an oligonucleotide, the amide formed from the reaction of compounds 47 and 64 is added to a solid support using procedures described in Example 7. The oligonucleotide synthesis is then completed using procedures described in Example 9 in 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	2321
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	2321
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	2321
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	2321

681257	$\mathbf{GalNAc_3\text{-}7_{a}\text{-}_{o'}}T_{es}G_{eo}{}^{m}C_{eo}T_{eo}{}^{m}C_{eo}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{eo}G_{eo}T_{es}T_{es}{}^{m}C_{e}$	$GalNAc_3$-7a	PO	2321
681258	$\mathbf{GalNAc_3\text{-}13_{a}\text{-}_{o'}}T_{es}G_{eo}{}^{m}C_{eo}T_{eo}{}^{m}C_{eo}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{eo}G_{eo}T_{es}T_{es}{}^{m}C_{e}$	$GalNAc_3$-13a	PO	2321
681260	$T_{es}G_{eo}{}^{m}C_{eo}T_{eo}{}^{m}C_{eo}{}^{m}C_{ds}G_{ds}T_{ds}T_{ds}G_{ds}G_{ds}T_{ds}G_{ds}{}^{m}C_{ds}T_{ds}T_{eo}G_{eo}T_{es}T_{es}{}^{m}C_{eo}\mathbf{A_{do'}\text{-}GalNAc_3\text{-}19}$	$GalNAc_3$-19a	A_d	2328

The structure of $GalNAc_3$-7_a was shown in Example 48.

Treatment

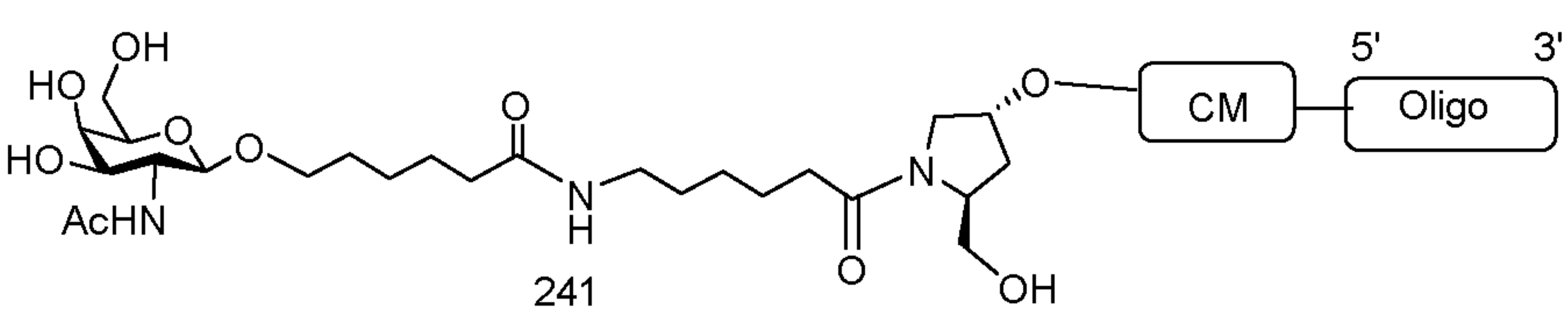
Male transgenic mice that express human Apo(a) were each injected subcutaneously once with an oligonucleotide and dosage listed in Table 119 or with PBS. Each treatment group consisted of 4 animals. Blood was drawn the day before dosing to determine baseline levels of Apo(a) protein in plasma and at 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

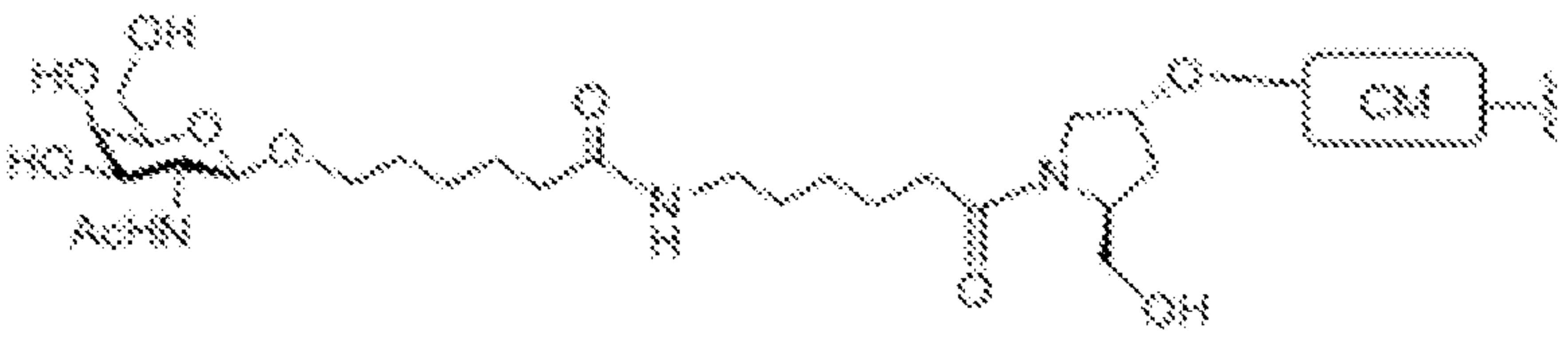
ISIS No.	Dosage (mg/kg)	Apo(a) at 1 week (% BL)
PBS	n/a	143
494372	50	58
681251	10	15
681255	10	14
681256	10	17
681257	10	24
681258	10	22
681260	10	26

Example 109: Synthesis of oligonucleotides comprising a $GalNAc_1$-28 or $GalNAc_1$-29 conjugate

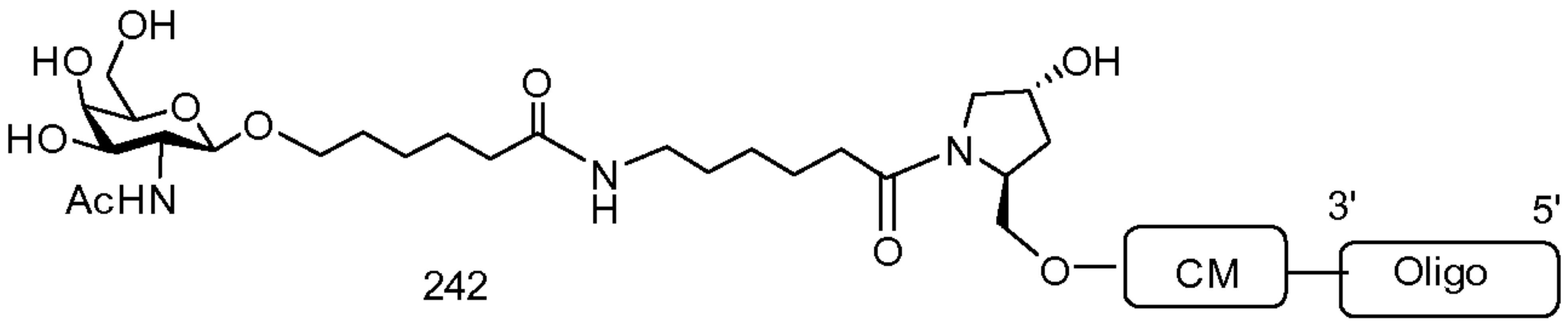


241

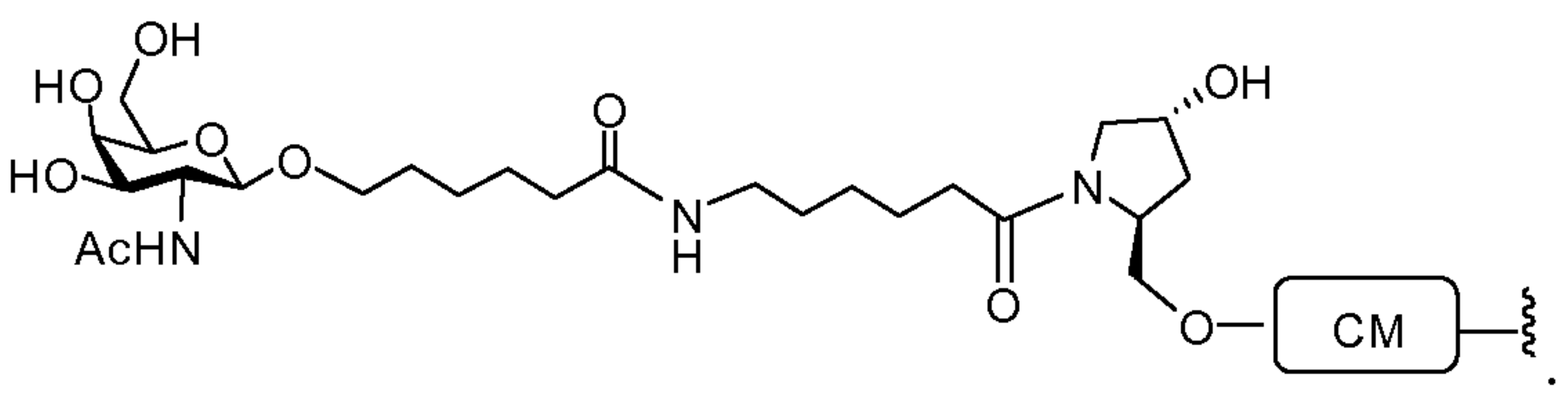
Oligonucleotide 241 is synthesized using procedures similar to those described in Example 71 to form the phosphoramidite intermediate, followed by procedures described in Example 10 to synthesize the oligonucleotide. The $GalNAc_1$ cluster portion ($GalNAc_1$-28_a) of the conjugate group $GalNAc_1$-28 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:



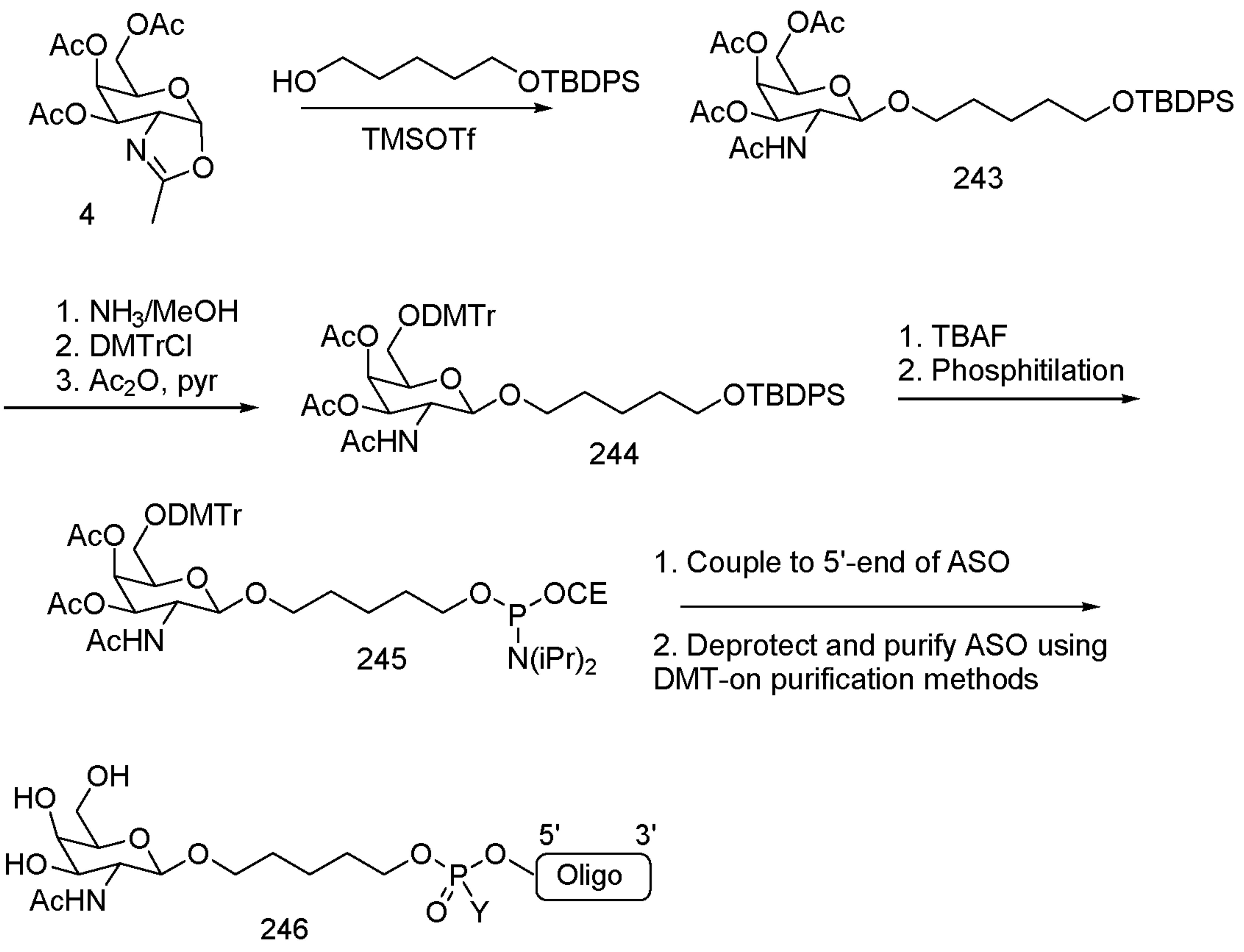
In order to add the GalNAc$_1$ conjugate group to the 3'-end of an oligonucleotide, procedures similar to those described in Example 71 are used to form the hydroxyl intermediate, which is then added to the solid support using procedures described in Example 7. The oligonucleotide synthesis is then completed using procedures described in Example 9 in order to form oligonucleotide 242.



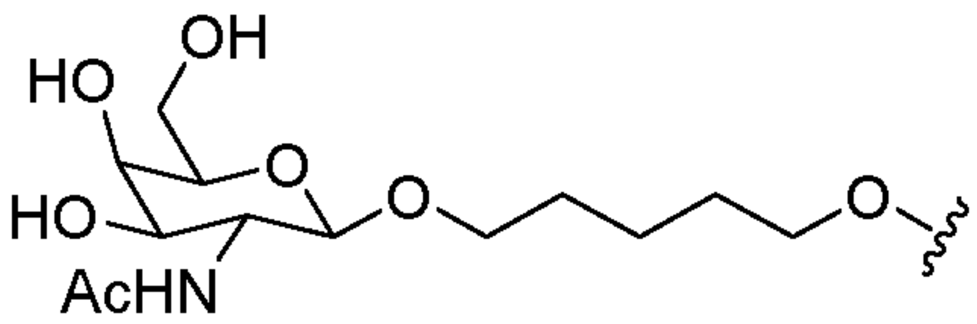
The GalNAc$_1$ cluster portion (GalNAc$_1$-29$_a$) of the conjugate group GalNAc$_1$-29 can be combined with any cleavable moiety present on the oligonucleotide to provide a variety of conjugate groups. The structure of GalNAc$_1$-29 (GalNAc$_1$-29$_a$-CM) is shown below:



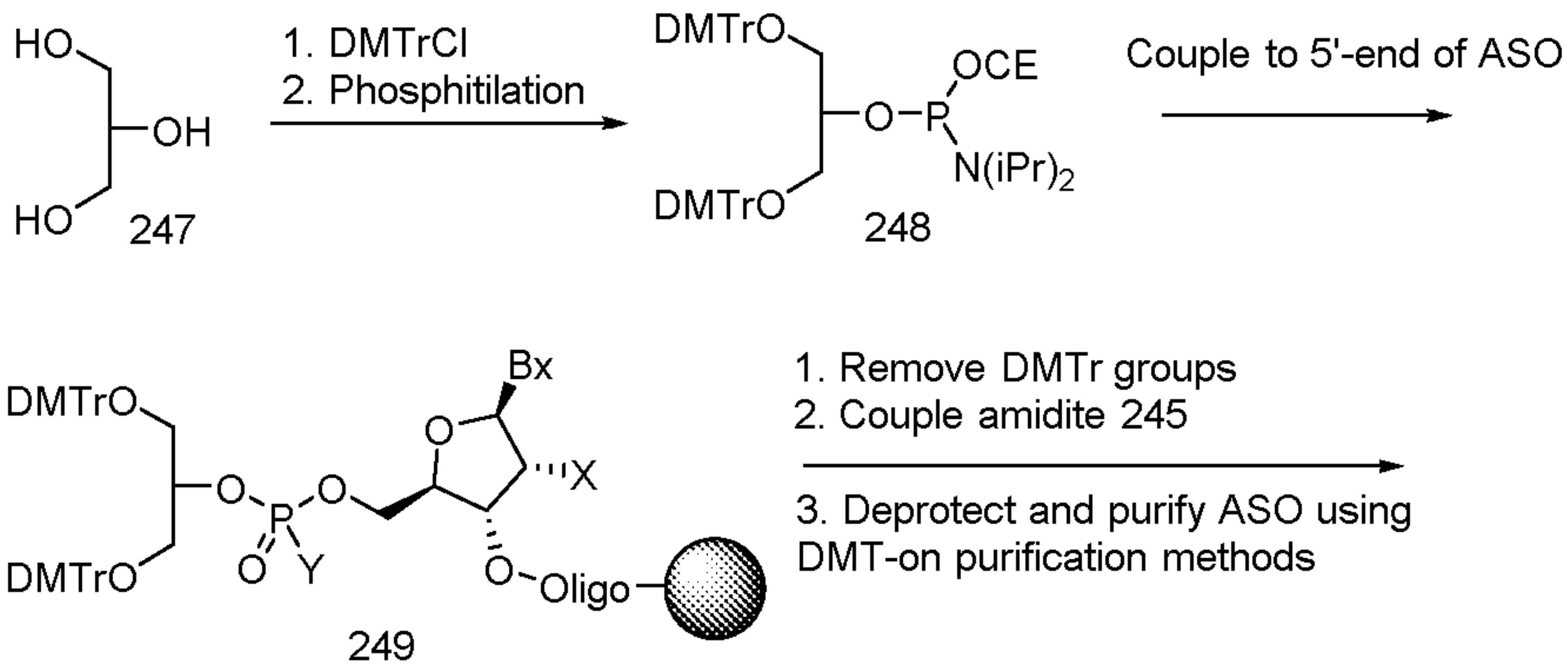
Example 110: Synthesis of oligonucleotides comprising a GalNAc$_1$-30 conjugate

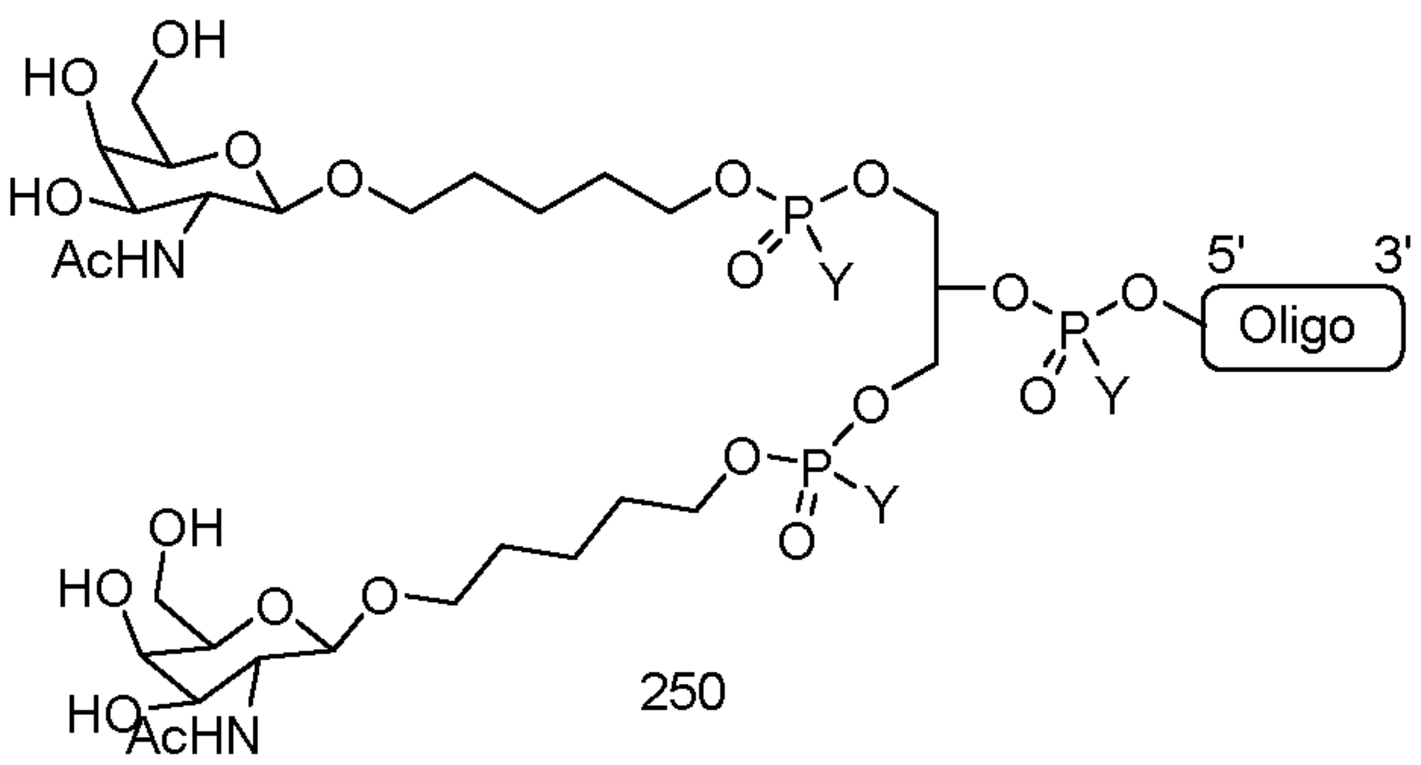


Oligonucleotide 246 comprising a $GalNAc_1$-30 conjugate group, wherein Y is selected from O, S, a substituted or unsubstituted C_1-C_{10} alkyl, amino, substituted amino, azido, alkenyl or alkynyl, is synthesized as shown above. The $GalNAc_1$ cluster portion ($GalNAc_1$-30_a) of the conjugate group $GalNAc_1$-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:

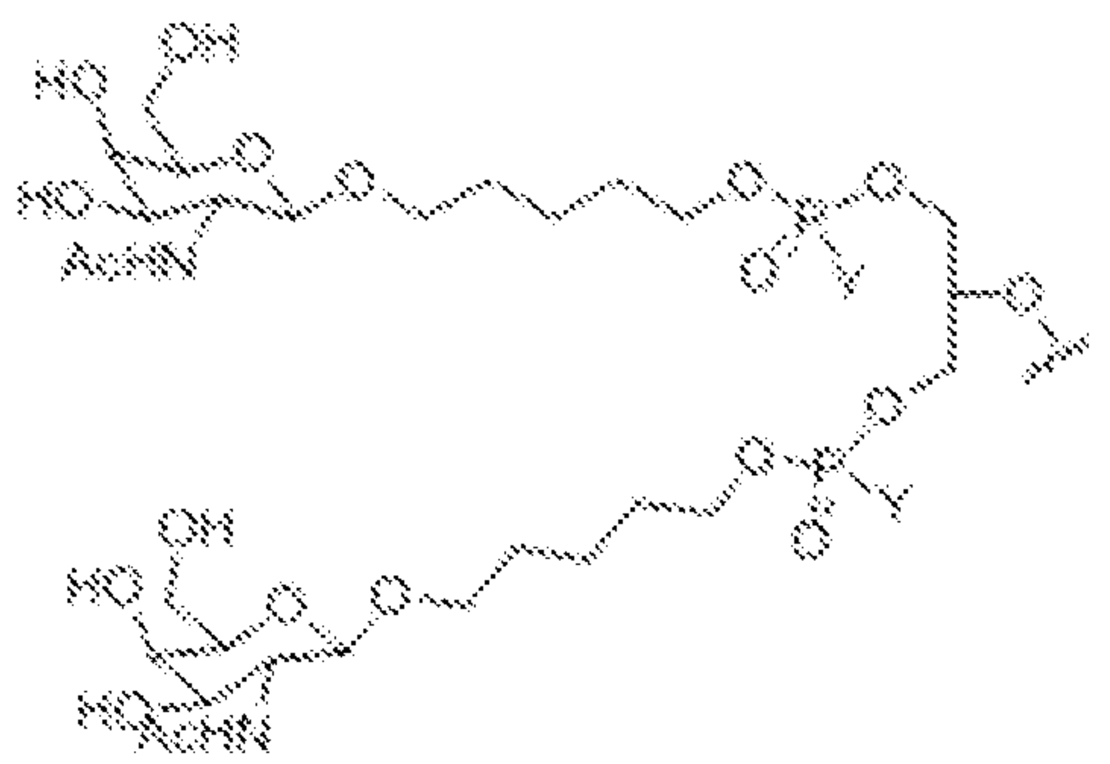


Example 111: Synthesis of oligonucleotides comprising a $GalNAc_2$-31 or $GalNAc_2$-32 conjugate

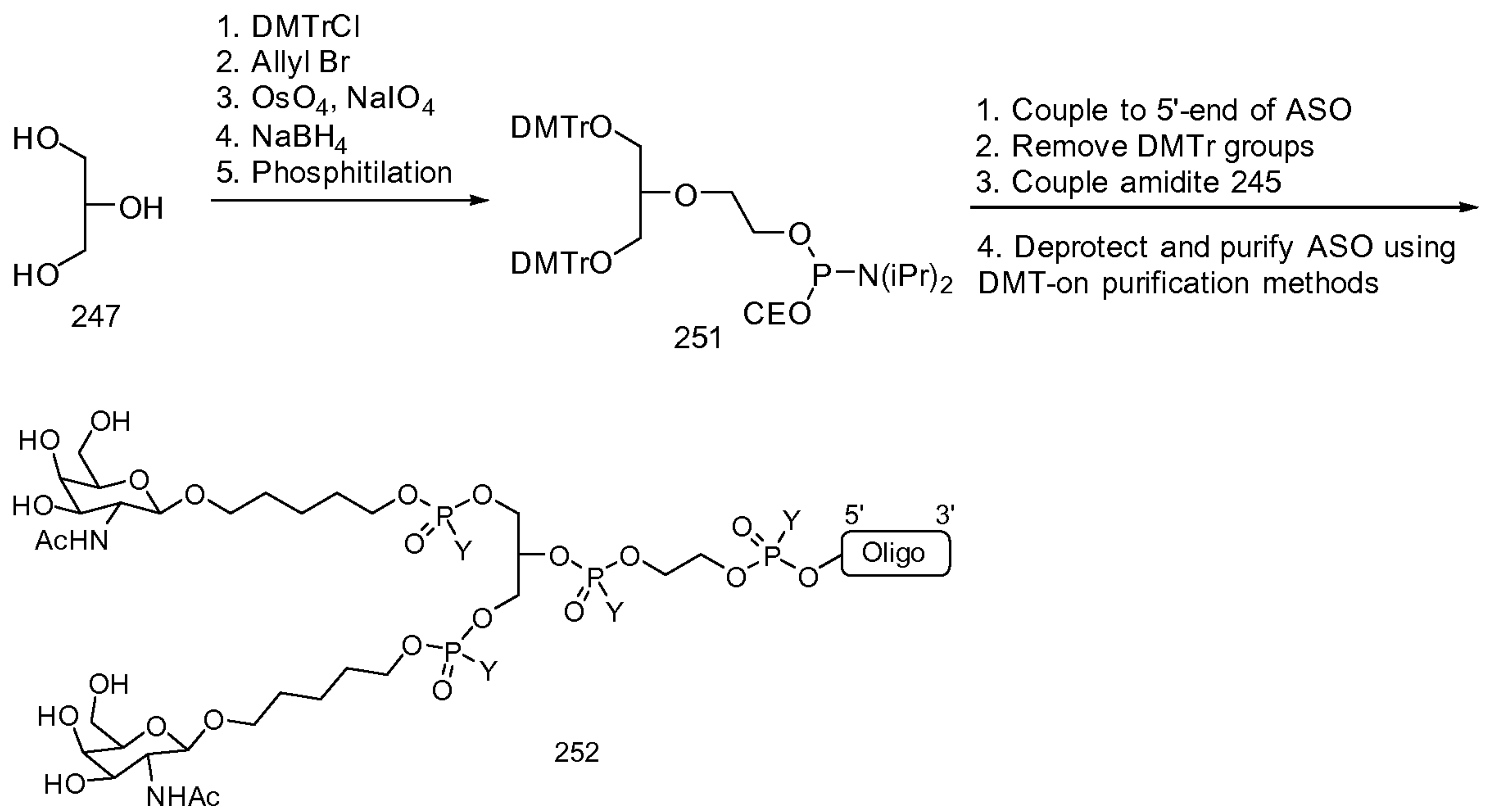




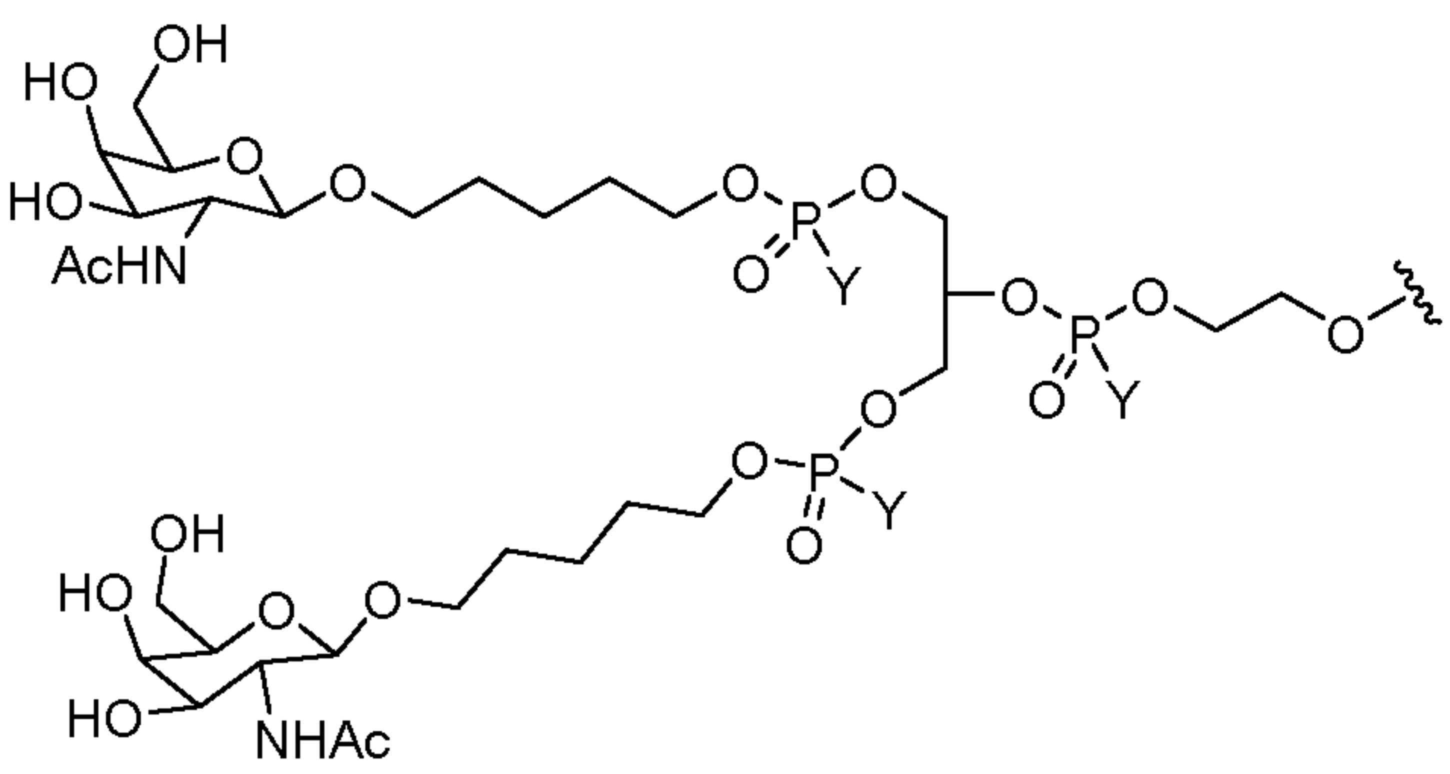
Oligonucleotide 250 comprising a $GalNAc_2$-31 conjugate group, wherein Y is selected from O, S, a substituted or unsubstituted C_1-C_{10} alkyl, amino, substituted amino, azido, alkenyl or alkynyl, is synthesized as shown above. The $GalNAc_2$ cluster portion ($GalNAc_2$-31_a) of the conjugate group $GalNAc_2$-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$_{a-o'}$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$_1$-25$_a$	A$_d$	2306
711462	**GalNAc$_1$-25$_{a-o'}$**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$_1$-25$_a$	PO	2304
711463	**GalNAc$_1$-25$_{a-o'}$**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$_1$-25$_a$	PO	2304
711465	**GalNAc$_1$-26$_{a-o'}$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$_1$-26$_a$	A$_d$	2306
711466	**GalNAc$_1$-26$_{a-o'}$**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$_1$-26$_a$	PO	2304
711467	**GalNAc$_1$-26$_{a-o'}$**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$_1$-26$_a$	PO	2304
711468	**GalNAc$_1$-28$_{a-o'}$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$_1$-28$_a$	A$_d$	2306
711469	**GalNAc$_1$-28$_{a-o'}$**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$_1$-28$_a$	PO	2304
711470	**GalNAc$_1$-28$_{a-o'}$**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$_1$-28$_a$	PO	2304
713844	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$_{eo'}$-**GalNAc$_1$-27$_a$**	GalNAc$_1$-27$_a$	PO	2304
713845	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$_{eo'}$-**GalNAc$_1$-27$_a$**	GalNAc$_1$-27$_a$	PO	2304
713846	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$_1$-27$_a$**	GalNAc$_1$-27$_a$	A$_d$	2305
713847	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$_{eo'}$-**GalNAc$_1$-29$_a$**	GalNAc$_1$-29$_a$	PO	2304

713848	$G_{es}\ {}^{m}C_{eo}\ T_{eo}\ T_{eo}\ {}^{m}C_{eo}\ A_{ds}\ G_{ds}\ T_{ds}\ {}^{m}C_{ds}\ A_{ds}\ T_{ds}\ G_{ds}\ A_{ds}\ {}^{m}C_{ds}\ T_{ds}\ T_{eo}\ {}^{m}C_{eo}\ {}^{m}C_{es}\ T_{es}\ T_{eo'}$-**GalNAc$_1-29_a$**	GalNAc$_1$-29$_a$	PO	2304
713849	$G_{es}\ {}^{m}C_{es}\ T_{es}\ T_{es}\ {}^{m}C_{es}\ A_{ds}\ G_{ds}\ T_{ds}\ {}^{m}C_{ds}\ A_{ds}\ T_{ds}\ G_{ds}\ A_{ds}\ {}^{m}C_{ds}\ T_{ds}\ T_{es}\ {}^{m}C_{es}\ {}^{m}C_{es}\ T_{es}\ T_{eo}$ **$A_{do'}$-GalNAc$_1$-29$_a$**	GalNAc$_1$-29$_a$	A_d	2305
713850	$G_{es}\ {}^{m}C_{eo}\ T_{eo}\ T_{eo}\ {}^{m}C_{eo}\ A_{ds}\ G_{ds}\ T_{ds}\ {}^{m}C_{ds}\ A_{ds}\ T_{ds}\ G_{ds}\ A_{ds}\ {}^{m}C_{ds}\ T_{ds}\ T_{eo}\ {}^{m}C_{eo}\ {}^{m}C_{es}\ T_{es}\ T_{eo}$ **$A_{do'}$-GalNAc$_1$-29$_a$**	GalNAc$_1$-29$_a$	A_d	2305

Example 113: Antisense oligonucleotides targeting growth hormone receptor and comprising a GalNAc cluster

The oligonucleotides in Table 121 were designed to target human growth hormone receptor (GHR).

Table 121

Sequences (5' to 3')	SEQ ID No.
GalNAc$_3$-3-${}^{m}C_{es}{}^{m}C_{es}A_{es}{}^{m}C_{es}{}^{m}C_{es}T_{ds}T_{ds}T_{ds}G_{ds}G_{ds}G_{ds}T_{ds}G_{ds}A_{ds}A_{ds}T_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$	703
GalNAc$_3$-3- ${}^{m}C_{es}{}^{m}C_{eo}A_{eo}{}^{m}C_{eo}{}^{m}C_{eo}T_{ds}T_{ds}T_{ds}G_{ds}G_{ds}G_{ds}T_{ds}G_{ds}A_{ds}A_{ds}T_{eo}A_{eo}G_{es}{}^{m}C_{es}A_{e}$	703
GalNAc$_3$-7- ${}^{m}C_{es}{}^{m}C_{es}A_{es}{}^{m}C_{es}{}^{m}C_{es}T_{ds}T_{ds}T_{ds}G_{ds}G_{ds}G_{ds}T_{ds}G_{ds}A_{ds}A_{ds}T_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$	703
GalNAc$_3$-7- ${}^{m}C_{es}{}^{m}C_{eo}A_{eo}{}^{m}C_{eo}{}^{m}C_{eo}T_{ds}T_{ds}T_{ds}G_{ds}G_{ds}G_{ds}T_{ds}G_{ds}A_{ds}A_{ds}T_{eo}A_{eo}G_{es}{}^{m}C_{es}A_{e}$	703
GalNAc$_3$-10- ${}^{m}C_{es}{}^{m}C_{es}A_{es}{}^{m}C_{es}{}^{m}C_{es}T_{ds}T_{ds}T_{ds}G_{ds}G_{ds}G_{ds}T_{ds}G_{ds}A_{ds}A_{ds}T_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$	703
GalNAc$_3$-10- ${}^{m}C_{es}{}^{m}C_{eo}A_{eo}{}^{m}C_{eo}{}^{m}C_{eo}T_{ds}T_{ds}T_{ds}G_{ds}G_{ds}G_{ds}T_{ds}G_{ds}A_{ds}A_{ds}T_{eo}A_{eo}G_{es}{}^{m}C_{es}A_{e}$	703
GalNAc$_3$-13- ${}^{m}C_{es}{}^{m}C_{es}A_{es}{}^{m}C_{es}{}^{m}C_{es}T_{ds}T_{ds}T_{ds}G_{ds}G_{ds}G_{ds}T_{ds}G_{ds}A_{ds}A_{ds}T_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$	703
GalNAc$_3$-13- ${}^{m}C_{es}{}^{m}C_{eo}A_{eo}{}^{m}C_{eo}{}^{m}C_{eo}T_{ds}T_{ds}T_{ds}G_{ds}G_{ds}G_{ds}T_{ds}G_{ds}A_{ds}A_{ds}T_{eo}A_{eo}G_{es}{}^{m}C_{es}A_{e}$	703
${}^{m}C_{es}{}^{m}C_{es}A_{es}{}^{m}C_{es}{}^{m}C_{es}T_{ds}T_{ds}T_{ds}G_{ds}G_{ds}G_{ds}T_{ds}G_{ds}A_{ds}A_{ds}T_{es}A_{es}G_{es}{}^{m}C_{es}A_{e}$ **-GalNAc$_3$-19**	703
${}^{m}C_{es}{}^{m}C_{eo}A_{eo}{}^{m}C_{eo}{}^{m}C_{eo}T_{ds}T_{ds}T_{ds}G_{ds}G_{ds}G_{ds}T_{ds}G_{ds}A_{ds}A_{ds}T_{eo}A_{eo}G_{es}{}^{m}C_{es}A_{e}$**-GalNAc$_3$-19**	703

Example 114: Antisense inhibition of human growth hormone receptor in Hep3B cells by MOE gapmers

Antisense oligonucleotides were designed targeting a growth hormone receptor (GHR) nucleic acid and were tested for their effects on GHR 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 Hep3B 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 GHR mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3437_MGB (forward sequence CGAGTTCAGTGAGGTGCTCTATGT, designated herein as SEQ ID NO: 2329; reverse sequence AAGAGCCATGGAAAGTAGAAATCTTC, designated herein as SEQ ID NO: 2330; probe sequence TTCCTCAGATGAGCCAATT, designated herein as SEQ ID NO: 2331) was used to measure mRNA levels. GHR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of GHR, relative to untreated control cells.

The newly designed chimeric antisense oligonucleotides in the Tables below were designed as 5-10-5 MOE or 3-10-4 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. 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. 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 GHR mRNA, designated herein as SEQ ID NO: 1 (GENBANK Accession No. NM_000163.4) or the human GHR genomic sequence, designated herein as SEQ ID NO: 2 (GENBANK Accession No. NT_006576.16 truncated from nucleotides 42411001 to 42714000). '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 122

Inhibition of GHR mRNA by 5-10-5 MOE gapmers targeting exonic regions of 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
523266	164	183	Exon 1	ACCTCCGAGCTTCGCCTCTG	64	3040	3059	20
523267	171	190	Exon-exon junction	CTGTAGGACCTCCGAGCTTC	31	n/a	n/a	21
523268	178	197	Exon-exon junction	TCCATACCTGTAGGACCTCC	37	n/a	n/a	22
523271	206	225	Exon 2	TGCCAAGGTCAACAGCAGCT	80	144990	145009	23
523272	213	232	Exon 2	CTGCCAGTGCCAAGGTCAAC	53	144997	145016	24
523273	220	239	Exon 2	CTTGATCCTGCCAGTGCCAA	49	145004	145023	25
523274	227	246	Exon 2	AGCATCACTTGATCCTGCCA	67	145011	145030	26
523275	234	253	Exon 2	CAGAAAAAGCATCACTTGAT	0	145018	145037	27
523276	241	260	Exon 2	TCACTTCCAGAAAAAGCATC	1	145025	145044	28
523284	361	380	Exon 4	GTCTCTCGCTCAGGTGAACG	48	268024	268043	29
523285	368	387	Exon 4	TGAAAAAGTCTCTCGCTCAG	15	268031	268050	30
523286	375	394	Exon 4	AGTGGCATGAAAAAGTCTCT	14	268038	268057	31

523287	382	401	Exon 4	TCTGTCCAGTGGCATGAAAA	4	268045	268064	32
523301	625	644	Exon 6	GGATCTGGTTGCACTATTTC	36	n/a	n/a	33
523302	632	651	Exon 6	AATGGGTGGATCTGGTTGCA	28	278926	278945	34
523303	647	666	Exon 6	AGTCCAGTTGAGGGCAATGG	26	278941	278960	35
523304	654	673	Exon 6	TCAGTAAAGTCCAGTTGAGG	0	278948	278967	36
523305	675	694	Exon 6	GAATCCCAGTTAAACTGACG	19	278969	278988	37
523306	682	701	Exon 6	TCTGCATGAATCCCAGTTAA	39	278976	278995	38
523309	736	755	Exon 6	ATCCATCCTTTCTGAATATC	34	279030	279049	39
523310	743	762	Exon 6	CAGAACCATCCATCCTTTCT	31	279037	279056	40
523311	750	769	Exon 6	CATACTCCAGAACCATCCAT	44	279044	279063	41
523312	757	776	Exon 6	TGAAGTTCATACTCCAGAAC	23	279051	279070	42
523313	764	783	Exon 6	TTTGTATTGAAGTTCATACT	6	279058	279077	43
523314	771	790	Exon 6	TTACTTCTTTGTATTGAAGT	0	279065	279084	44
523315	778	797	Exon 6	GTTTCATTTACTTCTTTGTA	3	279072	279091	45
523316	785	804	Exon 6	CCATTTAGTTTCATTTACTT	0	279079	279098	46
523317	792	811	Exon 4-exon 5 junction	TCATTTTCCATTTAGTTTCA	19	n/a	n/a	47
523323	862	881	Exon 7	ACACGCACTTCATATTCCTT	63	290360	290379	48
523324	869	888	Exon 7	GGATCTCACACGCACTTCAT	80	290367	290386	49
523328	926	945	Exon 7	AAGTGTTACATAGAGCACCT	56	290424	290443	50
523329	933	952	Exon 7	TCTGAGGAAGTGTTACATAG	53	290431	290450	51
523330	957	976	Exon 7	CTTCTTCACATGTAAATTGG	32	290455	290474	52
523331	964	983	Exon 5-exon 6 junction	TAGAAATCTTCTTCACATGT	4	n/a	n/a	53
523332	971	990	Exon 5-exon 6 junction	TGGAAAGTAGAAATCTTCTT	9	n/a	n/a	54
523333	978	997	Exon 8	AGAGCCATGGAAAGTAGAAA	46	292532	292551	55
523334	985	1004	Exon 8	ATAATTAAGAGCCATGGAAA	0	292539	292558	56

Table 123

Inhibition of GHR mRNA by 5-10-5 MOE gapmers targeting exonic regions of 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
523421	2072	2091	exon 10	CAGTTGGTCTGTGCTCACAT	76	298489	298508	57
533002	207	226	exon 2	GTGCCAAGGTCAACAGCAGC	63	144991	145010	58
533003	208	227	exon 2	AGTGCCAAGGTCAACAGCAG	62	144992	145011	59
533004	225	244	exon 2	CATCACTTGATCCTGCCAGT	53	145009	145028	60
533005	226	245	exon 2	GCATCACTTGATCCTGCCAG	80	145010	145029	61
533006	228	247	exon 2	AAGCATCACTTGATCCTGCC	75	145012	145031	62

533007	229	248	exon 2	AAAGCATCACTTGATCCTGC	61	145013	145032	63
533019	867	886	exon 7	ATCTCACACGCACTTCATAT	35	290365	290384	64
533020	868	887	exon 7	GATCTCACACGCACTTCATA	47	290366	290385	65
533021	870	889	exon 7	TGGATCTCACACGCACTTCA	86	290368	290387	66
533022	871	890	exon 7	TTGGATCTCACACGCACTTC	70	290369	290388	67
533037	1360	1379	exon 10	TCCAGAATGTCAGGTTCACA	59	297777	297796	68
533038	1361	1380	exon 10	CTCCAGAATGTCAGGTTCAC	74	297778	297797	69
533039	1363	1382	exon 10	GTCTCCAGAATGTCAGGTTC	45	297780	297799	70
533040	1364	1383	exon 10	AGTCTCCAGAATGTCAGGTT	51	297781	297800	71
533042	1525	1544	exon 10	GCTTGGATAACACTGGGCTG	41	297942	297961	72
533043	1526	1545	exon 10	TGCTTGGATAACACTGGGCT	46	297943	297962	73
533044	1528	1547	exon 10	TCTGCTTGGATAACACTGGG	55	297945	297964	74
533045	1529	1548	exon 10	CTCTGCTTGGATAACACTGG	47	297946	297965	75
533046	1530	1549	exon 10	TCTCTGCTTGGATAACACTG	54	297947	297966	76
533047	1744	1763	exon 10	CAGAGTGAGACCATTTCCGG	47	298161	298180	77
533048	1745	1764	exon 10	GCAGAGTGAGACCATTTCCG	60	298162	298181	78
533049	1747	1766	exon 10	TGGCAGAGTGAGACCATTTC	65	298164	298183	79
533050	1748	1767	exon 10	TTGGCAGAGTGAGACCATTT	47	298165	298184	80
533051	1749	1768	exon 10	CTTGGCAGAGTGAGACCATT	30	298166	298185	81
533066	2685	2704	exon 10	CAGTGTGTAGTGTAATATAA	53	299102	299121	82
533067	2686	2705	exon 10	ACAGTGTGTAGTGTAATATA	68	299103	299122	83
533068	2688	2707	exon 10	ACACAGTGTGTAGTGTAATA	62	299105	299124	84
533069	2689	2708	exon 10	TACACAGTGTGTAGTGTAAT	55	299106	299125	85
533070	2690	2709	exon 10	GTACACAGTGTGTAGTGTAA	50	299107	299126	86
533071	3205	3224	exon 10	TGTACCTTATTCCCTTCCTG	68	299622	299641	87
533072	3206	3225	exon 10	TTGTACCTTATTCCCTTCCT	61	299623	299642	88
533073	3208	3227	exon 10	TCTTGTACCTTATTCCCTTC	60	299625	299644	89
533074	3209	3228	exon 10	TTCTTGTACCTTATTCCCTT	46	299626	299645	90

Table 124

Inhibition of GHR mRNA by 5-10-5 MOE gapmers targeting intronic and exonic regions of 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
532174	n/a	n/a	Intron 1	ACATGTACCCAAACCAACAC	37	18731	18750	91
533086	3210	3229	Exon 10	CTTCTTGTACCTTATTCCCT	72	299627	299646	92
533087	3212	3231	Exon 10	TGCTTCTTGTACCTTATTCC	77	299629	299648	93
533088	3213	3232	Exon 10	ATGCTTCTTGTACCTTATTC	63	299630	299649	94
533089	3215	3234	Exon 10	AAATGCTTCTTGTACCTTAT	67	299632	299651	95
533090	3216	3235	Exon 10	AAAATGCTTCTTGTACCTTA	50	299633	299652	96
533091	3217	3236	Exon 10	CAAAATGCTTCTTGTACCTT	44	299634	299653	97

533092	3518	3537	Exon 10	CTTCTGAATGCTTGCTTTGA	29	299935	299954	98
533093	3519	3538	Exon 10	TCTTCTGAATGCTTGCTTTG	47	299936	299955	99
533094	3521	3540	Exon 10	TTTCTTCTGAATGCTTGCTT	63	299938	299957	100
533095	3522	3541	Exon 10	TTTTCTTCTGAATGCTTGCT	51	299939	299958	101
533096	3523	3542	Exon 10	TTTTTCTTCTGAATGCTTGC	34	299940	299959	102
533097	4041	4060	Exon 10	TGCGATAAATGGGAAATACT	36	300458	300477	103
533098	4042	4061	Exon 10	CTGCGATAAATGGGAAATAC	52	300459	300478	104
533099	4043	4062	Exon 10	TCTGCGATAAATGGGAAATA	41	300460	300479	105
533100	4045	4064	Exon 10	GGTCTGCGATAAATGGGAAA	40	300462	300481	106
533101	4046	4065	Exon 10	AGGTCTGCGATAAATGGGAA	39	300463	300482	107
533102	4048	4067	Exon 10	AAAGGTCTGCGATAAATGGG	34	300465	300484	108
533103	4049	4068	Exon 10	AAAAGGTCTGCGATAAATGG	35	300466	300485	109
533104	4050	4069	Exon 10	AAAAAGGTCTGCGATAAATG	15	300467	300486	110
533115	n/a	n/a	Intron 1	CATGAAGGCCACTCTTCCAA	63	12777	12796	111
533116	n/a	n/a	Intron 1	CCATGAAGGCCACTCTTCCA	78	12778	12797	112
533117	n/a	n/a	Intron 1	CCCATGAAGGCCACTCTTCC	71	12779	12798	113
533118	n/a	n/a	Intron 1	TGCCCATGAAGGCCACTCTT	66	12781	12800	114
533119	n/a	n/a	Intron 1	TTGCCCATGAAGGCCACTCT	60	12782	12801	115
533120	n/a	n/a	Intron 1	GTTGCCCATGAAGGCCACTC	74	12783	12802	116
533121	n/a	n/a	Intron 1	GGTCTTTCATGAATCAAGCT	79	17927	17946	117
533122	n/a	n/a	Intron 1	TGGTCTTTCATGAATCAAGC	83	17928	17947	118
533123	n/a	n/a	Intron 1	ATGGTCTTTCATGAATCAAG	83	17929	17948	119
533124	n/a	n/a	Intron 1	TGATGGTCTTTCATGAATCA	78	17931	17950	120
533125	n/a	n/a	Intron 1	CTGATGGTCTTTCATGAATC	82	17932	17951	121
533126	n/a	n/a	Intron 1	GCTGATGGTCTTTCATGAAT	74	17933	17952	122
533127	n/a	n/a	Intron 1	GTACCCAAACCAACACTAAT	57	18727	18746	123
533128	n/a	n/a	Intron 1	TGTACCCAAACCAACACTAA	65	18728	18747	124
533129	n/a	n/a	Intron 1	ATGTACCCAAACCAACACTA	64	18729	18748	125
533130	n/a	n/a	Intron 1	GACATGTACCCAAACCAACA	63	18732	18751	126
533131	n/a	n/a	Intron 1	AGACATGTACCCAAACCAAC	81	18733	18752	127
533132	n/a	n/a	Intron 1	AGGAATGGAAAACCAAATAT	49	26494	26513	128
533133	n/a	n/a	Intron 1	CAGGAATGGAAAACCAAATA	74	26495	26514	129
						121986	122005	
533134	n/a	n/a	Intron 1	TCAGGAATGGAAAACCAAAT	73	26496	26515	130
						121987	122006	
533135	n/a	n/a	Intron 1	ACTCAGGAATGGAAAACCAA	77	26498	26517	131
						113032	113051	
						121989	122008	
533136	n/a	n/a	Intron 1	AACTCAGGAATGGAAAACCA	79	26499	26518	132
						113033	113052	
						121990	122009	
533137	n/a	n/a	Intron 1	TAACTCAGGAATGGAAAACC	67	26500	26519	133
						113034	113053	

						121991	122010	
533138	n/a	n/a	Intron 1	CAAAATTACTGCAGTCACAG	67	39716	39735	134
533139	n/a	n/a	Intron 1	ACAAAATTACTGCAGTCACA	81	39717	39736	135
533140	n/a	n/a	Intron 1	TACAAAATTACTGCAGTCAC	81	39718	39737	136
533141	n/a	n/a	Intron 1	CATACAAAATTACTGCAGTC	67	39720	39739	137
533142	n/a	n/a	Intron 1	ACATACAAAATTACTGCAGT	48	39721	39740	138
533143	n/a	n/a	Intron 1	AACATACAAAATTACTGCAG	53	39722	39741	139
533144	n/a	n/a	Intron 1	TTTTAGTATGAACCTTAAAA	0	42139	42158	140
533145	n/a	n/a	Intron 1	CTTTTAGTATGAACCTTAAA	38	42140	42159	141
533146	n/a	n/a	Intron 1	TCTTTTAGTATGAACCTTAA	57	42141	42160	142
533147	n/a	n/a	Intron 1	AATCTTTTAGTATGAACCTT	60	42143	42162	143
533148	n/a	n/a	Intron 1	CAATCTTTTAGTATGAACCT	70	42144	42163	144
533149	n/a	n/a	Intron 1	ACAATCTTTTAGTATGAACC	60	42145	42164	145
533150	n/a	n/a	Intron 1	AAGTTATGTGACTCTGAGCA	67	43174	43193	146
533151	n/a	n/a	Intron 1	CAAGTTATGTGACTCTGAGC	67	43175	43194	147
533152	n/a	n/a	Intron 1	TCAAGTTATGTGACTCTGAG	63	43176	43195	148
533153	n/a	n/a	Intron 1	AGTTCTCCATTAGGGTTCTG	83	50948	50967	149
533154	n/a	n/a	Intron 1	TAGTTCTCCATTAGGGTTCT	76	50949	50968	150
533155	n/a	n/a	Intron 1	ATAGTTCTCCATTAGGGTTC	51	50950	50969	151
533156	n/a	n/a	Intron 1	AAGCAGGTTGGCAGACAGAC	79	53467	53486	152
533157	n/a	n/a	Intron 1	GAAGCAGGTTGGCAGACAGA	60	53468	53487	153
533158	n/a	n/a	Intron 1	GGAAGCAGGTTGGCAGACAG	67	53469	53488	154
533159	n/a	n/a	Intron 1	TCTTCTTGTGAGCTGGCTTC	61	64882	64901	155
533160	n/a	n/a	Intron 1	GTCTTCTTGTGAGCTGGCTT	83	64883	64902	156
533161	n/a	n/a	Intron 1	AGTCTTCTTGTGAGCTGGCT	81	64884	64903	157

Table 125

Inhibition of GHR mRNA by 5-10-5 MOE gapmers targeting intronic and exonic regions of 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
533133	n/a	n/a	Intron 1	CAGGAATGGAAAACCAAATA	76	26495	26514	129
						121986	122005	
533134	n/a	n/a	Intron 1	TCAGGAATGGAAAACCAAAT	83	26496	26515	130
						121987	122006	
533174	n/a	n/a	Intron 1	TAAGTCTTCTTGTGAGCTGG	73	64886	64905	158
533175	n/a	n/a	Intron 1	TTAAGTCTTCTTGTGAGCTG	58	64887	64906	159
533176	n/a	n/a	Intron 1	ATTAAGTCTTCTTGTGAGCT	51	64888	64907	160
533177	n/a	n/a	Intron 1	TCTCTTCCACTCACATCCAT	72	65989	66008	161
533178	n/a	n/a	Intron 1	GTCTCTTCCACTCACATCCA	86	65990	66009	162
533179	n/a	n/a	Intron 1	AGTCTCTTCCACTCACATCC	80	65991	66010	163

533180	n/a	n/a	Intron 1	TAAGTATTTGTAGCAGTTGC	31	78195	78214	164
533181	n/a	n/a	Intron 1	CTAAGTATTTGTAGCAGTTG	14	78196	78215	165
533182	n/a	n/a	Intron 1	GCTAAGTATTTGTAGCAGTT	59	78197	78216	166
533183	n/a	n/a	Intron 1	TGGCTAAGTATTTGTAGCAG	34	78199	78218	167
533184	n/a	n/a	Intron 1	TTGGCTAAGTATTTGTAGCA	18	78200	78219	168
533185	n/a	n/a	Intron 1	TTTGGCTAAGTATTTGTAGC	21	78201	78220	169
533186	n/a	n/a	Intron 1	AAAATGTCAACAGTGCATAG	61	80636	80655	170
533187	n/a	n/a	Intron 1	CAAAATGTCAACAGTGCATA	78	80637	80656	171
533188	n/a	n/a	Intron 1	CCAAAATGTCAACAGTGCAT	85	80638	80657	172
533189	n/a	n/a	Intron 1	GCCCAAAATGTCAACAGTGC	82	80640	80659	173
533190	n/a	n/a	Intron 1	GGCCCAAAATGTCAACAGTG	60	80641	80660	174
533191	n/a	n/a	Intron 1	TGGCCCAAAATGTCAACAGT	31	80642	80661	175
533192	n/a	n/a	Intron 1	CAGAATCTTCTCTTTGGCCA	66	98624	98643	176
533193	n/a	n/a	Intron 1	GCAGAATCTTCTCTTTGGCC	81	98625	98644	177
533194	n/a	n/a	Intron 1	TGCAGAATCTTCTCTTTGGC	72	98626	98645	178
533195	n/a	n/a	Intron 1	TTTGCAGAATCTTCTCTTTG	33	98628	98647	179
533196	n/a	n/a	Intron 1	ATTTGCAGAATCTTCTCTTT	27	98629	98648	180
533197	n/a	n/a	Intron 1	AATTTGCAGAATCTTCTCTT	38	98630	98649	181
533198	n/a	n/a	Intron 1	ATAAAGCTATGCCATAAAGC	37	99478	99497	182
533199	n/a	n/a	Intron 1	CATAAAGCTATGCCATAAAG	14	99479	99498	183
533200	n/a	n/a	Intron 1	CCATAAAGCTATGCCATAAA	30	99480	99499	184
533201	n/a	n/a	Intron 1	GACCATAAAGCTATGCCATA	54	99482	99501	185
533202	n/a	n/a	Intron 1	TGACCATAAAGCTATGCCAT	64	99483	99502	186
533203	n/a	n/a	Intron 1	CTGACCATAAAGCTATGCCA	61	99484	99503	187
533204	n/a	n/a	Intron 1	CAAAAAGTTGAGCTGAGAAA	0	101078	101097	188
533205	n/a	n/a	Intron 1	CCAAAAAGTTGAGCTGAGAA	28	101079	101098	189
533206	n/a	n/a	Intron 1	CCCAAAAAGTTGAGCTGAGA	52	101080	101099	190
533207	n/a	n/a	Intron 1	CACCCAAAAAGTTGAGCTGA	60	101082	101101	191
533208	n/a	n/a	Intron 1	ACACCCAAAAAGTTGAGCTG	34	101083	101102	192
533209	n/a	n/a	Intron 1	TACACCCAAAAAGTTGAGCT	36	101084	101103	193
533210	n/a	n/a	Intron 1	CTTTTAATGGCACCCAAGCA	41	103566	103585	194
533211	n/a	n/a	Intron 1	GCTTTTAATGGCACCCAAGC	54	103567	103586	195
533212	n/a	n/a	Intron 1	TGCTTTTAATGGCACCCAAG	67	103568	103587	196
533213	n/a	n/a	Intron 1	AATGCTTTTAATGGCACCCA	73	103570	103589	197
533214	n/a	n/a	Intron 1	AAATGCTTTTAATGGCACCC	73	103571	103590	198
533215	n/a	n/a	Intron 1	GAAATGCTTTTAATGGCACC	41	103572	103591	199
533216	n/a	n/a	Intron 1	TAATTCTTAAGGGCCCTCTG	36	106963	106982	200
533217	n/a	n/a	Intron 1	ATAATTCTTAAGGGCCCTCT	45	106964	106983	201
533218	n/a	n/a	Intron 1	CATAATTCTTAAGGGCCCTC	50	106965	106984	202
533219	n/a	n/a	Intron 1	AGCATAATTCTTAAGGGCCC	48	106967	106986	203
533220	n/a	n/a	Intron 1	TAGCATAATTCTTAAGGGCC	52	106968	106987	204
533221	n/a	n/a	Intron 1	TTAGCATAATTCTTAAGGGC	28	106969	106988	205
533222	n/a	n/a	Intron 1	AGGAATGGAAAACCAAACAT	13	113028	113047	206

533223	n/a	n/a	Intron 1	CAGGAATGGAAAACCAAACA	64	113029	113048	207
533224	n/a	n/a	Intron 1	TCAGGAATGGAAAACCAAAC	61	113030	113049	208
533225	n/a	n/a	Intron 1	AGGAATGGAAAACCAAATAC	18	121985	122004	209
533226	n/a	n/a	Intron 1	CATGACTATGTTCTGGCAAG	37	125591	125610	210
533227	n/a	n/a	Intron 1	ACATGACTATGTTCTGGCAA	44	125592	125611	211
533228	n/a	n/a	Intron 1	CACATGACTATGTTCTGGCA	63	125593	125612	212
533229	n/a	n/a	Intron 1	GTCACATGACTATGTTCTGG	47	125595	125614	213
533230	n/a	n/a	Intron 1	GGTCACATGACTATGTTCTG	49	125596	125615	214
533231	n/a	n/a	Intron 1	TGGTCACATGACTATGTTCT	30	125597	125616	215
533232	n/a	n/a	Intron 2	CTGAATTCTGAGCTCTGGAA	73	145428	145447	216
533233	n/a	n/a	Intron 2	CCTGAATTCTGAGCTCTGGA	88	145429	145448	217
533234	n/a	n/a	Intron 2	GCCTGAATTCTGAGCTCTGG	92	145430	145449	218
533235	n/a	n/a	Intron 2	AAGCCTGAATTCTGAGCTCT	83	145432	145451	219
533236	n/a	n/a	Intron 2	CAAGCCTGAATTCTGAGCTC	68	145433	145452	220
533237	n/a	n/a	Intron 2	ACAAGCCTGAATTCTGAGCT	81	145434	145453	221
533238	n/a	n/a	Intron 2	GGATCTCAGCTGCAATTCTT	72	146235	146254	222
533239	n/a	n/a	Intron 2	AGGATCTCAGCTGCAATTCT	53	146236	146255	223
533240	n/a	n/a	Intron 2	GAGGATCTCAGCTGCAATTC	69	146237	146256	224
533241	n/a	n/a	Intron 2	CAGAGGATCTCAGCTGCAAT	69	146239	146258	225
533242	n/a	n/a	Intron 2	GCAGAGGATCTCAGCTGCAA	76	146240	146259	226
533243	230	249	Exon 2	AAAAGCATCACTTGATCCTG	23	145014	145033	227

Table 126

Inhibition of GHR mRNA by 3-10-4 MOE gapmers targeting intronic and exonic regions of 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
539284	206	222	Exon 2	CAAGGTCAACAGCAGCT	62	144990	145006	228
539285	207	223	Exon 2	CCAAGGTCAACAGCAGC	74	144991	145007	229
539286	208	224	Exon 2	GCCAAGGTCAACAGCAG	73	144992	145008	230
539290	869	885	Exon 7	TCTCACACGCACTTCAT	29	290367	290383	231
539291	870	886	Exon 7	ATCTCACACGCACTTCA	51	290368	290384	232
539292	871	887	Exon 7	GATCTCACACGCACTTC	56	290369	290385	233
539299	n/a	n/a	Intron 1	CTTTCATGAATCAAGCT	63	17927	17943	234
539300	n/a	n/a	Intron 1	TCTTTCATGAATCAAGC	49	17928	17944	235
539301	n/a	n/a	Intron 1	GTCTTTCATGAATCAAG	61	17929	17945	236
539302	n/a	n/a	Intron 1	GGTCTTTCATGAATCAA	93	17930	17946	237
539303	n/a	n/a	Intron 1	ATGGTCTTTCATGAATC	74	17932	17948	238
539304	n/a	n/a	Intron 1	GATGGTCTTTCATGAAT	56	17933	17949	239
539305	n/a	n/a	Intron 1	TATATCAATATTCTCCC	42	21820	21836	240
539306	n/a	n/a	Intron 1	TTATATCAATATTCTCC	33	21821	21837	241

539307	n/a	n/a	Intron 1	GTTATATCAATATTCTC	12	21822	21838	242
539308	n/a	n/a	Intron 1	TTTCTTTAGCAATAGTT	21	22518	22534	243
539309	n/a	n/a	Intron 1	CTTTCTTTAGCAATAGT	38	22519	22535	244
539310	n/a	n/a	Intron 1	GCTTTCTTTAGCAATAG	39	22520	22536	245
539311	n/a	n/a	Intron 1	AGGAATGGAAAACCAAA	18	26497	26513	246
						113031	113047	
						121988	122004	
539312	n/a	n/a	Intron 1	CAGGAATGGAAAACCAA	40	26498	26514	247
						113032	113048	
						121989	122005	
539313	n/a	n/a	Intron 1	TCAGGAATGGAAAACCA	49	26499	26515	248
						113033	113049	
						121990	122006	
539314	n/a	n/a	Intron 1	TCTCCATTAGGGTTCTG	87	50948	50964	249
539315	n/a	n/a	Intron 1	TTCTCCATTAGGGTTCT	57	50949	50965	250
539316	n/a	n/a	Intron 1	GTTCTCCATTAGGGTTC	73	50950	50966	251
539317	n/a	n/a	Intron 1	AGGTTGGCAGACAGACA	73	53466	53482	252
539318	n/a	n/a	Intron 1	CAGGTTGGCAGACAGAC	84	53467	53483	253
539319	n/a	n/a	Intron 1	GCAGGTTGGCAGACAGA	85	53468	53484	254
539320	n/a	n/a	Intron 1	CTTCTTGTGAGCTGGCT	87	64884	64900	255
539321	n/a	n/a	Intron 1	TCTTCTTGTGAGCTGGC	89	64885	64901	256
539322	n/a	n/a	Intron 1	GTCTTCTTGTGAGCTGG	87	64886	64902	257
539323	n/a	n/a	Intron 1	AGTCTTCTTGTGAGCTG	70	64887	64903	258
539324	n/a	n/a	Intron 1	TCTTCCACTCACATCCA	65	65990	66006	259
539325	n/a	n/a	Intron 1	CTCTTCCACTCACATCC	78	65991	66007	260
539326	n/a	n/a	Intron 1	TCTCTTCCACTCACATC	68	65992	66008	261
539327	n/a	n/a	Intron 1	GTCTCTTCCACTCACAT	74	65993	66009	262
539328	n/a	n/a	Intron 1	ATAGATTTTGACTTCCC	57	72107	72123	263
539329	n/a	n/a	Intron 1	CATAGATTTTGACTTCC	35	72108	72124	264
539330	n/a	n/a	Intron 1	GCATAGATTTTGACTTC	53	72109	72125	265
539331	n/a	n/a	Intron 1	AAAATGTCAACAGTGCA	86	80639	80655	266
539332	n/a	n/a	Intron 1	CAAAATGTCAACAGTGC	73	80640	80656	267
539333	n/a	n/a	Intron 1	CCAAAATGTCAACAGTG	34	80641	80657	268
539334	n/a	n/a	Intron 1	CCCAAAATGTCAACAGT	66	80642	80658	269
539335	n/a	n/a	Intron 1	CATGACTATGTTCTGGC	67	125594	125610	270
539336	n/a	n/a	Intron 1	ACATGACTATGTTCTGG	42	125595	125611	271
539337	n/a	n/a	Intron 1	CACATGACTATGTTCTG	29	125596	125612	272
539338	n/a	n/a	Intron 2	GAATTCTGAGCTCTGGA	77	145429	145445	273
539339	n/a	n/a	Intron 2	TGAATTCTGAGCTCTGG	84	145430	145446	274
539340	n/a	n/a	Intron 2	CTGAATTCTGAGCTCTG	80	145431	145447	275
539341	n/a	n/a	Intron 2	CCTGAATTCTGAGCTCT	84	145432	145448	276
539342	n/a	n/a	Intron 2	GCCTGAATTCTGAGCTC	84	145433	145449	277
539343	n/a	n/a	Intron 2	AGCCTGAATTCTGAGCT	80	145434	145450	278

539344	n/a	n/a	Intron 2	ATATTGTAATTCTTGGT	0	148059	148075	279
539345	n/a	n/a	Intron 2	GATATTGTAATTCTTGG	20	148060	148076	280
539346	n/a	n/a	Intron 2	TGATATTGTAATTCTTG	13	148061	148077	281
539347	n/a	n/a	Intron 2	CTGATATTGTAATTCTT	8	148062	148078	282
539348	n/a	n/a	Intron 2	CCTGATATTGTAATTCT	67	148063	148079	283
539349	n/a	n/a	Intron 2	GCCTGATATTGTAATTC	73	148064	148080	284
539350	n/a	n/a	Intron 2	TGCCTGATATTGTAATT	32	148065	148081	285
539351	n/a	n/a	Intron 2	AATTATGTGCTTTGCCT	58	148907	148923	286
539352	n/a	n/a	Intron 2	CAATTATGTGCTTTGCC	82	148908	148924	287
539353	n/a	n/a	Intron 2	TCAATTATGTGCTTTGC	68	148909	148925	288
539354	n/a	n/a	Intron 2	GTCAATTATGTGCTTTG	80	148910	148926	289
539355	n/a	n/a	Intron 2	GCCATCACCAAACACCA	94	150972	150988	290
539356	n/a	n/a	Intron 2	TGCCATCACCAAACACC	84	150973	150989	291
539357	n/a	n/a	Intron 2	TTGCCATCACCAAACAC	74	150974	150990	292
539358	n/a	n/a	Intron 2	TGGTGACTCTGCCTGAT	85	151387	151403	293
539359	n/a	n/a	Intron 2	CTGGTGACTCTGCCTGA	86	151388	151404	294

Table 127

Inhibition of GHR mRNA by 5-10-5 MOE gapmers targeting intron 1 of SEQ ID NO: 2

ISIS NO	Sequence	% inhibition	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO
523561	TATTTCAGAAAGACTTTCTG	11	10373	10392	295
523562	AGGAAAAAATCAAGGAGTTA	8	11173	11192	296
523563	TATTTACTGAACACCTATTC	12	11973	11992	297
523564	GCCCATGAAGGCCACTCTTC	70	12780	12799	298
523565	ACCTATAAATAAAGTGAGGA	0	13581	13600	299
523566	GTTTCATAACCTGCTAATAA	40	14451	14470	300
523567	ATGTGCCTTACAGTTATCAG	36	15251	15270	301
523568	TTCTGAATTTAGAATTATAG	0	16051	16070	302
523569	GTTTATAATCTAGCAGTTAC	26	17130	17149	303
523570	GATGGTCTTTCATGAATCAA	62	17930	17949	304
523571	CATGTACCCAAACCAACACT	65	18730	18749	305
523572	TAAAATACAGCCTACATCAT	0	19637	19656	306
523573	CCATCACTACAACAAACTCA	39	20451	20470	307
523574	ATCTGAAATGATCCCCTTTC	33	21283	21302	308
523575	TGTTGCCCCTCCAAAAAGAC	12	22144	22163	309
523576	ATTAAAATTTTAAATGATGT	0	22944	22963	310
523577	CTCAGGAATGGAAAACCAAA	71	26497 113031 121988	26516 113050 122007	311
523578	AAAATTCTAGAAGATAACAT	0	27838	27857	312

523579	CTAGAAGTCCTAGCCAGAGT	2	28748	28767	313
523580	AACCGATATCACAGAAATAC	0	29548	29567	314
523581	AAGATAGACAGTAACATAAT	0	30348	30367	315
523582	GCACTACAAGAACTGCTTAA	40	31172	31191	316
523583	TTTCCAGACAAAGAATTCAG	6	31978	31997	317
523584	GTAGACAGCCTTTCTGGAAC	20	32827	32846	318
523585	CATCCTACATAGTGGCTGTG	47	33635	33654	319
523586	CAGAACAGTGTGTGGAGACT	8	34452	34471	320
523587	AGCTTTAAAAATACCTCTGC	52	35466	35485	321
523588	CCCAGGTACTTGCTCTCAGA	22	36266	36285	322
523589	TTACACCTGATTCTAGAAAT	30	37066	37085	323
523590	CTTTTCTCTACAACCTCACA	34	38094	38113	324
523591	TAGTAGTTTGAATTTCAAAG	1	38909	38928	325
523592	ATACAAAATTACTGCAGTCA	60	39719	39738	326
523593	GCCACTGCCAAAAAGGAGGA	30	40519	40538	327
523594	TGACAGAAACAGAGCTATGA	33	41342	41361	328
523595	ATCTTTTAGTATGAACCTTA	65	42142	42161	329
523596	AGTTATGTGACTCTGAGCAC	63	43173	43192	330
523597	ACTATGCCCTAGTTACTTCT	29	43973	43992	331
523598	TATAGTGGAAGTGATAGATC	0	44812	44831	332
523599	TGTTTTCTGAAATGGAATGT	0	45733	45752	333
523600	GCTGTAAATGTAATGAGTGT	34	46553	46572	334
523601	GAGAGAAGCCATGGCCCTAG	20	47392	47411	335
523602	CTCTCTTTCCCAGAACAAGA	32	48210	48229	336
523603	TCCAAAATGTCCAGTATAAT	33	50072	50091	337
523604	GTTCTCCATTAGGGTTCTGG	74	50947	50966	338
523605	TTAGTCACCCATCCACCACT	41	51747	51766	339
523606	CATGAATTCACCGAGTTAGG	51	52573	52592	340
523607	AGCAGGTTGGCAGACAGACA	62	53466	53485	341
523608	GAAAGACTTAAATTTTCACA	0	54306	54325	342
523609	TAGTAGAGGAAAAGGAGAAT	0	55730	55749	343
523610	AAACAGGGTCTGGAGTGGAC	3	61243	61262	344
523611	CAAGCTGATAATTAAAAAGA	0	62462	62481	345
523612	ATAAAGATACATTTTCTGGG	8	63277	63296	346
523613	CAGGATTCTTCCTGCCTGGC	47	64085	64104	347
523614	AAGTCTTCTTGTGAGCTGGC	71	64885	64904	348
523615	CTCTTCCACTCACATCCATT	63	65988	66007	349
523616	CCTATATCAGAAGACAAATG	5	66806	66825	350
523617	TCAAAACCCTGCCAAGGTAC	44	67662	67681	351
523618	TCATATTCTACTTCTGTTTA	11	68462	68481	352
523619	CATTCCAGTGTTTCAGTAAG	13	69262	69281	353
523620	GGCCTGGAATTAATCCTCAG	49	70114	70133	354
523621	AATGCCCTCTCCCTGTGCCT	48	70925	70944	355

523622	TTTATAATCAACCTTTGCTA	9	71741	71760	356
523623	ATATAACTACTTAAAATAAT	0	72541	72560	357
523624	TTAGCCAGGATATGGTTGCC	50	73350	73369	358
523625	CTACCTCCATCAAAGAAAAT	0	74190	74209	359
523626	GCATGCATAGATAAGTTTGA	20	74990	75009	360
523627	ATGAGAGTAAATGGATTTTC	10	75790	75809	361
523628	TTGGCAATCCTTGCTTAAAA	34	76598	76617	362
523629	GAATTAAGCCAGACTTATTT	3	77398	77417	363
523630	GGCTAAGTATTTGTAGCAGT	55	78198	78217	364
523631	TTGCCTGTGTGCAACTGGCG	0	79005	79024	365
523632	GTGGCCTTAGTAGGCCAGCT	0	79827	79846	366
523633	CCCAAAATGTCAACAGTGCA	70	80639	80658	367
523634	TTAAGCCTTCAATTTGAAAA	0	81455	81474	368
523635	TGCTCAGAAGGTTGAGCATA	0	82261	82280	369
523636	TTAATGCTTTCCCAAAGCTC	35	83061	83080	370
523637	AAAAGACTTCATACCTTTAC	52	83884	83903	371

Table 128

Inhibition of GHR mRNA by 5-10-5 MOE gapmers targeting intron 1 of SEQ ID NO: 2

ISIS NO	Sequence	% inhibition	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO
532146	GGCCCCCTGGCACAACAGGA	60	3097	3116	372
532147	TCTAGGGTGATTCAGGTGGA	62	4537	4556	373
532148	CTTAGATTAATGCAAAACAA	25	4875	4894	374
532149	AGGCAGAGGAGGGTGGAACC	34	6246	6265	375
532150	AGTCTAATGAGATCTGATGG	76	6499	6518	376
532151	GCTGAAATGAGTTAAGACTT	89	6737	6756	377
532152	ACTTTGGACTGTGGATTTTT	78	6765	6784	378
532153	GCATATTTACACAATGCCTG	84	6871	6890	379
532154	GGAAATGCCTGGATGTCCAG	27	7241	7260	380
532155	CTGCTGATTTTGGAATGGAG	68	10660	10679	381
532156	ACTGAACACCTATTCTATGG	51	11968	11987	382
532157	TTTACTGAACACCTATTCTA	23	11971	11990	383
532158	CCCTCAAATTATCCACAAAC	89	12053	12072	384
532159	CTTCTAAATGTTTCCAAGGC	63	12186	12205	385
532160	TTACATCCTGTAGGCTAATT	82	12469	12488	386
532161	CCACTAGCCTGGCCAGACTT	73	12487	12506	387
532162	CTGGTAGATGATCTCAAGTT	84	13351	13370	388
532163	AAAGAATTGAGTTATAAATC	23	13670	13689	389
532164	AACTCATCTCTGGCCAGCAG	89	14361	14380	390
532165	CAACATCATTGTATTTTCTG	33	14965	14984	391

532166	TCTTAGCTTACCAATGAGGA	81	15085	15104	392
532167	TTCCCAGAGCCAAAGCTCAA	77	15982	16001	393
532168	TTTGGCCAATCCCAGCTTAT	59	16253	16272	394
532169	GTTTGCAAATCTTCATTCAC	71	16447	16466	395
532170	CAATAGTCCCTGAGGCTTGG	74	16476	16495	396
532171	TTTCCCCAGATTAAATGCCC	85	17650	17669	397
532172	TTCAATAATGCAGTTATTAT	0	18308	18327	398
532173	AAATTCTTGGGCTTAAGCAC	69	18638	18657	399
532174	ACATGTACCCAAACCAACAC	71	18731	18750	91
532175	TGATCCAAATTCAGTACCTA	82	18752	18771	400
532176	GATGATCCAAATTCAGTACC	54	18754	18773	401
532177	CAATATTCATCTTTATATTC	25	19106	19125	402
532178	ATTGCTCTTAAGATAAGTAA	41	19661	19680	403
532179	CAGCTCCCTGAATATCTCTT	74	19783	19802	404
532180	ACTTCACAAATATATTATAA	0	19885	19904	405
532181	GTACAGTCAACTTTACTTCA	89	19899	19918	406
532182	CAATTCCCACTCTTGTCAAC	55	20288	20307	407
532183	TCAACTGCTTTCTGGAGCAG	66	21215	21234	408
532184	ACTGCTGAGCACCTCCAAAA	73	21454	21473	409
532185	CTTAGATTCCTGGTTTATCA	78	21587	21606	410
532186	AGTTATATCAATATTCTCCC	88	21820	21839	411
532187	TATACCATCTTCCCCATAAA	32	22038	22057	412
532188	GGCTTTCTTTAGCAATAGTT	86	22518	22537	413
532189	TACCAGGGATGTAGGTTTAC	82	29050	29069	414
532190	TCACAGCTGAATTCTATCTG	80	29323	29342	415
532191	GGAGATGGACAAATTCCTGC	77	29470	29489	416
532192	CTAGACATGTCATCAAGACA	19	30294	30313	417
532193	CAAATTAATAAAACAATTAC	10	30385	30404	418
532194	TATTCTTATATCAGACAAAA	30	30532	30551	419
532195	TCAAGGGATCCCTGCCATTC	32	32361	32380	420
532196	CGTCAAGGGATCCCTGCCAT	47	32363	32382	421
532197	GGCACTCCCAGTCTCCAGCT	83	34138	34157	422
532198	TTTCTCCAGCAGAAGTGTCA	60	34845	34864	423
532199	AAGTCCTCTTCCGCCTCCCT	82	36023	36042	424
532200	GGAATTTACCAAAAACAGTT	63	36721	36740	425
532201	AGTTAGGTATTGTCCATTTT	74	37032	37051	426
532202	ACATGGGTATCTTCTAGGAA	77	37111	37130	427
532203	TCAGTTTCAGAGAGACAAAA	41	37276	37295	428
532204	TTTGCCAGGTCCTATGTCGA	69	37656	37675	429
532205	ATTCCCTTTTCTCTACAACC	70	38099	38118	430
532206	ATGATAAGAGCCAAGATTTG	13	38994	39013	431
532207	GAAAAAAGGTCCACTGTGGT	49	40356	40375	432
532208	CCTGTCCTGGAATAGTTTCA	49	41164	41183	433

532209	TAGAAAAGTAAATAAGGAAT	15	41501	41520	434
532210	TTATAAAACTATGCAATAGG	0	41889	41908	435
532211	TTATTTCATATTTCCAGAAA	0	42675	42694	436
532212	CATGAATTACAGCTAAAGAT	20	42741	42760	437
532213	TTGCATGTATGTGTTTCTGA	62	43518	43537	438
532214	TCAATCTCTTTATACCCTTA	75	43765	43784	439
532215	TCTTCAATCTCTTTATACCC	58	43768	43787	440
532216	CTATGCCCTAGTTACTTCTA	47	43972	43991	441
532217	AAAGAGAATCTCTTCCTTTT	27	44070	44089	442
532218	TCATTAAAGATTATTATAAC	0	44222	44241	443
532219	TTTGGATGAGTGGAAGGCTA	0	44528	44547	444
532220	GGAAATGGCCTTTTTCCTTA	72	45400	45419	445
532221	GGAGAAGCCCTCTGCCTGTA	60	46477	46496	446
532222	AAACCATATTGTCCACCAGA	84	46510	46529	447

Table 129

Inhibition of GHR mRNA by 5-10-5 MOE gapmers targeting intron 1 of SEQ ID NO: 2

ISIS NO	Sequence	% inhibition	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO
532223	CTCAAACCATATTGTCCACC	90	46513	46532	448
532224	GTGTAAATAGTGACTTGTAC	76	50123	50142	449
532225	TGAGGCACAGGAAAGTTAAC	52	50719	50738	450
532226	AGCTATAGTTCTCCATTAGG	74	50954	50973	451
532227	TTACTTGCTGACTAAGCCAT	69	51071	51090	452
532228	GTTTGTCAACTCAACATCAA	73	51215	51234	453
532229	GACTATTTGTATATATATAC	33	51491	51510	454
532230	ATGACTATTTGTATATATAT	11	51493	51512	455
532231	ACTCTTCCTTATATTTGCTC	76	51778	51797	456
532232	ATACACTGACTTTTAACATT	67	52039	52058	457
532233	CTTAGAAACAGTAGTTTCAT	42	52124	52143	458
532234	CTGAGCTTTGCCTTAAGAAT	79	52633	52652	459
532235	CACCAGACAGCAGGTAGAGC	81	53540	53559	460
532236	GAGATGGAGTAGAAGGCAAA	43	55926	55945	461
532237	TAGGAAAGGAAGAATACACT	33	63881	63900	462
532238	TAGACCAGGAAGGGTGAGAG	27	64376	64395	463
532239	AAGTTGGATCTGGCATGCAT	64	64574	64593	464
532240	AAAGTTGGATCTGGCATGCA	70	64575	64594	465
532241	CCATAACTCTTCTAACTGGG	84	64643	64662	466
532242	ATATTAAAGTTTGAGAACTA	37	65080	65099	467
532243	CTTAACTACAAAATGCTGGA	71	66164	66183	468
532244	TGAGCAGCTGTCCTCAGTTC	43	67061	67080	469

532245	GAGTTCATAAAAGTTTTACT	26	67251	67270	470
532246	CTATCCACACCATTCCATAA	73	69203	69222	471
532247	AACATCTAAGTAATGCAAAC	58	69223	69242	472
532248	TTTGCATTCAAAGCCCTGGG	91	69565	69584	473
532249	TCCATATTATAGGCTATGAT	73	69889	69908	474
532250	ATTTTATGATAATGTAAAAC	27	69942	69961	475
532251	GAGATCACATTTTCTGAGTA	50	70352	70371	476
532252	ACCTCCCTAGGATTACCTCA	56	71617	71636	477
532253	AAAATCTGATTTATAATCAA	40	71750	71769	478
532254	AGCATAGATTTTGACTTCCC	92	72107	72126	479
532255	AAAGTCATATACACAGGTCT	53	72584	72603	480
532256	CTCATAGCAAATTCCCAGAA	66	73689	73708	481
532257	CAACATGGAGGCTAGCATGT	55	74112	74131	482
532258	AGACTAAGTGGCCTGAATGT	52	74317	74336	483
532259	ACCTACCATGTCACTCTCAA	61	74418	74437	484
532260	AACTTTCTTGTGTTTTATCA	9	75511	75530	485
532261	TTTGCAAGACAAAGAAATGA	31	75915	75934	486
532262	CATGCAAAGTGTTCCTCTTC	63	76024	76043	487
532263	AGTGCTTTGCTTTCTCTTAT	79	76047	76066	488
532264	GAACAAGAAACACTTGGTAA	44	76555	76574	489
532265	AGTGTTCCAATTAAATGGCA	34	76643	76662	490
532266	AAACAATGCCCTTGTAGTGA	57	76703	76722	491
532267	TATTCTAGGTTTTGAGGTGA	60	76752	76771	492
532268	ATATTCTAGGTTTTGAGGTG	24	76753	76772	493
532269	GTTTTCCATTCTTTAAGAAA	41	76896	76915	494
532270	AGCAATCCATTGATTGTATG	59	77044	77063	495
532271	AATTATGGCAAAATGGAAAA	37	77076	77095	496
532272	ACATTTGCTTATGAGACTAT	62	77638	77657	497
532273	GCAGAGATAATCCTATGATG	42	77841	77860	498
532274	TCCATCTGTTACCTCTCTGT	77	78122	78141	499
532275	TTTGCCTGAAGGGCAGAACC	40	79478	79497	500
532276	GAAAAAATCAGATTTTCACA	0	79664	79683	501
532277	AACTTAATTTAATCATTTCT	0	79959	79978	502
532278	TTTGGTTGTCATGAGTTGAG	67	80756	80775	503
532279	TTCCATCTCTAGGGCACTTT	74	80900	80919	504
532280	AGAGCTTATTTTCAAAATTC	36	80920	80939	505
532281	ATAAAGAGCAAACAAACATA	42	81524	81543	506
532282	TATAAATTCCTTGGTCTGAT	33	82835	82854	507
532283	AAAATATAAATTCCTTGGTC	13	82839	82858	508
532284	TTTTATAACAGCCTCTGACA	38	82959	82978	509
532285	AAAAGACCATGTTGCTTATT	72	83179	83198	510
532286	ATAGTCAGTCAGAATGTGGT	72	83330	83349	511
532287	TGCCTTAGCTTGGAAAAGAC	78	83897	83916	512

532288	AGGGCTAGCTGATGCCTCTC	69	84026	84045	513
532289	TTGGACTGGGCTCAAACAGA	72	84381	84400	514
532290	AAAGTCAGGCTAGAGGGACT	49	85713	85732	515
532291	TCCTTGTTTTCTTGTAATGA	50	85945	85964	516
532292	ACACCAGAGGAAGGAAATCA	44	86554	86573	517
532293	GATGTACACCATTTTGAATT	15	86629	86648	518
532294	TGCTCTGGCCTAGCCTATGT	62	86901	86920	519
532295	CAGAGGTGTCTCCCAAGAAA	60	89940	89959	520
532296	AAAGAGAATGGATCAAAGCT	36	91930	91949	521
532297	GATTTGCAGAACAAATCTTG	37	93332	93351	522
532298	TGGTTATGAAGGTTGGACCA	52	94839	94858	523
532299	TGGCTAATTAATGGGCAATT	63	95292	95311	524

Table 130

Inhibition of GHR mRNA by 5-10-5 MOE gapmers targeting intron 1 of SEQ ID NO: 2

ISIS NO	Sequence	% inhibition	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO
532300	CTGTGCCATATTGCCTCTAA	87	95471	95490	525
532301	GATTTCAACCAGCTCACCTG	48	95510	95529	526
532302	GCAAAAGGGAACCCTGAAGC	71	95564	95583	527
532303	CTAAGTGTTATAACAAACAC	43	96137	96156	528
532304	GTCCATTGGTATAAAACTCA	84	96282	96301	529
532305	TTTCAATACAATAAGATTTA	34	96793	96812	530
532306	GTCCTTAGACCCCTCAATGG	62	96987	97006	531
532307	GAGGATTTATTCATCTAGGC	68	97806	97825	532
532308	CAGTGGGAGGATCAGATATC	46	97870	97889	533
532309	ATCCCATCCAGCAGCTGGAC	67	98132	98151	534
532310	AACTTGGGATGAGTTACTGA	56	98653	98672	535
532311	GAAGGCTACCTAAAAGAAAT	43	98810	98829	536
532312	AAAGAAATATTCACAACATT	39	99096	99115	537
532313	ATGCTTATACTGCTGCTGTA	69	99791	99810	538
532314	TCCTCACTTCAATCACCTTT	70	99819	99838	539
532315	CTCTTTCTTCATAAATAAGT	33	100809	100828	540
532316	TGGTAATCTGTGTCCCTTTA	96	101242	101261	541
532317	TAATAAAAAAGTTTGAAACA	41	102549	102568	542
532318	GGTGGTGGCAAGAGAAAAAT	56	103015	103034	543
532319	CAAAAGGCCCTTTTTACATG	28	103034	103053	544
532320	ACTCTACTGGTACCAATTTA	31	103173	103192	545
532321	TCTGAACTTTTATGCTCTGT	76	103606	103625	546
532322	AACTTTTGCCTGGGCATCCA	16	104067	104086	547
532323	TGACTCCATGTCTCACATCC	66	104392	104411	548

532324	TTACTTCCTAGATACAACAG	53	104541	104560	549
532325	CTGGCCCCCATGATTCAATT	44	104835	104854	550
532326	AAGACTGGCCCCCATGATTC	49	104839	104858	551
532327	TGTCACTGGTCTGTGTATTT	60	106233	106252	552
532328	ACAGAGTAGATTTAGCATAA	23	106980	106999	553
532329	TAAACAGGTGTACTATTACA	27	107030	107049	554
532330	GCTTTATCAACTAAGTTTAT	22	107716	107735	555
532331	CAGAACTTCTTTTAAAATTG	8	107763	107782	556
532332	GAATACAGACATACCTTGAA	25	108514	108533	557
532333	CCATGACAACAATTTCAGAG	58	109486	109505	558
532334	ACAAATAGCAATGAATGGGT	45	110878	110897	559
532335	CAACAAATAGCAATGAATGG	47	110880	110899	560
532336	GTACACAAATCAGTAGCTCT	72	115087	115106	561
532337	CTATGTCAAAAAGACTGAAA	4	116370	116389	562
532338	ATATACAGAACATTTCATCC	13	116743	116762	563
532339	AGAATAGATAAGAACTCACC	32	117195	117214	564
532340	AGGAAAGATACAGTCATTTT	5	117507	117526	565
532341	GCACAAAGAACACCTGGGAA	43	117781	117800	566
532342	CAAGAAGTCTGGGATTATGT	0	117938	117957	567
532343	GTTAGTTATTAAGCTAATCA	48	118245	118264	568
532344	AACCATTATTTATAGGCTAA	14	119127	119146	569
532345	CCAGAATGCGATCACTTCTT	76	120826	120845	570
532346	CCAGAAATTATCCTCCTCTC	70	121209	121228	571
532347	AGGGAAATGCAAATTAAAAC	20	122479	122498	572
532348	GCATCAAGATACAGAAAAAT	24	122751	122770	573
532349	GAATGTTTATGAGATTTTTC	0	123571	123590	574
532350	GCCAATTATATTGCCACATT	23	124413	124432	575
532351	ATACTTGCTTATGTAGAAAT	45	124589	124608	576
532352	TAATACTTGCTTATGTAGAA	3	124591	124610	577
532353	GAACACATGGCATTCTGATA	36	125178	125197	578
532354	CAGAATTTGCAGTATAAATC	0	126051	126070	579
532355	TATGTTTTGAAATCTTATTT	0	126157	126176	580
532356	ACTCACTGCTACCTCATTAA	11	126998	127017	581
532357	AAGCAGTGATAGGGTATCTG	59	127080	127099	582
532358	ATGAGGCCTATTACAATGGA	14	127170	127189	583
532359	CTGGAGTCTCATGAGGCCTA	53	127180	127199	584
532360	TGACTATCAGCCTTTTAATC	45	127663	127682	585
532361	TTCAGAGAACAACCTTTGAA	0	127959	127978	586
532362	AGCCATGTGTGATCTGATGT	53	128813	128832	587
532363	GAAATTTACTCCAAACTAGC	17	128992	129011	588
532364	AACATCCAGACCACCATCTA	35	130094	130113	589
532365	GTACCAAACCATTCATGCTC	56	131036	131055	590
532366	AGTACCAAACCATTCATGCT	24	131037	131056	591

532367	TTATAGAGCTTGAGATTGAC	7	132165	132184	592
532368	AGTCCATTATAGAGCTTGAG	58	132171	132190	593
532369	AACCATGAGATGCAATGCAG	40	132498	132517	594
532370	AGGATTGAGAATCGCTGATT	42	133168	133187	595
532371	TCTAAAGCATGGCCAGGATT	48	133182	133201	596
532372	GGGACTGAGTATTGATACTT	44	133222	133241	597
532373	AGAAGTAGGGTGTTCCAGAT	29	133523	133542	598
532374	AGAAATAGTCTTCCTACTAA	0	133547	133566	599
532375	GCCTCCTTTAAGCTTCTATG	22	134240	134259	600
532376	GGCCTGCCTTTACTTTCCCA	36	134598	134617	601

Table 131

Inhibition of GHR mRNA by 5-10-5 MOE gapmers targeting introns 1 and 2 of SEQ ID NO: 2

ISIS NO	SEQ ID NO: 1 Start Site	SEQ ID NO: 1 Stop Site	Sequence	Target region	% inhibition	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO
523638	n/a	n/a	ACCTCAGTGGACTCTTTCCA	Intron 1	4	84684	84703	602
523639	n/a	n/a	CAAACCTAAGTTCAAGTCCT	Intron 1	62	85523	85542	603
523640	n/a	n/a	AGTTTCACTTCTTGAATCAA	Intron 1	38	86373	86392	604
523641	n/a	n/a	AAGATCAAATGAGGTCAAGG	Intron 1	30	87181	87200	605
523642	n/a	n/a	TAGATACAAATTTCATCACA	Intron 1	23	88063	88082	606
523643	n/a	n/a	ATTCCTAAAATAGGAGCAGG	Intron 1	45	88870	88889	607
523644	n/a	n/a	TTTTTATGTTGTATAAGATA	Intron 1	0	89670	89689	608
523645	n/a	n/a	GTTCAGCCAATACATGAGTA	Intron 1	48	90473	90492	609
523646	n/a	n/a	CCAGAGGGAGTTCATTACCA	Intron 1	62	91273	91292	610
523647	n/a	n/a	TCTCTCTAATTCAACCTTAT	Intron 1	44	92107	92126	611
523648	n/a	n/a	ATAATCCTCAGACCTCTTTA	Intron 1	29	92925	92944	612
523649	n/a	n/a	CACTGTGGCAGAATTCCAAG	Intron 1	28	93762	93781	613
523650	n/a	n/a	ACACCTTGGTGCCTAGAAGC	Intron 1	54	94581	94600	614
523651	n/a	n/a	GTAGCAATGACACCTAAGAA	Intron 1	58	95394	95413	615
523652	n/a	n/a	TTTAAAATAATAAATGCTTA	Intron 1	0	96194	96213	616
523653	n/a	n/a	TCATTTGGTCCTTAGACCCC	Intron 1	27	96994	97013	617
523654	n/a	n/a	TTATTCATCTAGGCCGAGTG	Intron 1	57	97800	97819	618
523655	n/a	n/a	TTGCAGAATCTTCTCTTTGG	Intron 1	65	98627	98646	619
523656	n/a	n/a	ACCATAAAGCTATGCCATAA	Intron 1	63	99481	99500	620
523657	n/a	n/a	GGCAAGGAGCACAATAGGAC	Intron 1	20	100281	100300	621
523658	n/a	n/a	ACCCAAAAAGTTGAGCTGAG	Intron 1	66	101081	101100	622
523659	n/a	n/a	TAGATTTTCAGACTCTTTCT	Intron 1	46	101887	101906	623
523660	n/a	n/a	AATTTCAATATTGTTGTGTT	Intron 1	0	102760	102779	624
523661	n/a	n/a	ATGCTTTTAATGGCACCCAA	Intron 1	69	103569	103588	625
523662	n/a	n/a	CATGTCTCACATCCAGGTCA	Intron 1	37	104386	104405	626

523663	n/a	n/a	TTCACTGGAGTAGACTTTTA	Intron 1	45	105255	105274	627
523664	n/a	n/a	CTTATAAGGGAGGTCTGGTA	Intron 1	41	106147	106166	628
523665	n/a	n/a	GCATAATTCTTAAGGGCCCT	Intron 1	71	106966	106985	629
523666	n/a	n/a	CCACAGAACTTCTTTTAAAA	Intron 1	27	107766	107785	630
523667	n/a	n/a	GGTGACCATGATTTTAACAA	Intron 1	25	108566	108585	631
523668	n/a	n/a	AACAGCTGCATGACAATTTT	Intron 1	50	109382	109401	632
523669	n/a	n/a	AGAAACAGAATCAGTGACTT	Intron 1	44	110403	110422	633
523670	n/a	n/a	CAGATTCCAGAGAAAAGCCA	Intron 1	14	111203	111222	634
523671	n/a	n/a	TGTGAGAAGAACTCTATCAC	Intron 1	12	112030	112049	635
523672	n/a	n/a	CTCACAAATCACCACTAAAG	Intron 1	31	112842	112861	636
523673	n/a	n/a	CAACGAGTGGATAAAGAAAC	Intron 1	28	113646	113665	637
523674	n/a	n/a	ATAAAACTGGATCCTCATCT	Intron 1	13	114446	114465	638
523675	n/a	n/a	ATTAAAACTCTCAGCAAAAT	Intron 1	0	115450	115469	639
523676	n/a	n/a	AAAGACTGAAAGAACACAAA	Intron 1	0	116361	116380	640
523677	n/a	n/a	TATCTGCTGCCTTCAGGAGA	Intron 1	0	117168	117187	641
523678	n/a	n/a	TTTGAATTAACCCAATTCAA	Intron 1	0	117999	118018	642
523679	n/a	n/a	TCTTAATTTACAACAGAGGA	Intron 1	25	118821	118840	643
523680	n/a	n/a	AGAAAAGTGACAGGCTTCCC	Intron 1	31	119659	119678	644
523681	n/a	n/a	ATGTTCCTTGAAGATCCCAA	Intron 1	37	120478	120497	645
523682	n/a	n/a	ATGAATAACACTTGCCACAA	Intron 1	0	121379	121398	646
523683	n/a	n/a	GTATGTTTATCACAGCACAG	Intron 1	56	122180	122199	647
523684	n/a	n/a	AAACACTGCAATATTAGGTT	Intron 1	34	123031	123050	648
523685	n/a	n/a	GATTGGTGCTTTTCAAACTG	Intron 1	39	123936	123955	649
523686	n/a	n/a	ATTTGTAAGACAAACATGAA	Intron 1	9	124764	124783	650
523687	n/a	n/a	TCACATGACTATGTTCTGGC	Intron 1	72	125594	125613	651
523688	n/a	n/a	AGTCCTGTCCACACTATTAA	Intron 1	6	126415	126434	652
523689	n/a	n/a	CTGGGCTCTGCCTGCTGAAC	Intron 1	17	127217	127236	653
523690	n/a	n/a	AAAACCCTTAAGTATTTCCT	Intron 1	12	128054	128073	654
523691	n/a	n/a	CTCTGTTTCAAACCCCCCAG	Intron 1	21	128854	128873	655
523692	n/a	n/a	GGACAGAACACCAATCACAA	Intron 1	18	129654	129673	656
523693	n/a	n/a	ACCTACCCTTCAAAGTCACG	Intron 1	0	130486	130505	657
523694	n/a	n/a	TTCAGTTCCCAGGAGGCTTA	Intron 1	5	131286	131305	658
523695	n/a	n/a	TTTTGCAATGTCTAGCAATT	Intron 1	0	132086	132105	659
523696	n/a	n/a	ATTAAGATCAGAAAATATTA	Intron 1	0	132953	132972	660
523697	n/a	n/a	TTAATGAGATATTTTGCACC	Intron 1	34	133858	133877	661
523698	n/a	n/a	GAGAGGTTAAGTAAATCTCC	Intron 1	0	134678	134697	662
523699	n/a	n/a	CAGACTCAAATTTGAAAATT	Intron 1	14	135500	135519	663
523700	n/a	n/a	GATAAGGCAATAATACAGCC	Intron 1	1	136306	136325	664
523701	n/a	n/a	ATCATTTGCCAATTTCTGTG	Intron 1	28	137133	137152	665
523702	n/a	n/a	CAAGAAGAAAAGATGCAAAA	Intron 1	0	138035	138054	666
523703	n/a	n/a	AATTTATTTCCTTCCTATGA	Intron 1	0	138857	138876	667
523704	n/a	n/a	TTTTGGAAATGTGAGAAACG	Intron 1	0	139771	139790	668
523705	n/a	n/a	AAACACATGAGAAAAGATGA	Intron 1	0	140593	140612	669

523706	n/a	n/a	TGTTGGCTCAGTGGGAATGA	Intron 1	0	141412	141431	670
523707	n/a	n/a	TGAACAGGTTTGCATTTCTC	Intron 1	42	142229	142248	671
523708	n/a	n/a	TCCTAGGTGAACAGGCTATG	Intron 1	38	143029	143048	672
523709	n/a	n/a	CCCTAATCAGGCTGAAATAA	Intron 1	0	143829	143848	673
523710	n/a	n/a	AGGGCCAGTAAGGTTTGCTT	Intron 1	12	144631	144650	674
523711	n/a	n/a	AGCCTGAATTCTGAGCTCTG	Intron 2	88	145431	145450	675
523712	n/a	n/a	AGAGGATCTCAGCTGCAATT	Intron 2	71	146238	146257	676
523713	n/a	n/a	GAAAATCCCTGCTCAAGTGC	Intron 2	67	147262	147281	677
523714	n/a	n/a	TGCCTGATATTGTAATTCTT	Intron 2	90	148062	148081	678

Table 132

Inhibition of GHR mRNA by 5-10-5 MOE gapmers targeting introns 1 and 2 of SEQ ID NO: 2

ISIS NO	Sequence	Target Region	% inhibition	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO
532377	CTCATACAGTGAAGTCTTCA	Intron 1	73	135431	135450	679
532378	CTCACTAAGCTTGATTCACT	Intron 1	67	135818	135837	680
532379	GATACAGAAATCCCAGTGAC	Intron 1	46	136111	136130	681
532380	TGTGCTTGGGTGTACAGGCA	Intron 1	71	136282	136301	682
532381	TCAAGCACTTACATCATATG	Intron 1	42	136377	136396	683
532382	AGGGTTAGTTATTACACTTA	Intron 1	60	136576	136595	684
532383	AGGCTTCATGTGAGGTAACA	Intron 1	58	136996	137015	685
532384	TGAAAGCTTAGTACAAGAAG	Intron 1	51	138048	138067	686
532385	CTCTCCTCTTGGAGATCCAG	Intron 1	58	138782	138801	687
532386	GCTGAGATTTCTCTCCTCTT	Intron 1	78	138792	138811	688
532387	CTTTTGCTGAGATTTCTCTC	Intron 1	58	138797	138816	689
532388	GAACATATGTCCATAGAATG	Intron 1	57	141700	141719	690
532389	GAACAGGCTATGTAATCAAA	Intron 1	68	143021	143040	691
532390	TTTTTATTACTGTGCAAACC	Intron 1	41	143878	143897	692
532391	ACTGAGGGTGGAAATGGAAA	Intron 2	23	145059	145078	693
532392	ATGCCATACTTTTCATTTCA	Intron 2	87	146351	146370	694
532393	TCTTTAAAGATTTCCTATGC	Intron 2	66	146367	146386	695
532394	TCACAATTAAATTATGTTTA	Intron 2	47	149858	149877	696
532395	TTTGCCATCACCAAACACCA	Intron 2	94	150972	150991	697
532396	TCAGAATGCTGAAGGATGGG	Intron 2	70	152208	152227	698
532397	ACAATTGCAGGAGAGAACTG	Intron 2	57	152296	152315	699
532398	GTTCAGTCACCTGGAAAGAG	Intron 2	62	152549	152568	700
532399	CGGAGTTCAGTCACCTGGAA	Intron 2	77	152553	152572	701
532400	AATCTAAAGTTCAATGTCCA	Intron 2	77	152752	152771	702
532401	CCACCTTTGGGTGAATAGCA	Intron 2	95	153921	153940	703
532402	CAACATCAAAAGTTTCCACC	Intron 2	81	153936	153955	704
532403	AAGCTTCTATCAACCAACTG	Intron 2	87	154093	154112	705

532404	ACCATTTTCTAATAATTCAC	Intron 2	46	154502	154521	706
532405	ACCTGCACTTGGACAACTGA	Intron 2	60	154727	154746	707
532406	GTCAGTGCTTTGGTGATGTA	Intron 2	11	155283	155302	708
532407	TAGAAGCACAGGAACTAGAG	Intron 2	68	155889	155908	709
532408	TTTAATTTTATTAGAAGCAC	Intron 2	14	155900	155919	710
532409	GAGCAAGAATTAAGAAAATC	Intron 2	29	155973	155992	711
532410	CTCTGCAGTCATGTACACAA	Intron 2	93	156594	156613	712
532411	GCTTGGTTTGTCAATCCTTT	Intron 2	95	156889	156908	713
532412	GTTCTCAAGCAGGAGCCATT	Intron 2	70	157330	157349	714
532413	AGGGTGATCTTCCAAAACAA	Intron 2	87	158612	158631	715
532414	TCTCCTATGCTTCCTTTAAT	Intron 2	25	158813	158832	716
532415	GACATAAATATGTTCACTGA	Intron 2	81	159216	159235	717
532416	TTACTGAGTGACAGTACAGT	Intron 2	65	161588	161607	718
532417	CCAGGCACCAGCACAGGCAC	Intron 2	47	161950	161969	719
532418	TTAATGTCAGTAGAAAGCTG	Intron 2	0	162349	162368	720
532419	GCAGGTGGAAAGAAGATGTC	Intron 2	50	162531	162550	721
532420	GCCAGGGTCTTTACAAAGTT	Intron 2	93	162751	162770	722
532421	CATTACCTTTGTACATGTAC	Intron 2	83	164839	164858	723
532422	GAAGCAACTTCTCTGAGGTC	Intron 2	68	165040	165059	724
532423	GCCTGGCAAGAAGGGCCCTT	Intron 2	56	165856	165875	725
532424	ACACATGTTTTTAAATTTAT	Intron 2	21	166241	166260	726
532425	TCACAATGCACTAAAAGAAA	Intron 2	53	168760	168779	727
532426	TCCCAATGACTTACTGTAGA	Intron 2	78	169073	169092	728
532427	TAAGCATTTATGGAGGAATG	Intron 2	46	169134	169153	729
532428	TGAGGTGGGTGGCCAACAGG	Intron 2	66	170081	170100	730
532429	GTTTTTCATTTTGATTGCAG	Intron 2	88	170158	170177	731
532430	AGCTCAAGTGTTTTTCATTT	Intron 2	64	170167	170186	732
532431	CAATGTCACAGCTGTTTCCT	Intron 2	62	170272	170291	733
532432	GAACTTTGGAGGCTTTTAGA	Intron 2	55	170703	170722	734
532433	TGTATGCCCCAAACTCCCAT	Intron 2	83	171431	171450	735
532434	ACACAAATAAGGGAATAATA	Intron 2	24	171549	171568	736
532435	TAGTTCAGCCACTATGGAAA	Intron 2	47	171926	171945	737
532436	CTCCAAATTCCAGTCCTAGG	Intron 2	93	172746	172765	738
532437	AGTTGGCACTGCTATATCAG	Intron 2	66	173668	173687	739
532438	GGCCTTAGATTGTAAGTTTT	Intron 2	69	174122	174141	740
532439	TTTTAGTATTATTGTAGGAA	Intron 2	16	174188	174207	741
532440	TTTCATTAATGAAACCTGAT	Intron 2	39	174812	174831	742
532441	CCCTCAGCTGCCTCTTCAAT	Intron 2	51	175014	175033	743
532442	TATTGTATCCTGGCCCCTAA	Intron 2	68	175689	175708	744
532443	AGAACAAGAGCCTAGAAGTA	Intron 2	35	176592	176611	745
532444	GTGACTATGTCACTGAATTT	Intron 2	14	176918	176937	746
532445	GCCCTACCCAGCAGCCTGTG	Intron 2	79	177540	177559	747
532446	CAAACATAAAGAGAGTTCCA	Intron 2	79	177811	177830	748

532447	CTTTAAATGAAGTAGAGCTC	Intron 2	0	178090	178109	749
532448	CTGTTCAAAGAATGCAGGCC	Intron 2	70	178905	178924	750
532449	GTCTAGCCTAACAGAGATAT	Intron 2	47	179137	179156	751
532450	AAAGAGTGATGTCTAGCCTA	Intron 2	55	179147	179166	752
532451	CACTTCTTACTCCTTTGAGG	Intron 2	50	179631	179650	753
532452	TTCCACAAGAAACTCAGTTT	Intron 2	56	181514	181533	754
532453	AGAAATGCCAAAGATAGCTC	Intron 2	56	182105	182124	755

Table 133

Inhibition of GHR mRNA by 5-10-5 MOE gapmers targeting intron 2 of SEQ ID NO: 2

ISIS NO	Sequence	% inhibition	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO
533249	AGCAGAGGATCTCAGCTGCA	84	146241	146260	756
533250	AATCCCTGCTCAAGTGCTAC	75	147259	147278	757
533251	AAATCCCTGCTCAAGTGCTA	71	147260	147279	758
533252	AAAATCCCTGCTCAAGTGCT	73	147261	147280	759
533253	AGAAAATCCCTGCTCAAGTG	56	147263	147282	760
533254	AAGAAAATCCCTGCTCAAGT	58	147264	147283	761
533255	CAAGAAAATCCCTGCTCAAG	46	147265	147284	762
533256	CTGATATTGTAATTCTTGGT	91	148059	148078	763
533257	CCTGATATTGTAATTCTTGG	90	148060	148079	764
533258	GCCTGATATTGTAATTCTTG	94	148061	148080	765
533259	ATGCCTGATATTGTAATTCT	91	148063	148082	766
533260	AATGCCTGATATTGTAATTC	74	148064	148083	767
533261	CAATGCCTGATATTGTAATT	76	148065	148084	768
533262	AATTATGTGCTTTGCCTGCA	92	148904	148923	769
533263	CAATTATGTGCTTTGCCTGC	83	148905	148924	770
533264	TCAATTATGTGCTTTGCCTG	83	148906	148925	771
533265	TGTCAATTATGTGCTTTGCC	91	148908	148927	772
533266	ATGTCAATTATGTGCTTTGC	83	148909	148928	773
533267	GATGTCAATTATGTGCTTTG	74	148910	148929	774
533268	CTGGTGACTCTGCCTGATGA	77	151385	151404	775
533269	GCTGGTGACTCTGCCTGATG	87	151386	151405	776
533270	TGCTGGTGACTCTGCCTGAT	89	151387	151406	777
533271	GCTGCTGGTGACTCTGCCTG	94	151389	151408	778
533272	GGCTGCTGGTGACTCTGCCT	77	151390	151409	779
533273	TGGCTGCTGGTGACTCTGCC	82	151391	151410	780
533274	GCTGAAGGATGGGCATCCAG	85	152201	152220	781
533275	TGCTGAAGGATGGGCATCCA	85	152202	152221	782
533276	ATGCTGAAGGATGGGCATCC	78	152203	152222	783
533277	GAATGCTGAAGGATGGGCAT	66	152205	152224	784

533278	AGAATGCTGAAGGATGGGCA	81	152206	152225	785
533279	CAGAATGCTGAAGGATGGGC	85	152207	152226	786
533280	TCCAGTAGTCAATATTATTT	87	153001	153020	787
533281	ATCCAGTAGTCAATATTATT	85	153002	153021	788
533282	TATCCAGTAGTCAATATTAT	69	153003	153022	789
533283	GTTATCCAGTAGTCAATATT	77	153005	153024	790
533284	GGTTATCCAGTAGTCAATAT	85	153006	153025	791
533285	TGGTTATCCAGTAGTCAATA	86	153007	153026	792
533286	CAACTTGAGGACAATAAGAG	35	155591	155610	793
533287	TCAACTTGAGGACAATAAGA	62	155592	155611	794
533288	CTCAACTTGAGGACAATAAG	86	155593	155612	795
533289	AACTCAACTTGAGGACAATA	82	155595	155614	796
533290	TAACTCAACTTGAGGACAAT	66	155596	155615	797
533291	ATAACTCAACTTGAGGACAA	87	155597	155616	798
533292	CAGGAAGAAAGGAACCTTAG	77	156391	156410	799
533293	CCAGGAAGAAAGGAACCTTA	84	156392	156411	800
533294	ACCAGGAAGAAAGGAACCTT	86	156393	156412	801
533295	AGACCAGGAAGAAAGGAACC	74	156395	156414	802
533296	TAGACCAGGAAGAAAGGAAC	59	156396	156415	803
533297	ATAGACCAGGAAGAAAGGAA	65	156397	156416	804
533298	TACAATGCACAGGACACGCC	73	157198	157217	805
533299	CTACAATGCACAGGACACGC	85	157199	157218	806
533300	GCTACAATGCACAGGACACG	83	157200	157219	807
533301	ATGCTACAATGCACAGGACA	89	157202	157221	808
533302	TATGCTACAATGCACAGGAC	82	157203	157222	809
533303	ATATGCTACAATGCACAGGA	84	157204	157223	810
533304	CTGATATTTATTGCTGTACG	76	158006	158025	811
533305	CTCTGATATTTATTGCTGTA	80	158008	158027	812
533306	TCTCTGATATTTATTGCTGT	86	158009	158028	813
533307	GTCTCTGATATTTATTGCTG	80	158010	158029	814
533308	CCAGAAGAATTACCCATGCA	85	165550	165569	815
533309	TCCAGAAGAATTACCCATGC	84	165551	165570	816
533310	TTCCAGAAGAATTACCCATG	81	165552	165571	817
533311	TCTTCCAGAAGAATTACCCA	58	165554	165573	818
533312	ATCTTCCAGAAGAATTACCC	64	165555	165574	819
533313	CATCTTCCAGAAGAATTACC	58	165556	165575	820
533314	TTTCTGCAGTATCCTAGCCT	78	166350	166369	821
533315	GTTTCTGCAGTATCCTAGCC	88	166351	166370	822
533316	AGTTTCTGCAGTATCCTAGC	86	166352	166371	823
533317	TCAGTTTCTGCAGTATCCTA	88	166354	166373	824
533318	TTCAGTTTCTGCAGTATCCT	87	166355	166374	825
533319	TTTCAGTTTCTGCAGTATCC	80	166356	166375	826
533320	GTTTCCATTTTCTTGATTCC	70	169601	169620	827

533321	TGTTTCCATTTTCTTGATTC	54	169602	169621	828
533322	GTGTTTCCATTTTCTTGATT	55	169603	169622	829
533323	TGGTGTTTCCATTTTCTTGA	73	169605	169624	830
533324	ATGGTGTTTCCATTTTCTTG	76	169606	169625	831
533325	AATGGTGTTTCCATTTTCTT	78	169607	169626	832

Table 134
Inhibition of GHR mRNA by 5-10-5 MOE gapmers targeting introns 2 and 3 of SEQ ID NO: 2

ISIS NO	Sequence	Target region	% inhibition	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO
533326	AACCCATTTCATCCATTTAA	Intron 2	93	175369	175388	833
533327	GAACCCATTTCATCCATTTA	Intron 2	83	175370	175389	834
533328	GGAACCCATTTCATCCATTT	Intron 2	92	175371	175390	835
533329	TAGGAACCCATTTCATCCAT	Intron 2	91	175373	175392	836
533330	GTAGGAACCCATTTCATCCA	Intron 2	95	175374	175393	837
533331	GGTAGGAACCCATTTCATCC	Intron 2	92	175375	175394	838
533332	TGAGGGATTGCCTCAGTAGC	Intron 2	66	179616	179635	839
533333	TTGAGGGATTGCCTCAGTAG	Intron 2	72	179617	179636	840
533334	TTTGAGGGATTGCCTCAGTA	Intron 2	67	179618	179637	841
533335	CCTTTGAGGGATTGCCTCAG	Intron 2	74	179620	179639	842
533336	TCCTTTGAGGGATTGCCTCA	Intron 2	66	179621	179640	843
533337	CTCCTTTGAGGGATTGCCTC	Intron 2	76	179622	179641	844
533338	AACTTAGGACTTGGGACATT	Intron 2	64	184575	184594	845
533339	TAACTTAGGACTTGGGACAT	Intron 2	54	184576	184595	846
533340	CTAACTTAGGACTTGGGACA	Intron 2	63	184577	184596	847
533341	CACTAACTTAGGACTTGGGA	Intron 2	82	184579	184598	848
533342	TCACTAACTTAGGACTTGGG	Intron 2	77	184580	184599	849
533343	GTCACTAACTTAGGACTTGG	Intron 2	83	184581	184600	850
533344	TGGGCTAGATCAGGATTGGT	Intron 2	81	188617	188636	851
533345	ATGGGCTAGATCAGGATTGG	Intron 2	70	188618	188637	852
533346	CATGGGCTAGATCAGGATTG	Intron 2	64	188619	188638	853
533347	ACCATGGGCTAGATCAGGAT	Intron 2	82	188621	188640	854
533348	TACCATGGGCTAGATCAGGA	Intron 2	88	188622	188641	855
533349	CTACCATGGGCTAGATCAGG	Intron 2	87	188623	188642	856
533350	ATGAGCTTAGCAGTCACTTA	Intron 2	83	189482	189501	857
533351	CATGAGCTTAGCAGTCACTT	Intron 2	87	189483	189502	858
533352	CCATGAGCTTAGCAGTCACT	Intron 2	92	189484	189503	859
533353	GTCTCAGCAAACCTGGGATA	Intron 2	84	190283	190302	860
533354	TGTCTCAGCAAACCTGGGAT	Intron 2	82	190284	190303	861
533355	ATGTCTCAGCAAACCTGGGA	Intron 2	81	190285	190304	862
533356	GAATGTCTCAGCAAACCTGG	Intron 2	76	190287	190306	863

533357	GGAATGTCTCAGCAAACCTG	Intron 2	82	190288	190307	864
533358	AGGAATGTCTCAGCAAACCT	Intron 2	85	190289	190308	865
533359	TACAGACATAGCTCTAACCT	Intron 2	79	191139	191158	866
533360	ATACAGACATAGCTCTAACC	Intron 2	79	191140	191159	867
533361	GATACAGACATAGCTCTAAC	Intron 2	71	191141	191160	868
533362	TGGATACAGACATAGCTCTA	Intron 2	79	191143	191162	869
533363	CTGGATACAGACATAGCTCT	Intron 2	82	191144	191163	870
533364	GCTGGATACAGACATAGCTC	Intron 2	95	191145	191164	871
533365	ACACTGTTTGTGAGGGTCAA	Intron 2	87	191939	191958	872
533366	AACACTGTTTGTGAGGGTCA	Intron 2	81	191940	191959	873
533367	CAACACTGTTTGTGAGGGTC	Intron 2	85	191941	191960	874
533368	AACAACACTGTTTGTGAGGG	Intron 2	65	191943	191962	875
533369	AAACAACACTGTTTGTGAGG	Intron 2	76	191944	191963	876
533370	CAAACAACACTGTTTGTGAG	Intron 2	67	191945	191964	877
533371	TTCAAGTTTAGGATCTGCAG	Intron 2	73	196536	196555	878
533372	CTTCAAGTTTAGGATCTGCA	Intron 2	88	196537	196556	879
533373	GCTTCAAGTTTAGGATCTGC	Intron 2	86	196538	196557	880
533374	GGGCTTCAAGTTTAGGATCT	Intron 2	67	196540	196559	881
533375	AGGGCTTCAAGTTTAGGATC	Intron 2	66	196541	196560	882
533376	CAGGGCTTCAAGTTTAGGAT	Intron 2	74	196542	196561	883
533377	TGTGGCTTTAATTCACTAAT	Intron 2	84	198145	198164	884
533378	ATGTGGCTTTAATTCACTAA	Intron 2	86	198146	198165	885
533379	TATGTGGCTTTAATTCACTA	Intron 2	79	198147	198166	886
533380	GGTATGTGGCTTTAATTCAC	Intron 2	83	198149	198168	887
533381	TGGTATGTGGCTTTAATTCA	Intron 2	81	198150	198169	888
533382	GTGGTATGTGGCTTTAATTC	Intron 2	86	198151	198170	889
533383	TCTGTGTTCAGTTGCATCAC	Intron 2	75	199817	199836	890
533384	TTCTGTGTTCAGTTGCATCA	Intron 2	82	199818	199837	891
533385	GTTCTGTGTTCAGTTGCATC	Intron 2	86	199819	199838	892
533386	GTACTCATGAGGAGGCACTT	Intron 2	81	201413	201432	893
533387	GGTACTCATGAGGAGGCACT	Intron 2	82	201414	201433	894
533388	TGGTACTCATGAGGAGGCAC	Intron 2	78	201415	201434	895
533389	ATTGGTACTCATGAGGAGGC	Intron 2	64	201417	201436	896
533390	AATTGGTACTCATGAGGAGG	Intron 2	47	201418	201437	897
533391	CAATTGGTACTCATGAGGAG	Intron 2	54	201419	201438	898
533392	AAACTCTGCAACTCCAACCC	Intron 2	69	205549	205568	899
533393	GAAACTCTGCAACTCCAACC	Intron 2	64	205550	205569	900
533394	GGAAACTCTGCAACTCCAAC	Intron 2	83	205551	205570	901
533395	ATGGAAACTCTGCAACTCCA	Intron 2	88	205553	205572	902
533396	CATGGAAACTCTGCAACTCC	Intron 2	70	205554	205573	903
533397	TCATGGAAACTCTGCAACTC	Intron 2	69	205555	205574	904
533398	ACATCTGGATGTGAGGCTCG	Intron 3	64	210559	210578	905
533399	CACATCTGGATGTGAGGCTC	Intron 3	84	210560	210579	906

533400	GTCACATCTGGATGTGAGGC	Intron 3	75	210562	210581	907
533401	TGTCACATCTGGATGTGAGG	Intron 3	51	210563	210582	908
533402	CTGTCACATCTGGATGTGAG	Intron 3	30	210564	210583	909

Table 135
Inhibition of GHR mRNA by 5-10-5 MOE gapmers targeting introns 2 and 3 of SEQ ID NO: 2

ISIS NO	Sequence	Target region	% inhibition	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO
523715	GTCAATTATGTGCTTTGCCT	Intron 2	91	148907	148926	910
523716	ACATTCAAAATTCTTCCTTG	Intron 2	50	149787	149806	911
523717	ATCCTGCATATATTTTATTG	Intron 2	20	150588	150607	912
523718	CTGCTGGTGACTCTGCCTGA	Intron 2	77	151388	151407	913
523719	AATGCTGAAGGATGGGCATC	Intron 2	66	152204	152223	914
523720	TTATCCAGTAGTCAATATTA	Intron 2	71	153004	153023	915
523721	TCTCATGTTAAAGTTCTTAA	Intron 2	48	153831	153850	916
523722	TGCACTTGGACAACTGATAG	Intron 2	29	154724	154743	917
523723	ACTCAACTTGAGGACAATAA	Intron 2	88	155594	155613	918
523724	GACCAGGAAGAAAGGAACCT	Intron 2	72	156394	156413	919
523725	TGCTACAATGCACAGGACAC	Intron 2	80	157201	157220	920
523726	TCTGATATTTATTGCTGTAC	Intron 2	73	158007	158026	921
523727	ATGCTTCCTTTAATAAATGT	Intron 2	0	158807	158826	922
523728	AACATTTAGAACCTAGGAGA	Intron 2	20	159610	159629	923
523729	CAAGCTTGCAAGTAGGAAAA	Intron 2	51	160410	160429	924
523730	CCAGGCTGTTCATGCCAAGG	Intron 2	26	161248	161267	925
523731	CCTGCCAAGGGCAAGCCAGG	Intron 2	17	162064	162083	926
523732	TTTCACCTGGTGACTGGAAG	Intron 2	51	163019	163038	927
523733	ATTTTCTACCATCAAAGAGA	Intron 2	4	163943	163962	928
523734	GATTAAGTTTTCTTTAAAAA	Intron 2	0	164746	164765	929
523735	CTTCCAGAAGAATTACCCAT	Intron 2	56	165553	165572	930
523736	CAGTTTCTGCAGTATCCTAG	Intron 2	77	166353	166372	931
523737	TATTTTGAAAATGAGATTCA	Intron 2	0	167195	167214	932
523738	GTGGCCCGAGTAAAGATAAA	Intron 2	21	167995	168014	933
523739	CCTGTCAATCCTCTTATATG	Intron 2	37	168804	168823	934
523740	GGTGTTTCCATTTTCTTGAT	Intron 2	65	169604	169623	935
523741	ACAGGGTCAAAAGTTCACTT	Intron 2	44	170407	170426	936
523742	TAGGAAAGCTGAGAGAATCC	Intron 2	35	171207	171226	937
523743	AGCATATGAAAAAATACTCA	Intron 2	0	172101	172120	938
523744	CTTCAGAAATCAGCATCTGA	Intron 2	45	172937	172956	939
523745	TTACAAGTGACAGTGTTTGT	Intron 2	28	173737	173756	940
523746	ATCAGACCCTGAAGAATTTA	Intron 2	29	174560	174579	941
523747	AGGAACCCATTTCATCCATT	Intron 2	83	175372	175391	942

523748	CACATTGGTAACTTAAAGTT	Intron 2	18	176263	176282	943
523749	TATTATCTGACTCATTTCTG	Intron 2	16	177072	177091	944
523750	AAATAAGACAAAGAAAATTC	Intron 2	0	177872	177891	945
523751	TTTTAAAAATAACCAATTCA	Intron 2	0	178788	178807	946
523752	CTTTGAGGGATTGCCTCAGT	Intron 2	66	179619	179638	947
523753	ACAGTCCTCATGAACAGATT	Intron 2	37	180513	180532	948
523754	ACTATCATTAATAATATTGT	Intron 2	0	181323	181342	949
523755	ATCTAGATTTGCCTTATAAG	Intron 2	27	182123	182142	950
523756	TGGTTGAGGAAGACAGTCTC	Intron 2	16	182962	182981	951
523757	TGGCTCATAACTTCCTTAGC	Intron 2	43	183762	183781	952
523758	ACTAACTTAGGACTTGGGAC	Intron 2	72	184578	184597	953
523759	CTTATAGCATTACTAAGTGG	Intron 2	49	185403	185422	954
523760	TGGTGGCAGGAGAGAGGGAA	Intron 2	48	186203	186222	955
523761	TTTGCCAGGAAATCTTGAAA	Intron 2	35	187003	187022	956
523762	ATAACTTTTCTCTGAAATTT	Intron 2	8	187803	187822	957
523763	CCATGGGCTAGATCAGGATT	Intron 2	59	188620	188639	958
523764	TGAGCTTAGCAGTCACTTAG	Intron 2	62	189481	189500	959
523765	AATGTCTCAGCAAACCTGGG	Intron 2	62	190286	190305	960
523766	GGATACAGACATAGCTCTAA	Intron 2	75	191142	191161	961
523767	ACAACACTGTTTGTGAGGGT	Intron 2	66	191942	191961	962
523768	TCTATTTTCTAATAGCTGTT	Intron 2	49	192742	192761	963
523769	GGCCCCACCTCTGACCTTCA	Intron 2	7	193542	193561	964
523770	TGGTAAAGCTAGAAAAAAAA	Intron 2	0	194346	194365	965
523771	AAGTGGTAAATATGATCACA	Intron 2	23	195159	195178	966
523772	GGCTTCAAGTTTAGGATCTG	Intron 2	52	196539	196558	967
523773	TTGTTGACACTCTCTTTTGG	Intron 2	18	197348	197367	968
523774	GTATGTGGCTTTAATTCACT	Intron 2	71	198148	198167	969
523775	AATTAGTTGTTTTGGCAAAT	Intron 2	14	198988	199007	970
523776	CTGTGTTCAGTTGCATCACG	Intron 2	75	199816	199835	971
523777	AATGTGGAAGTTTCCTAACA	Intron 2	15	200616	200635	972
523778	TTGGTACTCATGAGGAGGCA	Intron 2	58	201416	201435	973
523779	TTTCTCTGTGTTTAAAATTG	Intron 2	13	202308	202327	974
523780	GTAAAGCACAATGAACAAAA	Intron 2	21	203115	203134	975
523781	ATCACAGATCTTTGCTACAA	Intron 2	51	203915	203934	976
523782	TCCTGCCTTTCTGAACCAAA	Intron 2	50	204721	204740	977
523783	TGGAAACTCTGCAACTCCAA	Intron 2	58	205552	205571	978
523784	ACACAGTAGGGAACAATTTT	Intron 2	8	206412	206431	979
523785	AGACAGATGGTGAAATGATG	Intron 2	0	207219	207238	980
523786	AAACAGAAAGAGAAGAAAAC	Intron 2	0	208117	208136	981
523787	CTTAGATAAATACTTCAAGA	Intron 3	0	208938	208957	982
523788	AGCCACTTCTTTTACAACCT	Intron 3	0	209742	209761	983
523789	TCACATCTGGATGTGAGGCT	Intron 3	80	210561	210580	984
523790	GACTGAAACTTAAAGGTGGG	Intron 3	7	211399	211418	985

523791	AAAGATGTGCAATCATCTAA	Intron 3	44	212204	212223	986

Table 136
Inhibition of GHR mRNA by 3-10-4 MOE gapmers targeting introns 2 and 3 of SEQ ID NO: 2

ISIS NO	Sequence	Target region	% inhibition	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO
539360	GCTGGTGACTCTGCCTG	Intron 2	95	151389	151405	987
539361	TGCTGGTGACTCTGCCT	Intron 2	95	151390	151406	988
539362	CTGCTGGTGACTCTGCC	Intron 2	93	151391	151407	989
539363	AGTAGTCAATATTATTT	Intron 2	31	153001	153017	990
539364	CAGTAGTCAATATTATT	Intron 2	13	153002	153018	991
539365	CCAGTAGTCAATATTAT	Intron 2	34	153003	153019	992
539366	CCTTTGGGTGAATAGCA	Intron 2	64	153921	153937	993
539367	ACCTTTGGGTGAATAGC	Intron 2	78	153922	153938	994
539368	CACCTTTGGGTGAATAG	Intron 2	40	153923	153939	995
539369	CAACTTGAGGACAATAA	Intron 2	38	155594	155610	996
539370	TCAACTTGAGGACAATA	Intron 2	63	155595	155611	997
539371	CTCAACTTGAGGACAAT	Intron 2	81	155596	155612	998
539372	CAGGAAGAAAGGAACCT	Intron 2	70	156394	156410	999
539373	CCAGGAAGAAAGGAACC	Intron 2	59	156395	156411	1000
539374	ACCAGGAAGAAAGGAAC	Intron 2	43	156396	156412	1001
539375	TGCAGTCATGTACACAA	Intron 2	93	156594	156610	1002
539376	CTGCAGTCATGTACACA	Intron 2	91	156595	156611	1003
539377	TCTGCAGTCATGTACAC	Intron 2	87	156596	156612	1004
539378	TGGTTTGTCAATCCTTT	Intron 2	95	156889	156905	1005
539379	TTGGTTTGTCAATCCTT	Intron 2	97	156890	156906	1006
539380	CTTGGTTTGTCAATCCT	Intron 2	97	156891	156907	1007
539381	TACAATGCACAGGACAC	Intron 2	65	157201	157217	1008
539382	CTACAATGCACAGGACA	Intron 2	85	157202	157218	1009
539383	GCTACAATGCACAGGAC	Intron 2	96	157203	157219	1010
539384	GATATTTATTGCTGTAC	Intron 2	43	158007	158023	1011
539385	TGATATTTATTGCTGTA	Intron 2	35	158008	158024	1012
539386	CTGATATTTATTGCTGT	Intron 2	38	158009	158025	1013
539387	AGGGTCTTTACAAAGTT	Intron 2	61	162751	162767	1014
539388	CAGGGTCTTTACAAAGT	Intron 2	65	162752	162768	1015
539389	CCAGGGTCTTTACAAAG	Intron 2	88	162753	162769	1016
539390	TTCTGCAGTATCCTAGC	Intron 2	72	166352	166368	1017
539391	TTTCTGCAGTATCCTAG	Intron 2	53	166353	166369	1018
539392	GTTTCTGCAGTATCCTA	Intron 2	84	166354	166370	1019
539393	AGTTTCTGCAGTATCCT	Intron 2	78	166355	166371	1020
539394	CAGTTTCTGCAGTATCC	Intron 2	77	166356	166372	1021
539395	CAAATTCCAGTCCTAGG	Intron 2	60	172746	172762	1022

539396	CCAAATTCCAGTCCTAG	Intron 2	75	172747	172763	1023
539397	TCCAAATTCCAGTCCTA	Intron 2	62	172748	172764	1024
539398	AACCCATTTCATCCATT	Intron 2	82	175372	175388	1025
539399	GAACCCATTTCATCCAT	Intron 2	86	175373	175389	1026
539400	GGAACCCATTTCATCCA	Intron 2	84	175374	175390	1027
539401	GCTTCATGTCTTTCTAG	Intron 2	88	189119	189135	1028
539402	TGCTTCATGTCTTTCTA	Intron 2	77	189120	189136	1029
539403	GTGCTTCATGTCTTTCT	Intron 2	95	189121	189137	1030
539404	TGAGCTTAGCAGTCACT	Intron 2	92	189484	189500	1031
539405	CATGAGCTTAGCAGTCA	Intron 2	82	189486	189502	1032
539406	TACAGACATAGCTCTAA	Intron 2	45	191142	191158	1033
539407	ATACAGACATAGCTCTA	Intron 2	53	191143	191159	1034
539408	GATACAGACATAGCTCT	Intron 2	67	191144	191160	1035
539409	TGTGGCTTTAATTCACT	Intron 2	70	198148	198164	1036
539410	ATGTGGCTTTAATTCAC	Intron 2	40	198149	198165	1037
539411	TATGTGGCTTTAATTCA	Intron 2	35	198150	198166	1038
539412	TGTTCAGTTGCATCACG	Intron 2	84	199816	199832	1039
539413	GTGTTCAGTTGCATCAC	Intron 2	80	199817	199833	1040
539414	TGTGTTCAGTTGCATCA	Intron 2	74	199818	199834	1041
539415	CATCTGGATGTGAGGCT	Intron 3	82	210561	210577	1042
539416	ACATCTGGATGTGAGGC	Intron 3	86	210562	210578	1043
539417	CACATCTGGATGTGAGG	Intron 3	55	210563	210579	1044
539418	TCAGGTAATTTCTGGAA	Intron 3	35	219019	219035	1045
539419	CTCAGGTAATTTCTGGA	Intron 3	44	219020	219036	1046
539420	TCTCAGGTAATTTCTGG	Intron 3	31	219021	219037	1047
539421	TTGCTTATTTACCTGGG	Intron 3	0	225568	225584	1048
539422	TTTGCTTATTTACCTGG	Intron 3	38	225569	225585	1049
539423	TTTTGCTTATTTACCTG	Intron 3	33	225570	225586	1050
539424	ATGATGTTACTACTACT	Intron 3	29	229618	229634	1051
539425	AATGATGTTACTACTAC	Intron 3	10	229619	229635	1052
539426	CAATGATGTTACTACTA	Intron 3	0	229620	229636	1053
539427	CCCCTAGAGCAATGGTC	Intron 3	67	232826	232842	1054
539428	CCCCCTAGAGCAATGGT	Intron 3	65	232827	232843	1055
539429	TCCCCCTAGAGCAATGG	Intron 3	45	232828	232844	1056
539430	TCAATTGCAGATGCTCT	Intron 3	78	237675	237691	1057
539431	CTCAATTGCAGATGCTC	Intron 3	82	237676	237692	1058
539432	GCTCAATTGCAGATGCT	Intron 3	92	237677	237693	1059
539433	AGCTCAATTGCAGATGC	Intron 3	85	237678	237694	1060
539434	GTATATTCAGTCCAAGG	Intron 3	73	248231	248247	1061
539435	AGTATATTCAGTCCAAG	Intron 3	70	248232	248248	1062
539436	CAGTATATTCAGTCCAA	Intron 3	40	248233	248249	1063

Table 137

Inhibition of GHR mRNA by 5-10-5 MOE gapmers targeting introns 1 and 3 of SEQ ID NO: 2

ISIS NO	Sequence	Target region	% inhibition	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO
532502	GAGTATTTCAGGCTGGAAAA	Intron 3	43	214623	214642	1064
533404	GTAACTCAGGAATGGAAAAC	Intron 1	56	26501	26520	1065
				113035	113054	
				121992	122011	
533405	AGTAACTCAGGAATGGAAAA	Intron 1	41	26502	26521	1066
				113036	113055	
				121993	122012	
533406	AAGTAACTCAGGAATGGAAA	Intron 1	43	26503	26522	1067
				113037	113056	
				121994	122013	
533407	GAGATTTCAAATAAATCTCA	Intron 1	0	143207	143226	1068
				143235	143254	
				143263	143282	
				143291	143310	
				143319	143338	
				143347	143366	
				143375	143394	
				143403	143422	
				143431	143450	
				143459	143478	
533408	TGAGATTTCAAATAAATCTC	Intron 1	11	143208	143227	1069
				143236	143255	
				143264	143283	
				143292	143311	
				143320	143339	
				143348	143367	
				143376	143395	
				143404	143423	
				143432	143451	
				143460	143479	
533409	GTGAGATTTCAAATAAATCT	Intron 1	0	143209	143228	1070
				143237	143256	
				143265	143284	
				143293	143312	
				143321	143340	
				143349	143368	
				143377	143396	

				143405	143424	
				143433	143452	
				143461	143480	
533410	TGTGAGATTTCAAATAAATC	Intron 1	0	143210	143229	1071
				143238	143257	
				143266	143285	
				143294	143313	
				143322	143341	
				143350	143369	
				143378	143397	
				143406	143425	
				143434	143453	
				143462	143481	
533411	TTGTGAGATTTCAAATAAAT	Intron 1	10	143183	143202	1072
				143211	143230	
				143239	143258	
				143267	143286	
				143295	143314	
				143323	143342	
				143351	143370	
				143379	143398	
				143407	143426	
				143435	143454	
				143463	143482	
533412	TTTGTGAGATTTCAAATAAA	Intron 1	0	143184	143203	1073
				143212	143231	
				143240	143259	
				143296	143315	
				143324	143343	
				143352	143371	
				143380	143399	
				143464	143483	
533413	CTTTGTGAGATTTCAAATAA	Intron 1	20	143185	143204	1074
				143213	143232	
				143241	143260	
				143297	143316	
				143325	143344	
				143353	143372	
				143381	143400	
				143465	143484	
533414	ACTTTGTGAGATTTCAAATA	Intron 1	57	143186	143205	1075
				143214	143233	
				143242	143261	

				143298	143317	
				143326	143345	
				143354	143373	
				143382	143401	
				143466	143485	
533415	CACTTTGTGAGATTTCAAAT	Intron 1	69	143187	143206	1076
				143215	143234	
				143243	143262	
				143299	143318	
				143327	143346	
				143355	143374	
				143383	143402	
				143467	143486	
533895	AGTATTTCAGGCTGGAAAAA	Intron 3	35	214622	214641	1077
533896	TGAGTATTTCAGGCTGGAAA	Intron 3	55	214624	214643	1078
533897	TCTGAGTATTTCAGGCTGGA	Intron 3	71	214626	214645	1079
533898	ATCTGAGTATTTCAGGCTGG	Intron 3	77	214627	214646	1080
533899	TATCTGAGTATTTCAGGCTG	Intron 3	58	214628	214647	1081
533900	TTTTGTGTTATGCCTTGAGG	Intron 3	51	221483	221502	1082
533901	TTTTTGTGTTATGCCTTGAG	Intron 3	55	221484	221503	1083
533902	ATTTTTGTGTTATGCCTTGA	Intron 3	57	221485	221504	1084
533903	ATATTTTTGTGTTATGCCTT	Intron 3	56	221487	221506	1085
533904	AATATTTTTGTGTTATGCCT	Intron 3	61	221488	221507	1086
533905	AAATATTTTTGTGTTATGCC	Intron 3	18	221489	221508	1087
533906	TTGCTTATTTACCTGGGTAA	Intron 3	58	225565	225584	1088
533907	TTTGCTTATTTACCTGGGTA	Intron 3	64	225566	225585	1089
533908	TTTTGCTTATTTACCTGGGT	Intron 3	77	225567	225586	1090
533909	CCTTTTGCTTATTTACCTGG	Intron 3	69	225569	225588	1091
533910	GCCTTTTGCTTATTTACCTG	Intron 3	69	225570	225589	1092
533911	TGCCTTTTGCTTATTTACCT	Intron 3	55	225571	225590	1093
533912	ATGATGTTACTACTACTCAA	Intron 3	60	229615	229634	1094
533913	AATGATGTTACTACTACTCA	Intron 3	48	229616	229635	1095
533914	CAATGATGTTACTACTACTC	Intron 3	57	229617	229636	1096
533915	TCCAATGATGTTACTACTAC	Intron 3	69	229619	229638	1097
533916	TTCCAATGATGTTACTACTA	Intron 3	74	229620	229639	1098
533917	ATTCCAATGATGTTACTACT	Intron 3	74	229621	229640	1099
533918	CCCCTAGAGCAATGGTCTAG	Intron 3	71	232823	232842	1100
533919	CCCCCTAGAGCAATGGTCTA	Intron 3	44	232824	232843	1101
533920	TCCCCCTAGAGCAATGGTCT	Intron 3	54	232825	232844	1102
533921	TATCCCCCTAGAGCAATGGT	Intron 3	62	232827	232846	1103
533922	ATATCCCCCTAGAGCAATGG	Intron 3	50	232828	232847	1104
533923	AATATCCCCCTAGAGCAATG	Intron 3	61	232829	232848	1105
533924	GCTCACATTTGGAAGACAGT	Intron 3	68	233623	233642	1106

533925	GGCTCACATTTGGAAGACAG	Intron 3	74	233624	233643	1107
533926	AGGCTCACATTTGGAAGACA	Intron 3	56	233625	233644	1108
533927	AGAGGCTCACATTTGGAAGA	Intron 3	34	233627	233646	1109
533928	TAGAGGCTCACATTTGGAAG	Intron 3	18	233628	233647	1110
533929	TTAGAGGCTCACATTTGGAA	Intron 3	19	233629	233648	1111
533930	CTCAATTGCAGATGCTCTGA	Intron 3	66	237673	237692	1112
533931	GCTCAATTGCAGATGCTCTG	Intron 3	72	237674	237693	1113
533932	AGCTCAATTGCAGATGCTCT	Intron 3	74	237675	237694	1114
533933	AAAGCTCAATTGCAGATGCT	Intron 3	66	237677	237696	1115
533934	TAAAGCTCAATTGCAGATGC	Intron 3	59	237678	237697	1116
533935	ATAAAGCTCAATTGCAGATG	Intron 3	23	237679	237698	1117
533936	GTGAGTCCATTAAACCTCTT	Intron 3	73	244873	244892	1118
533937	TGTGAGTCCATTAAACCTCT	Intron 3	73	244874	244893	1119
533938	ACTGTGAGTCCATTAAACCT	Intron 3	17	244876	244895	1120
533939	AACTGTGAGTCCATTAAACC	Intron 3	19	244877	244896	1121
533940	GAACTGTGAGTCCATTAAAC	Intron 3	28	244878	244897	1122
533941	ATATTGAAAGGCCCATCAAA	Intron 3	13	246498	246517	1123
533942	AATATTGAAAGGCCCATCAA	Intron 3	31	246499	246518	1124
533943	AAATATTGAAAGGCCCATCA	Intron 3	51	246500	246519	1125
533944	GAAAATATTGAAAGGCCCAT	Intron 3	22	246502	246521	1126
533945	GGAAAATATTGAAAGGCCCA	Intron 3	42	246503	246522	1127
533946	AGGAAAATATTGAAAGGCCC	Intron 3	28	246504	246523	1128
533947	GTATATTCAGTCCAAGGATC	Intron 3	65	248228	248247	1129
533948	AGTATATTCAGTCCAAGGAT	Intron 3	63	248229	248248	1130
533949	CAGTATATTCAGTCCAAGGA	Intron 3	67	248230	248249	1131
533950	AACAGTATATTCAGTCCAAG	Intron 3	56	248232	248251	1132
533951	AAACAGTATATTCAGTCCAA	Intron 3	60	248233	248252	1133
533952	AAAACAGTATATTCAGTCCA	Intron 3	59	248234	248253	1134
533953	TCTATTGTTGCCACCTTTAT	Intron 3	45	252838	252857	1135
533954	TTCTATTGTTGCCACCTTTA	Intron 3	52	252839	252858	1136
533955	TTTCTATTGTTGCCACCTTT	Intron 3	46	252840	252859	1137
533956	AGTTTCTATTGTTGCCACCT	Intron 3	59	252842	252861	1138
533957	CAGTTTCTATTGTTGCCACC	Intron 3	41	252843	252862	1139
533958	CCAGTTTCTATTGTTGCCAC	Intron 3	48	252844	252863	1140

Table 138

Inhibition of GHR mRNA by 5-10-5 MOE gapmers targeting intron 3 of SEQ ID NO: 2

ISIS NO	Sequence	% inhibition	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO
532454	GCAGAACTGATTGCTTACTT	78	182862	182881	1141
532455	AGGTCATAAGATTTTCATTT	48	183533	183552	1142

532456	GCCTCTGGCCATAAAGAAAT	54	183578	183597	1143
532457	AAAGTTTAAGAGGCACCCCA	31	184508	184527	1144
532458	GAATAAGCACAAAAGTTTAA	28	184519	184538	1145
532459	GAACCAAATAAACCTCTCTT	52	185452	185471	1146
532460	ATGTTGAAATTTGATCCCCA	79	185763	185782	1147
532461	TGTGAGAGCTCACTCACTAT	42	186134	186153	1148
532462	CTTGTGAGAGCTCACTCACT	72	186136	186155	1149
532463	ACATGGTGGCAGGAGAGAGG	42	186206	186225	1150
532464	CTAGAAAGAAACTACCTGAG	12	186341	186360	1151
532465	AACTTCAGTTGTAAAATAAT	27	187044	187063	1152
532466	GAAAAGGATTTTGAGATTTC	43	188897	188916	1153
532467	CTTAGCTGTCAAGGCCCTTT	80	189084	189103	1154
532468	TGTGCTTCATGTCTTTCTAG	88	189119	189138	1155
532469	CCCTTGAACATGCTATCCTT	85	189256	189275	1156
532470	CTTGCAGGGATGCATCTCAG	87	189625	189644	1157
532471	TCTCTTGCACATCTAATTTC	82	189656	189675	1158
532472	CTTCCAGCACAACCCATCAC	77	190109	190128	1159
532473	GTAACTACATTCCCTTTATC	52	190860	190879	1160
532474	AGTAACTACATTCCCTTTAT	58	190861	190880	1161
532475	CAGATAGCACAGGGCTAAAA	84	190979	190998	1162
532476	AGAATCAGGAATGTTTGCCT	86	192904	192923	1163
532477	TGACTCAATCATTTAGACTT	45	192990	193009	1164
532478	TCAACAGTCAATGGACTTGT	71	193042	193061	1165
532479	AATTTCTACTGCTATGATGC	75	194806	194825	1166
532480	ATGGTTCCAAATTTCTATCT	86	195704	195723	1167
532481	CTGTATGGCTTTAAGTATTC	63	196756	196775	1168
532482	AACTTATGAACTGTTCACCA	86	198307	198326	1169
532483	AATAAGCTTGAAGTCTGAAG	63	199520	199539	1170
532484	TAGTTATCTAACTGCCCAAT	77	199544	199563	1171
532485	TTCTGCAAAGCTTCCCAGTA	72	200314	200333	1172
532486	ACAACTTCAAGCTTCACATA	65	200599	200618	1173
532487	GAATCAATGTTCTGGCAAGA	52	201842	201861	1174
532488	CAGCCTTTCAGCTGTGAAAG	52	204181	204200	1175
532489	AACAATGCCAAGAAATCTAT	74	204369	204388	1176
532490	CCCACAGTAACAATGCCAAG	90	204377	204396	1177
532491	TTTTACCTCCCAGTGAAACT	34	205896	205915	1178
532492	TAATTGTTGATCCATGATGT	5	208856	208875	1179
532493	GTTGGAGAGACAAGTTTAAC	29	208975	208994	1180
532494	AGTCATAAAATTCAAATTAT	39	209537	209556	1181
532495	GGCCTTGGGCACACTTTCTC	82	207510	207529	1182
			210189	210208	
532496	AAGTTTTTATTGAAGTTAAT	0	212551	212570	1183
532497	AAGAAAAATTAGGAAGCTAG	31	212649	212668	1184

532498	CAGGGAGATAAGTTTATTCA	61	212797	212816	1185
532499	ATTTAATACACATTGGAATA	15	213390	213409	1186
532500	GTAGGACTATTTATGATTCC	86	213914	213933	1187
532501	CACTCTCTTGGGCTGTTAAG	82	214479	214498	1188
532502	GAGTATTTCAGGCTGGAAAA	66	214623	214642	1064
532503	TTGTTTGAGTTCCAAAAGAA	39	214932	214951	1189
532504	TTTGCCATGAGACACACAAT	77	215932	215951	1190
532505	CACCAAACCTCAGAGACATG	80	216468	216487	1191
532506	CCACTGTTAAGTGATGCATG	83	217480	217499	1192
532507	CTCTCAGGTAATTTCTGGAA	86	219019	219038	1193
532508	GCTCCTCACAATGACCCTTT	84	219452	219471	1194
532509	GGGACTGGCACTGGTAATTT	56	220062	220081	1195
532510	CTAACCATTAGTTACTGTAT	69	220558	220577	1196
532511	GGATTTTAGGTTCTTGCTGT	51	221588	221607	1197
532512	TGAATCATATACTGATATCA	63	222914	222933	1198
532513	TTGAGGTATTAAATTTTAAA	0	223001	223020	1199
532514	AGTTTGTAATGTAGTGATTT	19	223156	223175	1200
532515	AAATATTTGATAGCTCACAT	18	224409	224428	1201
532516	AGAAATATTTGATAGCTCAC	57	224411	224430	1202
532517	CCACATTTCAAATGTTCTCT	80	224717	224736	1203
532518	GCAGGAAGAGTGGCATGGAC	59	224750	224769	1204
532519	CACTTATCCAAATGCAGAGA	82	225742	225761	1205
532520	CAAGGTAATGGGAGGCTAGC	47	225903	225922	1206
532521	ATAGTCAAAGCTAAGGATAT	4	226177	226196	1207
532522	GTAATTTCATTCATGCTTCC	67	226804	226823	1208
532523	GTCCACATTCAGCTGTGTGT	72	231912	231931	1209
532524	TCATTCAGGAAATTCTGCTA	62	232286	232305	1210
532525	AACATGTCTCATTCAGGAAA	71	232294	232313	1211
532526	TAACATGTCTCATTCAGGAA	85	232295	232314	1212
532527	AGATTCCTCAAATTCAGTGA	66	232389	232408	1213
532528	TAAGCGGAAAAGGAGAAAAG	0	233684	233703	1214
532529	AAAGCAAGAGAATTCCTAAA	32	234203	234222	1215
532530	AATGAACCTTTAACTTAGTA	40	234876	234895	1216

Table 139

Inhibition of GHR mRNA by 5-10-5 MOE gapmers targeting introns 3-8 and intron-exonic regions of SEQ ID NO: 2

ISIS NO	Sequence	Target region	% inhibition	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO
523792	AAAGCTTTGTGGATAAAGTT	Intron 3	44	213025	213044	1217
523793	GAAGGAAAGGTTCTGTGGAA	Intron 3	38	213825	213844	1218
523794	CTGAGTATTTCAGGCTGGAA	Intron 3	84	214625	214644	1219

523795	TTGAATTATCCCTTTAAAAA	Intron 3	38	215446	215465	1220
523796	TTTAGAATGGTTTGGCATAC	Intron 3	66	216365	216384	1221
523797	GATATGTCCACATTGATTAG	Intron 3	65	218132	218151	1222
523798	ATTATTTAAGCTTCTACTTT	Intron 3	44	218973	218992	1223
523799	ATACATGGCAATTAAAAGAT	Intron 3	26	219886	219905	1224
523800	TGAGATAGTGTGGGAAATAT	Intron 3	18	220686	220705	1225
523801	TATTTTTGTGTTATGCCTTG	Intron 3	73	221486	221505	1226
523802	TTATTAACTAGAATATGCCT	Intron 3	16	223110	223129	1227
523803	GATTATTCTATTTTTATTTT	Intron 3	33	223948	223967	1228
523804	AGGAAGAGTGGCATGGACAT	Intron 3	43	224748	224767	1229
523805	CTTTTGCTTATTTACCTGGG	Intron 3	84	225568	225587	1230
523806	TTTATATTATTAATATCATT	Intron 3	31	226371	226390	1231
523807	GGTACATGGCTTTTAAGTGG	Intron 3	53	227218	227237	1232
523808	AATATTGGTCAGGTTTAAGA	Intron 3	28	228018	228037	1233
523809	ATTTCATCTCTTTCTTAGTT	Intron 3	45	228818	228837	1234
523810	CCAATGATGTTACTACTACT	Intron 3	89	229618	229637	1235
523811	GTTCCCCCAACCCCTTGGAA	Intron 3	28	230418	230437	1236
523812	TATAGGAAGTGAGATGTATG	Intron 3	46	231218	231237	1237
523813	ATTATTCTAGAAGAAGATTT	Intron 3	12	232018	232037	1238
523814	ATCCCCCTAGAGCAATGGTC	Intron 3	79	232826	232845	1239
523815	GAGGCTCACATTTGGAAGAC	Intron 3	69	233626	233645	1240
523816	TACACAAATCCAAGGCAGAG	Intron 3	57	234447	234466	1241
523817	AGGAAGAGTGGGAGTGTTAC	Intron 3	35	235258	235277	1242
523818	GTCCCTGACTAGGCATTTTG	Intron 3	43	236071	236090	1243
523819	AAGCTCAATTGCAGATGCTC	Intron 3	80	237676	237695	1244
523820	CTGTGAGTCCATTAAACCTC	Intron 3	81	244875	244894	1245
523821	TGAAATGTGGCTAGTGTGAC	Intron 3	51	245701	245720	1246
523822	AAAATATTGAAAGGCCCATC	Intron 3	68	246501	246520	1247
523823	AATGTCAATAGTGCCCTATT	Intron 3	48	247431	247450	1248
523824	ACAGTATATTCAGTCCAAGG	Intron 3	82	248231	248250	1249
523825	TGTCTATTTAAGTTTGTTGC	Intron 3	45	250001	250020	1250
523826	TTCAAGTACTGTCATGAATA	Intron 3	47	251214	251233	1251
523827	TTTCTTTTTCTTAAACTAAG	Intron 3	11	252041	252060	1252
523828	GTTTCTATTGTTGCCACCTT	Intron 3	70	252841	252860	1253
523829	AAGGCCACATATTATAGTAT	Intron 3	29	253698	253717	1254
523830	ACCTGAACTATTAATTTCTT	Intron 3	19	255397	255416	1255
523831	GAATGGGCTGAGTAGTTGAA	Intron 3	47	256197	256216	1256
523832	TGATGAACATTGCTAATTTG	Intron 3	26	257018	257037	1257
523833	ATCTTGCCTCGATGAAAGTT	Intron 3	17	257818	257837	1258
523834	TTAAGTGGCACAGCCATGAT	Intron 3	9	258774	258793	1259
523835	AATGAGTTAAGTTGGAACAC	Intron 3	25	261294	261313	1260
523836	TCCTTAGTAGAATGCCTGGA	Intron 3	57	263338	263357	1261
523837	TATGTAGAAAAATAAGCTGG	Intron 3	0	266514	266533	1262

523838	GCCGAGGCAGGCACCTGAGT	Intron 3	43	267375	267394	1263
523839	TGGTACCTATATTGAGAGGT	Intron 4	46	269052	269071	1264
523840	TTAAGGAAAAATATAGTATA	Intron 4	7	269854	269873	1265
523841	TTATTTATGTGTCAGGGATG	Intron 4	28	270668	270687	1266
523842	CAAAAGTTAAGTGCTTTAGG	Intron 4	10	271468	271487	1267
523843	TTCATAGATGTCTAAGGAAT	Intron 4	32	273341	273360	1268
523844	ACCTGTGATTTACCTATTTC	Exon 5- intron 5 junction	18	274185	274204	1269
523845	TGCCTAGAAAACCACATAAA	Intron 5	38	274985	275004	1270
523846	AAACATCCTCAAAGGTACCT	Intron 5	64	275808	275827	1271
523847	CTTCCCTGAGACACACACAT	Intron 5	35	276617	276636	1272
523848	CTTCTTCAATCTTCTCATAC	Intron 5	33	278288	278307	1273
523849	TACCATTTTCCATTTAGTTT	Exon 6- intron 6 junction	7	279088	279107	1274
523850	ATTGGCATCTTTTTCAGTGG	Intron 6	34	279902	279921	1275
523851	TCAAGCTCACGGTTGGAGAC	Intron 6	36	280799	280818	1276
523852	AAATGAAATCAGTATGTTGA	Intron 6	0	281622	281641	1277
523853	TGATTTATCACAAAGGTGCT	Intron 6	29	282437	282456	1278
523854	AAAACAGTAGAAAAGATTAA	Intron 6	14	284073	284092	1279
523855	CTACATCACAGCAGTCAGAA	Intron 6	23	285187	285206	1280
523856	AAAAGATGTAAGTGTGACAT	Intron 6	28	286349	286368	1281
				286919	286938	
523857	TTACAAGAACTGCTAAAGGG	Intron 6	15	287151	287170	1282
523858	ATAAAGAAAAAGTTAACTGA	Intron 6	9	287982	288001	1283
523859	AGATAATATACTTCTTCTAT	Intron 6	4	288809	288828	1284
523860	CCTTCTTCACATGTAAATTG	Exon 7- intron 7 junction	19	290456	290475	1285
523861	TTTCTATGTAGCTTGTGGTT	Intron 7	30	291258	291277	1286
523862	AGGCAGAGTTTTTATTGATA	Intron 7	19	292058	292077	1287
523863	ATAGTCACCAGCCTAAGCCT	Intron 8	28	292858	292877	1288
523864	AGACTTTTAGCATGCTTGAC	Intron 8	56	293658	293677	1289
523865	TTTACAGCCCTACAGTTCTA	Intron 8	7	294464	294483	1290
523866	CCAGAGAACCTGACTCCAAA	Intron 8	6	295330	295349	1291
523867	CAGAAGAAAATATTAGACAG	Intron 8	10	296993	297012	1292

Table 140

Inhibition of GHR mRNA by 5-10-5 MOE gapmers targeting introns 3-8 of SEQ ID NO: 2

ISIS NO	Sequence	Target Region	% inhibition	SEQ ID NO: 2 Start Site	SEQ ID NO: 2 Stop Site	SEQ ID NO

532531	TATTATACTTCTAAATTCCC	Intron 3	70	236716	236735	1293
532532	TAAAAGCAAGAAAAAGGAAC	Intron 3	52	236889	236908	1294
532533	CCTAATTTATATGAACAAAC	Intron 3	56	237177	237196	1295
532534	TGCAATGCCTTAGCCTAAAA	Intron 3	86	238087	238106	1296
532535	CACCACCATTATTACACTAC	Intron 3	75	238186	238205	1297
532536	AAATAAATCAGATTATTATA	Intron 3	52	238242	238261	1298
532537	CTTAGATCTGTGCTGTCCAA	Intron 3	81	245758	245777	1299
532538	GTTAGTGTTAGATTCTTTGA	Intron 3	67	246152	246171	1300
532539	CATGCTCACGGCTGTGTTAC	Intron 3	66	246248	246267	1301
532540	CCCATCAAATACTGAGTTCT	Intron 3	86	246487	246506	1302
532541	GAAAGTAGTGATTAATGAGA	Intron 3	38	247012	247031	1303
532542	ATTAATCAACAAGTGGCATT	Intron 3	72	247203	247222	1304
532543	TTTAATTTTAGGGTTTAGAG	Intron 3	48	248344	248363	1305
532544	CTTGCTACCACTAGAGCCTT	Intron 3	69	248694	248713	1306
532545	ACCACTGACTTATATCATTT	Intron 3	58	248743	248762	1307
532546	TTCCCCATTGCTAATTTTGT	Intron 3	48	251601	251620	1308
532547	TCCTGAAACTTAGTAGCTGG	Intron 3	83	253147	253166	1309
532548	TGTCTTAAAAAGGAATAAAA	Intron 3	52	253785	253804	1310
532549	CCTATAATAAAGTATTGTCT	Intron 3	70	253800	253819	1311
532550	ATGTAAAATGGTATAGCTAC	Intron 3	50	254040	254059	1312
532551	AACCCTCACACACTTCTGTT	Intron 3	71	254064	254083	1313
532552	ATTCTGCATAAGCAGTGTTT	Intron 3	53	254246	254265	1314
532553	TTACTACCCTGAAGAAGAAC	Intron 3	35	254314	254333	1315
532554	AAGACCTATAACTTACTACC	Intron 3	49	254326	254345	1316
532555	TTTCACAAGATTTACTTGGT	Intron 3	77	254641	254660	1317
532556	CAGTTGTGATTGTCAACCTA	Intron 3	77	257073	257092	1318
532557	AATCTTGCCTCGATGAAAGT	Intron 3	57	257819	257838	1319
532558	TGGCCTAAATGTATCAGTTA	Intron 3	66	259157	259176	1320
532559	AGGCTTTGGGTAAAATCTTT	Intron 3	67	259184	259203	1321
532560	TATGATTTTTAAAGATTAAA	Intron 3	20	261419	261438	1322
532561	GTACAGTGAAAAAGATGTGT	Intron 3	56	263666	263685	1323
532562	GACAGGTATGAAGCAAAACA	Intron 3	64	267033	267052	1324
532563	TGAGCTGAGGGTCTTTGCCG	Intron 3	61	267391	267410	1325
532564	AGGCTGAGTTGTACACAAAC	Intron 4	52	269422	269441	1326
532565	ATGAGGAGGCTGAGTTGTAC	Intron 4	43	269428	269447	1327
532566	TCATAAAGTGGGCCCAGCTT	Intron 4	70	270044	270063	1328
532567	ACTCCTAATCCCTCAGTTTT	Intron 4	62	270492	270511	1329
532568	TTTACATGCAAGGAGCTGAG	Intron 4	61	271047	271066	1330
532569	TAATGCCCTTTCTCCCTACT	Intron 4	60	271215	271234	1331
532570	CCTGTTTAGATTATCCCAAA	Intron 4	62	271763	271782	1332
532571	CATGATTCACAGAATTTCTC	Intron 4	56	271831	271850	1333
532572	AGTTAGAAAACTCAAAGTAT	Intron 4	2	271915	271934	1334
532573	TCAAATGTACTTAGCATAAG	Intron 4	9	271947	271966	1335

532574	ATATCAAATGTACTTAGCAT	Intron 4	59	271950	271969	1336
532575	AAAGTTCAGAAGAGGGAATG	Intron 4	51	273233	273252	1337
532576	AATTCCCATCTGAGTAGTTT	Intron 4	56	273440	273459	1338
532577	GTCCCCTAATTTCAGGCTAA	Intron 4	31	273471	273490	1339
532578	CTATGTCAAATGAAACAAAA	Intron 5	38	274205	274224	1340
532579	TGATTATGCTTTGTGATAAA	Intron 5	42	274624	274643	1341
532580	TCCAGCTGACTAGGAGGGCT	Intron 5	7	275732	275751	1342
532581	CATACCAGTCTCCTCGCTCA	Intron 5	0	276738	276757	1343
532582	ATATAACAGAATCCAACCAT	Intron 5	47	277045	277064	1344
				278361	278380	
532583	TGCAAAATGGCCAAACTACA	Intron 5	56	277577	277596	1345
532584	TCTTCCTAGCCACATGTGAT	Intron 5	32	278227	278246	1346
532585	TACCATGCTCTCTAATTGCC	Intron 6	47	279624	279643	1347
532586	AGTGATCTGTGCCAGGCTGC	Intron 6	65	279848	279867	1348
532587	AAGTTACAGAACAGATATCT	Intron 6	61	280012	280031	1349
532588	GTATTGTGAAAATAGTACTG	Intron 6	45	280226	280245	1350
532589	AAACACTATCAAGCTCACGG	Intron 6	54	280807	280826	1351
532590	TTCAAGAAAAGTCTTCAAAT	Intron 6	24	280831	280850	1352
532591	GGATCATTTCCCCATGCATG	Intron 6	52	280982	281001	1353
532592	ATATTATATTAAGAAAAATG	Intron 6	4	281422	281441	1354
532593	CTCCCATGTTCATTACTTAT	Intron 6	49	281587	281606	1355
532594	CATGACATTGGTTTGGGCAA	Intron 6	43	282229	282248	1356
532595	AATGTTGTTGGGAAAATTGG	Intron 6	42	282383	282402	1357
532596	AGCTGCAGGATACAAAGTCA	Intron 6	49	282986	283005	1358
532597	ATATCCTTTCATGATAAAAA	Intron 6	31	283354	283373	1359
532598	ATGGGCTAATATCTCTGATA	Intron 6	50	283590	283609	1360
532599	ACATTACTAATAATTAGAGA	Intron 6	0	285236	285255	1361
532600	ATAAAAACATATGAAAGTAT	Intron 6	12	287093	287112	1362
532601	TTCTGAATTAAATCTATTAG	Intron 6	16	287408	287427	1363
532602	TTACATTTTTGCAAATTTAT	Intron 6	31	287472	287491	1364
532603	TGAACAGTTGATTAACAAAG	Intron 6	15	287887	287906	1365
532604	AAGTTATTGGTTTACTAGAT	Intron 6	0	288598	288617	1366
532605	TTGGAAAAGGTCCTAGAAAA	Intron 6	24	289808	289827	1367
532606	CATGACAGAAACTTCTTAGA	Intron 7	25	292035	292054	1368
532607	CCATACTTGCTGACAAATAT	Intron 8	39	294389	294408	1369

Example 115: Dose-dependent antisense inhibition of human GHR in Hep3B cells by MOE gapmers

Gapmers from the studies described above exhibiting significant *in vitro* inhibition of GHR mRNA were selected and tested at various doses in Hep3B cells. 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. Cells were plated at a density of 20,000 cells per well and transfected using electroporation with 0.625 μM, 1.25 μM, 2.50 μM, 5.00 μM and 10.00 μM concentrations of antisense oligonucleotide, as specified in the Tables below. After a treatment period of approximately 16 hours, RNA was isolated from the cells and GHR mRNA levels were measured by quantitative real-time PCR. Human primer probe set RTS3437_MGB was used to measure mRNA levels. GHR mRNA levels were adjusted according to total RNA content, as measured by RIBOGREEN®. Results are presented as percent inhibition of GHR, relative to untreated control cells.

The half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is also presented. GHR mRNA levels were significantly reduced in a dose-dependent manner in antisense oligonucleotide treated cells.

Table 141

ISIS No	0.625 μM	1.250 μM	2.50 μM	5.00 μM	10.00 μM	IC_{50} (μM)
523271	41	61	73	86	92	0.8
523274	20	36	64	80	92	1.8
523324	35	45	68	91	90	1.2

Table 142

ISIS No	0.625 μM	1.250 μM	2.50 μM	5.00 μM	10.00 μM	IC_{50} (μM)
523604	21	42	68	58	86	2.0
523577	6	22	56	66	91	2.7
523614	14	44	61	84	87	1.9
523564	4	26	48	67	86	2.8
523633	30	43	71	82	84	1.4
523571	2	9	38	55	82	3.9

Table 143

ISIS No	0.625 μM	1.250 μM	2.50 μM	5.00 μM	10.00 μM	IC_{50} (μM)
523570	25	50	64	77	88	1.5
523592	27	42	59	79	88	1.7
523595	21	50	62	76	90	1.6
523596	36	47	62	75	77	1.4
523607	49	62	71	82	84	0.5
523615	20	49	63	83	91	1.6
523630	4	28	54	79	78	2.6
523661	4	34	48	73	79	2.7

523665	4	28	54	73	79	2.7
523687	30	56	61	78	81	1.4
523711	42	66	78	94	95	0.7
523712	6	37	60	72	89	2.3
523713	4	32	55	72	85	2.5
523714	59	75	88	95	97	0.2

Table 144

ISIS No	0.625 μM	1.250 μM	2.50 μM	5.00 μM	10.00 μM	IC_{50} (μM)
523655	26	33	60	67	78	2.1
523656	19	33	45	69	87	2.4
523658	0	42	62	67	79	3.1
523715	78	90	92	93	95	<0.6
523718	30	46	67	84	92	1.4
523723	56	69	83	92	94	0.3
523725	45	64	79	89	95	0.6
523726	32	48	77	88	89	1.2
523736	0	64	75	90	96	1.5
523747	48	64	80	91	92	0.6
523758	25	39	61	74	84	1.9
523766	7	37	66	81	93	2.0
523776	26	54	72	78	83	1.3
523789	62	68	81	85	90	0.2

Table 145

ISIS No	0.625 μM	1.250 μM	2.50 μM	5.00 μM	10.00 μM	IC_{50} (μM)
523719	24	46	65	84	93	1.5
523720	18	49	72	85	93	1.5
523724	43	61	77	91	91	0.7
523735	8	42	63	81	93	2.0
523740	37	58	72	83	88	1.0
523752	9	29	52	72	86	2.5
523763	8	32	57	70	80	2.6
523764	43	52	67	77	79	0.9
523765	24	48	62	88	4	1.5
523767	49	62	67	72	82	0.6
523772	29	39	54	62	61	2.7
523774	28	59	63	88	91	1.2
523778	25	32	63	78	84	1.9

523783	0	22	53	72	88	2.8

Table 146

ISIS No	0.625 μM	1.250 μM	2.50 μM	5.00 μM	10.00 μM	IC_{50} (μM)
532151	57	69	76	85	88	<0.6
532153	23	43	54	80	86	1.8
532158	46	58	81	87	87	0.6
532160	17	26	55	76	92	2.2
532162	14	46	71	83	93	1.7
532164	37	76	82	90	93	0.6
532171	41	81	67	81	83	<0.6
532181	56	81	84	89	93	0.2
532186	26	65	75	83	91	1.1
532188	51	68	80	89	93	<0.6
532189	24	31	52	75	86	2.1
532197	0	40	66	85	93	2.1
532199	24	37	50	73	87	2.1
532222	12	41	67	84	94	1.8

Table 147

ISIS No	0.625 μM	1.250 μM	2.50 μM	5.00 μM	10.00 μM	IC_{50} (μM)
532175	41	54	76	84	89	0.9
532223	53	69	75	88	94	<0.6
532235	43	58	67	77	82	0.8
532241	39	53	62	73	87	1.2
532248	49	65	72	85	93	0.6
532254	52	62	85	87	92	<0.6
532300	20	29	49	66	78	2.7
532304	26	39	66	78	90	1.7
532316	41	66	76	86	94	0.7
532395	32	56	84	93	97	1.0
532401	47	80	92	96	98	<0.6
532411	73	90	94	97	98	<0.6
532420	38	49	82	85	97	1.0
532436	37	58	75	90	96	0.9

Table 148

ISIS No	0.625 µM	1.250 µM	2.50 µM	5.00 µM	10.00 µM	IC_{50} (µM)
532410	66	83	92	94	97	<0.6
532468	45	68	78	93	94	0.6
532469	0	17	56	76	92	2.8
532470	10	34	62	84	94	2.0
532475	13	36	52	64	87	2.5
532476	34	64	73	79	93	0.9
532480	28	54	67	78	87	1.4
532482	21	39	69	83	92	1.7
532490	42	60	68	84	93	0.9
532500	37	50	63	81	87	1.2
532506	13	41	66	75	89	1.9
532507	47	59	71	86	89	0.7
532508	0	31	73	83	89	2.2
532526	31	56	78	79	88	1.1

Table 149

ISIS No	0.625 µM	1.250 µM	2.50 µM	5.00 µM	10.00 µM	IC_{50} (µM)
532495	59	74	81	87	95	<0.6
532501	49	53	71	83	84	0.7
532534	53	75	85	91	97	<0.6
532535	0	34	61	84	92	2.6
532537	49	67	80	90	94	<0.6
532540	59	70	87	93	95	<0.6
532547	57	71	81	91	92	<0.6
532555	48	36	61	72	85	1.3
532556	33	57	67	86	90	1.1

Table 150

ISIS No	0.625 µM	1.250 µM	2.50 µM	5.00 µM	10.00 µM	IC_{50} (µM)
523421	32	57	81	82	88	1.0
533006	46	43	69	83	91	1.0
533121	53	75	75	88	93	<0.6
533122	65	77	82	90	93	<0.6
533123	39	71	84	91	95	0.6
533125	49	61	81	85	91	0.6

533131	3	57	59	82	90	1.9
533136	32	65	62	81	88	1.1
533139	13	51	72	90	94	1.5
533140	36	66	39	87	92	1.2
533153	50	65	83	89	90	<0.6
533156	43	64	74	85	90	0.7
533160	57	80	87	91	95	<0.6
533161	54	62	81	89	92	<0.6

Table 151

ISIS No	0.625 µM	1.250 µM	2.50 µM	5.00 µM	10.00 µM	IC_{50} (µM)
533234	50	70	86	93	95	<0.6
533237	5	45	63	84	93	1.9
533233	43	55	76	90	95	0.8
533179	31	63	75	87	87	1.0
533178	53	67	76	89	94	<0.6
533187	5	15	53	79	86	2.7
533188	49	68	83	89	94	<0.6
533271	45	66	85	92	94	0.6
533134	22	45	64	81	89	1.6
533258	52	72	88	93	95	<0.6
533235	50	54	75	82	90	0.7
533262	23	54	78	91	96	1.2
533189	48	66	78	82	88	<0.6
533193	38	53	72	77	91	1.0

Table 152

ISIS No	0.625 µM	1.250 µM	2.50 µM	5.00 µM	10.00 µM	IC_{50} (µM)
533259	63	78	84	90	92	<0.6
533291	25	57	75	86	96	1.2
533256	67	76	90	95	95	<0.6
533269	42	75	82	94	97	0.6
533265	67	78	91	95	97	<0.6
533318	16	45	77	87	95	1.5
533257	55	84	91	96	96	<0.6
533280	34	62	80	91	91	0.9
533301	52	77	84	93	96	<0.6
533316	41	50	79	93	94	0.9
533270	62	71	88	94	97	<0.6

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
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JUMBO APPLICATIONS/PATENTS

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NOTE POUR LE TOME / VOLUME NOTE:

WHAT IS CLAIMED:

1. A compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 8 to 80 linked nucleosides complementary within nucleobases 30-51, 63-82, 103-118, 143-159, 164-197, 206-259, 361-388, 554-585, 625-700, 736-776, 862-887, 923-973, 978-996, 1127-1142, 1170-1195, 1317-1347, 1360-1383, 1418-1449, 1492-1507, 1524-1548, 1597-1634, 1641-1660, 1683-1698, 1744-1768, 1827-1860, 1949-2002, 2072-2092, 2095-2110, 2306-2321, 2665-2683, 2685-2719, 2739-2770, 2859-2880, 2941-2960, 2963-2978, 3037-3052, 3205-3252, 3306-3332, 3371-3386, 3518-3542, 3975-3990, 4041-4087, 4418-4446, 4528-4546, 7231-7246, 7570-7585, 8395-8410, 9153-9168, 9554-9569, 9931-9946, 10549-10564, 11020-11035, 11793-11808, 12214-12229, 12474-12489, 12905-12920, 13400-13415, 13717-13732, 14149-14164, 14540-14555, 15264-15279, 15849-15864, 16530-16545, 17377-17392, 17581-17596, 17943-17958, 18353-18368, 18636-18651, 19256-19271, 19814-19829, 20365-20380, 20979-20994, 21566-21581, 22150-22165, 22803-22818, 29049-29064, 29554-29569, 30245-30260, 30550-30565, 30915-30930, 31468-31483, 32366-32381, 32897-32912, 33187-33202, 33780-33795, 34407-34422, 34846-34861, 35669-35684, 36312-36327, 36812-36827, 37504-37519, 38841-38856, 40250-40265, 40706-40721, 40922-40937, 41424-41439, 41999-42014, 42481-42496, 42700-42715, 43291-43306, 43500-43515, 43947-43962, 44448-44463, 45162-45177, 46010-46025, 46476-46491, 47447-47462, 47752-47767, 48001-48016, 48423-48438, 50195-50210, 50470-50485, 51104-51119, 51756-51771, 52015-52030, 52230-52245, 52588-52603, 53532-53547, or 54645-54660 of SEQ ID NO: 1, and wherein said modified oligonucleotide is at least 85%, 90%, 95%, or 100% complementary to SEQ ID NO: 1.
2. A compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide, wherein the modified oligonucleotide consists of 8 to 80 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases 100% complementary to an equal length portion of nucleobases 30-51, 63-82, 103-118, 143-159, 164-197, 206-259, 361-388, 554-585, 625-700, 736-776, 862-887, 923-973, 978-996, 1127-1142, 1170-1195, 1317-1347, 1360-1383, 1418-1449, 1492-1507, 1524-1548, 1597-1634, 1641-1660, 1683-1698, 1744-1768, 1827-1860, 1949-2002, 2072-2092, 2095-2110, 2306-2321, 2665-2683, 2685-2719, 2739-2770, 2859-2880, 2941-2960, 2963-2978, 3037-3052, 3205-3252, 3306-3332, 3371-3386, 3518-3542, 3975-3990, 4041-4087, 4418-4446, 4528-4546, 7231-7246, 7570-7585, 8395-8410, 9153-9168, 9554-9569, 9931-9946, 10549-10564, 11020-11035, 11793-11808, 12214-12229, 12474-12489, 12905-12920, 13400-13415, 13717-13732, 14149-14164, 14540-14555, 15264-15279, 15849-15864, 16530-16545, 17377-17392, 17581-17596, 17943-17958, 18353-18368, 18636-18651, 19256-19271, 19814-19829, 20365-20380, 20979-20994, 21566-21581, 22150-22165, 22803-22818, 29049-29064, 29554-29569, 30245-30260, 30550-30565,

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3. A compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 8 to 80 linked nucleosides complementary within nucleobases 2571-2586, 2867-3059, 3097-3116, 3341-3695, 4024-4039, 4446-4894, 5392-5817, 6128-6265, 6499-6890, 7231-7246, 8395-8410, 9153-9168, 9554-9569, 9931-9946, 10549-10564, 10660-10679, 11020-11035, 11793-12229, 12469-12920, 13351-13415, 13717-13732, 14149-14164, 14361-14555, 14965-15279, 15849-16001, 16253-16272, 16447-16545, 17130-17149, 17377-17669, 17927-17958, 18353-18368, 18636-18773, 19661-19918, 20288-20470, 20979-20994, 21215-21606, 21820-21837, 22150-22165, 22518-22536, 22803-22818, 26494-26522, 29049-29069, 29323-29489, 30550-30565, 30915-31191, 31468-31483, 32363-32382, 32827-33202, 33635-33795, 34138-34157, 34407-34422, 34845-34864, 35466-35485, 35669-35684, 36023-36042, 36266-36327, 36721-36827, 37032-37130, 37276-37295, 37504-37675, 38094-38118, 38841-38856, 39716-40538, 40706-40937, 41164-41183, 41342-41439, 42141-42164, 42700-42760, 43173-43537, 43765-46025, 46476-46532, 48423-48438, 50072-50210, 50470-50485, 50719-51234, 51747-51797, 52015-52143, 52230-52245, 52573-52652, 53466-54660, 54886-54901, 63751-64662, 64882-65099, 65363-65378, 65600-65615, 65988-66183, 66566-66581, 66978-67080, 67251-67270, 67662-67929, 68727-68742, 69203-69242, 69565-69620, 69889-70145, 70352-70584, 70925-71071, 71314-71329, 71617-71769, 72107-72241, 72584-72670, 73061-73076, 73350-73369, 73689-73723, 74107-74131, 74317-74557, 74947-75009, 75192-75207, 75979-76066, 76410-77095, 77292-77307, 77638-77869, 78122-78326, 79006-79021, 79478-79505, 80277-80292, 80575-80939, 81207-81222, 81524-81543, 81761-81776, 82233-82248, 82738-83198, 83330-83416, 83884-84063, 84381-85964, 86220-86392, 86554-86655, 86901-86920, 87181-87262, 88063-88082, 88293-88308, 88605-88967, 89160-89175, 89940-90255, 90473-90528, 91073-91088, 91273-91292, 91647-91662, 91930-92126, 92356-92371, 93190-93443, 93762-94111, 94374-94389, 94581-94653, 94839-94858, 95292-95583, 95829-95844, 96137-96503, 96793-97013, 97539-97554, 97800-97889, 98132-98151, 98624-98672, 98810-99115, 99258-99273, 99478-99503, 99791-99858, 100281-100300, 100406-100421, 100742-100828, 101080-101103, 101242-101320, 101788-101906, 102549-102568, 103566-103625, 104067-104086, 104277-104858, 105255-105274, 106147-106364, 106632-106647, 106964-107735, 108514-108788, 109336-109505, 109849-109864, 110403-110442, 110701-110974, 111203-

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4. A compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 8 to 80 linked nucleosides having a nucleobase sequence comprising a portion of at least 8 contiguous nucleobases 100% complementary to an equal length portion of nucleobases2571-2586, 2867-3059, 3097-3116, 3341-3695, 4024-4039, 4446-4894, 5392-5817, 6128-6265, 6499-6890, 7231-7246, 8395-8410, 9153-9168, 9554-9569, 9931-9946, 10549-10564, 10660-10679, 11020-11035, 11793-12229, 12469-12920, 13351-13415, 13717-13732, 14149-14164, 14361-14555, 14965-15279, 15849-16001, 16253-16272, 16447-16545, 17130-17149, 17377-17669, 17927-17958, 18353-18368, 18636-18773, 19661-19918, 20288-20470, 20979-20994, 21215-21606, 21820-21837, 22150-22165, 22518-22536, 22803-22818, 26494-26522, 29049-29069, 29323-29489, 30550-30565, 30915-31191, 31468-31483, 32363-32382, 32827-33202, 33635-33795, 34138-34157, 34407-34422, 34845-34864, 35466-35485, 35669-35684, 36023-36042, 36266-36327, 36721-36827, 37032-37130, 37276-37295, 37504-37675, 38094-38118, 38841-38856, 39716-40538, 40706-40937, 41164-41183, 41342-41439, 42141-42164, 42700-42760, 43173-43537, 43765-46025, 46476-46532, 48423-48438, 50072-50210, 50470-50485, 50719-51234, 51747-51797, 52015-52143, 52230-52245, 52573-52652, 53466-54660, 54886-54901, 63751-64662, 64882-65099, 65363-65378, 65600-65615, 65988-66183, 66566-66581, 66978-67080, 67251-67270, 67662-67929, 68727-68742, 69203-69242, 69565-69620, 69889-70145, 70352-70584, 70925-71071, 71314-71329, 71617-71769, 72107-72241, 72584-72670, 73061-73076, 73350-73369, 73689-73723, 74107-74131, 74317-74557, 74947-75009, 75192-75207, 75979-76066, 76410-77095, 77292-77307, 77638-77869, 78122-78326, 79006-79021, 79478-79505, 80277-80292, 80575-80939, 81207-81222, 81524-81543, 81761-81776, 82233-82248, 82738-83198, 83330-83416, 83884-84063, 84381-85964, 86220-86392, 86554-86655, 86901-86920, 87181-87262, 88063-88082, 88293-88308, 88605-88967, 89160-89175, 89940-90255, 90473-90528, 91073-91088, 91273-91292, 91647-91662, 91930-92126, 92356-92371, 93190-93443, 93762-94111, 94374-94389, 94581-94653, 94839-94858, 95292-95583, 95829-95844, 96137-96503, 96793-97013, 97539-

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5. A compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide is targeted to an intron of a GHR nucleic acid.

6. A compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 8 to 80 linked nucleosides complementary within nucleotides 72107-72126, 153004-153019, 153921-153940 , 155597-155612, 155594-155613, 159252-159267, 213425-213440, or 248234-248249 of SEQ ID NO: 2.

7. A compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 8 to 80 linked nucleosides having a nucleobase sequence comprising any one of SEQ ID NOs: 479, 703, 918, 1800, 1904, 2122, 2127, and 2194.

8. A compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide has a nucleobase sequence comprising any one of SEQ ID NOs: 479, 703, 918, 1800, 1904, 2122, 2127, and 2194.

9. 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: 20-2295.

10. 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: 20-2295.
11. 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: 20-2295.
12. 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: 20-2295.
13. 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: 20-2295.
14. 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 sequences of SEQ ID NOs: 20-2295.
15. A compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of the nucleobase sequences of SEQ ID NOs: 20-2295.

16. A compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide has a nucleobase sequence comprising at least an 8 nucleobase portion of any of SEQ ID NOs: 479, 703, 918, 1800, 1904, 2122, 2127, and 2194.

17. A compound comprising a 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 NOs: 479, 703, or 918, 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 the 5' wing segment comprises a 2'-O-methoxyethyl sugar; wherein each nucleoside of the 3' wing segment comprises a 2'-O-methoxyethyl sugar; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

18. A compound comprising a modified oligonucleotide and a conjugate group, wherein the modified oligonucleotide consists of 16 linked nucleosides having a nucleobase sequence consisting of the

sequence recited in SEQ ID NOs: 1800, 1904, 2122, 2127, or 2194, wherein the modified oligonucleotide comprises each nucleoside has either a MOE sugar modification, an (S)-cEt sugar modification, or a deoxy modification; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.

19. The compound of any one of claims 1-18, wherein the oligonucleotide is at least 80%, 85%, 90%, 95%, or 100% complementary to SEQ ID NOs: 1 or 2.
20. The compound of any one of claims 1-19, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage, at least one modified sugar, or at least one modified nucleobase.

21. The compound of claim 20, wherein the modified internucleoside linkage is a phosphorothioate internucleoside linkage.

22. The compound of claim 20, wherein the modified oligonucleotide comprises at least 1 phosphodiester internucleoside linkage.

23. The compound of claim 20, wherein the modified oligonucleotide comprises at least 2 phosphodiester internucleoside linkages.

24. The compound of claim 20, wherein the modified oligonucleotide comprises at least 3 phosphodiester internucleoside linkages.

25. The compound of claim 20, wherein the modified oligonucleotide comprises at least 4 phosphodiester internucleoside linkages.

26. The compound of claim 20, wherein the modified oligonucleotide comprises at least 5 phosphodiester internucleoside linkages.

27. The compound of claim 20, wherein the modified oligonucleotide comprises at least 6 phosphodiester internucleoside linkages.

28. The compound of claim 20, wherein the modified oligonucleotide comprises at least 7 phosphodiester internucleoside linkages.

29. The compound of any of claims 22-28, wherein each internucleoside linkage of the modified oligonucleotide is selected from a phosphodiester internucleoside linkage and a phosphorothioate internucleoside linkage.

30. The compound of claim 20, wherein each internucleoside linkage of the modified oligonucleotide comprises is a phosphorothioate internucleoside linkage.

31. The compound of any one of claims 20-30, wherein the modified sugar is a bicyclic sugar.

32. The compound of claim 31, wherein the bicyclic sugar is selected from the group consisting of: 4'-(CH_2)-O-2' (LNA); 4'-(CH_2)$_2$-O-2' (ENA); and 4'-CH(CH_3)-O-2' (cEt).

33. The compound of any one of claims 20-30, wherein the modified sugar is 2'-O-methoxyethyl.

34. The compound of any one of claims 20-33, wherein the modified nucleobase is a 5-methylcytosine.

35. The compound of any one of claims 1-34, wherein the modified oligonucleotide comprises:
    (a) a gap segment consisting of linked deoxynucleosides;
    (b) a 5' wing segment consisting of linked nucleosides; and
    (c) a 3' wing segment consisting of linked nucleosides;

    wherein the gap segment is positioned immediately adjacent to and between the 5' wing segment and the 3' wing segment and wherein each nucleoside of each wing segment comprises a modified sugar.
36. The compound of any one of claims 1-34, wherein the modified oligonucleotide comprises of nucleosides comprising either a MOE sugar modification, an (S)-cEt sugar modification, or a deoxy modification; wherein each internucleoside linkage is a phosphorothioate linkage; and wherein each cytosine is a 5-methylcytosine.
37. The compound of any one of claims 1-36, wherein the compound is single-stranded.
38. The compound of any one of claims 1-36, wherein the compound is double-stranded
39. The compound of any one of claims 1-38, wherein the compound comprises ribonucleotides.
40. The compound of any one of claims 1-38, wherein the compound comprises deoxyribonucleotides.
41. The compound of any one of claims 1-40, wherein the modified oligonucleotide consists of 10 to 30 linked nucleosides.
42. The compound of any one of claims 1-40, wherein the modified oligonucleotide consists of 12 to 30 linked nucleosides.

43. The compound of any one of claims 1-40, wherein the modified oligonucleotide consists of 15 to 30 linked nucleosides.
44. A compound consisting of ISIS 523723, ISIS 532254, ISIS 532401, ISIS 541767, ISIS 541875, ISIS 542112, ISIS 542118, or ISIS 542185, and a conjugate group.

45. The compound of any of claims 1 to 44, wherein the conjugate group is linked to the modified oligonucleotide at the 5' end of the modified oligonucleotide.

46. The compound of any of claims 1 to 44, wherein the conjugate group is linked to the modified oligonucleotide at the 3' end of the modified oligonucleotide.

47. The compound of any of claims 1-46, wherein the conjugate group comprises exactly one ligand.

48. The compound of any of claims 1-46, wherein the conjugate group comprises exactly two ligands.

49. The compound of any of claims 1-46, wherein the conjugate group comprises three or more ligands.

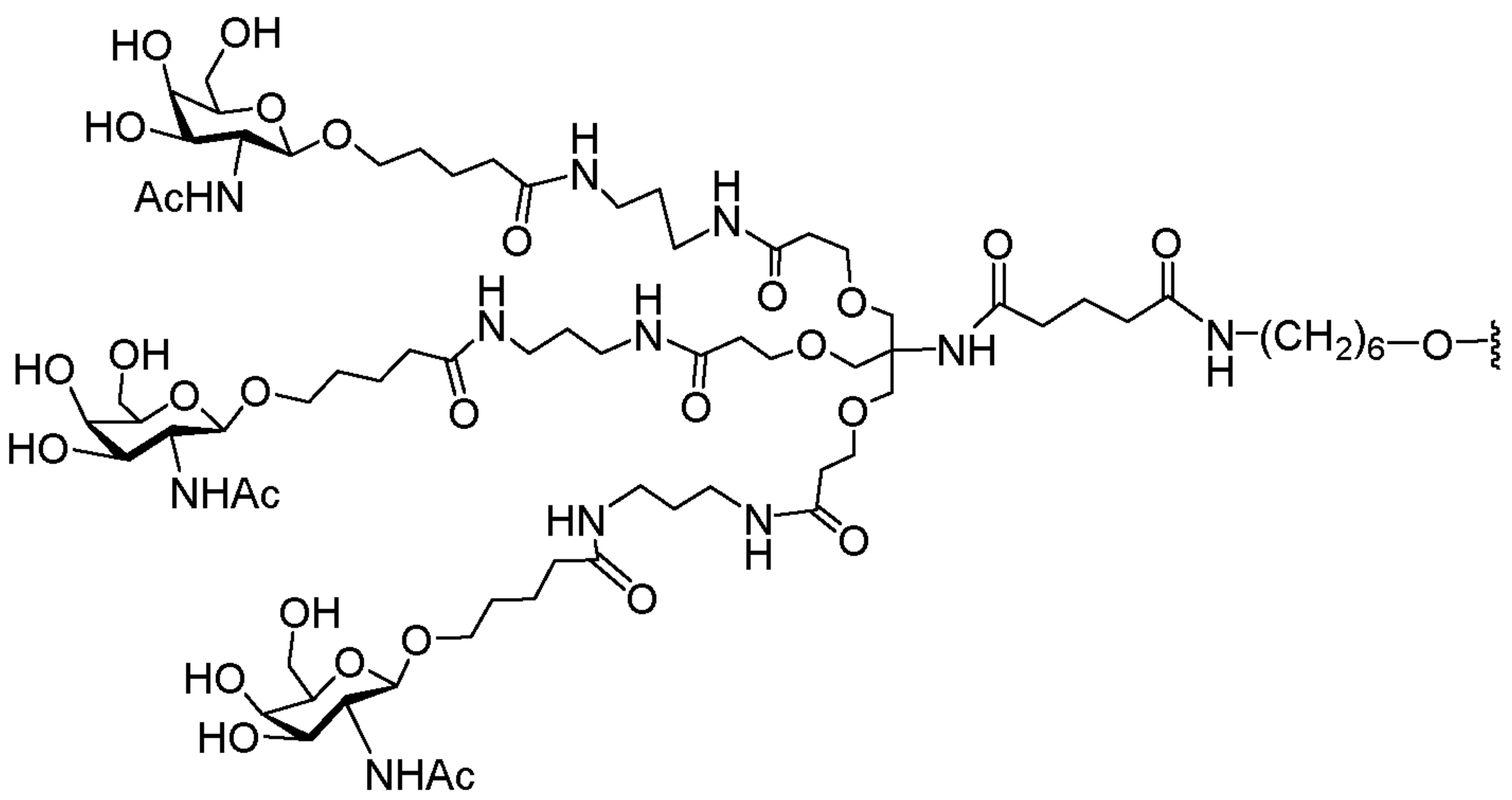
50. The compound of any of claims 1-46, wherein the conjugate group comprises exactly three ligands.

51. The compound of any of claims 47-50, wherein each ligand is selected from among: a polysaccharide, modified polysaccharide, mannose, galactose, a mannose derivative, a galactose derivative, D-mannopyranose, L-Mannopyranose, D-Arabinose, L-Galactose, D-xylofuranose, L-xylofuranose, D-glucose, L-glucose, D-Galactose, L-Galactose, α-D-Mannofuranose, β-D-Mannofuranose, α-D-Mannopyranose, β-D-Mannopyranose, α-D-Glucopyranose, β-D-Glucopyranose, α-D-Glucofuranose, β-D-Glucofuranose, α-D-fructofuranose, α-D-fructopyranose, α-D-Galactopyranose, β -D-Galactopyranose, α-D-Galactofuranose, β -D-Galactofuranose, glucosamine, sialic acid, α-D-galactosamine, N-Acetylgalactosamine, 2-Amino-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-β-D-glucopyranose, 2-Deoxy-2-methylamino-L-glucopyranose, 4,6-Dideoxy-4-formamido-2,3-di-*O*-methyl-D-mannopyranose, 2-Deoxy-2-

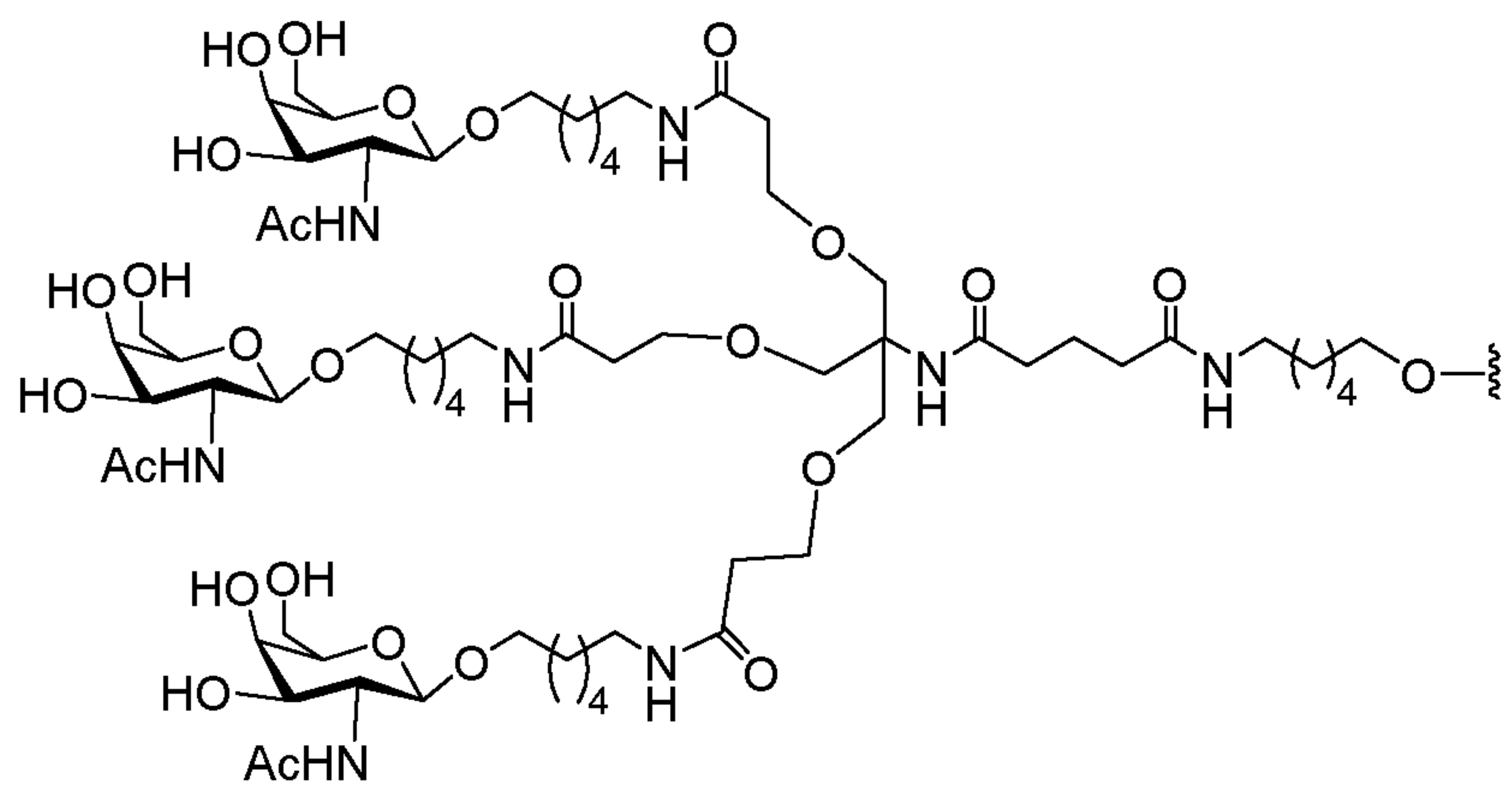
sulfoamino-D-glucopyranose, *N*-Glycoloyl-α-neuraminic acid, 5-thio-β-D-glucopyranose, methyl 2,3,4-tri-*O*-acetyl-1-thio-6-*O*-trityl-α-D-glucopyranoside, 4-Thio-β-D-galactopyranose, ethyl 3,4,6,7-tetra-*O*-acetyl-2-deoxy-1,5-dithio-α-D-*gluco*-heptopyranoside, 2,5-Anhydro-D-allononitrile, ribose, D-ribose, D-4-thioribose, L-ribose, L-4-thioribose.

52. The compound of claim 51, wherein each ligand is N-acetyl galactosamine.

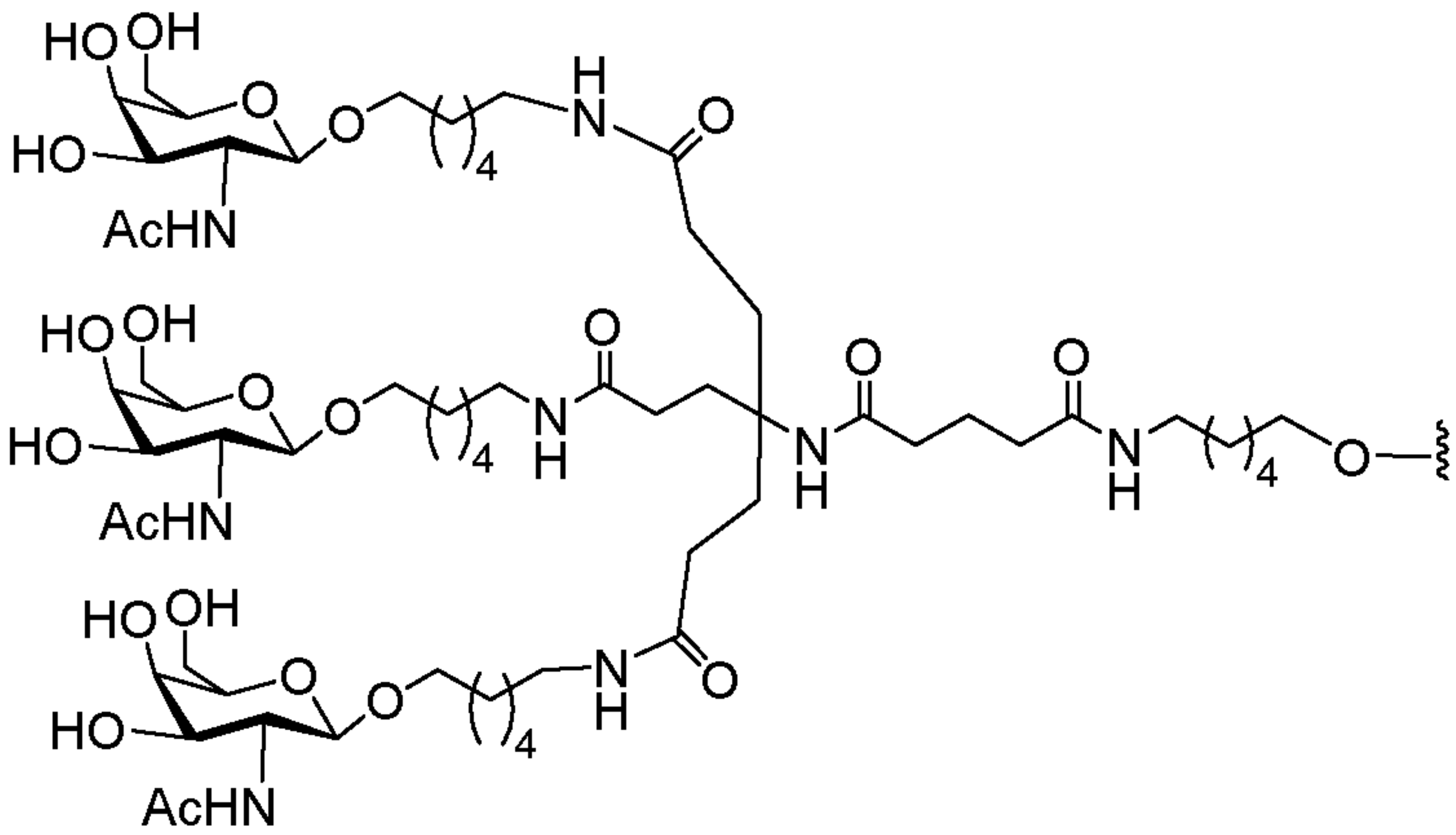
53. The compound of any of claims 1 to 46, wherein the conjugate group comprises:



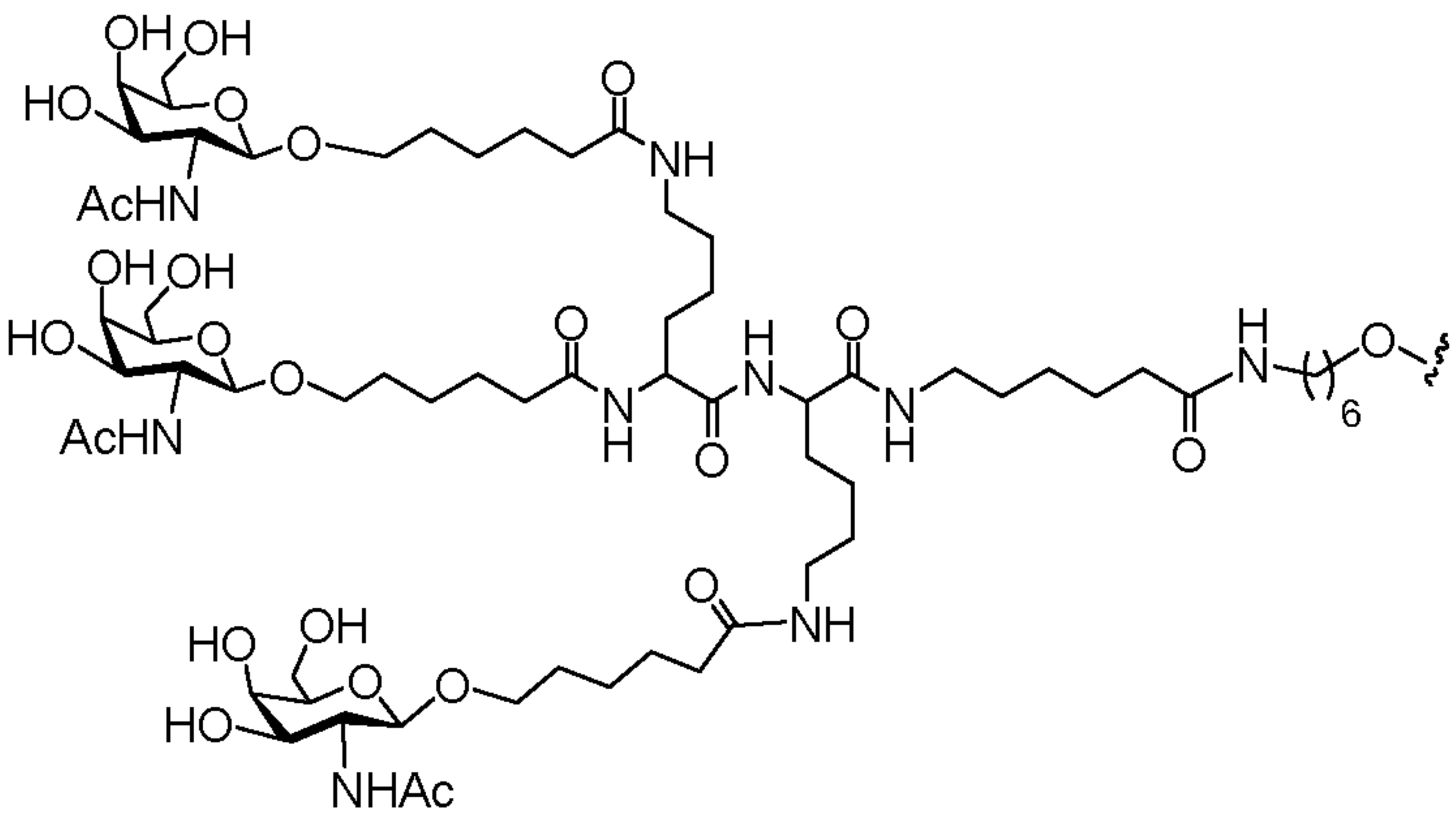
54. The compound of any of claims 1 to 46, wherein the conjugate group comprises:



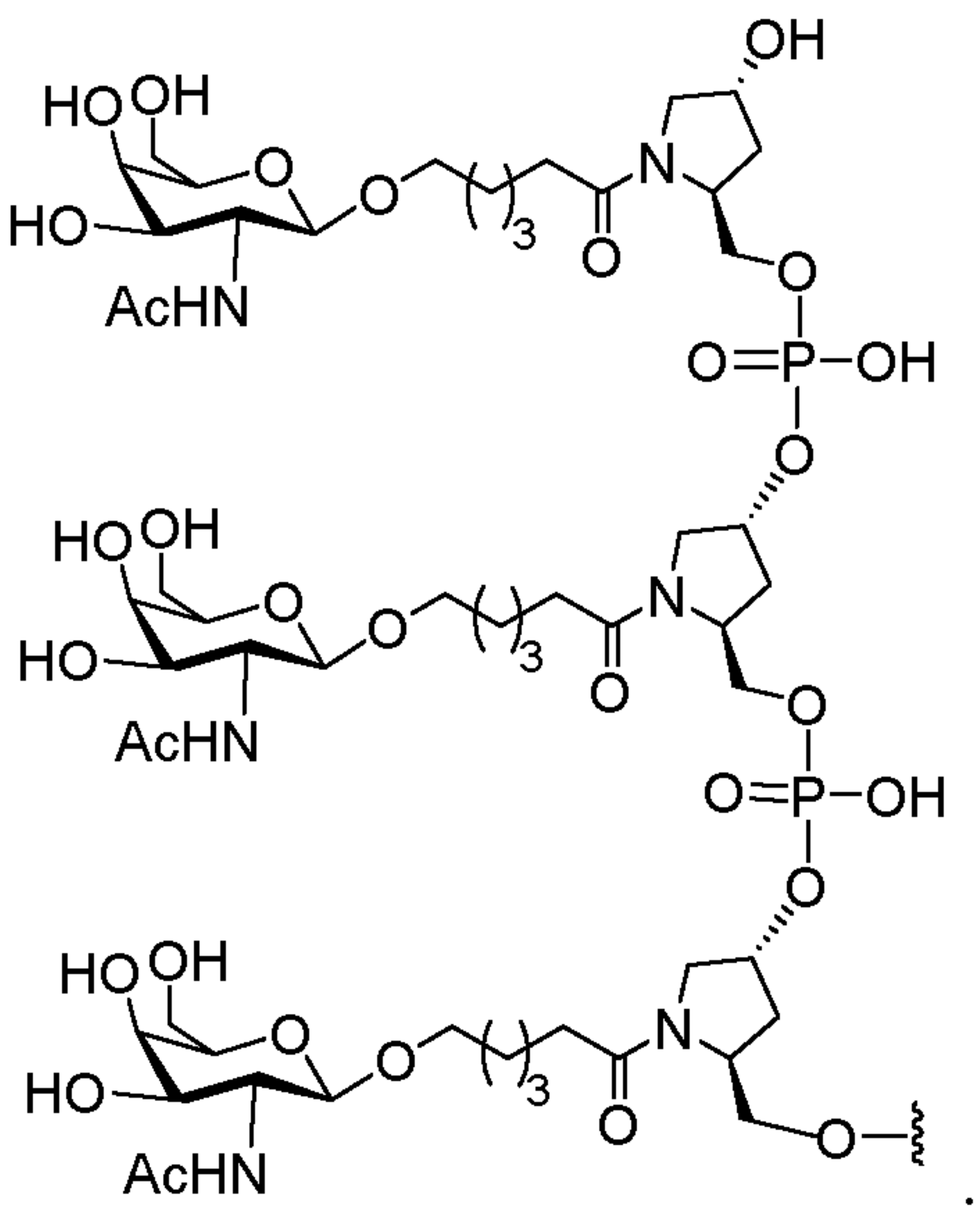
55. The compound of any of claims 1 to 46, wherein the conjugate group comprises:



56. The compound of any of claims 1 to 46, wherein the conjugate group comprises:

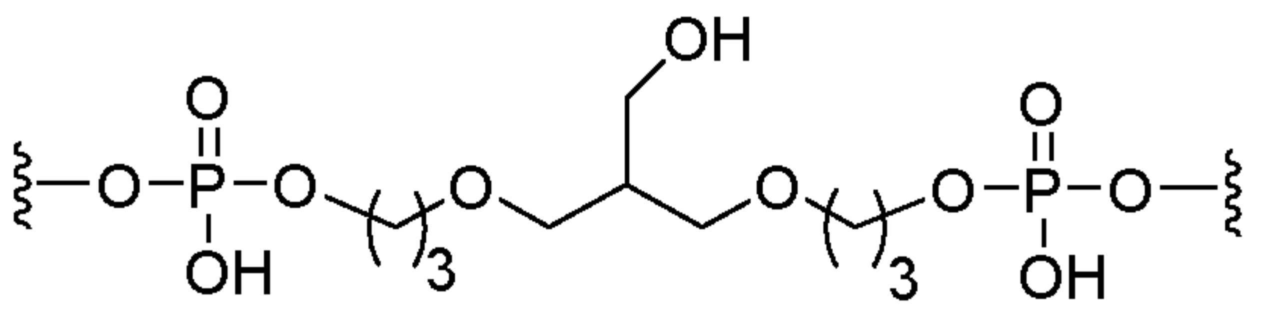
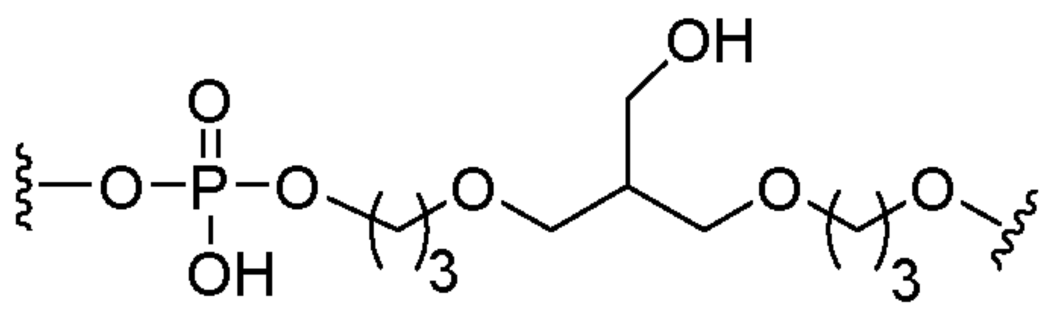


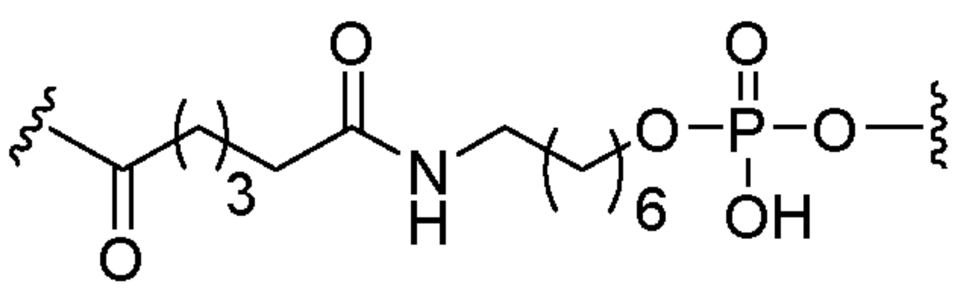
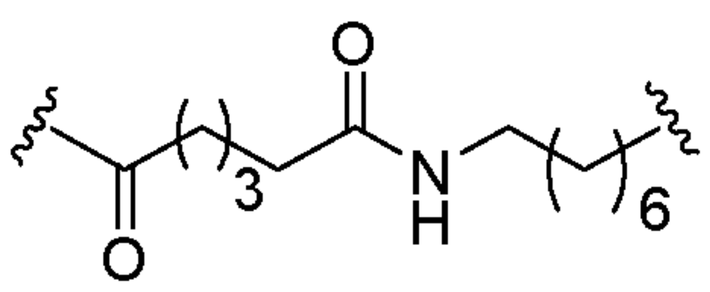
57. The compound of any of claims 1 to 46, wherein the conjugate group comprises:

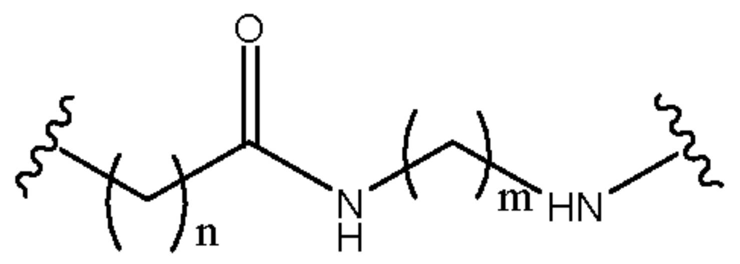
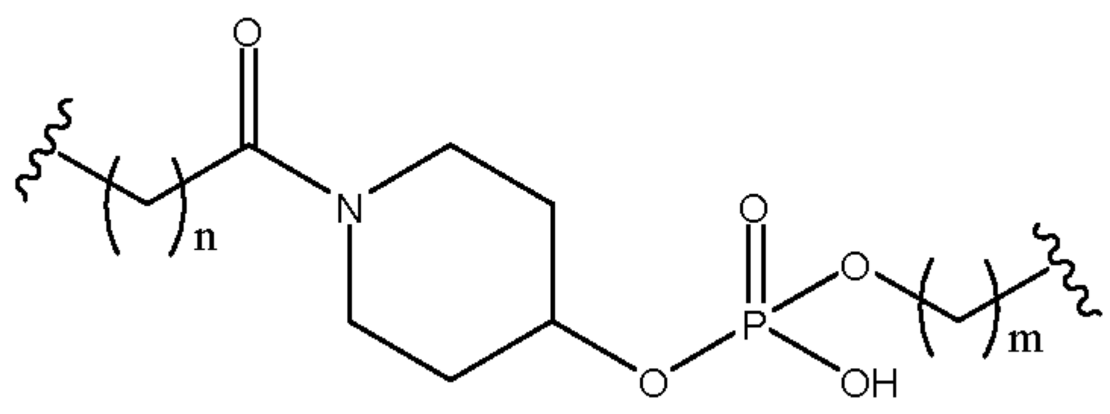


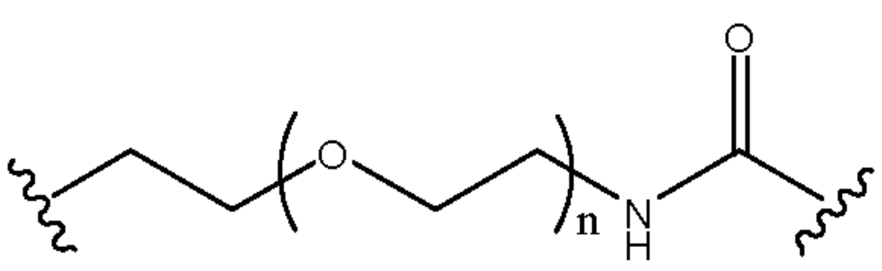
58. The compound of any of claims 46 to 52, wherein the conjugate group comprises at least one phosphorus linking group or neutral linking group.

59. The compound of any of claims 1 to 52, wherein the conjugate group comprises a structure selected from among:

 , 

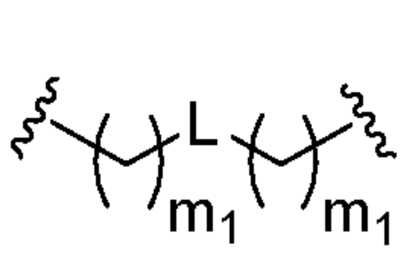
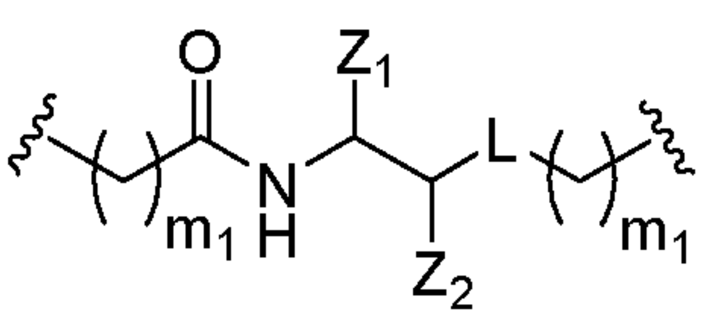
 and  .

 ;  ;

 ;

wherein n is from 1 to 12; and
wherein m is from 1 to 12.

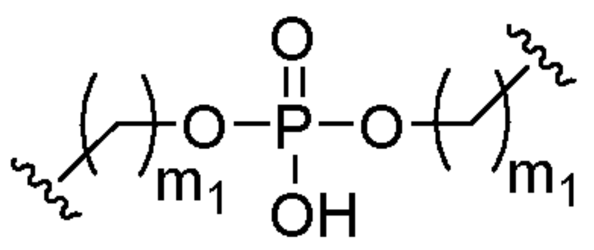
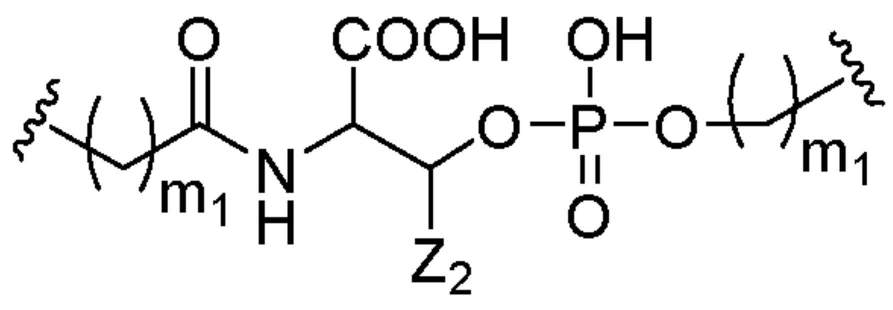
60. The compound of any of claims 1 to 52, wherein the conjugate group has a tether having a structure selected from among:

 and 

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.

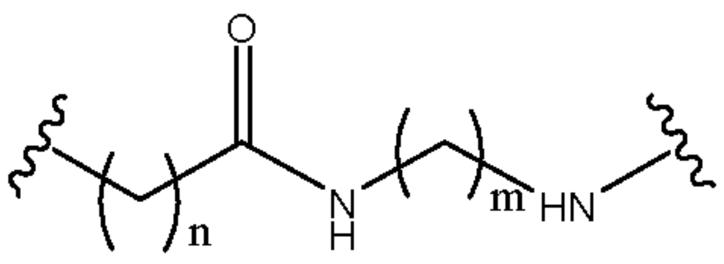
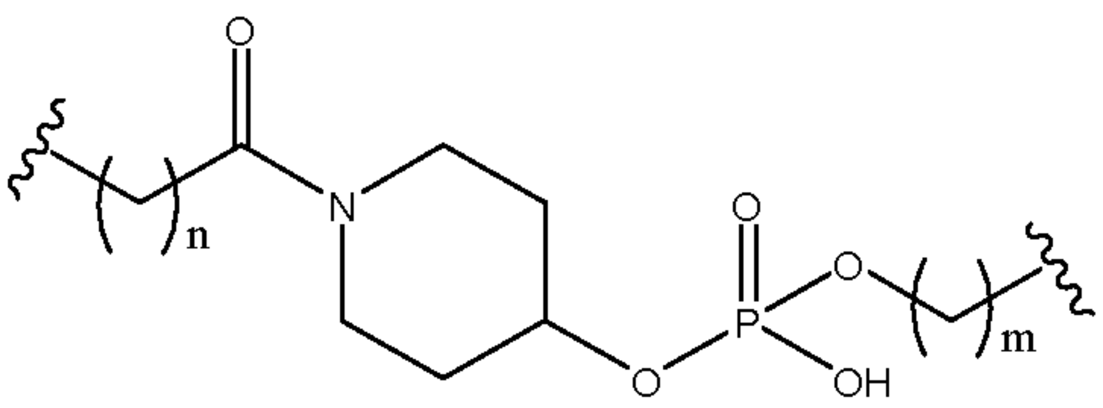
61. The compound of claim 60, wherein conjugate group has a tether having a structure selected from among:

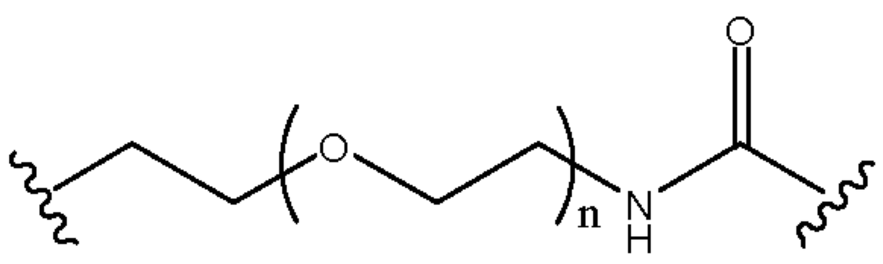
 and 

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.

62. The compound of any of claims 45 to 52, wherein the conjugate group has tether having a structure selected from among:

 ;  ;

 ;

wherein n is from 1 to 12; and
wherein m is from 1 to 12.

63. The compound of any of claims 1 to 62, wherein the conjugate group is covalently attached to the modified oligonucleotide.

64. The compound of any of claims 1 to 63, wherein the compound has a structure represented by the formula:

$$A—B—C—D\left(—E—F\right)_q$$

wherein

A is the modified 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.

65. The compound of any of claims 1 to 63, wherein the compound has a structure represented by the formula:

$$A\left(—B—\right)_{n_2}\left(—C—\right)_{n_1}\left(—D—\right)_{n_3}\left(—E—F\right)_q$$

wherein:

A is the modified 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;

each n is independently 0 or 1; and

q is an integer between 1 and 5.

66. The compound of any of claims 1 to 63, wherein the compound has a structure represented by the formula:

$$A—B—C\left(—E—F\right)_q$$

wherein

A is the modified 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.

67. The compound of any of claims 1 to 63, wherein the compound has a structure represented by the formula:

$$A - C - D \left(- E - F \right)_q$$

wherein

A is the modified 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.

68. The compound of any of claims 1 to 63, wherein the compound has a structure represented by the formula:

$$A - C \left(- E - F \right)_q$$

wherein

A is the modified 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.

69. The compound of any of claims 1 to 63, wherein the compound has a structure represented by the formula:

$$A - B - D \left(E - F \right)_q$$

wherein
A is the modified 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.

70. The compound of any of claims 1 to 63, wherein the compound has a structure represented by the formula:

$$A - B \left(E - F \right)_q$$

wherein
A is the modified oligonucleotide;
B is the cleavable moiety;
each E is a tether;
each F is a ligand; and
q is an integer between 1 and 5.

71. The compound of any of claims 1 to 63, wherein the compound has a structure represented by the formula:

$$A - D \left(E - F \right)_q$$

wherein

A is the modified oligonucleotide;

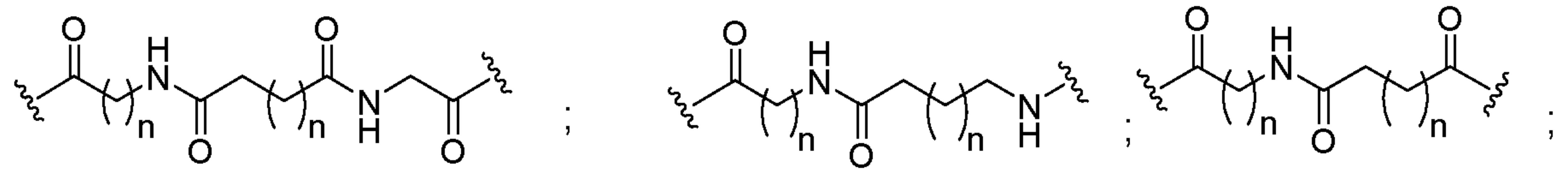
D is the branching group;

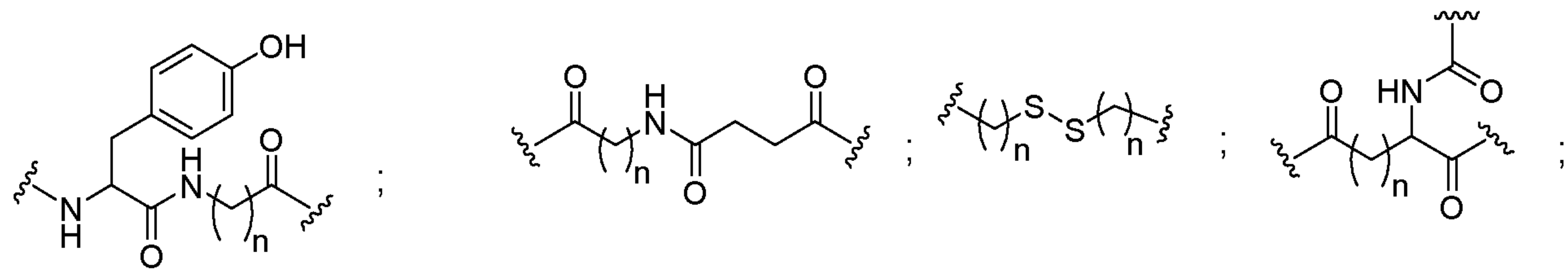
each E is a tether;

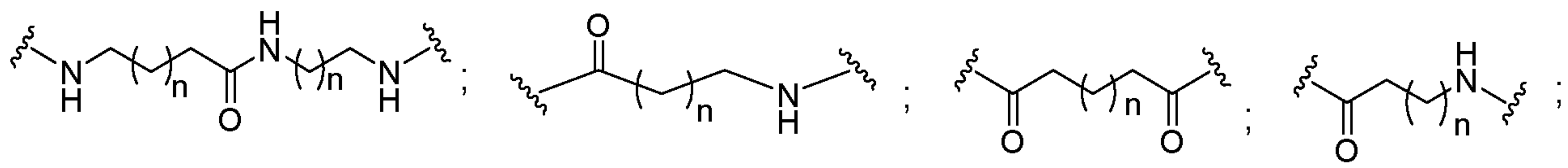
each F is a ligand; and

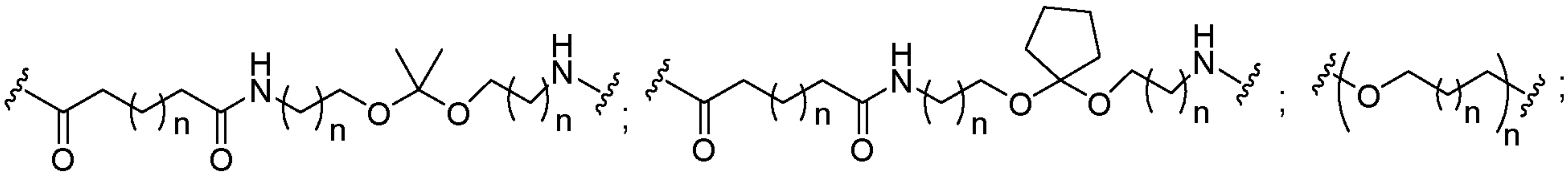
q is an integer between 1 and 5.

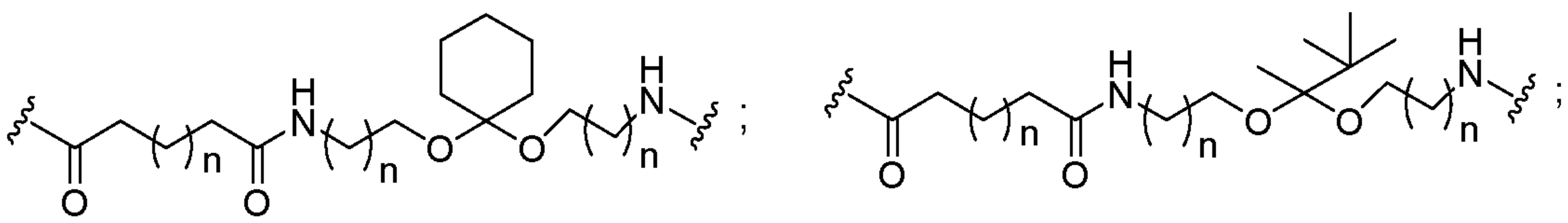
72. The compound of any of claims 64 to 71, wherein the conjugate linker has a structure selected from among:

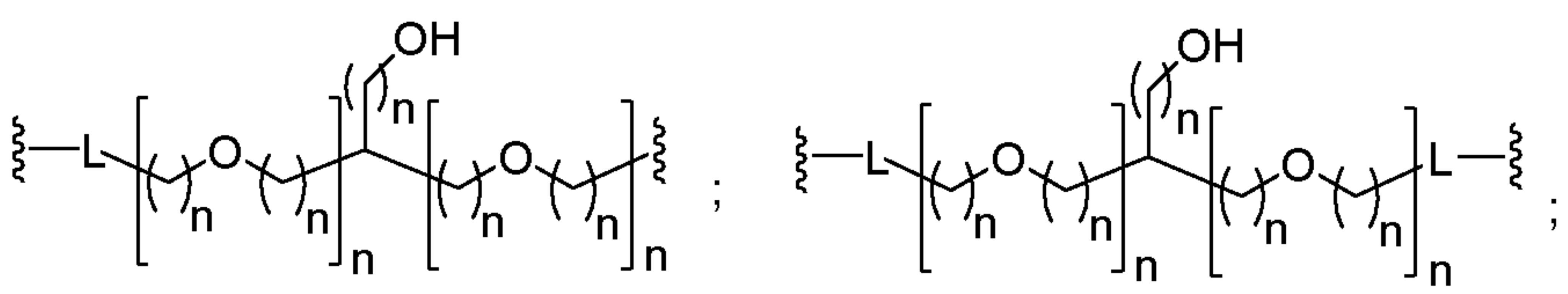


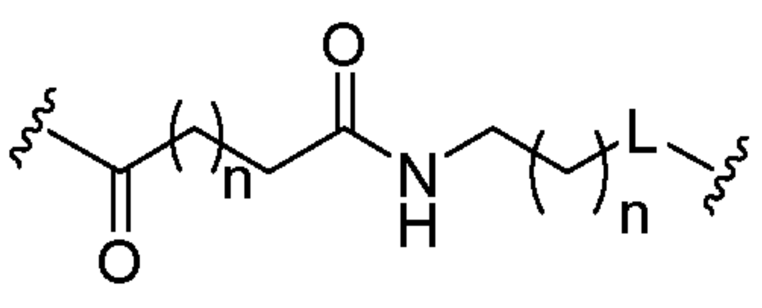
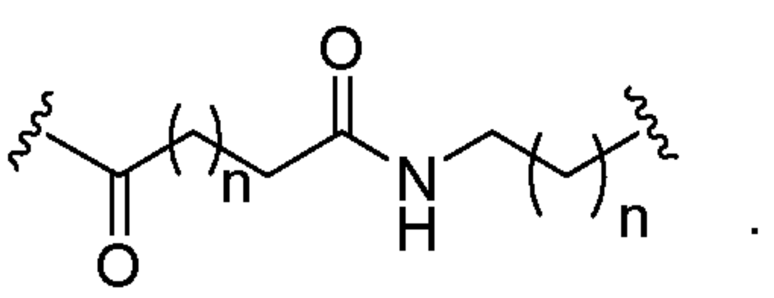






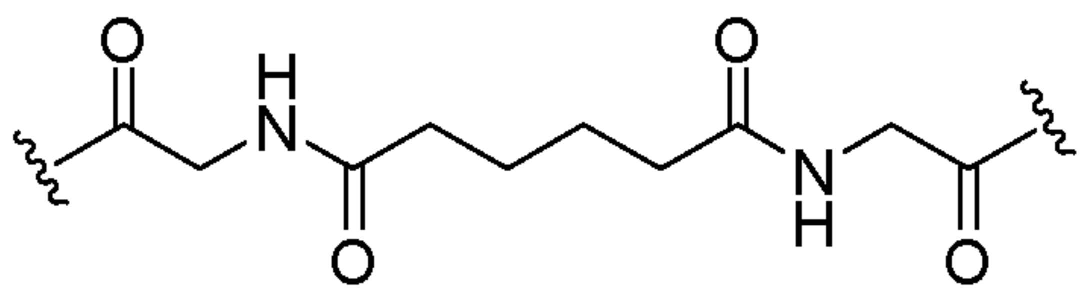
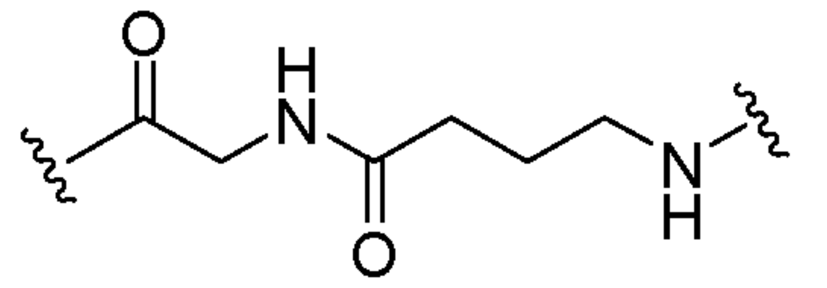
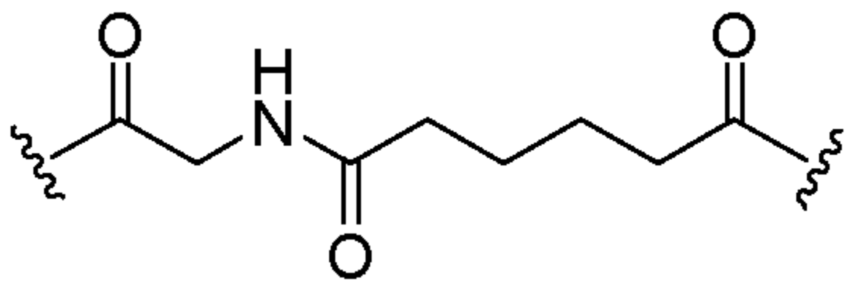


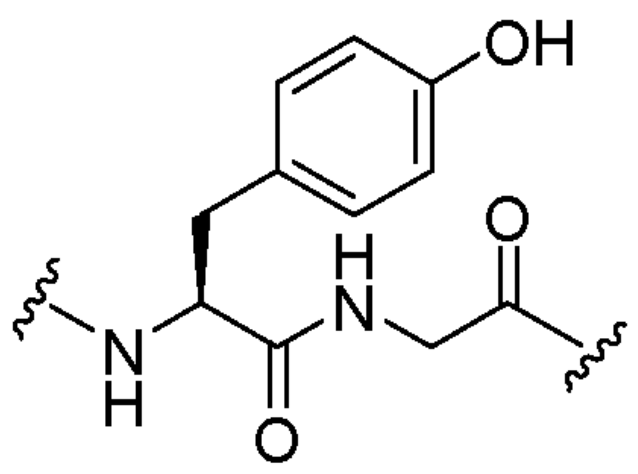
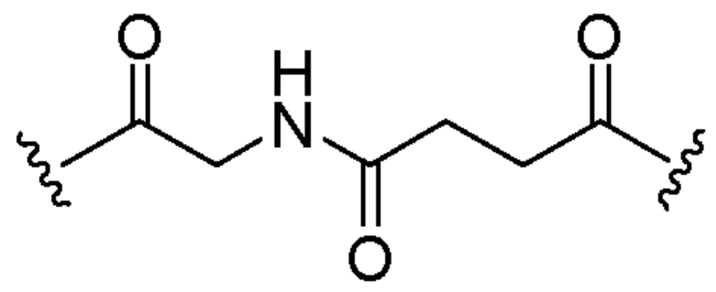
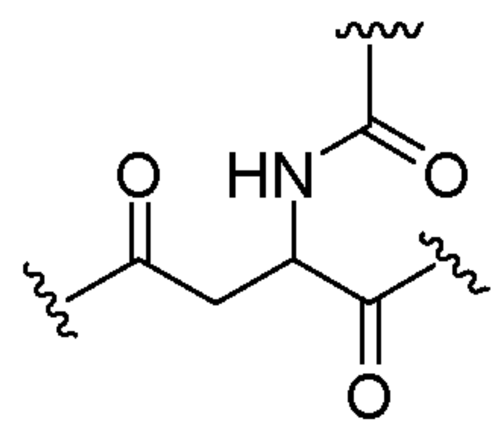


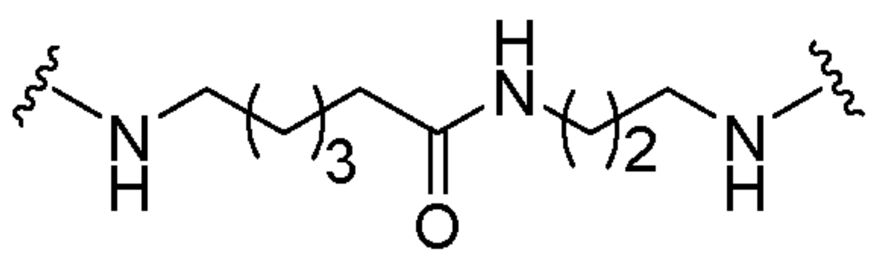
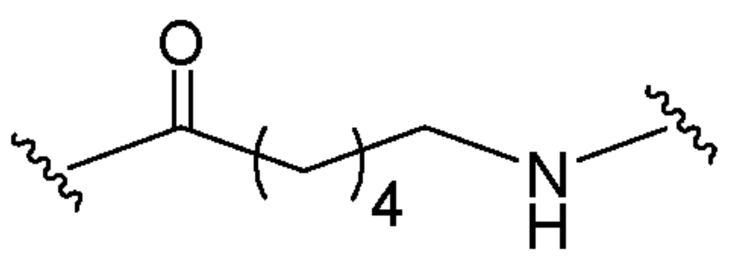
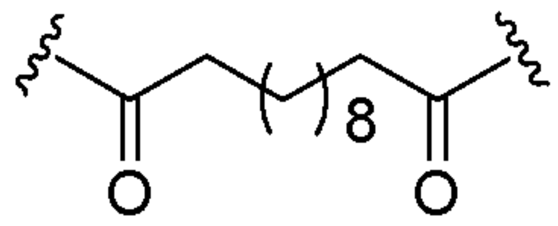
 and  .

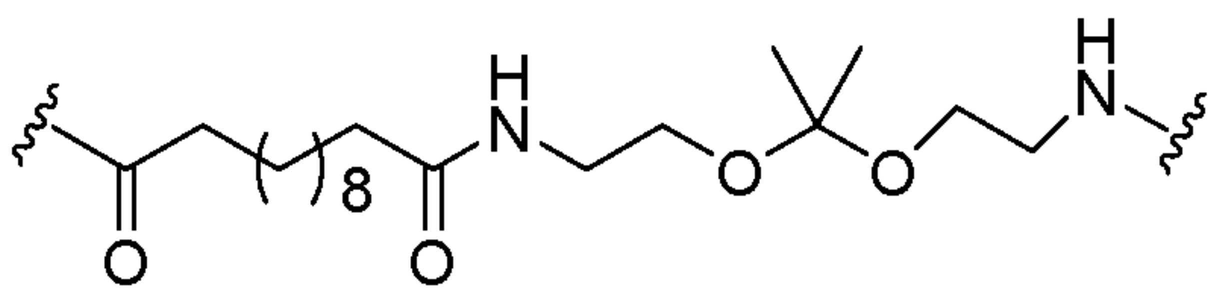
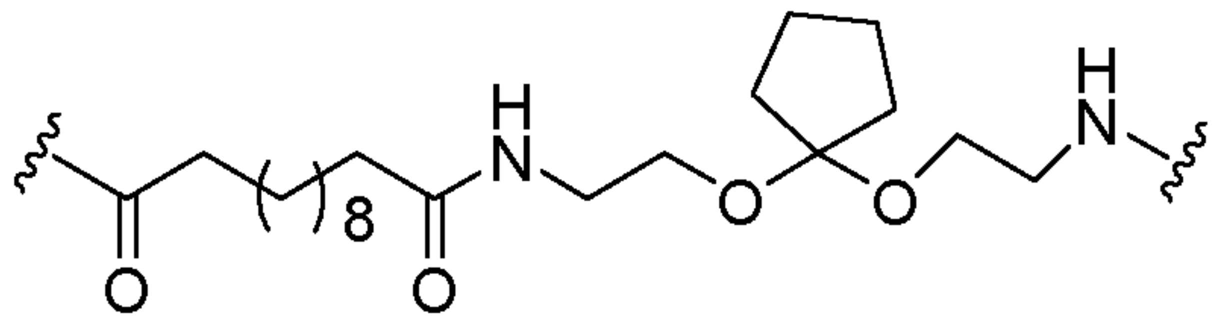
wherein each L is, independently, a phosphorus linking group or a neutral linking group; and each n is, independently, from 1 to 20.

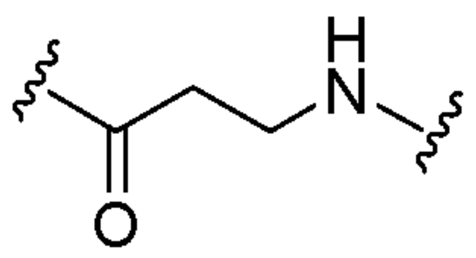
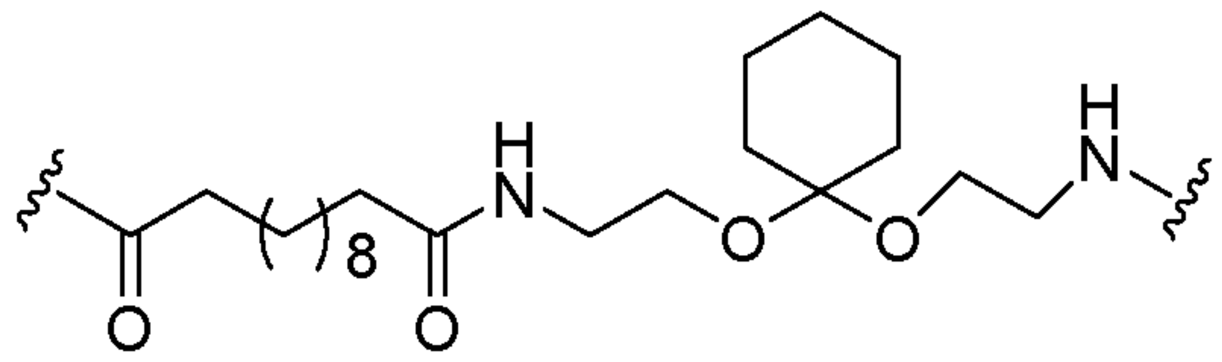
73. The compound of any of claims 64 to 72, wherein the conjugate linker has a structure selected from among:

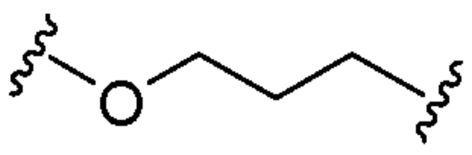
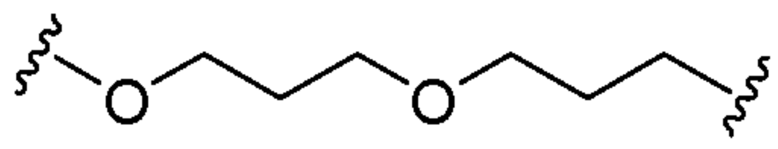
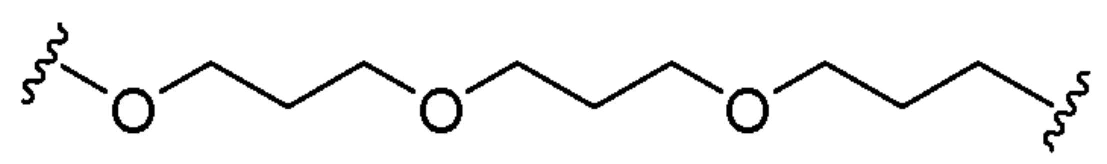
 ;  ;  ;

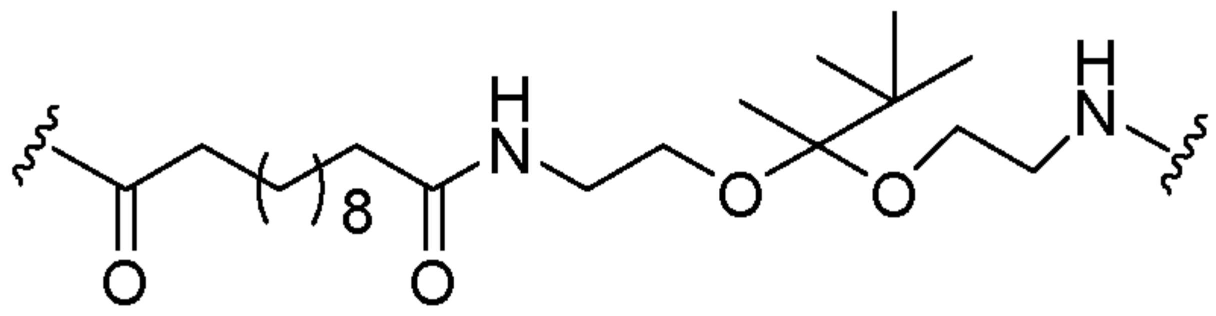
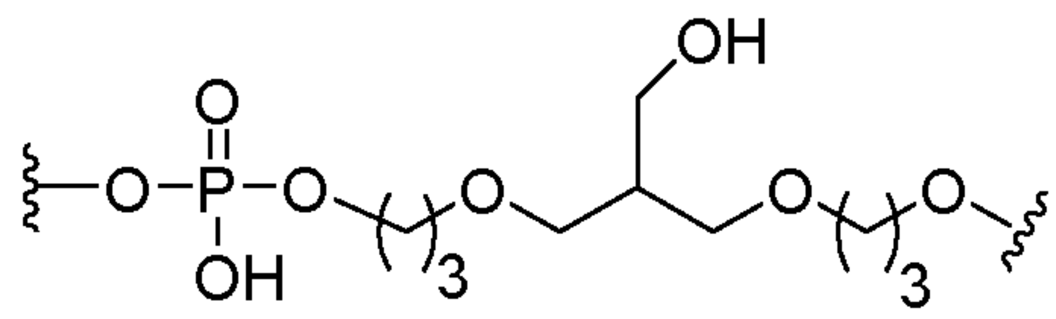
 ;  ;  ;

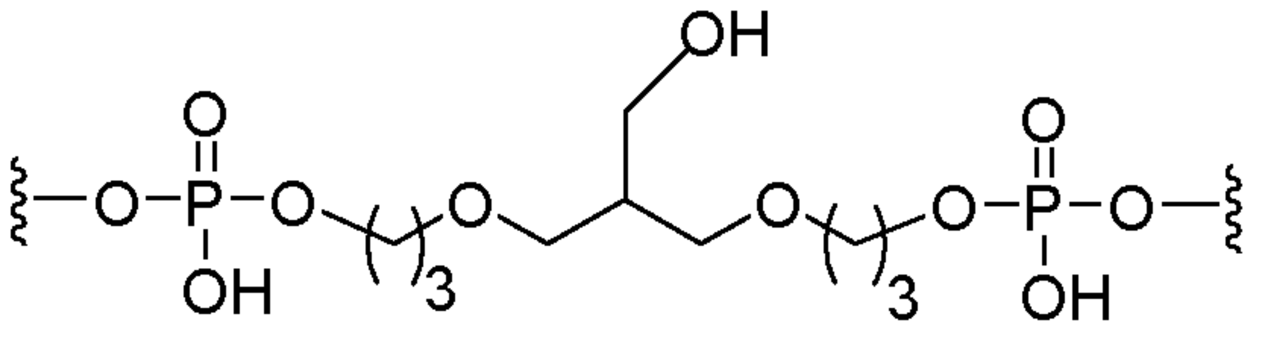
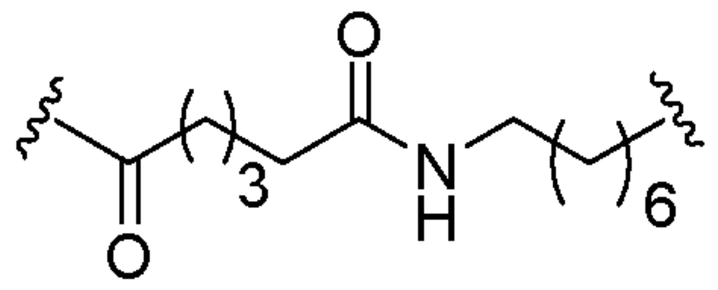
 ;  ;  ;

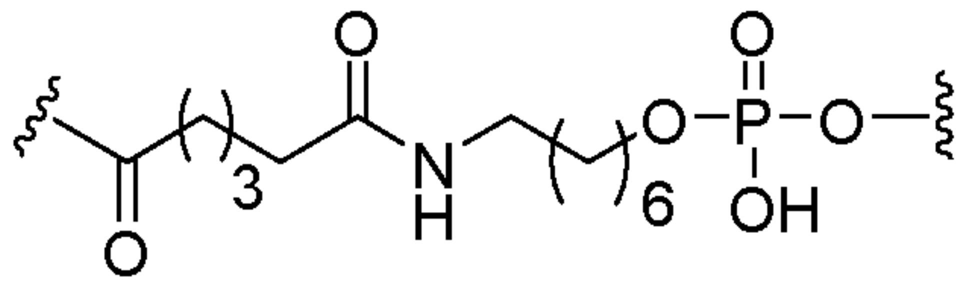
 ;  ;

 ; 

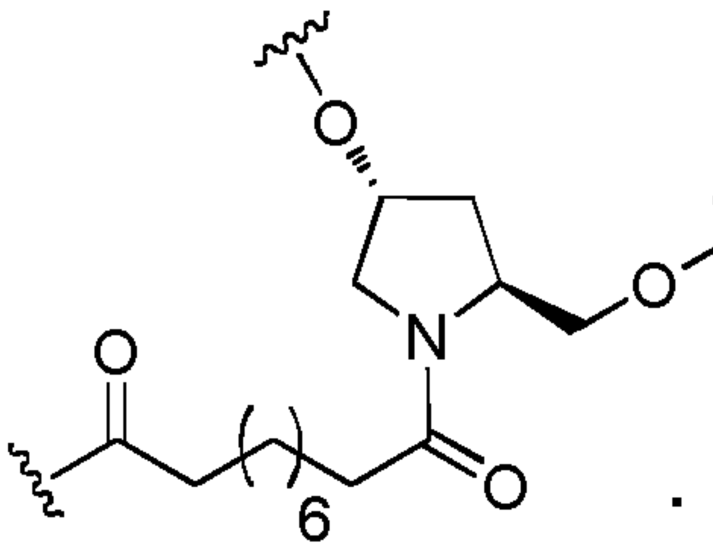
 ;  ;  ;

 ;  ;

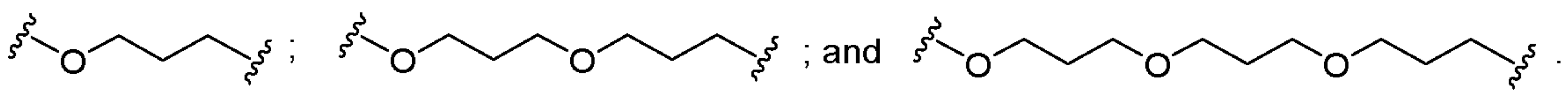
 ;  and

 .

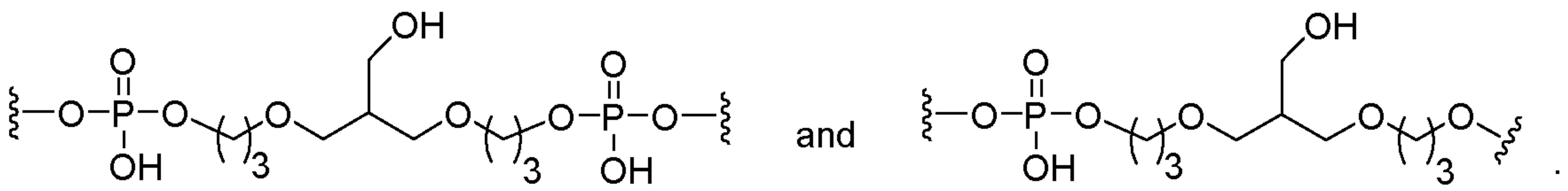
74. The compound of any of claims 64 to 72, wherein the conjugate linker has the followingstructure:



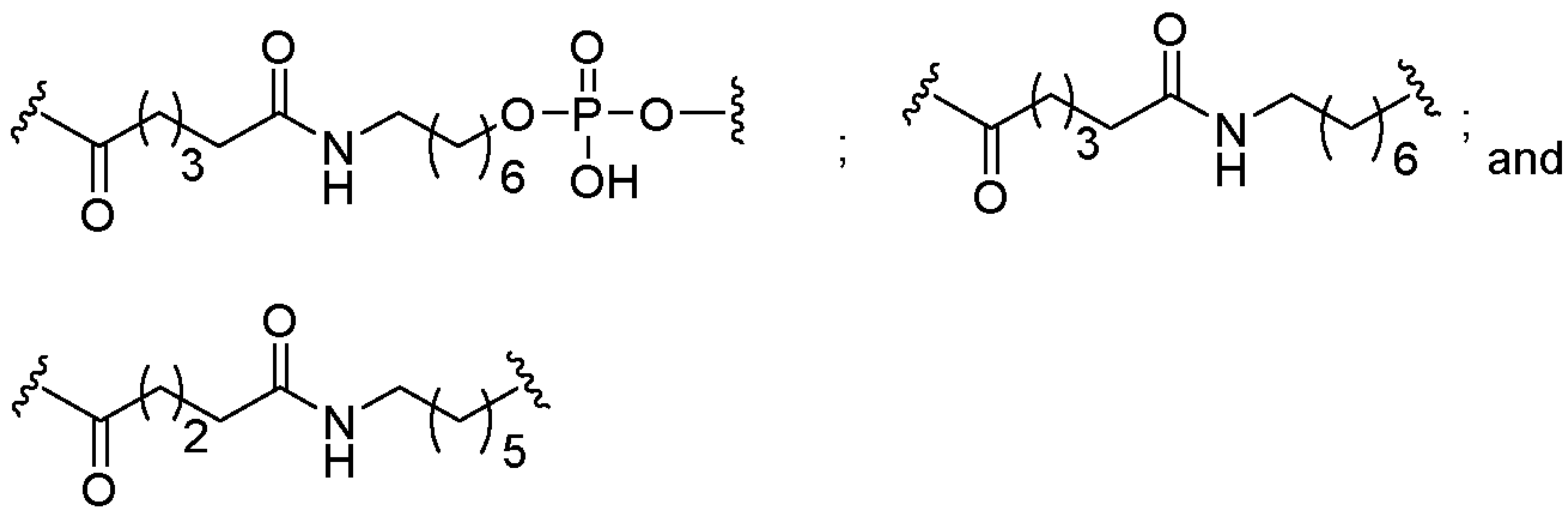
75. The compound of any of claims 64 to 72, wherein the conjugate linker has a structure selected from among:



76. The compound of any of claims 64 to 72, wherein the conjugate linker has a structure selected from among:



77. The compound of any of claims 64 to 72, wherein the conjugate linker has a structure selected from among:



78. The compound of any of claims 64 to 77, wherein the conjugate linker comprises a pyrrolidine.

79. The compound of any of claims 64 to 77, wherein the conjugate linker does not comprise a pyrrolidine.

80. The compound of any of claims 64 to 79, wherein the conjugate linker comprises PEG.

81. The compound of any of claims 64 to 80, wherein the conjugate linker comprises an amide.

82. The compound of any of claims 64 to 80, wherein the conjugate linker comprises at least two amides.

83. The compound of any of claims 64 to 80, wherein the conjugate linker does not comprise an amide.

84. The compound of any of claims 64 to 83, wherein the conjugate linker comprises a polyamide.

85. The compound of any of claims 64 to 84, wherein the conjugate linker comprises an amine.

86. The compound of any of claims 64 to 85, wherein the conjugate linker comprises one or more disulfide bonds.

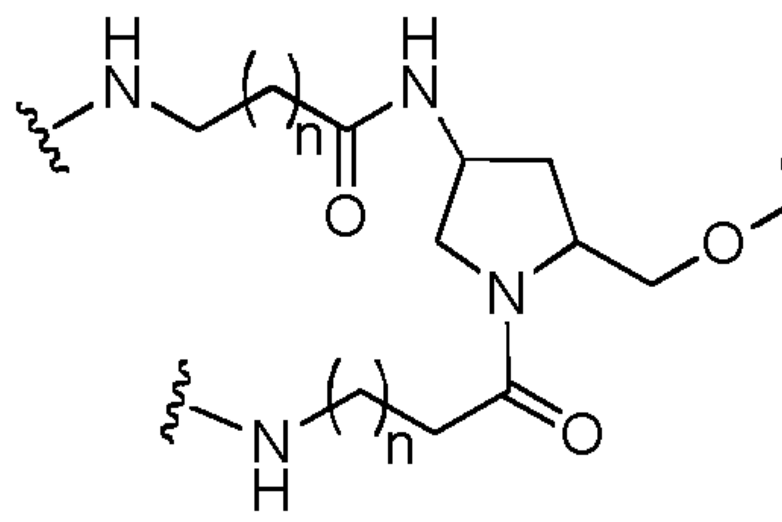
87. The compound of any of claims 64 to 86, wherein the conjugate linker comprises a protein binding moiety.

88. The compound of claim 87, wherein the protein binding moiety comprises a lipid.

89. The compound of claim 87, wherein the protein binding moiety is selected from among: 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.

90. The compound of claim 87, wherein the protein binding moiety is selected from among: a C16 to C22 long chain saturated or unsaturated fatty acid, cholesterol, cholic acid, vitamin E, adamantane or 1-pentafluoropropyl.

91. The compound of any of claims 67 to 93, wherein the conjugate linker has a structure selected from among: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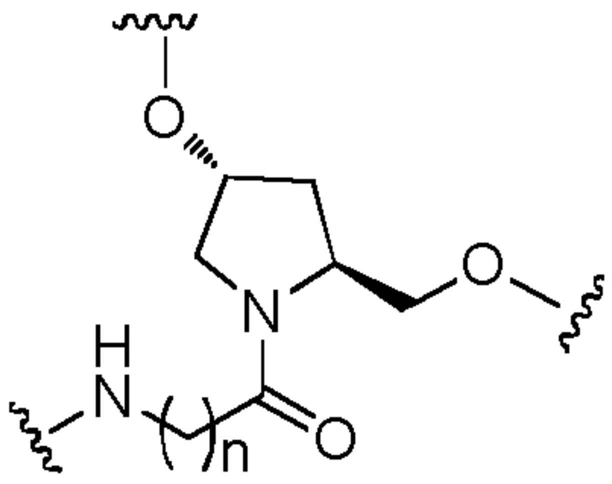
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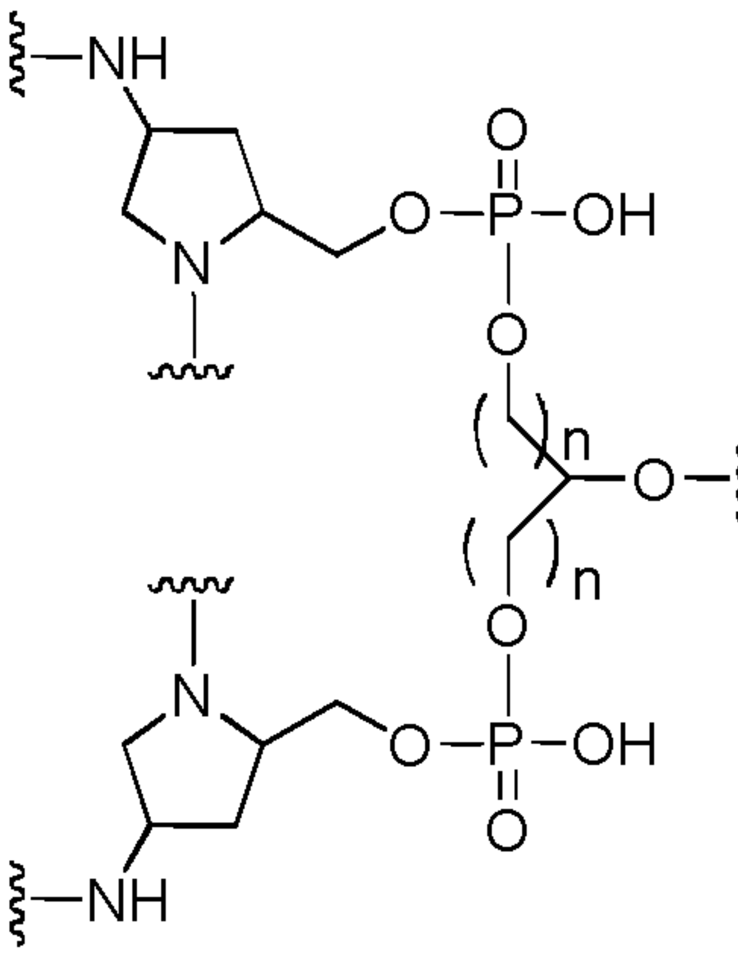
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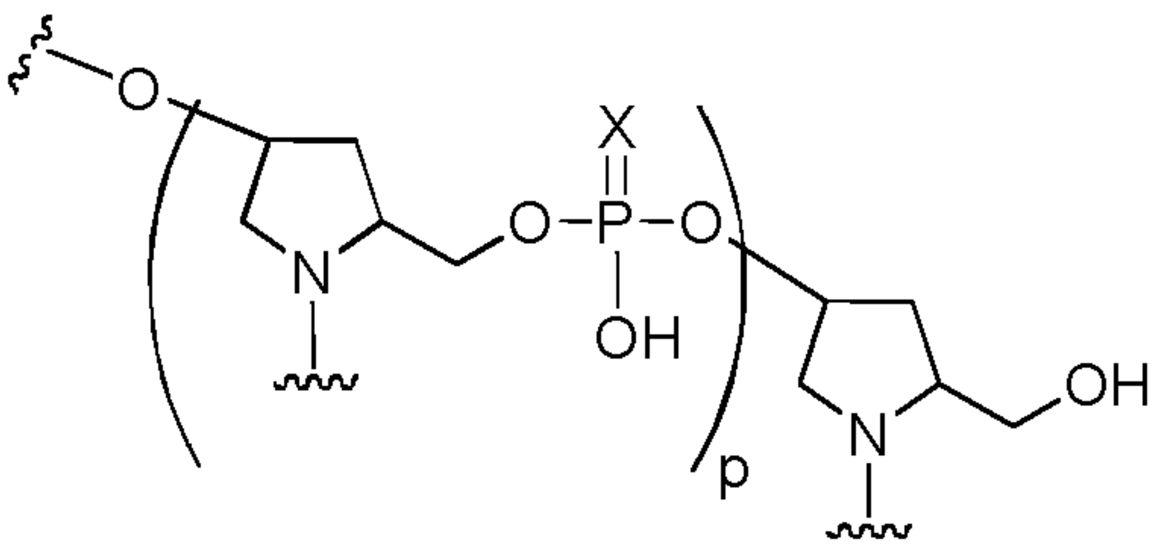
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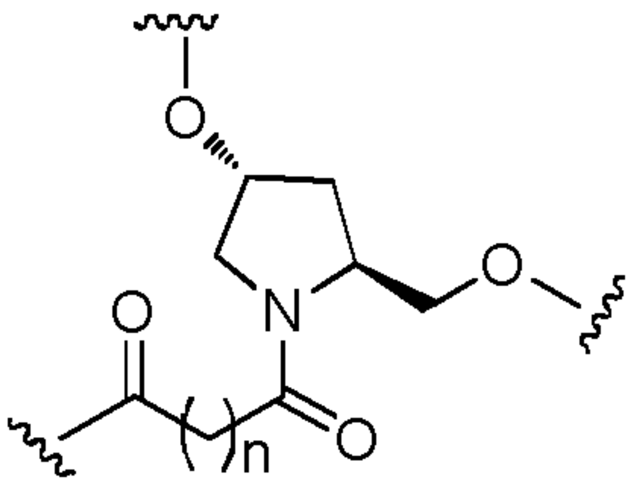
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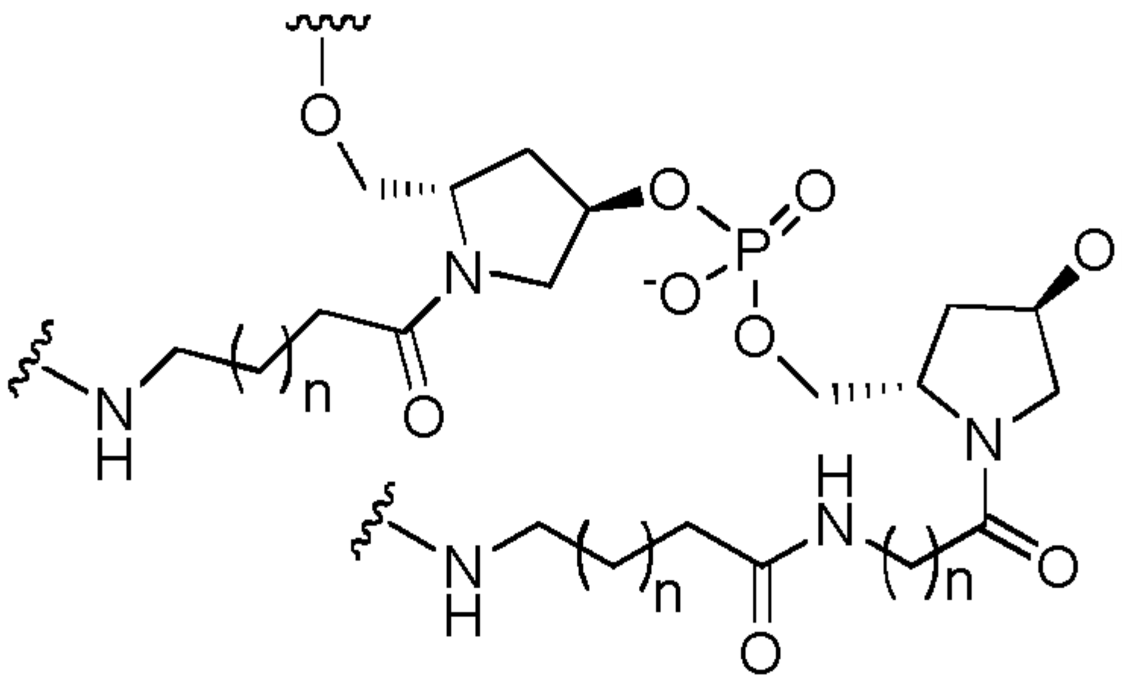
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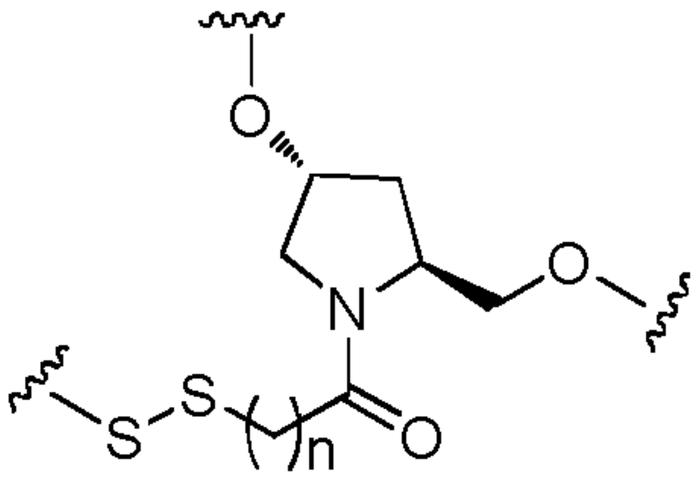
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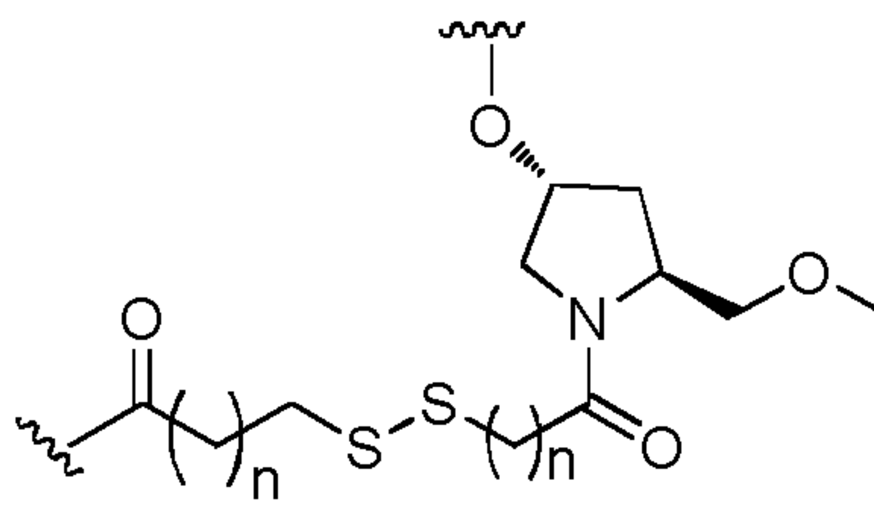
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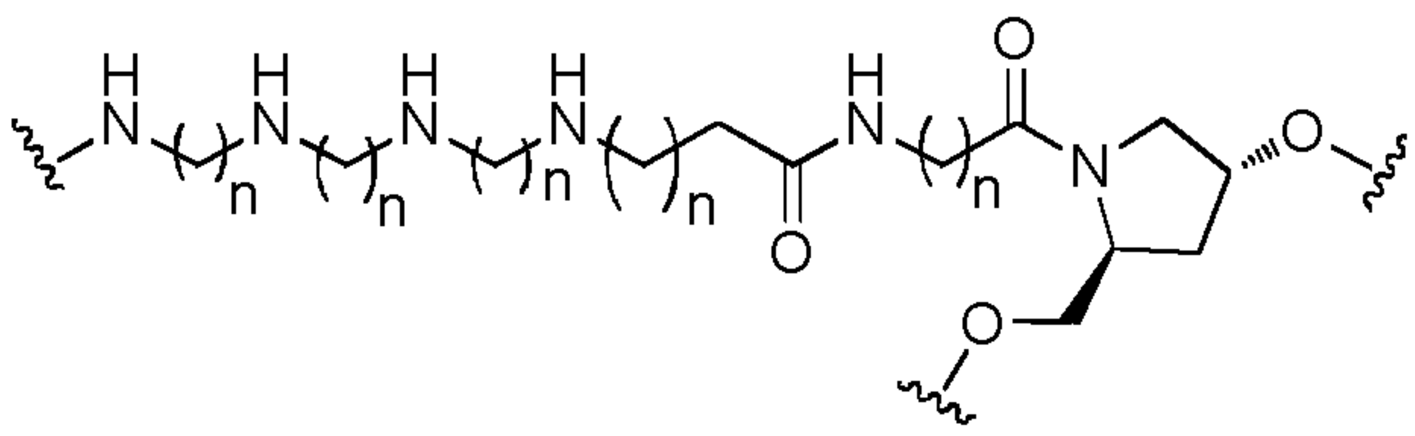
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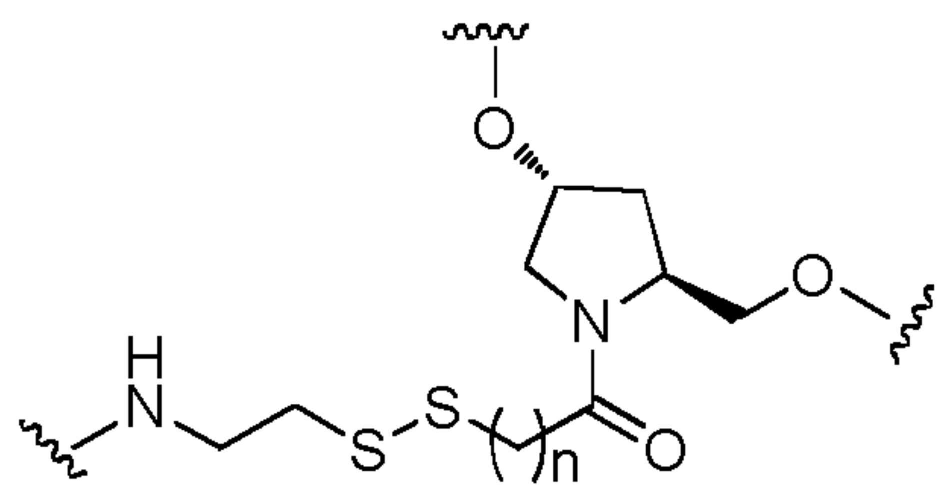
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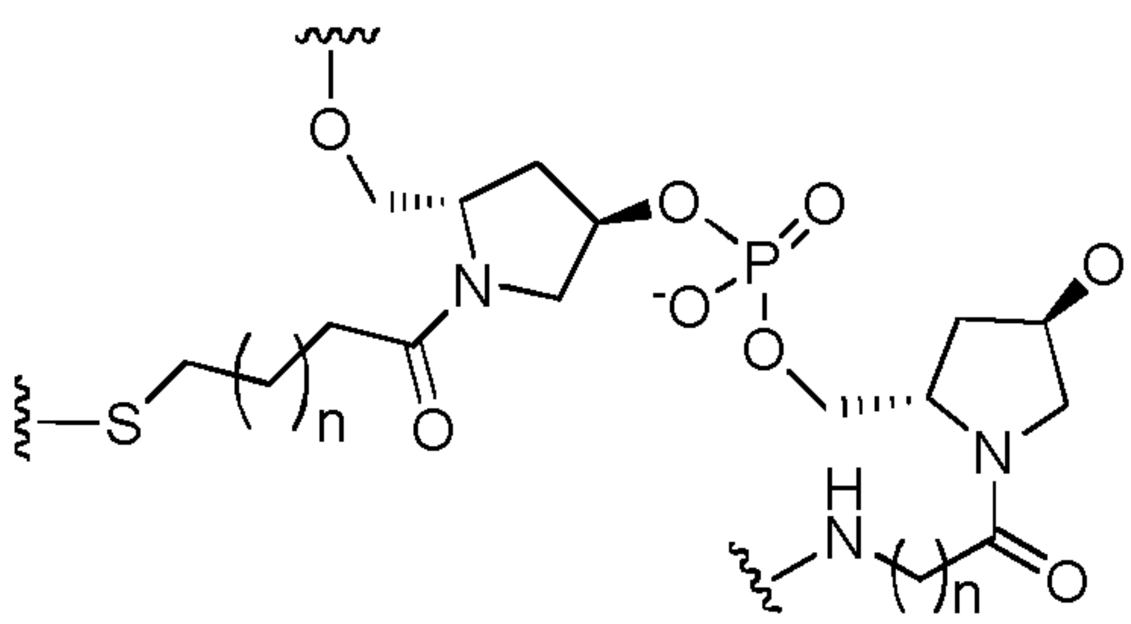
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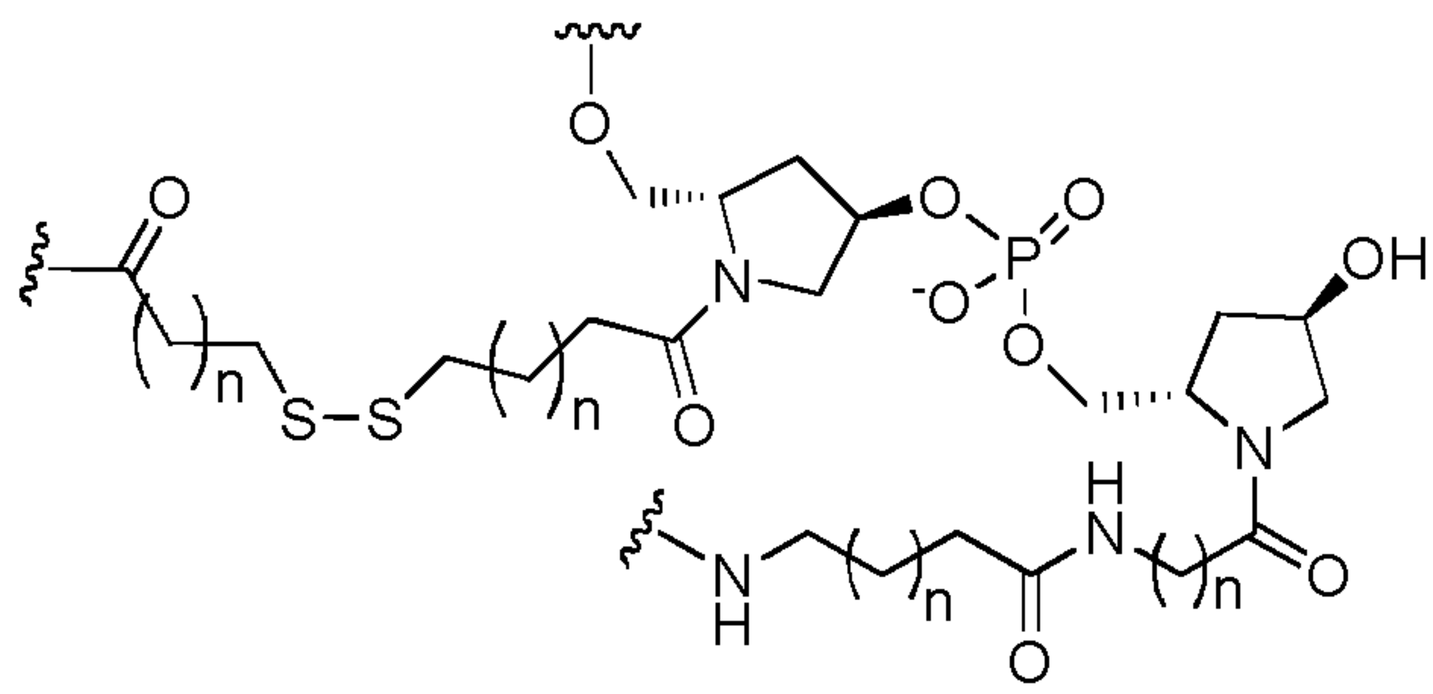
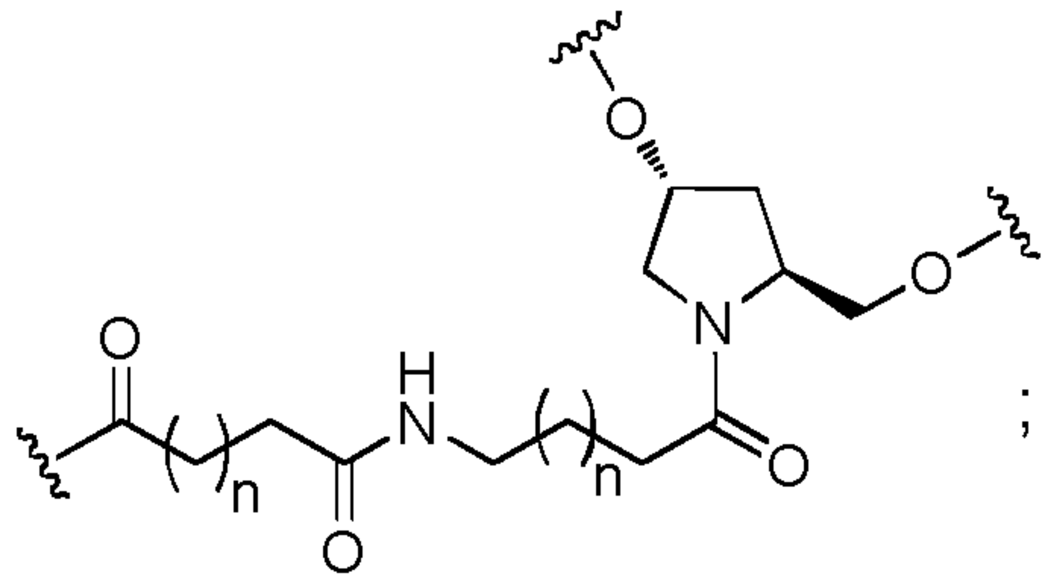


wherein each n is, independently, is from 1 to 20; and p is from 1 to 6.

92. The compound of any of claims 64 to 91, wherein the conjugate linker has a structure selected from among: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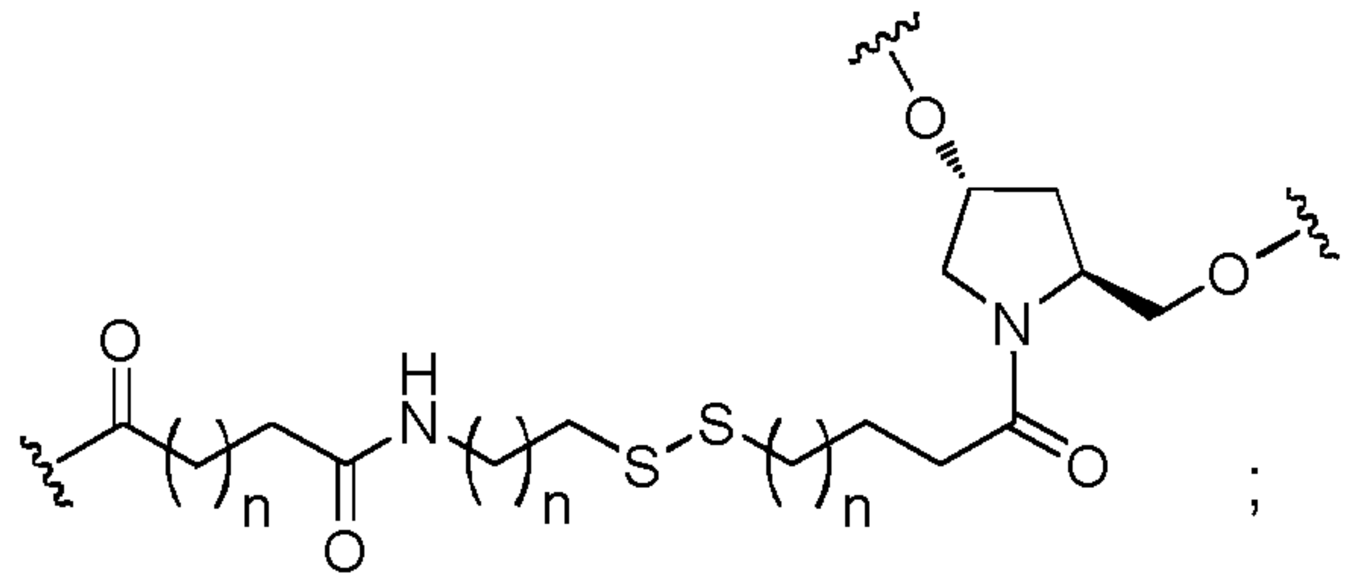
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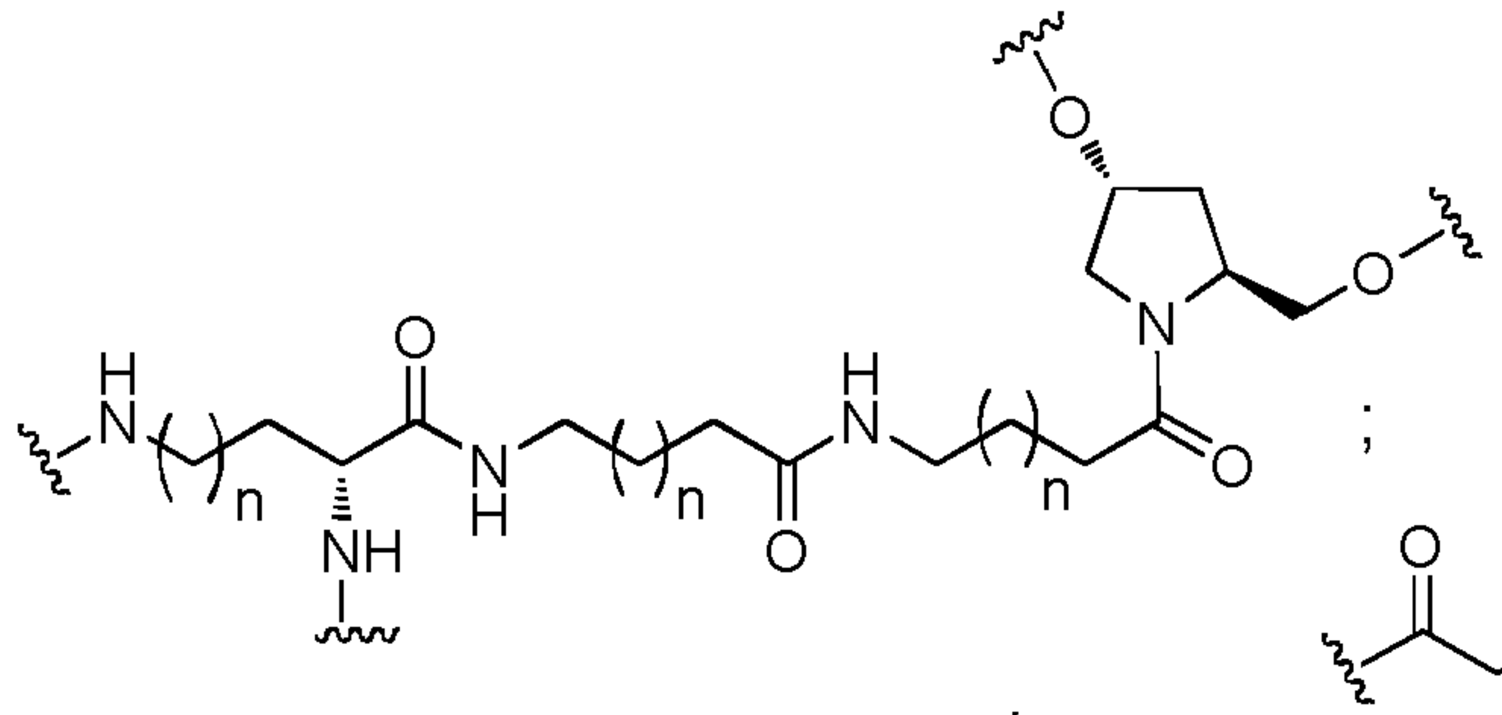
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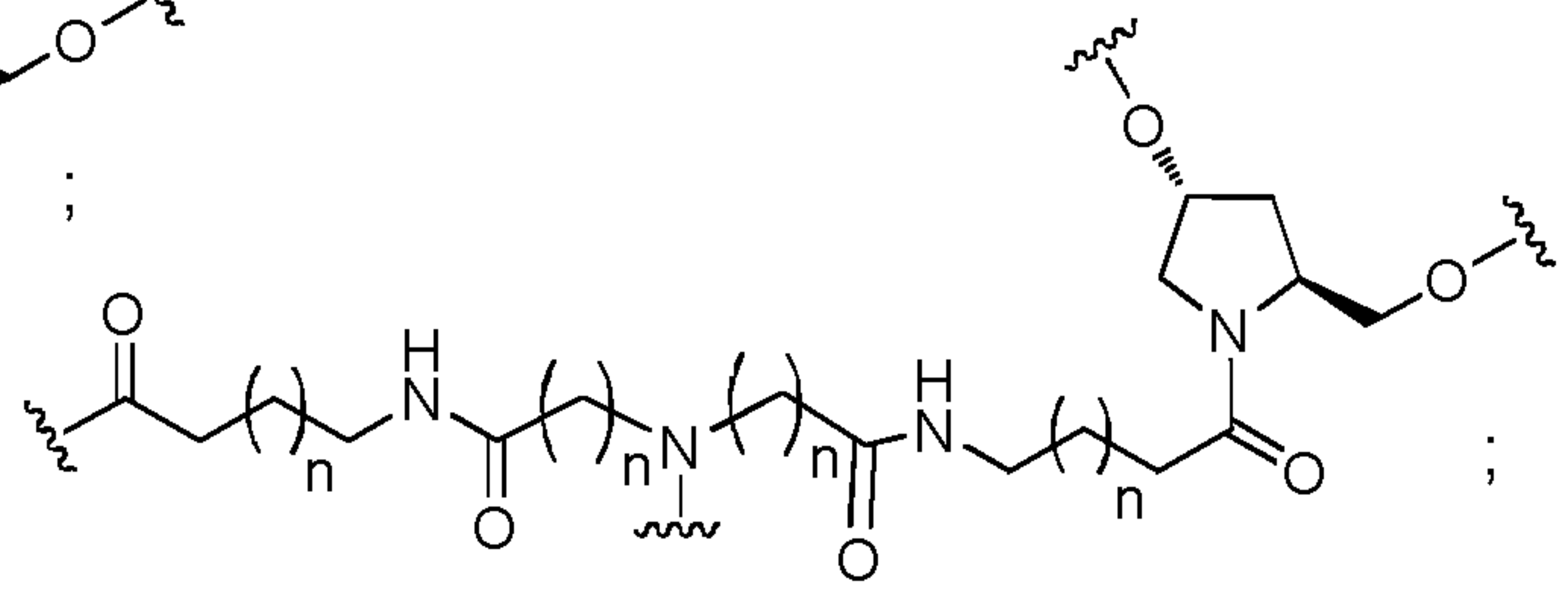
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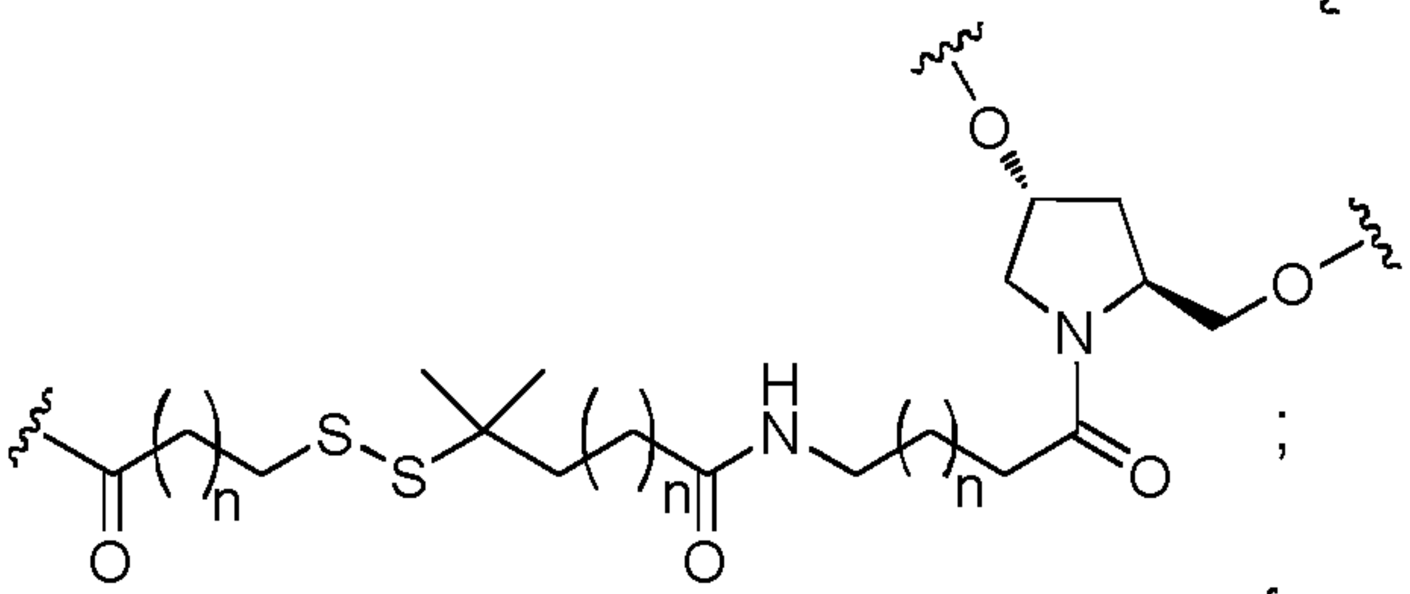
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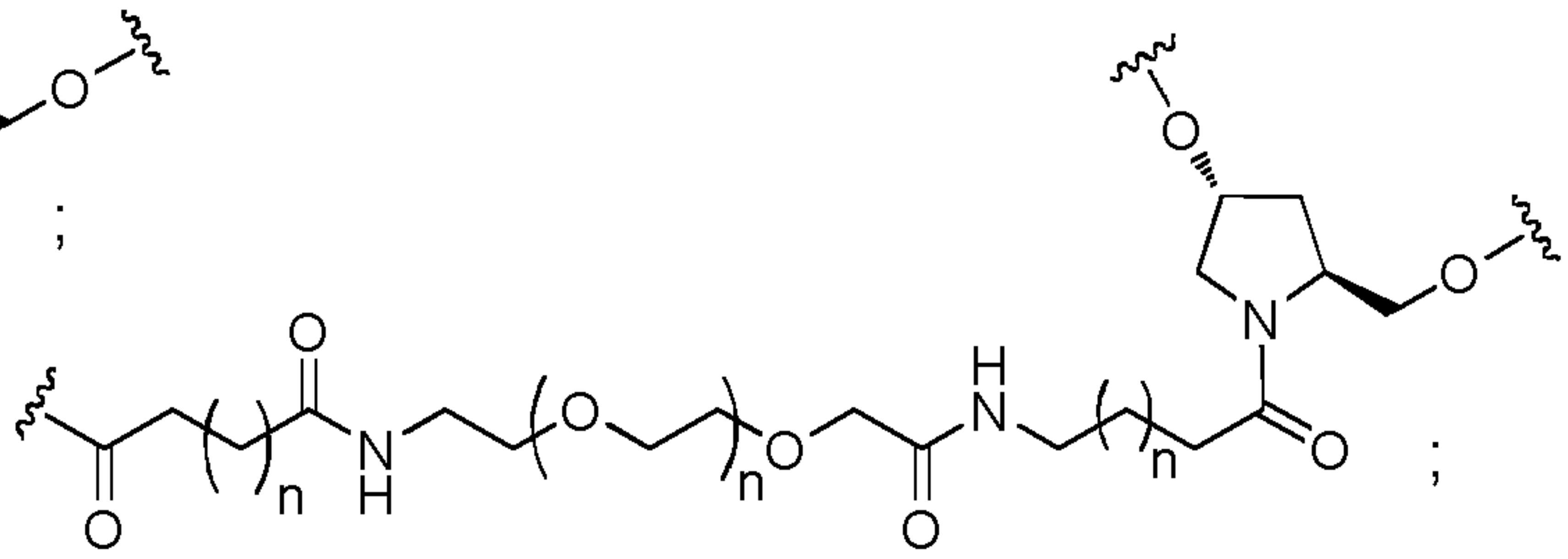
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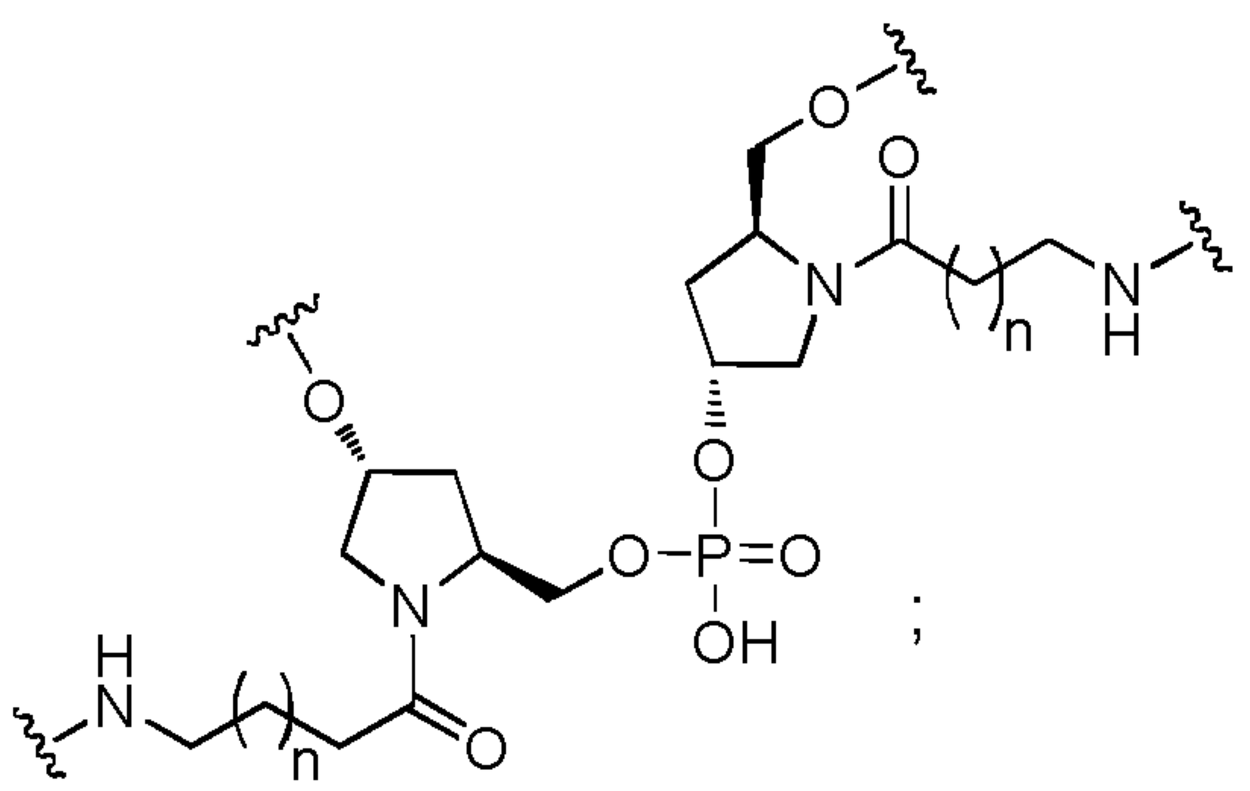
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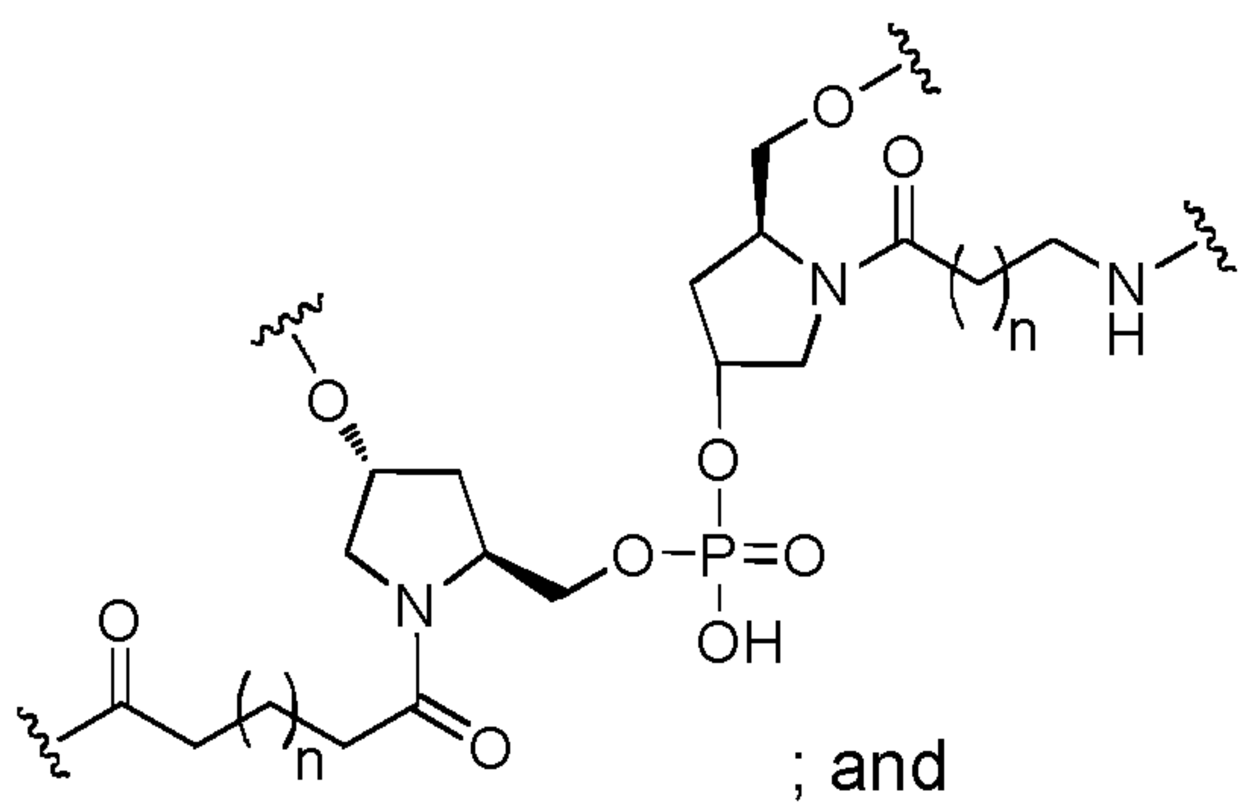
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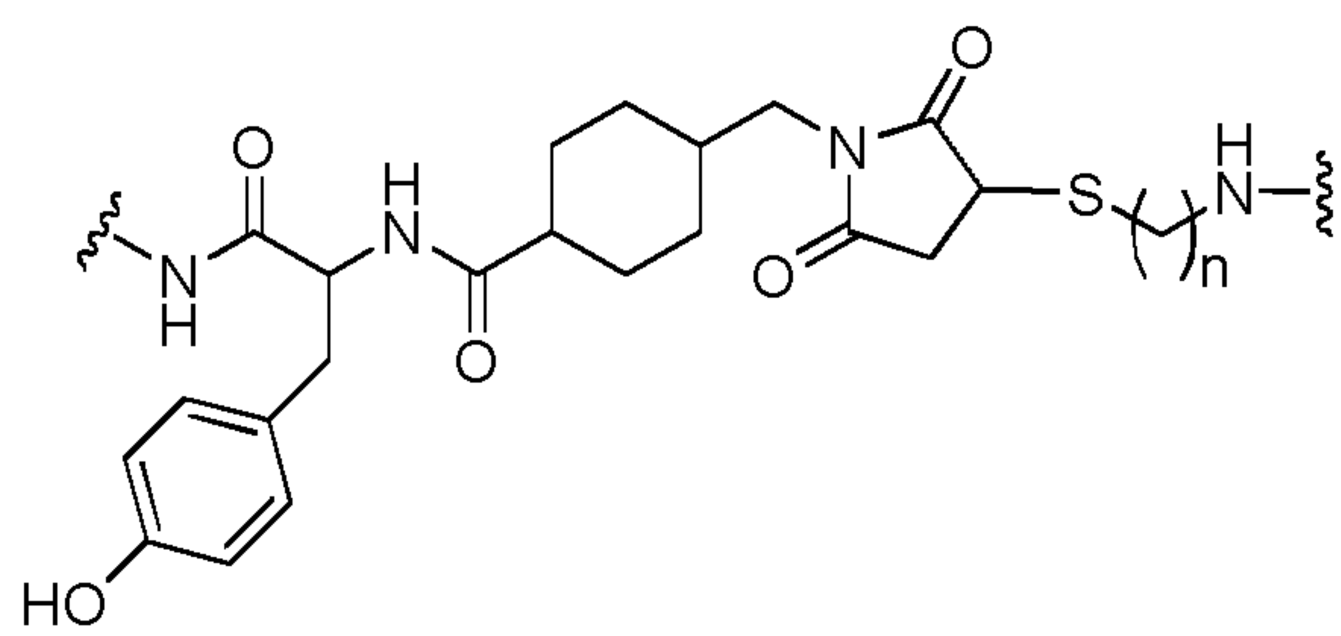
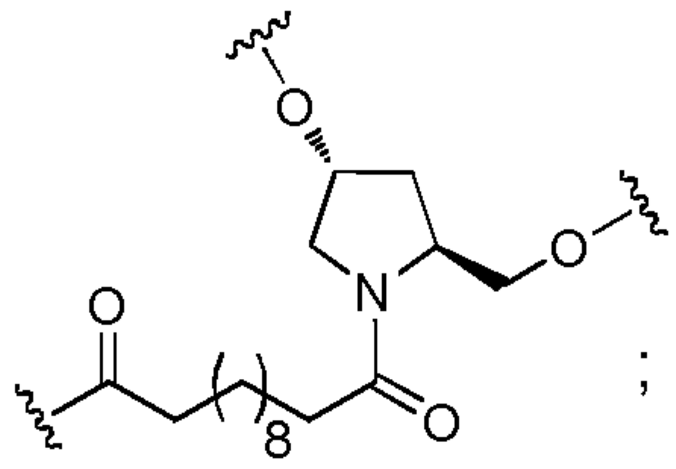
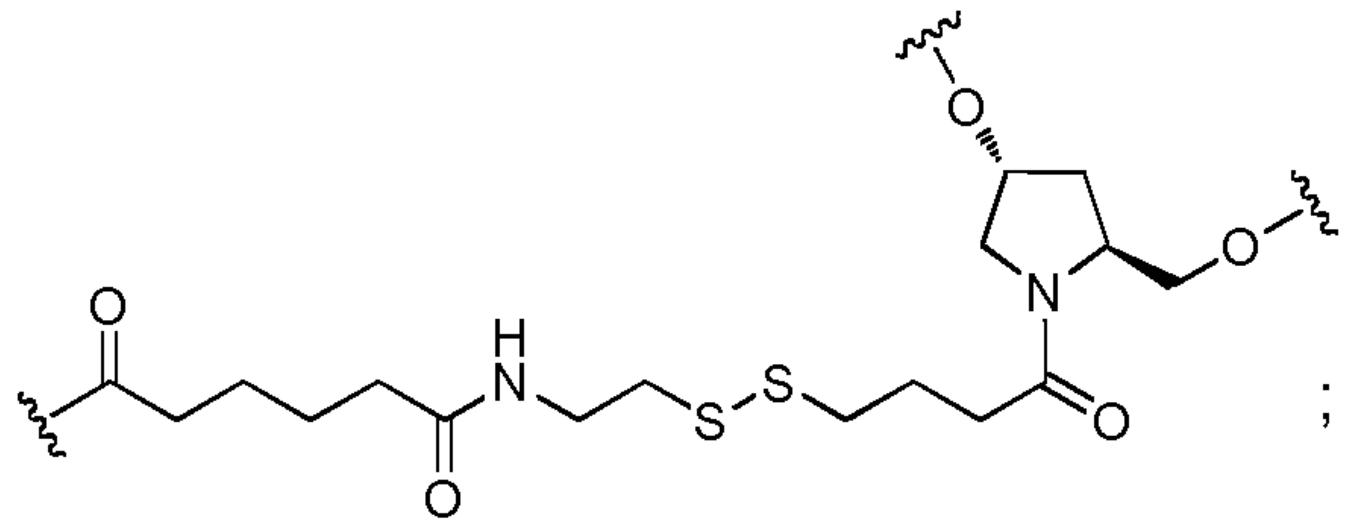
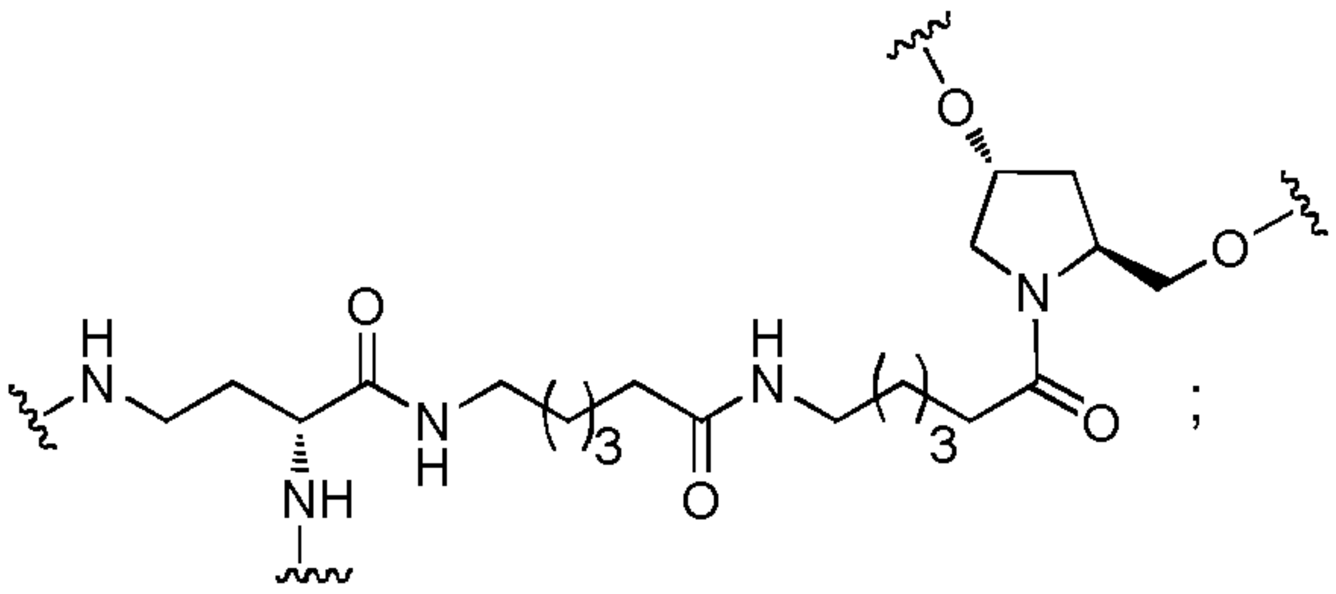
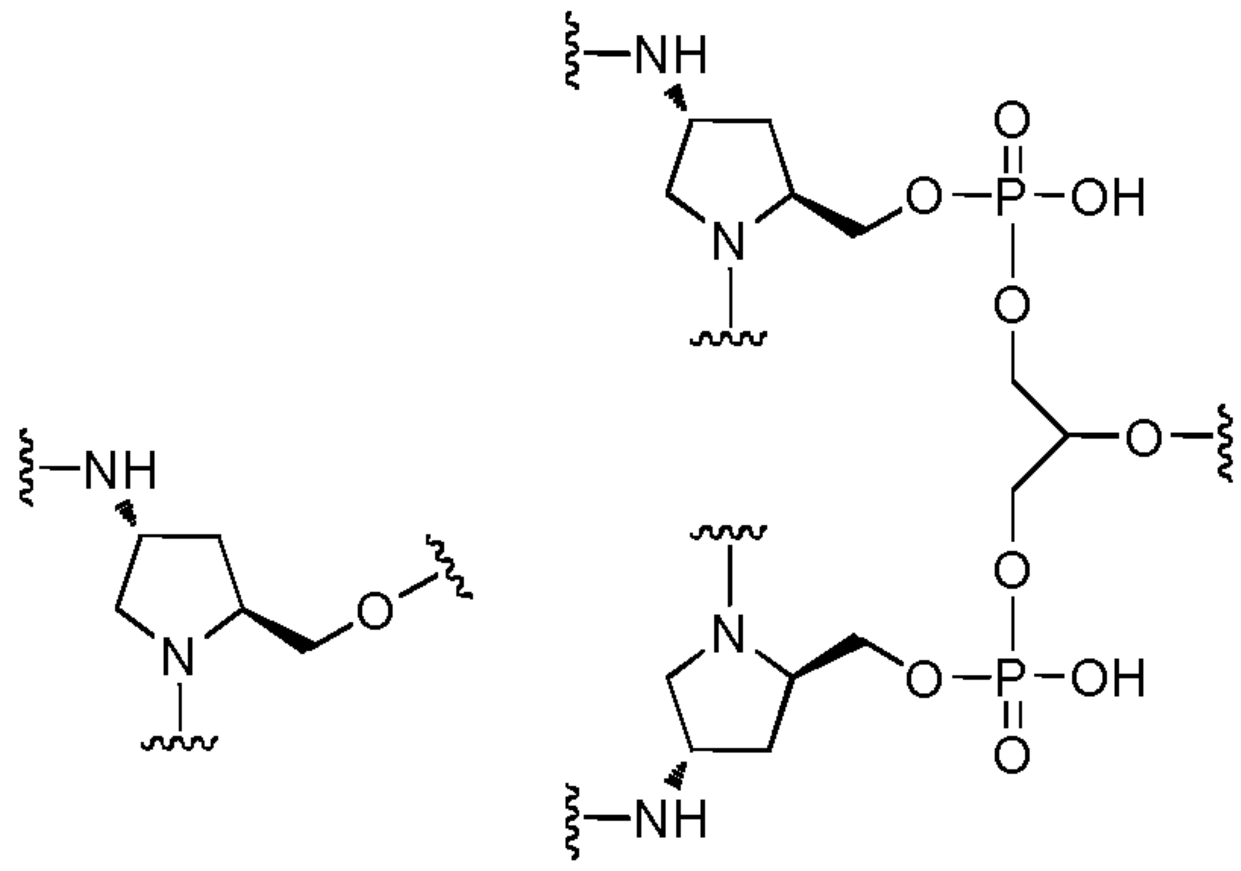
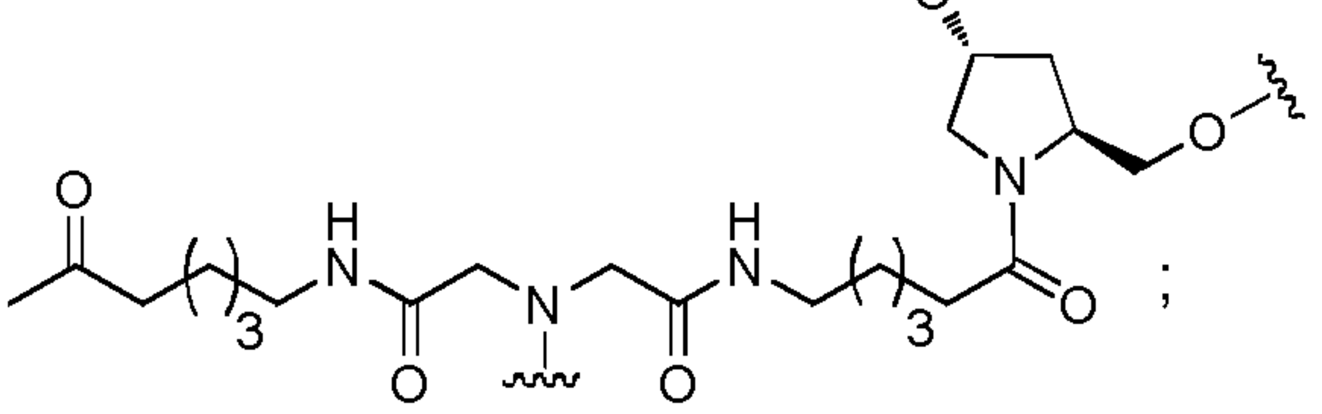
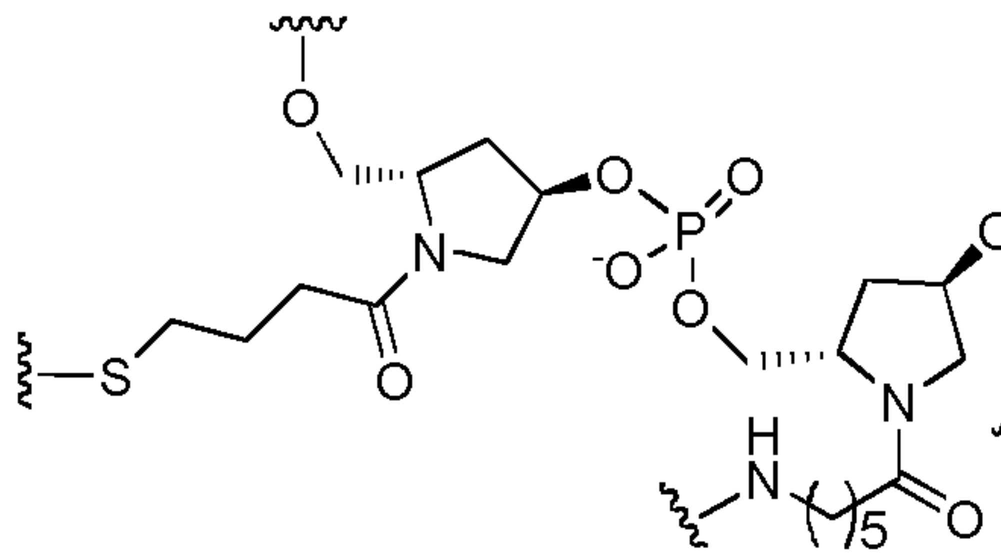


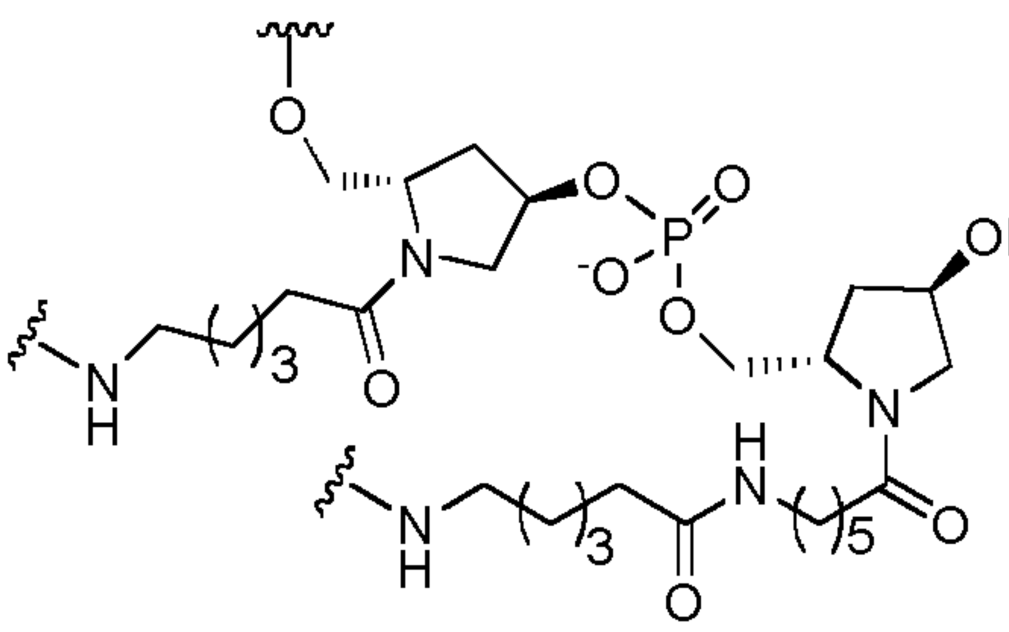
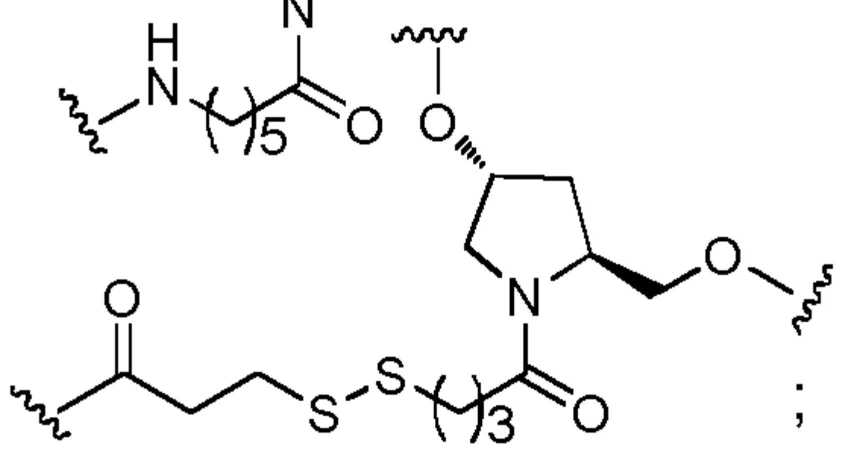
wherein each n is, independently, from 1 to 20.

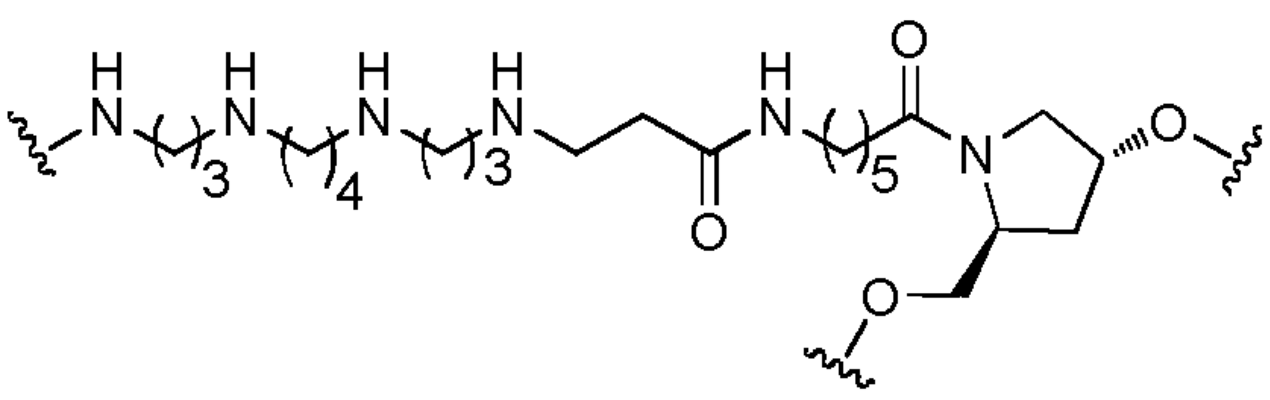
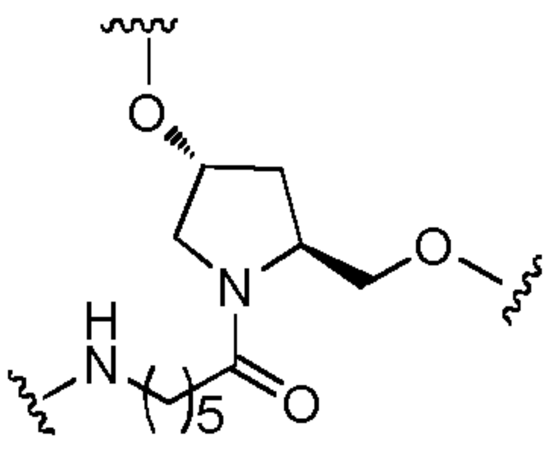
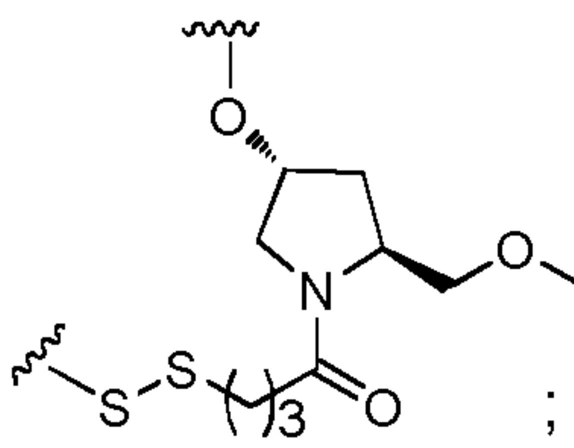
93. The compound of any of claims 64 to 92, wherein the conjugate linker has a structure selected from among:

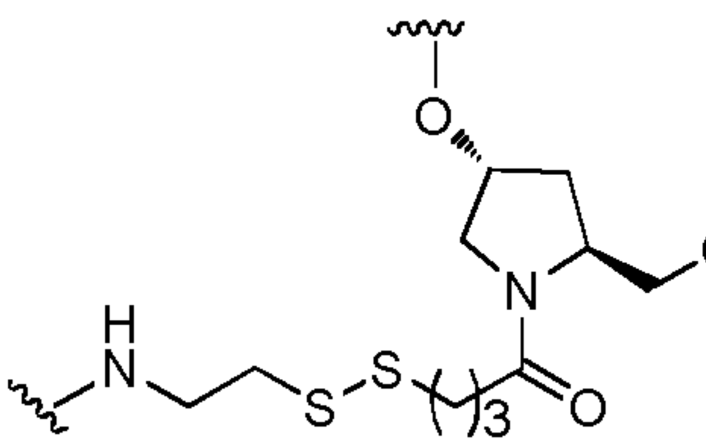
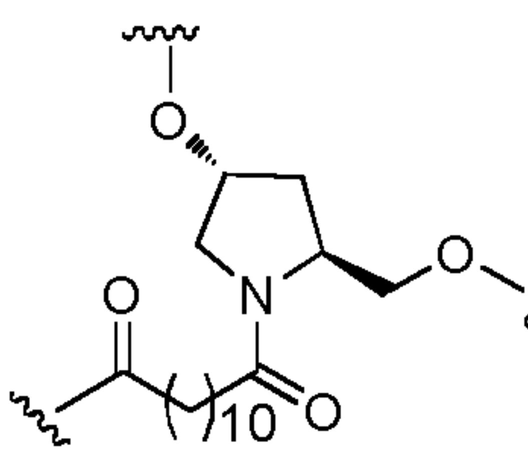
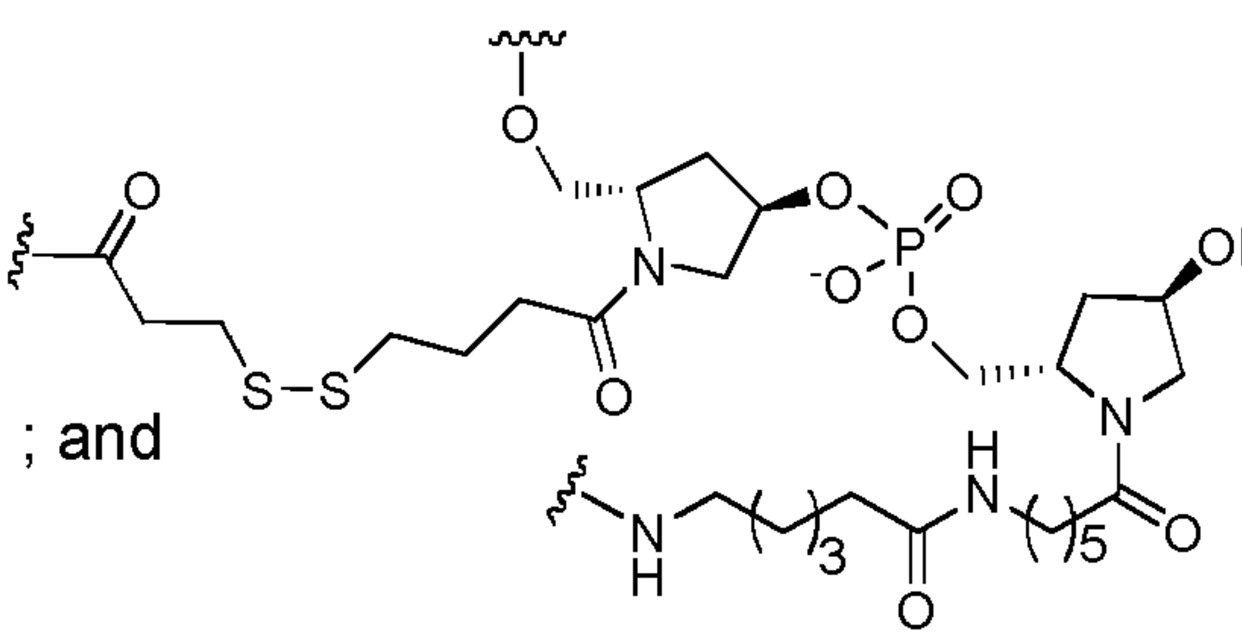
 ;  ;

 ;  ;

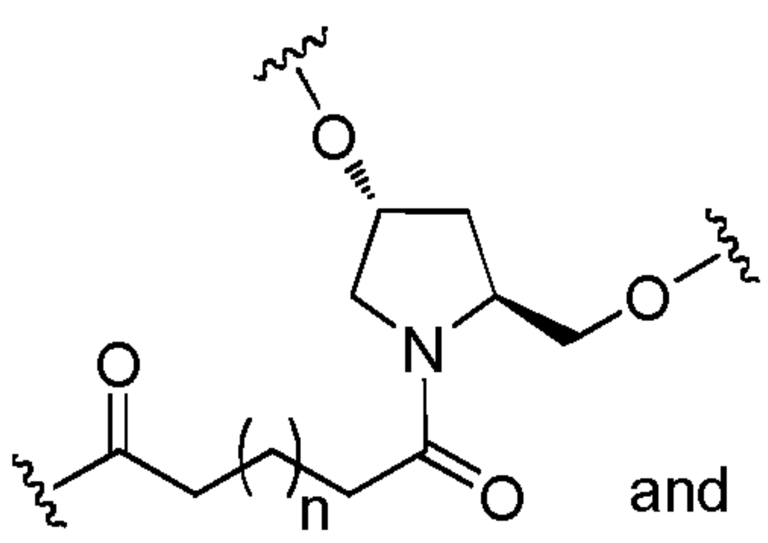
 ; 

 ;  ;

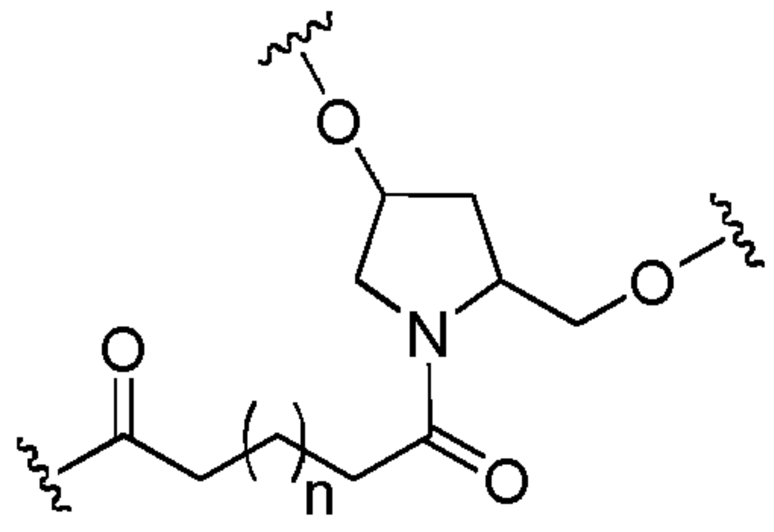
 ;  ;  ;

 ;  ; and  .

94. The compound of any of claims 64 to 92, wherein the conjugate linker has a structure selected from among:

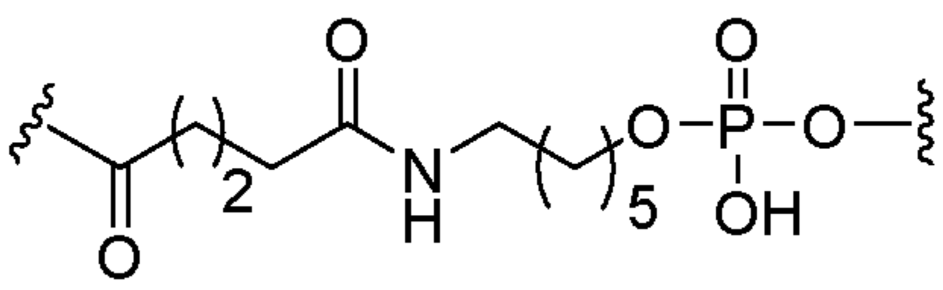


and

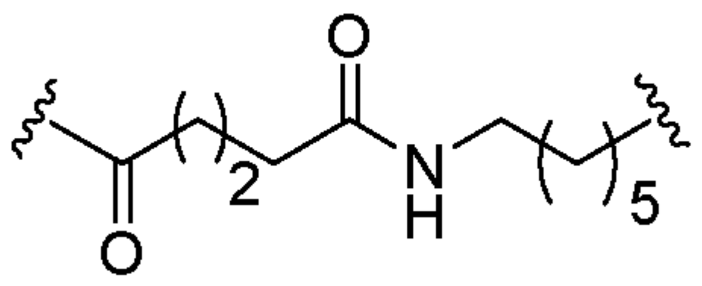


wherein n is from 1 to 20.

95. The compound of any of claims 64 to 92, wherein the conjugate linker has a structure selected from among:

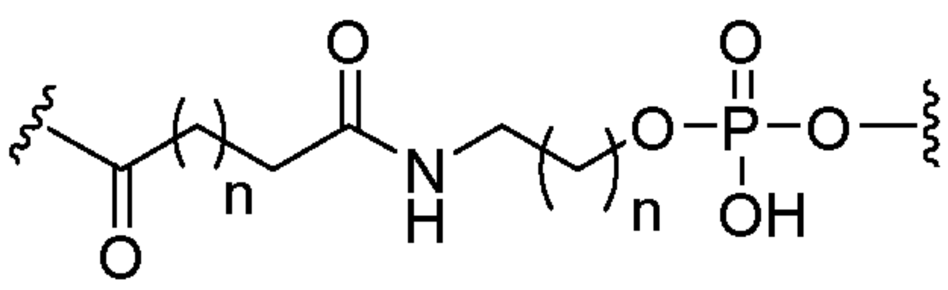


and

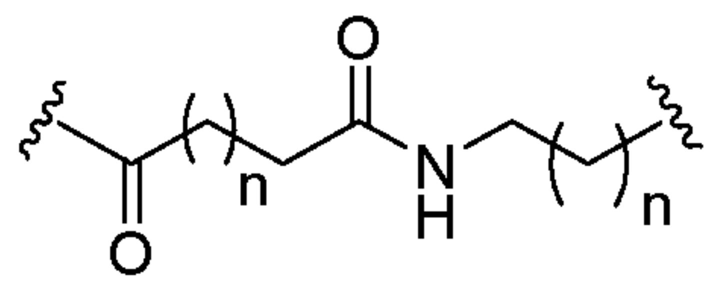


.

96. The compound of any of claims 64 to 92, wherein the conjugate linker has a structure selected from among:



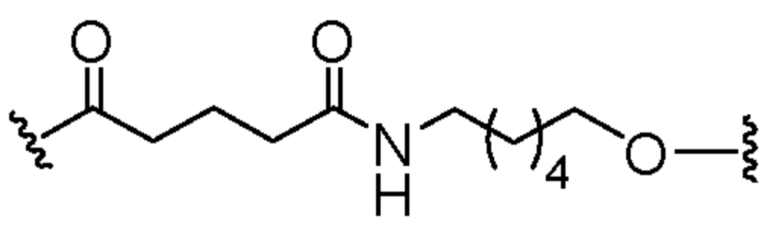
and



;

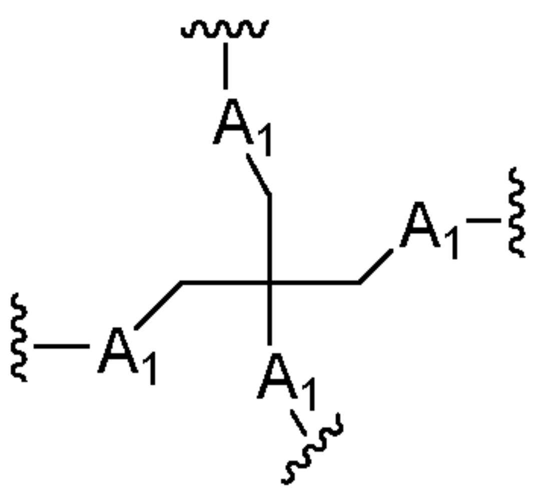
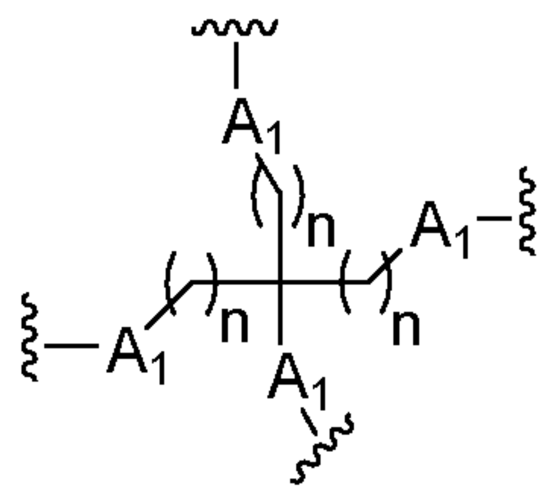
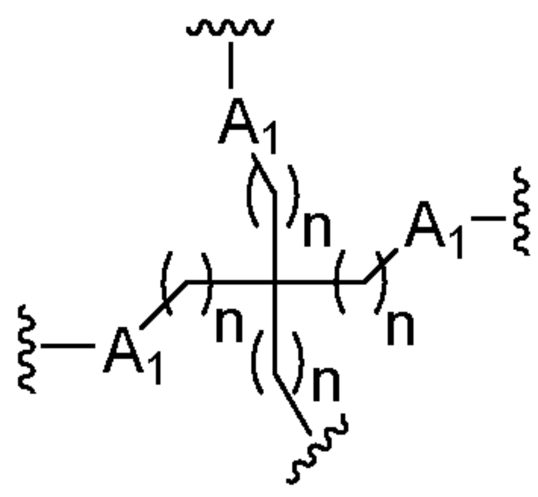
wherein each n is independently, 0, 1, 2, 3, 4, 5, 6, or 7.

97. The compound of any of claims 64 to 92, wherein the conjugate linker has the following structure:



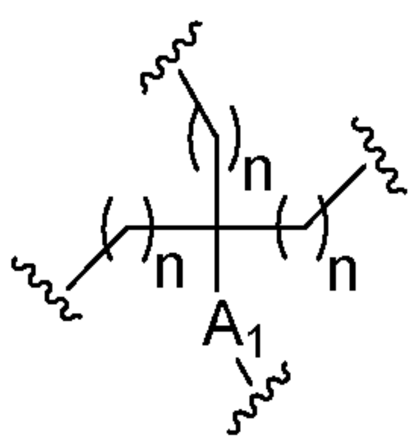
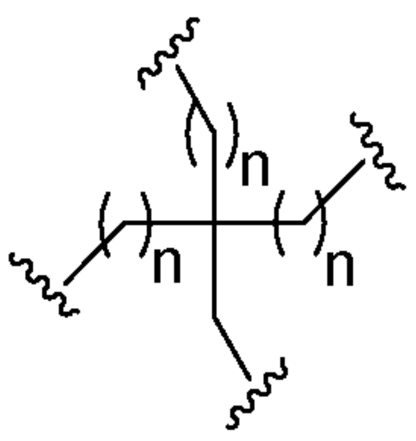
.

98. The compound of any of claims 64 to 97, wherein the branching group has one of the following structures:

 ;  and 

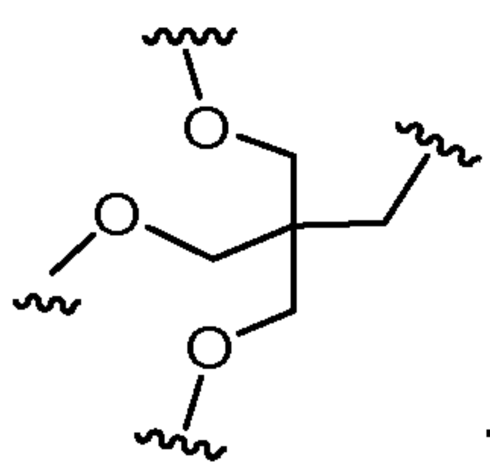
wherein each A_1 is independently, O, S, C=O or NH; and
each n is, independently, from 1 to 20.

99. The compound of any of claims 64 to 97, wherein the branching group has one of the following structures:

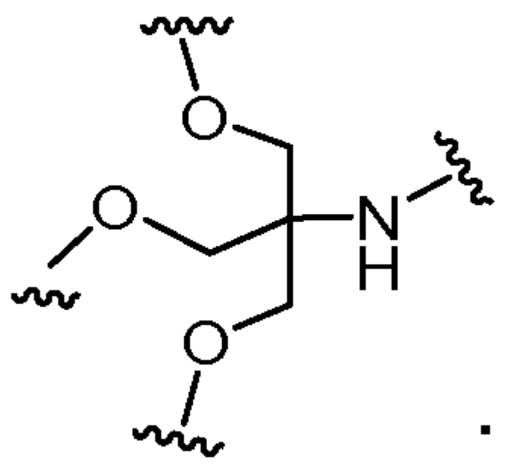
 and 

wherein each A_1 is independently, O, S, C=O or NH; and
each n is, independently, from 1 to 20.

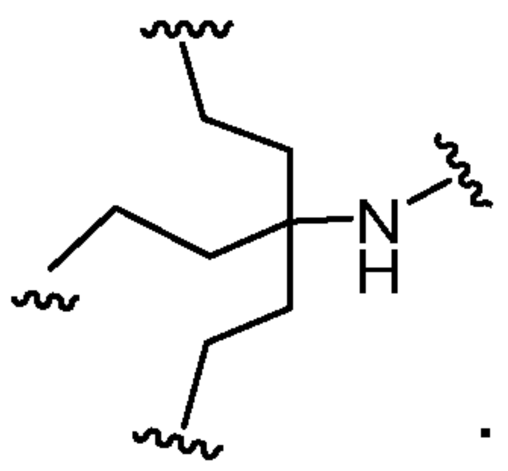
100. The compound of any of claims 64 to 97, wherein the branching group has the following structure:

 .

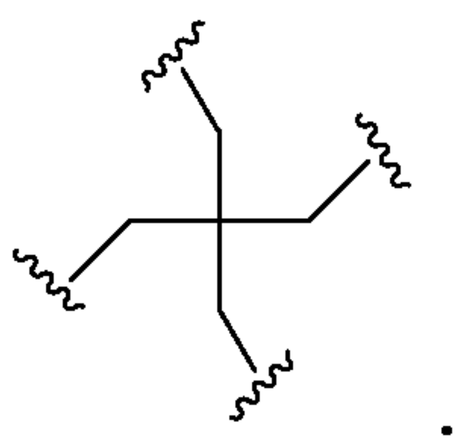
101. The compound of any of claims 64 to 97, wherein the branching group has the following structure:



102. The compound of any of claims 64 to 97, wherein the branching group has the following structure:

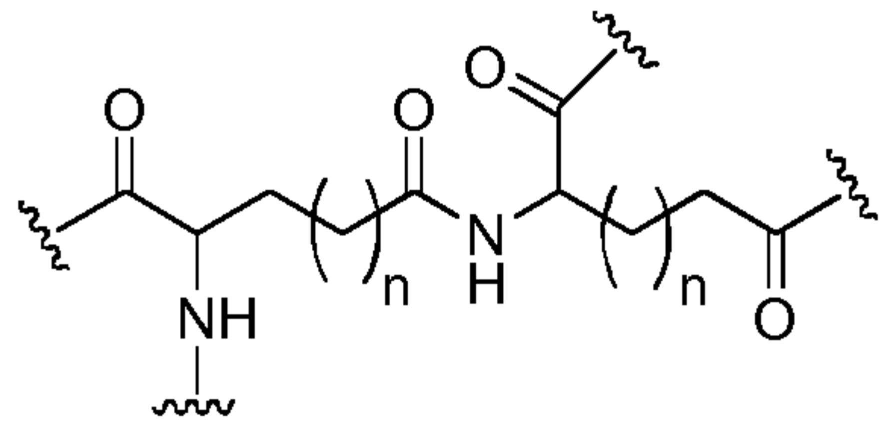
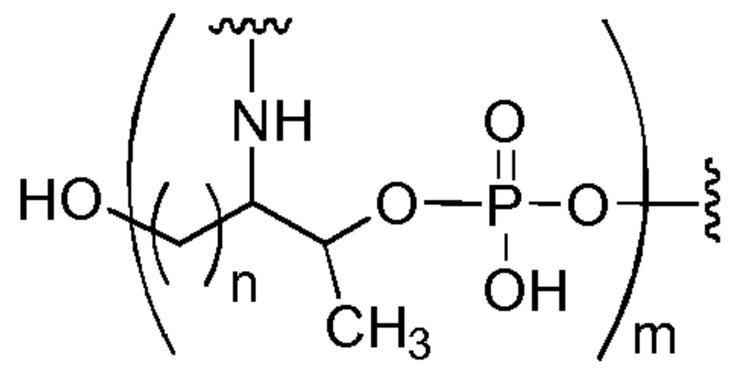
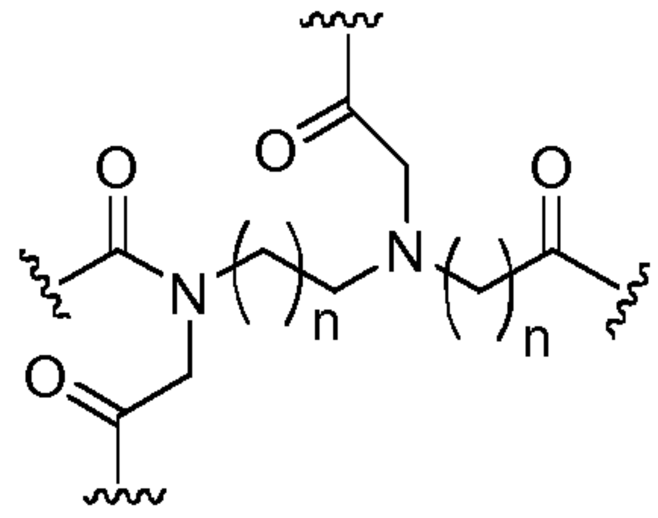
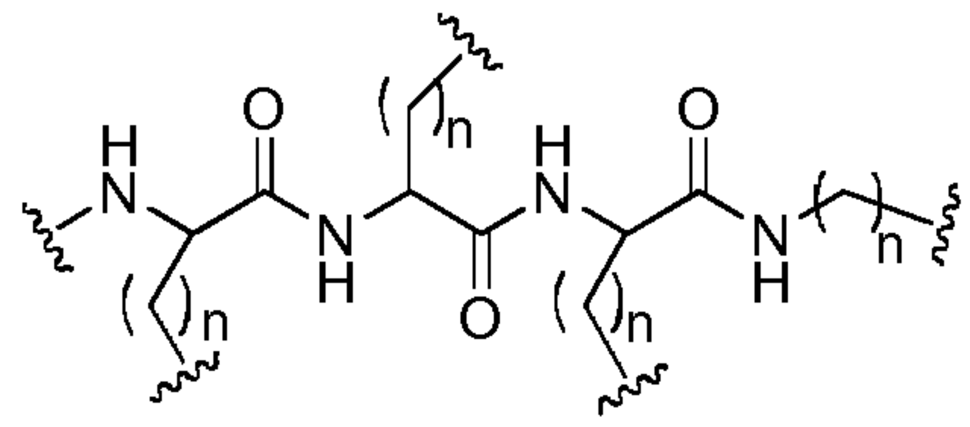
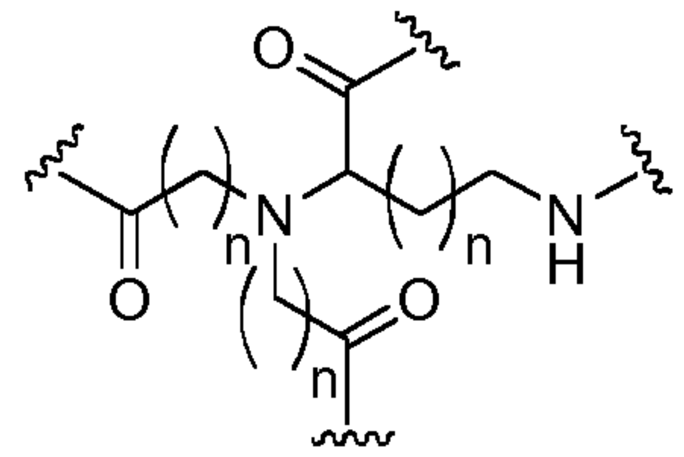
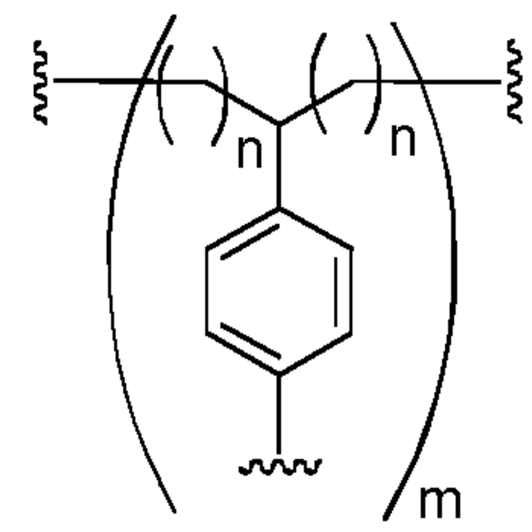
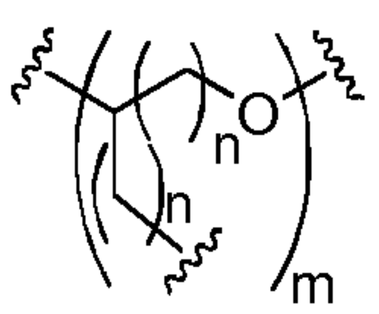
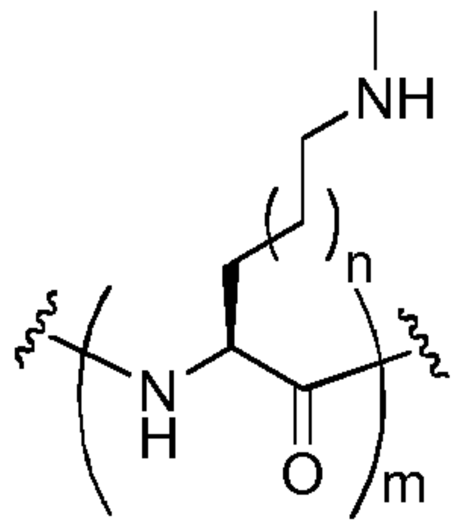
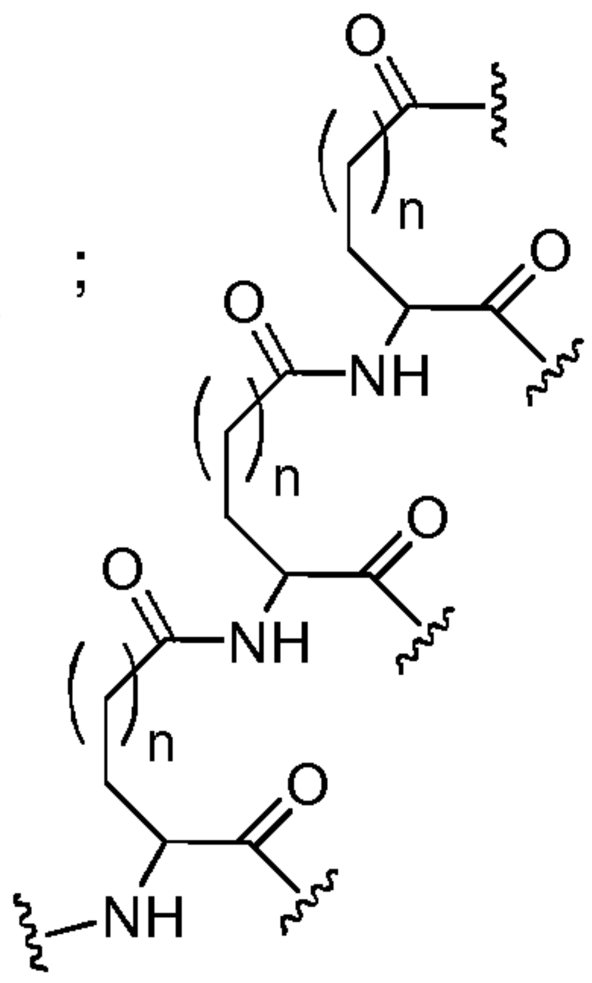
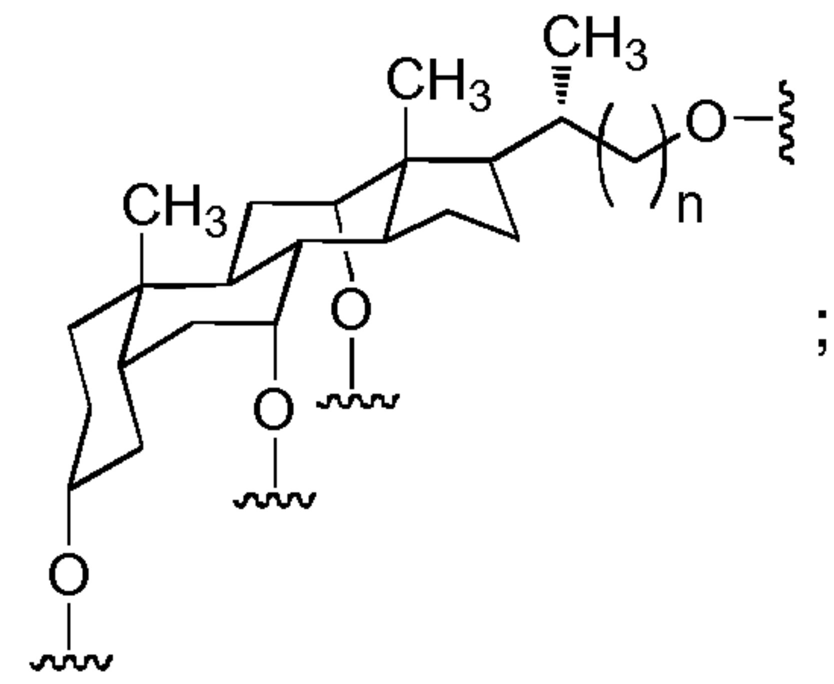
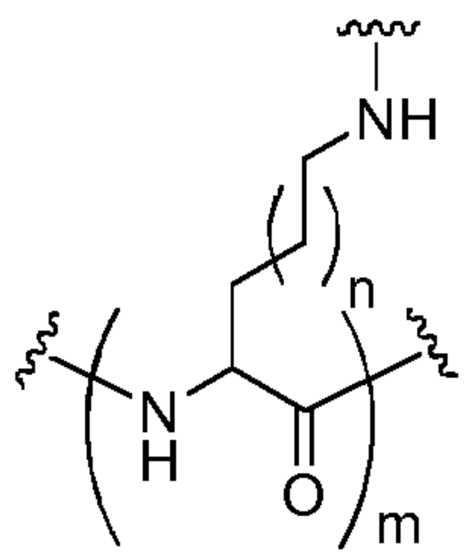


103. The compound of any of claims 64 to 97, wherein the branching group has the following structure:



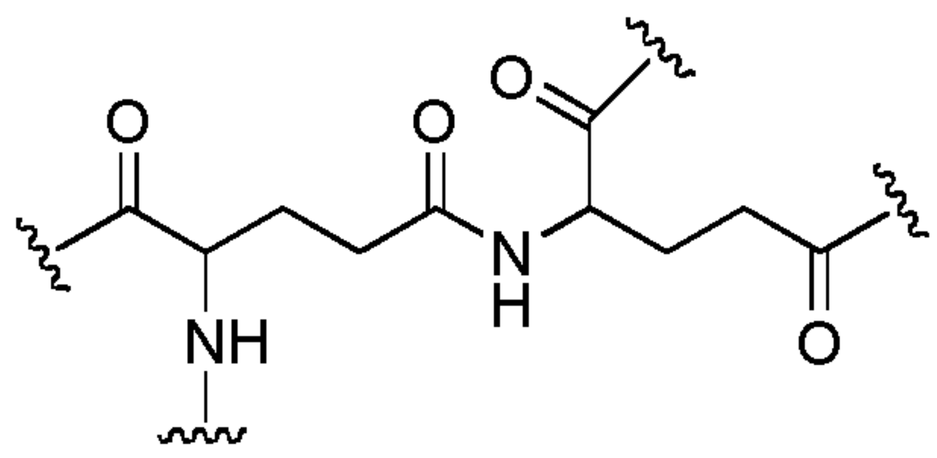
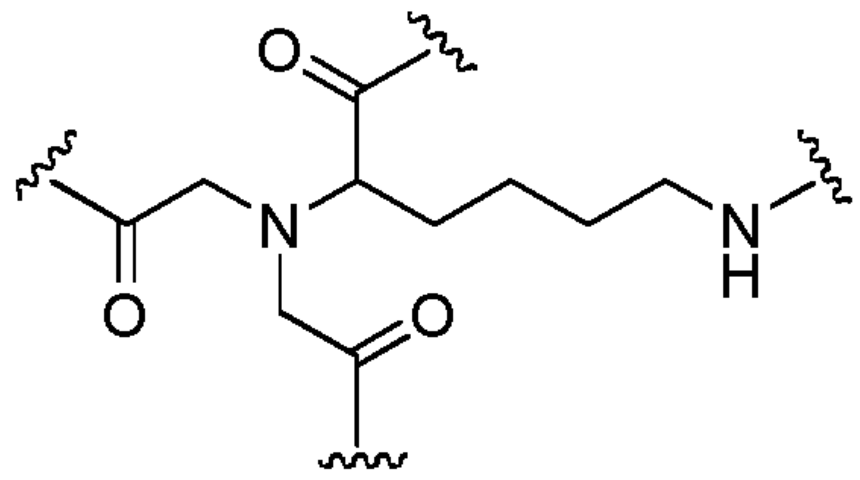
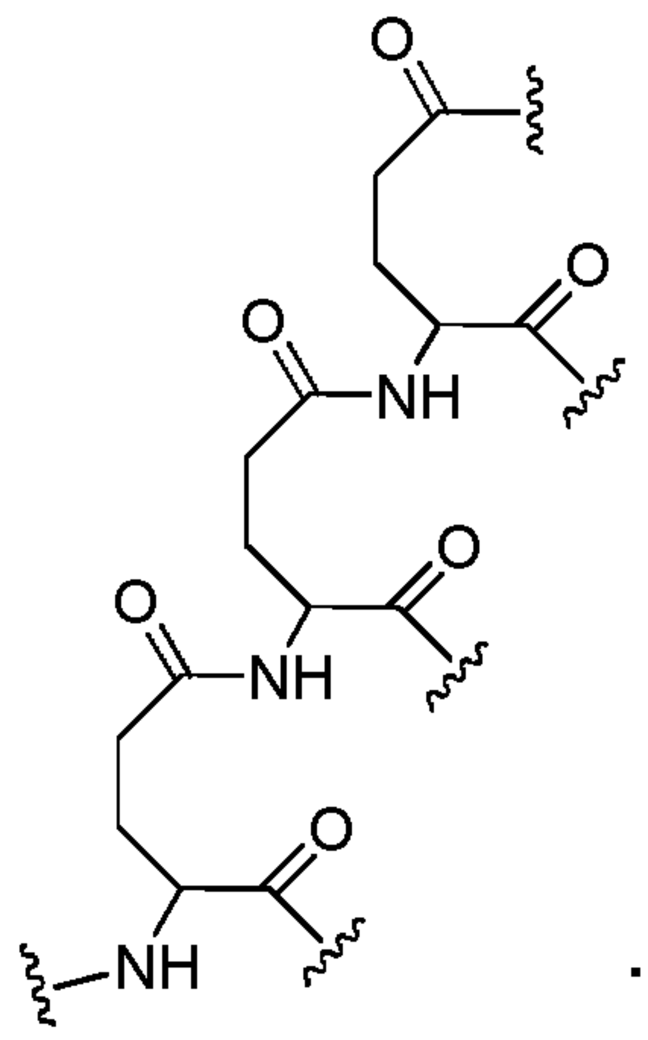
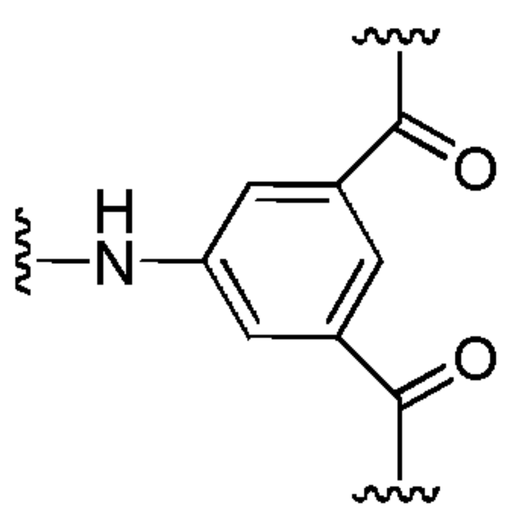
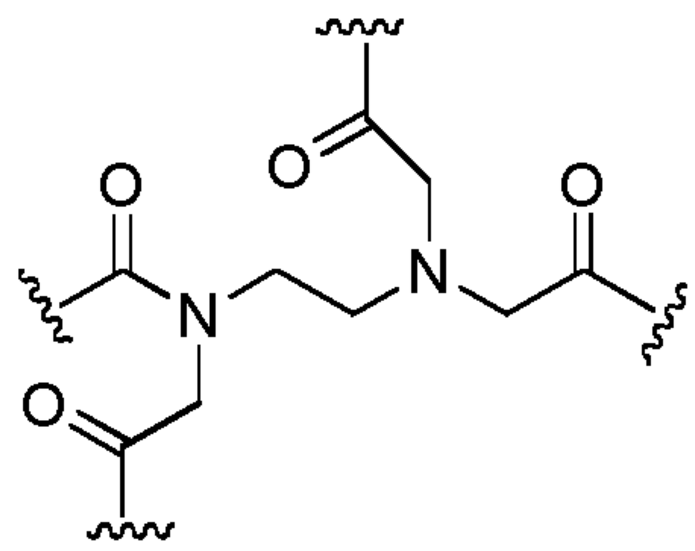
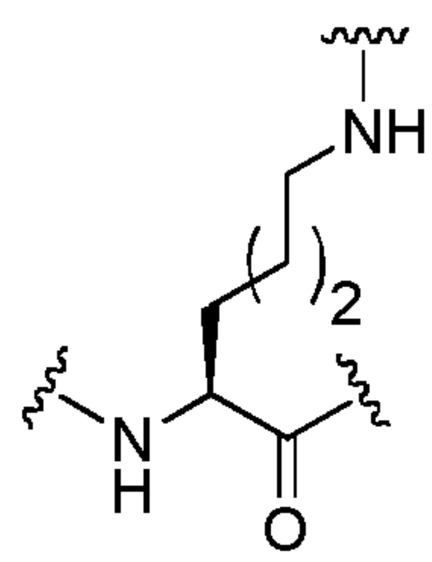
104. The compound of any of claims 64 to 97, wherein the branching group comprises an ether.

105. The compound of any of claims 64 to 97, wherein the branching group has the following structure:

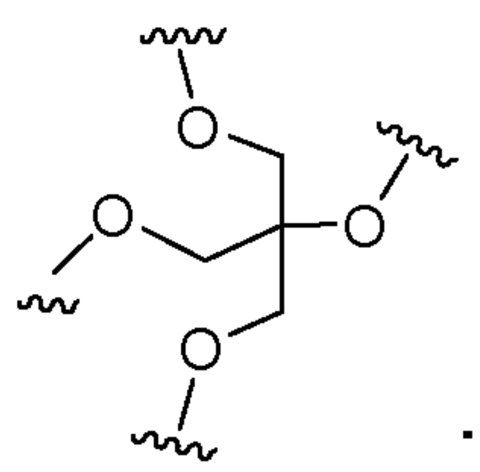

;

;

;

;

;

;

;

;

;

; and


each n is, independently, from 1 to 20; and
m is from 2 to 6.

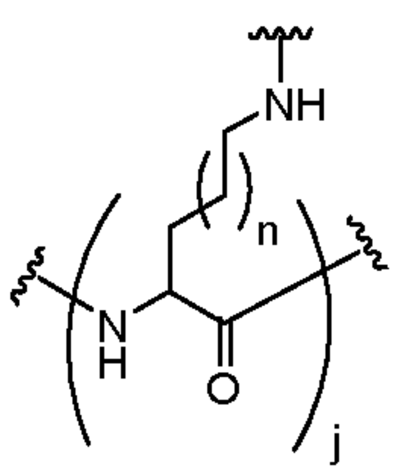
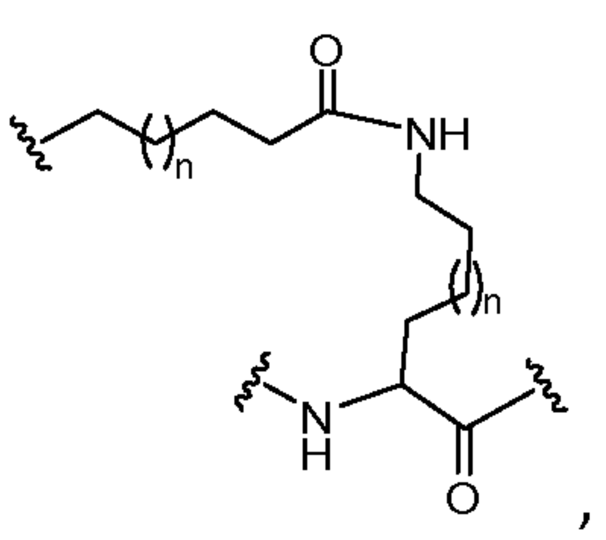
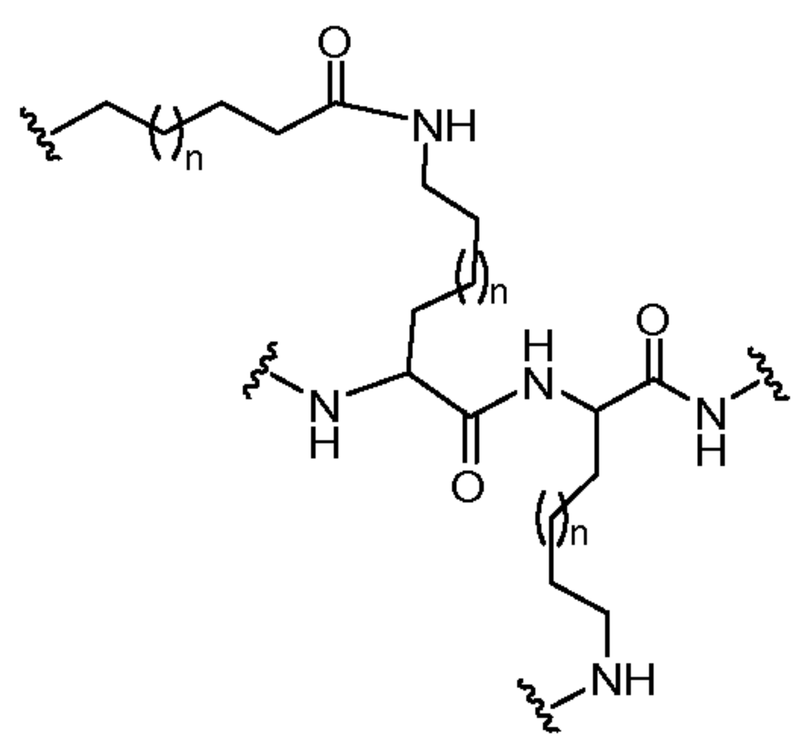
106. The compound of any of claims 64 to 97, wherein the branching group has the following structure:

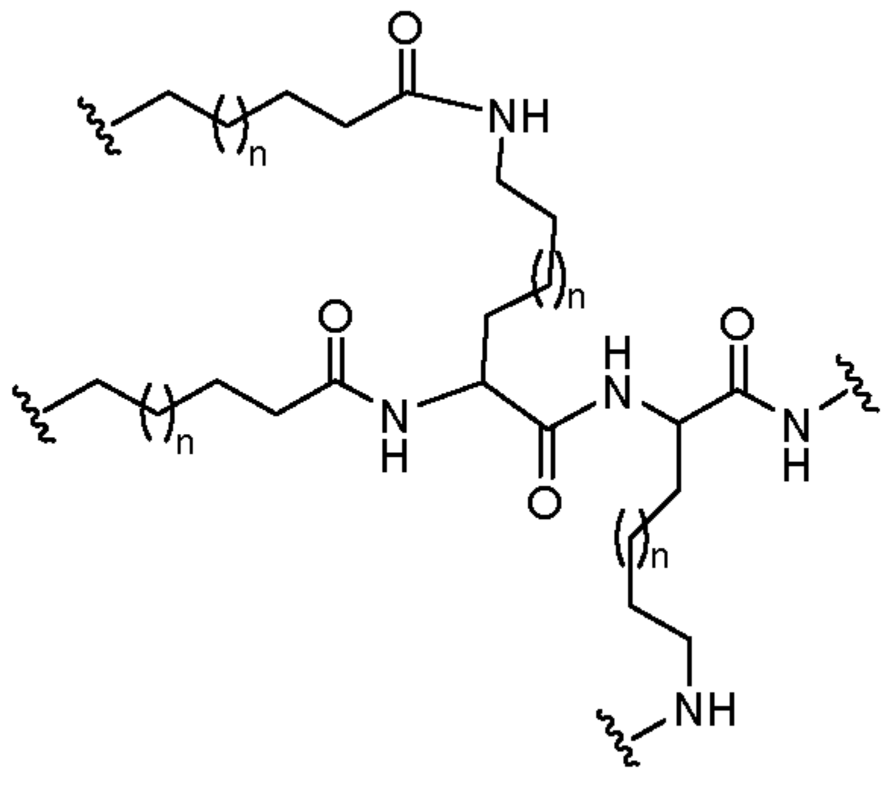
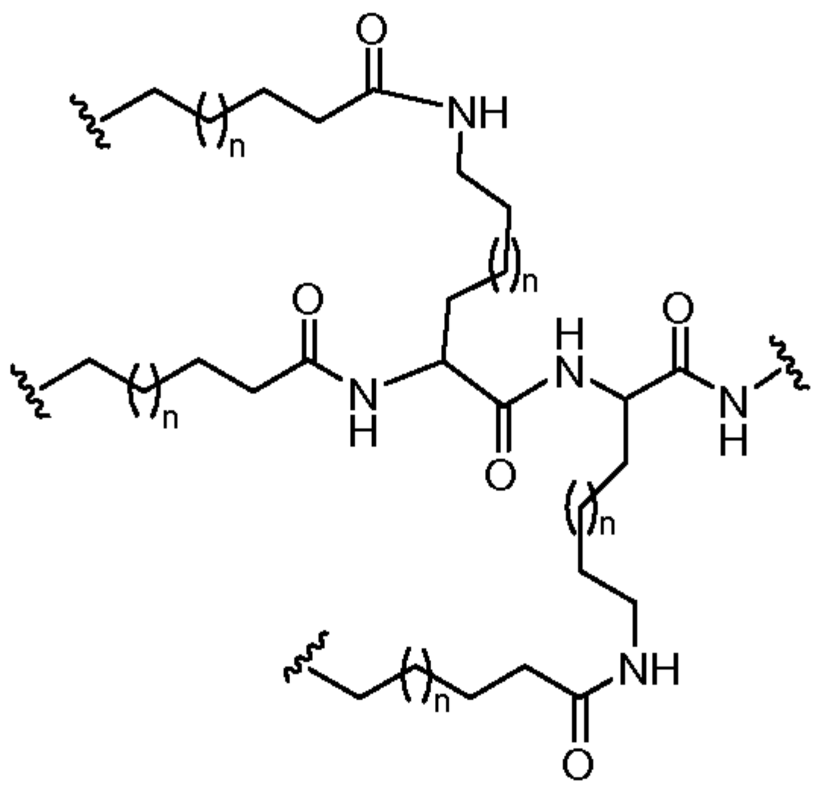
 ;  ;  ;  ;  ; and  .

107. The compound of any of claims 64 to 97, wherein the branching group has the following structure:

 .

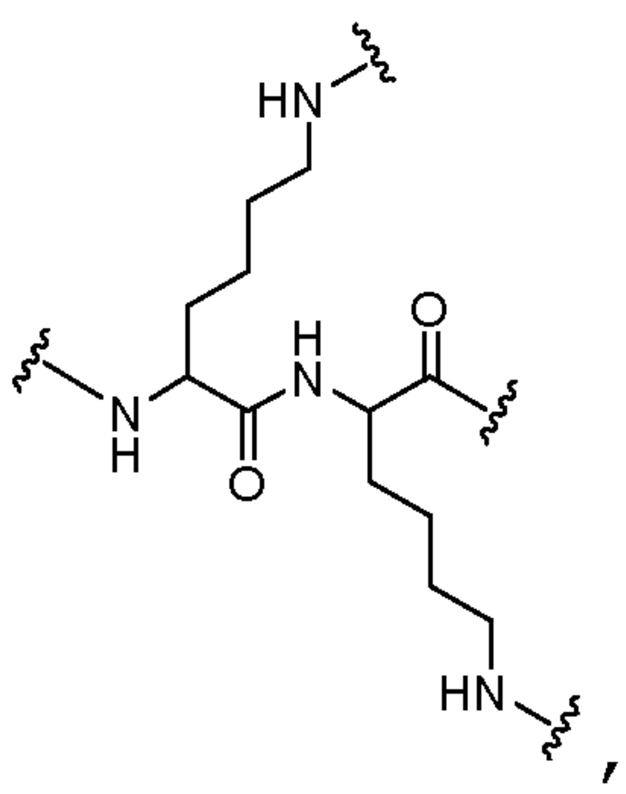
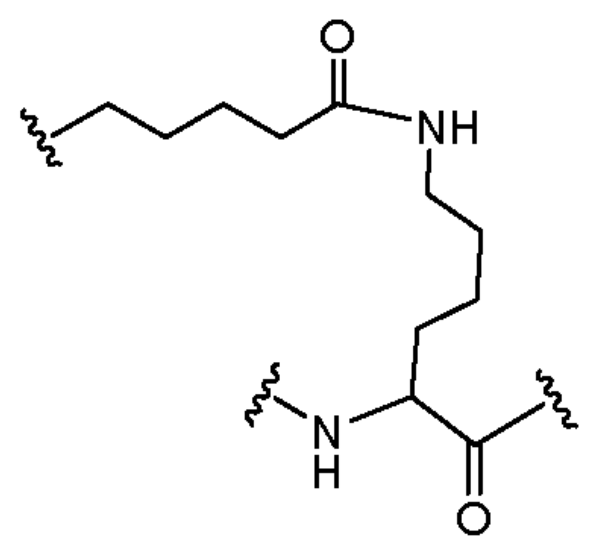
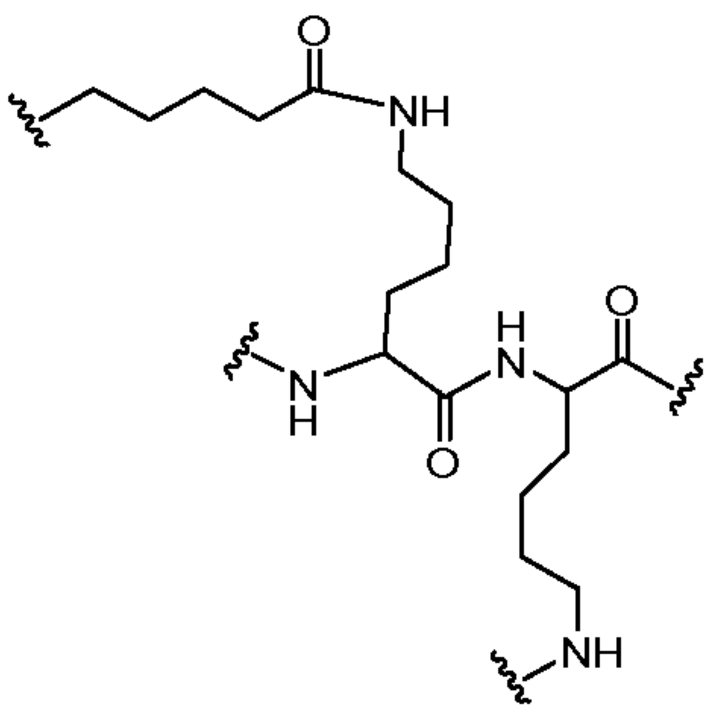
108. The compound of any of claims 64 to 97, wherein the branching group comprises:

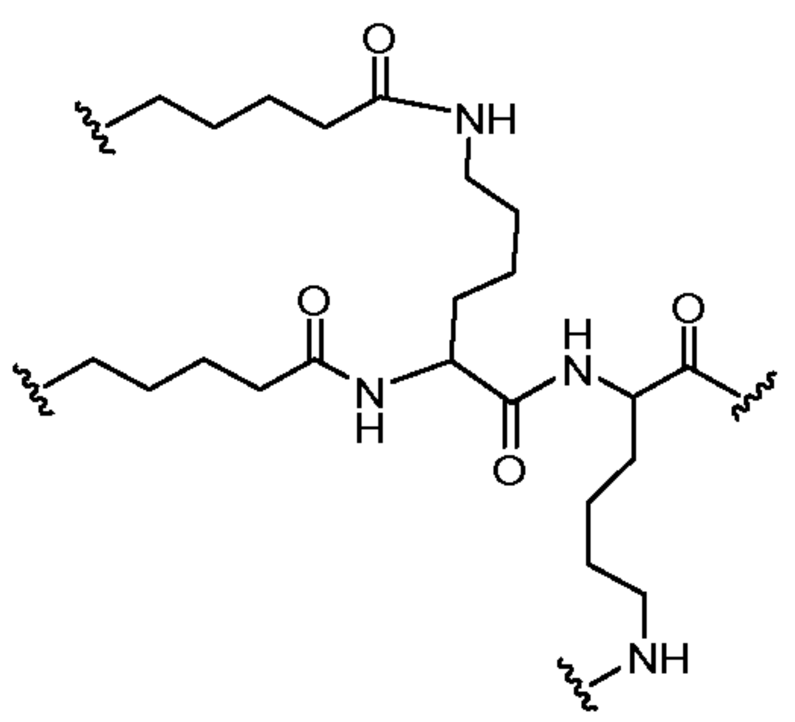
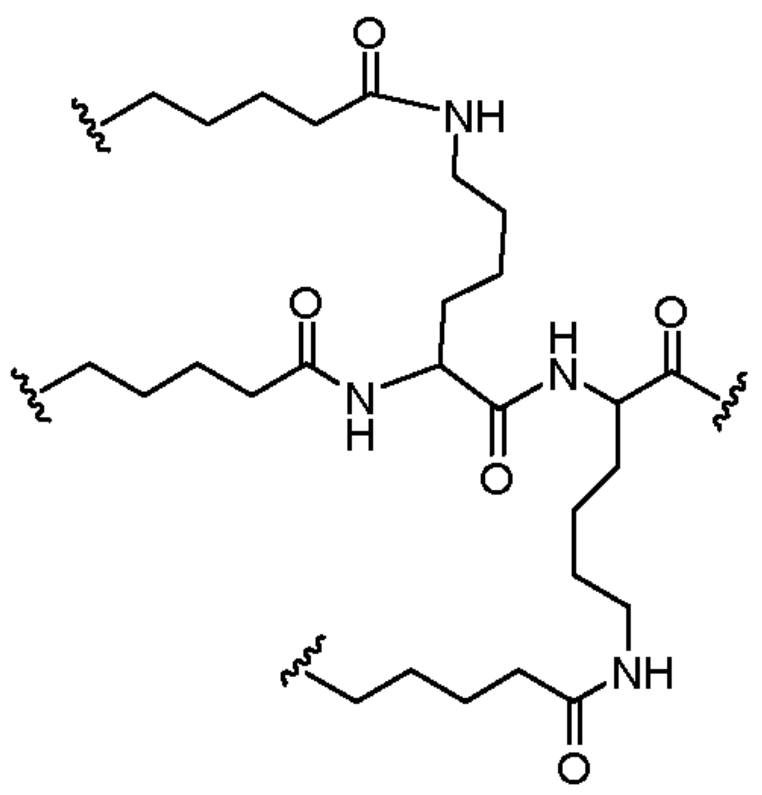
 ,  ,  ,

 , or  ;

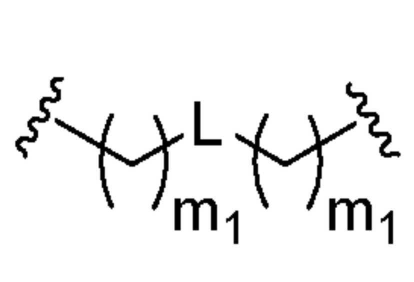
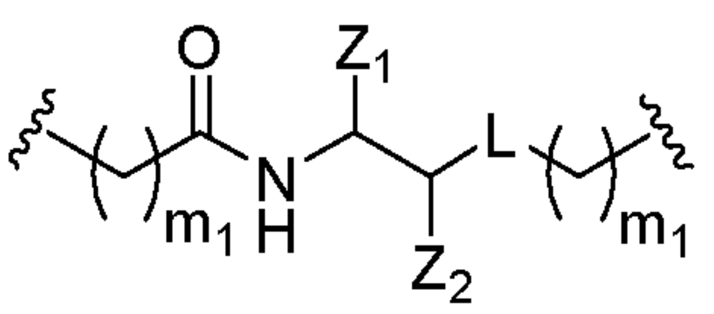
wherein each j is an integer from 1 to 3; and
wherein each n is an integer from 1 to 20.

109. The compound of any of claims 64 to 97, wherein the branching group comprises:

 ,  ,  ,

 , or  .

110. The compound of any of claims 64 to 109, wherein each tether is selected from among:

 and 

wherein L is selected from a phosphorus linking group and a neutral linking group;

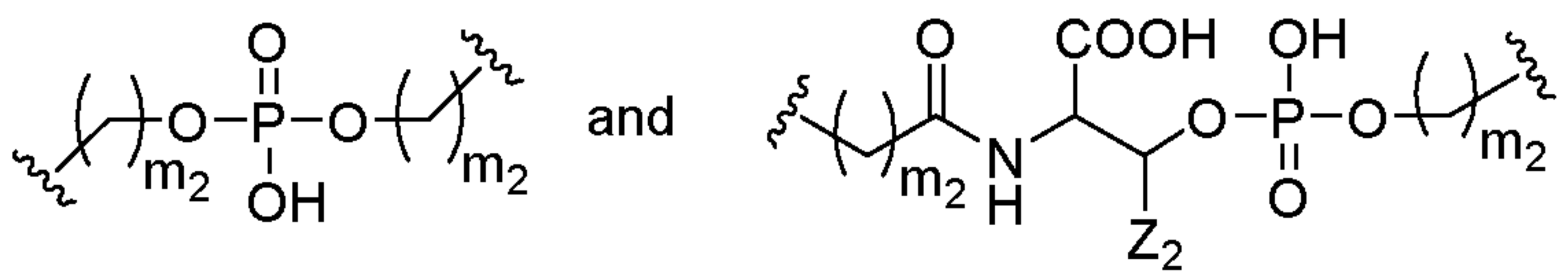
Z_1 is C(=O)O-R_2;

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.

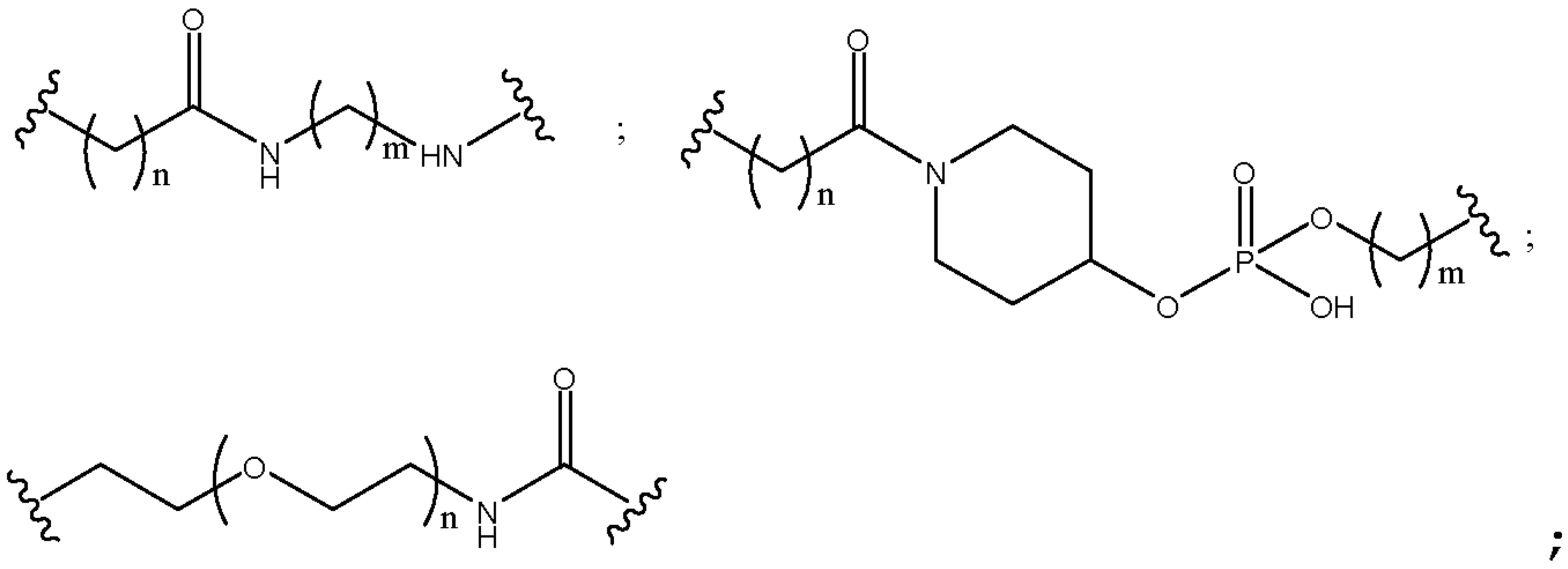
111. The compound of any of claims 64 to 109, wherein each tether is selected from among:



wherein Z_2 is H or CH_3; and

each m_2 is, independently, from 0 to 20 wherein at least one m_2 is greater than 0 for each tether.

112. The compound of any of claims 64 to 109, wherein each tether is selected from among:



wherein n is from 1 to 12; and

wherein m is from 1 to 12.

113. The compound of any of claims 64 to 109, wherein at least one tether comprises ethylene glycol.

114. The compound of any of claims 64 to 109 or 111, wherein at least one tether comprises an amide.

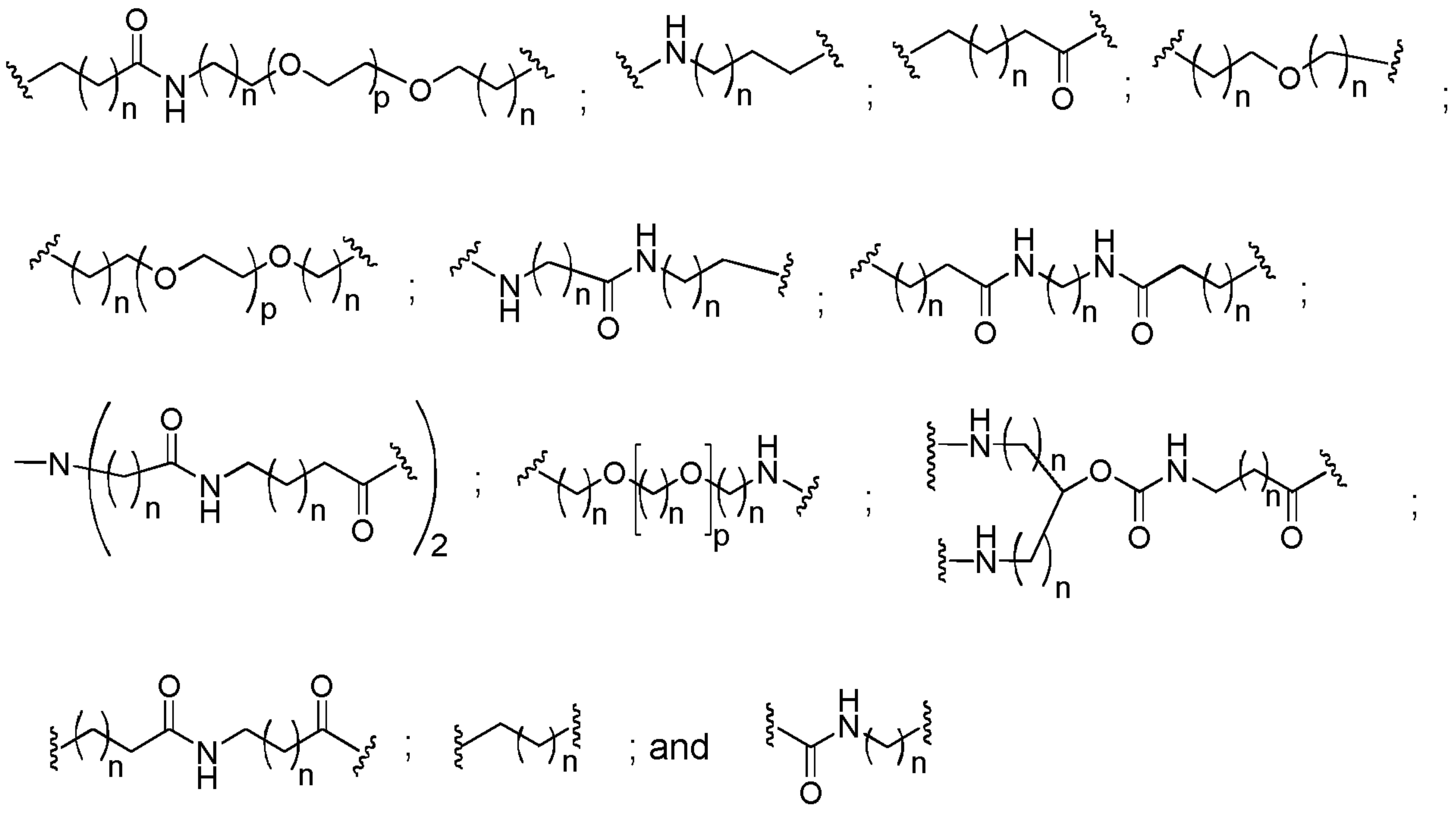
115. The compound of any of claims 64 to 109 or 111, wherein at least one tether comprises a polyamide.

116. The compound of any of claims 64 to 109 or 111, wherein at least one tether comprises an amine.

117. The compound of any of claims 64 to 109 or 111, wherein at least two tethers are different from one another.

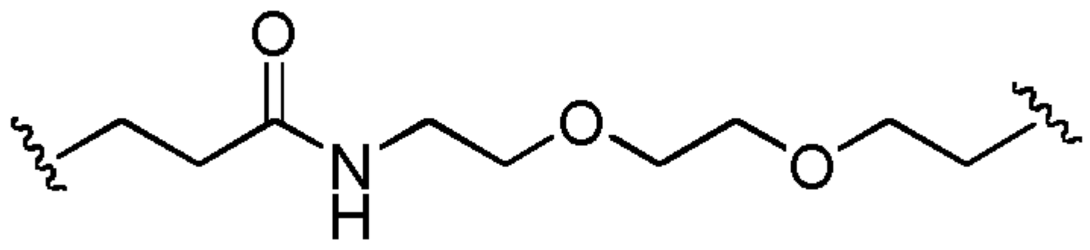
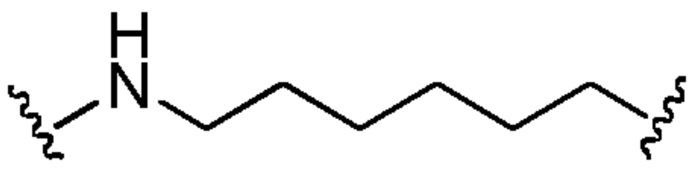
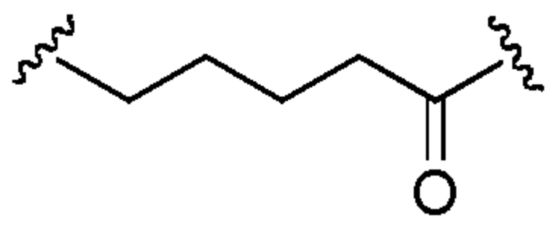
118. The compound of any of claims 64 to 109 or 111, wherein all of the tethers are the same as one another.

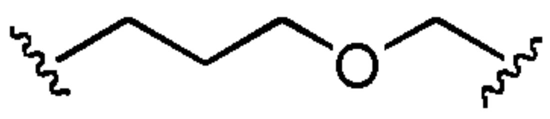
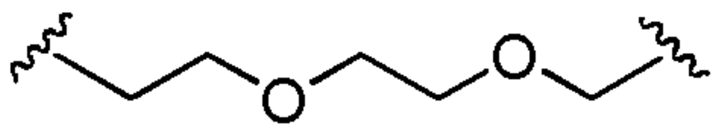
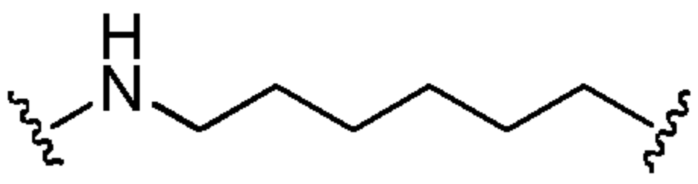
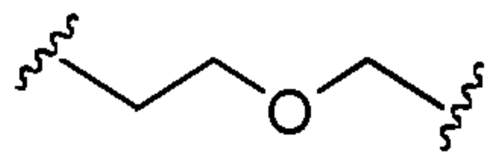
119. The compound of any of claims 64 to 109, wherein each tether is selected from among:



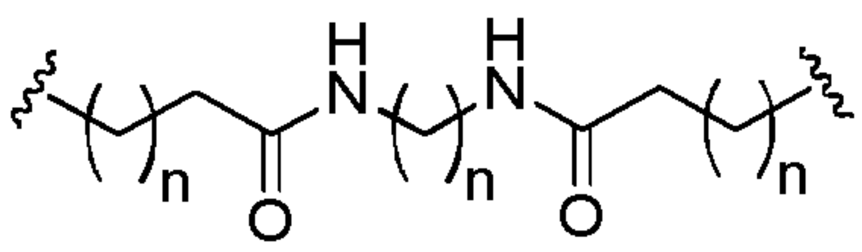
wherein each n is, independently, from 1 to 20; and
each p is from 1 to about 6.

120. The compound of any of claims 64 to 109, wherein each tether is selected from among:

 ;  ;  ;

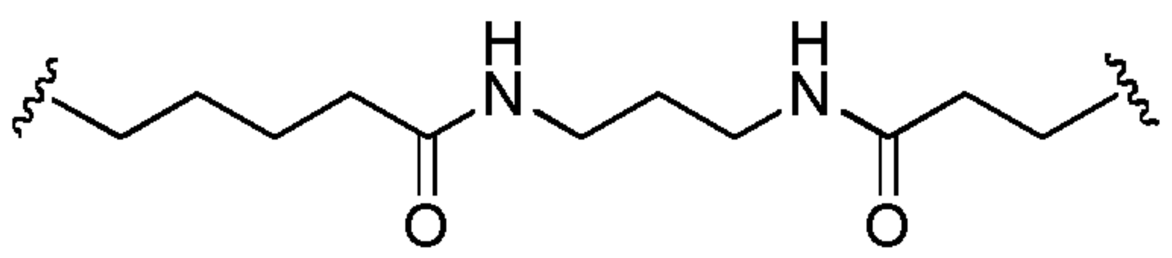
 ;  ;  ; and  .

121. The compound of any of claims 64 to 109, wherein each tether has the following structure:

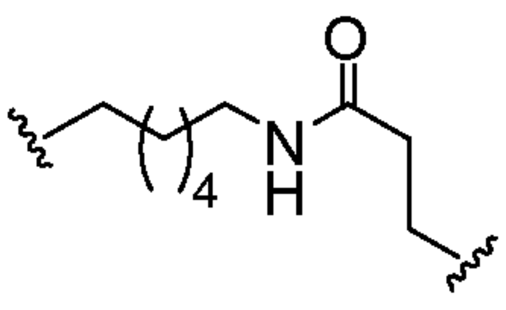
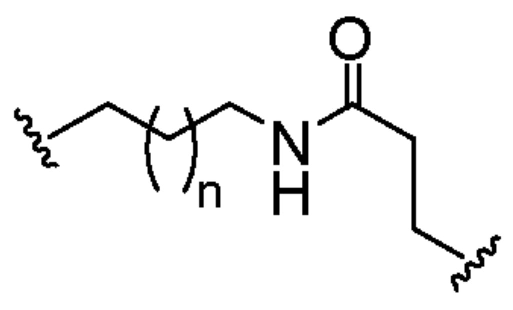


wherein each n is, independently, from 1 to 20.

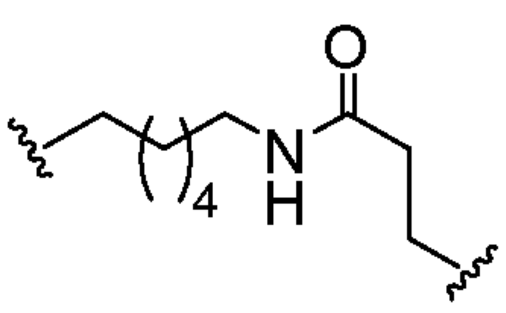
122. The compound of any of claims 64 to 109, wherein each tether has the following structure:



123. The compound of any of claims 64 to 109, wherein the tether has a structure selected from among:

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

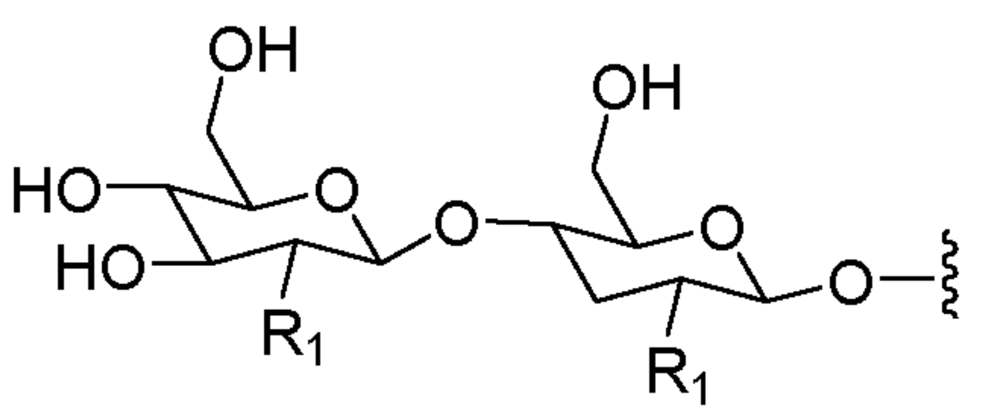
124. The compound of any of claims 64 to 109, wherein the tether has a structure selected from among:

.

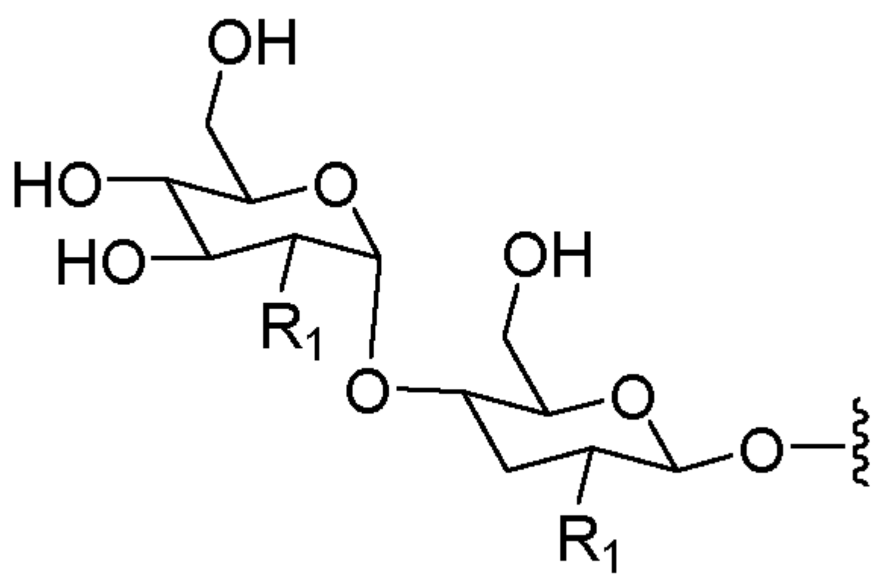
125. The compound of any of claims 121 to 124, wherein the ligand is galactose.

126. The compound of any of claims 121 to 124, wherein the ligand is mannose-6-phosphate.

127. The compound of any of claims 121 to 124, wherein each ligand is selected from among:

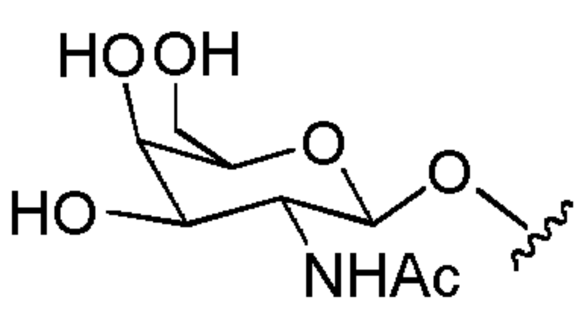
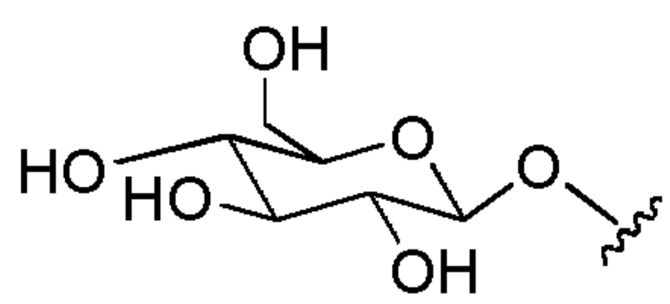
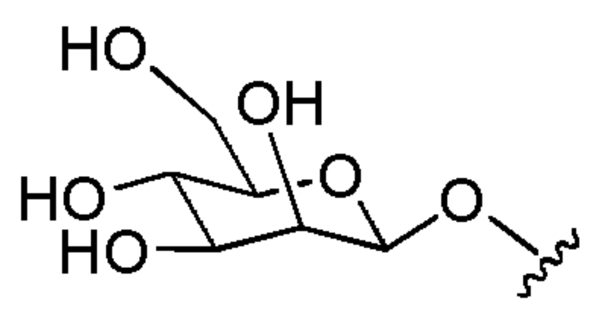
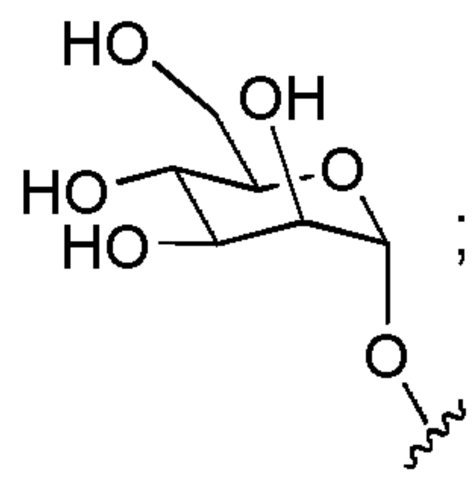
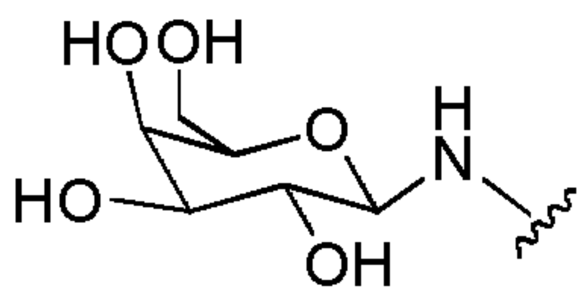
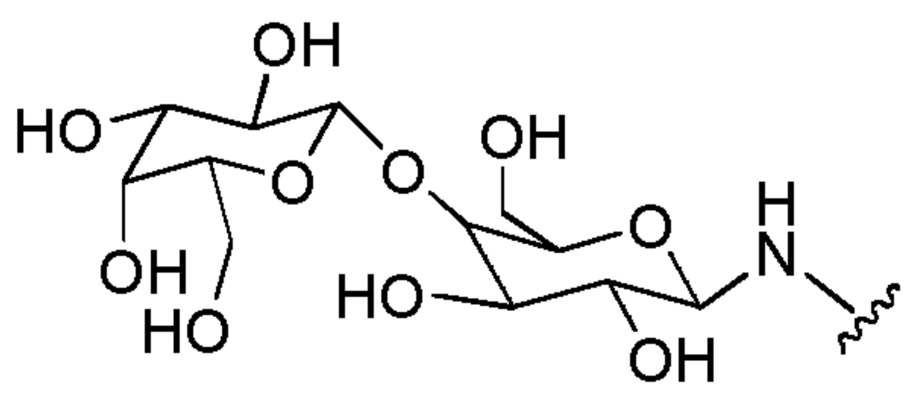
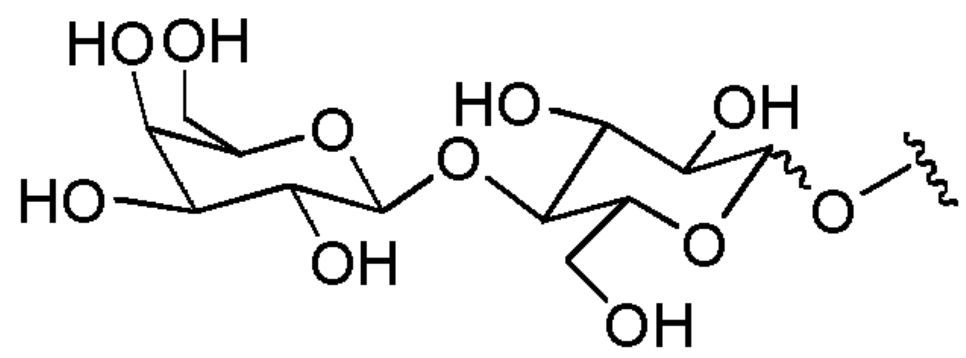
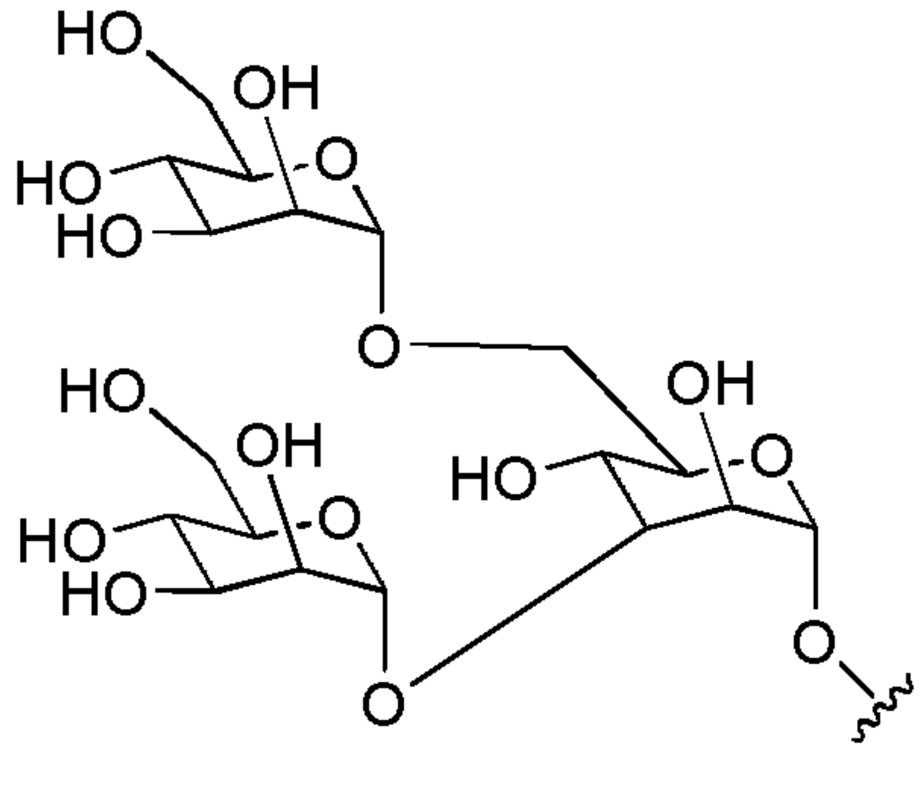


and

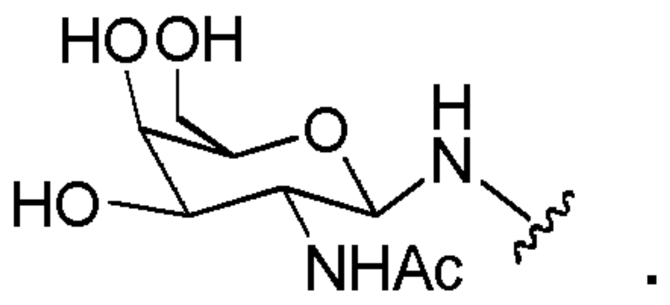


wherein each R_1 is selected from OH and NHCOOH.

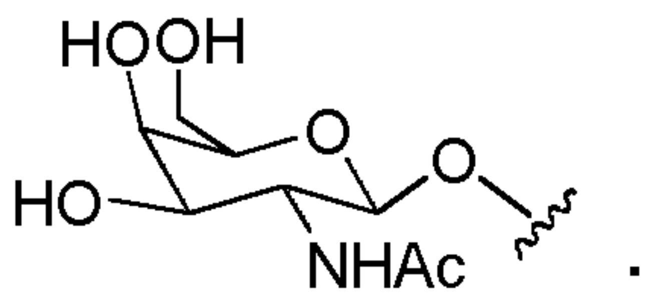
128. The compound of any of claims 121 to 124, wherein each ligand is selected from among:


;

;

;

;

;

;

; and

.

129. The compound of any of claims 121 to 124, wherein each ligand has the following structure:

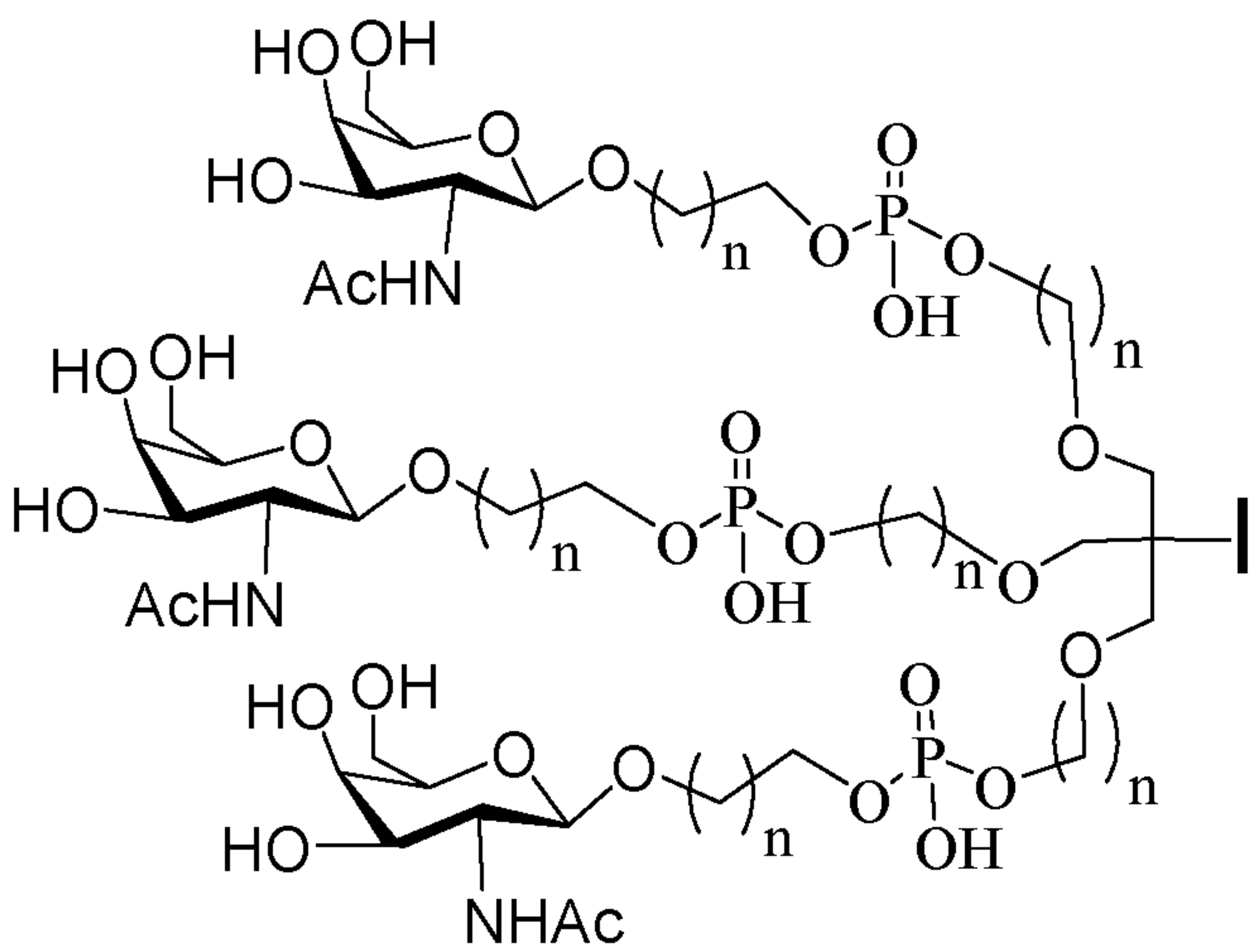


130. The conjugated antisense compound of any of claims 121 to 124, wherein each ligand has the following structure:



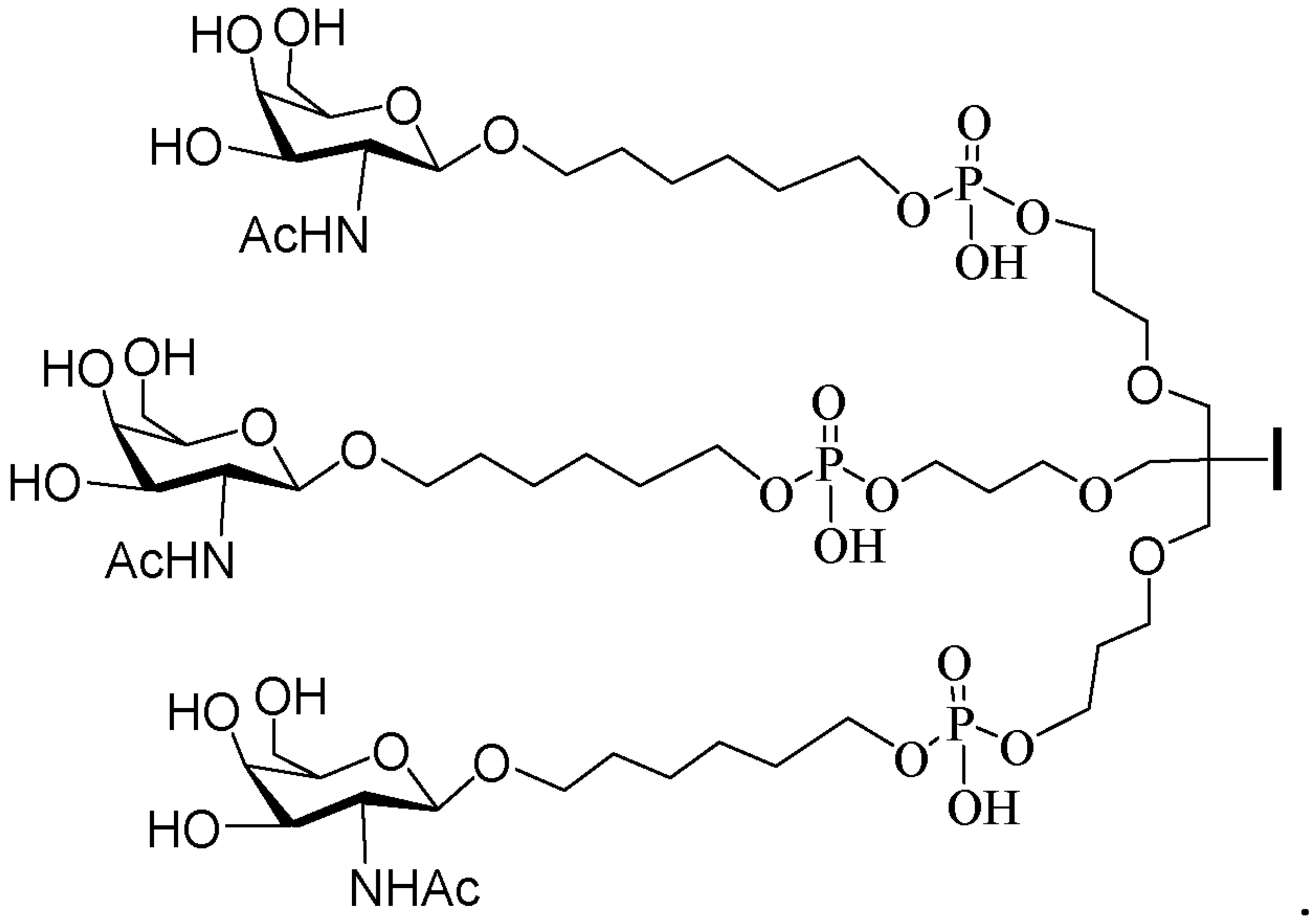
131. The compound of any preceding claim, wherein the conjugate group comprises a cell-targeting moiety.

132. The compound of claim 131, wherein the conjugate group comprises a cell-targeting moiety having the following structure:

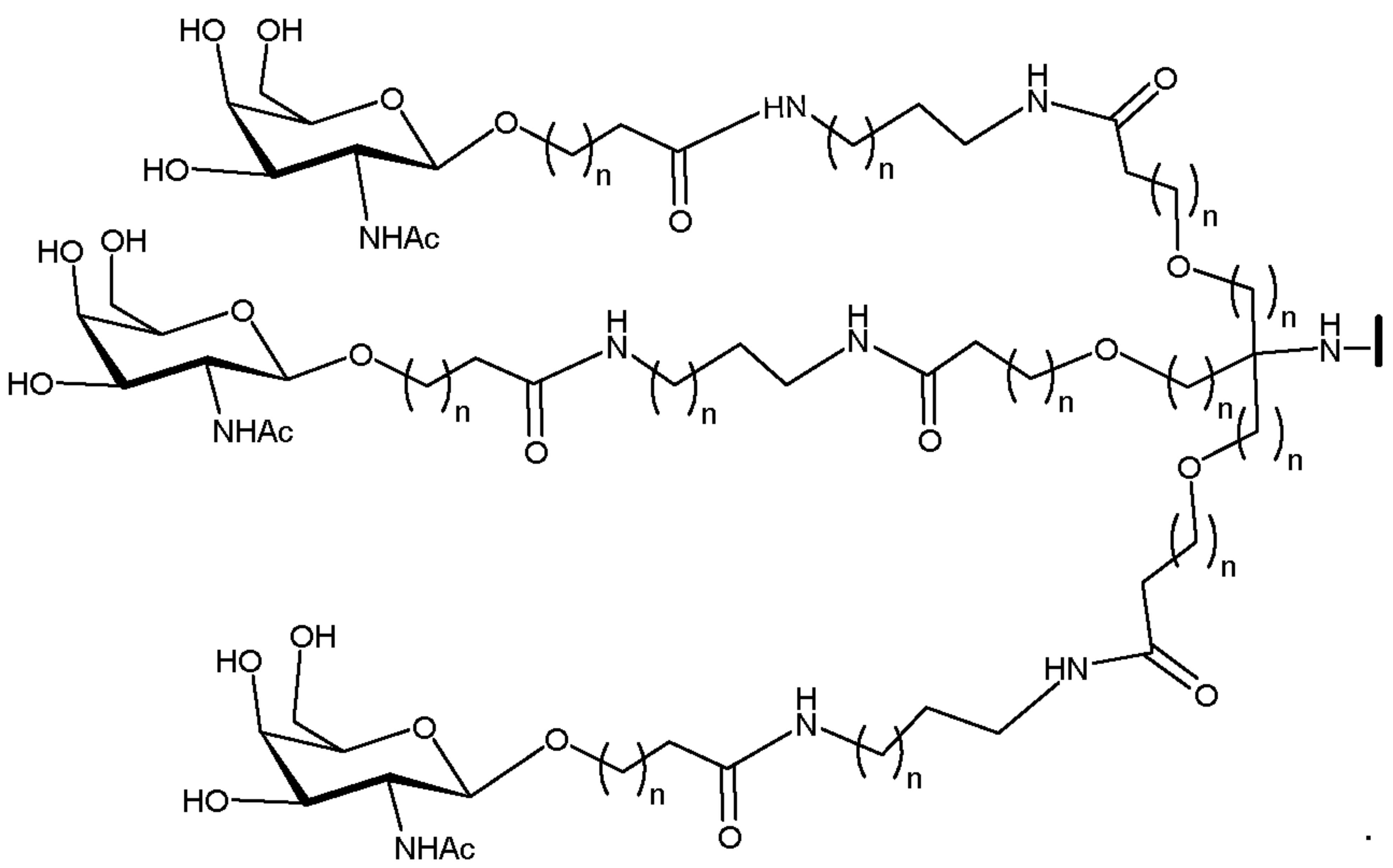


wherein each n is, independently, from 1 to 20.

133. The compound of claim 131, wherein the cell-targeting moiety comprises:

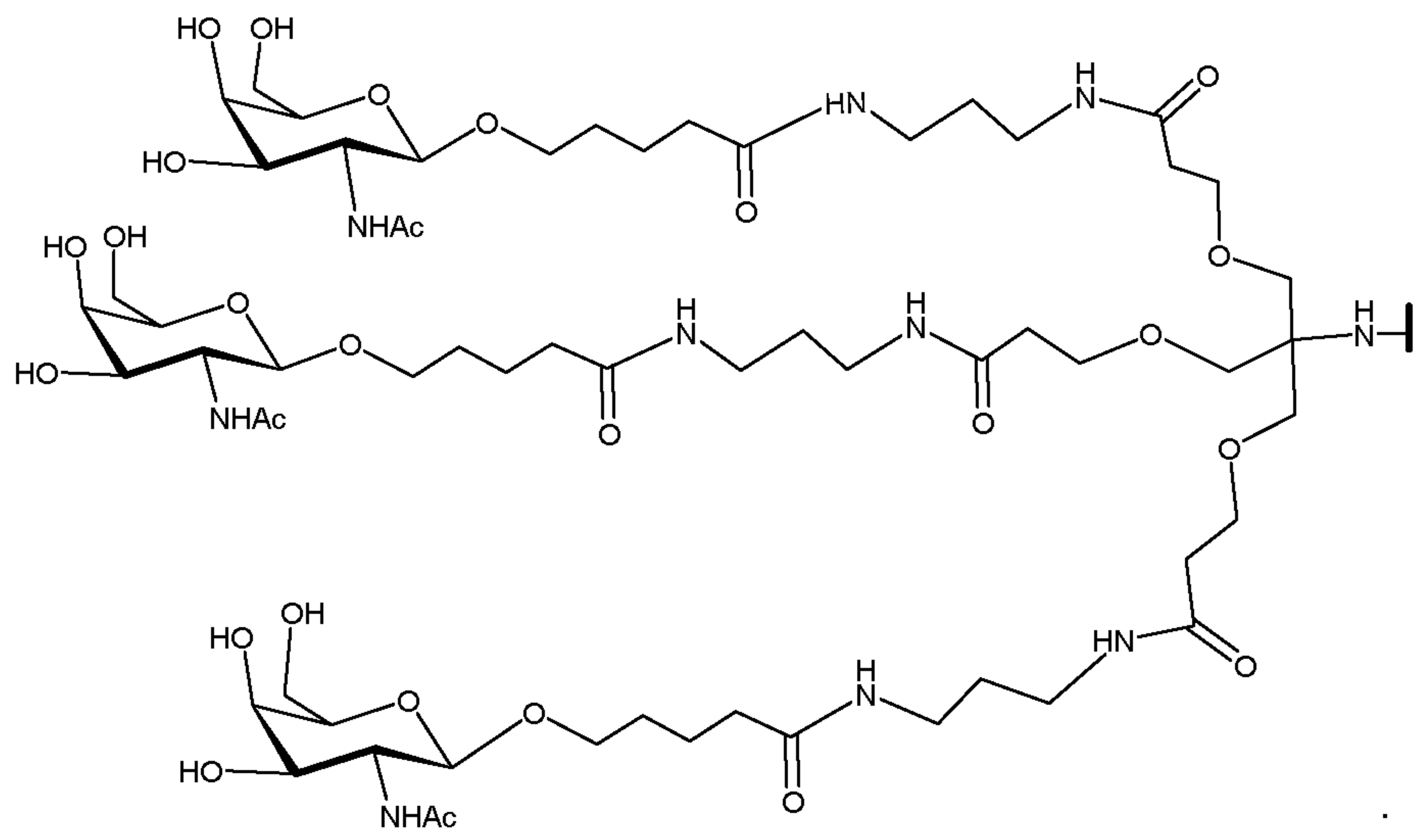


134. The compound of claim 131, wherein the cell-targeting moiety comprises:

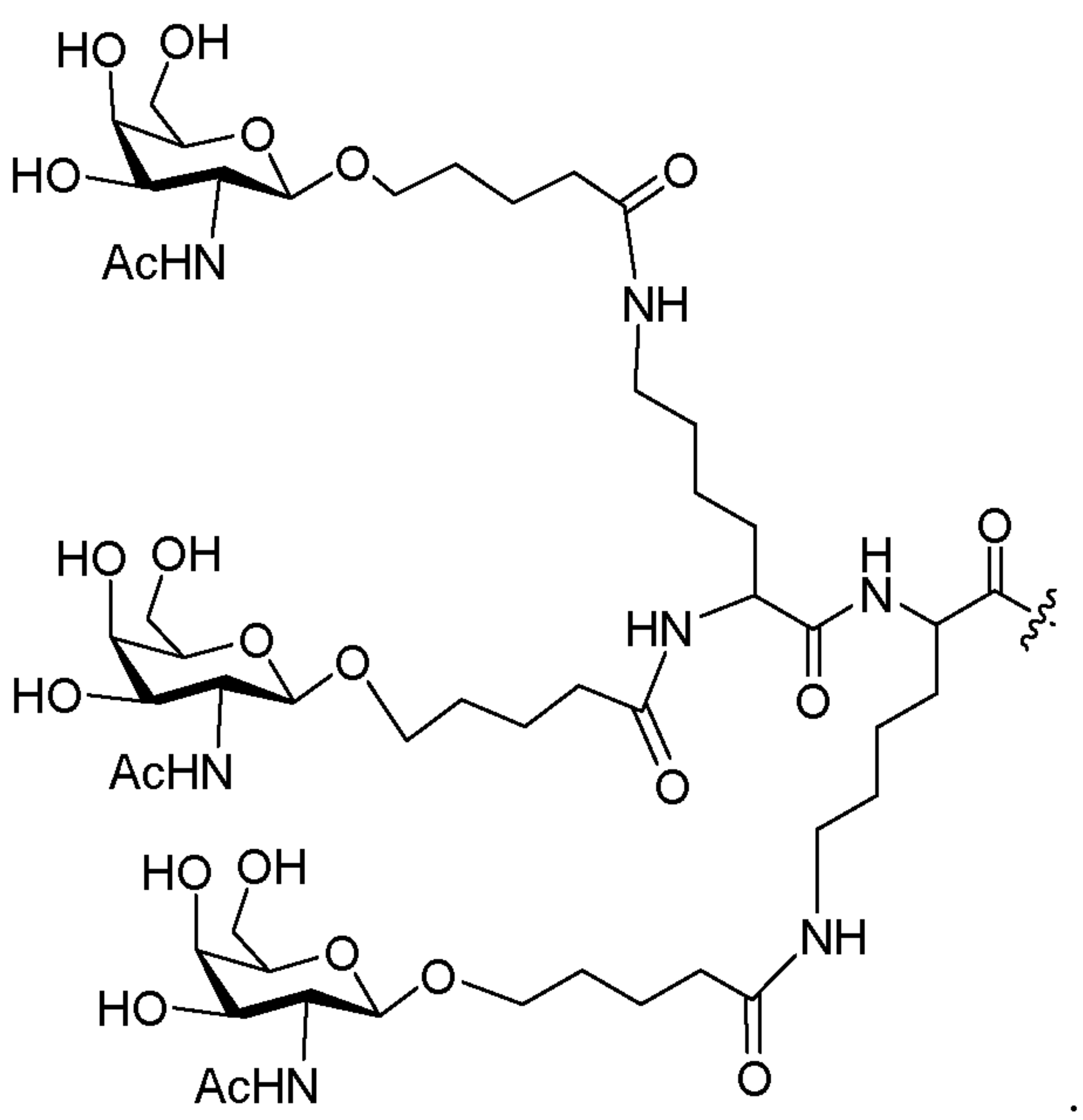


wherein each n is, independently, from 1 to 20.

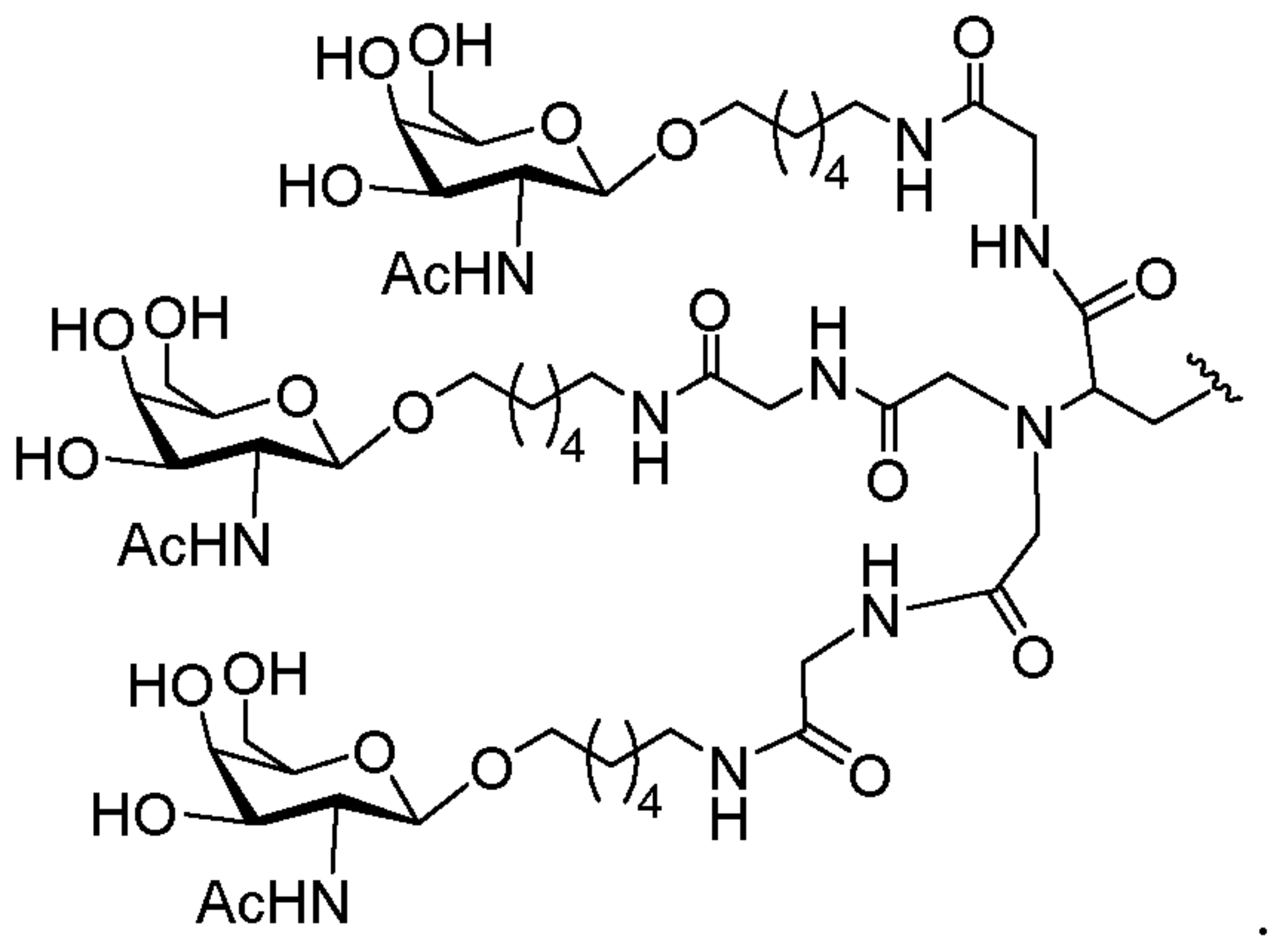
135. The compound of claim 131, wherein the cell-targeting moiety comprises:



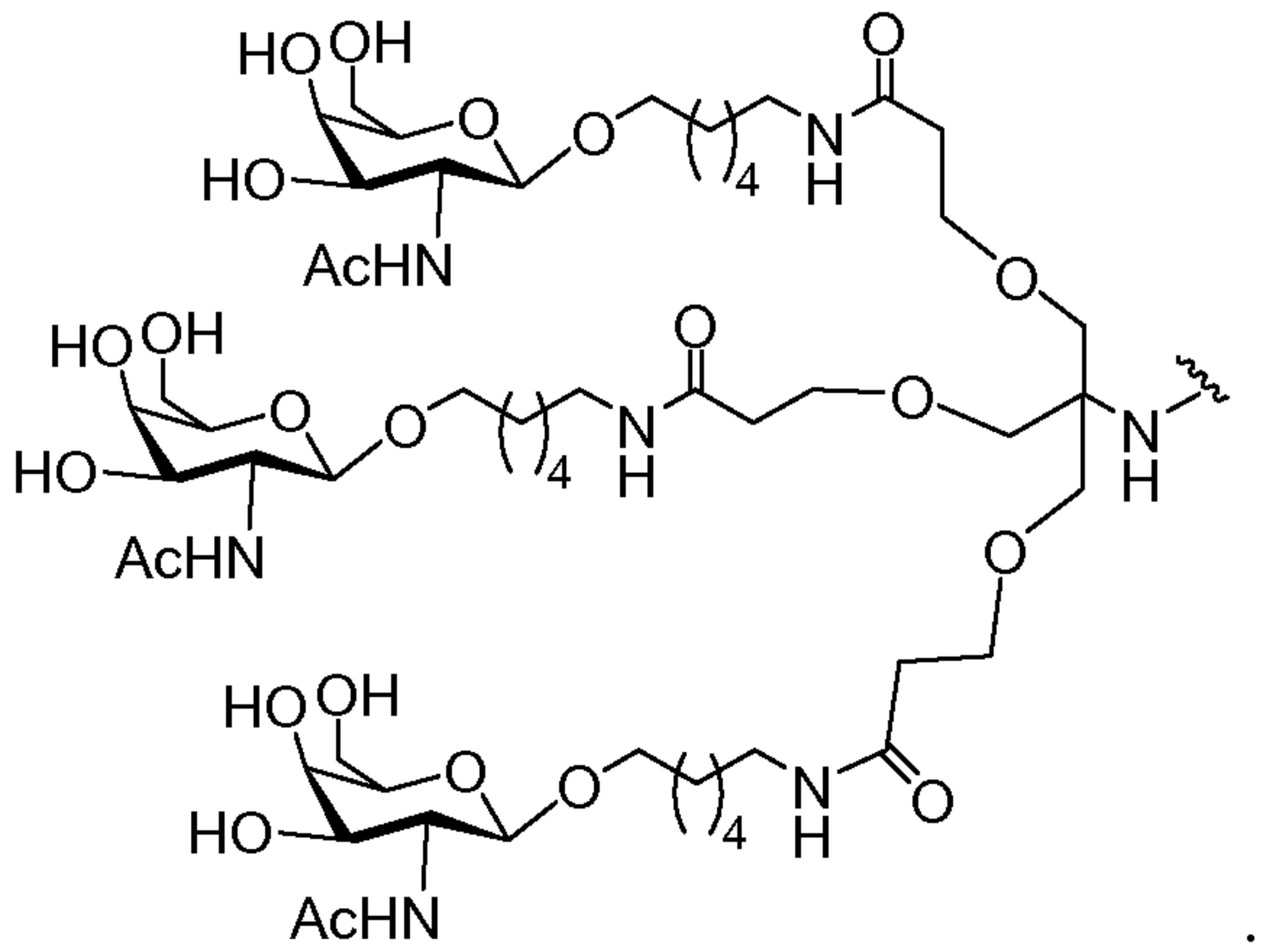
136. The compound of claim 131, wherein the cell-targeting moiety comprises:



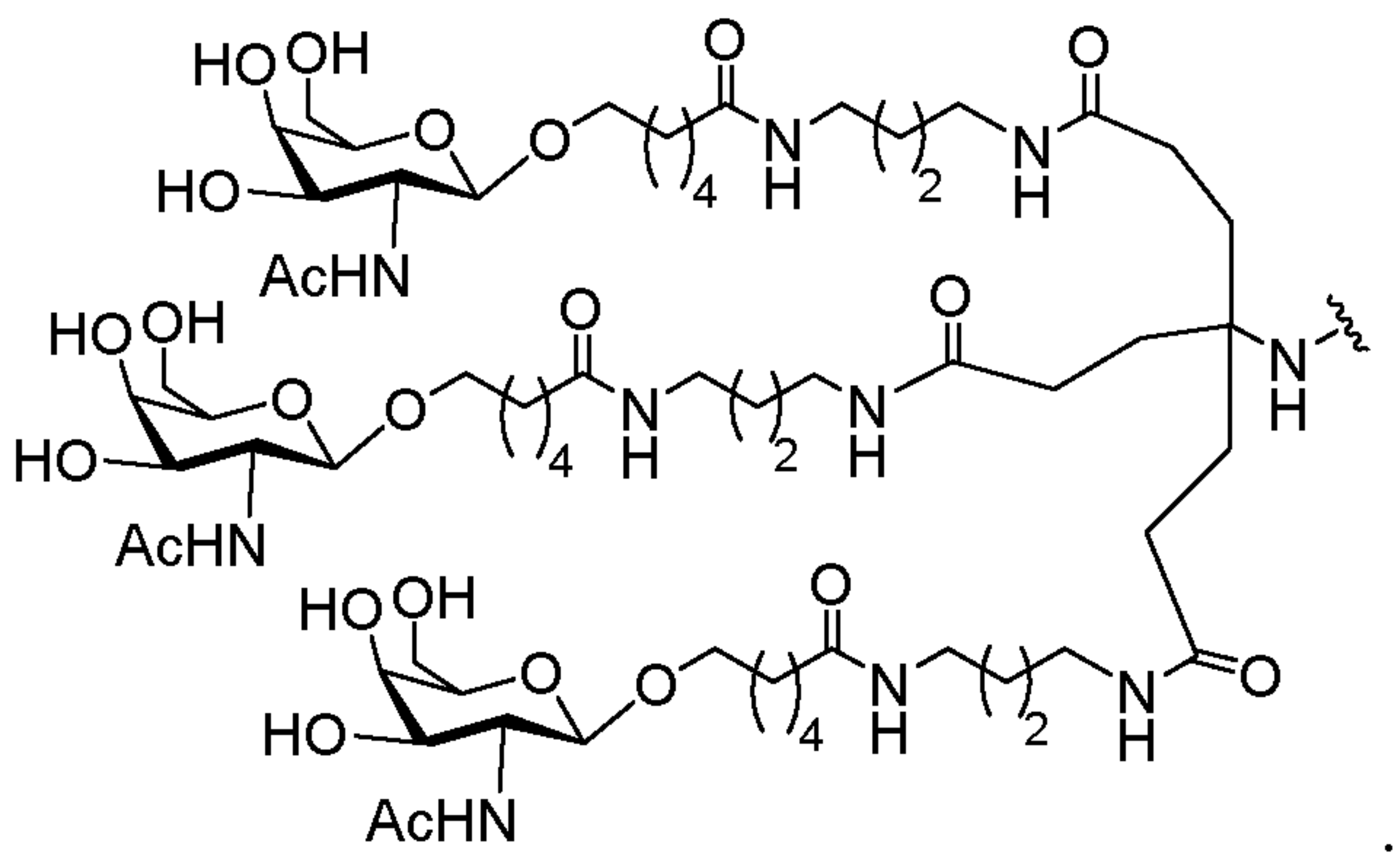
137. The compound of claim 131, wherein the cell-targeting moiety comprises:



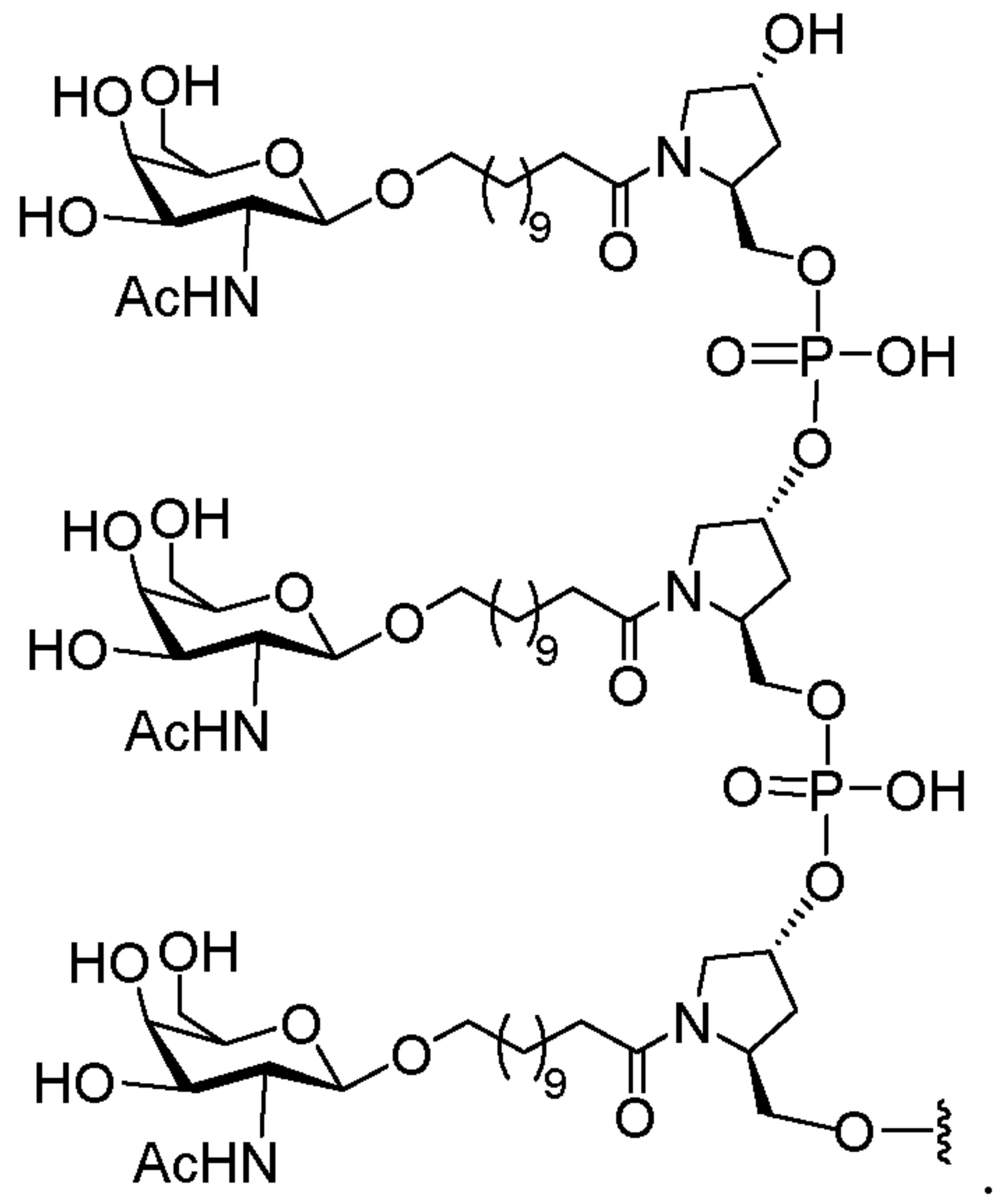
138. The compound of claim 131, wherein the cell-targeting moiety comprises:



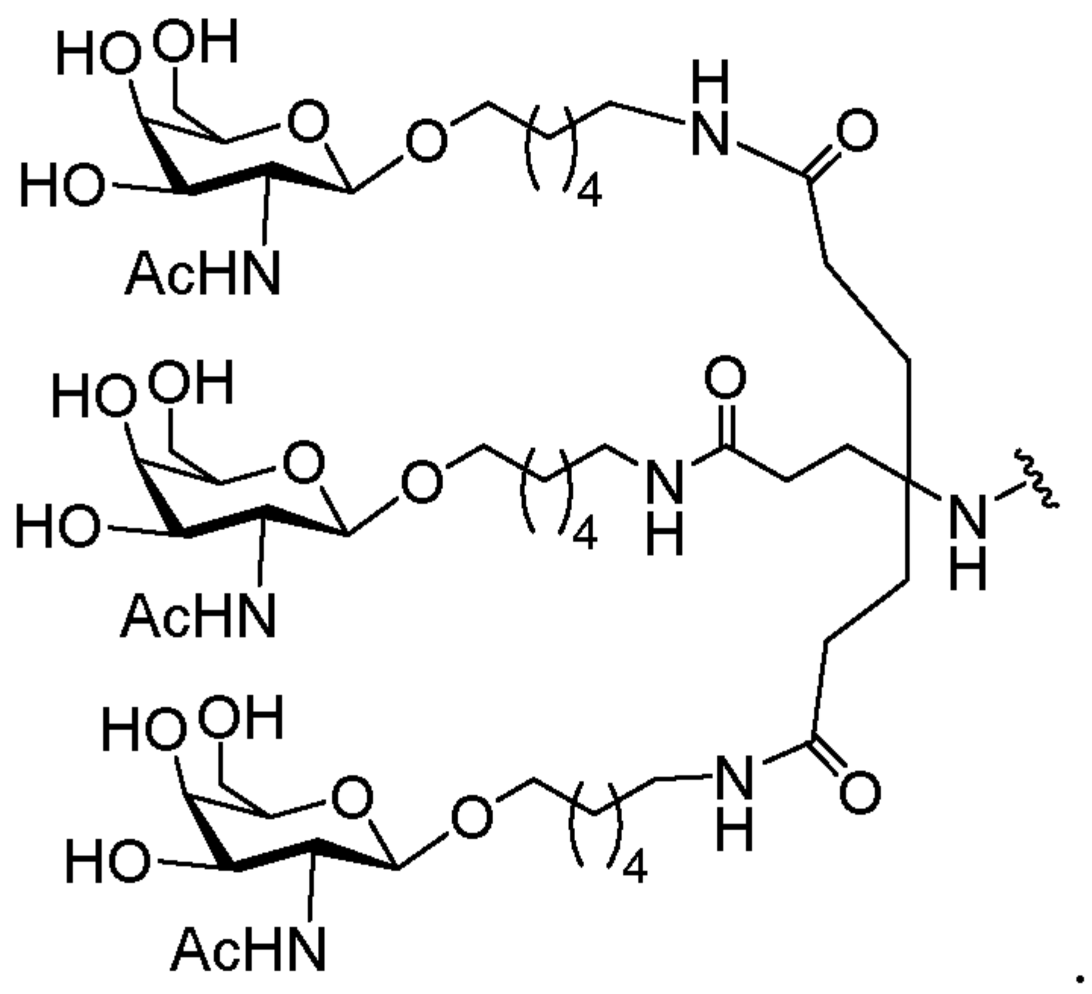
139. The compound of claim 131, wherein the cell-targeting moiety comprises:



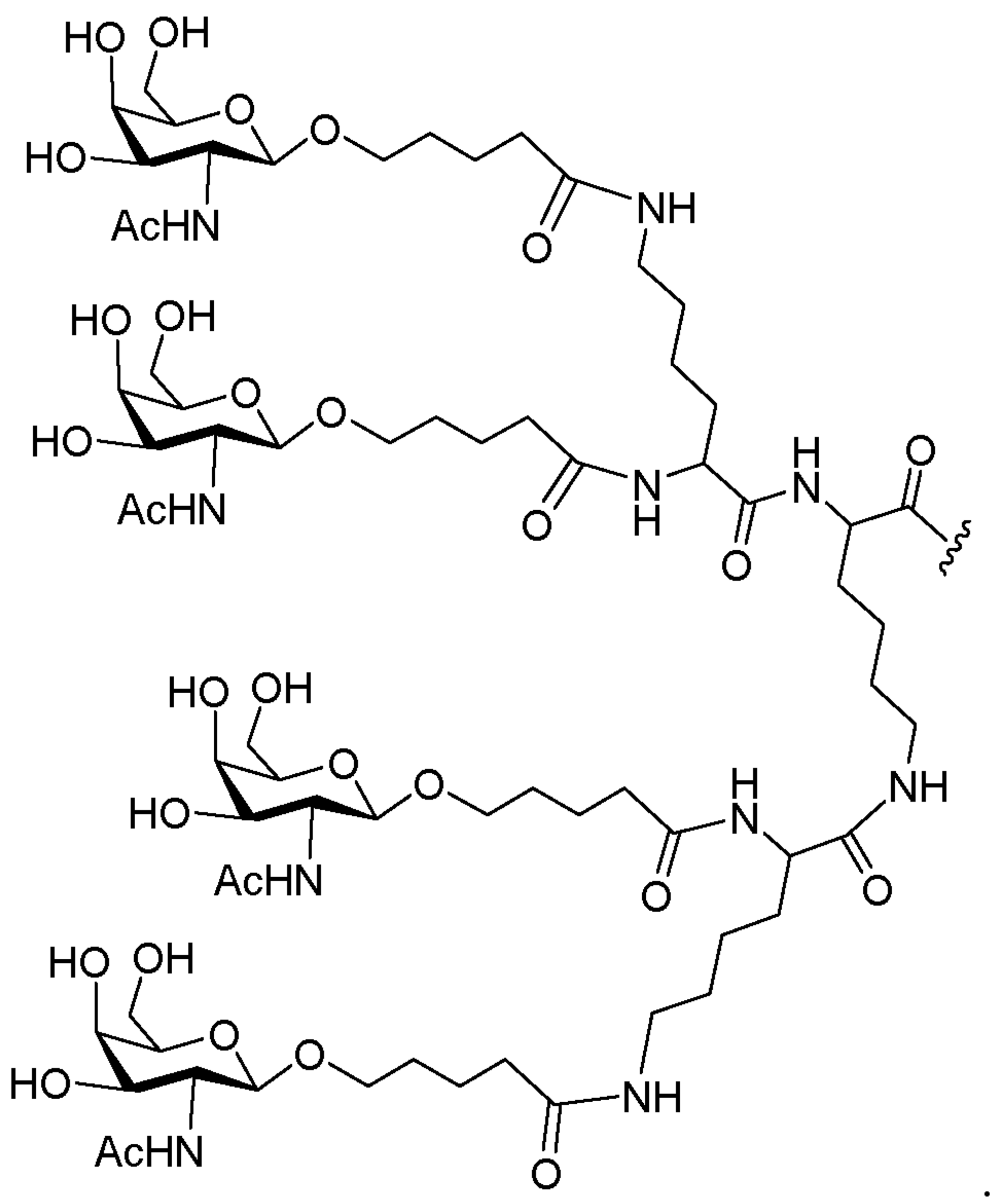
140. The compound of claim 131, wherein the cell-targeting moiety comprises:



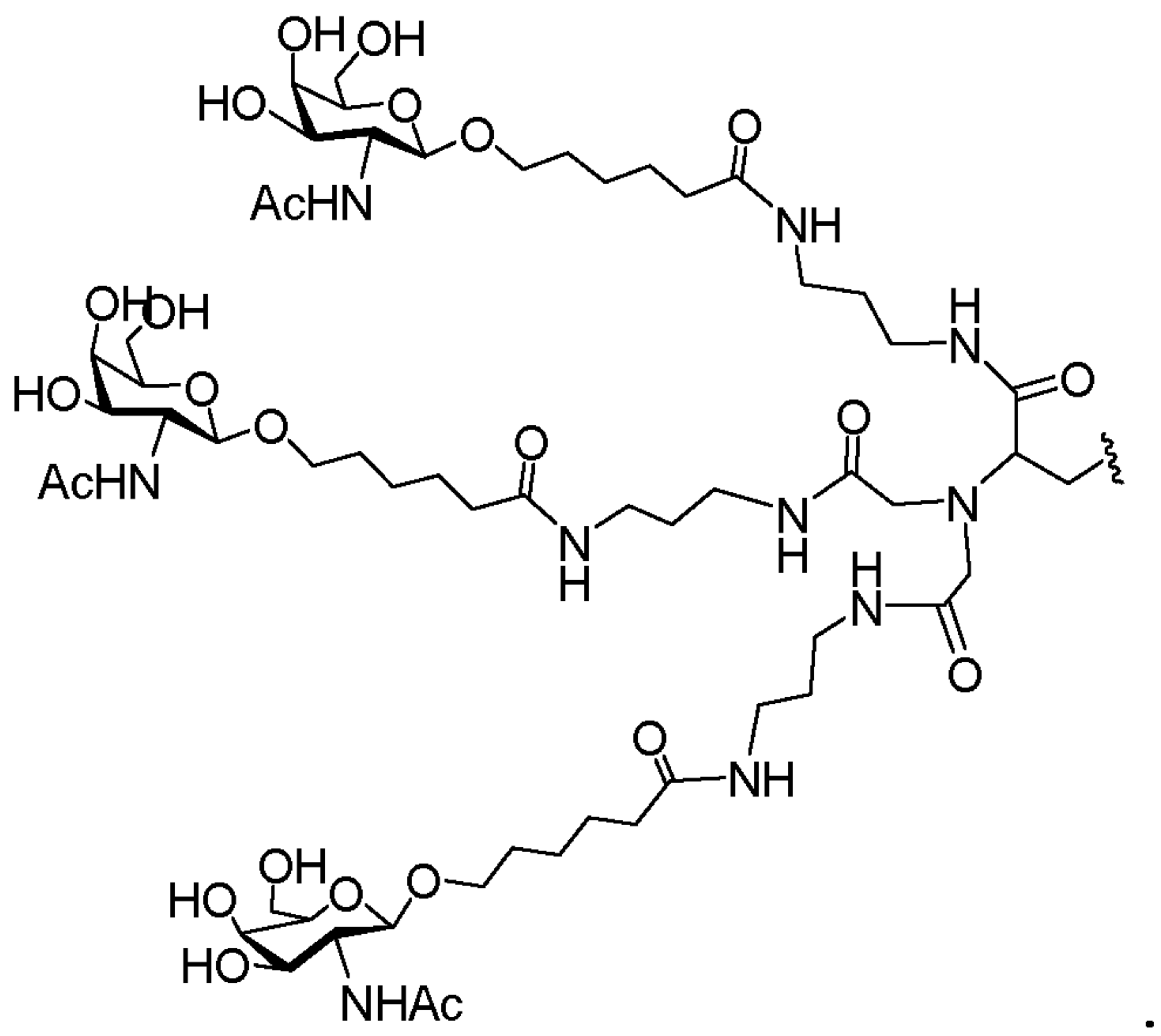
141. The compound of claim 131, wherein the cell-targeting moiety comprises:



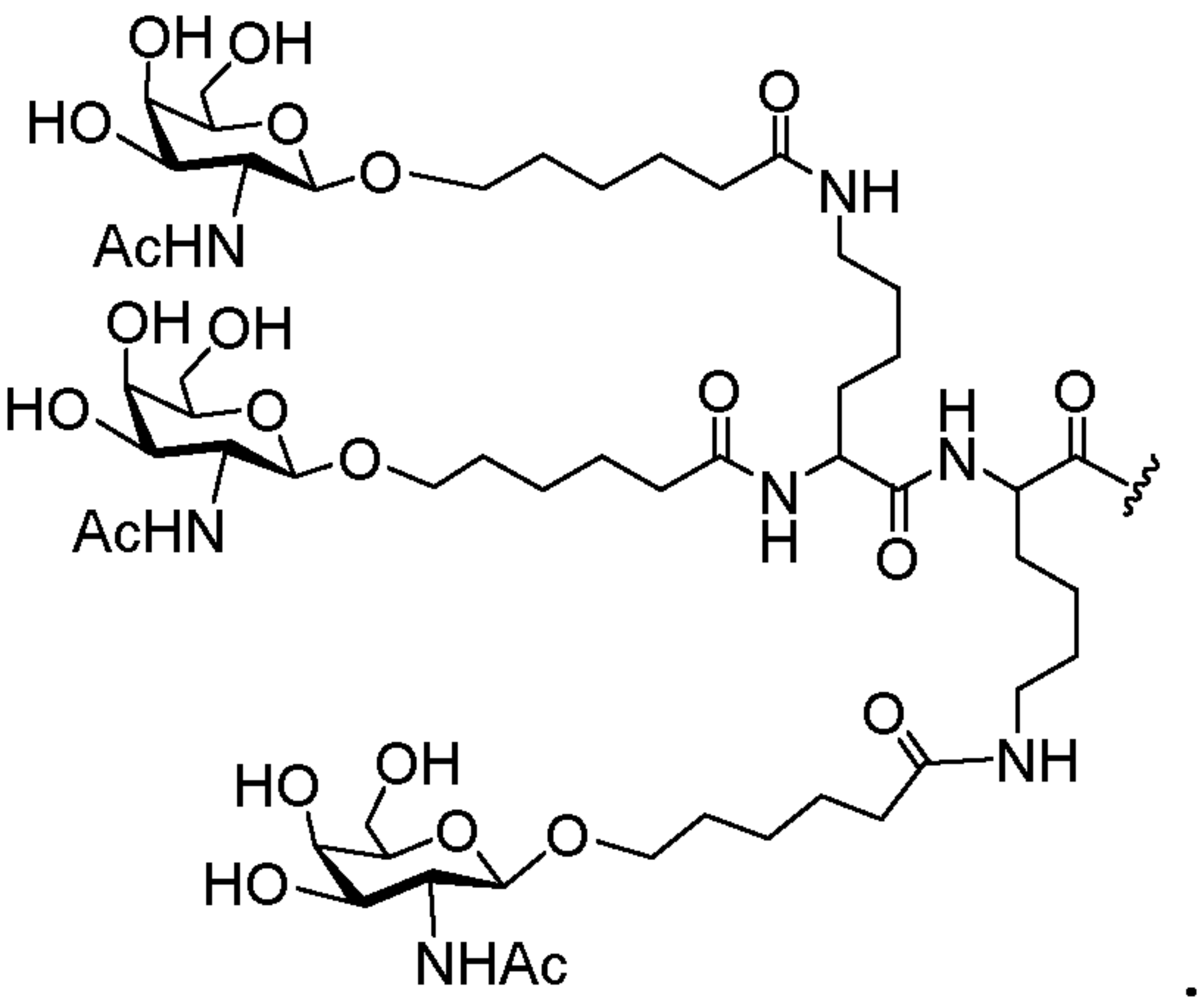
142. The compound of claim 131, wherein the cell-targeting moiety comprises:



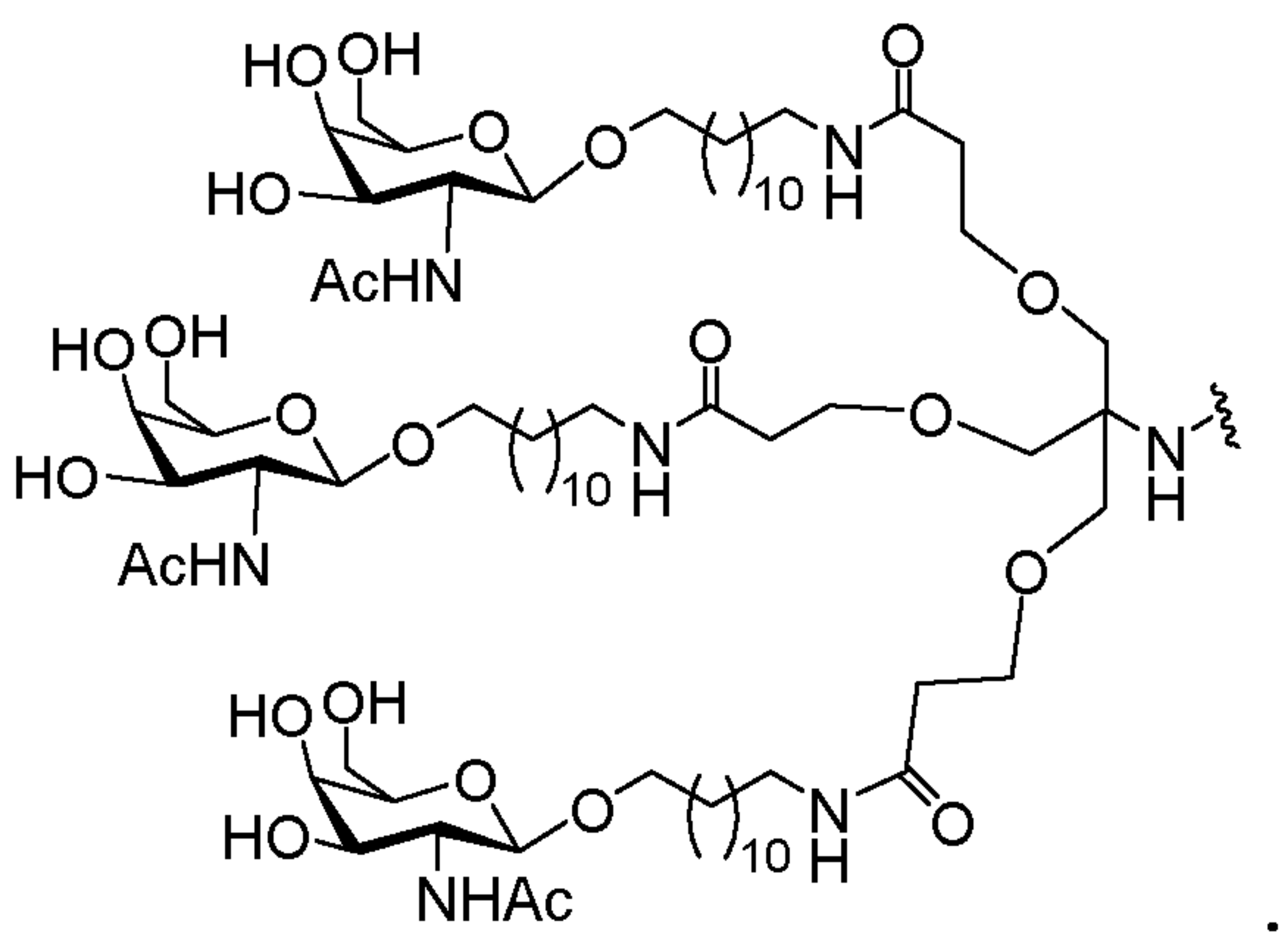
143. The compound of claim 131, wherein the cell-targeting moiety comprises:



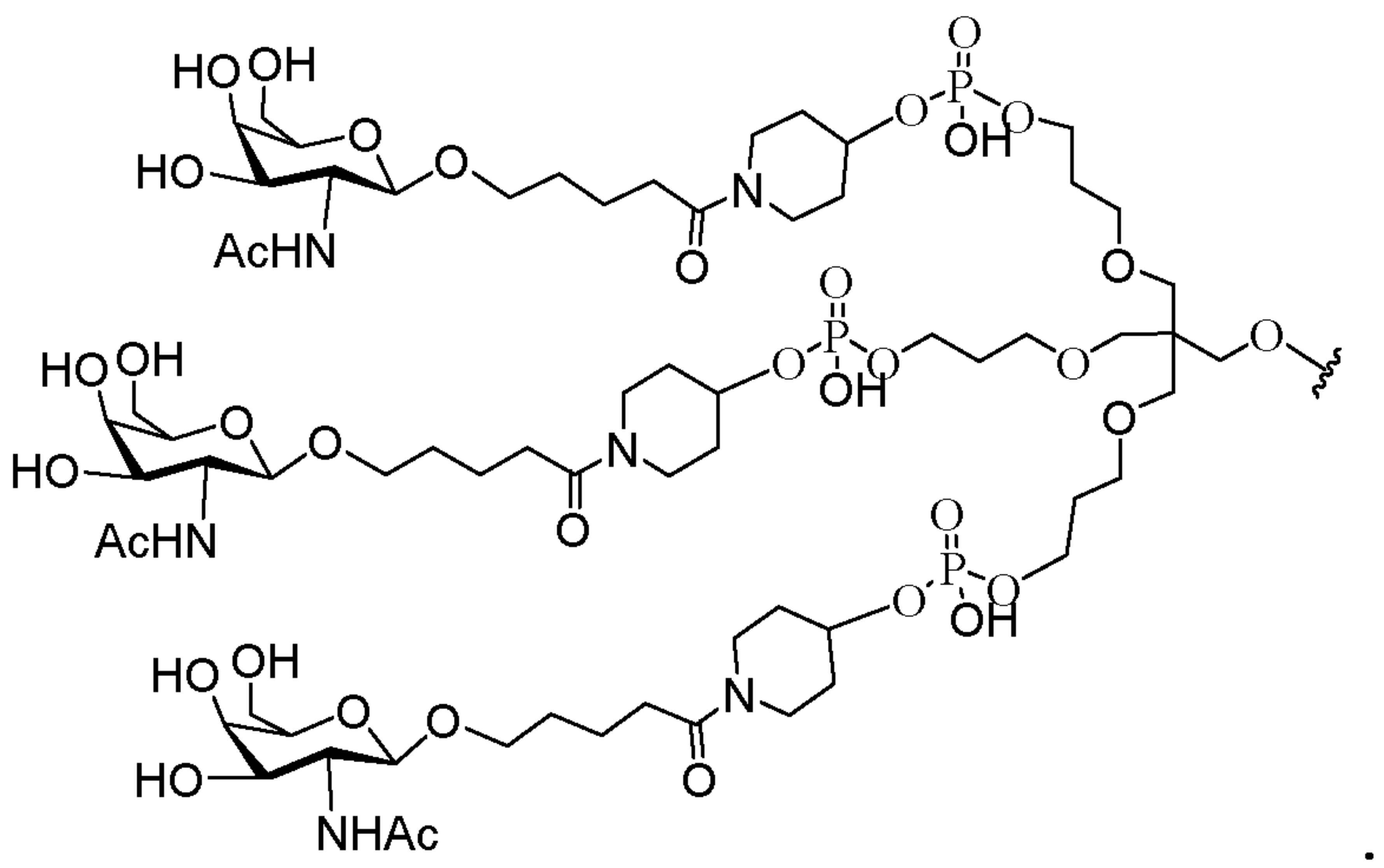
144. The compound of claim 131, wherein the cell-targeting moiety comprises:



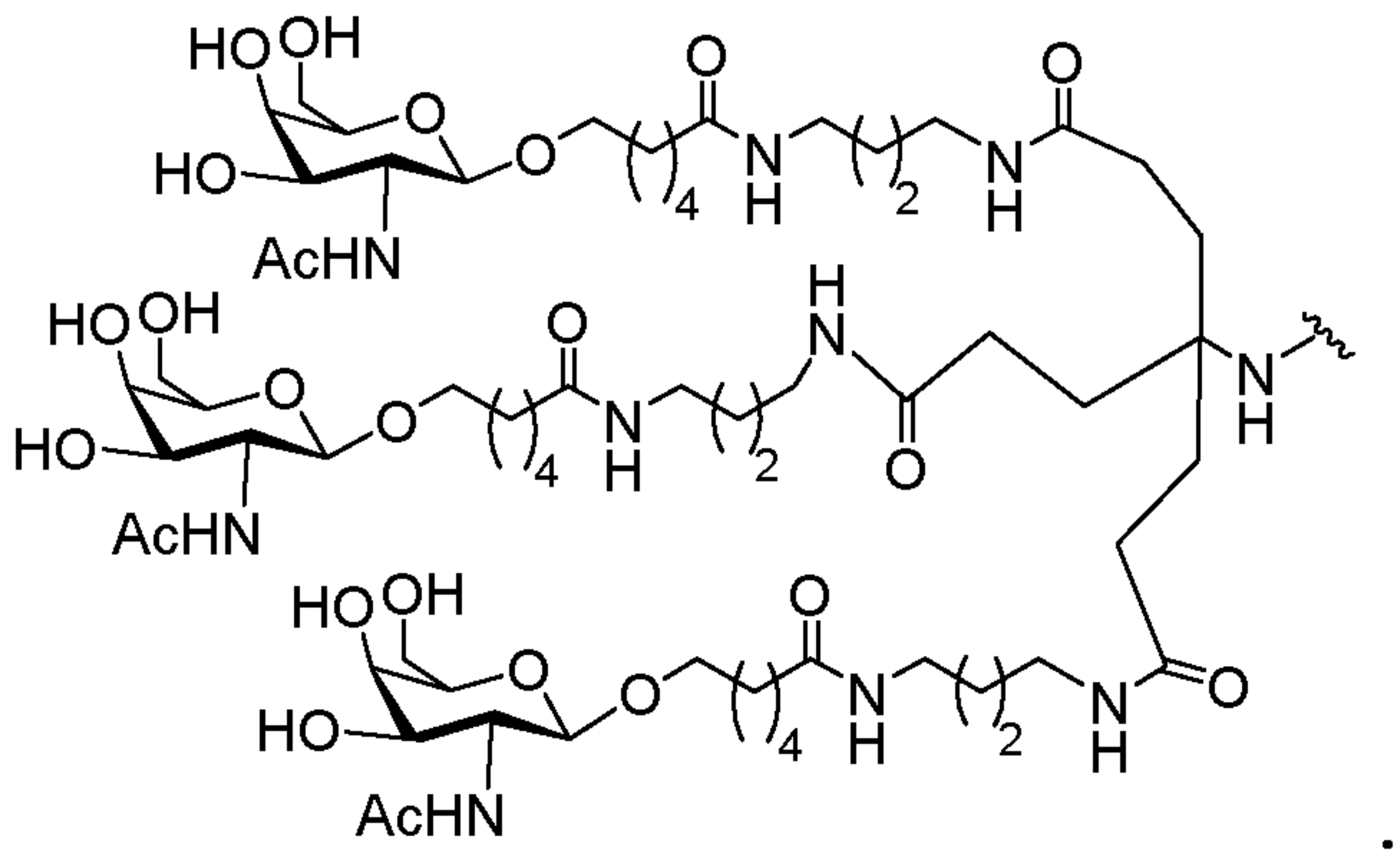
145. The compound of claim 131, wherein the cell-targeting moiety comprises:



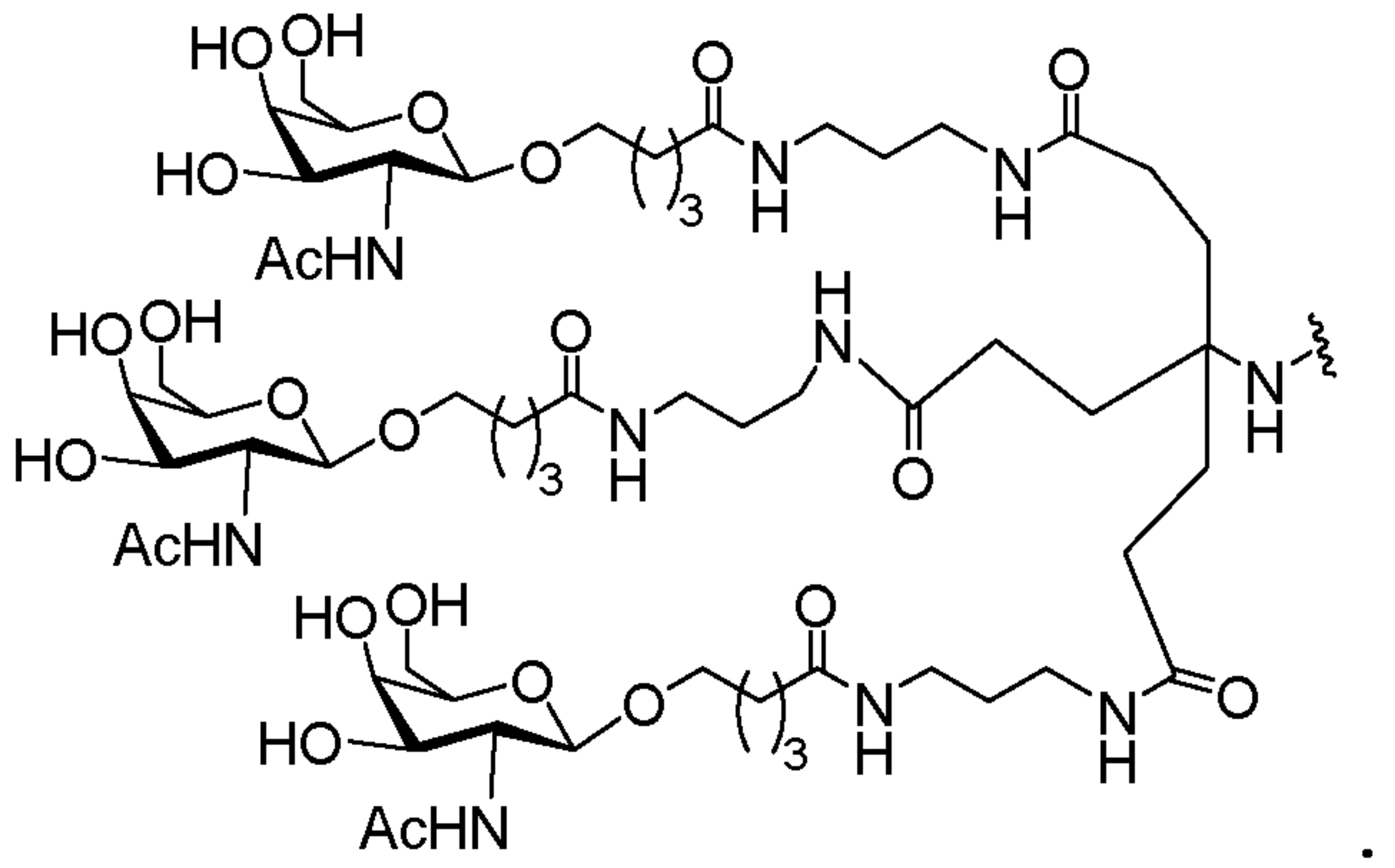
The compound of claim 131, wherein the cell-targeting moiety comprises:



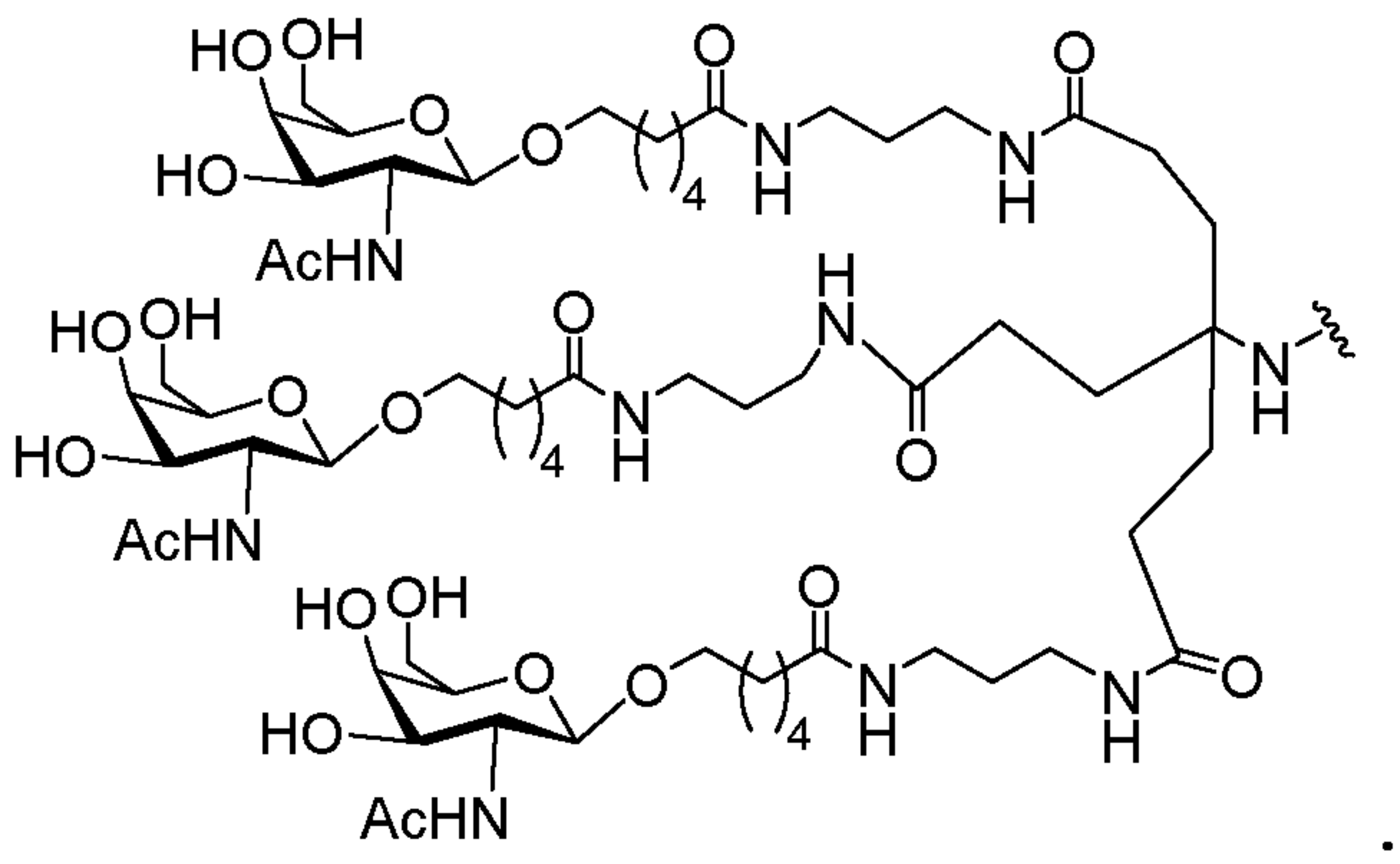
146. The compound of claim 131, wherein the cell-targeting moiety has the following structure:



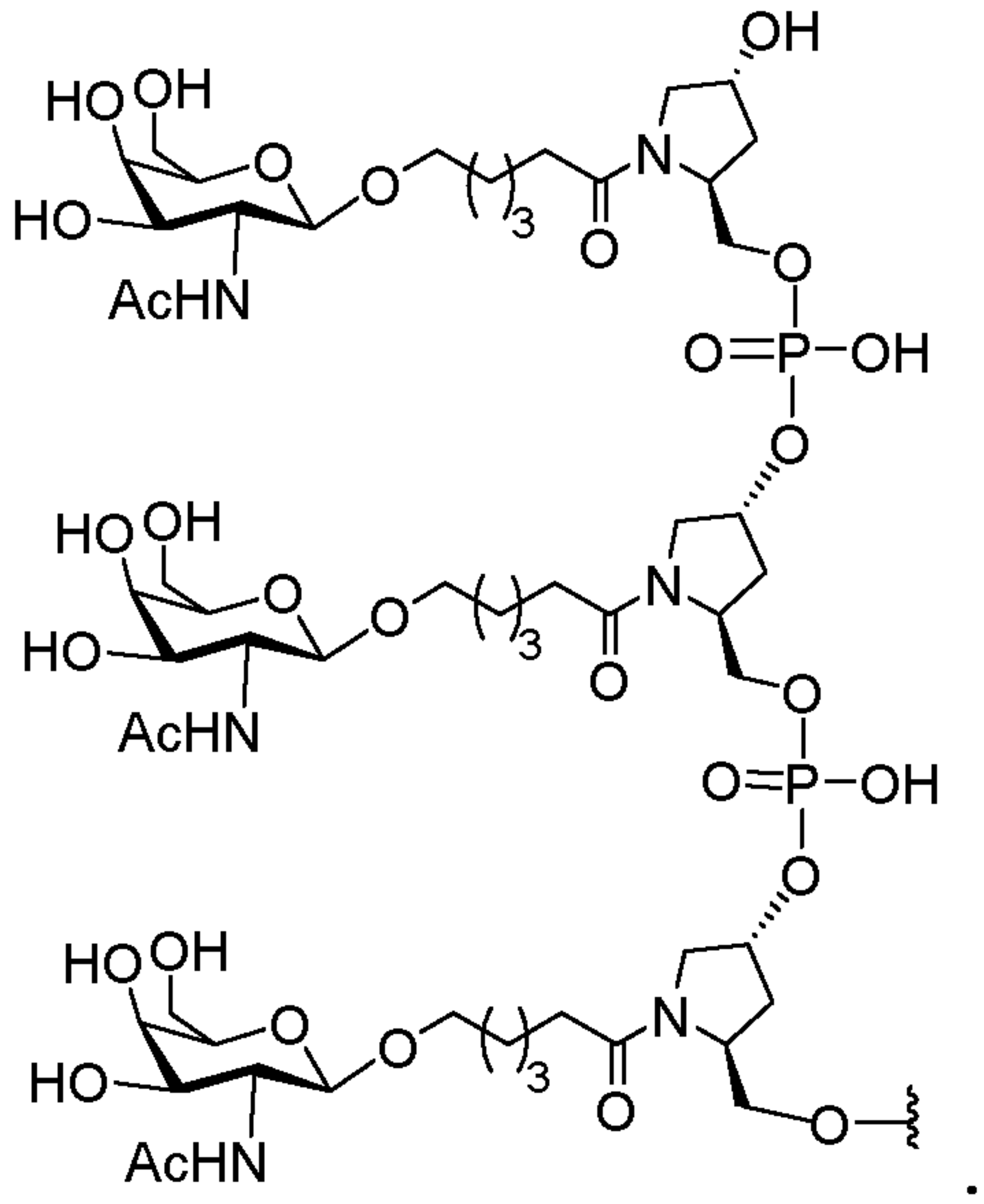
147. The compound of claim 131, wherein the cell-targeting moiety has the following structure:



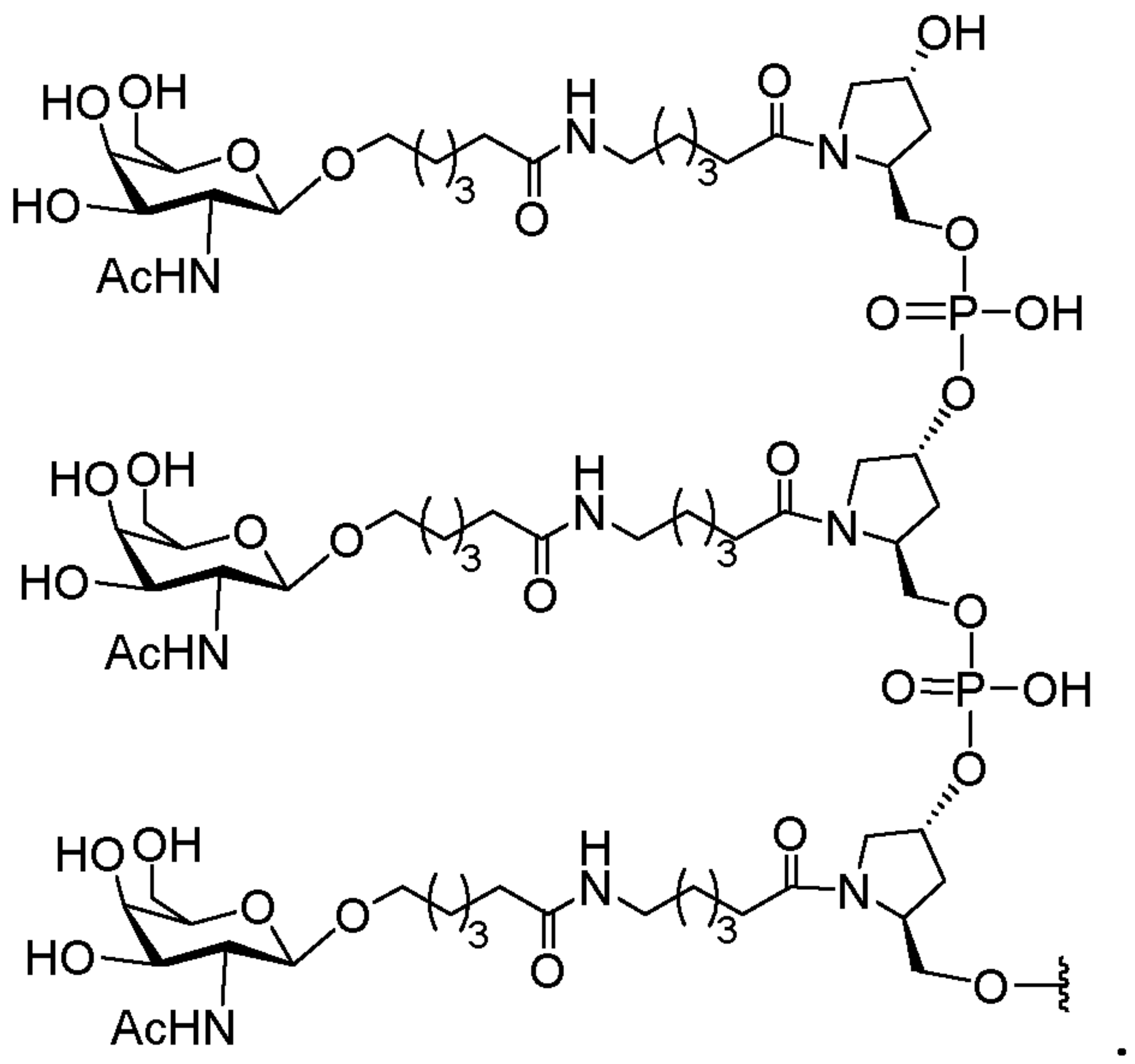
148. The compound of claim 131, wherein the cell-targeting moiety has the following structure:



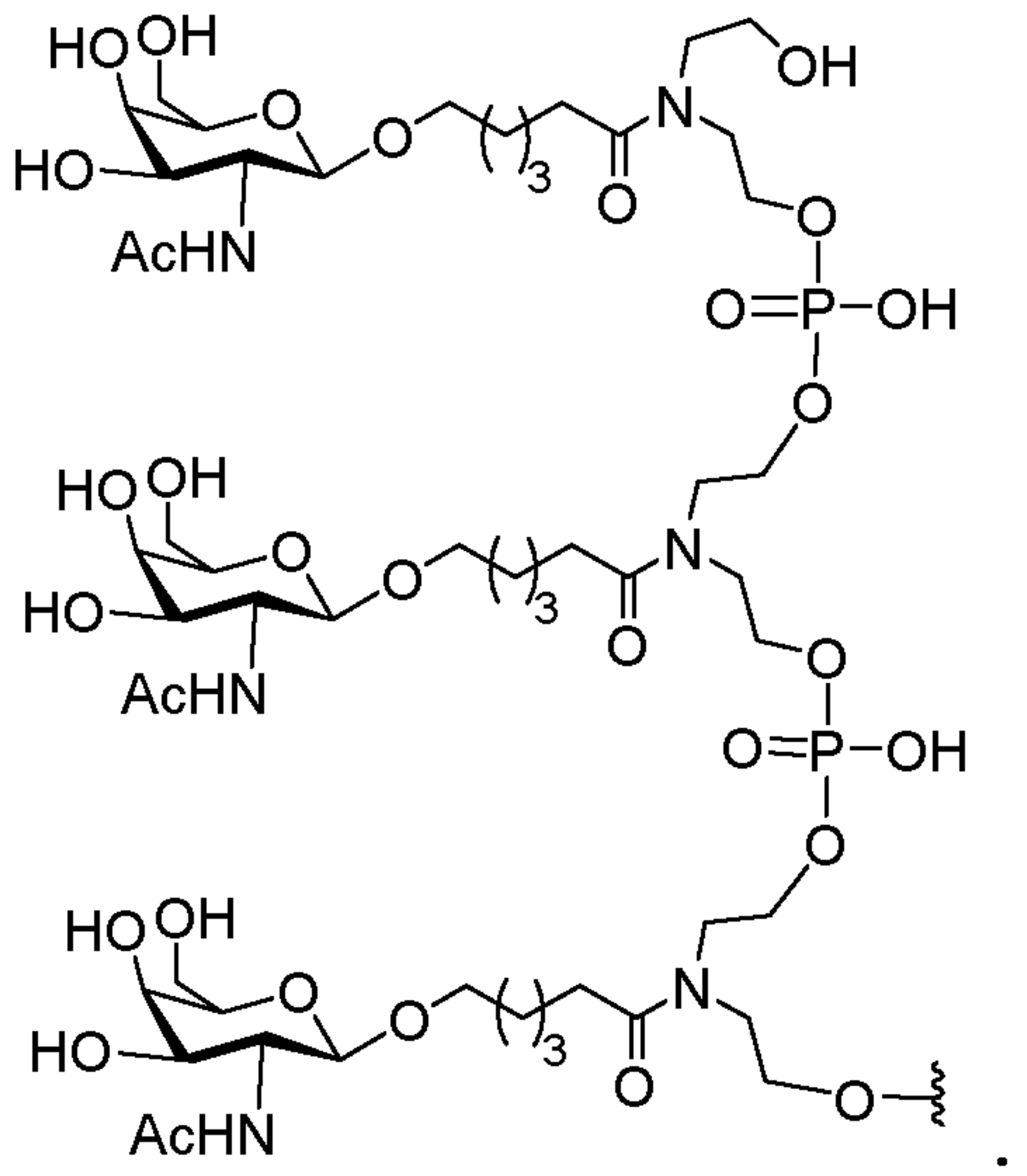
149. The compound of claim 131, wherein the cell-targeting moiety comprises:



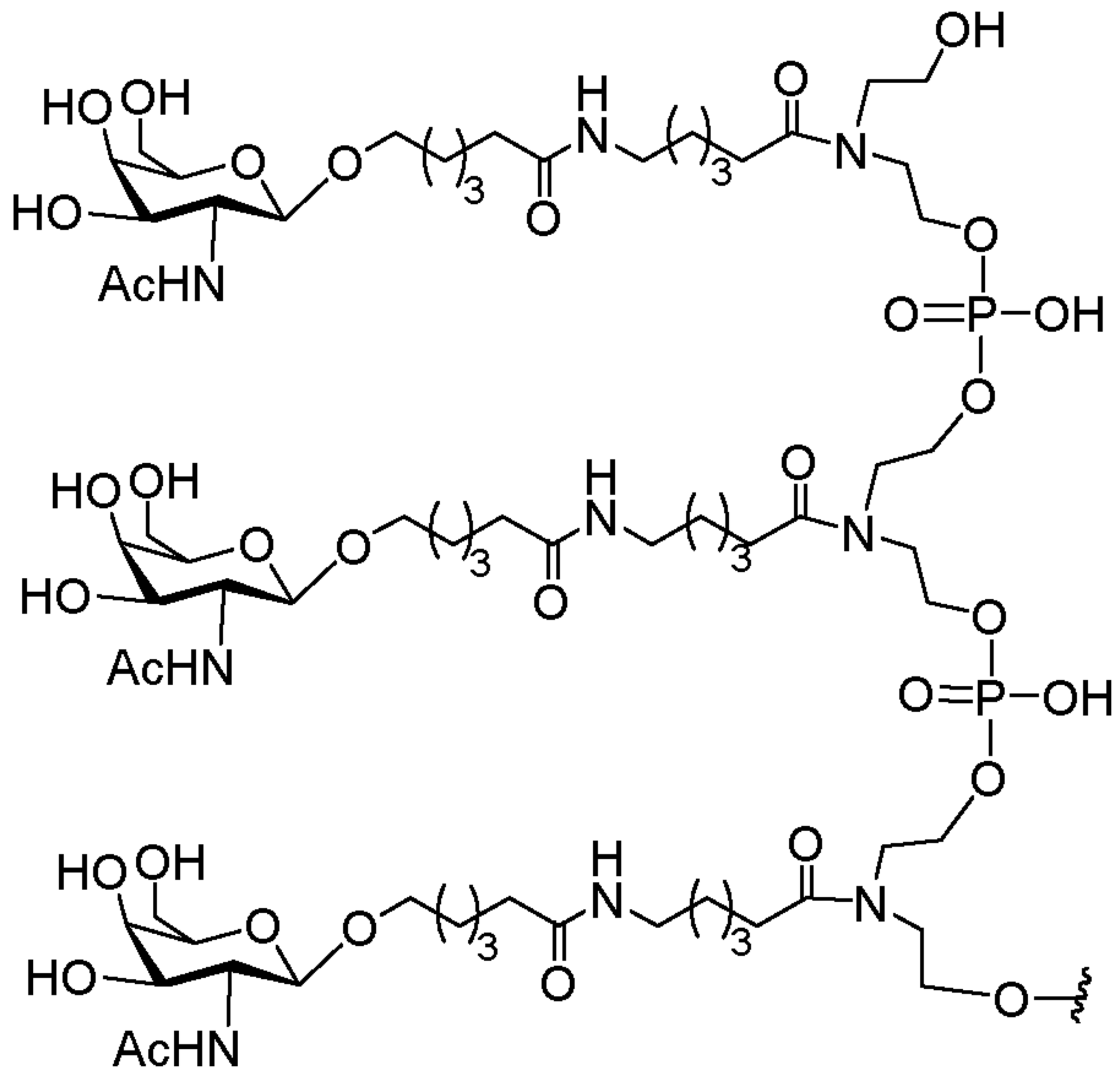
150. The compound of claim 131, wherein the cell-targeting moiety comprises:



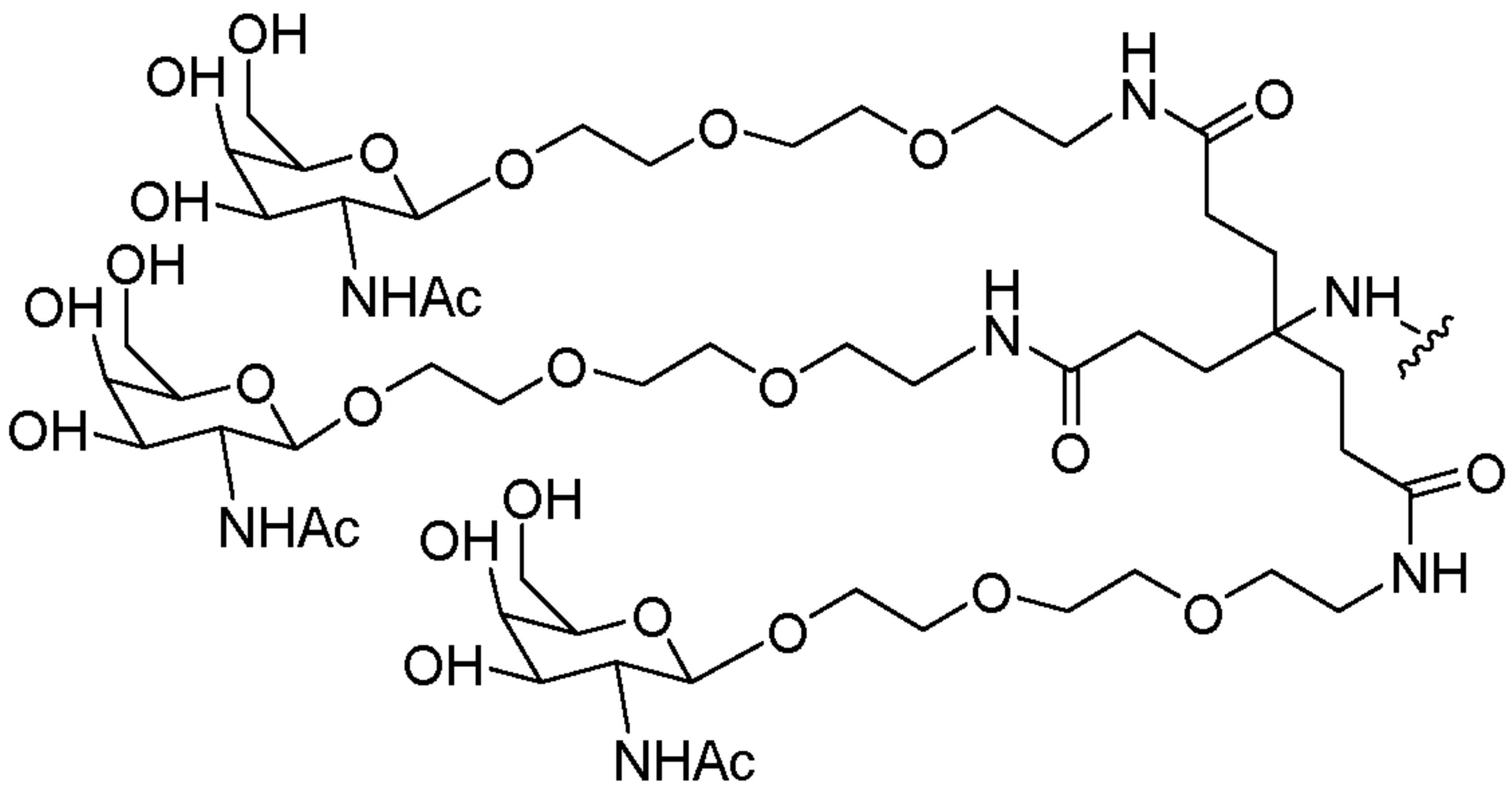
151. The compound of claim 131, wherein the cell-targeting moiety comprises:



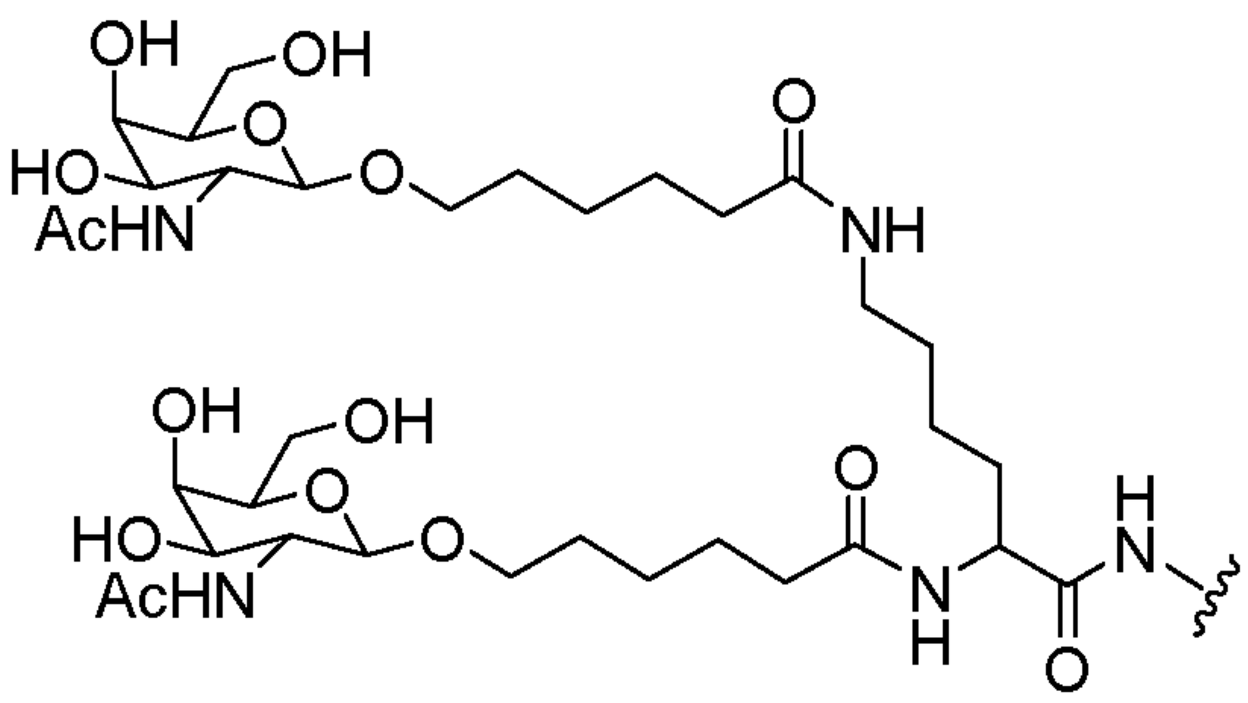
152. The compound of claim 131, wherein the cell-targeting moiety comprises:



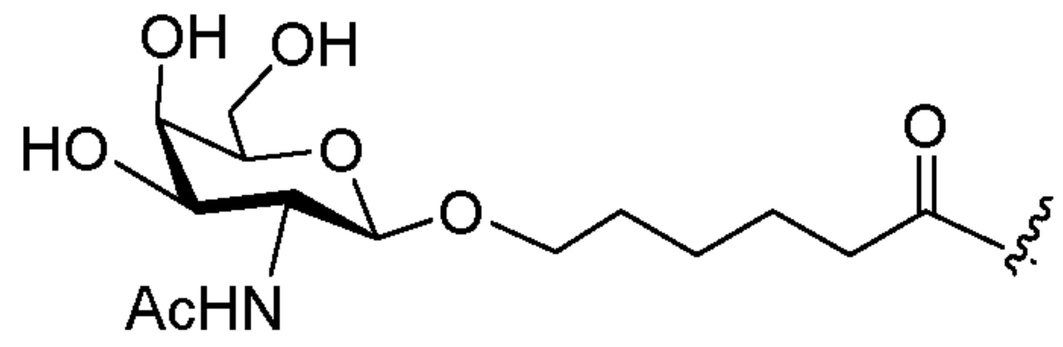
153. The compound of claim 131, wherein the cell-targeting moiety comprises:



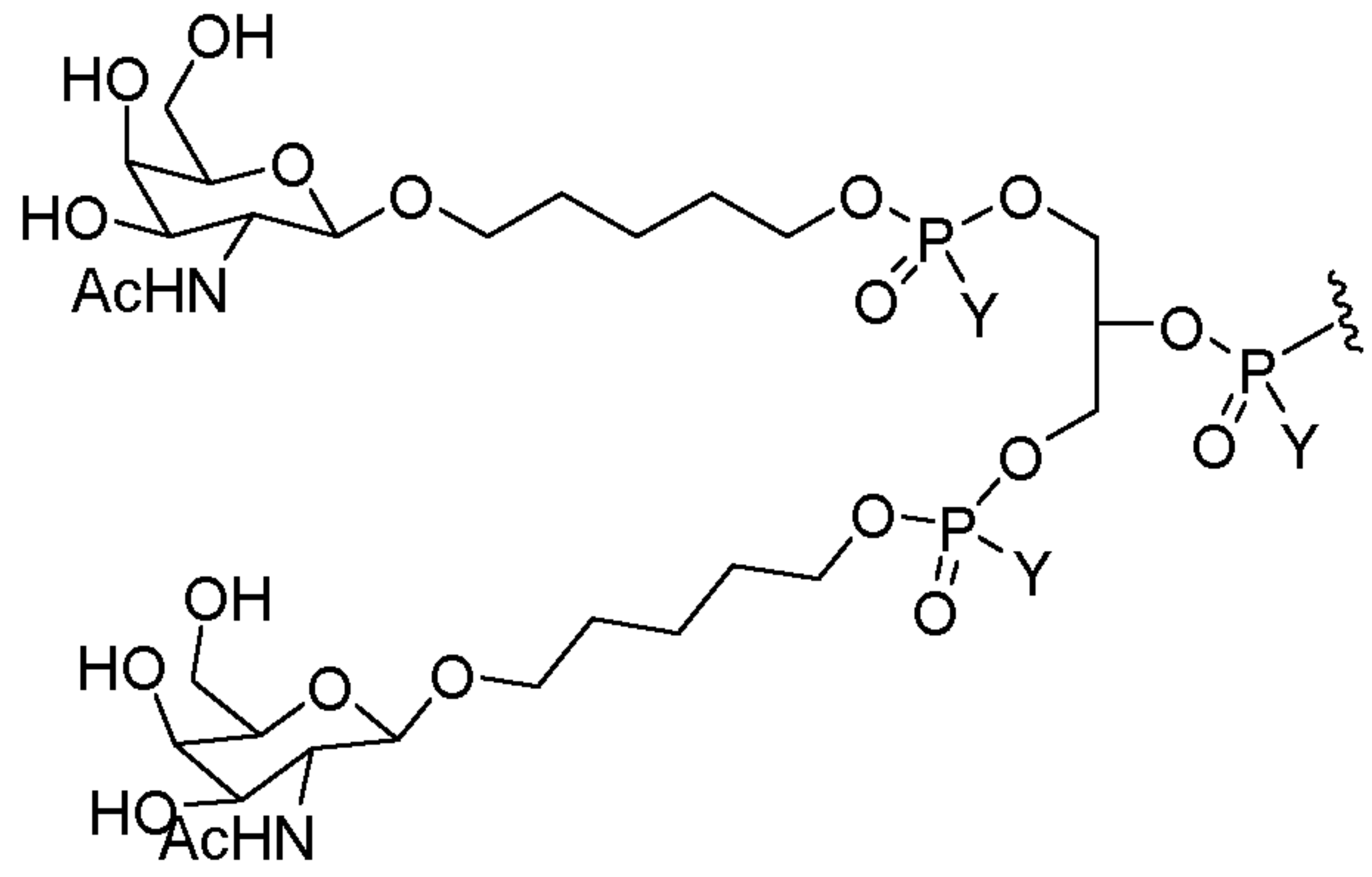
154. The compound of claim 131, wherein the cell-targeting moiety comprises:



155. The compound of claim 131, wherein the cell-targeting moiety comprises:

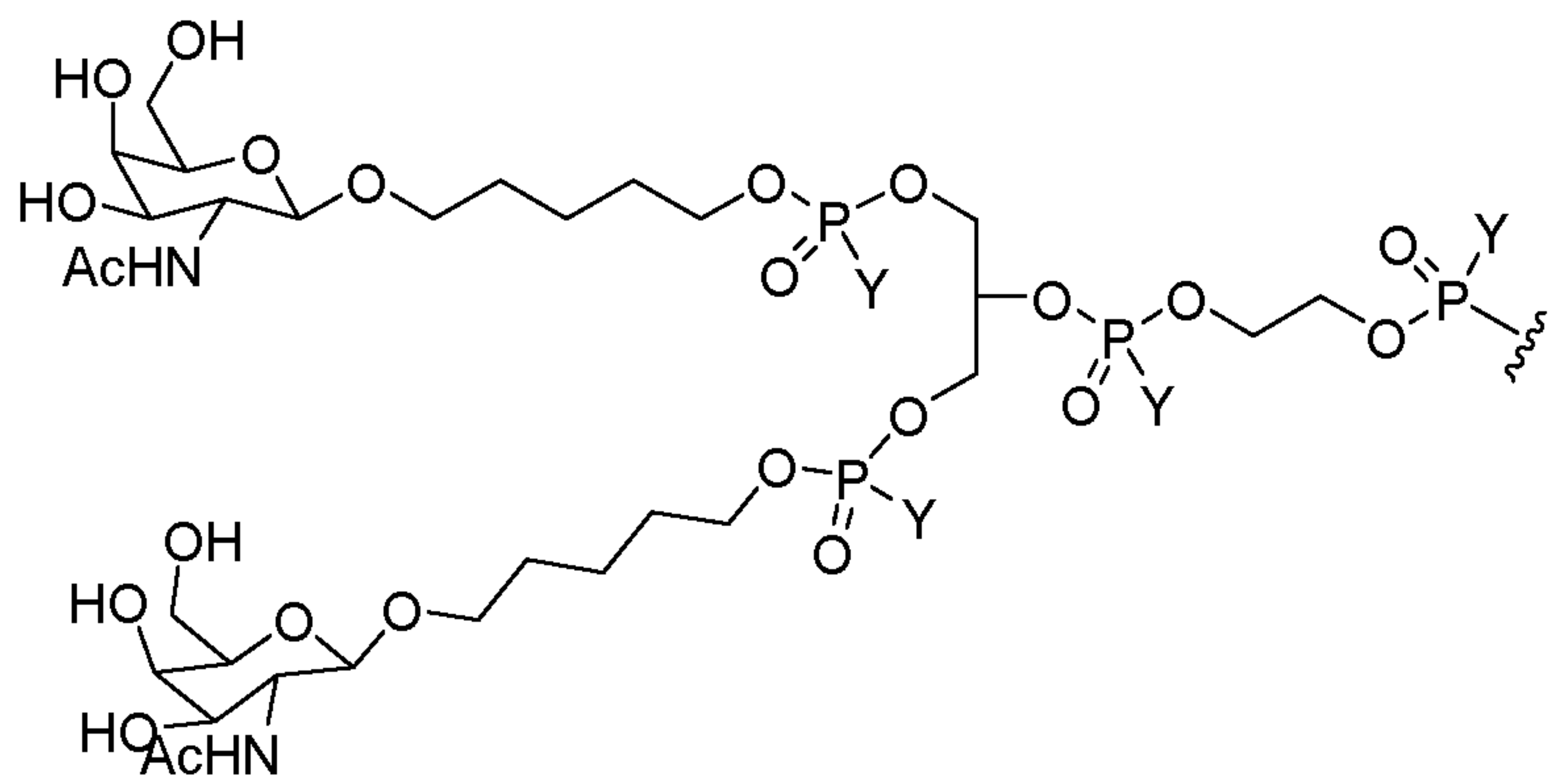


156. The compound of claim 131, wherein the cell-targeting moiety comprises:



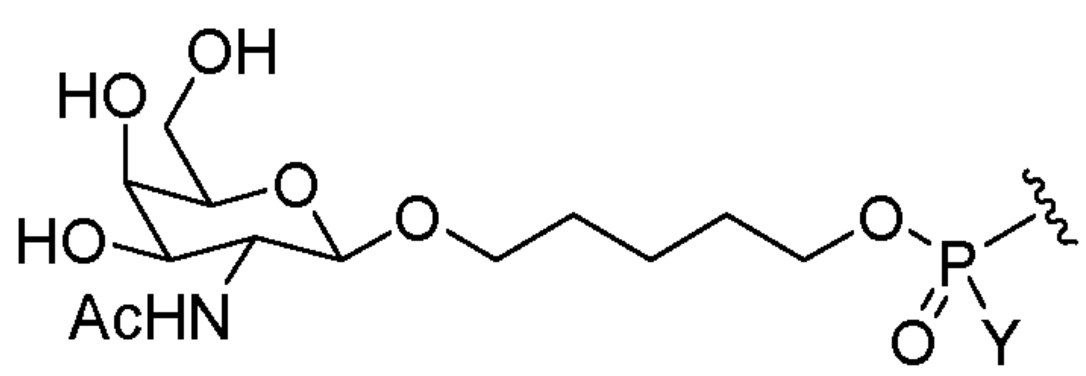
wherein each Y is selected from O, S, a substituted or unsubstituted C_1-C_{10} alkyl, amino, substituted amino, azido, alkenyl or alkynyl.

157. The compound of claim 131, wherein the conjugate group comprises:



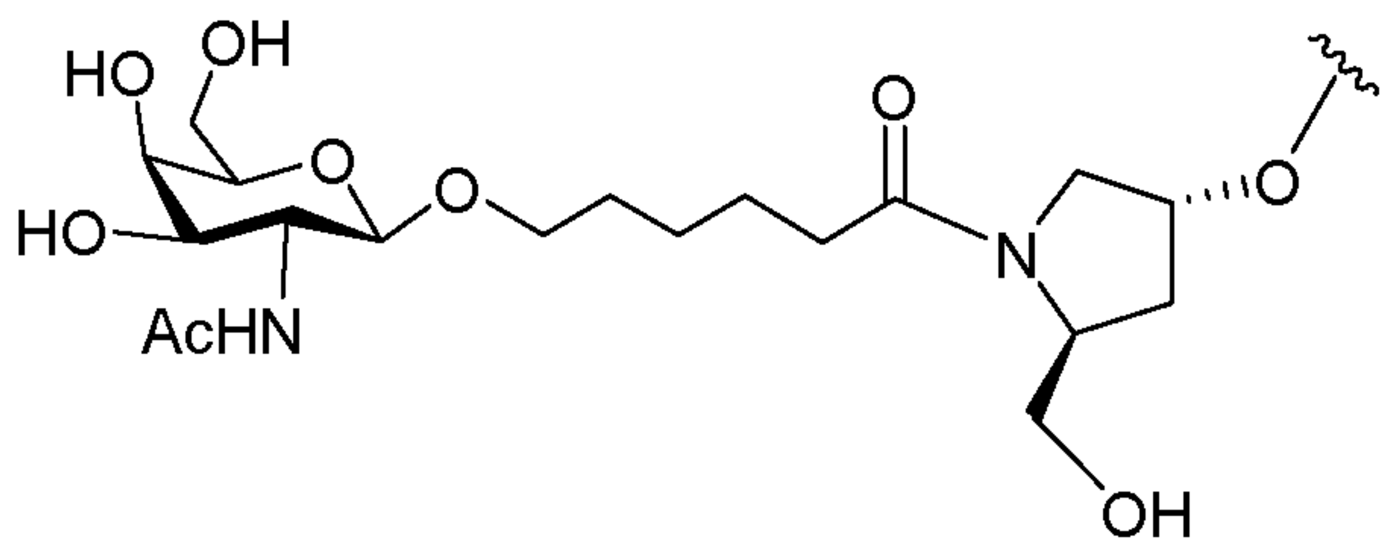
wherein each Y is selected from O, S, a substituted or unsubstituted C_1-C_{10} alkyl, amino, substituted amino, azido, alkenyl or alkynyl.

158. The compound of claim 131, wherein the cell-targeting moiety has the following structure:

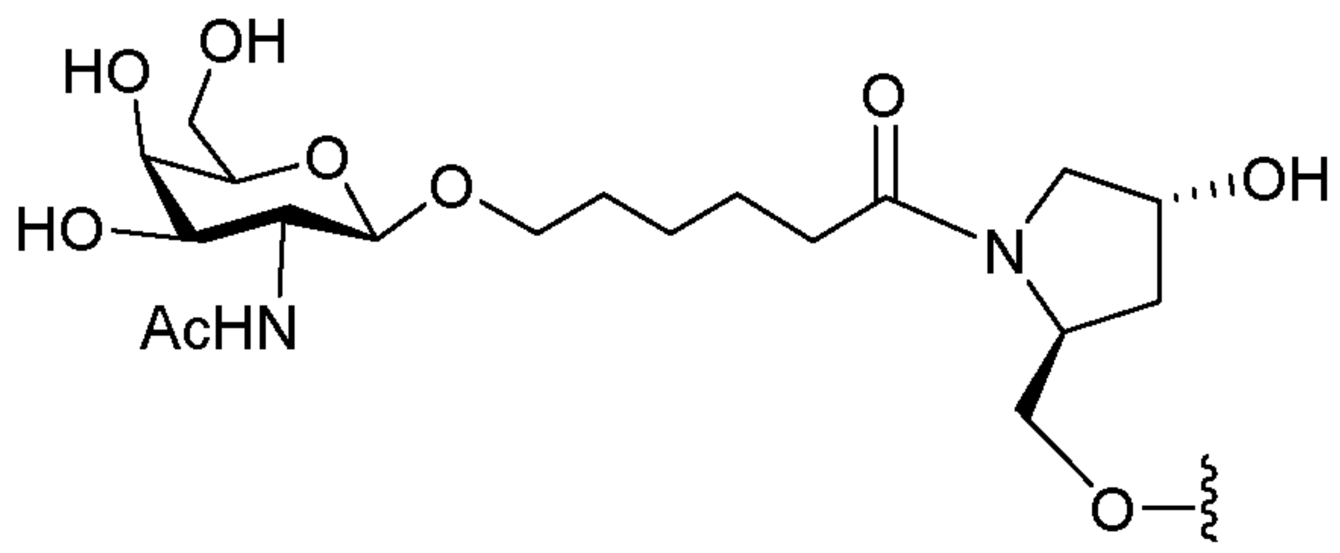


wherein each Y is selected from O, S, a substituted or unsubstituted C_1-C_{10} alkyl, amino, substituted amino, azido, alkenyl or alkynyl.

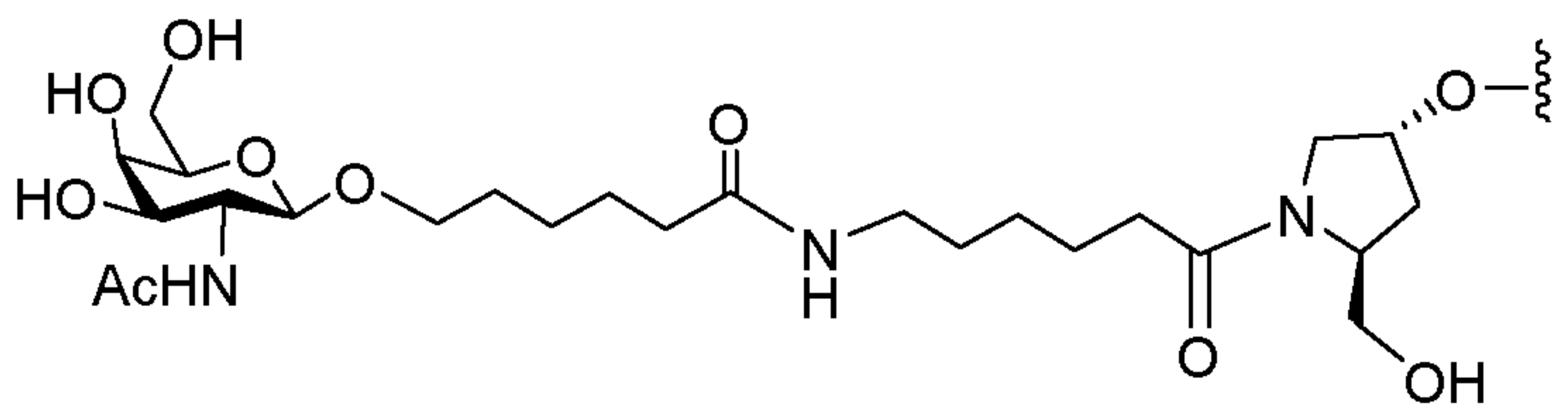
159. The compound of any preceding claim, wherein the conjugate group comprises:



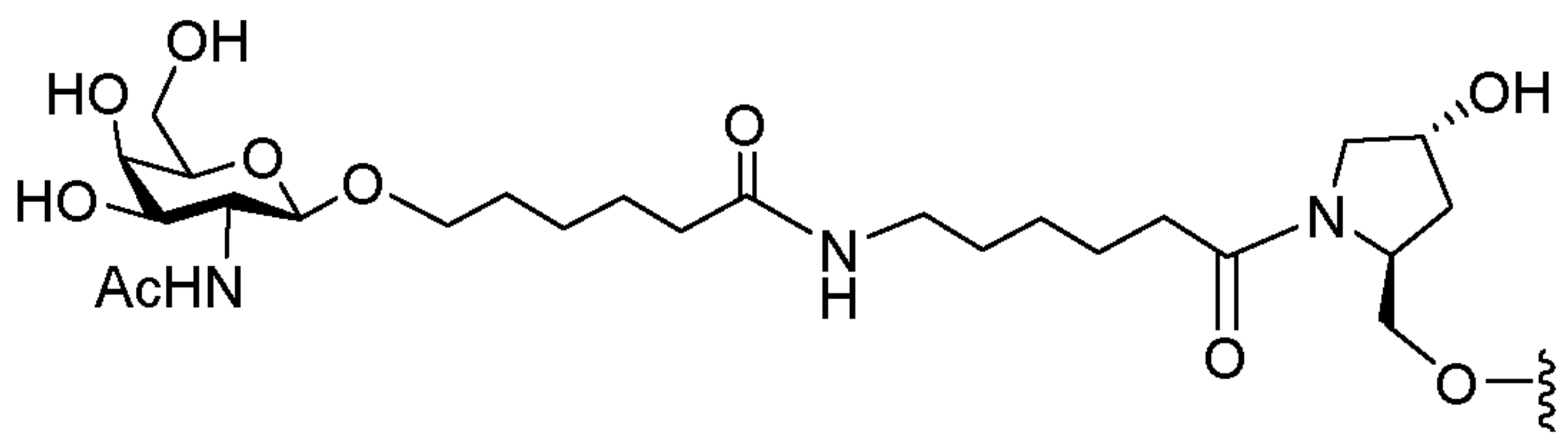
160. The compound of any preceding claim, wherein the conjugate group comprises:



161. The compound of any preceding claim, wherein the conjugate group comprises:



162. The compound of any preceding claim, wherein the conjugate group comprises:



163. The compound of any preceding claim, wherein the conjugate group comprises a cleavable moiety selected from among: a phosphodiester, an amide, or an ester.

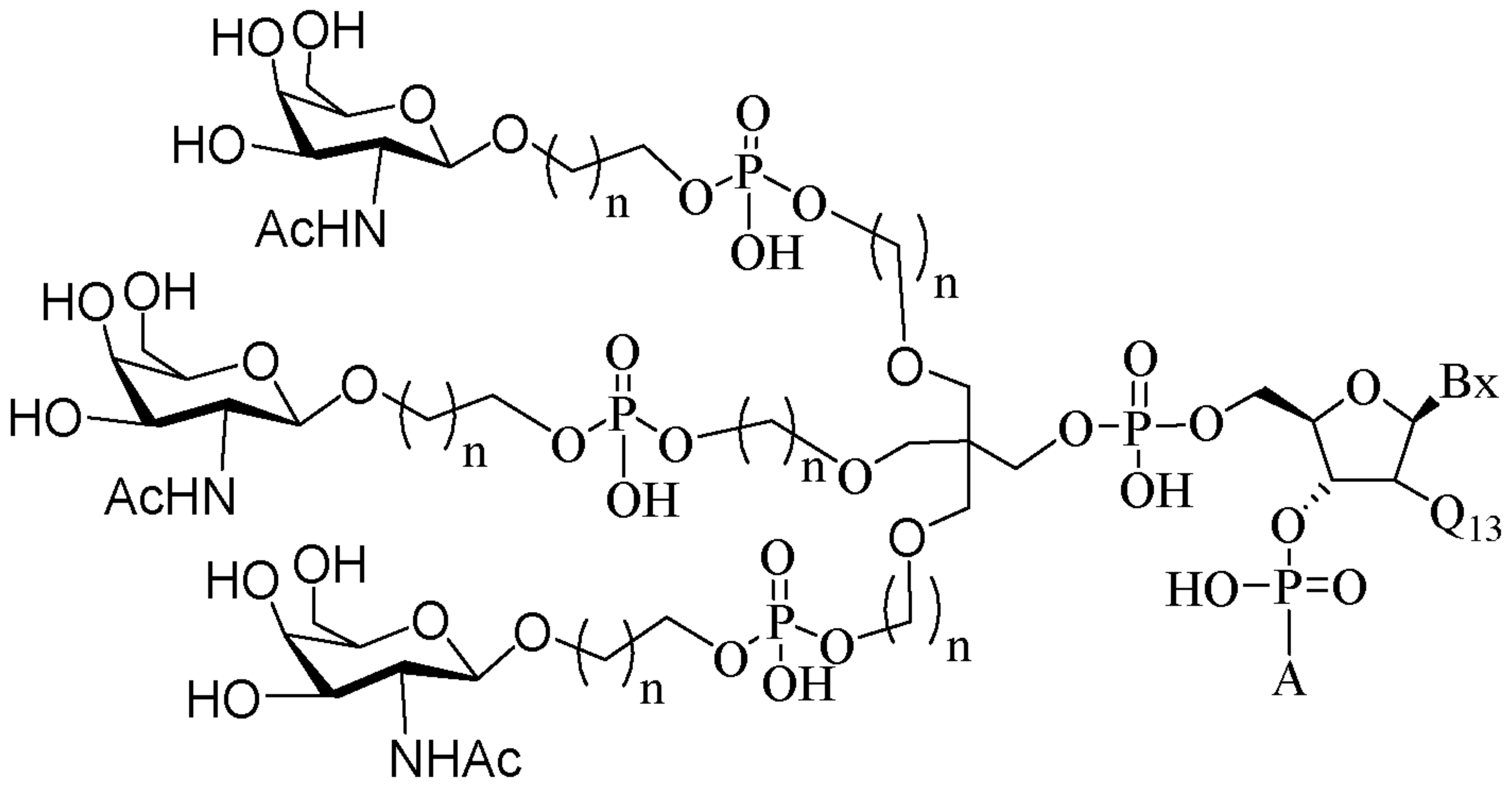
164. The compound of any preceding claim, wherein the conjugate group comprises a phosphodiester cleavable moiety.

165. The compound of any preceding claim, wherein the conjugate group does not comprise a cleavable moiety, and wherein the conjugate group comprises a phosphorothioate linkage between the conjugate group and the oligonucleotide.

166. The compound of any preceding claim, wherein the conjugate group comprises an amide cleavable moiety.

167. The compound of any preceding claim, wherein the conjugate group comprises an ester cleavable moiety.

168. The compound of any preceding claim, wherein the compound has the following structure:



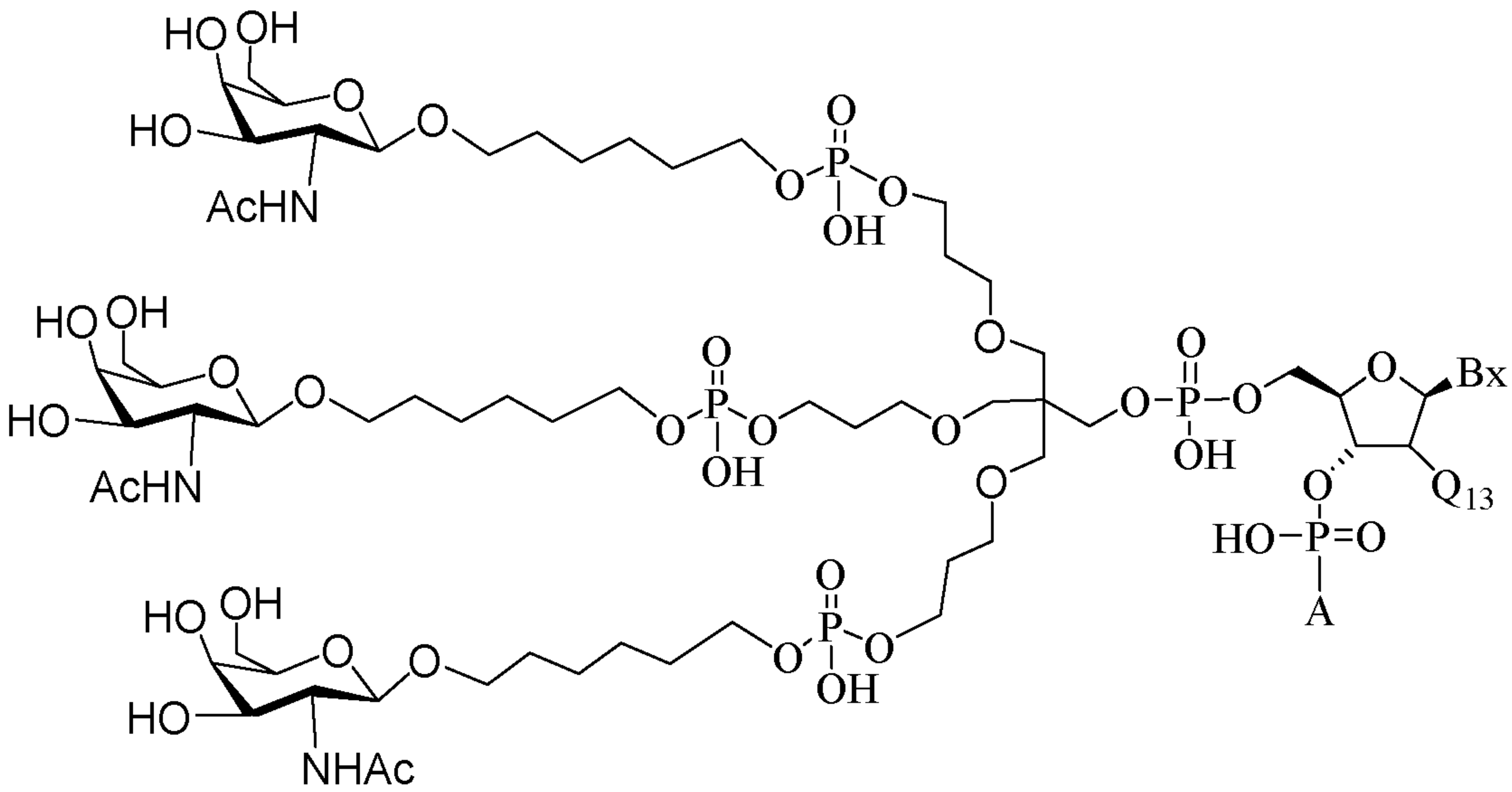
wherein each n is, independently, from 1 to 20;

Q_{13} is H or $O(CH_2)_2$-OCH_3;

A is the modified oligonucleotide; and

Bx is a heterocyclic base moiety.

169. The compound of any preceding claim, wherein the compound has the following structure:



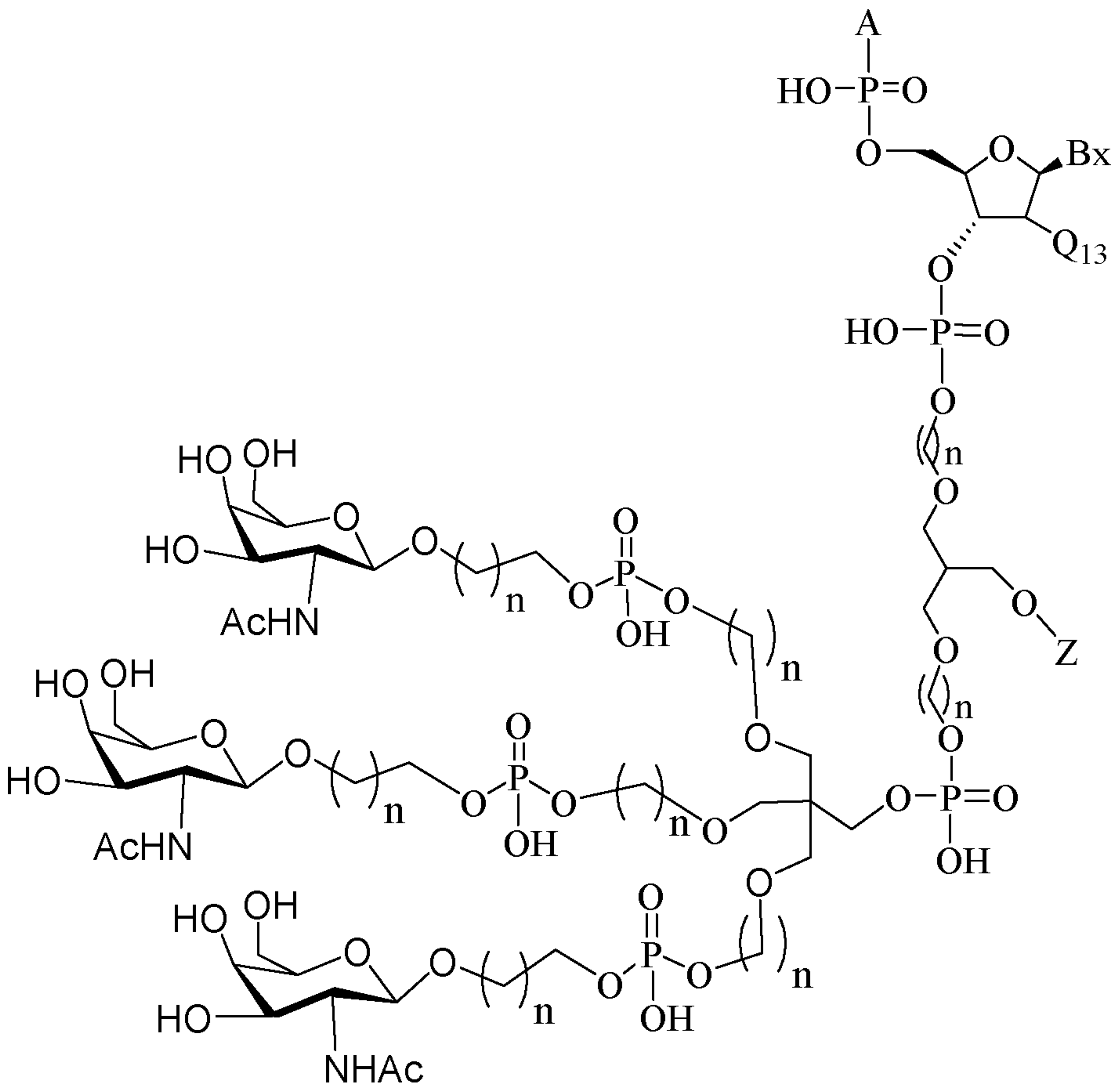
wherein each n is, independently, from 1 to 20;

Q_{13} is H or $O(CH_2)_2$-OCH_3;

A is the modified oligonucleotide; and

Bx is a heterocyclic base moiety.

170. The compound of any preceding claim, wherein the compound has the following structure:



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

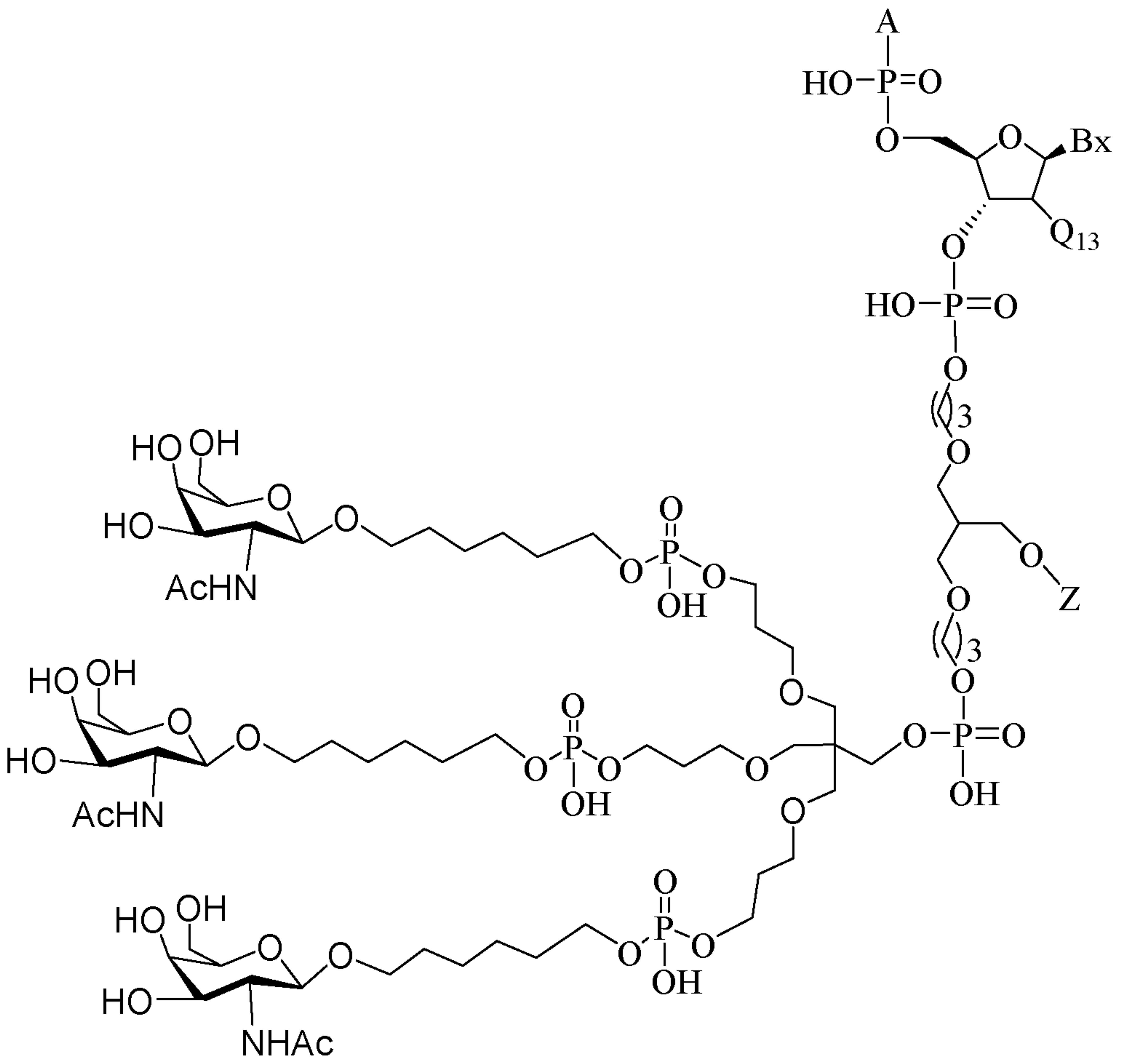
Q_{13} is H or $O(CH_2)_2$-OCH_3;

A is the modified oligonucleotide;

Z is H or a linked solid support; and

Bx is a heterocyclic base moiety.

171. The compound of any preceding claim, wherein the compound has the following structure:



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

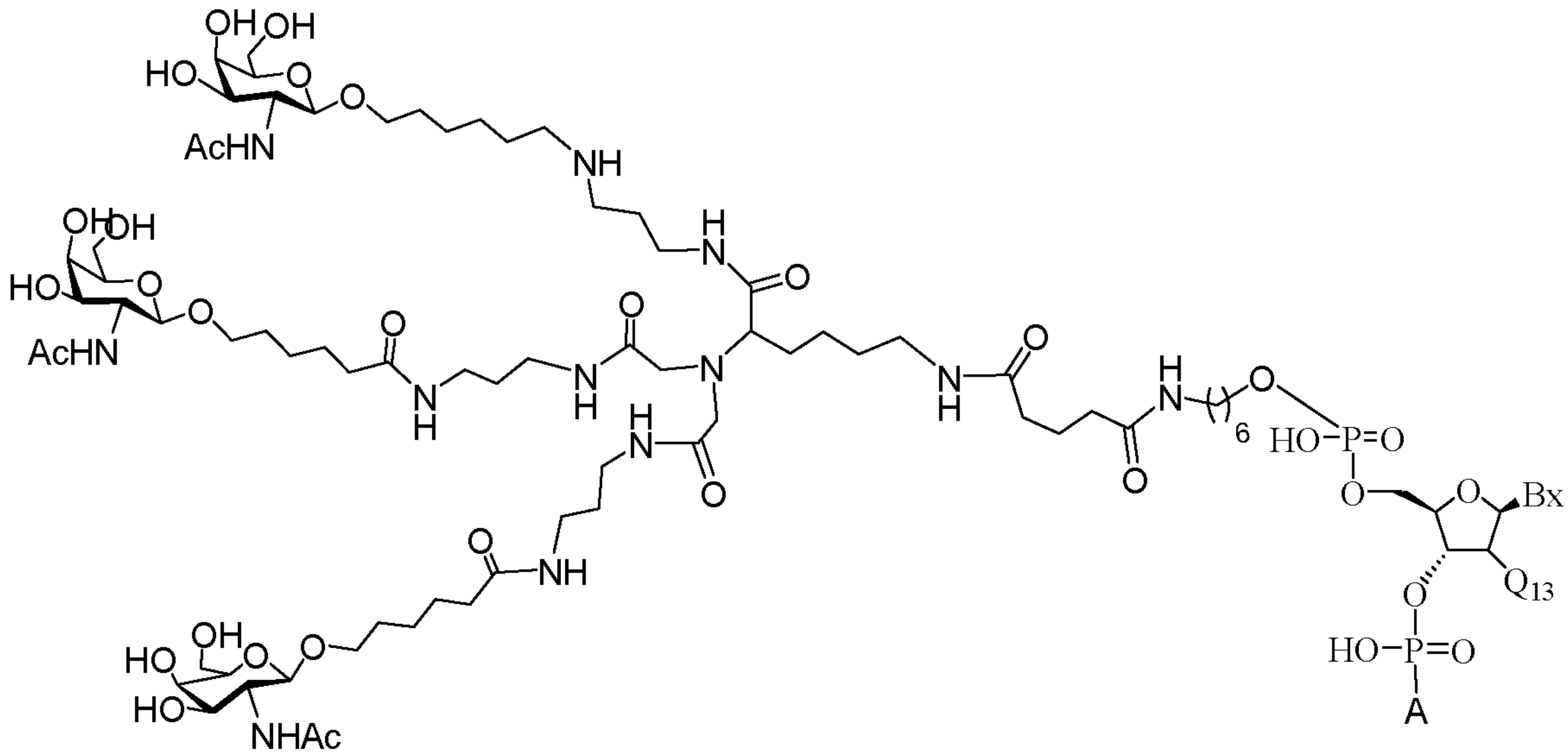
Q_{13} is H or $O(CH_2)_2$-OCH_3;

A is the modified oligonucleotide;

Z is H or a linked solid support; and

Bx is a heterocyclic base moiety.

172. The compound of any preceding claim, wherein the compound has the following structure:

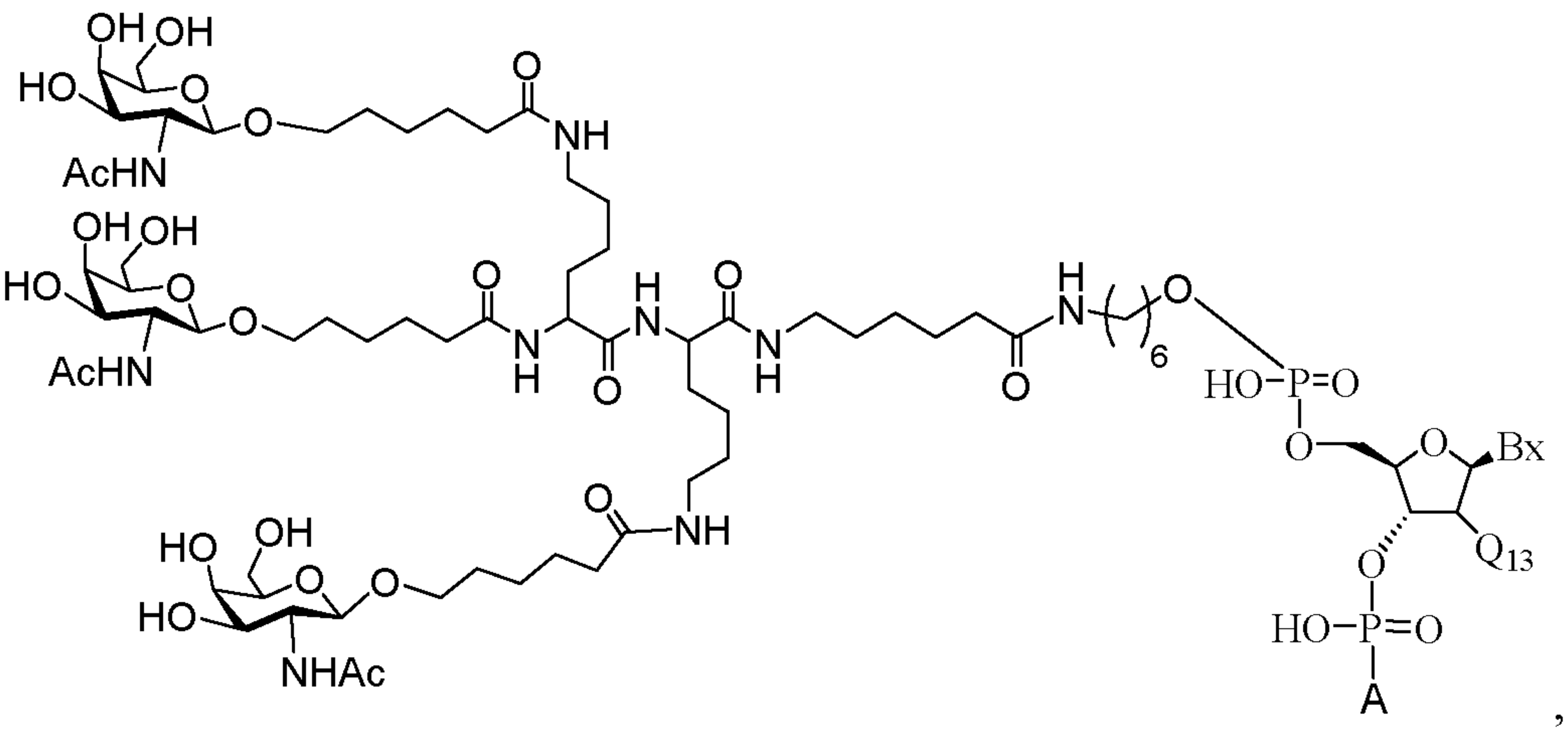


wherein Q_{13} is H or $O(CH_2)_2$-OCH_3;

A is the modified oligonucleotide;and

Bx is a heterocyclic base moiety.

173. The compound of any preceding claim, wherein the compound has the following structure:

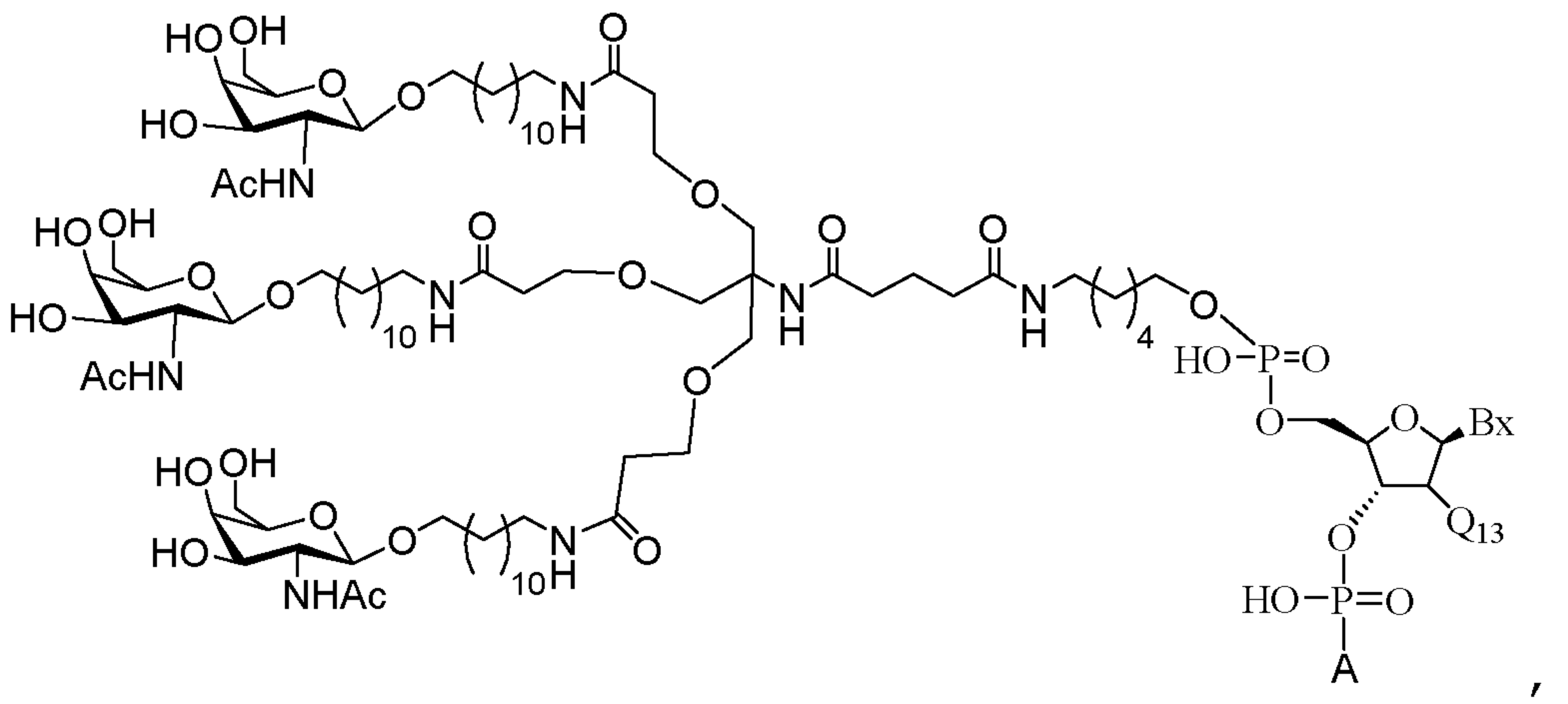


wherein Q_{13} is H or $O(CH_2)_2$-OCH_3;

A is the modified oligonucleotide;and

Bx is a heterocyclic base moiety.

174. The compound of any preceding claim, wherein the compound has the following structure:

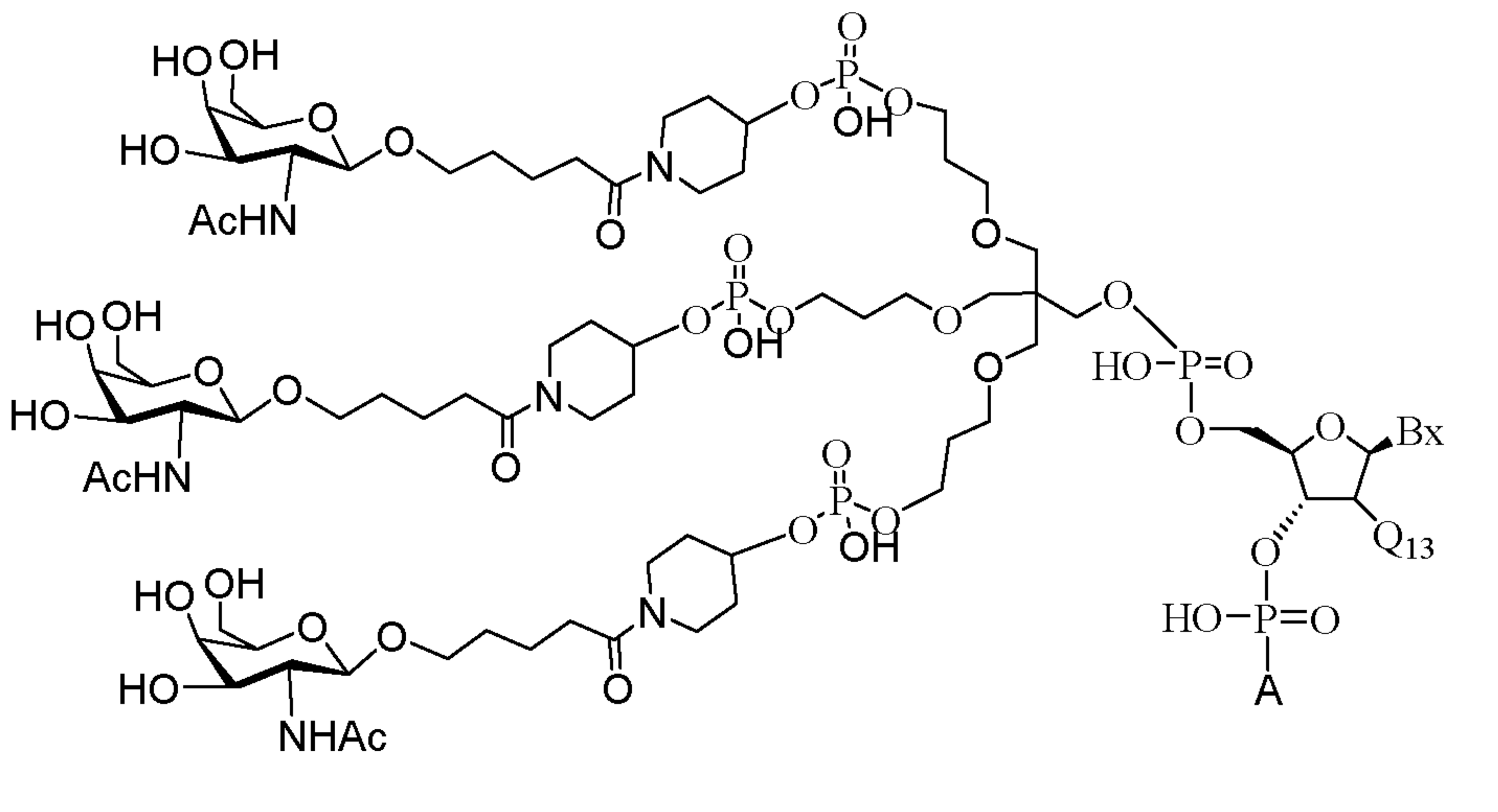


wherein Q_{13} is H or $O(CH_2)_2\text{-}OCH_3$;

A is the modified oligonucleotide; and

Bx is a heterocyclic base moiety.

175. The compound of any preceding claim, wherein the compound has the following structure:

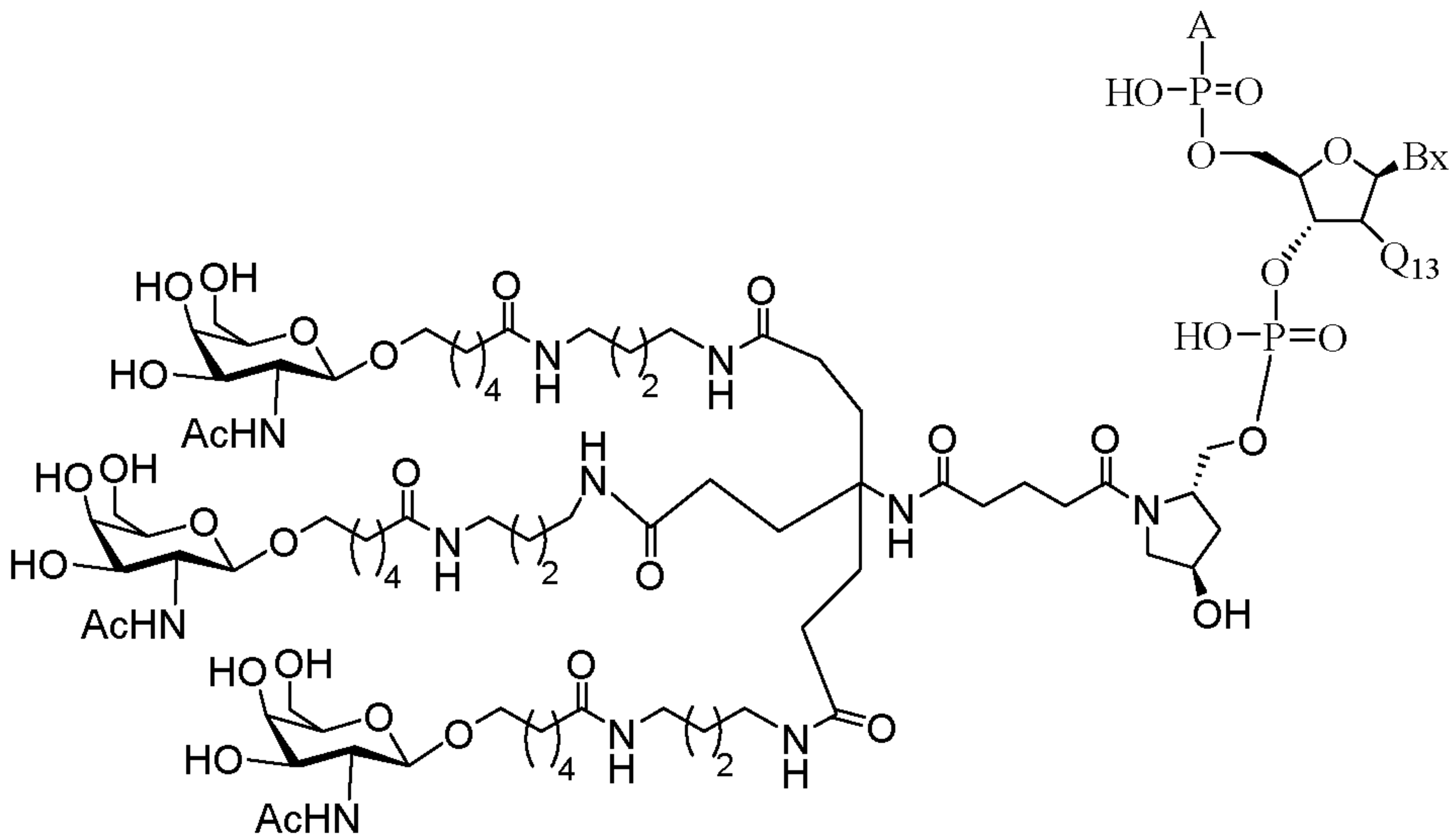


wherein Q_{13} is H or $O(CH_2)_2\text{-}OCH_3$;

A is the modified oligonucleotide; and

Bx is a heterocyclic base moiety.

176. The compound of any preceding claim, wherein the compound has the following structure:

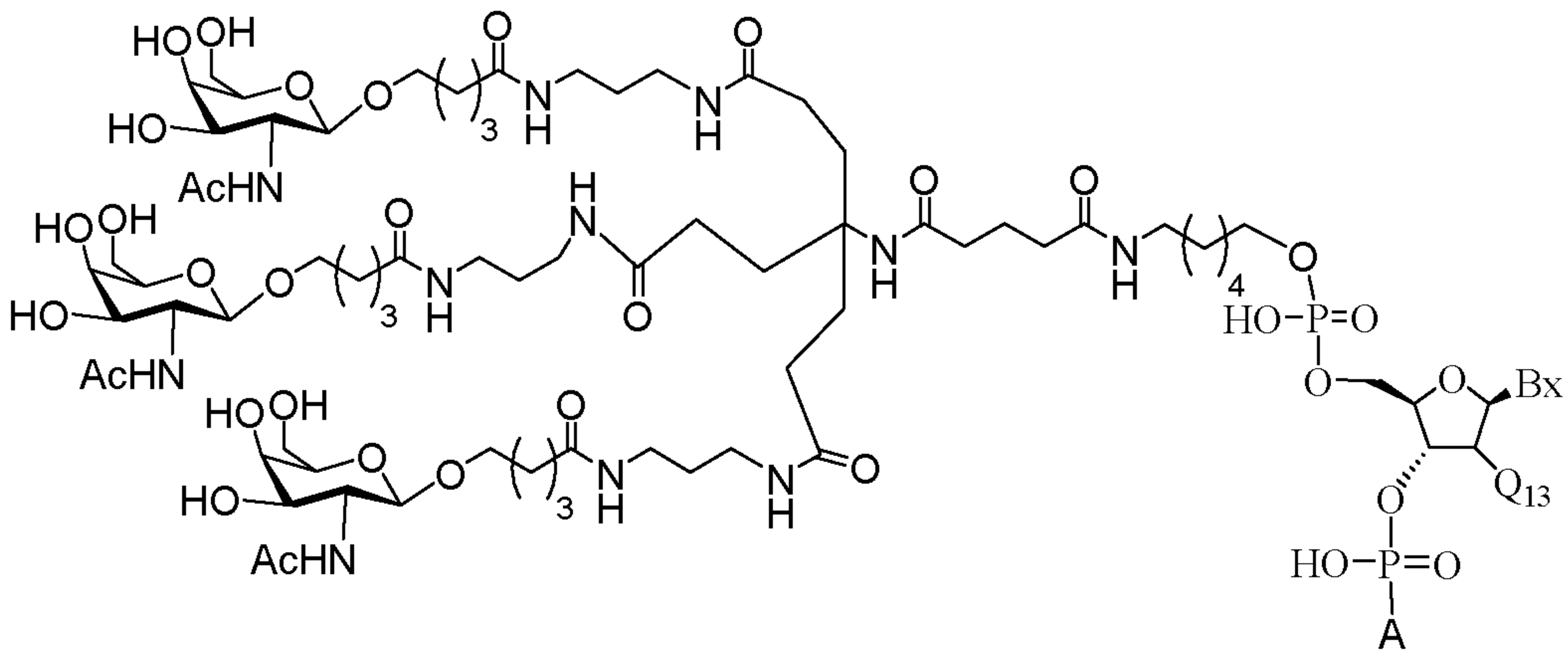


wherein Q_{13} is H or $O(CH_2)_2$-OCH_3;

A is the modified oligonucleotide; and

Bx is a heterocyclic base moiety.

177. The compound of any preceding claim, wherein the compound has the following structure:

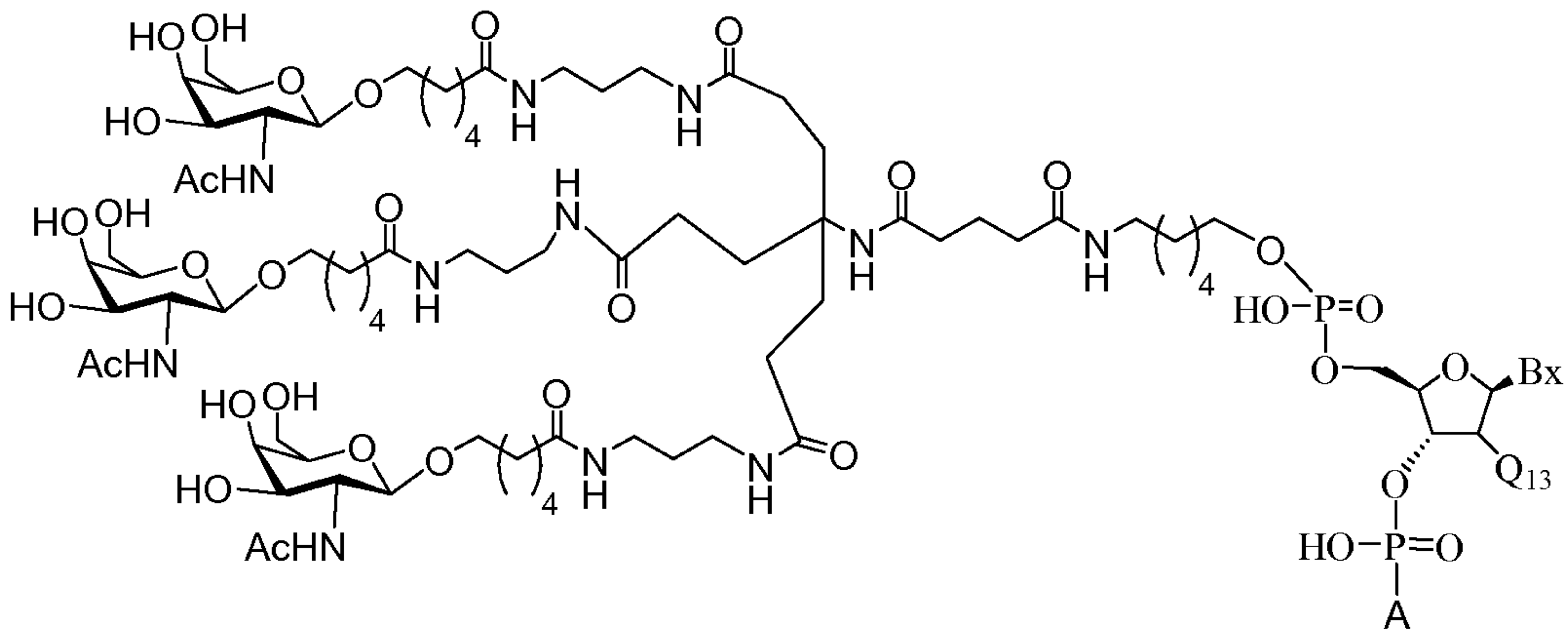


wherein Q_{13} is H or $O(CH_2)_2$-OCH_3;

A is the modified oligonucleotide; and

Bx is a heterocyclic base moiety.

178. The compound of any preceding claim, wherein the compound has the following structure:

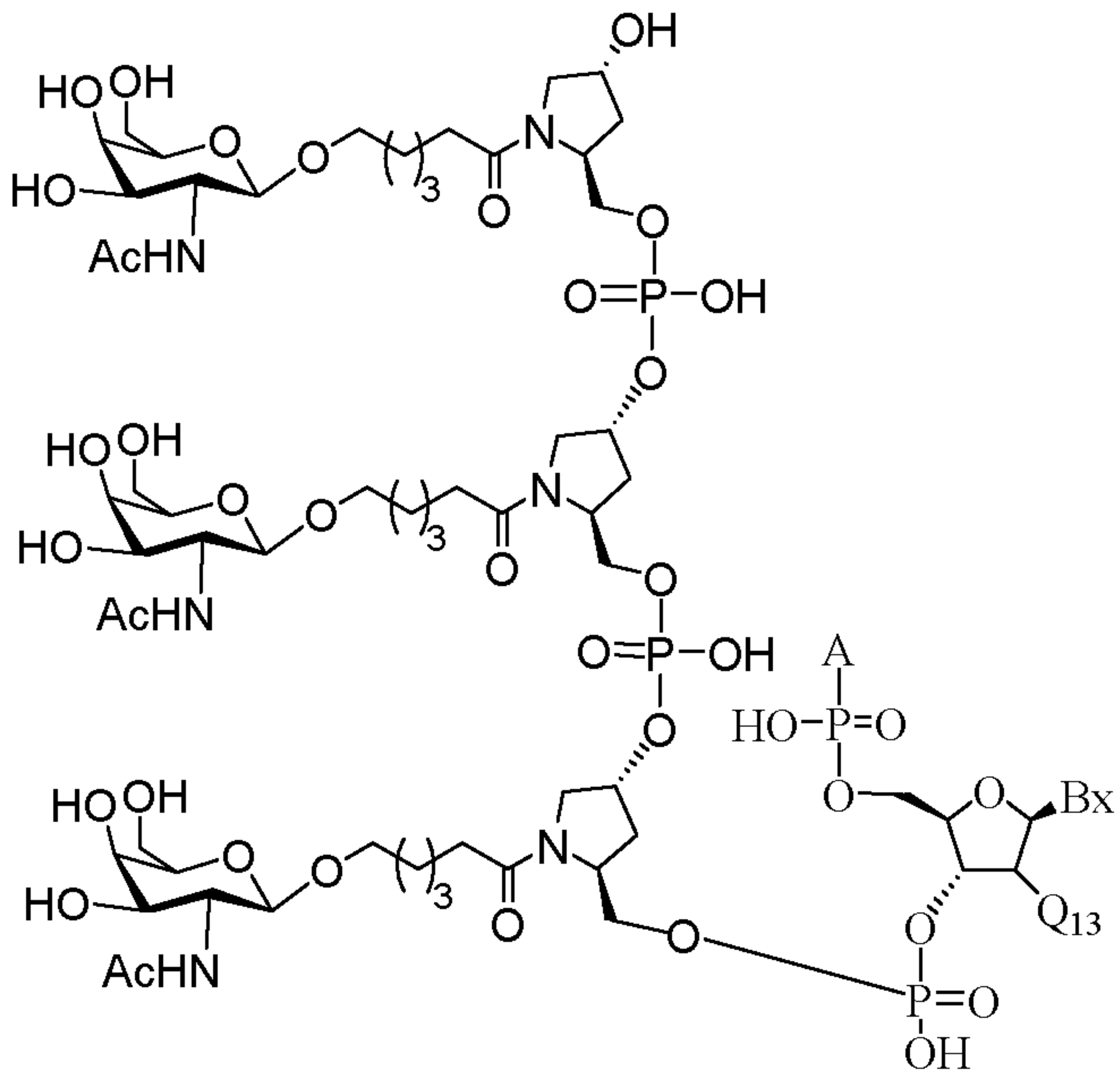


wherein Q_{13} is H or $O(CH_2)_2\text{-}OCH_3$;

A is the modified oligonucleotide; and

Bx is a heterocyclic base moiety.

179. The compound of any preceding claim, wherein the compound has the following structure:

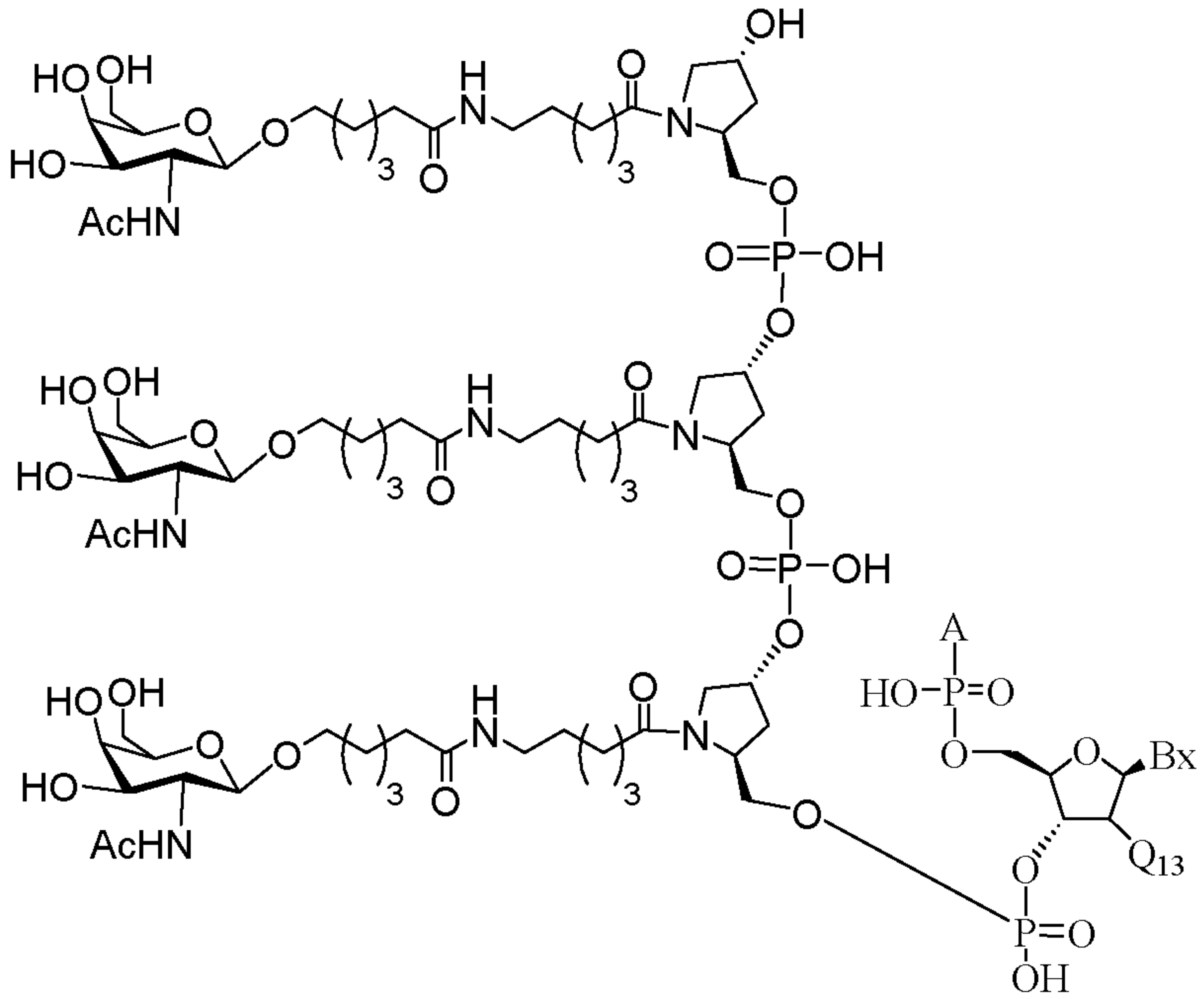


wherein Q_{13} is H or $O(CH_2)_2\text{-}OCH_3$;

A is the modified oligonucleotide; and

Bx is a heterocyclic base moiety.

180. The compound of any preceding claim, wherein the compound has the following structure:

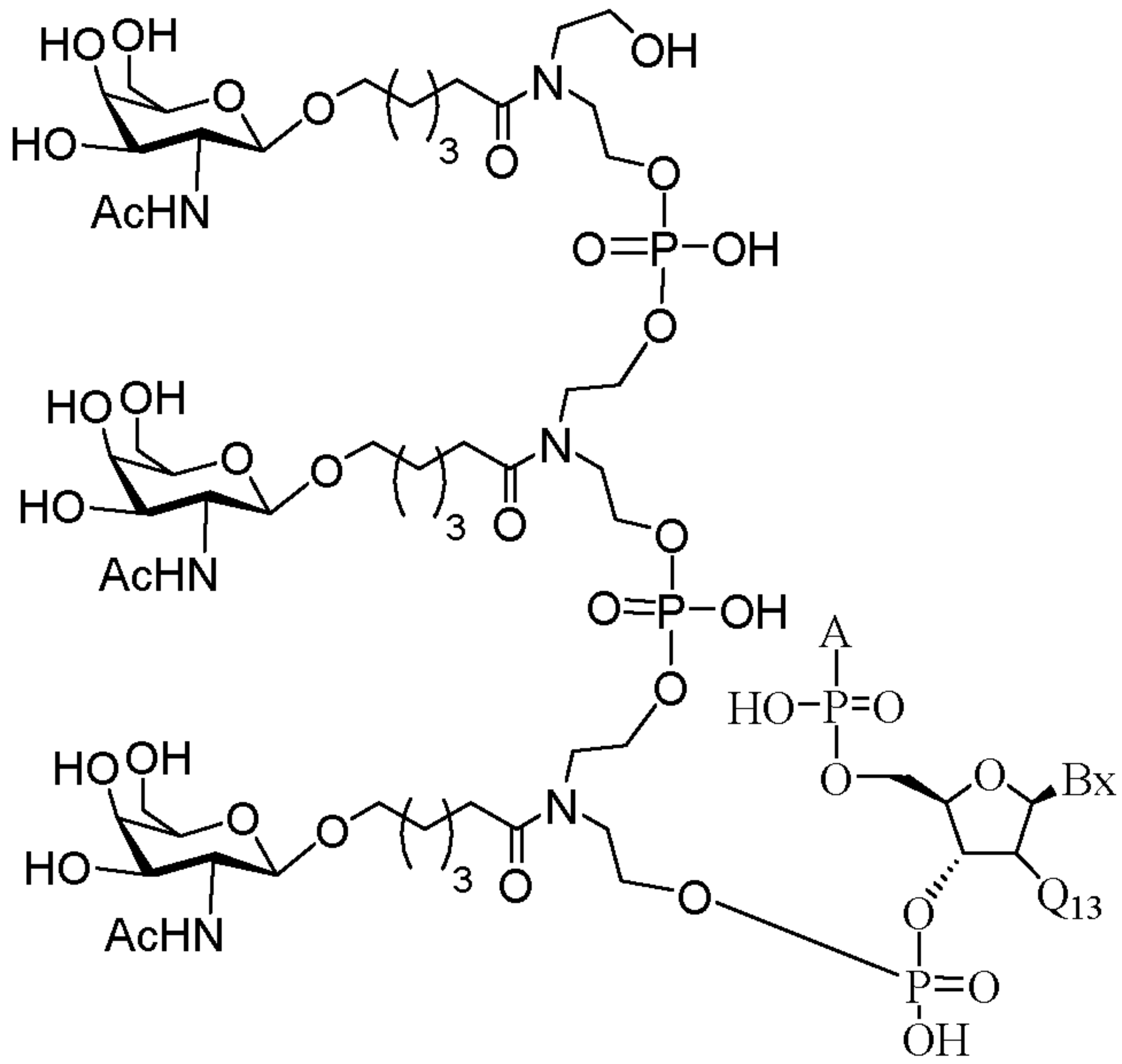


wherein Q_{13} is H or $O(CH_2)_2$-OCH_3;

A is the modified oligonucleotide; and

Bx is a heterocyclic base moiety.

181. The compound of any preceding claim, wherein the compound has the following structure:

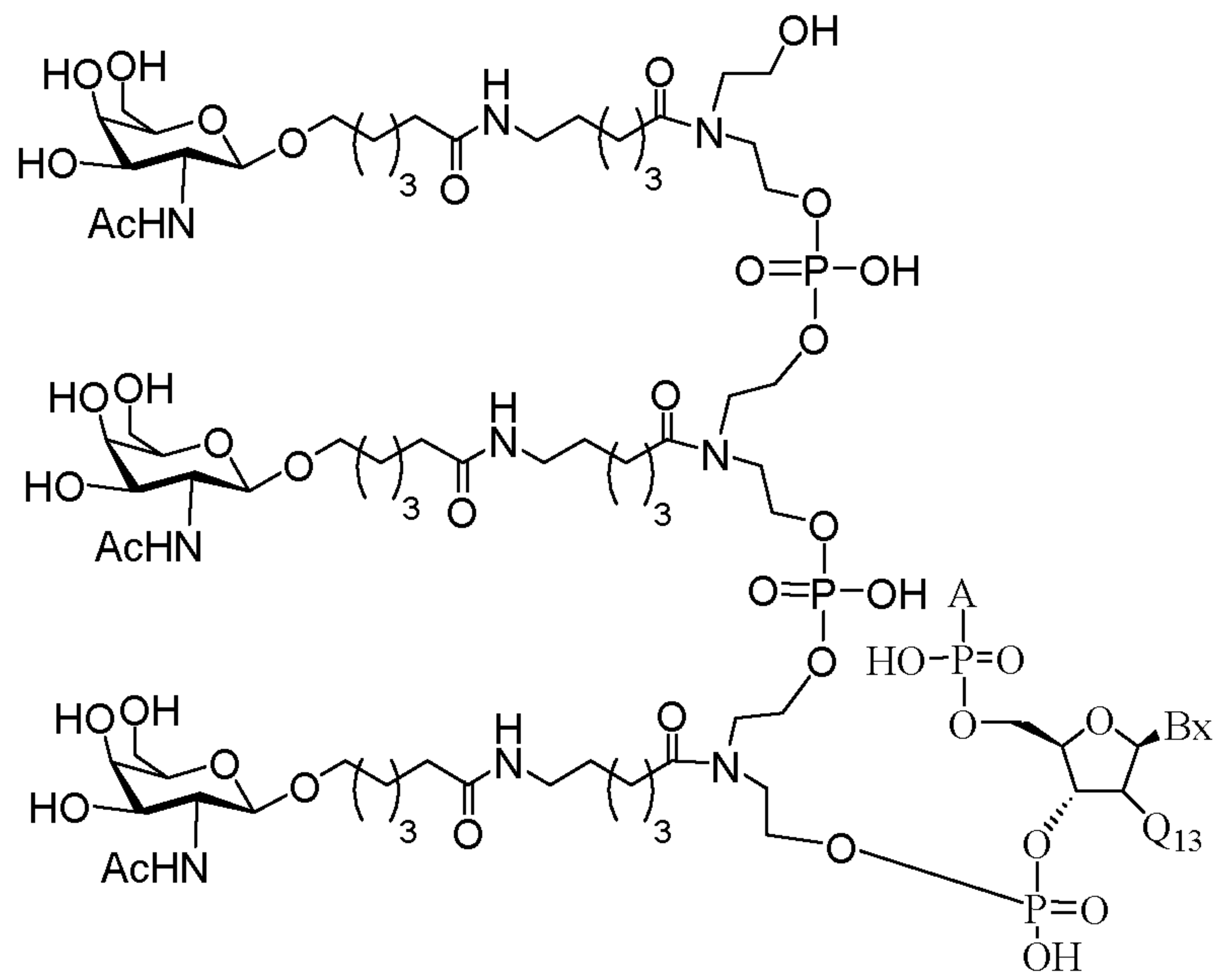


wherein Q_{13} is H or $O(CH_2)_2$-OCH_3;

A is the modified oligonucleotide; and

Bx is a heterocyclic base moiety.

182. The compound of any preceding claim, wherein the compound has the following structure:

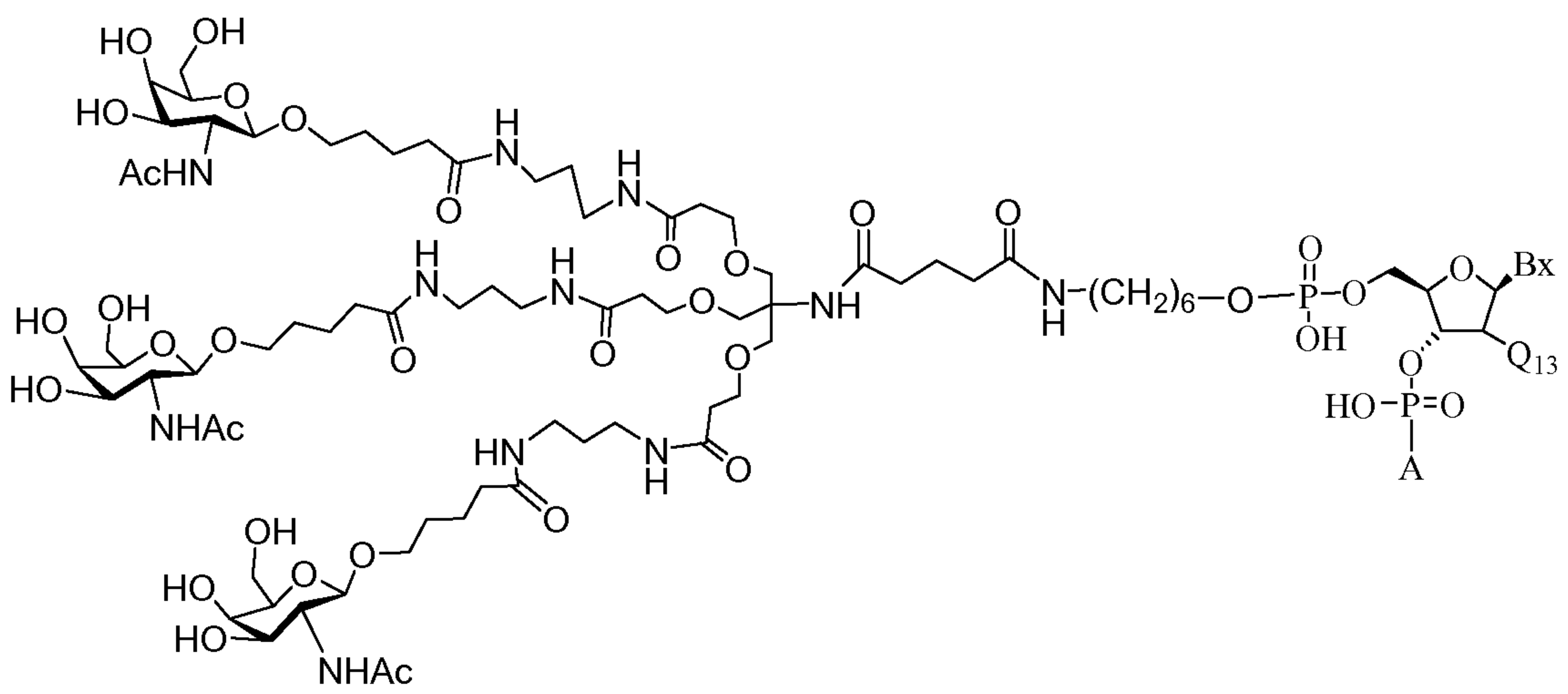


wherein Q_{13} is H or $O(CH_2)_2$-OCH_3;

A is the modified oligonucleotide; and

Bx is a heterocyclic base moiety.

183. The compound of any preceding claim, wherein the conjugate group comprises:

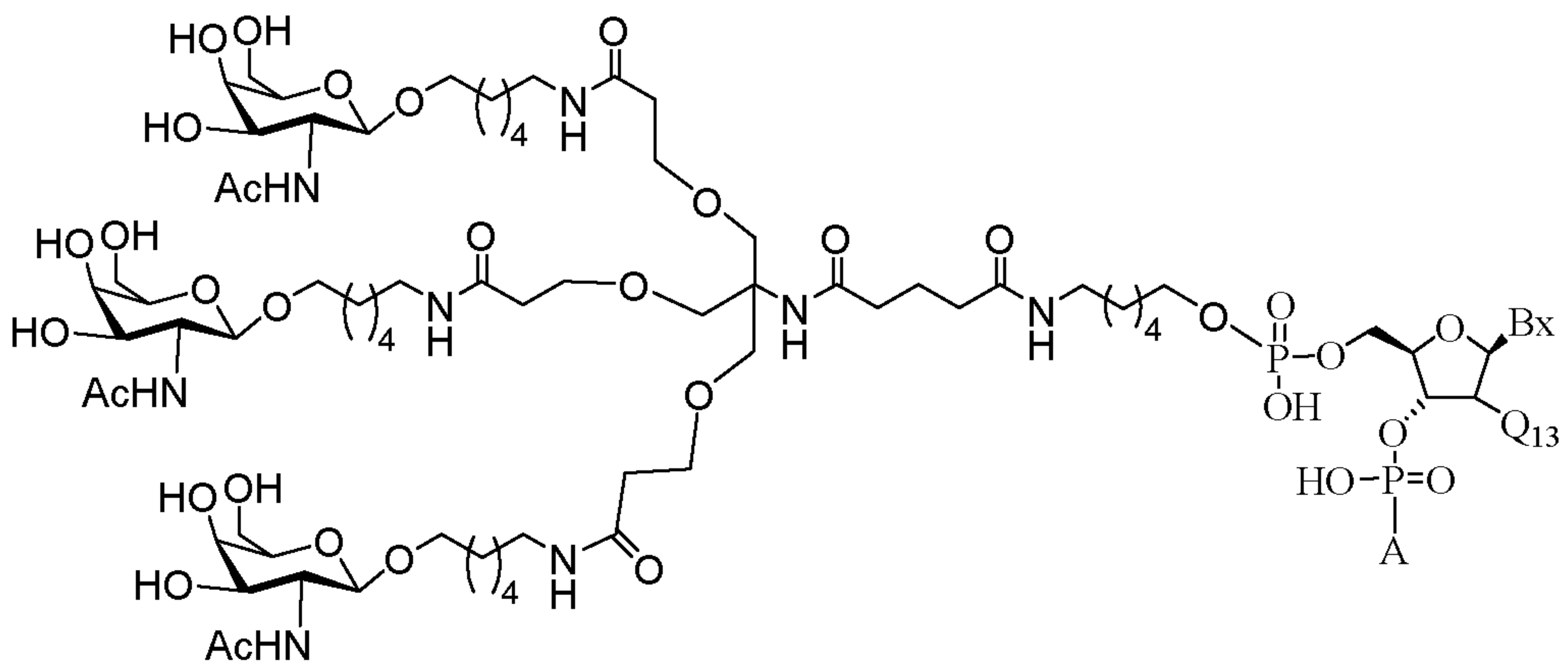


wherein Q_{13} is H or $O(CH_2)_2$-OCH_3;

A is the modified oligonucleotide; and

Bx is a heterocyclic base moiety.

184. The compound of any preceding claim, wherein the conjugate group comprises:

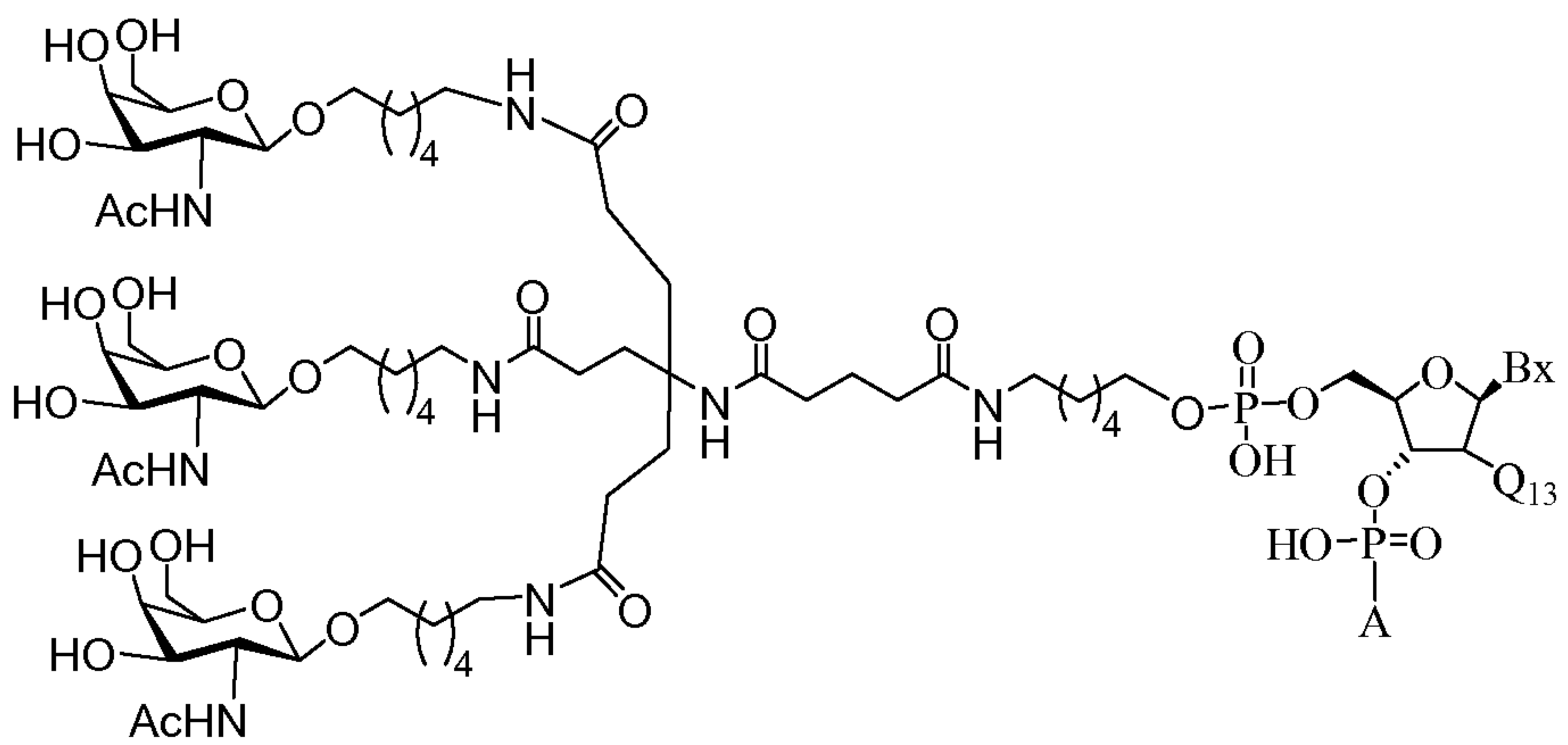


wherein Q_{13} is H or $O(CH_2)_2$-OCH_3;

A is the modified oligonucleotide; and

Bx is a heterocyclic base moiety.

185. The compound of any preceding claim, wherein the conjugate group comprises:



wherein Q_{13} is H or $O(CH_2)_2$-OCH_3;

A is the modified oligonucleotide; and

Bx is a heterocyclic base moiety.

186. The compound of any preceding claim, wherein B_x is selected from among from adenine, guanine, thymine, uracil, or cytosine, or 5-methyl cytosine.

187. The compound of any preceding claim, wherein B_x is adenine.

188. The compound of any preceding claim, wherein B_x is thymine.

189. The compound of any preceding claim, wherein Q_{13} is $O(CH_2)_2$-OCH_3.

190. The compound of any preceding claim, wherein Q_{13} is H.

191. A compound consisting of a conjugate group and a modified oligonucleotide according to the following formula: : mCes mCes Aes mCes mCes Tds Tds Tds Gds Gds Gds Tds Gds Ads Ads Tes Aes Ges mCes Ae; wherein,

A = an adenine,

mC = a 5'-methylcytosine

G = a guanine,

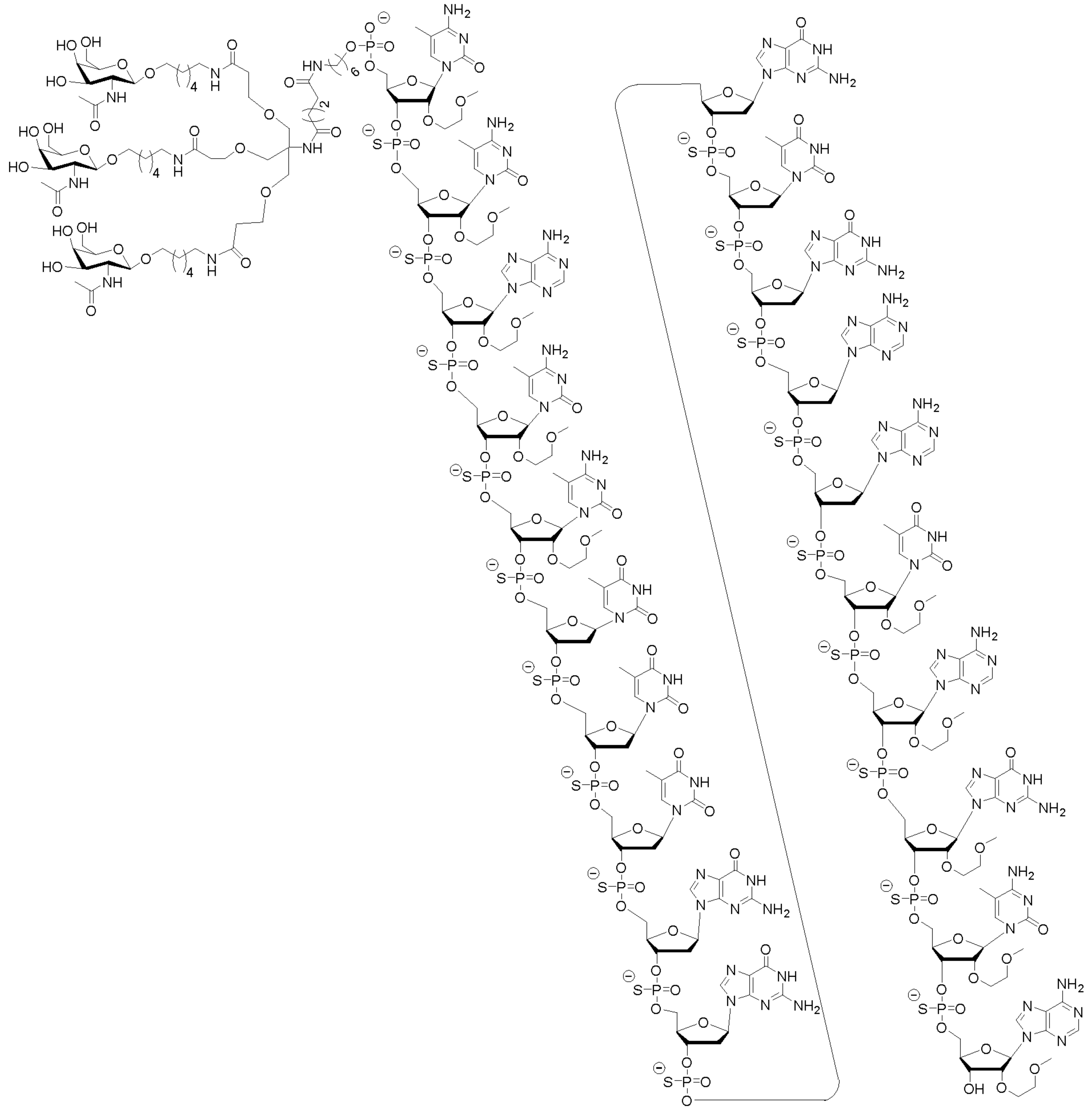
T = a thymine,

e = a 2'-O-methoxyethyl modified nucleoside,

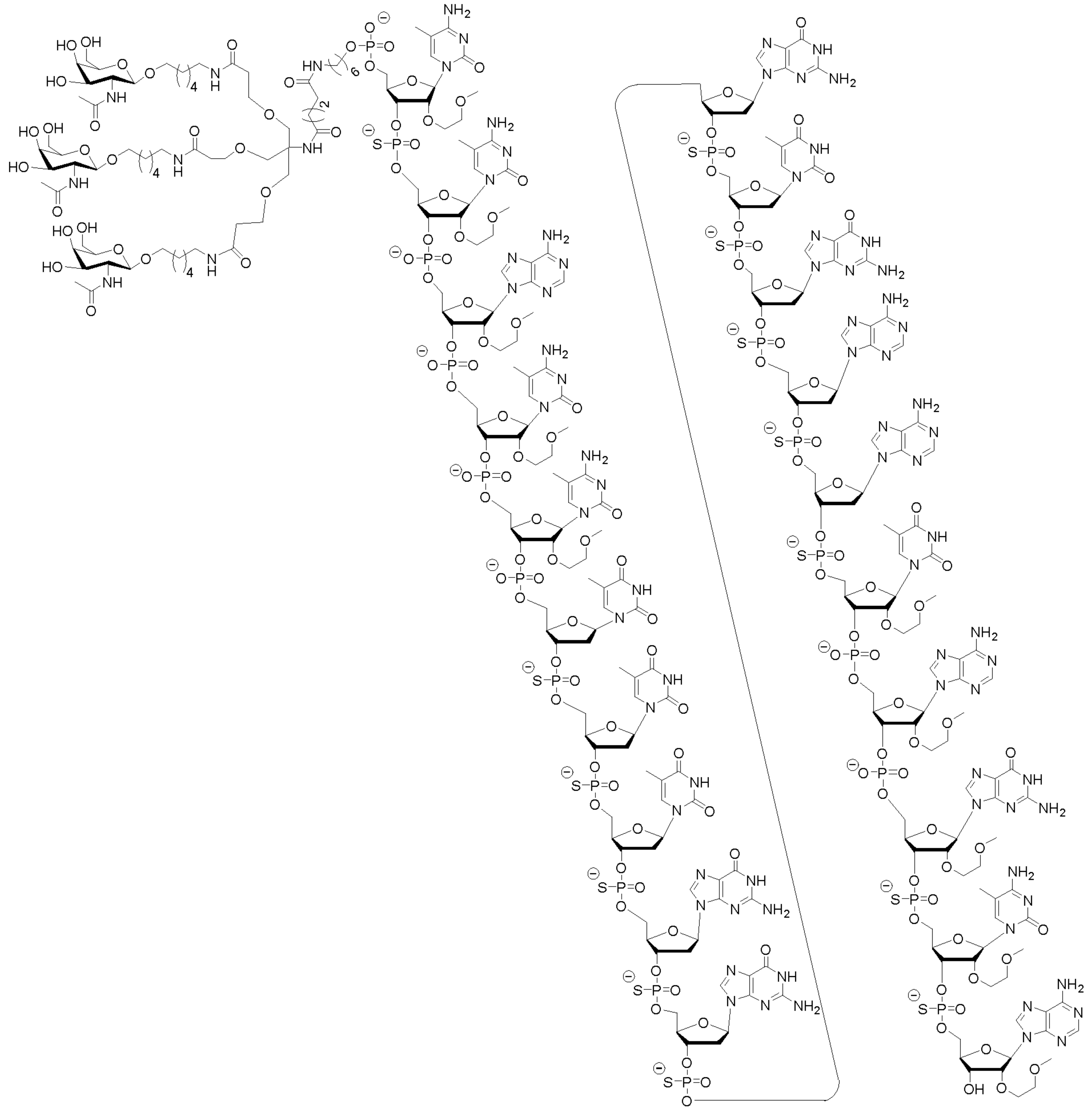
d = a 2'-deoxynucleoside, and

s = a phosphorothioate internucleoside linkage.

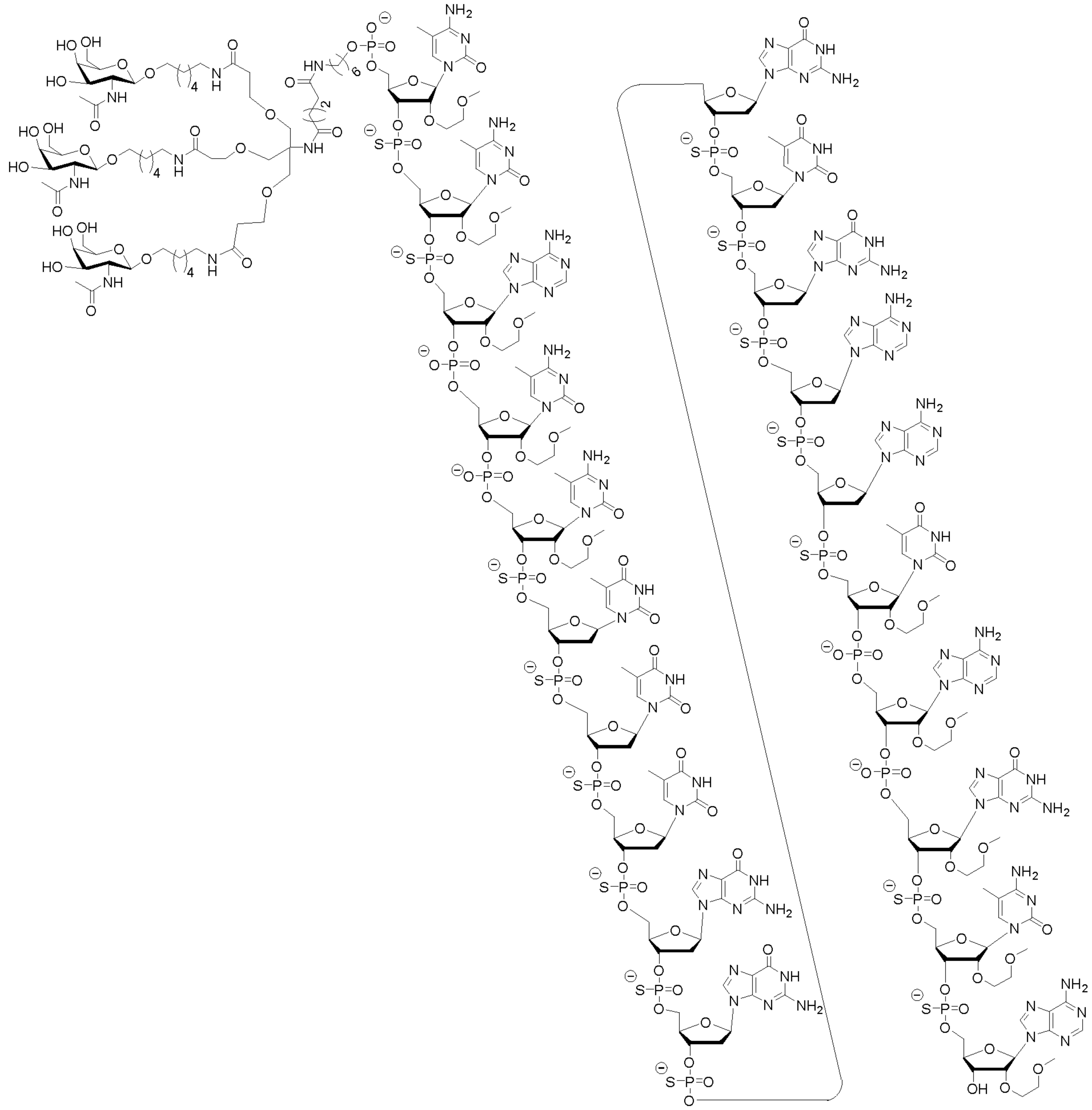
192. A compound consisting of a conjugate group and a modified oligonucleotide according to the following formula:



193. A compound consisting of a conjugate group and a modified oligonucleotide according to the following formula:



194. A compound consisting of a conjugate group and a modified oligonucleotide according to the following formula:



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

196. A prodrug comprising the compound of any of claims any preceding claim.

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

198. A prodrug comprising the compound or composition of any preceding claim.

199. A method comprising administering to an animal the compound or composition of any preceding claim.

200. The method of claim 198, wherein the animal is a human.

201. A method of treating a disease associated with excess growth hormone in a human comprising administering to the human a therapeutically effective amount of the compound or composition of any preceding claim, thereby treating the disease associated with excess growth hormone.

202. The method of claim 200, wherein the disease associated with excess growth hormone is acromegaly.

203. The method of claim 200, wherein the treatment reduces IGF-1 levels.

204. A method of preventing a disease associated with excess growth hormone in a human comprising administering to the human a therapeutically effective amount of a compound or composition of any preceding claim, thereby preventing the disease associated with excess growth hormone.

205. The method of claim 203, wherein the disease associated with excess growth hormone is acromegaly.

206. The method of any of claims 198-204, comprising co-administering the compound or composition and a second agent.

207. The method of claim 205, wherein the compound or composition and the second agent are administered concomitantly.

208. A method of reducing growth hormone receptor (GHR) levels in a human comprising administering to the human a therapeutically effective amount of the compound or composition of any preceding claim, thereby reducing GHR levels in the human.

209. The method of claim 207, wherein the human has a disease associated with excess growth hormone.

210. The method of claim 207, wherein the disease associated with excess growth hormone is acromegaly.

211. The method of any one of claims 206-209, comprising co-administering the compound or composition and a second agent.

212. The method of claim 210, wherein the compound or composition and the second agent are administered concomitantly.

213. Use of the compound or composition of any preceding claim for treating, prevening, or ameliorating a disease associated with excess growth hormone.

214. The use of claim 212, wherein the disease associated with excess growth hormone is acromegaly.