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(54) **Title:** TARGETED COMPOSITIONS

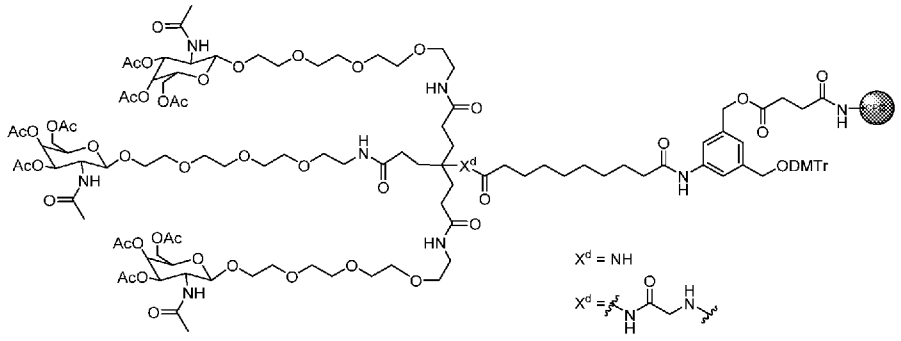

Figure 1: Intermediate compound of formula Ie, wherein a targeting ligand/linker is bound to a solid phase support, and wherein Pg^1 is the protecting group DMTr.

(57) **Abstract:** The invention provides certain nucleic acids (e.g., double stranded siRNA molecules), as well as conjugates that comprise a targeting moiety, a double stranded siRNA, and optional linking groups. Certain embodiments also provide synthetic methods useful for preparing the conjugates. The conjugates are useful to target therapeutic double stranded siRNA to the liver and to treat liver diseases including hepatitis (e.g. hepatitis B and hepatitis D).

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TARGETED COMPOSITIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

This patent application claims the benefit of priority of U.S. application serial No. 62/525,071, filed June 26, 2017 and of U.S. application serial No. 62/484,247, filed April 11, 2017, which applications are herein incorporated by reference.

BACKGROUND

A number of diseases are specific to the liver, for example Hepatitis B and nonalcoholic steatohepatitis (NASH). Accordingly, it would be beneficial to have therapeutic compositions that can be targeted primarily to the liver, kidney, heart, pancreas or other organs in living subjects.

Nucleic acids, including siRNA are useful as therapeutic agents.

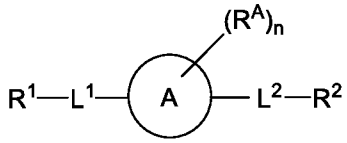
Currently there is a need for compositions and methods that can be used to deliver (e.g. target) therapeutic nucleic acids, such as double stranded siRNA, in living subjects.

BRIEF SUMMARY

The invention provides nucleic acid molecules (e.g., therapeutic double stranded siRNA molecules), as well as compounds, compositions and methods that can be used to target such nucleic acids (e.g. to the liver).

Accordingly, in one aspect this invention provides a double stranded siRNA molecule selected from the group consisting of siRNA 1 (SEQ ID NO:1 and 2), 2 (SEQ ID NO:3 and 4), 3 (SEQ ID NO:5 and 6), 4 (SEQ ID NO:7 and 8), 5 (SEQ ID NO:9 and 10), 6 (SEQ ID NO:11 and 12), 7 (SEQ ID NO:13 and 14), 8 (SEQ ID NO:15 and 16), 9 (SEQ ID NO:17 and 18), 10 (SEQ ID NO:19 and 20), 11 (SEQ ID NO:21 and 22), 12 (SEQ ID NO:23 and 24), 13 (SEQ ID NO:25 and 26), 14 (SEQ ID NO:27 and 28), 15 (SEQ ID NO:29 and 30), 16 (SEQ ID NO:31 and 32), 17 (SEQ ID NO:33 and 34), 18 (SEQ ID NO:35 and 36), 19 (SEQ ID NO:37 and 38), 20 (SEQ ID NO:39 and 40), 21 (SEQ ID NO:41 and 42), 22 (SEQ ID NO:43 and 44), 23 (SEQ ID NO:45 and 46), 24 (SEQ ID NO:47 and 48), 25 (SEQ ID NO:49 and 50), 26 (SEQ ID NO:51 and 52), 27 (SEQ ID NO:53 and 54), 28 (SEQ ID NO:55 and 56), 29 (SEQ ID NO:57 and 58), 30 (SEQ ID NO:59 and 60), 31 (SEQ ID NO:61 and 62), 32 (SEQ ID NO:63 and 64), 33 (SEQ ID NO:65 and 66), 34 (SEQ ID NO:67 and 68), 35 (SEQ ID NO:69 and 70), 36 (SEQ ID NO:71 and 72) and 37 (SEQ ID NO:73 and 74).

Another aspect this invention provides a compound of formula I



(I)

wherein:

R^1 a is targeting ligand;

L^1 is absent or a linking group;

L^2 is absent or a linking group;

R^2 is a double stranded siRNA molecule selected from the double stranded siRNA of Table 1;

the ring A is absent, a 3-20 membered cycloalkyl, a 5-20 membered aryl, a 5-20 membered heteroaryl, or a 3-20 membered heterocycloalkyl;

each R^A is independently selected from the group consisting of hydrogen, hydroxy, CN, F, Cl, Br, I, -C_{1-2} alkyl-OR^B, C_{1-10} alkyl C_{2-10} alkenyl, and C_{2-10} alkynyl; wherein the C_{1-10} alkyl C_{2-10} alkenyl, and C_{2-10} alkynyl are optionally substituted with one or more groups independently selected from halo, hydroxy, and C_{1-3} alkoxy;

R^B is hydrogen, a protecting group, a covalent bond to a solid support, or a bond to a linking group that is bound to a solid support; and

n is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

or a salt thereof.

Another aspect of the invention provides GalNAc conjugates that comprise one of the siRNAs described herein, which conjugates are not limited to conjugates that comprise the ligand-linkers disclosed herein. For example, an aspect of the invention provides a GalNAc conjugate of Formula X:

A-B-C

(X)

wherein A is a targeting ligand;

B is an optional linker; and

C is an siRNA molecule described herein.

The therapeutic double stranded siRNA described herein, as well as, compounds and compositions comprising such siRNA, may be used to treat Hepatitis B virus and Hepatitis B virus/Hepatitis D virus.

The invention also provides synthetic intermediates and methods disclosed herein that are useful to prepare compounds of formula I.

Other objects, features, and advantages of the present invention will be apparent to one of skill in the art from the following detailed description and figures.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Illustrates an intermediate compound of formula Ie, wherein a targeting ligand/linker is bound to a solid phase support, and wherein Pg^1 is the protecting group DMTr.

Figure 2: Illustrates a representative compound of formula Id wherein a targeting ligand is bound to a solid phase support, with a nucleic acid covalently bound.

Figure 3: Illustrates a representative compound of formula Id, wherein a targeting ligand-nucleic acid conjugate has been cleaved from a solid phase support and deprotected to provide the compound of formula I.

In the application, including Figures, Examples and Schemes, it is to be understood that an oligonucleotide can be a double stranded siRNA molecule as described in Table 1.

DETAILED DESCRIPTION

As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

The term "conjugate" as used herein includes compounds of formula (I) that comprise an oligonucleotide (e.g., an siRNA molecule) linked to a targeting ligand. Thus, the terms compound and conjugate may be used herein interchangeably.

The term "small-interfering RNA" or "siRNA" as used herein refers to double stranded RNA (*i.e.,* duplex RNA) that is capable of reducing or inhibiting the expression of a target gene or sequence (*e.g.,* by mediating the degradation or inhibiting the translation of mRNAs which are complementary to the siRNA sequence) when the siRNA is in the same cell as the target gene or sequence. The siRNA may have substantial or complete identity to the target gene or sequence, or may comprise a region of mismatch (*i.e.,* a mismatch motif). In certain embodiments, the siRNAs may be about 19-25 (duplex) nucleotides in length, and is preferably about 20-24, 21-22, or 21-23 (duplex) nucleotides in length. siRNA duplexes may comprise 3' overhangs of about 1 to about 4 nucleotides or about 2 to about 3 nucleotides and 5' phosphate termini. Examples of siRNA include, without limitation, a double-stranded polynucleotide

molecule assembled from two separate stranded molecules, wherein one strand is the sense strand and the other is the complementary antisense strand.

In certain embodiments, the 5' and/or 3' overhang on one or both strands of the siRNA comprises 1-4 (*e.g.*, 1, 2, 3, or 4) modified and/or unmodified deoxythymidine (t or dT) nucleotides, 1-4 (*e.g.*, 1, 2, 3, or 4) modified (*e.g.*, 2'OMe) and/or unmodified uridine (U) ribonucleotides, and/or 1-4 (*e.g.*, 1, 2, 3, or 4) modified (*e.g.*, 2'OMe) and/or unmodified ribonucleotides or deoxyribonucleotides having complementarity to the target sequence (e.g., 3'overhang in the antisense strand) or the complementary strand thereof (e.g., 3' overhang in the sense strand).

Preferably, siRNA are chemically synthesized. siRNA can also be generated by cleavage of longer dsRNA (*e.g.*, dsRNA greater than about 25 nucleotides in length) with the *E. coli* RNase III or Dicer. These enzymes process the dsRNA into biologically active siRNA (*see*, *e.g.*, Yang *et al.*, *Proc. Natl. Acad. Sci. USA,* 99:9942-9947 (2002); Calegari *et al.*, *Proc. Natl. Acad. Sci. USA,* 99:14236 (2002); Byrom *et al.*, *Ambion TechNotes,* 10(1):4-6 (2003); Kawasaki *et al.*, *Nucleic Acids Res.,* 31:981-987 (2003); Knight *et al.*, *Science,* 293:2269-2271 (2001); and Robertson *et al.*, *J. Biol. Chem.,* 243:82 (1968)). Preferably, dsRNA are at least 50 nucleotides to about 100, 200, 300, 400, or 500 nucleotides in length. A dsRNA may be as long as 1000, 1500, 2000, 5000 nucleotides in length, or longer. The dsRNA can encode for an entire gene transcript or a partial gene transcript. In certain instances, siRNA may be encoded by a plasmid (*e.g.*, transcribed as sequences that automatically fold into duplexes with hairpin loops).

The phrase "inhibiting expression of a target gene" refers to the ability of a siRNA of the invention to silence, reduce, or inhibit expression of a target gene. To examine the extent of gene silencing, a test sample (*e.g.*, a biological sample from an organism of interest expressing the target gene or a sample of cells in culture expressing the target gene) is contacted with a siRNA that silences, reduces, or inhibits expression of the target gene. Expression of the target gene in the test sample is compared to expression of the target gene in a control sample (*e.g.*, a biological sample from an organism of interest expressing the target gene or a sample of cells in culture expressing the target gene) that is not contacted with the siRNA. Control samples (*e.g.*, samples expressing the target gene) may be assigned a value of 100%. In particular embodiments, silencing, inhibition, or reduction of expression of a target gene is achieved when the value of the test sample relative to the control sample (*e.g.*, buffer only, an siRNA sequence that targets a different gene, a scrambled siRNA sequence, *etc.*) is about 100%, 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%,

85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5%, or 0%. Suitable assays include, without limitation, examination of protein or mRNA levels using techniques known to those of skill in the art, such as, *e.g.,* dot blots, Northern blots, *in situ* hybridization, ELISA, immunoprecipitation, enzyme function, as well as phenotypic assays known to those of skill in the art.

The term "synthetic activating group" refers to a group that can be attached to an atom to activate that atom to allow it to form a covalent bond with another reactive group. It is understood that the nature of the synthetic activating group may depend on the atom that it is activating. For example, when the synthetic activating group is attached to an oxygen atom, the synthetic activating group is a group that will activate that oxygen atom to form a bond (e.g. an ester, carbamate, or ether bond) with another reactive group. Such synthetic activating groups are known. Examples of synthetic activating groups that can be attached to an oxygen atom include, but are not limited to, acetate, succinate, triflate, and mesylate. When the synthetic activating group is attached to an oxygen atom of a carboxylic acid, the synthetic activating group can be a group that is derivable from a known coupling reagent (e.g. a known amide coupling reagent). Such coupling reagents are known. Examples of such coupling reagents include, but are not limited to, N,N'-Dicyclohexylcarbodimide (DCC), hydroxybenzotriazole (HOBt), N-(3-Dimethylaminopropyl)-N'-ethylcarbonate (EDC), (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP), benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) or O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU).

An "effective amount" or "therapeutically effective amount" of a therapeutic nucleic acid such as siRNA is an amount sufficient to produce the desired effect, *e.g.*, an inhibition of expression of a target sequence in comparison to the normal expression level detected in the absence of a siRNA. In particular embodiments, inhibition of expression of a target gene or target sequence is achieved when the value obtained with a siRNA relative to the control (*e.g.,* buffer only, an siRNA sequence that targets a different gene, a scrambled siRNA sequence, *etc.*) is about 100%, 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5%, or 0%. Suitable assays for measuring the expression of a target gene or target sequence include, but are not limited to, examination of protein or mRNA levels using techniques known to those of skill in the art,

such as, *e.g.,* dot blots, Northern blots, *in situ* hybridization, ELISA, immunoprecipitation, enzyme function, as well as phenotypic assays known to those of skill in the art.

The term "nucleic acid" as used herein refers to a polymer containing at least two nucleotides (*i.e.*, deoxyribonucleotides or ribonucleotides) in either single- or double-stranded form and includes DNA and RNA. "Nucleotides" contain a sugar deoxyribose (DNA) or ribose (RNA), a base, and a phosphate group. Nucleotides are linked together through the phosphate groups. "Bases" include purines and pyrimidines, which further include natural compounds adenine, thymine, guanine, cytosine, uracil, inosine, and natural analogs, and synthetic derivatives of purines and pyrimidines, which include, but are not limited to, modifications which place new reactive groups such as, but not limited to, amines, alcohols, thiols, carboxylates, and alkylhalides. Nucleic acids include nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, and which have similar binding properties as the reference nucleic acid. Examples of such analogs and/or modified residues include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2'-O-methyl ribonucleotides, and peptide-nucleic acids (PNAs). Additionally, nucleic acids can include one or more UNA moieties.

The term "nucleic acid" includes any oligonucleotide or polynucleotide, with fragments containing up to 60 nucleotides generally termed oligonucleotides, and longer fragments termed polynucleotides. A deoxyribooligonucleotide consists of a 5-carbon sugar called deoxyribose joined covalently to phosphate at the 5' and 3' carbons of this sugar to form an alternating, unbranched polymer. DNA may be in the form of, *e.g.*, antisense molecules, plasmid DNA, pre-condensed DNA, a PCR product, vectors, expression cassettes, chimeric sequences, chromosomal DNA, or derivatives and combinations of these groups. A ribooligonucleotide consists of a similar repeating structure where the 5-carbon sugar is ribose. RNA may be in the form, for example, of small interfering RNA (siRNA), Dicer-substrate dsRNA, small hairpin RNA (shRNA), asymmetrical interfering RNA (aiRNA), microRNA (miRNA), mRNA, tRNA, rRNA, tRNA, viral RNA (vRNA), and combinations thereof. Accordingly, in the context of this invention, the terms "polynucleotide" and "oligonucleotide" refer to a polymer or oligomer of nucleotide or nucleoside monomers consisting of naturally-occurring bases, sugars and intersugar (backbone) linkages. The terms "polynucleotide" and "oligonucleotide" also include polymers or oligomers comprising non-naturally occurring monomers, or portions thereof, which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of properties such as, for

example, enhanced cellular uptake, reduced immunogenicity, and increased stability in the presence of nucleases.

Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.,* degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, *Nucleic Acid Res.,* 19:5081 (1991); Ohtsuka *et al.*, *J. Biol. Chem.,* 260:2605-2608 (1985); Rossolini *et al.*, *Mol. Cell. Probes,* 8:91-98 (1994)).

The term "gene" refers to a nucleic acid (*e.g.*, DNA or RNA) sequence that comprises partial length or entire length coding sequences necessary for the production of a polypeptide or precursor polypeptide.

"Gene product," as used herein, refers to a product of a gene such as an RNA transcript or a polypeptide.

As used herein, the term "alkyl", by itself or as part of another substituent, means, unless otherwise stated, a straight or branched chain hydrocarbon radical, having the number of carbon atoms designated (i.e., C_{1-8} means one to eight carbons). Examples of alkyl groups include methyl, ethyl, n-propyl, iso-propyl, n-butyl, t-butyl, iso-butyl, sec-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. The term "alkenyl" refers to an unsaturated alkyl radical having one or more double bonds. Similarly, the term "alkynyl" refers to an unsaturated alkyl radical having one or more triple bonds. Examples of such unsaturated alkyl groups include vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butynyl, and the higher homologs and isomers.

The term "alkylene" by itself or as part of another substituent means a divalent radical derived from an alkane (including straight and branched alkanes), as exemplified by $-CH_2CH_2CH_2CH_2-$ and $-CH(CH_3)CH_2CH_2-$.

The term "cycloalkyl," "carbocyclic," or "carbocycle" refers to hydrocarbon ringsystem having 3 to 20 overall number of ring atoms (e.g., 3-20 membered cycloalkyl is a cycloalkyl with 3 to 20 ring atoms, or C_{3-20} cycloalkyl is a cycloalkyl with 3-20 carbon ring atoms) and for a 3-5 membered cycloalkyl being fully saturated or having no more than one double bond between ring vertices and for a 6 membered cycloalkyl or larger being fully saturated or having no more than two double bonds between ring vertices. As used herein, "cycloalkyl," "carbocyclic," or "carbocycle" is also meant to refer to bicyclic, polycyclic and spirocyclic

hydrocarbon ring system, such as, for example, bicyclo[2.2.1]heptane, pinane, bicyclo[2.2.2]octane, adamantane, norborene, spirocyclic C_{5-12} alkane, etc. As used herein, the terms, "alkenyl," "alkynyl," "cycloalkyl,", "carbocycle," and "carbocyclic," are meant to include mono and polyhalogenated variants thereof.

The term "heterocycloalkyl," "heterocyclic," or "heterocycle" refers to a saturated or partially unsaturated ring system radical having the overall having from 3-20 ring atoms (e.g., 3-20 membered heterocycloalkyl is a heterocycloalkyl radical with 3-20 ring atoms, a C_{2-19} heterocycloalkyl is a heterocycloalkyl having 3-10 ring atoms with between 2-19 ring atoms being carbon) that contain from one to ten heteroatoms selected from N, O, and S, wherein the nitrogen and sulfur atoms are optionally oxidized, nitrogen atom(s) are optionally quaternized, as ring atoms. Unless otherwise stated, a "heterocycloalkyl," "heterocyclic," or "heterocycle" ring can be a monocyclic, a bicyclic, spirocyclic or a polycylic ring system. Non limiting examples of "heterocycloalkyl," "heterocyclic," or "heterocycle" rings include pyrrolidine, piperidine, N-methylpiperidine, imidazolidine, pyrazolidine, butyrolactam, valerolactam, imidazolidinone, hydantoin, dioxolane, phthalimide, piperidine, pyrimidine-2,4(1H,3H)-dione, 1,4-dioxane, morpholine, thiomorpholine, thiomorpholine-S-oxide, thiomorpholine-S,S-oxide, piperazine, pyran, pyridone, 3-pyrroline, thiopyran, pyrone, tetrahydrofuran, tetrhydrothiophene, quinuclidine, tropane, 2-azaspiro[3.3]heptane, (1R,5S)-3-azabicyclo[3.2.1]octane, (1s,4s)-2-azabicyclo[2.2.2]octane, (1R,4R)-2-oxa-5-azabicyclo[2.2.2]octane and the like A "heterocycloalkyl," "heterocyclic," or "heterocycle" group can be attached to the remainder of the molecule through one or more ring carbons or heteroatoms. A "heterocycloalkyl," "heterocyclic," or "heterocycle" can include mono- and poly-halogenated variants thereof.

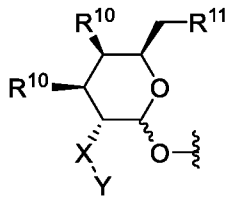
The terms "alkoxy," and “alkylthio”, are used in their conventional sense, and refer to those alkyl groups attached to the remainder of the molecule via an oxygen atom (“oxy”) or thio grou, and further include mono- and poly-halogenated variants thereof.

The terms "halo" or "halogen," by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. The term “(halo)alkyl” is meant to include both a “alkyl” and “haloalkyl” substituent. Additionally, the term "haloalkyl," is meant to include monohaloalkyl and polyhaloalkyl. For example, the term "C_{1-4} haloalkyl" is mean to include trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, difluoromethyl, and the like.

The term "aryl" means a carbocyclic aromatic group having 6-14 carbon atoms, whether or not fused to one or more groups. Examples of aryl groups include phenyl, naphthyl, biphenyl and the like unless otherwise stated.

The term "heteroaryl" refers to aryl ring(s) that contain from one to five heteroatoms selected from N, O, and S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A heteroaryl group can be attached to the remainder of the molecule through a heteroatom. Examples of heteroaryl groups include pyridyl, pyridazinyl, pyrazinyl, pyrimindinyl, triazinyl, quinolinyl, quinoxalinyl, quinazolinyl, cinnolinyl, phthalaziniyl, benzotriazinyl, purinyl, benzimidazolyl, benzopyrazolyl, benzotriazolyl, benzisoxazolyl, isobenzofuryl, isoindolyl, indolizinyl, benzotriazinyl, thienopyridinyl, thienopyrimidinyl, pyrazolopyrimidinyl, imidazopyridines, benzothiaxolyl, benzofuranyl, benzothienyl, indolyl, quinolyl, isoquinolyl, isothiazolyl, pyrazolyl, indazolyl, pteridinyl, imidazolyl, triazolyl, tetrazolyl, oxazolyl, isoxazolyl, thiadiazolyl, pyrrolyl, thiazolyl, furyl, thienyl and the like.

The term saccharide includes monosaccharides, disaccharides and trisaccharides. The term includes glucose, sucrose fructose, galactose and ribose, as well as deoxy sugars such as deoxyribose and amino sugar such as galactosamine. Saccharide derivatives can conveniently be prepared as described in International Patent Applications Publication Numbers WO 96/34005 and 97/03995. A saccharide can conveniently be linked to the remainder of a compound of formula I through an ether bond, a thioether bond (e.g. an S-glycoside), an amine nitrogen (e.g., an *N*-glycoside), or a carbon-carbon bond (e.g. a C-glycoside). In one embodiment the saccharide can conveniently be linked to the remainder of a compound of formula I through an ether bond. In one embodiment the term saccharide includes a group of the formula:



wherein:

X is NR^3, and Y is selected from $-(C=O)R^4$, $-SO_2R^5$, and $-(C=O)NR^6R^7$; or X is -(C=O)- and Y is NR^8R^9;

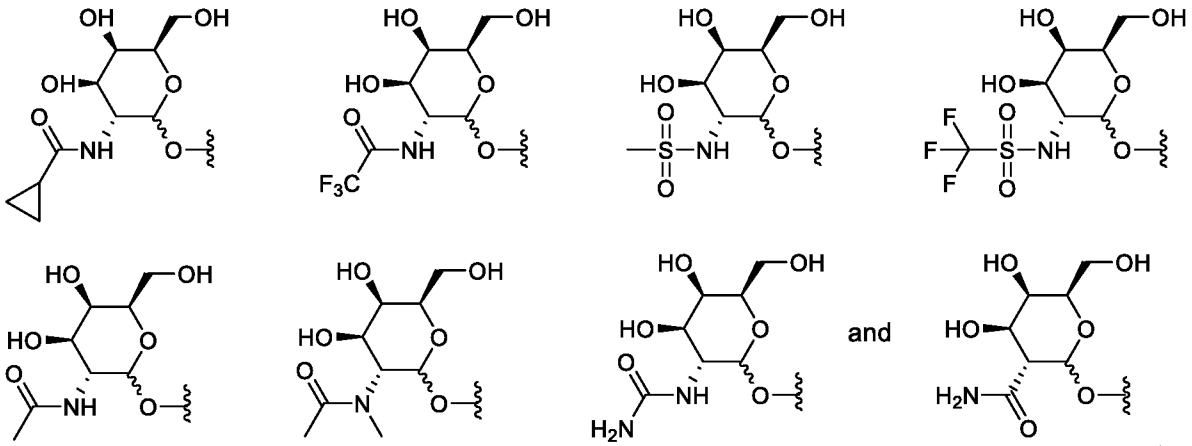
R^3 is hydrogen or $(C_1\text{-}C_4)$alkyl;

R^4, R^5, R^6, R^7 , R^8 and R^9 are each independently selected from the group consisting of hydrogen, $(C_1\text{-}C_8)$alkyl, $(C_1\text{-}C_8)$haloalkyl, $(C_1\text{-}C_8)$alkoxy and $(C_3\text{-}C_6)$cycloalkyl that is

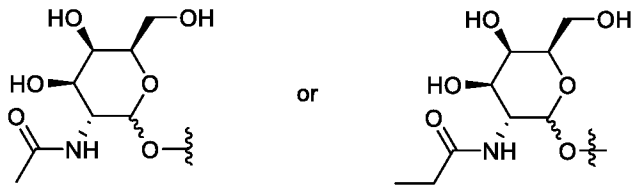
optionally substituted with one or more groups independently selected from the group consisting of halo, (C_1-C_4)alkyl, (C_1-C_4)haloalkyl, (C_1-C_4)alkoxy and (C_1-C_4)haloalkoxy;

R^{10} is -OH, -NR^8R^9 or – F; and

R^{11} is -OH, -NR^8R^9, -F or 5 membered heterocycle that is optionally substituted with one or more groups independently selected from the group consisting of halo, hydroxyl, carboxyl, amino, (C_1-C_4)alkyl, (C_1-C_4)haloalkyl, (C_1-C_4)alkoxy and (C_1-C_4)haloalkoxy. In another embodiment the saccharide can be selected from the group consisting of:



In another embodiment the saccharide can be:



N-Acetylgalactosamine (GalNAc) GalPro.

The term "animal" includes mammalian species, such as a human, mouse, rat, dog, cat, hamster, guinea pig, rabbit, livestock, and the like.

The term "lipid" refers to a group of organic compounds that include, but are not limited to, esters of fatty acids and are characterized by being insoluble in water, but soluble in many organic solvents. They are usually divided into at least three classes: (1) "simple lipids," which include fats and oils as well as waxes; (2) "compound lipids," which include phospholipids and glycolipids; and (3) "derived lipids" such as steroids.

The term "lipid particle" includes a lipid formulation that can be used to deliver a therapeutic nucleic acid (*e.g.*, siRNA) to a target site of interest (*e.g.*, cell, tissue, organ, and the like). In preferred embodiments, the lipid particle of the invention is a nucleic acid-lipid particle, which is typically formed from a cationic lipid, a non-cationic lipid (e.g., a phospholipid), a conjugated lipid that prevents aggregation of the particle (e.g., a PEG-lipid),

and optionally cholesterol. Typically, the therapeutic nucleic acid (*e.g.*, siRNA) may be encapsulated in the lipid portion of the particle, thereby protecting it from enzymatic degradation.

The term "electron dense core", when used to describe a lipid particle of the present invention, refers to the dark appearance of the interior portion of a lipid particle when visualized using cryo transmission electron microscopy ("cryoTEM"). Some lipid particles of the present invention have an electron dense core and lack a lipid bilayer structure. Some lipid particles of the present invention have an electron dense core, lack a lipid bilayer structure, and have an inverse Hexagonal or Cubic phase structure. While not wishing to be bound by theory, it is thought that the non-bilayer lipid packing provides a 3 -dimensional network of lipid cylinders with water and nucleic on the inside, i.e., essentially, a lipid droplet interpenetrated with aqueous channels containg the nucleic acid.

As used herein, the term "SNALP" refers to a stable nucleic acid-lipid particle. A SNALP is a particle made from lipids (*e.g.*, a cationic lipid, a non-cationic lipid, and a conjugated lipid that prevents aggregation of the particle), wherein the nucleic acid (*e.g.*, siRNA) is fully encapsulated within the lipid. In certain instances, SNALP are extremely useful for systemic applications, as they can exhibit extended circulation lifetimes following intravenous (i.v.) injection, they can accumulate at distal sites (*e.g.*, sites physically separated from the administration site), and they can mediate siRNA expression at these distal sites. The nucleic acid may be complexed with a condensing agent and encapsulated within a SNALP as set forth in PCT Publication No. WO 00/03683, the disclosure of which is herein incorporated by reference in its entirety for all purposes.

The lipid particles of the invention (*e.g.*, SNALP) typically have a mean diameter of from about 30 nm to about 150 nm, from about 40 nm to about 150 nm, from about 50 nm to about 150 nm, from about 60 nm to about 130 nm, from about 70 nm to about 110 nm, from about 70 nm to about 100 nm, from about 80 nm to about 100 nm, from about 90 nm to about 100 nm, from about 70 to about 90 nm, from about 80 nm to about 90 nm, from about 70 nm to about 80 nm, or about 30 nm, 35 nm, 40 nm, 45 nm, 50 nm, 55 nm, 60 nm, 65 nm, 70 nm, 75 nm, 80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 105 nm, 110 nm, 115 nm, 120 nm, 125 nm, 130 nm, 135 nm, 140 nm, 145 nm, or 150 nm, and are substantially non-toxic. In addition, nucleic acids, when present in the lipid particles of the present invention, are resistant in aqueous solution to degradation with a nuclease. Nucleic acid-lipid particles and their method of preparation are disclosed in, *e.g.*, U.S. Patent Publication Nos. 20040142025 and

20070042031, the disclosures of which are herein incorporated by reference in their entirety for all purposes.

As used herein, "lipid encapsulated" can refer to a lipid particle that provides a therapeutic nucleic acid such as an siRNA with full encapsulation, partial encapsulation, or both. In a preferred embodiment, the nucleic acid (*e.g.,* siRNA) is fully encapsulated in the lipid particle (*e.g.*, to form a SNALP or other nucleic acid-lipid particle).

The term "lipid conjugate" refers to a conjugated lipid that inhibits aggregation of lipid particles. Such lipid conjugates include, but are not limited to, PEG-lipid conjugates such as, *e.g.,* PEG coupled to dialkyloxypropyls (*e.g.*, PEG-DAA conjugates), PEG coupled to diacylglycerols (*e.g.*, PEG-DAG conjugates), PEG coupled to cholesterol, PEG coupled to phosphatidylethanolamines, and PEG conjugated to ceramides (*see, e.g.,* U.S. Patent No. 5,885,613), cationic PEG lipids, polyoxazoline (POZ)-lipid conjugates, polyamide oligomers (*e.g.,* ATTA-lipid conjugates), and mixtures thereof. Additional examples of POZ-lipid conjugates are described in PCT Publication No. WO 2010/006282. PEG or POZ can be conjugated directly to the lipid or may be linked to the lipid via a linker moiety. Any linker moiety suitable for coupling the PEG or the POZ to a lipid can be used including, *e.g.,* non-ester containing linker moieties and ester-containing linker moieties. In certain preferred embodiments, non-ester containing linker moieties, such as amides or carbamates, are used. The disclosures of each of the above patent documents are herein incorporated by reference in their entirety for all purposes.

The term "amphipathic lipid" refers, in part, to any suitable material wherein the hydrophobic portion of the lipid material orients into a hydrophobic phase, while the hydrophilic portion orients toward the aqueous phase. Hydrophilic characteristics derive from the presence of polar or charged groups such as carbohydrates, phosphate, carboxylic, sulfato, amino, sulfhydryl, nitro, hydroxyl, and other like groups. Hydrophobicity can be conferred by the inclusion of apolar groups that include, but are not limited to, long-chain saturated and unsaturated aliphatic hydrocarbon groups and such groups substituted by one or more aromatic, cycloaliphatic, or heterocyclic group(s). Examples of amphipathic compounds include, but are not limited to, phospholipids, aminolipids, and sphingolipids.

Representative examples of phospholipids include, but are not limited to, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyloleoyl phosphatidylcholine, lysophosphatidylcholine, lysophosphatidylethanolamine, dipalmitoylphosphatidylcholine, dioleoylphosphatidylcholine, distearoylphosphatidylcholine, and dilinoleoylphosphatidylcholine. Other compounds lacking

in phosphorus, such as sphingolipid, glycosphingolipid families, diacylglycerols, and β-acyloxyacids, are also within the group designated as amphipathic lipids. Additionally, the amphipathic lipids described above can be mixed with other lipids including triglycerides and sterols.

The term "neutral lipid" refers to any of a number of lipid species that exist either in an uncharged or neutral zwitterionic form at a selected pH. At physiological pH, such lipids include, for example, diacylphosphatidylcholine, diacylphosphatidylethanolamine, ceramide, sphingomyelin, cephalin, cholesterol, cerebrosides, and diacylglycerols.

The term "non-cationic lipid" refers to any amphipathic lipid as well as any other neutral lipid or anionic lipid.

The term "anionic lipid" refers to any lipid that is negatively charged at physiological pH. These lipids include, but are not limited to, phosphatidylglycerols, cardiolipins, diacylphosphatidylserines, diacylphosphatidic acids, N-dodecanoyl phosphatidylethanolamines, N-succinyl phosphatidylethanolamines, N-glutarylphosphatidylethanolamines, lysylphosphatidylglycerols, palmitoyloleyolphosphatidylglycerol (POPG), and other anionic modifying groups joined to neutral lipids.

The term "hydrophobic lipid" refers to compounds having apolar groups that include, but are not limited to, long-chain saturated and unsaturated aliphatic hydrocarbon groups and such groups optionally substituted by one or more aromatic, cycloaliphatic, or heterocyclic group(s). Suitable examples include, but are not limited to, diacylglycerol, dialkylglycerol, N-N-dialkylamino, 1,2-diacyloxy-3-aminopropane, and 1,2-dialkyl-3-aminopropane.

The terms "cationic lipid" and "amino lipid" are used interchangeably herein to include those lipids and salts thereof having one, two, three, or more fatty acid or fatty alkyl chains and a pH-titratable amino head group (*e.g.*, an alkylamino or dialkylamino head group). The cationic lipid is typically protonated (*i.e.*, positively charged) at a pH below the pK_a of the cationic lipid and is substantially neutral at a pH above the pK_a. The cationic lipids of the invention may also be termed titratable cationic lipids. In some embodiments, the cationic lipids comprise: a protonatable tertiary amine (*e.g.*, pH-titratable) head group; C_{18} alkyl chains, wherein each alkyl chain independently has 0 to 3 (*e.g.*, 0, 1, 2, or 3) double bonds; and ether, ester, or ketal linkages between the head group and alkyl chains. Such cationic lipids include, but are not limited to, DSDMA, DODMA, DLinDMA, DLenDMA, γ-DLenDMA, DLin-K-DMA, DLin-K-C2-DMA (also known as DLin-C2K-DMA, XTC2, and C2K), DLin-

K-C3-DMA, DLin-K-C4-DMA, DLen-C2K-DMA, γ-DLen-C2K-DMA, DLin-M-C2-DMA (also known as MC2), DLin-M-C3-DMA (also known as MC3) and (DLin-MP-DMA)(also known as 1-B11).

The term "alkylamino" includes a group of formula –N(H)R, wherein R is an alkyl as defined herein.

The term "dialkylamino" includes a group of formula $–NR_2$, wherein each R is independently an alkyl as defined herein.

The term "salts" includes any anionic and cationic complex, such as the complex formed between a cationic lipid and one or more anions. Non-limiting examples of anions include inorganic and organic anions, *e.g.,* hydride, fluoride, chloride, bromide, iodide, oxalate (*e.g.,* hemioxalate), phosphate, phosphonate, hydrogen phosphate, dihydrogen phosphate, oxide, carbonate, bicarbonate, nitrate, nitrite, nitride, bisulfite, sulfide, sulfite, bisulfate, sulfate, thiosulfate, hydrogen sulfate, borate, formate, acetate, benzoate, citrate, tartrate, lactate, acrylate, polyacrylate, fumarate, maleate, itaconate, glycolate, gluconate, malate, mandelate, tiglate, ascorbate, salicylate, polymethacrylate, perchlorate, chlorate, chlorite, hypochlorite, bromate, hypobromite, iodate, an alkylsulfonate, an arylsulfonate, arsenate, arsenite, chromate, dichromate, cyanide, cyanate, thiocyanate, hydroxide, peroxide, permanganate, and mixtures thereof. In particular embodiments, the salts of the cationic lipids disclosed herein are crystalline salts.

The term "acyl" includes any alkyl, alkenyl, or alkynyl wherein the carbon at the point of attachment is substituted with an oxo group, as defined below. The following are non-limiting examples of acyl groups: -C(=O)alkyl, -C(=O)alkenyl, and -C(=O)alkynyl.

The term "fusogenic" refers to the ability of a lipid particle, such as a SNALP, to fuse with the membranes of a cell. The membranes can be either the plasma membrane or membranes surrounding organelles, *e.g.*, endosome, nucleus, *etc.*

As used herein, the term "aqueous solution" refers to a composition comprising in whole, or in part, water.

As used herein, the term "organic lipid solution" refers to a composition comprising in whole, or in part, an organic solvent having a lipid.

"Distal site," as used herein, refers to a physically separated site, which is not limited to an adjacent capillary bed, but includes sites broadly distributed throughout an organism.

"Serum-stable" in relation to nucleic acid-lipid particles such as SNALP means that the particle is not significantly degraded after exposure to a serum or nuclease assay that would

significantly degrade free DNA or RNA. Suitable assays include, for example, a standard serum assay, a DNAse assay, or an RNAse assay.

"Systemic delivery," as used herein, refers to delivery of lipid particles that leads to a broad biodistribution of an active agent such as an siRNA within an organism. Some techniques of administration can lead to the systemic delivery of certain agents, but not others. Systemic delivery means that a useful, preferably therapeutic, amount of an agent is exposed to most parts of the body. To obtain broad biodistribution generally requires a blood lifetime such that the agent is not rapidly degraded or cleared (such as by first pass organs (liver, lung, *etc.*) or by rapid, nonspecific cell binding) before reaching a disease site distal to the site of administration. Systemic delivery of lipid particles can be by any means known in the art including, for example, intravenous, subcutaneous, and intraperitoneal. In a preferred embodiment, systemic delivery of lipid particles is by intravenous delivery.

"Local delivery," as used herein, refers to delivery of an active agent such as an siRNA directly to a target site within an organism. For example, an agent can be locally delivered by direct injection into a disease site, other target site, or a target organ such as the liver, heart, pancreas, kidney, and the like.

When used herein to describe the ratio of lipid:siRNA, the term "lipid" refers to the total lipid in the particle.

It will be appreciated by those skilled in the art that compounds of the invention having a chiral center may exist in and be isolated in optically active and racemic forms. Some compounds may exhibit polymorphism. It is to be understood that the present invention encompasses any racemic, optically-active, polymorphic, or stereoisomeric form, or mixtures thereof, of a compound of the invention, which possess the useful properties described herein, it being well known in the art how to prepare optically active forms (for example, by resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase.

When a bond in a compound formula herein is drawn in a non-stereochemical manner (e.g. flat), the atom to which the bond is attached includes all stereochemical possibilities. Unless otherwise specifically noted, when a bond in a compound formula herein is drawn in a defined stereochemical manner (e.g. bold, bold-wedge, dashed or dashed-wedge), it is to be understood that the atom to which the stereochemical bond is attached is enriched in the absolute stereoisomer depicted. In one embodiment, the compound may be at least 51% the absolute stereoisomer depicted. In another embodiment, the compound may be at least 60% the absolute stereoisomer depicted. In another embodiment, the compound may be at least

80% the absolute stereoisomer depicted. In another embodiment, the compound may be at least 90% the absolute stereoisomer depicted. In another embodiment, the compound may be at least 95 the absolute stereoisomer depicted. In another embodiment, the compound may be at least 99% the absolute stereoisomer depicted.

Unless stated otherwise herein, the term "about", when used in connection with a value or range of values, means plus or minus 5% of the stated value or range of values.

Generating siRNA Molecules

siRNA can be provided in several forms including, *e.g.*, as one or more isolated small-interfering RNA (siRNA) duplexes, as longer double-stranded RNA (dsRNA), or as siRNA or dsRNA transcribed from a transcriptional cassette in a DNA plasmid. In some embodiments, siRNA may be produced enzymatically or by partial/total organic synthesis, and modified ribonucleotides can be introduced by *in vitro* enzymatic or organic synthesis. In certain instances, each strand is prepared chemically. Methods of synthesizing RNA molecules are known in the art, *e.g.,* the chemical synthesis methods as described in Verma and Eckstein (1998) or as described herein.

Methods for isolating RNA, synthesizing RNA, hybridizing nucleic acids, making and screening cDNA libraries, and performing PCR are well known in the art (*see, e.g.,* Gubler and Hoffman, *Gene*, 25:263-269 (1983); Sambrook *et al., supra*; Ausubel *et al., supra*), as are PCR methods (*see,* U.S. Patent Nos. 4,683,195 and 4,683,202; *PCR Protocols: A Guide to Methods and Applications* (Innis *et al.*, eds, 1990)). Expression libraries are also well known to those of skill in the art. Additional basic texts disclosing the general methods of use in this invention include Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (2nd ed. 1989); Kriegler, *Gene Transfer and Expression: A Laboratory Manual* (1990); and *Current Protocols in Molecular Biology* (Ausubel *et al.*, eds., 1994). The disclosures of these references are herein incorporated by reference in their entirety for all purposes.

Typically, siRNA are chemically synthesized. The oligonucleotides that comprise the siRNA molecules of the invention can be synthesized using any of a variety of techniques known in the art, such as those described in Usman *et al.*, *J. Am. Chem. Soc.*, 109:7845 (1987); Scaringe *et al.*, *Nucl. Acids Res.*, 18:5433 (1990); Wincott *et al.*, *Nucl. Acids Res.*, 23:2677-2684 (1995); and Wincott *et al., Methods Mol. Bio.*, 74:59 (1997). The synthesis of oligonucleotides makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end and phosphoramidites at the 3'-end. As a non-limiting example, small scale syntheses can be conducted on an Applied Biosystems synthesizer using a 0.2 μmol

scale protocol. Alternatively, syntheses at the 0.2 μmol scale can be performed on a 96-well plate synthesizer from Protogene (Palo Alto, CA). However, a larger or smaller scale of synthesis is also within the scope of this invention. Suitable reagents for oligonucleotide synthesis, methods for RNA deprotection, and methods for RNA purification are known to those of skill in the art.

siRNA molecules can be assembled from two distinct oligonucleotides, wherein one oligonucleotide comprises the sense strand and the other comprises the antisense strand of the siRNA. For example, each strand can be synthesized separately and joined together by hybridization or ligation following synthesis and/or deprotection.

Embodiments of the Invention

Table 1 in Example 25 describes a series of chemically modified siRNA duplexes (sense and antisense strands shown) that target the Hepatitis B virus (abbreviated as "HBV"). As described herein, a compound of the invention may comprise such a siRNA (i.e., siRNA 1-37).

Accordingly, one aspect of the invention is a nucleic acid molecule selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71 and SEQ ID NO:73.

Another aspect of this invention is a nucleic acid molecule selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72 and SEQ ID NO:74.

One aspect of the invention is a composition comprising a nucleic acid molecule described herein, or a combination thereof.

One aspect of the invention provides a double stranded siRNA molecule selected from the group consisting of siRNA 1 (SEQ ID NO:1 and 2), 2 (SEQ ID NO:3 and 4), 3 (SEQ ID NO:5 and 6), 4 (SEQ ID NO:7 and 8), 5 (SEQ ID NO:9 and 10), 6 (SEQ ID NO:11 and 12), 7 (SEQ ID NO:13 and 14), 8 (SEQ ID NO:15 and 16), 9 (SEQ ID NO:17 and 18), 10 (SEQ ID NO:19 and 20), 11 (SEQ ID NO:21 and 22), 12 (SEQ ID NO:23 and 24), 13 (SEQ ID NO:25 and 26), 14 (SEQ ID NO:27 and 28), 15 (SEQ ID NO:29 and 30), 16 (SEQ ID NO:31 and 32), 17 (SEQ ID NO:33 and 34), 18 (SEQ ID NO:35 and 36), 19 (SEQ ID NO:37 and 38), 20 (SEQ ID NO:39 and 40), 21 (SEQ ID NO:41 and 42), 22 (SEQ ID NO:43 and 44), 23 (SEQ ID NO:45 and 46), 24 (SEQ ID NO:47 and 48), 25 (SEQ ID NO:49 and 50), 26 (SEQ ID NO:51 and 52), 27 (SEQ ID NO:53 and 54), 28 (SEQ ID NO:55 and 56), 29 (SEQ ID NO:57 and 58), 30 (SEQ ID NO:59 and 60), 31 (SEQ ID NO:61 and 62), 32 (SEQ ID NO:63 and 64), 33 (SEQ ID NO:65 and 66), 34 (SEQ ID NO:67 and 68), 35 (SEQ ID NO:69 and 70), 36 (SEQ ID NO:71 and 72) and 37 (SEQ ID NO:73 and 74).

Another aspect of the invention provides a composition comprising a double stranded siRNA molecule described herein.

In one embodiment, the composition is a pharmaceutical composition that comprises a pharmaceutically acceptable carrier.

One aspect of the invention is a compound of formula I, as set forth about in the Summary of the Invention, or a salt thereof.

In one embodiment of the compound of formula I, R^1 a is targeting ligand;

L^1 is absent or a linking group;

L^2 is absent or a linking group;

R^2 is a double stranded siRNA molecule selected from the double stranded siRNA of Table 1;

the ring A is absent, a 3-20 membered cycloalkyl, a 5-20 membered aryl, a 5-20 membered heteroaryl, or a 3-20 membered heterocycloalkyl;

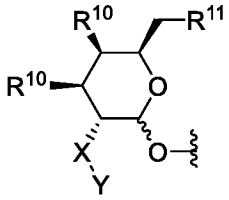
each R^A is independently selected from the group consisting of hydrogen, hydroxy, CN, F, Cl, Br, I, -C_{1-2} alkyl-OR^B and C_{1-8} alkyl that is optionally substituted with one or more groups independently selected from halo, hydroxy, and C_{1-3} alkoxy;

R^B is hydrogen, a protecting group, a covalent bond to a solid support, or a bond to a linking group that is bound to a solid support; and

n is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In one embodiment R^1 is $-C(H)_{(3-p)}(L^3\text{-saccharide})_p$, wherein each L^3 is independently a linking group; p is 1, 2, or 3; and saccharide is a monosaccharide or disaccharide.

In one embodiment the saccharide is:



wherein:

X is NR^3, and Y is selected from $-(C=O)R^4$, $-SO_2R^5$, and $-(C=O)NR^6R^7$; or X is -(C=O)- and Y is NR^8R^9;

R^3 is hydrogen or $(C_1\text{-}C_4)$alkyl;

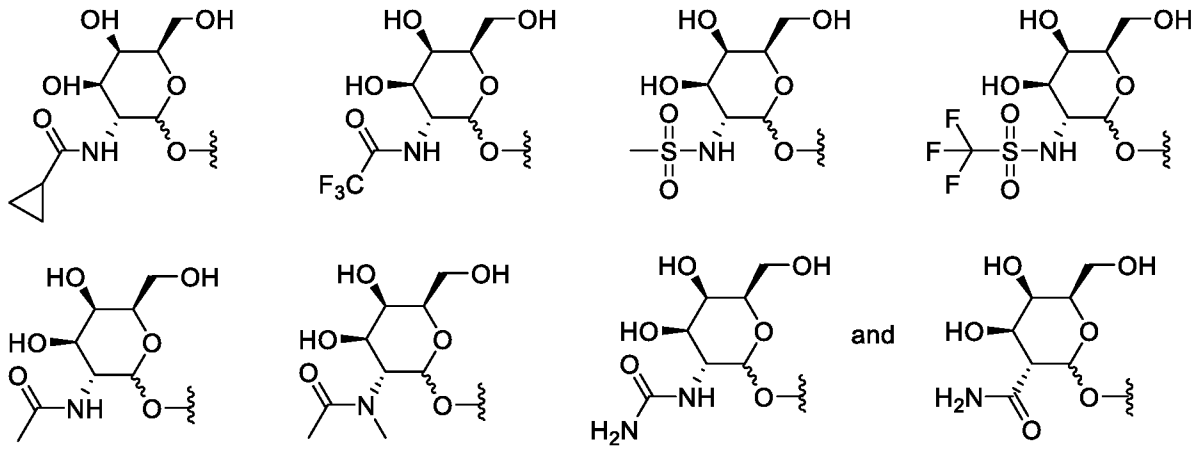
R^4, R^5, R^6, R^7 , R^8 and R^9 are each independently selected from the group consisting of hydrogen, $(C_1\text{-}C_8)$alkyl, $(C_1\text{-}C_8)$haloalkyl, $(C_1\text{-}C_8)$alkoxy and $(C_3\text{-}C_6)$cycloalkyl that is optionally substituted with one or more groups independently selected from the group consisting of halo, $(C_1\text{-}C_4)$alkyl, $(C_1\text{-}C_4)$haloalkyl, $(C_1\text{-}C_4)$alkoxy and $(C_1\text{-}C_4)$haloalkoxy;

R^{10} is -OH, $-NR^8R^9$ or – F; and

R^{11} is -OH, $-NR^8R^9$, -F or 5 membered heterocycle that is optionally substituted with one or more groups independently selected from the group consisting of halo, hydroxyl, carboxyl, amino, $(C_1\text{-}C_4)$alkyl, $(C_1\text{-}C_4)$haloalkyl, $(C_1\text{-}C_4)$alkoxy and $(C_1\text{-}C_4)$haloalkoxy;

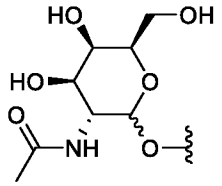
or a salt thereof.

In one embodiment the saccharide is selected from the group consisting of:

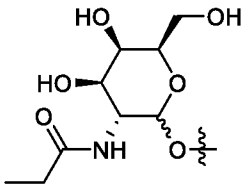


and salts thereof.

In one embodiment the saccharide is:



or

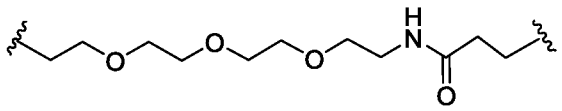


N-Acetylgalactosamine (GalNAc) GalPro

In one embodiment each L^3 is independently a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 0 to 50 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O-, -NR^X-, -NR^X-C(=O)-, -C(=O)-NR^X- or –S-, and wherein R^X is hydrogen or (C_1-C_6)alkyl, and wherein the hydrocarbon chain, is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C_1-C_6)alkoxy, (C_3-C_6)cycloalkyl, (C_1-C_6)alkanoyl, (C_1-C_6)alkanoyloxy, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

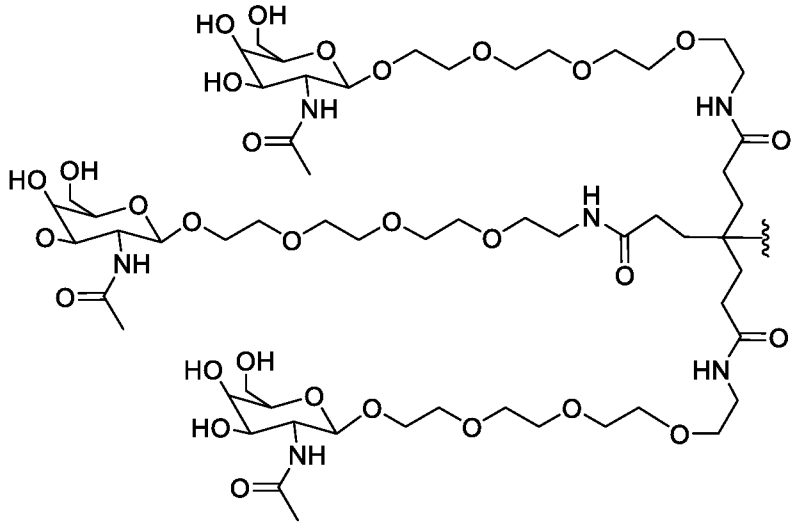
In one embodiment each L^3 is independently a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 20 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O-, -NR^X-, -NR^X-C(=O)-, -C(=O)-NR^X- or –S-, and wherein R^X is hydrogen or (C_1-C_6)alkyl, and wherein the hydrocarbon chain, is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C_1-C_6)alkoxy, (C_3-C_6)cycloalkyl, (C_1-C_6)alkanoyl, (C_1-C_6)alkanoyloxy, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

In one embodiment L^3 is:



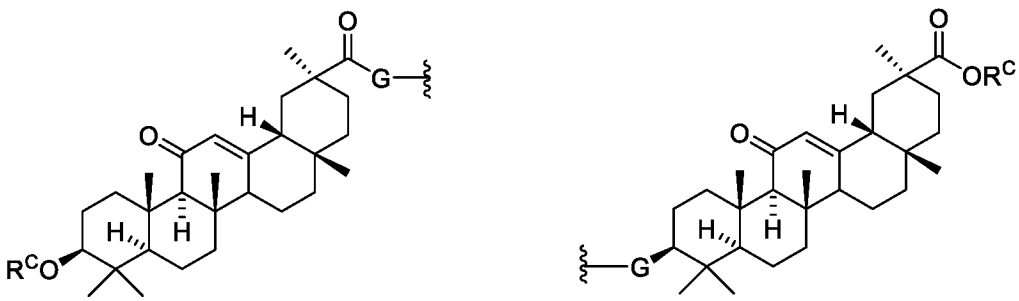
or a salt thereof.

In one embodiment R^1 is:



or a salt thereof.

In one embodiment R^1 is:

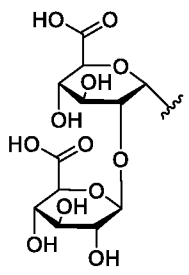


wherein G is –NH- or –O-;

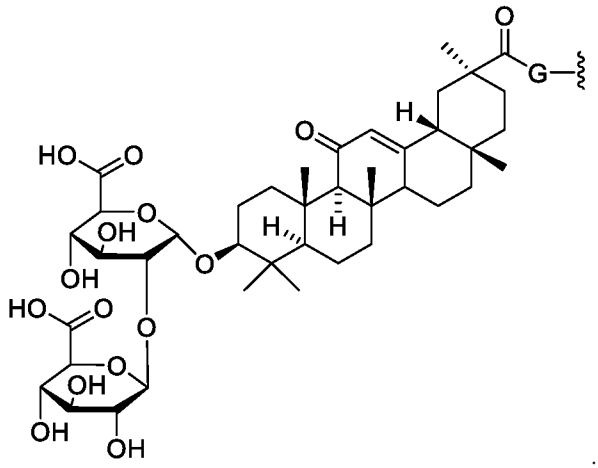
R^C is hydrogen, (C_1-C_8)alkyl, (C_1-C_8)haloalkyl, (C_1-C_8)alkoxy, (C_1-C_6)alkanoyl, (C_3-C_{20})cycloalkyl, (C_3-C_{20})heterocycle, aryl, heteroaryl, monosaccharide, disaccharide or trisaccharide; and wherein the cycloalkyl, heterocyle, ary, heteroaryl and saccharide are optionally substituted with one or more groups independently selected from the group consisting of halo, carboxyl, hydroxyl, amino, (C_1-C_4)alkyl, (C_1-C_4)haloalkyl, (C_1-C_4)alkoxy and (C_1-C_4)haloalkoxy;

or a salt thereof.

In one embodiment R^C is:



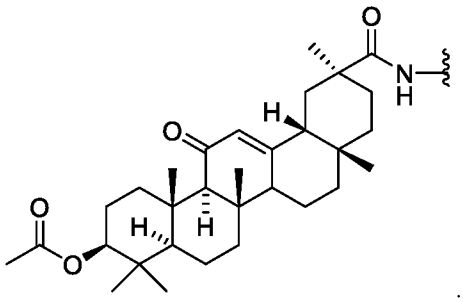
In one embodiment R^1 is:



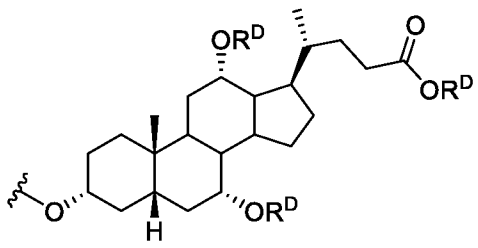
In one embodiment R^C is:

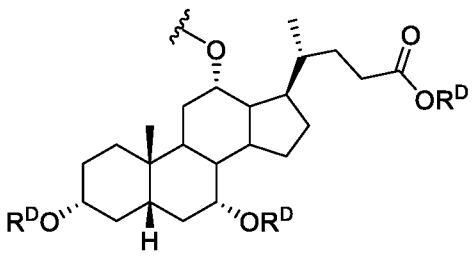
In one embodiment G is –NH-.

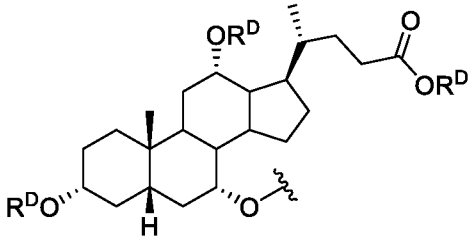
In one embodiment R^1 is:



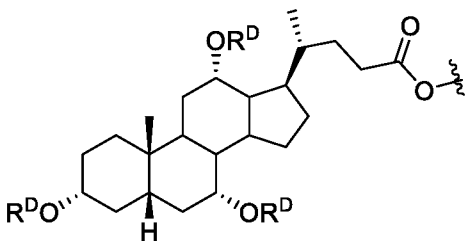
In one embodiment R^1 is:







or



wherein each R^D is independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, (C_9-C_{20})alkylsilyl, $(R^W)_3Si$-, (C_2-C_6)alkenyl, tetrahydropyranyl, (C_1-C_6)alkanoyl, benzoyl, aryl(C_1-C_3)alkyl, TMTr (Trimethoxytrityl), DMTr (Dimethoxytrityl), MMTr (Monomethoxytrityl), and Tr (Trityl); and

each R^W is independently selected from the group consisting of (C_1-C_4)alkyl and aryl.

In one embodiment linking groups L^1 and L^2 are independently a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 50 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O-, -NR^X-, -NR^X-C(=O)-, -C(=O)-NR^X- or –S-, and wherein R^X is hydrogen or (C_1-C_6)alkyl, and wherein the hydrocarbon chain, is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C_1-C_6)alkoxy, (C_3-C_6)cycloalkyl, (C_1-C_6)alkanoyl, (C_1-C_6)alkanoyloxy, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

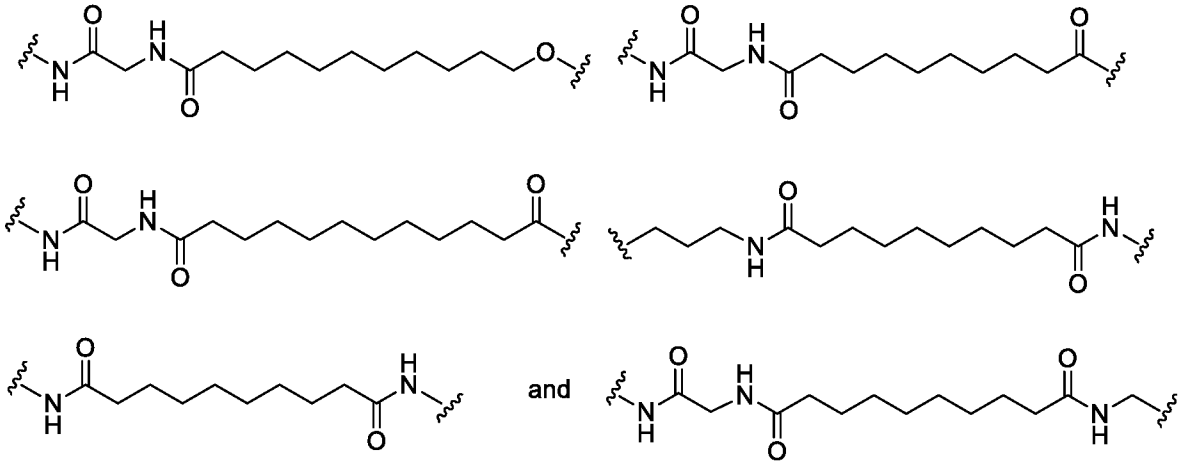
In one embodiment L^1 and L^2 are independently a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 20 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O-, -NR^X-, -NR^X-C(=O)-, -C(=O)-NR^X- or –S-, and wherein R^X is hydrogen or (C_1-C_6)alkyl, and wherein the hydrocarbon chain, is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C_1-C_6)alkoxy, (C_3-C_6)cycloalkyl, (C_1-C_6)alkanoyl, (C_1-C_6)alkanoyloxy, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

In one embodiment L^1 and L^2 are independently, a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 14 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced –O-, -NR^X-, -NR^X-C(=O)-, -C(=O)-NR^X- or –S-, and wherein R^X is hydrogen or (C_1-C_6)alkyl, and wherein the hydrocarbon chain, is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C_1-C_6)alkoxy, (C_3-C_6)cycloalkyl, (C_1-C_6)alkanoyl, (C_1-C_6)alkanoyloxy, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

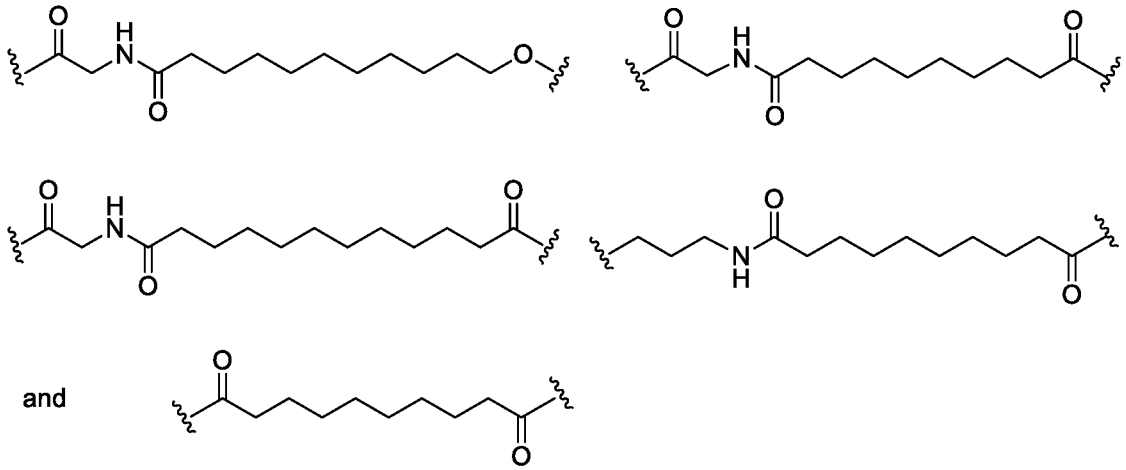
In one embodiment L^1 is connected to R^1 through -NH-, -O-, -S-, -(C=O)-, -(C=O)-NH-, -NH-(C=O)-, -(C=O)-O-, -NH-(C=O)-NH-, or –NH-(SO_2)-.

In one embodiment L^2 is connected to R^2 through -O-.

In one embodiment L^1 is selected from the group consisting of:



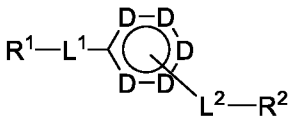
In one embodiment L^1 is selected from the group consisting of:



and salts thereof.

In one embodiment L^2 is $–CH_2-O-$ or $–CH_2-CH_2-O-$.

In one embodiment a compound of formula I has the following formula Ia:



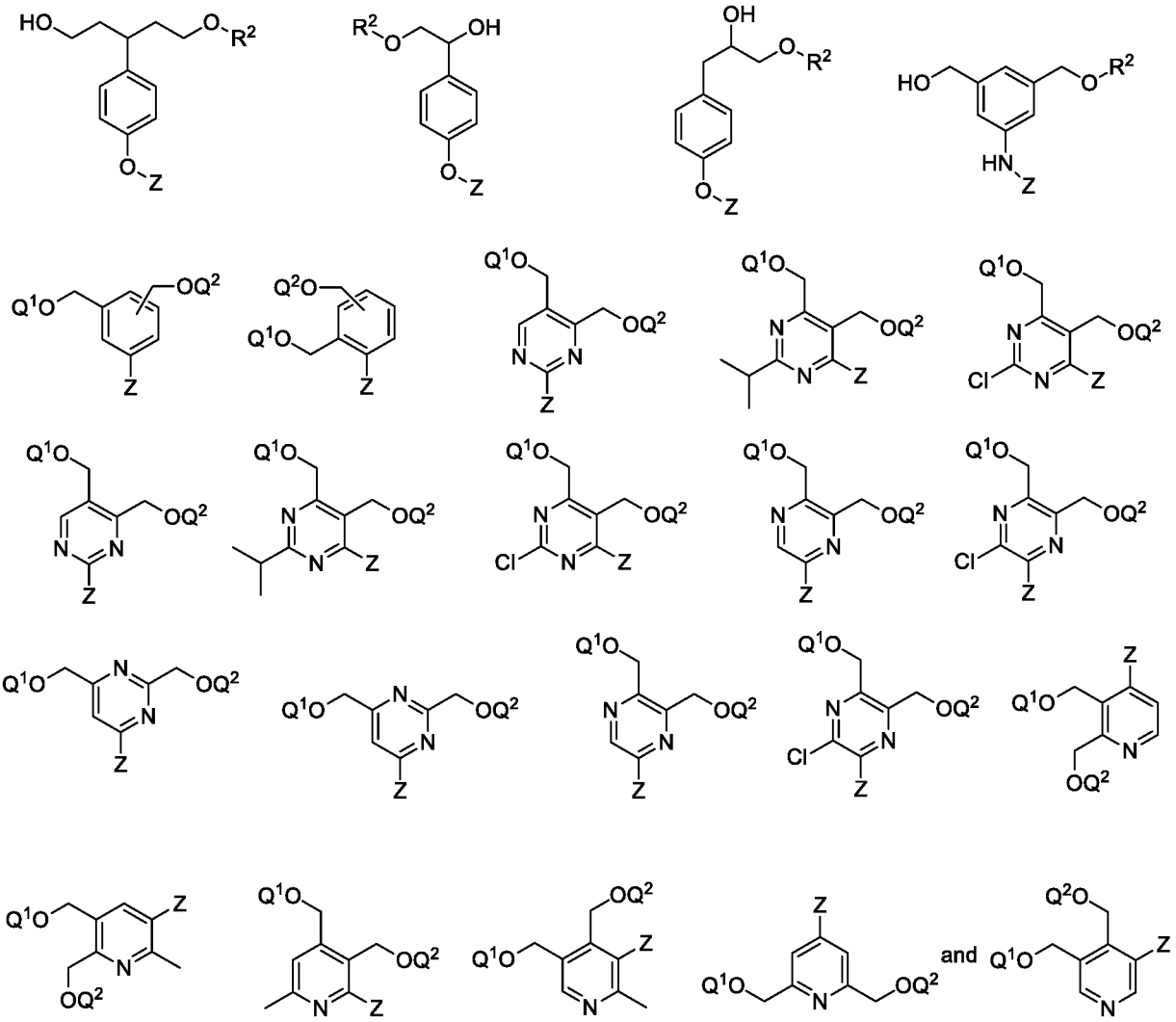
(Ia)

wherein:

each D is independently selected from the group consisting of  and –N=;

or a salt thereof.

In one embodiment a compound of formula Ia is selected from the group consisting of:



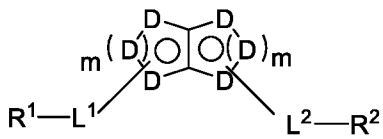
wherein:

Q^1 is hydrogen and Q^2 is R^2; or Q^1 is R^2 and Q^2 is hydrogen;

Z is $–L^1\text{-}R^1$;

and salts thereof.

In one embodiment a compound of formula I has the following formula Ib:



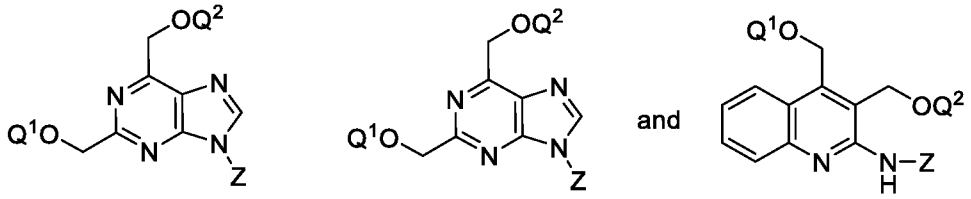
(Ib)

wherein:

each D is independently selected from the group consisting of  and –N=;

each m is independently 1 or 2; or a salt thereof.

In one embodiment a compound of formula Ib is selected from the group consisting of:



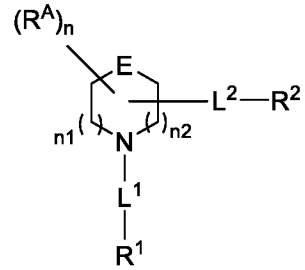
wherein:

Q^1 is hydrogen and Q^2 is R^2; or Q^1 is R^2 and Q^2 is hydrogen;

Z is $–L^1\text{-}R^1$;

and salts thereof.

In one embodiment a compound of formula I has the following formula (Ic):



(Ic)

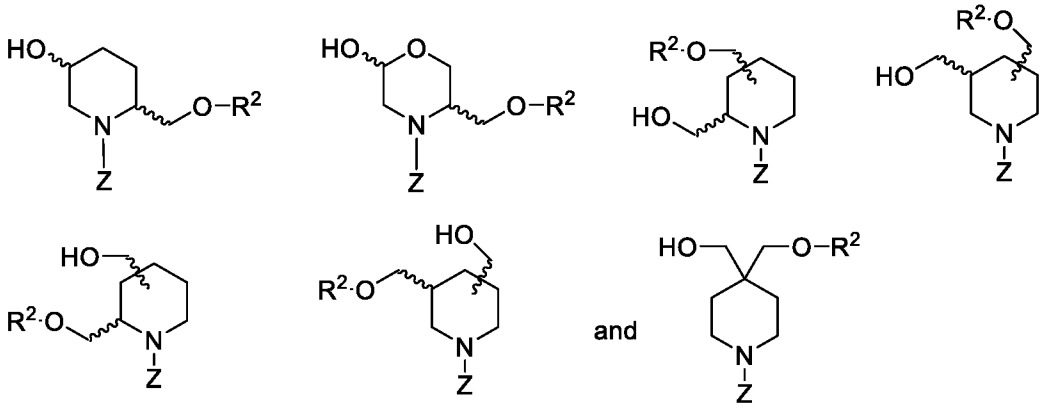
wherein E is –O- or $\text{-}CH_2\text{-}$;

n is selected from the group consisting of 0, 1, 2, 3, and 4; and

n1 and n2 are each independently selected from the group consisting of 0, 1, 2, and 3;

or a salt thereof.

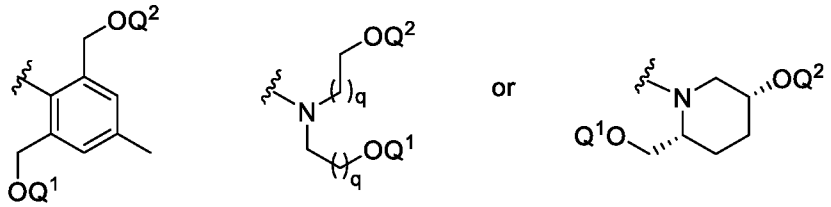
In certain embodiments a compound of formula (Ic) is selected from the group consisting of:



wherein Z is $–L^1\text{-}R^1$;

and salts thereof.

In one embodiment the -A-L^2-R^2 moiety is:



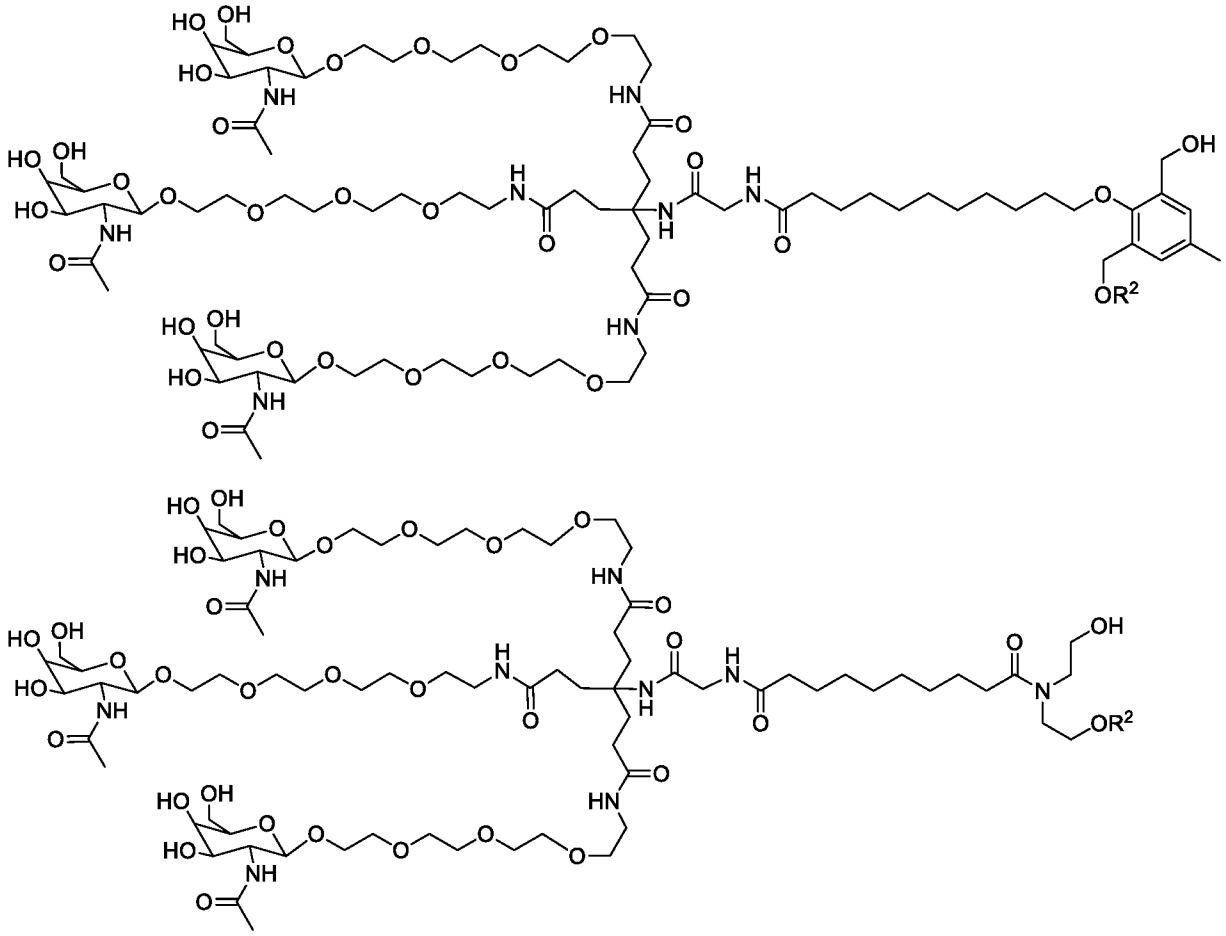
wherein:

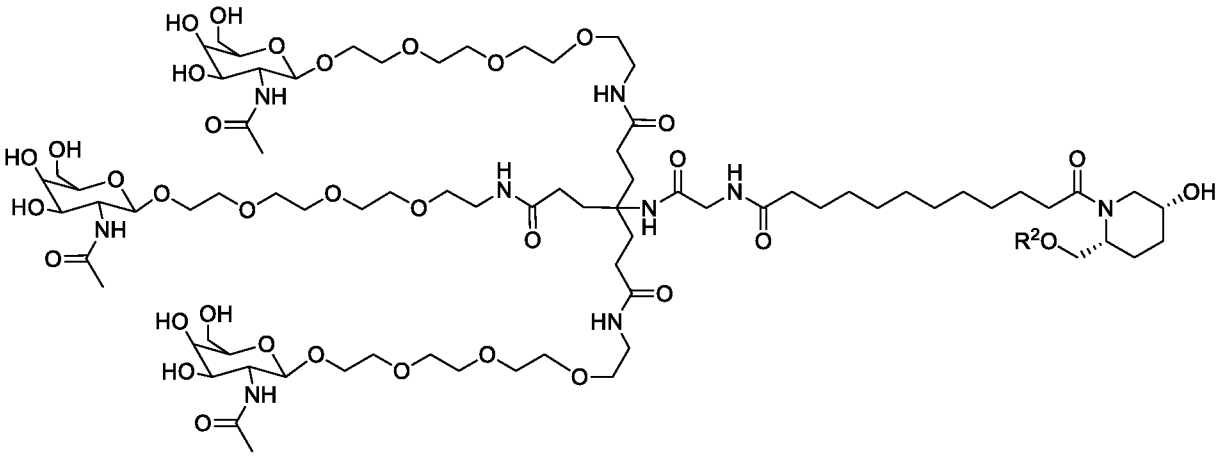
Q^1 is hydrogen and Q^2 is R^2; or Q^1 is R^2 and Q^2 is hydrogen; and

each q is independently 0, 1, 2, 3, 4 or 5;

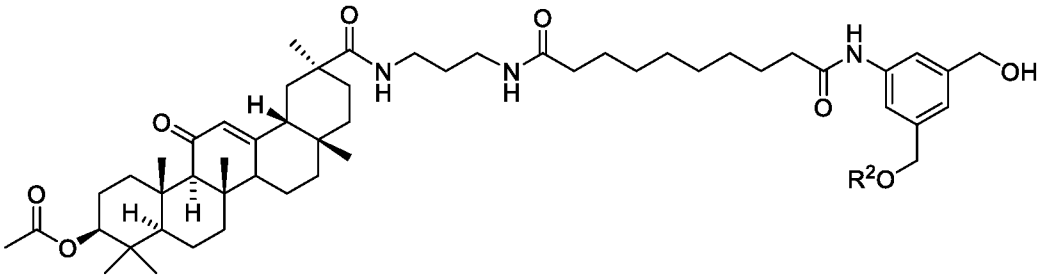
or a salt thereof.

In one embodiment a compound of formula (I) is selected from the group consisting of:



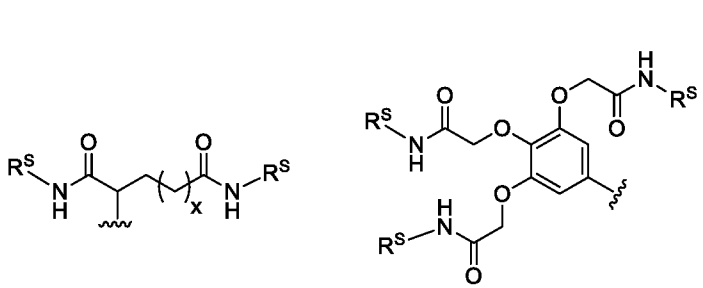


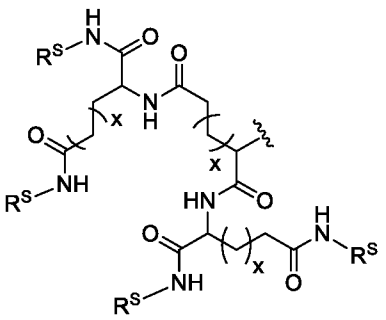
and

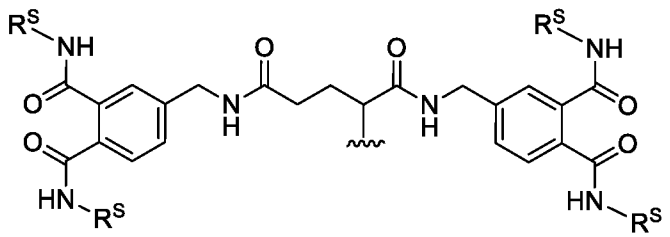


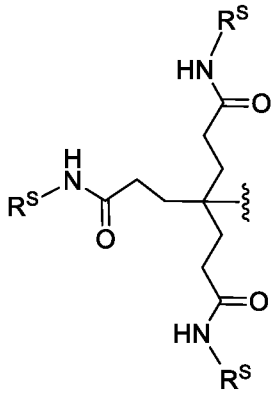
and salts thereof.

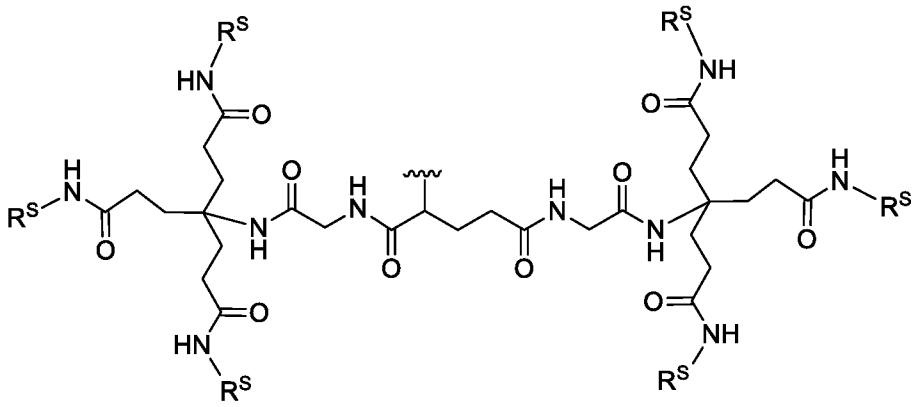
In one embodiment R^1 is selected from the group consisting of:

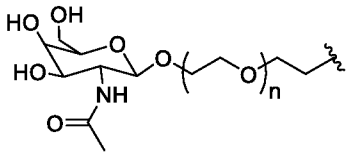








and 

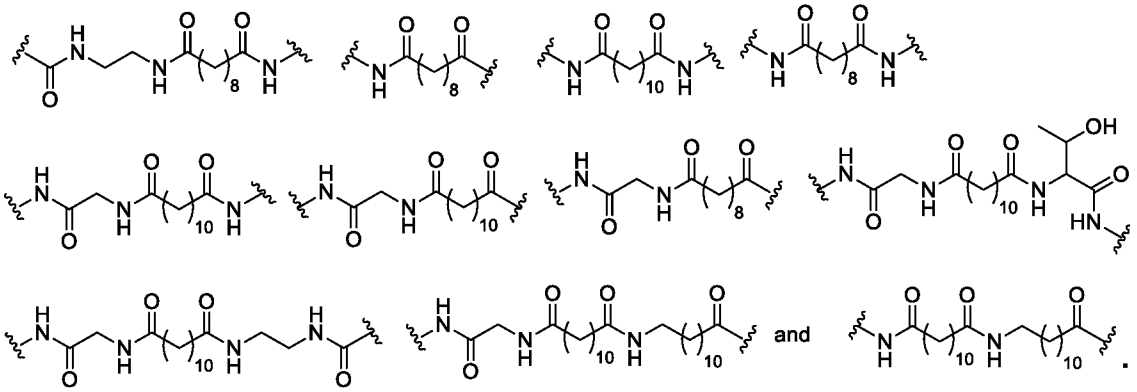


wherein R^S is ;

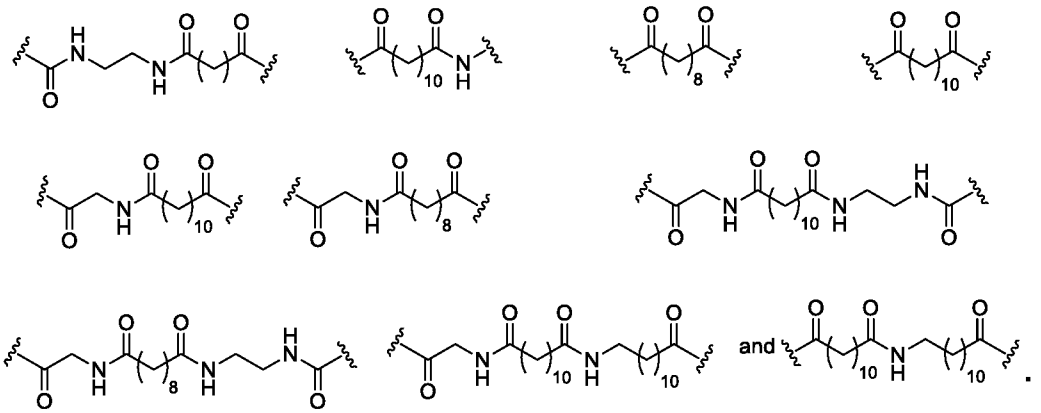
n is 2, 3, or 4;

x is 1 or 2.

In one embodiment L^1 is selected from the group consisting of:



In one embodiment L^1 is selected from the group consisting of:



In one embodiment A is absent, phenyl, pyrrolidinyl, or cyclopentyl.

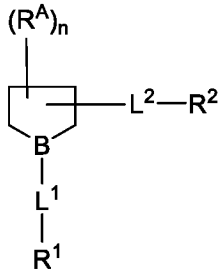
In one embodiment L^2 is C_{1-4} alkylene-O- that is optionally substituted with hydroxy.

In one embodiment L^2 is $–CH_2O-$, $-CH_2CH_2O-$, or $-CH(OH)CH_2O-$.

In one embodiment each R^A is independently hydroxy or C_{1-8} alkyl that is optionally substituted with hydroxyl.

In one embodiment each R^A is independently selected from the group consisting of hydroxy, methyl and $–CH_2OH$.

In one embodiment a compound of formula I has the following formula (Ig):



(Ig)

wherein B is –N- or -CH-;

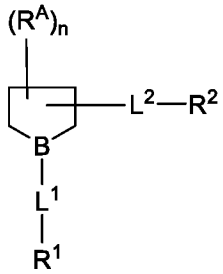
L^1 is absent or –NH-;

L^2 is C_{1-4} alkylene-O- that is optionally substituted with hydroxyl or halo;

n is 0, 1, or 2;

or a salt thereof.

In one embodiment a compound of formula I has the following formula (Ig):



(Ig)

wherein B is –N- or -CH-;

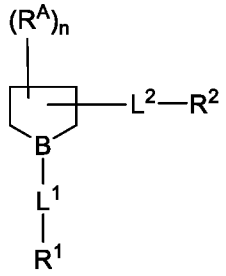
L^1 is absent or –NH-;

L^2 is C_{1-4} alkylene-O- that is optionally substituted with hydroxyl or halo;

n is 0, 1, 2, 3, 4, 5, 6, or 7;

or a salt thereof.

In one embodiment a compound of formula I has the following formula (Ig):



(Ig)

wherein B is –N- or -CH-;

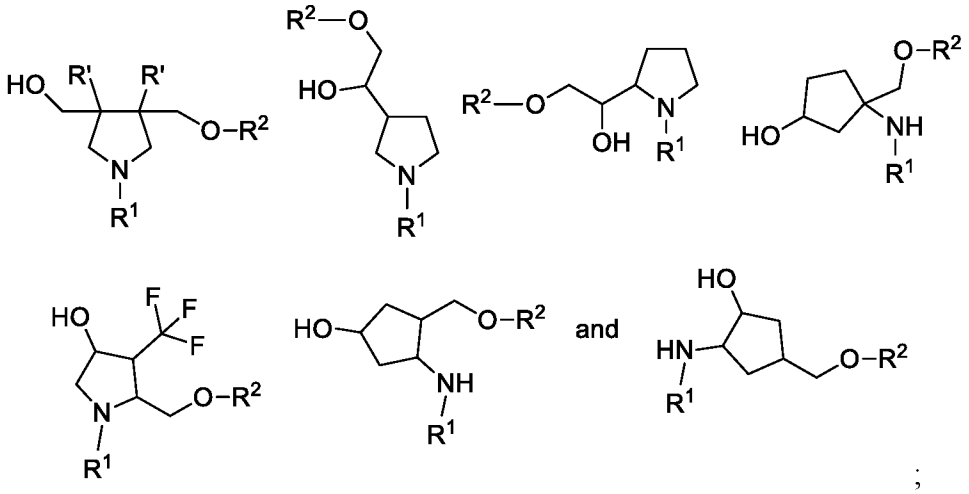
L^1 is absent or –NH-;

L^2 is C_{1-4} alkylene-O- that is optionally substituted with hydroxyl or halo;

n is 0, 1, 2, 3, or 4;

or a salt thereof.

In one embodiment a compound of formula Ig is selected from the group consisting of:

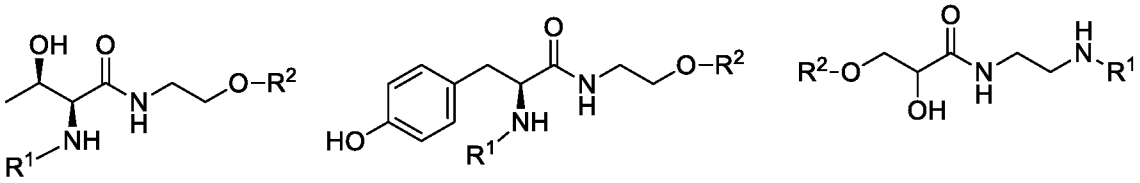


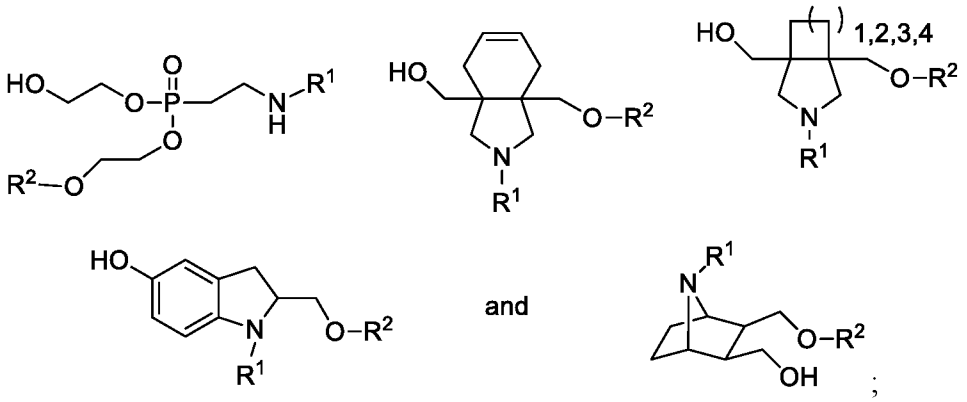
;

wherein R' is C_{1-9} alkyl, C_{2-9} alkenyl or C_{2-9} alkynyl; wherein the C_{1-9} alkyl, C_{2-9} alkenyl or C_{2-9} alkynyl are optionally substituted with halo or hydroxyl;

and salts thereof.

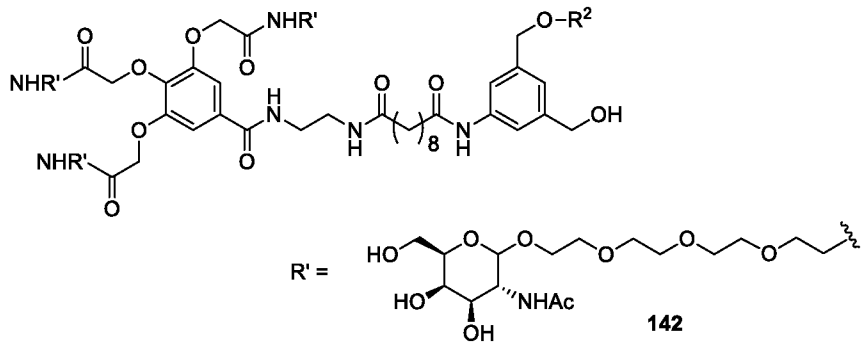
In one embodiment a compound of formula I is selected from the group consisting of:

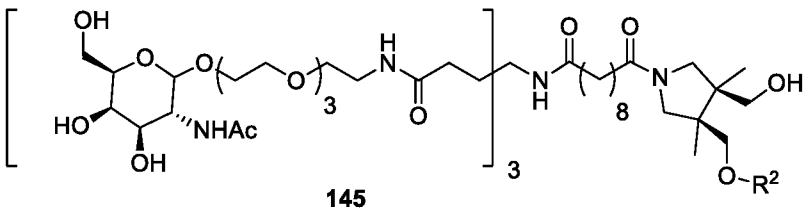


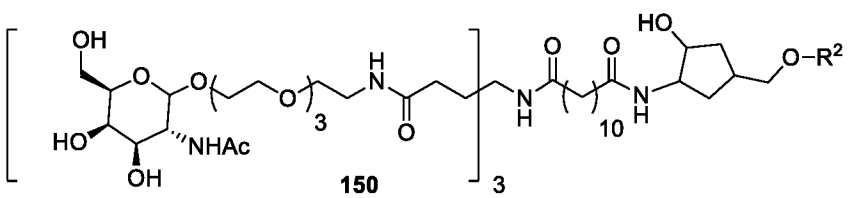


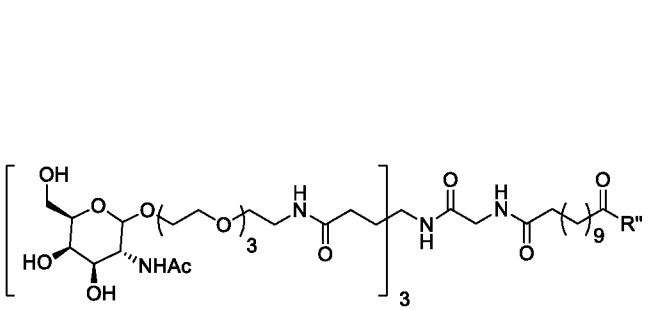
and salts thereof.

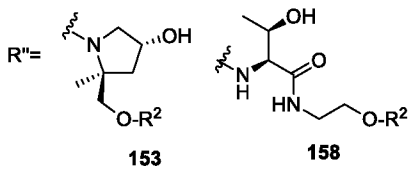
In one embodiment the compound of formula I or the salt thereof is selected from the group consisting of:



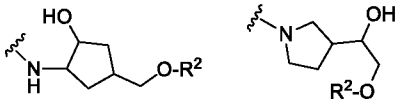




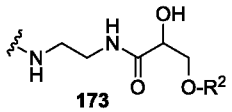




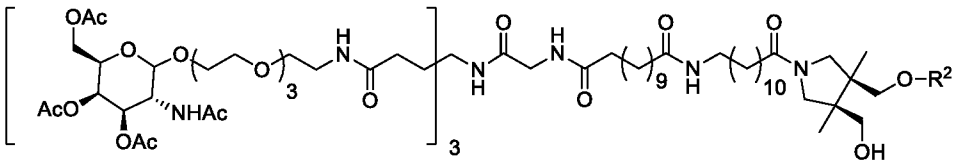
153 158



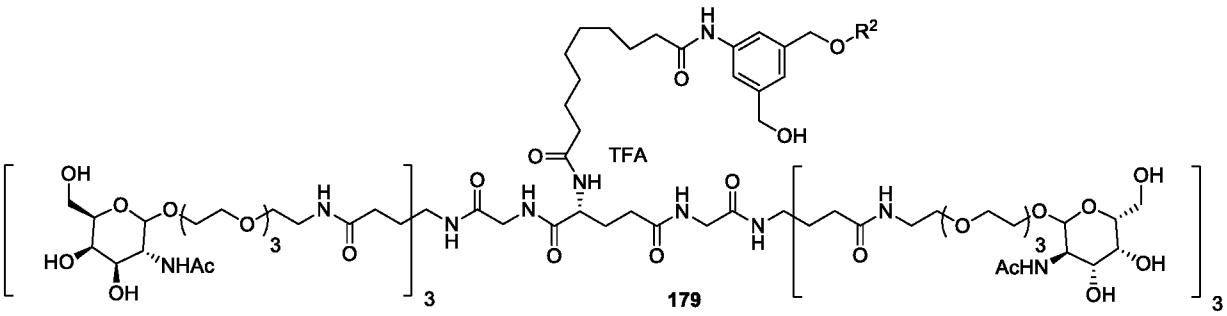
163 168



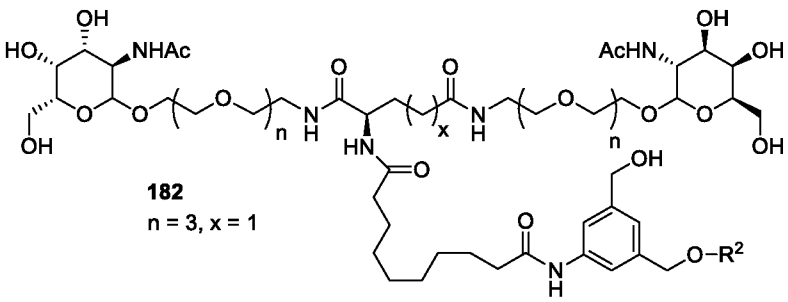
173



176

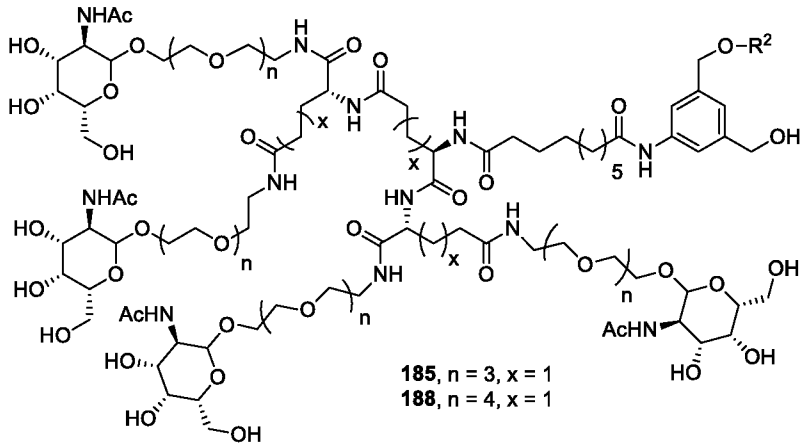


179

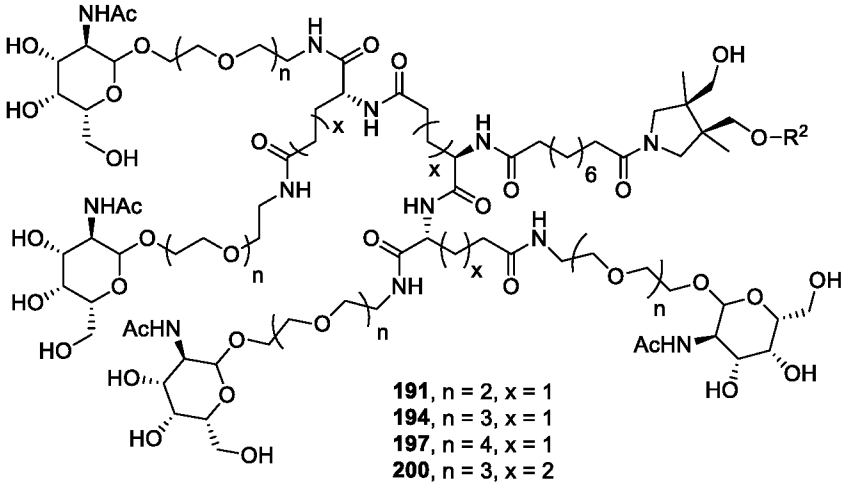


182

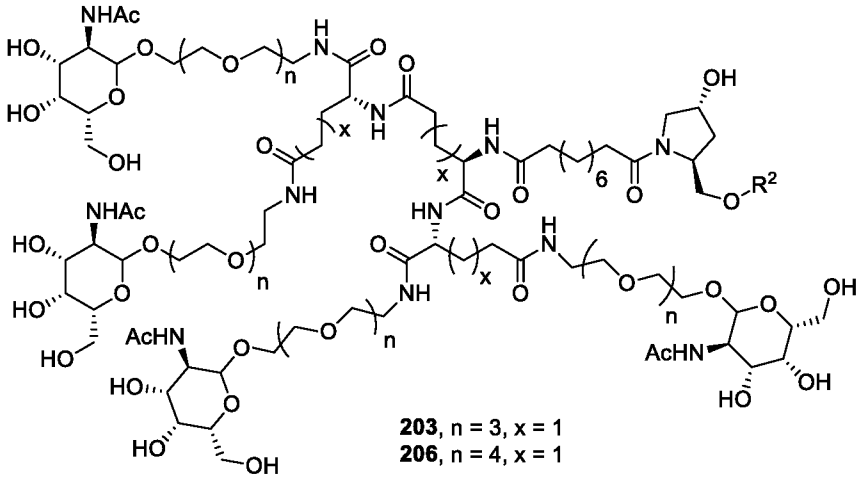
n = 3, x = 1



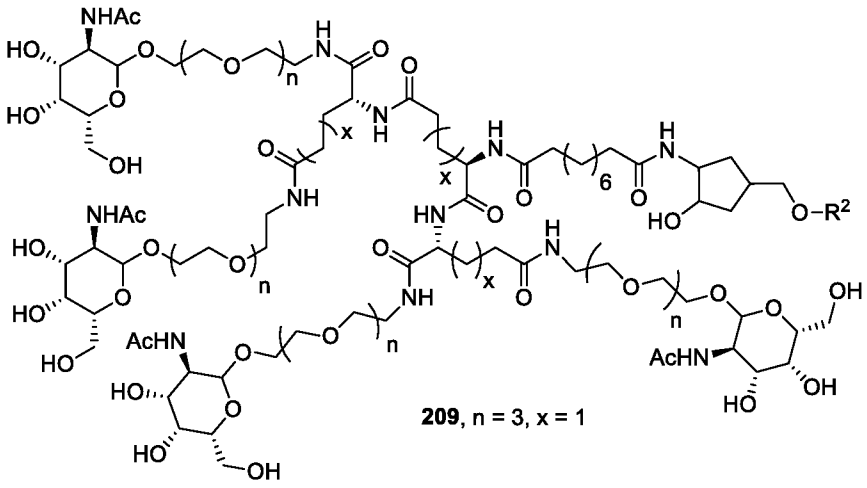
185, n = 3, x = 1
188, n = 4, x = 1



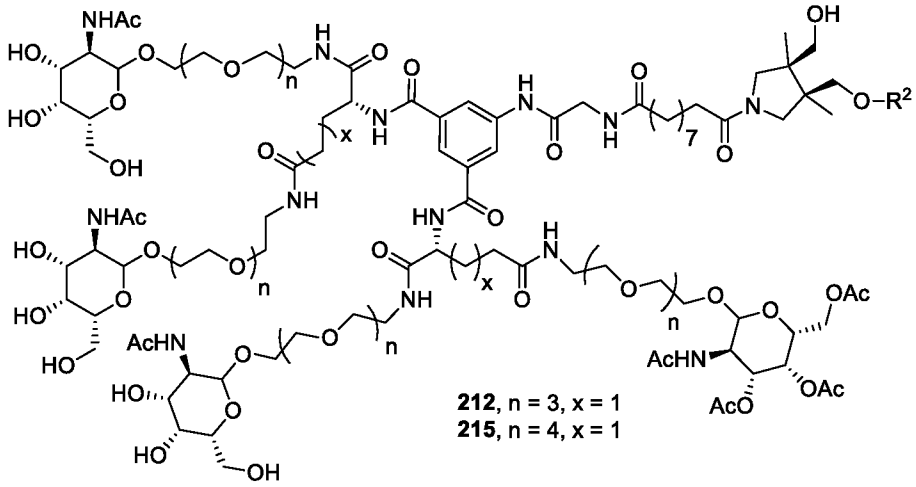
191, n = 2, x = 1
194, n = 3, x = 1
197, n = 4, x = 1
200, n = 3, x = 2



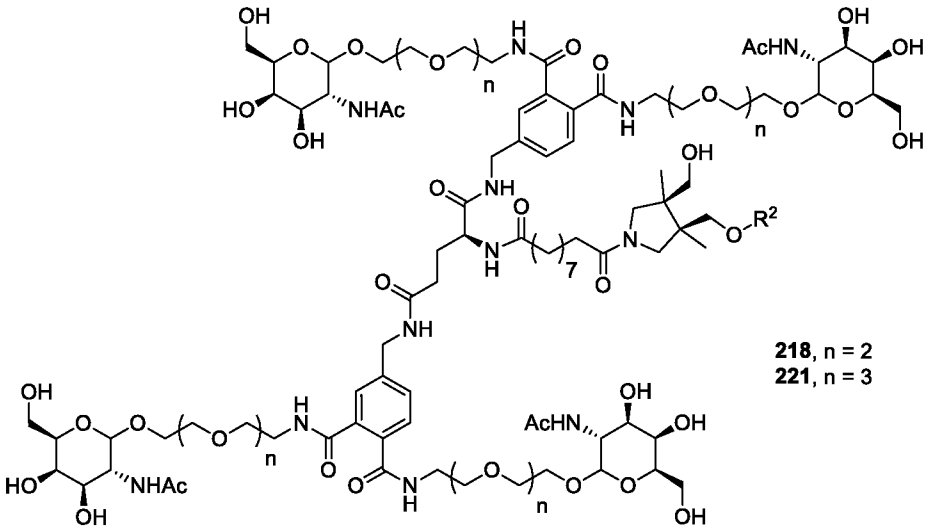
203, n = 3, x = 1
206, n = 4, x = 1



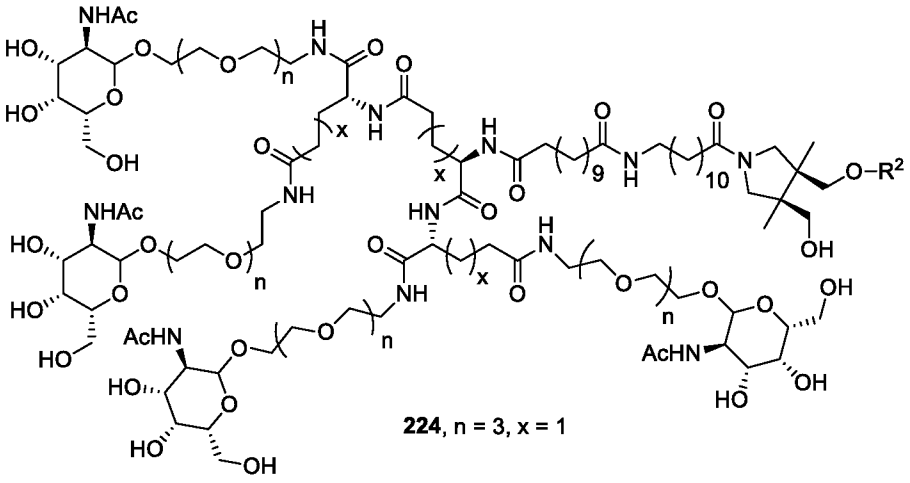
209, n = 3, x = 1



212, n = 3, x = 1
215, n = 4, x = 1

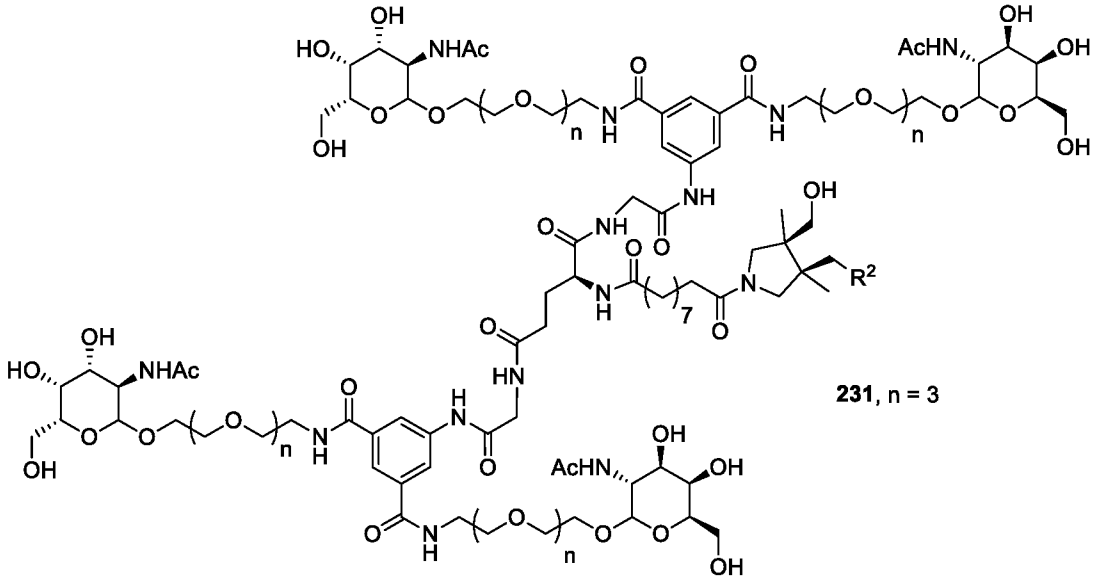


218, n = 2
221, n = 3



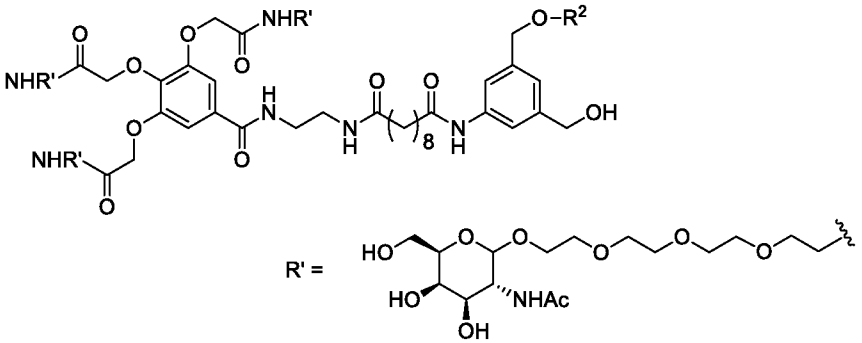
224, n = 3, x = 1

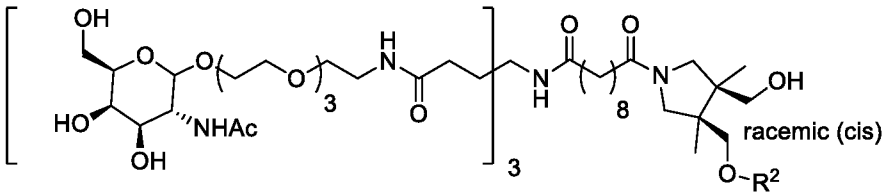
; and

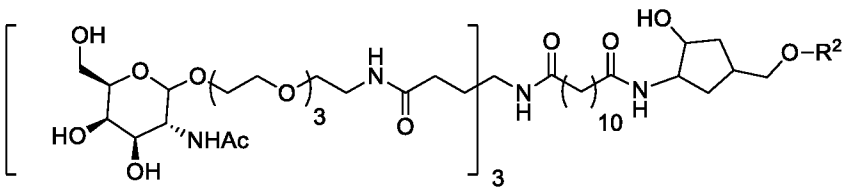


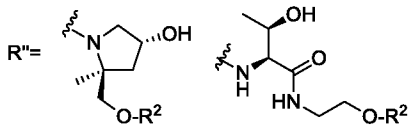
231, n = 3

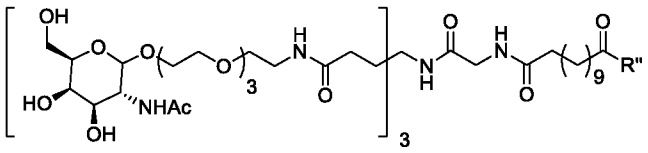
In one embodiment the compound of formula I or the salt thereof is selected from the group consisting of:

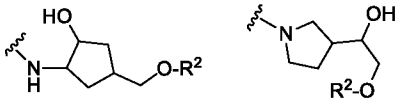


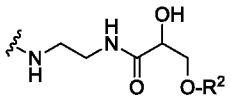

racemic (cis)

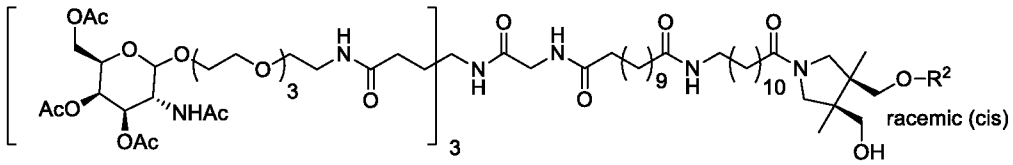


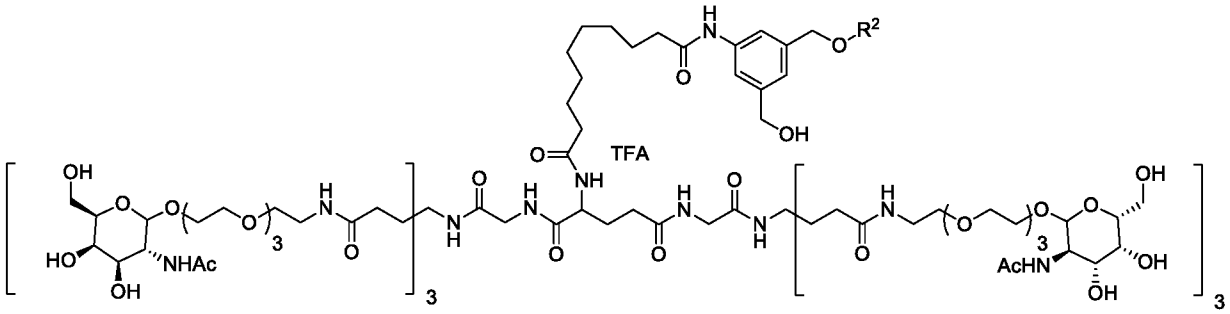

R"=

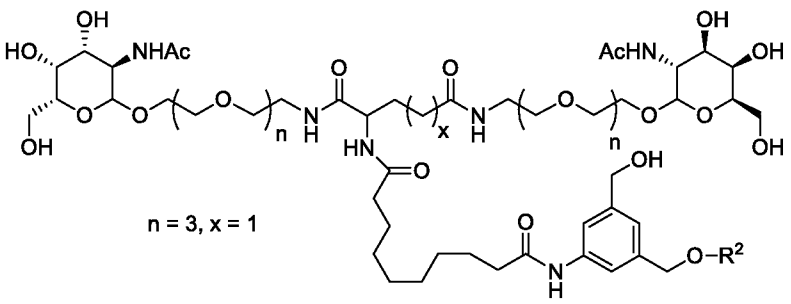


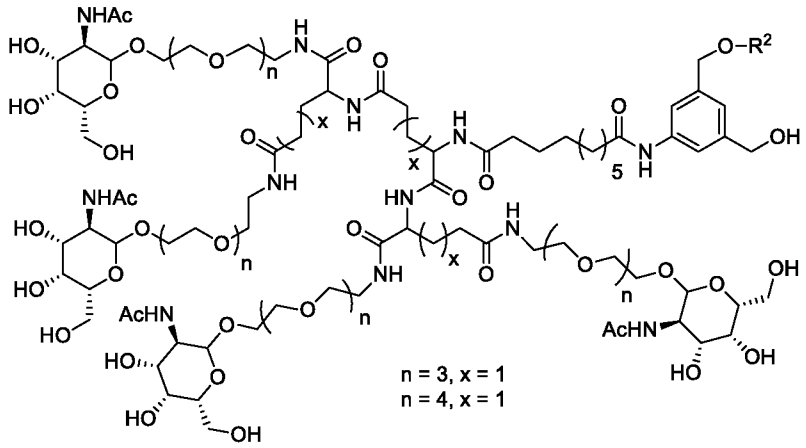


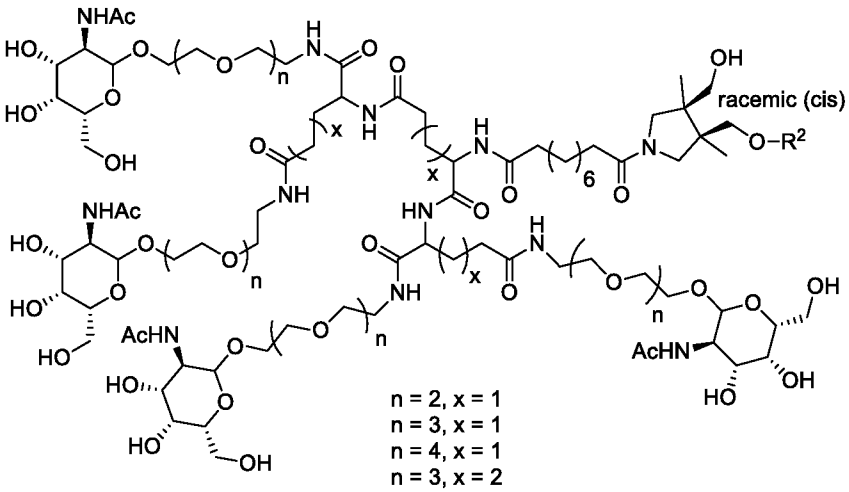


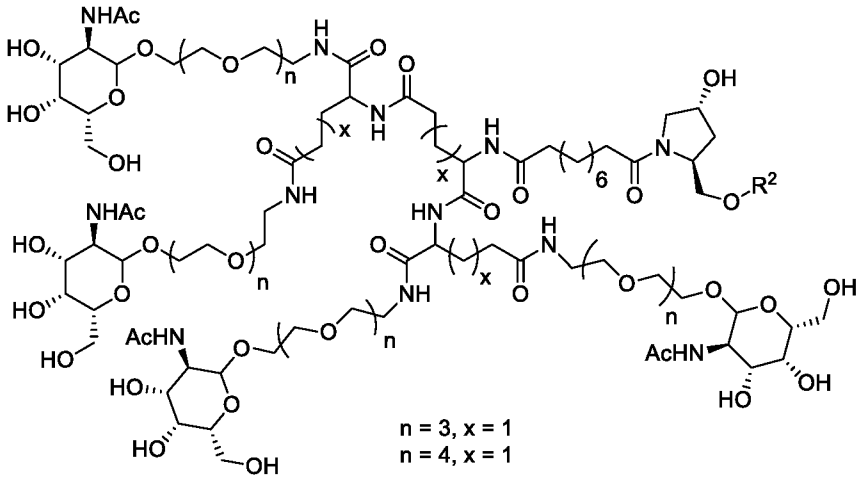

racemic (cis)

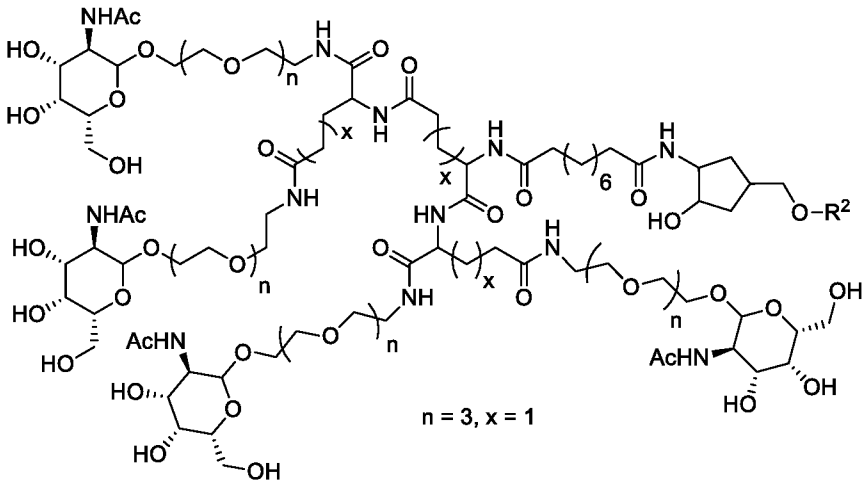

TFA

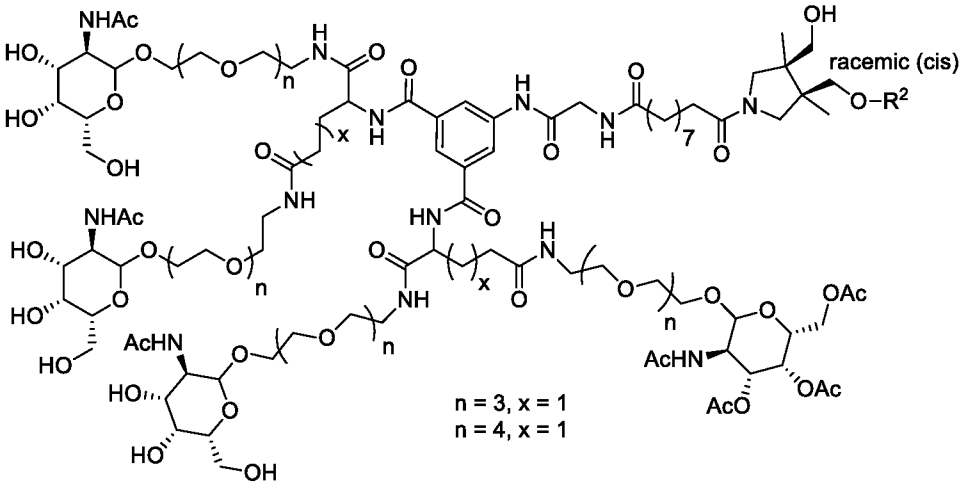

n = 3, x = 1

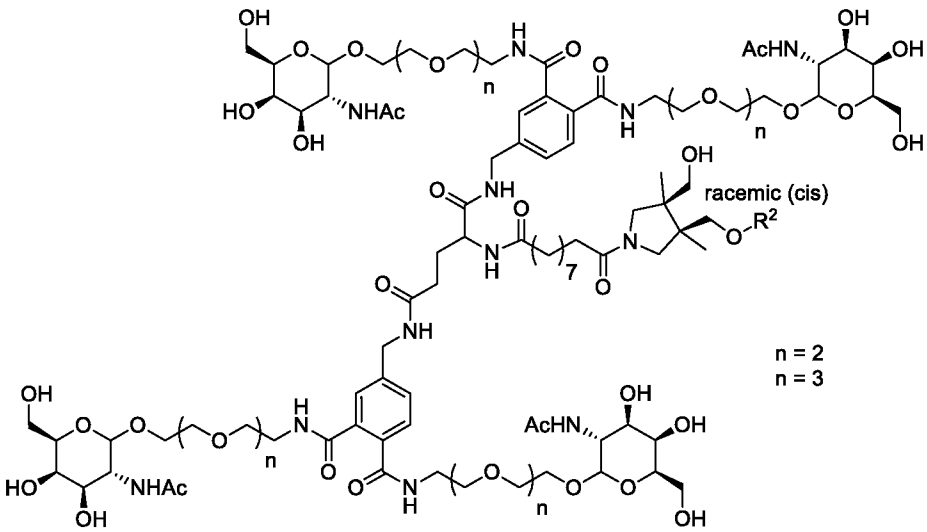

n = 3, x = 1
n = 4, x = 1

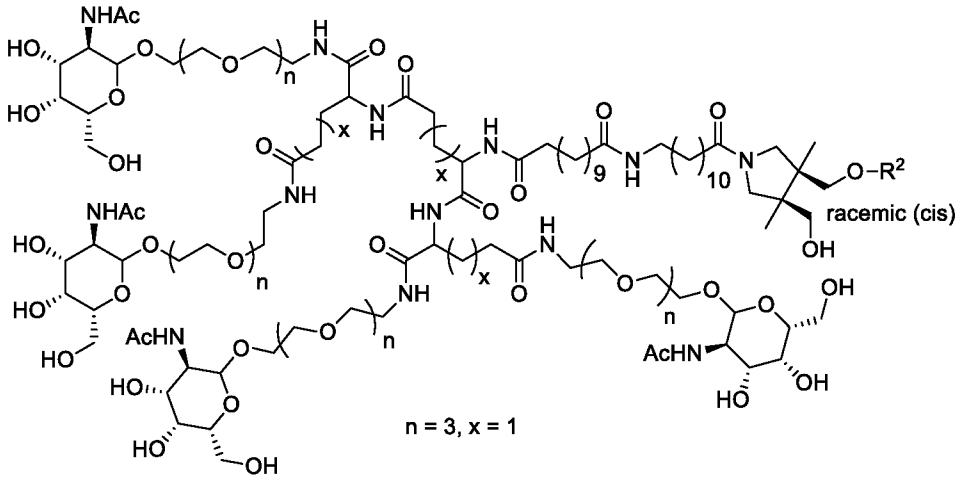

racemic (cis)
n = 2, x = 1
n = 3, x = 1
n = 4, x = 1
n = 3, x = 2


n = 3, x = 1
n = 4, x = 1

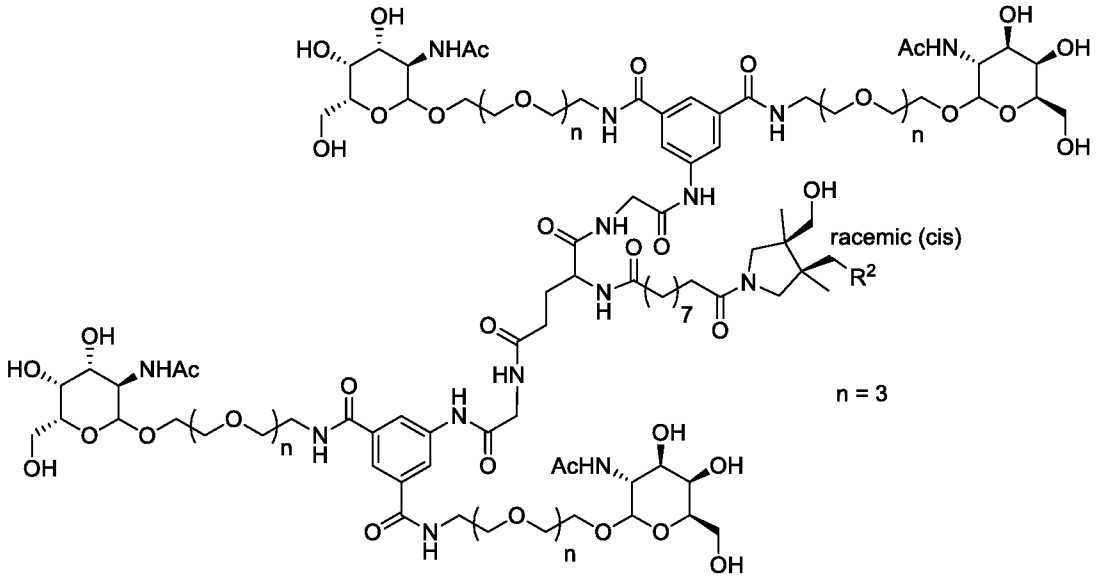

n = 3, x = 1


racemic (cis)
n = 3, x = 1
n = 4, x = 1


racemic (cis)
n = 2
n = 3

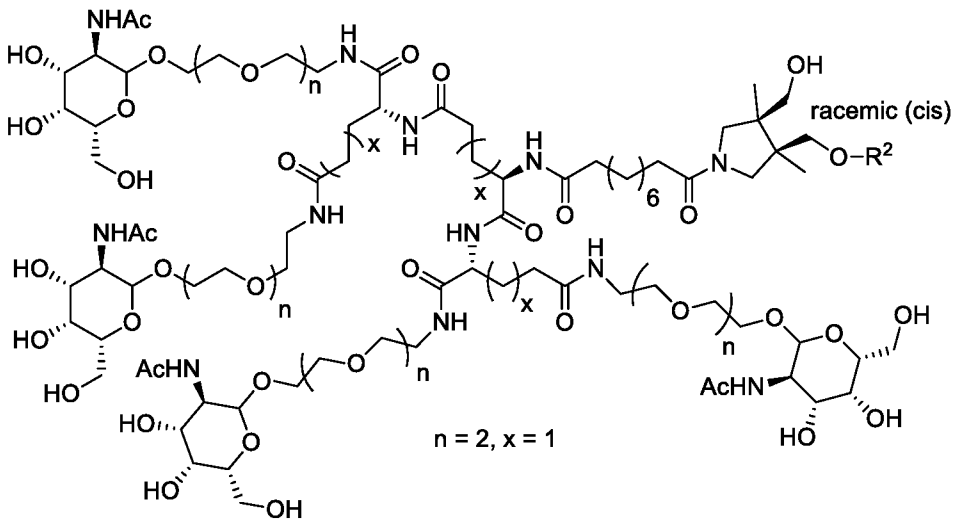


; and



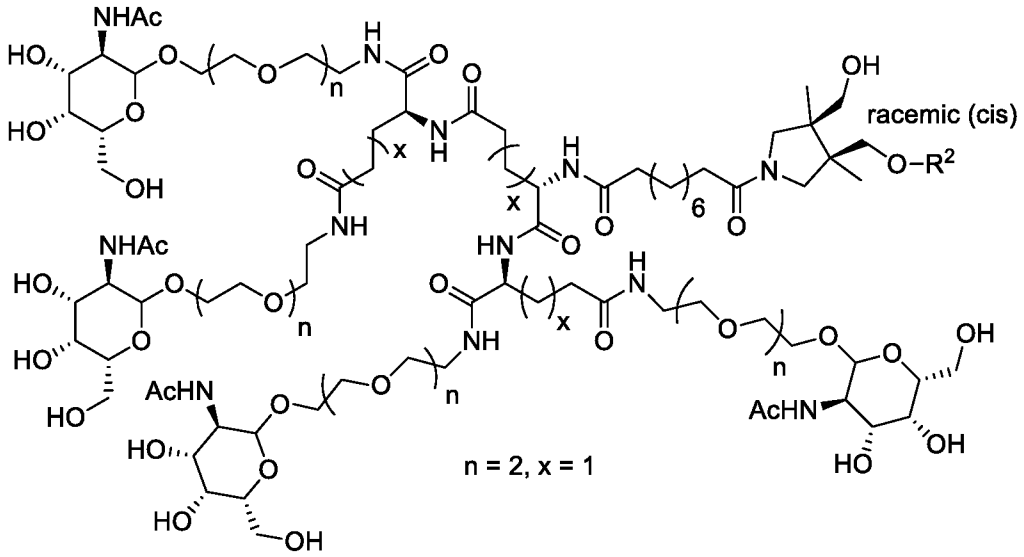
or pharmaceutically acceptable salts thereof, wherein R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of Table 1.

In one embodiment the compound of formula I is:



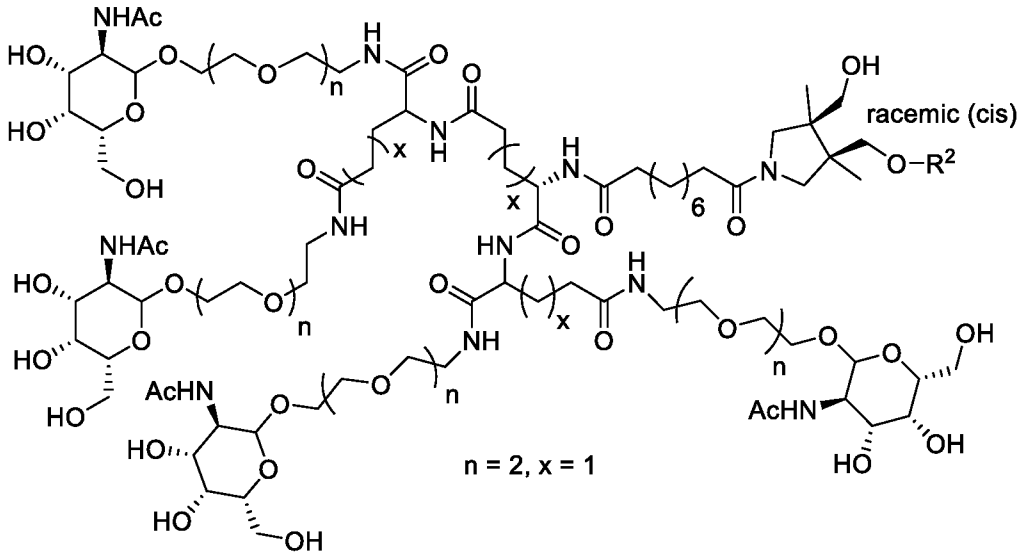
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule (e.g. a double stranded siRNA molecule selected from the double stranded siRNA molecules of Table 1).

In one embodiment the compound of formula I is:



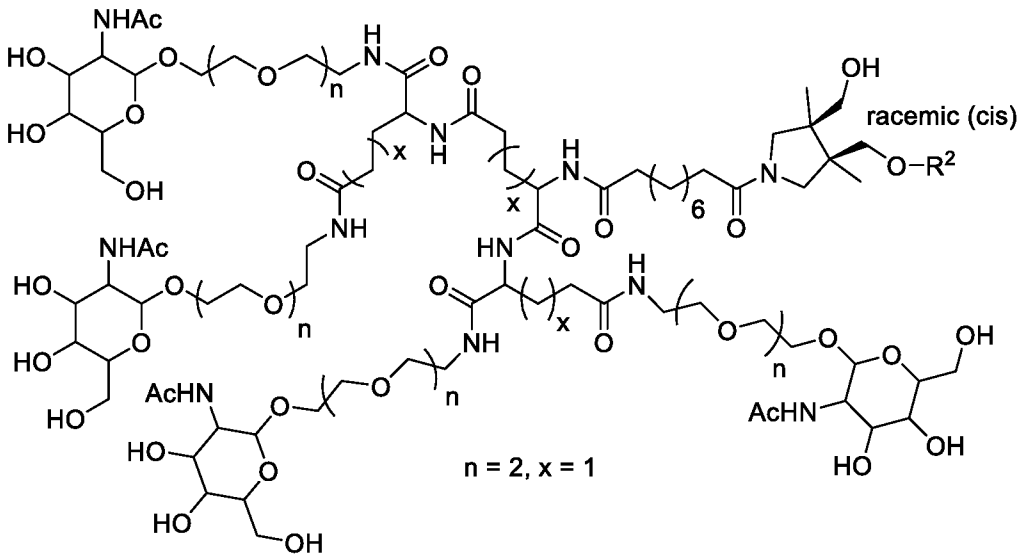
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule (e.g. a double stranded siRNA molecule selected from the double stranded siRNA molecules of Table 1).

In one embodiment the compound of formula I is:



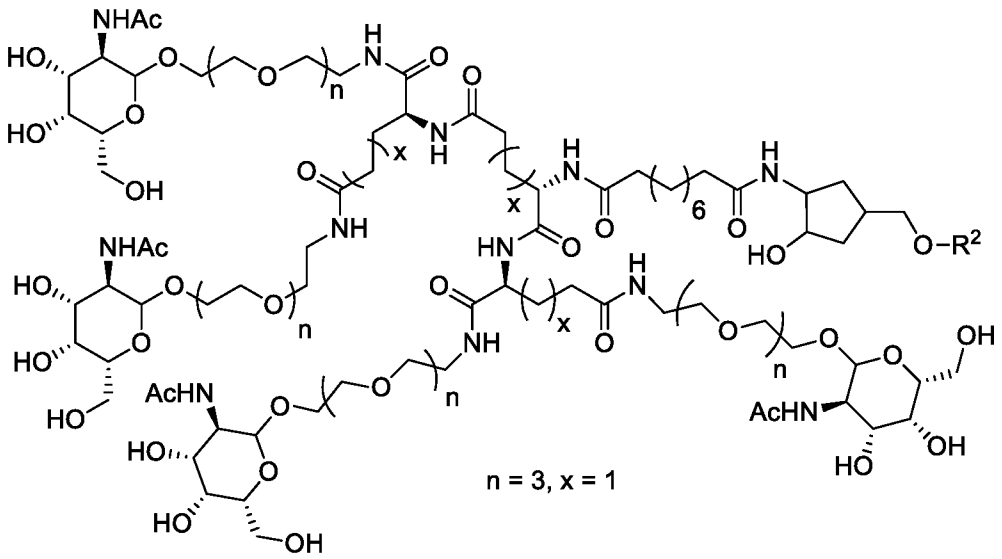
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule (e.g. a double stranded siRNA molecule selected from the double stranded siRNA molecules of Table 1).

In one embodiment the compound of formula I is:



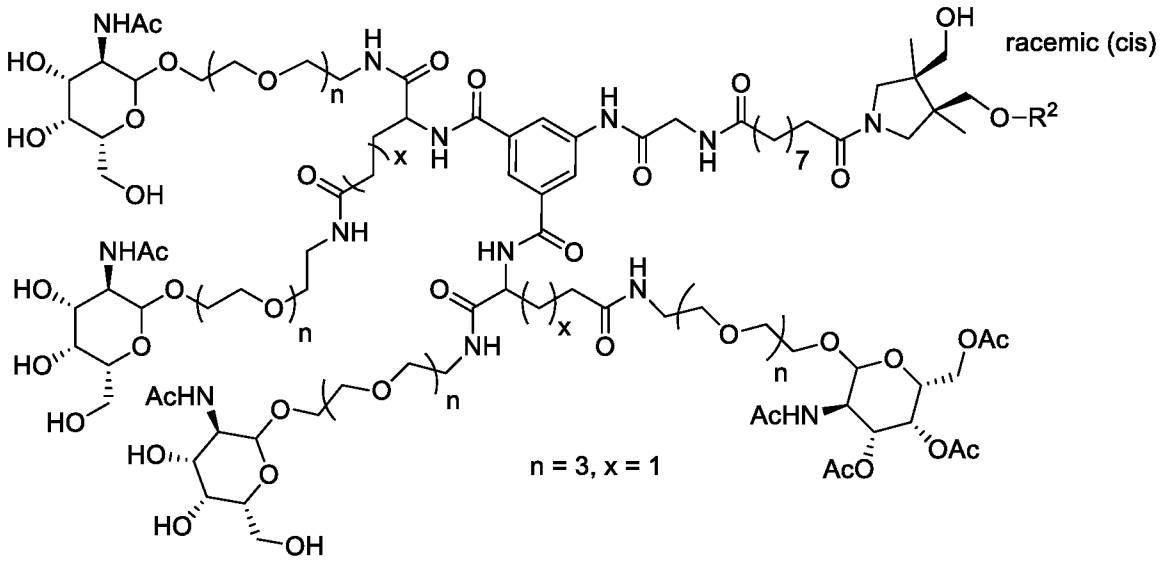
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule (e.g. a double stranded siRNA molecule selected from the double stranded siRNA molecules of Table 1).

In one embodiment the compound of formula I is:



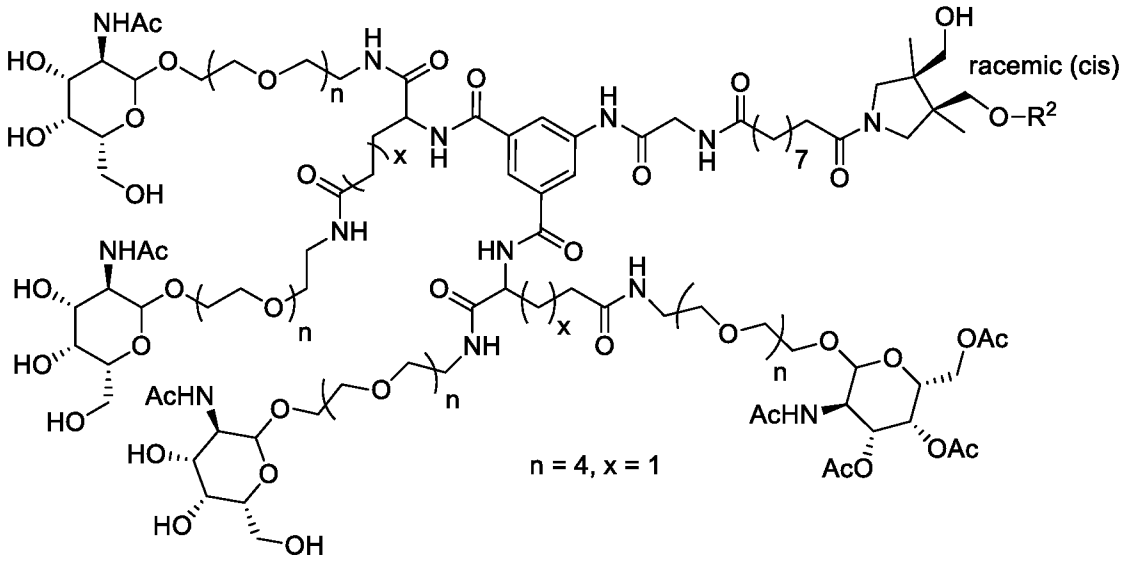
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule (e.g. a double stranded siRNA molecule selected from the double stranded siRNA molecules of Table 1).

In one embodiment the compound of formula I is:



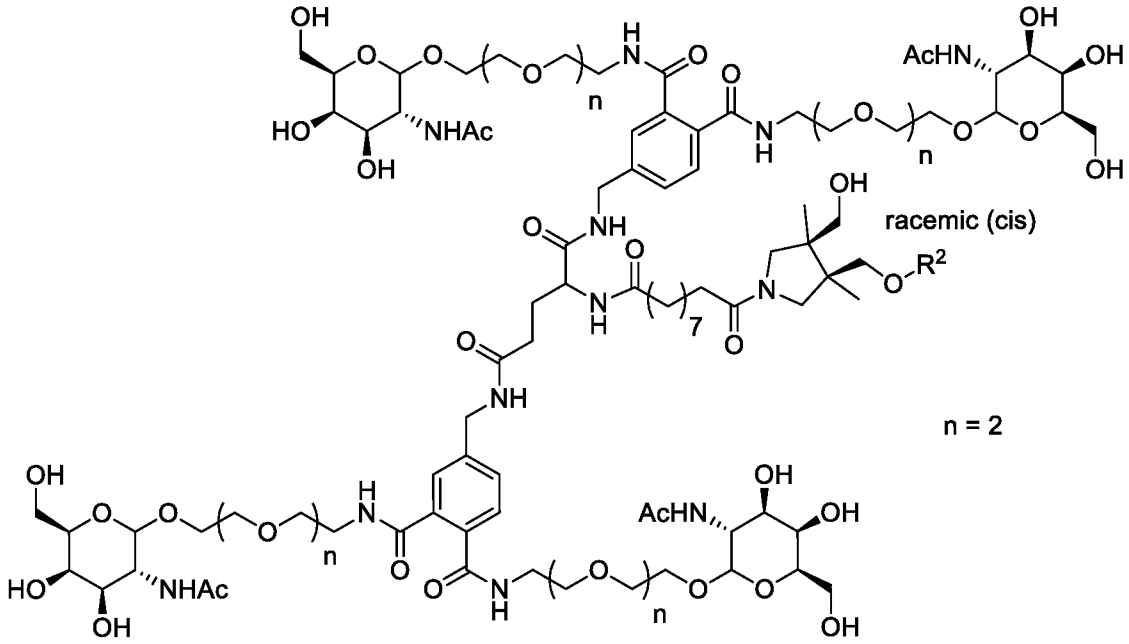
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule (e.g. a double stranded siRNA molecule selected from the double stranded siRNA molecules of Table 1).

In one embodiment the compound of formula I is:



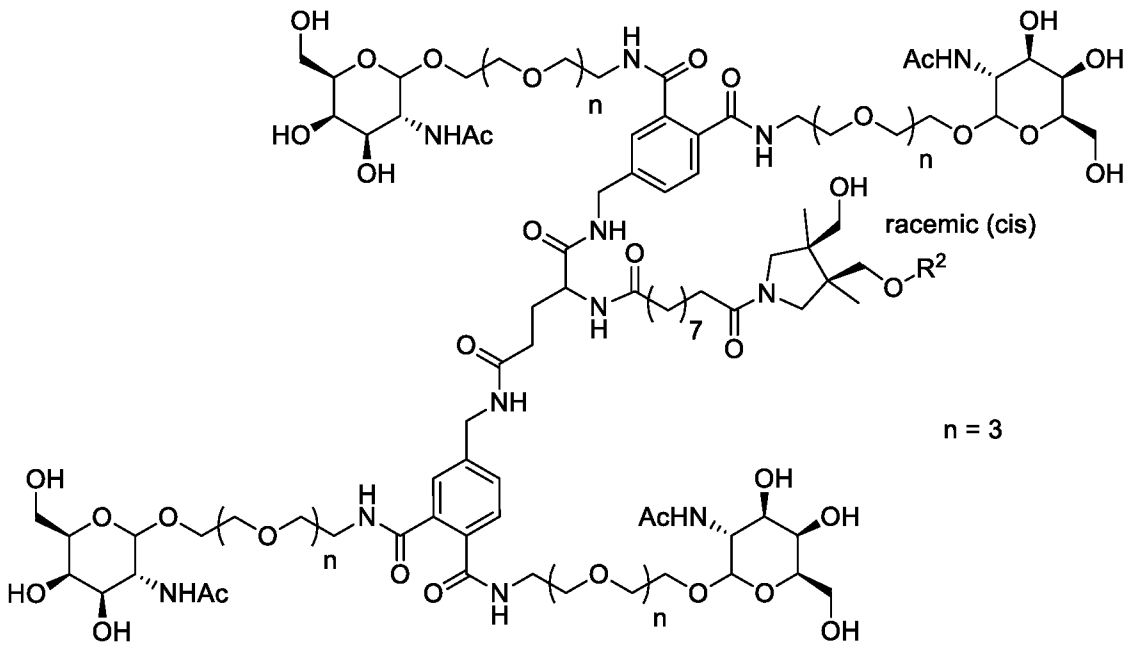
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule (e.g. a double stranded siRNA molecule selected from the double stranded siRNA molecules of Table 1).

In one embodiment the compound of formula I is:



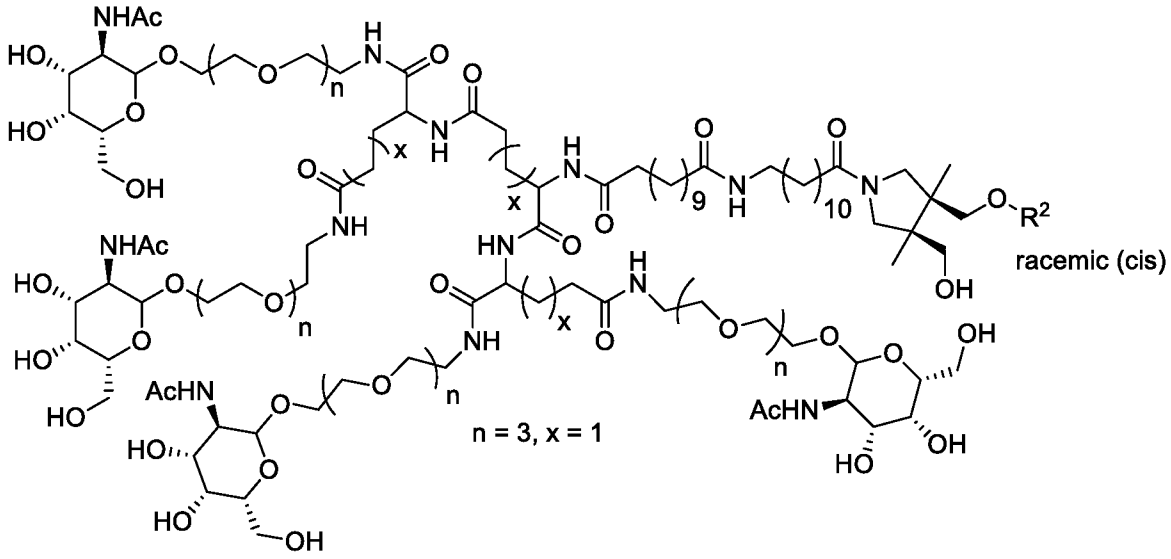
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule (e.g. a double stranded siRNA molecule selected from the double stranded siRNA molecules of Table 1).

In one embodiment the compound of formula I is:



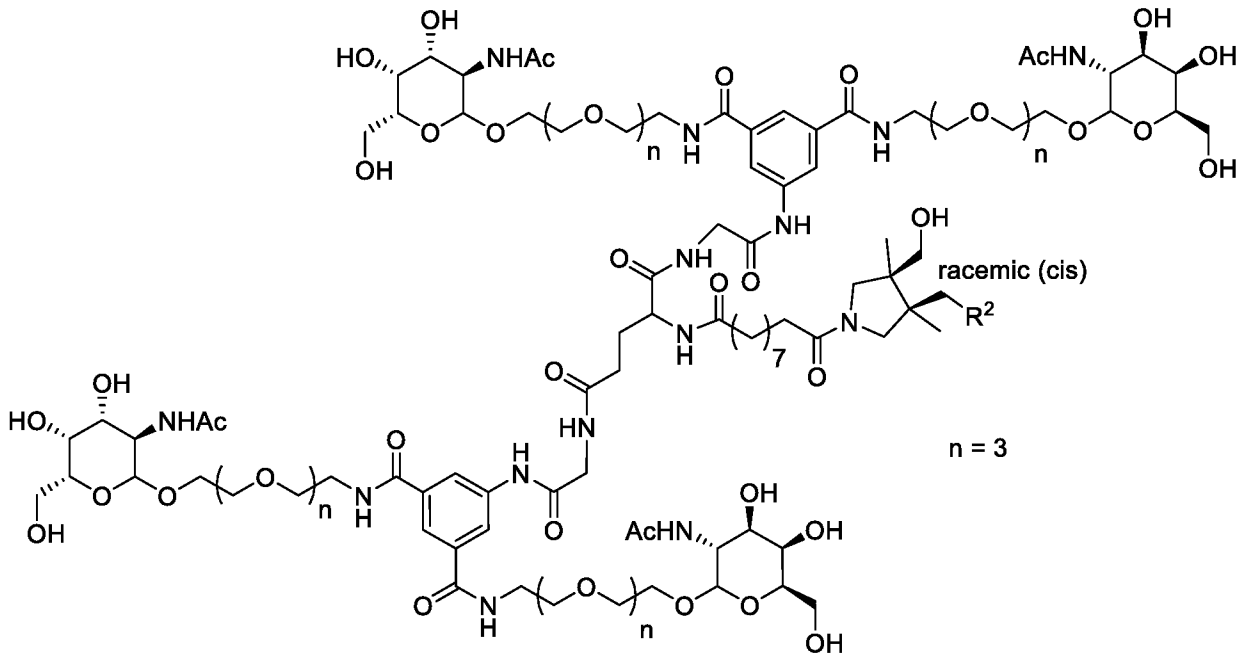
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule (e.g. a double stranded siRNA molecule selected from the double stranded siRNA molecules of Table 1).

In one embodiment the compound of formula I is:



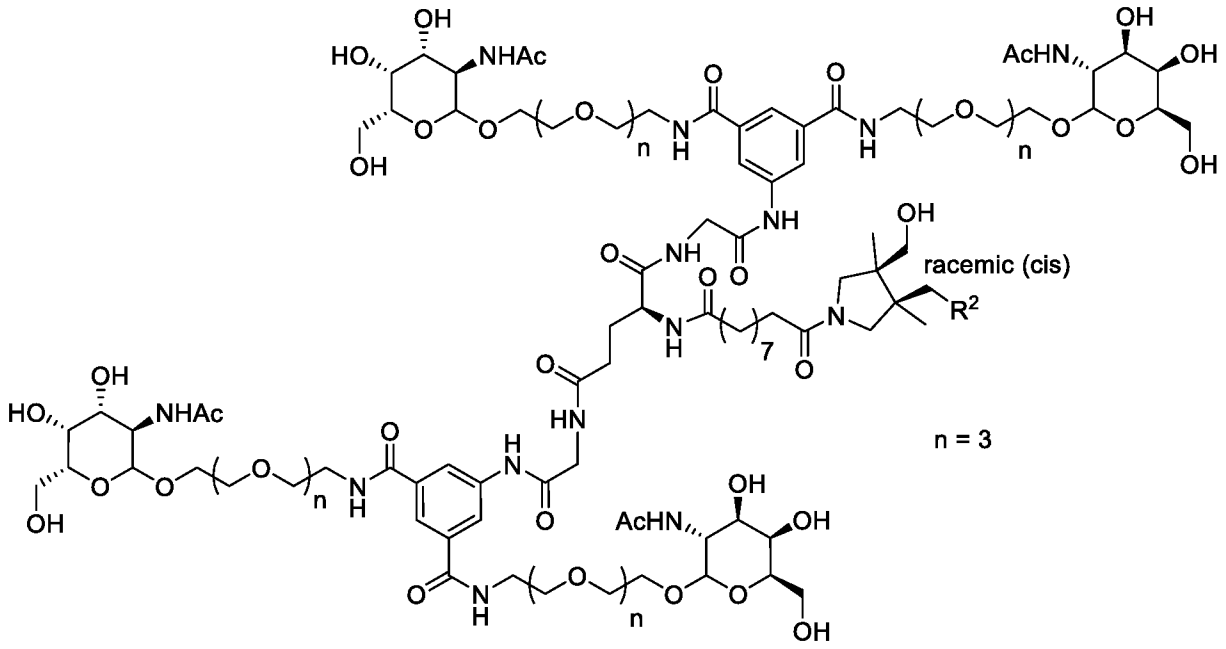
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule (e.g. a double stranded siRNA molecule selected from the double stranded siRNA molecules of Table 1).

In one embodiment the compound of formula I is:



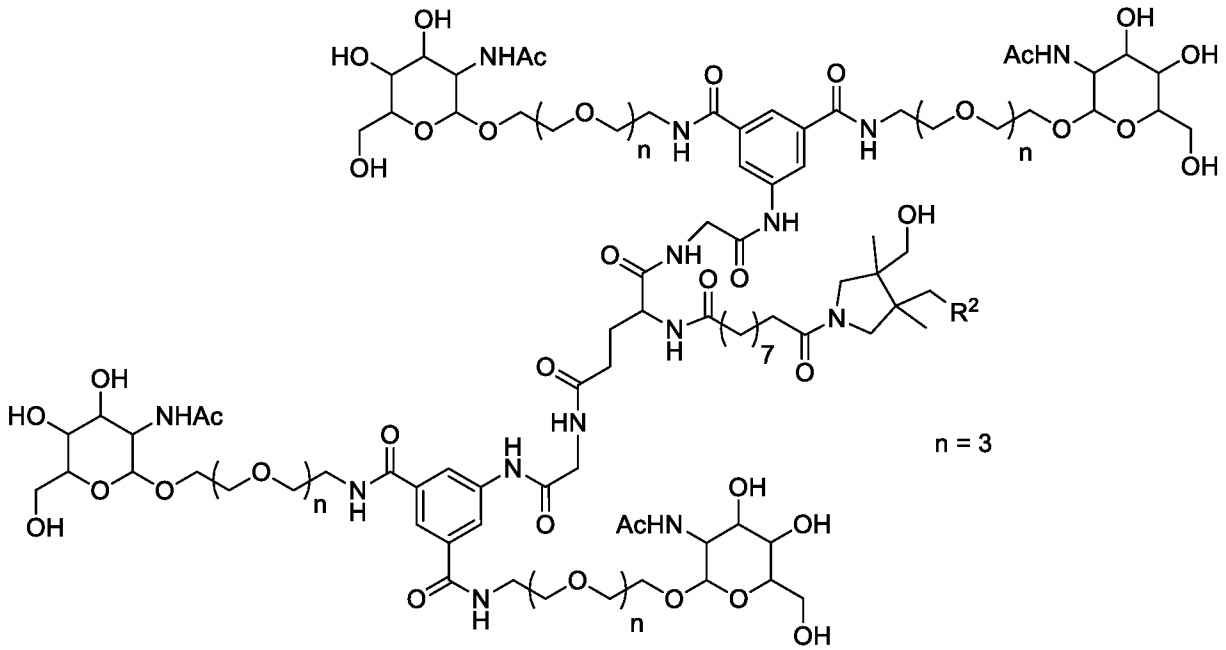
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule (e.g. a double stranded siRNA molecule selected from the double stranded siRNA molecules of Table 1).

In one embodiment the compound of formula I is:



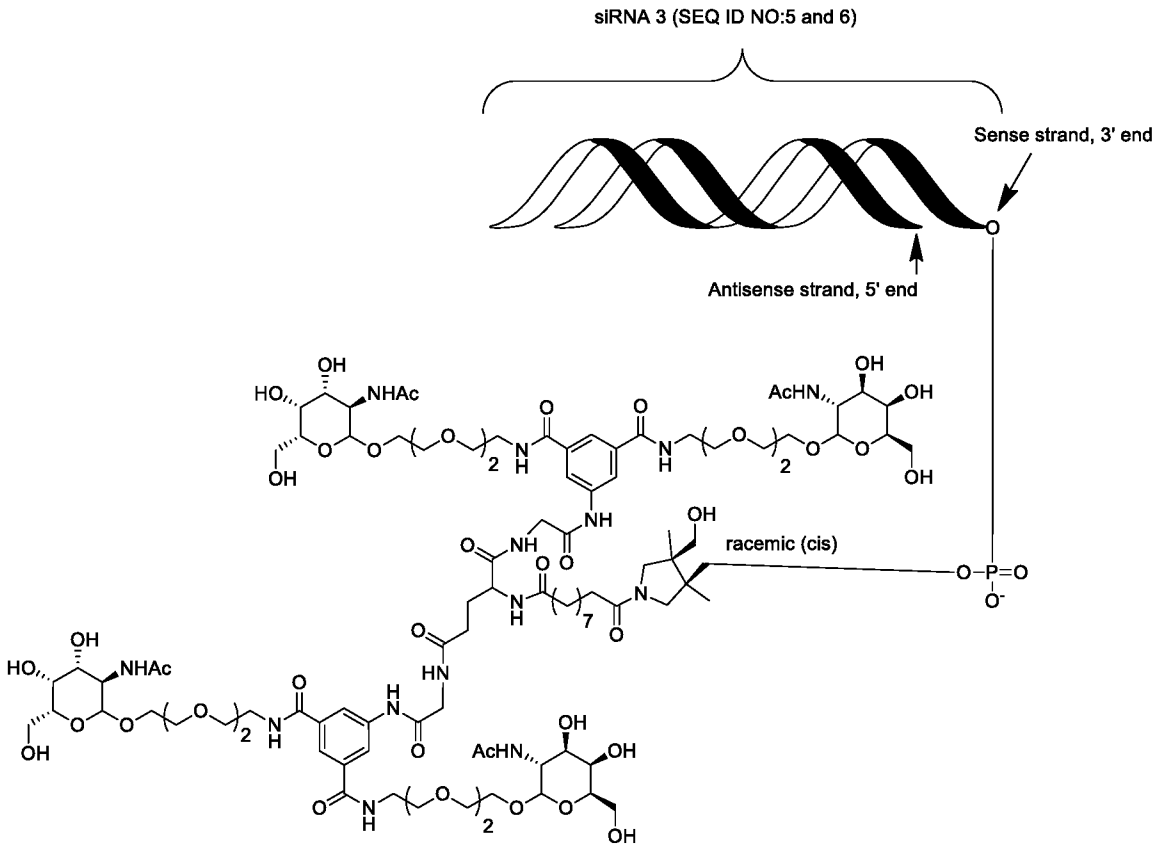
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule (e.g. a double stranded siRNA molecule selected from the double stranded siRNA molecules of Table 1).

In one embodiment the compound of formula I is:



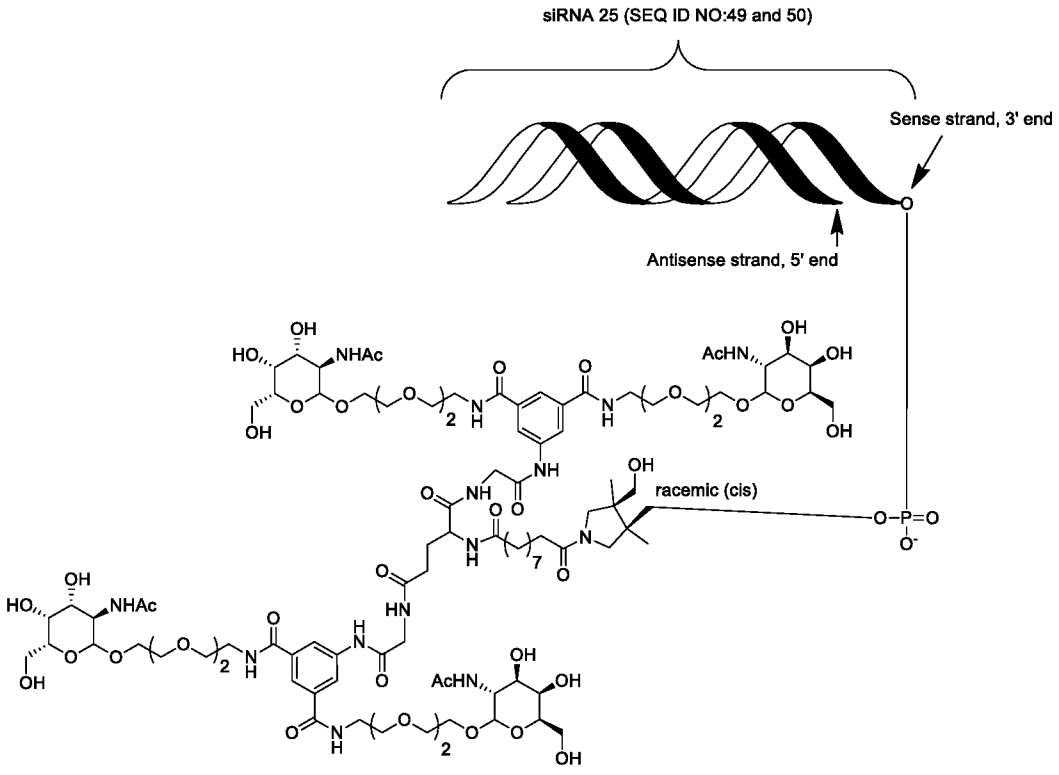
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule (e.g. a double stranded siRNA molecule selected from the double stranded siRNA molecules of Table 1).

In one embodiment the compound of formula I is:



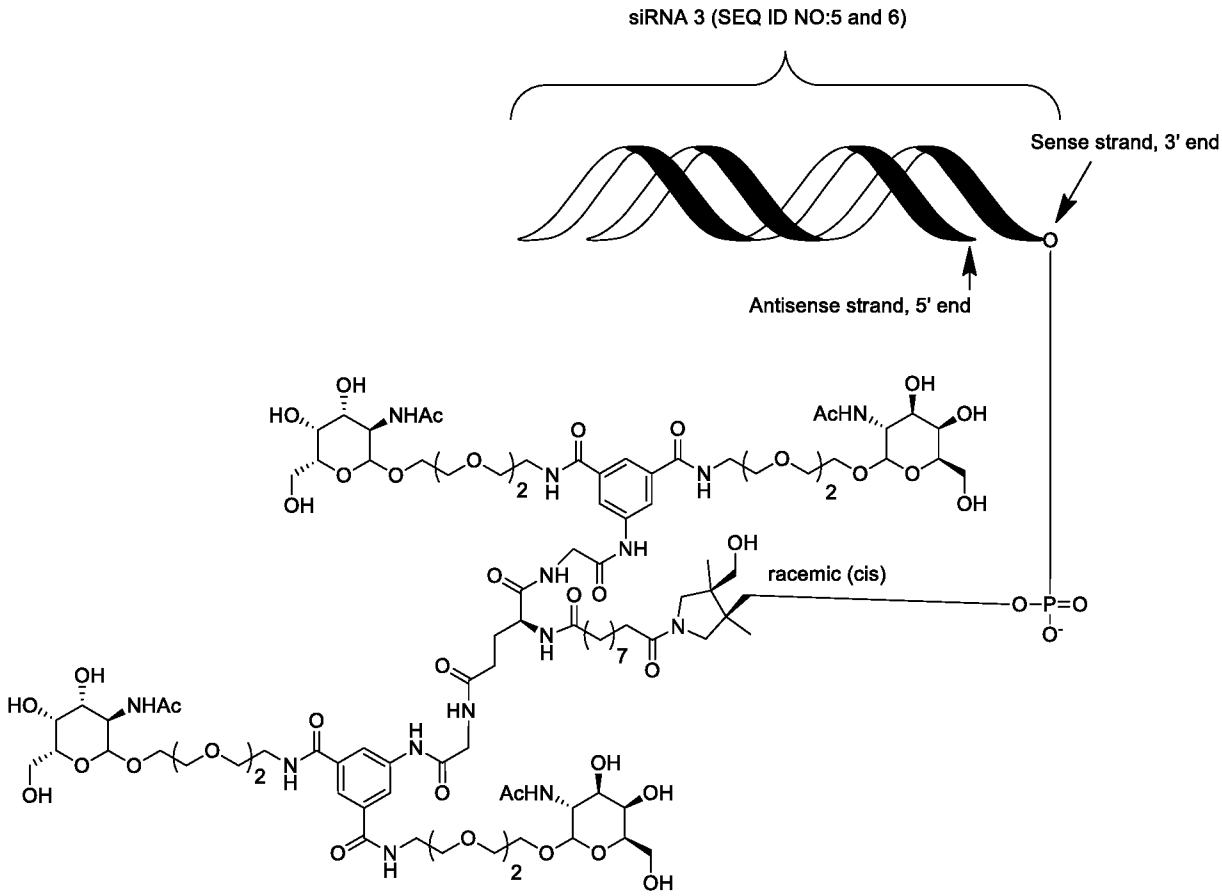
or a pharmaceutically acceptable salt thereof.

In one embodiment the compound of formula I is:



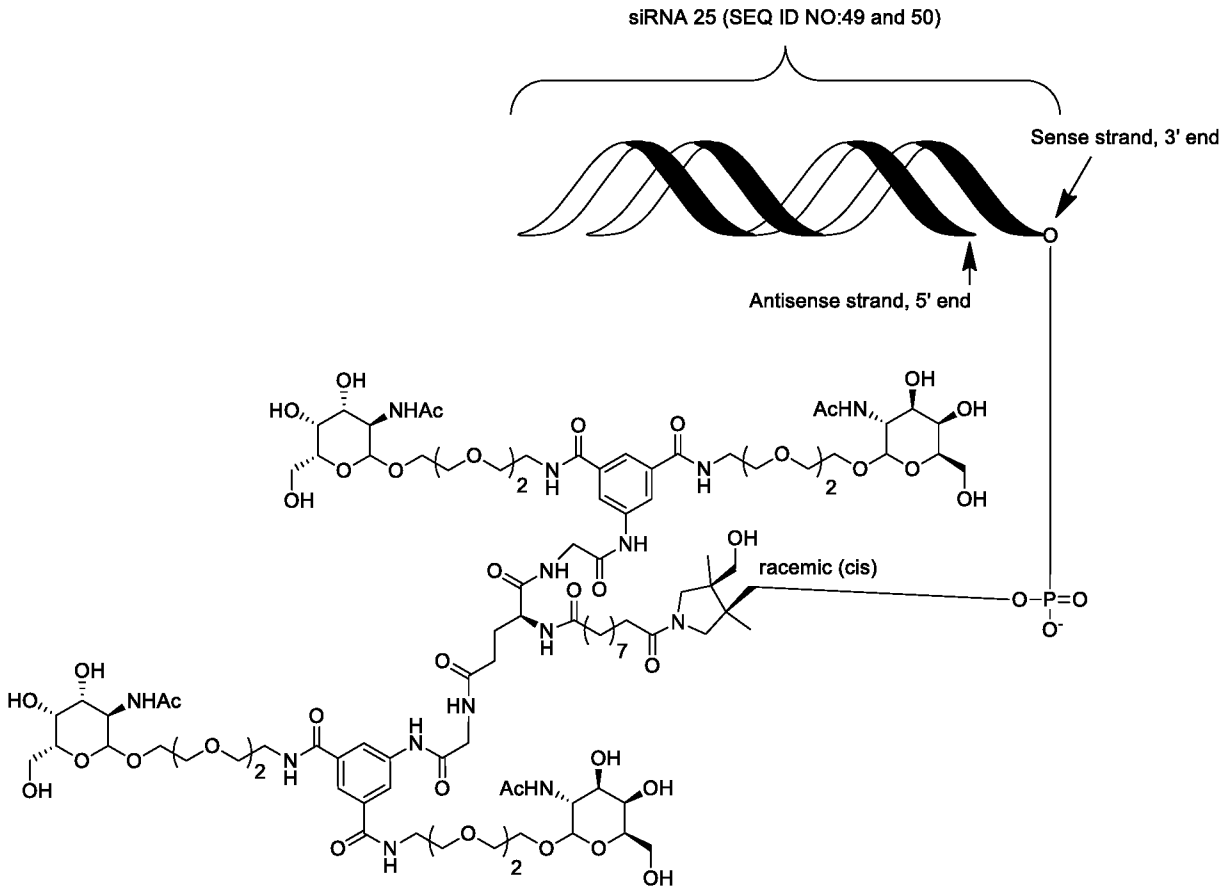
or a pharmaceutically acceptable salt thereof.

In one embodiment the compound of formula I is:



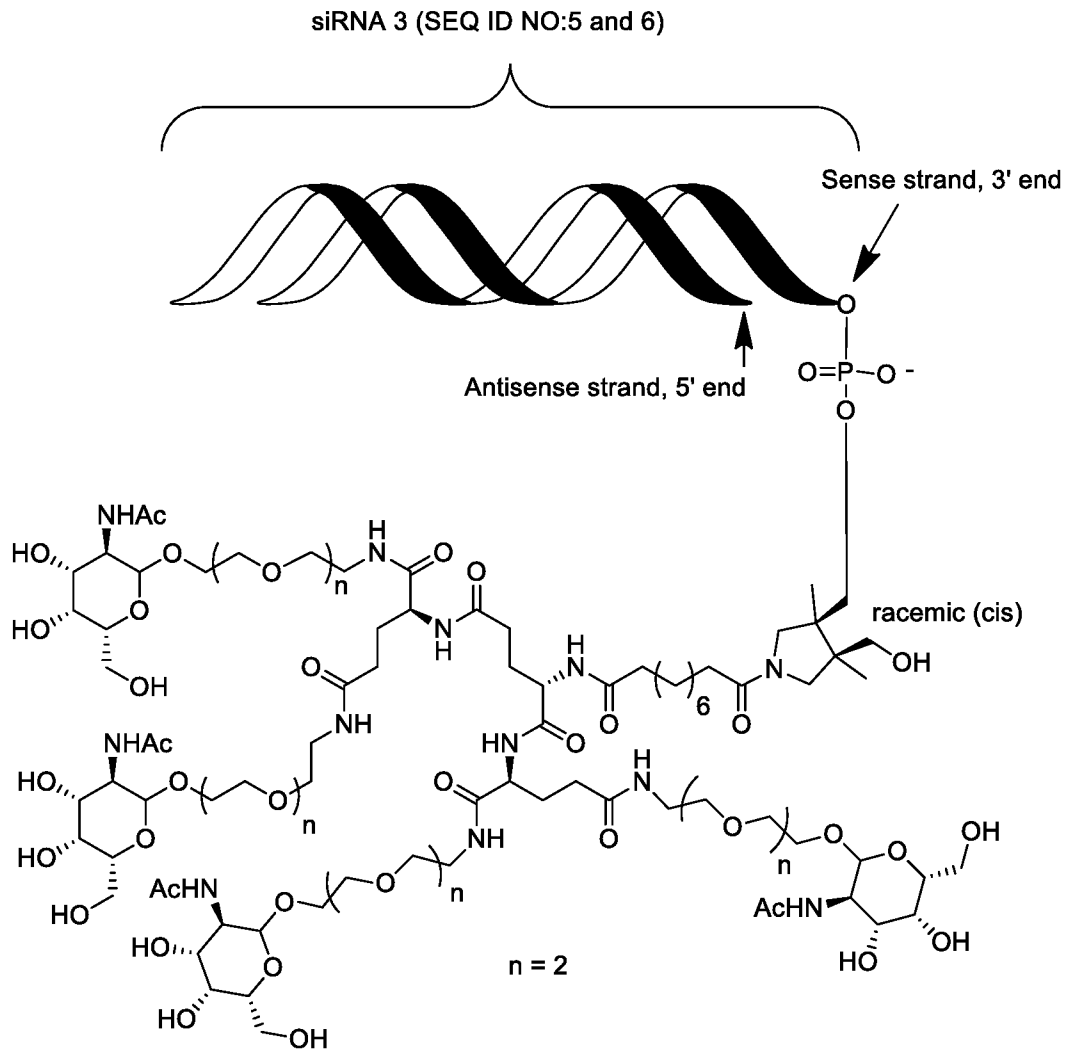
or a pharmaceutically acceptable salt thereof.

In one embodiment the compound of formula I is:



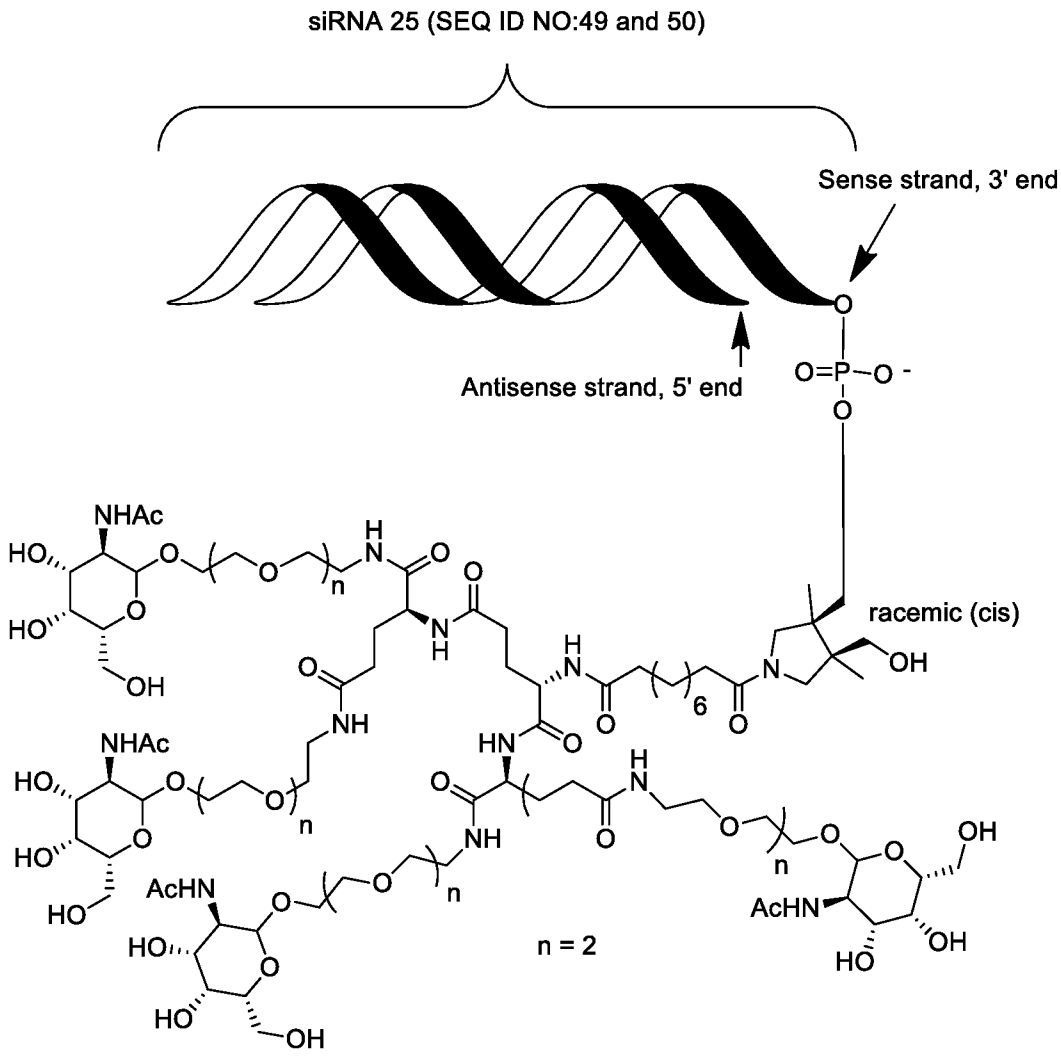
or a pharmaceutically acceptable salt thereof.

In one embodiment the compound of formula I is:



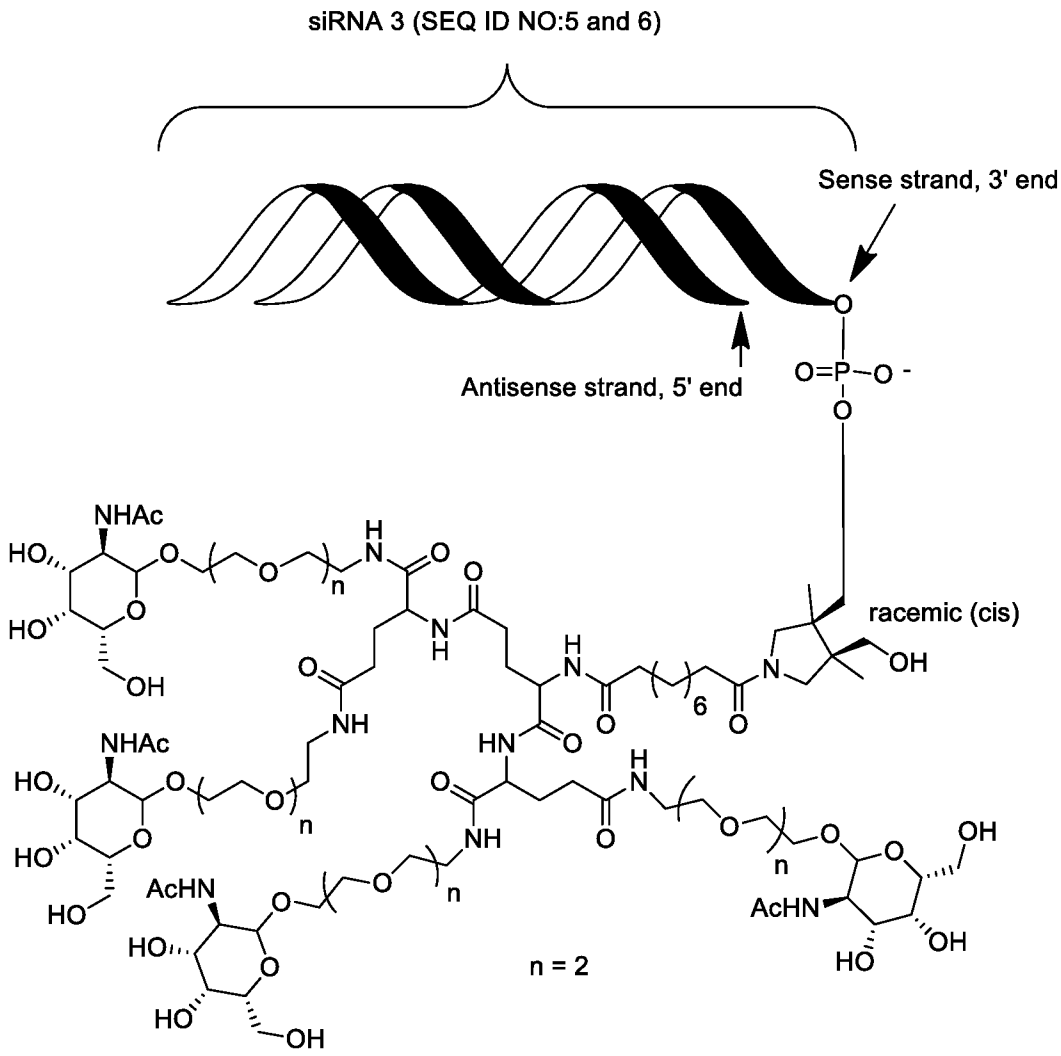
or a pharmaceutically acceptable salt thereof.

In one embodiment the compound of formula I is:



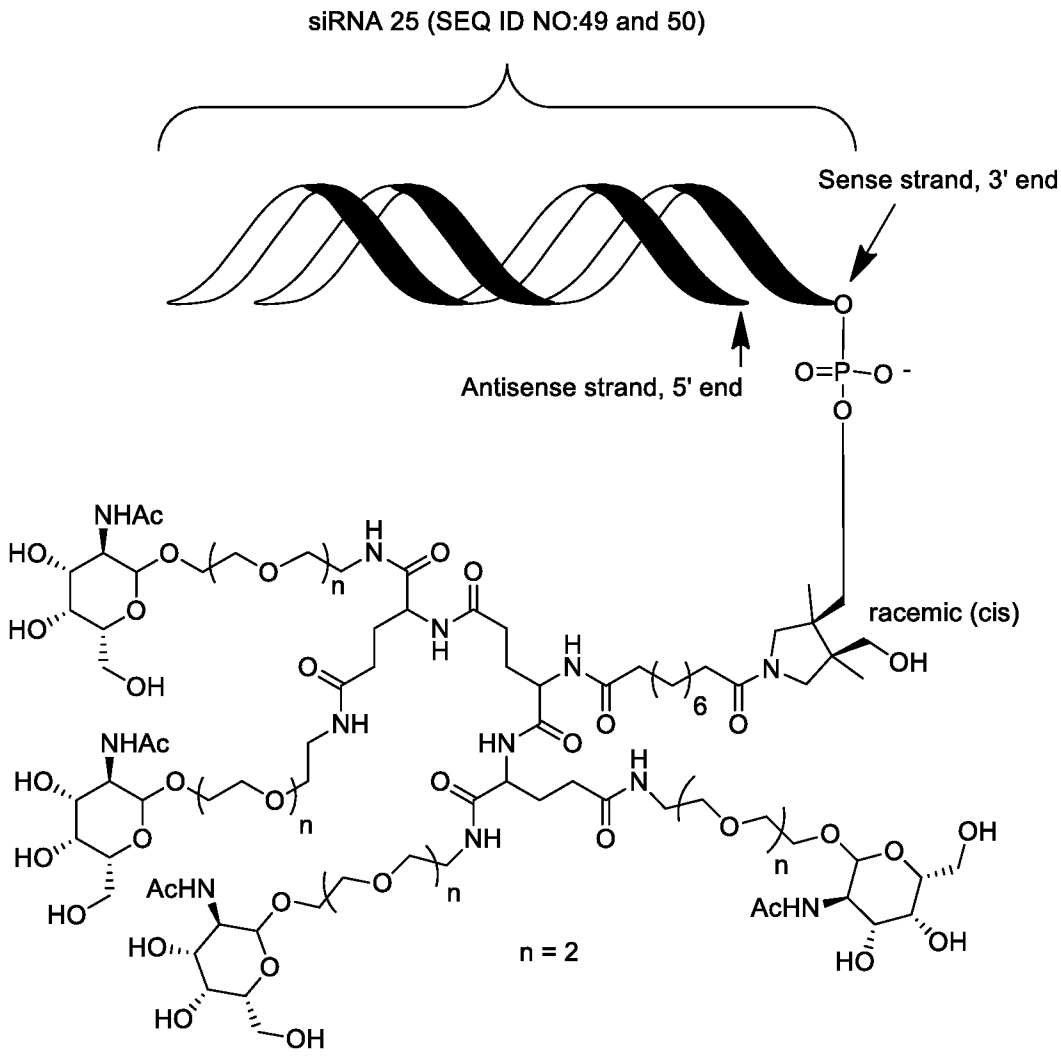
or a pharmaceutically acceptable salt thereof.

In one embodiment the compound of formula I is:



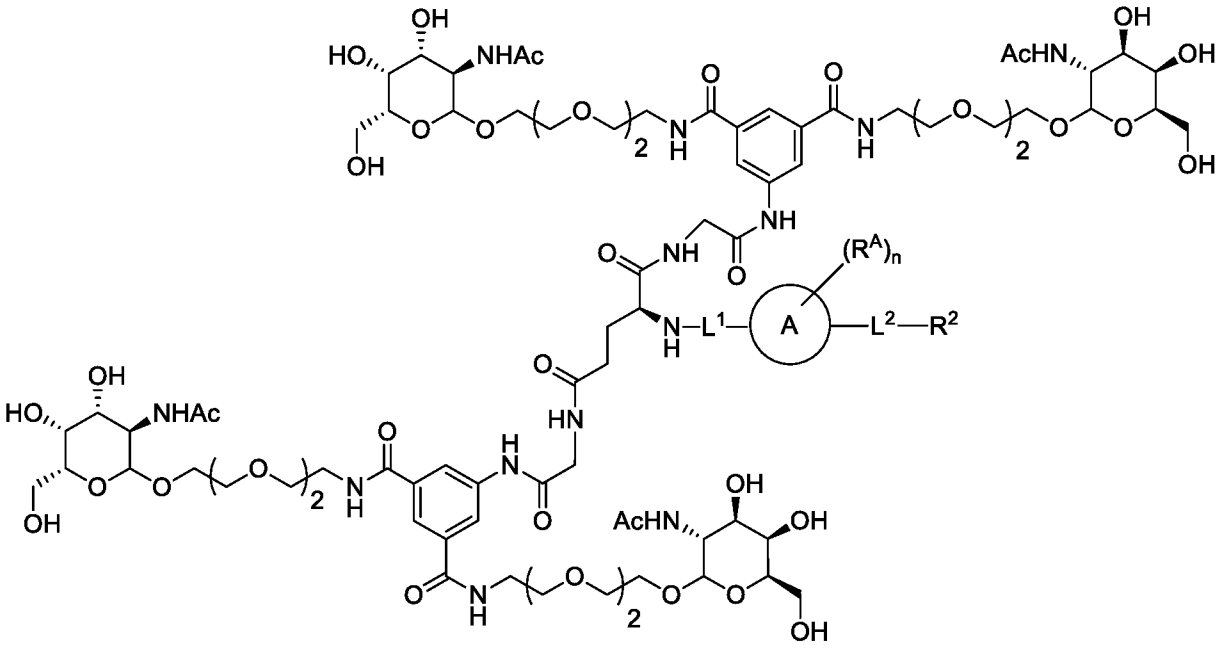
or a pharmaceutically acceptable salt thereof.

In one embodiment the compound of formula I is:



or a pharmaceutically acceptable salt thereof.

In one embodiment the invention provides a compound of formula (I):



(I)

wherein:

L^1 is absent or a linking group;

L^2 is absent or a linking group;

R^2 is a nucleic acid;

the ring A is absent, a 3-20 membered cycloalkyl, a 5-20 membered aryl, a 5-20 membered heteroaryl, or a 3-20 membered heterocycloalkyl;

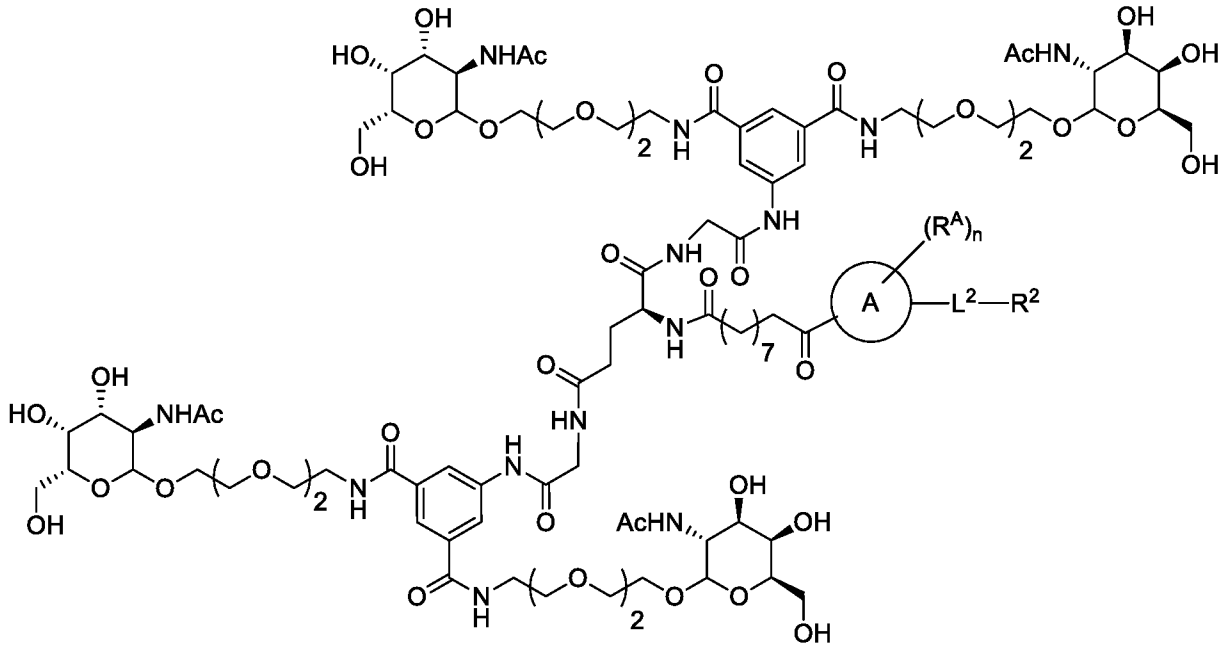
each R^A is independently selected from the group consisting of hydrogen, hydroxy, CN, F, Cl, Br, I, -C_{1-2} alkyl-OR^B, C_{1-10} alkyl C_{2-10} alkenyl, and C_{2-10} alkynyl; wherein the C_{1-10} alkyl C_{2-10} alkenyl, and C_{2-10} alkynyl are optionally substituted with one or more groups independently selected from halo, hydroxy, and C_{1-3} alkoxy;

R^B is hydrogen, a protecting group, a covalent bond to a solid support, or a bond to a linking group that is bound to a solid support; and

n is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

or a salt thereof.

In one embodiment the invention provides a compound of formula:



wherein:

L^2 is absent or a linking group;

R^2 is a nucleic acid;

the ring A is absent, a 3-20 membered cycloalkyl, a 5-20 membered aryl, a 5-20 membered heteroaryl, or a 3-20 membered heterocycloalkyl;

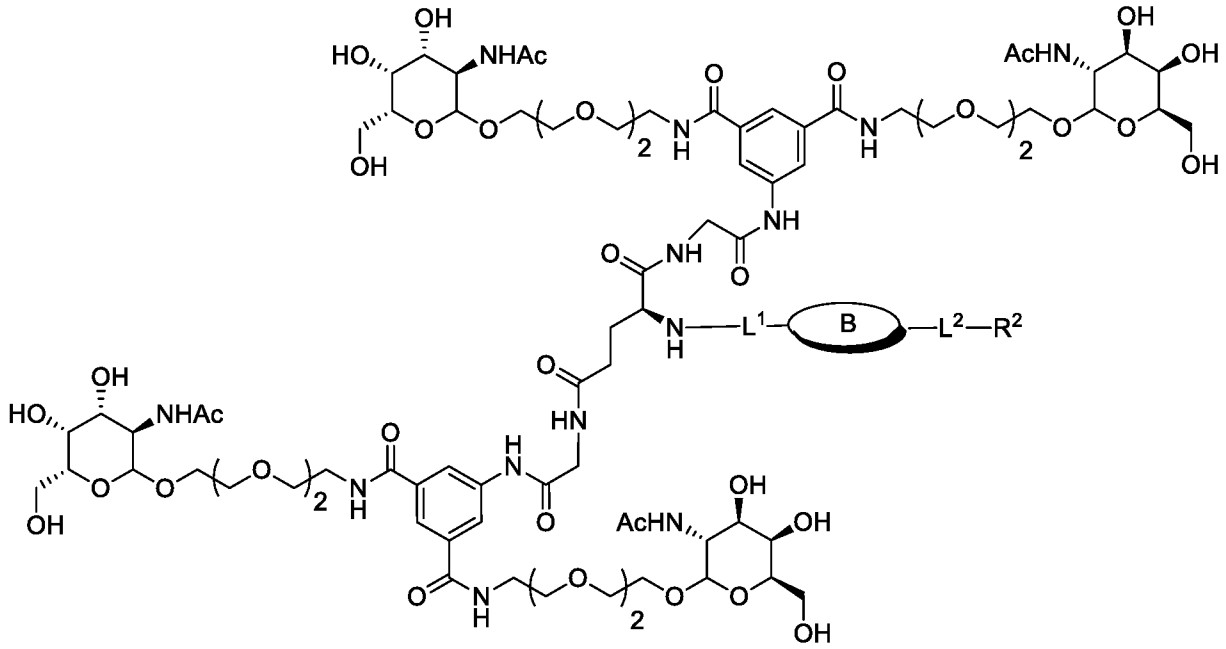
each R^A is independently selected from the group consisting of hydrogen, hydroxy, CN, F, Cl, Br, I, -C_{1-2} alkyl-OR^B, C_{1-10} alkyl C_{2-10} alkenyl, and C_{2-10} alkynyl; wherein the C_{1-10} alkyl C_{2-10} alkenyl, and C_{2-10} alkynyl are optionally substituted with one or more groups independently selected from halo, hydroxy, and C_{1-3} alkoxy;

R^B is hydrogen, a protecting group, a covalent bond to a solid support, or a bond to a linking group that is bound to a solid support; and

n is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

or a salt thereof.

In one embodiment the invention provides a compound of formula:



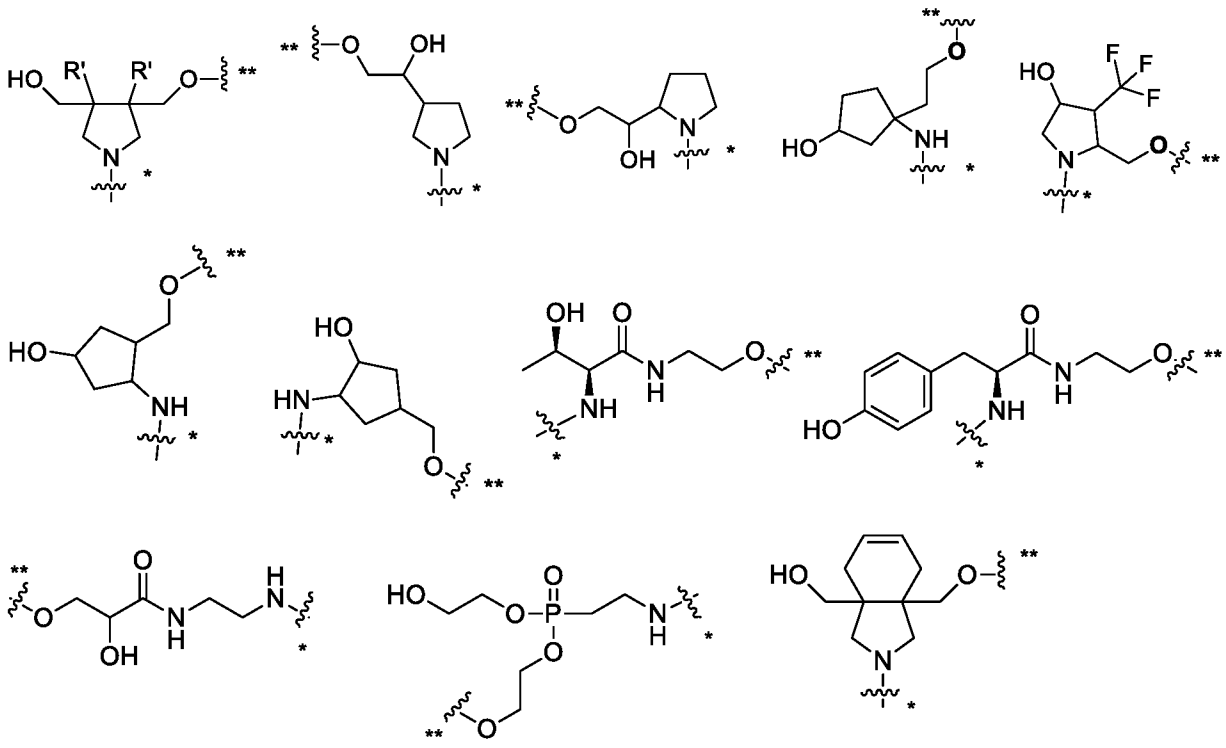
wherein:

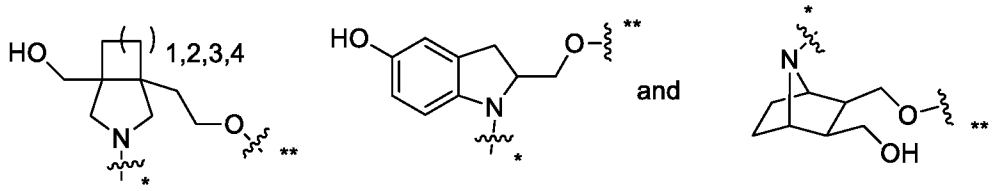
L^1 is absent or a linking group;

L^2 is absent or a linking group;

R^2 is a nucleic acid;

B is divalent and is selected from the group consisting of:





wherein:

each R' is independently C_{1-9} alkyl, C_{2-9} alkenyl or C_{2-9} alkynyl; wherein the C_{1-9} alkyl, C_{2-9} alkenyl or C_{2-9} alkynyl are optionally substituted with halo or hydroxyl;

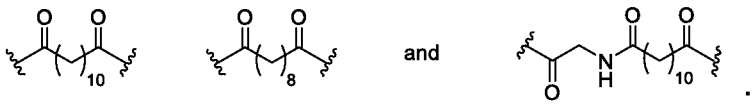
the valence marked with * is attached to L^1 or is attached to R^1 if L^1 is absent; and

the valence marked with ** is attached to L^2 or is attached to R^2 if L^2 is absent;

or a salt thereof.

In one embodiment L^1 and L^2 are independently a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 50 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O-, -NR^X-, -NR^X-C(=O)-, -C(=O)-NR^X- or –S-, and wherein R^X is hydrogen or (C1-C6)alkyl, and wherein the hydrocarbon chain, is optionally substituted with one or more substituents selected from (C1-C6)alkoxy, (C3-C6)cycloalkyl, (C1-C6)alkanoyl, (C1-C6)alkanoyloxy, (C1-C6)alkoxycarbonyl, (C1-C6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

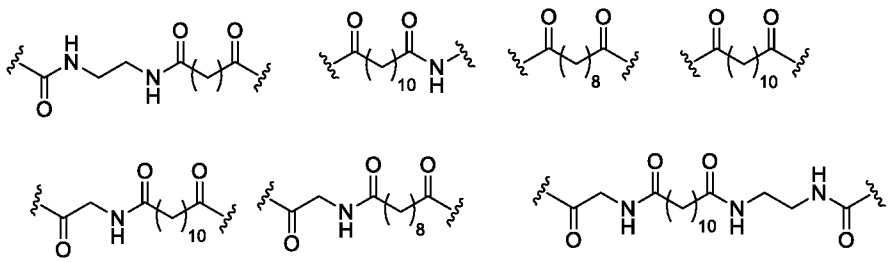
In one embodiment L^1 is selected from the group consisting of:

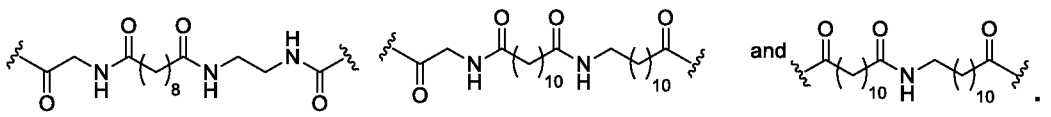


or a salt thereof.

In one embodiment L^1 is connected to B^1 through a linkage selected from the group consisting of: -O-, -S-, -(C=O)-, -(C=O)-NH-, -NH-(C=O), -(C=O)-O-, -NH-(C=O)-NH-, or –NH-(SO_2)-.

In one embodiment L^1 is selected from the group consisting of:



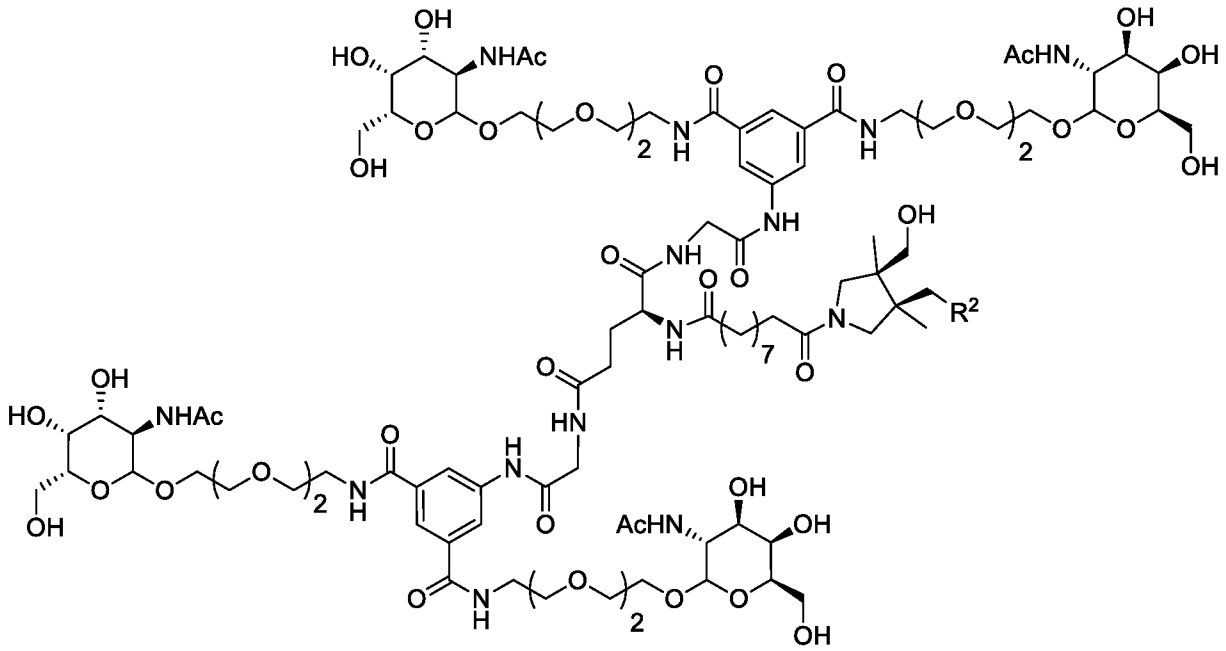


In one embodiment L^2 is connected to R^2 through -O-.

In one embodiment L^2 is C_{1-4} alkylene-O- that is optionally substituted with hydroxy.

In one embodiment L^2 is absent.

In one embodiment the invention provides a compound,



or a salt thereof wherein R^2 is a nucleic acid.

One aspect of this invention is pharmaceutical composition comprising a compound of formula I, and a pharmaceutically acceptable carrier.

Another aspect of this invention is a method to deliver a double stranded siRNA to the liver of an animal comprising administering a compound of formula I or a pharmaceutically acceptable salt thereof, to the animal.

Another aspect of this invention is a method to treat a disease or disorder (e.g., a liver disease or a viral infection, such as a hepatitis B viral infection) in an animal comprising administering a compound of formula I or a pharmaceutically acceptable salt thereof, to the animal.

Certain embodiments of the invention provide a compound of formula (I) or a pharmaceutically acceptable salt thereof for use in medical therapy.

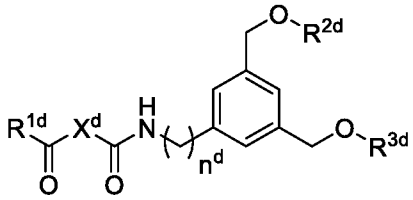
Certain embodiments of the invention provide a compound of formula (I) or a pharmaceutically acceptable salt thereof for the prophylactic or therapeutic treatment of a

disease or disorder (e.g., a liver disease or a viral infection, such as a hepatitis B virus infection) in an animal.

Certain embodiments of the invention provide the use of a compound of formula (I) or a pharmaceutically acceptable salt thereof to prepare a medicament for treating a disease or disorder (e.g., a liver disease or a viral infection, such as a hepatitis B virus infection) in an animal.

In certain embodiments, the animal is a mammal, such as a human (e.g., an HBV infected patient).

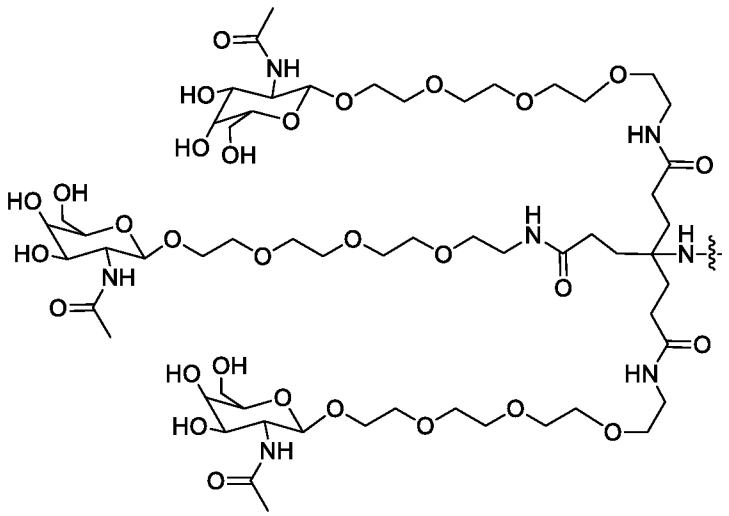
In one embodiment a compound of formula I has the following formula (Id):



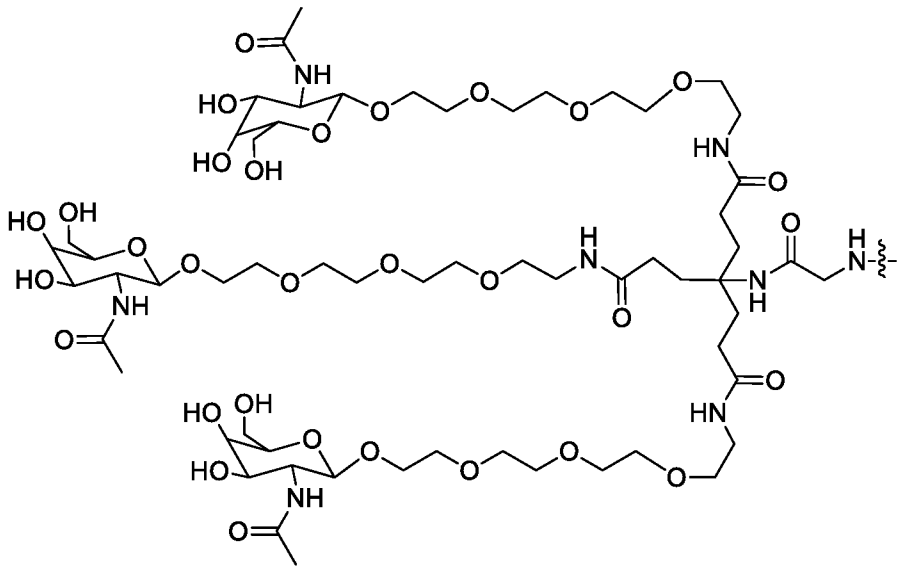
(Id)

wherein:

R^{1d} is selected from:



and



X^d is C_{2-10} alkylene;

n^d is 0 or 1;

R^{2d} is a double stranded siRNA molecule selected from the double stranded siRNA of Table 1; and

R^{3d} is H, a protecting group, a covalent bond to a solid support, or a bond to a linking group that is bound to a solid support.

In one embodiment R^{3d} includes a linking group that joins the remainder of the compound of formula Id to a solid support. The nature of the linking group is not critical provided the compound is a suitable intermediate for preparing a compound of formula Id wherein R^{2d} is a double stranded siRNA molecule selected from the double stranded siRNA of Table 1.

In one embodiment the linker in R^{3d} has a molecular weight of from about 20 daltons to about 1,000 daltons.

In one embodiment the linker in R^{3d} has a molecular weight of from about 20 daltons to about 500 daltons.

In one embodiment the linker in R^{3d} separates the solid support from the remainder of the compound of formula I by about 5 angstroms to about 40 angstroms, inclusive, in length.

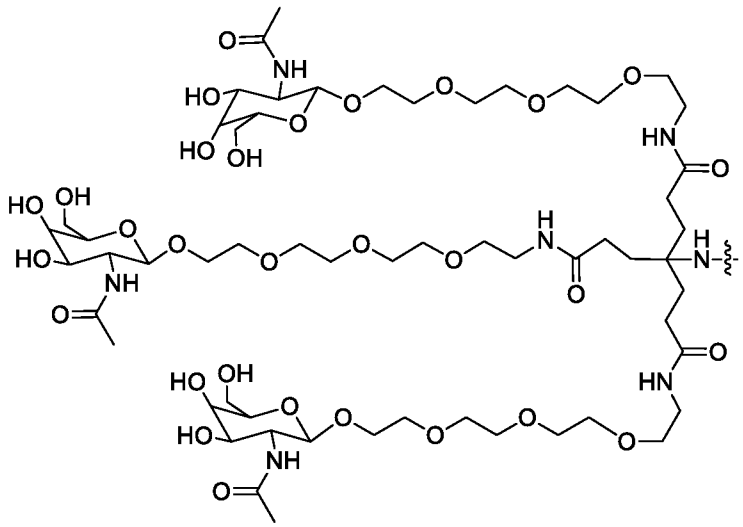
In one embodiment the linker in R^{3d} is a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 2 to 15 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms is optionally replaced by (-O-) or (-N(H)-), and wherein the chain is optionally substituted on carbon with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C_1-C_6)alkoxy, (C_3-C_6)cycloalkyl, (C_1-C_6)alkanoyl, (C_1-C_6)alkanoyloxy, (C_1-

C_6)alkoxycarbonyl, (C_1-C_6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

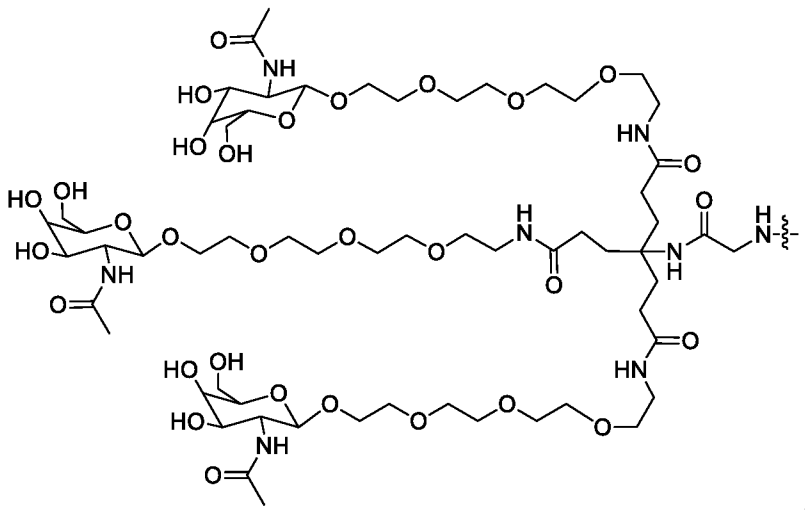
In one embodiment the linker in R^{3d} is a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 2 to 10 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms is optionally replaced by (-O-) or (-N(H)-), and wherein the chain is optionally substituted on carbon with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C_1-C_6)alkoxy, (C_3-C_6)cycloalkyl, (C_1-C_6)alkanoyl, (C_1-C_6)alkanoyloxy, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

In one embodiment the linker in R^{3d} is $-C(=O)CH_2CH_2C(=O)N(H)-$.

In one embodiment R^{1d} is:



In one embodiment R^{1d} is:



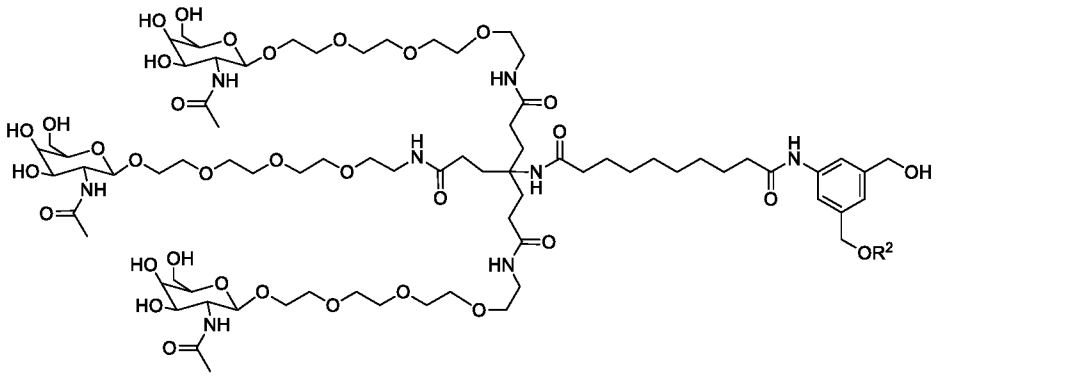
In one embodiment X^d is C_8alkylene.

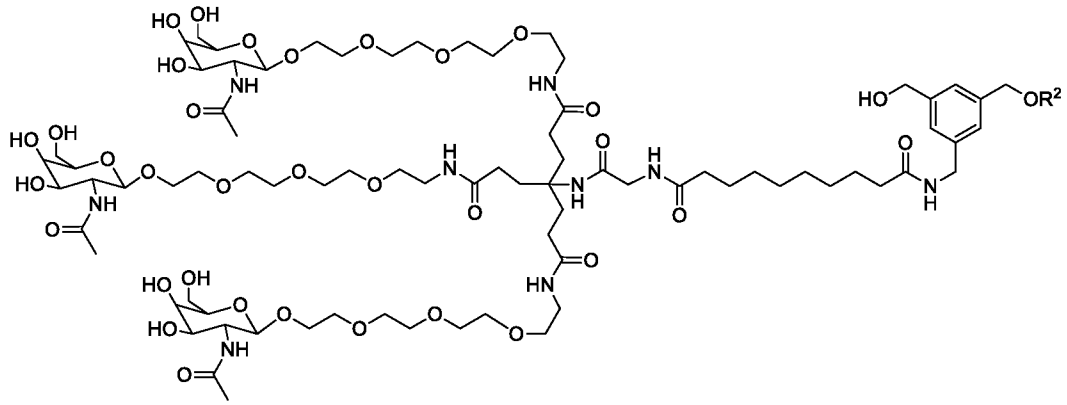
In one embodiment n^d is 0.

In one embodiment R^{2d} is an siRNA.

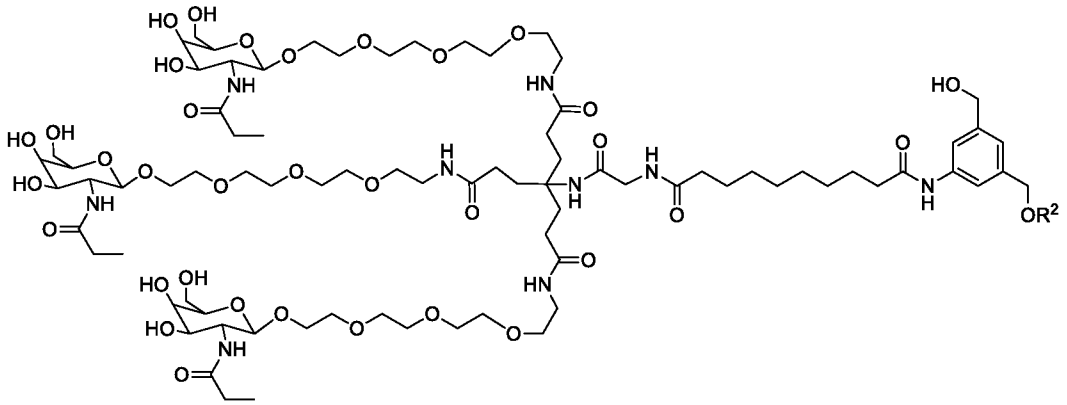
In one embodiment R^{3d} is H.

In another embodiment a compound of (Id) or the salt thereof is selected from the group consisting of:





and



and salts thereof.

One aspect of this invention is a pharmaceutical composition comprising a compound of formula (Id), and a pharmaceutically acceptable carrier.

One aspect of this invention is a method to deliver is a double stranded siRNA to the liver of an animal comprising administering a compound of formula (Id) or a pharmaceutically acceptable salt thereof, to the animal.

Another aspect of this invention is a method to treat a disease or disorder (e.g., a viral infection, such as a hepatitis B viral infection) in an animal comprising administering a compound of formula (Id) or a pharmaceutically acceptable salt thereof, to the animal.

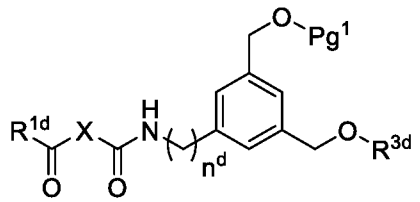
Certain embodiments of the invention provide a compound of formula (Id) or a pharmaceutically acceptable salt thereof for use in medical therapy.

Certain embodiments of the invention provide a compound of formula (Id) or a pharmaceutically acceptable salt thereof for the prophylactic or therapeutic treatment of a disease or disorder (e.g., a viral infection, such as a hepatitis B virus infection) in an animal.

Certain embodiments of the invention provide the use of a compound of formula (Id) or a pharmaceutically acceptable salt thereof to prepare a medicament for treating a disease or disorder (e.g., a viral infection, such as a hepatitis B virus infection) in an animal.

In certain embodiments, the animal is a mammal, such as a human (e.g., an HBV infected patient).

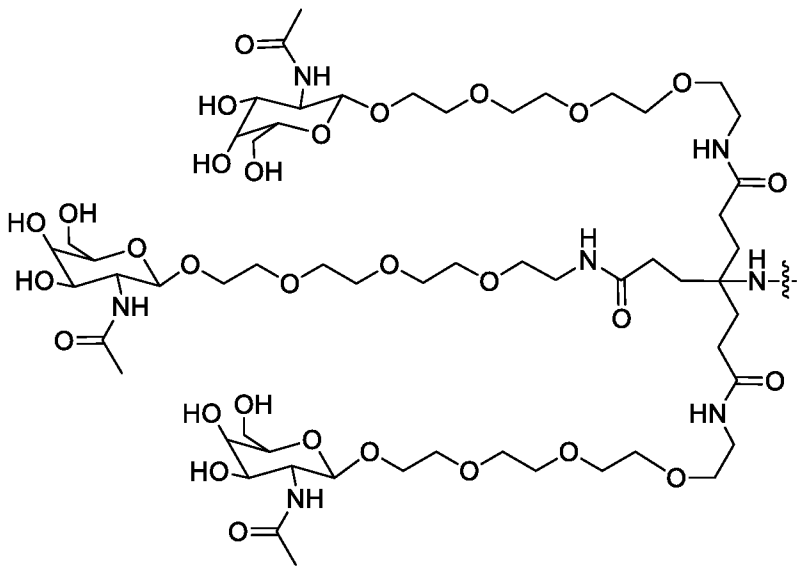
The invention also provides synthetic intermediates and methods disclosed herein that are useful to prepare compounds of formula (Id). For example, the invention includes an intermediate compound of formula Ie:



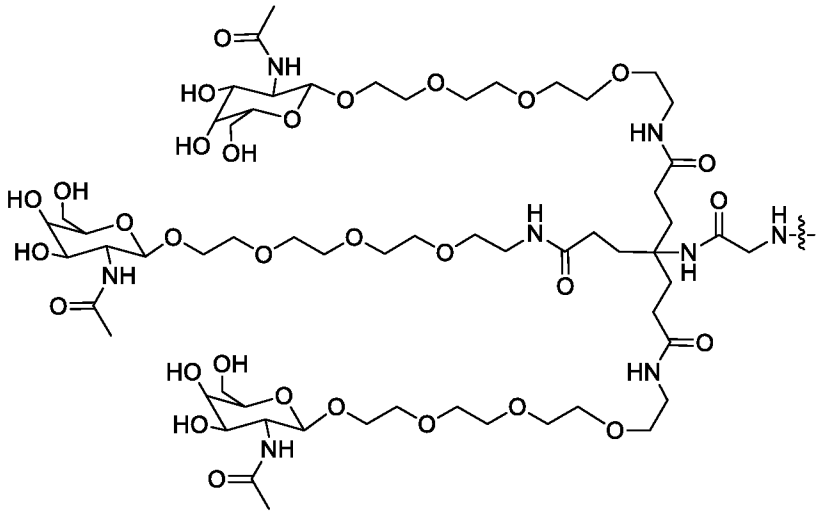
(Ie)

or a salt thereof, wherein:

R^{1d} is selected from:



and



X^d is C_{2-8} alkylene;

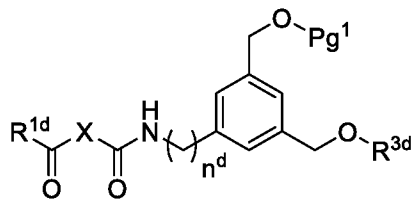
n^d is 0 or 1;

Pg^1 is H or a suitable protecting group; and

R^{3d} is H, a protecting group, a covalent bond to a solid support, or a bond to a linking group that is bound to a solid support. Figure 1 illustrates a representative intermediate compound of formula (Ie), wherein a targeting ligand/linker is bound to a solid phase support, and wherein Pg^1 is the protecting group DMTr.

In one embodiment Pg^1 is TMTr (Trimethoxytrityl), DMTr (Dimethoxytrityl), MMTr (Monomethoxytrityl), or Tr (Trityl).

The invention also provides a method to prepare a compound of formula (Id) as described herein comprising subjecting a corresponding compound of formula (Ie):



(Ie)

wherein:

X^d is C_{2-8} alkylene;

n^d is 0 or 1;

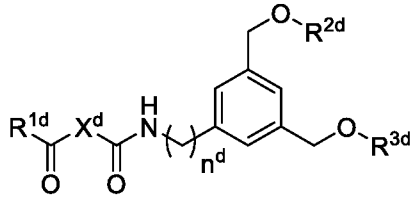
Pg^1 is H; and

R^{3d} is a covalent bond to a solid support or a bond to a linking group that is bound to a solid support, to solid phase nucleic acid synthesis conditions to provide a corresponding

compound of formula Id wherein R^{2d} is a double stranded siRNA molecule selected from the double stranded siRNA molecules of Table 1.

In one embodiment the method further comprises removing the compound from the solid support to provide the corresponding compound of formula Id wherein R^{3d} is H.

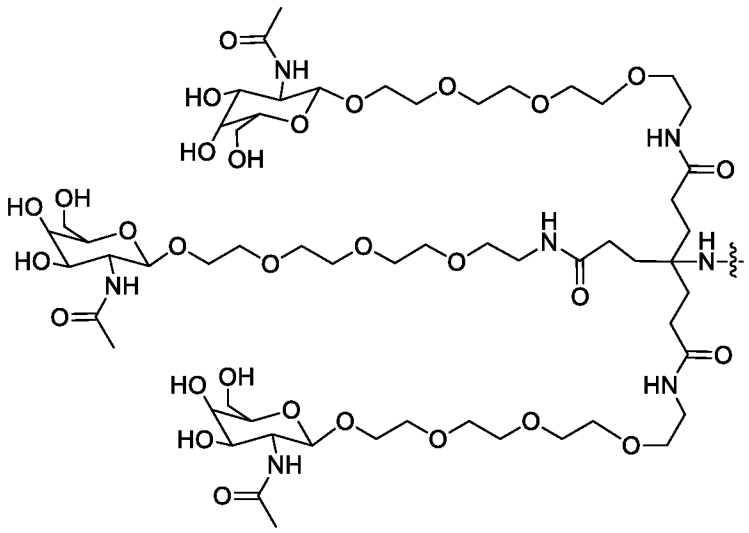
In one embodiment the compound is not a compound formula Id:



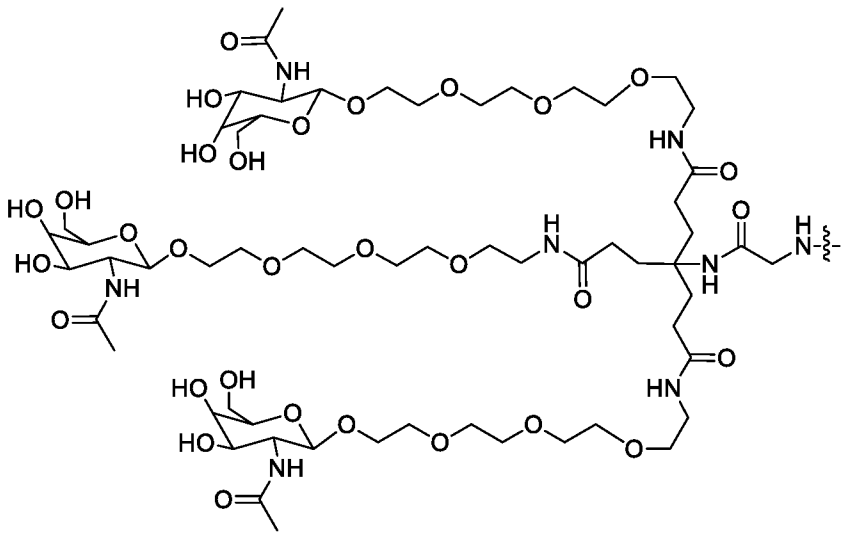
(Id)

or a salt thereof, wherein:

R^{1d} is selected from:



and



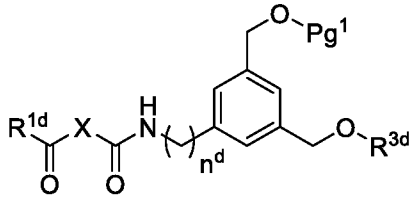
X^d is C_{2-10} alkylene;

N^d is 0 or 1;

R^{2d} is a double stranded siRNA molecule selected from the double stranded siRNA molecules of Table 1; and

R^{3d} is H, a protecting group, a covalent bond to a solid support, or a bond to a linking group that is bound to a solid support.

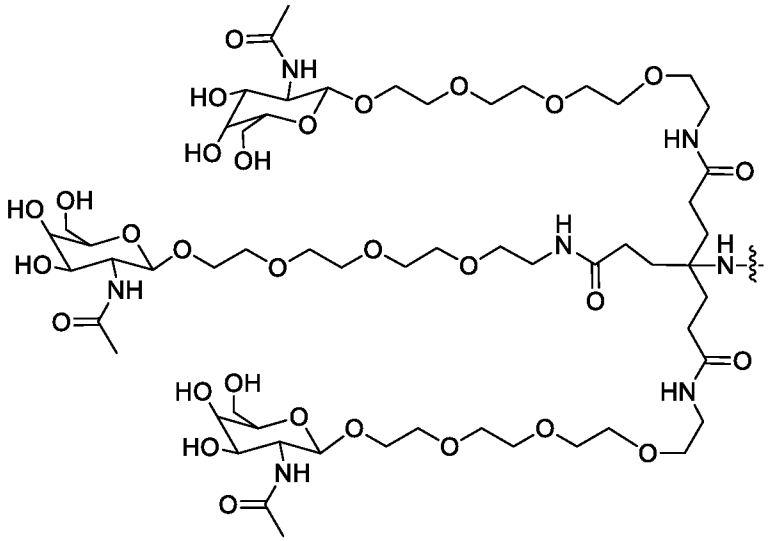
In one embodiment the compound is not a compound formula Ie:



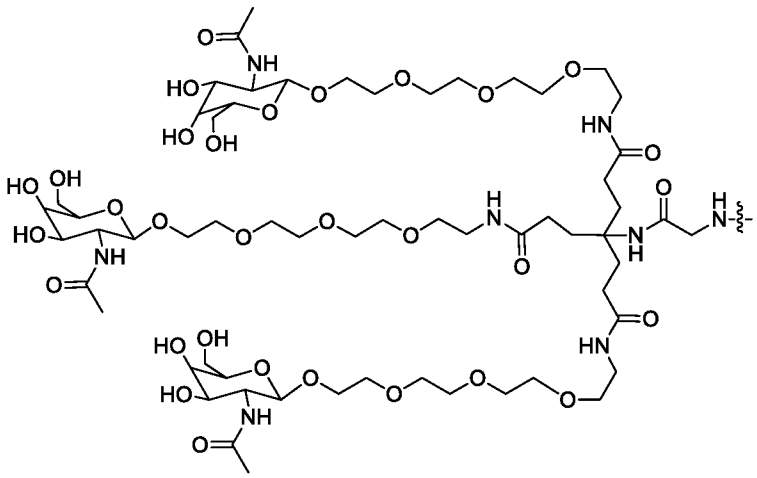
(Ie)

or a salt thereof, wherein:

R^{1d} is selected from:



and



X^d is C_{2-8} alkylene;

n^d is 0 or 1;

Pg^1 is H or a suitable protecting group; and

R^{3d} is H, a protecting group, a covalent bond to a solid support, or a bond to a linking group that is bound to a solid support.

In one embodiment R^{3d} is H.

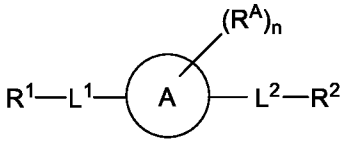
In one embodiment R^{3d} is a covalent bond to a solid support.

In one embodiment R^{3d} is a bond to a linking group that is bound to a solid support, wherein the linking group is a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 2 to 15 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms is optionally replaced by (-O-) or (-N(H)-), and wherein the chain is optionally substituted on carbon with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C_1-C_6)alkoxy, (C_3-C_6)cycloalkyl, (C_1-C_6)alkanoyl, (C_1-C_6)alkanoyloxy, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

In one embodiment R^{3d} is a bond to a linking group that is bound to a solid support, wherein the linking group is a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 2 to 10 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms is optionally replaced by (-O-) or (-N(H)-), and wherein the chain is optionally substituted on carbon with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C_1-C_6)alkoxy, (C_3-C_6)cycloalkyl, (C_1-C_6)alkanoyl, (C_1-C_6)alkanoyloxy, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

In one embodiment R^{3d} is a bond to a linking group that is bound to a solid support, wherein the linking group is –C(=O)CH_2CH_2C(=O)N(H)-.

In one embodiment the invention provides a compound of formula (I):



(I)

wherein:

R^1 is H or a synthetic activating group;

L^1 is absent or a linking group;

L^2 is absent or a linking group;

R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of Table 1;

the ring A is absent, a 3-20 membered cycloalkyl, a 5-20 membered aryl, a 5-20 membered heteroaryl, or a 3-20 membered heterocycloalkyl;

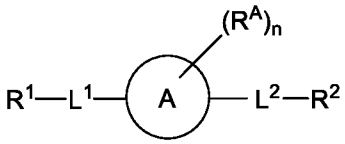
each R^A is independently selected from the group consisting of hydrogen, hydroxy, CN, F, Cl, Br, I, -C_{1-2} alkyl-OR^B, C_{1-10} alkyl C_{2-10} alkenyl, and C_{2-10} alkynyl; wherein the C_{1-10} alkyl C_{2-10} alkenyl, and C_{2-10} alkynyl are optionally substituted with one or more groups independently selected from halo, hydroxy, and C_{1-3} alkoxy;

R^B is hydrogen, a protecting group, a covalent bond to a solid support, or a bond to a linking group that is bound to a solid support; and

n is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

or a salt thereof.

In one embodiment the invention provides a compound of formula (I):



(I)

wherein:

R^1 a is targeting ligand;

L^1 is absent or a linking group;

L^2 is absent or a linking group;

R^2 is H or a synthetic activating group;

the ring A is absent, a 3-20 membered cycloalkyl, a 5-20 membered aryl, a 5-20 membered heteroaryl, or a 3-20 membered heterocycloalkyl;

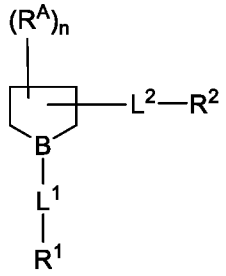
each R^A is independently selected from the group consisting of hydrogen, hydroxy, CN, F, Cl, Br, I, -C_{1-2} alkyl-OR^B, C_{1-10} alkyl C_{2-10} alkenyl, and C_{2-10} alkynyl; wherein the C_{1-10} alkyl C_{2-10} alkenyl, and C_{2-10} alkynyl are optionally substituted with one or more groups independently selected from halo, hydroxy, and C_{1-3} alkoxy;

R^B is hydrogen, a protecting group, a covalent bond to a solid support, or a bond to a linking group that is bound to a solid support; and

n is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

or a salt thereof.

In one embodiment the invention provides a compound of formula (Ig):



(Ig)

wherein:

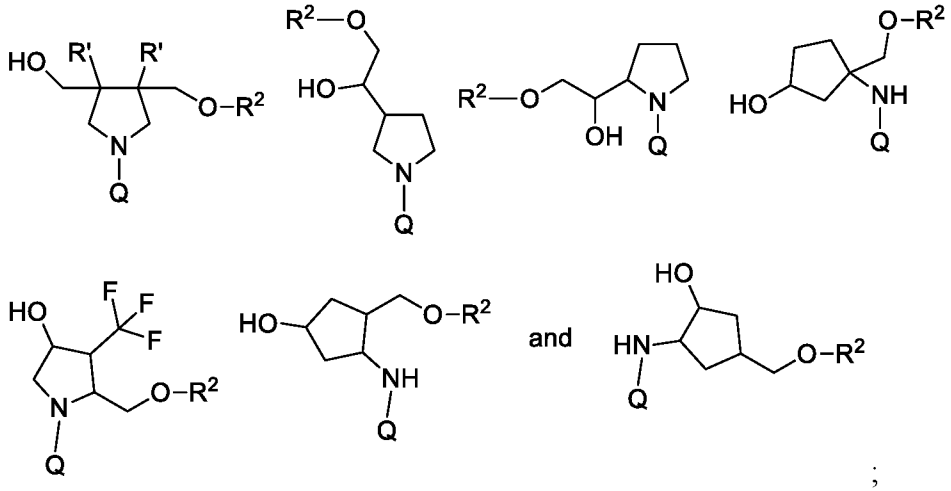
B is –N- or -CH-;

L^2 is C_{1-4} alkylene-O- that is optionally substituted with hydroxyl or halo; and

n is 0, 1, 2, 3, 4, 5, 6, or 7;

or a salt thereof.

In one embodiment the invention provides a compound selected from the group consisting of:



;

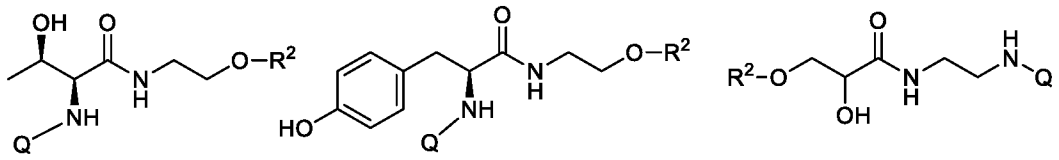
wherein:

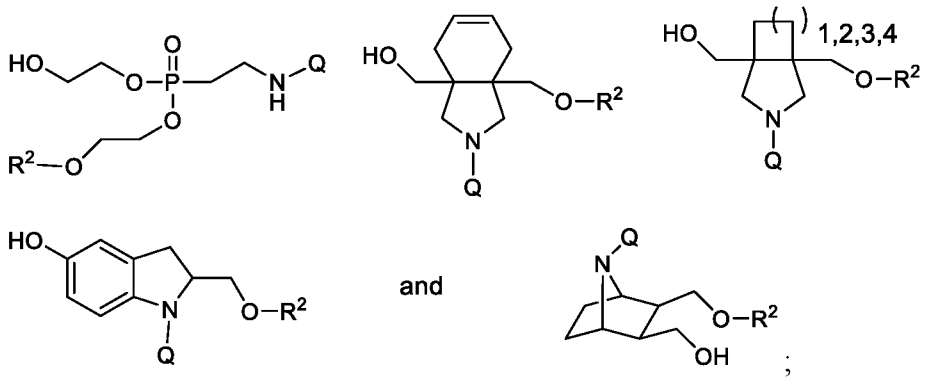
Q is $–L^1-R^1$; and

R' is C_{1-9} alkyl, C_{2-9} alkenyl or C_{2-9} alkynyl; wherein the C_{1-9} alkyl, C_{2-9} alkenyl or C_{2-9} alkynyl are optionally substituted with halo or hydroxyl;

and salts thereof.

In one embodiment the invention provides a compound selected from the group consisting of:

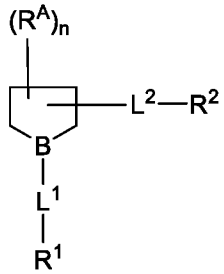




;

wherein: Q is $–L^1-R^1$; and salts thereof.

In one embodiment the invention provides a compound of formula (Ig):



(Ig)

wherein:

B is –N- or -CH-;

L^1 is absent or a linking group;

L^2 is C_{1-4} alkylene-O- that is optionally substituted with hydroxyl or halo;

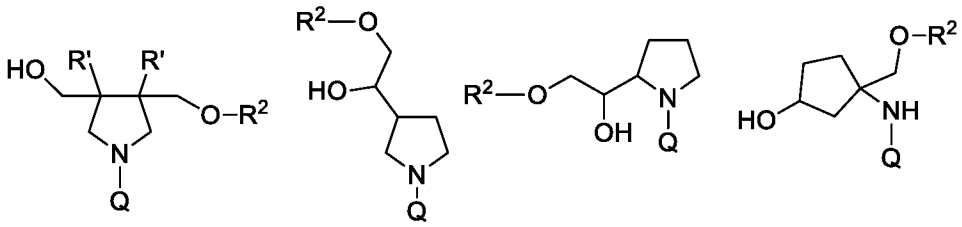
n is 0, 1, 2, 3, 4, 5, 6, or 7;

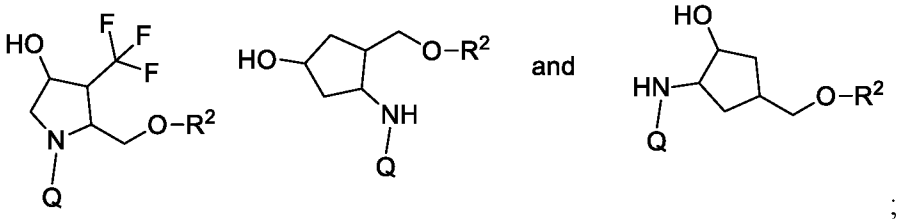
R^1 is H or a synthetic activating group; and

R^2 is H or a synthetic activating group;

or a salt thereof.

In one embodiment the invention provides a compound selected from the group consisting of:





;

wherein Q is $-L^1-R^1$;

L^1 is absent or a linking group;

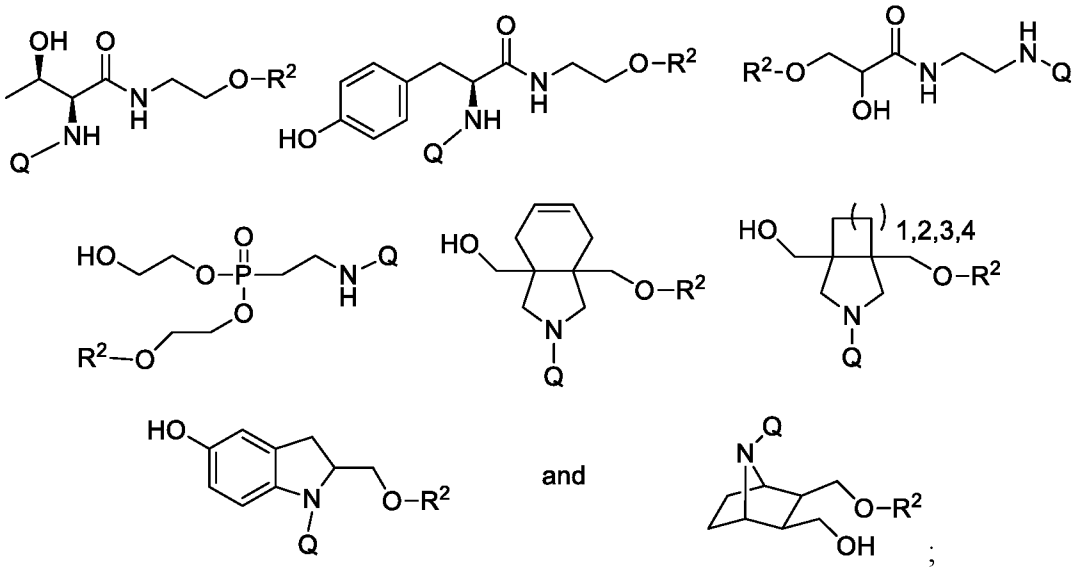
R' is C_{1-9} alkyl, C_{2-9} alkenyl or C_{2-9} alkynyl; wherein the C_{1-9} alkyl, C_{2-9} alkenyl or C_{2-9} alkynyl are optionally substituted with halo or hydroxyl;

R^1 is H or a synthetic activating group; and

R^2 is H or a synthetic activating group;

or a salt thereof.

In one embodiment the invention provides a compound selected from the group consisting of:



;

wherein:

Q is $-L^1-R^1$;

L^1 is absent or a linking group;

R^1 is H or a synthetic activating group; and

R^2 is H or a synthetic activating group;

or a salt thereof.

In one embodiment R^1 is H or a synthetic activating group derivable from DCC, HOBt, EDC, BOP, PyBOP or HBTU.

In one embodiment R^2 is H, acetate, triflate, mesylate or succinate.

In one embodiment R^1 is a synthetic activating group derivable from DCC, HOBt, EDC, BOP, PyBOP or HBTU.

In one embodiment R^2 is acetate, triflate, mesylate or succinate.

In one embodiment L^1 is a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 5 to 20 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced –O-, -NH-, -NH-C(=O)-, -C(=O)-NH- or –S-.

In one embodiment the invention provides a compound of formula (XX):

(XX)

wherein:

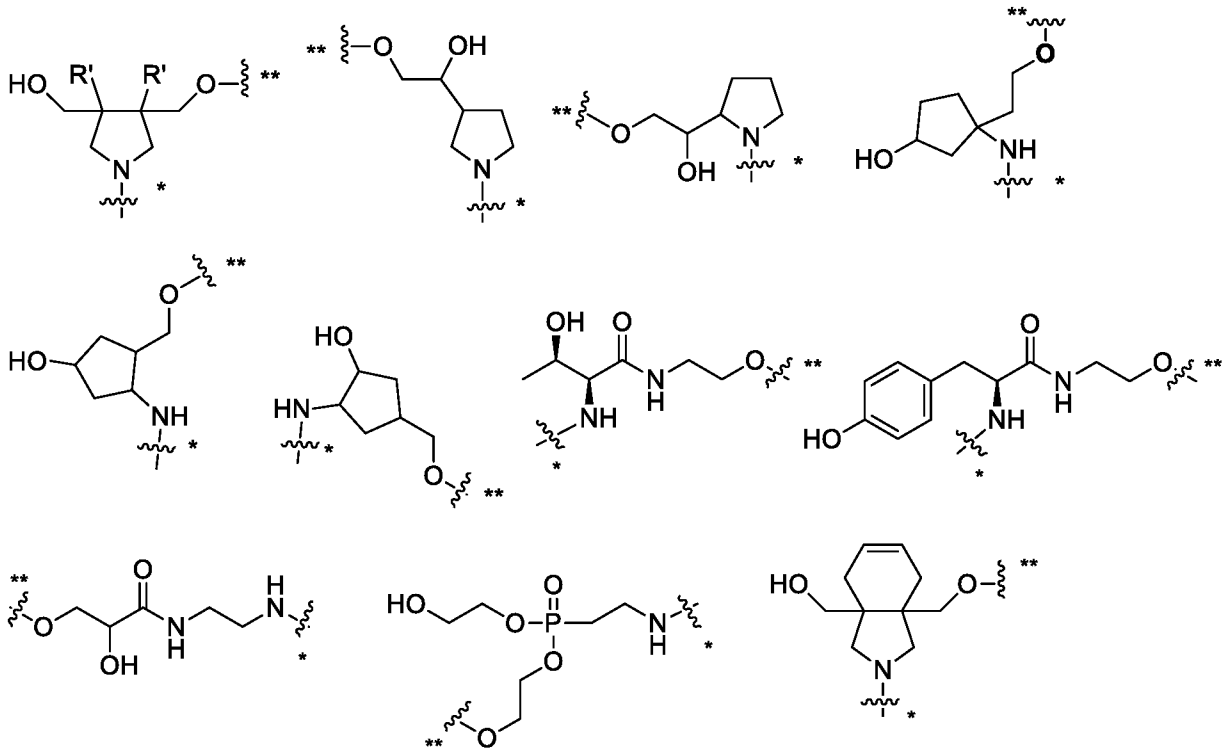
R^1 a is targeting ligand;

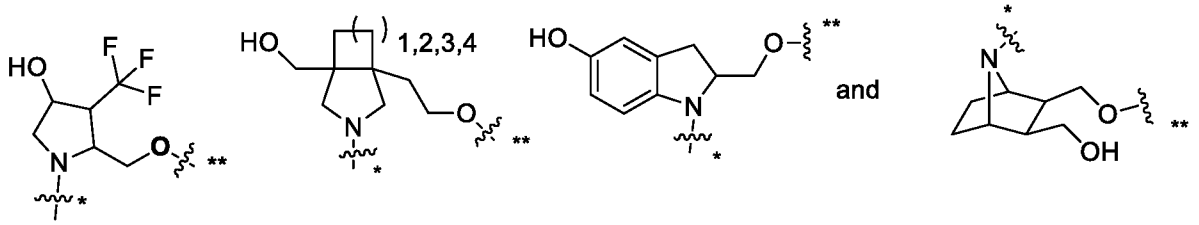
L^1 is absent or a linking group;

L^2 is absent or a linking group;

R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of Table 1;

B is divalent and is selected from the group consisting of:





wherein:

each R' is independently C_{1-9} alkyl, C_{2-9} alkenyl or C_{2-9} alkynyl; wherein the C_{1-9} alkyl, C_{2-9} alkenyl or C_{2-9} alkynyl are optionally substituted with halo or hydroxyl;

the valence marked with * is attached to L^1 or is attached to R^1 if L^1 is absent; and

the valence marked with ** is attached to L^2 or is attached to R^2 if L^2 is absent;

or a salt thereof.

In one embodiment R^1 comprises 2-8 saccharides.

In one embodiment R^1 comprises 2-6 saccharides.

In one embodiment R^1 comprises 2-4 saccharides.

In one embodiment R^1 comprises 3-8 saccharides.

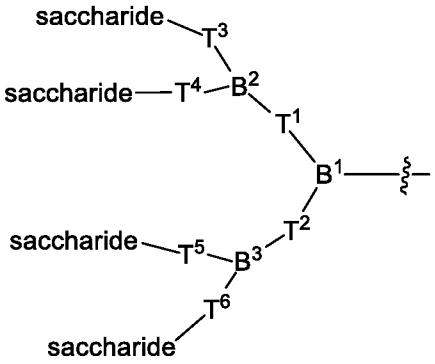
In one embodiment R^1 comprises 3-6 saccharides.

In one embodiment R^1 comprises 3-4 saccharides.

In one embodiment R^1 comprises 3 saccharides.

In one embodiment R^1 comprises 4 saccharides.

In one embodiment R^1 has the following formula:



wherein:

B^1 is a trivalent group comprising about 1 to about 20 atoms and is covalently bonded to L^1, T^1, and T^2.

B^2 is a trivalent group comprising about 1 to about 20 atoms and is covalently bonded to T^1, T^3, and T^4;

B^3 is a trivalent group comprising about 1 to about 20 atoms and is covalently bonded to T^2, T^5, and T^6;

T^1 is absent or a linking group;

T^2 is absent or a linking group;

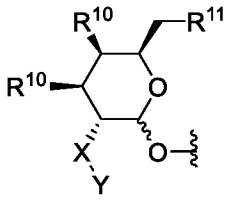
T^3 is absent or a linking group;

T^4 is absent or a linking group;

T^5 is absent or a linking group; and

T^6 is absent or a linking group

In one embodiment each saccharide is independently selected from:



wherein:

X is NR^3, and Y is selected from $-(C=O)R^4$, $-SO_2R^5$, and $-(C=O)NR^6R^7$; or X is -(C=O)- and Y is NR^8R^9;

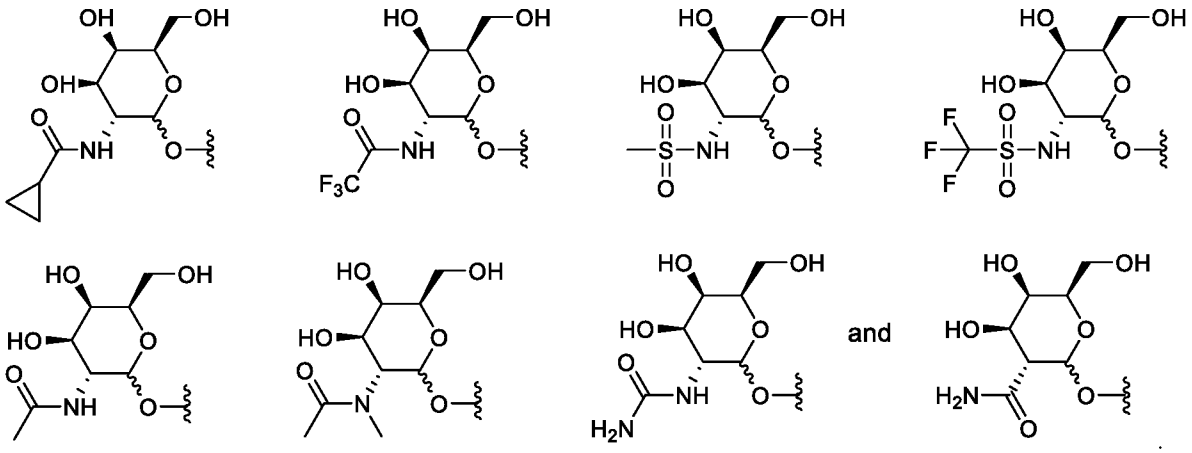
R^3 is hydrogen or $(C_1\text{-}C_4)$alkyl;

R^4, R^5, R^6, R^7 , R^8 and R^9 are each independently selected from the group consisting of hydrogen, $(C_1\text{-}C_8)$alkyl, $(C_1\text{-}C_8)$haloalkyl, $(C_1\text{-}C_8)$alkoxy and $(C_3\text{-}C_6)$cycloalkyl that is optionally substituted with one or more groups independently selected from the group consisting of halo, $(C_1\text{-}C_4)$alkyl, $(C_1\text{-}C_4)$haloalkyl, $(C_1\text{-}C_4)$alkoxy and $(C_1\text{-}C_4)$haloalkoxy;

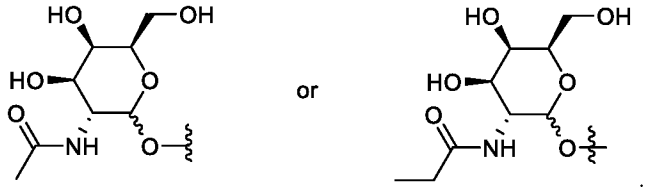
R^{10} is -OH, $-NR^8R^9$ or – F; and

R^{11} is -OH, $-NR^8R^9$, -F or 5 membered heterocycle that is optionally substituted with one or more groups independently selected from the group consisting of halo, hydroxyl, carboxyl, amino, $(C_1\text{-}C_4)$alkyl, $(C_1\text{-}C_4)$haloalkyl, $(C_1\text{-}C_4)$alkoxy and $(C_1\text{-}C_4)$haloalkoxy.

In one embodiment each saccharide is independently selected from the group consisting of:



In one embodiment each saccharide is independently:



In one embodiment one of T^1 and T^2 is absent.

In one embodiment both T^1 and T^2 are absent.

In one embodiment each of T^1, T^2, T^3, T^4, T^5, and T^6 is independently absent or a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 50 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O-, -NR^X-, -NR^X-C(=O)-, -C(=O)-NR^X- or –S-, and wherein R^X is hydrogen or (C1-C6)alkyl, and wherein the hydrocarbon chain, is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C1-C6)alkoxy, (C3-C6)cycloalkyl, (C1-C6)alkanoyl, (C1-C6)alkanoyloxy, (C1-C6)alkoxycarbonyl, (C1-C6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

In one embodiment each of T^1, T^2, T^3, T^4, T^5, and T^6 is independently absent or a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 20 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O-, -NR^X-, -NR^X-C(=O)-, -C(=O)-NR^X- or –S-, and wherein R^X is hydrogen or (C1-C6)alkyl, and wherein the hydrocarbon chain, is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C1-C6)alkoxy, (C3-C6)cycloalkyl, (C1-C6)alkanoyl, (C1-C6)alkanoyloxy, (C1-C6)alkoxycarbonyl, (C1-C6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

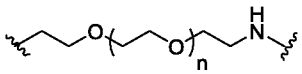
In one embodiment each of T^1, T^2, T^3, T^4, T^5, and T^6 is independently absent or a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 50 carbon atoms, or a salt thereof, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O- or -NR^X-, and wherein R^X is hydrogen or (C_1-C_6)alkyl, and wherein the hydrocarbon chain, is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from halo, hydroxy, and oxo (=O).

In one embodiment each of T^1, T^2, T^3, T^4, T^5, and T^6 is independently absent or a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 20 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon

chain is optionally replaced by –O- and wherein the hydrocarbon chain, is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from halo, hydroxy, and oxo (=O).

In one embodiment each of T^1, T^2, T^3, T^4, T^5, and T^6 is independently absent or a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 20 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O- and wherein the hydrocarbon chain, is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from halo, hydroxy, and oxo (=O).

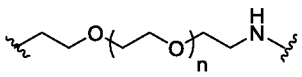
In one embodiment at least one of T^3, T^4, T^5, and T^6 is:



wherein:

n = 1, 2, 3.

In one embodiment each of T^3, T^4, T^5, and T^6 is independently selected from the group consisting of:



wherein:

n = 1, 2, 3.

In one embodiment at least one of T^1 and T^2 is glycine

In one embodiment each of T^1 and T^2 is glycine.

In one embodiment B^1 is a trivalent group comprising 1 to 15 atoms and is covalently bonded to L^1, T^1, and T^2.

In one embodiment B^1 is a trivalent group comprising 1 to 10 atoms and is covalently bonded to L^1, T^1, and T^2.

In one embodiment B^1 comprises a (C_1-C_6)alkyl.

In one embodiment B^1 comprises a C_{3-8} cycloalkyl.

In one embodiment B^1 comprises a silyl group.

In one embodiment B^1 comprises a D- or L-amino acid.

In one embodiment B^1 comprises a saccharide.

In one embodiment B^1 comprises a phosphate group.

In one embodiment B^1 comprises a phosphonate group.

In one embodiment B^1 comprises an aryl.

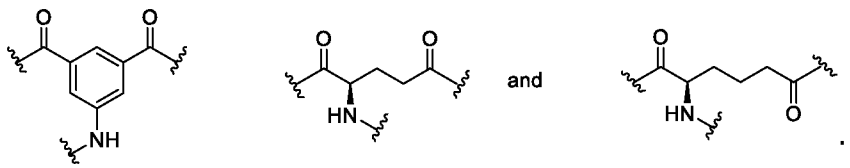
In one embodiment B^1 comprises a phenyl ring.

In one embodiment B^1 is a phenyl ring.

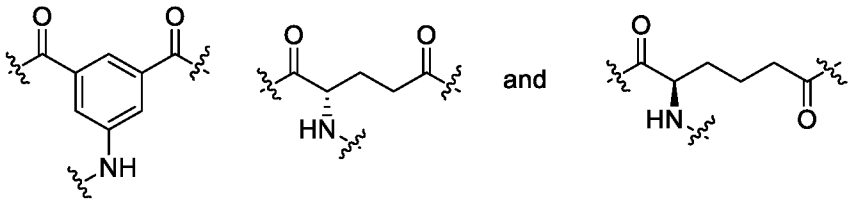
In one embodiment B^1 is CH.

In one embodiment B^1 comprises a heteroaryl.

In one embodiment B^1 is selected from the group consisting of:



In one embodiment B^1 is selected from the group consisting of:



In one embodiment B^2 is a trivalent group comprising 1 to 15 atoms and is covalently bonded to L^1, T^1, and T^2.

In one embodiment B^2 is a trivalent group comprising 1 to 10 atoms and is covalently bonded to L^1, T^1, and T^2.

In one embodiment B^2 comprises a $(C_1\text{-}C_6)$alkyl

In one embodiment B^2 comprises a $C_{3\text{-}8}$ cycloalkyl.

In one embodiment B^2 comprises a silyl group.

In one embodiment B^2 comprises a D- or L-amino acid.

In one embodiment B^2 comprises a saccharide.

In one embodiment B^2 comprises a phosphate group.

In one embodiment B^2 comprises a phosphonate group.

In one embodiment B^2 comprises an aryl.

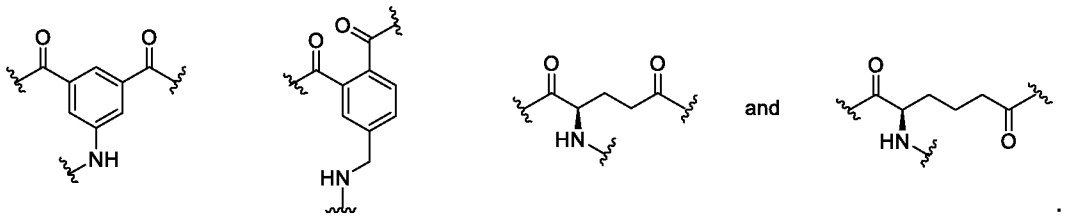
In one embodiment B^2 comprises a phenyl ring.

In one embodiment B^2 is a phenyl ring.

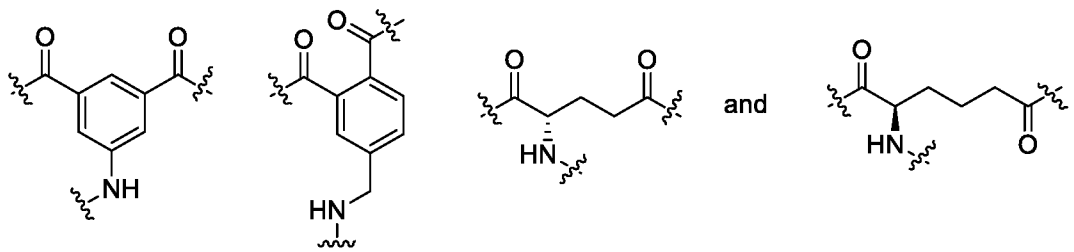
In one embodiment B^2 is CH.

In one embodiment B^2 comprises a heteroaryl.

In one embodiment B^2 is selected from the group consisting of:



In one embodiment B^2 is selected from the group consisting of:



or a salt thereof.

In one embodiment B^3 is a trivalent group comprising 1 to 15 atoms and is covalently bonded to L^1, T^1, and T^2.

In one embodiment B^3 is a trivalent group comprising 1 to 10 atoms and is covalently bonded to L^1, T^1, and T^2.

In one embodiment B^3 comprises a $(C_1\text{-}C_6)$alkyl.

In one embodiment B^3 comprises a $C_{3\text{-}8}$ cycloalkyl.

In one embodiment B^3 comprises a silyl group.

In one embodiment B^3 comprises a D- or L-amino acid.

In one embodiment B^3 comprises a saccharide.

In one embodiment B^3 comprises a phosphate group.

In one embodiment B^3 comprises a phosphonate group.

In one embodiment B^3 comprises an aryl.

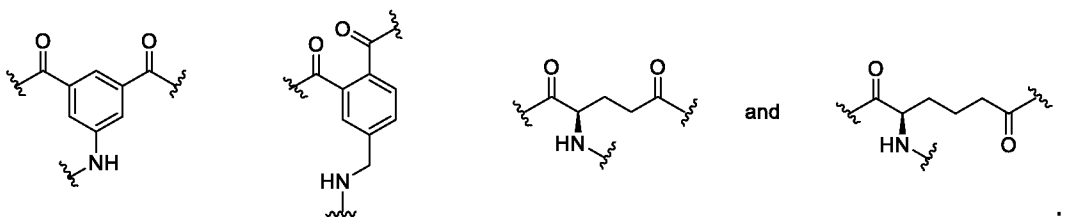
In one embodiment B^3 comprises a phenyl ring.

In one embodiment B^3 is a phenyl ring.

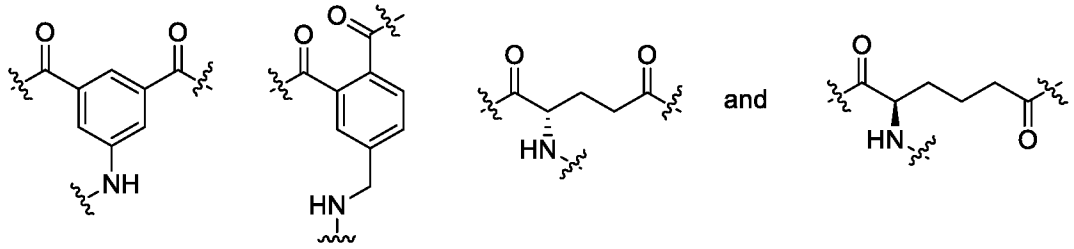
In one embodiment B^3 is CH.

In one embodiment B^3 comprises a heteroaryl.

In one embodiment B^3 is selected from the group consisting of:



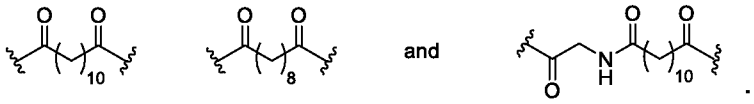
In one embodiment B^3 is selected from the group consisting of:



or a salt thereof.

In one embodiment L^1 and L^2 are independently a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 50 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O-, -NR^X-, -NR^X-C(=O)-, -C(=O)-NR^X- or –S-, and wherein R^X is hydrogen or (C1-C6)alkyl, and wherein the hydrocarbon chain, is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C1-C6)alkoxy, (C3-C6)cycloalkyl, (C1-C6)alkanoyl, (C1-C6)alkanoyloxy, (C1-C6)alkoxycarbonyl, (C1-C6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

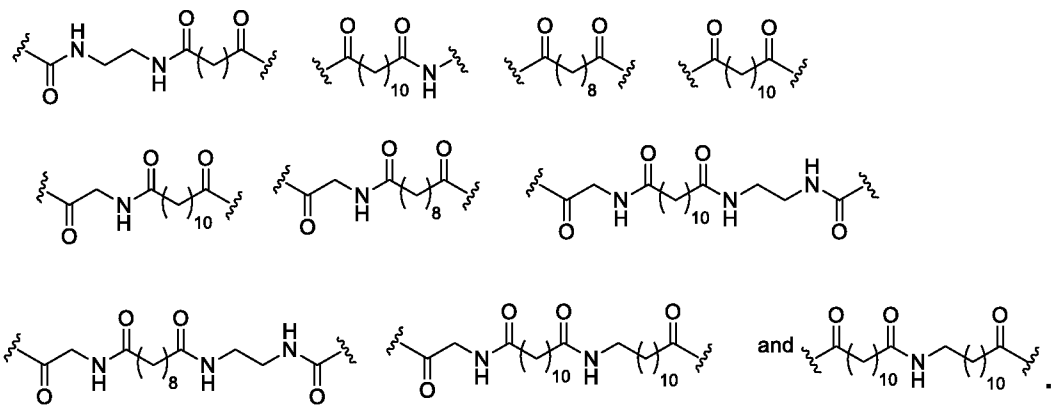
In one embodiment L^1 is selected from the group consisting of:



or a salt thereof.

In one embodiment L^1 is connected to B^1 through a linkage selected from the group consisting of: -O-, -S-, -(C=O)-, -(C=O)-NH-, -NH-(C=O), -(C=O)-O-, -NH-(C=O)-NH-, or –NH-(SO_2)-.

In one embodiment L^1 is selected from the group consisting of:



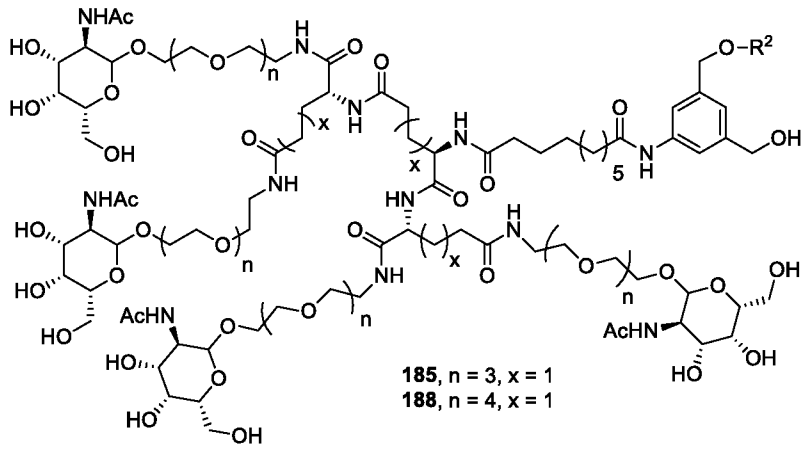
In one embodiment L^2 is connected to R^2 through -O-.

In one embodiment L^2 is C_{1-4} alkylene-O- that is optionally substituted with hydroxy.

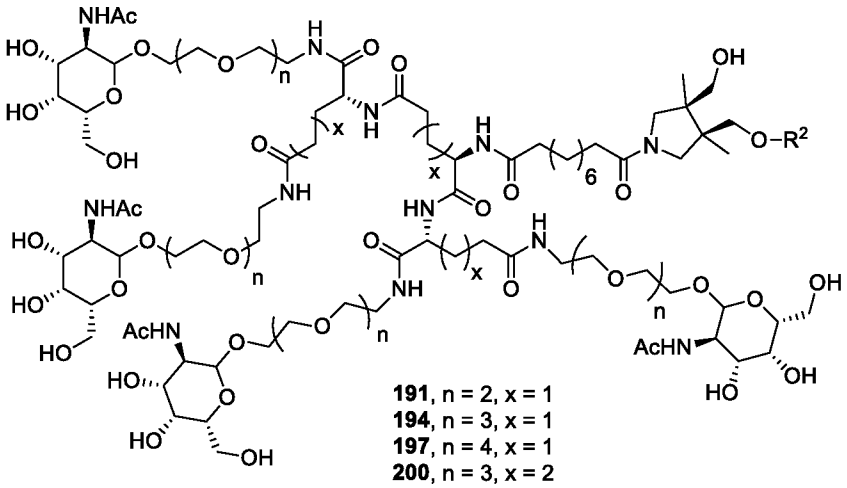
In one embodiment L^2 is connected to R^2 through -O-.

In one embodiment L^2 is absent.

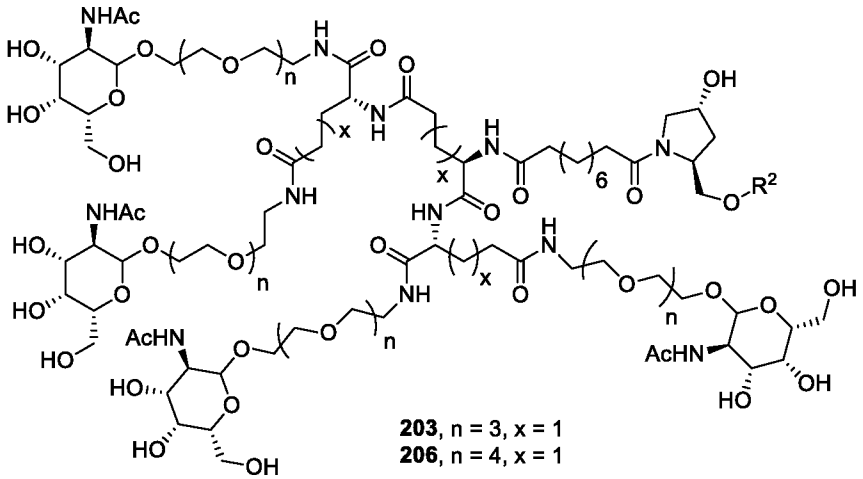
In one embodiment the invention provides a compound or salt selected from the group consisting of:



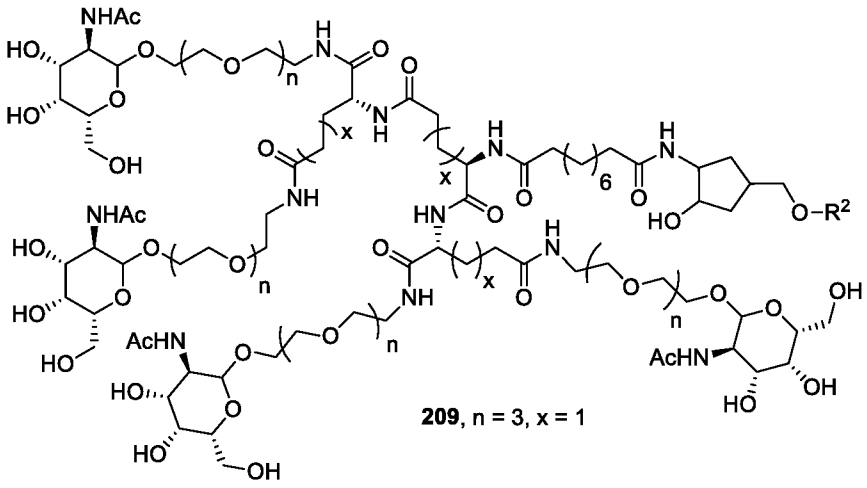
185, n = 3, x = 1
188, n = 4, x = 1



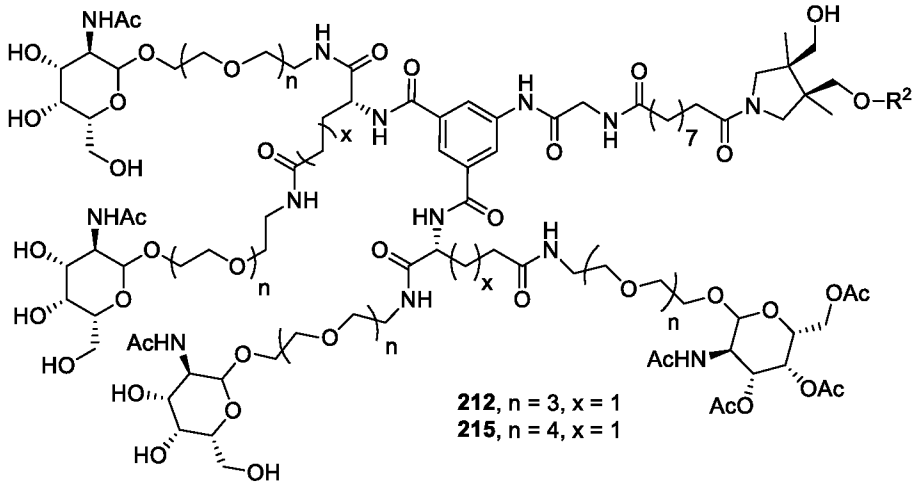
191, n = 2, x = 1
194, n = 3, x = 1
197, n = 4, x = 1
200, n = 3, x = 2



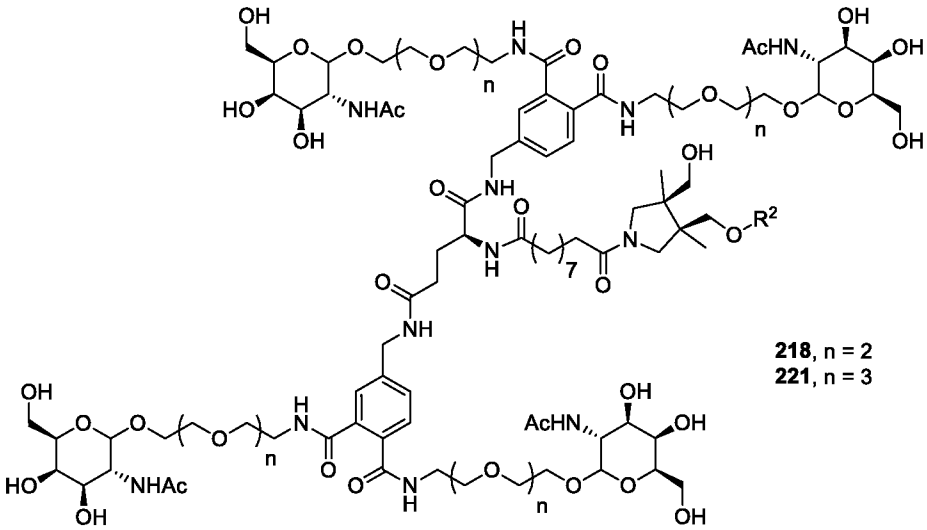
203, n = 3, x = 1
206, n = 4, x = 1



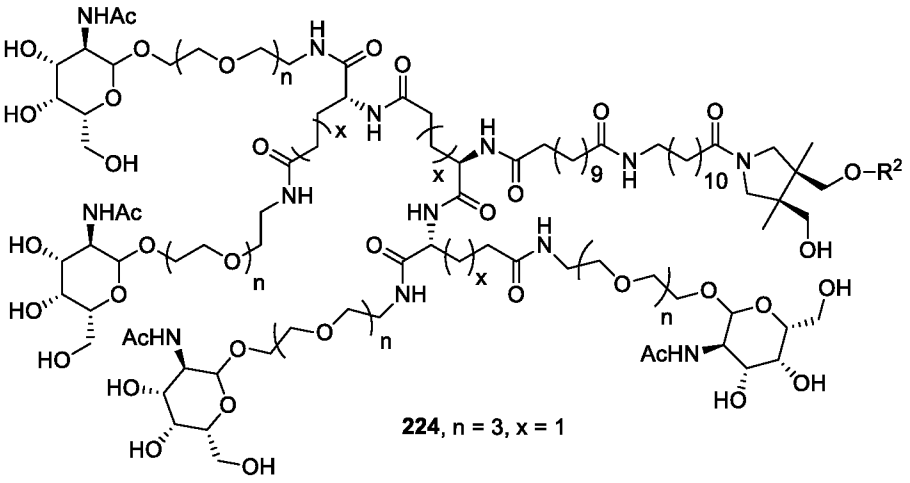
209, n = 3, x = 1



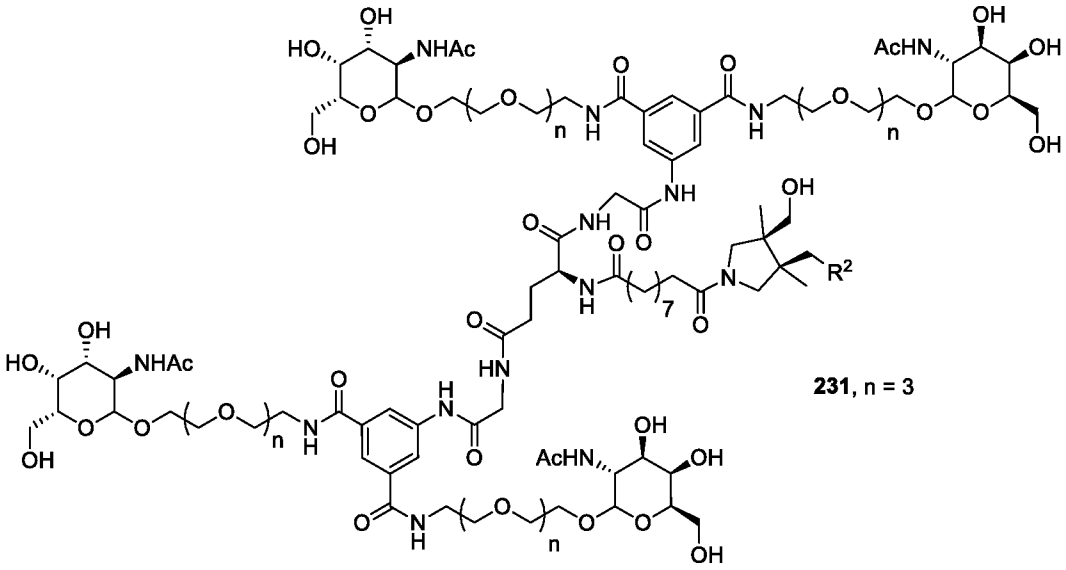
212, n = 3, x = 1
215, n = 4, x = 1



218, n = 2
221, n = 3



224, n = 3, x = 1

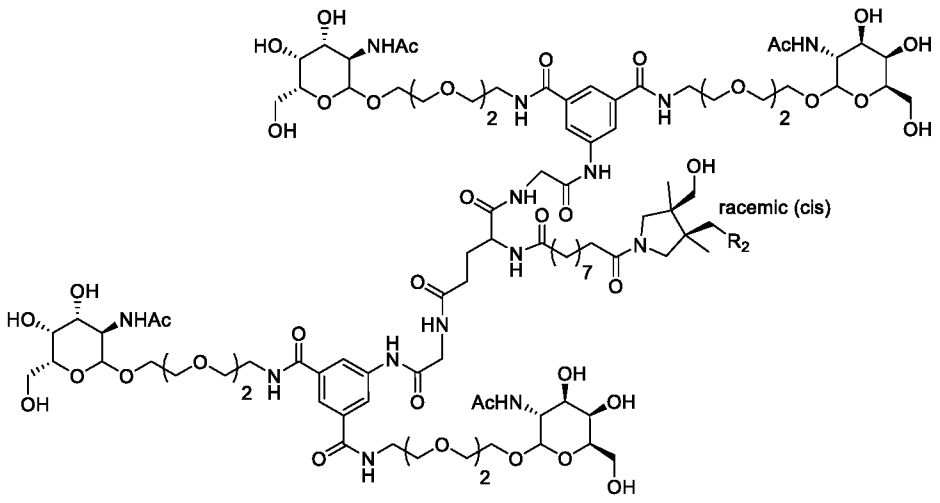


231, n = 3

and ;

and pharmaceutically acceptable salts thereof, wherein R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of Table 1.

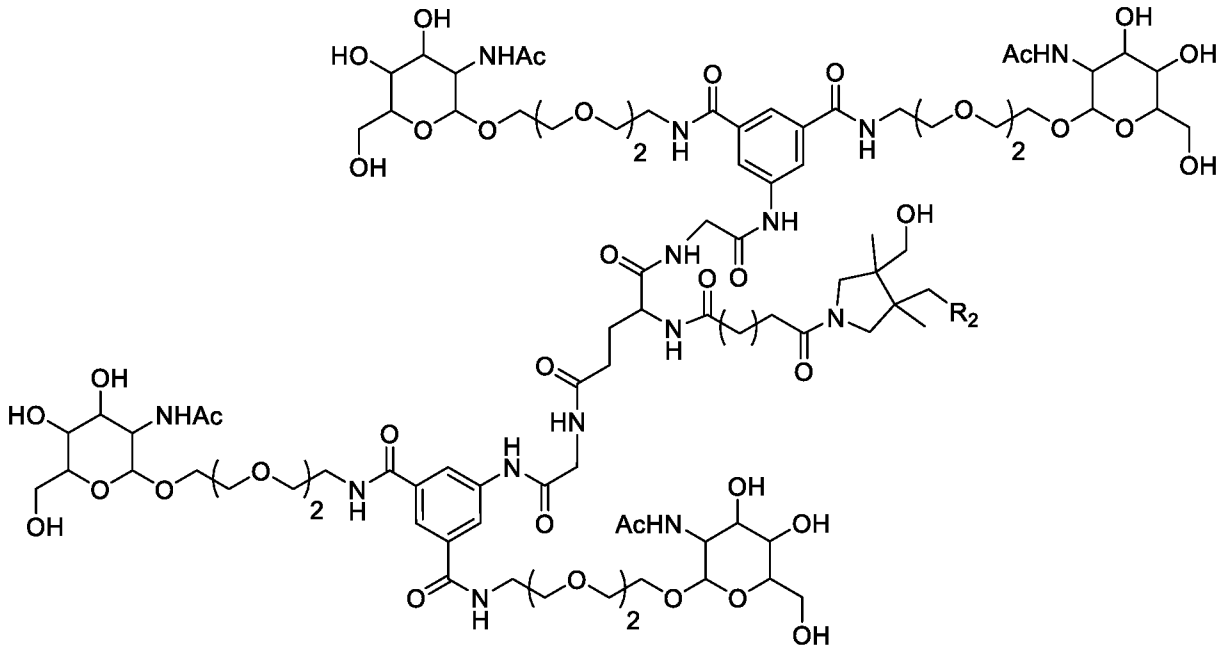
In one embodiment the invention provides a compound of formula:



racemic (cis)

or a salt thereof wherein R^2 is a nucleic acid.

In one embodiment the invention provides a compound of formula:

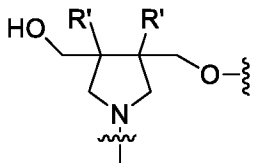


or a salt thereof wherein R^2 is a nucleic acid.

In one embodiment, the nucleic acid molecule (e.g., siRNA) is attached to the reminder of the compound through the oxygen of a phosphate at the 3'-end of the sense strand.

In one embodiment the compound or salt is administered subcutaneously.

When a compound comprises a group of the following formula:



there are four stereoisomers possible on the ring, two *cis* and two *trans*. Unless otherwise noted, the compounds of the invention include all four stereoisomers about such a ring. In one embodiment, the two R' groups are in a *cis* conformation. In one embodiment, the two R' groups are in a *trans* conformation.

One aspect of the invention is a nucleic acid-lipid particle comprising:

(a) one or more double stranded siRNA molecules selected from the double stranded siRNA molecules of Table 1;

(b) a cationic lipid; and

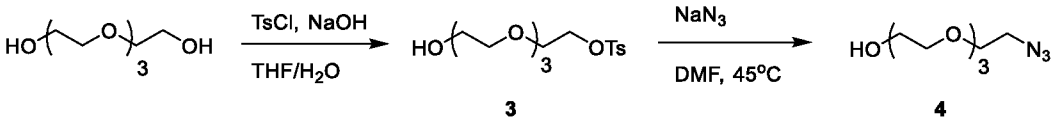
(c) a non-cationic lipid.

Examples

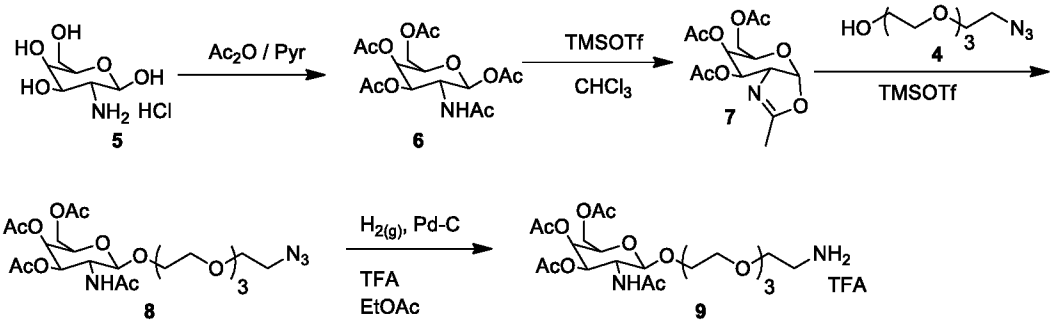
The present invention will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit the invention in any manner. Those of skill in the art will readily recognize a variety of noncritical parameters which can be changed or modified to yield essentially the same results. It is understood that in one embodiment the oligonucleotide is a double stranded siRNA molecule as described in Table 1.

Example 1. Synthesis of conjugate 1

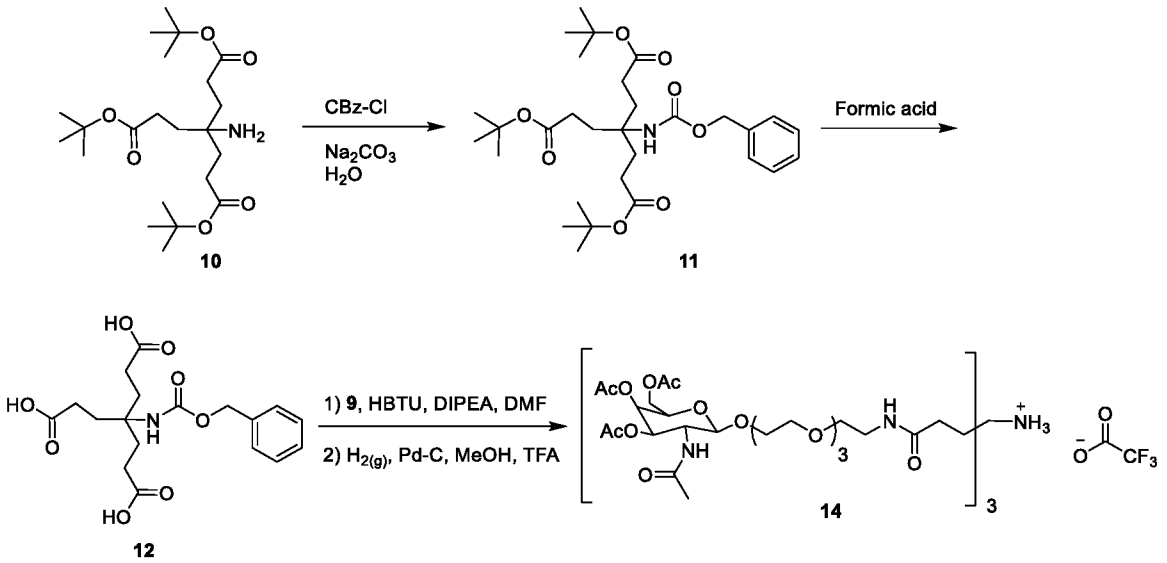
Scheme 1.



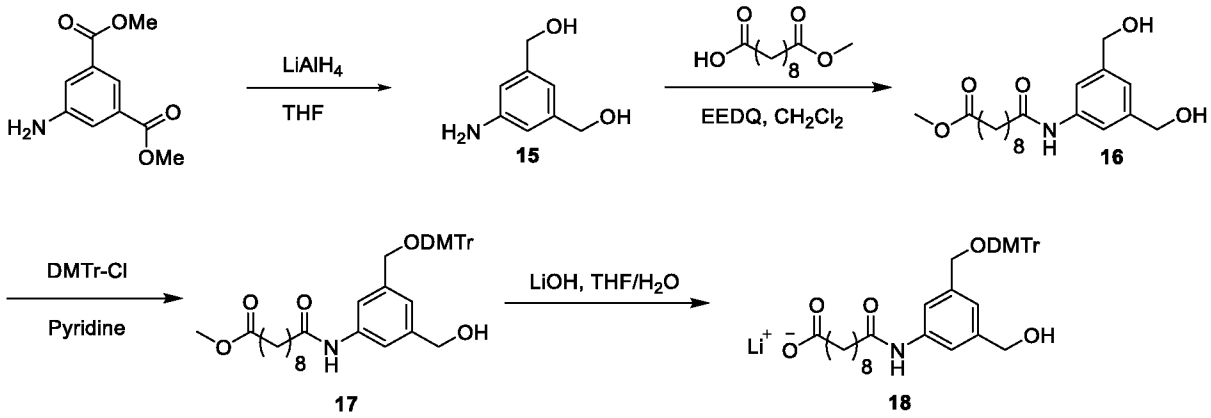
Scheme 2.



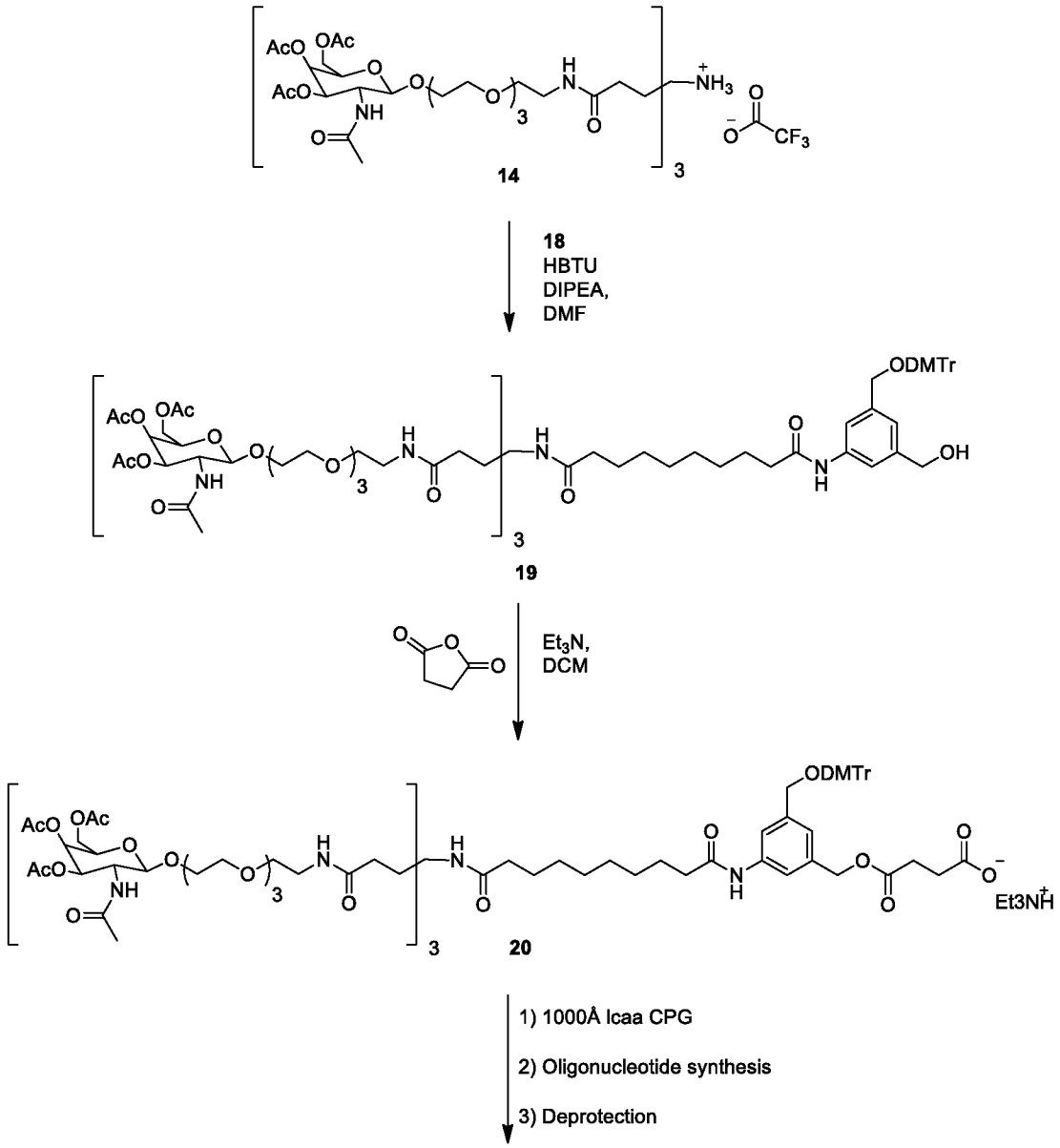
Scheme 3.

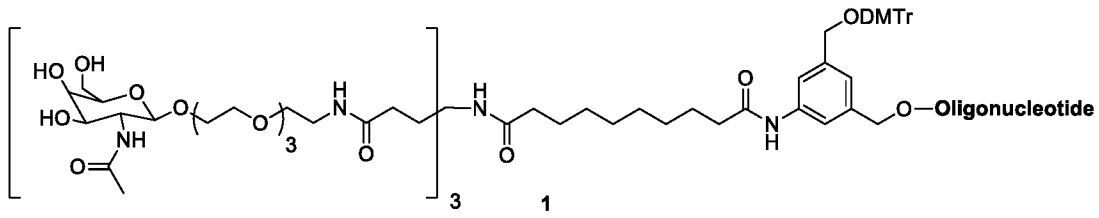


Scheme 4.



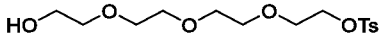
Scheme 5.





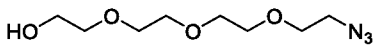
1

Step 1. Preparation of 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate 3



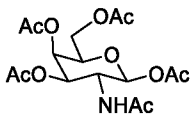
A solution of tetraethylene glycol (934 g, 4.8 mol) in THF (175mL) and aqueous NaOH (5M, 145 mL) was cooled (0°C) and treated with *p*-Toluensulfonyl chloride (91.4 g, 480 mmol) dissolved in THF (605 mL) and then stirred for two hours (0°C). The reaction mixture was diluted with water (3L) and extracted (3x 500mL) with CH_2Cl_2. The combined extracts were washed with water and brine then dried ($MgSO_4$), filtered and concentrated to afford 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate **3** (140 g, 84%) as a pale yellow oil. R_f (0.57, 10% MeOH-CH_2Cl_2).

Step 2. Preparation of 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-ol 4



A solution of **3** (140 g, 403 mmol) in DMF (880 mL) was treated with sodium azide (131 g, 2.02 mol) and heated (45°C) overnight. A majority of the DMF was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (500 mL) and washed (3x 500 mL) with brine then dried ($MgSO_4$), filtered and concentrated. The residue was passed through a short bed of silica (5% MeOH-CH_2Cl_2) and concentrated to yield 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-ol **4** (65g, 74%) as a yellow oil. R_f (0.56, 10% MeOH-CH_2Cl_2).

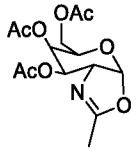
Step 3. Preparation of peracetylated galactosamine 6



D-Galactosamine hydrochloride **5** (250 g, 1.16 mol) in pyridine (1.5 L) was treated with acetic anhydride (1.25 L, 13.2 mol) over 45 minutes. After stirring overnight the reaction mixture was divided into three 1 L portions. Each 1 L portion was poured into 3 L of ice water and mixed for one hour. After mixing the solids were filtered off, combined, frozen over liquid

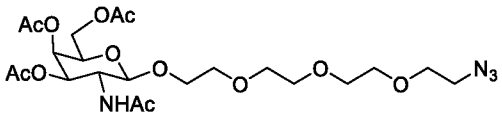
nitrogen and then lyophilized for five days to yield peracetylated galactosamine **6** (369.4 g, 82%) as a white solid. Rf (0.58, 10% MeOH-CH_2Cl_2).

Step 4. Preparation of (3aR,5R,6R,7R,7aR)-5-(acetoxymethyl)-2-methyl-3a,6,7,7a-tetrahydro-5H-pyrano[3,2-d]oxazole-6,7-diyl diacetate 7



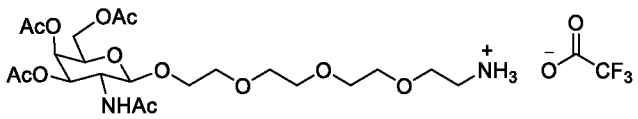
A solution of per-acetylated galactosamine **6** (8.45 g, 21.7 mmol) in $CHCl_3$ (320 mL) was treated dropwise with TMSOTf (4.32 mL, 23.9 mmol). After stirring (1.5 hr, 40°C) the reaction was quenched by the addition of triethylamine (5 mL) and concentrated to dryness to afford compound **7** as a pale yellow glass (7.2 g, Quant.). The product was used without further purification. Rf (0.59, 10% MeOH-CH_2Cl_2).

Step 5. Preparation of (2R,3R,4R,5R,6R)-5-acetamido-2-(acetoxymethyl)-6-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethoxy)tetrahydro-2H-pyran-3,4-diyl diacetate 8



Compound **7** (7.2 g, 21.7 mmol) and 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-ol **4** (2.65 g, 15.2 mmol) were azeotroped (3x) from toluene (150 mL) to remove traces of water. The dried material was dissolved in 1,2-dichloroethane (150 mL), cooled (~5°C) and treated with TMSOTf (784 µL, 4.34 mmol). After stirring overnight the reaction was quenched by the addition of triethylamine (5 mL) and concentrated. The residue was purified by chromatography (1% → 5% MeOH-CH_2Cl_2) to afford **8** (7.12 g, 85%) as a brown oil. Rf (0.3, 10% MeOH-CH_2Cl_2).

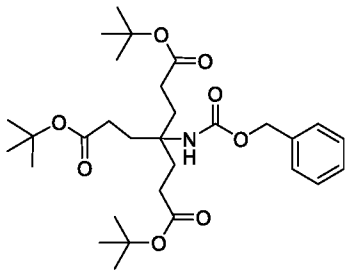
Step 6. Preparation of 2-(2-(2-(2-(((2R,3R,4R,5R,6R)-3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethoxy)ethoxy)ethan-1-aminium 2,2,2-trifluoroacetate 9



A solution of the azide **8** (7.12 g, 13 mmol) in EtOAc (150 mL) and trifluoroacetic acid (2 mL) was treated with palladium on charcoal (1.5 g, 10% w/w wet basis). The reaction

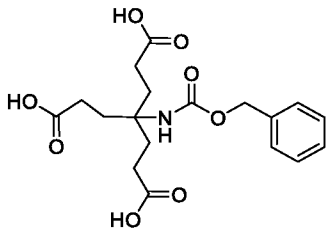
mixture was then purged with hydrogen and stirred vigorously overnight. After purging with nitrogen, the mixture was filtered through Celite, rinsing with MeOH. The filtrate was concentrated and purified via chromatography (5% → 10% → 20% MeOH-CH_2Cl_2) to afford **9** (5.8 g, 72%) as a brown oil. Rf (0.34, 15% MeOH-CH_2Cl_2).

Step 7. Preparation of di-tert-butyl 4-(((benzyloxy)carbonyl)amino)-4-(3-(tert-butoxy)-3-oxopropyl)heptanedioate 11



To a solution of di-tert-butyl 4-amino-4-(3-(tert-butoxy)-3-oxopropyl)heptanedioate **10** (13.5 g, 33 mmol), 25% $Na_2CO_{3\,(aq)}$ (150 mL) and dichloromethane (300 mL) was added slowly benzyl chloroformate (14 mL, 98 mmol). The solution was stirred vigorously overnight (16h) at room temperature. Upon completion, additional dichloromethane (100 mL) was added and the dichloromethane layer was separated. The aqueous layer was extracted with dichloromethane (2 x 100 mL). The combine dichloromethane extracts were dried on magnesium sulfate, filtered and concentrated to dryness. The product **11** was isolated as a colorless oil that required no further purification (15.8 g, 88%). Rf (0.7, 1:1 EtOAc-Hexane).

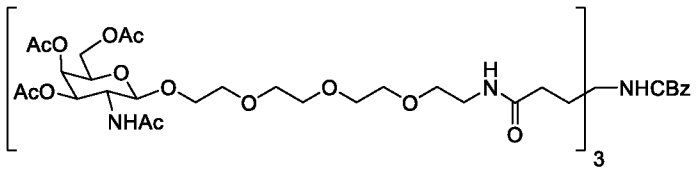
Step 8. Preparation of 4-(((benzyloxy)carbonyl)amino)-4-(2-carboxyethyl)heptanedioic acid 12



12

A solution of **11** (15.6 g, 28.8 mmol) in formic acid (50 mL) was stirred at room temperature for 2 hours. The solution was concentrated to dryness and dissolved in ethyl acetate (~25 mL). Upon standing, the product crystallized as a colorless solid. The solid was filtered, washed with ethyl acetate and air dried to afford **12** as a colorless solid (10.2 g, 93%). Rf (0.1, 10% MeOH-CH_2Cl_2).

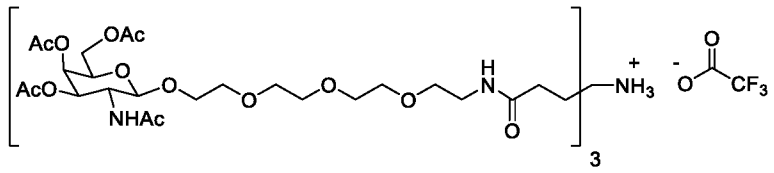
Step 9. Preparation of compound 13



13

A solution of **12** (793 mg, 2.08 mmol) and **9** (5.8 g, 9.36 mmol) in DMF (50mL) was treated with BOP (3.67 g, 8.32 mmol) then *N*,*N*-diisopropylethylamine (4.31 mL, 25 mmol). After stirring overnight the mixture was concentrated to dryness and subjected to chromatography (1% → 2% → 5% → 10% → 15% MeOH-CH_2Cl_2) to afford **13** (5.71 g [crude], >100% - contained coupling by-products that did not affect the next step). Rf (0.45, 10% MeOH-CH_2Cl_2).

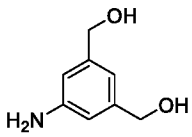
Step 10. Preparation of compound 14



14

Compound **13** (5.7 g) was dissolved in MeOH (150 mL) and TFA (1.5 mL) and treated with palladium on charcoal (1 g, 10% w/w wet basis). The reaction mixture was then purged with hydrogen and stirred vigorously overnight. After purging with nitrogen, the mixture was filtered through Celite, rinsing with MeOH. The filtrate was concentrated and purified via chromatography (5% → 10% → 20% MeOH-CH_2Cl_2) to afford **14** as a brown oil (2.15 g, 56% over two steps). Rf (0.32, 10% MeOH-CH_2Cl_2).

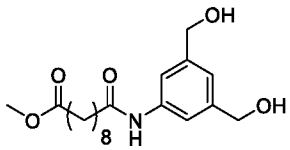
Step 11. Preparation of (5-amino-1,3-phenylene)dimethanol 15



A solution of dimethyl 5-aminoisophthalate (20.0 g, 96 mmol) in THF (350 mL) was added, dropwise, to a refluxing mixture of 3.75 eq $LiAlH_4$ (13.6 g, 358 mmol) in THF (440 mL) over one hour. The mixture was stirred at reflux for a further two hours, then cooled to room temperature and quenched by the careful addition of MeOH (27 mL) then water (40 mL). After stirring the quenched mixture for two hours it was filtered and concentrated to dryness.

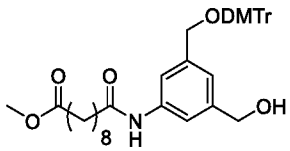
The residue was recrystallized (2X) from EtOAc to afford **15** as brownish-yellow crystals (10.2 g, 70 %).

Step 12. Preparation of methyl 10-((3,5-bis(hydroxymethyl)phenyl)amino)-10-oxodecanoate 16



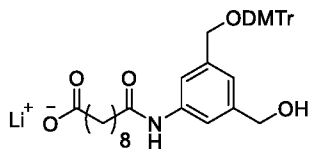
A solution of methyl sebacate (3.8 g, 17 mmol), **15** (2.5 g, 17 mmol) and EEDQ (8.1 g, 33 mmol) in 2:1 dichloromethane / methanol (200 mL) was stirred at room temperature for 2 hours. Upon completion the solution was concentrated to dryness. The solid obtained was triturated with dichloromethane (50 mL) and filtered. The solid was rinsed with cold dichloromethane and air dried to afford **16** as a colorless solid (4.3 g, 72%). Rf (0.33, EtOAc).

Step 13. Preparation of methyl 10-((3-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-(hydroxymethyl)phenyl)amino)-10-oxodecanoate 17



To a solution of **16** (4.3 g, 12 mmol) in pyridine (50 mL) was added 4,4'-(chloro(phenyl)methylene)bis(methoxybenzene) (4.1 g, 12 mmol). The solution was stirred under nitrogen overnight at room temperature. Upon completion the solution was concentrated to dryness and the residue was purified by column chromatography (0.5% → 0.75% → 1% → 1.5% MeOH-CH_2Cl_2) to afford **17** as a yellow solid (2.9 g, 35%). Rf (0.6, 10% MeOH-CH_2Cl_2).

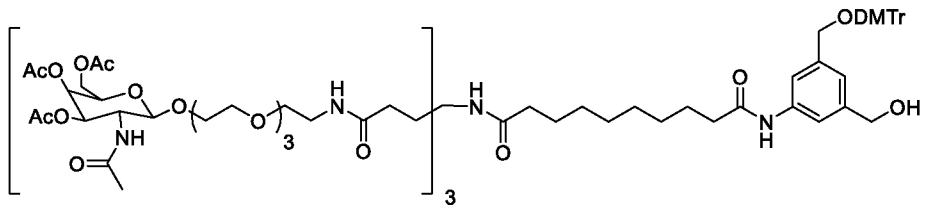
Step 14. Preparation of lithium 10-((3-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-(hydroxymethyl)phenyl)amino)-10-oxodecanoate 18



To a solution of **17** (2.9 g, 4.3 mmol) in THF (60 mL) was added water (15 mL) and lithium hydroxide (112 mg, 4.7 mmol). The solution was stirred overnight at room

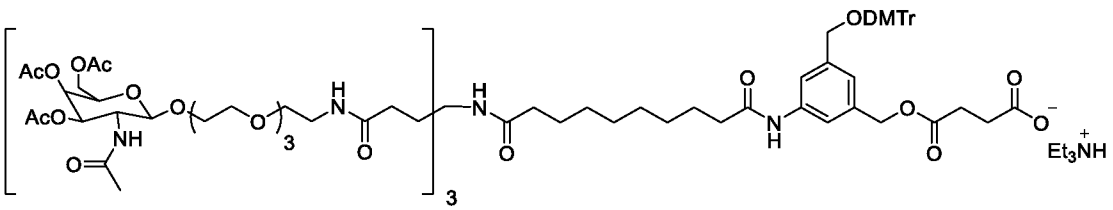
temperature. Upon completion the solution was concentrated to remove the THF. The remaining aqueous solution was flash frozen on liquid nitrogen and lyophilized overnight to afford a colorless solid (2.9 g, quant.). Rf (0.3, 10% MeOH-CH_2Cl_2).

Step 15. Preparation of compound 19



To a solution 14 (454 mg, 0.67 mmol), 18 (1.25 g, 0.67 mmol) and HBTU (381 mg, 1.0 mmol) in anhydrous DMF (25 mL) was added *N,N*-diisopropylethylamine (0.35 mL, 2.0 mmol). The solution was stirred overnight at room temperature. Upon completion, the solution was poured into ethyl acetate (250 mL) and washed with brine (3 x 200 mL). The ethyl acetate layer was dried on magnesium sulfate, filtered and concentration to dryness. Purification by column chromatography (5% → 7.5% → 10% → 15% MeOH in CH_2Cl_2) afforded **19** as a pale orange foam (1.5 g, 94%). Rf (0.25, 10% MeOH-CH_2Cl_2).

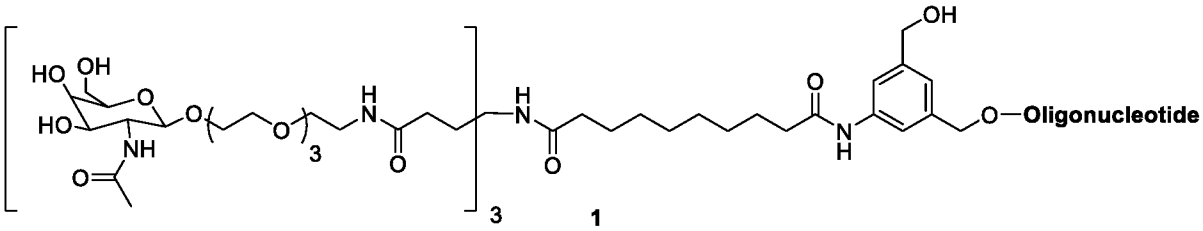
Step 16. Preparation of compound 20



A solution of compound **19** (1.5 g, 0.6 mmol), succinic anhydride (120 mg, 1.2 mmol), DMAP (220 mg, 1.8 mmol) and trimethylamine (250 µL, 1.8 mmol) in anhydrous CH_2Cl_2 (50 mL) was stirred overnight at room temperature. Upon completion, the solution was concentrated to dryness and filtered through a short plug of silica (100% CH_2Cl_2 → 15% MeOH in CH_2Cl_2) to afford the product **20** as a light beige foam (1.1 g, 70%). Mass *m/z* (ES-TOF MS) 727.7 $[M + 3H - DMTr]^+$, 1091.1 [M + 2H - DMTr]. ^1H NMR (400 MHz, $CDCl_3$) δ 8.92 (br s, 1H), 7.78 (s, 1H), 7.49-7.47 (m, 3H), 7.41 (br s, 1H), 7.38-7.34 (m, 5H), 7.32-7.26 (m, 4H), 7.24-7.08 (br s, 3H), 7.08 (s, 1H), 6.90-6.80 (m, 7H), 5.31 (d, 3H, J = 2.7Hz), 5.12 (s, 2H), 5.06 (dd, 3H, J = 11.2, 3.2 Hz), 4.78 (d, 3H, J = 8.5 Hz), 4.24-4.08 (m, 12H), 3.95-3.88 (m, 7H), 3.85-3.76 (m, 4H), 3.78 (s, 6H), 3.68-3.56 (m, 34H), 3.54-3.44 (m, 8H), 3.41-3.33 (m,

6H), 2.70-2.60 (m, 4H), 2.52-2.30 (m, 30H), 2.24-2.16 (m, 8H), 2.14 (s, 9H), 2.04 (s, 9H), 2.02-1.96 (m, 6H), 1.98 (s, 9H), 1.96 (s, 9H), 1.74-1.52 (m, 4H), 1.36-1.24 (m, 12H).

Step 17. Preparation of conjugate 1

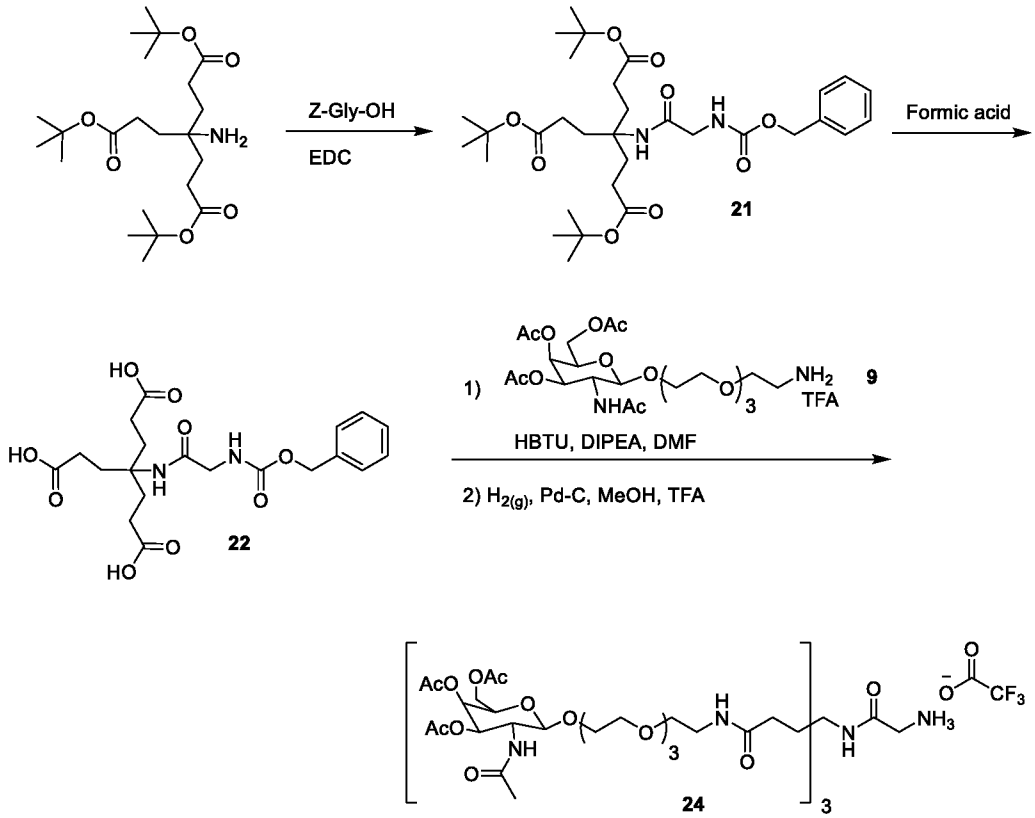


1

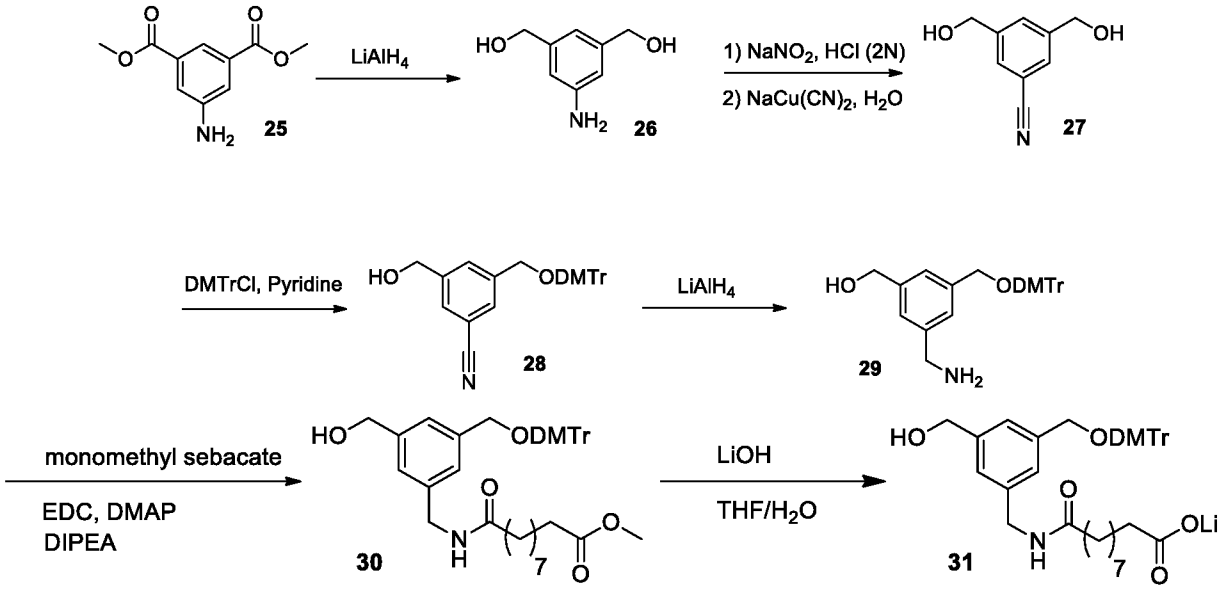
The succinate **20** was loaded onto 1000Å LCAA (long chain aminoalkyl) CPG (control pore glass) using standard amide coupling chemistry. A solution of diisopropylcarbodiimide (52.6 µmol), N-hydroxy succinimide (0.3 mg, 2.6 µmol) and pyridine (10 µL) in anhydrous acetonitrile (0.3 mL) was added to **20** (20.6 mg, 8 µmol) in anhydrous dichloromethane (0.2 mL). This mixture was added to LCAA CPG (183 mg). The suspension was gently mixed overnight at room temperature. Upon disappearance of **20** (HPLC), the reaction mixture was filtered and the CPG was washed with 1 mL of each dichloromethane, acetonitrile, a solution of 5% acetic anhydride / 5% N-methylimidazole / 5% pyridine in THF, then THF, acetonitrile and dichloromethane. The CPG was then dried overnight under high vacuum. Loading was determined by standard DMTr assay by UV/Vis (504 nm) to be 25 µmol/g. The resulting GalNAc loaded CPG solid support was employed in automated oligonucleotide synthesis using standard procedures. Nucleotide deprotection followed by removal from the solid support (with concurrent galactosamine acetate deprotection) afforded the GalNAc-oligonucleotide conjugate **1** as a representative example.

Example 2: Synthesis of conjugate 34

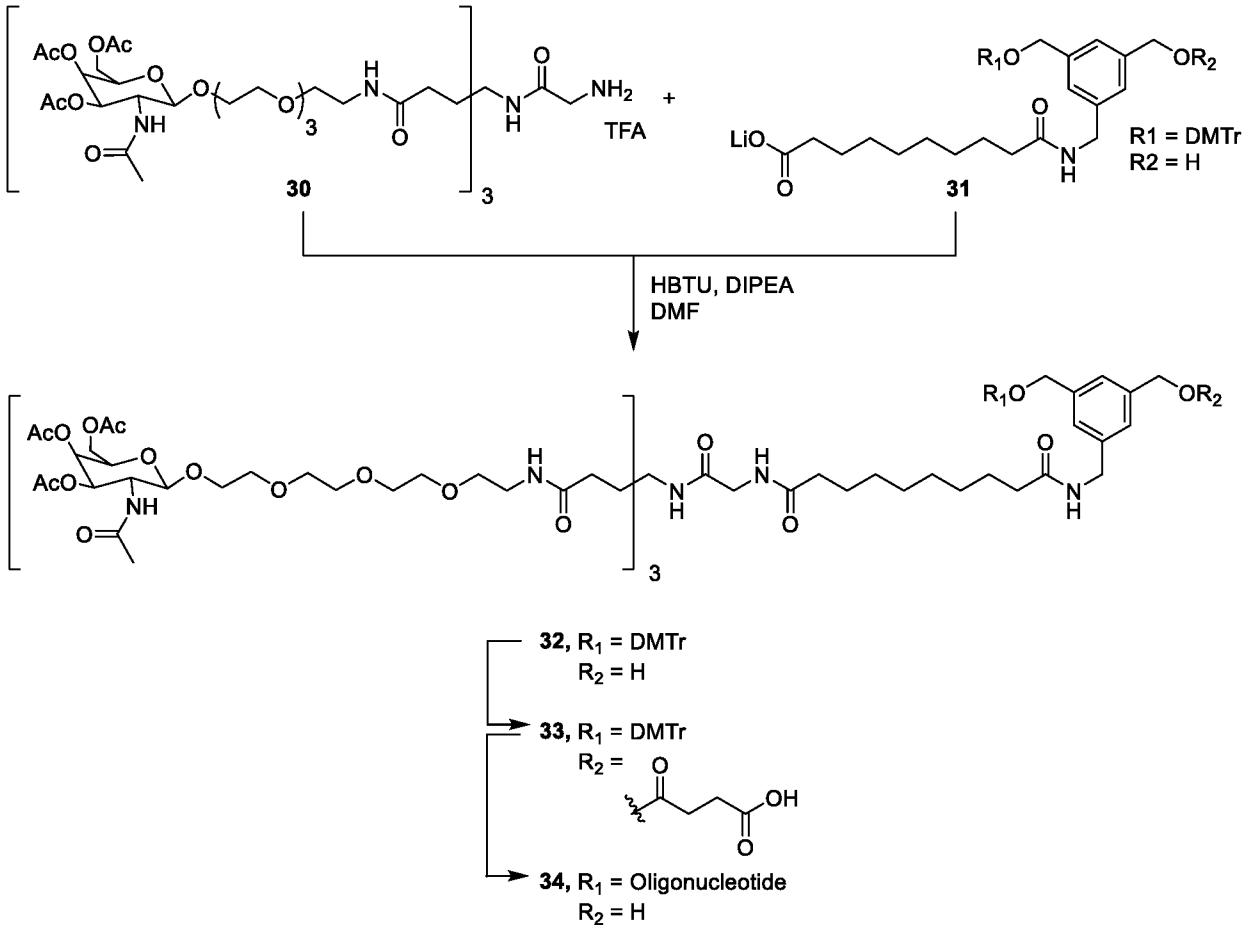
Scheme 6.



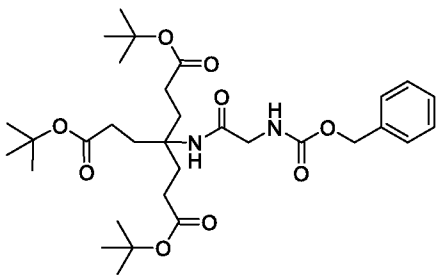
Scheme 7.



Scheme 8.



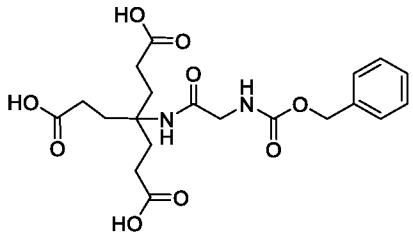
Step 1. Preparation of di-tert-butyl 4-(2-(((benzyloxy)carbonyl)amino)acetamido)-4-(3-(tert-butoxy)-3-oxopropyl)heptanedioate 21



A solution of di-tert-butyl 4-amino-4-(3-(tert-butoxy)-3-oxopropyl)heptanedioate (25 g, 60 mmol) and Z-glycine (18.9 g, 90.2 mmol,) in CH_2Cl_2 (300 mL) was treated successively with EDC (23 g, 120 mmol), Diisopropylethylamine (32 mL, 180 mmol) and DMAP (Cat. 17 mg). After stirring (16h) the reaction mixture was poured into $NaHCO_3$ (Sat. Aq.), extracted with CH_2Cl_2, washed with brine, dried ($MgSO_4$), filtered and concentrated to afford di-tert-butyl 4-(2-(((benzyloxy)carbonyl)amino)acetamido)-4-(3-(tert-butoxy)-3-

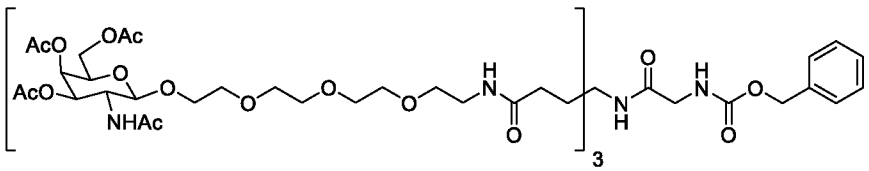
oxopropyl)heptanedioate **21** as an amorphous solid and was used without further processing (36 g, quant.). Rf (0.85, 10% MeOH-CH_2Cl_2).

Step 2. Preparation of 4-(2-(((benzyloxy)carbonyl)amino)acetamido)-4-(2-carboxyethyl)heptanedioic acid 22



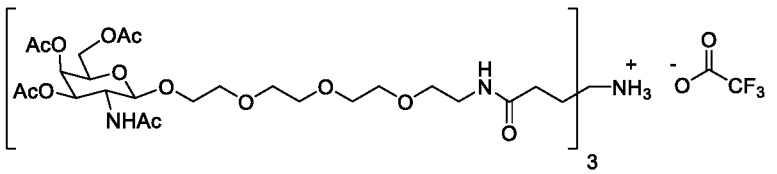
A solution of di-tert-butyl 4-(2-(((benzyloxy)carbonyl)amino)acetamido)-4-(3-(tert-butoxy)-3-oxopropyl)heptanedioate **21** (59.3mmol, 36g) was stirred in neat formic acid (150mL) for 72 hours. Upon completion, the formic acid was removed under reduced pressure and the crude solid was dried overnight on high-vacuum to yield **22** as a colorless solid (15.9 g, 61%). Rf (0.15, 10% MeOH-CH_2Cl_2).

Step 3. Preparation of compound 23



A solution of **22** (6.2 g, 14.1 mmol) and 2-(2-(2-(2-(((2R,3R,4R,5R,6R)-3-acetamido-4,5-diacetoxy-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethoxy)ethoxy)ethan-1-aminium 2,2,2-trifluoroacetate (35 g, 56.5 mmol) in DMF (250mL) was treated with BOP (25 g, 56.5 mmol) then *N*,*N*-diisopropylethylamine (29 mL, 170 mmol). After stirring overnight the mixture was concentrated to dryness and subjected to chromatography (100% CH_2Cl_2 to 15% MeOH-CH_2Cl_2) to afford compound **23** (24.6 g, 89%). Rf (0.55, 15% MeOH-CH_2Cl_2).

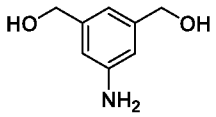
Step 4. Preparation of compound 24



Compound **23** (24.6 g) was dissolved in MeOH (200 mL) and TFA (1.5 mL) and purged with nitrogen. Palladium on charcoal (1 g, 10% w/w wet basis) was added and then the

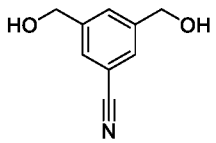
reaction mixture was purged with hydrogen and stirred vigorously overnight. Upon completion, the reaction was purged with nitrogen, filtered through Celite and rinsed with MeOH. The filtrate was concentrated and purified by column chromatography on silica gel 60 (gradient: 5% → 10% → 20% MeOH-CH_2Cl_2) to afford **24** as a pale brown viscous oil (23 g). Rf (0.32, 10% MeOH-CH_2Cl_2).

Step 5. Preparation of (5-amino-1,3-phenylene)dimethanol 26



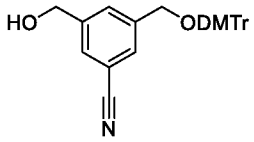
A suspension of lithium aluminum hydride (13.6 g, 358 mmol) in anhydrous tetrahydrofuran (450 mL) was brought to reflux under a nitrogen atmosphere and treated, dropwise, with a solution of dimethyl-5-aminoisophthalte **25** (20 g, 96 mmol) in anhydrous tetrahydrofuran (350 mL). After the addition was complete the mixture was heated to reflux for an additional 2 hours. Upon completion, the solution was cooled to room temperature and quenched by the slow addition of MeOH (27 mL) then water (40 mL). After stirring for 2 hours the mixture was filtered, concentrated and recrystallized from EtOAc to yield (5-amino-1,3-phenylene)dimethanol **26** as off-white crystals (10.2 g, 70%). Rf 0.5 (15% MeOH-CH_2Cl_2).

Step 6. Preparation of 3,5-bis(hydroxymethyl)benzonitrile 27



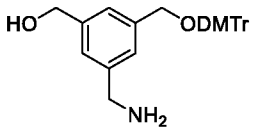
A solution of **26** (5 g, 33 mmol) in 2N hydrochloric acid (100 mL) was cooled to 0^0C and treated with a cold solution of sodium nitrite (3.53 g, 36mmol) in water (50 mL). The reaction mixture was maintained at a temperature $\leq 5^0$C for 30min then treated with a solution of copper(I) cyanide (3.19 g, 35.6mmol) and sodium cyanide (3.53 g, 72mmol) in water (50 mL) in a single portion. After stirring overnight at room temperature the mixture was filtered, extracted with dichloromethane (3 x 100 mL), concentrated and used without further purification. The diol, 3,5-bis(hydroxymethyl)benzonitrile **27** was obtained as a yellow solid (2.19 g, 41%). Rf 0.75 (15% MeOH-CH_2Cl_2).

Step 7. Preparation of 3-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-(hydroxymethyl)benzonitrile 28



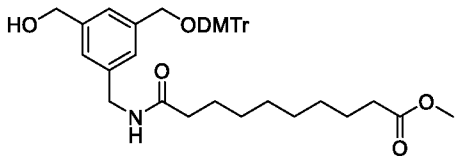
A solution of 3,5-bis(hydroxymethyl)benzonitrile **27** (538 mg, 3.3 mmol) in pyridine (14 mL) was treated with 4,4′-Dimethoxytrityl chloride (1.17 g, 3.46 mmol) and stirred overnight at room temperature. Once complete, the mixture was concentrated and dispersed in diethyl ether (25 mL), filtered and concentrated. The crude product was purified by column chromatography of silica gel 60 (gradient: 10% to 50% EtOAc-Hexane) to yield the **28** as a yellow solid (725 mg, 47%). Rf 0.5 (1:1 EtOAc-hexane).

Step 8. Preparation of (3-(aminomethyl)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)phenyl)methanol 29



A solution of the **28** (100 mg, 0.22 mmol) in methyl tetrahydrofuran (5 mL) was cooled to 0^{0}C and treated slowly with lithium aluminum hydride (0.64 mmol = 0.28mL of a 2.3M solution in MeTHF). After stirring for one hour the reaction was quenched by the addition of methanol (1 mL) then water (0.3 mL) and stirred for 30min. The mixture was filtered and concentrated, to yield (3-(aminomethyl)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)phenyl)methanol **29** (78 mg, 77%). Rf 0.15 (10% MeOH-CH_2Cl_2).

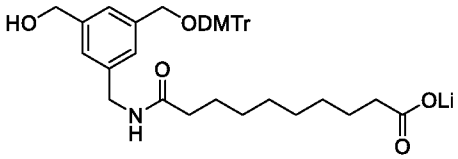
Step 9. Preparation of methyl 10-((3-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-(hydroxymethyl)benzyl)amino)-10-oxodecanoate 30



A solution of (3-(aminomethyl)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)-methyl)phenyl)methanol **29** (78 mg, 0.17 mmol) and monomethyl sebacate (38 mg, 0.17 mmol,) in dichloromethane (5 mL) were treated successively with EDC (48 mg, 0.25 mmol),

DMAP (cat., 5 mg) and diisopropylethylamine (57 μL, 0.33 mmol). After stirring (3.5 hr) the reaction mixture was poured into saturated sodium bicarbonate solution (50 mL). The sodium bicarbonate solution was extracted with dichloromethane (3 x 50 mL), washed with brine (50 mL), dried on magnesium sulfate, filtered and concentrated to dryness. The crude material was purified by column chromatography on silica gel 60 (gradient: 2% to 5% MeOH-CH_2Cl_2) to afford methyl 10-((3-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-(hydroxymethyl)benzyl)amino)-10-oxodecanoate **30** as a yellow oil (57 mg, 53%). Rf 0.45 (10% MeOH-CH_2Cl_2).

Step 10. Preparation of lithium 10-((3-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-(hydroxymethyl)benzyl)amino)-10-oxodecanoate 31

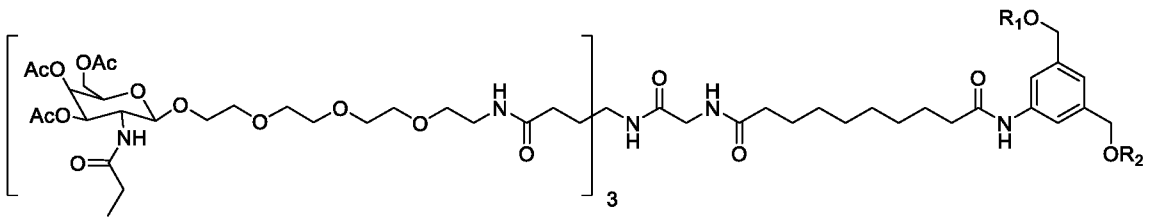


Compound **30** (188 mg, 0.28 mmol) was dissolved in tetrahydrofuran (5 mL) and treated with a solution of LiOH (7mg, 0.30 mmol) in water (1 mL). Upon completion, the tetrahydrofuran was removed *in vacuo* and the remaining aqueous mixture was frozen and lyophilized to afford lithium 10-((3-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-(hydroxymethyl)benzyl)amino)-10-oxodecanoate **31** as a colorless solid (180 mg, 99%). Rf 0.45 (10% MeOH-CH_2Cl_2).

Step 11. Preparation of compounds 32, 33, and 34

Compounds **32**, **33** and **34** were prepared according to same procedure used to synthesize compounds **19**, **20**, and **1** respectfully.

Example 3. Synthesis of conjugate 36



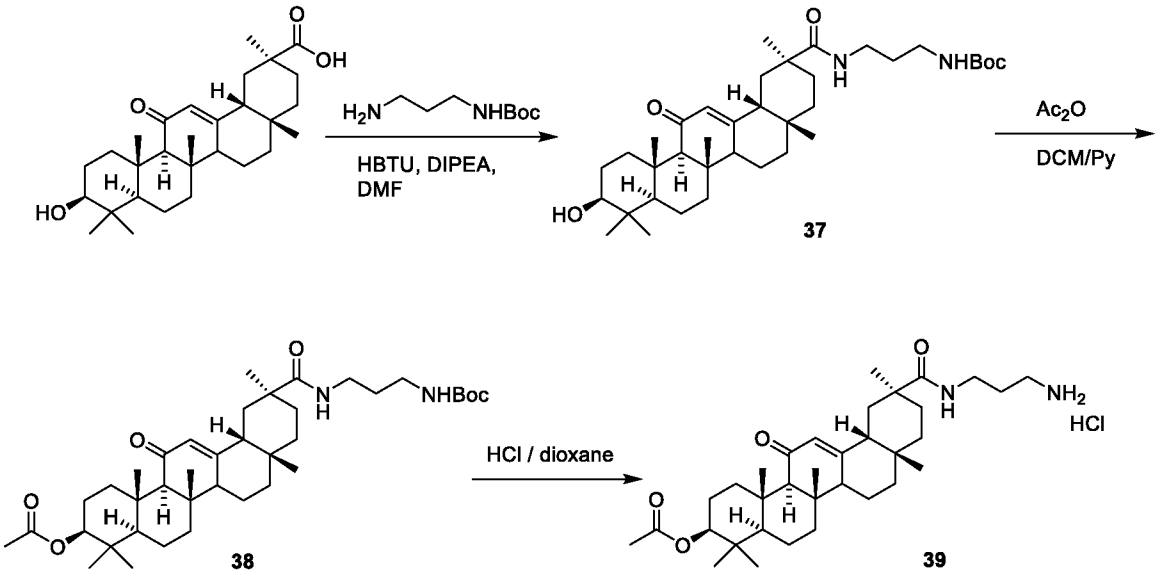
36, R_1 = Oligonucleotide
R_2 = H

Step 1. Preparation of conjugate 36

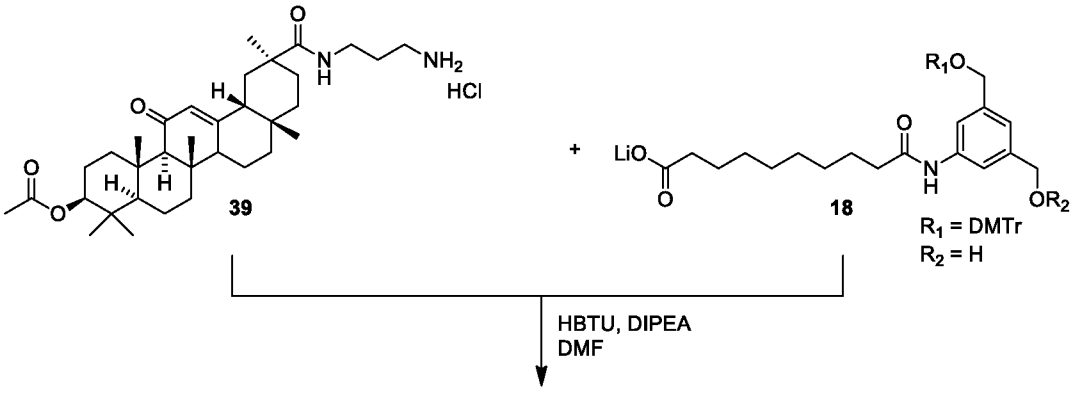
Conjugate **36** was prepared using identical procedures as used to synthesize compound **34** and all corresponding intermediates. The only exception being the synthesis of compound **6** where propanoic anhydride was used in place of acetic anhydride.

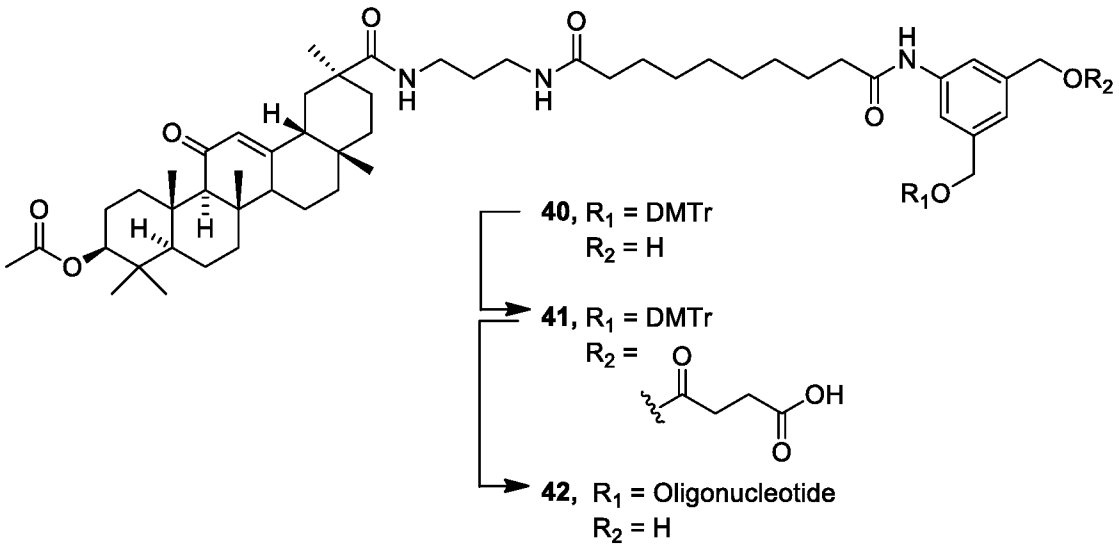
Example 4. Synthesis of conjugate 42

Scheme 9.

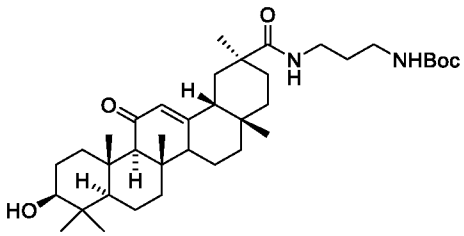


Scheme 10.



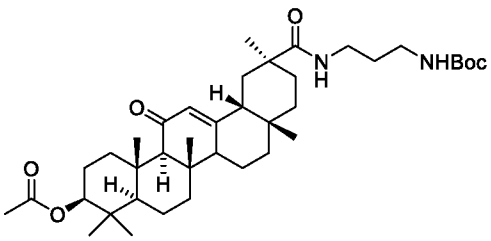


Step 1. Preparation of compound 37



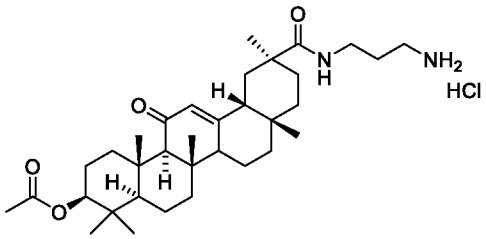
A solution of 18β-glycyrrhetinic acid (2.5 g, 5.3 mmol), tert-butyl (3-aminopropyl)carbamate (1.1 g, 6.4 mmol) and HBTU (3.0 g, 8.0 mmol) in *N*,*N*-dimethylformamide (20 mL) was added diisopropylethylamine (2.75 mL, 15.9 mmol). The solution was stirred overnight at room temperature. Upon completion, the solution was concentrated in vacuo to dryness. The residue was purified by column chromatography on silica gel 60 (gradient: 2% to 5% MeOH/CH_2Cl_2) to afford the product as a colorless solid (2.1 g, 63%).

Step 2. Preparation of compound 38



To a solution of **37** (2.1 g, 3.3 mmol) and triethylamine (3.5 mL, 10 mmol) in dichloromethane (25 mL) was added acetic anhydride (850 µL, 5.3 mmol) and DMAP (5 mg). The solution was stirred overnight at room temperature. Upon completion, the solution was concentrated to dryness and dissolved in ethyl acetate (100 mL), washed with water (100 mL), dried on magnesium sulfate, filtered and concentrated to dryness to afford a pale brown foam (1.9 g, 85%).

Step 3. Preparation of compound 39



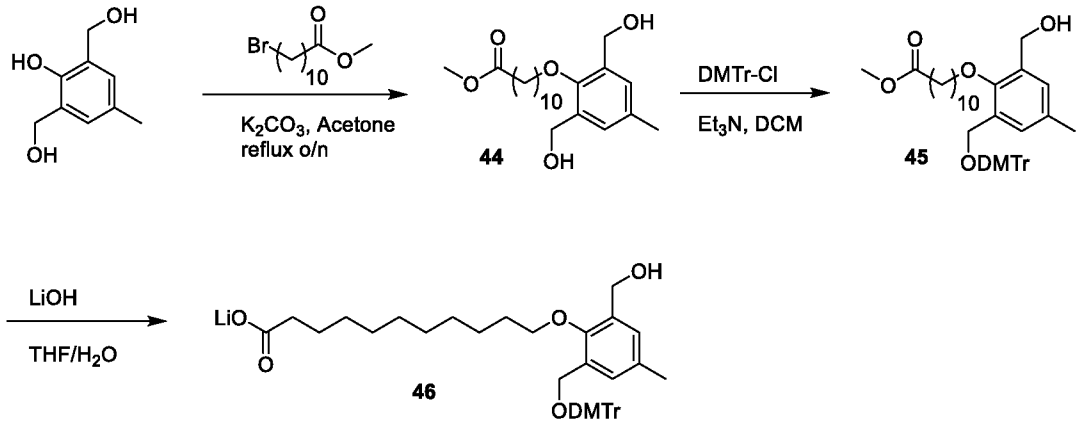
To a solution of 38 (1.5 g, 2.3 mmol) in anhydrous dioxane (25 mL) was added 2M Hydrogen chloride in dioxane (25 mL). The solution was stirred overnight at room temperature then concentrated in vacuo to dryness to afford a light brown solid (1.3 g, 96%).

Step 4. Preparation of compounds 40, 41 and 42

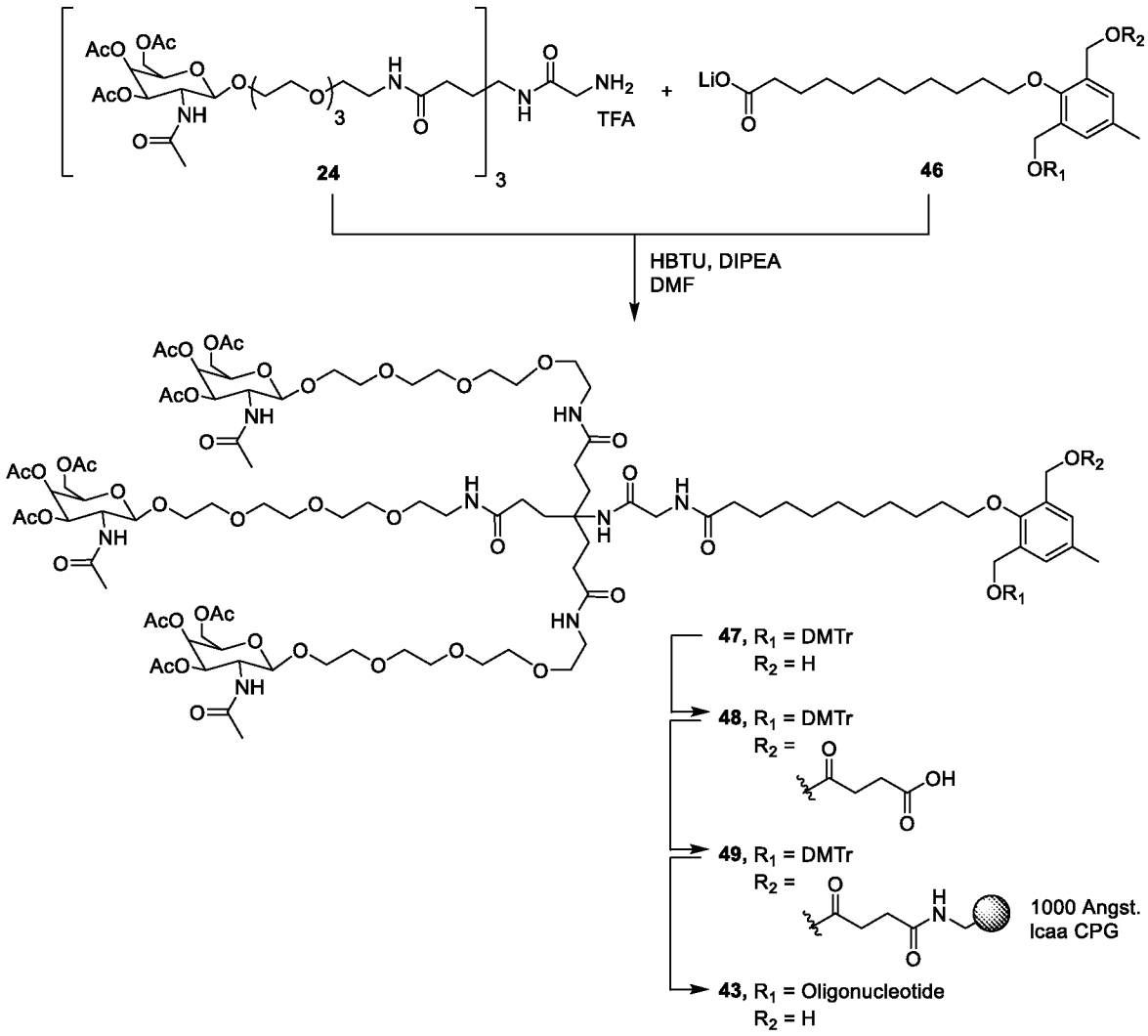
Compounds **40**, **41** and **42** were prepared according to the same procedure used to synthesize compounds **19**, **20**, and **1** respectfully.

Example 5. Synthesis of Conjugate 43

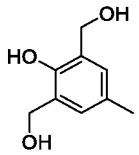
Scheme 11.



Scheme 12.



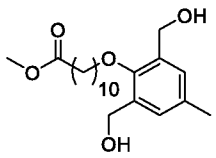
Step 1. Preparation of methyl 11-(2,6-bis(hydroxymethyl)-4-methylphenoxy)undecanoate 44



To a solution of 2,6-bis(hydroxymethyl)-p-cresol (2.7 g, 16.3 mmol), methyl 11-bromoundecanoate (5.0 g, 17.9 mmol) and potassium carbonate (4.5 g, 32.6 mmol) in acetone (100 mL) was refluxed for 16 hours. Upon completion the solution was concentrated *in vacuo* to dryness, suspended in ethyl acetate (150 mL) and washed with water (2 x 100 mL) and brine (100 mL). The ethyl acetate layer was dried on magnesium sulfate, filtered and concentrated *in vacuo* to dryness. The residue was purified by column chromatography on silica gel 60

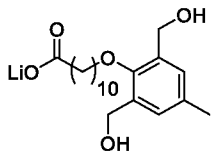
(gradient 100 % Hex → 50% EtOAc/Hex) to afford methyl 11-(2,6-bis(hydroxymethyl)-4-methylphenoxy)undecanoate **44** as a colorless oil (1.6 g, 27%).

Step 2. Preparation of methyl 11-(2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-6-(hydroxymethyl)-4-methylphenoxy)undecanoate 45



To a solution of methyl 11-(2,6-bis(hydroxymethyl)-4-methylphenoxy)undecanoate **44** (1.5 g, 4.1 mmol) in anhydrous pyridine (20 mL) was added 4,4′-Dimethoxytrityl chloride (1.4 g, 4.1 mmol). The solution was stirred overnight at room temperature. Upon completion the solution was concentrated *in vacuo* to dryness and purified by column chromatography on silica gel 60 (0.5 to 1% MeOH in CH_2Cl_2) to afford Methyl 11-(2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-6-(hydroxymethyl)-4-methylphenoxy)undecanoate **45** as a pale yellow solid (1.1 g, 40%).

Step 3. Preparation of lithium 11-(2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-6-(hydroxymethyl)-4-methylphenoxy)undecanoate 46



To a solution of Methyl 11-(2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-6-(hydroxymethyl)-4-methylphenoxy)undecanoate **45** (1.1 g, 1.7 mmol) in anhydrous tetrahydrofuran (40 mL) and water (10 mL) was added lithium hydroxide (44 mg, 1.8 mmol). The solution was concentrated *in vacuo* to remove all tetrahydrofuran. The remaining aqueous solution was flash frozen on liquid nitrogen then lyophilized overnight to afford lithium 11-(2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-6-(hydroxymethyl)-4-methylphenoxy)undecanoate **46** as a pale pink solid (1.1 g, 94%).

Step 4. Preparation of Compound 47

A solution of **10** (1.33 g, 0.66 mmol), **46** (0.5 g, 0.73 mmol), HBTU (400 mg, 1 mmol) in *N*,*N*-dimethylformamide (25 mL) was added diisopropylethylamine (0.35 mL, 2 mmol). The solution was stirred overnight (18 hours) at room temperature. Upon completion, the solvent

was remove in vacuo and the residue was purified by column chromatography on silica gel (gradient: 100% CH_2Cl_2 - 5% - 10% - 15% MeOH in CH_2Cl_2) to afford **47** as a colorless solid (710 mg, 41%).

Step 5. Preparation of Compound 48

To a solution of **47** (0.71 g, 0.3 mmol), triethylamine (0.4 mL, 3.0 mmol) and polystyrene-DMAP (3 mmol/g loading, 200 mg, 0.6 mmol) in dichloromethane (15 mL) was added succinic anhydride (60 mg, 0.6 mmol). The solution was stirred overnight at room temperature and upon completion filtered and concentrated *in vacuo* to dryness. The residue was purified by column chromatography on silica gel 60 (gradient 5% to 20% MeOH in CH_2Cl_2) to afford the **48** as a pale yellow solid (570 mg, 70%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.91 (m, 1H), 7.86-7.76 (m, 6H), 7.45-7.40 (m, 2H), 7.36-7.14 (m, 10H), 7.10 (s, 1H), 6.91 (d, *J* = 8.9 Hz, 4H), 5.21 (d, J = 3.3 Hz, 3H), 5.01 (s, 2H), 4.97 (dd, J = 11.2, 3.4 Hz, 3H), 4.56 (d, J = 8.5 Hz, 3H), 4.06-3.98 (m, 11H), 3.93-3.84 (m, 3H), 3.81-3.72 (m, 3H), 3.74 (s, 6H), 3.65-3.46 (m, 38H), 3.40-3.35 (m, 6H), 3.20-3.16 (m, 6H), 2.56-2.44 (m, 4H), 2.33 (s, 3H), 2.15-2.08 (m, 2H), 2.10 (s, 9H), 2.04-1.96 (m, 6H), 1.89 (s, 9H), 1.82-1.76 (m, 4H), 1.77 (s, 9H), 1.54-1.34 (m, 4H), 1.28-1.10 (m, 12H),

Step 6. Preparation of compound 49

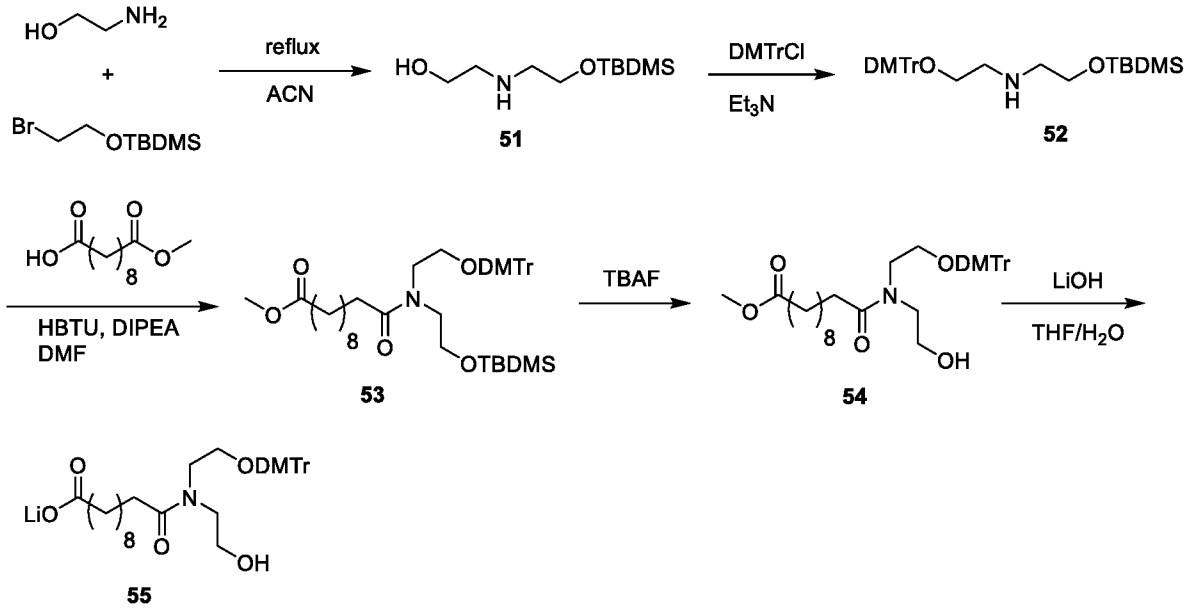
To a solution of **48** (100 mg, 40 µmol), *N*-Hydroxysuccinimide (30 mg/mL soln in acetonitrile, 50 µL, 13 µmol), *N*,*N*'-Diisopropylcarbodiimide (40 µL, 264 µmol) and pyridine (50 µL) in dichloromethane (2 mL) and acetonitrile (3 mL) was added 1000 Å lcaa CPG (prime synthesis, 920 mg). The solution was stirred overnight at room temperature on an orbital shaker. TLC analysis of the reaction solution showed only partial consumption of the activated *N*-Hydroxysuccinic ester so additional CPG (500 mg) was added. The solution was stirred again overnight. Upon completion, the CPG was filtered and washed with dichloromethane (25 mL), acetonitrile (25 mL) and tetrahydrofuran (25 mL). The unreacted amine residues on the CPG were acetylated (capped) by adding a 1:1 solution of acetic anhydride in acetonitrile (3 mL) and 10% N-methylimidazole / 10% pryridine in tetrahydrofuran (3 mL). The suspension was left for 2 hours then filtered and rinsed with equal parts tetrahydrofuran (25 mL), acetonitrile (25 mL) and dichloromethane (25 mL). The loaded CPG **49** was dried under high vacuum overnight. The ligand loading efficiency was determined to be 22 µmole/g using a standard DMT loading assay (3% trichloroacetic acid in CH_2Cl_2, UV-VIS, A_{504}).

Step 7. Preparation of conjugate 43

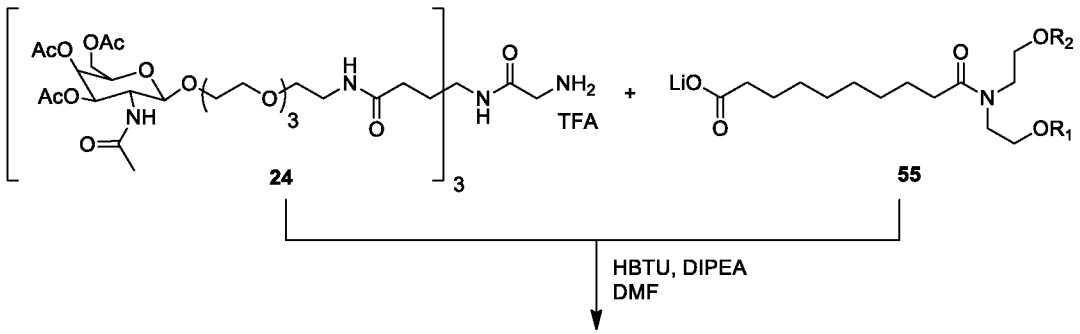
The resulting GalNAc loaded CPG solid support **49** was employed in automated oligonucleotide synthesis using standard procedures. Nucleotide deprotection followed by removal from the solid support (with concurrent galactosamine acetate deprotection) afforded a GalNAc-oligonucleotide conjugate **43**.

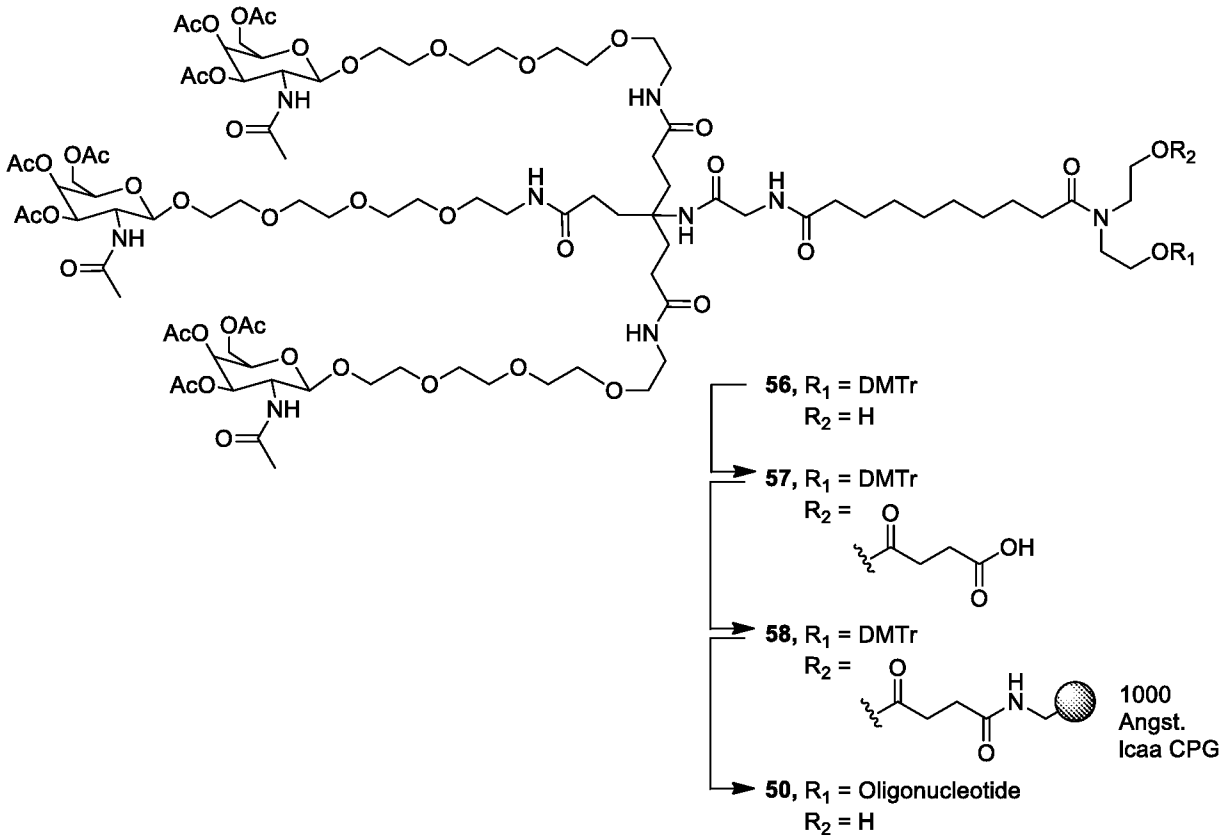
Example 6. Synthesis of Conjugate 50

Scheme 13.

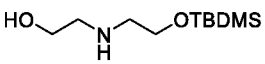


Scheme 14.



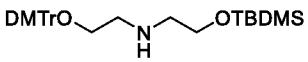


Step 1. Preparation of 2-((2-((tert-butyldimethylsilyl)oxy)ethyl)amino)ethan-1-ol 51



A solution of ethanolamine (77 mL, 1.25 mol) and (2-bromoethoxy)-*tert*-butyl dimethylsilane (15 g, 62.7 mmol) in anhydrous acetonitrile (200 mL) was refluxed for 3 hours. Upon completion the reaction was cooled to room temperature, diluted with water (400 mL) and extracted with ethyl acetate (3 x 150 mL). The combined ethyl acetate extracts were dried on magnesium sulfate, filtered and concentrated in vacuo to dryness. The residue was purified by filtration through a pad of silica first with 50% ethyl acetate/hexanes then 50% MeOH/EtOAc to afford **51** as a pale yellow oil (14 g, 100%).

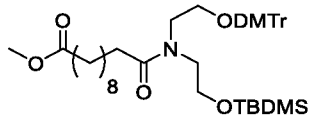
Step 2. Preparation of 2-(bis(4-methoxyphenyl)(phenyl)methoxy)-N-(2-((tert-butyldimethylsilyl)oxy)ethyl)ethan-1-amine 52



To a solution of 2-((2-((tert-butyldimethylsilyl)oxy)ethyl)amino)ethan-1-ol **51** (14 g, 64 mmol) and triethylamine (17.5 mL, 128 mmol) in anhydrous dichloromethane (250 mL) was added 4,4′-Dimethoxytrityl chloride (24 g, 70 mmol). The solution was stirred overnight at

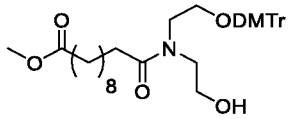
room temperature then concentrated in vacuo to dryness. The residue was dissolved in ethyl acetate (300 mL) and washed with water (250 mL) and brine (250 mL). The ethyl acetate was dried on magnesium sulfate, filtered and concentrated in vacuo to dryness. Purification by column chromatography on silica gel 60 (1% to 5% MeOH in CH_2Cl_2) afforded **52** as a pale yellow viscous oil (13 g, 39%).

Step 3. Preparation of methyl 10-((2-(bis(4-methoxyphenyl)(phenyl)methoxy)ethyl)(2-((tert-butyldimethylsilyl)oxy)ethyl)amino)-10-oxodecanoate 53



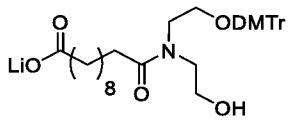
A solution of 2-(bis(4-methoxyphenyl)(phenyl)methoxy)-N-(2-((tert-butyldimethylsilyl)oxy)ethyl)ethan-1-amine **52** (5.4 g, 10.3 mmol), monomethyl sebacate (2.2 g, 10.3 g), HBTU (4.9 g, 12.9 mmol), DIPEA (5.3 mL, 30.9 mmol) in *N,N*-dimethylformamide (100 mL) was stirred for 3 hours at room temperature. Upon completion, the solution was poured into water (400 mL) and extracted with ethyl acetate (1 x 500 mL). The ethyl acetate extract was washed with brine (2 x 250 mL), dried on magnesium sulfate, filtered and concentrated in vacuo to dryness. Purification by column chromatography on silica gel 60 (10% to 25% ethyl acetate in hexanes) afforded **53** as a viscous yellow oil (6.5 g, 87%).

Step 4. Preparation of methyl 10-((2-(bis(4-methoxyphenyl)(phenyl)methoxy)ethyl)(2-hydroxyethyl)amino)-10-oxodecanoate 54



To a solution of methyl 10-((2-(bis(4-methoxyphenyl)(phenyl)methoxy)ethyl)(2-((tert-butyldimethylsilyl)oxy)ethyl)amino)-10-oxodecanoate **53** (2.0 g, 2.8 mmol) and triethylamine (1 mL) in anhydrous tetrahydrofuran (20 mL) was added TBAF (1M in THF, 3.4 mL, 3.3 mmol). The solution was stirred for 6h, but only partial conversion observed by TLC (5% MeOH in CH_2Cl_2). Additional 1.7 mL TBAF added and the solution was stirred overnight at room temperature. Upon completion, the solution was concentrated in vacuo and purified by column chromatography on silica gel 60 (10% to 50% EtOAc in hexanes then 100% EtOAc) to afford **54** as a viscous colorless oil (0.5 g, 29%).

Step 5. Preparation of lithium 10-((2-(bis(4-methoxyphenyl)(phenyl)methoxy)ethyl)(2-hydroxyethyl)amino)-10-oxodecanoate 55



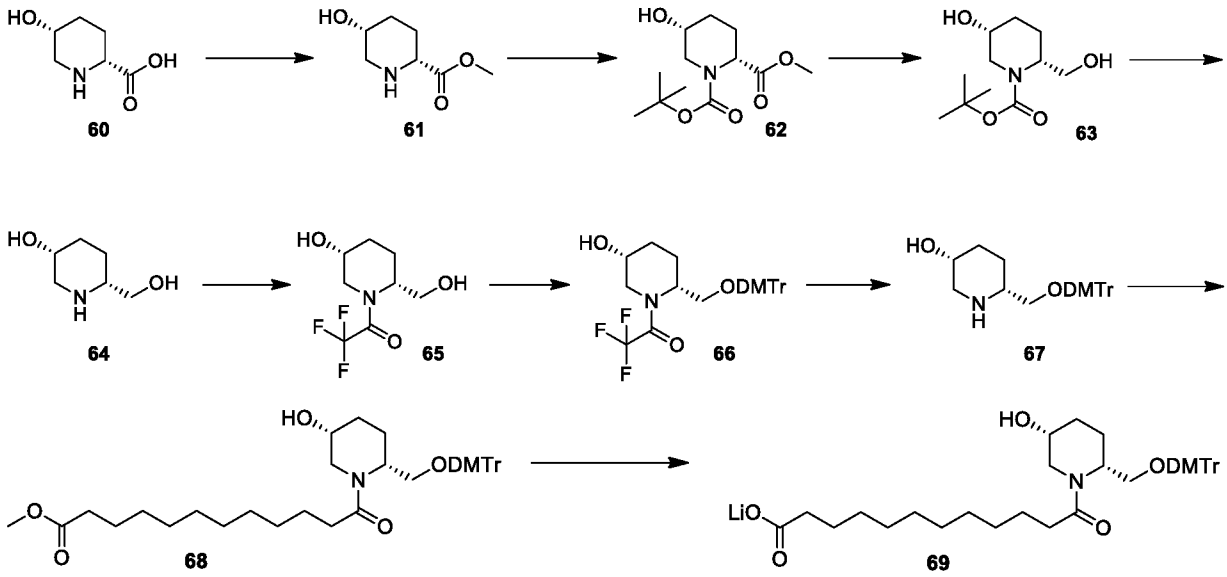
To a solution of methyl 10-((2-(bis(4-methoxyphenyl)(phenyl)methoxy)ethyl)(2-hydroxyethyl)amino)-10-oxodecanoate **54** (0.5 g, 0.83 mmol) in THF (40 mL) was added water (10 mL) and lithium hydroxide (24 mg, 1.0 mmol). The solution was stirred overnight at room temperature then concentrated in vacuo to remove the THF. The remaining aqueous solution was flash frozen on liquid nitrogen and lyophilized to afford **55** as a colorless solid (485 mg, 95%).

Step 6. Preparation of compounds 56, 57, 58 and 50

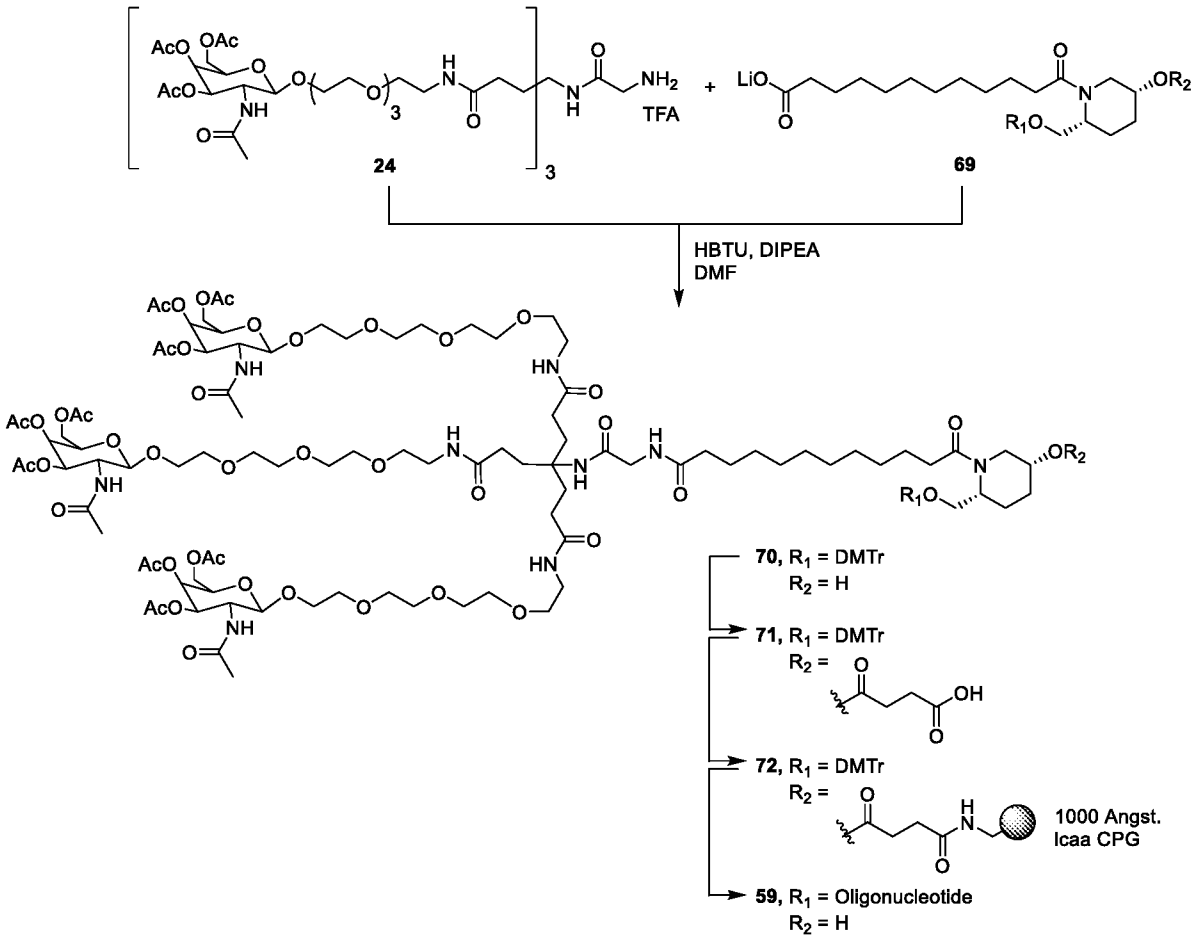
Compounds **56**, **57**, **58** and **50** were prepared using the identical procedures to those used to synthesize compounds **47**, **48**, **49** and **43** respectfully.

Example 7. Synthesis of conjugate 59

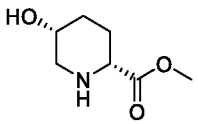
Scheme 15.



Scheme 16.

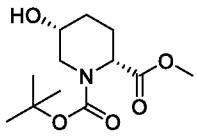


Step 1. Preparation of methyl (2R,5R)-5-hydroxypiperidine-2-carboxylate 61



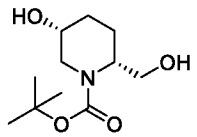
(2R,5R)-5-hydroxypiperidine-2-carboxylic acid **60** (3.5 g, 24.1 mmol) was stirred in MeOH (50 mL). HCl (g) was bubbled through the solution for 2 mins and the reaction stirred at reflux for 1.5 h. The reaction was concentrated in-vacuo to give methyl (2R,5R)-5-hydroxypiperidine-2-carboxylate **61** in quantitative yield which was used without further purification.

Step 2. Preparation of 1-(tert-butyl) 2-methyl (2R,5R)-5-hydroxypiperidine-1,2-dicarboxylate 62



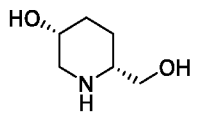
Methyl (2R,5R)-5-hydroxypiperidine-2-carboxylate **61** (24.1 mmol) and TEA (7.2 mL, 53.02 mmol) were stirred in DCM (100 mL) at RT. Di-*tert*-butyl-di-carbonate (5.7 g, 26.5 mmol) was added in portions and the reaction stirred for 2 h. The reaction was diluted with DCM (100 mL) and washed sequentially with 1 M HCl (2 x 75 mL), saturated $NaHCO_3$ (2 x 75 mL), H_2O (2 x 75 mL) and saturated NaCl solution (2 x75 mL). The organics were separated, dried (Na_2SO_4) and concentrated in-vacuo to give 1-(tert-butyl) 2-methyl (2R,5R)-5-hydroxypiperidine-1,2-dicarboxylate **62** (5.53 g, 88%) which was used without further purification.

Step 3. Preparation of tert-butyl (2R,5R)-5-hydroxy-2-(hydroxymethyl)piperidine-1-carboxylate 63



(2R,5R)-1-(tert-Butoxycarbonyl)-5-hydroxypiperidine-2-carboxylic acid **62** (5.53 g, 21.4 mmol) was stirred in THF at 0°C. $LiBH_4$ (3.0 M solution in THF)(8.9 mL, 27.7 mmol) was added dropwise over 1 hr. The reaction was allowed to warm to RT and stirring continued for 16 h. Reaction was quenched with 1M NaOH, THF removed in-vacuo and the aqueous exhaustively extracted with EtOAc (10 x 100 mL). The combined organics were washed with H_2O (50 mL), saturated NaCl solution (2 x 50 mL), dried (Na_2SO_4) and concentrated in-vacuo to give *tert*-butyl (2R,5R)-5-hydroxy-2-(hydroxymethyl)piperidine-1-carboxylate **63** (2.4 g, 49.0 %) which was used without further purification.

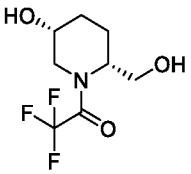
Step 4. Preparation of (3R,6R)-6-(hydroxymethyl)piperidin-3-ol 64



tert-Butyl (2R,5R)-5-hydroxy-2-(hydroxymethyl)piperidine-1-carboxylate **63** (2.4 g, 10.4 mmol) was stirred in Et_2O at RT. HCl (g) was bubbled through for 45 secs and the reaction stirred at RT for 45 mins. The reaction was concentrated in-vacuo and dried under hi-

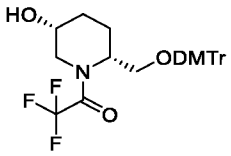
vac to afford (3R,6R)-6-(hydroxymethyl)piperidin-3-ol **64**. The product was used without further purification.

Step 5. Preparation of 2,2,2-trifluoro-1-((2R,5R)-5-hydroxy-2-(hydroxymethyl)piperidin-1-yl)ethan-1-one 65



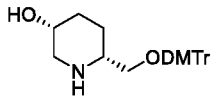
Crude (3R,6R)-6-(hydroxymethyl)piperidin-3-ol **64** from the previous reaction was stirred in MeCN (50 mL) with TEA (3.5 mL, 25.2 mmol) at RT. Ethyl trifluoroacetate (3 mL, 25.2 mmol) was added and the reaction stirred at RT for 16 hr, then concentrated in-vacuo to give 2,2,2-trifluoro-1-((2R,5R)-5-hydroxy-2-(hydroxymethyl)piperidin-1-yl)ethan-1-one **65**. The product was used without further purification.

Step 6. Preparation of 1-((2R,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-hydroxypiperidin-1-yl)-2,2,2-trifluoroethan-1-one 66



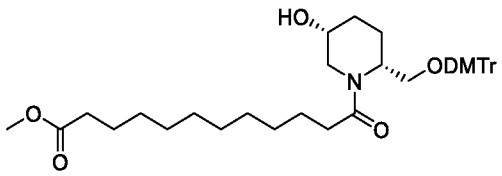
Crude 2,2,2-trifluoro-1-((2R,5R)-5-hydroxy-2-(hydroxymethyl)piperidin-1-yl)ethan-1-one **65** from the previous reaction was stirred in DCM with TEA (50 mL) at RT. 4,4'-Dimethoxytrityl chloride (DMTrCl) (3.87 g, 11.44 mmol) was added in one portion and the reaction stirred at RT for 3 hours. The reaction was diluted with DCM (50 mL) and washed sequentially with saturated $NaHCO_3$ (2 x 75 mL), H_2O (2 x 75 mL) and saturated NaCl solution (2 x75 mL). The organics were separated, dried (Na_2SO_4), concentrated in-vacuo and purified by column chromatography (100% hexanes – 60% EtOAc/Hexanes) (0.1 % TEA) to give 1-((2R,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-hydroxypiperidin-1-yl)-2,2,2-trifluoroethan-1-one **66** (3.14 g, 57%)

Step 7. Preparation of (3R,6R)-6-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-piperidin-3-ol 67



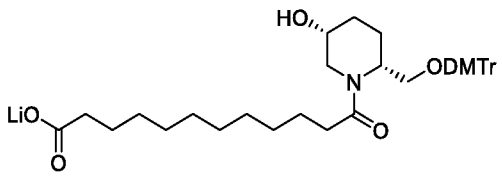
1-((2R,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-hydroxypiperidin-1-yl)-2,2,2-trifluoroethan-1-one **66** (3.14 g, 6.0 mmol) was stirred in MeOH (50 mL) at RT. KOH (672 mg, 12 mmol) was added and the reaction stirred at RT for 16 hours. Additional KOH (300 mg, 6 mmol) was added and stirring continued for an additional 24 h. The reaction was concentrated in-vacuo, taken up in DCM (150 mL), washed with H_2O (4 x 50 mL), dried (Na_2SO_4) and concentrated in-vacuo to give (3R,6R)-6-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)piperidin-3-ol **67** (2.34 g, 90%) which was used without further purification.

Step 8. Preparation of methyl 12-((2R,5R)-2-((bis(4-methoxyphenyl)(phenyl)-methoxy)methyl)-5-hydroxypiperidin-1-yl)-12-oxododecanoate 68



(3R,6R)-6-((Bis(4-methoxyphenyl)(phenyl)methoxy)methyl)piperidin-3-ol **67** (2.34 g, 5.34 mmol) was stirred in DCM (75 mL) at RT. Triethylamine (2.2 mL, 16.2 mmol), HATU (3.5 g, 9.2 mmol) and 12-methoxy-12-oxododecanoic acid (1.32 g, 5.4 mmol) were added and the reaction stirred at RT for 3 h. The resultant solid precipitate was removed by filtration, the filtrate concentrated in-vacuo and the residue purified by column chromatography (2.5 %MeOH/DCM, 0.1% TEA) to give methyl 12-((2R,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-hydroxypiperidin-1-yl)-12-oxododecanoate **68** in quantitative yield.

Step 9. Preparation of lithium 12-((2R,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)-methyl)-5-hydroxypiperidin-1-yl)-12-oxododecanoate 69



Methyl 12-((2R,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-hydroxypiperidin-1-yl)-12-oxododecanoate **68** (5.4 mmol) and LiOH (140 mg, 5.94 mmol) were stirred in THF:H_2O (1:1, 100 mL) at RT for 48 h. The THF was removed in-vacuo, the aqueous frozen and lyophilized to give lithium 12-((2R,5R)-2-((bis(4-

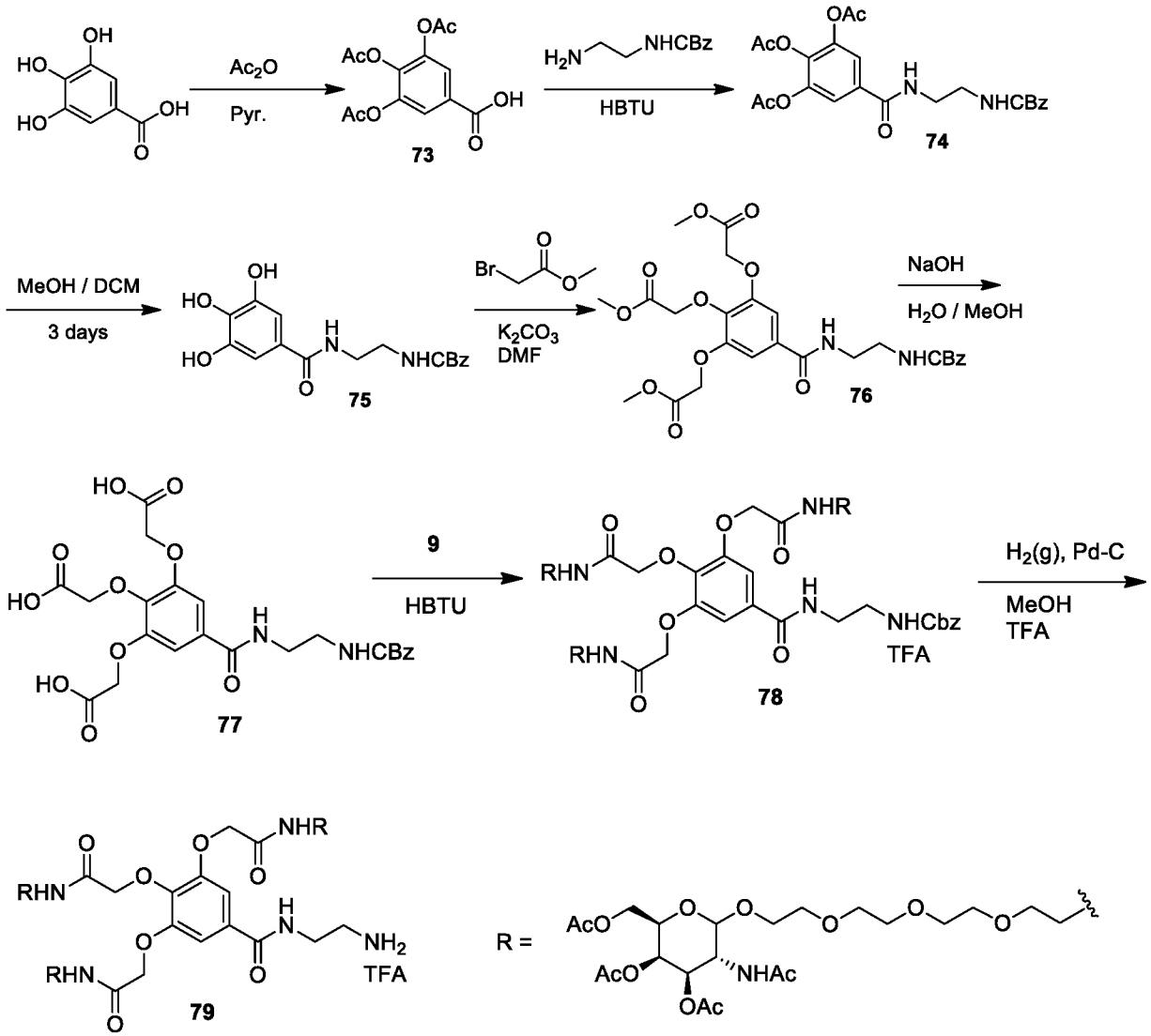
methoxyphenyl)(phenyl)methoxy)methyl)-5-hydroxypiperidin-1-yl)-12-oxododecanoate **69** (3.2 g, 91 %). Which was used in subsequent reactions without additional purification.

Step 10. Preparation of compounds 70, 71, 72, and 59

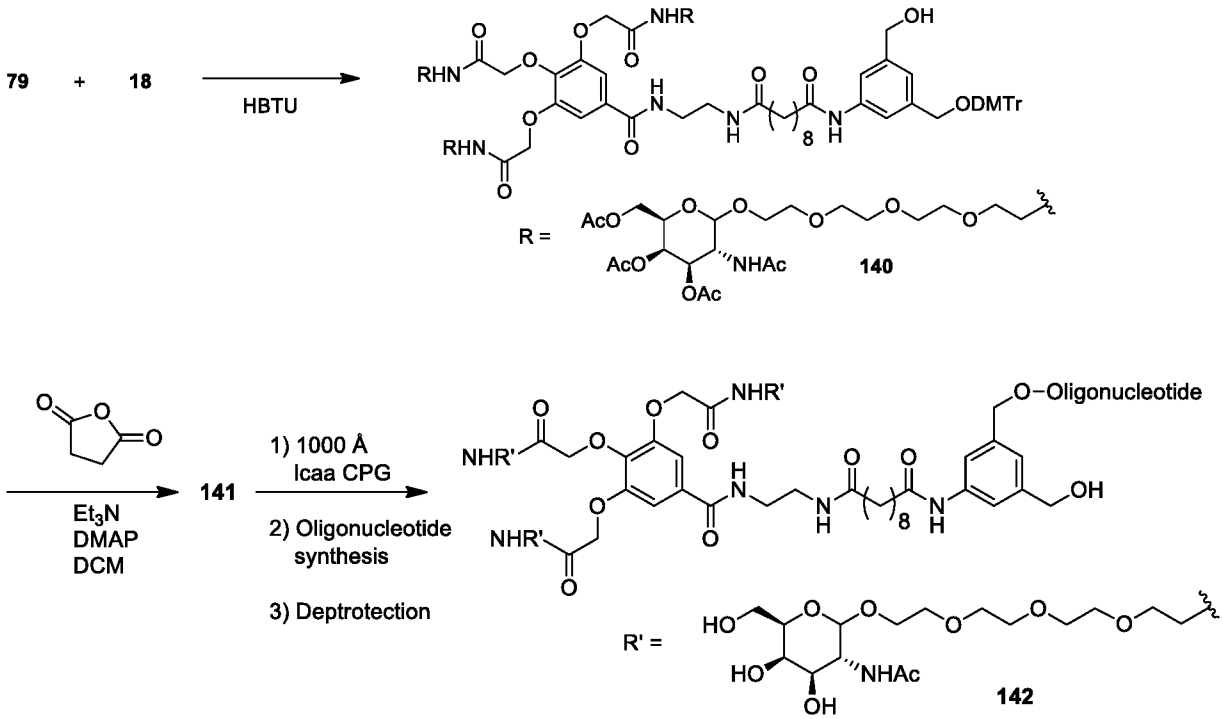
Compounds **70**, **71**, **72** and **59** were prepared using the identical procedures to those used to synthesize compounds **47**, **48**, **49** and **43** respectfully.

Example 8. Synthesis of conjugate 142

Scheme 17.



Scheme 18.



Step 1. Preparation of 3,4,5-Triacetoxybenzoic acid 73

To a solution of Gallic acid (20 g) in pyridine (50 mL) and acetic anhydride (50 mL). The solution was stirred overnight at room temperature then poured into ice water (1 L). The solution was made acidic with concentrated hydrochloric acid where upon a colorless solid precipitated. The solid was collected via filtration and washed with water (5 x 100 mL). The wet solid was frozen on liquid nitrogen and freeze dried to afford 3,4,5-triacetoxybenzoic acid (26 g, 75%).

Step 2. Preparation of 5-((2-((2-Oxo-2-phenyl-1λ^2-ethyl)amino)ethyl)carbamoyl)benzene-1,2,3-triyl triacetate 74

To a solution of 3,4,5-triacetoxybenzoic acid (10 g, 33.8 mmol), N-carbobenzoxy-1,2-diaminoethane hydrochloride (5.3 g, 33.8 mmol) and HBTU (13.5 g, 35.5 mmol) in DMF (200 mL) was added DIPEA (17.5 mL, 101 mmol). The solution was stirred for 16 hours then diluted with ethyl acetate (250 mL), washed with brine (3 x 200 mL), dried on magnesium sulfate, filtered and concentrated *in vacuo* to dryness. The crude product was purified by column chromatography on silica gel (Gradient 1% to 5% MeOH in DCM) to afford 5-((2-((2-Oxo-2-phenyl-1λ^2-ethyl)amino)ethyl)carbamoyl)benzene-1,2,3-triyl triacetate as an off white solid (5.5 g).

Step 3. Preparation of 3,4,5-Trihydroxy-N-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)ethyl)benzamide 75

A solution of 5-((2-((2-Oxo-2-phenyl-1λ^2-ethyl)amino)ethyl)carbamoyl)benzene-1,2,3-triyl triacetate (5 g, 1.1 mmol) in 1:1 MeOH / CH_2Cl_2 (100 mL) was stirred for 3 days at room temperature. Upon completion the solvent was removed to afford 3,4,5-Trihydroxy-N-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)ethyl)benzamide as a colorless solid (4 g, quantitative).

Step 4. Preparation of Trimethyl 2,2',2''-((5-((2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)ethyl)carbamoyl)benzene-1,2,3-triyl)tris(oxy))triacetate 76

A solution of 3,4,5-Trihydroxy-N-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)ethyl)benzamide (4 g, 11.6 mmol), methyl bromoacetate (7.7 g, 46.4 mmol) and potassium carbonate (9.6 g, 69.4 mmol) in DMF (100 mL) was stirred overnight at 60 °C. Upon completion the solution was cooled to room temperature, diluted with ethyl acetate (200 mL), washed with water (200 mL), brine (3 x 100 mL), dried on magnesium sulfate, filtered and concentrated in vacuo to dryness. The crude product was purified by column chromatography on silica gel (Gradient 2% to 10% MeOH in DCM) to afford trimethyl 2,2',2''-((5-((2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)ethyl)carbamoyl)benzene-1,2,3-triyl)tris(oxy))-triacetate as a beige solid (5 g, 79%)

Step 5. Preparation of 2,2',2''-((5-((2-((2-Oxo-2-phenyl-1λ^2-ethyl)amino)ethyl)-carbamoyl)benzene-1,2,3-triyl)tris(oxy))triacetic acid 77

A solution of trimethyl 2,2',2''-((5-((2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)ethyl)-carbamoyl)benzene-1,2,3-triyl)tris(oxy))triacetate (5 g, 9.2 mmol) and 1M NaOH (30 mL) in methanol (100 mL) was stirred for 2 hours at room temperature. Upon completion the reaction was concentrated to remove the methanol and diluted with water (75 mL). The mixture was cooled to 0°C, acidified with 2M HCl and extracted with ethyl acetate (5 x 150 mL). The combined ethyl acetate extracts were dried on magnesium sulfate, filtered and concentrated in vacuo to dryness to afford 2,2',2''-((5-((2-((2-Oxo-2-phenyl-1λ^2-ethyl)amino)ethyl)carbamoyl)benzene-1,2,3-triyl)tris(oxy))triacetic acid as a colorless solid (2.3 g, 50%).

Step 6. Preparation of Compound 78

Compound **78** was prepared from compounds **9** (2.75 g, 4.3 mmol) and **77** (0.5 g, 0.96 mmol) using an identical procedure to that used for compound **13**. Yield: 600 mg.

Step 7. Preparation of Compound 79

Compound **79** was prepared from compounds **78** (0.6 g) using an identical procedure to that used for compound **14**. Yield: 500 mg.

Step 8. Preparation of compound 140

Compound **140** was prepared from compound **79** (500 mg, 0.25 mmol) and compound **18** (175 mg, 0.25 mmol) using an identical procedure to that used for compound **19**. Yield: 250 mg, 44%.

Step 9. Preparation of compound 141

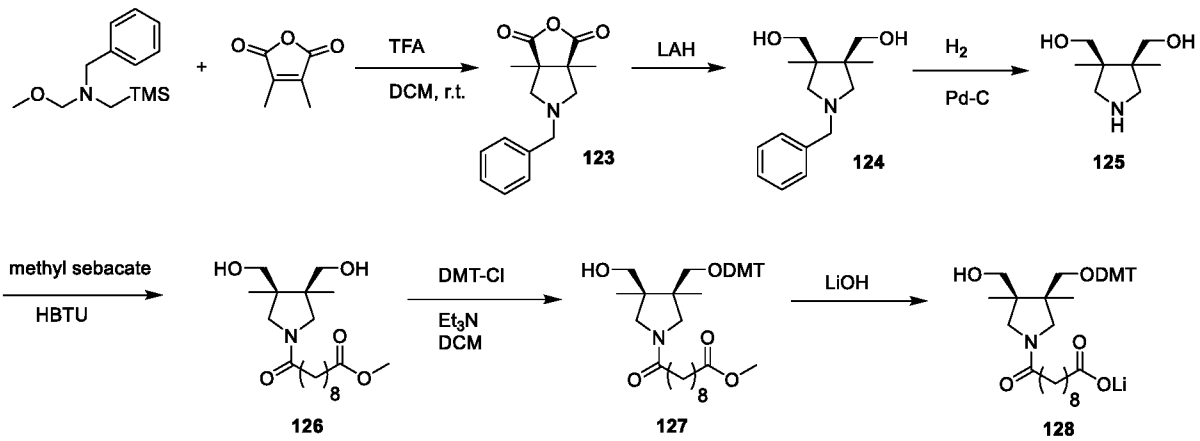
Compound **141** was prepared from compound **140** (250 mg, 0.11 mmol) using an identical procedure to that used for compound **20**. Yield: 200 mg.

Step 10. Preparation of conjugate 142

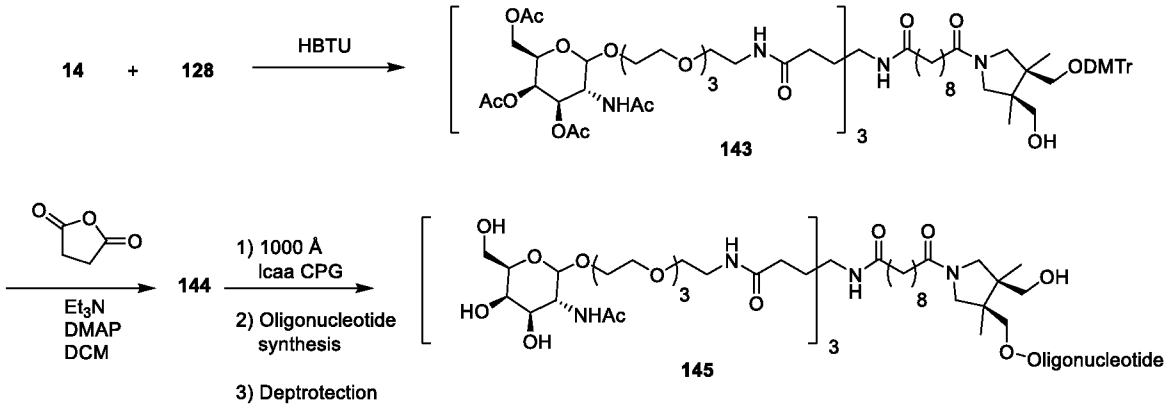
Conjugate **142** was prepared from compound **141** (200 mg) and 1000A lcaa CPG (1.8 g) using an identical procedure to that used for compound **1**. Yield: 1.9 g, 22 μmol/g CPG loading. The resulting GalNAc loaded CPG solid support was employed in automated oligonucleotide synthesis using standard procedures. Nucleotide deprotection followed by removal from the solid support (with concurrent galactosamine acetate deprotection) afforded the GalNAc-oligonucleotide conjugate **142.**

Example 9. Synthesis of conjugate 145

Scheme 19.



Scheme 20.



Step 1. Preparation of Racemic (cis) 5-Benzyl-3a,6a-dimethyltetrahydro-1H-furo[3,4-c]pyrrole-1,3(3aH)-dione 123

To a cooled solution (0°C) of 3,4-dimethylfuran-2,5-dione (3 g, 24 mmol) and N-benzyl-1-methoxy-N-((trimethylsilyl)methyl)methanamine (7 g, 29.8 mmol) in dichloromethane (75 mL) was slowly added trifluoroacetic acid (75 µL). Stir overnight allowing the solution to slowly warm to room temperature as the ice bath melted. The reaction mixture was concentrated to dryness, dissolved in ethyl acetate (100 mL), washed with saturated sodium bicarbonate (2 x 100mL), dried on magnesium sulfate, filtered and concentrated to dryness. Purification by column chromatography on silica gel (gradient: 20% ethyl acetate in hexanes to 100% ethyl acetate) afforded racemic (cis) 5-Benzyl-3a,6a-dimethyltetrahydro-1H-furo[3,4-c]pyrrole-1,3(3aH)-dione as a yellow oil (3.5 g, 56%).

Step 2. Preparation of Racemic (cis) 1-Benzyl-3,4-dimethylpyrrolidine-3,4-diyl)dimethanol 124

To a cooled (0°C) solution of (3aR,6aS)-5-Benzyl-3a,6a-dimethyltetrahydro-1H-furo[3,4-c]pyrrole-1,3(3aH)-dione (3.5 g, 13.4 mmol) in anhydrous diethyl ether (50 mL) was added slowly lithium aluminum hydride pellets (1.5 g, 40 mmol) over three portions. The solution was stirred overnight warming to room temperature as the ice water bath melted. Upon completion, the reaction was cooled to 0°C and very slowly quenched with 1.5 mL of 5M NaOH followed by 1.5 mL of water. Stir for 30 minutes then add magnesium sulfate and filter. The filtrate was concentrated to afford racemic (cis) 1-Benzyl-3,4-dimethylpyrrolidine-3,4-diyl)dimethanol as a colorless oil (2.7 g).

Step 3. Preparation of Racemic (cis) 3,4-Dimethylpyrrolidine-3,4-diyl)dimethanol 125

To a solution of ((3R,4S)-1-Benzyl-3,4-dimethylpyrrolidine-3,4-diyl)dimethanol (10 g, 40 mmol) in methanol (10 mL) was added 10% palladium on activated charcoal wet (1 g). The solution was stirred vigorously under a hydrogen atmosphere for 16 hours. Upon completion the solution was filtered through Celite, and concentrated to dryness to afford racemic (cis) 3,4-Dimethylpyrrolidine-3,4-diyl)dimethanol as a colorless solid (5.5 g, 86%).

Step 4. Preparation of Racemic (cis) Methyl 10-(3,4-bis(hydroxymethyl)-3,4-dimethylpyrrolidin-1-yl)-10-oxodecanoate 126

Compound **126** was prepared from compound **125** (1.3 g, 8.2 mmol) and monomethyl sebacate (1.8 g, 8.2 mmol) using an identical procedure to that used for compound **17**. Yield: 1.8 g, 61%.

Step 5. Preparation of Racemic (cis) Methyl 10-(3-((bis(4-methoxyphenyl-)(phenyl)methoxy)-methyl)-4-(hydroxymethyl)-3,4-dimethylpyrrolidin-1-yl)-10-oxodecanoate 127

Compound **127** was prepared from compound **126** (1.8 g, 5.0 mmol) and 4,4′-Dimethoxytrityl chloride (1.7 g, 5.0 mmol) using an identical procedure to that used for compound **18**. Yield: 1.4 g, 42%.

Step 6. Preparation of Racemic (cis) Lithium 10-(3-((bis(4-methoxyphenyl)-(phenyl)methoxy)-methyl)-4-(hydroxymethyl)-3,4-dimethylpyrrolidin-1-yl)-10-oxodecanoate 128

To a solution of compound **127** (3.0 g, 4.6 mmol) in THF (50 mL) and water (50 mL) was added lithium hydroxide (121 mg, 5.0 mmol). The solution was stirred for 4 hours at room temperature then concentrated to remove the THF. The remaining aqueous solution was freeze dried overnight to afford a pale pink solid (2.9 g, quantitative) .

Step 7. Preparation of compound 143

Compound **143** was prepared from compound **128** (270 mg, 0.42 mmol) and compound **14** (800 mg, 0.42 mmol) using an identical procedure to that used for compound **19**. Yield: 900 mg, 87%.

Step 8. Preparation of compound 144

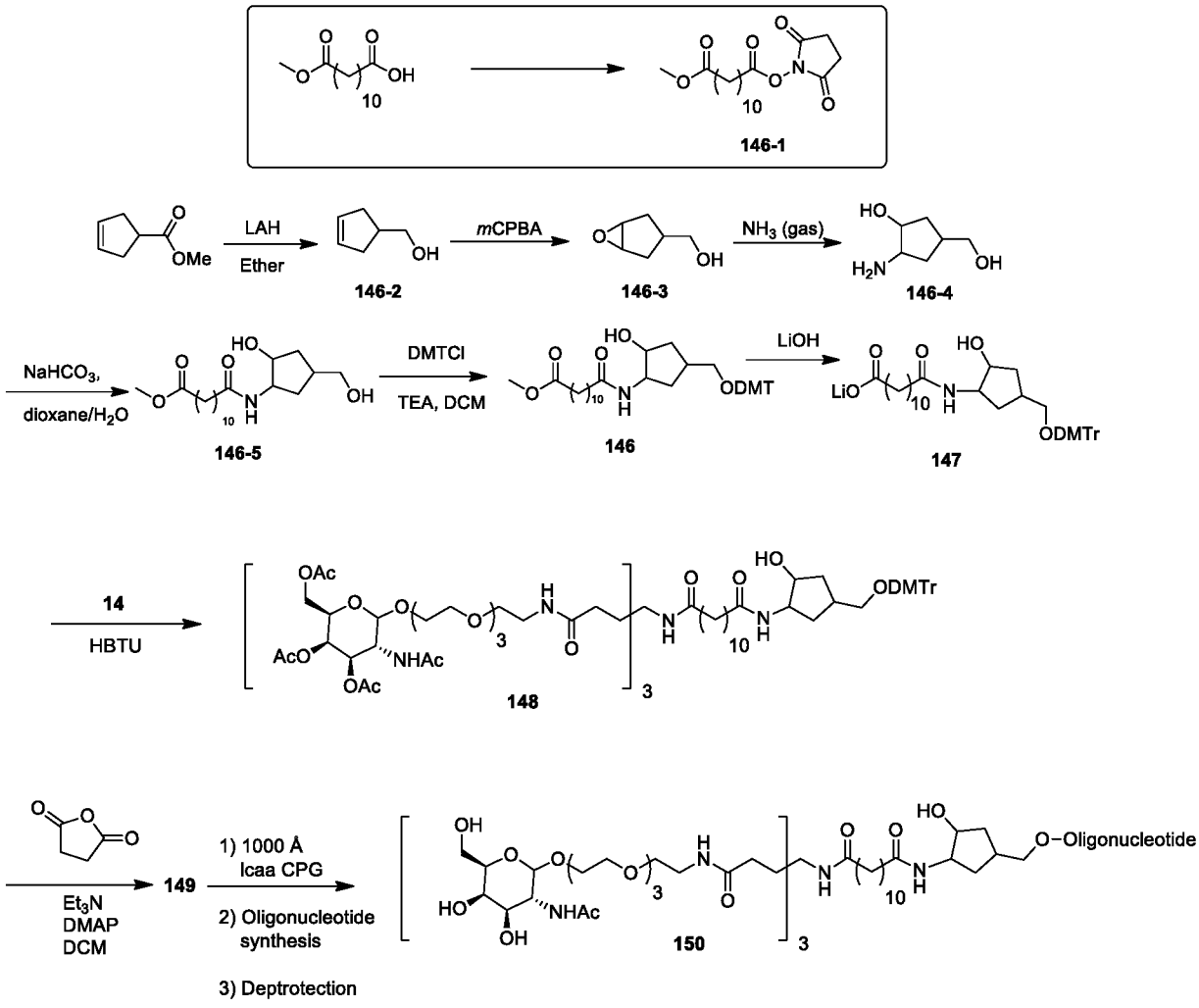
Compound **144** was prepared from compound **143** (500 mg, 0.2 mmol) using an identical procedure to that used for compound **20**. Yield: 200 mg.

Step 9. Preparation of conjugate 145

Conjugate **145** was prepared from compound **144** (200 mg) and 1000A lcaa CPG (1.8 g) using an identical procedure to that used for compound **1**. Yield: 1.9 g, 20 µmol/g CPG loading. The resulting GalNAc loaded CPG solid support was employed in automated oligonucleotide synthesis using standard procedures. Nucleotide deprotection followed by removal from the solid support (with concurrent galactosamine acetate deprotection) afforded the GalNAc-oligonucleotide conjugate **145.**

Example 10. Synthesis of conjugate 150

Scheme 21.



Step 1. Preparation of 146-1

To a solution of mono methyl ester of dodecanedioic acid (12.2 g, 50.0 mmol) in dichloromethane (300 mL) was added N-hydroxysuccinimide (6.10g, 53.0 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (10.52g, 55.0 mmol). The cloudy mixture was stirred overnight at room temperature and the reaction became a clear solution. TLC indicated the reaction was completed. The organics were washed with saturated NH_4Cl (300 mL) and brine (100 mL). The organic layer was separated, dried over $MgSO_4$ and concentrated to dryness to pure 1-(2,5-dioxopyrrolidin-1-yl) 12-methyl dodecanedioate **146-1** as a white solid (16.7g, 97.8%).

Step 2. Preparation of cyclopent-3-en-1-ylmethanol 146-2

To a suspension of lithium aluminum hydride (15.2g, 0.40 mol) in anhydrous ether (1 L) at 0°C under nitrogen, was added the solution of methyl cyclopent-3-enecarboxylate (50 g, 0.40 mol) in ether (300 mL) dropwise over 5 hrs. The suspension was stirred at room temperature overnight. TLC indicated the completion of the reaction. The reaction was re-cooled to 0°C. Saturated solution of Na_2SO_4 (32 mL) was added dropwise to quench the reaction. After the addition was complete, the mixture was stirred for another 3 hrs and was filtered through a pad of celite. Evaporation of solvent afforded cyclopent-3-enylmethanol **146-2** (37.3 g, 95 %) as a colorless liquid.

Step 3. Preparation of (6-oxabicyclo[3.1.0]hexan-3-yl)methanol 146-3

To a solution of cyclopent-3-enylmethanol **146-2** (4.0 g, 41 mmol) in dichloromethane (150 mL) at 0°C was added 3-chloroperbenzoic acid (10 g, 45 mmol, 77% purity) by portion. The reaction was stirred overnight. Dichloromethane (150 mL) was added. The organics was washed with sodium thiosulfate (12 g in 10 mL water), followed by saturated $NaHCO_3$ (40 mL). This was repeated till all the remaining 3-chloroperbenzoic acid was washed away. The organic was dried over $MgSO_4$. Evaporation of solvent gave a mixture of *cis*- and *trans*- 6-oxabicyclo[3.1.0]hexan-3-ylmethanol **146-3** (2.6 g, 57 %) as a yellow oil. GC-MS: *m/z* 114 (5) (M^+), 95 (15), 88 (100), 81 (15).

Step 4. Preparation of 2-amino-4-(hydroxymethyl)cyclopentan-1-ol 146-4

To a solution of 6-oxabicyclo[3.1.0]hexan-3-ylmethanol **146-3** (2.0g, 17.6 mmol) in methanol (20 mL) at 0°C was purged ammonia gas for 10 min. The reaction was stirred at room temperature overnight. TLC indicated the incompletion of the reaction. Methanol was

removed and $NH_3 \cdot H_2O$ (50 mL) was added and this was stirred at room temperature over a week. TLC confirmed the completion of the reaction. Water was removed by azeotropically with ethanol to afford 2-amino-4-(hydroxymethyl)cyclopentanol **146-4** (2.1 g, 91%) as a yellow oil.

Step 5. Preparation of Methyl 12-(2-hydroxy-4-(hydroxymethyl)cyclopentylamino)-12-oxododecanoate 146-5

Compound **146-5** was prepared from 2-amino-4-(hydroxymethyl)cyclopentanol **146-4** and 1-(2,5-dioxopyrrolidin-1-yl) 12-methyl dodecanedioate **146-1**, using the same procedure as described in the synthesis of 12-(2-(*tert*-butoxycarbonylamino)ethylamino)-12-oxododecanoate (3-2). Methyl 12-(2-hydroxy-4-(hydroxymethyl)cyclopentylamino)-12-oxododecanoate **146-5** was obtained in 87.4% yield as an off-white solid.

Step 6. Preparation of compound 147

Compound **147** was prepared quantitatively from compound **146** (1.4 g, 2.33mmol) using an identical procedure to that used for compound **18**.

Step 7. Preparation of compound 148

Compound **148** was prepared from compound **147** (150mg, 0.23mmol) and compound **14** (431mg, 0.23mmol) using an identical procedure to that used for compound **19**. Yield: 460mg, 84%.

Step 8. Preparation of compound 149

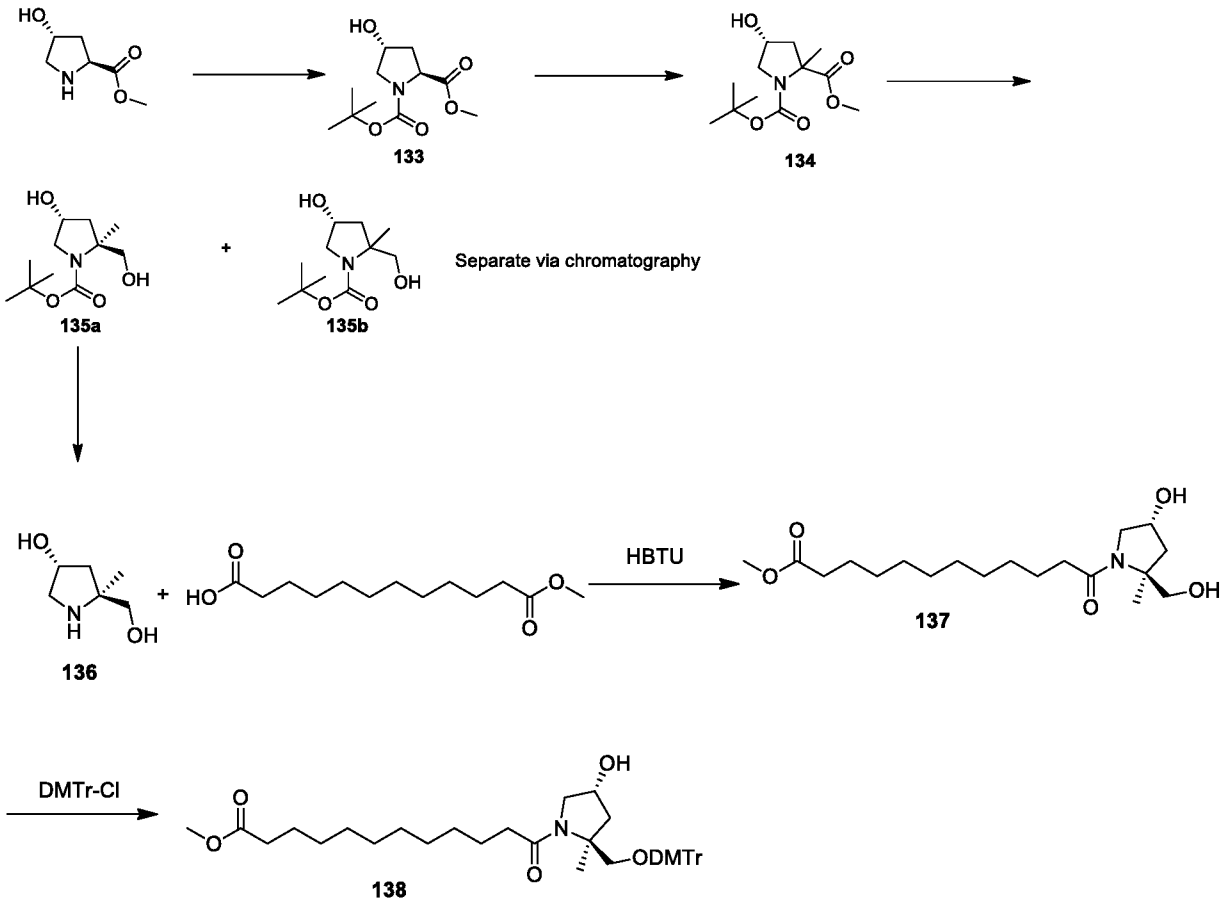
Compound **149** was prepared from compound **148** (460mg, 0.19mmol) using an identical procedure to that used for compound **20**. Yield: 436mg, 91%.

Step 9. Preparation of conjugate 150

Compound **150** was prepared from compound **149** (436mg) and 1000A lcaa CPG (2.62g) using an identical procedure to that used for compound **1**. Yield: 2.7g, 21.3μmol/g CPG loading. The resulting GalNAc loaded CPG solid support was employed in automated oligonucleotide synthesis using standard procedures. Nucleotide deprotection followed by removal from the solid support (with concurrent galactosamine acetate deprotection) afforded the GalNAc-oligonucleotide conjugate **150.**

Example 11. Synthesis of conjugates 153, 158, 163, 168 and 173

Scheme 22.



Step 1. Preparation of 1-(tert-butyl) 2-methyl (2S,4R)-4-hydroxypyrrolidine-1,2-dicarboxylate (133)

Methyl (2S,4R)-4-hydroxypyrrolidine-2-carboxylate (25.9 g, 46 mmol), BOC anhydride (65.9 g, 302.5 mmol) and TEA (42 ml, 302.5 mmol) were stirred in DCM at RT for 16 h. The organics were washed sequentially with 1M HCl (x2), saturated $NaHCO_3$ (x2), H_2O and brine, dried and concentrated in-vacuo to give 1-(tert-butyl) 2-methyl (2S,4R)-4-hydroxypyrrolidine-1,2-dicarboxylate (**133**) (58.1g, 85%).

Step 2. Preparation of 1-(tert-butyl) 2-methyl (4R)-4-hydroxy-2-methylpyrrolidine-1,2-dicarboxylate (134)

1-(tert-butyl) 2-methyl (2S,4R)-4-hydroxypyrrolidine-1,2-dicarboxylate (**133**) (5g, 20.4 mmol) and MeI (12 g, 84.5 mmol) were stirred in anhydrous THF at -40°C. LDA (2.0 M solution in THF) (37.5 mL, 75 mmol) was added dropwise. The reaction was allowed to warm to RT and stirred for 4 h then quenched with saturated NH_4Cl. The reaction was extracted with

EtOAc, washed with H_2O and brine, dried (Na_2SO_4) and concentrated in-vacuo. The residue was purified by column chromatography 50:50 EtOAc//hexanes to give 1-(tert-butyl) 2-methyl (4R)-4-hydroxy-2-methylpyrrolidine-1,2-dicarboxylate **(134)** as a racemic mixture (3.6 g, 68%)

Step 3. Preparation of tert-butyl (2S,4R)-4-hydroxy-2-(hydroxymethyl)-2-methylpyrrolidine-1-carboxylate (135a)

1-(Tert-butyl) 2-methyl (4R)-4-hydroxy-2-methylpyrrolidine-1,2-dicarboxylate **(134)** (19g, 73.5 mmol) was stirred in anhydrous THF under N_2. $LiBH_4$ solution (48 ml, 96 mmol) was added dropwise and the reaction stirred at RT for 48 h. The reaction was quenched with 1M NaOH, the THF removed in-vacuo and the residual extracted with EtOAc (4 x 100ml). The organics were washed with H_2O and brine, dried (Na_2SO_4) and concentrated in-vacuo. The residue was purified by column chromatography (5% MeOH/DCM) to give tert-butyl (2S,4R)-4-hydroxy-2-(hydroxymethyl)-2-methylpyrrolidine-1-carboxylate (**135a**) as the major product (8g, 47%). Structure assigned according to literature references.

Step 4. Preparation of (3R,5S)-5-(hydroxymethyl)-5-methylpyrrolidin-3-ol hydrochloride (136)

tert-Butyl (2S,4R)-4-hydroxy-2-(hydroxymethyl)-2-methylpyrrolidine-1-carboxylate (**135a**) (8g, 34.6 mmol) was stirred in EtOAc at RT and gaseous HCl applied for approximately two minutes. The reaction was stirred for one hour then concentrated in-vacuo and dried under high vacuum to give (3R,5S)-5-(hydroxymethyl)-5-methylpyrrolidin-3-ol hydrochloride **(136)** in quantitative fashion.

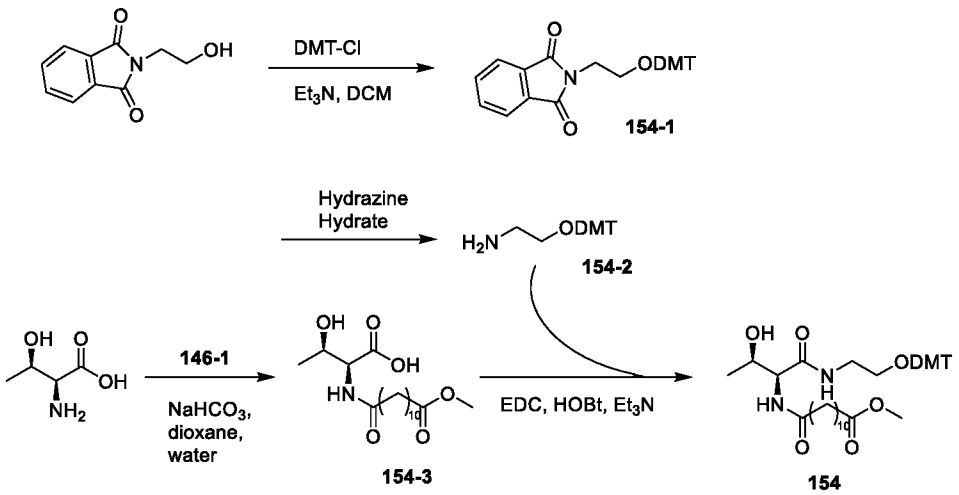
Step 5. Preparation of methyl 12-((2S,4R)-4-hydroxy-2-(hydroxymethyl)-2-methylpyrrolidin-1-yl)-12-oxododecanoate (137)

(3R,5S)-5-(Hydroxymethyl)-5-methylpyrrolidin-3-ol hydrochloride **(136)** (7.9 g, 47.4 mmol), 12-methoxy-12-oxododecanoic acid (11.5 g, 47.4 mmol), HBTU (36 g, 76 mmol) and TEA 20 mL, 142.2 mmol) were stirred in DCM at RT for 16h. The precipitate was removed by filtration and the organics washed with 1M HCl (x2), saturated $NaHCO_3$ (x2), H_2O and brine. After drying the organics were concentrated in-vacuo and purified by column chromatography (5%MeOH/DCM) to give methyl 12-((2S,4R)-4-hydroxy-2-(hydroxymethyl)-2-methylpyrrolidin-1-yl)-12-oxododecanoate (**137**) (3.1 g, 18.3 %).

Step 6. Preparation of methyl 12-((2S,4R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)-methyl)-4-hydroxy-2-methylpyrrolidin-1-yl)-12-oxododecanoate (138)

Methyl 12-((2S,4R)-4-hydroxy-2-(hydroxymethyl)-2-methylpyrrolidin-1-yl)-12-oxododecanoate (**137**) (3.1 g, 9.0 mmol), DMTr-Cl (2.8 g, 8.2 mmol) and TEA (1.1 ml, 8.2 mmol) were stirred in DC< at RT for 16 h. The reaction was concentrated in-vacuo and the residue purified by column chromatography (5% MeOH/DCM, 0.1%TEA) to give methyl 12-((2S,4R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxy-2-methylpyrrolidin-1-yl)-12-oxododecanoate (**138**) (2.7 g, 45.5 mmol).

Scheme 23



Step 7. Preparation of Compound 154-1

To a solution of *N*-(2-hydroxyethyl)phthalimide (4.80 g, 25.0 mmol) and 4,4'-dimethoxytrityl chloride (8.8 g, 26.0 mmol) in dichloromethane (200 mL) at 0°C under nitrogen, was added triethylamine (10.4 mL, 74.6 mmol) dropwise. The reaction mixture was stirred at room temperature for 3 hrs. TLC indicated the completion of the reaction. The organic layer was washed with brine (100 mL), dried over $MgSO_4$, and concentrated to dryness. This was used directly for the next reaction without purification.

Step 8. Preparation of Compound 154-2

2-(2-(Bis(4-methoxyphenyl)(phenyl)methoxy)ethyl)isoindoline-1,3-dione (**154-1**) obtained above and hydrazine monohydrate (3.6 mL, 74 mmol) in ethanol (100 mL) was stirred overnight at room temperature. TLC indicated the completion of the reaction. The precipitate was filtered out. The filtrate was evaporated. The residue was taken up by ethyl

acetate (100 mL). The organic solution was washed with 10% NaOH, water and brine, and dried over $MgSO_4$. Evaporation of solvent afforded 2-(bis(4-methoxyphenyl)(phenyl)methoxy)ethanamine (**154-2**) as a yellow liquid (8.11g, 89.3% yield over two steps). This was used for the next reaction without further purification.

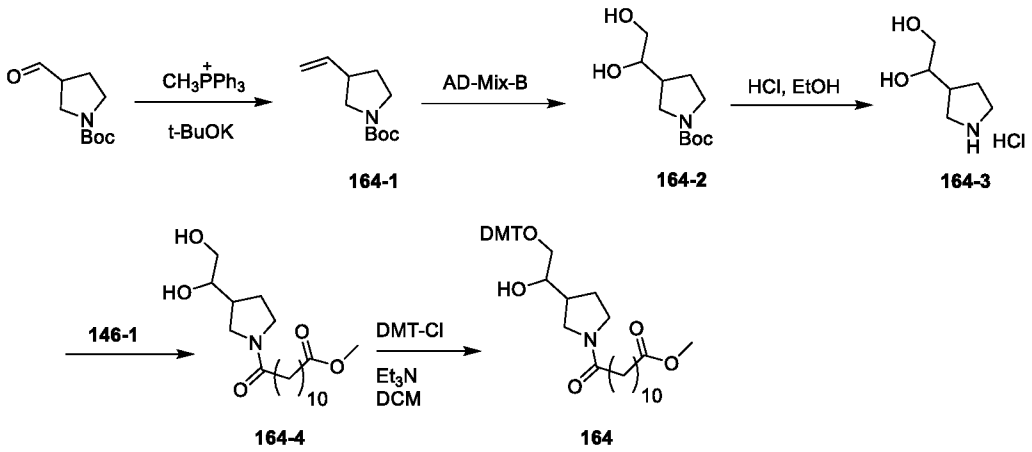
Step 9. Preparation of Compound 154-3

To a solution of L-threonine (1.19g, 10.0 mmol) and $NaHCO_3$ (2.3g, 27 mmol) in water (20 mL) and dioxane (10 mL), was added 1-(2,5-dioxopyrrolidin-1-yl) 12-methyl dodecanedioate **146-1** (3.1g, 9.1 mmol) in dioxane (10 mL) dropwise. The reaction mixture was stirred at room temperature overnight. 4N HCl (10 mL) was added. The precipitate was collected by filtration and washed with water (3 x 10 mL). The solid was dried over P_2O_5 in a desiccator to afford (2S,3R)-3-hydroxy-2-(12-methoxy-12-oxododecanamido)butanoic acid **154-3** as an off-white solid (2.84g, 82.2%). LC-MS (ESI): *m/z*: 346 (100), ($M + H^+$).

Step 10. Preparation of Compound 154

(2S,3R)-3-hydroxy-2-(12-methoxy-12-oxododecanamido)butanoic acid **154-3** (2.47g, 7.15 mmol), 2-(bis(4-methoxyphenyl)(phenyl)methoxy)ethanamine **154-2** (2.60g, 7.15 mmol), EDC (1.64g, 8.58 mmol), 1-hydroxybenzotriazole (HOBt) (1.16g, 8.58 mmol) and TEA (2.4 mL, 17.2 mmol) were stirred in dichloromethane (72 mL) at room temperature for 2 hrs. Water (30 mL) was added. The organic layer was separated and washed with brine (2 x30 mL). Evaporation of solvent followed by column chromatography (30% ethyl acetate/hexanes -50% ethyl acetate/hexanes) afforded methyl 12-((2S,3R)-1-(2-(bis(4-methoxyphenyl)(phenyl)methoxy)ethylamino)-3-hydroxy-1-oxobutan-2-ylamino)-12-oxododecanoate **154** as a waxy yellow semi-solid (2.60g, 52.6%). ^{1}HNMR (400MHz, acetone-d6, ppm): δ 7.51 (t, J = 5.5 Hz, 1H), 7.45-7.49 (m, 2H), 7.28-7.36 (m, 6H), 7.21 (tt, J = 7.2, 1.2 Hz, 1H), 7.08 (d, J = 8.1 Hz, 1H), 6.88 (dt, J = 8.9, 2.5 Hz, 4H), 4.39 (dd, J = 8.2, 3.0 Hz, 1H), 4.20-4.27 (m, 1H), 3.78 (s, 6H), 3.60 (s, 1H), 3.35-3.52 (m, 2H), 3.07-3.16 (m, 2H), 2.23-2.37 (m, 4H), 1.53-1.65 (m, 4H), 1.23-1.36 (m, 12H), 1.10 (d, J = 6.4 Hz, 3H).

Scheme 24



Step 11. Preparation of Compound 164-1

To a suspension of potassium t-butoxide (14.6 g, 130 mol) in THF (120 mL)/ether (360 mL) was added methyltriphenylphosphonium bromide (46.6 g,130 mmol). The mixture was refluxed for 2 hrs and then cooled to 0°C. *tert*-butyl 2-formylpyrrolidine-1-carboxylate (13.0g, 65.2 mmol) in ether (50 mL) was added dropwise. The reaction mixture was stirred at 0°C and then quenched by the addition of water (250 mL). The organic layer was separated and the aqueous was extracted with ether (250 mL). The combined extract was dried over $MgSO_4$. Evaporation of solvent, followed by column chromatography purification (5% ethyl acetae/hexanes) gave *tert*-butyl 3-vinylpyrrolidine-1-carboxylate **164-1** (11.5g, 89.4%) as a colorless liquid. GC-MS: *m/z*: 197 (2) (M^+), 141 (40), 124 (30), 57 (100).

Step 12. Preparation of Compound 164-2

To a mixture of *t*-BuOH (140 mL) and water (70 mL), was charged AD-mix-β (47.4 g) and methanesulfonamide (2.89 g, 30.4 mmol). The mixture was stirred at room temperature for 30 min and was then cooled to 0°C. *tert*-Butyl 3-vinylpyrrolidine-1-carboxylate **164-1** (6.00g, 30.4 mmol) was added. The reaction was stirred at room temperature overnight. The reaction mixture was cooled to 0°C. Sodium thiosulfate pentahydrate (96 g, 387 mmol) was added and the temperature was allowed to warm to room temperature. Water (700mL) was added and the mixture was extracted with ethyl acetate (500 mL). The extract was washed with water (2 x 50 mL) and brine (50 mL), and dried over $MgSO_4$. Evaporation of solvent, followed by column chromatography (2% methanol/dichloromethane - 7% methanol/dichloromethane) gave *tert*-butyl 3-(1,2-dihydroxyethyl)pyrrolidine-1-carboxylate **164-2** (5.4 g, 77%) as a light brown oil.

Step 13. Preparation of Compound 164-3

To a solution of *tert*-butyl 3-(1,2-dihydroxyethyl)pyrrolidine-1-carboxylate **164-2** (3.1g, 13.4 mmol) in ethanol (10 mL) was added 3N HCl (30 mL, 90 mmol). The reaction mixture was stirred at room temperature overnight. TLC indicated the completion of the reaction. Ethanol was evaporated. Toluene was added and evaporated. This was repeated three times to give 1-(pyrrolidin-3-yl)ethane-1,2-diol hydrochloride **164-3** (2.0g, 89%) as a brown oil. LC-MS (ESI): *m/z*: 132 (100), ($M + H^+$, free amine).

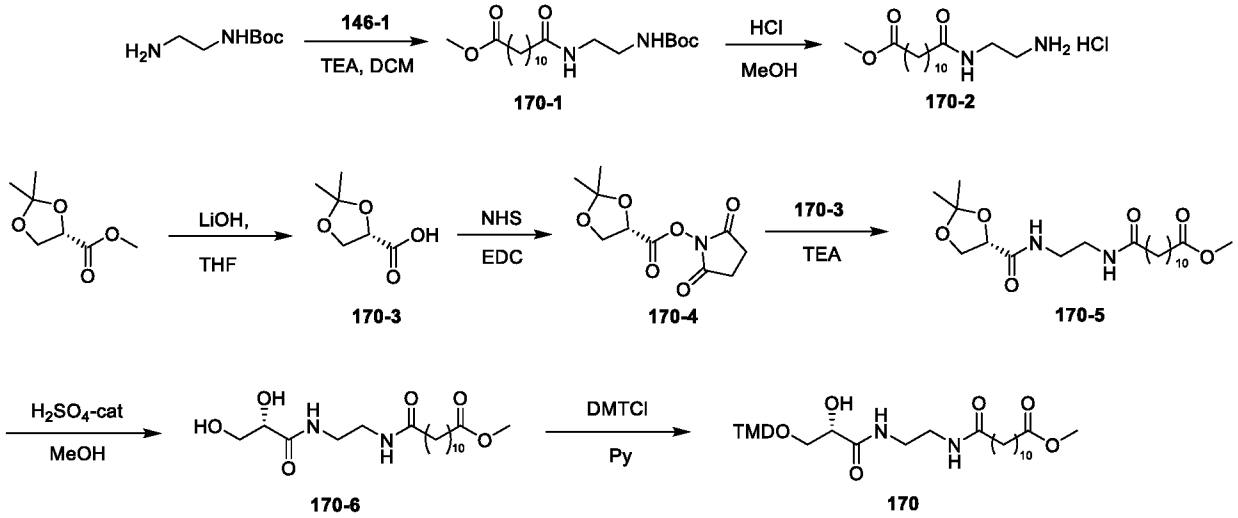
Step 14 Preparation of Compound 164-4

To a solution of 1-(pyrrolidin-3-yl)ethane-1,2-diol hydrochloride **164-2** (2.0g, 12 mmol) in water (30 mL) was added $NaHCO_3$ (3.7g, 44 mmol) by portion. Dioxane (20 mL) was then added. To the above solution was added 1-(2,5-dioxopyrrolidin-1-yl) 12-methyl dodecanedioate **146-1** (3.7g, 11 mmol) in dioxane (30 mL). The reaction mixture was stirred overnight. This was extracted with ethyl acetate (3 x100 mL). The combined extract was washed with 0.5N HCl (50 mL) and brine (50 mL), and dried over $MgSO_4$.

Step 15. Preparation of Compound 164

This substance was prepared from methyl 12-(3-(1,2-dihydroxyethyl)pyrrolidin-1-yl)-12-oxododecanoate **164-4** and 4,4-dimethoxytrityl chloride (1 eq) using the same procedure as described in the synthesis of 2-(2-(bis(4-methoxyphenyl)(phenyl)methoxy)ethyl)isoindoline-1,3-dione **138**. The product was purified by column chromatography (1.5% methanol/dichloromethane). Methyl 12-(3-(2-(bis(4-methoxyphenyl)(phenyl)methoxy)-1-hydroxyethyl)pyrrolidin-1-yl)-12-oxododecanoate **164** was obtained in 51% yield as a yellow oil. ^{1}HNMR (400MHz, acetone-d6, ppm): δ 7.49-7.54 (m, 2H), 7.35-7.40 (m, 4H), 7.28-7.34 (m, 2H), 7.19-7.25 (m, 1H), 6.86-6.91 (m, 4H), 4.11-4.20 (m, 1H), 3.79 (s, 6H), 3.68-3.77 (m, 1H), 3.60 (s, 3H), 3.29-3.59 (m, 3H), 3.06-3.20 (m, 3H), 2.33-2.55 (m, 1H), 2.29 (t, J = 7.4 Hz, 2H), 2.19 (t, J = 7.6 Hz, 2H), 1.65-2.0 (m, 2H), 1.51-1.62 (m, 4H), 1.26-1.35 (m, 12H).

Scheme 25



Step 16. Preparation of Compound 170-1

To a solution of *tert*-butyl 2-aminoethylcarbamate (2.88g, 18.0 mmol) and triethylamine (2.98g, 29.4 mmol) in dichloromethane (100 mL), was added 1-(2,5-dioxopyrrolidin-1-yl) 12-methyl dodecanedioate (**146-1**) (5.09g, 14.9 mmol) in dichloromethane (50 mL) dropwise at room temperature. The reaction mixture was stirred overnight and TLC indicated the completion of the reaction. 100 mL brine was added and the organic layer was separated. The organic layer was washed with 0.5N HCl (150 mL), brine (2 x 100 mL) and dried over $MgSO_4$. Evaporation of solvent gave pure methyl 12-(2-(*tert*-butoxycarbonylamino)ethylamino)-12-oxododecanoate **170-1** (5.85g 100%) as a white solid.

Step 17. Preparation of Compound 170-2

To a solution of 12-(2-(*tert*-butoxycarbonylamino)ethylamino)-12-oxododecanoate **170-1** (5.55g, 14.4 mmol) in methanol (100 mL) at 0°C, was added thionyl chloride (3.3 mL, 45.5 mmol) dropwise. The reaction was then stirred at room temperature overnight. TLC indicated the completion of the reaction. The solvent and volatile organics were evaporated. The residue was then co-evaporated with heptanes twice to give methyl 12-(2-aminoethylamino)-12-oxododecanoate hydrochloride **170-2** quantitatively as a white solid. LC-MS (ESI): *m/z*: 287 (100), ($M + H^+$, free amine).

Step 18. Preparation of Compound 170-3

(-)-Methyl (S)-2,2-dimethyl-1,3-dioxolane-4-carboxylate (5.01g, 31.2 mmol) and $LiOH{\cdot}H_2O$ (2.55g, 60.8 mmol) in THF (50 mL) and water (50 mL) was stirred overnight. TLC indicated the completion of the reaction. THF was evaporated and the aqueous was acidified

with 1N HCl to pH = 1. This was extracted with ethyl acetate (5 x 50 mL). The combined extract was dried over $MgSO_4$. Evaporation of solvent gave (S)-2,2-dimethyl-1,3-dioxolane-4-carboxylic acid **170-3** (2.93g, 64.3%) as a light yellow liquid.

Step 19. Preparation of Compound 170-4

Compound **170-4** was synthesized from (S)-2,2-dimethyl-1,3-dioxolane-4-carboxylic acid **170-3** and N-hydroxysuccinimide in 86% yield, using the same procedure as described in the synthesis of 1-(2,5-dioxopyrrolidin-1-yl) 12-methyl dodecanedioate **146-1**. (S)-2,5-Dioxopyrrolidin-1-yl 2,2-dimethyl-1,3-dioxolane-4-carboxylate **170-4** was obtained in 86% yield as a white solid.

Step 20. Preparation of Compound 170-5

To a suspension of methyl 12-(2-aminoethylamino)-12-oxododecanoate hydrochloride **170-2** (14.4 mmol) and (S)-2,5-dioxopyrrolidin-1-yl 2,2-dimethyl-1,3-dioxolane-4-carboxylate **170-4** (3.80g, 15.6 mmol) in dichloromethane (100 mL) was added triethylamine (6 mL, 43.0 mmol) in dichloromethane (25 mL) over 4 hrs at 0°C. The reaction mixture was then stirred at room temperature overnight. LC-MS indicated that the starting material **170-2** was completely converted. The organic layer was washed with brine (50 mL), 1N HCl (50 mL), brine (50 mL), dried over $MgSO_4$ and concentrated to dryness to afford (S)-methyl 12-(2-(2,2-dimethyl-1,3-dioxolane-4-carboxamido)ethylamino)-12-oxododecanoate **170-5** (5.93g, 99.3%) as a white solid.

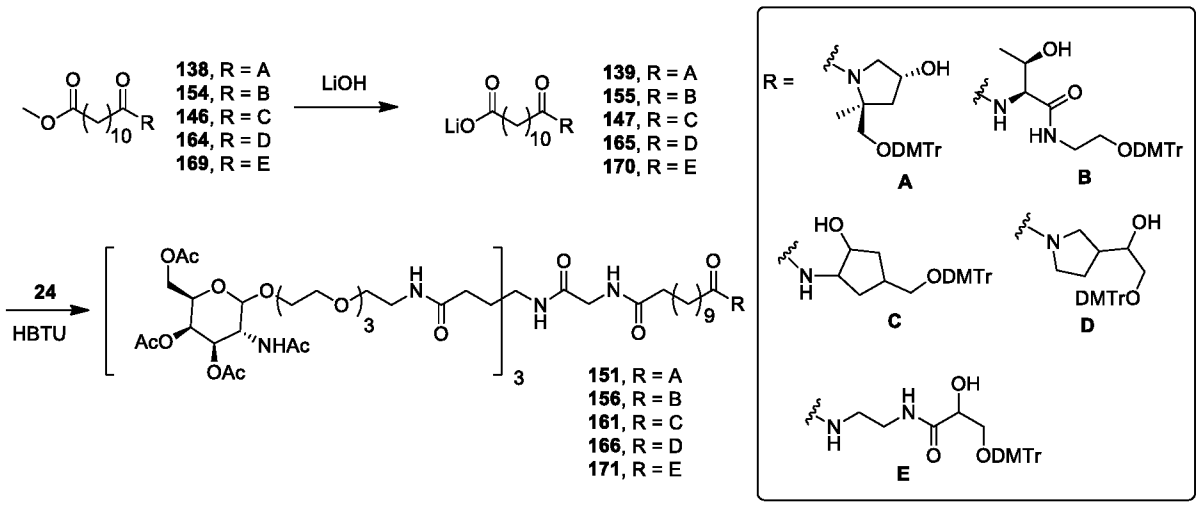
Step 21. Preparation of Compound 170-6

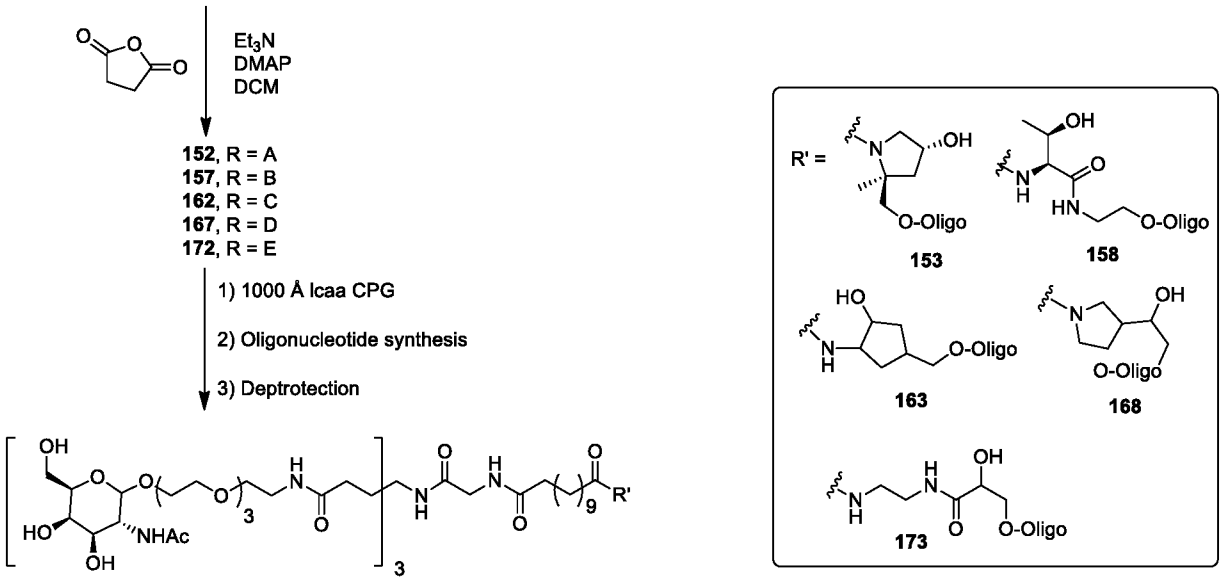
To a solution of (S)-methyl 12-(2-(2,2-dimethyl-1,3-dioxolane-4-carboxamido)ethylamino)-12-oxododecanoate **170-5** (5.93g, 14.3 mmol) was added one drop of concentrated sulfuric acid. This was refluxed for 6 hrs and then cooled to room temperature. The solid was collected through filtration and washed twice with cold methanol. The solid was dried in the air (3.32g). The second crop (0.42g) was obtained from the mother liquid to give (S)-methyl 12-(2-(2,3-dihydroxypropanamido)ethylamino)-12-oxododecanoate **170-6** (3.74g in total, 69.4%) as a white crystal. LC-MS (ESI): *m/z*: 375 (100), $(M + H^+)$. ^{1}HNMR (400MHz, DMSO-d6, ppm): δ 7.79 (br, 2H), 5.49 (d, J = 5.3 Hz, 1H), 4.66 (t, J = 5.8 Hz, 1H), 3.83-3.88 (m, 1H), 3.55-3.61 (m, 4H), 3.41-3.47 (m, 1H), 3.05-3.15 (m, 4H), 2.29 (t, J = 7.4 Hz, 2H), 2.03 (t, J = 7.6 Hz, 2H), 1.42-1.52 (m, 4H), 1.18-1.29 (m, 12H).

Step 22. Preparation of Compound 170

To a solution of (S)-methyl 12-(2-(2,3-dihydroxypropanamido)ethylamino)-12-oxododecanoate **170-6** (2.99g, 7.99 mmol) in dry pyridine (57.5 mL) under nitrogen, was added 4,4'-dimethoxytrityl chloride (2.84g, 8.38 mmol) in one portion. The reaction was stirred at room temperature for two days. Methanol (5 mL) was added to quench the reaction. Pyridine was evaporated. Toluene was added and then evaporated. This was repeated three times. Water (100 mL) was added and this was extracted with ethyl acetate (5 x 250 mL). The extracts were combined and dried over MgSO4. Evaporation of solvent, followed by column chromatography (1%methanol/dichloromethane-3% methanol/dichloromethane) gave (S)-methyl 12-(2-(3-(bis(4-methoxyphenyl)(phenyl)methoxy)-2-hydroxypropanamido)-ethylamino)-12-oxododecanoate **170** (1.70g, 31.4%) as a viscous oil. ^{1}HNMR (400MHz, acetone-d6, ppm): δ 7.64-7.70 (br, 1H), 7.47-7.51 (m, 2H), 7.33-7.37 (m, 4H), 7.26-7.32 (m, 2H), 7.20 (dt, J = 7.3, 2.1 Hz, 1H), 7.11 (br, 1H), 6.86 (d, J = 8.7 Hz, 4H), 4.84 (br, 1H), 4.21 (dd, J = 5.1, 3.8 Hz, 1H), 3.78 (s, 6H), 3.60 (s, 1H), 3.25-3.42 (m, 6H), 2.28 (t, J = 7.4 Hz, 2H), 1.48-1.62 (m, 4H), 1.21-1.34 (m, 12H).

Scheme 26.





Step 23. Preparation of compounds 139, 155, 160, 165 and 170

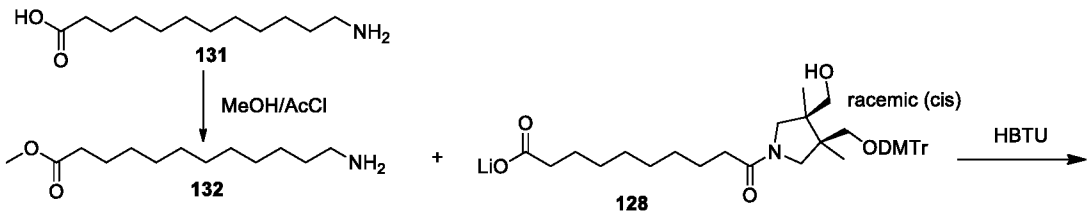
Compounds **139, 155**, **160**, **165** and **170** were prepared from compounds **138, 154**, **159**, **164** and **169** using an identical procedure to that used for compound **18**.

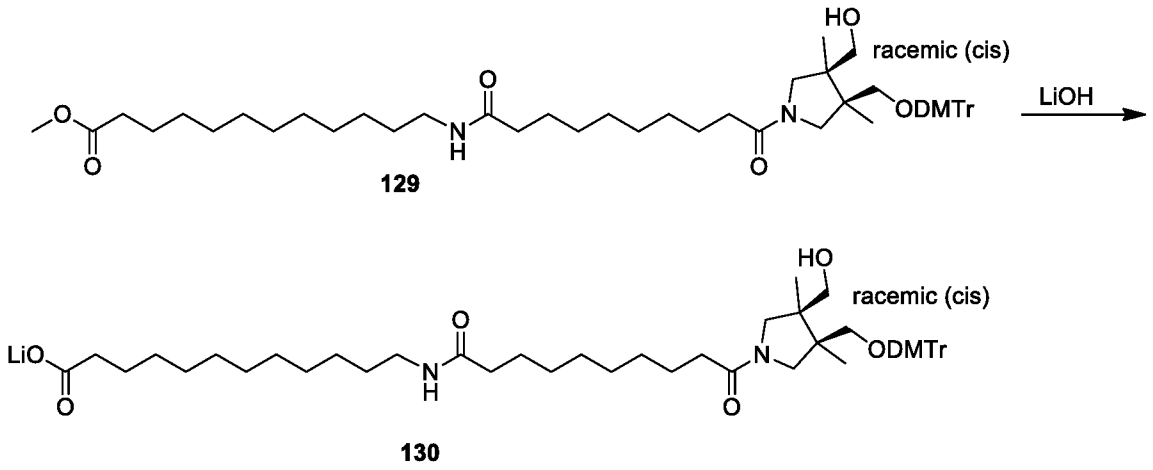
Step 24. Preparation of conjugates 153, 158, 163, 168 and 173

Conjugates **153, 158, 163, 168 and 173** were prepared from compound **139**, **154**, **159**, **164** and **169** using an identical procedure to that used for compound **1**.

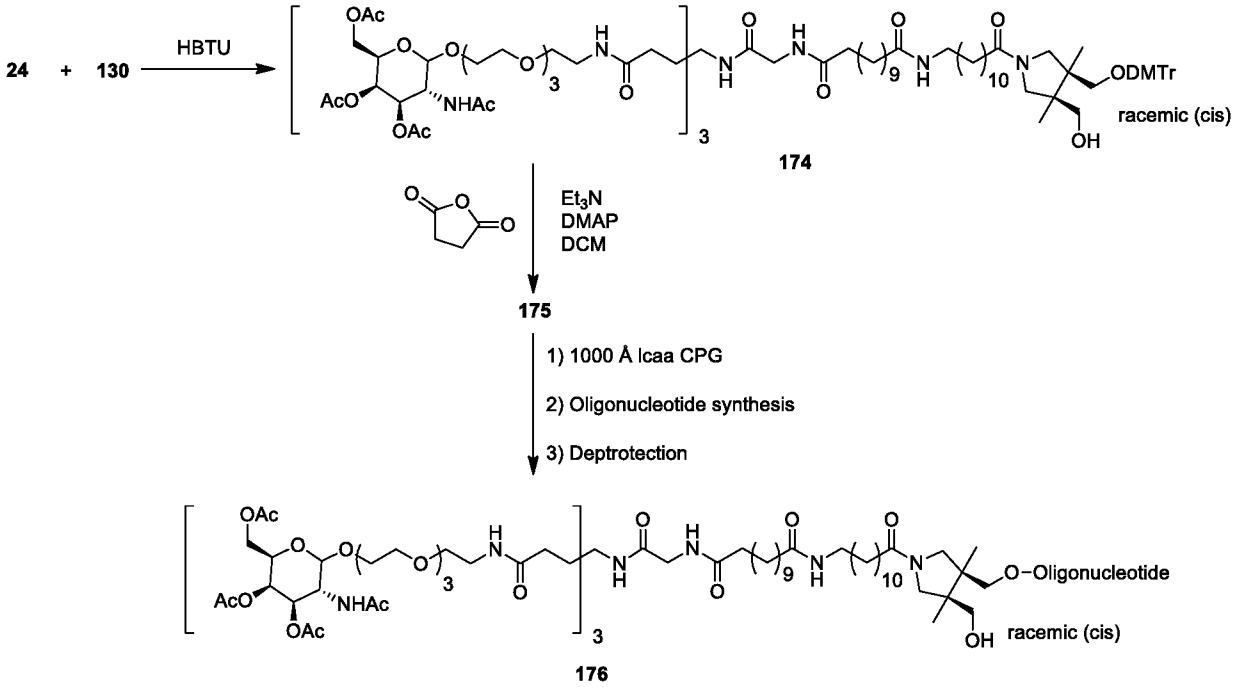
Example 12. Synthesis of conjugate 176

Scheme 27.





Scheme 28.



Step. 1. Preparation of methyl 12-aminododecanoate 132

12-aminoundecanoic acid (**131**) (10g, 4.64 mmol) was stirred in MeOH at RT. Acetyl chloride (856µL, 12 mmol) was added dropwise and the reaction stirred for 1.5 hr. The solvent was removed in-vacuo, the residue taken up in MTBE and chilled in the fridge overnight. The resultant precipitate was collected by filtration, washed with ice cold MTBE and dried under high vacuum to afford methyl 12-aminododecanoate **132**.

Step 2. Preparation of Racemic (cis) Methyl 12-(12-(10-(3-((bis(4-methoxyphenyl)-(phenyl)methoxy)methyl)-4-(hydroxymethyl)-3,4-dimethylpyrrolidin-1-yl)-10-oxodecanamido)dodecanamido)dodecanoate 129

Lithium racemic (cis) 10-(3-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-(hydroxymethyl)-3,4-dimethylpyrrolidin-1-yl)-10-oxodecanoate (**128**) (2g, 3.1 mmol), of methyl 12-aminododecanoate (**132**) (778 mg, 3.1 mmol), HBTU (1.2 g, 3.1 mmol) and TEA (1.4 mL, 10 mmol) were stirred in DCM at RT O/N. The precipitate was removed by filtration, the filtrate concentrated in-vacuo and the residue purified by column chromatography (5% MeOH, DCM). TLC showed two close running spots with identical mass that were assigned as geometric isomers and pooled together to give of Methyl 12-(12-(10-((3R,4S)-3-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-(hydroxymethyl)-3,4-dimethylpyrrolidin-1-yl)-10-oxodecanamido)dodecanamido)dodecanoate (**129**) in quantitative fashion.

Step 3. Preparation of Racemic (cis) Lithium 12-(12-(10-(-3-((bis(4-methoxyphenyl)(phenyl)-methoxy)methyl)-4-(hydroxymethyl)-3,4-dimethylpyrrolidin-1-yl)-10-oxodecanamido)-dodecanamido)dodecanoate 130

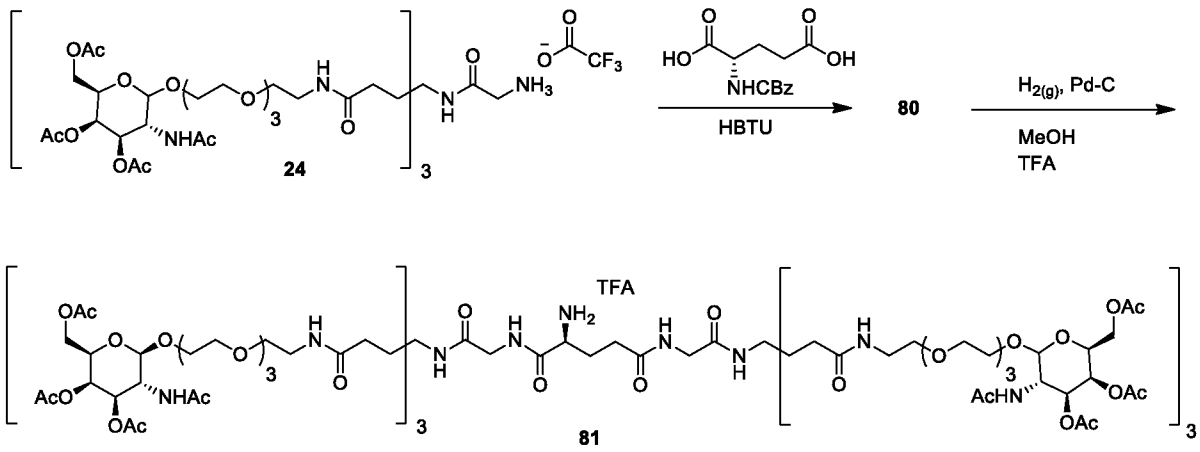
Racemic (cis) methyl 12-(12-(10-(3-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-(hydroxymethyl)-3,4-dimethylpyrrolidin-1-yl)-10-oxodecanamido)dodecanamido)dodecanoate (**129**) (3.1 mmol) was stirred in THF:H_2O (50:50) with LiOH (88 mg, 3.7 mmol) at RT O/N. Reaction was confirmed by TLC and the THF removed in-vacuo. The aqueous solution was frozen in liquid N_2 and lyophilized for 48 hours to give racemic (cis) Lithium 12-(12-(10-(3-((bis(4-methoxyphenyl)(phenyl)-methoxy)methyl)-4-(hydroxymethyl)-3,4-dimethylpyrrolidin-1-yl)-10-oxodecanamido)-dodecanamido)dodecanoate **130** quantitatively.

Step 4. Preparation of conjugate 176

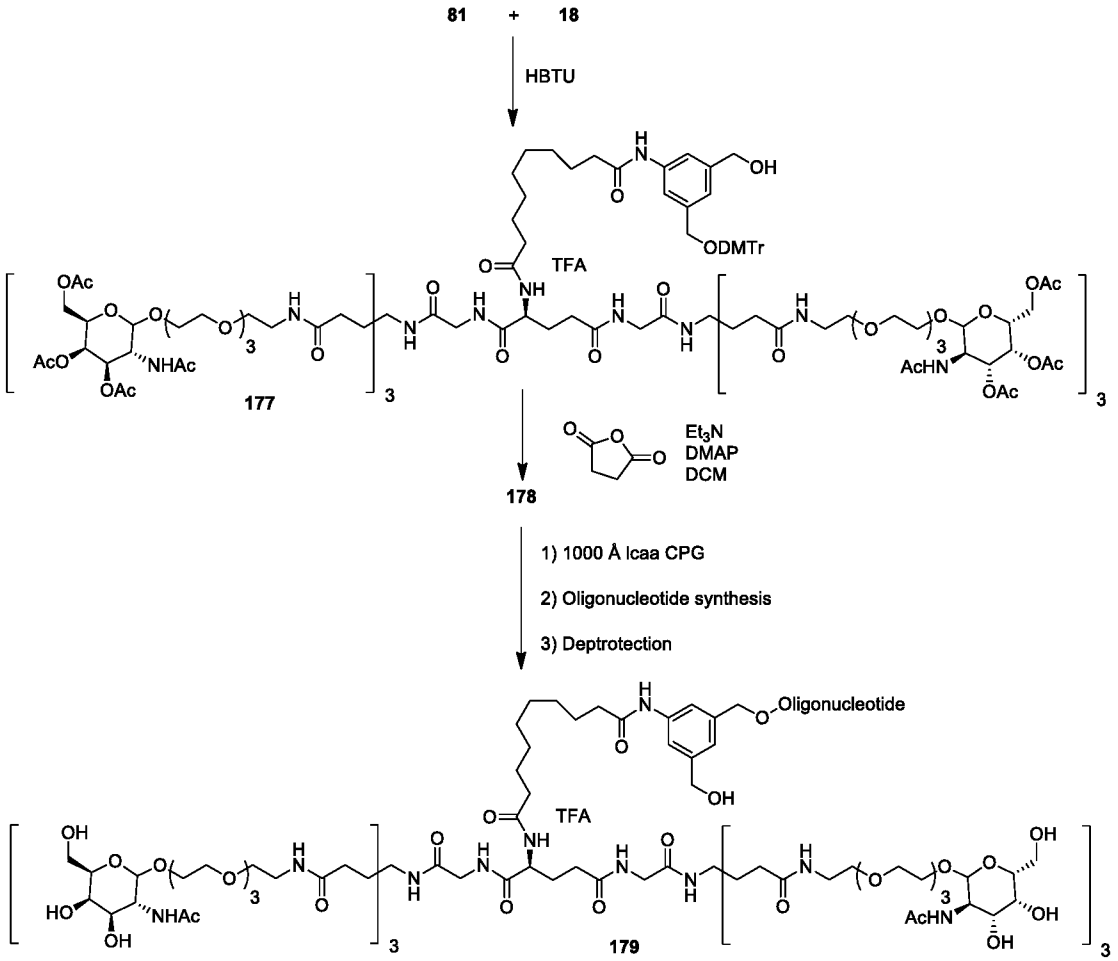
Conjugate **176** was prepared from compounds **24** and **130** using an identical procedure to that used for compound **1**.

Example 13. Synthesis of conjugate 179

Scheme 29.



Scheme 30.



Step 1. Preparation of compound 80

Compound **24** (2g, 0.86 mmol), N-carbobenzoxy-L-glutamic acid (120 mg, 0.43 mmol), HBTU (326 mg, 0.86 mmol) and TEA (353 µL, 2.6 mmol) were stirred in DCM at RT O/N. The mixture was concentrated in-vacuo and purified by column chromatography to give compound **80** (2.88 g, 83%).

Step 2. Preparation of compound 81

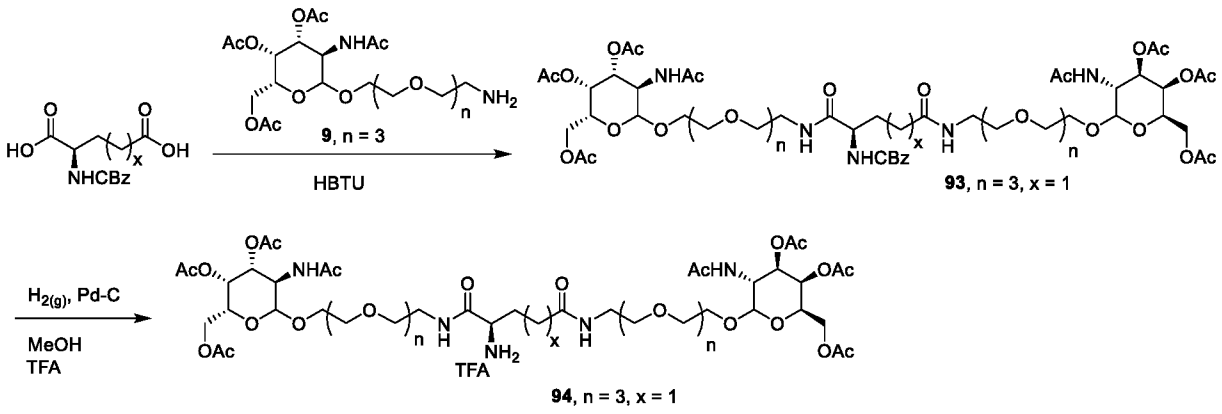
Compound **81** was prepared from compounds **80** (670 mg, 0.17 mmol) using an identical procedure to that used for compound **14**. The compound was used crude in subsequent reactions and the yield taken as quantitative.

Step 3. Preparation of conjugate179

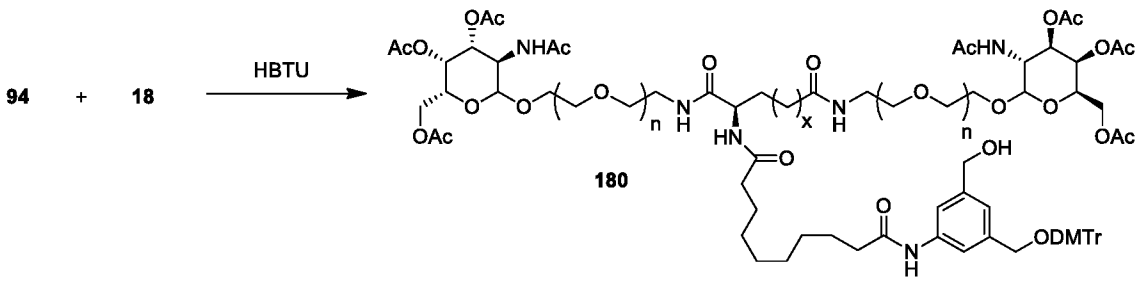
Conjugate **179** was prepared from compounds **18** and **81** using an identical procedure to that used for compound **1**.

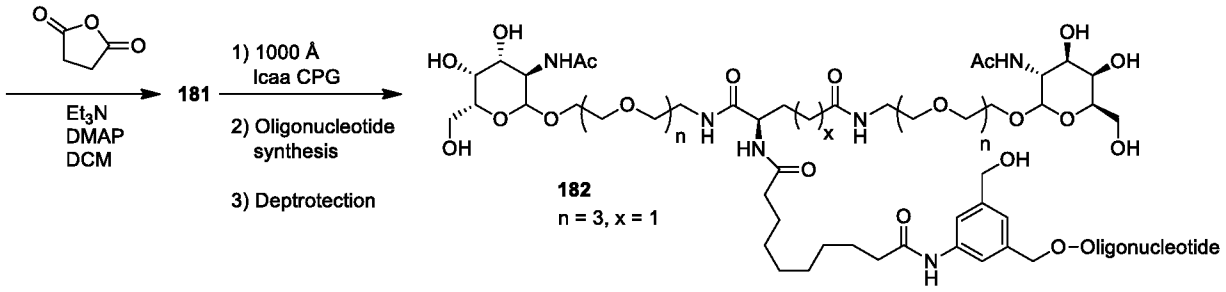
Example 14. Synthesis of conjugate 182

Scheme 31.



Scheme 32.





Step 1. Preparation of compound 93

Compound **93** was prepared from (2-oxo-2-phenyl-1λ^2-ethyl)-D-glutamic acid (2.25 g, 8.1 mmol) and **9** (13 g, 21 mmol) using an identical procedure to that used for compound **89**. Yield: 11.2 g.

Step 2. Preparation of compound 94

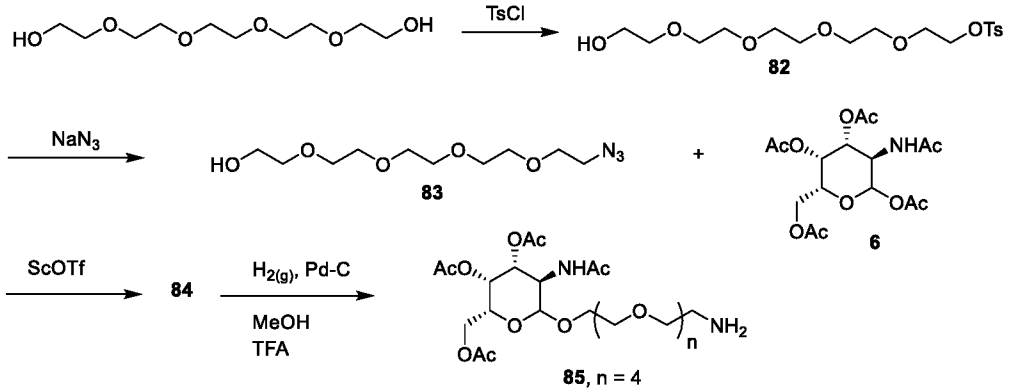
Compound **94** was prepared from compound **93** (11.1 g) using an identical procedure to that used for compound **90**. Yield: 10.2 g.

Step 3. Preparation of conjugate 182

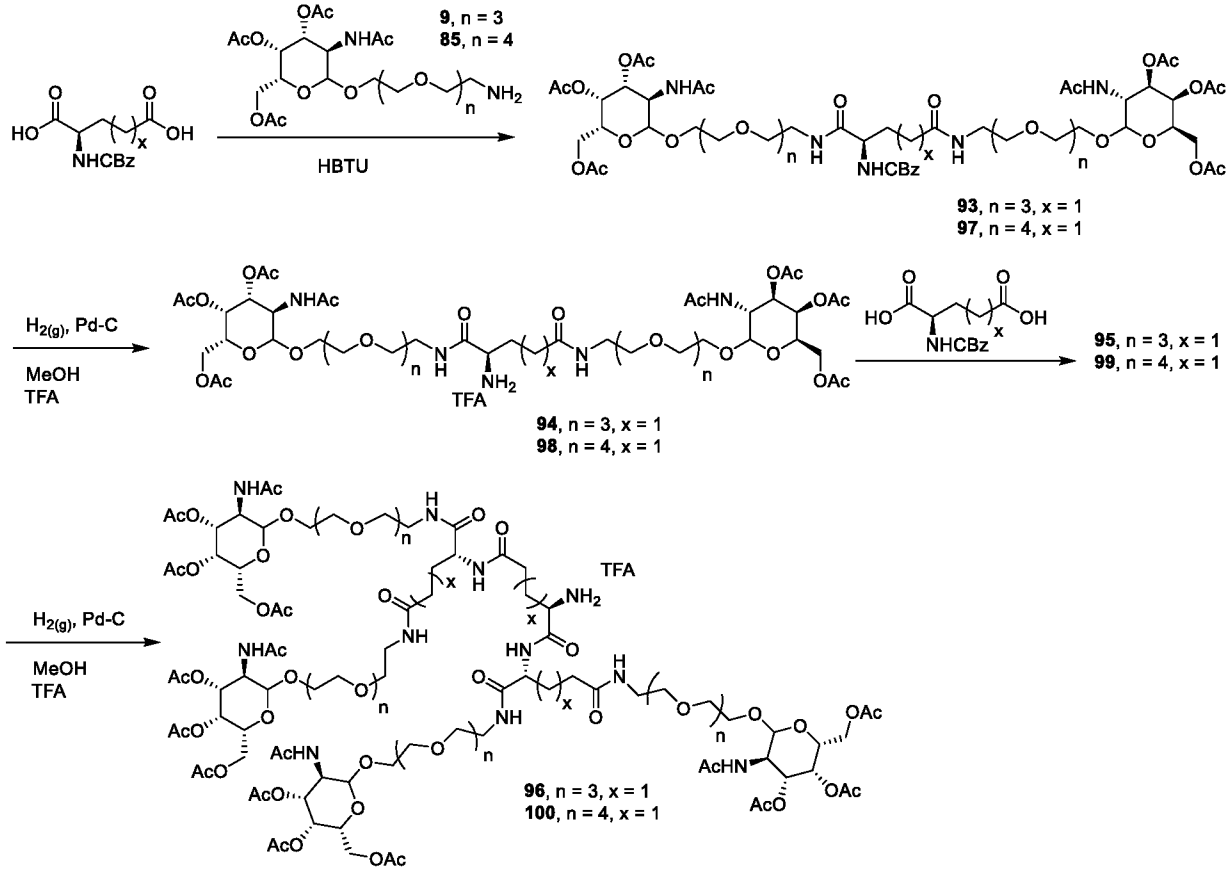
Conjugate **182** was prepared from compounds **18** and **94** using an identical procedure to that used for compound **1**.

Example 15. Synthesis of conjugates 185 and 188

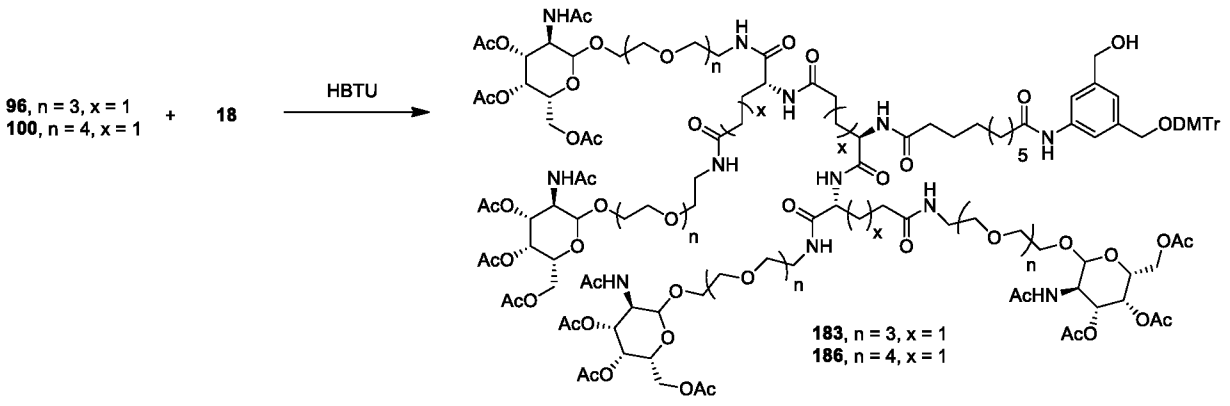
Scheme 33.

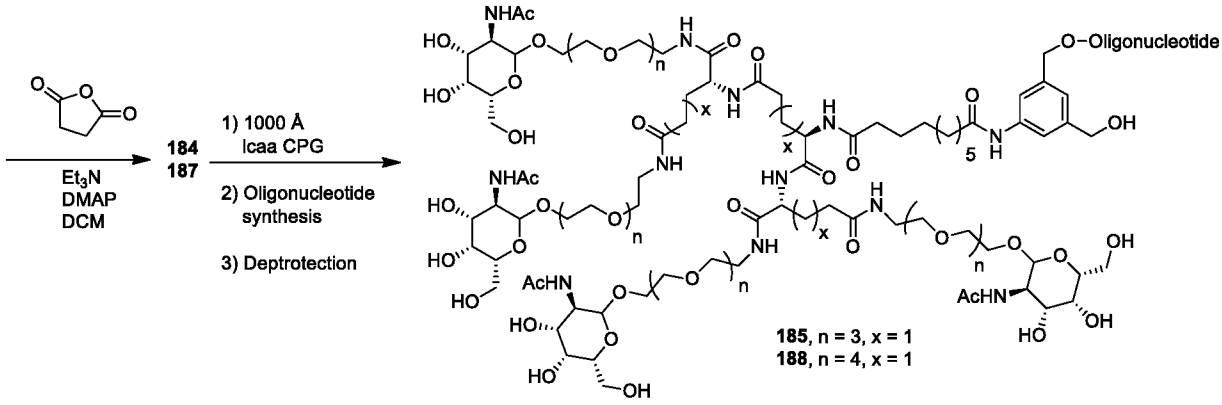


Scheme 34.



Scheme 35.





Step 1. Preparation of 14-Hydroxy-3,6,9,12-tetraoxatetradecyl 4-methylbenzenesulfonate 82

A solution of pentaethylene glycol (35g, 147mmol), TEA (41mL, 294mmol) and trimethylamine-HCl (1.4g, 14.7mmol) in CH_2Cl_2 (600mL) was treated with tosyl chloride (29.4g, 154mmol). After stirring (18h) the reaction mixture was washed with H_2O-brine (1:1), dried ($MgSO_4$), filtered, concentrated and subjected to chromatography to yield **82** (24.6g, 43%) as a pale yellow oil. Rf 0.8 (10% CH_3OH-CH_2Cl_2).

Step 2. 14-azido-3,6,9,12-tetraoxatetradecan-1-ol 83

14-azido-3,6,9,12-tetraoxatetradecan-1-ol **(83)** was prepared from **82** (24.6g, 62.7mmol) and sodium azide (7.13g, 110mmol) using an identical procedure to that used for compound **4**. Yield: 14.8g, 90%.

Step 3. Preparation of compound 84

A solution of GalNAc **6** (12.2g, 31.4mmol) and HO-PEG-N_3 **83** (9.2g, 35mmol) in 1,2-dichloroethane (150mL) was treated with $Sc(OTf)_3$ (771mg, 1.6mmol). After stirring (85°C, 2hr) the reaction was cooled (RT), quenched by the addition of TEA (40mL) and concentrated. The crude material was subjected to chromatography to yield **84** (11.16g, 60%) as a pale yellow foam. Rf 0.7 (10% CH_3OH-CH_2Cl_2).

Step 4. Preparation of compound 85

A solution of **84** (11.16g, 18.8mmol) and Pd/C (1.1g, 10% - wet support) in EtOAc (120mL) was treated with TFA (4.32mL, 56.5mmol) and purged with H_2. After stirring vigorously (4.5h) the reaction was purged with N_2, filtered through Celite and concentrated.

The crude material was subjected to chromatography to yield **85** (5.77g, 45%) as a colorless foam. Rf 0.5 (10% CH_3OH-CH_2Cl_2).

Step 5. Preparation of compound 95

Compound **95** was prepared from (2-oxo-2-phenyl-1λ^2-ethyl)-D-glutamic acid **(**1.04 g, 3.7 mmol) and compound **94 (**10.2 g) using an identical procedure to that used for compound **91**. Yield: 7.2 g.

Step 6. Preparation of compound 96

Compound **96** was prepared from compound **95 (**11.1 g) using an identical procedure to that used for compound **92**. Yield: 6.5 g.

Step 7. Preparation of compound 97

Compound **97** was prepared from (2-oxo-2-phenyl-1λ^2-ethyl)-D-glutamic acid (2g, 7.1mmol) and **85** (12.1g, 17.8mmol) using an identical procedure to that used for compound **89**. Yield: 10g, quantitative.

Step 8. Preparation of compound 98

Compound **98** was prepared from compound **97** (10g, 7.2mmol) using an identical procedure to that used for compound **90**. Yield: 3.5g, 36%.

Step 9. Preparation of compound 99

Compound **99** was prepared quantitatively from (2-oxo-2-phenyl-1λ^2-ethyl)-D-glutamic acid **(**350 mg, 1.25 mmol) and compound **98** (2.86 mg, 2.5mmol) using an identical procedure to that used for compound **91**.

Step 10. Preparation of compound 100

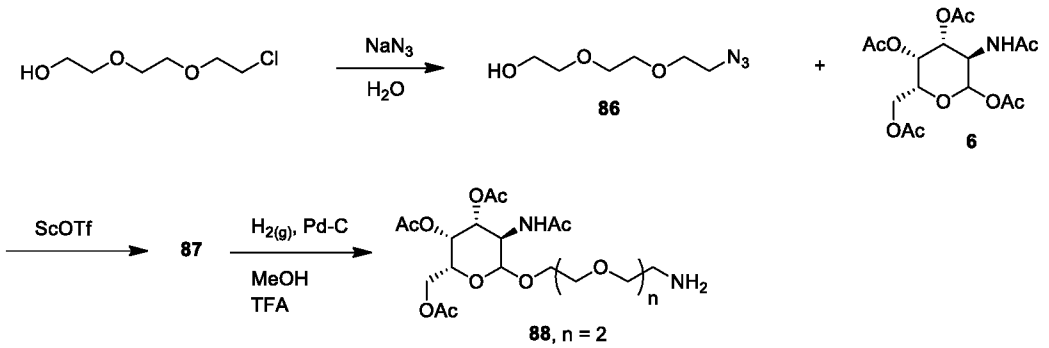
Compound **100** was prepared quantitatively from compound **99** (3.2 g, 1.25 mmol) using an identical procedure to that used for compound **92.**

Step 11. Preparation of conjugates 185 and 188

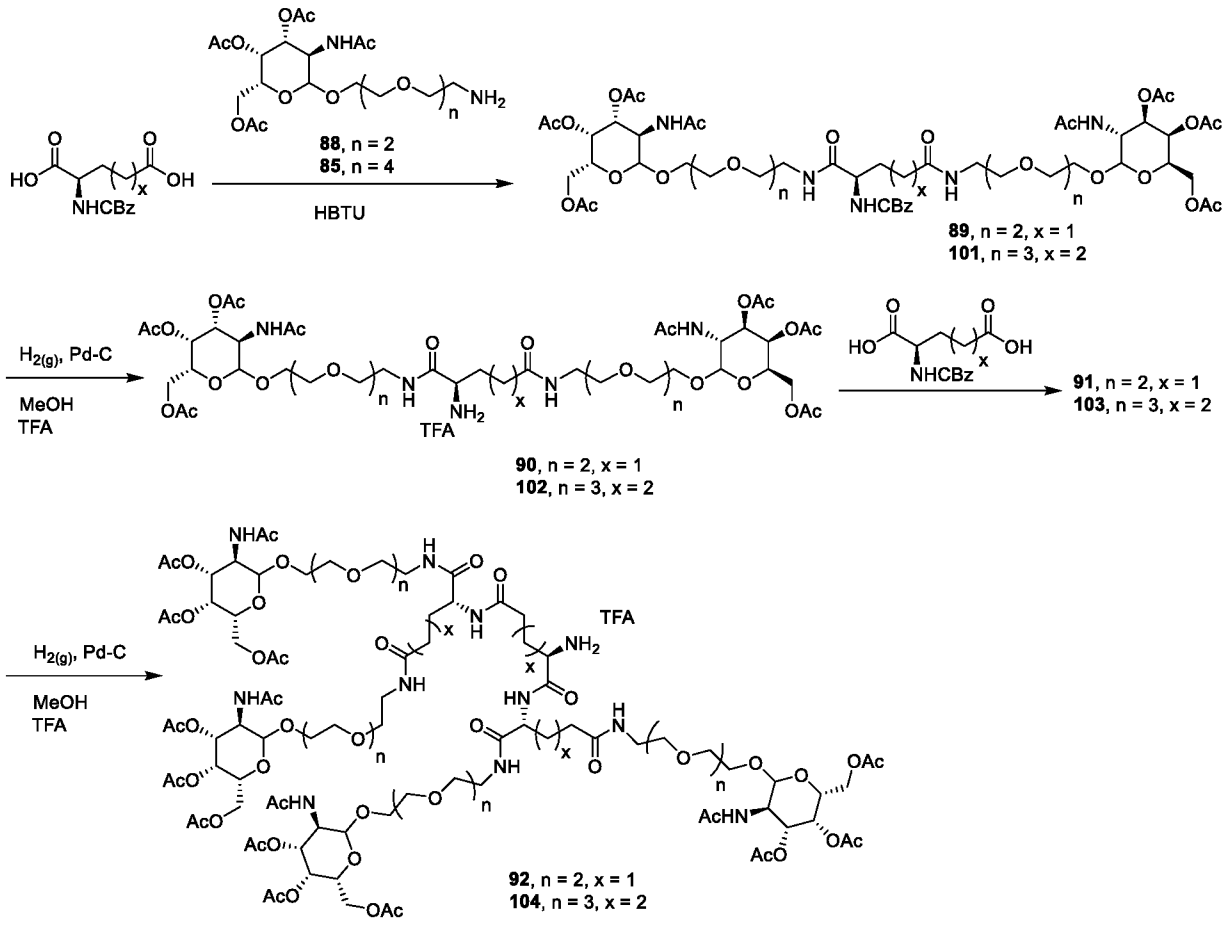
Conjugate **185** and **188** were prepared from compounds **18** and **96** or **18** and **100** using an identical procedure to that used for compound **1**.

Example 16. Synthesis of conjugates 191, 194, 197 and 200

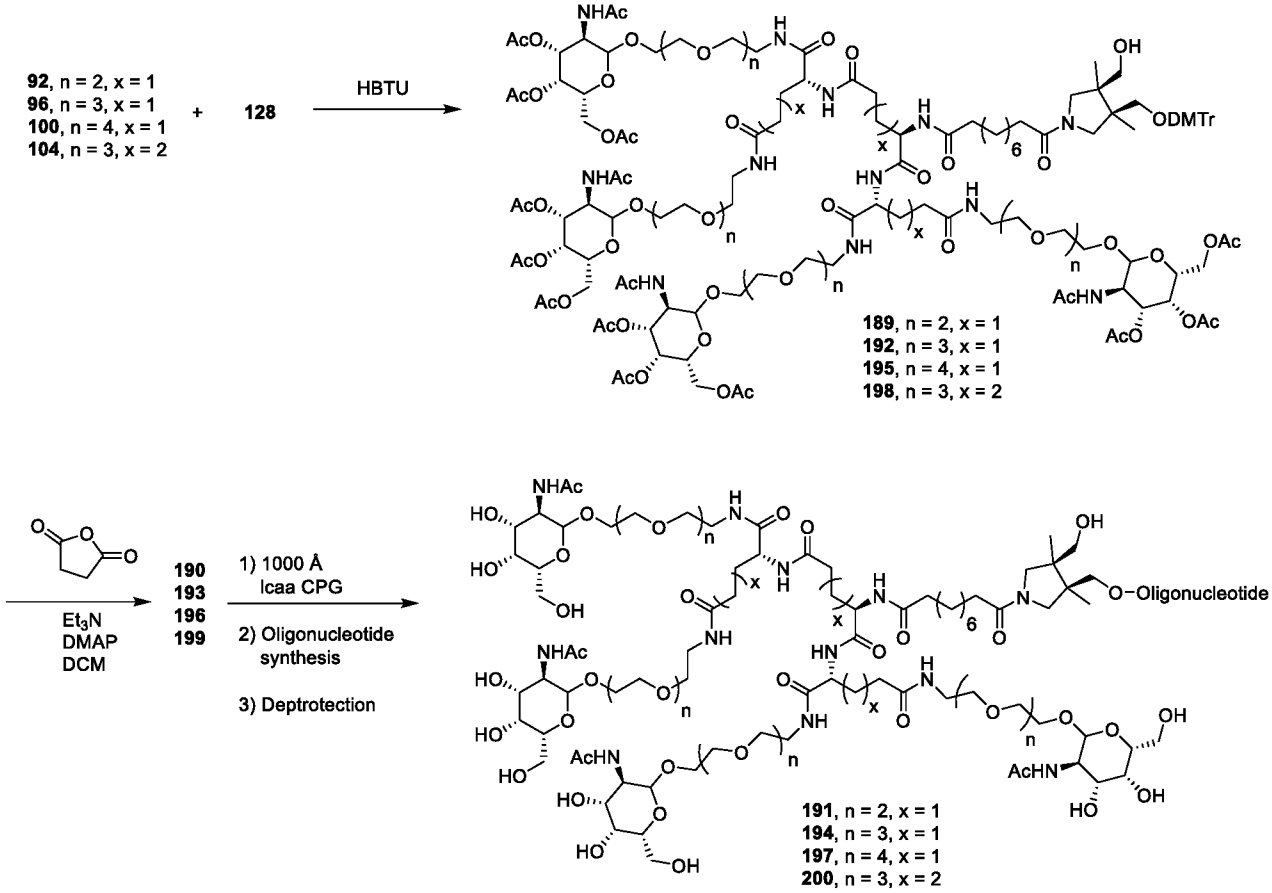
Scheme 36



Scheme 37.



Scheme 38.



Step 1. Preparation of 2-(2-(2-azidoethoxy)ethoxy)ethan-1-ol 86

To a solution of 2-(2-(2-chloroethoxy)ethoxy)ethan-1-ol (13 g, 77 mmol) in water (200 mL) is added sodium azide (10 g, 154 mmol). The reaction was heated to 100°C for 18 hours. The reaction is cooled to room temperature and poured into a 1L separatory funnel and extracted with dichloromethane (3 x 200 mL). The combine dichloromethane extracts are dried on magnesium sulfate, filtered and concentrated to dryness to afford 2-(2-(2-azidoethoxy)ethoxy)ethan-1-ol as a colorless oil (11.7 g).

Step 2. Preparation of compound 87

Compound **87** is prepared from **86** (4.95g, 28.3mmol) and **6** (10g, 25.7mmol) using an identical procedure to that used for compound **84**. Yield: 10g, 77%.

Step 3. Preparation of compound 88

Compound **88**is prepared from **87** (10g, 19.8mmol) using an identical procedure to that used for compound **85**. Yield: 7.63g, 65%.

Step 4. Preparation of compound 89

A solution of **88** (2g, 3.38mmol) and Z-glutamic acid (427mg, 1.52mmol) in CH_2Cl_2 (50mL) is treated with HBTU (1.41g, 3.7mmol) and Hünig's base (1.77mL, 10.1mmol). After stirring (18h) the mixture is concentrated and subjected to chromatography to yield **89** (871mg, 48%) as a colorless foam. Rf 0.5 (10% CH_3OH-CH_2Cl_2).

Step 5. Preparation of compound 90

A solution of **89** (870mg, 0.72mmol) and Pd/C (90mg, 10% - wet support) in EtOAc (10mL) is treated with TFA (84µL, 1.1mmol) and purged with H_2. After stirring vigorously (2h) the reaction is purged with N_2, filtered through Celite and concentrated. The crude material is used without further processing and yielded **90** (850mg, quantitative) as a colorless foam. Rf 0.25 (10% CH_3OH-CH_2Cl_2).

Step 6. Preparation of compound 91

A solution of **90** (850mg, 0.72mmol) and Z-glutamic acid (91mg, 0.32mmol) in CH_2Cl_2 (10mL) is treated with HBTU (300mg, 0.79mmol) and Hünig's base (502µL, 2.9mmol). After stirring (1.5h) the mixture is diluted with CH_2Cl_2 and washed with $NaHCO_3$ (Sat. Aq.), dried ($MgSO_4$), filtered and concentrated. The crude material is subjected to chromatography to yield **91** (590mg, 76%) as a colorless foam. Rf 0.5 (10% CH_3OH-CH_2Cl_2).

Step 7. Preparation of compound 92

A solution of **91** (590mg, 0.25mmol) and Pd/C (100mg, 10% - wet support) in CH_3OH (30mL) is treated with TFA (29µL, 0.37mmol) and purged with H_2. After stirring (3h) the mixture is purged with N_2, then filtered through Celite and concentrated. The crude material is used without further processing and yielded **92** (600mg, quantitative) as a colorless foam. Rf 0.1 (10% CH_3OH-CH_2Cl_2).

Step 8. Preparation of compound 101

Compound **101** is prepared from (R)-2-((2-oxo-2-phenyl-1l2-ethyl)amino)hexanedioic acid (2.51g, 8.6 mmol) and **9** (11g, 17.2 mmol) using an identical procedure to that used for compound **89**. Yield: 4.2 g, 37%.

Step 9. Preparation of compound 102

Compound **102** is prepared from compound **101** (4.2g, 3.2 mmol) using an identical procedure to that used for compound **90**. Yield: 2.1 g, 47%.

Step 10. Preparation of compound 103

Compound **103** is prepared from (R)-2-((2-oxo-2-phenyl-1l2-ethyl)amino)hexanedioic acid (265 mg, 0.9 mmol) and compound **102** (2.1 g, 1.8 mmol) using an identical procedure to that used for compound **91**. Yield: (560 mg, 24 %).

Step 11. Preparation of compound 104

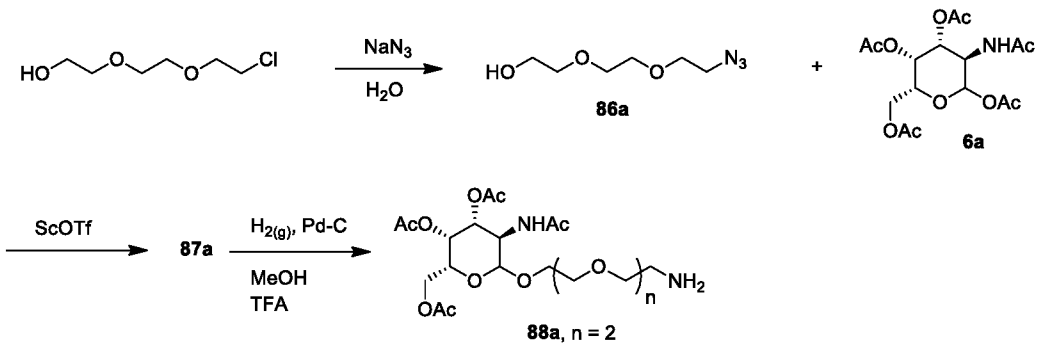
Compound **104** is prepared quantitatively from compound **103** (560 mg) using an identical procedure to that used for compound **92**. The compound is used without purification.

Step 12. Preparation of conjugates 191, 194, and 197

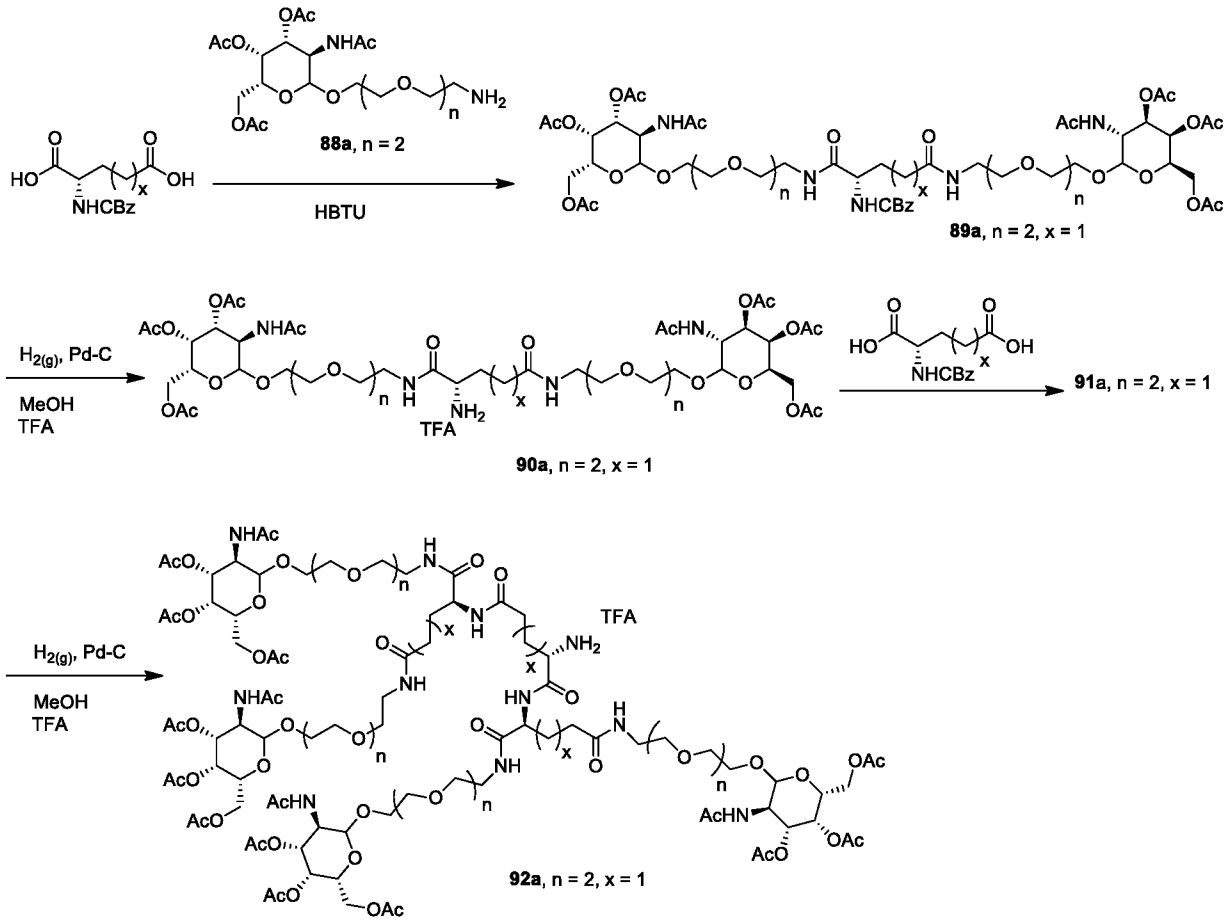
Conjugates **191, 194, and 197** are prepared from compound **128** and **92, 96, and 100** using an identical procedure to that used for compound **1**.

Example 16a. Synthesis of conjugates 191a

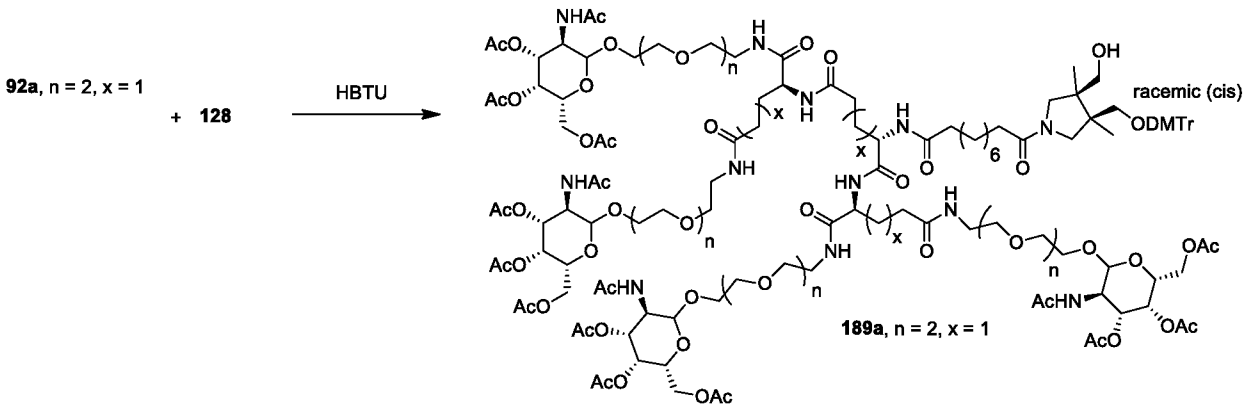
Scheme 36a

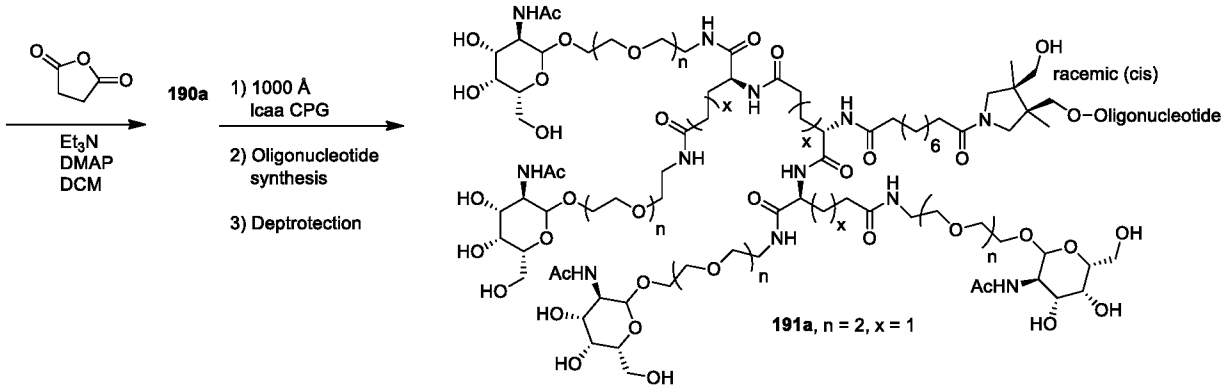


Scheme 37a.



Scheme 38a.





Step 1. Preparation of 2-(2-(2-azidoethoxy)ethoxy)ethan-1-ol 86a

To a solution of 2-(2-(2-chloroethoxy)ethoxy)ethan-1-ol (13 g, 77 mmol) in water (200 mL) was added sodium azide (10 g, 154 mmol). The reaction was heated to 100°C for 18 hours. The reaction was cooled to room temperature and poured into a 1L separatory funnel and extracted with dichloromethane (3 x 200 mL). The combine dichloromethane extracts were dried on magnesium sulfate, filtered and concentrated to dryness to afford 2-(2-(2-azidoethoxy)ethoxy)ethan-1-ol as a colorless oil (11.7 g).

Step 2. Preparation of compound 87a

Compound **87a** was prepared from **86a** (4.95g, 28.3mmol) and **6a** (10g, 25.7mmol) using an identical procedure to that used for compound **84**. Yield: 10g, 77%.

Step 3. Preparation of compound 88a

Compound **88a** was prepared from **87a** (10g, 19.8mmol) using an identical procedure to that used for compound **85**. Yield: 7.63g, 65%.

Step 4. Preparation of compound 89a

A solution of **88a** (2g, 3.38mmol) and Z—L-glutamic acid (427mg, 1.52mmol) in CH_2Cl_2 (50mL) was treated with HBTU (1.41g, 3.7mmol) and Hünig's base (1.77mL, 10.1mmol). After stirring (18h) the mixture was concentrated and subjected to chromatography to yield **89a** (871mg, 48%) as a colorless foam. Rf 0.5 (10% CH_3OH-CH_2Cl_2).

Step 5. Preparation of compound 90a

A solution of **89a** (870mg, 0.72mmol) and Pd/C (90mg, 10% - wet support) in EtOAc (10mL) was treated with TFA (84µL, 1.1mmol) and purged with H_2. After stirring vigorously

(2h) the reaction was purged with N_2, filtered through Celite and concentrated. The crude material was used without further processing and yielded **90a** (850mg, quantitative) as a colorless foam. Rf 0.25 (10% CH_3OH-CH_2Cl_2).

Step 6. Preparation of compound 91a

A solution of **90a** (850mg, 0.72mmol) and Z-glutamic acid (91mg, 0.32mmol) in CH_2Cl_2 (10mL) was treated with HBTU (300mg, 0.79mmol) and Hünig's base (502µL, 2.9mmol). After stirring (1.5h) the mixture diluted with CH_2Cl_2 and washed with $NaHCO_3$ (Sat. Aq.), dried ($MgSO_4$), filtered and concentrated. The crude material was subjected to chromatography to yield **91a** (590mg, 76%) as a colorless foam. Rf 0.5 (10% CH_3OH-CH_2Cl_2).

Step 7. Preparation of compound 92a

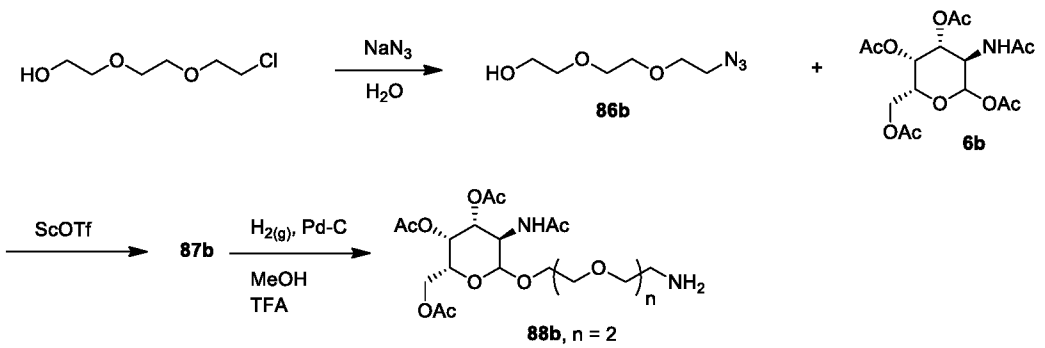
A solution of **91a** (590mg, 0.25mmol) and Pd/C (100mg, 10% - wet support) in CH_3OH (30mL) was treated with TFA (29µL, 0.37mmol) and purged with H_2. After stirring (3h) the mixture was purged with N_2, then filtered through Celite and concentrated. The crude material was used without further processing and yielded **92a** (600mg, quantitative) as a colorless foam. Rf 0.1 (10% CH_3OH-CH_2Cl_2).

Step 8. Preparation of conjugate 191a,

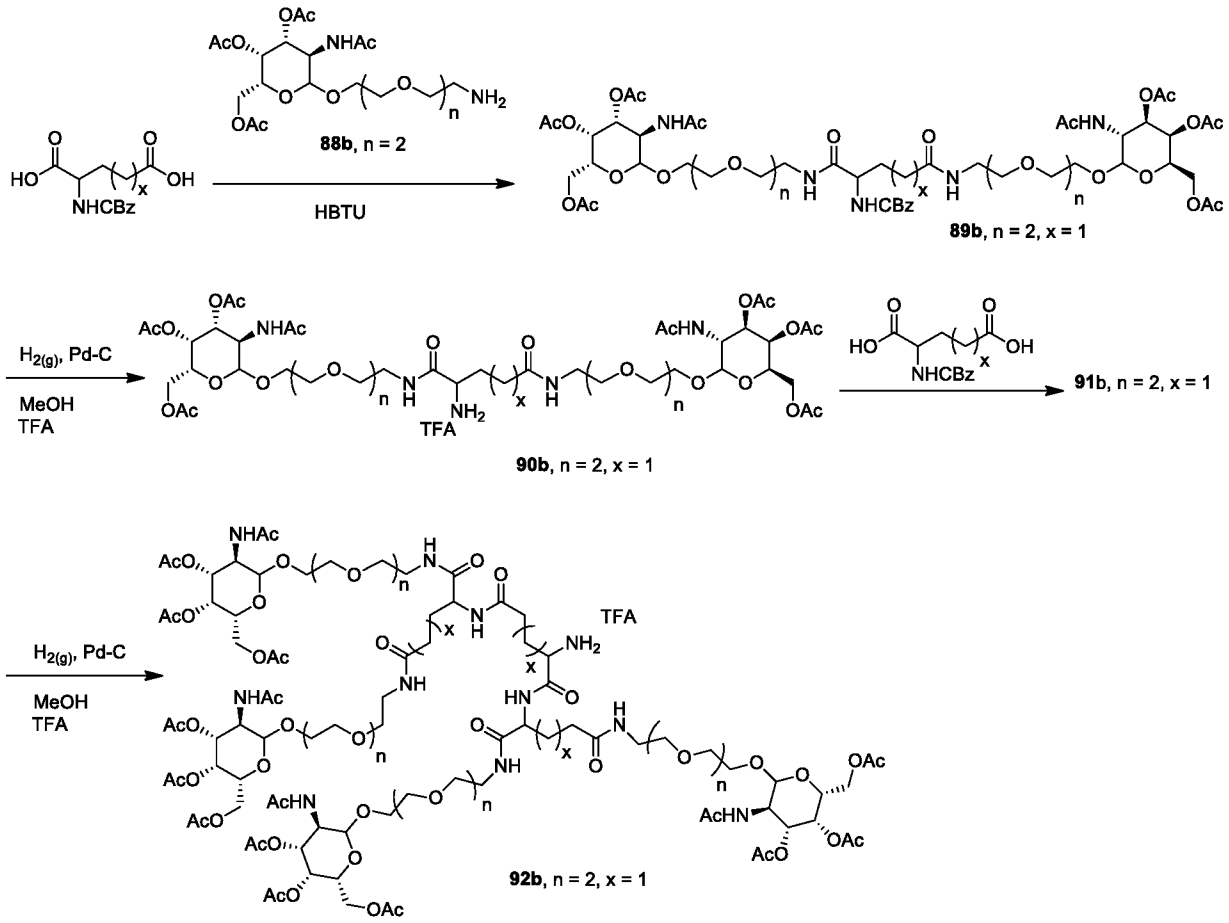
Conjugate **191a** was prepared from compound **128** and compound **92a** using an identical procedure to that used for compound **1**.

Example 16b. Synthesis of conjugates 191b

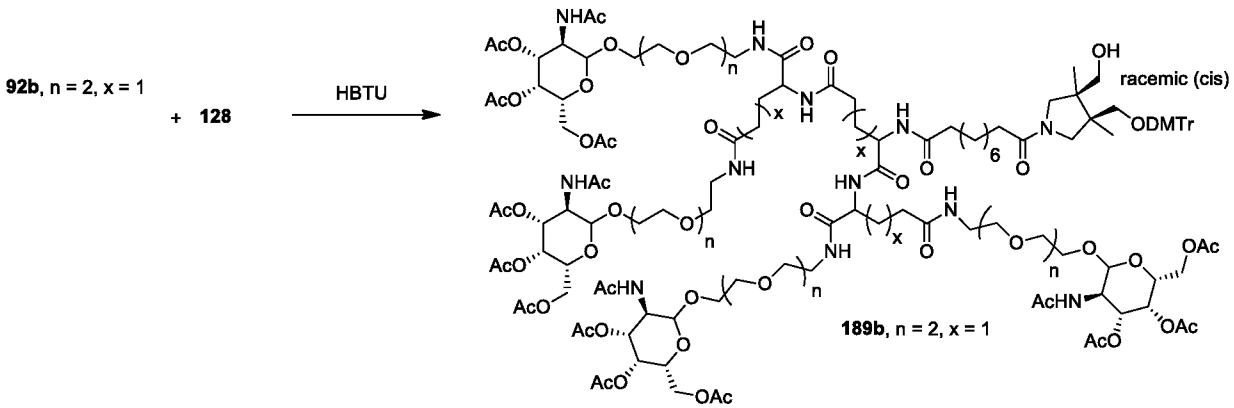
Scheme 36b

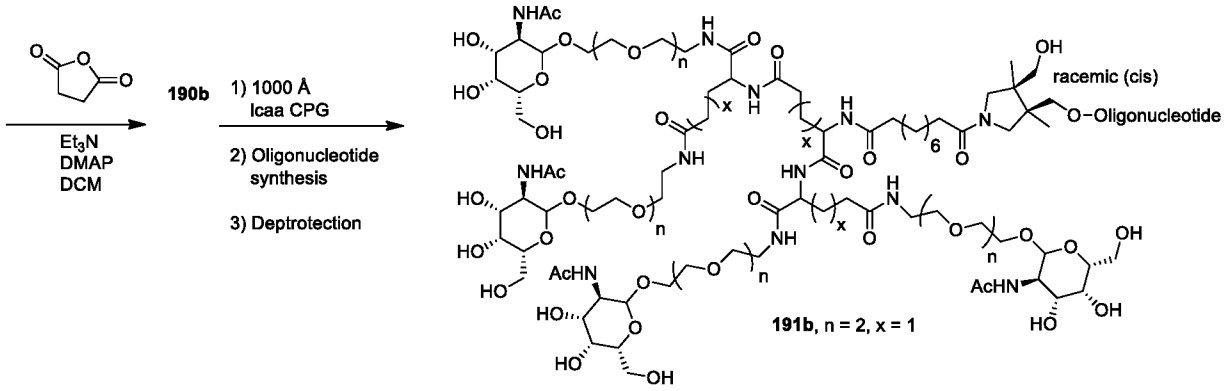


Scheme 37b.



Scheme 38b.





Step 1. Preparation of 2-(2-(2-azidoethoxy)ethoxy)ethan-1-ol 86b

To a solution of 2-(2-(2-chloroethoxy)ethoxy)ethan-1-ol (13 g, 77 mmol) in water (200 mL) is added sodium azide (10 g, 154 mmol). The reaction was heated to 100°C for 18 hours. The reaction was cooled to room temperature and poured into a 1L separatory funnel and extracted with dichloromethane (3 x 200 mL). The combine dichloromethane extracts were dried on magnesium sulfate, filtered and concentrated to dryness to afford 2-(2-(2-azidoethoxy)ethoxy)ethan-1-ol as a colorless oil (11.7 g).

Step 2. Preparation of compound 87b

Compound **87a** is prepared from **86b** (4.95g, 28.3mmol) and **6b** (10g, 25.7mmol) using an identical procedure to that used for compound **84**. Yield: 10g, 77%.

Step 3. Preparation of compound 88b

Compound **88a** is prepared from **87b** (10g, 19.8mmol) using an identical procedure to that used for compound **85**. Yield: 7.63g, 65%.

Step 4. Preparation of compound 89b

A solution of **88b** (2g, 3.38mmol) and racemic Z-glutamic acid (427mg, 1.52mmol) in CH_2Cl_2 (50mL) is treated with HBTU (1.41g, 3.7mmol) and Hünig's base (1.77mL, 10.1mmol). After stirring (18h) the mixture was concentrated and subjected to chromatography to yield **89b** (871mg, 48%) as a colorless foam. Rf 0.5 (10% CH_3OH-CH_2Cl_2).

Step 5. Preparation of compound 90b

A solution of **89b** (870mg, 0.72mmol) and Pd/C (90mg, 10% - wet support) in EtOAc (10mL) is treated with TFA (84µL, 1.1mmol) and purged with H_2. After stirring vigorously (2h) the reaction is purged with N_2, filtered through Celite and concentrated. The crude material is used without further processing and yielded **90b** (850mg, quantitative) as a colorless foam. Rf 0.25 (10% CH_3OH-CH_2Cl_2).

Step 6. Preparation of compound 91b

A solution of **90b** (850mg, 0.72mmol) and Z-glutamic acid (91mg, 0.32mmol) in CH_2Cl_2 (10mL) is treated with HBTU (300mg, 0.79mmol) and Hünig's base (502µL, 2.9mmol). After stirring (1.5h) the mixture is diluted with CH_2Cl_2 and washed with $NaHCO_3$ (Sat. Aq.), dried ($MgSO_4$), filtered and concentrated. The crude material is subjected to chromatography to yield **91b** (590mg, 76%) as a colorless foam. Rf 0.5 (10% CH_3OH-CH_2Cl_2).

Step 7. Preparation of compound 92b

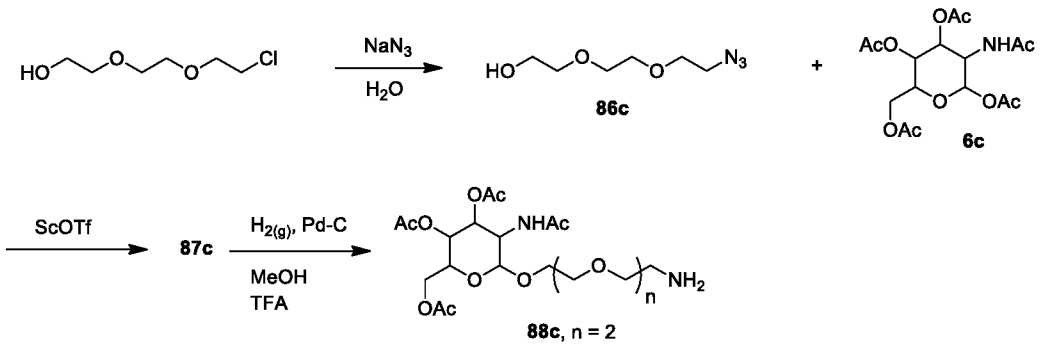
A solution of **91b** (590mg, 0.25mmol) and Pd/C (100mg, 10% - wet support) in CH_3OH (30mL) is treated with TFA (29µL, 0.37mmol) and purged with H_2. After stirring (3h) the mixture is purged with N_2, then filtered through Celite and concentrated. The crude material is used without further processing and yielded **92b** (600mg, quantitative) as a colorless foam. Rf 0.1 (10% CH_3OH-CH_2Cl_2).

Step 8. Preparation of conjugate 191b

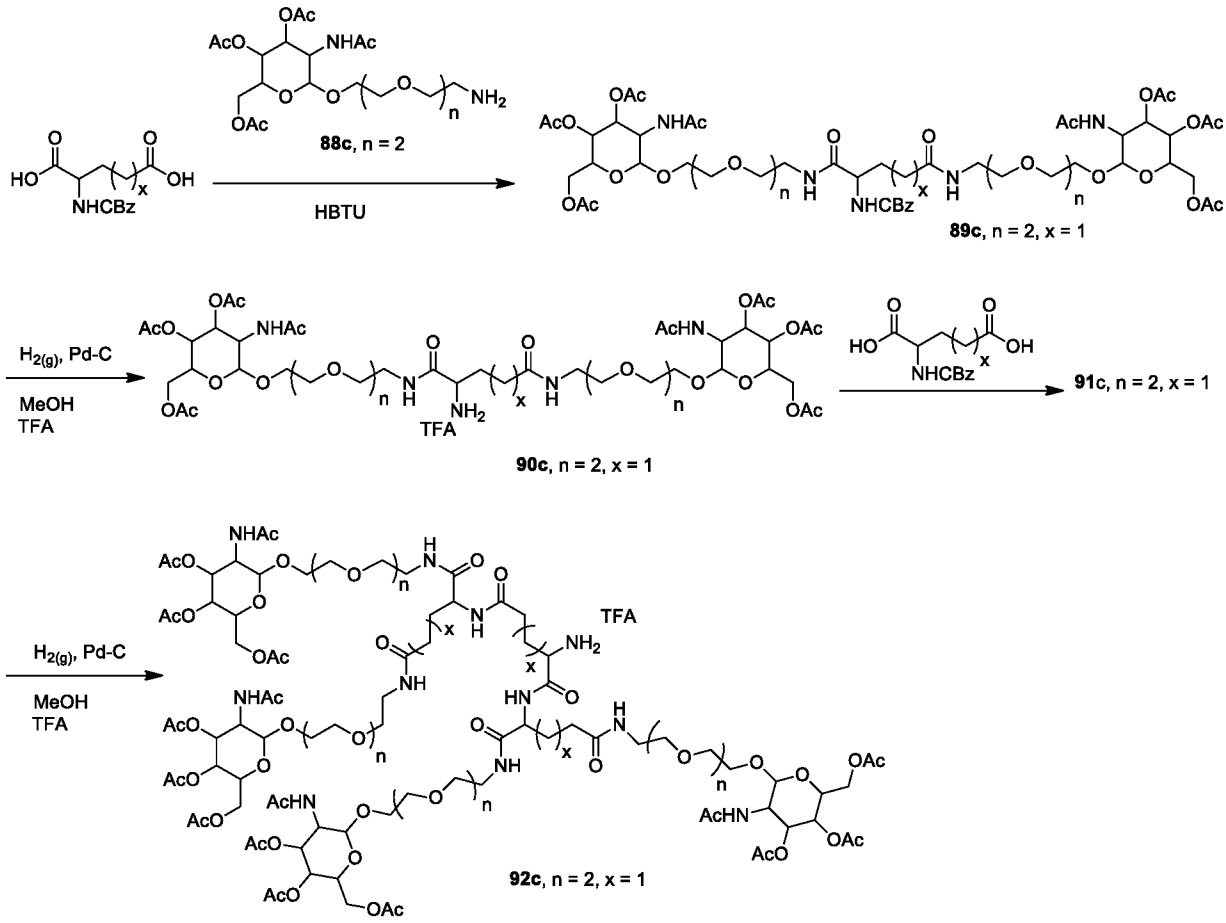
Conjugate **191b** is prepared from compound **128** and compound **92b** using an identical procedure to that used for compound **1**.

Example 16c. Synthesis of conjugates 191c

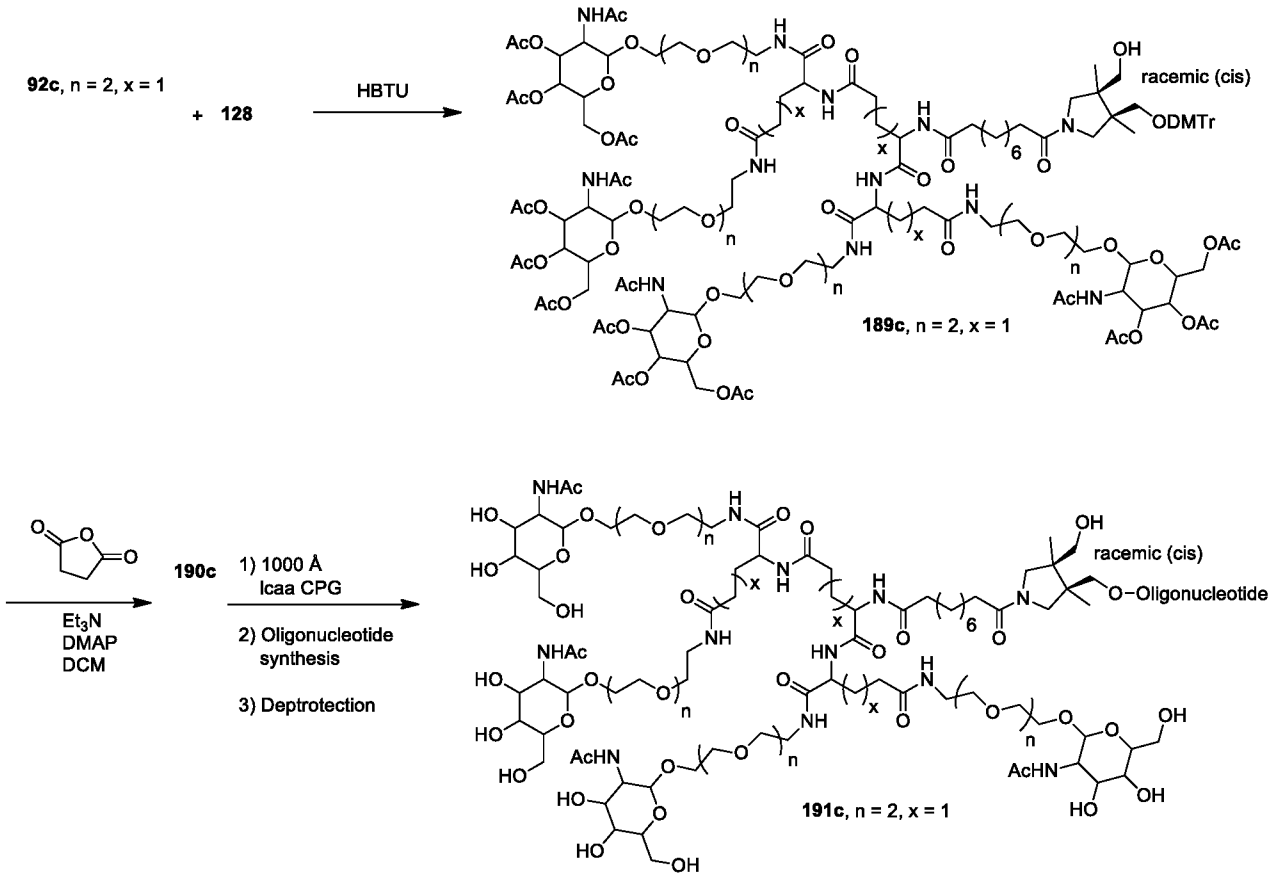
Scheme 36c



Scheme 37c.



Scheme 38c.



Step 1. Preparation of 2-(2-(2-azidoethoxy)ethoxy)ethan-1-ol 86c

To a solution of 2-(2-(2-chloroethoxy)ethoxy)ethan-1-ol (13 g, 77 mmol) in water (200 mL) is added sodium azide (10 g, 154 mmol). The reaction was heated to 100°C for 18 hours. The reaction was cooled to room temperature and poured into a 1L separatory funnel and extracted with dichloromethane (3 x 200 mL). The combine dichloromethane extracts were dried on magnesium sulfate, filtered and concentrated to dryness to afford 2-(2-(2-azidoethoxy)ethoxy)ethan-1-ol as a colorless oil (11.7 g).

Step 2. Preparation of compound 87c

Compound **87c** is prepared from **86c** (4.95g, 28.3mmol) and **6c** (10g, 25.7mmol) using an identical procedure to that used for compound **84**. Yield: 10g, 77%.

Step 3. Preparation of compound 88c

Compound **88c** is prepared from **87c** (10g, 19.8mmol) using an identical procedure to that used for compound **85**. Yield: 7.63g, 65%.

Step 4. Preparation of compound 89c

A solution of **88c** (2g, 3.38mmol) and racemic Z-glutamic acid (427mg, 1.52mmol) in CH_2Cl_2 (50mL) is treated with HBTU (1.41g, 3.7mmol) and Hünig's base (1.77mL, 10.1mmol). After stirring (18h) the mixture was concentrated and subjected to chromatography to yield **89c** (871mg, 48%) as a colorless foam. Rf 0.5 (10% CH_3OH-CH_2Cl_2).

Step 5. Preparation of compound 90c

A solution of **89c** (870mg, 0.72mmol) and Pd/C (90mg, 10% - wet support) in EtOAc (10mL) is treated with TFA (84µL, 1.1mmol) and purged with H_2. After stirring vigorously (2h) the reaction is purged with N_2, filtered through Celite and concentrated. The crude material is used without further processing and yielded **90c** (850mg, quantitative) as a colorless foam. Rf 0.25 (10% CH_3OH-CH_2Cl_2).

Step 6. Preparation of compound 91c

A solution of **90c** (850mg, 0.72mmol) and Z-glutamic acid (91mg, 0.32mmol) in CH_2Cl_2 (10mL) is treated with HBTU (300mg, 0.79mmol) and Hünig's base (502µL, 2.9mmol). After stirring (1.5h) the mixture is diluted with CH_2Cl_2 and washed with $NaHCO_3$ (Sat. Aq.), dried ($MgSO_4$), filtered and concentrated. The crude material is subjected to chromatography to yield **91c** (590mg, 76%) as a colorless foam. Rf 0.5 (10% CH_3OH-CH_2Cl_2).

Step 7. Preparation of compound 92c

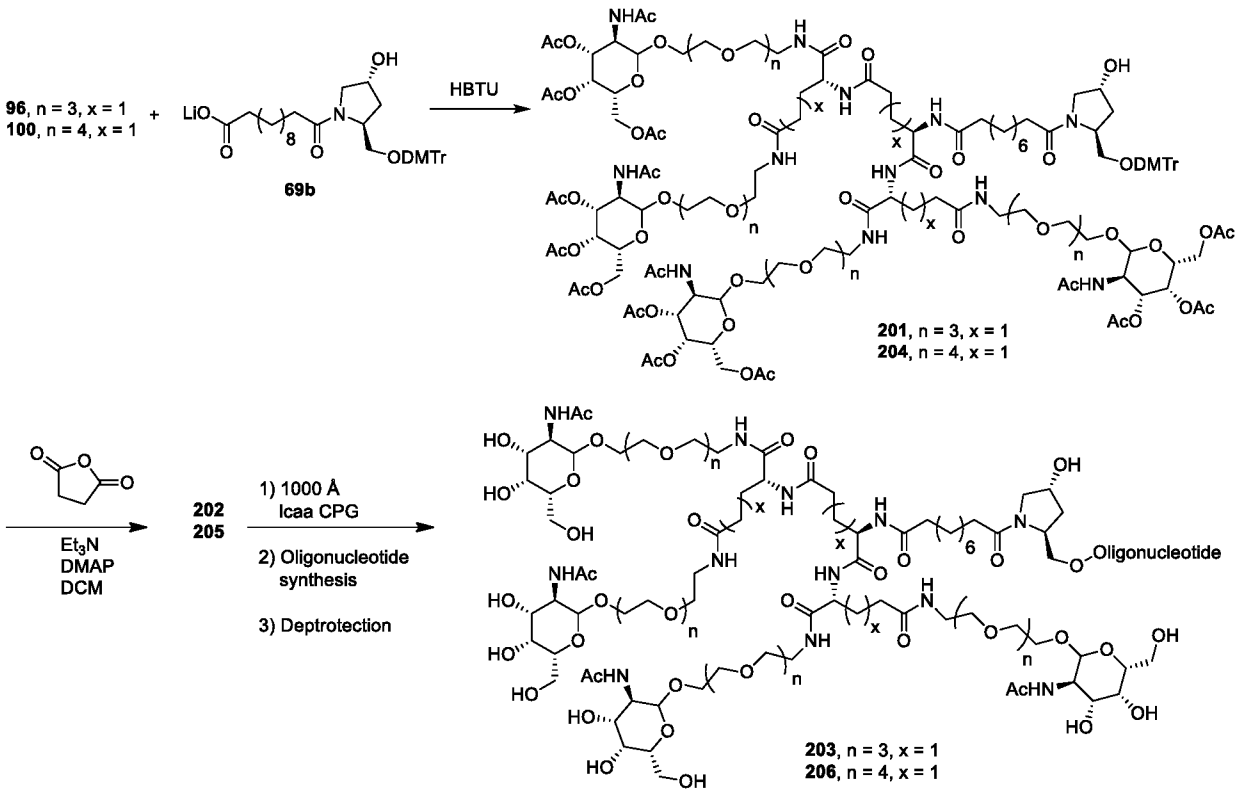
A solution of **91c** (590mg, 0.25mmol) and Pd/C (100mg, 10% - wet support) in CH_3OH (30mL) is treated with TFA (29µL, 0.37mmol) and purged with H_2. After stirring (3h) the mixture is purged with N_2, then filtered through Celite and concentrated. The crude material is used without further processing and yielded **92c** (600mg, quantitative) as a colorless foam. Rf 0.1 (10% CH_3OH-CH_2Cl_2).

Step 8. Preparation of conjugate 191c

Conjugate **191c** is prepared from compound **128** and compound **92c** using an identical procedure to that used for compound **1**.

Example 17. Synthesis of conjugates 203 and 206

Scheme 39.



Step 1. Preparation of compound 69b

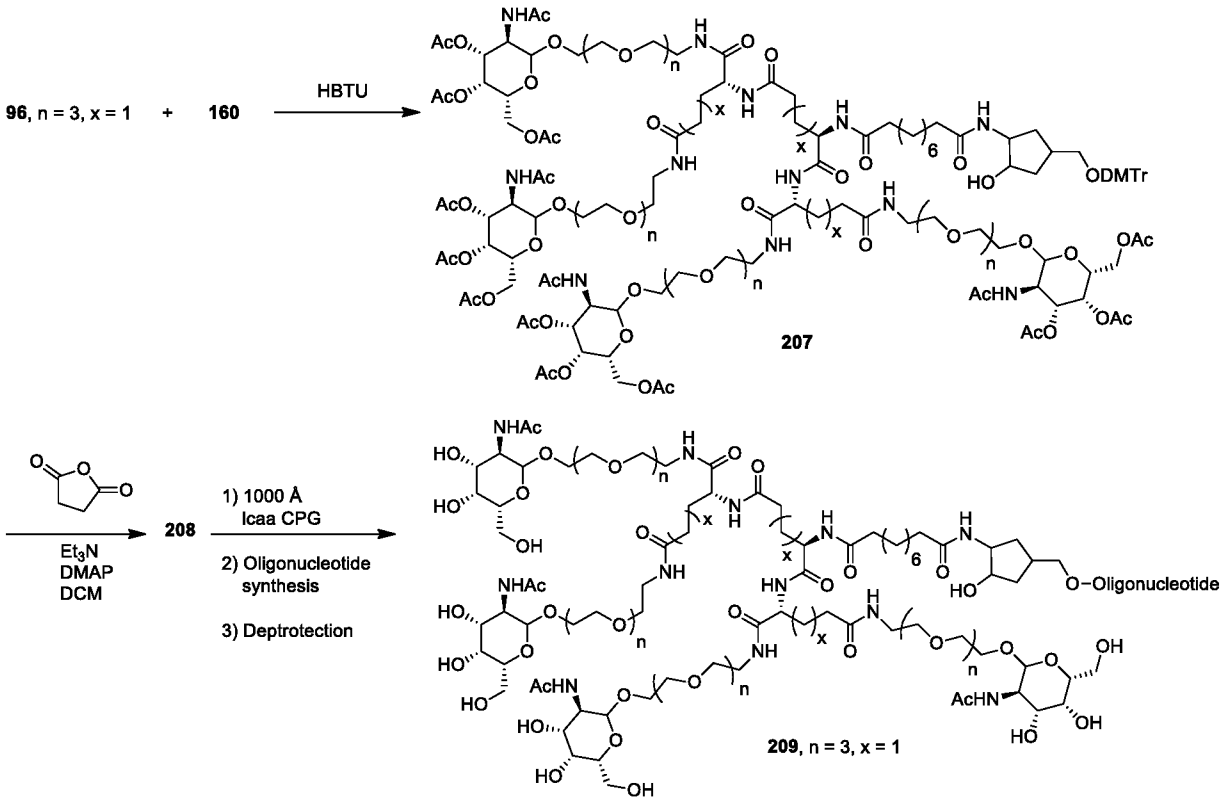
Compound **69b** was prepared from (2S,4R)-4-Hydroxypyrrolidine-2-carboxylic acid using an identical procedure to that used for compound **69**.

Step 2. Preparation of conjugates 203 and 206

Conjugates **203** and **206** were prepared from compound **96** and **100** using an identical procedure to that used for compound **1**.

Example 18. Synthesis of conjugate 209

Scheme 40.

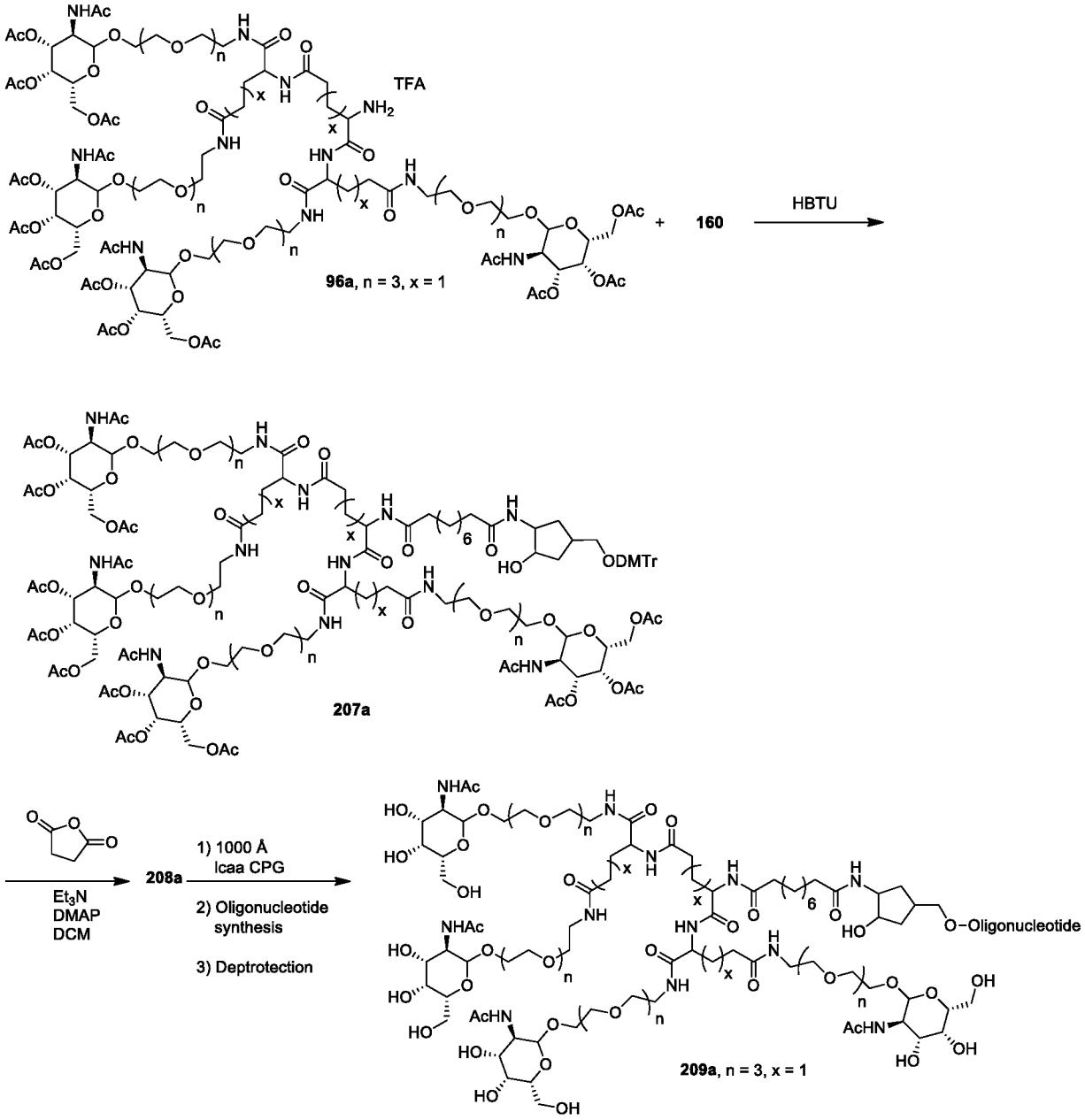


Step 1. Preparation of conjugate 209

Conjugate **209** was prepared from compound **96** and **160** using an identical procedure to that used for compound **1**.

Example 18a. Synthesis of conjugate 209a

Scheme 40a.

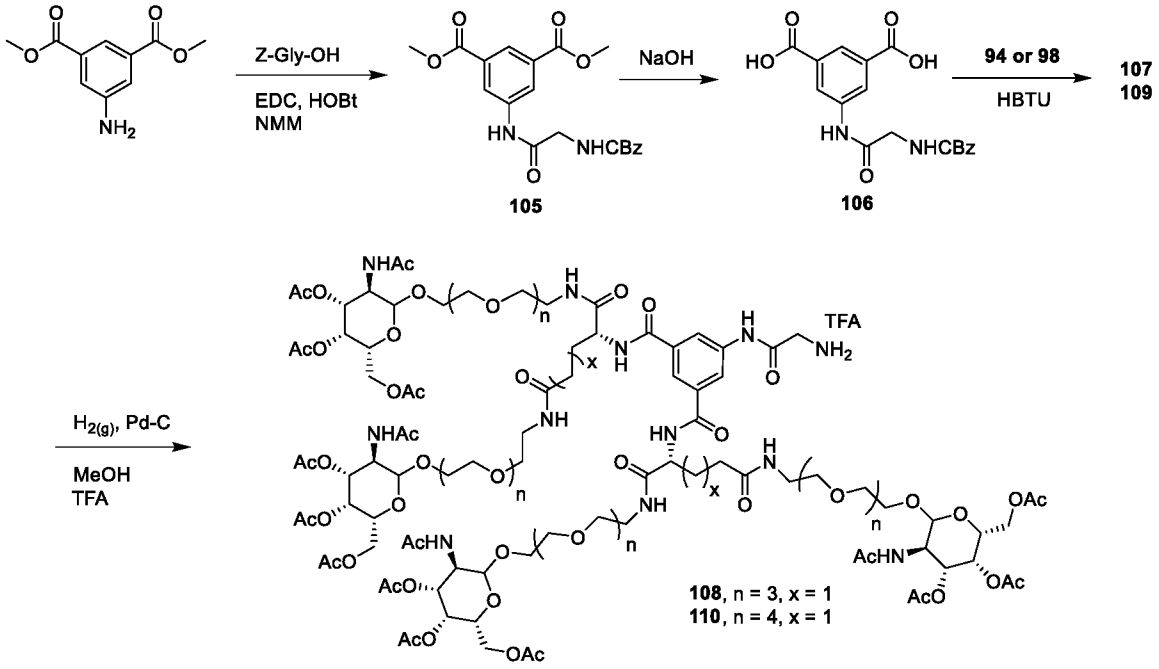


Step 1. Preparation of conjugate 209a

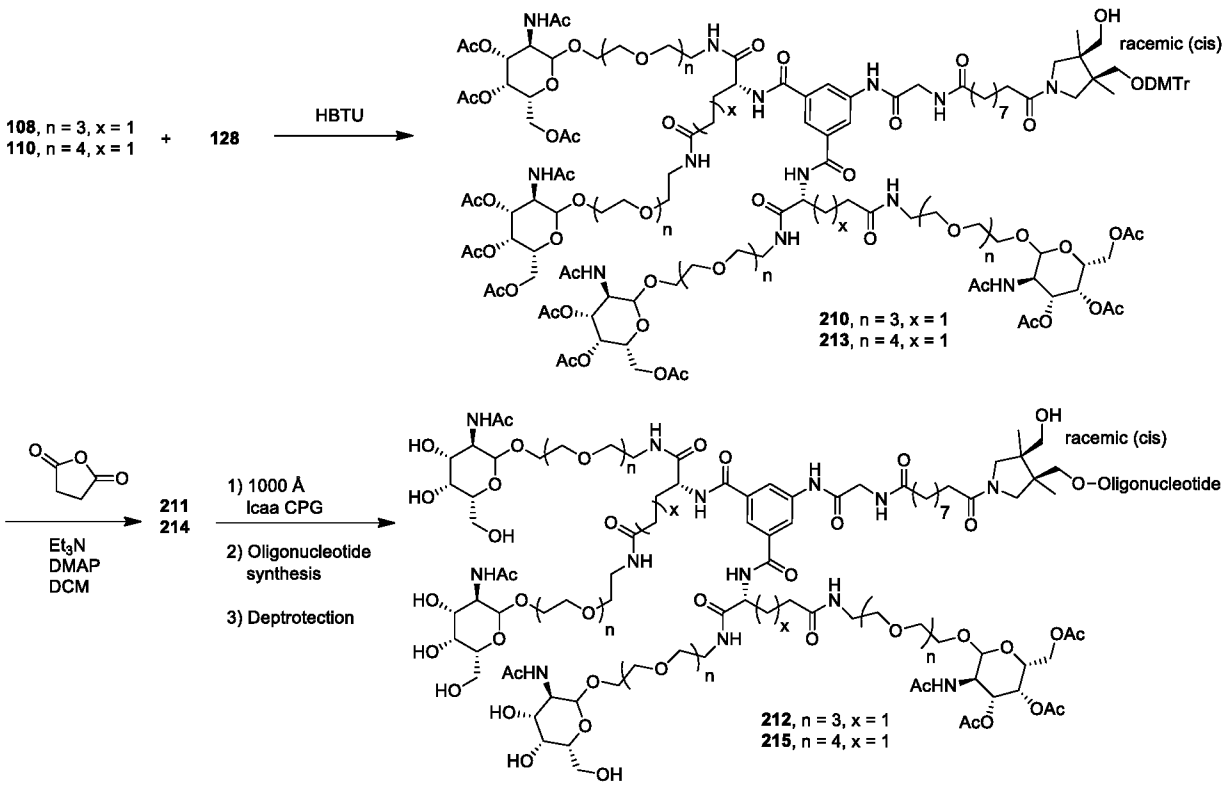
Conjugate **209a** is prepared from compound **96a** and **160** using an identical procedure to that used for compound **1**.

Example 19. Synthesis of conjugates 212 and 215

Scheme 41.



Scheme 42.



Step 1. Preparation of Dimethyl 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)-isophthalate 105

A solution of dimethyl 5-aminoisophthalate (5 g, 24 mmol), Z-Gly-OH (5 g, 24 mmol), EDC (5 g, 26.3 mmol), HOBt (3.6 g, 26.3 mmol), NMM (2.9 mL, 26.3 mmol) in DMF (50 mL) was stirred overnight at room temperature. Upon completion, the reaction mixture was diluted with ethyl acetate (250 mL) and washed with each 1M HCl (2 x 100 mL), saturated sodium bicarbonate (1 x 100 mL) and brine (2 x 100 mL). Dry on magnesium sulfate, filter and concentrate to dryness to afford Dimethyl 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)isophthalate as a colorless solid (7.2 g, 79%).

Step 2. Preparation of 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)isophthalic acid 106

To a solution of methyl 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)isophthalate (7.2 g) in methanol (25 mL) and THF (25 mL) was added 1M NaOH (25 mL). The solution was stirred at room temperature for 2 hours then concentrated to remove THF and MeOH. The aqueous solution remaining was diluted with water (75 mL), cooled on an ice water bath and acidified to pH = 1 with 6M HCl. The solid was filtered and washed with water (3 x 100 mL). The solid was freeze dried to afford 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)-isophthalic acid (6.9 g, quantitative) .

Step 3. Preparation of compound 107

Compound **107** was prepared from 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)isophthalic acid **106** (200 mg, 0.54 mmol) and **94** (1.7 g, 1.3 mmol) using an identical procedure to that used for compound **95**. Yield: 600 mg.

Step 4. Preparation of compound 108

Compound **108** was prepared from compound **107** (600 mg) using an identical procedure to that used for compound **96**. Yield: 650 mg, quantitative.

Step 5. Preparation of compound 109

Compound **109** was prepared from 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)isophthalic acid **106** (180 mg, 0.48 mmol) and **98** (1.5 g, 1.1 mmol) using an identical procedure to that used for compound **99**. Yield: 900 mg.

Step 6. Preparation of compound 110

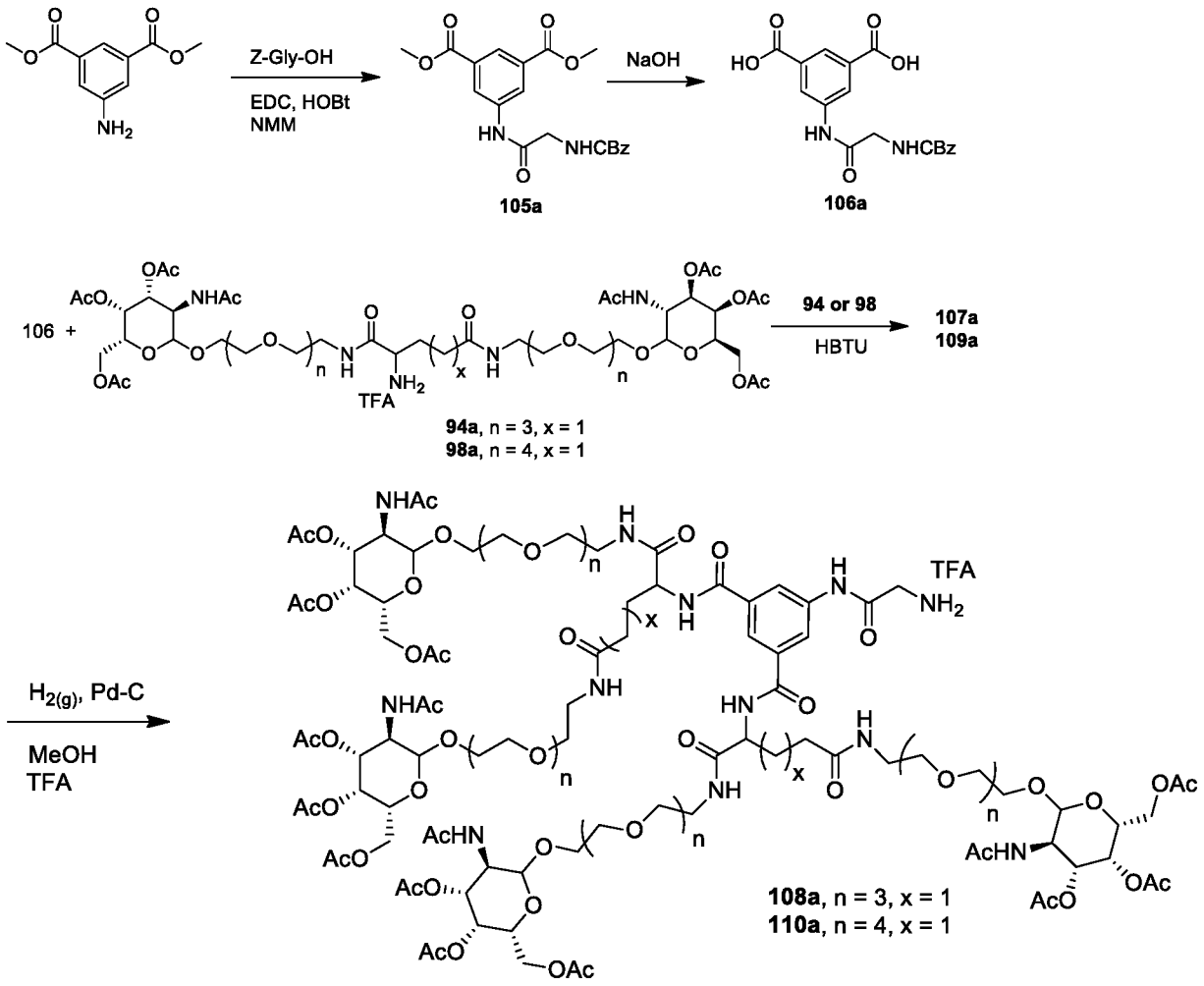
Compound **110** was prepared from compound **109** (900 mg) using an identical procedure to that used for compound **100**. Yield: 920 mg, quantitative.

Step 7. Preparation of conjugates 212 and 215

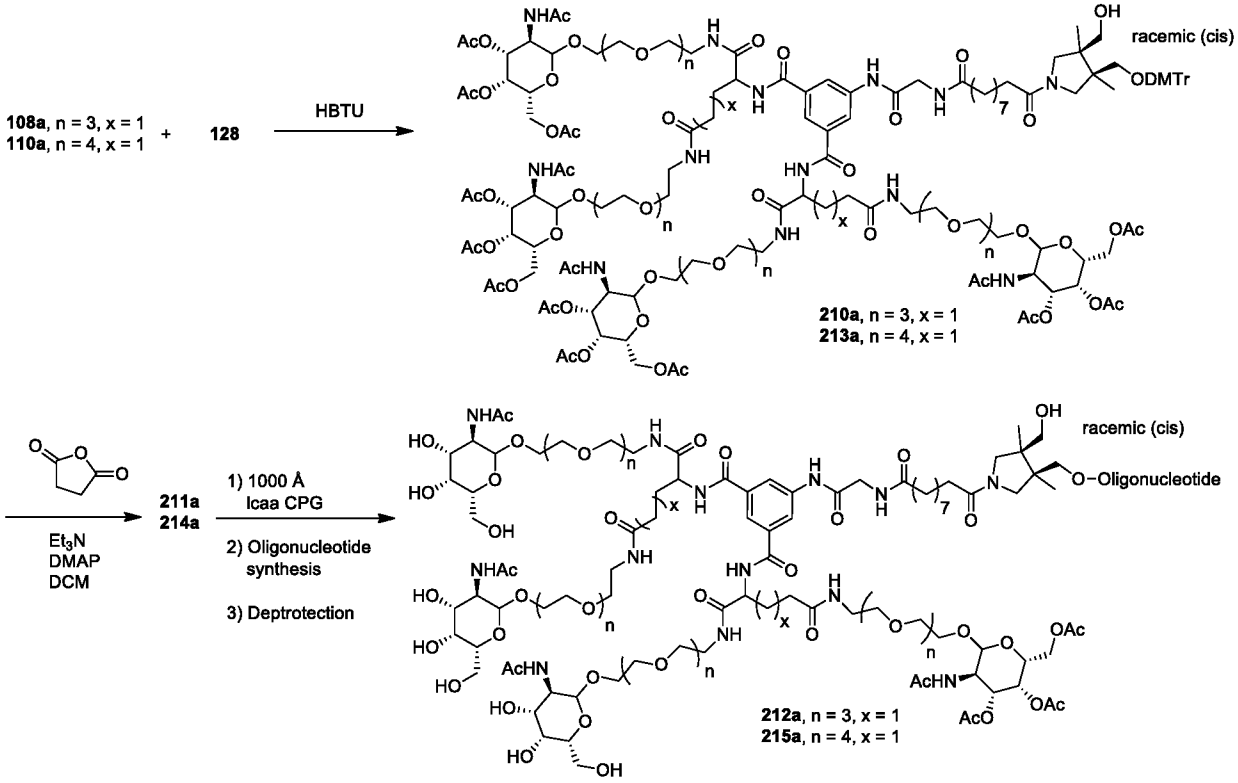
Conjugates **212** and **215** were prepared from compound **128** and **108** or **110** using an identical procedure to that used for compound **1**.

Example 19a. Synthesis of conjugates 212a and 215a

Scheme 41a.



Scheme 42a.



Step 1. Preparation of Dimethyl 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)-isophthalate 105a

A solution of dimethyl 5-aminoisophthalate (5 g, 24 mmol), Z-Gly-OH (5 g, 24 mmol), EDC (5 g, 26.3 mmol), HOBt (3.6 g, 26.3 mmol), NMM (2.9 mL, 26.3 mmol) in DMF (50 mL) is stirred overnight at room temperature. Upon completion, the reaction mixture is diluted with ethyl acetate (250 mL) and washed with each 1M HCl (2 x 100 mL), saturated sodium bicarbonate (1 x 100 mL) and brine (2 x 100 mL). Dry on magnesium sulfate, filter and concentrate to dryness to afford Dimethyl 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)isophthalate as a colorless solid (7.2 g, 79%).

Step 2. Preparation of 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)isophthalic acid 106a

To a solution of methyl 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)isophthalate (7.2 g) in methanol (25 mL) and THF (25 mL) is added 1M NaOH (25 mL). The solution is stirred at room temperature for 2 hours then concentrated to remove THF and MeOH. The aqueous solution remaining is diluted with water (75 mL), cooled on an ice water bath and acidified to pH = 1 with 6M HCl. The solid is filtered and washed with water (3 x 100 mL).

The solid is freeze dried to afford 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)-isophthalic acid (6.9 g, quantitative) .

Step 3. Preparation of compound 107a

Compound **107a** is prepared from 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)isophthalic acid **106a** (200 mg, 0.54 mmol) and **94a** (1.7 g, 1.3 mmol) using an identical procedure to that used for compound **95**. Yield: 600 mg.

Step 4. Preparation of compound 108a

Compound **108a** is prepared from compound **107a** (600 mg) using an identical procedure to that used for compound **96a**. Yield: 650 mg, quantitative.

Step 5. Preparation of compound 109a

Compound **109a** is prepared from 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)isophthalic acid **106a** (180 mg, 0.48 mmol) and **9a8** (1.5 g, 1.1 mmol) using an identical procedure to that used for compound **99**. Yield: 900 mg.

Step 6. Preparation of compound 110a

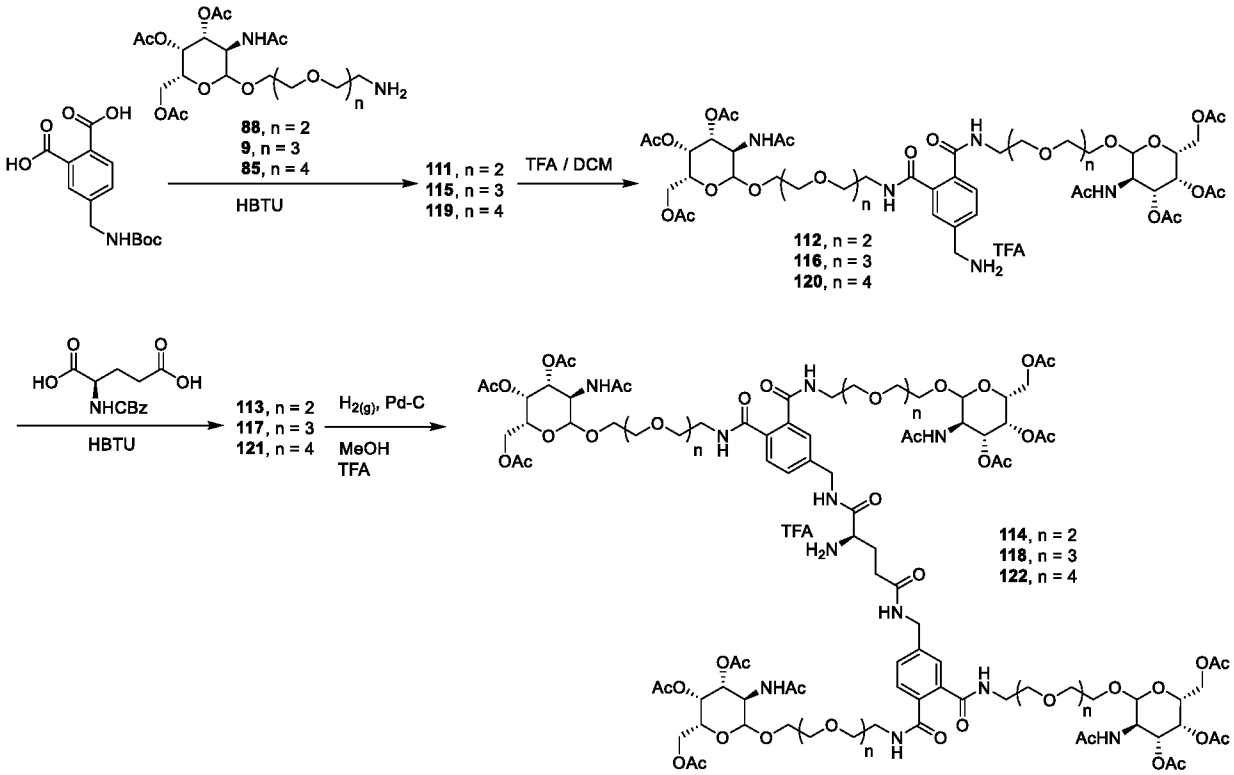
Compound **110a** is prepared from compound **109** (900 mg) using an identical procedure to that used for compound **100**. Yield: 920 mg, quantitative.

Step 7. Preparation of conjugates 212a and 215a

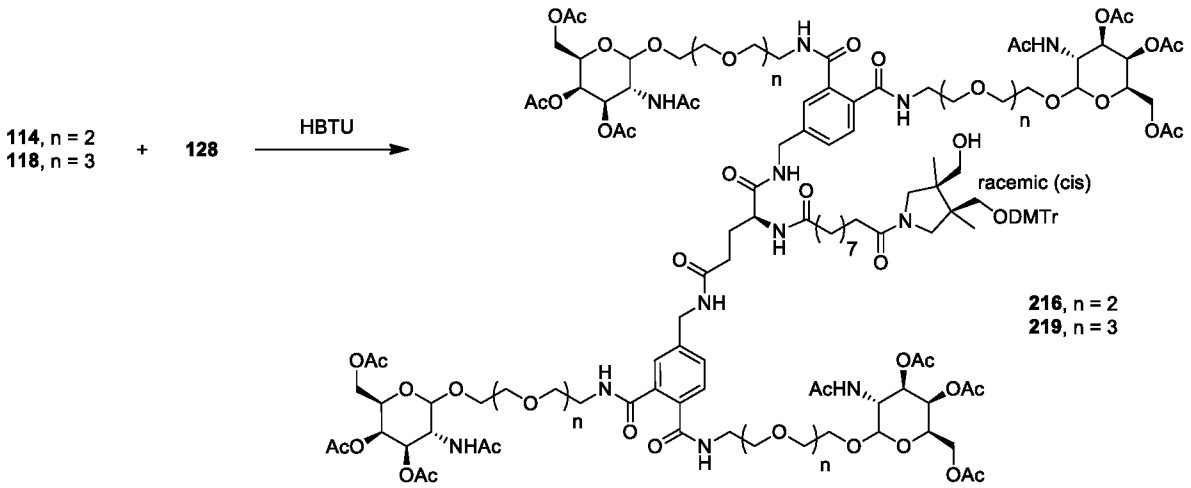
Conjugates **212a** and **21a5** are prepared from compound **128** and **108a** or **110a** using an identical procedure to that used for compound **1**.

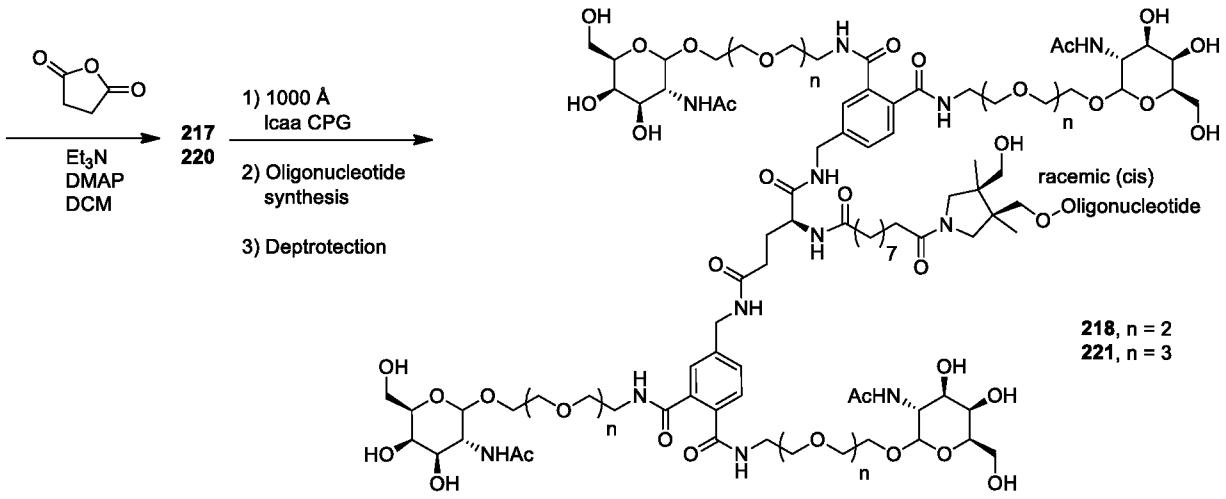
Example 20. Synthesis of conjugates 218 and 221

Scheme 43.



Scheme 44.





Step 1. Preparation of compound 111

Compound **111** was prepared from 4-(((tert-butoxycarbonyl)amino)methyl)phthalic acid (1.13g, 3.84mmol) and **88** (5g, 8.44mmol) using an identical procedure to that used for compound **89**. Yield: 2.21g, 49%.

Step 2. Preparation of compound 112

A solution of **111** (2.21g, 1.87mmol) in CH_2Cl_2 (40mL) was slowly treated with TFA (5mL). After stirring (2h) the mixture was concentrated and subjected to chromatography to yield **112** (1.08g, 47%) as a colorless foam. Rf 0.1 (10% CH_3OH-CH_2Cl_2).

Step 3. Preparation of compound 113

Compound **113** was prepared from compound **112** (1.08g, 0.88mmol) and (2-oxo-2-phenyl-1λ^2-ethyl)-D-glutamic acid (112mg, 0.39mmol) using an identical procedure to that used for compound **91**. Yield: 600mg, 62%.

Step 4. Preparation of compound 114

Compound **114** was prepared from compound **113** using an identical procedure to that used for compound **92**.

Step 5. Preparation of compound 115

Compound **115** was prepared from 4-(((tert-butoxycarbonyl)amino)methyl)phthalic acid (3.94g, 13.3mmol) and **9** (18.2g, 29.4mmol) using an identical procedure to that used for compound **93**. Yield: 9.02g, 53%.

Step 6. Preparation of compound 116

Compound **116** was prepared from compound **115** (8g, 6.3mmol) using an identical procedure to that used for compound **112**. Yield: 3.23g, 39%.

Step 7. Preparation of compound 117

Compound **117** was prepared from compound **116** (3.23g, 2.45mmol) and (2-oxo-2-phenyl-1λ^2-ethyl)-D-glutamic acid (192mg, 1.1mmol) using an identical procedure to that used for compound **95**. Yield: 2.22g, 34%.

Step 8. Preparation of compound 118

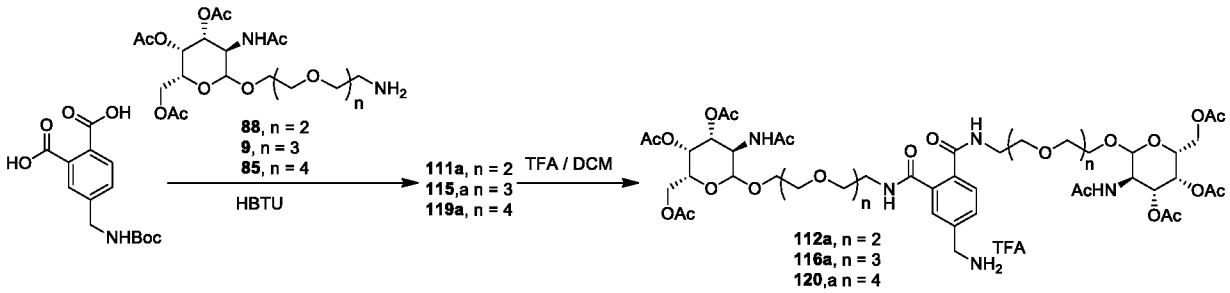
Compound **118** was prepared from compound **117** (2.22g, 0.84mmol) using an identical procedure to that used for compound **96**. Yield: 2.02g, 91%.

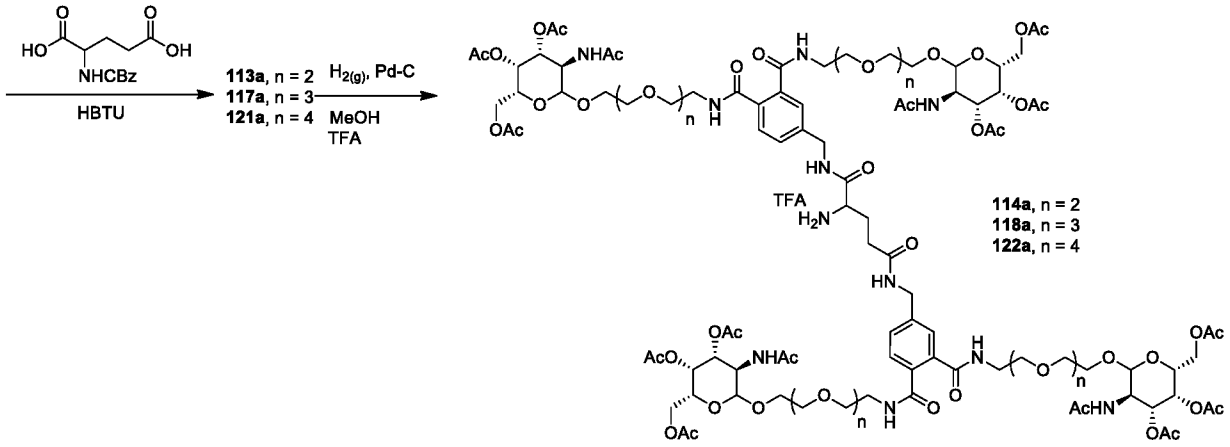
Step 9. Preparation of conjugates 218 and 221

Conjugates **218** and **221** were prepared from compounds **128** and **114** or **118** using an identical procedure to that used for compound **1**.

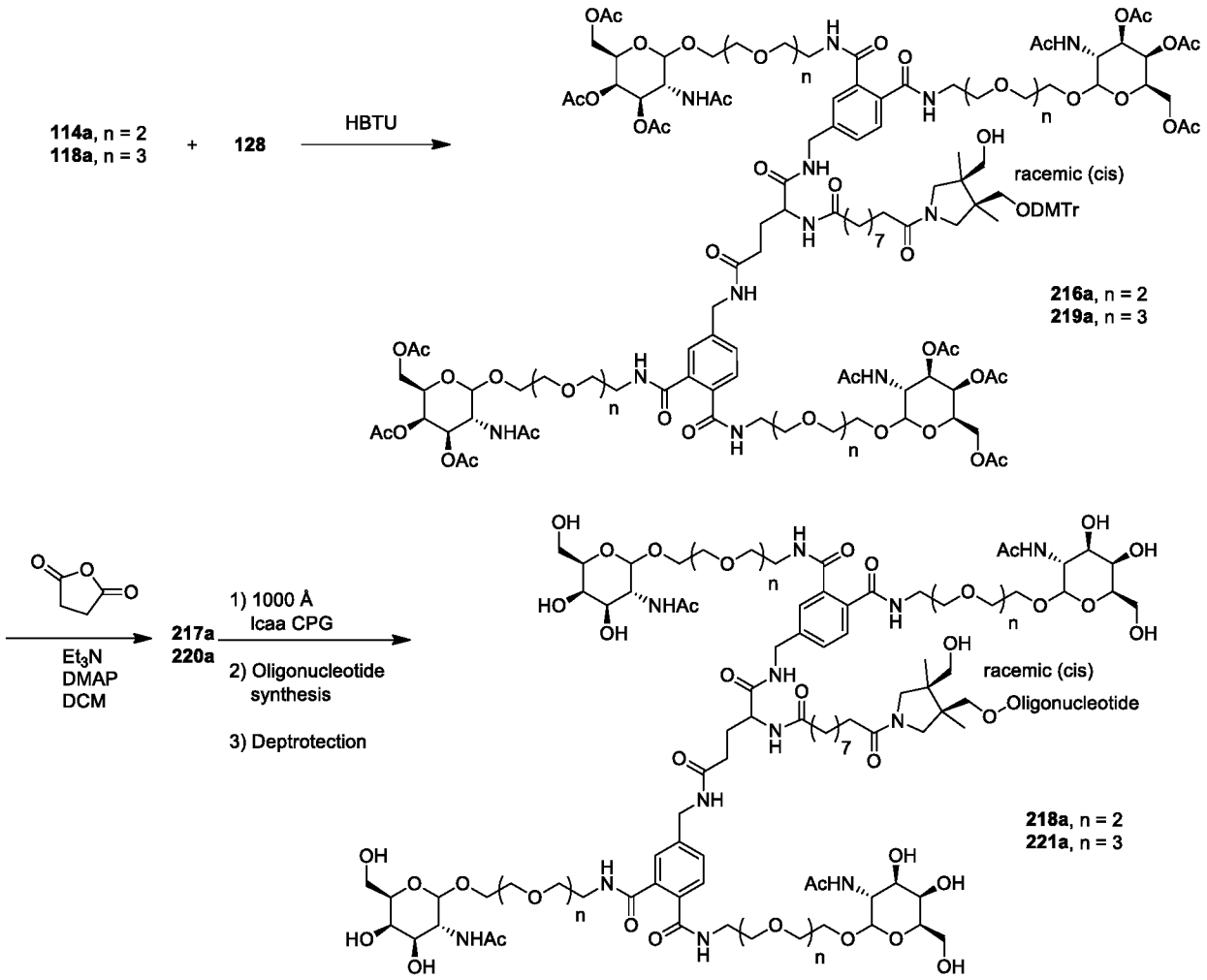
<u>Example 20a. Synthesis of conjugates 218a and 221a</u>

Scheme 43a.




HBTU
113a, n = 2
117a, n = 3
121a, n = 4
H2(g), Pd-C
MeOH
TFA
114a, n = 2
118a, n = 3
122a, n = 4

Scheme 44a.


114a, n = 2
118a, n = 3
128
HBTU
racemic (cis)
ODMTr
216a, n = 2
219a, n = 3
Et3N
DMAP
DCM
217a
220a
1) 1000 Å
Icaa CPG
2) Oligonucleotide
synthesis
3) Deptrotection
Oligonucleotide
218a, n = 2
221a, n = 3

Step 1. Preparation of compound 111a

Compound **111a** is prepared from 4-(((tert-butoxycarbonyl)amino)methyl)phthalic acid (1.13g, 3.84mmol) and **88** (5g, 8.44mmol) using an identical procedure to that used for compound **89**. Yield: 2.21g, 49%.

Step 2. Preparation of compound 112a

A solution of **111a** (2.21g, 1.87mmol) in CH_2Cl_2 (40mL) is slowly treated with TFA (5mL). After stirring (2h) the mixture is concentrated and subjected to chromatography to yield **112a** (1.08g, 47%) as a colorless foam. Rf 0.1 (10% CH_3OH-CH_2Cl_2).

Step 3. Preparation of compound 113a

Compound **113a** is prepared from compound **112a** (1.08g, 0.88mmol) and (2-oxo-2-phenyl-1λ^2-ethyl)-D-glutamic acid (112mg, 0.39mmol) using an identical procedure to that used for compound **91**. Yield: 600mg, 62%.

Step 4. Preparation of compound 114a

Compound **114a** is prepared from compound **113a** using an identical procedure to that used for compound **92**.

Step 5. Preparation of compound 115a

Compound **115a** is prepared from 4-(((tert-butoxycarbonyl)amino)methyl)phthalic acid (3.94g, 13.3mmol) and **9** (18.2g, 29.4mmol) using an identical procedure to that used for compound **93**. Yield: 9.02g, 53%.

Step 6. Preparation of compound 116a

Compound **116a** is prepared from compound **115a** (8g, 6.3mmol) using an identical procedure to that used for compound **11a**. Yield: 3.23g, 39%.

Step 7. Preparation of compound 117a

Compound **117a** is prepared from compound **116a** (3.23g, 2.45mmol) and (2-oxo-2-phenyl-1λ^2-ethyl)glutamic acid (192mg, 1.1mmol) using an identical procedure to that used for compound **95**. Yield: 2.22g, 34%.

Step 8. Preparation of compound 118a

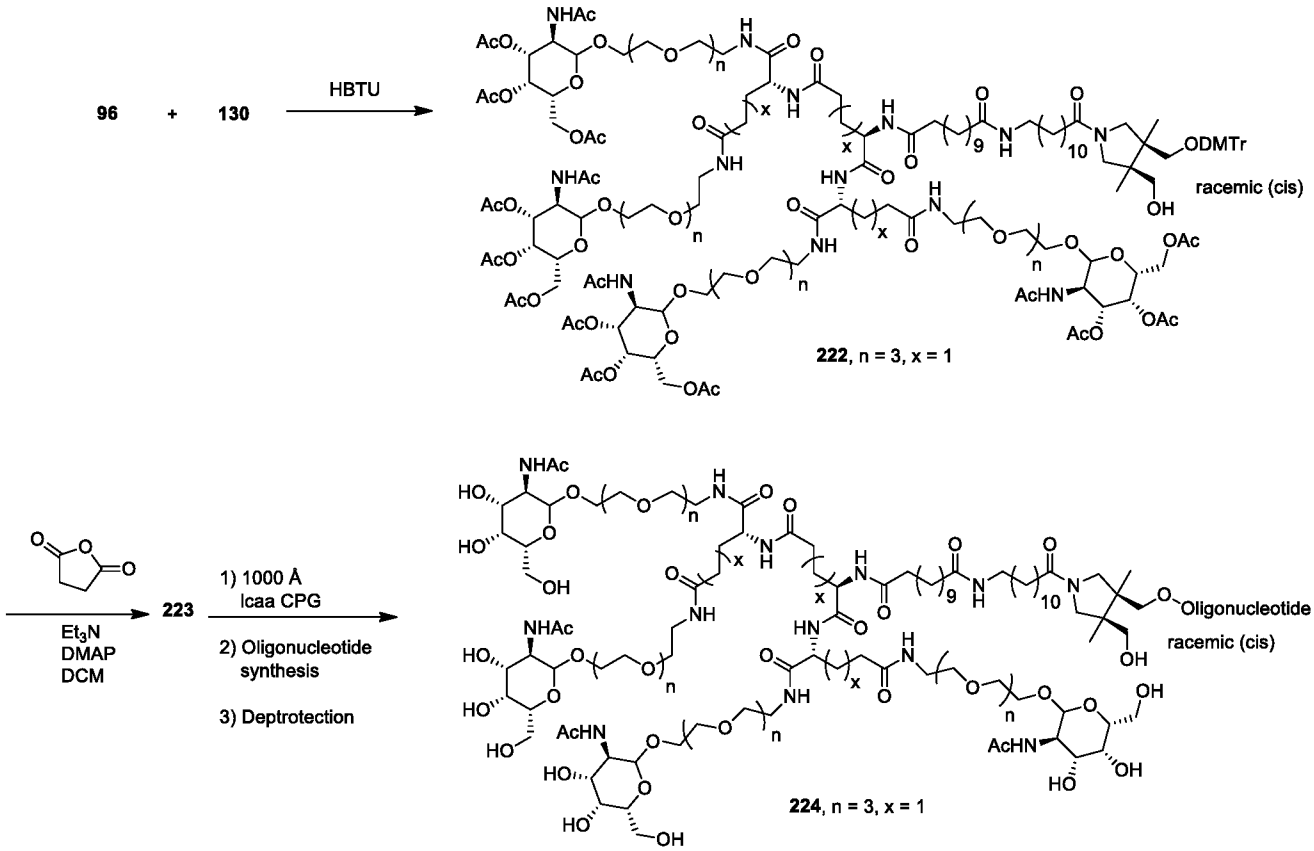
Compound **118a** is prepared from compound **117a** (2.22g, 0.84mmol) using an identical procedure to that used for compound **96**. Yield: 2.02g, 91%.

Step 9. Preparation of conjugates 21a8 and 221a

Conjugates **218a** and **22a1** are prepared from compounds **128** and **114a** or **118a** using an identical procedure to that used for compound **1**.

Example 21. Synthesis of conjugate 224

Scheme 45.

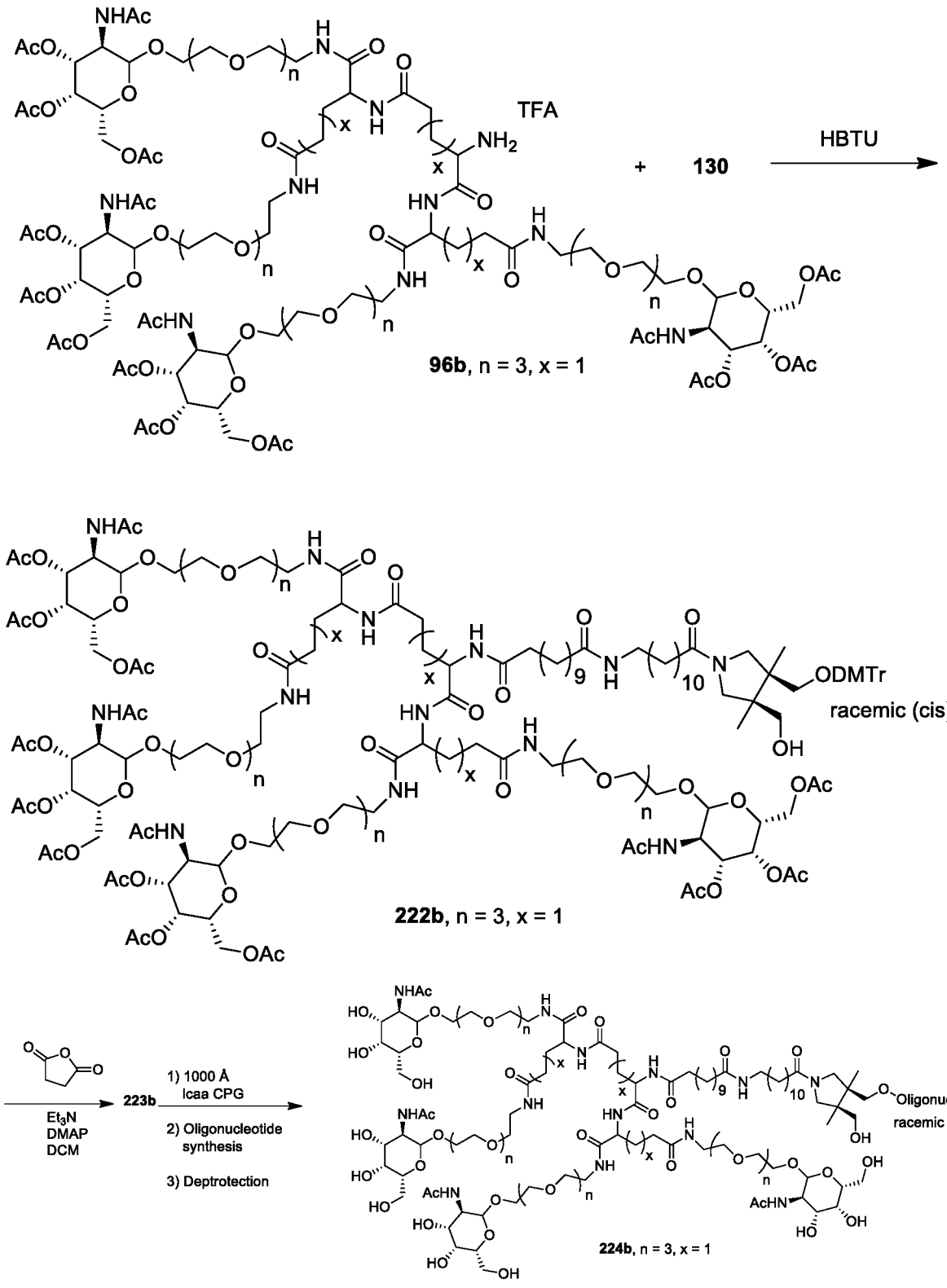


Step 1. Preparation of compounds 224

Conjugate **224** was prepared from compounds **96** and **130** using an identical procedure to that used for compound **1**.

Example 21a. Synthesis of conjugate 224b

Scheme 45a.

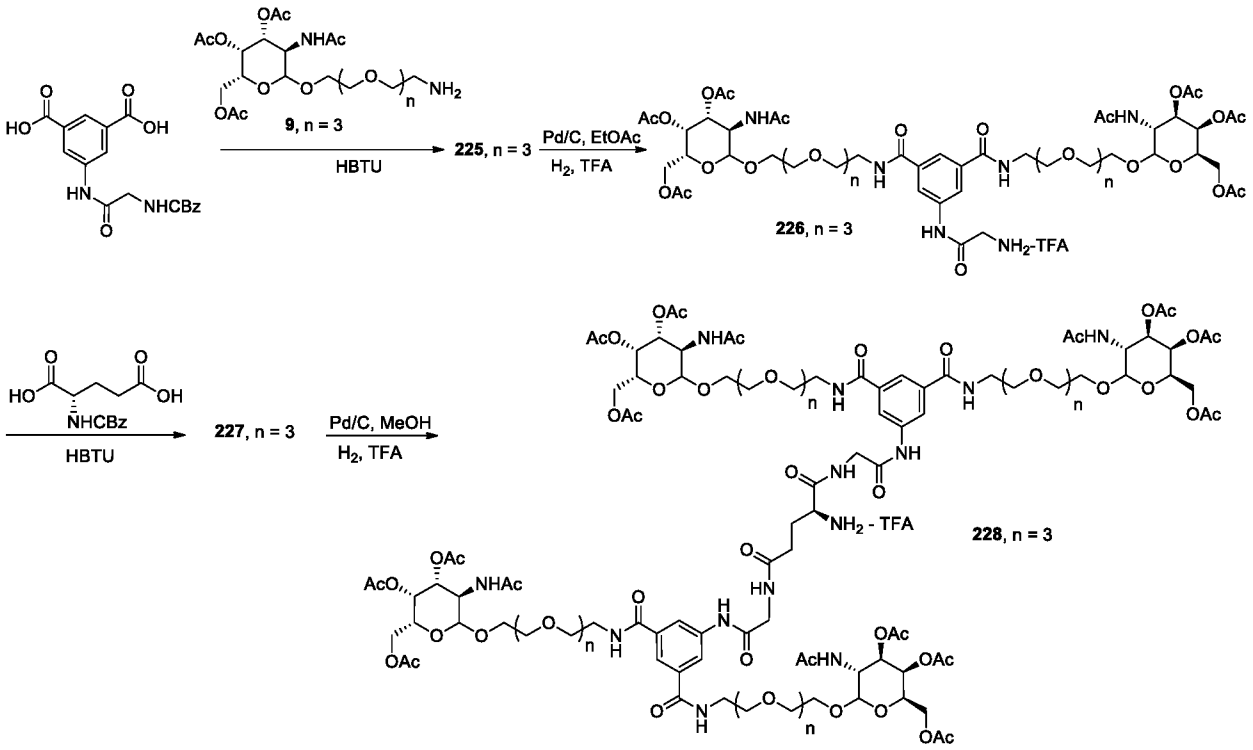


Step 1. Preparation of compounds 224b

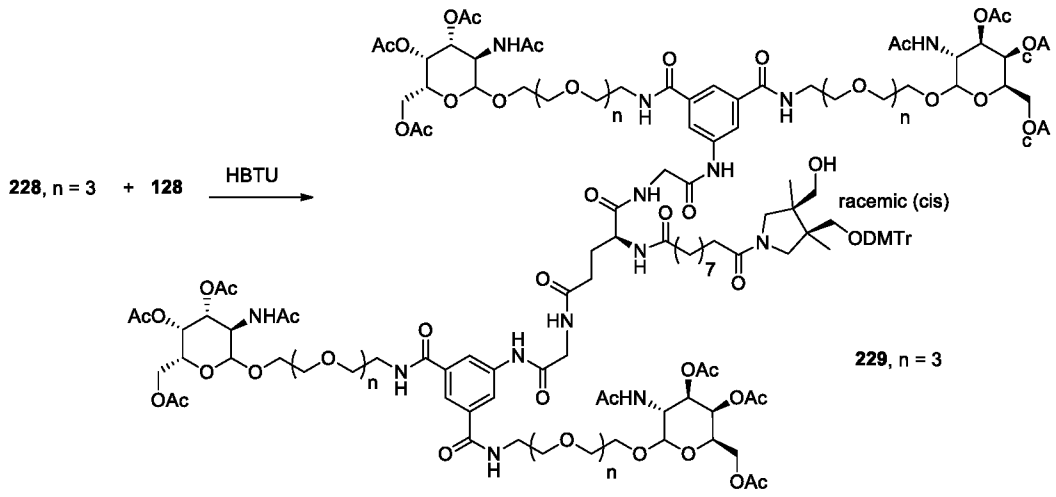
Conjugate **224b** is prepared from compounds **96b** and **130** using an identical procedure to that used for compound **1**.

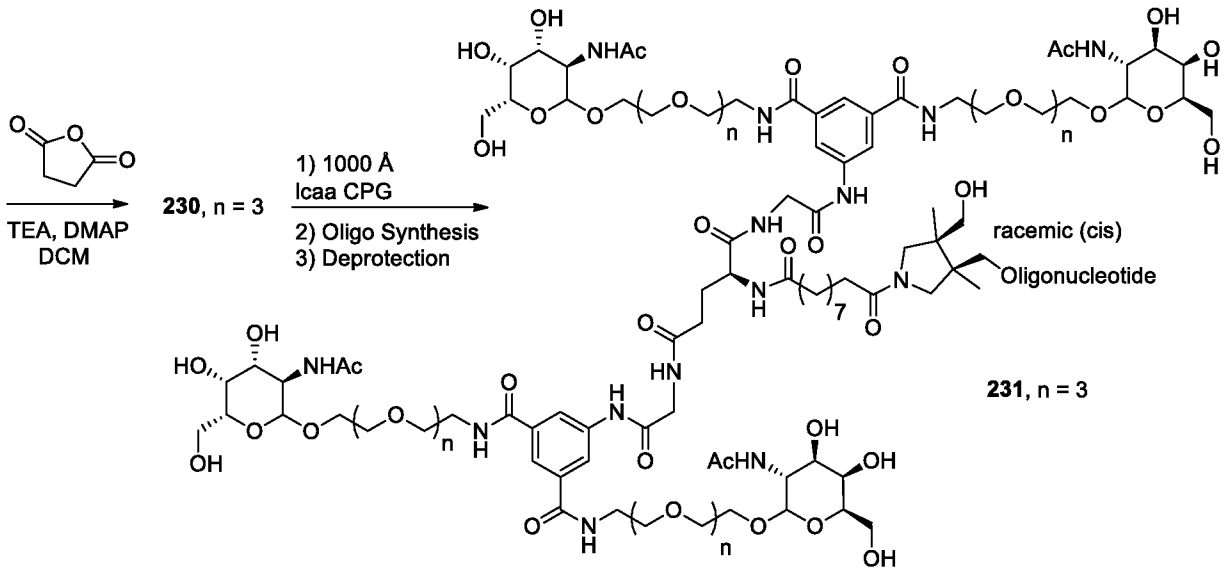
Example 22 Synthesis of Conjugate 231

Scheme 46



Scheme 47





Step 1 Preparation of compound 225

Compound **225** was prepared from 5-(2-aminoacetamido)isophthalic acid **106** (560mg, 1.5mmol) and **9** (2.24g, 3.6mmol) using an identical procedure to that used for **89**. Yield 1.6g, 80%.

Step 2 Preparation of compound 226

Compound **226** was prepared in the same fashion as **14**. Yield 1.22g, 78%.

Step 3 Preparation of compound 227

Compound **227** was prepared in the same fashion as **89**, from Z-glutamic acid (108mg, 0.38mmol) and **226** (1.22g, 0.92mmol). Yield 471mg, 45%.

Step 4 Preparation of compound 228

Compound **228** was prepared in the same fashion as **14**. Yield 460mg, Quant.

Step 5 Preparation of compound 229

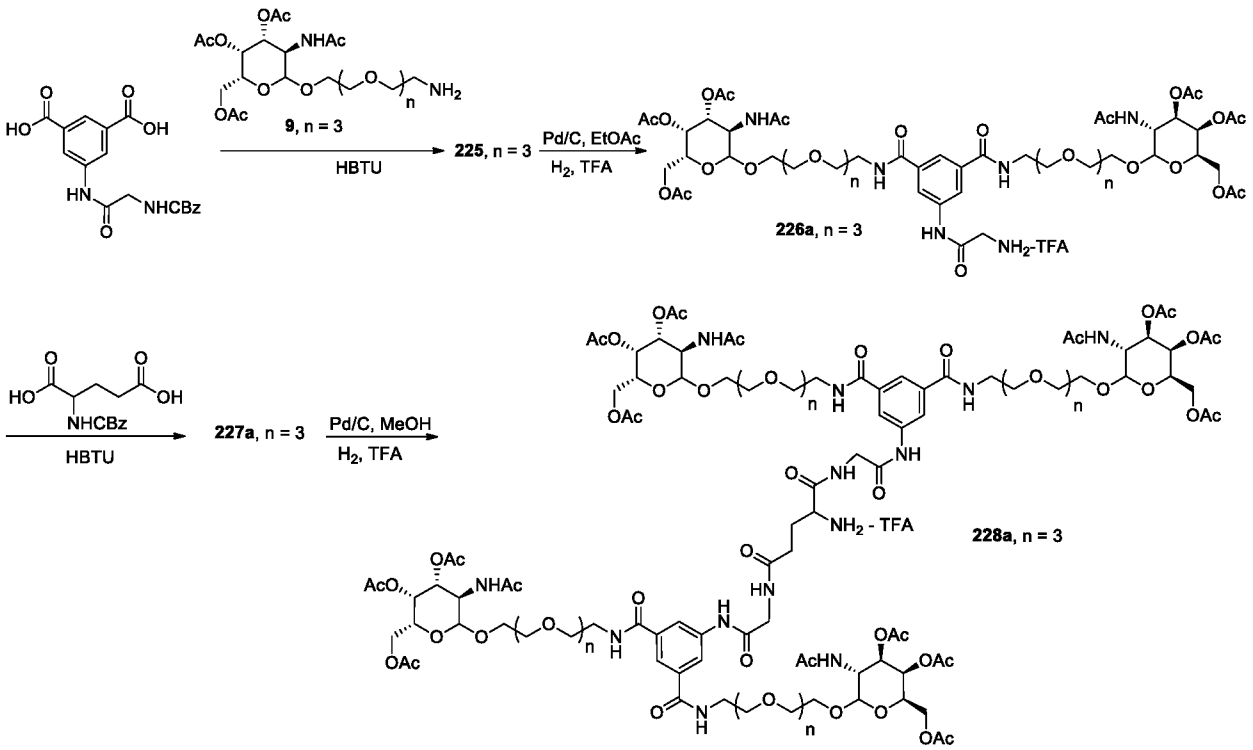
Compound **229** was prepared from **228** (460mg, 0.17mmol) and 128 (125mg, 0.19mmol) in the same fashion as **89**. Yield 365mg, 66%.

Step 6 Preparation of compound 231

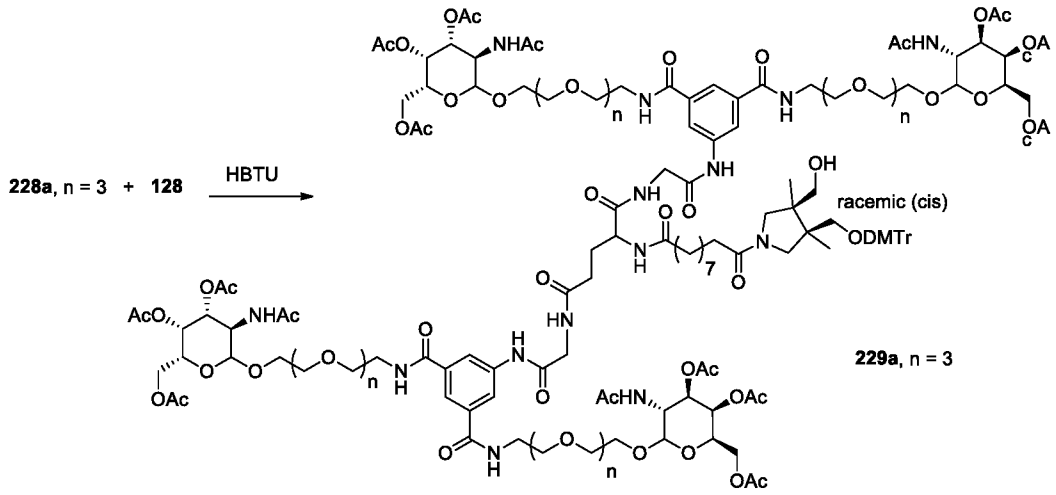
Conjugate **231** was prepared using an identical procedure to that used for compound **1**.

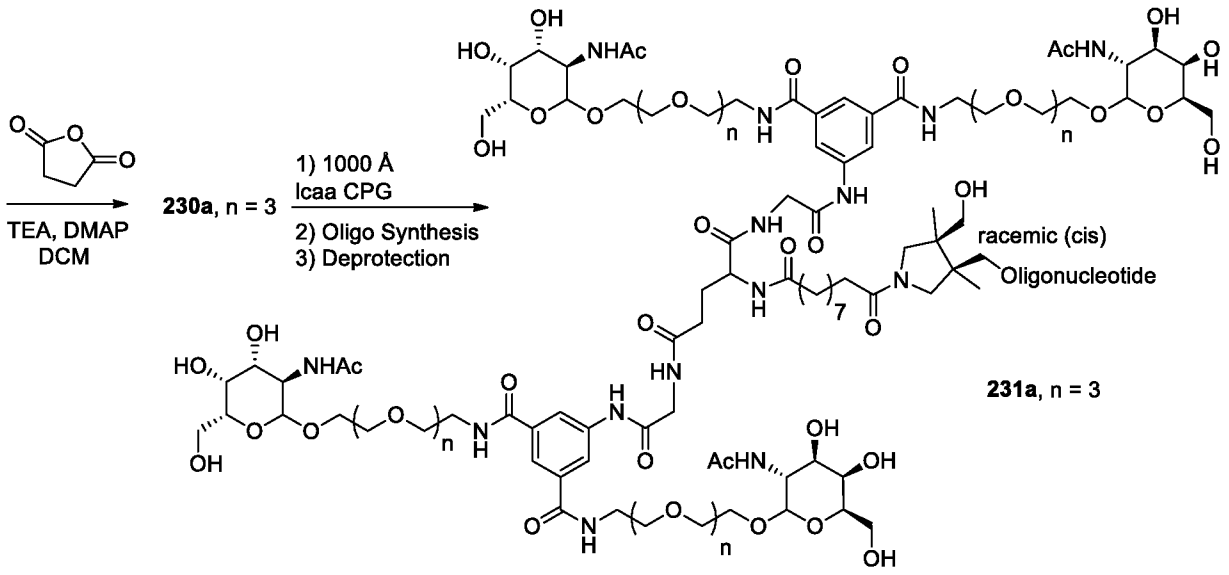
Example 22a Synthesis of Conjugate 231a

Scheme 46a



Scheme 47a





Step 1 Preparation of compound 225a

Compound **225a** is prepared from 5-(2-aminoacetamido)isophthalic acid **106** (560mg, 1.5mmol) and **9** (2.24g, 3.6mmol) using an identical procedure to that used for **89**. Yield 1.6g, 80%.

Step 2 Preparation of compound 226a

Compound **226a** is prepared in the same fashion as **14**. Yield 1.22g, 78%.

Step 3 Preparation of compound 227a

Compound **227a** is prepared in the same fashion as **89**, from Z-glutamic acid (108mg, 0.38mmol) and **226a** (1.22g, 0.92mmol). Yield 471mg, 45%.

Step 4 Preparation of compound 228a

Compound **228a** is prepared in the same fashion as **14**. Yield 460mg, Quant.

Step 5 Preparation of compound 229a

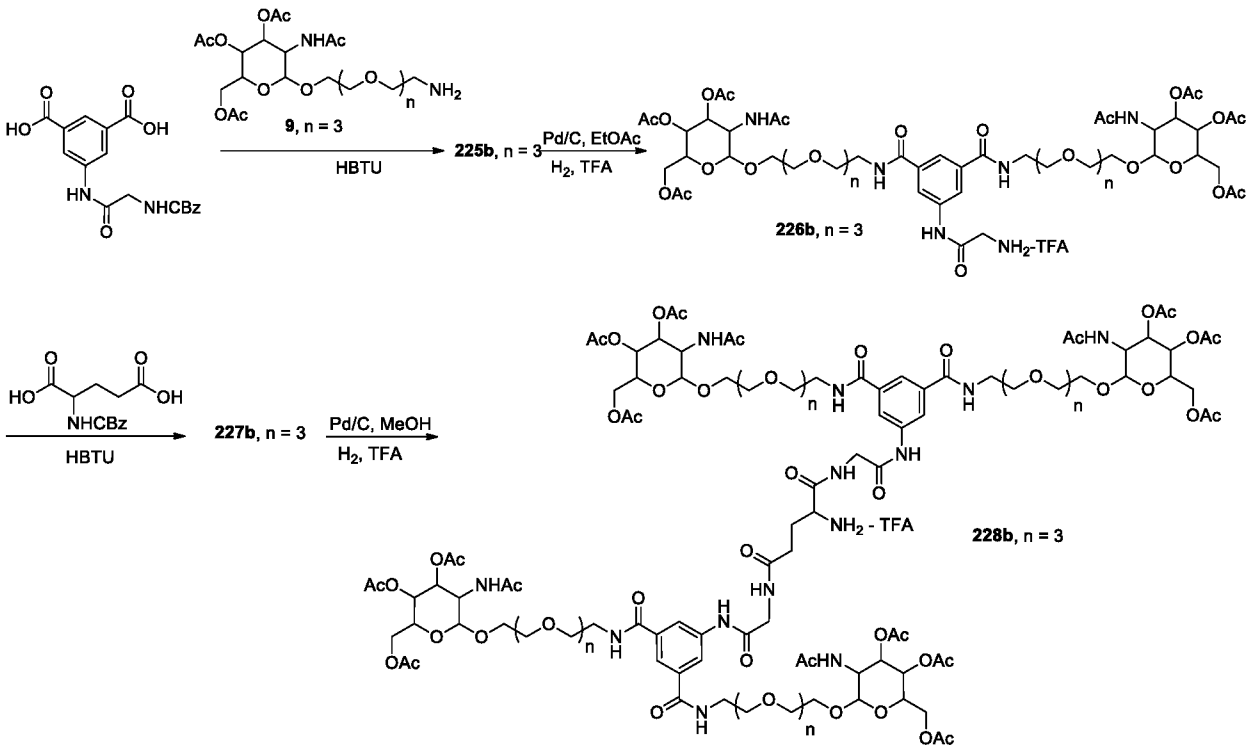
Compound **229a** is prepared from **228a** (460mg, 0.17mmol) and 128 (125mg, 0.19mmol) in the same fashion as **89**. Yield 365mg, 66%.

Step 6 Preparation of compound 231a

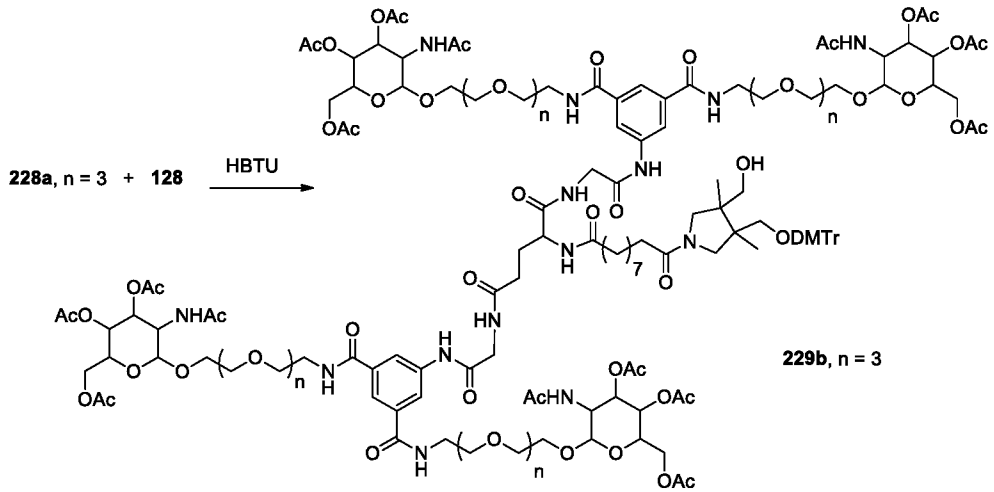
Conjugate **231a** is prepared using an identical procedure to that used for compound **1**.

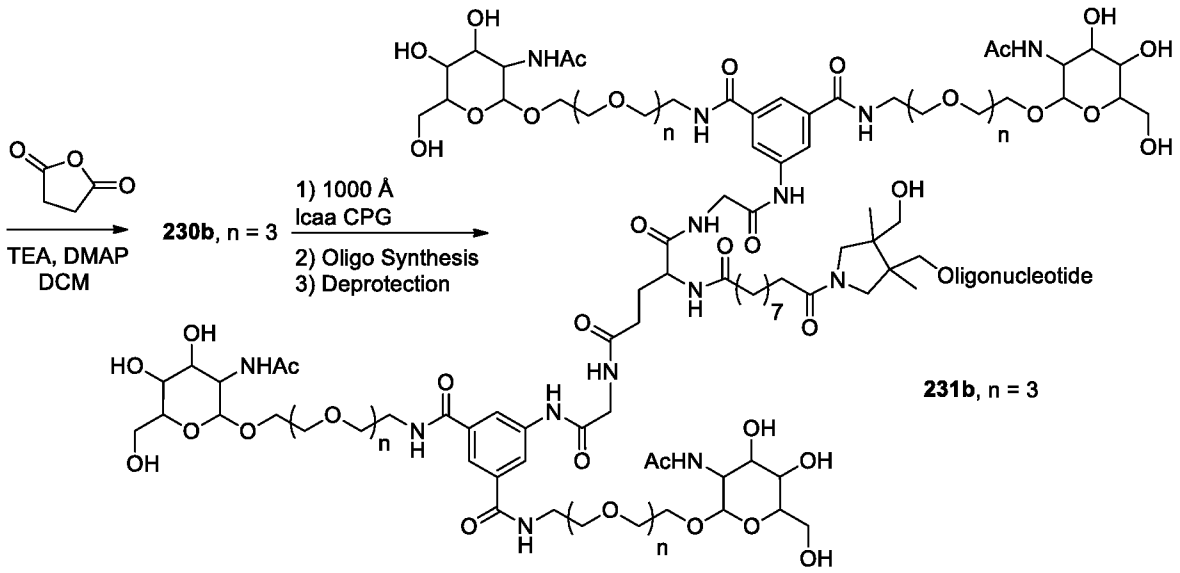
Example 22b Synthesis of Conjugate 231b

Scheme 46b



Scheme 47b





Step 1 Preparation of compound 225b

Compound **225b** is prepared from 5-(2-aminoacetamido)isophthalic acid **106** (560mg, 1.5mmol) and **9** (2.24g, 3.6mmol) using an identical procedure to that used for **89**. Yield 1.6g, 80%.

Step 2 Preparation of compound 226b

Compound **226b** is prepared in the same fashion as **14**. Yield 1.22g, 78%.

Step 3 Preparation of compound 227b

Compound **227b** is prepared in the same fashion as **89**, from Z-glutamic acid (108mg, 0.38mmol) and **226b** (1.22g, 0.92mmol). Yield 471mg, 45%.

Step 4 Preparation of compound 228b

Compound **228b** is prepared in the same fashion as **14**. Yield 460mg, Quant.

Step 5 Preparation of compound 229b

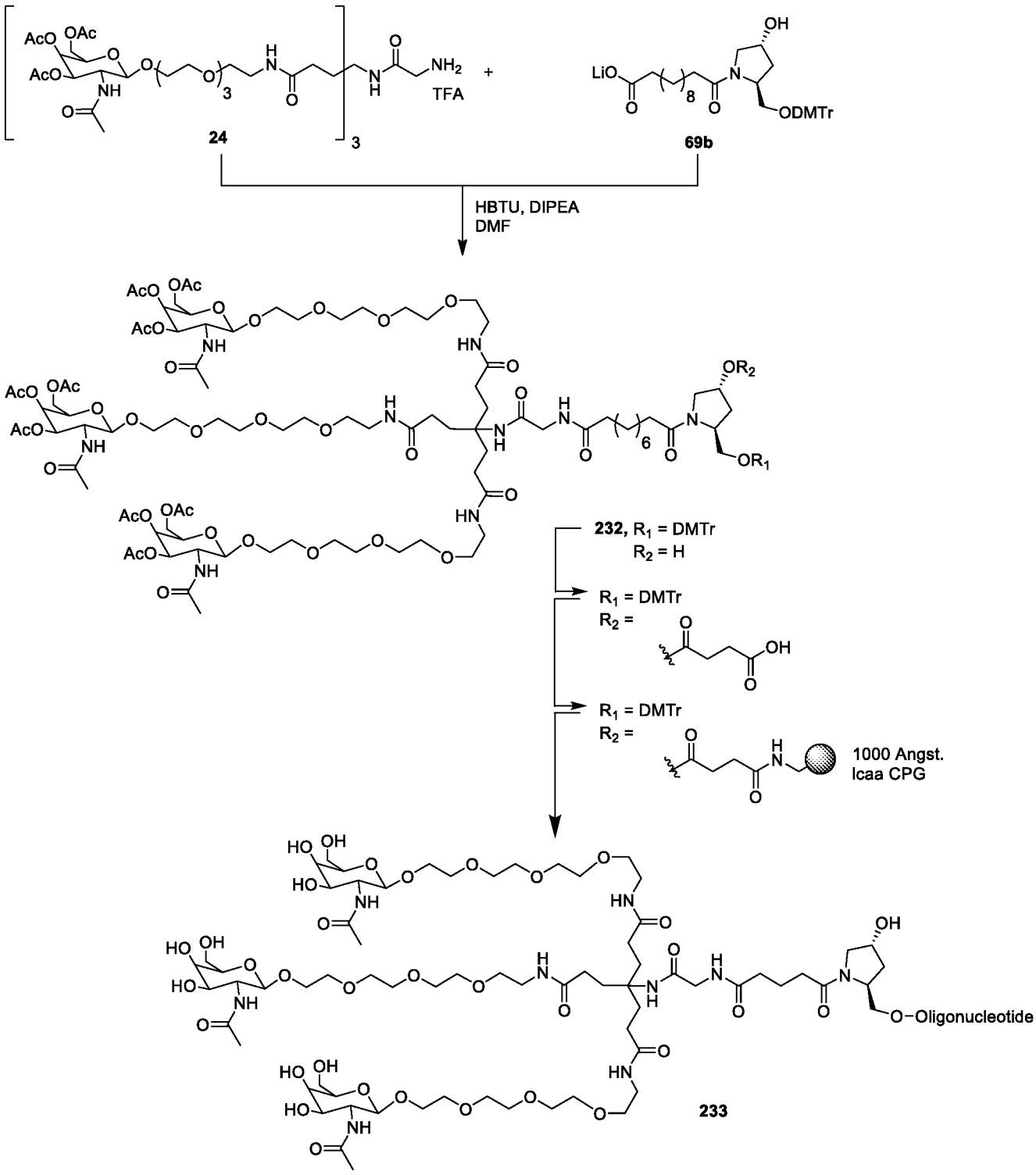
Compound **229b** is prepared from **228b** (460mg, 0.17mmol) and 128 (125mg, 0.19mmol) in the same fashion as **89**. Yield 365mg, 66%.

Step 6 Preparation of compound 231b

Conjugate **231b** is prepared using an identical procedure to that used for compound **1**.

Example 23. Synthesis of conjugate 233

Scheme 48



Step 1. Preparation of compound 232

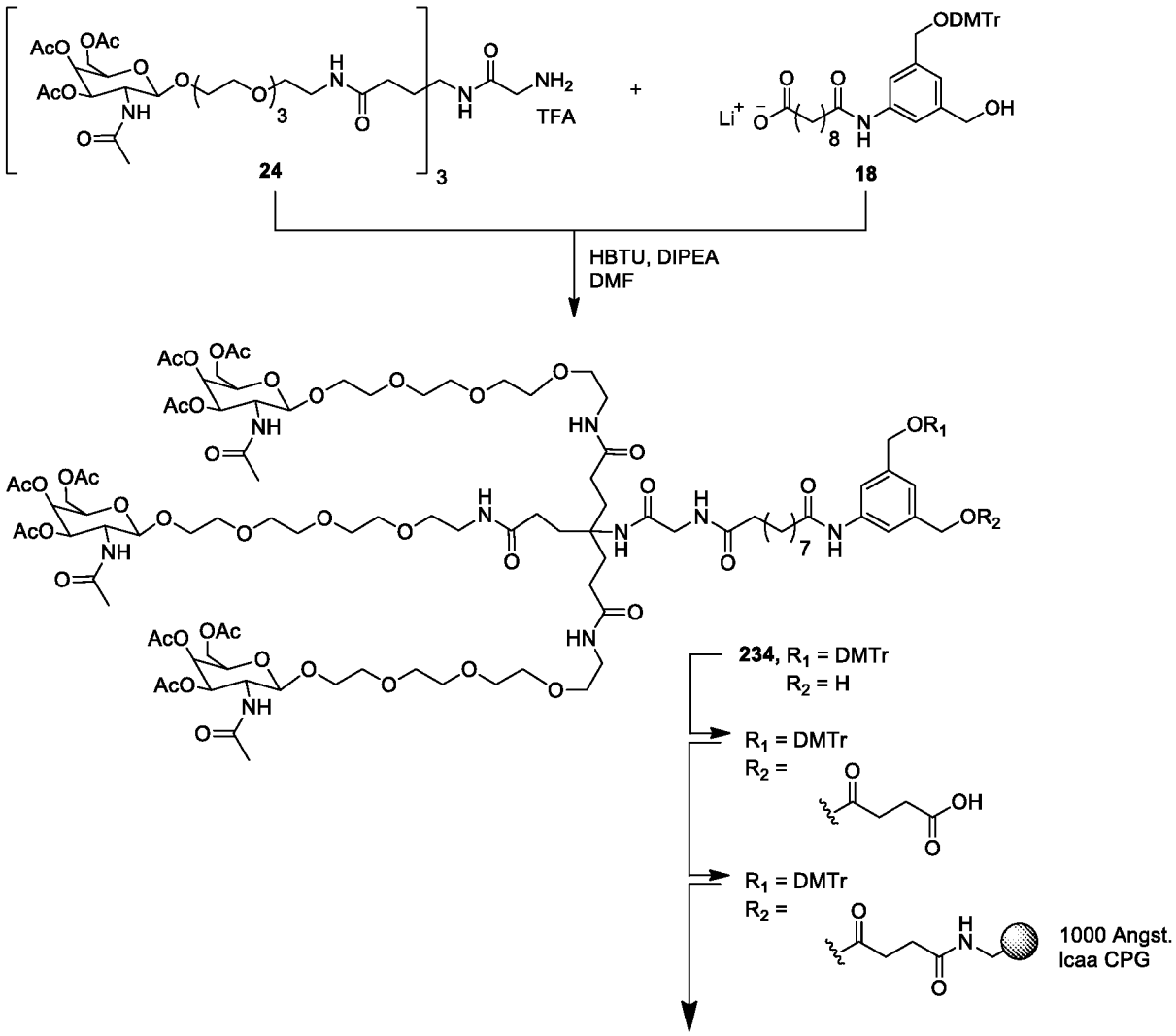
Compound **232** was prepared from compound **24** (650 mg, 0.33 mmol) and compound **69b** (175 mg, 0.33 mmol) using an identical procedure to that used for compound **19**. Yield: 380 mg, 47%.

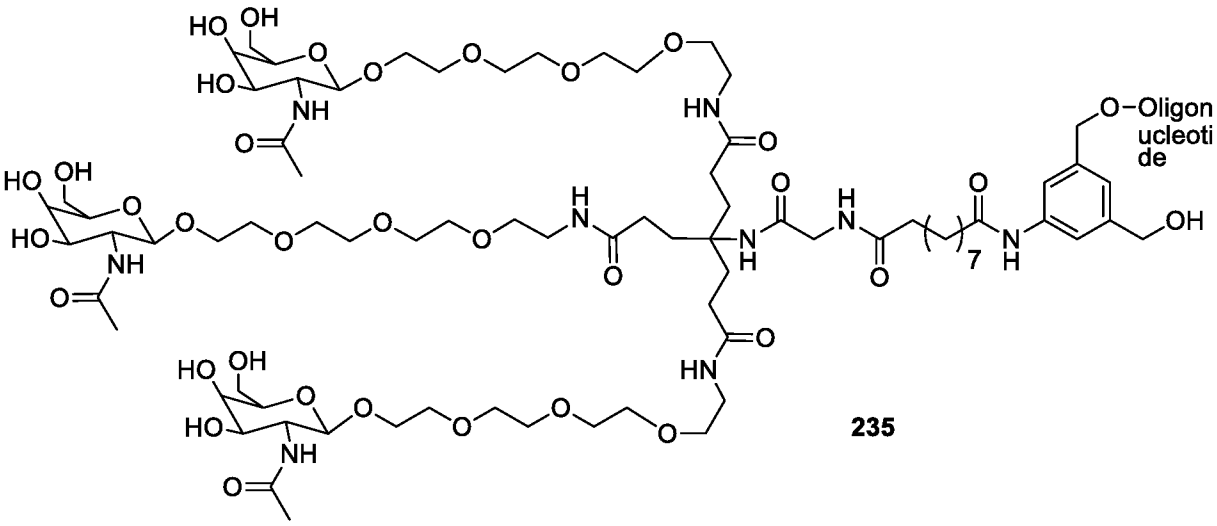
Step 2. Preparation of compound 233

Compound **233** was prepared from compound **232** using identical procedures to that used for compound **1**.

Example 24. Synthesis of conjugate 235

Scheme 49





Step 1. Preparation of compound 234

Compound **234** was prepared from compound **24** (1.1 g, 0.55 mmol) and compound **18** (175 mg, 0.33 mmol) using an identical procedure to that used for compound **19**. Yield: 685 mg, 51%.

Step 2. Preparation of compound 235

Compound **235** was prepared from compound **234** using identical procedures to that used for compound **1**.

Example 25. In vivo testing of HBV siRNA conjugates

Chronic HBV infection is a worldwide disease with progressing damage to the liver. Current treatments available may reduce the viral DNA but have had little effect on the viral antigens that contribute greatly to the disease progression. Thus, siRNAs to target HBV to reduce the viral antigens were designed.

Chemically modified HBV siRNA described in Table 1 conjugated to GalNAc ligands were tested for in vivo activity in an established mouse model of HBV infection. In the AAV-HBV1.2 C57BL/6 mouse model, stable and persistent HBV expression is achieved after injection of an adeno-associated virus (AAV) vector encoding an over-genomic length sequence of HBV, leading to hepatic expression of HBV RNA and proteins and the secretion of viral and sub-viral particles into the blood.

The AAV-HBV1.2 construct used in these studies was based on details provided in Dion, S., et al., Journal of Virology, 2013, 87(10): 5554–5563. All animal-related procedures were conducted according to written operating procedures, in accordance with Canadian

Council on Animal Care (CCAC) Guidelines on Good Animal Practices, and approved by the local Institutional Animal Care and Use Committee (IACUC).

Each animal was inoculated with 1E11 vector genomes (VG) of AAV-HBV1.2 vector. Prior to treatment, all animals were test bled and serum HBsAg levels determined for individual animals to confirm established HBV expression.

siRNA treatment: Groups of mice (typically n = 5) were administered a single 3 mg/kg dose of HBV siRNA conjugate once on Day 0 (1 dose per animal) via subcutaneous injection in the scapular region. One group of animals administered vehicle only (saline) served as controls.

Collections: All mice were test bled on Day 0, prior to treatment, and at defined time points after test article administration (for example on study days 7, 14, 21, 28, 35, 42, 49, 56, 63, and 70) to determine maximum reductions in serum HBsAg levels and the duration of pharmacologic activity.

Analysis: HBsAg levels in serum samples were determined using the Biorad EIA GS HBsAg 3.0 kit (BioRad, catalog no. 32591) as per the manufacturer's instructions. Pooled serum from each treatment group was used to determine the group mean HBsAg levels at individual time points. Data was analyzed and expressed as HBsAg levels relative to pre-treatment baseline (% relative to Day 0).

Results: Results from testing each of the chemically modified HBV siRNA described in Table 1 are presented in Table 2. Values represent % HBsAg levels (relative to Day 0 baseline) on Days 7, 14, 21, 28, 42, 49, 56 and 70 post treatment.

Table 1. Chemically Modified HBV siRNA duplexes

siRNA Number	Sense strand SEQ ID NO	Sense strand 5' – 3'	Antisense strand SEQ ID NO	Antisense strand 5'-3'
1	SEQ ID NO:1	csgsugugCaCUUcgcuucaccu	SEQ ID NO:2	asGsgugAaGCgaagUgCacacgsgsuUU
2	SEQ ID NO:3	usgsCaCUUcgcuucaccu	SEQ ID NO:4	asGsgugAaGCgaagUgCacascsgU
3	SEQ ID NO:5	usgscaCUUcgcuucaccu	SEQ ID NO:6	asGsgugaagcgaagUgCacascsgU
4	SEQ ID NO:7	usgscaCUUCgcuucaccu	SEQ ID NO:8	asGsgugAagcgaagUgCacascsgU
5	SEQ ID NO:9	CscsGuGuGcACUucGcuuCacc	SEQ ID NO:10	gsGsUgAaGcgAaguGcAcAcGgsusc
6	SEQ ID NO:11	cscsguguGcACUucgcuucacc	SEQ ID NO:12	gsGsugaAgCGaaguGcAcacggsusc
7	SEQ ID NO:13	cscsguGuGcAcUucgcuucacc	SEQ ID NO:14	gsGsugaAgCGaaguGcAcacggsusc
8	SEQ ID NO:15	cscsguguGcACUucgcuuCacc	SEQ ID NO:16	gsGsugaAgCgaaguGcAcacGgsusc
9	SEQ ID NO:17	cscsgugugcACUucgcuucacc	SEQ ID NO:18	gsGsugaagcgaaguGcAcacggsusc

siRNA Number	Sense strand SEQ ID NO	Sense strand 5' – 3'	Antisense strand SEQ ID NO	Antisense strand 5'-3'
10	SEQ ID NO:19	cscsguguGcacuucgcuucacc	SEQ ID NO:20	gsgsugaAgCGaagugcacacggsusc
11	SEQ ID NO:21	CscsGuGuGcACUucGcuuCacc	SEQ ID NO:22	gsGsUgAaGcgAaguGcAcAcGgsuscUU
12	SEQ ID NO:23	cscsguguGcACUucgcuucacc	SEQ ID NO:24	gsGsugaAgCGaaguGcAcacggsuscUU
13	SEQ ID NO:25	cscsguGuGcAcUucgcuucacc	SEQ ID NO:26	gsGsugaAgCGaaguGcAcacggsuscUU
14	SEQ ID NO:27	cscsguguGcACUucgcuuCacc	SEQ ID NO:28	gsGsugaAgCgaaguGcAcacGgsuscUU
15	SEQ ID NO:29	GsusGcACUucGcuuCacc	SEQ ID NO:30	gsGsUgAaGcgAaguGcAcAcsGsgU
16	SEQ ID NO:31	GsusGcACUucGcuuCacc	SEQ ID NO:32	gsGsUgAaGcgAaguGcAcAcsGsg
17	SEQ ID NO:33	GsusGcACUucGcuuCacc	SEQ ID NO:34	gsGsUgAaGcgAaguGcAcsAscsGsg
18	SEQ ID NO:35	CscsGuGuGcACUucGcuuCaca	SEQ ID NO:36	usGsUgAaGcgAaguGcAcAcGgsusc
19	SEQ ID NO:37	CscsGuGuGcACUucGcuuCaca	SEQ ID NO:38	usGsUgAaGcgAaguGcAcAcGgsuscUU
20	SEQ ID NO:39	cscsguguGcACUucgcuucaca	SEQ ID NO:40	usGsugaAgCGaaguGcAcacggsuscUU
21	SEQ ID NO:41	cscsguGuGcAcUucgcuucaca	SEQ ID NO:42	usGsugaAgCGaaguGcAcacggsuscUU
22	SEQ ID NO:43	cscsguguGcACUucgcuuCaca	SEQ ID NO:44	usGsugaAgCgaaguGcAcacGgsuscUU
23	SEQ ID NO:45	cscsgugugcACUucgcuucaca	SEQ ID NO:46	usGsugaagcgaaguGcAcacggsuscUU
24	SEQ ID NO:47	gsusGcACUucgcuucaca	SEQ ID NO:48	usGsugaAgCGaaguGcAcacsgsgU
25	SEQ ID NO:49	gsusgcACUucgcuucaca	SEQ ID NO:50	usGsugaagcgaaguGcAcacsgsgU
26	SEQ ID NO:51	gsusGcaCUucgcuucaca	SEQ ID NO:52	usGsugaagcgaaguGcAcacsgsgU
27	SEQ ID NO:53	GsusGcACUucGcuuCaca	SEQ ID NO:54	usGsUgAaGcgAaguGcAcAcsGsg
28	SEQ ID NO:55	uscsgcuuCaCCUcugcacgucg	SEQ ID NO:56	csGsacgUgCAgaggUgAagcgasasgUU
29	SEQ ID NO:57	uscsgcuuCaCCUcugcacguca	SEQ ID NO:58	usGsacgUgCAgaggUgAagcgasasgUU
30	SEQ ID NO:59	uscsgcUuCaCcUcugcacguca	SEQ ID NO:60	usGsacgUgCAgaggUgAagcgasasgUU
31	SEQ ID NO:61	ususCaCCUcugcacguca	SEQ ID NO:62	usGsacgUgCAgaggUgAagcsgsaU
32	SEQ ID NO:63	ususcaCCUcugcacguca	SEQ ID NO:64	usGsacgugcagaggUgAagcsgsaU
33	SEQ ID NO:65	ususCaCCUcugcacguca	SEQ ID NO:66	usGsacgUgcagaggUgAagcsgsaU
34	SEQ ID NO:67	ususuaCuAgUGCcaUuuguuca	SEQ ID NO:68	usGsAaCaAauGgcaCuAgUaAascsu
35	SEQ ID NO:69	ususuaCuAgUGCcaUuuguuca	SEQ ID NO:70	usGsAaCaAauGgcaCuAgUaAascsuUU
36	SEQ ID NO:71	ususuacuAgUGCcauuuguuca	SEQ ID NO:72	usGsaacAaAUggcaCuAguaaascsuUU
37	SEQ ID NO:73	ususuaCuAgUgCcauuuguuca	SEQ ID NO:74	usGsaacAaAUggcaCuAguaaascsuUU

2'-O-Methyl nucleotides = lower case; 2'-Fluoro nucleotides = UPPER CASE;
Phosphorothioate linker = s; Unmodified = UPPER CASE

Table 2. Serum HBsAg levels in mice after single subcutaneous administration (3 mg/kg) of GalNAc conjugated siRNA from Table 1.

HBsAg data expressed as percent of baseline (Day 0) values

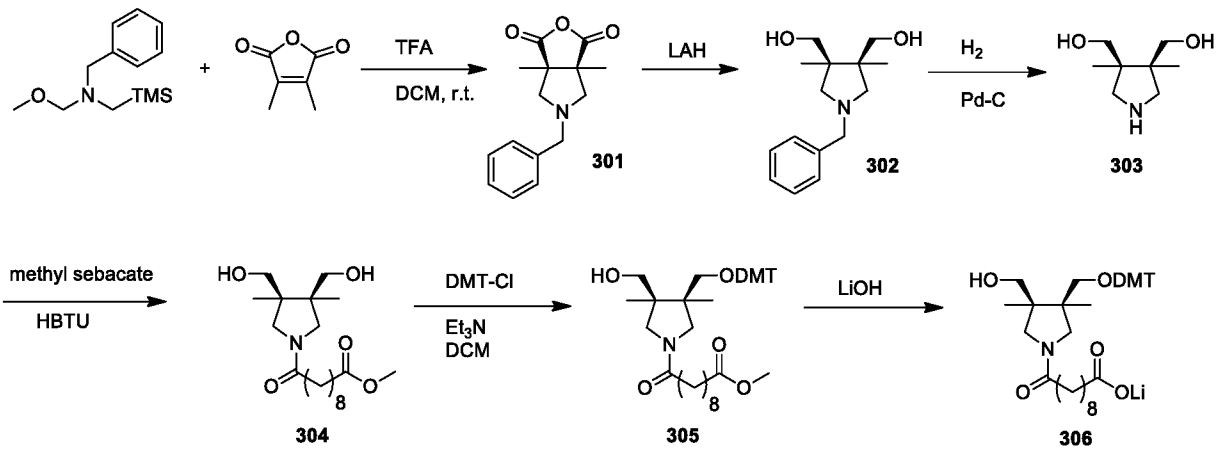
siRNA Number	Ligand Cpd #	Day 7	Day 14	Day 21	Day 28	Day 42	Day 49	Day 56	Day 70
Saline		95.7	118.6	101.0	111.4	115.3		112.2	106.5
1	145	3.1	1.0	22.8	1.3		3.2		7.3
2	145	1.3	0.5	0.4	0.7		3.4		6.5
5	235	12.7	7.0	9.6	21.3	59.7		74.6	98.8
5	233	16.6	8.2	11.0	12.6	24.4		32.2	60.3
5	145	4.3	1.2	1.7	2.4		11.9		24.5
6	233	20.1	10.4	10.9	13.5	30.8		46.0	67.5
7	233	20.7	12.4	10.5	13.6	24.9		46.2	77.0
8	233	18.1	10.5	11.5	12.1	26.0		37.6	64.9
9	145	10.0	2.7	2.0	3.6		6.8		17.0
10	145	16.7	16.7	16.0	16.7		74.4		97.3
11	233	20.5	14.8	16.0	23.9	65.2		80.2	
12	233	18.4	11.6	12.2	14.1	23.6		67.1	72.6
12	145	5.1	1.1	1.2	1.0	2.2		4.5	8.2
13	233	20.7	10.1	11.6	13.2	21.1		39.9	72.3
14	233	16.5	8.0	11.0	11.8	28.8		48.0	90.0
15	145	6.3	3.5	8.4	11.4		89.7		83.1
16	145	4.0	3.4	9.7	14.8		85.1		88.9
17	145	2.4	0.6	0.7	1.1		6.3		15.1
18	233	2.5	1.0	1.3	2.6	11.2		24.5	55.6
19	233	1.9	0.8	1.5	2.6	6.5		12.9	23.4
19	145	1.7	0.6	0.7	1.4	3.8		7.3	15.0
19	200	1.8	0.9	1.4	2.2	5.4		10.2	27.5
19	197	2.0	0.8	1.4	2.1	3.1		8.4	14.2
19	194	2.8	1.8	2.2	4.0	10.7		26.0	37.3
20	145	2.7	0.5	0.7	1.0	4.7		9.3	11.3
20	215	3.4	1.5	1.7	1.7	1.9		4.5	6.2
20	194	1.4	0.5	0.3	0.7	1.2		3.0	6.0
20	197	3.4	0.6	1.0	1.3	2.1		4.9	8.2
20	212	3.2	0.8	1.0	1.9	2.4		4.9	7.5

siRNA Number	Ligand Cpd #	Day 7	Day 14	Day 21	Day 28	Day 42	Day 49	Day 56	Day 70
20	191	3.3	1.4	1.4	2.1	1.9		1.2	3.4
21	215	2.5	1.1	1.9	2.6	3.8		7.8	9.8
22	233	2.5	2.0	3.1	6.1	12.2		30.4	61.9
23	215	1.6	0.3	0.3	0.3	0.4		1.0	1.7
24	197	1.9	0.4	0.4	0.4	0.8		1.7	3.2
25	197	2.1	2.2	0.9	0.5	0.9		2.0	2.2
27	145	0.3	0.3	1.6	7.4		71.1		100.1
28	145	11.4	6.7	7.1	9.6	20.8		27.1	36.7
29	145	2.9	1.7	2.1	3.3	7.9		21.4	18.2
30	145	10.0	3.8	3.5	5.9	13.7		19.0	28.8
34	233	13.2	7.4	8.9	16.8	55.2		60.5	
35	233	11.6	8.5	14.0	19.5	58.4		82.0	
36	145	11.3	8.5	11.6	12.5	36.6		49.7	64.7
37	145	27.8	21.6	25.9	31.1	49.9		43.3	64.5

Table 2 identifies the compound numbers (column 2) and the corresponding oligonucleotide (column 1) for the HBV siRNA conjugates that were tested.

Example 26 Synthesis of conjugate 320

Scheme 50 Preparation of activated linker



Step 1. Preparation of Racemic (cis) 5-Benzyl-3a,6a-dimethyltetrahydro-1H-furo[3,4-c]pyrrole-1,3(3aH)-dione 301

To a cooled solution (0°C) of 3,4-dimethylfuran-2,5-dione (3 g, 24 mmol) and N-benzyl-1-methoxy-N-((trimethylsilyl)methyl)methanamine (7 g, 29.8 mmol) in dichloromethane (75 mL) was slowly added trifluoroacetic acid (75 µL). Stir overnight

allowing the solution to slowly warm to room temperature as the ice bath melted. The reaction mixture was concentrated to dryness, dissolved in ethyl acetate (100 mL), washed with saturated sodium bicarbonate (2 x 100mL), dried on magnesium sulfate, filtered and concentrated to dryness. Purification by column chromatography on silica gel (gradient: 20% ethyl acetate in hexanes to 100% ethyl acetate) afforded (3aR,6aS)-5-Benzyl-3a,6a-dimethyltetrahydro-1H-furo[3,4-c]pyrrole-1,3(3aH)-dione as a yellow oil (3.5 g, 56%).

Step 2. Preparation of Racemic (cis) (1-Benzyl-3,4-dimethylpyrrolidine-3,4-diyl)dimethanol 302

To a cooled (0°C) solution of (3aR,6aS)-5-Benzyl-3a,6a-dimethyltetrahydro-1H-furo[3,4-c]pyrrole-1,3(3aH)-dione (3.5 g, 13.4 mmol) in anhydrous diethyl ether (50 mL) was added slowly lithium aluminum hydride pellets (1.5 g, 40 mmol) over three portions. The solution was stirred overnight warming to room temperature as the ice water bath melted. Upon completion, the reaction was cooled to 0°C and very slowly quenched with 1.5 mL of 5M NaOH followed by 1.5 mL of water. Stir for 30 minutes then add magnesium sulfate and filter. The filtrate was concentrated to afford ((3R,4S)-1-Benzyl-3,4-dimethylpyrrolidine-3,4-diyl)dimethanol as a colorless oil (2.7 g).

Step 3. Preparation of Racemic (cis) (3,4-Dimethylpyrrolidine-3,4-diyl)dimethanol 303

To a solution of ((3R,4S)-1-Benzyl-3,4-dimethylpyrrolidine-3,4-diyl)dimethanol (10 g, 40 mmol) in methanol (10 mL) was added 10% palladium on activated charcoal wet (1 g). The solution was stirred vigorously under a hydrogen atmosphere for 16 hours. Upon completion the solution was filtered through Celite, and concentrated to dryness to afford ((3R,4S)-3,4-Dimethylpyrrolidine-3,4-diyl)dimethanol as a colorless solid (5.5 g, 86%).

Step 4. Preparation of Racemic (cis) Methyl 10-(3,4-bis(hydroxymethyl)-3,4-dimethylpyrrolidin-1-yl)-10-oxodecanoate 304

A solution of **3** (1.3 g, 8.2 mmol) and monomethyl sebacate (1.8 g, 8.2 mmol) in CH_2Cl_2 (100mL) was treated with HBTU (3.41g, 9.02mmol) and Hunig's base (5.71mL, 32.8mmol). After stirring overnight the mixture was washed with $NaHCO_3$ (sat. aq.), water and brine, then dried ($MgSO_4$), filtered and concentrated. The crude material was subjected to chromatography (gradient: 0% CH_3OH-CH_2Cl_2 to 20%) to yield 4 (1.8g, 61%).

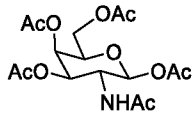
Step 5. Preparation of Racemic (cis) Methyl 10-(3-((bis(4-methoxyphenyl)(phenyl)-methoxy)methyl)-4-(hydroxymethyl)-3,4-dimethylpyrrolidin-1-yl)-10-oxodecanoate 305

A solution of **304** (1.8 g, 5.0 mmol) and 4,4′-Dimethoxytrityl chloride (1.7 g, 5.0 mmol) in pyridine (180mL) was stirred overnight. The pyridine was then removed under reduced pressure and the crude material was subjected to chromatography (gradient: 0% CH_3OH-CH_2Cl_2 to 10%) to yield **5** (1.4 g, 42%) as a yellow oil.

Step 6. Preparation of Racemic (cis) Lithium 10-(3-((bis(4-methoxyphenyl)-(phenyl)methoxy)methyl)-4-(hydroxymethyl)-3,4-dimethylpyrrolidin-1-yl)-10-oxodecanoate 306

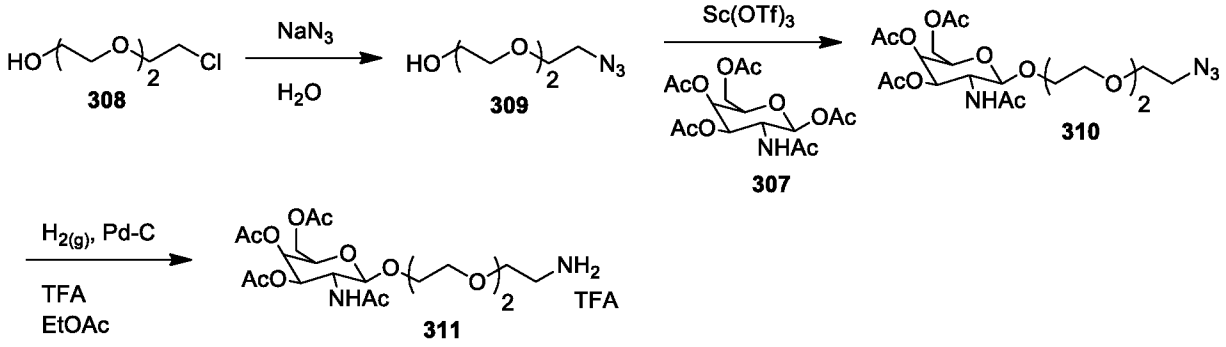
To a solution of compound **305** (3.0 g, 4.6 mmol) in THF (50 mL) and water (50 mL) was added lithium hydroxide (121 mg, 5.0 mmol). The solution was stirred for 4 hours at room temperature then concentrated to remove the THF. The remaining aqueous solution was freeze dried overnight to afford a pale pink solid (2.9 g, quantitative). Compound **306** was prepared as a mixture of two *cis*-diastereomers.

Scheme 51 Synthesis of peracetylated galactosamine 307



D-Galactosamine hydrochloride (250 g, 1.16 mol) in pyridine (1.5 L) was treated with acetic anhydride (1.25 L, 13.2 mol) over 45 minutes. After stirring overnight the reaction mixture was divided into three 1 L portions. Each 1 L portion was poured into 3 L of ice water and mixed for one hour. After mixing the solids were filtered off, combined, frozen over liquid nitrogen and then lyophilized for five days to yield peracetylated galactosamine **7** (369.4 g, 82%) as a white solid. Rf (0.58, 10% MeOH-CH_2Cl_2).

Scheme 52 Synthesis of GalNAc monomer



Step 1 Preparation of compound 309

A solution of 2-[2-(2-chloroethoxy)]ethanol **308** (100g, 593mmol) in water (1L) was treated with NaN_3 (77g, 1.19mol) and heated (90°C). After stirring (72 hours) the solution was cooled (RT) and extracted (4x) with CH_2Cl_2. The combined organics were washed with brine, dried ($MgSO_4$), filtered, concentrated and used without further processing. Compound **9** (88.9g, 86%) was obtained as a pale yellow oil.

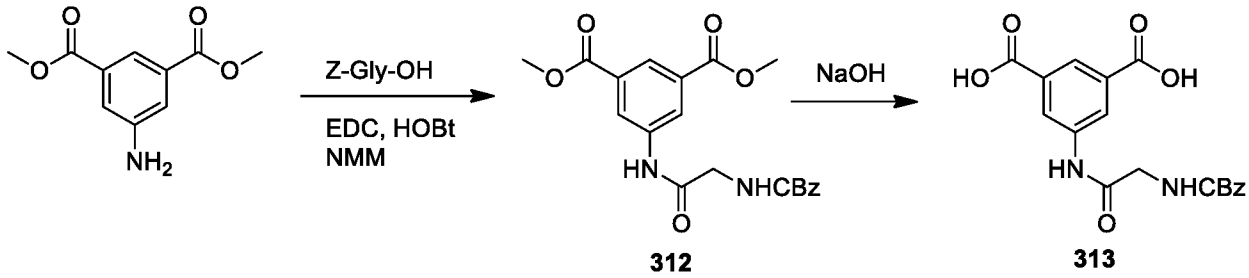
Step 2 Preparation of compound 310

A solution of **7** (2.76g, 7.1mmol) and **309** (1.37g, 7.8mmol) in 1,2-dichloroethane (40mL) was treated with $Sc(OTf)_3$ (174mg, 0.36mmol) and heated (85°C). After stirring (2 hours) the mixture was cooled (RT) and quenched by the addition of TEA (4mL) and concentrated. The crude material was subjected to chromatography to yield **310** (3.03g, 85%) as a pale yellow foam.

Step 3 Preparation of compound 311

A solution of **310** (3.02g, 5.99mmol) and Pd/C (300mg, 10% Pd loading - wet support) in EtOAc (30mL) was treated with TFA (576µL, 7.5mmol). The reaction mixture was purged with hydrogen gas (45min) then purged with nitrogen gas (10min), then filtered through celite. The filtrate was concentrated and then subjected to chromatography to yield **311** (2.67g, 75%) as a brown foam.

Scheme 53 Synthesis of aromatic core



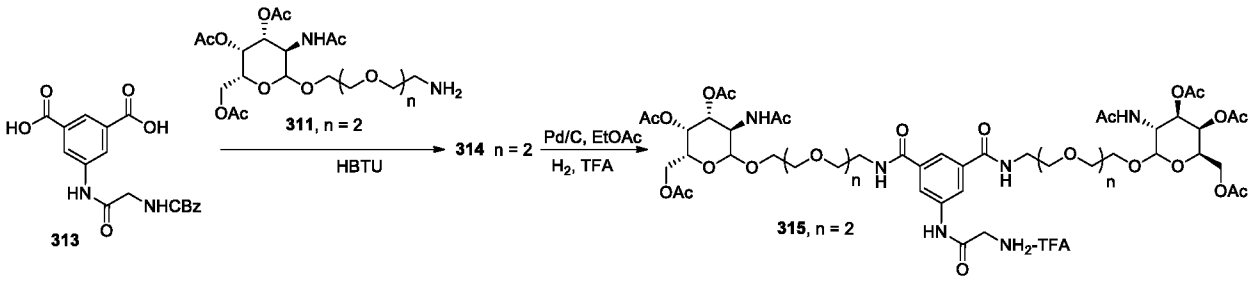
Step 1. Preparation of Dimethyl 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)-isophthalate 312

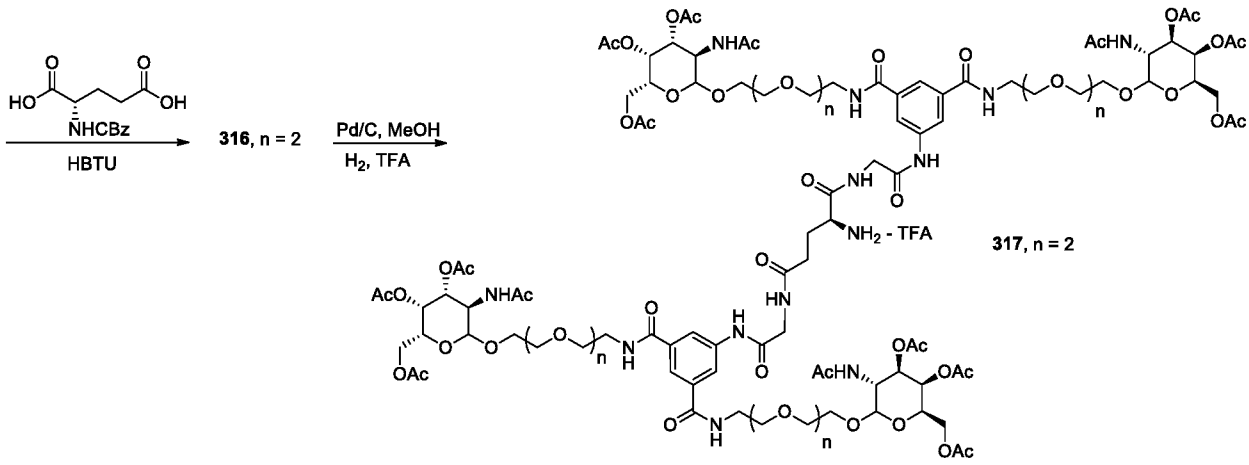
A solution of dimethyl 5-aminoisophthalate (5 g, 24 mmol), Z-Gly-OH (5 g, 24 mmol), EDC (5 g, 26.3 mmol), HOBt (3.6 g, 26.3 mmol), NMM (2.9 mL, 26.3 mmol) in DMF (50 mL) was stirred overnight at room temperature. Upon completion, the reaction mixture was diluted with ethyl acetate (250 mL) and washed with each 1M HCl (2 x 100 mL), saturated sodium bicarbonate (1 x 100 mL) and brine (2 x 100 mL). Dry on magnesium sulfate, filter and concentrate to dryness to afford Dimethyl 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)-acetamido)isophthalate as a colorless solid (7.2 g, 79%).

Step 2. Preparation of 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)isophthalic acid 313

To a solution of methyl 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)isophthalate (7.2 g) in methanol (25 mL) and THF (25 mL) was added 1M NaOH (25 mL). The solution was stirred at room temperature for 2 hours then concentrated to remove THF and MeOH. The aqueous solution remaining was diluted with water (75 mL), cooled on an ice water bath and acidified to pH = 1 with 6M HCl. The solid was filtered and washed with water (3 x 100 mL). The solid was freeze dried to afford 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)-isophthalic acid (6.9 g, quantitative) .

Scheme 54: Preparation of tetramer





Step 1 Preparation of compound 314

A solution of **313** (2.09g, 5.6mmol) and **311** (8.34g, 14.07mmol) in CH_2Cl_2 (150mL) was treated with HBTU (6.4g, 16.9mmol) and Hunig's base (7.35mL, 42.2mmol). After stirring (overnight) the reaction mixture was poured into $NaHCO_3$ (sat. aq.) then washed with water and brine, dried ($MgSO_4$), filtered and concentrated. The crude material was subjected to chromatography (gradient 1-12% CH_3OH-CH_2Cl_2) to yield 6 (3.97g, 55%) as a pale yellow foam.

Step 2 Preparation of compound 315

Compound **314** (3.92g, 3.07mmol), Pd/C (400mg, 10% loading – wet support) and trifluoroacetic acid (308µL, 4mmol) was purged with H_2. After stirring under H_2 (overnight), the mixture was purged with N_2 (15-20 min) then filtered through celite and concentrated. The crude material was subjected to chromatography to yield 7 (3.36g, 86%) as a white to cream colored foam.

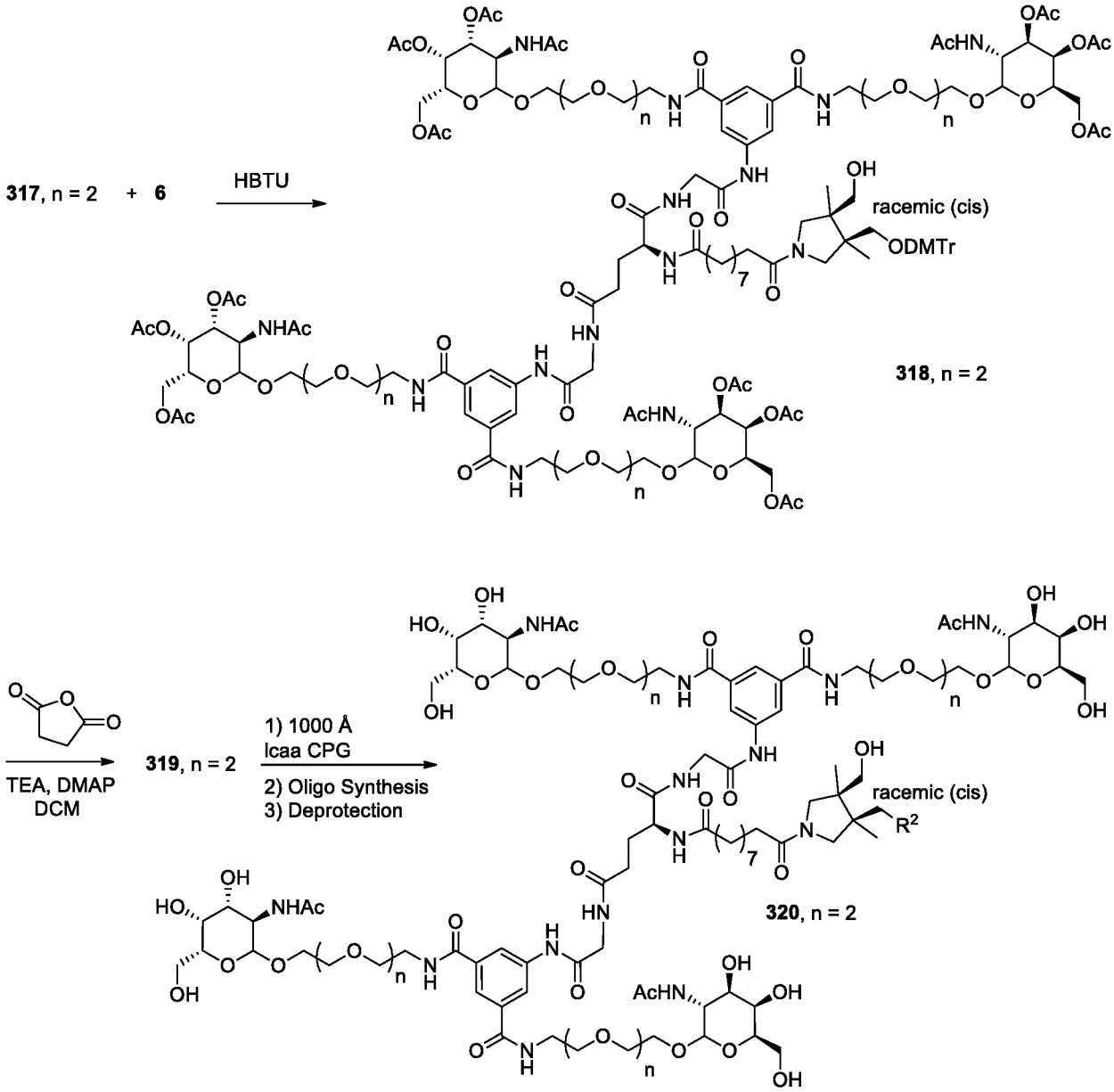
Step 3 Preparation of compound 316

Compound **316** was prepared in the same fashion as **314**, from Z-glutamic acid (306mg, 1.09mmol) and **315** (3.3g, 2.6mmol). Yield 1.66g, 60%.

Step 4 Preparation of compound 317

Compound **317** was prepared in the same fashion as **315**. Yield 1.65g, Quant.

Scheme 55 Preparation of complete conjugate



Step 1 Preparation of compound 318

A solution of **317** (1.91g, 0.75mmol) in CH_2Cl_2 (100mL) was treated first with Hunig's base (392μL, 2.25mmol) then **6** (a mixture of two *cis*-diastereomers, 509mg, 0.79mmol) followed by HBTU (356mg, 0.94mmol). After stirring (overnight) the solution was poured into $NaHCO_3$ (sat. aq.) then washed with water and brine, dried ($MgSO_4$), filtered and concentrated. The crude material was subjected to chromatography to yield **318** (1.19g, 52%) as a white foam.

Step 2 Preparation of compound 319

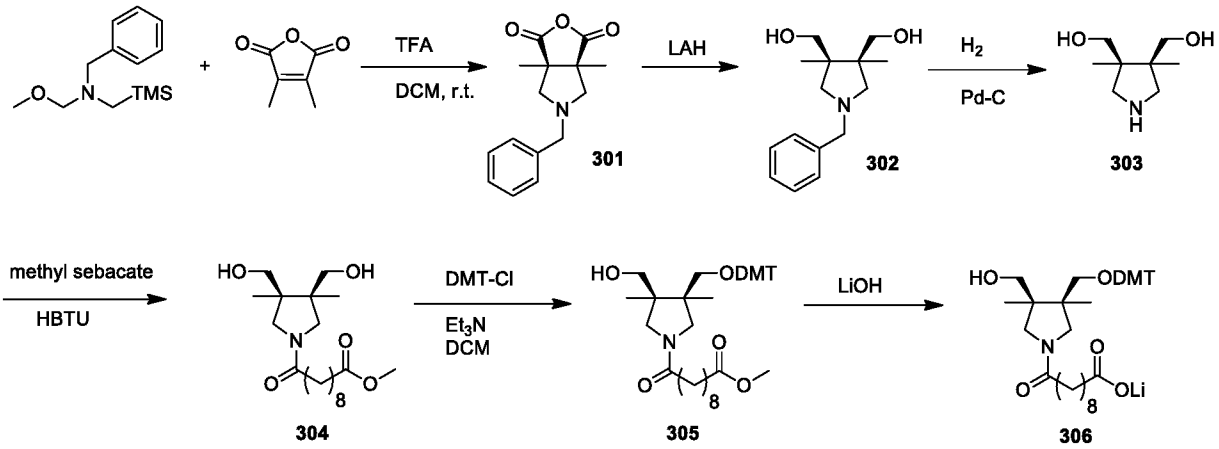
A solution of **318** (1.19g, 0.39mmol) in 1,2 dichloroethane (100mL) was treated with TEA (542µL, 3.9mmol), DMAP (238mg, 1.95mmol) and succinic anhydride (195mg, 1.95mmol) and heated (85°C). After stirring (2.5 hours) the solution was removed from heat and treated with CH_3OH (10mL) and allowed to stir (1 hour). After stirring the mixture was poured into $NaHCO_3$ (sat. aq.) then washed with brine, dried ($MgSO_4$), filtered and concentrated. The residue obtained was used without further processing. Yield = 1.4g, Quant.

Step 3 Preparation of conjugate 320

The succinate **319** was loaded onto 1000Å LCAA (long chain aminoalkyl) CPG (control pore glass) using standard amide coupling chemistry. A solution of diisopropylcarbodiimide (52.6 µmol), N-hydroxy succinimide (0.3 mg, 2.6 µmol) and pyridine (10 µL) in anhydrous acetonitrile (0.3 mL) was added to **319** (20.6 mg, 8 µmol) in anhydrous dichloromethane (0.2 mL). This mixture was added to LCAA CPG (183 mg). The suspension was gently mixed overnight at room temperature. Upon disappearance of **319** (HPLC), the reaction mixture was filtered and the CPG was washed with 1 mL of each dichloromethane, acetonitrile, a solution of 5% acetic anhydride / 5% N-methylimidazole / 5% pyridine in THF, then THF, acetonitrile and dichloromethane. The CPG was then dried overnight under high vacuum. Loading was determined by standard DMTr assay by UV/Vis (504 nm) to be 19 µmol/g. The resulting GalNAc loaded CPG solid support was employed in automated oligonucleotide synthesis using standard procedures. Nucleotide deprotection followed by removal from the solid support (with concurrent galactosamine acetate deprotection) afforded the GalNAc-oligonucleotide conjugate **320**.

Example 27 Synthesis of conjugate 520

Scheme 56 Preparation of activated linker



Step 1. Preparation of Racemic (cis) 5-Benzyl-3a,6a-dimethyltetrahydro-1H-furo[3,4-c]pyrrole-1,3(3aH)-dione 301

To a cooled solution (0°C) of 3,4-dimethylfuran-2,5-dione (3 g, 24 mmol) and N-benzyl-1-methoxy-N-((trimethylsilyl)methyl)methanamine (7 g, 29.8 mmol) in dichloromethane (75 mL) was slowly added trifluoroacetic acid (75 µL). Stir overnight allowing the solution to slowly warm to room temperature as the ice bath melted. The reaction mixture was concentrated to dryness, dissolved in ethyl acetate (100 mL), washed with saturated sodium bicarbonate (2 x 100mL), dried on magnesium sulfate, filtered and concentrated to dryness. Purification by column chromatography on silica gel (gradient: 20% ethyl acetate in hexanes to 100% ethyl acetate) afforded (3aR,6aS)-5-Benzyl-3a,6a-dimethyltetrahydro-1H-furo[3,4-c]pyrrole-1,3(3aH)-dione as a yellow oil (3.5 g, 56%).

Step 2. Preparation of Racemic (cis) (1-Benzyl-3,4-dimethylpyrrolidine-3,4-diyl)dimethanol 302

To a cooled (0°C) solution of (3aR,6aS)-5-Benzyl-3a,6a-dimethyltetrahydro-1H-furo[3,4-c]pyrrole-1,3(3aH)-dione (3.5 g, 13.4 mmol) in anhydrous diethyl ether (50 mL) was added slowly lithium aluminum hydride pellets (1.5 g, 40 mmol) over three portions. The solution was stirred overnight warming to room temperature as the ice water bath melted. Upon completion, the reaction was cooled to 0°C and very slowly quenched with 1.5 mL of 5M NaOH followed by 1.5 mL of water. Stir for 30 minutes then add magnesium sulfate and filter. The filtrate was concentrated to afford ((3R,4S)-1-Benzyl-3,4-dimethylpyrrolidine-3,4-diyl)dimethanol as a colorless oil (2.7 g).

Step 3. Preparation of Racemic (cis) (3,4-Dimethylpyrrolidine-3,4-diyl)dimethanol 303

To a solution of ((3R,4S)-1-Benzyl-3,4-dimethylpyrrolidine-3,4-diyl)dimethanol (10 g, 40 mmol) in methanol (10 mL) was added 10% palladium on activated charcoal wet (1 g). The solution was stirred vigorously under a hydrogen atmosphere for 16 hours. Upon completion the solution was filtered through Celite, and concentrated to dryness to afford ((3R,4S)-3,4-Dimethylpyrrolidine-3,4-diyl)dimethanol as a colorless solid (5.5 g, 86%).

Step 4. Preparation of Racemic (cis) Methyl 10-(3,4-bis(hydroxymethyl)-3,4-dimethylpyrrolidin-1-yl)-10-oxodecanoate 304

A solution of **3** (1.3 g, 8.2 mmol) and monomethyl sebacate (1.8 g, 8.2 mmol) in CH_2Cl_2 (100mL) was treated with HBTU (3.41g, 9.02mmol) and Hunig's base (5.71mL, 32.8mmol). After stirring overnight the mixture was washed with $NaHCO_3$ (sat. aq.), water and brine, then dried ($MgSO_4$), filtered and concentrated. The crude material was subjected to chromatography (gradient: 0% CH_3OH-CH_2Cl_2 to 20%) to yield 4 (1.8g, 61%).

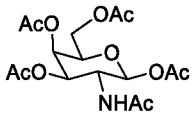
Step 5. Preparation of Racemic (cis) Methyl 10-(3-((bis(4-methoxyphenyl)(phenyl)-methoxy)methyl)-4-(hydroxymethyl)-3,4-dimethylpyrrolidin-1-yl)-10-oxodecanoate 305

A solution of **304** (1.8 g, 5.0 mmol) and 4,4′-Dimethoxytrityl chloride (1.7 g, 5.0 mmol) in pyridine (180mL) was stirred overnight. The pyridine was then removed under reduced pressure and the crude material was subjected to chromatography (gradient: 0% CH_3OH-CH_2Cl_2 to 10%) to yield **5** (1.4 g, 42%) as a yellow oil.

Step 6. Preparation of Racemic (cis) Lithium 10-(3-((bis(4-methoxyphenyl)-(phenyl)methoxy)methyl)-4-(hydroxymethyl)-3,4-dimethylpyrrolidin-1-yl)-10-oxodecanoate 306

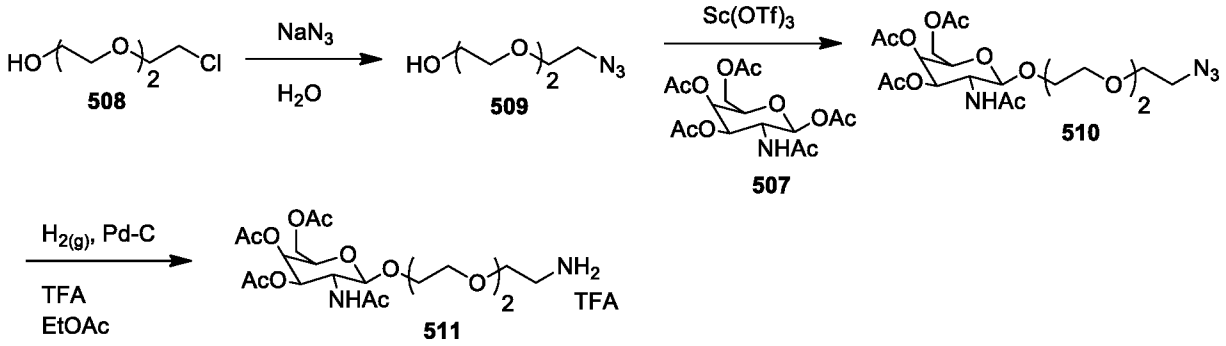
To a solution of compound **305** (3.0 g, 4.6 mmol) in THF (50 mL) and water (50 mL) was added lithium hydroxide (121 mg, 5.0 mmol). The solution was stirred for 4 hours at room temperature then concentrated to remove the THF. The remaining aqueous solution was freeze dried overnight to afford a pale pink solid (2.9 g, quantitative). Compound **306** was prepared as a mixture of two *cis*-diastereomers.

Scheme 57 Synthesis of peracetylated galactosamine 507



Galactosamine hydrochloride (250 g, 1.16 mol) in pyridine (1.5 L) is treated with acetic anhydride (1.25 L, 13.2 mol) over 45 minutes. After stirring overnight the reaction mixture is divided into three 1 L portions. Each 1 L portion is poured into 3 L of ice water and mixed for one hour. After mixing the solids are filtered off, combined, frozen over liquid nitrogen and then lyophilized for five days to yield peracetylated galactosamine **507** (369.4 g, 82%) as a white solid. Rf (0.58, 10% MeOH-CH_2Cl_2).

Scheme 58 Synthesis of GalNAc monomer



Step 1 Preparation of compound 509

A solution of 2-[2-(2-chloroethoxy)]ethanol **508** (100g, 593mmol) in water (1L) is treated with NaN_3 (77g, 1.19mol) and heated (90°C). After stirring (72 hours) the solution is cooled (RT) and extracted (4x) with CH_2Cl_2. The combined organics are washed with brine, dried ($MgSO_4$), filtered, concentrated and used without further processing. Compound **509** (88.9g, 86%) is obtained as a pale yellow oil.

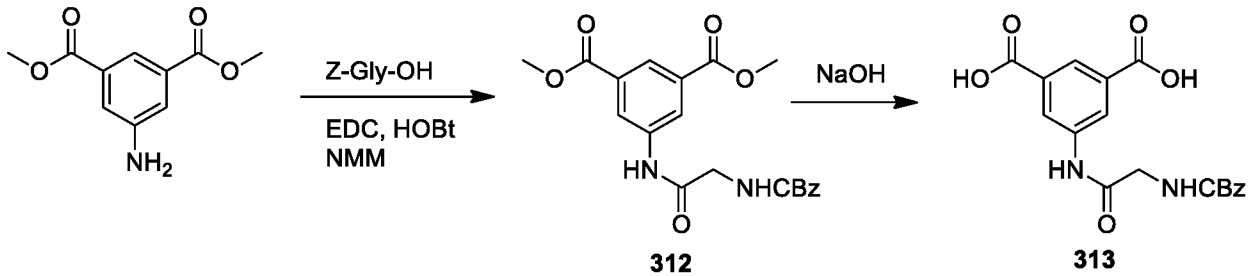
Step 2 Preparation of compound 510

A solution of **507** (2.76g, 7.1mmol) and **509** (1.37g, 7.8mmol) in 1,2-dichloroethane (40mL) is treated with $Sc(OTf)_3$ (174mg, 0.36mmol) and heated (85°C). After stirring (2 hours) the mixture is cooled (RT) and quenched by the addition of TEA (4mL) and concentrated. The crude material is subjected to chromatography to yield **510** (3.03g, 85%) as a pale yellow foam.

Step 3 Preparation of compound 511

A solution of **510** (3.02g, 5.99mmol) and Pd/C (300mg, 10% Pd loading - wet support) in EtOAc (30mL) is treated with TFA (576μL, 7.5mmol). The reaction mixture is purged with hydrogen gas (45min) then purged with nitrogen gas (10min), then filtered through celite. The filtrate is concentrated and then subjected to chromatography to yield **511** (2.67g, 75%) as a brown foam.

Scheme 59 Synthesis of aromatic core



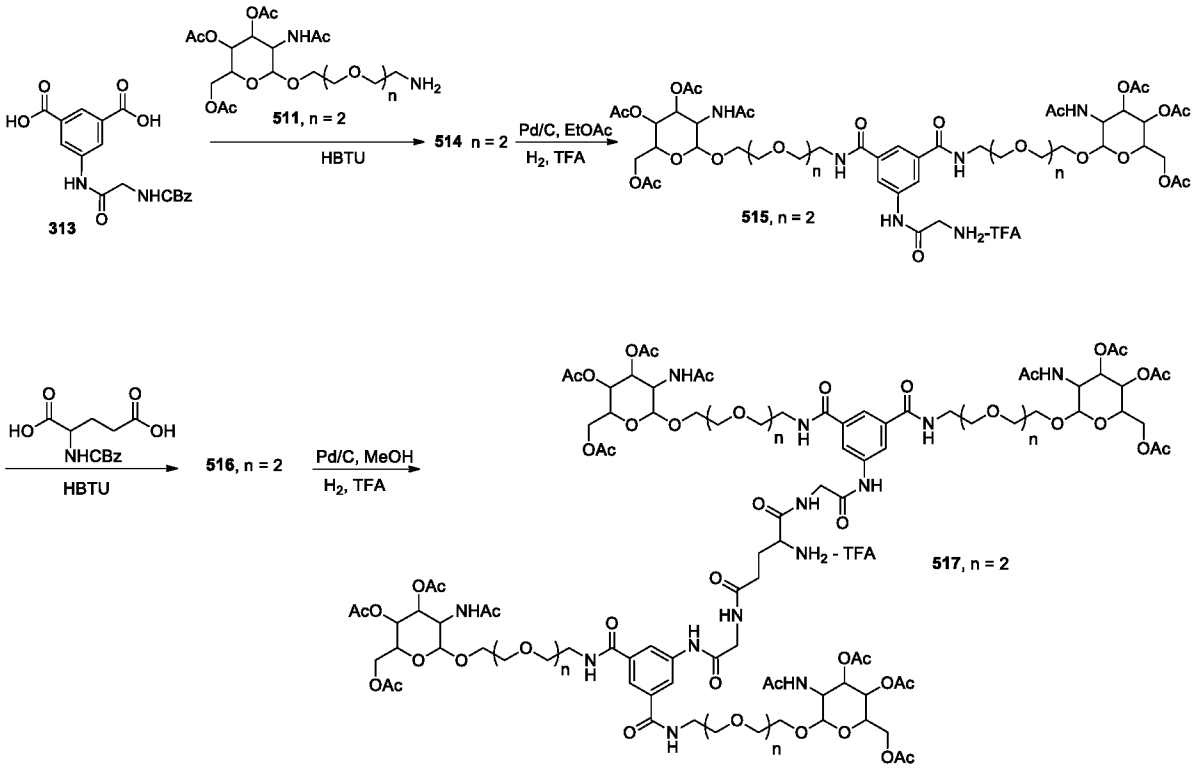
Step 1. Preparation of Dimethyl 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)-isophthalate 312

A solution of dimethyl 5-aminoisophthalate (5 g, 24 mmol), Z-Gly-OH (5 g, 24 mmol), EDC (5 g, 26.3 mmol), HOBt (3.6 g, 26.3 mmol), NMM (2.9 mL, 26.3 mmol) in DMF (50 mL) was stirred overnight at room temperature. Upon completion, the reaction mixture was diluted with ethyl acetate (250 mL) and washed with each 1M HCl (2 x 100 mL), saturated sodium bicarbonate (1 x 100 mL) and brine (2 x 100 mL). Dry on magnesium sulfate, filter and concentrate to dryness to afford Dimethyl 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)-acetamido)isophthalate as a colorless solid (7.2 g, 79%).

Step 2. Preparation of 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)isophthalic acid 313

To a solution of methyl 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)isophthalate (7.2 g) in methanol (25 mL) and THF (25 mL) was added 1M NaOH (25 mL). The solution was stirred at room temperature for 2 hours then concentrated to remove THF and MeOH. The aqueous solution remaining was diluted with water (75 mL), cooled on an ice water bath and acidified to pH = 1 with 6M HCl. The solid was filtered and washed with water (3 x 100 mL). The solid was freeze dried to afford 5-(2-((2-oxo-2-phenyl-1λ^2-ethyl)amino)acetamido)-isophthalic acid (6.9 g, quantitative) .

Scheme 60: Preparation of tetramer



Step 1 Preparation of compound 514

A solution of **313** (2.09g, 5.6mmol) and **511** (8.34g, 14.07mmol) in CH_2Cl_2 (150mL) is treated with HBTU (6.4g, 16.9mmol) and Hunig's base (7.35mL, 42.2mmol). After stirring (overnight) the reaction mixture is poured into $NaHCO_3$ (sat. aq.) then washed with water and brine, dried ($MgSO_4$), filtered and concentrated. The crude material is subjected to chromatography (gradient 1-12% CH_3OH-CH_2Cl_2) to yield 6 (3.97g, 55%) as a pale yellow foam.

Step 2 Preparation of compound 515

Compound **514** (3.92g, 3.07mmol), Pd/C (400mg, 10% loading – wet support) and trifluoroacetic acid (308µL, 4mmol) is purged with H_2. After stirring under H_2 (overnight), the mixture is purged with N_2 (15-20 min) then filtered through celite and concentrated. The crude material is subjected to chromatography to yield 7 (3.36g, 86%) as a white to cream colored foam.

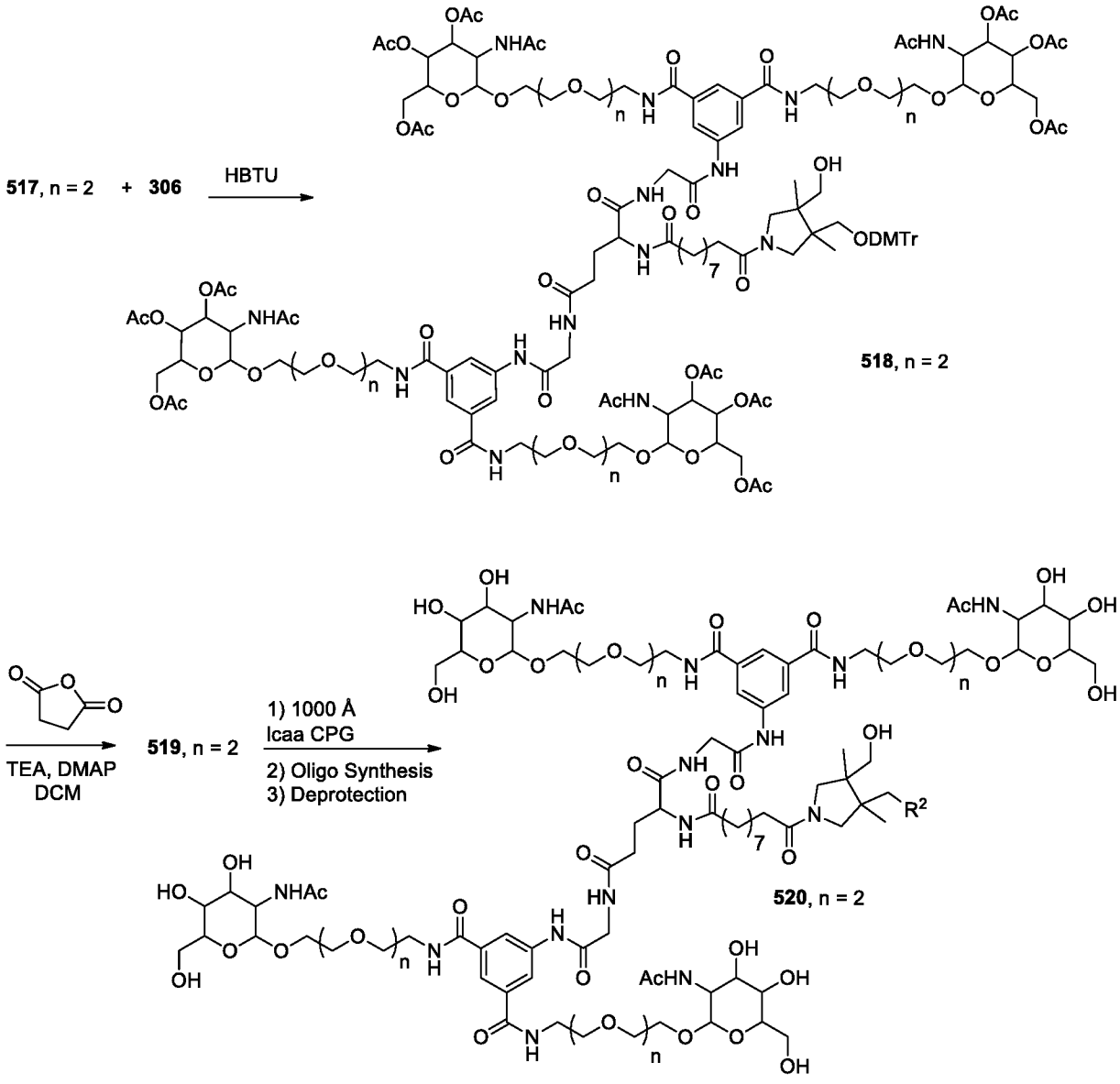
Step 3 Preparation of compound 516

Compound **516** is prepared in the same fashion as **514**, from Z-glutamic acid (306mg, 1.09mmol) and **515** (3.3g, 2.6mmol). Yield 1.66g, 60%.

Step 4 Preparation of compound 517

Compound **517** is prepared in the same fashion as **515**. Yield 1.65g, Quant.

Scheme 61 Preparation of complete conjugate



Step 1 Preparation of compound 518

A solution of **517** (1.91g, 0.75mmol) in CH_2Cl_2 (100mL) is treated first with Hunig's base (392μL, 2.25mmol) then **306** (a mixture of two *cis*-diastereomers, 509mg, 0.79mmol)

followed by HBTU (356mg, 0.94mmol). After stirring (overnight) the solution was poured into $NaHCO_3$ (sat. aq.) then washed with water and brine, dried ($MgSO_4$), filtered and concentrated. The crude material is subjected to chromatography to yield **518** (1.19g, 52%) as a white foam.

Step 2 Preparation of compound 519

A solution of **518** (1.19g, 0.39mmol) in 1,2 dichloroethane (100mL) is treated with TEA (542µL, 3.9mmol), DMAP (238mg, 1.95mmol) and succinic anhydride (195mg, 1.95mmol) and heated (85°C). After stirring (2.5 hours) the solution is removed from heat and treated with CH_3OH (10mL) and allowed to stir (1 hour). After stirring the mixture is poured into $NaHCO_3$ (sat. aq.) then washed with brine, dried ($MgSO_4$), filtered and concentrated. The residue obtained is used without further processing. Yield = 1.4g, Quant.

Step 3 Preparation of conjugate 520

The succinate **519** is loaded onto 1000Å LCAA (long chain aminoalkyl) CPG (control pore glass) using standard amide coupling chemistry. A solution of diisopropylcarbodiimide (52.6 µmol), N-hydroxy succinimide (0.3 mg, 2.6 µmol) and pyridine (10 µL) in anhydrous acetonitrile (0.3 mL) is added to **519** (20.6 mg, 8 µmol) in anhydrous dichloromethane (0.2 mL). This mixture is added to LCAA CPG (183 mg). The suspension was gently mixed overnight at room temperature. Upon disappearance of **519** (HPLC), the reaction mixture is filtered and the CPG is washed with 1 mL of each dichloromethane, acetonitrile, a solution of 5% acetic anhydride / 5% N-methylimidazole / 5% pyridine in THF, then THF, acetonitrile and dichloromethane. The CPG is then dried overnight under high vacuum. Loading was determined by standard DMTr assay by UV/Vis (504 nm) to be 19 µmol/g. The resulting GalNAc loaded CPG solid support is employed in automated oligonucleotide synthesis using standard procedures. Nucleotide deprotection followed by removal from the solid support (with concurrent galactosamine acetate deprotection) affords the GalNAc-oligonucleotide conjugate **520**.

Example 28. In vivo testing of TTR siRNA conjugates

Compound **320**, wherein R^2 comprises the modified TTR siRNA described in Table 3, was tested for *in vivo* activity in a wild-type mouse model of TTR knock-down. In the present example, Compound **320**, wherein R^2 comprises the modified TTR siRNA, is demonstrated as

a possible treatment for the orphan disease of TTR (Transthyretin) amyloidosis. In those afflicted with this disease, the misfolding and aggregation of the Transthyretin protein is associated with disease progression. By using this siRNA-GalNAc conjugate, the amount of misfolded/aggregated protein in the patient can be reduced, with a potential result of halting the progression of the disease. Accordingly, certain embodiments provide compound **320**, wherein the R^2 comprises the modified TTR siRNA, and uses thereof to treat transthyretin amyloidosis.

Table 3. Chemically Modified TTR siRNA duplexes

siRNA Number	Sense strand SEQ ID NO	Sense strand 5' – 3'	Antisense strand SEQ ID NO	Antisense strand 5'-3'
40	SEQ ID NO:75	AsasCaGuGuUCUuGcUcUaUaA	SEQ ID NO:76	usUsaUaGaGcAagaAcAcUgUususu

2'-O-Methyl nucleotides = lower case; 2'-Fluoro nucleotides = UPPER CASE;
Phosphorothioate linker = s; Unmodified = UPPER CASE

Both the TTR siRNA sequence and animal model were described by Nair *et al.*, *J. Am. Chem. Soc.*, *36(49)*, 16958–16961 (2014). All animal-related procedures were conducted according to written operating procedures, in accordance with Canadian Council on Animal Care (CCAC) Guidelines on Good Animal Practices, and approved by the local Institutional Animal Care and Use Committee (IACUC).

siRNA treatment: Female C57BL/6 mice (n = 4) were administered a single 2 mg/kg dose of compound **320** (R^2 comprises the modified TTR siRNA) once on Day 0 (1 dose per animal) via subcutaneous injection in the scapular region. One group of animals administered vehicle only (PBS) served as controls.

Collections: All animals were test bled at defined time points after test article administration (days 2, 4, 7, 9, 14 and 21) to determine maximum reductions in plasma TTR levels and the duration of pharmacologic activity.

Analysis: TTR protein levels in plasma samples were determined using the Abnova Prealbumin (Mouse) ELISA kit (Cedar Lane, catalogue number KA2070) as per the manufacturer's instructions. TTR plasma protein values were calculated for the individual plasma samples and the average of each group was determined. From these averages, the TTR protein levels relative to control (% relative to PBS treated animals) were determined.

Results: Results from testing are presented in Table 4. Values represent % TTR protein levels (relative to PBS Control) on Days 2, 4, 7, 9, 14 and 21 post treatment.

Table 4. Plasma TTR protein levels in mice after single subcutaneous administration (2 mg/kg) of GalNAc conjugated siRNA from Table 3. TTR protein data expressed as percent of PBS treated mouse values

siRNA Number	Ligand Cpd #	Day 2	Day 4	Day 7	Day 9	Day 14	Day 21
40	320	36.6	15.7	17.2	17.7	36.9	59.2

Conclusion: Animals treated with Compound **320**, wherein R^2 comprises the modified TTR siRNA described in Table 3, exhibited a marked knockdown of target mRNA and protein with maximal knock down of TTR protein occurring between days 4 and 9 post subcutaneous injection.

Example. 29. In vivo testing of HBV siRNA conjugates

Chemically modified HBV siRNA described in Table 1 in Example 25, conjugated to GalNAc ligands, were tested for *in vivo* activity in an established mouse model of HBV infection. In the AAV-HBV1.2 C57BL/6 mouse model, stable and persistent HBV expression is achieved after injection of an adeno-associated virus (AAV) vector encoding an over-genomic length sequence of HBV, leading to hepatic expression of HBV RNA and proteins and the secretion of viral and sub-viral particles into the blood.

The AAV-HBV1.2 construct used in these studies was based on details provided in Dion *et al.*, *Journal of Virology*, *87(10)*, 5554–5563 (2013). All animal-related procedures were conducted according to written operating procedures, in accordance with Canadian Council on Animal Care (CCAC) Guidelines on Good Animal Practices, and approved by the local Institutional Animal Care and Use Committee (IACUC).

Each animal was inoculated with 1E11 vector genomes (VG) of AAV-HBV1.2 vector. Prior to treatment, all animals were test bled and serum HBsAg levels determined for individual animals to confirm established HBV expression.

siRNA treatment: Groups of mice (typically n = 5) were administered a single 3 mg/kg dose of HBV siRNA conjugate once on Day 0 (1 dose per animal) via subcutaneous injection in the scapular region. One group of animals administered vehicle only (saline) served as controls.

Collections: All mice were test bled on Day 0, prior to treatment, and at defined time points after test article administration (for example on study days 7, 14, 21, 28, 42, 56, and 70)

to determine maximum reductions in serum HBsAg levels and the duration of pharmacologic activity.

Analysis: HBsAg levels in serum samples were determined using the Biorad EIA GS HBsAg 3.0 kit (BioRad, catalog no. 32591) as per the manufacturer's instructions. Pooled serum from each treatment group was used to determine the group mean HBsAg levels at individual timepoints. Data was analysed and expressed as HBsAg levels relative to pre-treatment baseline (% relative to Day 0).

Results: Results from testing each of the chemically modified HBV siRNA described in Table 1 are presented in Table 5. Values represent % HBsAg levels (relative to Day 0 baseline) on Days 7, 14, 21, 28, 42, 56 and 70 post treatment.

Table 5. Serum HBsAg levels in mice after single subcutaneous administration (3 mg/kg) of GalNAc conjugated siRNA from Table 1 in Example 25. HBsAg data expressed as percent of baseline (Day 0) values

siRNA Number	Ligand Cpd #	Day 7	Day 14	Day 21	Day 28	Day 42	Day 56	Day 70
2	194	7.0	4.1	4.2	5.6	10.1	17.2	29.5
3	194	5.8	2.4	1.8	2.3	4.6	10.6	12.9
3	191a	1.7	0.3	0.3	0.3	0.5	0.9	2.3
3	320	3.1	0.5	0.5	0.5	0.8	1.6	3.6
4	194	5.5	3.1	3.2	4.4	6.0	9.5	16.2
20	231	5.3	2.2	1.9	3.4	4.8	9.8	17.4
20	320	2.6	1.0	1.1	1.3	3.1	6.4	
25	191a	1.9	0.2	0.2	0.3	0.5	1.1	1.8
25	320	1.1	0.1	0.3	0.4	1.4	2.9	3.5
26	194	10.4	3.2	2.7	3.0	4.0	6.3	12.3
31	194	13.3	7.0	8.0	11.7	17.7	25.6	36.7
32	194	13.7	5.7	8.2	11.6	16.6	25.0	46.5
33	194	14.4	8.0	10.8	14.4	24.3	41.8	65.2

Each of the 13 compounds tested caused serum HBV surface antigen reduction after a single dose of subcutaneously-administered treatment, with maximum effect obtained at Day 14 or 21. The four compounds showing the greatest reductions were compound **191a**, wherein

the oligonucleotide comprised siRNA 3 or 25, and compound **320**, wherein R^2 comprised siRNA 3 or 25. These four compounds were notable for a more rapid reduction (≥97%) at the first time point (Day 7), greater maximal reduction (≥99%), and a more sustained reductive effect (still ≥97% at Day 56, 8 weeks after treatment).

CLAIMS

WHAT IS CLAIMED IS:

1. A nucleic acid molecule selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71 and SEQ ID NO:73.

2. A nucleic acid molecule selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72 and SEQ ID NO:74.

3. A composition comprising a nucleic acid molecule of claim 1, a nucleic acid molecule of claim 2, or a combination thereof.

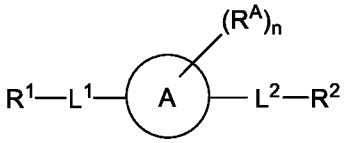
4. A double stranded siRNA molecule selected from the group consisting of siRNA 1 (SEQ ID NO:1 and 2), 2 (SEQ ID NO:3 and 4), 3 (SEQ ID NO:5 and 6), 4 (SEQ ID NO:7 and 8), 5 (SEQ ID NO:9 and 10), 6 (SEQ ID NO:11 and 12), 7 (SEQ ID NO:13 and 14), 8 (SEQ ID NO:15 and 16), 9 (SEQ ID NO:17 and 18), 10 (SEQ ID NO:19 and 20), 11 (SEQ ID NO:21 and 22), 12 (SEQ ID NO:23 and 24), 13 (SEQ ID NO:25 and 26), 14 (SEQ ID NO:27 and 28), 15 (SEQ ID NO:29 and 30), 16 (SEQ ID NO:31 and 32), 17 (SEQ ID NO:33 and 34), 18 (SEQ ID NO:35 and 36), 19 (SEQ ID NO:37 and 38), 20 (SEQ ID NO:39 and 40), 21 (SEQ ID NO:41 and 42), 22 (SEQ ID NO:43 and 44), 23 (SEQ ID NO:45 and 46), 24 (SEQ ID NO:47 and 48), 25 (SEQ ID NO:49 and 50), 26 (SEQ ID NO:51 and 52), 27 (SEQ ID NO:53 and 54), 28 (SEQ ID NO:55 and 56), 29 (SEQ ID NO:57 and 58), 30 (SEQ ID NO:59 and 60), 31 (SEQ

ID NO:61 and 62), 32 (SEQ ID NO:63 and 64), 33 (SEQ ID NO:65 and 66), 34 (SEQ ID NO:67 and 68), 35 (SEQ ID NO:69 and 70), 36 (SEQ ID NO:71 and 72) and 37 (SEQ ID NO:73 and 74).

5. A composition comprising a double stranded siRNA molecule of claim 4.

6. The composition of claim 3 or 5, wherein the composition is a pharmaceutical composition that comprises a pharmaceutically acceptable carrier.

7. A compound of formula (I):



(I)

wherein:

R^1 a is targeting ligand;

L^1 is absent or a linking group;

L^2 is absent or a linking group;

R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4;

the ring A is absent, a 3-20 membered cycloalkyl, a 5-20 membered aryl, a 5-20 membered heteroaryl, or a 3-20 membered heterocycloalkyl;

each R^A is independently selected from the group consisting of hydrogen, hydroxy, CN, F, Cl, Br, I, -C_{1-2} alkyl-OR^B, C_{1-10} alkyl C_{2-10} alkenyl, and C_{2-10} alkynyl; wherein the C_{1-10} alkyl C_{2-10} alkenyl, and C_{2-10} alkynyl are optionally substituted with one or more groups independently selected from halo, hydroxy, and C_{1-3} alkoxy;

R^B is hydrogen, a protecting group, a covalent bond to a solid support, or a bond to a linking group that is bound to a solid support; and

n is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

or a salt thereof.

8. The compound of claim 7, wherein:

R^1 a is targeting ligand;

L^1 is absent or a linking group;

L^2 is absent or a linking group;

R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4;

the ring A is absent, a 3-20 membered cycloalkyl, a 5-20 membered aryl, a 5-20 membered heteroaryl, or a 3-20 membered heterocycloalkyl;

each R^A is independently selected from the group consisting of hydrogen, hydroxy, CN, F, Cl, Br, I, -C_{1-2} alkyl-OR^B and C_{1-8} alkyl that is optionally substituted with one or more groups independently selected from halo, hydroxy, and C_{1-3} alkoxy;

R^B is hydrogen, a protecting group, a covalent bond to a solid support, or a bond to a linking group that is bound to a solid support; and

n is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

or a salt thereof.

9. The compound of claim 8 wherein R^1 is $-C(H)_{(3-p)}(L^3\text{-saccharide})_p$,

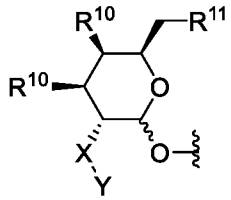
wherein each L^3 is independently a linking group;

p is 1, 2, or 3; and

saccharide is a monosaccharide or disaccharide

or a salt thereof.

10. The compound of claim 9 wherein the saccharide is:



wherein:

X is NR^3, and Y is selected from -$(C=O)R^4$, -SO_2R^5, and -$(C=O)NR^6R^7$; or X is -(C=O)- and Y is NR^8R^9;

R^3 is hydrogen or $(C_1\text{-}C_4)$alkyl;

R^4, R^5, R^6, R^7, R^8 and R^9 are each independently selected from the group consisting of hydrogen, $(C_1\text{-}C_8)$alkyl, $(C_1\text{-}C_8)$haloalkyl, $(C_1\text{-}C_8)$alkoxy and $(C_3\text{-}C_6)$cycloalkyl that is

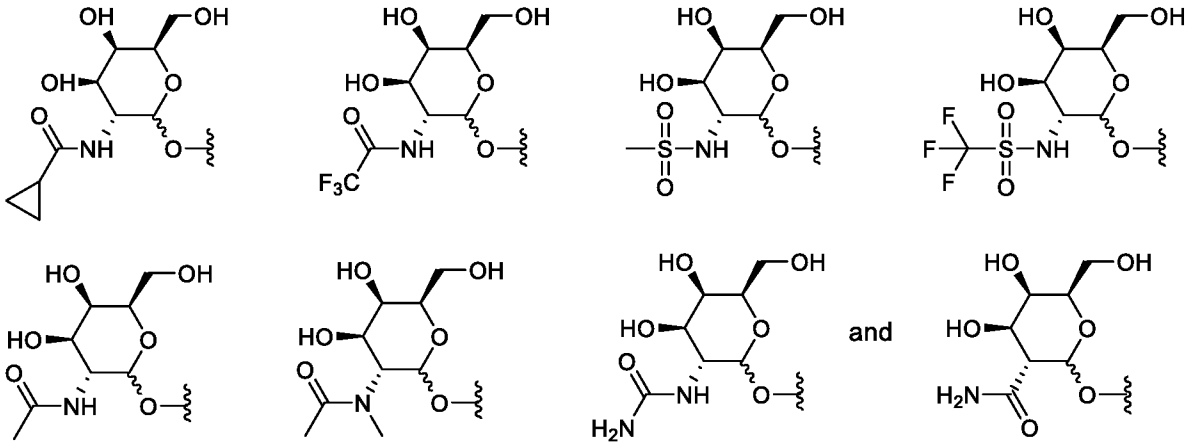
optionally substituted with one or more groups independently selected from the group consisting of halo, (C_1-C_4)alkyl, (C_1-C_4)haloalkyl, (C_1-C_4)alkoxy and (C_1-C_4)haloalkoxy;

R^{10} is -OH, -NR^8R^9 or – F; and

R^{11} is -OH, -NR^8R^9, -F or 5 membered heterocycle that is optionally substituted with one or more groups independently selected from the group consisting of halo, hydroxyl, carboxyl, amino, (C_1-C_4)alkyl, (C_1-C_4)haloalkyl, (C_1-C_4)alkoxy and (C_1-C_4)haloalkoxy;

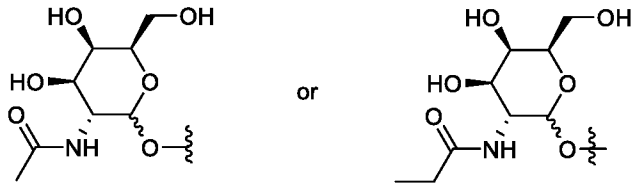
or a salt thereof.

11. The compound of claim 9 or 10, wherein the saccharide is selected from the group consisting of:



or a salt thereof.

12. The compound of any one of claims 9–11, wherein the saccharide is:



N-Acetylgalactosamine (GalNAc) GalPro

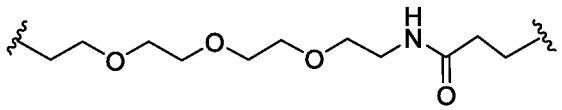
or a salt thereof.

13. The compound of any one of claims 9-12 or a salt thereof, wherein each L^3 is independently a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 0 to 50 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O-, -NR^X-, -NR^X-C(=O)-, -C(=O)-NR^X- or –S-, and wherein R^X is hydrogen or (C_1-C_6)alkyl, and wherein the hydrocarbon chain,

is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C_1-C_6)alkoxy, (C_3-C_6)cycloalkyl, (C_1-C_6)alkanoyl, (C_1-C_6)alkanoyloxy, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

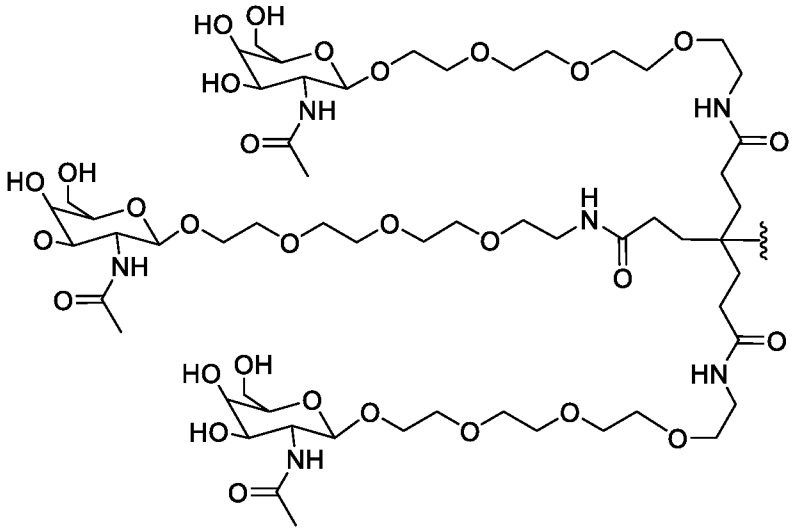
14. The compound of any one of claims 9-13 or a salt thereof, wherein each L^3 is independently a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 20 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O-, -NR^X-, -NR^X-C(=O)-, -C(=O)-NR^X- or –S-, and wherein R^X is hydrogen or (C_1-C_6)alkyl, and wherein the hydrocarbon chain, is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C_1-C_6)alkoxy, (C_3-C_6)cycloalkyl, (C_1-C_6)alkanoyl, (C_1-C_6)alkanoyloxy, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

15. The compound of any one of claims 9-14, wherein L^3 is:



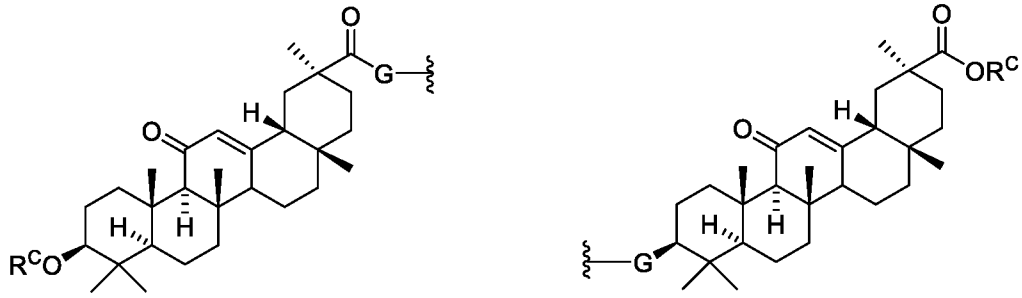
or a salt thereof.

16. The compound of any one of claims 8-15, wherein R^1 is:



or a salt thereof.

17. The compound of claim 8, wherein R^1 is:



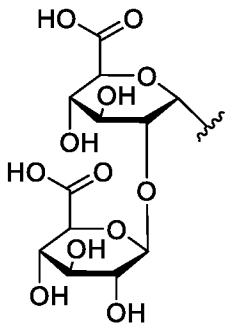
wherein:

G is –NH- or –O-;

R^C is hydrogen, (C_1-C_8)alkyl, (C_1-C_8)haloalkyl, (C_1-C_8)alkoxy, (C_1-C_6)alkanoyl, (C_3-C_{20})cycloalkyl, (C_3-C_{20})heterocycle, aryl, heteroaryl, monosaccharide, disaccharide or trisaccharide; and wherein the cycloalkyl, heterocyle, ary, heteroaryl and saccharide are optionally substituted with one or more groups independently selected from the group consisting of halo, carboxyl, hydroxyl, amino, (C_1-C_4)alkyl, (C_1-C_4)haloalkyl, (C_1-C_4)alkoxy and (C_1-C_4)haloalkoxy;

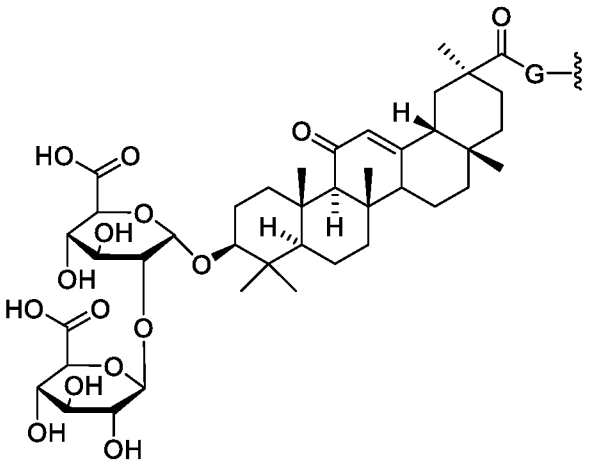
or a salt thereof.

18. The compound of claim 17, wherein R^C is:



or a salt thereof.

19. The compound of any one of claims 8, 17 and 18, wherein R^1 is:

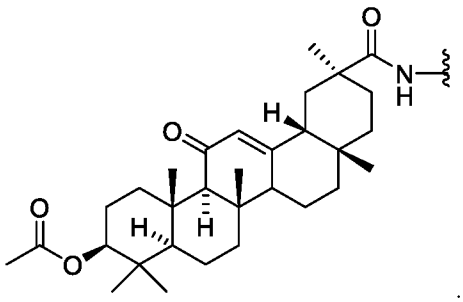


or a salt thereof.

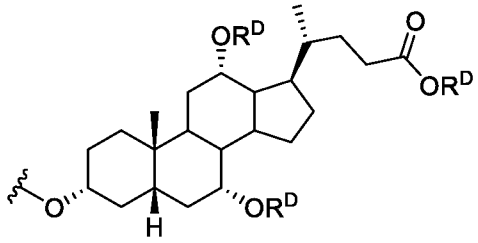
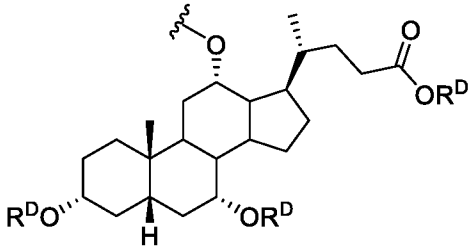
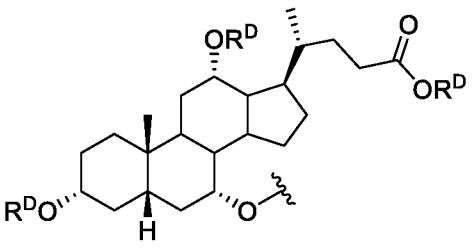
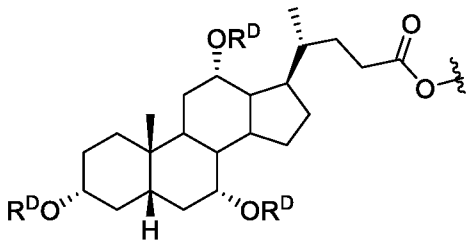
20. The compound of claim 17 or a salt thereof, wherein R^C is:

21. The compound of any one of claims 17–20 or a salt thereof, wherein G is –NH-.

22. The compound of any one of claims 8, 17, 20 and 21 or a salt thereof, wherein R^1 is:



23. The compound of claim 8 or a salt thereof, wherein R^1 is:




or


wherein each R^D is independently selected from the group consisting of hydrogen, (C_1-C_6)alkyl, (C_9-C_{20})alkylsilyl, $(R^W)_3Si$-, (C_2-C_6)alkenyl, tetrahydropyranyl, (C_1-C_6)alkanoyl, benzoyl, aryl(C_1-C_3)alkyl, TMTr (Trimethoxytrityl), DMTr (Dimethoxytrityl), MMTr (Monomethoxytrityl), and Tr (Trityl); and

each R^W is independently selected from the group consisting of (C_1-C_4)alkyl and aryl.

24. The compound of any one of claims 8-23 or a salt thereof, wherein L^1 and L^2 are independently a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 50 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O-, -NR^X-, -NR^X-C(=O)-, -C(=O)-NR^X- or –S-, and wherein R^X is hydrogen or (C_1-C_6)alkyl, and wherein the hydrocarbon chain, is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C_1-C_6)alkoxy, (C_3-C_6)cycloalkyl, (C_1-C_6)alkanoyl, (C_1-C_6)alkanoyloxy, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

25. The compound of any one of claims 8-24 or a salt thereof, wherein L^1 and L^2 are independently a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 20 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O-, -NR^X-, -NR^X-C(=O)-, -C(=O)-NR^X- or –S-, and wherein R^X is hydrogen or (C_1-C_6)alkyl, and wherein the hydrocarbon chain, is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C_1-

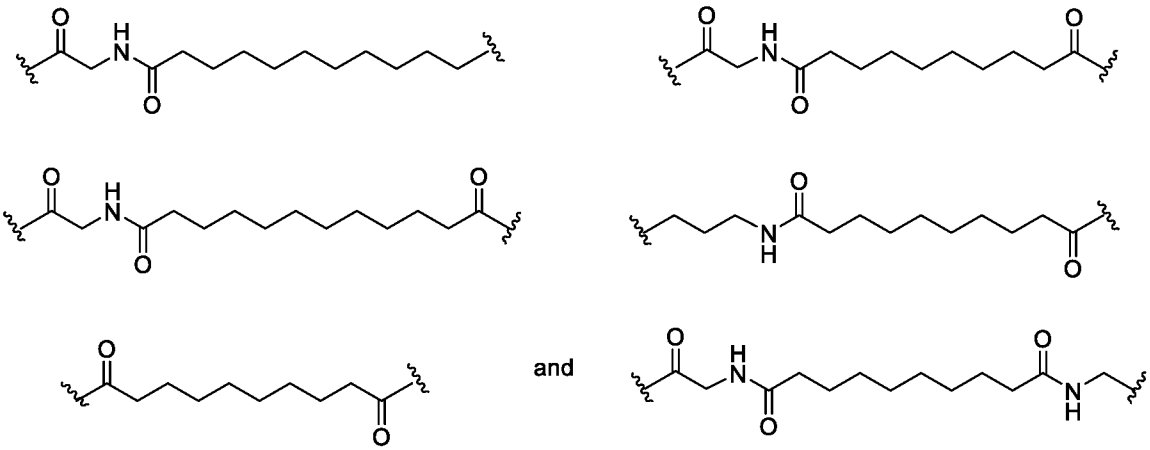
C_6)alkoxy, (C_3-C_6)cycloalkyl, (C_1-C_6)alkanoyl, (C_1-C_6)alkanoyloxy, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

26. The compound of any one of claims 8-25 or a salt thereof, wherein L^1 and L^2 are independently, a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 14 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced –O-, -NR^X-, -NR^X-C(=O)-, -C(=O)-NR^X- or –S-, and wherein R^X is hydrogen or (C_1-C_6)alkyl, and wherein the hydrocarbon chain, is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C_1-C_6)alkoxy, (C_3-C_6)cycloalkyl, (C_1-C_6)alkanoyl, (C_1-C_6)alkanoyloxy, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

27. The compound of any one of claims 8-26 or a salt thereof, wherein L^1 is connected to R^1 through -NH-, -O-, -S-, -(C=O)-, -(C=O)-NH-, -NH-(C=O)-, -(C=O)-O-, -NH-(C=O)-NH-, or –NH-(SO_2)-.

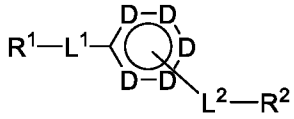
28. The compound of any one of claims 8-27 or a salt thereof, wherein L^2 is connected to R^2 through -O-.

29. The compound of any one of claims 8–28 or a salt thereof, wherein L^1 is selected from the group consisting of:



30. The compound of any one of claims 8–29 or a salt thereof, wherein L^2 is $–CH_2-O-$ or $–CH_2-CH_2-O-$.

31. The compound of claim 8 which is a compound formula (Ia):



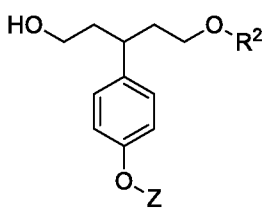
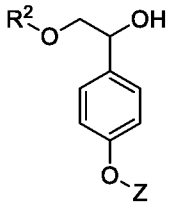
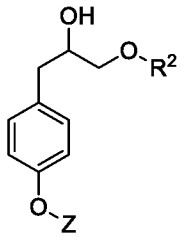
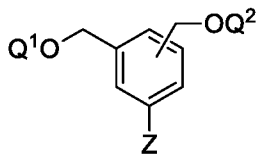
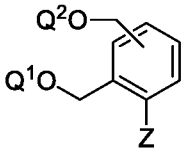
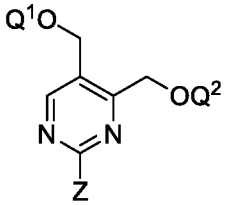
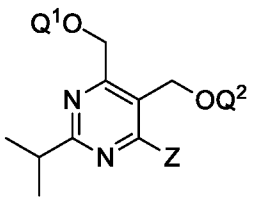
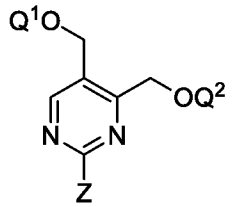
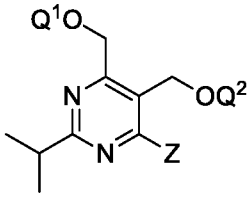
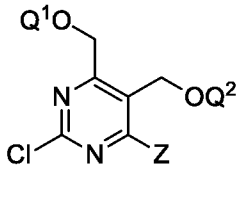
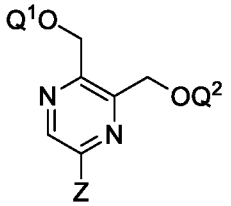
(Ia)

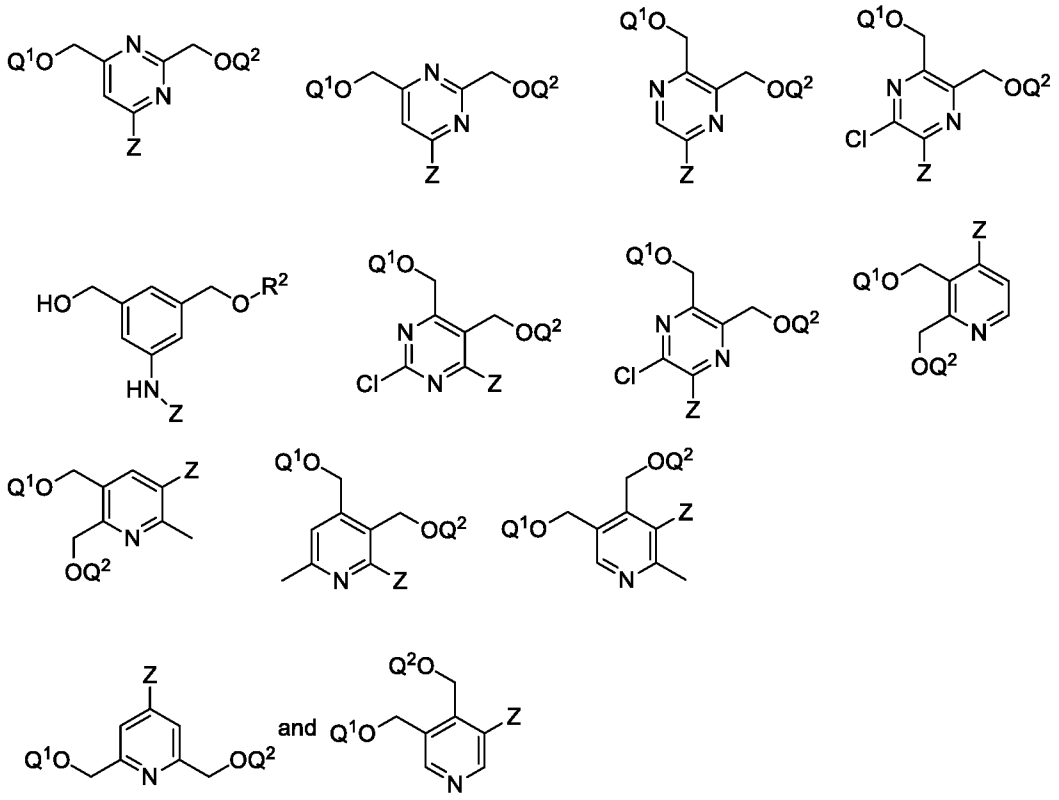
wherein:

each D is independently selected from the group consisting of  and –N=;

or a salt thereof.

32. The compound of claim 8 or claim 31, or a salt thereof, that is selected from the group consisting of:



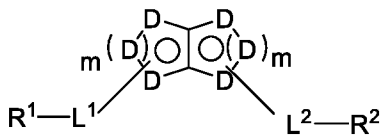
wherein:

Q^1 is hydrogen and Q^2 is R^2; or Q^1 is R^2 and Q^2 is hydrogen; and

Z is $–L^1\text{-}R^1$;

and salts thereof.

33. The compound of claim 8 or a salt thereof, which is a compound formula (Ib):



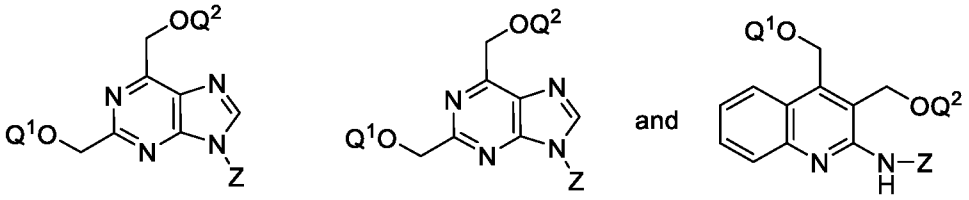
(Ib)

wherein:

each D is independently selected from the group consisting of  and –N=; and

each m is independently 1 or 2.

34. The compound of claim 8 or claim 33, or a salt thereof, that is selected from the group consisting of:



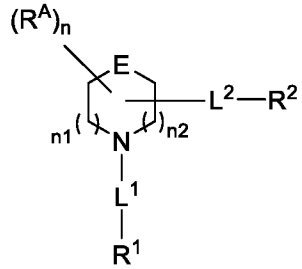
wherein:

Q^1 is hydrogen and Q^2 is R^2; or Q^1 is R^2 and Q^2 is hydrogen; and

Z is $–L^1\text{-}R^1$;

and salts thereof.

35. The compound of claim 8 or a salt thereof, which is a compound formula (Ic):



(Ic)

wherein:

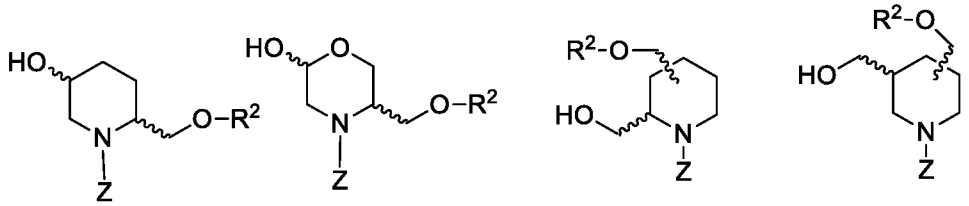
E is –O- or $\text{-}CH_2\text{-}$;

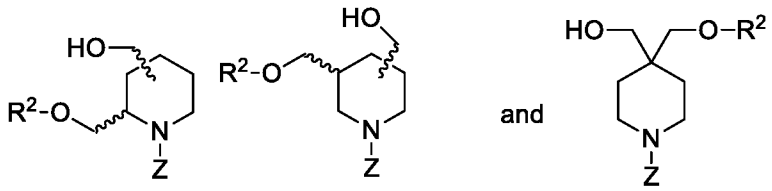
n is selected from the group consisting of 0, 1, 2, 3, and 4; and

n1 and n2 are each independently selected from the group consisting of 0, 1, 2, and 3;

or a salt thereof.

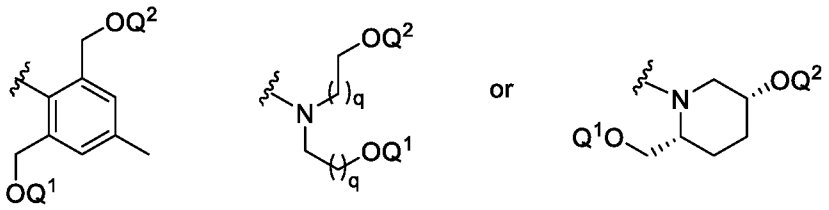
36. The compound of claim 8 or claim 35, or a salt thereof, that is selected from the group consisting of:





wherein: Z is $-L^1-R^1$; and salts thereof.

37. The compound of claim 8 or a salt thereof, wherein the $-A-L^2-R^2$ moiety is:

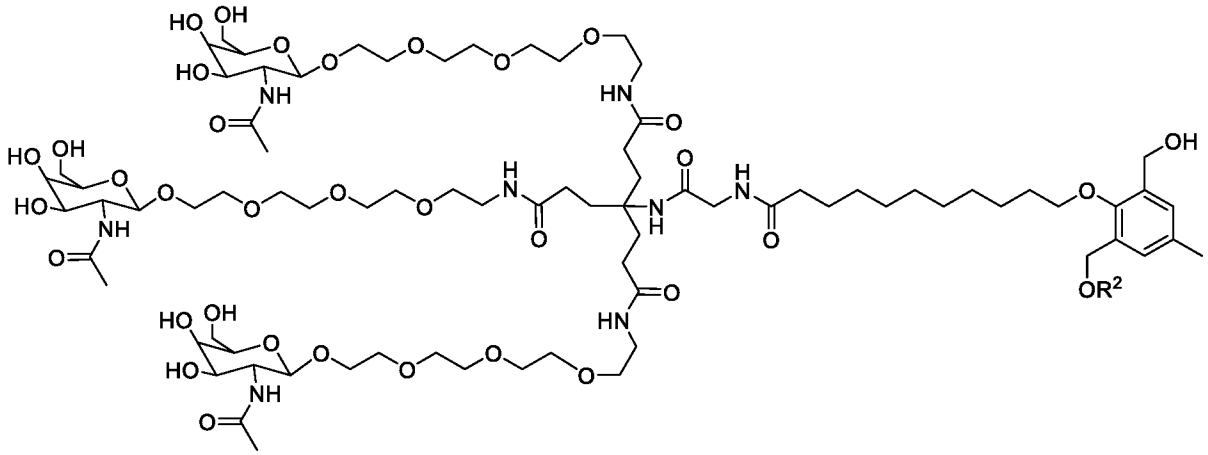


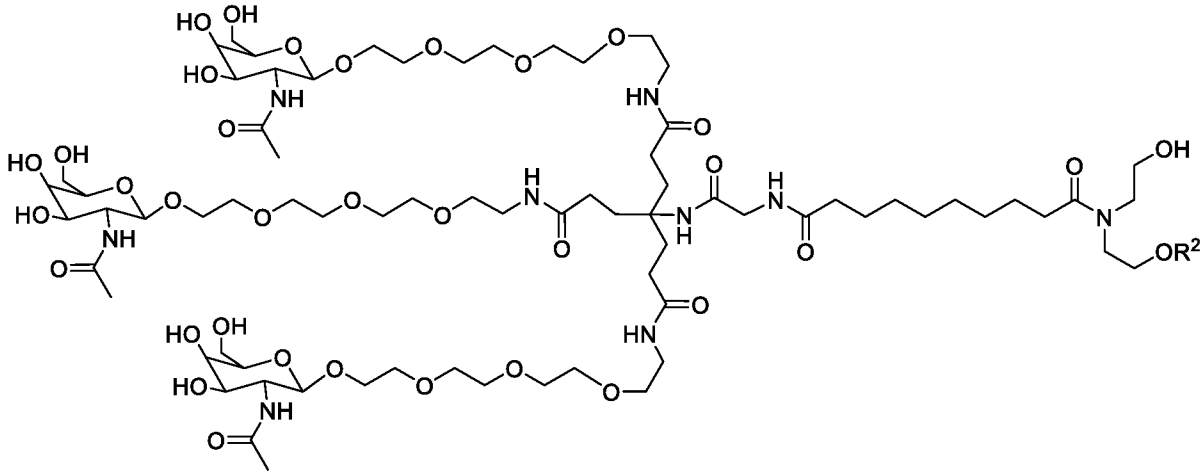
wherein:

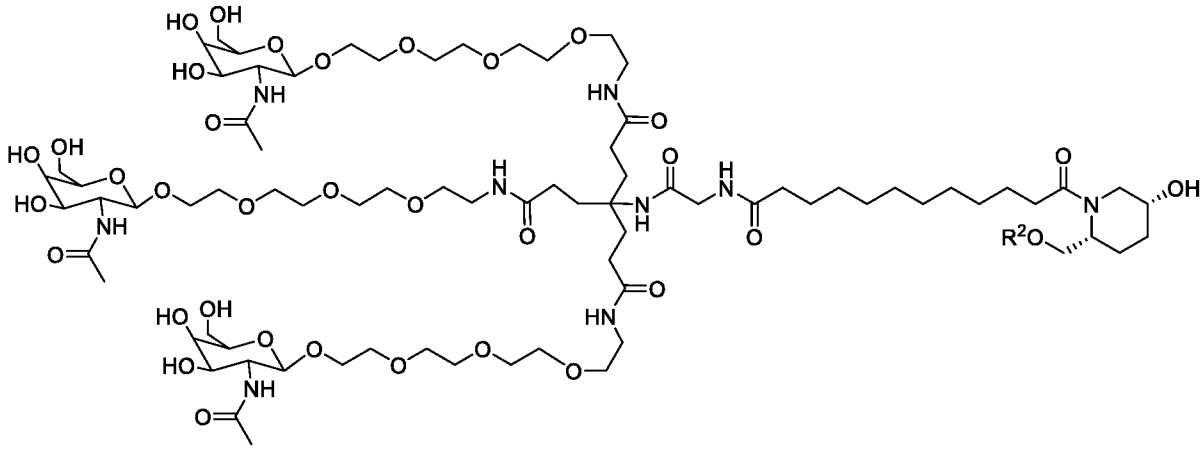
Q^1 is hydrogen and Q^2 is R^2; or Q^1 is R^2 and Q^2 is hydrogen; and

each q is independently 0, 1, 2, 3, 4 or 5.

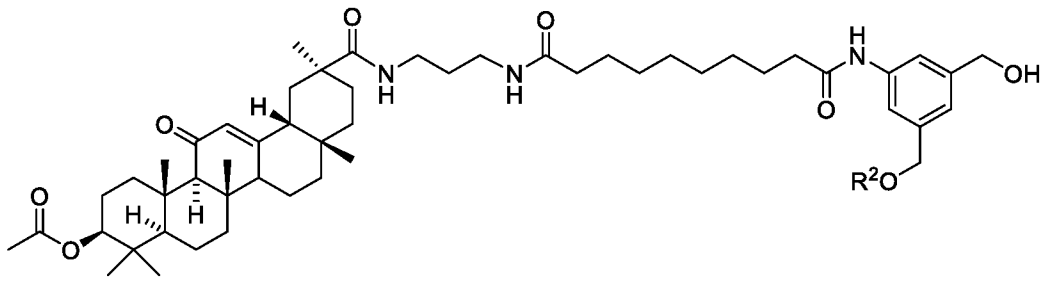
38. The compound of claim 8 or a salt thereof that is selected from the group consisting of:





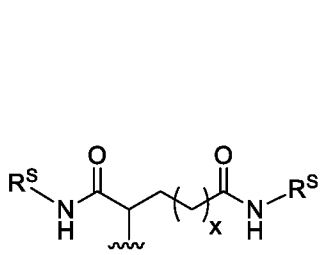


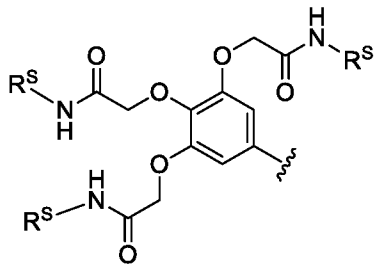
and

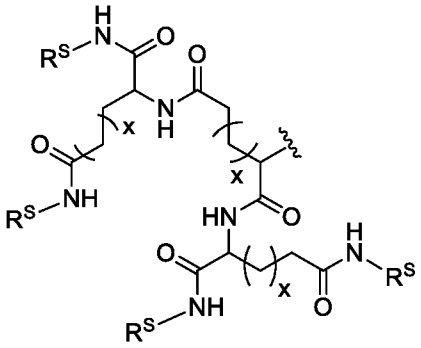


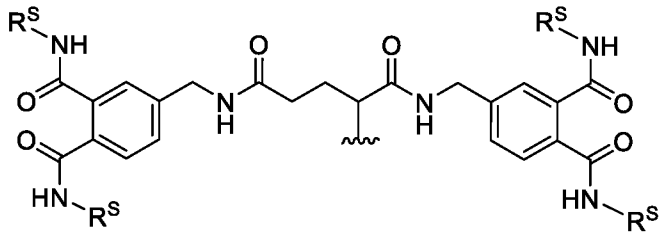
and salts thereof.

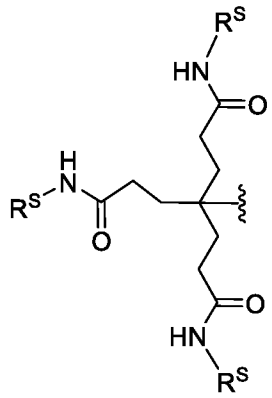
39. The compound of claim 7 or a salt thereof, wherein R^1 is selected from the group consisting of:



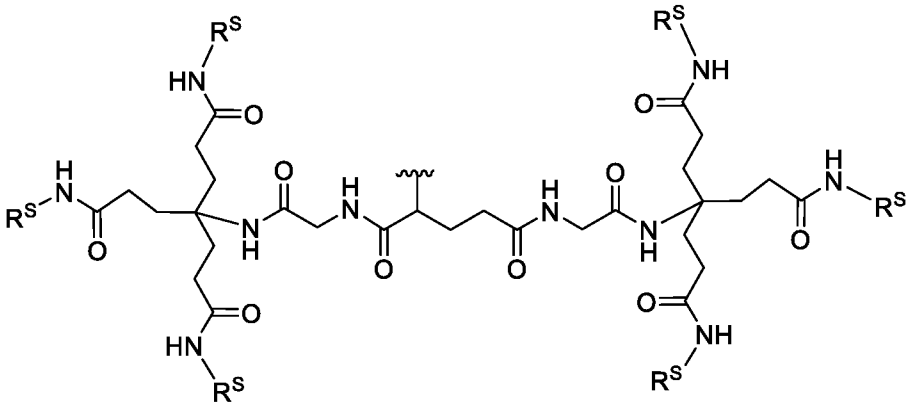




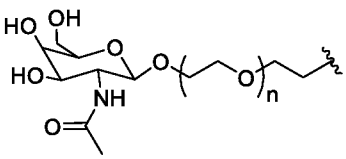




and



wherein:

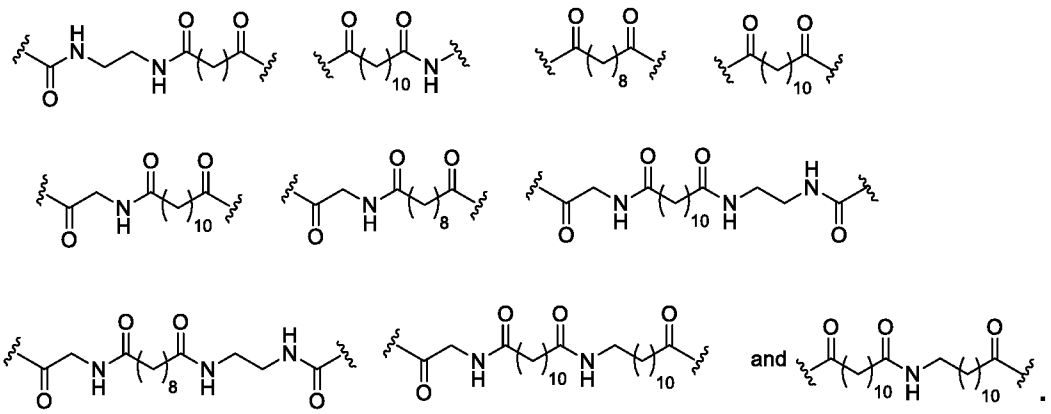


R^S is ;

n is 2, 3, or 4; and

x is 1 or 2.

40. The compound of claim 7 or 39, or a salt thereof, wherein L^1 is selected from the group consisting of:



41. The compound of any one of claims 7, 39 and 40 or a salt thereof, wherein A is absent, phenyl, pyrrolidinyl, or cyclopentyl.

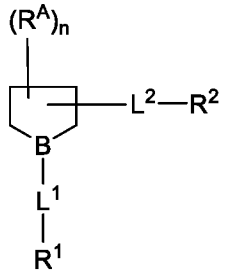
42. The compound of any one of claims 7 and 39-41 or a salt thereof, wherein L^2 is C_{1-4} alkylene-O- that is optionally substituted with hydroxy.

43. The compound of any one of claims 7 and 39-42 or a salt thereof, wherein L^2 is –CH_2O-, -CH_2CH_2O-, or -$CH(OH)CH_2O$-.

44. The compound of any one of claims 7 and 39-43 or a salt thereof, wherein each R^A is independently hydroxy or C_{1-8} alkyl that is optionally substituted with hydroxyl.

45. The compound of any one of claims 7 and 39-44 or a salt thereof, wherein each R^A is independently selected from the group consisting of hydroxy, methyl and –CH_2OH.

46. The compound of claim 7 or a salt thereof that is a compound formula (Ig):



(Ig)

wherein:

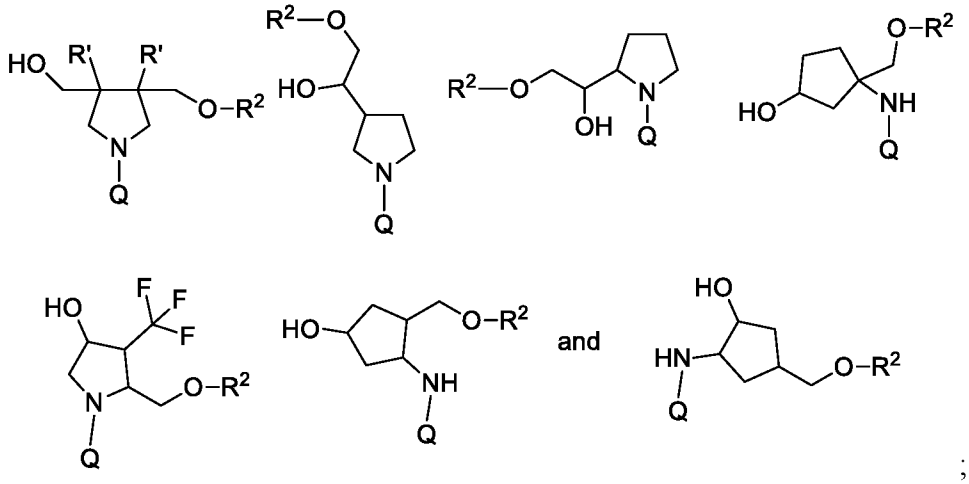
B is –N- or -CH-;

L^2 is C_{1-4} alkylene-O- that is optionally substituted with hydroxyl or halo; and

n is 0, 1, 2, 3, 4, 5, 6, or 7;

or a salt thereof.

47. The compound of claim 7 or 46, or a salt thereof that is selected from the group consisting of:



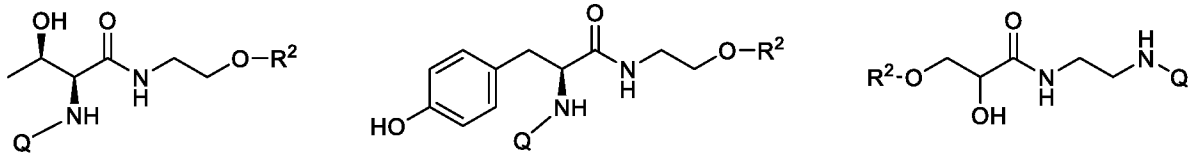
;

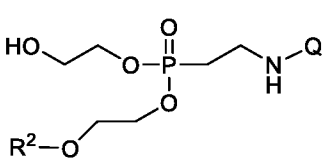
wherein Q is $–L^1-R^1$; and

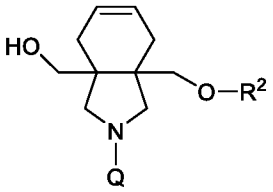
R' is C_{1-9} alkyl, C_{2-9} alkenyl or C_{2-9} alkynyl; wherein the C_{1-9} alkyl, C_{2-9} alkenyl or C_{2-9} alkynyl are optionally substituted with halo or hydroxyl;

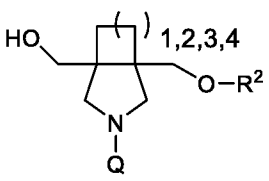
and salts thereof.

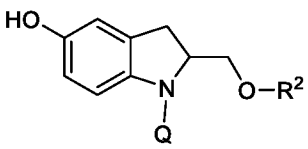
48. The compound of claim 7 or a salt thereof that is selected from the group consisting of:



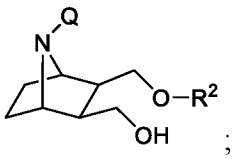








and

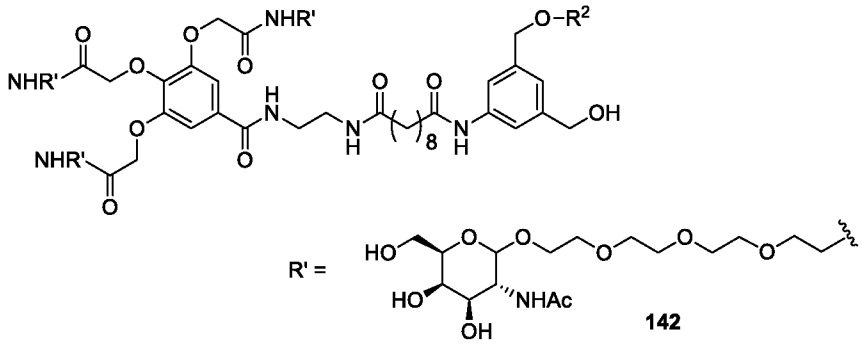


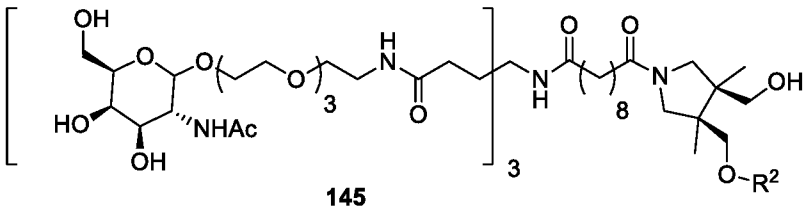
;

wherein Q is $-L^1-R^1$;

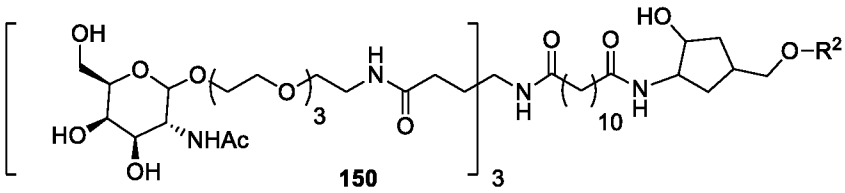
and salts thereof.

49. The compound of claim 7 or a salt thereof that is selected from the group consisting of:

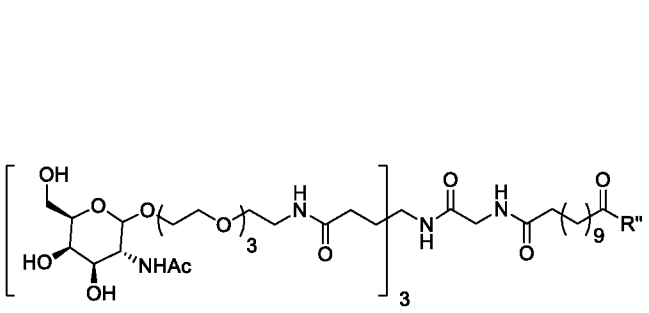


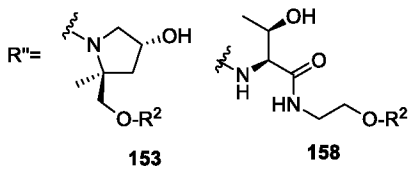


145

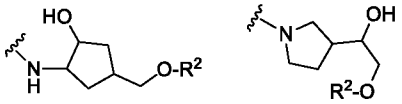


150

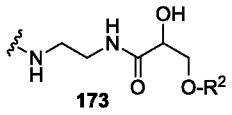




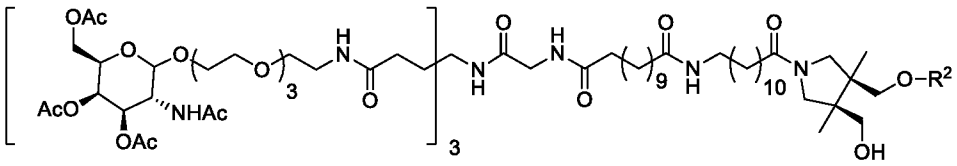
153 158



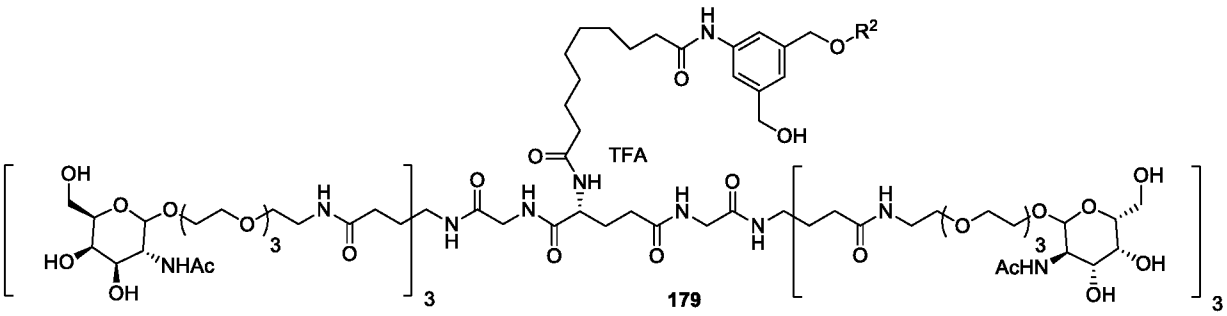
163 168



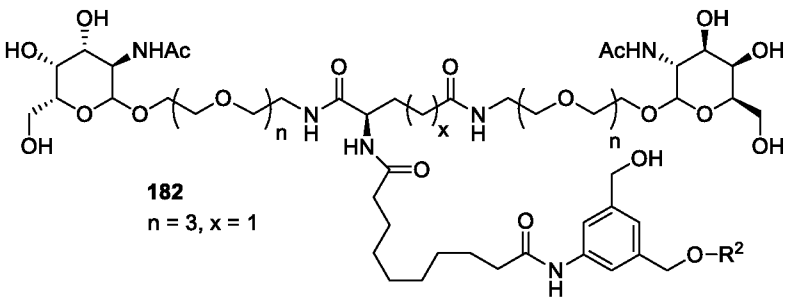
173



176

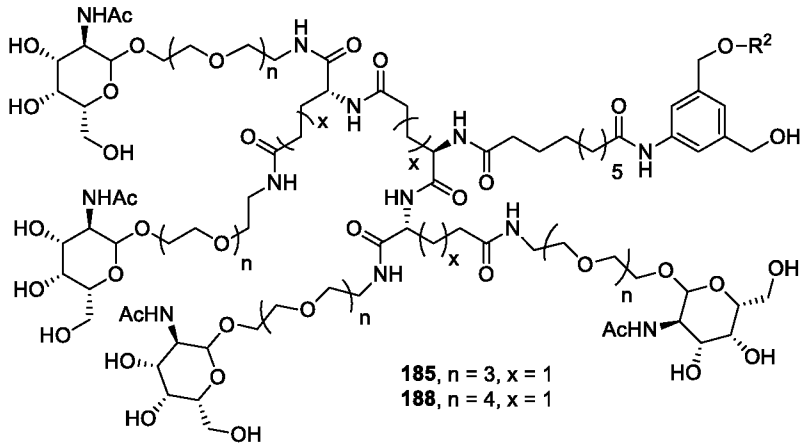


179

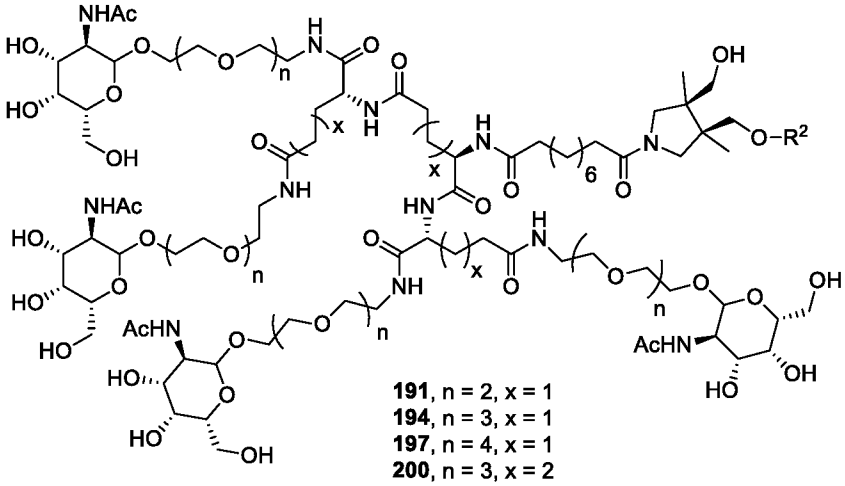


182

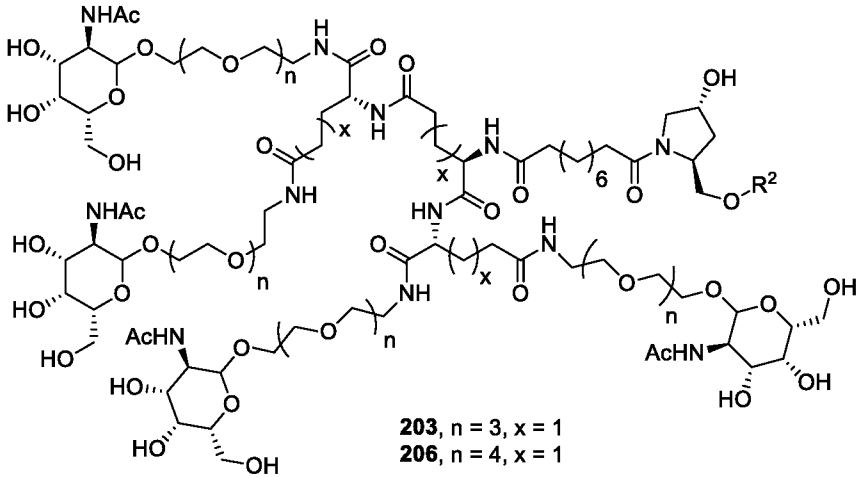
n = 3, x = 1



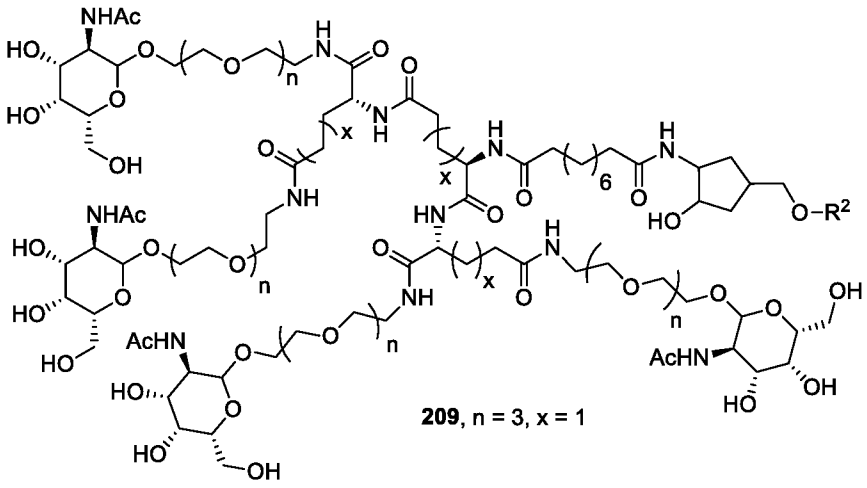
185, n = 3, x = 1
188, n = 4, x = 1



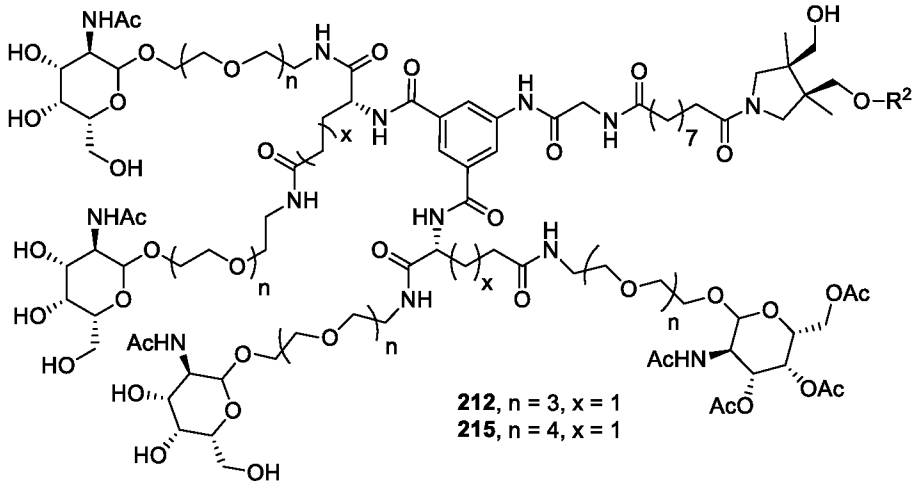
191, n = 2, x = 1
194, n = 3, x = 1
197, n = 4, x = 1
200, n = 3, x = 2



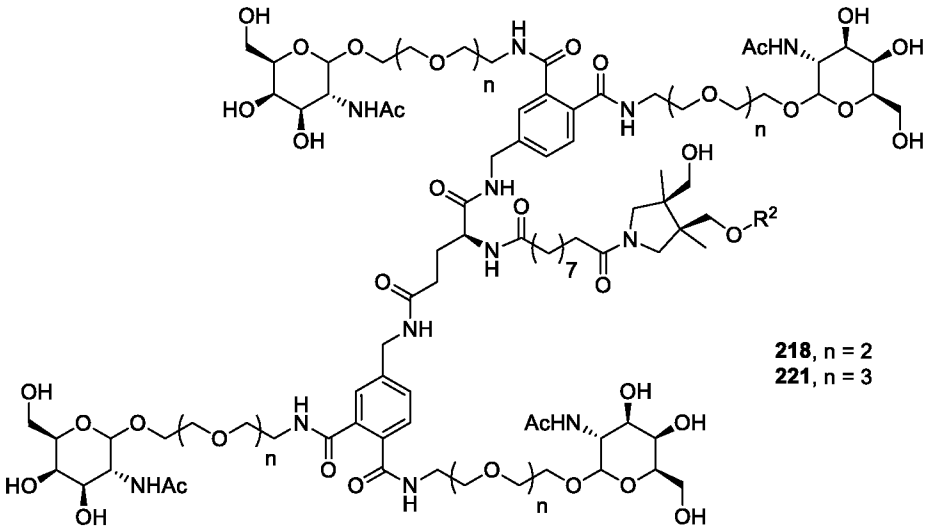
203, n = 3, x = 1
206, n = 4, x = 1



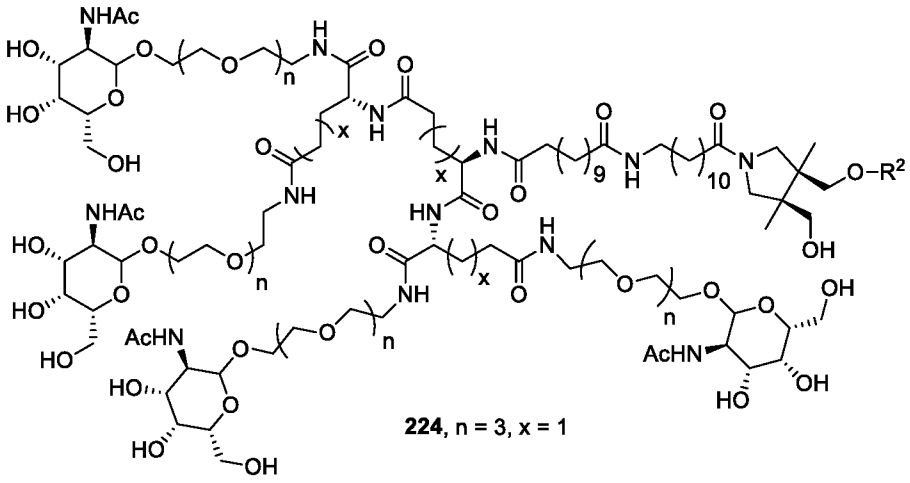
209, n = 3, x = 1



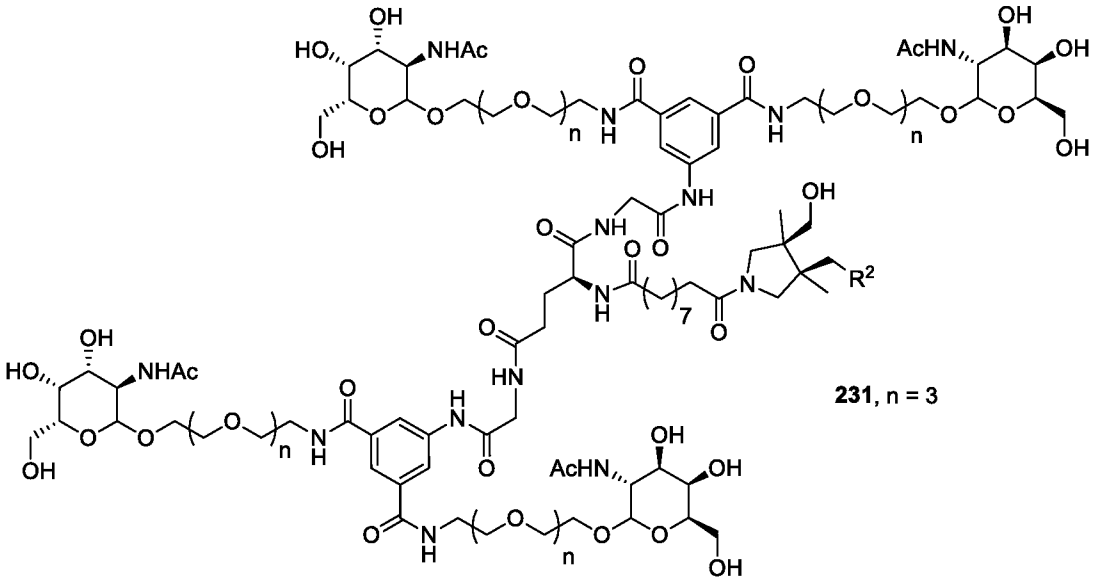
212, n = 3, x = 1
215, n = 4, x = 1



218, n = 2
221, n = 3



224, n = 3, x = 1



231, n = 3

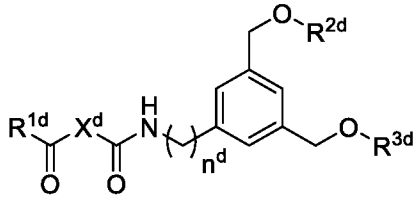
and

and pharmaceutically acceptable salts thereof, wherein R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4.

50. A pharmaceutical composition comprising a compound as described in any one of claims 1-49, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

51. A method to deliver a siRNA to the liver of an animal comprising administering a compound of formula I as described in any one of claims 1-49 or a pharmaceutically acceptable salt thereof, to the animal.

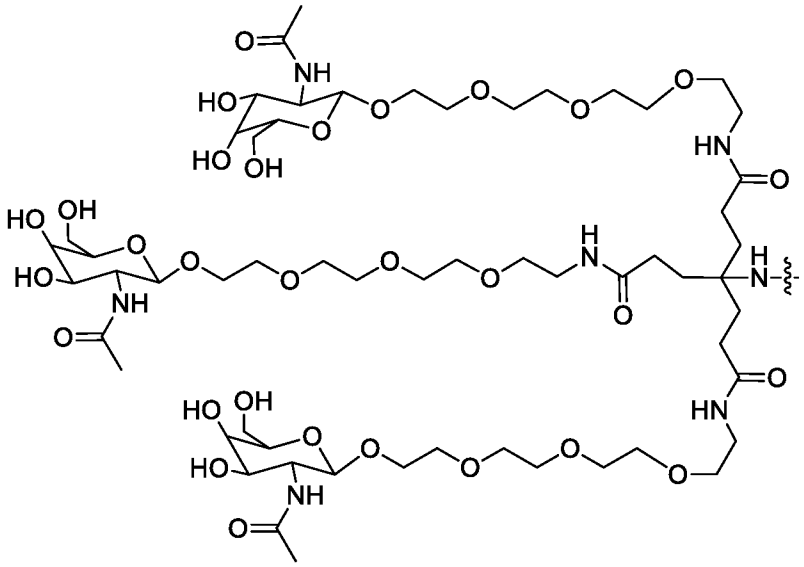
52. The compound of claim 8 or a salt thereof, which is a compound formula (Id):



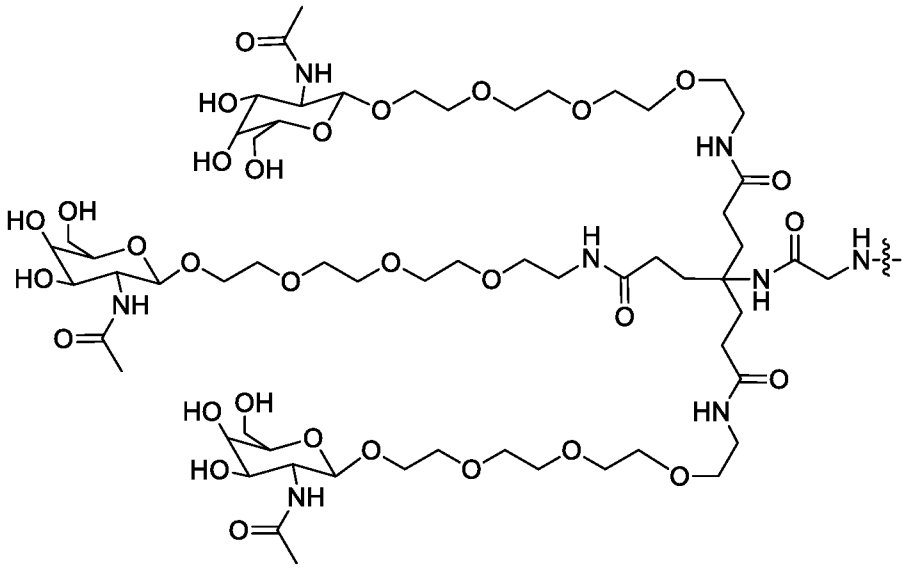
(Id)

wherein:

R^{1d} is selected from:



and



X^d is C_{2-10} alkylene;

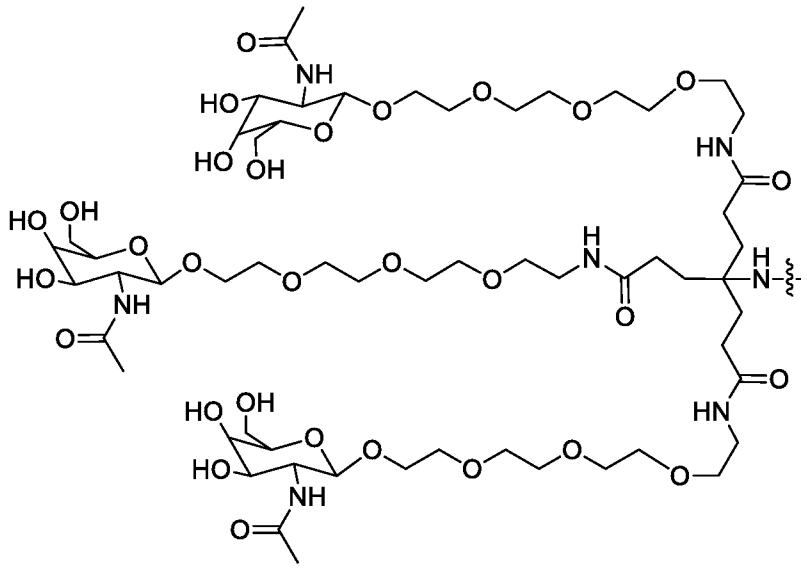
n^d is 0 or 1;

R^{2d} is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4; and

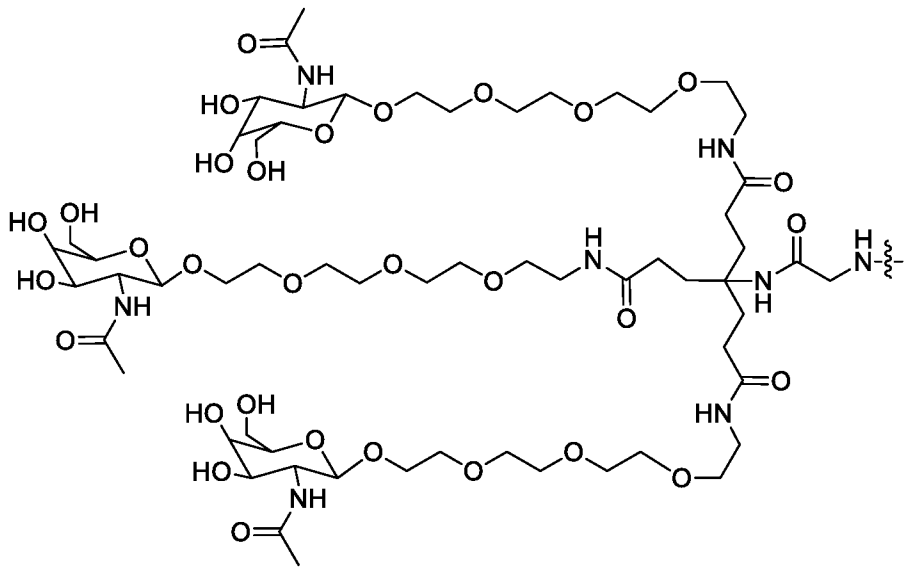
R^{3d} is H, a protecting group, a covalent bond to a solid support, or a bond to a linking group that is bound to a solid support

or a salt thereof.

53. The compound of claim 52 or a salt thereof, wherein R^{1d} is:



54. The compound of claim 52 or a salt thereof, wherein R^{1d} is:



55. The compound or salt of any one of claims 52-54, wherein X^d is C_8alkylene.

56. The compound or salt of any one of claims 52-54, wherein n^d is 0.

57. The compound or salt of any one of claims 52-56, wherein R^{3d} is H.

58. The compound or salt of any one of claims 52-56, wherein R^{3d} is a covalent bond to a solid support.

59. The compound of any one of claims 52-56, or a salt thereof, wherein R^{3d} is a bond to a linking group that is bound to a solid support, wherein the linking group is a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 2 to 15 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms is optionally replaced by (-O-) or (-N(H)-), and wherein the chain is optionally substituted on carbon with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C_1-C_6)alkoxy, (C_3-C_6)cycloalkyl, (C_1-C_6)alkanoyl, (C_1-C_6)alkanoyloxy, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

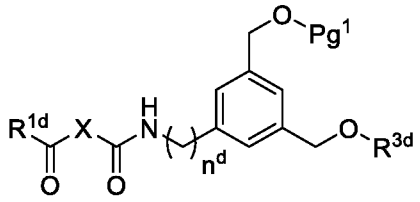
60. The compound of any one of claims 52-56, or a salt thereof, wherein R^{3d} is a bond to a linking group that is bound to a solid support, wherein the linking group is a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 2 to 10 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms is optionally replaced by (-O-) or (-N(H)-), and wherein the chain is optionally substituted on carbon with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C_1-C_6)alkoxy, (C_3-C_6)cycloalkyl, (C_1-C_6)alkanoyl, (C_1-C_6)alkanoyloxy, (C_1-C_6)alkoxycarbonyl, (C_1-C_6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

61. The compound of any one of claims 52-56, or a salt thereof, wherein R^{3d} is a bond to a linking group that is bound to a solid support, wherein the linking group is $-C(=O)CH_2CH_2C(=O)N(H)-$.

62. A pharmaceutical composition comprising a compound as described in any one of claims 52-61 or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

63. A method to deliver a siRNA to the liver of an animal comprising administering a compound of formula Id as described in any one of claims 52-61, or a pharmaceutically acceptable salt thereof, to the animal.

64. A method to prepare a compound of formula (Id) as described claim 48, or a salt thereof, comprising subjecting a corresponding compound of formula (Ie):



(Ie)

wherein:

X^d is C_{2-8} alkylene;

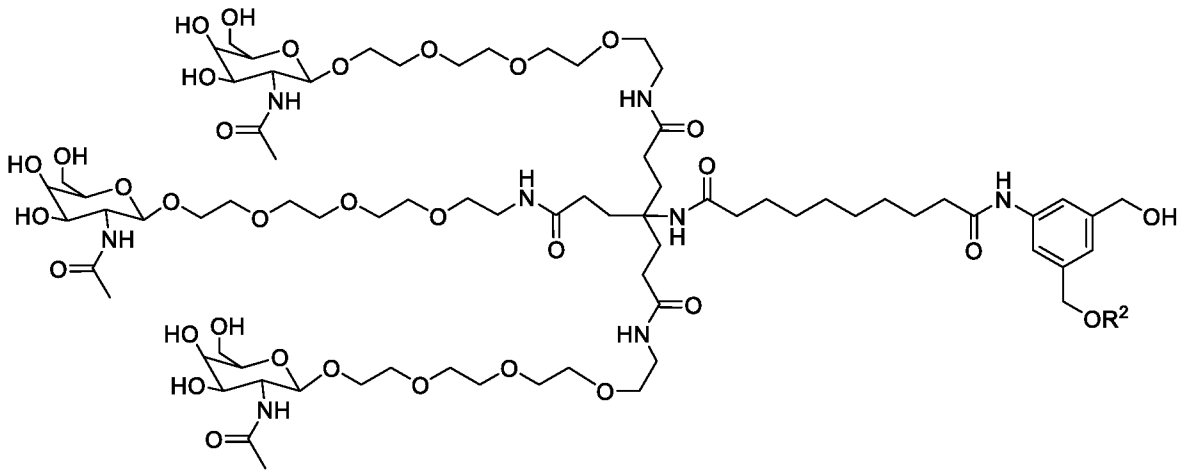
n^d is 0 or 1;

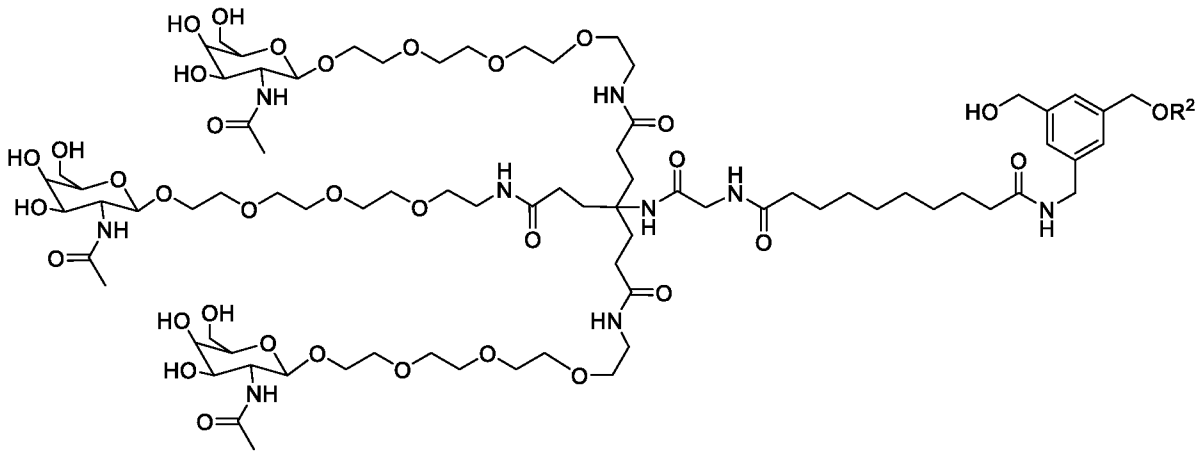
Pg^1 is H; and

R^{3d} is a covalent bond to a solid support or a bond to a linking group that is bound to a solid support, to solid phase nucleic acid synthesis conditions to provide a corresponding compound of formula Id wherein R^{2d} is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4.

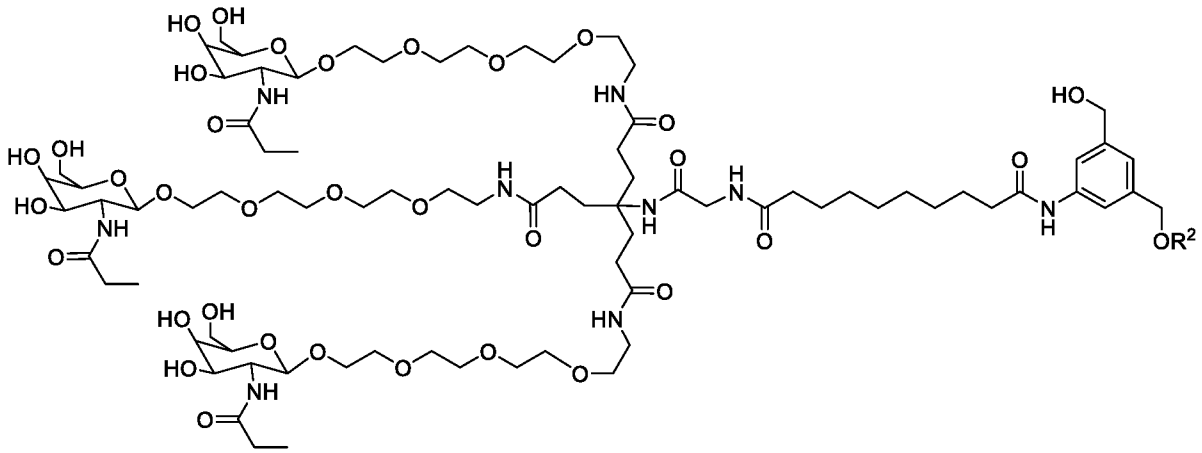
65. The method of claim 64 further comprising removing the compound from the solid support to provide the corresponding compound of formula Id wherein R^{3d} is H.

66. The compound of claim 52 or a salt thereof that is selected from the group consisting of:



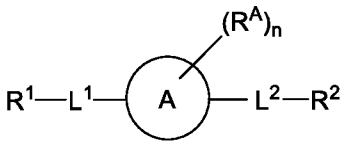


and



and salts thereof.

67. A compound of formula (I):



(I)

wherein:

R^1 is H or a synthetic activating group;

L^1 is absent or a linking group;

L^2 is absent or a linking group;

R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4;

the ring A is absent, a 3-20 membered cycloalkyl, a 5-20 membered aryl, a 5-20 membered heteroaryl, or a 3-20 membered heterocycloalkyl;

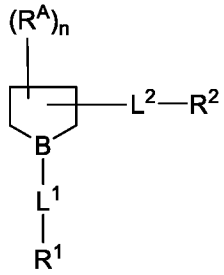
each R^A is independently selected from the group consisting of hydrogen, hydroxy, CN, F, Cl, Br, I, -C_{1-2} alkyl-OR^B, C_{1-10} alkyl C_{2-10} alkenyl, and C_{2-10} alkynyl; wherein the C_{1-10} alkyl C_{2-10} alkenyl, and C_{2-10} alkynyl are optionally substituted with one or more groups independently selected from halo, hydroxy, and C_{1-3} alkoxy;

R^B is hydrogen, a protecting group, a covalent bond to a solid support, or a bond to a linking group that is bound to a solid support; and

n is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

or a salt thereof.

68. The compound of claim 67 or a salt thereof that is a compound of formula (Ig):



(Ig)

wherein:

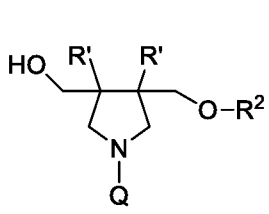
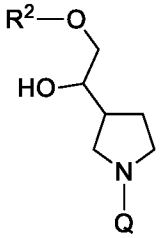
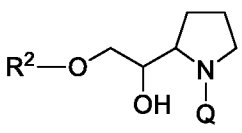
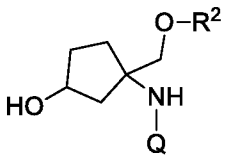
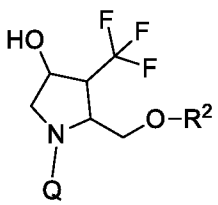
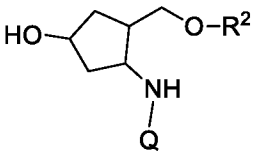
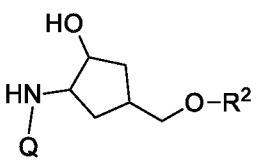
B is –N- or -CH-;

L^2 is C_{1-4} alkylene-O- that is optionally substituted with hydroxyl or halo; and

n is 0, 1, 2, 3, 4, 5, 6, or 7;

or a salt thereof.

69. The compound of claim 67 or a salt thereof that is selected from the group consisting of:







and

;

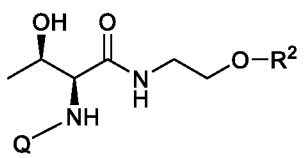
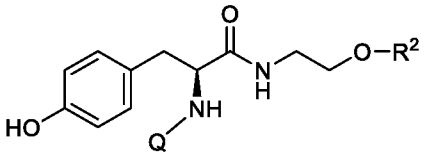
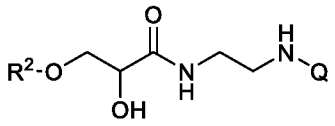
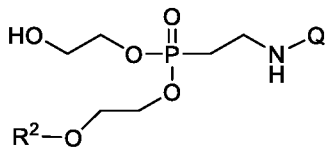
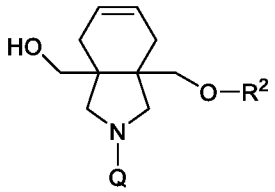
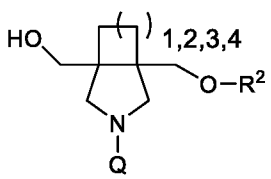
wherein:

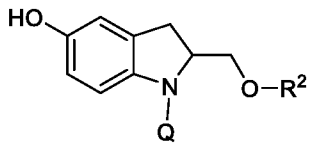
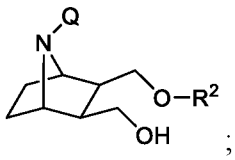
Q is $-L^1-R^1$; and

R' is C_{1-9} alkyl, C_{2-9} alkenyl or C_{2-9} alkynyl; wherein the C_{1-9} alkyl, C_{2-9} alkenyl or C_{2-9} alkynyl are optionally substituted with halo or hydroxyl;

and salts thereof.

70. The compound of claim 67 or a salt thereof that is selected from the group consisting of:


and

;

wherein: Q is $-L^1-R^1$; and salts thereof.

71. The compound of any one of claims 66-73 or a salt thereof, wherein R^1 is H or a synthetic activating group derivable from DCC, HOBt, EDC, BOP, PyBOP or HBTU.

72. The compound of any one of claims 66-73 or a salt thereof, wherein R^1 is a synthetic activating group derivable from DCC, HOBt, EDC, BOP, PyBOP or HBTU.

73. The compound of any one of claims 66-77 or a salt thereof, wherein L^1 is a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 5 to 20 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced –O-, -NH-, -NH-C(=O)-, -C(=O)-NH- or –S-.

74. A compound of formula (XX):

(XX)

wherein:

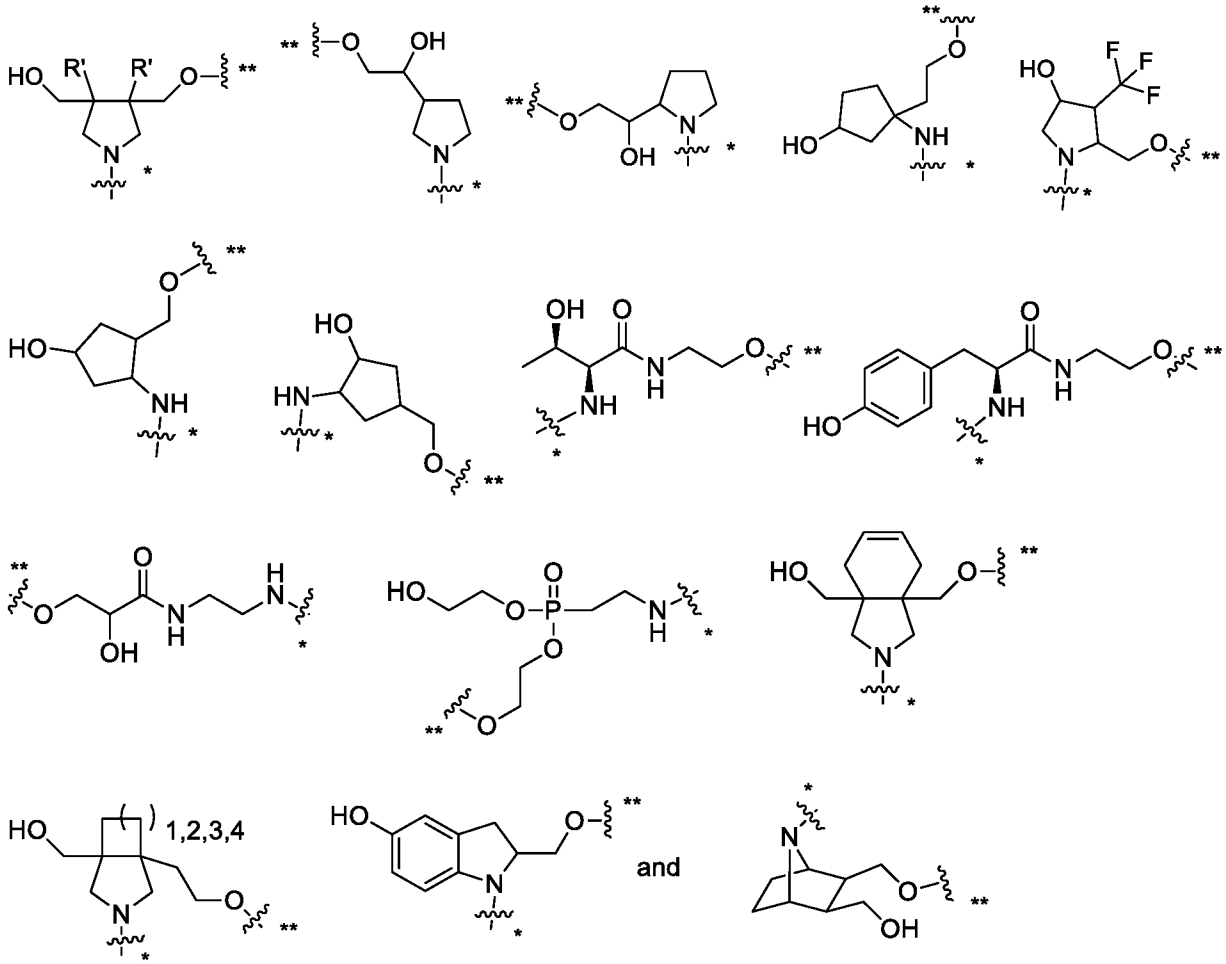
R^1 a is targeting ligand;

L^1 is absent or a linking group;

L^2 is absent or a linking group;

R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4;

B is divalent and is selected from the group consisting of:



wherein:

each R' is independently C_{1-9} alkyl, C_{2-9} alkenyl or C_{2-9} alkynyl; wherein the C_{1-9} alkyl, C_{2-9} alkenyl or C_{2-9} alkynyl are optionally substituted with halo or hydroxyl;

the valence marked with * is attached to L^1 or is attached to R^1 if L^1 is absent; and

the valence marked with ** is attached to L^2 or is attached to R^2 if L^2 is absent;
or a salt thereof.

75. The compound of claim 74 or a salt thereof, wherein the targeting ligand R^1 comprises 2-8 saccharides.

76. The compound of claim 74 or a salt thereof, wherein the targeting ligand R^1 comprises 2-4 saccharides.

77. The compound of claim 74 or a salt thereof, wherein the targeting ligand R^1 comprises 3-8 saccharides.

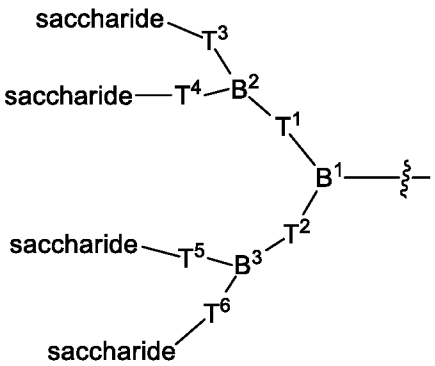
78. The compound of claim 74 or a salt thereof, wherein the targeting ligand R^1 comprises 3-6 saccharides.

79. The compound of claim 74 or a salt thereof, wherein the targeting ligand R^1 comprises 3-4 saccharides.

80. The compound of claim 74 or a salt thereof, wherein the targeting ligand R^1 comprises 3 saccharides.

81. The compound of claim 74 or a salt thereof, wherein the targeting ligand R^1 comprises 4 saccharides.

82. The compound of any one of claims 7-8, 31-36, 38-39, 40-44, 46-48, 67-69, and 74 or a salt thereof, wherein the targeting moiety R^1 has the following formula:



wherein:

B^1 is a trivalent group comprising about 1 to about 20 atoms and is covalently bonded to L^1, T^1, and T^2.

B^2 is a trivalent group comprising about 1 to about 20 atoms and is covalently bonded to T^1, T^3, and T^4;

B^3 is a trivalent group comprising about 1 to about 20 atoms and is covalently bonded to T^2, T^5, and T^6;

T^1 is absent or a linking group;

T^2 is absent or a linking group;

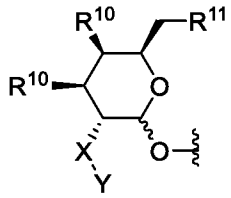
T^3 is absent or a linking group;

T^4 is absent or a linking group;

T^5 is absent or a linking group; and

T^6 is absent or a linking group.

83. The compound of claim 82 or a salt thereof, wherein each saccharide is independently selected from:



wherein:

X is NR^3, and Y is selected from $-(C=O)R^4$, $-SO_2R^5$, and $-(C=O)NR^6R^7$; or X is -(C=O)- and Y is NR^8R^9;

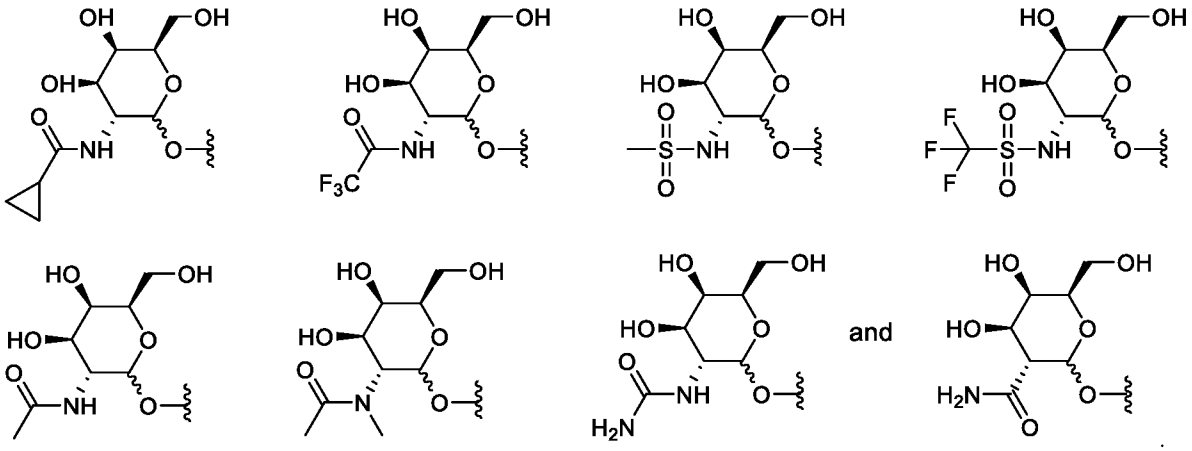
R^3 is hydrogen or $(C_1\text{-}C_4)$alkyl;

R^4, R^5, R^6, R^7 , R^8 and R^9 are each independently selected from the group consisting of hydrogen, $(C_1\text{-}C_8)$alkyl, $(C_1\text{-}C_8)$haloalkyl, $(C_1\text{-}C_8)$alkoxy and $(C_3\text{-}C_6)$cycloalkyl that is optionally substituted with one or more groups independently selected from the group consisting of halo, $(C_1\text{-}C_4)$alkyl, $(C_1\text{-}C_4)$haloalkyl, $(C_1\text{-}C_4)$alkoxy and $(C_1\text{-}C_4)$haloalkoxy;

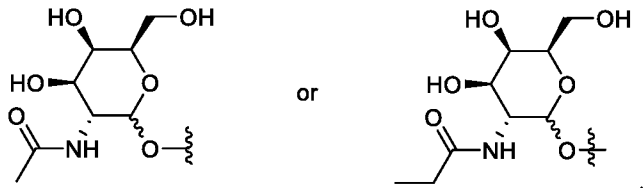
R^{10} is -OH, $-NR^8R^9$ or – F; and

R^{11} is -OH, $-NR^8R^9$, -F or 5 membered heterocycle that is optionally substituted with one or more groups independently selected from the group consisting of halo, hydroxyl, carboxyl, amino, $(C_1\text{-}C_4)$alkyl, $(C_1\text{-}C_4)$haloalkyl, $(C_1\text{-}C_4)$alkoxy and $(C_1\text{-}C_4)$haloalkoxy.

84. The compound of claim 82 or a salt thereof, wherein each the saccharide is independently selected from the group consisting of:



85. The compound of claim 82 or a salt thereof, wherein each saccharide is independently:



86. The compound of any one of claims 82-85 or a salt thereof, wherein one of T^1 and T^2 is absent.

87. The compound of any one of claims 82-85 or a salt thereof, wherein both T^1 and T^2 are absent.

88. The compound of any one of claims 82-85 or a salt thereof, wherein each of T^1, T^2, T^3, T^4, T^5, and T^6 is independently absent or a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 50 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O-, $-NR^X-$, $-NR^X-C(=O)-$, $-C(=O)-NR^X-$ or –S-, and wherein R^X is hydrogen or (C1-C6)alkyl, and wherein the hydrocarbon chain, is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C1-C6)alkoxy, (C3-C6)cycloalkyl, (C1-C6)alkanoyl, (C1-C6)alkanoyloxy, (C1-C6)alkoxycarbonyl, (C1-C6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

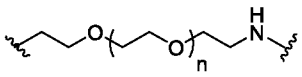
89. The compound of any one of claims 82-85 or a salt thereof, wherein each of T^1, T^2, T^3, T^4, T^5, and T^6 is independently absent or a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 20 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O-, -NR^X-, -NR^X-C(=O)-, -C(=O)-NR^X- or –S-, and wherein R^X is hydrogen or (C1-C6)alkyl, and wherein the hydrocarbon chain, is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from (C1-C6)alkoxy, (C3-C6)cycloalkyl, (C1-C6)alkanoyl, (C1-C6)alkanoyloxy, (C1-C6)alkoxycarbonyl, (C1-C6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

90. The compound of any one of claims 82-85 or a salt thereof, wherein each of T^1, T^2, T^3, T^4, T^5, and T^6 is independently absent or a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 50 carbon atoms, or a salt thereof, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O- or -NR^X-, and wherein R^X is hydrogen or (C_1-C_6)alkyl, and wherein the hydrocarbon chain, is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from halo, hydroxy, and oxo (=O).

91. The compound of any one of claims 82-85 or a salt thereof, wherein each of T^1, T^2, T^3, T^4, T^5, and T^6 is independently absent or a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 20 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O- and wherein the hydrocarbon chain, is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from halo, hydroxy, and oxo (=O).

92. The compound of any one of claims 82-85 or a salt thereof, wherein each of T^1, T^2, T^3, T^4, T^5, and T^6 is independently absent or a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 20 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O- and wherein the hydrocarbon chain, is optionally substituted with one or more (e.g. 1, 2, 3, or 4) substituents selected from halo, hydroxy, and oxo (=O).

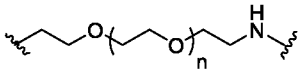
93. The compound of any one of claims 82-85 or a salt thereof, where at least one of T^3, T^4, T^5, and T^6 is:



wherein:

n = 1, 2, 3.

94. The compound of any one of claims 82-85 or a salt thereof, where each of T^3, T^4, T^5, and T^6 is independently selected from the group consisting of:



wherein:

n = 1, 2, 3.

95. The compound of any one of claims 82-85 or a salt thereof, wherein at least one of T^1 and T^2 is glycine.

96. The compound of any one of claims 82-85 or a salt thereof, wherein each of T^1 and T^2 is glycine.

97. The compound of any one of claims 82-96 or a salt thereof, wherein B^1 is a trivalent group comprising 1 to 15 atoms and is covalently bonded to L^1, T^1, and T^2.

98. The compound of any one of claims 82-96 or a salt thereof, wherein B^1 is a trivalent group comprising 1 to 10 atoms and is covalently bonded to L^1, T^1, and T^2.

99. The compound of any one of claims 82-96 or a salt thereof, wherein B^1 comprises a $(C_1\text{-}C_6)$alkyl

100. The compound of any one of claims 82-96 or a salt thereof, wherein B^1 comprises a $C_{3\text{-}8}$ cycloalkyl.

101. The compound of any one of claims 82-96 or a salt thereof, wherein B^1 comprises a silyl group.

102. The compound of any one of claims 82-96 or a salt thereof, wherein B^1 comprises a D- or L-amino acid.

103. The compound of any one of claims 82-96 or a salt thereof, wherein B^1 comprises a saccharide.

104. The compound of any one of claims 82-96 or a salt thereof, wherein B^1 comprises a phosphate group.

105. The compound of any one of claims 82-96 or a salt thereof, wherein B^1 comprises a phosphonate group.

106. The compound of any one of claims 82-96 or a salt thereof, wherein B^1 comprises an aryl.

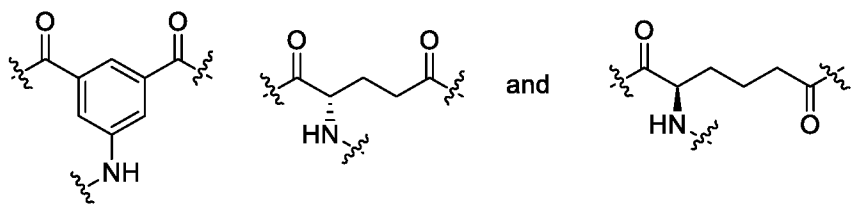
107. The compound of any one of claims 82-96 or a salt thereof, wherein B^1 comprises a phenyl ring.

108. The compound of any one of claims 82-96 or a salt thereof, wherein B^1 is a phenyl ring.

109. The compound of any one of claims 82-96 or a salt thereof, wherein B^1 is CH.

110. The compound of any one of claims 82-96 or a salt thereof, wherein B^1 comprises a heteroaryl.

111. The compound of any one of claims 82-96 or a salt thereof, wherein B^1 is:



112. The compound of any one of claims 82-111 or a salt thereof, wherein B^2 is a trivalent group comprising 1 to 15 atoms and is covalently bonded to L^1, T^1, and T^2.

113. The compound of any one of claims 82-111 or a salt thereof, wherein B^2 is a trivalent group comprising 1 to 10 atoms and is covalently bonded to L^1, T^1, and T^2.

114. The compound of any one of claims 82-111 or a salt thereof, wherein B^2 comprises a $(C_1\text{-}C_6)$alkyl.

115. The compound of any one of claims 82-111 or a salt thereof, wherein B^2 comprises a $C_{3\text{-}8}$ cycloalkyl.

116. The compound of any one of claims 82-111 or a salt thereof, wherein B^2 comprises a silyl group.

117. The compound of any one of claims 82-111 or a salt thereof, wherein B^2 comprises a D- or L-amino acid.

118. The compound of any one of claims 82-111 or a salt thereof, wherein B^2 comprises a saccharide.

119. The compound of any one of claims 82-111 or a salt thereof, wherein B^2 comprises a phosphate group.

120. The compound of any one of claims 82-111 or a salt thereof, wherein B^2 comprises a phosphonate group.

121. The compound of any one of claims 82-111 or a salt thereof, wherein B^2 comprises an aryl.

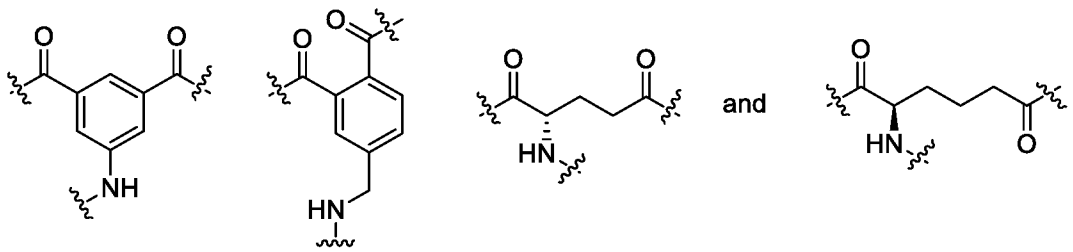
122. The compound of any one of claims 82-111 or a salt thereof, wherein B^2 comprises a phenyl ring.

123. The compound of any one of claims 82-111 or a salt thereof, wherein B^2 is a phenyl ring.

124. The compound of any one of claims 82-111 or a salt thereof, wherein B^2 is CH.

125. The compound of any one of claims 82-111 or a salt thereof, wherein B^2 comprises a heteroaryl.

126. The compound of any one of claims 82-111 or a salt thereof, wherein B^2 is selected from the group consisting of:



127. The compound of any one of claims 82-126 or a salt thereof, wherein B^3 is a trivalent group comprising 1 to 15 atoms and is covalently bonded to L^1, T^1, and T^2.

128. The compound of any one of claims 82-126 or a salt thereof, wherein B^3 is a trivalent group comprising 1 to 10 atoms and is covalently bonded to L^1, T^1, and T^2.

129. The compound of any one of claims 82-126 or a salt thereof, wherein B^3 comprises a $(C_1\text{-}C_6)$alkyl.

130. The compound of any one of claims 82-126 or a salt thereof, wherein B^3 comprises a $C_{3\text{-}8}$ cycloalkyl.

131. The compound of any one of claims 82-126 or a salt thereof, wherein B^3 comprises a silyl group.

132. The compound of any one of claims 82-126 or a salt thereof, wherein B^3 comprises a D- or L-amino acid.

133. The compound of any one of claims 82-126 or a salt thereof, wherein B^3 comprises a saccharide.

134. The compound of any one of claims 82-126 or a salt thereof, wherein B^3 comprises a phosphate group.

135. The compound of any one of claims 82-126 or a salt thereof, wherein B^3 comprises a phosphonate group.

136. The compound of any one of claims 82-126 or a salt thereof, wherein B^3 comprises an aryl.

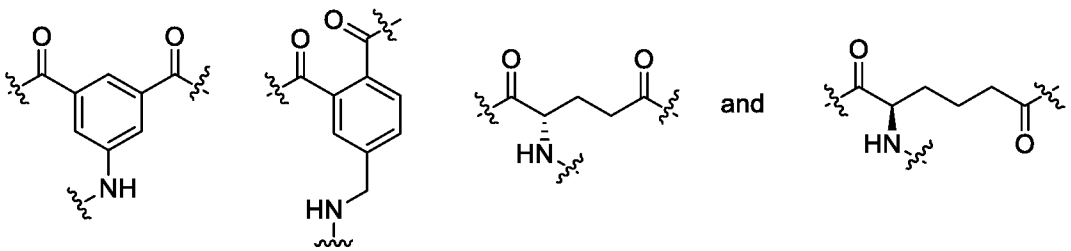
137. The compound of any one of claims 82-126 or a salt thereof, wherein B^3 comprises a phenyl ring.

138. The compound of any one of claims 82-126 or a salt thereof, wherein B^3 is a phenyl ring.

139. The compound of any one of claims 82-126 or a salt thereof, wherein B^3 is CH.

140. The compound of any one of claims 82-126 or a salt thereof, wherein B^3 comprises a heteroaryl.

141. The compound of any one of claims 82-126 or a salt thereof, wherein B^3 is selected from the group consisting of:

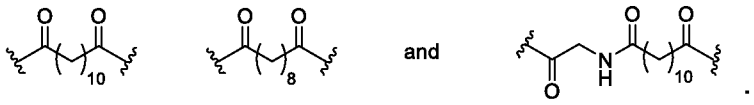


or a salt thereof.

142. The compound of any one of claims 87-146 or a salt thereof, wherein L^1 and L^2 are independently a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon

chain, having from 1 to 50 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O-, -NR^X-, -NR^X-C(=O)-, -C(=O)-NR^X- or –S-, and wherein R^X is hydrogen or (C1-C6)alkyl, and wherein the hydrocarbon chain, is optionally substituted with one or more substituents selected from (C1-C6)alkoxy, (C3-C6)cycloalkyl, (C1-C6)alkanoyl, (C1-C6)alkanoyloxy, (C1-C6)alkoxycarbonyl, (C1-C6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

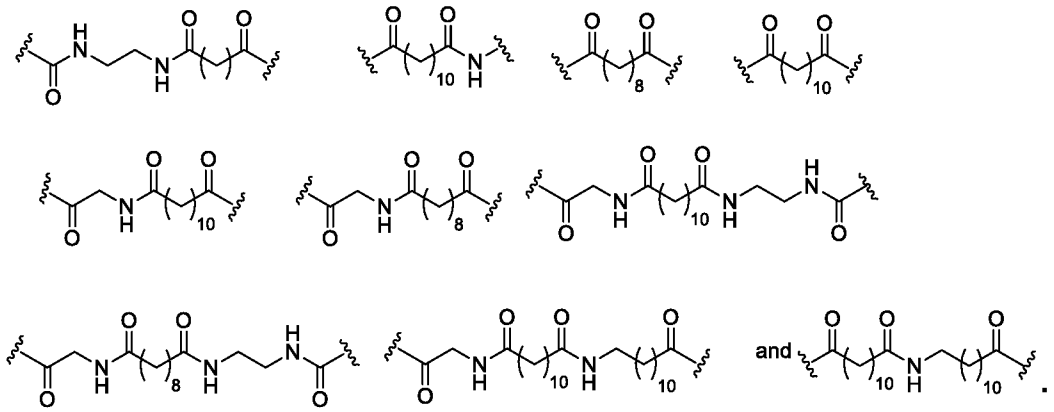
143. The compound of any one of claims 82-141 or a salt thereof, L^1 is selected from the group consisting of:



or a salt thereof.

144. The compound of any one of claims 82-141 or a salt thereof, wherein L^1 is connected to B^1 through a linkage selected from the group consisting of: -O-, -S-, -(C=O)-, -(C=O)-NH-, -NH-(C=O), -(C=O)-O-, -NH-(C=O)-NH-, or –NH-(SO_2)-.

145. The compound of any one of claims 87-141 or a salt thereof, wherein L^1 is selected from the group consisting of:



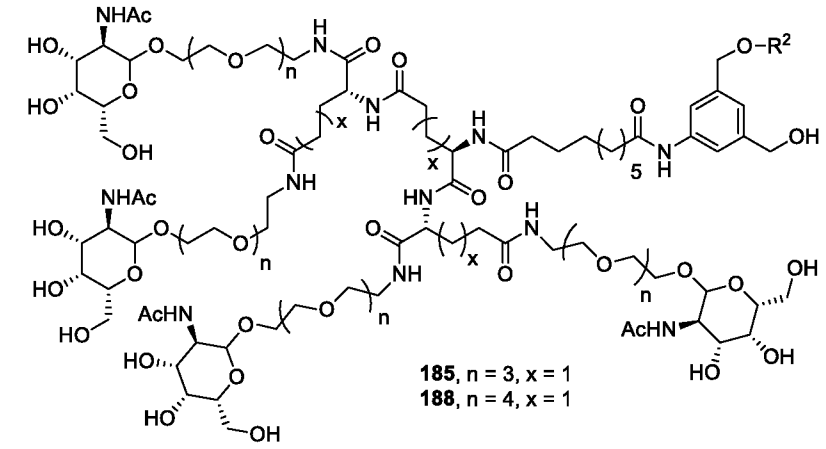
146. The compound of any one of claims 82-145 or a salt thereof, wherein L^2 is connected to R^2 through -O-.

147. The compound of any one of claims 82-145 or a salt thereof, wherein L^2 is C_{1-4} alkylene-O- that is optionally substituted with hydroxy.

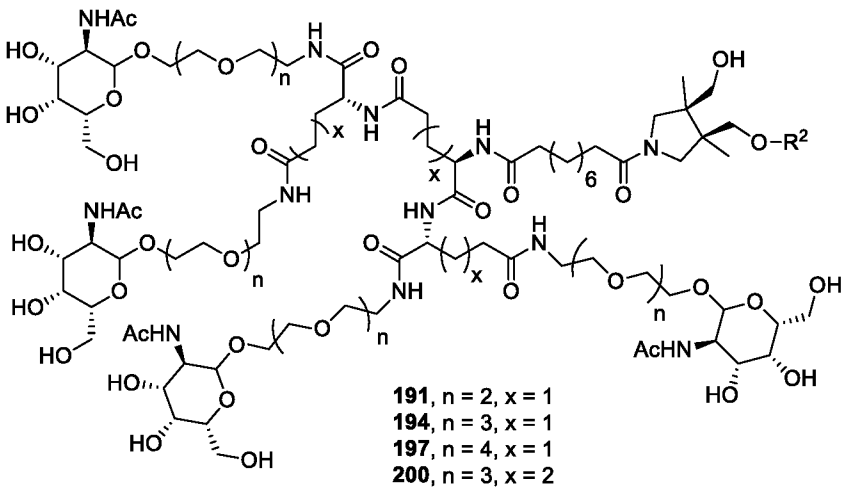
148. The compound of any one of claims 82-145 or a salt thereof, wherein L^2 is connected to R^2 through -O-.

149. The compound of any one of claims 82-145 or a salt thereof, wherein L^2 is absent.

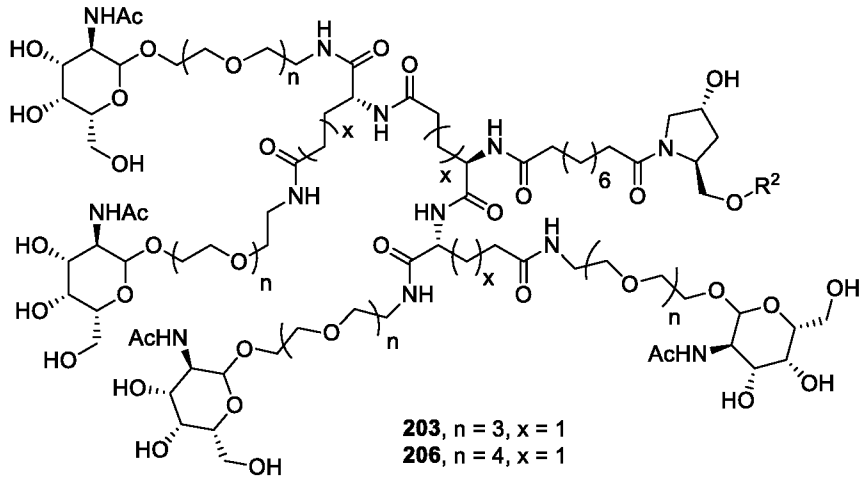
150. The compound of claim 87 or a salt thereof that is selected from the group consisting of:



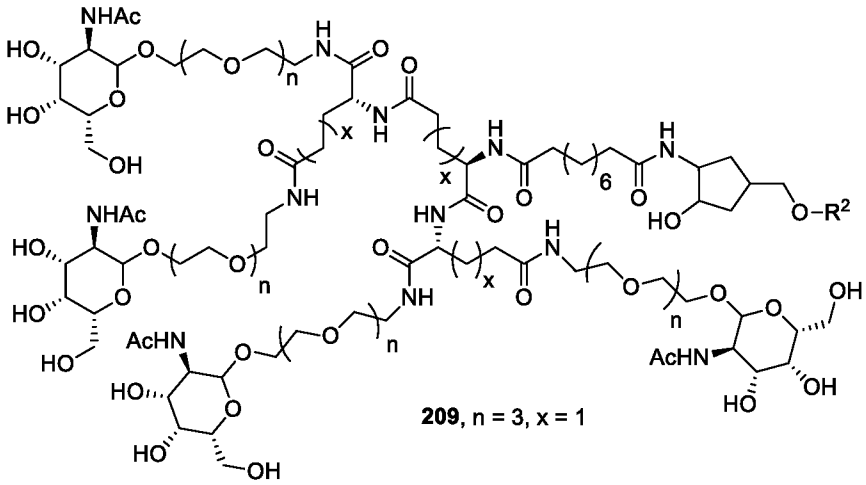
185, n = 3, x = 1
188, n = 4, x = 1



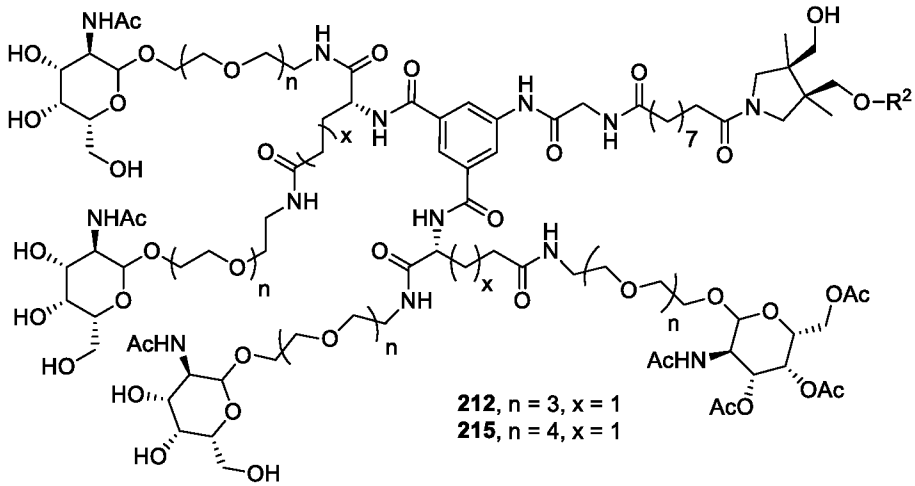
191, n = 2, x = 1
194, n = 3, x = 1
197, n = 4, x = 1
200, n = 3, x = 2



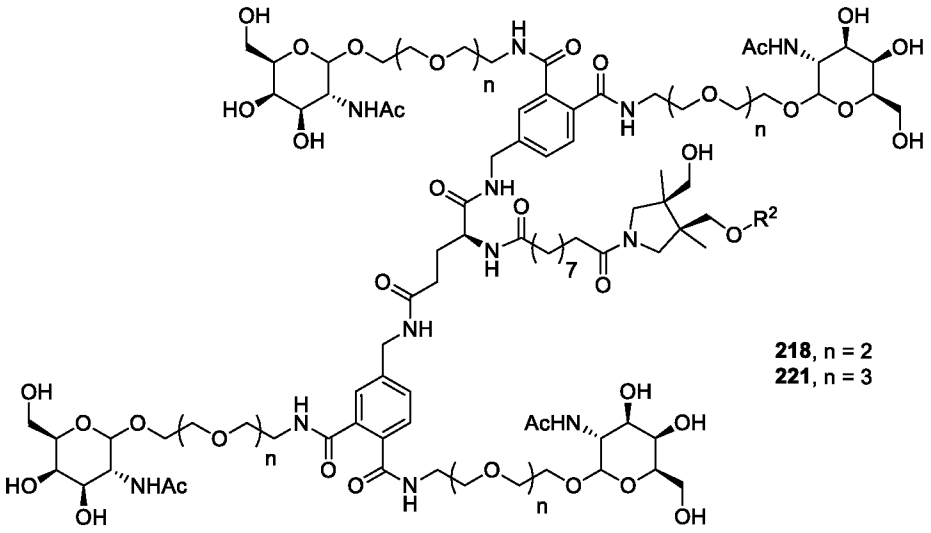
203, n = 3, x = 1
206, n = 4, x = 1



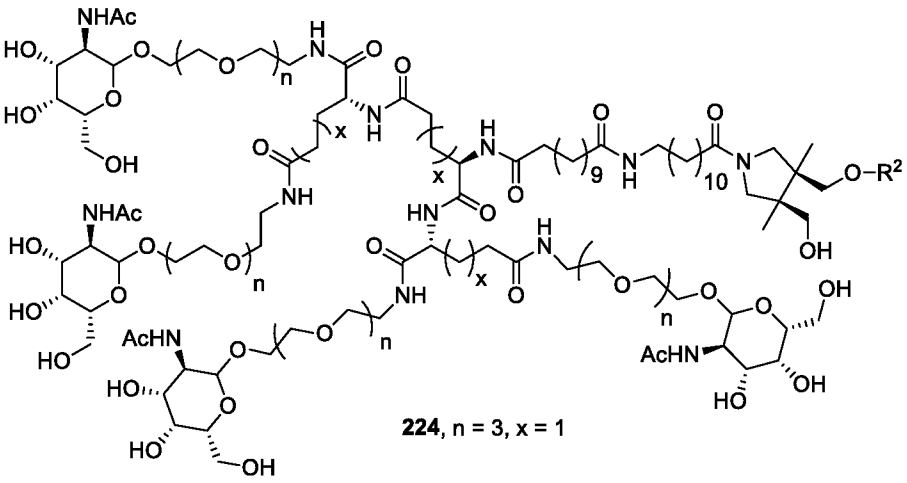
209, n = 3, x = 1



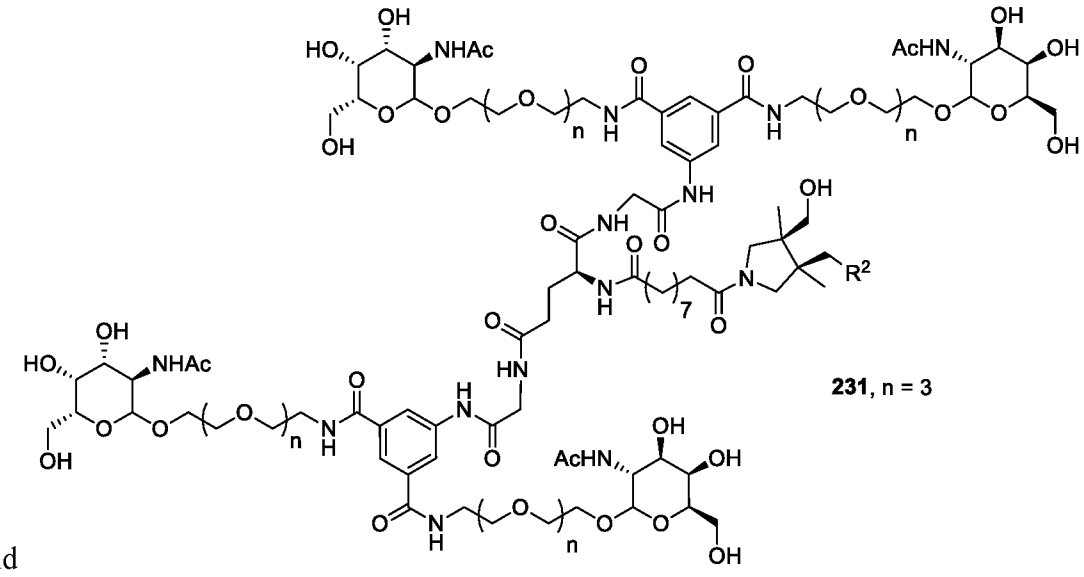
212, n = 3, x = 1
215, n = 4, x = 1



218, n = 2
221, n = 3



224, n = 3, x = 1

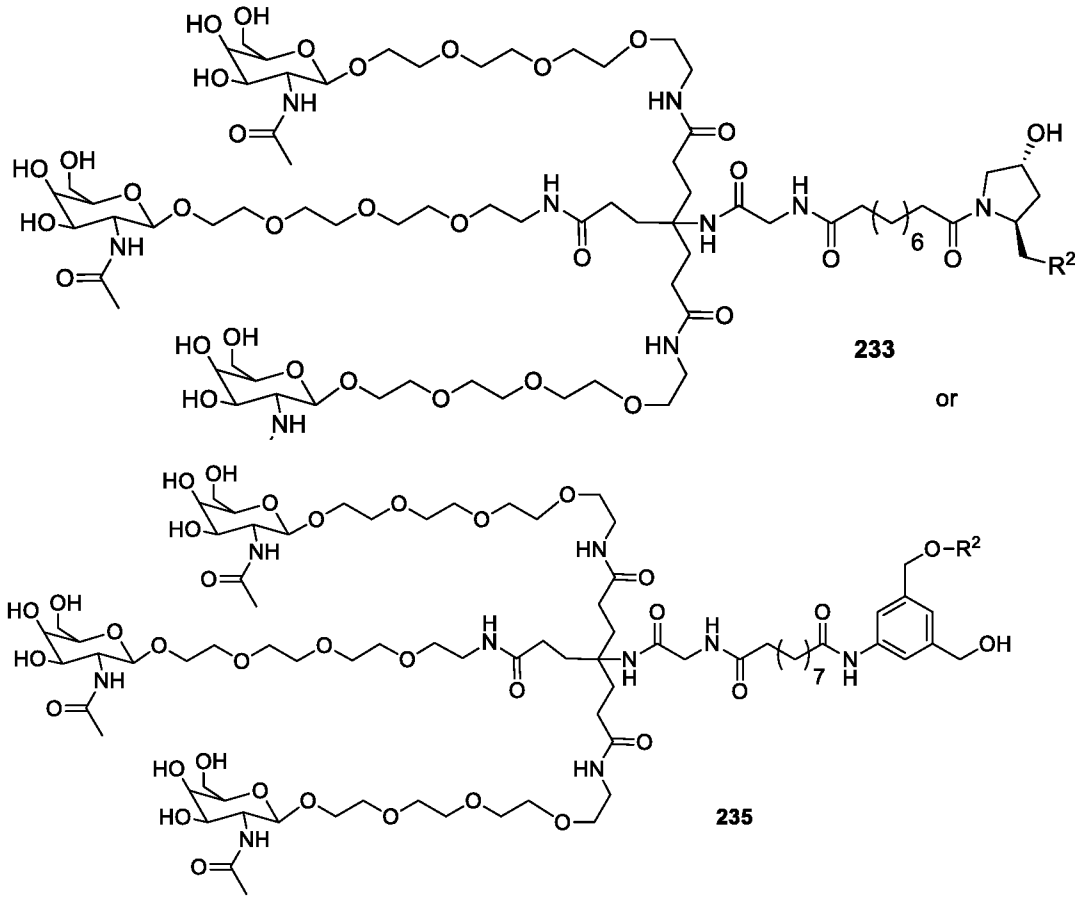


231, n = 3

and ;

and pharmaceutically acceptable salts thereof, wherein R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4.

151. The compound,



233

or

235

or a salt thereof, wherein R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4.

152. A GalNAc conjugate of Formula X:

A-B-C

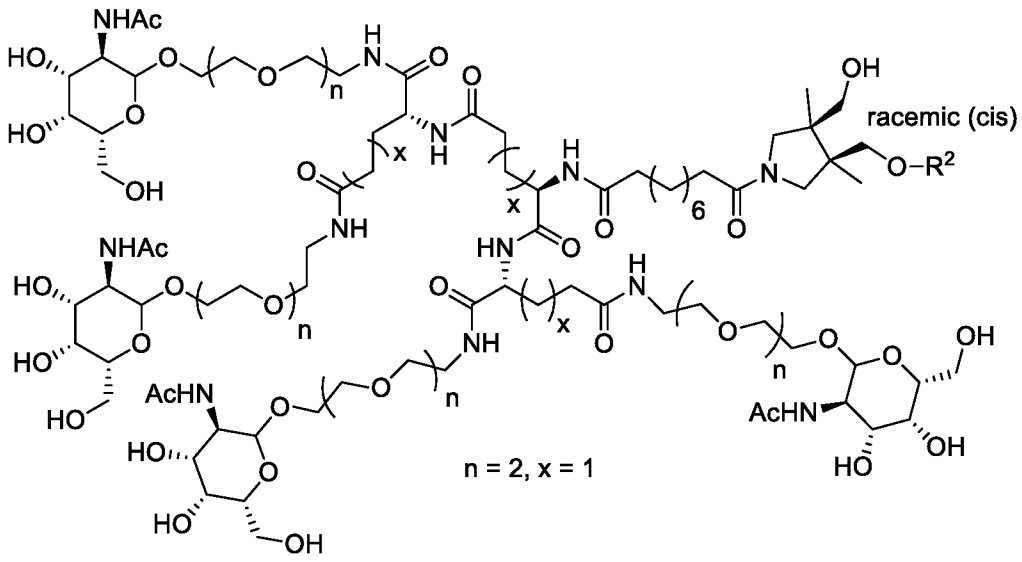
(X)

wherein A is a targeting ligand;

B is an optional linker; and

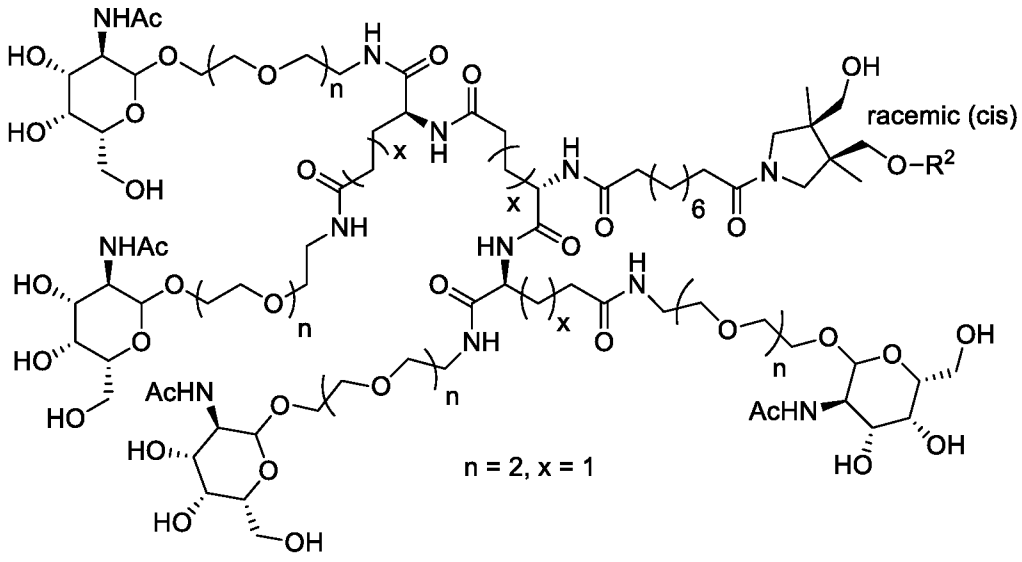
C is an siRNA molecule of claim 4.

153. A compound of formula:



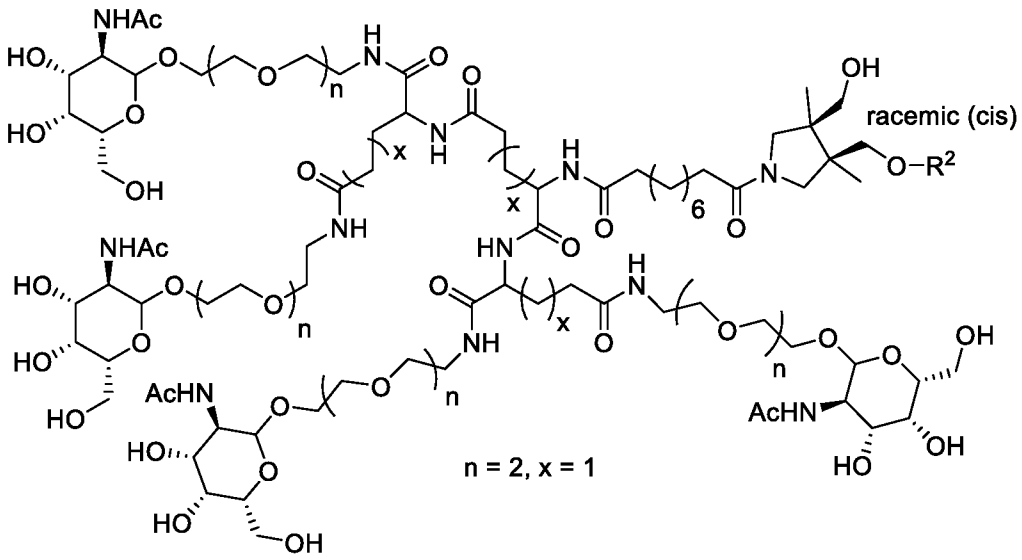
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4.

154. A compound of formula:



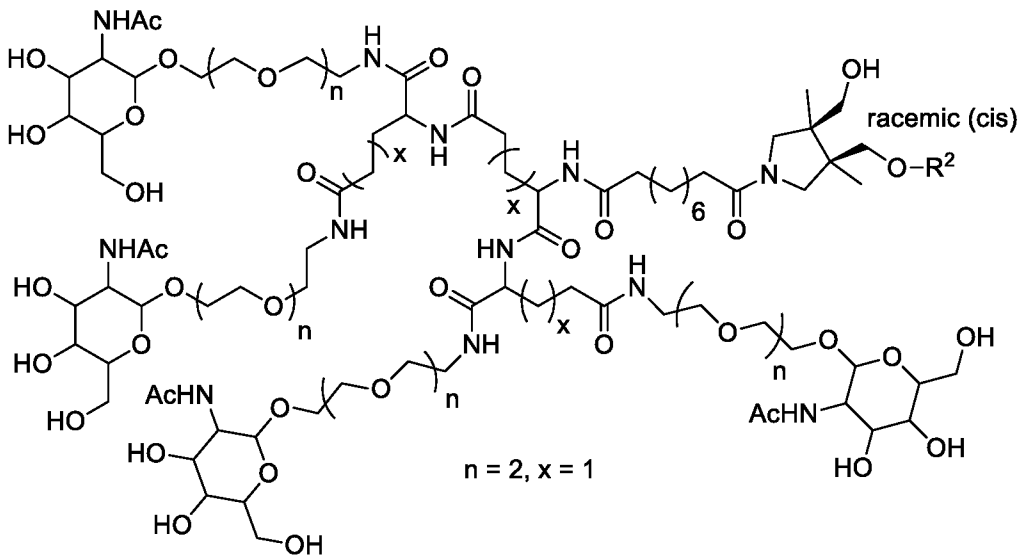
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4.

155. A compound of formula:



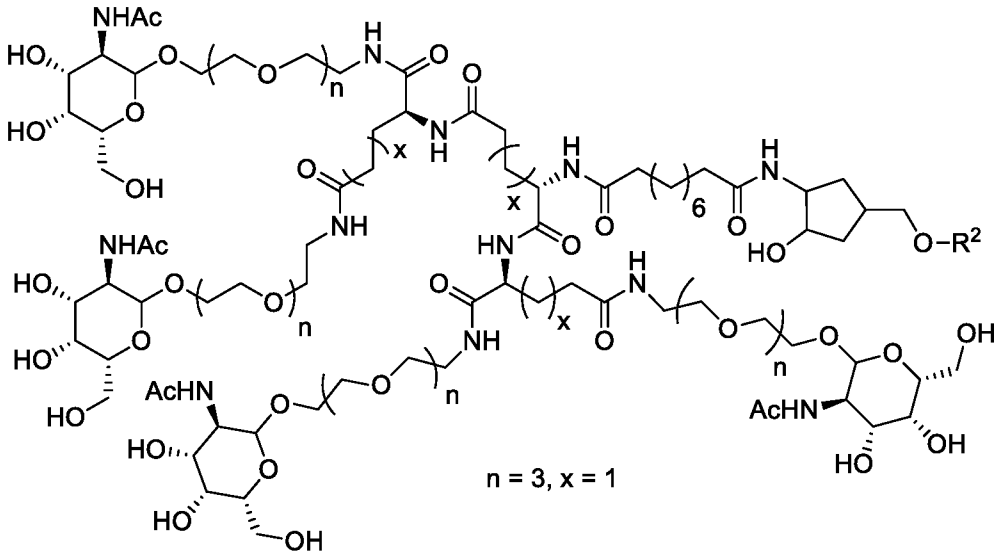
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4.

156. A compound of formula:



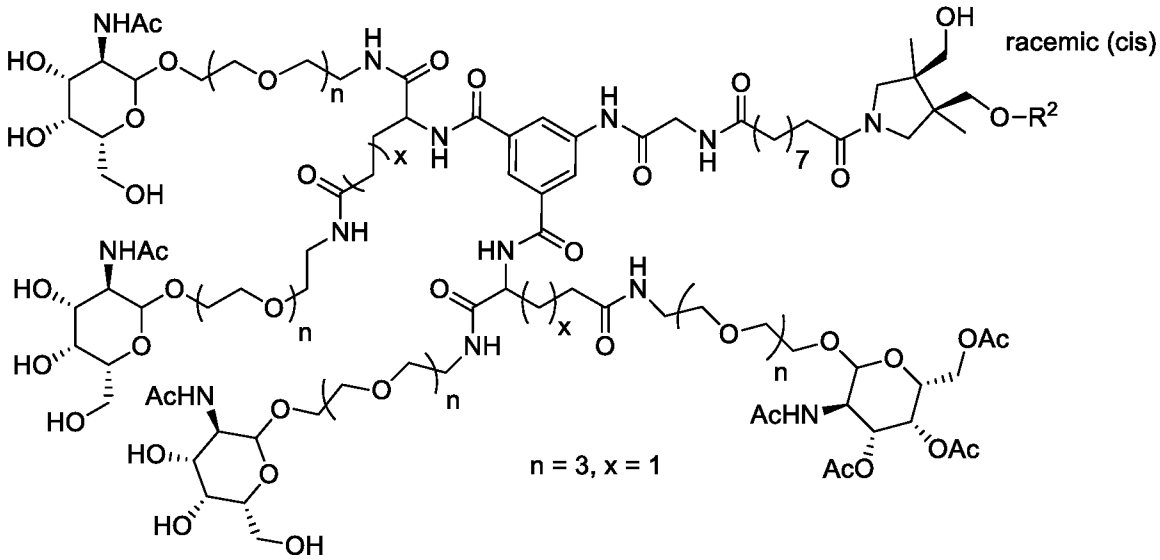
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4.

157. A compound of formula:



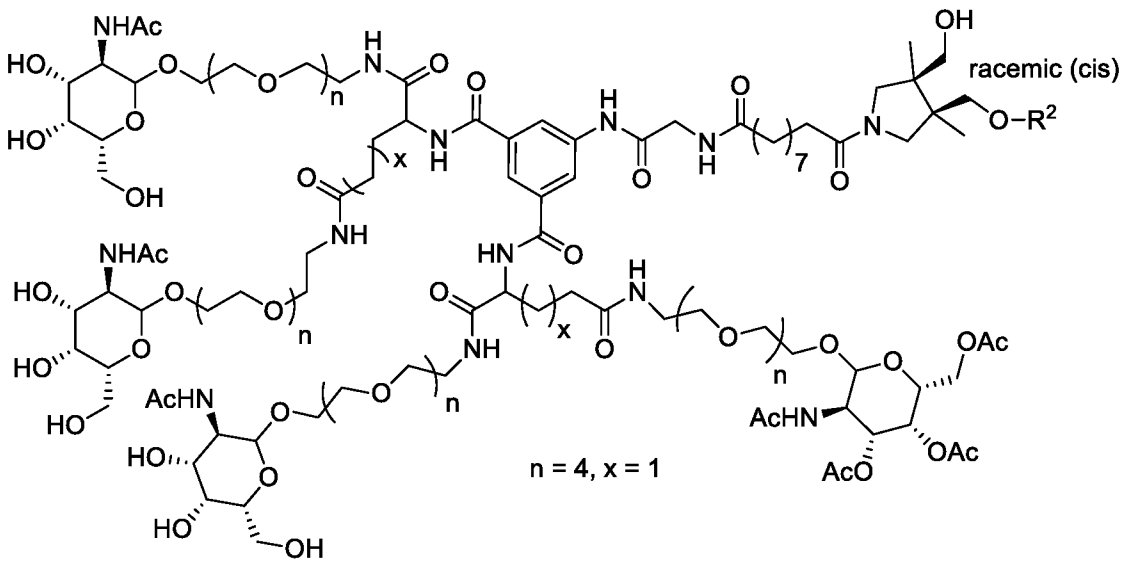
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4.

158. A compound of formula:



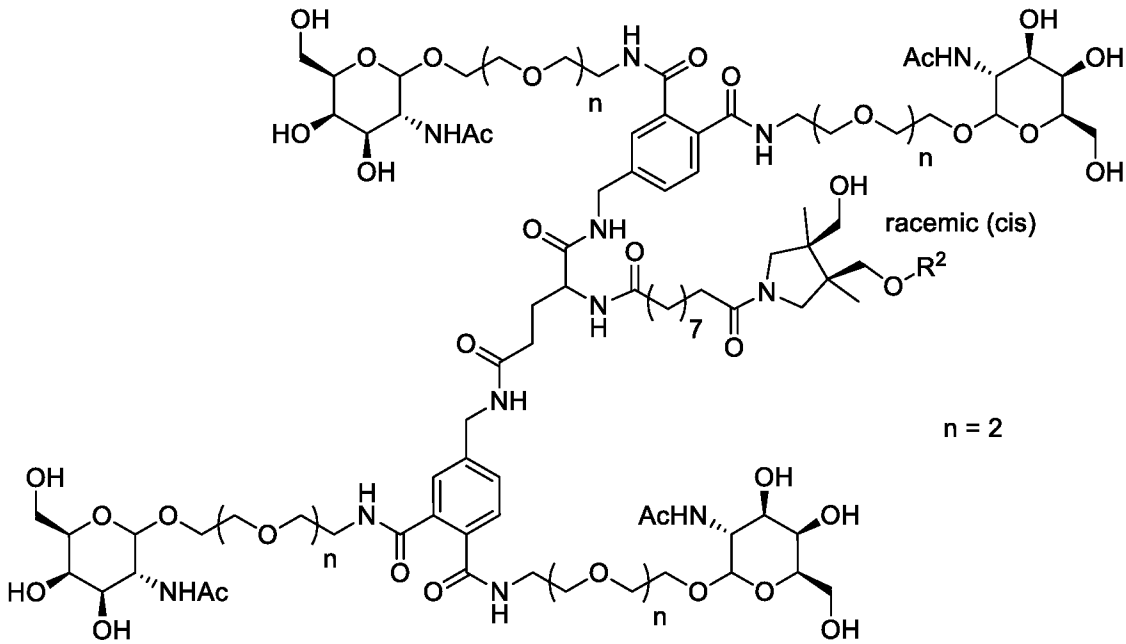
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4.

159. A compound of formula:



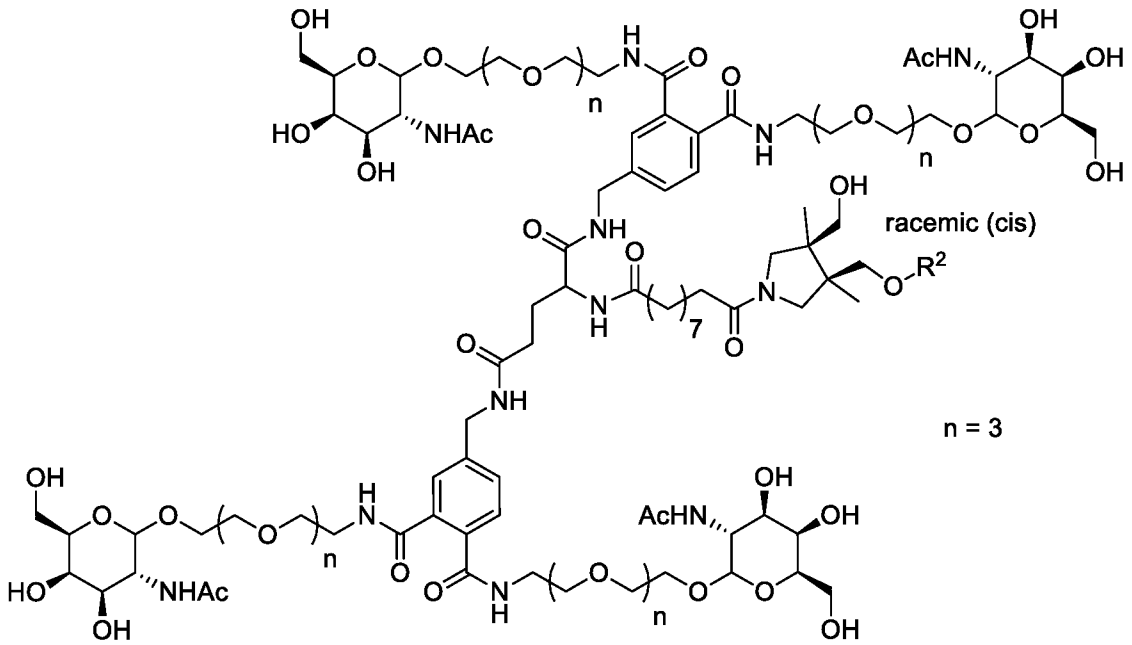
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4.

160. A compound of formula:



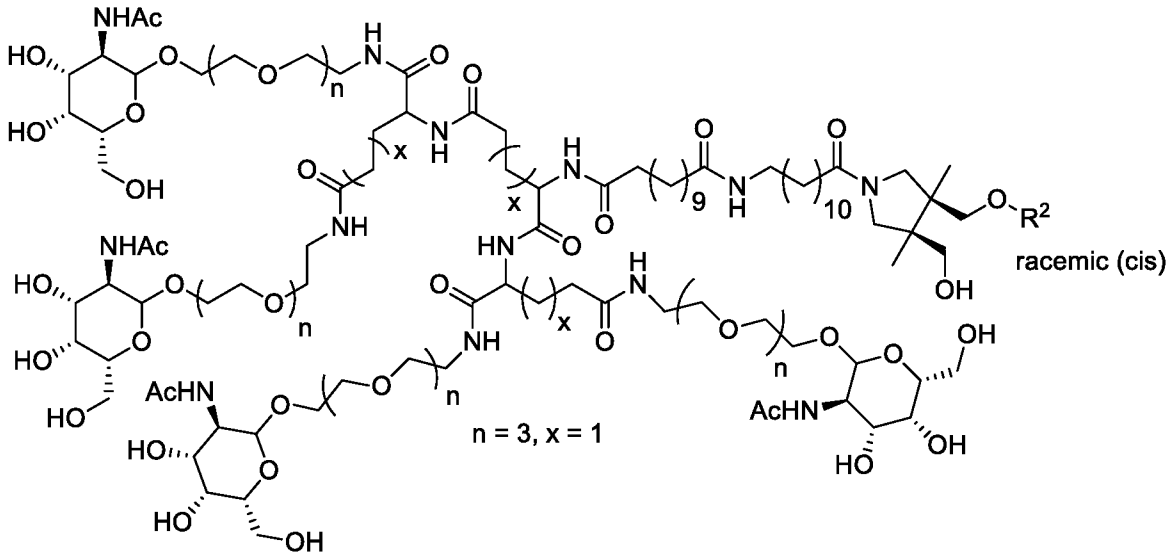
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4.

161. A compound of formula:



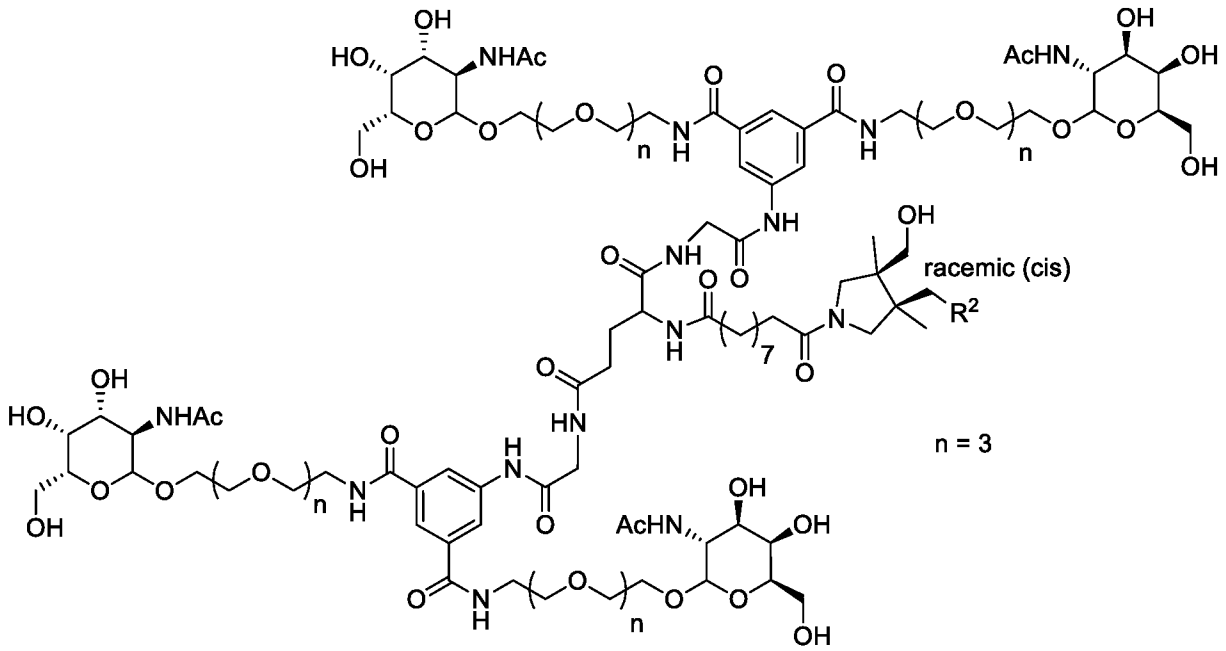
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4.

162. A compound of formula:



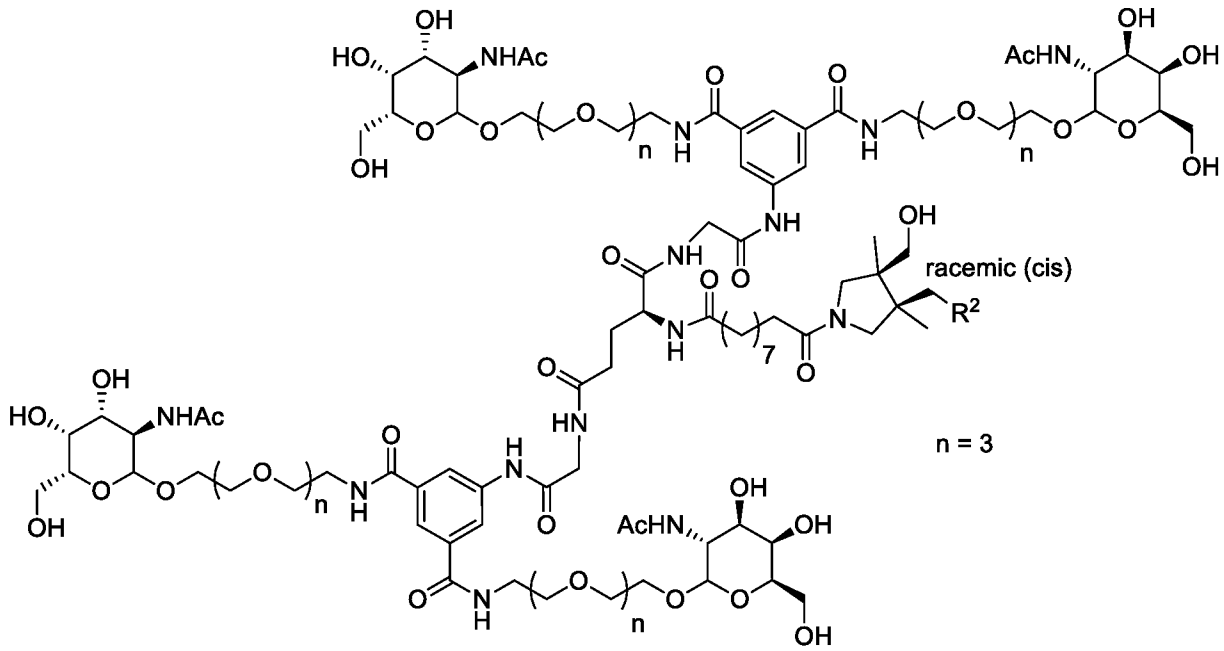
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4.

163. A compound of formula:



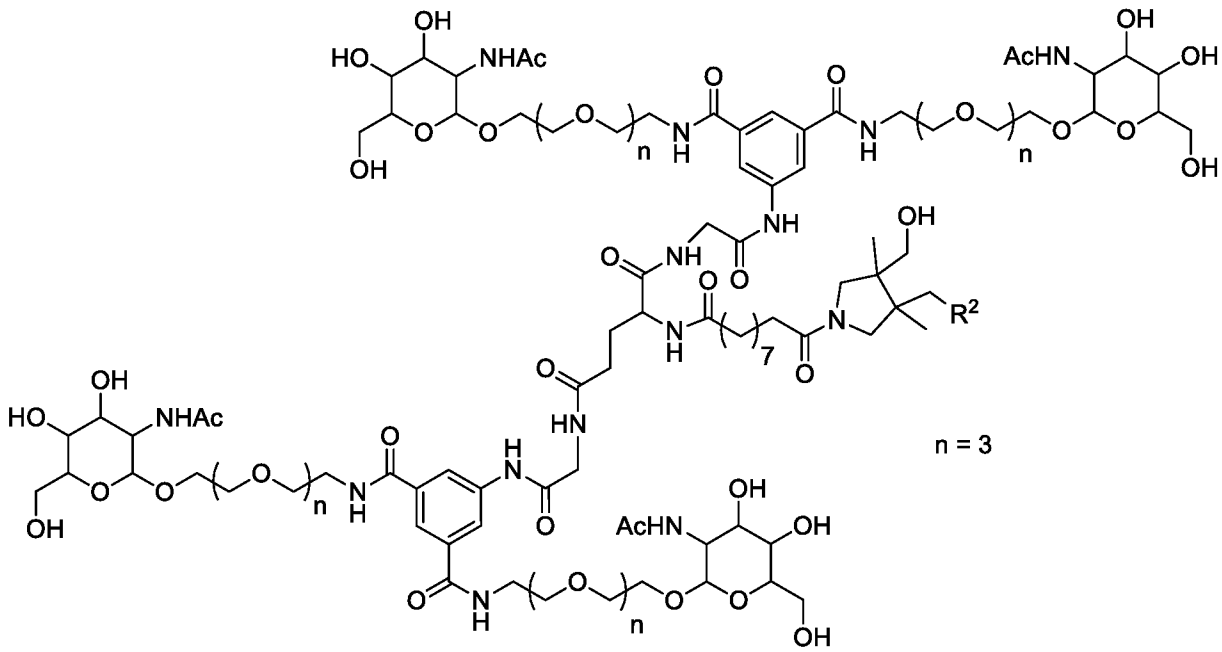
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4.

164. A compound of formula:



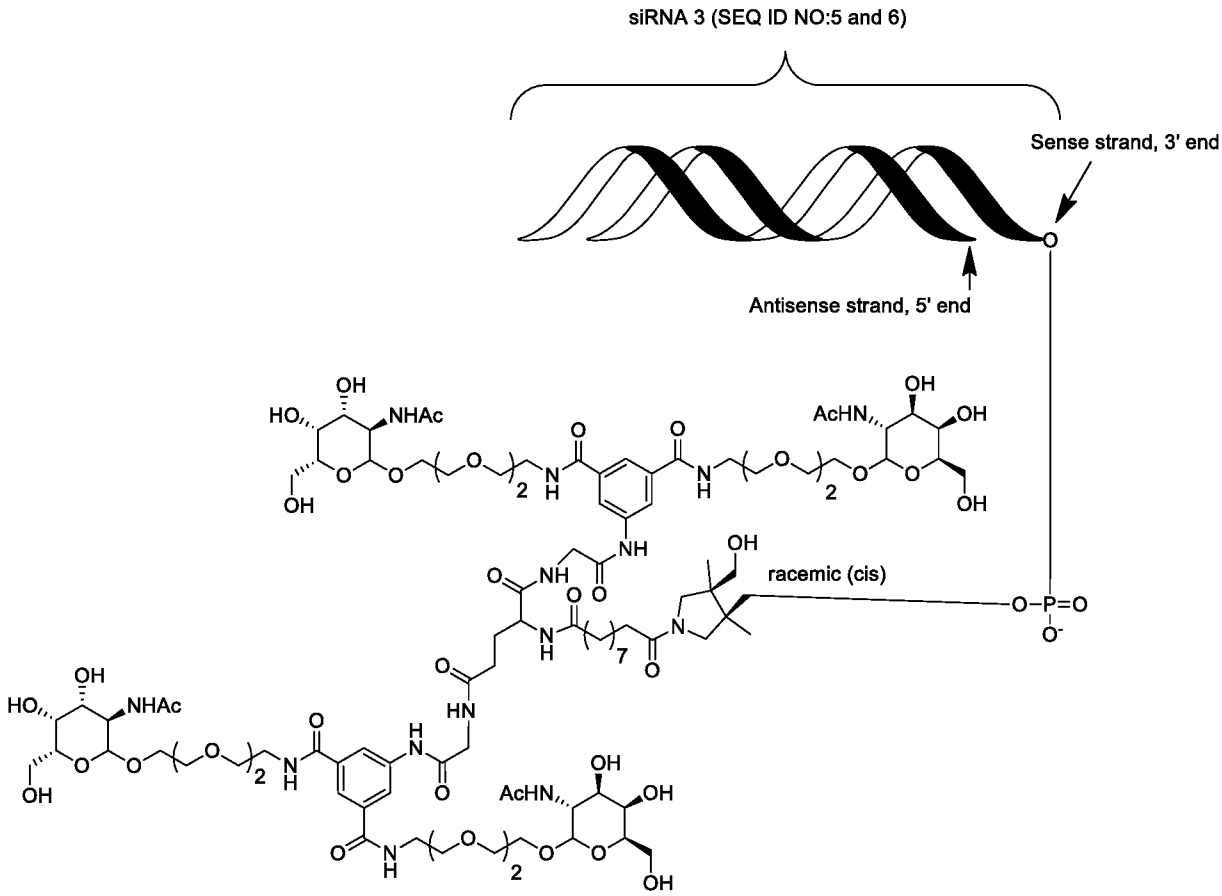
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4.

165. A compound of formula:



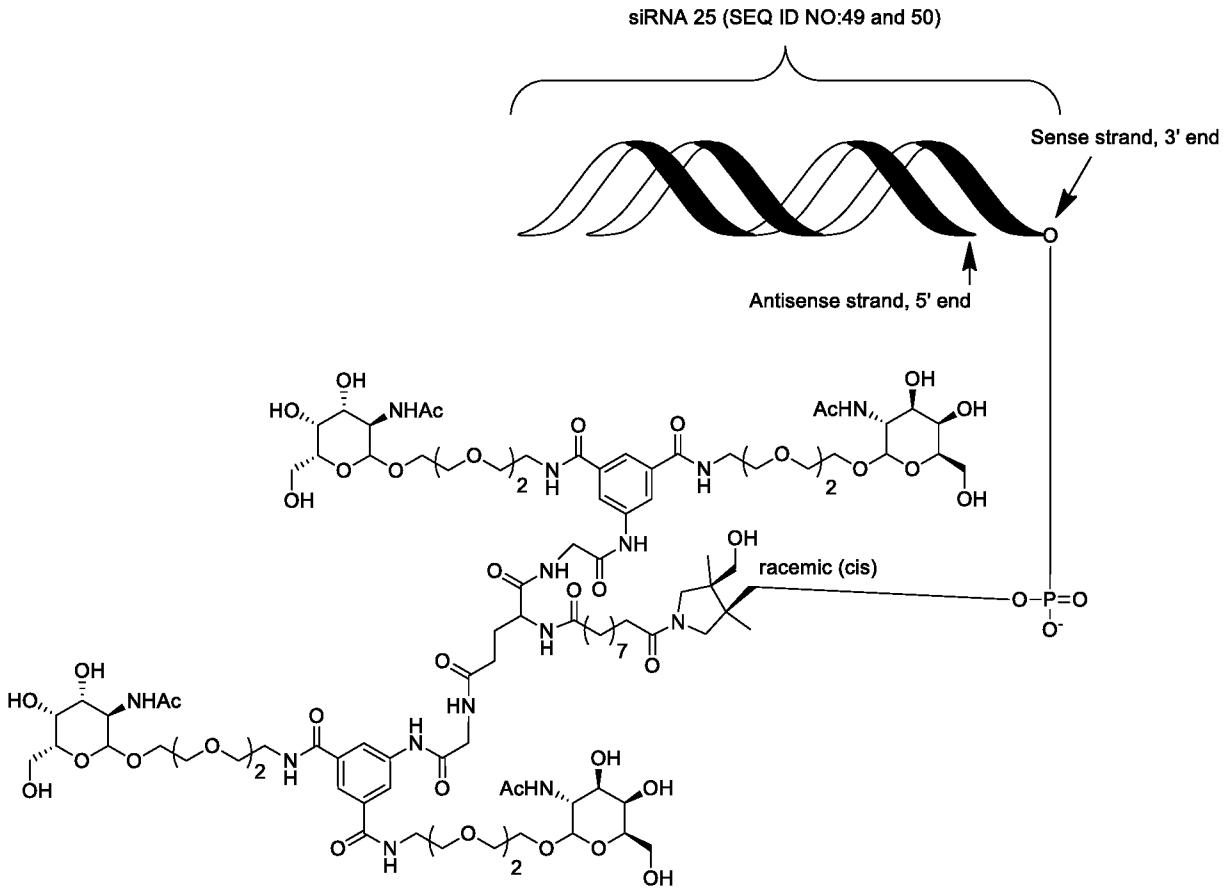
or a pharmaceutically acceptable salt thereof, wherein R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4.

166. A compound of formula:



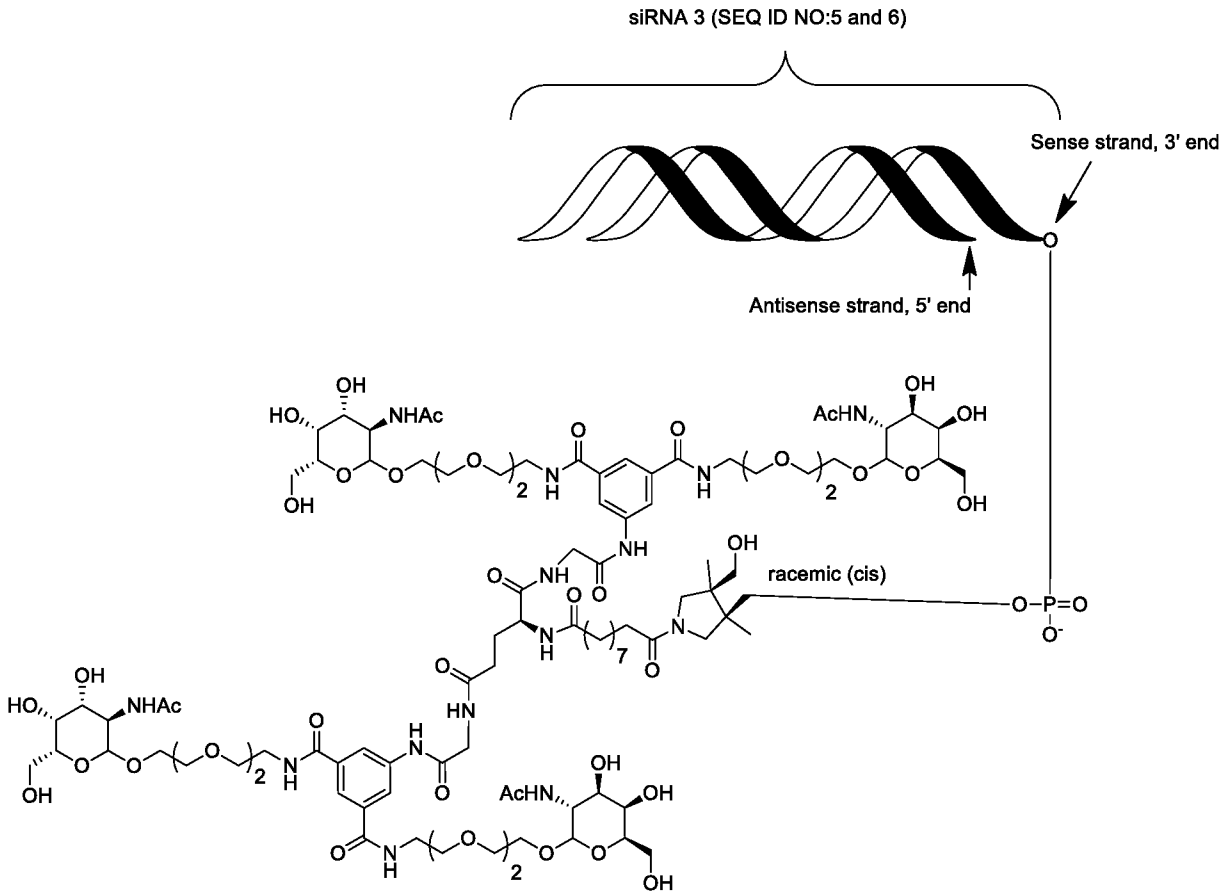
or a pharmaceutically acceptable salt thereof.

167. A compound of formula:



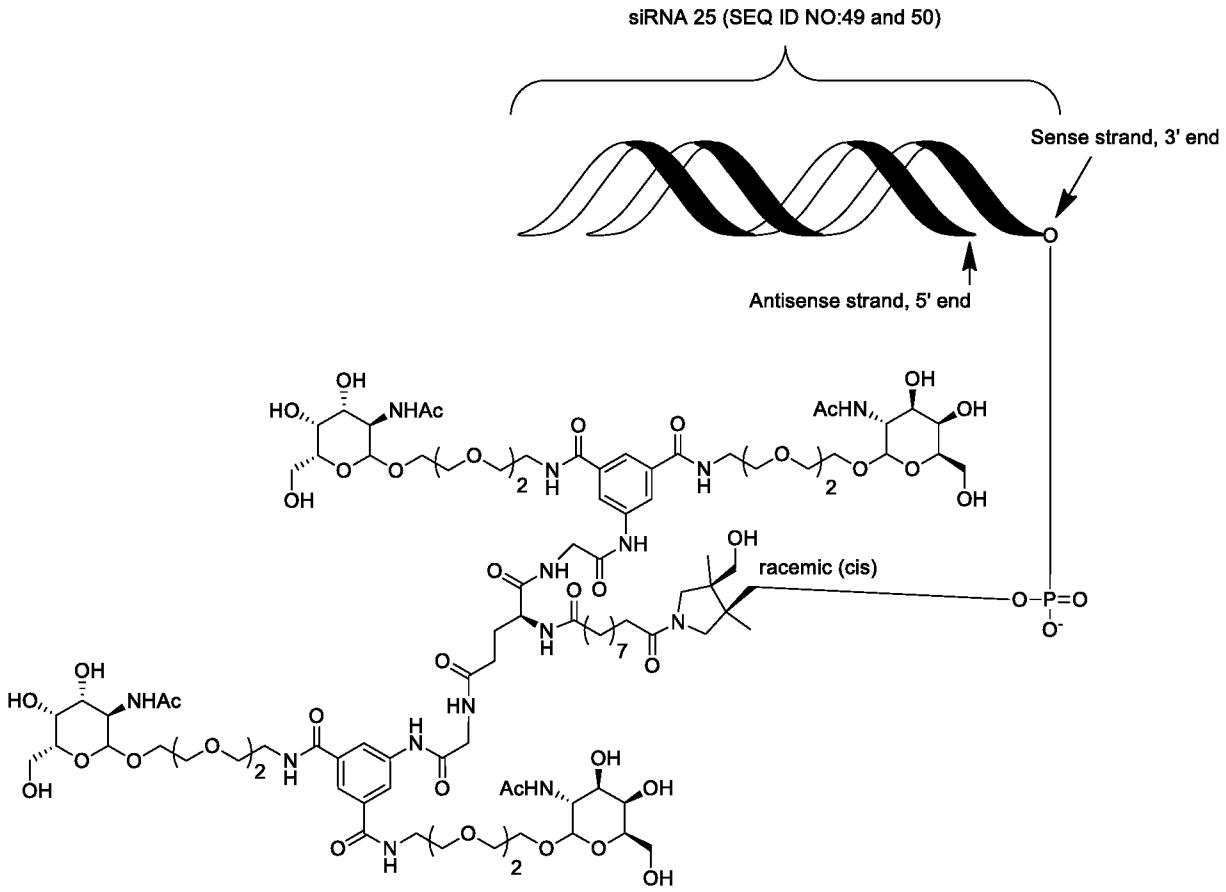
or a pharmaceutically acceptable salt thereof.

168. A compound of formula:



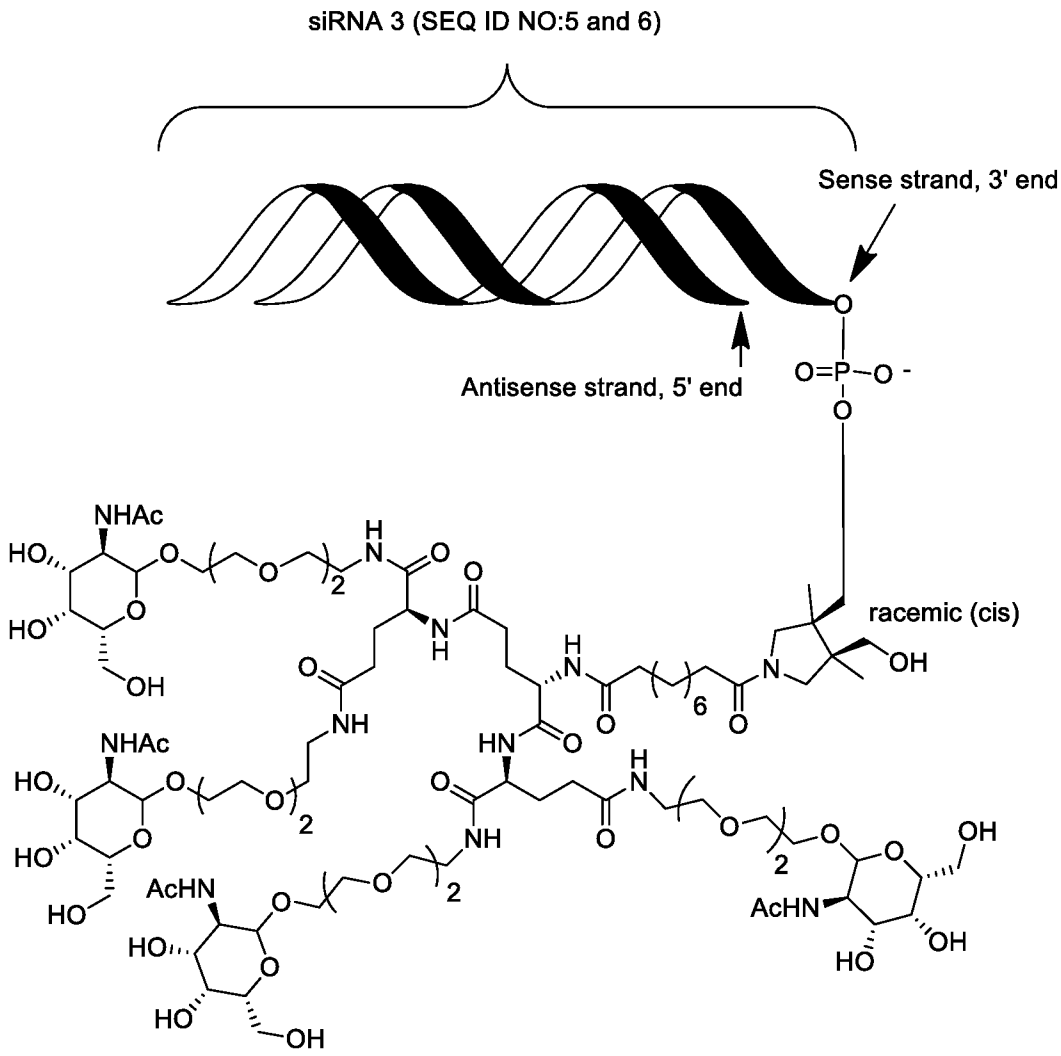
or a pharmaceutically acceptable salt thereof.

169. A compound of formula:



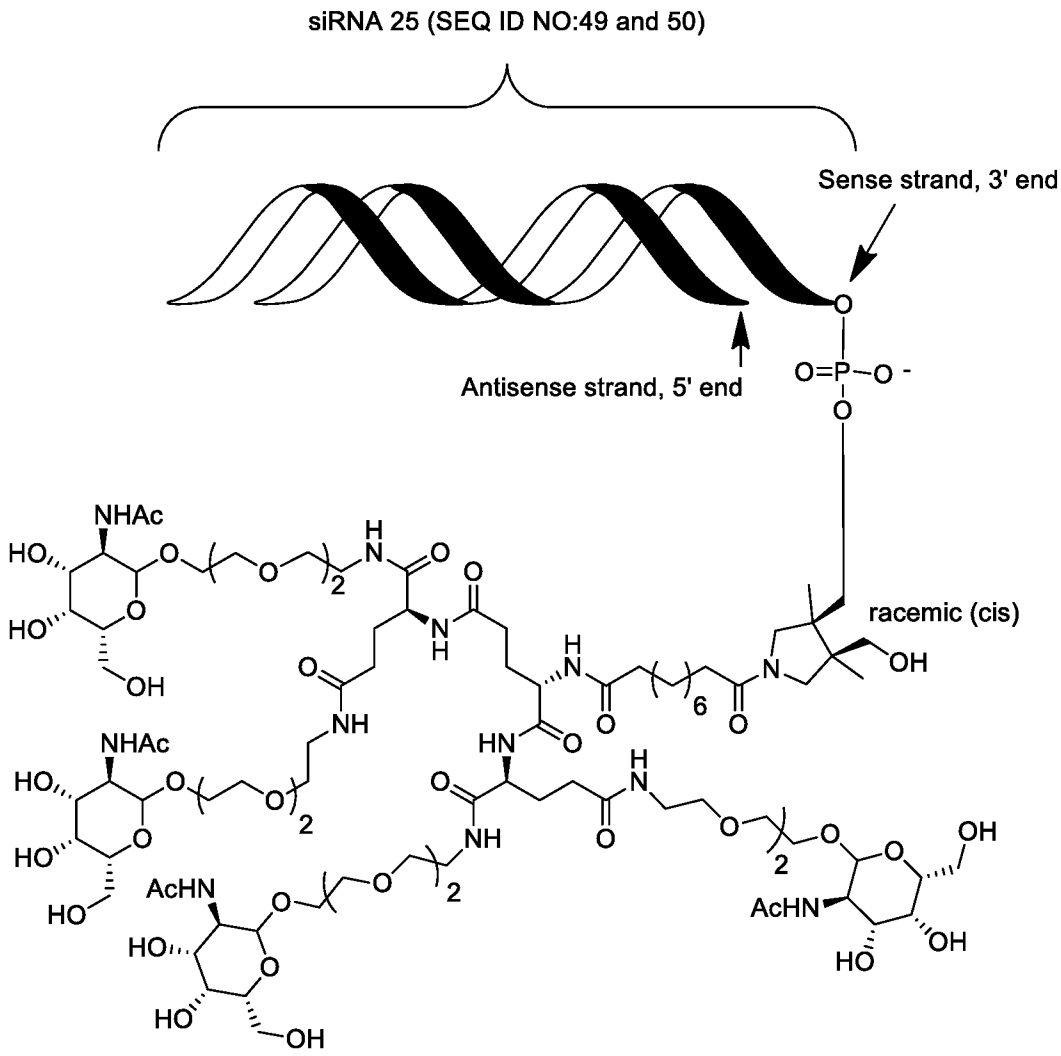
or a pharmaceutically acceptable salt thereof.

170. A compound of formula:



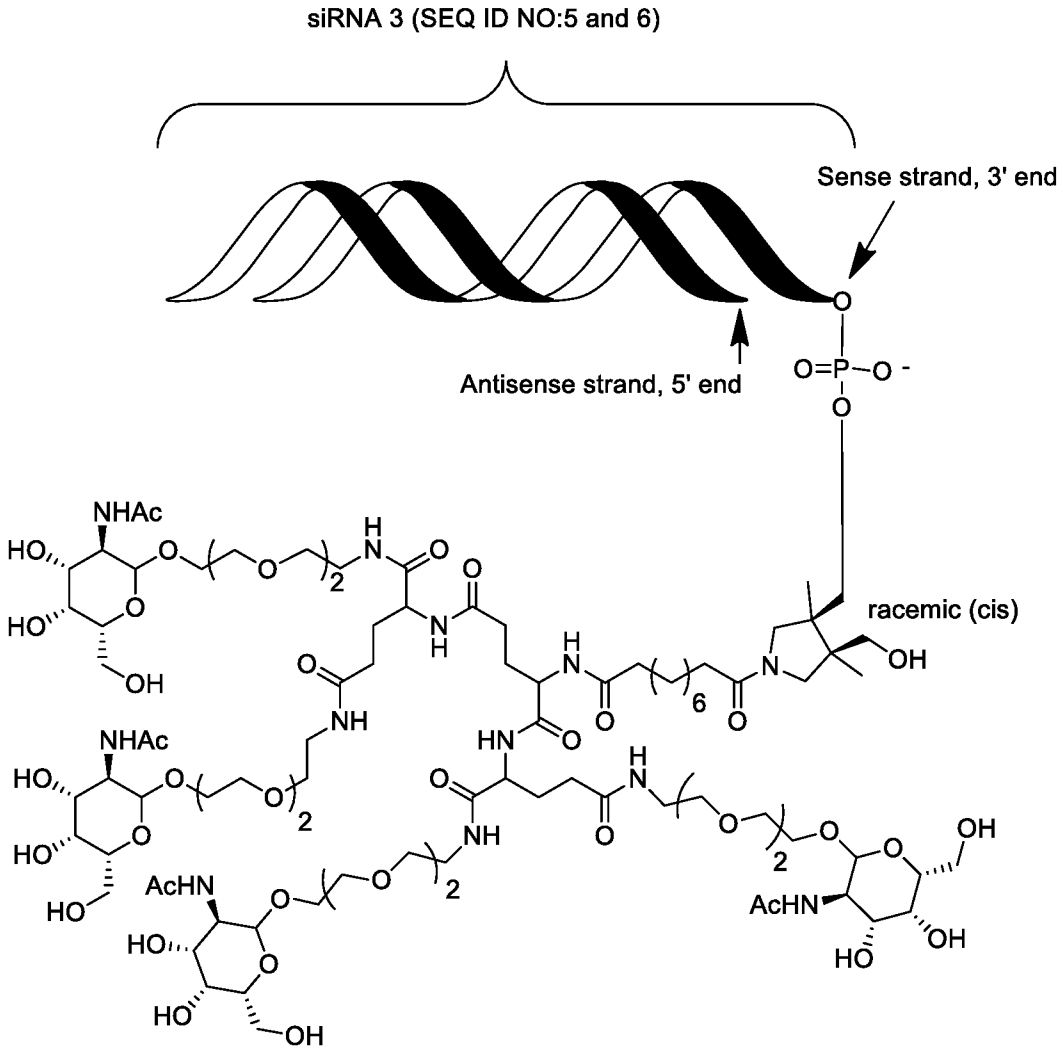
or a pharmaceutically acceptable salt thereof.

171. A compound of formula:



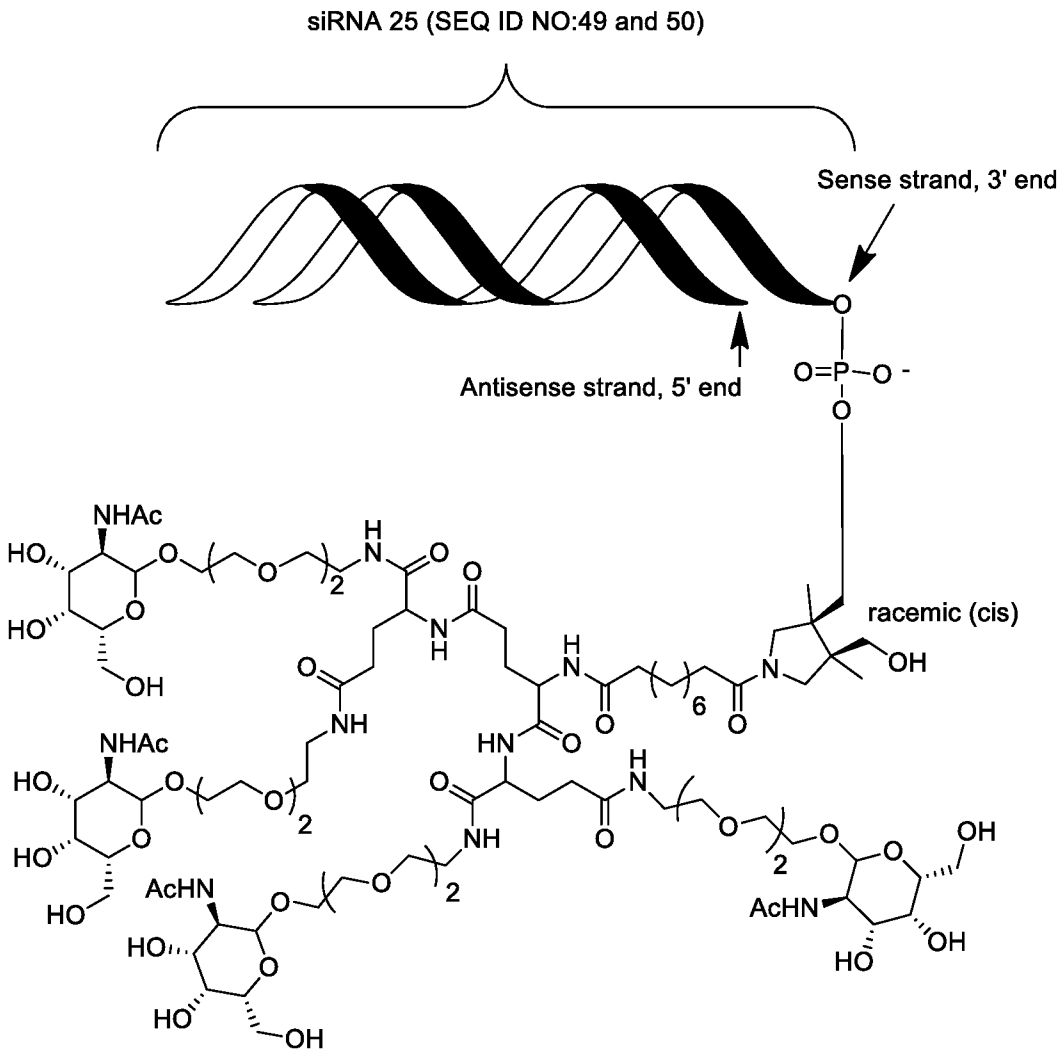
or a pharmaceutically acceptable salt thereof.

172. A compound of formula:



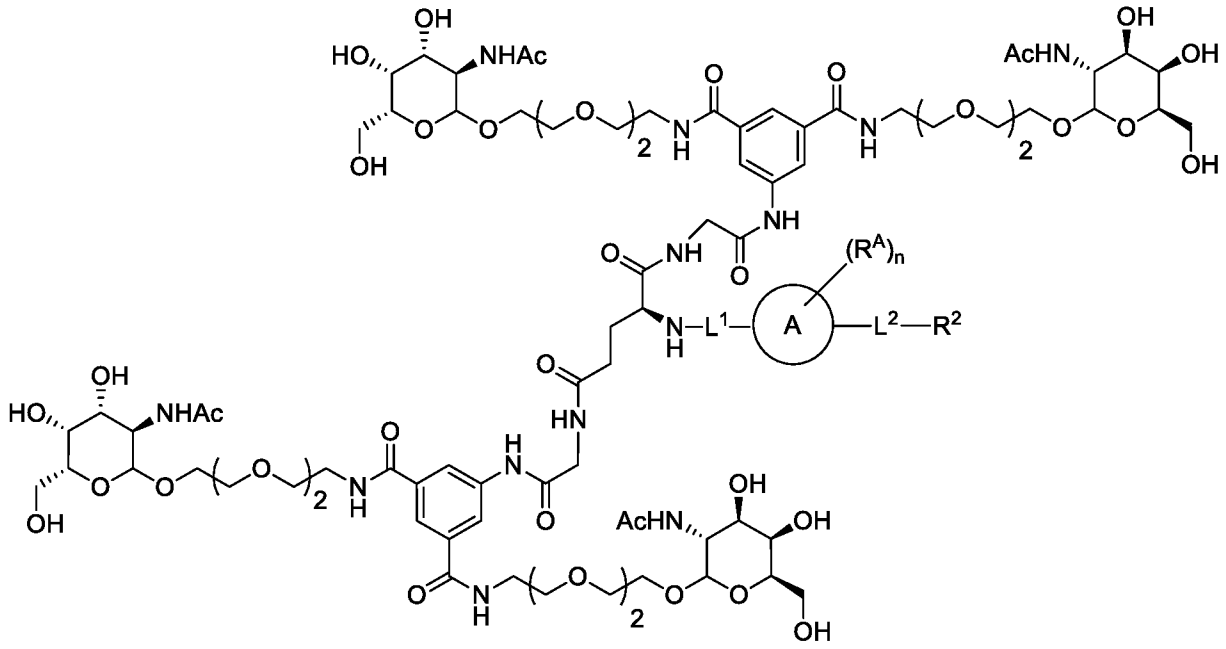
or a pharmaceutically acceptable salt thereof.

173. A compound of formula:



or a pharmaceutically acceptable salt thereof.

174. A compound of formula (I):



(I)

wherein:

L^1 is absent or a linking group;

L^2 is absent or a linking group;

R^2 is a nucleic acid;

the ring A is absent, a 3-20 membered cycloalkyl, a 5-20 membered aryl, a 5-20 membered heteroaryl, or a 3-20 membered heterocycloalkyl;

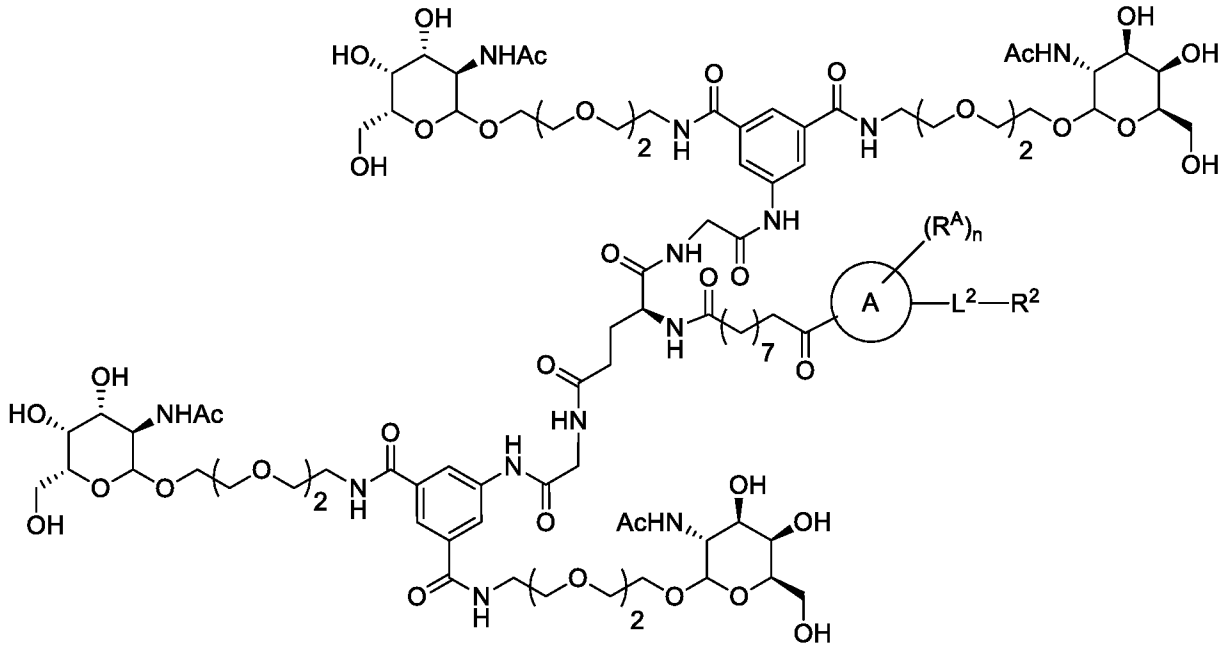
each R^A is independently selected from the group consisting of hydrogen, hydroxy, CN, F, Cl, Br, I, -C_{1-2} alkyl-OR^B, C_{1-10} alkyl C_{2-10} alkenyl, and C_{2-10} alkynyl; wherein the C_{1-10} alkyl C_{2-10} alkenyl, and C_{2-10} alkynyl are optionally substituted with one or more groups independently selected from halo, hydroxy, and C_{1-3} alkoxy;

R^B is hydrogen, a protecting group, a covalent bond to a solid support, or a bond to a linking group that is bound to a solid support; and

n is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

or a salt thereof.

175. A compound of formula (Ia):



(Ia)

wherein:

L^2 is absent or a linking group;

R^2 is a nucleic acid;

the ring A is absent, a 3-20 membered cycloalkyl, a 5-20 membered aryl, a 5-20 membered heteroaryl, or a 3-20 membered heterocycloalkyl;

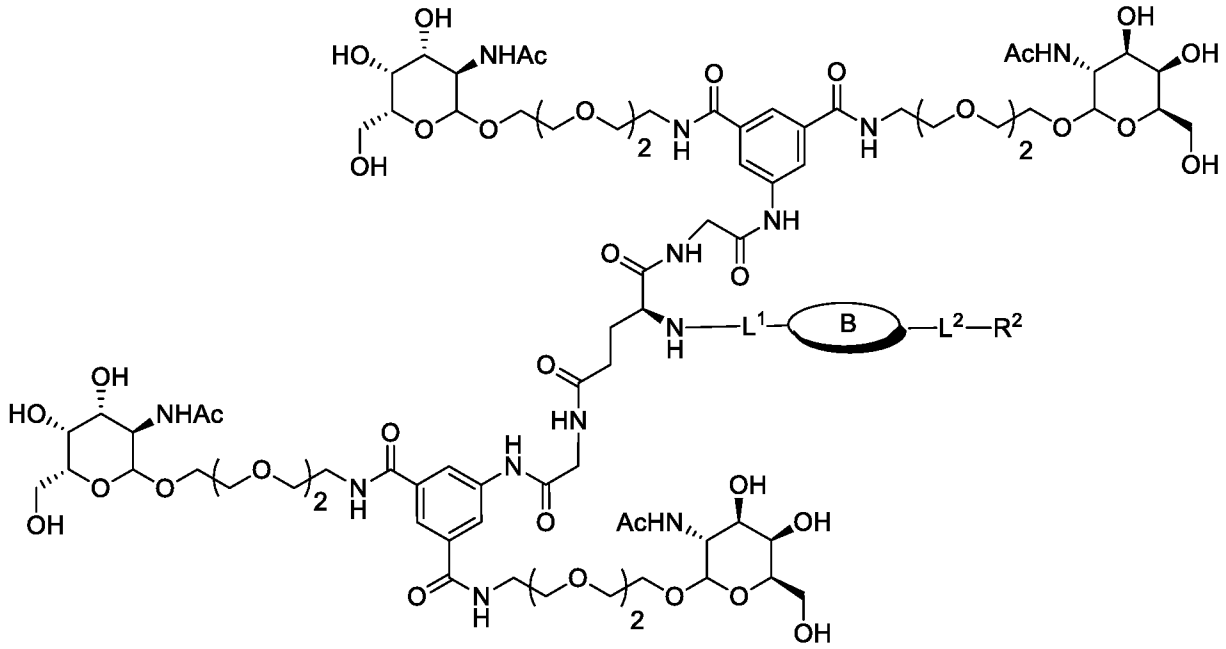
each R^A is independently selected from the group consisting of hydrogen, hydroxy, CN, F, Cl, Br, I, -C_{1-2} alkyl-OR^B, C_{1-10} alkyl C_{2-10} alkenyl, and C_{2-10} alkynyl; wherein the C_{1-10} alkyl C_{2-10} alkenyl, and C_{2-10} alkynyl are optionally substituted with one or more groups independently selected from halo, hydroxy, and C_{1-3} alkoxy;

R^B is hydrogen, a protecting group, a covalent bond to a solid support, or a bond to a linking group that is bound to a solid support; and

n is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

or a salt thereof.

176. A compound of formula (XX):



(II)

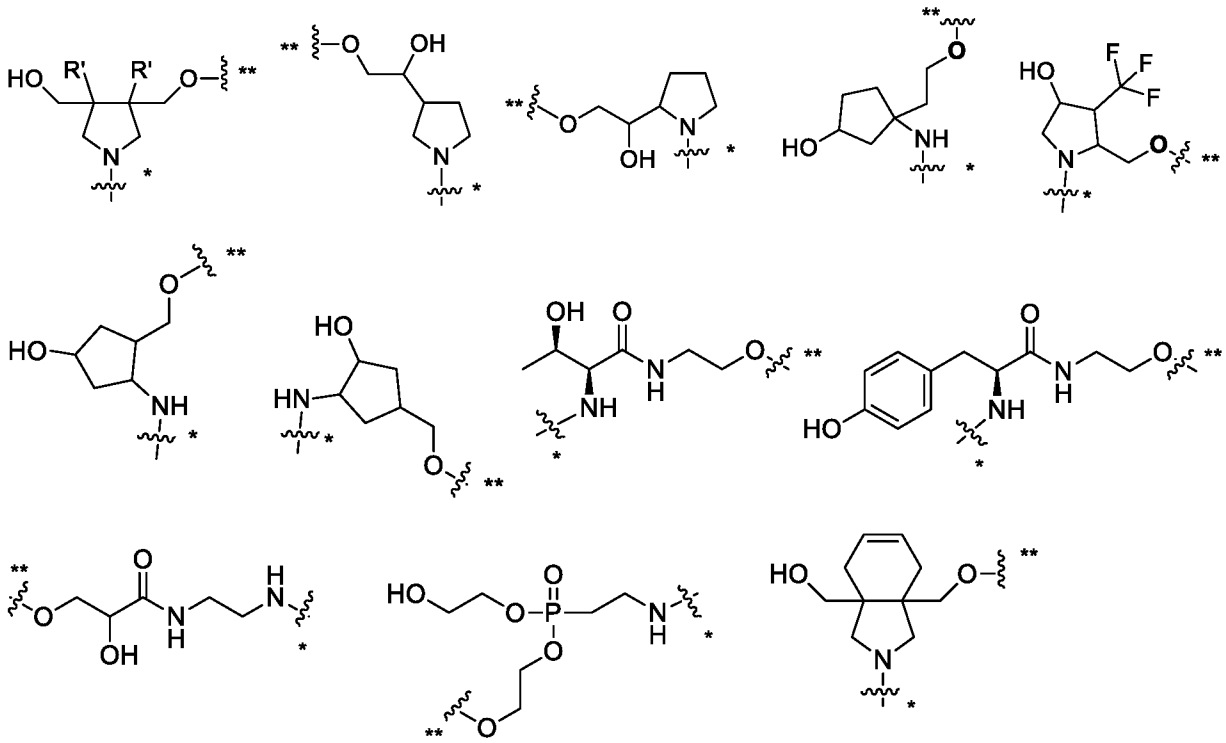
wherein:

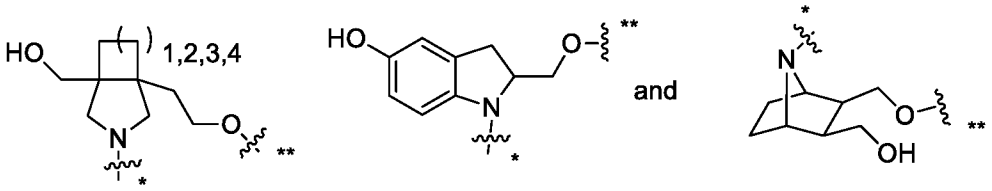
L^1 is absent or a linking group;

L^2 is absent or a linking group;

R^2 is a nucleic acid;

B is divalent and is selected from the group consisting of:





wherein:

each R' is independently C_{1-9} alkyl, C_{2-9} alkenyl or C_{2-9} alkynyl; wherein the C_{1-9} alkyl, C_{2-9} alkenyl or C_{2-9} alkynyl are optionally substituted with halo or hydroxyl;

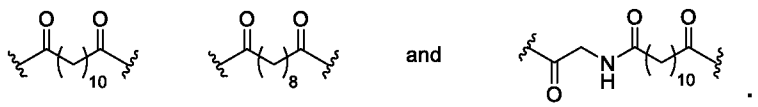
the valence marked with * is attached to L^1 or is attached to R^1 if L^1 is absent; and

the valence marked with ** is attached to L^2 or is attached to R^2 if L^2 is absent;

or a salt thereof.

177. The compound of claim 175 or a salt thereof, wherein L^1 and L^2 are independently a divalent, branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 50 carbon atoms, wherein one or more (e.g. 1, 2, 3, or 4) of the carbon atoms in the hydrocarbon chain is optionally replaced by –O-, -NR^X-, -NR^X-C(=O)-, -C(=O)-NR^X- or –S-, and wherein R^X is hydrogen or (C1-C6)alkyl, and wherein the hydrocarbon chain, is optionally substituted with one or more substituents selected from (C1-C6)alkoxy, (C3-C6)cycloalkyl, (C1-C6)alkanoyl, (C1-C6)alkanoyloxy, (C1-C6)alkoxycarbonyl, (C1-C6)alkylthio, azido, cyano, nitro, halo, hydroxy, oxo (=O), carboxy, aryl, aryloxy, heteroaryl, and heteroaryloxy.

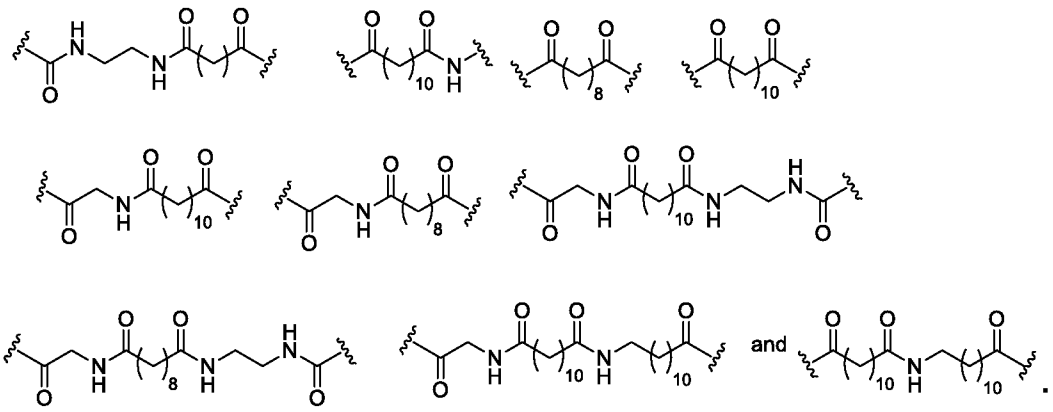
178. The compound of claim 176 or a salt thereof, L^1 is selected from the group consisting of:



or a salt thereof.

179. The compound of claim 176 or a salt thereof, wherein L^1 is connected to B^1 through a linkage selected from the group consisting of: -O-, -S-, -(C=O)-, -(C=O)-NH-, -NH-(C=O), -(C=O)-O-, -NH-(C=O)-NH-, or –NH-(SO_2)-.

180. The compound of claim 176 or a salt thereof, wherein L^1 is selected from the group consisting of:

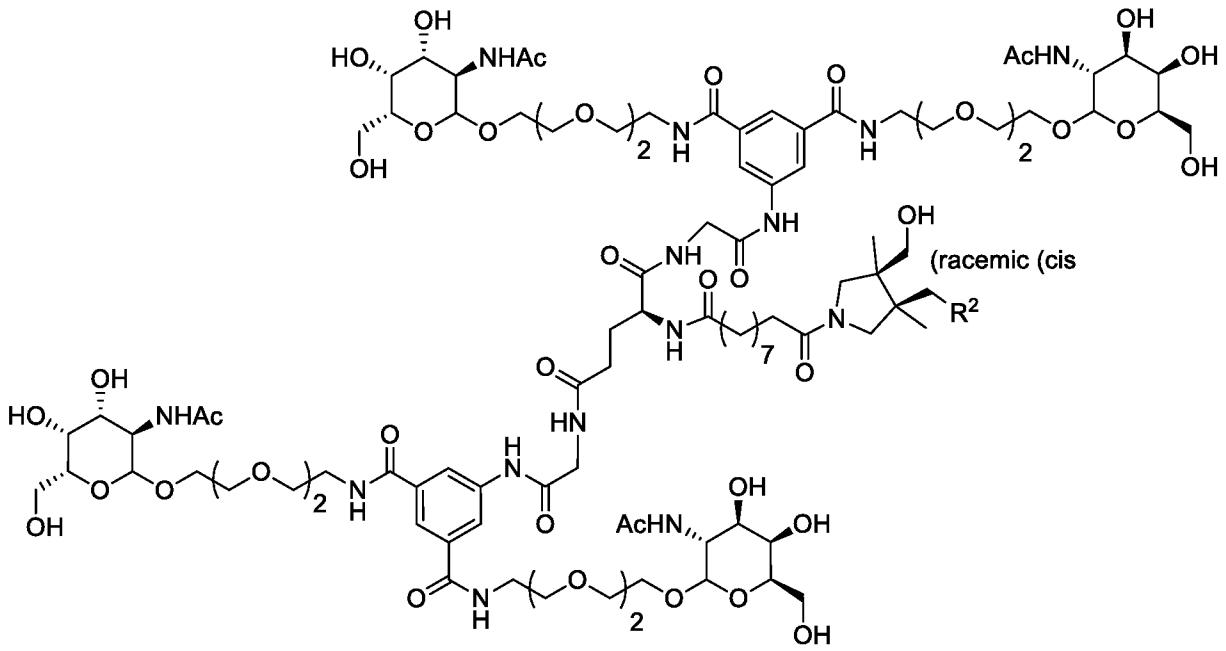


181. The compound of claim 176 or a salt thereof, wherein L^2 is connected to R^2 through -O-.

182. The compound of claim 176 or a salt thereof, wherein L^2 is C_{1-4} alkylene-O- that is optionally substituted with hydroxy.

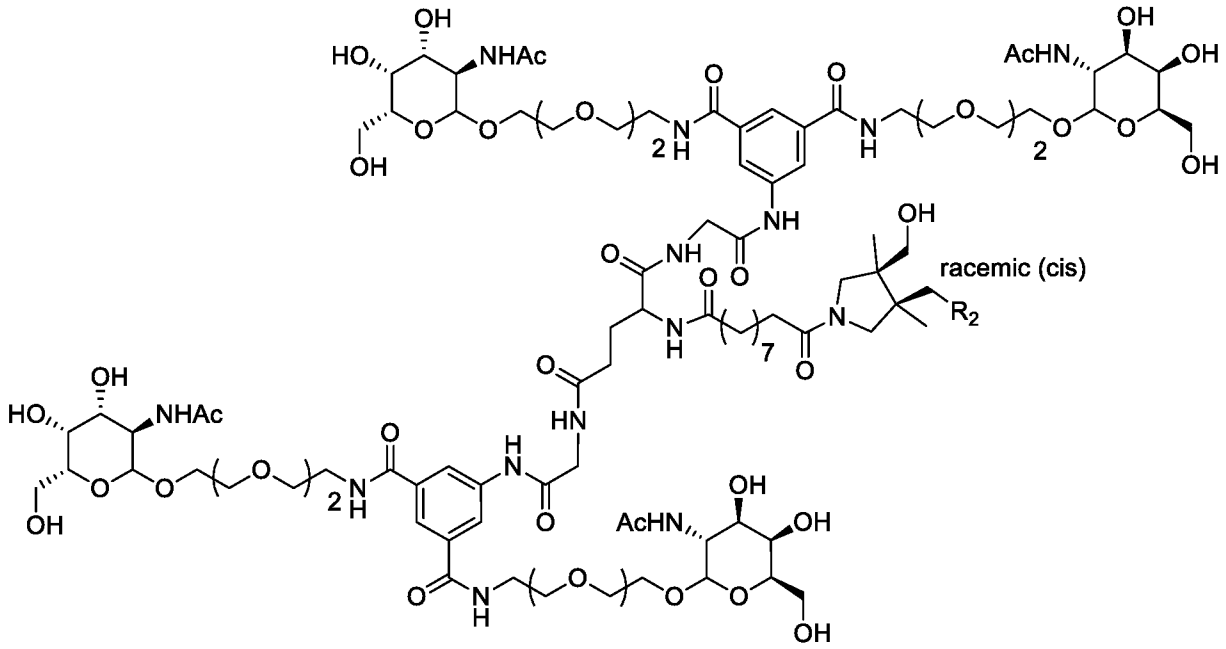
183. The compound of claim 176 or a salt thereof, wherein L^2 is absent.

184. The compound,



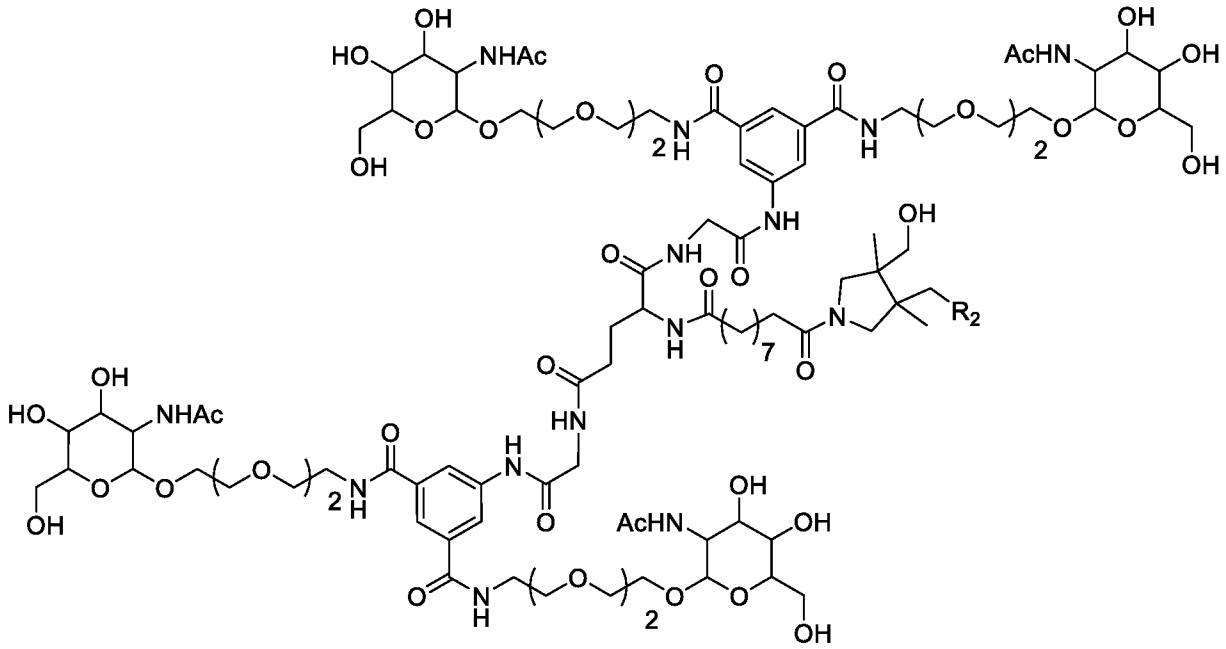
or a salt thereof wherein R^2 is a nucleic acid.

185. The compound,



or a salt thereof wherein R^2 is a nucleic acid.

186. The compound,



or a salt thereof wherein R^2 is a nucleic acid.

187. The compound of any one of claims 174-186 wherein R^2 is a double stranded siRNA molecule selected from the double stranded siRNA molecules of claim 4.

188. A pharmaceutical composition comprising a compound as described in any one of claims 174-186, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

189. A method to deliver a siRNA to the liver of an animal comprising administering a compound of formula I as described in any one of claims 174-186, or a pharmaceutically acceptable salt thereof, to the animal.

190. A method to treat a hepatitis B viral infection in an animal comprising administering an effective amount of a compound of formula I or Id as described in any one of claims 1-49, 52-61 or 174-186, or a pharmaceutically acceptable salt thereof, to the animal.

191. The method of claim 190, wherein the compound of formul I or Id, or a pharmaceutically acceptable salt thereof, is administered subcutaneously.

192. A compound of formula I or Id as described in any one of claims 1-49, 52-61 or 174-186, or a pharmaceutically acceptable salt thereof, for use in medical therapy.

193. A compound of formula I or Id as described in any one of claims 1-49, 52-61 or 174-186, or a pharmaceutically acceptable salt thereof, for the prophylactic or therapeutic treatment of a hepatitis B virus infection in an animal.

194. The use of a compound of formula I or Id as described in any one of claims 1-49, 52-61 or 174-186, or a pharmaceutically acceptable salt thereof, to prepare a medicament for treating a hepatitis B virus infection in an animal.

195. The method, compound or use of any one of claims 190-194, wherein the animal is a human.

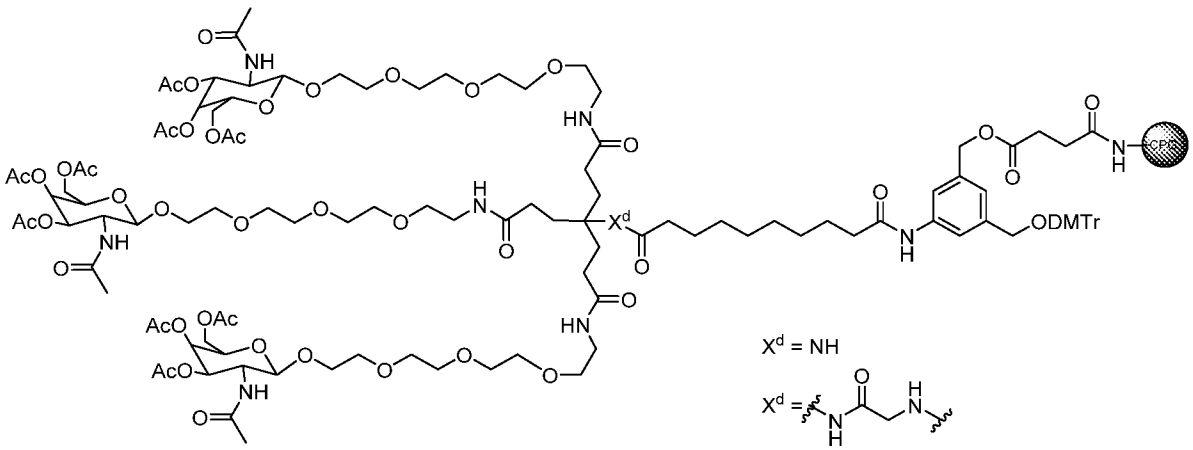


Figure 1: Intermediate compound of formula Ie, wherein a targeting ligand/linker is bound to a solid phase support, and wherein Pg^1 is the protecting group DMTr.

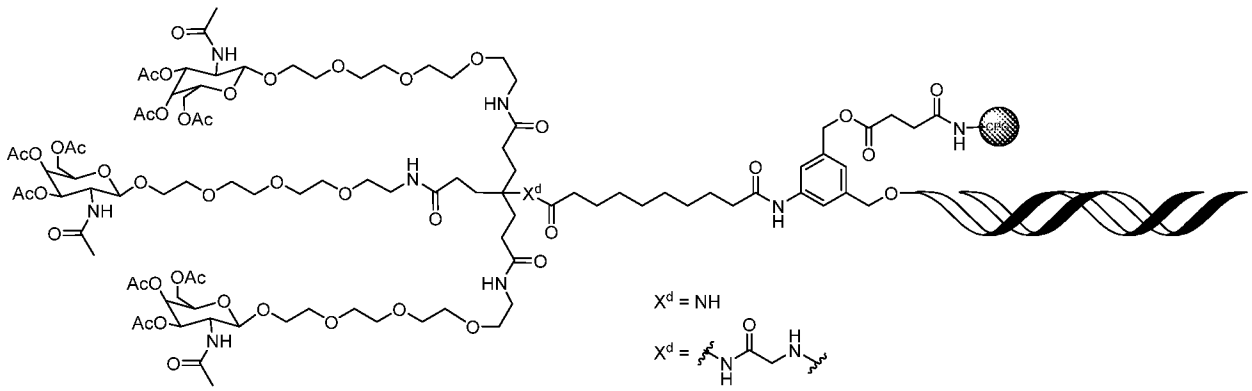


Figure 2: Representative compound of formula Id wherein a targeting ligand is bound to a solid phase support, with an oligonucleotide covalently bound.

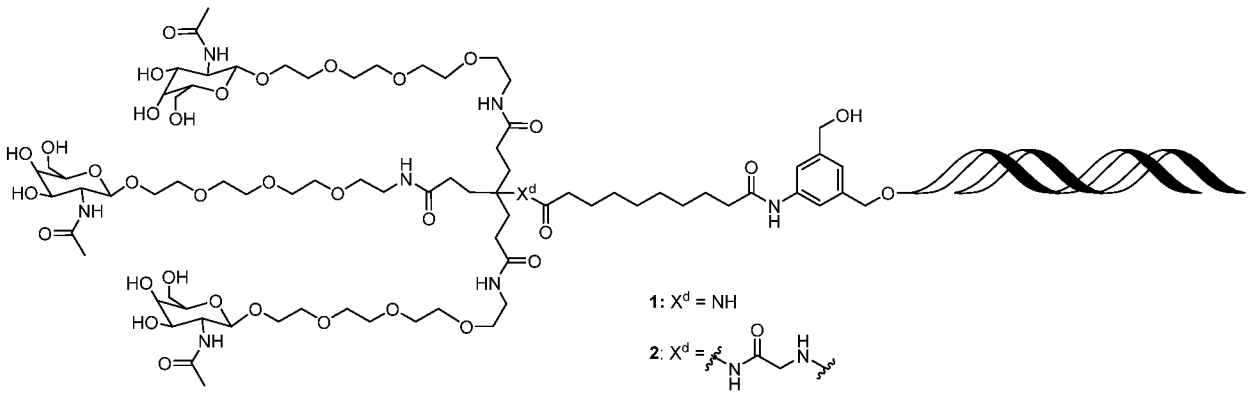


Figure 3: Representative compound of formula Id, wherein a targeting ligand-oligo nucleotide conjugate has been cleaved from a solid phase support and deprotected.