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(54) Title: LIPID NANOPARTICLE (LNP) DELIVERY SYSTEMS AND FORMULATIONS

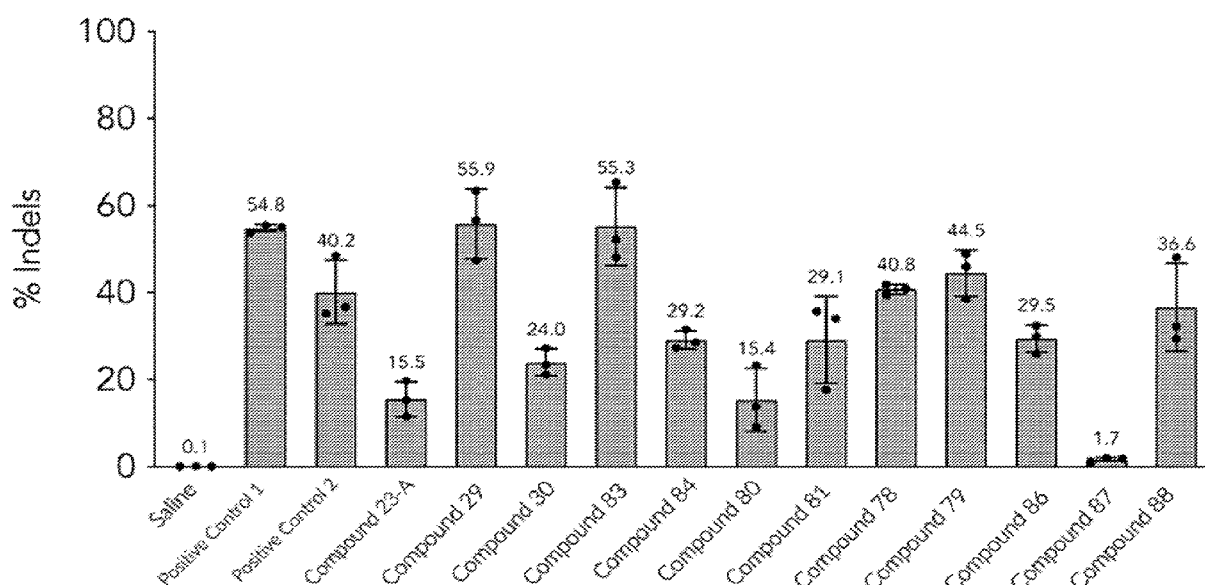


FIG. 1

(57) Abstract: The present disclosure describes compositions, nanoparticles (such as lipid nanoparticles), and/or lipid nanoparticle compositions and methods of their use.



Published:

- *without international search report and to be republished
upon receipt of that report (Rule 48.2(g))*
- *with sequence listing part of description (Rule 5.2(a))*

LIPID NANOPARTICLE (LNP) DELIVERY SYSTEMS AND FORMULATIONS**CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] The present application claims priority to and the benefits of U.S. Provisional Application No. 63/497,059, filed April 19, 2023, and U.S. Provisional Application No. 63/586,456, filed September 29, 2023, the contents of each of which are herein incorporated by reference in their entirety.

SEQUENCE LISTING

[0002] This application includes and incorporates by reference in its entirety a Sequence Listing XML in the required .xml format. The Sequence Listing XML file that has been electronically filed contains the information of the nucleotide and/or amino acid sequences disclosed in the patent application using the symbols and format in accordance with the requirements of 37 C.F.R. §§1.832 through 1.834.

[0003] The Sequence Listing XML filed herewith serves as the electronic copy required by §1.834(b)(1).

[0004] The Sequence Listing XML is identified as follows: “272059-545516_Sequence-listing.xml” (4,562 bytes in size), which was created on April 12, 2024, at 5:45 p.m.

FIELD OF THE INVENTION

[0005] The present disclosure describes compounds, compositions, lipid nanoparticles, and methods for delivering constructs and polynucleotides.

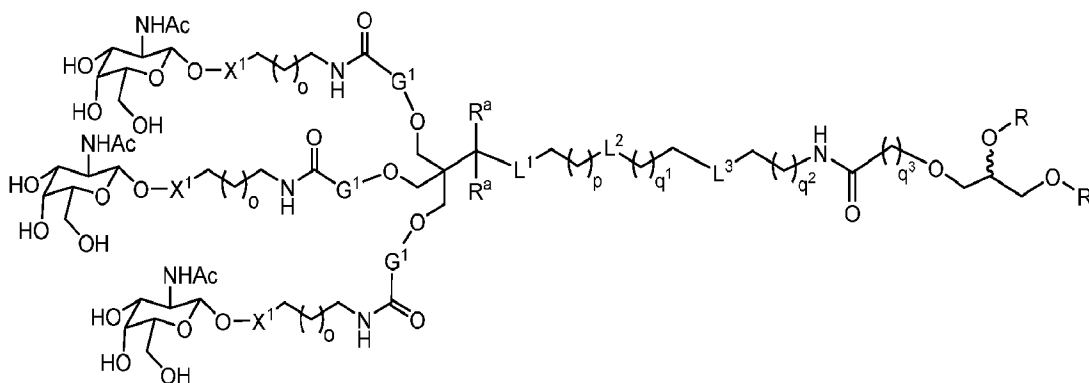
BACKGROUND

[0006] GalNAc-based ligands, which bind to the asialoglycoprotein receptor (ASGPR), have recently been developed in lipid nanoparticles systems to deliver nucleic acids to the liver. The editing activity achieved by GalNAc-LNP system has paved the way of nucleic acid delivery and further develop as potential gene therapeutics delivery system. Extensive structure-activity relationship (SAR) studies have reported that different linkers influence the GalNAc-based lipids and their delivery activity. Accordingly, there is a need to develop GalNAc-based lipids that can be incorporated into LNP compositions.

SUMMARY

[0007] The present disclosure provides lipid nanoparticle (LNP) compositions. In some embodiments, the LNP compositions comprise a lipid that comprises a compound described herein. In some embodiments, the compound described herein is a GalNAc-based lipid.

[0008] An aspect of the present disclosure provides compounds of Formula I:

**Formula I**

wherein,

each X^1 is independently $-(CH_2)_r-$, $-(CH_2CH_2O)_s-$, $-(OCH_2CH_2)_s-$, $-((CH_2)_m-C(O)NH)-$, or $-(C(O)NH-(CH_2)_m)-$,

each subscript “m” is independently 1-5;

each subscript “o” is independently 1-5;

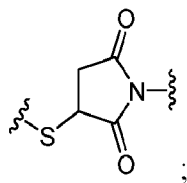
each subscript “r” is independently 1-10;

each subscript “s” is independently 1-5;

each G^1 is independently a C_1 - C_6 substituted or unsubstituted alkylene;

each R^a is independently H or a C_1 - C_6 substituted or unsubstituted alkyl;

L^1 is $-NHC(O)-$, $-C(O)NH-$, $-NHC(O)O-$, $-OC(O)NH-$, $-NHC(O)NH-$, $-S-S-$, or



subscript “p” is independently 1-5;

L^2 is $-O-(CH_2CH_2O)_n-$, $-(CH_2CH_2O)_n-O-$, or $-(OCH_2CH_2O)_n-$,

each subscript “n” is independently 1-200;

subscript “ q^1 ” is independently 0-5;

subscript “ q^2 ” is independently 0-5;

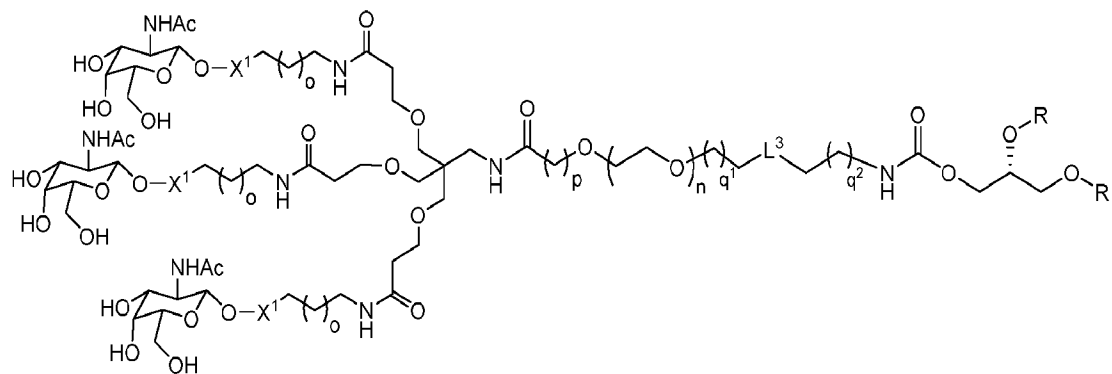
L^3 is absent, $-NHC(O)-$, or $-C(O)NH-$;

subscript “ q^3 ” is independently 0-5;

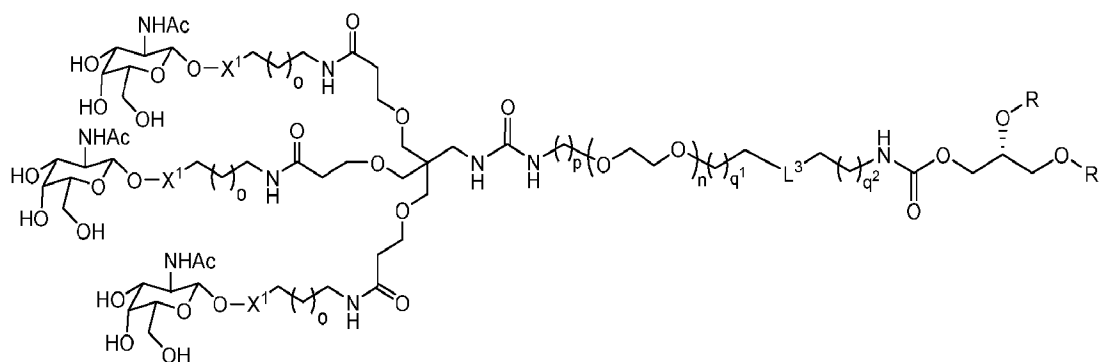
each R is independently a C_{12} - C_{20} substituted or unsubstituted alkyl; and

the “” indicates an *R* configuration or an *S* configuration.

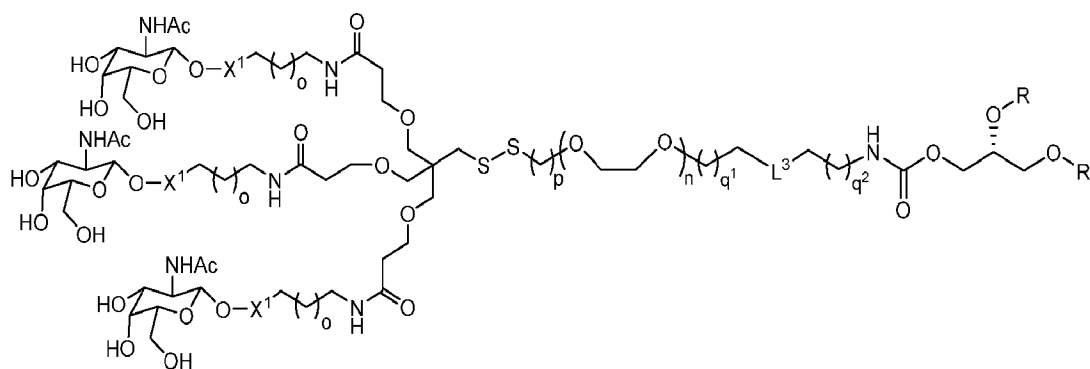
[0009] Another aspect of the disclosure provides compounds selected from the group consisting of Formula I-c-5, I-c-6, I-c-7, and I-c-8:



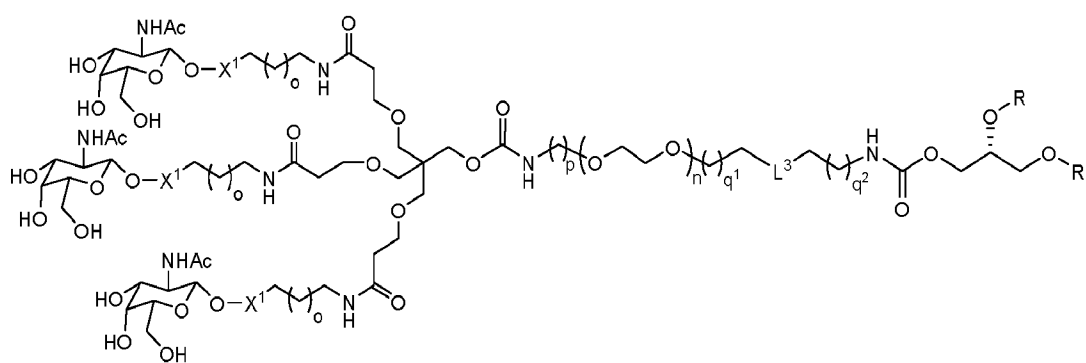
Formula I-c-5,



Formula I-c-6,



Formula I-c-7,



Formula I-c-8,

wherein,

each X^1 is independently $-(CH_2)_r-$, $-(CH_2CH_2O)_s-$, $-(OCH_2CH_2)_s-$, $-((CH_2)_m-C(O)NH)-$, or $-(C(O)NH-(CH_2)_m)-$;

each subscript “m” is independently 1-5;

each subscript “s” is independently 1-5;

each subscript “o” is independently 1-5;

each subscript “r” is independently 1-10;

each subscript “p” is independently 1-5;

each subscript “n” is independently 1-200;

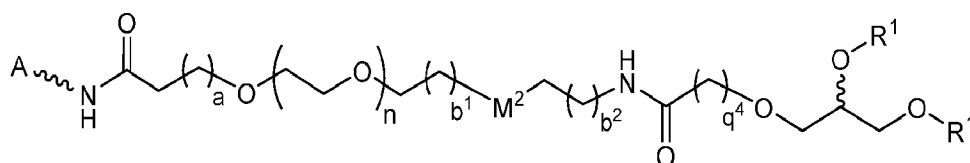
each subscript “q¹” is independently 0-5;

each subscript “q²” is independently 0-5;

each L^3 is independently absent, $-NHC(O)-$, or $-C(O)NH-$; and

each R is independently a C₁₂-C₂₀ substituted or unsubstituted alkyl.

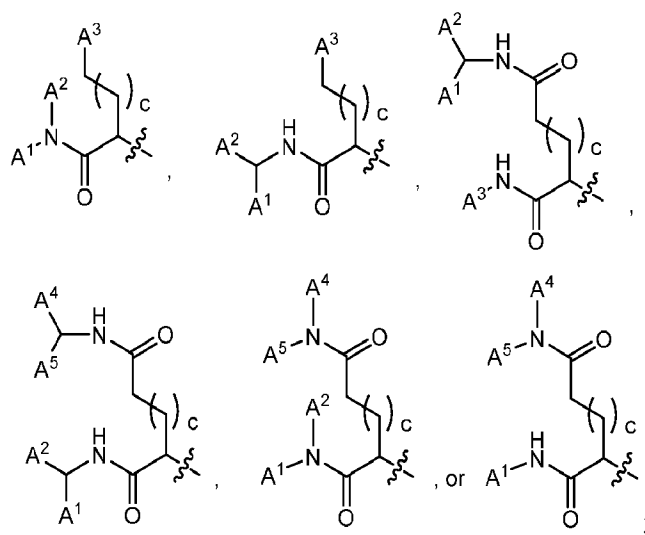
[0010] Another aspect of the present disclosure provides compounds of Formula II:



Formula II

wherein:

A is:



each A^1 , A^2 , A^3 , A^4 , and A^5 is independently ;

each Y¹ is independently –NH–, –NHC(O)O–, –OC(O)NH–, –NHC(O)–, or –C(O)NH–;

each Y² is independently absent, –(CH₂)_t–, –(CH₂CH₂O)_t–, or –(OCH₂CH₂)_t–;

each subscript “t” is independently 1-5;

each Y³ is independently absent, –NHC(O)–, or –C(O)NH–;

each Y⁴ is independently absent or –(CH₂)_t–;

each subscript “t” is independently 1-10;

each subscript “d” is independently 0-5;

each subscript “c” is independently 0-5;

subscript “n” is independently 1-200;

subscript “a” is 1-5;

subscript “b¹” is independently 0-5;

M² is absent, –NHC(O)–, or –C(O)NH–;

subscript “b²” is independently 0-5;

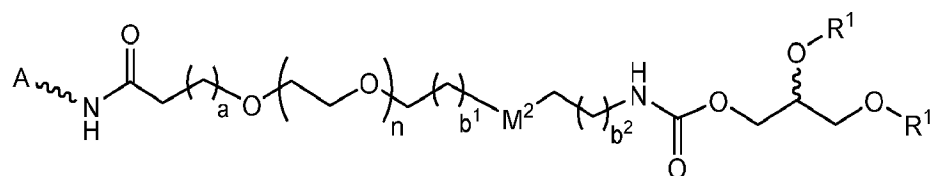
subscript “q⁴” is independently 0-6;

each R¹ is independently a C₁₂-C₂₀ substituted or unsubstituted alkyl; and

each of the “~” bonds independently indicate an *R* configuration or an *S* configuration;

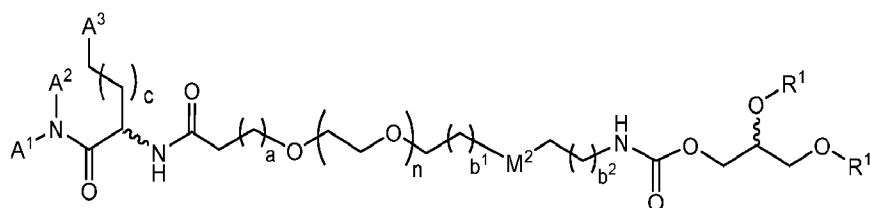
with the proviso that (1) A¹ and A² are the same and (2) at least one of Y², Y³ and Y⁴ is present.

[0011] In some embodiments, a compound of Formula II is a compound of Formula II-a:

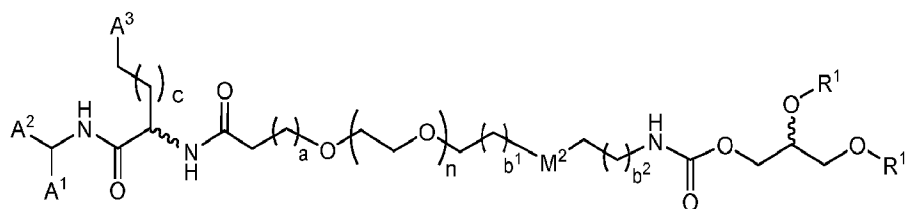
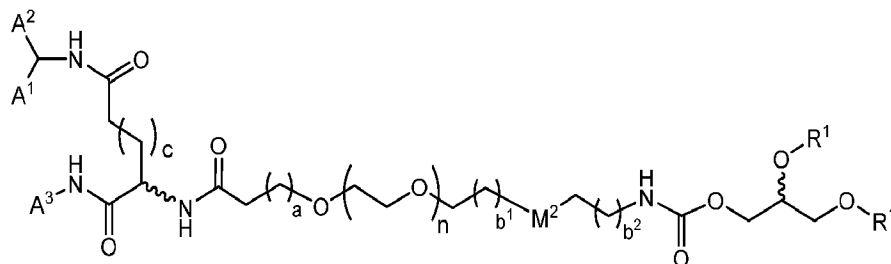
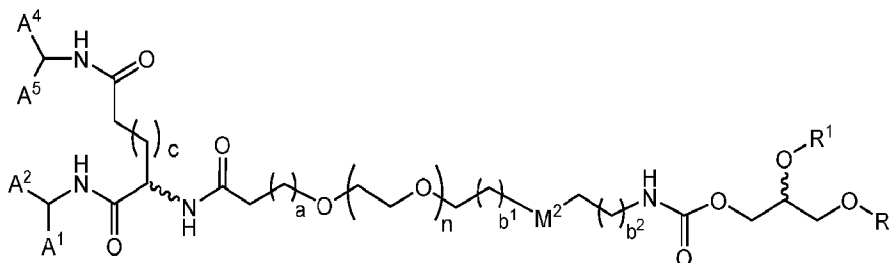
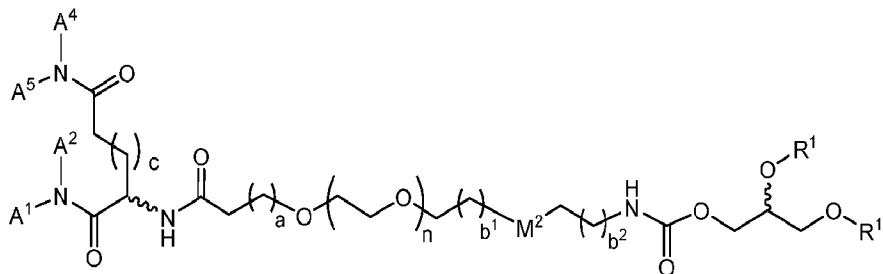
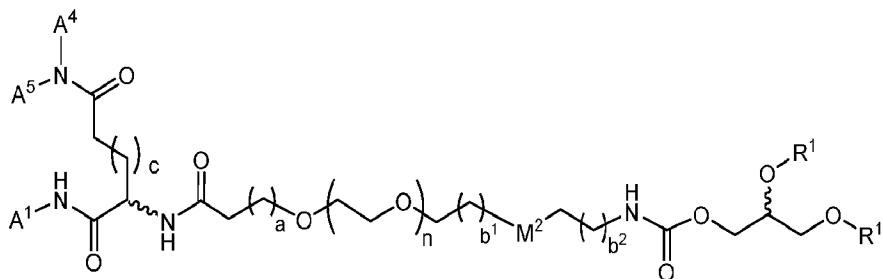


Formula II-a.

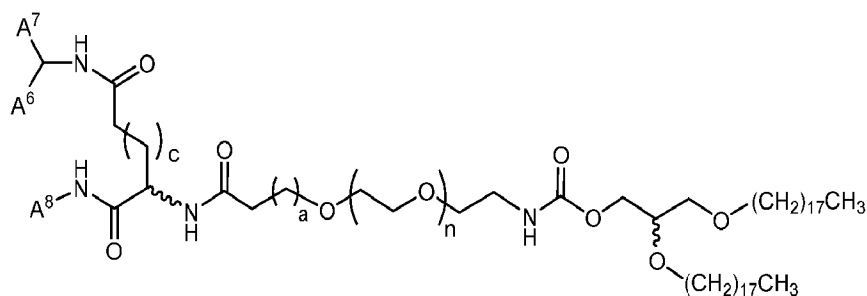
[0012] In some embodiments, a compound of Formula II-a is a compound of Formula II-a-1, Formula II-a-2, Formula II-a-3, Formula II-a-4, Formula II-a-5, or Formula II-a-6:



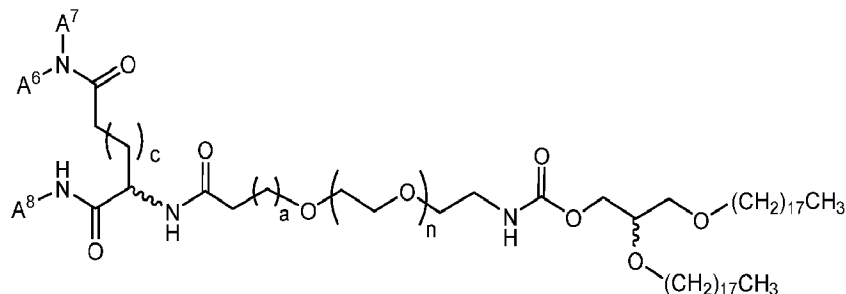
Formula II-a-1,

**Formula II-a-2,****Formula II-a-3,****Formula II-a-4,****Formula II-a-5, or****Formula II-a-6.**

[0013] Another aspect of the disclosure provides a compound of Formula III or Formula IV:

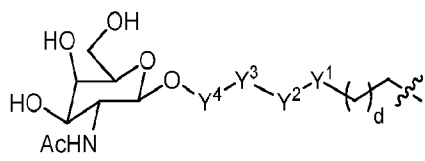


Formula III, or



Formula IV

wherein,



each A^6 , A^7 , and A^8 is independently

each Y^1 is independently absent, $-NHC(O)O-$, or $-OC(O)NH-$;

each Y^2 and Y^4 is independently $-(CH_2)_r-$;

each subscript "r" is independently 1-10;

each Y^3 is independently absent, $-NHC(O)-$, or $-C(O)NH-$;

each subscript "d" is independently 1-5;

each subscript "c" is independently 1-5;

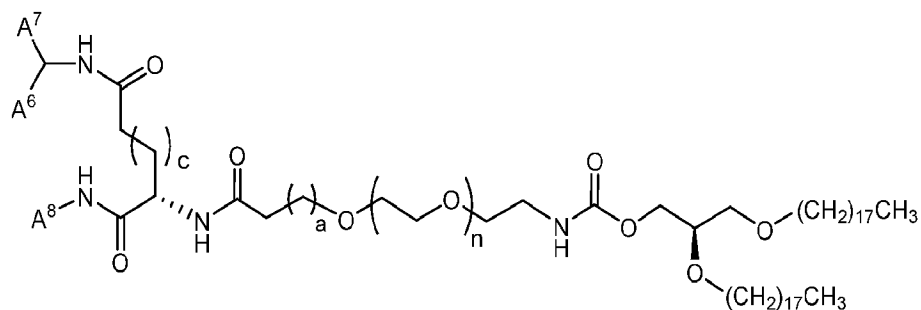
subscript "a" is 1-5;

subscript "n" is independently 10-100; and

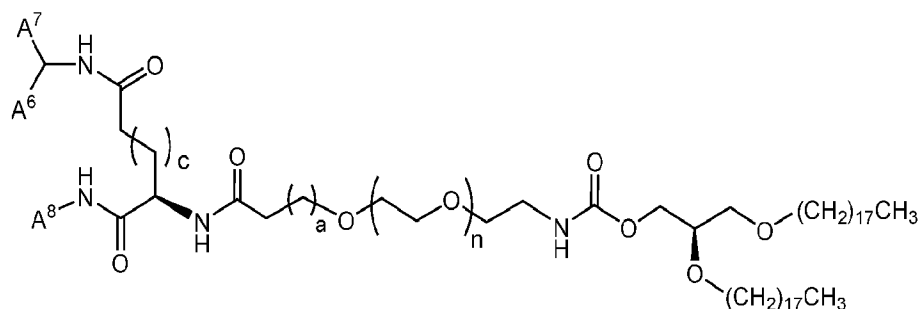
each of the "wavy" bonds independently indicate an *R* configuration or an *S* configuration;

with the proviso that at least one of Y^1 or Y^3 is present.

[0014] In some embodiments a compound of Formula III is a compound Formula III-a or Formula III-b:

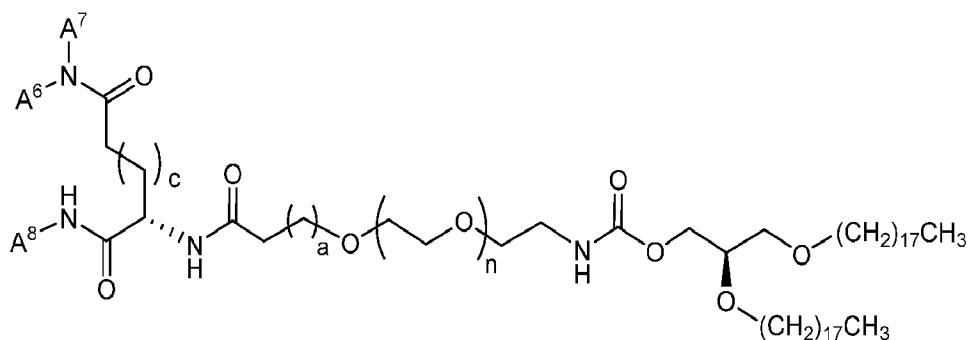


Formula III-a, or

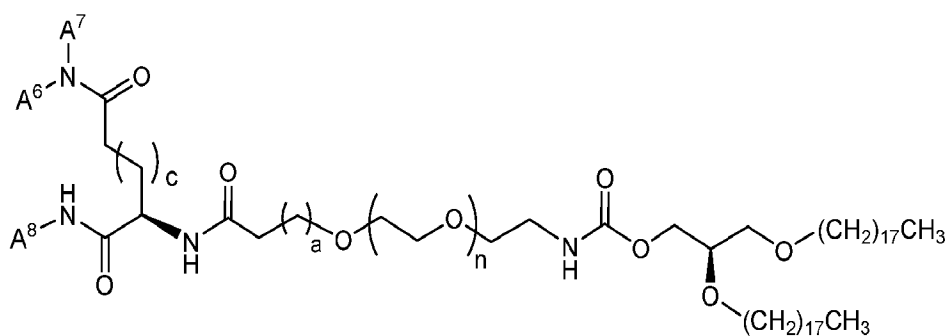


Formula III-b.

[0015] In some embodiments, a compound of Formula IV is a compound of Formula IV-a or Formula IV-b:



Formula IV-a, or



Formula IV-b.

[0016] In another aspect, the present disclosure provides a lipid nanoparticle (LNP) composition comprising:

a lipid that comprises a compound as described herein;

a cationic lipid;
a phospholipid;
a PEG-containing lipid; and
a sterol.

[0017] Another aspect of the present disclosure provides a pharmaceutical composition comprising the LNP composition described herein.

[0018] Another aspect of the present disclosure provides a method for delivering polynucleotides into a target cell comprising:

introducing into the target cell at least one lipid nanoparticle comprising a GalNAc-based lipid that comprises any one of the compounds described herein and one or more polynucleotides.

[0019] Another aspect of the present disclosure provides a method for delivering a prime editing system into a target cell comprising:

introducing into the target cell at least one lipid nanoparticle comprising a GalNAc-based lipid that comprises any one of the compounds described herein and one or more components of a prime editing system.

[0020] Other aspects, embodiments, and features will be apparent from the following description and the claims.

INCORPORATION BY REFERENCE

[0021] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] The novel features of the methods and compositions provided herein are set forth with particularity in the appended claims. A better understanding of the features and advantages of the methods and compositions provided herein will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the methods and compositions provided herein are utilized, and the accompanying drawings of which:

[0023] **FIG. 1** shows Indel% activity for compounds disclosed herein.

[0024] **FIG. 2** shows prime editing % in a dose response study for compounds disclosed herein.

DETAILED DESCRIPTION

[0025] Provided herein are compounds of Formula I to Formula IV. Compositions, lipid nanoparticles, and pharmaceutical compositions provided herein can comprise the compounds described herein. Methods for delivering said compositions and lipid nanoparticles are also provided herein.

[0026] The following description and examples illustrate embodiments of the present disclosure in detail. It is to be understood that this disclosure is not limited to the particular embodiments described herein and as such can vary. Those of skill in the art will recognize that there are numerous variations and modifications of this disclosure, which are encompassed within its scope. Although various features of the present disclosure can be described in the context of a single embodiment, the features can also be provided separately or in any suitable combination. Conversely, although the present disclosure can be described herein in the context of separate embodiments for clarity, the present disclosure can also be implemented in a single embodiment.

[0027] As used herein, the following definitions shall apply unless otherwise indicated.

[0028] I. DEFINITIONS

[0029] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. As used herein, the singular forms “a”, “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms “including”, “includes”, “having”, “has”, “with”, or variants thereof are used herein, they mean “comprising”.

[0030] Unless otherwise specified, the words “comprising”, “comprise”, “comprises”, “having”, “have”, “has”, “including”, “includes”, “include”, “containing”, “contains” and “contain” are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[0031] Reference to “some embodiments”, “an embodiment”, “one embodiment”, or “other embodiments” means that a particular feature or characteristic described in connection with the embodiments is included in at least one or more embodiments, but not necessarily all embodiments, of the present disclosure.

[0032] The term “about” or “approximately” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on

how the value is measured or determined, *e.g.*, the limitations of the measurement system.

For example, “about” can mean within 1 standard deviation, per the practice in the art.

Alternatively, “about” can mean a range of up to 20%, up to 10%, up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated, the term “about” meaning within an acceptable error range for the particular value should be assumed.

[0033] The term “between” means the range of numbers including the first and the last number in a range.

[0034] The term “substantially” as used herein may refer to a value approaching 100% of a given value. In some embodiments, the term may refer to an amount that may be at least about 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 99.99% of a total amount. In some embodiments, the term may refer to an amount that may be about 100% of a total amount.

[0035] For purposes of this disclosure, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed. Additionally, general principles of organic chemistry are described in "Organic Chemistry," Thomas Sorrell, University Science Books, Sausalito: 1999, and "March's Advanced Organic Chemistry," 5th Ed., Ed.: Smith, M.B. and March, J., John Wiley and Sons, New York: 2001, the entire contents of which are hereby incorporated by reference.

[0036] As described herein, compounds of the disclosure optionally may be substituted with one or more substituents, such as are illustrated generally above, or as exemplified by particular classes, subclasses, and species of the disclosure.

[0037] As used herein, the term "hydroxyl" or "hydroxy" refers to an –OH moiety.

[0038] As used herein, an “alkyl” refers to a straight or branched hydrocarbon chain radical consisting solely of carbon and hydrogen atoms, which is saturated or unsaturated (*e.g.*, contains one or more double and/or triple bonds), having, for example, from one to twenty-four carbon atoms (C₁-C₂₄ alkyl), one to twenty carbon atoms (C₁-C₂₀ alkyl), one to eighteen carbon atoms (C₁-C₁₈ alkyl), one to sixteen carbon atoms (C₁-C₁₆ alkyl), one to twelve carbon atoms (C₁-C₁₂ alkyl), one to eight carbon atoms (C₁-C₈ alkyl), one to six carbon atoms (C₁-C₆ alkyl). An alkyl group is attached to the rest of the molecule by a single bond, *e.g.*, methyl, ethyl, n propyl, 1-methylethyl (iso propyl), n butyl, n pentyl, 1, 1 dimethylethyl (t butyl), 3 methylhexyl, 2 methylhexyl, ethenyl, prop 1 enyl, but-1-enyl, pent-1-enyl, penta-1,4-dienyl,

ethynyl, propynyl, butynyl, pentynyl, hexynyl, and the like. In some embodiments, an alkyl group is substituted.

[0039] "Alkylene" or "alkylene chain" refers to a straight or branched divalent hydrocarbon chain linking the rest of the molecule to a radical group, consisting solely of carbon and hydrogen, which is saturated or unsaturated (*e.g.*, contains one or more double (alkenylene) and/or triple bonds (alkynylene)), and having, for example, from one to twenty-four carbon atoms (C₁-C₂₄ alkynylene), one to twenty carbon atoms (C₁-C₂₀ alkynylene), one to eighteen carbon atoms (C₁-C₁₈ alkynylene), one to sixteen carbon atoms (C₁-C₁₆ alkynylene), one to twelve carbon atoms (C₁-C₁₂ alkynylene), one to eight carbon atoms (C₁-C₈ alkynylene), one to six carbon atoms (C₁-C₆ alkynylene), two to four carbon atoms (C₂-C₄ alkylene), or one to two carbon atoms (C₁-C₂ alkylene). Examples of alkylene groups include, methylene, ethylene, propylene, n-butylene, ethenylene, propenylene, n-butenylene, propynylene, n-butynylene, and the like. The alkylene is attached to the rest of the molecule through a single or double bond and to the radical group through a single or double bond. The points of attachment of the alkylene to the rest of the molecule and to the radical group can be through one carbon or any two carbons within the chain. In some embodiments, an alkylene is substituted.

[0100] As used herein, a "carbonyl" refers to -C(O)-.

[0101] As used herein, a "cycloalkyl" refers to a stable non aromatic monocyclic or polycyclic hydrocarbon radical consisting solely of carbon and hydrogen atoms, which may include fused or bridged ring systems, having from three to fifteen carbon atoms, three to ten carbon atoms, etc. and which is saturated or unsaturated and attached to the rest of the molecule by a single bond. Monocyclic radicals include, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. Polycyclic radicals include, for example, adamantyl, norbornyl, decalyl, 7,7 dimethyl bicyclo[2.2.1]heptanyl, and the like.

[0102] As used herein, an "oxo" refers to =O.

[0103] As used herein, the term "substituted," whether preceded by the term "optionally" or not, refers generally to the replacement of hydrogen atoms in a given structure with the radical of a specified substituent. Specific substituents are described above in the definitions and below in the description of compounds and examples thereof. Unless otherwise indicated, an optionally substituted group can have a substituent at each substitutable position of the group, and when more than one position in any given structure can be substituted with more than one substituent selected from a specified group, the substituent can be either the same or

different at every position. As one of ordinary skill in the art will recognize, combinations of substituents envisioned by this disclosure are those combinations that result in the formation of stable or chemically feasible compounds. In some embodiments, a "substituted" group (*e.g.*, alkyl or alkylene) has at least one hydrogen atom replaced by a bond to a non-hydrogen atoms such as, but not limited to: a halogen atom such as F, Cl, Br, and I; oxo groups (=O); hydroxyl groups (-OH); alkoxy groups (-OR^x, where R^x is C₁-C₁₂ alkyl or cycloalkyl); carboxyl groups (-OC(=O)R^y or -C(=O)OR^y, where R^y is H, C₁-C₁₂ alkyl or cycloalkyl); amine groups (-NR^xR^y, where R^x and R^y are each independently H, C₁-C₁₂ alkyl or cycloalkyl); C₁-C₁₂ alkyl groups; and cycloalkyl groups. In some embodiments the substituent is a C₁-C₁₂ alkyl group. In other embodiments, the substituent is a cycloalkyl group. In other embodiments, the substituent is a halo group, such as fluoro. In other embodiments, the substituent is an oxo group. In other embodiments, the substituent is a hydroxyl group. In other embodiments, the substituent is an alkoxy group. In other embodiments, the substituent is a carboxyl group. In other embodiments, the substituent is an amine group.

[0104] Unless otherwise stated, structures depicted herein also are meant to include all isomeric (*e.g.*, enantiomeric, diastereomeric, and geometric (or conformational)) forms of the structure; for example, the R and S configurations for each asymmetric center, (Z) and (E) double bond isomers, and (Z) and (E) conformational isomers. Therefore, single stereochemical isomers as well as enantiomeric, diastereomeric, and geometric (or conformational) mixtures of the present compounds are within the scope of the disclosure. Unless otherwise stated, all tautomeric forms of the compounds of the disclosure are within the scope of the disclosure. Additionally, unless otherwise stated, structures depicted herein also are meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of hydrogen by deuterium or tritium, or the replacement of a carbon by a ¹³C- or ¹⁴C-enriched carbon are within the scope of this disclosure. Such compounds are useful, for example, as analytical tools or probes in biological assays, or as therapeutic agents.

[0105] Compounds disclosed herein may be "isomerically pure" compounds. As used herein, the term "isomerically pure" refers to an isomeric form of a compound that is substantially free from other isomeric forms of the compound (*e.g.*, substantially free from other stereoisomers (*e.g.*, enantiomers, diastereomers, geometric (or conformational) isomers, etc.), constitutional isomers, isotopomers, etc.). For example, an "isomerically pure"

compound having at least one asymmetric center of a particular configuration (*e.g.*, R or S configuration) is substantially free from other isomeric forms of the compound having a different configuration at the at least one asymmetric center. An "isomerically pure" compound comprises more than 75% by weight, more than 80% by weight, more than 85% by weight, more than 90% by weight, more than 91% by weight, more than 92% by weight, more than 93% by weight, more than 94% by weight, more than 95% by weight, more than 96% by weight, more than 97% by weight, more than 98% by weight, more than 98.5% by weight, more than 99% by weight, more than 99.2% by weight, more than 99.5% by weight, more than 99.6% by weight, more than 99.7% by weight, more than 99.8% by weight, or more than 99.9% by weight, of a single isomer of the compound based on the total weight of all isomers of the compound that are present.

[0106] Chemical structures and nomenclature are derived from ChemDraw, version 18.0.0.231, Cambridge, MA.

[0107] It also will be appreciated that certain of the compounds of the present disclosure can exist in free form for treatment, or where appropriate, as a pharmaceutically acceptable derivative (*e.g.*, a salt) thereof. According to the present disclosure, a pharmaceutically acceptable derivative includes, but is not limited to, pharmaceutically acceptable prodrugs, salts, esters, salts of such esters, or any other adduct or derivative that upon administration to a patient in need is capable of providing, directly or indirectly, a compound as otherwise described herein, or a metabolite or residue thereof.

[0108] As used herein, the term "pharmaceutically acceptable" means approved or approvable by a regulatory agency of the Federal or a state government or the corresponding agency in countries other than the United States, or that is listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals, and more particularly, in humans.

[0109] As used herein, the term "pharmaceutically acceptable salt" refers to a salt of a compound of the disclosure that is pharmaceutically acceptable and that possesses the desired pharmacological activity of the parent compound. In particular, such salts are non-toxic may be inorganic or organic acid addition salts and base addition salts. Specifically, such salts include: (1) acid addition salts, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or formed with organic acids such as acetic acid, propionic acid, hexanoic acid, cyclopentanepropionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, 3-(4-hydroxybenzoyl) benzoic acid,

cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethane-disulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, 4-chlorobenzenesulfonic acid, 2-naphthalenesulfonic acid, 4-toluenesulfonic acid, camphorsulfonic acid, 4-methylbicyclo[2.2.2]-oct-2-ene-1-carboxylic acid, glucoheptonic acid, 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, hydroxynaphthoic acid, salicylic acid, stearic acid, muconic acid, and the like; or (2) salts formed when an acidic proton present in the parent compound either is replaced by a metal ion, *e.g.*, an alkali metal ion, an alkaline earth ion, or an aluminum ion; or coordinates with an organic base such as ethanolamine, diethanolamine, triethanolamine, N-methylglucamine and the like. Salts further include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium, and the like; and when the compound contains a basic functionality, salts of non-toxic organic or inorganic acids, such as hydrochloride, hydrobromide, tartrate, mesylate, acetate, maleate, oxalate and the like. The term "pharmaceutically acceptable cation" refers to an acceptable cationic counter-ion of an acidic functional group. Such cations are exemplified by sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium cations, and the like. See, *e.g.*, Berge, et al., J. Pharm. Sci. (1977) 66(1): 1–79.

[0110] The term “lipid” refers to a group of organic compounds that include, but are not limited to, esters of fatty acids and are generally characterized by being poorly soluble in water, but soluble in many organic solvents.

[0111] A “lipid nanoparticle” may refer to particles having at least one dimension in the nanometers range (*e.g.*, 1-1,000 nm) and include the compounds described herein. In some embodiments, lipid nanoparticles (LNPs) can be included in compositions that are used to deliver at least one polynucleotide and/or construct according to this disclosure. In some embodiments, the lipid nanoparticles of the disclosure comprise at least one polynucleotide and/or one or more components of a prime editing system. In some embodiments, the lipid nanoparticles can include one or more additional lipid components. In some embodiments, the at least one polynucleotide and/or construct may be encapsulated in the lipid portion of the lipid nanoparticle or an aqueous space enveloped by some or all of the lipid portion of the lipid nanoparticle.

[0112] In some embodiments, the lipid nanoparticles can 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.

[0113] As used herein, the term “biodegradable” refers to materials that, when introduced into cells, are broken down by cellular machinery (*e.g.*, enzymatic degradation) or by hydrolysis into components that cells can either reuse or dispose of without significant toxic effect(s) on the cells. In certain embodiments, components generated by breakdown of a biodegradable material do not induce inflammation and/or other adverse effects *in vivo*. In some embodiments, biodegradable materials are enzymatically broken down. Alternatively or additionally, in some embodiments, biodegradable materials are readily broken down and eliminated.

[0114] As used herein, “cationic lipids” refer to lipids that are positively charged at the head group. Cationic lipids typically contain three domains: a hydrophilic headgroup, a hydrophobic domain, and a linker. Cationic lipids are an amphiphilic entity with the head group having hydrophilicity and lipid tails possessing hydrophobicity.

[0115] As used herein, a “cell” can generally refer to a biological cell. A cell can be the basic structural, functional and/or biological unit of a living organism. A cell can originate from any organism having one or more cells. Some non-limiting examples include: a prokaryotic cell, eukaryotic cell, a bacterial cell, an archaeal cell, a cell of a single-cell eukaryotic organism, a protozoa cell, a cell from a plant, an animal cell, a cell from an invertebrate animal (*e.g.*, fruit fly, cnidarian, echinoderm, nematode, etc.), a cell from a vertebrate animal (*e.g.*, fish, amphibian, reptile, bird, mammal), a cell from a mammal (*e.g.*, a pig, a cow, a goat, a sheep, a rodent, a rat, a mouse, a non-human primate, a human, etc.), et cetera. Sometimes a cell may not originate from a natural organism (*e.g.*, a cell can be synthetically made, sometimes termed an artificial cell).

[0116] In some embodiments, the cell is a human cell. A cell may be of or derived from different tissues, organs, and/or cell types. In some embodiments, the cell is a primary cell. In some embodiments, the term primary cell means a cell isolated from an organism, *e.g.*, a mammal, which is grown in tissue culture (*e.g.*, *in vitro*) for the first time before subdivision and transfer to a subculture. In some non-limiting examples, mammalian primary cells can be modified through introduction of one or more polynucleotides, polypeptides, and/or prime editing compositions (*e.g.*, through transfection, transduction, electroporation and the like) and further passaged. In some embodiments, a cell is not isolated from an organism but forms

part of a tissue or organ of an organism, *e.g.*, a mammal. In some embodiments, the cell is a stem cell. In some embodiments, the cell is a human stem cell.

[0117] The term “construct” refers to refers to a polynucleotide or a portion of a polynucleotide, comprising one or more nucleic acid sequences encoding one or more transcriptional products and/or proteins. A construct may be a recombinant nucleic acid molecule or a part thereof. In some embodiments, the one or more nucleic acid sequences of a construct are operably linked to one or more regulatory sequences, for example, transcriptional initiation regulatory sequences. In some embodiments, a construct is a vector, a plasmid, or a portion thereof. In some embodiments, a construct comprises DNA. In some embodiments, a construct comprises RNA. In some embodiments, a construct is double stranded. In some embodiments, a construct is single stranded. In some embodiments, a construct comprises an expression cassette. An expression cassette means a polynucleotide comprising a nucleic acid sequence that encodes one or more transcriptional products and is operably linked to at least one transcriptional regulatory sequence, *e.g.*, a promoter.

[0118] The term “polynucleotide” or “nucleic acid molecule” can be any polymeric form of nucleotides, including DNA, RNA, a hybridization thereof, or RNA-DNA chimeric molecules. For example, a polynucleotide can be a cDNA, genomic DNA, mRNA, tRNA, rRNA, microRNA, antisense DNA or RNA, plasmid DNA, microRNA inhibitors, mRNA-interfering complementary RNA (micRNA), multivalent RNA, etc. In some embodiments, a polynucleotide is double stranded, *e.g.*, a double-stranded DNA in a gene. In some embodiments, a polynucleotide is single-stranded or substantially single-stranded, *e.g.*, single-stranded DNA or an mRNA. In some embodiments, a polynucleotide is a cell-free nucleic acid molecule. In some embodiments, a polynucleotide circulates in blood. In some embodiments, a polynucleotide is a cellular nucleic acid molecule. In some embodiments, a polynucleotide is a cellular nucleic acid molecule in a cell circulating in blood.

[0119] Polynucleotides can have any three-dimensional structure. The following are nonlimiting examples of polynucleotides: a gene or gene fragment (for example, a probe, primer, EST or SAGE tag), an exon, an intron, intergenic DNA (including, without limitation, heterochromatic DNA), messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), a ribozyme, cDNA, a recombinant polynucleotide, a branched polynucleotide, a plasmid, a vector, isolated DNA, isolated RNA, sgRNA, guide RNA, a nucleic acid probe, a primer, an snRNA, a long non-coding RNA, a snoRNA, a siRNA, a miRNA, a tRNA-derived small RNA (tsRNA), an antisense RNA, an shRNA, or a small rDNA-derived RNA (srRNA).

[0120] In some embodiments, a polynucleotide comprises deoxyribonucleotides, ribonucleotides or analogs thereof. In some embodiments, a polynucleotide comprises modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure can be imparted before or after assembly of the polynucleotide. The sequence of nucleotides can be interrupted by non-nucleotide components. A polynucleotide can be further modified after polymerization, such as by conjugation with a labeling component.

[0121] In some embodiments, a polynucleotide is composed of a specific sequence of four nucleotide bases: adenine (A); cytosine (C); guanine (G); thymine (T); and uracil (U) for thymine when the polynucleotide is RNA. In some embodiments, the polynucleotide may comprise one or more other nucleotide bases, such as inosine (I), which is read by the translation machinery as guanine (G).

[0122] In some embodiments, a polynucleotide may be modified. As used herein, the terms “modified” or “modification” refers to chemical modification with respect to the A, C, G, T and U nucleotides. In some embodiments, modifications may be on the nucleoside base and/or sugar portion of the nucleosides that comprise the polynucleotide. In some embodiments, the modification may be on the internucleoside linkage (*e.g.*, phosphate backbone). In some embodiments, multiple modifications are included in the modified nucleic acid molecule. In some embodiments, a single modification is included in the modified nucleic acid molecule.

[0123] The terms “protein” and “polypeptide” can be used interchangeably to refer to a polymer of two or more amino acids joined by covalent bonds (*e.g.*, an amide bond) that can adopt a three-dimensional conformation. In some embodiments, a protein or polypeptide comprises at least 10 amino acids, 15 amino acids, 20 amino acids, 30 amino acids or 50 amino acids joined by covalent bonds (*e.g.*, amide bonds). In some embodiments, a protein comprises at least two amide bonds. In some embodiments, a protein comprises multiple amide bonds. In some embodiments, a protein comprises an enzyme, enzyme precursor proteins, regulatory protein, structural protein, receptor, nucleic acid binding protein, a biomarker, a member of a specific binding pair (*e.g.*, a ligand or aptamer), or an antibody. In some embodiments, a protein may be a full-length protein (*e.g.*, a fully processed protein having certain biological function). In some embodiments, a protein may be a variant or a fragment of a full-length protein. A variant of a protein or enzyme, for example a variant reverse transcriptase, comprises a polypeptide having an amino acid sequence that is about 60% identical, about 70% identical, about 80% identical, about 90% identical, about 95%

identical, about 96% identical, about 97% identical, about 98% identical, about 99% identical, about 99.5% identical, or about 99.9% identical to the amino acid sequence of a reference protein.

[0124] In some embodiments, a protein comprises one or more protein domains or subdomains. As used herein, the term “polypeptide domain”, “protein domain”, or “domain” when used in the context of a protein or polypeptide, refers to a polypeptide chain that has one or more biological functions, *e.g.*, a catalytic function, a protein-protein binding function, or a protein-DNA function. In some embodiments, a protein comprises multiple protein domains. In some embodiments, a protein comprises multiple protein domains that are naturally occurring. In some embodiments, a protein comprises multiple protein domains from different naturally occurring proteins. A protein that comprises amino acid sequences from different origins or naturally occurring proteins may be referred to as a fusion, or chimeric protein.

[0125] In some embodiments, a protein or polypeptide includes naturally occurring amino acids (*e.g.*, one of the twenty amino acids commonly found in peptides synthesized in nature, and known by the one letter abbreviations A, R, N, C, D, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y and V). In some embodiments, a protein or polypeptides includes non-naturally occurring amino acids (*e.g.*, amino acids which is not one of the twenty amino acids commonly found in peptides synthesized in nature, including synthetic amino acids, amino acid analogs, and amino acid mimetics). In some embodiments, a protein or polypeptide is modified.

[0126] In some embodiments, a protein comprises an isolated polypeptide. The term “isolated” means free or removed to varying degrees from components which normally accompany it as found in the natural state or environment. For example, a polypeptide naturally present in a living animal is not isolated, and the same polypeptide partially or completely separated from the coexisting materials of its natural state is isolated.

[0127] In some embodiments, a protein is present within a cell, a tissue, an organ, or a virus particle. In some embodiments, a protein is present within a cell or a part of a cell (*e.g.*, a bacteria cell, a plant cell, or an animal cell). In some embodiments, the cell is in a tissue, in a subject, or in a cell culture. In some embodiments, the cell is a microorganism (*e.g.*, a bacterium, fungus, protozoan, or virus). In some embodiments, a protein is present in a mixture of analytes (*e.g.*, a lysate). In some embodiments, the protein is present in a lysate from a plurality of cells or from a lysate of a single cell.

[0128] The term “encode” as it is applied to polynucleotides refers to a polynucleotide

which is said to “encode” another polynucleotide, a polypeptide, or an amino acid if, in its native state or when manipulated by methods well known to those skilled in the art, it can be used as a polynucleotide synthesis template, *e.g.*, transcribed into an RNA, reverse transcribed into a DNA or cDNA, and/or translated to produce an amino acid, or a polypeptide or fragment thereof. In some embodiments, a polynucleotide comprising three contiguous nucleotides form a codon that encodes a specific amino acid. In some embodiments, a polynucleotide comprises one or more codons that encode a polypeptide. In some embodiments, a polynucleotide comprising one or more codons comprises a mutation in a codon compared to a wild-type reference polynucleotide. In some embodiments, the mutation in the codon encodes an amino acid substitution in a polypeptide encoded by the polynucleotide as compared to a wild-type reference polypeptide.

[0129] II. LIPID NANOPARTICLES (LNPs)

[0130] The present disclosure provides lipid nanoparticle (LNP) compositions. In some embodiments, the LNP compositions comprise an N-acetylgalactosamine (“GalNAc”) based lipid that comprises a compound described herein. In some embodiments, the LNP compositions comprise a GalNAc-based lipid that is or comprises a compound described herein, a cationic lipid, a phospholipid, a polyethylene glycol-lipid, and a sterol.

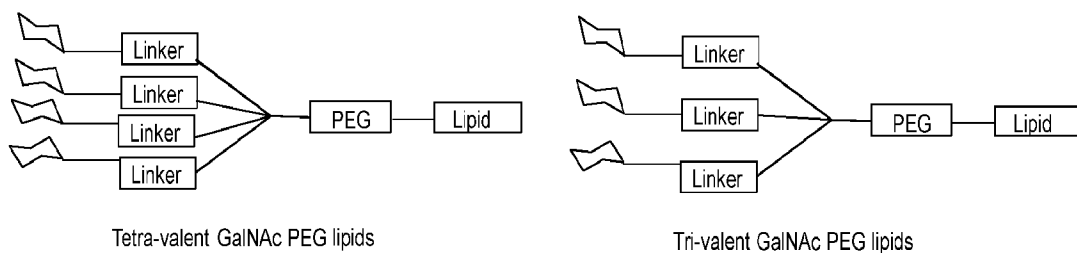
[0131] A. Compounds

[0132] The present disclosure provides lipid nanoparticle (LNP) compositions. In some embodiments, the LNP compositions comprise a GalNAc-based lipid that is or comprises a compound described herein.

[0133] As used herein, when describing GalNAc-based lipids, ligands, and compounds, in both writing and chemical drawings, “Ac” refers to an acetyl –(C(O)CH₃) functional group and is not to be confused with the element actinium.

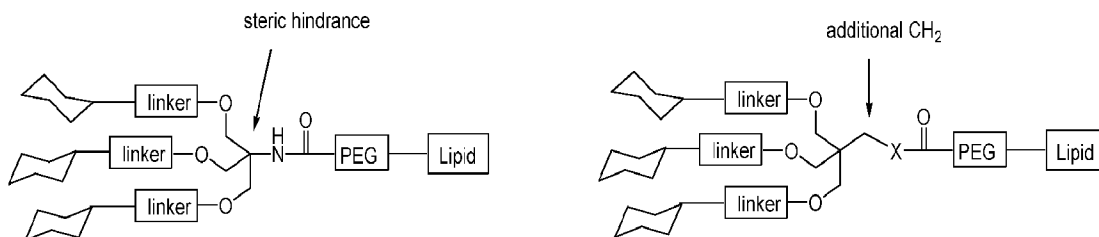
[0134] GalNAc-based ligands, which bind to the asialoglycoprotein receptor (ASGPR), have recently been developed in lipid nanoparticles systems to deliver nucleic acids to the liver. The editing activity achieved by GalNAc-LNP system has paved the way of nucleic acid delivery and further develop as potential gene therapeutics delivery system. Extensive SAR studies have reported that different linkers influence the GalNAc-based lipids and their delivery activity. Accordingly, there is a need to develop GalNAc-based lipids that can be incorporated into LNP composition.

[0135] The present disclosure provides a new series of GalNAc-based lipids which include a PEG moiety. In here, the compounds of Formula I and II disclosed herein employed three major design strategies: 1) insertion of CH₂ motif between NH₂ and a carbon atom; 2) insertion of a biodegradable carbamide motif within the linker; and/or 3) employed different amino acid as linkers. PEG moieties were selected to bridge the GalNAc-based moieties with lipids. Using these strategies, a series of tri-valent-GalNAc and tetra-valent GalNAc PEG lipids are disclosed herein, as shown in Scheme 1 below.



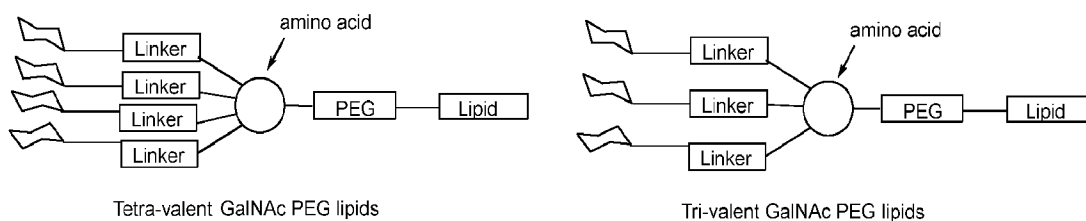
Scheme 1

[0136] As shown in Scheme 2 below, the introduction of a CH₂ motif between the amide and the GalNAc-based ligands provides less steric hindrance on the primary amine and can make the coupling easier and increase the yield. Furthermore, the primary amine can be replaced with other moieties, such as O, S, and S-S.



Scheme 2

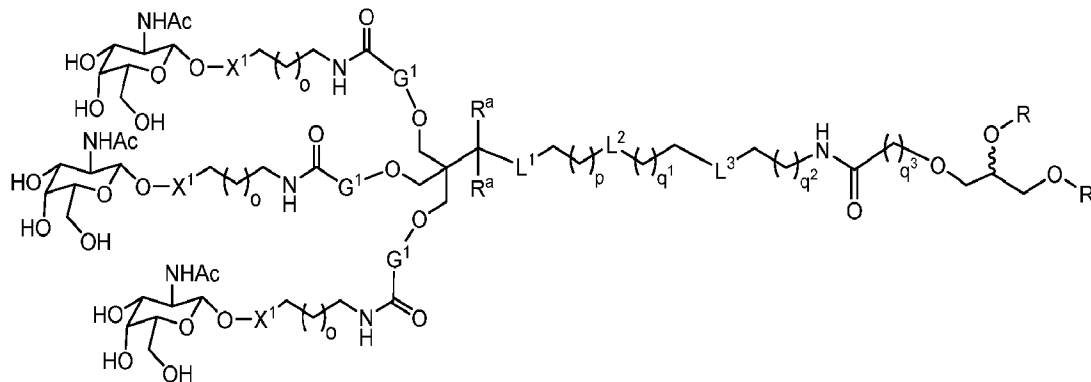
[0137] As shown in Scheme 3 below, the introduction of a biodegradable carbamide motif can be incorporated into to linker, basic and acidic amino acids, such as Lys and Glu can be selected to conjugate with tri-valent and tetra-valent GalNAc, and PEG-based lipits can be conjugated with alpha amine (*R* or *S* isomer) of the amine acids.



Scheme 3

[0138] (i) *Compounds of Formula I*

[0139] In some embodiments, the present disclosure provides compounds of Formula I:



Formula I

wherein,

each X^1 is independently $-(CH_2)_r-$, $-(CH_2CH_2O)_s-$, $-(OCH_2CH_2)_s-$, $-((CH_2)_m-C(O)NH)-$, or $-(C(O)NH-(CH_2)_m)-$,

each subscript "m" is independently 1-5;

each subscript "o" is independently 1-5;

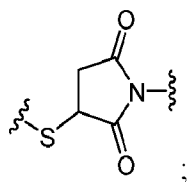
each subscript "r" is independently 1-10;

each subscript "s" is independently 1-5;

each G^1 is independently a C_1 - C_6 substituted or unsubstituted alkylene;

each R^a is independently H or a C_1 - C_6 substituted or unsubstituted alkyl;

L^1 is $-NHC(O)-$, $-C(O)NH-$, $-NHC(O)O-$, $-OC(O)NH-$, $-NHC(O)NH-$, $-S-S-$, or



subscript "p" is independently 1-5;

L^2 is $-O-(CH_2CH_2O)_n-$, $-(CH_2CH_2O)_n-O-$, or $-(OCH_2CH_2O)_n-$,

each subscript "n" is independently 1-200;

subscript " q^1 " is independently 0-5;

subscript " q^2 " is independently 0-5;

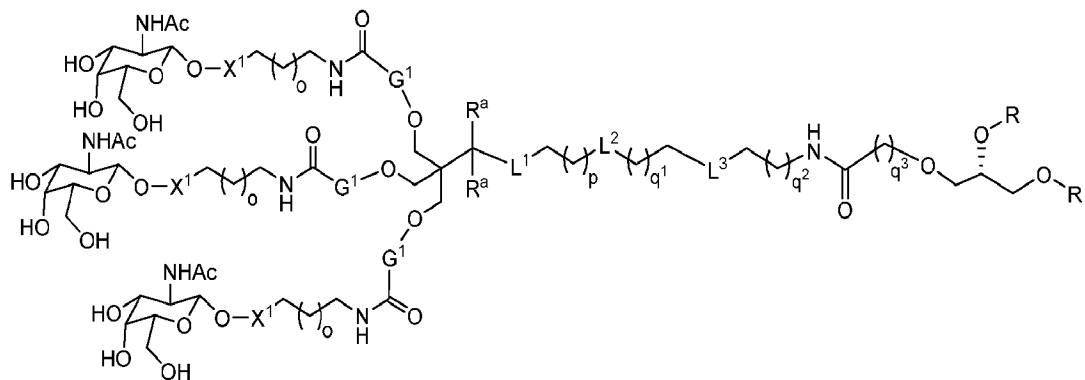
L^3 is absent, $-NHC(O)-$, or $-C(O)NH-$;

subscript " q^3 " is independently 0-5;

each R is independently a C_{12} - C_{20} substituted or unsubstituted alkyl; and

the "wavy" indicates an *R* configuration or an *S* configuration.

[0140] In some embodiments, the compound of Formula I is a compound of Formula I-a:



Formula I-a.

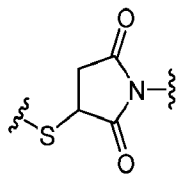
[0141] In some embodiments, each X^1 is independently $-(CH_2)_r-$, $-(CH_2CH_2O)_s-$, $-(OCH_2CH_2)_s-$, $-((CH_2)_m-C(O)NH)-$, or $-(C(O)NH-(CH_2)_m)-$. In some embodiments, each subscript “m” is independently 1-5. In some embodiments, each subscript “r” is independently 1-10. In some embodiments, each subscript “s” is independently 1-5. In some embodiments, each X^1 is independently $-(CH_2)_r-$. In some embodiments, each subscript “r” is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, each X^1 is independently $-(CH_2CH_2O)_s-$. In some embodiments, each X^1 is independently $-(OCH_2CH_2)_s-$. In some embodiments, each subscript “s” is independently 1, 2, 3, 4, or 5. In some embodiments, each X^1 is independently $-((CH_2)_m-C(O)NH)-$. In some embodiments, each X^1 is independently $-(C(O)NH-(CH_2)_m)-$. In some embodiments, each subscript “m” is independently 1, 2, 3, 4, or 5.

[0142] In some embodiments, each subscript “o” is independently 1, 2, 3, 4, or 5.

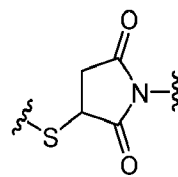
[0143] In some embodiments, each G^1 is independently a C_1 - C_6 substituted or unsubstituted alkylene. In some embodiments, each G^1 is independently a C_1 - C_6 substituted alkylene. In some embodiments, each G^1 is independently a C_1 - C_6 unsubstituted alkylene. In some embodiments, each G^1 is independently a C_1 ($-CH_2-$), C_2 ($-CH_2CH_2-$), C_3 (e.g., $-(CH_2)_3-$), C_4 (e.g., $-(CH_2)_4-$), C_5 (e.g., $-(CH_2)_5-$), or C_6 (e.g., $-(CH_2)_6-$) unsubstituted alkyl.

[0144] In some embodiments, each R^a is independently H or a C_1 - C_6 substituted or unsubstituted alkyl. In some embodiments, each R^a is independently H. In some embodiments, each R^a is independently a C_1 - C_6 substituted or unsubstituted alkyl. In some embodiments, each R^a is independently a C_1 - C_6 substituted alkyl. In some embodiments, each R^a is independently a C_1 - C_6 unsubstituted alkyl.

[0145] In some embodiments, L^1 is $-\text{NHC(O)}-$, $-\text{C(O)NH}-$, $-\text{NHC(O)O}-$, $-\text{OC(O)NH}-$, $-\text{NHC(O)NH}-$, $-\text{S-S}-$, or



$\text{NHC(O)NH}-$, $-\text{S-S}-$, or $-\text{NHC(O)NH}-$. In some embodiments, L^1 is $-\text{NHC(O)}-$. In some embodiments, L^1 is $-\text{C(O)NH}-$. In some embodiments, L^1 is $-\text{NHC(O)O}-$. In some embodiments, L^1 is $-\text{OC(O)NH}-$. In some embodiments, L^1 is $-\text{NHC(O)NH}-$. In some



embodiments, L^1 is $-\text{S-S}-$. In some embodiments, L^1 is

[0146] In some embodiments, subscript “p” is independently 1, 2, 3, 4, or 5.

[0147] In some embodiments, L^2 is $-\text{O}-(\text{CH}_2\text{CH}_2\text{O})_n-$, $-(\text{CH}_2\text{CH}_2\text{O})_n-\text{O}-$, or $-(\text{OCH}_2\text{CH}_2\text{O})_n-$. In some embodiments, L^2 is $-\text{O}-(\text{CH}_2\text{CH}_2\text{O})_n-$. In some embodiments, L^2 is $-(\text{CH}_2\text{CH}_2\text{O})_n-\text{O}-$. In some embodiments, L^2 is $-(\text{OCH}_2\text{CH}_2\text{O})_n-$. In some embodiments, each subscript “n” is independently 1-200, 10-200, 10-100, 10-50, 10-40, 10-30, 10-20, or 100-200. In some embodiments, each subscript “n” is independently 10-200. In some embodiments, each subscript “n” is independently 100-200. In some embodiments, each subscript “n” is independently 10, 20, 30, 40, or 50. In some embodiments, the quantity of each subscript “n” depends on the molecular weight average of the moiety, such that the PEG-like moiety results in a molecular weight average of about 100-2000 Daltons (Da), about 1000-2000 Da, about 1000 Da, or about 2000 Da.

[0148] In some embodiments, subscript “q¹” is independently 0, 1, 2, 3, 4, or 5. In some embodiments, subscript “q²” is independently 0, 1, 2, 3, 4, or 5. In some embodiments, subscript “q³” is independently 0, 1, 2, 3, 4, or 5.

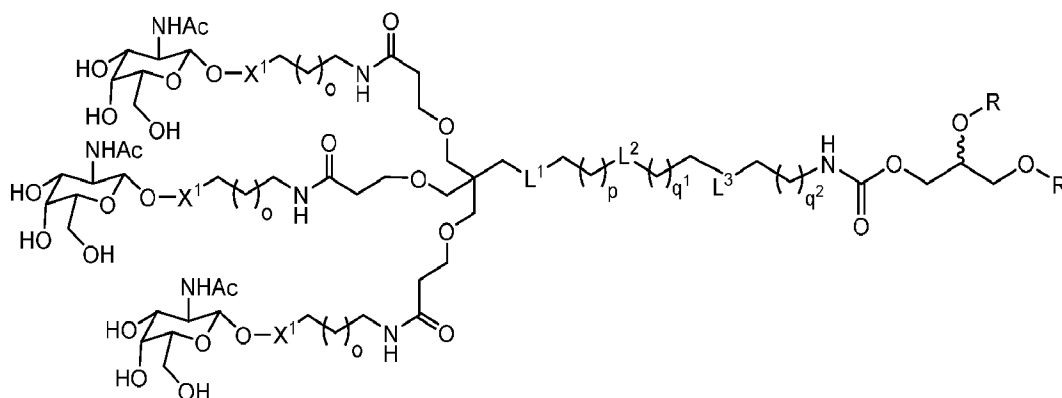
[0149] In some embodiments, L^3 is absent, $-\text{NHC(O)}-$, or $-\text{C(O)NH}-$. In some embodiments, L^3 is absent. In some embodiments, L^3 is $-\text{NHC(O)}-$. In some embodiments, L^3 is $-\text{C(O)NH}-$.

[0150] In some embodiments, each R is independently a C_{12} - C_{20} substituted or unsubstituted alkyl. In some embodiments, each R is independently a C_{12} - C_{20} substituted alkyl. In some embodiments, each R is independently a C_{12} - C_{20} unsubstituted alkyl. In some embodiments, each R is independently a C_{14} - C_{18} unsubstituted alkyl. In some embodiments, each R is independently a C_{14} unsubstituted alkyl. In some embodiments, each R is independently a C_{16} unsubstituted alkyl. In some embodiments, each R is independently a C_{18} unsubstituted alkyl. In some embodiments, each R is a C_{12} alkyl, C_{14} alkyl, C_{15} alkyl, C_{16}

alkyl, C₁₇ alkyl, C₁₈ alkyl, C₁₉ alkyl, or C₂₀ alkyl. In some embodiments, each R is n-dodecanyl, n-tridecanyl, n-tetradecanyl, or n-pentadecanyl.

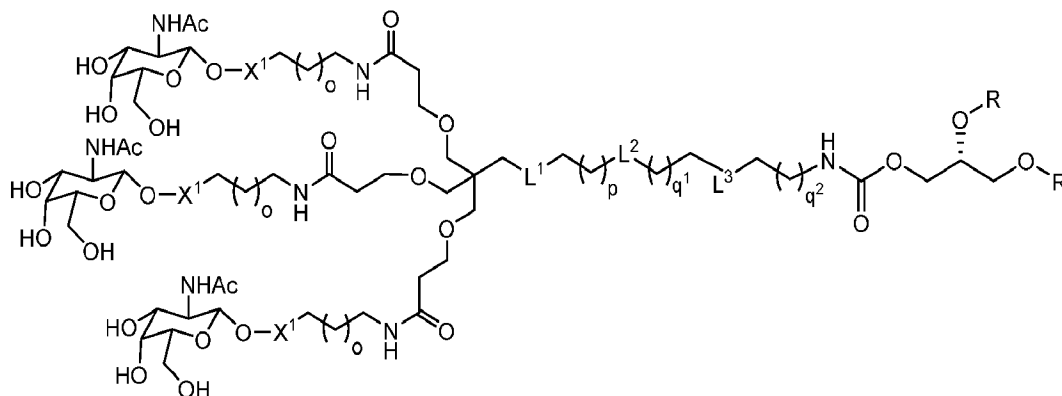
[0151] In some embodiments, the “” indicates an *R* configuration or an *S* configuration. In some embodiments, the “” indicates an *R* configuration. In some embodiments, the “” indicates an *S* configuration.

[0152] In some embodiments, the compound of Formula I is a compound of Formula I-b:



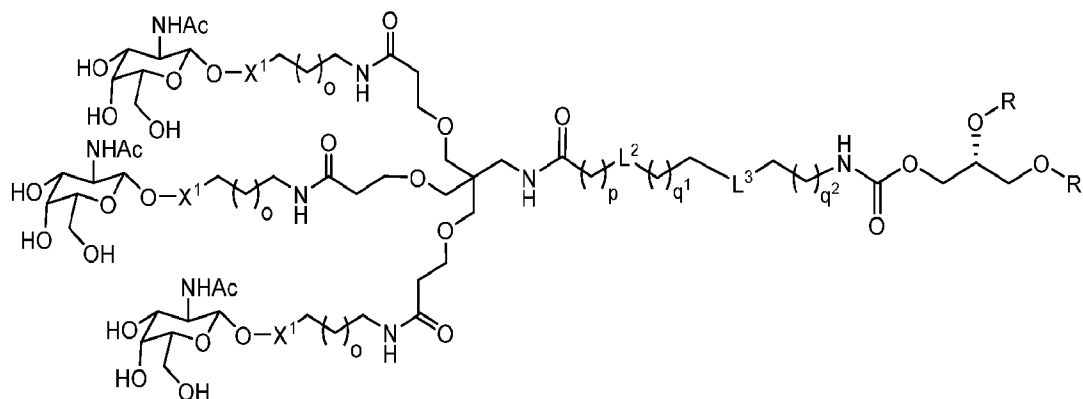
Formula I-b.

[0153] In some embodiments, the compound of Formula I is a compound of Formula I-c:

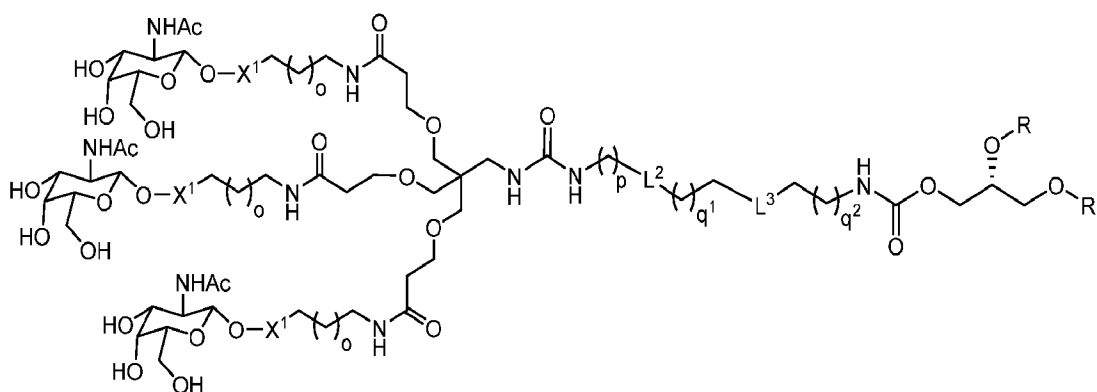


Formula I-c.

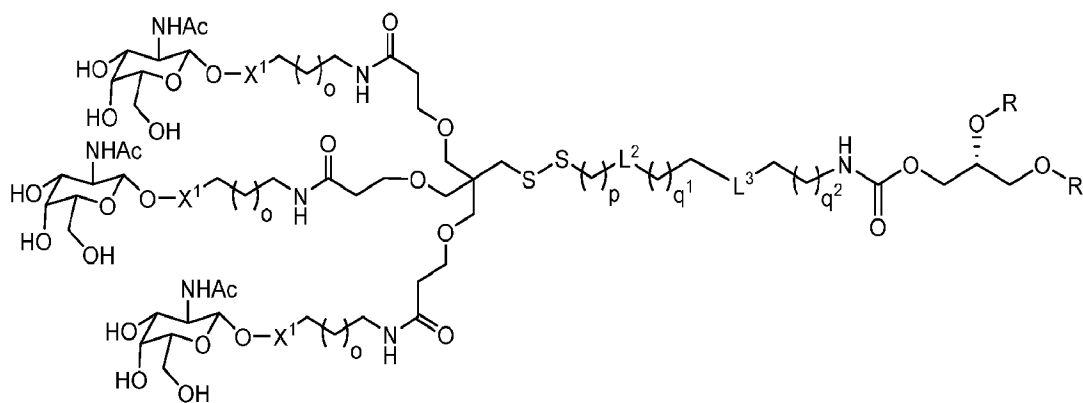
[0154] In some embodiments, the compound of Formula I is any one of a compound of Formula I-c-1:

**Formula I-c-1.**

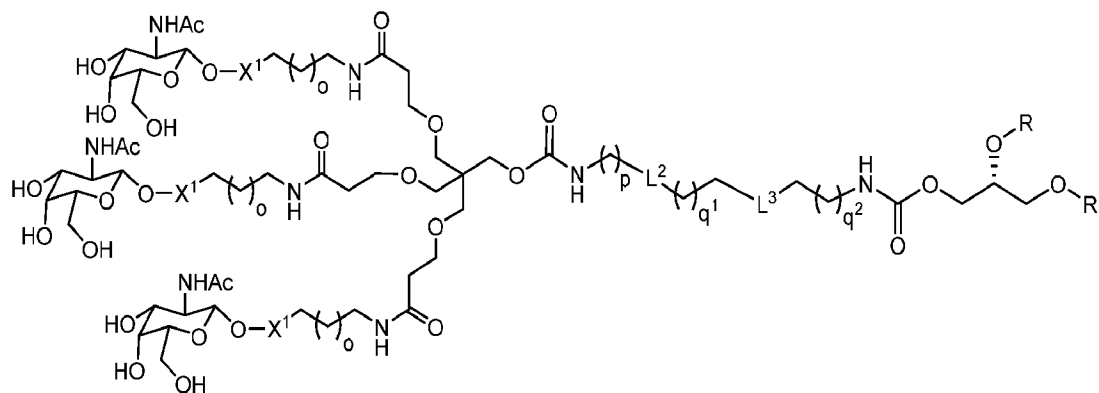
[0155] In some embodiments, the compound of Formula I is any one of a compound of Formula I-c-2:

**Formula I-c-2.**

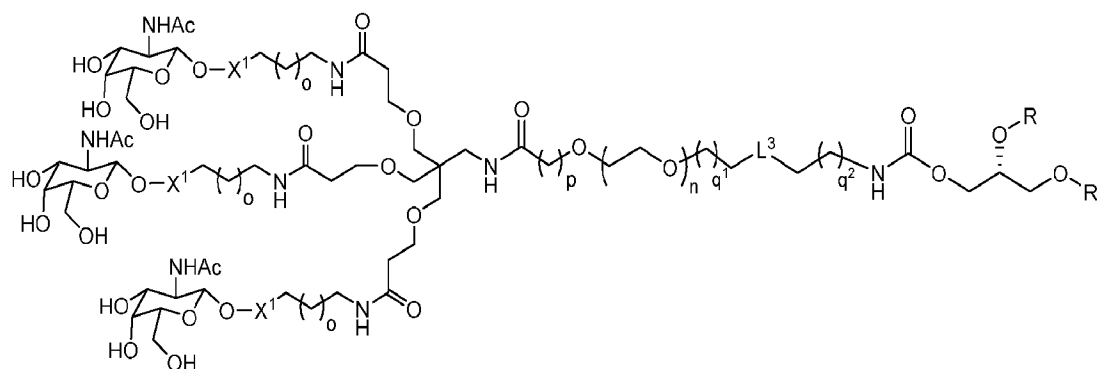
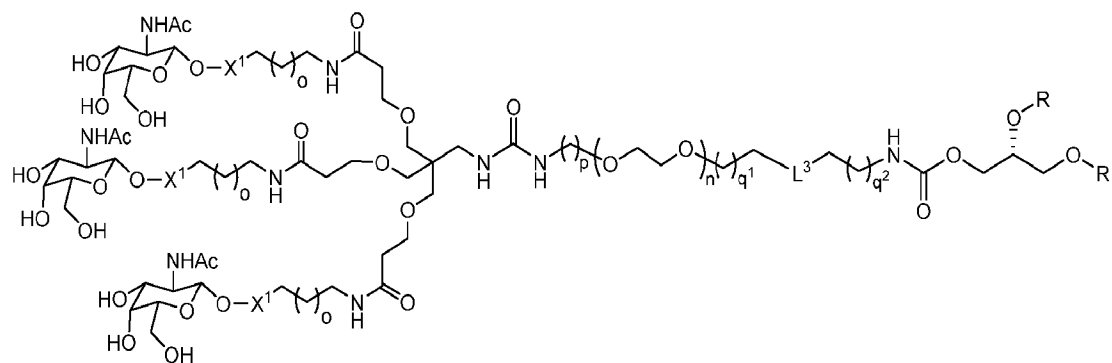
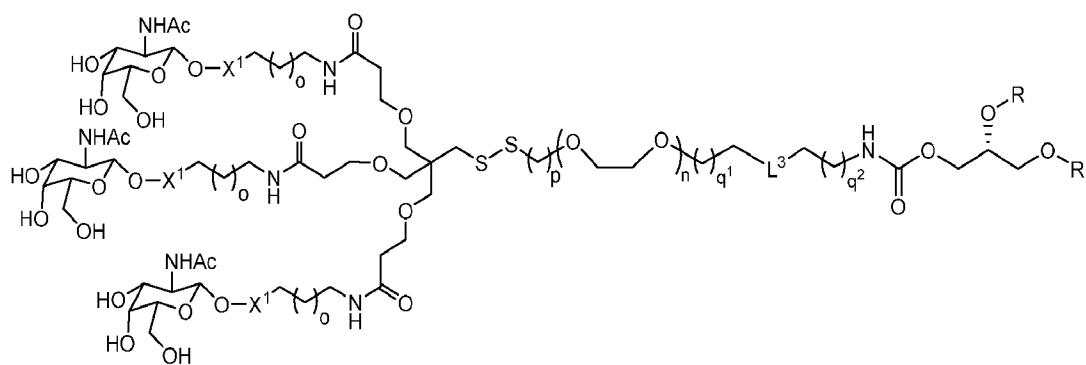
[0156] In some embodiments, the compound of Formula I is any one of a compound of Formula I-c-3:

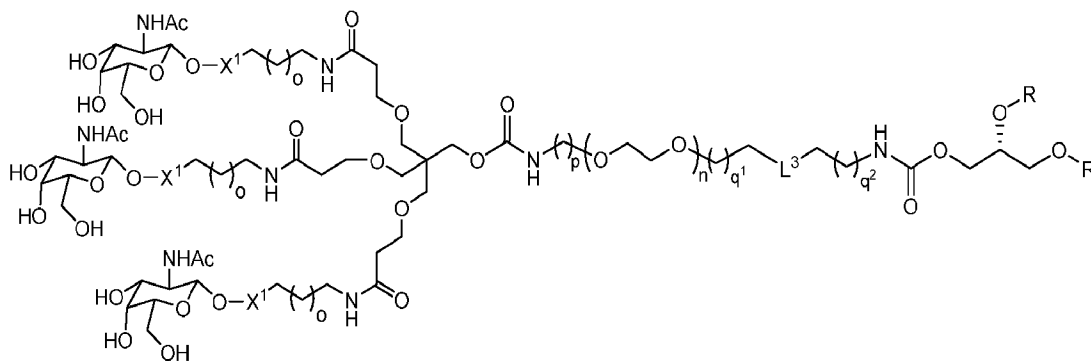
**Formula I-c-3.**

[0157] In some embodiments, the compound of Formula I is any one of a compound of Formula I-c-4:

**Formula I-c-4.**

[0158] In some embodiments, the disclosure provides a compound selected from the group consisting of Formula I-c-5, I-c-6, I-c-7, and I-c-8:

**Formula I-c-5,****Formula I-c-6,****Formula I-c-7,**

**Formula I-c-8**

wherein,

each X^1 is independently $-(CH_2)_r-$, $-(CH_2CH_2O)_s-$, $-(OCH_2CH_2)_s-$, $-((CH_2)_m-C(O)NH)-$, or $-(C(O)NH-(CH_2)_m)-$;

each subscript “m” is independently 1-5;

each subscript “s” is independently 1-5;

each subscript “o” is independently 1-5;

each subscript “r” is independently 1-10;

each subscript “p” is independently 1-5;

each subscript “n” is independently 1-200;

each subscript “q¹” is independently 0-5;

each subscript “q²” is independently 0-5;

each L^3 is independently absent, $-NHC(O)-$, or $-C(O)NH-$; and

each R is independently a C_{12} - C_{20} substituted or unsubstituted alkyl.

[0159] In some embodiments, each X^1 is independently $-(CH_2)_r-$, $-(CH_2CH_2O)_s-$, $-(OCH_2CH_2)_s-$, $-((CH_2)_m-C(O)NH)-$, or $-(C(O)NH-(CH_2)_m)-$. In some embodiments, each subscript “m” is independently 1-5. In some embodiments, each subscript “r” is independently 1-10. In some embodiments, each subscript “s” is independently 1-5. In some embodiments, each X^1 is independently $-(CH_2)_r-$. In some embodiments, each subscript “r” is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, each X^1 is independently $-(CH_2CH_2O)_s-$. In some embodiments, each X^1 is independently $-(OCH_2CH_2)_s-$. In some embodiments, each subscript “s” is independently 1, 2, 3, 4, or 5. In some embodiments, each X^1 is independently $-((CH_2)_m-C(O)NH)-$. In some embodiments, each X^1 is independently $-(C(O)NH-(CH_2)_m)-$. In some embodiments, each subscript “m” is independently 1, 2, 3, 4, or 5.

[0160] In some embodiments, each subscript “o” is independently 1, 2, 3, 4, or 5.

[0161] In some embodiments, each subscript “p” is independently 1, 2, 3, 4, or 5.

[0162] In some embodiments, each subscript “n” is independently 1-200. In some embodiments, each subscript “n” is independently 10-200. In some embodiments, each subscript “n” is independently 100-200. In some embodiments, each subscript “n” is independently 100. In some embodiments, each subscript “n” is independently 200.

[0163] In some embodiments, each subscript “q¹” is independently 0, 1, 2, 3, 4, or 5. In some embodiments, each subscript “q²” is independently 0, 1, 2, 3, 4, or 5. In some embodiments, each subscript “q³” is independently 0, 1, 2, 3, 4, or 5.

[0164] In some embodiments, each L³ is absent, –NHC(O)–, or –C(O)NH–. In some embodiments, each L³ is absent. In some embodiments, each L³ is –NHC(O)–. In some embodiments, each L³ is –C(O)NH–.

[0165] In some embodiments, each R is independently a C₁₂-C₂₀ substituted or unsubstituted alkyl. In some embodiments, each R is independently a C₁₂-C₂₀ substituted alkyl. In some embodiments, each R is independently a C₁₂-C₂₀ unsubstituted alkyl. In some embodiments, each R is independently a C₁₄-C₁₈ unsubstituted alkyl. In some embodiments, each R is independently a C₁₄ unsubstituted alkyl. In some embodiments, each R is independently a C₁₆ unsubstituted alkyl. In some embodiments, each R is independently a C₁₈ unsubstituted alkyl. In some embodiments, each R is a C₁₂ alkyl, C₁₄ alkyl, C₁₅ alkyl, C₁₆ alkyl, C₁₇ alkyl, C₁₈ alkyl, C₁₉ alkyl, or C₂₀ alkyl. In some embodiments, each R is n-dodecanyl, n-tridecanyl, n-tetradecanyl, or n-pentadecanyl.

[0166] In some embodiments, each X¹ is –((CH₂)_m–C(O)NH)–, each subscript “m” is independently 2, and each subscript “o” is independently 1.

[0167] In some embodiments, each X¹ is –(CH₂CH₂O)_s–, each subscript “s” is independently 2, and each subscript “o” is independently 1.

[0168] In some embodiments, the compound is a compound of Formula I-c-7 and, optionally, each X¹ is –(CH₂)_r–, each subscript “r” is independently 5, and each subscript “o” is 1. In some embodiments, the compound is a compound of Formula I-c-7 and each X¹ is –(CH₂)_r–, each subscript “r” is independently 5, and each subscript “o” is 1.

[0169] In some embodiments, the compound is a compound of Formula I-c-5 and L² is –O–(CH₂CH₂O)_n–, and subscript “n” is 100 to 200.

[0170] In some embodiments, the compound is a compound of Formula I-c-6, I-c-7, or I-c-8, and each L² is –(OCH₂CH₂O)_n– and each subscript “n” is 100 to 200.

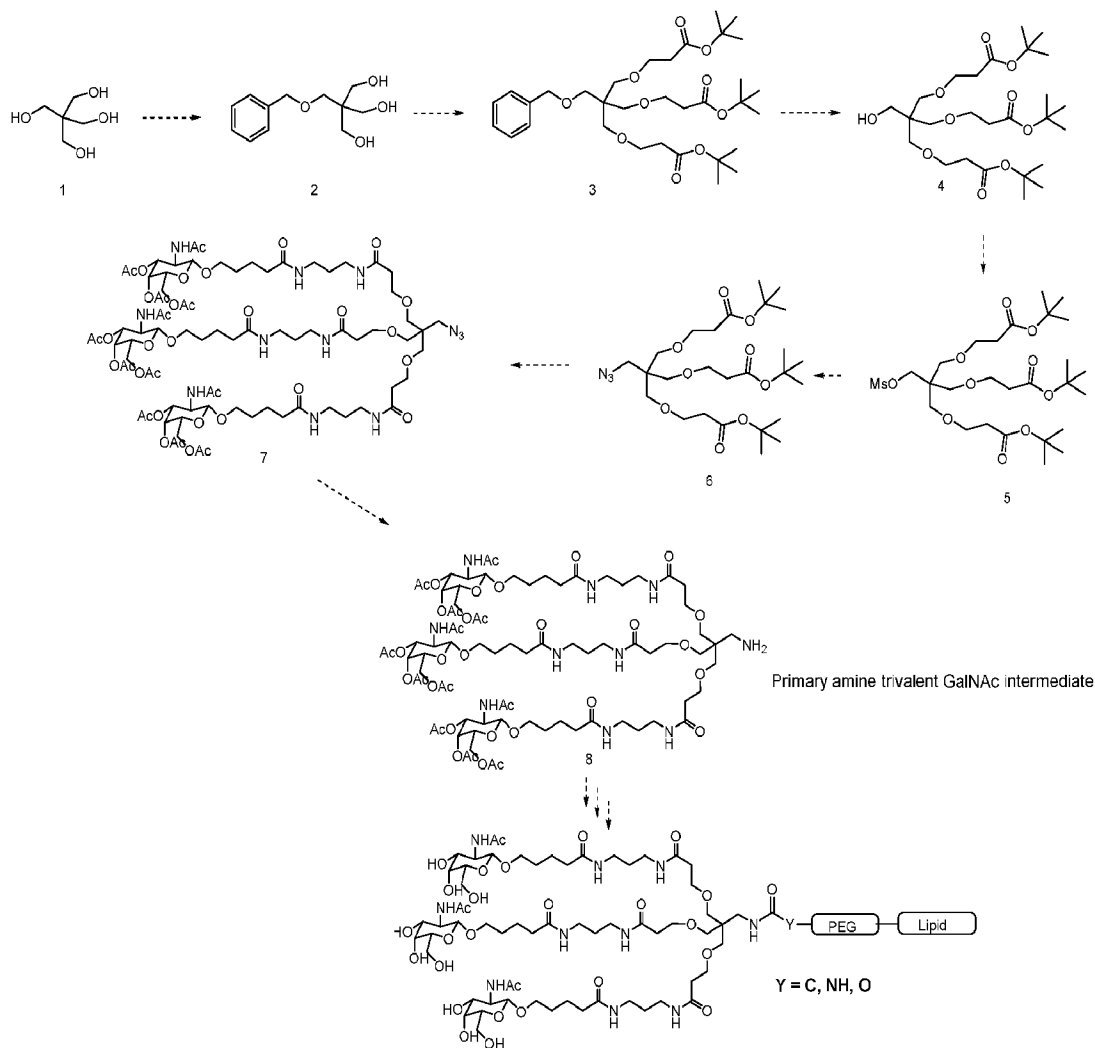
[0171] In some embodiments, in Formula I-c-4, subscript “p” is 4, subscript “q¹” is 0, and subscript “q²” is 0.

[0172] In some embodiments, in Formulas I-c-6, I-c-7, and I-c-8, each subscript “p” is 2, subscript “q¹” is 0, and subscript “q²” is 0.

[0173] In some embodiments, in Formula I-a-4, subscript “p” is 4, subscript “q¹” is 1, and subscript “q²” is 4.

[0174] (ii) *General Synthetic Schemes of Formula I*

[0175] Compounds of Formula I of the present disclosure can be synthesized according to the following general synthetic.



Scheme 4

[0176] Scheme 4 shows a general synthetic scheme of 2,2-Bis(hydroxymethyl)propane-1,3-diol (1) based GalNAc PEG lipids.

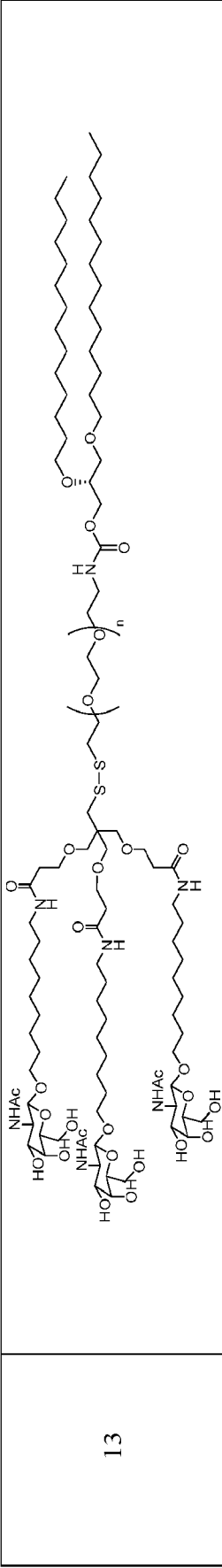
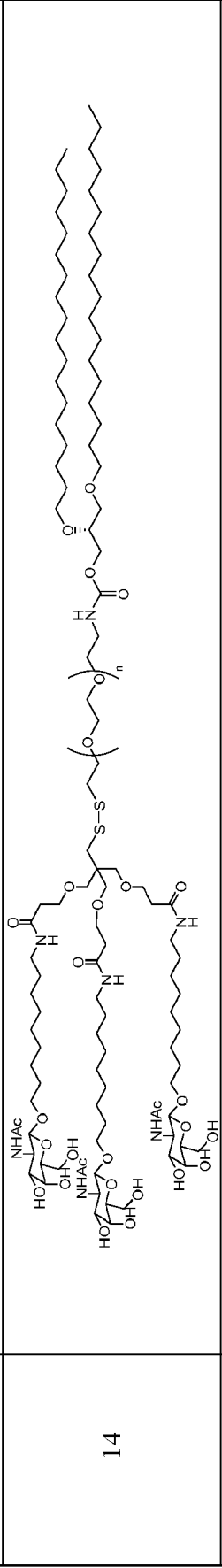
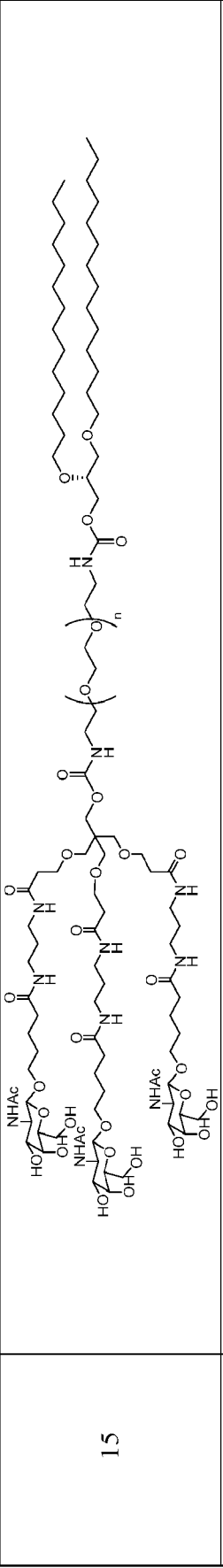
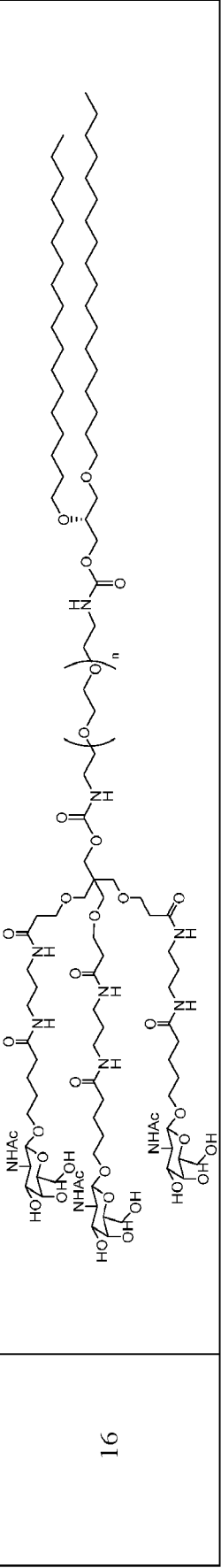
[0177] The abovementioned synthetic scheme can be used to synthesize the compounds shown in Table 1.

[0178] **Table 1. Representative Compounds of Formula I.**

Structure	
1	
2	
3	
4	

5	
6	
7	
8	

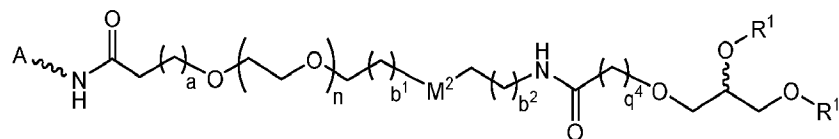
9	
10	
11	
12	

13	
14	
15	
16	

wherein each subscript “n” is between 10-200.

[0179] (iii) *Compounds of Formula II*

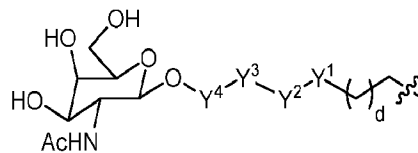
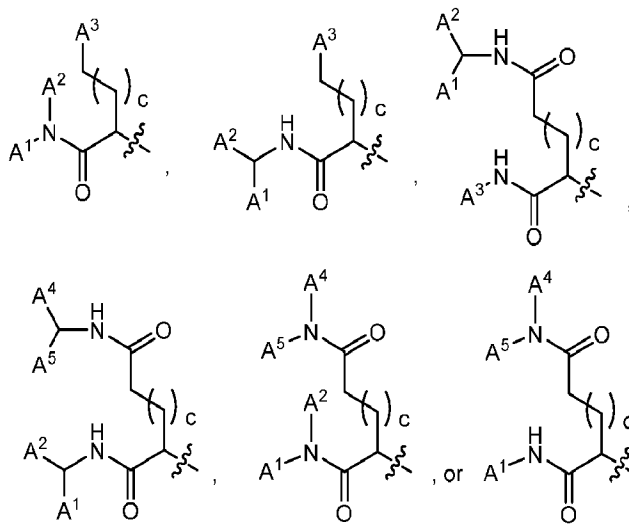
[0180] Another aspect of the present disclosure provides compounds of Formula II:



Formula II

wherein:

A is:



each A^1 , A^2 , A^3 , A^4 , and A^5 is independently

each Y^1 is independently $-\text{NH}-$, $-\text{NHC}(\text{O})\text{O}-$, $-\text{OC}(\text{O})\text{NH}-$, $-\text{NHC}(\text{O})-$, or $-\text{C}(\text{O})\text{NH}-$;

$\text{C}(\text{O})\text{NH}-$;

each Y^2 is independently absent, $-(\text{CH}_2)_t-$, $-(\text{CH}_2\text{CH}_2\text{O})_t-$, or $-(\text{OCH}_2\text{CH}_2)_t-$;

each subscript “t” is independently 1-5;

each Y^3 is independently absent, $-\text{NHC}(\text{O})-$, or $-\text{C}(\text{O})\text{NH}-$;

each Y^4 is independently absent or $-(\text{CH}_2)_t-$;

each subscript “r” is independently 1-10;

each subscript “d” is independently 0-5;

each subscript “c” is independently 0-5;

subscript “n” is independently 1-200;

subscript “a” is 1-5;

subscript “b¹” is independently 0-5;

M^2 is absent, $-\text{NHC}(\text{O})-$, or $-\text{C}(\text{O})\text{NH}-$;

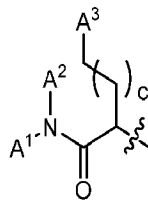
subscript “b²” is independently 0-5;

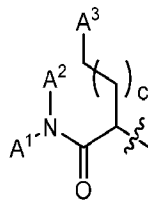
subscript “q⁴” is independently 0-6;

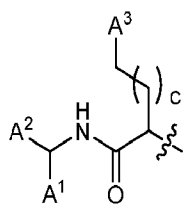
each R¹ is independently a C₁₂-C₂₀ substituted or unsubstituted alkyl; and

each of the “” bonds independently indicate an *R* configuration or an *S* configuration;

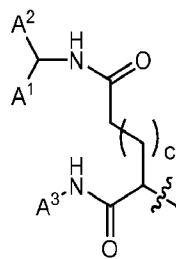
with the proviso that at least one of Y², Y³ and Y⁴ is present.



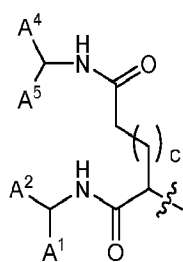
[0181] In some embodiments, A is: . In some embodiments, A is:



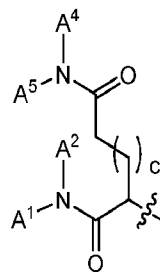
. In some embodiments, A is:



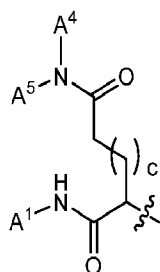
. In some embodiments, A is:



. In some embodiments, A is:

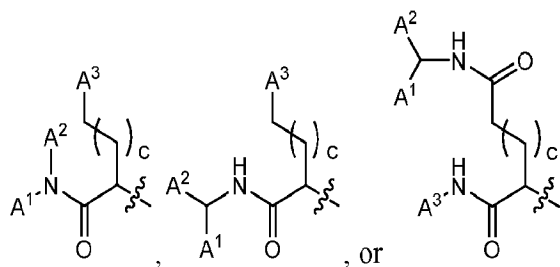


. In some embodiments, A is:



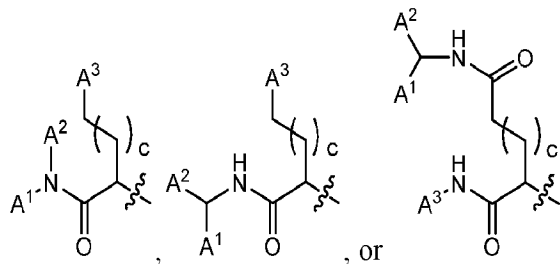
[0182] In some embodiments, each subscript “c” is independently 0, 1, 2, 3, 4, or 5. In some embodiments, each subscript “c” is independently 0. In some embodiments, each subscript “c” is independently 1.

[0183] In some embodiments, A¹ and A² are the same. In some embodiments, A¹ and A² are different. In some embodiments, A¹, A², and A³ are the same. In some embodiments, A¹, A², and A³ are different. In some embodiments, A¹, A², A⁴, and A⁵ are the same. In some embodiments, A¹, A², A⁴, and A⁵ are different. In some embodiments, A¹, A⁴, and A⁵ are the same. In some embodiments, A¹, A⁴, and A⁵ are different.



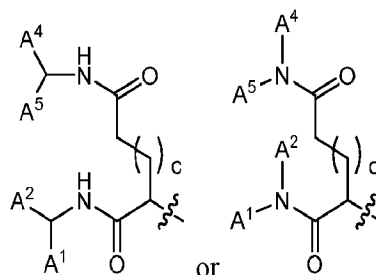
[0184] In some embodiments, when A is:

A¹, A², and A³ are the same.



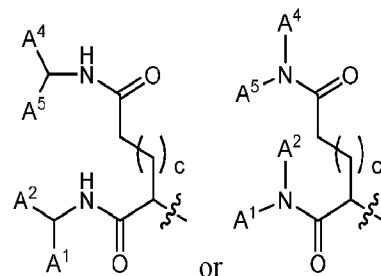
[0185] In some embodiments, when A is:

A¹ and A² are the same and both different from A³.



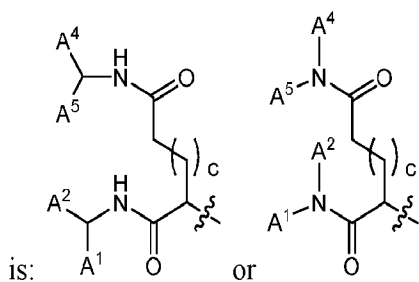
[0186] In some embodiments, when A is:

A¹, A², A⁴, and A⁵ are the same.

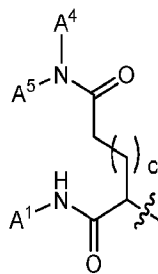


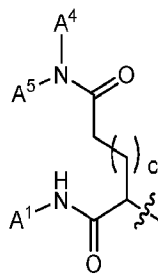
A⁵ are the same. In some embodiments, when A is:

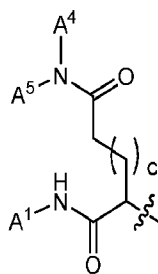
A¹, A², and A⁴ are the same and A⁵ is different from A¹, A², and A⁴.

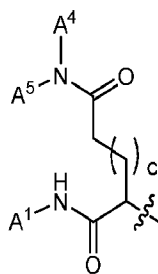


is: A¹, A², and A⁵ are the same and A⁴ is different from A¹, A², and A⁵.

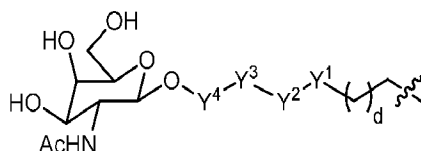


[0187] In some embodiments, when A is: , A¹, A⁴, and A⁵ are the same. In



some embodiments, when A is: , A⁴ and A⁵ are the same and A¹ is different.

[0188] In some embodiments, each A¹, A², A³, A⁴, and A⁵ is independently



. In some embodiments, each subscript “d” is independently 0, 1, 2, 3, 4, or 5.

[0189] In some embodiments, each Y¹ is independently –NH–, –NHC(O)O–, –OC(O)NH–, –NHC(O)–, or –C(O)NH–. In some embodiments, each Y¹ is independently –NH–. In some embodiments, each Y¹ is independently –NHC(O)O–. In some embodiments, each Y¹ is independently –OC(O)NH–. In some embodiments, each Y¹ is independently –NHC(O)–. In some embodiments, each Y¹ is independently –C(O)NH–.

[0190] In some embodiments, each Y² is independently absent, –(CH₂)_r–, –(CH₂CH₂O)_t–, or –(OCH₂CH₂)_t–. In some embodiments, each Y² is independently absent. In some embodiments, each Y² is independently –(CH₂)_r–. In some embodiments, each Y² is independently –(CH₂)_r– and each subscript “r” is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, each Y² is independently –(CH₂CH₂O)_t–. In some embodiments, each Y² is independently –(CH₂CH₂O)_t– and each subscript “t” is independently 1, 2, 3, 4, or 5. In some embodiments, each Y² is independently –(OCH₂CH₂)_t– and each subscript “t” is independently 1, 2, 3, 4, or 5.

[0191] In some embodiments, each Y³ is independently absent, –NHC(O)–, or –C(O)NH–. In some embodiments, each Y³ is independently absent. In some embodiments, each Y³ is independently –NHC(O)–. In some embodiments, each Y³ is independently –C(O)NH–.

[0192] In some embodiments, each Y⁴ is independently absent or –(CH₂)_r–. In some embodiments, each Y⁴ is independently absent. In some embodiments, each Y⁴ is

independently $-(CH_2)_r-$. In some embodiments, each Y^4 is independently $-(CH_2)_r-$ and each subscript “r” is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[0193] In some embodiments, each subscript “n” is independently 1-200, 10-200, 10-100, 10-50, 10-40, 10-30, 10-20, or 100-200. In some embodiments, each subscript “n” is independently 10-200. In some embodiments, each subscript “n” is independently 100-200. In some embodiments, each subscript “n” is independently 10, 20, 30, 40, or 50. In some embodiments, the quantity of each subscript “n” depends on the molecular weight average of the moiety, such that the PEG-like moiety results in a molecular weight average of about 100-2000 Daltons (Da), about 1000-2000 Da, about 1000 Da, or about 2000 Da.

[0194] In some embodiments, subscript “a” is independently 1, 2, 3, 4, or 5.

[0195] In some embodiments, subscript “b¹” is independently 0, 1, 2, 3, 4, or 5. In some embodiments, subscript “b²” is independently 0, 1, 2, 3, 4, or 5.

[0196] In some embodiments, M^2 is absent, $-NHC(O)-$, or $-C(O)NH-$. In some embodiments, M^2 is absent. In some embodiments, M^2 is $-NHC(O)-$. In some embodiments, M^2 is $-C(O)NH-$.

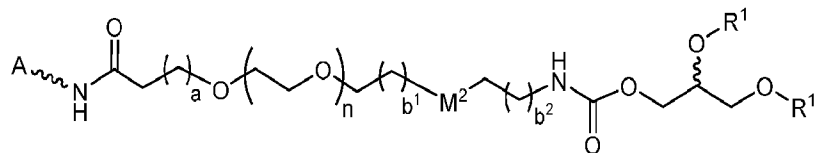
[0197] In some embodiments, subscript “q⁴” is independently 0, 1, 2, 3, 4, 5, or 6.

[0198] In some embodiments, each R^1 is independently a C_{12} - C_{20} substituted or unsubstituted alkyl. In some embodiments, each R^1 is independently a C_{12} - C_{20} substituted alkyl. In some embodiments, each R^1 is independently a C_{12} - C_{20} unsubstituted alkyl. In some embodiments, each R^1 is independently a C_{14} - C_{18} unsubstituted alkyl. In some embodiments, each R^1 is independently a C_{14} unsubstituted alkyl. In some embodiments, each R^1 is independently a C_{16} unsubstituted alkyl. In some embodiments, each R^1 is independently a C_{18} unsubstituted alkyl. In some embodiments, each R^1 is a C_{12} alkyl, C_{14} alkyl, C_{15} alkyl, C_{16} alkyl, C_{17} alkyl, C_{18} alkyl, C_{19} alkyl, or C_{20} alkyl. In some embodiments, each R^1 is n-dodecanyl, n-tridecanyl, n-tetradecanyl, or n-pentadecanyl.

[0199] In some embodiments, in Formula II, each of the “” bonds independently indicate an *R* configuration or an *S* configuration. In some embodiments, in Formula II, each of the “” bonds independently indicate an *R* configuration. In some embodiments, in Formula II, each of the “” bonds independently indicate an *S* configuration. In some embodiments, in Formula II, the “” bond next to the “A” moiety indicates an *S* configuration and the other “” bond indicates an *R* configuration. In some embodiments, in Formula II, the “” bond next to the “A” moiety indicates an *R* configuration and the other “” bond indicates an *R* configuration. In some embodiments, in Formula II, the “

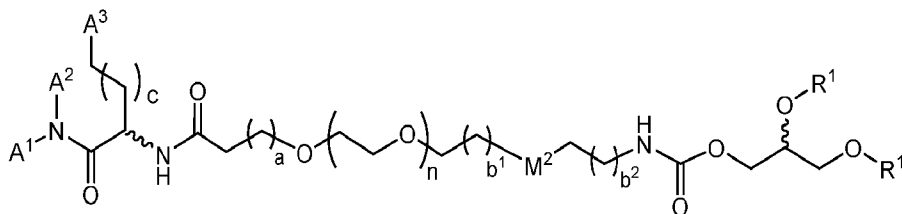
~~~~~” bond next to the “A” moiety indicates an *S* configuration and the other “~~~~~” bond indicates an *S* configuration.

[0200] In some embodiments, the compound of Formula II is a compound of Formula II-a:



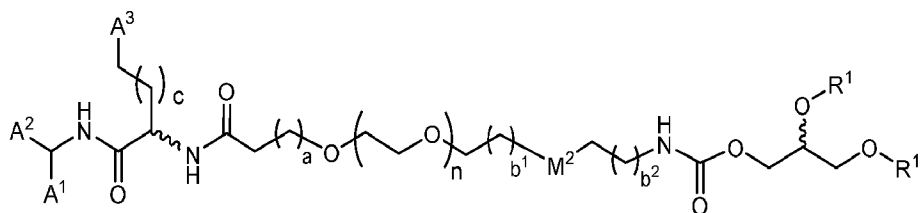
**Formula II-a.**

[0201] In some embodiments, the compound of Formula II is a compound of Formula II-a-1:



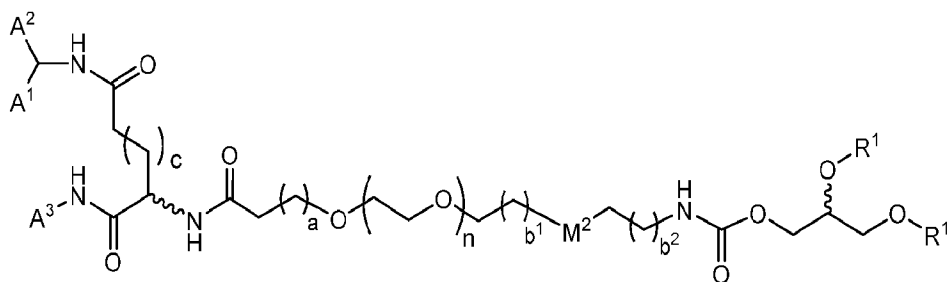
**Formula II-a-1.**

[0202] In some embodiments, the compound of Formula II is a compound of Formula II-a-2:



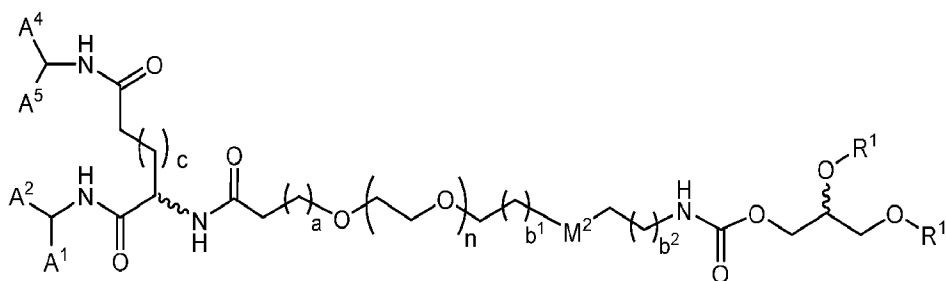
**Formula II-a-2.**

[0203] In some embodiments, the compound of Formula II is a compound of Formula II-a-3:

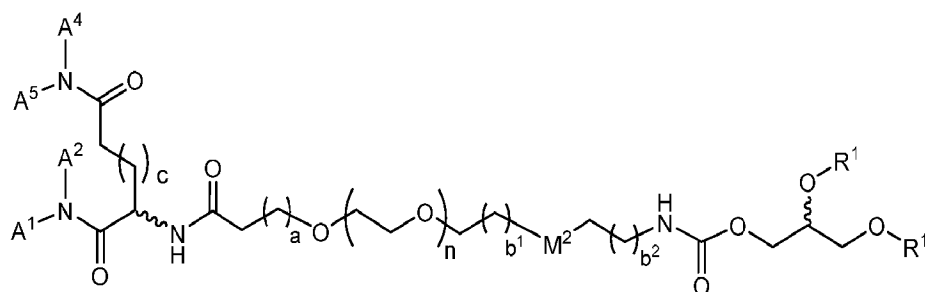


**Formula II-a-3.**

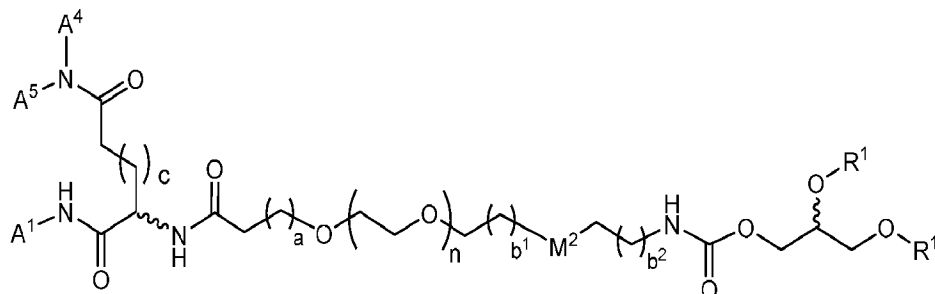
[0204] In some embodiments, the compound of Formula II is a compound of Formula II-a-4:

**Formula II-a-4.**

[0205] In some embodiments, the compound of Formula II is a compound of Formula II-a-5:

**Formula II-a-5.**

[0206] In some embodiments, the compound of Formula II is a compound of Formula II-a-6:

**Formula II-a-6.**

[0207] In some embodiments, in the compound of Formula II, Formula II-a, Formula II-a-1, Formula II-a-2, or Formula II-a-3, A<sup>1</sup>, A<sup>2</sup>, and A<sup>3</sup> are the same. In some embodiments, in the compound of Formula II, Formula II-a, Formula II-a-1, Formula II-a-2, or Formula II-a-3, A<sup>1</sup> and A<sup>2</sup> are different from A<sup>3</sup>.

[0208] In some embodiments, in the compound of Formula II, Formula II-a, Formula II-a-4, or Formula II-a-5, A<sup>1</sup>, A<sup>2</sup>, A<sup>4</sup>, and A<sup>5</sup> are the same. In some embodiments, in the compound of Formula II, Formula II-a, Formula II-a-4, or Formula II-a-5, A<sup>1</sup>, A<sup>2</sup>, and A<sup>4</sup> are the same. In some embodiments, in the compound of Formula II, Formula II-a, Formula II-a-4, or Formula II-a-5, A<sup>1</sup>, A<sup>2</sup>, and A<sup>4</sup> are the same and A<sup>5</sup> is different from A<sup>1</sup>, A<sup>2</sup>, and A<sup>4</sup>. In some

embodiments, in the compound of Formula II, Formula II-a, Formula II-a-4, or Formula II-a-5,  $A^1$ ,  $A^2$ , and  $A^5$  are the same. In some embodiments, in the compound of Formula II, Formula II-a, Formula II-a-4, or Formula II-a-5,  $A^1$ ,  $A^2$ , and  $A^5$  are the same and  $A^4$  is different from  $A^1$ ,  $A^2$ , and  $A^5$ .

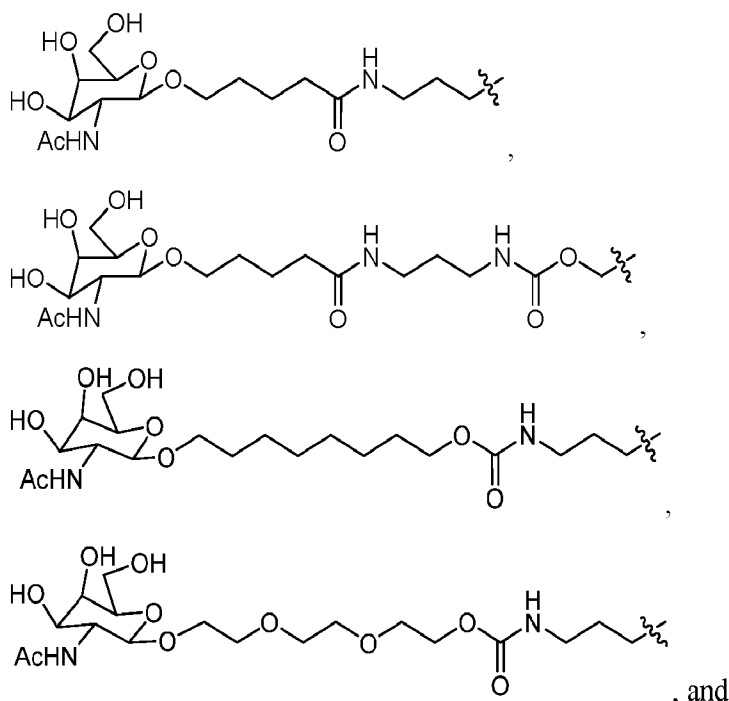
[0209] In some embodiments, in the compound of Formula II, Formula II-a, or Formula II-a-6,  $A^1$ ,  $A^4$ , and  $A^5$  are the same. In some embodiments, in the compound of Formula II, Formula II-a, or Formula II-a-6  $A^4$  and  $A^5$  are the same and  $A^1$  is different from  $A^4$  and  $A^5$ .

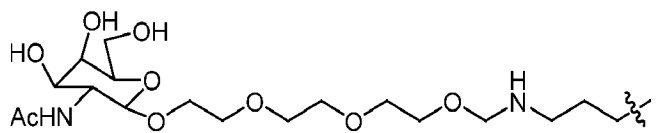
[0210] In some embodiments, each of  $Y^1$  and  $Y^4$  are present and each of  $Y^2$  and  $Y^3$  are absent. In some embodiments, each  $Y^1$  is  $-C(O)NH-$  and each  $Y^4$  is  $-(CH_2)_r-$ . In some embodiments, each  $Y^1$  is  $-OC(O)NH-$  and each  $Y^4$  is  $-(CH_2)_r-$ . In some embodiments, each subscript “r” is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[0211] In some embodiments, each of  $Y^1$ ,  $Y^2$ ,  $Y^3$ , and  $Y^4$  are present. In some embodiments, each  $Y^1$  is independently  $-NHC(O)O-$ ; each  $Y^2$  is independently  $-(CH_2)_r-$ ; each  $Y^3$  is independently  $-C(O)NH-$ ; and each  $Y^4$  is independently  $-(CH_2)_r-$ . In some embodiments, each subscript “r” is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[0212] In some embodiments, each of  $Y^1$  and  $Y^2$  are present and each of  $Y^3$  and  $Y^4$  are absent. In some embodiments, each  $Y^1$  is independently  $-OC(O)NH-$  and each  $Y^2$  is independently  $-(CH_2CH_2O)_r-$ . In some embodiments, each subscript “r” is independently 1, 2, 3, 4, or 5.

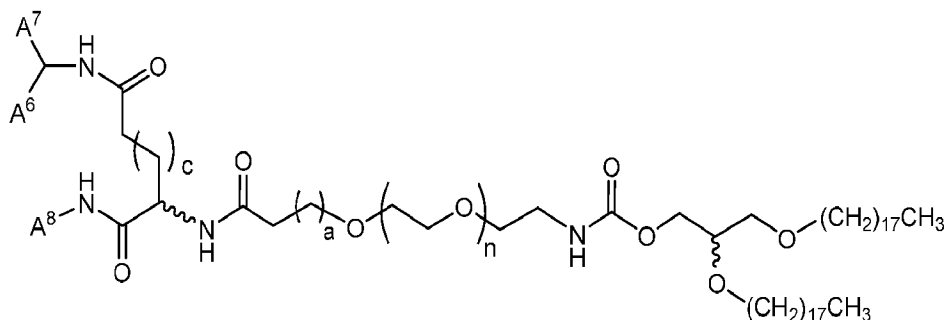
[0213] In some embodiments, each of  $A^1$ ,  $A^2$ ,  $A^3$ ,  $A^4$ , and  $A^5$  is selected from the group consisting of:



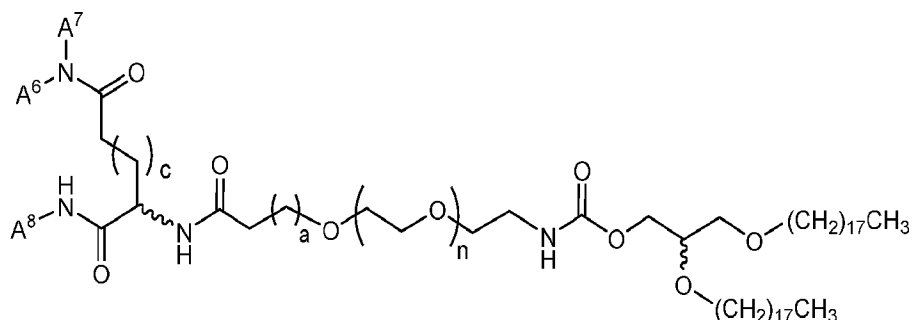


**[0214]** (iii.A) *Compounds of Formula III or Formula IV*

**[0215]** Another aspect of the disclosure provides a compound of Formula III or Formula IV:

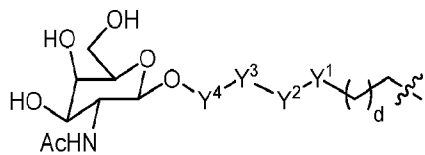


**Formula III, or**



### Formula IV

wherein,



each  $A^6$ ,  $A^7$ , and  $A^8$  is independently

each Y<sup>1</sup> is independently absent, -NHC(O)O-, or -OC(O)NH-;

each  $Y^2$  and  $Y^4$  is independently  $-(CH_2)_r$ ;

each subscript “r” is independently 1-10;

each Y<sup>3</sup> is independently absent, -NHC(O)-, or -C(O)NH-;

each subscript “d” is independently 1-5;

each subscript “c” is independently 1-5;

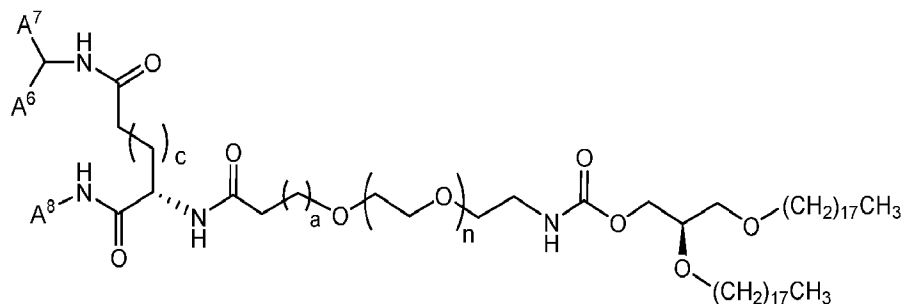
subscript “a” is 1-5;

subscript “n” is independently 35-46; and

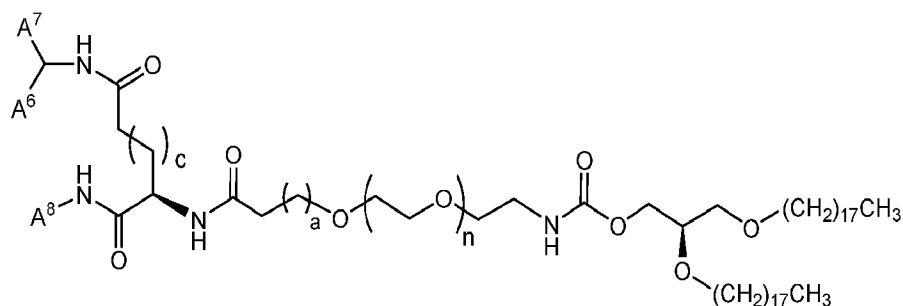
each of the “” bonds independently indicate an *R* configuration or an *S* configuration;

with the proviso that at least one of Y<sup>1</sup> or Y<sup>3</sup> is present.

[0216] In some embodiments, the compound of Formula III is a compound of Formula III-a or Formula III-b:

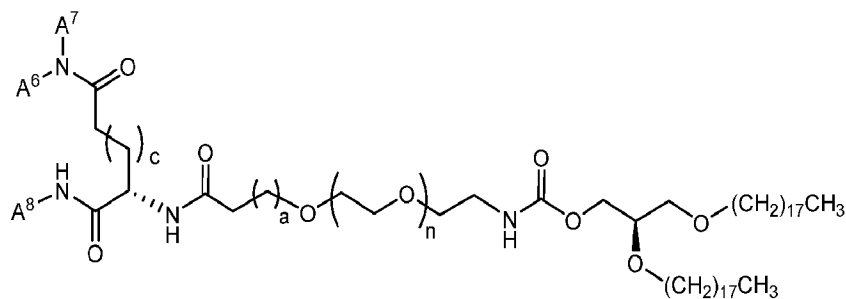


**Formula III-a, or**

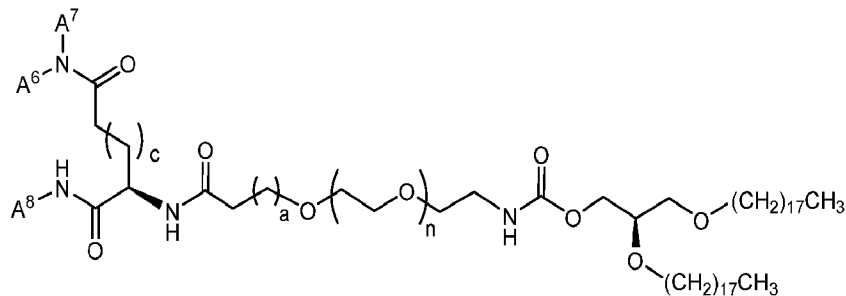


**Formula III-b.**

[0217] In some embodiments, the compound of Formula IV is a compound of Formula IV-a or Formula IV-b:



**Formula IV-a, or**



**Formula IV-b.**

[0218] In some embodiments, each of the variables,  $Y^1$ ,  $Y^2$ ,  $Y^3$ ,  $Y^4$ , subscript “a”, “c”, “d” and “n” have values as described above for Formula II.

[0219] In some embodiments, each of the variables  $A^6$ ,  $A^7$ , and  $A^8$  variables are as described above for  $A^1$  through  $A^5$  for Formula II.

[0220] In some embodiments,  $A^6$ ,  $A^7$ , and  $A^8$  are the same. In some embodiments,  $A^6$ ,  $A^7$ , and  $A^8$  are each different. In some embodiments,  $A^6$  and  $A^7$  are different. In some embodiments,  $A^6$  and  $A^7$  are different.

[0221] In some embodiments, subscript “a” is 3. In some embodiments, subscript “a” is 1.

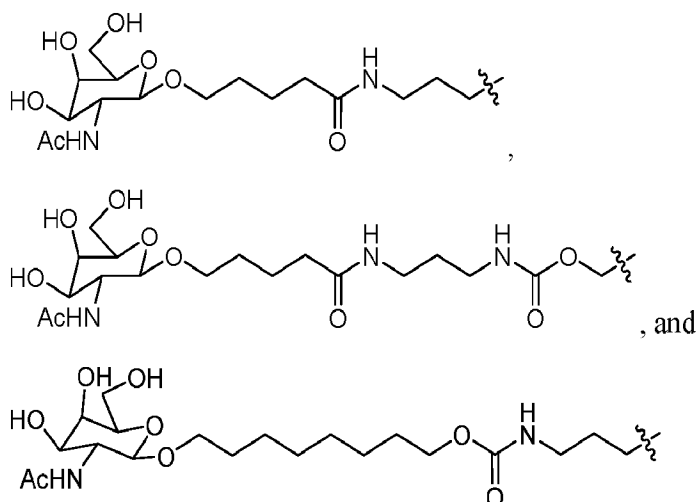
[0222] In some embodiments, subscript “n” is independently between 34-46 or in a range of 34-46.

[0223] In some embodiments, subscript “n” is independently between 34-36 or in a range of 34-36. In some embodiments, subscript “n” is independently 34, 35, or 36.

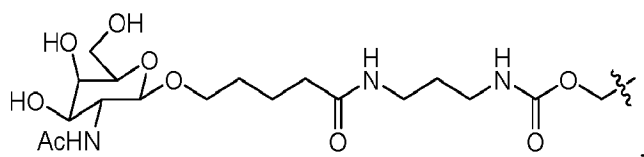
[0224] In some embodiments, subscript “n” is independently between 44-46 or in a range of 44-46. In some embodiments, subscript “n” is independently 44, 45, or 46.

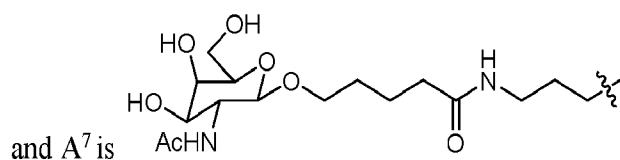
[0225] In some embodiments, subscript “c” is 1.

[0226] In some embodiments, each of  $A^6$ ,  $A^7$ , and  $A^8$  is selected from the group consisting of:

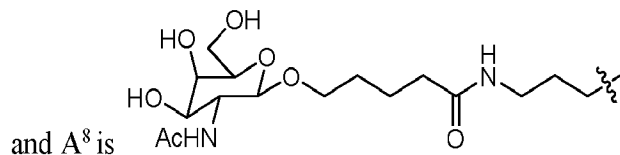
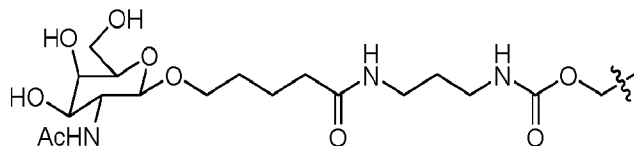


[0227] In some embodiments,  $A^6$  and  $A^8$  are each

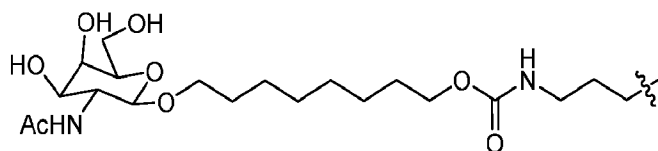




[0228] In some embodiments, A<sup>6</sup> and A<sup>7</sup> are each independently

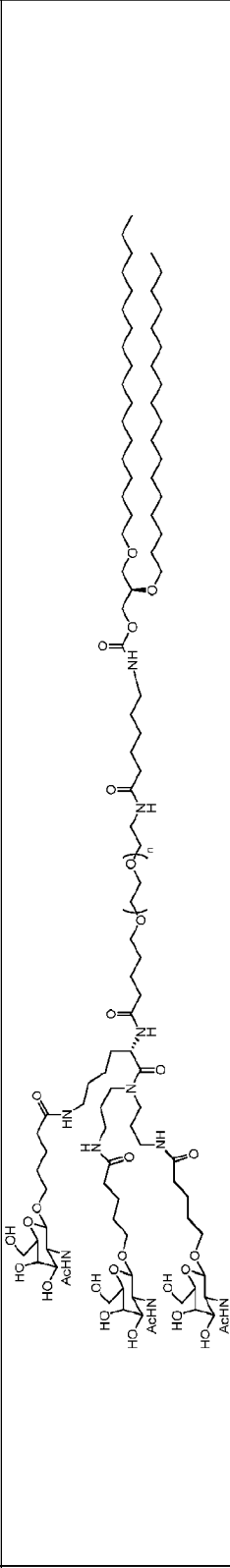
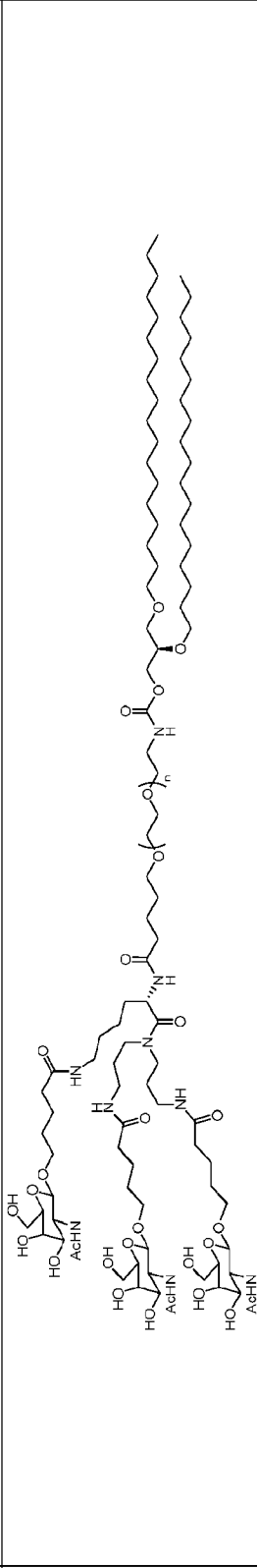
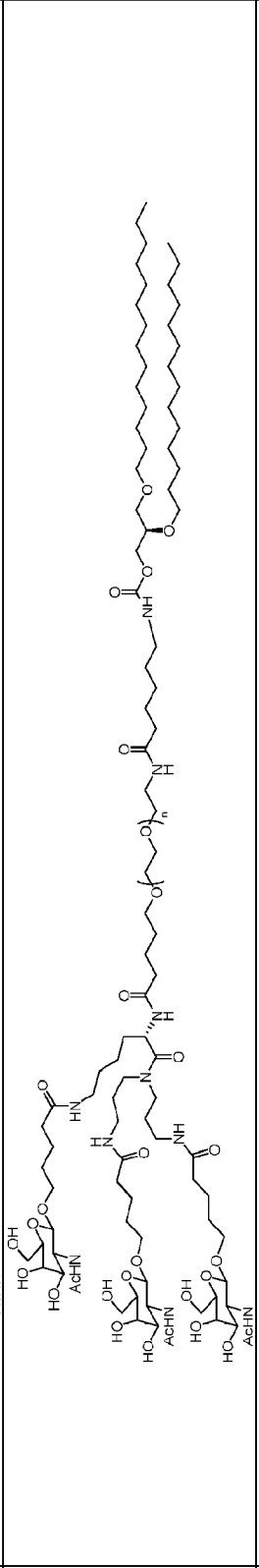
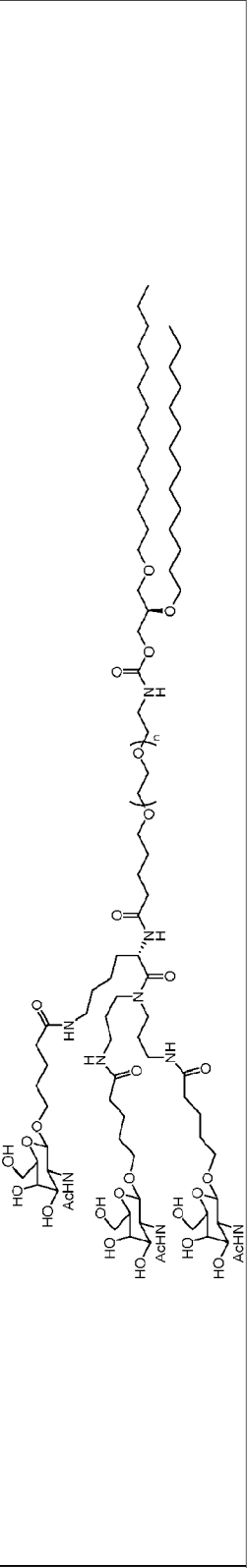


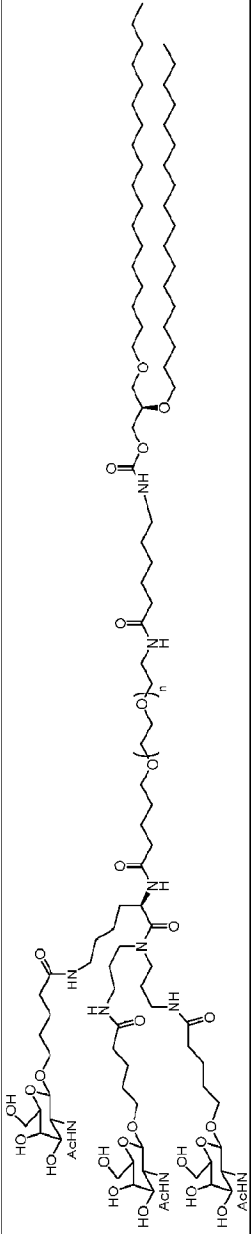
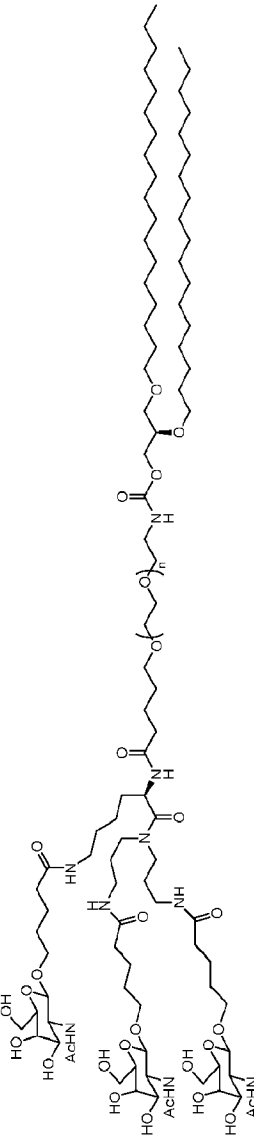
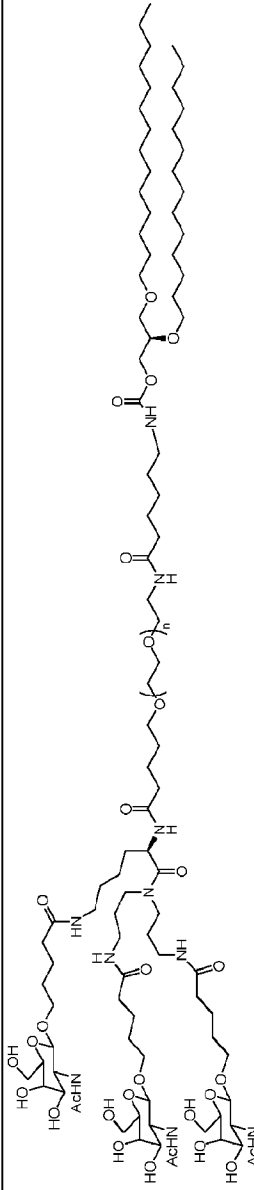
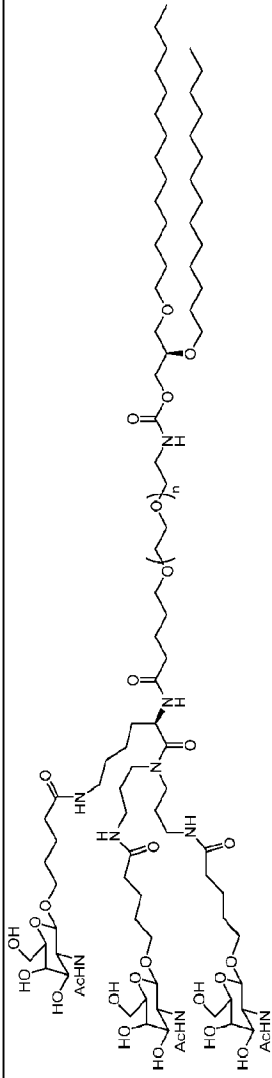
[0229] In some embodiments, A<sup>6</sup>, A<sup>7</sup>, and A<sup>8</sup> are each independently

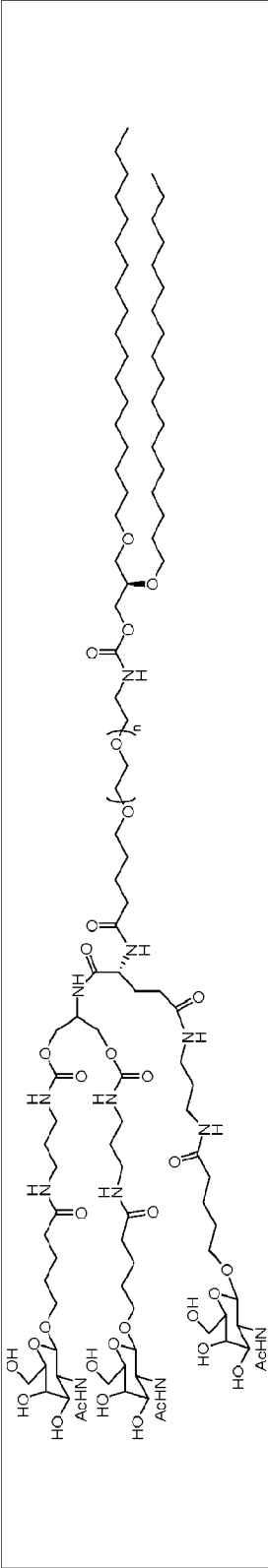
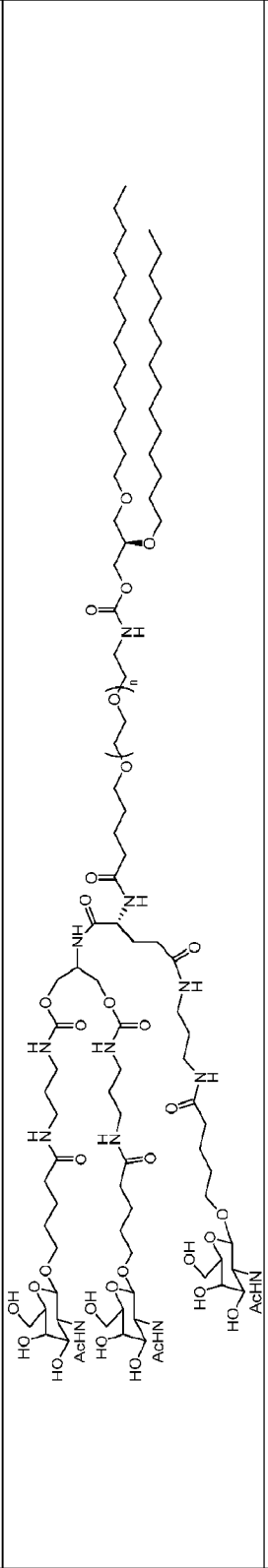
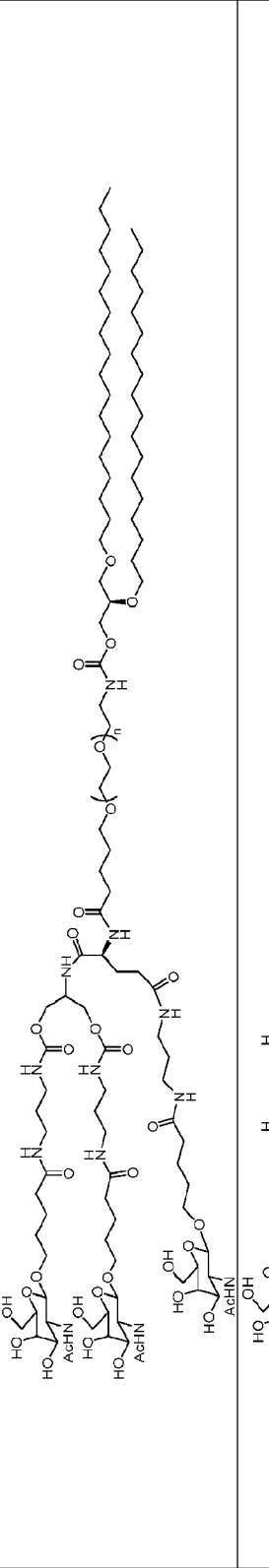
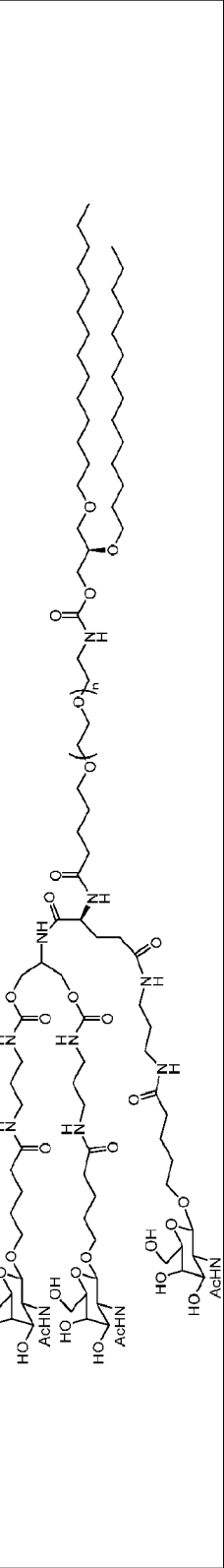


[0230] Representative compounds of Formula II, Formula III, or Formula IV are shown in Table 2.

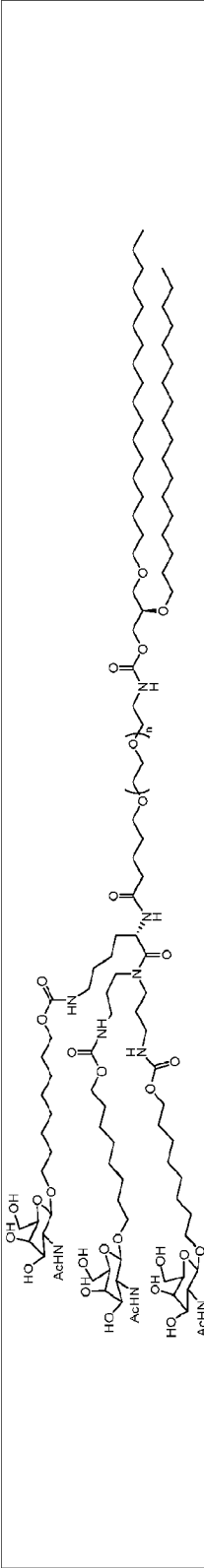
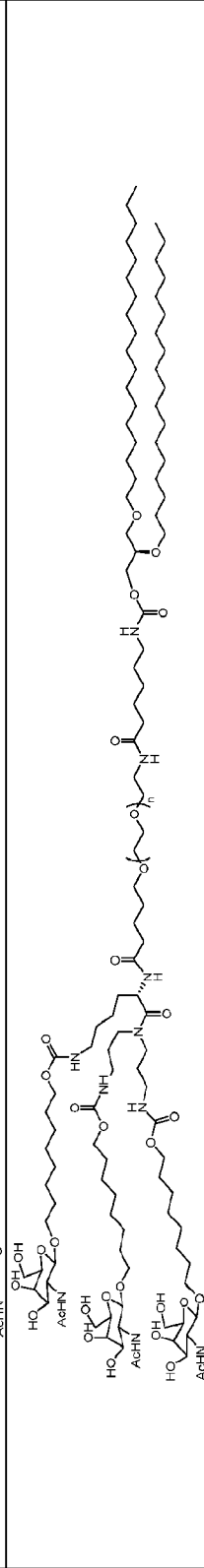
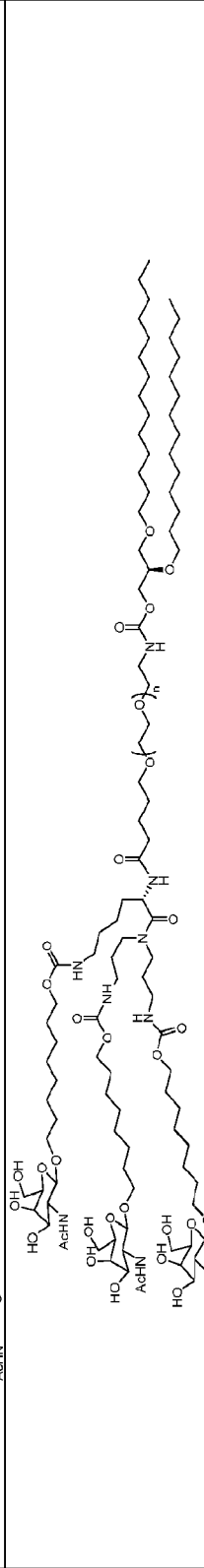
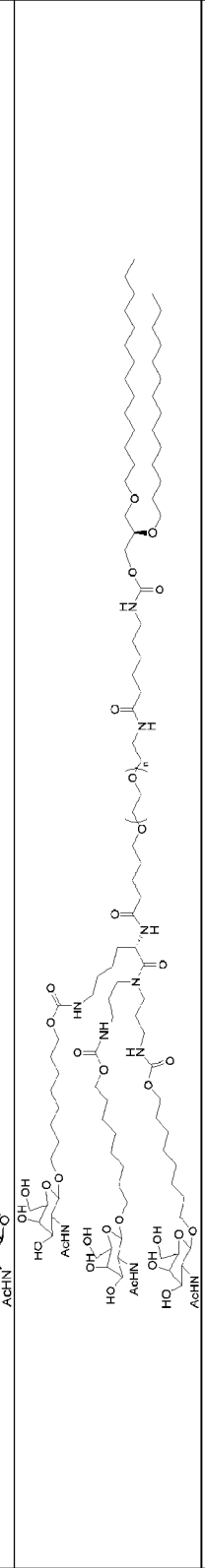
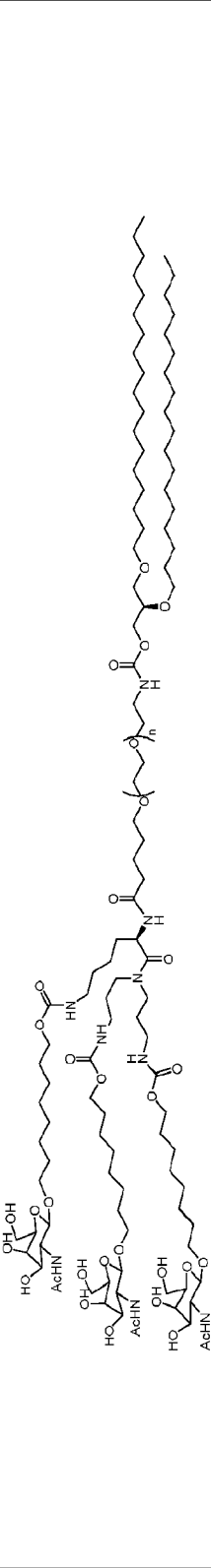
[0231] **Table 2. Representative Compounds of Formula II, Formula III, or Formula IV.**

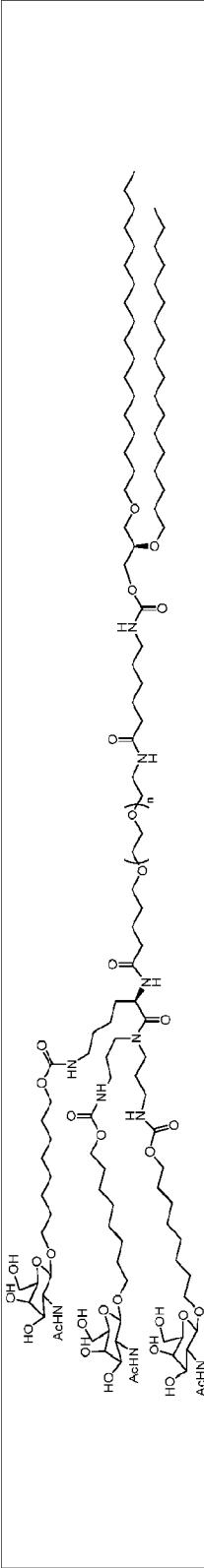
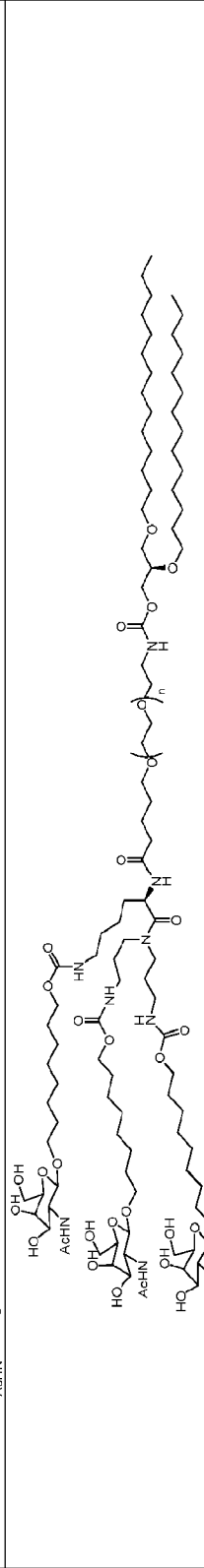
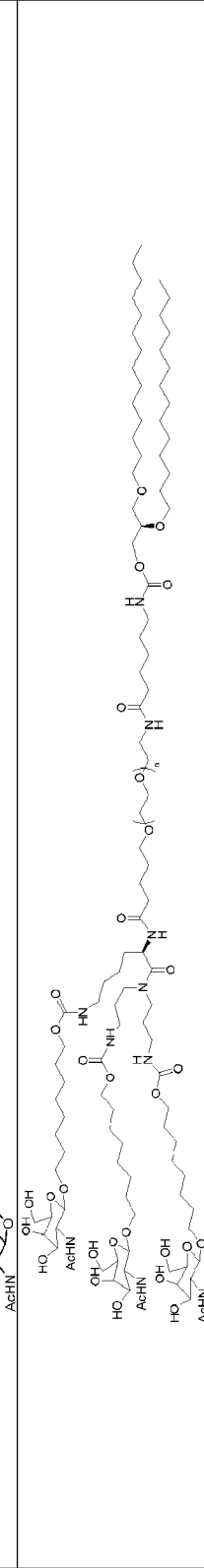
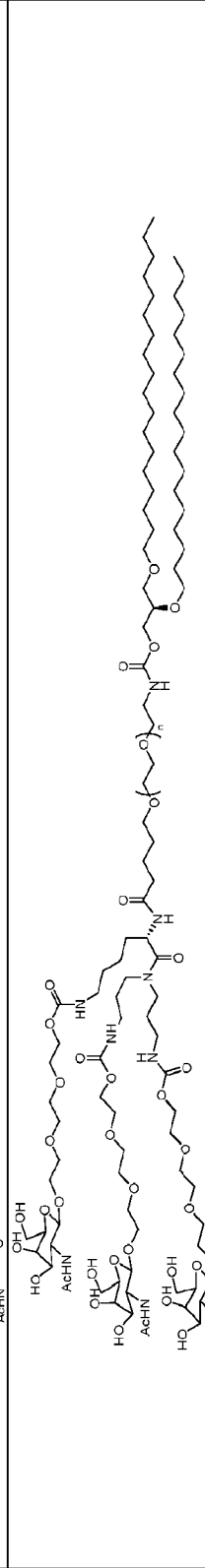
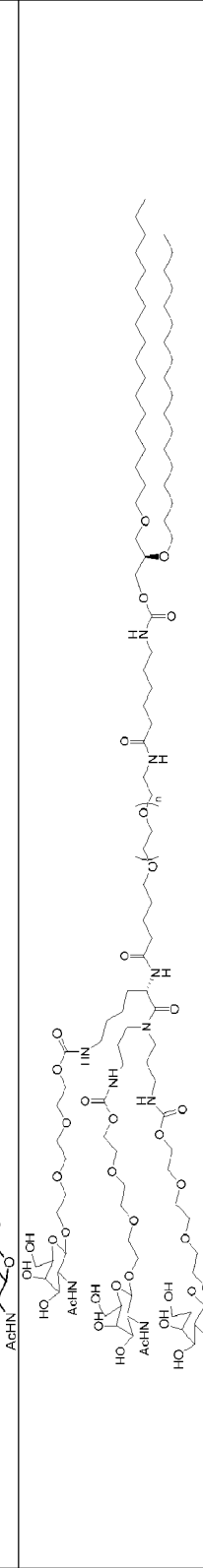
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| 20           |  |

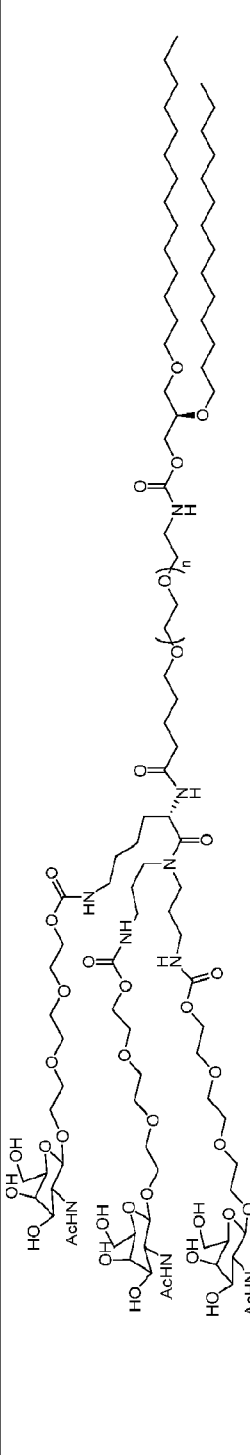
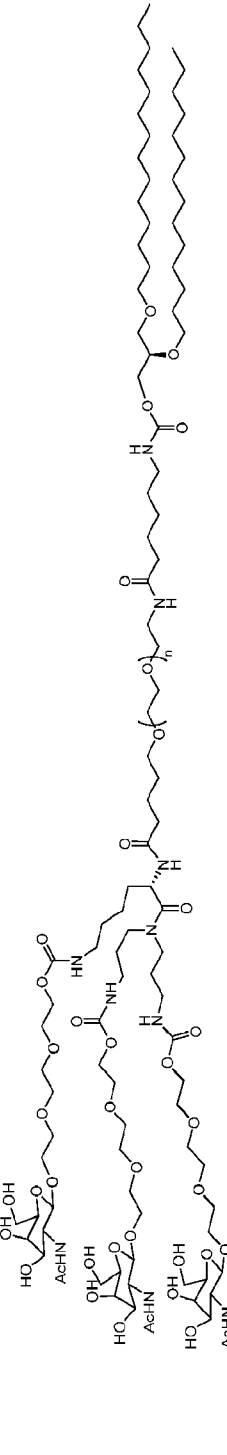
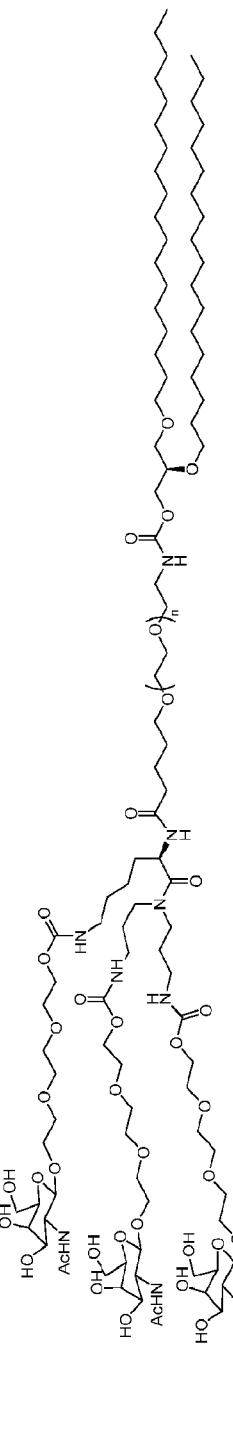
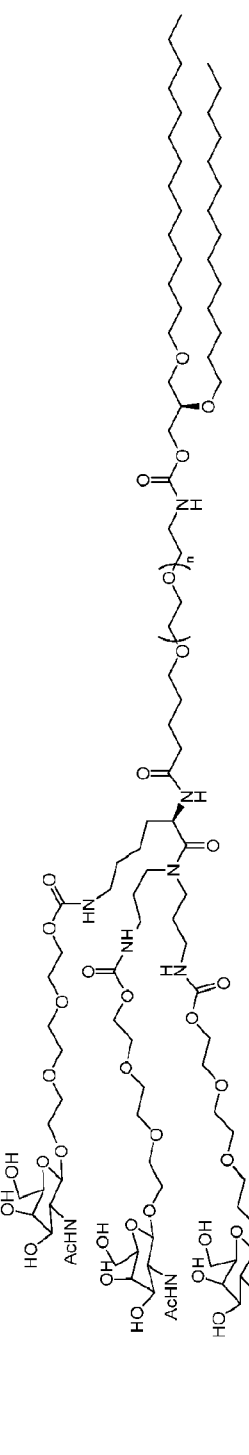
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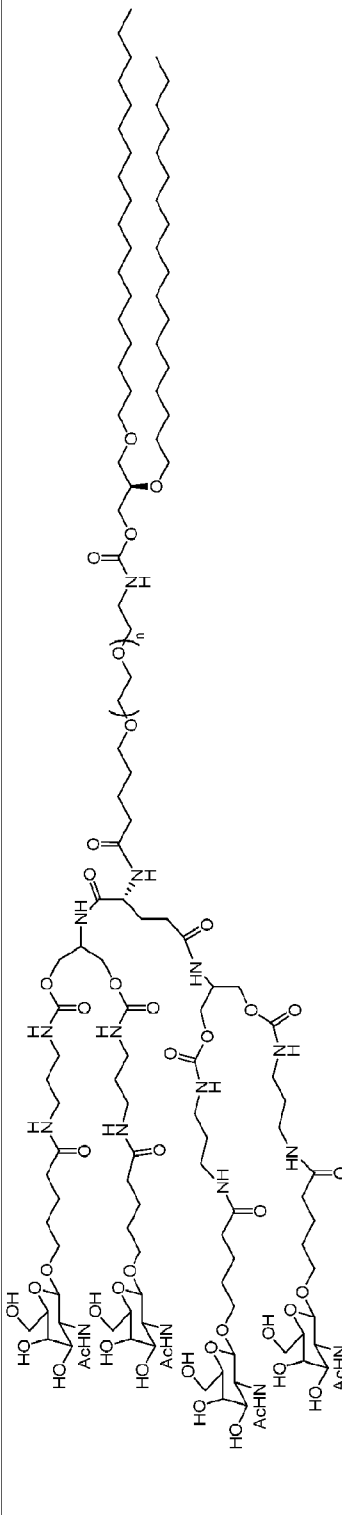
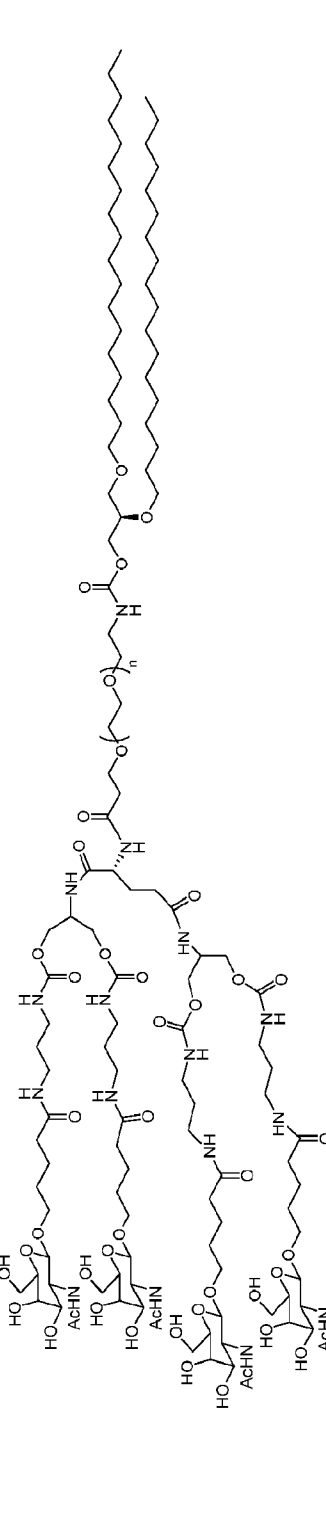
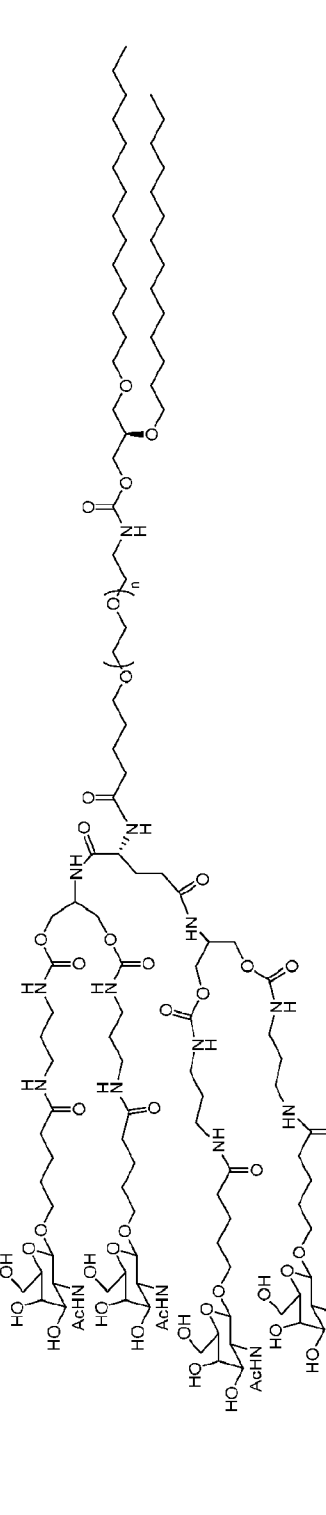
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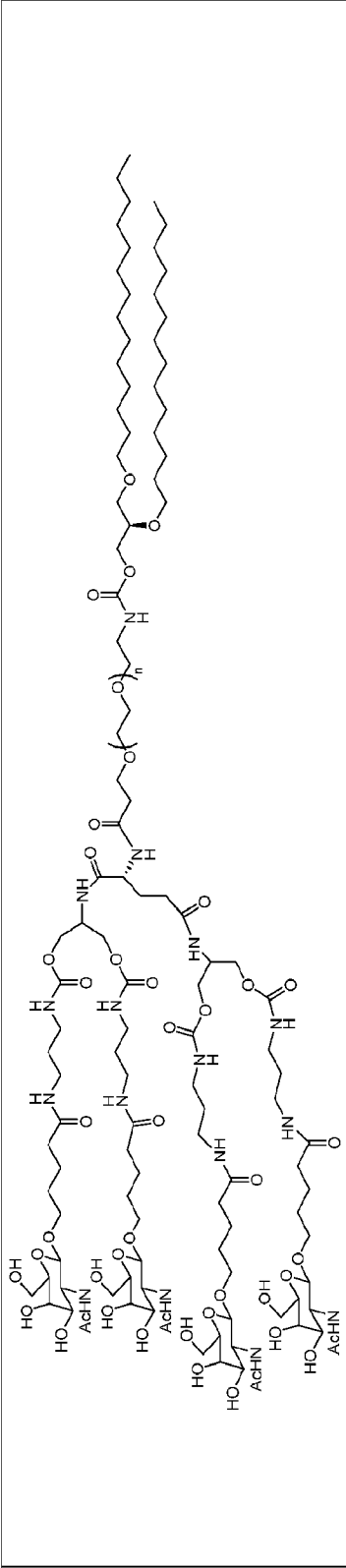
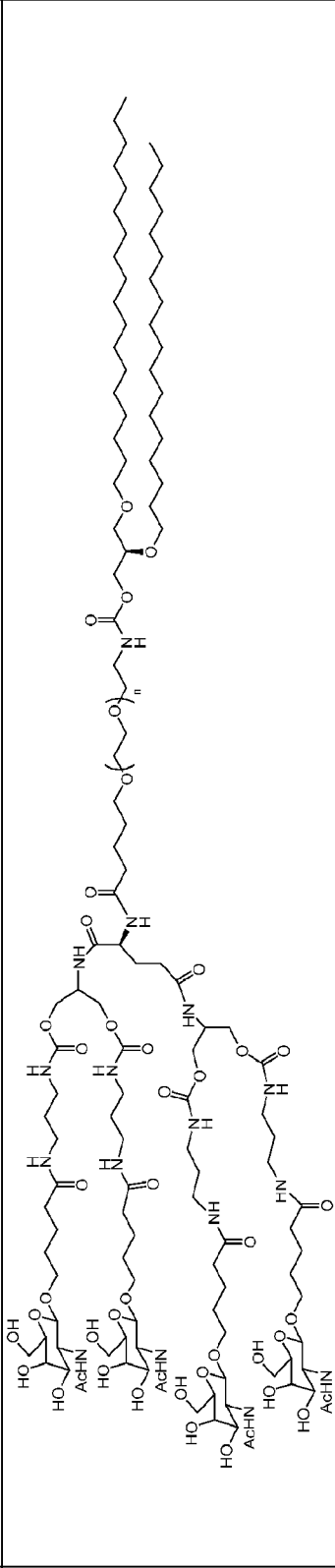
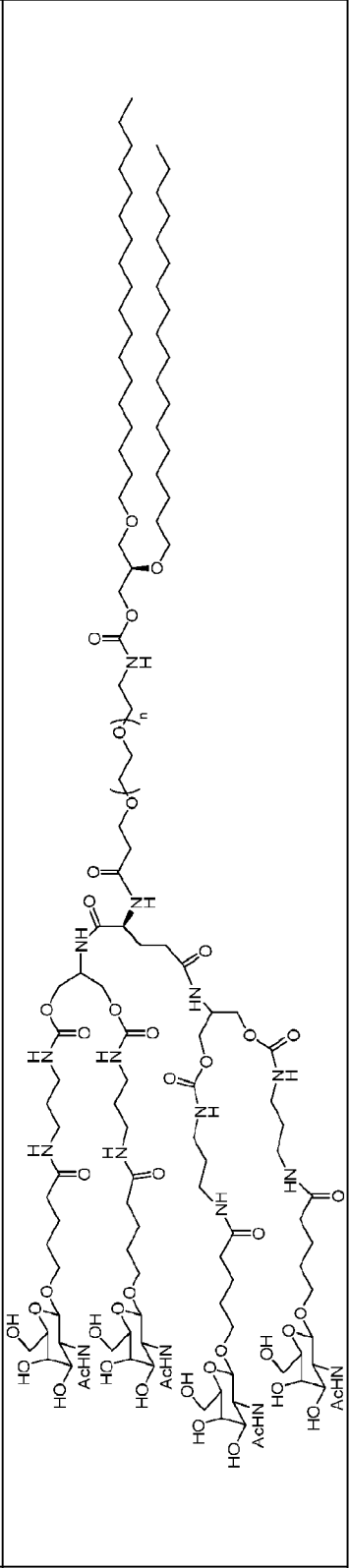
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| 32 |  |

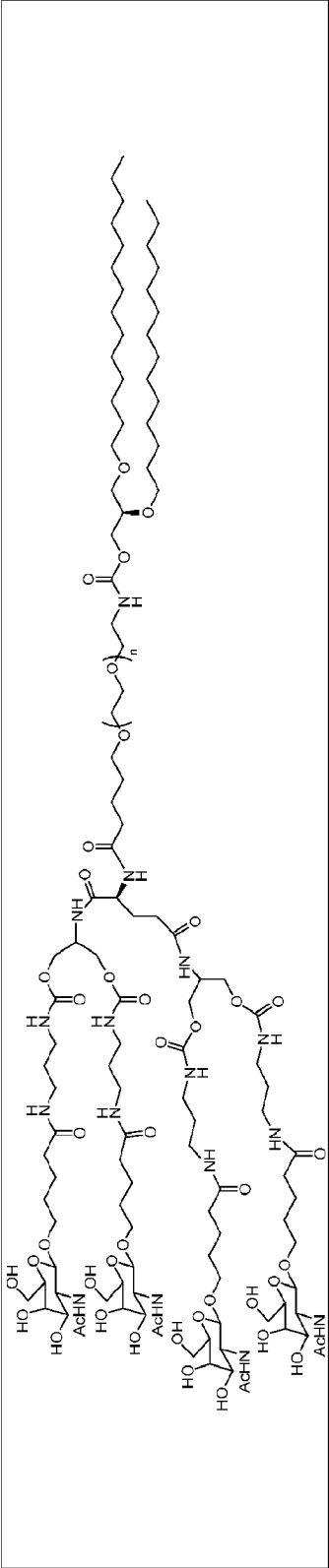
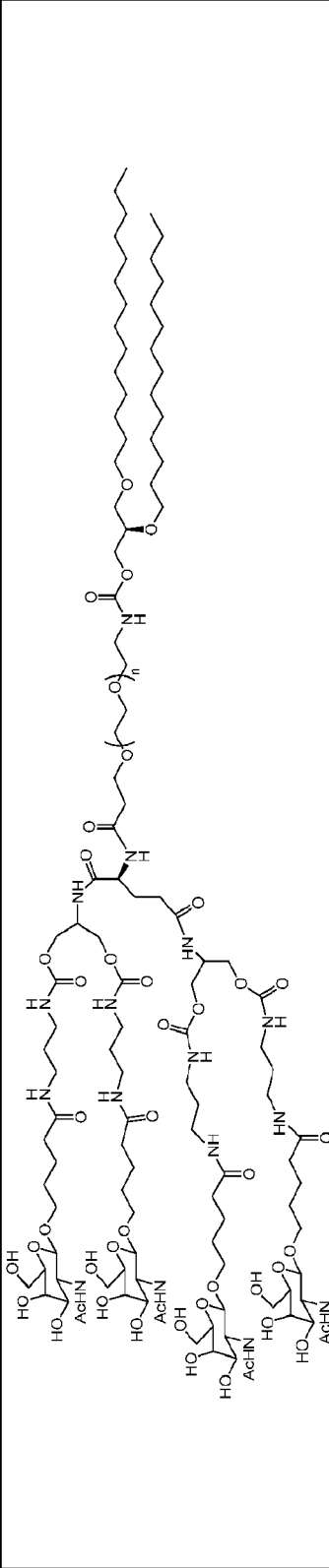
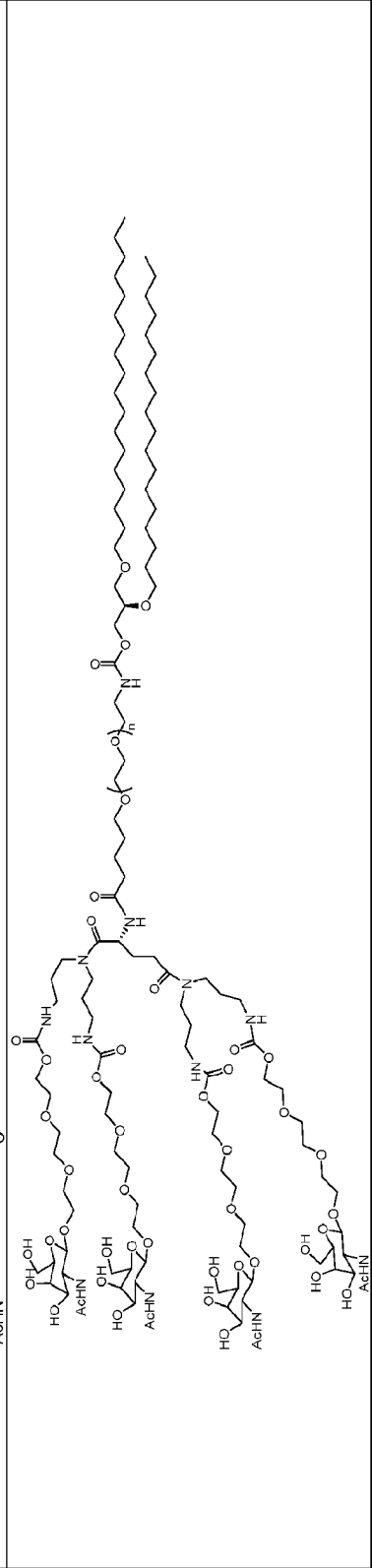
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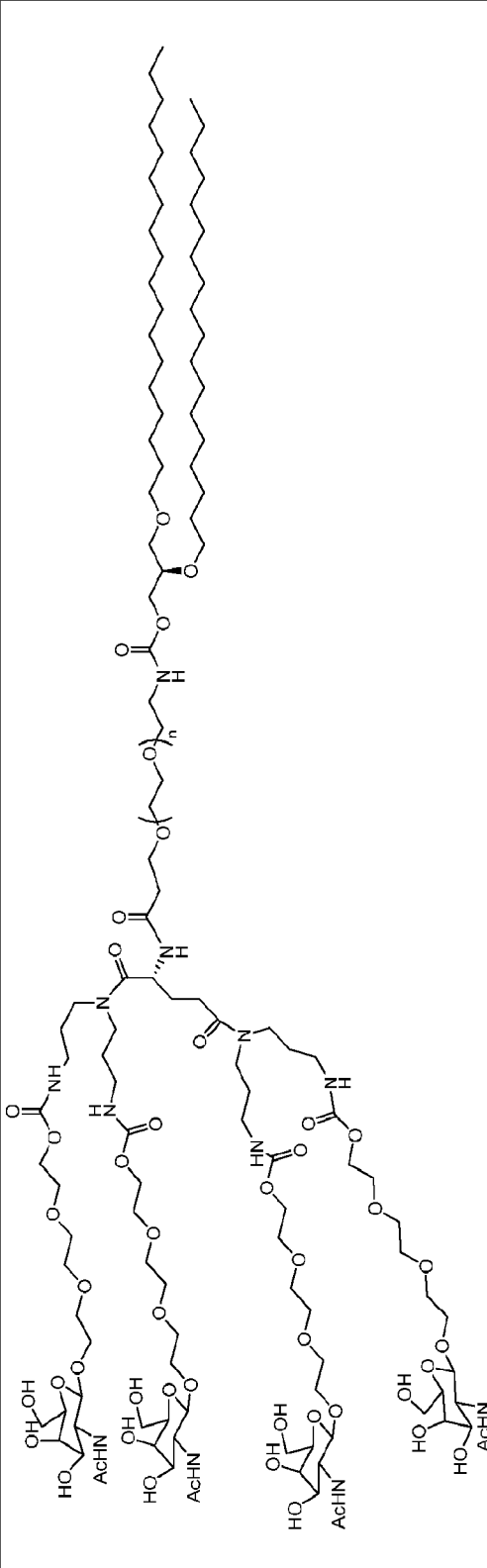
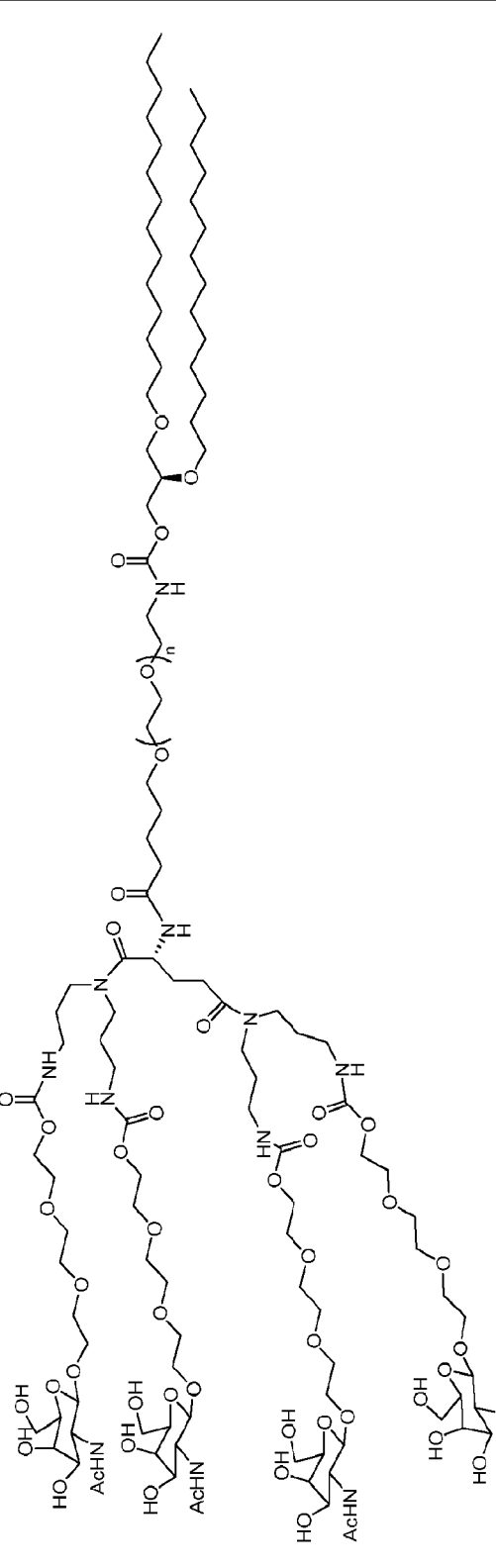
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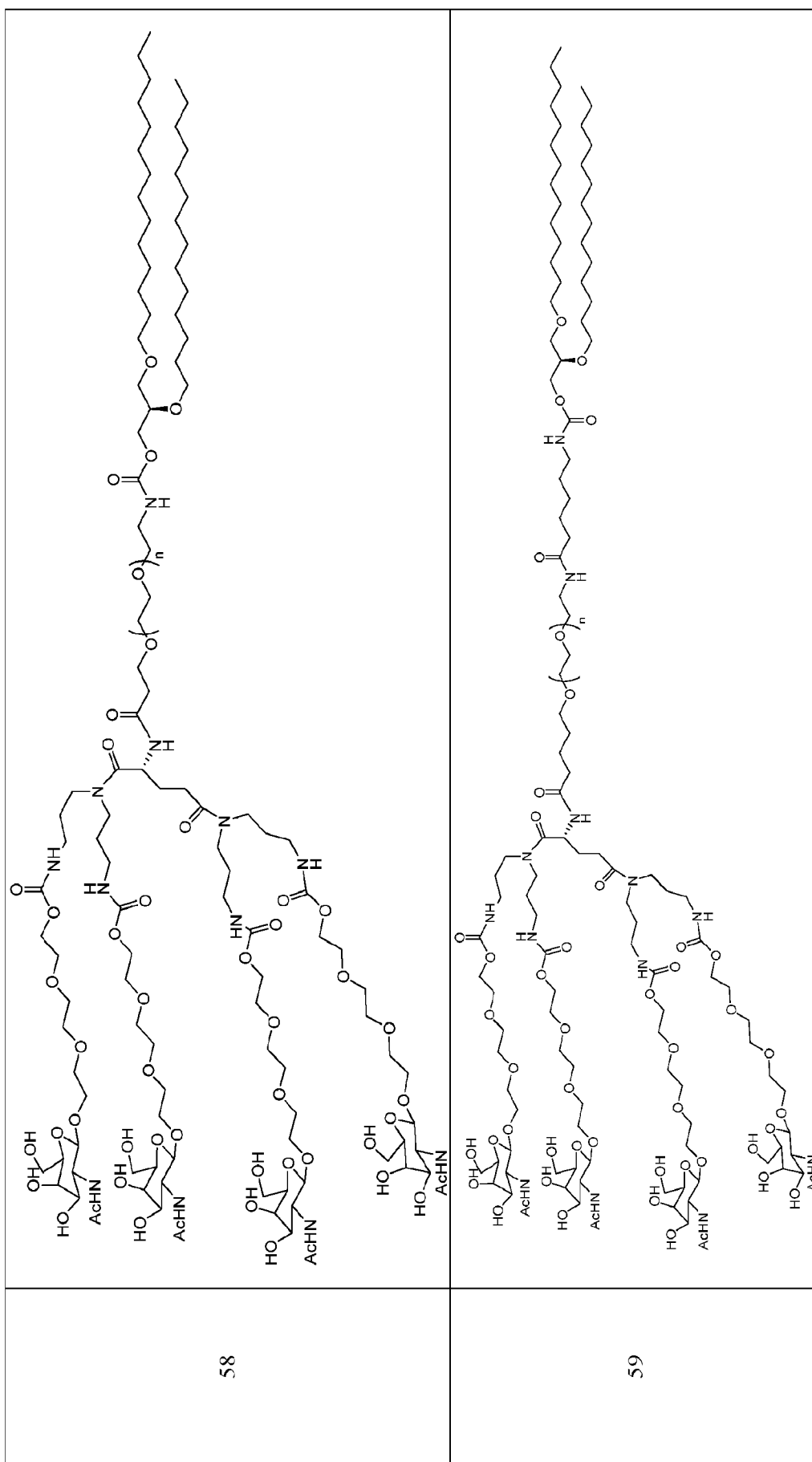
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| <p>46</p> |  |

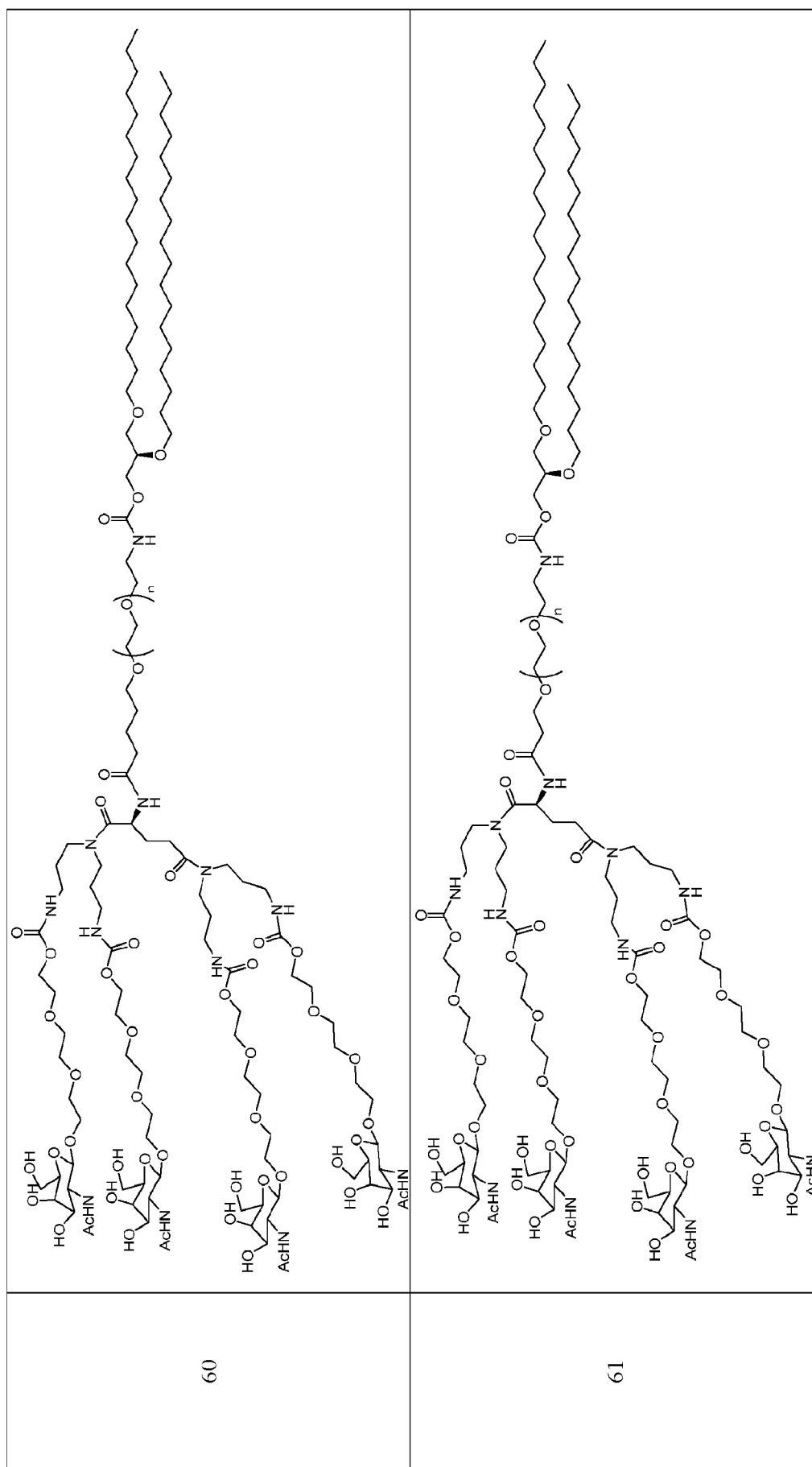
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|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  <p>Chemical structure 47: A complex molecule featuring a central core with multiple hydroxyl groups and acetyl groups. It includes a long alkyl chain, a polyether chain, and a complex amide linkage.</p> |  <p>Chemical structure 48: A complex molecule featuring a central core with multiple hydroxyl groups and acetyl groups. It includes a long alkyl chain, a polyether chain, and a complex amide linkage.</p> |  <p>Chemical structure 49: A complex molecule featuring a central core with multiple hydroxyl groups and acetyl groups. It includes a long alkyl chain, a polyether chain, and a complex amide linkage.</p> |
| 47                                                                                                                                                                                                                                                                                            | 48                                                                                                                                                                                                                                                                                            | 49                                                                                                                                                                                                                                                                                             |

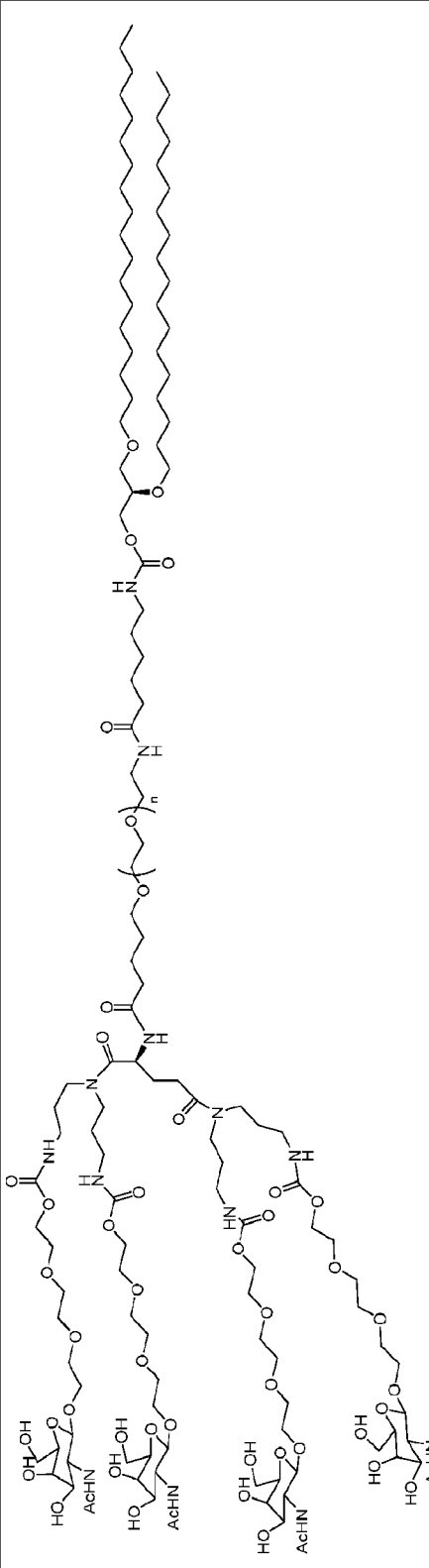
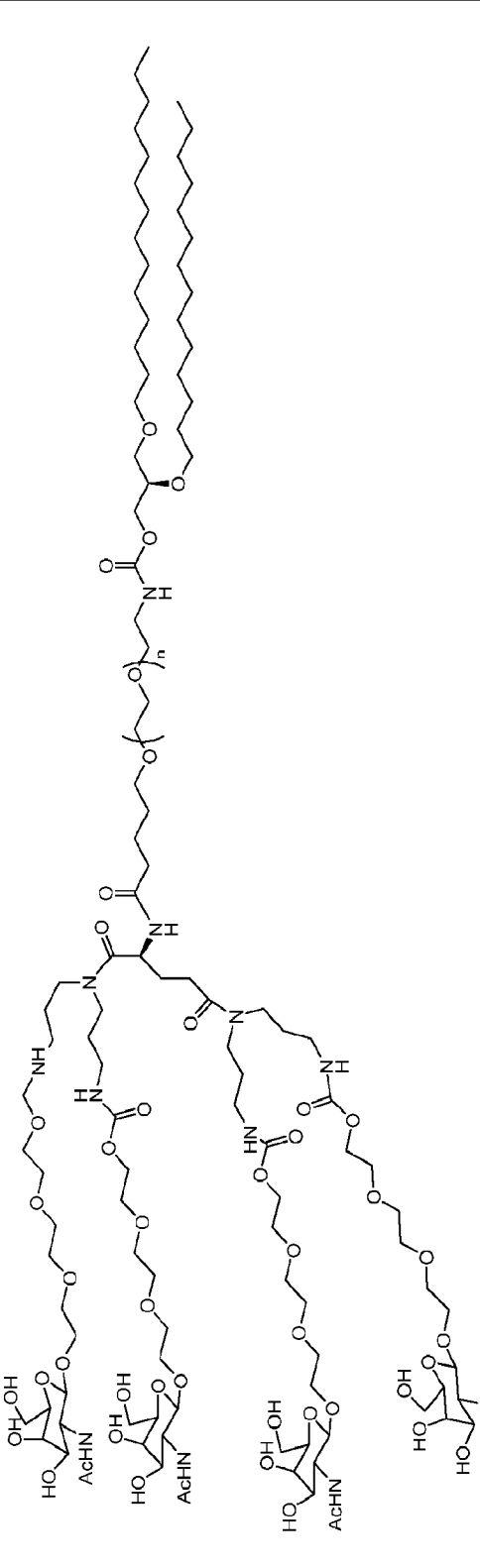
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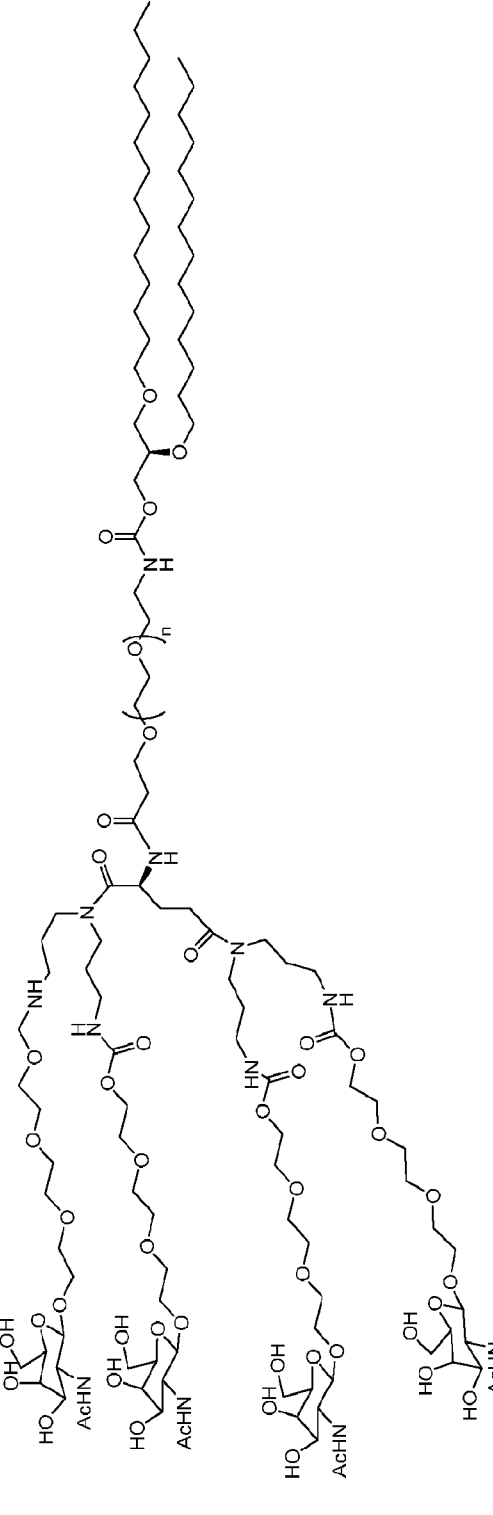
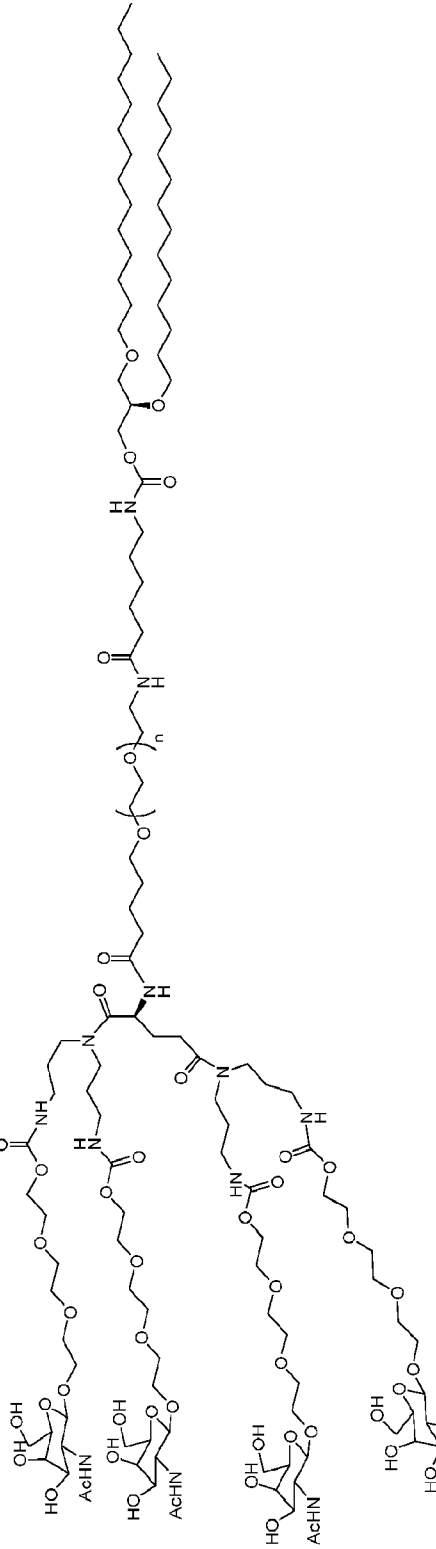
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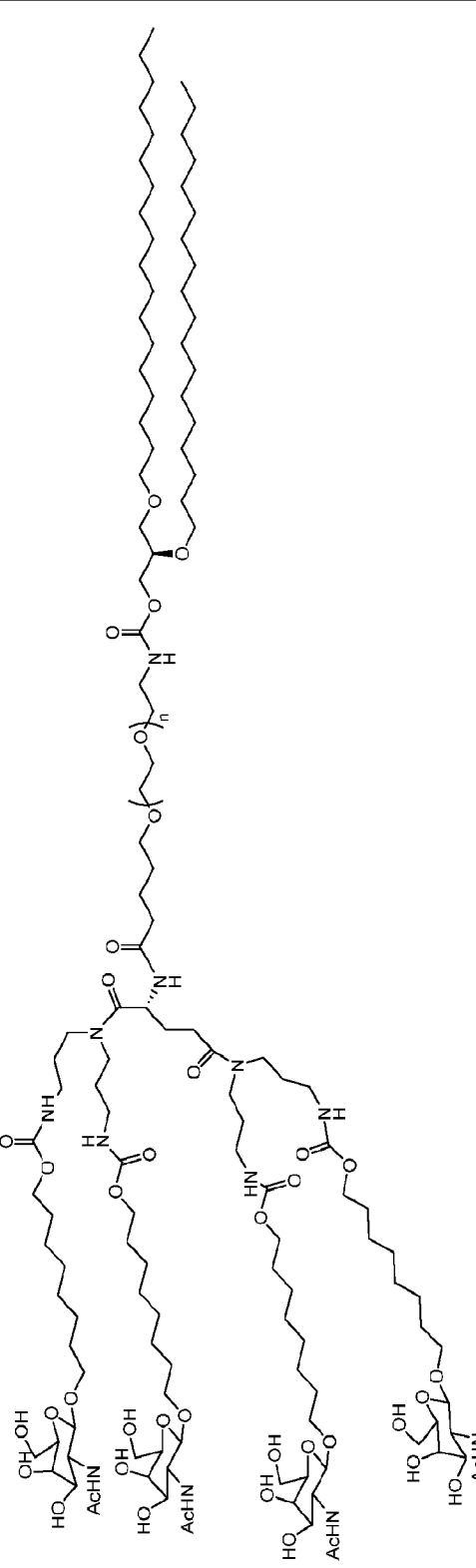
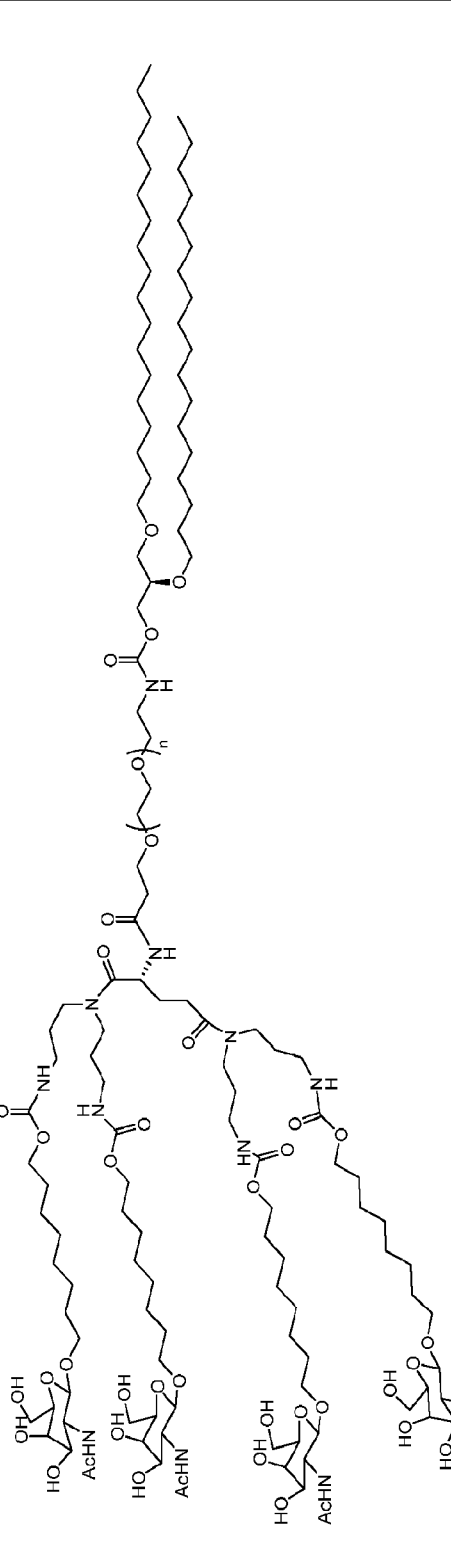
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| 56                                                                                 | 57                                                                                  |

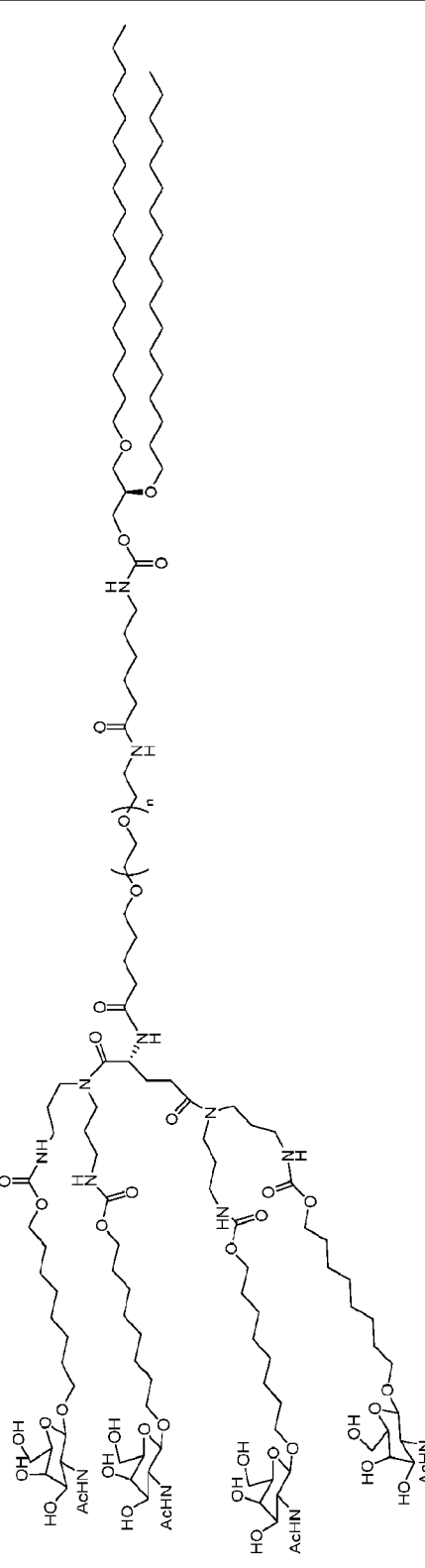
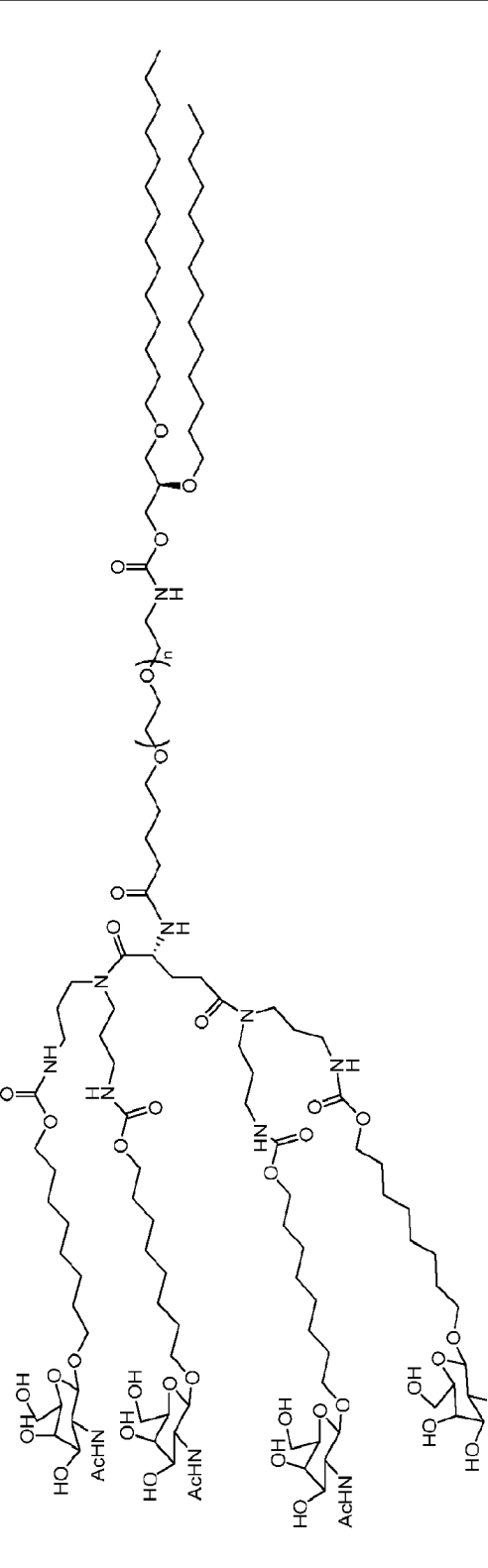


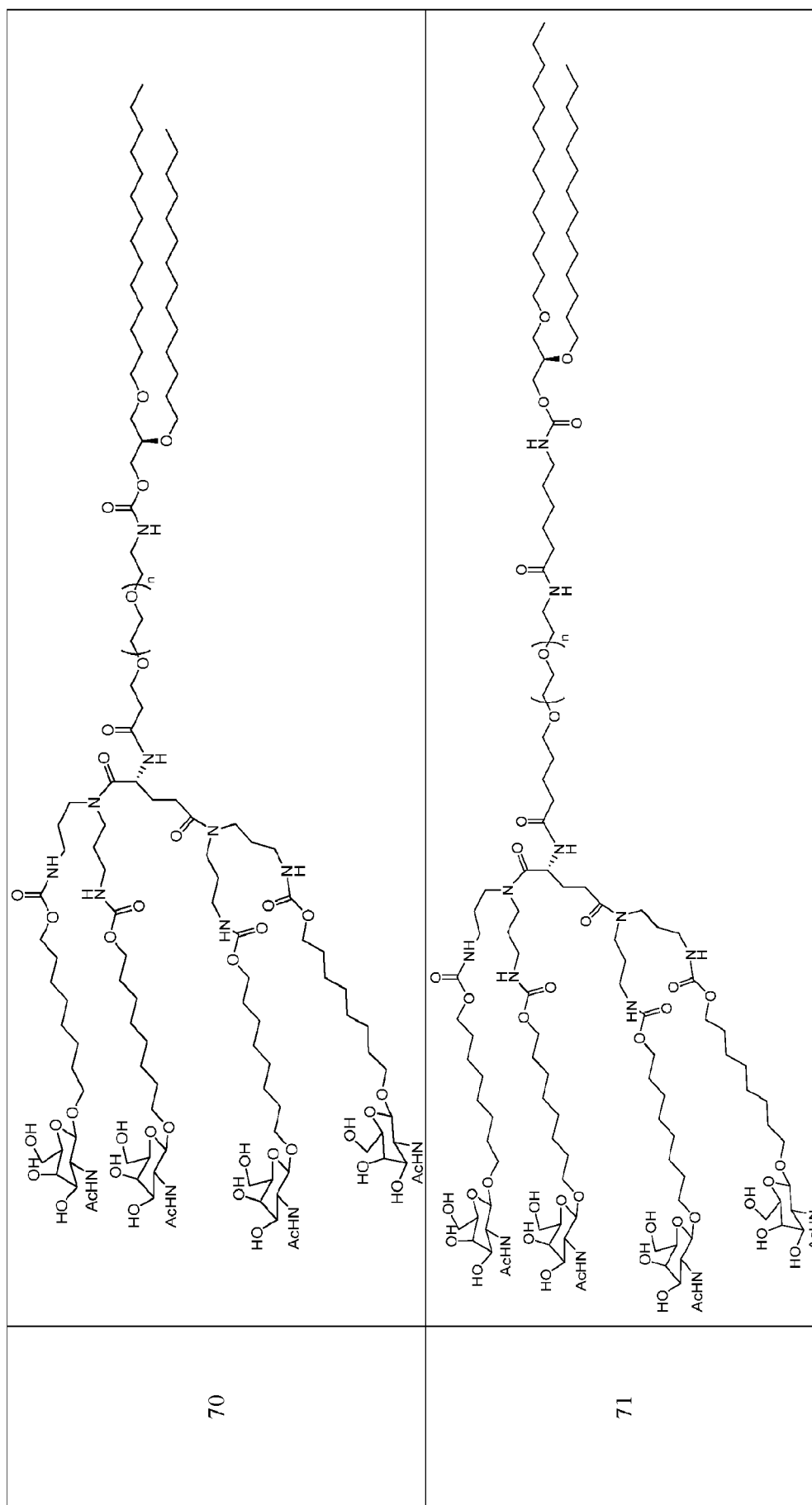


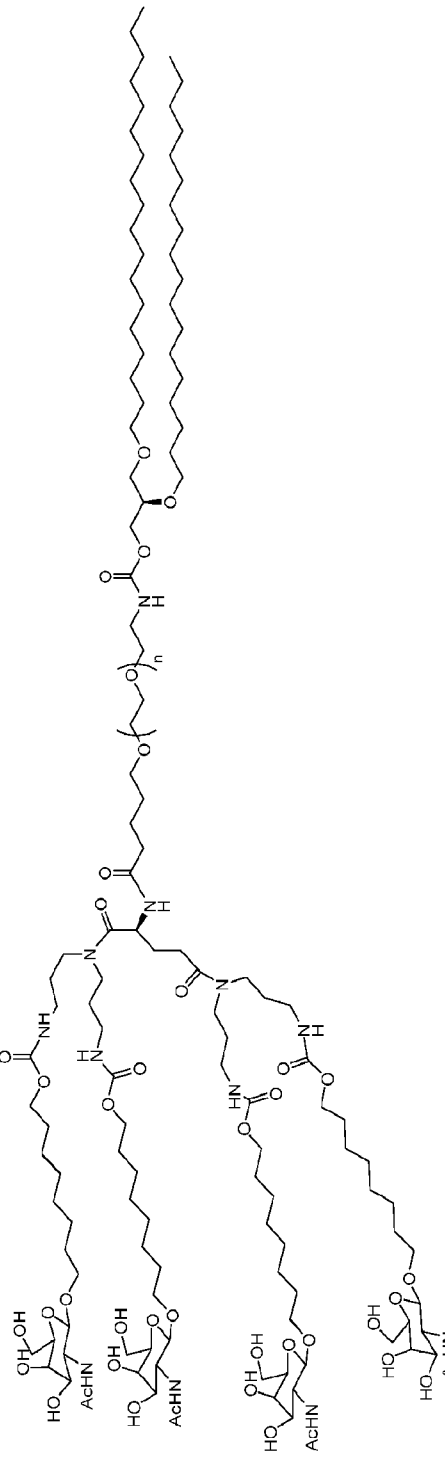
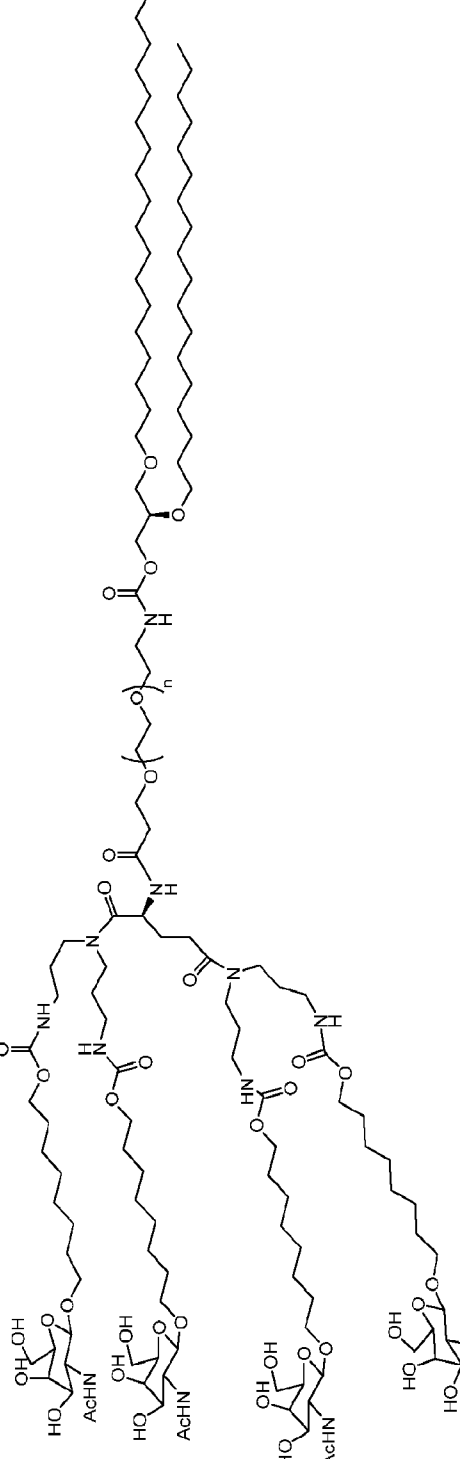
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|  <p>Chemical structure 62: A complex molecule featuring a central core with multiple hydroxyl groups and acetyl groups. It is linked via amide bonds to a long, flexible chain containing a repeating unit with an ether linkage and a terminal long alkyl chain.</p> |  <p>Chemical structure 63: A complex molecule similar to 62, but with a different arrangement of the flexible chain and the terminal long alkyl chain, resulting in a different overall shape and functional group distribution.</p> |
| 62                                                                                                                                                                                                                                                                                                                                                      | 63                                                                                                                                                                                                                                                                                                                      |

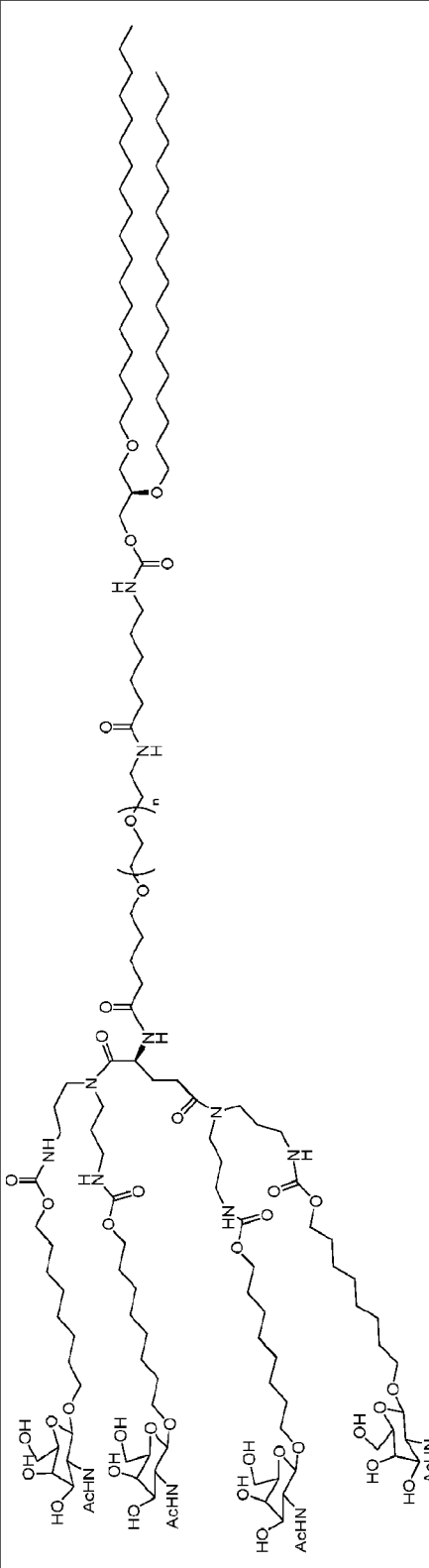
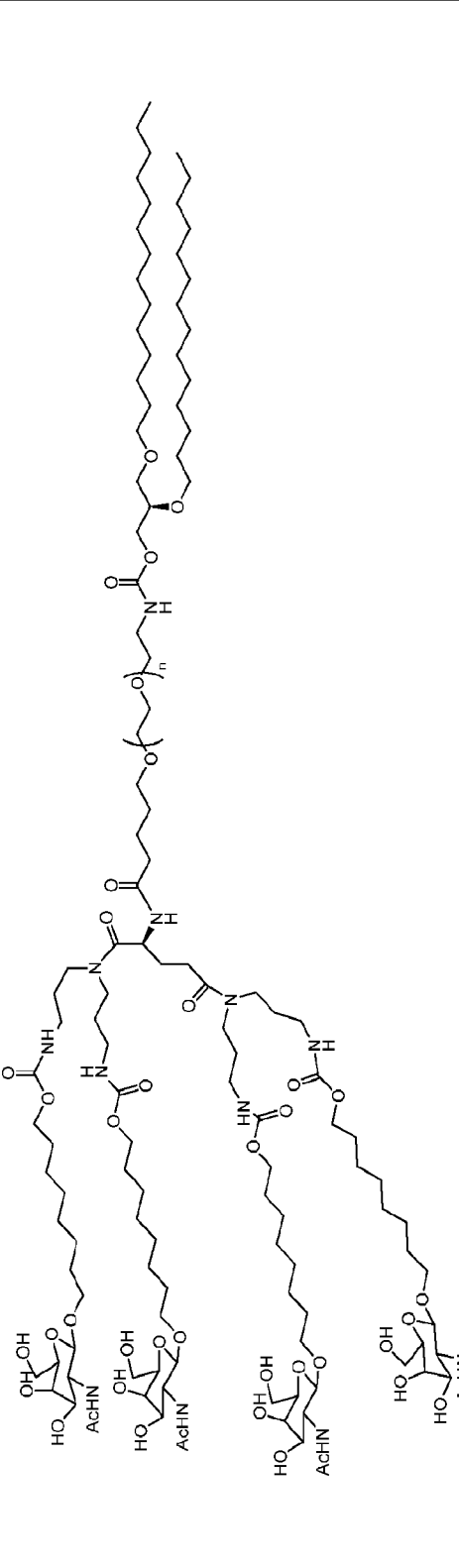
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| 64                                                                                 | 65                                                                                  |

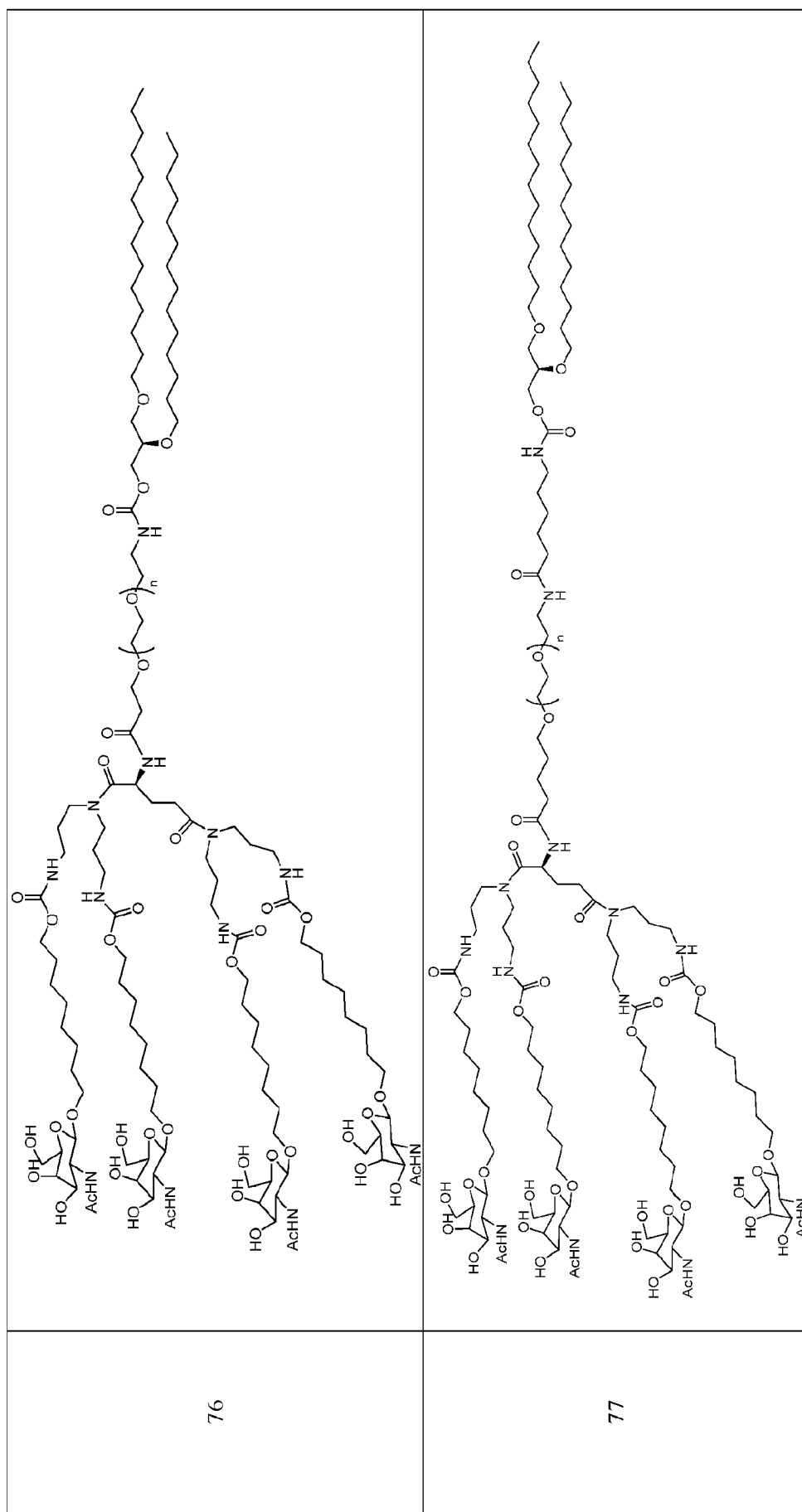
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| 66                                                                                 | 67                                                                                  |

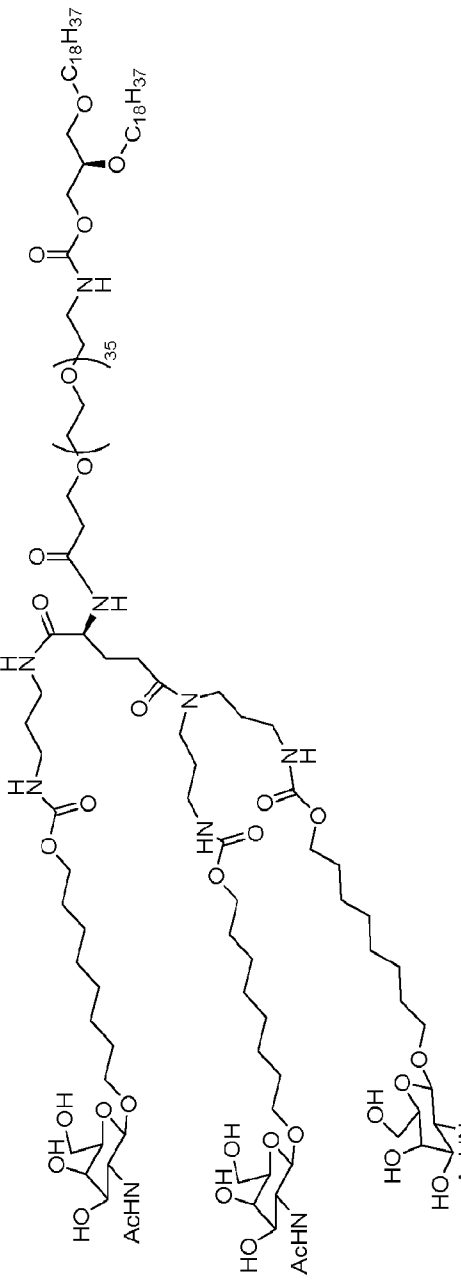
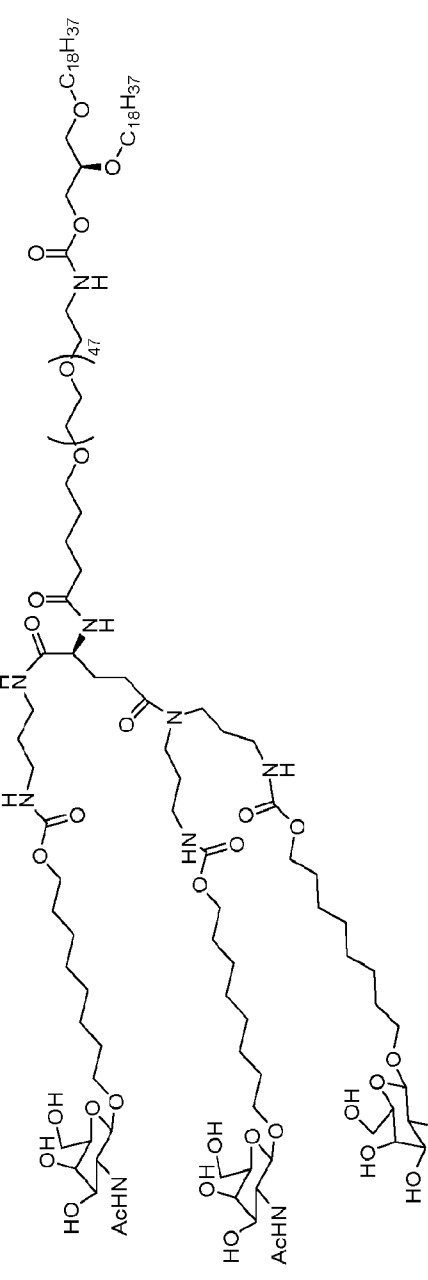
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|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  <p>Chemical structure 68: A complex molecule featuring a central core with multiple hydroxyl groups and acetyl groups. It is linked via amide bonds to a long, flexible chain containing a repeating unit of a polyether. The chain terminates in a long, straight alkyl chain.</p> |  <p>Chemical structure 69: A complex molecule similar to 68, but with a different arrangement of the polyether chain and the terminal alkyl chain. It features a central core with hydroxyl and acetyl groups, linked via amide bonds to a polyether chain that terminates in a long, straight alkyl chain.</p> |
| 68                                                                                                                                                                                                                                                                                                                                                                     | 69                                                                                                                                                                                                                                                                                                                                                                                                 |

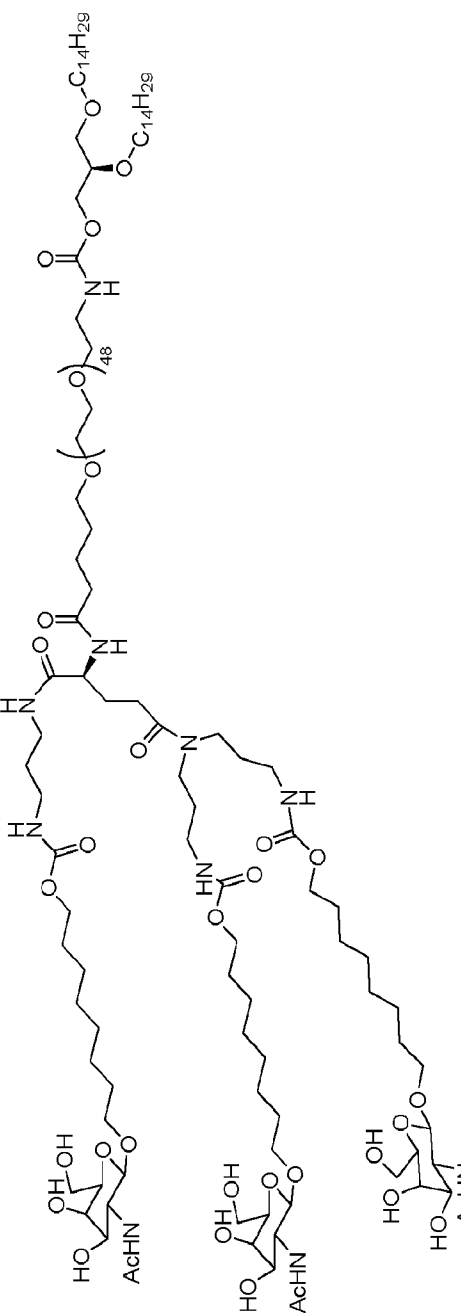
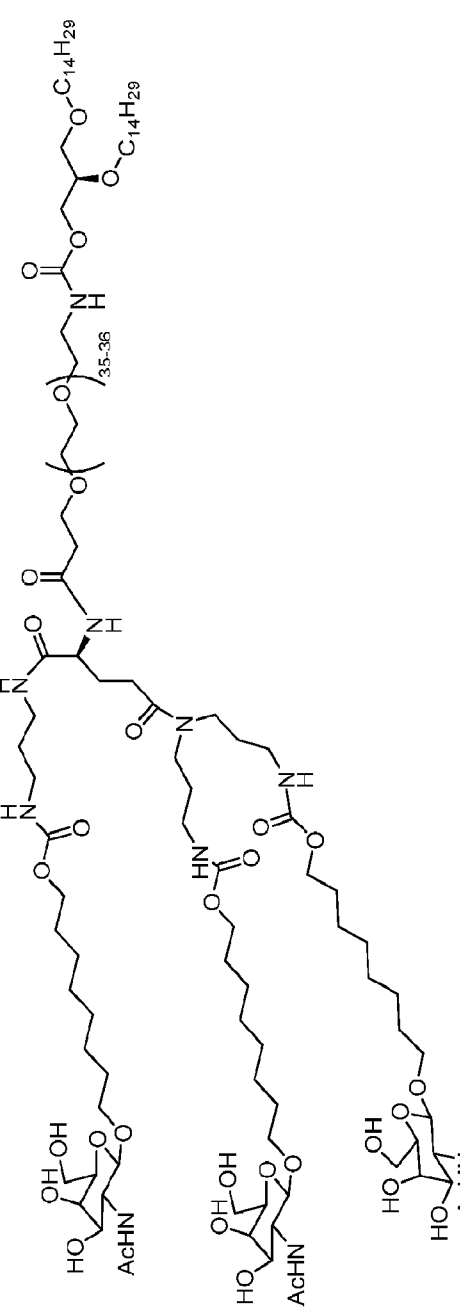


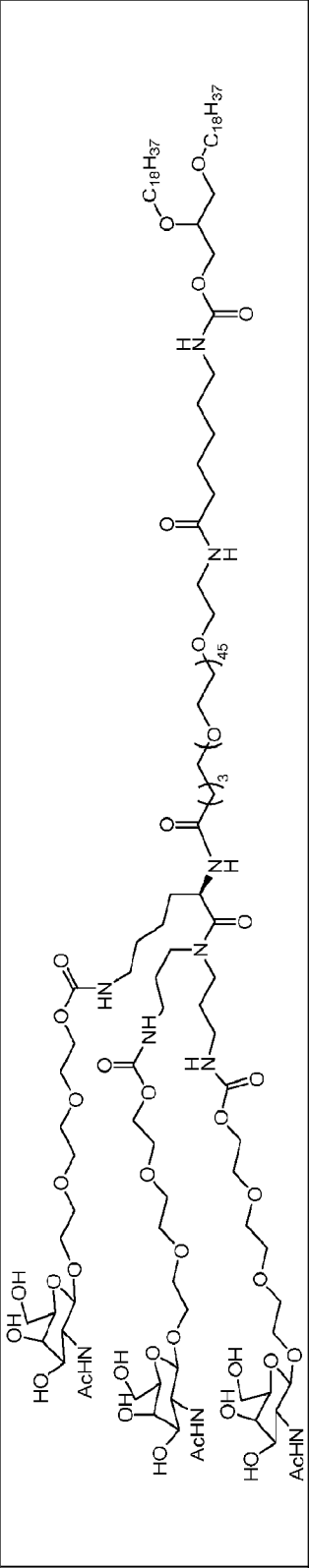
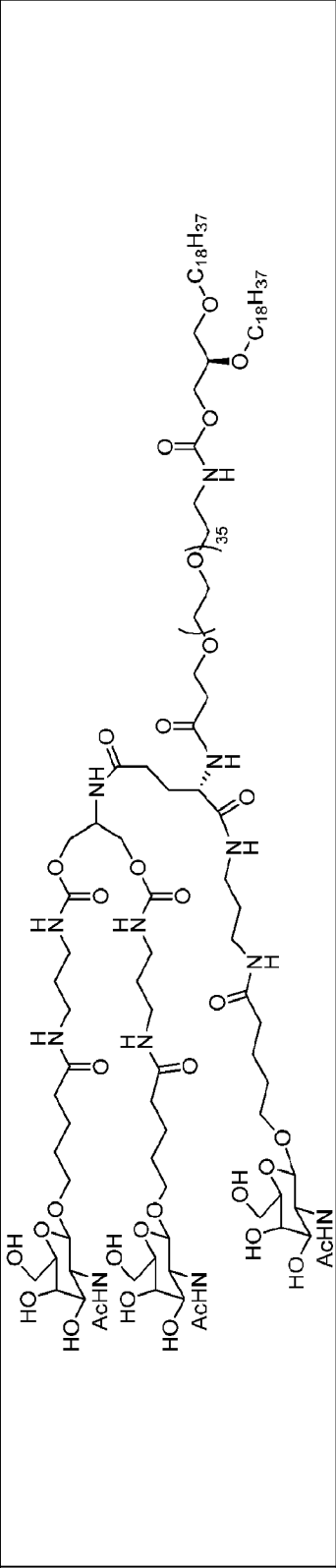
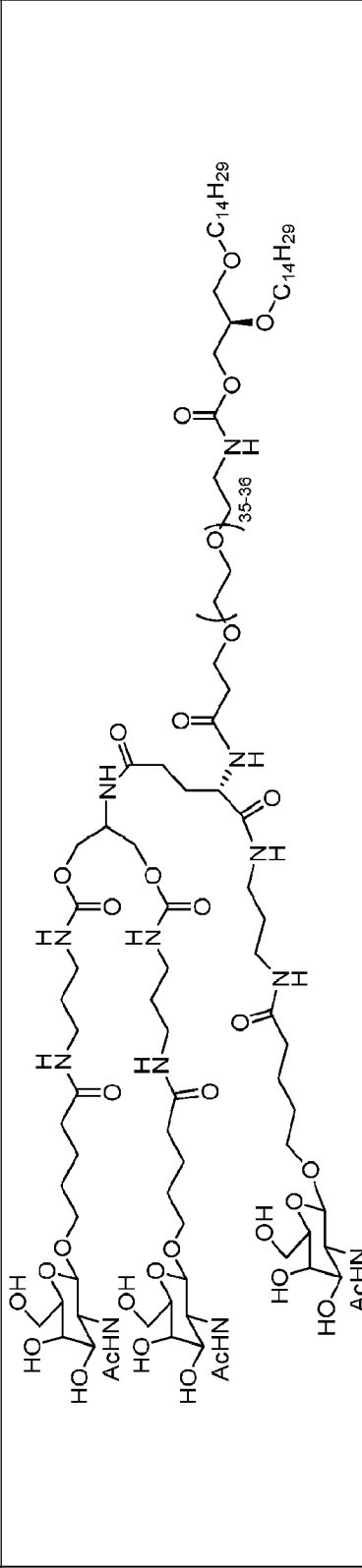
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|  <p>Chemical structure 72: A complex molecule featuring a central core with multiple amide and ester linkages. It includes a long, branched aliphatic chain on the left, a central amide linkage, and a long, branched aliphatic chain on the right. The structure is highly symmetrical and contains several hydroxyl groups and amide bonds.</p> |  <p>Chemical structure 73: A complex molecule featuring a central core with multiple amide and ester linkages. It includes a long, branched aliphatic chain on the left, a central amide linkage, and a long, branched aliphatic chain on the right. The structure is highly symmetrical and contains several hydroxyl groups and amide bonds.</p> |
| 72                                                                                                                                                                                                                                                                                                                                                                                                                                   | 73                                                                                                                                                                                                                                                                                                                                                                                                                                    |

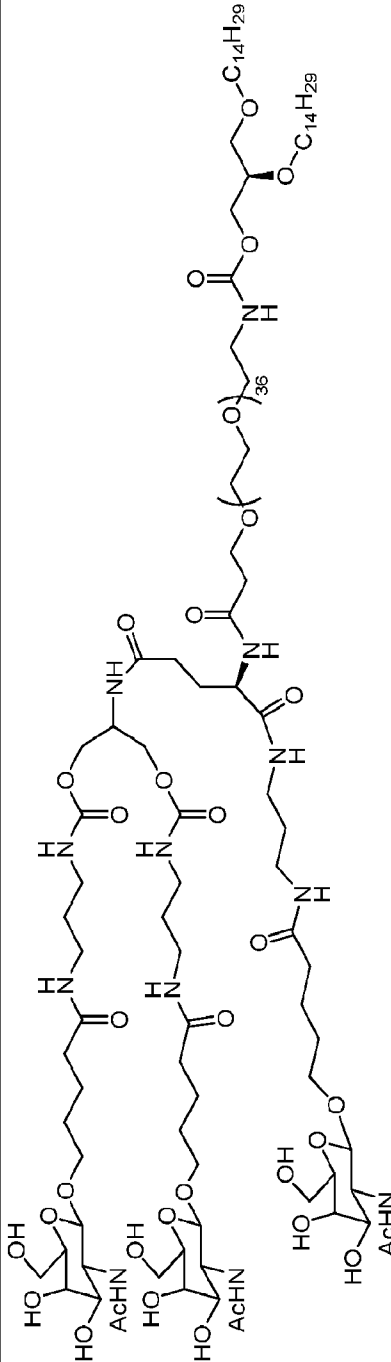
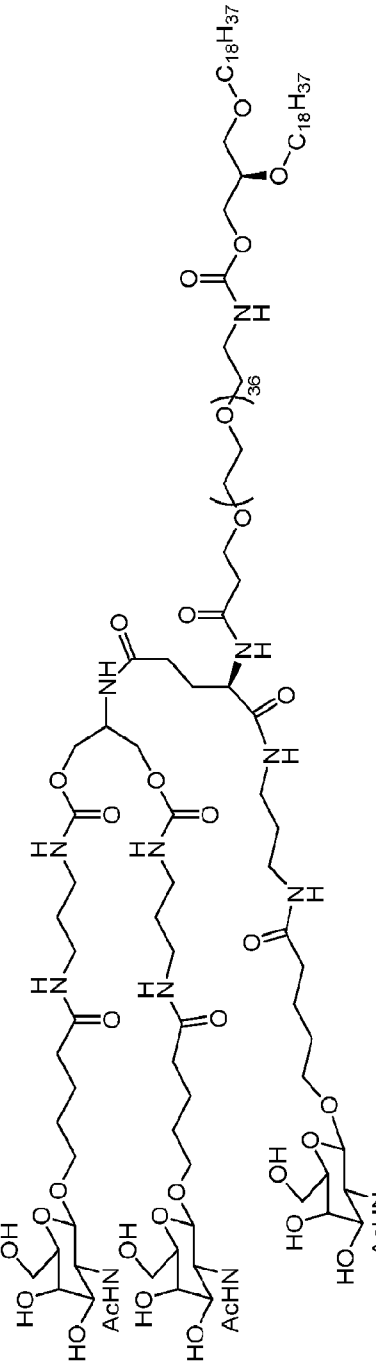
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|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  <p>Chemical structure 74: A complex molecule featuring a central core with multiple amide and ether linkages. It includes a long alkyl chain, a polyether segment, and several sugar units (likely glucose) attached via glycosidic bonds. The structure is highly branched and contains various functional groups including hydroxyl, amine, and carbonyl groups.</p> |  <p>Chemical structure 75: A complex molecule similar to 74, but with a different arrangement of the polyether segment and the sugar units. It also features a long alkyl chain and various functional groups, including hydroxyl, amine, and carbonyl groups.</p> |
| 74                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 75                                                                                                                                                                                                                                                                                                                                                    |

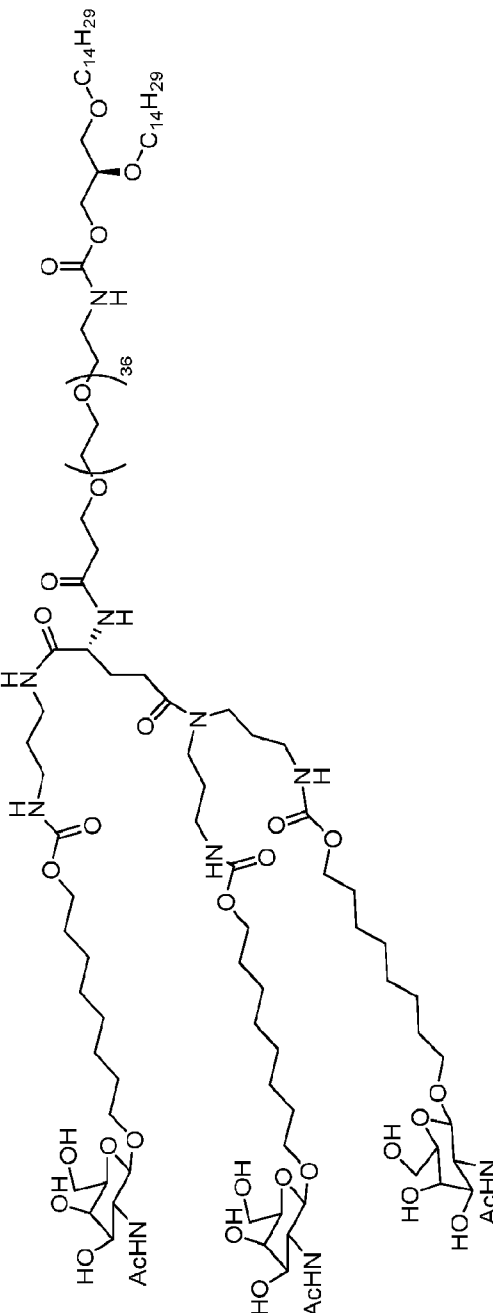
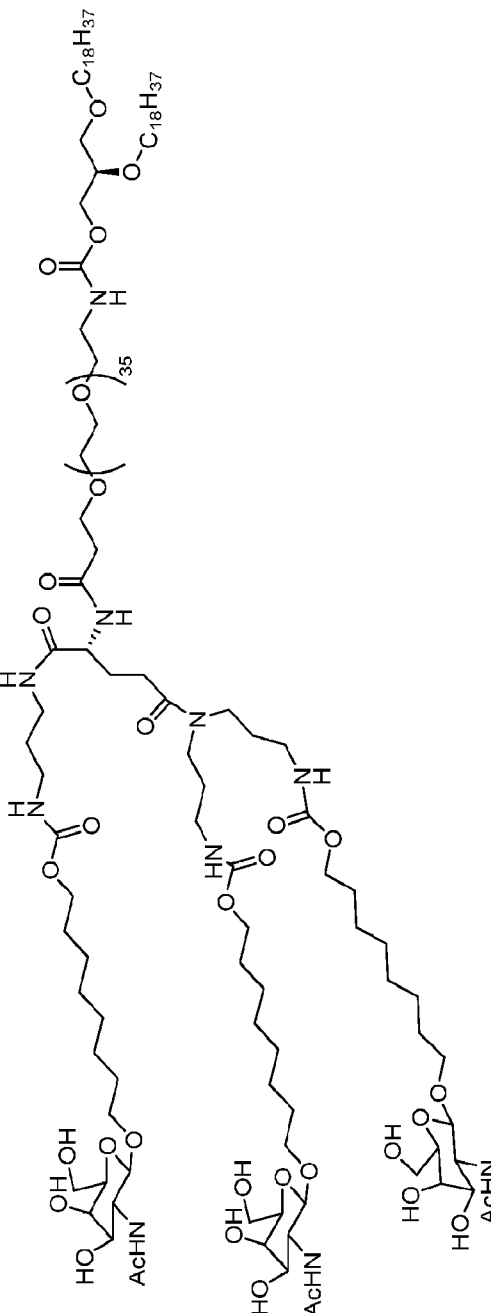


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|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  <p>Chemical structure 87: A complex molecule featuring a central core with multiple side chains. The side chains include long alkyl groups, branched alkyl groups, and a branched alkyl group with a terminal hydroxyl group. The molecule is labeled with 'IZ' and 'ZI' at various positions.</p> |  <p>Chemical structure 88: A complex molecule featuring a central core with multiple side chains. The side chains include long alkyl groups, branched alkyl groups, and a branched alkyl group with a terminal hydroxyl group. The molecule is labeled with 'IZ' and 'ZI' at various positions.</p> |
| 87                                                                                                                                                                                                                                                                                                                                                                                    | 88                                                                                                                                                                                                                                                                                                                                                                                     |

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|  | 90 |

wherein each subscript "n" is independently 10-200.

[0232] In some embodiments, the compounds of Table 2 each have a subscript “n” of between 35-47. In some embodiments, the compounds of Table 2 each have a subscript “n” of 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, or 47. In some embodiments, the compounds of Table 2 each have a subscript “n” of between 35-37. In some embodiments, the compounds of Table 2 each have a subscript “n” of between 44-46.

**[0233] B. LNP Compositions**

[0234] The present disclosure provides lipid nanoparticle (LNP) compositions.

[0235] Delivering payload to a specific tissue in the human body, such as liver, is a major challenge in novel lipid nanoparticle (LNP) development. Incorporation of biodegradable groups can enhance the gene editing level and reduce the potential toxicity of LNPs.

[0236] In some embodiments, the LNP compositions comprise a GalNAc-based lipid that comprises a compound described herein. In some embodiments, a GalNAc-based lipid is or comprises a compound described herein (*e.g.*, a compound of Formulae I or II).

[0237] In some embodiments, a lipid nanoparticle (LNP) composition comprises:

- GalNAc-based lipid that comprises a compound as described herein;
- a cationic lipid (also referred to as an ionizable lipid);
- a phospholipid (also referred to as a helper lipid);
- a PEG-containing lipid; and
- a sterol.

[0238] In some embodiments, a lipid nanoparticle (LNP) composition comprises:

- about 0.001-5 mol% of a GalNAc-based lipid that comprises a compound as described herein, based on the total lipid presented in the LNP composition;
- about 35-60 mol% of a cationic lipid (also referred to as an ionizable lipid), based on the total lipid presented in the LNP composition;
- about 10-45 mol% of a phospholipid (also referred to as a helper lipid), based on the total lipid presented in the LNP composition;
- about 0.5-3 mol% of a PEG-containing lipid, based on the total lipid presented in the LNP composition; and
- about 15-50 mol% of a sterol, based on the total lipid presented in the LNP composition.

[0239] In some embodiments, a lipid nanoparticle (LNP) composition comprises:

- about 0.05-1 mol% of a GalNAc-based lipid that comprises a compound as described herein, based on the total lipid presented in the LNP composition;

about 35-55 mol% of a cationic lipid (also referred to as an ionizable lipid), based on the total lipid presented in the LNP composition;

about 10-20 mol% of a phospholipid (also referred to as a helper lipid), based on the total lipid presented in the LNP composition;

about 1-2.5 mol% of a PEG-containing lipid, based on the total lipid presented in the LNP composition; and

about 30-45 mol% of a sterol, based on the total lipid presented in the LNP composition.

[0240] In some embodiments, a lipid nanoparticle (LNP) composition comprises about 0.0001, 0.0005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, or 5 mol% of a GalNAc-based lipid that comprises a compound as described herein, based on the total lipid presented in the LNP composition. In some embodiments, a lipid nanoparticle (LNP) composition comprises about 35, 40, 45, 50, 55, or 60 mol% of a cationic lipid (also referred to as an ionizable lipid), based on the total lipid presented in the LNP composition. In some embodiments, a lipid nanoparticle (LNP) composition comprises about 10, 12.5, 15, 17.5, 20, 25, 30, 35, 40, or 45 mol% of a phospholipid (also referred to as a helper lipid), based on the total lipid presented in the LNP composition. In some embodiments, a lipid nanoparticle (LNP) composition comprises about 0.5, 1, 1.25, 1.5, 1.75, 2, 2.5, or 3 mol% of a PEG-containing lipid, based on the total lipid presented in the LNP composition. In some embodiments, a lipid nanoparticle (LNP) composition comprises about 15, 20, 25, 30, 35, 40, 45, or 50 mol% of a sterol, based on the total lipid presented in the LNP composition.

[0241] (iv). *Cationic Lipid*

[0242] In some embodiments, a LNP composition comprises a cationic lipid. In some embodiments, a cationic lipid is referred to as an ionizable lipid. In some embodiments, a cationic lipid comprises an amino lipid. In some embodiments, a cationic lipid comprises one or more nitrogen atoms which are positively charged. In some embodiments, a cationic lipid includes, but is not limited to, N,N-dioleoyl-N,N-dimethylammonium chloride ("DODAC"); N-(2,3-dioleoyloxy)propyl-N,N,N-triethylammonium chloride ("DOTMA"); N,N-distearyl-N,N-dimethylammonium bromide ("DDAB"); N-(2,3-dioleoyloxy)propyl-N,N,N-trimethylammonium chloride ("DOTAP"); 1,2-Dioleoyloxy-3-trimethylaminopropane chloride salt ("DOTAP.C1"); 3 $\beta$ -(N-(N',N'-dimethylaminoethane)-carbamoyl)cholesterol ("DC-Choi"); N-(1-(2,3-dioleoyloxy)propyl)-N-2-(sperminecarboxamido)ethyl-N,N-dimethylammonium trifluoroacetate ("DOSPA"); dioctadecylamidoglycyl carboxyspermine

("DOGS"), 1,2-dioleoyl- sn-3-phosphoethanolamine ("DOPE"), 1,2-dioleoyl-3-dimethylammonium propane ("DODAP"), N, N-dimethyl-2,3-dioleoyloxy)propylamine ("DODMA"), N-(1, 2- dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide ("DMRIE"), 3-(didodecylamino)-N1,N1,4-tridodecyl-1-piperazineethanamine (KL10), N1-[2-(didodecylamino)ethyl]-N1,N4,N4-tridodecyl-1,4-piperazinediethanamine (KL22), 14,25-ditridecyl-15,18,21,24-tetraaza-octatriacontane (KL25), 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLin-DMA), 2,2-dilinoley-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (DLin-MC3-DMA), 2,2-dilinoley-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), 1,2-dioleoyloxy-N,N-dimethylaminopropane (DODMA), 2-({8-[(33)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA), (2R)-2-({8-[(3P)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2R)), and (2S)-2-({8-[(33)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2S)). Additionally, a number of commercial preparations of cationic lipids can be used, such as, *e.g.*, LIPOFECTIN (including DOTMA and DOPE, available from GIBCO/BRL), and LIPOFECTAMINE (comprising DOSPA and DOPE, available from GIBCO/BRL).

**[0243] (v). Phospholipids**

**[0244]** In some embodiments, a LNP composition comprises a phospholipid. In some embodiments, a phospholipid is referred to as a helper lipid. In some embodiments, a phospholipid includes, but is not limited to, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl serine, phosphatidic acid, 2-lysophosphatidyl choline, and sphingomyelin. In some embodiments, a fatty acid moiety includes but is not limited to lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, erucic acid, phytanic acid, arachidic acid, arachidonic acid, eicosapentaenoic acid, behenic acid, docosapentaenoic acid, and docosahexaenoic acid. Non-natural species including natural species with modifications and substitutions including branching, oxidation, cyclization, and alkynes are also contemplated. For example, a phospholipid may be functionalized with or cross-linked to one or more alkynes (*e.g.*, an alkenyl group in which one or more double bonds is replaced with a triple bond).

**[0245]** Other phospholipids include, but are not limited to, 1,2-distearoyl-snglycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dilinoley-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), 1,2-dimyristoyl-sn-glycerophosphocholine

(DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-diundecanoyl-sn-glycerophosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl 1-sn-glycero-3-phosphocholine (OChemPC), 1-hexadecyl sn-glycero-3-phosphocholine (C<sub>16</sub> Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosaheptaenoyl-sn-glycero-3-phosphocholine, 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosaheptaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (1-glycerol) sodium salt (DOPG), dipalmitoylphosphatidylglycerol (DPPG), palmitoyl-oleoylphosphatidylethanolamine (POPE), distearoyl-phosphatidylethanolamine (DSPE), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), 1-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE), 1-stearoyl-2-oleoylphosphatidylcholine (SOPC), sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyl-oleoyl phosphatidylcholine, lysophosphatidylcholine, lysophosphatidylethanolamine (LPE), or combinations thereof.

**[0246] (vi). Polyethylene glycol-lipid**

**[0247]** In some embodiments, a LNP composition comprises a polyethylene glycol-lipid (PEG-lipids). In some embodiments, PEG lipids include PEG conjugated to saturated or unsaturated C<sub>6</sub>-C<sub>20</sub> alkyl chains. In some embodiments, polyethylene glycol (PEG) lipids include PEG-modified lipids such as PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, and PEG-modified dialkylglycerols. In some embodiments, polyethylene glycol lipids include DMG-PEG, DLPE-PEGs, DMPE-PEGs, DPPC-PEGs, and DSPE-PEGs. In some embodiments, the polyethylene glycol lipid's name is followed by XXX, intended to signify the molecular weight of the polyethylene glycol moiety. For example, if the polyethylene glycol lipid is DMG-PEGXXX, the "XXX" represents the molecular weight, such as molecular weight of the polyethylene glycol moiety, *e.g.*, DMG-PEG1000, DMG-PEG2000, DMG-PEG3000, or DMG-PEG5000.

**[0248] (vii). Sterol**

**[0249]** In some embodiments, a LNP composition comprises a sterol. Sterols are known in the art and typically refer to compounds having a perhydrocyclopentanophenanthrene ring system with one or more OH substituents. Examples of sterols include, but are not limited to, cholesterol, campesterol, ergosterol, sitosterol, and the like. In some embodiments, the sterol is cholesterol. In some embodiments, a cholesterol is modified. In some embodiments, a cholesterol is an oxidized cholesterol. In some embodiments, a cholesterol is esterified cholesterol. In some embodiments, the sterol is a cholesterol-based lipid such as PEGylated cholesterol, DC-Choi (N,N-dimethyl-N-ethylcarboxamidocholesterol), 1,4-bis(3-N-oleylamino-propyl)piperazine, or combinations thereof. Exemplary sterols include, but are not limited to, 25-hydroxycholesterol (25-OH), 20 $\alpha$ -hydroxycholesterol (20 $\alpha$ -OH), 27-hydroxycholesterol, 6-keto-5 $\alpha$ -hydroxycholesterol, 7-ketocholesterol, 7 $\beta$ -hydroxycholesterol, 7 $\alpha$ -hydroxycholesterol, 7 $\beta$ -25-dihydroxycholesterol, beta-sitosterol, stigmasterol, brassicasterol, campesterol, or combinations thereof.

**[0250] (viii). Polynucleotides**

**[0251]** In some embodiments, the LNP composition further comprises at least one polynucleotide.

**[0252]** The at least one polynucleotide present in the compositions described herein can be a DNA, cDNA, and RNA of all types. In some embodiments, the at least one polynucleotide is a double stranded DNA, single-stranded DNA, complexed DNA, encapsulated DNA, naked RNA, encapsulated RNA, messenger RNA (mRNA), tRNA, short interfering RNA (siRNA), double stranded RNA (dsRNA), micro-RNA (miRNA), antisense RNA (asRNA), or combinations thereof. The at least one polynucleotide can also be a DNA construct, such as expression vectors, expression vectors encoding a desired gene product, and the like. In some embodiments, the at least one polynucleotide is an mRNA. In some embodiments, the at least one polynucleotide is or encodes one or more components of a prime editing system.

**[0253] (ix). Prime Editing System**

**[0254]** In some embodiments, the LNP composition further comprises one or more components of a prime editing system.

**[0255]** The term “prime editing system” or “prime editing composition” refers to compositions involved in the methods of prime editing. A prime editing system may include a prime editor, *e.g.*, a prime editor fusion protein, and one or more prime editing guide RNAs (PEgRNAs). A prime editing system may further comprise additional elements. For example, a prime editing system may comprise a prime editor, a PEgRNA, and a second strand nick

guide RNA (ngRNA). Components of a prime editing system may be combined to form a complex for prime editing, or may be kept separately, *e.g.*, for administration purposes. In some embodiments, a prime editing system may include one or more prime editors and one or more PEGRNAs. In some embodiments, a prime editing system may include (i) one or more polynucleotides encoding one or more prime editor polypeptides and (ii) one or more PEGRNAs, or one or more polynucleotides encoding one or more PEGRNAs.

**[0256]** The term “prime editing” refers to programmable editing of a target DNA using a prime editor complexed with a PEGRNA to incorporate an intended nucleotide edit into the target DNA through target-primed DNA synthesis. In prime editing, a target DNA may comprise a double stranded DNA molecule having two complementary strands. The prime editing process may search specific targets and edit the endogenous sequence of a target DNA, *e.g.*, a target gene. In some embodiments, the spacer sequence of a PEGRNA comprises complementarity to a target strand of the target gene, and can anneal with the target strand. The PEGRNA may form a complex with a prime editor, which may generate a nick in the target gene on the edit strand which is the complementary strand of the target strand. The prime editing complex may then use a free 3' end formed at the nick site of the edit strand to initiate DNA synthesis, where a primer binding site (PBS) of the PEGRNA complexes with the free 3' end, and a single stranded DNA is synthesized using an editing template of the PEGRNA as a template. The editing template may comprise one or more nucleotide edits compared to the endogenous target gene sequence. Accordingly, the newly-synthesized single stranded DNA also comprises the nucleotide edit(s) encoded by the editing template. Through removal of an endogenous sequence on the edit strand of the target gene and incorporation of the newly-synthesized single stranded DNA, the intended nucleotide edit(s) are incorporated into the target gene.

**[0257]** The term “prime editor (PE)” refers to the polypeptide or polypeptide components involved in prime editing. In various embodiments, a prime editor includes a polypeptide domain having DNA binding activity and a polypeptide domain having DNA polymerase activity. In some embodiments, the polypeptide domain having DNA binding activity is a polypeptide domain having programmable DNA binding activity. In some embodiments, the prime editor further comprises a polypeptide domain having nuclease activity. In some embodiments, the polypeptide domain having DNA binding activity comprises a nuclease domain or nuclease activity. In some embodiments, the polypeptide domain having nuclease activity comprises a nickase, or a fully active nuclease. As used herein, the term “nickase” refers to a nuclease capable of cleaving only one strand of a double-stranded DNA target. In

some embodiments, the prime editor comprises a polypeptide domain that is an inactive nuclease. In some embodiments, the polypeptide domain having programmable DNA binding activity comprises a nucleic acid guided DNA binding domain, for example, a CRISPR-Cas protein, for example, a Cas9 nickase, a Cpf1 nickase, or another CRISPR-Cas nuclease. In some embodiments, the polypeptide domain having DNA polymerase activity comprises a template-dependent DNA polymerase, for example, a DNA-dependent DNA polymerase or an RNA-dependent DNA polymerase. In some embodiments, the DNA polymerase is a reverse transcriptase. In some embodiments, the prime editor comprises additional polypeptides or polypeptide domains involved in prime editing, for example, a polypeptide domain having 5' endonuclease activity, *e.g.*, a 5' endogenous DNA flap endonucleases (*e.g.*, FEN1), for helping to drive the prime editing process towards the edited product formation. In some embodiments, the prime editor further comprises an RNA-protein recruitment polypeptide, for example, a MS2 coat protein.

**[0258]** In some embodiments, polypeptide domains of a prime editor may be fused or linked by a peptide linker to form a fusion protein. In other embodiments, a prime editor comprises one or more polypeptide domains provided in trans as separate proteins, which are capable of being associated to each other through non-peptide linkages or through aptamers or recruitment sequences. For example, a prime editor may comprise a DNA binding domain and a reverse transcriptase domain associated with each other by an RNA-protein recruitment aptamer, *e.g.*, a MS2 aptamer, which may be linked to a PEGRNA. Prime editor polypeptide components may be encoded by one or more polynucleotides in whole or in part. In some embodiments, a single polynucleotide, construct, or vector encodes the prime editor fusion protein. In some embodiments, multiple polynucleotides, constructs, or vectors each encode a polypeptide domain or portion of a domain of a prime editor, or a portion of a prime editor fusion protein. For example, a prime editor may comprise an N-terminal portion fused to an intein-N and a C-terminal portion fused to an intein-C, each of which is individually encoded by a vector.

**[0259]** In some embodiments, a prime editor polypeptide is fused to one or more nuclear localization signals. In some embodiments, a prime editor polypeptide is fused to a polypeptide permeant domain to promote uptake by the cell. In some embodiments, the permeant domain is a peptide, a peptidomimetic, or a non-peptide carrier.

**[0260]** In some embodiments, a prime editor polypeptide is produced in vitro or by host cells. In some embodiments, a prime editor polypeptide is prepared by in vitro synthesis. Various commercial synthetic apparatuses can be used. In some embodiments, a prime editor

polypeptide is isolated and purified in accordance with recombinant synthesis methods, for example, by expression in a host cell and the lysate purified using HPLC, exclusion chromatography, gel electrophoresis, affinity chromatography, or other purification technique.

[0261] The term “prime editing guide RNA”, or “PEgRNA”, refers to a guide polynucleotide that comprises one or more intended nucleotide edits for incorporation into the target double stranded DNA. In some embodiments, the PEgRNA associates with and directs a prime editor to incorporate the one or more intended nucleotide edits into the target gene via prime editing.

[0262] In some embodiments, a PEgRNA comprises a spacer that is complementary or substantially complementary to a search target sequence on a target strand of the target gene. In some embodiments, the PEgRNA comprises a gRNA core that associates with a DNA binding domain, *e.g.*, a CRISPR-Cas protein domain, of a prime editor. In some embodiments, a PEgRNA comprises an extension arm that comprises an editing template and a primer binding site (PBS). In some embodiments, the editing template comprises one or more intended nucleotide edits to be incorporated in the target DNA by prime editing. In some embodiments, the editing template comprises substantial or partial complementarity to the editing target sequence except at the position of the intended nucleotide edits to be incorporated into the target gene. In some embodiments, the PBS comprises complementarity or substantial complementarity to a free 3' end on the edit strand of the target gene at a nick site generated by the prime editor.

[0263] In some embodiments, a PEgRNA consists of RNA. In some embodiments, a PEgRNA is a chimeric or hybrid PEgRNA that comprises an RNA portion (*e.g.*, including the spacer and the gRNA core) and a DNA portion (*e.g.*, the extension arm comprising the editing template that includes a strand of DNA).

[0264] In some embodiments, a PEgRNA comprises a single polynucleotide molecule that comprises a spacer, a gRNA core, and an extension arm. In some embodiments, a PEgRNA comprises multiple polynucleotide molecules, for example, two polynucleotide molecules.

[0265] In some embodiments, the prime editing system comprises a PEgRNA, a prime editor, and further comprises a second strand nick guide polynucleotide, *e.g.*, a nick guide RNA (ngRNA). In some embodiments, a ngRNA comprises a spacer (referred to as a ngRNA spacer or ng spacer) and a gRNA core, wherein the ng spacer comprises a region of complementarity to the edit strand, and wherein the gRNA core can interact with a Cas, *e.g.*, Cas9, of a prime editor. Without wishing to be bound by any particular theory, an ngRNA

may bind to the edit strand and direct a Cas nickase to generate a nick on the non-edit strand (or target strand).

[0266] In some embodiments, a prime editing system comprises a first prime editing guide RNA (PEgRNA), a second PEgRNA, and one or more prime editors (a dual prime editing system). In some embodiments, dual prime editing involves two different PEgRNAs each complexed with a prime editor. In some embodiments, the prime editor is the same for each of the PEgRNA-prime editor complexes. In some embodiments, the prime editor is different for each of the PEgRNA-prime editor complexes. In some embodiments, the first PEgRNA and the second PEgRNA each comprises a spacer, a gRNA core, and an extension arm comprising a PBS and an editing template. In some embodiments, each of the two PEgRNAs comprises a spacer comprising a region of complementarity to a distinct search target sequence of the double stranded target DNA, wherein the two distinct search target sequences are on the two complementary strands of the double stranded target DNA. In some embodiments, the two PEgRNAs each can direct a prime editor to initiate the prime editing process on the two complementary strands of the double stranded target DNA. In some embodiments, the editing template of the first PEgRNA and the editing template of the second PEgRNA comprises a region of complementarity to each other. In some embodiments, the editing template of the first PEgRNA and the editing template of the second PEgRNA each comprises a region of complementarity to a distinct sequence of the double stranded target DNA.

[0267] In some embodiments, a prime editing system comprises (i) a prime editor or one or more polynucleotides encoding the prime editor, and (ii) a PEgRNA or one or more polynucleotides encoding the PEgRNA. In some embodiments, a prime editing system comprises (i) a prime editor or one or more polynucleotides encoding the prime editor, (ii) a PEgRNA or one or more polynucleotides encoding the PEgRNA, and (iii) a ngRNA or one or more polynucleotides the ngRNA. In some embodiments, a prime editing system is a dual prime editing system that comprises (i) a prime editor or one or more polynucleotides encoding the prime editor, (ii) a first PEgRNA or one or more polynucleotides encoding the PEgRNA, and (iii) a second PEgRNA or one or more polynucleotides encoding the second PEgRNA. In some embodiments, the one or more polynucleotides encoding the prime editor, the PEgRNA, the ngRNA, the first PEgRNA, and/or the second PEgRNA is a part of, or encoded by, an expression cassette, a construct, or a vector. In some embodiments, the vector is a non-viral vector. In some embodiments, the one or more polynucleotides is operably linked to a regulatory element, *e.g.*, a transcriptional control element, such as a promoter. In

some embodiments, the polynucleotide is operably linked to multiple control elements. Depending on the expression system utilized, any of a number of suitable transcription and translation control elements, including constitutive and inducible promoters, transcription enhancer elements, transcription terminators, etc. may be used in the expression vector (*e.g.*, U6 promoter, H1 promoter). Prime editing system components may be encoded individually by separate polynucleotides, or two or more prime editing system components may be encoded by a single polynucleotide. In some embodiments, a prime editor polypeptide and one or more PEGRNA(s) or ngRNA are encoded by a single polynucleotide. In some embodiments, the polynucleotide encodes a prime editor fusion protein comprising a DNA binding domain and a DNA polymerase domain. In some embodiments, the polynucleotide encodes a DNA polymerase domain of a prime editor. In some embodiments, the polynucleotide encodes a DNA binding domain of a prime editor. In some embodiments, the polynucleotide encodes a portion of a prime editor protein, for example, a N-terminal portion of a prime editor fusion protein connected to an intein-N. In some embodiments, the polynucleotide encodes a portion of a prime editor protein, for example, a C-terminal portion of a prime editor fusion protein connected to an intein-C. In some embodiments, the polynucleotide encodes a PEGRNA. In some embodiments, the polynucleotide encodes two or more components of a prime editing composition, for example, a prime editor fusion protein and a PEGRNA.

**[0268]** In some embodiments, a prime editing system comprises one or more polynucleotides that encode prime editor components and/or the first PEGRNA and/or the second PEGRNA. In some embodiments, a prime editing system comprises a polynucleotide encoding a fusion protein comprising a DNA binding domain and a DNA polymerase domain. In some embodiments, a prime editing system comprises (i) a polynucleotide encoding a fusion protein comprising a DNA binding domain and a DNA polymerase domain, (ii) a first PEGRNA or a polynucleotide encoding the first PEGRNA, and (iii) a second PEGRNA or a polynucleotide encoding the second PEGRNA. In some embodiments, a prime editing system comprises (i) a polynucleotide encoding a DNA binding domain of a prime editor, *e.g.*, a Cas9 nickase, (ii) a polynucleotide encoding a DNA polymerase domain of a prime editor, *e.g.*, a reverse transcriptase, (iii) a first PEGRNA or a polynucleotide encoding the first PEGRNA, and (iv) a second PEGRNA or a polynucleotide encoding the second PEGRNA.

**[0269] (x). *Pharmaceutical compositions***

**[0270]** Disclosed herein are pharmaceutical compositions comprising any of the compounds, compositions and/or nanoparticles described herein, and one or more pharmaceutically acceptable excipient.

**[0271]** The compounds and LNP compositions described herein can be formulated into pharmaceutical compositions that further comprise a pharmaceutically acceptable carrier, diluent, adjuvant or vehicle. In one embodiment, the present disclosure provides a pharmaceutical composition comprising a compound described herein, and a pharmaceutically acceptable carrier, diluent, adjuvant or vehicle. In one embodiment, the present disclosure provides a pharmaceutical composition comprising a LNP composition described herein, and a pharmaceutically acceptable carrier, diluent, adjuvant or vehicle.

**[0272]** In some embodiments, a pharmaceutical composition comprises any of the compounds described herein, at least one polynucleotide, and one or more pharmaceutically acceptable excipients. In some embodiments, a pharmaceutical composition comprises any of the compounds described herein, one or more components of a prime editing system, and one or more pharmaceutically acceptable excipients. In some embodiments, a pharmaceutical composition comprises any of the compositions described herein and one or more pharmaceutically acceptable excipients. In some embodiments, a pharmaceutical composition comprises any of the lipid nanoparticles described herein and one or more pharmaceutically acceptable excipients.

**[0273]** The term “pharmaceutical composition”, as used herein, refers to a composition formulated for pharmaceutical use. In some embodiments, the pharmaceutical composition comprises additional agents, *e.g.*, for specific delivery, increasing half-life, or other therapeutic compounds.

**[0274]** In some embodiments, a pharmaceutically-acceptable excipient comprises any vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (*e.g.*, lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the compound from one site (*e.g.*, the delivery site) of the body, to another site (*e.g.*, organ, tissue or portion of the body). A pharmaceutically acceptable carrier is “acceptable” in the sense of being compatible with the other ingredients of the formulation and not injurious to a tissue and/or subject (*e.g.*, physiologically compatible, sterile, physiologic pH, etc.).

**[0275]** A pharmaceutically acceptable carrier may contain inert ingredients that do not unduly inhibit the biological activity of the compounds. The pharmaceutically acceptable

carriers should be biocompatible, *e.g.*, non-toxic, non-inflammatory, non-immunogenic or devoid of other undesired reactions or side-effects upon the administration to a subject.

Standard pharmaceutical formulation techniques can be employed.

[0276] The pharmaceutically acceptable carrier, adjuvant, or vehicle, as used herein, includes any and all solvents, diluents, or other liquid vehicle, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's Pharmaceutical Sciences, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980) discloses various carriers used in formulating pharmaceutically acceptable compositions and known techniques for the preparation thereof. Except insofar as any conventional carrier medium is incompatible with the compounds described herein, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutically acceptable composition, the use of such conventional carrier medium is contemplated to be within the scope of this disclosure.

[0277] Some examples of materials that can serve as pharmaceutically acceptable carriers include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins (such as human serum albumin), buffer substances (such as twin 80, phosphates, glycine, sorbic acid, or potassium sorbate), partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes (such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, or zinc salts), colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, methylcellulose, hydroxypropyl methylcellulose, wool fat, sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil and soybean oil; glycols; such a propylene glycol or polyethylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents. Preservatives

and antioxidants can also be present in the composition, according to the judgment of the formulator.

[0278] Formulations of the pharmaceutical compositions described herein can be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient(s) into association with an excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single- or multi-dose unit. Pharmaceutical formulations can additionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired.

[0279] The pharmaceutical compositions described herein may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. As used herein, the term "parenteral" includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intraocular, intrahepatic, intralesional and intracranial injection or infusion techniques.

[0280] The the pharmaceutical compositions described herein may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents also may be added.

[0281] Alternatively, the pharmaceutical compositions described herein may be administered in the form of suppositories for rectal or vaginal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient that is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum or vaginal cavity to release the drug. Such materials include cocoa butter, polyethylene glycol or a suppository wax that is solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

[0282] The pharmaceutical compositions described herein also may be administered topically, especially when the target of treatment includes areas or organs readily accessible

by topical application, including diseases of the eye, skin, or lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

[0283] Topical application for the lower intestinal tract can be effected in a rectal suppository formulation or in a suitable enema formulation. Topically-transdermal patches also may be used.

[0284] For topical applications, the pharmaceutical compositions described herein may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds or LNP compositions described herein include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical compositions described herein can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

[0285] For ophthalmic use, the pharmaceutical compositions described herein may be formulated, *e.g.*, as micronized suspensions in isotonic, pH adjusted sterile saline or other aqueous solution, or, preferably, as solutions in isotonic, pH adjusted sterile saline or other aqueous solution, either with or without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the pharmaceutical compositions described herein may be formulated in an ointment such as petrolatum. The pharmaceutical compositions described herein also may be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

[0286] Liquid dosage forms for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan,

and mixtures thereof. Besides inert diluents, the oral compositions also can include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

[0287] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions, may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation also may be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables.

[0288] The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions that can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[0289] Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form also may comprise buffering agents.

[0290] Solid compositions of a similar type also may be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric

coatings and other coatings well known in the pharmaceutical formulating art. Solid dosage forms optionally may contain opacifying agents. These solid dosage forms also can be of a composition such that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. Solid compositions of a similar type also may be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

[0291] The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the LNP composition may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms also may comprise, as is normal practice, additional substances other than inert diluents, *e.g.*, tableting lubricants and other tableting aids such as a magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms also may comprise buffering agents. They may optionally contain opacifying agents and also can be of a composition such that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes.

[0292] (xi). *Delivery*

[0293] Also disclosed herein are methods for delivering polynucleotides or prime editing system(s) into a target cell by utilizing the LNP compositions described herein.

[0294] In some embodiments, the LNP compositions described herein are used to deliver a prime editing system, a DNA plasmid encoding prime editor components, a DNA plasmid encoding a PEGRNA and optionally an ngRNA, a DNA plasmid encoding a PEGRNA pair, a DNA plasmid encoding a prime editor fusion protein, or one or more DNA plasmids encoding one or more of a prime editor fusion protein, a PEGRNA, an ngRNA, or a PEGRNA pair.

[0295] In some embodiments, the LNP compositions described herein are used to deliver an mRNA encoding prime editor components, an mRNA encoding prime editor fusion protein, a PEGRNA and optionally ngRNA, or a PEGRNA pair.

[0296] In some embodiments, the LNP compositions described herein are used to deliver a prime editor protein, a prime editor fusion protein, a PEgRNA and optionally ngRNA, or a PEgRNA pair.

[0297] In some embodiments, a method for delivering polynucleotides into a cell comprising:

introducing into the cell at least one lipid nanoparticle comprising a GalNAc-based lipid that comprises any of the compounds described herein and one or more polynucleotides.

[0298] In some embodiments, the polynucleotides are DNA or mRNA.

[0299] In some embodiments, a method for delivering a prime editing system into a cell comprising:

introducing into the cell at least one lipid nanoparticle comprising a GalNAc-based lipid that comprises any of the compounds described herein and one or more components of a prime editing system.

[0300] In some embodiments, the one or more components of a prime editing system comprises a construct encoding prime editor components. In some embodiments, the construct is a DNA plasmid encoding prime editor components.

[0301] In some embodiments, the one or more components of a prime editing system comprises a construct encoding a prime editing guide RNA (PEgRNA), and optionally a nick guide RNA (ngRNA). In some embodiments, the one or more components of a prime editing system comprises a construct encoding a PEgRNA pair, wherein the PEgRNA comprises a first PEgRNA and/or a second PEgRNA. In some embodiments, the construct is a DNA plasmid encoding a prime editing guide RNA (PEgRNA), and optionally a nick guide RNA (ngRNA). In some embodiments, the construct is a DNA plasmid encoding a PEgRNA pair, wherein the PEgRNA comprises a first PEgRNA and/or a second PEgRNA.

[0302] In some embodiments, the prime editor components comprise a prime editing guide RNA (PEgRNA), and optionally a nick guide RNA (ngRNA). In some embodiments, the prime editor components comprise a PEgRNA pair, wherein the PEgRNA comprises a first PEgRNA and/or a second PEgRNA.

[0303] In some embodiments, the one or more components of a prime editing system comprises a construct encoding a prime editor fusion protein. In some embodiments, the construct is a DNA plasmid encoding a prime editor fusion protein.

[0304] In some embodiments, the one or more components of a prime editing system comprises one or more constructs encoding one or more of a prime editor fusion protein,

PEgRNA, ngRNA, or a PEgRNA pair. In some embodiments, the one or more constructs are one or more DNA plasmids encoding one or more of a prime editor fusion protein, PEgRNA, ngRNA, or a PEgRNA pair.

[0305] In some embodiments, the one or more components of a prime editing system comprises an mRNA encoding prime editor components. In some embodiments, the one or more components of a prime editing system comprises an mRNA encoding a prime editor fusion protein.

[0306] In some embodiments, the prime editor components comprise a prime editing guide RNA (PEgRNA), and optionally a nick guide RNA (ngRNA). In some embodiments, the prime editor components comprise a PEgRNA pair, wherein the PEgRNA comprises a first PEgRNA and/or a second PEgRNA.

[0307] In some embodiments, the one or more components of a prime editing system comprises a PEgRNA, and optionally an ngRNA. In some embodiments, the one or more components of a prime editing system comprises a PEgRNA pair. In some embodiments, the one or more components of a prime editing system comprises a prime editor protein, a prime editor fusion protein, a PEgRNA, a ngRNA, or a PEgRNA pair.

[0308] In some embodiments, the LNP composition is formulated to deliver at least one polynucleotide or one or more components of the prime editing system to target cells.

[0309] In some embodiments, wherein the target cells are or comprise liver cells, bone marrow cells, lung cells, eye cells, muscle cells, or cells in the central nervous system.

[0310] In some embodiments, the first and second lipid nanoparticles are the same. In some embodiments, the first and second lipid nanoparticles are different.

[0311] In some embodiments, the first and second lipid nanoparticles are introduced into the cell simultaneously. In some embodiments, the first and second lipid nanoparticles are introduced into the cell sequentially.

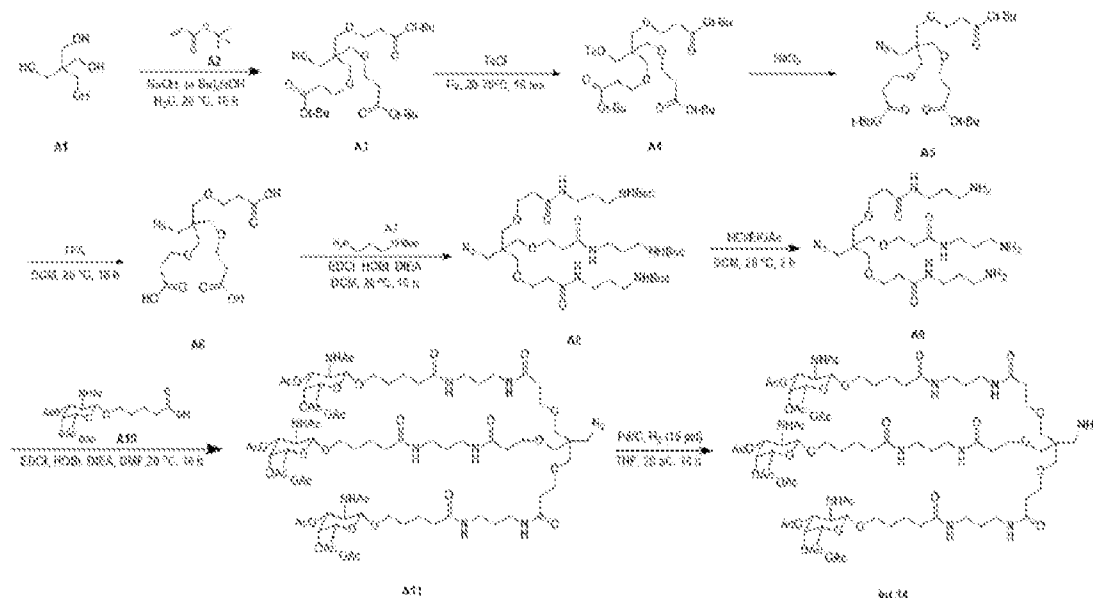
[0312] Delivery to the cells can be in vitro, via ex vivo administration, or via in vivo administration.

[0313] In some embodiments, the target cells are or comprise human cells, mammalian cells, liver cells (*e.g.*, hepatocytes), bone marrow cells (*e.g.*, bone marrow monocytes), lung cells, eye cells, muscle cells, or cells in the central nervous system.

## EXAMPLES

**[0314] General LC/MS Method:** Mobile phase A was 0.04% trifluoroacetic acid in water, mobile phase B was 0.02% trifluoroacetic acid in acetonitrile. The flow rate was 1 ml/min. The column used for chromatography was a Halo C18- 30\*3mm (5  $\mu$ m particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection as well as positive electrospray ionization. MS range was 50-2000.

### [0315] Example 1. Preparation of Int-14



**Scheme 5: Synthesis of Int-14**

### [0316] Preparation of Compound A3

**[0317]** To a solution of 2,2-bis(hydroxymethyl)propane-1,3-diol (**A1**, 30 g, 220.35 mmol) and (n-Bu)<sub>4</sub>NOH (11.43 g, 22.04 mmol, 50% purity) in tert-butyl acrylate (**A2**, 90.37 g, 705.13 mmol) was added slowly NaOH (1.76 g, 44.07 mmol) in H<sub>2</sub>O (3.4 mL) at 20°C. The mixture was stirred at 20 °C for 16 h. TLC (PE : EtOAc = 5:1, R<sub>f</sub> = 0.5) showed the Reactant 1 was consumed. One new spot was detected. The mixture was added water (1000 ml) solution and extracted with ethyl acetate (6 × 200 ml). Then combined organic phase was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (SiO<sub>2</sub>, PE:EtOAc = 1:0 to 0:1) to give desired compound **A3** (50 g, 47 %) as a colorless oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  3.70 - 3.59 (m, 8H), 3.44 (s, 6H), 2.46 (t, *J* = 6.4 Hz, 6H), 1.46 (s, 26H).

### [0318] Preparation of Compound A4

**[0319]** To a solution of compound **A3** (40 g, 83.58 mmol) in Pyridine (1500 mL) was added slowly TsCl (26.53 g, 376.12 mmol) at 20°C. The mixture was stirred at 70 °C for 16 hrs.

TLC (PE : EtOAc = 5:1, R<sub>f</sub> = 0.7) showed the Reactant 1 was consumed. One new spot was detected. The mixture was added water (1000 ml) solution and extracted with ethyl acetate (6 × 200 ml). Then combined organic phase was washed with brine (1000 mL × 3), dried over anhydride Na<sub>2</sub>SO<sub>4</sub>, concentrated under reduced pressure. The residue was purified by silica gel column chromatography (SiO<sub>2</sub>, PE:EtOAc = 1:0 to 0:1) to give desired compound **A4** (38 g, 67% yield) as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>-d) δ 7.77 (d, *J* = 8.4 Hz, 2H), 7.34 (d, *J* = 8.0 Hz, 2H), 4.00 (s, 2H), 3.53 (t, *J* = 6.4 Hz, 6H), 3.33 (s, 6H), 2.46 (s, 3H), 2.37 (t, *J* = 6.4 Hz, 6H), 1.44 (s, 27H).

**[0320] Preparation of Compound A5**

**[0321]** To a solution of compound **A4** (38 g, 56.31 mmol) in DMSO (400 mL) and H<sub>2</sub>O (40 mL) was added slowly NaN<sub>3</sub> (10.04 g, 154.44 mmol) at 20°C. The mixture was stirred at 120 °C for 40 hrs. TLC (PE : EtOAc = 3:1, R<sub>f</sub> = 0.7) showed the Reactant 1 was consumed. One new spot was detected. The reaction mixture was added H<sub>2</sub>O (50 mL) and sat. NaOH solution, adjusted pH to 10 and extracted with EtOAc (3 x 800 mL). The combined organic layers were washed with brine (800 × 3 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (SiO<sub>2</sub>, PE:EtOAc = 1:0 to 0:1) to give desired compound **A5** (26 g, 85% yield) as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 3.62 (t, *J* = 6.4 Hz, 6H), 3.35 (s, 6H), 3.31 (s, 2H), 2.46 (t, *J* = 6.4 Hz, 6H), 1.46 (s, 27H).

**[0322] Preparation of Compound A6**

**[0323]** A solution of Cpd.**A5** (26 g, 47.65 mmol) in TFA (30 mL) and DCM (150 mL) was stirred at 20°C. The mixture was stirred at 20 °C for 16 h. TLC (PE : EtOAc = 3:1, R<sub>f</sub> = 0.2) showed the Reactant 1 was consumed. One new spot was detected. The reaction mixture was quenched by H<sub>2</sub>O (1000 mL) and extracted with EtOAc (3 × 800 mL). The combined organic layers were washed with brine (1000 × 3 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure. The reaction mixture was concentrated under reduced pressure to give Cpd.**A6** (20 g, crude) as a brown oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.55 - 6.12 (m, 4H), 3.69 (t, *J* = 6.4 Hz, 6H), 3.45 - 3.22 (m, 6H), 2.62 (t, *J* = 6.0 Hz, 6H).

**[0324] Preparation of Compound A8**

**[0325]** To a solution of Cpd.**A6** (16.5 g, 43.73 mmol) and Cpd.**A7** (30.48 g, 174.91 mmol) in DCM (200 mL) was added HOBt (23.63 g, 174.91 mmol), EDCI (33.53 g, 174.91 mmol) and DIEA (33.91 g, 262.36 mmol) at 20 °C. The mixture was stirred at 20 °C for 16 hrs. TLC (DCM : MeOH = 10:1, R<sub>f</sub> = 0.1) showed the Reactant 1 was consumed. One new spot was detected. The reaction mixture was quenched by H<sub>2</sub>O (400 mL) and extracted with DCM (3 ×

200 mL). The combined organic layers were washed with brine (1000 mL× 3), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM:MeOH=1:0 to 1:1) to give Cpd. **A8** (23 g, 62.17% yield) as a brown oil. <sup>1</sup>H NMR (400 MHz, MeOD-d<sub>4</sub>) δ 3.64 (t, *J* = 6.0 Hz, 6H), 3.33 (s, 6H), 3.29 (br s, 2H), 3.22 (t, *J* = 6.8 Hz, 6H), 3.07 (t, *J* = 6.8 Hz, 6H), 2.41 (t, *J* = 6.0 Hz, 6H), 1.68 - 1.61 (m, 3H), 1.44 (s, 33H).

**[0326] Preparation of Compound A9**

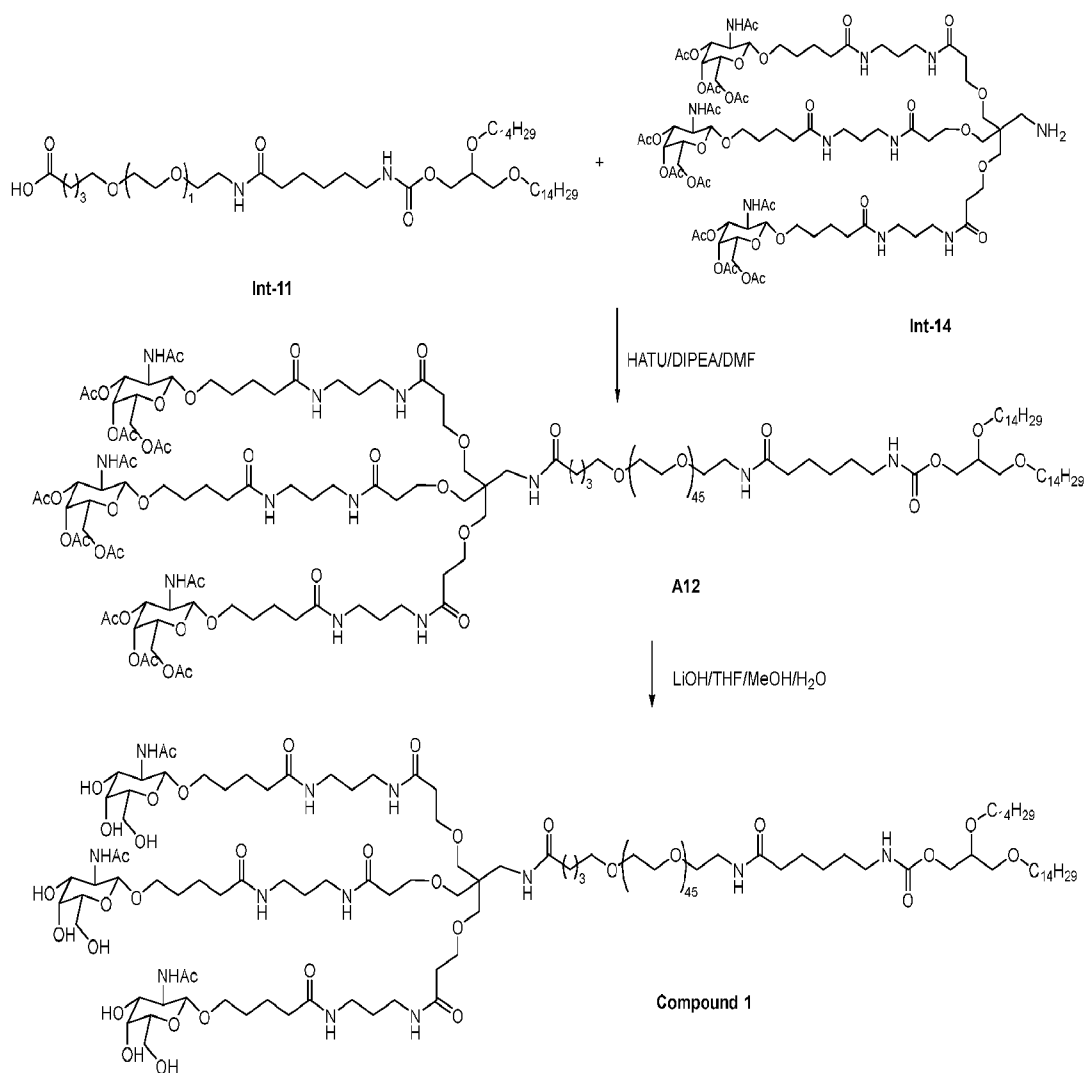
**[0327]** To a solution of Cpd.**A8** (23 g, 27.19 mmol) in EtOAc (200 mL) was added HCl/EtOAc (4 M, 40 mL) at 20 °C. The mixture was stirred at 20 °C for 2 h. TLC (DCM : MeOH = 5:1, R<sub>f</sub> = 0.01) showed the Reactant 1 was consumed. One new spot was detected. The reaction mixture was filtered, and the filter cake was dried under reduced pressure to give Cpd.**A9** (20 g, crude) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 8.26 - 8.04 (m, 12H), 3.55 (br t, *J* = 6.4 Hz, 6H), 3.22 (s, 6H), 3.17 - 3.06 (m, 6H), 2.82 - 2.71 (m, 6H), 2.32 (br t, *J* = 6.4 Hz, 6H), 1.71 (quin, *J* = 6.8 Hz, 6H).

**[0328] Preparation of Compound A11**

**[0329]** To a solution of Cpd.**A9** (10 g, 18.33 mmol) and Cpd. **A10** (32.80 g, 73.32 mmol) in DMF (300 mL) was added HATU (27.87 g, 73.32 mmol) and DIEA (23.69 g, 183.30 mmol) at 20 °C. The mixture was stirred at 20 °C for 16 h. TLC (DCM : MeOH = 8:1, R<sub>f</sub> = 0.3) showed the Reactant 1 was consumed. One new spot was detected. indicated Reactant 1 was consumed, and one major new spot with lower polarity was detected. The reaction mixture was quenched by H<sub>2</sub>O (1000 mL) and extracted with EtOAc (3 × 800 mL). The combined organic layers were washed with brine (1000 × 3 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (SiO<sub>2</sub>, DCM: MeOH= 1:0 to 0:1) to give Cpd. **A11** (5 g, 15% yield) as a brown oil. <sup>1</sup>H NMR (400 MHz, MeOD-d<sub>4</sub>) δ 5.30 (d, *J* = 3.2 Hz, 3H), 5.02 (dd, *J* = 3.2, 11.2 Hz, 3H), 4.52 (d, *J* = 8.4 Hz, 3H), 4.18 - 4.07 (m, 6H), 4.07 - 3.93 (m, 6H), 3.88 - 3.78 (m, 3H), 3.61 (t, *J* = 6.0 Hz, 6H), 3.54 - 3.44 (m, 3H), 3.30 (s, 6H), 3.27 (dt, *J* = 1.2, 3.2 Hz, 6H), 3.22 - 3.14 (m, 12H), 2.38 (t, *J* = 6.0 Hz, 6H), 2.20 - 2.13 (m, 6H), 2.11 (s, 9H), 1.99 (s, 9H), 1.90 (d, *J* = 7.6 Hz, 18H), 1.73 - 1.53 (m, 18H).

**[0330] Preparation of Compound Int-14**

**[0331]** To a solution of Cpd.**A11** (3 g, 1.64 mmol) in THF (48 mL) was added Pd/C (1.6 g, 10% purity) at 20 °C. The mixture was stirred at 20 °C for 16 h. TLC indicated the Reactant 1 was consumed, and one new spot was detected. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give **Int-14** (2.3 g, 77% yield) as an off-white solid. LCMS:  $t_R = 1.732$  min, 97.74% purity,  $m/z = 1808.0$  (M+H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  8.07 - 7.72 (m, 9H), 5.27 (d,  $J = 3.2$  Hz, 3H), 5.03 (dd,  $J = 3.2, 11.2$  Hz, 3H), 4.55 (d,  $J = 8.4$  Hz, 3H), 4.09 (s, 8H), 3.99 - 3.87 (m, 3H), 3.83 - 3.72 (m, 3H), 3.66 (br t,  $J = 6.4$  Hz, 2H), 3.63 - 3.53 (m, 6H), 3.52 - 3.41 (m, 4H), 3.32 (s, 5H), 3.10 (quin,  $J = 6.0$  Hz, 12H), 2.64 (br s, 2H), 2.35 (br t,  $J = 6.0$  Hz, 6H), 2.17 (s, 9H), 2.11 (br t,  $J = 6.8$  Hz, 6H), 2.06 (s, 9H), 1.95 (s, 9H), 1.84 (s, 9H), 1.62 - 1.47 (m, 18H).

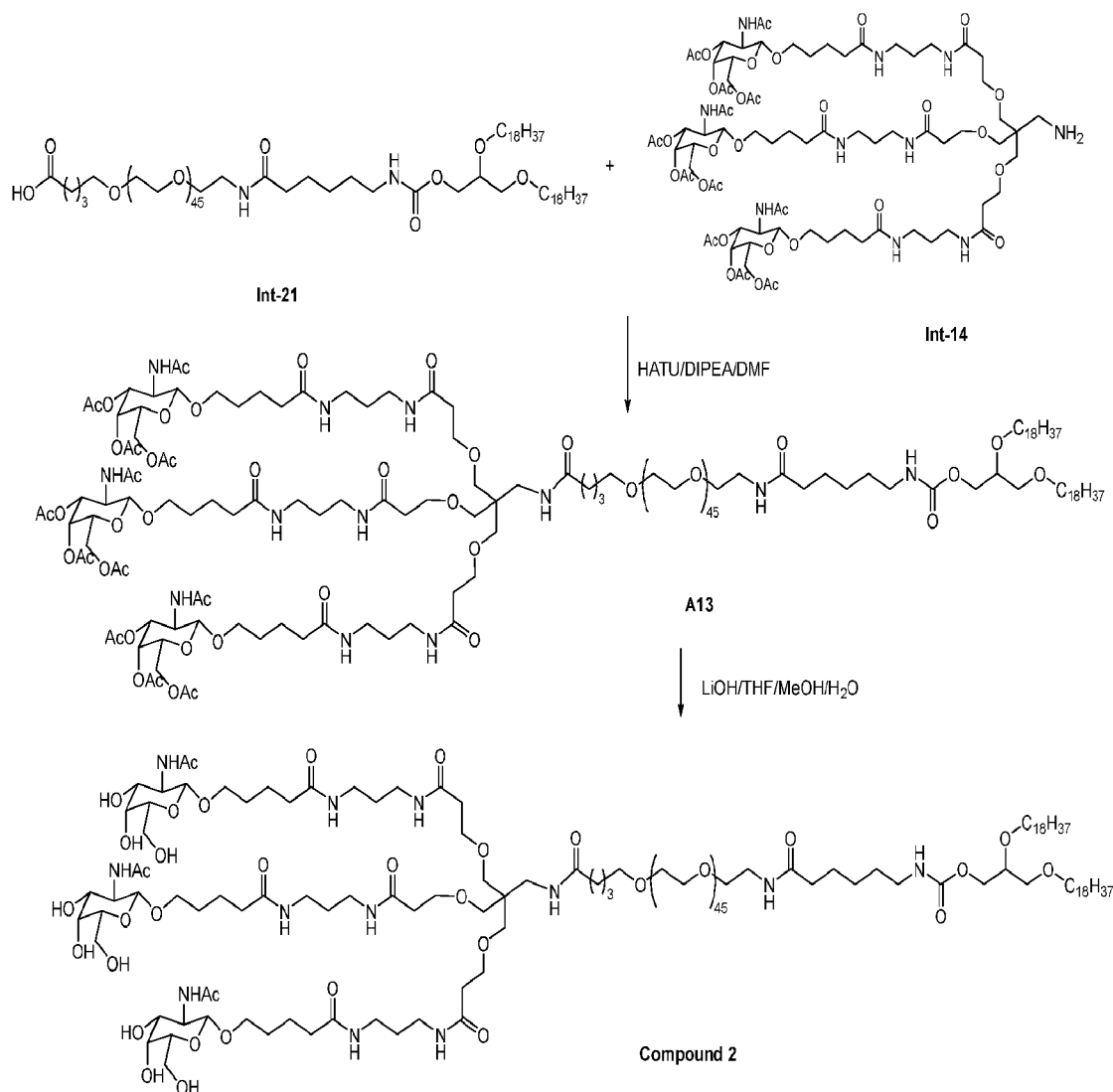
**[0332] Example 2. Preparation of Compound 1****Scheme 6: Synthesis of Compound 1**

**[0333] Preparation of A12**

**[0334]** To a solution of DMG PEG<sub>45</sub> acid (**Int-11**, 276 mg, 1.0 equiv) in DMF (4.0 mL), HATU (45 mg, 1.2 equiv) and DIPEA (0.5 mL) were added, the mixture was stirred for 30 mins at room temperature, then **Int-14** (180 mg, 1.0 equiv) was added, the resulting mixture was stirred for 48 hours at room temperature, the solvents were removed under vacuum, the water bath temp is 55-60 ° C, DCM was added, washed with brine, dried with MgSO<sub>4</sub>, filtered, concentrated, the crude was purified with combi-flash (24 g column, 0-20% MeOH in DCM), collected 197 mg desired product. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>), δ, 8.69 (s, 1H), 8.31 (s, 1H), 7.54-7.40(m, 3H), 7.21-7.08(m, 3H), 6.86-6.92(m, 3H), 5.35-5.43(s, 3H), 5.27-5.16 (m, 3H), 4.67 (d, *J* = 8.4 Hz, 1H), 3.77- 4.20(m, 20 H), 3.04-3.78 (m, 184H), 2.16 (s, 9H), 2.06 (s, 9H), 2.01(s, 9H), 1.97(s, 9H), 1.69-1.45(m, 40H), 1.27 (s, br, 43H), 0.89 (d, *J* = 7.0 Hz, 1H).

**[0335] Preparation of Compound 1**

**[0336]** To a solution of **A12** (185 mg) in THF (2.0 mL) and MeOH (2.0 mL), H<sub>2</sub>O (1.0 mL) was added, followed by addition of LiOH (24 mg), the mixture was stirred at room temperature for 4 hours, cooled with ice water, the pH was adjusted to 5-6 with 50% HCL aqueous solution, concentrated, the crude product was uploaded to C-18 column (24 g), eluted with 100% water in 15 mins, then raise the ACN to 100%, then replaced ACN with 100% MeOH, the major peak was eluted out at 50 mins, after concentration, collected 101 mg desired product. Calculated average MW: 4053. Observed MALDI-TOF average (MS+44): 4097. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>), 4.34-4.42(d, 3H), 4.01-4.17(m, 3H), 3.72-4.0(m, 17H), 3.44-3.73(m, 210H), 3.17-3.43(m, 39H), 3.06-3.17(m, 3H), 2.38-2.51(m, 6H), 2.16-2.35(m, 11H), 2.01(s, 9H), 1.25-1.81(m, 80H), 0.89 (d, *J* = 7.0 Hz, 1H).

**[0337] Example 3. Preparation of Compound 2****Scheme 7: Synthesis of Compound 2****[0338] Preparation of A13**

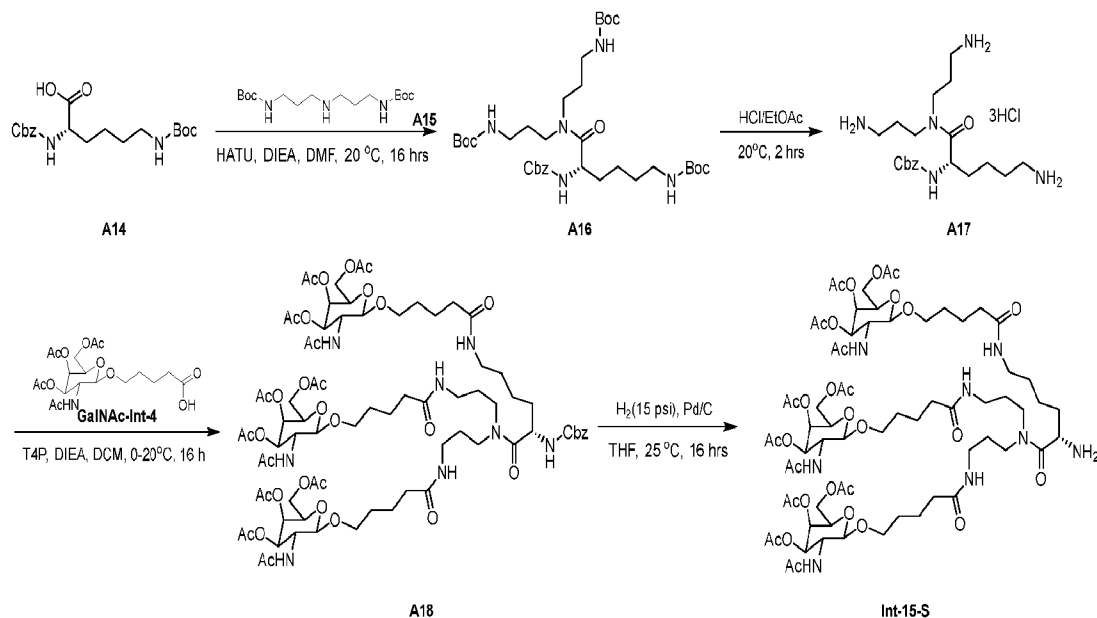
**[0339]** To a solution of **Int-21** (150 mg, 1.0 equiv) in DMF (4.0 mL), HATU (38 mg, 1.2 equiv) was added, followed by addition of DIPEA (0.5 mL), the mixture was stirred for 30 mins at room temperature, then **Int-14** was added, the resulting mixture was stirred for 48 hrs at room temperature, the solvents were evaporated under vacuum, the water bath temperature is around 55 - 60 °C, DCM was added and washed with brine, dried with  $\text{MgSO}_4$ , filtered, concentrated, the crude product was purified with combi-flash (24 g column, 0-20% MeOH in DCM), collected 41 mg desired product. From  $^1\text{H}$  NMR, diagnostic peaks three sugar  $\text{CH}_3\text{COO}$ , single peak of  $\text{NHCOCH}_3$ , lipid tail  $\text{CH}_3$ , and PEG ( $\text{OCH}_2\text{CH}_2\text{O}$ ) was observed.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ),  $\delta$  8.69 (s, 1H), 8.31 (s, 1H), 7.54-7.40(m, 3H), 7.21-7.08(m, 3H), 6.86-6.92(m, 3H), 5.35-5.43(s, 3H), 5.27- 5.16 (m, 3H), 4.67 (d,  $J = 8.4$  Hz, 1H), 3.77-

4.20(m, 20 H), 3.04-3.78 (m, 184H), 2.46 (s, br, 8H), 2.16 (s, 9H), 2.06 (s, 9H), 2.01(s, 9H), 1.97(s, 9H), 1.69-1.46(m, 58H), 1.27 (s, br, 46H), 0.89 (d,  $J = 7.0$  Hz, 1H).

#### [0340] Preparation of Compound 2

[0341] To a solution of **A13** (41 mg in THF (2.0 mL), MeOH (2.0 mL) and H<sub>2</sub>O (1.0 mL), LiOH (20 mg) was added, the mixture was stirred for 4 hrs at room temperature, cooled with ice bath, pH was adjusted to 5-6 with 50% HCl aq solution, concentrated, the crude product was dissolved in water, loaded on C-18 column, eluted with water (flow rate: 15 mL/min), after salt peak appeared, then raise the ACN from 0 - 100%, then change ACN to 100% MeOH, the desired product peak was collected and obtained 17 mg pure compound. Observed MALDI-TOF average MW: 4200 matches the calculated MW: 4200. From H NMR, diagnostic peaks sugar CH<sub>3</sub>COO was deprotected, single peak of NHCOCH<sub>3</sub>, lipid tail CH<sub>3</sub>, and PEG (OCH<sub>2</sub>CH<sub>2</sub>O) was observed. Calculated average MW: 4179.3. Observed MALDI-TOF average MS: 4267.3 <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>), 4.67 (d,  $J = 8.4$  Hz, 3H), 3.77- 4.20(m, 20 H), 3.04-3.78 (m, 184H), 2.46 (s, br, 8H), 2.01(s, 9H), 1.97(s, 9H), 1.69-1.46(m, 58H), 1.27 (s, br, 46H), 0.89 (d,  $J = 7.0$  Hz, 1H).

#### [0342] Example 4. Preparation of Compound 17



Scheme 8: Synthesis of Int-15-S

#### [0343] Preparation of Compound A16

[0344] To a solution of **Cpd.A14** (6.31 g, 16.59 mmol) and **Cpd.A15** (5 g, 15.09 mmol) in DMF (50 mL) was added HATU (8.60 g, 22.63 mmol) and DIEA (5.85 g, 45.26 mmol) at 20 °C. The mixture was stirred at 20 °C for 16 hr. TLC showed Reactant 1 was consumed completely and one new spot was detected. The mixture was quenched with H<sub>2</sub>O (50 mL) and the aqueous phase was extracted with EtOAc (50 mL x 3). The combined

organic layers were separated, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (SiO<sub>2</sub>, PE:EtOAc = 1:0 to 2:1) to give **Cpd.A16** (6.5 g, 62% yield) as a yellowish oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.40 - 7.28 (m, 5H), 5.53 (br d, *J* = 7.6 Hz, 1H), 5.26 (br s, 1H), 5.22 - 5.15 (m, 1H), 5.15 - 5.03 (dd, *J* = 7.6, 12.0 Hz, 2H), 4.81 - 4.48 (br s, 1H), 4.63 - 4.55 (m, 1H), 3.59 (m, 1H), 3.38 (m, 1H), 3.32 - 3.16 (m, 3H), 3.10 (br s, 4H), 3.00 - 2.96 (m, 1H), 1.90 - 1.78 (m, 2H), 1.70 - 1.58 (m, 4H), 1.49 - 1.38 (m, 31H).

**[0345] Preparation of Compound A17**

**[0346]** To a solution of **Cpd.A16** (49 g, 70.62 mmol) in DCM (400 mL) was added HCl/EtOAc (4 M, 40 mL). The mixture was stirred at 20 °C for 2 hrs. TLC indicated Reactant 1 was consumed completely and one new spot formed. The mixture was concentrated under reduced pressure to give **Cpd.A17** (20 g, 72% yield) as a white solid. <sup>1</sup>H NMR (400 MHz, MeOD-d<sub>6</sub>) δ 7.45 - 7.25 (m, 5H), 5.09 (q, *J* = 12 Hz, 2H), 4.52 - 4.43 (m, 1H), 3.61 - 3.53 (m, 4H), 3.50 - 3.39 (m, 1H), 3.11 - 3.01 (m, 2H), 2.98 - 2.84 (m, 4H), 2.24 - 2.05 (m, 2H), 1.98 - 1.88 (m, 2H), 1.81 - 1.65 (m, 4H), 1.59 - 1.43 (m, 2H).

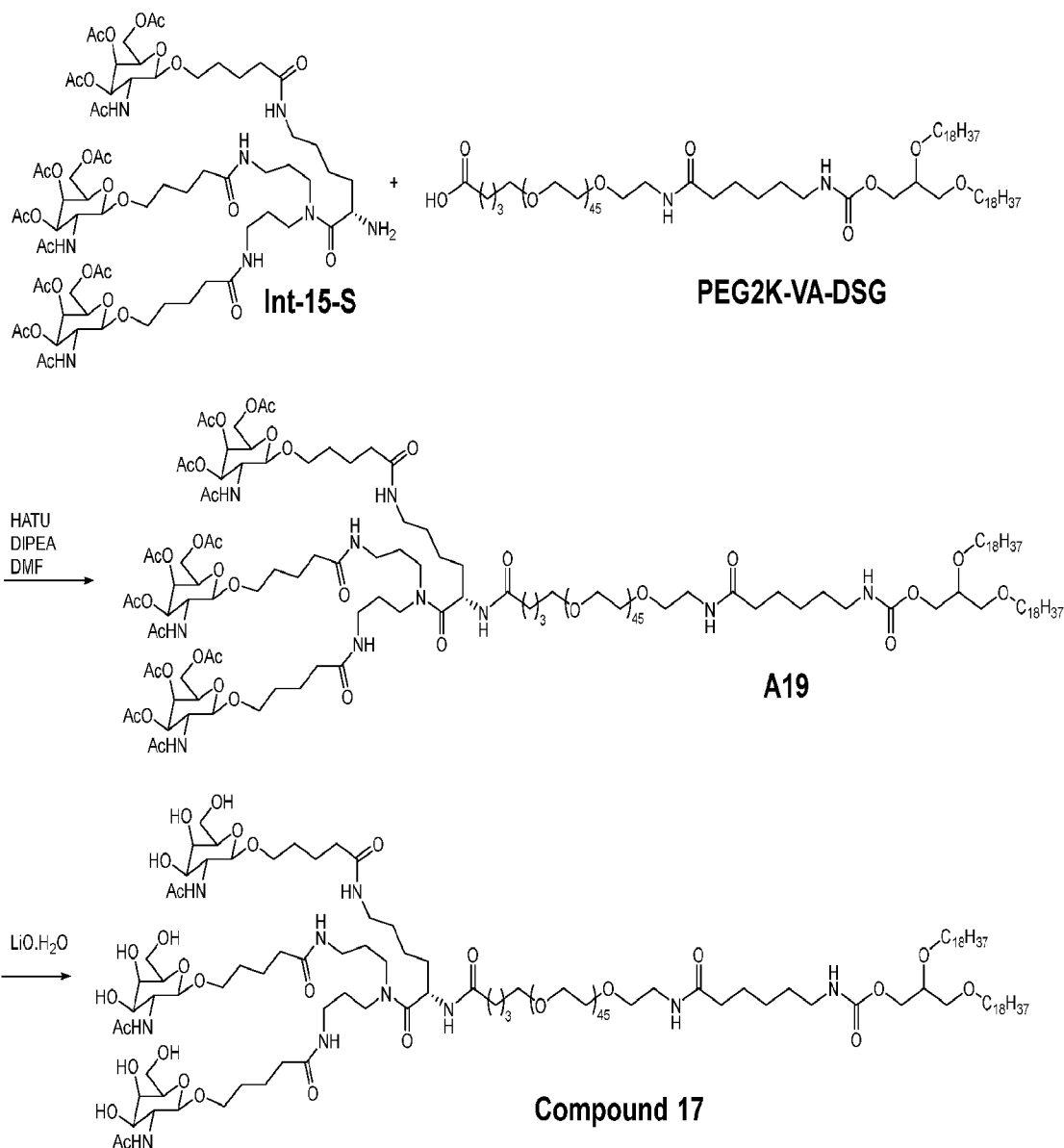
**[0347] Preparation of Compound A18**

**[0348]** A mixture of **GalNac-Int-4** (13.79 g, 30.82 mmol), **Cpd.A17** (5 g, 9.94 mmol, 3HCl), DIEA (12.85 g, 99.42 mmol, 17.32 mL) in DCM (200 mL) was degassed and purged with N<sub>2</sub> for 3 times, was added T4P (25.07 g, 34.80 mmol, 50% purity) at 0 °C and then the mixture was stirred at 20 °C for 16 h under N<sub>2</sub> atmosphere. TLC showed Reactant 1 was consumed completely and one new spot was detected. The mixture was quenched with H<sub>2</sub>O (50 mL) and the aqueous phase was extracted with DCM (30 mL x 3). The combined organic layers were separated, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM:MeOH = 1/0 to 5/1) for twice to give **Cpd.A18** (9 g, 54% yield) as a brown solid. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 7.92 - 7.62 (m, 6H), 7.53 - 7.22 (m, 5H), 5.21 (d, *J* = 3.2 Hz, 3H), 4.98 (dt, *J* = 2.4, 10.8 Hz, 5H), 4.49 (d, *J* = 8.4 Hz, 3H), 4.35 - 4.18 (m, 1H), 4.13 - 3.95 (m, 9H), 3.87 (q, *J* = 9.6 Hz, 3H), 3.76 - 3.66 (m, 3H), 3.65 - 3.55 (m, 1H), 3.46 - 3.36 (m, 3H), 3.29 (br d, *J* = 5.2 Hz, 2H), 3.21 - 2.84 (m, 8H), 2.10 (s, 8H), 2.07 - 2.01 (m, 5H), 1.99 (s, 9H), 1.89 (s, 9H), 1.77 (s, 9H), 1.61 - 1.31 (m, 18H).

**[0349] Preparation of Compound Int-15-S**

**[0350]** To a solution of **Cpd.A18** (3.0 g, 1.79 mmol) in THF (150 mL) was added Pd/C (1.5 g, 1.41 mmol, 10% purity) at 25 °C. The mixture was stirred at 25 °C for 16 hrs under H<sub>2</sub> (15 psi). LCMS indicated the Reactant 1 was consumed, and the desired ms was detected. The

reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give **GalNac-Int-15-S** (2.3 g, 1.49 mmol) as a gray solid. **LCMS**:  $t_R = 1.766$  min, 97.29% purity,  $m/z = 1547.1$  ( $M+H$ )<sup>+</sup>. **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  7.90 - 7.65 (m, 6H), 5.21 (d,  $J = 3.2$  Hz, 3H), 4.97 (dd,  $J = 3.2, 11.2$  Hz, 3H), 4.48 (d,  $J = 8.4$  Hz, 3H), 4.02 (s, 8H), 3.93-3.81 (m, 3H), 3.77 - 3.66 (m, 3H), 3.60 (m, 4H), 3.51 - 3.33 (m, 6H), 3.28 - 3.14 (m, 2H), 3.12 - 2.91 (m, 7H), 2.10 (s, 9H), 2.07 - 2.01 (m, 6H), 2.00 (s, 9H), 1.89 (s, 9H), 1.77 (s, 9H), 1.69 - 1.60 (m, 2H), 1.59 - 1.39 (m, 16H), 1.35 - 1.32 (m, 2H).



**Scheme 9: Synthesis of Compound 17**

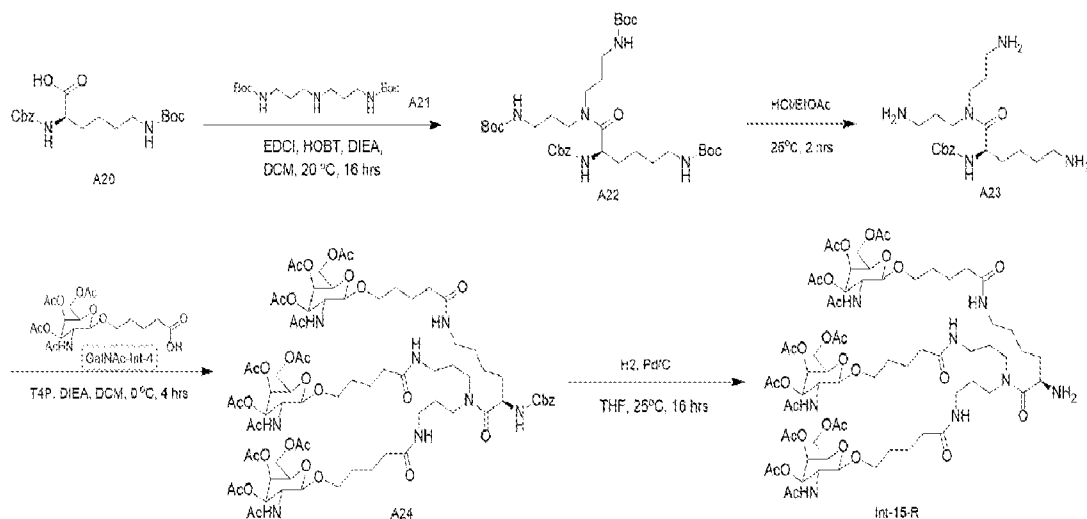
**[0351] Preparation of A19**

**[0352]** To a solution of **PEG2K-VA-DSG** (98 mg, 0.035 mmol) in DMF (1.0 mL), HATU (14 mg, 0.035 mmol) was added, followed by the addition of DIPEA (0.02 mL), the mixture

was stirred for 15 mins at room temperature, then **Int-15-S** (50 mg, 0.032 mmol) was added, the resulting mixture was stirred for 48 hours at room temperature, the solvents were removed under vacuum, the crude product was dissolved in DCM, washed with brine, dried with MgSO<sub>4</sub>, filtered, concentrated, the crude product was purified with Combi-flash (24 g gold normal phase column, 0-50% MeOH in DCM), collected 100 mg product (72 % yield). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.38 (d, J = 3.3 Hz, 3H), 5.33 – 5.15 (m, 2H), 4.85 (s, 1H), 4.68 (q, J = 7.8 Hz, 2H), 4.15 (dd, J = 17.7, 10.5 Hz, 9H), 3.94 (d, J = 6.6 Hz, 5H), 3.75 (dt, J = 13.9, 6.9 Hz, 5H), 3.67 (s, 162H), 3.63 – 3.42 (m, 20H), 3.15 (q, J = 7.5 Hz, 8H), 2.25 – 1.88 (m, 36H), 1.63 (ddt, J = 29.0, 14.2, 7.0 Hz, 25H), 1.47 (dq, J = 10.5, 6.4 Hz, 30H), 1.28 (s, 52H), 0.90 (t, J = 6.7 Hz, 6H).

**[0353] Preparation of Compound 17**

**[0354]** To a solution of **A19** (100 mg) in THF (2.0 mL) and MeOH (2.0 mL), H<sub>2</sub>O (1.0 mL) was added, followed by addition of LiOH (28 mg), the mixture was stirred at room temperature for 4 hours, cooled with ice water, the pH was adjusted to 5-6 with 10% HCl aqueous solution, concentrated, the crude product was uploaded to C-18 column (24 g), eluted with 100% water in 15 mins, then raise the ACN to 100%, then replaced ACN with 100% MeOH, the major peak was eluted out at 50 mins, after concentration, collected 40 mg of Compound 17 with 43 % yield. <sup>1</sup>H NMR (400 MHz, MeOD) δ 4.44 – 4.33 (m, 3H), 4.00 – 3.71 (m, 23H), 3.66 (s, 167H), 3.58 – 3.43 (m, 30H), 3.24 – 3.06 (m, 19H), 2.35 – 2.17 (m, 12H), 2.03 – 1.98 (m, 9H), 1.76 – 1.50 (m, 34H), 1.31 (s, 52H), 0.92 (t, J = 6.7 Hz, 6H). MS (MALDI\_TOF) m/z [M+Na]<sup>+</sup> calculated for 4051.05 ; found for 4051.57.

**[0355] Example 5. Preparation of Compound 19****Scheme 10: Synthesis of Int-15-R****[0356] Preparation of Compound A22**

**[0357]** To a mixture of **Cpd. 20** (20 g, 60.34 mmol) and **Cpd.21** (25.25 g, 66.38 mmol) in DMF (300 mL) was added DIEA (23.40 g, 181.02 mmol) and HATU (34.42 g, 90.51 mmol) at 20 °C under N<sub>2</sub>. The mixture was stirred 20 °C for 16 hrs. The reaction mixture was quenched by H<sub>2</sub>O (200 mL) and extracted with EtOAc (3 × 200mL). The combined organic layers were washed with brine (300 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (SiO<sub>2</sub>, PE:EtOAc = 1:0 to 0:1) to give **Cpd.A22** (40 g, 96% yield) as white solid. <sup>1</sup>H NMR (MHz, CDCl<sub>3</sub>-d) δ 7.43 - 7.30 (m, 5H), 5.51 (br d, *J* = 8.0 Hz, 1H), 5.25 (br s, 1H), 5.18 - 5.13 (m, 1H), 5.12 - 5.02 (m, 2H), 4.69 (br d, *J* = 2.8 Hz, 1H), 4.62 - 4.53 (m, 1H), 3.69 - 3.52 (m, 1H), 3.48 - 3.35 (m, 1H), 3.34 - 3.17 (m, 3H), 3.10 (br s, 4H), 3.02 - 2.87 (m, 1H), 1.92 - 1.75 (m, 2H), 1.74 - 1.56 (m, 5H), 1.56 - 1.39 (m, 31H).

**[0358] Preparation of Compound A23**

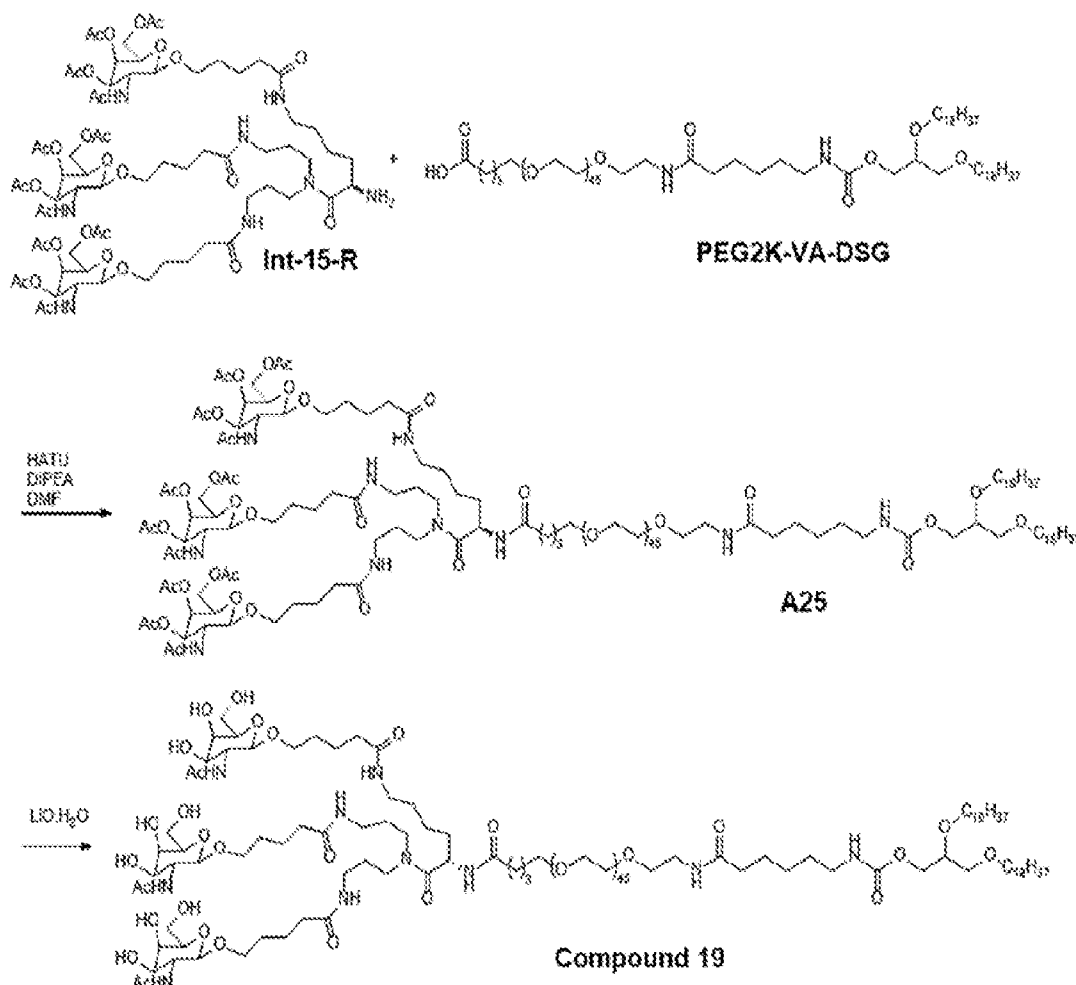
**[0359]** To a solution of **Cpd.A22** (20 g, 28.82 mmol, 1 eq) in DCM (60 mL) was added HCl/EtOAc (4 M, 20 mL). The mixture was stirred at 25 °C for 2 hrs. The mixture was concentrated under reduced pressure to give **Cpd.A23** (14 g, 87% yield, 3HCl) as white solid. <sup>1</sup>H NMR (400 MHz, MeOD-d<sub>4</sub>) δ 7.46 - 7.21 (m, 5H), 5.19 - 4.96 (m, 2H), 4.58 - 4.46 (m, 1H), 3.50 - 3.38 (m, 2H), 3.73 - 3.36 (m, 3H), 3.13 - 3.02 (m, 2H), 3.01 - 2.87 (m, 4H), 2.09 (dt, *J* = 8.0, 15.6 Hz, 2H), 2.00 - 1.91 (m, 2H), 1.82 - 1.64 (m, 4H), 1.59 - 1.42 (m, 2H).

**[0360] Preparation of Compound A24**

**[0361]** A mixture of **GalNAc-Int-4** (13.79 g, 30.82 mmol), **Cpd.A23** (5.00 g, 9.94 mmol, 3HCl), DIEA (8.99 g, 69.60 mmol) in DCM (200 mL) at 25 °C, was added T4P (25.07 g, 34.80 mmol) at 0 °C and then the mixture was stirred at 20 °C for 4 hrs. TLC (eluted with DCM: MeOH = 8:1,  $R_f$  = 0.4) showed the starting material was consumed and one new spot formed. The mixture was quenched with water (100 ml) and extracted with DCM (30 mL  $\times$  3). The combined organic layers were separated, and the organic layer was washed with brine (40 mL  $\times$  6), dried over Na<sub>2</sub>SO<sub>4</sub>. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM: MeOH = 1:0 to 8:1) twice to get **Cpd.A24** (2.8 g, 16.67% yield) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 7.85-7.66 (m, 6H), 7.44 (br d,  $J$  = 8.0 Hz, 1H), 7.39-7.26 (m, 5H), 5.20 (d,  $J$  = 3.6 Hz, 3H), 5.00 (br d,  $J$  = 2.4 Hz, 2H), 4.96 (dd,  $J$  = 3.2, 11.2 Hz, 3H), 4.48 (d,  $J$  = 8.4 Hz, 3H), 4.26 (br d,  $J$  = 6.0 Hz, 1H), 4.01 (s, 10H), 3.90-3.80 (m, 3H), 3.74-3.65 (m, 3H), 3.39 (br dd,  $J$  = 3.2, 9.6 Hz, 4H), 3.15-2.91 (m, 8H), 2.09 (s, 9H), 2.07-2.01 (m, 6H), 1.99 (s, 9H), 1.88 (s, 9H), 1.76 (s, 9H), 1.57-1.38 (m, 18H), 1.37-1.22 (m, 4H)

**[0362] Preparation of Int-15-R**

**[0363]** A mixture of **Cpd.A24** (2.8 g, 1.666 mmol), Pd/C (2.79 g, 2.62 mmol, 10% purity) in THF (30 mL) was degassed and purged with H<sub>2</sub> (15 psi) for 3 times, and then the mixture was stirred at 25 °C for 12 hrs under H<sub>2</sub>(15 psi) atmosphere. TLC (eluted with DCM: MeOH = 5:1,  $R_f$  = 0.2) showed the starting material was consumed and one new spot formed. The mixture was filtered, and the filtrate was concentrated under reduced pressure to get **Int-15-R** (2 g, 77.64% yield) was obtained as a gray solid. LCMS:  $t_R$  = 1.759 min, 100% purity,  $m/z$  = 1548.7 (M+H)<sup>+</sup>



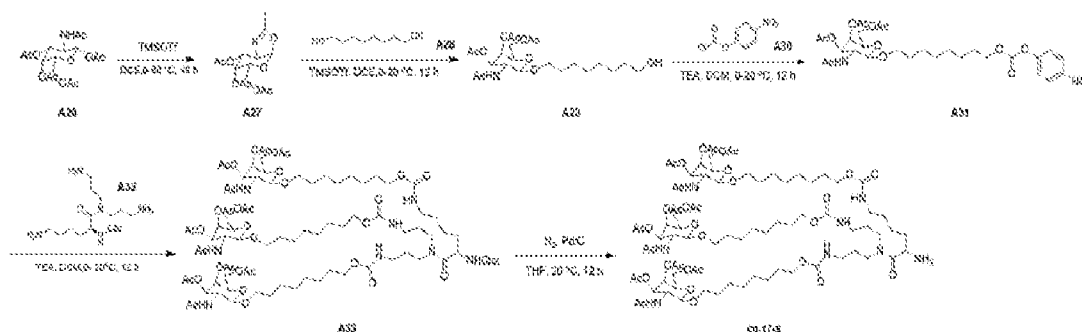
Scheme 12: Synthesis of Compound 19

**[0364] Preparation of A25**

**[0365]** To a solution of PEG2K-VA-DSG (604 mg, 0.194 mmol) in 5 mL of DMF, HATU (74 mg (0.19 mmol) and DIPEA (0.09 mL, 0.4 mmol) were added, the mixture was stirred for 15-30 min at room temperature. The reaction mixture was turned into a yellow solution. Then 250 mg (0.16 mmol) of Int-15-R was added and stirred overnight. Next day reaction was concentrated and diluted with DCM and washed with brine and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated again. The crude was purified using a gold column using 50% MeOH in DCM and DCM as mobile phases to recover 390 mg (0.09 mmol, 55 %) of final product as a white oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.38 (d, J = 3.3 Hz, 3H), 5.33 – 5.15 (m, 2H), 4.85 (s, 1H), 4.68 (q, J = 7.8 Hz, 2H), 4.15 (dd, J = 17.7, 10.5 Hz, 9H), 3.94 (d, J = 6.6 Hz, 5H), 3.75 (dt, J = 13.9, 6.9 Hz, 5H), 3.67 (s, 162H), 3.63 – 3.42 (m, 20H), 3.15 (q, J = 7.5 Hz, 8H), 2.25 – 1.88 (m, 36H), 1.63 (ddt, J = 29.0, 14.2, 7.0 Hz, 25H), 1.47 (dq, J = 10.5, 6.4 Hz, 30H), 1.28 (s, 52H), 0.90 (t, J = 6.7 Hz, 6H).

**[0366] Preparation of Compound 19**

**[0367]** To a solution of **A25** (140 mg) in THF (2.0 mL) and MeOH (2.0 mL), H<sub>2</sub>O (1.0 mL) was added, followed by addition of LiOH (30 mg), the mixture was stirred at room temperature for 4 hours. cooled with ice water, the pH was adjusted to 5-6 with 10% HCL aqueous solution, concentrated, the crude product was uploaded to C-18 column (24 g), eluted with 100% water in 15 mins, then raise the ACN to 100%, then replaced ACN with 100% MeOH, the major peak was eluted out at 50 mins, after concentration, collected 80 mg of Compound 19 with 62 % yield. <sup>1</sup>H NMR (400 MHz, MeOD) δ 4.44 – 4.33 (m, 3H), 4.00 – 3.71 (m, 23H), 3.66 (s, 167H), 3.58 – 3.43 (m, 30H), 3.24 – 3.06 (m, 19H), 2.35 – 2.17 (m, 12H), 2.03 – 1.98 (m, 9H), 1.76 – 1.50 (m, 34H), 1.31 (s, 52H), 0.92 (t, J = 6.7 Hz, 6H). MS (MALDI\_TOF) m/z [M+Na]<sup>+</sup> calculated for 4051.05; found for 4051.57.

**[0368] Example 6. Preparation of Compound 29****Scheme 11: Synthesis of Int-17-S****[0369] Preparation of Compound A27**

**[0370]** To a solution of Compound **A26** (100 g, 256.84 mmol) in DCE (1500 mL) was added TMSOTf (85.63 g, 385.25 mmol, 69.62 mL) at 0 °C. The reaction mixture was warmed to 50 °C and stirred for 1.5 h. Then the mixture was cooled to 20 °C and stirred for 14.5 h. TLC (eluted with DCM: methanol = 10:1, R<sub>f</sub> = 0.6) showed the starting material was consumed and a new main spot formed. The mixture was quenched with addition of sodium bicarbonate solution (1500 mL) and extracted with dichloromethane (500 mL × 3). The combined organic phase was washed with brine (500 mL × 2), dried over anhydrous sodium sulfate, filtered and the filtrate was concentrated under reduced pressure to get Compound **A26** (92 g, crude) as yellowish oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm 5.92 (d, J = 6.8 Hz, 1H), 5.39 (t, J = 2.8 Hz, 1H), 4.84 (dd, J = 3.2, 7.2 Hz, 1H), 4.20-4.09 (m, 2H), 4.04 (dd, J = 5.6, 11.2 Hz, 1H), 3.93 (t, J = 7.2 Hz, 1H), 2.05 (s, 3H), 2.00 (s, 6H), 1.98 (s, 3H).

**[0371] Preparation of Compound A29**

[0372] To a solution of Cpd.**A28** (33.30 g, 227.75 mmol) in DCE (500 mL) was added Cpd.**A27** (15 g, 45.55 mmol) and 4A MOLECULAR SIEVE (15 g) was added TMSOTf (5.06 g, 22.78 mmol) at 20 °C. The mixture was stirred at 20 °C for 12 h. TLC (eluted with petroleum ether: ethyl acetate = 0:1, R<sub>f</sub> = 0.2) showed the starting material was consumed and one new spot was formed. The mixture was quenched with addition of sodium bicarbonate solution (1000 mL) and extracted with dichloromethane (500 mL × 3). The combined organic phase was washed with brine (500 mL × 2), dried over anhydrous sodium sulfate, the mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (SiO<sub>2</sub>, eluted with petroleum ether:ethyl acetate = 1: 0 to 0:1) to get Cpd.**A29** (33 g, 78.15% yield) as a brown oil. LCMS: t<sub>R</sub> = 1.801 min, 45.4% purity, m/z = 946.3 (M+H)<sup>+</sup>.

[0373] **Preparation of Compound A31**

[0374] To a solution of TEA (2.98 g, 29.44 mmol) and Cpd.**A29** (7 g, 14.72 mmol) in DCM (100 mL) was added Cpd.**A30** (3.26 g, 16.19 mmol) at 0 °C and stirred at 20°C. The mixture was stirred at 20 °C for 12 h. LCMS indicated the Reactant 1 was consumed, and the desired ms was detected. The reaction mixture was quenched by H<sub>2</sub>O (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (SiO<sub>2</sub>, PE:EtOAc = 1:0 to 0:1) to give A31 (5.6 g, 59% yield) as a colorless oil. <sup>1</sup>H NMR ( 400 MHz, CDCl<sub>3</sub>) δ 8.34 - 8.25 (m, 2H), 7.47 - 7.37 (m, 2H), 5.45 - 5.37 (m, 2H), 5.35 - 5.32 (m, 2H), 4.73 (d, J = 8.4 Hz, 1H), 4.31 (t, J = 6.8 Hz, 2H), 4.24 - 4.18 (m, 1H), 3.98 - 3.87 (m, 3H), 3.50 (td, J = 6.8, 9.6 Hz, 1H), 2.16 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H), 1.82 - 1.72 (m, 2H), 1.61 (s, 1H), 1.49 - 1.31 (m, 9H).

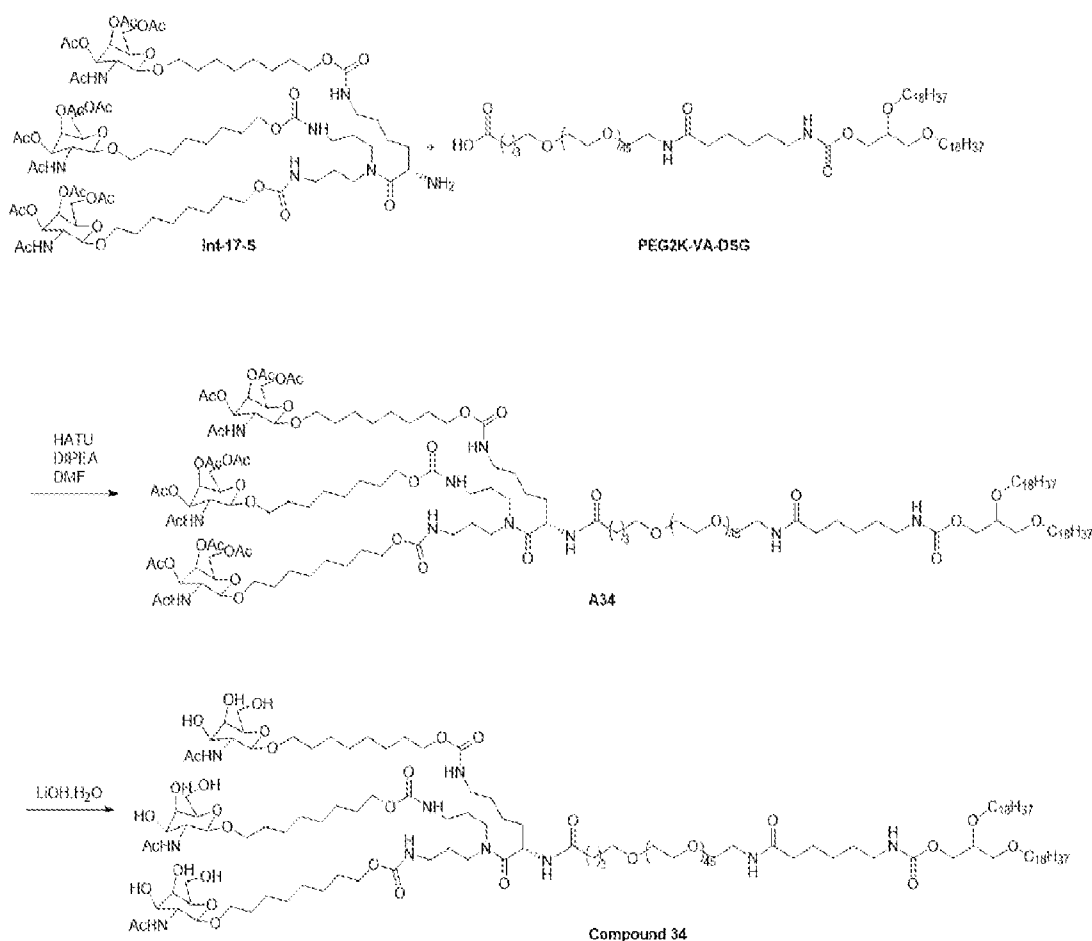
[0375] **Preparation of Compound A33**

[0376] To a solution of Cpd.**A31** (22.83 g, 35.63 mmol) in DCM (50 mL) was added TEA (10.14 g, 100.22 mmol) and Cpd.**A32** (5.6 g, 11.14 mmol) at 0 °C. The mixture was stirred at 20 °C for 16 h. TLC (DCM:MeOH = 10:1, R<sub>f</sub>=0.4) indicated the Reactant 1 was consumed, and the desired MS was detected. The reaction mixture was quenched by H<sub>2</sub>O (100 mL) and extracted with DCM (3 × 100 mL). The combined organic layers were washed with brine (100 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (SiO<sub>2</sub>, DCM:MeOH = 1:0 to 0:1) for twice to give Cpd.**A33** (2.5 g, 12% yield) as a white solid. <sup>1</sup>H NMR ( 400 MHz, CDCl<sub>3</sub>) δ 7.41 - 7.29 (m, 5H), 6.01 - 5.56 (m, 6H), 5.41 - 5.27 (m, 7H), 5.18 - 5.04 (m, 3H),

5.01 - 4.82 (m, 1H), 4.78 - 4.67 (m, 3H), 4.59 (br d,  $J = 4.0$  Hz, 1H), 4.23 - 4.08 (m, 9H), 4.06 - 3.77 (m, 15H), 3.72 - 3.38 (m, 1H), 3.36 - 3.06 (m, 8H), 3.05 - 2.96 (m, 1H), 2.15 (s, 8H), 2.05 (s, 10H), 2.00 (s, 9H), 1.96 (s, 9H), 1.66 - 1.46 (m, 18H), 1.41-1.22 (m, 28H).

**[0377] Preparation of Int-17-S.**

**[0378]** A mixture of Cpd. **A33** (1.8 g, 948.32  $\mu\text{mol}$ ), Pd/C (900.00 mg, 845.71  $\mu\text{mol}$ , 10% purity) in THF (100 mL) was degassed and purged with  $\text{H}_2$  (15 psi) for 3 times, and then the mixture was stirred at 20 °C for 12 h under  $\text{H}_2$  atmosphere. LC-MS showed Reactant 1 was consumed completely and one main peak with desired m/z was detected. The mixture was filtered, and the filtrate was concentrated under reduced pressure to get **Int-17-S** (1.5 g, 89.67% yield) as a gray solid. **LCMS:** observed 882.9 ( $1/2\text{M}+\text{H}$ )<sup>+</sup>  **$^1\text{H}$  NMR** (400 MHz, DMSO)  $\delta$  7.81 (d,  $J = 9.2$  Hz, 3H), 7.23-6.94 (m, 3H), 5.21 (d,  $J = 3.2$  Hz, 3H), 4.96 (dd,  $J = 3.2, 11.2$  Hz, 3H), 4.48 (d,  $J = 8.4$  Hz, 3H), 4.09-3.98 (m, 9H), 3.96-3.81 (m, 9H), 3.69 (td,  $J = 6.0, 9.6$  Hz, 3H), 3.60 (br t,  $J = 6.0$  Hz, 2H), 3.45-3.34 (m, 4H), 3.29-3.18 (m, 2H), 3.12-3.04 (m, 1H), 3.02-2.88 (m, 6H), 2.10 (s, 9H), 1.99 (s, 9H), 1.89 (s, 9H), 1.76 (s, 9H), 1.68-1.60 (m, 2H), 1.58-1.41 (m, 15H), 1.37-1.16 (m, 30H).



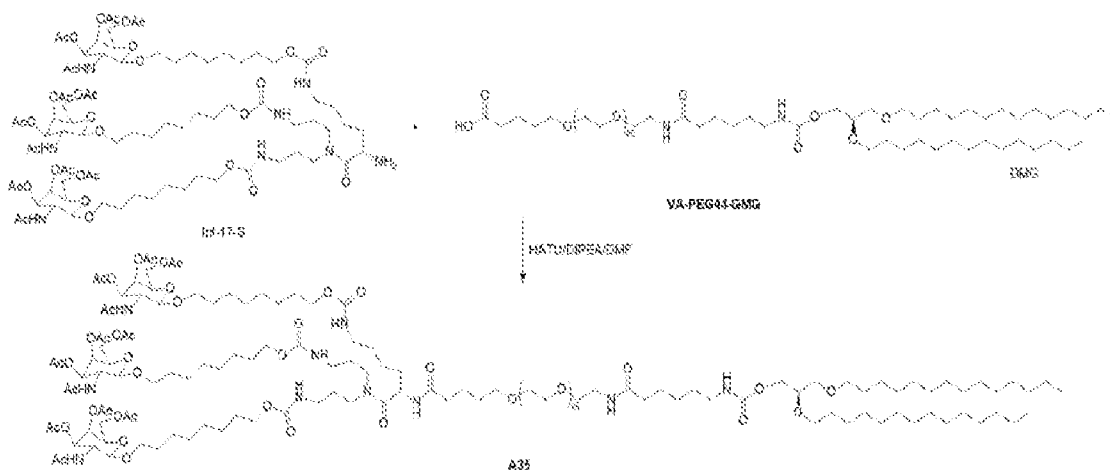
**Scheme 12: Synthesis of Compound 34**

**[0379] Preparation of Compound A34**

**[0380]** To a solution of VA-PEG2k-DSG acid (270 mg, 0.093 mmol) in DMF (2.0 mL), HATU (36 mg, 0.093 mmol) was added, followed by the addition of DIPEA (0.044 mL), the mixture was stirred for 15 mins at room temperature. Then Int-17-S (150 mg, 0.058 mmol) was added, the resulting mixture was stirred for 48 hours at room temperature, the solvents were removed under vacuum, the crude product was dissolved in DCM, washed with brine, dried with  $\text{MgSO}_4$ , filtered, concentrated, the crude product was purified with Combi-flash (24 g gold normal phase column, 0-50% MeOH in DCM), collected 400 mg product.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.37 (d,  $J$  = 11.6 Hz, 2H), 4.74 (d,  $J$  = 8.4 Hz, 3H), 4.30 – 3.80 (m, 10H), 3.67 (s, 120H), 3.62 – 3.42 (m, 8H), 2.25 – 1.94 (m, 36H), 1.28 (s, 71H), 0.90 (t, 6H).

**[0381] Preparation of Compound 34**

**[0382]** A solution of A34 (310 mg, 0.067 mmol) in THF (2.0 mL) and MeOH (2.0 mL) was cooled to 0 C  $\text{H}_2\text{O}$  (1.0 mL) was added with LiOH (84 mg, 2.01 mmol), the mixture was stirred at room temperature for 4 hours, cooled with ice water, the pH was adjusted to 5-6 with 1N HCL aqueous solution, concentrated, the crude product was uploaded to C-18 column (24 g), eluted with 100% water in 15 mins, then raise the ACN to 100%, then replaced ACN with 100% MeOH, the major peak was eluted out at 50 mins, after concentration, collected 124 mg of Compound 34 with 43 % yield.  $^1\text{H}$  NMR (400 MHz, MeOD)  $\delta$  4.60 (s, 6H), 4.39 (d,  $J$  = 8.4 Hz, 3H), 4.11 – 3.74 (m, 24H), 3.66 (s, 190H), 3.61 – 3.44 (m, 23H), 3.20 – 3.04 (m, 8H), 2.26 (dt,  $J$  = 25.1, 7.3 Hz, 4H), 2.00 (s, 9H), 1.33 (d,  $J$  = 17.1 Hz, 71H), 0.92 (t, 6H).  $[\text{M}+\text{Na}]^+$  calculated for 4223.77 ; found for 4223.72,  $n=44$ .

**[0383] Example 7. Preparation of Compound A35**

**[0384]** To a solution of VA-PEG2k-DMG acid (276 mg, 0.093 mmol, 1.2 equiv) in DMF (2.0 mL), HATU (45mg, 0.093 mmol, 1.2 equiv) was added, followed by the addition of

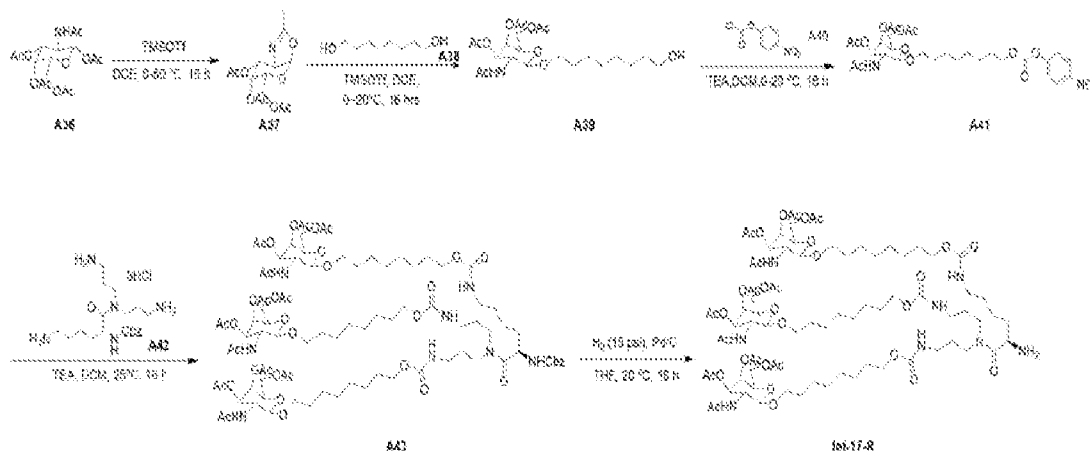
DIPEA (0.2 mL), the mixture was stirred for 15 mins at room temperature. Then **Int-17-S** (211 mg, 1.0 equiv) was added, the resulting mixture was stirred for 48 hours at room temperature, the solvents were removed under vacuum, the crude product was dissolved in DCM, washed with brine, dried with  $\text{MgSO}_4$ , filtered, concentrated, the crude product was purified with Combi-flash (24 g gold normal phase column, 0-50% MeOH in DCM), collected 240 mg product.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.38-5.31 (m, 6H), 4.72 (m, 3H), 4.17 – 3.82 (m, 24H), 3.66-3.43 (m, 190H), 3.07 (m, 7H), 2.21 (m, 6H), 2.16(s, 9H), 2.07(s, 9H), 2.02(s, 9H), 1.98(s, 9H), 1.70-1.51(m, 35H), 1.34-1.27(m, 68H), 0.90(t, 6H).

**[0385] Example 8. Preparation of Compound 35**



**[0386]** To a solution of **A35** (220 mg) in THF (2.0 mL) and MeOH (2.0 mL),  $\text{H}_2\text{O}$  (1.0 mL) was added, followed by the addition of LiOH (50 mg), the resulting mixture was stirred for 4 hours at room temperature, then pH was adjusted to 5-6 with 50% HCl aqueous solution with ice water cooling, concentrated, the crude product was dissolved in water, loaded on C-18 column, eluted with water for 15 mins, then raise the ACN from 0-100% in 15 mins, switched to 100% MeOH, the pure 127 mg of product was collected.  $^1\text{H}$  NMR (400 MHz, MeOD)  $\delta$  4.40 (d,  $J = 8.4$  Hz, 3H), 4.04 (m, 8H), 3.94-3.78 (m, 17H), 3.66-3.60 (m, 203H), 3.09 (m, 8H), 2.29-2.22 (m, 5H), 2.00 (s, 9H), 1.71-1.53 (m, 31H), 1.35-1.31(m, 69H), 0.92 (t, 6H).  $[\text{M}+\text{Na}]^+$  calculated for 4135.2 ; found for 4155.7.

[0387] Preparation of Int-17-R



Scheme 13: Synthesis of Int-17-R

[0388] Preparation of Compound A37

[0389] To a solution of Cpd.A36 (50 g, 128.42 mmol) in DCE (750 mL) was added TMSOTf (42.81 g, 192.63 mmol, 34.81 mL) at 0 °C. The reaction mixture was warmed to 50 °C and stirred for 1.5 h. Then the mixture was stirred at 20 °C for 14.5 h. TLC (DCM:MeOH= 10:1, R<sub>f</sub>=0.6) showed the Reactant 1 consumed. A new spot was detected. The reaction mixture was quenched by saturated NaHCO<sub>3</sub> solution (1000 mL) and extracted with DCM (3 × 500 mL). The combined organic layers were washed with brine (500 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure to give Cpd.A37 (37 g, 87% yield) as a brown oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 6.00 (d, *J* = 6.8 Hz, 1H), 5.47 (t, *J* = 3.2 Hz, 1H), 4.92 (dd, *J* = 3.2, 7.6 Hz, 1H), 4.24 (br dd, *J* = 2.8, 6.0 Hz, 1H), 4.23 - 4.17 (m, 1H), 4.13 (d, *J* = 6.0 Hz, 1H), 4.03 - 3.97 (m, 1H), 2.13 (s, 3H), 2.08 (s, 6H), 2.06 (d, *J* = 1.2 Hz, 3H).

[0390] Preparation of Compound A39

[0391] To a solution of Cpd.A38 (9.99 g, 68.33 mmol) and Cpd.A37 (15 g, 45.55 mmol) in DCE (30 mL) was added TMSOTf (20.25 g, 91.10 mmol) at 0 °C and stirred at 20 °C. The mixture was stirred at 20 °C for 16 hrs. TLC (DCM:MeOH=10:1, R<sub>f</sub> = 0.5) indicated the Reactant 1 was consumed, and a new spot was detected. The reaction mixture was quenched by saturated NaHCO<sub>3</sub> solution (500 mL) and extracted with DCM (3 × 300 mL). The combined organic layers were washed with brine (500 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (SiO<sub>2</sub>, DCM: MeOH = 1:0 to 0:1) to give Cpd.A39 (7 g, 32% yield) as a brown oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.39 - 5.23 (m, 3H), 4.18 - 4.09 (m, 4H), 3.98 - 3.87 (m, 3H), 3.65 (br t, *J* = 6.0 Hz, 2H), 3.53 - 3.47 (m, 1H), 2.15 (s, 3H), 2.05 (d, *J* = 1.2 Hz, 3H), 2.01 (s, 3H), 1.96 (s, 3H), 1.61 - 1.55 (m, 4H), 1.33 (br s, 8H).

**[0392] Preparation of Compound A41**

**[0393]** To a solution of TEA (2.98 g, 29.44 mmol) and Cpd.**A39** (7 g, 14.72 mmol) in DCM (100 mL) was added Cpd.**A40** (3.26 g, 16.19 mmol) at 0 °C and stirred at 20 °C. The mixture was stirred at 20 °C for 16 h. TLC (PE:EtOAc = 1:1, R<sub>f</sub> = 0.6) indicated the Reactant 1 was consumed, and one new spot was detected. The reaction mixture was quenched by H<sub>2</sub>O (50 mL) and extracted with DCM (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (SiO<sub>2</sub>, PE:EtOAc = 1:0 to 0:1) to give Cpd.**A41** (5.6 g, 59 % yield) as a colorless oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.34 - 8.25 (m, 2H), 7.47 - 7.37 (m, 2H), 5.45 - 5.37 (m, 2H), 5.35 - 5.32 (m, 2H), 4.73 (d, *J* = 8.4 Hz, 1H), 4.31 (t, *J* = 6.8 Hz, 2H), 4.24 - 4.18 (m, 1H), 3.98 - 3.87 (m, 3H), 3.50 (td, *J* = 6.8, 9.6 Hz, 1H), 2.16 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H), 1.82 - 1.72 (m, 2H), 1.61 (s, 1H), 1.49 - 1.31 (m, 9H).

**[0394] Preparation of Compound A43**

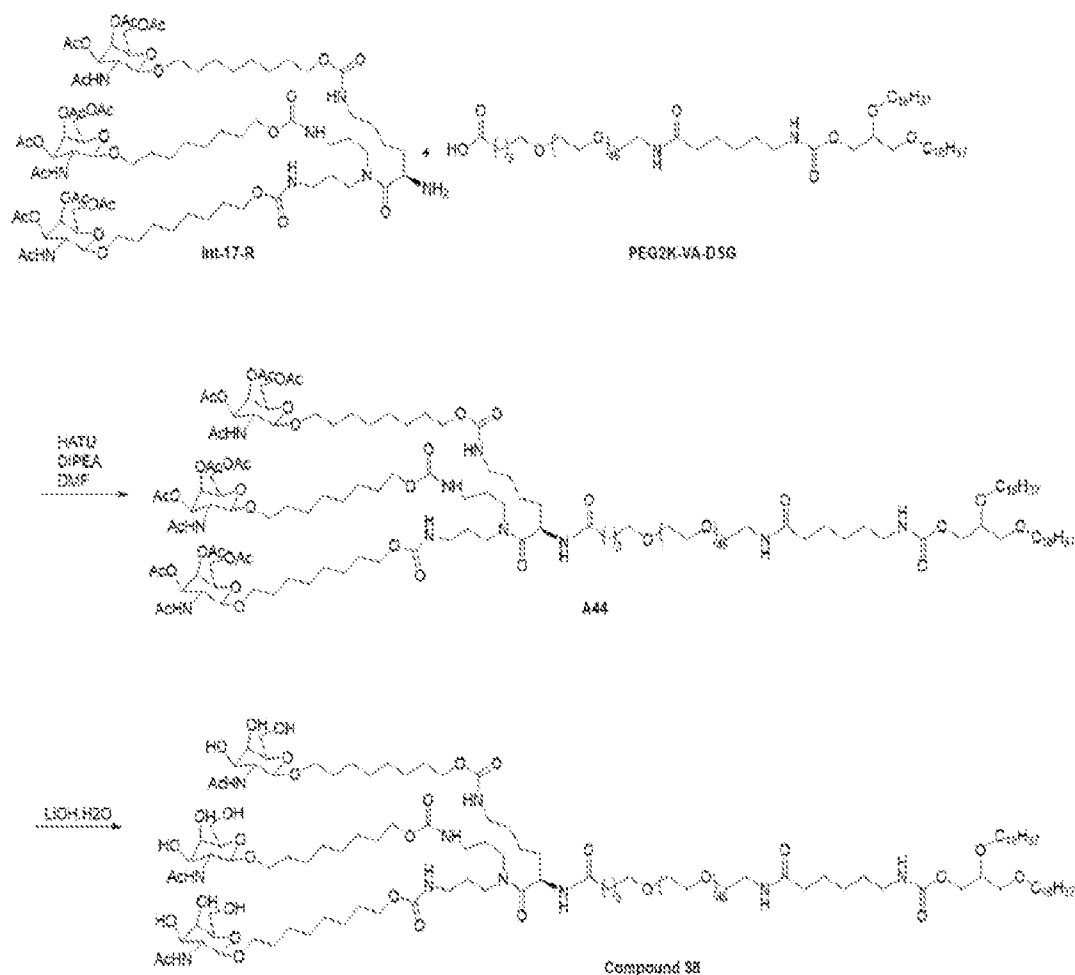
**[0395]** To a solution of Cpd.**A41** (4.9 g, 7.64 mmol) in DCM (50 mL) was added TEA (2.42 g, 13.86 mmol) and Cpd.**A42** (1.2 g, 2.38 mmol, 3HCl) at 25 °C. The mixture was stirred at 25 °C for 16 h. TLC (DCM:MeOH = 10:1, R<sub>f</sub> = 0.4) indicated the Reactant 1 was consumed, and the desired ms was detected. The reaction mixture was quenched by H<sub>2</sub>O (100 mL) and extracted with DCM (3 x 50 mL). The combined organic layers were washed with brine (100 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (SiO<sub>2</sub>, DCM:MeOH = 20:1 to 0:1) for twice to give Cpd.**A43** (3.5 g, 44% yield) as a yellow solid. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 7.81 (d, *J* = 9.2 Hz, 3H), 7.44 (br d, *J* = 7.6 Hz, 1H), 7.40 - 7.22 (m, 5H), 7.19 - 7.09 (m, 1H), 6.99 (td, *J* = 5.2, 18.8 Hz, 2H), 5.21 (d, *J* = 3.2 Hz, 3H), 5.06 - 4.90 (m, 5H), 4.48 (d, *J* = 8.4 Hz, 3H), 4.32 - 4.20 (m, 1H), 4.10 - 3.96 (m, 9H), 3.96 - 3.81 (m, 9H), 3.71 (td, *J* = 6.0, 10.0 Hz, 3H), 3.46 - 3.36 (m, 3H), 3.32 - 3.24 (m, 2H), 3.08 (br dd, *J* = 6.0, 7.2 Hz, 1H), 3.02 - 2.95 (m, 2H), 2.95 - 2.84 (m, 4H), 2.10 (s, 9H), 1.99 (s, 9H), 1.89 (s, 9H), 1.76 (s, 10H), 1.70 - 1.63 (m, 1H), 1.61 - 1.40 (m, 17H), 1.38 - 1.12 (m, 30H).

**[0396] Preparation of Int-17-R**

**[0397]** To a solution of Cpd.**A43** (3.5 g, 2.08 mmol) in THF (100 mL) was added Pd/C (1.8 g, 10% purity) at 20 °C and stirred at 20 °C for 16 h under H<sub>2</sub> (15 psi). TLC indicated the Reactant 1 was consumed, and a new spot was detected. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give Int-17-R (2.03 g, 92% yield) as a gray solid. LCMS: R<sub>t</sub> = 2.438 min, 95.49% purity, m/z = 1764.1 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz,

DMSO-d<sub>6</sub>)  $\delta$  7.87 (d,  $J$  = 9.2 Hz, 3H), 7.34 - 6.91 (m, 3H), 5.27 (d,  $J$  = 3.2 Hz, 3H), 5.03 (dd,  $J$  = 3.2, 11.2 Hz, 3H), 4.54 (d,  $J$  = 8.4 Hz, 3H), 4.15 - 4.04 (m, 9H), 4.02 - 3.85 (m, 9H), 3.76 (td,  $J$  = 6.0, 9.6 Hz, 3H), 3.67 (br t,  $J$  = 6.4 Hz, 10H), 3.47 (td,  $J$  = 6.4, 9.6 Hz, 5H), 3.38 (s, 7H), 3.34 - 3.21 (m, 2H), 3.19 - 3.09 (m, 1H), 3.08 - 2.88 (m, 6H), 2.17 (s, 9H), 2.06 (s, 10H), 2.02 - 1.92 (m, 10H), 1.84 - 1.83 (m, 8H), 1.75 - 1.66 (m, 3H), 1.65 - 1.47 (m, 16H), 1.45 - 1.21 (m, 30H).

### [0398] Preparation of Compound 38



### Scheme 14: Synthesis of Compound 38

#### [0399] Preparation of A44

[0400] To a solution of PEG2k-VA-DSG acid (270 mg, 0.093 mmol) in DMF (2.0 mL), HATU (36 mg, 0.093 mmol) was added, followed by the addition of DIPEA (0.044 mL), the mixture was stirred for 15 mins at room temperature, then Int-17-R (150 mg, 0.058 mmol) was added, the resulting mixture was stirred for 48 hours at room temperature, the solvents were removed under vacuum, the crude product was dissolved in DCM, washed with brine, dried with MgSO<sub>4</sub>, filtered, concentrated, the crude product was purified with Combi-flash (24 g gold normal phase column, 0-50% MeOH in DCM), collected 370 mg product. <sup>1</sup>H

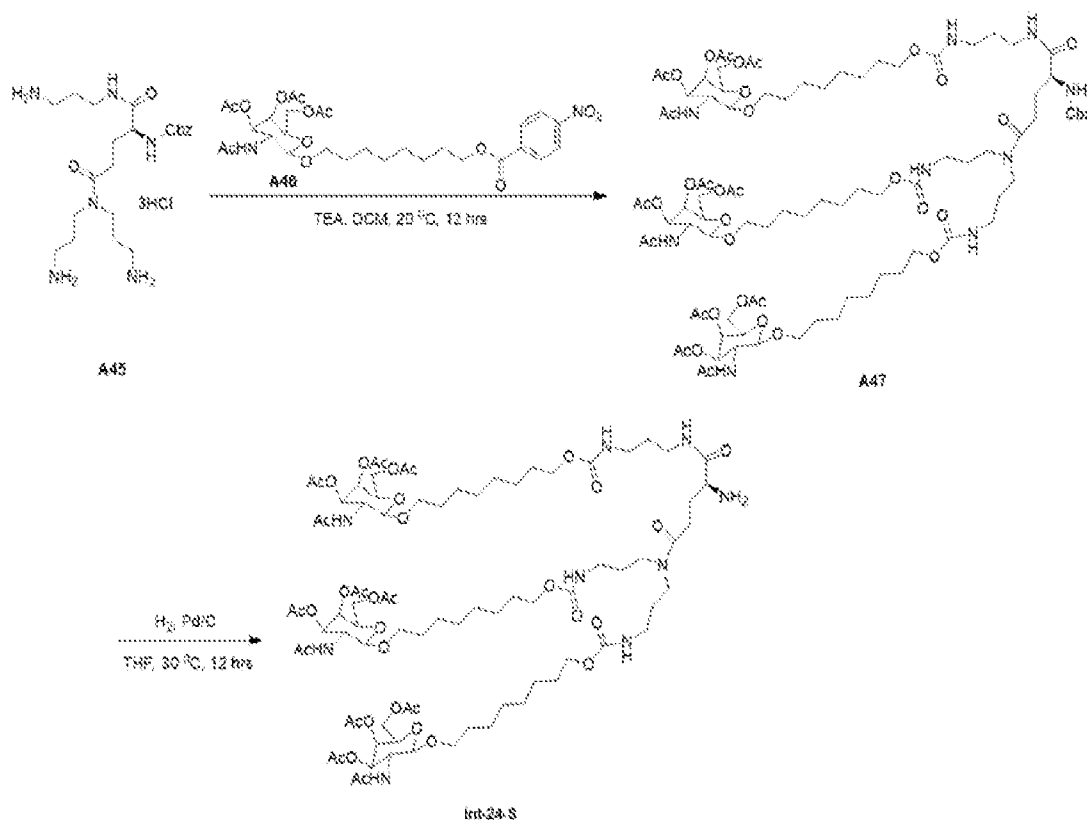
NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.37 (d, J = 11.6 Hz, 2H), 4.74 (d, J = 8.4 Hz, 3H), 4.30 – 3.80 (m, 10H), 3.67 (s, 120H), 3.62 – 3.42 (m, 8H), 2.25 – 1.94 (m, 36H), 1.28 (s, 71H), 0.90 (t, 6H).

**[0401] Preparation of Compound 38**

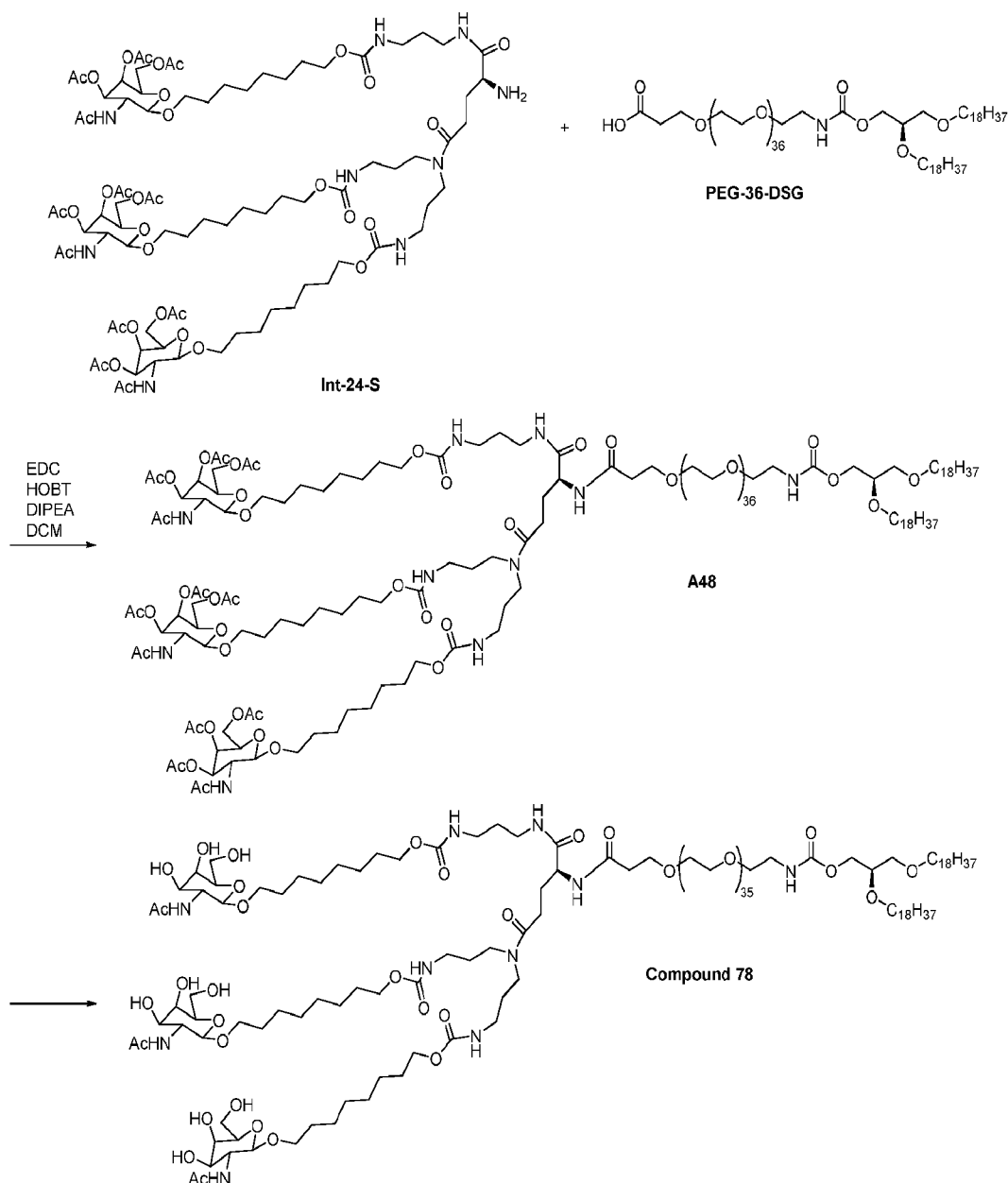
**[0402]** A solution of **A44** (370 mg, 0.08 mmol) in THF (2.0 mL) and MeOH (2.0 mL) was cooled to 0°C H<sub>2</sub>O (1.0 mL) was added with LiOH (100 mg, 2.4 mmol), the mixture was stirred at room temperature for 4 hours, cooled with ice water, the pH was adjusted to 5-6 with 1N HCl aqueous solution, concentrated, the crude product was uploaded to C-18 column (24 g), eluted with 100% water in 15 mins, then raise the ACN to 100%, then replaced ACN with 100% MeOH, the major peak was eluted out at 50 mins, after concentration, collected 130 mg Compound 38 with 38 % yield. <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  4.60 (s, 6H), 4.39 (d, J = 8.4 Hz, 3H), 4.11 – 3.74 (m, 24H), 3.66 (s, 190H), 3.61 – 3.44 (m, 23H), 3.20 – 3.04 (m, 8H), 2.26 (dt, J = 25.1, 7.3 Hz, 4H), 2.00 (s, 9H), 1.33 (d, J = 17.1 Hz, 71H), 0.92 (t, 6H).

[M+Na]<sup>+</sup> calculated for 4223.77; found for 4223.69, n=44.

**[0403] Example 9. Preparation of Compound 78**



**Scheme 15: Synthesis of Int-24-S**



Scheme 16: Synthesis of Compound 78

**[0404] Preparation of Compound A47**

**[0405]** To a solution of Cpd. **A45** (1.8 g, 3.02 mmol, 4HCl) in DCM (20 mL) was added TEA (3.05 g, 30.18 mmol, 4.20 mL) and Cpd. **A46** (6.03 g, 9.66 mmol) at 0 °C. The mixture was stirred at 20 °C for 12 hrs. TLC (eluted with DCM: MeOH = 10:1, R<sub>f</sub> = 0.5) showed the starting material was consumed and one new spot formed. The mixture was quenched with water (50 ml) and extracted with DCM (20 mL × 3). The combined organic layers were separated, and the organic layer was washed with brine (10 mL × 6), dried over Na<sub>2</sub>SO<sub>4</sub>. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM: MeOH = 1/0 to 19/1) for twice to get Cpd. **A47** (2.1 g, 35.59% yield) as a yellowish solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ ppm

7.89-7.77 (m, 4H), 7.44-7.30 (m, 5H), 7.10 (br s, 1H), 7.04-6.94 (m, 2H), 5.21 (d,  $J = 3.2$  Hz, 3H), 5.01 (s, 2H), 4.96 (dd,  $J = 3.2, 11.2$  Hz, 3H), 4.48 (d,  $J = 8.4$  Hz, 3H), 4.08-3.99 (m, 10H), 3.94-3.80 (m, 10H), 3.69 (td,  $J = 6.0, 9.6$  Hz, 3H), 3.40 (td,  $J = 6.4, 9.6$  Hz, 3H), 3.19 (br s, 4H), 3.08-3.01 (m, 3H), 2.99-2.88 (m, 7H), 2.28 (br t,  $J = 6.8$  Hz, 2H), 2.10 (s, 9H), 1.99 (s, 9H), 1.89 (s, 9H), 1.76 (s, 9H), 1.64 - 1.58 (m, 2H), 1.55-1.41 (m, 16H), 1.24 (br s, 24H).

**[0406] Preparation of Int-24-S**

**[0407]** A mixture of Cpd. **A47** (1.9 g, 971.80  $\mu$ mol), Pd/C (1.03 g, 971.80  $\mu$ mol, 10% purity) in THF (200 mL) was degassed and purged with H<sub>2</sub> (15 psi) for 3 times, and then the mixture was stirred at 30 °C for 12 hrs under H<sub>2</sub> atmosphere. LC-MS showed Cpd. **A47** was consumed completely and one main peak with desired MS was detected. The mixture was filtered, and the filtrate was concentrated under reduced pressure to get **Int-24-S** (1 g, 56.51% yield) as a black oil. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  7.88 (br t,  $J = 5.6$  Hz, 1H), 7.81 (d,  $J = 9.2$  Hz, 3H), 7.12 (br d,  $J = 4.4$  Hz, 1H), 7.05-6.93 (m, 2H), 5.21 (d,  $J = 3.2$  Hz, 3H), 4.96 (dd,  $J = 3.2, 11.2$  Hz, 3H), 4.48 (d,  $J = 8.4$  Hz, 3H), 4.02 (s, 9H), 3.93-3.88 (m, 6H), 3.69 (td,  $J = 6.0, 9.6$  Hz, 3H), 3.60 (br t,  $J = 6.4$  Hz, 4H), 3.40 (td,  $J = 6.8, 9.6$  Hz, 3H), 3.21 (br d,  $J = 6.4$  Hz, 4H), 3.16-3.11 (m, 1H), 3.09-3.03 (m, 2H), 3.00-2.87 (m, 6H), 2.30 (br dd,  $J = 6.4, 13.6$  Hz, 2H), 2.10 (s, 9H), 1.99 (s, 9H), 1.89 (s, 9H), 1.76 (s, 9H), 1.66-1.60 (m, 2H), 1.57-1.39 (m, 18H), 1.25 (br s, 24H). LCMS:  $t_R = 3.879$  min, 100% purity,  $m/z = 911.0$  (M/2+H)<sup>+</sup>

**[0408] Preparation of A48**

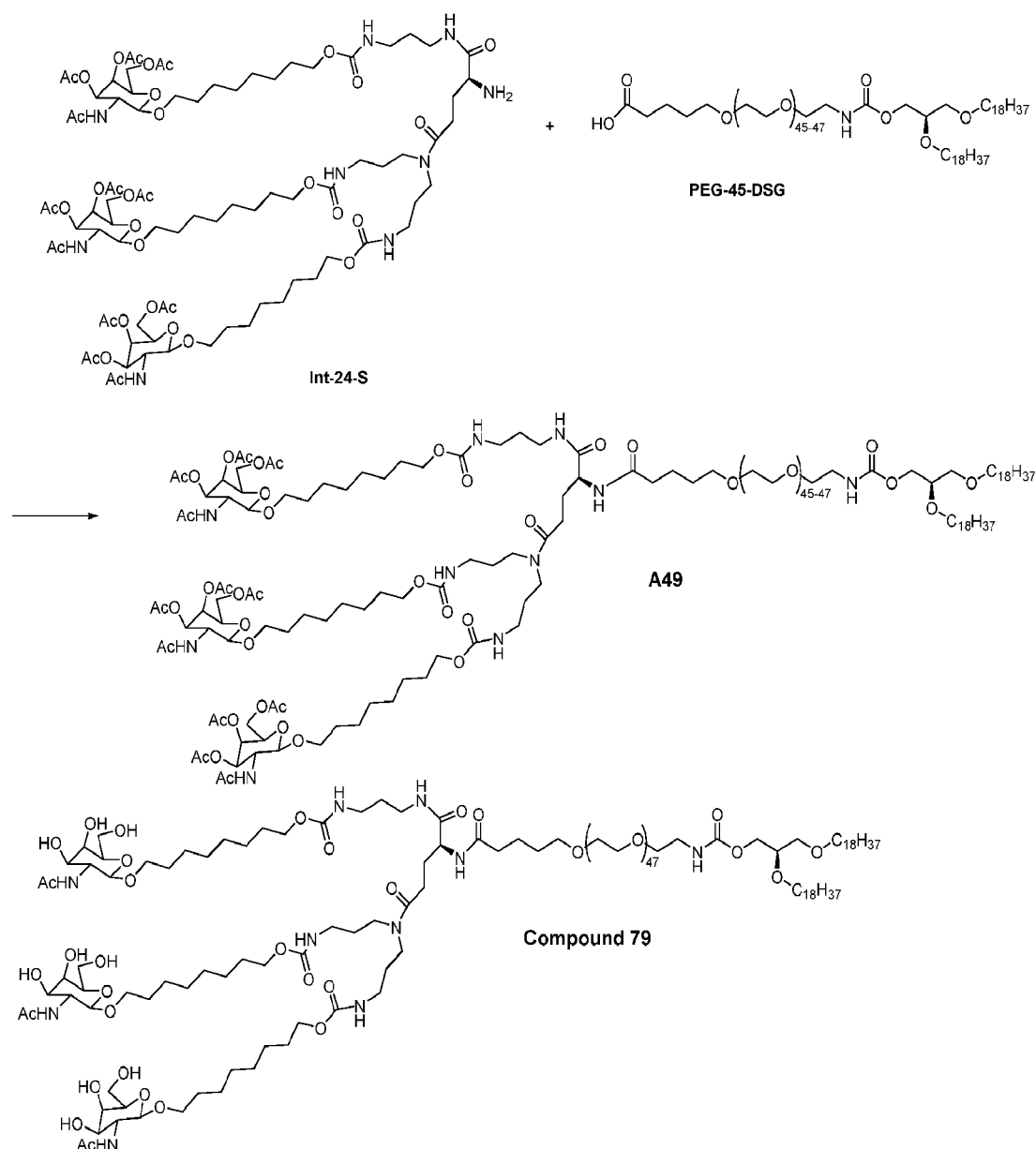
**[0409]** To a solution of **PEG36-DSG** (128 mg, 0.055 mmol, 1.0 equiv) and **Int-24-S** (100 mg, 0.055 mmol, 1.0 equiv) in DCM (2.0 mL), HOBt (10 mg, 0.065 mmol, 1.2 equiv), EDC (12 mg, 0.066 mmol, 1.2 equiv) and DIPEA (0.3 mL, 0.16 mmol, 3 equiv) were added, the resulting mixture was stirred for 48 h at room temperature, additional 10 mL of DCM was added, washed with brine, dried with anhydrous MgSO<sub>4</sub>. Filtered, concentrated, the crude product was purified with reverse phase Combi Flash (Gold C18 gel column, eluted with 100% MeOH) collected 200 mg (88% yield) desired product. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  5.40 – 5.27 (m, 6H), 4.73 (d,  $J = 8.3$  Hz, 2H), 4.25 – 4.11 (m, 7H), 4.05 (d,  $J = 6.8$  Hz, 5H), 3.92 (d,  $J = 21.8$  Hz, 8H), 3.67 (s, 124H), 3.57 (t,  $J = 5.0$  Hz, 4H), 3.47 (dd,  $J = 20.9, 6.3$  Hz, 9H), 2.19 – 1.94 (m, 32H), 1.68 – 1.58 (m, 43H), 1.30 (d,  $J = 19.3$  Hz, 80H), 0.90 (t,  $J = 6.6$  Hz, 6H).

**[0410] Preparation of Compound 78**

**[0411]** The compound **A48** (200 mg, 0.048 mmol) was dissolved in 3 mL of 7N NH<sub>3</sub> (1.45 mmol, 30 equiv) in MeOH solution and transferred to a pressure tube and stirred at room

temperature for 24 h. Then reaction was concentrated via rotovap and purified by reverse phase chromatography (C18 column) with water and acetonitrile/methanol as the mobile phases. Final product was recovered as a white solid 58.7 mg with a yield of 54.7 %.  $^1\text{H}$  NMR (400 MHz, MeOD)  $\delta$  4.60 (s, 1H), 4.39 (d,  $J$  = 8.4 Hz, 3H), 4.06 (dt,  $J$  = 20.1, 6.1 Hz, 6H), 3.96 – 3.72 (m, 18H), 3.66 (s, 14H), 3.51 (ddt,  $J$  = 16.8, 11.1, 6.0 Hz, 14H), 3.25 (q,  $J$  = 6.5 Hz, 2H), 3.18 – 3.05 (m, 7H), 2.00 (s, 9H), 1.84 – 1.79 (m, 2H), 1.72 (dt,  $J$  = 13.3, 6.6 Hz, 4H), 1.66 – 1.52 (m, 15H), 1.34 (d,  $J$  = 17.1 Hz, 86H), 0.95 – 0.90 (m, 6H). MS (MALDI\_TOF)  $m/z$   $[\text{M}+\text{Na}]^+$  calculated for 3743.39 ; found for 3743.78,  $n=35$

**[0412] Example 10. Preparation of Compound 79**



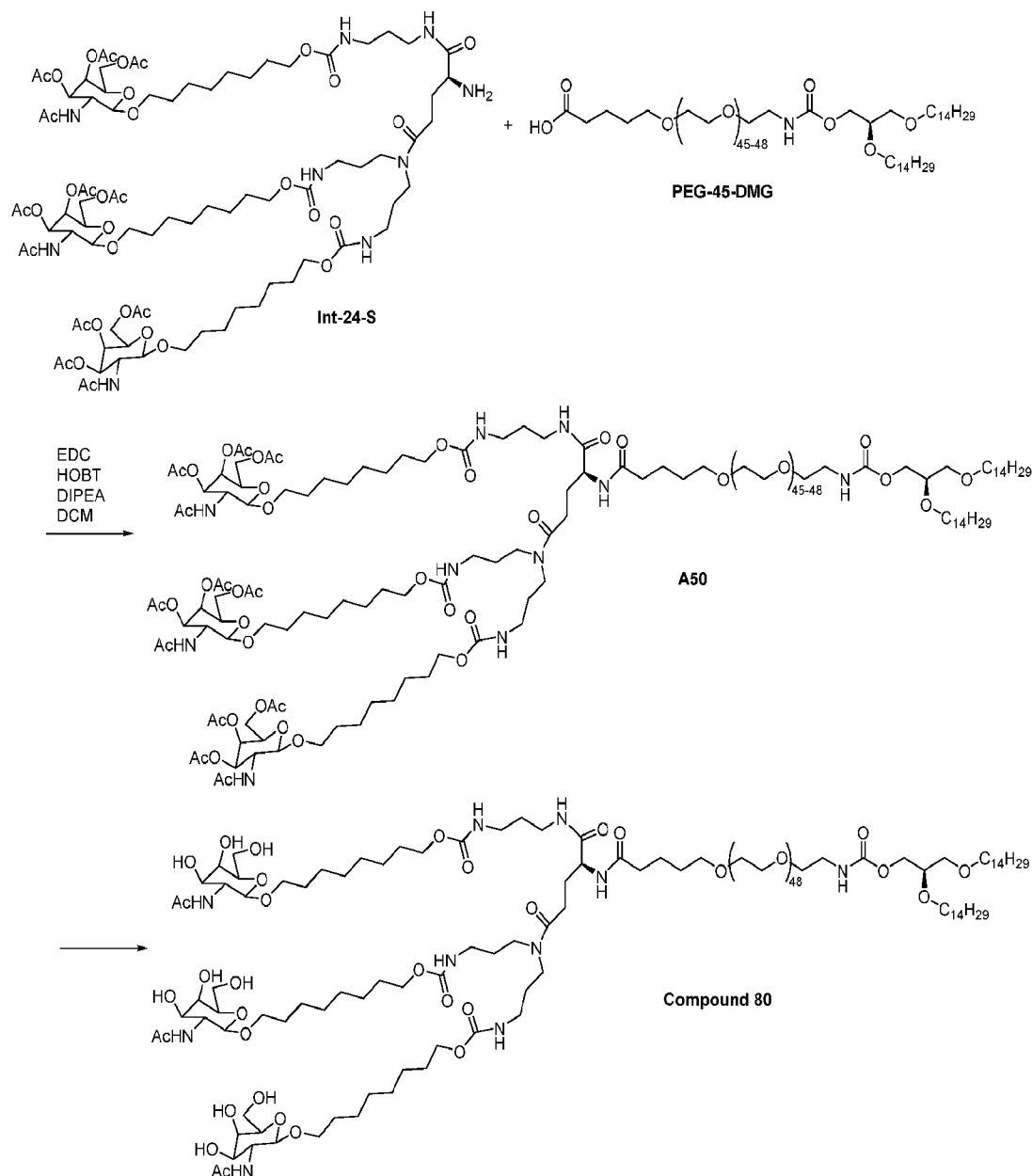
**Scheme 15: Synthesis of Compound 79**

**[0413] Preparation of A49**

**[0414]** To a solution of **PEG-45-DSG** (150 mg, 0.055 mmol, 1.0 equiv) and **Int-24-S** (100 mg, 0.055 mmol, 1.0 equiv) in DCM (2.0 mL), HOBt (10 mg, 0.066 mmol, 1.2 equiv), EDC (12.6 mg, 0.066 mmol, 1.2 equiv) and DIPEA (0.3 mL, 0.16 mmol, 3 equiv) were added, the resulting mixture was stirred for 48 h at room temperature, additional 10 mL of DCM was added, washed with brine, dried with anhydrous MgSO<sub>4</sub>. Filtered, concentrated, the crude product was purified with normal phase Combi Flash (Gold column, eluted with 0- 100% MeOH) collected 210 mg (84% yield) desired product. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.35 (dd, *J* = 21.5, 7.4 Hz, 3H), 4.73 (d, *J* = 8.4 Hz, 1H), 4.05 (d, *J* = 6.4 Hz, 4H), 4.01 – 3.85 (m, 5H), 3.67 (s, 98H), 3.60 – 3.53 (m, 4H), 3.47 (dt, *J* = 13.9, 6.3 Hz, 7H), 3.26 – 3.06 (m, 6H), 2.20 – 1.93 (m, 22H), 1.60 (s, 35H), 1.47 (d, *J* = 6.7 Hz, 8H), 1.30 (d, *J* = 17.0 Hz, 61H), 0.90 (t, *J* = 6.7 Hz, 6H).

**[0415] Preparation of Compound 79**

**[0416]** The compound **A49** (210 mg, 0.046 mmol) was dissolved in 3 mL of 7N NH<sub>3</sub> (24 mg, 1.39 mmol, 30 equiv) in MeOH solution and transferred to a pressure tube and stirred at room temperature for 24 h. Then reaction was concentrated via rotavapor and purified by reverse phase chromatography (C18 column) with water and acetonitrile/methanol as the mobile phases. Final product was recovered as a white solid 94 mg with a yield of 48.8 %. <sup>1</sup>H NMR (400 MHz, MeOD) δ 4.60 (s, 2H), 4.39 (d, *J* = 8.4 Hz, 3H), 4.34 – 4.28 (m, 1H), 4.05 (dt, *J* = 13.1, 6.3 Hz, 8H), 3.97 – 3.74 (m, 17H), 3.66 (s, 190H), 3.58 – 3.42 (m, 18H), 3.26 (d, *J* = 6.4 Hz, 2H), 3.11 (dd, *J* = 8.5, 4.9 Hz, 9H), 2.49 (d, *J* = 8.8 Hz, 2H), 2.32 (t, *J* = 7.3 Hz, 2H), 2.22 (t, *J* = 7.5 Hz, 2H), 2.00 (s, 9H), 1.61 (dt, *J* = 22.6, 15.9, 8.0 Hz, 30H), 1.31 (s, 89H), 0.95 – 0.89 (m, 6H). MS (MALDI\_TOF) *m/z* [M+H]<sup>+</sup> calculated for 4280.45 ; found for 4281.09, n=47

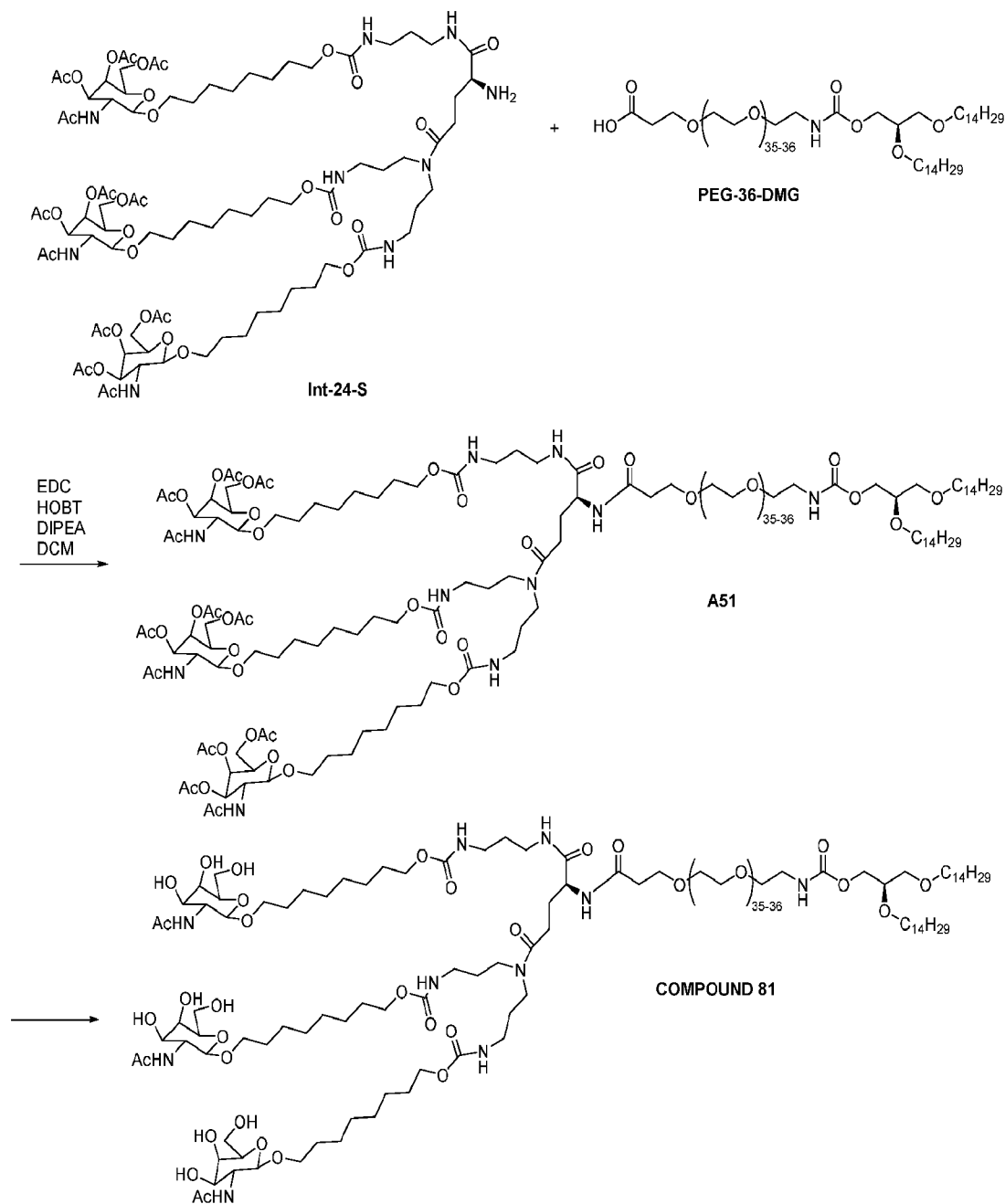
**[0417] Example 11. Preparation of Compound 80****Scheme 16: Synthesis of Compound 80****[0418] Preparation of A50**

**[0419]** To a solution of **PEG-45-DMG** (145.8 mg, 0.055 mmol, 1.0 equiv) and **Int-24-S** (100 mg, 0.055 mmol, 1.0 equiv) in DCM (2.0 mL), HOBT (10 mg, 0.066 mmol, 1.2 equiv), EDC (12.6 mg, 0.066 mmol, 1.2 equiv) and DIPEA (0.3 mL, 0.16 mmol, 3 equiv) were added, the resulting mixture was stirred for 48 h at room temperature, additional 10 mL of DCM was added, washed with brine, dried with anhydrous  $\text{MgSO}_4$ . Filtered, concentrated, the crude product was purified with normal phase Combi Flash (Gold column, eluted with 0-100% MeOH) collected 210 mg (84% yield) desired product.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.35 (d,  $J = 27.5$  Hz, 3H), 4.73 (d,  $J = 8.7$  Hz, 2H), 4.26 – 4.14 (m, 4H), 4.00 (d,  $J = 36.9$  Hz,

11H), 3.67 (s, 128H), 3.48 (dd,  $J = 12.9, 6.5$  Hz, 11H), 3.33 (s, 4H), 3.27 – 3.02 (m, 10H), 2.21 – 1.90 (m, 31H), 1.58 (dd,  $J = 12.3, 7.0$  Hz, 24H), 1.48 (s, 8H), 1.30 (d,  $J = 18.7$  Hz, 67H), 0.90 (t,  $J = 6.6$  Hz, 6H).

**[0420] Preparation of Compound 80**

**[0421]** The compound **A50** (180 mg, 0.04 mmol, 1 equiv) was dissolved in 3 mL of 7N  $\text{NH}_3$  (20.6 mg, 1.21 mmol, 30 equiv) in MeOH solution and transferred to a pressure tube and stirred at room temperature for 24 h. Then reaction was concentrated via rotovap and purified by reverse phase chromatography (C18 column) with water and acetonitrile/methanol as the mobile phases. Final product was recovered as a white solid 114 mg with a yield of 69 %.  $^1\text{H}$  NMR (400 MHz, MeOD)  $\delta$  4.60 (s, 2H), 4.39 (d,  $J = 8.4$  Hz, 3H), 4.17 – 3.99 (m, 9H), 3.97 – 3.72 (m, 18H), 3.66 (s, 191H), 3.62 – 3.43 (m, 26H), 3.37 (t,  $J = 5.4$  Hz, 7H), 3.25 (t,  $J = 6.9$  Hz, 3H), 3.18 – 3.07 (m, 10H), 2.49 (q,  $J = 8.2$  Hz, 3H), 2.32 (t,  $J = 7.3$  Hz, 3H), 2.22 (t,  $J = 7.5$  Hz, 3H), 2.10 (s, 1H), 2.00 (s, 9H), 1.84 – 1.50 (m, 34H), 1.34 (d,  $J = 16.2$  Hz, 71H), 0.92 (t,  $J = 6.7$  Hz, 6H). MS (MALDI\_TOF)  $m/z$   $[\text{M}+\text{H}]^+$  calculated for 4212.29 ; found for 4212.95,  $n=48$

**[0422] Example 12. Preparation of Compound 81****Scheme 17: Synthesis of Compound 81****[0423] Preparation of A51**

**[0424]** To a solution of **PEG-45-DMG** (122.4 mg, 0.055 mmol, 1.0 equiv) and **Int-24-S** (100 mg, 0.055 mmol, 1.0 equiv) in DCM (2.0 mL), HOBt (10 mg, 0.066 mmol, 1.2 equiv), EDC (12.6 mg, 0.066 mmol, 1.2 equiv) and DIPEA (0.3 mL, 0.16 mmol, 3 equiv) were added, the resulting mixture was stirred for 48 h at room temperature, additional 10 mL of DCM was added, washed with brine, dried with anhydrous  $\text{MgSO}_4$ . Filtered, concentrated, the crude product was purified with reverse phase Combi Flash (Gold C18 gel column, eluted with 100% MeOH) collected 200 mg (90.3% yield) desired product.  $^1\text{H}$  NMR (400 MHz,

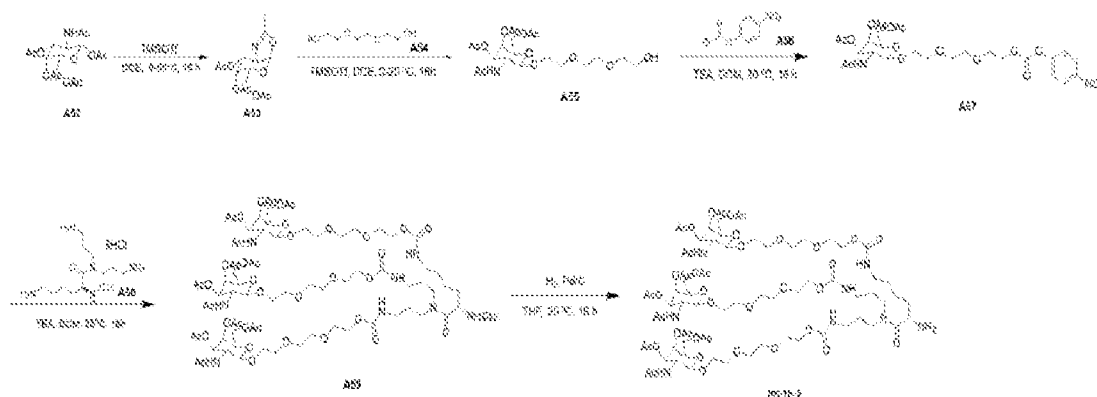
CDCl<sub>3</sub>)  $\delta$  5.41 – 5.27 (m, 6H), 4.73 (d,  $J$  = 8.4 Hz, 2H), 4.24 – 4.11 (m, 8H), 4.05 (d,  $J$  = 7.1 Hz, 5H), 4.00 – 3.77 (m, 10H), 3.66 (s, 129H), 3.57 (q,  $J$  = 5.9 Hz, 4H), 3.53 – 3.42 (m, 9H), 3.41 – 3.08 (m, 11H), 2.20 – 1.93 (m, 34H), 1.58 (dd,  $J$  = 12.5, 7.3 Hz, 16H), 1.47 (d,  $J$  = 6.7 Hz, 2H), 1.30 (d,  $J$  = 19.0 Hz, 66H), 0.90 (t,  $J$  = 6.7 Hz, 6H).

#### [0425] Preparation of Compound 81

[0426] The compound **A51** (200 mg, 0.05 mmol, 1 equiv) was dissolved in 3 mL of 7N NH<sub>3</sub> (25.3 mg, 1.49 mmol, 30 equiv) in MeOH solution and transferred to a pressure tube and stirred at room temperature for 24 h. Then reaction was concentrated via rotovap and purified by reverse phase chromatography (C18 column) with water and acetonitrile/methanol as the mobile phases. Final product was recovered as a white solid 90.3 mg with a yield of 49.8 %.

<sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  4.60 (s, 1H), 4.39 (d,  $J$  = 8.4 Hz, 3H), 4.17 – 4.13 (m, 1H), 4.05 (dt,  $J$  = 13.2, 6.4 Hz, 7H), 3.98 – 3.76 (m, 18H), 3.66 (s, 142H), 3.60 (d,  $J$  = 3.5 Hz, 3H), 3.58 – 3.43 (m, 14H), 3.13 (dq,  $J$  = 13.6, 6.6 Hz, 7H), 2.55 (t,  $J$  = 6.1 Hz, 2H), 2.52 – 2.46 (m, 1H), 2.00 (s, 9H), 1.83 – 1.68 (m, 6H), 1.57 (t,  $J$  = 6.6 Hz, 16H), 1.34 (d,  $J$  = 17.0 Hz, 71H), 0.95 – 0.89 (m, 6H). MS (MALDI\_TOF)  $m/z$  [M+Na]<sup>+</sup> calculated for 3631.26 ; found for 3631.68,  $n=35$ .

#### [0427] Example 13. Preparation of Int-16-S



#### [0428] Preparation of Compound A56

[0429] To a solution of Compound **A52** (100 g, 256.84 mmol) in DCE (1500 mL) was added TMSOTf (85.63 g, 385.25 mmol, 69.62 mL) at 0 °C. The reaction mixture was warmed to 50 °C and stirred for 1.5 h. Then the mixture was cooled to 20 °C and stirred for 14.5 h. TLC (eluted with DCM: methanol = 10:1,  $R_f$  = 0.6) showed the starting material was consumed and a new main spot formed. The mixture was quenched with addition of sodium bicarbonate solution (1500 mL) and extracted with dichloromethane (500 mL  $\times$  3). The combined organic phase was washed with brine (500 mL  $\times$  2), dried over anhydrous sodium sulfate, filtered and the filtrate was concentrated under reduced pressure to get Cpd. **A53** (90 g, crude) as yellowish oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  ppm 6.22 (d,  $J$  = 3.6 Hz, 1H), 5.99

(d,  $J = 6.8$  Hz, 1H), 5.46 (t,  $J = 3.6$  Hz, 1H), 4.91 (dd,  $J = 3.2, 7.5$  Hz, 1H), 4.24 (t,  $J = 2.8$  Hz, 1H), 4.13-4.08 (m, 2H), 4.00 (dt,  $J = 1.6, 7.1$  Hz, 1H), 2.12 (s, 3H), 2.07 (s, 6H), 2.05 (d,  $J = 1.2$  Hz, 3H).

**[0430] Preparation of Compound A55**

**[0431]** To a mixture of Cpd. **A53** (29 g, 88.07 mmol), Cpd. **A54** (52.90 g, 352.26 mmol) and 4A MOLECULAR SIEVE (29 g) in DCE (1600 mL) was added TMSOTf (29.36 g, 132.10 mmol, 23.87 mL) at 0 °C, and then the mixture was stirred at 20 °C for 16 h under N<sub>2</sub> atmosphere. LCMS showed 8% of Compound A53 remained. 50% desired compound was detected. The mixture was quenched with addition of sodium bicarbonate solution (1000 mL) and extracted with dichloromethane (500 mL  $\times$  3). The combined organic phase was washed with brine (500 mL  $\times$  2), dried over anhydrous sodium sulfate, the mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM : MeOH = 1:0 to 1:2) to get Cpd. **A55** (33 g, 78.15% yield) as a yellowish oil. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 7.81 (d,  $J = 9.2$  Hz, 1H), 5.21 (d,  $J = 3.6$  Hz, 1H), 4.96 (dd,  $J = 3.2, 11.2$  Hz, 1H), 4.60 (t,  $J = 5.2$  Hz, 1H), 4.55 (d,  $J = 8.4$  Hz, 1H), 4.06-3.99 (m, 3H), 3.82-3.74 (m, 1H), 3.63-3.55 (m, 2H), 3.54-3.47 (m, 8H), 3.44-3.39 (m, 2H), 2.11 (s, 3H), 2.00 (s, 3H), 1.89 (s, 3H), 1.77 (s, 3H).

**[0432] Preparation of Compound A57**

**[0433]** To a solution of Compound **A55** (20 g, 41.71 mmol) in DCM (120 mL) was added TEA (6.33 g, 62.57 mmol, 8.71 mL) and Cpd. **A56** (12.61 g, 62.57 mmol) at 20 °C. The mixture was stirred at 20 °C for 16 h. LC-MS showed 10% of Compound A55 remained. 79% of desired compound was detected. The mixture was quenched with water (300 mL) and extracted with DCM (60 mL  $\times$  3). The combined organic layers were separated, and the organic layer was washed with brine (30 mL  $\times$  6), dried over Na<sub>2</sub>SO<sub>4</sub>. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM: MeOH = 1/0 to 10/1) to get Compound **A57** (15 g, 55.79% yield) as a yellowish oil. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 8.41-8.27 (m, 2H), 7.80 (d,  $J = 9.2$  Hz, 1H), 7.65-7.48 (m, 2H), 5.22 (d,  $J = 3.2$  Hz, 1H), 4.98 (dd,  $J = 3.4, 11.2$  Hz, 1H), 4.57 (d,  $J = 8.4$  Hz, 1H), 4.44-4.31 (m, 2H), 4.04 (s, 3H), 3.96-3.87 (m, 1H), 3.83-3.70 (m, 3H), 3.63-3.48 (m, 7H), 2.15-2.09 (m, 3H), 2.05-1.97 (m, 3H), 1.90 (s, 3H), 1.78 (s, 3H).

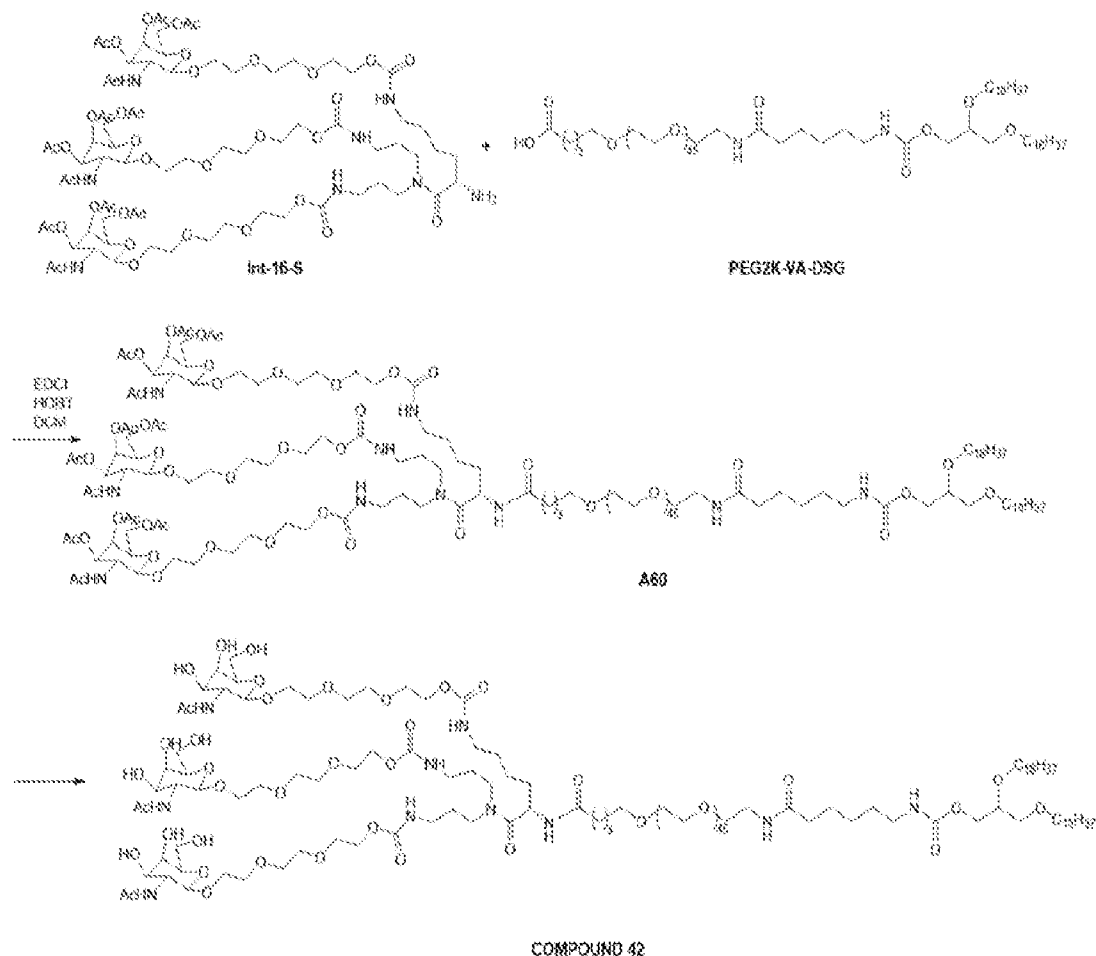
**[0434] Preparation of Compound A59**

**[0435]** To a solution of Compound **A58** (1.3 g, 2.58 mmol, 3HCl) in DCM (10 mL) was added TEA (2.62 g, 25.85 mmol, 3.60 mL) and Compound **A57** (5.33 g, 8.27 mmol) at 25

°C. The mixture was stirred at 25 °C for 16 h. LC-MS showed Compound **A57** was consumed completely and one main peak with desired MS was detected. The mixture was quenched with water (20 ml) and extracted with DCM (20 mL × 3). The combined organic layers were separated, and the organic layer was washed with brine (20 mL × 3), dried over Na<sub>2</sub>SO<sub>4</sub>. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM: MeOH = 1/0 to 19/1) for twice to get Compound **A59** (2.3 g, 46.59% yield) as a yellowish solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ ppm 7.80 (d, *J* = 9.2 Hz, 3H), 7.49-7.24 (m, 7H), 7.21-7.09 (m, 2H), 5.22 (d, *J* = 3.6 Hz, 3H), 5.01 (s, 2H), 4.98 (dd, *J* = 3.2, 11.2 Hz, 3H), 4.56 (d, *J* = 8.4 Hz, 3H), 4.32-4.22 (m, 1H), 4.11-3.98 (m, 15H), 3.89 (td, *J* = 8.8, 11.2 Hz, 3H), 3.82-3.75 (m, 3H), 3.65-3.42 (m, 29H), 3.15-2.99 (m, 3H), 2.97-2.85 (m, 4H), 2.11 (s, 9H), 2.00 (s, 9H), 1.90 (s, 9H), 1.78 (s, 9H), 1.60-1.45 (m, 4H), 1.40-1.23 (m, 4H).

**[0436] Preparation of Int-16-S**

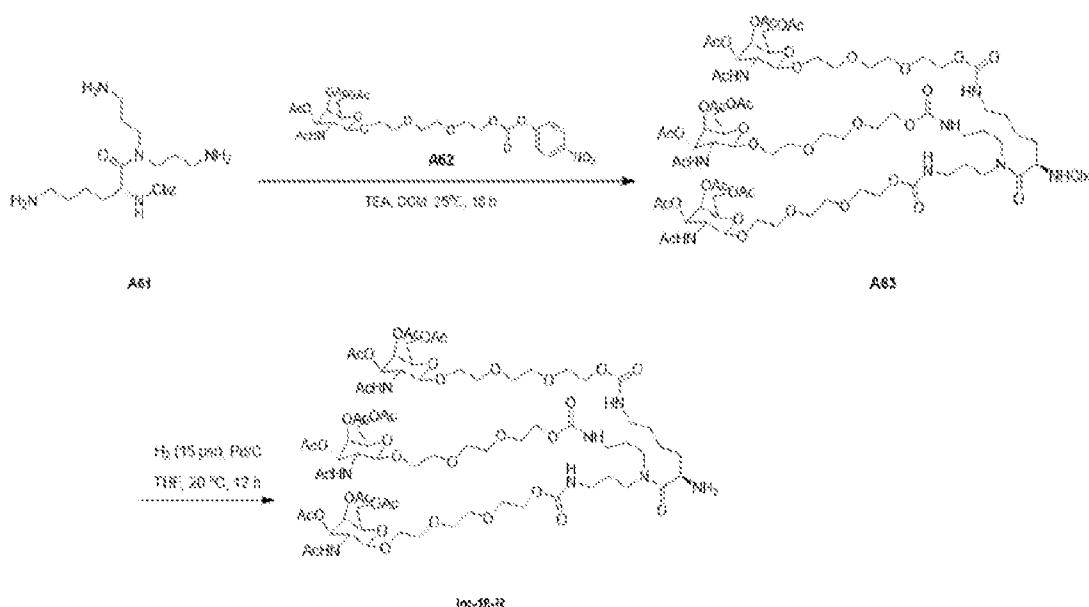
**[0437]** A mixture of Compound **A59** (2.3 g, 1.20 mmol) and Pd/C (1.15 g, 1.08 mmol, 10% purity) in THF (100 mL) was degassed and purged with H<sub>2</sub> (15 psi) for 3 times, and then the mixture was stirred at 20 °C for 16 h under H<sub>2</sub> atmosphere. LCMS showed Reactant 1 was consumed completely and one main peak with desired MS was detected. The mixture was filtered, and the filtrate was concentrated under reduced pressure to get **Int-16-S** (1.4 g, 65.47% yield) as a gray solid. **LCMS**: *t*<sub>R</sub> = 1.840 min, 100% purity, *m/z* = 1776.0 (M+H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ ppm 7.79 (d, *J* = 9.2 Hz, 3H), 7.31 (br t, *J* = 5.2 Hz, 1H), 7.21-7.09 (m, 2H), 5.21 (d, *J* = 3.6 Hz, 3H), 4.97 (dd, *J* = 3.2, 11.2 Hz, 3H), 4.55 (d, *J* = 8.4 Hz, 3H), 4.03 (s, 16H), 3.92-3.83 (m, 3H), 3.82-3.73 (m, 3H), 3.60-3.48 (m, 28H), 3.42 (br d, *J* = 5.2 Hz, 1H), 3.36 (br d, *J* = 6.8 Hz, 1H), 3.24 (br dd, *J* = 8.4, 16.8 Hz, 2H), 3.12-3.05 (m, 1H), 3.03-2.97 (m, 2H), 2.93 (br d, *J* = 6.0 Hz, 4H), 2.10 (s, 9H), 2.00 (s, 9H), 1.89 (s, 9H), 1.77 (s, 9H), 1.67-1.62 (m, 2H), 1.59 - 1.54 (m, 2H), 1.41-1.18 (m, 6H).

**[0438] Example 14. Preparation of Compound 42****Scheme 18: Synthesis of Compound 42****[0439] Preparation of A60**

**[0440]** To a solution of **PEG2k-VA-DSG** acid (250 mg, 0.084 mmol), **Int-16-S** (150 mg, 0.084 mmol) in DCM (2.0 mL), DIPEA (0.05 mL, 0.25 mmol) was added, followed by the addition of EDCI (17 mg, 0.084 mmol), and finally HOBT (13 mg, 0.084 mmol). The resulting mixture was stirred for 48 hours at room temperature. Then reaction was diluted with DCM and washed with brine and dried over MgSO<sub>4</sub>, and filtered, concentrated, the crude product was purified with Combi-flash (24 g gold normal phase column, 0-50% MeOH in DCM), collected 260 mg product with 67% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.44 – 5.31 (m, 3H), 5.15 – 4.99 (m, 2H), 4.81 (d, *J* = 8.7 Hz, 2H), 4.36 – 4.06 (m, 8H), 4.00 – 3.80 (m, 4H), 3.67 (s, 120H), 3.61 – 3.41 (m, 10H), 3.22 – 3.07 (m, 4H), 2.22 – 1.96 (m, 36H), 1.74 – 1.45 (m, 36H), 1.28 (s, 48H), 0.89 (t, *J* = 7.9 Hz, 6H).

**[0441] Preparation of Compound 42**

**[0442]** A solution of **A60** (260 mg, 0.056 mmol) in THF (2.0 mL) and MeOH (2.0 mL) was cooled to 0°C H<sub>2</sub>O (1.0 mL) was added with LiOH (70.58 mg, 1.68 mmol), the mixture was stirred at room temperature for 4 hours, cooled with ice water, the pH was adjusted to 5-6 with 1N HCl aqueous solution, concentrated, the crude product was uploaded to C-18 column (24 g), eluted with 100% water in 15 mins, then raise the ACN to 100%, then replaced ACN with 100% MeOH, the major peak was eluted out at 50 mins, after concentration, collected 100 mg of desired product with 41 % yield. <sup>1</sup>H NMR (400 MHz, MeOD) δ 4.60 (s, 1H), 4.50 (d, *J* = 8.4 Hz, 3H), 4.20 (d, *J* = 5.2 Hz, 6H), 4.00 – 3.93 (m, 5H), 3.88 – 3.72 (m, 14H), 3.60 (s, 200H), 3.58 – 3.43 (m, 17H), 3.20 – 3.06 (m, 8H), 2.01 (d, *J* = 1.0 Hz, 8H), 1.62 (dddt, *J* = 37.6, 22.3, 15.3, 8.0 Hz, 18H), 1.31 (s, 64H), 0.95 – 0.88 (m, 6H). [M+Na]<sup>+</sup> calculated for 4235.59; found for 4235.31, n=44.

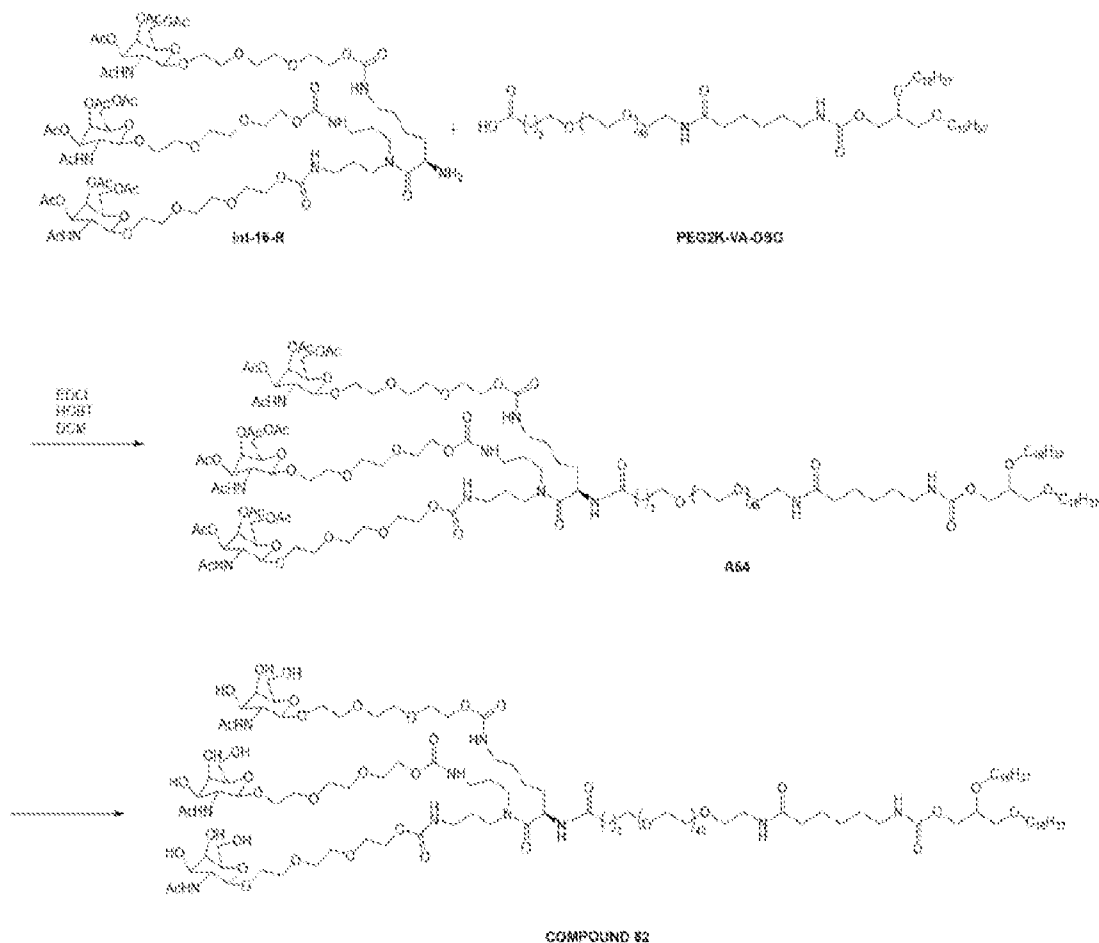
**[0443] Example 15. Preparation of Int-16-R:****[0444] Preparation of Compound A63**

**[0445]** To a solution of **Cpd. A61** (1 g, 1.99 mmol, 3HCl) and **Cpd.A62** (3.97 g, 6.16 mmol) in DCM (30 mL) was added TEA (2.01 g, 19.88 mmol) at 25 °C and stirred at 25°C for 16 h. TLC indicated the Reactant 1 was consumed, and a new spot was detected. The reaction mixture was quenched by H<sub>2</sub>O (50 mL) and extracted with DCM (3 x 50 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (SiO<sub>2</sub>, DCM:MeOH = 1:0 to 0:1) for twice to give **Cpd.A63** (3.5 g, 46% yield) as a yellow oil. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 7.80 (d, *J* = 9.2 Hz, 3H),

7.44 (br d,  $J = 7.6$  Hz, 1H), 7.38 (br s, 6H), 7.21 - 7.07 (m, 2H), 5.22 (d,  $J = 3.2$  Hz, 3H), 5.05 - 4.92 (m, 5H), 4.56 (d,  $J = 8.4$  Hz, 3H), 4.33 - 4.20 (m, 1H), 4.12 - 3.97 (m, 16H), 3.89 (td,  $J = 8.8, 11.2$  Hz, 3H), 3.82 - 3.74 (m, 3H), 3.65 - 3.41 (m, 29H), 3.29 (br s, 1H), 3.16 - 2.83 (m, 8H), 2.11 (s, 9H), 2.03 - 1.99 (m, 9H), 1.90 (s, 9H), 1.78 (s, 9H), 1.60 - 1.45 (m, 4H), 1.40 - 1.24 (m, 4H).

**[0446] Preparation of Int-16-R**

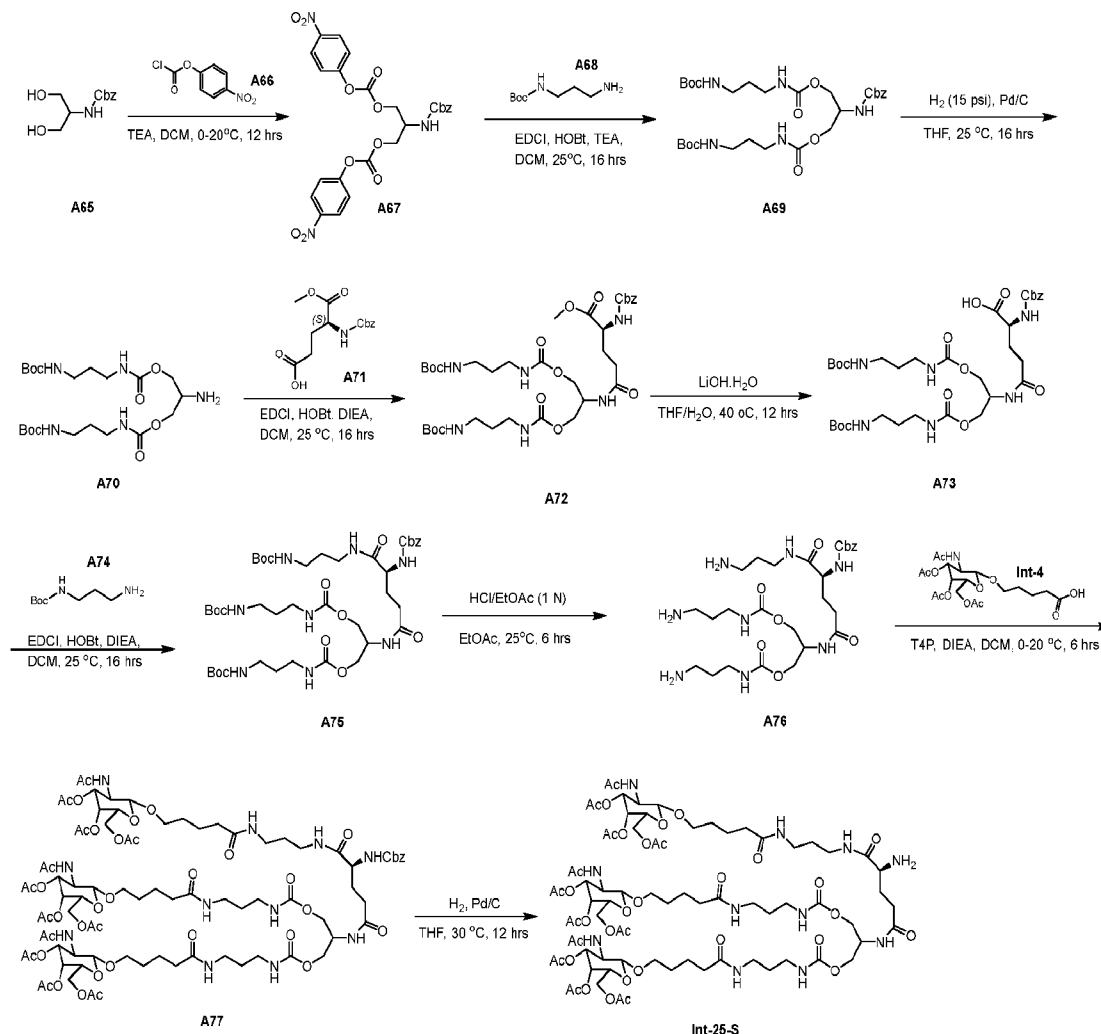
**[0447]** To a solution of **Cpd.A63** (750 mg, 392.69  $\mu$ mol) in THF (150 mL) was added Pd/C (375.00 mg, 352.38  $\mu$ mol, 10% purity) at 20 °C and stirred at 20°C for 12 h under H<sub>2</sub> (15 psi). TLC (DCM: MeOH = 10:1 R<sub>f</sub> = 0.4) indicated the Reactant 1 was consumed, a new spot was detected. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give **Int-16-R** (2.0 g, 71.70% yield) as a gray solid. **LCMS**:  $t_R = 1.846$  min, 100.00% purity,  $m/z = 1776.0$  (M+H)<sup>+</sup>. **<sup>1</sup>H NMR** (400 MHz, DMSO- $d_6$ )  $\delta$  7.80 (br d,  $J = 9.2$  Hz, 3H), 7.31 (br t,  $J = 5.2$  Hz, 1H), 7.21 - 7.08 (m, 2H), 5.21 (d,  $J = 3.2$  Hz, 3H), 4.97 (dd,  $J = 3.2, 11.1$  Hz, 3H), 4.55 (d,  $J = 8.4$  Hz, 3H), 4.03 (s, 15H), 3.93 - 3.83 (m, 3H), 3.83 - 3.72 (m, 3H), 3.65 - 3.46 (m, 27H), 3.42 (br d,  $J = 6.8$  Hz, 1H), 3.36 (br d,  $J = 7.2$  Hz, 1H), 3.29 - 3.16 (m, 2H), 3.13 - 3.04 (m, 1H), 3.03 - 2.97 (m, 2H), 2.93 (br d,  $J = 5.6$  Hz, 4H), 2.17 - 2.07 (m, 9H), 2.00 (s, 9H), 1.95 - 1.85 (m, 9H), 1.82 - 1.72 (m, 10H), 1.72 - 1.47 (m, 6H), 1.46 - 1.14 (m, 5H).

**[0448] Example 16. Preparation of Compound 82****[0449] Preparation of A64**

**[0450]** To a solution of **PEG2K-VA-DSG** acid (243 mg, 0.084 mmol), **Int-16-R** (150 mg, 0.084 mmol) in DCM (2.0 mL), DIPEA (0.05 mL, 0.25 mmol) was added, followed by the addition of EDCI (17 mg, 0.084 mmol), and finally HOBt (13 mg, 0.084 mmol). The resulting mixture was stirred for 48 hours at room temperature. Then reaction was diluted with DCM and washed with brine and dried over  $\text{MgSO}_4$ , and filtered, concentrated, the crude product was purified with Combi-flash (24 g gold normal phase column, 0-50% MeOH in DCM), collected 330 mg product with 84% yield.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.44 – 5.33 (m, 2H), 5.16 – 5.03 (m, 2H), 4.81 (dd,  $J = 8.5, 6.7$  Hz, 3H), 4.33 – 4.09 (m, 10H), 3.97 – 3.79 (m, 7H), 3.66 (s, 144H), 3.58 (q,  $J = 5.3$  Hz, 8H), 3.47 (ddd,  $J = 15.2, 7.8, 5.9$  Hz, 8H), 3.28 – 3.08 (m, 5H), 2.26 – 1.92 (m, 26H), 1.72 (s, 13H), 1.27 (s, 54H), 0.90 (t,  $J = 6.7$  Hz, 5H).

**[0451] Preparation of Compound 82**

**[0452]** A solution of **A64** (330mg, 0.071 mmol) in THF (3.0 mL) and MeOH (3.0 mL) was cooled to 0°C. H<sub>2</sub>O (1.5 mL) was added with LiOH (90 mg, 2.13 mmol), the mixture was stirred at room temperature for 4 hours, cooled with ice water, the pH was adjusted to 5-6 with 1N HCl aqueous solution, concentrated, the crude product was uploaded to C-18 column (24 g), eluted with 100% water in 15 mins, then raise the ACN to 100%, then replaced ACN with 100% MeOH, the major peak was eluted out at 50 mins, after concentration, collected 70 mg of desired product with 23 % yield. <sup>1</sup>H NMR (400 MHz, MeOD) δ 4.60 (s, 6H), 4.50 (d, *J* = 8.5 Hz, 3H), 4.19 (d, *J* = 5.1 Hz, 6H), 3.96 (t, *J* = 9.9 Hz, 5H), 3.90 – 3.79 (m, 7H), 3.66 (s, 200H), 3.59 – 3.45 (m, 19H), 3.20 – 3.07 (m, 8H), 2.26 (dt, *J* = 27.1, 7.3 Hz, 4H), 2.01 (s, 9H), 1.62 (ddt, *J* = 45.4, 22.0, 7.6 Hz, 20H), 1.31 (s, 65H), 0.92 (t, *J* = 6.7 Hz, 6H). [M+Na]<sup>+</sup> calculated for 4235.59; found for 4235.12, n=44.

**[0453] Example 17. Preparation of Int-25-S****Scheme 19: Synthesis of Int-25-S**

**[0454] Preparation of Compound A67**

**[0455]** To a solution of Cpd.A66 (78.75 g, 390.69 mmol) in DCM (400 mL) was drop wise Cpd.A65 (40 g, 177.59 mmol) in DCM (200 mL) and Pyridine (42.14 g, 532.76 mmol) at 0°C. The mixture was stirred at 20 °C for 12 hrs. TLC (eluted with Petroleum ether:Ethyl acetate = 3:1, R<sub>f</sub> = 0.5) showed the starting material was consumed and one new spot formed. The mixture was quenched with water (600 ml) and extracted with DCM (200 mL × 3). The combined organic layers were separated, and the organic layer was washed with brine (100 mL), dried over Na<sub>2</sub>SO<sub>4</sub>. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (SiO<sub>2</sub>, Petroleum ether:Ethyl acetate=1:0 to 1:2) to get Cpd.A67 (70 g, 71.42% yield) as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm 8.33-8.19 (m, 4H), 7.37 (s, 8H), 5.33 (br s, 1H), 5.16 (s, 2H), 4.53-4.41 (m, 4H).

**[0456] Preparation of Compound A69**

**[0457]** To a solution of Cpd.A68 (46.11 g, 264.65 mmol, 46.21 mL) and Cpd.A67 (70 g, 126.02 mmol) in DCM (800 mL) was added TEA (38.26 g, 378.07 mmol, 52.62 mL). The mixture was stirred at 20 °C for 16 hrs. TLC (eluted with DCM: MeOH = 10:1, R<sub>f</sub> = 0.47) showed the starting material was consumed and one new spot formed. The mixture was quenched with water (400 ml) and extracted with DCM (150 mL × 3). The combined organic layers were separated, and the organic layer was washed with brine (100 mL × 2), dried over Na<sub>2</sub>SO<sub>4</sub>. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM: MeOH =1:0 to 10:1) to get Cpd.A69 (39 g, 49.46% yield) as a yellowish solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ ppm 7.40-7.31 (m, 5H), 7.11 (br t, *J* = 5.2 Hz, 2H), 6.75 (br t, *J* = 5.2 Hz, 2H), 5.02 (s, 2H), 3.98 (br d, *J* = 5.6 Hz, 2H), 3.93-3.82 (m, 3H), 3.02-2.81 (m, 8H), 1.48 (quin, *J* = 6.8 Hz, 4H), 1.37 (s, 18H)

**[0458] Preparation of Compound A70**

**[0459]** A mixture of Cpd.A69 (15 g, 23.98 mmol), Pd/C (7.66 g, 7.20 mmol, 10% purity,) in THF (1500 mL) was degassed and purged with H<sub>2</sub>(15 psi) for 3 times, and then the mixture was stirred at 20 °C for 16hr under H<sub>2</sub> atmosphere. LC-MS showed Cpd.5 was consumed completely and one main peak with desired m/z was detected. The mixture was filtered, and the filtrate was concentrated under reduced pressure to get Cpd.A70 (9.5 g, 80.61% yield) as a gray solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ ppm 7.07 (br t, *J* = 5.6 Hz, 2H), 6.75 (br s, 2H), 3.94-3.79 (m, 4H), 2.93 (qd, *J* = 6.4, 19.2 Hz, 9H), 1.57-1.45 (m, 6H), 1.37 (s, 18H).

**[0460] Preparation of Compound A72**

**[0461]** To a solution of Cpd.A71 (1.2 g, 4.06 mmol), DIEA (2.10 g, 16.26 mmol, 2.83 mL) and Cpd.A70 (2.20 g, 4.47 mmol) in DCM (20 mL) was added EDCI (1.56 g, 8.13 mmol) and HOBt (1.10 g, 8.13 mmol). The mixture was stirred at 25 °C for 16 hrs. LC-MS showed Cpd.6 was consumed completely and one main peak with desired m/z was detected. The mixture was quenched with water (40 ml) and extracted with DCM (15 mL × 3). The combined organic layers were separated, and the organic layer was washed with brine (10 mL × 2), dried over Na<sub>2</sub>SO<sub>4</sub>. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM: MeOH = 1/0 to 3/7) to get Cpd.A72 (2.4 g, 76.81% yield) as a colorless solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ ppm 7.90 (br d, *J* = 8.0 Hz, 1H), 7.75 (br d, *J* = 7.6 Hz, 1H), 7.43-7.22 (m, 5H), 7.08 (br d, *J* = 4.0 Hz, 2H), 6.74 (br s, 2H), 5.03 (s, 2H), 4.10-4.04 (m, 2H), 3.99-3.84 (m, 4H), 3.63 (s, 3H), 2.92 (qd, *J* = 6.4, 18.4 Hz, 8H), 2.17 (br t, *J* = 7.2 Hz, 2H), 1.96-1.89 (m, 1H), 1.77 (br dd, *J* = 8.4, 13.8 Hz, 1H), 1.55-1.44 (m, 4H), 1.37 (s, 18H).

**[0462] Preparation of Compound A73**

**[0463]** To a solution of Cpd.A72 (5.8 g, 7.54 mmol) in THF (60 mL) and H<sub>2</sub>O (30 mL) was added LiOH.H<sub>2</sub>O (1.27 g, 30.17 mmol) at 20 °C. The mixture was stirred at 40 °C for 12 hrs. TLC (eluted with DCM: MeOH = 10:1, *R*<sub>f</sub> = 0.23) showed the starting material was consumed and one new spot formed. The THF was removed in vacuo and the residue dissolved in DCM (30 ml). The residue was added citric acid 10% w/v (100 ml) adjust pH less than 4 and the mixture was stirred for 5 min. Then the residue was extracted with DCM (30 × 2mL) and the mixture was filtered and the filtrate was concentrated under reduced pressure to get Cpd.A73 (4 g, 70.25% yield) as a colorless oil. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ ppm 7.90 (br d, *J* = 8.0 Hz, 1H), 7.56 (br d, *J* = 8.0 Hz, 1H), 7.43-7.27 (m, 5H), 7.09 (br s, 2H), 6.74 (br t, *J* = 5.2 Hz, 2H), 5.03 (d, *J* = 2.8 Hz, 2H), 4.12-4.04 (m, 1H), 3.99-3.83 (m, 5H), 3.32 (br s, 2H), 3.03-2.81 (m, 8H), 2.17 (br t, *J* = 7.2 Hz, 2H), 1.96 (br dd, *J* = 5.6, 13.6 Hz, 1H), 1.82-1.68 (m, 1H), 1.48 (quin, *J* = 6.8 Hz, 4H), 1.37 (s, 18H).

**[0464] Preparation of Compound A75**

**[0465]** To a solution of Cpd.A74 (3.23 g, 18.55 mmol, 3.24 mL), Cpd.A73 (4 g, 5.30 mmol) and DIEA (3.42 g, 26.50 mmol, 4.62 mL) in DCM (45 mL) was added EDCI (3.56 g, 18.55 mmol) and HOBt (2.51 g, 18.55 mmol) at 20 °C. The mixture was stirred at 25 °C for 16 hrs. TLC (eluted with DCM: MeOH = 10:1, *R*<sub>f</sub> = 0.43) showed the starting material was consumed and one new spot formed. The mixture was quenched with water (60 ml) and extracted with DCM (15 mL × 3). The combined organic layers were separated, and the

organic layer was washed with brine (10 mL  $\times$  2), dried over Na<sub>2</sub>SO<sub>4</sub>. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM: MeOH = 1/0 to 10/1) to get Cpd.A75 (3.7 g, 76.64% yield) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 7.94-7.80 (m, 2H), 7.43-7.26 (m, 5H), 7.09 (br s, 2H), 6.82-6.71 (m, 3H), 5.02 (s, 2H), 4.00-3.83 (m, 5H), 3.17 (d, *J* = 5.2 Hz, 2H), 3.04 (q, *J* = 6.4 Hz, 2H), 2.98-2.86 (m, 10H), 2.20-2.06 (m, 2H), 1.93-1.81 (m, 1H), 1.76-1.64 (m, 1H), 1.48 (quin, *J* = 6.8 Hz, 6H), 1.41-1.30 (m, 27H).

**[0466] Preparation of Compound A76**

**[0467]** A mixture of Cpd.A75 (3.7 g, 4.06 mmol) in Ethyl acetate (40 mL) was added HCl/Ethyl acetate (4 M, 10 mL), and then the mixture was stirred at 25 °C for 6hr. LCMS showed Cpd.A75 was consumed completely and one main peak with desired *m/z* was detected. The mixture was concentrated under reduced pressure to give Cpd.A76 (3.26 g, crude) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 8.20 (br t, *J* = 5.6 Hz, 1H), 8.01 (br s, 9H), 7.49 (br d, *J* = 7.6 Hz, 1H), 7.38-7.29 (m, 5H), 5.12-4.95 (m, 2H), 4.07 (br d, *J* = 4.4 Hz, 5H), 3.95-3.84 (m, 3H), 3.14 (q, *J* = 6.4 Hz, 2H), 3.04 (q, *J* = 6.4 Hz, 4H), 2.81-2.70 (m, 5H), 2.22-2.07 (m, 2H), 1.94-1.82 (m, 1H), 1.71 (qd, *J* = 7.2, 14.3 Hz, 6H), 1.59 (s, 1H).

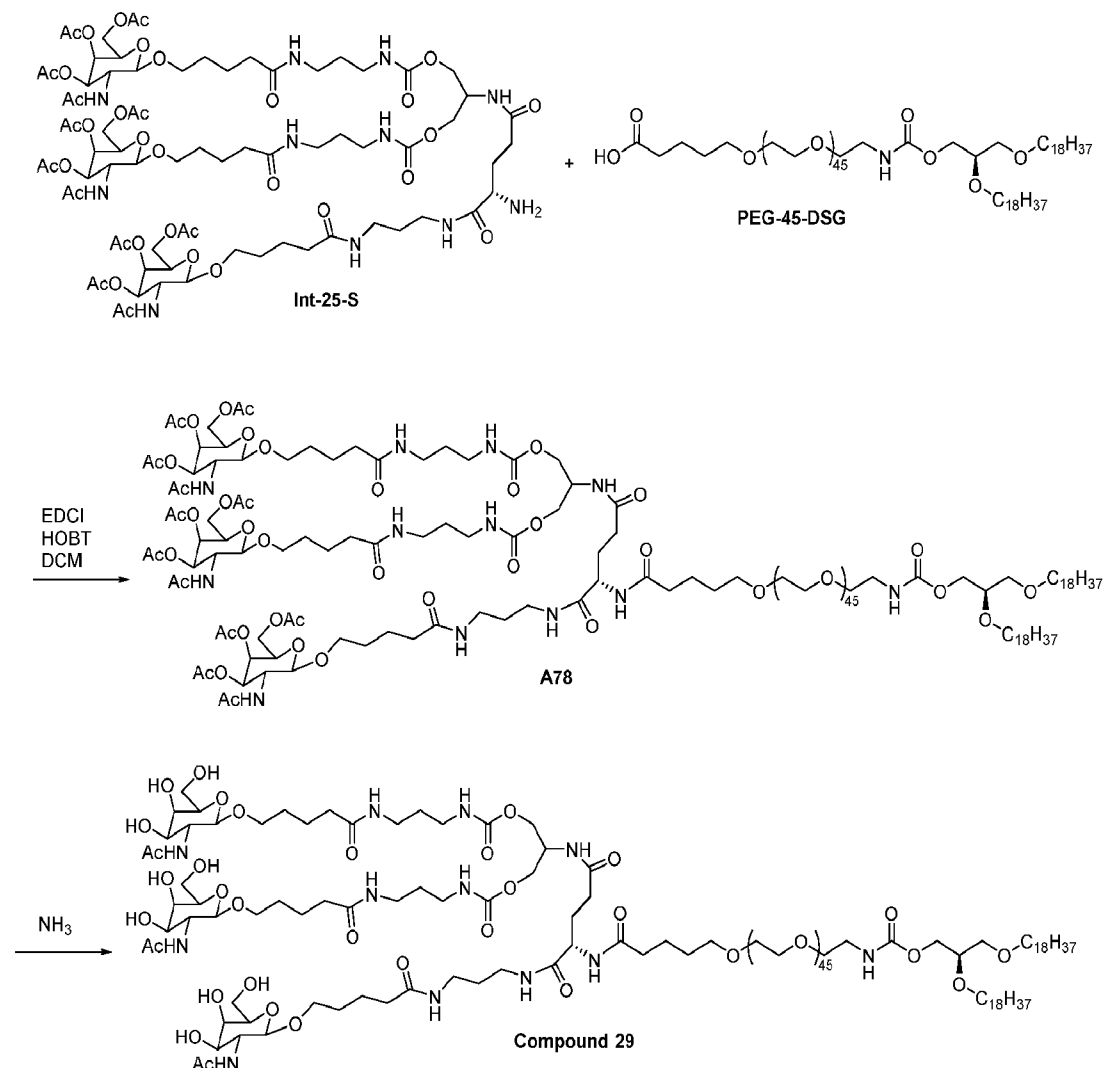
**[0468] Preparation of Compound A77**

**[0469]** To a solution of Int-4 (7.29 g, 16.30 mmol), DIEA (5.85 g, 45.27 mmol) and Cpd.A76 (3.26 g, 4.53 mmol, 3HCl) in DCM (120 mL) was added then was added T4P (13.05 g, 18.11 mmol, 50% purity) at 0 °C. The mixture was stirred at 20 °C for 6 hrs. LC-MS showed Cpd.A76 was consumed completely and one main peak with desired MS or desired mass was detected. The mixture was quenched with water (100 ml) and extracted with DCM (40 mL  $\times$  3). The combined organic layers were separated, and the organic layer was washed with brine (30 mL  $\times$  6), dried over Na<sub>2</sub>SO<sub>4</sub>. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM: MeOH = 1:0 to 7:1) in twice to get Cpd.A77 (1.6 g, 53.33% yield) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 7.95-7.67 (m, 9H), 7.47-7.30 (m, 6H), 7.12 (br s, 2H), 5.22 (d, *J* = 3.2 Hz, 3H), 5.10-4.92 (m, 5H), 4.49 (d, *J* = 8.4 Hz, 3H), 4.10-4.00 (m, 10H), 3.99-3.94 (m, 2H), 3.91-3.84 (m, 5H), 3.77-3.64 (m, 3H), 3.46-3.37 (m, 3H), 3.11-2.89 (m, 13H), 2.11 (s, 10H), 2.07-2.02 (m, 6H), 2.00 (s, 9H), 1.90 (s, 10H), 1.78 (s, 9H), 1.61-1.34 (m, 20H).

**[0470] Preparation of Compound Int-25-S**

**[0471]** A mixture of Cpd.A77 (1.6 g, 0.84 mmol), Pd/C (896.66 mg, 10% purity) in THF (200 mL) was degassed and purged with H<sub>2</sub>(15 psi) for 3 times, and then the mixture was stirred at 30 °C for 12hr under H<sub>2</sub> atmosphere. LC-MS showed Cpd.A77 was consumed completely and one main peak with desired MS was detected. The mixture was filtered, and the filtrate was concentrated under reduced pressure to get **Int-25-S** (1.48 g, 99 % yield) as a gray solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ ppm 7.95-7.69 (m, 8H), 7.12 (br s, 2H), 5.37 (dd, *J* = 2.0, 6.0 Hz, 1H), 5.21 (d, *J* = 3.6 Hz, 3H), 4.97 (dd, *J* = 3.6, 11.3 Hz, 3H), 4.49 (d, *J* = 8.4 Hz, 3H), 4.26 (t, *J* = 7.2 Hz, 1H), 4.08-4.00 (m, 9H), 3.95 (br s, 2H), 3.93-3.84 (m, 5H), 3.84-3.76 (m, 2H), 3.75-3.66 (m, 3H), 3.44-3.38 (m, 3H), 3.12-2.92 (m, 13H), 2.45-2.40 (m, 1H), 2.15 (br d, *J* = 6.8 Hz, 1H), 2.10 (s, 9H), 2.03 (br d, *J* = 7.2 Hz, 6H), 2.00 (s, 9H), 1.89 (s, 8H), 1.77 (s, 9H), 1.57-1.38 (m, 20H). **LCMS:** *t*<sub>R</sub> = 1.768 min, 100% purity, *m/z* = 883.3 (M/2+H)<sup>+</sup>

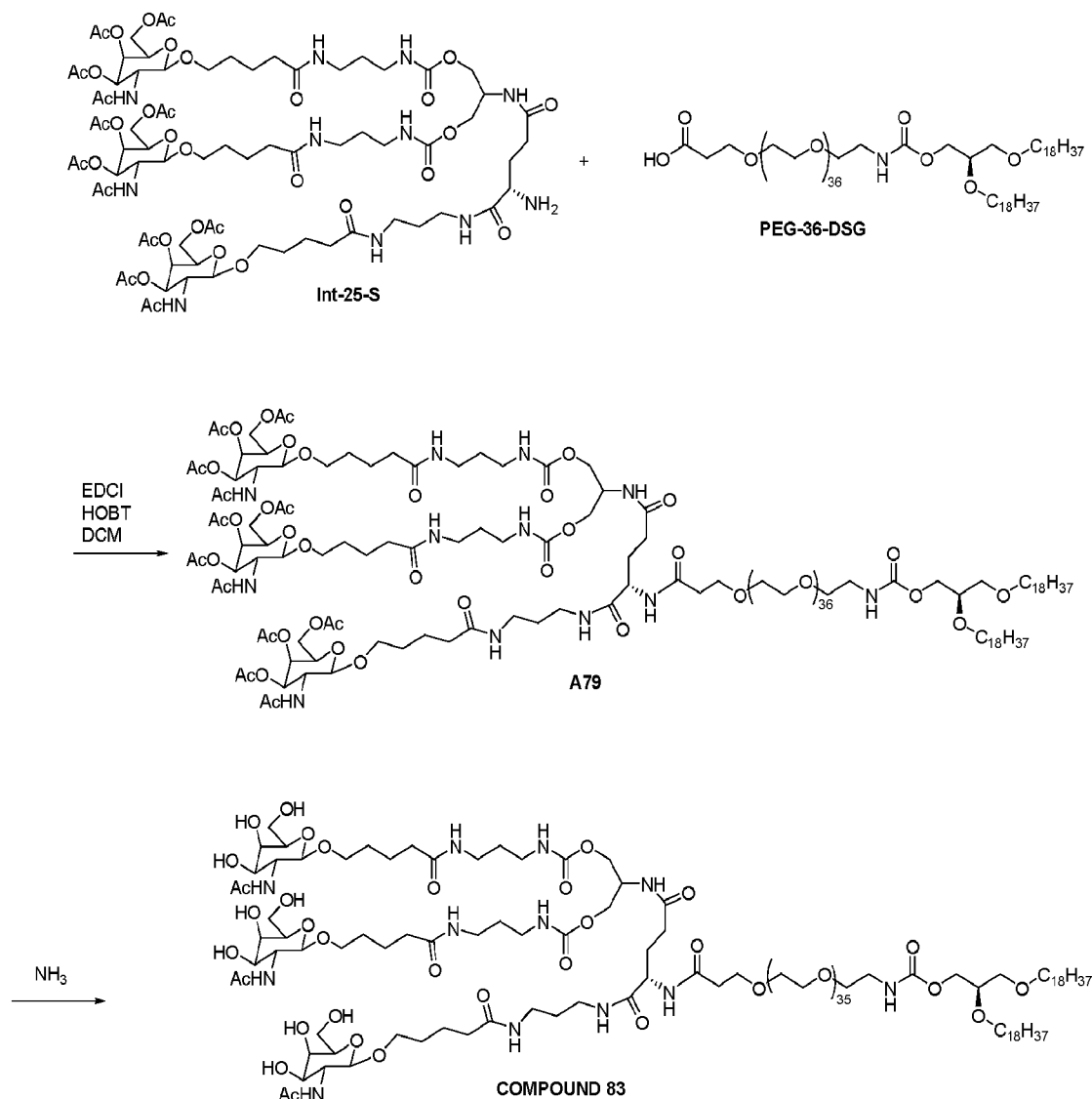
**[0472] Method of LCMS:** LC/MS (The gradient was 0%-60 B in 0-2.5 min, maintains 60% B in 2.5-3.0 min, and 60% B-0%B in 3.0-3.01min, 0% B in 3.01-3.5 min the flow rate was 1 ml/min. Mobile phase A was 0.04% trifluoroacetic acid in water, mobile phase B was 0.02% trifluoroacetic acid in acetonitrile. The column used for chromatography was a Luna LC- C18 50\*2 mm (5 um particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection as well as positive electrospray ionization. MS range was 100-2000.

**[0473] Example 18. Preparation of Compound 29****Scheme 20: Synthesis of Compound 29****[0474] Preparation of A78**

**[0475]** To a solution of **PEG-45-DSG** (156.7 mg, 0.057 mmol, 1.0 equiv) and **Int-25-S** (100 mg, 0.057 mmol, 1.0 equiv) in **DCM** (2.0 mL), **HOBT** (10.4 mg, 0.068 mmol, 1.2 equiv), **EDC** (13 mg, 0.068 mmol, 1.2 equiv) and **DIPEA** (0.17 mmol, 0.3 mL, 1.0 mL) were added, the resulting mixture was stirred for 48 h at room temperature, additional 10 mL of **DCM** was added, washed with brine, dried with anhydrous **MgSO<sub>4</sub>**. Filtered, concentrated, the crude product was purified with C18 reverse phase Combi Flash (Gold C18 gel column, eluted with 100% MeOH) collected 180 mg (86% yield) desired product. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.38 (d, *J* = 3.4 Hz, 2H), 5.22 (d, *J* = 11.2 Hz, 2H), 4.64 (d, *J* = 8.8 Hz, 2H), 4.15 (ddd, *J* = 15.8, 11.4, 5.9 Hz, 9H), 3.95 (d, *J* = 7.0 Hz, 4H), 3.85 (d, *J* = 4.7 Hz, 1H), 3.67 (s, 128H), 3.55 – 3.42 (m, 9H), 3.39 – 3.13 (m, 9H), 2.26 – 1.90 (m, 30H), 1.28 (s, 51H), 0.90 (t, *J* = 6.7 Hz, 6H).

**[0476] Preparation of Compound 29**

**[0477]** The compound **A78** (230 mg, 0.051 mmol, 1 equiv) was dissolved in 3 mL of 7N NH<sub>3</sub> (26 mg, 1.53 mmol, 30 equiv) in MeOH solution and transferred to a pressure tube and stirred at room temperature for 24 h. Then reaction was concentrated via rotovap and purified by reverse phase chromatography (C18 column) with water and acetonitrile/methanol as the mobile phases. Final product was recovered as a white solid 50 mg with a yield of 23.7 %. <sup>1</sup>H NMR (400 MHz, DMSO)  $\delta$  7.95 – 7.70 (m, 6H), 7.62 (d,  $J$  = 9.0 Hz, 3H), 7.12 (d,  $J$  = 6.1 Hz, 2H), 4.63 – 4.43 (m, 9H), 4.22 (d,  $J$  = 8.4 Hz, 3H), 4.01 – 3.85 (m, 7H), 3.76 – 3.61 (m, 13H), 3.51 (s, 207H), 3.19 (t,  $J$  = 5.8 Hz, 5H), 3.08 – 2.90 (m, 14H), 2.17 – 2.00 (m, 11H), 1.80 (s, 9H), 1.56 – 1.34 (m, 31H), 1.24 (s, 67H), 0.86 (t,  $J$  = 6.6 Hz, 6H). MS (MALDI\_TOF)  $m/z$  [M+H]<sup>+</sup> calculated for 4268.31 ; found for 4269.01,  $n=47$

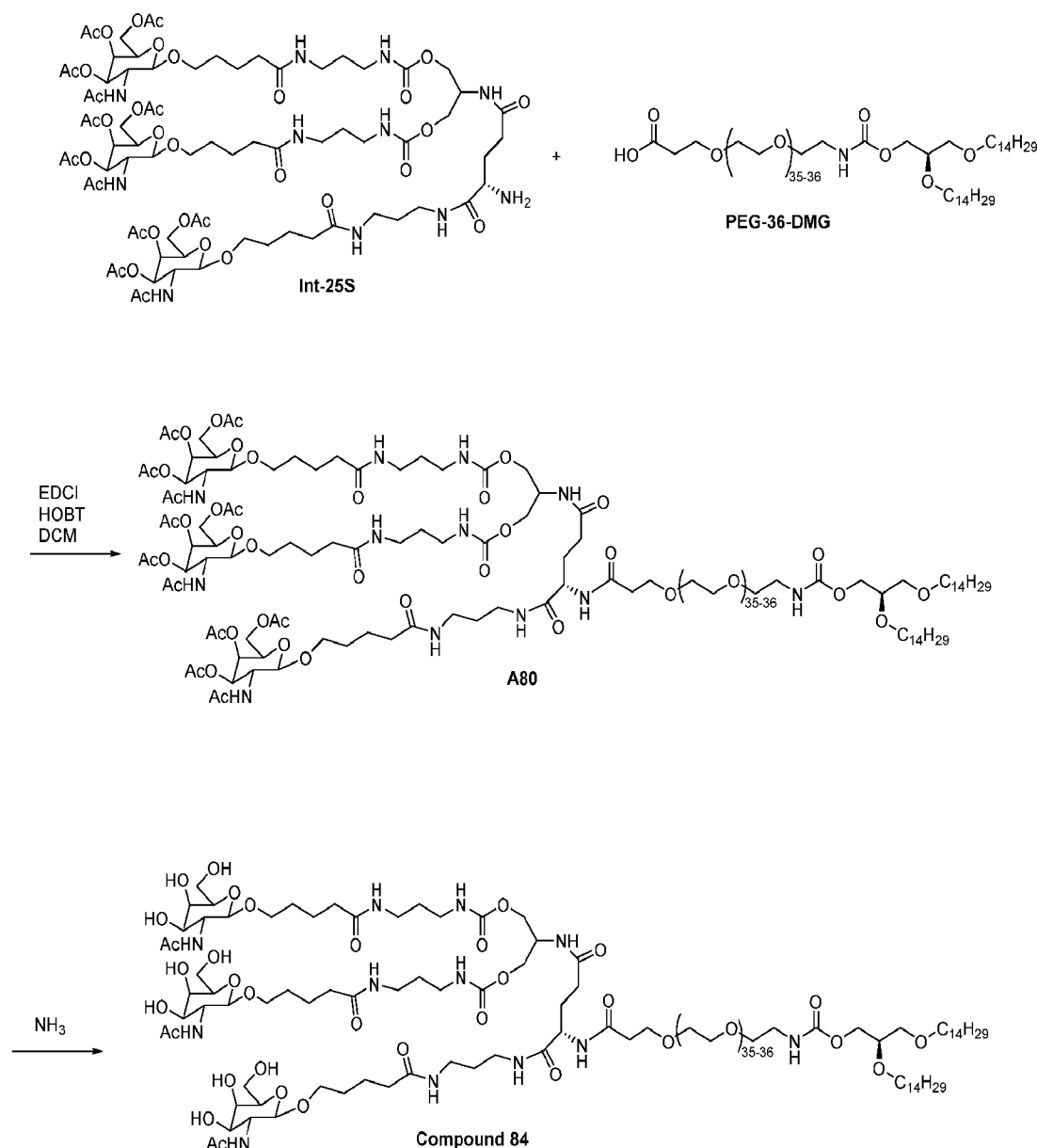
**[0478] Example 19. Preparation of Compound 83****Scheme 21: Synthesis of Compound 83**

**[0479] Preparation of A79**

**[0480]** To a solution of **PEG-36-DSG** (132 mg, 0.057 mmol, 1.0 equiv) and **Int-25-S** (100 mg, 0.057 mmol, 1.0 equiv) in DCM (2.0 mL), HOBt (10.4 mg, 0.068 mmol, 1.2 equiv), EDC (13 mg, 0.068 mmol, 1.2 equiv) and DIPEA (22 mg, 0.17 mmol, 3 equiv) were added, the resulting mixture was stirred for 48 h at room temperature, additional 10 mL of DCM was added, washed with brine, dried with anhydrous MgSO<sub>4</sub>. Filtered, concentrated, the crude product was purified with C18 reverse phase Combi Flash (Gold C18 gel column, eluted with 100% MeOH) collected 180 mg (86% yield) desired product. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.38 (d, *J* = 3.4 Hz, 2H), 5.21 (d, *J* = 10.9 Hz, 2H), 4.64 (d, *J* = 8.6 Hz, 2H), 4.44 (s, 1H), 4.14 (ddd, *J* = 21.4, 11.0, 6.0 Hz, 12H), 3.95 (d, *J* = 6.8 Hz, 4H), 3.81 (dt, *J* = 24.5, 5.4 Hz, 2H), 3.67 (s, 140H), 3.60 – 3.16 (m, 30H), 2.61 (t, *J* = 6.2 Hz, 1H), 2.55 (s, 1H), 2.17 (s, 8H), 2.07 (s, 8H), 2.02 (s, 7H), 1.98 (d, *J* = 2.7 Hz, 8H), 1.72 (s, 38H), 1.57 (t, *J* = 6.8 Hz, 7H), 1.28 (s, 64H), 0.90 (t, *J* = 6.7 Hz, 6H).

**[0481] Preparation of Compound 83**

**[0482]** The compound **A79** (180 mg, 0.044 mmol, 1 equiv) was dissolved in 3 mL of 7N NH<sub>3</sub> in MeOH (22.5 mg, 1.32 mmol, 30 equiv) solution and transferred to a pressure tube and stirred at room temperature for 24 h. Then reaction was concentrated via rotovap and purified by reverse phase chromatography (C18 column) with water and acetonitrile/methanol as the mobile phases. Final product was recovered as a white solid 42.7 mg with a yield of 26.4 %. <sup>1</sup>H NMR (400 MHz, DMSO) δ 8.04 (d, *J* = 7.8 Hz, 1H), 7.88 (d, *J* = 5.9 Hz, 2H), 7.77 – 7.69 (m, 3H), 7.62 (d, *J* = 9.0 Hz, 3H), 7.13 (s, 3H), 4.59 – 4.52 (m, 5H), 4.46 (d, *J* = 4.3 Hz, 3H), 4.22 (d, *J* = 8.4 Hz, 3H), 4.00 – 3.85 (m, 6H), 3.73 – 3.60 (m, 9H), 3.51 (s, 117H), 3.04 (ddd, *J* = 31.9, 26.7, 6.3 Hz, 16H), 2.05 (s, 8H), 1.80 (s, 9H), 1.49 (dt, *J* = 18.6, 8.9 Hz, 21H), 1.24 (s, 49H), 0.86 (t, *J* = 6.6 Hz, 6H). MS (MALDI-TOF) *m/z* [M+Na]<sup>+</sup> calculated for 3689.55 ; found for 3689.74, *n*=35

**[0483] Example 20. Preparation of Compound 84****Scheme 22: Synthesis of Compound 84****[0484] Preparation of A80**

**[0485]** To a solution of **PEG-36-DMG** (126 mg, 0.057 mmol, 1.0 equiv) and **Int-25-S** (100 mg, 0.057 mmol, 1.0 equiv) in DCM (2.0 mL), HOBT (10.4 mg, 0.068 mmol, 1.2 equiv), EDC (13. mg, 0.068 mmol, 1.2 equiv) and DIPEA (0.2 mL) were added, the resulting mixture was stirred for 48 h at room temperature, additional 10 mL of DCM was added, washed with brine, dried with anhydrous  $\text{MgSO}_4$ . Filtered, concentrated, the crude product was purified with C18 reverse phase Combi Flash (Gold C18 gel column, eluted with 100% MeOH) collected 180 mg (86% yield) desired product.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.37 (d,  $J = 3.3$  Hz, 3H), 5.21 (d,  $J = 10.8$  Hz, 2H), 4.63 (d,  $J = 8.8$  Hz, 2H), 4.44 (s, 1H), 4.16 (dtd,  $J = 21.4$ , 11.2, 6.3 Hz, 14H), 3.95 (d,  $J = 6.8$  Hz, 5H), 3.77 (s, 3H), 3.66 (s, 150H), 3.59 – 3.15 (m,

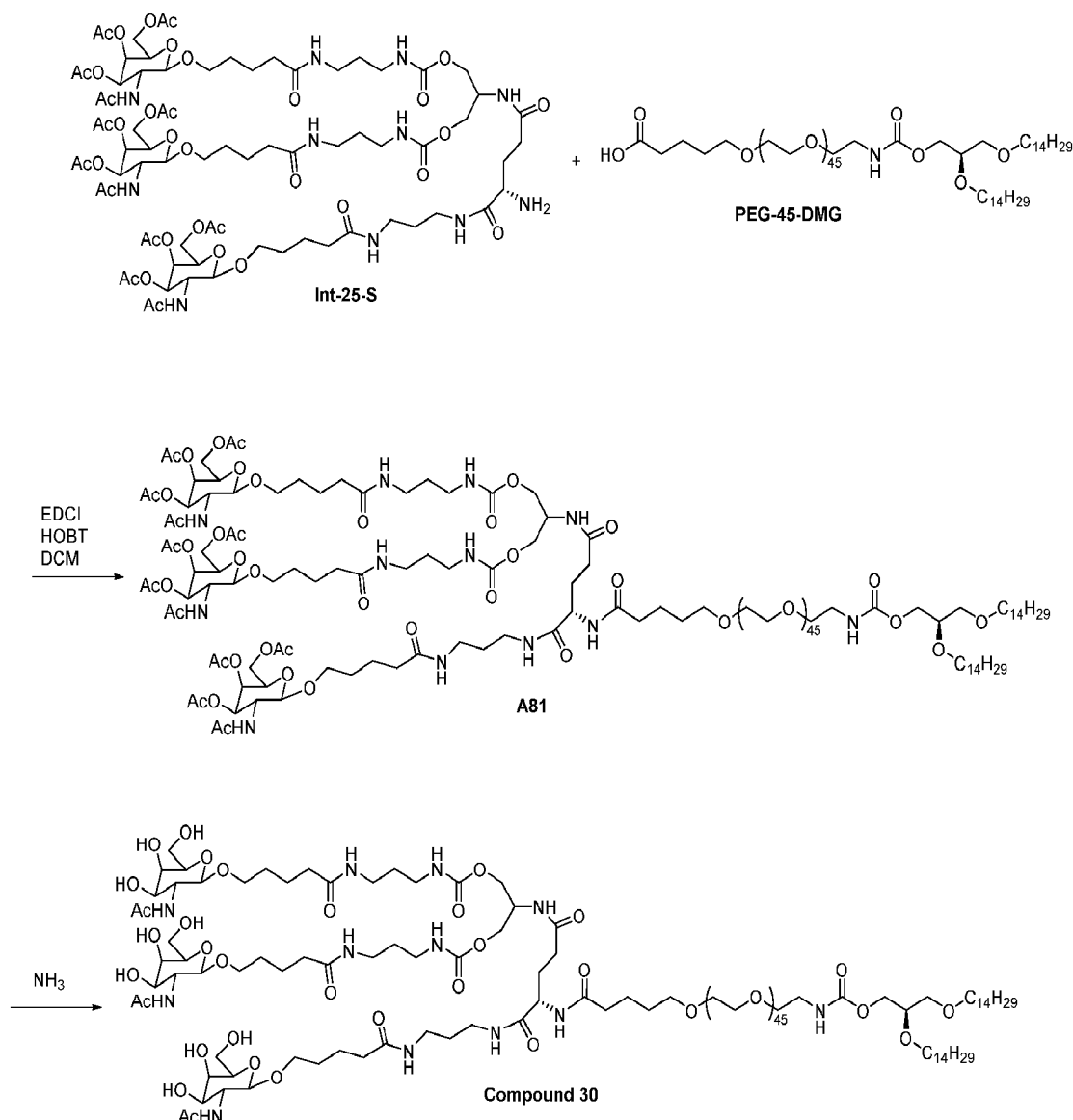
30H), 2.17 (s, 9H), 2.07 (s, 9H), 2.02 (s, 9H), 1.98 (d,  $J = 2.8$  Hz, 9H), 1.80 (s, 22H), 1.69 (s, 12H), 1.57 (t,  $J = 6.8$  Hz, 7H), 1.28 (s, 50H), 0.90 (t,  $J = 6.7$  Hz, 6H).

**[0486] Preparation of Compound 84**

**[0487]** The compound **A80** (120 mg, 0.03 mmol, 1 equiv) was dissolved in 3 mL of 7N NH<sub>3</sub> in MeOH (15.4 mg, 0.9 mmol, 30 equiv) solution and transferred to a pressure tube and stirred at room temperature for 24 h. Then reaction was concentrated via rotovap and purified by reverse phase chromatography (C18 column) with water and acetonitrile/methanol as the mobile phases. Final product was recovered as a white solid 62.5 mg with a yield of 57.5 %.

<sup>1</sup>H NMR (400 MHz, DMSO)  $\delta$  8.04 (d,  $J = 8.0$  Hz, 1H), 7.96 – 7.82 (m, 2H), 7.73 (q,  $J = 5.3$  Hz, 3H), 7.63 (s, 2H), 7.14 (d,  $J = 7.5$  Hz, 3H), 4.64 – 4.51 (m, 6H), 4.46 (d,  $J = 4.3$  Hz, 3H), 4.21 (t,  $J = 9.7$  Hz, 4H), 4.07 – 3.56 (m, 23H), 3.51 (s, 167H), 3.21 – 2.95 (m, 14H), 2.40 (t,  $J = 6.6$  Hz, 2H), 2.04 (d,  $J = 8.1$  Hz, 8H), 1.80 (s, 9H), 1.56 – 1.37 (m, 23H), 1.24 (s, 47H), 0.92 – 0.83 (m, 6H). MS (MALDI\_TOF)  $m/z$  [M+Na]<sup>+</sup> calculated for 3577.33 ; found for 3577.65,  $n=35$

[0488] **Example 21. Preparation of Compound 30**



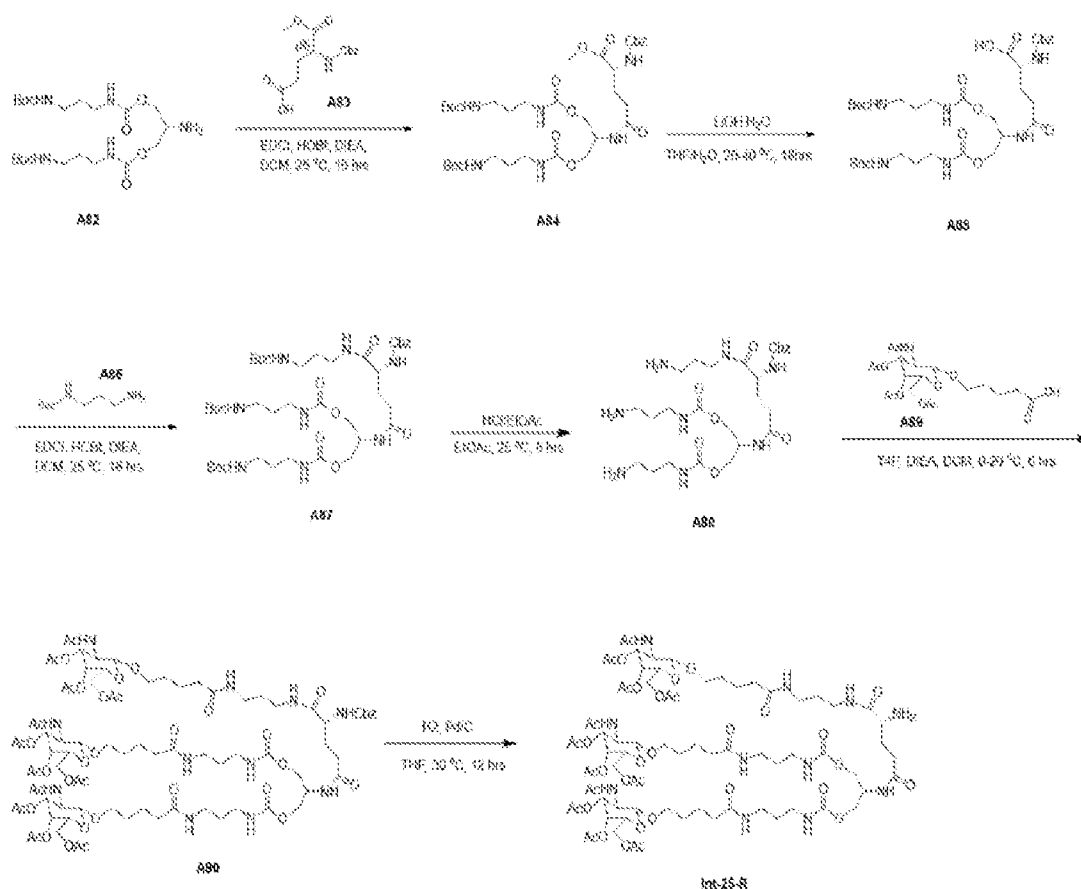
[0489] **Preparation of A81**

[0490] To a solution of **PEG-45-DMG** (150.4 mg, 0.057 mmol, 1.0 equiv) and Int-25S (100 mg, 0.057 mmol, 1.0 equiv) in DCM (2.0 mL), HOBT (10.4 mg, 0.068 mmol, 1.2 equiv), EDC (13 mg, 0.068 mmol, 1.2 equiv) and DIPEA (0.3 mL, 0.17 mmol, 3 equiv) were added, the resulting mixture was stirred for 48 h at room temperature, additional 10 mL of DCM was added, washed with brine, dried with anhydrous MgSO<sub>4</sub>. Filtered, concentrated, the crude product was purified with C18 reverse phase Combi Flash (Gold C18 gel column, eluted with 100% MeOH) collected 180 mg (86% yield) desired product. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.38 (d, *J* = 3.4 Hz, 3H), 5.21 (d, *J* = 11.2 Hz, 2H), 4.64 (d, *J* = 8.3 Hz, 2H), 4.15 (ddd, *J* = 15.6, 11.4, 5.8 Hz, 13H), 3.95 (d, *J* = 7.3 Hz, 6H), 3.67 (s, 202H), 3.62 – 3.43 (m, 24H), 3.38 – 3.10 (m, 14H), 2.19 – 1.95 (m, 37H), 1.75 – 1.59 (m, 46H), 1.28 (s, 49H), 0.90 (t, *J* = 6.7 Hz, 6H).

**[0491] Preparation of Compound 30**

**[0492]** The compound A81 (100 mg, 0.023 mmol, 1 equiv) was dissolved in 3 mL of 7N  $\text{NH}_3$  in MeOH (12 mg, 0.7 mmol, 30 equiv) solution and transferred to a pressure tube and stirred at room temperature for 24 h. Then reaction was concentrated via rotovap and purified by reverse phase chromatography (C18 column) with water and acetonitrile/methanol as the mobile phases. Final product was recovered as a white solid 65 mg with a yield of 71.1 %.  $^1\text{H}$  NMR (400 MHz, DMSO)  $\delta$  7.91 (dd,  $J = 17.7, 7.9$  Hz, 2H), 7.83 – 7.70 (m, 4H), 7.62 (d,  $J = 9.0$  Hz, 3H), 7.12 (d,  $J = 6.1$  Hz, 2H), 4.63 – 4.51 (m, 6H), 4.47 (d,  $J = 4.3$  Hz, 3H), 4.22 (d,  $J = 8.4$  Hz, 3H), 4.00 – 3.84 (m, 7H), 3.74 – 3.62 (m, 12H), 3.51 (s, 215H), 3.19 (t,  $J = 5.8$  Hz, 4H), 3.08 – 2.88 (m, 15H), 2.22 – 2.00 (m, 13H), 1.80 (s, 9H), 1.48 (dt,  $J = 15.9, 8.4$  Hz, 33H), 1.24 (s, 53H), 0.86 (t,  $J = 6.6$  Hz, 6H). MS (MALDI\_TOF)  $m/z$   $[\text{M}+\text{H}]^+$  calculated for 4156.09 ; found for 4156.97,  $n=48$ .

**[0493] Example 22. Preparation of Int-25-R**



**Scheme 23: Synthesis of Int-25-R**

**[0494] Preparation of Compound A84**

**[0495]** To a solution of Cpd.A83 (2.7 g, 9.14 mmol), DIEA (4.73 g, 36.57 mmol, 6.37 mL) and Cpd.A82 (4.94 g, 10.06 mmol) in DCM (50 mL) was added EDCI (3.51 g, 18.29 mmol) and HOBt (2.47 g, 18.29 mmol). The mixture was stirred at 25 °C for 16hr. TLC (eluted with

petroleum ether: ethyl acetate = 0:1,  $R_f$  = 0.22) showed the starting material was consumed and a new main spot formed. The mixture was quenched with water (40 ml) and extracted with DCM (15 mL  $\times$  3). The combined organic layers were separated, and the organic layer was washed with brine (10 mL  $\times$  2), dried over  $\text{Na}_2\text{SO}_4$ . The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography ( $\text{SiO}_2$ , DCM: MeOH = 1:0 to 3:7) to get Cpd.A84 (5.6 g, 80% yield) as a colorless solid.  $^1\text{H NMR}$  (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  = 7.90 (br d,  $J$  = 8.0 Hz, 1H), 7.75 (d,  $J$  = 7.9 Hz, 1H), 7.44-7.23 (m, 5H), 7.08 (br d,  $J$  = 4.4 Hz, 2H), 6.75 (br t,  $J$  = 5.2 Hz, 2H), 5.07-5.01 (m, 2H), 4.07 (br d,  $J$  = 5.6 Hz, 1H), 3.99-3.84 (m, 4H), 3.63 (s, 3H), 2.98-2.84 (m, 8H), 2.17 (br t,  $J$  = 7.8 Hz, 2H), 1.97-1.89 (m, 1H), 1.83-1.70 (m, 1H), 1.48 (quin,  $J$  = 7.2 Hz, 4H), 1.36 (s, 18H).

**[0496] Preparation of Compound A85**

**[0497]** To a solution of Cpd.A84 (5.60 g, 7.28 mmol) in THF (50 mL) and  $\text{H}_2\text{O}$  (25 mL) was added  $\text{LiOH}\cdot\text{H}_2\text{O}$  (1.22 g, 29.13 mmol) at 20 °C. The mixture was stirred at 40 °C for 16 hrS. TLC (eluted with DCM: MeOH = 10:1,  $R_f$  = 0.23) showed the starting material was consumed and one new spot formed. The THF was removed in vacuo and the residue dissolved in DCM (30 ml). The residue was added citric acid 10% w/v (100 ml) adjust pH less than 4 and the mixture was stirred for 5 min. Then the residue was extracted with DCM: MeOH = 10:1 (25  $\times$  3mL) and the mixture were filtered, and the filtrate was concentrated under reduced pressure to get Cpd.A85 (5.3 g, 96.40% yield) as a colorless oil.  $^1\text{H NMR}$  (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  ppm 7.90 (br d,  $J$  = 7.6 Hz, 1H), 7.59 (br d,  $J$  = 8.0 Hz, 1H), 7.42-7.28 (m, 5H), 7.09 (br d,  $J$  = 4.4 Hz, 2H), 6.85-6.67 (m, 2H), 5.03 (d,  $J$  = 2.4 Hz, 2H), 4.11-4.06 (m, 1H), 3.99-3.92 (m, 3H), 3.91-3.84 (m, 2H), 3.03-2.83 (m, 8H), 2.18 (br t,  $J$  = 7.2 Hz, 2H), 1.95 (br d,  $J$  = 5.2 Hz, 1H), 1.91 (s, 1H), 1.83-1.69 (m, 1H), 1.48 (quin,  $J$  = 7.2 Hz, 4H), 1.37 (s, 18H).

**[0498] Preparation of Compound A87**

**[0499]** To a solution of Cpd.A86 (3.55 g, 20.36 mmol, 3.56 mL) in DCM (50 mL) was added DIEA (3.63 g, 28.09 mmol, 4.89 mL) and Cpd.A87 (5.3 g, 7.02 mmol), then was added EDCI (4.04 g, 21.06 mmol) and HOBt (2.85 g, 21.06 mmol). The mixture was stirred at 25 °C for 16 hrs. TLC (eluted with DCM: MeOH = 10:1,  $R_f$  = 0.43) showed the starting material was consumed and one new spot formed. The mixture was quenched with water (60 ml) and extracted with DCM (15 mL  $\times$  3). The combined organic layers were separated, and the organic layer was washed with brine (10 mL  $\times$  2), dried over  $\text{Na}_2\text{SO}_4$ . The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by

column chromatography (SiO<sub>2</sub>, DCM: MeOH =1:0 to 10:1) to get Cpd.**A87** (4 g, 62.53% yield) as a white solid. <sup>1</sup>H NMR ( 400 MHz, DMSO-*d*<sub>6</sub>) δ ppm 7.93-7.82 (m, 2H), 7.41-7.28 (m, 5H), 7.09 (br s, 2H), 6.82-6.66 (m, 3H), 5.02 (s, 2H), 4.07 (br s, 1H), 3.99-3.84 (m, 5H), 3.07-3.00 (m, 2H), 2.99-2.85 (m, 10H), 2.20-2.04 (m, 2H), 1.85 (br dd, *J* = 8.8, 14.4 Hz, 1H), 1.71 (br dd, *J* = 6.0, 14.0 Hz, 1H), 1.48 (td, *J* = 6.9, 13.6 Hz, 7H), 1.37 (d, *J* = 2.4 Hz, 27H).

**[0500] Preparation of Compound A88**

**[0501]** A mixture of Cpd.**A87** (4 g, 4.39 mmol) in Ethyl acetate (40 mL), then was added HCl/Ethyl acetate (4 M, 10 mL), and then the mixture was stirred at 25 °C for 6 hrs. LC-MS showed Cpd.**A87** was consumed completely and one main peak with desired *m/z* was detected. The mixture was concentrated under reduced pressure to give Cpd.**A88** (4 g, crude) as a white solid. <sup>1</sup>H NMR ( 400 MHz, DMSO-*d*<sub>6</sub>) δ ppm 8.21 (br t, *J* = 5.6 Hz, 1H), 8.02 (br s, 9H), 7.49 (br d, *J* = 7.6 Hz, 1H), 7.41-7.28 (m, 5H), 5.09-4.96 (m, 2H), 4.13-4.06 (m, 1H), 3.98 (br s, 1H), 3.95-3.85 (m, 2H), 3.18-3.10 (m, 2H), 3.04 (q, *J* = 6.4 Hz, 4H), 2.82-2.71 (m, 5H), 2.23-2.07 (m, 2H), 1.93-1.81 (m, 1H), 1.71 (qd, *J* = 6.8, 14.4 Hz, 6H), 1.59 (s, 1H), 1.37 (d, *J* = 2.4 Hz, 1H)

**[0502] Preparation of Compound A90**

**[0503]** To a solution of Cpd.**A88** (3.85 g, 8.61 mmol) and Cpd.**A89** (2 g, 2.78 mmol, 3HCl) in DCM (70 mL) was added DIEA (2.51 g, 19.44 mmol, 3.39 mL), then was added T4P (7.00 g, 9.72 mmol, 50% purity) at 0 °C. The mixture was stirred at 20 °C for 6hr. LC-MS showed Cpd.**A88** was consumed completely and one main peak with desired *m/z* or desired mass was detected. The mixture was quenched with water (100 ml) and extracted with DCM (40 mL × 3). The combined organic layers were separated, and the organic layer was washed with brine (30 mL × 6), dried over Na<sub>2</sub>SO<sub>4</sub>. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM: MeOH =1:0 to 7:1) for twice to get Cpd.**A90** (1.6 g, 53.33% yield) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ ppm 7.97-7.67 (m, 8H), 7.48-7.25 (m, 6H), 7.12 (br s, 2H), 5.21 (d, *J* = 3.2 Hz, 3H), 5.06-4.91 (m, 5H), 4.48 (d, *J* = 8.4 Hz, 3H), 4.02 (s, 10H), 3.94 (br d, *J* = 5.2 Hz, 2H), 3.92-3.82 (m, 6H), 3.70 (m, 3H), 3.47-3.37 (m, 3H), 3.13-2.85 (m, 13H), 2.10 (s, 9H), 2.07-2.01 (m, 6H), 1.99 (s, 9H), 1.89 (s, 9H), 1.77 (s, 9H), 1.59-1.34 (m, 20H).

**[0504] Preparation of Int-25-R**

**[0505]** A mixture of Cpd.**A90** (2.0 g, 1.10 mmol), Pd/C (1.2 g, 10% purity) in THF (100 mL) was degassed and purged with H<sub>2</sub> (15 psi) for 3 times, and then the mixture was stirred at 30 °C for 12 hrs. under H<sub>2</sub> atmosphere. LC-MS showed Cpd.**A90** was consumed completely and one main peak with desired MS was detected. The mixture was filtered, and the filtrate

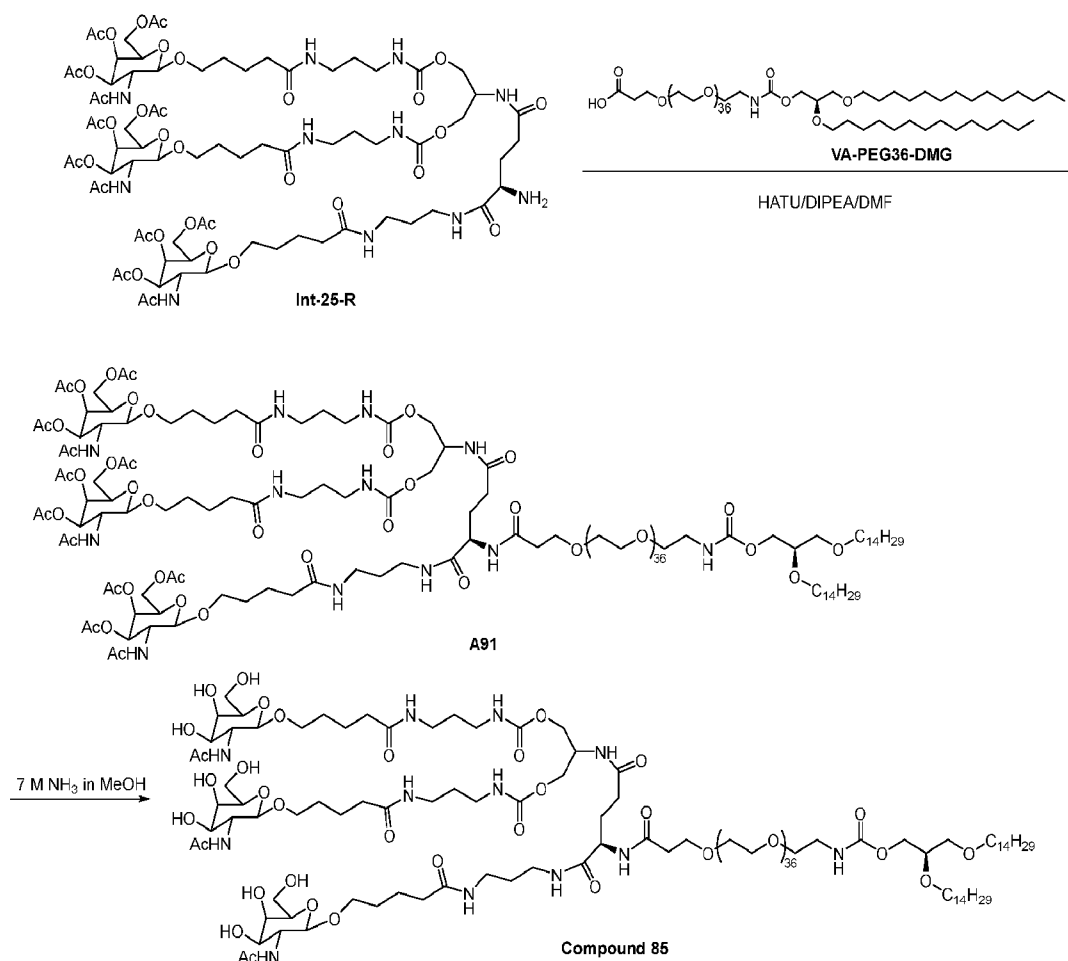
was concentrated under reduced pressure to get **Int-25-R** (1.19 g, 78 % yield) as a gray solid.

LCMS:  $t_R = 1.769$  min, 94.8% purity,  $m/z = 883.3$  ( $M/2+H$ )<sup>+</sup>

**[0506] Method of LC/MS:** LC/MS (The gradient was 0%-60 B in 0-2.5 min, maintains 60% B in 2.5-3.0 min, and 60% B-0%B in 3.0-3.01min, 0% B in 3.01-3.5 min the flow rate was 1 ml/min. Mobile phase A was 0.04% trifluoroacetic acid in water, mobile phase B was 0.02% trifluoroacetic acid in acetonitrile. The column used for chromatography was a Luna LC- C18 50\*2 mm (5 um particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection as well as positive electrospray ionization. MS range was 100-2000.

**[0507] <sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 7.94-7.67 (m, 8H), 7.12 (br s, 2H), 5.21 (d,  $J = 3.2$  Hz, 3H), 4.96 (dd,  $J = 3.2, 11.2$  Hz, 3H), 4.48 (d,  $J = 8.4$  Hz, 3H), 4.26 (t,  $J = 7.2$  Hz, 1H), 4.08-3.99 (m, 10H), 3.96 (br d,  $J = 9.6$  Hz, 2H), 3.92-3.82 (m, 5H), 3.74-3.65 (m, 3H), 3.44-3.37 (m, 4H), 3.12-2.88 (m, 13H), 2.42 (s, 1H), 2.15 (br d,  $J = 8.0$  Hz, 1H), 2.10 (s, 9H), 2.07-2.02 (m, 6H), 2.00 (s, 9H), 1.89 (s, 9H), 1.77 (s, 9H), 1.56-1.39 (m, 20H)

**[0508] Example 23. Preparation of Compound 85**



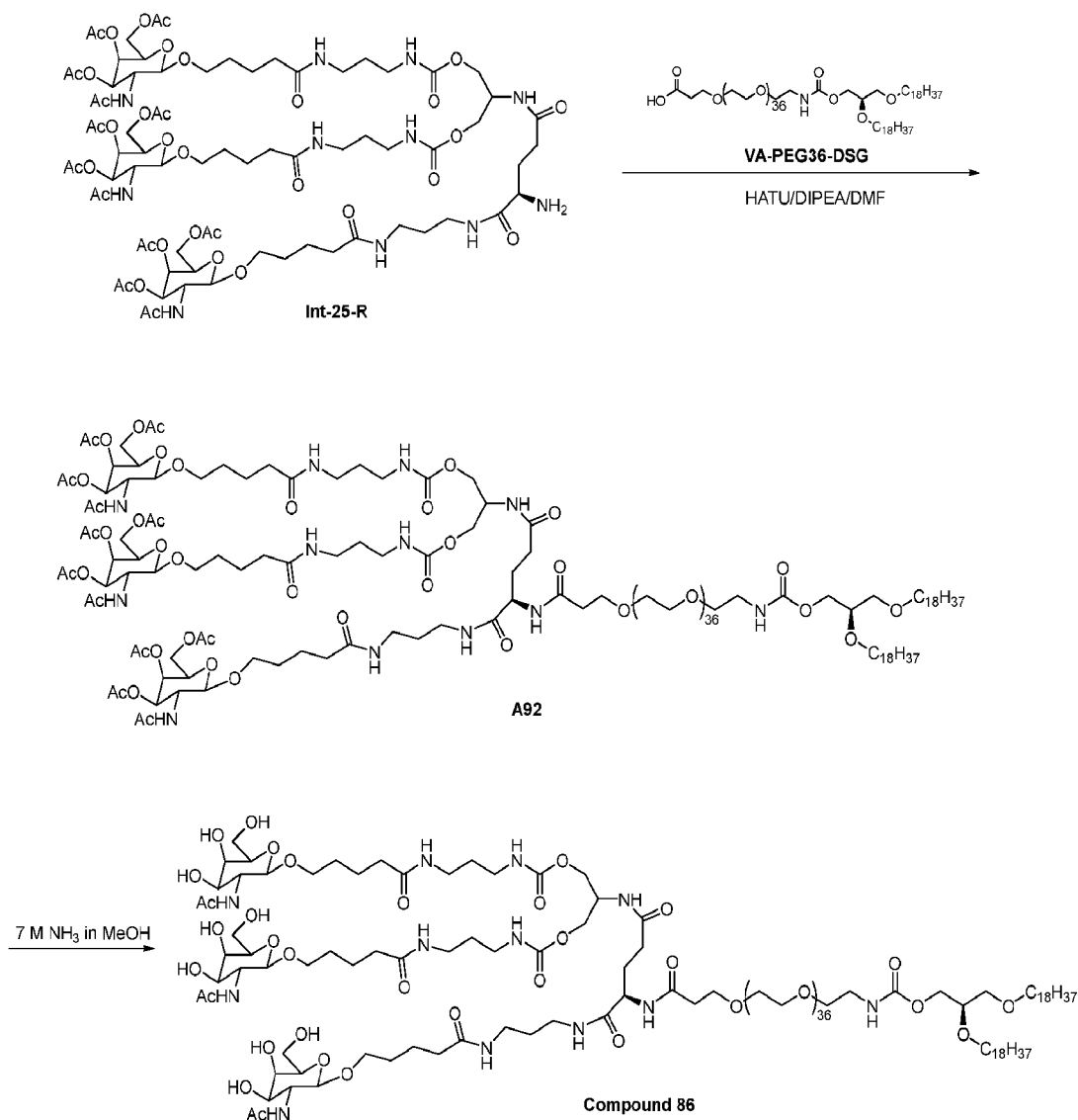
**Scheme 24: Synthesis of Compound 85**

**[0509] Preparation of A91**

**[0510]** To a solution of **PEG-36-DMG** (134 mg, 1.0 equiv) in DMF (2.0 mL), HATU (35 mg, 1.5 equiv) and DIPEA (0.2 mL) were added, the mixture was stirred 15 mins at room temperature, then **Int-25-R** (105 mg, 1.0 equiv) was added, the resulting mixture was stirred over weekend at room temperature, the solvents were evaporated under vacuum, the crude product was purified with Combi Flash (24 g gold column, eluted with 0-50% MeOH in DCM), collected 37 mg desired product <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$ , 5.38 (s, 3H), 5.22(s, 3H), 4.63(m, 2H), 4.14 (m, 15H), 3.95(m, 6H), 3.66(s, 88H), 3.57-3.43 (m, 12H), 3.45-3.21 (m, 15H), 2.17 (s, 9H), 2.07(s, 9H), 2.02(s, 9H), 1.98(s, 9H), 1.81-1.56(m, 44H), 1.27( s, 52H), 0.90(t, 6H)

**[0511] Preparation of Compound 85**

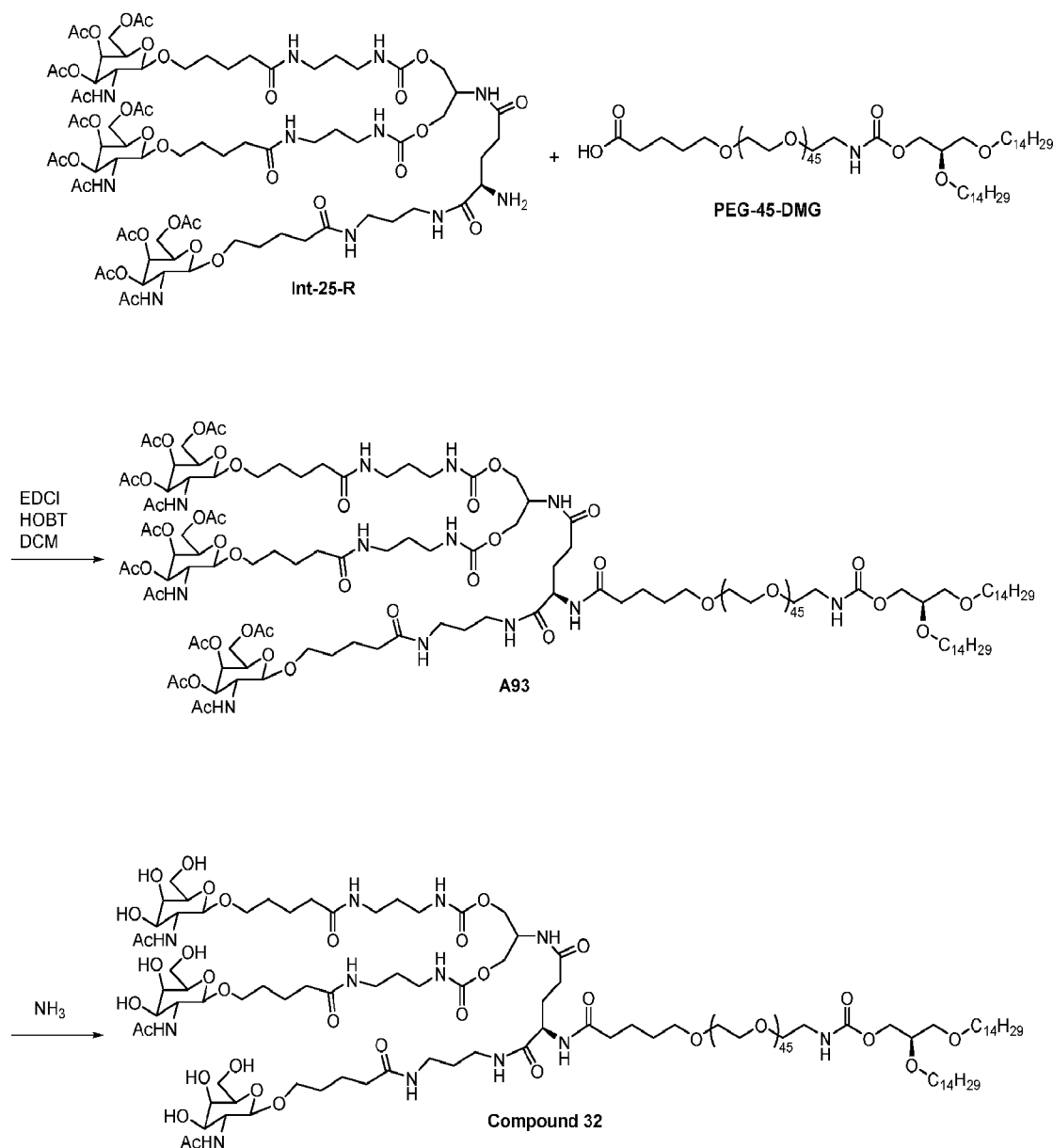
**[0512]** A solution of **A91** (41 mg) in MeOH (2.0 mL) was added into pressure tube, followed by addition of 2 mL of 7M NH<sub>3</sub> in MeOH, the mixture was sealed and stirred overnight at room temperature. The reaction mixture was concentrated, the crude product was dissolved in water and was uploaded 24 g C-18 column, eluted with water, then increase ACN from 0 to 100%, then replaced ACN with MeOH, the desired product 18 mg was collected. <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  4.60 (s, 3H), 4.38 (d,  $J$  = 8.4 Hz, 3H), 3.97-3.77 (m, 18H), 3.66 (s, 140 H), 3.51(m, 15H), 3.24(m, 8H), 3.15(m, 5H), 2.56(m, 2H), 2.35(m, 4H), 2.23(m, 6H), 2.01(s, 9H), 1.70 (m, 12H), (1.59 (m, 12H), 1.31(s, 72H), 0.92 (t,  $J$  = 6.5 Hz, 6H). MS (MALDI\_TOF)  $m/z$  [M+Na]<sup>+</sup> calculated for 3553 ; found for 3579,  $n=35$ .

**[0513] Example 24. Preparation of Compound 86****Scheme 25: Synthesis of Compound 86****[0514] Preparation of A92**

**[0515]** To a solution of **PEG-36-DSG** (140 mg, 1.0 equiv) in DMF (2.0 mL), HATU (35 mg, 1.5 equiv) and DIPEA (0.2 mL) were added, the mixture was stirred 15 mins at room temperature, then **Int-25-R** (105 mg, 1.0 equiv) was added, the resulting mixture was stirred over weekend at room temperature, the solvents were evaporated under vacuum, the crude product was purified with Combi Flash (24 g gold column, eluted with 0-50% MeOH in DCM), collected 41 mg desired product, containing additional intermediates. <sup>1</sup>H NMR (400 MHz, MeOD) δ, 5.38 (s, 3H), 5.20(s, 6H), 4.66(m, 4H), 4.17 (m, 21H), 3.95(m, 10H), 3.66(s, 262H), 3.57-3.44 (m, 26H), 3.39-3.21 (m, 21H), 2.17 (s, 11H), 2.07(s, 12H), 2.02(s, 12H), 1.99(s, 12H), 1.81-1.57(m, 56H), 1.28( s, 82H), 0.90(t, 9H)

**[0516] Preparation of Compound 86**

**[0517]** A solution of **A92** (41 mg) in MeOH (2.0 mL) was added into pressure tube, followed by addition of 2 mL of 7M NH<sub>3</sub> in MeOH, the mixture was sealed and stirred overnight at room temperature. The reaction mixture was concentrated, the crude product was dissolved in water and was uploaded 24 g C-18 column, eluted with water, then increase ACN from 0 to 100%, then replaced ACN with MeOH, the desired product (19 mg) was collected. <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  4.38 (d,  $J$  = 8.4 Hz, 3H), 4.17-4.07 (m, 7H), 3.97-3.76 (m, 18H), 3.66 (s, 176H), 3.56-3.49(m, 16H), 3.49-3.14(m, 15H), 2.56 (m, 2H), 2.25(m, 2H), 1.71-1.57 (m, 24H), 1.31 (s, 66H), 0.92 (t,  $J$  = 6.7 Hz, 6H). MS (MALDI-TOF)  $m/z$  [M+Na]<sup>+</sup> calculated for 3666 ; found for 3687, n=35

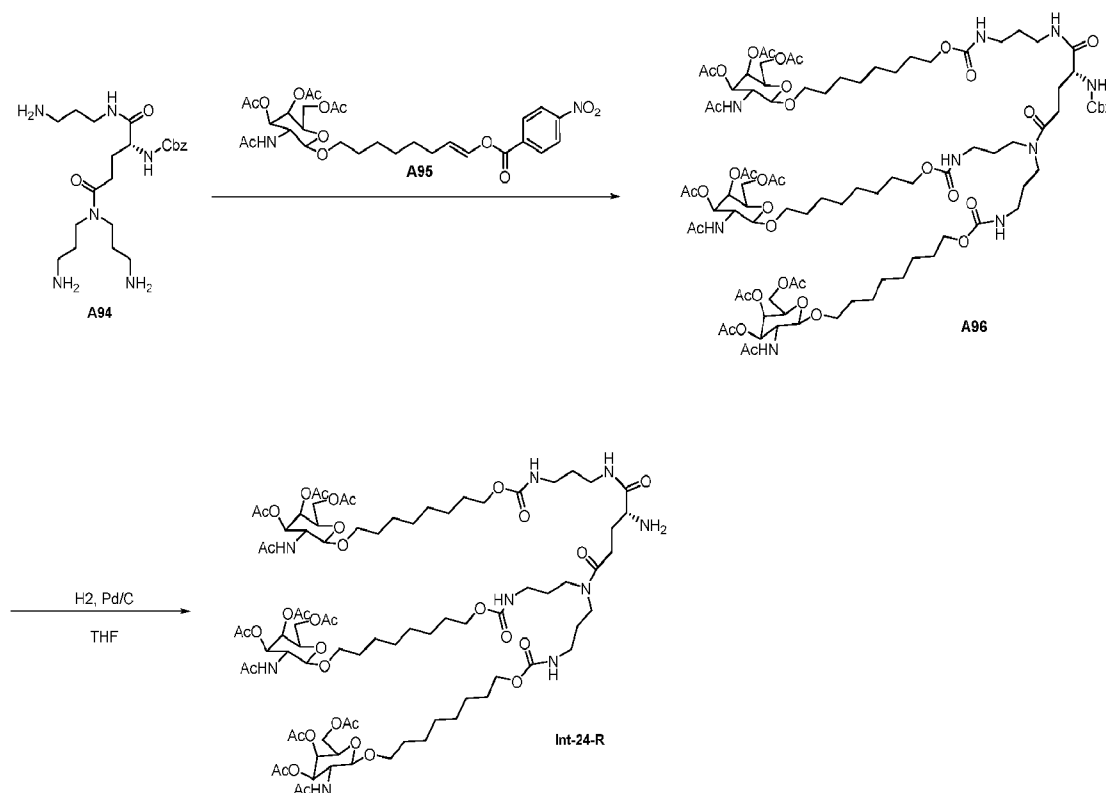
**[0518] Example 24. Preparation of Compound 32****Scheme 26: Synthesis of Compound 32**

**[0519] Preparation of A93**

**[0520]** To a solution of **PEG-45-DMG** (150.4 mg, 0.057 mmol, 1.0 equiv) and **Int-25-R** (1.0 equiv) in DCM (2.0 mL), HOBt (10.4 mg, 0.068 mmol, 1.2 equiv), EDC (13 mg, 0.068 mmol, 1.2 equiv) and DIPEA (0.3 mL, 0.17 mmol, 30 equiv) were added, the resulting mixture was stirred for 48 h at room temperature, additional 10 mL of DCM was added, washed with brine, dried with anhydrous MgSO<sub>4</sub>. Filtered, concentrated, the crude product was purified with C18 reverse phase Combi Flash (Gold C18 gel column, eluted with 100% MeOH) collected 150 mg (60% yield) desired product. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.24 (s, 1H), 7.12 (d, *J* = 13.2 Hz, 2H), 6.40 (s, 2H), 5.37 (d, *J* = 3.3 Hz, 1H), 5.31 – 5.14 (m, 2H), 4.88 (s, 1H), 4.68 (d, *J* = 8.7 Hz, 2H), 4.14 (pt, *J* = 11.3, 4.8 Hz, 8H), 3.95 (s, 3H), 3.83 (dd, *J* = 5.7, 4.1 Hz, 1H), 3.66 (s, 146H), 3.57 (dd, *J* = 8.8, 4.1 Hz, 8H), 3.52 – 3.42 (m, 10H), 3.39 – 3.09 (m, 17H), 2.42 – 2.21 (m, 6H), 2.19 – 1.94 (m, 20H), 1.61 (ddq, *J* = 47.1, 16.3, 8.6 Hz, 20H), 1.27 (s, 42H), 0.89 (t, *J* = 6.6 Hz, 6H).

**[0521] Preparation of Compound 32**

**[0522]** The compound **A93** (150 mg, 0.034 mmol, 1 equiv) was dissolved in 3 mL of 7N NH<sub>3</sub> in MeOH (17.4 mg, 1.02 mmol, 30 equiv) solution and transferred to a pressure tube and stirred at room temperature for 24 h. Then reaction was concentrated via rotovap and purified by reverse phase chromatography (C18 column) with water and acetonitrile/methanol as the mobile phases. Final product was recovered as a white solid 40 mg with a yield of 29.1 %. <sup>1</sup>H NMR (400 MHz, MeOD) δ 4.60 (s, 3H), 4.38 (d, *J* = 8.4 Hz, 3H), 3.98 – 3.90 (m, 6H), 3.89 – 3.72 (m, 11H), 3.66 (s, 234H), 3.58 – 3.44 (m, 20H), 3.27 – 3.20 (m, 8H), 3.19 – 3.09 (m, 8H), 2.33 (t, *J* = 7.3 Hz, 4H), 2.23 (s, 9H), 2.00 (s, 8H), 1.69 (dd, *J* = 13.0, 6.3 Hz, 15H), 1.61 (d, *J* = 16.1 Hz, 16H), 1.32 (s, 60H), 0.92 (d, *J* = 7.0 Hz, 6H). MS (MALDI-TOF) *m/z* [M+H]<sup>+</sup> calculated for 4112.07 ; found for 4112.92, *n*=47

**[0523] Example 25. Preparation of Int-24-R****Scheme 27: Synthesis of Int-24-R****[0524] Preparation of Compound A96**

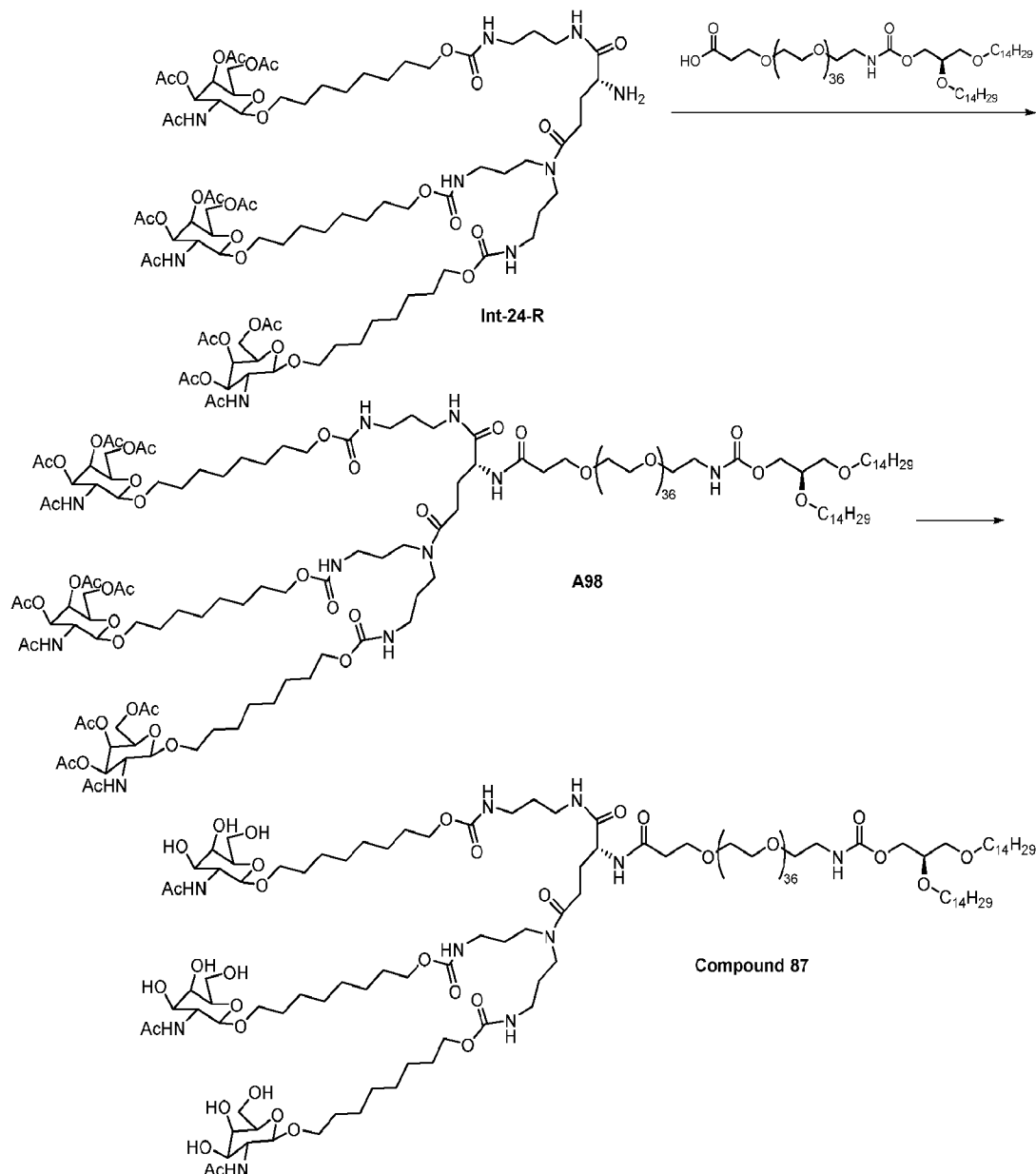
**[0525]** To a solution of **Cpd.A94** (2 g, 3.35 mmol) in DCM (80 mL) was added **Cpd.A95** (6.66 g, 10.40 mmol) and TEA (3.39 g, 33.53 mmol, 4.67 mL) at 25 °C. The mixture was stirred at 25 °C for 12hr. LCMS showed the desired was observed. The mixture was poured into H<sub>2</sub>O, extracted with DCM (100 mL × 3). The combined organic layers were washed with brine (100 mL × 3), dried over anhydride Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM: MeOH = 100:1 to 100:7) for twice to afford **GalNac-Int-24-R\_3** (1.73 g, 884.85 μmol, 26.39% yield) was obtained as a white solid. LCMS:  $t_R = 2.784$  min, 93.298% purity,  $m/z = 978.7$  (1/2M+H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ ppm 7.92-7.72 (m, 4H), 7.46-7.25 (m, 5H), 7.16-6.89 (m, 3H), 5.76 (s, 1H), 5.21 (d,  $J = 3.2$  Hz, 3H), 5.05-4.92 (m, 5H), 4.48 (d,  $J = 8.4$  Hz, 3H), 4.06-3.99 (m, 10H), 3.95-3.82 (m, 10H), 3.69 (td,  $J = 6.0, 9.6$  Hz, 3H), 3.40 (td,  $J = 6.4, 9.6$  Hz, 3H), 3.19 (br s, 3H), 3.08-2.86 (m, 8H), 2.35-2.23 (m, 2H), 2.10 (s, 9H), 2.00-1.97 (m, 10H), 1.89 (s, 8H), 1.76 (s, 9H), 1.58-1.41 (m, 16H), 1.25 (br s, 24H).

**[0526] Preparation of Int-24-R**

[0527] To a solution of **A96** (1.06 g, 542.16  $\mu\text{mol}$ ) in THF (100 mL) was added Pd/C (500 mg, 469.84  $\mu\text{mol}$ , 10% purity) in THF (100 mL) with a balloon filled with hydrogen. The mixture was stirred at 25 °C for 12hr. LCMS showed the desired compound. The mixture was degassed with nitrogen and filtered through celite with a wash of THF, the filtrate was collected and concentrated under reduce pressure to give **Int-24-R** (725 mg, 398.13  $\mu\text{mol}$ , 73.43% yield) as a light gray solid. LCMS:  $t_R$  = 3.903 min, 100% purity,  $m/z$  = 911.0

(1/2M+H)<sup>+</sup> <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ ).  $\delta$  ppm 7.87-7.78 (m, 4H), 7.16-7.07 (m, 1H), 7.04-6.92 (m, 2H), 5.21 (d,  $J$  = 3.2 Hz, 3H), 4.96 (dd,  $J$  = 3.6, 11.2 Hz, 3H), 4.48 (d,  $J$  = 8.4 Hz, 3H), 4.04-4.00 (m, 8H), 3.95-3.82 (m, 10H), 3.69 (td,  $J$  = 6.0, 9.6 Hz, 3H), 3.40 (td,  $J$  = 6.4, 10.0 Hz, 3H), 3.25-3.17 (m, 4H), 3.11-3.03 (m, 3H), 2.99-2.89 (m, 6H), 2.33-2.27 (m, 2H), 2.10 (s, 9H), 1.99 (s, 9H), 1.89 (s, 9H), 1.76 (s, 9H), 1.65-1.60 (m, 2H), 1.56-1.43 (m, 18H), 1.25 (br s, 26H).

[0528] **Method of LC/MS:** LC/MS (The gradient was 90% B in 0-0.4 min, 90% B-100% B in 0.4-3.4 min, and maintains 100% B in 3.40-3.85 min, and maintains 90% B in 3.86-4.5 min, the flow rate was 0.8 ml/min. Mobile phase A was 0.05% trifluoroacetic acid in water, mobile phase B was 0.05% trifluoroacetic acid in acetonitrile. The column used for chromatography was a Luna LC- C18 50\*2 mm (5  $\mu\text{m}$  particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection as well as positive electrospray ionization. MS range was 100-2000.

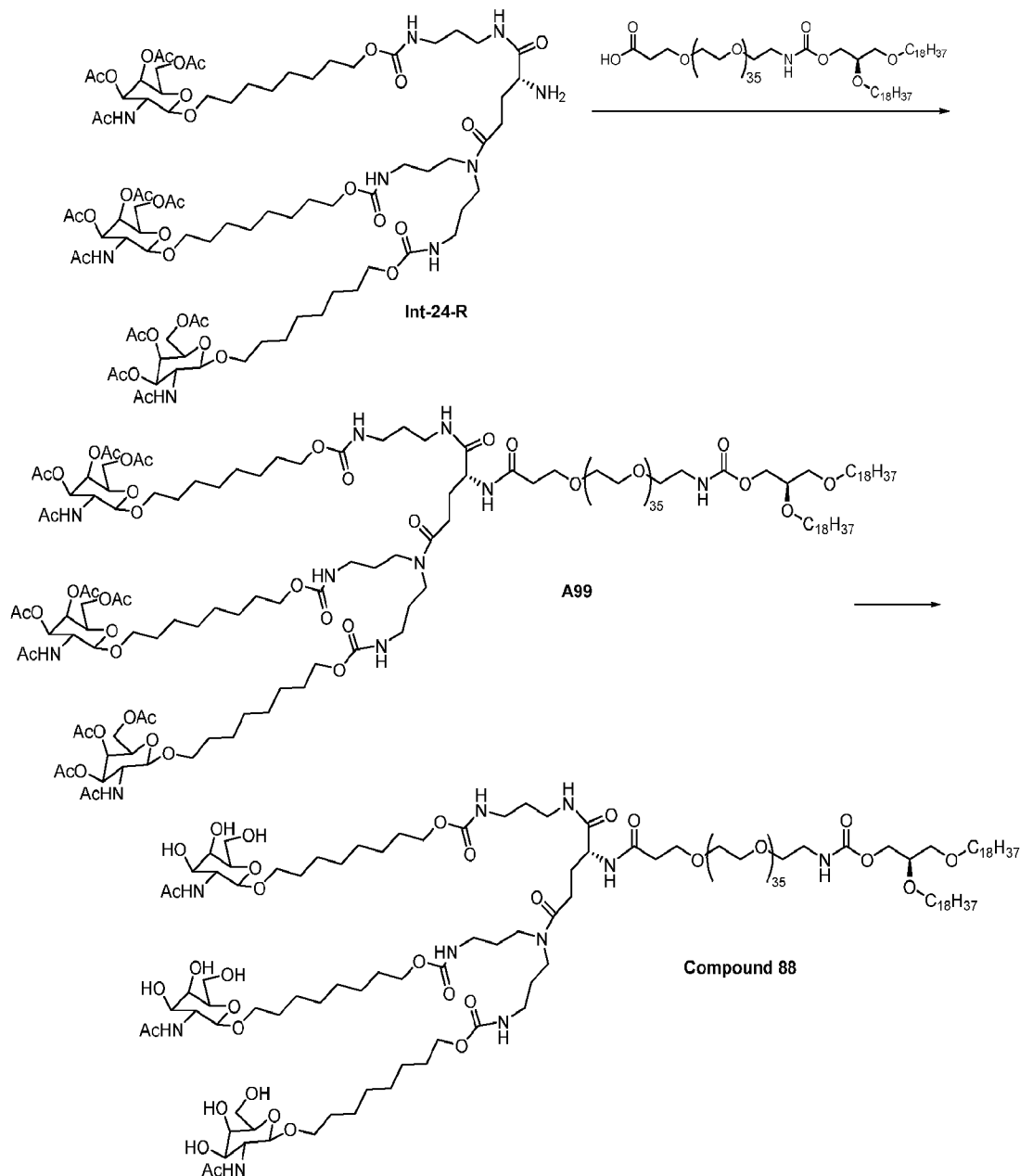
**[0529] Example 26. Preparation of Compound 87****Scheme 28: Synthesis of Compound 87****[0530] Preparation of A98**

**[0531]** To a solution of PEG36-DMG (134 mg, 1.0 equiv) in DMF (2.0 mL), HATU (35 mg, 1.5 equiv) and DIPEA (0.2 mL) were added, the mixture was stirred 15 mins at room temperature, then **Int-24-R** (109 mg, 1.0 equiv) was added, the resulting mixture was stirred over weekend at room temperature, the solvents were evaporated under vacuum, the crude product was purified with Combi Flash (24 g gold column, eluted with 0-50% MeOH in DCM), collected 87 mg desired product containing minor impurities, that was used for the next step without further purification. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ, 5.38 (s, 3H), 5.2(m, 4H), 4.71(m, 2H), 4.20-3.91 (m, 22H), 3.66(s, 130H), 3.58-3.38 (m, 25H), 3.13-3.08 (m,

10H), 2.16 (s, 9H), 2.06(s, 9H), 2.01(s, 9H), 1.97(s, 9H), 1.60-1.47(m, 68H), 1.32-1.27( s, 65H), 0.90(t, 6H).

**[0532] Preparation of Compound 87**

**[0533]** A solution of **A98** (81 mg) in MeOH (2.0 mL) was added into pressure tube, followed by addition of 3 mL of 7M NH<sub>3</sub> in MeOH, the mixture was sealed and stirred overnight at room temperature. The reaction mixture was concentrated, the crude product was dissolved in water and was uploaded 24 g C-18 column, eluted with water, then increase ACN from 0 to 100%, then replaced ACN with MeOH, the desired product (57 mg) was collected. <sup>1</sup>H NMR (400 MHz, MeOD) δ 4.38 (d, *J* = 8.4 Hz, 3H), 4.17-4.07 (m, 7H), 3.97-3.76 (m, 18H), 3.66 (s, 176H), 3.56-3.49(m, 16H), 3.49-3.14(m, 15H), 2.56 (m, 2H), 2.25(m, 2H), 1.71-1.57 (m, 24H), 1.31 (s, 66H), 0.92 (t, *J* = 6.7 Hz, 6H). MS (MALDI\_TOF) *m/z* [M+Na]<sup>+</sup> calculated for 3610.5 ; found for 3631.7

**[0534] Example 27. Preparation of Compound 88****Scheme 29: Synthesis of Compound 88****[0535] Preparation of A99**

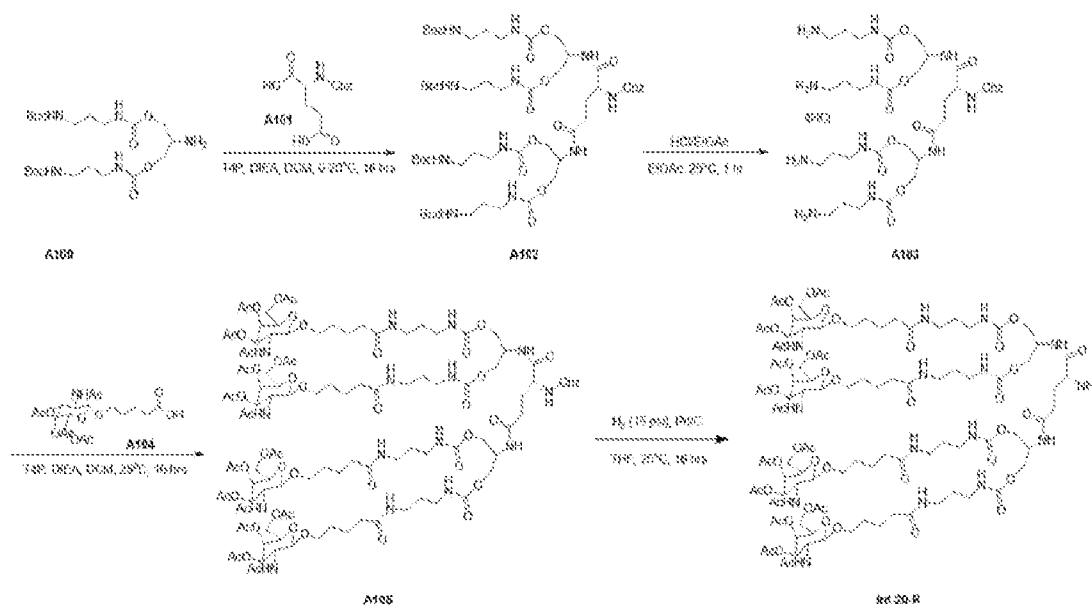
**[0536]** To a solution of PEG36-DSG (140 mg, 1.0 equiv) in DMF (2.0 mL), HATU (35 mg, 1.5 equiv) and DIPEA (0.2 mL) were added, the mixture was stirred 15 mins at room temperature, then **Int-24-R** (109 mg, 1.0 equiv) was added, the resulting mixture was stirred over weekend at room temperature, the solvents were evaporated under vacuum, the crude product was purified with Combi Flash (24 g gold column, eluted with 0-50% MeOH in DCM), collected 94 mg desired product containing minor impurities. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ, 5.38 (s, 3H), 5.34(m, 4H), 4.72(m, 2H), 4.20-3.77 (m, 27H), 3.66(s, 14H), 3.57-

3.10 (m, 38H), 2.16 (s, 9H), 2.07(s, 9H), 2.02(s, 9H), 1.98(s, 9H), 1.71-1.46(m, 56H), 1.32-1.27(s, 90H), 0.90(t, 6H).

#### [0537] Preparation of Compound 88

[0538] A solution of **A99** (41 mg) in MeOH (2.0 mL) was added into pressure tube, followed by addition of 2 mL of 7M NH<sub>3</sub> in MeOH, the mixture was sealed and stirred overnight at room temperature. The reaction mixture was concentrated, the crude product was dissolved in water and was uploaded 24 g C-18 column, eluted with water, then increase ACN from 0 to 100%, then replaced ACN with MeOH, the desired product (56 mg) was collected. <sup>1</sup>H NMR (400 MHz, MeOD) δ 4.40 (d, *J* = 8.4 Hz, 3H), 4.12-4.01 (m, 7H), 3.95-3.76 (m, 18H), 3.66 (s, 147H), 3.56-3.48(m, 16H), 3.17-3.09(m, 7H), 2.56 (m, 2H), 2.00(s, 9H), 1.70-1.56 (m, 19H), 1.36-1.31 (s, 84H), 0.92 (t, *J* = 6.7 Hz, 6H). MS (MALDI\_TOF) *m/z* [M+Na]<sup>+</sup> calculated for 3722.4 ; found for 3743.8.

#### [0539] Example 28. Preparation of Int-20-R



Scheme 30: Synthesis of Int-20-R

#### [0540] Preparation of Compound A102

[0541] To a solution of Cpd.**A101** (1.54 g, 5.49 mmol), DIEA (9.46 g, 73.23 mmol), HOBt (4.95 g, 36.62 mmol) and Cpd.**A100** (6 g, 12.21 mmol) in DCM (80 mL) was added EDCI (7.02 g, 36.62 mmol) at 0 °C and stirred at 20°C. The mixture was stirred at 20 °C for 16 h. TLC indicated the Reactant 1 was consumed, and a new spot was detected. The reaction mixture was quenched by H<sub>2</sub>O (100 mL) and extracted with DCM (3 x 100 mL). The combined organic layers were washed with brine (100 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was purified by silica

gel column chromatography (SiO<sub>2</sub>, PE:EtOAc = 1:0 to 0:1) to give Cpd.**A102** (7 g, 47% yield) as a colorless oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.42 - 7.22 (m, 5H), 7.00 - 6.71 (m, 1H), 6.07 - 5.54 (m, 4H), 5.01 (s, 5H), 4.49 - 3.88 (m, 11H), 3.08 (br d, *J* = 4.0 Hz, 15H), 2.41 - 2.14 (m, 2H), 2.04 - 1.83 (m, 2H), 1.67 - 1.48 (m, 9H), 1.36 (s, 41H).

**[0542] Preparation of Compound A103**

**[0543]** To a solution of Cpd.**A102** (4 g, 3.26 mmol) in DCM (40 mL) was added HCl/EtOAc (4 M, 6.51 mL) at 25 °C. The mixture was stirred at 25 °C for 1 h. LCMS indicated the Reactant 1 was consumed, and the desired ms was detected. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give Cpd.**A103** (2.5 g, 93% yield, 4HCl) as a white solid. LCMS: Rt = 0.976 min, 95.48% purity, *m/z* = 1764.1 (M+H)<sup>+</sup>. LC/MS (The gradient was 0%-60 B in 0-2.5 min, maintains 60% B in 2.5-3.0 min, and 60% B-0%B in 3.0-3.01min, 0% B in 3.01-3.5 min the flow rate was 1 ml/min. Mobile phase A was 0.04% trifluoroacetic acid in water, mobile phase B was 0.02% trifluoroacetic acid in acetonitrile. The column used for chromatography was a Luna LC- C18 50\*2 mm (5 μm particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection as well as positive electrospray ionization. MS range was 100-2000.

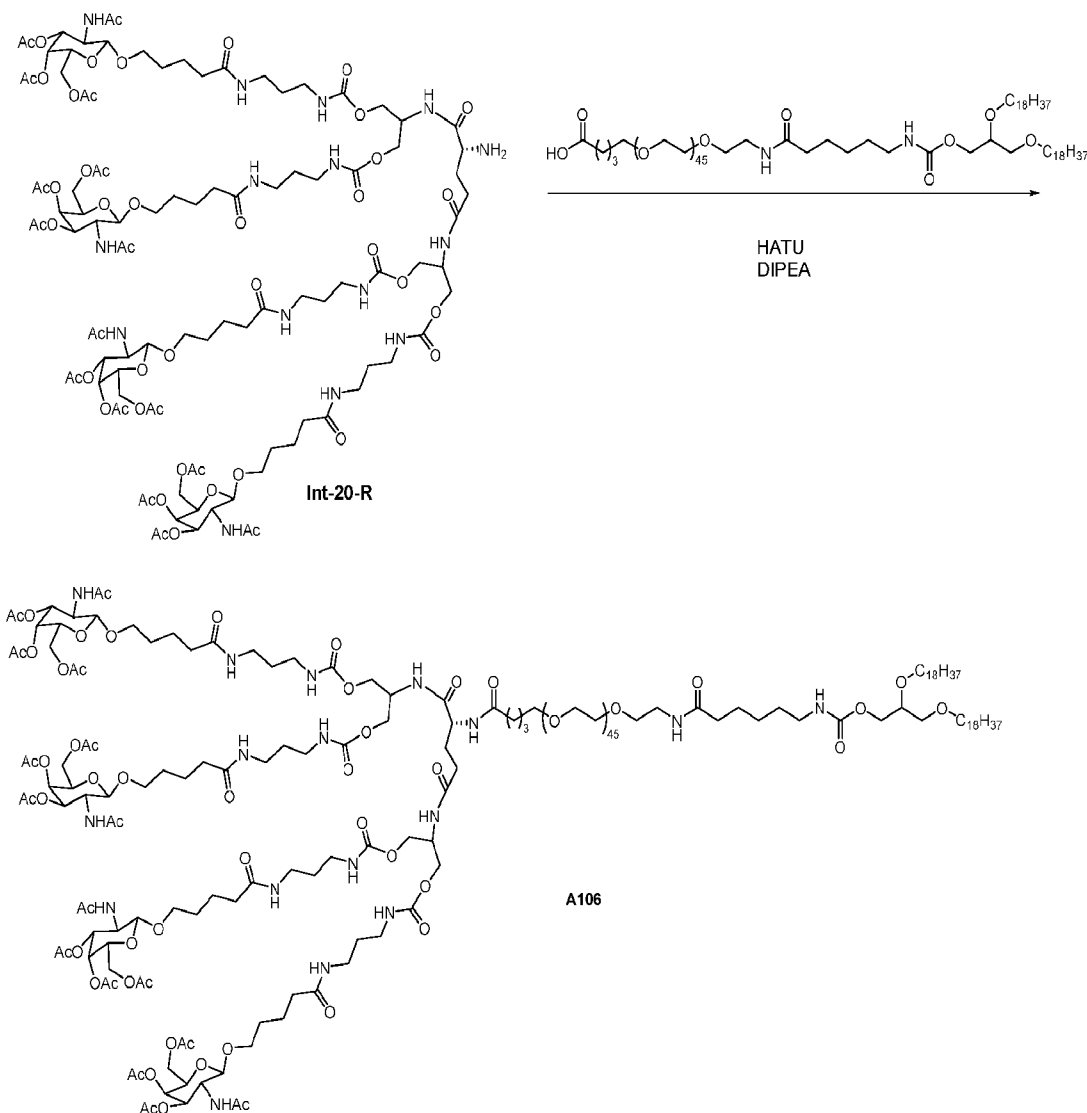
**[0544] Preparation of Compound A105**

**[0545]** To a solution of Cpd.**A104** (2.32 g, 5.18 mmol), Cpd.**A103** (1.2 g, 1.23 mmol, 4HCl) and DIEA (1.59 g, 12.32 mmol) in DCM (30 mL) was added T4P (3.73 g, 5.18 mmol, 50% purity) at 0 °C and stirred at 25°C. The mixture was stirred at 25 °C for 16 h. TLC (DCM:MeOH=10:1, R<sub>f</sub>= 0.15) indicated the Reactant 1 was consumed, and a new spot was detected. The reaction mixture was quenched by H<sub>2</sub>O (50 mL) and extracted with DCM (3 × 30 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (SiO<sub>2</sub>, DCM : MeOH = 1:0 to 0:1) for twice to give Cpd.**6** (1.9 g, 58% yield) as colorless solid. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 8.09 - 7.88 (m, 2H), 7.88 - 7.78 (m, 4H), 7.73 (br s, 4H), 7.41 - 7.31 (m, 5H), 7.26 - 6.97 (m, 4H), 5.22 (br s, 4H), 4.97 (br d, *J* = 10.0 Hz, 4H), 4.49 (br d, *J* = 8.0 Hz, 4H), 4.09 - 3.99 (m, 17H), 3.94 - 3.85 (m, 8H), 3.76 - 3.63 (m, 5H), 3.47 - 3.25 (m, 16H), 3.09 - 2.90 (m, 16H), 2.11 (s, 12H), 2.08 - 1.97 (m, 20H), 1.90 (s, 13H), 1.78 (s, 11H), 1.52 - 1.42 (m, 20H).

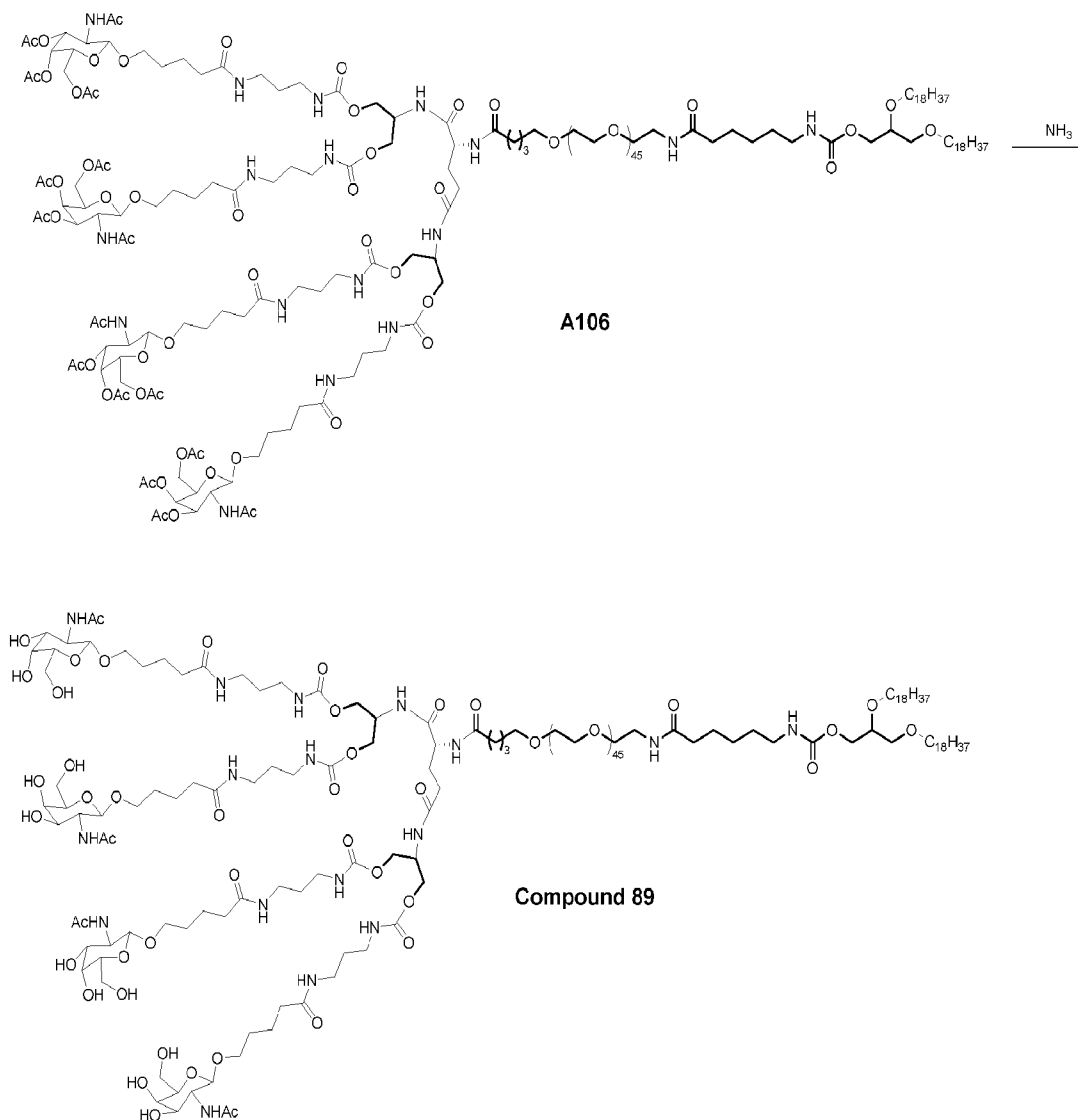
**[0546] Preparation of Int-20-R**

**[0547]** To a solution of Cpd.**A105** (1.9 g, 746.39 μmol) in THF (180 mL) was added Pd/C (900 mg, 10% purity) under H<sub>2</sub>(15 psi) at 25 °C and stirred at 25 °C for 16 hrs. LCMS indicated the Reactant 1 was consumed, and the desire ms was detected. The reaction mixture

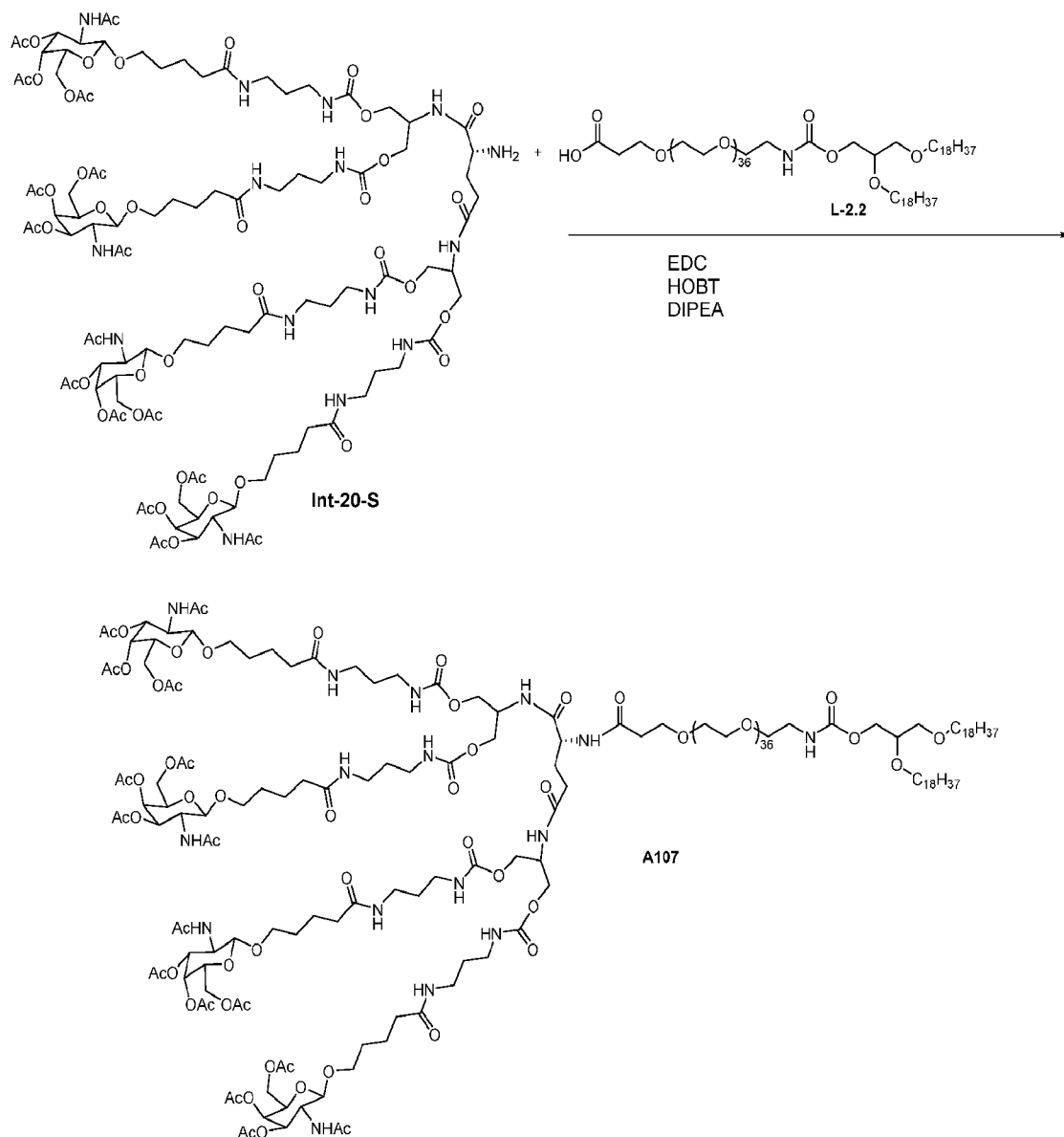
was filtered and the filtrate was concentrated under reduced pressure to give **Int-20-R** (1.10 g, 61% yield) as a gray solid. **LCMS**:  $R_t = 1.926$  min, 100% purity,  $m/z = 1206.7$   $(1/2M+H)^+$ . **LC/MS** (The gradient was 0%-60 B in 0-2.5 min, maintains 60% B in 2.5-3.0 min, and 60% B-0%B in 3.0-3.01min, 0% B in 3.01-3.5 min the flow rate was 1 ml/min. Mobile phase A was 0.04% trifluoroacetic acid in water, mobile phase B was 0.02% trifluoroacetic acid in acetonitrile. The column used for chromatography was a Luna LC- C18 50\*2 mm (5  $\mu$ m particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection as well as positive electrospray ionization. MS range was 100-2000.  **$^1H$  NMR** (400 MHz, DMSO- $d_6$ )  $\delta$  7.86 - 7.80 (m, 4H), 7.74 (br d,  $J = 4.0$  Hz, 4H), 7.36 - 6.95 (m, 4H), 5.21 (d,  $J = 3.2$  Hz, 4H), 4.96 (dd,  $J = 3.2, 11.2$  Hz, 4H), 4.48 (d,  $J = 8.4$  Hz, 4H), 4.09 - 3.93 (m, 20H), 3.92 - 3.80 (m, 7H), 3.74 - 3.67 (m, 4H), 3.49 - 3.34 (m, 8H), 3.19 - 2.80 (m, 18H), 2.10 (s, 12H), 2.07 - 1.96 (m, 22H), 1.89 (s, 12H), 1.77 (s, 12H), 1.53 - 1.43 (m, 24H).

**[0548] Example 29. Preparation of A106**

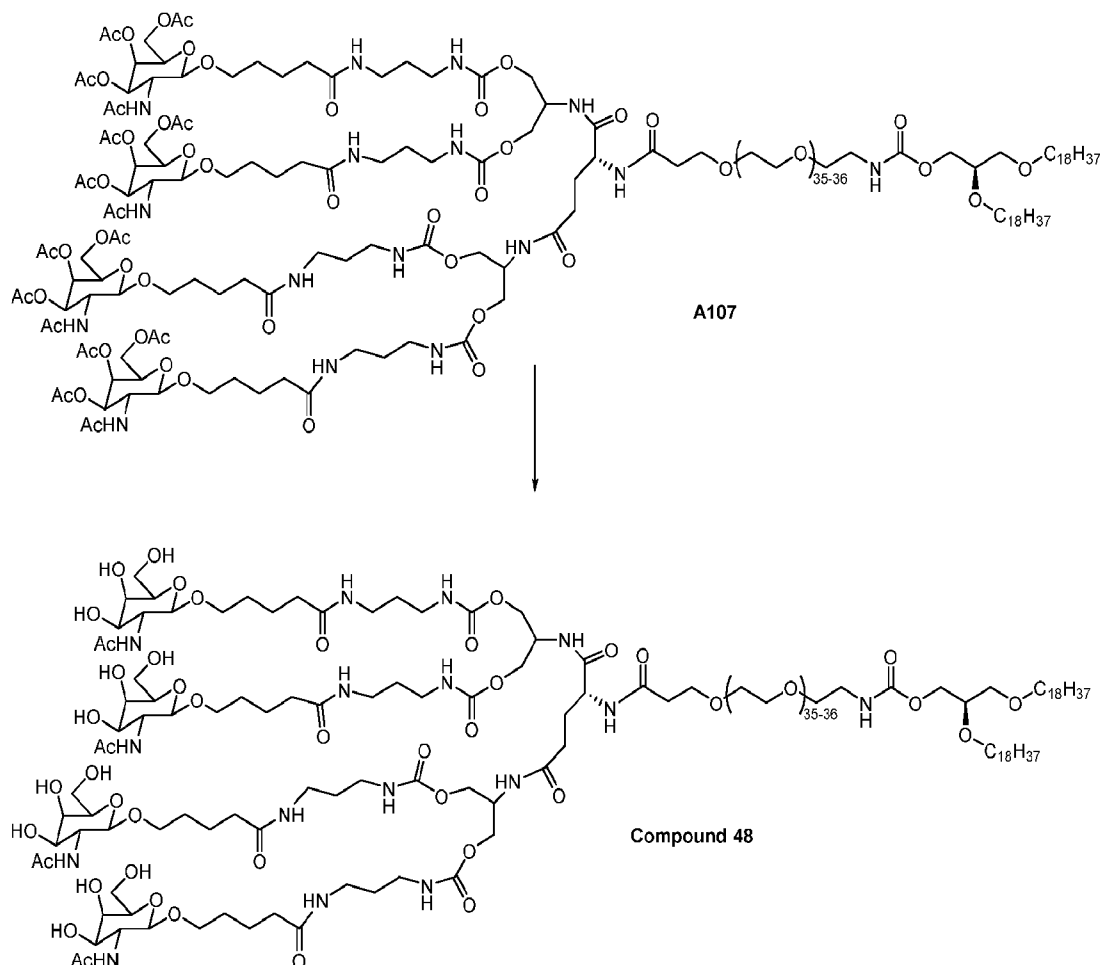
**[0549]** To a solution of PEG45-DSG (157.63 mg, 1.0 equiv) in DMF (2.0 mL), HATU (22.83 mg, 1.2 equiv) and DIPEA (0.2 mL) were added, the mixture was stirred 15 mins at room temperature, then **Int-20-R** (120 mg, 1.0 equiv) was added, the resulting mixture was stirred for 48 h at room temperature, the solvents were evaporated under vacuum, additional 10 mL of DCM was added, washed with brine, dried with anhydrous MgSO<sub>4</sub>. Filtered, concentrated, the crude product was purified with Combi Flash (Gold silica gel column, eluted with MeOH in DCM (0-50%), collected 250 mg (95% yield) desired product. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.38 (d, *J* = 3.3 Hz, 2H), 5.23 (d, *J* = 11.2 Hz, 2H), 4.84 (s, 1H), 4.66 (s, 2H), 4.28 – 4.07 (m, 13H), 3.96 (s, 4H), 3.84 (t, *J* = 4.9 Hz, 1H), 3.67 (s, 163H), 3.54 – 3.40 (m, 11H), 3.31 (s, 3H), 3.19 (t, *J* = 6.5 Hz, 7H), 2.23 – 1.89 (m, 48H), 1.56 (dt, *J* = 16.1, 7.9 Hz, 45H), 1.28 (s, 63H), 0.90 (t, *J* = 6.7 Hz, 6H).

**[0550] Example 30. Preparation of Compound 89**

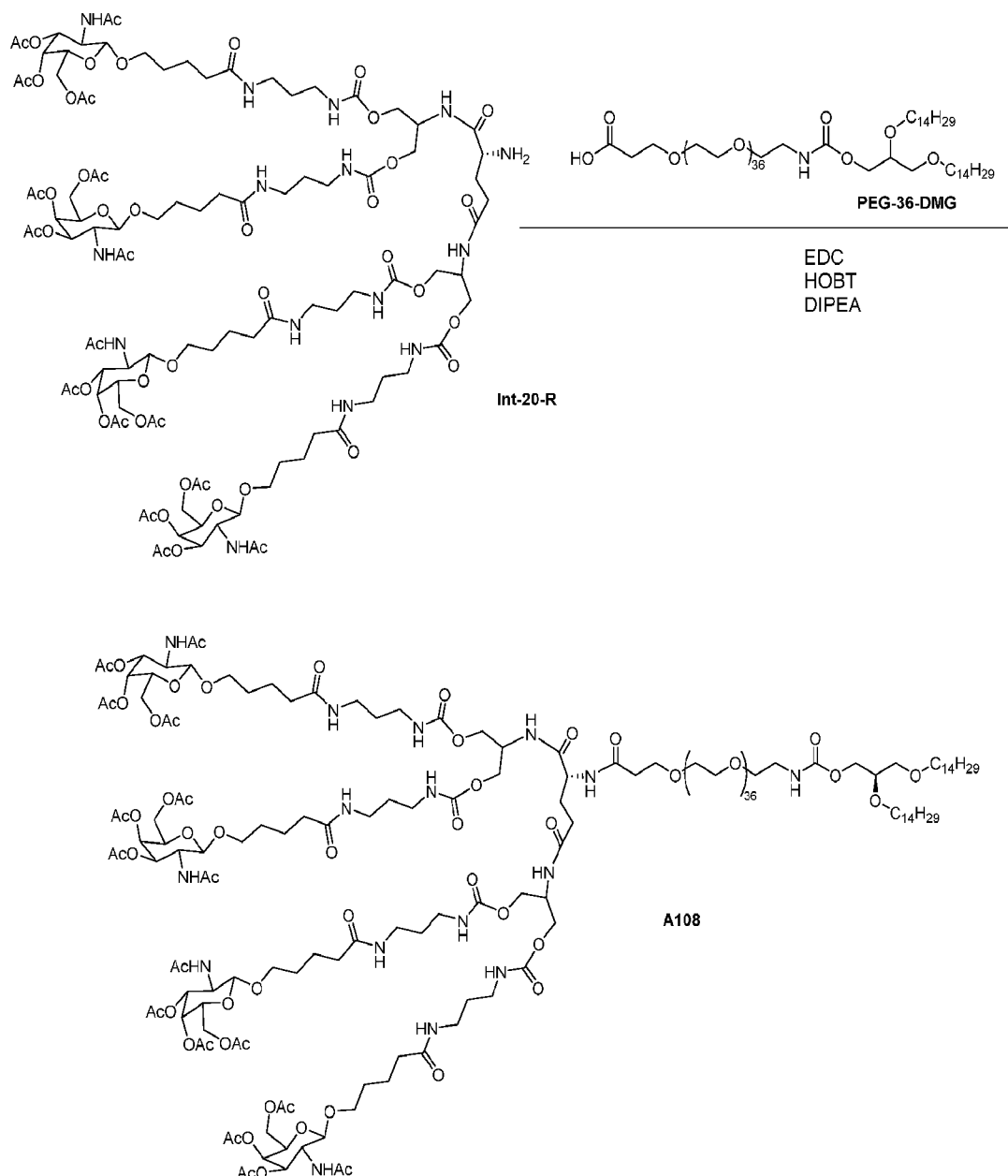
**[0551]** To a solution of **A106** (100 mg, 1 eq) was dissolved in 3 mL of 7N NH<sub>3</sub> in MeOH solution (excess) and stirred at room temperature in a pressure tube overnight. Subsequently, reaction was concentrated and loaded on C-18 column, eluted with water, ACN and MeOH as mobile phases. The product was eluted with 100% MeOH. <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  4.38 (d,  $J$  = 8.4 Hz, 4H), 4.27 – 4.04 (m, 10H), 3.99 – 3.75 (m, 21H), 3.66 (s, 194H), 3.52 (tdd,  $J$  = 12.4, 9.9, 5.8 Hz, 22H), 3.23 (d,  $J$  = 5.2 Hz, 7H), 3.16 (t,  $J$  = 6.8 Hz, 9H), 2.23 (td,  $J$  = 7.5, 3.1 Hz, 10H), 2.01 (s, 12H), 1.70 (p,  $J$  = 6.7 Hz, 18H), 1.59 (p,  $J$  = 7.4 Hz, 18H), 1.31 (s, 72H), 0.92 (t,  $J$  = 6.6 Hz, 6H). MS (MALDI-TOF)  $m/z$  [M+Na]<sup>+</sup> calculated for 4791.88 ; found for 4791.88.05,  $n=45$ .

**[0552] Example 31. Preparation of A107**

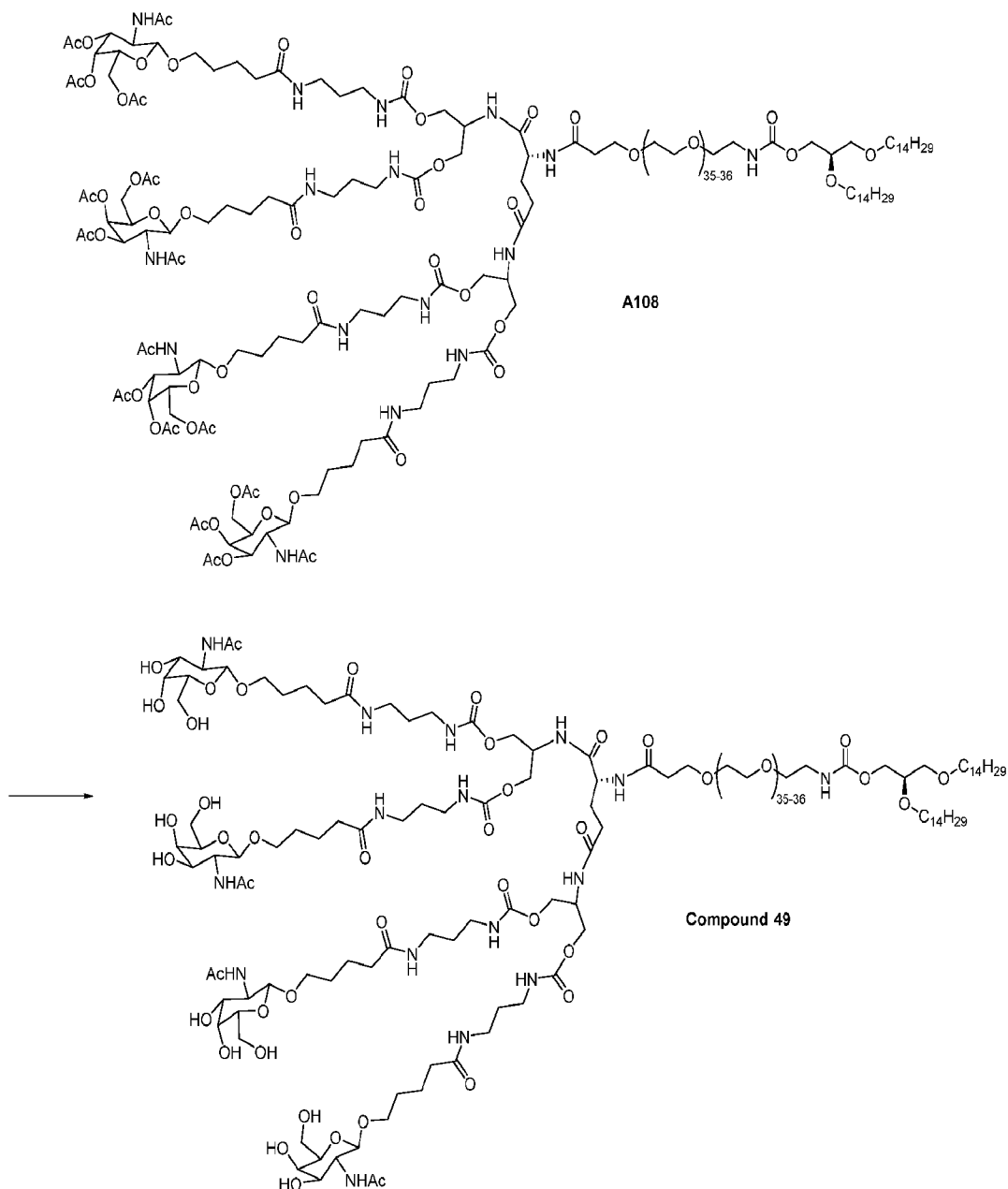
**[0553]** To a solution of L-2.2 (146.6 mg, 0.06 mmol), Int-20-S (120 mg, 0.05 mmol) in DCM (2.0 mL), DIPEA (0.05 mL, 0.25 mmol) was added, followed by the addition of EDCI (11 mg, 0.06 mmol), and finally HOBT (9 mg, 0.06 mmol). The resulting mixture was stirred for 48 hours at room temperature. Then reaction was diluted with DCM and washed with brine and dried over  $\text{MgSO}_4$ , and filtered, concentrated, the crude product was purified with Combi-flash (24 g gold normal phase column, 0-50% MeOH in DCM), collected 100 mg product with 44% yield.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.38 (d,  $J$  = 3.3 Hz, 3H), 5.30 – 5.18 (m, 3H), 4.69 – 4.59 (m, 2H), 4.17 (ddd,  $J$  = 21.1, 10.7, 6.3 Hz, 14H), 3.95 (d,  $J$  = 7.0 Hz, 5H), 3.88 – 3.75 (m, 3H), 3.66 (s, 139H), 3.57 (dd,  $J$  = 6.4, 3.6 Hz, 6H), 3.54 – 3.41 (m, 8H), 3.39 (d,  $J$  = 5.4 Hz, 2H), 3.31 (s, 4H), 3.21 (s, 5H), 2.24 – 1.91 (m, 48H), 1.69 (d,  $J$  = 7.0 Hz, 11H), 1.63 – 1.54 (m, 11H), 1.48 (s, 5H), 0.90 (t,  $J$  = 6.7 Hz, 6H).

**[0554] Example 32. Preparation of Compound 48**

**[0555]** To a solution of **A107** (100 mg, 1 eq) was dissolved in 3 mL of 7N  $\text{NH}_3$  in MeOH solution (excess) and stirred at room temperature in a pressure tube overnight. Subsequently, reaction was concentrated and loaded on C-18 column, eluted with water, ACN and MeOH as mobile phases. The product was eluted with 100% MeOH.  $^1\text{H}$  NMR (400 MHz, MeOD)  $\delta$  4.38 (d,  $J = 8.4$  Hz, 4H), 4.27 – 4.04 (m, 10H), 3.99 – 3.75 (m, 21H), 3.66 (s, 194H), 3.52 (tdd,  $J = 12.4, 9.9, 5.8$  Hz, 22H), 3.23 (d,  $J = 5.2$  Hz, 7H), 3.16 (t,  $J = 6.8$  Hz, 9H), 2.23 (td,  $J = 7.5, 3.1$  Hz, 10H), 2.01 (s, 12H), 1.70 (p,  $J = 6.7$  Hz, 18H), 1.59 (p,  $J = 7.4$  Hz, 18H), 1.31 (s, 72H), 0.92 (t,  $J = 6.6$  Hz, 6H). MS (MALDI-TOF)  $m/z$   $[\text{M}+\text{Na}]^+$  calculated for 4210.08; found for 4212.0,  $n=35$

**[0556] Example 33. Preparation of A108**

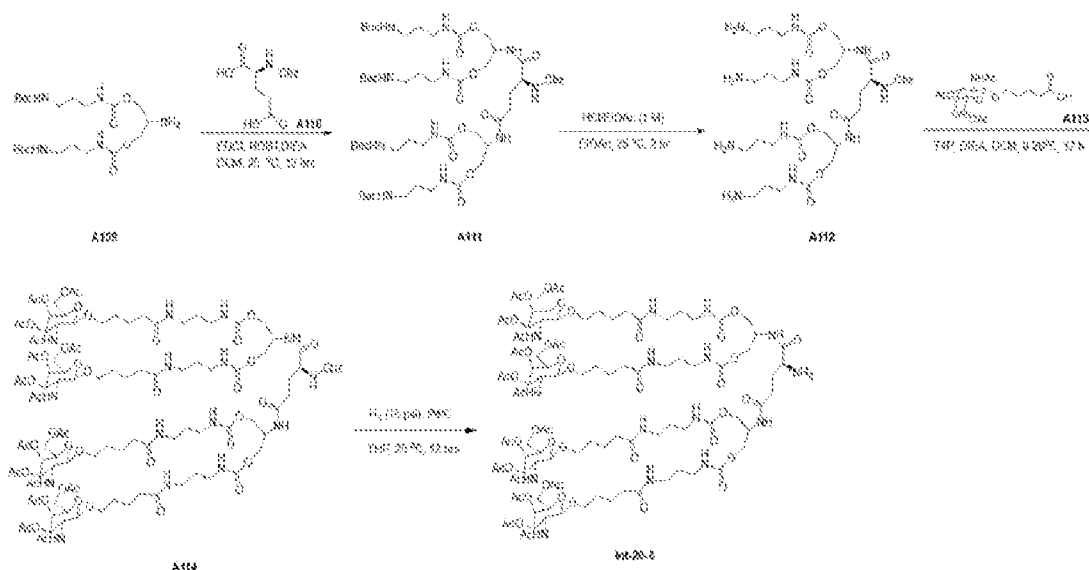
**[0557]** To a solution of **PEG45-DSG** (97 mg, 0.041 mmol, 1.0 equiv) and **Int-20-R** (100 mg, 0.041 mmol, 1.0 equiv) in DCM (2.0 mL), HOBT (7.6 mg, 0.05 mmol, 1.2 equiv), EDC (9.5 mg, 0.05 mmol, 1.2 equiv) and DIPEA (0.2 mL, 0.12 mmol, 3 equiv) were added, the resulting mixture was stirred for 48 h at room temperature, additional 10 mL of DCM was added, washed with brine, dried with anhydrous  $\text{MgSO}_4$ . Filtered, concentrated, the crude product was purified with C18 reverse phase Combi Flash (Gold C18 gel column, eluted with 100% MeOH) collected 103 mg (52% yield) desired product.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.38 (d,  $J = 3.4$  Hz, 3H), 5.21 (d,  $J = 11.3$  Hz, 4H), 4.63 (d,  $J = 8.1$  Hz, 3H), 4.32 – 4.09 (m, 17H), 3.96 (d,  $J = 6.3$  Hz, 7H), 3.67 (s, 133H), 3.61 – 3.38 (m, 15H), 3.21 (s, 7H), 2.54 (s, 2H), 2.26 – 1.87 (m, 48H), 1.65 (s, 54H), 1.28 (s, 48H), 0.90 (t,  $J = 6.6$  Hz, 6H).

**[0558] Example 34. Preparation of Compound 49**

**[0559]** The compound **A108** (103 mg, 0.02 mmol, 1 equiv) was dissolved in 3 mL of 7N  $\text{NH}_3$  in MeOH (11.4 mg, 0.7 mmol, 30 equiv) solution and transferred to a pressure tube and stirred at room temperature for 24 h. Then reaction was concentrated via rotovap and purified by reverse phase chromatography (C18 column) with water and acetonitrile/methanol as the mobile phases. Final product was recovered as a white solid 61.4 mg with a yield of 66.9%.  $^1\text{H}$  NMR (400 MHz, MeOD)  $\delta$  4.58 (s, 1H), 4.38 (d,  $J$  = 8.4 Hz, 4H), 4.25 – 4.02 (m, 10H), 3.99 – 3.89 (m, 7H), 3.86 (d,  $J$  = 3.3 Hz, 4H), 3.78 (dd,  $J$  = 6.1, 4.5 Hz, 9H), 3.66 (s, 154H), 3.52 (td,  $J$  = 10.4, 5.7 Hz, 18H), 3.23 (d,  $J$  = 5.3 Hz, 7H), 3.16 (d,  $J$  = 6.9 Hz, 9H), 2.58 – 2.44 (m, 2H), 2.33 (d,  $J$  = 7.6 Hz, 2H), 2.24 (d,  $J$  = 7.4 Hz, 8H), 2.01 (s, 12H), 1.70 (p,  $J$  =

7.1 Hz, 15H), 1.60 (q,  $J = 6.6$  Hz, 12H), 1.32 (s, 53H), 0.92 (t,  $J = 6.7$  Hz, 6H). MS (MALDI\_TOF)  $m/z$   $[M+Na]^+$  calculated for 4097.38 ; found for 4097.05,  $n=35$ .

**[0560] Example 35. Preparation of Int-20-S**



**[0561] Preparation of Compound A111**

**[0562]** A mixture of Cpd. **A109** (4 g, 8.14 mmol), Cpd. **A110** (1.10 g, 3.91 mmol), DIEA (6.31 g, 48.82 mmol), HOBt (3.30 g, 24.41 mmol) and EDCI (4.68 g, 24.41 mmol) in DCM (40 mL) was degassed and purged with  $N_2$  for 3 times, and then the mixture was stirred at 25°C for 12 hrs under  $N_2$  atmosphere. TLC (eluted with DCM: MeOH =10: 1,  $R_f$ =0.50) showed Compound **A109** was consumed and one new spot formed. The reaction mixture was diluted with  $H_2O$  (30 mL) and extracted with DCM (40 mL  $\times$  3). The combined organic layers were washed with brine 100 mL (50 mL  $\times$  2), dried over  $Na_2SO_4$ , filtered, and concentrated under reduced pressure to give a residue, which was purified by column chromatography ( $SiO_2$ , DCM: MeOH=1:0 to 10:1) to get Cpd. **A111** (3 g, 2.44 mmol, 30.01% yield) as white solid.  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  7.40-7.30 (m, 5H), 6.04-5.68 (m, 4H), 5.25- 4.96 (m, 6H), 4.49-4.37 (m, 2H), 4.31-4.07 (m, 10H), 3.45-2.93 (m, 18H), 2.40-2.22 (m, 2H), 1.75-1.56 (m, 10H), 1.43 (s, 36H).

**[0563] Preparation of Compound A112**

**[0564]** A mixture of Cpd. **A111** (2.2 g, 1.79 mmol), HCl/EtOAc (1 M, 10 mL) in DCM (15 mL) was degassed and purged with  $N_2$  for 3 times, and then the mixture was stirred at 25 °C for 2 hr under  $N_2$  atmosphere. TLC (eluted with DCM: MeOH =10: 1,  $R_f$ =0.1) showed Cpd. **A111** was consumed and one new spot formed. The reaction mixture was concentrated under reduced pressure to give Cpd. **A112** (1.7 g, 1.75 mmol, 97.48% yield, 4 HCl) as white

solid. <sup>1</sup>H NMR (400 MHz, MeOD) δ 7.41-7.29 (m, 5H), 5.11 (s, 2H), 4.25- 4.04 (m, 11H), 3.25-3.17 (m, 8H), 2.99 (br t, J = 7.2 Hz, 8H), 2.41-2.30 (m, 2H), 1.97-1.78 (m, 10H).

**[0565] Preparation of Compound A114**

**[0566]** To a mixture of Cpd. **A112** (1.70 g, 1.74 mmol, 4HCl) and Cpd. **A113** (3.28 g, 7.32 mmol), DIEA (1.80 g, 13.95 mmol) in DCM (20 mL) was added T4P (2.51 g, 6.98 mmol) in one portion at 0 °C under N<sub>2</sub>. The mixture was stirred at 0 °C for 0.5 h, then heated to 20 °C and stirred for 12 hrs. TLC (eluted with DCM: MeOH=10: 1, R<sub>f</sub>=0.50) showed Cpd. **A112** was consumed and one new spot formed. The reaction mixture was diluted with H<sub>2</sub>O (20 mL) and extracted with DCM 60 mL (20 mL × 3). The combined organic layers were washed with brine 100 mL (25 mL × 4), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure to give a residue, which was purified by column chromatography (SiO<sub>2</sub>, DCM; MeOH=1:0 to 10:1) to get Cpd. **A114** (2 g, 46% yield) as white solid. <sup>1</sup>H NMR (400 MHz, DMSO) δ 7.99 (br d, J = 4.0 Hz, 1H), 7.94-7.88 (m, 1H), 7.81 (br d, J = 9.2 Hz, 4H), 7.72 (br s, 4H), 7.35 (br s, 6H), 7.12 (br s, 4H), 5.21 (br d, J = 3.2 Hz, 4H), 5.05-4.91 (m, 6H), 4.48 (br d, J = 8.4 Hz, 4H), 4.08-3.96 (m, 18H), 3.93-3.80 (m, 8H), 3.74-3.64 (m, 4H), 3.44-3.36 (m, 4H), 3.06-2.92 (m, 16H), 2.10 (s, 12H), 2.03 (br d, J = 8.0 Hz, 10H), 1.99 (s, 12H), 1.89 (s, 12H), 1.77 (s, 12H), 1.60-1.38 (m, 26H).

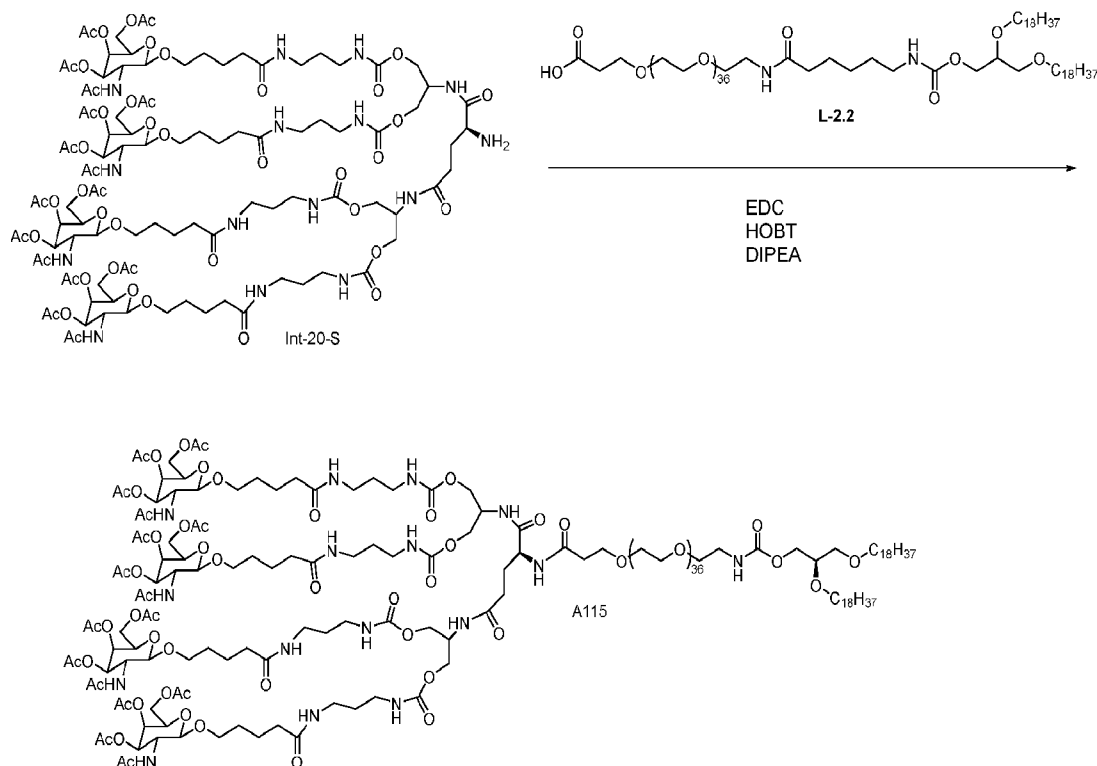
**[0567] Preparation of Compound Int-20-S**

**[0568]** A mixture of Cpd. **A114** (2 g, 785.68 μmol), Pd/C (1 g, 469.84 μmol, 10% purity) in THF (200 mL) was degassed and purged with H<sub>2</sub> for 3 times, and then the mixture was stirred at 25 °C for 12hrs under H<sub>2</sub> atmosphere. LCMS showed Cpd. **A114** was consumed and one main peak with desired MS was detected. The reaction mixture was filtered and concentrated under reduced pressure to give **Int-20-S** (1.2 g, 63% yield) as white solid. <sup>1</sup>H NMR (400 MHz, DMSO) δ 8.63 (br d, J = 3.6 Hz, 1H), 8.40-8.17 (m, 2H), 8.16-8.05 (m, 1H), 7.89-7.82 (m, 4H), 7.80-7.66 (m, 4H), 7.13 (br s, 4H), 5.21 (br s, 4H), 5.03-4.88 (m, 4H), 4.49 (br d, J = 8.4 Hz, 4H), 4.02 (br s, 18H), 3.95-3.81 (m, 8H), 3.78 (br d, J = 7.2 Hz, 1H), 3.74-3.66 (m, 4H), 3.44-3.38 (m, 4H), 3.07-2.91 (m, 16H), 2.10 (s, 12H), 2.03 (br d, J = 6.8 Hz, 10H), 1.99 (br s, 12H), 1.89 (s, 12H), 1.77 (s, 12H), 1.58-1.38 (m, 26H). LCMS: t<sub>R</sub> = 1.895 min, 96% purity, m/z = 1206.7 (M/2+H)<sup>+</sup>

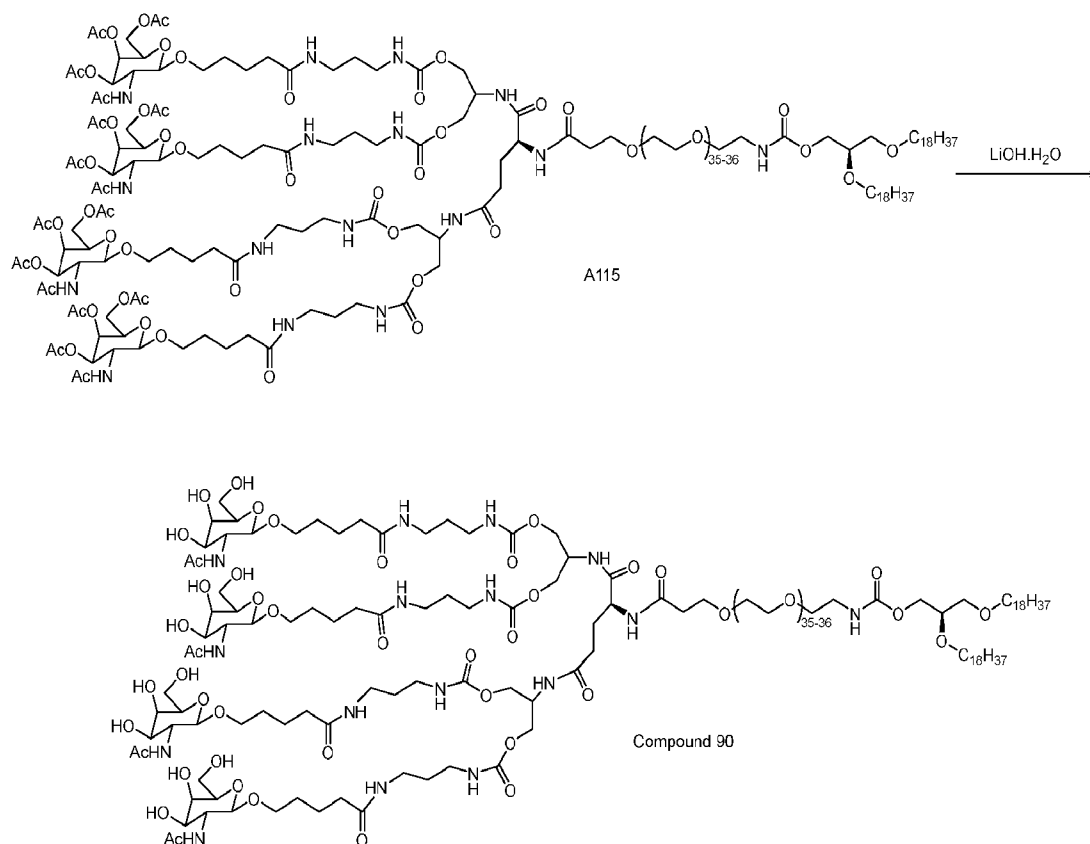
**[0569] Method of LCMS:** LC/MS (The gradient was 0%-60 B in 0-2.5 min, maintains 60% B in 2.5-3.0 min, and 60% B-0%B in 3.0-3.01min, 0% B in 3.01-3.5 min the flow rate was 1 ml/min. Mobile phase A was 0.04% trifluoroacetic acid in water, mobile phase B was 0.02% trifluoroacetic acid in acetonitrile. The column used for chromatography was a Luna LC- C18 50\*2 mm (5 μm particles). Detection methods are diode array (DAD) and evaporative light

scattering (ELSD) detection as well as positive electrospray ionization. MS range was 100-2000.

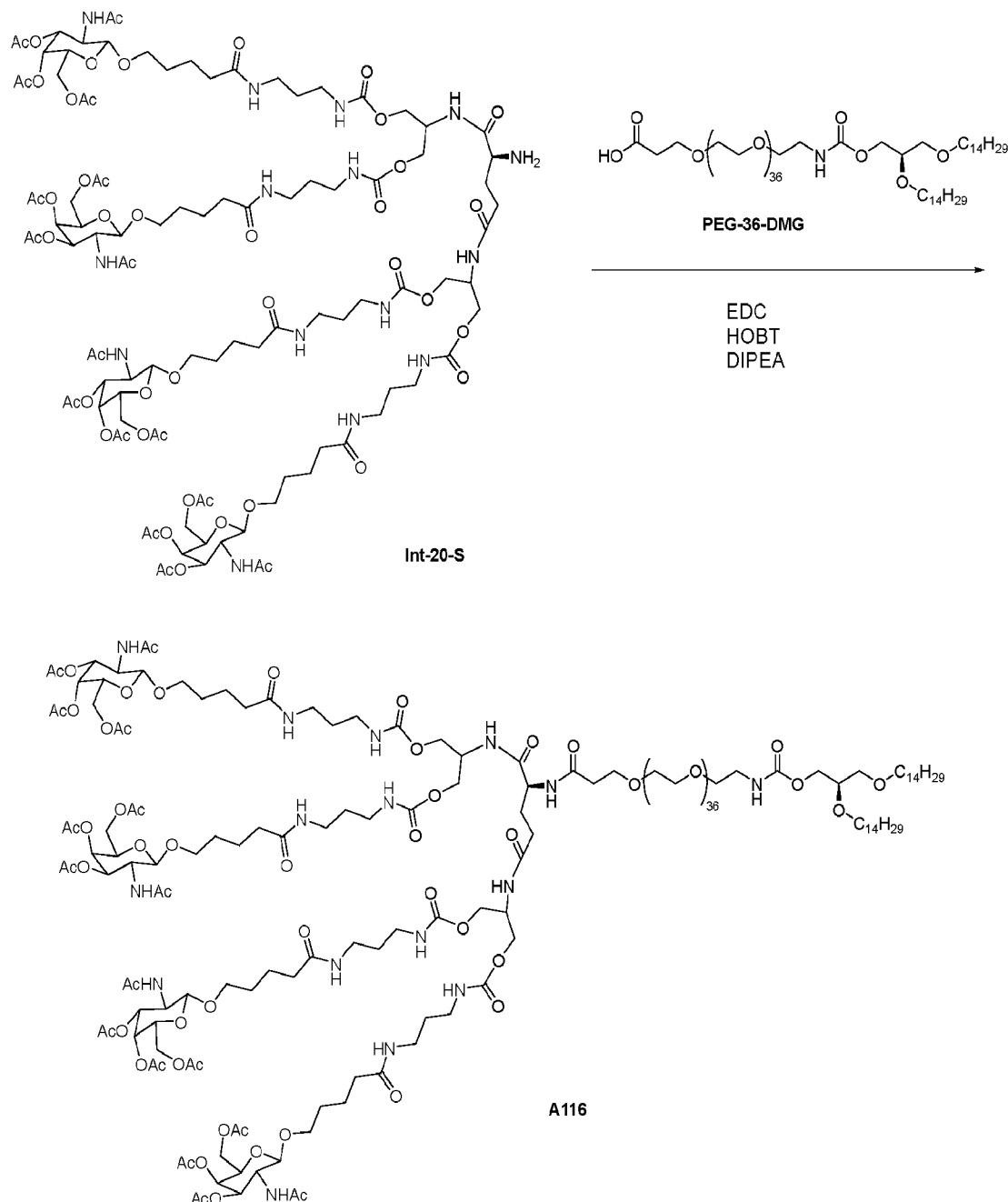
**[0570] Example 36. Preparation of A115**



**[0571]** To a solution of **PEG2000-DSG** (146.6 mg, 0.06 mmol), **Int-20-S** (120 mg, 0.05 mmol) in DCM (2.0 mL), DIPEA (0.05 mL, 0.25 mmol) was added, followed by the addition of EDCI (11 mg, 0.06 mmol), and finally HOBT (9 mg, 0.06 mmol). The resulting mixture was stirred for 48 hours at room temperature. Then reaction was diluted with DCM and washed with brine and dried over MgSO<sub>4</sub>, and filtered, concentrated, the crude product was purified with Combi-flash (24 g gold normal phase column, 0-50% MeOH in DCM), collected 170 mg product with 70% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.38 (d, *J* = 3.4 Hz, 3H), 5.22 (d, *J* = 11.0 Hz, 3H), 4.64 (d, *J* = 8.1 Hz, 2H), 4.17 (dtd, *J* = 15.8, 11.3, 7.3 Hz, 15H), 3.95 (s, 5H), 3.84 (t, *J* = 4.9 Hz, 1H), 3.77 (s, 2H), 3.67 (s, 106H), 3.60 – 3.37 (m, 13H), 3.31 (s, 6H), 3.17 (d, *J* = 34.9 Hz, 7H), 2.24 – 1.91 (m, 48H), 1.61 – 1.54 (m, 10H), 1.47 (d, *J* = 6.7 Hz, 5H), 1.28 (s, 52H), 0.90 (t, *J* = 6.8 Hz, 6H).

**[0572] Preparation of Compound 90**

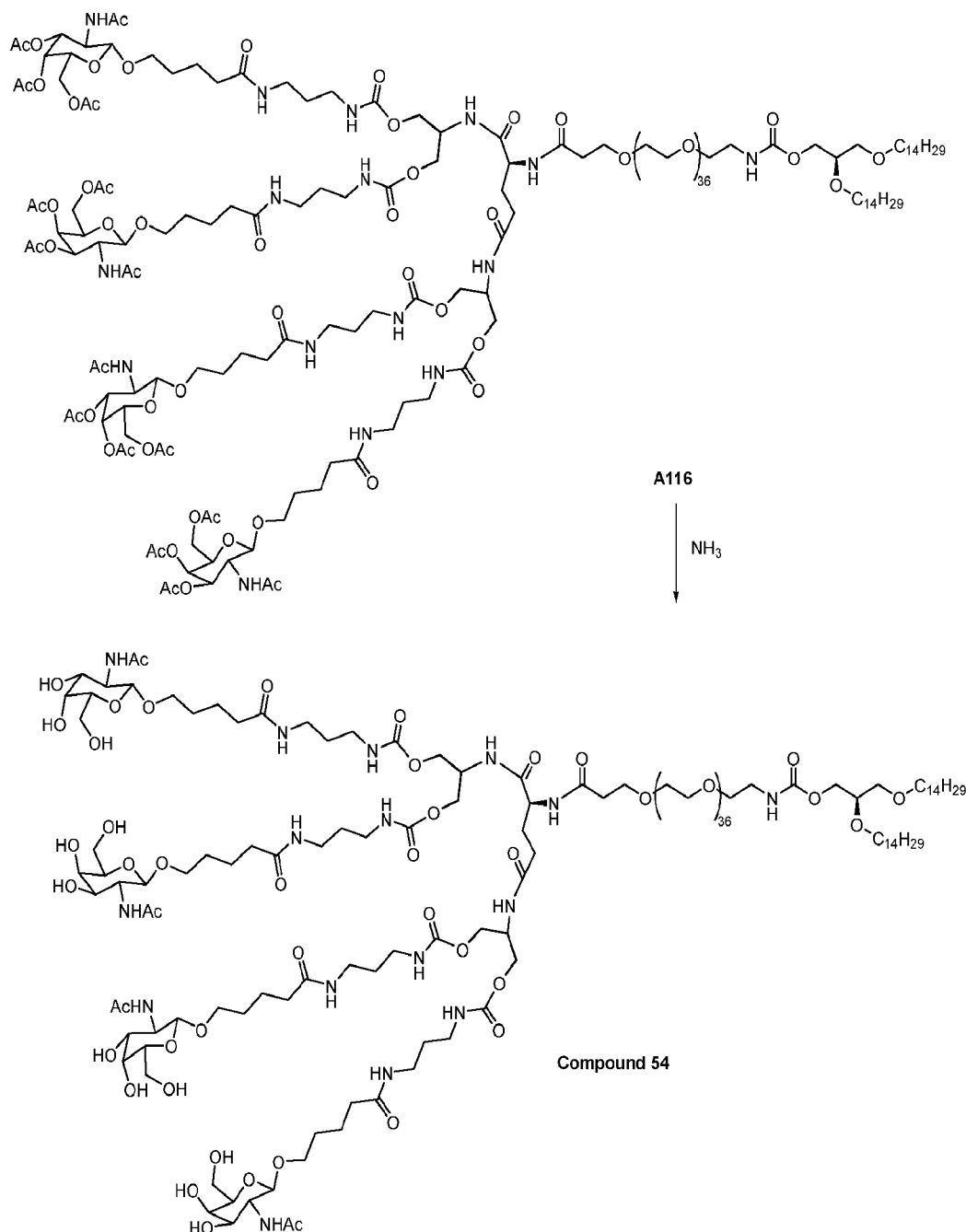
**[0573]** To a solution of **A115** (85 MG, 0.018 mmol) in THF (2 mL) and MeOH (2 mL), a pre-made 1 mL of LiOH (23 mg) aqueous solution was added with ice water cooling, after addition, the ice water cooling bath was removed, the resulting reaction mixture was stirred for 4 hrs at room temperature, then pH was adjusted to 5-6 with 1N HCl aqueous under ice water cooling, concentrated, the crude product was dissolved in water, loaded on C-18 column, eluted with 5% Acetonitrile in water in 15 mins, then raise Acetonitrile to 10% in 15 mins, switched to 100% MeOH to recover product with 95% HPLC purity. <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  4.38 (d,  $J$  = 8.4 Hz, 4H), 4.13 (ddt,  $J$  = 23.6, 11.2, 5.4 Hz, 10H), 3.97 (s, 1H), 3.86 (d,  $J$  = 3.3 Hz, 4H), 3.78 (p,  $J$  = 5.2 Hz, 9H), 3.66 (s, 159H), 3.52 (qd,  $J$  = 11.4, 6.3 Hz, 18H), 3.27 – 3.20 (m, 8H), 3.16 (t,  $J$  = 6.9 Hz, 9H), 2.56 (d,  $J$  = 4.2 Hz, 2H), 2.34 (t,  $J$  = 7.5 Hz, 2H), 2.23 (t,  $J$  = 7.4 Hz, 8H), 2.01 (s, 12H), 1.70 (p,  $J$  = 7.3 Hz, 15H), 1.60 (q,  $J$  = 6.6 Hz, 12H), 1.31 (s, 66H), 0.92 (t,  $J$  = 6.7 Hz, 6H). MS (MALDI\_TOF)  $m/z$   $[M+Na]^+$  calculated for 4207.5; found for 42017.3,  $n=35$ .

**[0574] Example 37. Preparation of A116**

**[0575]** To a solution of **-PEG45-DSG** (97 mg, 0.04 mmol, 1.0 equiv) and **Int-20-S** (100 mg, 0.04 mmol, 1.0 equiv) in DCM (2.0 mL), HOBT (8 mg, 0.05 mmol, 1.2 equiv), EDC (9.5 mg, 0.05 mmol, 1.2 equiv) and DIPEA (0.2 mL, 0.12 mmol, 3 equiv) were added, the resulting mixture was stirred for 48 h at room temperature, additional 10 mL of DCM was added, washed with brine, dried with anhydrous  $\text{MgSO}_4$ . Filtered, concentrated, the crude product was purified with C18 reverse phase Combi Flash (Gold C18 gel column, eluted with 100% MeOH) collected 120 mg (61% yield) desired product.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  6.95 – 6.52 (m, 5H), 6.29 (s, 1H), 5.38 (d,  $J = 3.3$  Hz, 3H), 5.21 (d,  $J = 10.8$  Hz, 3H), 4.63 (d,

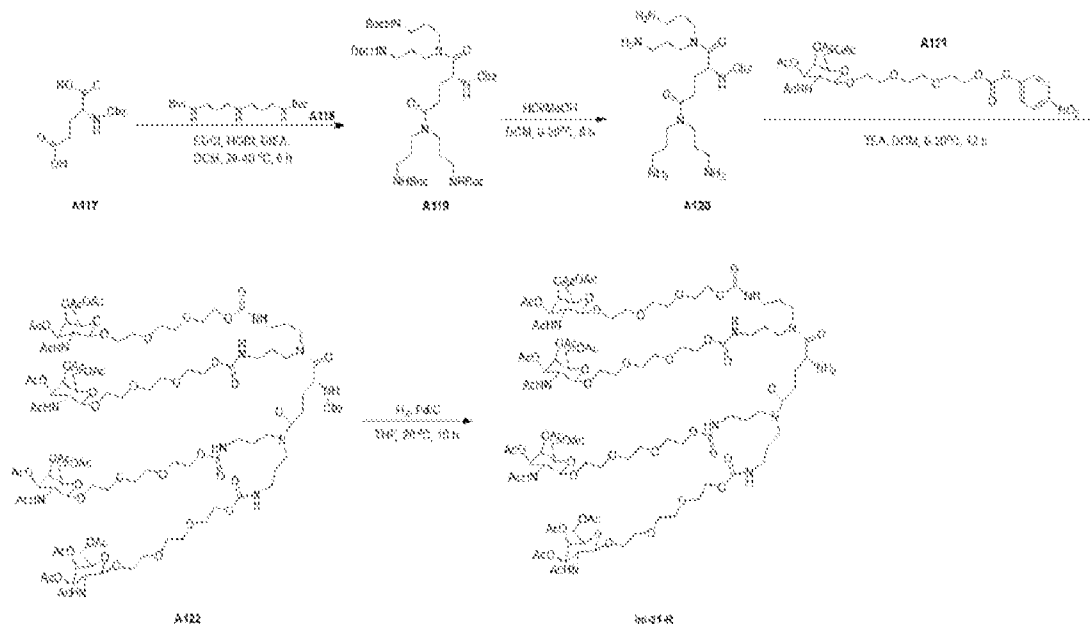
$J = 8.6$  Hz, 3H), 3.95 (s, 8H), 3.89 – 3.68 (m, 8H), 3.67 (s, 130H), 3.60 – 3.15 (m, 30H), 2.53 (s, 2H), 2.26 – 1.91 (m, 49H), 1.67 (s, 32H), 1.28 (s, 47H), 0.90 (t,  $J = 6.7$  Hz, 6H).

**[0576] Example 39. Preparation of Compound 54**



**[0577]** To a solution of **A116** (120 mg, 1 eq) was dissolved in 3 mL of 7N  $\text{NH}_3$  in MeOH solution (excess) and stirred at room temperature in a pressure tube overnight. Subsequently, reaction was concentrated and loaded on C-18 column, eluted with water, ACN and MeOH as mobile phases. The product was eluted with 100% MeOH.  $^1\text{H}$  NMR (400 MHz, MeOD)  $\delta$  4.38 (d,  $J = 8.4$  Hz, 4H), 4.24 – 4.03 (m, 11H), 3.94 (dt,  $J = 9.9, 6.3$  Hz, 8H), 3.86 (d,  $J = 3.3$  Hz, 4H), 3.82 – 3.77 (m, 8H), 3.66 (s, 154H), 3.51 (ddd,  $J = 16.9, 10.9, 6.9$  Hz, 16H), 3.20 (dt,  $J = 33.8, 6.8$  Hz, 17H), 2.59 – 2.53 (m, 2H), 2.34 (t,  $J = 7.5$  Hz, 2H), 2.23 (t,  $J = 7.3$  Hz,

8H), 2.01 (s, 12H), 1.70 (p,  $J = 7.2$  Hz, 17H), 1.60 (q,  $J = 6.5$  Hz, 12H), 1.32 (s, 49H), 0.92 (t,  $J = 6.7$  Hz, 6H). MS (MALDI\_TOF)  $m/z$   $[M+Na]^+$  calculated for 4097.87; found for 4097.10,  $n=35$

**[0578] Example 40.****Preparation of Int-21-R****[0579] Preparation of Compound A119**

**[0580]** To a solution of Cpd.A117 (3.39 g, 12.07 mmol), Cpd.A118 (10 g, 30.17 mmol) in DCM (100 mL) was added EDCI (6.94 g, 36.20 mmol), HOBt (4.89 g, 36.20 mmol) and DIEA (9.36 g, 72.41 mmol) at 20°C. The mixture was stirred at 40 °C for 6 h. LCMS showed reaction was ok. The mixture was cooled down and poured into H<sub>2</sub>O (50 mL). The aqueous phase was extracted with DCM (3 × 50 mL). The combined organic phase was washed with brine (30 mL × 2), dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure. The crude product was purified by flash silica gel column chromatography (DCM: MeOH= 1:0 to 10:1) to give Cpd.A119 (8.6 g, 78.47% yield) as colorless gum. <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  = 7.43 - 7.23 (m, 5H), 5.16 - 5.00 (m, 2H), 4.59 (br dd,  $J = 1.2, 9.2$  Hz, 1H), 3.77 - 3.59 (m, 1H), 3.55 - 3.45 (m, 1H), 3.37 (br d,  $J = 4.8$  Hz, 2H), 3.28 - 3.18 (m, 4H), 3.12 (br t,  $J = 6.4$  Hz, 2H), 3.07 - 2.96 (m, 6H), 2.65 - 2.50 (m, 1H), 2.48 - 2.36 (m, 1H), 2.12 - 2.02 (m, 1H), 1.86 (br d,  $J = 8.0$  Hz, 2H), 1.75 - 1.63 (m, 6H), 1.44 (s, 36H).

**[0581] Preparation of Compound A120**

**[0582]** To a solution of Cpd.A119 (10.00 g, 11.01 mmol) in MeOH (100 mL) was added HCl/MeOH (4 M, 22.22 mL) at 0 °C. The mixture was stirred at 20 °C for 8 h. LCMS showed reactant 1 was consumed and one main peak desired. The reaction mixture was concentrated under reduced pressure to give Cpd.A120 (6.8 g, 94% yield, 4HCl) as white solid. <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  = 7.42 - 7.23 (m, 5H), 5.15 - 5.01 (m, 2H), 4.73 - 4.57 (m, 1H), 3.95 - 3.75

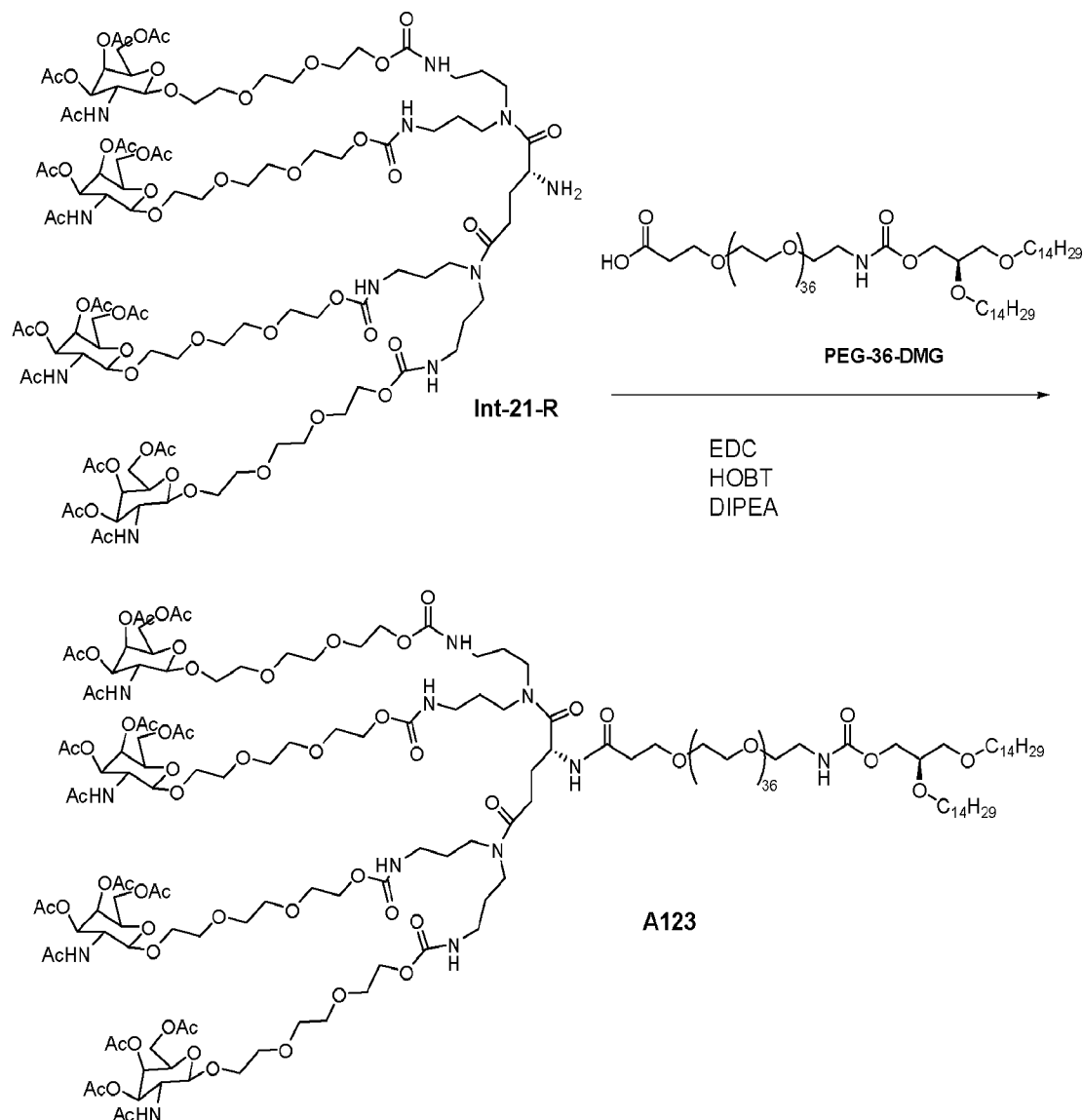
(m, 1H), 3.62 - 3.38 (m, 7H), 3.15 - 3.03 (m, 2H), 3.02 - 2.85 (m, 6H), 2.77 - 2.46 (m, 2H), 2.21 - 2.02 (m, 3H), 1.95 (td,  $J = 7.6, 14.8$  Hz, 6H), 1.88 - 1.76 (m, 1H).

**[0583] Preparation of Compound A122**

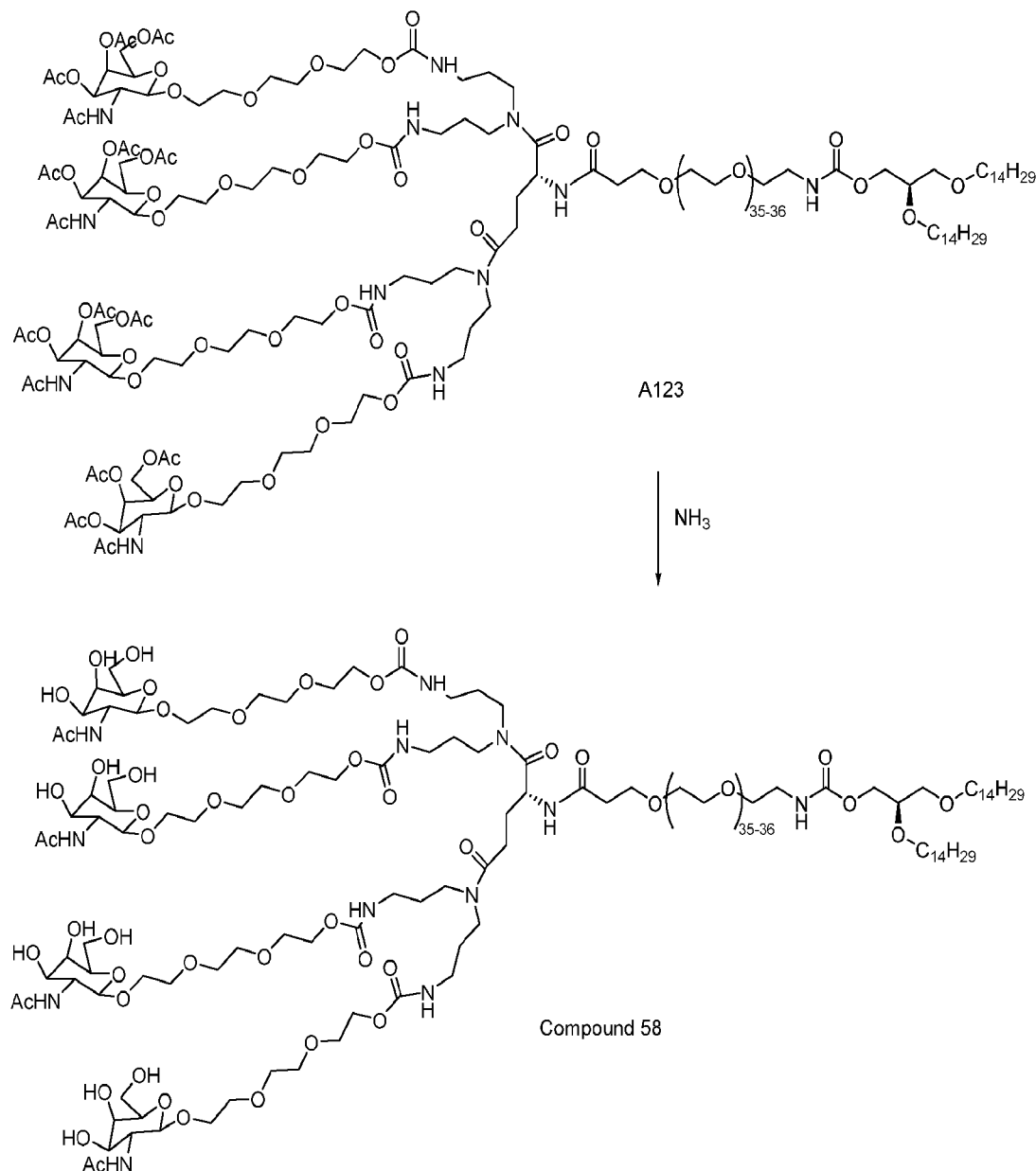
**[0584]** To a solution of Cpd.**A121** (7.45 g, 11.56 mmol) in DCM (20 mL) was added TEA (2.79 g, 27.53 mmol) and Cpd.**A120** (2 g, 2.75 mmol, 6HCl) at 0 °C. The mixture was stirred at 20 °C for 12h. TLC (eluted with DCM:MeOH = 10:1,  $R_f = 0.5$ ) showed the starting material was consumed and one new spot formed. The mixture was quenched with water (50 ml) and extracted with DCM (10 mL  $\times$  3). The combined organic layers were separated, and the organic layer was washed with brine (10 mL  $\times$  6), dried over Na<sub>2</sub>SO<sub>4</sub>. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (SiO<sub>2</sub>, DCM: MeOH = 1:0 to 19:1) for twice to get Cpd.**A122** (2.6 g, 37.33% yield) as a yellowish solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 7.80 (br d,  $J = 9.2$  Hz, 4H), 7.51-7.07 (m, 10H), 5.21 (br d,  $J = 2.4$  Hz, 4H), 5.05-4.92 (m, 6H), 4.55 (br d,  $J = 8.4$  Hz, 4H), 4.36 (br s, 1H), 4.03 (br s, 21H), 3.94-3.83 (m, 5H), 3.81-3.74 (m, 4H), 3.65-3.42 (m, 38H), 3.28-3.13 (m, 5H), 3.11-2.84 (m, 10H), 2.10 (s, 12H), 2.00 (s, 12H), 1.89 (s, 12H), 1.77 (s, 12H), 1.69-1.49 (m, 8H).

**[0585] Preparation of Int-21-R**

**[0586]** To a solution of Cpd.**A122** (1.8 g, 711.59  $\mu$ mol) in THF (150 mL) was added Pd/C (1.20 g, 1.12 mmol, 10% purity) at 20 °C and stirred at 20°C for 16 h. LCMS indicated the Cpd.**A122** was consumed, and the desired mass was detected. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give the desired product to give **Int-21-R** (1.2 g, 70% yield) as a gray solid. **LCMS**:  $R_t = 1.986$  min, 99.04% purity,  $m/z = 1198.7$  (1/2M+H)<sup>+</sup>. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  ppm 7.79 (br d,  $J = 9.2$  Hz, 4H), 7.38 - 7.01 (m, 4H), 5.21 (d,  $J = 3.2$  Hz, 4H), 4.97 (dd,  $J = 3.2, 11.2$  Hz, 4H), 4.55 (d,  $J = 8.4$  Hz, 4H), 4.26 (t,  $J = 7.2$  Hz, 1H), 4.03 (s, 20H), 3.93-3.83 (m, 4H), 3.82-3.72 (m, 4H), 3.65-3.44 (m, 39H), 3.30-3.15 (m, 6H), 3.05-2.88 (m, 8H), 2.45-2.39 (m, 1H), 2.18-2.14 (m, 1H), 2.10 (s, 11H), 2.00 (s, 12H), 1.89 (s, 12H), 1.77 (s, 12H), 1.73-1.43 (m, 12H). **LCMS**:  $t_R = 1.986$  min, 99.04% purity,  $m/z = 1198.7$  (1/2M+H)<sup>+</sup> **LC/MS** (The gradient was 0%-60 B in 0-2.5 min, maintains 60% B in 2.5-3.0 min, and 60% B-0%B in 3.0-3.01min, 0% B in 3.01-3.5 min the flow rate was 1 ml/min. Mobile phase A was 0.04% trifluoroacetic acid in water, mobile phase B was 0.02% trifluoroacetic acid in acetonitrile. The column used for chromatography was a Luna LC- C18 50\*2 mm (5  $\mu$ m particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection as well as positive electrospray ionization. MS range was 100-2000.

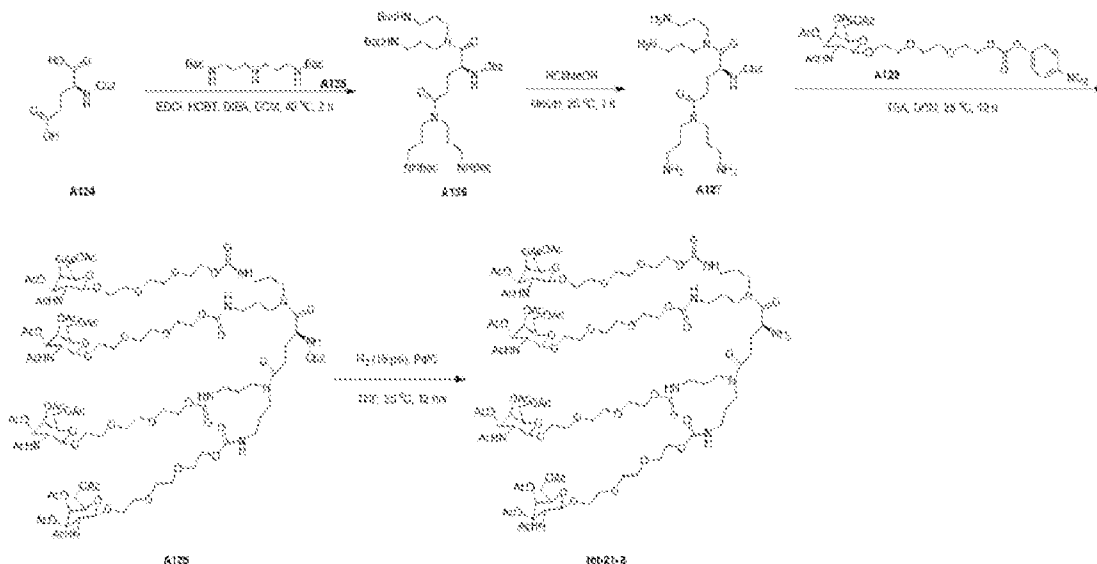
**[0587] Example 41. Preparation of A123**

**[0588]** To a solution of **PEG36-DMG** (117 mg, 0.05 mmol, 1.0 equiv) and **Int-21-R** (120 mg, 0.05 mmol, 1.0 equiv) in DCM (2.0 mL), HOBT (8.4 mg, 0.055 mmol, 1.2 equiv), EDC (10.6 mg, 0.055 mmol, 1.2 equiv) and DIPEA (equiv) were added, the resulting mixture was stirred for 48 h at room temperature, additional 10 mL of DCM was added, washed with brine, dried with anhydrous  $\text{MgSO}_4$ . Filtered, concentrated, the crude product was purified with C18 reverse phase Combi Flash (Gold C18 gel column, eluted with 100% MeOH) collected 120 mg (67% yield) desired product.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  6.70 – 6.54 (m, 2H), 6.44 (s, 1H), 6.00 – 5.91 (m, 1H), 5.82 (s, 1H), 5.40 – 5.28 (m, 4H), 5.11 (d,  $J$  = 11.2 Hz, 3H), 4.96 (s, 1H), 4.81 (d,  $J$  = 8.1 Hz, 3H), 4.02 – 3.81 (m, 13H), 3.67 (s, 170H), 3.59 – 3.34 (m, 16H), 3.22 (d,  $J$  = 30.3 Hz, 8H), 2.63 – 2.46 (m, 2H), 2.34 (s, 1H), 2.24 – 1.94 (m, 44H), 1.67 (s, 28H), 1.58 (t,  $J$  = 6.9 Hz, 5H), 1.28 (s, 50H), 0.90 (t,  $J$  = 6.7 Hz, 6H).

**[0589] Preparation of Compound 58**

**[0590]** The compound **A123** (160 mg, 35 mmol, 1 equiv) was dissolved in 3 mL of 7N  $\text{NH}_3$  in MeOH (18 mg, 1.04 mmol, 30 equiv) solution and transferred to a pressure tube and stirred at room temperature for 24 h. Then reaction was concentrated via rotovap and purified by reverse phase chromatography (C18 column) with water and acetonitrile/methanol as the mobile phases. Final product was recovered as a white solid 111.9 mg with a yield of 78.5 %.

$^1\text{H}$  NMR (400 MHz, MeOD)  $\delta$  4.49 (dd,  $J = 8.5, 2.4$  Hz, 4H), 4.26 – 4.13 (m, 10H), 4.04 – 3.77 (m, 23H), 3.66 (s, 180H), 3.57 – 3.45 (m, 16H), 3.22 – 3.07 (m, 10H), 2.01 (s, 11H), 1.77 (dt,  $J = 13.3, 7.0$  Hz, 6H), 1.58 (d,  $J = 7.1$  Hz, 5H), 1.32 (s, 43H), 0.92 (t,  $J = 6.7$  Hz, 6H). MS (MALDI\_TOF)  $m/z$   $[\text{M}+\text{Na}]^+$  calculated for 4079.34 ; found for 4079.87,  $n=35$ .

**[0591] Example 44. Preparation of Int-21-S****[0592] Preparation of Compound A126**

**[0593]** To a solution of Cpd. **A124** (10.18 g, 36.20 mmol) in DCM (300 mL) was added EDCI (20.82 g, 108.61 mmol), HOBt (14.68 g, 108.61 mmol) and DIEA (28.08 g, 217.23 mmol, 37.84 mL). Then Cpd. **A125** (30 g, 90.51 mmol) was added, and the mixture was stirred at 40 °C for 2 h. TLC (eluted with DCM: MeOH = 10: 1,  $R_f$  = 0.30) showed Cpd. **A124** was consumed and one new spot formed. The reaction mixture was diluted with H<sub>2</sub>O 300 mL and extracted with DCM 1200 mL (400 mL  $\times$  3). The combined organic layers were washed with brine 1000 mL (500 mL  $\times$  2), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated under reduced pressure to give a residue, which was purified by column chromatography (SiO<sub>2</sub>, DCM: MeOH = 1/0 to 10/1) to get Cpd. **A126** (27 g, 29.73 mmol, 82.12% yield) as colorless oil. <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  = 7.43-7.22 (m, 5H), 5.07 (br d,  $J$  = 4.2 Hz, 2H), 4.64-4.51 (m, 1H), 3.77-3.60 (m, 1H), 3.55-3.44 (m, 1H), 3.42-3.34 (m, 2H), 3.26 (br s, 4H), 3.12 (br d,  $J$  = 6.4 Hz, 2H), 3.02 (br d,  $J$  = 5.6 Hz, 6H), 2.65-2.51 (m, 1H), 2.49-2.35 (m, 1H), 2.10-2.02 (m, 1H), 1.87 (br s, 2H), 1.77-1.64 (m, 6H), 1.62-1.55 (m, 1H), 1.44 (s, 36H).

**[0594] Preparation of Compound A127**

**[0595]** To a solution of Cpd. **A126** (9 g, 9.91 mmol) in MeOH (100 mL) was added HCl/MeOH (4 M, 20 mL). The mixture was stirred at 25 °C for 2 h. TLC (eluted with DCM: MeOH = 10: 1,  $R_f$  = 0) showed Cpd. **A126** was consumed and one new spot formed. The reaction mixture was concentrated under reduced pressure to remove solvent to give Cpd. **A127** (6.7 g, 9.23 mmol, 93.10% yield, 4HCl) as white solid. <sup>1</sup>H NMR (400 MHz, MeOD)  $\delta$  7.43-7.24 (m, 5H), 5.16-5.02 (m, 2H), 4.74-4.58 (m, 1H), 3.92-3.78 (m, 1H), 3.65-

3.37 (m, 7H), 3.10 (br t,  $J = 6.8$  Hz, 2H), 3.01-2.85 (m, 6H), 2.75-2.55 (m, 2H), 2.21-2.03 (m, 3H), 2.00-1.89 (m, 5H), 1.87-1.73 (m, 1H).

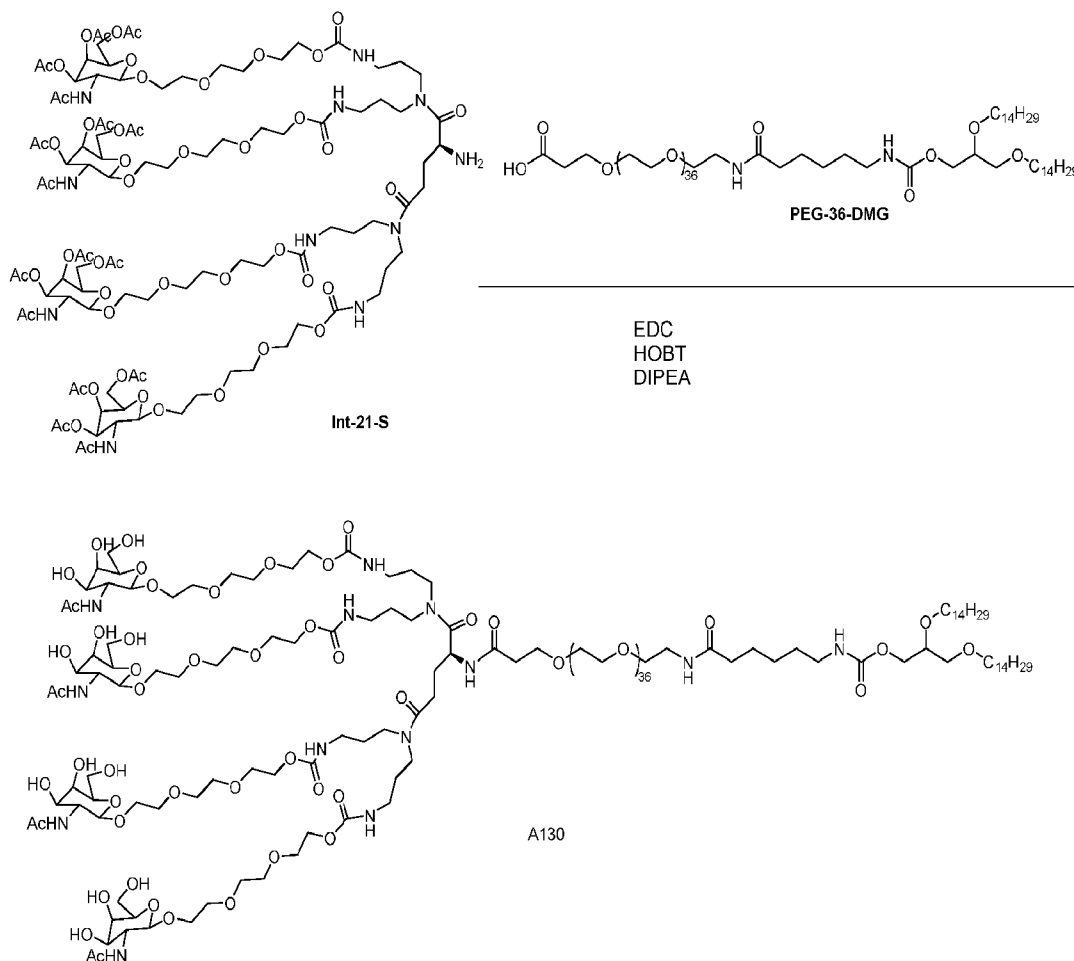
**[0596] Preparation of Compound A129**

**[0597]** A mixture of Cpd.**A127** (2 g, 2.75 mmol, 4HCl), Cpd.**A128** (7.45 g, 11.56 mmol), TEA (2.79 g, 27.53 mmol) in DCM (15 mL) was degassed and purged with N<sub>2</sub> for 3 times, and then the mixture was stirred at 25 °C for 12 h under N<sub>2</sub> atmosphere. TLC (eluted with DCM: MeOH =10: 1,  $R_f$ =0.20) showed Cpd.**A127** was consumed and one new spot formed. The reaction mixture was diluted with H<sub>2</sub>O (20 mL) and extracted with DCM 60 mL (20 mL  $\times$  3). The combined organic layers were washed with brine 100 mL (25 mL  $\times$  4), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue, which was purified by column chromatography (SiO<sub>2</sub>, DCM: MeOH=1:0 to 10:1) for twice to get Cpd. **A129** (2 g, 28.72% yield) as yellow solid. <sup>1</sup>HNMR (400 MHz, DMSO)  $\delta$  7.80 (d,  $J = 9.2$  Hz, 4H), 7.46-7.18 (m, 8H), 7.12 (br s, 2H), 5.21 (d,  $J = 3.2$  Hz, 4H), 5.01-4.97 (m, 3H), 4.96 (d,  $J = 3.2$  Hz, 2H), 4.55 (d,  $J = 8.4$  Hz, 4H), 4.03 (s, 21H), 3.93-3.83 (m, 4H), 3.82-3.73 (m, 4H), 3.63-3.43 (m, 40H), 3.25-3.14 (m, 4H), 3.12-2.84 (m, 11H), 2.10 (s, 12H), 2.00 (s, 12H), 1.89 (s, 12H), 1.77 (s, 12H), 1.67-1.43 (m, 8H).

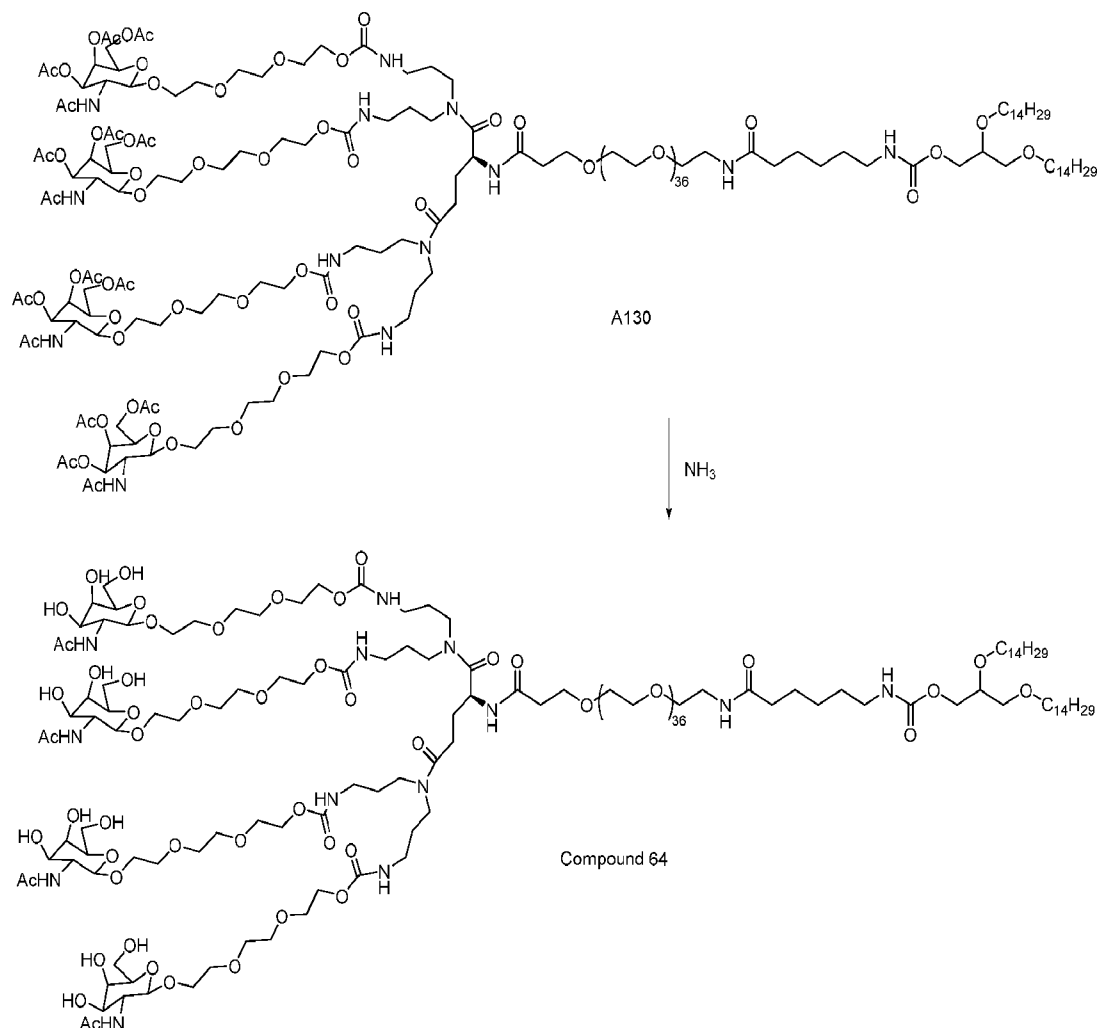
**[0598] Preparation of Int-21-S**

**[0599]** A mixture of Cpd.**A129** (2 g, 790.66  $\mu$ mol), Pd/C (800.00 mg, 751.74  $\mu$ mol, 10% purity) in THF (200 mL) was degassed and purged with H<sub>2</sub> for 3 times, and then the mixture was stirred at 25 °C for 12 hr under H<sub>2</sub> atmosphere. LCMS showed Cpd.**A129** was consumed and one main peak with desired m/z was detected. The reaction mixture was filtered and concentrated under reduced pressure to give **Int-21-S** (1.4 g, 73.92% yield) as grey solid. <sup>1</sup>HNMR (400 MHz, DMSO)  $\delta$  = 7.80 (br d,  $J = 9.2$  Hz, 4H), 7.33-7.20 (m, 2H), 7.14 (br s, 2H), 5.22 (d,  $J = 3.2$  Hz, 4H), 4.98 (m, 4H), 4.56 (d,  $J = 8.4$  Hz, 4H), 4.03 (s, 20H), 3.93-3.84 (m, 4H), 3.82-3.75 (m, 4H), 3.64-3.46 (m, 43H), 3.27-3.19 (m, 4H), 3.04-2.90 (m, 8H), 2.11 (s, 12H), 2.00 (s, 12H), 1.89 (s, 12H), 1.78 (s, 12H), 1.77-1.73 (m, 4H), 1.69- 1.54 (m, 8H). LCMS:  $t_R = 1.972$  min, 95% purity, m/z = 1198.6 (M/2+H)<sup>+</sup>.

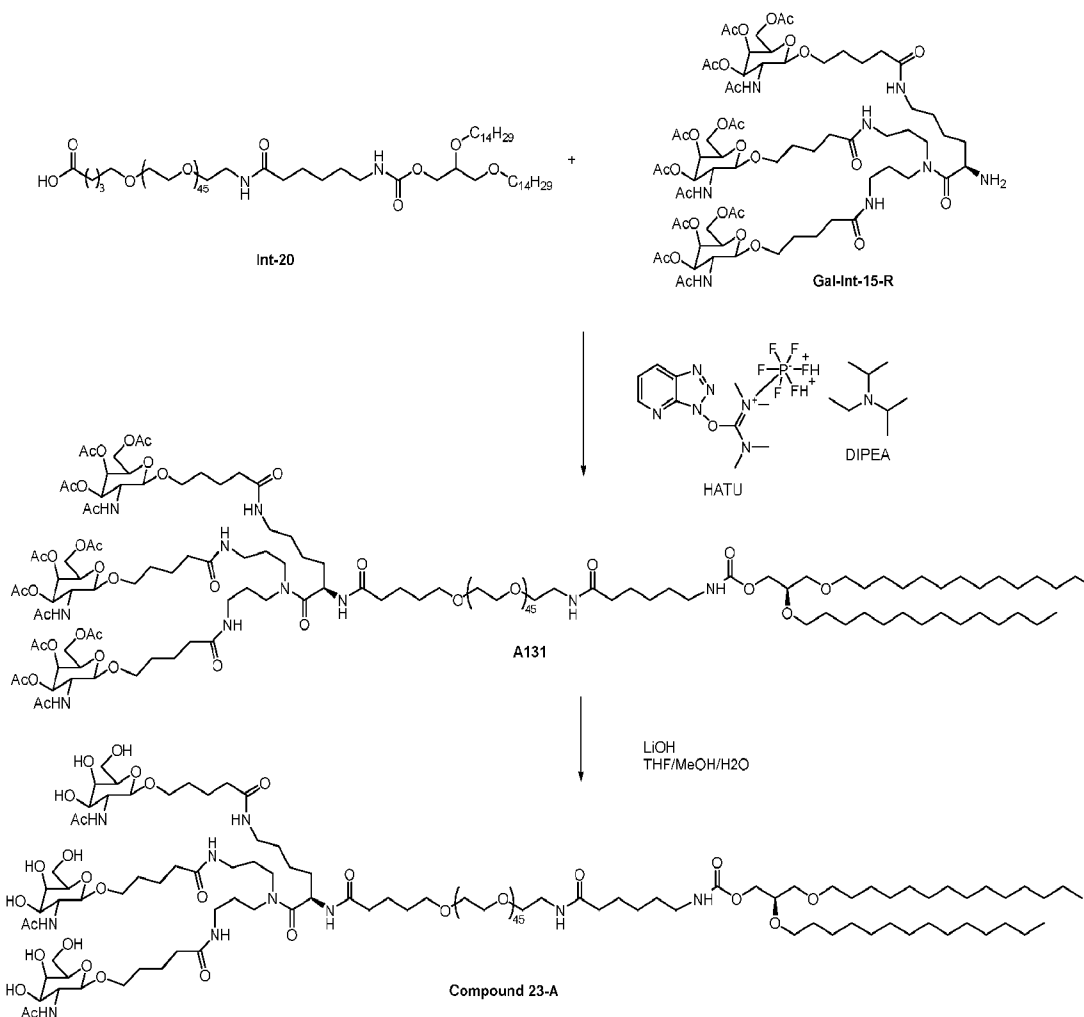
**[0600] Method of LCMS:** LC/MS (The gradient was 0%-60 B in 0-2.5 min, maintains 60% B in 2.5-3.0 min, and 60% B-0%B in 3.0-3.01min, 0% B in 3.01-3.5 min the flow rate was 1 ml/min. Mobile phase A was 0.04% trifluoroacetic acid in water, mobile phase B was 0.02% trifluoroacetic acid in acetonitrile. The column used for chromatography was a Luna LC- C18 50\*2 mm (5  $\mu$ m particles). Detection methods are diode array (DAD) and evaporative light scattering (ELSD) detection as well as positive electrospray ionization. MS range was 100-2000.

**[0601] Example 45. Preparation of A130**

**[0602]** To a solution of **PEG36-DMG** (117 mg, 0.05 mmol, 1.0 equiv) and **Int-21-S** (120 mg, 0.05 mmol, 1.0 equiv) in DCM (2.0 mL), HOBT (8.4 mg, 0.055 mmol, 1.2 equiv), EDC (10.6 mg, 0.055 mmol, 1.2 equiv) and DIPEA (0.3 mL, 0.15 mmol, 3 equiv) were added, the resulting mixture was stirred for 48 h at room temperature, additional 10 mL of DCM was added, washed with brine, dried with anhydrous  $\text{MgSO}_4$ . Filtered, concentrated, the crude product was purified with C18 reverse phase Combi Flash (Gold C18 gel column, eluted with 100% MeOH) collected 112 mg (47.4% yield) desired product.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  6.68 – 6.53 (m, 2H), 6.46 (s, 1H), 6.29 (s, 1H), 6.02 – 5.80 (m, 2H), 5.40 – 5.26 (m, 4H), 5.10 (q,  $J = 12.5$  Hz, 4H), 4.96 (s, 1H), 4.81 (d,  $J = 8.2$  Hz, 3H), 4.32 – 4.07 (m, 21H), 3.98 – 3.91 (m, 7H), 3.88 – 3.78 (m, 7H), 3.66 (d,  $J = 2.9$  Hz, 18H), 3.61 – 3.37 (m, 17H), 3.22 (d,  $J = 38.0$  Hz, 10H), 2.55 (q,  $J = 14.5$  Hz, 2H), 2.33 (s, 1H), 2.17 (s, 11H), 2.07 (s, 12H), 2.00 (d,  $J = 12.0$  Hz, 22H), 1.72 (s, 29H), 1.57 (t,  $J = 7.0$  Hz, 5H), 1.28 (s, 51H), 0.90 (t,  $J = 6.6$  Hz, 6H).

**[0603] Preparation of Compound 64**

**[0604]** The compound **A130** (112 mg, 0.024 mmol, 1 equiv) was dissolved in 3 mL of 7N NH<sub>3</sub> in MeOH (12 mg, 0.7 mmol, 30 equiv) solution and transferred to a pressure tube and stirred at room temperature for 24 h. Then reaction was concentrated via rotovap and purified by reverse phase chromatography (C18 column) with water and acetonitrile/methanol as the mobile phases. Final product was recovered as a white solid 76 mg with a yield of 75.9 %. <sup>1</sup>H NMR (400 MHz, MeOD) δ 4.49 (dd, *J* = 8.5, 2.4 Hz, 4H), 4.26 – 4.13 (m, 10H), 4.04 – 3.77 (m, 23H), 3.66 (s, 180H), 3.57 – 3.45 (m, 16H), 3.22 – 3.07 (m, 10H), 2.01 (s, 11H), 1.77 (dt, *J* = 13.3, 7.0 Hz, 6H), 1.58 (d, *J* = 7.1 Hz, 5H), 1.32 (s, 43H), 0.92 (t, *J* = 6.7 Hz, 6H). MS (MALDI\_TOF) *m/z* [M+Na]<sup>+</sup> calculated for 4079.34 ; found for 4079.84, *n*=35.

**[0605] Example 46. Preparation of Compound 23-A**

**[0606]** To a solution of Int-20 (321 mg, 1.0 equiv) in DMF (4.0 mL), HATU (44 mg, 1.2 equiv) was added, followed by the addition of DIPEA (0.5 mL), the mixture was stirred for 30 mins at room temperature, then Gal-Int-15-R (154 mg, 1.0 equiv) was added. The resulting mixture was stirred for 48 hours at room temperature, the solvents were removed under vacuum, the crude product was dissolved in DCM, washed with brine, dried with MgSO<sub>4</sub>, filtered, concentrated, the crude product was purified with Combi-flash (24 g gold normal phase column, 0-30% MeOH in DCM), collected 187 mg of A131, confirmed by H NMR.

**[0607]** To a solution of A131 (187 mg, 1.0 equiv) in THF (2.0 mL) and MeOH (2.0 mL), H<sub>2</sub>O (1.0 mL) was added, followed by addition of LiOH (50 mg). The mixture was stirred for 4 hours at room temperature, cooled with ice water, the pH was adjusted to 5-6, concentrated under vacuum, the crude product was dissolved in water, loaded on C-18 column, eluted with water in 10 mins, raised the ACN to 100% for around 20 mins, then ACN was replaced with 100% Methanol. The desired product was collected, confirmed by H NMR and MALDI. <sup>1</sup>H NMR (400 MHz, MeOD) δ 4.38 (dd, J = 8.5, 1.3 Hz, 3H), 4.21 – 3.72 (m, 20H), 3.66 (s, 190H),

3.62 – 3.40 (m, 20H), 3.15 (dt, J = 33.5, 7.1 Hz, 8H), 2.33 – 2.18 (m, 10H), 2.02 – 1.99 (m, 9H), 1.78 – 1.49 (m, 30H), 1.31 (s, 52H), 0.98 – 0.87 (m, 6H). MS (MALDI\_TOF) m/z [M+Na]<sup>+</sup> calculated for 3897.84 ; found for 3897.09.

**[0608] LNP Formulations.**

**[0609]** The compounds described and exemplified herein can be formulated into lipid nanoparticle (LNP) formulations. For example, the compounds can be formulated into LNP formulations as set out below:

**[0610] Procedure for LNP Formulation:**

- Thaw RNA stocks on ice, pipette up and down a few times to mix the solution.
- Thaw lipid stocks at room temperature, warm up lipid stocks at 37°C if necessary, vortex a few seconds to mix the solution.
- Prepare a 10mM citrate buffer in DI water using 100mM Citrate buffer pH 5 and keep on ice. If acetate buffer is used, the concentration of final buffer is 50 nM.
- Prepare lipid mixture using fresh EtOH.
- Prepare RNA mixture. The citrate (or acetate) buffer should be added right before formulation to minimize RNA degradation.
- Fill in the appropriate size syringes with the lipid and RNA mixtures. Use the lowest volume syringe possible to minimize loss.
- Insert Ignite cartridge into Ignite.
  - A dilution cartridge is used when adding a ligand to the LNPs. (Ligand/LNP ratio 1/1, flow rate = 12 mL/min)
- Formulate the LNP.
- Refrigerate the formulated LNPs for 15 to 60 mins. This is to allow LNPs to form.
- Meanwhile, set up the dialysis vessel with adequate volumes of 1X PBS and set it on the magnetic stirrer with a stirring bar.
- Hydrate appropriately sized dialysis cassettes (20K MWCO) for 5 min.
- Add LNPs into the dialysis cassettes and remove the air before closing the cassettes. Dialyze for 2 hours.
  - The dialysis volume should be at least 200 times more than the total LNP volume being dialyzed.
  - For LNP screen, add the LNPs to a Pierce 48-well microdialysis plate (1 mL, 20K MWCO) using 2.5 mL of 1X PBS. Change 1X PBS after one hour.

- While the LNPs are dialyzing, prepare the Amicon Ultra Centrifugal filter tubes (100K NMWL) by washing them with 1-5 mL (depending on size) of molecular grade water. This removes the glycerin used to stabilize the membrane.
- Once the dialysis is done, transfer LNPs into the previously cleaned Amicon centrifugation tubes and spin at 2,000 RCF (or G) for less than 30 min. Over concentrating LNPs should be avoided as much as possible, and centrifuging should be done in increments of between 3 – 5 minutes.
- Collect the concentrated LNPs using a syringe and pass them through a 0.2 µm sterile filter.

[0611] To analyze the LNP Formulation one can use the Stunner Protocol and/or Ribogreen Assay Protocol.

[0612] *In vivo Study*

[0613] The LNP formulations can also be evaluated via the activity of the payload, *e.g.*, mRNA and PEGRNA when dosed to animals. An exemplary dosing protocol is shown below.

[0614] **Animal Dosing Protocol:**

[0615] Prior to dosing on the day of study initiation, all animals will be weighed, and health status confirmed by visual inspection. Animals will be identified using a series of stripes made with non-toxic marker on the tail of each animal and recorded on the corresponding cage card.

[0616] Dose volumes will be calculated for each animal at a rate of 15 mL/kg. Mice will be dosed by a single, intravenous injection into the lateral tail vein. To facilitate IV placement, animals will be briefly placed under a heat lamp to dilate the tail vein.

[0617] Animals will be monitored for the entire injection period to ensure proper delivery of test article. Once the full dose is administered, the needle will be removed, and gauze applied to the injection site until hemostasis is confirmed. Following test article administration, animals will be returned to their respective cages and monitored for any adverse effects.

[0618] Following dosing of test materials, animals will be observed for changes to general health status and behavior. Abnormal observations will be recorded with date and time.

[0619] **Terminal Collection and Tissue Processing:**

[0620] All animals will be euthanized upon study completion by asphyxiation with carbon dioxide followed by cardiocentesis to ensure death.

[0621] Blood samples will be collected via cardiocentesis immediately following euthanasia. Whole blood will be collected into serum separator tubes and allowed to clot at room temperature for approximately 30 minutes and a maximum of 1 hour until processing for serum. Blood samples will then be stored in 96-well plates at -80 °C.

[0622] Immediately following blood collection, the livers will be collected from each mouse (gall bladders excised from livers) and each tissue placed into a separate pre-labeled 15mL jar. All samples will be flash frozen on liquid nitrogen. Any abnormal findings during the collection will be recorded. Frozen samples will be stored at -80°C.

[0623] gDNA will be isolated following MA150E-QuickExtract™ DNA Extraction Solution. 5mg of tissue will be weighted and 100 uL of QuickExtract enzyme will be added. Samples will be vortexed for 15 seconds. The tubes will be transferred to a heat block at 65°C and incubated for 6 minutes while shaking at 1000 rpm. The tube will then be transferred to a heat block at 98°C and incubated for 2 minutes, while shaking at 1000 rpm. The final gDNA extracts will be stored at -20°C.

[0624] **Example 47. LNP Formulation Evaluation**

[0625] To evaluate the delivery capabilities of LNP formulations, the loaded RNA (*e.g.*, PEgRNA) activity. For example, by measuring insertion-deletion mutations (indels%).

[0626] LNP-GalNAc compositions for in vivo studies:

[0627] GalNAc-based LNPs were prepared using a 5-component lipid system consisting of an ionizable lipid, a helper lipid (*e.g.*, DSPC, DOPE), cholesterol, DMG-PEG-2K and a GalNAc-PEG lipid. Each system contained a different GalNAc lipid. The mol % of ionizable lipid varied between 35-55%, the mol % of helper lipid varied between 10-20%, the mol % of cholesterol varied between 30-45%, the mol % of DMG-PEG-2K varied between 1-2.5% and the mol % of GalNAc-PEG lipid varied between 0.05-1%.

[0628] LNP Formulations Method

[0629] The lipid components were dissolved in 100% ethanol with the lipid component molar ratios described herein. The guide RNA(s) and mRNA were combined and dissolved in 50 mM acetate, pH 5.0, resulting in a concentration of total RNA cargo of approximately 0.1 mg/mL. The LNPs were formulated with an ionizable/RNA weight ratio of 15. The LNPs were formed by microfluidics mixing using a NanoAssemblr® Ignite™ from Precision Nanosystems, according to manufacturer's protocol. A 3:1 ratio of aqueous to organic solvent was maintained during mixing at differential flow rates. The LNPs were held for 30-60 min at 4°C before dialyzing them using a Slide-A-Lyzer™ Dialysis Cassette, 20K MWCO, for two hours at room temperature in 1X PBS. LNPs were then concentrated using an Amicon Ultra Centrifugal filter tube (100K NMWL). The resulting LNPs passed through a 0.2 µm sterile filter and stored at 4°C.

[0630] LNP Composition analysis:

[0631] Dynamic Light Scattering (DLS) was used to characterize the size and polydispersity index (PDI) of an LNP. The PDI represents the particle size distribution (around the mean) in a population; a uniform population will have a PDI of zero. 2  $\mu$ L of each sample were used to measure LNP size and PDI using the Unchained Labs Stunner instrument, without further dilution. A fluorescence-based assay (Ribogreen®, ThermoFisher Scientific) was used to determine total RNA concentration as well as free RNA. LNP samples were diluted with 1X TE buffer to determine free RNA or with 1X TE containing 0.2% Triton-X 100 to determine total RNA. Standard curves were prepared by utilizing the starting RNA solution used to make the compositions and diluted in 1X TE buffer + / - 0.2% Triton-X 100 buffer. Diluted RiboGreen® dye (according to the manufacturer's instructions) was added to each condition and incubate for 10-20 minutes at 37°C, in the absence of light. A SpectraMax i3x Microplate Reader was used to read the samples with excitation and emission wavelengths set to 488 and 525 nm, respectively. The total RNA and free RNA were calculated from the appropriate standard curve.

[0632] LNP delivery in vivo:

[0633] WT (Balb/C or C57Bl/6) or ApoE<sup>-/-</sup> female mice ranging from 5-8 weeks of age were used in each study. Prior to dosing, all animals were weighed, and health status was confirmed by visual inspection. Animals were identified using a series of stripes made with non-toxic marker on the tail of each animal and recorded on the corresponding cage card. Dose volumes were calculated for each animal at a rate of 10-20 mL/kg. Mice were dosed by a single, intravenous injection into the lateral tail vein. To facilitate IV placement, animals were briefly placed under a heat lamp to dilate the tail vein. Animals were monitored for the entire injection period to ensure proper delivery of test article. Once the full dose was administered, the needle was removed, and gauze was applied to the injection site until hemostasis was confirmed. Following test article administration, animals were returned to their respective cages and monitored periodically for any adverse effects for at least 24 h. All animals were euthanized upon study completion by asphyxiation with carbon dioxide followed by cardiocentesis to ensure death. Blood samples were collected via cardiocentesis immediately following euthanasia. Whole blood was collected into serum separator tubes and allowed to clot at room temperature for 30-60 minutes until processing for serum. Blood samples were stored in 96-well plates at -80 °C. Immediately following blood collection, the livers were collected from each mouse (gall bladders excised from livers), flashed frozen in liquid nitrogen, and stored at -80 °C. Any abnormal findings during the collection were recorded. Tissue samples were processed to measure editing by Next-Generation Sequencing

(NGS). gDNA was isolated according to MA150E-QuickExtract™ DNA Extraction Solution protocol. 5mg of tissue was weighted and 100  $\mu$ L of QuickExtract enzyme was added. Samples were vortexed for 15 seconds. The tubes were transferred to a heat block at 65 °C and incubated for 6 minutes while shaking at 1000 rpm. The tubes were then be transferred to a heat block at 98°C and incubated for 2 minutes, while shaking at 1000 rpm. The final gDNA extracts was stored at -20°C. The first PCR reaction on the gDNA used paired primers (Forward primer- CAGCAGGTTTGGAGTCAGC (SEQ ID NO.: 1 and reverse primer- ACTCTAGTTCTCACATCTGGA (SEQ ID NO.: 2) and adapters (ACACTCTTTCCCTACACGACGCTCTTCCGATCTT (SEQ ID NO.: 3), TGGAGTTCAGACGTGTGCTCTTCCGATCT (SEQ ID NO.: 4)) in a 20  $\mu$ L reaction with Q5 Hot Start High-Fidelity 2X Master Mix (NEB). This reaction was run on a thermocycler at 98°C for 30 seconds, then 98°C for 10 seconds, 63°C for 20 seconds, 72°C for 30 seconds for 30 cycles total, followed by last step of 72°C for 2 minutes. PCR1 reactions were stored at 4°C. Following the first PCR reaction, a second PCR reaction was set up with barcoded primers, 1  $\mu$ L of the first PCR reaction, and Q5 Hot Start High-Fidelity 2X Master Mix (NEB), which underwent 7 cycles of amplification. The second PCR reactions were pooled and run on a 2% agarose E-gel for DNA purification. The DNA samples were then run on the Miseq for sequencing. NGS results were analyzed using JupyterHub notebooks and CRISPResso to measure percent correction and percent of indels for each mutation in the Rhodopsin gene. The percent correction and percent indel values were determined based on the percentage of sequencing reads.

**[0634]** FIG. 1 shows delivery capabilities of various LNP formulations that contain various GalNAc lipids by measuring percent indel values in a PE2 nuclease TTR guide system (1 mg/kg total RNA).

**[0635]** Positive Control 1 is (R)-2,3-bis(octadecyloxy)propyl ((S)-127-(((2R,3R,4R,5R,6R)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-116-(4-(5-(((2R,3R,4R,5R,6R)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)pentanamido)butyl)-118-(3-(5-(((2R,3R,4R,5R,6R)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)pentanamido)propyl)-114,117,123-trioxo-3,6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90,93,96,99,102,105,108,111-heptatriacontaoxa-115,118,122-triazaheptacosahexyl)carbamate.

**[0636]** Positive Control 2 is ammonium 155-(((2R,3R,4R,5R,6R)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-144-(2-((2-(2-(((2R,3R,4R,5R,6R)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-

yl)oxy)ethoxy)ethoxy)ethyl)amino)-2-oxoethyl)-143-((2-(2-(2-(((2R,3R,4R,5R,6R)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethoxy)ethyl)carbamoyl)-4,137,146-trioxo-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51,54,57,60,63,66,69,72,75,78,81,84,87,90,93,96,99,102,105,108,111,114,117,120,123,126,129,132,135,150,153-hexatetracontaoxa-3,138,144,147-tetraazapentapentacontahectyl ((R)-2,3-bis(stearoyloxy)propyl) phosphate.

**[0637] Example 48. LNP Formulation Evaluation**

**[0638]** The delivery capabilities of LNP formulations were also shown via in vivo prime editing efficiency of various prime editing systems when delivered with different LNP formulations that contain various GalNAc lipids disclosed herein.

**[0639]** The experiment was conducted in a similar manner to the experiment described in Example 47.

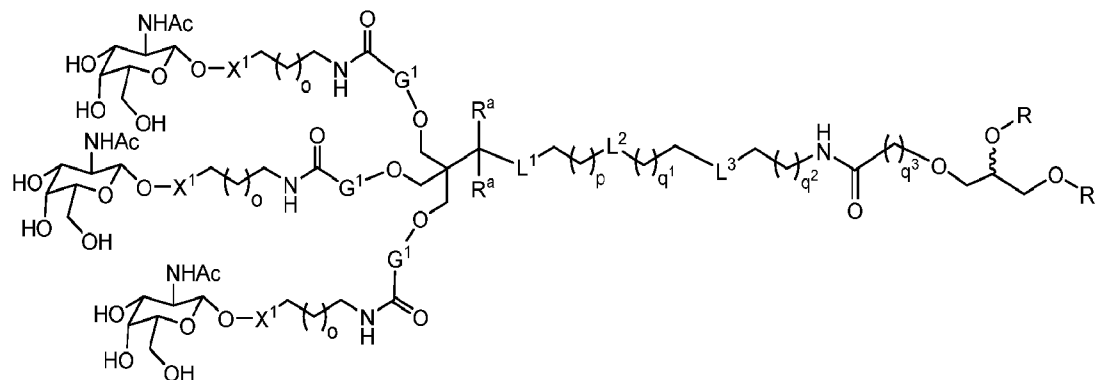
**[0640]** FIG. 2 shows in vivo prime editing efficiency of Pcsk9 PE2 when delivered via LNP formulations that contain GalNAc lipids Compound 29 or Compound 83 to ApoE KO female mice at various doses.

**[0641]** Prime editing systems, including PE2 and PE3, are described in Anzalone, A.V., Randolph, P.B., Davis, J.R. *et al.*, “Search-and-replace genome editing without double-strand breaks or donor DNA,” *Nature* 576, 149–157 (2019); and in International PCT Application Publication No.: WO 2020/191234A1, the contents of each of which are herein incorporated by reference in their entirety.

### **OTHER EMBODIMENTS**

**[0642]** The above examples are to be understood as illustrative examples. Further examples are envisaged, which include combinations of features as indicated in the following list, which contains various envisaged claim dependencies for the claims originally filed with this application. Hence, in addition to the description above, this list provides basis for examples having a combination of features of claims filed herewith. Hence, the following list also discloses embodiments of the invention.

[0643] Embodiment 1. A compound of Formula I:



**Formula I**

wherein,

each  $X^1$  is independently  $-(CH_2)_r-$ ,  $-(CH_2CH_2O)_s-$ ,  $-(OCH_2CH_2)_s-$ ,  $-((CH_2)_m-C(O)NH)-$ , or  $-(C(O)NH-(CH_2)_m)-$ ,

each subscript “m” is independently 1-5;

each subscript “o” is independently 1-5;

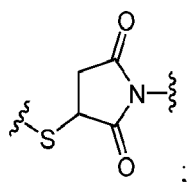
each subscript “r” is independently 1-10;

each subscript “s” is independently 1-5;

each  $G^1$  is independently a  $C_1$ - $C_6$  substituted or unsubstituted alkylene;

each  $R^a$  is independently H or a  $C_1$ - $C_6$  substituted or unsubstituted alkyl;

$L^1$  is  $-NHC(O)-$ ,  $-C(O)NH-$ ,  $-NHC(O)O-$ ,  $-OC(O)NH-$ ,  $-NHC(O)NH-$ ,  $-S-S-$ , or



subscript “p” is independently 1-5;

$L^2$  is  $-O-(CH_2CH_2O)_n-$ ,  $-(CH_2CH_2O)_n-O-$ , or  $-(OCH_2CH_2O)_n-$ ,

each subscript “n” is independently 1-200;

subscript “q<sup>1</sup>” is independently 0-5;

subscript “q<sup>2</sup>” is independently 0-5;

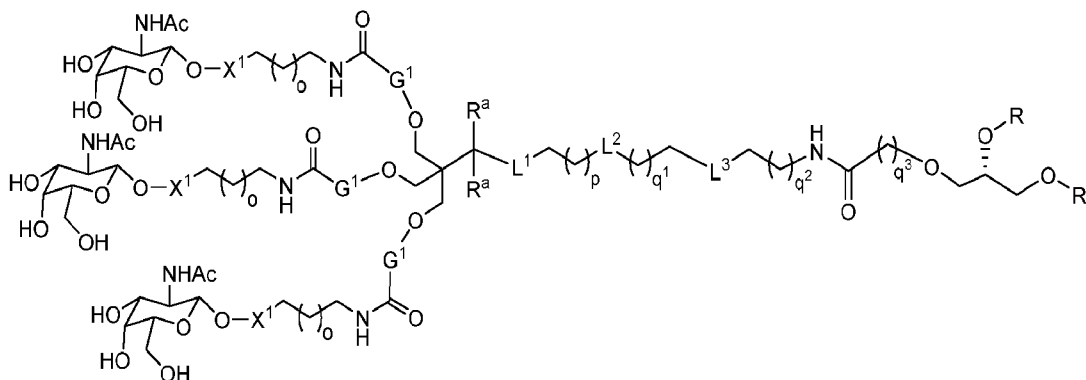
$L^3$  is absent,  $-NHC(O)-$ , or  $-C(O)NH-$ ;

subscript “q<sup>3</sup>” is independently 0-5;

each R is independently a  $C_{12}$ - $C_{20}$  substituted or unsubstituted alkyl; and

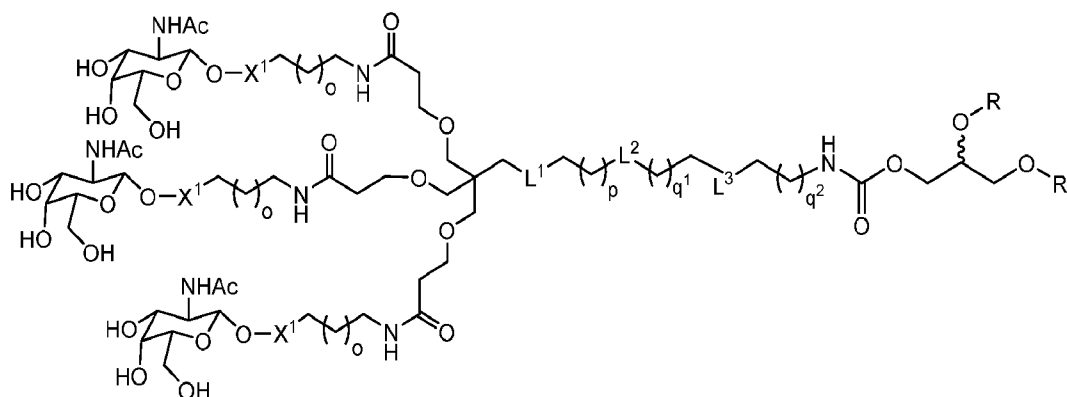
the “~” indicates an *R* configuration or an *S* configuration.

**[0644]** Embodiment 2. The compound of Embodiment 1, wherein the compound of Formula I is a compound of Formula I-a:



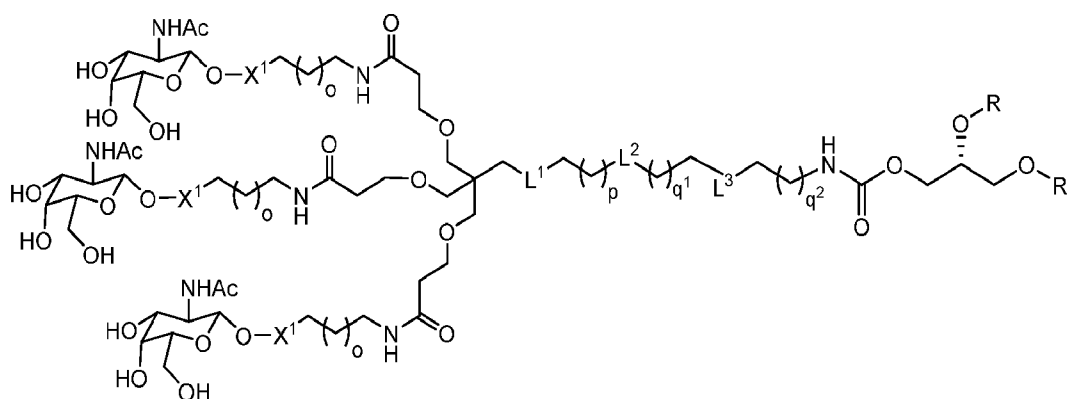
### Formula I-a

**[0645]** Embodiment 3. The compound of Embodiment 1, wherein the compound of Formula I is a compound of Formula I-b:



**Formula I-b.**

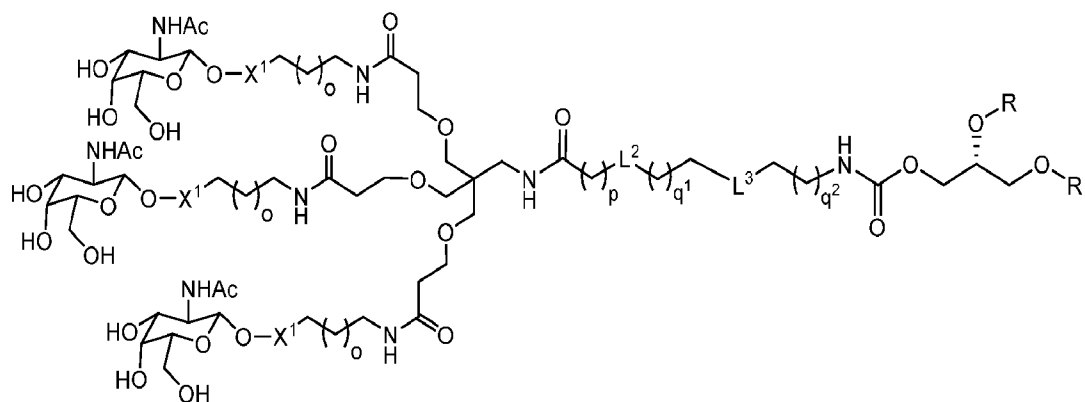
**[0646]** Embodiment 4. The compound of Embodiment 1, wherein the compound of Formula I is a compound of Formula I-c:



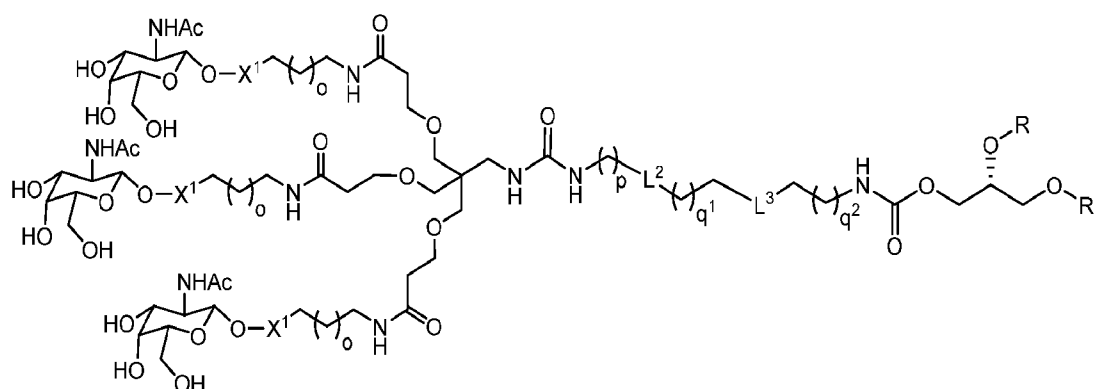
**Formula I-c.**

**[0647]** Embodiment 5. The compound of any one of Embodiments 1-4, wherein L<sup>1</sup> is –NHC(O).

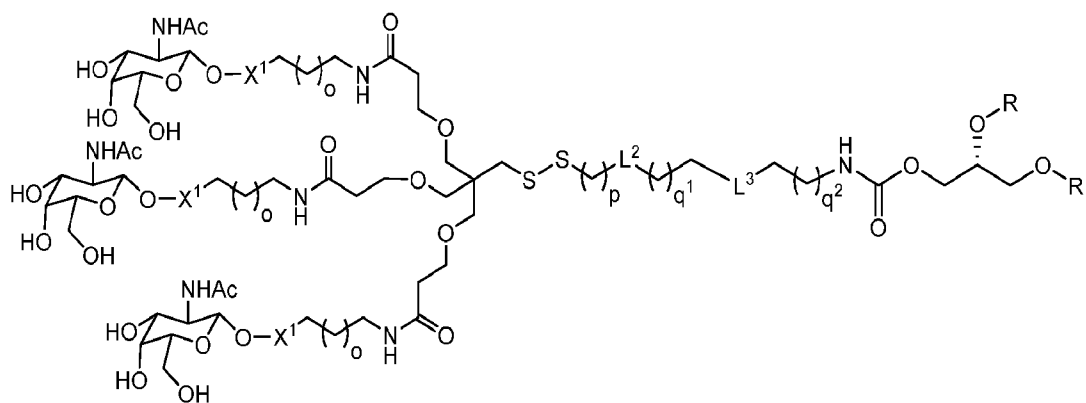
- [0648] Embodiment 6. The compound of any one of Embodiments 1-4, wherein  $L^1$  is  $-NHC(O)NH-$ .
- [0649] Embodiment 7. The compound of any one of Embodiments 1-4, wherein  $L^1$  is  $-S-S-$ .
- [0650] Embodiment 8. The compound of any one of Embodiments 1-4, wherein  $L^1$  is  $-OC(O)NH-$ .
- [0651] Embodiment 9. The compound of any one of Embodiments 1-8, wherein each  $X^1$  is  $-((CH_2)_m-C(O)NH)$ .
- [0652] Embodiment 10. The compound of any one of Embodiments 1-8, wherein each  $X^1$  is  $-(CH_2CH_2O)_s$ .
- [0653] Embodiment 11. The compound of any one of Embodiments 1-8, wherein each  $X^1$  is  $-(CH_2)_r-$  and each subscript "r" is 5.
- [0654] Embodiment 12. The compound of any one of Embodiments 1-11, wherein each subscript "o" is 1.
- [0655] Embodiment 13. The compound of any one of Embodiments 1-12, wherein  $L^2$  is  $-O-(CH_2CH_2O)_n-$ .
- [0656] Embodiment 14. The compound of any one of Embodiments 1-12, wherein  $L^2$  is  $-(OCH_2CH_2O)_n-$ .
- [0657] Embodiment 15. The compound of Embodiment 13 or 14, wherein each subscript "n" is 100 to 200.
- [0658] Embodiment 16. The compound of any one of Embodiments 1-15, wherein  $L^3$  is absent.
- [0659] Embodiment 17. The compound of any one of Embodiments 1-15, wherein  $L^3$  is present and is  $-NHC(O)-$ .
- [0660] Embodiment 18. The compound of any one of Embodiments 1-17, wherein each R is independently a  $C_{14}$ - $C_{18}$  unsubstituted alkyl.
- [0661] Embodiment 19. The compound of any one of Embodiments 1-18, wherein the compound of Formula I is any one of a compound of Formula I-c-1 to I-c-4:



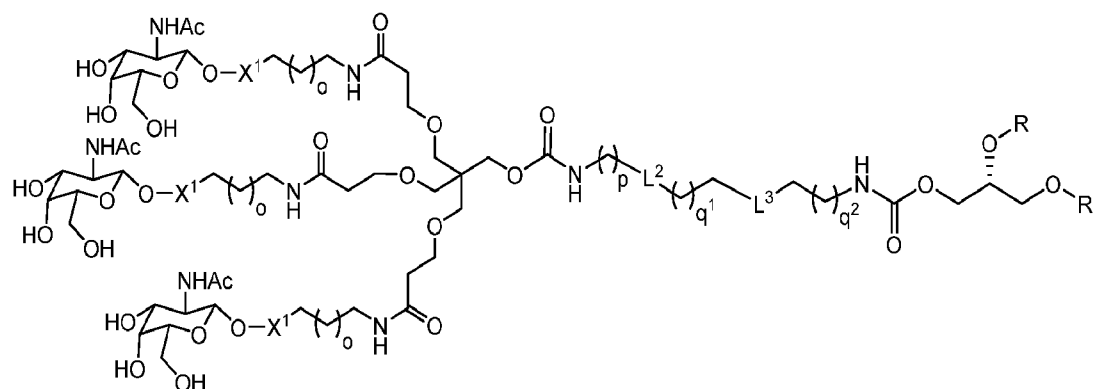
**Formula I-c-1,**



**Formula I-c-2,**

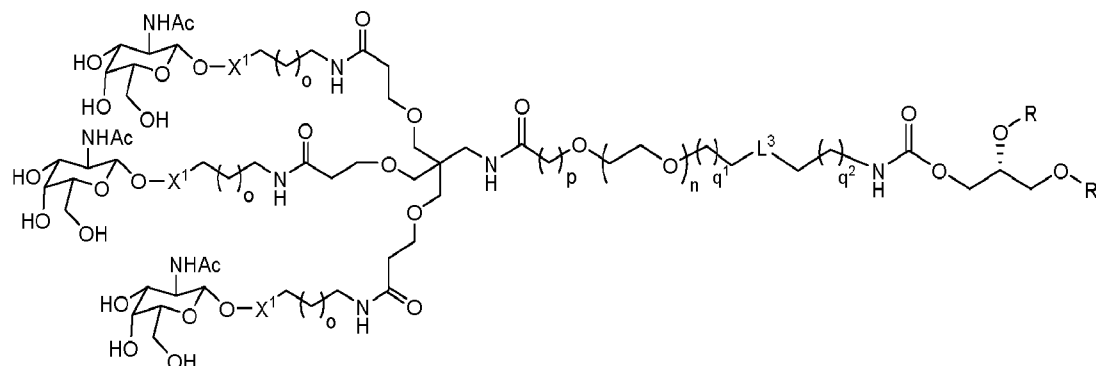


**Formula I-c-3, or**

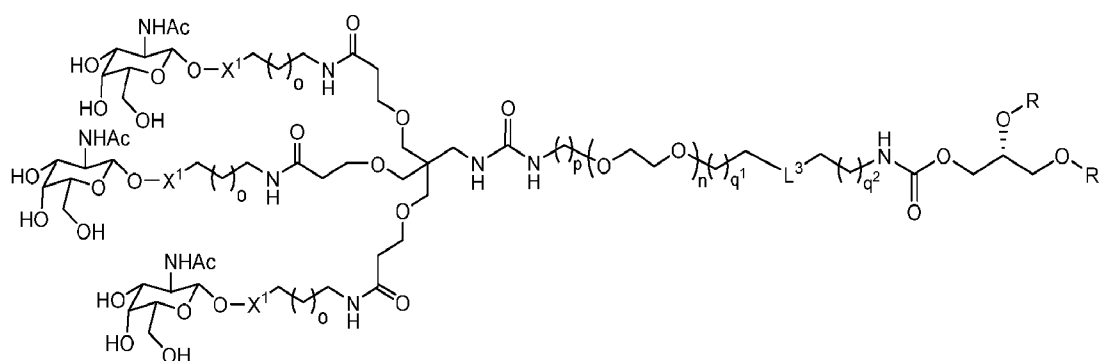


**Formula I-c-4.**

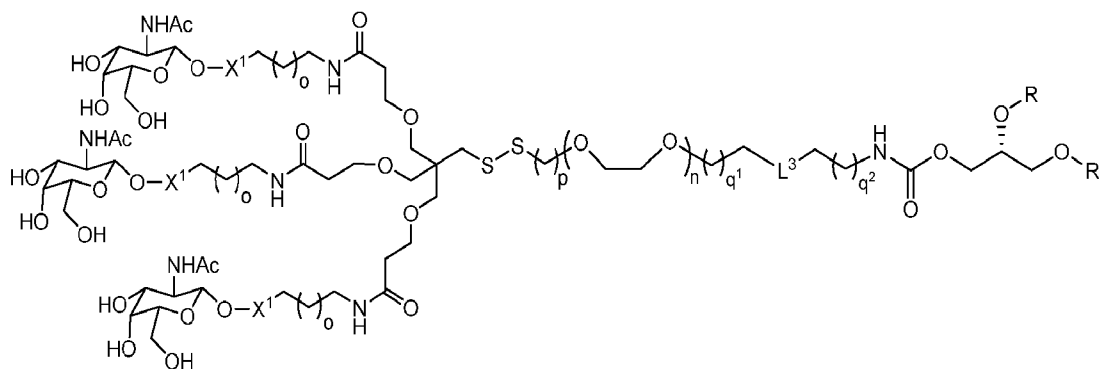
[0662] Embodiment 20. A compound selected from the group consisting of Formula I-c-5, I-c-6, I-c-7, and I-c-8:



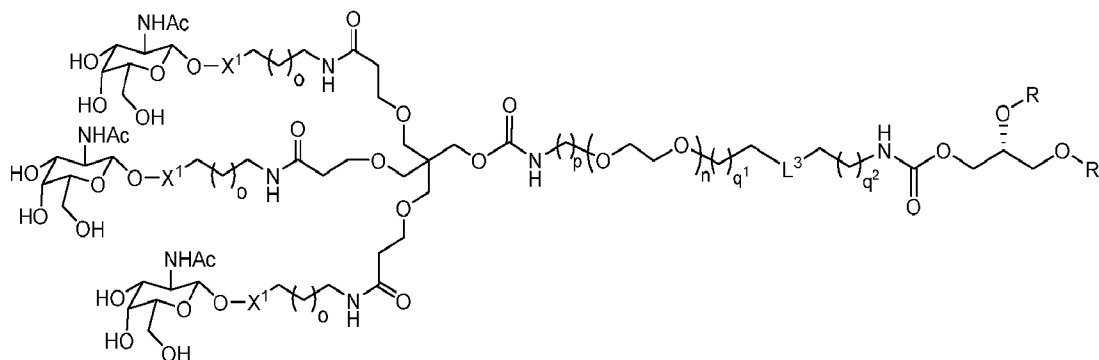
**Formula I-c-5,**



**Formula I-c-6,**



**Formula I-c-7,**



**Formula I-c-8**

wherein,

each  $X^1$  is independently  $-(CH_2)_r-$ ,  $-(CH_2CH_2O)_s-$ ,  $-(OCH_2CH_2)_s-$ ,  $-((CH_2)_m-C(O)NH)-$ , or  $-(C(O)NH-(CH_2)_m)-$ ;

each subscript “m” is independently 1-5;

each subscript “s” is independently 1-5;

each subscript “o” is independently 1-5;

each subscript “r” is independently 1-10;

each subscript “p” is independently 1-5;

each subscript “n” is independently 1-200;

each subscript “q<sup>1</sup>” is independently 0-5;

each subscript “q<sup>2</sup>” is independently 0-5;

each  $L^3$  is independently absent,  $-NHC(O)-$ , or  $-C(O)NH-$ ; and

each R is independently a C<sub>12</sub>-C<sub>20</sub> substituted or unsubstituted alkyl.

**[0663]** Embodiment 21. The compound of Embodiment 20, wherein each  $X^1$  is  $-((CH_2)_m-C(O)NH)-$ , each subscript “m” is independently 2, and each subscript “o” is independently 1.

**[0664]** Embodiment 22. The compound of Embodiment 20, wherein each  $X^1$  is  $-(CH_2CH_2O)_s-$ , each subscript “s” is independently 2, and each subscript “o” is independently 1.

**[0665]** Embodiment 23. The compound of Embodiment 20, wherein the compound is a compound of Formula I-c-7 and, optionally, each  $X^1$  is  $-(CH_2)_r-$ , each subscript “r” is independently 5, and each subscript “o” is 1.

**[0666]** Embodiment 24. The compound of Embodiment 20, wherein the compound is a compound of Formula I-c-5,  $L^2$  is  $-O-(CH_2CH_2O)_n-$ , and subscript “n” is 100 to 200.

**[0667]** Embodiment 25. The compound of Embodiment 20, wherein the compound is a compound of Formula I-c-6, I-c-7, or I-c-8, each  $L^2$  is  $-(OCH_2CH_2O)_n-$  and each subscript “n” is 100 to 200.

**[0668]** Embodiment 26. The compound of any one of Embodiments 20-25, wherein  $L^3$  is absent.

**[0669]** Embodiment 27. The compound of Embodiment 26, wherein in Formula I-c-4, subscript “p” is 4, subscript “q<sup>1</sup>” is 0, and subscript “q<sup>2</sup>” is 0.

**[0670]** Embodiment 28. The compound of Embodiment 26, wherein in Formulas I-c-6, I-c-7, and I-c-8, each subscript “p” is 2, subscript “q<sup>1</sup>” is 0, and subscript “q<sup>2</sup>” is 0.

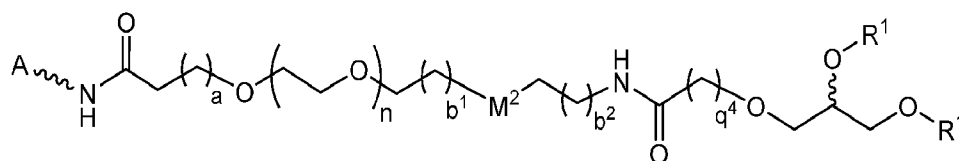
[0671] Embodiment 29. The compound of any one of Embodiments 20-25, wherein  $L^3$  is present and is  $-NHC(O)-$ .

[0672] Embodiment 30. The compound of Embodiment 29, wherein in Formula I-a-4, subscript “p” is 4, subscript “q<sup>1</sup>” is 1 and subscript “q<sup>2</sup>” is 4.

[0673] Embodiment 31. The compound of any one of Embodiments 20-30, wherein each R is independently a C<sub>14</sub>-C<sub>18</sub> unsubstituted alkyl.

[0674] Embodiment 32. A compound selected from the group consisting of compounds shown in Table 1.

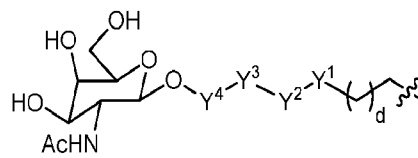
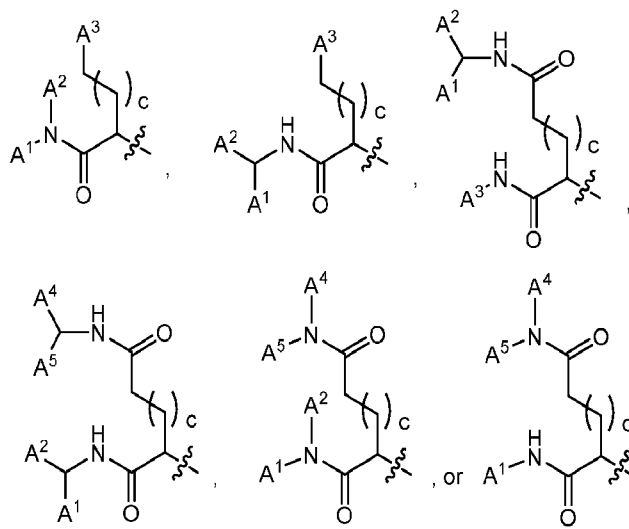
[0675] Embodiment 33. A compound of Formula II:



**Formula II**

wherein:

A is:



each A<sup>1</sup>, A<sup>2</sup>, A<sup>3</sup>, A<sup>4</sup>, and A<sup>5</sup> is independently

each Y<sup>1</sup> is independently  $-NH-$ ,  $-NHC(O)O-$ ,  $-OC(O)NH-$ ,  $-NHC(O)-$ , or  $-C(O)NH-$ ;

each Y<sup>2</sup> is independently absent,  $-(CH_2)_t-$ ,  $-(CH_2CH_2O)_t-$ , or  $-(OCH_2CH_2)_t-$ ;

each subscript “t” is independently 1-5;

each Y<sup>3</sup> is independently absent,  $-NHC(O)-$ , or  $-C(O)NH-$ ;

each Y<sup>4</sup> is independently absent or  $-(CH_2)_t-$ ;

each subscript “r” is independently 1-10;

each subscript “d” is independently 0-5;

each subscript “c” is independently 0-5;

subscript “n” is independently 1-200;

subscript “a” is 1-5;

subscript “b<sup>1</sup>” is independently 0-5;

M<sup>2</sup> is absent, -NHC(O)-, or -C(O)NH-;

subscript “b<sup>2</sup>” is independently 0-5;

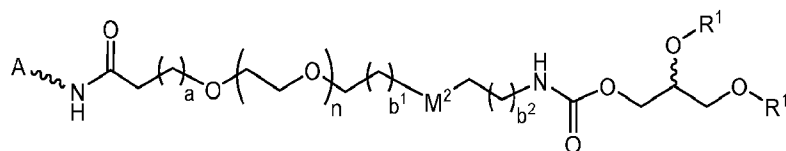
subscript “q<sup>4</sup>” is independently 0-6;

each R<sup>1</sup> is independently a C<sub>12</sub>-C<sub>20</sub> substituted or unsubstituted alkyl; and

each of the “*w*” bonds independently indicate an *R* configuration or an *S* configuration;

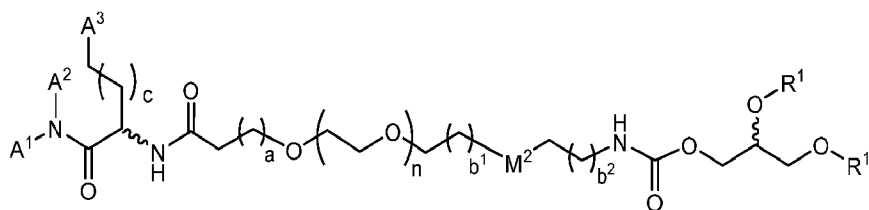
with the proviso that at least one of Y<sup>2</sup>, Y<sup>3</sup> and Y<sup>4</sup> is present.

[0676] Embodiment 34. The compound of Embodiment 33, wherein the compound of Formula II is a compound of Formula II-a:

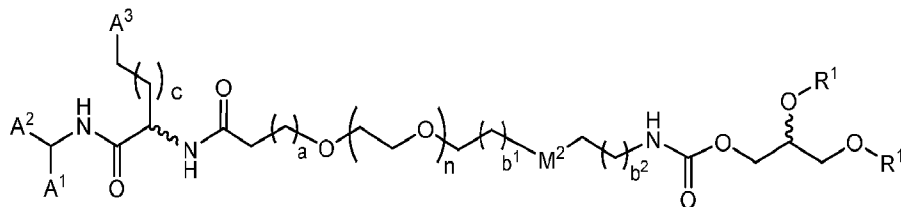


**Formula II-a.**

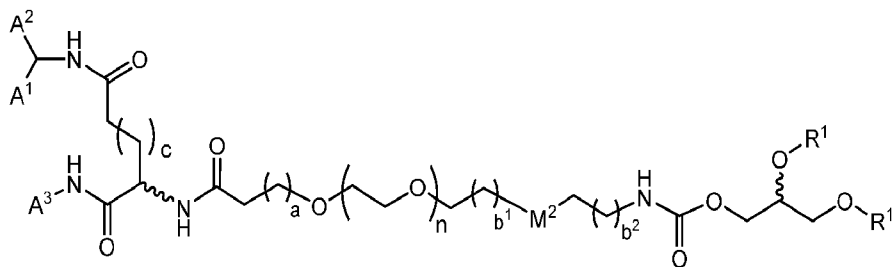
[0677] Embodiment 35. The compound of Embodiment 33 or 34, wherein the compound of Formula II is a compound of Formula II-a-1, Formula II-a-2, Formula II-a-3, Formula II-a-4, Formula II-a-5, or Formula II-a-6:



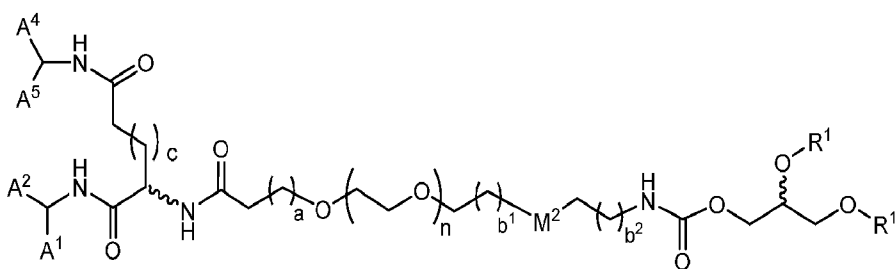
**Formula II-a-1,**



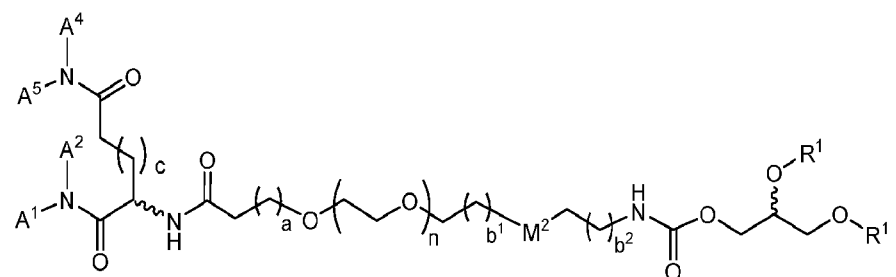
**Formula II-a-2,**



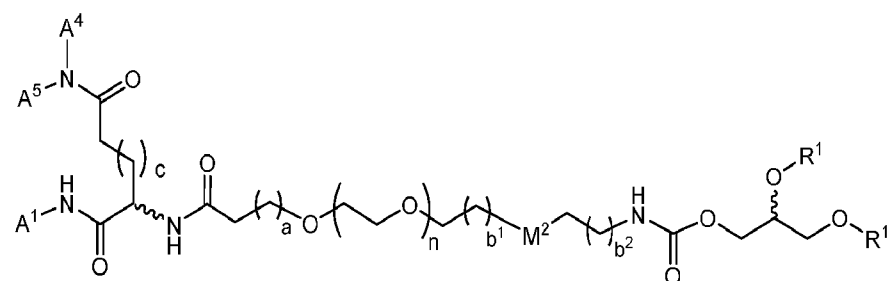
**Formula II-a-3,**



**Formula II-a-4,**



**Formula II-a-5, or**



**Formula II-a-6.**

- [0678]** Embodiment 36. The compound of any one of Embodiments 33-35, wherein  $A^1$ ,  $A^2$ , and  $A^3$  are the same.
- [0679]** Embodiment 37. The compound of any one of Embodiments 33-35, wherein  $A^1$  and  $A^2$  are different from  $A^3$ .
- [0680]** Embodiment 38. The compound of any one of Embodiments 33-35, wherein  $A^1$ ,  $A^2$ ,  $A^4$ , and  $A^5$  are the same.
- [0681]** Embodiment 39. The compound of any one of Embodiments 33-35, wherein  $A^1$ ,  $A^2$ , and  $A^5$  are the same.

[0682] Embodiment 40. The compound of any one of Embodiments 33-39, wherein each of  $Y^1$  and  $Y^4$  are present and each of  $Y^2$  and  $Y^3$  are absent.

[0683] Embodiment 41. The compound of Embodiment 40, wherein each  $Y^1$  is  $-C(O)NH-$  and each  $Y^4$  is  $-(CH_2)_r-$ .

[0684] Embodiment 42. The compound of Embodiment 40, wherein each  $Y^1$  is  $-OC(O)NH-$  and each  $Y^4$  is  $-(CH_2)_r-$ .

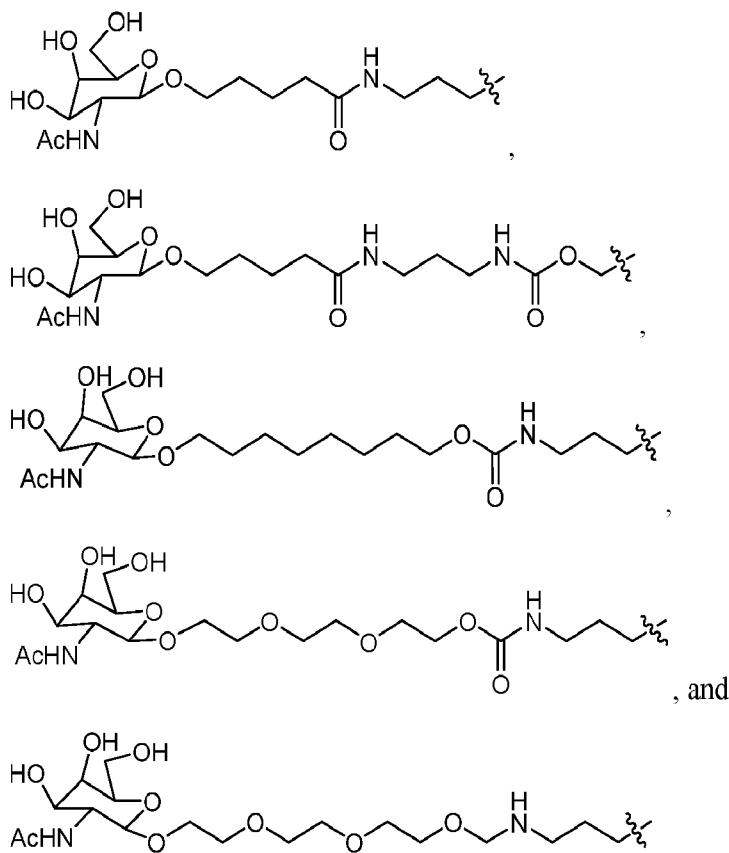
[0685] Embodiment 43. The compound of any one of Embodiments 33-39, wherein each of  $Y^1$ ,  $Y^2$ ,  $Y^3$ , and  $Y^4$  are present.

[0686] Embodiment 44. The compound of Embodiment 43, wherein each  $Y^1$  is independently  $-NHC(O)O-$ ; each  $Y^2$  is independently  $-(CH_2)_r-$ ; each  $Y^3$  is independently  $-C(O)NH-$ ; and each  $Y^4$  is independently  $-(CH_2)_r-$ .

[0687] Embodiment 45. The compound of any one of Embodiments 33-39, wherein each of  $Y^1$  and  $Y^2$  are present and each of  $Y^3$  and  $Y^4$  are absent.

[0688] Embodiment 46. The compound of Embodiment 45, wherein each  $Y^1$  is independently  $-OC(O)NH-$  and each  $Y^2$  is independently  $-(CH_2CH_2O)_t-$ .

[0689] Embodiment 47. The compound of any one of Embodiments 33-46, wherein each of  $A^1$ ,  $A^2$ ,  $A^3$ ,  $A^4$ , and  $A^5$  is selected from the group consisting of:

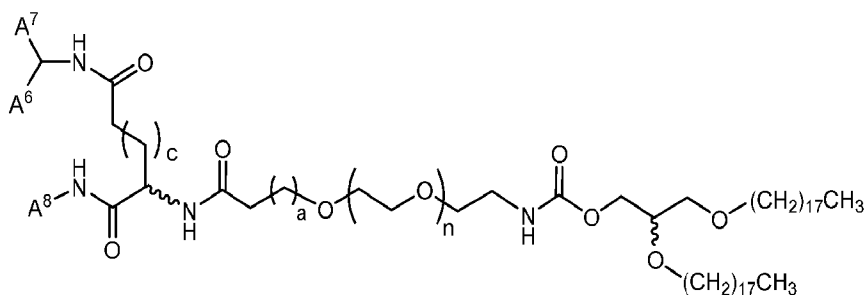


[0690] Embodiment 48. The compound of any one of Embodiments 33-47, wherein  $M^2$  is absent.

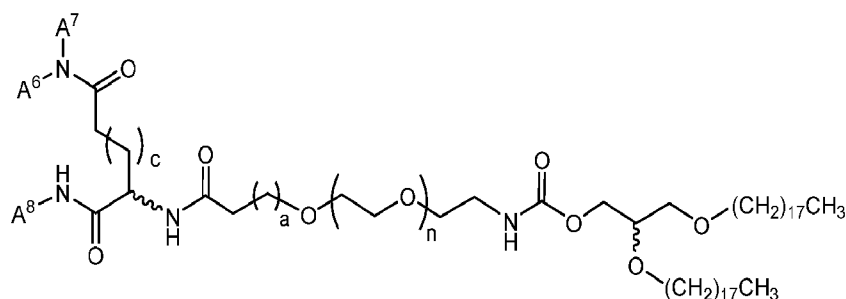
[0691] Embodiment 49. The compound of any one of Embodiments 33-47, wherein  $M^2$  is  $-\text{NHC(O)}-$ .

[0692] Embodiment 50. The compound of any one of Embodiments 33-49, wherein each  $R^1$  is independently a  $C_{14}$ - $C_{18}$  unsubstituted alkyl.

[0693] Embodiment 51. A compound of Formula III or Formula IV:

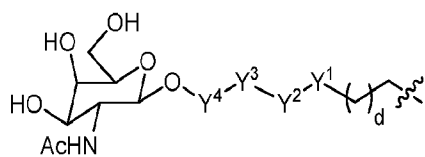


**Formula III, or**



**Formula IV**

wherein,



each  $A^6$ ,  $A^7$ , and  $A^8$  is independently

each  $Y^1$  is independently absent,  $-\text{NHC(O)}O-$ , or  $-\text{OC(O)}\text{NH}-$ ;

each  $Y^2$  and  $Y^4$  is independently  $-(\text{CH}_2)_r-$ ;

each subscript " $r$ " is independently 1-10;

each  $Y^3$  is independently absent,  $-\text{NHC(O)}-$ , or  $-\text{C(O)}\text{NH}-$ ;

each subscript " $d$ " is independently 1-5;

each subscript " $c$ " is independently 1-5;

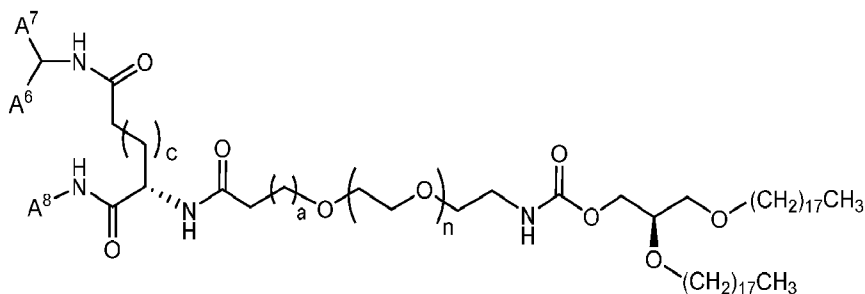
subscript " $a$ " is 1-5;

subscript " $n$ " is independently 10-100; and

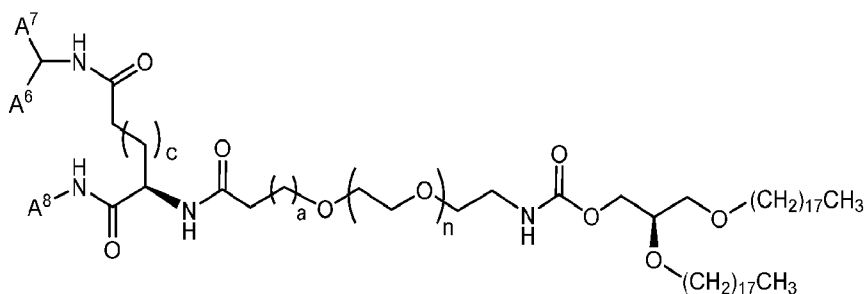
each of the “*wavy*” bonds independently indicate an *R* configuration or an *S* configuration;

with the proviso that at least one of Y<sup>1</sup> or Y<sup>3</sup> is present.

[0694] Embodiment 52. The compound of Embodiment 51, wherein the compound of Formula III is a compound of Formula III-a or Formula III-b:

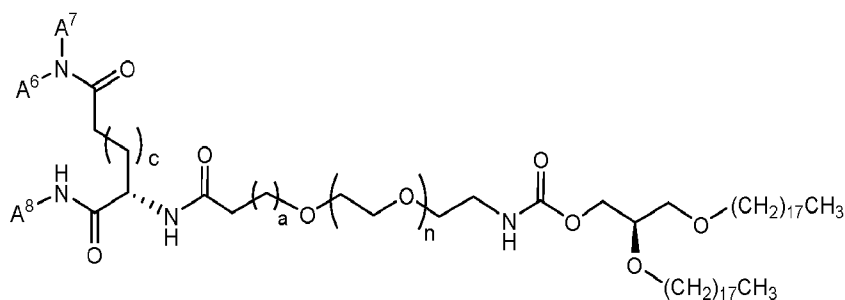


**Formula III-a, or**

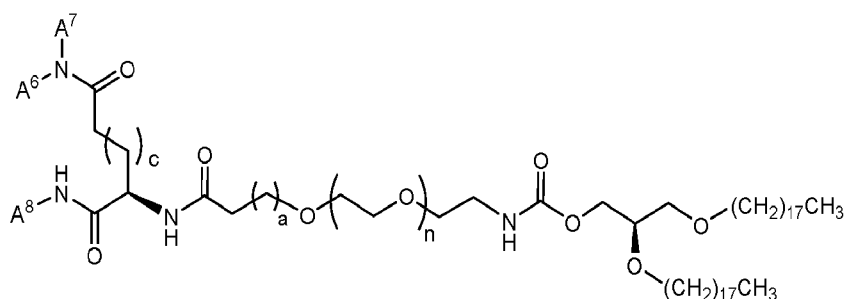


**Formula III-b.**

[0695] Embodiment 53. The compound of Embodiment 51, wherein the compound of Formula IV is a compound of Formula IV-a or Formula IV-b:



**Formula IV-a, or**



**Formula IV-b.**

[0696] Embodiment 54. The compound of any one of Embodiments 51-53, wherein subscript “a” is 3.

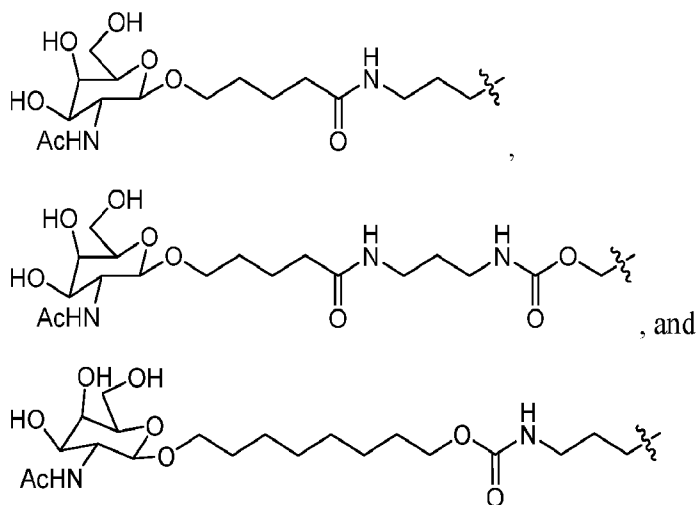
[0697] Embodiment 55. The compound of any one of Embodiments 51-53, wherein subscript “a” is 1.

[0698] Embodiment 56. The compound of any one of Embodiments 51-55, wherein subscript “n” is independently 35-46.

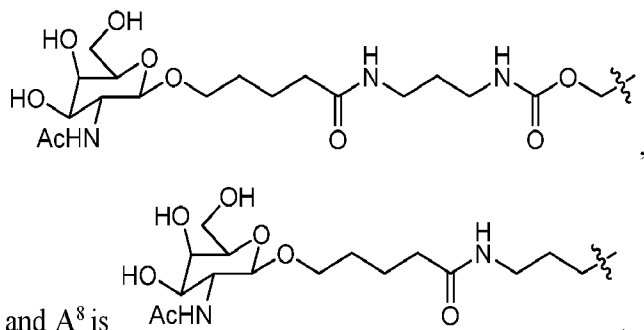
[0699] Embodiment 57. The compound of any one of Embodiments 51-55, wherein subscript “n” is independently between 34-36, in a range of 34-36, between 44-46, or in a range of 44-46.

[0700] Embodiment 58. The compound of any one of Embodiments 51-57, wherein subscript “c” is 1.

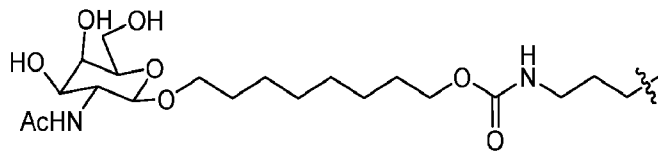
[0701] Embodiment 59. The compound of any one of Embodiments 51-58, wherein each of A<sup>6</sup>, A<sup>7</sup>, and A<sup>8</sup> is independently selected from the group consisting of:



[0702] Embodiment 60. The compound of any one of Embodiments 51-58, wherein A<sup>6</sup> and A<sup>7</sup> are each independently



[0703] Embodiment 61. The compound of any one of Embodiments 51-58, wherein A<sup>6</sup>, A<sup>7</sup>, and A<sup>8</sup> are each



[0704] Embodiment 62. A compound selected from the group consisting of compounds of Table 2.

[0705] Embodiment 63. A pharmaceutically acceptable salt of a compound of any one of Embodiments 1-62.

[0706] Embodiment 64. A lipid nanoparticle (LNP) composition comprising a lipid that comprises a compound of any one of Embodiments 1-62.

[0707] Embodiment 65. A lipid nanoparticle (LNP) composition comprising:

a lipid that comprises a compound of any one of Embodiments 1-62;

a cationic lipid;

a phospholipid;

a PEG-containing lipid; and

a sterol.

[0708] Embodiment 66. The LNP composition of Embodiment 65, further comprising a prime editor comprising a DNA binding domain and a DNA polymerase domain, or one or more polynucleotides encoding the prime editor.

[0709] Embodiment 67. The LNP composition of Embodiment 65 or 66, further comprising a prime editing guide RNA (PEgRNA) or a polynucleotide encoding the PEgRNA.

[0710] Embodiment 68. The LNP composition of any one of Embodiments 65-67, further comprising a nick guide RNA (ngRNA) or a polynucleotide encoding the ngRNA.

[0711] Embodiment 69. The LNP composition of any one of Embodiments 65-68, further comprising a second PEgRNA or a polynucleotide encoding the second PEgRNA.

[0712] Embodiment 70. A pharmaceutical composition comprising the LNP composition of any one of Embodiments 65-69.

[0713] Embodiment 71. A method for delivering polynucleotides into a target cell comprising:

introducing into the target cell at least one lipid nanoparticle comprising a lipid that comprises the compound of any one of Embodiments 1-62 and one or more polynucleotides.

[0714] Embodiment 72. A method for delivering a prime editing system into a target cell comprising:

introducing into the target cell at least one lipid nanoparticle comprising a lipid that comprises the compound of any one of Embodiments 1-62 and one or more components of a prime editing system.

[0715] Embodiment 73. The method of Embodiment 72, wherein the one or more components of a prime editing system comprises a construct encoding prime editor components.

[0716] Embodiment 74. The method of Embodiment 72, wherein the one or more components of a prime editing system comprises an mRNA encoding prime editor components.

[0717] Embodiment 75. The method of Embodiment 73 or 74, wherein the prime editor components comprise a prime editing guide RNA (PEgRNA), and optionally a nick guide RNA (ngRNA).

[0718] Embodiment 76. The method of Embodiment 73 or 74, wherein the prime editor components comprise a PEgRNA pair, wherein the PEgRNA comprises a first PEgRNA and/or a second PEgRNA.

[0719] Embodiment 77. The method of Embodiment 72, wherein the one or more components of a prime editing system comprises a construct encoding a prime editor fusion protein or an mRNA encoding a prime editor fusion protein.

[0720] Embodiment 78. The method of Embodiment 72, wherein the one or more components of a prime editing system comprises one or more constructs encoding one or more of a prime editor fusion protein, PEgRNA, ngRNA, or a PEgRNA pair.

[0721] Embodiment 79. The method of Embodiment 72, wherein the one or more components of a prime editing system comprises a prime editor fusion protein, a PEgRNA, a ngRNA, or a PEgRNA pair.

[0722] Embodiment 80. The LNP composition of any one of Embodiments 65-69, wherein the LNP composition is formulated to deliver the at least one polynucleotide or one or more components of the prime editing system to target cells.

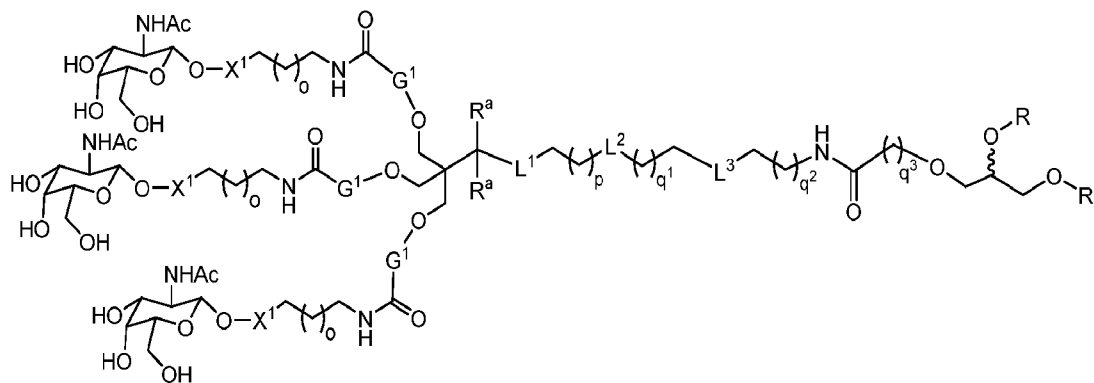
[0723] Embodiment 81. The method of any one of Embodiments 71-79 or the LNP composition of Embodiment 80, wherein the target cells are or comprise human cells, mammalian cells, liver cells, bone marrow cells, lung cells, eye cells, muscle cells, or cells in the central nervous system.

[0724] It is to be understood that while the disclosure has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not

limit the scope of the disclosure, which is defined by the scope of the appended claims.  
Other aspects, advantages, and modifications are within the scope of the following claims.

## WHAT IS CLAIMED IS

1. A compound of Formula I:



**Formula I**

wherein,

each X<sup>1</sup> is independently  $-(CH_2)_r-$ ,  $-(CH_2CH_2O)_s-$ ,  $-(OCH_2CH_2)_s-$ ,  $-((CH_2)_m-C(O)NH)-$ , or  $-(C(O)NH-(CH_2)_m)-$ ,

each subscript "m" is independently 1-5;

each subscript "o" is independently 1-5;

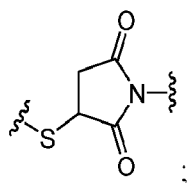
each subscript "r" is independently 1-10;

each subscript "s" is independently 1-5;

each G<sup>1</sup> is independently a C<sub>1</sub>-C<sub>6</sub> substituted or unsubstituted alkylene;

each R<sup>a</sup> is independently H or a C<sub>1</sub>-C<sub>6</sub> substituted or unsubstituted alkyl;

L<sup>1</sup> is  $-NHC(O)-$ ,  $-C(O)NH-$ ,  $-NHC(O)O-$ ,  $-OC(O)NH-$ ,  $-NHC(O)NH-$ ,  $-S-S-$ , or



subscript "p" is independently 1-5;

L<sup>2</sup> is  $-O-(CH_2CH_2O)_n-$ ,  $-(CH_2CH_2O)_n-O-$ , or  $-(OCH_2CH_2O)_n-$ ,

each subscript "n" is independently 1-200;

subscript "q<sup>1</sup>" is independently 0-5;

subscript "q<sup>2</sup>" is independently 0-5;

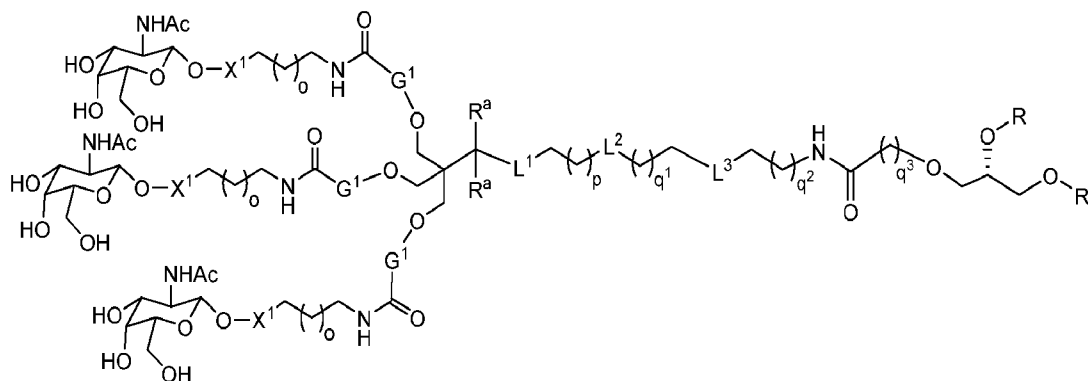
L<sup>3</sup> is absent,  $-NHC(O)-$ , or  $-C(O)NH-$ ;

subscript "q<sup>3</sup>" is independently 0-5;

each R is independently a C<sub>12</sub>-C<sub>20</sub> substituted or unsubstituted alkyl; and

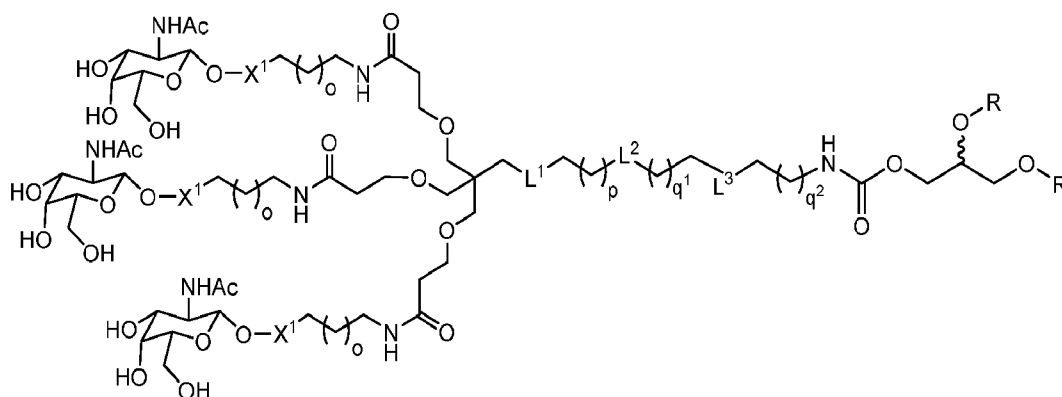
the “*wavy*” indicates an *R* configuration or an *S* configuration.

2. The compound of claim 1, wherein the compound of Formula I is a compound of Formula I-a:



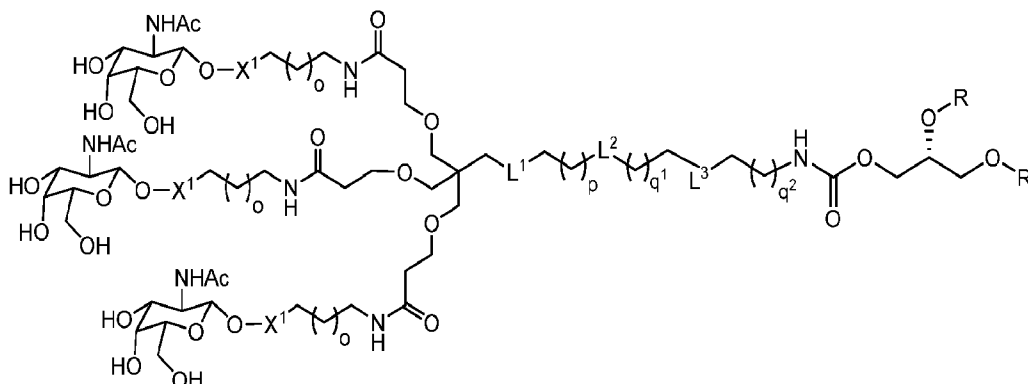
**Formula I-a**

3. The compound of claim 1, wherein the compound of Formula I is a compound of Formula I-b:



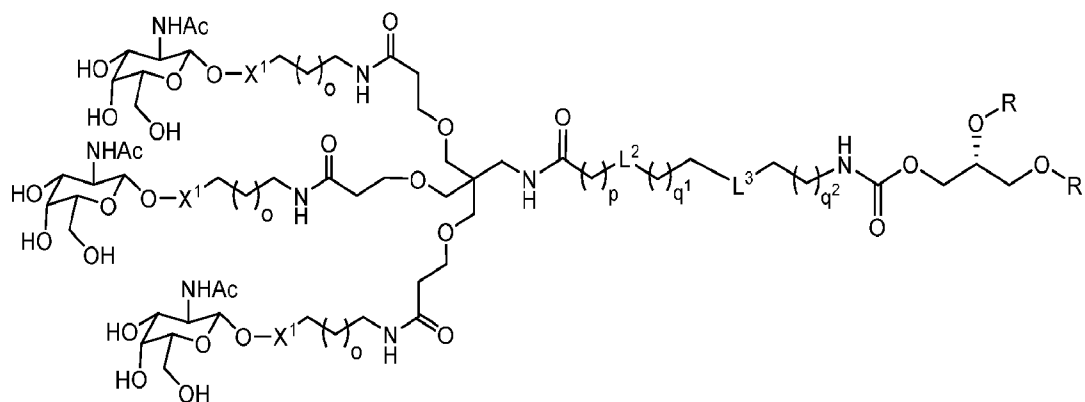
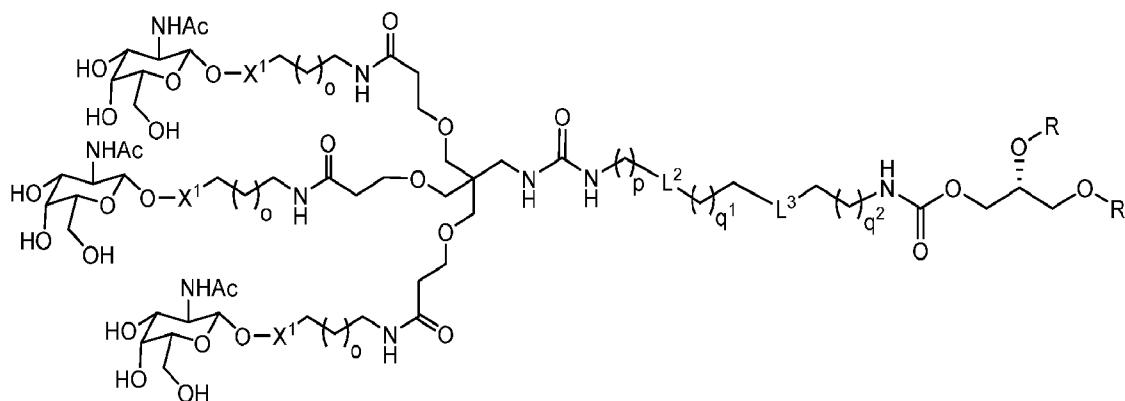
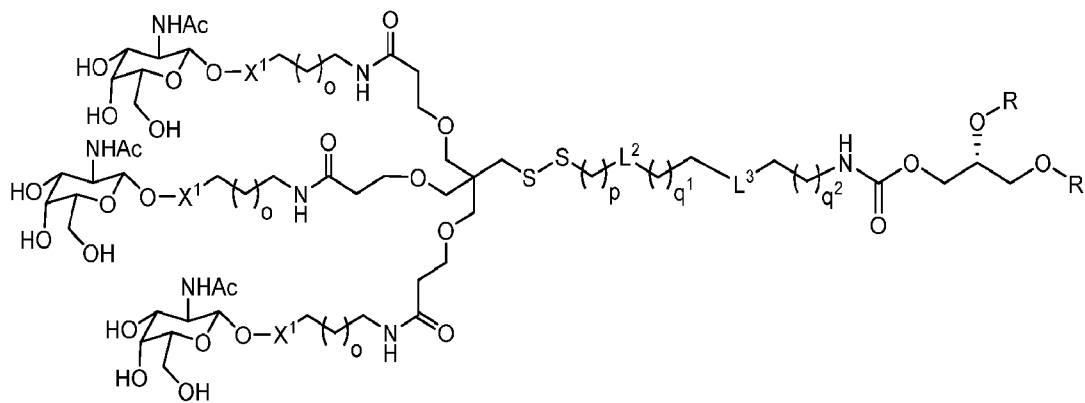
**Formula I-b.**

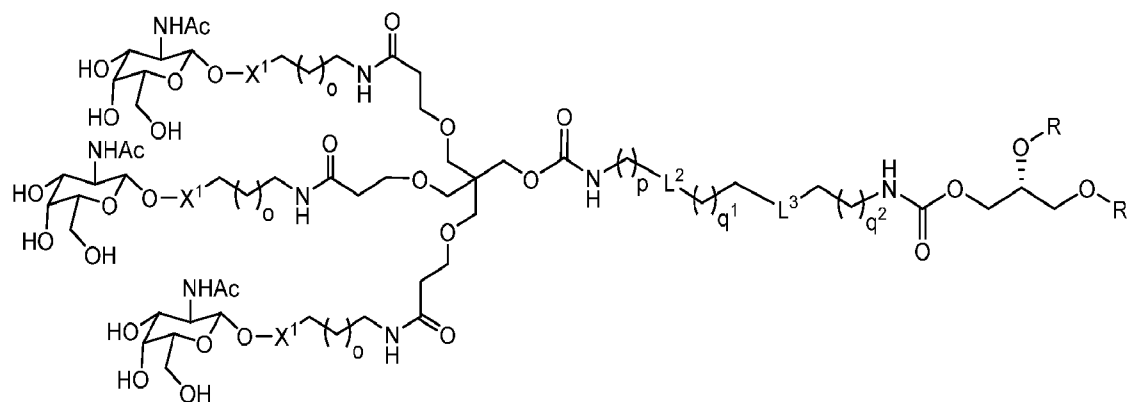
4. The compound of claim 1, wherein the compound of Formula I is a compound of Formula I-c:



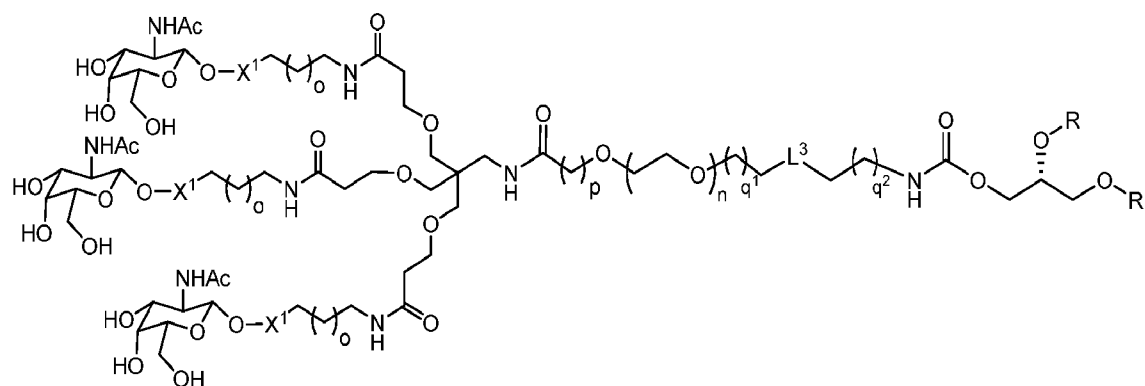
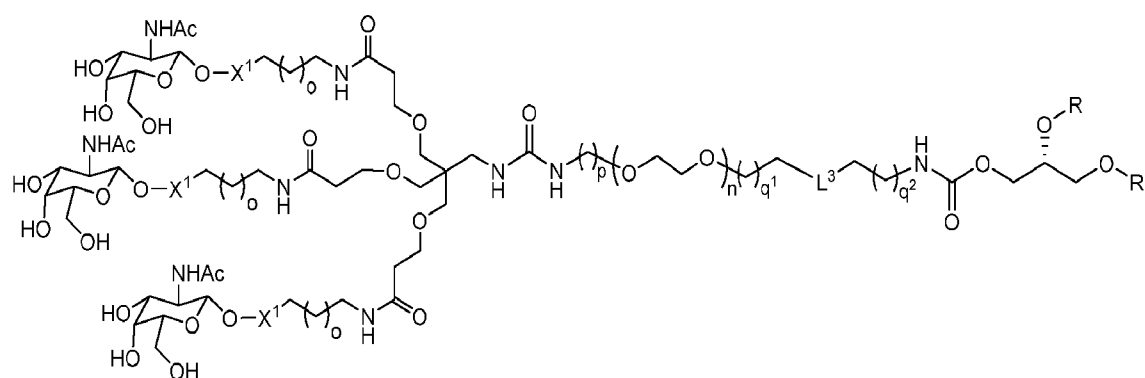
**Formula I-c.**

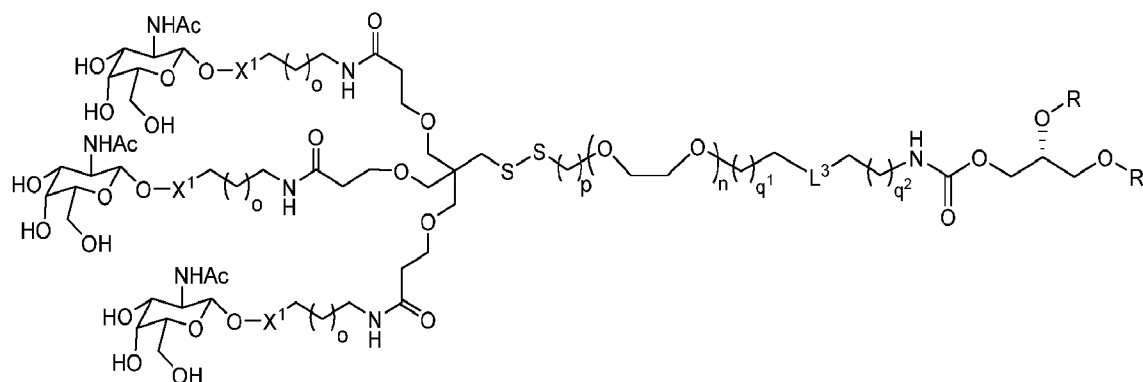
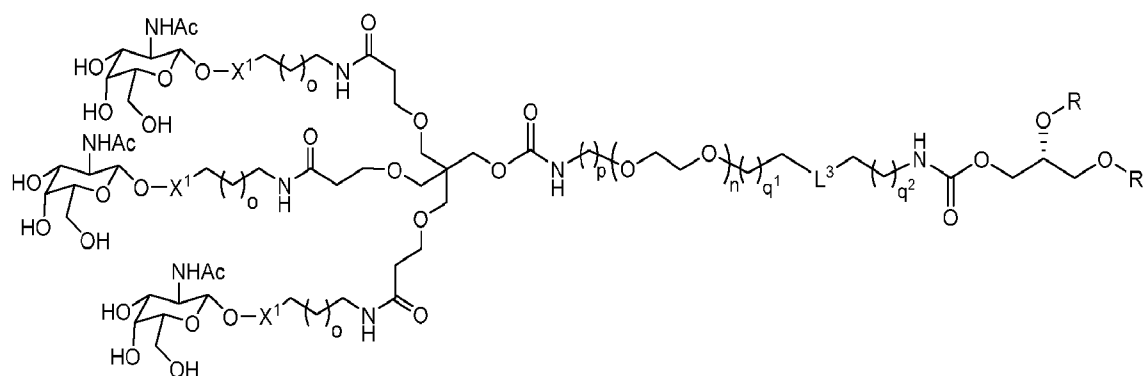
5. The compound of any one of claims 1-4, wherein  $L^1$  is  $-\text{NHC}(\text{O})-$ .
6. The compound of any one of claims 1-4, wherein  $L^1$  is  $-\text{NHC}(\text{O})\text{NH}-$ .
7. The compound of any one of claims 1-4, wherein  $L^1$  is  $-\text{S}-\text{S}-$ .
8. The compound of any one of claims 1-4, wherein  $L^1$  is  $-\text{OC}(\text{O})\text{NH}-$ .
9. The compound of any one of claims 1-8, wherein each  $X^1$  is  $-\text{((CH}_2\text{)}_m-\text{C}(\text{O})\text{NH})-$ .
10. The compound of any one of claims 1-8, wherein each  $X^1$  is  $-(\text{CH}_2\text{CH}_2\text{O})_s-$ .
11. The compound of any one of claims 1-8, wherein each  $X^1$  is  $-(\text{CH}_2)_r-$  and each subscript "r" is 5.
12. The compound of any one of claims 1-11, wherein each subscript "o" is 1.
13. The compound of any one of claims 1-12, wherein  $L^2$  is  $-\text{O}-(\text{CH}_2\text{CH}_2\text{O})_n-$ .
14. The compound of any one of claims 1-12, wherein  $L^2$  is  $-(\text{OCH}_2\text{CH}_2\text{O})_n-$ .
15. The compound of claim 13 or 14, wherein each subscript "n" is 100 to 200.
16. The compound of any one of claims 1-15, wherein  $L^3$  is absent.
17. The compound of any one of claims 1-15, wherein  $L^3$  is present and is  $-\text{NHC}(\text{O})-$ .
18. The compound of any one of claims 1-17, wherein each R is independently a  $\text{C}_{14}$ - $\text{C}_{18}$  unsubstituted alkyl.
19. The compound of any one of claims 1-18, wherein the compound of Formula I is any one of a compound of Formula I-c-1 to I-c-4:

**Formula I-c-1,****Formula I-c-2,****Formula I-c-3, or**

**Formula I-c-4.**

20. A compound selected from the group consisting of Formula I-c-5, I-c-6, I-c-7, and I-c-8:

**Formula I-c-5,****Formula I-c-6,**

**Formula I-c-7, or****Formula I-c-8**

wherein,

each  $X^1$  is independently  $-(CH_2)_r-$ ,  $-(CH_2CH_2O)_s-$ ,  $-(OCH_2CH_2)_s-$ ,  $-((CH_2)_m-C(O)NH)-$ , or  $-(C(O)NH-(CH_2)_m)-$ ;

each subscript “m” is independently 1-5;

each subscript “s” is independently 1-5;

each subscript “o” is independently 1-5;

each subscript “r” is independently 1-10;

each subscript “p” is independently 1-5;

each subscript “n” is independently 1-200;

each subscript “q<sup>1</sup>” is independently 0-5;

each subscript “q<sup>2</sup>” is independently 0-5;

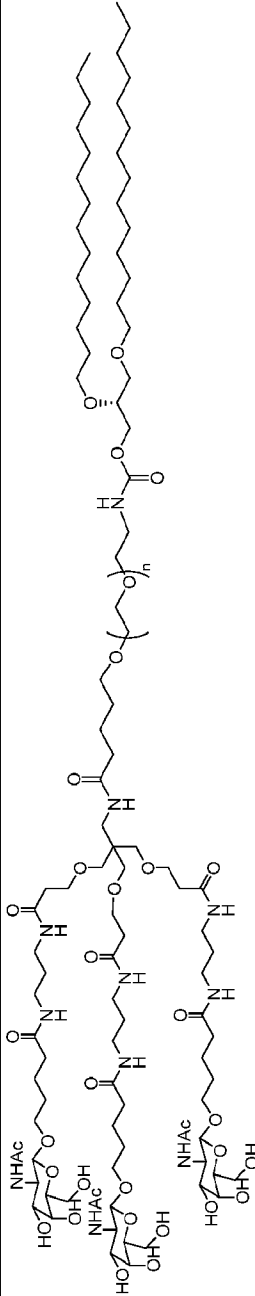
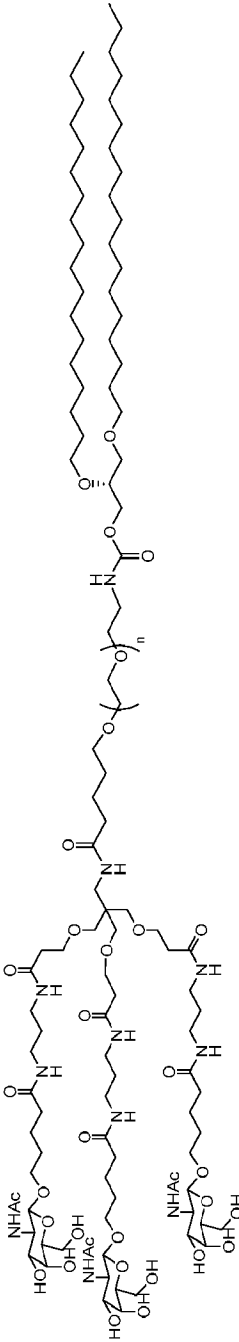
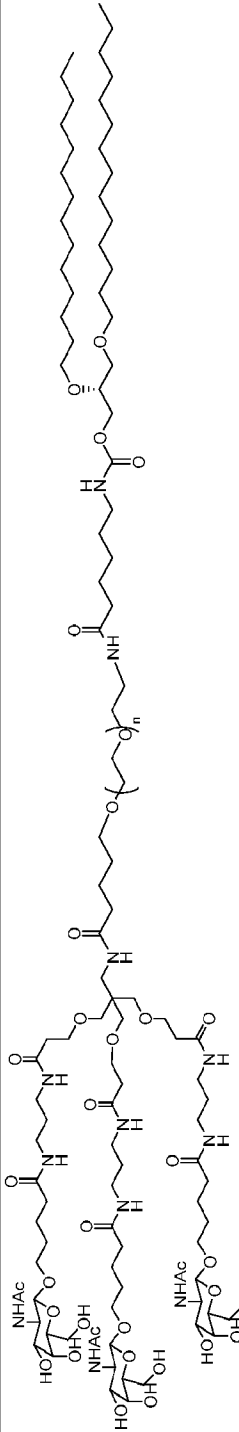
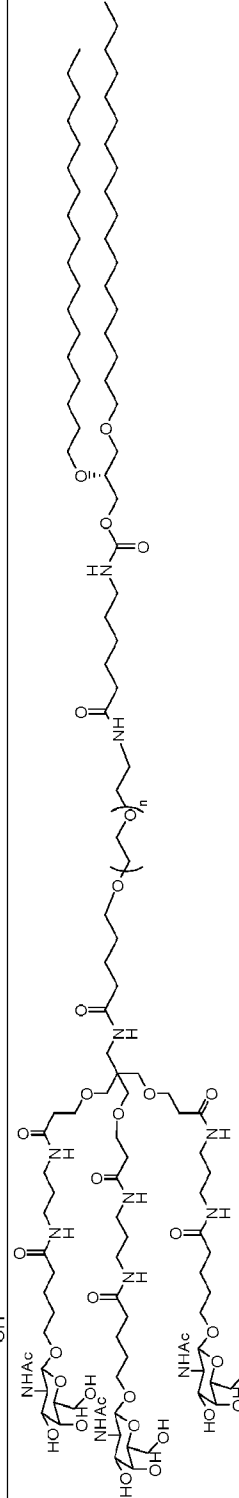
each  $L^3$  is independently absent,  $-NHC(O)-$ , or  $-C(O)NH-$ ; and

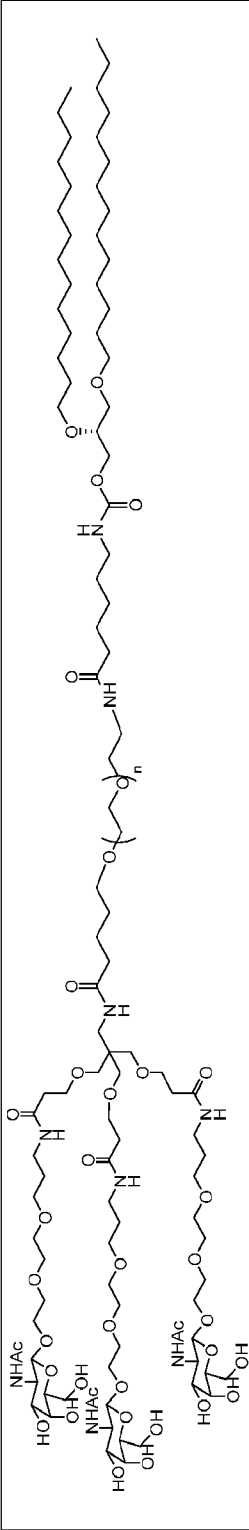
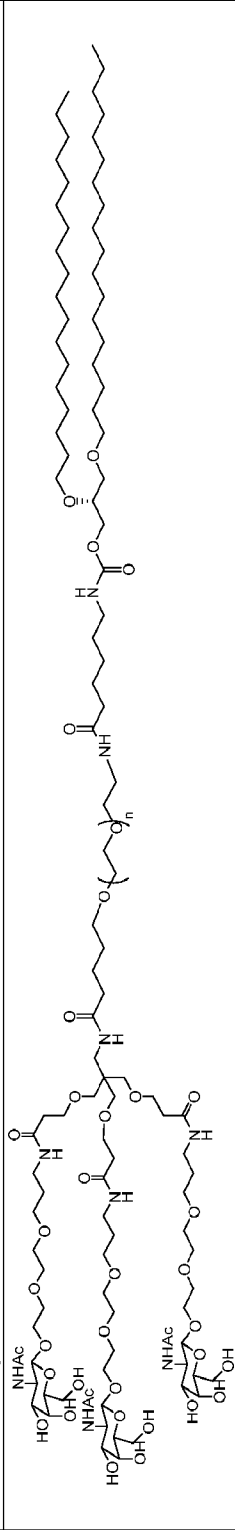
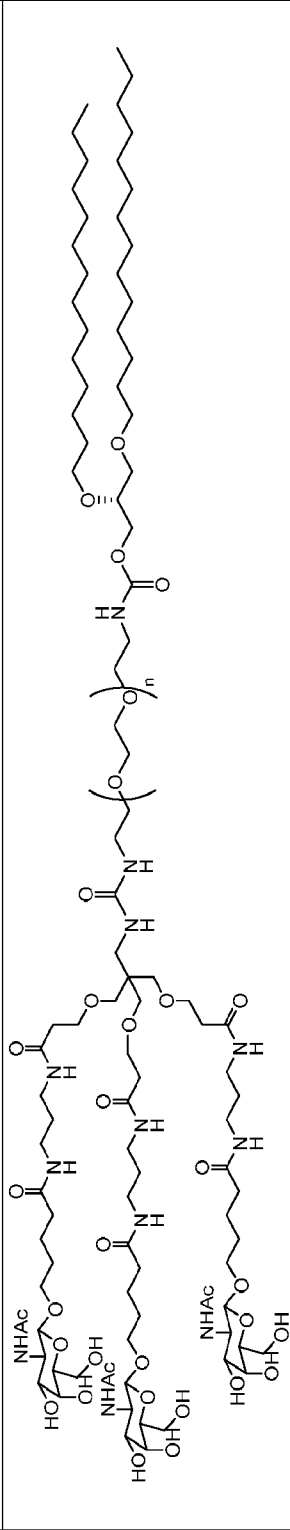
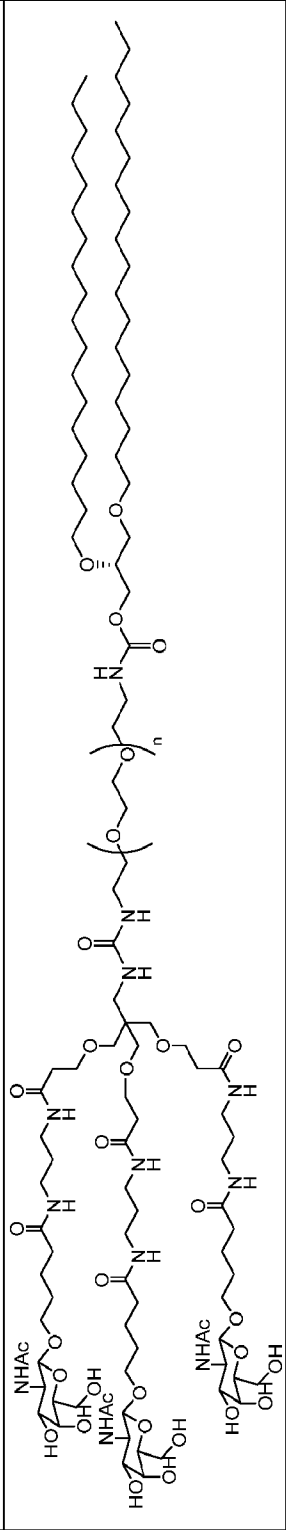
each R is independently a  $C_{12}$ - $C_{20}$  substituted or unsubstituted alkyl.

21. The compound of claim 20, wherein each  $X^1$  is  $-((CH_2)_m-C(O)NH)-$ , each subscript “m” is independently 2, and each subscript “o” is independently 1.

22. The compound of claim 20, wherein each  $X^1$  is  $-(CH_2CH_2O)_s-$ , each subscript “s” is independently 2, and each subscript “o” is independently 1.
23. The compound of claim 20, wherein the compound is a compound of Formula I-c-7 and, optionally, each  $X^1$  is  $-(CH_2)_r-$ , each subscript “r” is independently 5, and each subscript “o” is 1.
24. The compound of claim 20, wherein the compound is a compound of Formula I-c-5,  $L^2$  is  $-O-(CH_2CH_2O)_n-$ , and subscript “n” is 100 to 200.
25. The compound of claim 20, wherein the compound is a compound of Formula I-c-6, I-c-7, or I-c-8, each  $L^2$  is  $-(OCH_2CH_2O)_n-$  and each subscript “n” is 100 to 200.
26. The compound of any one of claims 20-25, wherein  $L^3$  is absent.
27. The compound of claim 26, wherein in Formula I-c-4, subscript “p” is 4, subscript “q<sup>1</sup>” is 0., and subscript “q<sup>2</sup>” is 0.
28. The compound of claim 26, wherein in Formulas I-c-6, I-c-7, and I-c-8, each subscript “p” is 2, subscript “q<sup>1</sup>” is 0, and subscript “q<sup>2</sup>” is 0.
29. The compound of any one of claims 20-25, wherein  $L^3$  is present and is  $-NHC(O)-$ .
30. The compound of claim 29, wherein in Formula I-a-4, subscript “p” is 4, subscript “q<sup>1</sup>” is 1 and subscript “q<sup>2</sup>” is 4.
31. The compound of any one of claims 20-30, wherein each R is independently a C<sub>14</sub>-C<sub>18</sub> unsubstituted alkyl.

32. A compound selected from the group consisting of:

| Compound No. | Structure                                                                            |
|--------------|--------------------------------------------------------------------------------------|
| 1            |    |
| 2            |    |
| 3            |   |
| 4            |  |

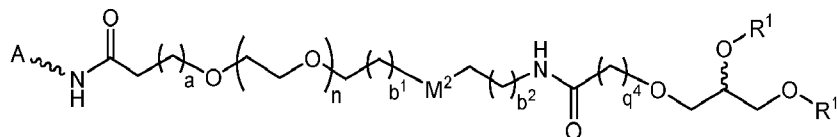
|   |                                                                                      |
|---|--------------------------------------------------------------------------------------|
| 5 |    |
| 6 |    |
| 7 |   |
| 8 |  |

|    |  |
|----|--|
| 9  |  |
| 10 |  |
| 11 |  |
| 12 |  |

|    |  |
|----|--|
| 13 |  |
| 14 |  |
| 15 |  |
| 16 |  |

wherein each subscript “n” is between 10-200.

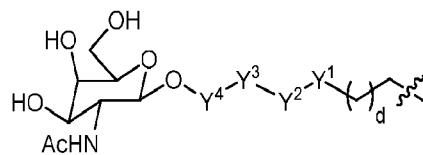
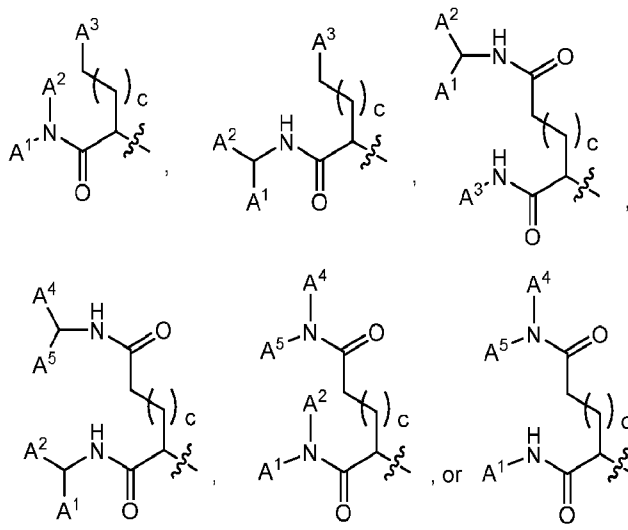
33. A compound of Formula II:



**Formula II**

wherein:

A is:



each  $A^1$ ,  $A^2$ ,  $A^3$ ,  $A^4$ , and  $A^5$  is independently

each  $Y^1$  is independently  $-\text{NH}-$ ,  $-\text{NHC}(\text{O})\text{O}-$ ,  $-\text{OC}(\text{O})\text{NH}-$ ,  $-\text{NHC}(\text{O})-$ , or  $-\text{C}(\text{O})\text{NH}-$ ;

each  $Y^2$  is independently absent,  $-(\text{CH}_2)_t-$ ,  $-(\text{CH}_2\text{CH}_2\text{O})_t-$ , or  $-(\text{OCH}_2\text{CH}_2)_t-$ ;

each subscript “t” is independently 1-5;

each  $Y^3$  is independently absent,  $-\text{NHC}(\text{O})-$ , or  $-\text{C}(\text{O})\text{NH}-$ ;

each  $Y^4$  is independently absent or  $-(\text{CH}_2)_r-$ ;

each subscript “r” is independently 1-10;

each subscript “d” is independently 0-5;

each subscript “c” is independently 0-5;

subscript “n” is independently 1-200;

subscript “a” is 1-5;

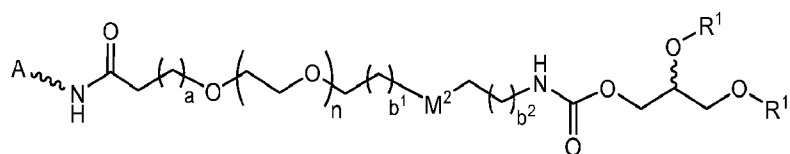
subscript “b<sup>1</sup>” is independently 0-5;

$M^2$  is absent,  $-\text{NHC}(\text{O})-$ , or  $-\text{C}(\text{O})\text{NH}-$ ;

subscript “b<sup>2</sup>” is independently 0-5;

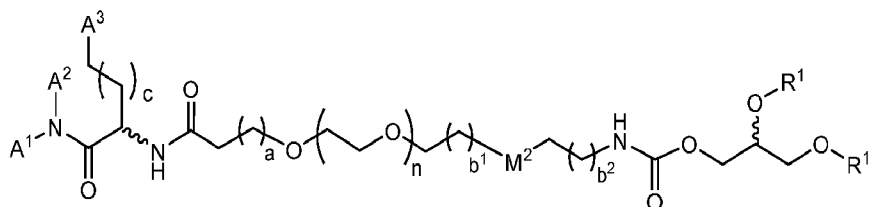
subscript “q<sup>4</sup>” is independently 0-6;  
 each R<sup>1</sup> is independently a C<sub>12</sub>-C<sub>20</sub> substituted or unsubstituted alkyl; and  
 each of the “” bonds independently indicate an *R* configuration or an *S* configuration;  
 with the proviso that at least one of Y<sup>2</sup>, Y<sup>3</sup> and Y<sup>4</sup> is present.

34. The compound of claim 33, wherein the compound of Formula II is a compound of Formula II-a:

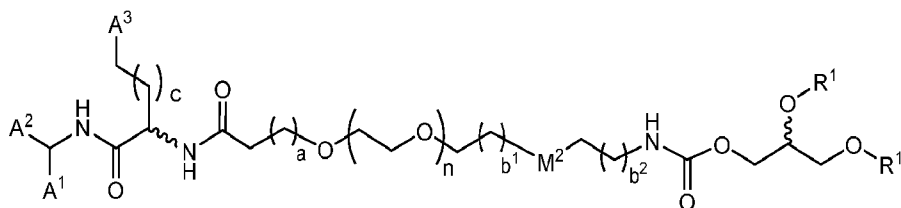


**Formula II-a.**

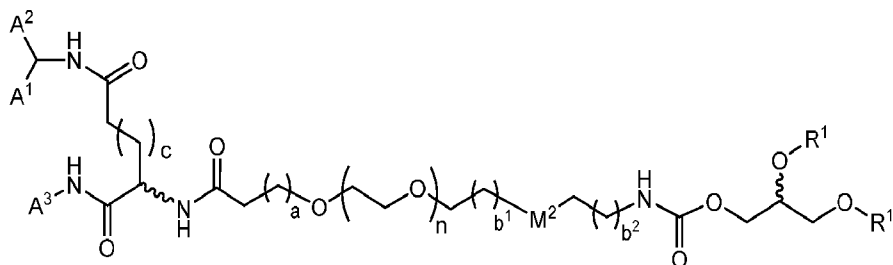
35. The compound of claim 33 or 34, wherein the compound of Formula II is a compound of Formula II-a-1, Formula II-a-2, Formula II-a-3, Formula II-a-4, Formula II-a-5, or Formula II-a-6:



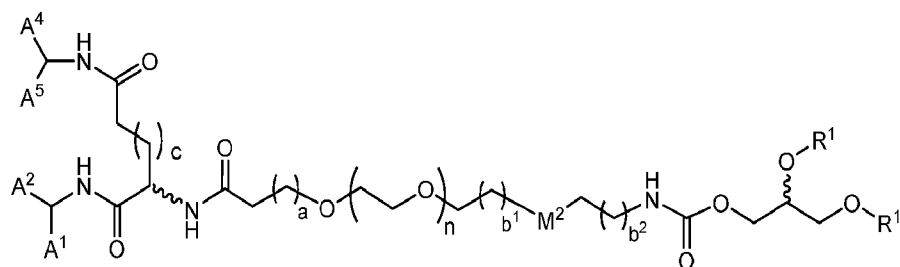
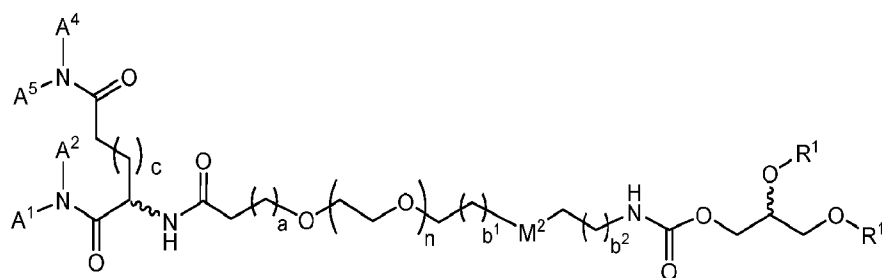
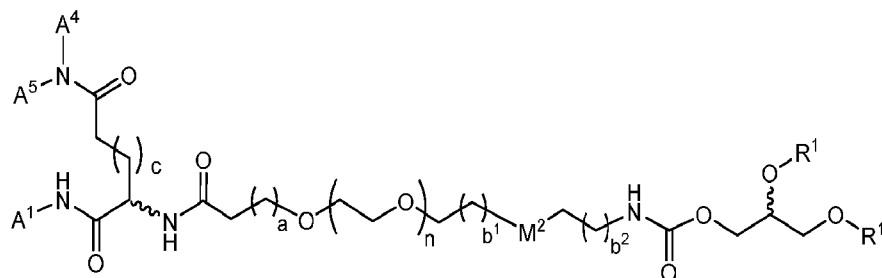
**Formula II-a-1,**



**Formula II-a-2,**

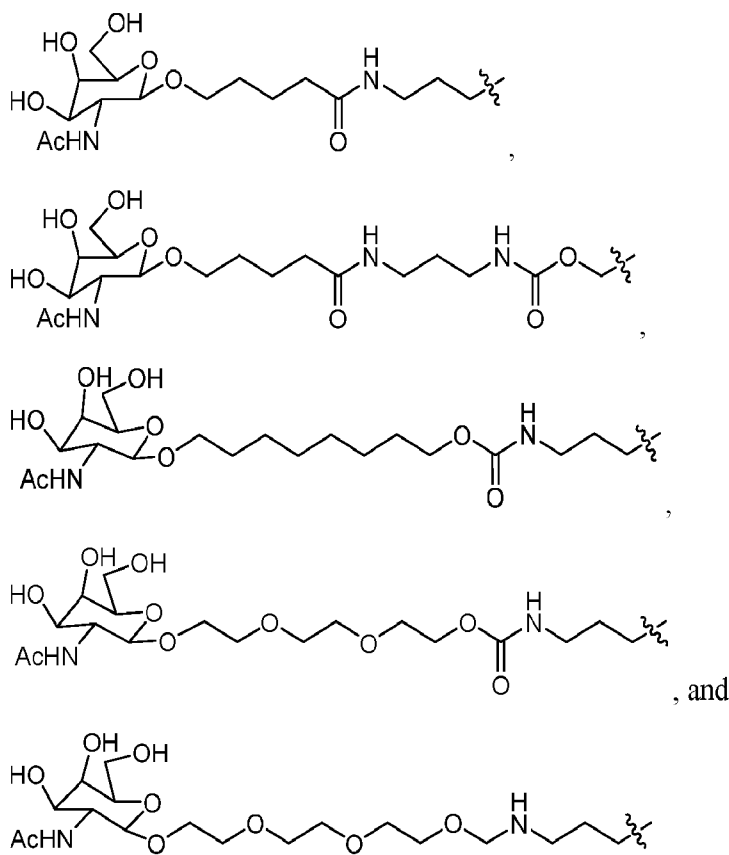


**Formula II-a-3,**

**Formula II-a-4,****Formula II-a-5, or****Formula II-a-6.**

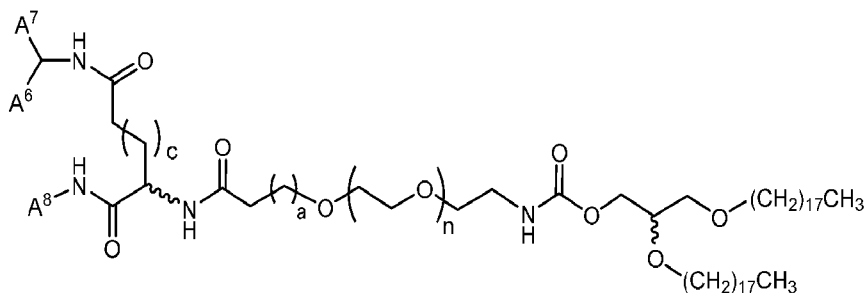
36. The compound of any one of claims 33-35, wherein  $A^1$ ,  $A^2$ , and  $A^3$  are the same.
37. The compound of any one of claims 33-35, wherein  $A^1$  and  $A^2$  are different from  $A^3$ .
38. The compound of any one of claims 33-35, wherein  $A^1$ ,  $A^2$ ,  $A^4$ , and  $A^5$  are the same.
39. The compound of any one of claims 33-35, wherein  $A^1$ ,  $A^2$ , and  $A^5$  are the same.
40. The compound of any one of claims 33-39, wherein each of  $Y^1$  and  $Y^4$  are present and each of  $Y^2$  and  $Y^3$  are absent.
41. The compound of claim 40, wherein each  $Y^1$  is  $-C(O)NH-$  and each  $Y^4$  is  $-(CH_2)_r-$ .
42. The compound of claim 40, wherein each  $Y^1$  is  $-OC(O)NH-$  and each  $Y^4$  is  $-(CH_2)_r-$ .

43. The compound of any one of claims 33-39, wherein each of  $Y^1$ ,  $Y^2$ ,  $Y^3$ , and  $Y^4$  are present.
44. The compound of claim 43, wherein each  $Y^1$  is independently  $-\text{NHC(O)O}-$ ; each  $Y^2$  is independently  $-(\text{CH}_2)_r-$ ; each  $Y^3$  is independently  $-\text{C(O)NH}-$ ; and each  $Y^4$  is independently  $-(\text{CH}_2)_r-$ .
45. The compound of any one of claims 33-39, wherein each of  $Y^1$  and  $Y^2$  are present and each of  $Y^3$  and  $Y^4$  are absent.
46. The compound of claim 45, wherein each  $Y^1$  is independently  $-\text{OC(O)NH}-$  and each  $Y^2$  is independently  $-(\text{CH}_2\text{CH}_2\text{O})_t-$ .
47. The compound of any one of claims 33-46, wherein each of  $A^1$ ,  $A^2$ ,  $A^3$ ,  $A^4$ , and  $A^5$  is selected from the group consisting of:

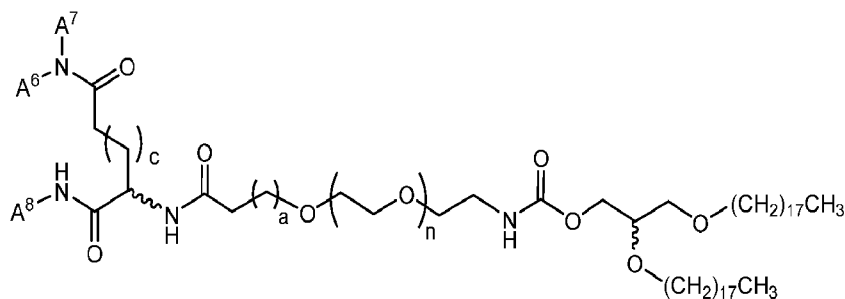


48. The compound of any one of claims 33-47, wherein  $M^2$  is absent.

49. The compound of any one of claims 33-47, wherein  $M^2$  is  $-\text{NHC}(\text{O})-$ .
50. The compound of any one of claims 33-49, wherein each  $R^1$  is independently a  $\text{C}_{14}$ - $\text{C}_{18}$  unsubstituted alkyl.
51. A compound of Formula III or Formula IV:

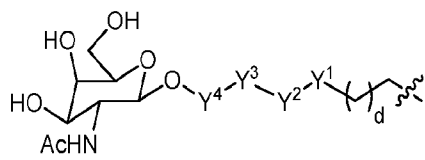


Formula III, or



Formula IV

wherein,



each  $A^6$ ,  $A^7$ , and  $A^8$  is independently

each  $Y^1$  is independently absent,  $-\text{NHC}(\text{O})\text{O}-$ , or  $-\text{OC}(\text{O})\text{NH}-$ ;

each  $Y^2$  and  $Y^4$  is independently  $-(\text{CH}_2)_r-$ ;

each subscript “r” is independently 1-10;

each  $Y^3$  is independently absent,  $-\text{NHC}(\text{O})-$ , or  $-\text{C}(\text{O})\text{NH}-$ ;

each subscript “d” is independently 1-5;

each subscript “c” is independently 1-5;

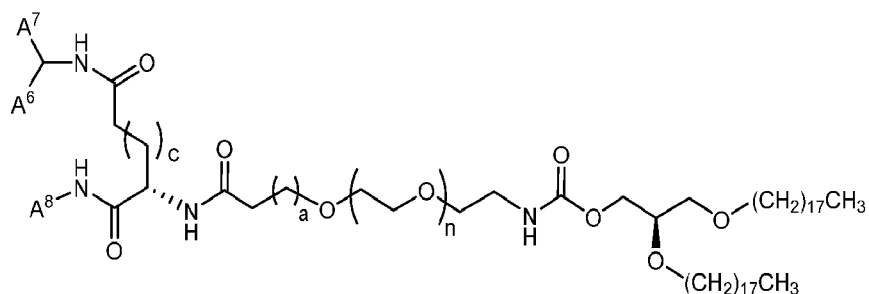
subscript “a” is 1-5;

subscript “n” is independently 10-100; and

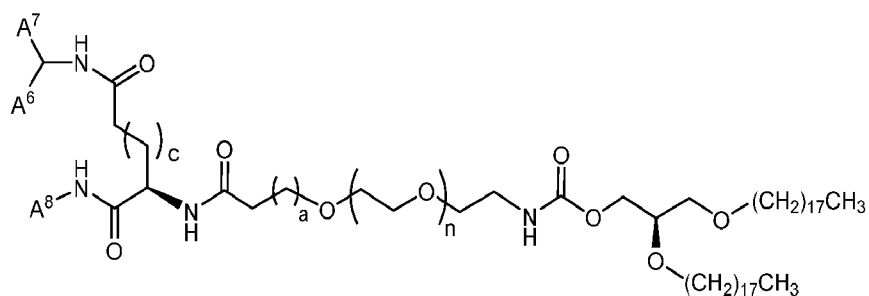
each of the “ $\sim$ ” bonds independently indicate an *R* configuration or an *S* configuration;

with the proviso that at least one of  $Y^1$  or  $Y^3$  is present.

52. The compound of claim 51, wherein the compound of Formula III is a compound of Formula III-a or Formula III-b:

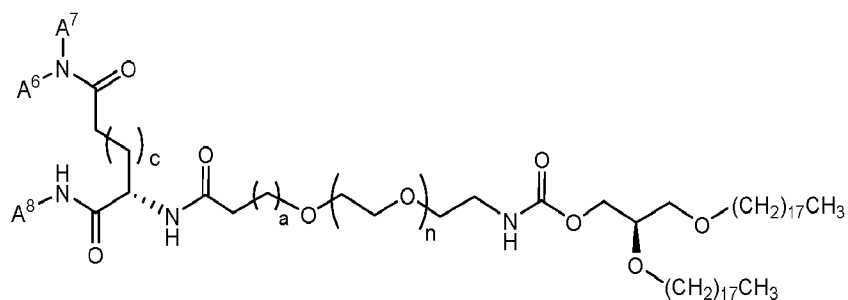


**Formula III-a, or**

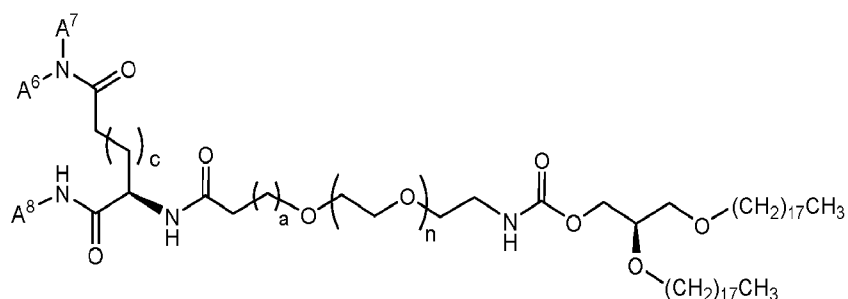


**Formula III-b.**

53. The compound of claim 51, wherein the compound of Formula IV is a compound of Formula IV-a or Formula IV-b:

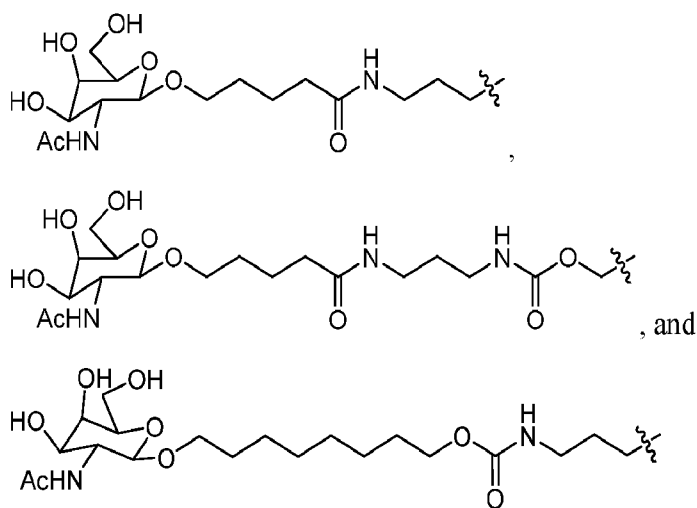


**Formula IV-a, or**

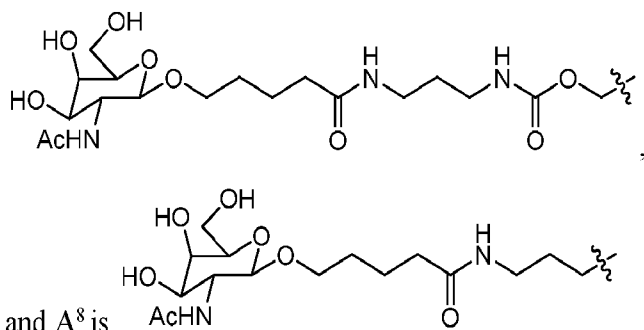


**Formula IV-b.**

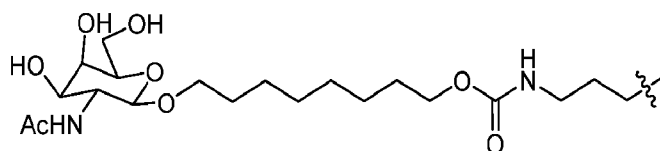
54. The compound of any one of claims 51-53, wherein subscript "a" is 3.
55. The compound of any one of claims 51-53, wherein subscript "a" is 1.
56. The compound of any one of claims 51-55, wherein subscript "n" is independently 34-46.
57. The compound of any one of claims 51-55, wherein subscript "n" is independently between 34-36, in a range of 34-36, between 44-46 or in a range of 44-46.
58. The compound of any one of claims 51-57, wherein subscript "c" is 1.
59. The compound of any one of claims 51-58, wherein each of A<sup>6</sup>, A<sup>7</sup>, and A<sup>8</sup> is independently selected from the group consisting of:



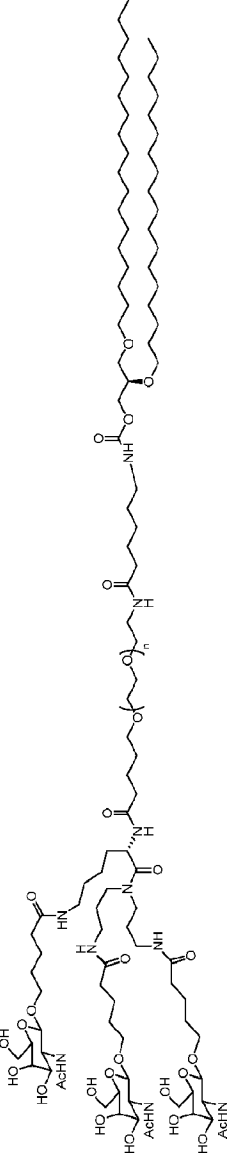
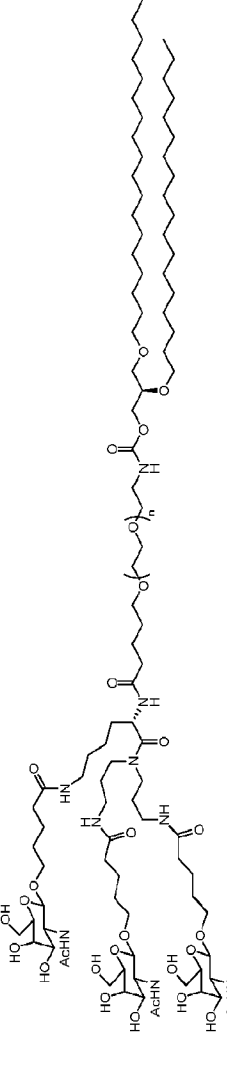
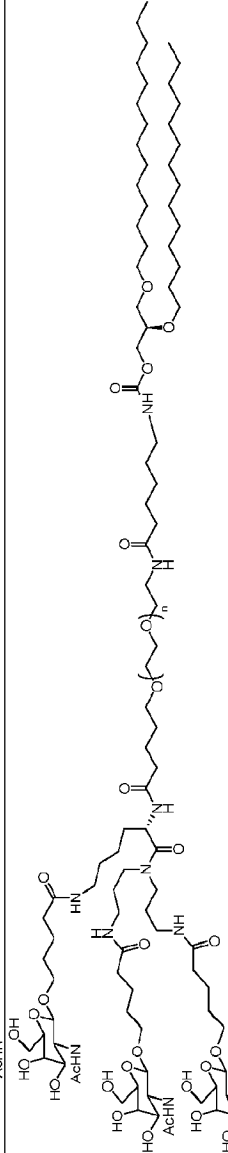
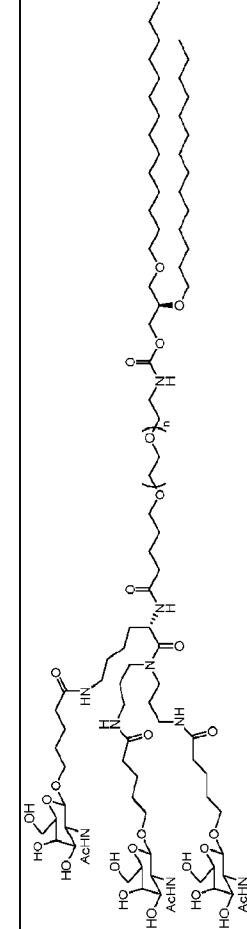
60. The compound of any one of claims 51-58, wherein A<sup>6</sup> and A<sup>7</sup> are each independently

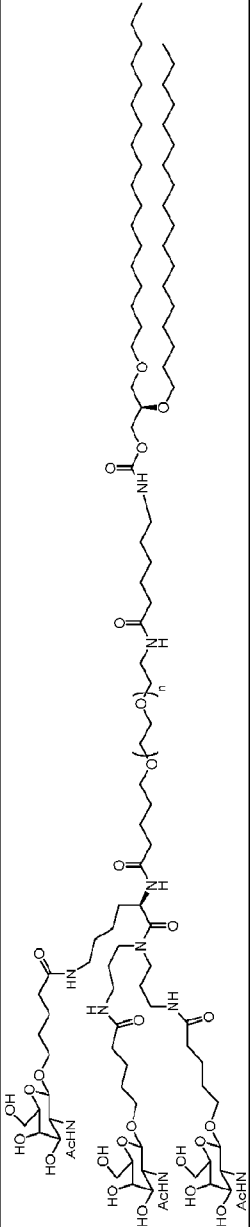
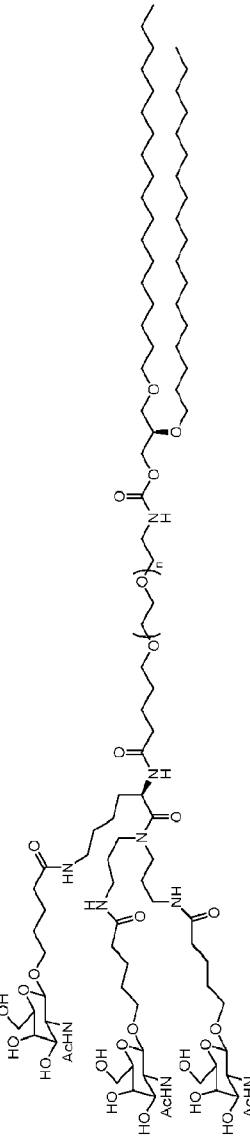
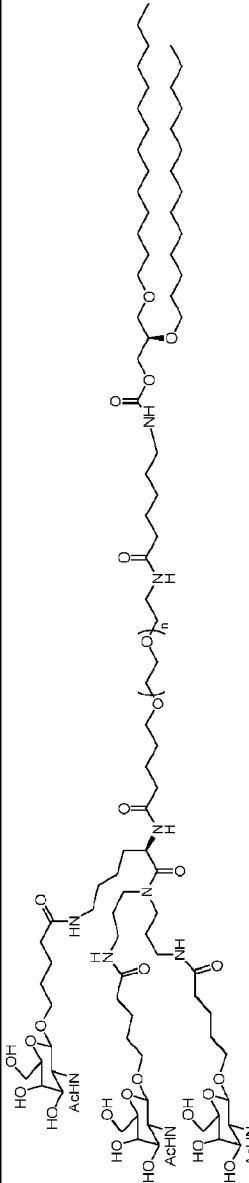
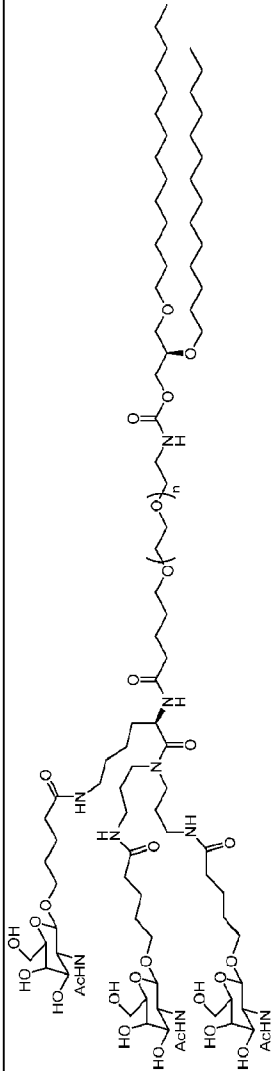


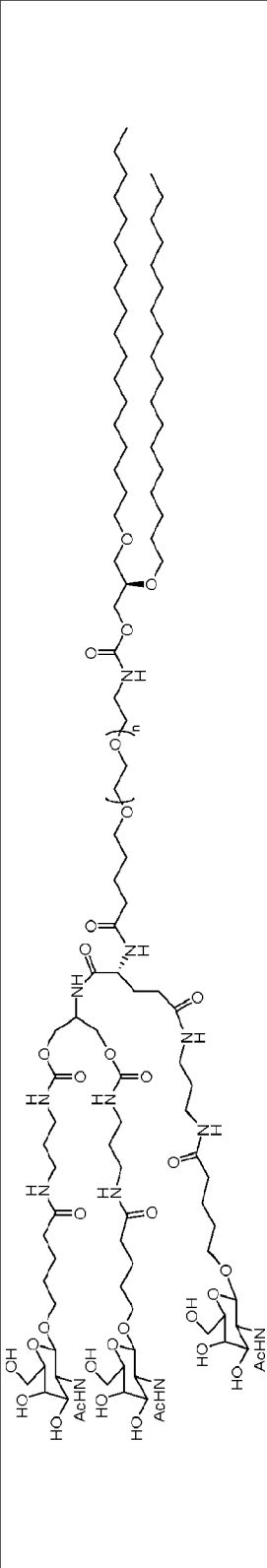
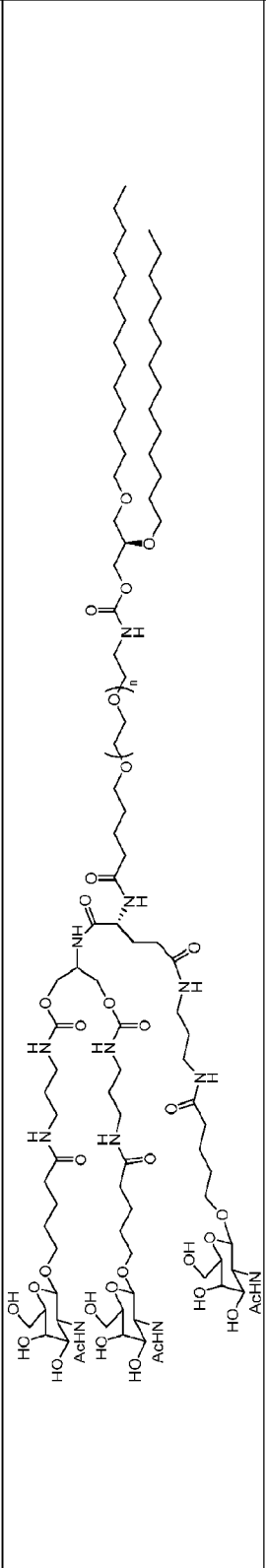
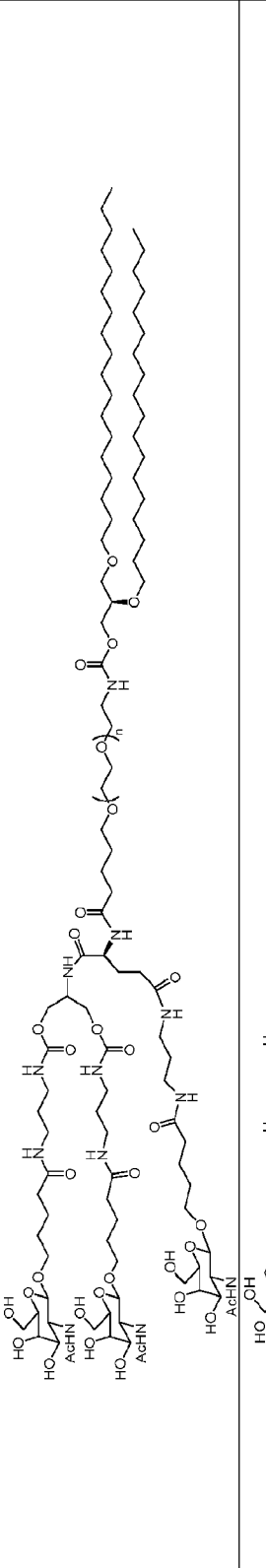
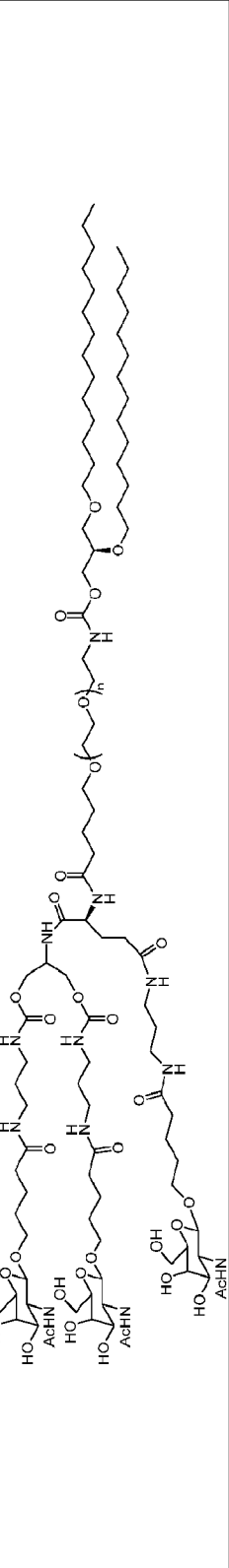
61. The compound of any one of claims 51-58, wherein A<sup>6</sup>, A<sup>7</sup>, and A<sup>8</sup> are each independently

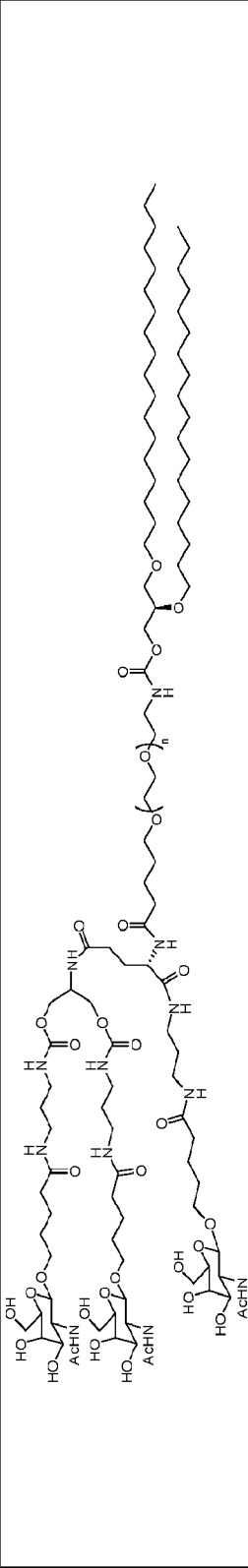
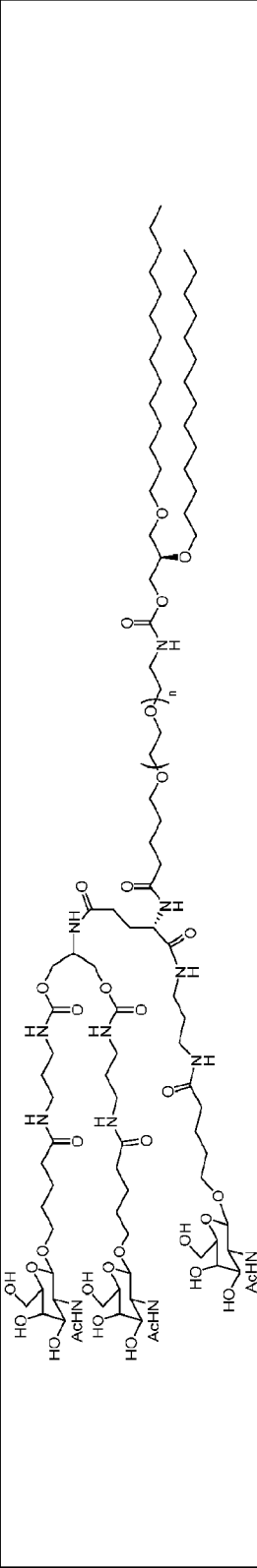
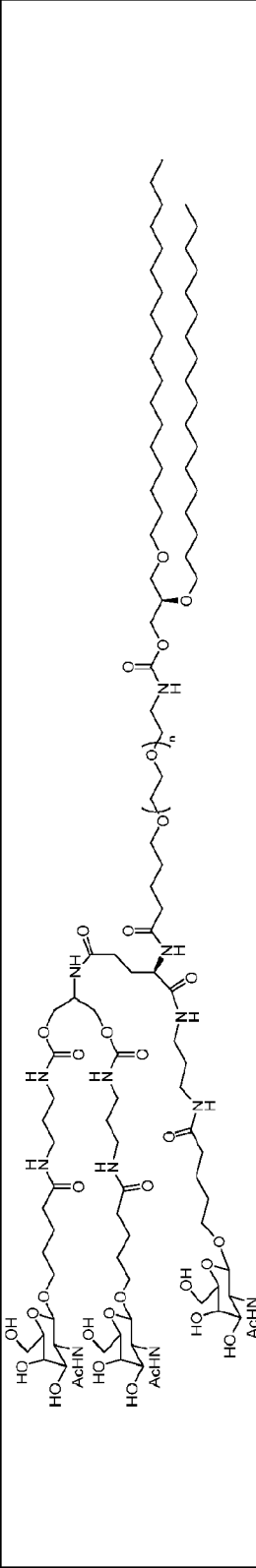
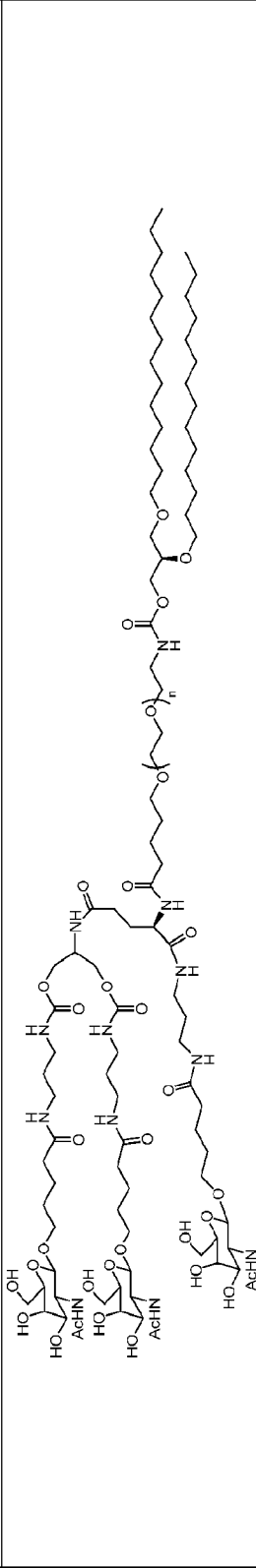


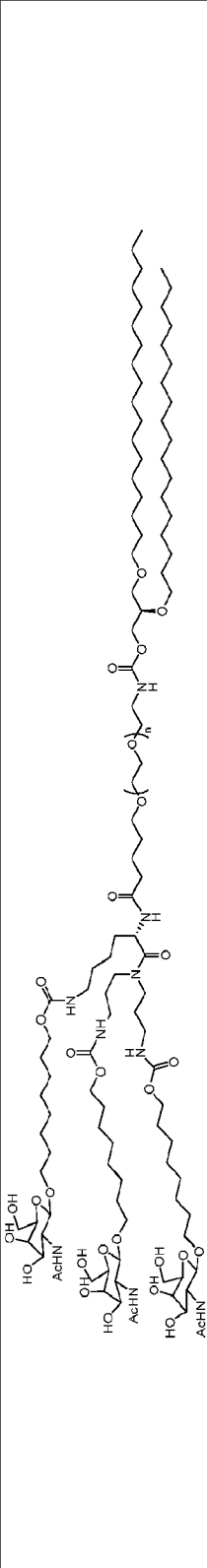
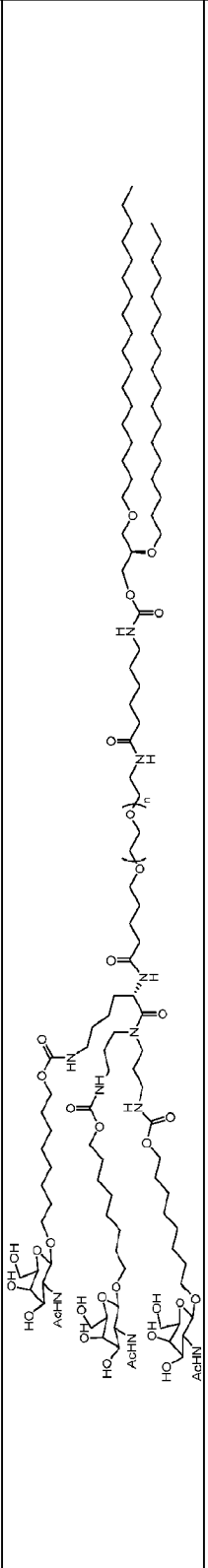
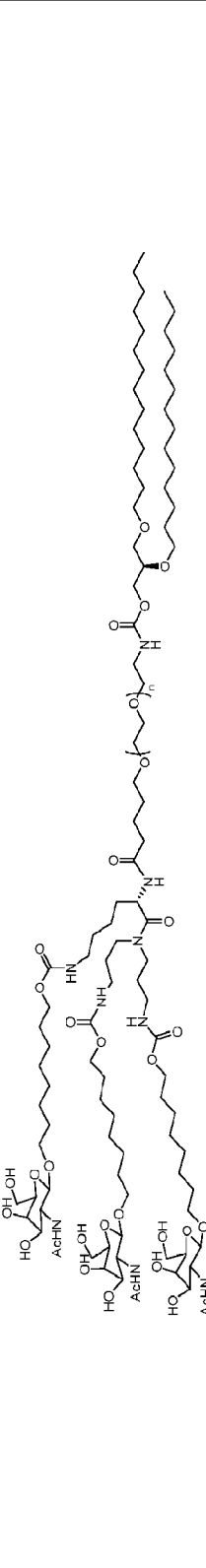
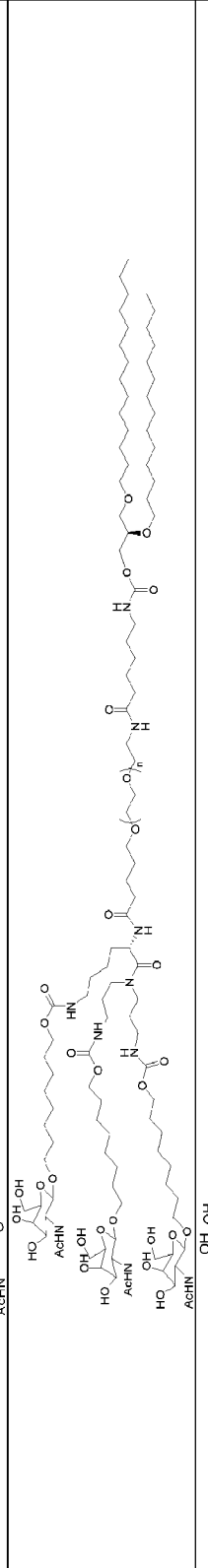
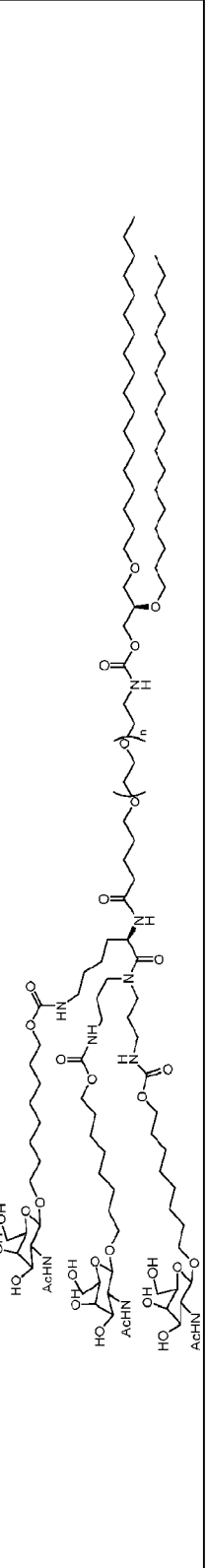
62. A compound selected from the group consisting of:

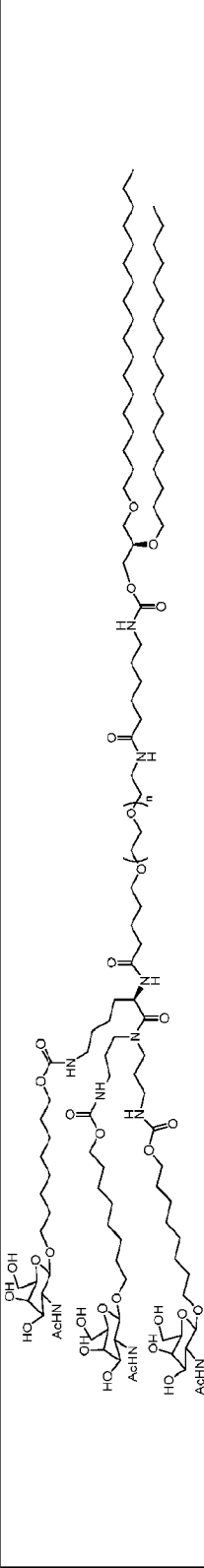
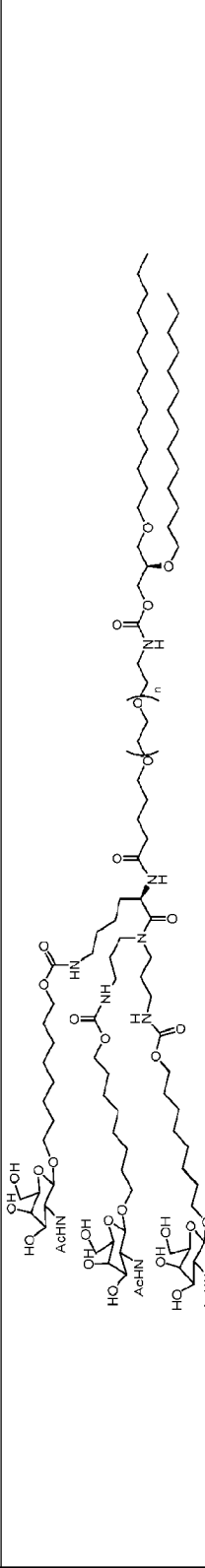
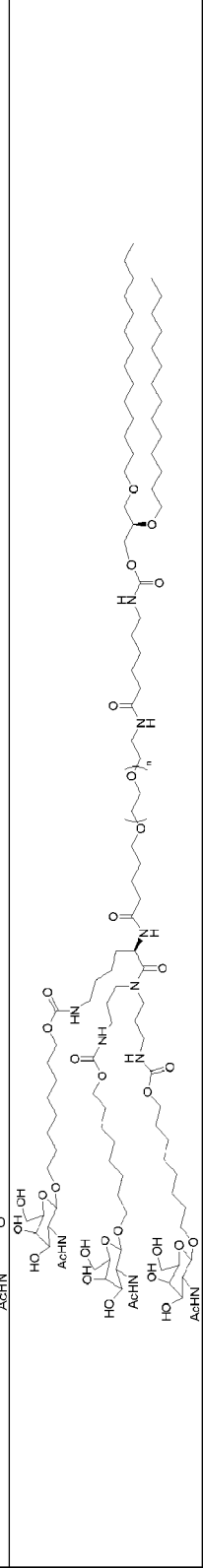
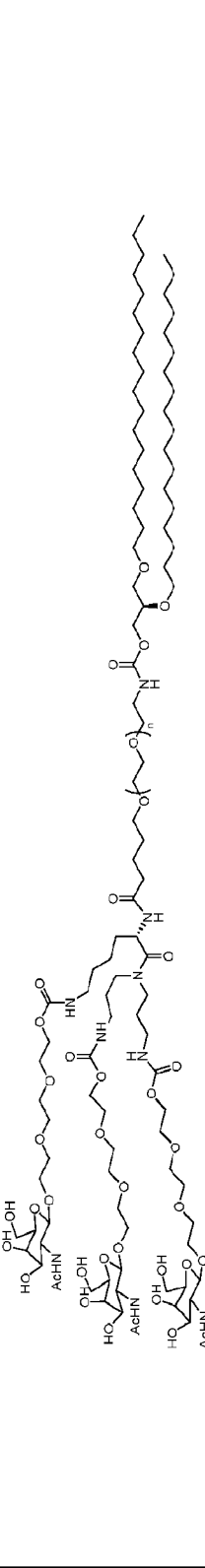
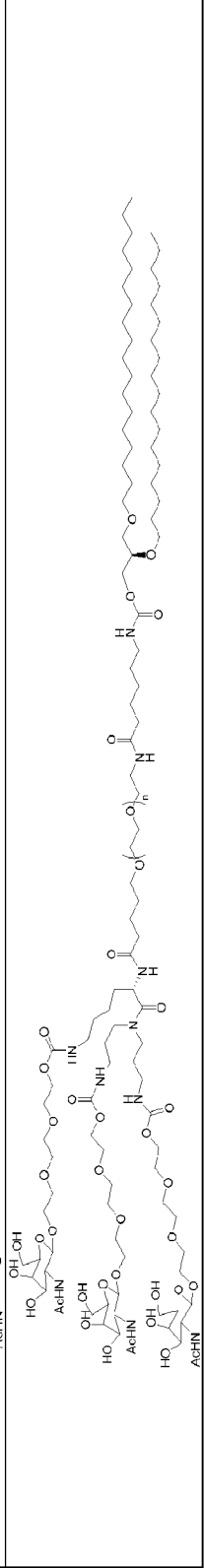
| Compound No. | Structure                                                                            |
|--------------|--------------------------------------------------------------------------------------|
| 17           |    |
| 18           |    |
| 19           |   |
| 20           |  |

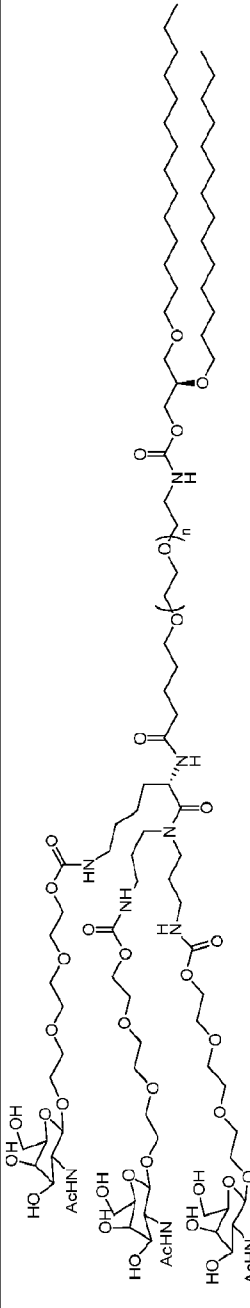
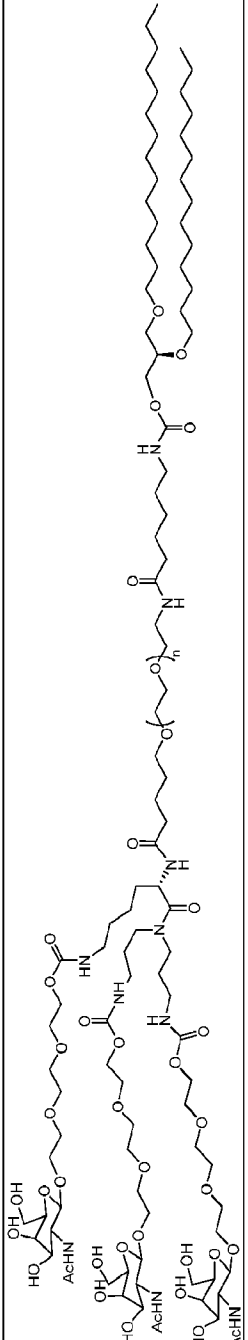
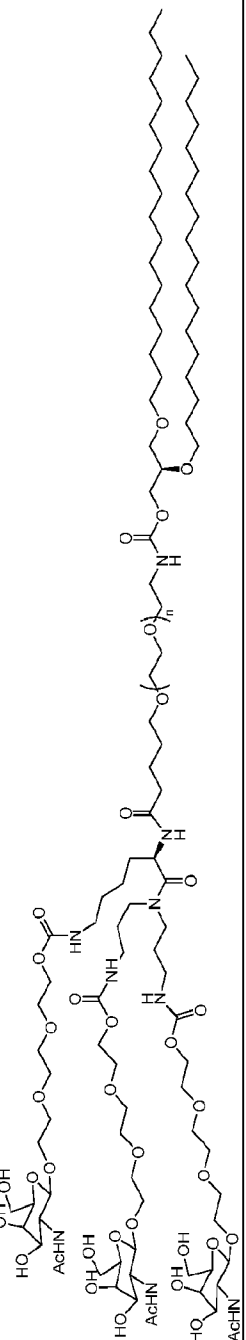
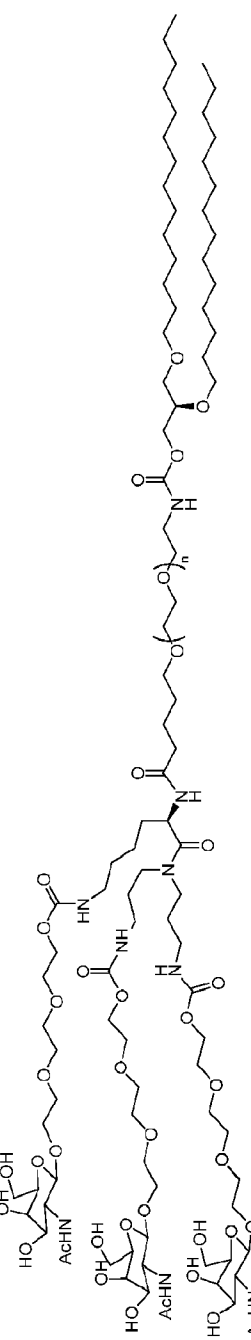
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| 22 |    |
| 23 |   |
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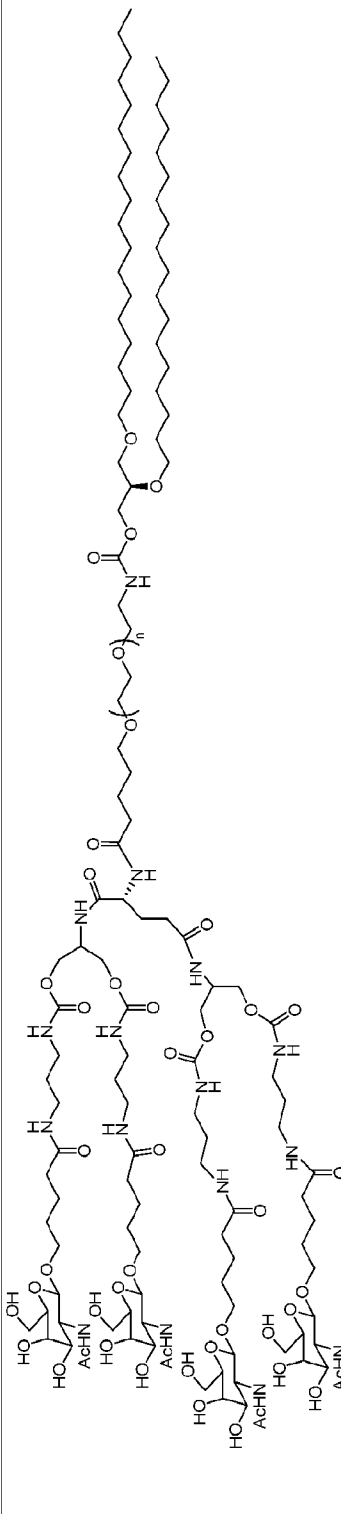
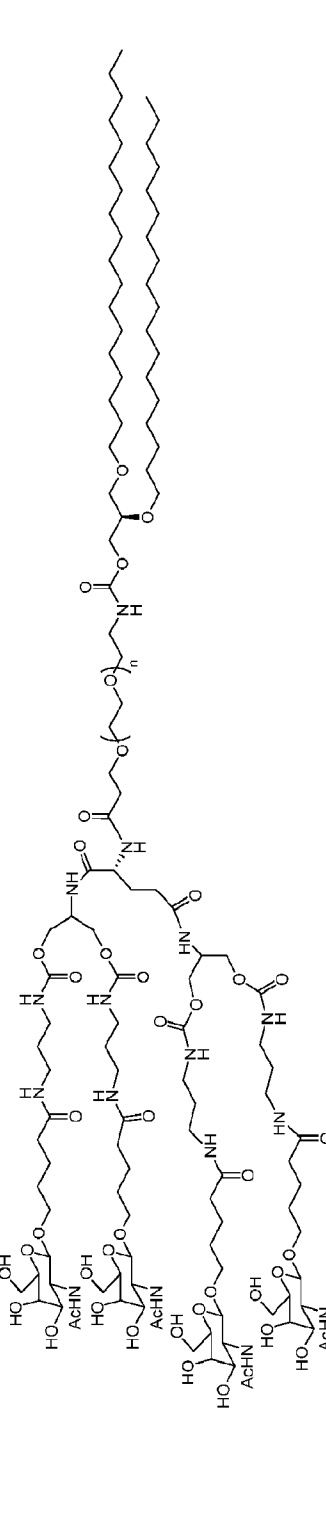
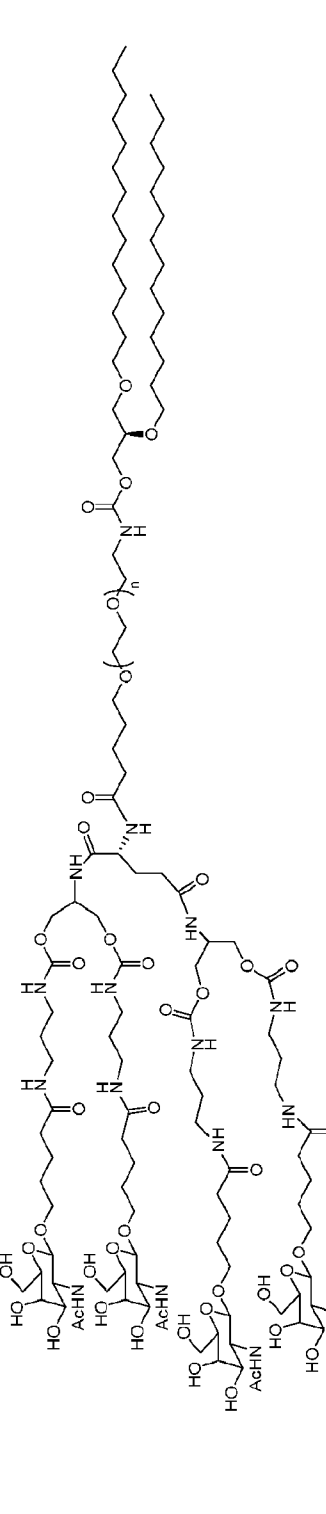
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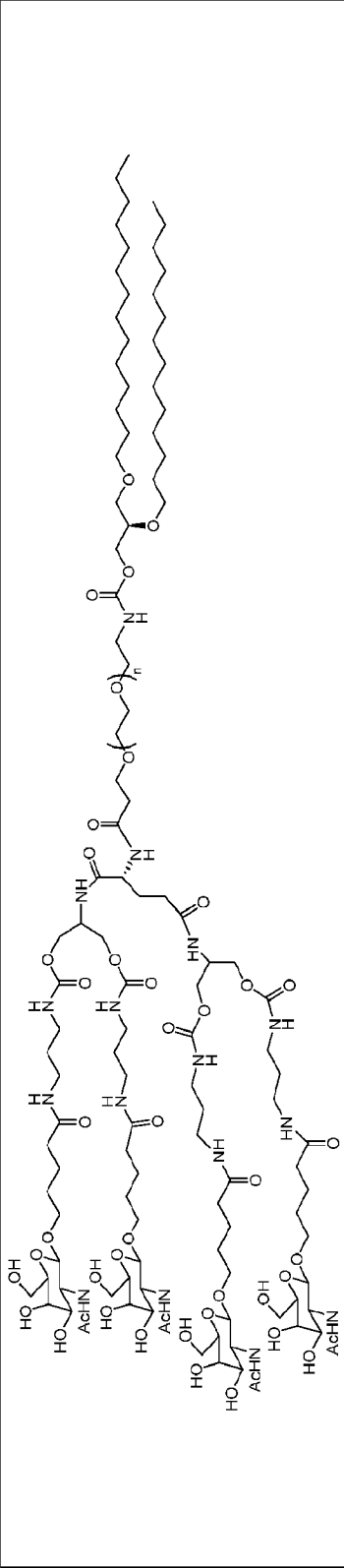
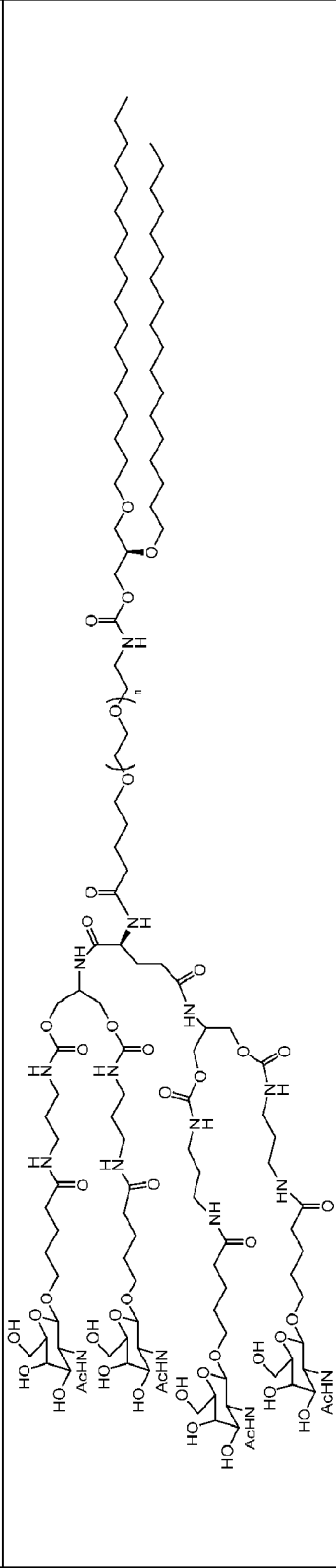
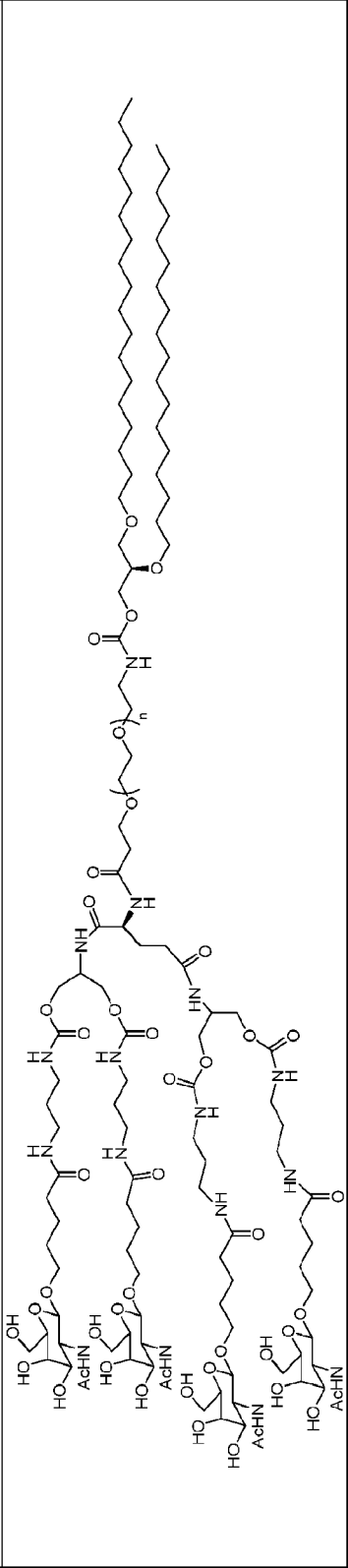
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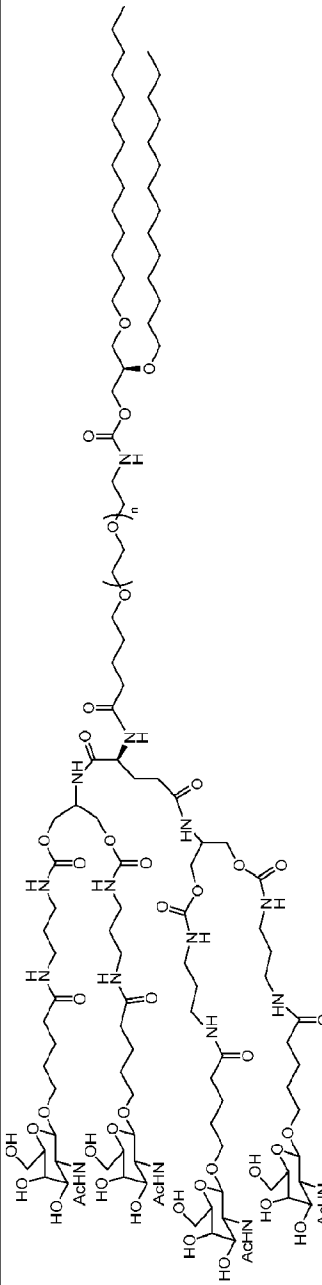
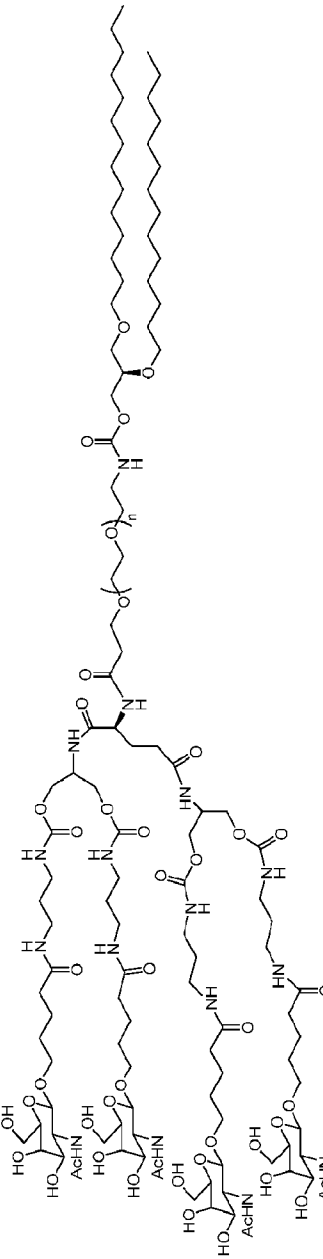
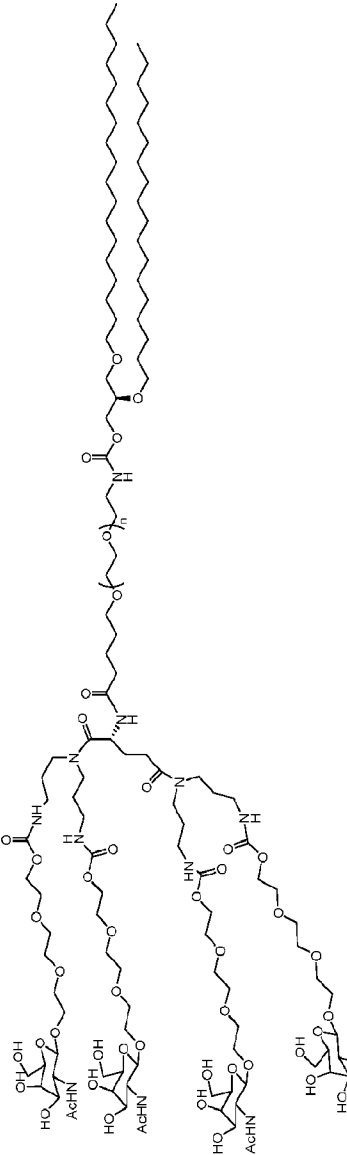
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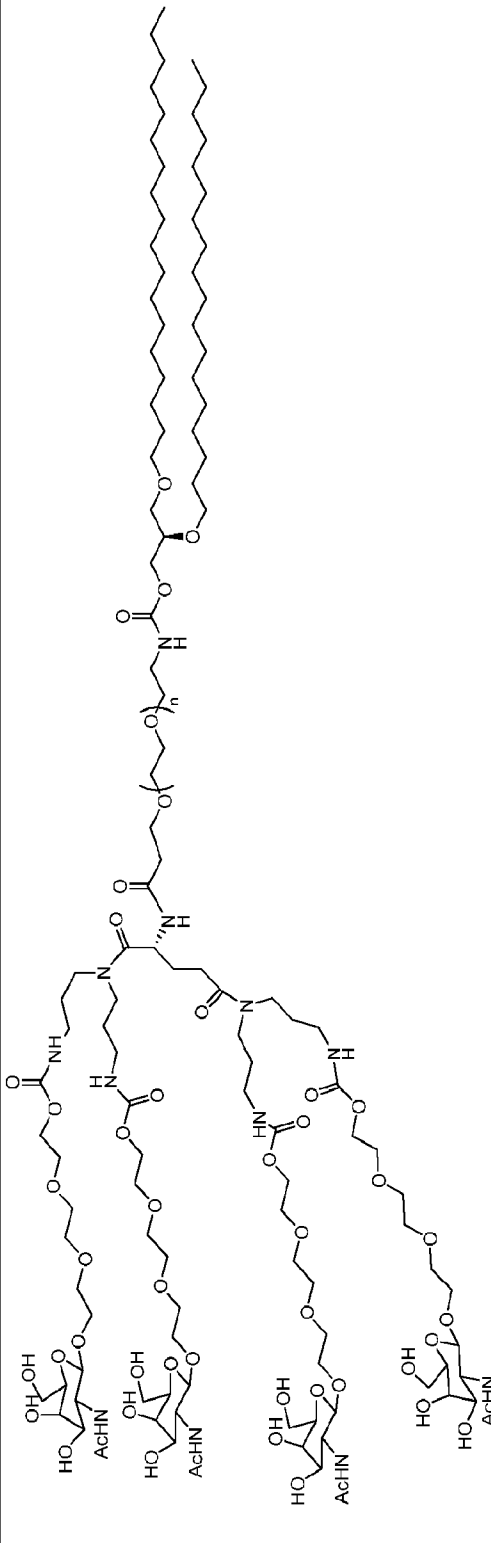
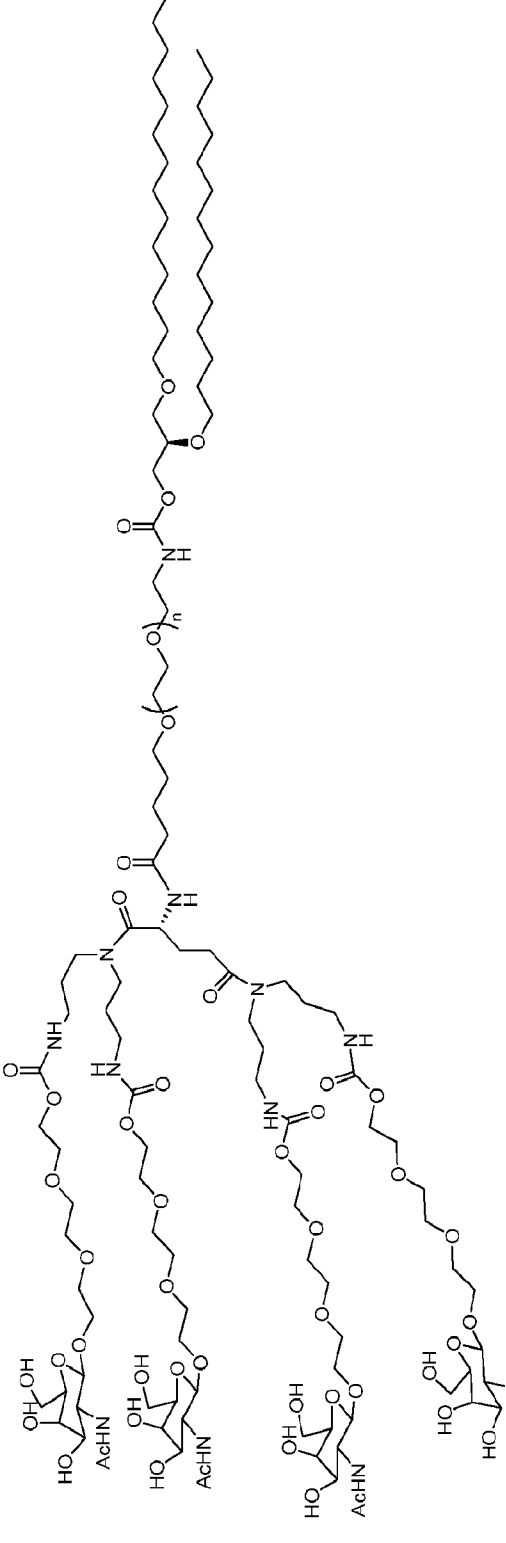
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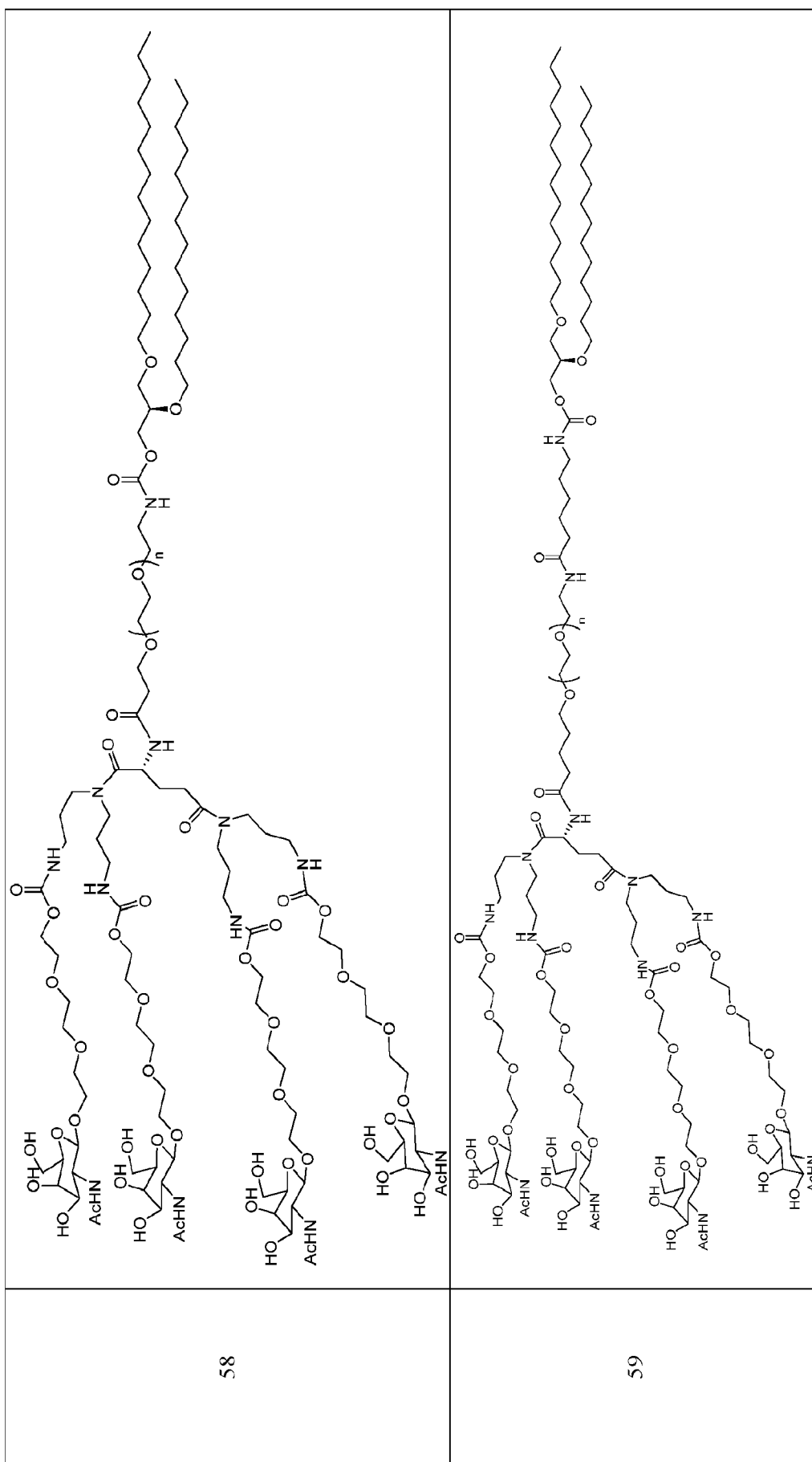
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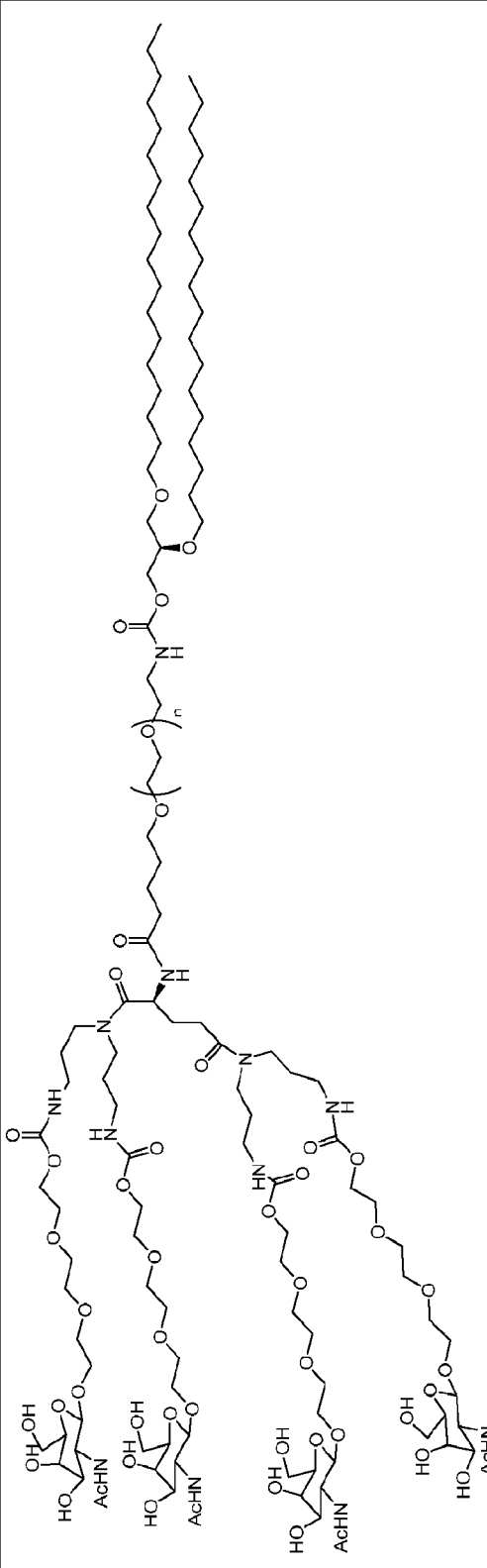
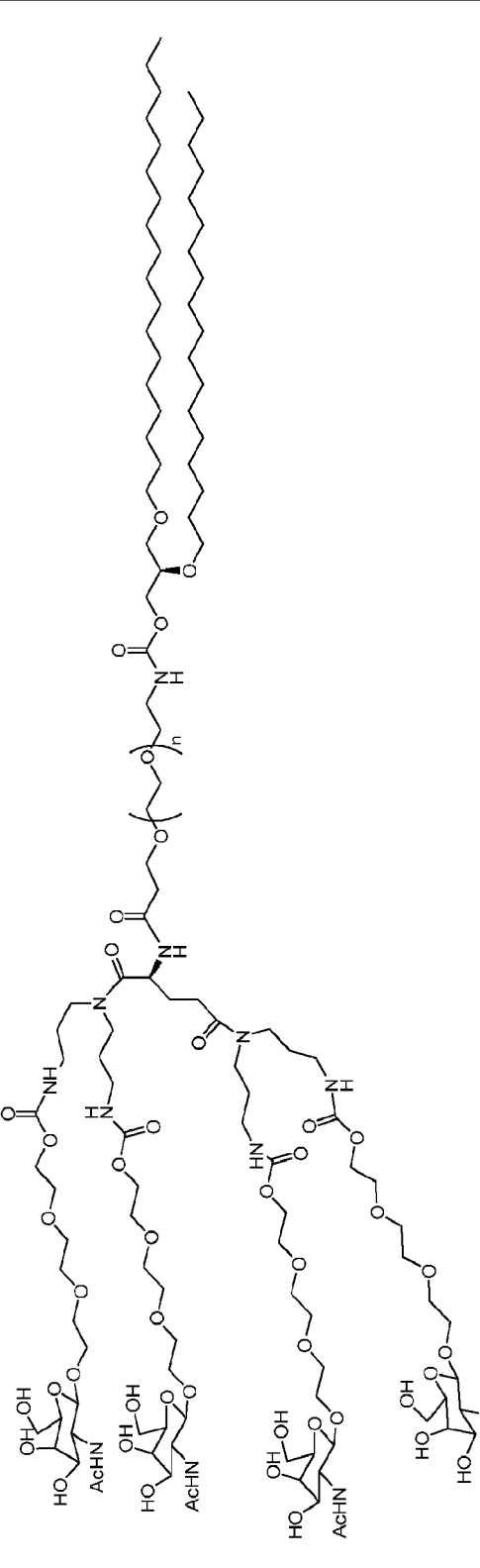
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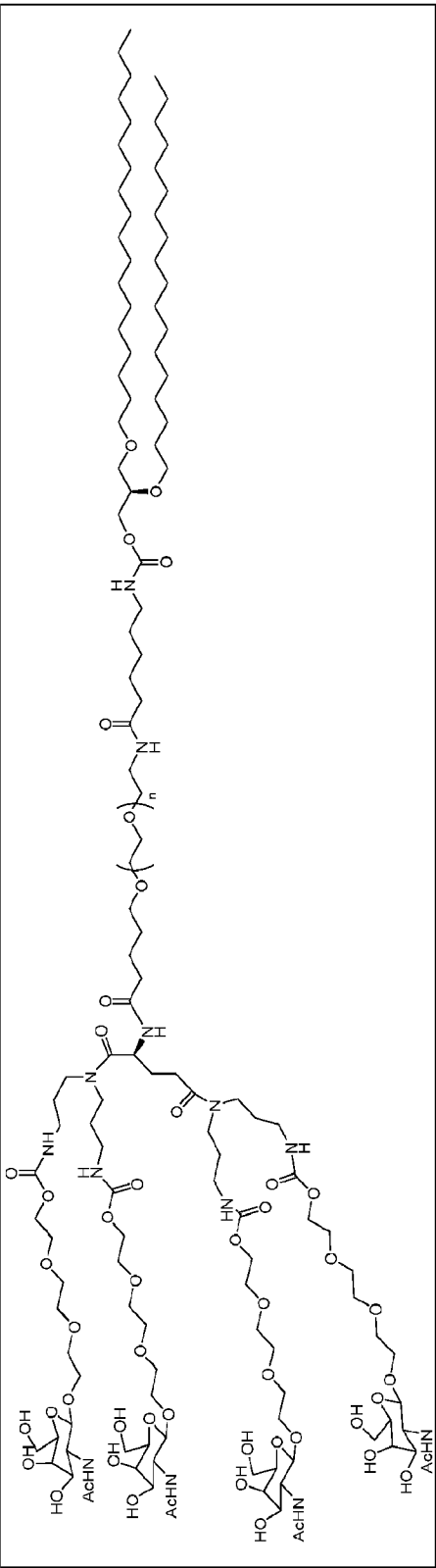
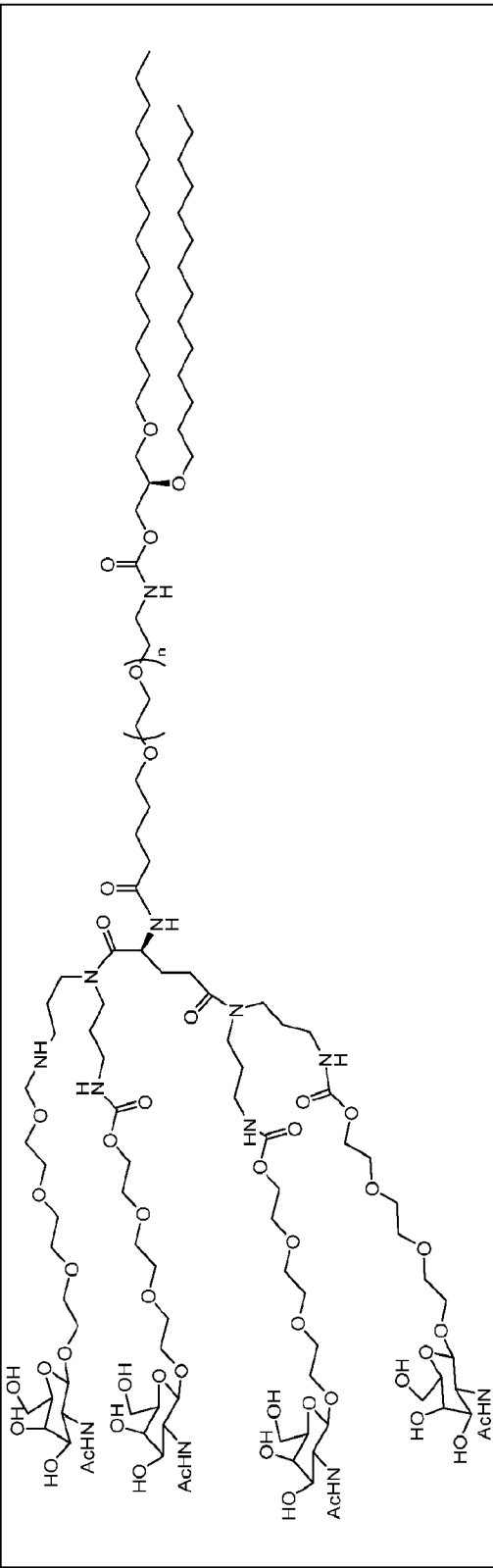
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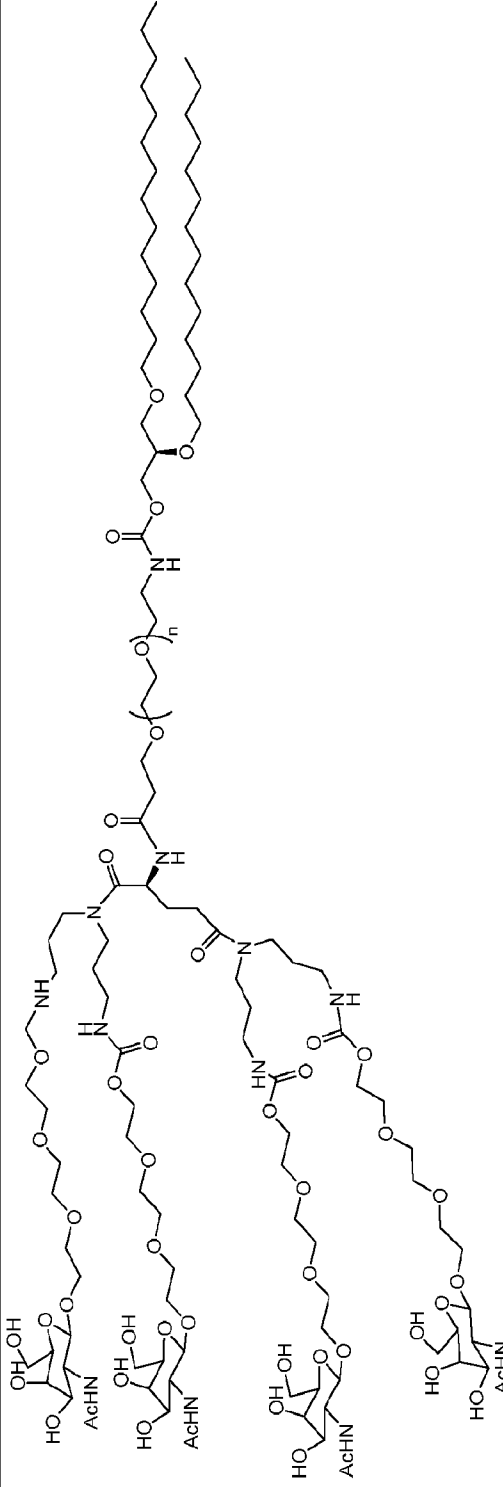
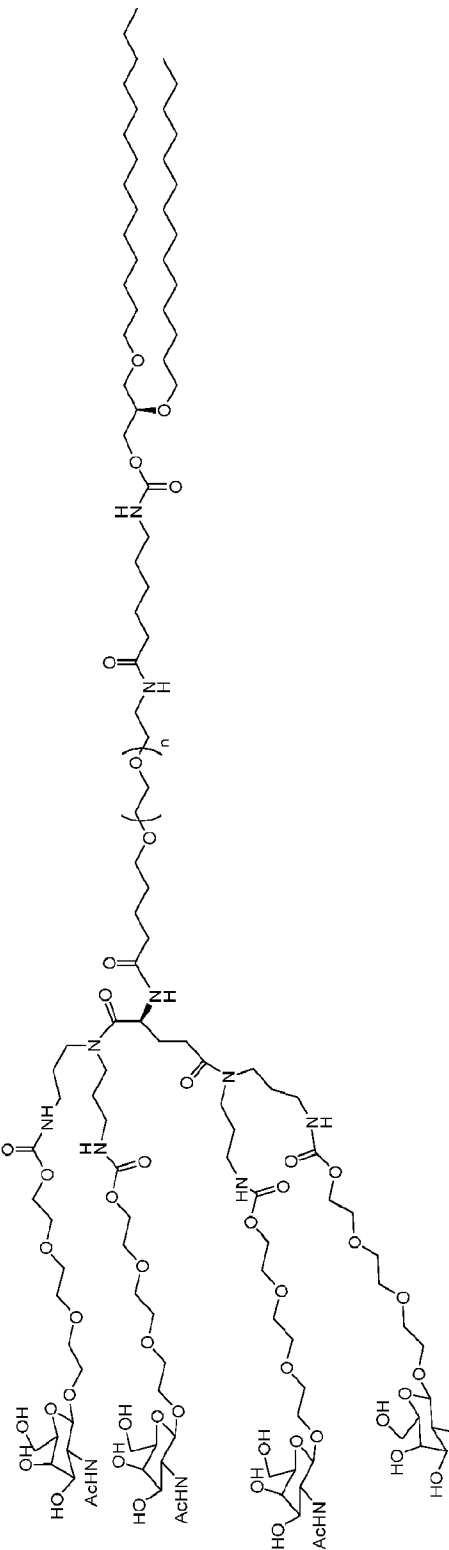
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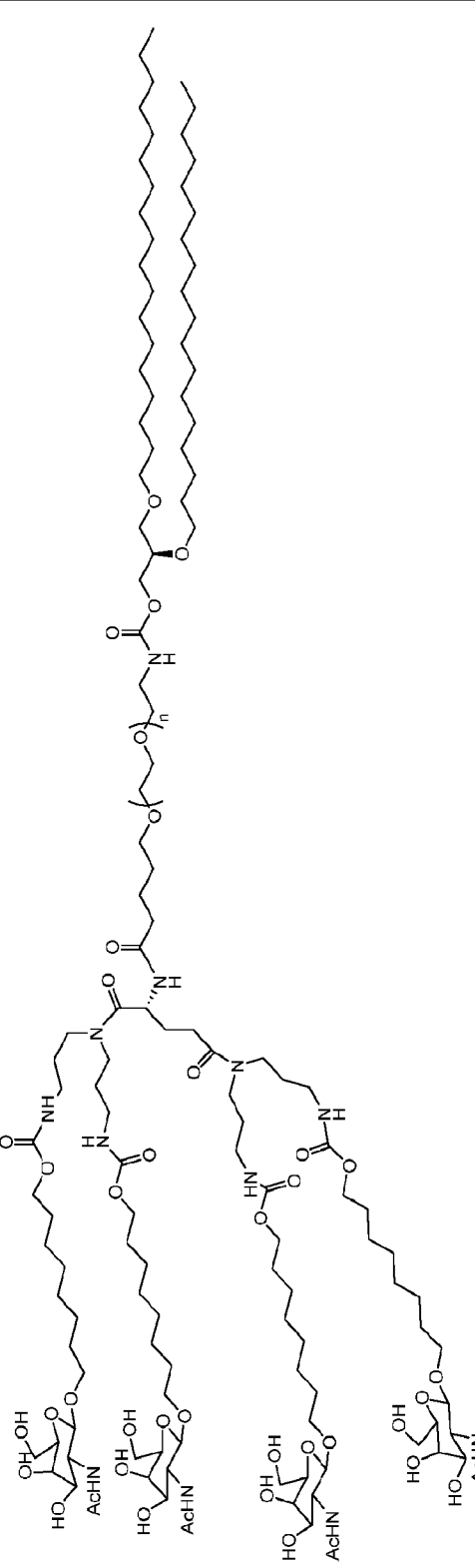
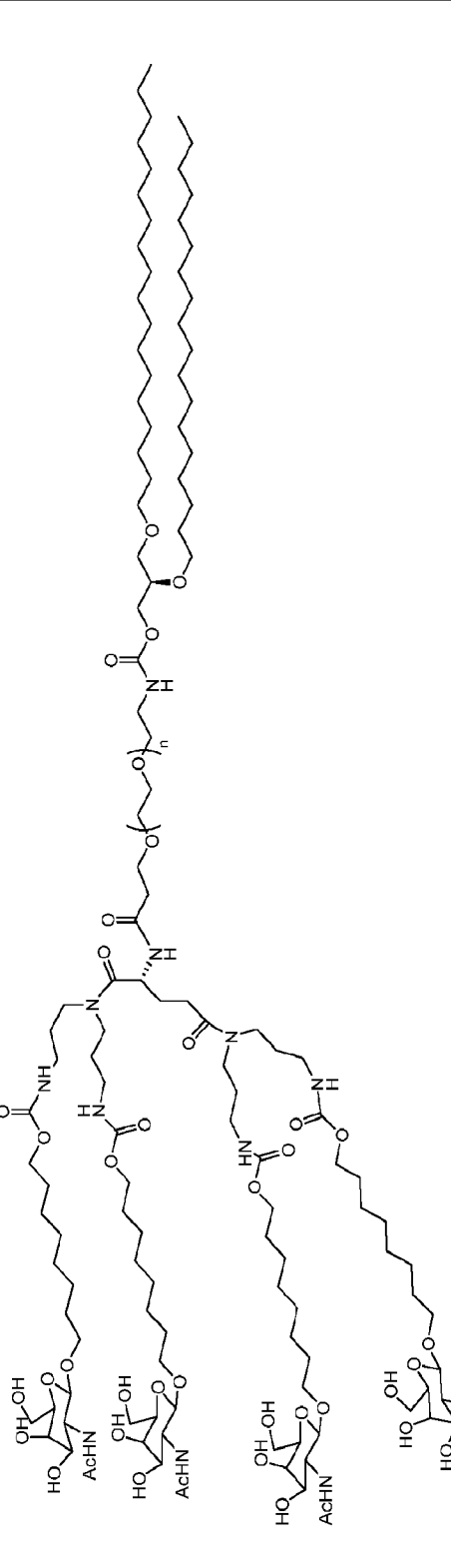
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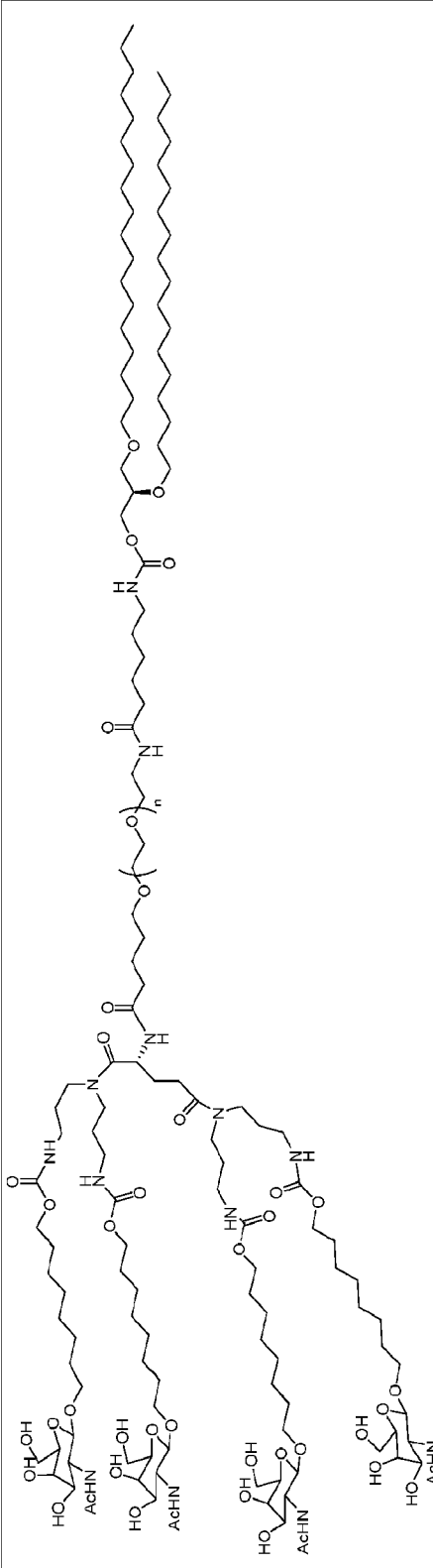
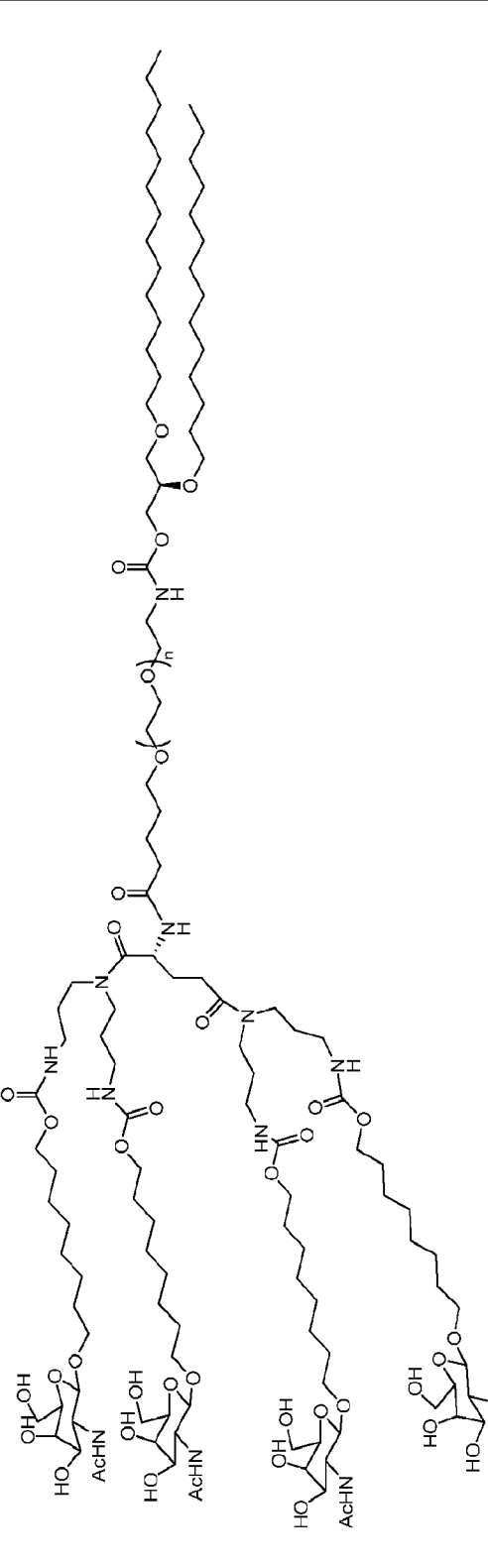


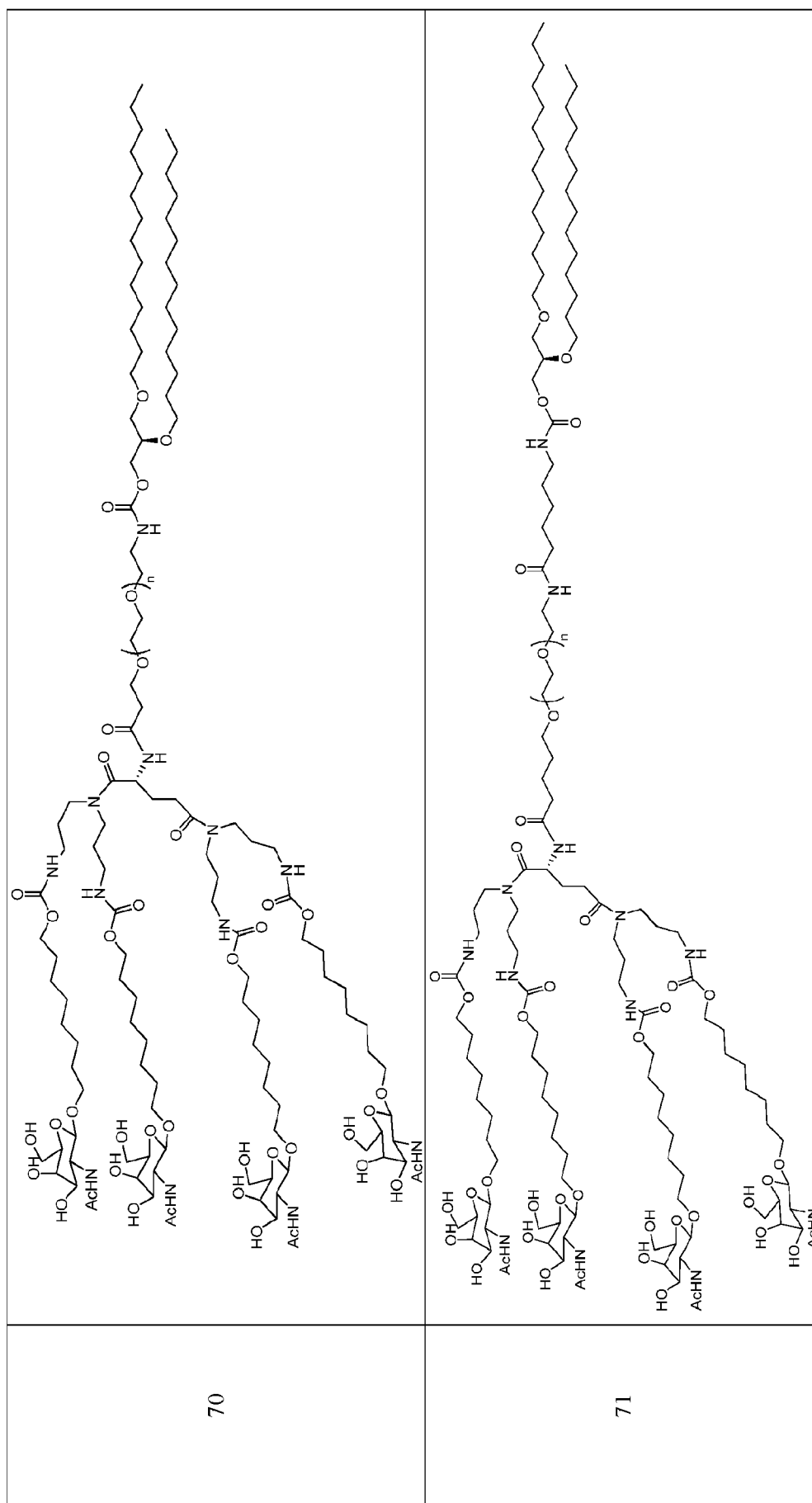
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|  <p>Chemical structure 60: A complex molecule featuring a central core with multiple amide and ether linkages. It includes a long alkyl chain, a polyether segment, and several sugar units (likely glucose) attached via acetal or glycosidic bonds. The structure is highly branched and contains various functional groups including hydroxyl, amide, and ether.</p> |  <p>Chemical structure 61: A complex molecule similar to 60, but with a different arrangement of the central core and side chains. It also features a long alkyl chain, a polyether segment, and sugar units. The branching and functional group distribution differ from structure 60.</p> |
| 60                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 61                                                                                                                                                                                                                                                                                                                                                                             |

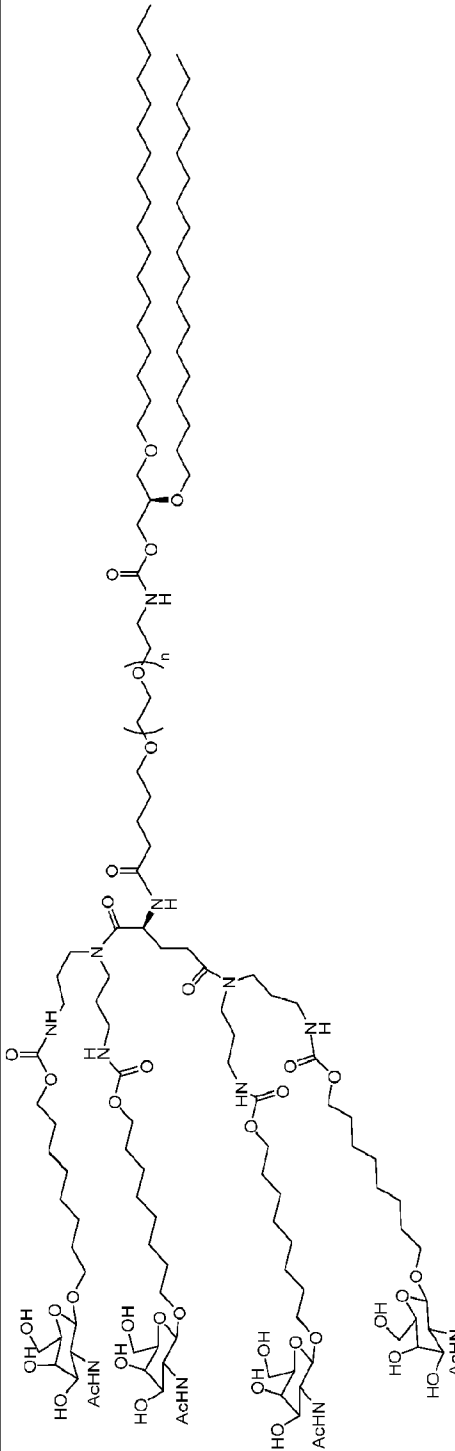
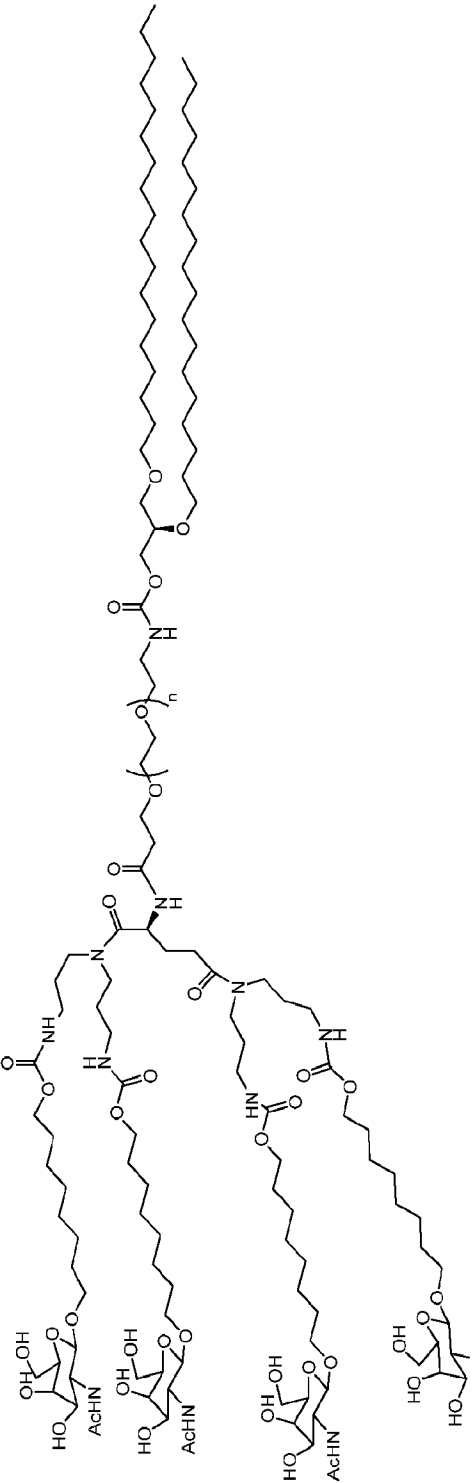
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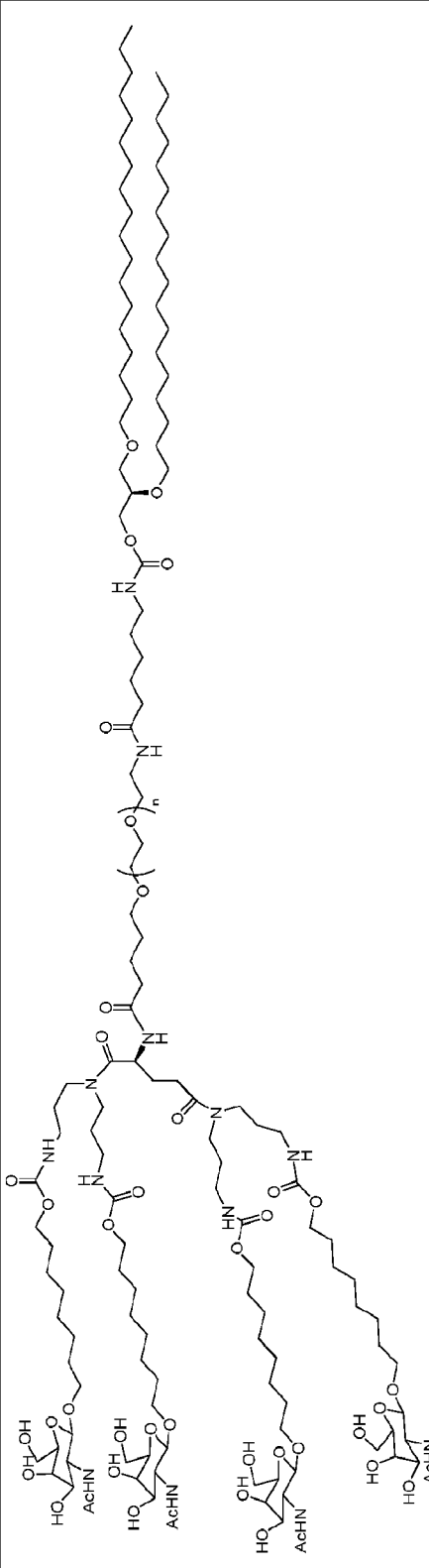
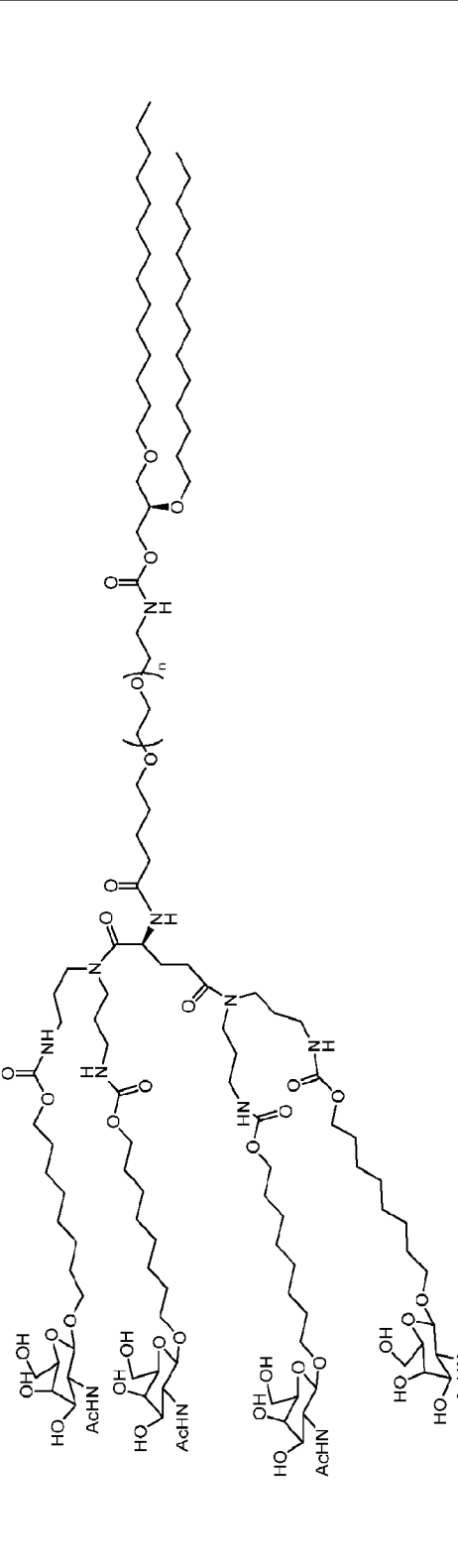
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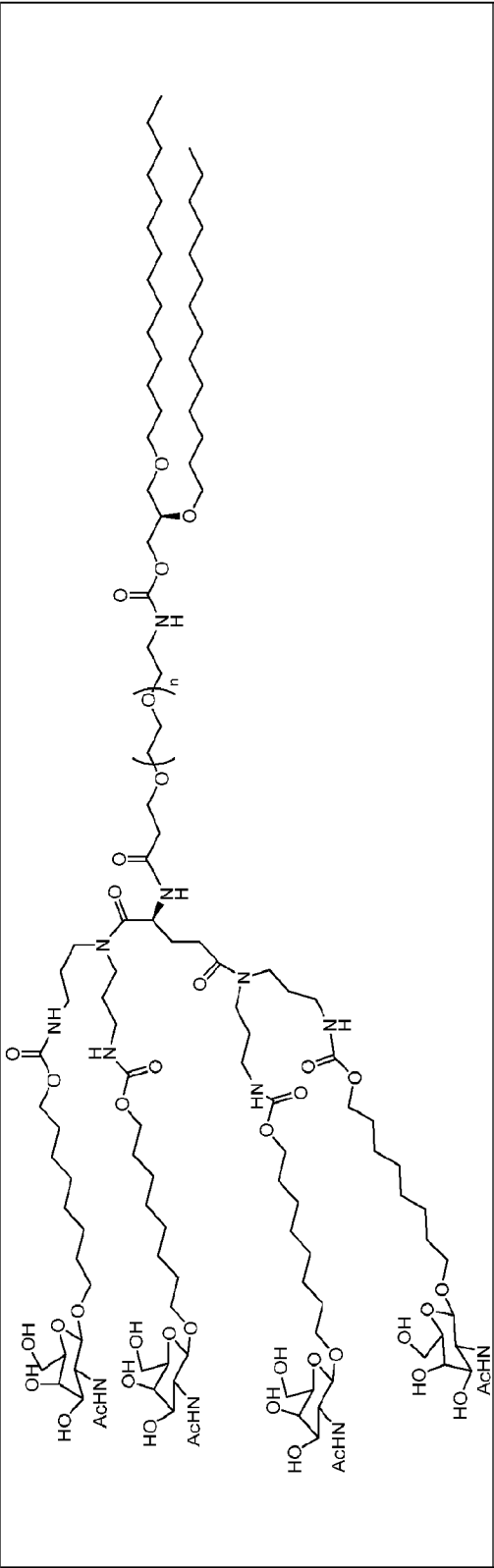
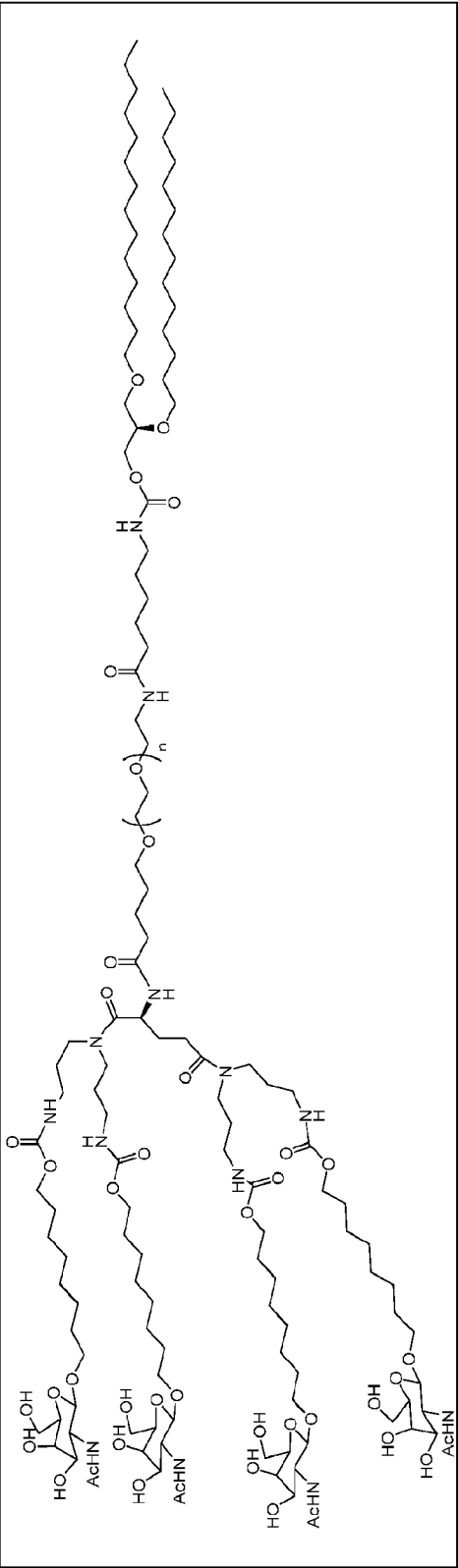
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|  <p>Chemical structure 66: A complex molecule featuring a central core with multiple amide and ether linkages. It includes a long alkyl chain, a polyether segment, and several sugar units (likely glucose) attached via glycosidic bonds. The structure is highly branched and contains various functional groups including hydroxyl, amide, and ether.</p> |  <p>Chemical structure 67: A complex molecule similar to 66, but with a different arrangement of the sugar units and the polyether segment. It also features a long alkyl chain and a central core with amide and ether linkages. The structure is highly branched and contains various functional groups including hydroxyl, amide, and ether.</p> |
| 66                                                                                                                                                                                                                                                                                                                                                                                                                                              | 67                                                                                                                                                                                                                                                                                                                                                                                                                                     |

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|  <p>Chemical structure 68: A complex molecule featuring four acetylcholine (AChN) units linked by ester bonds to a central polyether chain. The chain includes a long alkyl group, a repeating unit with a carbonyl and a hydroxyl group, and a terminal long alkyl group.</p> |  <p>Chemical structure 69: A complex molecule featuring four acetylcholine (AChN) units linked by ester bonds to a central polyether chain. The chain includes a long alkyl group, a repeating unit with a carbonyl and a hydroxyl group, and a terminal long alkyl group.</p> |
| 68                                                                                                                                                                                                                                                                                                                                                               | 69                                                                                                                                                                                                                                                                                                                                                                |

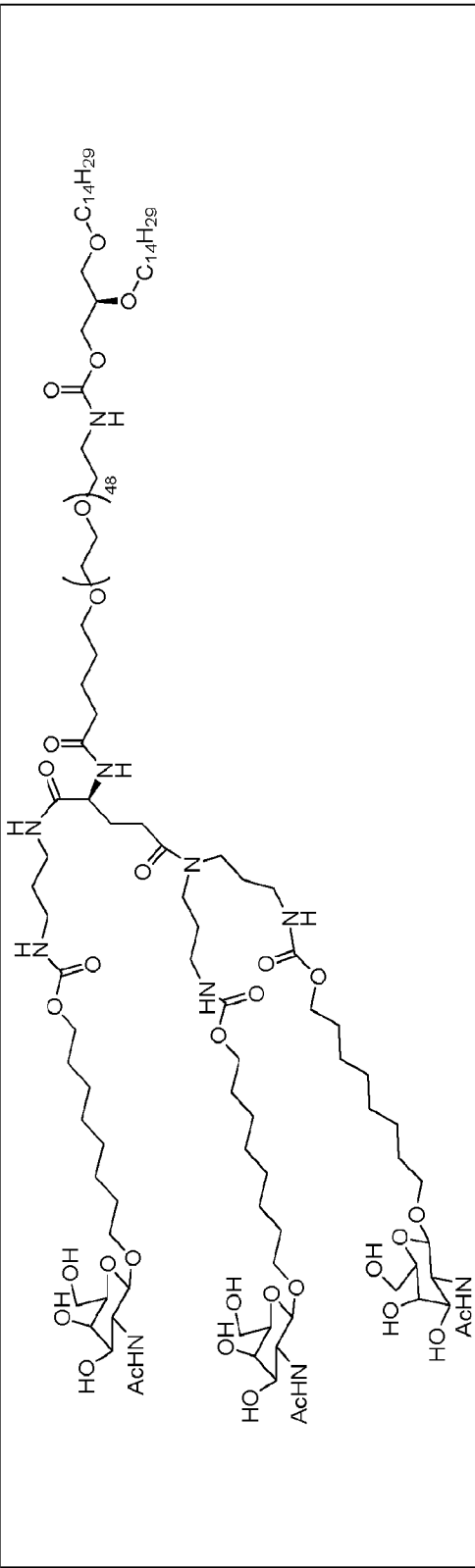
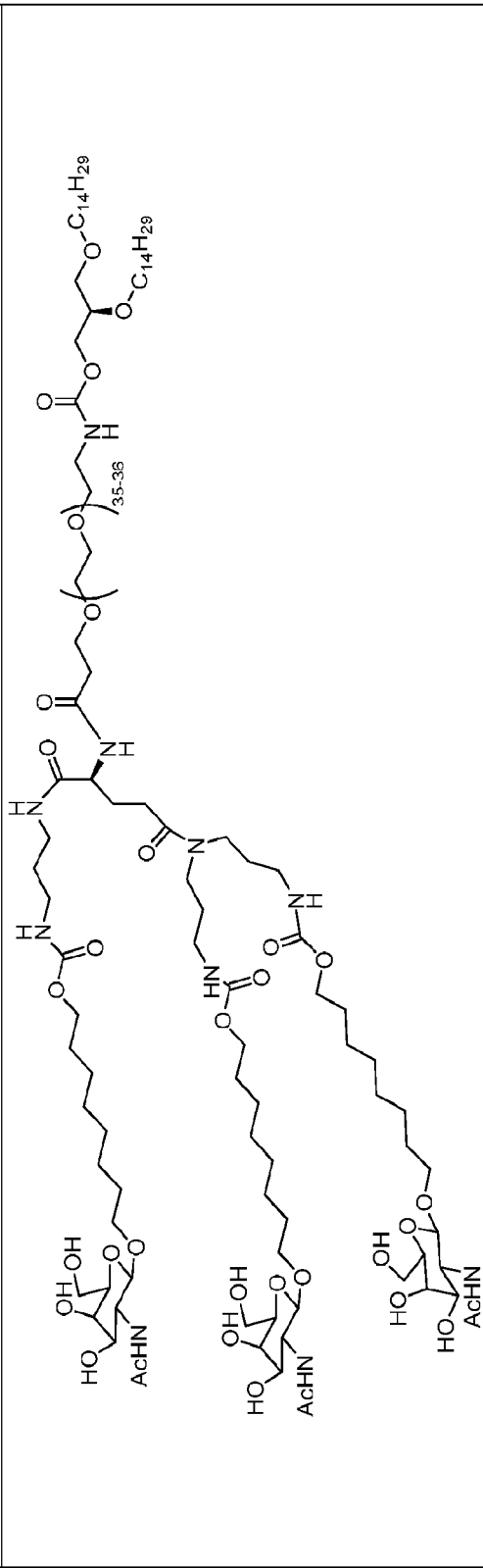


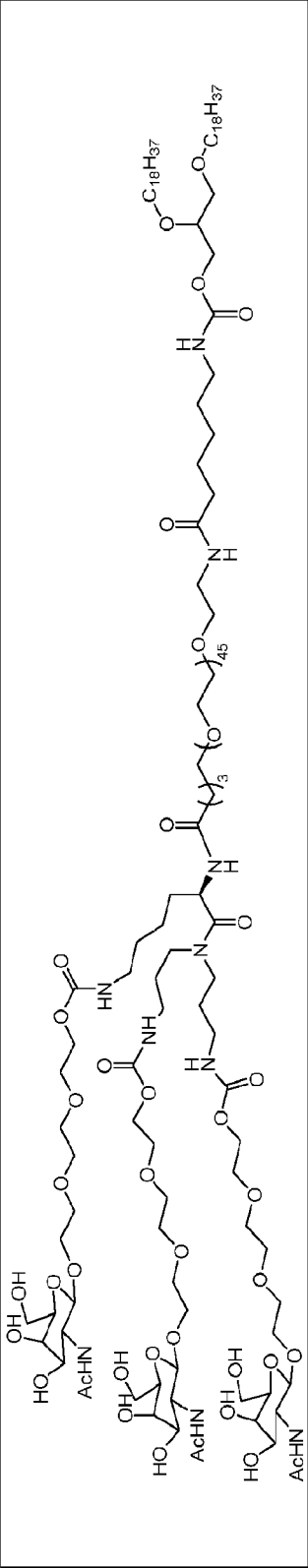
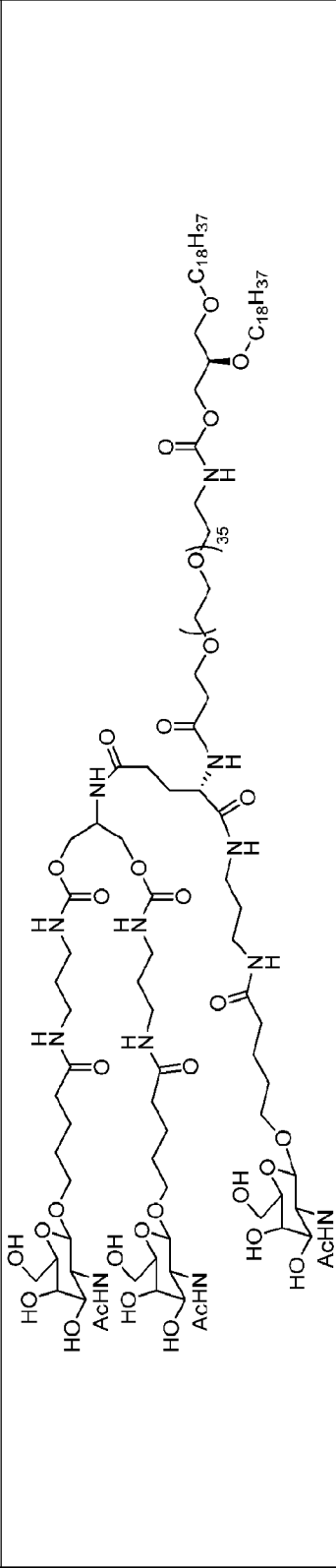
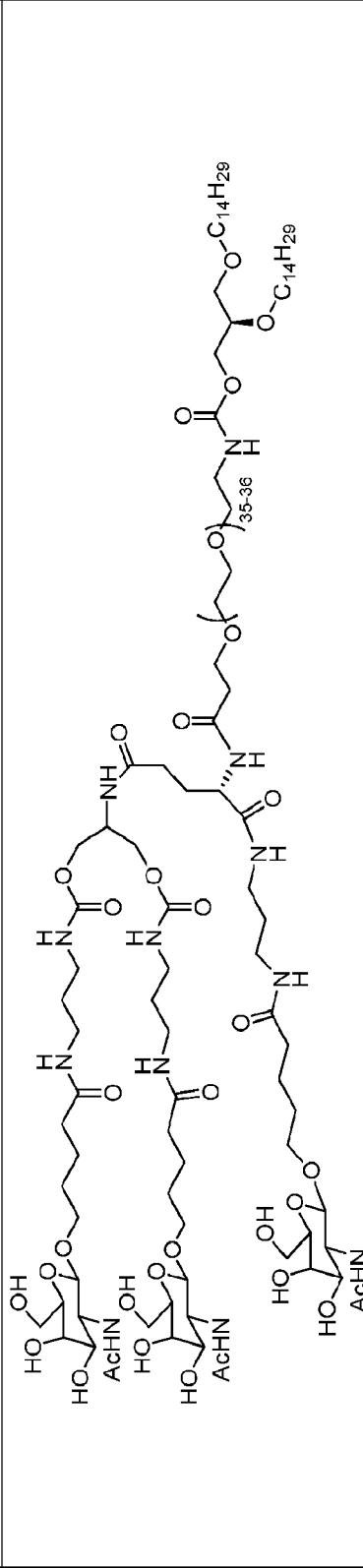
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|  <p>Chemical structure 72 is a complex molecule. It features a central core with multiple hydroxyl groups and acetyl groups. The structure includes a long alkyl chain with an ether linkage and a repeating unit with a subscript 'c'. The molecule is highly branched and contains several functional groups, including amide and ester linkages.</p> |  <p>Chemical structure 73 is a complex molecule, similar to 72, but with a different arrangement of the long alkyl chain and ether linkage. It also features a central core with multiple hydroxyl groups and acetyl groups, and a repeating unit with a subscript 'c'.</p> |
| 72                                                                                                                                                                                                                                                                                                                                                                                                                                        | 73                                                                                                                                                                                                                                                                                                                                                             |

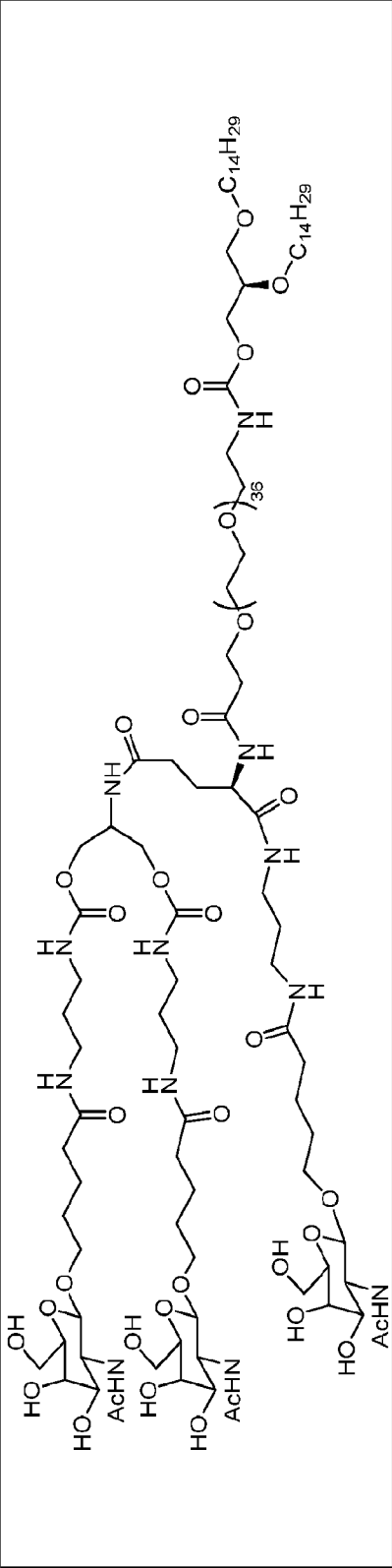
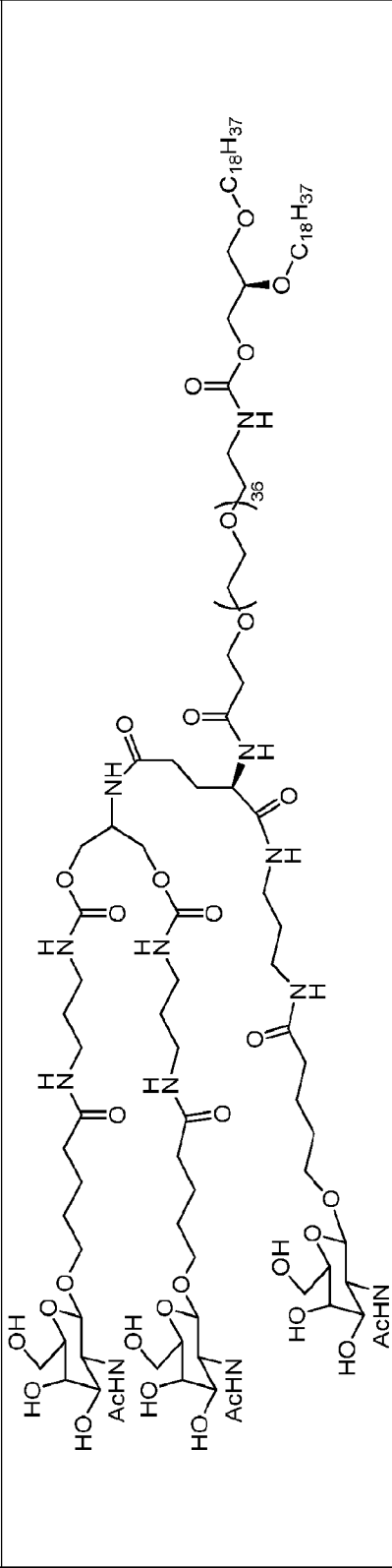
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|  <p>Chemical structure 74: A complex molecule featuring a central core with multiple amide and ether linkages. It includes a long alkyl chain, a polyether segment, and four sugar units (likely glucose) attached via glycosidic bonds. The structure is highly branched and contains several chiral centers.</p> |  <p>Chemical structure 75: A complex molecule similar to 74, but with a different arrangement of the sugar units and the polyether segment. It also features a long alkyl chain and multiple amide and ether linkages. The structure is highly branched and contains several chiral centers.</p> |
| 74                                                                                                                                                                                                                                                                                                                                                                                                   | 75                                                                                                                                                                                                                                                                                                                                                                                  |

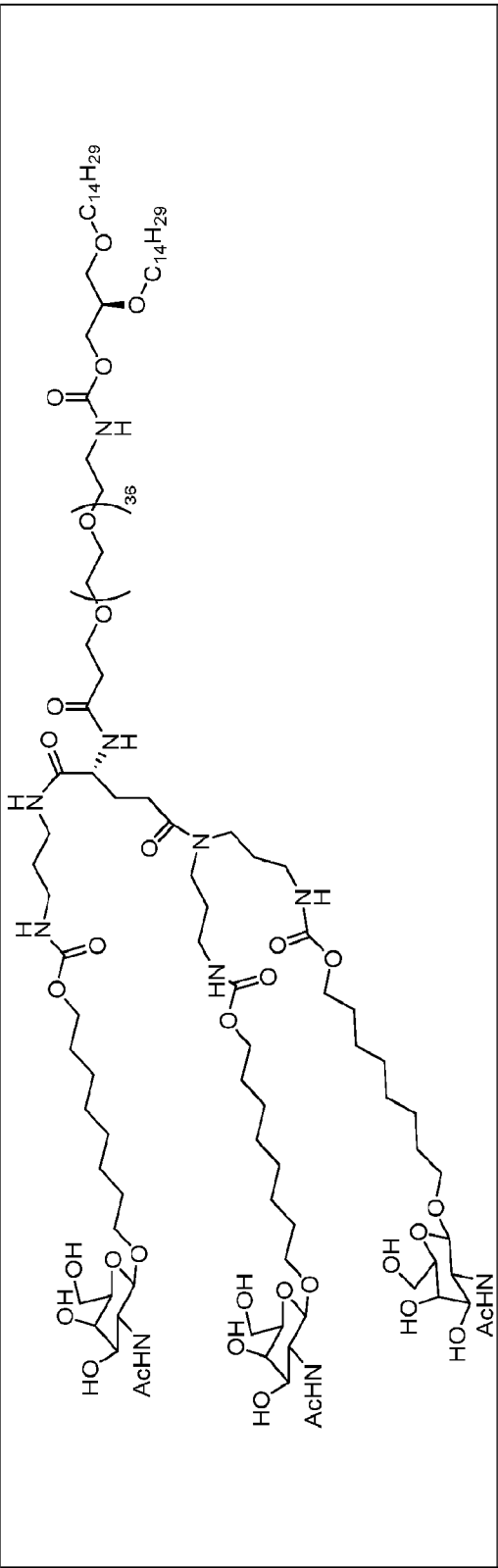
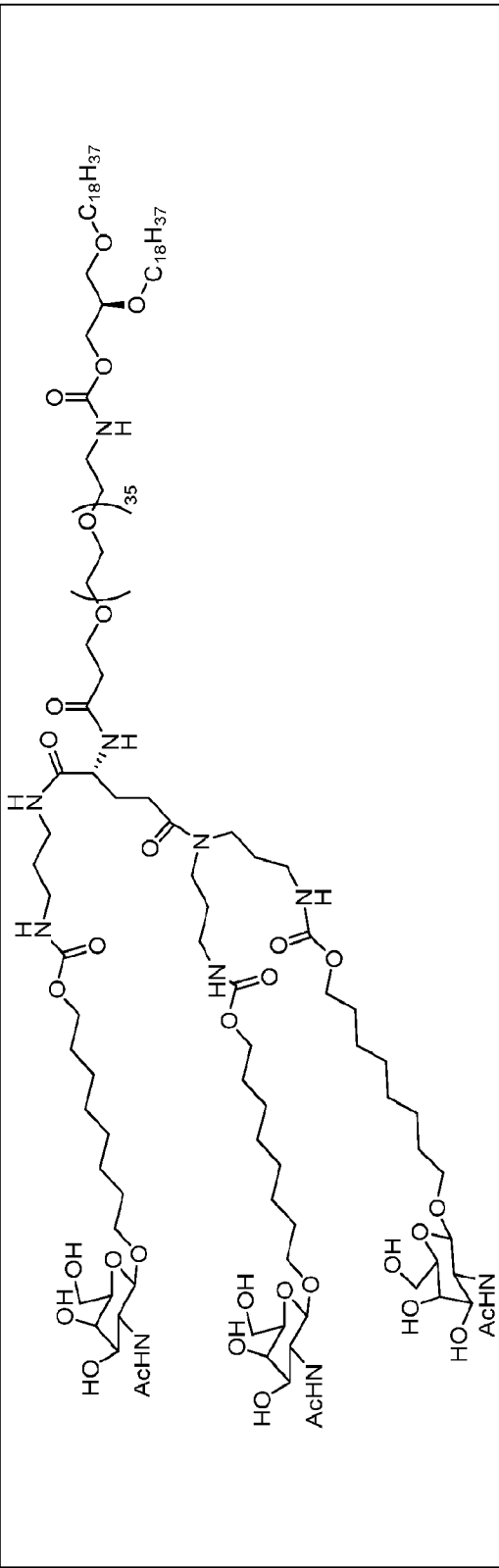
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| <p>Chemical structure 78: A complex molecule featuring a central core with multiple long alkyl chains and sugar moieties. The structure includes a repeating unit with a subscript 35, indicating a polymer chain. The molecule is terminated with a long alkyl chain (C<sub>18</sub>H<sub>37</sub>) and a sugar moiety (AcHN).</p>                                                        | 78 |
| <p>Chemical structure 79: A complex molecule similar to 78, but with a different repeating unit (subscript 47) and a different termination group (C<sub>18</sub>H<sub>37</sub>). The structure includes a repeating unit with a subscript 47, indicating a polymer chain. The molecule is terminated with a long alkyl chain (C<sub>18</sub>H<sub>37</sub>) and a sugar moiety (AcHN).</p> | 79 |

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| <p>89</p> | <p>90</p> |
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wherein each subscript "n" is between 10-200.

63. A pharmaceutically acceptable salt of a compound of any one of claims 1-62.
64. A lipid nanoparticle (LNP) composition comprising a lipid that comprises a compound of any one of claims 1-62.
65. A lipid nanoparticle (LNP) composition comprising:  
a lipid that comprises a compound of any one of claims 1-62;  
a cationic lipid;  
a phospholipid;  
a PEG-containing lipid; and  
a sterol.
66. The LNP composition of claim 65, further comprising a prime editor comprising a DNA binding domain and a DNA polymerase domain, or one or more polynucleotides encoding the prime editor.
67. The LNP composition of claim 65 or 66, further comprising a prime editing guide RNA (PEgRNA) or a polynucleotide encoding the PEgRNA.
68. The LNP composition of any one of claims 65-67, further comprising a nick guide RNA (ngRNA) or a polynucleotide encoding the ngRNA.
69. The LNP composition of any one of claims 65-68, further comprising a second PEgRNA or a polynucleotide encoding the second PEgRNA.
70. A pharmaceutical composition comprising the LNP composition of any one of claims 65-69.
71. A method for delivering polynucleotides into a target cell comprising:  
introducing into the target cell at least one lipid nanoparticle comprising a lipid that comprises the compound of any one of claims 1-62 and one or more polynucleotides.
72. A method for delivering a prime editing system into a target cell comprising:

introducing into the target cell at least one lipid nanoparticle comprising a lipid that comprises the compound of any one of claims 1-62 and one or more components of a prime editing system.

73. The method of claim 72, wherein the one or more components of a prime editing system comprises a construct encoding prime editor components.

74. The method of claim 72, wherein the one or more components of a prime editing system comprises an mRNA encoding prime editor components.

75. The method of claim 73 or 74, wherein the prime editor components comprise a prime editing guide RNA (PEgRNA), and optionally a nick guide RNA (ngRNA).

76. The method of claim 72 or 74, wherein the prime editor components comprise a PEgRNA pair, wherein the PEgRNA comprises a first PEgRNA and/or a second PEgRNA.

77. The method of claim 72, wherein the one or more components of a prime editing system comprises a construct encoding a prime editor fusion protein or an mRNA encoding a prime editor fusion protein.

78. The method of claim 72, wherein the one or more components of a prime editing system comprises one or more constructs encoding one or more of a prime editor fusion protein, PEgRNA, ngRNA, or a PEgRNA pair.

79. The method of claim 72, wherein the one or more components of a prime editing system comprises a prime editor fusion protein, a PEgRNA, a ngRNA, or a PEgRNA pair.

80. The LNP composition of any one of claims 65-69, wherein the LNP composition is formulated to deliver the at least one polynucleotide or one or more components of the prime editing system to target cells.

81. The method of any one of claims 71-79 or the LNP composition of claim 80, wherein the target cells are or comprise human cells, mammalian cells, liver cells, bone marrow cells, lung cells, eye cells, muscle cells, or cells in the central nervous system.

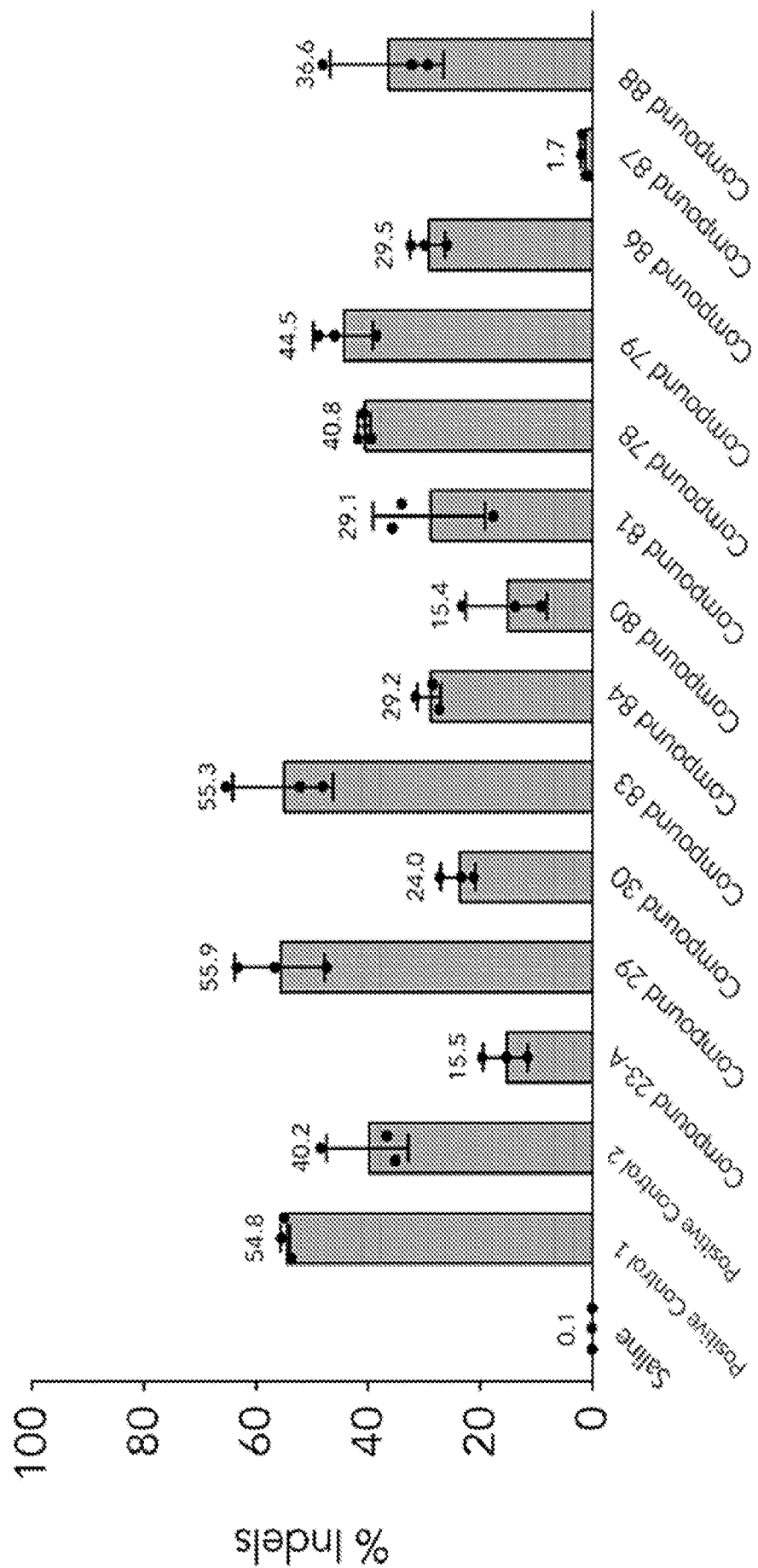


FIG. 1

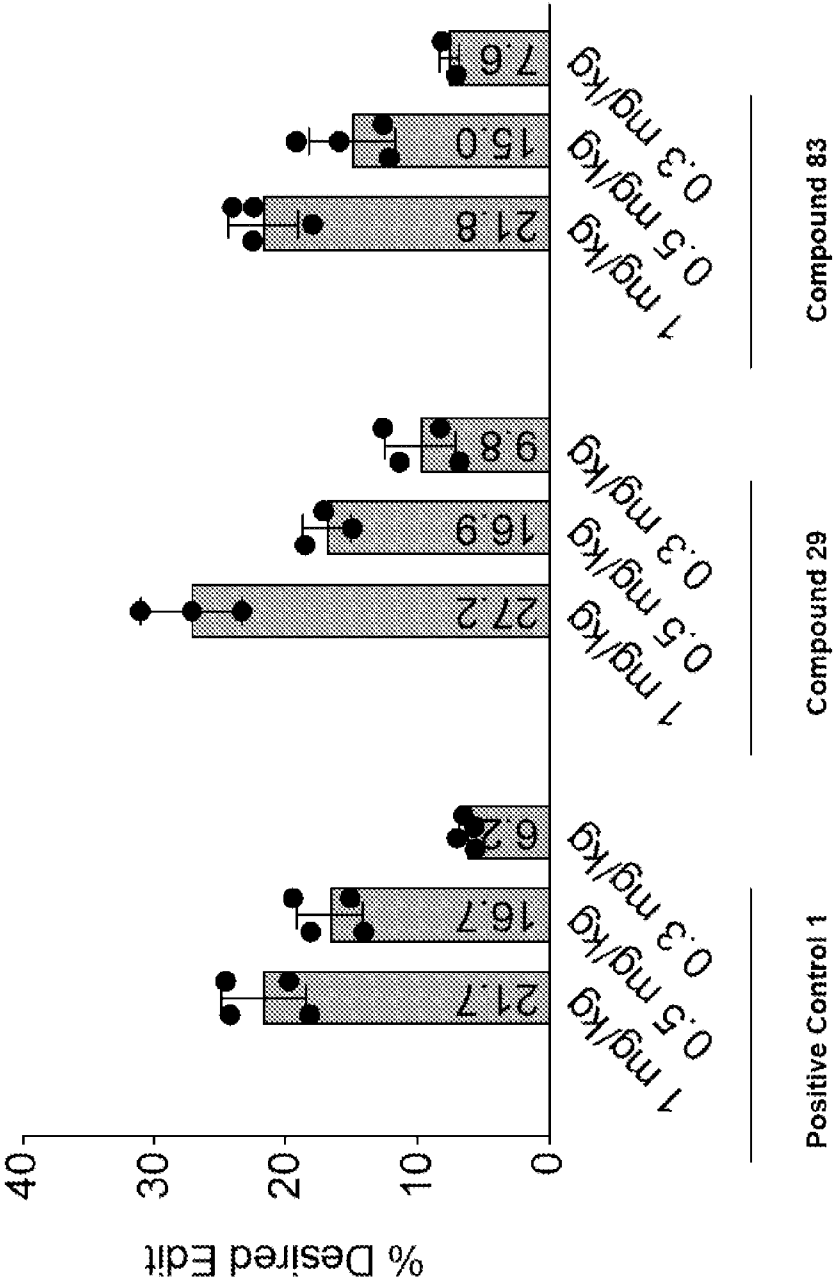


FIG. 2