

DEGRADABLE LIPID FOR ACTIVE MOLECULE DELIVERY AND LIPID NANOPARTICLE THEREOF

Technical Field

The present invention provides an ionizable lipid compound having an optimized carbon chain length and an ionizable amine head structure so as to achieve efficient delivery of an active molecule including, but not limited to, nucleic acids, proteins, small molecule drugs and the like. The present invention further relates to a lipid nanoparticle (LNP) comprising the ionizable lipid compound and the active molecule, and a pharmaceutical composition comprising the lipid nanoparticle.

Background Art

Biomacromolecules such as nucleic acids and proteins have great advantages and potential in the regulation of gene expression and the prevention and treatment of malignant diseases; however, biomacromolecules are a class of charged molecules with strong hydrophilicity, which are difficult to penetrate the cell membrane into the cell, and in addition, nucleic acid drugs such as RNAs are easily degraded by nucleases, which limits their stability *in vivo* and further limits the druggability of RNAs.

A lipid nanoparticle (LNP) is generally composed of four components: a cationic or ionizable lipid, a phospholipid, cholesterol and a PEG lipid. The LNP can encapsulate nucleic acid drug molecules such as RNAs and protect them from nuclease degradation, and has a high degree of stability and biocompatibility, and thus it has become a promising drug delivery system and has been clinically applied.

The drug delivery efficiency of the LNP is affected by its composition. In the LNP, the amine head part (amine head) of the cationic lipid or ionizable lipid can promote the binding and encapsulation of the LNP and a biomacromolecule, through electrostatic interaction with the biomacromolecule, and improve the efficiency of LNP uptake by cells. Studies have shown that the hydrophobic carbon chain length of cationic lipids or ionizable lipids affects stability of LNPs and the efficiency of drug encapsulation and delivery. For example, Min Qiu et al. (*PNAS* 2022, 119, e2116271119) reported that the delivery efficiency of LNPs increases with the increase of the length of lipid hydrophobic carbon chain, and for example, a lipid molecule with a hydrophobic chain of 16 carbon atoms show the highest nucleic acid delivery efficiency as compared with lipid molecules with hydrophobic chains of 12-14 carbon atoms; Chun Chan et al. (*Curr Top Med Chem* 2021, 21, 92-114) also reported that lipid molecules with a carbon chain length of 12 to 18 carbon atoms provide sufficient fluidity and rigidity for LNPs; and Shuai Liu

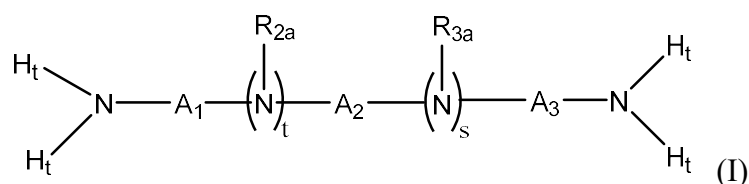
et al. (*Nature Mater*, 2021, 20, 701-710) showed in Fig. 2e of this document that the carbon chain length affects the delivery efficiency of nucleic acid drugs, and lipid molecules with a carbon chain length of 10, 11 and 12 carbon atoms have higher delivery efficiency.

In summary, the hydrophobic carbon chain length of cationic lipids or ionizable lipids affects the formation of LNPs and the drug delivery efficiency. Therefore, a balance of these factors needs to be considered when designing LNPs to improve their delivery efficiency and stability *in vivo*.

Summary of the Invention

The present invention provides an ionizable lipid compound with an optimized hydrophobic carbon chain length and an amine head. The inventors of the present invention have found that unlike cationic lipids or ionizable lipid compounds having a carbon chain length of 12 to 18 carbon atoms in the prior art, further decreasing the hydrophobic carbon chain length instead leads to increased delivery efficiency for the ionizable lipid compound of this structure of the present invention. For this reason, the present invention is completed by the inventors of the present invention.

In a first aspect, the present invention provides a lipid compound of formula (I),



wherein

R_{2a} and R_{3a} are each independently hydrogen, a monovalent aliphatic group, a monovalent heteroaliphatic group, a monovalent aromatic group, a monovalent heteroaromatic group, or H_t ;

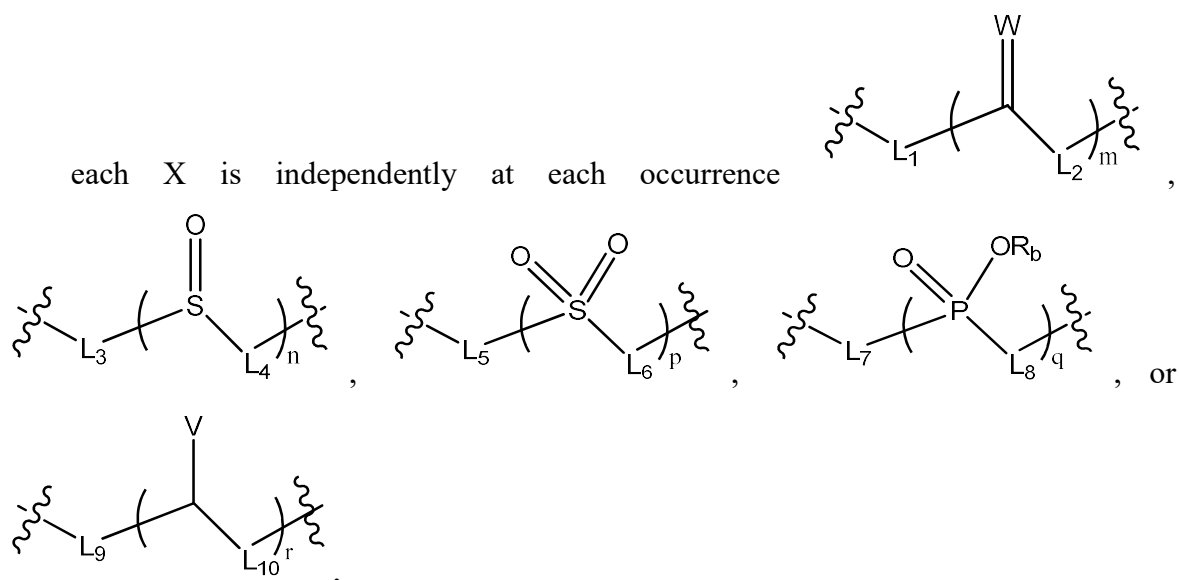
t and s are each independently 0 or 1, and when t or s is zero, it means that the part is directly a single bond, provided that t and s are not both 0;

A_1 , A_2 , and A_3 are each independently a single bond, a divalent aliphatic group, a divalent heteroaliphatic group, a divalent aromatic group, or a divalent heteroaromatic group, or a combination of two of the above;

each H_t is independently at each occurrence $-\text{R}_1-\text{X}-\text{R}_2-\text{Y}-\text{R}_3-\text{Z}-\text{R}_4$,

wherein

each R_1 is independently at each occurrence a divalent aliphatic group, a divalent heteroaliphatic group, a divalent aromatic group, or a divalent heteroaromatic group;



wherein

m, n, p, q, and r are each independently 1-6;

W is O, S, or NR_c;

L₁, L₃, L₅, L₇, and L₉ are directly connected to R₁ or R₂ and are each independently a single bond, O, S, or NR_d;

L₂, L₄, L₆, L₈, and L₁₀ are each independently a bond, O, S, or NR_e;

V is an aliphatic group, OR_f, SR_g, or NR_hR_i,

wherein R_b, R_c, R_d, R_e, R_f, R_g, R_h, and R_i are each independently hydrogen, hydroxyl, an oxyaliphatic group, a monovalent aliphatic group, a monovalent heteroaliphatic group, a monovalent aromatic group, or a monovalent heteroaromatic group;

Y and Z are each independently at each occurrence S or O;

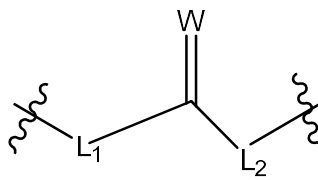
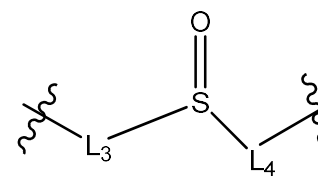
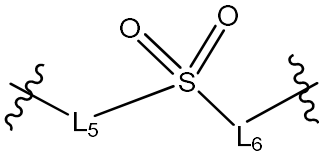
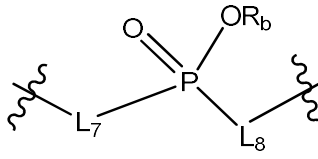
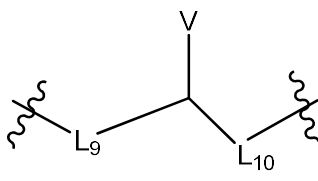
each R₂ is independently at each occurrence a single bond, a divalent aliphatic group, a divalent heteroaliphatic group, a divalent aromatic group, or a divalent heteroaromatic group;

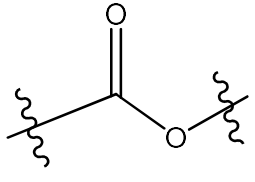
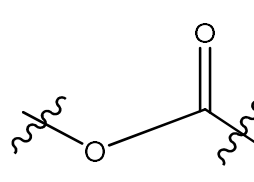
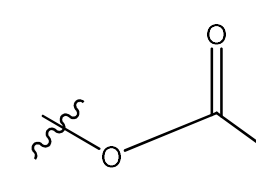
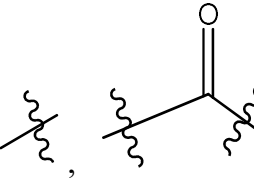
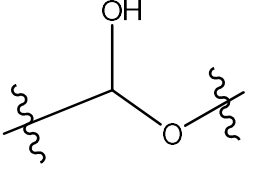
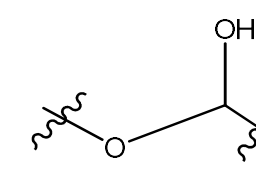
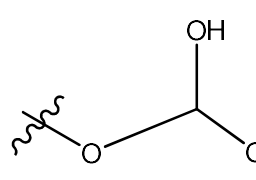
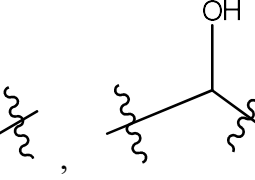
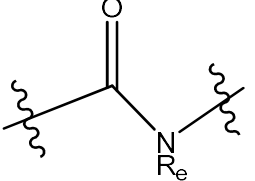
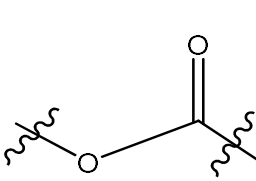
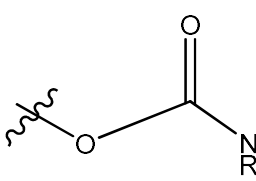
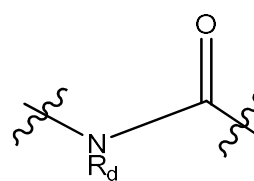
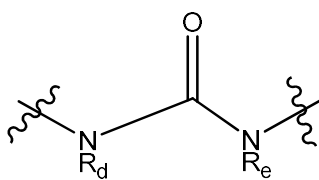
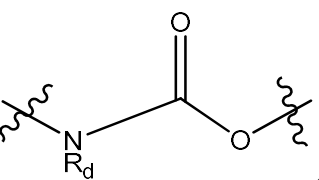
each R₃ is independently at each occurrence a single bond, a divalent aliphatic group, a divalent heteroaliphatic group, a divalent aromatic group, or a divalent heteroaromatic group;

each R₄ is independently at each occurrence a hydrophobic group selected from - (CH₂CH₂O)_y-C₃-C₈ alkyl, -(CH₂CH₂O)_y-C₃-C₈ alkenyl or -(CH₂CH₂O)_y-C₃-C₈ alkynyl, wherein y is 0 or 1 or 2.

In some embodiments, t and s are both 0; or t is 0 and s is 1; or t is 1 and s is 0.

In some embodiments, R₁ is a C₁₋₆ (e.g., C₁₋₄) divalent aliphatic group or a C₁₋₆ (e.g., C₁₋₄) divalent heteroaliphatic group, preferably a C₁₋₄ divalent alkyl or a C₁₋₄ divalent heteroalkyl.

In some embodiments, X is , , , , or , wherein each variable is as defined for formula (I). In a further embodiment, L₁, L₃, L₅, L₇, and L₉ are connected to R₁ and are each independently a single bond, O, S, or NH. In a further embodiment,

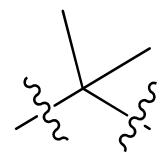
X is , , , , , , , , , , , , , or , wherein R_d and R_e are as defined for formula (I). In some embodiments, R^d and R^e are each independently H or a C₁₋₄ monovalent aliphatic group, preferably H or C₁₋₄ monovalent alkyl.

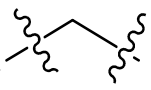
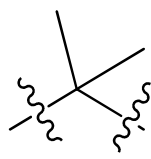
In some embodiments, Y and Z are both S; or Y is S and Z is O; or Y is O and Z is S; or Y and Z are both O.

In some embodiments, each R₂ is independently at each occurrence a single bond or a C₁₋₆ divalent aliphatic group (e.g., a C₁₋₄ divalent aliphatic group, preferably a C₁₋₄ divalent alkyl, more preferably a C₁₋₂ divalent alkyl).

In some embodiments, each R₃ is independently at each occurrence a single bond or a C₁₋₆ divalent aliphatic group (e.g., a C₁₋₄ divalent aliphatic group, preferably a C₁₋₄ divalent alkyl). In

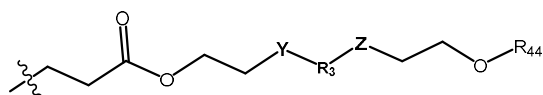
a further embodiment, each R₃ is independently at each occurrence a single bond, or



or methylene () , preferably .

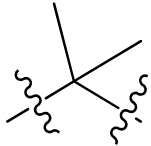
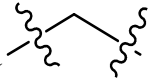
In some embodiments, each R₄ is independently at each occurrence C₃-C₈ alkyl, C₃-C₈ alkenyl, C₃-C₈ alkynyl, -(CH₂CH₂O)-C₃-C₈ alkyl, -(CH₂CH₂O)-C₃-C₈ alkenyl, -(CH₂CH₂O)-C₃-C₈ alkynyl, -(CH₂CH₂O)₂-C₃-C₈ alkyl, -(CH₂CH₂O)₂-C₃-C₈ alkenyl or -(CH₂CH₂O)₂-C₃-C₈ alkynyl. In some embodiments, each R₄ is independently at each occurrence C₄-C₈ alkyl, C₄-C₈ alkenyl, C₄-C₈ alkynyl, -(CH₂CH₂O)-C₄-C₈ alkyl, -(CH₂CH₂O)-C₄-C₈ alkenyl, -(CH₂CH₂O)₂-C₄-C₈ alkynyl, -(CH₂CH₂O)₂-C₄-C₈ alkyl, -(CH₂CH₂O)₂-C₄-C₈ alkenyl or -(CH₂CH₂O)₂-C₄-C₈ alkynyl. In some embodiments, the alkyl in the C₄-C₈ alkyl, -(CH₂CH₂O)-C₄-C₈ alkyl or -(CH₂CH₂O)₂-C₄-C₈ alkyl is a linear alkyl; the alkenyl in the C₄-C₈ alkenyl, -(CH₂CH₂O)-C₄-C₈ alkenyl or -(CH₂CH₂O)₂-C₄-C₈ alkenyl is a linear alkenyl; and the alkynyl in the C₄-C₈ alkynyl, -(CH₂CH₂O)-C₄-C₈ alkynyl or -(CH₂CH₂O)₂-C₄-C₈ alkynyl is a linear alkynyl. In some embodiments, each R₄ is independently at each occurrence -(CH₂CH₂O)-(CH₂)₃CH₃, -(CH₂CH₂O)₂-(CH₂)₃CH₃, -(CH₂CH₂O)-(CH₂)₄CH₃, -(CH₂CH₂O)₂-(CH₂)₄CH₃, -(CH₂CH₂O)-(CH₂)₅CH₃, -(CH₂CH₂O)₂-(CH₂)₅CH₃, -(CH₂CH₂O)-(CH₂)₆CH₃, -(CH₂CH₂O)₂-(CH₂)₆CH₃, -(CH₂CH₂O)-(CH₂)₇CH₃ or -(CH₂CH₂O)₂-(CH₂)₇CH₃.

In some embodiments, each Ht is independently at each occurrence



, wherein Y and Z are both S; or Y is S and Z is O; or

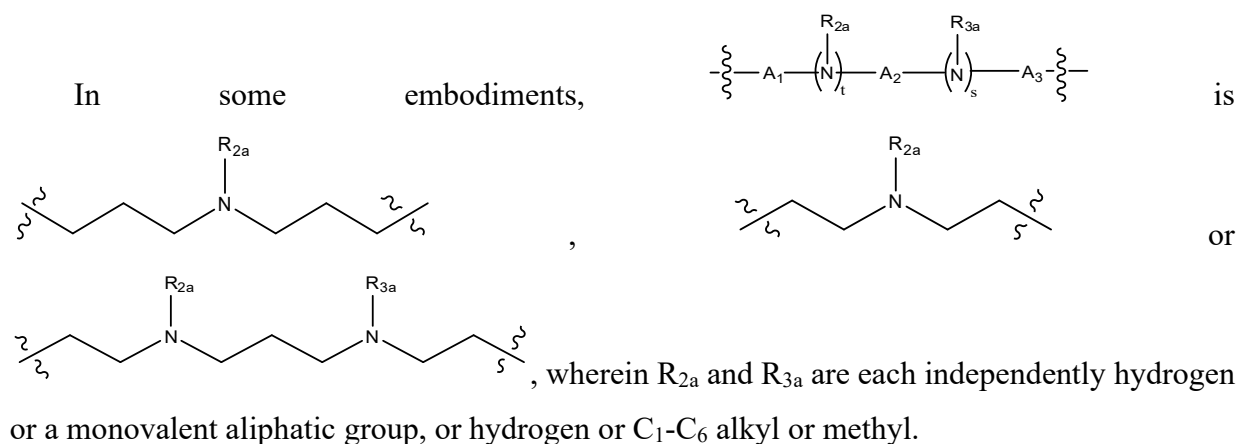
Y is O and Z is S; or Y and Z are both O; each R₃ is independently at each occurrence a single

bond, or , or methylene () , and R₄₄ is C₄-C₈ alkyl, C₄-C₈ alkenyl or C₄-C₈ alkynyl; and in some embodiments, each R₄ is independently at each occurrence -(CH₂)₃CH₃, -(CH₂CH₂O)-(CH₂)₃CH₃, -(CH₂CH₂O)-(CH₂)₄CH₃, -(CH₂CH₂O)-(CH₂)₅CH₃, -(CH₂CH₂O)-(CH₂)₆CH₃ or -(CH₂CH₂O)-(CH₂)₇CH₃.

In the present invention, A₁, A₂ and A₃ form an amine head of the lipid, which undergoes an addition reaction with a hydrophobic lipid tail to obtain a series of lipids. Since the amine head can be protonated, such lipid nanoparticles are positively charged as a whole, so they can undergo an electrostatic interaction with negatively charged mRNAs, cell membranes and

lysosomal membranes, such that such lipids can effectively encapsulate and deliver mRNAs. In some embodiments, the pKa value of the amine head is greater than 4, preferably greater than 6, and more preferably greater than 8.

In some embodiments, t is 1, s is 0, A₂ is a single bond, and A₁ and A₃ are each independently a divalent aliphatic group or a divalent heteroaliphatic group; in a further embodiment, A₁ and A₃ are each independently a C₁-C₆ divalent aliphatic group (e.g., a C₁-C₄ divalent aliphatic group, preferably C₁-C₄ divalent alkyl); and in a further embodiment, A₁ and A₃ are each independently -CH₂CH₂- or -CH₂CH₂CH₂-, and R_{2a} is hydrogen or a monovalent aliphatic group, or is hydrogen or C₁-C₆ alkyl or methyl. In some embodiments, t is 1, s is 1, A₁, A₂ and A₃ are each independently a divalent aliphatic group or a divalent heteroaliphatic group; in a further embodiment, A₁, A₂ and A₃ are each independently a C₁-C₆ divalent aliphatic group (e.g., a C₁-C₄ divalent aliphatic group, preferably C₁-C₄ divalent alkyl); and in a further embodiment, A₁, A₂ and A₃ are each independently -CH₂CH₂- or -CH₂CH₂CH₂-, and R_{2a} and R_{3a} are each independently hydrogen or a monovalent aliphatic group, or is hydrogen or C₁-C₆ alkyl or methyl.



In some embodiments, the lipid of formula (I) is

YX8	
YX17	
YX31	

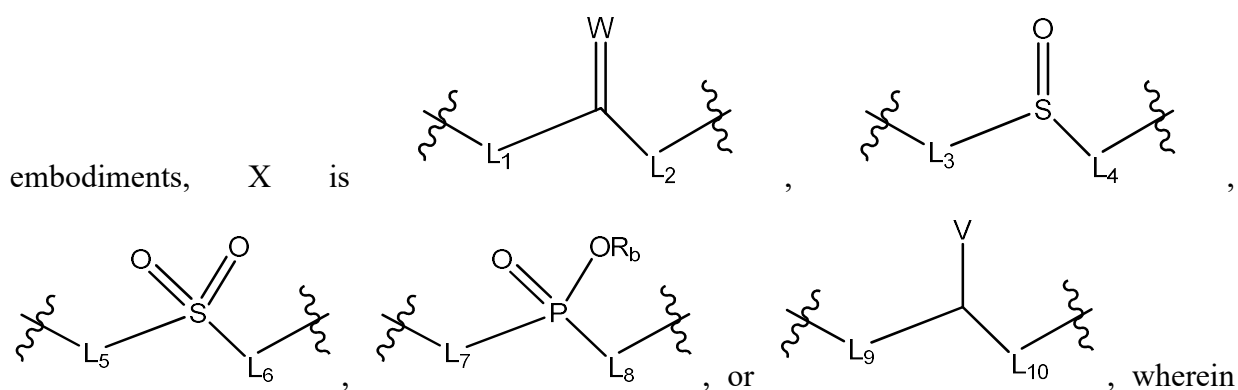
YX27	
YX28	
YX35	
YX29	
YX33	

The lipid of formula (I) of the present invention is biodegradable due to the fact that the Ht group contains a corresponding ketal unit (especially a corresponding thioketal unit when Y and Z are both S). Such a lipid can efficiently bind an mRNA and assemble to form a nanostructure, and after entering a diseased cell, the lipid selectively responds to a highly oxidative environment in the cell and degrades, thus resulting in efficient release of the mRNA and expression of a corresponding protein.

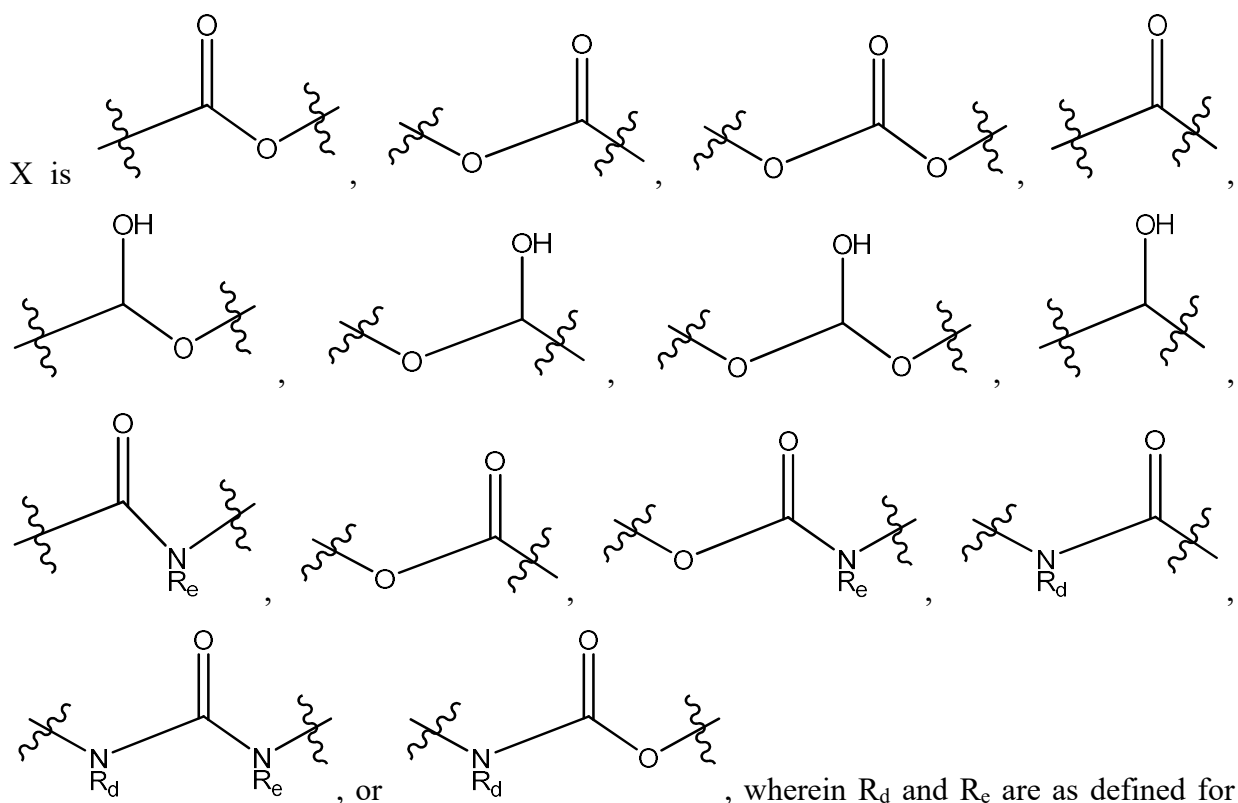
In a second aspect, the present invention provides a method for preparing a lipid of formula (I) in which R₁ is ethylene, the method comprising:

mixing and reacting an acrylic compound of formula (II), i.e. CH₂=CH-X-R₂-Y-R₃-Z-R₄ (II), in which each variable is as defined for formula (I), with a hydrophilic amine at a molar ratio, wherein the molar ratio of the hydrophilic amine to the acrylate compound of formula (II) is greater than 4, for example 4.3:1 or 4.5:1, to ensure that the reaction is fully completed; and then optionally, purifying the product by column chromatography to obtain the desired lipid of formula (I);

wherein the variables R₂, X, Y, R₃, Z, and R₄ are as defined for formula (I). In some



each variable is as defined for formula (I). In a further embodiment, L₁, L₃, L₅, L₇, and L₉ are connected to R₁ and are each independently a single bond, O, S, or NH. In a further embodiment,



wherein R_d and R_e are as defined for formula (I). In some embodiments, R^d and R^e are each independently H or a C₁₋₄ monovalent aliphatic group, preferably H or C₁₋₄ monovalent alkyl.

In some embodiments, in method (a), the product is purified by column chromatography to obtain the desired lipid of formula (I), wherein the column chromatographic purification method is well known in the art.

In some embodiments, in the present invention, A₁, A₂ and A₃, together with the N to which they are attached, form an amine head of the lipid, which undergoes an addition reaction with a hydrophobic lipid tail to obtain a series of lipids. Since the amine head can be protonated, such lipid nanoparticles are positively charged as a whole, so they can undergo an electrostatic interaction with negatively charged mRNAs, cell membranes and lysosomal membranes, such that such lipids can effectively encapsulate and deliver mRNAs.

[illegible]


C=COC(CCCOC(R44))YR3ZCCCOR44
, and the remaining variables are as defined for formula (I).

In some embodiments, the reaction is carried out at a temperature of 60-85°C, such as at 70°C for 24-96 hours or longer.

9

Other lipids of the present invention can be prepared by the above synthesis routes and other routes known in the art using other suitable starting materials. The methods listed above may include one or more additional steps to add or remove appropriate protecting groups to finally allow for the synthesis of lipids. In addition, various synthesis steps can be carried out in an alternative order or sequence to obtain the desired materials. Synthetic chemical transformation and protective group methods (protection and deprotection) which can be used to synthesize suitable lipids are known in the art.

In a third aspect, the present invention provides a pharmaceutical composition comprising a pharmaceutical carrier and a lipid nanoparticle (LNP), wherein the lipid nanoparticle comprises the lipid of formula (I) according to the present invention and a pharmaceutically active molecule.

In some embodiments, the pharmaceutically active molecule is a small molecule drug, a protein, a peptide, a nucleic acid, a saccharide, or a combination thereof. In a further embodiment, the pharmaceutically active molecule is an mRNA. In still a further embodiment, the mRNA may be a firefly luciferase mRNA which can be expressed as luciferase.

In some embodiments, the lipid nanoparticle has a particle size distribution of 50 to 500 nm.

In some embodiments, the lipid and the pharmaceutically active molecule are bound via a non-covalent interaction, a covalent bond or both.

In some embodiments, the lipid nanoparticle further comprises other lipids selected from cholesterol, dioleoylphosphatidylethanolamine (DOPE, CAS: 4004-05-1), and distearoylphosphatidylethanolamine-polyethylene glycol 2000 (DSPE-PEG2000, CAS: 147867-65-0), or 1,2-dimyristoyl-RAC-glycero-3-methoxy polyethylene glycol 2000 (DMG-PEG2000, CAS: 160743-62-4), or a combination thereof. Furthermore, replacing DOPE with the same ratio of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC, CAS: 4235-95-4) or distearoyl phosphatidylcholine (DSPC, CAS: 816-94-4) can also achieve similar delivery effects. In a further embodiment, the lipid nanoparticles further contain cholesterol, dioleoylphosphatidylethanolamine, and distearoylphosphatidylethanolamine-polyethylene glycol 2000 or 1,2-dimyristoyl-RAC-glycero-3-methoxy polyethylene glycol 2000. The molar ratio of lipid:cholesterol:DOPE:DSPE-PEG2000 in the examples of the present invention is 50:38.5:10:1.5.

In some embodiments, the lipid nanoparticles of the present invention are prepared by the method comprising: dissolving the ionizable lipid compound of the present invention, cholesterol, DOPE and DSPE-PEG2000 in absolute ethanol, and dissolving the pharmaceutically active molecule (the mass of lipids being 5-20 times the mass thereof (the N/P ratio of lipids to nucleic acids being in the range of 5:1-20:1)) in 100 mM sodium acetate buffer (pH = 5.2). The ethanol solution and the sodium acetate buffer solution were mixed and prepared on a microfluidic

machine at a flow rate of 1:3. In a further embodiment, the pharmaceutically active molecule is an mRNA.

In a fourth aspect, the present invention relates to a method for regulating gene expression comprising delivering a nucleic acid to a cell, the method comprising: contacting the cell with the pharmaceutical composition of the third aspect under conditions sufficient to cause uptake of the nucleic acid into the cell. In some embodiments, the cell is contacted *in vitro* or *ex vivo*. In some embodiments, the cell is contacted *in vivo*. In some embodiments, the regulation of gene expression is sufficient to treat a disease or condition, such as cancer.

The present invention also relates to a method of treating a disease or condition in a patient, the method comprising administering to a patient in need thereof a pharmaceutically effective amount of the pharmaceutical composition described in the present application, wherein the pharmaceutical composition comprises a therapeutic nucleic acid or protein for the disease or condition.

Definitions

The term “aliphatic group” refers to a saturated or unsaturated, linear or branched, acyclic, cyclic or polycyclic hydrocarbon moiety. Examples include, but are not limited to, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, and cycloalkynyl moieties.

The term “alkyl” includes hydrocarbon groups selected from linear and branched saturated hydrocarbon groups containing 1 to 30, such as 1 to 24, 1 to 18, such as 1 to 12, further such as 1 to 10, and still further such as 1 to 8 or 1 to 6 or 1 to 4 carbon atoms. Examples of monovalent alkyl or alkyl include, but are not limited to, methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl, octadecyl, nonadecyl, eicosyl, triacontyl, etc. Examples of divalent alkyl, i.e. alkylene, include, but are not limited to, methylene, ethylene, propylene, butylene, pentylene, hexylene, heptylene, octylene, nonylene, decylene, undecylene, dodecylene, tridecylene, tetradecylene, pentadecylene, hexadecylene, heptadecylene, octadecylene, nonadecylene, eicosylene, triacotylene, etc.

A monovalent group is a group formed by removing a hydrogen atom from the corresponding hydrocarbon moiety. A divalent group is a group formed by removing two hydrogen atoms from the corresponding hydrocarbon moiety.

The term “alkenyl” includes hydrocarbon groups selected from linear and branched hydrocarbon groups containing at least one C=C double bond and 2 to 30, such as 2 to 24, 2 to 18, such as 2 to 8, further such as 2 to 6 carbon atoms. Examples of alkenyl, such as C2-6 alkenyl, include, but are not limited to, ethenyl/vinyl, prop-1-enyl, prop-2-enyl, 2-methylprop-1-enyl, but-1-enyl, but-2-enyl, but-3-enyl, buta-1,3-dienyl, 2-methylbuta-1,3-dienyl, hex-1-enyl, hex-2-

enyl, hex-3-enyl, hex-4-enyl, hexa-1,3-dienyl, dodecenyl, tetradecenyl, hexadecenyl, octadecenyl, etc.

The term “alkynyl” includes hydrocarbon groups selected from linear and branched hydrocarbon groups containing at least one $C\equiv C$ triple bond and 2 to 30, such as 2 to 24, 2 to 18, such as 2 to 8, further such as 2 to 6 carbon atoms. Examples of alkynyl, such as C2-6 alkynyl, include but are not limited to ethynyl, 1-propynyl, 2-propynyl (propargyl), 1-butynyl, 2-butynyl, and 3-butynyl.

The term “cycloalkyl” includes hydrocarbon groups selected from saturated cyclic hydrocarbon groups containing monocyclic and polycyclic (e.g., bicyclic and tricyclic) groups, including fused, bridged or spirocyclic alkyl. The cycloalkyl may contain 3 to 30, 3 to 12, for example 3 to 10, further for example 3 to 8, further for example 3 to 6, 3 to 5 or 3 to 4 carbon atoms. Furthermore, by way of example, the cycloalkyl may be selected from monocyclic groups containing 3 to 12, such as 3 to 10, further such as 3 to 8, 3 to 6 carbon atoms. Examples of monocyclic cycloalkyl include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl, cycloundecyl, and cyclododecyl. The term “cycloalkenyl” refers to a non-aromatic, cyclic hydrocarbon moiety containing at least one double bond, such as cyclohexenyl and cyclohexenylene. The term “cycloalkynyl” refers to a non-aromatic, cyclic hydrocarbon moiety containing at least one triple bond, such as cyclooctynyl and cyclooctynylene. Similarly, cycloalkylene, cycloalkenylene, and cycloalkynylene are corresponding divalent groups.

The term “heteroaliphatic group” refers to an aliphatic moiety containing at least one heteroatom selected from N, O, P, B, S, Si, Sb, Al, Sn, As, Se, and Ge. The heteroaliphatic group of the present invention includes alkyl, alkenyl or alkynyl containing at least one heteroatom selected from N, O, P, B, S, Si, Sb, Al, Sn, As, Se, and Ge, and a cycloalkyl, cycloalkenyl or cycloalkynyl moiety containing at least one heteroatom selected from N, O, P, B, S, Si, Sb, Al, Sn, As, Se, and Ge. The cyclic heteroaliphatic group includes a 3- to 7-membered monocyclic heteroaliphatic group and a 7- to 12-membered bicyclic heteroaliphatic group, and the cyclic heteroaliphatic groups contain 1-3 or more, for example, 1-3 heteroatoms selected from oxygen, nitrogen and sulfur. In some embodiments, the cyclic heteroaliphatic group is a 3- to 7-membered monocyclic heteroaliphatic group containing 1-3 heteroatoms selected from oxygen, nitrogen and sulfur. Cyclic heteroaliphatic groups include, but are not limited to, pyrrolidinyl, imidazolidinyl, piperidinyl, piperazinyl, pyranyl, morpholinyl, oxiranyl, azirinyl, thiiranyl, azetidiny, oxetanyl, thietanyl, dithietanyl, dihydropyridine, tetrahydropyridine, thiomorpholine, homopiperazinyl, homopiperidinyl, azepanyl, oxepanyl, thiepanyl, oxathianyl, dioxepanyl, oxathiepanyl, oxazepanyl, dithiepanyl, thiazepanyl, diazepanyl, thiazinanyl, oxazepine,

diazepine, thiazepine, dihydrothienyl, dihydropyranyl, dihydrofuranyl, tetrahydrofuranyl, tetrahydrothienyl, tetrahydropyranyl, tetrahydrothiapyranyl, pyrrolinyl, indolinyl, dioxanyl, dioxolanyl, pyrazolinyl, pyrazolidinyl, dithianyl, dithiolanyl, pyrimidonyl, dioxo-thiomorpholinyl, azabicyclo[3.1.0]hexyl, azabicyclo[4.1.0]heptyl, azabicyclo[2.2.2]hexyl, etc. A typical heteroaliphatic group is heteroalkyl, i.e. an alkyl group containing at least one heteroatom such as N, O or S, e.g. C₁₋₆ alkyl containing one N atom, C₁₋₆ alkyl containing one O atom, or C₁₋₆ alkyl containing one S atom; or C₁₋₄ heteroalkyl containing one N atom, C₁₋₄ heteroalkyl containing one O atom, or C₁₋₄ heteroalkyl containing one S atom.

The term “oxyaliphatic group” refers to a -O-aliphatic group. Examples of the oxyaliphatic group include methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, sec-butoxy, and tert-butoxy.

The term “aryl (aromatic group)” refers to a C₆ monocyclic, C₁₀ bicyclic, C₁₄ tricyclic, C₂₀ tetracyclic, or C₂₄ pentacyclic aromatic ring system. Examples of aryl include phenyl, phenylene, naphthyl, naphthylene, anthryl, anthrylene, pyrenyl, and pyrenylene.

The term “heteroaryl (heteroaromatic group)” refers to aromatic 5- to 8-membered monocyclic, 8- to 12-membered bicyclic, 11- to 14-membered tricyclic, and 15- to 20-membered tetracyclic ring systems having one or more heteroatoms (such as O, N, S, or Se). Examples of heteroaryl include furanyl, furanylene, fluorenyl, fluorenylene, pyrrolyl, pyrrolylene, thienyl, thienylene, oxazolyl, azolylene, imidazolyl, imidazolylene, benzimidazolyl, benzimidazolylene, thiazolyl, thiazolylene, pyridyl, pyridylene, pyrimidinyl, pyrimidinylene, quinazolinyl, quinazolinylene, quinolinyl, quinolinylene, isoquinolinyl, isoquinolinylene, indolyl, and indolylene.

Unless otherwise specified, the aliphatic group, heteroaliphatic group, oxyaliphatic group, alkyl, alkylene, alkenyl, alkenylene, alkynyl, alkynylene, cycloalkyl, cycloalkylene, cycloalkenyl, cycloalkenylene, cycloalkynyl, cycloalkynylene, heterocycloalkyl, heterocycloalkylene, heterocycloalkenyl, heterocycloalkenylene, aryl, and heteroaryl mentioned in the present application include both substituted and unsubstituted moieties. Possible substituents on the cycloalkyl, cycloalkylene, cycloalkenyl, cycloalkenylene, cycloalkynyl, cycloalkynylene, heterocycloalkyl, heterocycloalkylene, heterocycloalkenyl, heterocycloalkenylene, aryl, and heteroaryl include, but are not limited to, C₁-C₁₀ alkyl, C₂-C₁₀ alkenyl, C₂-C₁₀ alkynyl, C₃-C₂₀ cycloalkyl, C₃-C₂₀ cycloalkenyl, C₂-C₂₀ heterocycloalkyl, C₃-C₂₀ heterocycloalkenyl, C₁-C₁₀ alkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, amino, C₁-C₁₀ alkylamino, C₂-C₂₀ dialkylamino, arylamino, diarylamino, C₁-C₁₀ alkylsulfonamido, arylsulfonamido, C₁-C₁₀ alkylimino, arylimino, C₁-C₁₀ alkylsulfonimido, arylsulfonimido, hydroxyl, halo, thio, C₁-C₁₀ alkylthio, arylthio, C₁-C₁₀ alkylsulfonyl, arylsulfonyl, acylamino,

aminoacyl, aminothioacyl, amido, amidino, guanidine, ureido, thioureido, cyano, nitro, nitroso, azido, acyl, thioacyl, acyloxy, carboxyl, and carboxylate. In another aspect, possible substituents on the aliphatic group, heteroaliphatic group, oxyaliphatic group, alkyl, alkylene, alkenyl, alkenylene, alkynyl, and alkynylene include all the substituents listed above, except C₁-C₁₀ alkyl. Cycloalkyl, cycloalkylene, cycloalkenyl, cycloalkenylene, heterocycloalkyl, heterocycloalkylene, heterocycloalkenyl, heterocycloalkenylene, aryl, and heteroaryl may also be fused with one another.

The term “pharmaceutically active molecule” refers to any chemical substance intended for medical diagnosis, cure, treatment or prevention of diseases, including small molecule drugs such as chemotherapeutic drugs, and macromolecule drugs such as oligopeptides, oligosaccharides, oligonucleotides, peptides, proteins or nucleic acids.

Brief Description of the Drawings

Fig. 1a shows whole-body bioluminescence imaging (*in vivo* imaging system, IVIS) 6 hours after delivery of firefly luciferase (Fluc) mRNAs by LNPs containing lipid compounds with different hydrophobic chain lengths, where mice were administered via tail vein (dose: 150 µg/kg); and Fig. 1b shows the expression level of luciferase and bioluminescence intensity in liver tissue.

Fig. 2a shows whole-body bioluminescence imaging (*in vivo* imaging system, IVIS) 6 hours after delivery of firefly luciferase mRNAs by LNPs containing lipids with different chain lengths, where mice were administered intramuscularly (dose: 250 µg/kg); and Fig. 2b shows the expression level of luciferase and bioluminescence intensity at tissue level.

Fig. 3 shows the expression level of luciferase and bioluminescence intensity in liver tissue 6 hours after delivery of firefly luciferase mRNAs by YX27 lipid nanoparticles under different N/P conditions, where mice were administered via tail vein (dose: 150 µg/kg).

Fig. 4a shows whole-body bioluminescence imaging (*in vivo* imaging system, IVIS) 6 hours after delivery of YX27, SM102 and D-Lin-MC3-DMA (MC3) lipid nanoparticles, where mice were administered via tail vein (dose: 150 µg/kg); and Fig. 4b shows the expression level of luciferase and bioluminescence intensity in liver tissue.

Fig. 5 shows a plot of the expression level of luciferase and bioluminescence intensity in liver tissue over time after delivery of firefly luciferase mRNAs by YX27 lipid nanoparticles.

Fig. 6 shows the bioluminescence intensity of liver tissue 6 hours after delivery of firefly luciferase mRNAs by lipid nanoparticles with different molecular structures.

Detailed Description of Embodiments

The present invention will be further described in detail in conjunction with specific embodiments, and examples are given only to illustrate the present invention, but not to limit the scope of the present invention. The examples provided below can be used as a guide for further improvement by those of ordinary skill in the art and are not intended to limit the present invention in any way.

For all the quantitative experiments in the following examples, three repeated experiments are set up and the results are averaged.

The experimental methods in the following examples are all conventional unless otherwise specified. The materials, reagents, etc., used in the following examples can all be obtained from commercial sources unless otherwise specified.

In the following examples, cholesterol is a product from Macklin, with article number C10006595, CAS: 57-88-5.

In the following examples, both DOPE (dioleoylphosphatidylethanolamine, CAS: 4004-05-1) and DMG-PEG₂₀₀₀ (CAS: 147867-65-) are products from Aveto (Shanghai) Pharmaceutical Technology Co., Ltd.

The firefly luciferase mRNA in the following examples is a product from Shanghai Hongene Technology Development Co., Ltd.

In the following examples, Balb/c mice are products from Beijing Vital River Laboratory Animal Technology Co., Ltd.

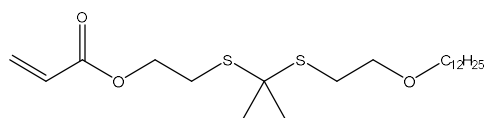
Examples

Example 1: Synthesis of ionizable lipid compounds

An ionizable lipid compound was synthesized by a Michael addition reaction between a corresponding hydrophilic amine compound and a corresponding hydrophobic tail, wherein the hydrophobic tail was a ketal-containing acrylate, specifically a thioketal-containing acrylate.

Hydrophobic tail compound **TK** is synthesized or prepared as follows:

TK12 is 2-((2-((2-(dodecyloxy)ethyl)thio)propan-2-yl)thio)ethyl acrylate



, i.e. the compound of formula I-1 in Example 2 of CN110101665A, and ¹H NMR of **TK-12** was: ¹H NMR (300 MHz, CDCl₃) δ 6.18 (s, 1H), 5.97 (d, J = 10.2 Hz, 1H), 4.26 (s, 2H), 3.51 (s, 4H), 3.34 (s, 6H), 2.87 (s, 1H), 2.74 (s, 1H), 1.55 (s, 4H), 1.50 – 1.39 (m, 1H), 1.25 (s, 6H), 0.86 (s, 1H).

TK2, TK4, TK6, TK8, and TK10 were synthesized in a manner similar to that in Examples

1 and 2 of CN110101665A, using 2,2'-(propane-2,2-diylbis(sulfanediyl))bis(ethan-1-ol) and a corresponding short-chain haloalkane Halo-C_nH_{2n+1} as

starting materials to prepare , which was then reacted with acryloyl halide (such as acryloyl chloride) to obtain the corresponding TK2, TK4, TK6, TK8 and TK10, in which

TK10 is 2-((2-((2-(decyloxy)ethyl)thio)propan-2-yl)thio)ethyl acrylate , and ¹H NMR of **TK10** was as follows: ¹H NMR (300 MHz, CDCl₃) δ 6.45(d, 1H), 6.13(m, 1H), 5.86(d, 1H), 4.31(t, 2H), 3.58(t, 2H), 3.43(t, 2H), 2.91(t, 2H), 2.82(t, 2H), 1.61 - 1.26(m, 23H), 0.87(t, 3H).

TK8 is 2-((2-((2-(octyloxy)ethyl)thio)propan-2-yl)thio)ethyl acrylate , and ¹H NMR of **TK8** was as follows: ¹H NMR (300 MHz, CDCl₃) δ 6.40(d, 1H), 6.13(m, 1H), 5.86(d, 1H), 4.31(t, 2H), 3.58(t, 2H), 3.43(t, 2H), 2.91(t, 2H), 2.82(t, 2H), 1.61 - 1.27(m, 17H), 0.87(t, 3H).

TK6 is 2-((2-((2-(hexyloxy)ethyl)thio)propan-2-yl)thio)ethyl acrylate , and ¹H NMR of **TK6** was as follows: ¹H NMR (300 MHz, CDCl₃) δ 6.41(d, 1H), 6.12(m, 1H), 5.83(d, 1H), 4.32(t, 2H), 3.59(t, 2H), 3.46(t, 2H), 2.92(t, 2H), 2.82(t, 2H), 1.61(m, 8H), 1.31(m, 6H), 0.88(t, 3H).

TK4 is 2-((2-((2-(butyloxy)ethyl)thio)propan-2-yl)thio)ethyl acrylate , and ¹H NMR of **TK4** was as follows: ¹H NMR (300 MHz, CDCl₃) δ 6.41(d, 1H), 6.12(t, 1H), 5.83(d, 1H), 4.32(d, 2H), 3.59(d, 2H), 3.46(d, 2H), 2.92(d, 2H), 2.82(d, 2H), 1.61–1.38 (m, 10H), 0.89(d, 3H).

The synthesis of the ionizable lipid compound **YX** of the present invention can be found in CN110101665A, etc.

YX9, i.e. the compound of formula II-10 in Example 5 of CN110101665A, was synthesized from the hydrophilic amine compound 3,3'-diamino-N-methyldipropylamine (CAS: 105-83-9) and TK12 in a similar manner to the method in Example 5 of CN110101665A, except that the molar ratio of the hydrophilic amine compound to TK12 was 4.3:1. ¹H NMR of **YX9** was as

follows: ^1H NMR (300 MHz, CDCl_3) δ 4.21(t, 8H), 3.57(t, 8H), 3.42(t, 8H), 2.85 - 2.76(m, 24H), 2.44(t, 12H), 2.29(s, 4H), 2.18(s, 3H), 1.59 - 1.25(m, 114H), 0.87(t, 12H).

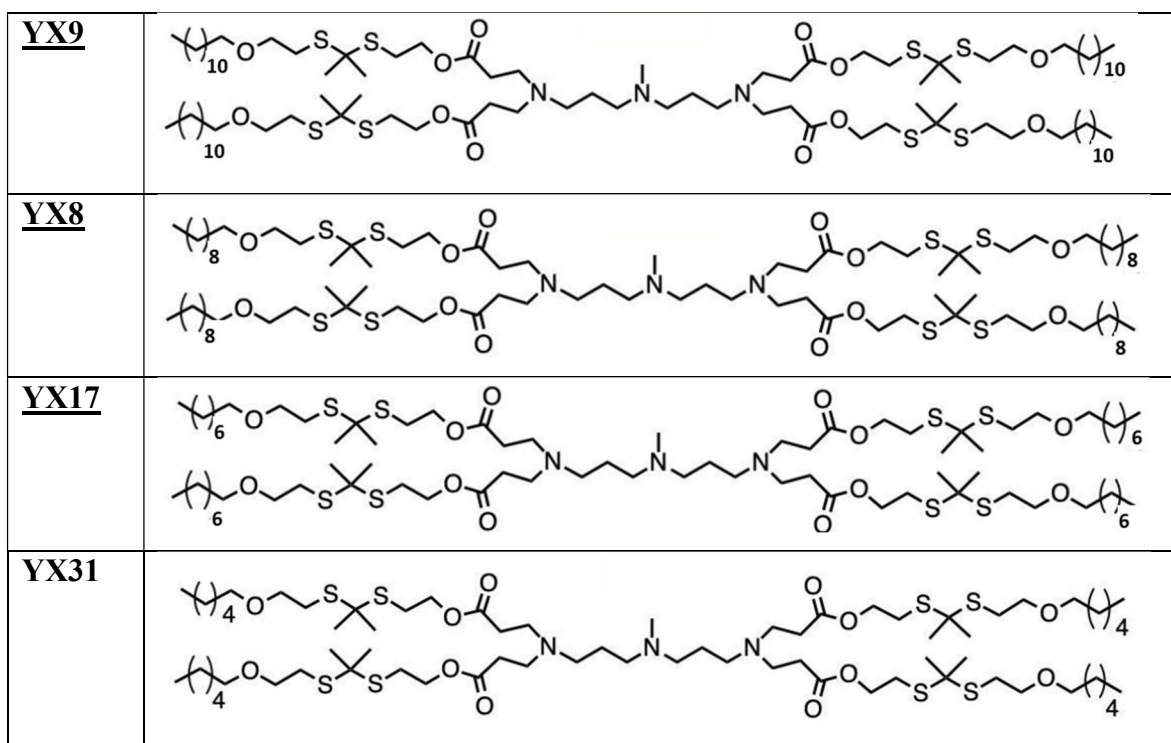
YX8 was synthesized from hydrophilic amine compound 3,3'-diamino-N-methyldipropylamine and TK10, wherein the hydrophilic amine compound 3,3'-diamino-N-methyldipropylamine and TK10 were mixed at a molar ratio of 4.3:1 and heated at 70°C for 72 h. The crude product was purified on a silica gel column with dichloromethane/methanol as an eluent to obtain ionizable lipid compound YX8. ^1H NMR of **YX8** was as follows: ^1H NMR (300 MHz, CDCl_3) δ 4.21(t, 8H), 3.57(t, 8H), 3.42(t, 8H), 2.85 - 2.76(m, 24H), 2.44(t, 12H), 2.28(s, 4H), 2.19(s, 3H), 1.60 - 1.25(m, 100H), 0.87(t, 12H).

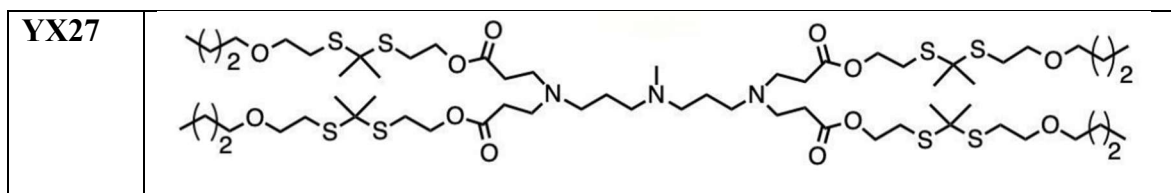
YX17, YX27 and YX31 were synthesized by mixing hydrophilic amine compound 3,3'-diamino-N-methyldipropylamine with a corresponding hydrophobic tail compound at a molar ratio of 4.3:1.

^1H NMR of **YX17** was as follows: ^1H NMR (300 MHz, CDCl_3) δ 4.21(t, 8H), 3.58(t, 8H), 3.43(t, 8H), 2.85 - 2.77(m, 24H), 2.45(m, 12H), 2.28(s, 4H), 2.18(s, 3H), 1.60 - 1.28(m, 83H), 0.87(t, 12).

^1H NMR of **YX27** was as follows: ^1H NMR (300 MHz, CDCl_3) δ 4.22(t, 8H), 3.58(t, 8H), 3.44(t, 8H), 2.86 - 2.77(m, 24H), 2.45(t, 12H), 2.28(t, 4H), 2.18(s, 3H), 1.60 - 1.38(m, 45H), 0.92(t, 12H).

^1H NMR of **YX31** was as follows: ^1H NMR (300 MHz, CDCl_3) δ 4.22(t, 8H), 3.58(t, 8H), 3.43(t, 8H), 2.86 - 2.77(m, 24H), 2.45(t, 12H), 2.28(t, 4H), 2.18(s, 3H), 1.60(m, 37H), 1.29(s, 25H), 0.88(t, 12H).





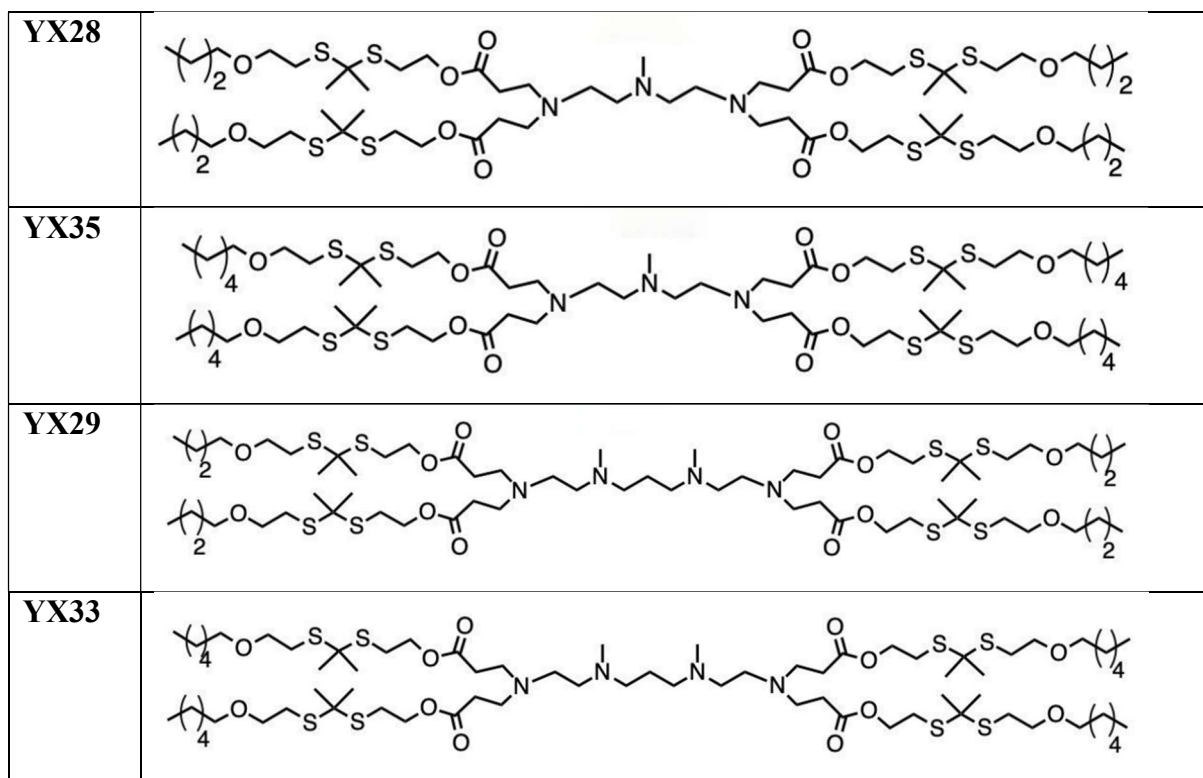
Ionizable lipid compounds YX28, YX35, YX29 and YX33 were synthesized from hydrophilic amine compound N-methyl-2,2-diaminodiethylamine (CAS No. 4097-88-5) or N,N'-bis(2-aminoethyl)-N,N'-dimethyl-1,3-propanediamine (CAS No. 152829-22-6) and corresponding hydrophobic tail compounds, in a manner similar to the above.

¹H NMR of **YX28** was as follows: ¹H NMR (300 MHz, CDCl₃) δ 4.22(t, 8H), 3.58(t, 8H), 3.44(t, 8H), 2.86 - 2.77(m, 24H), 2.55 - 2.46(m, 16H), 2.23(s, 3H), 1.60 - 1.38(m, 41H), 0.91(t, 12H).

¹H NMR of **YX29** was as follows: ¹H NMR (300 MHz, CDCl₃) δ 4.22(t, 8H), 3.58(t, 8H), 3.44(t, 8H), 2.86 - 2.77(m, 24H), 2.56(t, 4H), 2.46(t, 12H), 2.34(t, 4H), 2.22(s, 6H), 1.6 - 1.38(m, 50H), 0.92(t, 12H).

¹H NMR of **YX33** was as follows: ¹H NMR (300 MHz, CDCl₃) δ 4.22(t, 8H), 3.58(t, 8H), 3.43(t, 8H), 2.86 - 2.77(m, 24H), 2.57(s, 4H), 2.46(t, 12H), 2.35(s, 4H), 2.23(s, 6H), 1.60 - 1.29(m, 60 H), 0.88(t, 12H).

¹H NMR of **YX35** was as follows: ¹H NMR (300 MHz, CDCl₃) δ 4.22(t, 8H), 3.58(t, 8H), 3.43(t, 8H), 2.86 - 2.77(m, 24H), 2.55 - 2.46(m, 16H), 2.23(s, 3H), 1.60(m, 35H), 1.29(m, 24H), 0.89(t, 12H).



Example 2: Encapsulation of firefly luciferase mRNAs and preparation of lipid nanoparticles

In order to achieve mRNA delivery at animal level, the inventors prepared mRNA/lipid nanoparticles using the method as follows: formulating each of the ionizable lipid compounds (YX8, YX17, YX31, YX27, YX28, YX35, YX29 or YX33) prepared in Example 1, cholesterol, DOPE and DMG-PEG2000 into a 10 mg/mL ethanol solution. Taking YX27 as an example, when preparing an mRNA/LNP complex in a single time, YX27 solution (1.6 mg) was measured and mixed with cholesterol, DOPE and DMG-PEG2000 according to a molar ratio of 50:38.5:10:1.5, and the total volume of the solution was allowed to reach 450 μ l by supplementing ethanol. 150 μ g of firefly luciferase mRNAs were taken and added to a sodium acetate buffer (50 mM, pH = 5.2), and the total volume of the solution was 1350 μ l. The ionizable lipid compound solution and the mRNA solution were mixed by introducing into a microfluidic machine (speed: 0.2 mL/min of ethanol solution, and 0.6 mL/min of sodium acetate buffer solution) to prepare lipid nanoparticles encapsulating mRNAs, which were dialyzed against PBS buffer for 2 hours, and the obtained samples were directly used for animal experiments. The ratio of the ionizable lipid compound YX27 to mRNAs was calculated according to the molar ratio (N/P) of the nitrogen atoms in YX27 to the phosphorus atoms in the phosphate skeleton of the mRNAs. In this example, N/P was 7.5.

For lipid nanoparticles encapsulating mRNAs, Quant-it™ RiboGreen RNA Assay Kit (ThermoFisher Scientific, USA) was used to determine the concentration of free RNAs therein, and on this basis, a method for determining the RNA encapsulation efficiency of the nanoparticle composition was established. Samples were diluted in TE buffer (Solarbio, T1120, pH 8.0) to a concentration of about 5 μ g/mL. 50 μ L of the diluted sample was transferred to a polystyrene 96-well plate and 50 μ L of TE buffer or 50 μ L of 2% Triton X-100 solution (Solarbio, T8200) was added to the wells. The plate was incubated at a temperature of 37°C for 10 minutes. Reagents were diluted 1:200 in TE buffer and 100 μ L of this solution was added to each well. The fluorescence intensity can be measured using a fluorescence plate reader (BIOTEK/Synergy H1) at an excitation wavelength of 480 nm and an emission wavelength of 520 nm, and the percentage of free RNAs was determined by dividing the fluorescence intensity of the intact sample (without addition of Triton X-100) by the fluorescence value of the lysed sample (caused by addition of Triton X-100).

The LNP comprising YX27 in this example was tested as follows: an average particle size of 185 nm, PDI of 0.148, and a zeta potential of -7.87 mV.

Example 3: Animal experiment of delivery of mRNAs by lipid nanoparticles of the present invention

In order to verify the delivery effect of the above Fluc mRNA/LNP preparation, YX27 LNPs encapsulating 150 µg/kg Fluc mRNAs were injected into 6-8 week-old female Balb/c mice by administration via tail vein, or YX27 LNPs encapsulating 250 µg/kg Fluc mRNAs were injected into the hind legs of mice by intramuscular administration; after 6 hours, 200 µl of D-Luciferin (with a mass fraction of 30%) was injected by intraperitoneal administration, and the expression and intensity of luciferase in the whole body, liver and muscle administration sites were detected by PerkinElmer IVIS Lumina III small animal *in vivo* optical imaging system. In order to study the effect of the hydrophobic chain length of lipid compounds on mRNA delivery efficiency, LNP samples encapsulating Fluc mRNAs were prepared using lipid compounds YX9, YX8, YX17 and YX31 respectively according to YX27 experimental operation, and the efficiency of luciferase expression in mice was studied. The study found that the mRNA delivery efficiency of the lipid compounds containing 4 and 6 carbon atoms at hydrophobic carbon chain tail structures was significantly superior to other lipid molecules.

The results of *in vivo* luciferase imaging luminescence intensity after using different lipid compounds to deliver Fluc mRNAs were shown in the table below and Figs. 1 and 2:

Lipid No.	Average intensity	Standard deviation	Average intensity	Standard deviation
	Administration via tail vein/liver luminescence intensity		Intramuscular administration/muscle luminescence intensity	
YX9	2.04E+09	7.83E+08	7.85E+07	3.79E+07
YX8	4.04E+09	2.42E+09	8.42E+07	2.93E+06
YX17	3.91E+09	1.16E+09	2.20E+08	1.74E+07
YX31	1.04E+10	6.7E+08	7.70E+08	2.36E+08
YX27	1.2E+10	4.13E+09	5.93E+08	1.62E+08

Unit: p/s

Example 4: Effect of the N/P ratio of YX27 dosage forms on mRNA delivery

In order to study the effect of the mixing ratio (N/P ratio) of ionizable lipids and mRNAs as the LNP components on the mRNA delivery efficiency, mixed samples of YX27 and luciferase mRNAs at N/P ratios of 7.5 and 9 were prepared according to the operation steps of Example 2, the mRNA delivery and the expression and intensity of luciferase in the whole body, liver and

other sites were studied by administration via tail vein (the delivery results are shown in Fig. 3 and the table below).

Fluc mRNA dose	Average intensity	Standard deviation	Average intensity	Standard deviation
	N/P = 7.5		N/P = 9	
25 µg/kg	1.37E+09	6.29E+08	9.54E+08	2.49E+08
50 µg/kg	3.15E+09	1.76E+09	3.29E+09	6.17E+08
150 µg/kg	1.20E+10	4.15E+09	1.05E+10	3.69E+09

Example 5: Comparing the effect of YX27 with SM102 and MC3 on mRNA delivery

LNPs containing SM102 or MC3 as the cationic lipid and luciferase mRNAs at an N/P ratio of 7.5 were prepared according to the operation steps of Example 2, in which the dose of Fluc mRNAs was 25 µg/kg, 50 µg/kg or 150 µg/kg respectively; and then the mRNA delivery by the prepared LNPs and the expression and intensity of luciferase in the whole body, liver and other sites were studied by administration via tail vein. The delivery results are shown in Fig. 4 and the table below.

Fluc mRNA dose	Average intensity	Standard deviation	Average intensity	Standard deviation	Average intensity	Standard deviation
	SM102		MC3		YX27	
25 µg/kg	1.20E+09	1.02E+08	4.28E+08	6.06E+07	1.38E+09	6.27E+08
50 µg/kg	2.02E+09	2.99E+08	8.40E+08	2.18E+08	3.16E+09	1.75E+09
150 µg/kg	5.65E+09	1.45E+09	7.16E+09	1.09E+09	1.20E+10	4.12E+09

Unit: p/s

It can be seen from the above table that the ionizable lipid YX27 of the present invention exhibits superior delivery effect than the commercial cationic lipid SM102 or MC3 at each mRNA dose.

Example 6: Time-dependent experiments of mRNA delivery and expression

In order to verify the expression efficiency of luciferase after administration of Fluc mRNA/LNPs as a function of time, YX27 solution encapsulating 150 µg/kg of Fluc mRNAs was injected into 6-8 week-old female Balb/c mice by administration via tail vein; 2h, 4h, 6h, 18h and 24 h after administration respectively, 200 µl of D-Luciferin (with a mass fraction of 30%) was injected by intraperitoneal administration, and the expression and intensity of luciferase in

the whole body and liver site were detected by PerkinElmer IVIS Lumina III small animal *in vivo* optical imaging system. The delivery results are shown in Fig. 5 and the table below. It can be seen from the imaging results that the *in vivo* expression reached the peak at 6 h after the mRNA delivery.

Time after administration	Average intensity	Standard deviation
2h	1.47E+09	1.32E+08
4h	3.01E+09	1.72E+09
6h	3.51E+09	1.37E+09
18h	1.03E+09	7.34E+08
24h	5.49E+08	2.07E+08

Unit: p/s

Example 7: Evaluation of mRNA delivery efficiency of YX lipid compounds with different structures

LNPs containing SM102, YX27, YX28, YX29, YX33, YX31 or YX35 as the ionizable lipid compound and luciferase mRNAs at an N/P ratio of 7.5 were prepared according to the operation steps of Example 2, in which the dose of Fluc mRNAs was 150 µg/kg. The results of *in vivo* luciferase imaging luminescence intensity after delivery of Fluc mRNAs using different lipid compounds are shown in Fig. 6 and the table below. It can be found that when the number of carbon contained was 4 or 6, lipids having different amine heads can achieve efficient mRNA delivery, and the efficiency was close to that of commercial ionizable lipid SM102.

Lipid No.	Average intensity	Standard deviation	Average intensity	Standard deviation
	50 µg/kg		150 µg/kg	
SM102	2.02E+09	2.99E+08	5.65E+09	1.45E+09
YX27	3.16E+09	1.75E+09	1.20E+10	4.12E+09
YX28	1.88E+09	8.69E+08	5.29E+09	1.62E+09
YX29	1.20E+09	2.06E+08	4.24E+09	1.39E+09
YX33	8.19E+08	5.26E+08	2.97E+09	1.01E+09
YX31	1.69E+09	2.42E+08	1.02E+10	6.64E+08

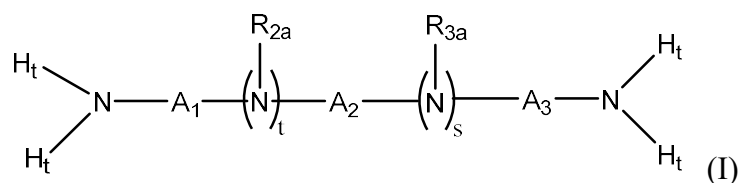
YX35	1.50E+09	6.90E+08	2.83E+09	7.57E+08
------	----------	----------	----------	----------

Unit: p/s

The above description is only preferred embodiments of the present invention; however, the scope of protection of the present invention is not limited thereto. Any changes or substitutions readily conceivable to those familiar with the technical field within the technical scope disclosed by the present invention should be covered by the scope of protection of the present invention. Therefore, the scope of protection of the present invention should be based on the scope of protection of the claims.

Claims

1. A lipid compound of formula (I),



wherein

R_{2a} and R_{3a} are each independently hydrogen, a monovalent aliphatic group, a monovalent heteroaliphatic group, a monovalent aromatic group, a monovalent heteroaromatic group, or H_t ;

t and s are each independently 0 or 1, and when t or s is zero, it means that the part is directly a single bond, provided that t and s are not both 0;

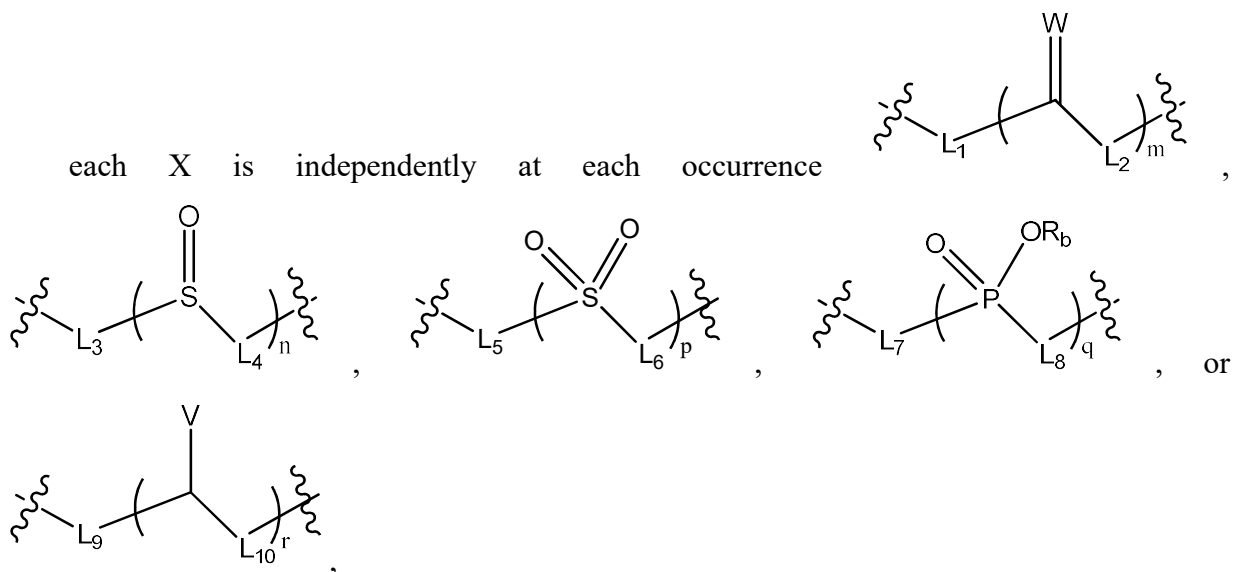
A_1 , A_2 , and A_3 are each independently a single bond, a divalent aliphatic group, a divalent heteroaliphatic group, a divalent aromatic group, or a divalent heteroaromatic group, or a combination of two of the above;

each H_t is independently at each occurrence $-\text{R}_1-\text{X}-\text{R}_2-\text{Y}-\text{R}_3-\text{Z}-\text{R}_4$,

wherein

each R_1 is independently at each occurrence a divalent aliphatic group, a divalent heteroaliphatic group, a divalent aromatic group, or a divalent heteroaromatic group;

each X is independently at each occurrence



wherein

m , n , p , q , and r are each independently 1-6;

W is O , S , or NR_c ;

L_1 , L_3 , L_5 , L_7 , and L_9 are directly connected to R_1 or R_2 and are each independently a single bond, O , S , or NR_d ;

L_2 , L_4 , L_6 , L_8 , and L_{10} are each independently a bond, O , S , or NR_e ;

V is an aliphatic group, OR_f , SR_g , or NR_hR_i ,

wherein R_b , R_c , R_d , R_e , R_f , R_g , R_h , and R_i are each independently hydrogen, hydroxy, an oxyaliphatic group, a monovalent aliphatic group, a monovalent heteroaliphatic group, a monovalent aromatic group, or a monovalent heteroaromatic group;

Y and Z are each independently at each occurrence S or O ;

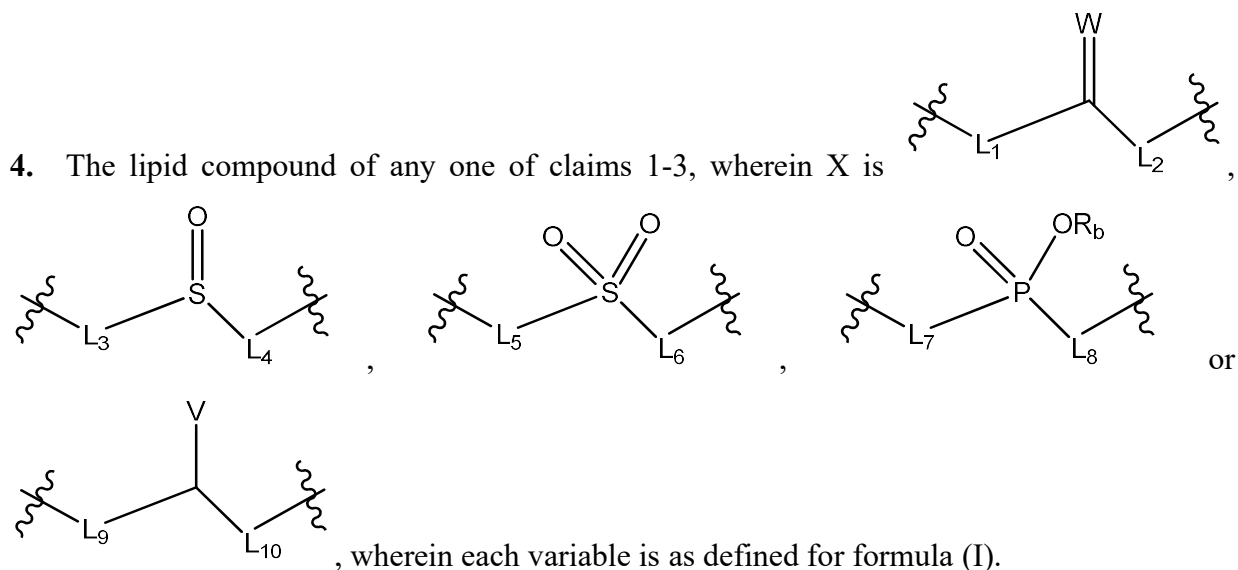
each R_2 is independently at each occurrence a single bond, a divalent aliphatic group, a divalent heteroaliphatic group, a divalent aromatic group, or a divalent heteroaromatic group;

each R_3 is independently at each occurrence a single bond, a divalent aliphatic group, a divalent heteroaliphatic group, a divalent aromatic group, or a divalent heteroaromatic group;

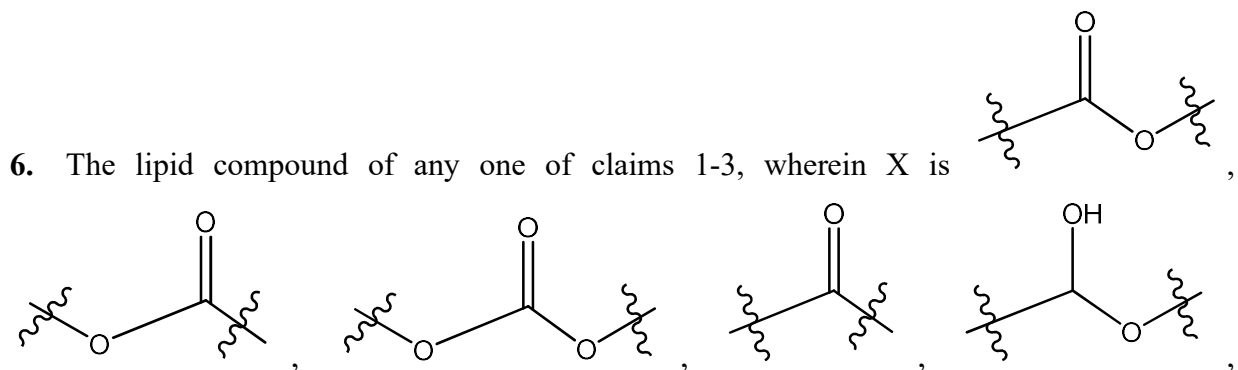
each R_4 is independently at each occurrence a hydrophobic group selected from $-(CH_2CH_2O)_y-C_3-C_8$ alkyl, $-(CH_2CH_2O)_y-C_3-C_8$ alkenyl or $-(CH_2CH_2O)_y-C_3-C_8$ alkynyl, wherein y is 0 or 1 or 2.

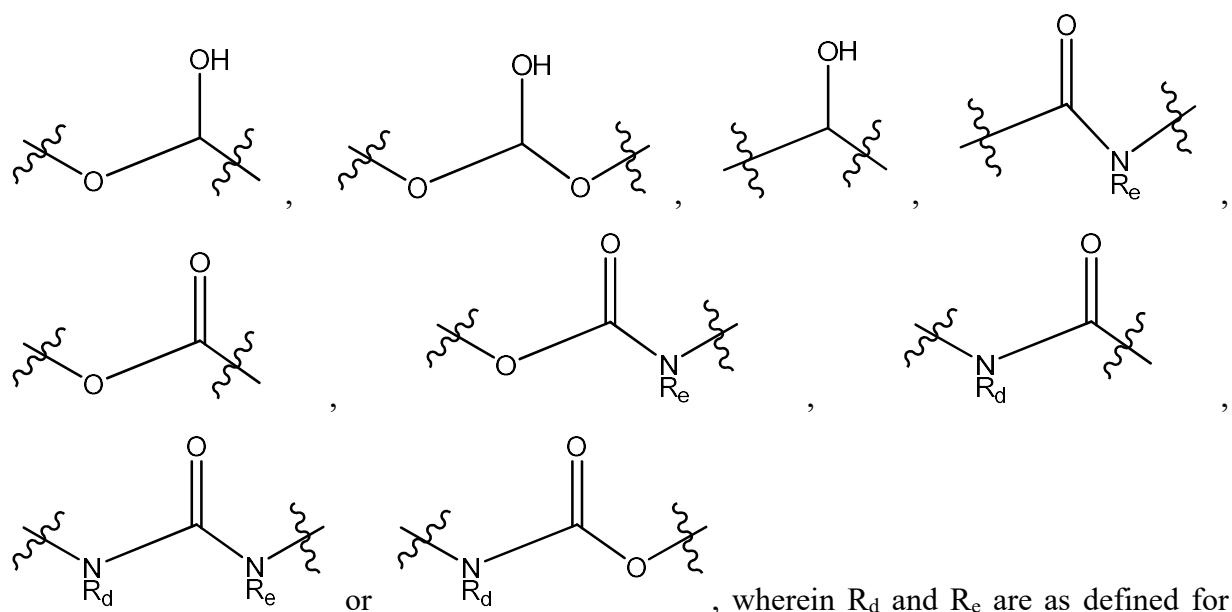
2. The lipid compound of claim 1, wherein t and s are both 0; or t is 0 and s is 1; or t is 1 and s is 0.

3. The lipid compound of claim 1 or 2, wherein R_1 is a C_{1-6} (e.g., C_{1-4}) divalent aliphatic group or a C_{1-6} (e.g., C_{1-4}) divalent heteroaliphatic group, preferably a C_{1-4} divalent alkyl or a C_{1-4} divalent heteroalkyl.

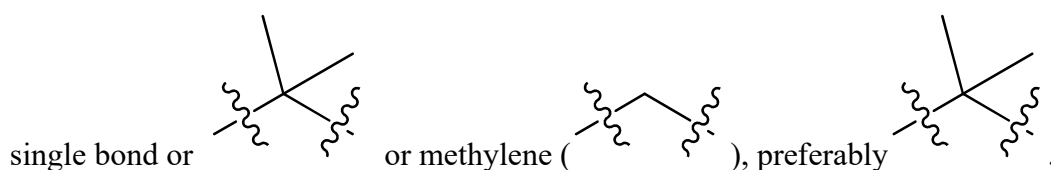


5. The lipid compound of claim 4, wherein L_1 , L_3 , L_5 , L_7 , and L_9 are connected to R_1 and are each independently a single bond, O , S , or NH .





7. The lipid compound of claim 6, wherein R^d and R^e are each independently H or a C_{1-4} monovalent aliphatic group, preferably H or C_{1-4} monovalent alkyl.
8. The lipid compound of any one of claims 1-7, wherein Y and Z are both S; or Y is S and Z is O; or Y is O and Z is S; or Y and Z are both O.
9. The lipid compound of any one of claims 1-8, wherein each R_2 is independently at each occurrence a single bond or a C_{1-6} divalent aliphatic group.
10. The lipid compound of claim 9, wherein each R_2 is independently at each occurrence C_{1-4} divalent aliphatic group, preferably C_{1-4} divalent alkyl, more preferably C_{1-2} divalent alkyl.
11. The lipid compound of any one of claims 1-10, wherein each R_3 is independently at each occurrence a single bond or C_{1-6} divalent aliphatic groups (e.g., C_{1-4} divalent aliphatic group, preferably C_{1-4} divalent alkyl).
12. The lipid compound of claim 11, wherein each R_3 is independently at each occurrence a

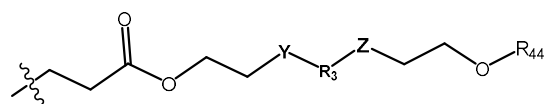


13. The lipid compound of any one of claims 1-12, wherein
 - each R_4 is independently at each occurrence C_3 - C_8 alkyl, C_3 - C_8 alkenyl, C_3 - C_8 alkynyl, - (CH_2CH_2O) - C_3 - C_8 alkyl, - (CH_2CH_2O) - C_3 - C_8 alkenyl, - (CH_2CH_2O) - C_3 - C_8 alkynyl, - $(CH_2CH_2O)_2$ - C_3 - C_8 alkyl, - $(CH_2CH_2O)_2$ - C_3 - C_8 alkenyl or - $(CH_2CH_2O)_2$ - C_3 - C_8 alkynyl; or
 - each R_4 is independently at each occurrence C_4 - C_8 alkyl, C_4 - C_8 alkenyl, C_4 - C_8 alkynyl, - (CH_2CH_2O) - C_4 - C_8 alkyl, - (CH_2CH_2O) - C_4 - C_8 alkenyl, - $(CH_2CH_2O)_2$ - C_4 - C_8 alkynyl, - $(CH_2CH_2O)_2$ - C_4 - C_8 alkyl, - $(CH_2CH_2O)_2$ - C_4 - C_8 alkenyl or - $(CH_2CH_2O)_2$ - C_4 - C_8 alkynyl.

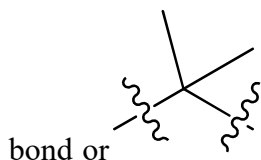
14. The lipid compound of claim 13, wherein the alkyl in the C₄-C₈ alkyl, -(CH₂CH₂O)-C₄-C₈ alkyl or -(CH₂CH₂O)₂-C₄-C₈ alkyl is a linear alkyl; the alkenyl in the C₄-C₈ alkenyl, -(CH₂CH₂O)-C₄-C₈ alkenyl or -(CH₂CH₂O)₂-C₄-C₈ alkenyl is a linear alkenyl; and the alkynyl in the C₄-C₈ alkynyl, -(CH₂CH₂O)-C₄-C₈ alkynyl or -(CH₂CH₂O)₂-C₄-C₈ alkynyl is a linear alkynyl.

15. The lipid compound of claim 13, wherein each R₄ is independently at each occurrence - (CH₂CH₂O)-(CH₂)₃CH₃, -(CH₂CH₂O)₂-(CH₂)₃CH₃, -(CH₂CH₂O)-(CH₂)₄CH₃, -(CH₂CH₂O)₂-(CH₂)₄CH₃, -(CH₂CH₂O)-(CH₂)₅CH₃, -(CH₂CH₂O)₂-(CH₂)₅CH₃, -(CH₂CH₂O)-(CH₂)₆CH₃, -(CH₂CH₂O)₂-(CH₂)₆CH₃, -(CH₂CH₂O)-(CH₂)₇CH₃ or -(CH₂CH₂O)₂-(CH₂)₇CH₃.

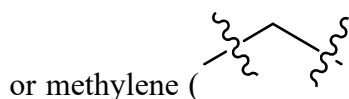
16. The lipid compound of claim 1, wherein each Ht is independently at each occurrence



, wherein Y and Z are both S; or Y is S and Z is O; or Y is O and Z is S; or Y and Z are both O; each R₃ is independently at each occurrence a single



bond or



or methylene (

), and R₄₄ is C₄-C₈ alkyl, C₄-C₈ alkenyl or C₄-C₈ alkynyl.

17. The lipid compound of claim 16, wherein each R₄₄ is independently at each occurrence - (CH₂)₃CH₃, -(CH₂)₄CH₃, -(CH₂)₅CH₃, -(CH₂)₆CH₃ or -(CH₂)₇CH₃.

18. The lipid compound of claim 1, wherein

t is 1, s is 0, A₂ is a single bond, and A₁ and A₃ are each independently a divalent aliphatic group or a divalent heteroaliphatic group; or

t is 1, s is 0, A₂ is a single bond, and A₁ and A₃ are each independently a C₁-C₆ divalent aliphatic group (e.g., C₁-C₄ divalent aliphatic group, preferably C₁-C₄ divalent alkyl); or

t is 1, s is 0, A₂ is a single bond, A₁ and A₃ are each independently -CH₂CH₂- or -CH₂CH₂CH₂-, and

R_{2a} is hydrogen or a monovalent aliphatic group, or is hydrogen or C₁-C₆ alkyl or methyl.

19. The lipid compound of claim 1, wherein

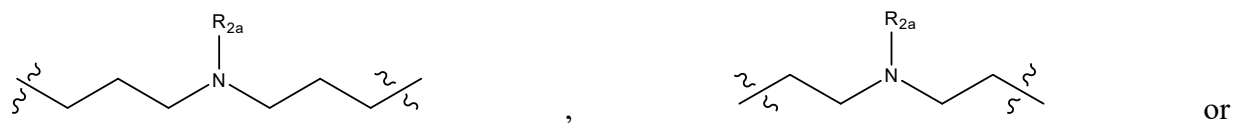
t is 1, s is 1, and A₁, A₂ and A₃ are each independently a divalent aliphatic group or a divalent heteroaliphatic group; or

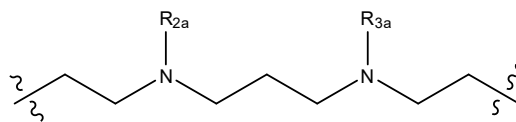
t is 1, s is 1, and A₁, A₂ and A₃ are each independently a C₁-C₆ divalent aliphatic group (e.g., C₁-C₄ divalent aliphatic group, preferably C₁-C₄ divalent alkyl); or

t is 1, s is 1, and A₁, A₂ and A₃ are each independently -CH₂CH₂- or -CH₂CH₂CH₂-, and

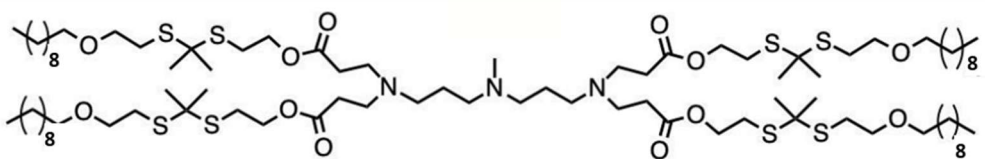
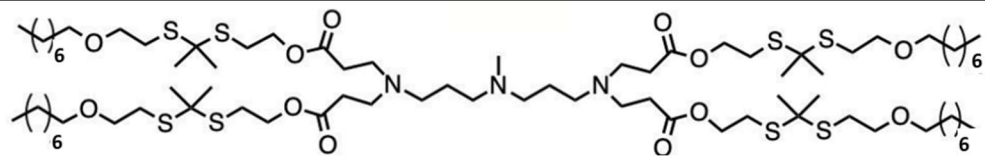
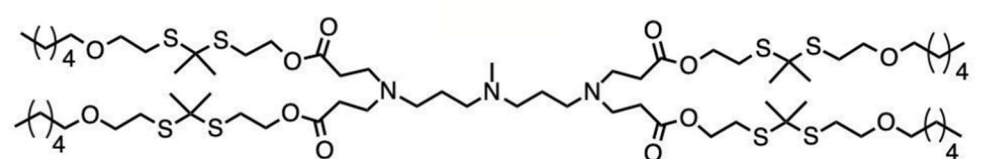
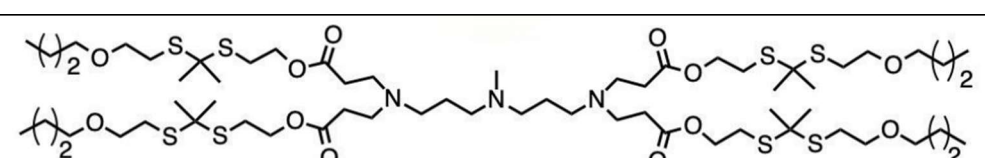
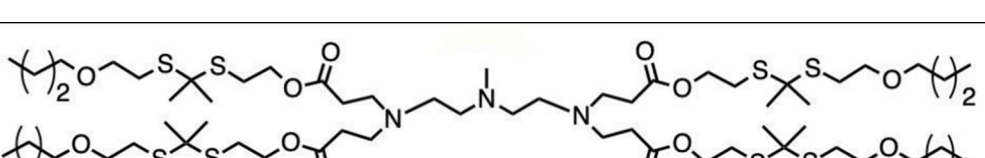
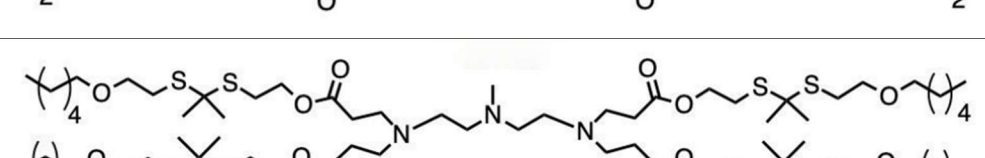
R_{2a} and R_{3a} are each independently hydrogen or a monovalent aliphatic group, or hydrogen or C₁-C₆ alkyl or methyl.

20. The lipid compound of claim 1, wherein $\sim A_1-(N^{R_{2a}})_t-A_2-(N^{R_{3a}})_s-A_3\sim$ is



 , wherein R_{2a} and R_{3a} are each independently hydrogen or a monovalent aliphatic group, or hydrogen or C₁-C₆ alkyl.

21. The lipid compound of claim 1, wherein the lipid of formula (I) is

YX8	
YX17	
YX31	
YX27	
YX28	
YX35	
YX29	