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(54) Title: NOVEL COMPOUNDS AND USE THEREOF FOR TARGETED DELIVERY

(57) Abstract: Disclosed herein are novel compounds and compositions involving the same, lipid particles including the novel compounds, as well as additional lipids such as phospholipids, structural lipids, and PEG lipids, and the use of the compounds or the lipid particles in the delivery of a therapeutic agent to a subject, targeted organs, or targeted cells.

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NOVEL COMPOUNDS AND USE THEREOF FOR TARGETED DELIVERY

[0001] FIELD OF THE DISCLOSURE

[0002] The present disclosure provides novel compounds and lipid particles comprising such compounds. The present disclosure also provides use of said compounds and lipid particles for targeted delivery. The present disclosure also provides methods of preparation for said compounds and lipid particles.

[0003] TECHNICAL BACKGROUND

[0004] Therapeutic nucleic acids, e.g., RNA has great application prospects in the prevention and treatment of many diseases or disorders. Various types of nucleic acids (e.g., small interfering RNA (siRNA), microRNA (miRNA), small activating RNAs (saRNA), single guide RNA (sgRNA), messenger RNA (mRNA), self-amplifying RNA, circRNA, antisense oligonucleotide (ASO), ribonucleoproteins (RNP), ssDNA and dsDNA) can be used to regulate specific gene(s) at DNA level, mRNA level or protein level, and accordingly the precise expression regulation of such gene(s) can be realized.

[0005] However, the problems currently faced by therapeutic nucleic acids are:

[0006] 1. how to protect nucleic acids from nuclease digestion;

[0007] 2. how to effectively deliver nucleic acids to the intracellular;

[0008] 3. how to increase efficiency of nucleic acids in endosomal escape;

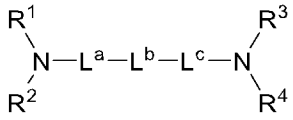
[0009] 4. how to deliver therapeutic nucleic acids to targeted cells and/or tissues of interest.

[0010] Therefore, there remains a need to develop compounds, compositions and methods to facilitate the delivery of therapeutic nucleic acids.

[0011] SUMMARY

[0012] Disclosed here are novel compounds and lipid particles comprising such compounds. Disclosed here is also use of said compounds and lipid particles for targeted delivery. Disclosed here are also methods of preparation for said compounds and lipid particles.

[0013] In one aspect, the present disclosure provides a compound having Formula (I) below:



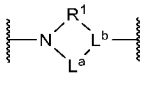
(I),

or a pharmaceutically acceptable salt thereof,

wherein

L^a and L^c are each independently selected from the group consisting of a bond, alkyl, alkenyl, alkynyl and heteroalkyl, wherein the alkyl, alkenyl, alkynyl and heteroalkyl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, cyano and amino;

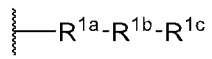
L^b is selected from the group consisting of a bond, $-NR^a-$, -O-, -S-, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, cycloalkyl, heterocyclyl, aryl and heteroaryl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, cycloalkyl, heterocyclyl, aryl and heteroaryl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, cyano and amino, or

L^b is covalently connected with R^1 to form  ;

provided that when two of L^a, L^b and L^c are bonds, then the remaining one is not a bond;

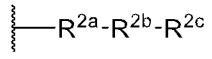
R^a is selected from the group consisting of hydrogen, R^5, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, cycloalkyl, heterocyclyl, aryl and heteroaryl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, cycloalkyl, heterocyclyl, aryl and heteroaryl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, cyano, cycloalkyl, heterocyclyl, aryl, heteroaryl and R^6;

R^1 is a group having Formula (I-a) below:



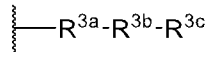
(I-a),

R^2 is a group having Formula (I-b) below:



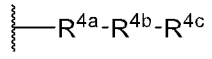
(I-b),

R^3 is a group having Formula (I-c) below:



(I-c),

R^4 is a group having Formula (I-d) below:



(I-d),

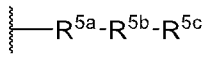
wherein

each of R^{1a}, R^{2a}, R^{3a} and R^{4a} is independently a bond or alkyl;

each of R^{1b}, R^{2b}, R^{3b} and R^{4b} is independently a bond, alkyl or heteroalkyl, wherein the alkyl and heteroalkyl are optionally substituted by one or more groups independently selected from the group consisting of hydroxyl, amino, oxo, thioxo and imino; and

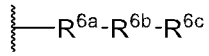
each of R^{1c}, R^{2c}, R^{3c} and R^{4c} is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, oxo, cyano, cycloalkyl, heterocyclyl, aryl and heteroaryl, and the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally interrupted by one or more groups independently selected from the group consisting of cycloalkyl, heterocyclyl, aryl and heteroaryl; and

R^5 is a group having Formula (I-e) below:



(I-e),

each R^6 is independently a group having Formula (I-f) below:



(I-f),

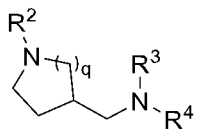
wherein

each of R^{5a} and R^{6a} is independently a bond or alkyl;

each of R^{5b} and R^{6b} is independently a bond, alkyl or heteroalkyl, wherein the alkyl and heteroalkyl is optionally substituted by one or more groups independently selected from the group consisting of hydroxyl, amino, oxo, thioxo and imino; and

each of R^{5c} and R^{6c} is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl is optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, oxo and cyano, and the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally interrupted by one or more groups independently selected from the group consisting of cycloalkyl, heterocyclyl, aryl and heteroaryl.

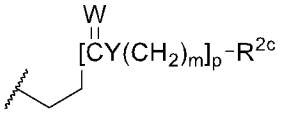
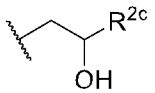
[0014] In one aspect, the present disclosure provides a compound having Formula (A) below:

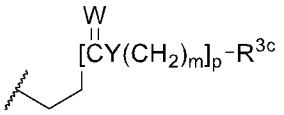
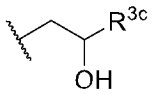


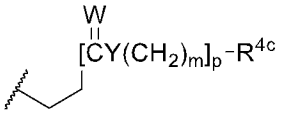
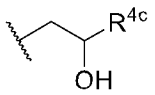
(A),

or a pharmaceutically acceptable salt thereof,

wherein

R^2 is  or  ;

R^3 is  or  ;

R^4 is  or  ;

each W is independently selected from O, S or NR^b;

each Y is independently selected from O, S, NR^c, $N(R^c)Z(W)$, $N(R^c)N(R^c)$ or $N(R^c)N(R^c)Z(W)$;

each Z is independently selected from C, S or S(O);

each m is independently 0 or 1;

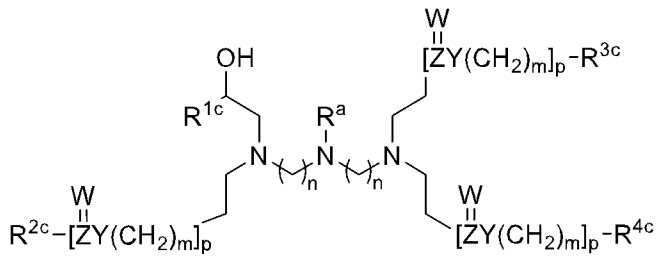
each p is independently 1 or 2;

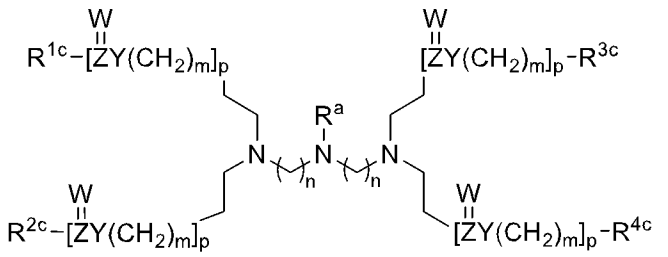
q is 1, 2 or 3;

each of R^b and R^c is independently selected from hydrogen, alkyl, alkyloxycarbonyl, alkylacyl, arylacyl or sulfonyl; and

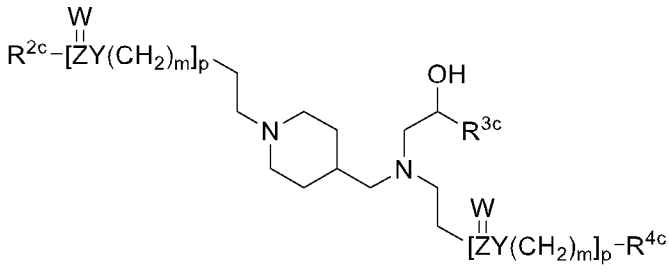
each of R^{2c}, R^{3c} and R^{4c} is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, oxo and cyano, and the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally interrupted by one or more groups independently selected from the group consisting of cycloalkyl, heterocyclyl, aryl and heteroaryl.

[0015] In one aspect, the present disclosure provides a compound having Formula (B), Formula (C), Formula (D) or Formula (E):

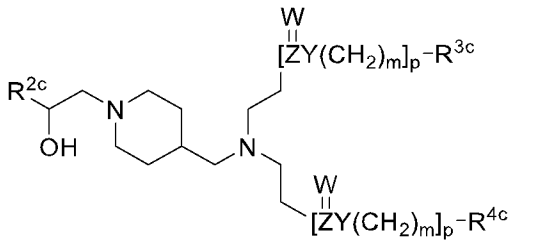
 (B),



(C),



(D),



(E),

wherein

each W is independently selected from O, S or NR^b;

each Y is independently selected from O, S, NR^c, $N(R^c)Z(W)$, $N(R^c)N(R^c)$ or $N(R^c)N(R^c)Z(W)$;

each Z is independently selected from C, S or S(O);

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

each m is independently 0, 1, 2 or 3;

each p is independently 1, 2, 3 or 4; and

each of R^b and R^c is independently selected from hydrogen, alkyl, alkyloxycarbonyl, acyl or sulfonyl.

[0016] In one aspect, the present disclosure provides a lipid particle comprising a compound having Formular (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E), or a pharmaceutically acceptable salt thereof. In one aspect, the present disclosure provides a loaded lipid particle comprising the lipid particle of the present disclosure and a therapeutic agent. In one aspect, the present disclosure provides a pharmaceutical composition comprising the loaded lipid particle of the present disclosure, and a pharmaceutically acceptable excipient.

[0017] In one aspect, the present disclosure provides a method of delivering a therapeutic agent to a subject, comprising the step of:

i) encapsulating a therapeutic agent into the lipid particle of the present disclosure; and

ii) administering the lipid particle encapsulating the therapeutic agent to the subject.

[0018] In one aspect, the present disclosure provides a method of delivering a therapeutic agent to a subject, comprising administering to the subject the loaded lipid particle of the present disclosure, or the pharmaceutical composition of the present disclosure.

[0019] In one aspect, the present disclosure provides a method of treating a disease or disorder in a subject comprising administering to the subject an effective amount of the loaded lipid particle of the present disclosure, or the pharmaceutical composition of the present disclosure.

[0020] In one aspect, the present disclosure provides use of the lipid particle of the present disclosure, the loaded lipid particle of the present disclosure or the pharmaceutical composition of the present disclosure for the manufacture of a medicament for a treatment of a disease or disorder.

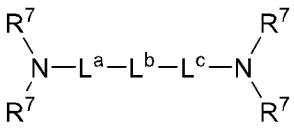
[0021] In one aspect, the present disclosure provides a method of expressing a protein in a subject comprising:

i) encapsulating a polynucleotide encoding the protein into the lipid particle of the present disclosure; and

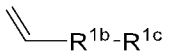
ii) administering the lipid particle encapsulating the polynucleotide to the subject.

[0022] In one aspect, the present disclosure provides a method for preparation of the compound having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E), or a pharmaceutically acceptable salt thereof, comprising:

A) mixing a compound having Formula (II) below and a compound having Formula (III) below:



(II),



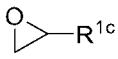
(III),

wherein

each R^7 is independently selected from the group consisting of hydrogen, alkyl, heteroalkyl, haloalkyl and hydroxyalkyl;

each L^a, L^b, L^c, R^{1b} and R^{1c} has the same definition as above; and
B) refluxing the mixture until completion of reaction, and obtaining the compound having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E).

[0023] In one aspect, the present disclosure provides a method for preparation of the compound having Formular (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E), or a pharmaceutically acceptable salt thereof, comprising:
i) mixing a compound having Formula (II) and a compound having Formula (IV) below:


(IV),

wherein R^{1c} has the same definition as above;
ii) mixing a product of step (i) and a compound having Formula (III); and
iii) refluxing the mixture until completion of reaction, and obtaining the compound having Formular (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E) thereby.

[0024] **DESCRIPTION OF THE DRAWINGS**

[0025] FIG. 1 shows changes of physical characteristics of test lipid particles in two weeks.

[0026] FIG. 2 shows *in vitro* and *in vivo* delivering properties of test lipid particles at day 0 and day 14.

[0027] FIG. 3 shows *in vitro* and *in vivo* delivering properties of formulations 1 to 4.

[0028] FIG. 4 shows *in vitro* delivering properties and cell viability of test formulations.

[0029] FIG. 5 shows A) *in vivo* imaging of fluorescence intensity in mice for FLuc mRNA delivering by test formulations; and B) *in vivo* delivering properties of test formulations.

[0030] FIG. 6 shows *in vitro* delivering properties and cell viability of lipid particles comprising test compounds.

[0031] FIG. 7 shows A) *in vivo* imaging of fluorescence intensity in mice for FLuc mRNA delivering by lipid particles comprising test compounds; and B) *in vivo* delivering properties to muscle of lipid particles comprising test compounds.

[0032] FIG. 8 shows A) imaging of liver, spleen and lung after *in vivo* delivering by lipid particles comprising test compounds; B) *in vivo* delivering properties to liver of lipid particles comprising test compounds; and C) FLuc mRNA delivering distribution of tissues for test liver targeting lipid particles.

[0033] FIG. 9 shows A) imaging of liver, spleen and lung after *in vivo* delivering by lipid particles comprising test compounds; B) *in vivo* delivering properties to spleen of lipid particles comprising test compounds; and C) FLuc mRNA delivering distribution of tissues for test spleen targeting lipid particles.

[0034] FIG. 10 shows A) imaging of liver, spleen and lung after *in vivo* delivering by lipid particles comprising test compounds; B) *in vivo* delivering properties to lung of lipid particles comprising test compounds; and C) FLuc mRNA delivering distribution of tissues for test lung targeting lipid particles.

[0035] FIG. 11 shows hEPO expression level after *in vivo* delivering by lipid particle comprising test compounds.

[0036] FIG. 12 shows changed ratio of test group to control group of drug concentration in plasma and lung douche after *in vivo* delivering by test lipid particle.

[0037] FIG. 13 shows antibody titers induced by a single immunization with *in vivo* RAV-G mRNA vaccine delivering by test lipid particle.

[0038] FIG. 14 shows recognizing ability of test antibody-conjugated lipid particle formulations toward different cell surface markers.

[0039] **DETAILED DESCRIPTION**

[0040] Reference will now be made in detail to certain embodiments of the present disclosure, examples of which are illustrated in the accompanying structures and formulas. While the present disclosure will be described in conjunction with the enumerated embodiments, it will be understood that they are not intended to limit the present disclosure to those embodiments. On the contrary, the present disclosure is intended to cover all alternatives, modifications, and equivalents, which may be included within the scope of the present disclosure as defined by the claims. One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present disclosure.

The present disclosure is in no way limited to the methods and materials described. In the event that one or more of the incorporated references and similar materials differs from or contradicts this application, including but not limited to defined terms, term usage, described techniques, or the like, the present disclosure controls. All references, patents, patent applications cited in the present disclosure are hereby incorporated by reference in their entireties.

[0041] *Definitions*

[0042] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the following terms are intended to have the following meanings:

[0043] As used in the specification and claims, the singular forms "a", "an", and "the" and the like includes plural references unless the context clearly dictates otherwise. Thus, for example, reference to "a compound" includes both a single compound and a plurality of different compounds.

[0044] The term "about" as used herein intends to indicate that the values quoted are not to be construed as absolute, and measurement error, inter-batches variation and/or inter-apparatus variation should also be taken into account.

[0045] The words "comprise," "comprising," "include," "including," and "includes" when used in this specification and in the following claims are intended to specify the presence of stated features, integers, components, or steps, but they do not preclude the presence or addition of one or more other features, integers, components, steps, or groups thereof.

[0046] It is to be understood that the "compound" of present disclosure can exist in solvated as well as un-solvated forms, such as, for example, hydrated forms, solid forms, and the present disclosure is intended to encompass all such solvated and unsolvated forms. It is further to be understood that the "compound" of present disclosure can exist in forms of pharmaceutically acceptable salts. In some embodiments, the "compound" of present disclosure is an ionizable lipid. In some embodiments, the "compound" of present disclosure can exist as a cationic lipid at physiological pH.

[0047] Definitions of specific functional groups and chemical terms are described in more detail below. 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., inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in Organic Chemistry, Thomas Sorrell, 2nd Edition, University Science Books, Sausalito, 2006; Smith and March March's Advanced Organic Chemistry, 6th Edition, John Wiley & Sons, Inc., New York, 2007; Larock, Comprehensive Organic Transformations, 3rd Edition, VCH Publishers, Inc., New York, 2018; Carruthers, Some Modern Methods of Organic Synthesis, 4th Edition, Cambridge University Press, Cambridge, 2004; the entire contents of each of which are incorporated herein by reference.

[0048] At various places in the present disclosure, linking substituents are described. Where the structure clearly requires a linking group, the Markush variables listed for that group are understood to be linking groups. For example, if the structure requires a linking group and the Markush group definition for that variable lists "alkyl", then it is understood that the "alkyl" represents a linking alkylene group.

[0049] When a bond to a substituent is shown to cross a bond connecting two atoms in a ring, then such substituent may be bonded to any atom in the ring. When a substituent is listed without indicating the atom via which such substituent is bonded to the rest of the compound of a given formula, then such substituent may be bonded via any atom in such formula. Combinations of substituents and/or variables are permissible, but only if such combinations result in stable compounds.

[0050] When any variable (e.g., R^i) occurs more than one time in any constituent or formula for a compound, its definition at each occurrence is independent of its definition at every other occurrence. Thus, for example, if a group is shown to be substituted with 0-2 R^i moieties, then the group may optionally be substituted with up to two R^i moieties and R^i at each occurrence is selected independently from the definition of R^i. Also, combinations of substituents and/or variables are permissible, but only if such combinations result in stable compounds.

[0051] As used herein, the term "C_{i-j}" indicates a range of the carbon atoms numbers, wherein i and j are integers and the range of the carbon atoms numbers includes the endpoints (i.e., i and j) and each integer point in between, and wherein j is greater than i. For examples, C_{1-6} indicates a range of one to six carbon atoms,

including one carbon atom, two carbon atoms, three carbon atoms, four carbon atoms, five carbon atoms and six carbon atoms. In some embodiments, the term "C_{1-24}" indicates 1 to 24, particularly 2 to 24, particularly 4 to 24, particularly 6 to 24, particularly 8 to 22, particularly 10 to 20, particularly 10 to 18 or particularly 12 to 18 carbon atoms.

[0052] As used herein, the term "alkyl", whether as part of another term or used independently, refers to a saturated linear or branched-chain hydrocarbon radical, which may be optionally substituted independently with one or more substituents described below. The term "C_{i-j} alkyl" refers to an alkyl having i to j carbon atoms. In some embodiments, alkyl groups contain 1 to 24 carbon atoms. In some embodiments, alkyl groups contain 1 to 23 carbon atoms. In some embodiments, alkyl groups contain 1 to 22 carbon atoms. In some embodiments, alkyl groups contain 1 to 21 carbon atoms. In some embodiments, alkyl groups contain 1 to 20 carbon atoms, 1 to 19 carbon atoms, 1 to 18 carbon atoms, 1 to 17 carbon atoms, 1 to 16 carbon atoms, 1 to 15 carbon atoms, 1 to 14 carbon atoms, 1 to 13 carbon atoms, or 1 to 12 carbon atoms. In some embodiments, alkyl groups contain 12 to 18 carbon atoms. In some embodiments, alkyl groups contain 12 to 17 carbon atoms, 12 to 16 carbon atoms, 12 to 15 carbon atoms or 12 to 14 carbon atoms. In some embodiments, alkyl groups contain 12 to 19 carbon atoms, 12 to 20 carbon atoms, 12 to 21 carbon atoms, 12 to 22 carbon atoms, 12 to 23 carbon atoms or 12 to 24 carbon atoms. In some embodiments, alkyl groups contain 1 carbon atom, 2 carbon atoms, 3 carbon atoms, 4 carbon atoms, 5 carbon atoms, 6 carbon atoms, 7 carbon atoms, 8 carbon atoms, 9 carbon atoms, 10 carbon atoms, 11 carbon atoms, 12 carbon atoms, 13 carbon atoms, 14 carbon atoms, 15 carbon atoms, 16 carbon atoms, 17 carbon atoms, 18 carbon atoms, 19 carbon atoms, 20 carbon atoms, 21 carbon atoms, 22 carbon atoms, 23 carbon atoms or 24 carbon atoms. Examples of "C_{1-10} alkyl" include, but are not limited to, methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, and decyl. Examples of "C_{1-6} alkyl" are methyl, ethyl, propyl, isopropyl, n-butyl, i-butyl, s-butyl, t-butyl, n-pentyl, 2-pentyl, 3-pentyl, 2-methyl-2-butyl, 3-methyl-2-butyl, 3-methyl-1-butyl, 2-methyl-1-butyl, 1-hexyl, 2-hexyl, 3-hexyl, 2-methyl-2-pentyl, 3-methyl-2-pentyl, 4-methyl-2-pentyl, 3-methyl-3-pentyl, 2-methyl-3-pentyl, 2,3-dimethyl-2-butyl, 3,3-dimethyl-2-butyl, and the like.

[0053] The alkyl groups can be further substituted by substituents which independently replace one or more hydrogen atoms on one or more carbons of the alkyl groups. Examples of such substituents can include, but are not limited to, acyl, alkyl, alkenyl, alkynyl, oxo, halogen, hydroxyl, alkoxyl, haloalkyl, haloalkoxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, phosphate, phosphonato, phosphinato, amino (including alkylamino, dialkylamino, arylamino, diarylamino and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfmyl, sulfonate, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, nitro, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety. Alkenyl, alkynyl, cycloalkyl, heterocyclyl, aryl and heteroaryl groups as described below may also be similarly substituted.

[0054] As used herein, the term "alkenyl", whether as part of another term or used independently, refers to linear or branched-chain hydrocarbon radical having at least one carbon-carbon double bond, which may be optionally substituted independently with one or more substituents described herein, and includes radicals having "*cis*" and "*trans*" orientations, or alternatively, "*E*" and "*Z*" orientations. In some embodiments, alkenyl groups contain 2 to 24 carbon atoms. In some embodiments, alkenyl groups contain 2 to 23 carbon atoms. In some embodiments, alkenyl groups contain 2 to 22 carbon atoms, 2 to 21 carbon atoms, 2 to 20 carbon atoms, 2 to 19 carbon atoms, 2 to 18 carbon atoms, 2 to 17 carbon atoms, 2 to 16 carbon atoms, 2 to 15 carbon atoms, 2 to 14 carbon atoms, 2 to 13 carbon atoms, 2 to 12 carbon atoms, 2 to 11 carbon atoms, and in some embodiments, alkenyl groups contain 2 carbon atoms. In some embodiments, alkenyl groups contain 12 to 18 carbon atoms. In some embodiments, alkenyl groups contain 12 to 17 carbon atoms, 12 to 16 carbon atoms, 12 to 15 carbon atoms or 12 to 14 carbon atoms. In some embodiments, alkenyl groups contain 12 to 19 carbon atoms, 12 to 20 carbon atoms, 12 to 21 carbon atoms, 12 to 22 carbon atoms, 12 to 23 carbon atoms or 12 to 24 carbon atoms. In some embodiments, alkenyl groups contain 2 carbon atoms, 3 carbon atoms, 4 carbon atoms, 5 carbon atoms, 6 carbon atoms, 7 carbon atoms, 8 carbon atoms, 9 carbon atoms, 10 carbon atoms, 11 carbon atoms, 12 carbon atoms, 13 carbon atoms, 14 carbon atoms,

15 carbon atoms, 16 carbon atoms, 17 carbon atoms, 18 carbon atoms, 19 carbon atoms, 20 carbon atoms, 21 carbon atoms, 22 carbon atoms, 23 carbon atoms or 24 carbon atoms. In some embodiments, alkenyl groups contain one or more "*Z*" carbon-carbon double bond. Examples of alkenyl group include, but are not limited to, ethylenyl (or vinyl), propenyl, butenyl, pentenyl, 1-methyl-2 buten-1-yl, 5-hexenyl, and the like. In some embodiments, an alkenyl group has at least one carbon-carbon double bond. In some embodiments, an alkenyl group has at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten carbon-carbon double bonds. In some embodiments, two or more of carbon-carbon double bonds in an alkenyl group are conjugated. In some embodiments, two or more of carbon-carbon double bonds in an alkenyl group are not conjugated. In some embodiments, two or more of carbon-carbon double bonds in an alkenyl group are isolated, cumulated or conjugated. The term "alkenyl", whether as part of another term or used independently, is also meant to include linear or branched-chain hydrocarbon radical having at least one carbon-carbon double bond and at least one carbon-carbon triple bond.

[0055] As used herein, the term "alkynyl", whether as part of another term or used independently, refers to a linear or branched hydrocarbon radical having at least one carbon-carbon triple bond, which may be optionally substituted independently with one or more substituents described herein. In some embodiments, alkynyl groups contain 2 to 24 carbon atoms. In some embodiments, alkynyl groups contain 2 to 23 carbon atoms. In some embodiments, alkynyl groups contain 2 to 22 carbon atoms, 2 to 21 carbon atoms, 2 to 20 carbon atoms, 2 to 19 carbon atoms, 2 to 18 carbon atoms, 2 to 17 carbon atoms, 2 to 16 carbon atoms, 2 to 15 carbon atoms, 2 to 14 carbon atoms, 2 to 13 carbon atoms, 2 to 12 carbon atoms, 2 to 10 carbon atoms, and in some embodiments, alkynyl groups contain 2 carbon atoms. In some embodiments, alkynyl groups contain 12 to 18 carbon atoms. In some embodiments, alkynyl groups contain 12 to 17 carbon atoms, 12 to 16 carbon atoms, 12 to 15 carbon atoms or 12 to 14 carbon atoms. In some embodiments, alkynyl groups contain 12 to 19 carbon atoms, 12 to 20 carbon atoms, 12 to 21 carbon atoms, 12 to 22 carbon atoms, 12 to 23 carbon atoms or 12 to 24 carbon atoms. In some embodiments, alkynyl groups contain 2 carbon atoms, 3 carbon atoms, 4 carbon atoms, 5 carbon atoms, 6 carbon atoms, 7 carbon atoms, 8 carbon atoms, 9 carbon atoms, 10 carbon atoms, 11 carbon atoms, 12 carbon atoms, 13

carbon atoms, 14 carbon atoms, 15 carbon atoms, 16 carbon atoms, 17 carbon atoms, 18 carbon atoms, 19 carbon atoms, 20 carbon atoms, 21 carbon atoms, 22 carbon atoms, 23 carbon atoms or 24 carbon atoms. Examples of alkynyl group include, but are not limited to, ethynyl, 1-propynyl, 2-propynyl, and the like. In some embodiments, an alkynyl group has at least one carbon-carbon triple bond. In some embodiments, an alkynyl group has at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten carbon-carbon triple bonds. In some embodiments, two or more of carbon-carbon triple bonds in an alkynyl group are conjugated. In some embodiments, two or more of carbon-carbon triple bonds in an alkynyl group are not conjugated. The term "alkynyl", whether as part of another term or used independently, is also meant to include linear or branched-chain hydrocarbon radical having at least one carbon-carbon triple bond and at least one carbon-carbon double bond.

[0056] As used herein, the term "alkoxyl", whether as part of another term or used independently, refers to an alkyl group, as previously defined, attached to the parent molecule through an oxygen atom. The term "C_{i-j} alkoxyl" means that the alkyl moiety of the alkoxy group has i to j carbon atoms. In some embodiments, alkoxy groups contain 1 to 10 carbon atoms. In some embodiments, alkoxy groups contain 1 to 9 carbon atoms. In some embodiments, alkoxy groups contain 1 to 8 carbon atoms, 1 to 7 carbon atoms, 1 to 6 carbon atoms, 1 to 5 carbon atoms, 1 to 4 carbon atoms, 1 to 3 carbon atoms, or 1 to 2 carbon atoms. Examples of "C_{1-6} alkoxyl" include, but are not limited to, methoxy, ethoxy, propoxy (e.g., *n*-propoxy and isopropoxy), *t*-butoxy, neopentoxy, *n*-hexoxy, and the like.

[0057] As used herein, the term "amino" refers to $-NH_2$. In some embodiments, amino may be substituted by any possible substituents on nitrogen.

[0058] As used herein, the term "aryl", whether as part of another term or used independently, refers to monocyclic and polycyclic ring systems having a total of 5 to 20 ring members, wherein at least one ring in the system is aromatic and wherein each ring in the system contains 3 to 12 ring members. Examples of "aryl" include, but are not limited to, phenyl, biphenyl, naphthyl, anthracyl and the like, which may bear one or more substituents. Also included within the scope of the term "aryl", as it is used herein, is a group in which an aromatic ring is fused to one or more additional rings. In the case of polycyclic ring system, only one of the rings needs to be aromatic (e.g.,

2,3-dihydroindole), although all of the rings may be aromatic (e.g., quinoline). The second ring can also be fused, bridged or spiro. Examples of polycyclic aryl include, but are not limited to, benzofuranyl, indanyl, phthalimidyl, naphthimidyl, phenanthridinyl, or tetrahydronaphthyl, and the like. Aryl groups can be substituted at one or more ring positions with substituents as described above.

[0059] As used herein, the term "cycloalkyl", whether as part of another term or used independently, refer to a monovalent non-aromatic, saturated or partially unsaturated monocyclic and polycyclic ring system, in which all the ring atoms are carbon and which contains at least three ring forming carbon atoms. In some embodiments, the cycloalkyl may contain 3 to 12 ring forming carbon atoms, 3 to 11 ring forming carbon atoms, 3 to 10 ring forming carbon atoms, 3 to 9 ring forming carbon atoms, 3 to 8 ring forming carbon atoms, 3 to 7 ring forming carbon atoms, 3 to 6 ring forming carbon atoms, 3 to 5 ring forming carbon atoms, 3 to 4 ring forming carbon atoms, 4 to 12 ring forming carbon atoms, 4 to 11 ring forming carbon atoms, 4 to 10 ring forming carbon atoms, 4 to 9 ring forming carbon atoms, 4 to 8 ring forming carbon atoms, 4 to 7 ring forming carbon atoms, 4 to 6 ring forming carbon atoms, 4 to 5 ring forming carbon atoms. Cycloalkyl groups may be saturated or partially unsaturated. Cycloalkyl groups may be substituted. In some embodiments, the cycloalkyl group may be a saturated cyclic alkyl group. In some embodiments, the cycloalkyl group may be a partially unsaturated cyclic alkyl group that contains at least one double bond or triple bond in its ring system.

[0060] In some embodiments, the cycloalkyl group may be monocyclic or polycyclic. Examples of monocyclic cycloalkyl group include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, 1-cyclopent-1-enyl, 1-cyclopent-2-enyl, 1-cyclopent-3-enyl, cyclohexyl, 1-cyclohex-1-enyl, 1-cyclohex-2-enyl, 1-cyclohex-3-enyl, cyclohexadienyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl, cycloundecyl and cyclododecyl.

[0061] In some embodiments, the cycloalkyl group may be saturated or partially unsaturated polycyclic (e.g., bicyclic and tricyclic) carbocyclic ring system, which can be arranged as a fused-, spiro- or bridged-ring system. As used herein, the term "fused-ring" refers to a ring system having two rings sharing two adjacent atoms, the term "spiro-ring" refers to a ring systems having two rings connected through one single common atom, and the term "bridged-ring" refers to a ring system with two rings

sharing three or more atoms. Examples of fused carbocyclyl include, but are not limited to, naphthyl, benzopyrenyl, anthracenyl, acenaphthenyl, fluorenyl and the like. Examples of spiro carbocyclyl include, but are not limited to, spiro[5.5]undecanyl, spiro-pentadienyl, spiro[3.6]-decanyl, and the like. Examples of bridged carbocyclyl include, but are not limited to bicyclo[1,1,1]pentenyl, bicyclo[2,2,1]heptenyl, bicyclo[2,2,1]heptanyl, bicyclo[2,2,2]octanyl, bicyclo[3,3,1]nonanyl, bicyclo[3,3,3]undecanyl, and the like.

[0062] As used herein, the term "cyano" refers to -CN.

[0063] As used herein, the term "oxo" refers to =O, which substitutes two hydrogen atoms connected to one atom. For example, an oxo substituted ethyl is $CH_3C(=O)$- or -$C(=O)CH_2$-.

[0064] As used herein, the term "thioxo" refers to =S, which substitutes two hydrogen atoms connected to one atom. For example, an thioxo substituted ethyl is $CH_3C(=S)$- or -$C(=S)CH_2$-.

[0065] As used herein, the term "imino" refers to =NR, which substitutes two hydrogen atoms connected to one atom, wherein R refers to hydrogen or any possible substituent on nitrogen. For example, an imino substituted ethyl is $CH_3C(=NR)$- or -$C(=NR)CH_2$-.

[0066] As used herein, the term "halogen" refers to an atom selected from fluorine (or fluoro), chlorine (or chloro), bromine (or bromo) and iodine (or iodo).

[0067] As used herein, the term "haloalkyl", whether as part of another term or used independently, refers to an alkyl group having one or more halogen substituents. Examples of haloalkyl group include, but are not limited to, trifluoromethyl (-CF_3), pentafluoroethyl (-C_2F_5), difluoromethyl (-CHF_2), trichloromethyl (-CCl_3), dichloromethyl (-$CHCl_2$), pentachloroethyl (-C_2Cl_5), and the like.

[0068] As used herein, the term "hydroxyalkyl", whether as part of another term or used independently, refers to an alkyl group having one or more hydroxyl substituents. Examples of hydroxyalkyl group include, but are not limited to, hydroxymethyl (-CH_2OH), 1-hydroxyethyl (-$CH(OH)CH_3$), 2-hydroxyethyl (-CH_2CH_2OH), 1,2-dihydroxyethyl (-$CH(OH)CH_2OH$), 1-hydroxy-*n*-propyl (-$CH(OH)CH_2CH_3$), 2-hydroxy-*n*-propyl (-$CH_2CH(OH)CH_3$), 3-hydroxy-*n*-propyl (-$CH_2CH_2CH_2OH$), 2-hydroxypropan-2-yl (-$C(OH)(CH_3)_2$), 1-hydroxypropan-2-yl (-$C(CH_3)CH_2OH$), 1,2-dihydroxypropyl (-$CH(OH)CH(OH)CH_3$), 1,3-dihydroxypropyl

(-CH(OH)CH_2CH_2OH), 2,3-dihydroxypropyl (-CH_2CH(OH)CH_2OH), 1,2,3-trihydroxypropyl (-CH(OH)CH(OH)CH_2OH), and the like.

[0069] As used herein, the term "haloalkoxyl", whether as part of another term or used independently, refers to an alkoxyl group having one or more halogen substituents. As a result, the term "halo-C_{i-j} alkoxyl", whether as part of another term or used independently, refers to a C_{i-j} alkoxyl group having one or more halogen substituents. Examples of haloalkoxyl include, but are not limited to, -O-CF_3, -O-C_2F_5, -O-CHF_2, -O-CCl_3, -O-$CHCl_2$, -O-C_2Cl_5, and the like.

[0070] As used herein, the term "heteroatom" refers to nitrogen (N), oxygen (O), sulfur (S), and includes any oxidized form of nitrogen or sulfur, and any quaternized form of a basic nitrogen (including N-oxides).

[0071] As used herein, the term "heteroalkyl", "heteroalkenyl", or "heteroalkynyl", whether as part of another term or used independently, refers to an alkyl, alkenyl, or alkynyl group containing one or more heteroatoms. As a result, the term "hetero-C_{i-j} alkyl", "hetero-C_{i-j} alkenyl", or "hetero-C_{i-j} alkynyl", whether as part of another term or used independently, refers to a C_{i-j} alkyl, C_{i-j} alkenyl, or C_{i-j} alkynyl containing one or more heteroatoms. For example, the term "hetero-C_{1-6} alkyl", whether as part of another term or used independently, refers to a C_{1-6} alkyl containing one or more heteroatoms. In some embodiments, a heteroalkyl, heteroalkenyl or heteroalkynyl group contains at least one heteroatom. In some embodiments, a heteroalkyl, heteroalkenyl or heteroalkynyl group contains at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine or at least ten heteroatoms. In some embodiments, two or more heteroatoms in a heteroalkyl, heteroalkenyl or heteroalkynyl group are the same. In some embodiments, two or more heteroatoms in a heteroalkyl, heteroalkenyl or heteroalkynyl group are different. In some embodiments, two or more heteroatoms in a heteroalkyl, heteroalkenyl or heteroalkynyl group are directly bonded. In some embodiments, two or more heteroatoms in a heteroalkyl, heteroalkenyl or heteroalkynyl group are not directly bonded.

[0072] As used herein, the term "heteroaryl", whether as part of another term or used independently, refers to an aryl group having, in addition to carbon atoms, one or more heteroatoms. The heteroaryl group can be monocyclic. Examples of monocyclic heteroaryl include, but are not limited to, thienyl, furanyl, pyrrolyl,

imidazolyl, pyrazolyl, triazolyl, tetrazolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiazolyl, isothiazolyl, thiadiazolyl, pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, indolizinyl, purinyl, naphthyridinyl, benzofuranyl and pteridinyl. The heteroaryl group also includes polycyclic groups in which a heteroaromatic ring is fused to one or more aryl, heteroaryl, cycloaliphatic, or heterocyclyl rings, where the radical or point of attachment is on the heteroaromatic ring or the other ring. Examples of polycyclic heteroaryl include, but are not limited to, indolyl, isoindolyl, benzothienyl, benzofuranyl, benzo[1,3]dioxolyl, dibenzofuranyl, indazolyl, benzimidazolyl, benzthiazolyl, quinolyl, isoquinolyl, dihydroquinolinyl, dihydroisoquinolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, 4H-quinolizinyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, and the like.

[0073] As used herein, the term "heterocyclyl" refers to a saturated or partially unsaturated carbocyclyl group in which one or more ring atoms are heteroatoms independently selected from oxygen, sulfur, nitrogen, phosphorus, and the like, the remaining ring atoms being carbon, wherein one or more ring atoms may be optionally substituted independently with one or more substituents. In some embodiments, the heterocyclyl is a saturated heterocyclyl. In some embodiments, the heterocyclyl is a partially unsaturated heterocyclyl having one or more double bonds in its ring system. In some embodiments, the heterocyclyl may contains any oxidized form of carbon, nitrogen or sulfur, and any quaternized form of a basic nitrogen. The heterocyclyl radical may be carbon linked or nitrogen linked where such is possible. In some embodiments, the heterocycle is carbon linked. In some embodiments, the heterocycle is nitrogen linked. For example, a group derived from pyrrole may be pyrrol-1-yl (nitrogen linked) or pyrrol-3-yl (carbon linked). Further, a group derived from imidazole may be imidazol-1-yl (nitrogen linked) or imidazol-3-yl (carbon linked).

[0074] Heterocyclyl group may be monocyclic. Examples of monocyclic heterocyclyl include, but are not limited to oxetanyl, 1,1-dioxothietanylpyrrolidyl, tetrahydrofuryl, tetrahydropyranyl, tetrahydrothienyl, azetidinyl, pyrrolyl, furanyl, thienyl, pyrazolyl, imidazolyl, triazolyl, oxazolyl, thiazolyl, piperidyl, piperazinyl, morpholinyl, pyridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazinyl, pyridonyl, pyrimidonyl, pyrazinonyl, pyrimidonyl, pyridazonyl, pyrrolidinyl, triazinonyl, and the like.

[0075] Heterocyclyl group may be polycyclic, including the fused-, spiro- and bridged-ring systems. The fused heterocyclyl group includes radicals wherein the heterocyclyl radicals are fused with a saturated, partially unsaturated, or fully unsaturated (i.e., aromatic) carbocyclic or heterocyclic ring. Examples of fused heterocyclyl include, but are not limited to, phenyl fused-ring or pyridinyl fused-ring, such as quinolinyl, isoquinolinyl, quinoxalinyl, quinolizinyl, quinazolinyl, azaindolizinyl, pteridinyl, chromenyl, isochromenyl, indolyl, isoindolyl, indolizinyl, indazolyl, purinyl, benzofuranyl, isobenzofuranyl, benzimidazolyl, benzothienyl, benzothiazolyl, carbazolyl, phenazinyl, phenothiazinyl, phenanthridinyl, imidazo[1,2-a]pyridinyl, [1,2,4]triazolo[4,3-a]pyridinyl, [1,2,3]triazolo[4,3-a]pyridinyl groups, and the like. Examples of spiro heterocyclyl include, but are not limited to, spiropyranyl, spirooxazinyl, 5-aza-spiro[2.4]heptanyl, 6-aza-spiro[2.5]octanyl, 6-aza-spiro[3.4]octanyl, 2-oxa-6-aza-spiro[3.3]heptanyl, 2-oxa-6-aza-spiro[3.4]octanyl, 6-aza-spiro[3.5]nonanyl, 7-aza-spiro[3.5]nonanyl, 1-oxa-7-aza-spiro[3.5]nonanyl and the like. Examples of bridged heterocyclyl include, but are not limited to, 3-aza-bicyclo[3,1,0]hexanyl, 8-aza-bicyclo[3,2,1]octanyl, 1-aza-bicyclo[2,2,2]octanyl, 2-aza-bicyclo[2,2,1]heptanyl, 1,4-diazabicyclo[2,2,2]octanyl, and the like.

[0076] As used herein, the term "hydroxyl" or "hydroxy" refers to -OH.

[0077] As used herein, the term "sulfhydryl" refers to -SH.

[0078] As used herein, the term "alkyloxycarbonyl" refers to alkyl-O-C(=O)-. In some embodiments, an alkyloxycarbonyl may be further substituted on alkyl by any possible substituents described above. Examples of alkyloxycarbonyl include, but not limited to, *t*-butoxylcarbonyl, benzyloxycarbonyl, allyloxycarbonyl and 9-fluorenylmethyloxycarbonyl.

[0079] As used herein, the term "sulfonyl" refers to R-SO_2-, wherein R is hydrogen or any possible substituents on sulfur. Examples of sulfonyl include, but not limited to, *p*-toluenesulfonyl, *p*-bromobenzenesulfonyl, 2- or 4-nitrobenzenesulfonyl, trifluoromethanesulfonyl, methanesulfonyl and 5-(dimethylamino)naphthalene-1-sulfonyl.

[0080] As used herein, the term "acyl", whether as part of another term or used independently, refers to RC(=O)-, wherein R is hydrogen or any possible substituents on carbon. Examples of acyl include, but not limited to, formyl, acetyl, trifluoroacetyl and benzoyl. The term "alkylacyl" refers to alkyl-C(=O)-, wherein the alkyl is

optionally substituted by any possible substituents described above. The term "arylacyl" refers to aryl-C(O=)-, wherein the aryl is optionally substituted by any possible substituents described above.

[0081] As used herein, the term "partially unsaturated" refers to a radical that includes at least one double or triple bond. The term "partially unsaturated" is intended to encompass rings having multiple sites of unsaturation, but is not intended to include aromatic (i.e., fully unsaturated) moieties.

[0082] As used herein, the term "substituted", whether preceded by the term "optionally" or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent. It will be understood that "substitution", "substituted by" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and that the substitution results in a stable or chemically feasible compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. Unless otherwise indicated, an "optionally substituted" group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted by more than one substituent selected from a specified group, the substituent may be either the same or different at every position. It will be understood by those skilled in the art that substituents can themselves be substituted, if appropriate. Unless specifically stated as "unsubstituted", references to chemical moieties herein are understood to include substituted variants. For example, reference to an "aryl" group or moiety implicitly includes both substituted and unsubstituted variants.

[0083] As used herein, the term "interrupted", whether preceded by the term "optionally" or not, means that one or more covalent bonds of the designated moiety are replaced with a suitable linking group, but not at the termini. In some embodiments, the replaced bond is a carbon-carbon bond. In some embodiments, the replaced bond is a carbon-heteroatom bond. Unless otherwise indicated, an "optionally interrupted" group may have a suitable linking group at each replaceable position of the group, and when more than one position in any given structure may be interrupted by more than one linking group selected from a specified group, the linking group may be either the same or different at every position. In some embodiments, an alkyl "interrupted" by a cycloalkyl refers to alkyl-cycloalkyl-alkyl. In some

embodiments, an alkenyl "interrupted" by a cycloalkyl refers to alkenyl-cycloalkyl-alkyl, alkenyl-cycloalkyl-alkenyl, alkenyl-cycloalkyl-alkynyl, alkyl-cycloalkyl-alkenyl or alkynyl-cycloalkyl-alkenyl. In some embodiments, an alkynyl "interrupted" by a cycloalkyl refers to alkynyl-cycloalkyl-alkyl, alkynyl-cycloalkyl-alkenyl, alkynyl-cycloalkyl-alkynyl, alkyl-cycloalkyl-alkynyl or alkenyl-cycloalkyl-alkynyl. In some embodiments, an alkyl "interrupted" by a heterocyclyl refers to alkyl-heterocyclyl-alkyl. In some embodiments, an alkenyl "interrupted" by a heterocyclyl refers to alkenyl-heterocyclyl-alkyl, alkenyl-heterocyclyl-alkenyl, alkenyl-heterocyclyl-alkynyl, alkyl-heterocyclyl-alkenyl or alkynyl-heterocyclyl-alkenyl. In some embodiments, an alkynyl "interrupted" by a heterocyclyl refers to alkynyl-heterocyclyl-alkyl, alkynyl-heterocyclyl-alkenyl, alkynyl-heterocyclyl-alkynyl, alkyl-heterocyclyl-alkynyl or alkenyl-heterocyclyl-alkynyl. In some embodiments, an alkyl "interrupted" by an aryl refers to alkyl-aryl-alkyl. In some embodiments, an alkenyl "interrupted" by an aryl refers to alkenyl-aryl-alkyl, alkenyl-aryl-alkenyl, alkenyl-aryl-alkynyl, alkyl-aryl-alkenyl or alkynyl-aryl-alkenyl. In some embodiments, an alkynyl "interrupted" by an aryl refers to alkynyl-aryl-alkyl, alkynyl-aryl-alkenyl, alkynyl-aryl-alkynyl, alkyl-aryl-alkynyl or alkenyl-aryl-alkynyl. In some embodiments, an alkyl "interrupted" by a heteroaryl refers to alkyl-heteroaryl-alkyl. In some embodiments, an alkenyl "interrupted" by a heteroaryl refers to alkenyl-heteroaryl-alkyl, alkenyl-heteroaryl-alkenyl, alkenyl-heteroaryl-alkynyl, alkyl-heteroaryl-alkenyl or alkynyl-heteroaryl-alkenyl. In some embodiments, an alkynyl "interrupted" by a heteroaryl refers to alkynyl-heteroaryl-alkyl, alkynyl-heteroaryl-alkenyl, alkynyl-heteroaryl-alkynyl, alkyl-heteroaryl-alkynyl or alkenyl-heteroaryl-alkynyl.

[0084] As used herein, the term "pharmaceutically acceptable" refers to those compounds, lipid particles, loaded lipid particles, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and other animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. In some embodiments, compounds, lipid particles, loaded lipid particles, materials, compositions, and/or dosage forms that are pharmaceutically acceptable refer to those approved by a regulatory agency (such as U.S. Food and Drug Administration, National Medical Products Administration or European Medicines Agency) or listed in generally recognized pharmacopoeia (such as

U.S. Pharmacopoeia, China Pharmacopoeia or European Pharmacopoeia) for use in animals, and more particularly in humans.

[0085] As used herein, "pharmaceutically acceptable salts" or "pharmaceutical salts" refers to derivatives of a compound wherein the parent compound is modified by converting an existing acidic moiety (e.g., carboxyl and the like) or base moiety (e.g., amine, alkali and the like) to its salt form. In many cases, compounds of present disclosure are capable of forming acid addition salts and/or base salts by virtue of the presence of amino, alkali or groups similar thereto. And the "pharmaceutically acceptable salts" include acid addition salts or base salts that retain biological effectiveness and properties of the parent compound, which typically are not biologically or otherwise undesirable. Pharmaceutically acceptable salts are well known in the art. For example, Berge et al. describes pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences (1977) 66: 1-19. Pharmaceutically acceptable salts of the compounds provided herein include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, lactic acid, trifluoracetic acid, benzoic acid, cinnamic acid, mandelic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, malonic acid, fumaric acid, citric acid, malic acid, maleic acid, tartaric acid, succinic acid, or methanesulfonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, besylate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. In some embodiments, inorganic acids from which salts can be derived include, for example, hydrochloric acid salt, sulfuric acid

salt, phosphoric acid salt, and the like. In some embodiments, organic acids from which salts can be derived include, for example, maleic acid salt, fumaric acid salt, oxalic acid salt, p-toluenesulfonic acid salt, succinic acid salt, L-(+)-tartaric acid salt, mono-adipic acid salt, hemi-adipic acid salt, and the like.

[0086] The term "pharmaceutical composition" refers to a mixture of one or more compounds of the present disclosure or one or more lipid particles or loaded lipid particles of present disclosure, with other chemical components, such as pharmaceutically acceptable diluent, excipient or carrier. The purpose of a pharmaceutical composition is to facilitate administration of compounds, lipid particles or loaded lipid particles to a subject.

[0087] The term "pharmaceutically acceptable excipient" or "pharmaceutically acceptable carrier" as used herein refers to a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a compound provided herein from one location, body fluid, tissue, organ (interior or exterior), or portion of the body, to another location, body fluid, tissue, organ, or portion of the body. Pharmaceutically acceptable excipients or carriers can be vehicles, diluents, excipients, or other materials that can be used to contact the tissues of an animal without excessive toxicity or adverse effects. Non-limiting examples of pharmaceutically acceptable excipients or carriers include sugars such as lactose, glucose and sucrose; starches such as com starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, com oil and soybean oil; glycols, such as polyethylene glycol and propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate; coloring agents; releasing agents; coating agents; sweetening, flavoring and perfuming agents; preservatives; antioxidants; ion exchangers; alumina; aluminum stearate; lecithin; self-emulsifying drug delivery systems (SEDDS) such as d-α-tocopherol polyethyleneglycol 1000 succinate; surfactants used in pharmaceutical dosage forms such as Tweens or other similar polymeric delivery matrices; serum proteins such as

human serum albumin; glycine; sorbic acid; 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, and zinc salts; colloidal silica; magnesium trisilicate; polyvinyl pyrrolidone; cellulose-based substances; polyacrylates; waxes; and polyethylene-polyoxypropylene-block polymers. Cyclodextrins such as α-, β-, and γ-cyclodextrin, or chemically modified derivatives such as hydroxyalkylcyclodextrins, including 2- and 3-hydroxypropyl-cyclodextrins, or other solubilized derivatives can also be used to enhance delivery of compounds described herein. Pharmaceutically acceptable excipients or carriers that can be employed in present disclosure includes those generally known in the art, such as those disclosed in "Remington Pharmaceutical Sciences" Mack Pub. Co., New Jersey (1991), which is incorporated herein by reference.

[0088] As used herein, "administration" of a disclosed compound, lipid particles or loaded lipid particles encompasses the delivery to a subject of a compound, lipid particles or loaded lipid particles as described herein, or a prodrug or other pharmaceutically acceptable derivative thereof, using any suitable formulation or route of administration, as discussed herein.

[0089] As used herein, the term "delivering", "deliver" or "delivery" means providing an entity to a destination. For example, delivering a therapeutic and/or prophylactic agent to a subject may involve administering a lipid particle composition comprising the therapeutic and/or prophylactic agent to the subject (e.g, by an intravenous, intramuscular, intradermal, or subcutaneous route). Administration of lipid particle or a composition comprising the lipid particle to a mammal or mammalian cell may involve contacting one or more cells with the lipid particle or the composition.

[0090] As used herein, the term "enhanced delivery" means delivery of more (e.g., at least 1.5 fold more, at least 2-fold more, at least 3-fold more, at least 4-fold more, at least 5-fold more, at least 6-fold more, at least 7-fold more, at least 8-fold more, at least 9-fold more, at least 10-fold more or at least 100-fold more) of a therapeutic and/or prophylactic agent by a lipid particle to a target tissue of interest (e.g., liver, lung, spleen or muscle) or a target cell of interest (e.g., liver cell, lung cell, spleen cell or muscle cell) compared to the level of delivery of a therapeutic and/or prophylactic agent by a control lipid particle (e.g., lipid particle comprising DLin-MC3-DMA) to a target tissue of interest (e.g., liver, lung, spleen or muscle) or a target cell of interest (e.g.,

liver cell, lung cell, spleen cell or muscle cell). The level of delivering a therapeutic and/or prophylactic agent to a particular tissue or cell may be measured by comparing the amount of therapeutic and/or prophylactic agent in a tissue or cell to the amount of total therapeutic and/or prophylactic agent in said tissue or cell, comparing the amount of therapeutic and/or prophylactic agent in a tissue or cell to the weight of said tissue, comparing the amount of protein produced in a tissue or cell to the amount of total protein in said tissue or cell, comparing the amount of protein produced in a tissue to the weight of said tissue or cell, or comparing the amount of therapeutic and/or prophylactic agent delivered to a tissue or cell to the amount of total administrated therapeutic and/or prophylactic agent. It will be understood that the enhanced delivery of a therapeutic and/or prophylactic agent to a target tissue or cell need not be determined in a subject being treated, it may be determined in a surrogate such as an animal model (e.g., a mice or rat model). In certain embodiments, a lipid particle composition including a compound having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E) has substantively the same level of delivery enhancement regardless of administration routes. For example, certain compounds disclosed herein exhibit similar delivery enhancement when they are used for delivering a therapeutic and/or prophylactic agent either intravenously or intramuscularly.

[0091] As used herein, the term "selective delivery," "selectively deliver," or "selectively delivering" means delivery of more (e.g., at least 1.5 fold more, at least 2-fold more, at least 3- fold more, at least 4-fold more, at least 5-fold more, at least 6-fold more, at least 7-fold more, at least 8-fold more, at least 9-fold more, at least 10-fold more) of a therapeutic and/or prophylactic agent by a lipid particle to a target tissue of interest (e.g., liver, lung, spleen or muscle) or a target cell of interest (e.g., liver cell, lung cell, spleen cell or muscle cell) compared to an off-target tissue (e.g., liver, lung, spleen or muscle) or an off-target cell of interest (e.g., liver cell, lung cell, spleen cell or muscle cell). The level of delivery of a therapeutic and/or prophylactic agent to a particular tissue or cell may be measured by comparing the amount of therapeutic and/or prophylactic agent in a tissue or cell to the amount of total therapeutic and/or prophylactic agent in said tissue or cell, comparing the amount of therapeutic and/or prophylactic agent in a tissue or cell to the weight of said tissue or cell, comparing the amount of protein produced in a tissue or cell to the amount of total protein in said tissue or cell, or comparing the amount of protein produced in a tissue or cell to the

weight of said tissue or cell, . It will be understood that the ability of a lipid particle to specifically deliver a therapeutic and/or prophylactic agent to a target tissue or cell need not be determined in a subject being treated, it may be determined in a surrogate such as an animal model (e.g., a mice or rat model).

[0092] The term "effective amount" "pharmaceutically effective amount" or "therapeutically effective amount" refers to the amount of a therapeutic and/or prophylactic agent delivered to a tissue or cell, or a compound or pharmaceutical composition described herein that is sufficient to prevent, treat, reduce and/or ameliorate the symptoms and/or underlying causes of any disorder or disease in a subject, or the amount of an agent sufficient to produce a desired effect on target cells, e.g., reduction of cell migration, an increase or inhibition of expression of a target nucleic acid in a cell in comparison to the normal expression level of the nucleic acid detected in the absence of the therapeutic and/or prophylactic agent delivered to a tissue or cell, or a compound or pharmaceutical composition described herein. In one embodiment, a "pharmaceutically effective amount" or "therapeutically effective amount" is an amount sufficient to reduce or eliminate a symptom of a disease. In another embodiment, a pharmaceutically or therapeutically effective amount is an amount sufficient to overcome the disease itself. In certain specific embodiments, a "pharmaceutically effective amount" or "therapeutically effective amount" is an amount effective for detectable killing or inhibition of the growth or spread of cancer cells, reducing in the size or number of tumors; or other measure of the level, stage, progression or severity of the cancer. The pharmaceutically or therapeutically effective amount will vary depending upon the subject and the condition being treated, the weight and age of the subject, the severity of the condition, the particular composition or excipient chosen, the dosing regimen to be followed, timing of administration, the manner of administration and the like, all of which can be determined readily by one of ordinary skill in the art. The full therapeutic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. The specific dose will vary depending on, for example, the particular compounds chosen, the species of the subject and their age/existing health conditions or risk for health conditions, the dosing regimen to be followed, the severity of the disease, whether it is administered in combination with other agents, timing of administration, the tissue to which it is administered, and the

physical delivery system in which it is carried. Thus, a pharmaceutically or therapeutically effective amount may be administered in one or more administrations. For example, and without limitation, a pharmaceutically or therapeutically effective amount of an agent, in the context of treating cancer, refers to an amount of the agent that alleviates, ameliorates, palliates, or eliminates one or more symptoms of cancer in the patient.

[0093] As used herein, the terms "treatment", "treat" and "treating" refer to reversing, alleviating, delaying the onset of, or inhibiting the progress of a disease or disorder, or one or more symptoms thereof, as described herein. In some embodiments, treatment may be administered after one or more symptoms have developed. In other embodiments, treatment may be administered in the absence of symptoms. For example, treatment may be administered to a susceptible individual prior to the onset of symptoms (e.g., in light of a history of symptoms and/or in light of genetic or other susceptibility factors). Treatment may also be continued after symptoms have resolved, for example to present or delay their recurrence.

[0094] The term "subject" to which administration is contemplated includes, but is not limited to, humans (i.e., a male or female of any age group, e.g., a pediatric subject (e.g., infant, child, adolescent) or adult subject (e.g., young adult, middle-aged adult or senior adult)) and/or other primates (e.g., cynomolgus monkeys, rhesus monkeys); mammals, including commercially relevant mammals such as cattle, pigs, horses, sheep, goats, rabbits, hamsters, mice, cats, and/or dogs; and/or birds, including commercially relevant birds such as chickens, ducks, geese, quail, and/or turkeys. In some embodiments, a subject has been or may be diagnosed with a disease or disorder. In some embodiments, a subject has not been diagnosed with a disease or disorder.

[0095] *Lipid*

[0096] In one aspect, the present disclosure provides novel compounds useful as lipids.

[0097] A lipid has at least one features of the following: a hydrophilic head group with varying pKa, a cationic, mono-, di-, tri-amine, oligoamine/polyamine, an imidazole, a pyridine, a guanidinium, and hydrophobic tails. In some embodiments, a lipid is an ionizable lipid. In some embodiments, a lipid is a cationic lipid.

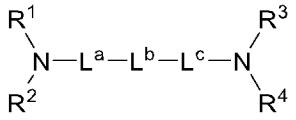
[0098] As used herein, the term "cationic lipid" includes lipids having an amino head and one or more aliphatic chains, which may be protonated to form a cationic lipid

as physiological pH. In some embodiments, a cationic lipid is an amino lipid. Lipids having one or more protonatable or deprotonatable group, or which are zwitterionic are also included. In some embodiments, a lipid of the present disclosure has at least one protonatable group, such that the lipid is positively charged at a first pH at or below physiological pH (e.g., at or below pH 7.4), and neutral at a second pH (e.g., at or above physiological pH). In some embodiments, a lipid of the present disclosure has at least two or at least three protonatable groups. It shall be understood that the addition or removal of protons as a function of pH is an equilibrium process, and that the reference to a charged (e.g., protonated) or a neutral lipid refers to the nature of the predominant species (e.g., more than 50%, 60%, 70%, 80%, 90%, 95% or 99%) and does not require that all of the lipid be present in the charged or neutral form.

[0099] In some embodiments, cationic lipids of the present disclosure have a pKa of the protonatable group in the range of about 4 to about 11. In some embodiments, lipids have a pKa of about 4 to about 7, about 5 to about 7, or about 5.5 to about 6.8, when incorporated into lipid particles. In some embodiments, lipids having such pKa will be cationic at a lower pH, while particles will be largely (though not completely) surface neutralized at physiological pH (e.g., at pH 7.4). In some embodiments, at least some nucleic acid associated with the outside surface of the particle comprising lipids having such pKa will lose its electrostatic interaction at physiological pH and be removed by simple dialysis; thus greatly reducing the particle's susceptibility to clearance. pKa measurements of lipids within lipid particles can be performed, for example, by using the fluorescent probe 2-(p-toluidino)-6-napthalene sulfonic acid (TNS), using method described in Cullis et al., (1986) *Chem Phys Lipids* 40, 127-144.

[0100] In some embodiments, lipids of the present disclosure are advantageously used in lipid particles. In some embodiments, a lipid particle is used in *in vivo* delivery of therapeutic agents to cells. In some embodiments, a lipid particle is used in *in vivo* delivery of therapeutic agents to a tissue.

[0101] In another aspect, a lipid of the present disclosure is a compound having Formula (I) below:



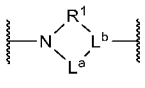
(I),

or a pharmaceutically acceptable salt thereof,

wherein

L^a and L^c are each independently selected from the group consisting of a bond, alkyl, alkenyl, alkynyl and heteroalkyl, wherein the alkyl, alkenyl, alkynyl and heteroalkyl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, cyano and amino;

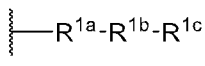
L^b is selected from the group consisting of a bond, $-NR^a-$, -O-, -S-, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, cycloalkyl, heterocyclyl, aryl and heteroaryl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, cycloalkyl, heterocyclyl, aryl and heteroaryl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, cyano and amino, or

L^b is covalently connected with R^1 to form  ;

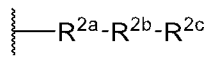
provided that when two of L^a, L^b and L^c are bonds, then the remaining one is not a bond;

R^a is selected from the group consisting of hydrogen, R^5, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, cycloalkyl, heterocyclyl, aryl and heteroaryl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, cycloalkyl, heterocyclyl, aryl and heteroaryl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, cyano, cycloalkyl, heterocyclyl, aryl, heteroaryl and R^6;

R^1 is a group having Formula (I-a) below:

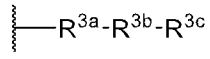
 (I-a),

R^2 is a group having Formula (I-b) below:



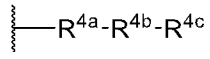
(I-b),

R^3 is a group having Formula (I-c) below:



(I-c),

R^4 is a group having Formula (I-d) below:



(I-d),

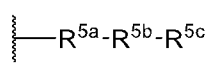
wherein

each of R^{1a}, R^{2a}, R^{3a} and R^{4a} is independently a bond or alkyl;

each of R^{1b}, R^{2b}, R^{3b} and R^{4b} is independently a bond, alkyl or heteroalkyl, wherein the alkyl and heteroalkyl are optionally substituted by one or more groups independently selected from the group consisting of hydroxyl, amino, oxo, thioxo and imino; and

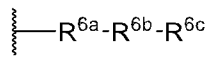
each of R^{1c}, R^{2c}, R^{3c} and R^{4c} is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, oxo, cyano, cycloalkyl, heterocyclyl, aryl and heteroaryl, and the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally interrupted by one or more groups independently selected from the group consisting of cycloalkyl, heterocyclyl, aryl and heteroaryl; and

R^5 is a group having Formula (I-e) below:



(I-e),

each R^6 is independently a group having Formula (I-f) below:



(I-f),

wherein

each of R^{5a} and R^{6a} is independently a bond or alkyl;

each of R^{5b} and R^{6b} is independently a bond, alkyl or heteroalkyl, wherein the alkyl and heteroalkyl is optionally substituted by one or more groups independently selected from the group consisting of hydroxyl, amino, oxo, thioxo and imino; and

each of R^{5c} and R^{6c} is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl is optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, oxo and cyano, and the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally interrupted by one or more groups independently selected from the group consisting of cycloalkyl, heterocyclyl, aryl and heteroaryl.

[0102] In some embodiments, L^a and L^c are each independently an alkyl. In some embodiments, L^a and L^c are each independently a C_{1-6} alkyl.

[0103] In some embodiments, L^a and L^c are each independently methyl, ethyl or propyl.

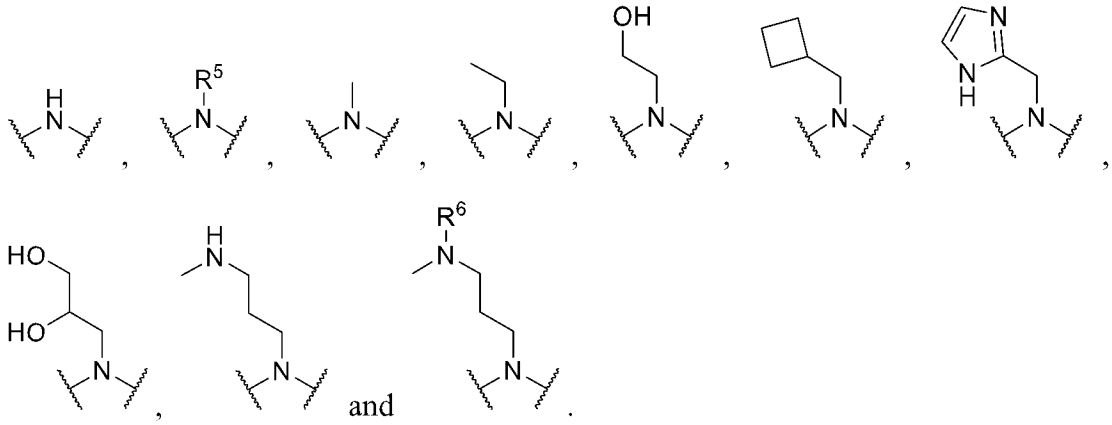
[0104] In some embodiments, L^b is $-NR^a-$.

[0105] In some embodiments, R^a is selected from the group consisting of hydrogen, R^5, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, cycloalkyl, heterocyclyl, aryl and heteroaryl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, cycloalkyl, heterocyclyl, aryl and heteroaryl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, cyano, cycloalkyl, heterocyclyl, aryl heteroaryl and R^6.

[0106] In some embodiments, R^a is C_{1-6} heteroalkyl.

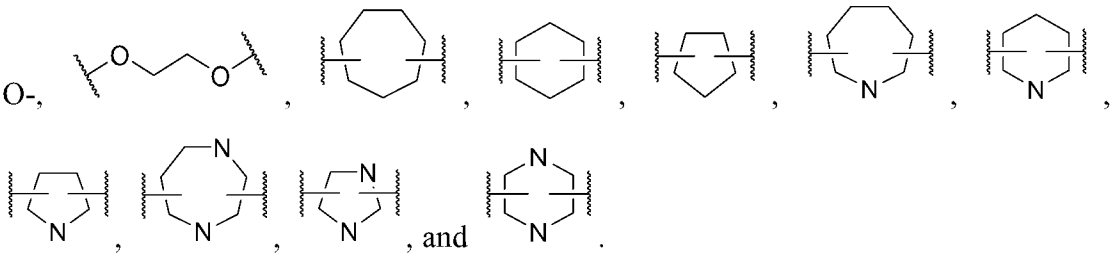
[0107] In some embodiments, R^a is C_{1-6} alkyl optionally substituted by one or more groups independently selected from the group consisting of hydroxyl, cycloalkyl and heteroaryl.

[0108] In some embodiments, L^b is selected from the group consisting of

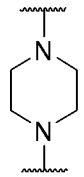


[0109] In some embodiments, L^b is selected from the group consisting of -S-, -O-, -O-alkyl-O-, cycloalkyl and heterocyclyl.

[0110] In some embodiments, L^b is selected from the group consisting of -S-, -O-,

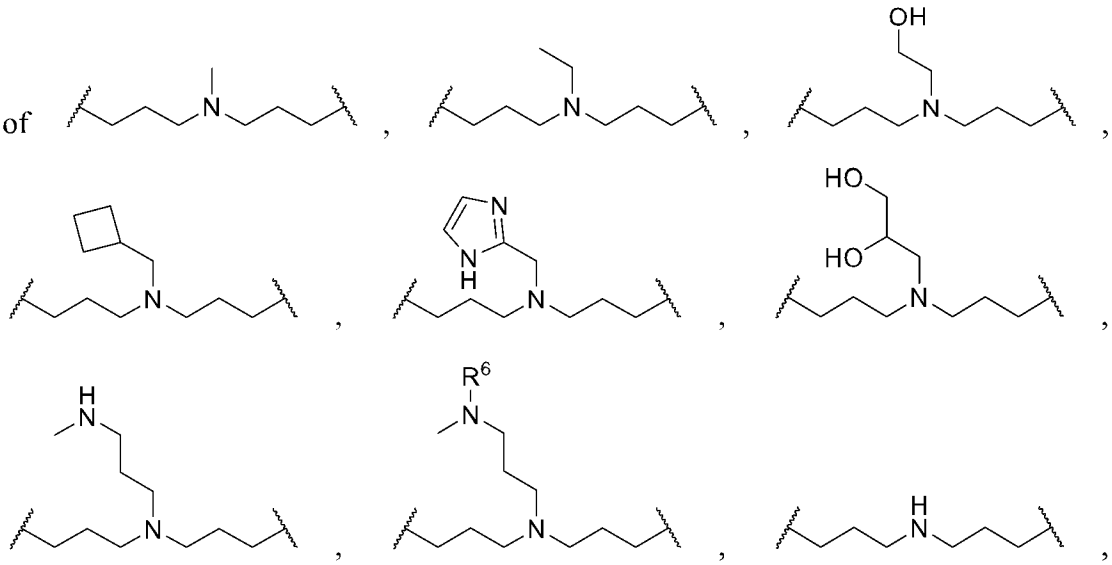


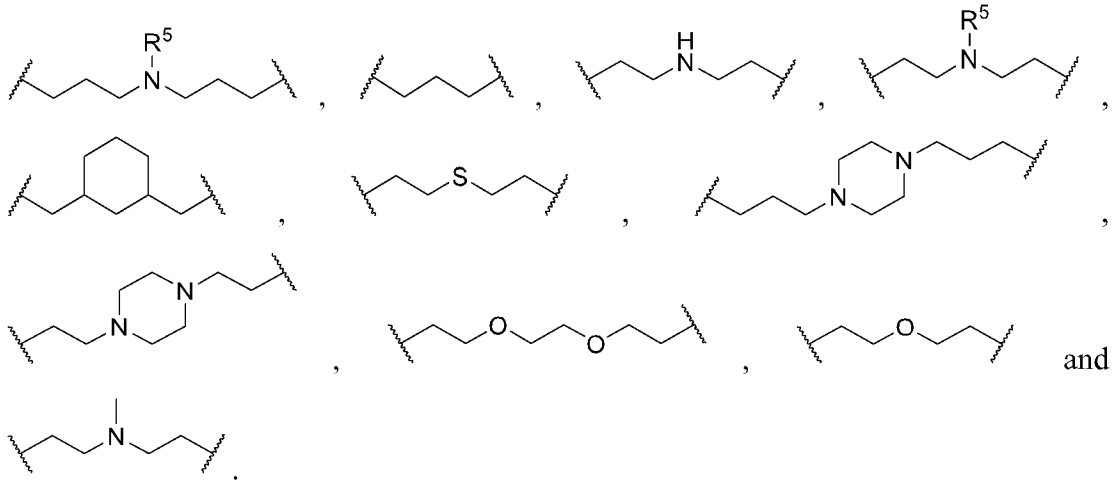
[0111] In some embodiments, L^b is

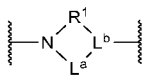


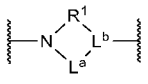
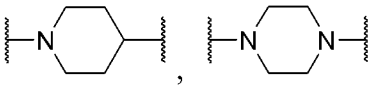
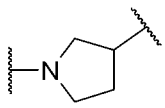
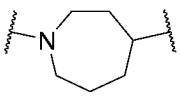
.

[0112] In some embodiments, $-L^a-L^b-L^c-$ is selected from the group consisting of





[0113] In some embodiments, L^b is alkyl or heteroalkyl covalently connected with R^1 to form  , L^a is a bond, alkyl or heteroalkyl, and R^1 is a bond, alkyl or heteroalkyl.

[0114] In some embodiments,  is  ,  or  .

[0115] In some embodiments, L^c is methyl.

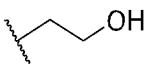
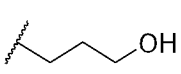
[0116] In some embodiments, at least two of R^1, R^2, R^3, R^4, R^5 and R^6 are the same.

[0117] In some embodiments, at least three of R^1, R^2, R^3, R^4, R^5 and R^6 are the same.

[0118] In some embodiments, at least four of R^1, R^2, R^3, R^4, R^5 and R^6 are the same.

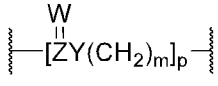
[0119] In some embodiments, at least two of R^1, R^2, R^3 and R^4 are different.

[0120] In some embodiments, one or more of R^1, R^2, R^3 and R^4 is independently alkyl optionally substituted by one or more hydroxyl.

[0121] In some embodiments, one or more of R^1, R^2, R^3 and R^4 is independently methyl,  or  .

[0122] In some embodiments, one or more of R^{1a}, R^{2a}, R^{3a}, R^{4a}, R^{5a} and R^{6a} is independently a bond, methyl, ethyl, propyl, butyl or pentyl.

[0123] In some embodiments, one or more of R^{1b}, R^{2b}, R^{3b}, R^{4b}, R^{5b} and R^{6b} is independently selected from the group consisting of alkyl and heteroalkyl, wherein the alkyl and heteroalkyl is optionally substituted by one or more groups independently selected from the group consisting of hydroxyl, oxo, thioxo and imino.

[0124] In some embodiments, one or more of R^{1b}, R^{2b}, R^{3b}, R^{4b}, R^{5b} and R^{6b} is independently  , wherein

each W is independently selected from O, S or NR^b;

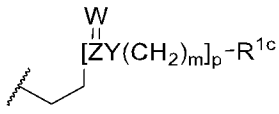
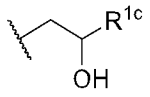
each Y is independently selected from O, S, NR^c, $N(R^c)Z(W)$, $N(R^c)N(R^c)$ or $N(R^c)N(R^c)Z(W)$;

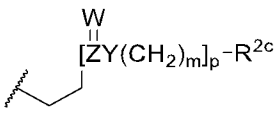
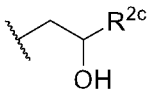
each Z is independently selected from C, S or S(O);

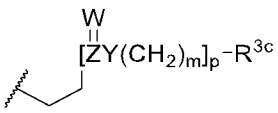
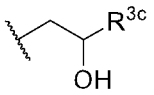
each m is independently 0, 1, 2 or 3;

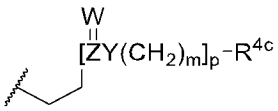
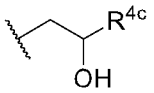
each p is independently 1, 2, 3 or 4; and

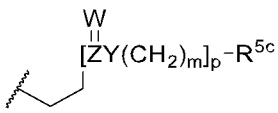
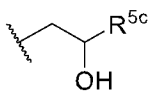
each of R^b and R^c is independently selected from hydrogen, alkyl, alkyloxycarbonyl, alkylacyl, arylacyl and sulfonyl.

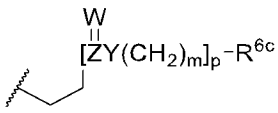
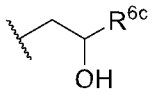
[0125] In some embodiments, R^1 is  or  ;

R^2 is  or  ;

R^3 is  or  ;

R^4 is  or  ;

R^5, if exists, is  or  ;

R^6, if exists, is  or  ;

each W is independently selected from O, S or NR^b;

each Y is independently selected from O, S, NR^c, $N(R^c)Z(W)$, $N(R^c)N(R^c)$ or $N(R^c)N(R^c)Z(W)$;

each Z is independently selected from C, S or S(O);

each m is independently 0 or 1;

each p is independently 1 or 2;

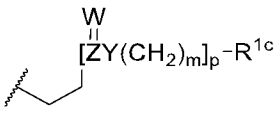
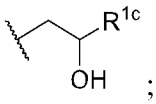
each of R^b and R^c is independently selected from hydrogen, alkyl, alkyloxycarbonyl, alkylacyl, arylacyl and sulfonyl; and

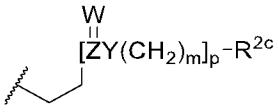
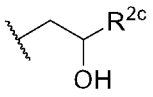
each of R^{1c}, R^{2c}, R^{3c}, R^{4c}, R^{5c} and R^{6c}, if exists, is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, oxo and cyano, and the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally interrupted by one or more groups independently selected from the group consisting of cycloalkyl, heterocyclyl, aryl and heteroaryl.

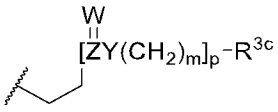
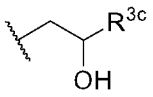
[0126] In some embodiments,

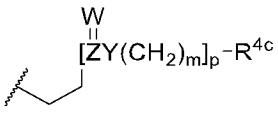
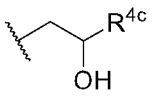
L^b is $-NR^a-$;

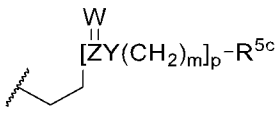
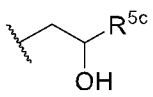
R^a is selected from the group consisting of hydrogen, R^5, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, cyano, cycloalkyl, heterocyclyl, aryl, heteroaryl and R^6;

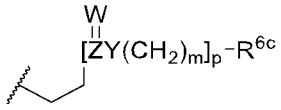
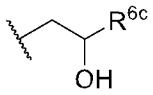
R^1 is  or  ;

R^2 is  or  ;

R^3 is  or  ;

R^4 is  or  ;

R^5, if exists, is  or  ;

R^6, if exists, is  or  ;

each W is independently selected from O, S or NR^b;

each Y is independently selected from O, S, NR^c, $N(R^c)Z(W)$, $N(R^c)N(R^c)$ or $N(R^c)N(R^c)Z(W)$;

each Z is independently selected from C, S or S(O);

each m is independently 0 or 1;

each p is 1 or 2;

each of R^b and R^c is independently selected from hydrogen, alkyl, alkyloxycarbonyl, alkylacyl, arylacyl and sulfonyl; and

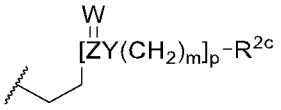
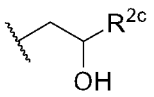
each of R^{1c}, R^{2c}, R^{3c}, R^{4c}, R^{5c} and R^{6c}, if exists, is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, oxo and cyano, and the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally interrupted by one or more groups independently selected from the group consisting of cycloalkyl, heterocyclyl, aryl and heteroaryl.

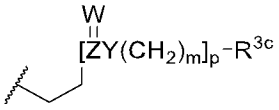
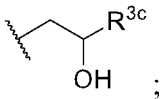
[0127] In some embodiments,

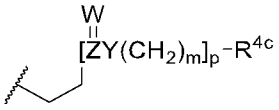
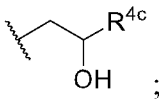
L^b is $-NR^a-$;

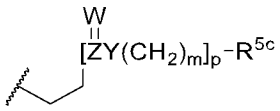
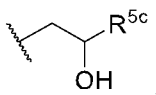
R^a is selected from the group consisting of R^5, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are substituted by R^6 and optionally substituted by one or more additional groups independently selected from the group consisting of halogen, hydroxyl, cyano, cycloalkyl, heterocyclyl, aryl and heteroaryl;

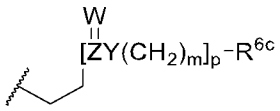
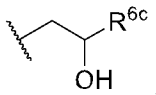
R^1 is selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, cyano, cycloalkyl, heterocyclyl, aryl and heteroaryl;

R^2 is  or  ;

R^3 is  or  ;

R^4 is  or  ;

R^5, if exists, is  or  ;

R^6, if exists, is  or  ;

each W is independently selected from O, S or NR^b;

each Y is independently selected from O, S, NR^c, $N(R^c)Z(W)$, $N(R^c)N(R^c)$ or $N(R^c)N(R^c)Z(W)$;

each Z is independently selected from C, S or S(O);

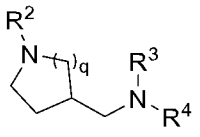
each m is independently 0 or 1;

each p is 1 or 2;

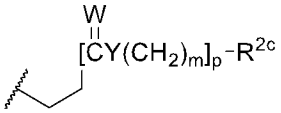
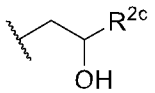
each of R^b and R^c is independently selected from hydrogen, alkyl, alkyloxycarbonyl, alkylacyl, arylacyl or sulfonyl; and

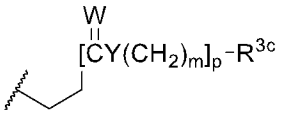
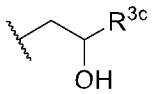
each of R^{2c}, R^{3c}, R^{4c}, R^{5c} and R^{6c}, if exists, is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, oxo and cyano, and the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally interrupted by one or more groups independently selected from the group consisting of cycloalkyl, heterocyclyl, aryl and heteroaryl.

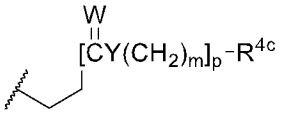
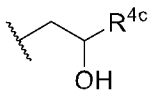
[0128] In some embodiments, the compound has Formula (A) below:

 (A),

wherein

R^2 is  or  ;

R^3 is  or  ;

R^4 is  or  ;

each W is independently selected from O, S or NR^b;

each Y is independently selected from O, S, NR^c, $N(R^c)Z(W)$, $N(R^c)N(R^c)$ or $N(R^c)N(R^c)Z(W)$;

each Z is independently selected from C, S or S(O);

each m is independently 0 or 1;

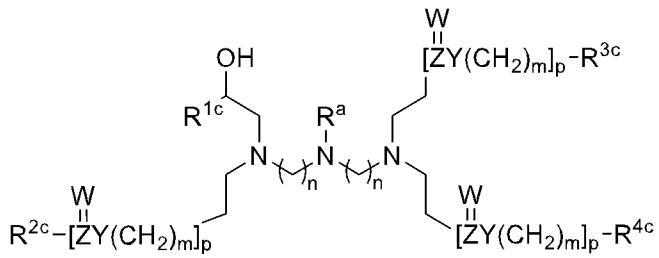
each p is independently 1 or 2;

q is 1, 2 or 3;

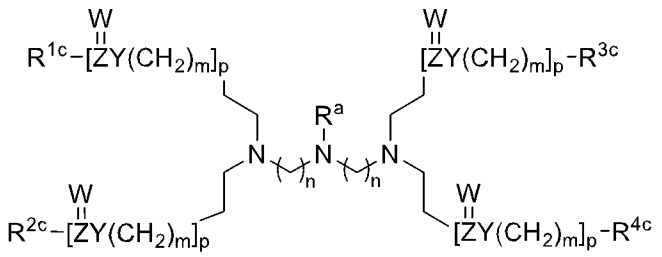
each of R^b and R^c is independently selected from hydrogen, alkyl, alkyloxycarbonyl, alkylacyl, arylacyl or sulfonyl; and

each of R^{2c}, R^{3c} and R^{4c} is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, oxo and cyano, and the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally interrupted by one or more groups independently selected from the group consisting of cycloalkyl, heterocyclyl, aryl and heteroaryl.

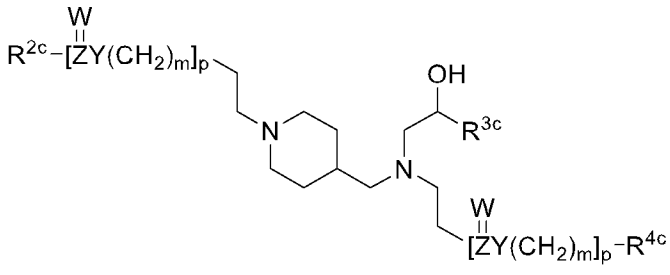
[0129] In some embodiments, the compound has Formula (B), Formula (C), Formula (D) or Formula (E):



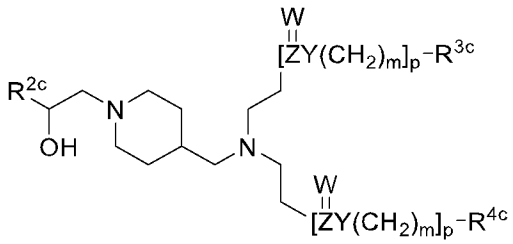
(B),



(C),



(D),



(E),

wherein

each W is independently selected from O, S or NR^b;

each Y is independently selected from O, S, NR^c, $N(R^c)Z(W)$, $N(R^c)N(R^c)$ or $N(R^c)N(R^c)Z(W)$;

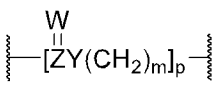
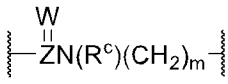
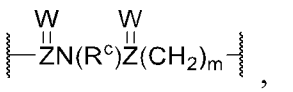
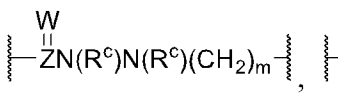
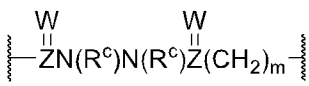
each Z is independently selected from C, S or S(O);

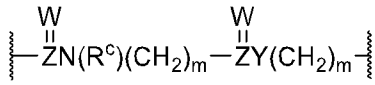
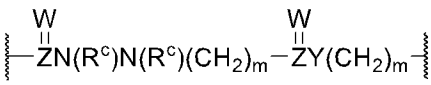
each n is independently 0, 1, 2, 3, 4 or 5;

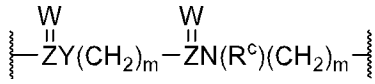
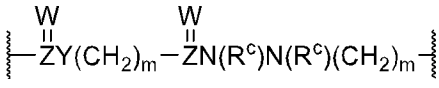
each m is independently 0, 1, 2 or 3;

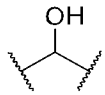
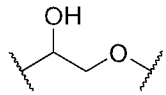
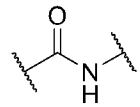
each p is independently 1, 2, 3 or 4; and

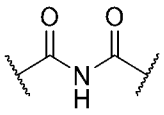
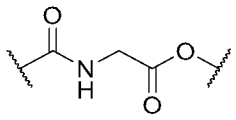
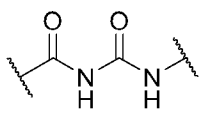
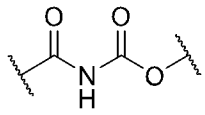
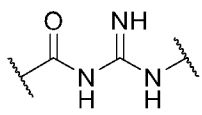
each of R^b and R^c is independently selected from hydrogen, alkyl, alkyloxycarbonyl, acyl or sulfonyl.

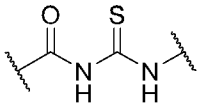
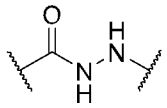
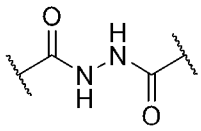
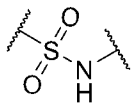
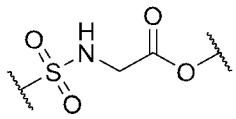
[0130] In some embodiments, each of  is independently selected from the group consisting of  ,  ,  , 

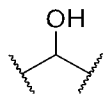
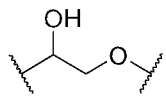
 ,  ,

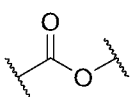
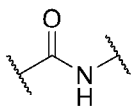
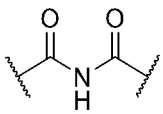
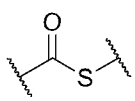
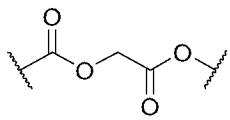
 and  .

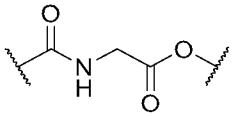
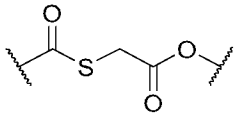
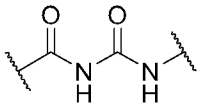
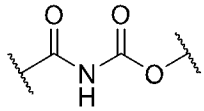
[0131] In some embodiments, R^{1b}, R^{2b}, R^{3b}, R^{4b}, R^{5b} and R^{6b} is independently selected from the group consisting of  ,  ,  ,

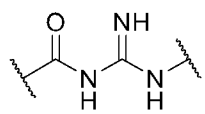
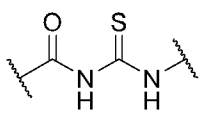
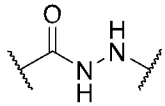
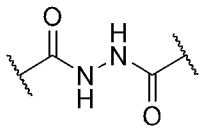
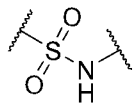
 ,  ,  ,  ,  ,

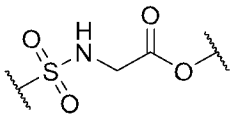
 ,  ,  ,  and  .

[0132] In some embodiments, one or more of R^{1b}, R^{2b}, R^{3b}, R^{4b}, R^{5b} and R^{6b} is independently selected from the group consisting of  ,  ,

 ,  ,  ,  ,  ,

 ,  ,  ,  ,

 ,  ,  ,  ,  and

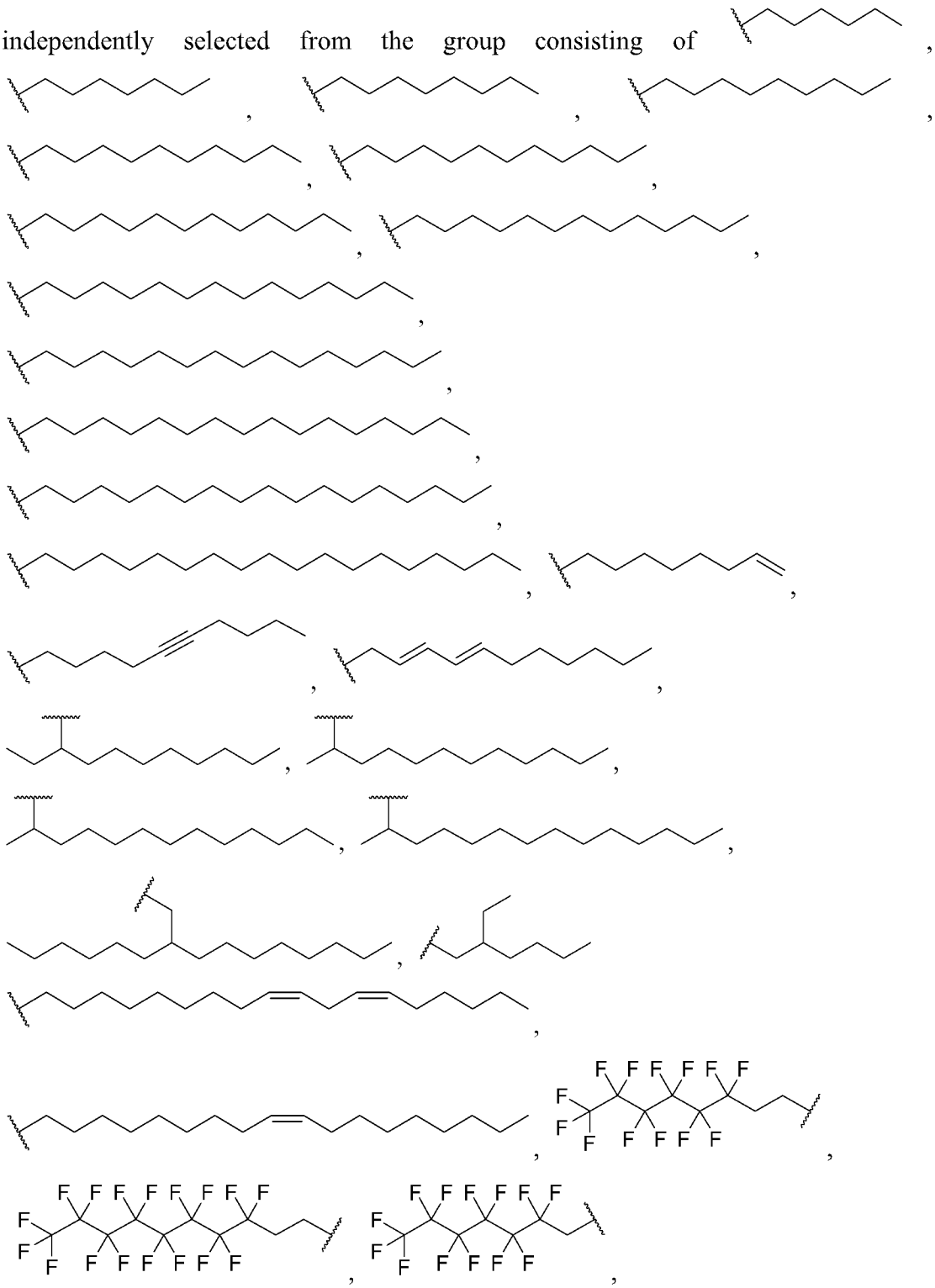
 .

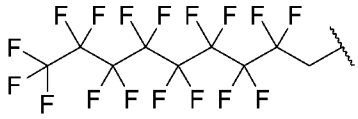
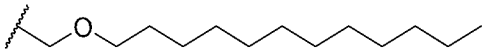
[0133] In some embodiments, one or more of R^{1c}, R^{2c}, R^{3c}, R^{4c}, R^{5c} and R^{6c} is independently selected from the group consisting of alkyl, alkenyl and alkynyl, wherein the alkyl, alkenyl and alkynyl are optionally substituted with one or more halogens.

[0134] In some embodiments, one or more of R^{1c}, R^{2c}, R^{3c}, R^{4c}, R^{5c} and R^{6c} is independently selected from the group consisting of $-CH_2-O-$alkyl, $-CH_2-O-$alkenyl, $-CH_2-O-$alkynyl, $-CH_2-O-$heteroalkyl, $-CH_2-O-$heteroalkenyl and $-CH_2-O-$heteroalkynyl.

[0135] In some embodiments, each of the alkyl, alkenyl and alkynyl independently contains 6 to 18 carbon atoms.

[0136] In some embodiments, each of the alkenyl independently contains one or more Z-olefin.

[0137] In some embodiments, one or more of R^{1c}, R^{2c}, R^{3c}, R^{4c}, R^{5c} and R^{6c} is independently selected from the group consisting of 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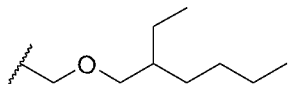
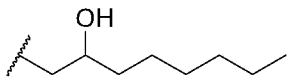
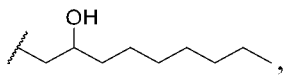
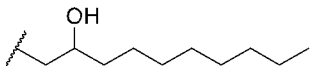
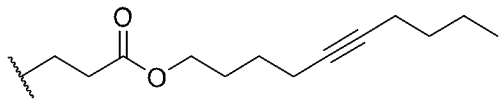

.

[0138] In some embodiments, one or more of R^1, R^2, R^3, R^4, R^5 and R^6 is independently selected from the group consisting of  ,
 ,
 ,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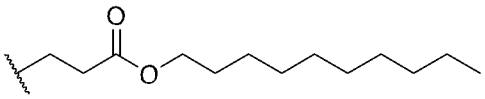
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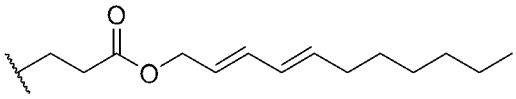
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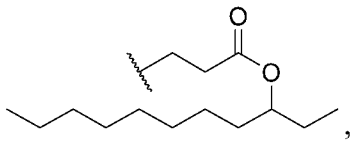
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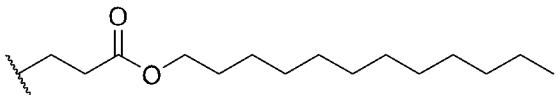
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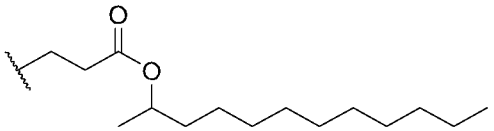
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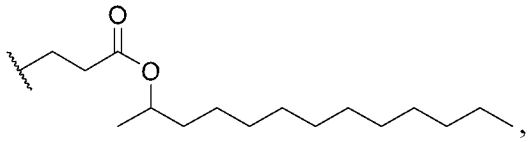
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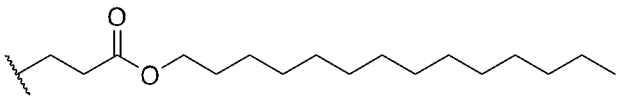
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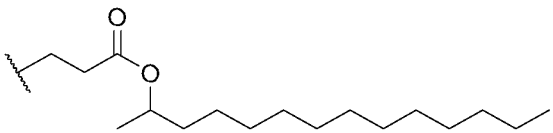
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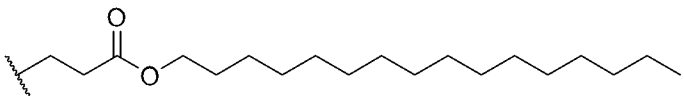
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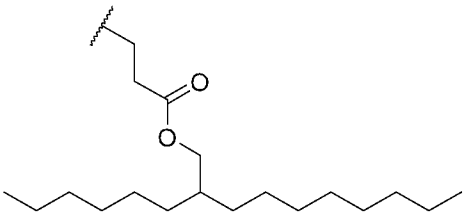
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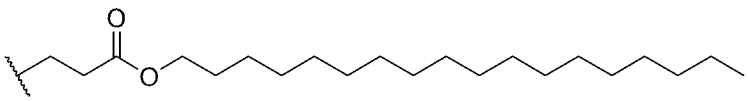
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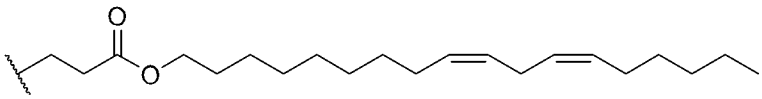
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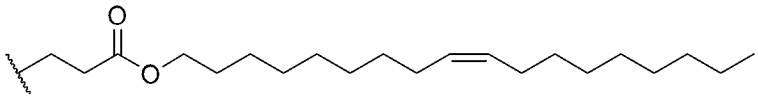
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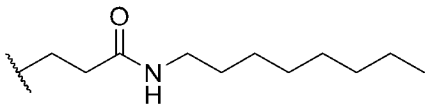
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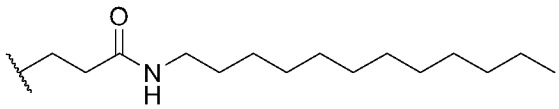
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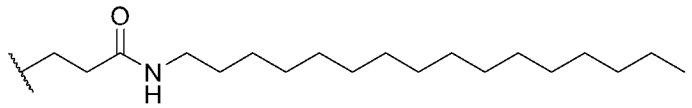
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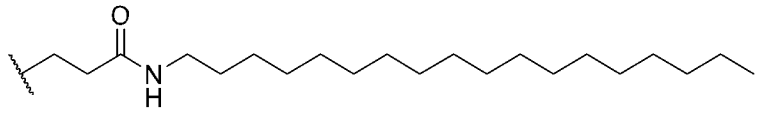
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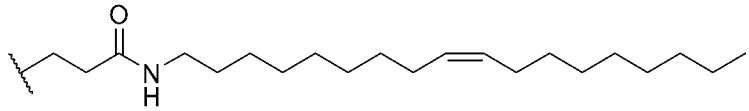
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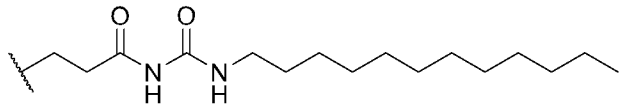
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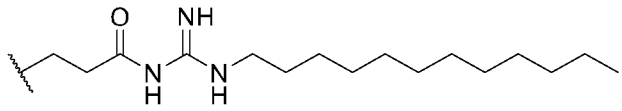
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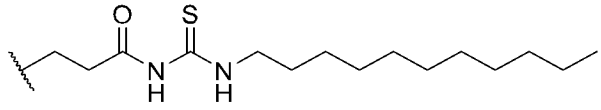
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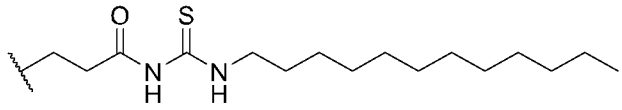
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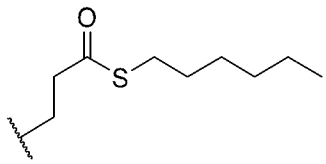
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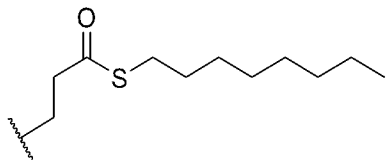
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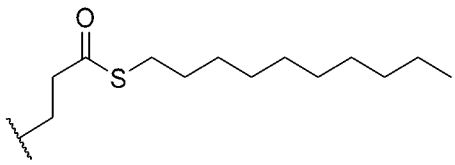
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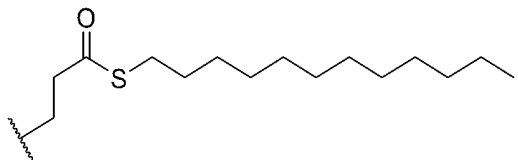
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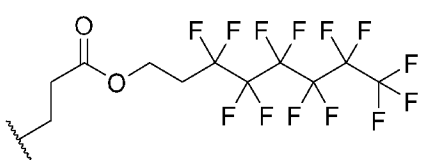
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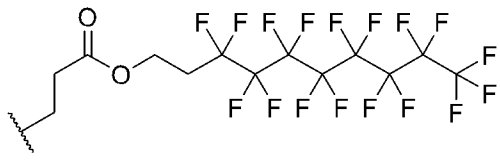
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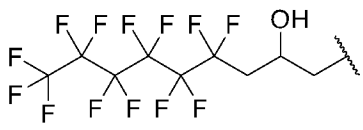
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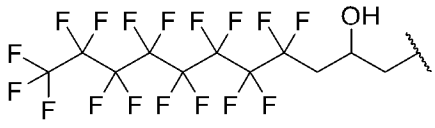
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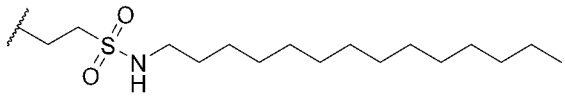
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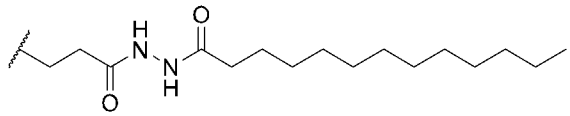
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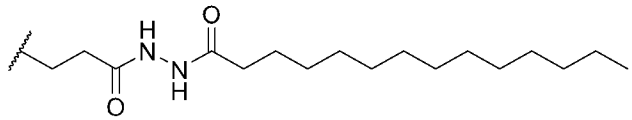
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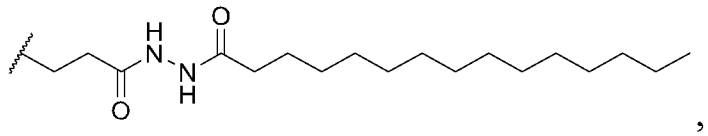
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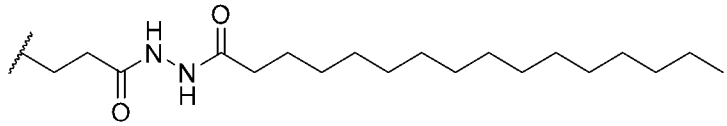
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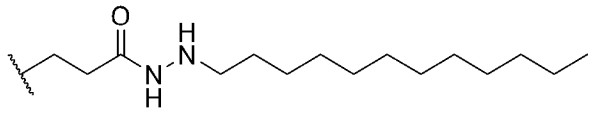
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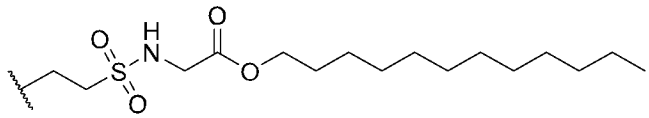
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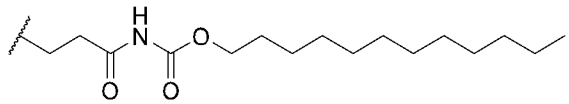
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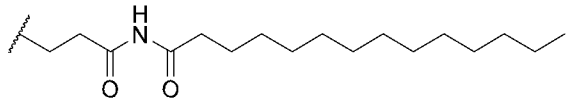
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[0139] In some embodiments, each of -R^{1b}-R^{1c}, -R^{2b}-R^{2c}, -R^{3b}-R^{3c}, -R^{4b}-R^{4c}, -R^{5b}-R^{5c} and -R^{6b}-R^{6c}, if exists, does not comprise two heteroatoms directly bonded to each other.

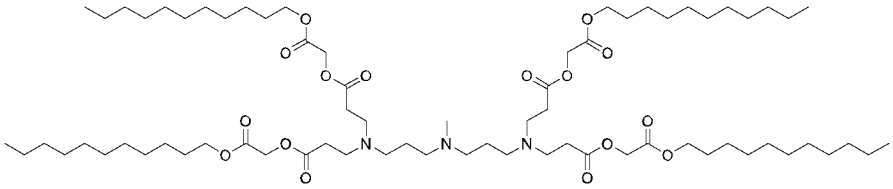
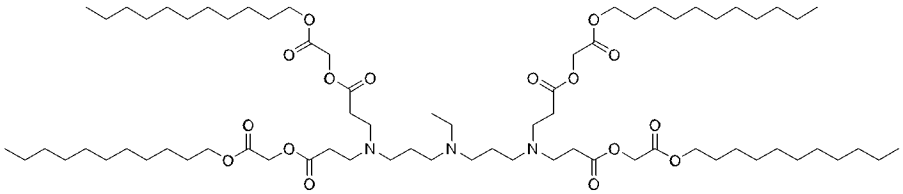
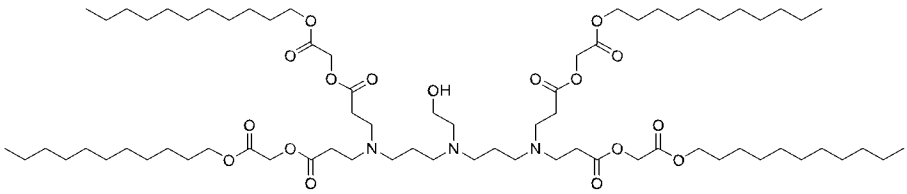
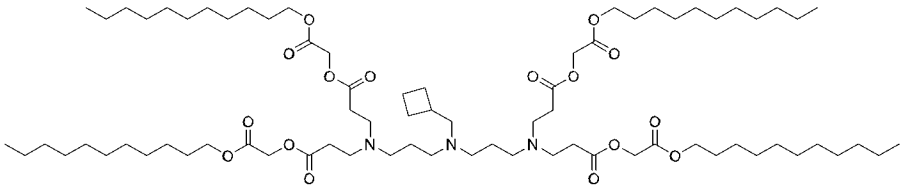
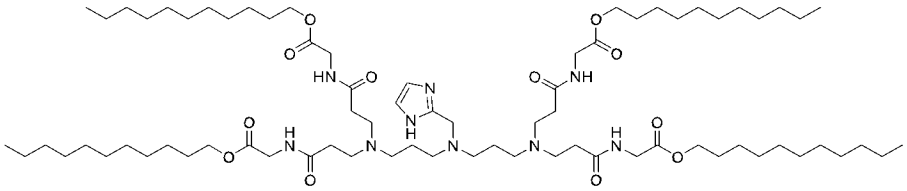
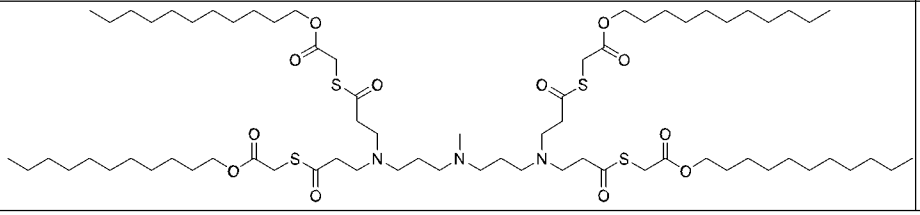
[0140] In some embodiments, one or more of -R^{1b}-R^{1c}, -R^{2b}-R^{2c}, -R^{3b}-R^{3c}, -R^{4b}-R^{4c}, -R^{5b}-R^{5c} and -R^{6b}-R^{6c}, if exists, comprises one or more groups independently selected from the group consisting of -N(R^c)-N(R^c)-, -N(R^c)-S(O)-, -N(R^c)-$S(O)_2$-, -N(R^c)-O-, -S(O)-N(R^c)-, -$S(O)_2$-N(R^c)-, -S(O)-O-, -$S(O)_2$-O-, -O-N(R^c)-, -O-S(O)-, -O-$S(O)_2$- and -O-O-.

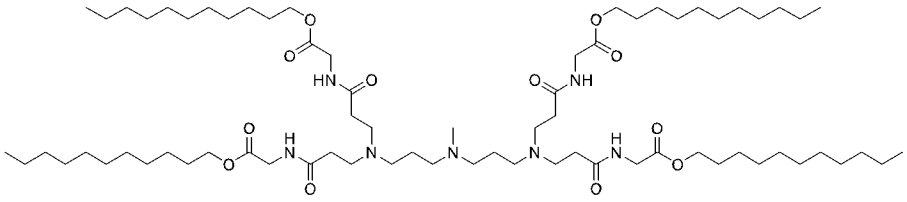
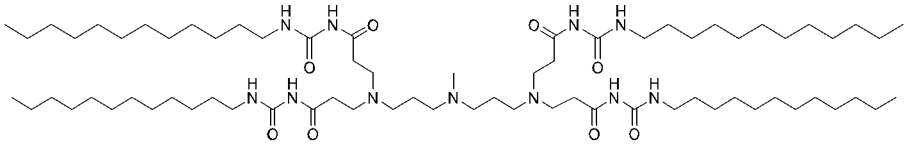
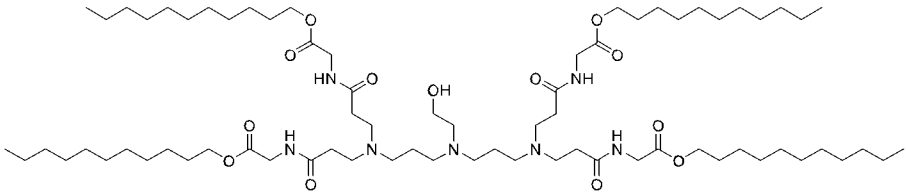
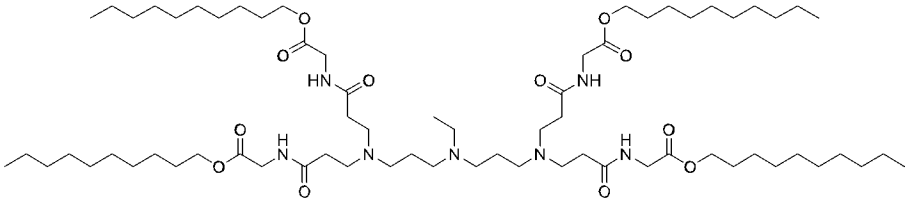
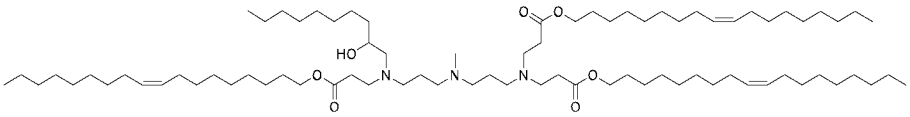
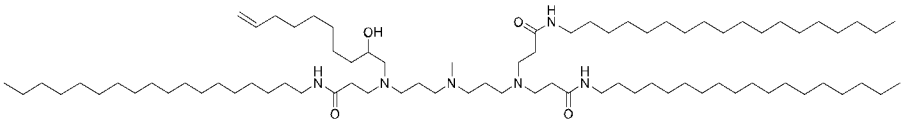
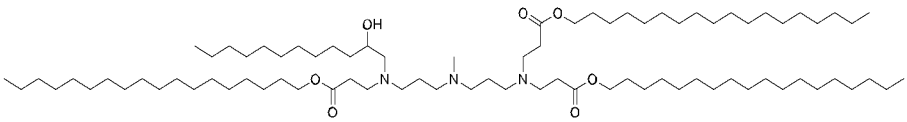
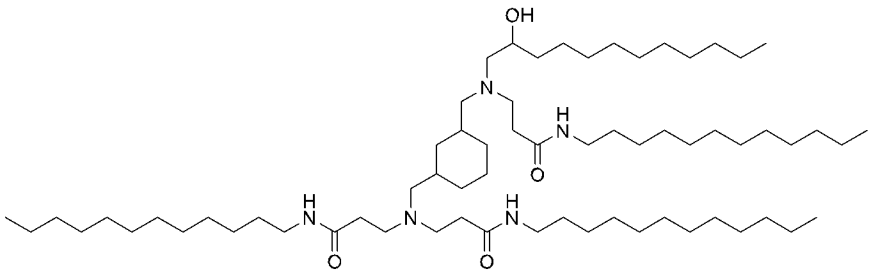
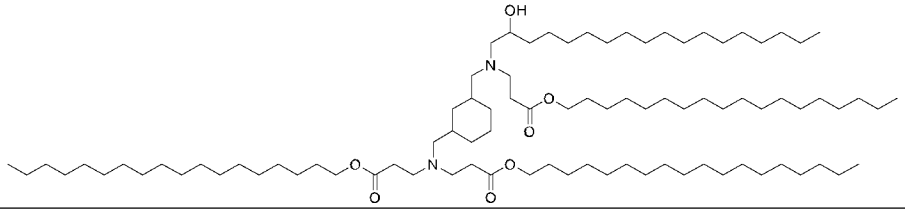
[0141] In some embodiments, one or more of -R^{1b}-R^{1c}, -R^{2b}-R^{2c}, -R^{3b}-R^{3c}, -R^{4b}-R^{4c}, -R^{5b}-R^{5c} and -R^{6b}-R^{6c}, if exists, comprises -N(R^c)-N(R^c)- or -$S(O)_2$-N(R^c)-.

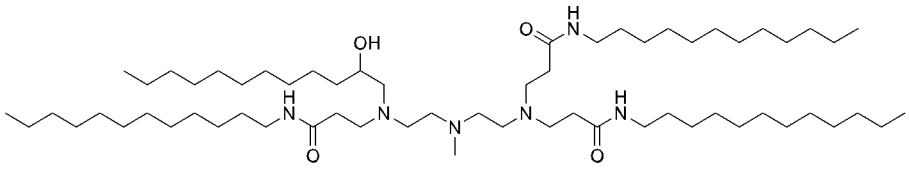
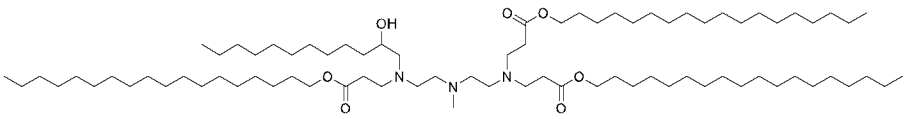
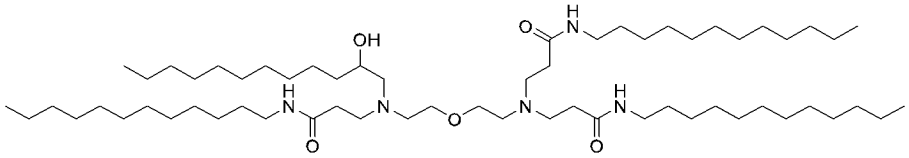
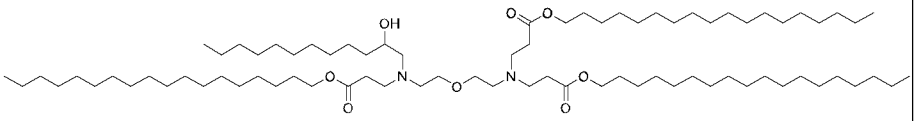
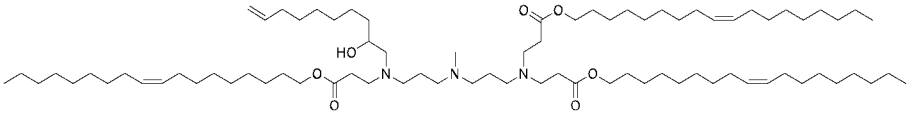
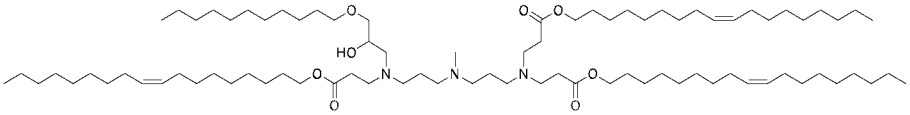
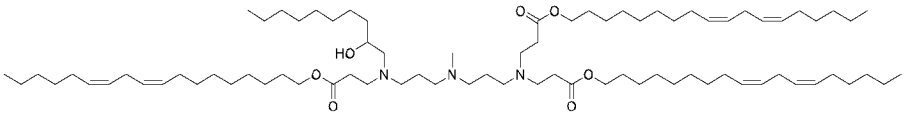
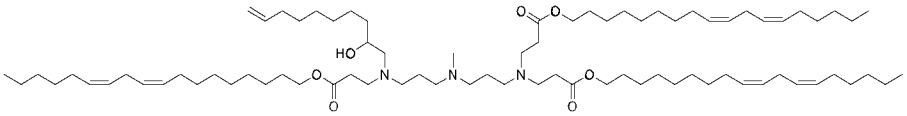
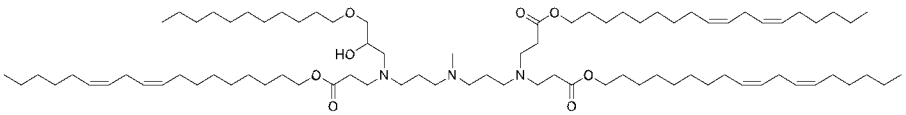
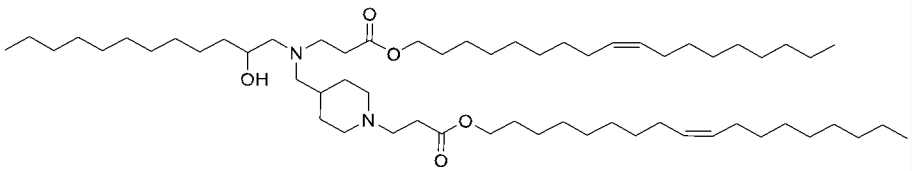
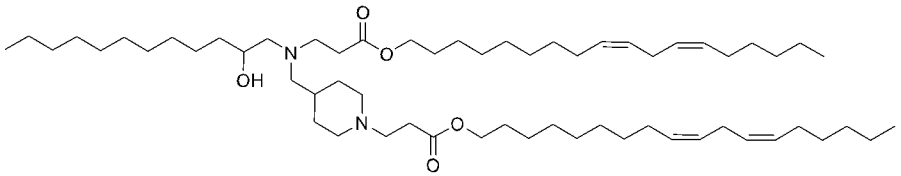
[0142] In some embodiments, a compound having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E) is a compound listed in Table 1.

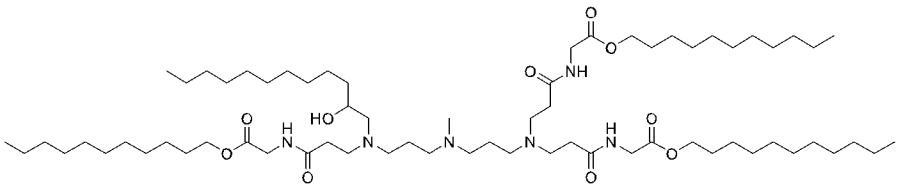
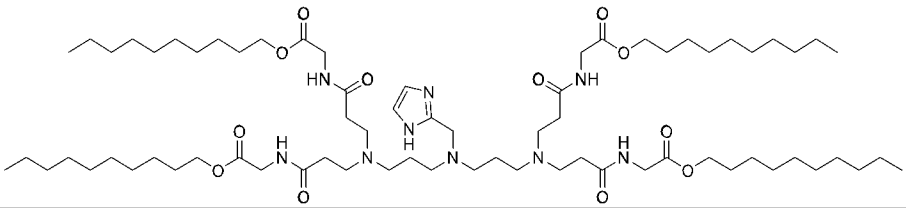
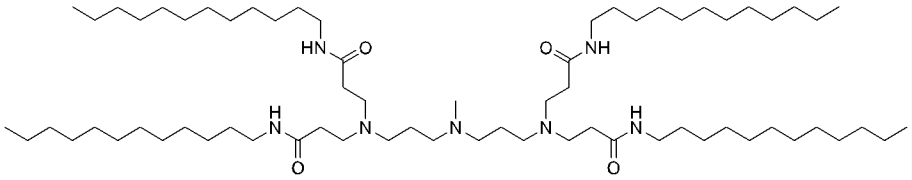
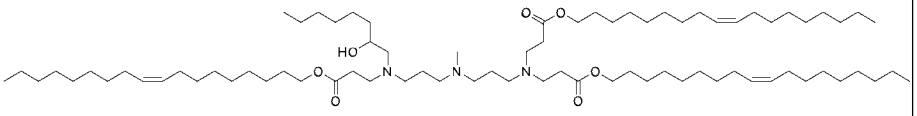
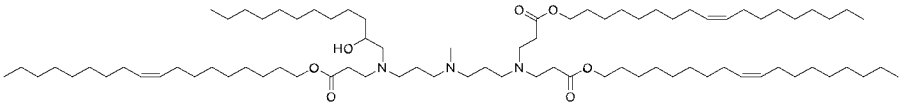
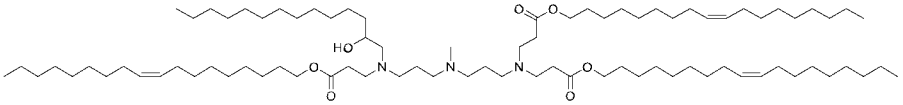
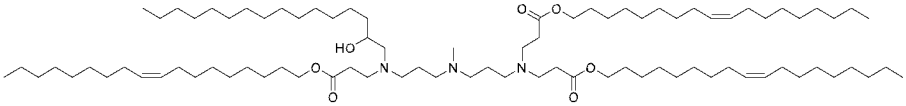
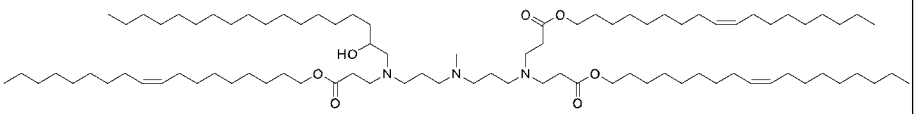
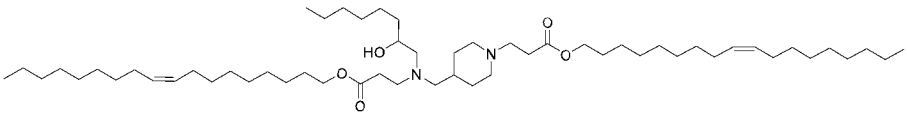
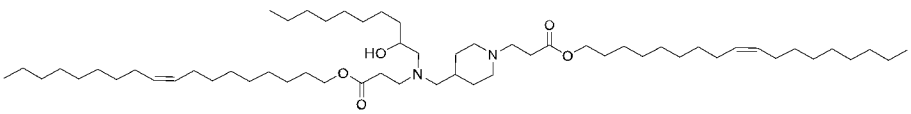
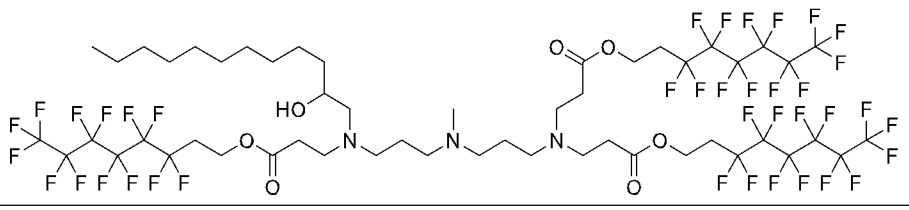
[0143] For the purpose of illustration, exemplary compounds of the present disclosure and their Structure Code are set forth in Table 1 below.

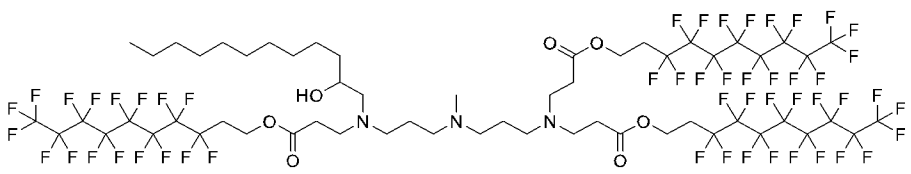
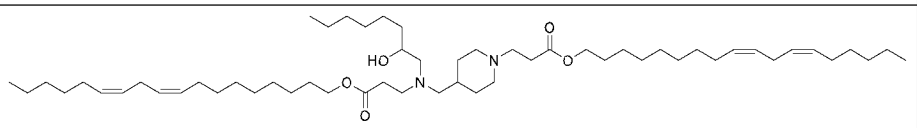
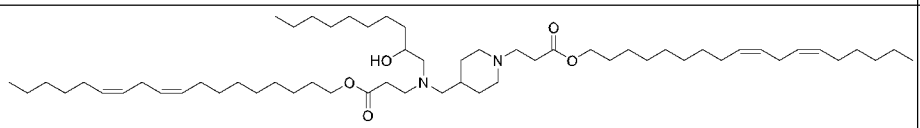
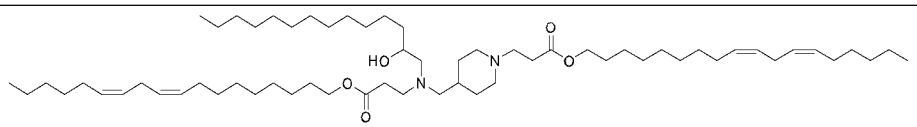
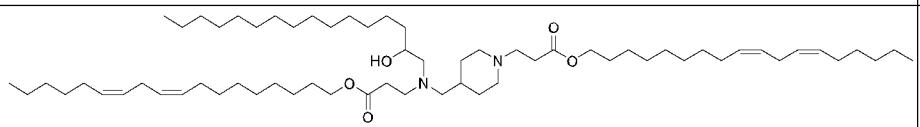
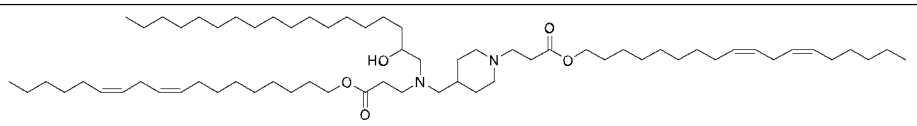
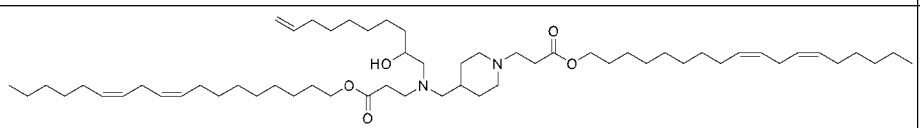
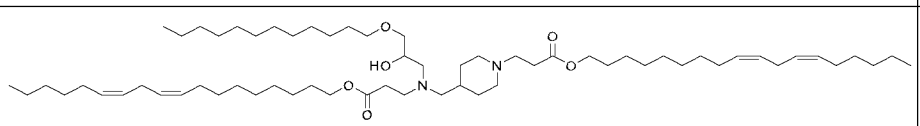
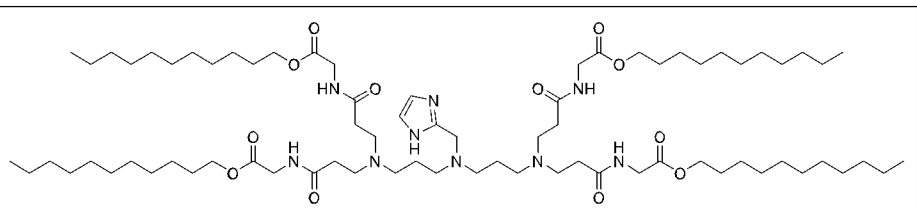
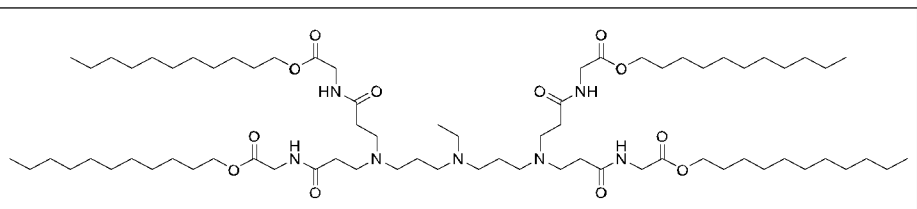
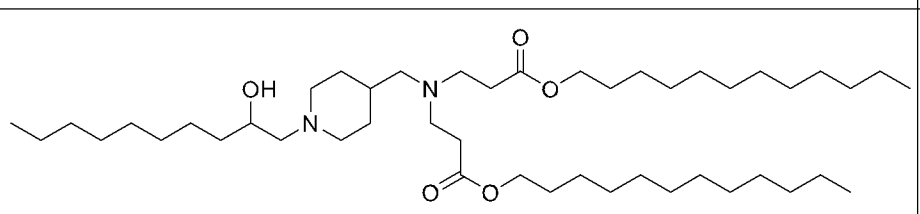
Table 1 Exemplary Compounds and Structure Code Thereof

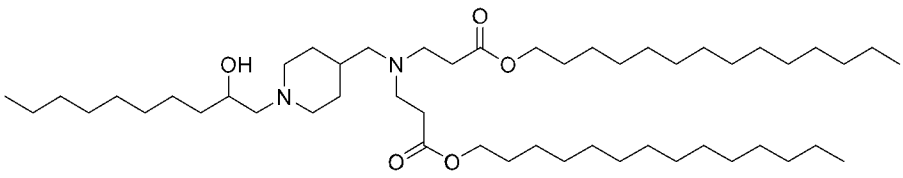
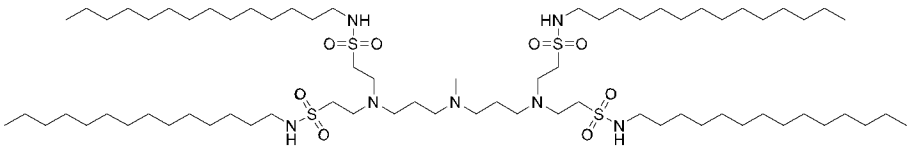
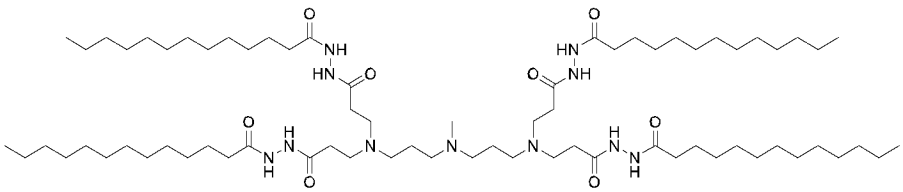
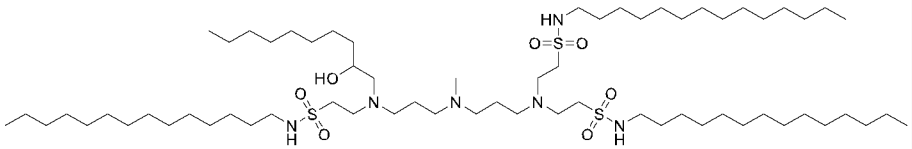
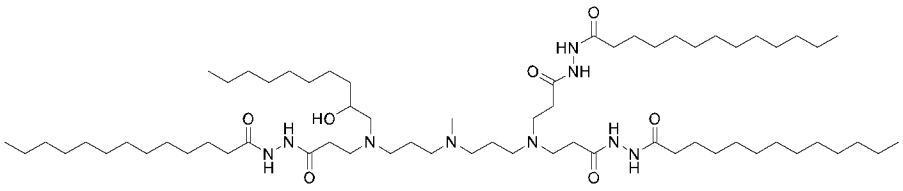
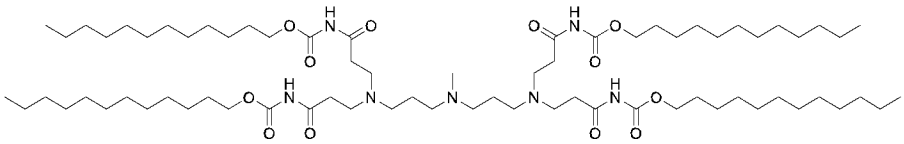
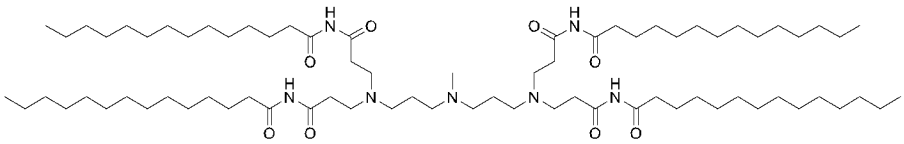
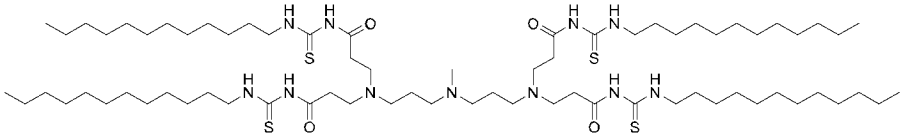
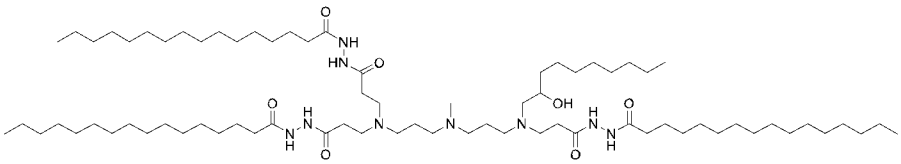
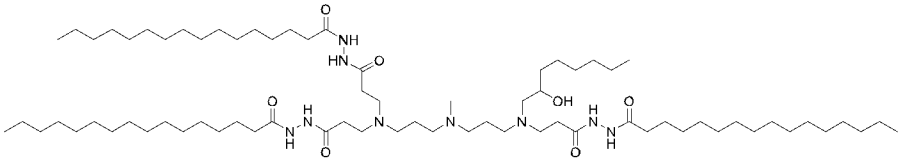
Compd. No.	Structure	Structure Code
1		A1O11C
2		A2O11C
3		A3O11C
4		A4O11C
5		A5O11C
6		A1S11E

7		A1N11F
8		A1NCN12
9		A3N11F
10		A2N10F
11		A1EP10O18B
12		A1EP10AN18
13		A1EP12O18
14		A13EP12N12
15		A13EP18O18

16		A1EP12N12
17		A1EP12O18
18		A19EP12N12
19		A19EP12O18
20		A1EP10AO18B
21		A1EPO15O18B
22		A1EP10O18A
23		A1EP10AO18A
24		A1EPO15O18A
25		A10AEP12O18B
26		A10AEP12O18A

27		A1EP12N11F
28		A5N10F
29		A1N12
30		A1EP8O18B
31		A1EP12O18B
32		A1EP14O18B
33		A1EP16O18B
34		A1EP18O18B
35		A10AEP8O18B
36		A10AEP10O18B
37		A1EP12O8F13

38		A1EP12O8F17
39		A10AEP8O18A
40		A10AEP10O18A
41		A10AEP14O18A
42		A10AEP16O18A
43		A10AEP18O18A
44		A10AEP10AO18A
45		A10AEPO15O18A
46		A5N11F
47		A2N11F
48		A10BEP10O12

49		A10BEP10O14
50		A1SO2N14
51		A1NNCC13
52		A1EP10SO2N14
53		A1EP10NNCC13
54		A1NCO12
55		A1NCC14
56		A1NSN12
57		A1EP10NNCC16
58		A1EP8NNCC16

[0144] Compounds provided herein are described with reference to both generic formulae and specific compounds. In addition, the compounds of the present disclosure may exist in a number of different forms or derivatives, including but not limited to, stereoisomers, racemic mixtures, regioisomers, tautomers, salts, prodrugs, soft drugs, active metabolic derivatives (active metabolites), solvated forms, different crystal forms or polymorphs, all within the scope of the present disclosure.

[0145] The compounds of present disclosure can comprise one or more asymmetric centers, and thus can exist in various stereoisomeric forms, e.g., enantiomers and/or diastereomers. Thus, the compounds of present disclosure and compositions thereof may be in the form of an individual enantiomer, diastereomer or geometric isomer, or may be in the form of a mixture of stereoisomers. In certain embodiments, the compounds of the present disclosure are enantiopure compounds. In certain embodiments, mixtures of enantiomers or diastereomers are provided.

[0146] The term "enantiomer" refers to two stereoisomers of a compound which are non-superimposable mirror images of one another. The term "diastereomer" refers to a pair of optical isomers which are not mirror images of one another. Diastereomers have different physical properties, e.g., melting points, boiling points, spectral properties, and reactivities.

[0147] Furthermore, certain compounds, as described herein may have one or more double bonds that can exist as either the Z or E isomer, unless otherwise indicated. The present disclosure additionally encompasses the compounds as individual isomers substantially free of other isomers and alternatively, as mixtures of various isomers, e.g., racemic mixtures of enantiomers. In addition to the above-mentioned compounds *per se*, this disclosure also encompasses compositions comprising one or more compounds.

[0148] As used herein, the term "isomers" includes any and all geometric isomers and stereoisomers. For example, "isomers" include *cis*- and *trans*-isomers, *E*- and *Z*- isomers, *R*- and *S*-enantiomers, diastereomers, (*D*)-isomers, (*L*)-isomers, racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. For instance, a stereoisomer may, in some embodiments, be provided substantially free of one or more corresponding stereoisomers, and may also be referred to as "stereochemically enriched".

[0149] Where a particular enantiomer is preferred, it may, in some embodiments be provided substantially free of the opposite enantiomer, and may also be referred to as "optically enriched". "Optically enriched", as used herein, means that the compound is made up of a significantly greater proportion of one enantiomer. In certain embodiments, the compound is made up of at least about 90% by weight of a preferred enantiomer. In other embodiments, the compound is made up of at least about 95%, 98%, or 99% by weight of a preferred enantiomer. Preferred enantiomers may be isolated from racemic mixtures by any method known to those skilled in the art, including chiral high performance liquid chromatography (HPLC) and the formation and crystallization of chiral salts or prepared by asymmetric syntheses. See, for example, Jacques, *et al.*, Enantiomers, Racemates and Resolutions (Wiley Interscience, New York, 1981); Wilen, S.H., *et al.*, Tetrahedron 33:2725 (1977); Eliel, E.L. Stereochemistry of Carbon Compounds (McGraw-Hill, NY, 1962); Wilen, S.H. Tables of Resolving Agents and Optical Resolutions p. 268 (E.L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, IN 1972).

[0150] The compounds of the present disclosure may also exist in different tautomeric forms, and all such forms are embraced within the scope of the present disclosure. The term "tautomer" or "tautomeric form" refers to structural isomers of different energies which are interconvertible via a low energy barrier. The presence and concentrations of the isomeric forms will depend on the environment the compound is found in and may be different depending upon, for example, whether the compound is a solid or is in an organic or aqueous solution. By way of examples, proton tautomers (also known as prototropic tautomers) include interconversions via migration of a proton, such as keto-enol, amide-imidic acid, lactam-lactim, imine-enamine isomerizations and annular forms where a proton can occupy two or more positions of a heterocyclic system. Valence tautomers include interconversions by reorganization of some of the bonding electrons. Tautomers can be in equilibrium or sterically locked into one form by appropriate substitution. Compounds of the present disclosure identified by name or structure as one particular tautomeric form are intended to include other tautomeric forms unless otherwise specified.

[0151] The present disclosure is also intended to include all isotopes of atoms in the compounds. Isotopes of an atom include atoms having the same atomic number but different mass numbers. For example, unless otherwise specified, hydrogen,

carbon, nitrogen, oxygen, phosphorous, sulfur, fluorine, chlorine, bromide or iodine in the compounds of present disclosure are meant to also include their isotopes, such as but not limited to ^{1}H, ^{2}H, ^{3}H, ^{11}C, ^{12}C, ^{13}C, ^{14}C, ^{14}N, ^{15}N, ^{16}O, ^{17}O, ^{18}O, ^{31}P, ^{32}P, ^{32}S, ^{33}S, ^{34}S, ^{36}S, ^{17}F, ^{18}F, ^{19}F, ^{35}Cl, ^{37}Cl, ^{79}Br, ^{81}Br, ^{124}I, ^{127}I and ^{131}I. In some embodiments, hydrogen includes protium, deuterium and tritium. In some embodiments, carbon includes ^{12}C and ^{13}C.

[0152] *Synthesis of Compounds*

[0153] Synthesis of the compounds provided herein, including pharmaceutically acceptable salts thereof, are illustrated in the synthetic schemes in the examples. The compounds provided herein can be prepared using any known organic synthesis techniques and can be synthesized according to any of numerous possible synthetic routes, and thus these schemes are illustrative only and are not meant to limit other possible methods that can be used to prepare the compounds provided herein. Additionally, the steps in the Schemes are for better illustration and can be changed as appropriate. The embodiments of the compounds in examples were synthesized for the purposes of research and potentially submission to regulatory agencies.

[0154] The reactions for preparing compounds of the present disclosure can be carried out in suitable solvents, which can be readily selected by one skilled in the art of organic synthesis. Suitable solvents can be substantially non-reactive with the starting materials (reactants), the intermediates, or products at the temperatures at which the reactions are carried out, e.g. temperatures that can range from the solvent's freezing temperature to the solvent's boiling temperature. A given reaction can be carried out in one solvent or a mixture of more than one solvent. Depending on the particular reaction step, suitable solvents for a particular reaction step can be selected by one skilled in the art.

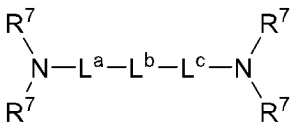
[0155] Preparation of compounds of the present disclosure can involve the protection and deprotection of various chemical groups. The need for protection and deprotection, and the selection of appropriate protecting groups, can be readily determined by one skilled in the art. The chemistry of protecting groups can be found, for example, in T. W. Greene and P. G. M. Wuts, Protective Groups in Organic Synthesis, 3rd Ed., Wiley & Sons, Inc., New York (1999), which is incorporated herein by reference in its entirety.

[0156] Reactions can be monitored according to any suitable method known in the art. For example, product formation can be monitored by spectroscopic means, such as nuclear magnetic resonance spectroscopy (e.g. ^{1}H or ^{13}C), infrared spectroscopy, spectrophotometry (e.g. UV-visible), mass spectrometry, or by chromatographic methods such as high performance liquid chromatography (HPLC), liquid chromatography-mass spectroscopy (LCMS), or thin layer chromatography (TLC). Compounds can be purified by one skilled in the art by a variety of methods, including high performance liquid chromatography (HPLC) ("Preparative LC-MS Purification: Improved Compound Specific Method Optimization" Karl F. Blom, Brian Glass, Richard Sparks, Andrew P. Combs J. Combi. Chem. 2004, 6(6), 874-883, which is incorporated herein by reference in its entirety), and normal phase silica chromatography.

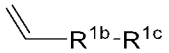
[0157] The structures of the compounds in the examples are characterized by nuclear magnetic resonance (NMR) or/and liquid chromatography-mass spectrometry (LC-MS). NMR chemical shift (δ) is given in the unit of 10^{-6} (ppm). ^{1}H-NMR spectra is recorded in $CDCl_3$, CD_3OD or DMSO-d_6 solutions (reported in ppm) on a Bruker instrument (400 MHz or 500 MHz), using tetramethylsilane (TMS) as the reference standard (0.0 ppm).

[0158] In one aspect, the present disclosure provides a method for preparation of the compound having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E), or a pharmaceutically acceptable salt thereof, comprising:

A) mixing a compound having Formula (II) below and a compound having Formula (III) below:



(II),



(III),

wherein

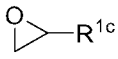
each R^7 is independently selected from the group consisting of hydrogen, alkyl, heteroalkyl, haloalkyl and hydroxyalkyl;

each L^a, L^b, L^c, R^{1b} and R^{1c} has the same definition as above; and

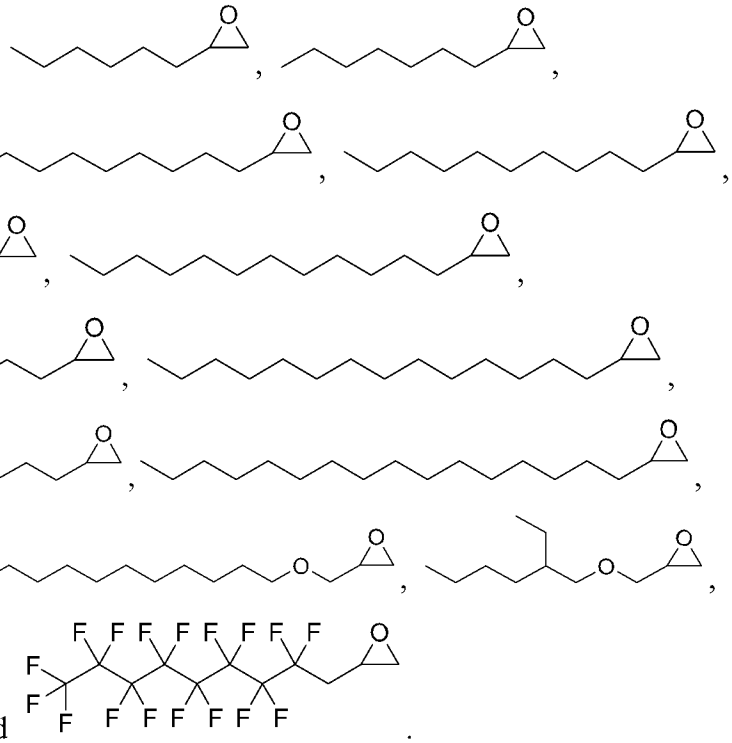
B) refluxing the mixture until completion of reaction, and obtaining the compound

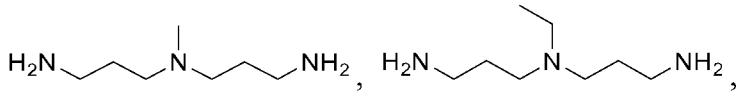
having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E).

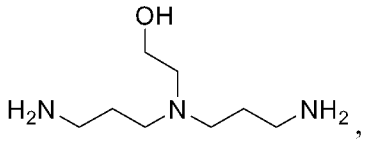
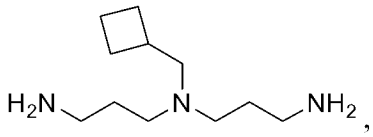
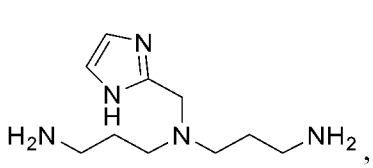
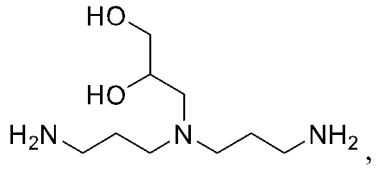
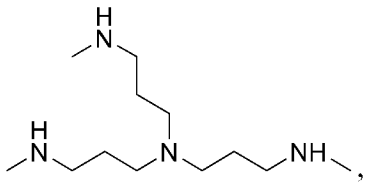
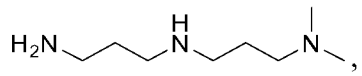
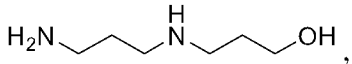
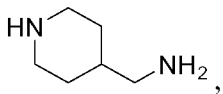
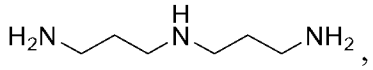
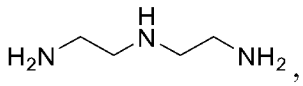
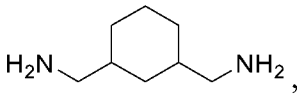
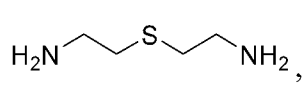
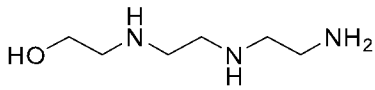
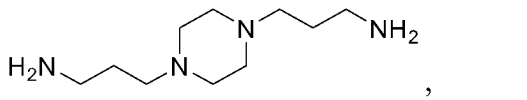
[0159] In one aspect, the present disclosure provides a method for preparation of the compound having Formular (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E), or a pharmaceutically acceptable salt thereof, comprising:
i) mixing a compound having Formula (II) and a compound having Formula (IV) below:

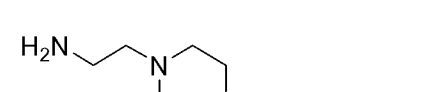

(IV),

wherein R^{1c} has the same definition as above;
ii) mixing a product of step (i) and a compound having Formula (III); and
iii) refluxing the mixture until completion of reaction, and obtaining the compound having Formular (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E) thereby.

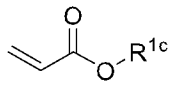
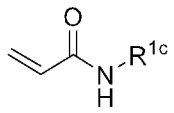
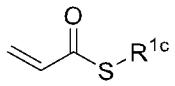
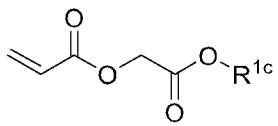
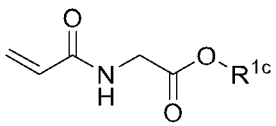
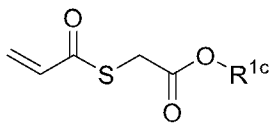
[0160] In some embodiments, the compound having Formula (IV) is selected from the group consisting of 
and .

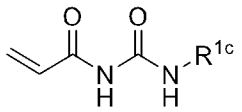
[0161] In some embodiments, the compound having Formula (II) is selected from the group consisting of 



and .

[0162] In some embodiments, the compound having Formula (III) is selected from the group consisting of  ,  ,  ,  ,  ,  ,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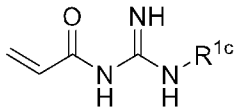
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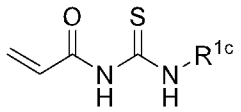
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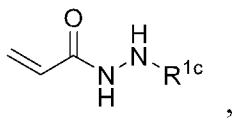
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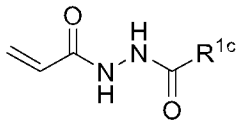
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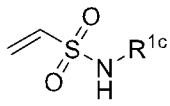
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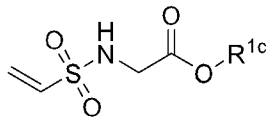
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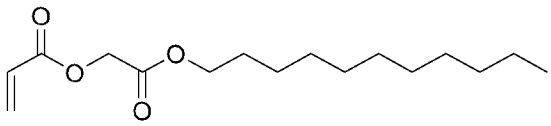
[0163] In some embodiments, the compound having Formula (III) is selected from the group consisting of: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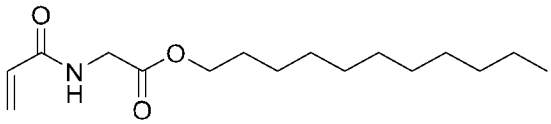
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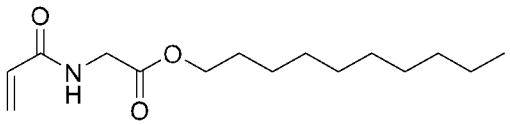
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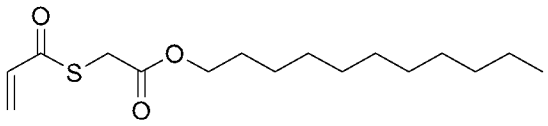
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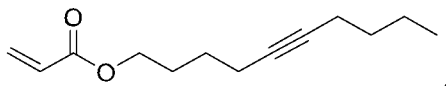
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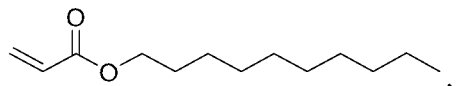
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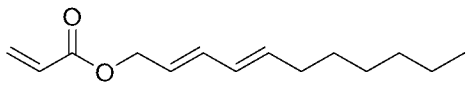
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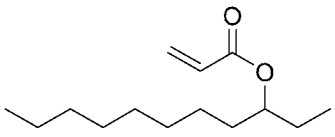
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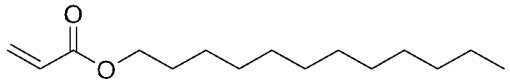
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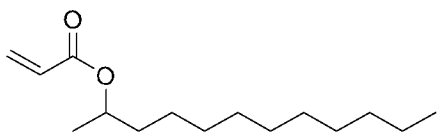
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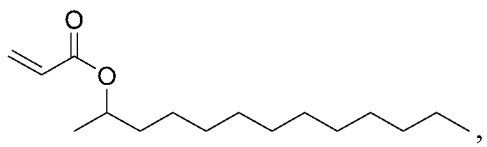
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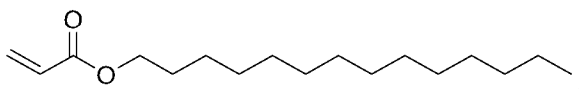
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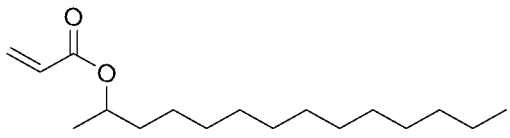
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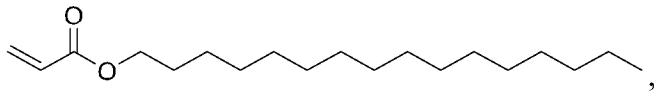

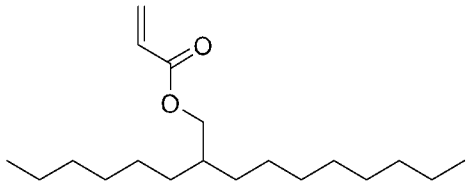

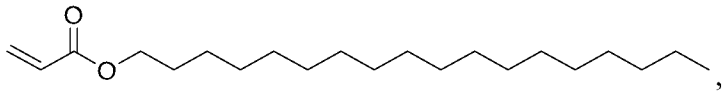

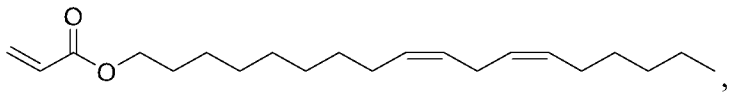

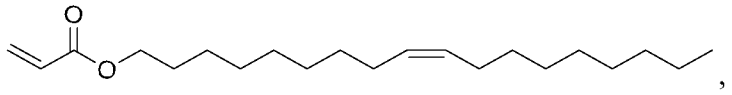

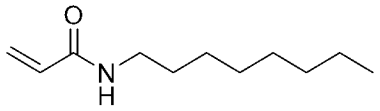

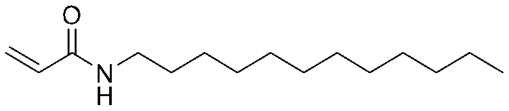

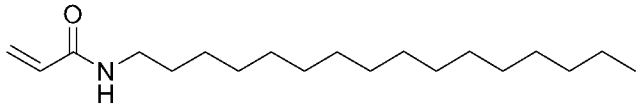

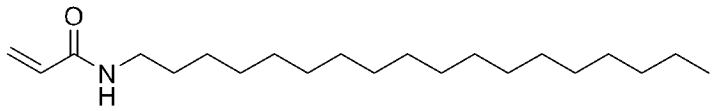

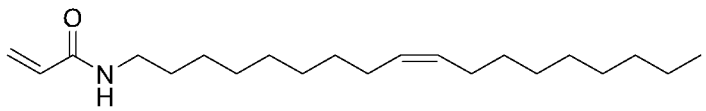

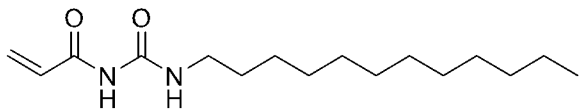

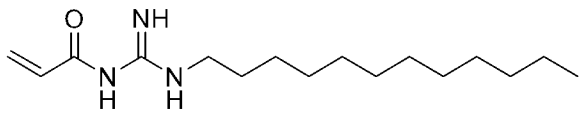

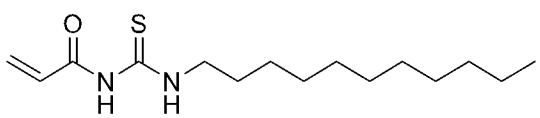

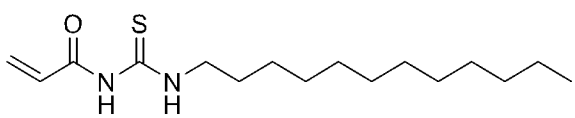

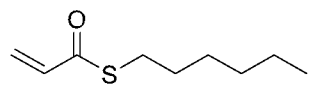

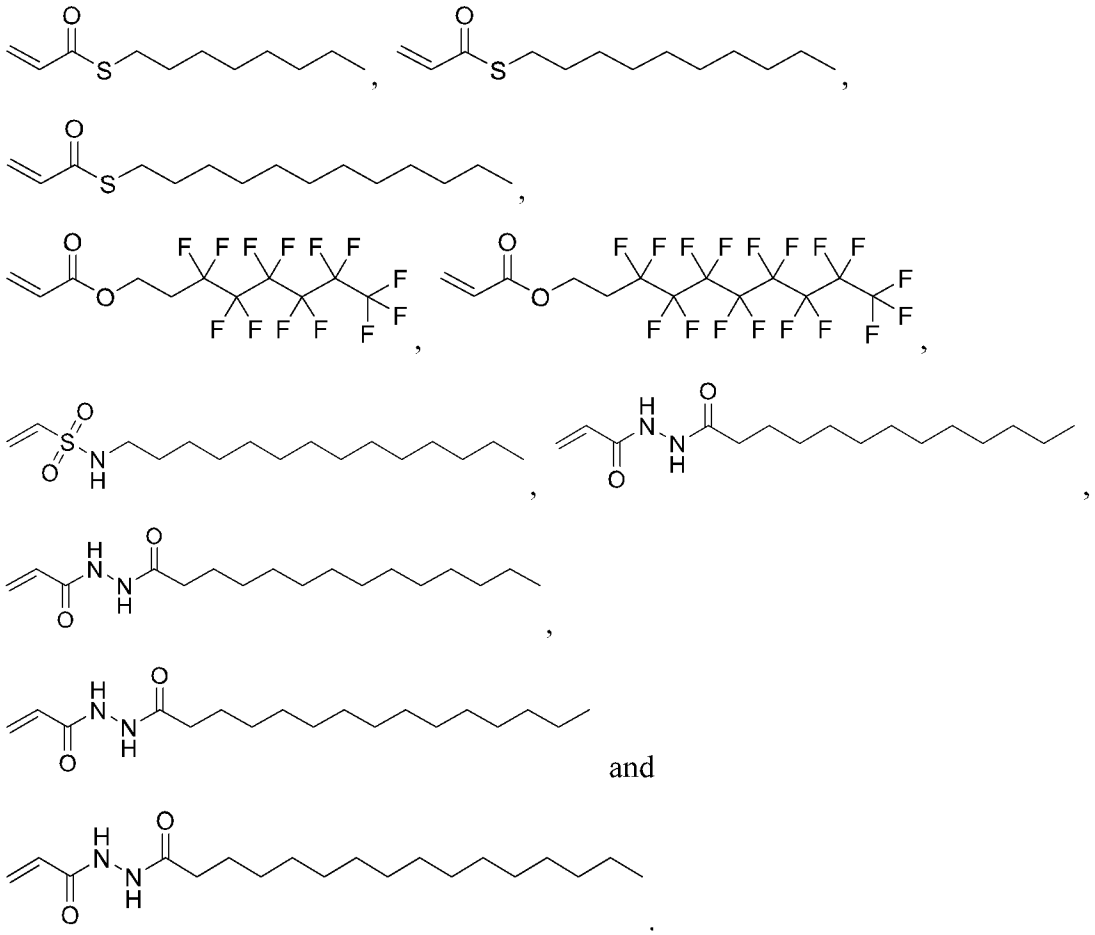

and

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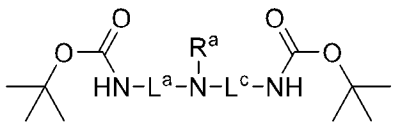
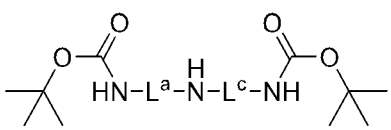
[0164] In some embodiments, the method further comprising a method for preparation of the compound having Formula (II), wherein L^b is $-NR^a-$ and R^a is not hydrogen, from a compound having Formula (V) below:



(V),

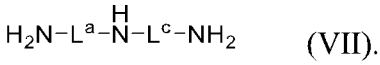
wherein R^a is not hydrogen.

[0165] In some embodiments, the method further comprising a method for preparation of the compound having Formula (V), wherein R^a is not hydrogen, from a compound having Formula (VI) below:



(VI).

[0166] In some embodiments, the method further comprising a method for preparation of the compound having Formula (VI) from a compound having Formula (VII) below:



(VII).

[0167] Unless otherwise specified, the reactions of the present disclosure were typically done under a positive pressure of nitrogen or argon or with a drying tube in anhydrous solvents, and the reaction flasks were typically fitted with rubber septa for the introduction of substrates and reagents via syringe. Glassware was oven dried and/or heat dried.

[0168] *Lipid Particles*

[0169] The present disclosure also provides lipid particles comprising one or more of the lipids described above. Lipid particles include, but are not limited to, lipid nanoparticles (LNPs), liposomes, lipoplexes, and lipopolyplex (LPP). In some embodiments, the lipid particles are lipid nanoparticles. The present disclosure also provides a method for preparation of the lipid particles.

[0170] The lipid particles of the present disclosure may further comprise one or more additional lipids and/or other components such as a sterol. Other lipids may be included in the lipid particles of the present disclosure for a variety of purposes, such as to prevent lipid oxidation or to attach ligands onto the particle surface. Any lipid may be present in lipid particles of the present disclosure, including amphipathic, neutral, cationic, and anionic lipids, which can be used alone or in combination. Examples of additional lipid components that may be present are described below.

[0171] In some embodiments, the lipid particles comprise a lipid of the present disclosure (e.g., compound having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E), or any compound of Table 1). In some embodiments, the lipid particles comprise two or more lipids of the present disclosure. In some embodiments, the lipid of the present disclosure (e.g., compound having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E), or any compound of Table 1) is in a molar fraction of about 5 to 75%, about 10 to 75%, about 15 to 75%, about 20 to 75%, about 25 to 75%, about 30 to 75%, about 35 to 75%, about 40 to 75%, about 10 to 70%, about 15 to 70%, about 20 to 70%, about 25 to 70%, about 30 to 70%, about 35 to 70%, about 40 to 70%, about 10 to 65%, about 15 to 65%, about 20 to 65%,

about 25 to 65%, about 30 to 65%, about 35 to 65%, about 40 to 65%, about 45 to 65%, about 10 to 60%, about 15 to 60%, about 20 to 60%, about 25 to 60%, about 30 to 60%, about 35 to 60%, about 40 to 60%, about 45 to 60%, about 10 to 55%, about 15 to 55%, about 20 to 55%, about 25 to 55%, about 30 to 55%, about 35 to 55%, about 40 to 55%, about 10 to 50%, about 15 to 50%, about 20 to 50%, about 25 to 50%, about 30 to 50%, about 35 to 50%, about 40 to 50%, about 10 to 45%, about 15 to 45%, about 20 to 45%, about 25 to 45%, about 30 to 45%, about 35 to 45%, about 40 to 45%, about 10 to 40%, about 15 to 40%, about 20 to 40%, about 25 to 40%, about 30 to 40%, about 35 to 40%, about 45 to 50%, about 45 to 55%, about 45 to 60%, about 45 to 65%, about 45 to 70%, about 45 to 75%, about 50 to 55%, about 50 to 60%, about 55 to 65%, about 55 to 70%, about 55 to 75%, about 55 to 60%, about 55 to 65%, about 55 to 70%, about 55 to 75%, about 60 to 65%, about 60 to 70%, or about 60 to 75% of the total lipids present in the lipid particle. In some embodiments, the lipid of the present disclosure (e.g., compound having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E), or any compound of Table 1 is in a molar fraction of about 10 to 75%, about 40 to 75% or about 40 to 70% of the total lipids present in the lipid particle. In some embodiments, the lipid of the present disclosure (e.g., compound having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E), or any compound of Table 1) is in a molar fraction of about 40-50% of the total lipids present in the lipid particle. In some embodiments, the lipid of the present disclosure (e.g., compound having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E), or any compound of Table 1) is in a molar fraction of about 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69% or 70% of the total lipids present in the lipid particle.

[0172] In some embodiments, the lipid particles comprise a neutral lipid. The term "neutral lipid" refers to any of a number of lipid species that exist either in an uncharged or neutral zwitterionic form at a selected pH. In some embodiments, the neutral lipid is a phospholipid. Examples of phospholipid include, but not limited to, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-Distearoyl-sn-glycero-3-phosphorylethanolamine (DSPE), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC),

1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (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-sn-glycero-3-phosphocholine (OChemsPC), 1-hexadecyl-sn-glycero-3-phosphocholine (Cl6 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-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-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), sphingomyelin, and any mixtures thereof. In some embodiments, the lipid particles comprise one neutral lipid. In some embodiments, the lipid particles comprise two or more neutral lipids. In some embodiments, the neutral lipid is selected from the group consisting of 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), and any mixtures thereof. In some embodiments, the neutral lipid is in a molar fraction of about 1 to 40%. In some embodiments, the neutral lipid is in a molar fraction of about 1 to 35%, about 5 to 30%, about 5 to 25%, about 5 to 20%, about 5 to 15%, about 4 to 15%, about 3 to 15%, about 2 to 15%, about 1 to 15%, about 5 to 10%, about 4 to 10%, about 3 to 10%, about 2 to 10% or about 1 to 10% of the total lipids present in the lipid particle. In some embodiments, the neutral lipid is in a molar fraction of about 1 to 35%, about 2 to 15% or about 3 to 15% of the total lipids present in the lipid particle. In some embodiments, the neutral lipid is in a molar fraction of about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 15%, 20%, 25% or 30% of the total lipids present in the lipid particle.

[0173] In some embodiments, the lipid particles comprise a structural lipid. In some embodiments, the structural lipid is a sterol, a sterol derivative or any mixtures thereof. Examples of a sterol or a sterol derivative include, but are not limited to, cholesterol, fecosterol, sitosterol, beta-sitosterol, ergosterol, campesterol, stigmasterol,

brassicasterol, tomatidine, tomatine, ursolic acid, alpha-tocopherol, and mixtures thereof. In some embodiments, the structural lipid is cholesterol. In some embodiments, the structural lipid includes cholesterol and a corticosteroid (such as prednisolone, dexamethasone, prednisone, and hydrocortisone), or a combination thereof. In some embodiments, the lipid particle comprises cholesterol. In some embodiments, the structural lipid is in a molar fraction of about 5 to 50% of the total lipids present in the lipid particle. In some embodiments, the structural lipid is in a molar fraction of about 10 to 50%, about 15 to 50%, about 20 to 50%, about 21 to 50%, about 22 to 50%, about 23 to 50%, about 24 to 50%, about 25 to 50%, about 26 to 50%, about 27 to 50%, about 28 to 50%, about 29 to 50%, about 30 to 50%, about 31 to 50%, about 32 to 50%, about 33 to 50%, about 34 to 50%, about 35 to 50%, about 36 to 50%, about 37 to 50%, about 38 to 50%, about 39 to 50%, or about 40 to 50% of the total lipids present in the lipid particle. In some embodiments, the structural lipid is in a molar fraction of about 10 to 45%, about 15 to 45%, about 25 to 45%, about 30 to 45% or about 35 to 45% of the total lipids present in the lipid particle. In some embodiments, the structural lipid is in a molar fraction of about 15 to 50%, about 20 to 50%, or about 22 to 50% of the total lipids present in the lipid particle. In some embodiments, the structural lipid is in a molar fraction of about 20%, 20.1%, 20.2%, 20.3%, 20.4%, 20.5%, 20.6%, 20.7%, 20.8%, 20.9%, 21%, 21.1%, 21.2%, 21.3%, 21.4%, 21.5%, 21.6%, 21.7%, 21.8%, 21.9%, 22%, 22.1%, 22.2%, 22.3%, 22.4%, 22.5%, 22.6%, 22.7%, 22.8%, 22.9%, 23%, 23.1%, 23.2%, 23.3%, 23.4%, 23.5%, 23.6%, 23.7%, 23.8%, 23.9%, 24%, 24.1%, 24.2%, 24.3%, 24.4%, 24.5%, 24.6%, 24.7%, 24.8%, 24.9%, 25%, 25.1%, 25.2%, 25.3%, 25.4%, 25.5%, 25.6%, 25.7%, 25.8%, 25.9%, 26%, 26.1%, 26.2%, 26.3%, 26.4%, 26.5%, 26.6%, 26.7%, 26.8%, 26.9%, 27%, 27.1%, 27.2%, 27.3%, 27.4%, 27.5%, 27.6%, 27.7%, 27.8%, 27.9%, 28%, 28.1%, 28.2%, 28.3%, 28.4%, 28.5%, 28.6%, 28.7%, 28.8%, 28.9%, 29%, 29.1%, 29.2%, 29.3%, 29.4%, 29.5%, 29.6%, 29.7%, 29.8%, 29.9%, 30%, 30.1%, 30.2%, 30.3%, 30.4%, 30.5%, 30.6%, 30.7%, 30.8%, 30.9%, 31%, 31.1%, 31.2%, 31.3%, 31.4%, 31.5%, 31.6%, 31.7%, 31.8%, 31.9%, 32%, 32.1%, 32.2%, 32.3%, 32.4%, 32.5%, 32.6%, 32.7%, 32.8%, 32.9%, 33%, 33.1%, 33.2%, 33.3%, 33.4%, 33.5%, 33.6%, 33.7%, 33.8%, 33.9%, 34%, 34.1%, 34.2%, 34.3%, 34.4%, 34.5%, 34.6%, 34.7%, 34.8%, 34.9%, 35%, 35.1%, 35.2%, 35.3%, 35.4%, 35.5%, 35.6%, 35.7%, 35.8%, 35.9%, 36%, 36.1%, 36.2%, 36.3%, 36.4%, 36.5%, 36.6%, 36.7%, 36.8%,

36.9%, 37%, 37.1%, 37.2%, 37.3%, 37.4%, 37.5%, 37.6%, 37.7%, 37.8%, 37.9%, 38%, 38.1%, 38.2%, 38.3%, 38.4%, 38.5%, 38.6%, 38.7%, 38.8%, 38.9%, 39%, 39.1%, 39.2%, 39.3%, 39.4%, 39.5%, 39.6%, 39.7%, 39.8%, 39.9%, 40%, 40.1%, 40.2%, 40.3%, 40.4%, 40.5%, 40.6%, 40.7%, 40.8%, 40.9%, 41%, 41.1%, 41.2%, 41.3%, 41.4%, 41.5%, 41.6%, 41.7%, 41.8%, 41.9%, 42%, 42.1%, 42.2%, 42.3%, 42.4%, 42.5%, 42.6%, 42.7%, 42.8%, 42.9%, 43%, 43.1%, 43.2%, 43.3%, 43.4%, 43.5%, 43.6%, 43.7%, 43.8%, 43.9%, 44%, 44.1%, 44.2%, 44.3%, 44.4%, 44.5%, 44.6%, 44.7%, 44.8%, 44.9%, 45%, 45.1%, 45.2%, 45.3%, 45.4%, 45.5%, 45.6%, 45.7%, 45.8%, 45.9%, 46%, 46.1%, 46.2%, 46.3%, 46.4%, 46.5%, 46.6%, 46.7%, 46.8%, 46.9%, 47%, 47.1%, 47.2%, 47.3%, 47.4%, 47.5%, 47.6%, 47.7%, 47.8%, 47.9%, 48%, 48.1%, 48.2%, 48.3%, 48.4%, 48.5%, 48.6%, 48.7%, 48.8%, 48.9%, 49%, 49.1%, 49.2%, 49.3%, 49.4%, 49.5%, 49.6%, 49.7%, 49.8%, 49.9%, 50%, 50.1%, 50.2%, 50.3%, 50.4%, 50.5%, 50.6%, 50.7%, 50.8%, 50.9%, 51%, 51.1%, 51.2%, 51.3%, 51.4%, 51.5%, 51.6%, 51.7%, 51.8%, 51.9%, 52%, 52.1%, 52.2%, 52.3%, 52.4%, 52.5%, 52.6%, 52.7%, 52.8%, 52.9%, 53%, 53.1%, 53.2%, 53.3%, 53.4%, 53.5%, 53.6%, 53.7%, 53.8%, 53.9%, 54%, 54.1%, 54.2%, 54.3%, 54.4%, 54.5%, 54.6%, 54.7%, 54.8%, 54.9% or 55% of the total lipids present in the lipid particle.

[0174] In some embodiments, the lipid particles comprise a lipid selected to reduce aggregation of lipid particles during formation, which may result from steric stabilization of particles which prevents charge-induced aggregation during formation. Examples of lipids that reduce aggregation of particles during formation include, but not limited to, polyethylene glycol (PEG)-modified lipids, monosialoganglioside Gm1, and polyamide oligomers (PAO). Other compounds with uncharged, hydrophilic, steric-barrier moieties, which prevent aggregation during formulation, like PEG or Gm1 can also be coupled to lipids for use as in the methods and compositions of the present disclosure. In some embodiments, lipids that reduce aggregation of particles during formation is a surfactant. In some embodiments, the surfactant is a PEG-modified lipid. Examples of PEG-modified lipid include, but not limited to, PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, PEG-modified dialkylglycerols, and mixtures thereof. In some embodiments, the PEG-modified lipid is 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol (DMG-PEG), 1,2-distearoyl-rac-glycero-3-methoxypolyethylene glycol (DSG-PEG),

N-(methylpolyoxyethylene-carbonyl)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (PEG-DSPE), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[(polyethylene glycol)] (DOPE-PEG), or any mixtures thereof. Typically, the lipid component selected to reduce aggregation is in a molar fraction of about 1 to 15% of the total lipids present in the lipid particle. In some embodiments, the lipid component selected to reduce aggregation is in a molar fraction of about 1 to 10%, about 1 to 7%, about 1 to 5% or about 0.5 to 5% of the total lipids present in the lipid particle. In some embodiments, the lipid component selected to reduce aggregation is in a molar fraction of about 1 to 5%, about 1 to 2.5% or about 1.5 to 2% of the total lipids present in the lipid particle. In some embodiments, the lipid component selected to reduce aggregation is in a molar fraction of about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 1.1%, 1.2%, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, 1.9%, 2%, 2.1%, 2.2%, 2.3%, 2.4%, 2.5%, 2.6%, 2.7%, 2.8%, 2.9%, 3%, 3.1%, 3.2%, 3.3%, 3.4%, 3.5%, 3.6%, 3.7%, 3.8%, 3.9%, or 4% of the total lipids present in the lipid particle.

[0175] As used herein, the term "PEG", whether as part of another term or used independently, refers to polyethylene glycol. The term "PEGm" or "PEG-m", wherein m is an integer, refers to a polyethylene glycol molecule or moiety with molecular weight of m. For example, "PEG2000" or "PEG-2000" refers to a polyethylene glycol molecule or moiety with molecular weight of 2000. In some embodiments, a PEG is a PEG700, PEG800, PEG900, PEG1000, PEG1100, PEG1200, PEG1300, PEG1400, PEG1500, PEG1600, PEG1700, PEG1800, PEG1900, PEG2000, PEG2100, PEG2200, PEG2300, PEG2400, PEG2500, PEG2600, PEG2700, PEG2800, PEG2900 or PEG3000. In some embodiments, a PEG is a PEG2000.

[0176] In some embodiments, the lipid particle of the present disclosure comprises: a compound having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E) in a molar fraction of about 10-65%; a neutral lipid of the present disclosure in a molar fraction of about 5-30%; a structural lipid of the present disclosure in a molar fraction of about 15-50%; a lipid component selected to reduce aggregation of the present disclosure in a molar fraction of about 0.5-5%, based on the total lipids present in the lipid particle.

[0177] In some embodiments, the lipid particle of the present disclosure comprises: a compound having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E) in a molar fraction of about 20-65%; a neutral lipid of the

present disclosure in a molar fraction of about 5-25%; a structural lipid of the present disclosure in a molar fraction of about 25-50%; a lipid component selected to reduce aggregation of the present disclosure in a molar fraction of about 1-5%, based on the total lipids present in the lipid particle.

[0178] In some embodiments, the lipid particle of the present disclosure comprises: a compound having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E) in a molar fraction of about 40-65%; a neutral lipid of the present disclosure in a molar fraction of about 5-15%; a structural lipid of the present disclosure in a molar fraction of about 25-50%; a lipid component selected to reduce aggregation of the present disclosure in a molar fraction of about 1-2.5%, based on the total lipids present in the lipid particle.

[0179] In some embodiments, the lipid particle of the present disclosure comprises: a compound having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E) in a molar fraction of about 40-50%; a neutral lipid of the present disclosure in a molar fraction of about 5-15%; a structural lipid of the present disclosure in a molar fraction of about 40-50%; a lipid component selected to reduce aggregation of the present disclosure in a molar fraction of about 1.5-2%, based on the total lipids present in the lipid particle.

[0180] In some embodiments, the lipid particle of the present disclosure comprises: a compound having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E) in a molar fraction of about 10-75%; a neutral lipid of the present disclosure in a molar fraction of about 1-35%; a structural lipid of the present disclosure in a molar fraction of about 15-50%; a lipid component selected to reduce aggregation of the present disclosure in a molar fraction of about 0.5-5%, based on the total lipids present in the lipid particle.

[0181] In some embodiments, the lipid particle of the present disclosure comprises: a compound having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E) in a molar fraction of about 40-75%; a neutral lipid of the present disclosure in a molar fraction of about 2-15%; a structural lipid of the present disclosure in a molar fraction of about 20-50%; a lipid component selected to reduce aggregation of the present disclosure in a molar fraction of about 1-5%, based on the total lipids present in the lipid particle.

[0182] In some embodiments, the lipid particle of the present disclosure comprises: a compound having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E) in a molar fraction of about 40-70%; a neutral lipid of the present disclosure in a molar fraction of about 3-15%; a structural lipid of the present disclosure in a molar fraction of about 20-50%; a lipid component selected to reduce aggregation of the present disclosure in a molar fraction of about 1-2.5%, based on the total lipids present in the lipid particle.

[0183] In some embodiments, the lipid particle of the present disclosure comprises: a compound having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E) in a molar fraction of about 40-70%; a neutral lipid of the present disclosure in a molar fraction of about 3-15%; a structural lipid of the present disclosure in a molar fraction of about 22-50%; a lipid component selected to reduce aggregation of the present disclosure in a molar fraction of about 1.5-2.5%, based on the total lipids present in the lipid particle.

[0184] In some embodiments, the lipid particle of the present disclosure comprises:

a compound having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E) in a molar fraction of about 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69% or 70%;

a neutral lipid of the present disclosure in a molar fraction of about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 15%, 20%, 25% or 30%;

a structural lipid of the present disclosure in a molar fraction of about 20%, 20.1%, 20.2%, 20.3%, 20.4%, 20.5%, 20.6%, 20.7%, 20.8%, 20.9%, 21%, 21.1%, 21.2%, 21.3%, 21.4%, 21.5%, 21.6%, 21.7%, 21.8%, 21.9%, 22%, 22.1%, 22.2%, 22.3%, 22.4%, 22.5%, 22.6%, 22.7%, 22.8%, 22.9%, 23%, 23.1%, 23.2%, 23.3%, 23.4%, 23.5%, 23.6%, 23.7%, 23.8%, 23.9%, 24%, 24.1%, 24.2%, 24.3%, 24.4%, 24.5%, 24.6%, 24.7%, 24.8%, 24.9%, 25%, 25.1%, 25.2%, 25.3%, 25.4%, 25.5%, 25.6%, 25.7%, 25.8%, 25.9%, 26%, 26.1%, 26.2%, 26.3%, 26.4%, 26.5%, 26.6%, 26.7%, 26.8%, 26.9%, 27%, 27.1%, 27.2%, 27.3%, 27.4%, 27.5%, 27.6%, 27.7%, 27.8%, 27.9%, 28%, 28.1%, 28.2%, 28.3%, 28.4%, 28.5%, 28.6%, 28.7%, 28.8%, 28.9%, 29%,

29.1%, 29.2%, 29.3%, 29.4%, 29.5%, 29.6%, 29.7%, 29.8%, 29.9%, 30%, 30.1%, 30.2%, 30.3%, 30.4%, 30.5%, 30.6%, 30.7%, 30.8%, 30.9%, 31%, 31.1%, 31.2%, 31.3%, 31.4%, 31.5%, 31.6%, 31.7%, 31.8%, 31.9%, 32%, 32.1%, 32.2%, 32.3%, 32.4%, 32.5%, 32.6%, 32.7%, 32.8%, 32.9%, 33%, 33.1%, 33.2%, 33.3%, 33.4%, 33.5%, 33.6%, 33.7%, 33.8%, 33.9%, 34%, 34.1%, 34.2%, 34.3%, 34.4%, 34.5%, 34.6%, 34.7%, 34.8%, 34.9%, 35%, 35.1%, 35.2%, 35.3%, 35.4%, 35.5%, 35.6%, 35.7%, 35.8%, 35.9%, 36%, 36.1%, 36.2%, 36.3%, 36.4%, 36.5%, 36.6%, 36.7%, 36.8%, 36.9%, 37%, 37.1%, 37.2%, 37.3%, 37.4%, 37.5%, 37.6%, 37.7%, 37.8%, 37.9%, 38%, 38.1%, 38.2%, 38.3%, 38.4%, 38.5%, 38.6%, 38.7%, 38.8%, 38.9%, 39%, 39.1%, 39.2%, 39.3%, 39.4%, 39.5%, 39.6%, 39.7%, 39.8%, 39.9%, 40%, 40.1%, 40.2%, 40.3%, 40.4%, 40.5%, 40.6%, 40.7%, 40.8%, 40.9%, 41%, 41.1%, 41.2%, 41.3%, 41.4%, 41.5%, 41.6%, 41.7%, 41.8%, 41.9%, 42%, 42.1%, 42.2%, 42.3%, 42.4%, 42.5%, 42.6%, 42.7%, 42.8%, 42.9%, 43%, 43.1%, 43.2%, 43.3%, 43.4%, 43.5%, 43.6%, 43.7%, 43.8%, 43.9%, 44%, 44.1%, 44.2%, 44.3%, 44.4%, 44.5%, 44.6%, 44.7%, 44.8%, 44.9%, 45%, 45.1%, 45.2%, 45.3%, 45.4%, 45.5%, 45.6%, 45.7%, 45.8%, 45.9%, 46%, 46.1%, 46.2%, 46.3%, 46.4%, 46.5%, 46.6%, 46.7%, 46.8%, 46.9%, 47%, 47.1%, 47.2%, 47.3%, 47.4%, 47.5%, 47.6%, 47.7%, 47.8%, 47.9%, 48%, 48.1%, 48.2%, 48.3%, 48.4%, 48.5%, 48.6%, 48.7%, 48.8%, 48.9%, 49%, 49.1%, 49.2%, 49.3%, 49.4%, 49.5%, 49.6%, 49.7%, 49.8%, 49.9%, 50%, 50.1%, 50.2%, 50.3%, 50.4%, 50.5%, 50.6%, 50.7%, 50.8%, 50.9%, 51%, 51.1%, 51.2%, 51.3%, 51.4%, 51.5%, 51.6%, 51.7%, 51.8%, 51.9%, 52%, 52.1%, 52.2%, 52.3%, 52.4%, 52.5%, 52.6%, 52.7%, 52.8%, 52.9%, 53%, 53.1%, 53.2%, 53.3%, 53.4%, 53.5%, 53.6%, 53.7%, 53.8%, 53.9%, 54%, 54.1%, 54.2%, 54.3%, 54.4%, 54.5%, 54.6%, 54.7%, 54.8%, 54.9% or 55%;

a lipid component selected to reduce aggregation of the present disclosure in a molar fraction of about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 1.1%, 1.2%, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, 1.9%, 2%, 2.1%, 2.2%, 2.3%, 2.4%, 2.5%, 2.6%, 2.7%, 2.8%, 2.9%, 3%, 3.1%, 3.2%, 3.3%, 3.4%, 3.5%, 3.6%, 3.7%, 3.8%, 3.9%, or 4%,

based on the total lipids present in the lipid particle.

[0185] In some embodiments, the lipid particle of the present disclosure comprises components in molar fractions as listed in the table below.

A compound having Formula (I), Formula (A), Formula (B), Formula (C), Formula (D) or Formula (E)	**A neutral lipid**	**A structural lipid**	**A lipid component selected to reduce aggregation**
49%	8%	40.7%	2.3%
46%	8%	43.9%	2.1%
46%	12%	39.7%	2.3%
50%	10%	38.5%	1.5%
40%	6%	52.4%	1.6%
40%	8%	50.1%	1.9%
43%	6%	49.1%	1.9%
43%	8%	47.4%	1.6%
46%	6%	45.9%	2.1%
46%	8%	43.6%	2.4%
46%	10%	42.4%	1.6%
46%	12%	40.1%	1.9%
49%	6%	42.6%	2.4%
49%	8%	40.9%	2.1%
49%	12%	37.4%	1.6%
44%	7%	47.5%	1.5%
45%	6%	47.5%	1.5%
45%	8%	45.3%	1.7%
47%	7%	44.4%	1.6%
47%	8%	43.5%	1.5%
46%	8%	43.9%	2.1%
43%	8%	47.4%	1.6%
46%	10%	42.4%	1.6%
45%	8%	45.3%	1.7%
47%	7%	44.4%	1.6%
45%	6%	47.4%	1.6%
70%	4%	23.9%	2.1%

[0186] In some embodiments, the lipid particles comprise a targeting moiety. In some embodiments, the targeting moiety is conjugated to the lipid particle. In some embodiments, the targeting moiety is conjugated to the lipid particle directly or via a linker. In some embodiments, the targeting moiety is an antibody or a ligand.

[0187] Lipid particles may be characterized by a variety of methods. For example, microscopy (e.g., transmission electron microscopy (TEM) or scanning electron microscopy (SEM)) may be used to examine the morphology and size distribution of a lipid particle composition. Dynamic light scattering (DLS) or potentiometry (e.g., potentiometric titrations) may be used to measure zeta potentials. DLS may also be utilized to determine particle sizes. Instruments such as the Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, Worcestershire, UK) may also be used to measure multiple characteristics of a lipid particle, such as particle size, polydispersity index, and zeta potential. The error of measurement by DLS may depend on various factor such as scattering angle and multiple scattering. The typical error of measurement by DLS is 5%.

[0188] The mean size of lipid particles may be between 10s of nm and 100s of nm, e.g., measured by DLS. For example, the mean size of lipid particles may be from about 40 nm to about 150 nm, such as about 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. In some embodiments, the mean size of lipid particles may be from about 50 nm to about 100 nm, from about 50 nm to about 90 nm, from about 50 nm to about 80 nm, from about 50 nm to about 70 nm, from about 50 nm to about 60 nm, from about 60 nm to about 100 nm, from about 60 nm to about 90 nm, from about 60 nm to about 80 nm, from about 60 nm to about 70 nm, from about 70 nm to about 100 nm, from about 70 nm to about 90 nm, from about 70 nm to about 80 nm, from about 80 nm to about 100 nm, from about 80 nm to about 90 nm, or from about 90 nm to about 100 nm. In some embodiments, the mean size of lipid particles may be from about 70 nm to about 100 nm. In some embodiment, the mean size of lipid particles may be about 80 nm. In some embodiments, the mean size of lipid particles may be about 100 nm.

[0189] Lipid particles may be relatively homogenous. A polydispersity index (PDI) may be used to indicate the homogeneity of lipid particles, e.g., the particle size distribution of the lipid particles. A small (e.g., less than 0.3) PDI generally indicates

a narrow particle size distribution. In some embodiments, the lipid particles of the present disclosure may have a PDI from about 0 to about 0.30, such as 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.20, 0.21, 0.22, 0.23, 0.24, 0.25, 0.26, 0.27, 0.28, 0.29 or 0.30. In some embodiments, the PDI of the lipid particles of the present disclosure may be from about 0.05 to about 0.20.

[0190] The zeta potential of lipid particles may be used to indicate the electrokinetic potential. For example, the zeta potential may describe the surface charge of the lipid particles. Lipid particles with relatively low charges, positive or negative, are generally desirable, as more highly charged species may interact undesirably with cells, tissues, and other elements in the body. In some embodiments, the zeta potential of the lipid particles of the present disclosure may be from about -10 mV to about +25 mV, from about -10 mV to about +20 mV, from about -10 mV to about +15 mV, from about -10 mV to about +10 mV, from about -10 mV to about +5 mV, from about -10 mV to about 0 mV, from about -10 mV to about -5 mV, from about -5 mV to about +25 mV, from about -5 mV to about +20 mV, from about -5 mV to about +15 mV, from about -5 mV to about +10 mV, from about -5 mV to about +5 mV, from about -5 mV to about 0 mV, from about 0 mV to about +25 mV, from about 0 mV to about +20 mV, from about 0 mV to about +15 mV, from about 0 mV to about +10 mV, from about 0 mV to about +5 mV, from about +5 mV to about +25 mV, from about +5 mV to about +20 mV, from about +5 mV to about +15 mV, or from about +5 mV to about +10 mV.

[0191] *Compositions*

[0192] In another aspect, the present disclosure also provides a loaded lipid particle, which refers to a composition comprising one or more lipid particles as discussed above and one or more therapeutic agents. In some embodiments, the pharmaceutical compositions further comprise a pharmaceutically acceptable excipient.

[0193] In some embodiments, the therapeutic agent is encapsulated within an aqueous interior of the lipid particle. In some embodiments, the therapeutic agent is present within one or more lipid layers of the lipid particle. In some embodiments, the therapeutic agent is bound to the exterior or interior lipid surface of the lipid particle.

[0194] As used herein, the term "therapeutic agents" include any molecule or compound capable of exerting a desired effect on a cell, tissue, organ, or subject. Such

effects may be biological, physiological, or cosmetic. Therapeutic agents may be any type of molecule or compound, including but not limited to nucleic acids, peptides and polypeptides, e.g., antibodies, such as polyclonal antibodies, monoclonal antibodies, antibody fragments, humanized antibodies, recombinant antibodies, recombinant human antibodies, and Primatized™ antibodies, cytokines, growth factors, apoptotic factors, differentiation-inducing factors, cell surface receptors and their ligands, hormones, and small molecules, including small organic molecules or compounds. In some embodiments, the therapeutic agent is a nucleic acid.

[0195] As used herein, the term "nucleic acid" is meant to include any oligonucleotide or polynucleotide. Fragments containing up to 50 nucleotides are generally termed oligonucleotides, and longer fragments are called polynucleotides. In some embodiments, oligonucleotides has 10-50 nucleotides in length. In some embodiments, oligonucleotides has 10-40 nucleotides in length. In some embodiments, oligonucleotides has 20-30 nucleotides in length. In some embodiments, oligonucleotides has 20-30 nucleotides in length. In some embodiments, oligonucleotides has 10, 11, 12, 13, 14, 15, 15, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 nucleotides in length.

[0196] As used herein, the terms "polynucleotide" and "oligonucleotide" refer to a polymer or oligomer of nucleotide or nucleoside monomers consisting of naturally occurring bases, sugars and intersugar (backbone) linkages. The terms "polynucleotide" and "oligonucleotide" also includes polymers or oligomers comprising non-naturally occurring monomers, or portions thereof, which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of properties such as, for example, enhanced cellular uptake and increased stability in the presence of nucleases. As used herein, the term "nucleotides" contain a sugar deoxyribose (DNA) or ribose (RNA), a base, and a phosphate group. Nucleotides are linked together through the phosphate groups. "Bases" include purines and pyrimidines, which further include natural compounds adenine, thymine, guanine, cytosine, uracil, inosine, and natural analogs, and synthetic derivatives of purines and pyrimidines, which include, but are not limited to, modifications which place new reactive groups such as, but not limited to, amines, alcohols, thiols, carboxylates, and alkylhalides.

[0197] The nucleic acid that is present in a composition (e.g., a loaded lipid particle comprising a nucleic acid) of the present disclosure includes any form of nucleic acid that is known. The nucleic acids used herein include, but not limited to, single-stranded DNA or RNA, or double-stranded DNA or RNA, and DNA-RNA hybrids. Examples of double-stranded DNA include, but not limited to, structural genes, genes including control and termination regions, and self-replicating systems such as viral or plasmid DNA. Examples of double-stranded RNA include, but not limited to, siRNA and other RNA interference reagents. Single-stranded nucleic acids include, but not limited to, antisense oligonucleotides, ribozymes, microRNA, and triplex-forming oligonucleotides. The nucleic acids used herein also include nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, or non-naturally occurring, and which have similar binding properties as the reference nucleic acid. Examples of such analogs include, but not limited to, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2'-O-methyl ribonucleotides, and peptide-nucleic acids (PNAs). Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), alleles, orthologs, single nucleotide polymorphisms, and complementary sequences as well as the sequence explicitly indicated. The nucleic acid that is present in a composition (e.g., a lipid particle comprising a nucleic acid) of the present disclosure may include one or more modifications.

[0198] Nucleic acids of the present disclosure may be of various lengths, generally dependent upon the particular form of nucleic acid. In some embodiments, the nucleic acid has 10-5000 nucleotides in length. In some embodiments, the nucleic acid has about 4000 nucleotides in length. In some embodiments, the nucleic acid has about 3000 nucleotides in length. In some embodiments, the nucleic acid has about 2500 nucleotides in length. In some embodiments, the nucleic acid has about 2000, 1900, 1800, 1700, 1600, 1500, 1400, 1300, 1200, 1100, 1000, 900, 800, 750, 700, 650, 600, 550, 450, 400, 350, 300, 250, 240, 230, 220, 210, 200, 190, 180, 170, 160, 150, 140, 130, 120, 110, 100, 90, 80, 70, 60, 50, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11 or 10 in length.

[0199] In some embodiments, a therapeutic agent (e.g., nucleic acid) is an RNA. In some embodiments, a therapeutic agent (e.g., nucleic acid) is an DNA. In some embodiments, the therapeutic agent (e.g., nucleic acid) is small interfering RNA (siRNA), microRNA (miRNA), small activating RNAs (saRNA), single guide RNA (sgRNA), messenger RNA (mRNA), self-amplifying RNA, circRNA, antisense oligonucleotide (ASO), ribonucleoproteins (RNP), ssDNA, dsDNA, or any mixtures thereof.

[0200] In certain embodiments, a therapeutic agent is an mRNA. An mRNA may encode any polypeptide of interest, including any naturally or non-naturally occurring or otherwise modified polypeptide. A polypeptide encoded by an mRNA may be of any size and may have any secondary structure or activity. In some embodiments, a polypeptide encoded by an mRNA may be a "therapeutic protein", which refers to a polypeptide has a therapeutic effect when expressed in a cell. For example, a therapeutic protein may be a protein needed to augment or replace a naturally occurring protein of interest. In some embodiments, the polypeptide encoded by an mRNA is an antibody, an antigen, a cytokine, a coagulation factor or an enzyme, or a functional fragment thereof.

[0201] In some embodiments, a mRNA is designed to have 5' untranslated regions (UTR), open reading frame (ORF) and 3' UTR. In some embodiments, a mRNA can be prepared *in vitro* by any means well-known in the art. For example, the mRNA used in the exemplary embodiments disclosed in present can be prepared by using TranscriptAid T7 High Yield Transcription Kit (Thermo K0441). *In vitro* transcription was performed using the TranscriptAid T7 High Yield Transcription Kit, using linear double-strand DNA as a template for generating a mRNA of interest, with addition of a certain proportion of pseudouridine and capping reagents. *In vitro* transcription conditions: the reaction system was configured according to the kit instructions, reaction at 37 ℃ for 0.5-2 hours, the transcript was digested for 30 minutes by DNase, and the transcript was purified with Monarch RNA Cleanup Kit (NEB T2040L).

Table 2. mRNA Sequences of Interest

SEQ ID NO: 1_Fluc mRNA

AUGGAAGAUGCCAAAAACAUUAAGAAGGGCCCAGCGCCAUUCUACCCA
CUCGAAGACGGGACCGCCGGCGAGCAGCUGCACAAAGCCAUGAAGCGC
UACGCCCUGGUGCCCGGCACCAUCGCCUUUACCGACGCACAUAUCGAG
GUGGACAUUACCUACGCCGAGUACUUCGAGAUGAGCGUUCGGCUGGC
AGAAGCUAUGAAGCGCUAUGGGCUGAAUACAAACCAUCGGAUCGUGG
UGUGCAGCGAGAAUAGCUUGCAGUUCUUCAUGCCCGUGUUGGGUGCC
CUGUUCAUCGGUGUGGCUGUGGCCCCAGCUAACGACAUCUACAACGAG
CGCGAGCUGCUGAACAGCAUGGGCAUCAGCCAGCCCACCGUCGUAUUC
GUGAGCAAGAAAGGGCUGCAAAAGAUCCUCAACGUGCAAAAGAAGCU
ACCGAUCAUACAAAAGAUCAUCAUCAUGGAUAGCAAGACCGACUACCA
GGGCUUCCAAAGCAUGUACACCUUCGUGACUUCCCAUUUGCCACCCGG
CUUCAACGAGUACGACUUCGUGCCCGAGAGCUUCGACCGGGACAAAAC
CAUCGCCCUGAUCAUGAACAGUAGUGGCAGUACCGGAUUGCCCAAGGG
CGUAGCCCUACCGCACCGCACCGCUUGUGUCCGAUUCAGUCAUGCCCG
CGACCCCAUCUUCGGCAACCAGAUCAUCCCCGACACCGCUAUCCUCAG
CGUGGUGCCAUUUCACCACGGCUUCGGCAUGUUCACCACGCUGGGCUA
CUUGAUCUGCGGCUUUCGGGUCGUGCUCAUGUACCGCUUCGAGGAGG
AGCUAUUCUUGCGCAGCUUGCAAGACUAUAAGAUUCAAUCUGCCCUGC
UGGUGCCCACACUAUUUAGCUUCUUCGCUAAGAGCACUCUCAUCGACA
AGUACGACCUAAGCAACUUGCACGAGAUCGCCAGCGGCGGGGCGCCGC
UCAGCAAGGAGGUAGGUGAGGCCGUGGCCAAACGCUUCCACCUACCAG
GCAUCCGCCAGGGCUACGGCCUGACAGAAACAACCAGCGCCAUUCUGA
UCACCCCCGAAGGGGACGACAAGCCUGGCGCAGUAGGCAAGGUGGUGC
CCUUCUUCGAGGCUAAGGUGGUGGACUUGGACACCGGUAAGACACUG
GGUGUGAACCAGCGCGGCGAGCUGUGCGUCCGUGGCCCCAUGAUCAUG
AGCGGCUACGUUAACAACCCCGAGGCUACAAACGCUCUCAUCGACAAG
GACGGCUGGCUGCACAGCGGCGACAUCGCCUACUGGGACGAGGACGAG
CACUUCUUCAUCGUGGACCGGCUGAAGAGCCUGAUCAAAUACAAGGGC
UACCAGGUAGCCCCAGCCGAACUGGAGAGCAUCCUGCUGCAACACCCC
AACAUCUUCGACGCCGGGGUCGCCGGCCUGCCCGACGACGAUGCCGGC
GAGCUGCCCGCCGCAGUCGUCGUGCUGGAACACGGUAAAACCAUGACC
GAGAAGGAGAUCGUGGACUAUGUGGCCAGCCAGGUUACAACCGCCAA
GAAGCUGCGCGGUGGUGUUGUGUUCGUGGACGAGGUGCCUAAAGGAC

UGACCGGCAAGUUGGACGCCCGCAAGAUCCGCGAGAUUCUCAUUAAGG CCAAGAAGGGCGGCAAGAUCGCCGUGUAA
SEQ ID NO: 2_hEPO mRNA
AUGGGGGUGCACGAAUGUCCUGCCUGGCUGUGGCUUCUCCUGUCCCUG CUGUCGCUCCCUCUGGGCCUCCCAGUCCUGGGCGCCCCACCACGCCUC AUCUGUGACAGCCGAGUCCUGGAGAGGUACCUCUUGGAGGCCAAGGA GGCCGAGAAUAUCACGACGGGCUGUGCUGAACACUGCAGCUUGAAUG AGAAUAUCACUGUCCCAGACACCAAAGUUAAUUUCUAUGCCUGGAAG AGGAUGGAGGUCGGGCAGCAGGCCGUAGAAGUCUGGCAGGGCCUGGC CCUGCUGUCGGAAGCUGUCCUGCGGGGCCAGGCCCUGUUGGUCAACUC UUCCCAGCCGUGGGAGCCCCUGCAGCUGCAUGUGGAUAAAGCCGUCAG UGGCCUUCGCAGCCUCACCACUCUGCUUCGGGCUCUGGGAGCCCAGAA GGAAGCCAUCUCCCCUCCAGAUGCGGCCUCAGCUGCUCCACUCCGAAC AAUCACUGCUGACACUUUCCGCAAACUCUUCCGAGUCUACUCCAAUUU CCUCCGGGGAAAGCUGAAGCUGUACACAGGGGAGGCCUGCAGGACAG GGGACAGAUGA
SEQ ID NO: 3_CTGF antibody mRNA
AUGGUGAGCCAGGCCCUGAGACUGCUGUGCCUGCUGCUCGGCCUGCAG GGCUGUCUGGCCGAGGGCCAGCUGGUGCAGAGCGGCGGAGGACUCGU GCACCCCGGCGGAUCUCUGAGACUGUCUUGUGCUGGCAGCGGAUUUAC AUUCAGCAGCUACGGCAUGCACUGGGUCAGACAGGCCCCUGGCAAAGG CCUGGAAUGGGUGUCCGGCAUCGGCACCGGCGGCGGCACAUACAGCAC CGACAGCGUGAAGGGCAGAUUCACCAUCAGCCGGGACAACGCCAAGAA UAGCCUGUACCUGCAGAUGAACAGCCUGCGGGCCGAGGAUAUGGCCGU GUACUACUGCGCCAGAGGAGAUUACUAUGGCUCUGGCUCCUUCUUCGA CUGCUGGGGCCAAGGCACCCUGGUGACCGUGUCCAGCGGCGGCGGCGG CAGCGGCGGCGGCGGAUCUGGCGGAGGCGGAAGCGACAUCCAGAUGAC CCAGUCCCCUAGCAGCCUGAGCGCCAGCGUGGGCGACCGGGUGACCAU CACCUGUAGAGCCUCUCAGGGAAUUAGCAGCUGGCUGGCCUGGUAUCA GCAGAAACCUGAGAAGGCCCCUAAGUCCCUGAUCUACGCCGCUUCUAG CCUGCAGAGCGGCGUGCCAAGCAGAUUCAGCGGAUCUGGCAGCGGCAC CGACUUCACCCUGACAAUCAGCUCCCUGCAACCUGAGGAUUUUGCUAC AUACUACUGCCAGCAAUACAACAGCUACCCCCCCACCUUCGGCCAGGG

CACAAAGCUGGAAAUCAAGGAGCCCAAGUCCUGUGAUAAGACCCACAC
AUGCCCUCCAUGUCCUGCUCCUGAGCUGCUGGGCGGACCUUCUGUGUU
UCUGUUCCCCCCCAAGCCUAAGGACACCCUGAUGAUCAGCAGAACCCC
UGAAGUGACCUGCGUGGUCGUGGACGUGUCCCACGAAGAUCCUGAGG
UGAAGUUCAACUGGUAUGUGGAUGGCGUGGAAGUGCACAAUGCUAAA
ACCAAGCCCAGAGAGGAACAGUACAACUCAACAUACAGAGUGGUGUC
UGUUCUCACAGUGCUGCACCAGGACUGGCUGAACGGCAAGGAAUACA
AGUGCAAGGUGUCCAACAAGGCCCUGCCCGCCCCUAUCGAGAAGACAA
UCAGCAAAGCCAAGGGCCAACCUCGGGAGCCUCAGGUGUACACCCUGC
CUCCAAGCAGAGAGGAAAUGACCAAAAACCAGGUCAGCCUGACAUGCC
UGGUGAAAGGCUUCUACCCCAGCGACAUCGCCGUGGAAUGGGAGAGC
AACGGCCAGCCUGAGAACAACUACAAGACAACCCCACCUGUGCUGGAC
AGCGACGGCAGCUUCUUCCUGUACAGCAAGCUGACCGUGGACAAGAGC
CGGUGGCAGCAGGGCAACGUGUUUAGCUGCAGCGUGAUGCACGAGGC
CCUGCAUAAUCACUACACCCAGAAGAGCCUGAGCCUGUCUCCCGGAAA
AUACCCCUACGACGUGCCUGAUUACGCC
SEQ ID NO: 4_RAV-G mRNA
AUGAUCCCUCAGGCCCUGCUGUUCGUGCCCCUGCUGGUGUUUCCUCUG
UGCUUCGGCAAGUUCCCAAUCUACACCAUCCCAGAUAAGCUGGGACCU
UGGAGCCCUAUCGACAUUCACCACCUGAGUUGUCCUAACAACCUGGUG
GUCGAGGACGAGGGCUGUACCAAUCUGAGCGGCUUCUCCUACAUGGA
ACUGAAGGUGGGCUACAUCAGCGCCAUCAAGGUGAACGGCUUCACUU
GCACCGGCGUGGUGACCGAGGCCGAGACAUACACAAAUUUCGUUGGCU
ACGUGACCACCACCUUCAAGAGAAAGCACUUCCGCCCCACCCCUGACG
CCUGUCGGAGCGCUUACAACUGGAAGAUGGCCGGCGAUCCUAGAUACG
AGGAAAGUCUGCACAACCCCUAUCCUGAUUACCACUGGCUGAGAACCG
UGAAGACCACAAAGGAAAGCGUGGUGAUCAUUAGCCCUAGCGUGGCC
GAUCUGGACCCUUACGACAAGUCUCUCCAUAGCAGAGUGUUCCCUAGA
GGCAAGUGCAGCGGCAUAACAGUGAGCUCCGCCUACUGCAGCACCAAU
CACGACUACACCAUCUGGAUGCCUGAGAACCCUAGACUGGGUACAUCU
UGUGAUAUCUUCACAAACAGCAGAGGCAAACGGGCUUCUAAAGGCAG
CAAGACCUGUGGCUUUGUGGACGAGCGGGGACUGUACAAAUCUCUGA
AGGGCGCCUGCAAGCUGAAACUGUGCGGCGUGCUGGGCCUCCGGCUGA

UGGACGGCACCUGGGUCGCCAUCCAGACCAGCAACGAGACAAAGUGGU GCCCCCCCGAUCAGCUGGUGAAUCUGCACGAUUUCCACAGCGACGAAA UCGAGCAUCUGGUGGUGGAAGAACUGGUUAAAAAGCGGGAAGAGUGC CUGGAUGCUCUGGAAAGCAUCAUGACAACCAAAUCCGUGAGCUUCCGG AGGCUGAGCCACCUGAGAAAGCUGGUUCCCGGCUUCGGCAAGGCCUAU ACCAUCUUUAACAAGACACUGAUGGAAGCCGACGCCCACUACAAGAGC GUCCGGACCUGGAACGAGAUCAUCCCUAGCAAGGGCUGCCUGAGAGUG GGCGGAAGAUGCCACCCCCACGUGAACGGCGUGUUUUUCAACGGCAUC AUCCUGGGCCCUGACGGCCACGUGCUGAUCCCUGAGAUGCAGUCCAGC CUGCUGCAGCAGCACAUGGAACUGCUGGAGAGCUCUGUGAUCCCCCUG AUGCACCCUCUGGCCGACCCCAGCACAGUGUUUAAGGACGGCGACGAG GUGGAGGACUUCGUGGAAGUGCACCUGCCUGACGUGCAUAAGCAGGU GAGCGGCGUGGACCUGGGACUGCCAAACUGGGGAAAAGACGUGCUGA UGGGCGCUGGCGUGCUGACCGCCCUGAUGCUGAUGAUCUUCCUGAUGA CAUGCUGCAGAAGAACCAACCGGGCCGAGAGCAUCCAACACAGCCUGG GCGAGACCGGCAGAAAGGUGUCGGUCACCUCUCAGUCUGGAAGAGUG AUCAGCUCUUGGGAGAGCUACAAGAGCGGAGGAGAAACCAAACUGUG AUAA
SEQ ID NO: 5_TROP2 CAR mRNA
AUGGUGUCCCAGGCGCUGCGCCUGCUGUGCCUGCUGCUUGGACUGCAG GGCUGCCUGGCCCAGGUGCAGCUCCAGCAGUCGGGCUCCGAGCUGAAG AAGCCGGGCGCCUCGGUGAAGGUUUCAUGCAAAGCUUCUGGCUACACC UUCACCAACUACGGCAUGAACUGGGUCAAGCAGGCGCCGGGACAGGGC UUGAAAUGGAUGGGCUGGAUUAACACGUACACAGGCGAGCCUACUUA CACCGACGACUUUAAAGGCCGCUUCGCGUUCAGUUUGGACACCUCCGU GUCCACUGCAUACCUGCAAAUUAGCUCUCUGAAGGCGGAUGACACCGC CGUGUAUUUCUGUGCUCGUGGAGGCUUCGGCUCCUCAUACUGGUACU UCGACGUGUGGGGCCAGGGGUCGUUGGUGACCGUAUCCUCUGGGGGU GGCGGAUCCGGCGGGGGCGGUUCUGGAGGCGGUGGUAGCGAUAUCCA GCUAACCCAGAGUCCCUCGUCUCUCAGCGCAUCUGUGGGUGAUCGUGU CAGCAUCACCUGUAAGGCUUCCCAGGAUGUCUCUAUCGCGGUAGCUUG GUACCAGCAGAAGCCCGGGAAAGCCCCCAAGCUGCUUAUAUACUCCGC UAGCUACCGGUACACCGGCGUGCCUGACAGGUUUUCGGGCUCCGGCUC

UGGAACGGACUUCACCCUGACCAUCUCUUCCCUUCAGCCGGAAGAUUU
CGCUGUUUAUUACUGUCAGCAGCACUACAUCACUCCCCUGACCUUCGG
AGCCGGCACCAAGGUGGAGAUCAAGCGCGCCCUUUCAAAUUCCAUCAU
GUAUUUCAGUCACUUUGUCCCGGUGUUCCUGCCAGCGAAGCCAACCAC
CACGCCCGCUCCUCGACCCCCCACACCGGCUCCGACUAUUGCGAGCCA
GCCACUUAGCUUGCGCCCUGAGGCCUGUCGCCCCGCGGCCGGCGGAGC
CGUCCACACGCGCGGUCUGGACAUCUAUAUCUGGGCCCCUCUGGCUGG
UACCUGCGGGGUCCUGUUACUAUCGCUGGUGAUCACUCGAGUGAAAU
UUUCCCGCUCCGCGGACGCACCUGCCUACCAACAGGGGCAGAACCAGC
UCUACAACGAGCUCAACCUAGGGAGAAGGGAGGAGUACGAUGUGCUC
GACAAGCGCCGCGGGCGUGACCCGGAGAUGGGCGGCAAGCCCCGGCGU
AAGAAUCCUCAGGAGGGCCUCUACAACGAGCUGCAGAAGGACAAGAU
GGCCGAGGCCUACAGCGAAAUCGGUAUGAAGGGCGAGCGCCGGCGCGG
UAAGGGCCACGACGGCCUGUAUCAGGGUCUGUCCACCGCCACUAAGGA
CACCUACGACGCUCUGCAUAUGCAGGCCCUGCCACCCCGCUAGUAG
SEQ ID NO: 6_tdTomato mRNA
AUGGUGAGCAAGGGCGAGGAGGUCAUCAAAGAGUUCAUGCGCUUCAA
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GCGAGGGCGAGGGCCGCCCCUACGAGGGCACCCAGACCGCCAAGCUGA
AGGUGACCAAGGGCGGCCCCCUGCCCUUCGCCUGGGACAUCCUGUCCC
CCCAGUUCAUGUACGGCUCCAAGGCGUACGUGAAGCACCCCGCCGACA
UCCCCGAUUACAAGAAGCUGUCCUUCCCCGAGGGCUUCAAGUGGGAGC
GCGUGAUGAACUUCGAGGACGGCGGUCUGGUGACCGUGACCCAGGAC
UCCUCCCUGCAGGACGGCACGCUGAUCUACAAGGUGAAGAUGCGCGGC
ACCAACUUCCCCCCCGACGGCCCCGUAAUGCAGAAGAAGACCAUGGGC
UGGGAGGCCUCCACCGAGCGCCUGUACCCCCGCGACGGCGUGCUGAAG
GGCGAGAUCCACCAGGCCCUGAAGCUGAAGGACGGCGGCCACUACCUG
GUGGAGUUCAAGACCAUCUACAUGGCCAAGAAGCCCGUGCAACUGCCC
GGCUACUACUACGUGGACACCAAGCUGGACAUCACCUCCCACAACGAG
GACUACACCAUCGUGGAACAGUACGAGCGCUCCGAGGGCCGCCACCAC
CUGUUCCUGGGGCAUGGCACCGGCAGCACCGGCAGCGGCAGCUCCGGC
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AUCGAGGGCGAGGGCGAGGGCCGCCCCUACGAGGGCACCCAGACCGCC
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GCCGACAUCCCCGAUUACAAGAAGCUGUCCUUCCCCGAGGGCUUCAAG
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CCAGGACUCCUCCCUGCAGGACGGCACGCUGAUCUACAAGGUGAAGAU
GCGCGGCACCAACUUCCCCCCCGACGGCCCCGUAAUGCAGAAGAAGAC
CAUGGGCUGGGAGGCCUCCACCGAGCGCCUGUACCCCCGCGACGGCGU
GCUGAAGGGCGAGAUCCACCAGGCCCUGAAGCUGAAGGACGGCGGCCA
CUACCUGGUGGAGUUCAAGACCAUCUACAUGGCCAAGAAGCCCGUGCA
ACUGCCCGGCUACUACUACGUGGACACCAAGCUGGACAUCACCUCCCA
CAACGAGGACUACACCAUCGUGGAACAGUACGAGCGCUCCGAGGGCCG
CCACCACCUGUUCCUGUACGGCAUGGACGAGCUGUACAAGUAG

Table 3. UTRs

UTR	Sequence
SEQ ID NO: 7 (5'UTR-1)	ACAUUUGCUUCUGACACAACUGUGUUCACUAGCAAC CUCAAACAGACACC
SEQ ID NO: 8 (5'UTR-2)	ACCGCCGAGACCGCGUCCGCCCCGCGAGCACAGAGCC UCGCCUUUGCCGAUCCGCCGCCCGUCCACACCCGCCG CCAGCUCACC
SEQ ID NO: 9 (5'UTR-3)	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUA UAAGAGCCACC
SEQ ID NO: 10 (3'UTR-1)	GCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGG GCCUCCCCCCCAGCCCCUCCUCCCCUUCCUGCACCCGU ACCCCCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGG CA
SEQ ID NO: 11 (3'UTR-2)	GCUCGCUUUCUUGCUGUCCAAUUUCUAUUAAAGGUU CCUUUGUUCCCUAAGUCCAACUACUAAACUGGGGGGA UAUUAUGAAGGGCCUUGAGCAUCUGGAUUCUGCCUA AUAAAAAACAUUUAUUUUCAUUGCAAUUGCCAUGUG

	UAUGUGGGUUCGCCCACAUACUCUGAUGAUCCCCAA UCGUGGCGUGUCGGCCUGCUUCGGCAGGCACUGGCG CCGGGAUCAUUCAUGGCAA
SEQ ID NO: 12 (3'UTR-3)	GCUCGCUUUCUUGCUGUCCAAUUUCUAUUAAAGGUU CCUUUGUUCCCUAAGUCCAACUACUAAACUGGGGGGA UAUUAUGAAGGGCCUUGAGCAUCUGGAUUCUGCCUA AUAAAAAACAUUUAUUUUCAUUGC
SEQ ID NO: 13 (3'UTR-4)	GCUCGCUUUCUUGCUGUCCAAUUUCUAUUAAAGGUU CCUUUGUUCCCUAAGUCCAACUACUAAACUGGGGGGA UAUUAUGAAGGGCCUUGAGCAUCUGGAUUCUGCCUA AUAAAAAACAUUUAUUUUCAUUGCGCUCGCUUUCUU GCUGUCCAAUUUCUAUUAAAGGUUCCUUUGUUCCCU AAGUCCAACUACUAAACUGGGGGAUAUUAUGAAGGG CCUUGAGCAUCUGGAUUCUGCCUAAUAAAAAACAUU UAUUUUCAUUGC

[0202] In some embodiments, a therapeutic agent is an siRNA. An siRNA may be capable of selectively knocking down or down regulating expression of a gene of interest. For example, an siRNA could be selected to silence a gene associated with a particular disease, disorder, or condition upon administration to a subject in need thereof of a lipid particle composition including the siRNA. An siRNA may comprise a sequence that is complementary to an mRNA sequence that encodes a gene or protein of interest. In some embodiments, the siRNA may be an immunomodulatory siRNA.

[0203] In some embodiments, a therapeutic agent is a vaccine, a compound (e.g., a nucleic acid that encodes a polypeptide, or a polypeptide) that elicits an immune response. Vaccines include compounds and preparations that are capable of providing immunity against one or more conditions related to infectious diseases and can include mRNAs encoding infectious disease derived antigens and/or epitopes. Vaccines also include compounds and preparations that direct an immune response against cancer cells and can include mRNAs encoding tumor cell derived antigens, epitopes, and/or neoepitopes.

[0204] The "efficiency of encapsulation" of a therapeutic agent refers to the amount of therapeutic agent that is encapsulated or otherwise associated with a lipid

particle after preparation, relative to the initial amount provided. The encapsulation efficiency is desirably high (e.g., more than 80%, more than 85%, more than 90% or more than 95%). The encapsulation efficiency may be measured, for example, by comparing the amount of therapeutic agent in a solution containing the lipid particle before and after breaking up the lipid particle with one or more organic solvents or detergents. Fluorescence may be used to measure the amount of free therapeutic agent (e.g, RNA) in a solution. In some embodiments, the encapsulation efficiency of a therapeutic agent may be at least 50%, for example, at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%. In some embodiments, the encapsulation efficiency may be at least 80%. In certain embodiments, the encapsulation efficiency may be at least 90%.

[0205] As used herein, "encapsulation", "encapsulated", "encapsulating" and "loaded", and "associated" may refer to complete, substantial, or partial enclosure, confinement, surrounding, or encasement. As used herein, "encapsulation" or "association" may refer to the process of confining an individual nucleic acid within a lipid particle and/or establishing a physiochemical relationship between an individual nucleic acid and a lipid particle.

[0206] Nucleic acids and polynucleotides may include one or more alternative components, as described herein, which impart useful properties including increased stability and/or the lack of a substantial induction of the innate immune response of a cell into which the polynucleotide is introduced. For example, an alternative polynucleotide or nucleic acid exhibits reduced degradation in a cell into which the polynucleotide or nucleic acid is introduced, relative to a corresponding unaltered polynucleotide or nucleic acid. These alternative species may enhance the efficiency of protein production, intracellular retention of the polynucleotides, and/or viability of contacted cells, as well as possess reduced immunogenicity.

[0207] The amount of a therapeutic agent in lipid particles may depend on the size, composition, desired target and/or application, or other properties of the lipid particles as well as on the properties of the therapeutic agent. For example, the amount of an RNA useful in a lipid particle may depend on the size, sequence, and other characteristics of the RNA. The relative amounts of a therapeutic agent and other elements (e.g., lipids) in a lipid particle may also vary. In some embodiments, the mass ratio of the lipids (e.g., cationic lipid, neutral lipid, structural lipid such as sterol

and a lipid selected to reduce aggregation such as a surfactant) to a therapeutic agent (e.g., RNA) in a loaded lipid particle may be from about 5:1 to about 60:1, such as about 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, and 60:1. For example, the mass ratio of lipids to a therapeutic agent may be from about 10:1 to about 50:1. In some embodiments, the mass ratio of lipids to a therapeutic agent is about 40:1. In some embodiments, the mass ratio of lipids to a therapeutic agent is about 20:1. In some embodiments, the mass ratio of lipids to a therapeutic agents is calculated by total mass of cationic lipid, neutral lipid, structural lipid such as sterol and a surfactant without solvents divided by mass of a dry therapeutic agent (e.g., RNA). The amount of a therapeutic agent in a lipid particle composition may, for example, be measured using absorption spectroscopy (e.g., ultraviolet-visible spectroscopy).

[0208] In another aspect, the present disclosure also provides a pharmaceutical composition comprising the loaded lipid particles provided herein and a pharmaceutically acceptable excipient.

[0209] The pharmaceutically acceptable excipient are conventional medicinal excipients in the art which can be prepared in a manner well known in the pharmaceutical art. Some examples of materials which can serve as pharmaceutically acceptable excipient or carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) alcohol, such as ethyl alcohol and propane alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations such as acetone.

[0210] The pharmaceutical compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for

example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like.

[0211] The form of pharmaceutical compositions depends on a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

[0212] The pharmaceutical compositions can be formulated for oral, nasal, rectal, percutaneous, intravenous, or intramuscular administration. In accordance to the desired route of administration, the pharmaceutical compositions can be formulated in the form of tablets, capsule, pill, dragee, powder, granule, sachets, cachets, lozenges, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), spray, ointment, paste, cream, lotion, gel, patches, inhalant, or suppository.

[0213] The pharmaceutical compositions can be formulated to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art. In some embodiments, the pharmaceutical composition is formulated in a sustained released form. In some embodiments, the prolonged period of time can be about 1 hour to 24 hours, 2 hours to 12 hours, 3 hours to 8 hours, 4 hours to 6 hours, 1 to 2 days or more. In certain embodiments, the prolonged period of time is at least about 4 hours, at least about 8 hours, at least about 12 hours, or at least about 24 hours. The pharmaceutical composition can be formulated in the form of tablet. For example, release rate of the active agent can not only be controlled by dissolution of the active agent in gastrointestinal fluid and subsequent diffusion out of the tablet or pills independent of pH, but can also be influenced by physical processes of disintegration and erosion of the tablet. In some embodiments, polymeric materials as disclosed in "Medical Applications of Controlled Release," Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); "Controlled Drug Bioavailability," Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, J Macromol. Sci. Rev. Macromol Chem. 23:61; see also Levy et al., 1985, Science 228:190; During et al., 1989, Ann. Neurol. 25:351; Howard et al., 1989, J. Neurosurg. 71:105 can be used for sustained release. The above references are incorporated herein by reference in their entirety.

[0214] In certain embodiments, the pharmaceutical compositions of present disclosure may be administrated at dosage level sufficient to deliver from about 0.0001 mg/kg to about 10 mg/kg (e.g. from about 0.0001 mg/kg to about 10 mg/kg, from about

0.001 mg/kg to about 10 mg/kg, from about 0.005 mg/kg to about 10 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.05 mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, from about 1 mg/kg to about 10 mg/kg, from about 2 mg/kg to about 10 mg/kg, from about 5 mg/kg to about 10 mg/kg, from about 0.0001 mg/kg to about 5 mg/kg, from about 0.001 mg/kg to about 5 mg/kg, from about 0.005 mg/kg to about 5 mg/kg, from about 0.01 mg/kg to about 5 mg/kg, from about 0.05 mg/kg to about 5 mg/kg, from about 0.1 mg/kg to about 5 mg/kg, from about 1 mg/kg to about 5 mg/kg, from about 2 mg/kg to about 5 mg/kg, from about 0.0001 mg/kg to about 2.5 mg/kg, from about 0.001 mg/kg to about 2.5 mg/kg, from about 0.005 mg/kg to about 2.5 mg/kg, from about 0.01 mg/kg to about 2.5 mg/kg, from about 0.05 mg/kg to about 2.5 mg/kg, from about 0.1 mg/kg to about 2.5 mg/kg, from about 1 mg/kg to about 2.5 mg/kg, from about 2 mg/kg to about 2.5 mg/kg, from about 0.0001 mg/kg to about 1 mg/kg, from about 0.001 mg/kg to about 1 mg/kg, from about 0.005 mg/kg to about 1 mg/kg, from about 0.01 mg/kg to about 1 mg/kg, from about 0.05 mg/kg to about 1 mg/kg, from about 0.1 mg/kg to about 1 mg/kg, from about 0.0001 mg/kg to about 0.25 mg/kg, from about 0.001 mg/kg to about 0.25 mg/kg, from about 0.005 mg/kg to about 0.25 mg/kg, from about 0.01 mg/kg to about 0.25 mg/kg, from about 0.05 mg/kg to about 0.25 mg/kg, or from about 0.1 mg/kg to about 0.25 mg/kg) of the therapeutical agent in a given dose, wherein a dose of 1 mg/kg provides 1 mg of a therapeutic and/or prophylactic per 1 kg of subject body weight.

[0215] In certain embodiments, the pharmaceutical compositions can be formulated in a unit dosage form. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier. In some embodiments, a dose of about 0.001 mg/kg to about 10 mg/kg of a therapeutical agent may be administered. In other embodiments, a dose of about 0.005 mg/kg to about 2.5 mg/kg of a therapeutical agent may be administered. In certain embodiments, a dose of about 0.1 mg/kg to about 1 mg/kg a therapeutical agent may be administered. In other embodiments, a dose of about 0.05 mg/kg to about 0.25 mg/kg a therapeutical agent may be administered. A dose may be administered one or more times per day, in the same or a different amount, to obtain a desired level of therapeutic, diagnostic, prophylactic, or imaging effect. The desired dosage may be delivered, for example,

three times a day, two times a day, once a day, every other day, every third day, every week, every two weeks, every three weeks, or every four weeks.

[0216] In some embodiments, the loaded lipid particle comprising therapeutic agents or the pharmaceutical composition thereof is useful in development of nucleic acid vaccine, protein replacement therapies, nucleic acid immunotherapy, and the like. In some embodiments, the loaded lipid particle comprising therapeutic agents or the pharmaceutical composition thereof is useful in treating a disease or disorder. In some embodiments, the loaded lipid particle comprising therapeutic agents or the pharmaceutical composition thereof is useful in the manufacture of a medicament for a treatment of a disease or disorder.

[0217] *Use of Lipid Particles*

[0218] In another aspect, the present disclosure provides a method of delivering a therapeutic agent to a subject. In some embodiments, the method comprises the step of:

i) encapsulating a therapeutic agent into the lipid particle of the present disclosure; and ii) administering the lipid particle encapsulating the therapeutic agent to the subject.

[0219] In another aspect, the present disclosure provides a method of delivering a therapeutic agent to a subject. In some embodiments, the method comprises administering to the subject the loaded lipid particle of the present disclosure or the pharmaceutical composition comprising the loaded lipid particle.

[0220] In some embodiments, the therapeutic agent is delivered to cells in the subject, wherein the cells are selected from the group consisting of muscle cells, lung cells, liver cells, spleen cells, kidney cells, heart cells, skin cells, hair cells, nail cells, bone cells, artery cells, vein cells, diaphragm cells, larynx cells, stomach cells, intestine cells, ureter cells, bladder cells, lymph node cells, bone marrow cells, thymus cells, brain cells, spinal cord cells, nerve cells, pituitary gland cells, thyroid cells, adrenal cells, penis cells, vagina cells, prostate cells and uterus cells. In some embodiments, the therapeutic agent is delivered to cells in the subject, wherein the cells are selected from the group consisting of liver cells, lung cells, spleen cells, muscle cells and brain cells.

[0221] In another aspect, the present disclosure provides a method of treating a disease or disorder in a subject comprising administering to the subject an effective

amount of the loaded lipid particle of the present disclosure, or the pharmaceutical composition comprising the loaded lipid particle.

[0222] In another aspect, the present disclosure provides use of the lipid particle of the present disclosure, the loaded lipid particle of the present disclosure or the pharmaceutical composition comprising the loaded lipid particle for the manufacture of a medicament for a treatment of a disease or disorder.

[0223] In another aspect, the present disclosure provides a method of expressing a protein in a subject. In some embodiments, the method comprises:

i) encapsulating a polynucleotide encoding the protein into the lipid particle of the present disclosure; and

ii) administering the lipid particle encapsulating the polynucleotide to the subject.

[0224] In some embodiments, the protein is an antibody, an antigen, a cytokine, a coagulation factor or an enzyme, or a functional fragment thereof.

[0225] As used herein, "expression" of a nucleic acid sequence refers to translation of a nucleic acid (e.g., an mRNA) into a polypeptide or protein and/or post-translational modification of a polypeptide or protein.

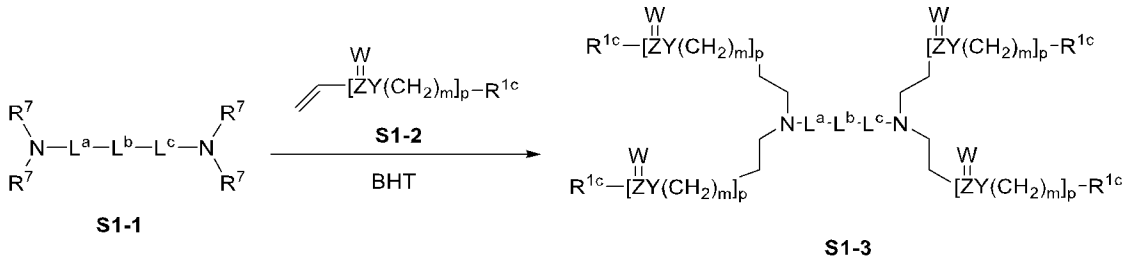
[0226] **EXAMPLES**

[0227] For the purpose of illustration, the following examples are included. However, it is to be understood that these examples do not limit the invention and are only meant to suggest a method of practicing the present disclosure. Persons skilled in the art will recognize that the chemical reactions described may be readily adapted to prepare a number of other compounds of the present disclosure, and alternative methods for preparing the compounds of the present disclosure are deemed to be within the scope of the present disclosure. For example, the synthesis of non-exemplified compounds according to the present disclosure may be successfully performed by modifications apparent to those skilled in the art, e.g., by appropriately protecting interfering groups, by utilizing other suitable reagents known in the art other than those described, and/or by making routine modifications of reaction conditions. Alternatively, other reactions disclosed herein or known in the art will be recognized as having applicability for preparing other compounds of the present disclosure.

[0228] For illustrative purposes, the following shows general synthetic schemes for preparing the compounds of the present disclosure as well as key intermediates. Those skilled in the art will appreciate that other synthetic schemes may be used to

synthesize the inventive compounds. Although specific starting materials and reagents are depicted in the General Schemes and discussed below, other starting materials and reagents can be easily substituted to provide a variety of derivatives and/or reaction conditions. In addition, many of the compounds prepared by the methods described below can be further modified in light of this disclosure using conventional chemistry well known to those skilled in the art.

[0229] Example 1: General Scheme 1



wherein

each W is independently selected from O, S and NH;

each Y is independently selected from O, S and NH;

each n is independently 1, 2 or 3;

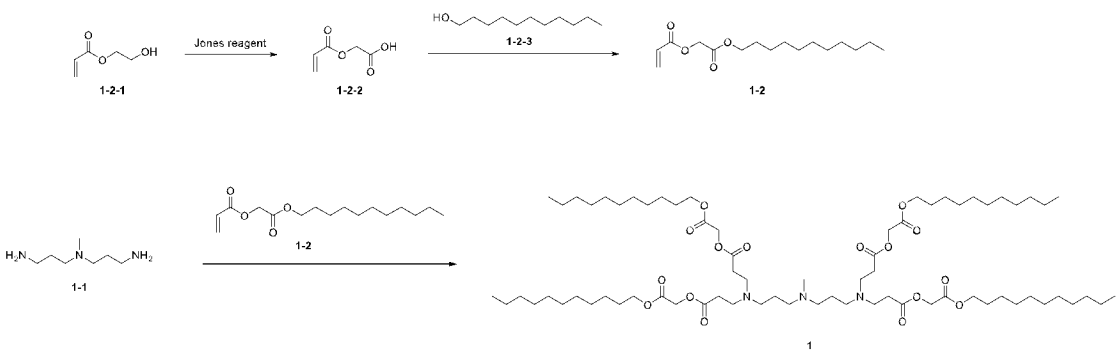
each m is 0 or 1;

each p is 1 or 2.

[0230] *General Procedure*

[0231] A mixture of compound **S1-1**, **S1-2**, 2,6-di-*tert*-butyl-4-methylphenol (BHT), acetonitrile (ACN) and/or acetic acid was added into a sealed container. The mixture was heated at 60-80 ℃ for 18-36 hours. Then the mixture was cooled to room temperature and purified by column chromatography to afford desired lipid.

[0232] Example 1.1: Synthesis of Compound 1



[0233] **Step 1:** to a solution of compound **1-2-1** (11.6 g, 100 mmol) in acetone (500 mL) was added Jones reagent (20 g) under stirring in an ice bath. The mixture was allowed to warm to room temperature and kept at the same temperature for 3 hours. Then BHT (220 mg, 1.0 mmol) were added. The mixture was concentrated and extracted with ethyl ether. The compound **1-2-2** was used directly without further purification.

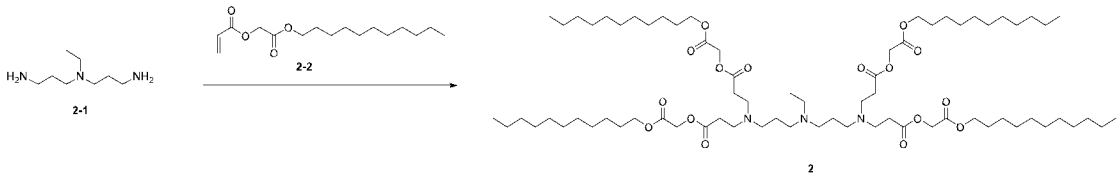
[0234] **Step 2:** to a solution of compound **1-2-2** (1.3 g, 10 mmol), compound **1-2-3** (1.7 g, 10 mmol) and 4-methylmorpholine (1.2 g, 10.5 mmol) in DCM (100 mL) were added dicyclohexylcarbodiimide (DCC, 2.1 g, 10 mmol) and 4-dimethylaminopyridine (DMAP, 0.12 g, 1 mmol) under stirring at room temperature. The mixture was stirred overnight and then filtered. To the filtrate was added BHT (22 mg, 0.1 mmol). The mixture was concentrated to afford crude product. The crude product was purified by column chromatography on silica (eluent: hexane with 0-10% ethyl acetate, v/v) and concentrated under reduced pressure to afford compound **1-2** as a colorless oil (1.8 g, 60% yield).

[0235] ^{1}H NMR of compound **1-2**: (400 MHz, Chloroform-d) δ 6.50 (d, J = 17.3 Hz, 1H), 6.21 (dd, J = 17.4, 10.5 Hz, 1H), 5.92 (d, J = 10.5 Hz, 1H), 4.68 (s, 2H), 4.16 (t, J = 6.7 Hz, 2H), 1.63 (dd, J = 7.4, 6.6 Hz, 3H), 1.27 (d, J = 19.2 Hz, 18H), 0.87 (t, J = 6.8 Hz, 3H).

[0236] **Step 3:** to a solution of compound **1-2** (280 mg, 1 mmol, with BHT) in ACN (2 mL) compound **1-1** (36 mg, 0.25 mmol) and acetic acid (1 drop) were added. The mixture was refluxed for 48 hours, until thin layer chromatography (TLC) indicated completion of reaction. The mixture was diluted with dichloromethane (DCM) (10 mL) and concentrated under reduced pressure to afford crude product. The crude product was purified by column chromatography on silica (eluent: DCM with 0-10% methanol, v/v) and concentrated under reduced pressure to afford compound **1** as a colorless oil (188 mg, 60% yield).

[0237] ^{1}H NMR of compound **1**: (400 MHz, Chloroform-d) δ 4.60 (s, 8H), 4.14 (t, J = 6.8 Hz, 8H), 2.80 (t, J = 7.3 Hz, 8H), 2.55 (t, J = 7.0 Hz, 8H), 2.52 – 2.15 (m, 8H), 1.63 (p, J = 6.9 Hz, 11H), 1.34 – 1.23 (m, 68H), 0.88 (t, J = 6.7 Hz, 12H).

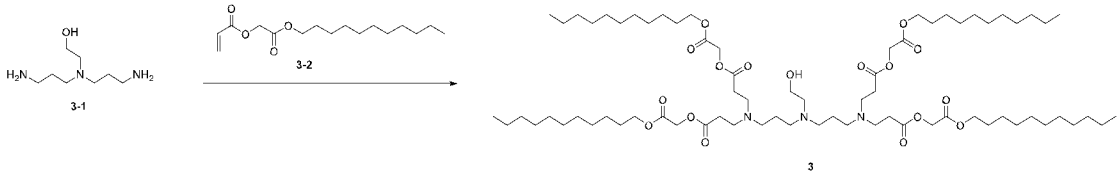
[0238] **Example 1.2: Synthesis of Compound 2**



[0239] To a solution of compound **2-2** (280 mg, 1 mmol, with BHT, prepared by the method of Example 1.1 Step 1 and 2) in ACN (2 mL) compound **2-1** (40 mg, 0.25 mmol) and acetic acid (1 drop) were added. The mixture was refluxed for 48 hours, until TLC indicated completion of reaction. The mixture was diluted with DCM (10 mL) and concentrated under reduced pressure to afford crude product. The crude product was purified by column chromatography on silica (eluent: DCM with 0-10% methanol, v/v) and concentrated under reduced pressure to afford compound **2** as a colorless oil (207 mg, 65% yield).

[0240] ^{1}H NMR of compound **2**: (400 MHz, Chloroform-d) δ 4.60 (s, 8H), 4.14 (t, J = 6.8 Hz, 8H), 2.79 (d, J = 7.1 Hz, 8H), 2.68 – 2.27 (m, 16H), 1.63 (t, J = 7.0 Hz, 12H), 1.28 (d, J = 16.5 Hz, 74H), 0.88 (t, J = 6.7 Hz, 14H).

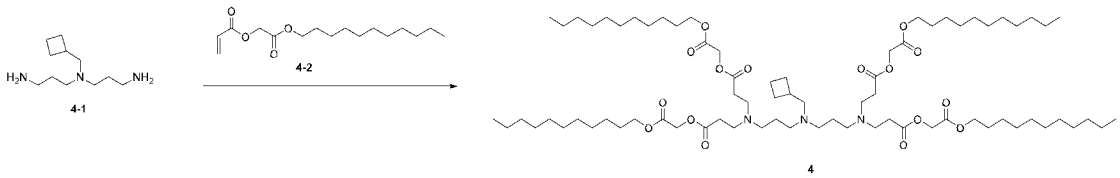
[0241] **Example 1.3: Synthesis of Compound 3**



[0242] To a solution of compound **3-2** (280 mg, 1 mmol, with BHT, prepared by the method of Example 1.1 Step 1 and 2) in ACN (2 mL) compound **3-1** (44 mg, 0.25 mmol) and acetic acid (1 drop) were added. The mixture was refluxed for 48 hours, until TLC indicated completion of reaction. The mixture was diluted with DCM (10 mL) and concentrated under reduced pressure to afford crude product. The crude product was purified by column chromatography on silica (eluent: DCM with 0-10% methanol, v/v) and concentrated under reduced pressure to afford compound **3** as a colorless oil (190 mg, 60% yield).

[0243] ^{1}H NMR of compound **3**: (400 MHz, Chloroform-d) δ 4.53 (s, 8H), 4.07 (t, J = 6.8 Hz, 9H), 2.73 (t, J = 6.9 Hz, 9H), 2.49 (t, J = 6.9 Hz, 10H), 2.40 (dt, J = 19.4, 6.8 Hz, 7H), 1.57 (q, J = 7.0 Hz, 12H), 1.20 (d, J = 8.0 Hz, 79H), 0.81 (t, J = 6.7 Hz, 15H).

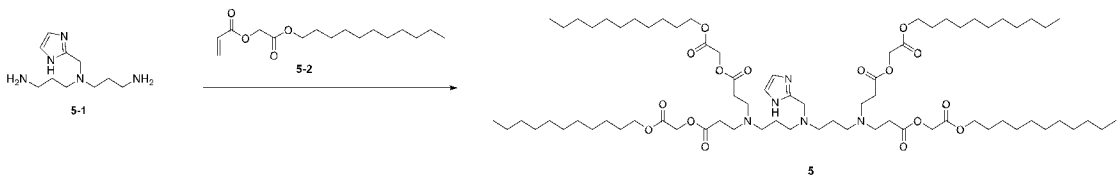
[0244] **Example 1.4: Synthesis of Compound 4**



[0245] To a solution of compound **4-2** (280 mg, 1 mmol, with BHT, prepared by the method of Example 1.1 Step 1 and 2) in ACN (2 mL) compound **4-1** (50 mg, 0.25 mmol) and acetic acid (1 drop) were added. The mixture was refluxed for 48 hours, until TLC indicated completion of reaction. The mixture was diluted with DCM (10 mL) and concentrated under reduced pressure to afford crude product. The crude product was purified by column chromatography on silica (eluent: DCM with 0-10% methanol, v/v) and concentrated under reduced pressure to afford compound **4** as a colorless oil (180 mg, 55% yield).

[0246] ^{1}H NMR of compound **4**: (400 MHz, Chloroform-d) δ 4.60 (s, 8H), 4.14 (t, J = 6.8 Hz, 8H), 2.98 (s, 2H), 2.79 (p, J = 7.0 Hz, 8H), 2.55 (t, J = 6.8 Hz, 9H), 2.38 (dt, J = 32.7, 7.2 Hz, 7H), 1.99 (s, 3H), 1.63 (p, J = 6.9 Hz, 12H), 1.28 (d, J = 16.2 Hz, 70H), 0.88 (t, J = 6.7 Hz, 13H).

[0247] **Example 1.5: Synthesis of Compound 5**

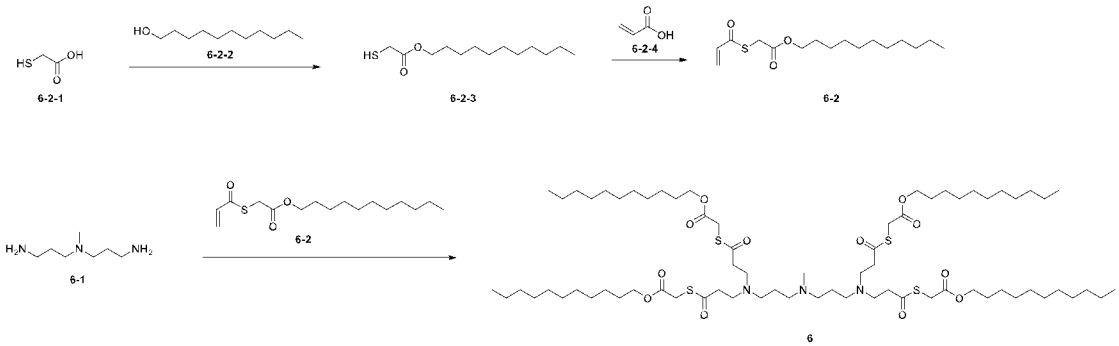


[0248] To a solution of compound **5-2** (280 mg, 1 mmol, with BHT, prepared by the method of Example 1.1 Step 1 and 2) in ACN (2 mL) compound **5-1** (53 mg, 0.25 mmol) and acetic acid (1 drop) were added. The mixture was refluxed for 48 hours, until TLC indicated completion of reaction. The mixture was diluted with DCM (10 mL) and concentrated under reduced pressure to afford crude product. The crude product was purified by column chromatography on silica (eluent: DCM with 0-10% methanol, v/v) and concentrated under reduced pressure to afford compound **5** as a colorless oil (166 mg, 50% yield).

[0249] ^{1}H NMR of compound **5**: (400 MHz, Chloroform-d) δ 6.96 (s, 1H), 6.91 (s, 1H), 4.60 (d, J = 6.9 Hz, 8H), 4.38 (t, J = 6.8 Hz, 1H), 4.20 (t, J = 6.8 Hz, 1H), 4.13

(t, J = 6.8 Hz, 9H), 3.66 (d, J = 10.7 Hz, 2H), 2.95 (t, J = 6.7 Hz, 2H), 2.78 (t, J = 7.3 Hz, 6H), 2.52 (t, J = 7.2 Hz, 6H), 2.39 (q, J = 10.6, 7.8 Hz, 6H), 1.63 (p, J = 6.9 Hz, 12H), 1.35 – 1.24 (m, 72H), 0.88 (t, J = 6.7 Hz, 14H).

[0250] Example 1.6: Synthesis of Compound 6



[0251] **Step 1:** A solution of compound **6-2-1** (0.92 g, 10 mmol), compound **6-2-2** (1.72 g, 10 mmol) and sulfuric acid (100 mg, 98%) in toluene (100 mL) were heated at 150 ℃ under N_2 atmosphere. The mixture was refluxed in Dean–Stark apparatus for 1.5 hours. The mixture was cooled to room temperature and washed with saturated aqueous $NaHCO_3$ solution twice followed by brine. The aqueous layer was extracted with DCM. The combined organic layer was concentrated to afford crude compound **6-2-3** and was used directly without further purification.

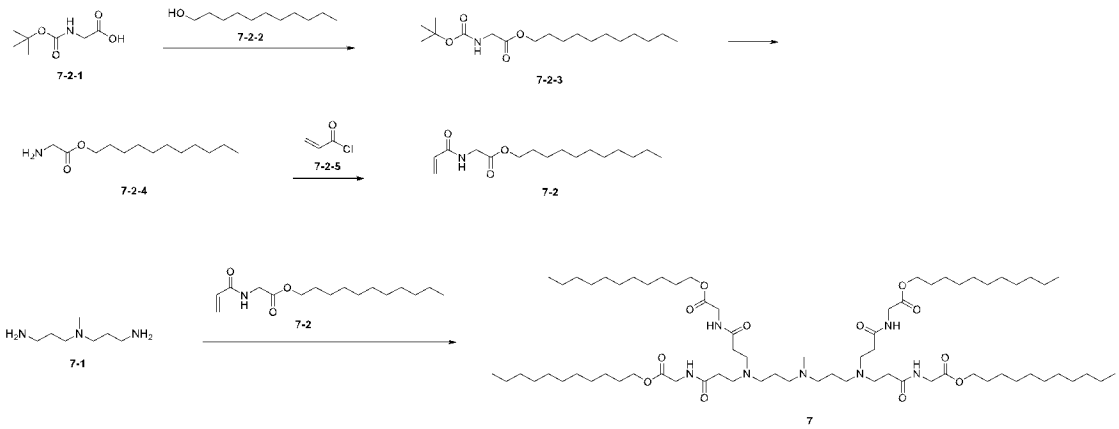
[0252] **Step 2:** to a solution of compound **6-2-3** (1.23 g, 5 mmol), compound **6-2-4** (0.36 g, 5 mmol) and 4-methylmorpholine (0.6 g, 5.03 mmol) in DCM (50 mL) were added DCC (1.05 g, 5 mmol) and DMAP (0.06 g, 0.5 mmol) under stirring at room temperature. The mixture was stirred overnight and then filtered. To the filtrate was added BHT (11 mg, 0.05 mmol). The mixture was concentrated to afford crude product. The crude product was purified by column chromatography on silica (eluent: hexane with 0-10% ethyl acetate, v/v) and concentrated under reduced pressure to afford compound **6-2** as a colorless oil (0.7 g, 48% yield).

[0253] **Step 3**: To a solution of compound **6-2** (300 mg, 1 mmol, with BHT) in ACN (2 mL) compound **6-1** (36 mg, 0.25 mmol) was added. The mixture was refluxed for 48 hours, until TLC indicated completion of reaction. The mixture was diluted with DCM (10 mL) and concentrated under reduced pressure to afford crude product. The crude product was purified by column chromatography on silica (eluent: DCM with 0-

10% methanol, v/v) and concentrated under reduced pressure to afford compound **6** as a colorless oil (151 mg, 45% yield).

[0254] ^{1}H NMR of compound **6**: (400 MHz, Chloroform-d) δ 4.10 (t, J = 6.9 Hz, 8H), 3.70 (s, 6H), 3.23 (s, 1H), 2.95 (s, 2H), 2.84 – 2.68 (m, 12H), 2.50 (q, J = 7.5, 6.9 Hz, 4H), 1.63 (t, J = 7.0 Hz, 9H), 1.36 – 1.19 (m, 75H), 0.91 – 0.75 (m, 15H).

[0255] **Example 1.7: Synthesis of Compound 7**



[0256] **Step 1:** to a solution of compound **7-2-1** (10 g, 57.1 mmol) and compound **7-2-2** (10.3 g, 59.9 mmol) in DCM (100 mL) were added DMAP (698 mg, 5.7 mmol). To the mixture was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 21.9 g, 114.2 mmol) in portions under N_2 atmosphere at <10 ℃. The mixture was allowed to warm to room temperature and stirred at the same temperature for 16 hours. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (100 mL). The aqueous layer was extracted with ethyl acetate (200 mL). The combined organic layer was washed with 5% aqueous citric acid solution (100 mL) and brine (100 mL), followed by brine (100 mL). The organic layer was dried over Na_2SO_4 and concentrated to afford 18.5 g crude compound **7-2-3**. The compound **7-2-3** was used directly without further purification.

[0257] ^{1}H NMR of compound **7-2-3**: (400 MHz, DMSO-d6) δ 7.15 (t, J = 6.2 Hz, 1H), 4.02 (t, J = 6.5 Hz, 2H), 3.64 (d, J = 6.2 Hz, 2H), 1.55 (t, J = 6.9 Hz, 2H), 1.38 (s, 8H), 1.34 (s, 2H), 1.24 (s, 19H), 0.85 (t, J = 6.5 Hz, 4H).

[0258] **Step 2:** to a solution of compound **7-2-3** (18.5 g, 56.1 mmol, crude) in ethyl acetate (93 mL) was added HCl (4 M in ethyl acetate, 93 mL) under N_2 atmosphere at <10 ℃. The mixture was allowed to warm to room temperature and stirred at the same temperature for 16 hours. TLC indicated the consumption of starting

materials. The mixture was cooled to 5 ℃ and stirred at the same temperature for 2 hours. The mixture was filtrated, and the residue was washed with ethyl acetate and dried to afford 12.5 g crude compound **7-2-4**. The compound **7-2-4** was used directly without further purification.

[0259] ^{1}H NMR of compound **7-2-4**: (400 MHz, DMSO-d6) δ 8.63 (s, 3H), 4.11 (t, J = 6.6 Hz, 2H), 3.73 (s, 2H), 1.57 (p, J = 6.7 Hz, 2H), 1.23 (s, 17H), 0.84 (t, J = 6.6 Hz, 3H).

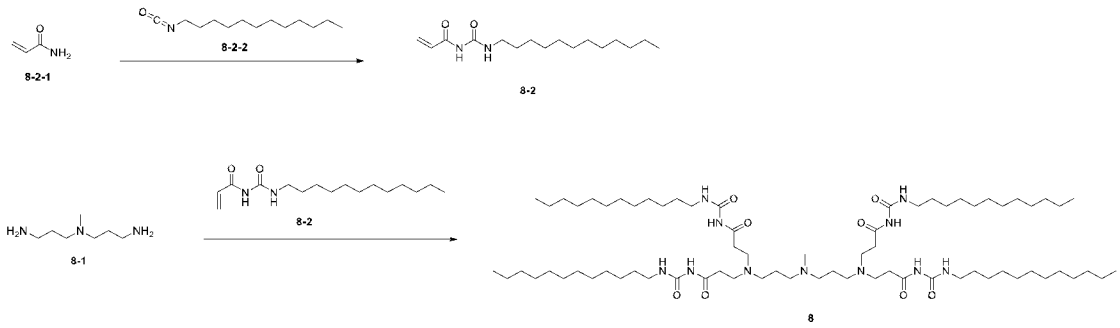
[0260] **Step 3:** to a solution of compound **7-2-4** (5 g, 18.9 mmol) and compound **7-2-5** (1.9 g, 20.7 mmol) in DCM (50 mL) was added DMAP (698 mg 5.7 mmol) under N_2 atmosphere at <10 ℃. To the mixture was added a solution of N,N-diisopropylethylamine (DIEA, 4.9 g, 37.8 mmol) in DCM (5 mL) dropwise at the same temperature. The mixture was allowed to warm to room temperature and stirred at the same temperature for 3 hours. TCL indicated the consumption of starting materials. The reaction was quenched with saturated aqueous $NaHCO_3$ solution (50 mL). The aqueous layer was extracted with ethyl acetate (100 mL). The combined organic layer was washed with 5% aqueous citric acid solution (50 mL) and brine (50 mL), followed by brine (100 mL). The organic layer was dried over Na_2SO_4 and concentrated to afford crude product. The crude product was purified by column chromatography on silica (eluent: petroleum ether with 20% ethyl acetate, v/v) and concentrated under reduced pressure to afford compound **7-2** (4.4 g, 27% yield for 3 steps).

[0261] ^{1}H NMR of compound **7-2**:(400 MHz, DMSO-d6) δ 8.51 (t, J = 6.0 Hz, 1H), 6.29 (dd, J = 17.1, 10.2 Hz, 1H), 6.11 (dd, J = 17.1, 2.1 Hz, 1H), 5.63 (dd, J = 10.2, 2.1 Hz, 1H), 4.04 (t, J = 6.6 Hz, 2H), 3.90 (d, J = 5.9 Hz, 2H), 1.57 (s, 2H), 1.24 (s, 16H), 0.85 (t, J = 6.6 Hz, 3H).

[0262] **Step 4:** to compound **7-1** (10 mg, 0.07 mmol) in a 2 mL container, compound **7-2** (97 mg, 0.34 mmol) was added under N_2 atmosphere, followed by ACN (0.2 mL), BHT (2 mg) and acetic acid (3 mg). The mixture was heated at 70 ℃ for 16 hours. TLC indicated incompletion of reaction. Then the mixture was heated at 85 ℃ for 24 hours, and TLC indicated completion of reaction. The mixture was purified by column chromatography on silica (eluent: DCM with 0-10% methanol and 0.5% NH_4OH, v/v) and concentrated under reduced pressure to afford compound **7** (18 mg, 24% yield).

[0263] ^{1}H NMR of compound **7**: (400 MHz, Chloroform-d) δ 7.71 (t, J = 5.6 Hz, 4H), 4.09 (t, J = 6.8 Hz, 8H), 3.98 (d, J = 5.6 Hz, 8H), 2.75 (t, J = 6.1 Hz, 8H), 2.51 (t, J = 7.1 Hz, 5H), 2.44 (t, J = 6.0 Hz, 9H), 1.71 (s, 3H), 1.63 (q, J = 7.0 Hz, 9H), 1.28 (d, J = 15.9 Hz, 67H), 0.88 (t, J = 6.8 Hz, 12H). HR-MS: $[M+H]^+$ = 1279.03769.

[0264] **Example 1.8: Synthesis of Compound 8**

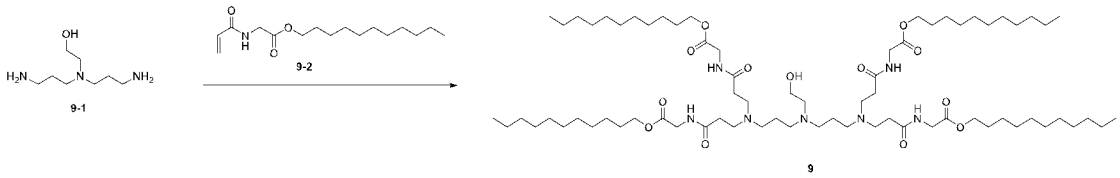


[0265] **Step 1:** to compound **8-2-1** (4.0 g, 56.3 mmol), BHT (1.25 g, 5.6 mmol) and compound **8-2-2** (6.0 g, 28.4 mmol) in a 100 mL container was added toluene (20 mL). The mixture was heated at 80 ℃ for 3 days. TLC (eluent: hexane/ethyl acetate=10/1) indicated substantial completion of reaction. The mixture was purified by column chromatography on silica (eluent: hexane with 0-33% ethyl acetate, v/v) and concentrated under reduced pressure to afford compound **8-2** (4.38 g, 55% yield).

[0266] **Step 2:** to compound **8-1** (20 mg, 0.14 mmol), compound **8-2** (187 mg, 0.66 mmol) and BHT (15 mg) in a 2 mL container acetic acid (3 μL) was added. The mixture was heated at 75 ℃ for 24 hours. Additional compound **8-2** (95 mg, 0.34 mmol) and acetic acid (3 μL) were added. Then the mixture was heated at 75 ℃ for another 24 hours, and TLC (eluent: DCM/MeOH/NH_4OH=10/1/1) indicated substantial completion of reaction. The mixture was purified by column chromatography on silica (eluent: DCM with 1-1.6% methanol and 0.5% NH_4OH, v/v) and concentrated under reduced pressure to afford compound **8** (134.8 mg, 76% yield).

[0267] ^{1}H NMR of compound **8**: (400 MHz, Chloroform-d) δ 10.05 (s, 4H), 8.41 (t, J = 5.7 Hz, 4H), 3.23 (q, J = 7.4, 6.9 Hz, 8H), 2.68 (t, J = 5.6 Hz, 8H), 2.53 (t, J = 5.6 Hz, 8H), 2.42 (s, 4H), δ 1.74 (m, 7H), 1.53 (p, J = 7.2 Hz, 8H), 1.27 (d, J = 11.4 Hz, 70H), 0.88 (t, J = 6.7 Hz, 12H). HR-MS: $[M+H]^+$ = 1275.20592.

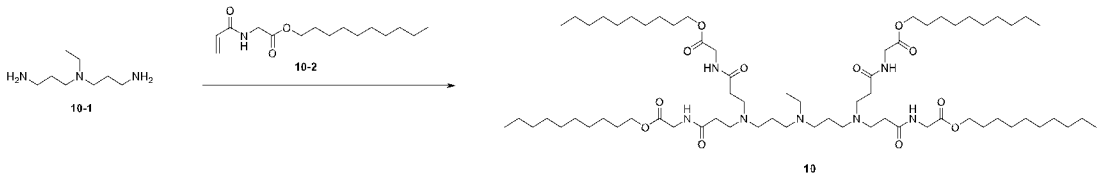
[0268] **Example 1.9: Synthesis of Compound 9**



[0269] To compound **9-1** (10 mg, 0.06 mmol) in a 2 mL container, compound **9-2** (97 mg, 0.34 mmol, prepared by the method of Example 1.7 Step 1 to 3) was added under N_2 atmosphere, followed by ACN (0.2 mL), BHT (2 mg) and acetic acid (2.5 mg). The mixture was heated at 73 ℃ for 40 hours. TLC indicated completion of reaction. The mixture was purified by column chromatography on silica (eluent: DCM with 0-10% methanol and 0.5% NH_4OH, v/v) and concentrated under reduced pressure to afford compound **9** (23 mg, 31% yield).

[0270] ^{1}H NMR of compound **9**: (400 MHz, Chloroform-d) δ 7.72 – 7.65 (m, 4H), 4.09 (t, J = 6.7 Hz, 8H), 3.98 (d, J = 5.5 Hz, 8H), 3.70 – 3.61 (m, 2H), 2.81-2.68 (m, 10H), 2.52 (t, J = 7.1 Hz, 4H), 2.44 (t, J = 6.1 Hz, 8H), 1.70-1.66 (m, 4H), 1.63 (p, J = 6.9 Hz, 8H), 1.38 – 1.19 (m, 64H), 0.88 (t, J = 6.7 Hz, 12H).

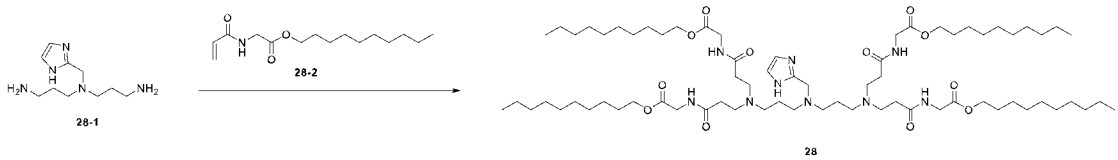
[0271] **Example 1.10: Synthesis of Compound 10**



[0272] To compound **10-1** (10 mg, 0.06 mmol) in a 2 mL container, compound **10-2** (93 mg, 0.35 mmol, prepared by similar method to Example 1.7 Step 1 to 3) was added under N_2 atmosphere, followed by ACN (0.2 mL), BHT (2 mg) and acetic acid (2.5 mg). The mixture was heated at 73 ℃ for 40 hours. TLC indicated completion of reaction. The mixture was purified by column chromatography on silica (eluent: DCM with 0-10% methanol and 0.5% NH_4OH, v/v) and concentrated under reduced pressure to afford compound **10** (18 mg, 25% yield).

[0273] ^{1}H NMR of compound **10**: (400 MHz, Chloroform-d) δ 7.74 (t, J = 5.6 Hz, 4H), 4.10 (t, J = 6.8 Hz, 8H), 3.99 (d, J = 5.4 Hz, 8H), 2.76 (t, J = 6.1 Hz, 8H), 2.47 (dt, J = 25.8, 6.7 Hz, 14H), 1.63 (q, J = 7.1 Hz, 10H), 1.31 (d, J = 7.7 Hz, 20H), 1.26 (s, 45H), 1.07-0.98(m,3H)0.88 (t, J = 6.7 Hz, 12H). HR-MS: $[M+H]^+$ = 1293.06248.

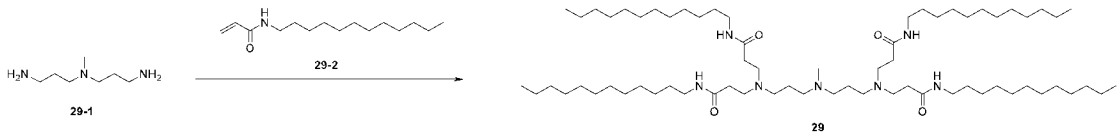
[0274] **Example 1.11: Synthesis of Compound 28**



[0275] To compound **28-1** (200 mg, 0.95 mmol), BHT (104 mg) and compound **28-2** (1.22 g, 4.5 mmol, prepared by similar method to Example 1.7 Step 1 to 3) in a 5 mL container, was added acetic acid (12 mg). The mixture was heated at 80 ℃ for 48 hours. TLC (eluent: DCM/MeOH/NH_4OH=10/1/1) indicated substantial completion of reaction. The mixture was purified by column chromatography on silica (eluent: DCM with 1.4-1.5% methanol and 0.5% NH_4OH, v/v) and concentrated under reduced pressure to afford compound **28** (490 mg, 27% yield).

[0276] ^{1}H NMR of compound **28**: (400 MHz, Chloroform-d) δ 7.91 (t, J = 5.7 Hz, 3H), 7.01 (s, 2H), 4.07 (t, J = 6.8 Hz, 8H), 3.97 (d, J = 5.5 Hz, 8H), 3.86 (s, 1H), 2.75 (t, J = 6.1 Hz, 8H), 2.62 (s, 4H), 2.45 (dt, J = 16.3, 6.5 Hz, 12H), 1.77 – 1.66 (m, 3H), 1.60 (q, J = 7.1 Hz, 9H), 1.28 (d, J = 12.6 Hz, 61H), 0.88 (t, J = 6.7 Hz, 12H). HR-MS: $[M+H]^+$ = 1290.00711.

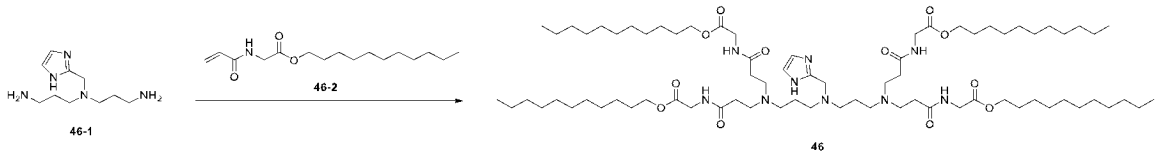
[0277] **Example 1.12: Synthesis of Compound 29**



[0278] To compound **29-1** (600 mg, 4.13 mmol), BHT (182 mg) and compound **29-2** (4.75 g, 19.8 mmol, prepared by the method of Example 2.4 Step 1) in a 5 mL container, were added acetic acid (50 mg) and ACN (0.6 mL). The mixture was heated at 90 ℃ for 2 days. TLC (eluent: DCM/MeOH/NH_4OH=10/1/1) indicated substantial completion of reaction. The mixture was purified by flash column chromatography on silica (eluent: DCM with 1.4-2.4% methanol and 0.5% NH_4OH, v/v) and concentrated under reduced pressure to afford compound **29** (2.80 g, 61% yield).

[0279] ^{1}H NMR of compound **29**: (400 MHz, Chloroform-*d*) δ 7.07 (t, *J* = 5.6 Hz, 4H), 3.28 – 3.11 (m, 8H), 2.70 (t, *J* = 6.2 Hz, 7H), 2.42 (dt, *J* = 17.1, 7.0 Hz, 8H), 2.33 (t, *J* = 6.1 Hz, 9H), 2.24 (s, 3H), 1.61 (q, *J* = 7.1 Hz, 4H), 1.49 (p, *J* = 7.4 Hz, 8H), 1.27 (d, *J* = 9.1 Hz, 68H), 0.88 (t, *J* = 6.7 Hz, 12H). HR-MS: $[M+H]^+$=1103.08503.

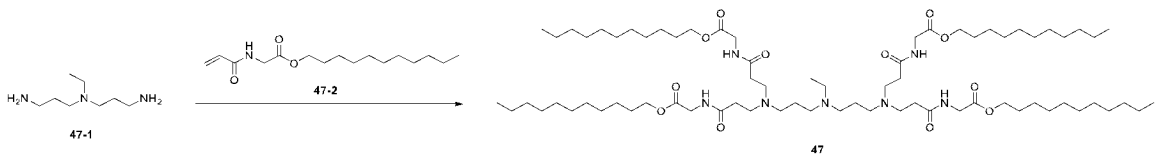
[0280] **Example 1.13: Synthesis of Compound 46**



[0281] To compound **46-1** (200 mg, 0.95 mmol), BHT (104 mg) and compound **46-2** (1.29 g, 4.55 mmol, prepared by the method of Example 1.7 Step 1 to 3) in a 5 mL container, was added acetic acid (12 mg). The mixture was heated at 80 ℃ for 48 hours. TLC (eluent: DCM/MeOH/NH_4OH=10/1/1) indicated substantial completion of reaction. The mixture was purified by flash column chromatography on silica (eluent: DCM with 1.3-1.4% methanol and 0.5% NH_4OH, v/v) and concentrated under reduced pressure to afford compound **46** (262 mg, 21% yield).

[0282] ^1H NMR of compound **46**: (400 MHz, Chloroform-d) δ 7.90 (t, J = 5.7 Hz, 2H), 7.02 (s, 2H), 4.07 (t, J = 6.8 Hz, 8H), 3.97 (d, J = 5.7 Hz, 9H), 2.75 (t, J = 6.0 Hz, 7H), 2.70 (s, 3H), 2.45 (dt, J = 12.4, 6.5 Hz, 14H), 1.73 (t, J = 6.7 Hz, 3H), 1.60 (q, J = 6.8 Hz, 9H), 1.28 (d, J = 11.9 Hz, 63H), 0.88 (t, J = 6.8 Hz, 12H). HR-MS: $[M+H]^+$=1345.06869.

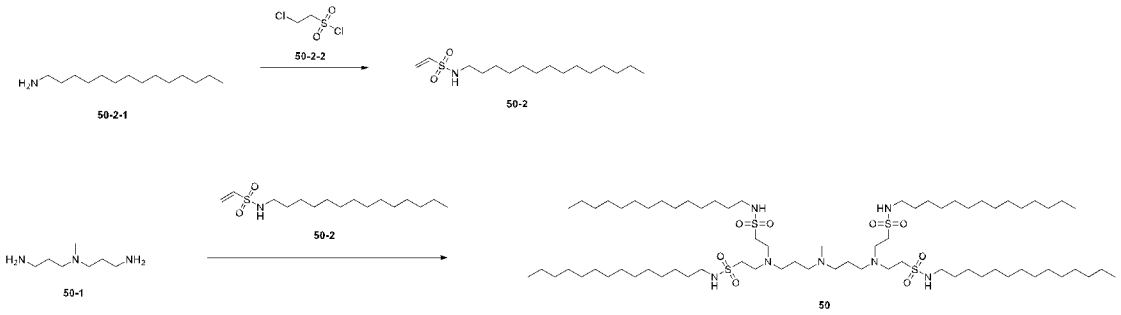
[0283] **Example 1.14: Synthesis of Compound 47**



[0284] To compound **47-1** (200 mg, 1.26 mmol), BHT (138 mg) and compound **47-2** (1.71 g, 6.03 mmol, prepared by the method of Example 1.7 Step 1 to 3) in a 5 mL container, was added acetic acid (15 mg). The mixture was heated at 80 ℃ for 50 hours. TLC (eluent: DCM/MeOH/NH_4OH=10/1/1) indicated substantial completion of reaction. The mixture was purified by flash column chromatography on silica (eluent: DCM with 1.6-1.9% methanol and 0.5% NH_4OH, v/v) and concentrated under reduced pressure to afford compound **47** (370 mg, 23% yield).

[0285] ^1H NMR of compound **47**: (400 MHz, Chloroform-*d*) δ 7.74 (t, *J* = 5.6 Hz, 4H), 4.10 (t, *J* = 6.8 Hz, 8H), 3.99 (d, *J* = 5.4 Hz, 8H), 2.76 (t, *J* = 6.1 Hz, 8H), 2.47 (dt, *J* = 25.8, 6.7 Hz, 14H), 1.63 (q, *J* = 7.1 Hz, 10H), 1.31 (d, *J* = 7.7 Hz, 20H), 1.26 (s, 45H), 1.07-0.98(m,3H)0.88 (t, *J* = 6.7 Hz, 12H). HR-MS: $[M+H]^+$=1293.06248.

[0286] **Example 1.15: Synthesis of Compound 50**



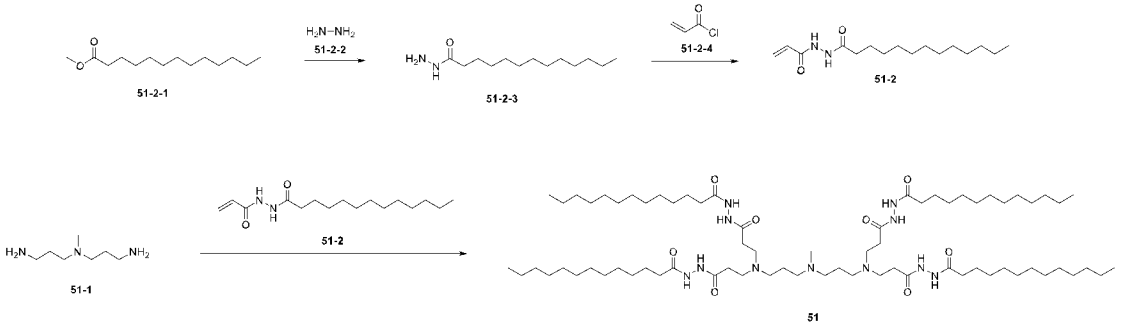
[0287] **Step 1**: to DCM (20 mL) were added compound **50-2-1** (1.6 g, 7.3 mmol) and triethylamine (1.86 g, 18.4 mmol) under N_2 atmosphere. The mixture was cooled to 5 ℃. Compound **50-2-2** (1.0 g, 6.1 mmol) was added dropwise. The mixture was allowed to warm to room temperature and stirred at the same temperature for 16 hours. TLC (eluent: DCM) indicated formation of product. The mixture was purified by column chromatography on silica to afford compound **50-2** (0.7 g, 37% yield).

[0288] ^{1}H NMR of compound **50-2**: (400 MHz, Chloroform-d) δ 6.51 (dd, J = 16.5, 9.9 Hz, 1H), 6.23 (d, J = 16.6 Hz, 1H), 5.93 (d, J = 9.9 Hz, 1H), 4.44 (s, 1H), 3.00 (q, J = 6.8 Hz, 2H), 1.53 (t, J = 7.2 Hz, 2H), 1.25 (s, 24H), 0.87 (t, J = 6.7 Hz, 3H).

[0289] **Step 2**: to a solution of compound **50-1** (20 mg, 0.14 mmol), and compound **50-2** (209 mg, 0.69 mmol) in ACN (0.4 mL), were added BHT (15 mg) and acetic acid (10 mg). The mixture was heated at 75 ℃ for 40 hours. TLC (eluent: DCM/MeOH/=10/1) indicated consumption of starting materials. The mixture was purified by column chromatography on silica and concentrated under reduced pressure to afford compound **50** (86 mg, 46% yield).

[0290] ^{1}H NMR of compound **50**: (400 MHz, Chloroform-d) δ 5.47 (s, 3H), 3.19 (t, J = 6.0 Hz, 8H), 3.08 (q, J = 6.6 Hz, 8H), 2.92 (t, J = 6.0 Hz, 8H), 2.80 (s, 3H), 2.56 (t, J = 6.8 Hz, 5H), 2.48 (s, 3H), 2.38 – 2.09 (m, 6H), 2.03 (s, 3H), 1.84 (s, 4H), 1.57 (p, J = 7.5 Hz, 8H), 1.25 (s, 90H), 0.97 – 0.81 (m, 12H).

[0291] **Example 1.16: Synthesis of Compound 51**



[0292] **Step 1**: to methanol (3 mL) were added compound **51-2-1** (1 g, 4.38 mmol) and compound **51-2-2** (2.8 g, 43.8 mmol) under N_2 atmosphere. The mixture was stirred at room temperature for 40 hours. TLC (eluent: DCM/MeOH=10/1) indicated incompletion consumption of starting materials. Compound **51-2-2** (2.8 g, 43.8 mmol) was added to the mixture. The mixture was heated at 60 ℃ for 16 hours. TLC (eluent: PE) indicated consumption of starting materials. The mixture was concentrated and diluted with water (10 mL). The mixture was stirred for 30 minutes and filtrated. The residue was washed and dried to afford compound **51-2-3** (540 mg).

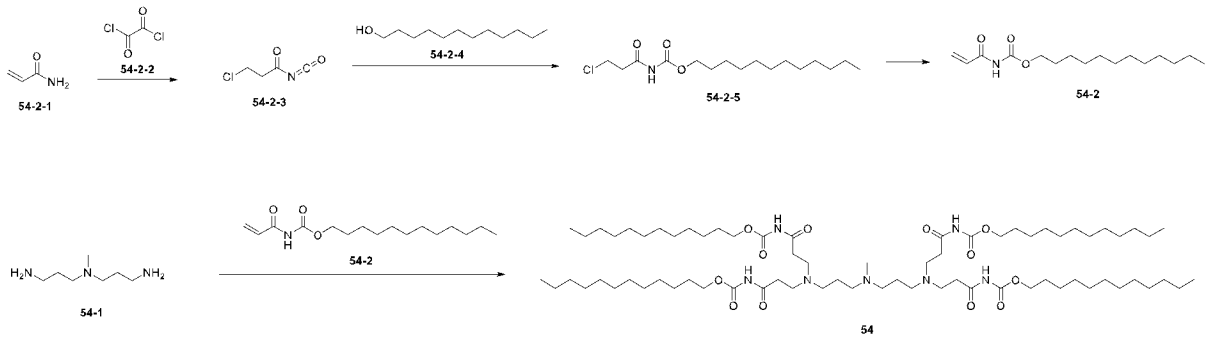
[0293] ^{1}H NMR of compound **51-2-3**: (400 MHz, Chloroform-d) δ 6.82 (s, 1H), 3.90 (s, 2H), 2.14 (t, J = 7.6 Hz, 2H), 1.62 (t, J = 7.3 Hz, 2H), 1.26 (d, J = 10.9 Hz, 18H), 0.87 (t, J = 6.7 Hz, 3H). HRMS: $[M+H]^+$ 229.22922.

[0294] **Step 2**: to DCM (20 mL) were added compound **51-2-3** (0.54 g, 2.4 mmol) and DIEA (0.62 g, 4.8 mmol) under N_2 atmosphere. The mixture was cooled to 5 ℃. compound **51-2-4** (0.26 g, 2.9 mmol) was added dropwise at 5-10 ℃. The mixture was allowed to warm to room temperature and stirred for 2 hours at the same temperature. TLC (eluent: DCM/MeOH=10/1) indicated consumption of starting materials. The mixture was purified by column chromatography on silica to afford crude product. The crude product was added into water (10 mL) and stirred for 30 minutes and dried to afford compound **51-2** (510 mg). HRMS：$[M+H]^+$ 283.24978.

[0295] **Step 3**: to a solution of compound **51-1** (20 mg, 0.14 mmol), and compound **51-2** (187 mg, 0.66 mmol) in ethanol (0.4 mL), were added BHT (15 mg) and acetic acid (10 mg). The mixture was heated at 80 ℃ for 16 hours. TLC (eluent: DCM/MeOH/NH_4OH=10/1/0.05) indicated consumption of starting materials. The mixture was purified by flash column chromatography on silica and concentrated under reduced pressure to afford compound **51** (86 mg, 49% yield).

[0296] ^{1}H NMR of compound **51**: (400 MHz, Chloroform-d) δ 2.89 – 2.56 (m, 5H), 2.45 (d, J = 6.1 Hz, 8H), 2.26 (q, J = 6.8, 5.9 Hz, 8H), 2.16 – 2.05 (m, 2H), 1.63 (t, J = 7.5 Hz, 7H), 1.56 – 1.43 (m, 2H), 1.37 – 1.11 (m, 59H), 0.95 – 0.73 (m, 12H). HRMS：$[M+H]^+$ 1275.09531.

[0297] **Example 1.17: Synthesis of Compound 54**



[0298] **Step 1:** to a solution of compound **54-2-2** (13 g, 102 mmol) in 1,2-dichloroethane (20 mL), were added a mixture of compound **54-2-1** (7.1 g, 100 mmol) in 1,2-dichloroethane (20 mL) at room temperature. The mixture was stirred for 2 hours at the same temperature. TLC indicated substantial consumption of starting materials.

[0299] **Step 2:** to the mixture of Step 1, was added a solution of compound **54-2-4** (10.2 g, 54.7 mmol) in 1,2-dichloroethane (20 mL) dropwise. The mixture was stirred for 1 hours. The mixture was diluted with DCM (50 mL) and washed with water (30 mL). The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography on silica (eluent: hexane with 0-5% ethyl acetate) and concentrated under reduced pressure to afford compound **54-2-5** (5.0 g, 31% yield for two steps).

[0300] **Step 3**: to a solution of compound **54-2-5** (5.0 g, 15.6 mmol) in ACN (125 mL) was added triethylamine (4.75 g, 47.0 mmol) at room temperature. The mixture was stirred at the same temperature for 1.5 hours. TLC indicated substantial consumption of starting materials. The mixture was concentrated at 40 ℃. Water (200 mL) was added to the mixture and the mixture was stirred for 0.5 hour. The mixture was filtrated and the residue was washed with ACN (15 mL) to afford compound **54-2** (3.5 g, 79% yield)

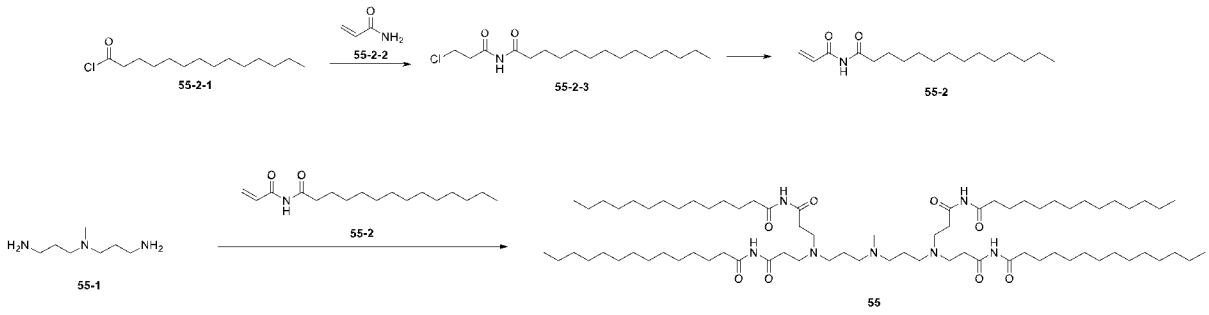
[0301] ^{1}H NMR of compound **54-2**: (400 MHz, Chloroform-*d*) δ 7.82 (s, 1H), 7.09 (dd, *J* = 17.0, 10.4 Hz, 1H), 6.52 (d, *J* = 17.0 Hz, 1H), 5.87 (d, *J* = 10.7 Hz, 1H), 4.16 (t, *J* = 6.7 Hz, 2H), 1.73 – 1.59 (m, 2H), 1.39 – 1.17 (m, 19H), 0.87 (t, *J* = 6.7 Hz, 3H). HR-MS: $[M+H]^+$=284.23291.

[0302] **Step 4**: a mixture of compound **54-1** (20 mg, 0.14 mmol), BHT (15 mg) and compound **54-2** (235 mg, 0.83 mmol) in THF (0.2 mL) was stirred at room temperature for 1 hours. TLC (eluent: DCM/MeOH/NH_4OH=10/1/0.05) indicated completion of reaction. The mixture was purified by flash column chromatography on

silica (eluent: DCM with 0-2.5% methanol and 0.5% NH_4OH) and concentrated under reduced pressure to afford compound **54** (127 mg, 76% yield).

[0303] ^{1}H NMR of compound **54**: (400 MHz, Chloroform-*d*) δ 9.00 (s, 3H), 4.06 (t, *J* = 6.9 Hz, 8H), 2.71 (dt, *J* = 29.1, 6.5 Hz, 15H), 2.46 (t, *J* = 7.2 Hz, 4H), 2.28 (d, *J* = 10.9 Hz, 4H), 2.11 (s, 4H), 1.59 (q, *J* = 6.9 Hz, 12H), 1.20 (d, *J* = 6.5 Hz, 72H), 0.81 (t, *J* = 6.7 Hz, 12H). HR-MS: $[M+H]^+$=1279.04739.

[0304] **Example 1.18: Synthesis of Compound 55**



[0305] **Step 1**: to compound **55-2-2** (0.2 g, 2.8 mmol) were added compound **55-2-1** (0.83 g, 3.4 mmol) and BHT (31 mg) under N_2 atmosphere. The mixture was stirred at 80 ℃ for 16 hours. TLC indicated consumption of starting materials. The mixture was cooled to room temperature, diluted with ACN (20 mL), stirred for 2 hours and filtrated. The residue was washed with ACN to afford compound **55-2-3** (0.8 g).

[0306] **Step 2**: to a solution of compound **55-2-3** (1.0 g, 3.14 mmol) in ACN (25 mL) was added triethylamine (0.95 g, 9.4 mmol) at room temperature. The mixture was stirred at the same temperature for 1.5 hours. TLC indicated substantial consumption of starting materials. The mixture was concentrated at 40 ℃. The mixture was purified by flash column chromatography on silica (eluent: petroleum ether with 0-6.3% ethyl acetate) and concentrated under reduced pressure to afford compound **55-2** (0.60 g, 68% yield).

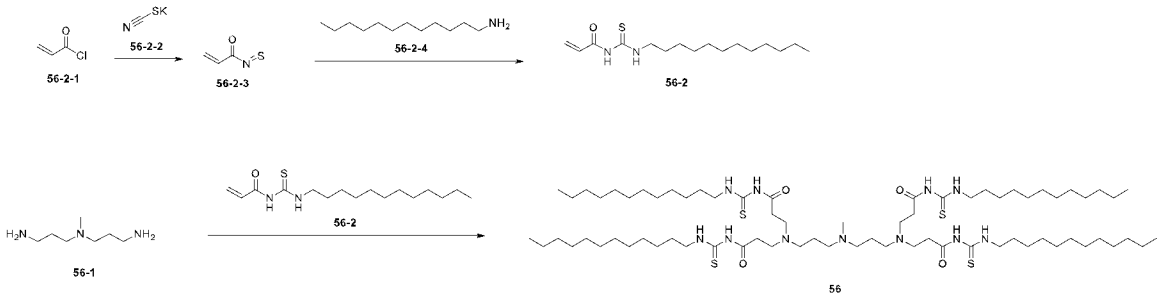
[0307] ^{1}H NMR of compound **55-2**: (400 MHz, Chloroform-*d*) δ 8.53 (s, 1H), 6.61 (dd, *J* = 17.1, 10.2 Hz, 1H), 6.49 (dd, *J* = 16.9, 1.5 Hz, 1H), 5.89 (dd, *J* = 10.1, 1.5 Hz, 1H), 2.71 (t, *J* = 7.5 Hz, 2H), 1.65 (h, *J* = 8.5, 8.0 Hz, 2H), 1.41 – 1.17 (m, 21H), 0.88 (t, *J* = 6.7 Hz, 3H).

[0308] **Step 3**: a mixture of compound **55-1** (20 mg, 0.14 mmol), BHT (15 mg) and compound **55-2** (250 mg, 0.89 mmol) in THF (2 mL) and DCM (1 mL) was stirred at room temperature for 0.5 hours. The mixture was purified by flash column

chromatography on silica (eluent: DCM with 0-3.3% methanol and 0.5% NH_4OH) and concentrated under reduced pressure to afford compound **55** (75 mg, 43% yield).

[0309] ^1H NMR of compound **55**: (400 MHz, Chloroform-*d*) δ 10.00 (s, 3H), 2.77 (t, *J* = 6.0 Hz, 8H), 2.67 (h, *J* = 6.0 Hz, 15H), 2.52 (t, *J* = 7.1 Hz, 4H), 2.32 (s, 4H), 2.25 – 2.10 (m, 4H), 1.60 (q, *J* = 9.9, 7.2 Hz, 12H), 1.25 (s, 76H), 0.93 – 0.81 (m, 12H). HR-MS: $[M/2+H]^+$=636.06442, $[M+H]^+$=1271.12962, $[M+Na]^+$=1294.11383.

[0310] **Example 1.19: Synthesis of Compound 56**



[0311] **Step 1**: to DCM (100 mL) were added compound **56-2-2** (9.72 g, 100 mmol) and PEG-350 (1 g) under N_2 atmosphere. The mixture was cooled to 0 ℃. To the mixture was added a solution of compound **56-2-1** (9.05 g, 100 mmol) in DCM (20 mL) dropwise for 1 hours at 0-5 ℃. The mixture was allowed to warm to room temperature and stirred for 2 hours. TLC (quenched with compound **56-2-4**, eluent: petroleum ether/ethyl acetate=10/1) indicated formation of product. The mixture was filtrated through Celite to afford crude compound **56-2-3**.

[0312] **Step 2**: compound **56-2-3** was cooled to 5 ℃ under N_2 atmosphere. To cooled compound **56-2-3** was added a solution of compound **56-2-4** (18.5 g, 100 mmol) in DCM (50 mL) dropwise at 5-10 ℃. The mixture was allowed to warm to room temperature and stirred for 2 hours. The mixture was purified by column chromatography on silica (eluent: petroleum ether with 2-10% ethyl acetate) and concentrated under reduced pressure to afford compound **56-2** (7.5 g, 25% yield)

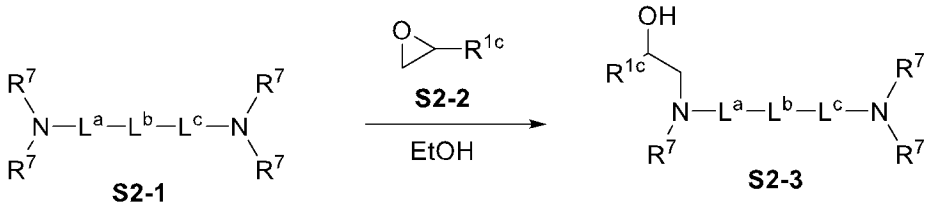
[0313] ^1H NMR of compound **56-2**: (400 MHz, Chloroform-*d*) δ 10.68 (t, *J* = 5.4 Hz, 1H), 9.28 (s, 1H), 6.50 (dd, *J* = 16.9, 1.0 Hz, 1H), 6.23 (dd, *J* = 16.9, 10.3 Hz, 1H), 5.92 (dd, *J* = 10.3, 1.1 Hz, 1H), 3.65 (td, *J* = 7.2, 5.3 Hz, 2H), 1.74 – 1.61 (m, 2H), 1.40 – 1.15 (m, 20H), 0.87 (t, *J* = 6.8 Hz, 3H).

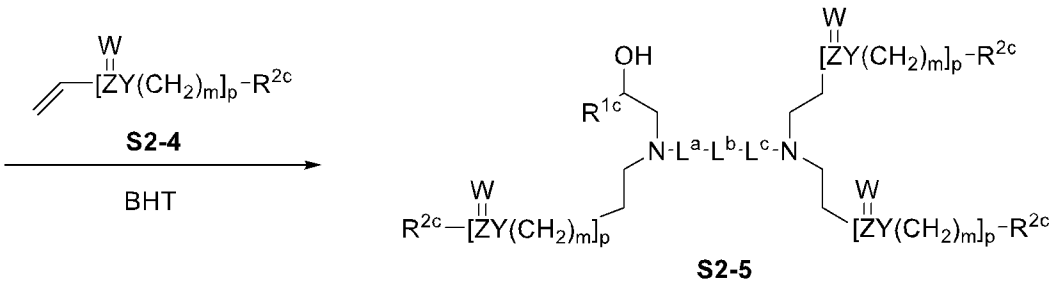
[0314] **Step 3**: to a solution of compound **56-1** (100 mg, 0.69 mmol), BHT (50 mg) and acetic acid (50 mg) in ACN (2 mL) was added compound **56-2** (1.12 g, 3.3

mmol) under N_2 atmosphere. The mixture was stirred at room temperature for 16 hours. TLC (eluent: DCM/methanol=10/1) indicated substantial consumption of starting materials. The mixture was purified by column chromatography on silica and concentrated under reduced pressure to afford compound **56** (850 mg, 65% yield).

[0315] ^{1}H NMR of compound **56**: (400 MHz, Chloroform-*d*) δ 10.70 – 10.29 (m, 8H), 3.57 (td, *J* = 7.3, 5.3 Hz, 8H), 3.00 – 2.83 (m, 4H), 2.73 (t, *J* = 5.8 Hz, 8H), 2.60 (t, *J* = 5.7 Hz, 8H), 2.53 (d, *J* = 8.6 Hz, 7H), 1.96 (q, *J* = 7.7 Hz, 4H), 1.63 (t, *J* = 7.3 Hz, 8H), 1.48 – 1.10 (m, 80H), 0.87 (t, *J* = 6.7 Hz, 13H). HRMS : $[M+H]^+$=1339.01158.

[0316] **Example 2: General Scheme 2**





wherein

each W is independently selected from O, S and NH;

each Y is independently selected from O, S and NH;

each n is independently 1, 2 or 3;

each m is 0 or 1;

each p is 1 or 2.

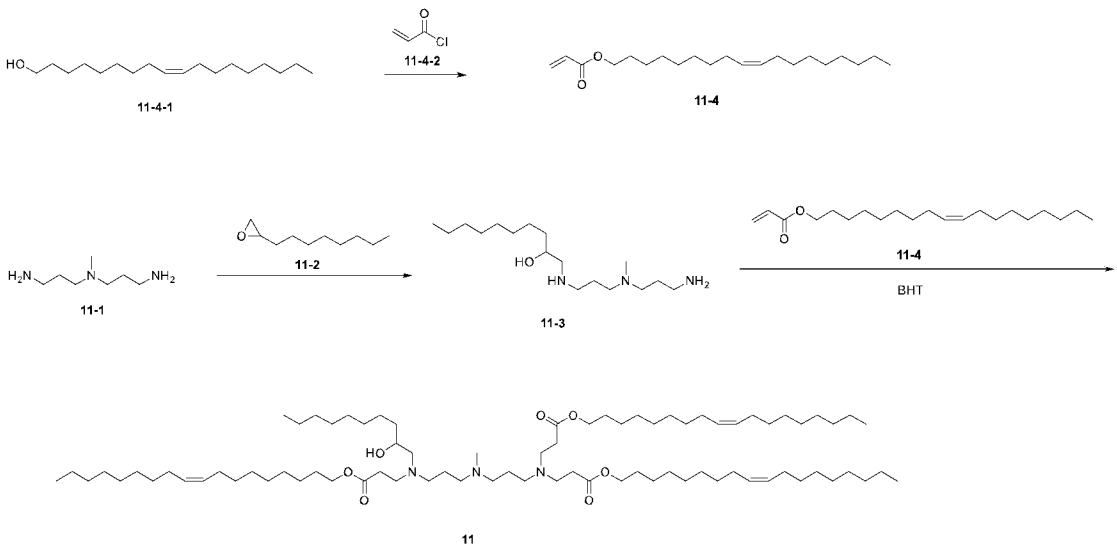
[0317] *General Procedure*

[0318] **General Step 1:** a solution of compound **S2-1** in ethanol was heated at 60-80 ℃. To the mixture compound **S2-2** was added. The mixture was cooled to room

temperature after completion of reaction, and then washed and dried to afford selective mono-substituted intermediate **S2-3**.

[0319] **General Step 2:** a mixture of intermediate **S2-3**, compound **S2-4** (with ACN and/or acetic acid) and BHT was added into a sealed container. The mixture was heated at 60-80 ℃ for 12-24 hours and then cooled to room temperature. The mixture was purified by column chromatography to afford compound **S2-5**.

[0320] **Example 2.1: Synthesis of Compound 11**



[0321] **Step 1:** to a container were added compound **11-4-1** (280 mg, 1.0 mmol) dry DCM (6 mL) and compound **11-4-2** (104 mg, 1.1 mmol) in sequence at room temperature. The mixture was stirred for 5 minutes before cooled in an ice bath. To the mixture was added triethylamine (211 mg, 2.1 mmol). The mixture was allowed to warm to room temperature and stirred at the same temperature for 5 hours. TLC (eluent: petroleum ether/DCM=3/1, $KMnO_4$ stain) indicated substantial completion of reaction. The mixture was purified by column chromatography on silica (eluent: petroleum ether with 0-33% DCM, v/v) and concentrated under reduced pressure to afford compound **11-4** as a colorless oil (220 mg, 65% yield).

[0322] ^{1}H NMR of compound **11-4**: (400 MHz, Chloroform-d) δ 6.33 (dd, J = 17.3, 1.6 Hz, 1H), 6.05 (dd, J = 17.4, 10.4 Hz, 1H), 5.74 (dd, J = 10.4, 1.5 Hz, 1H), 5.34 – 5.20 (m, 2H), 4.08 (t, J = 6.8 Hz, 2H), 1.94 (q, J = 6.4 Hz, 4H), 1.59 (p, J = 6.8 Hz, 2H), 1.22 (q, J = 8.0, 5.5 Hz, 24H), 0.81 (t, J = 6.7 Hz, 3H).

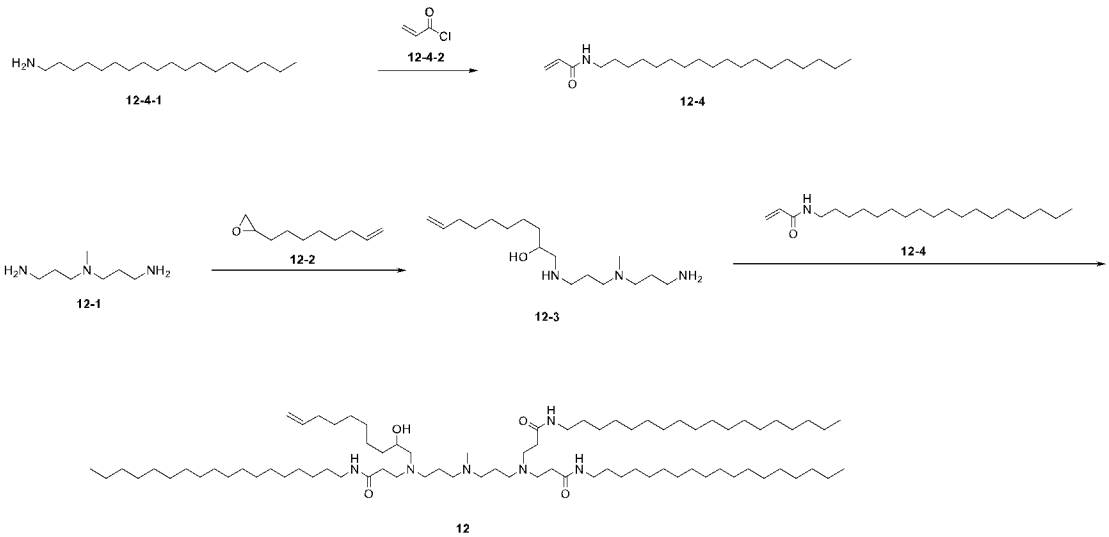
[0323] **Step 2:** to a solution of compound **11-1** (9.46 g, 65.13 mmol) in ethanol (600 mL) heated at 65 ℃ (internal), compound **11-2** (2.0 g, 12.80 mmol) was added.

The mixture was heated at 70-75℃ (external) overnight. The mixture was cooled to room temperature then concentrated under reduced pressure. The residue was diluted with DCM (500 mL), and brine (400 mL) was added thereto under stirring. The mixture was heated at 35 ℃ (internal) and stirred for 10 minutes then let stand and separated. The aqueous layer was extracted with DCM (500 mL) again, repeated for 4-5 times. TLC (eluent: DCM/MeOH=5:1, with 3 drops of NH_4OH) indicated completion of extraction. Combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford compound **11-3** (3.47 g, 97% yield).

[0324] **Step 3:** to compound **11-3** (80 mg, 0.27 mmol) in a 4 mL container were added compound **11-4** (308 mg, 0.95 mmol) and BHT (5 mg) under N_2 atmosphere. The mixture was heated at 70 ℃ for 48 hours. TLC indicated completion of reaction. The mixture was purified by column chromatography on silica (eluent: DCM with 0-10% methanol, v/v) and concentrated under reduced pressure to afford compound **11** (250 mg, 74% yield).

[0325] ^{1}H NMR of compound **11**: (400 MHz, Chloroform-d) δ 5.44 – 5.26 (m, 5H), 4.05 (q, J = 6.4 Hz, 6H), 3.58 (dd, J = 6.9, 3.4 Hz, 1H), 2.97 – 2.83 (m, 1H), 2.72 (dt, J = 24.8, 7.0 Hz, 5H), 2.57 (dt, J = 14.3, 7.3 Hz, 1H), 2.01 (q, J = 6.4 Hz, 12H), 1.61 (t, J = 7.0 Hz, 10H), 1.28 (q, J = 8.2, 4.7 Hz, 91H), 0.87 (t, J = 6.6 Hz, 14H).

[0326] **Example 2.2: Synthesis of Compound 12**



[0327] **Step 1:** to a container were added dry DCM (200 mL), $NaHCO_3$ (7.43 g, 88.5 mmol) compound **12-4-1** (9.6 g, 35.7 mmol) and compound **12-4-2** (4 g, 44.2 mmol) in sequence in an ice bath. The mixture was allowed to warm to room

temperature and stirred at the same temperature for 2 hours. TLC (I_2 stain) indicated completion of reaction. The reaction was quenched with water (200 mL) and stirred for 10 minutes. The organic layer was washed with saturated aqueous $NaHCO_3$ solution (200 mL) twice, followed by water (200 mL). The organic layer was dried and concentrated to afford crude compound **12-4** (11.4 g, 87%).

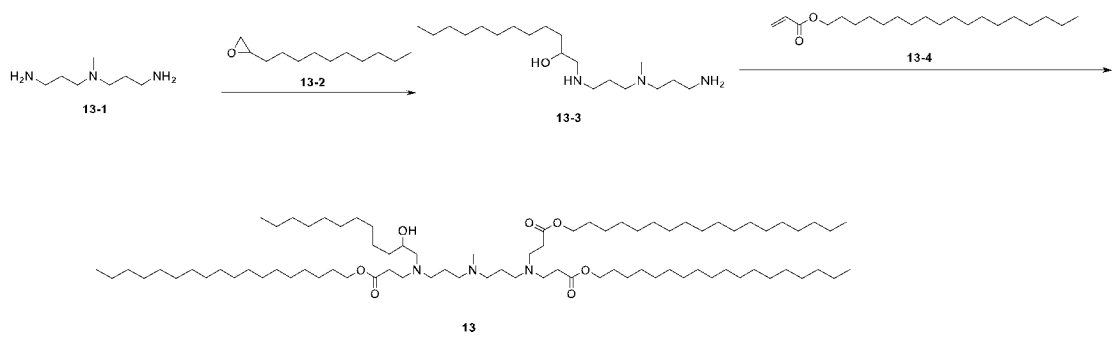
[0328] ^{1}H NMR of compound **12-4**: (400 MHz, Chloroform-d) δ 6.29 (dd, J = 17.0, 1.5 Hz, 1H), 6.10 (dd, J = 17.0, 10.3 Hz, 1H), 5.68 – 5.48 (m, 2H), 3.39 – 3.31 (m, 2H), 1.56 (p, J = 7.1 Hz, 2H), 1.28 (s, 29H), 0.90 (t, J = 6.7 Hz, 3H).

[0329] **Step 2:** to a solution of compound **12-1** (9.46 g, 65.13 mmol) in ethanol (600 mL) heated at 65 ℃ (internal), compound **12-2** (2.0 g, 12.97 mmol) was added. The mixture was heated at 70-75℃ (external) overnight. The mixture was cooled to room temperature then concentrated under reduced pressure. The residue was diluted with DCM (500 mL), and brine (400 mL) was added thereto under stirring. The mixture was heated at 35 ℃ (internal) and stirred for 10 minutes then let stand and separated. The aqueous layer was extracted with DCM (500 mL) again, repeated for 4-5 times. TLC (eluent: DCM/MeOH=5:1, with 3 drops of NH_4OH) indicated completion of extraction. Combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford compound **12-3** (3.4 g, 95% yield).

[0330] **Step 3:** to compound **12-3** (100 mg, 0.33 mmol) were added compound **12-4** (384 mg, 1.19 mmol) and BHT (5 mg). The mixture was heated for 48 hours. TLC indicated completion of reaction. The mixture was diluted with DCM (10 mL) and concentrated under reduced pressure. The residue was purified by column chromatography on silica (eluent: DCM with 0-10% methanol, v/v) and concentrated under reduced pressure to afford compound **12** as a colorless oil (140 mg, 45% yield).

[0331] ^{1}H NMR of compound **12**: (400 MHz, Chloroform-d) δ 5.88 – 5.71 (m, 1H), 5.49 – 5.25 (m, 6H), 5.05 – 4.81 (m, 2H), 4.05 (q, J = 6.4 Hz, 7H), 3.58 (td, J = 7.0, 3.6 Hz, 1H), 2.90 (dt, J = 14.5, 7.4 Hz, 1H), 2.73 (dt, J = 25.2, 6.9 Hz, 6H), 2.57 (dt, J = 14.4, 7.3 Hz, 2H), 2.43 (q, J = 7.0, 6.4 Hz, 13H), 2.36 – 1.91 (m, 27H), 1.61 (t, J = 7.1 Hz, 12H), 1.30 (ddd, J = 17.8, 10.8, 5.4 Hz, 96H), 0.88 (t, J = 6.7 Hz, 12H).

[0332] **Example 2.3: Synthesis of Compound 13**

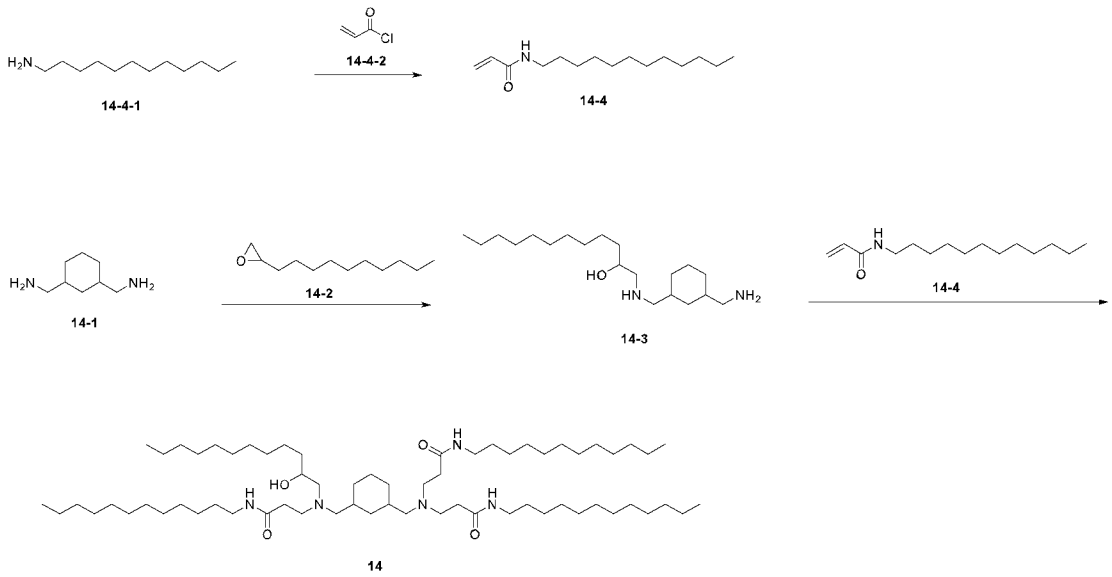


[0333] **Step 1:** to a solution of compound **13-1** (9.46 g, 65.13 mmol) in ethanol (600 mL) heated at 65 ℃ (internal), compound **13-2** (2.2 g, 11.94 mmol) was added. The mixture was heated at 70-75℃ (external) overnight. The mixture was cooled to room temperature then concentrated under reduced pressure. The residue was diluted with DCM (500 mL), and brine (400 mL) was added thereto under stirring. The mixture was heated at 35 ℃ (internal) and stirred for 10 minutes then let stand and separated. The aqueous layer was extracted with DCM (500 mL) again, repeated for 4-5 times. TLC (eluent: DCM/MeOH=5:1, with 3 drops of NH_4OH) indicated completion of extraction. Combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford compound **13-3** (3.5 g, 97% yield).

[0334] **Step 2:** to compound **13-3** (100 mg, 0.30 mmol) were added compound **13-4** (350 mg, 1.08 mmol) and BHT (5 mg). The mixture was heated for 48 hours. TLC indicated completion of reaction. The mixture was diluted with DCM (10 mL) and concentrated under reduced pressure. The residue was purified by column chromatography on silica (eluent: DCM with 0-5% methanol, v/v) and concentrated under reduced pressure to afford compound **13** as a colorless oil (198 mg, 70% yield).

[0335] ^1H NMR of compound **13**: (400 MHz, Chloroform-d) δ 4.05 (q, J = 6.6 Hz, 6H), 3.58 (s, 1H), 2.91 (dt, J = 14.3, 7.5 Hz, 1H), 2.72 (dt, J = 23.3, 6.7 Hz, 7H), 2.42 (h, J = 6.4 Hz, 10H), 2.24 (d, J = 48.6 Hz, 6H), 1.68 – 1.52 (m, 9H), 1.25 (s, 112H), 0.88 (t, J = 6.8 Hz, 12H).

[0336] **Example 2.4: Synthesis of Compound 14**



[0337] **Step 1:** to a container were added dry DCM (300 mL), $NaHCO_3$ (11.14 g, 132.6 mmol), compound **14-4-1** (14.15 g, 76.3 mmol) and compound **12-4-2** (6.0 g, 66.3 mmol) in sequence in an ice bath. The mixture was allowed to warm to room temperature and stirred at the same temperature for 2 hours. TLC (I_2 stain) indicated completion of reaction. The reaction was quenched with water (300 mL) and stirred for 10 minutes. The organic layer was washed with saturated aqueous $NaHCO_3$ solution (300 mL) twice, followed by water (300 mL). The organic layer was dried and concentrated to afford crude compound **14-4** (8.4 g, 47%).

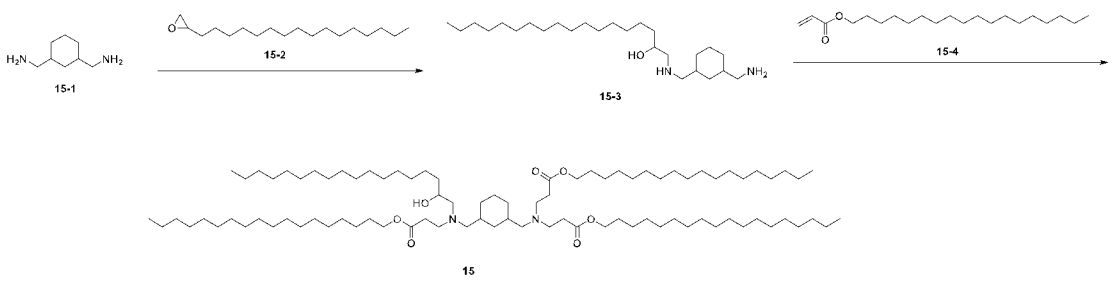
[0338] ^1H NMR of compound **14-4**: (400 MHz, Chloroform-d) δ 6.27 (d, J = 16.9 Hz, 1H), 6.09 (dd, J = 17.0, 10.2 Hz, 1H), 5.64 (t, J = 11.1 Hz, 2H), 3.32 (q, J = 6.8 Hz, 2H), 1.53 (p, J = 7.1 Hz, 2H), 1.26 (s, 24H), 0.88 (t, J = 6.6 Hz, 3H).

[0339] **Step 2:** to a solution of compound **14-1** (1.85 g, 13.01 mmol) in ethanol (100 mL) heated at 65 ℃ (internal), compound **14-2** (0.40 g, 2.17 mmol) was added. The mixture was heated at 70-75℃ (external) overnight. The mixture was cooled to room temperature then concentrated under reduced pressure. The residue was diluted with DCM (300 mL), and tap water (300 mL) was added thereto under stirring. The mixture was heated at 35 ℃ (internal) and stirred for 10 minutes then let stand and separated. Extraction was repeated for 3-4 times. TLC (eluent: DCM/MeOH=10:1, with 1 drop of NH_4OH) indicated completion of extraction. Combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford compound **14-3** (0.63 g, 68% yield).

[0340] **Step 3:** to compound **14-3** (20 mg, 0.06 mmol) in a 2 mL container were added compound **14-4** (51.6 mg, 0.22 mmol), BHT (14 mg), and acetic acid (3 μL). The mixture was heated at 80 ℃ for 60 hours. TLC (eluent: DCM/MeOH=10/1 with NH_4OH) indicated substantial completion of reaction. The mixture was purified by column chromatography on silica (eluent: DCM with 1-1.6% methanol and 0.5% NH_4OH, v/v) and concentrated under reduced pressure to afford compound **14** (17 mg, 27% yield).

[0341] ^1H NMR of compound **14**: (400 MHz, Chloroform-d) δ 6.93 (t, J = 5.6 Hz, 1H), 3.74 – 3.55 (m, 1H), 3.38 – 3.08 (m, 5H), 2.98 (dq, J = 15.1, 7.4 Hz, 1H), 2.69 (dddd, J = 35.5, 21.3, 13.6, 6.6 Hz, 5H), 2.49 – 2.11 (m, 11H), 2.07 – 1.59 (m, 4H), 1.46 (dh, J = 16.5, 8.5, 7.9 Hz, 10H), 1.26 (d, J = 8.9 Hz, 73H), 0.87 (t, J = 6.7 Hz, 13H).

[0342] **Example 2.5: Synthesis of Compound 15**



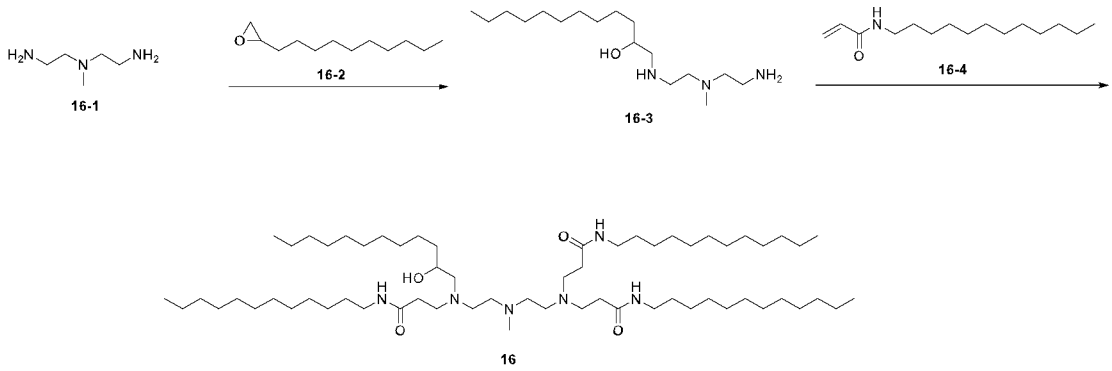
[0343] **Step 1:** to a solution of compound **15-1** (1.27 g, 8.93 mmol) in ethanol (100 mL) heated at 65 ℃ (internal), compound **15-2** (0.40 g, 1.49 mmol, 85% purity) was added. The mixture was heated at 70-75℃ (external) overnight. The mixture was cooled to room temperature then concentrated under reduced pressure. The residue was diluted with DCM (300 mL), and tap water (300 mL) was added thereto under stirring. The mixture was heated at 35 ℃ (internal) and stirred for 10 minutes then let stand and separated. Extraction was repeated for 3-4 times. TLC (eluent: DCM/MeOH=10:1, with 1 drop of NH_4OH) indicated completion of extraction. Combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford compound **15-3** (0.40 g, 35% yield).

[0344] **Step 2:** to compound **15-3** (20 mg, 0.05 mmol) in a 2 mL container were added compound **15-4** (58.5 mg, 0.18 mmol) and BHT (13 mg). The mixture was heated at 70 ℃ for 40 hours. TLC (eluent: DCM/MeOH=40/1) indicated substantial completion of reaction. The mixture was purified by flash column chromatography on

silica (eluent: DCM with 0-5% methanol, v/v) and concentrated under reduced pressure to afford compound **15** (45.6 mg, 54% yield).

[0345] ^{1}H NMR of compound **15**: (400 MHz, Chloroform-d) δ 4.05 (qd, J = 6.9, 2.3 Hz, 5H), 3.59 (s, 1H), 2.94 (s, 1H), 2.75 (tt, J = 19.9, 9.3 Hz, 4H), 2.41 (t, J = 7.1 Hz, 6H), 2.34 – 2.10 (m, 4H), 1.94 – 1.56 (m, 13H), 1.26 (s, 110H), 0.88 (t, J = 6.7 Hz, 12H).

[0346] **Example 2.6: Synthesis of Compound 16**

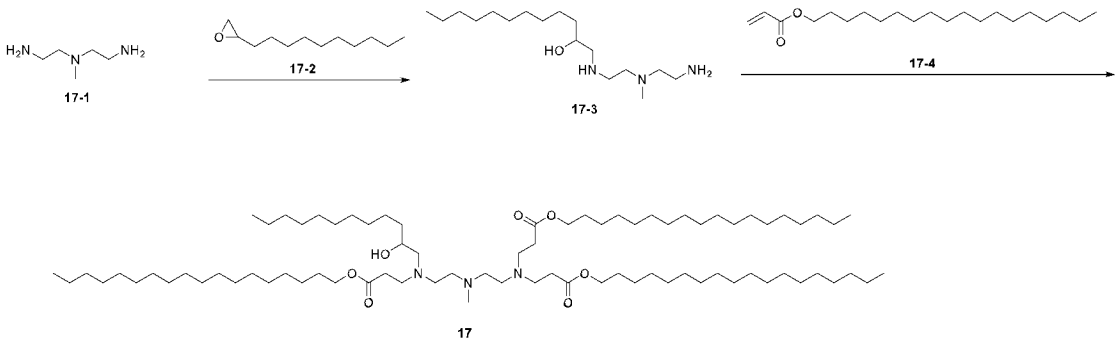


[0347] **Step 1:** to a solution of compound **16-1** (1.145 g, 9.77 mmol) in ethanol (100 mL) heated at 65 ℃ (internal), compound **16-2** (0.30 g, 1.63 mmol) was added. The mixture was heated at 70-75℃ (external) overnight. The mixture was cooled to room temperature then concentrated under reduced pressure. The residue was diluted with DCM (300 mL), and brine (300 mL) was added thereto under stirring. The mixture was heated at 35 ℃ (internal) and stirred for 10 minutes then let stand and separated. Extraction was repeated for 3-4 times. TLC (eluent: DCM/MeOH=5:1, with 2 drop of NH_4OH) indicated completion of extraction. Combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford compound **16-3** (0.47 g, 95% yield).

[0348] **Step 2:** to compound **16-3** (20 mg, 0.07 mmol) in a 2 mL container were added compound **16-4** (61 mg, 0.25 mmol, prepared by the method of Example 2.4 Step 1), BHT (15 mg), and acetic acid (3 μL). The mixture was heated at 80 ℃ for 105 hours. TLC (eluent: DCM/MeOH=10/1 with NH_4OH) indicated substantial completion of reaction. The mixture was purified by column chromatography on silica (eluent: DCM with 1-1.3% methanol and 0.5% NH_4OH, v/v) and concentrated under reduced pressure to afford compound **16** (45 mg, 66% yield).

[0349] ^{1}H NMR of compound **16**: (400 MHz, Chloroform-d) δ 7.12 – 6.96 (m, 1H), 6.93 (t, J = 5.5 Hz, 1H), 3.67 – 3.48 (m, 1H), 3.10 (dq, J = 26.9, 6.6 Hz, 6H), 2.91 – 2.81 (m, 1H), 2.54 (d, J = 37.5 Hz, 9H), 2.44 – 2.22 (m, 11H), 2.16 (dd, J = 14.0, 6.4 Hz, 2H), 2.08 – 1.85 (m, 2H), 1.49 – 1.30 (m, 9H), 1.20 (d, J = 11.0 Hz, 80H), 0.81 (t, J = 6.8 Hz, 14H).

[0350] **Example 2.7: Synthesis of Compound 17**

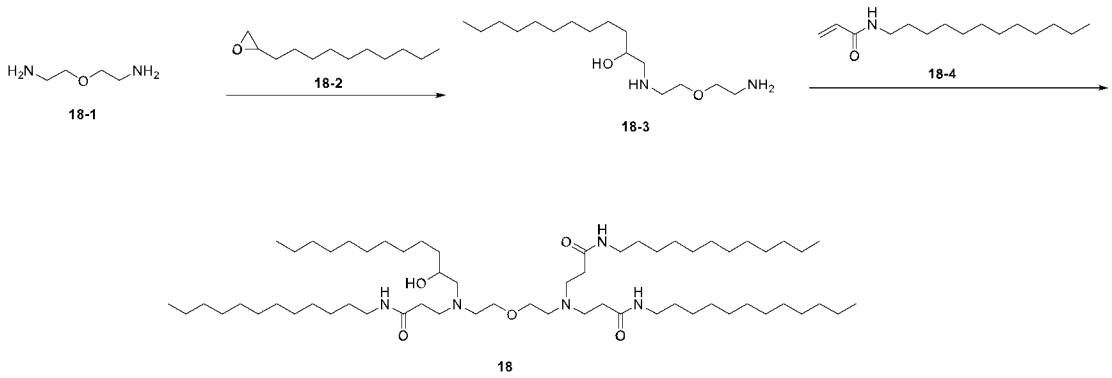


[0351] **Step 1:** compound **17-3** was prepared by the method of Example 2.6 Step 1.

[0352] **Step 2:** to compound **17-3** (19.6 mg, 0.07 mmol) in a 2 mL container were added compound **17-4** (78.6 mg, 0.24 mmol) and BHT (17.2 mg). The mixture was heated at 70 ℃ for 67 hours then at 90 ℃ for 23 hours. TLC (eluent: DCM/MeOH=20/1) indicated substantial completion of reaction. The mixture was purified by flash column chromatography on silica (eluent: DCM with 0-5% methanol, v/v) and concentrated under reduced pressure to afford compound **17** (40.8 mg, 49% yield).

[0353] ^{1}H NMR of compound **17**: (400 MHz, Chloroform-d) δ 4.05 (q, J = 7.2 Hz, 5H), 3.62 (dt, J = 11.9, 6.5 Hz, 1H), 2.94 (dt, J = 14.2, 7.3 Hz, 2H), 2.84 (q, J = 6.6 Hz, 2H), 2.77 (t, J = 6.9 Hz, 5H), 2.65 – 2.58 (m, 1H), 2.47 (dq, J = 13.8, 7.5, 7.0 Hz, 7H), 2.40 – 2.17 (m, 2H), 1.71 – 1.53 (m, 6H), 1.25 (s, 106H), 0.88 (t, J = 6.8 Hz, 12H).

[0354] **Example 2.8: Synthesis of Compound 18**

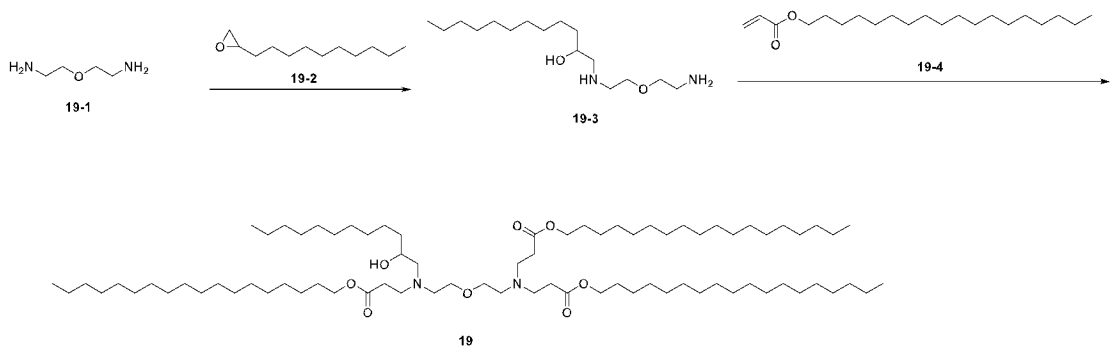


[0355] **Step 1:** to a solution of compound **18-1** (0.61 g, 5.86 mmol) in ethanol (100 mL) heated at 65 ℃ (internal), compound **18-2** (0.18 g, 0.98 mmol) was added. The mixture was heated at 70-75℃ (external) overnight. The mixture was cooled to room temperature then concentrated under reduced pressure. The residue was diluted with DCM (200 mL), and water (200 mL) was added thereto under stirring. The mixture was heated at 35 ℃ (internal) and stirred for 10 minutes then let stand and separated. Extraction was repeated for 3-4 times. TLC (eluent: DCM/MeOH=10:1, with 1 drop of NH_4OH) indicated completion of extraction. Combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford compound **18-3** (0.25 g, 50% yield).

[0356] **Step 2:** to compound **18-3** (20 mg, 0.07 mmol) in a 2 mL container were added compound **18-4** (59 mg, 0.25 mmol, prepared by the method of Example 2.4 Step 1) and BHT (17.2 mg). The mixture was heated at 70 ℃ for 67 hours then at 90 ℃ for 23 hours. TLC (eluent: DCM/MeOH=20/1) indicated substantial completion of reaction. The mixture was purified by flash column chromatography on silica (eluent: DCM with 0-5% methanol, v/v) and concentrated under reduced pressure to afford compound **18** (40.8 mg, 49% yield).

[0357] ^{1}H NMR of compound **18**: (400 MHz, Chloroform-d) δ 6.94 (t, J = 5.7 Hz, 2H), 6.77 (t, J = 5.8 Hz, 1H), 3.66 (d, J = 9.6 Hz, 1H), 3.51 (dhept, J = 10.1, 5.1 Hz, 4H), 3.24 – 3.13 (m, 6H), 3.02 (s, 2H), 2.89 – 2.72 (m, 6H), 2.71 – 2.52 (m, 4H), 2.37 (tq, J = 14.5, 9.0, 7.3 Hz, 7H), 1.54 – 1.38 (m, 9H), 1.26 (d, J = 8.7 Hz, 79H), 0.87 (t, J = 6.7 Hz, 15H).

[0358] **Example 2.9: Synthesis of Compound 19**

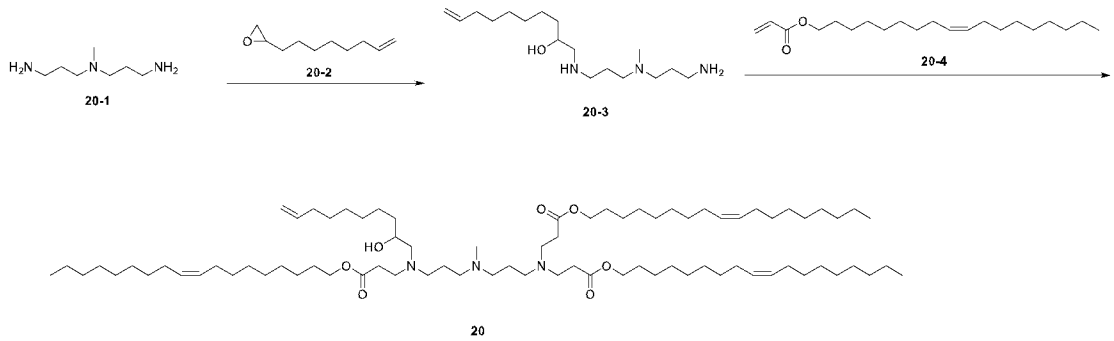


[0359] **Step 1:** compound **19-3** was prepared by the method of Example 2.8 Step 1.

[0360] **Step 2:** to compound **19-3** (20 mg, 0.07 mmol) in a 2 mL container were added compound **19-4** (85.1 mg, 0.26 mmol) and BHT (14.3 mg). The mixture was heated at 70 ℃ for 60 hours. TLC (eluent: DCM/MeOH=20/1) indicated substantial completion of reaction. The mixture was purified by flash column chromatography on silica (eluent: DCM with 0-5% methanol, v/v) and concentrated under reduced pressure to afford compound **19** (54.1 mg, 62% yield).

[0361] ^{1}H NMR of compound **19**: (400 MHz, Chloroform-d) δ 4.05 (q, J = 6.6 Hz, 5H), 3.60 (s, 1H), 3.49 (t, J = 6.2 Hz, 4H), 3.00 (s, 1H), 2.84 (t, J = 7.3 Hz, 5H), 2.67 (t, J = 6.0 Hz, 3H), 2.46 (t, J = 7.3 Hz, 7H), 2.39 – 2.26 (m, 2H), 1.61 (h, J = 6.1 Hz, 7H), 1.46 – 1.39 (m, 4H), 1.26 (s, 97H), 0.88 (t, J = 6.7 Hz, 12H).

[0362] **Example 2.10: Synthesis of Compound 20**



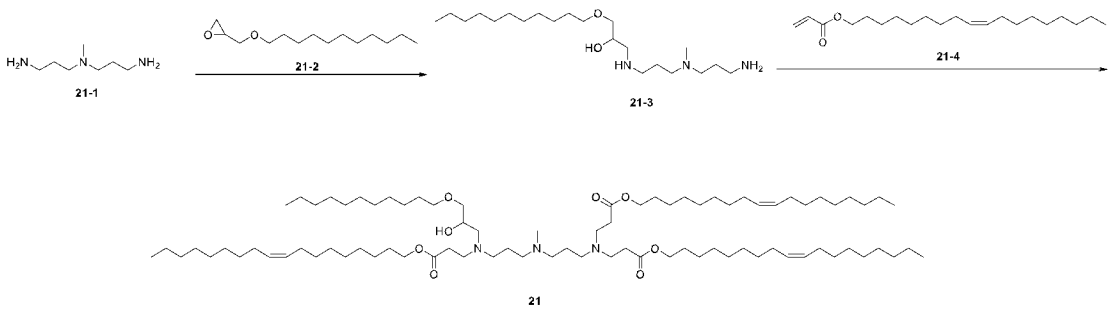
[0363] **Step 1:** compound **20-3** was prepared by the methods of Example 2.2 Step 2.

[0364] **Step 2:** to compound **20-3** (80 mg, 0.27 mmol) in a 4 mL container were added compound **20-4** (310 mg, 0.96 mmol, prepared by the method of Example 2.1 Step 1) and BHT (5 mg) under N_2 atmosphere. The mixture was heated at 70 ℃ for 48

hours. TLC indicated completion of reaction. The mixture was purified by column chromatography on silica (eluent: DCM with 0-10% methanol, v/v) and concentrated under reduced pressure to afford compound **20** (230 mg, 68% yield).

[0365] ^{1}H NMR of compound **20**: (400 MHz, Chloroform-d) δ 5.88 – 5.71 (m, 1H), 5.49 – 5.25 (m, 6H), 5.05 – 4.81 (m, 2H), 4.05 (q, J = 6.4 Hz, 7H), 3.58 (td, J = 7.0, 3.6 Hz, 1H), 2.90 (dt, J = 14.5, 7.4 Hz, 1H), 2.73 (dt, J = 25.2, 6.9 Hz, 6H), 2.57 (dt, J = 14.4, 7.3 Hz, 2H), 2.43 (q, J = 7.0, 6.4 Hz, 13H), 2.36 – 1.91 (m, 27H), 1.61 (t, J = 7.1 Hz, 12H), 1.30 (ddd, J = 17.8, 10.8, 5.4 Hz, 96H), 0.88 (t, J = 6.7 Hz, 12H).

[0366] **Example 2.11: Synthesis of Compound 21**

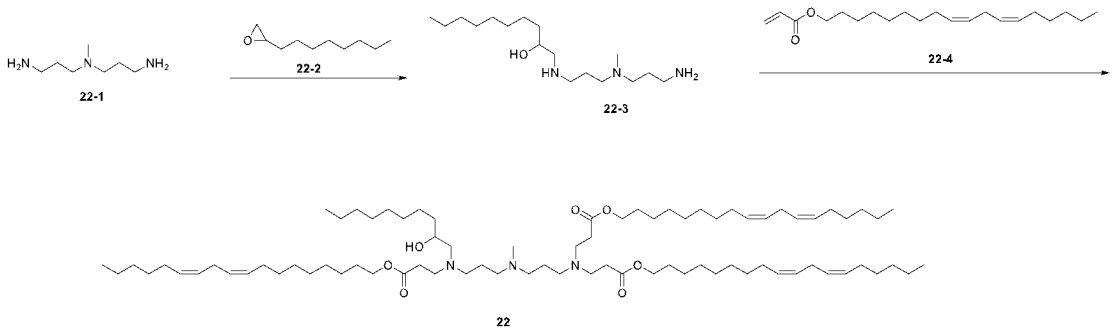


[0367] **Step 1:** to a solution of compound **21-1** (1.44 g, 9.91 mmol) in ethanol (100 mL) heated at 65 ℃ (internal), compound **21-2** (0.40 g, 1.75 mmol) was added. The mixture was heated at 70-75℃ (external) overnight. The mixture was cooled to room temperature then concentrated under reduced pressure. The residue was diluted with DCM (200 mL), and water (200 mL) was added thereto under stirring. The mixture was heated at 35 ℃ (internal) and stirred for 10 minutes then let stand and separated. Extraction was repeated for 3-4 times. TLC (eluent: DCM/MeOH=5:1, with 3 drop of NH_4OH) indicated completion of extraction. Combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford compound **21-3** (0.43 g, 66% yield).

[0368] **Step 2:** to compound **21-3** (100 mg, 0.27 mmol) in a 4 mL container were added compound **21-4** (270 mg, 0.94 mmol, prepared by the method of Example 2.1 Step 1) and BHT (5 mg) under N_2 atmosphere. The mixture was heated at 70 ℃ for 48 hours. TLC indicated completion of reaction. The mixture was purified by column chromatography on silica (eluent: DCM with 0-10% methanol, v/v) and concentrated under reduced pressure to afford compound **21** (220 mg, 67% yield).

[0369] ^{1}H NMR of compound **21**: (400 MHz, Chloroform-d) δ 5.45 – 5.21 (m, 5H), 4.05 (td, J = 6.8, 4.3 Hz, 6H), 3.89 – 3.74 (m, 1H), 3.53 – 3.34 (m, 4H), 2.98 – 2.82 (m, 1H), 2.74 (td, J = 7.0, 4.5 Hz, 6H), 2.62 – 2.35 (m, 16H), 2.00 (hept, J = 5.9, 5.2 Hz, 12H), 1.58 (dt, J = 18.9, 7.0 Hz, ^{12H}), 1.47 – 1.07 (m, 90H), 0.87 (t, J = 6.7 Hz, 13H).

[0370] **Example 2.12: Synthesis of Compound 22**

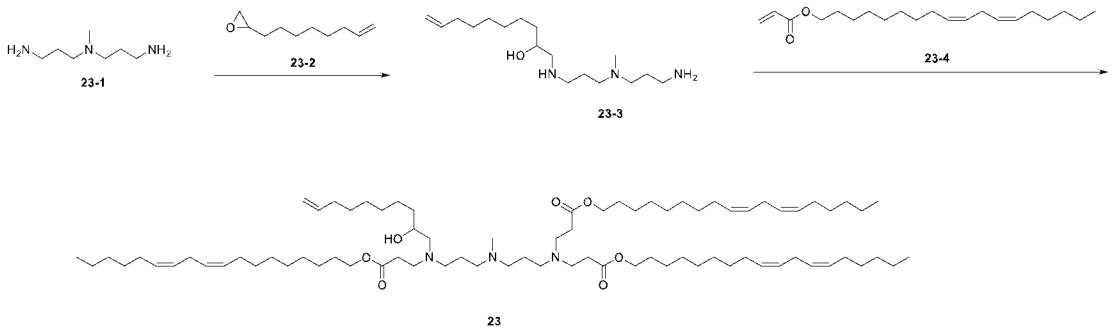


[0371] **Step 1:** compound **22-3** was prepared by the method of Example 2.1 Step 2.

[0372] **Step 2:** to compound **22-3** (100 mg, 0.33 mmol) in a 4 mL container were added compound **22-4** (350 mg, 1.09 mmol) and BHT (10 mg) under N_2 atmosphere. The mixture was heated at 70 ℃ for 48 hours. TLC indicated completion of reaction. The mixture was purified by column chromatography on silica (eluent: DCM with 0-10% methanol, v/v) and concentrated under reduced pressure to afford compound **22** (230 mg, 55% yield).

[0373] ^{1}H NMR of compound **22**: (400 MHz, Chloroform-d) δ 5.80 (ddt, J = 16.9, 10.2, 6.7 Hz, 1H), 5.46 – 5.23 (m, 13H), 5.05 – 4.88 (m, 2H), 4.17 – 3.94 (m, 6H), 3.58 (ddt, J = 10.4, 6.9, 3.4 Hz, 1H), 3.02 – 2.83 (m, 1H), 2.82 – 2.65 (m, 12H), 2.56 (q, J = 7.4, 6.8 Hz, 2H), 2.51 – 2.11 (m, 21H), 2.05 (q, J = 6.7 Hz, 16H), 1.61 (dd, J = 10.6, 4.3 Hz, 12H), 1.49 – 1.10 (m, 71H), 0.89 (t, J = 6.8 Hz, 12H).

[0374] **Example 2.13: Synthesis of Compound 23**

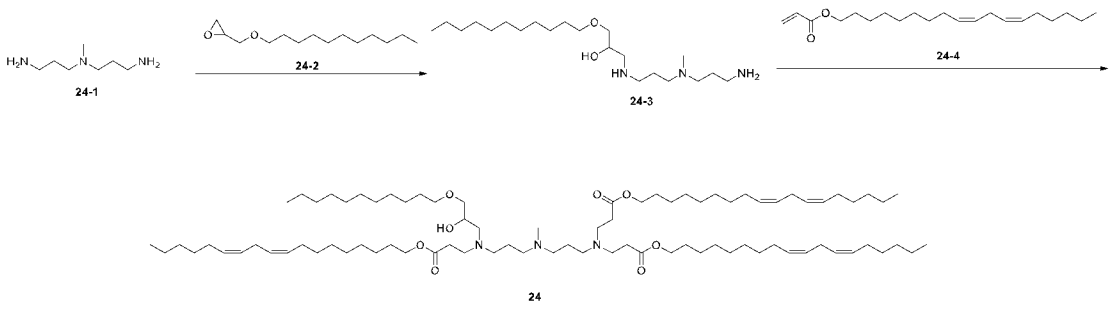


[0375] **Step 1:** compound **23-3** was prepared by the methods of Example 2.2 Step 2.

[0376] **Step 2:** to compound **23-3** (100 mg, 0.33 mmol) in a 4 mL container were added compound **23-4** (360 mg, 1.12 mmol) and BHT (10 mg) under N_2 atmosphere. The mixture was heated at 70 ℃ for 48 hours. TLC indicated completion of reaction. The mixture was purified by column chromatography on silica (eluent: DCM with 0-10% methanol, v/v) and concentrated under reduced pressure to afford compound **23** (221 mg, 56% yield).

[0377] ^{1}H NMR of compound **23**: (400 MHz, Chloroform-d) δ 5.45 – 5.26 (m, 12H), 4.05 (q, J = 7.1 Hz, 6H), 3.60 (s, 1H), 2.93 (dt, J = 13.9, 7.5 Hz, 3H), 2.75 (dt, J = 17.7, 6.7 Hz, 15H), 2.54 – 2.20 (m, 14H), 2.05 (q, J = 6.8 Hz, 14H), 1.88 (s, 5H), 1.62 (p, J = 6.7 Hz, 9H), 1.46 – 1.19 (m, 72H), 0.88 (td, J = 6.8, 4.5 Hz, 14H).

[0378] **Example 2.14: Synthesis of Compound 24**



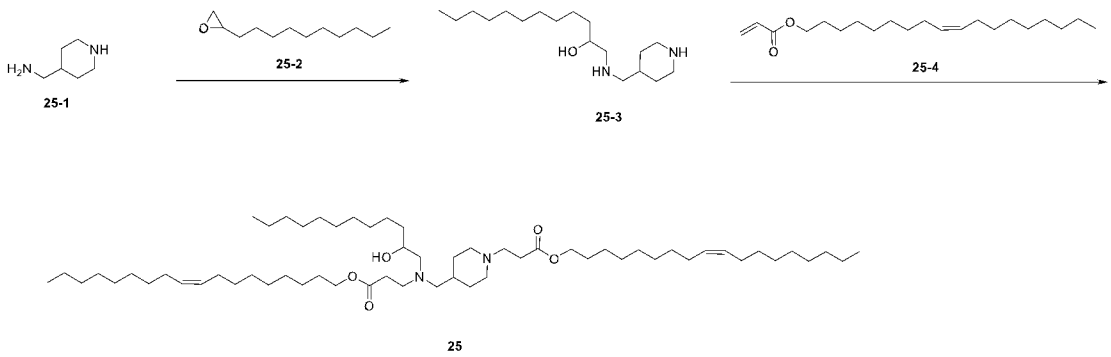
[0379] **Step 1:** compound **24-3** was prepared by the method of Example 2.11 Step 1.

[0380] **Step 2:** to compound **24-3** (120 mg, 0.32 mmol) in a 4 mL container were added compound **24-4** (350 mg, 1.09 mmol) and BHT (10 mg) under N_2 atmosphere. The mixture was heated at 70 ℃ for 48 hours. TLC indicated completion of reaction. The mixture was purified by column chromatography on silica (eluent:

DCM with 0-10% methanol, v/v) and concentrated under reduced pressure to afford compound **24** (205 mg, 50% yield).

[0381] ^{1}H NMR of compound **24**: (400 MHz, Chloroform-d) δ 5.48 – 5.24 (m, 14H), 4.06 (q, J = 7.2 Hz, 6H), 3.79 (dq, J = 9.9, 5.1 Hz, 1H), 2.87 (dq, J = 17.0, 9.7, 8.5 Hz, 3H), 2.77 (t, J = 6.4 Hz, 7H), 2.68 (dt, J = 13.0, 6.3 Hz, 5H), 2.44 (dt, J = 13.2, 7.7 Hz, 7H), 2.29 (d, J = 6.9 Hz, 4H), 2.13 – 2.03 (m, 12H), 1.59 (dt, J = 17.9, 6.9 Hz, 18H), 1.43 – 1.20 (m, 88H), 0.88 (td, J = 6.8, 4.1 Hz, 16H).

[0382] **Example 2.15: Synthesis of Compound 25**



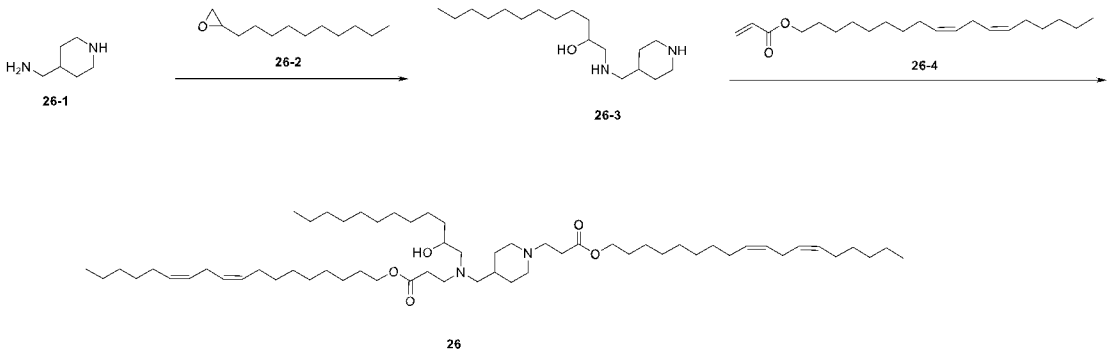
[0383] **Step 1:** to a solution of compound **25-1** (1.0 g, 8.76 mmol) in ethanol (100 mL) heated at 65 ℃ (internal), compound **25-2** (0.27 g, 1.46mmol) was added. The mixture was heated at 70-75℃ (external) overnight. The mixture was cooled to room temperature then concentrated under reduced pressure. The residue was diluted with DCM (200 mL), and dilute aqueous NaOH solution (200 mL) was added thereto under stirring. The mixture was heated at 35 ℃ (internal) and stirred for 10 minutes then let stand and separated. Extraction was repeated for 3-4 times. Combined organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford compound **25-3** (0.26 g, 60% yield).

[0384] **Step 2:** to compound **25-3** (120 mg, 0.40 mmol) in a 4 mL container were added compound **25-4** (350 mg, 0.96 mmol, prepared by the method of Example 2.1 Step 1) and BHT (10 mg) under N_2 atmosphere. The mixture was heated at 70 ℃ for 48 hours. TLC indicated completion of reaction. The mixture was purified by column chromatography on silica (eluent: DCM with 0-10% methanol, v/v) and concentrated under reduced pressure to afford compound **25** (215 mg, 56% yield).

[0385] ^{1}H NMR of compound **25**: (400 MHz, Chloroform-d) δ 5.51 – 5.21 (m, 5H), 4.05 (t, J = 7.1 Hz, 6H), 3.57 (s, 2H), 2.90 (dt, J = 25.7, 9.3 Hz, 5H), 2.64 (dt, J =

24.3, 7.2 Hz, 5H), 2.53 – 2.18 (m, 14H), 1.99 (dt, J = 16.0, 8.3 Hz, 15H), 1.76 (t, J = 14.1 Hz, 5H), 1.61 (t, J = 7.1 Hz, 10H), 1.49 – 1.00 (m, 106H), 0.87 (t, J = 6.6 Hz, 15H).

[0386] **Example 2.16: Synthesis of Compound 26**

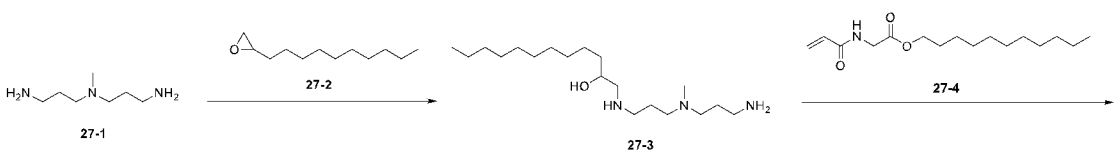


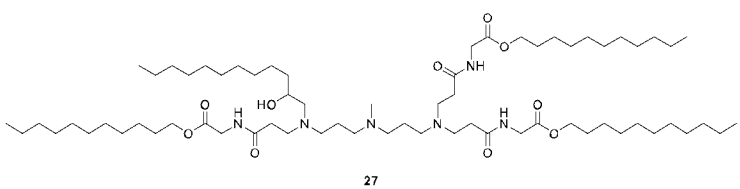
[0387] **Step 1:** compound **26-3** was prepared by the method of Example 2.15 Step 1.

[0388] **Step 2:** to compound **26-3** (120 mg, 0.40 mmol) in a 4 mL container were added compound **26-4** (309 mg, 0.96 mmol) and BHT (10 mg) under N_2 atmosphere. The mixture was heated at 70 ℃ for 48 hours. TLC indicated completion of reaction. The mixture was purified by column chromatography on silica (eluent: DCM with 0-6.3% methanol, v/v) and concentrated under reduced pressure to afford compound **26** (260 mg, 69% yield).

[0389] ^{1}H NMR of compound **26**: (400 MHz, Chloroform-d) δ 5.35 (tt, J = 11.3, 6.3 Hz, 8H), 4.06 (q, J = 6.8 Hz, 4H), 3.66 – 3.48 (m, 1H), 2.92 (dt, J = 13.1, 7.6 Hz, 2H), 2.77 (t, J = 6.5 Hz, 5H), 2.61 (dd, J = 12.6, 6.4 Hz, 3H), 2.48 – 2.22 (m, 7H), 2.05 (q, J = 6.8 Hz, 10H), 1.86 (d, J = 13.5 Hz, 2H), 1.62 (p, J = 7.1 Hz, 9H), 1.49 – 1.14 (m, 59H), 0.88 (td, J = 6.8, 4.3 Hz, 10H).

[0390] **Example 2.17: Synthesis of Compound 27**



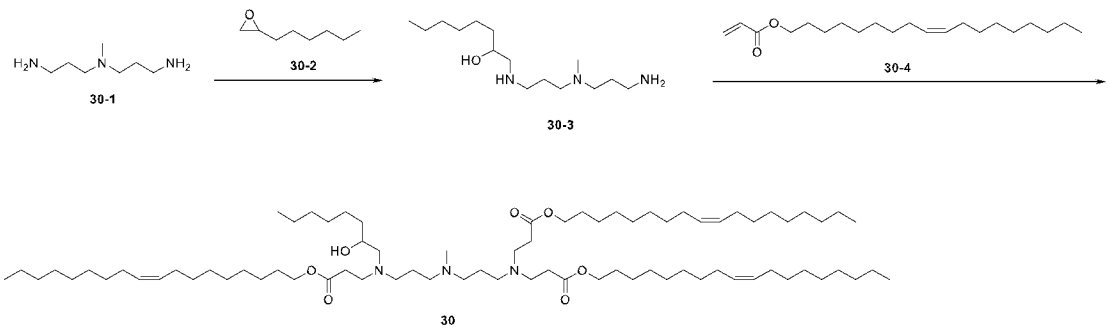


[0391] **Step 1:** compound **27-3** was prepared by the method of Example 2.3 Step 1.

[0392] **Step 2:** to compound **27-3** (400 mg, 1.2 mmol), BHT (53.4 mg) and compound **27-4** (1.24 g, 4.4 mmol, prepared by the method of Example 1.7 Step 1 to 3) in a 2 mL container was added acetic acid (14.6 mg). The mixture was heated at 80 ℃ for 42 hours. TLC (eluent: DCM/MeOH/NH_4OH=10/1/1) indicated substantial completion of reaction. The mixture was purified by flash column chromatography on silica (eluent: DCM with 1.4-24% methanol and 0.5% NH_4OH, v/v) and concentrated under reduced pressure to afford compound **27** (390 mg, 27% yield).

[0393] ^{1}H NMR of compound **27**: (400 MHz, Chloroform-d) δ 7.87 (t, J = 5.5 Hz, 1H), 7.68 (t, J = 5.5 Hz, 2H), 4.15-4.01(m, 6H), 4.01-3.88 (m, 4H), 3.68 (dt, J = 17.8, 8.6 Hz, 1H), 3.03-2.91 (m, 1H), 2.74 (t, J = 6.0 Hz, 4H), 2.66 (dt, J = 14.1, 7.3 Hz, 2H), 2.59-2.39 (m, 9H), 2.40 – 2.19 (m, 5H), 1.81-1.66 (m, 4H), 1.62 (p, J = 6.9 Hz, 6H), 1.52-1.04 (m, 68H), 0.87 (q, J = 5.9, 5.5 Hz, 12H). HR-MS: $[M+H]^+$ = 1180.00680.

[0394] **Example 2.18: Synthesis of Compound 30**



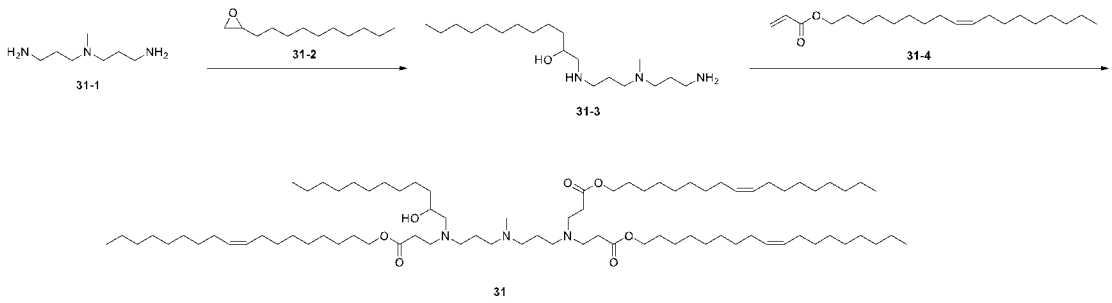
[0395] **Step 1:** compound **30-3** was prepared by similar method as Example 2.1 Step 2.

[0396] **Step 2:** to compound **30-3** (80 mg, 0.29 mmol) in a 4 mL container were added BHT (5 mg) and compound **30-4** (340 mg, 1.05 mmol, prepared by the method of Example 2.1 Step 1) under N_2 atmosphere. The mixture was heated at 70 ℃ for 48 hours. TLC indicated completion of reaction. The mixture was purified by flash column chromatography on silica (eluent: DCM with 0-10% methanol, v/v) and concentrated under reduced pressure to afford compound **30** (295 mg, 81% yield).

[0397] ^{1}H NMR of compound **30**: (400 MHz, Chloroform-d) δ 5.44 – 5.26 (m, 5H), 4.05 (q, J = 6.4 Hz, 6H), 3.58 (dd, J = 6.9, 3.4 Hz, 1H), 2.97 – 2.83 (m, 1H), 2.72

(dt, J = 24.8, 7.0 Hz, 5H), 2.57 (dt, J = 14.3, 7.3 Hz, 1H), 2.01 (q, J = 6.4 Hz, 12H), 1.61 (t, J = 7.0 Hz, 10H), 1.28 (q, J = 8.2, 4.7 Hz, 91H), 0.87 (t, J = 6.6 Hz, 14H). HR-MS: $[M+H]^+$=1240.15427.

[0398] Example 2.19: Synthesis of Compound 31

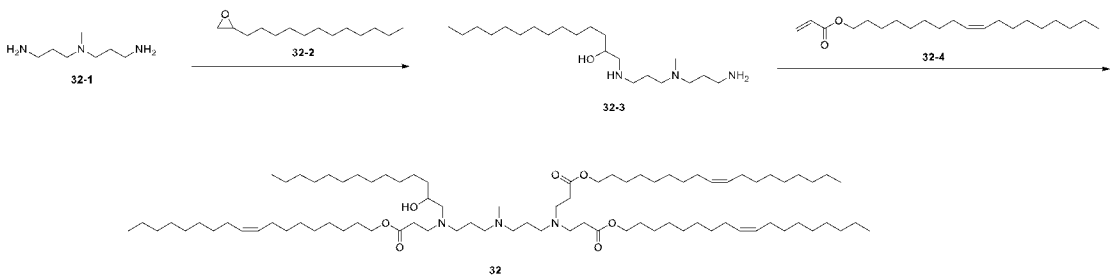


[0399] **Step 1:** compound **31-3** was prepared by the method of Example 2.3 Step 1.

[0400] **Step 2:** to compound **31-3** (80 mg, 0.24 mmol) in a 4 mL container were added BHT (5 mg) and compound **31-4** (308 mg, 0.95 mmol, prepared by the method of Example 2.1 Step 1) under N_2 atmosphere. The mixture was heated at 70 ℃ for 48 hours. TLC indicated completion of reaction. The mixture was purified by flash column chromatography on silica (eluent: DCM with 0-10% methanol, v/v) and concentrated under reduced pressure to afford compound **31** (250 mg, 74% yield).

[0401] ^{1}H NMR of compound **31**: (400 MHz, Chloroform-d) δ 5.49 – 5.22 (m, 6H), 4.05 (q, J = 6.5 Hz, 6H), 3.58 (td, J = 6.8, 3.4 Hz, 1H), 2.91 (dt, J = 14.3, 7.5 Hz, 1H), 2.72 (dt, J = 23.7, 6.9 Hz, 6H), 2.43 (q, J = 7.6 Hz, 13H), 2.34 – 2.12 (m, 6H), 2.00 (dh, J = 11.6, 6.5 Hz, 11H), 1.63 (s, 10H), 1.30 (dt, J = 16.3, 9.4 Hz, 91H), 0.88 (t, J = 6.7 Hz, 13H). $[M+H]^+$ = 1297.21294.

[0402] Example 2.20: Synthesis of Compound 32

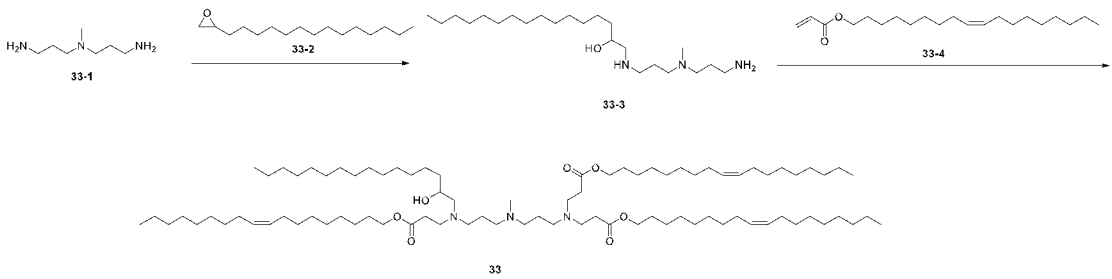


[0403] **Step 1:** compound **32-3** was prepared by similar method as Example 2.3 Step 1.

[0404] **Step 2:** to compound **32-3** (80 mg, 0.22 mmol) in a 4 mL container were added BHT (5 mg) and compound **32-4** (259 mg, 0.80 mmol, prepared by the method of Example 2.1 Step 1) under N_2 atmosphere. The mixture was heated at 70 ℃ for 48 hours. TLC indicated completion of reaction. The mixture was purified by flash column chromatography on silica (eluent: DCM with 0-10% methanol, v/v) and concentrated under reduced pressure to afford compound **32** (220 mg, 74% yield).

[0405] ^{1}H NMR of compound **32**: (400 MHz, Chloroform-d) δ 5.45 – 5.23 (m, 6H), 4.05 (q, J = 6.5 Hz, 6H), 3.58 (td, J = 6.7, 3.4 Hz, 1H), 2.90 (dt, J = 14.3, 7.5 Hz, 1H), 2.80 – 2.36 (m, 19H), 2.33 – 1.87 (m, 20H), 1.60 (q, J = 7.0 Hz, 11H), 1.51 – 1.06 (m, 102H), 0.88 (t, J = 6.7 Hz, 14H). $[M+H]^+$=1325.24099.

[0406] **Example 2.21: Synthesis of Compound 33**

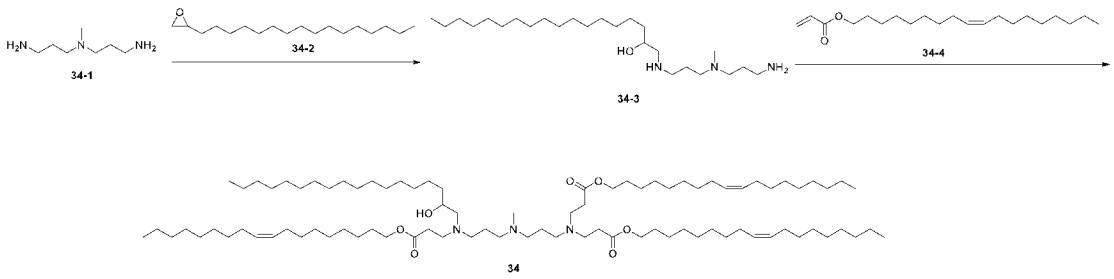


[0407] **Step 1:** compound **33-3** was prepared by similar method as Example 2.3 Step 1.

[0408] **Step 2:** to compound **33-3** (100 mg, 0.26 mmol) in a 4 mL container were added BHT (5 mg) and compound **33-4** (301 mg, 0.93 mmol, prepared by the method of Example 2.1 Step 1) under N_2 atmosphere. The mixture was heated at 70 ℃ for 48 hours. TLC indicated completion of reaction. The mixture was purified by flash column chromatography on silica (eluent: DCM with 0-10% methanol, v/v) and concentrated under reduced pressure to afford compound **33** (250 mg, 69% yield).

[0409] ^{1}H NMR of compound **33**: (400 MHz, Chloroform-d) δ 5.49 – 5.12 (m, 5H), 4.05 (q, J = 6.6 Hz, 6H), 3.58 (td, J = 6.9, 3.5 Hz, 1H), 2.91 (dt, J = 14.3, 7.5 Hz, 1H), 2.72 (dt, J = 23.2, 6.8 Hz, 6H), 2.64 – 2.52 (m, 2H), 2.52 – 2.19 (m, 17H), 2.01 (q, J = 6.4 Hz, 14H), 1.61 (t, J = 7.0 Hz, 11H), 1.28 (dd, J = 18.7, 7.3 Hz, 99H), 0.88 (t, J = 6.7 Hz, 13H).$[M+H]^+$ = 1297.21294.

[0410] **Example 2.22: Synthesis of Compound 34**

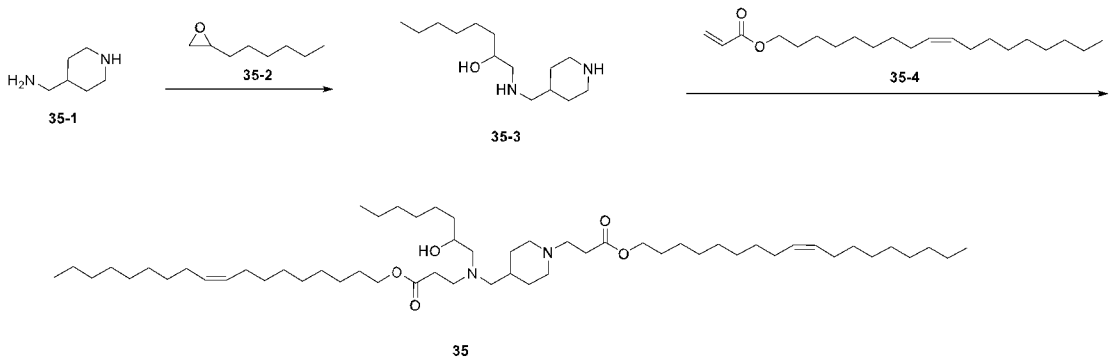


[0411] **Step 1:** compound **34-3** was prepared by similar method as Example 2.3 Step 1.

[0412] **Step 2:** to compound **34-3** (100 mg, 0.24 mmol) in a 4 mL container were added BHT (5 mg) and compound **34-4** (280 mg, 0.87 mmol, prepared by the method of Example 2.1 Step 1) under N_2 atmosphere. The mixture was heated at 70 ℃ for 48 hours. TLC indicated completion of reaction. The mixture was purified by flash column chromatography on silica (eluent: DCM with 0-10% methanol, v/v) and concentrated under reduced pressure to afford compound **34** (197 mg, 59% yield).

[0413] ^{1}H NMR of compound **34**: (400 MHz, Chloroform-d) δ 5.48 – 5.23 (m, 5H), 4.05 (q, J = 6.6 Hz, 6H), 3.58 (td, J = 7.5, 7.1, 3.7 Hz, 1H), 2.91 (dt, J = 14.5, 7.5 Hz, 1H), 2.83 – 2.14 (m, 26H), 2.01 (q, J = 6.4 Hz, 12H), 1.80 – 1.53 (m, 10H), 1.28 (dd, J = 20.0, 7.6 Hz, 109H), 0.87 (t, J = 6.7 Hz, 14H). $[M+H]^+$ = 1381.31640.

[0414] **Example 2.23: Synthesis of Compound 35**



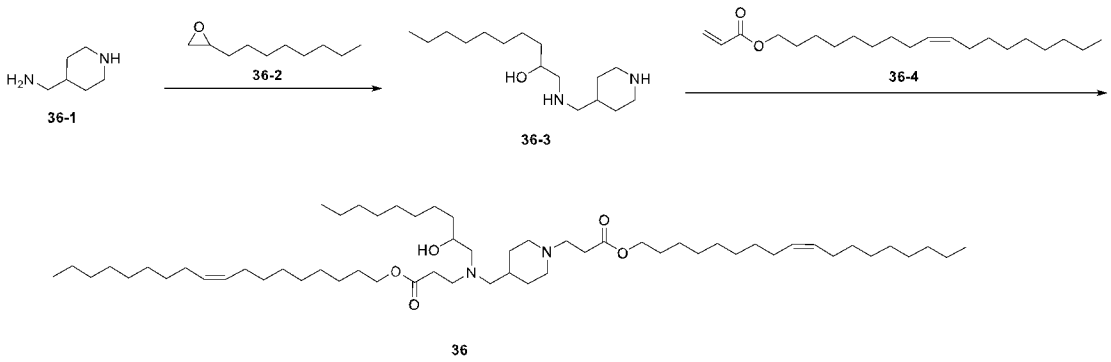
[0415] **Step 1:** compound **35-3** was prepared by similar method as Example 2.15 Step 1.

[0416] **Step 2:** to compound **35-3** (120 mg, 0.50 mmol) in a 4 mL container were added BHT (5 mg) and compound **35-4** (383 mg, 1.19 mmol, prepared by the method of Example 2.1 Step 1) under N_2 atmosphere. The mixture was heated at 70 ℃ for 48 hours. TLC indicated completion of reaction. The mixture was purified by flash

column chromatography on silica (eluent: DCM with 0-10% methanol, v/v) and concentrated under reduced pressure to afford compound **35** (188 mg, 42% yield).

[0417] ^{1}H NMR of compound **35**: (400 MHz, Chloroform-d) δ 5.51 – 5.21 (m, 6H), 4.05 (td, J = 6.8, 2.8 Hz, 6H), 3.57 (ddt, J = 10.4, 6.7, 3.4 Hz, 2H), 2.90 (ddt, J = 15.1, 11.7, 5.8 Hz, 5H), 2.75 – 2.17 (m, 17H), 2.01 (q, J = 5.9 Hz, 14H), 1.82 – 1.03 (m, 102H), 0.88 (t, J = 6.6 Hz, 14H). $[M+H]^+$=887.82994.

[0418] **Example 2.24: Synthesis of Compound 36**

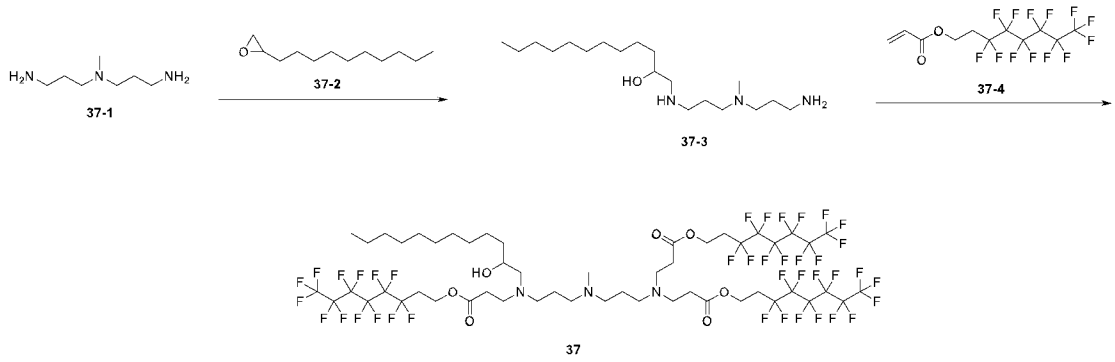


[0419] **Step 1:** compound **36-3** was prepared by similar method as Example 2.15 Step 1.

[0420] **Step 2:** to compound **36-3** (120 mg, 0.44 mmol) in a 4 mL container were added BHT (5 mg) and compound **36-4** (343 mg, 1.06 mmol, prepared by the method of Example 2.1 Step 1) under N_2 atmosphere. The mixture was heated at 70 ℃ for 48 hours. TLC indicated completion of reaction. The mixture was purified by flash column chromatography on silica (eluent: DCM with 0-10% methanol, v/v) and concentrated under reduced pressure to afford compound **36** (191 mg, 47% yield).

[0421] ^{1}H NMR of compound **36**: (400 MHz, Chloroform-d) δ 5.45 – 5.27 (m, 5H), 4.05 (td, J = 6.8, 2.7 Hz, 6H), 3.64 – 3.30 (m, 2H), 2.90 (ddt, J = 22.2, 11.1, 5.7 Hz, 5H), 2.72 – 2.18 (m, 18H), 1.99 (qd, J = 12.0, 11.2, 7.3 Hz, 15H), 1.83 – 1.55 (m, 15H), 1.52 – 1.06 (m, 105H), 0.87 (t, J = 6.7 Hz, 15H). $[M+H]^+$=915.84710.

[0422] **Example 2.25: Synthesis of Compound 37**

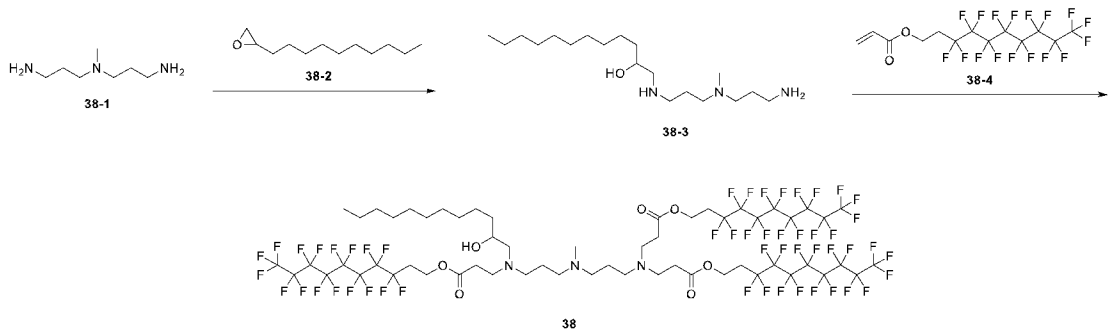


[0423] **Step 1:** compound **37-3** was prepared by similar method as Example 2.3 Step 1.

[0424] **Step 2:** to compound **37-3** (30 mg, 0.09 mmol) in a vial were added BHT (1 mg), compound **37-4** (137 mg, 0.32 mmol) and ACN (0.3 mL). The mixture was vented and decanted for N_2 atmosphere thrice. The mixture was heated at 70 ℃ for 2-3 days. TLC indicated completion of reaction. The mixture was purified by column chromatography on silica (eluent: DCM with 0-3% methanol, v/v) and concentrated under reduced pressure to afford compound **37** (110 mg, 76% yield).

[0425] ^{1}H NMR of compound **37**: (400 MHz, Chloroform-d) δ 4.37 (q, J = 6.3 Hz, 6H), 3.57 (s, 1H), 2.91 (dt, J = 14.3, 7.4 Hz, 1H), 2.83 – 2.63 (m, 5H), 2.63 – 2.08 (m, 25H), 1.25 (s, 23H), 0.87 (t, J = 6.8 Hz, 3H).

[0426] **Example 2.26: Synthesis of Compound 38**



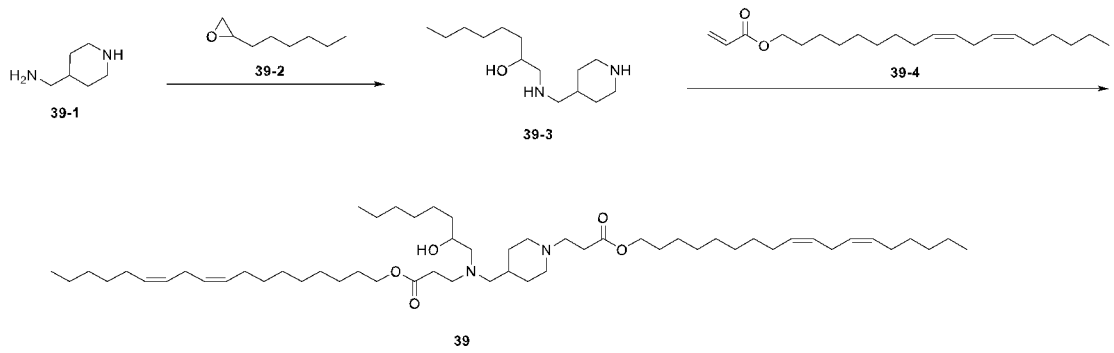
[0427] **Step 1:** compound **38-3** was prepared by similar method as Example 2.3 Step 1.

[0428] **Step 2:** to compound **38-3** (30 mg, 0.09 mmol) in a vial were added BHT (1 mg), compound **38-4** (170 mg, 0.33 mmol) and ACN (0.3 mL). The mixture was vented and decanted for N_2 atmosphere thrice. The mixture was heated at 70 ℃ for 2-3 days. TLC indicated completion of reaction. The mixture was purified by column

chromatography on silica (eluent: DCM with 0-3% methanol, v/v) and concentrated under reduced pressure to afford compound **38** (100 mg, 58% yield).

[0429] ^{1}H NMR of compound **38**: (400 MHz, Chloroform-d) δ 4.37 (q, J = 6.4 Hz, 6H), 3.57 (s, 1H), 2.91 (dt, J = 14.0, 7.4 Hz, 1H), 2.78 – 2.66 (m, 5H), 2.56 – 2.15 (m, 25H), 1.25 (s, 24H), 0.88 – 0.84 (m, 3H).

[0430] **Example 2.27: Synthesis of Compound 39**

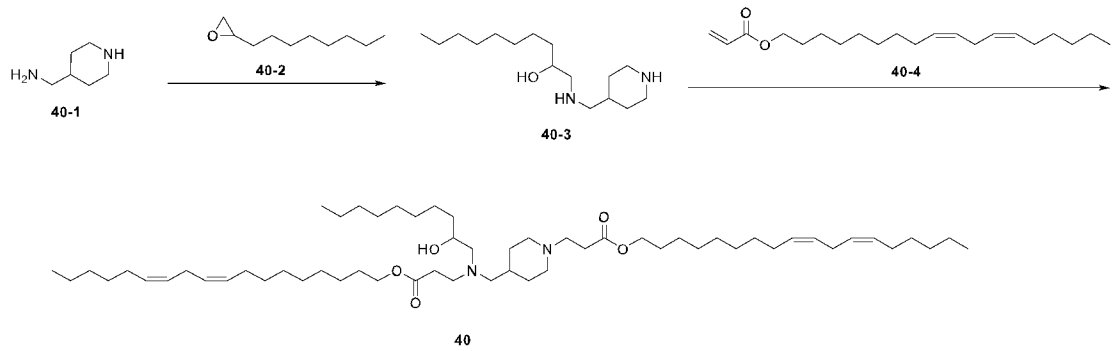


[0431] **Step 1:** compound **39-3** was prepared by similar method as Example 2.15 Step 1.

[0432] **Step 2:** to compound **39-3** (120 mg, 0.50 mmol) in a 5 mL container were added BHT (10.8 mg), compound **39-4** (380.8 mg, 1.19 mmol) at room temperature. The mixture was heated at 70 ℃ for 40 hours. TLC (eluent: DCM/MeOH=15/1) indicated substantial completion of reaction. The mixture was purified by automatic column chromatography on silica (eluent: DCM with 0-3% methanol, v/v) and concentrated under reduced pressure to afford compound **39** (190 mg, 43% yield).

[0433] ^{1}H NMR of compound **39**: (400 MHz, Chloroform-*d*) δ 5.45 – 5.25 (m, 8H), 4.06 (q, *J* = 6.7 Hz, 4H), 3.58 (td, *J* = 8.4, 7.5, 4.1 Hz, 1H), 2.92 (dt, *J* = 13.1, 7.6 Hz, 2H), 2.77 (t, *J* = 6.5 Hz, 5H), 2.60 (p, *J* = 5.6 Hz, 2H), 2.48 – 2.19 (m, 7H), 2.05 (q, *J* = 6.8 Hz, 9H), 1.85 (d, *J* = 12.7 Hz, 1H), 1.62 (p, *J* = 6.9 Hz, 7H), 1.54 – 1.42 (m, 3H), 1.40 – 1.21 (m, 46H), 0.89 (td, *J* = 6.8, 3.7 Hz, 10H). $[M+H]^+$=883.80336.

[0434] **Example 2.28: Synthesis of Compound 40**

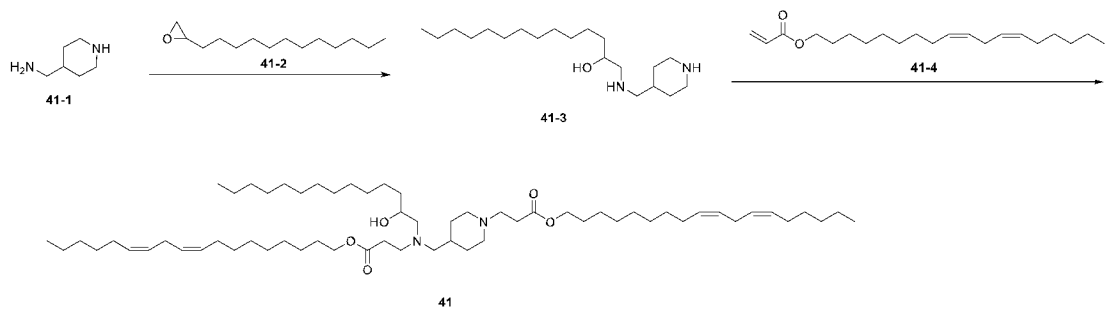


[0435] **Step 1:** compound **40-3** was prepared by similar method as Example 2.15 Step 1.

[0436] **Step 2:** to compound **40-3** (120 mg, 0.44 mmol) in a 5 mL container were added BHT (10.8 mg), compound **40-4** (341.3 mg, 1.06 mmol) at room temperature. The mixture was heated at 70 ℃ for 40 hours. TLC (eluent: DCM/MeOH=15/1) indicated substantial completion of reaction. The mixture was purified by automatic column chromatography on silica (eluent: DCM with 0-3% methanol, v/v) and concentrated under reduced pressure to afford compound **40** (250 mg, 62% yield).

[0437] ^{1}H NMR of compound **40**: (400 MHz, Chloroform-d) δ 5.45 – 5.23 (m, 8H), 4.06 (q, J = 6.7 Hz, 4H), 3.58 (td, J = 6.8, 3.5 Hz, 1H), 2.92 (dt, J = 13.1, 7.6 Hz, 2H), 2.77 (t, J = 6.5 Hz, 5H), 2.60 (dt, J = 12.6, 5.1 Hz, 2H), 2.48 – 2.19 (m, 7H), 2.05 (q, J = 6.8 Hz, 9H), 1.92 – 1.80 (m, 1H), 1.62 (p, J = 6.9 Hz, 7H), 1.54 – 1.16 (m, 55H), 0.88 (td, J = 6.8, 4.4 Hz, 10H). $[M+H]^+$=911.82438.

[0438] **Example 2.29: Synthesis of Compound 41**



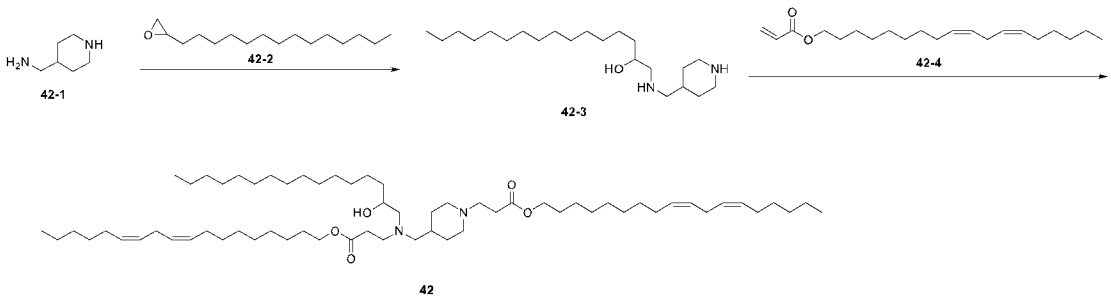
[0439] **Step 1:** compound **41-3** was prepared by similar method as Example 2.15 Step 1.

[0440] **Step 2:** to compound **41-3** (120 mg, 0.37 mmol) in a 5 mL container were added BHT (10.8 mg), compound **41-4** (282.6 mg, 0.88 mmol) at room

temperature. The mixture was heated at 70 ℃ for 40 hours. TLC (eluent: DCM/MeOH=15/1) indicated substantial completion of reaction. The mixture was purified by automatic column chromatography on silica (eluent: DCM with 0-3% methanol, v/v) and concentrated under reduced pressure to afford compound **41** (170 mg, 48% yield).

[0441] ^{1}H NMR of compound **41**: (400 MHz, Chloroform-*d*) δ 5.46 – 5.26 (m, 9H), 4.12 – 4.01 (m, 4H), 3.58 (s, 1H), 3.30 (s, 1H), 2.92 (dt, *J* = 14.4, 7.6 Hz, 2H), 2.77 (t, *J* = 6.5 Hz, 5H), 2.66 – 2.53 (m, 2H), 2.47 – 2.19 (m, 8H), 2.05 (q, *J* = 6.9 Hz, 9H), 1.89 (s, 1H), 1.61 (dd, *J* = 13.4, 6.6 Hz, 13H), 1.47 – 1.17 (m, 63H), 0.88 (td, *J* = 6.8, 4.1 Hz, 10H). $[M+H]^+$=967.88990.

[0442] **Example 2.30: Synthesis of Compound 42**

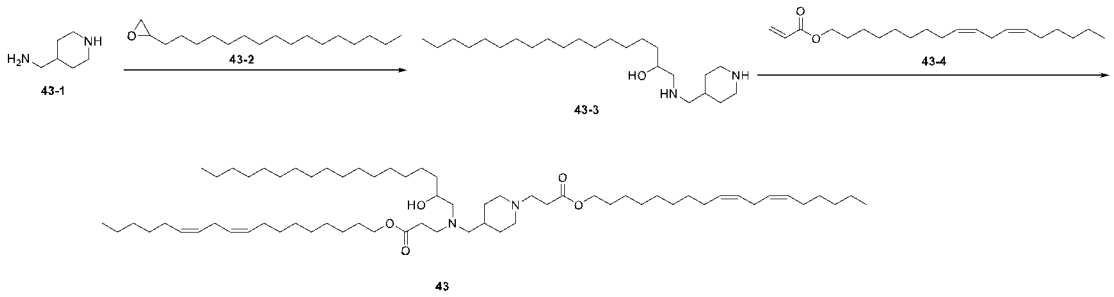


[0443] **Step 1:** compound **42-3** was prepared by similar method as Example 2.15 Step 1.

[0444] **Step 2:** to compound **42-3** (120 mg, 0.34 mmol) in a 5 mL container were added BHT (10.8 mg), compound **42-4** (260.3 mg, 0.81 mmol) at room temperature. The mixture was heated at 70 ℃ for 40 hours. TLC (eluent: DCM/MeOH=15/1) indicated substantial completion of reaction. The mixture was purified by automatic column chromatography on silica (eluent: DCM with 0-3% methanol, v/v) and concentrated under reduced pressure to afford compound **42** (203.7 mg, 60% yield).

[0445] ^{1}H NMR of compound **42**: (400 MHz, Chloroform-d) δ 5.46 – 5.24 (m, 9H), 4.06 (q, J = 6.9 Hz, 4H), 3.58 (tt, J = 7.4, 3.6 Hz, 1H), 3.33 (s, 1H), 2.92 (dt, J = 13.2, 7.5 Hz, 2H), 2.77 (t, J = 6.5 Hz, 5H), 2.60 (p, J = 5.7 Hz, 3H), 2.49 – 2.19 (m, 8H), 2.05 (q, J = 6.8 Hz, 9H), 1.87 (d, J = 13.1 Hz, 1H), 1.62 (p, J = 7.6, 6.9 Hz, 9H), 1.50 – 1.16 (m, 69H), 0.88 (td, J = 6.8, 4.1 Hz, 10H). $[M+H]^+$=995.91986.

[0446] **Example 2.31: Synthesis of Compound 43**

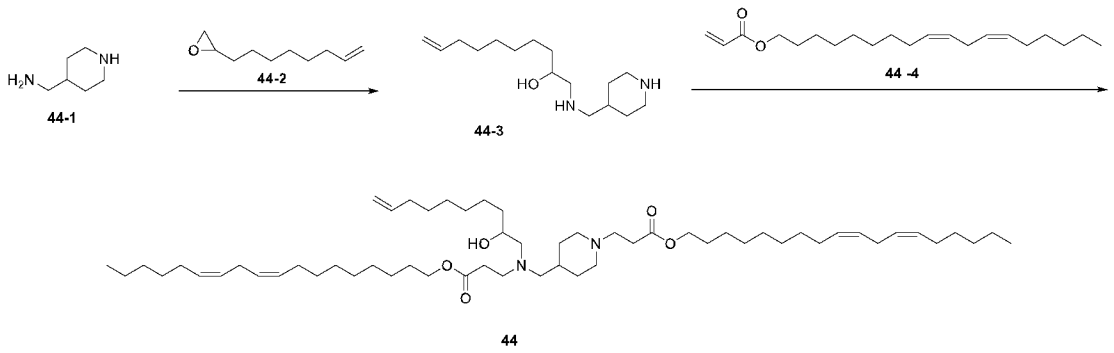


[0447] **Step 1:** compound **43-3** was prepared by similar method as Example 2.15 Step 1.

[0448] **Step 2:** to compound **43-3** (120 mg, 0.31 mmol) in a 5 mL container were added BHT (10.8 mg), compound **43-4** (241.2 mg, 0.75 mmol) at room temperature. The mixture was heated at 70 ℃ for 40 hours. TLC (eluent: DCM/MeOH=15/1) indicated substantial completion of reaction. The mixture was purified by automatic column chromatography on silica (eluent: DCM with 0-3% methanol, v/v) and concentrated under reduced pressure to afford compound **43** (212.1 mg, 66% yield).

[0449] ^{1}H NMR of compound **43**: (400 MHz, Chloroform-*d*) δ 5.46 – 5.24 (m, 10H), 4.06 (q, *J* = 6.8 Hz, 4H), 3.58 (td, *J* = 7.2, 3.8 Hz, 1H), 3.33 (s, 1H), 2.92 (dt, *J* = 13.2, 7.6 Hz, 2H), 2.77 (t, *J* = 6.5 Hz, 5H), 2.60 (dt, *J* = 12.9, 5.7 Hz, 3H), 2.46 – 2.20 (m, 7H), 2.05 (q, *J* = 6.8 Hz, 9H), 1.86 (d, *J* = 15.4 Hz, 2H), 1.62 (p, *J* = 6.9 Hz, 9H), 1.52 – 1.15 (m, 75H), 0.88 (td, *J* = 6.8, 4.2 Hz, 11H). $[M+H]^+$=1023.94772.

[0450] **Example 2.32: Synthesis of Compound 44**



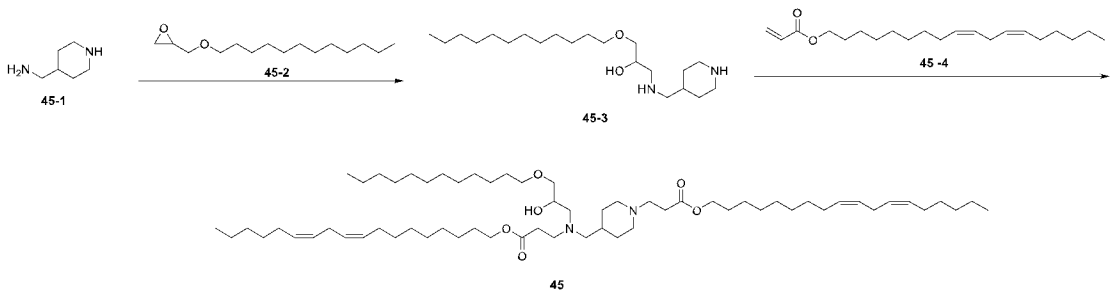
[0451] **Step 1:** compound **44-3** was prepared by similar method as Example 2.15 Step 1.

[0452] **Step 2:** to compound **44-3** (120 mg, 0.45 mmol) in a 5 mL container were added BHT (10.8 mg), compound **44-4** (343.87 mg, 1.07 mmol) at room

temperature. The mixture was heated at 70 ℃ for 40 hours. TLC (eluent: DCM/MeOH=15/1) indicated substantial completion of reaction. The mixture was purified by automatic column chromatography on silica (eluent: DCM with 0-3% methanol, v/v) and concentrated under reduced pressure to afford compound **44** (230 mg, 57% yield).

[0453] ^{1}H NMR of compound **44**: (400 MHz, Chloroform-*d*) δ 5.81 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H), 5.45 – 5.28 (m, 8H), 5.06 – 4.84 (m, 2H), 4.06 (q, *J* = 6.7 Hz, 4H), 3.58 (ddt, *J* = 10.4, 7.0, 3.2 Hz, 1H), 3.34 (s, 1H), 2.92 (dt, *J* = 13.2, 7.6 Hz, 2H), 2.77 (t, *J* = 6.5 Hz, 5H), 2.60 (dt, *J* = 12.6, 5.5 Hz, 3H), 2.47 – 2.21 (m, 7H), 2.05 (q, *J* = 6.7 Hz, 11H), 1.85 (d, *J* = 12.9 Hz, 1H), 1.62 (p, *J* = 6.8 Hz, 7H), 1.48 (q, *J* = 11.9, 10.8 Hz, 2H), 1.42 – 1.18 (m, 47H), 0.89 (t, *J* = 6.8 Hz, 7H). $[M+H]^+$=909.81634

[0454] **Example 2.33: Synthesis of Compound 45**

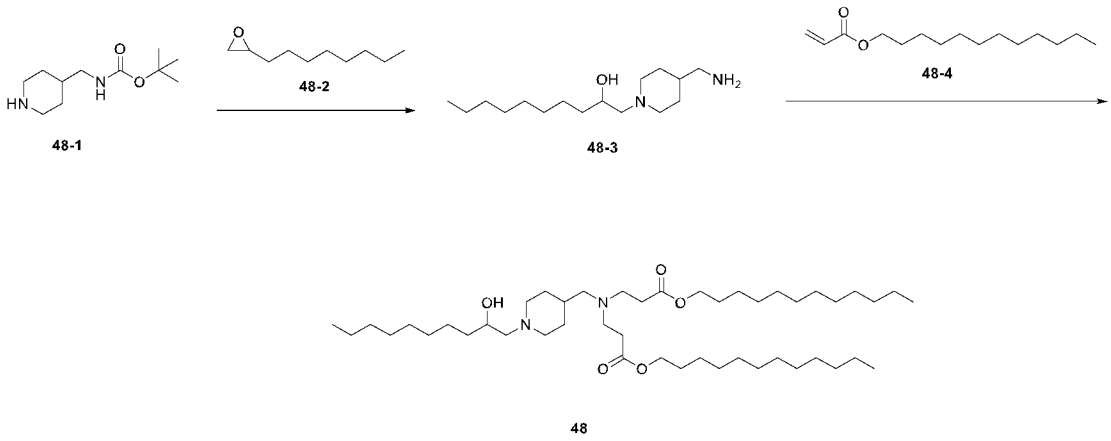


[0455] **Step 1:** compound **45-3** was prepared by similar method as Example 2.15 Step 1.

[0456] **Step 2:** to compound **45-3** (120 mg, 0.34 mmol) in a 5 mL container were added BHT (10.8 mg), compound **45-4** (258.86 mg, 0.81 mmol) at room temperature. The mixture was heated at 70 ℃ for 40 hours. TLC (eluent: DCM/MeOH=15/1) indicated substantial completion of reaction. The mixture was purified by automatic column chromatography on silica (eluent: DCM with 0-3% methanol, v/v) and concentrated under reduced pressure to afford compound **45** (184 mg, 55% yield).

[0457] ^{1}H NMR of compound **45**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.48 – 5.23 (m, 8H), 4.05 (td, *J* = 6.8, 5.3 Hz, 4H), 3.82 (q, *J* = 5.7 Hz, 1H), 3.48 – 3.34 (m, 3H), 2.90 (dt, *J* = 14.0, 7.1 Hz, 1H), 2.75 (dt, *J* = 15.0, 6.9 Hz, 8H), 2.64 – 2.35 (m, 10H), 2.05 (q, *J* = 6.9 Hz, 9H), 1.59 (dp, *J* = 20.6, 6.9 Hz, 7H), 1.41 – 1.17 (m, 50H), 0.88 (td, *J* = 6.9, 4.2 Hz, 9H). $[M+H]^+$=997.90395.

[0458] Example 2.34: Synthesis of Compound 48



[0459] **Step 1**: to a solution of compound **48-1** (1.3 g) in dry ethanol (6.5 mL) was added compound **48-2** (1.04 g) under stirring. The mixture was heated at 70 ℃ for 16 hours. TLC (eluent: DCM/methanol=15/1, I_2 stain) indicated formation of product.

[0460] **Step 2**: the mixture of Step 1 was concentrated under reduced pressure and diluted with ethyl acetate (10 mL). To the mixture was added HCl (10 mL, 4 M in ethyl acetate) dropwise. The mixture was stirred at room temperature overnight. The mixture was filtrate and the residue was dissolved with water and ethyl acetate. To the mixture was added saturated aqueous $NaHCO_3$ solution until pH = 10-12. The mixture was extracted with ethyl acetate. The combined organic layer was washed with brine and concentrated to afford compound **48-3** (1.32 g, 80% yield).

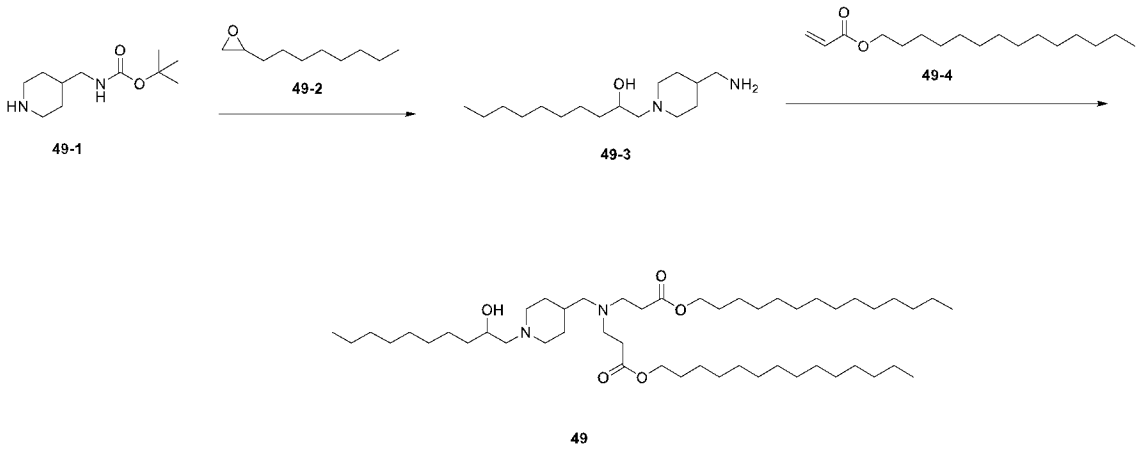
[0461] ^{1}H NMR of compound **48-3**: (400 MHz, Chloroform-d) δ 3.68 (ddt, J = 11.0, 7.8, 3.8 Hz, 1H), 3.07 (d, J = 11.4 Hz, 1H), 2.85 (d, J = 11.8 Hz, 1H), 2.59 (d, J = 5.8 Hz, 2H), 2.38 – 2.17 (m, 4H), 1.94 (td, J = 11.6, 2.5 Hz, 2H), 1.76 – 1.68 (m, 3H), 1.51 – 1.18 (m, 22H), 0.88 (t, J = 6.7 Hz, 4H).

[0462] **Step 3**: to the mixture of compound **48-3** (75 mg), BHT (31 mg) and compound **48-4** (160 mg) was added a drop of acetic acid. The mixture was heated 70 ℃ for 1 day in sealed tube. The mixture was cooled to room temperature and concentrated under reduce pressure. The residue was purified by column chromatography on silica (eluent: DCM with 0-5% methanol) to afford compound **48** (168 mg, 81% yield).

[0463] ^{1}H NMR of compound **48**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.03 (t, *J* = 6.8 Hz, 4H), 3.73 (dq, *J* = 13.6, 6.7, 4.6 Hz, 2H), 3.11 (d, *J* = 11.2 Hz, 1H), 2.92 (d, *J* = 11.5 Hz, 1H), 2.72 (t, *J* = 7.1 Hz, 4H), 2.43 – 2.27 (m, 7H), 2.22 (d, *J* = 7.0 Hz,

2H), 2.07 – 1.97 (m, 1H), 1.71 (dd, *J* = 13.4, 3.4 Hz, 2H), 1.61 (p, *J* = 6.8 Hz, 4H), 1.52 – 1.37 (m, 3H), 1.26 (d, *J* = 6.5 Hz, 49H), 0.87 (t, *J* = 6.7 Hz, 9H).

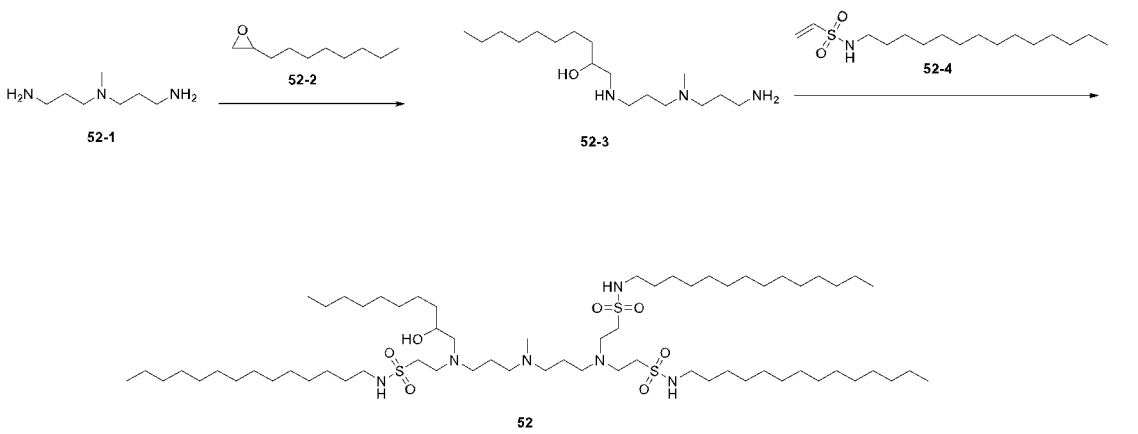
[0464] Example 2.35: Synthesis of Compound 49



[0465] To the mixture of compound **49-3** (75 mg, prepared by the method of Example 2.34 Step 1 to 2), BHT (31 mg) and compound **49-4** (179 mg) was added a drop of acetic acid. The mixture was heated 70 ℃ for 1 day in sealed tube. The mixture was cooled to room temperature and concentrated under reduce pressure. The residue was purified by column chromatography on silica (eluent: DCM with 0-5% methanol) to afford compound **49** (175 mg, 78% yield).

[0466] ^{1}H NMR of compound **49**: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.04 (t, *J* = 6.8 Hz, 4H), 3.75 (q, *J* = 6.0 Hz, 1H), 3.13 (d, *J* = 11.6 Hz, 2H), 2.94 (d, *J* = 11.4 Hz, 1H), 2.73 (t, *J* = 7.1 Hz, 4H), 2.46 – 2.29 (m, 7H), 2.22 (d, *J* = 7.0 Hz, 2H), 2.05 (t, *J* = 11.6 Hz, 1H), 1.77 – 1.67 (m, 2H), 1.61 (p, *J* = 6.9 Hz, 4H), 1.55 – 1.39 (m, 3H), 1.26 (d, *J* = 7.4 Hz, 58H), 0.88 (t, *J* = 6.6 Hz, 9H).

[0467] Example 2.36: Synthesis of Compound 52

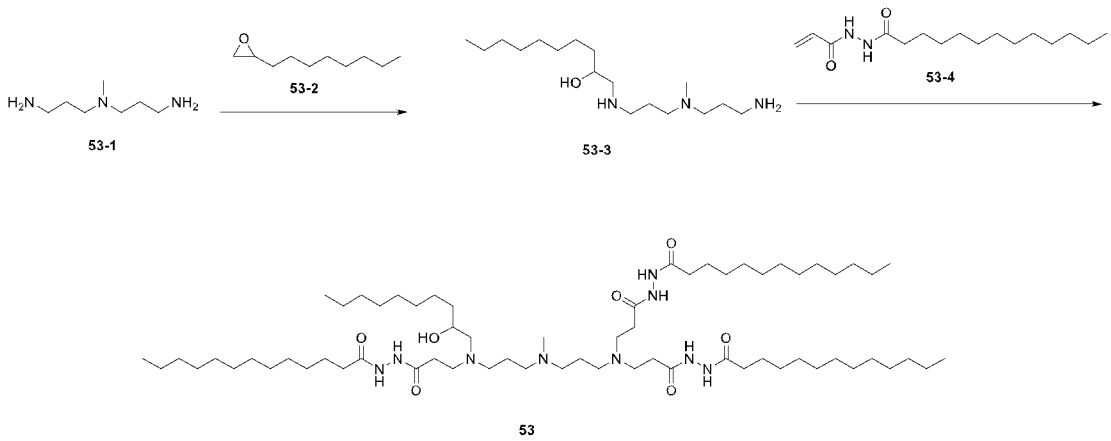


[0468] **Step 1:** compound **52-3** was prepared by the method of Example 2.1 Step 2.

[0469] **Step 2:** to a solution of compound **52-3** (30 mg, 0.096 mmol) compound **52-4** (117.3 mg, 0.386 mmol, prepared by the method of Example 1.15 Step 1) in ACN (0.2 mL) were added BHT (15 mg) and acetic acid (7 mg) at room temperature under N_2 atmosphere. The mixture was heated at 75 ℃ for 40 hours. TLC (eluent: DCM/MeOH=10/1) indicated substantial consumption of starting materials. The mixture was purified by column chromatography on silica and concentrated under reduced pressure to afford compound **52** (67 mg, 55% yield).

[0470] ^{1}H NMR of compound **52**: (400 MHz, Chloroform-d) δ 5.78 (t, J = 6.1 Hz, 1H), 3.65 (td, J = 8.7, 7.5, 4.0 Hz, 1H), 3.44 – 3.26 (m, 2H), 3.19 (dt, J = 19.3, 5.2 Hz, 7H), 3.07 (q, J = 7.1 Hz, 7H), 2.99 – 2.81 (m, 4H), 2.75 – 2.63 (m, 5H), 2.55 (dt, J = 28.9, 7.4 Hz, 3H), 2.46 – 2.20 (m, 2H), 2.00 (d, J = 11.9 Hz, 5H), 1.58 (dt, J = 12.5, 4.7 Hz, 6H), 1.26 (d, J = 5.9 Hz, 81H), 0.94 – 0.73 (m, 12H). $[M+H]^+$=1212.00789.

[0471] **Example 2.37: Synthesis of Compound 53**



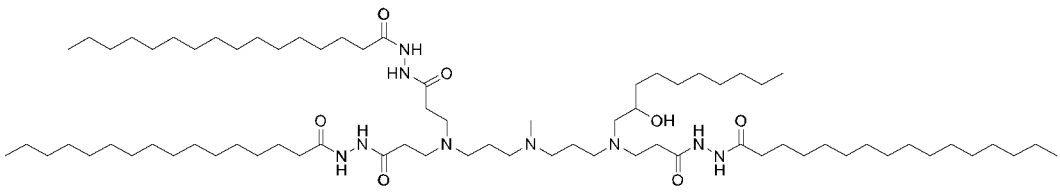
[0472] **Step 1:** compound **53-3** was prepared by the method of Example 2.1 Step 2.

[0473] **Step 2:** to a solution of compound **53-3** (30 mg, 0.096 mmol) compound **53-4** (101.2 mg, 0.36 mmol, prepared by the method of Example 1.16 Step 1 to 2) in ACN (0.2 mL) were added BHT (15 mg) and acetic acid (10 mg) at room temperature under N_2 atmosphere. The mixture was heated at 80 ℃ for 16 hours. TLC (eluent: DCM/MeOH=10/1+0.5% NH_4OH) indicated substantial consumption of starting materials. The mixture was purified by column chromatography on silica and concentrated under reduced pressure to afford compound **53** (50 mg, 43% yield).

[0474] ^{1}H NMR of compound **53**: (400 MHz, Chloroform-d) δ 3.70 (s, 1H), 3.07 – 2.87 (m, 1H), 2.68 (t, J = 5.6 Hz, 2H), 2.48 – 2.31 (m, 6H), 2.31 – 2.19 (m, 5H), 2.15 (d, J = 18.3 Hz, 2H), 1.62 (d, J = 8.0 Hz, 7H), 1.26 (d, J = 5.9 Hz, 48H), 0.94 – 0.76 (m, 12H). HRMS: $[M+H]^+$ 1149.01684.

[0475] **Example 2.38: Synthesis of Compounds 57 and 58**

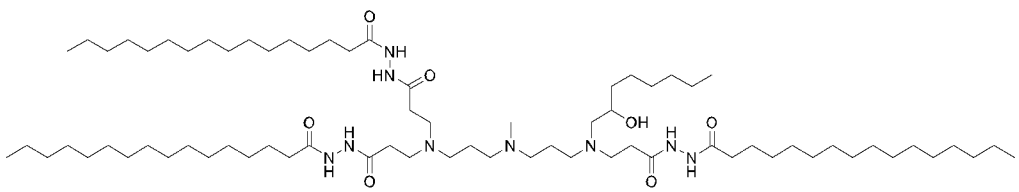
[0476] Compounds 57 and 58 were synthesized with similar method as Example 2.37 with different starting material and modified conditions.



57

[0477] 1-((3-((3-aminopropyl)(methyl)amino)propyl)amino)decan-2-ol (50 mg, 1.0 eq) and N'-acryloylpalmitohydrazide (193.7mg, 3.6eq) were added to 5 ml reaction flask, BHT (10mg), AcOH (6 μl) and 0.5 ml anhydrous ethanol were added. The mixture was stirred at 80 ℃ for 24 h. The crude product was purified by flash column chromatography (DCM ~ DCM/MeOH = 20/1 + 0.5% ammonia) to give targeting compound (78mg, 39.5% yield).

[0478] ^{1}H NMR of compound **57**: (400 MHz, DMSO-d_6) δ 9.88 – 9.65 (m, 5H), 3.53 (s, 1H), 3.05 (d, J = 33.9 Hz, 4H), 2.79 (s, 1H), 2.66 (d, J = 15.9 Hz, 7H), 2.37 – 2.18 (m, 7H), 2.10 (t, J = 7.4 Hz, 5H), 1.73 (s, 4H), 1.63 – 1.43 (m, 6H), 1.43 – 1.32 (m, 3H), 1.23 (s, 74H), 0.92 – 0.72 (m, 12H). HRMS: m/z Calcd for Chemical Formula: $C_{74}H_{147}N_9O_7$ $[M+H]^+$ 1275.1423, found 1275.17927.



58

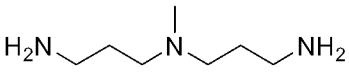
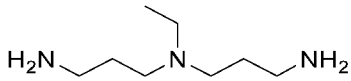
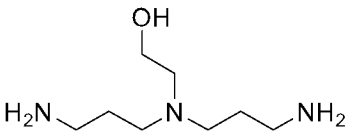
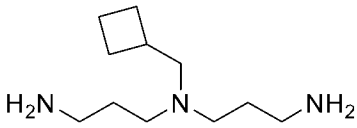
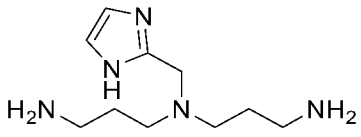
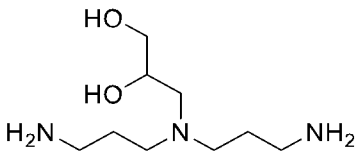
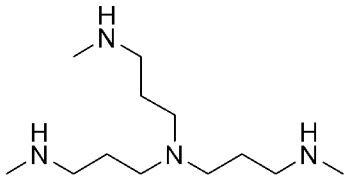
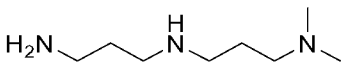
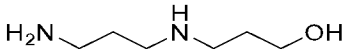
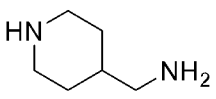
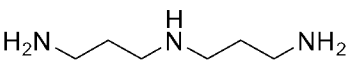
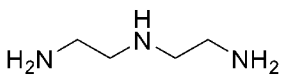
[0479] 1-(3-((3-aminopropyl)(methyl) amino) propyl) octyl-2-ol (80 mg, 1.0 eq) and N'-acryloylpalmitohydrazide (342mg, 3.6 eq) were added to 2 ml reaction flask, BHT (10 mg), AcOH (3 μl) and 0.8 ml anhydrous ethanol were added. The mixture was

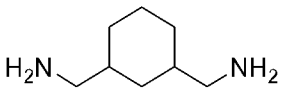
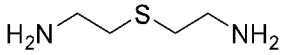
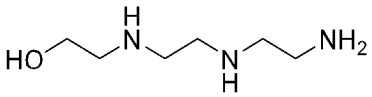
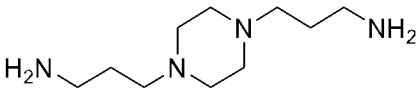
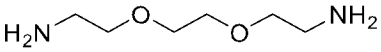
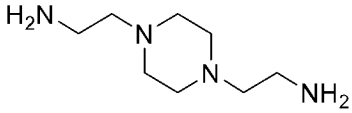
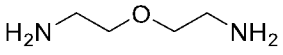
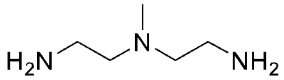
stirred at 80 ℃ for 24 h. The crude product was purified by flash column chromatography (DCM ~ DCM/MeOH = 50/1 + 0.5% ammonia) to give target compound (95mg, 26.04 % yield, HPLC(ELSD): 96.15%).

[0480] ^{1}H NMR of compound **58** (400 MHz, Chloroform-*d*) δ 3.70 (dt, *J* = 7.5, 3.6 Hz, 1H), 2.69 (t, *J* = 5.7 Hz, 4H), 2.59 (tdd, *J* = 11.8, 10.7, 8.5, 5.1 Hz, 3H), 2.50 – 2.34 (m, 10H), 2.27 (pd, *J* = 11.8, 10.9, 4.6 Hz, 12H), 2.11 (s, 3H), 1.62 (tt, *J* = 10.9, 6.9 Hz, 10H), 1.54 (s, 2H), 1.35 – 1.21 (m, 70H), 0.93 – 0.83 (m, 12H). HRMS: m/z Calcd for Chemical Formula: $C_{72}H_{143}N_9O_7$ $[M+H]^+$ 1247.1110, found 1247.13610.

[0481] Other compounds of the present disclosure were synthesized by similar methods with modified conditions and different starting materials. Exemplary starting materials were shown in Table 4-6.

Table 4. Exemplary Amino Starting Materials

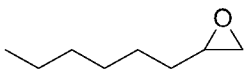
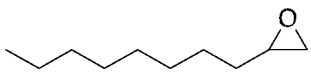
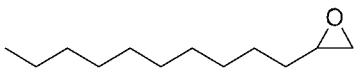
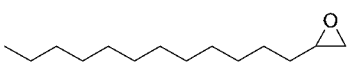
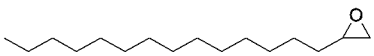
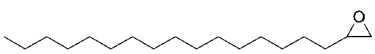
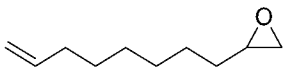
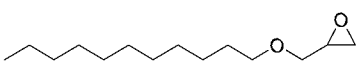
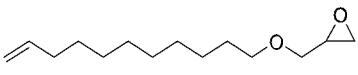
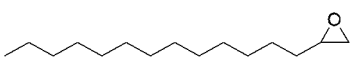
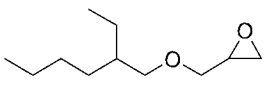
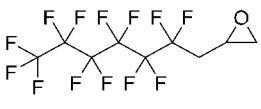
ID	Compound Structure	ID	Compound Structure
A1		A2	
A3		A4	
A5		A6	
A7		A8	
A9		A10	
A11		A12	

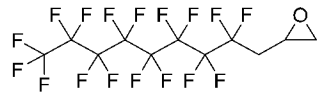
A13		A14	
A15		A16	
A17		A18	
A19		A20	

[0482] Each of the exemplary amino starting materials shown in Table 4 above can be used as an alternative for compound **S1-1** in General Scheme 1 or compound **S2-1** in General Scheme 2.

[0483] Each of the exemplary amino starting materials shown in Table 4 above is commercially available or can be synthesized by methods described below in Example 3.

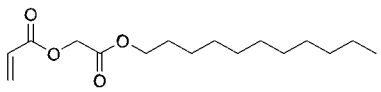
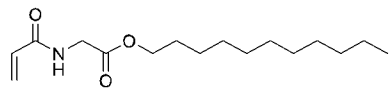
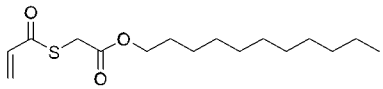
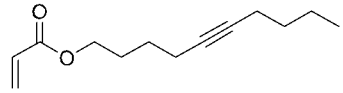
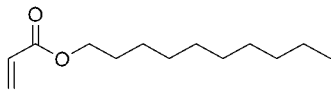
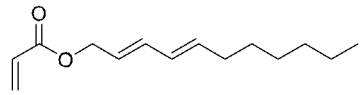
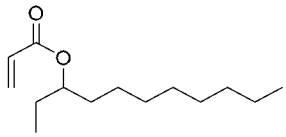
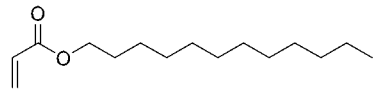
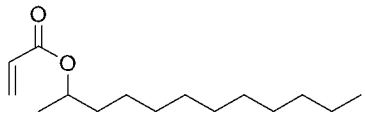
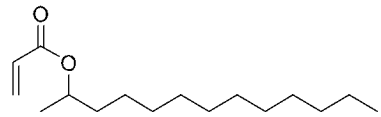
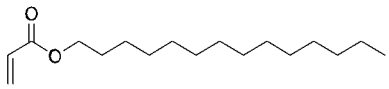
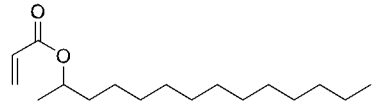
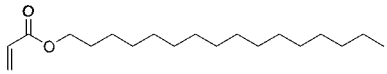
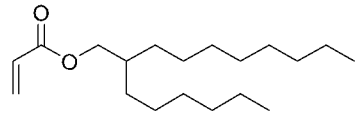
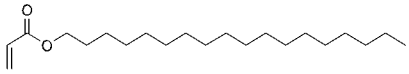
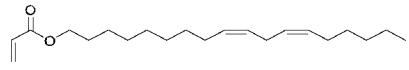
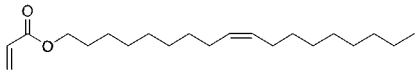
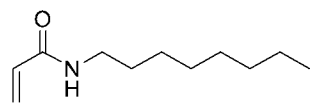
Table 5. Exemplary Epoxide Starting Materials

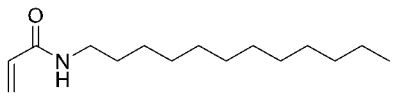
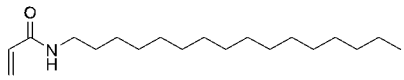
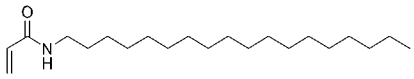
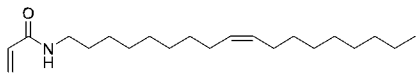
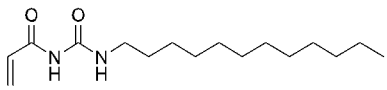
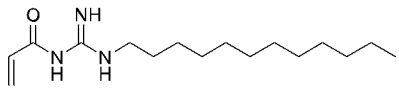
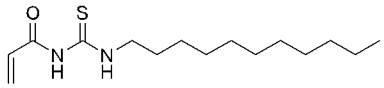
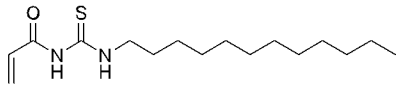
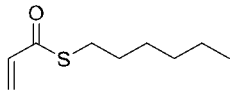
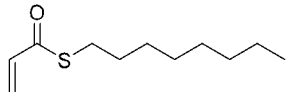
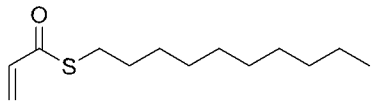
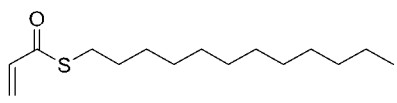
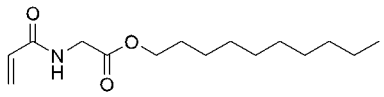
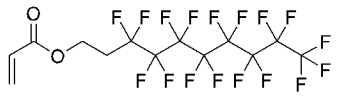
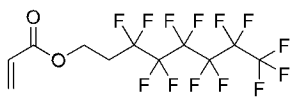
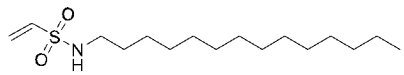
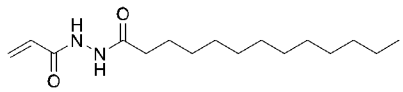
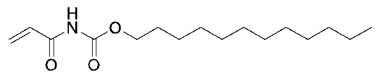
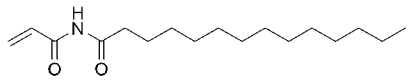
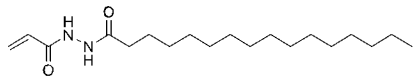
ID	**Compound Structure**	**ID**	**Compound Structure**
EP8		EP10	
EP12		EP14	
EP16		EP18	
EP10A		EPO15	
EPO15A		EP15	
EPO12A		EP9F13	

EP11F19			

[0484] Each of the exemplary epoxide starting materials shown in Table 5 above can be used as an alternative for compound **S2-2** in General Scheme 2.

Table 6. Exemplary Acrylic Starting Materials

ID	Compound Structure	ID	Compound Structure
O11C		N11F	
S11E		O10A	
O10		O11A	
O11B		O12	
O12A		O13A	
O14		O14A	
O16		O16A	
O18		O18A	
O18B		N8	

N12		N16	
N18		N18A	
NCN12		NNN12	
NSN11		NSN12	
S6		S8	
S10		S12	
N10F		O10F17	
O8F13		SO2N14	
NNCC13		NCO12	
NCC14		NNCC16	

[0485] Each of the exemplary acrylic starting materials shown in Table 6 above can be used as an alternative for compound **S1-2** in General Scheme 1 or compound **S2-4** in General Scheme 2.

[0486] Each of the exemplary acrylic starting materials shown in Table 6 above is commercially available or can be synthesized by methods described above.

[0487] Exemplary lipids synthesized by similar methods as General Scheme 1 or General Scheme 2 with selected starting materials shown in Table 4-6 and their Structure Code were shown in Table 7 below.

Table 7. Structure Code of Exemplary Lipids and Starting Materials Thereof

Amino	Epoxide	Acrylic	Product structure code	Amino	Epoxide	Acrylic	Product structure code
A1	N/A	O11C	A1O11C	A1	EP10	O18	A1EP10O18
A1	N/A	N11F	A1N11F	A1	EP10	O18A	A1EP10O18A
A1	N/A	S11E	A1S11E	A1	EP10	O18B	A1EP10O18B
A1	EP8	O10A	A1EP8O10A	A1	EP10	N8	A1EP10N8
A1	EP8	O10	A1EP8O10	A1	EP10	N12	A1EP10N12
A1	EP8	O11A	A1EP8O11A	A1	EP10	N16	A1EP10N16
A1	EP8	O11B	A1EP8O11B	A1	EP10	N18	A1EP10N18
A1	EP8	O12	A1EP8O12	A1	EP10	N18A	A1EP10N18A
A1	EP8	O12A	A1EP8O12A	A1	EP10	NCN12	A1EP10NCN12
A1	EP8	O13A	A1EP8O13A	A1	EP10	NNN12	A1EP10NNN12
A1	EP8	O14	A1EP8O14	A1	EP10	S6	A1EP10S6
A1	EP8	O14A	A1EP8O14A	A1	EP10	S8	A1EP10S8
A1	EP8	O16	A1EP8O16	A1	EP10	S10	A1EP10S10
A1	EP8	O16A	A1EP8O16A	A1	EP10	S12	A1EP10S12
A1	EP8	O18	A1EP8O18	A1	EPO15	O11C	A1EPO15O11C
A1	EP8	O18A	A1EP8O18A	A1	EPO15	N11F	A1EPO15N11F
A1	EP8	O18B	A1EP8O18B	A1	EPO15	S11E	A1EPO15S11E
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A1	EP8	N16	A1EP8N16	A1	EPO15	O11A	A1EPO15O11A
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A1	EP8	N18A	A1EP8N18A	A1	EPO15	O12	A1EPO15O12
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A1	EP8	S6	A1EP8S6	A1	EPO15	O14	A1EPO15O14
A1	EP8	S8	A1EP8S8	A1	EPO15	O14A	A1EPO15O14A
A1	EP8	S10	A1EP8S10	A1	EPO15	O16	A1EPO15O16
A1	EP8	S12	A1EP8S12	A1	EPO15	O16A	A1EPO15O16A
A1	EP10	O11C	A1EP1O11C	A1	EPO15	O18	A1EPO15O18
A1	EP10	N11F	A1EP10N11F	A1	EPO15	O18A	A1EPO15O18A
A1	EP10	S11E	A1EP10S11E	A1	EPO15	O18B	A1EPO15O18B
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A1	EP10	O11B	A1EP10O11B	A1	EPO15	N18	A1EPO15N18
A1	EP10	O12	A1EP10O12	A1	EPO15	N18A	A1EPO15N18A
A1	EP10	O12A	A1EP10O12A	A1	EPO15	NCN12	A1EPO15NCN12
A1	EP10	O13A	A1EP10O13A	A1	EPO15	NNN12	A1EPO15NNN12
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A1	EP10	O14A	A1EP10O14A	A1	EPO15	S8	A1EPO15S8
A1	EP10	O16	A1EP10O16	A1	EPO15	S10	A1EPO15S10
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A5	N/A	S11E	A5S11E	A5	EP10	O18B	A5EP10O18B
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A5	EP8	O10	A5EP8O10	A5	EP10	N12	A5EP10N12
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A5	EP8	O16	A5EP8O16	A5	EP10	S10	A5EP10S10
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A20	EP8	O10	A20EP8O10	A20	EP10	N12	A20EP10N12
A20	EP8	O11A	A20EP8O11A	A20	EP10	N16	A20EP10N16
A20	EP8	O11B	A20EP8O11B	A20	EP10	N18	A20EP10N18
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A20	EP8	O13A	A20EP8O13A	A20	EP10	NNN12	A20EP10NNN12
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A20	EP8	O16	A20EP8O16	A20	EP10	S10	A20EP10S10
A20	EP8	O16A	A20EP8O16A	A20	EP10	S12	A20EP10S12
A20	EP8	O18	A20EP8O18	A20	EPO15	O11C	A20EPO15O11C
A20	EP8	O18A	A20EP8O18A	A20	EPO15	N11F	A20EPO15N11F
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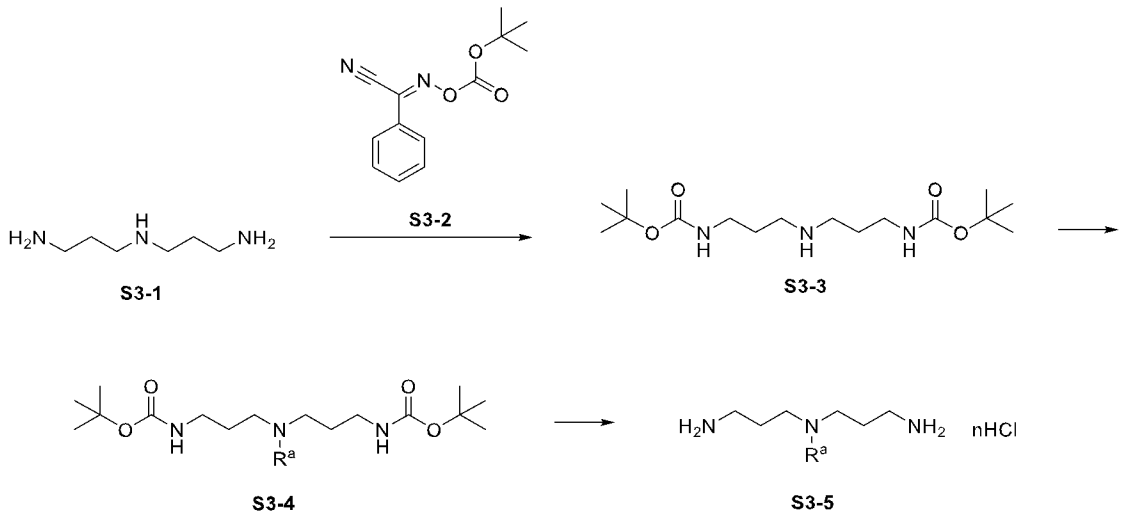
A20	EP8	N18A	A20EP8N18A	A20	EPO15	O12	A20EPO15O12
A20	EP8	NCN12	A20EP8NCN12	A20	EPO15	O12A	A20EPO15O12A
A20	EP8	NNN12	A20EP8NNN12	A20	EPO15	O13A	A20EPO15O13A
A20	EP8	S6	A20EP8S6	A20	EPO15	O14	A20EPO15O14
A20	EP8	S8	A20EP8S8	A20	EPO15	O14A	A20EPO15O14A
A20	EP8	S10	A20EP8S10	A20	EPO15	O16	A20EPO15O16
A20	EP8	S12	A20EP8S12	A20	EPO15	O16A	A20EPO15O16A
A20	EP10	O11C	A20EP10O11C	A20	EPO15	O18	A20EPO15O18
A20	EP10	N11F	A20EP10N11F	A20	EPO15	O18A	A20EPO15O18A
A20	EP10	S11E	A20EP10S11E	A20	EPO15	O18B	A20EPO15O18B
A20	EP10	O10A	A20EP10O10A	A20	EPO15	N8	A20EPO15N8
A20	EP10	O10	A20EP10O10	A20	EPO15	N12	A20EPO15N12
A20	EP10	O11A	A20EP10O11A	A20	EPO15	N16	A20EPO15N16
A20	EP10	O11B	A20EP10O11B	A20	EPO15	N18	A20EPO15N18
A20	EP10	O12	A20EP10O12	A20	EPO15	N18A	A20EPO15N18A
A20	EP10	O12A	A20EP10O12A	A20	EPO15	NCN12	A20EPO15NCN12
A20	EP10	O13A	A20EP10O13A	A20	EPO15	NNN12	A20EPO15NNN12
A20	EP10	O14	A20EP10O14	A20	EPO15	S6	A20EPO15S6
A20	EP10	O14A	A20EP10O14A	A20	EPO15	S8	A20EPO15S8
A20	EP10	O16	A20EP10O16	A20	EPO15	S10	A20EPO15S10
A20	EP10	O16A	A20EP10O16A	A20	EPO15	S12	A20EPO15S12

[0488] Example 3: Synthesis of Amino Starting Materials

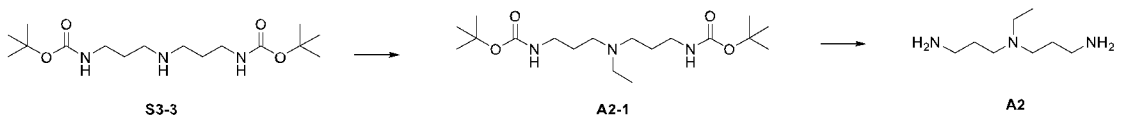


[0489] Example 3.1: Synthesis of Compound S3-3

[0490] To a solution of compound **S3-1** (26.25 g, 200 mmol) in tetrahydrofuran (THF) (800 mL) was added triethylamine (42.51 g, 420 mmol). The mixture was cooled in an ice bath and stirred. To the mixture was added a cold solution of compound S3-2 (98.53 g, 400 mmol) in THF (200 mL) dropwise under stirring in an ice bath. The mixture was allowed to warm to room temperature and kept at the same temperature overnight. The mixture was concentrated and purified by column chromatography on silica (eluent: petroleum ether with 0-50% ethyl acetate to ethyl acetate with 0-5% methanol and 0.5% NH_4OH, v/v) and concentrated under reduced pressure to afford compound **S3-3** (42 g, 63% yield).

[0491] ^1H NMR of compound **S3-3**: (400 MHz, Chloroform-*d*) δ 5.19 (s, 2H), 3.20 (q, J = 6.3 Hz, 4H), 2.66 (t, J = 6.5 Hz, 4H), 1.66 (p, J = 6.5 Hz, 4H), 1.43 (s, 18H).

[0492] Example 3.2: Synthesis of Compound A2



[0493] Step 1: to a solution of compound **S3-3** (13.24 g, 39.9 mmol) in ACN (100 mL) was added grinded K_2CO_3 (8.28 g, 59.9 mmol). The mixture was stirred for 30 minutes and then added ethyl iodide (6.23 g, 39.9 mmol) thereto. The mixture was stirred at room temperature overnight. The mixture was filtrated and the filtrate was concentrated and purified by column chromatography on silica (eluent: petroleum ether

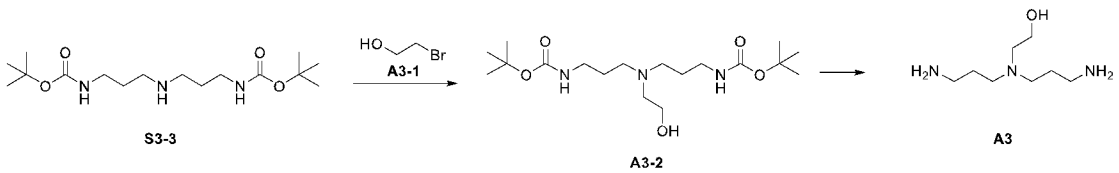
with 0-50% ethyl acetate to ethyl acetate with 0-5% methanol and 0.5% NH_4OH, v/v) and concentrated under reduced pressure to afford compound **A2-1** (9 g, 63% yield).

[0494] ^{1}H NMR of compound **A2-1**: (400 MHz, Chloroform-*d*) δ 5.34 (s, 2H), 3.16 (q, *J* = 6.3 Hz, 4H), 2.56 – 2.36 (m, 6H), 1.62 (p, *J* = 6.6 Hz, 4H), 1.42 (s, 18H), 1.01 (t, *J* = 7.1 Hz, 3H).

[0495] **Step 2:** to compound **A2-1** (9 g, 25 mmol) was added HCl (4 M in methanol, 50 mL). The mixture was stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure to afford crude compound **A2** (4.2 g, with water).

[0496] ^{1}H NMR of compound **A2**: (400 MHz, Deuterium Oxide) δ 2.98 – 2.27 (m, 10H), 1.60 (dtt, *J* = 14.4, 12.3, 7.1 Hz, 4H), 0.95 (dt, *J* = 18.1, 7.2 Hz, 3H).

[0497] **Example 3.3: Synthesis of Compound A3**



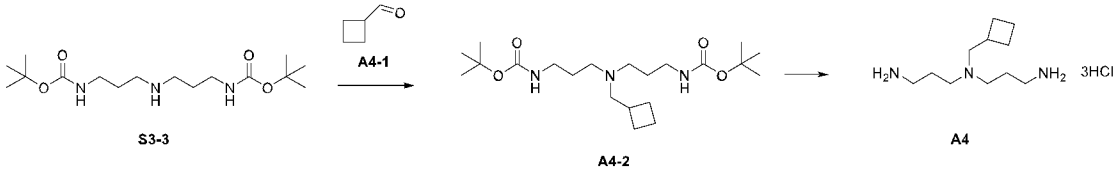
[0498] **Step 1:** to a solution of compound **S3-3** (13.24 g, 39.9 mmol) in ACN (100 mL) was added grinded K_2CO_3 (8.28 g, 59.9 mmol). The mixture was stirred for 30 minutes and then added compound **A3-1** (4.99 g, 39.9 mmol) and NaI (5.98 g, 39.9 mmol) thereto. The mixture was stirred at room temperature for 2 days. The mixture was filtrated and the filtrate was concentrated and purified by column chromatography on silica (eluent: petroleum ether with 0-50% ethyl acetate to ethyl acetate with 0-5% methanol, v/v) and concentrated under reduced pressure to afford compound **A3-2** (5.9 g, 40% yield).

[0499] ^{1}H NMR of compound **A3-2**: (400 MHz, Chloroform-*d*) δ 5.10 (t, *J* = 5.9 Hz, 2H), 3.65 (t, *J* = 5.2 Hz, 2H), 3.42 (s, 1H), 3.17 (q, *J* = 6.5 Hz, 4H), 2.67 (t, *J* = 5.2 Hz, 2H), 2.61 (t, *J* = 6.9 Hz, 4H), 1.70 (p, *J* = 6.8 Hz, 4H), 1.42 (s, 18H).

[0500] **Step 2:** to compound **A3-2** (5.24 g, 19 mmol) was added HCl (4 M in methanol, 30 mL). The mixture was stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure to afford crude compound **A3** (4.0 g, with water).

[0501] ^{1}H NMR of compound **A3**: (400 MHz, Methanol-d_4) δ 3.95 (t, *J* = 5.0 Hz, 2H), 3.50 – 3.33 (m, 6H), 3.10 (t, *J* = 7.5 Hz, 4H), 2.23 (p, *J* = 8.1 Hz, 4H).

[0502] Example 3.4: Synthesis of Compound A4



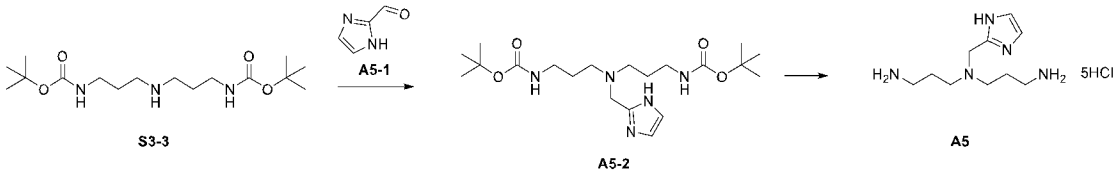
[0503] **Step 1:** to a solution of compound **S3-3** (13.26 g, 40 mmol) in DCM (200 mL) was added compound **A4-1** (3.37 g, 40 mmol) and acetic acid (0.2 mL). The mixture was stirred for 60 minutes in an ice bath and then added and $NaBH(CH_3COO)_3$ (12.72 g, 120 mmol) thereto. The mixture was stirred at room temperature overnight. The reaction was quenched with saturated $NaHCO_3$. The mixture was extracted with DCM. The combined organic layer was concentrated and purified by column chromatography on silica (eluent: petroleum ether with 0-50% ethyl acetate to ethyl acetate with 0-5% methanol, v/v) and concentrated under reduced pressure to afford compound **A4-2** (14 g, 88% yield).

[0504] ^{1}H NMR of compound **A4-2**: (400 MHz, Chloroform-*d*) δ 5.39 (s, 2H), 3.16 (q, *J* = 6.3 Hz, 4H), 2.46 (dd, *J* = 14.8, 8.1 Hz, 7H), 2.04 (qd, *J* = 8.0, 4.0 Hz, 2H), 1.95 – 1.76 (m, 2H), 1.64 (qd, *J* = 8.6, 4.2 Hz, 6H), 1.43 (s, 18H).

[0505] **Step 2:** to compound **A4-2** (14 g, 35 mmol) was added HCl (4 M in methanol, 100 mL). The mixture was stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure to afford crude compound **A4** (12 g, with water).

[0506] ^{1}H NMR of compound **A4**: (400 MHz, Methanol-d_4) δ 3.24 – 3.15 (m, 6H), 3.01 (t, *J* = 7.6 Hz, 4H), 2.79 (hept, *J* = 7.6 Hz, 1H), 2.21 – 2.06 (m, 6H), 2.00 – 1.78 (m, 4H).

[0507] **Example 3.5: Synthesis of Compound A5**



[0508] **Step 1:** to a solution of compound **S3-3** (13.26 g, 40 mmol) in DCM (200 mL) was added compound **A5-1** (3.84 g, 40 mmol) and acetic acid (0.2 mL). The mixture was stirred for 60 minutes in an ice bath and then added and $NaBH(CH_3COO)_3$

(12.72 g, 120 mmol) thereto. The mixture was stirred at room temperature overnight. The reaction was quenched with saturated $NaHCO_3$. The mixture was extracted with DCM. The combined organic layer was concentrated and purified by column chromatography on silica (eluent: petroleum ether with 0-50% ethyl acetate to ethyl acetate with 0-5% methanol, v/v) and concentrated under reduced pressure to afford compound **A5-2** (7.0 g, 42% yield).

[0509] ^{1}H NMR of compound **A5-2**: (400 MHz, Methanol-d_4) δ 7.03 (s, 2H), 3.69 (s, 2H), 3.06 (t, J = 6.9 Hz, 4H), 2.48 (t, J = 7.1 Hz, 4H), 1.63 (p, J = 6.9 Hz, 4H), 1.43 (s, 18H).

[0510] **Step 2:** to compound **A5-2** (7.0 g, 17 mmol) was added HCl (4 M in methanol, 60 mL). The mixture was stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure to afford crude compound **A5** (7.0 g, with water).

[0511] ^{1}H NMR of compound **A5**: (400 MHz, DMSO-d_6) δ 8.29 (t, J = 5.8 Hz, 5H), 7.77 (s, 2H), 4.50 (s, 2H), 3.33 – 2.66 (m, 8H), 2.21 – 1.94 (m, 4H).

[0512] **Example 4: Lipid Particles Comprising Different Neutral Lipids**

[0513] **Example 4.1: Preparation of mRNA Loaded Lipid Particles**

[0514] **Step 1:** FLuc mRNA was dissolved in citrate buffer (pH 4) and adjust the concentration of mRNA to 0.2 mg/mL, so that obtaining aqueous layer thereby.

[0515] **Step 2:** Test Compound, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) or 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), cholesterol and DMG-PEG2000 were dissolved in desired molar fractions (shown in below Table 8) in dry ethanol and the total concentration of lipids was adjusted to 10 mg/mL, thereby obtaining organic layer.

Table 8. Molar Fractions of Components in Lipid Particles

Lipid Particle No.	Test Compound (Compound 1)	Neutral lipid	Cholesterol	DMG-PEG2000
1	62.5%	9% (DOPE)	27%	1.5%
2	50%	10% (DSPC)	38.5%	1.5%

[0516] **Step 3:** The aqueous layer and organic layer were admixed in 3:1 ratio (v/v) by microfluidic device (NanoAssemblr® Ignite™) at total flow rate of 12 mL/min. The mixture was 10-fold diluted with PBS buffer (pH 7.4). Ethanol was separated by

tangential flow filtration (Repligen, TFF). The solution was concentrated to 0.1 mg/mL (mRNA concentration) and filtrated by 0.22 μm millipore filter to afford mRNA containing lipid particles.

[0517] Mean particle size and polymer dispersity index (PDI) of exemplary lipid particles were measured with DLS by Malvern Zetasizer.

[0518] Total RNA concentration and free RNA concentration were measured based on the measurement of fluorescence with RiboGreen® (ThermoFisher Scientific). The efficiency of encapsulation (EE) was calculated by

$$\text{efficiency of encapsulation} = \frac{\text{total RNA concentration} - \text{free RNA concentration}}{\text{total RNA concentration}} .$$

The total RNA concentration was measured by properly diluting test lipid particle with 1xTE buffer containing 0.2% Triton-X 100. The free RNA concentration was measured by properly diluting test lipid particle with 1xTE buffer.

[0519] FIG. 1 shows changes of the physical characteristics (Size: mean particle size; PDI: mean polymer dispersity index; and Encapsulation: efficiency of encapsulating RNA) of test lipid particles in two weeks. The physical characteristic parameter of each of these three physical characteristic parameters of the lipid particles on Day 0 is used as the basic value for the corresponding normalization. Each normalized value for Day 3, 5, 7, 10 and 15 is the ratio of the physical characteristic parameter of the lipid particles on that day to the physical characteristic parameter of the lipid particles on Day 0. The normalized value of each of the three physical characteristic parameters on Day 0 is 1.

[0520] **Example 4.2: *In vitro* Delivery Assay**

[0521] OVCAR3 cells in good growth condition were digested with 0.25% trypsin. The medium was re-suspended and mixed with pipette gun for 5 times. Cell suspension (20 μL) was taken on the counting plate and counted with Countstar cell counter. The cells were inoculated on the 96-well plate with 10,000 cells and 100 μL medium per well. Cells were cultured overnight and transfected with test lipid particles comprising 25ng FLuc mRNA. The test lipid particles and control were diluted with medium to total volume of 100 μL. The medium in cell culture plate was removed and 100 μL test lipid particles was added. Each test lipid particles transfected 2-3 wells. A blank control group was set up with 3 wells with 100 μL medium each well. The culture plates were put into the cell incubator. After cell transfection for 24 hours, the ONEGLO™ kit (Promega, item E7120) was melted at room temperature for 1 hour,

and 5X CellTiter-Fluor™ Reagent (20 μL per well) was added to the cell culture plate. The cell culture plate was placed in a shaker and incubated at 300 rpm at 37 ℃ for 30 min. The fluorescence was measured by microplate reader (EX390nm/EM505nm). Then ONEGLO™ Reagent (100 μL per well) was added at room temperature for 3 minutes, Luminescence was measured by microplate reader. The obtained relative luminescence units ("RLU") was shown in Fig. 2 (*in vitro*).

[0522] **Example 4.3: *In vivo* Delivery Assay**

[0523] Six-week-old male C57BL/6 mice, weighing about 20g, fed in the specific-pathogen free (SPF) feeding room were selected. All animal tests were carried out in strict accordance with the guidelines of National Institutes of Health and animal ethics. Two or three mice (n=2 or 3) were randomly selected in each group, and the FLuc mRNA containing lipid particles prepared as described above were administered via intramuscular injection ("IM") at the dosage of 0.25 mg/kg. After 6 hours, 100μL of 30mg/mL D-luciferin (potassium salt) was intraperitoneally injected into each mouse. After 10 minutes, the total fluorescence intensity of each mouse was observed and recorded by *in vivo* imaging system. And the total fluorescence intensity (p/s) was shown in Fig. 2 (*in vivo*).

[0524] FIG. 2 shows the *in vitro* and *in vivo* delivering properties of above test lipid particles at day 0 and day 14.

[0525] It can be seen that lipid particles comprising the disclosed compounds and different neutral lipids can effectively and safely deliver nucleic acids to a cell or a subject and stably express them.

[0526] **Example 5: Lipid Particles with Different Formulation**

[0527] Test lipid particles were prepared by the method of Example 4.1 Step 1 to 3 with molar fractions as shown in below Table 9. Table 10 shows the physical characteristics of test lipid particles.

Table 9. Molar Fractions of Components in Lipid Particles

Formulation No.	Test Compound	DSPC	Cholesterol	DMG-PEG2000
1	49% (Compound 1)	8%	40.7%	2.3%
2	46% (Compound 1)	8%	43.9%	2.1%
3	46% (Compound 1)	12%	39.7%	2.3%

4	50% (Compound 1)	10%	38.5%	1.5%
5	40% (Compound 22)	6%	52.4%	1.6%
6	40% (Compound 22)	8%	50.1%	1.9%
7	43% (Compound 22)	6%	49.1%	1.9%
8	43% (Compound 22)	8%	47.4%	1.6%
9	46% (Compound 22)	6%	45.9%	2.1%
10	46% (Compound 22)	8%	43.6%	2.4%
11	46% (Compound 22)	10%	42.4%	1.6%
12	46% (Compound 22)	12%	40.1%	1.9%
13	49% (Compound 22)	6%	42.6%	2.4%
14	49% (Compound 22)	8%	40.9%	2.1%
15	49% (Compound 22)	12%	37.4%	1.6%
16	44% (Compound 22)	7%	47.5%	1.5%
17	45% (Compound 22)	6%	47.5%	1.5%
18	45% (Compound 22)	8%	45.3%	1.7%
19	47% (Compound 22)	7%	44.4%	1.6%
20	47% (Compound 22)	8%	43.5%	1.5%

Table 10. Physical Characteristics of Test Lipid Particles

Formulation No.	**Particle size (nm)**	**Polydispersity index (PDI)**	**Encapsulation efficiency (EE, %)**
5	83.22	0.085	95
6	77.03	0.0945	92
7	79.67	0.0862	93.7
8	86.66	0.0749	93.1
9	82.52	0.067	93.1
10	76.1	0.0927	94.6
11	84.11	0.0659	94.7
12	77.97	0.0475	93.5
13	81.79	0.0719	96.1
14	81.25	0.0874	96.1
15	87.02	0.0664	95.2
16	103.8	0.0279	97.6
17	106.7	0.0384	97.5
18	96.22	0.0502	97

19	113.3	0.0223	97
20	115.8	0.0482	97.1

[0528] *In vitro* and *in vivo* delivering assay was performed by the method of Example 4.2 and 4.3. FIG. 3, FIG. 4 and FIG. 5 show the *in vitro* and *in vivo* delivering properties of test lipid particles, respectively.

[0529] **Example 6: Lipid Particles with Different Lipids (Test Compounds)**

[0530] The test lipid particles were prepared by the method of Example 4.1 Step 1 to 3 with certain molar fractions of Test Compound (46%), DSPC (8%), cholesterol (43.9%) and DMG-PEG2000 (2.1%) (Formulation A), or Test Compound (70%), DSPC (4%), cholesterol (23.9%) and DMG-PEG2000 (2.1%) (Formulation B), respectively. Table 11 shows the physical characteristics of test lipid particles.

Table 11. Physical Characteristics of Test Lipid Particles

Test Compound (Structure Code)	**Particle size (nm)**	**Polydispersity index (PDI)**	**Encapsulation efficiency (EE, %)**	**Formulation**
Compound 7 (A1N11F)	51.4	0.169	99	A
Compound 8 (A1NCN12)	86.21	0.163	99	A
Compound 9 (A3N11F)	50.5	0.145	99	A
Compound 12 (A1EP10AN18)	70.6	0.259	99	A
Compound 20 (A1EP10AO18B)	100.5	0.072	89	A
Compound 21 (A1EPO15O18B)	93.3	0.135	98	A
Compound 22 (A1EP10O18A)	92.6	0.042	96	A
Compound 23 (A1EP10AO18A)	89.5	0.054	96	A
Compound 24 (A1EPO15O18A)	88.1	0.117	97	A
Compound 25 (A10AEP12O18B)	69.8	0.069	94	A
Compound 26 (A10AEP12O18A)	78.4	0.094	93	A

Compound 27 (A1EP12N11F)	53.2	0.225	99	A
Compound 28 (A5N10F)	63.9	0.157	96	A
Compound 29 (A1N12)	72.7	0.199	99	A
Compound 31 (A1EP12O18B)	92.1	0.124	90	A
Compound 32 (A1EP14O18B)	95.7	0.097	91	A
Compound 33 (A1EP16O18B)	97.8	0.078	95	A
Compound 34 (A1EP18O18B)	94.2	0.117	96	A
Compound 35 (A10AEP8O18B)	69.4	0.079	95	A
Compound 36 (A10AEP10O18B)	72.7	0.074	94	A
Compound 40 (A10AEP10O18A)	76.8	0.083	93	A
Compound 41 (A10AEP14O18A)	76.9	0.069	92	A
Compound 43 (A10AEP18O18A)	74.1	0.093	90	A
Compound 44 (A10AEP10AO18A)	69.4	0.106	94	A
Compound 45 (A10AEPO15O18A)	66.5	0.069	96	A
Compound 46 (A5N11F)	64.8	0.152	97	A
Compound 47 (A2N11F)	47.9	0.16	99	A
Compound 53 (A1EP10NNCC13)	48.5	0.17	99.9	B
Compound 57 (A1EP10NNCC16)	51.7	0.107	99.7	B
Compound 58 (A1EP8NNCC16)	98.08	0.13	99.1	B

[0531] *In vitro* and *in vivo* delivering assay was performed by the method of Example 4.2 and 4.3 with DLin-MC3-DMA (DLin-MC3-DMA (50%), DSPC (10%), Cholesterol (38.5%) and DMG-PEG2000 (1.5%)) as positive control. *In vivo* delivering assay was performed via both tail intravenous injection and intramuscular injection.

FIG. 6 shows the *in vitro* delivering properties and cell viability of test lipid particles and FIG. 7 show the *in vivo* delivering properties of test lipid particles by muscular expression. Table 12 shows the distribution of *in vivo* delivering to liver, spleen and lung. FIG. 8, FIG. 9 and FIG. 10 show imaging of liver, spleen and lung, FLuc mRNA expression intensity and distribution of organs for exemplary lipid particles targeting liver, spleen and lung, respectively. The efficacy in lung (total flux, p/s) measured for LNPs with Compounds 53, 57 and 58 were 1.15 E+07, 1.51 E+08 and 1.80 E+08, respectively.

Table 12. FLuc mRNA Expression Intensity in Liver, Spleen and Lung

Test Compound (Structure Code)	**Distribution in liver (%)**	**Distribution in spleen (%)**	**Distribution in lung (%)**
Compound 7 (A1N11F)	0.15	10.4	89.45
Compound 8 (A1NCN12)	1	1	98
Compound 9 (A3N11F)	1.05	11.65	87.55
Compound 11 (A1EP10O18B)	99.1	0.5	0.15
Compound 12 (A1EP10AN18)	0.6	4.25	94.9
Compound 14 (A13EP12N12)	98.5	1.5	0
Compound 20 (A1EP10AO18B)	96.35	2	0.3
Compound 21 (A1EPO15O18B)	94.95	4.7	0.25
Compound 22 (A1EP10O18A)	98.5	1.5	0
Compound 23 (A1EP10AO18A)	98.5	1.5	0
Compound 24 (A1EPO15O18A)	96	3.65	0.2
Compound 25 (A10AEP12O18B)	73	17	10.5
Compound 26 (A10AEP12O18A)	47.5	52.5	0
Compound 27 (A1EP12N11F)	0.25	6.55	93.15

Compound 28 (A5N10F)	19.2	78.2	2.6
Compound 29 (A1N12)	0.5	10	89.6
Compound 30 (A1EP8O18B)	96.9	2.55	0.35
Compound 31 (A1EP12O18B)	98.05	1.5	0.3
Compound 32 (A1EP14O18B)	95.4	3.75	0.3
Compound 33 (A1EP16O18B)	95.2	4.4	0.25
Compound 34 (A1EP18O18B)	97.9	1.9	0.15
Compound 35 (A10AEP8O18B)	86	11.5	3.5
Compound 36 (A10AEP10O18B)	85	14.5	1
Compound 39 (A10AEP8O18A)	77	23	0
Compound 40 (A10AEP10O18A)	80	20	0
Compound 41 (A10AEP14O18A)	84	16	0
Compound 42 (A10AEP16O18A)	37.5	35.5	27
Compound 43 (A10AEP18O18A)	76.5	13.5	10
Compound 44 (A10AEP10AO18A)	11.5	72.5	16
Compound 45 (A10AEPO15O18A)	79	21	0
Compound 47 (A2N11F)	0.8	4.95	94.2
Compound 46 (A5N11F)	26.2	72.95	0.75
Compound 53 (A1EP10NNCC13)	1.35	0.84	97.81
Compound 57 (A1EP10NNCC16)	0.86	1.23	97.91
Compound 58 (A1EP8NNCC16)	0.18	0.55	99.27
Positive Control	96	4	0.5

[0532] Example 7: Nucleic Acids Delivering by Exemplary Lipid Particles

[0533] The test lipid particles comprising hEPO mRNA were prepared by the method of Example 4.1 Step 1 to 3 with certain molar fractions of Test Compound (46%), DSPC (8%), cholesterol (43.9%) and DMG-PEG2000 (2.1%).

[0534] Six-week-old male C57BL/6 mice, weighing about 20 g, fed in the SPF feeding room were selected. All animal tests were carried out in strict accordance with the guidelines of National Institutes of Health and animal ethics. Three mice (n=3) were randomly selected in each group, and the hEPO mRNA containing lipid particles prepared as described above were administered via intravenous injection (tail) at the dosage of 0.3 mg/kg. After 6 hours, sera were isolated from blood samples and analyzed by commercially available ELISA kit (ab274397, Abcam).

[0535] Using the above assays, lipid particles with exemplary test compounds (A1EP10AO18B, A1EP10O18B, A1EP10AO18A and A1EP10O18A), a positive control (DLin-MC3-DMA) and a negative control (PBS) were tested. The expression level of hEPO (mIU/mL) was shown in FIG. 11.

[0536] Example 8: Effectively Delivering Nucleic Acids to Lung

[0537] CTGF antibody analogue vector was constructed by connecting codon optimized CTGF antibody gene in the order of: heavy chain variable region, GGGGSGGGGSGGGGS, light chain variable region and Fc region. The 5' UTR and 3' UTR sequences are shown as set forth in SEQ ID NOs: 7-9 and SEQ ID NOs: 10-13, respectively. The test lipid particles comprising CTGF antibody encoding mRNA were prepared by the method of Example 4.1 Step 1 to 3 with certain molar fractions of Compound 8 (46%), DSPC (8%), cholesterol (43.9%) and DMG-PEG2000 (2.1%).

[0538] The test lipid particle comprising CTGF antibody mRNA was diluted to 0.25 mg/mL with PBS, and administered to C57 mice via intravenous injection (tail) at the dosage of 100 μL. The test lipid particle was administered twice a week, four time total. At the second day after the last administration, EDTA-anticoagulated whole blood and lung douche were collected. Concentrations of CTGF antibodies in plasma and lung douche were measured by following steps: 50ng CTGF protein was coated on a 96-well plate at 4°C overnight in carbonated buffer; washed the coated well 3 times with PBST; 100 μL 0.5% BSA-PBS buffer was added into the coated well and incubated at 37°C for 1 hour; washed the coated well 3 times with PBST; 100 μL of plasma, lung douche or CTGF antibody standard was added to the coated well and incubated at 37°C for 2

hours; washed the coated well 3 times with PBST; horseradish peroxidase (HRP) labeled anti-human antibody (Sino Biological) was added into the coated well and incubated at 37°C for 1 hour; washed the coated well 3 times with PBST; HRP chromogenic substrate solution (invitrogen) was added. FIG. 12 shows the fold change of the concentration of CTGF antibody in plasma and in lung douche of the test group mice to control group mice ("blank": treated with PBS).

[0539] Example 9: Effectively Delivering Nucleic Acids to Induce an Immune Response

[0540] The test lipid particles comprising RAV-G mRNA were prepared by the method of Example 4.1 Step 1 to 3 with molar fractions shown in Table 13.

[0541] Six-week-old male Balb/C mice, weighing about 20g, fed in the SPF feeding room were selected. All animal tests were carried out in strict accordance with the guidelines of the National Institutes of Health and animal ethics. 5μg of test lipid particles were administrated in each mouse at the dosage of 50μL per mouse, while the same volume of buffer 1 was administrated in negative control groups. At Day 7 and Day 14 post immunization, blood samples were harvested by retro-orbital bleeding and set at 4 °C for 30 minutes. Sera were isolated by centrifuging at 4000 rpm at 4 °C and stored in -80 °C stock for the ELISA titer testing later on.

[0542] 96-well ELISA plate was coated with 100 μL/well of 2 μg/mL RAV-G in PBS buffer, at 4 °C overnight. The plate was washed 3 times and blocked with 300μL/well of blocking buffer at 37 °C for 2 hours, and then washed 3 times. 100μL/well of serially diluted testing sera, in the ratios of 1:100, 1:400, 1:1600, 1:3200, 1:6400...1:2048000, were added and incubated at 37 °C for 60 minutes, and then washed four times. HRP-labeled anti-mouse IgG H&L antibody was diluted in 1000 folds and added with 100μL/well, incubated at 37°C for 60 minutes and washed 4 times. After color development of chromogenic substrate and the subsequent quenching, the signal was measured by absorbance 450nm using plate reader. As shown in FIG. 13, the antibody titers ("IgG Titer") from the testing groups, which were induced by a single immunization with different lipid particle delivering RAV-G mRNA vaccines, were higher than the positive control groups.

Table 13. Molar Fractions of Components in Lipid Particles

Formulation No.	Test Compound	DSPC	Cholesterol	DMG-PEG2000
Formulation 38	46% (Compound 22)	8%	43.9%	2.1%
Formulation 39	43% (Compound 22)	8%	47.4%	1.6%
Formulation 40	46% (Compound 22)	10%	42.4%	1.6%
Formulation 41	45% (Compound 22)	8%	45.3%	1.7%
Formulation 42	47% (Compound 22)	7%	44.4%	1.6%
Formulation 43	45% (Compound 22)	6%	47.4%	1.6%

[0543] Example 10: Antibody Conjugated LNP Targeting CD8+ T Cells.

[0544] The test lipid particles comprising tdTomato mRNA were prepared by the method of Example 4 Steps 1 to 3 with certain molar fractions of Compound 8 (46%), DSPC (8%), cholesterol (43.9%) and DMG-PEG2000 (2.1%).

[0545] The test lipid particle comprising tdTomato mRNA was conjugated with a mouse anti-CD8 antibody (LNP-CD8Ab) or a mouse Immunoglobulin G2B (LN-IgG2B). The lipid particle-antibody conjugates were diluted in PBS to 0.5mg/ml, and 150μL of the diluted lipid particle-antibody conjugates was administrated in C57 mice via intravenous injection (tail). Mice were sacrificed at 16 hours post injection, cut out and placed the spleen into the cell strainer (200 mesh). The cell suspension was prepared by mashing the spleen through the cell strainer using a syringe plunger. Cells were fluorescently labeled by different cell marker antibodies as listed in Table 14. 50μL of cell suspension (about 10^5 cells) was mixed with 0.25μL staining antibody and incubated in dark for 30 minutes on ice. Cells were washed with 1mL PBS and centrifuged at 400g for 30min, discarded the supernatant, and repeated these processes once. Cells were resuspended in 200mL 4% paraformaldehyde and analyzed by flow cytometry. FIG. 14 shows the recognizing ability of antibody-conjugated lipid particle formulations toward different cell surface markers. That is, the percentage of cells that were recognized by cell marker antibodies was shown in Fig. 14. In "T/ALL" section, among all observed spleen cells, about 24%, about 21%, about 21% and about 24% T cells were recognized by their corresponding cell surface markers in PBS, LNP (lipid particles comprising tdTomato mRNA), LNP-CD8Ab and LN-IgG2B treated test groups, respectively. In "$CD4^+$T/T" section, among all observed T cells, about 56%, about 63%, about 62% and about 66% $CD4^+$ T cells were recognized by their corresponding cell surface markers in PBS, LNP, LNP-CD8Ab and LN-IgG2B treated

test groups, respectively. In "$CD8^+$T/T" section, among all observed T cells, about 38%, about 31%, about 0% and about 28% $CD8^+$ T cells were recognized by their corresponding cell surface markers in PBS, LNP, LNP-CD8Ab and LN-IgG2B treated test groups, respectively. As shown in the results, among different formulations, CD8 antibody-labeled lipid particle s could efficiently bind to the surface of corresponding CD8+ cells, thus blocking the specific binding and labeling of said staining antibodies to those CD8+ cells, resulting in low or undetectable CD8+ cell signal in the test.

Table 14. Antibodies For Fluorescently Label

Antibody	Vendor	Catalog No.
Ms CD3e FITC 145-2C11 100μg	BD Biosciences	553061
Ms CD4 PerCP-Cy5.5 RM4-5 100μg	BD Biosciences	550954
Ms CD8a PE-Cy7 53-6.7 100μg	BD Biosciences	552877
Ms CD45 APC-Cy7 30-F11 100μg	BD Biosciences	557659

[0546] The TROP2-CAR mRNA was *in vitro* transcribed by the method described in present disclosure. 25μg of TROP2-CAR-Ab/LNP was administrated in C57 mice via intravenous injection (tail), while the same volume of PBS was served as the negative control. To prepare cell suspensions, mouse spleens were isolated at Day 3, Day 7 and Day 14 post injection, and meshed through a cell strainer (200 mesh) using a syringe plunger. 50μL of cell suspension (about 10^5 cells) was mixed with 0.25μL staining antibody and incubated in dark for 30 minutes on ice. Cells were washed with 1mL PBS and centrifuged at 400g for 30min, discarded the supernatant, and repeated these processes once. Cells were resuspended in 200mL 4% paraformaldehyde. The percentage of TROP2-CAR positive T cells was analyzed by flow cytometry.

[0547] **Example 11: TNS Protocol**

[0548] The test lipid nanoparticles were prepared by the method of Example 4.1 Steps 1 to 3 with molar fractions as shown in below Table 15.

Table 15. Molar Fractions of Components in Lipid Particles

Formulation No.	Test Compound	DSPC	Cholesterol	DMG-PEG2000
21	46% (Compound 22)	8%	43.9%	2.1%
22	46% (Compound 8)	8%	43.9%	2.1%

23	46% (Compound 53)	8%	43.9%	2.1%
24	46% (Compound 57)	8%	43.9%	2.1%

[0549] The obtained LNP/mRNA formulations were diluted by a series of buffer, containing 10 mM HEPES, 10 mM MES (4-morpholineethanesulfonic acid), 10 mM ammonium acetate and 130 mM NaCl, where the pH ranged from 2.5 to 11. TNS probe (200 μM stock in distilled water) was mixed with above solutions for 5 min with slight shaking, making the final volume of 100 μL that contained lipids and probe. The fluorescence of each well was measured by a Tecan plate reader with Ex = 321 nm and Em = 445 nm and data was normalized to the value of pH 2.5. The pH of half-maximum fluorescence indicated the pKa of formulation. The observed pKa values of the test lipid particles were shown in Table 16. It demonstrated that the test lipid nanoparticles have significant potential in delivering desired nucleic acids to the intracellular effectively.

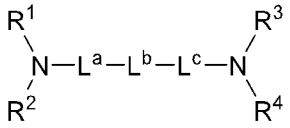
Table 16. TNS pKa of Components in Lipid Particles

Test Compound (Structure Code)	**pKa (TNS)**
Compound 8 (A1NCN12)	6.385
Compound 22 (A1EP10O18A)	6.280
Compound 53 (A1EP10NNCC13)	6.302
Compound 57 (A1EP10NNCC16)	6.756

[0550] The foregoing description is considered as illustrative only of the principles of the present disclosure. Further, since numerous modifications and changes will be readily apparent to those skilled in the art, it is not desired to limit the invention to the exact construction and process shown as described above. Accordingly, all suitable modifications and equivalents maybe considered to fall within the scope of the invention as defined by the claims that follow.

We claim:

1. A compound having Formula (I) below:



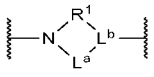
(I),

or a pharmaceutically acceptable salt thereof,

wherein

L^a and L^c are each independently selected from the group consisting of a bond, alkyl, alkenyl, alkynyl and heteroalkyl, wherein the alkyl, alkenyl, alkynyl and heteroalkyl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, cyano and amino;

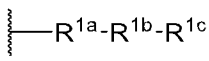
L^b is selected from the group consisting of a bond, -NR^a-, -O-, -S-, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, cycloalkyl, heterocyclyl, aryl and heteroaryl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, cycloalkyl, heterocyclyl, aryl and heteroaryl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, cyano and amino, or

L^b is covalently connected with R^1 to form  ;

provided that when two of L^a, L^b and L^c are bonds, then the remaining one is not a bond;

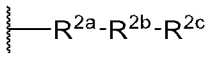
R^a is selected from the group consisting of hydrogen, R^5, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, cycloalkyl, heterocyclyl, aryl and heteroaryl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, cycloalkyl, heterocyclyl, aryl and heteroaryl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, cyano, cycloalkyl, heterocyclyl, aryl, heteroaryl and R^6;

R^1 is a group having Formula (I-a) below:



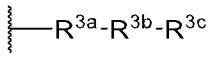
(I-a),

R^2 is a group having Formula (I-b) below:



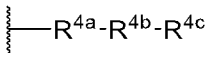
(I-b),

R^3 is a group having Formula (I-c) below:



(I-c),

R^4 is a group having Formula (I-d) below:



(I-d),

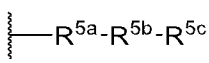
wherein

each of R^{1a}, R^{2a}, R^{3a} and R^{4a} is independently a bond or alkyl;

each of R^{1b}, R^{2b}, R^{3b} and R^{4b} is independently a bond, alkyl or heteroalkyl, wherein the alkyl and heteroalkyl are optionally substituted by one or more groups independently selected from the group consisting of hydroxyl, amino, oxo, thioxo and imino; and

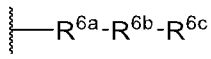
each of R^{1c}, R^{2c}, R^{3c} and R^{4c} is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, oxo, cyano, cycloalkyl, heterocyclyl, aryl and heteroaryl, and the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally interrupted by one or more groups independently selected from the group consisting of cycloalkyl, heterocyclyl, aryl and heteroaryl; and

R^5 is a group having Formula (I-e) below:



(I-e),

each R^6 is independently a group having Formula (I-f) below:



(I-f),

wherein

each of R^{5a} and R^{6a} is independently a bond or alkyl;

each of R^{5b} and R^{6b} is independently a bond, alkyl or heteroalkyl, wherein the alkyl and heteroalkyl is optionally substituted by one or more groups independently selected from the group consisting of hydroxyl, amino, oxo, thioxo and imino; and

each of R^{5c} and R^{6c} is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl is optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, oxo and cyano, and the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally interrupted by one or more groups independently selected from the group consisting of cycloalkyl, heterocyclyl, aryl and heteroaryl.

2. The compound of claim 1, or a pharmaceutically acceptable salt thereof, wherein L^a and L^c are each independently an alkyl, preferably a C_{1-6} alkyl.

3. The compound of claim 1 or 2, or a pharmaceutically acceptable salt thereof, wherein L^a and L^c are each independently methyl, ethyl or propyl.

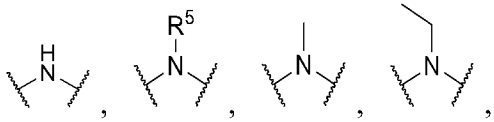
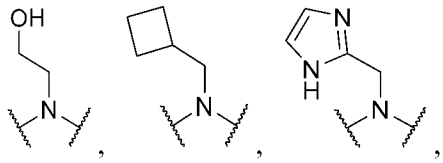
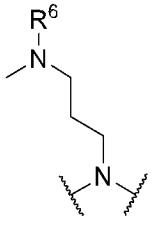
4. The compound of any one of claims 1 to 3, or a pharmaceutically acceptable salt thereof, wherein L^b is $-NR^a-$.

5. The compound of claim 4, or a pharmaceutically acceptable salt thereof, wherein R^a is selected from the group consisting of hydrogen, R^5, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, cycloalkyl, heterocyclyl, aryl and heteroaryl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, cycloalkyl, heterocyclyl, aryl and heteroaryl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, cyano, cycloalkyl, heterocyclyl, aryl heteroaryl and R^6.

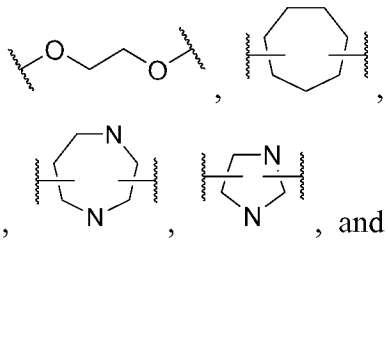
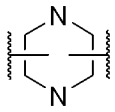
6. The compound of claim 4 or 5, or a pharmaceutically acceptable salt thereof, wherein R^a is C_{1-6} heteroalkyl.

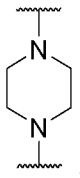
7. The compound of claim 4 or 5, or a pharmaceutically acceptable salt thereof, wherein

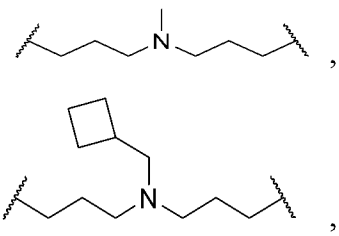
R^a is C_{1-6} alkyl optionally substituted by one or more groups independently selected from the group consisting of hydroxyl, cycloalkyl and heteroaryl.

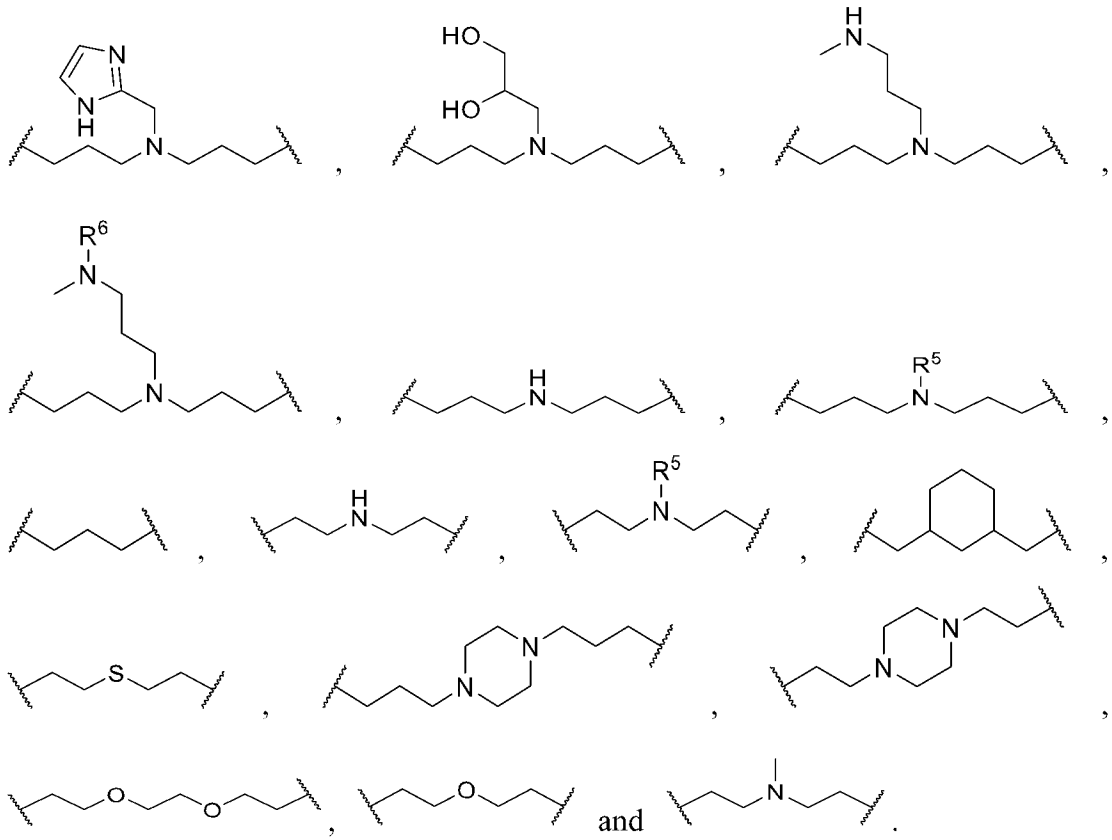
8. The compound of claim 4 or 5, or a pharmaceutically acceptable salt thereof, wherein L^b is selected from the group consisting of  ,  and  .

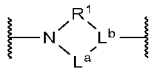
9. The compound of any one of claims 1 to 3, or a pharmaceutically acceptable salt thereof, wherein L^b is selected from the group consisting of -S-, -O-, -O-alkyl-O-, cycloalkyl and heterocyclyl.

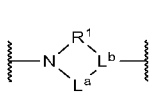
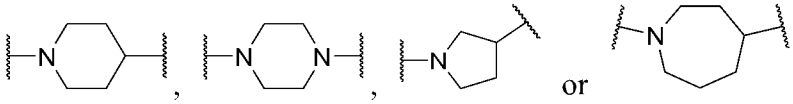
10. The compound of claim 9, or a pharmaceutically acceptable salt thereof, wherein L^b is selected from the group consisting of -S-, -O-,  , and  .

11. The compound of claim 10, or a pharmaceutically acceptable salt thereof, wherein L^b is  .

12. The compound of claim 1, or a pharmaceutically acceptable salt thereof, wherein -L^a-L^b-L^c- is selected from the group consisting of  .



13. The compound of any one of claims 1 to 3, or a pharmaceutically acceptable salt thereof, wherein L^b is alkyl or heteroalkyl covalently connected with R^1 to form  , L^a is a bond, alkyl or heteroalkyl, and R^1 is a bond, alkyl or heteroalkyl.

14. The compound of claim 13, or a pharmaceutically acceptable salt thereof, wherein  is  .

15. The compound of claim 14, or a pharmaceutically acceptable salt thereof, wherein L^c is methyl.

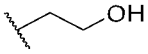
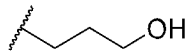
16. The compound of any one of claims 1 to 12, or a pharmaceutically acceptable salt thereof, wherein at least two of R^1, R^2, R^3, R^4, R^5 and R^6 are the same.

17. The compound of claim 16, or a pharmaceutically acceptable salt thereof, wherein at least three of R^1, R^2, R^3, R^4, R^5 and R^6 are the same.

18. The compound of claim 17, or a pharmaceutically acceptable salt thereof, wherein at least four of R^1, R^2, R^3, R^4, R^5 and R^6 are the same.

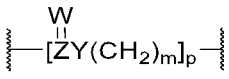
19. The compound of any one of claims 1 to 15, or a pharmaceutically acceptable salt thereof, wherein at least two of R^1, R^2, R^3 and R^4 are different.

20. The compound of any one of claims 1 to 19, or a pharmaceutically acceptable salt thereof, wherein one or more of R^1, R^2, R^3 and R^4 is independently alkyl optionally substituted by one or more hydroxyl.

21. The compound of claim 20, or a pharmaceutically acceptable salt thereof, wherein one or more of R^1, R^2, R^3 and R^4 is independently methyl,  or  .

22. The compound of any one of claims 1 to 21, or a pharmaceutically acceptable salt thereof, wherein one or more of R^{1a}, R^{2a}, R^{3a}, R^{4a}, R^{5a} and R^{6a} is independently a bond, methyl, ethyl, propyl, butyl or pentyl.

23. The compound of any one of claims 1 to 22, or a pharmaceutically acceptable salt thereof, wherein one or more of R^{1b}, R^{2b}, R^{3b}, R^{4b}, R^{5b} and R^{6b} is independently selected from the group consisting of alkyl and heteroalkyl, wherein the alkyl and heteroalkyl is optionally substituted by one or more groups independently selected from the group consisting of hydroxyl, oxo, thioxo and imino.

24. The compound of claim 23, or a pharmaceutically acceptable salt thereof, wherein one or more of R^{1b}, R^{2b}, R^{3b}, R^{4b}, R^{5b} and R^{6b} is independently  ,

wherein

each W is independently selected from O, S or NR^b;

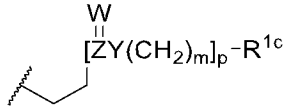
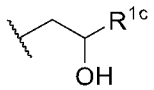
each Y is independently selected from O, S, NR^c, $N(R^c)Z(W)$, $N(R^c)N(R^c)$ or $N(R^c)N(R^c)Z(W)$;

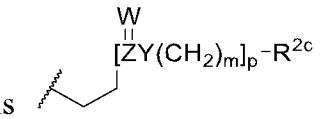
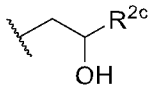
each Z is independently selected from C, S or S(O);

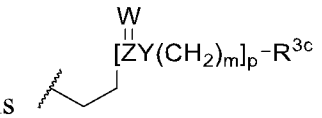
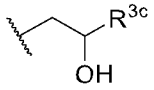
each m is independently 0, 1, 2 or 3;

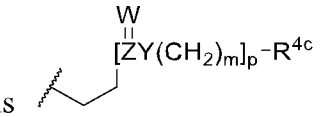
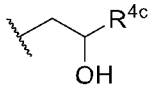
each p is independently 1, 2, 3 or 4; and

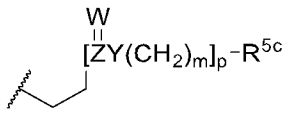
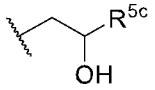
each of R^b and R^c is independently selected from hydrogen, alkyl, alkyloxycarbonyl, alkylacyl, arylacyl and sulfonyl.

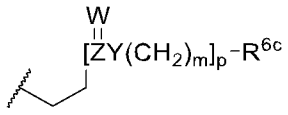
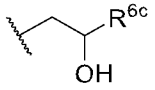
25. The compound of claim 1, or a pharmaceutically acceptable salt thereof, wherein R^1 is  or  ;

R^2 is  or  ;

R^3 is  or  ;

R^4 is  or  ;

R^5, if exists, is  or  ;

R^6, if exists, is  or  ;

each W is independently selected from O, S or NR^b;

each Y is independently selected from O, S, NR^c, $N(R^c)Z(W)$, $N(R^c)N(R^c)$ or $N(R^c)N(R^c)Z(W)$;

each Z is independently selected from C, S or S(O);

each m is independently 0 or 1;

each p is independently 1 or 2;

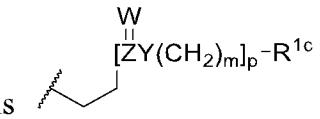
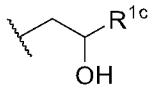
each of R^b and R^c is independently selected from hydrogen, alkyl, alkyloxycarbonyl, alkylacyl, arylacyl and sulfonyl; and

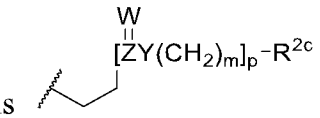
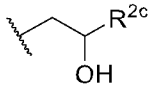
each of R^{1c}, R^{2c}, R^{3c}, R^{4c}, R^{5c} and R^{6c}, if exists, is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, oxo and cyano, and the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally interrupted by one or more groups independently selected from the group consisting of cycloalkyl, heterocyclyl, aryl and heteroaryl.

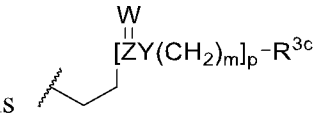
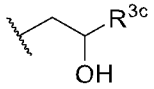
26. The compound of claim 1, or a pharmaceutically acceptable salt thereof, wherein

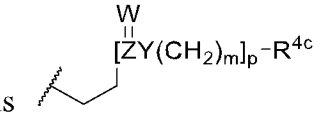
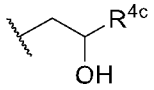
L^b is $-NR^a-$;

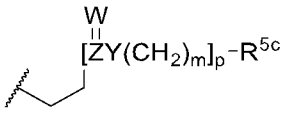
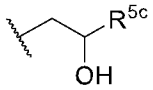
R^a is selected from the group consisting of hydrogen, R^5, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, cyano, cycloalkyl, heterocyclyl, aryl, heteroaryl and R^6;

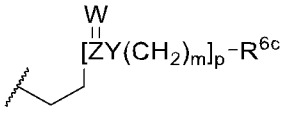
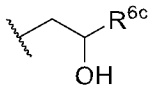
R^1 is  or  ;

R^2 is  or  ;

R^3 is  or  ;

R^4 is  or  ;

R^5, if exists, is  or  ;

R^6, if exists, is  or  ;

each W is independently selected from O, S or NR^b;

each Y is independently selected from O, S, NR^c, $N(R^c)Z(W)$, $N(R^c)N(R^c)$ or $N(R^c)N(R^c)Z(W)$;

each Z is independently selected from C, S or S(O);

each m is independently 0 or 1;

each p is 1 or 2;

each of R^b and R^c is independently selected from hydrogen, alkyl, alkyloxycarbonyl, alkylacyl, arylacyl and sulfonyl; and

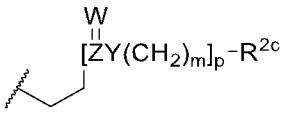
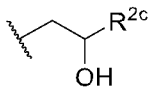
each of R^{1c}, R^{2c}, R^{3c}, R^{4c}, R^{5c} and R^{6c}, if exists, is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, oxo and cyano, and the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally interrupted by one or more groups independently selected from the group consisting of cycloalkyl, heterocyclyl, aryl and heteroaryl.

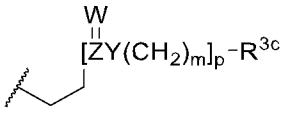
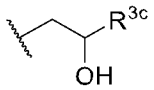
27. The compound of claim 1, or a pharmaceutically acceptable salt thereof, wherein L^b is $-NR^a-$;

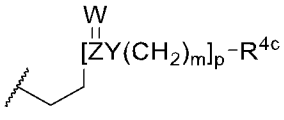
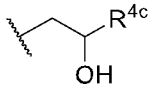
R^a is selected from the group consisting of R^5, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are substituted by R^6 and optionally substituted by one or more additional groups independently selected from the group consisting of halogen,

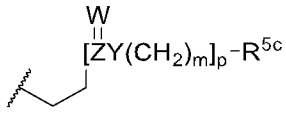
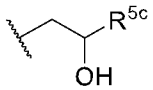
hydroxyl, cyano, cycloalkyl, heterocyclyl, aryl and heteroaryl;

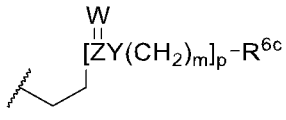
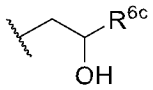
R^1 is selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, cyano, cycloalkyl, heterocyclyl, aryl and heteroaryl;

R^2 is  or  ;

R^3 is  or  ;

R^4 is  or  ;

R^5, if exists, is  or  ;

R^6, if exists, is  or  ;

each W is independently selected from O, S or NR^b;

each Y is independently selected from O, S, NR^c, $N(R^c)Z(W)$, $N(R^c)N(R^c)$ or $N(R^c)N(R^c)Z(W)$;

each Z is independently selected from C, S or S(O);

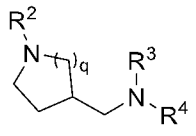
each m is independently 0 or 1;

each p is 1 or 2;

each of R^b and R^c is independently selected from hydrogen, alkyl, alkyloxycarbonyl, alkylacyl, arylacyl or sulfonyl; and

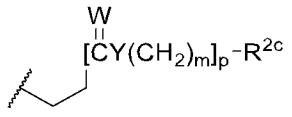
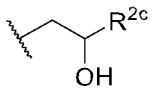
each of R^{2c}, R^{3c}, R^{4c}, R^{5c} and R^{6c}, if exists, is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, oxo and cyano, and the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally interrupted by one or more groups independently selected from the group consisting of cycloalkyl, heterocyclyl, aryl and heteroaryl.

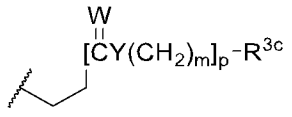
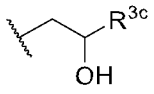
28. The compound of claim 1, or a pharmaceutically acceptable salt thereof, wherein the compound has Formula (A) below:

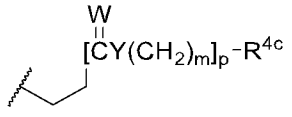
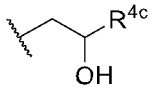


(A),

wherein

R^2 is  or  ;

R^3 is  or  ;

R^4 is  or  ;

each W is independently selected from O, S or NR^b;

each Y is independently selected from O, S, NR^c, $N(R^c)Z(W)$, $N(R^c)N(R^c)$ or $N(R^c)N(R^c)Z(W)$;

each Z is independently selected from C, S or S(O);

each m is independently 0 or 1;

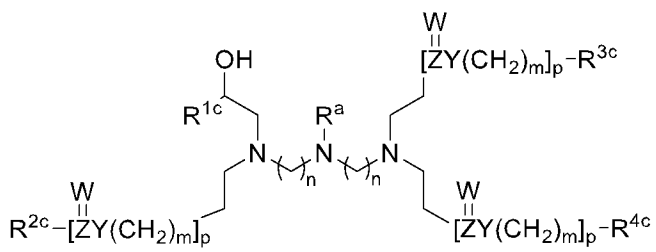
each p is independently 1 or 2;

q is 1, 2 or 3;

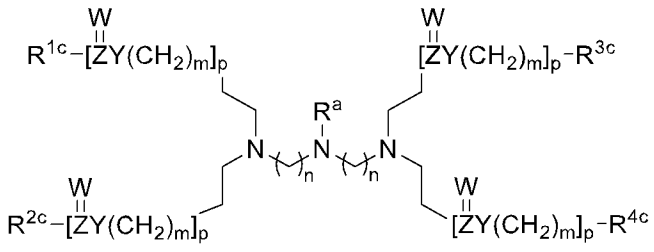
each of R^b and R^c is independently selected from hydrogen, alkyl, alkyloxycarbonyl, alkylacyl, arylacyl or sulfonyl; and

each of R^{2c}, R^{3c} and R^{4c} is independently selected from the group consisting of alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl, wherein the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally substituted by one or more groups independently selected from the group consisting of halogen, hydroxyl, oxo and cyano, and the alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl and heteroalkynyl are optionally interrupted by one or more groups independently selected from the group consisting of cycloalkyl, heterocyclyl, aryl and heteroaryl.

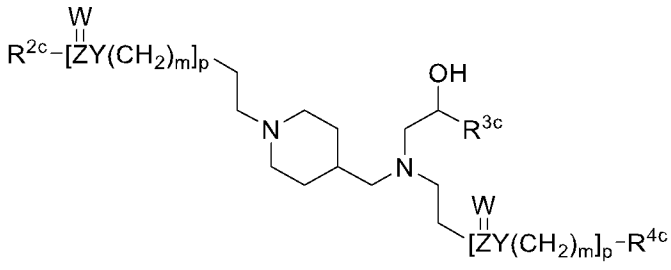
29. The compound of claim 23, or a pharmaceutically acceptable salt thereof, wherein the compound has Formula (B), Formula (C), Formula (D) or Formula (E):



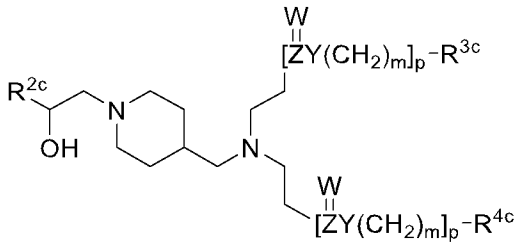
(B),



(C),



(D),



(E),

wherein

each W is independently selected from O, S or NR^b;

each Y is independently selected from O, S, NR^c, $N(R^c)Z(W)$, $N(R^c)N(R^c)$ or $N(R^c)N(R^c)Z(W)$;

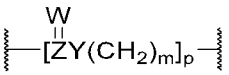
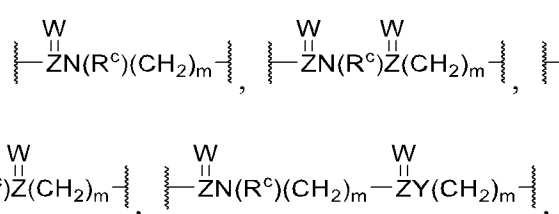
each Z is independently selected from C, S or S(O);

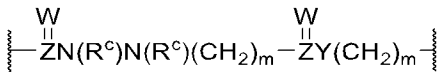
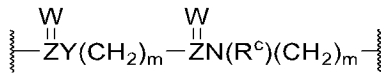
each n is independently 0, 1, 2, 3, 4 or 5;

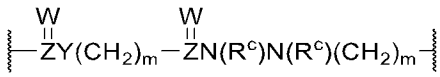
each m is independently 0, 1, 2 or 3;

each p is independently 1, 2, 3 or 4; and

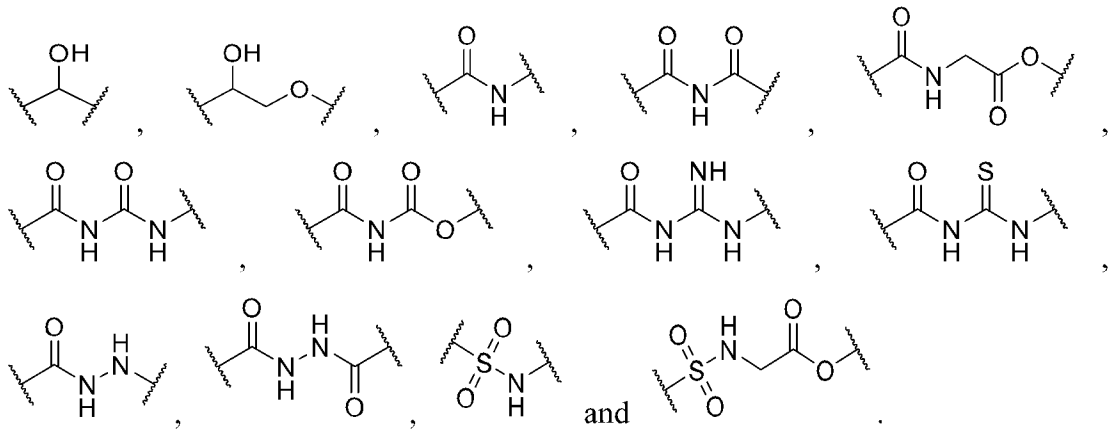
each of R^b and R^c is independently selected from hydrogen, alkyl, alkyloxycarbonyl, acyl or sulfonyl.

30. The compound of any one of claims 24 to 29, or a pharmaceutically acceptable salt thereof, wherein each of  is independently selected from the group consisting of 

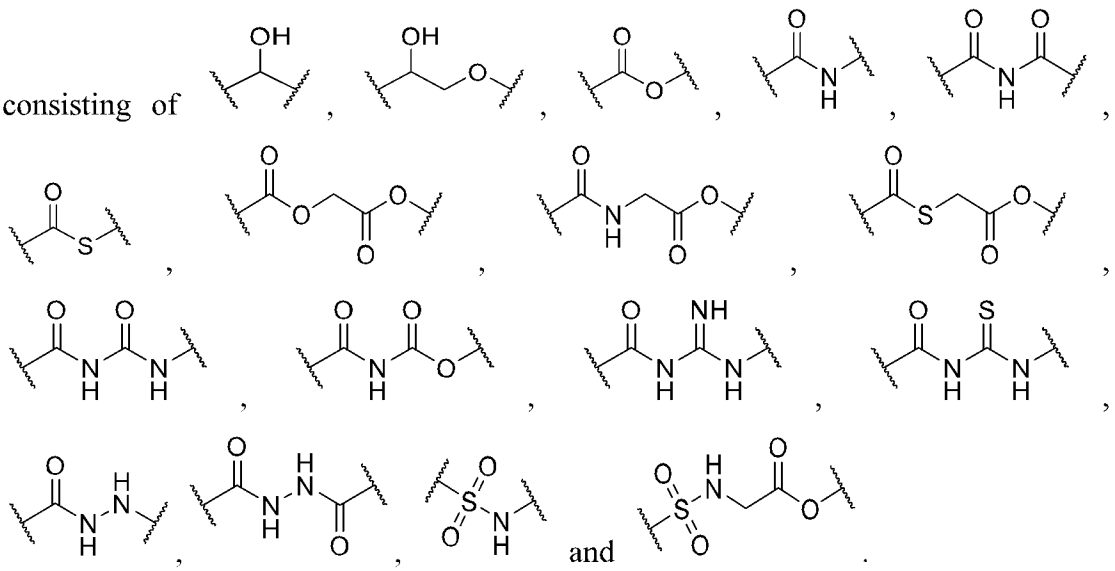
 ,  and

 .

31. The compound of claim 30, or a pharmaceutically acceptable salt thereof, wherein R^{1b}, R^{2b}, R^{3b}, R^{4b}, R^{5b} and R^{6b} is independently selected from the group consisting of



32. The compound of claim 23, or a pharmaceutically acceptable salt thereof, wherein one or more of R^{1b}, R^{2b}, R^{3b}, R^{4b}, R^{5b} and R^{6b} is independently selected from the group consisting of



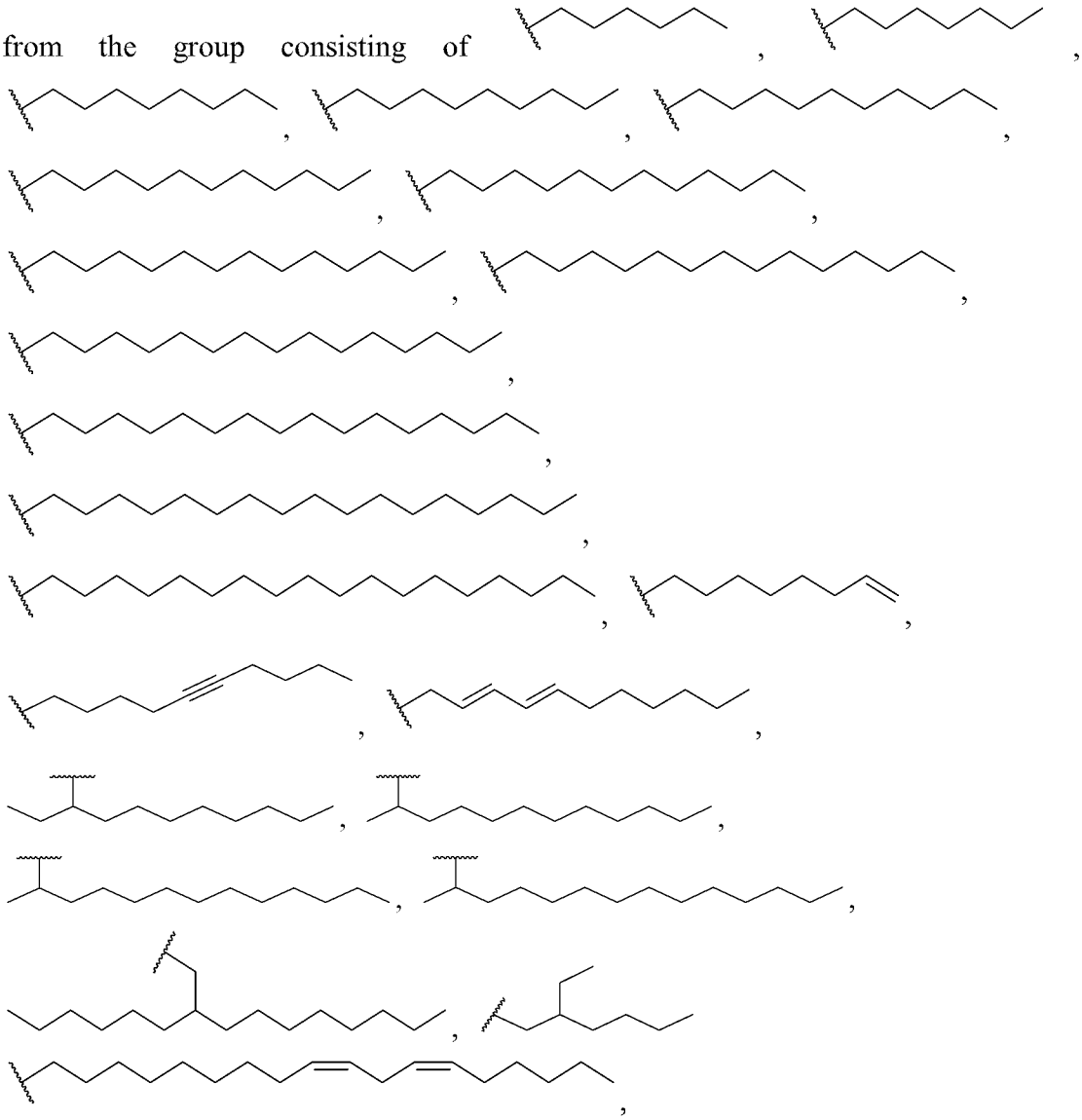
33. The compound of any one of claims 1 to 32, or a pharmaceutically acceptable salt thereof, wherein one or more of R^{1c}, R^{2c}, R^{3c}, R^{4c}, R^{5c} and R^{6c} is independently selected from the group consisting of alkyl, alkenyl and alkynyl, wherein the alkyl, alkenyl and alkynyl are optionally substituted with one or more halogens.

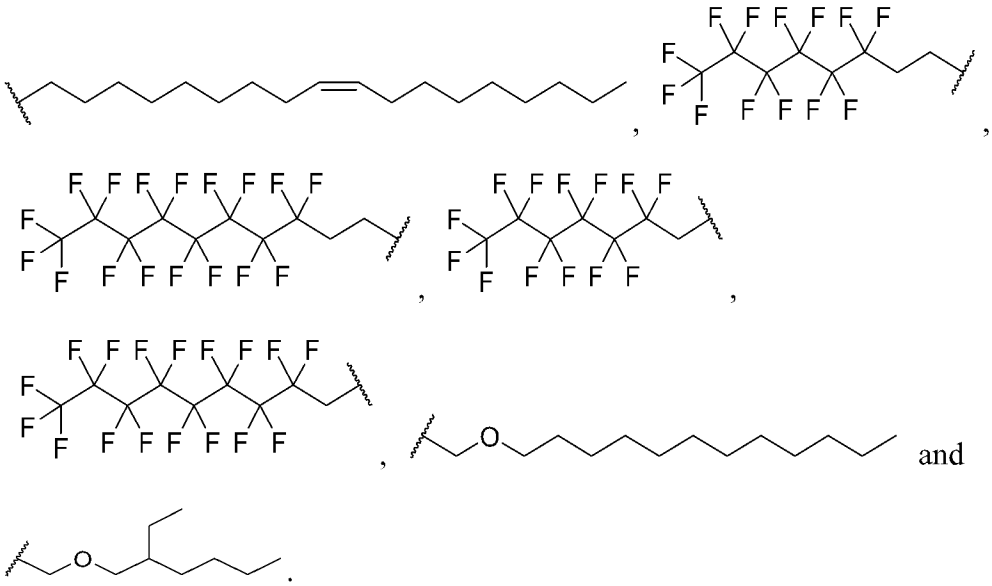
34. The compound of any one of claims 1 to 32, or a pharmaceutically acceptable salt

thereof, wherein one or more of R^{1c}, R^{2c}, R^{3c}, R^{4c}, R^{5c} and R^{6c} is independently selected from the group consisting of -CH_2-O-alkyl, -CH_2-O-alkenyl, -CH_2-O-alkynyl, -CH_2-O-heteroalkyl, -CH_2-O-heteroalkenyl and -CH_2-O-heteroalkynyl.

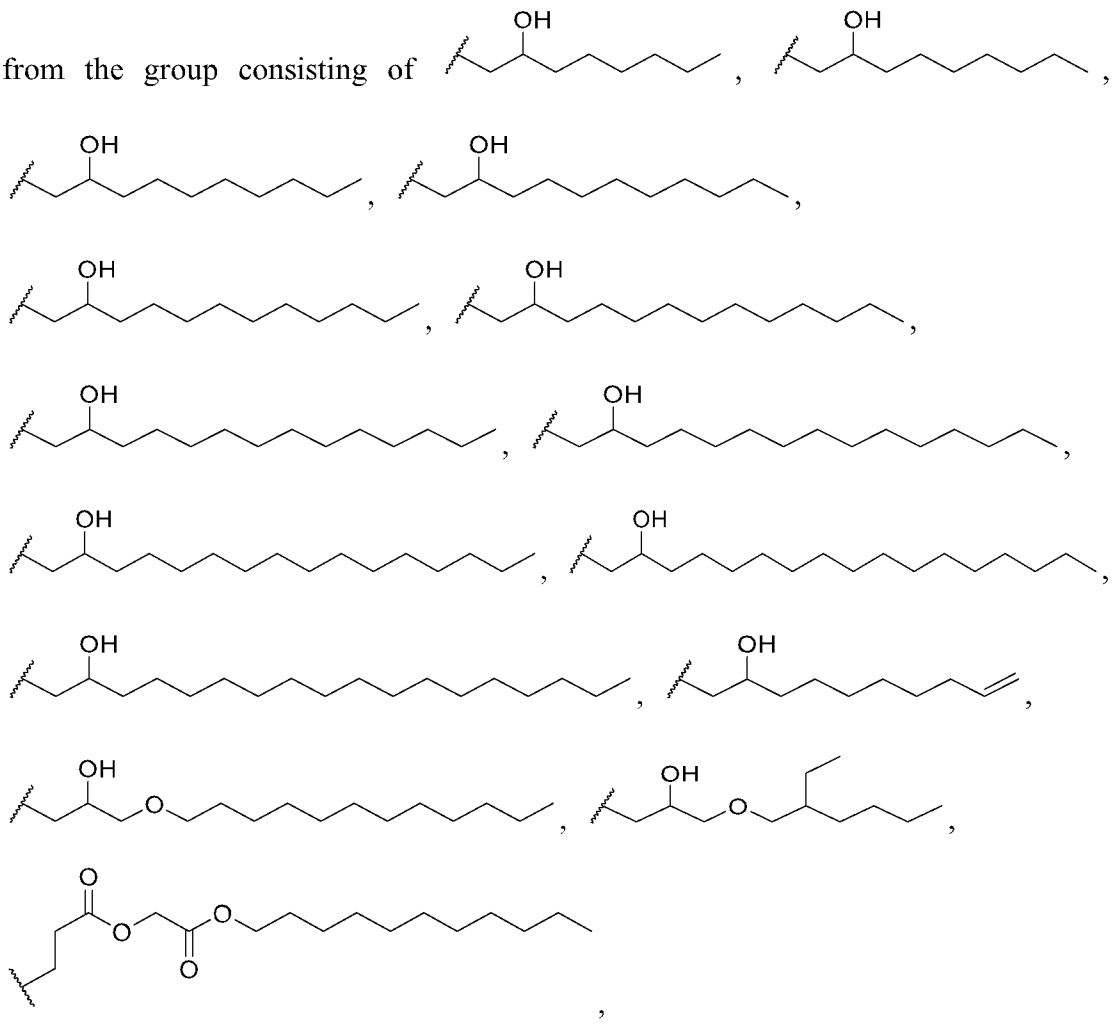
35. The compound of claim 33 or 34, or a pharmaceutically acceptable salt thereof, wherein each of the alkyl, alkenyl and alkynyl independently contains 6 to 18 carbon atoms.

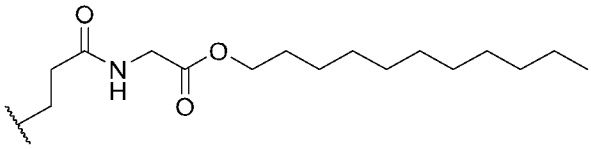
36. The compound of claim 33 or 34, or a pharmaceutically acceptable salt thereof, wherein each of the alkenyl independently contains one or more Z-olefin.

37. The compound of any one of claims 1 to 36, or a pharmaceutically acceptable salt thereof, wherein one or more of R^{1c}, R^{2c}, R^{3c}, R^{4c}, R^{5c} and R^{6c} is independently selected from the group consisting of 



38. The compound of any one of claims 1 to 37, or a pharmaceutically acceptable salt thereof, wherein one or more of R^1, R^2, R^3, R^4, R^5 and R^6 is independently selected from the group consisting of

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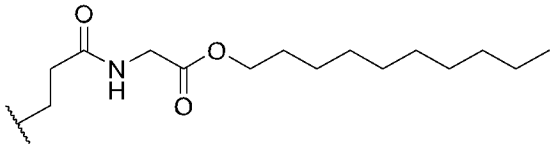
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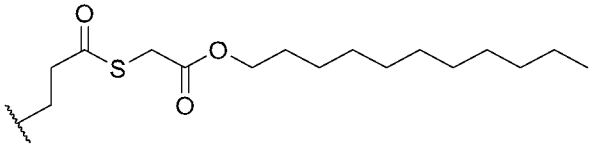
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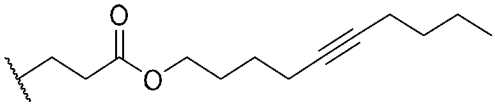
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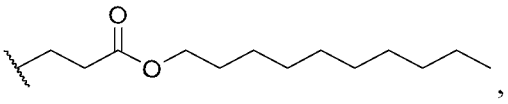
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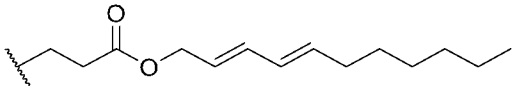
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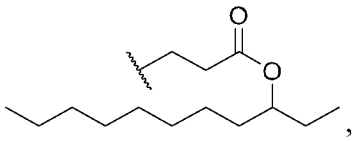
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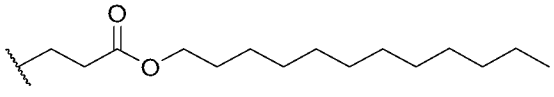
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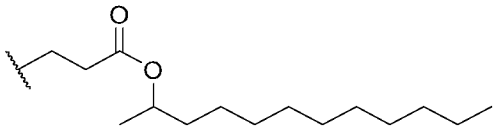
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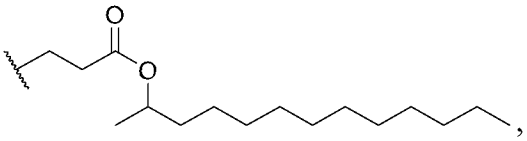
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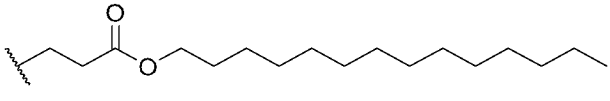
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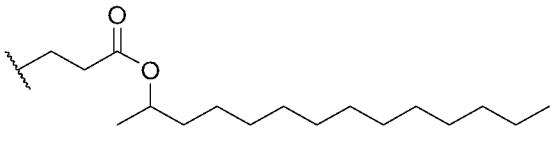
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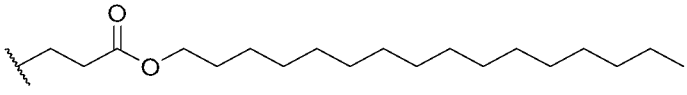
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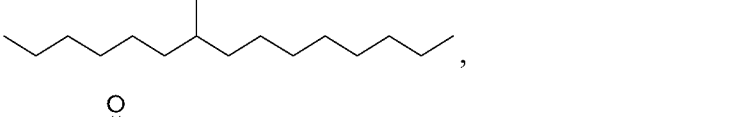
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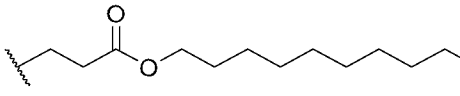
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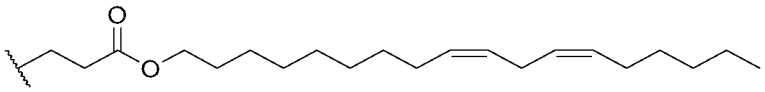
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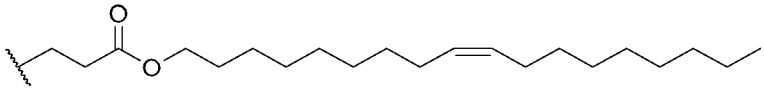
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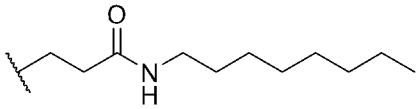
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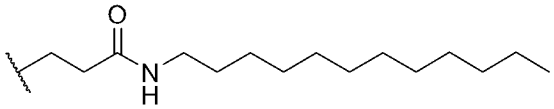
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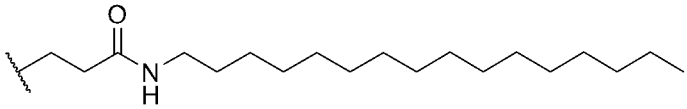
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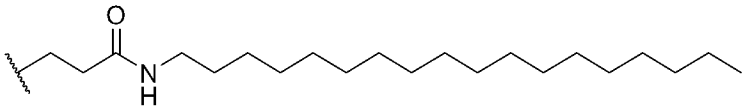
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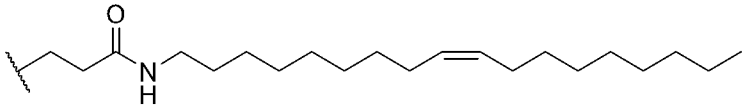
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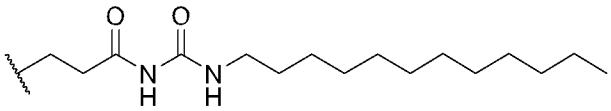
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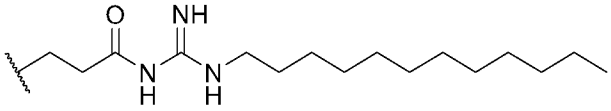
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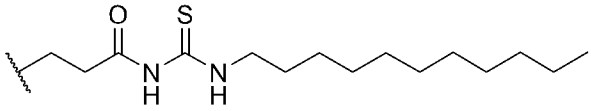
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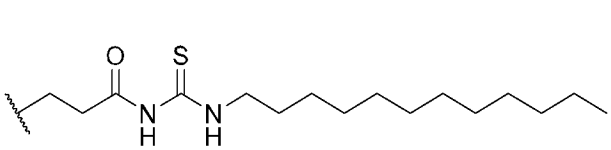
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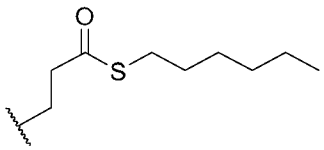
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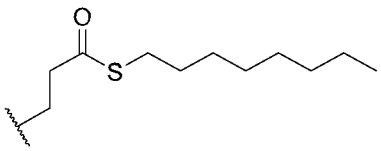
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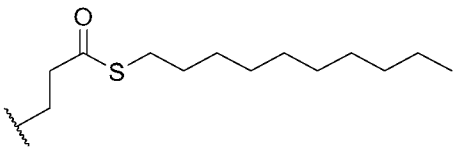
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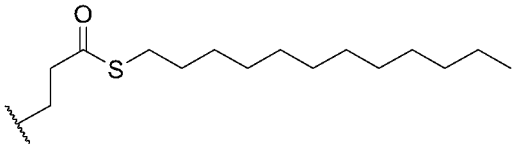
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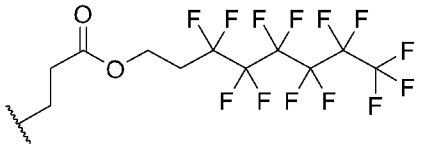
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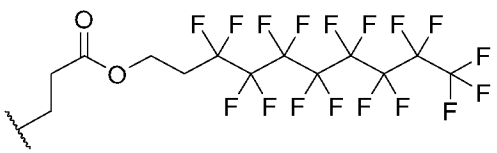
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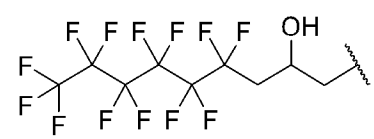
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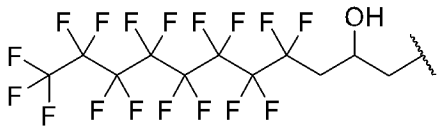
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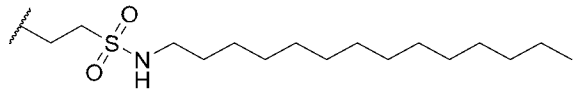
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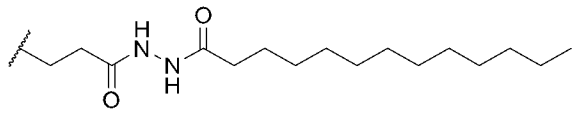
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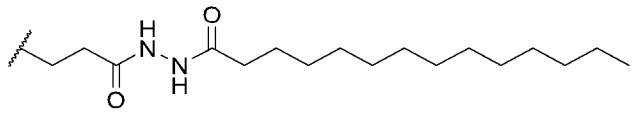
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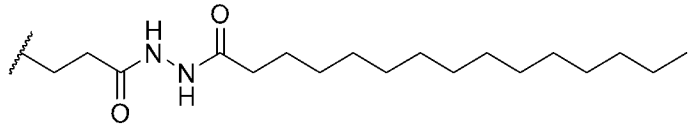
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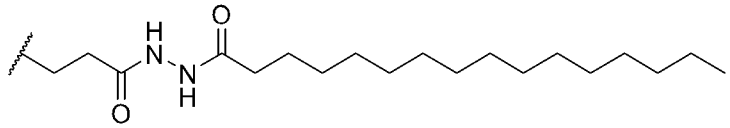
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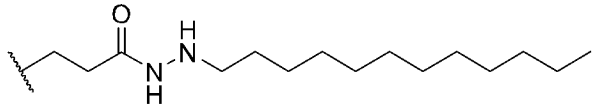
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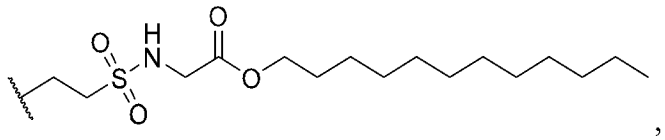
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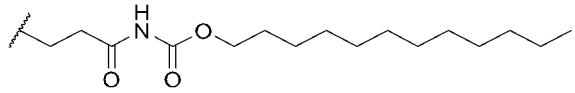
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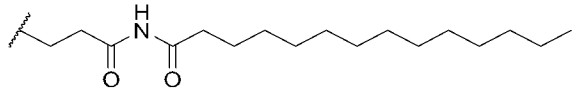
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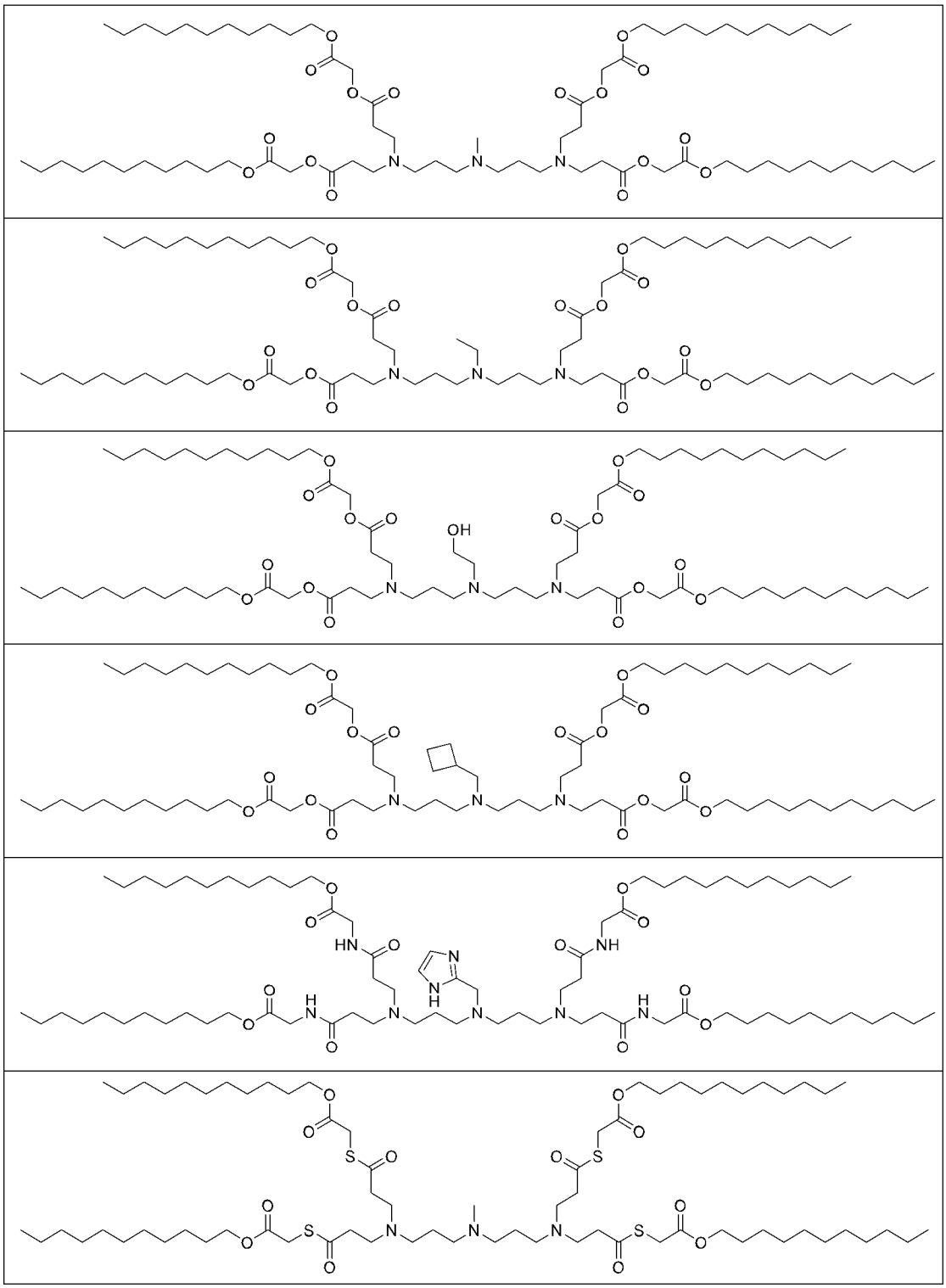
39. The compound of any one of claims 1 to 38, or a pharmaceutically acceptable salt thereof, wherein each of -R^{1b}-R^{1c}, -R^{2b}-R^{2c}, -R^{3b}-R^{3c}, -R^{4b}-R^{4c}, -R^{5b}-R^{5c} and -R^{6b}-R^{6c}, if exists, does not comprise two heteroatoms directly bonded to each other.

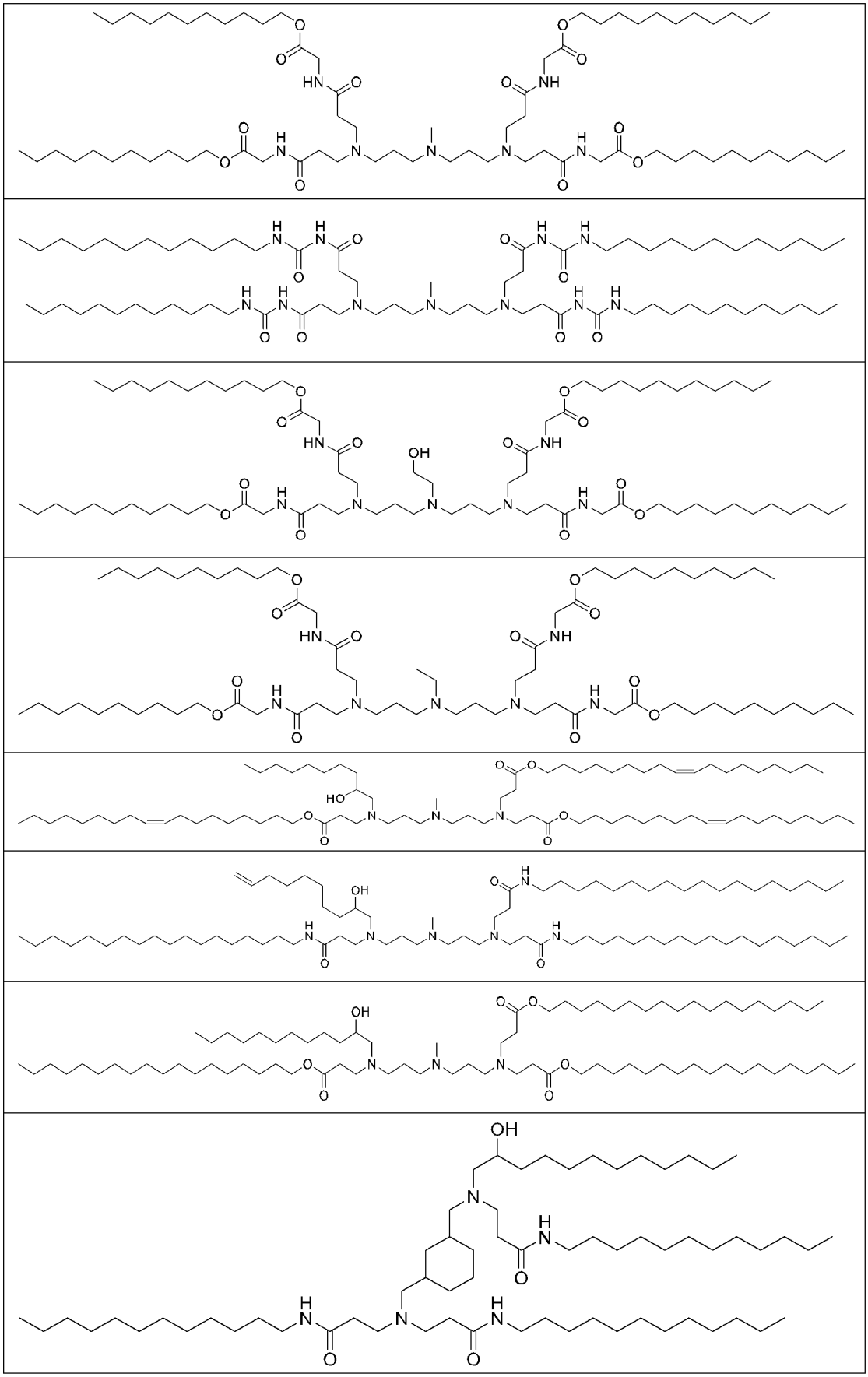
40. The compound of any one of claims 1 to 38, or a pharmaceutically acceptable salt thereof, wherein one or more of -R^{1b}-R^{1c}, -R^{2b}-R^{2c}, -R^{3b}-R^{3c}, -R^{4b}-R^{4c}, -R^{5b}-R^{5c} and -R^{6b}-R^{6c}, if exists, comprises one or more groups independently selected from the group consisting of -N(R^c)-N(R^c)-, -N(R^c)-S(O)-, -N(R^c)-S(O)$_2$-, -N(R^c)-O-, -S(O)-N(R^c)-, -S(O)$_2$-N(R^c)-, -S(O)-O-, -S(O)$_2$-O-, -O-N(R^c)-, -O-S(O)-, -O-S(O)$_2$- and -O-O-.

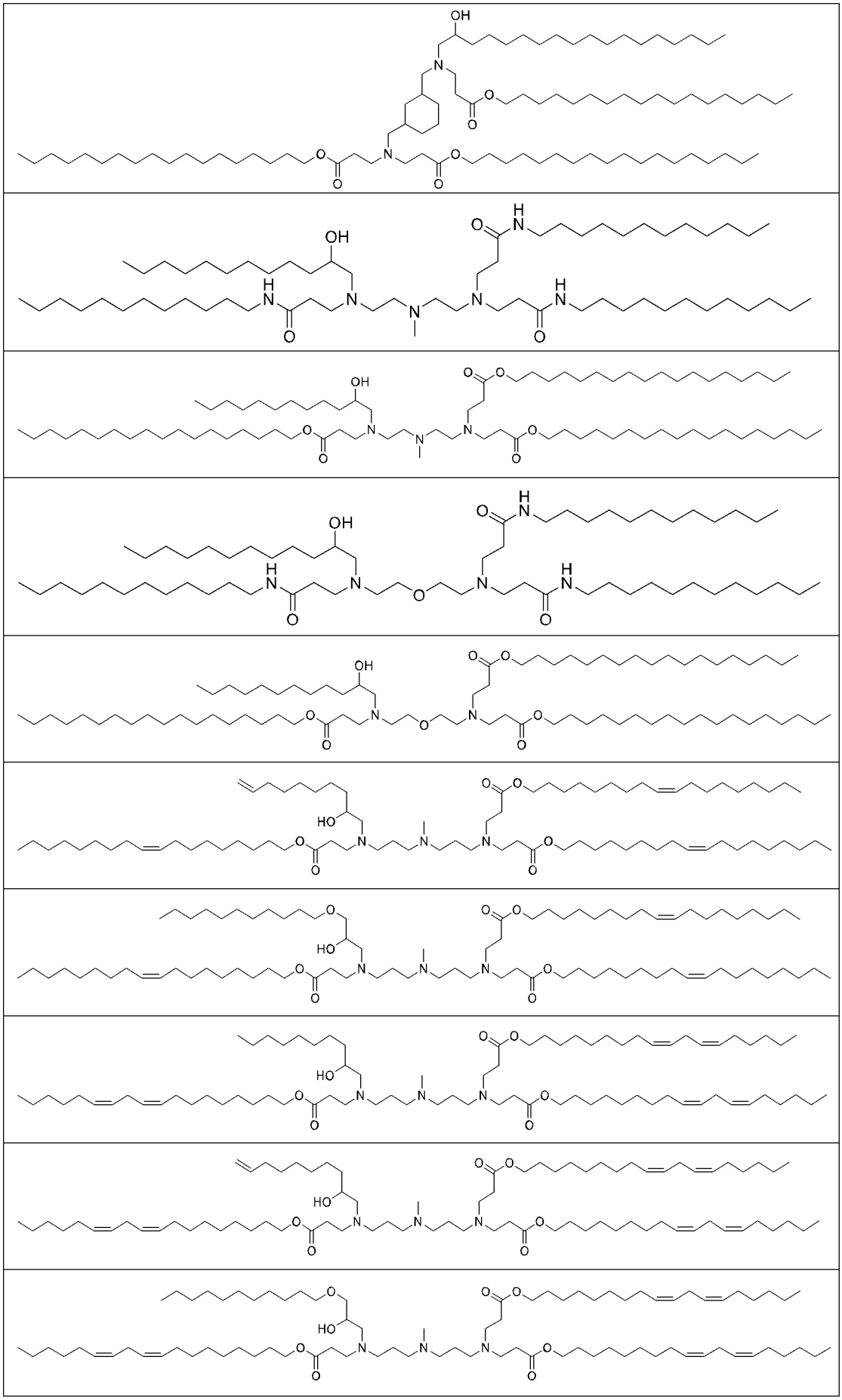
41. The compound of claim 40, or a pharmaceutically acceptable salt thereof, wherein one or more of -R^{1b}-R^{1c}, -R^{2b}-R^{2c}, -R^{3b}-R^{3c}, -R^{4b}-R^{4c}, -R^{5b}-R^{5c} and -R^{6b}-R^{6c}, if exists,

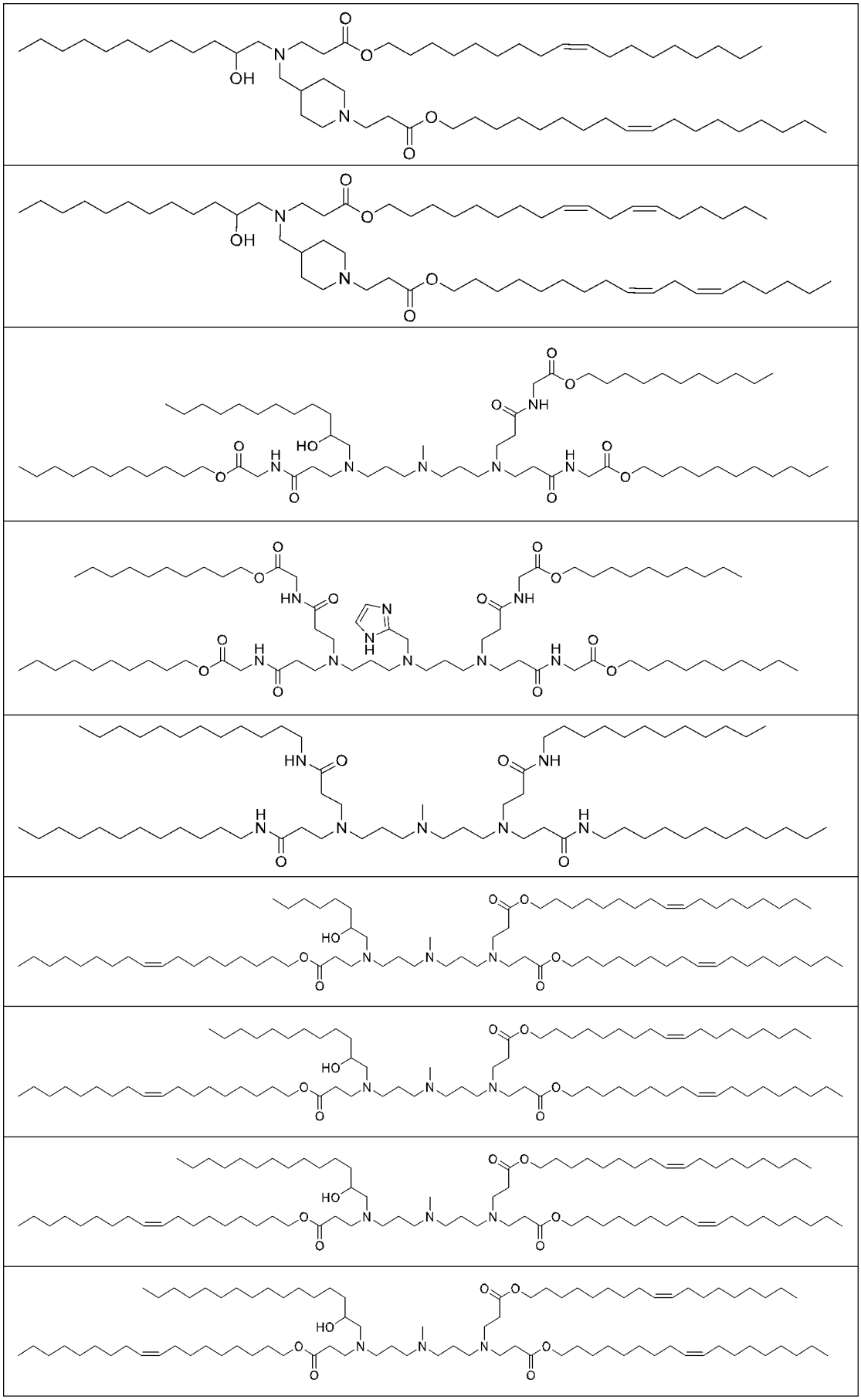
comprises -N(R^e)-N(R^e)- or -S(O)$_2$-N(R^e)-.

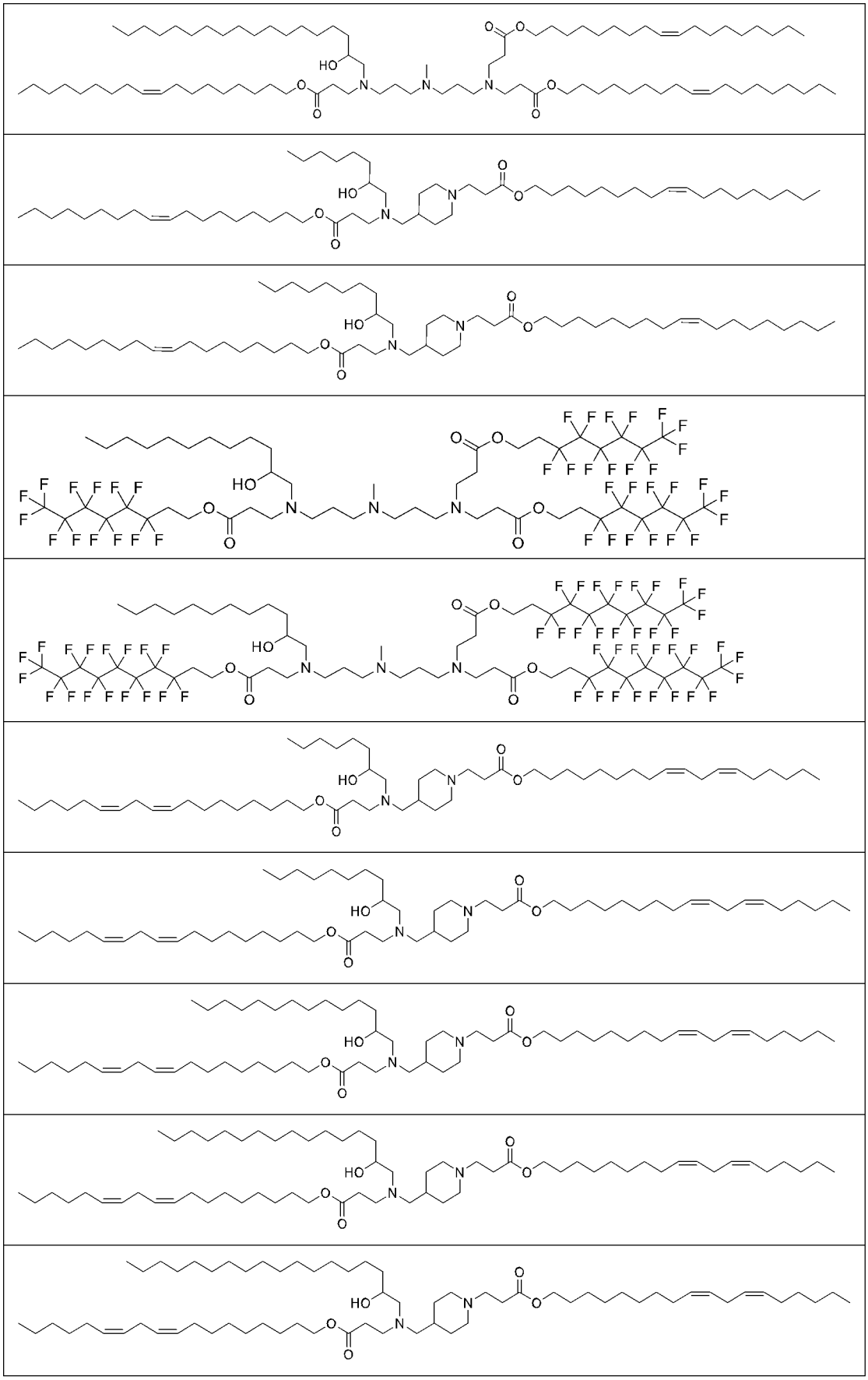
42. The compound of claim 1, or a pharmaceutically acceptable salt thereof, which is selected from any one listed in the table below:

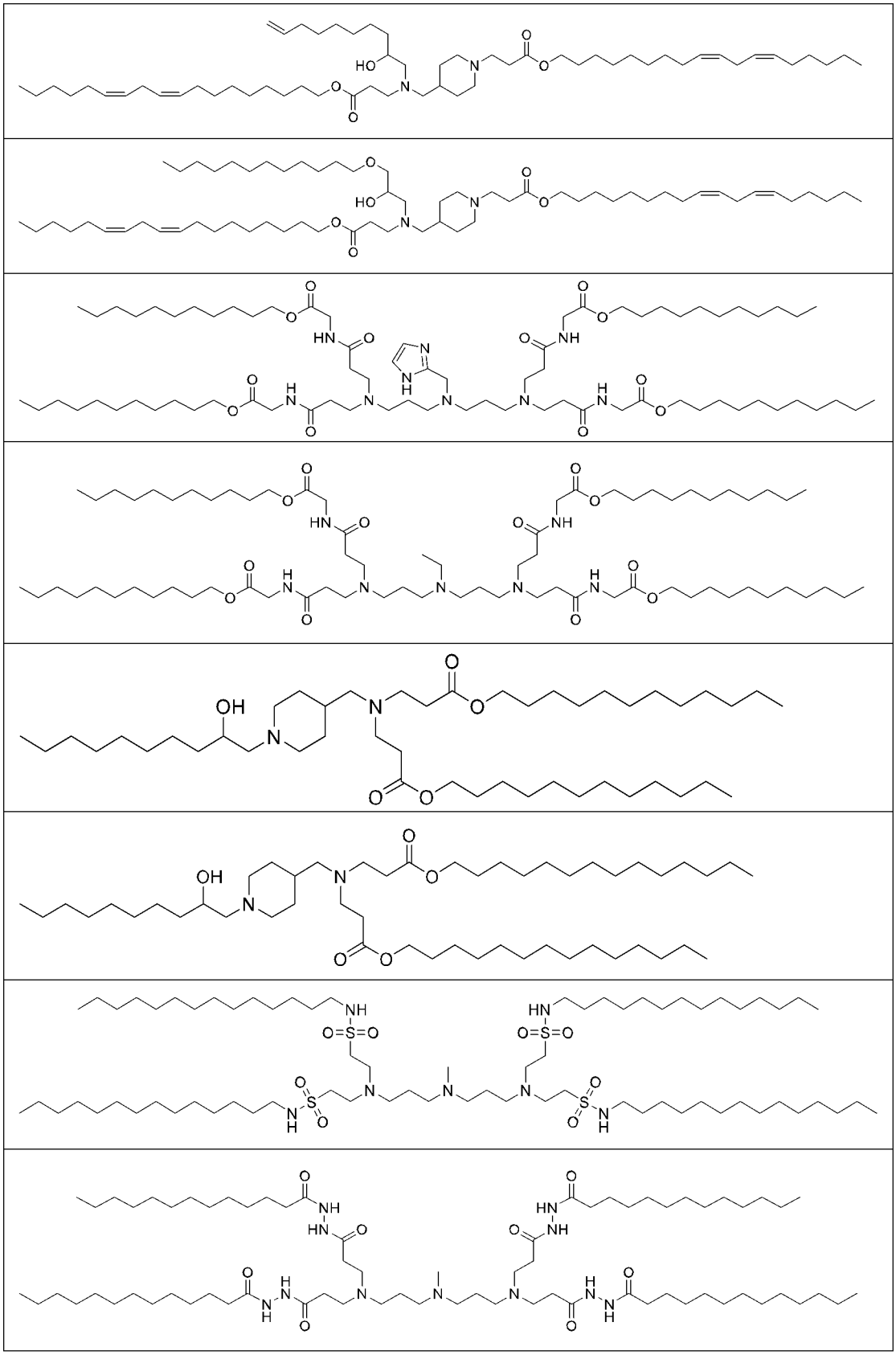


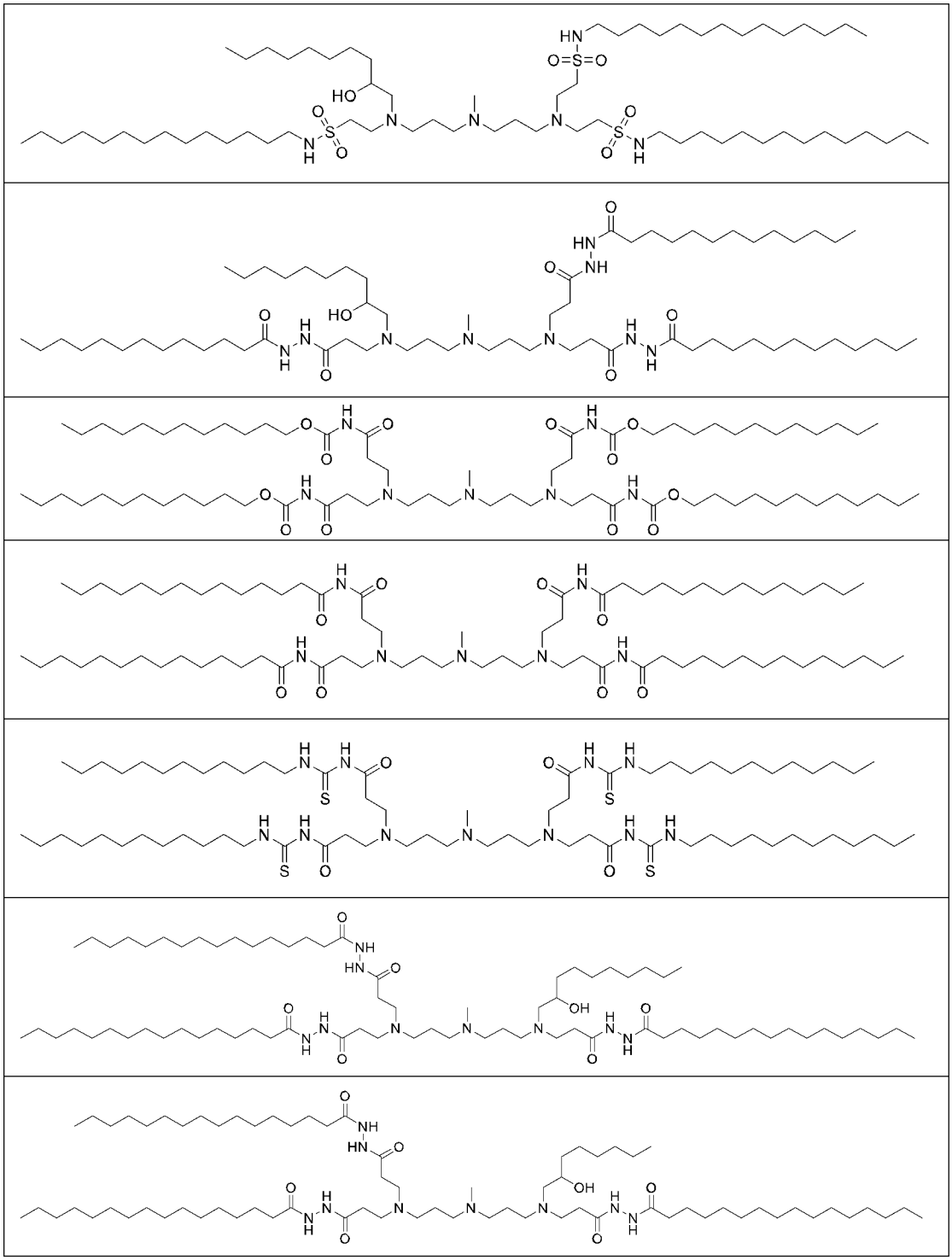












43. A lipid particle comprising the compound of any one of claims 1 to 42, or a pharmaceutically acceptable salt thereof.

44. The lipid particle of claim 43, wherein the lipid particle further comprises a neutral lipid.

45. The lipid particle of claim 44, wherein the neutral lipid is selected from the group consisting of 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dimyristoyl-sn-glycero-3-phosphocholine

(DMPC), 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-Distearoyl-sn-glycero-3-phosphorylethanolamine (DSPE), 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), 1,2-di-O-octadecenyl-5-glycero-3-phosphocholine (18:0 Diether PC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1-oleoyl-2-cholesterylhemisuccinoyl-5-glycero-3-phosphocholine (OChemsPC), and any mixtures thereof.

46. The lipid particle of any one of claims 43 to 45, wherein the lipid particle further comprises a sterol, a sterol derivative or any mixtures thereof.

47. The lipid particle of claim 46, wherein the sterol is cholesterol, β-sitosterol, stigmasterol, ergosterol, brassicasterol, fecosterol or campesterol.

48. The lipid particle of any one of claims 43 to 47, wherein the lipid particle further comprises a surfactant.

49. The lipid particle of claim 48, wherein the surfactant is selected from the group consisting of 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol (DMG-PEG), 1,2-distearoyl-rac-glycero-3-methoxypolyethylene glycol (DSG-PEG), N-(methylpolyoxyethylene-carbonyl)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (PEG-DSPE), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[(polyethylene glycol)] (DOPE-PEG), and any mixtures thereof.

50. The lipid particle of any one of claims 43 to 49, wherein the lipid particle comprising in a molar fraction of:

10-75% of the compound of any one of claims 1 to 42;

1-35% of a neutral lipid;

15-50% of a sterol, a sterol derivative or any mixtures thereof; and

0.5-5% of a surfactant,

based on the total amount of the lipid particle.

51. The lipid particle of any one of claims 43 to 50, wherein the lipid particle comprising in a molar fraction of:

40-75% of the compound of any one of claims 1 to 42;

2-15% of a neutral lipid;

20-50% of a sterol, a sterol derivative or any mixtures thereof; and

1-5% of a surfactant,

based on the total amount of the lipid particle.

52. The lipid particle of any one of claims 43 to 51, wherein the lipid particle comprising in a molar fraction of:

40-70 % of the compound of any one of claims 1 to 42;

3-15% of DSPC;

20-50% of cholesterol; and

1-2.5% of DMG-PEG,

based on the total amount of the lipid particle.

53. The lipid particle of any one of claims 43 to 52, wherein the lipid particle comprising in a molar fraction of:

40-70% of the compound of any one of claims 1 to 42;

3-15% of DSPC;

22-50% of cholesterol; and

1.5-2.5% of DMG-PEG,

based on the total amount of the lipid particle.

54. The lipid particle of any one of claims 43 to 53, wherein the lipid particle further comprises a targeting moiety conjugated to the lipid particle.

55. The lipid particle of claim 54, wherein the targeting moiety is conjugated to the lipid particle directly or via a linker.

56. The lipid particle of claim 54 or 55, wherein the targeting moiety is an antibody or a ligand.

57. A loaded lipid particle comprising the lipid particle of any one of claims 43-56 and a therapeutic agent.

58. The loaded lipid particle of claim 57, wherein the therapeutic agent is a nucleic acid.

59. The loaded lipid particle of claim 58, wherein the nucleic acid is selected from the group consisting of small interfering RNA (siRNA), microRNA (miRNA), small activating RNAs (saRNA), single guide RNA (sgRNA), messenger RNA (mRNA), self-amplifying RNA, circRNA, antisense oligonucleotide (ASO), ribonucleoproteins (RNP), ssDNA, dsDNA, and any mixtures thereof.

60. The loaded lipid particle of claim 59, wherein the nucleic acid is an mRNA encoding a therapeutical protein.

61. The loaded lipid particle of claim 60, wherein the therapeutical protein is an antibody, an antigen, a cytokine, a coagulation factor or an enzyme, or a functional fragment thereof.

62. The loaded lipid particle of claim 61, wherein the therapeutical protein is an antigen or a functional fragment thereof.

63. The loaded lipid particle of any one of claims 57 to 62, wherein a mass ratio of the lipids particle to the therapeutic agent is in a range of 10:1 to 50:1.

64. A pharmaceutical composition comprising the loaded lipid particle of any one of claims 57 to 63, and a pharmaceutically acceptable excipient.

65. A method of delivering a therapeutic agent to a subject, comprising the step of:

i) encapsulating a therapeutic agent into the lipid particle of any one of claims 43 to 56; and

ii) administering the lipid particle encapsulating the therapeutic agent to the subject.

66. A method of delivering a therapeutic agent to a subject, comprising administering to the subject the loaded lipid particle of any one of claims 57 to 63, or the pharmaceutical composition of claim 64.

67. The method of claim 65 or 66, wherein the therapeutic agent is delivered to cells in the subject, wherein the cells are selected from the group consisting of muscle cells, lung cells, liver cells, spleen cells, kidney cells, heart cells, skin cells, hair cells, nail cells, bone cells, artery cells, vein cells, diaphragm cells, larynx cells, stomach cells, intestine cells, ureter cells, bladder cells, lymph node cells, bone marrow cells, thymus cells, brain cells, spinal cord cells, nerve cells, pituitary gland cells, thyroid cells, adrenal cells, penis cells, vagina cells, prostate cells and uterus cells.

68. The method of claim 67, wherein the cells are selected from the group consisting of liver cells, lung cells, spleen cells, muscle cells and brain cells.

69. A method of treating a disease or disorder in a subject comprising administering to the subject an effective amount of the loaded lipid particle of any one of claims 57 to 63, or the pharmaceutical composition of claim 64.

70. Use of the lipid particle of any one of claims 43 to 56, the loaded lipid particle of any one of claims 57 to 63 or the pharmaceutical composition of claim 64 for the manufacture of a medicament for a treatment of a disease or disorder.

71. A method of expressing a protein in a subject comprising:

i) encapsulating a polynucleotide encoding the protein into the lipid particle of any one of claims 43 to 56; and

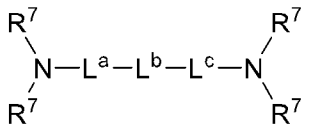
ii) administering the lipid particle encapsulating the polynucleotide to the subject.

72. The method of claim 71, wherein the protein is an antibody, an antigen, a cytokine, a coagulation factor or an enzyme, or a functional fragment thereof.

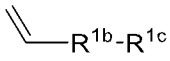
73. The method of claim 72, wherein the protein is an antigen or a functional fragment thereof.

74. A method for preparation of the compound of any one of claims 1 to 42, or a pharmaceutically acceptable salt thereof, comprising:

A) mixing a compound having Formula (II) below and a compound having Formula (III) below:



(II),



(III),

wherein

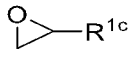
each R^7 is independently selected from the group consisting of hydrogen, alkyl, heteroalkyl, haloalkyl and hydroxyalkyl;

each L^a, L^b, L^c, R^{1b} and R^{1c} has the same definition as in claim 1; and

B) refluxing the mixture until completion of reaction, and obtaining the compound of any one of claims 1 to 42 thereby.

75. A method for preparation of the compound of any one of claims 1 to 42, or a pharmaceutically acceptable salt thereof, comprising:

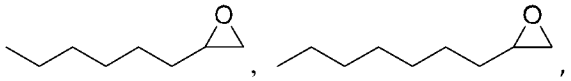
i) mixing a compound having Formula (II) and a compound having Formula (IV) below:

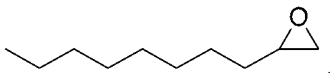
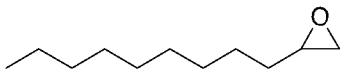
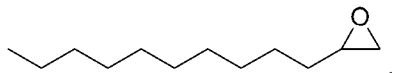

(IV),

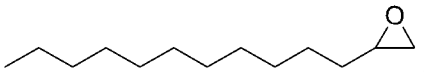
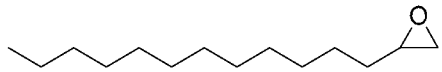
wherein R^{1c} has the same definition as in claim 1;

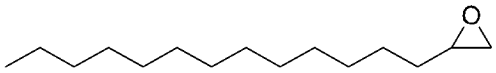
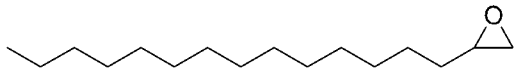
ii) mixing a product of step (i) and a compound having Formula (III); and

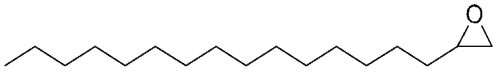
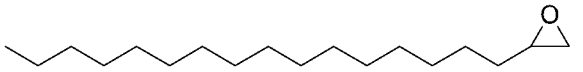
iii) refluxing the mixture until completion of reaction, and obtaining the compound of any one of claims 1 to 42 thereby.

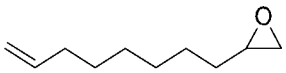
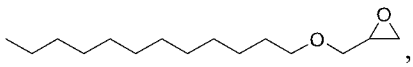
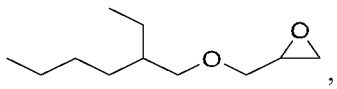
76. The method of claim 75, wherein the compound having Formula (IV) is selected from the group consisting of  , ,

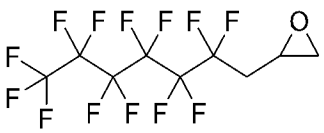
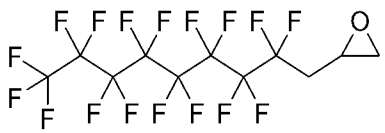
 ,  ,  ,

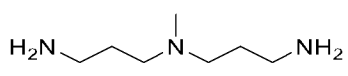
 ,  ,

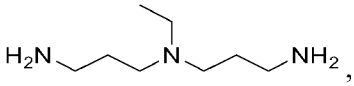
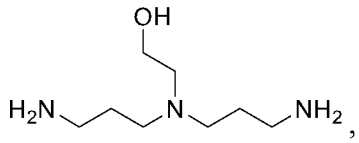
 ,  ,

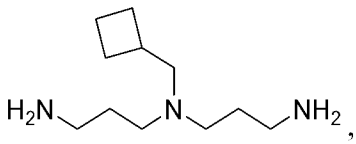
 ,  ,

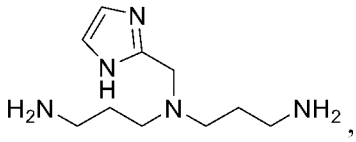
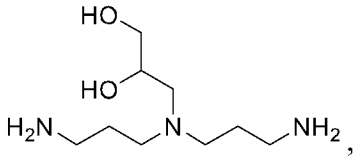
 ,  ,  ,

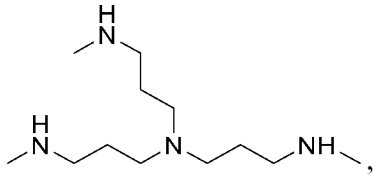
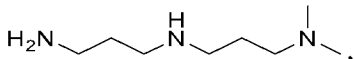
 and  .

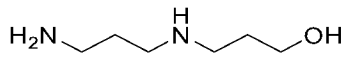
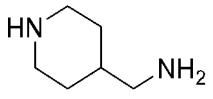
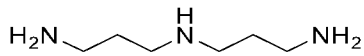
77. The method of any one of claims 74 to 76, wherein the compound having Formula (II) is selected from the group consisting of  ,

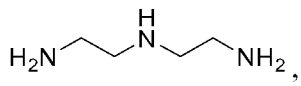
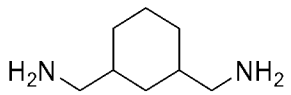
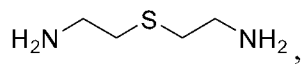
 ,  ,

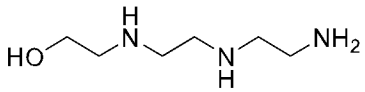
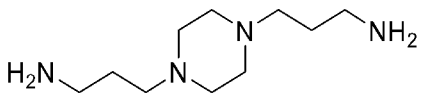
 ,

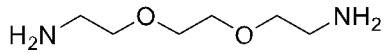
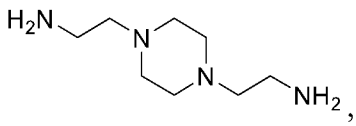
 ,  ,

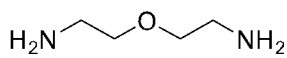
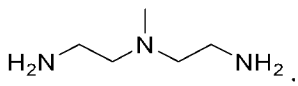
 ,  ,

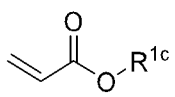
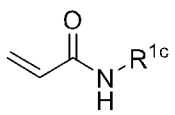
 ,  ,  ,

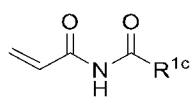
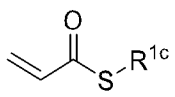
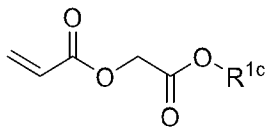
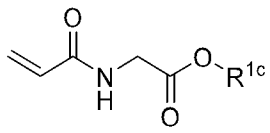
 ,  ,  ,

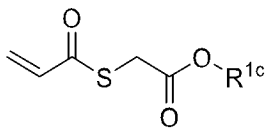
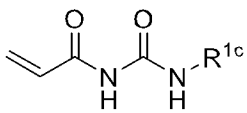
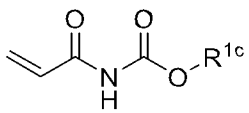
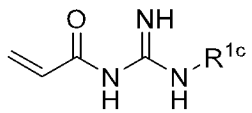
 ,  ,

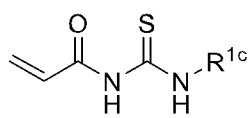
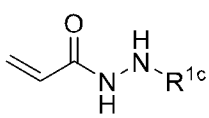
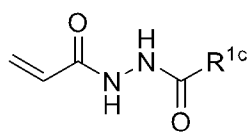
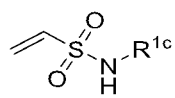
 ,  ,

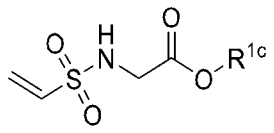
 and  .

78. The method of any one of claims 74 to 77, wherein the compound having Formula (III) is selected from the group consisting of  ,  ,

 ,  ,  ,  ,

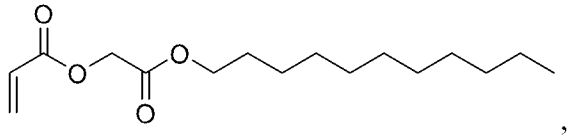
 ,  ,  ,  ,

 ,  ,  ,  and



.

79. The method of any one of claims 74 to 78, wherein the compound having Formula (III) is selected from the group consisting of:



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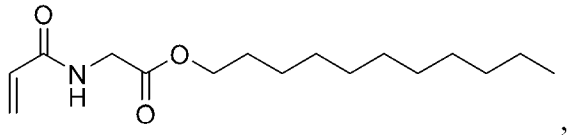

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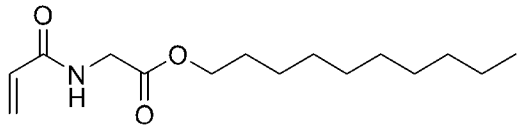

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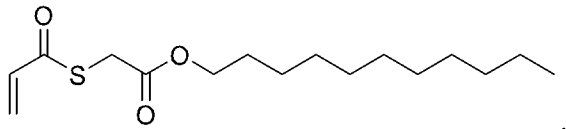

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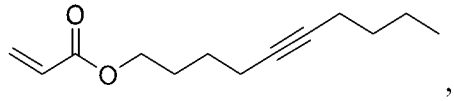

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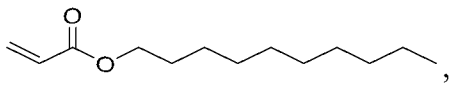

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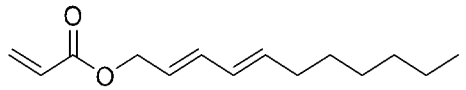

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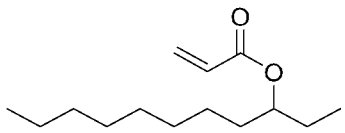

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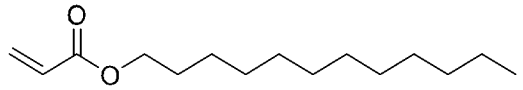

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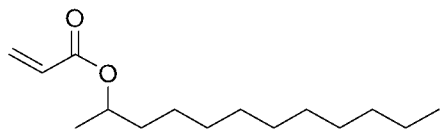

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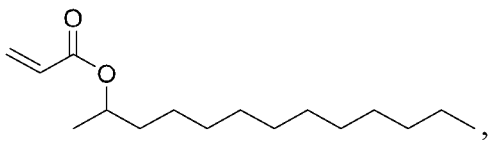

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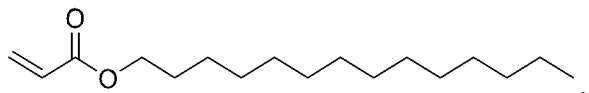

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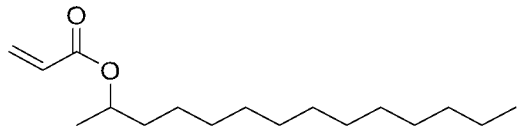

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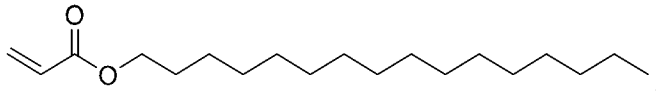
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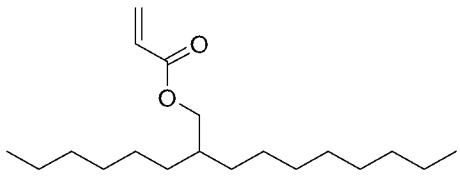
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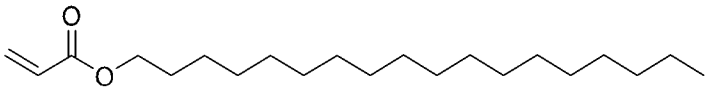
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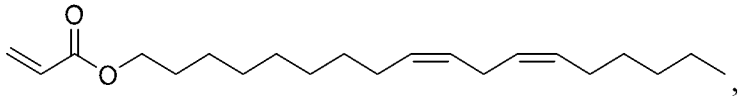
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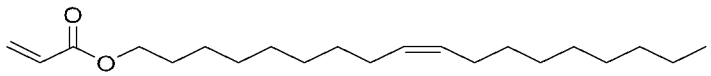
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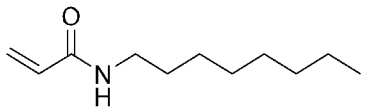
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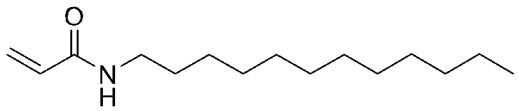
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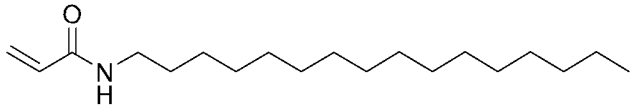
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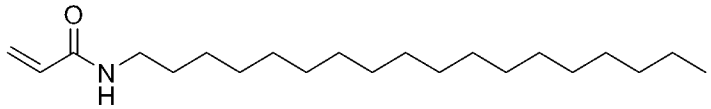
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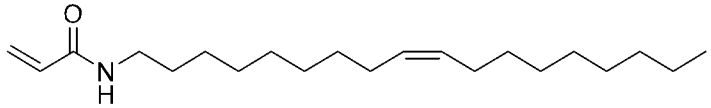
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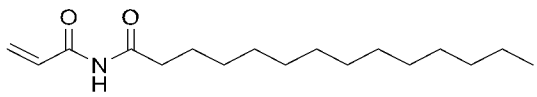
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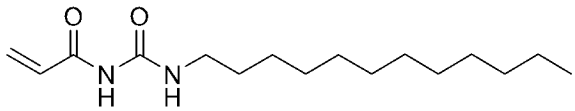
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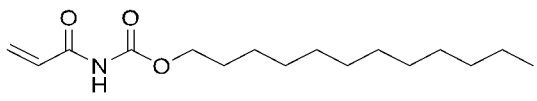
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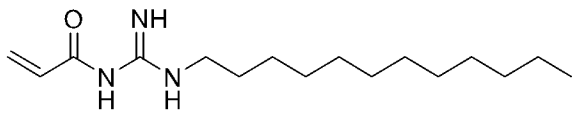
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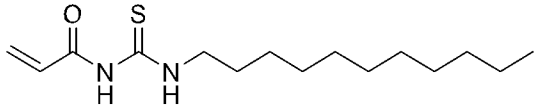
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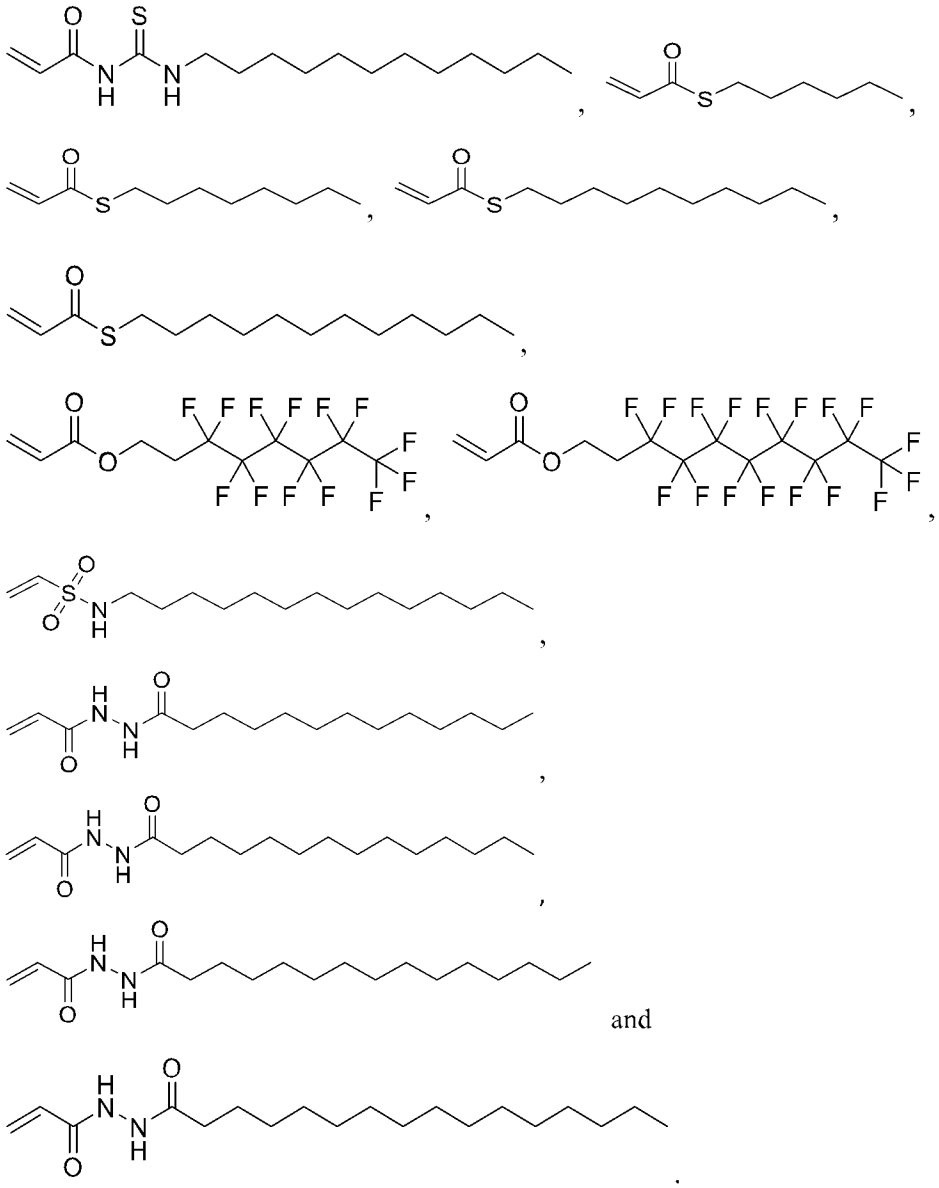
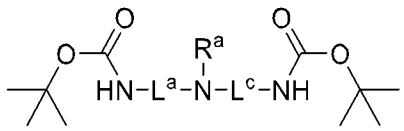


and

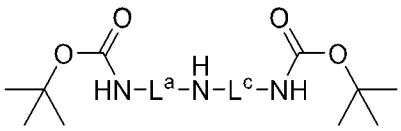
80. The method of any one of claims 74 to 79, wherein the method further comprising a method for preparation of the compound having Formula (II), wherein L^b is -NR^a- and R^a is not hydrogen, from a compound having Formula (V) below:



(V),

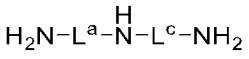
wherein R^a is not hydrogen.

81. The method of claim 80, wherein the method further comprising a method for preparation of the compound having Formula (V), wherein R^a is not hydrogen, from a compound having Formula (VI) below:



(VI).

82. The method of claim 81, wherein the method further comprising a method for preparation of the compound having Formula (VI) from a compound having Formula (VII) below:



(VII).

FIG. 1

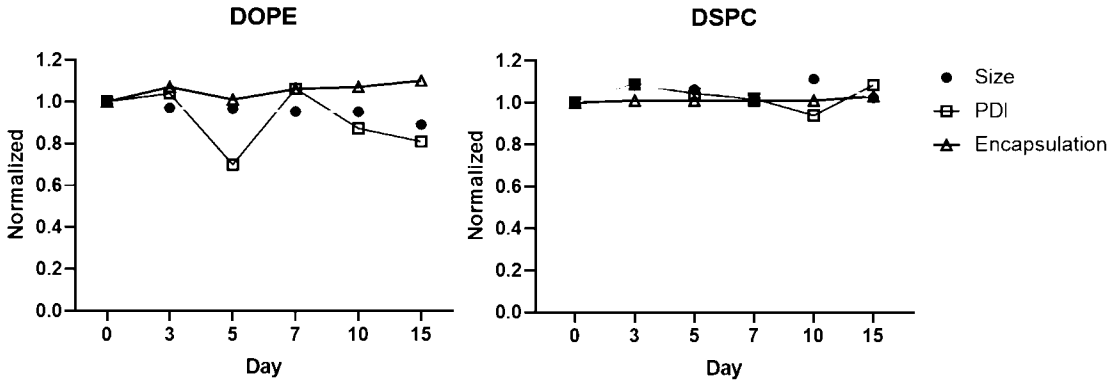

DOPE
DSPC
Normalized
Day
0
3
5
7
10
15
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Size
PDI
Encapsulation

FIG. 2

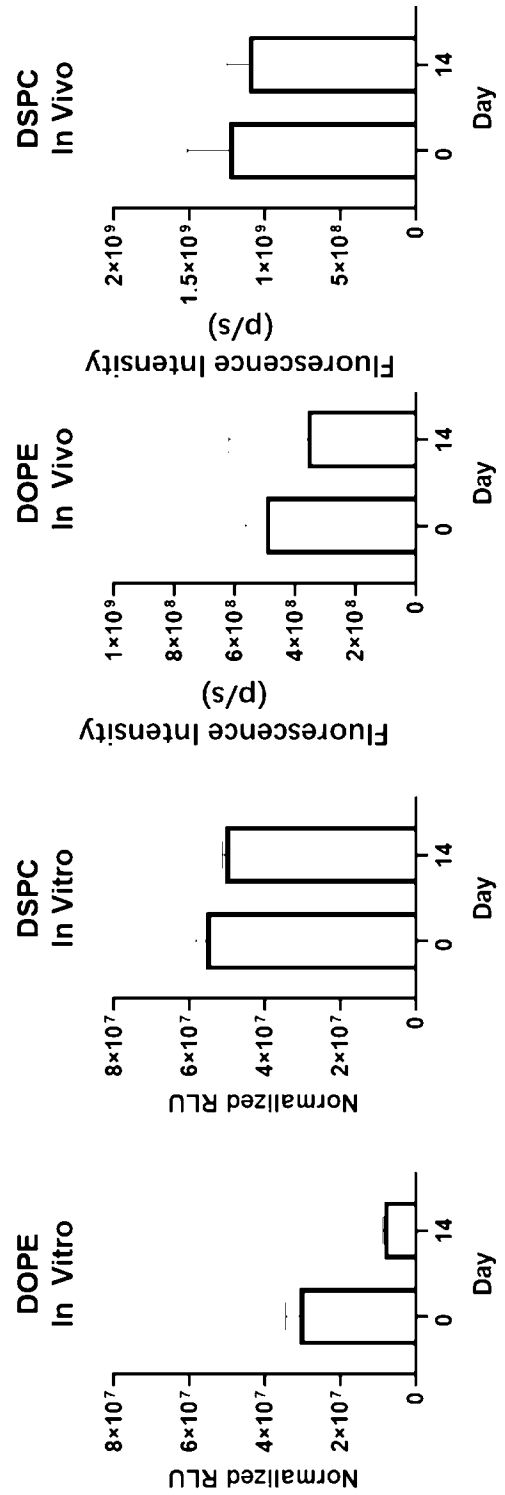

DOPE
In Vitro
Normalized RLU
8×10^7
6×10^7
4×10^7
2×10^7
0
0
14
Day
DSPC
In Vitro
Normalized RLU
8×10^7
6×10^7
4×10^7
2×10^7
0
0
14
Day
DOPE
In Vivo
Fluorescence Intensity
(p/s)
1×10^9
8×10^8
6×10^8
4×10^8
2×10^8
0
0
14
Day
DSPC
In Vivo
Fluorescence Intensity
(p/s)
2×10^9
1.5×10^9
1×10^9
5×10^8
0
0
14
Day

FIG. 3

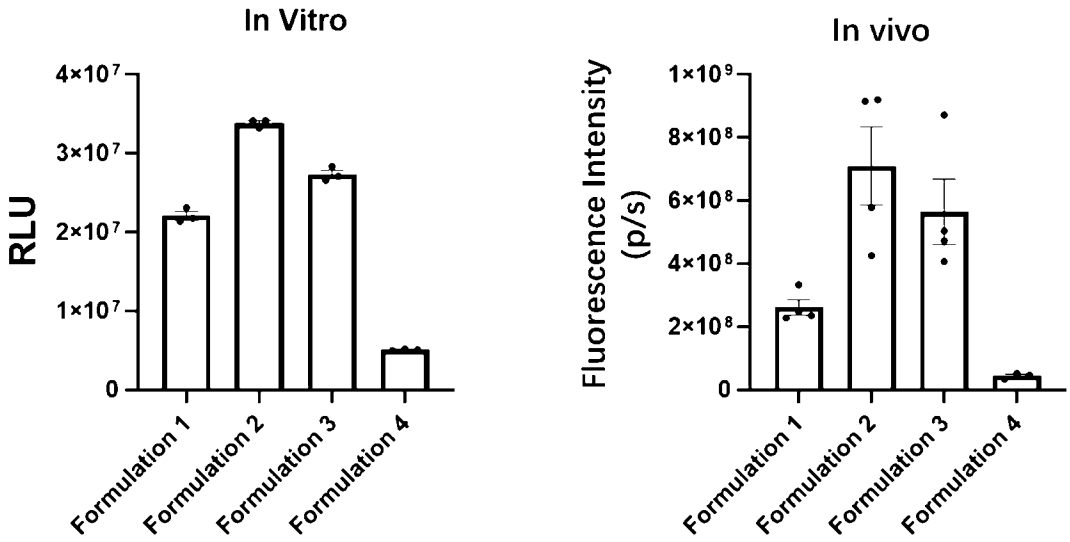

In Vitro
RLU
4×10⁷
3×10⁷
2×10⁷
1×10⁷
0
Formulation 1
Formulation 2
Formulation 3
Formulation 4
In vivo
Fluorescence Intensity (p/s)
1×10⁹
8×10⁸
6×10⁸
4×10⁸
2×10⁸
0
Formulation 1
Formulation 2
Formulation 3
Formulation 4

FIG. 4

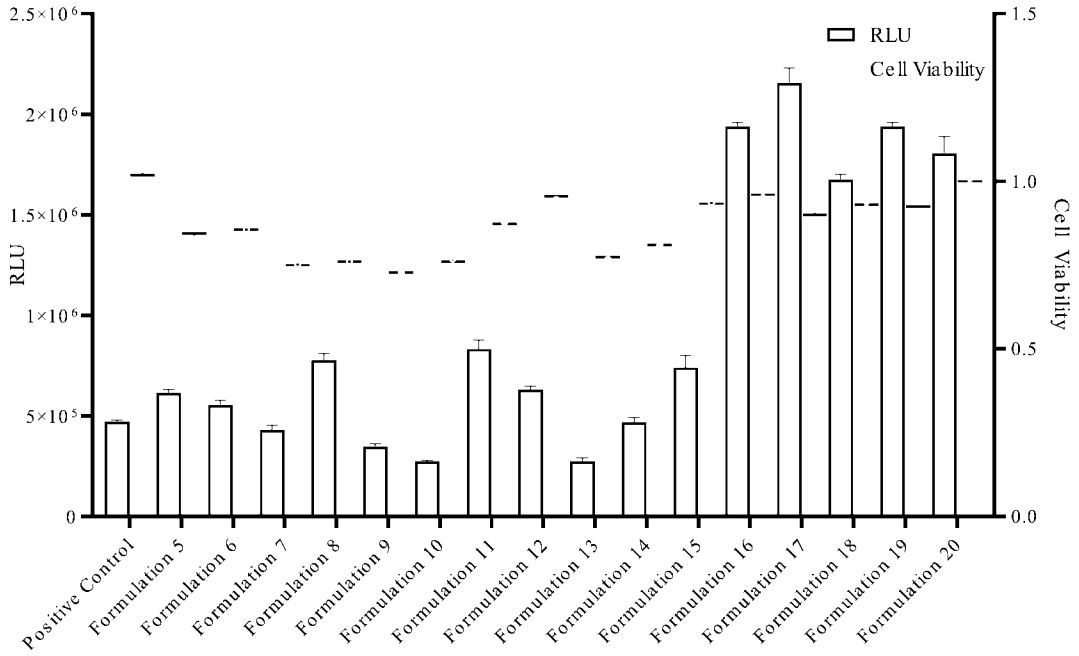

RLU
Cell Viability
2.5×10⁶
2×10⁶
1.5×10⁶
1×10⁶
5×10⁵
0
1.5
1.0
0.5
0.0
Positive Control
Formulation 5
Formulation 6
Formulation 7
Formulation 8
Formulation 9
Formulation 10
Formulation 11
Formulation 12
Formulation 13
Formulation 14
Formulation 15
Formulation 16
Formulation 17
Formulation 18
Formulation 19
Formulation 20

FIG. 5

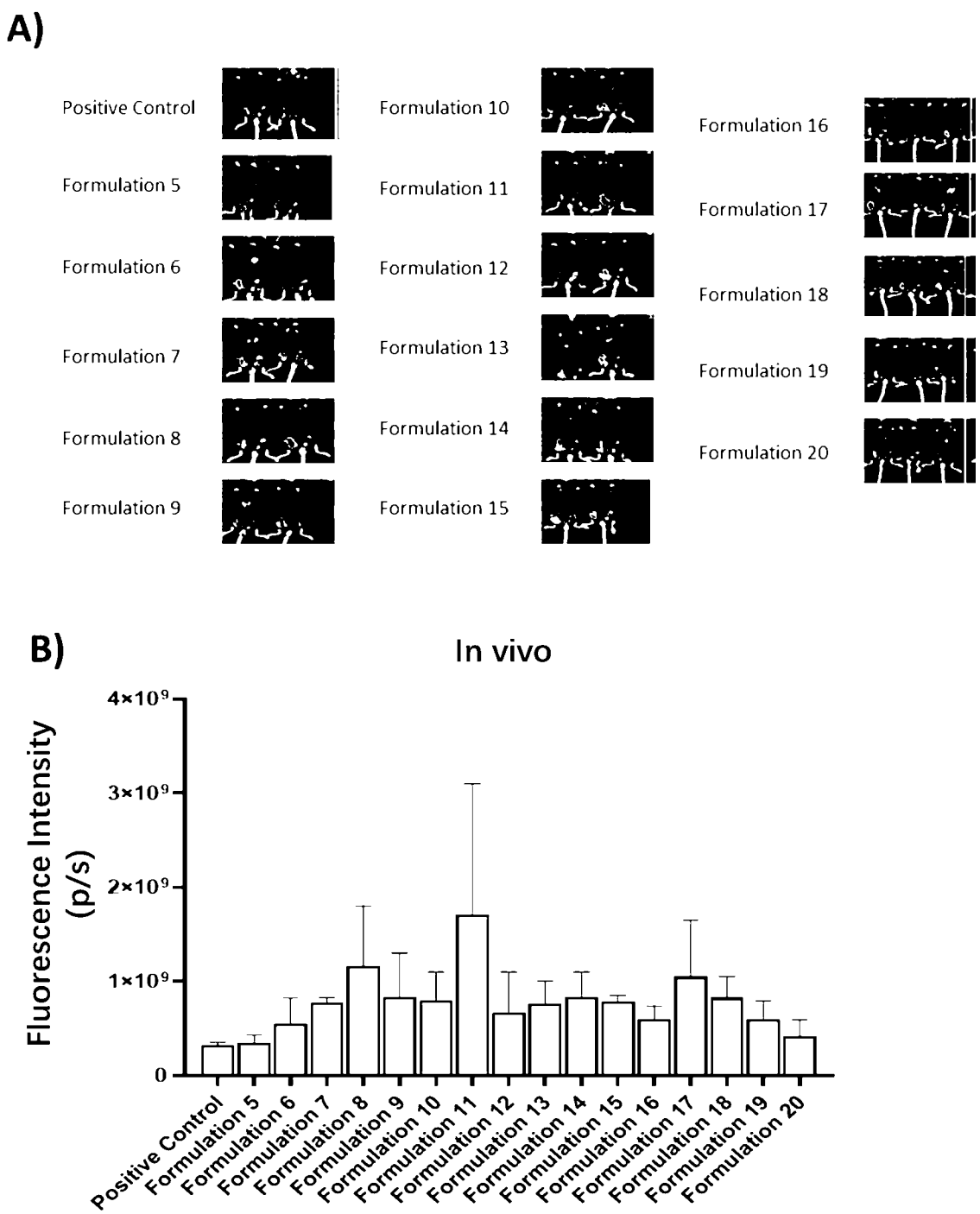

A)
Positive Control
Formulation 5
Formulation 6
Formulation 7
Formulation 8
Formulation 9
Formulation 10
Formulation 11
Formulation 12
Formulation 13
Formulation 14
Formulation 15
Formulation 16
Formulation 17
Formulation 18
Formulation 19
Formulation 20
B)
In vivo
Fluorescence Intensity (p/s)
4×10⁹
3×10⁹
2×10⁹
1×10⁹
0
Positive Control
Formulation 5
Formulation 6
Formulation 7
Formulation 8
Formulation 9
Formulation 10
Formulation 11
Formulation 12
Formulation 13
Formulation 14
Formulation 15
Formulation 16
Formulation 17
Formulation 18
Formulation 19
Formulation 20

FIG. 6

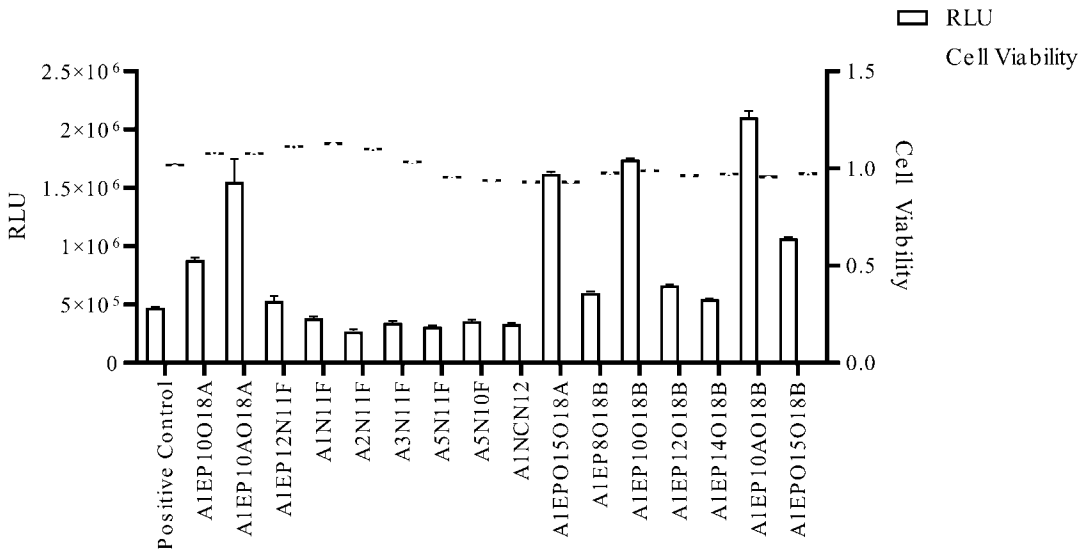
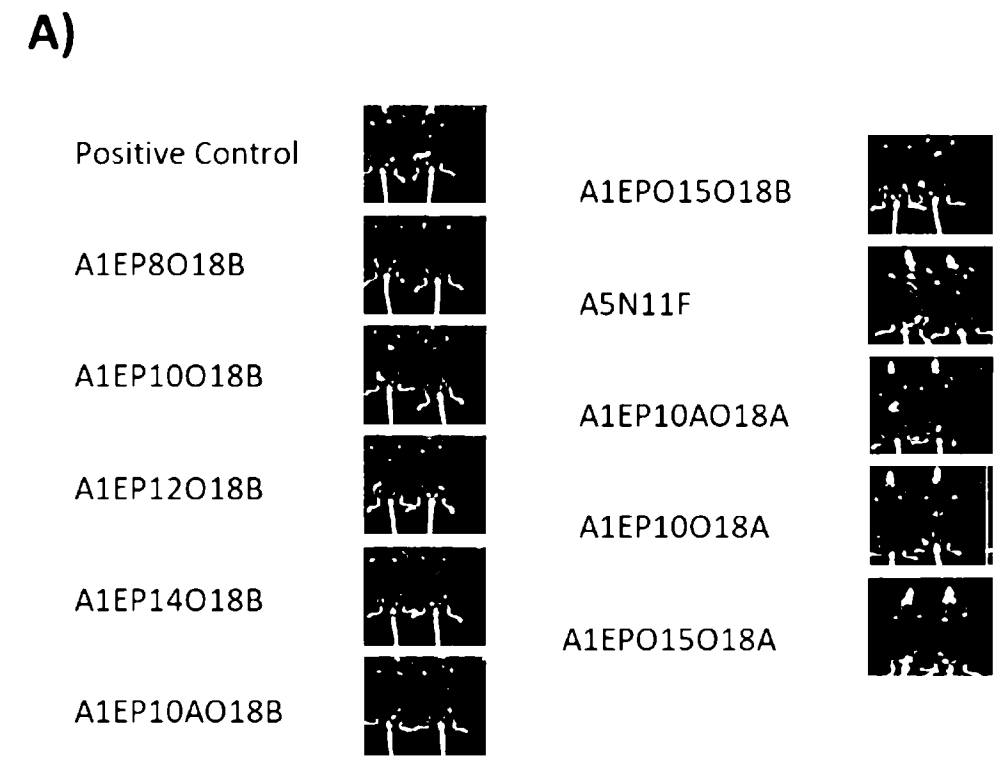

RLU
Cell Viability
2.5×10^6
2×10^6
1.5×10^6
1×10^6
5×10^5
0
1.5
1.0
0.5
0.0
Positive Control
A1EP10O18A
A1EP10AO18A
A1EP12N11F
A1N11F
A2N11F
A3N11F
A5N11F
A5N10F
A1NCN12
A1EPO15O18A
A1EP8O18B
A1EP10O18B
A1EP12O18B
A1EP14O18B
A1EP10AO18B
A1EPO15O18B

FIG. 7


A)
Positive Control
A1EP8O18B
A1EP10O18B
A1EP12O18B
A1EP14O18B
A1EP10AO18B
A1EPO15O18B
A5N11F
A1EP10AO18A
A1EP10O18A
A1EPO15O18A

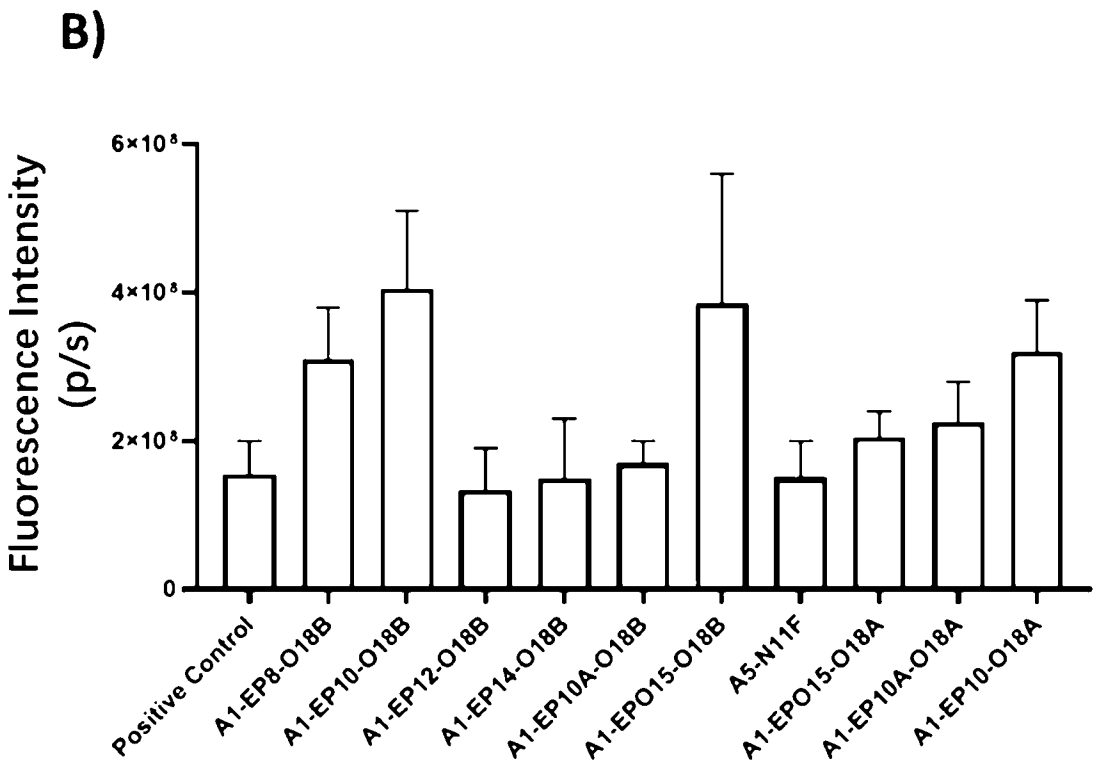

B)
Fluorescence Intensity (p/s)
6×10⁸
4×10⁸
2×10⁸
0
Positive Control
A1-EP8-O18B
A1-EP10-O18B
A1-EP12-O18B
A1-EP14-O18B
A1-EP10A-O18B
A1-EPO15-O18B
A5-N11F
A1-EPO15-O18A
A1-EP10A-O18A
A1-EP10-O18A

FIG. 8

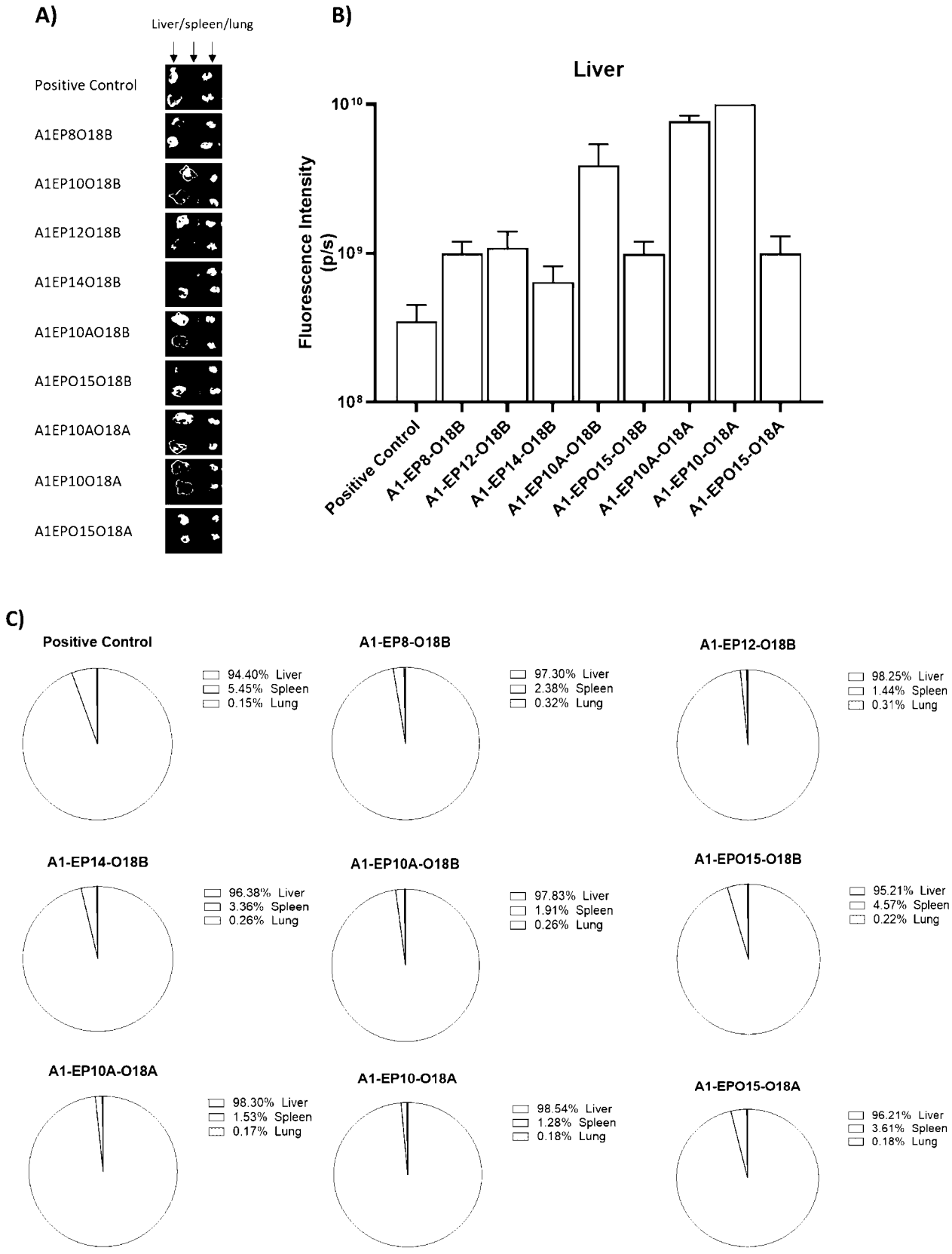

A)
Liver/spleen/lung
Positive Control
A1EP8O18B
A1EP10O18B
A1EP12O18B
A1EP14O18B
A1EP10AO18B
A1EPO15O18B
A1EP10AO18A
A1EP10O18A
A1EPO15O18A
B)
Liver
Fluorescence Intensity (p/s)
10^10
10^9
10^8
Positive Control
A1-EP8-O18B
A1-EP12-O18B
A1-EP14-O18B
A1-EP10A-O18B
A1-EPO15-O18B
A1-EP10A-O18A
A1-EP10-O18A
A1-EPO15-O18A
C)
Positive Control
94.40% Liver
5.45% Spleen
0.15% Lung
A1-EP8-O18B
97.30% Liver
2.38% Spleen
0.32% Lung
A1-EP12-O18B
98.25% Liver
1.44% Spleen
0.31% Lung
A1-EP14-O18B
96.38% Liver
3.36% Spleen
0.26% Lung
A1-EP10A-O18B
97.83% Liver
1.91% Spleen
0.26% Lung
A1-EPO15-O18B
95.21% Liver
4.57% Spleen
0.22% Lung
A1-EP10A-O18A
98.30% Liver
1.53% Spleen
0.17% Lung
A1-EP10-O18A
98.54% Liver
1.28% Spleen
0.18% Lung
A1-EPO15-O18A
96.21% Liver
3.61% Spleen
0.18% Lung

FIG. 9

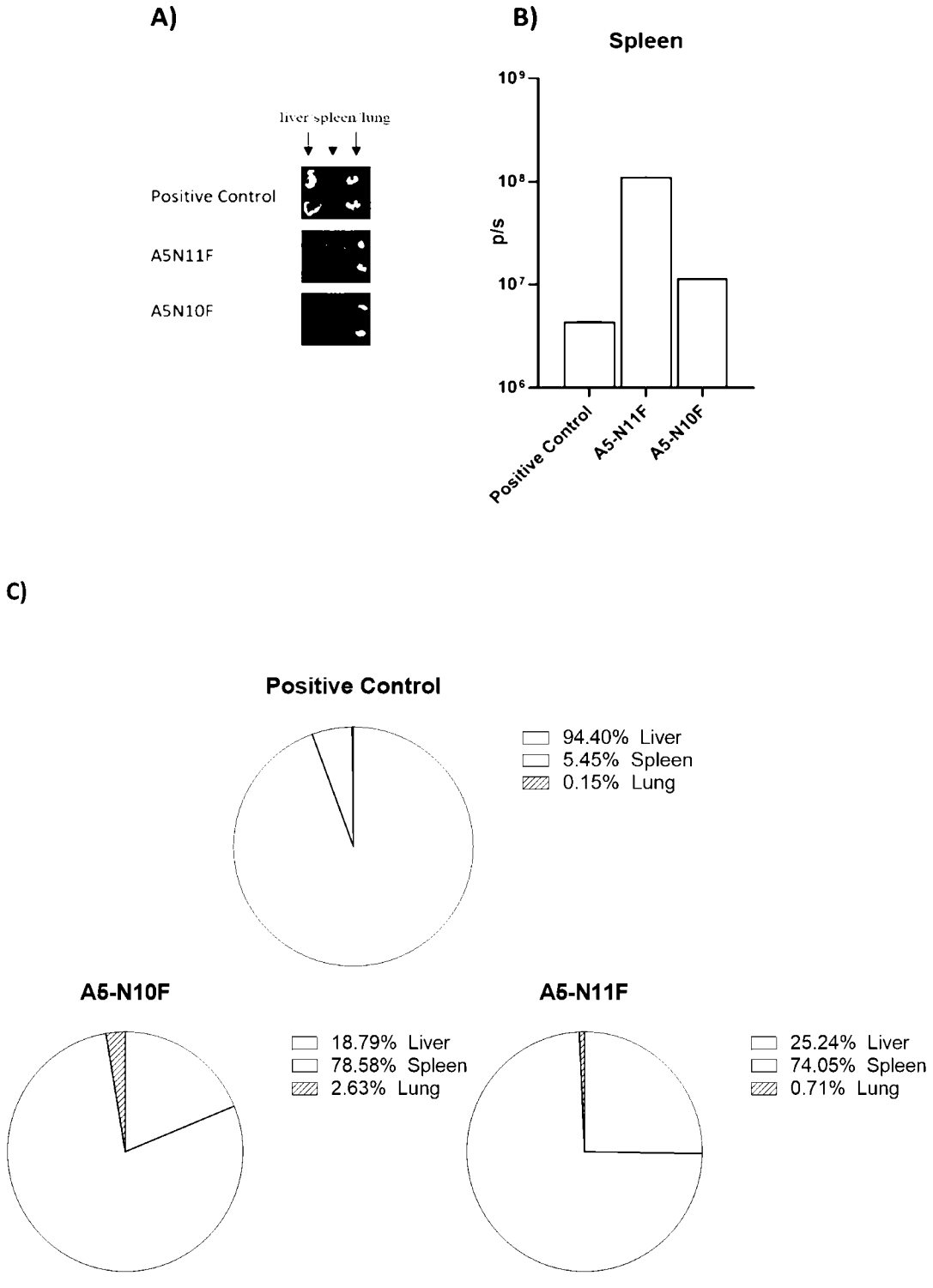

A)
liver spleen lung
Positive Control
A5N11F
A5N10F
B)
Spleen
10⁹
10⁸
10⁷
10⁶
p/s
Positive Control
A5-N11F
A5-N10F
C)
Positive Control
94.40% Liver
5.45% Spleen
0.15% Lung
A5-N10F
18.79% Liver
78.58% Spleen
2.63% Lung
A5-N11F
25.24% Liver
74.05% Spleen
0.71% Lung

FIG. 10

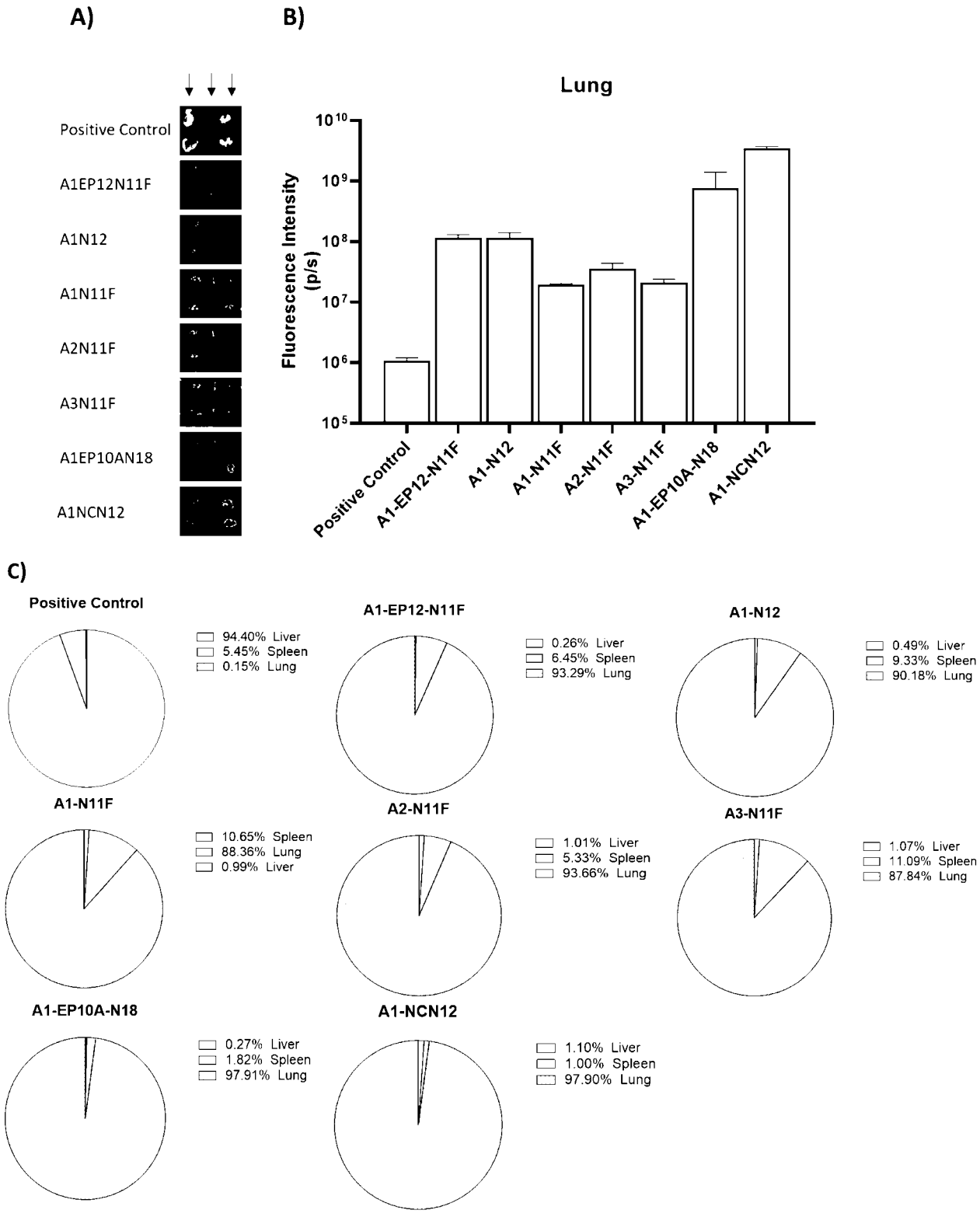

A)
B)
Positive Control
A1EP12N11F
A1N12
A1N11F
A2N11F
A3N11F
A1EP10AN18
A1NCN12
Lung
Fluorescence Intensity (p/s)
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
Positive Control
A1-EP12-N11F
A1-N12
A1-N11F
A2-N11F
A3-N11F
A1-EP10A-N18
A1-NCN12
C)
Positive Control
94.40% Liver
5.45% Spleen
0.15% Lung
A1-EP12-N11F
0.26% Liver
6.45% Spleen
93.29% Lung
A1-N12
0.49% Liver
9.33% Spleen
90.18% Lung
A1-N11F
10.65% Spleen
88.36% Lung
0.99% Liver
A2-N11F
1.01% Liver
5.33% Spleen
93.66% Lung
A3-N11F
1.07% Liver
11.09% Spleen
87.84% Lung
A1-EP10A-N18
0.27% Liver
1.82% Spleen
97.91% Lung
A1-NCN12
1.10% Liver
1.00% Spleen
97.90% Lung

FIG. 11

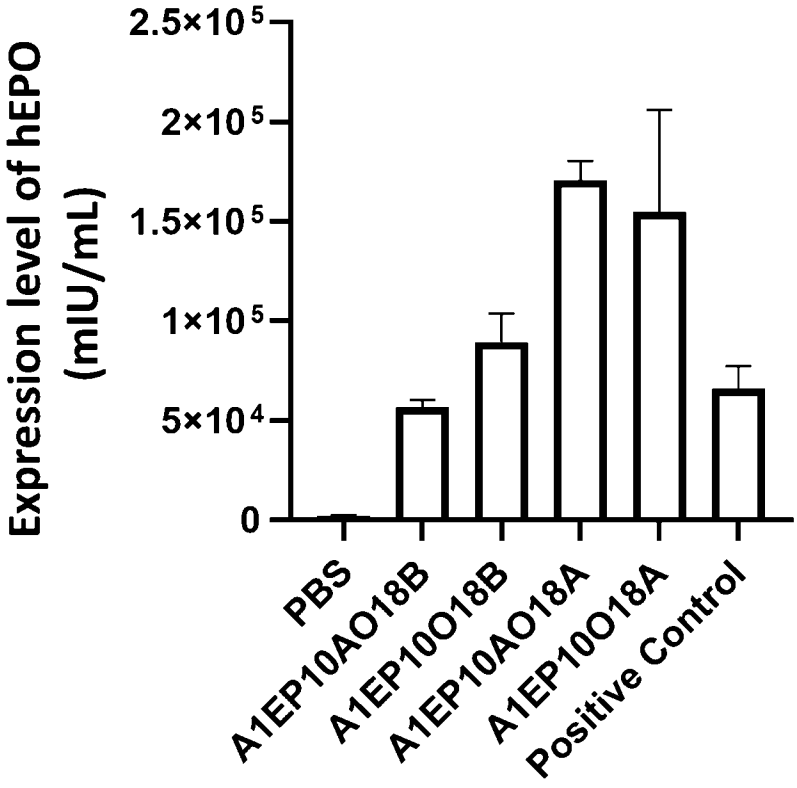

Expression level of hEPO (mIU/mL)
2.5×10^5
2×10^5
1.5×10^5
1×10^5
5×10^4
0
PBS
A1EP10AO18B
A1EP10O18B
A1EP10AO18A
A1EP10O18A
Positive Control

FIG. 12

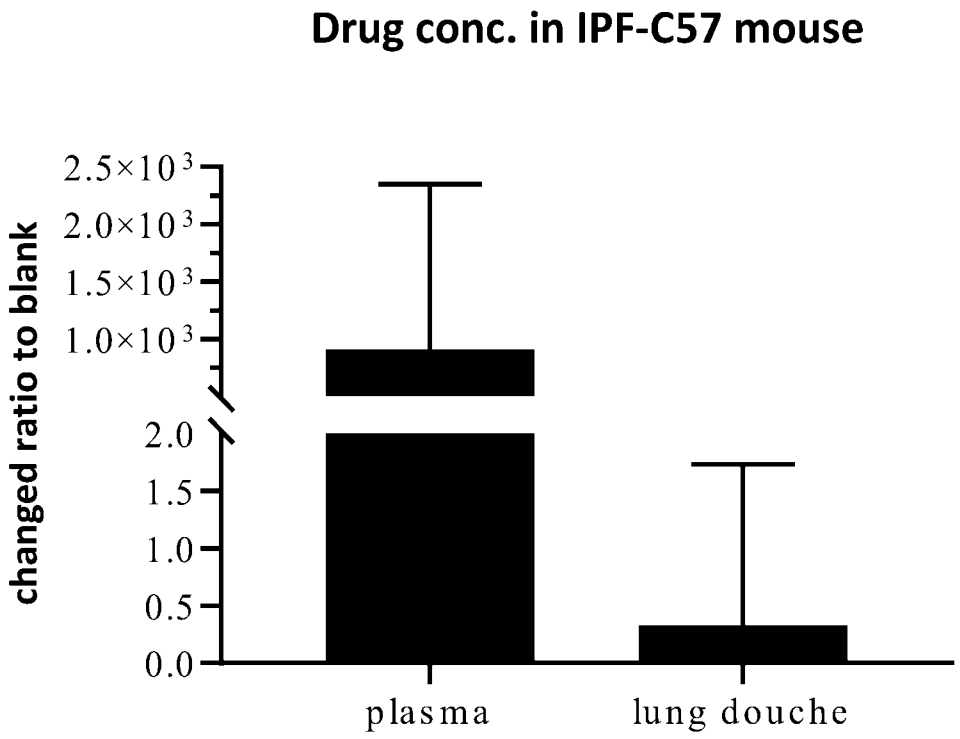

Drug conc. in IPF-C57 mouse
changed ratio to blank
2.5×10³
2.0×10³
1.5×10³
1.0×10³
2.0
1.5
1.0
0.5
0.0
plasma
lung douche

FIG. 13

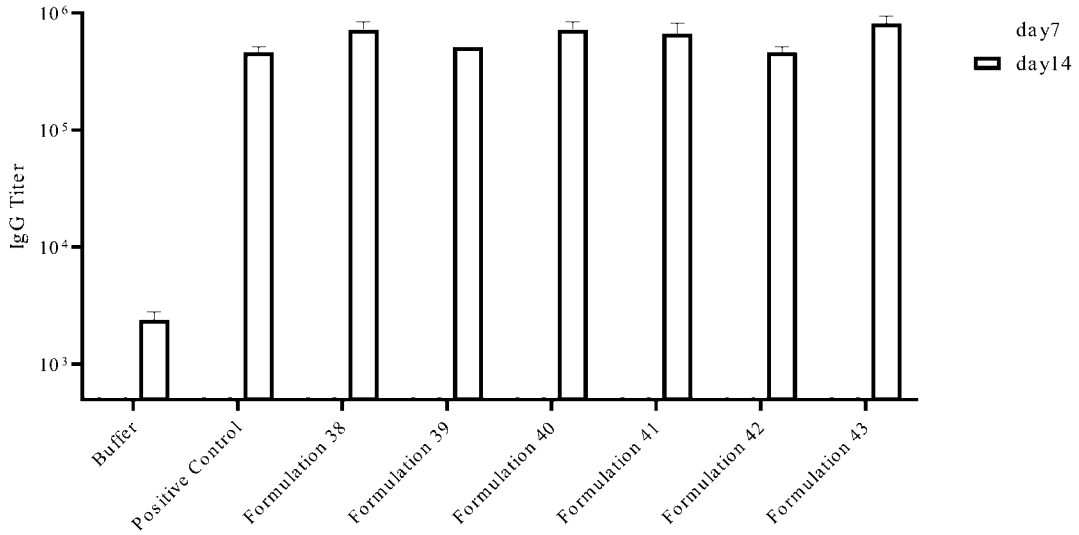
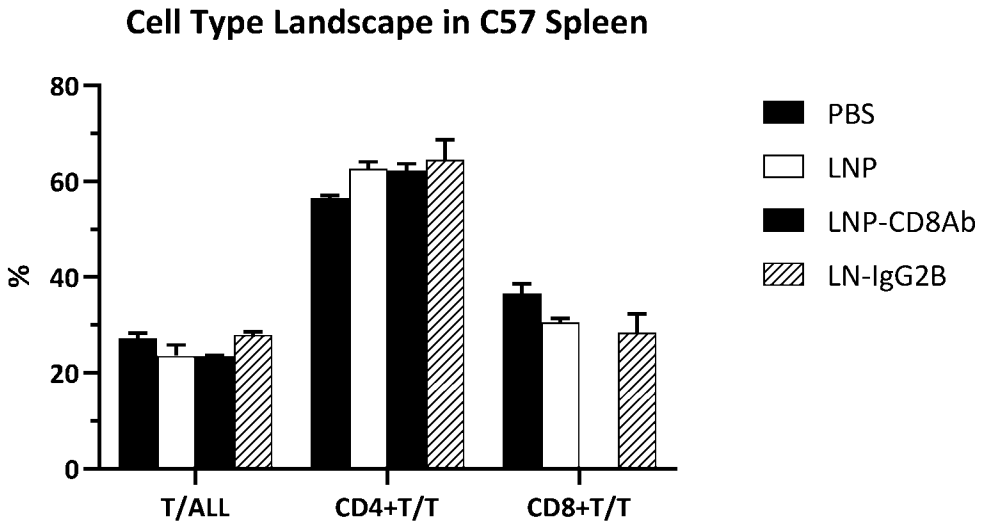

IgG Titer
10^6
10^5
10^4
10^3
day7
day14
Buffer
Positive Control
Formulation 38
Formulation 39
Formulation 40
Formulation 41
Formulation 42
Formulation 43

FIG. 14


Cell Type Landscape in C57 Spleen
%
80
60
40
20
0
T/ALL
CD4+T/T
CD8+T/T
PBS
LNP
LNP-CD8Ab
LN-IgG2B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2023/137998

A. CLASSIFICATION OF SUBJECT MATTER

C07C229/28(2006.01)i; C07C229/12(2006.01)i; C07C229/14(2006.01)i; C07D295/145(2006.01)i; A61K47/18(2017.01)i; A61K9/127(2006.01)i; C12N15/11(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: C07C, C07D, A61K, C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

REGISTRY (STN), CAPLUS (STN), VEN, CNTXT, CNKI, lipid particle, delivery, nucleic acid, RNA, STARNA THERAPEUTICS, structure search in STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014028487 A1 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 20 February 2014 (2014-02-20) the abstract, claims 1-200, figures (1-1) to (2-9)	1-82
X	US 2012009222 A1 (NGUYEN, David-Huy Nhu et al.) 12 January 2012 (2012-01-12) the abstract, claims 1-166, figures 1 to 3	1-82
X	WO 2006138380 A2 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY et al.) 28 December 2006 (2006-12-28) the abstract, claims 1-131, figures 1A and 1B	1-82
X	WO 2008042973 A2 (ALNYLAM PHARMACEUTICALS, INC. et al.) 10 April 2008 (2008-04-10) the abstract, claims 1-349	1-82
X	WO 2014186366 A1 (TUFTS UNIVERSITY) 20 November 2014 (2014-11-20) the abstract, claims 1-23	1-3, 12, 16, 19-25, 30-33, 35, 43, 54-57, 64, 66, 69-70

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 February 2024	**23 February 2024**

Name and mailing address of the ISA/CN	Authorized officer
CHINA NATIONAL INTELLECTUAL PROPERTY ADMINISTRATION **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088, China**	**YUAN,Yue** Telephone No. **(+86) 010-53962235**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2023/137998

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2018191750 A2 (THE BROAD INSTITUTE INC. et al.) 18 October 2018 (2018-10-18) the abstract, claims 1-98, description, pages 17-22	1-82
X	AKINC, Akin et al. "A combinatorial library of lipid-like materials for delivery of RNAi therapeutics" *Nature Biotechnology,* Vol. 26, No. 5, 27 April 2008 (2008-04-27), 561-569	1-5, 8-10, 12, 16-18, 22-27, 29-33, 35, 37-39, 43, 54-59, 66, 69-70
X	WHITEHEAD, Kathryn A. et al. "Degradable lipid nanoparticles with predictable in vivo siRNA delivery activity" *Nature Communications,* Vol. 5, 27 June 2014 (2014-06-27), 4277	1-5, 8-29, 32-33, 35, 37-39, 43-49, 57-59, 66, 69-70
X	HAJJ, Khalid A. et al. "Branched-Tail Lipid Nanoparticles Potently Deliver mRNA In Vivo due to Enhanced Ionization at Endosomal pH" *Small,* Vol. 15, No. 6, 31 December 2019 (2019-12-31), 1805097	1-5, 8, 12, 16-18, 22-27, 29, 32-33, 35, 37-39, 43-45, 57-59, 66, 69-70
X	ZHANG, Xinfu et al. "Biodegradable Amino-Ester Nanomaterials for Cas9 mRNA Delivery in Vitro and in Vivo" *ACS Applied Materials & Interfaces,* Vol. 9, No. 30, 07 July 2017 (2017-07-07), 25481-25487	1-5, 8, 12, 16-19, 22-27, 29, 32-33, 35-39, 43-45, 48-49, 54-60, 66, 69-70
PX	WO 2023233042 A1 (BIO-TRIP B.V.) 07 December 2023 (2023-12-07) the abstract, claims 1-23	1-82
PX	WO 2023125184 A1 (SOUTH CHINA UNIVERSITY OF TECHNOLOGY et al.) 06 July 2023 (2023-07-06) the abstract, claims 1-46	1-82
PX	WO 2023158738 A2 (TRUSTEES OF TUFTS COLLEGE et al.) 24 August 2023 (2023-08-24) the abstract, claims 1-156	1-82
PX	WO 2023172774 A1 (TRUSTEES OF TUFTS COLLEGE) 14 September 2023 (2023-09-14) the abstract, claims 1-72	1-82
PX	WO 2022260772 A1 (CARNEGIE MELLON UNIVERSITY) 15 December 2022 (2022-12-15) the abstract, claims 1-44	1-82
PX	WO 2023215467 A1 (CARNEGIE MELLON UNIVERSITY) 09 November 2023 (2023-11-09) the abstract, claims 1-49	1-82
PX	CN 116947785 A (WESTGENE BIOPHARMA CO., LTD.) 27 October 2023 (2023-10-27) the abstract, claims	1-82

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2023/137998

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed.
   b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),
      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2023/137998

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

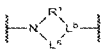
This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: **65-69, 71-73**
because they relate to subject matter not required to be searched by this Authority, namely:

The subject matter of claims 65-69, 71-73 relates to a method for treatment of human body by therapy as defined in PCT Rule 39.1(iv). This search has been carried out on the basis of the subject matter of the use in manufacture of medicaments for treating the alleged diseases.

2. ☑ Claims Nos.: **1-41**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

The compounds involved in claims 1-41 cover a large number of known compounds in the prior art. It is difficult for the examiner to search the prior art exhaustively according to the scope of the current claims. The search report is searched based on the scope that the -L ^{a}-L ^{b}-L ^{c}- is limited to the scope of claim 12,

 is limited to the scope of claim 14, the R ^{1}, R ^{2}, R ^{3} and R ^{4} are limited to the scope of claim 38.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2023/137998

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
WO	2014028487	A1	20 February 2014	CA	2884870	A1	20 February 2014
				CA	2884870	C	29 March 2022
				US	2016114042	A1	28 April 2016
				US	9439968	B2	13 September 2016
				EP	2882706	A1	17 June 2015
				US	2017152213	A1	01 June 2017
				US	2014161830	A1	12 June 2014
				US	9227917	B2	05 January 2016
US	2012009222	A1	12 January 2012	WO	2010062322	A2	03 June 2010
				WO	2010062322	A3	05 August 2010
WO	2006138380	A2	28 December 2006	WO	2006138380	A3	24 July 2008
				JP	2009143960	A	02 July 2009
				CA	2611944	A1	28 December 2006
				JP	2014169288	A	18 September 2014
				US	2016009657	A1	14 January 2016
				JP	2016027057	A	18 February 2016
				EP	2476756	A1	18 July 2012
				JP	2009501699	A	22 January 2009
				JP	5777846	B2	09 September 2015
				US	2011009641	A1	13 January 2011
				US	9006487	B2	14 April 2015
				EP	1912679	A2	23 April 2008
				EP	1912679	A4	29 July 2009
				AU	2006259415	A1	28 December 2006
				AU	2006259415	B2	30 August 2012
WO	2008042973	A2	10 April 2008	CA	3144493	A1	10 April 2008
				MX	2019003005	A	05 August 2019
				US	2009023673	A1	22 January 2009
				US	8034376	B2	11 October 2011
				ES	2611924	T3	11 May 2017
				MX	2022013691	A	13 December 2022
				CA	2665225	A1	10 April 2008
				CA	2665225	C	30 June 2015
				DK	2068886	T3	18 November 2013
				EP	2695608	A2	12 February 2014
				EP	2695608	A3	21 May 2014
				EP	2695608	B1	23 November 2016
				US	2012046478	A1	23 February 2012
				US	8642076	B2	04 February 2014
				MX	363224	B	15 March 2019
				IN	DEN2015	A	12 June 2015
				JP	2010505873	A	25 February 2010
				JP	5933163	B2	08 June 2016
				JP	2015227370	A	17 December 2015
				JP	2022164716	A	27 October 2022
				US	2023174459	A1	08 June 2023
				US	2014121393	A1	01 May 2014
				US	2018065918	A1	08 March 2018
				ES	2430206	T3	19 November 2013
				JP	2021011503	A	04 February 2021

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2023/137998

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
		JP 7127098 B2	29 August 2022
		AU 2007303205 A1	10 April 2008
		JP 2017036330 A	16 February 2017
		KR 20090088362 A	19 August 2009
		KR 101129509 B1	13 April 2012
		CA 2927045 A1	10 April 2008
		EP 2068886 A2	17 June 2009
		EP 2068886 A4	22 December 2010
		EP 2068886 B1	18 September 2013
		MX 2009003548 A	15 April 2009
		WO 2008042973 A3	28 August 2008
		EP 3192788 A1	19 July 2017
		US 2020361854 A1	19 November 2020
		US 11420931 B2	23 August 2022
		CA 2848238 A1	10 April 2008
		CA 2848238 C	19 July 2016
		JP 2019048887 A	28 March 2019
		JP 2013213036 A	17 October 2013
		JP 5833597 B2	16 December 2015
WO 2014186366 A1	20 November 2014	US 2016082126 A1	24 March 2016
		US 2019240288 A1	08 August 2019
		US 10792328 B2	06 October 2020
WO 2018191750 A2	18 October 2018	US 2020405639 A1	31 December 2020
		WO 2018191750 A3	29 November 2018
WO 2023233042 A1	07 December 2023	None	
WO 2023125184 A1	06 July 2023	None	
WO 2023158738 A2	24 August 2023	WO 2023158738 A3	02 November 2023
WO 2023172774 A1	14 September 2023	None	
WO 2022260772 A1	15 December 2022	None	
WO 2023215467 A1	09 November 2023	None	
CN 116947785 A	27 October 2023	None	