

LIVER ASIALOGLYCOPROTEIN RECEPTOR TARGETED LIPID AND USES THEREOF

FIELD OF THE INVENTION

The invention relates to lipids comprising an N-acetylgalactosamine that targets
5 liver asialoglycoprotein receptors.

BACKGROUND OF THE INVENTION

Compounds containing N-acetylgalactosamine moieties are known to bind to the
liver asialoglycoprotein receptor (ASGR). However, these compounds are typically fairly
hydrophobic and therefore less effective for drug or oligonucleotide delivery to a person
10 in need thereof.

H. Cui et al.; *ACS Omega* 2021, 6, 16259–16265 provides a review of liver-
targeted delivery of oligonucleotides with N-acetylgalactosamine conjugation.

K. Godbout et al.; *Pharmaceutics* 2022, 14, 2129 provides an overview of
delivery of RNAs to specific organs by lipid nanoparticles for gene therapy.

15 L. N. Kasiewicz et al.; *Nature Communications*, (2023) 14:2776 discloses GalNAc-
lipid nanoparticles to enable non-LDLR (low density lipoprotein receptor) dependent
hepatic delivery of a CRISPR base editing therapy.

Y. Zhou et al. *ACS Cent. Sci.* 2021, 7, 499–506; discloses triantennary
N-acetylgalactosamine conjugates as degraders for extracellular proteins.

20 CA 02930393 A1 discloses carbohydrate conjugates as delivery agents for
oligonucleotides.

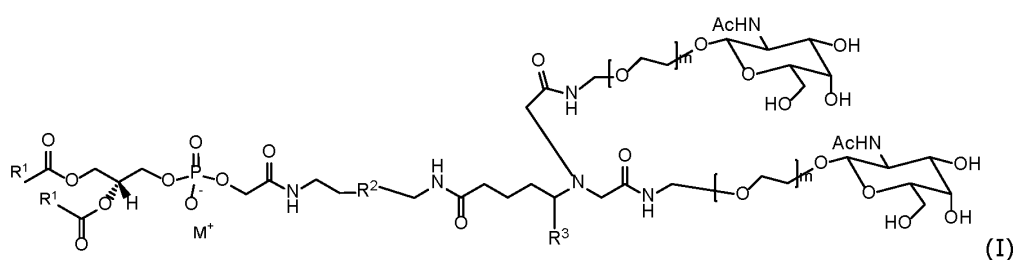
The present disclosure provides liver asialoglycoprotein receptor targeted lipid
compounds containing hydrophilic linker moieties that overcome certain drawbacks
associated with the hydrophobic lipid moiety. These compounds provide a method of
25 delivering therapeutic drugs or oligonucleotides to the liver via the liver
asialoglycoprotein receptors.

SUMMARY OF THE INVENTION

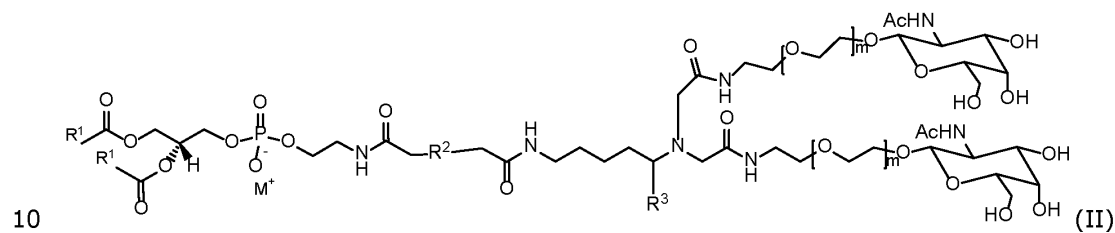
The present disclosure is directed to lipid compounds that are bound to three
N-acetylgalactosamine units. The lipid compounds target the liver asialoglycoprotein
30 receptor and are useful for delivery of therapeutic agents, such as drugs or
oligonucleotides, to a person in need thereof. According to a particular embodiment, the
lipid compounds are used to form lipid nanoparticles that deliver the therapeutic agents,
such as drugs or oligonucleotides, to a person in need thereof. According to another

embodiment, the lipid compounds are used to form liposomes that deliver the therapeutic agents, such as drugs or oligonucleotides, to a person in need thereof. According to another embodiment, the lipid compounds are used to form micelles that deliver the therapeutic agents, such as drugs or oligonucleotides, to a person in need thereof.

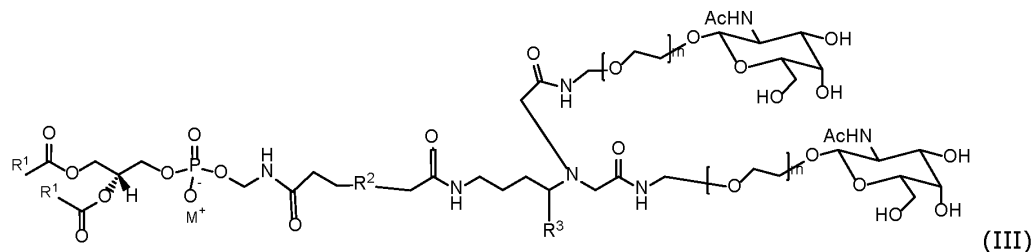
According to an embodiment, the compounds have the structure (I), (II), (III), or (IV), or a combination of any two or more thereof.



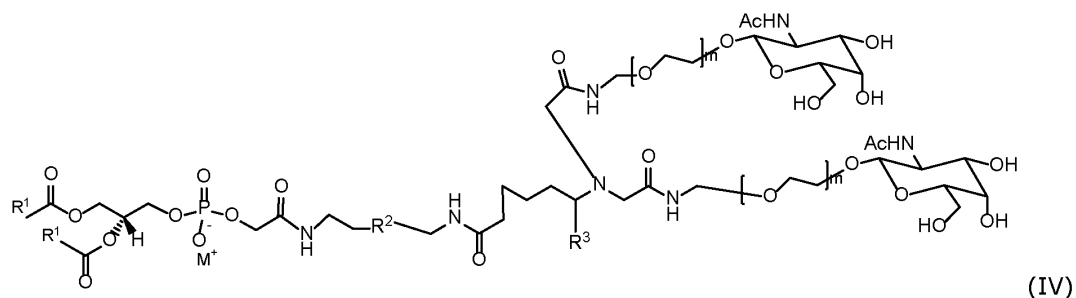
(I)



(II)

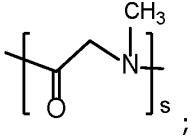


(III)

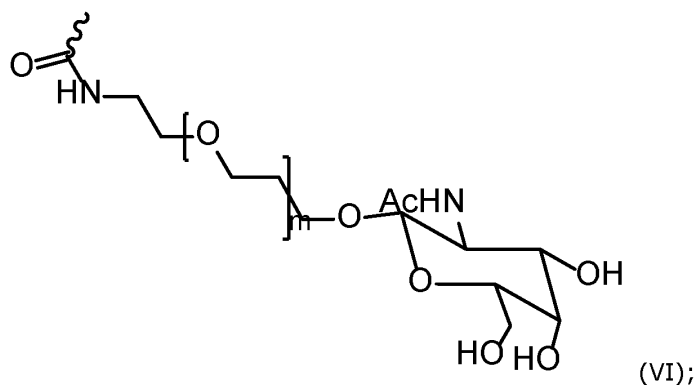
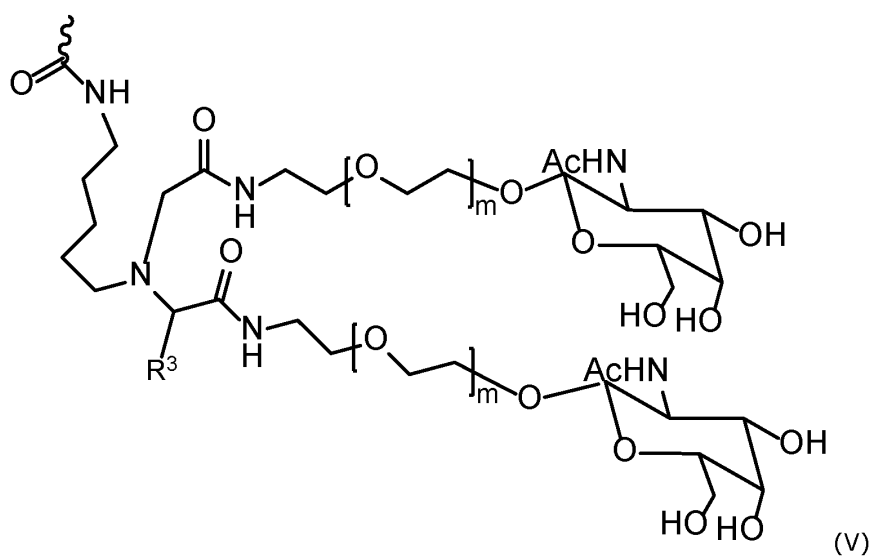


(IV)

In these structures, (I), (II), (III), and (IV), each R^1 is independently selected from aliphatic alkyl C_4 - C_{100} groups optionally substituted with one or more of alkenyl, alkynyl, hydroxyl, amide, ester, and/or ether groups;

R^2 is selected from $[-OCH_2CH_2-]_p$, $[-OCH_2CH_2-]_p-O-$, or  ;

5 R^3 is selected from Formula (V) and Formula (VI):



M^+ is selected from an alkali metal ion, an alkaline earth metal ion, or a primary, secondary, or tertiary ammonium ion; and

10 m , p , and s are independently selected from integers from 1 to 120.

According to an embodiment, the compounds having the structure (I), (II), (III), or (IV) do not have more than 3, 5, 7, 9, 11, 13, or 15 moieties of structure (VI). In

other words, the compounds having the structure (I), (II), (III), or (IV) do not have more than 3, 5, 7, 9, 11, 13, or 15 N-acetylgalactosamine moieties per molecule.

A liver asialoglycoprotein receptor-targeted therapeutic agent comprising a drug or oligonucleotide in a lipid nanoparticle, a liposome or a micelle including a compound
5 having the structure (I), (II), (III), or (IV), or a combination of any two or more thereof is provided.

A method of treatment, comprising administering the liver asialoglycoprotein receptor-targeted therapeutic agent to a patient in need thereof is also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

10 The Figure shows a high-performance liquid chromatogram of a compound according to an embodiment of the invention.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used herein, the terms "prevention", "prevent", "preventing", "suppression",
15 "suppress" and "suppressing" as used herein refer to a course of action (such as administering a compound or pharmaceutical composition) initiated prior to the onset of a symptom, aspect, or characteristics of a disease or condition so as to prevent or reduce such symptom, aspect, or characteristics. Such preventing and suppressing need not be absolute to be useful.

20 As used herein, the terms "treatment", "treat" and "treating" as used herein refers a course of action (such as administering a compound or pharmaceutical composition) initiated after the onset of a symptom, aspect, or characteristics of a disease or condition so as to eliminate or reduce such symptom, aspect, or characteristics. Such treating need not be absolute to be useful.

25 As used herein, the term "in need of treatment" as used herein refers to a judgment made by a caregiver that a patient requires or will benefit from treatment.

This judgment is made based on a variety of factors that are in the realm of a caregiver's expertise, but that includes the knowledge that the patient is ill, or will be ill, as the result of a disease or condition that is treatable by a method or compound of the
30 disclosure.

As used herein, the term "in need of prevention" as used herein refers to a judgment made by a caregiver that a patient requires or will benefit from prevention. This judgment is made based on a variety of factors that are in the realm of a caregiver's expertise, but that includes the knowledge that the patient will be ill or may

become ill, as the result of a disease or condition that is preventable by a method or compound of the disclosure.

As used herein, the terms "individual", "subject" or "patient" as used herein refers to any animal, including mammals, such as mice, rats, other rodents, rabbits, dogs, cats, swine, cattle, sheep, horses, or primates, and humans. The term may specify male or female or both or exclude male or female.

As used herein, the term "therapeutically effective amount" as used herein refers to an amount of a compound, either alone or as a part of a pharmaceutical composition, that is capable of having any detectable, positive effect on any symptom, aspect, or characteristics of a disease or condition. Such effect need not be absolute to be beneficial.

As used herein, the term "alkyl", whether used alone or as part of a substituent or linking group, includes straight hydrocarbon groups comprising from one to 100 carbon atoms. Thus, the phrase includes straight chain alkyl groups such as methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl and the like. The phrase also includes branched chain isomers of straight chain alkyl groups, including but not limited to, the following which are provided by way of example:
—CH(CH₃)₂, —CH(CH₃)(CH₂CH₃), —CH(CH₂CH₃)₂, —C(CH₃)₃, —C(CH₂CH₃)₃,
—CH₂CH(CH₃)₂, —CH₂CH(CH₃)(CH₂CH₃), —CH₂CH(CH₂CH₃)₂, —CHC(CH₃)₃,
—CH₂C(CH₂CH₃)₃, —CH(CH₃)CH(CH₃)(CH₂CH₃), —CH₂CH₂CH(CH₃)₂,
—CH₂CH₂CH(CH₃)(CH₂CH₃)—, CH₂CH₂CH(CH₂CH₃)₂, —CH₂CH₂C(CH₃)₃,
—CH₂CH₂C(CH₂CH₃)₃, —CH(CH₃)CH₂CH(CH₃)₂, —CH(CH₃)CH(CH₃)CH(CH₃)CH(CH₃)₂,
—CH(CH₂CH₃)CH(CH₃)CH(CH₃)(CH₂CH₃) and others. The phrase also includes cyclic alkyl groups such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl and such rings substituted with straight and branched chain alkyl groups as defined above. The phrase also includes polycyclic alkyl groups such as, but not limited to, adamantyl norbornyl, and bicyclo[2.2.2]octyl and such rings substituted with straight and branched chain alkyl groups as defined above.

As used herein, the term "alkylene", whether used alone or as part of a substituent group, includes any group obtained by removing a hydrogen atom from an alkyl group; an alkylene group forms two bonds with other groups.

As used herein, the term "alkenyl", whether used alone or as part of a substituent group, includes an alkyl group having at least one double bond between any two adjacent carbon atoms.

As used herein the term "alkynyl", whether used alone or as part of a substituent group, includes an alkyl group having at least one triple bond between any two adjacent carbon atoms.

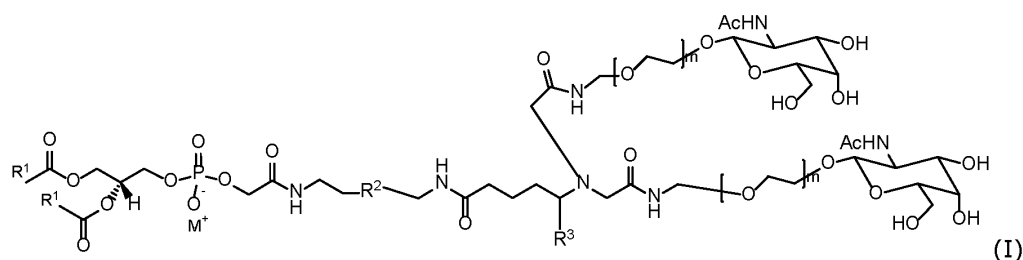
As used herein, the terms "unsubstituted alkyl," "unsubstituted alkenyl" and "unsubstituted alkenyl" refer to alkyl, alkenyl and alkynyl groups that do not contain heteroatoms.

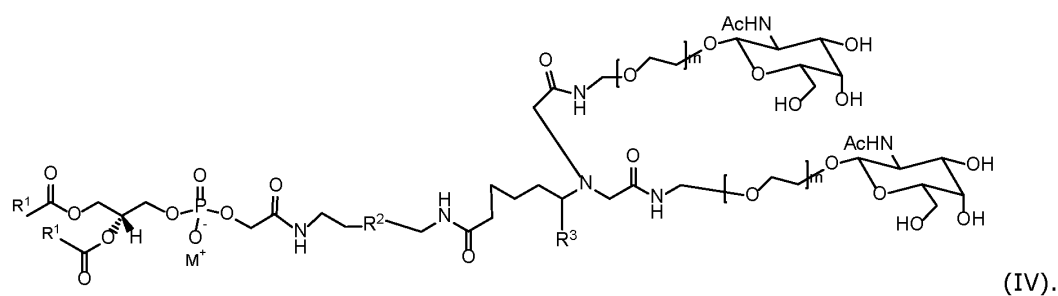
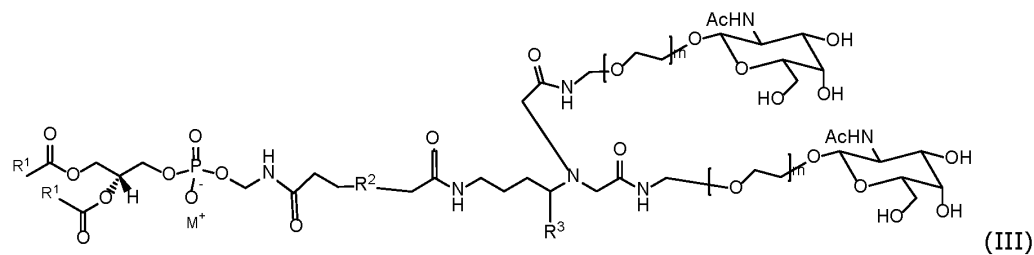
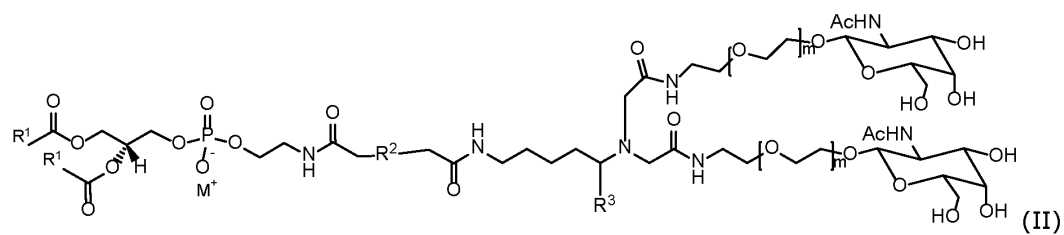
The phrases "substituted alkyl," "substituted alkenyl," and "substituted alkynyl" refer to alkyl, alkenyl, and alkynyl groups as defined above in which one or more bonds to a carbon(s) or hydrogen(s) are replaced by a bond to non-hydrogen or non-carbon atoms such as, but not limited to, a halogen atom in halides such as F, Cl, Br, and I; and oxygen atom in groups such as carbonyl, carboxyl, hydroxyl groups, alkoxy groups, aryloxy groups, and ester groups; a sulfur atom in groups such as thiol groups, alkyl and aryl sulfide groups, sulfone groups, sulfonyl groups, and sulfoxide groups; a nitrogen atom in groups such as amines, amides, alkylamines, dialkylamines, arylamines, alkylarylamines, diarylamines, N-oxides, imides, enamines imines, oximes, hydrazones, and nitriles; a silicon atom in groups such as in trialkylsilyl groups, dialkylarylsilyl groups, alkylarylsilyl groups, and triarylsilyl groups; and other heteroatoms in various other groups. Other alkyl groups include those in which one or more bonds to a carbon or hydrogen atom is replaced by a bond to an oxygen atom such that the substituted alkyl group contains a hydroxyl, alkoxy, aryloxy group, or heterocycloxy group. Still other alkyl groups include alkyl groups that have an amine, alkylamine, dialkylamine, arylamine, (alkyl)(aryl)amine, diarylamine, heterocyclamine, (alkyl)(heterocycl)-amine, (aryl)(heterocycl)amine, or diheterocyclamine group.

As used herein the term "alkynyl", whether used alone or as part of a substituent group, includes an alkyl group having at least one triple bond between any two adjacent carbon atoms.

Compounds

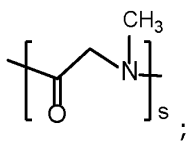
According to an embodiment, a compound having a structure (I), (II), (III), or (IV), or a combination of any two or more thereof is provided.





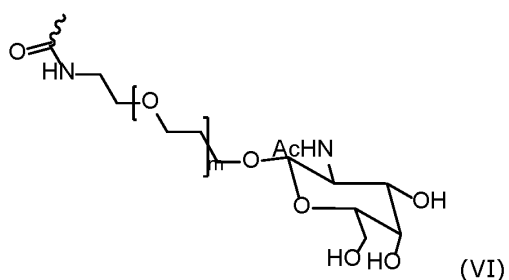
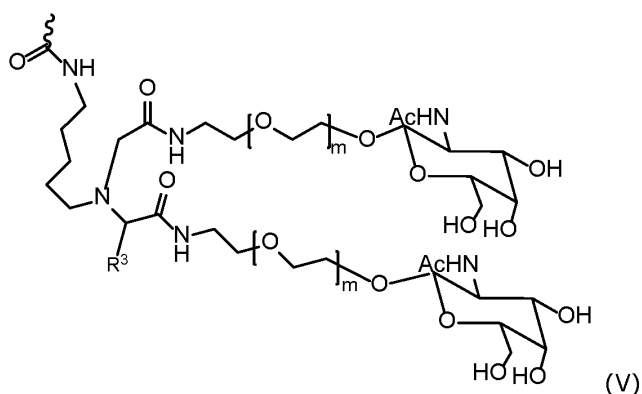
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In these structures, (I), (II), (III), and (IV), each R^1 is independently selected from aliphatic alkyl C_4 - C_{100} groups optionally substituted with one or more of alkenyl, alkynyl, hydroxyl, amide, ester, and/or ether groups;

R^2 is selected from $[-OCH_2CH_2-]_p$, $[-OCH_2CH_2-]_p-O-$, or  ;

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R^3 is selected from Formula (V) or (VI):

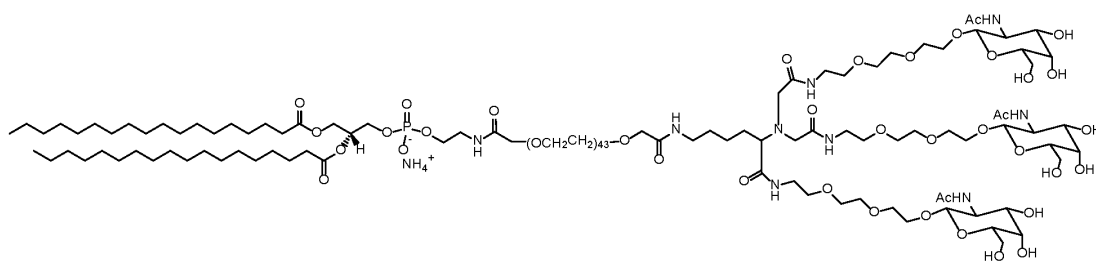


M^+ is selected from an alkali metal ion, an alkaline earth metal ion, or a primary, secondary or tertiary ammonium ion. Nonlimiting examples are Li^+ , Na^+ , K^+ , NH_4^+ , Ca^{2+} , Ba^{2+} , or Mg^{2+} . As is known in the art, the number of ions necessary to balance a charge on the compound is understood to be present.

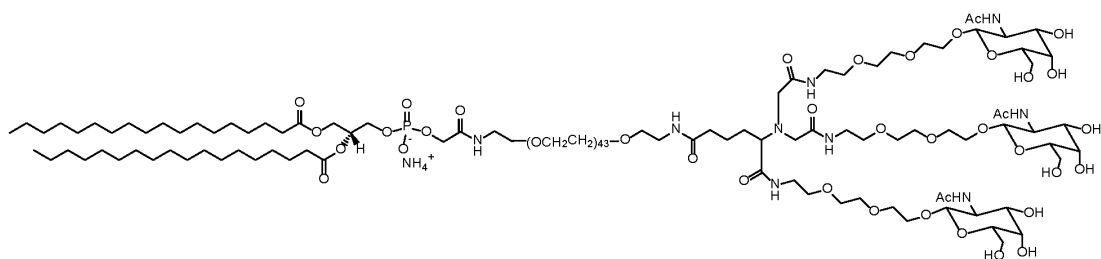
m , p , and s are independently selected from integers from 1 to 120.

According to some embodiments, the compounds do not have more than 3, 5, 7, 9, 11, 13, or 15 N-acetylgalactosamine moieties per molecule.

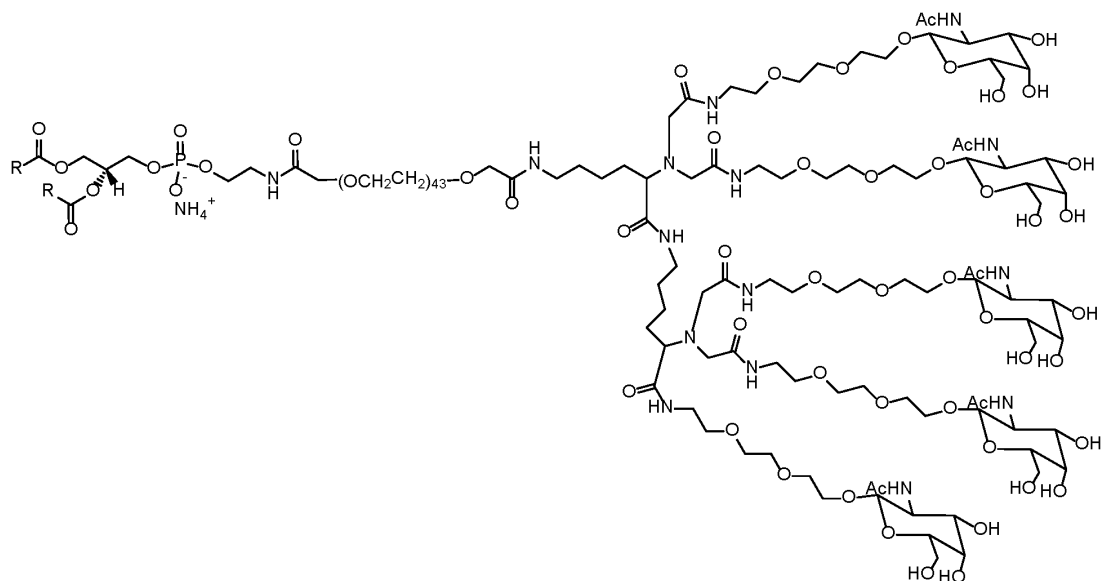
10 According to an embodiment, the compound (II) has structure:



According to another embodiment the compound (IV) has structure:

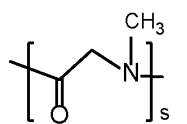


According to another embodiment, the compound (II) has structure:



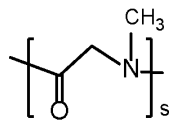
- According to some embodiments, R¹ is a substituted or an unsubstituted, straight
 5 chained or branched aliphatic or cyclic alkyl C₄-C₁₀₀ group, preferably C₁₀-C₈₀ group,
 more preferably C₁₀-C₅₀ group, most preferably an C₁₂-C₂₄ group. According to some
 other embodiments, R¹ may be substituted with at least one alkenyl group. According to
 another embodiment, R¹ may be substituted with at least one alkynyl group. According to
 an embodiment, R¹ may be substituted with at least hydroxyl group. According to an
 10 embodiment, R¹ may be substituted with at least one amide group. According to an
 embodiment, R¹ may be substituted with at least one ester group. Non-limiting examples
 of suitable R¹ moieties are C₁₂-C₂₄ straight chained alkyl groups. The C₁₂-C₂₄ groups
 may be branched. The C₁₂-C₂₄ groups may be cyclic.

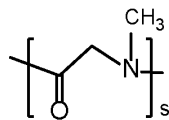
- According to an embodiment, R² is [-OCH₂CH₂-]_p or [-OCH₂CH₂-]_p-O-. Preferably
 15 R² is [-OCH₂CH₂-]_p-O-.

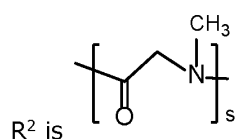


According to another embodiment, R² is

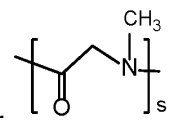
According to an embodiment, R^2 is $[-OCH_2CH_2-]_p$ or $[-OCH_2CH_2-]_p-O-$, and p is an integer from 1 to 120, from 6 to 120, or from 10 to 80, particularly from 20 to 70, more particularly from 30 to 60, most particularly from 40 to 50. According to an embodiment, p is 43. According to an embodiment, p is 43 and R^2 is $[-OCH_2CH_2-]_p$ or $[-OCH_2CH_2-]_p-O-$,
 5 and preferably p is 43 and R^2 is $[-OCH_2CH_2-]_p-O-$.

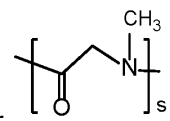


According to another embodiment, R^2 is  and s is an integer from 1 to 120, from 6 to 120, or from 10 to 80, particularly from 20 to 70, more particularly from 30 to 60, most particularly from 40 to 50. According to an embodiment, s is 43 and

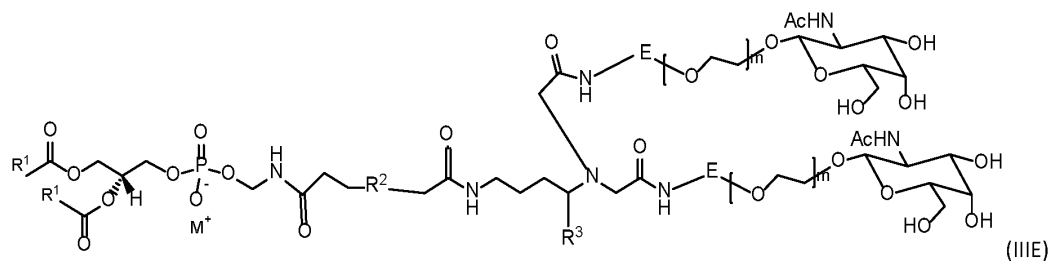
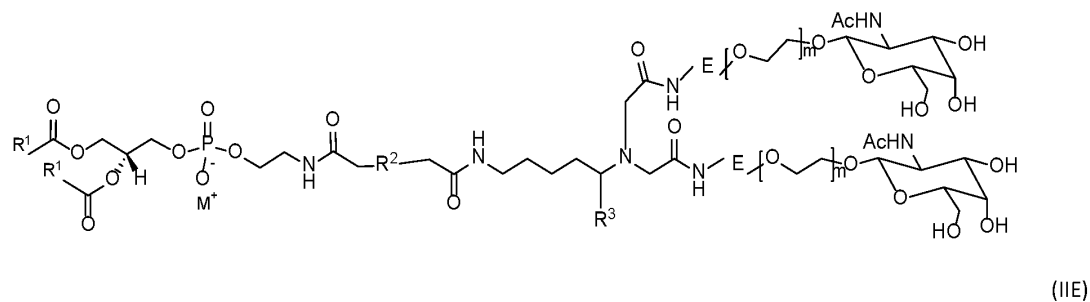
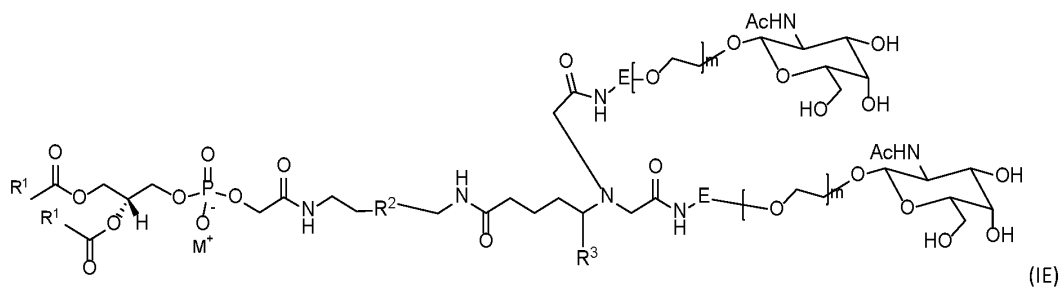


10 According to some embodiments, m is an integer selected from 1 to 50, particularly from 1 to 40, more particularly from 1 to 30, more particularly from 3 to 25, most particularly from 2 to 5.

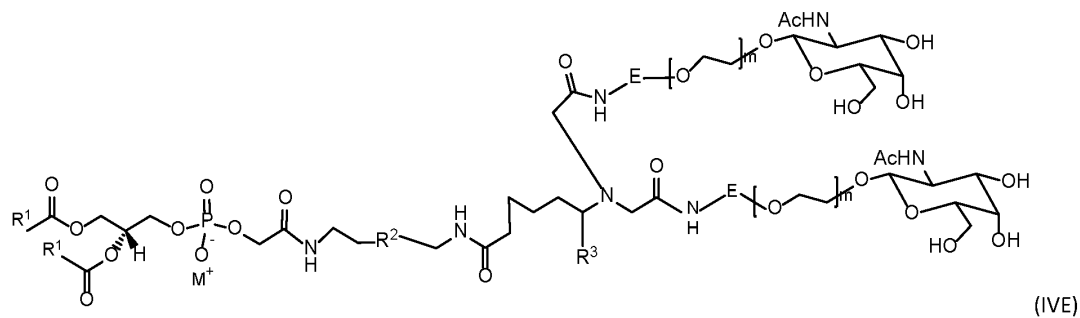


These R^2 groups $[-OCH_2CH_2-]_p$, $[-OCH_2CH_2-]_p-O-$, or  are hydrophilic and thus provide advantages in certain formulations. For example, without
 15 wishing to be bound by any particular theory, the hydrophilic nature of these R^2 groups improve stability of micelles, liposomes and lipid nanoparticles that are used to deliver aqueous and/or polar drugs or other therapeutic agents.

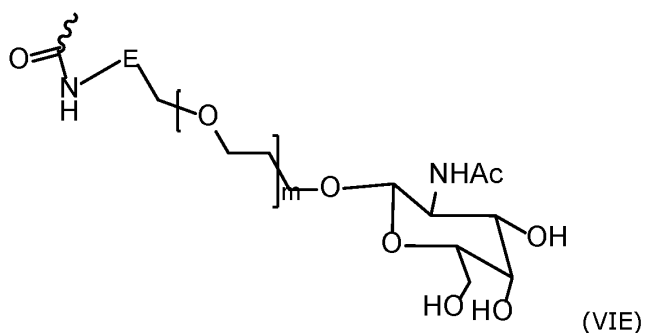
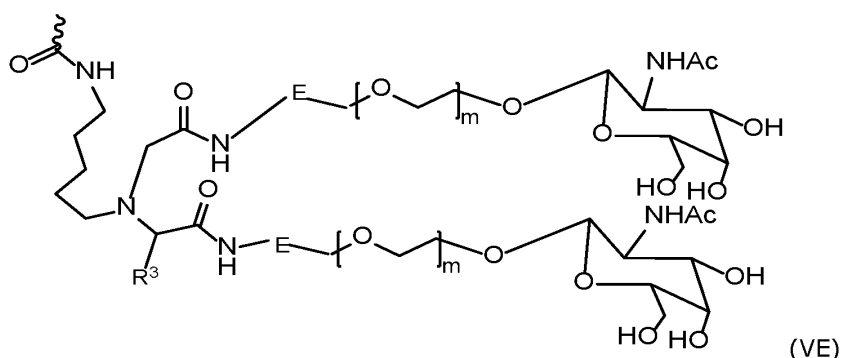
Additionally, or alternatively, in some embodiments the compound having a structure (I), (II), (III), or (IV), or a combination of any two or more thereof further
 20 comprising a spacer or extender group (E). As such, in some embodiments the compound has a structure (IE), (IIE), (IIIE) or (IVE), or a combination of any two or more thereof. As such, it should be understood that in some embodiments the compound has a structure (I), (II), (III), (IV), (IE), (IIE), (IIIE) or (IVE), or a combination of any two or more thereof. Without wishing to be bound by theory, the
 25 spacer or extender group (E) is believed to ease problems associated with steric hindrance during manufacture of the compound having a structure (I), (II), (III), or (IV), leading to improved yields of product, but without losing the beneficial hydrophilic properties of the compound.



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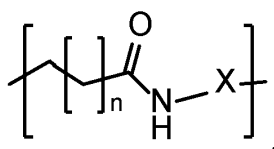
Additionally, or alternatively, R^3 , as described above, may also further comprising a spacer or extender group (E), such that R^3 is selected from Formula (VE) or (VIE):



Without wishing to be bound by theory, the spacer or extender group (E) is believed to ease problems associated with steric hindrance during manufacture of the compound having a structure (V) or (VI), leading to improved yields of product, but

5 without losing the beneficial hydrophilic properties of the compound.

Preferably, in accordance with some embodiments of the present invention, when the spacer or extender group (E) is present, E is

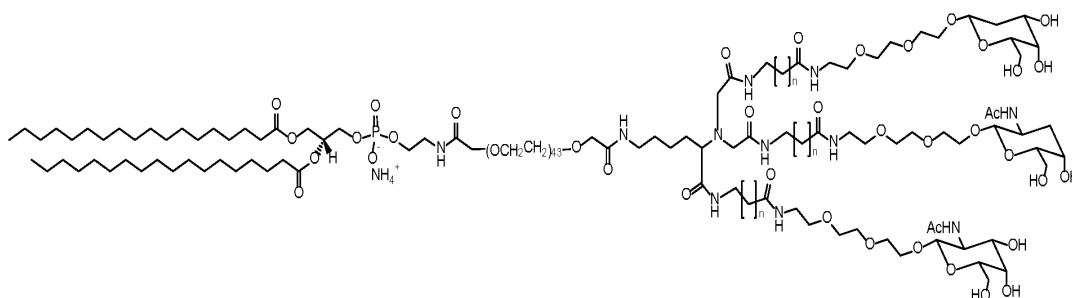


10 Wherein, n is an integer between the value 0 and 6, and X is an optional alkyl chain of between 0 and 4 carbons in length. Without wishing to be bound by theory, it is believed that overall shorter-chain versus longer-chain spacer or extender groups are preferable since this will result in a limited flexible conformation distribution in the final compound. As such, preferably n is 0, 1 or 2, more preferably 1 or 2. Furthermore,

15 preferably X is the alkyl chain -CH₂-, or -CH₂CH₂-.

Suitably, where the compound has a structure (IE), (IIE), (IIIE), or (IVE), such that the compound comprises the optional spacer or extender group (E), the R₃ group of the structure also comprises the spacer or extender group (E).

According to a further embodiment, the compound (i.e. further comprising the spacer or extender group (E)) has the structure:



- Reference to the structure (I), (II), (III), or (IV) throughout the description below
- 5 also encompass the embodiments wherein said structure or a combination of any two or more thereof further comprise the spacer or extender group (E) in accordance with structures (IE), (IIE), (IIIE), or (IVE) and/or where R_3 further comprises the spacer or extender group (E), as described above.

Liver Asialoglycoprotein Receptor-Targeted Therapeutic Agent

- 10 As is known in the art, the N-acetylgalactosamine moieties of the structures (I), (II), (III), or (IV) bond to liver asialoglycoprotein receptors and thus the liver uptakes the drug or oligonucleotide in a lipid nanoparticle, liposome or micelle which includes the compound of the structure (I), (II), (III), or (IV), or a combination of any two or more thereof via the asialoglycoprotein receptor (ASGPR) pathway.

- 15 According to some embodiments, RNA or DNA may be delivered to the liver in lipid nanoparticles (LNP). The present lipid compounds having structures (I), (II), (III), or (IV) are part of the lipids in the formulation of LNP. The outer layer of LNP will contain DSPE-PEG2K-triGalNAc with triantennary GalNAc (Example 1, below) targeting groups projecting out of the LNP. LNP with GalNAc will accumulate more on hepatic cell surfaces
- 20 and achieve the targeting delivery effect.

According to an embodiment, a liver asialoglycoprotein receptor-targeted therapeutic agent comprising a drug or oligonucleotide conjugated with and/or covalently bound to a compound having the structure (I), (II), (III), or (IV), or a combination of any two or more thereof is provided.

- 25 According to some embodiments, the compound having the structure (I), (II), (III), or (IV), or a combination of any two or more thereof may be in the form of a liposome.

According to some embodiments, the compound having the structure (I), (II), (III), or (IV), or a combination of any two or more thereof may be in the form of a micelle.

According to some embodiments, the present compounds having structure (I),
5 (II), (III), or (IV) are useful when used as part of liver asialoglycoprotein receptor-targeted therapeutic agents that are in the form of lipid nanoparticles (LNP) that are designed to specifically target the liver. As is known in the art, these compounds (I), (II), (III), or (IV) may be used in conjunction with other lipids to form the LNP that encapsulates a therapeutic agent that targets the liver. Non-limiting examples of other
10 lipids that may be used in conjunction with compounds having structure (I), (II), (III), or (IV) are ionizable cationic lipids, permanently charged cationic lipids, polyethylene glycol (PEG) lipids, and/or cholesterol.

Since the liver is able to synthesize proteins, delivery of various drugs or oligonucleotides via these LNPs that include compounds having the structure (I), (II),
15 (III), or (IV) are especially attractive, since the present compounds target the liver asialoglycoprotein receptor. Thus, these compounds can be useful to deliver gene therapy to a patient in need thereof.

Suitable drugs may be antibodies, enzymes, proteins, small molecules, nucleotides, and especially drugs that are used to treat diseases of the liver. These
20 include but are not limited to treatments for hepatitis A, B or C, medications for bile acid synthesis disorders, medications for cholestatic pruritus in alagille syndrome, medications for hepatic tumors, medications for hepatic coma, medications for hyperbilirubinemia, medications for variable causes of liver cirrhosis, medications for liver metastasis in adenocarcinoma, medications for metabolic dysfunction-associated
25 steatotic liver disease, medications for pancreatic exocrine dysfunction, medications for portal hypertension, medications to treat glycogen storage disorders, or medications for progressive familial intrahepatic cholestasis, medications for liver cancer. Specific examples may include cholic acid, maralixibat, odeixibat, sorafenib, phenobarbital, ursodiol, colchicine, azathioprine, obeticholic acid, floxuridine, pioglitazone, orlistat,
30 betaine, rosiglitazone, rosiglitazone, pancrelipase, propranolol, nadolol, odeixibat, doxorubicin. For example, many types of APIs (active pharmaceutical ingredient) can be included in a lipid particle, but the drug properties will dictate different composition and processing conditions. Hydrophilic drugs can be loaded into a liposome's aqueous core. Lipophilic drugs can be loaded into the liposomal bilayer or sequestered in a micelle.
35 Anionic drugs (oligonucleotides or proteins, for example) can be loaded into lipid nanoparticle. Both hydrophilic and hydrophobic drugs can be loaded into emulsion preparations that include the subject compounds.

Different size nucleic acids coding for expression or suppression of proteins, or editing of bases, codons, etc. through variable actions may be included in therapeutic agents that utilize the subject compounds. For example, some suitable oligonucleotides are ABE (adenine base editor), miRNA inhibitors, antisense oligonucleotides, ribozymes, DNAzymes, plasmids, immune stimulating nucleic acids, antagomir, antimir, mimic, supermir, and aptamers, allele-specific oligonucleotide, antisense oligonucleotide, ribonucleoproteins (RNP), mRNA, siRNA, sgRNA, gRNA, ss-siRNA.

According to some embodiments, CRISPR (clustered regularly interspaced short palindromic repeats) base editing therapy can be delivered the liver using the present N-acetylgalactosamine moieties of the structures (I), (II), (III), or (IV).

The therapeutic agents may be formulated into a composition for administration to a patient in need thereof. A formulated composition can assume a variety of states. In some examples, the composition may be at least partially crystalline, uniformly crystalline, and/or anhydrous (e.g., less than 80, 50, 30, 20, or 10% water). In another example, the formulation is in an aqueous phase, e.g., in a solution that includes water.

The aqueous phase or the crystalline compositions can, e.g., be incorporated into a delivery vehicle, e.g., a lipid nanoparticle. Generally, the composition is formulated in a manner that is compatible with the intended method of administration.

In some embodiments, the liver asialoglycoprotein receptor-targeted therapeutic agent comprising a drug or oligonucleotide in the form of a lipid nanoparticle, and/or in the form of a liposome including a compound having the structure (I), (II), (III), or (IV), or a combination of any two or more thereof may be prepared by at least one of the following methods: spray drying, lyophilization, vacuum drying, evaporation, fluid bed drying, or a combination of these techniques; or sonication with a lipid, freeze-drying, condensation and other self-assembly.

The liver asialoglycoprotein receptor-targeted therapeutic agent comprising a drug or oligonucleotide in the form of a lipid nanoparticle including a compound having the structure (I), (II), (III), or (IV); or a combination of any two or more thereof may be formulated into pharmaceutical compositions suitable for administration. The liver asialoglycoprotein receptor-targeted therapeutic agent comprising a drug or oligonucleotide and the compound having the structure (I), (II), (III), or (IV) may also be in the form of a lipid nanoparticle or may be in the form of a liposome. As is known in the art, liposomes have one or more rings of lipid bilayer surrounding an aqueous pocket, but lipid nanoparticles usually only have a single phospholipid outer layer that encapsulates the interior, which may be non-aqueous.

The therapeutic agent, e.g. drug and/or oligonucleotide preparation can be formulated for delivery in a membranous molecular assembly, e.g., a liposome or a micelle. As used herein, the term "liposome" refers to a vesicle composed of amphiphilic lipids arranged in at least one bilayer, e.g., one bilayer or a plurality of bilayers.

- 5 Liposomes include unilamellar and multilamellar vesicles that have a membrane formed from a lipophilic material comprising a compound having structure (I), (II), (III), or (IV), or a combination of any two or more thereof and an aqueous interior.

The interior aqueous or non-aqueous portion contains the therapeutic agent composition. The lipophilic material isolates the aqueous interior from an aqueous
10 exterior, which typically does not include the therapeutic agent composition, although in some examples, it may. Liposomes, micelles and lipid nanoparticles are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal or lipid nanoparticle or micelle membrane is structurally similar to biological membranes, when liposomes, lipid nanoparticles or micelles are applied to a tissue, the exterior layer
15 of the lipid particle fuses with bilayer of the cellular membranes. As the merging of the liposome, lipid nanoparticle, or micelle and cell progresses, the internal contents that include the therapeutic agent composition are delivered into the cell. As discussed herein, the compound having structure (I), (II), (III), or (IV) target the liver asialoglycoprotein receptor.

- 20 "Micelles" are defined herein as a particular type of molecular assembly in which amphipathic molecules are arranged in a spherical structure such that all the hydrophobic portions of the molecules are directed inward, leaving the hydrophilic portions in contact with the surrounding aqueous phase. The converse arrangement exists if the environment is hydrophobic in which inverted micelles are formed.

- 25 A micelle containing a drug can be prepared by a variety of methods. In one example, the lipid component is dissolved in a detergent, emulsifier, and/or surfactant so that micelles are formed with the lipid component. For example, the lipid component can include the present compound having structure (I), (II), (III), or (IV) or combination of any two or more thereof. The detergent can have a high critical micelle concentration
30 and may be nonionic. Exemplary detergents, emulsifier, and/or surfactants include cholate, CHAPS (3-((3-cholamidopropyl) dimethylammonio)-1-propanesulfonate), octylglucoside, deoxycholate, and lauroyl sarcosine. The drug preparation is then added to the micelles that include the lipid component, to yield a micelle preparation of a drug.

- A mixed micellar formulation suitable for delivery through transdermal
35 membranes may be prepared by mixing an aqueous solution of the oligonucleotide or drug composition, an alkali metal C8 to C22 alkyl sulphate, and micelle forming compounds. Exemplary micelle forming compounds include lecithin, hyaluronic acid,

pharmaceutically acceptable salts of hyaluronic acid, glycolic acid, lactic acid, chamomile extract, cucumber extract, oleic acid, linoleic acid, linolenic acid, monoolein, monooleates, monolaurates, borage oil, evening of primrose oil, menthol, trihydroxy oxo cholanyl glycine and pharmaceutically acceptable salts thereof, glycerin, polyglycerin, lysine, polylysine, triolein, polyoxyethylene ethers and analogues thereof, polidocanol alkyl ethers and analogues thereof, chenodeoxycholate, deoxycholate, and mixtures thereof.

The micelle forming compounds may be added at the same time or after addition of the alkali metal alkyl sulphate. Mixed micelles will form with substantially any kind of mixing of the ingredients, but vigorous mixing will provide smaller size micelles.

In one method a first micellar composition is prepared which contains the oligonucleotide or drug composition and at least the alkali metal alkyl sulphate. The first micellar composition is then mixed with at least three micelle forming compounds to form a mixed micellar composition. In another method, the micellar composition is prepared by mixing the oligonucleotide or drug composition, the alkali metal alkyl sulphate and at least one of the micelles forming compounds, followed by addition of the remaining micelle forming compounds, with vigorous mixing.

Phenol and/or m-cresol may be added to the mixed micellar composition to stabilize the formulation and protect against bacterial growth. Alternatively, phenol and/or m-cresol may be added with the micelle forming ingredients. An isotonic agent such as glycerin may also be added after formation of the mixed micellar composition.

For delivery of the micellar formulation as a spray, the formulation can be put into an aerosol dispenser and the dispenser is charged with a propellant. The propellant, which is under pressure, is in liquid form in the dispenser. The ratios of the ingredients are adjusted so that the aqueous and propellant phases become one, i.e., there is one phase. If there are two phases, it is necessary to shake the dispenser prior to dispensing a portion of the contents, e.g., through a metered valve. The dispensed dose of pharmaceutical agent is propelled from the metered valve in a fine spray. Propellants may include hydrogen-containing chlorofluorocarbons, hydrogen containing fluorocarbons, dimethyl ether and diethyl ether. In certain embodiments, HFA 134a (1, 1,1,2 tetrafluoroethane) may be used.

The specific concentrations of the essential ingredients can be determined by relatively straightforward experimentation. For absorption through the oral cavities, it is often desirable to increase, e.g., at least double or triple, the dosage for through injection or administration through the gastrointestinal tract.

Commonly used techniques for preparing lipid aggregates of appropriate size for use as delivery vehicles include sonication and freeze-thaw plus extrusion (see, e.g., Mayer, et al. Biochim. Bio phys. Acta 858: 161, 1986). Microfluidization, high pressure extrusion, high pressure homogenization or solvent injection can be used when
5 consistently small (50 to 200 nm) and relatively uniform aggregates are desired.

Lipid nanoparticle (LNP) is a vesicle formed of multiple lipid components and an oligonucleotide, where a complex interaction forms between the oligonucleotide and one or more of the lipidic components to create particle, which may have a solid core. Lipid nanoparticles may include four types of lipids. These are neutral phospholipids (helper
10 lipids), cationic lipids, sterol, and polymeric lipids. The present compounds (I), (II), (III), or (IV) may be used either as phospholipids or polymeric lipids. The purpose of the compounds having structure (I), (II), (III), or (IV) is thus to improve the stability of the nanoparticle. Lipid nanoparticles may be prepared by mixing solvent/lipid mixture including the present compounds (I), (II), (III), or (IV) with an aqueous buffer solution
15 containing protein(s), small molecules(s), enzyme(s) oligonucleotide(s) and/or drug(s). A 1:3 ratio of lipid mixture to aqueous buffer is generally used. The general method is to combine the lipid elements with the therapeutic agent (oligonucleotides, proteins, enzymes, or drugs) under controlled conditions to precipitate lipid nanoparticles. Non-limiting examples of suitable solvents are those that are miscible with water, including
20 C1-C4 alcohols, methanol, ethanol, propanols, butanols, for example. Suitable aqueous buffers are those that maintain a pH in the correct buffering range for the lipids and cargo present.

Such compositions include the liver asialoglycoprotein receptor-targeted therapeutic agent comprising a drug or oligonucleotide in the form of a lipid nanoparticle
25 including a compound having the structure (I), (II), (III), or (IV), or as a liposome, or as a micelle and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration.

30 The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated.

Methods of Treatment

According to an embodiment, a method of treatment is provided. The method
35 comprising administering the liver asialoglycoprotein receptor-targeted therapeutic agent to a patient in need thereof. Suitable administration to a subject in need thereof includes

a variety of routes. Non-limiting examples include: parenteral, intravenous, topical, rectal, anal, vaginal, nasal, pulmonary, or ocular.

The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and
5 upon the area to be treated. The target of the present liver asialoglycoprotein receptor-targeted therapeutic agent is the liver, and the administration method should therefore be chosen to enhance uptake by the liver. Administration may be topical (including ophthalmic, vaginal, rectal, intranasal, transdermal), oral or parenteral. Parenteral
10 administration includes intravenous drip, subcutaneous, intraperitoneal or intramuscular injection, or intrathecal or intraventricular administration.

Formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders.

Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

15 Compositions for oral administration include powders or granules, suspensions or solutions in water, syrups, elixirs or non-aqueous media, tablets, capsules, lozenges, or troches. In the case of tablets, carriers that can be used include lactose, sodium citrate and salts of phosphoric acid. Various disintegrants such as starch, and lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc, are commonly used in
20 tablets. For oral administration in capsule form, useful diluents are lactose and high molecular weight polyethylene glycols. When aqueous suspensions are required for oral use, the liver asialoglycoprotein receptor-targeted therapeutic agent comprising a drug or oligonucleotide in the form of a lipid nanoparticle, liposome or micelle including a compound having the structure (I), (II), (III), or (IV) can be combined with emulsifying
25 and suspending agents. If desired, certain sweetening and/or flavoring agents can be added.

According to some embodiments, compositions for intrathecal or intraventricular administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

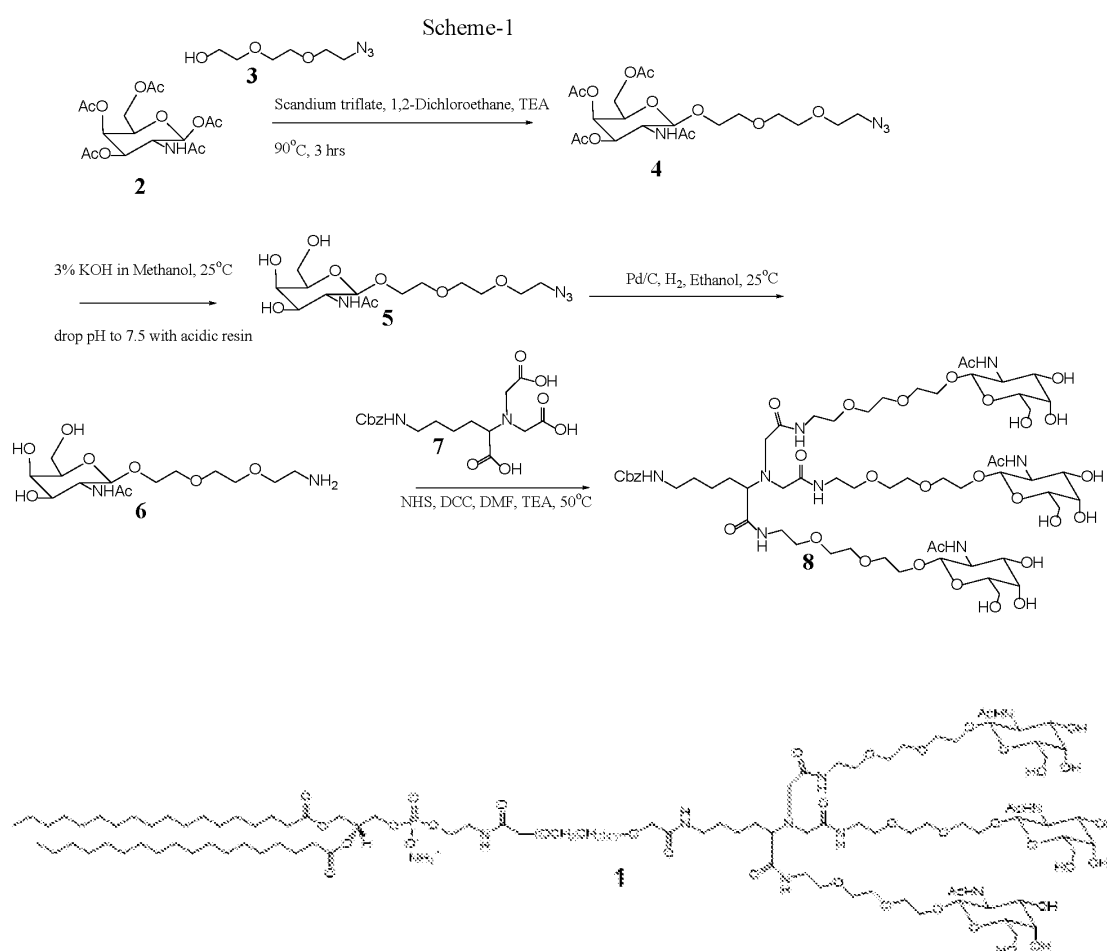
30 According to some embodiments, the administration of the pharmaceutical composition is parenteral, e.g., intravenous (e.g., as a bolus or as a diffusible infusion), intradermal, intraperitoneal, intramuscular, intrathecal, intraventricular, intracranial, subcutaneous, transmucosal, buccal, sublingual, endoscopic, rectal, oral, vaginal, topical, pulmonary, intranasal, urethral or ocular. Administration can be provided by the subject
35 or by another person, e.g., a health care provider. The medication can be provided in measured doses or in a dispenser which delivers a metered dose. Formulations for

parenteral administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives. Intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir. For intravenous use, the total concentration of solutes may be controlled to render the preparation isotonic.

According to some embodiments the administration of the pharmaceutical composition is intramuscular.

EXAMPLES

Example 1: Preparation of compound 1 (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000]-N-tri(N-acetylglactosamine), ammonium salt)



Experimental procedure:Synthesis of (2R,3R,4R,5R,6R)-5-acetamido-2-(acetoxymethyl)-6-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)tetrahydro-2H-pyran-3,4-diyl diacetate (4):

Tetraacetate GalNAc (2) (60.0 g, 154 mmol) and azido-Gly-alcohol (3) (29.7g,
 5 170 mmol) were suspended in 1,2-dichloroethane (1.7 L) in a dry 3 litter round bottom flask and scandium triflate (4.55 g, 9.25 mmol) was added to the reaction flask. The reaction flask was warmed to 90°C with stirring for 3 hours under nitrogen and the progress of the reaction checked by thin layer chromatography (TLC) (chloroform: methanol, 93:7, Rf 0.5). The reaction mixture was cooled to 10°C and quenched with
 10 triethyl amine (TEA) (86 mL, 616 mol) and concentrated. The crude product was purified via silica flash column chromatography to obtain Azido-Gly-GalNAc (4) (54.4 g, 70%) as a viscous oil. ¹H NMR (400 MHz, CDCl₃) δ 2.01 (s, 3H), 2.03 (s, 3H), 2.07 (s, 3H), 2.18 (s, 3H), 3.49 (t, J = 4.8 Hz, 2H), 3.65-3.75 (m, 9H), 3.85-3.95 (m, 3H), 4.13-4.29 (m, 3H), 4.81 (d, J = 8.5 Hz, 1H), 5.08 (dd, J = 3.4, 3.4, 1H), 5.34 (s, 1H, NH).

15 Synthesis of N-((2R,3R,4R,5R,6R)-2-(2-(2-(2-azidoethoxy) ethoxy)ethoxy)-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)acetamide (5):

The Azido-Gly-GalNAc (4) (10.0 g, 19.8 mmol) was dissolved in 3% Potassium hydroxide solution in methanol (methanolic KOH solution) (4.45 g, 79.0 mmol) and stirred for 3 hours. The status of the progress of the reaction was checked via TLC
 20 (chloroform: methanol: ammonium hydroxide: 65:30:6, Rf 0.3). Once the crude product (5) had formed the reaction mixture was neutralized with strong acidic resin to pH 7.5; subsequently the resin was filtered off and concentrated for the column. The crude product (5) was purified via silica flash column chromatography, yield 6.0 g (80%) as a colorless gel. ¹H NMR (400 MHz, CD₃OD) δ 2.00 (s, 3H), 3.40 (t, J = 5.1 Hz, 2H), 3.51 (t, J = 5.6 Hz, 1H), 3.60 (dd, J = 3.3, 3.4 Hz, 1H), 3.65-3.71 (m, 9H), 3.72-3.82 (m, 3H), 3.84 (d, J = 3.0 Hz, 1H), 3.90-4.00 (m, 2H). MS (ESI) m/z calcd. for C₂₀H₃₄N₂O₁₁ (M+H)⁺ : 479.50; found 478.0.

25 Synthesis of N-((2R,3R,4R,5R,6R)-2-(2-(2-(2-aminoethoxy) ethoxy)ethoxy)-4,5-dihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-3-yl) acetamide (6):

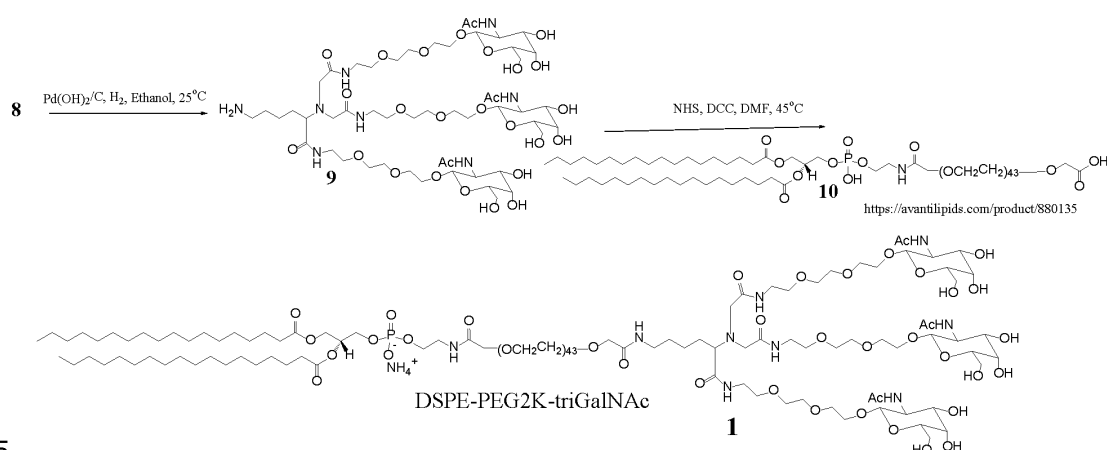
30 In a 400 ml Parr glass vessel, the Azido-GalNAc (5) (5.20 g, 13.4 mmol) was dissolved in ethanol/H₂O (4:1, 100 mL) and purged with nitrogen, 10% palladium on carbon (360 mg) was added to the vessel, the mixture was hydrogenated for 12 hours at room temperature (RT). The resultant mixture was filtered through the bed of Celite® (a diatomaceous earth ex. Imerys S.A., France) and concentrated. The crude amine-GalNAc
 35 product (6) was used for the next step without further any purification, yield 4.8 g

(quantitative) as a colorless gel. ^1H NMR (400 MHz, D_2O) δ 1.98 (s, 3H), 2.56-2.57 (m, 3H), 3.52-3.62, 3.66- (m, 12H), 3.67-3.90 (m, 3H), 4.33 (d, $J = 4$ Hz, 1H).

Synthesis of Benzyl (1-(((2S,3S,4S,5S,6S)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-12-(2-((2-(2-(((2S,3S,4S,5S,6S)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethoxy) ethyl)amino)-2-oxoethyl)-13-((2-(2-(((2S,3S,4S,5S,6S)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethoxy)ethyl)carbamoyl)-10-oxo-3,6-dioxo-9,12-diazaheptadecan-17-yl)carbamate (8):

Cbz-Lysine-Triacid (7) (2,2'-((5-(((benzyloxy)carbonyl)amino)-1-carboxypentyl)azanediyl)diacetic acid) (1.4 g, 3.5 mmol) was dissolved in dry dimethylformamide (DMF) and N-hydroxysuccinimide (NHS) (1.6 g, 14.1 mmol) was added to the solution and stirred for 10 minutes, then N,N'-dicyclohexylcarbodiimide (DCC) (2.33 g, 11.3 mmol) was added to the mixture and stirred for between 12 and 24 hours at 50°C . Amine-GalNAc (6) (4.1 g, 11.6 mmol) and triethyl amine (TEA) (2 mL, 14.0 mmol) were added and stirred the reaction mixture at 50°C for 24 hours. The progress of the reaction was checked by thin layer chromatography (TLC) (chloroform: methanol: water: 50:40:10, R_f 0.3) and solvent was removed under reduced pressure. The crude product (8) was purified by silica flash column chromatography to obtain yield 1.25 g of product (8) (51%) as a waxy white solid. ^1H NMR (400 MHz, $\text{CDCl}_3:\text{CD}_3\text{OD}$, 8:2) δ 1.23-1.43 (m, 2H), 1.42-1.51 (m, 2H), 1.52-1.70 (m, 2H), 2.01 (s, 9H), 3.07-3.16 (m, 3H), 3.21-3.28 (m, 1H) 3.36-3.43 (m, 6H), 3.44-3.70 (m, 36H), 3.71-3.80 (m, 12H), 3.81-3.95 (m, 3H) 4.42-4.50 (m, 3H), 5.03 (s, 2H), 7.31 (s, 5H).

Scheme-2



Synthesis of 2,2'-((1-((2-(2-(2-(((2S,3S,4S,5S,6S)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethoxy)ethyl)amino)-6-amino-1-oxohexan-2-yl)azanediyl)bis(N-(2-(2-(2-(((2S,3S,4S,5S,6S)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethoxy)ethyl)acetamide) (9):

In a 400 ml Parr glass vessel, Z-NTA-TriGalNAc (8) (2.00 g, 1.43 mmol) was dissolved in ethanol/H₂O (4:1, 60 mL) and purged with nitrogen, 10% palladium hydroxide on carbon (100 mg) was added to the vessel. The mixture was hydrogenated for 12 hours at room temperature (RT). The resultant mixture was filtered through the bed of Celite® and concentrated. The crude product (9) was used for the next step without any purification, yield 1.75 g (quantitative) as a semi solid. ¹H NMR (400 MHz, CDCl₃:CD₃OD, 8:2) δ 1.30-1.61 (m, 3H), 1.63-1.79 (m, 3H), 2.01 (s, 9H), 2.94-3.01 (m, 1H), 3.23-3.37 (m, 2H), 3.38-3.47 (m, 6H), 3.48-3.81 (m, 44H), 3.83-3.88 (m, 3H), 3.91-4.01 (m, 5H), 4.39-4.48 (m, 3H). TOF m/z calculated for C₅₂H₉₆N₈O₂₇ (M+H)⁺ : 1265.64; found 1265.6448.

Synthesis of 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000]-N-tri(N-acetylgalactosamine), ammonium salt (1), DSPE-PEG(2000)-triGalNAc):

1,2-Distearoyl-sn-glycero-3-phosphorylethanolamine (DSPE-PEG (2000)) Carboxylic acid (10) (2.0 g, 0.714 mmol) was dissolved in dry DMF and NHS (0.099 g, 0.857 mmol) was added whilst stirring the mixture and stirring was continued for 10 minutes prior to adding DCC (0.147 g, 0.714 mmol) to the reaction mixture and then stirred for 24 hours under nitrogen at 50°C. Lysine-triGalNAc (9) (0.60 g, 0.474 mmol) and TEA (0.3 mL, 2.15 mmol) were added and stirring was continued at 50°C for 24 hours. The progress of the reaction was checked by TLC (chloroform: methanol: water: 10:10:3, R_f 0.3) and solvent was removed under reduced pressure. The crude DSPE-PEG(2000)-triGalNAc final product (1) was purified via silica flash column chromatography, yield 0.70 g. The structure (1) was confirmed with ¹H and ³¹P NMR as well as high performance liquid chromatography (HPLC) with a mass spectrometer detector.

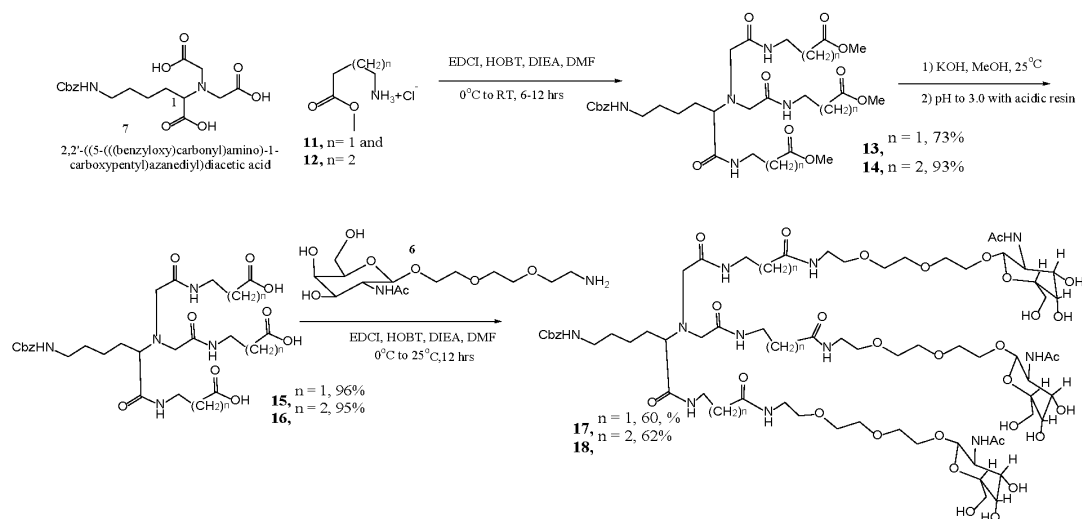
NMR conditions and results: ¹H NMR (400 MHz, CD₃OD) δ 0.92 (t, J = 7.0 Hz, 6H), 1.26-1.46 (m, 56H), 1.54-1.77 (m, 8H), 2.01 (s, 9H), 2.31-2.37 (q, J = 7.3 Hz, 4H), 3.22-3.28 (m, 3H), 3.30-3.33 (m, 3H), 3.39-3.55 (m, 15H), 5.56-5.73 (m, 200H), 3.74-3.81 (m, 5H), 3.82-3.87 (m, 4H), 3.91-4.01 (m, 12H), 4.18-4.25 (q, J = 6.7 Hz, 1H), 4.43-4.49 (m, 4H).

³¹P NMR (101MHz, CD₃OD) δ 0.1633.

Example 2: Preparation of compound 1 variants further comprising a spacer or extender group (E).

Preparation of a modified DSPE-PEG(2000)-triGalNAc product (1) as described above, where spacer or extender groups are included in the structure is now described below. The introduction of the spacer or extender groups occurs to provide improved yields of an equivalent to intermediate product (8) as described above and shown in step 4 of Scheme 1, which in turn provides improved yields of the desirable DSPE-PEG(2000)-triGalNAc final products in accordance with the present invention. Without wishing to be bound by theory it is believed that the carboxy acid at position-1 of the reactant Cbz-Lysine-Triacid (7), as shown above in scheme 1, is sterically hindered and that this could be a limiting factor for achieving improved yields of intermediate product (8). For the avoidance of doubt, prior to following the synthesis steps described below, steps 1 through 3 are carried out as described above in relation to Scheme 1.

Scheme-3



Synthesis of Methyl 10-(2-((3-methoxy-3-oxopropyl)amino)-2-oxoethyl)-9-((3-methoxy-3-oxopropyl)carbamoyl)-3,12-dioxo-1-phenyl-2-oxa-4,10,13-triazahexadecan-16-oate(13):

Cbz-Lysine-Triacid (7 as described above in Example 1) (5 g, 12.61 mmol) was dissolved in dry DMF and cooled to 5°C with an ice-bath under nitrogen, subsequently 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (9.43 g, 49.2 mmol) and 1-Hydroxybenzotriazole (HOBT) (7.73 g, 50.5 mmol) were added to the solution and stirred for 20 minutes. Then 4-methoxy-4-oxobutan-1-aminium chloride (11) (7.75 g, 55.5 mmol) and N,N-Diisopropylethylamine (DIEA) (20 mL, 114 mmol) were added to the mixture, warmed to room temperature (RT) and stirred for 12 hours at room

temperature (RT). The progress of the reaction was checked by TLC (chloroform: methanol: ammonium hydroxide: 90:10:1, Rf 0.5) and solvent was removed under reduced pressure. The crude product (13) was purified by silica flash column chromatography to obtain a yield of 6.0 g of product (13) (73%) as a viscous liquid. ¹H NMR (400 MHz, CDCl₃): δ 1.45-1.32 (m, 2H), 1.63-1.47 (m, 3H), 1.86-1.74 (m, 1H), 2.63-2.51 (m, 6H), 3.07-3.01 (m, 1H), 3.26-3.15 (m, 4H), 3.35-3.27 (m, 2H), 3.67-3.45 (m, 6H), 3.70 (m, 9H), 5.11 (s, 2H), 7.38-7.31 (m, 5H), 7.45 (m, 1H), 7.55 (m, 2H).

Synthesis of Methyl 10-(2-((4-methoxy-4-oxobutyl)amino)-2-oxoethyl)-9-((4-methoxy-4-oxobutyl)carbamoyl)-3,12-dioxo-1-phenyl-2-oxa-4,10,13-triazahetadecan-17-oate (14):

As an alternative to (13) described above, crude product (14) was prepared in a similar manner to (13), but (11) is replaced with (12, so that in this case n=2) and the progress of the reaction was checked by TLC (chloroform: methanol: ammonium hydroxide: 90:10:1, Rf 0.5) and solvent was removed under reduced pressure. The crude product (14) was purified by silica flash column chromatography to obtain product 14 (93%) as a viscous liquid. ¹H NMR (400 MHz, CDCl₃:CD₃OD:D₂O, 80:20:2): δ 1.39-1.23 (m, 2H), 1.54-1.42 (m, 3H), 1.72-1.60 (m, 1H), 1.85-1.79 (m, 6H), 2.37-2.29 (m, 6H), 3.03-2.96 (m, 1H), 3.16-3.08 (m, 4H), 3.30-3.18 (m, 8H), 3.6 (s, 9H), 5.04 (s, 2H), 7.39 (m, 5H), 7.91 (m, 1H), 8.17 (m, 1H).

Synthesis of 10-(2-((2-carboxyethyl)amino)-2-oxoethyl)-9-((2-carboxyethyl)carbamoyl)-3,12-dioxo-1-phenyl-2-oxa-4,10,13-triazaheptadecan-16-oic acid (15):

Intermediate product 13 (6.0 g, 8.69 mmol) as described above was dissolved in 3% methanolic KOH solution (93 mL, 2.2 g, 39.1 mmol) and stirred for 12 hours at 25°C and the progress of the reaction checked via TLC (chloroform: methanol: water: 50:40:10, Rf 0.4). Once the Cbz-Lysine-triacid product (15) had formed the reaction mixture was neutralized with strong acidic resin to pH 3.0. The resin was filtered off and concentrated and (15) used without any further purification. The yield of (15) obtained was 4.92 g (93%) as a white solid. ¹H NMR (400 MHz, D₂O) δ 1.22-1.05 (m, 2H), 1.42-1.132 (m, 2H), 1.54-1.44 (m, 2H), 2.54-2.43 (m, 6H), 3.20-2.97 (m, 5H), 3.45-3.27 (m, 8H), 5.00 (s, 2H), 7.32 (m, 5H).

Synthesis of 10-(2-((3-carboxypropyl)amino)-2-oxoethyl)-9-((3-carboxypropyl)carbamoyl)-3,12-dioxo-1-phenyl-2-oxa-4,10,13-triazaheptadecan-17-oic acid (16):

As an alternative to (15) described above, product (16) was prepared in a similar manner to (15), but in the preceding reaction step (11) was replaced with (12) (so that in this case n=2). In this case, the resin was filtered off and concentrated and (16) used

without any further purification. The yield of (16) obtained was 96% as a white solid. ¹H NMR (400 MHz, D₂O) δ 1.24-1.05 (m, 2H), 1.45-1.33 (m, 2H), 1.60-1.47 (m, 2H), 1.77-1.61 (m, 6H), 2.35-2.21 (m, 6H), 3.06-3.95 (m, 2H), 3.28-3.08 (m, 8H), 3.44-3.30 (m, 2H), 3.72-3.51 (m, 1H), 4.99 (s, 2H), 7.32 (m, 5H).

- 5 Synthesis of Benzyl(1-(((2S,3S,4S,5S,6S)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-16-(1-(((2S,3S,4S,5S,6S)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-10,14-dioxo-3,6-dioxo-9,13-diazapentadecan-15-yl)-17-(((3-(((2-((2-((2S,3S,4S,5S,6S)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethoxy)ethyl)amino)-3-oxopropyl)carbamoyl)-10,14-dioxo-3,6-dioxo-9,13,16-triazahenicosan-21-yl)carbamate (17). Azido-GalNAc:

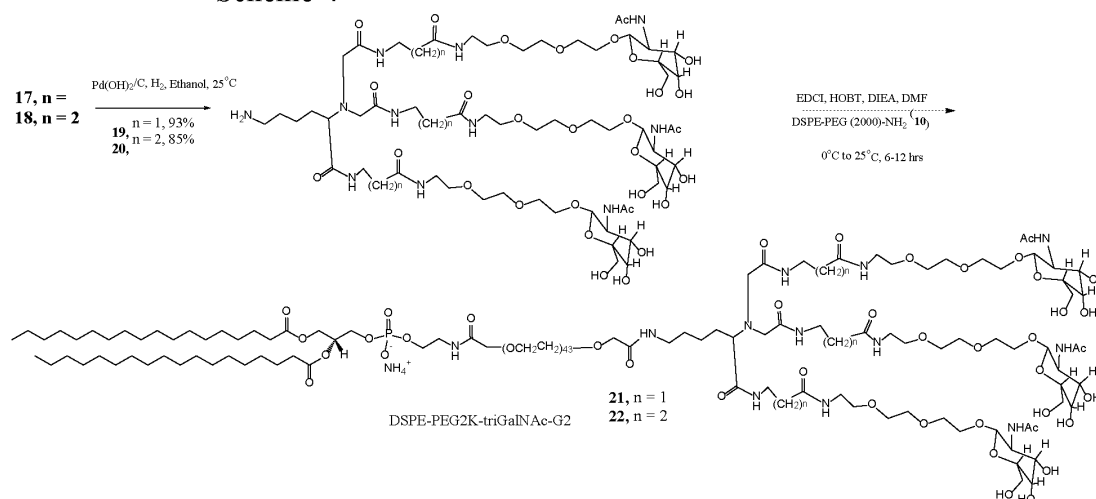
- The intermediate product Cbz-Lysine-triacid (15) (0.55 g, 5.6 mmol) was dissolved in dry DMF and cooled to 5°C with an ice-bath under nitrogen. EDCI (0.960 g, 4.99 mmol) and HOBT (0.960 g, 6.24 mmol) were added to the solution and stirred for 25 minutes. In the meantime, crude amine-GalNAc (6) (3.43 g, 5.62 mmol) as prepared above in Example 1 and DIEA (1.3 mL, 7.8 mmol) were dissolved in dry DMF and cooled to 5°C, and subsequently the activated tri-acid with EDCI-HOBT was added dropwise whilst stirring the solution of amine. The mixture was stirred for 12 hours at room temperature (RT). The progress of the reaction was checked by TLC (chloroform: methanol: ammonium hydroxide: 65:35:8, R_f 0.1) and solvent was removed under reduced pressure. The crude azido-GalNAc product (17) was purified by silica flash column chromatography to obtain a yield of 1.6 g of product (17) (60%) as a viscous liquid. ¹H NMR (400 MHz, CD₃OD): δ 1.43-1.58 (m, 2H), 1.55-1.47 (m, 2H), 1.75-1.58 (m, 2H), 2.01 (s, 9H), 2.54-2.41 (m, 6H), 3.22-3.09 (m, 2H), 3.43-3.36 (m, 7H), 3.58-3.44 (m, 17H), 3.80-3.59 (m, 32H), 3.88-3.83 (m, 3H), 4.02-3.91 (m, 6H), 4.45 (d, J = 8.4 Hz, 3H), 5.10 (s, 2H), 7.42-7.12 (m, 5H).

- 30 Synthesis of Benzyl (1-(((2S,3S,4S,5S,6S)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-17-(1-(((2S,3S,4S,5S,6S)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-10,15-dioxo-3,6-dioxo-9,14-diazahexadecan-16-yl)-18-(((4-((2-((2-((2S,3S,4S,5S,6S)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethoxy)ethyl)amino)-4-oxobutyl)carbamoyl)-10,15-dioxo-3,6-dioxo-9,14,17-triazadocosan-22-yl)carbamate (18):

- 35 As an alternative to (17) described above, product (18) was prepared in a similar manner to (17), but in the earlier reaction step (11) was replaced with (12) (so that in this case n=2), and the progress of the reaction was checked by TLC (chloroform: methanol: ammonium hydroxide: 65:35:8, R_f 0.1) and solvent was removed under

reduced pressure. The crude product (18) was purified by silica flash column chromatography to obtain a yield of the product 18 (60%) as a viscous liquid. ¹H NMR (400 MHz, CD₃OD): δ 1.43-1.58 (m, 2H), 1.55-1.47 (m, 2H), 1.75-1.58 (m, 2H), 1.88-1.77 (m, 6H), 2.01 (s, 9H), 2.30-2.22 (s, 6H), 3.16-3.09 (m, 2H), 3.30-3.17 (m, 7H), 3.42-3.36 (m, 6H), 3.58-3.47 (m, 12H), 3.81-3.68 (m, 10H), 3.88-3.83 (m, 3H), 4.02-3.90 (m, 5H), 4.45 (d, J = 9.1 Hz, 3H), 5.10 (s, 2H), 7.42-7.28 (m, 5H).

Scheme-4



Synthesis of 3,3'-((1-(((2S,3S,4S,5S,6S)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-15-(4-aminobutyl)-10,14-dioxo-16-(2-oxoethyl)-3,6-dioxo-9,13,16-triazaoctadecan-18-oyl)bis(azanediyl))bis(N-(2-(2-(((2S,3S,4S,5S,6S)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)ethoxy)ethoxy)ethyl)propanamide) (19):

In a 400 ml parr glass vessel, Azido-GalNAc (17) (1.4 g, 0.81 mmol) was dissolved in ethanol/H₂O (4:1, 50 mL) and purged with nitrogen, 20% palladium hydroxide on carbon (114 mg) was added to the vessel. The mixture was hydrogenated for 12 hours at room temperature. The resultant mixture was filtered through the bed of celite and concentrated. The crude product (19) was used for the next reaction step (to produce (21))without any further purification, yield of product (19) was 1.2 g (93%) as a colorless solid. ¹H NMR (400 MHz, D₂O) δ 1.25-1.47 (m, 2H), 1.73-1.50 (m, 4H), 1.99 (s, 9H), 2.99-2.19 (m, 6H), 2.87-2.71 (m, 2H), 3.40-3.14 (m, 13H), 3.78-3.66 (m, 8H) 3.67-3.44 (m, 34H), 3.86-3.80 (m, 3H), 4.03, 3.97 (m, 7H), 4.15 (d, J = 7.7 Hz, 3H).

Synthesis of 4,4'-((1-(((2S,3S,4S,5S,6S)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-16-(4-aminobutyl)-10,15-dioxo-17-(2-

oxoethyl)-3,6-dioxo-9,14,17-triazanonadecan-19-oyl)bis(azanediyl))bis(N-(2-(2-(2-
(((2S,3S,4S,5S,6S)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-
2-yl)oxy)ethoxy)ethoxy)ethyl)butanamide) (20):

20 was prepared according to the procedure 19, as described above, but in this
5 case but in the earlier reaction step (11) was replaced with (12) (so that in this case
n=2). The crude product (20) was used for the next reaction step (to produce
(22))without any further purification, and yield of product (20) (84%) as a white
powder. ¹H NMR (400 MHz, D₂O) δ 1.27-1.48 (m, 2H), 1.66-1.53 (m, 2H), 1.72-1.65 (m,
2H), 1.86-1.74 (m, 6H), 2.01 (s, 9H), 2.30-2.17 (m, 6H), 2.87-2.68 (m, 2H), 3.40-3.18
10 (m, 13H), 3.79-3.43 (m, 42H), 3.86-3.80 (m, 3H), 4.02, 3.95 (m, 7H), 4.14 (d, J = 8.0
Hz, 3H).

Synthesis of 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-
[carboxy(polyethylene glycol)-2000]-N-tri(N-acetylgalactosamine), ammonium salt (21
and 22), DSPE-PEG(2K)-triGalNAc-G2):

15 1,2-Distearoyl-sn-glycero-3-phosphorylethanolamine (DSPE-PEG (2000))
Carboxylic acid (10) (2.0 g, 0.714 mmol) as described above in Example 1 was dissolved
in dry DMF and NHS (0.099 g, 0.857 mmol) was added whilst stirring the mixture and
stirring was continued for 10 minutes prior to adding DCC (0.147 g, 0.714 mmol) to the
reaction mixture and then stirred for 24 hours under nitrogen at 50°C. TEA (0.3 mL, 2.15
20 mmol) and (19) or (20) were added to produce either (21) or (22) respectively and
stirring was continued at 50°C for 24 hours. The progress of the reaction was checked by
TLC (chloroform: methanol: water: 10:10:3, R_f 0.3) and solvent was removed under
reduced pressure. The crude DSPE-PEG(2000)-triGalNAc-G2 final product (21 or 22) was
purified via silica flash column chromatography. The structure (21 or 22) was confirmed
25 with ¹H and ³¹P NMR as well as high performance liquid chromatography (HPLC) with a
mass spectrometer detector.

HPLC conditions and results

Chromatographic conditions of DSPE-PEG (2000)-triGalNAc: Detector:
evaporative light scattering detector (ELSD), concentration: 0.1 mg/mL (delusion with
30 methanol), gradient: solvent A (80:20:0.5:0.5, methanol: water: ammonium hydroxide:
acetic acid) with solvent B (100:0.5, methanol: ammonium hydroxide), injection volume
= 10 µL. Flow rate = 0.5 mL/min. Column = Phenomenex Luna-C18, 5 µm 4.6 x 250
mm, column temperature: 60 °C and detector temperature: 40°C.

Result: 100% compound (1) The HPLC chromatogram is shown in the Figure.

35 Example 3 Preparation of Lipid Nanoparticles:

100 mg 18:1 TAP (1,2-dioleoyl-3-trimethylammonium-propane), 23.7 mg 18:0 PC, 46.5 mg cholesterol, and 30 mg of the Example 1 compound (1) are mixed together in ethanol at 25 mg/mL. cDNA is dissolved in RNase free PBS (phosphate-buffered saline) (pH=7.4). The two solutions are mixed together using microfluidics to produce
5 lipid nanoparticles. Ethanol is removed by solvent exchange with RNase free buffer (pH=7.4). The lipid nanoparticles are 70-100nm.

Example 4 Preparation of Lipid Nanoparticles:

141.5 mg DODMA (1,2-dioleoyloxy-3-dimethylaminopropane), 72.8 mg cholesterol, 37.2 mg 18:0 PC, and 28.4 mg of the Example 1 compound (1) are dissolved in ethanol
10 at 20 mg/mL. mRNA is dissolved in 100 mM citrate buffer, pH=4. The two solutions are combined under controlled mixing using microfluidics. Ethanol is removed and buffer pH is adjusted by dialysis.

Example 5 Preparation of Liposomes:

5.4 grams HSPC (L-alpha-phosphatidylcholine, hydrogenated), 1.8 grams
15 cholesterol, 1.8 grams of the Example 1 compound (1) are dissolved in chloroform. Chloroform is removed by rotary evaporation and further removed under vacuum to create a thin film. Lipid film is hydrated with 250 mM ammonium sulfate buffer (pH=5.4) at 25 mg/mL at 60°C. Hydrated lipids are extruded to a final particle size 80-120nm. Buffer is exchanged for phosphate buffered saline (pH=7.4) by tangential flow filtration.
20 Separately, doxorubicin hydrochloride salt is dissolved in PBS (phosphate buffered saline) at elevated temperature, then combined with liposome solution and incubated at 60°C. Unencapsulated doxorubicin is removed, and buffer exchanged for 10 mM histidine with 10% sucrose.

Example 6 Preparation of Liposomes:

25 29.8 mg 18:0 PC (1,2-distearoyl-sn-glycero-3-phosphocholine), 11 mg cholesterol, 10 mg of the Example 1 compound (1) and 10 mg of a hydrophobic API (active pharmaceutical ingredient) are mixed together in chloroform. Chloroform is removed under nitrogen to create a thin film. Lipid/API film is hydrated in phosphate-buffered saline (pH=7.4) and extruded to a size 115 nm. Unencapsulated API is removed
30 by size exclusion.

Example 7 Preparation of Micelles:

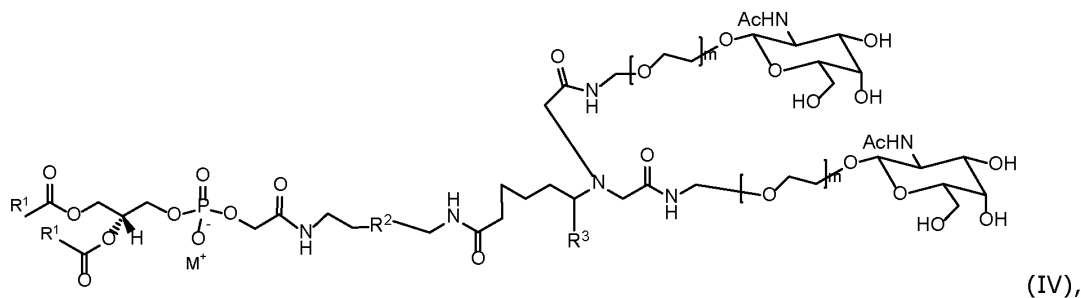
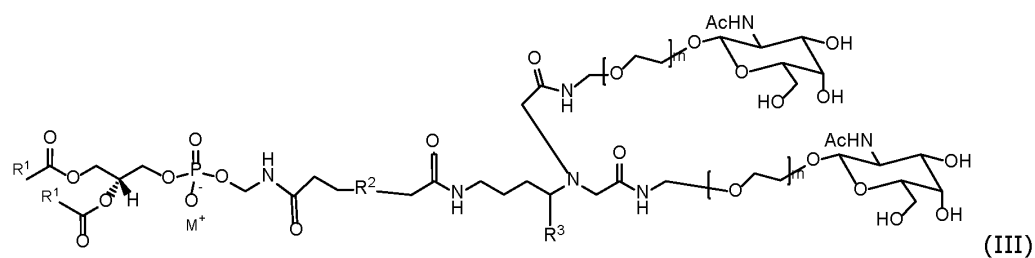
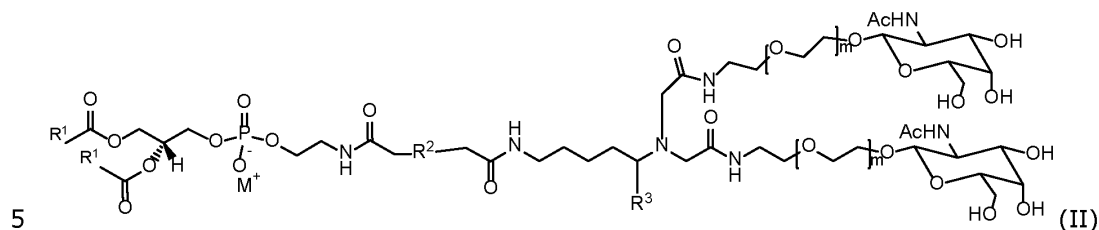
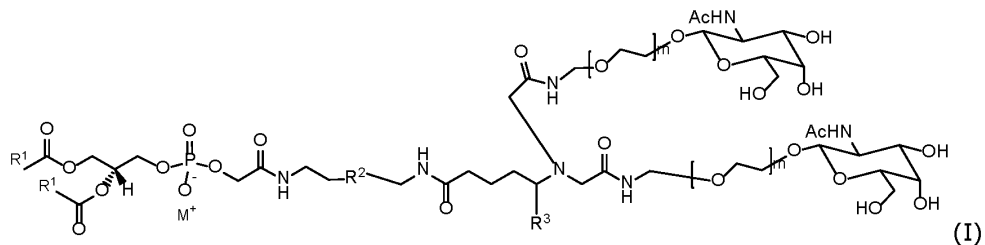
60 mg 18:1 PC (1,2-dioleoyl-sn-glycero-3-phosphocholine), 40 mg of the Example 1 compound (1), and 10 mg doxorubicin hydrochloride are dissolved in chloroform. Solvent is removed under nitrogen. Lipids are hydrated with phosphate-
35 buffered saline and sonicated to achieve micelles. Micelles are about 30 nm in diameter.

Within this specification embodiments have been described in a way which enables a clear and concise specification to be written, but it is intended and will be appreciated that embodiments may be variously combined or separated without departing from the invention. For example, it will be appreciated that all preferred
5 features described herein are applicable to all aspects of the invention described herein.

The foregoing description of various forms of the invention has been presented for purposes of illustration and description. It is not intended to be exhaustive or to limit the invention to the precise forms disclosed. Numerous modifications or variations are possible in light of the above teachings. The forms discussed were chosen and described
10 to provide the best illustration of the principles of the invention and its practical application to thereby enable one of ordinary skill in the art to utilize the invention in various forms and with various modifications as are suited to the particular use contemplated. All such modifications and variations are within the scope of the invention as determined by the appended claims when interpreted in accordance with the breadth
15 to which they are fairly, legally, and equitably entitled.

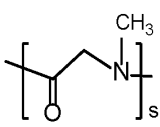
What is claimed is:

1. A compound comprising the structure (I), (II), (III), or (IV); or a combination of any two or more thereof:

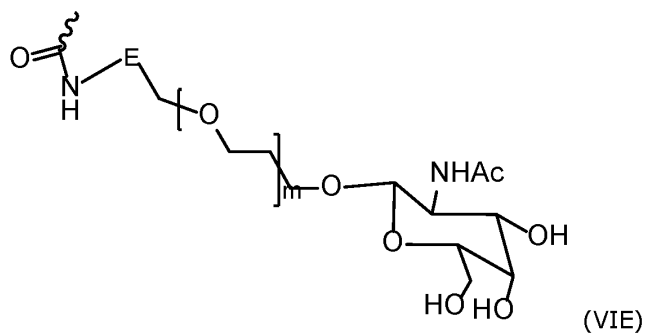
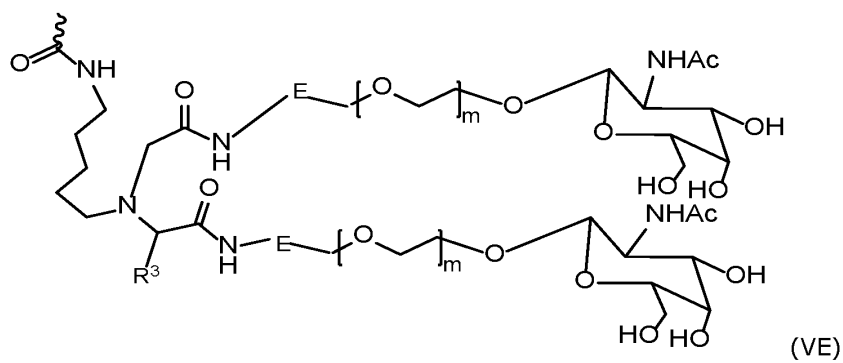
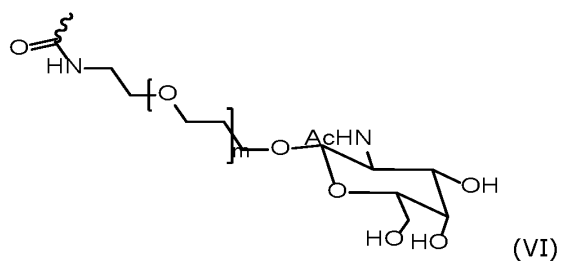
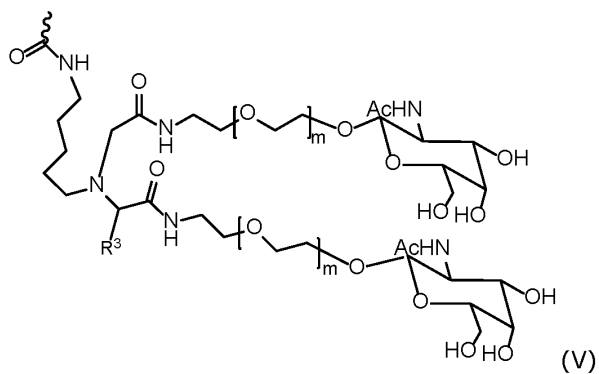


wherein:

- 10 each R^1 is independently selected from aliphatic alkyl C_4 - C_{100} groups optionally substituted with one or more of alkenyl, alkynyl, hydroxyl, amide, ester, and/or ether groups;

R^2 is selected from $[-OCH_2CH_2-]_p$, $[-OCH_2CH_2-]_p-O-$, or  ;

R^3 is selected from Formula (V) and Formula (VI), Formula (VE) and Formula (VIE):



M^+ is selected from an alkali metal ion, an alkaline earth metal ion, or a primary, secondary or tertiary ammonium ion;

m, p, and s are independently selected from integers from 1 to 120,

5 and wherein E is an optional spacer or extender group.

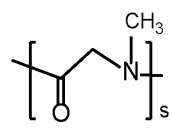
2. The compound according to claim 1, wherein the compound includes at most 15 N-acetylgalactosamine moieties per molecule.

10 3. The compound according to claim 1 or claim 2, wherein R^1 is an unsubstituted aliphatic alkyl C_4 - C_{100} group.

4. The compound according to any of claims 1-3, wherein R^2 is $[-OCH_2CH_2-]_p$ or $[-OCH_2CH_2-]_p-O-$.

15

5. The compound according to any of claims 1-3, wherein R^2 is



6. The compound according to any of claims 1-5, wherein p is an integer selected from 10 to 80, preferably from 20 to 70, more preferably from 30 to 60, most preferably from 40 to 50.

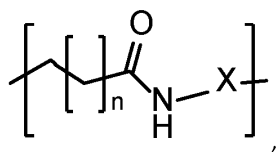
20

7. The compound according to any of claims 1-5, wherein s is an integer selected from 10 to 80, preferably from 20 to 70, more preferably from 30 to 60, most preferably from 40 to 50.

25

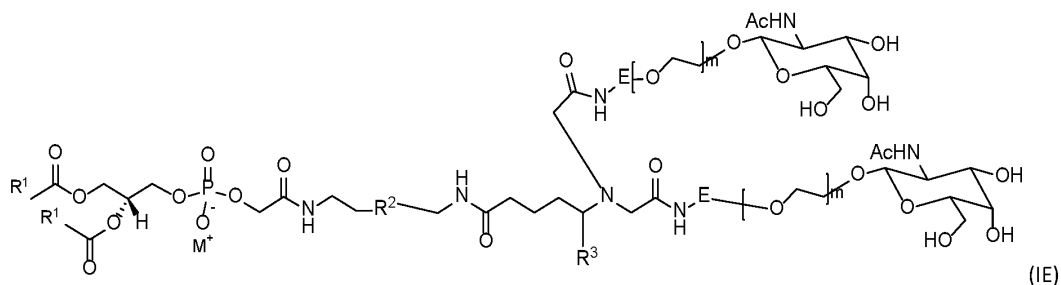
8. The compound according to any of claims 1-7, wherein m is an integer selected from 1 to 50, preferably from 1 to 20, more preferably from 1 to 10, most preferably from 2 to 5.

9. The compound according to any of claims 1-8, further comprising a spacer or extender group (E), where E has the structure:

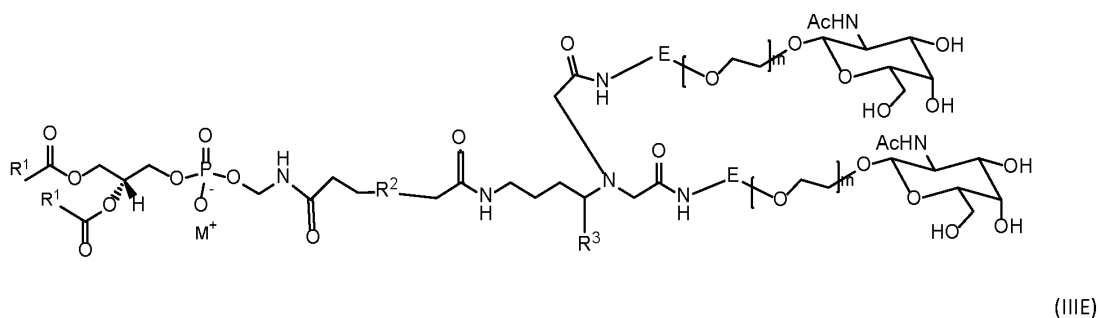
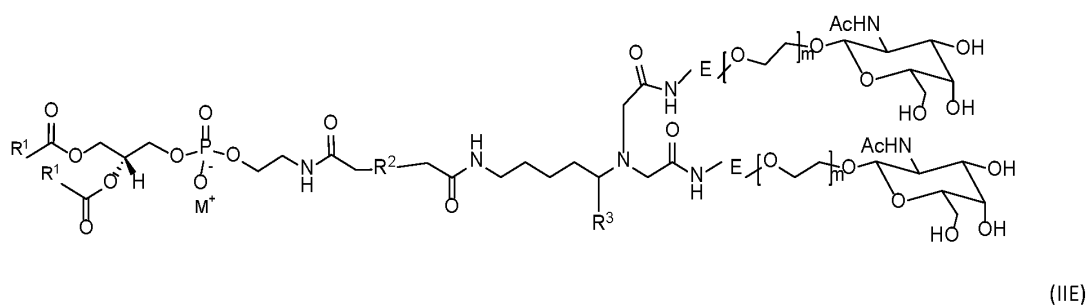


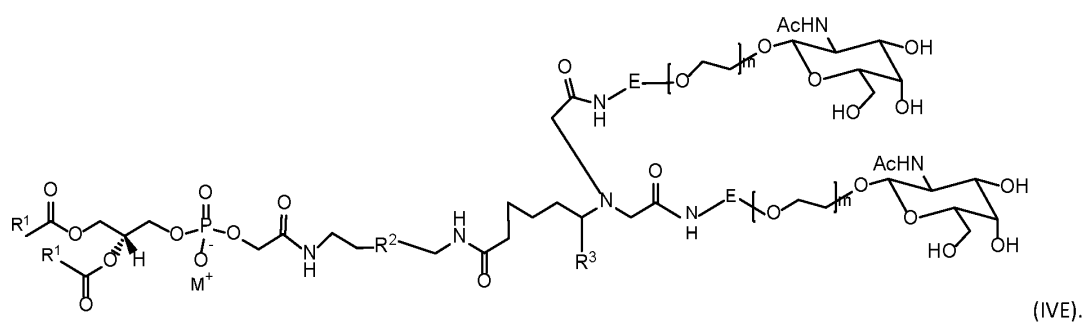
wherein, n is an integer between the value 0 and 6, and X is an alkyl chain of between 0 and 4 carbons in length.

10. The compound according to claim 9, comprising the structure (IE), (IIE), (IIIE), or (IVE); or a combination of any two or more thereof:

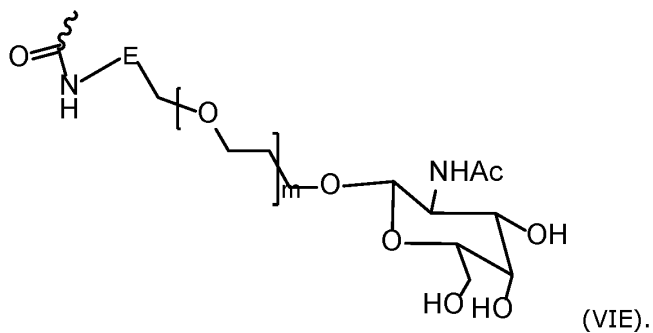
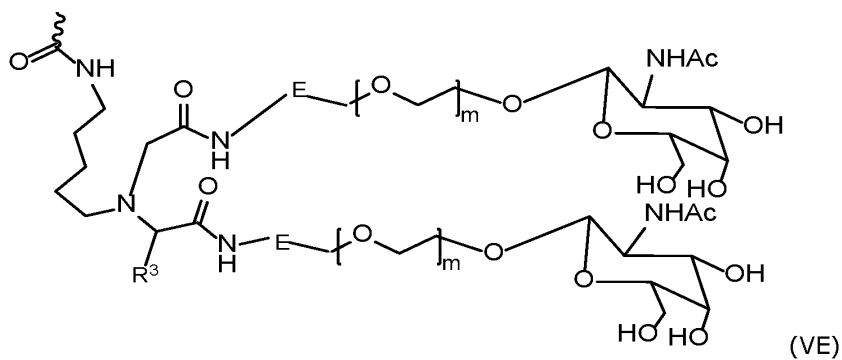


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11. The compound according to claim 9 or 10, wherein R^3 according to claim 1
 5 necessarily comprises a spacer or extender group (E), such that R^3 is selected from Formula (VE) and Formula (VIE):



10

12. A pharmaceutical composition comprising the compound of any one of claims 1 to
 11, an active pharmaceutical ingredient and pharmaceutically acceptable carrier.

13. A liver asialoglycoprotein receptor-targeted therapeutic agent comprising a drug oligonucleotide, antibody, enzyme, protein, small molecule, or nucleotide, in a form of a lipid nanoparticle, or in a form of a liposome, or in a form of a micelle comprising a compound in accordance with anyone of claims 1 to 11.

5

14. The liver asialoglycoprotein receptor-targeted therapeutic agent according claim 13, wherein the drug comprises at least one of medications for bile acid synthesis disorders, medications for cholestatic pruritus in alagille syndrome, medications for hepatic tumors, medications for hepatic coma, medications for hyperbilirubinemia, medications for liver
10 cirrhosis, medications for liver metastasis in adenocarcinoma, medications for metabolic dysfunction-associated steatotic liver disease, medications for pancreatic exocrine dysfunction, medications for portal hypertension, or medications for progressive familial intrahepatic cholestasis.

15 15. The liver asialoglycoprotein receptor-targeted therapeutic agent according to claim 12 or 13, wherein the oligonucleotide comprises at least one of ABE (adenine base editor), miRNA inhibitors, antisense oligonucleotides, ribozymes, DNazymes, plasmids, immune stimulating nucleic acids, antagomir, antimir, mimic, supermir, and aptamers, allele-specific oligonucleotide, antisense oligonucleotide, ribonucleoproteins (RNP),
20 mRNA, siRNA, sgRNA, gRNA, ss-siRNA, or combinations thereof.

16. A pharmaceutical composition comprising the liver asialoglycoprotein receptor-targeted therapeutic agent according to any of claims 13 to 15 and a pharmaceutically acceptable carrier.

25

17. A method of treatment, comprising administering the liver asialoglycoprotein receptor-targeted therapeutic agent or pharmaceutical composition according any of claims 12 to 16 to a patient in need thereof.

30