

LIPID COMPOUND AND THE COMPOSITION THEREOF

Technical field

[0001] The invention belongs to the field of biomedicine and biotechnology, and relates to a series of lipid compounds and therapeutic pharmaceutical delivery systems thereof.

Background

[0002] Exogenous biomolecules and some pharmaceutical molecules are hard to reach the cytoplasm through the cell membrane for curing effect. mRNA is one kind of biomolecule with negative charge, which has to overcome the barrier of cell membrane, for translating to protein and playing the biological function. Thus, delivering the biomolecules efficiently in vivo is an important challenge.

[0003] Lipid Nanoparticle (LNP) is one kind of new nucleic molecule delivery technology, which typically includes four components: (1) ionizable lipid, which combines with mRNA into a particle as large as bacteria, and releases mRNA from endosome to cytoplasm; (2) PEG-lipid, which improves the half-life of LNPs in blood; (3) cholesterol, which improves the stability of nanoparticles; (4), phospholipid, which is beneficial to form the double lipid structure (lipid bilayer). LNPs function to not only protect the mRNA from being decomposed by RNA enzyme (RNAses) or recognized by TLRs, but to also avoid the over-reaction of the innate immune system. The ionizable lipid can accelerate the cell uptake, and help the pharmaceutical molecules to release from endosome, achieving therapeutic effect.

[0004] The first LNP-siRNA medicine encapsulated by MC3 cationic lipid has been approved for marketing, proving that LNP can deliver the nucleic acid pharmaceuticals effectively in vivo, with an acceptable safety profile to some extent. In recent years, the study found that LNP also showed great application potential in the field of mRNA pharmaceutical and vaccine. The development direction of LNP delivery system is mainly focused on the ionizable lipid, the formula thereof and how to overcome the toxicity of some lipid preparations.

[0005] PCT/US2016/052352, published as WO2017/049245, discloses compounds and compositions and their use for intracellular delivery of therapeutic agents, including several novel lipid structures which can deliver the mRNA molecule to the target cell.

PCT/US2010/038224, published as WO2010/144740 discloses the chemical structure of MC3 which can encapsulate the siRNA pharmaceuticals with high efficiency, and avoid decomposition and removal during the delivery. Currently, the LNP delivery system is considered as a key technology for promoting nucleic acid pharmaceuticals into therapeutic application.

SUMMARY OF THE DISCLOSURE

[0006] For the current technical question, it was necessary to discover novel ionizable lipid compounds to improve the delivery efficiency and lower the toxicity of nucleic acid pharmaceuticals, such as mRNA and siRNA. The disclosure provides a series of novel ionizable lipid compounds which form the aliphatic chain by ester group of glycerol and ether group. The delivery effects of such lipids are better than the ionizable lipid of aliphatic chain. The novel ionizable lipids are combined with other lipid ingredients and formed into lipid nanoparticles which deliver mRNA or other pharmaceutical agents effectively in vivo where the intended biological function occurs. For example, delivering siRNA into a cell plays a role in gene silencing therapy; delivering mRNA into a cell can translate to protein or antigen efficiently for vaccine or pharmaceutical therapy; delivering antibody in vivo plays a role in therapy; and delivering Cas 9 mRNA in vivo plays a role in gene editing.

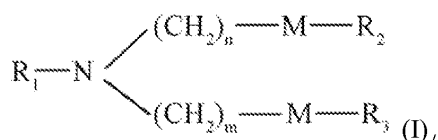
BRIEF DESCRIPTION OF THE DRAWINGS

- [0007]** Figure 1 is a graph depicting the weight change of male rat in LNP safety evaluation.
- [0008]** Figure 2 depicts the weight change of female rat in LNP safety evaluation.
- [0009]** Figure 3 depicts the food uptake change of male rat in LNP safety evaluation.
- [0010]** Figure 4 depicts the food uptake change of female rat in LNP safety evaluation.
- [0011]** Figure 5 depicts the IgG antibody titer of LNP-mRNA in an immunogenicity study.

DETAILED DESCRIPTION

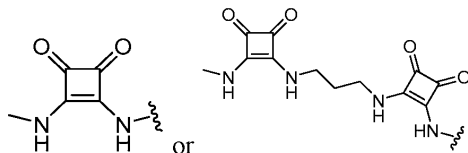
[0012] The disclosure provides a series of novel ionizable lipids, synthesis methods thereof, and pharmaceutical molecules mixed and encapsulated by a mixture comprising ionizable lipid, PEG lipid, structural lipid (such as, cholesterol) and phospholipid, thereby forming a nanoparticle delivery system which can be used for in vitro cell delivery and in vivo organ targeted cell delivery.

[0013] In one embodiment, the disclosure relates to a compound of the following formula (I):



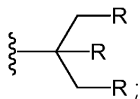
wherein R_1 is selected from $-R_1'-X$,

R_1' is $-(CH_2)_{0-6}-$, and X is amino, hydroxyl, ethynyl, cyano, $-C(O)(CH_2)_{1-3}NR_aR_b$, $-C(O)O(CH_2)_{1-3}NR_aR_b$, $-OC(O)(CH_2)_{1-3}NR_aR_b$, $-C(O)NH(CH_2)_{1-3}NR_aR_b$, $-NHC(O)(CH_2)_{1-3}NR_aR_b$, $-NHC(O)CH(NR_aR_b)(CH_2)_{1-3}NR_aR_b$, C_{3-7} cycloalkyl, 4-7 membered heterocyclic group, C_{6-10} aryl or 5-10 membered heteroaryl, the said cycloalkyl, heterocyclic group, aryl or heteroaryl are optionally substituted by the following groups: $-(CH_2)_{1-3}OH$, $-(CH_2)_{1-3}NR_aR_b$, $-(CH_2)_{1-3}C(O)NR_aR_b$; or X can also be:



R_a and R_b are independently selected from H, C_{1-3} alkyl, $-(CH_2)_{1-3}NH_2$, $-(CH_2)_{1-3}NH(CH_2)_{1-3}NH_2$; or R_a and R_b together with the nitrogen to which they are connected form a 5-10 membered heterocycle that includes 1-3 heteroatoms selected from N, O or S, said heterocycle is optionally substituted by the following groups: C_{1-6} alkyl, C_{1-6} alkyl halide, C_{1-6} alkyl hydroxyl group and C_{1-6} alkyl amino group;

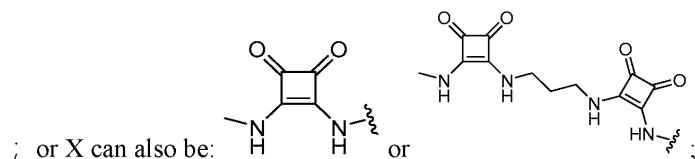
R_2 and R_3 are independently selected from H, C_{2-18} alkyl, C_{4-18} alkenyl or



each M is independently selected from $-\text{CH}_2-$, $-\text{CH}=\text{CH}-$, $-\text{NH}-$, $-\text{C}(\text{O})-$, $-\text{O}-$, $-\text{C}(\text{O})\text{O}-$, $-\text{OC}(\text{O})-$, $-\text{C}(\text{O})\text{NH}-$, or $-\text{NHC}(\text{O})-$;
 each R is independently selected from H, R' , $-\text{OR}^*$ or $-\text{R}''\text{OR}^*$;
 each R' is independently selected from C_{1-10} alkyl or C_{3-12} alkenyl;
 each R'' is independently selected from C_{1-10} alkyl or C_{3-12} alkenyl;
 each R^* is independently selected from C_{1-10} alkyl or C_{3-12} alkenyl;
 n, and m are independently an integer independently selected from 1 through 9;
 or a salt or an isomer thereof.

[0014] In one embodiment, the ionizable lipid is a compound of formula (I), wherein:

R' is $-(\text{CH}_2)_{2-3}-$, and X is hydroxyl, $-\text{C}(\text{O})(\text{CH}_2)_{2-3}\text{NR}_a\text{R}_b$, $-\text{C}(\text{O})\text{O}(\text{CH}_2)_{2-3}\text{NR}_a\text{R}_b$, $-\text{C}(\text{O})\text{NH}(\text{CH}_2)_{2-3}\text{NR}_a\text{R}_b$, or 5-10 heteroaryl which is optionally substituted by one or more of the following groups: $-(\text{CH}_2)_{2-3}\text{OH}$, $-(\text{CH}_2)_{2-3}\text{NR}_a\text{R}_b$, $-(\text{CH}_2)_{2-3}\text{C}(\text{O})\text{NR}_a\text{R}_b$

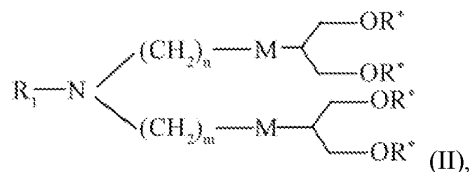


R_a , and R_b are independently selected from H, C_{1-3} alkyl, $-(\text{CH}_2)_{2-3}\text{NH}_2$, $-(\text{CH}_2)_{2-3}\text{NH}(\text{CH}_2)_{2-3}\text{NH}_2$; or the 5-10 membered heterocycle including 1-3 heteroatoms selected from N or O, which is formed together by R_a , R_b and their connected nitrogen, the said heterocycle is optionally substituted by the one or more of the following groups: C_{1-6} alkyl, C_{1-6} alkyl halide, C_{1-6} alkyl hydroxyl group and C_{1-6} alkyl amino group.

[0015] In one embodiment, the ionizable lipid is a compound of formula (I), wherein:

each M is independently selected from $-\text{CH}_2-$, $-\text{CH}=\text{CH}-$, $-\text{C}(\text{O})\text{O}-$, $-\text{OC}(\text{O})-$, $-\text{C}(\text{O})\text{NH}-$, or $-\text{NHC}(\text{O})-$.

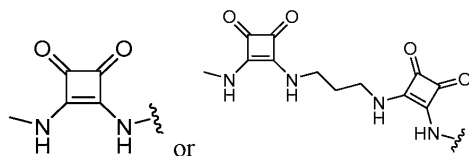
[0016] In one embodiment, the compound of formula (I) is a compound of formula (II):



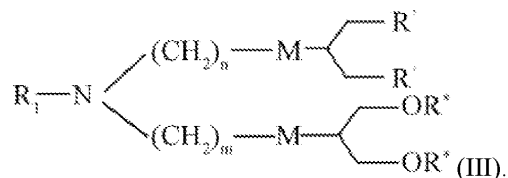
wherein:

each R^* is independently selected from C_{2-10} alkyl, preferably C_{6-10} alkyl, preferably C_6 alkyl.

- [0017] In one embodiment, the ionizable lipid is a compound of formula (II), wherein:
each M is independently selected from -C(O)O- or -OC(O)-, preferably -C(O)O-.
- [0018] In one embodiment, the ionizable lipid is a compound of formula (II), wherein:
R₁ is selected from -R₁'-X, R₁' is -(CH₂)₁₋₆-, and X is hydroxyl.
- [0019] In one embodiment, the ionizable lipid is a compound of formula (II), wherein:
R₁ is selected from -R₁'-X, R₁' is -(CH₂)₁₋₆-, and X is -C(O)(CH₂)₂₋₃NR_aR_b, -C(O)O(CH₂)₂₋₃NR_aR_b, -C(O)NH(CH₂)₂₋₃NR_aR_b,
R_a and R_b are independently selected from H, C₁₋₃ alkyl, -(CH₂)₂₋₃NH₂; or 5-10 membered heterocycle containing 1-3 heteroatoms selected from N or O, which is formed together by R_a and R_b and their connected nitrogen atom, preferably morpholinyl or piperidinyl, the said heterocycle is optionally substituted by C₁₋₆ alkyl hydroxyl.
- [0020] In one embodiment, the ionizable lipid is a compound of formula (II), wherein:
R₁ is selected from -R₁'-X, R₁' is -(CH₂)₁₋₆-, X is 5-6 membered heteroaromatic group, preferably triazolyl, said heteroaromatic group is optionally substituted by one or more of the the following groups: -(CH₂)₂₋₃OH, -(CH₂)₂₋₃NR_aR_b, and -(CH₂)₂₋₃C(O)NR_aR_b,
R_a and R_b are independently selected from H, C₁₋₃ alkyl, -(CH₂)₂₋₃NH₂, -(CH₂)₂₋₃NH(CH₂)₂₋₃NH₂, or 5-10 membered heterocycle containing 1-3 heteroatoms selected from N or O, which is formed together by R_a and R_b and their connected nitrogen atom, preferably morpholinyl, piperazinyl or piperidinyl, the said heterocycle is optionally substituted by one or more of the following groups: C₁₋₆ alkyl, and hydroxyl.
- [0021] In one embodiment, the ionizable lipid is a compound of formula (II), wherein:
R₁ is selected from -R₁'-X, R₁' is -(CH₂)₁₋₆-, and X is



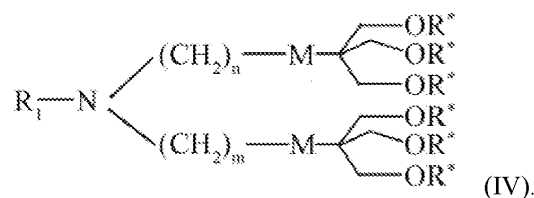
- [0022] In one embodiment, the ionizable lipid is a compound of formula (II), wherein:
each n is 7, m is 7.
- [0023] In one embodiment, the compound of formula (I) is a compound of formula (III):



[0024] In one embodiment, the ionizable lipid is a compound of formula (III), wherein: each R' is independently selected from C₁₋₁₀ alkyl, preferably C₂₋₈ alkyl.

[0025] In one embodiment, the ionizable lipid is a compound of formula (III), wherein: each M is independently selected from -C(O)O- or -OC(O)-, preferably -C(O)O-.

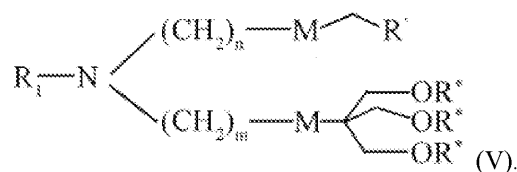
[0026] In one embodiment, the ionizable lipid is a compound of formula (I) is compound of formula (IV):



[0027] In one embodiment, the ionizable lipid is a compound of formula (IV), wherein: each R* is independently selected from C₂₋₁₀ alkyl, preferably C₆₋₁₀ alkyl, preferably C₆ alkyl.

[0028] In one embodiment, the ionizable lipid is a compound of formula (IV), wherein: each M is independently selected from -C(O)O- or -OC(O)-, preferably -C(O)O-.

[0029] In one embodiment, the ionizable lipid is a compound of formula (I) is a compound of formula (V):



[0030] In one embodiment, the ionizable lipid is a compound of formula (V), wherein: each R* is independently selected from C₂₋₁₀ alkyl, preferably C₆₋₁₀ alkyl, preferably C₆ alkyl.

[0031] In one embodiment, the ionizable lipid is a compound of formula (V), wherein: each M is independently selected from -CH=CH-, -C(O)O- or -OC(O)-, preferably -CH=CH- or -C(O)O-.

- [0032]** In one embodiment, the ionizable lipid is a compound of formula (V), wherein:
each R' is independently selected from C₁₋₁₀ alkyl or C₃₋₁₂ alkenyl, preferably C₁₀ alkyl or C₈ alkenyl.
- [0033]** In another embodiment, the compound is a salt of any of the prior embodiments.
- [0034]** In another embodiment, the compound is a stereoisomer of any of the prior embodiments.
- [0035]** In one embodiment, the said compound is selected from the following compounds, salts or stereoisomers thereof: A1, A5, A6, A7, A9, A10, A11, A12, A13, A15, A16, A17, A18, A19, A20, A21, A22, A23, A24, A25, A26, A27, A28, A29, A30, A31, A32, A34, A35, A36, A37, A38, A39, A40, A41, A42, A43, A44, A45, A46, A47, and 48.
- [0036]** In one embodiment, the disclosure related to a composition comprising a ionizable lipid compound according to the claim 1, in admixture with a PEG lipid, a structural lipid and a phospholipid.
- [0037]** In one embodiment, the phospholipids are selected from at least any one of the following groups: 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 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-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-0-octadecenyl-5'-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-5'-glycero-3-phosphocholine (OChemPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosaheptaenoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-phosphoethanol amine (DOPE), 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosaheptaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), dipalmitoylphosphatidylglycerol (DPPG), palmitoyl-oleoylphosphatidylethanolamine (POPE), distearoyl-phosphatidyl-ethanolamine (DSPE), dipalmitoyl phosphatidyl ethanolamine (DPPE),

dimyristoylphosphoethanolamine (DMPE), 1 -stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE), 1-stearoyl-2-oleoyl-phosphatidylcholine (SOPC), sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyl-oleoyl phosphatidylcholine, lysophosphatidylcholine, lysophosphatidylethanolamine (LPE). One or more of the recited phospholipids can be used in the mixture.

[0038] In one embodiment, the PEG lipid is selected from at least any one of the following groups: PEG-modified phosphatidylethanolamine, PEG- modified phosphatidic acid, PEG-modified ceramide, PEG-modified dialkylamine, PEG- modified diacylglycerol, PEG-modified dialkylglycerol. One or more PEG lipids can be used in the mixture.

[0039] In one embodiment, the structural lipid is selected from at least any one of the following groups: cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, ursolic acid, alpha-tocopherol. One or more structural lipids can be used in the mixture.

[0040] In one embodiment, in the composition, ionizable lipid compound is from 20% to 80%, PEG lipid is from 1% to 10%, structural lipid is from 10% to 50% and phospholipid is from 5% to 30%, each of these percentages being calculated based on mole percentage of all lipids in the composition. In another embodiment, ionizable lipid compound is 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80%, calculated based on mole percentage of all lipids in the composition. In another embodiment, PEG lipid is 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 5.5%, 6%, 6.5%, 7%, 7.5%, 8%, 8.5%, 9%, 9.5%, or 10%, calculated based on mole percentage of all lipids in the composition. In another embodiment, structural lipid is 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50%, calculated based on mole percentage of all lipids in the composition. In another embodiment, phospholipid is 5%, 10%, 15%, 20%, 25%, or 30%, calculated based on mole percentage of all lipids in the composition. .

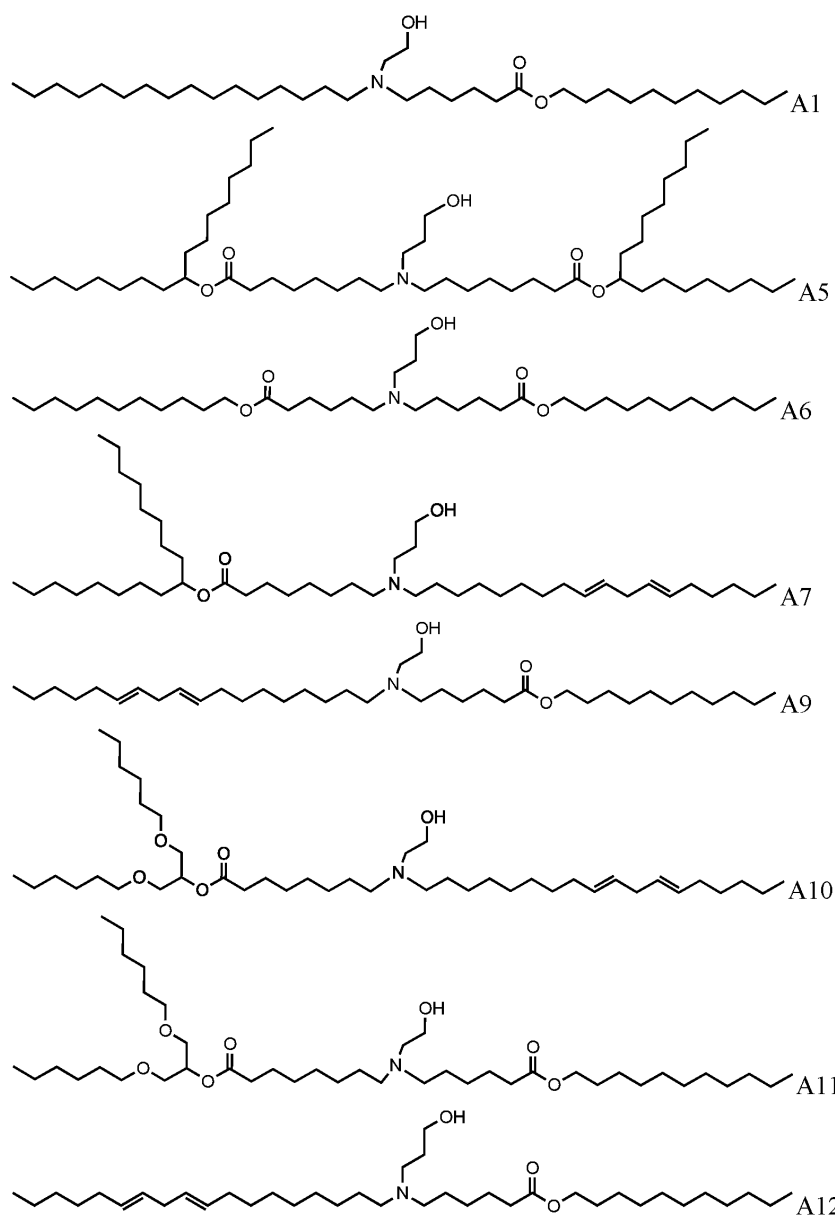
[0041] In one embodiment, the composition is in the form of a lipid nanoparticle.

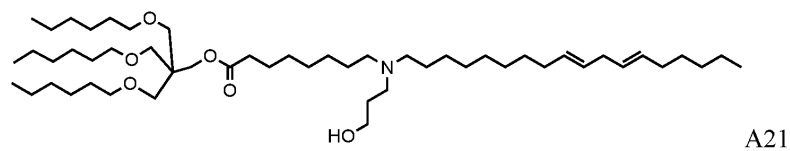
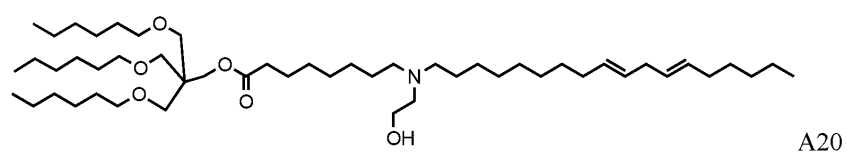
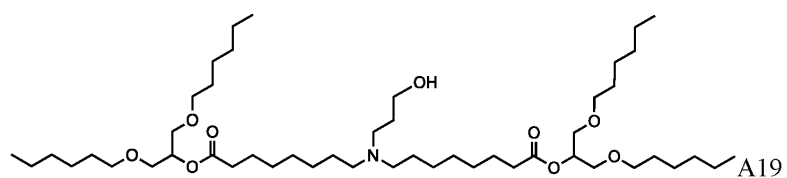
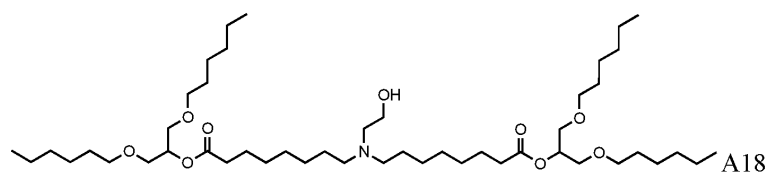
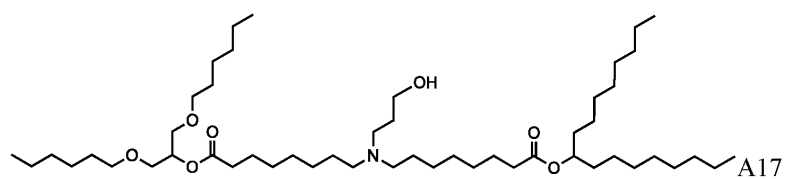
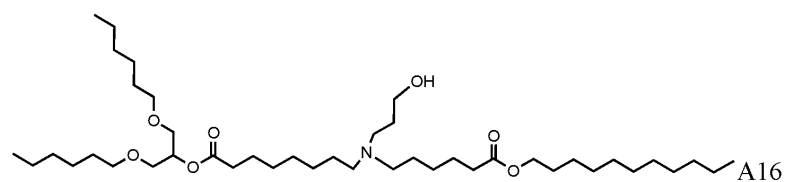
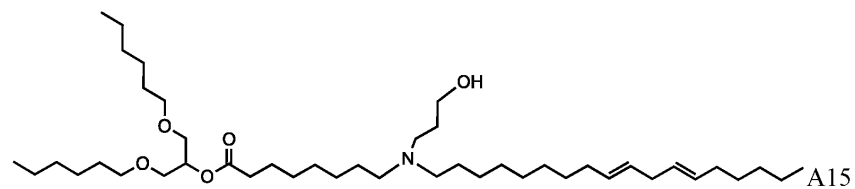
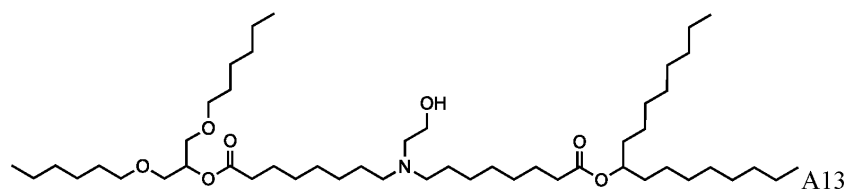
[0042] In another embodiment, the lipid nanoparticle also comprises active ingredient. The active ingredient can be selected from at least any one of: DNA, RNA, protein, or an active pharmaceutical molecule.

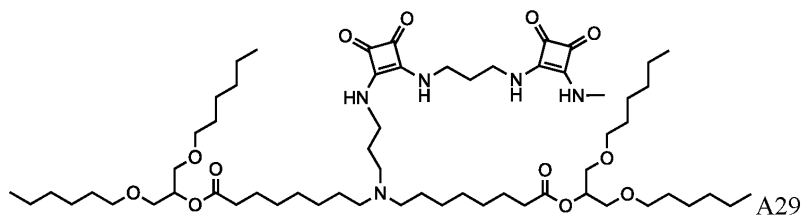
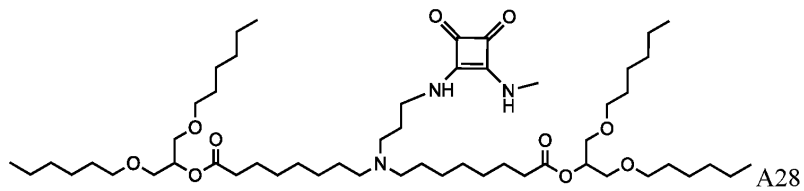
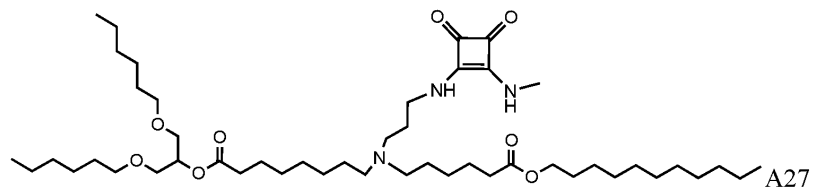
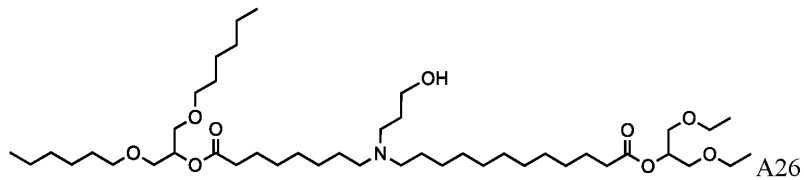
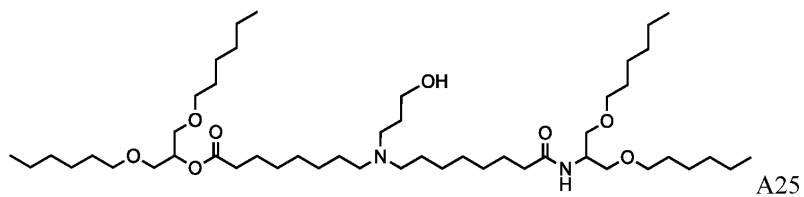
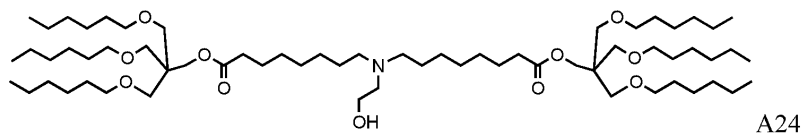
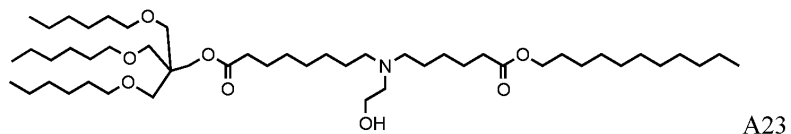
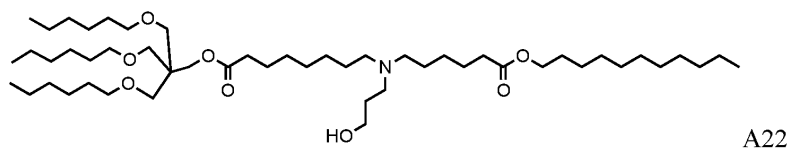
- [0043] In one embodiment, the RNA is selected from at least any one of: mRNA, siRNA, aiRNA, miRNA, dsRNA, aRNA, lncRNA, antisense nucleotide (ASO) or oligonucleotide.
- [0044] In one embodiment, the protein is selected from at least any one of: antibody, enzyme, recombinant protein, polypeptide and short chain polypeptide.
- [0045] The disclosure also relates to a method of producing lipid nanoparticles, comprising step (1): mixing the ionizable lipid compound, a PEG lipid, a structural lipid and a phospholipid in ethanol to form a lipid mixture.
- [0046] The method may further comprise step (2): mixing the lipid mixture with an active ingredient to form lipid nanoparticle by mixer.
- [0047] In one embodiment, the ionizable lipid compound, PEG lipid or PEG modified lipid, structural lipid and phospholipid are dissolved and mixed in ethanol, then mixed with an active ingredient by mixer to form lipid nanoparticle.
- [0048] In one embodiment, the disclosure relates to an ionizable compound for use in the production of lipid nanoparticle.
- [0049] In one embodiment, the lipid nanoparticle is neutral and uncharged in a neutral medium, and is positively charged after being protonated in an acidic medium.
- [0050] In one embodiment, the lipid nanoparticle is as defined in the specification.
- [0051] In one embodiment, disclosed is a pharmaceutical composition comprising the lipid nanoparticle and a pharmaceutically acceptable carrier.
- [0052] In one embodiment, the disclosure relates to the lipid nanoparticle or a pharmaceutical composition thereof for use in the production of medicine.
- [0053] In one embodiment, the medicine also comprises an active ingredient, wherein the active ingredient comprises at least any one of DNA, RNA, protein, and an active pharmaceutical molecule.
- [0054] In one embodiment, the RNA is selected from at least any one of: mRNA, siRNA, aiRNA, miRNA, dsRNA, aRNA, and lncRNA.
- [0055] In one embodiment, the invention relates to the lipid nanoparticle for use in the production of medicine, encapsulating an active ingredient into the said lipid nanoparticle.

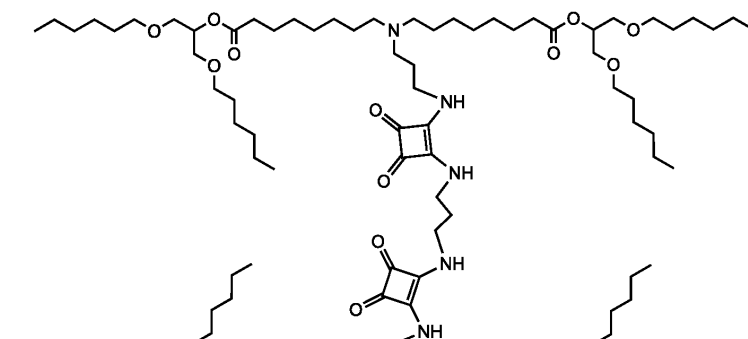
[0056] In one embodiment, the invention relates to the use of medicine, the medicine can be applied to a human by intravenous injection, intramuscular injection, subcutaneous injection, microneedle patch, oral administration, oral and nasal spray, or painting.

[0057] The structures of representative ionizable lipid compounds of the disclosure are shown as follows:

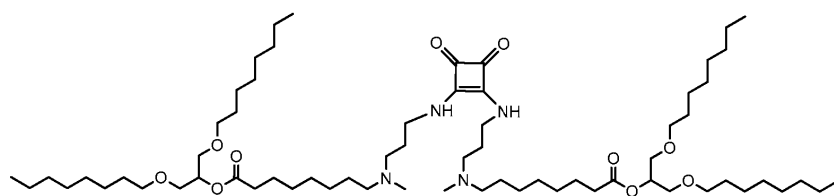




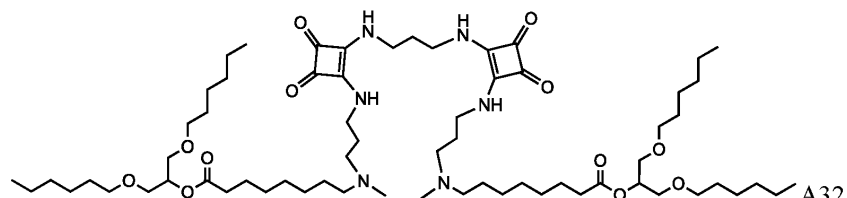




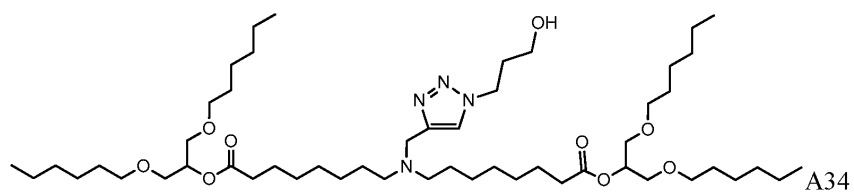
A30



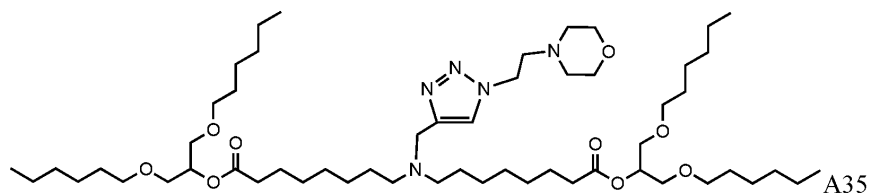
A31



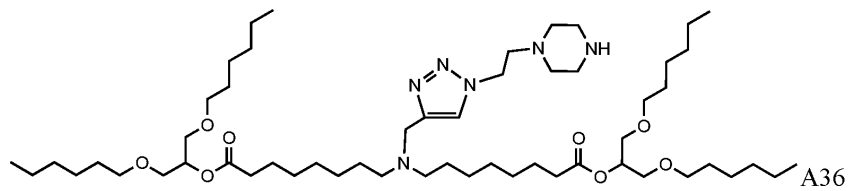
A32



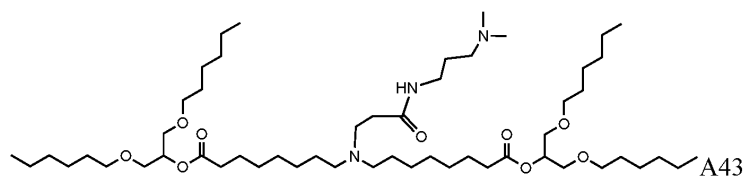
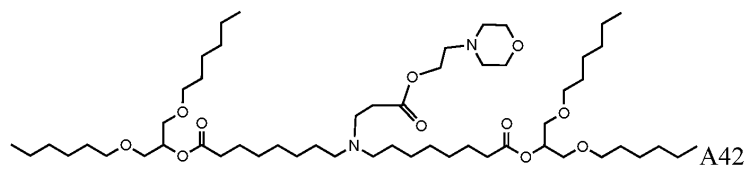
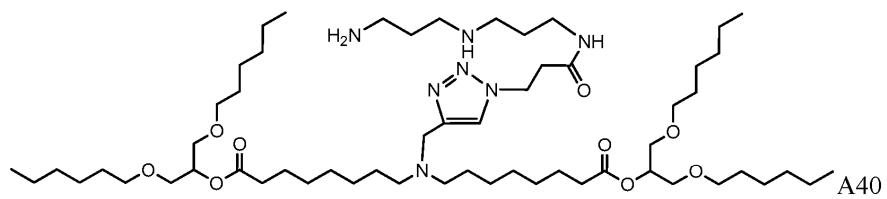
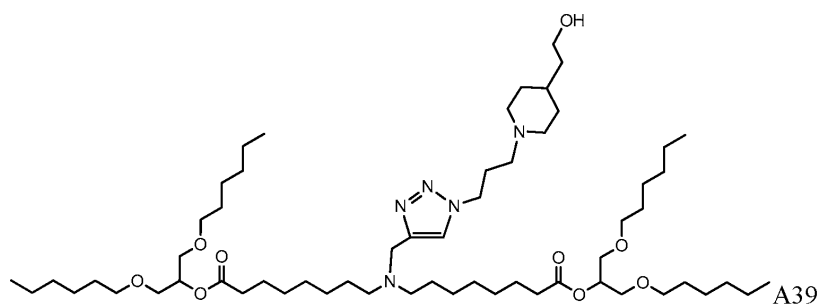
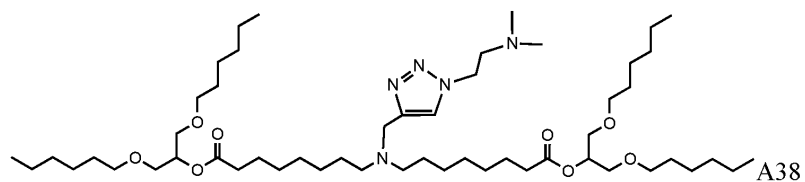
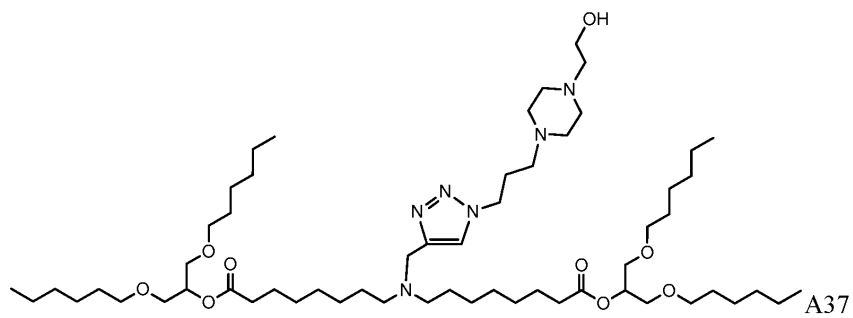
A34

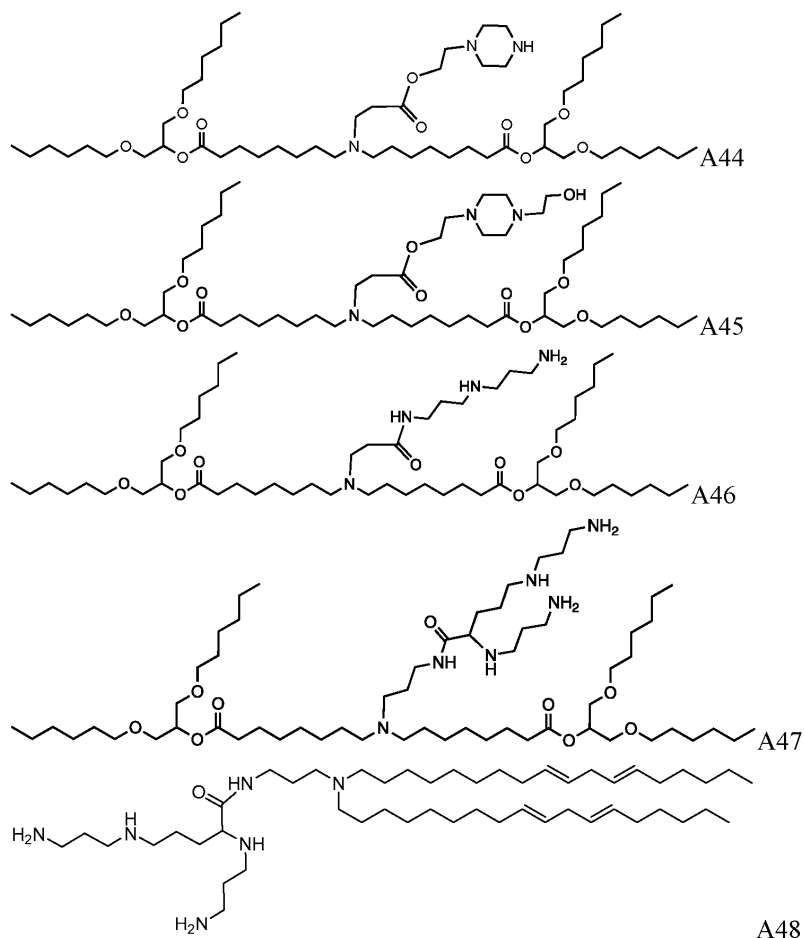


A35



A36





[0058] Compared with the prior art, such as PCT/US2016/052352 (published as WO2017/049245), and PCT/US2010/038224 (published as WO2010/144740), the ionizable lipids of the present disclosure differ from ionizable lipids disclosed in these documents in one or more of the following ways:

1. Different chemical structure: the 1 or 2 aliphatic chains that are connected to nitrogen (N) of tertiary amine contain an ester group formed with another saturated or unsaturated aliphatic chain having glycerol structure to become novel aliphatic chain with ether groups, the outcome shows that this transfection efficiency is higher than ionizable lipid having aliphatic chains lacking ether groups;
2. Different metabolites: the aliphatic chain of ionizable lipids of the disclosure consists of ester group, glycerol and short aliphatic chains. The metabolites of

such molecules are small molecule compounds, such as short fatty acids, fatty alcohols or ethers, which are capable of being metabolized and extracted more easily and hard to accumulate in vivo, as well as lower toxicity.

3. Novel alkynyl intermediate structure: the alkynyl intermediate is formed by propargylamine and brominated aliphatic chain, which can click react with many azido compounds to generate a series of novel ionizable aliphatic compounds.

4. Ionizable lipids of the disclosure are easy to synthesize and easy to obtain the raw materials: the original raw materials are glycerol, short fatty alcohols and fatty acids, which are relatively inexpensive and easy to synthesize.

DEFINITION

[0059] When the numeric range is listed, it includes each value and the subrange within the said range. For example, “C₁₋₆ alkyl” includes C₁, C₂, C₃, C₄, C₅, C₆, C₁₋₆, C₁₋₅, C₁₋₄, C₁₋₃, C₁₋₂, C₂₋₆, C₂₋₅, C₂₋₄, C₂₋₃, C₃₋₆, C₃₋₅, C₃₋₄, C₄₋₆, C₄₋₅ and C₅₋₆ alkyl.

[0060] The term “alkyl” refers to straight or branched saturated alkyl containing one or several carbon atoms (such as, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more carbon atoms). Specifically, “C₁₋₁₀ alkyl” refers to straight or branched saturated alkyl containing 1-10 carbon atoms. “C₂₋₁₈ alkyl” refers to randomly substituted straight or branched saturated alkyl containing 2-18 carbon atoms. Unless otherwise specified, the said alkyl in this specification refers to unsubstituted and substituted alkyl.

[0061] The term “alkenyl” refers to straight or branched alkyl containing two or more carbon atoms and at least one carbon-carbon double bond (such as, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more carbon atoms). The alkenyl can include one, two, three, four or more carbon-carbon double bond. Specifically, “C₃₋₁₂ alkenyl” refers to straight or branched saturated alkenyl containing 3-12 carbon atoms and at least one carbon-carbon double bond. Specifically, “C₄₋₁₈ alkenyl” refers to straight or branched saturated alkenyl containing 4-18 carbon atoms and at least one carbon-carbon double bond. Unless otherwise specified, the said alkenyl in this specification refers to unsubstituted and substituted alkenyl.

[0062] The term “halogen” refers to fluorine (F), chlorine (Cl), bromine (Br) and iodine (I).

- [0063]** The term “C₁₋₆ alkyl halide” refers to the above mentioned “C₁₋₆ alkyl” is substituted by one or more halogen groups. Exemplary said alkyl halide includes but not limited to -CF₃, -CH₂F, -CHF₂, -CHFCH₂F, -CH₂CHF₂, -CF₂CF₃, -CCl₃, -CH₂Cl, -CHCl₂, and 2,2,2-trifluoro-1,1-dimethyl-ethyl.
- [0064]** The term “C₃₋₇cycloalkyl” refers to non-aromatic cyclic hydrocarbon group containing 3-7 cyclocarbon atoms and 0 heteroatom. Exemplary cycloalkyl groups include but are not limited to: cyclopropyl (C3), cyclopropenyl (C3), cyclobutyl (C4), cyclobutenyl (C4), cyclopentyl (C5), cyclopentenyl (C5), cyclohexyl (C6), cyclohexenyl (C6), cyclohexadienyl (C6), cycloheptyl (C7), cycloheptenyl (C7), cycloheptadienyl (C7), cycloheptatrienyl (C7), etc. The cycloalkyl group can be optionally substituted by one or more substituents, for example, it is substituted by 1-5 substituents, 1-3 substituents or 1 substituent.
- [0065]** The term “4-10 membered heterocyclyl” refers to 4-10 membered non-aromatic ring system containing ring carbon atom and 1-3 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, sulfur, boron, phosphorus and silicon. Likewise, the term “4-7 membered heterocyclyl” and “5-10 membered heterocyclyl” are also have the same definition except that the total number of carbon atoms and heteroatoms varies for each grouping. In the heterocyclyl containing one or several nitrogen atoms, the point of attachment can be carbon or nitrogen atom as long as the valence allows. Exemplary 3 membered heterocyclyl groups containing one heteroatom include but are not limited to: aziridinyl, oxiranyl and thioenyl. Exemplary 4 membered heterocyclyl groups containing one heteroatom include but are not limited to: azetidiny, oxetanyl, and thietanyl. Exemplary 5 membered heterocyclyl groups containing one heteroatom include but are not limited to: tetrahydrofuranyl, dihydrofuryl, tetrahydrothienyl, dihydrothienyl, pyrrolidinyl, dihydropyrrolyl and pyrrolyl-2, 5-dione. Exemplary 5 membered heterocyclyl groups containing two heteroatoms include but are not limited to: dioxolanyl, oxasulfuranyl, disulfuranyl, and oxazolidin-2-ketone. Exemplary 5 membered heterocyclyl groups containing three heteroatoms include but are not limited to: triazoliny, oxadiazoliny and thiadiazoliny. Exemplary 6 membered heterocyclyl groups containing one heteroatom include but are not limited to: piperidinyl, tetrahydropyranyl, dihydropyridyl and thianyl. Exemplary 6 membered heterocyclyl groups containing two heteroatoms include but are not limited to: piperazinyl,

morpholinyl, dithianyl, dioxanyl. Exemplary 6 membered heterocyclyl groups containing three heteroatoms include but are not limited to: hexahydrotriazinyl. Exemplary 7 membered heterocyclyl groups containing one heteroatom include but are not limited to: azepanyl, oxepanyl and thiepanyl.

[0066] The term “C₆₋₁₀ aryl” refers to monocyclic or polycyclic (e.g., bicyclic) 4n+2 aromatic ring system (e.g., including 6 or 10 π electrons shared in a cyclic array) containing 6-10 ring carbon atoms and 0 heteroatom. In some embodiments, the aryl has six ring carbon atoms (“C₆ aryl”; e.g., phenyl). In some embodiments, the aryl has ten ring carbon atoms (“C₁₀ aryl”; e.g., naphthyl, e.g., 1-naphthyl and 2-naphthyl).

[0067] The term “5-10 membered heteroaryl” refers to 5-10 membered monocyclic or bicyclic 4n+2 aromatic ring system (e.g., including 6 or 10 π electrons shared in a cyclic array) containing ring carbon atom and 1-4 heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen and sulfur. In the heteroaryl containing one or more nitrogen atoms, the point of attachment could be carbon or nitrogen atom, as valence permits. The heteroaryl bicyclic system in one or two rings can include one or more heteroatoms. The heteroaryl also includes ring system fused by the above mentioned heteroaryl ring and one or more cycloalkyl or heterocyclyl wherein the point of attachment located on the said heteroaryl ring, and the number of carbon atoms still continue to represent the number of carbon atoms in the heteroaryl ring system. Exemplary 5 membered heteroaryl groups containing one heteroatom include but are not limited to: pyrrolyl, furanyl and thiophenyl. Exemplary 5 membered heteroaryl groups containing two heteroatoms include but are not limited to: imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl and isothiazolyl. Exemplary 5 membered heteroaryl groups containing three heteroatoms include but are not limited to: triazolyl, oxadiazolyl (e.g., 1,2,4-oxadiazolyl) and thiadiazolyl. Exemplary 5 membered heteroaryl groups containing four heteroatoms include but are not limited to: tetrazolyl. Exemplary 6 membered heteroaryl groups containing one heteroatom include but are not limited to: pyridinyl. Exemplary 6 membered heteroaryl groups containing two heteroatoms include but are not limited to: pyridazinyl, pyrimidinyl and pyrazinyl. Exemplary 6 membered heteroaryl groups containing three or four heteroatoms include but are not limited to: triazinyl and tetrazinyl. Exemplary 7 membered heteroaryl groups containing one heteroatom include but are not limited to: azepinyl, oxepinyl and thiepinyl. Exemplary 5, 6-bicyclic

heteroaryl groups include but are not limited to: indolyl, isoindolyl, indazolyl, benzotriazolyl, benzothienyl, isobenzothienyl, benzofuranyl, benzoisofuranyl, benzimidazolyl, benzoxazolyl, benzisoxazolyl, benzoxadiazolyl, benzothiazolyl, benzisothiazolyl, benzothiadiazolyl, indoliziny and purinyl. Exemplary 6, 6 bicyclic heteroaryl groups include but are not limited to: naphthyridinyl, pteridinyl, quinolinyl, isoquinolinyl, cinnolinyl, quinoxalinyl, phthalazinyl and quinazolinyl.

[0068] The term “isomer” refers to different compounds with the same molecular formula. The disclosure especially relates to stereoisomers, the term “stereoisomer” refers to isomers that are only different in the atom space arrangement.

[0069] In some situations, the invention's compounds can form into salts, which are also in the scope of the invention. The term “salt (one or more)” refers to acidic and/or basic salts formed by inorganic and/or organic acids and bases. The salts of compounds of the invention are preferably the pharmaceutically acceptable salts.

[0070] The term “pharmaceutically acceptable salts” refers to those carboxylate salts, amino acid addition salts of the compounds of the present invention which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of patients without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention.

[0071] Pharmaceutically acceptable base addition salts are formed with metals or amines, such as alkali and alkaline earth metal hydroxides, or of organic amines. Examples of metals used as cations are sodium, potassium, magnesium, calcium, and the like. Examples of suitable amines are N, N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, N-methylglucamine, and procaine.

[0072] The base addition salts of acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid in a conventional manner. The free acid forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid for purposes of the present invention.

[0073] Salts may be prepared from inorganic acids sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, nitrate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide such as hydrochloric, nitric, sulfuric, hydrobromic, hydriodic, phosphorus, and the like. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, nitrate, acetate, oxalate, valerate, oleate, palmitate, stearate, laurate, borate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate mesylate, glucoheptonate, lactobionate, laurylsulphonate and isethionate salts, and the like. Salts may also be prepared from organic acids, such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanolic acids, hydroxy alkanolic acids, alkanedioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, etc. and the like. Representative salts include acetate, propionate, caprylate, isobutyrate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, mandelate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, phthalate, benzenesulfonate, toluenesulfonate, phenylacetate, citrate, lactate, maleate, tartrate, methanesulfonate, and the like. Pharmaceutically acceptable salts may include cations based on the alkali and alkaline earth metals, such as sodium, lithium, potassium, calcium, magnesium and the like, as well as non-toxic ammonium, quaternary ammonium, and amine cations including, but not limited to, ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. Also contemplated are the salts of amino acids such as arginate, gluconate, galacturonate, and the like. (See, for example, Berge S. M. et al., "Pharmaceutical Salts," *J. Pharm. Sci.*, 1977; 66:1-19 which is incorporated herein by reference.)

[0074] Examples of pharmaceutically acceptable, non-toxic esters of the compounds of this invention include C₁-C₆ alkyl esters wherein the alkyl group is a straight or branched chain. Acceptable esters also include C₅-C₇ cycloalkyl esters as well as arylalkyl esters such as, but not limited to benzyl. C₁-C₄ alkyl esters are preferred. Esters of the compounds of the present invention may be prepared according to conventional methods "March's Advanced Organic Chemistry, 5th Edition". M. B. Smith & J. March, John Wiley & Sons, 2001.

[0075] Examples of pharmaceutically acceptable, non-toxic amides of the compounds of this invention include amides derived from ammonia, primary C₁-C₆ alkyl amines and

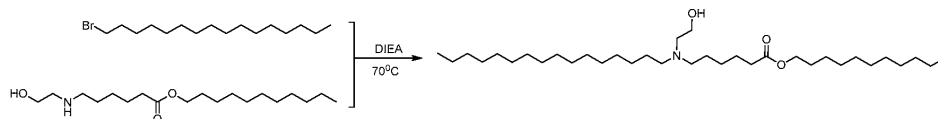
secondary C₁-C₆ dialkyl amines wherein the alkyl groups are straight or branched chain. In the case of secondary amines the amine may also be in the form of a 5- or 6-membered heterocycle containing one nitrogen atom. Amides derived from ammonia, C₁-C₃ alkyl primary amines and C₁-C₂ dialkyl secondary amines are preferred. Amides of the compounds of the invention may be prepared according to conventional methods such as "March's Advanced Organic Chemistry, 5th Edition". M. B. Smith & J. March, John Wiley & Sons, 2001.

[0076] The term “acceptable carrier” refers to suitable carrier using current materials for the purpose of the invention, without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio.

EXAMPLES

[0077] To make the purpose, technological protocol and benefit of the invention clearer, the present invention is illustrated in further details by the following examples, as well as drawings.

Example 1 - Synthesis of A1

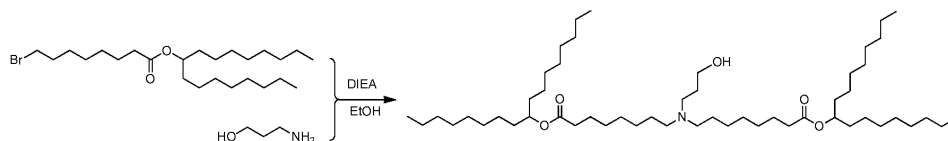


[0078] Hexadecane bromide (2.22 g, 7.28 mmol) was solved in 50mL absolute ethanol, N,N-diisopropylethylamine (DIEA, 1.17 g, 9.10 mmol) and alkanolamine compound (2 g, 6.07 mmol) were added, reacted at 80 °C for 18 h. After the reaction was completed, the solvent was removed by concentrating, the reaction was diluted with 200 mL ethyl acetate (EA), washed once with 200 mL water, extracted, the organic layer was dried over, concentrated, and passed through a silica gel column for purification (DCM:MeOH=3%--5%), 1.2 g oily product was obtained. MS(ES): m/z (M+H)⁺ 553.54. ¹HNMR (CDCl₃) δ :ppm : 4.06(t, 2H), 3.57(t, 2H), 2.62(bs, 2H), 2.50(br, 4H), 2.29(m, 2H), 1.68-1.25(m, 52H), 0.88(m, 6H).

[0079] The synthetic routes for the following examples and synthesis of A5-A7, A9-A13, A15-A32 were designed based on this example, most were as follows: a substitution

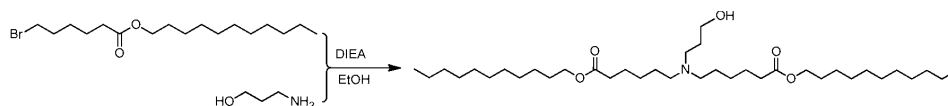
reaction occurs through a brominated compound or a ketene compound and a primary/secondary amine compound (e.g., alkanolamine compound).

Example 2 – Synthesis of A5



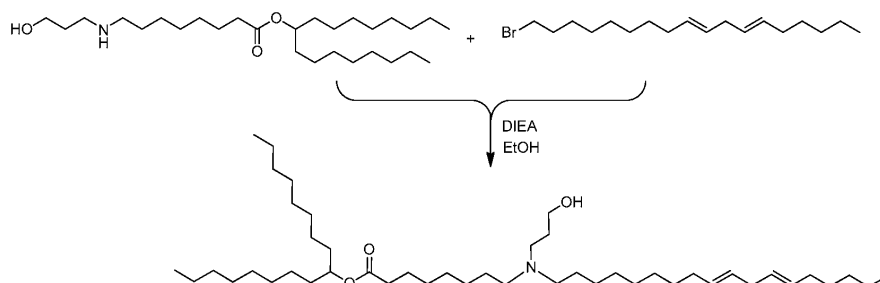
[0080] A5 was synthesized employing reaction steps similar to Example 1, 0.75 g oily product was obtained. MS(ES): m/z (M+H)⁺ 835.80. ¹HNMR (CDCl₃) δ :ppm : 4.87(m,2H), 3.79(t,2H), 2.67(br,2H), 2.45(br,4H), 2.27(t,4H), 1.70-1.25(m,78H), 0.90(m,12H).

Example 3 – Synthesis of A6

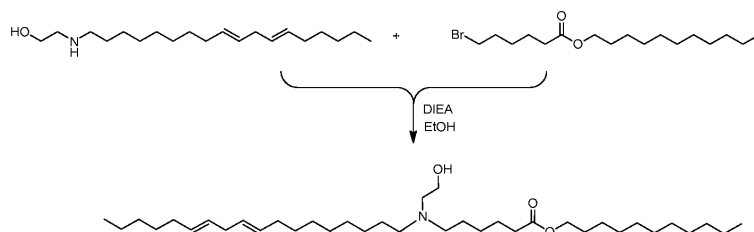


[0081] A6 was synthesized employing reaction steps similar to Example 1, 0.51 g oily product was obtained. MS(ES): m/z (M+H)⁺ 611.55. ¹HNMR (CDCl₃) δ :ppm : 4.05(t,4H), 3.78(m,2H), 2.65(t,2H), 2.43(br,4H), 2.29(m,4H), 1.69-1.31(m,50H), 0.90(m,6H).

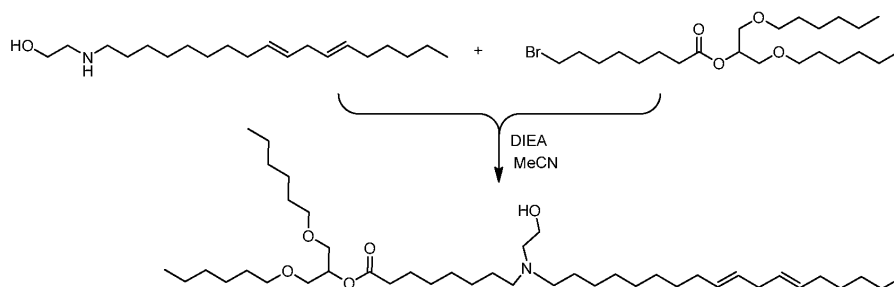
Example 4 - Synthesis of A7



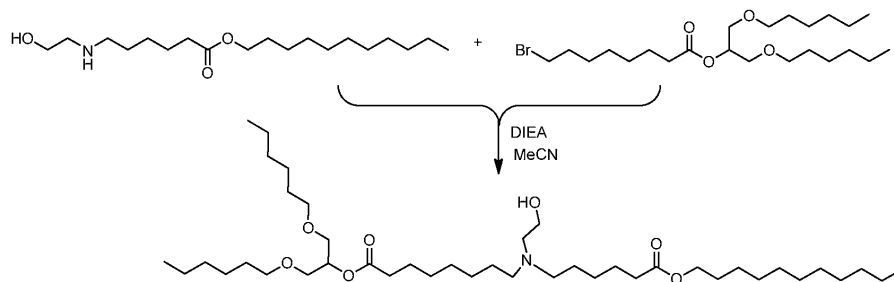
[0082] A7 was synthesized employing reaction steps similar to Example 1, 2.45 g oily product was obtained. MS(ES): m/z (M+H)⁺ 703.68. ¹HNMR (CDCl₃) δ :ppm : 5.38-5.31(m,4H), 4.86 (m,1H), 3.79(t,2H), 2.77(m,2H), 2.67(br,2H), 2.45(br,4H), 2.27(m,2H), 2.04(m,4H), 1.70-1.25(m,58H), 0.90(m,9H).

Example 5 - Synthesis of A9

[0083] A9 was synthesized employing reaction steps similar to Example 1, 1.6 g oily product was obtained. MS(ES): m/z (M+H)⁺ 577.54. ¹HNMR (CDCl₃) δ :ppm : 5.38-5.33(m,4H), 4.06(t,2H), 3.60(t,2H), 2.77(t,2H), 2.66(m,2H), 2.54 (bs,4H), 2.30(m,2H), 2.05(m,4H), 1.68-1.25(m,42H), 0.88(m,6H).

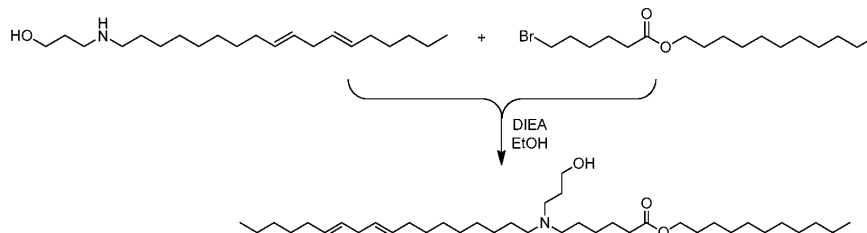
Example 6 - Synthesis of A10

[0084] A10 was synthesized employing reaction steps similar to Example 1, 0.4 g oily product was obtained. MS(ES): m/z (M+H)⁺ 693.63. ¹HNMR (CDCl₃) δ :ppm : 5.36(m,4H), 5.10(m,1H), 3.56(t,4H), 3.46-3.40(br,4H), 2.76(t,2H), 2.64(br,4H), 2.51(bs,4H), 2.32(m,2H), 2.05(m,6H), 1.67-1.25(m,44H), 0.88(m,9H).

Example 7 - Synthesis of A11

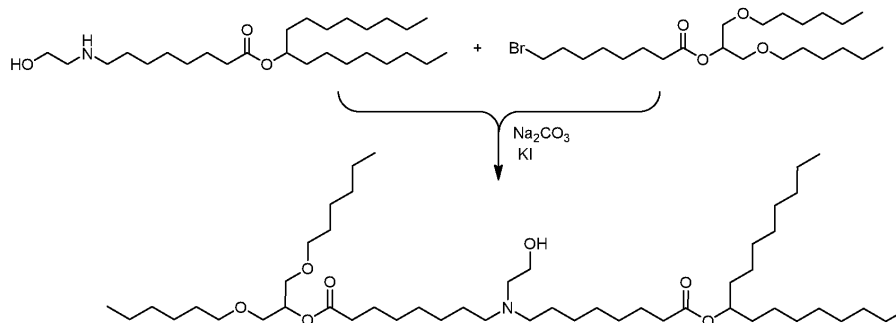
[0085] A11 was synthesized employing reaction steps similar to Example 1, 0.5 g oily product was obtained. MS(ES): m/z (M+H)⁺ 713.62. ¹HNMR (CDCl₃) δ :ppm : 5.10(m,1H), 4.05(d,4H), 4.03(t,4H), 3.54(m,2H), 3.43(s,2H), 3.16(t,2H), 3.10(br,4H), 2.32(t,4H), 1.66-1.27(m,50H), 0.88(m,9H).

Example 8 - Synthesis of A12

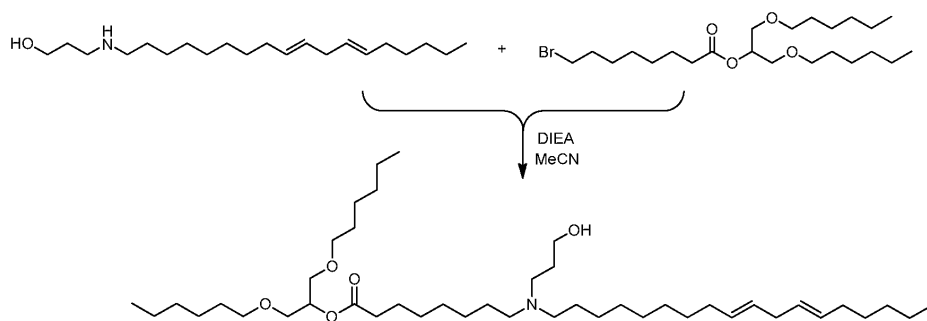


[0086] A12 was synthesized employing reaction steps similar to Example 1, 2.68 g oily product was obtained. MS(ES): m/z (M+H)⁺ 591.56, ¹HNMR (CDCl₃) δ :ppm : 5.37-5.35(m,4H), 4.05(t,2H), 3.78(t,2H), 2.77(t,2H), 2.64(m,2H), 2.41(bs,4H), 2.31(m,2H), 2.03(m,4H), 1.68-1.25(m,44H), 0.88(m,6H).

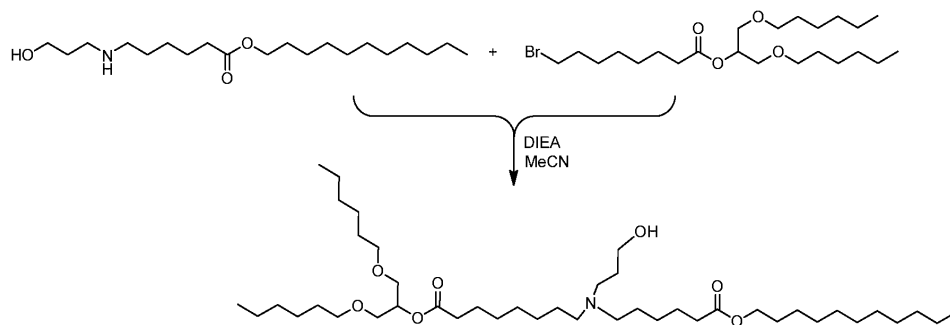
Example 9 - Synthesis of A13



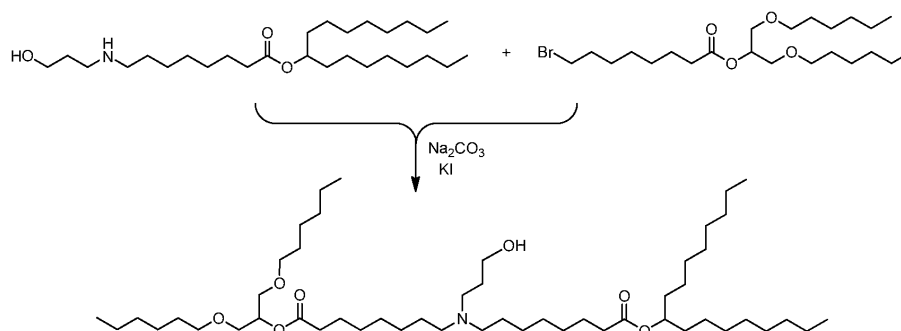
[0087] A13 was synthesized employing reaction steps similar to Example 1, 1.2 g oily product was obtained. MS(ES): m/z (M+H)⁺ 825.74 ; ¹HNMR (CDCl₃) δ :ppm : 5.12(m,1H), 4.86(m,1H), 3.65-3.40(m,10H), 2.72(br,2H), 2.60(br,4H), 2.34-2.26(m,4H), 1.62-1.25(m,64H), 0.88(m,12H).

Example 10 - Synthesis of A15

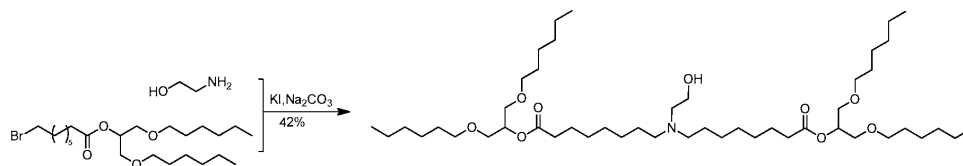
[0088] A15 was synthesized employing reaction steps similar to Example 1, 0.4 g oily product was obtained. MS(ES): m/z ($M+H$)⁺ 707.64. ¹HNMR (CDCl₃) δ :ppm : 5.34(m,4H), 5.10(m,1H), 3.79(t,2H), 3.56-3.41(m,8H), 2.80(t,2H), 2.77(t,2H), 2.46(br,4H), 2.32(m,2H), 2.05(m,4H), 1.67-1.25(m,46H), 0.88(m,9H).

Example 11 - Synthesis of A16

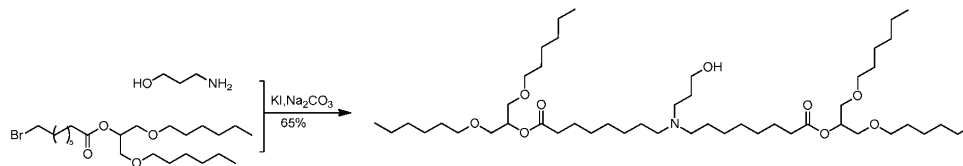
[0089] A16 was synthesized employing reaction steps similar to Example 1, 0.59 g oily product was obtained. MS(ES): m/z ($M+H$)⁺ 727.63. ¹HNMR (CDCl₃) δ :ppm : 5.10(m,1H), 4.05(dd,2H), 3.77(t,2H), 3.54(dd,4H), 3.46-3.38(m,4H), 3.19(t,2H), 3.01(br,4H), 2.32(t,4H), 1.66-1.27(m,52H), 0.88(m,9H).



Example 13 - Synthesis of A18



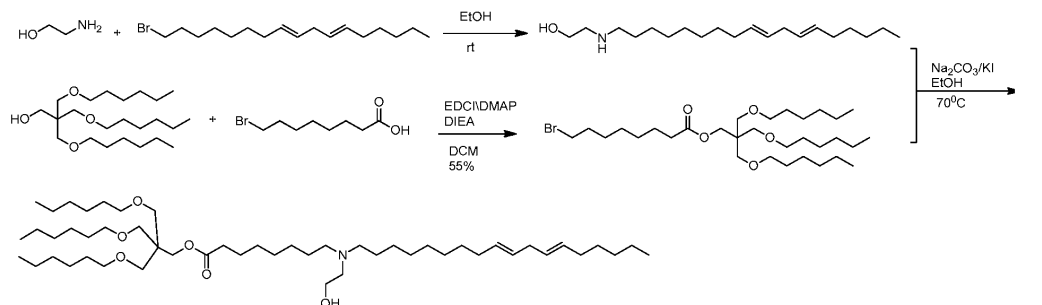
Example 14 - Synthesis of A19



[0092] A19 was synthesized employing reaction steps similar to Example 1, 2.3 g oily product was obtained. ¹HNMR (CDCl₃) δ: ppm.5.06(t, 2H), 3.72(t, 2H), 3.50-

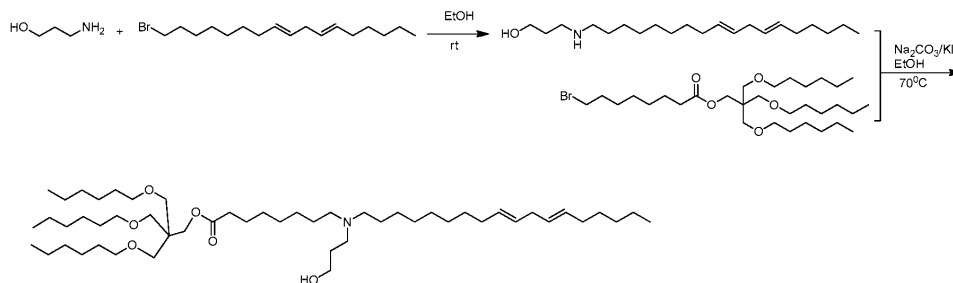
3.33(m, 16H), 2.27(t, 2H), 2.25-2.24(m, 8H), 1.56-
1.19(m, 52H), 0.88(m, 12H), MS(ES): m/z (M+H)⁺ 843.72.

Example 15 - Synthesis of A20

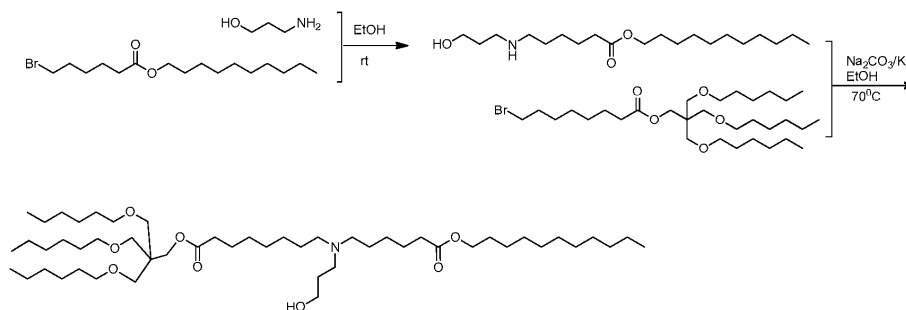


[0093] A20 was synthesized employing reaction steps similar to Example 1, 0.95 g oily product was obtained. MS (ES): m/z (MH⁺) 821.75; ¹H-NMR (400 MHz, CDCl₃) δ : ppm 5.29 (m, 4H), 4.03 (s, 2H), 3.51(t, 2H), 3.30-3.27(m, 12H), 2.70 (m, 2H), 2.57(t, 2H), 2.46 (br, 4H), 2.21(m, 2H), 1.30 -1.19(br. m, 52H), 0.89 (m, 12H).

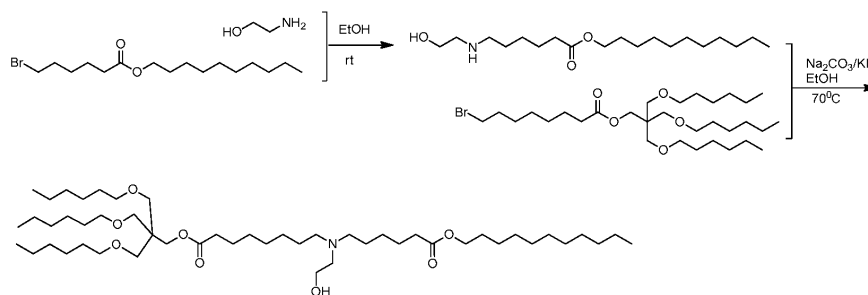
Example 16 - Synthesis of A21



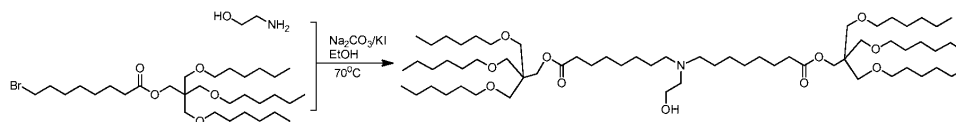
[0094] A21 was synthesized employing reaction steps similar to Example 1, 0.68 g oily product was obtained. MS (ES): m/z (MH⁺) 835.76; ¹H-NMR (400 MHz, CDCl₃) δ : ppm 5.35 (m, 4H), 4.10 (s, 2H), 3.79 (t, 2H), 3.30 (m, 12H), 2.76 (br, m, 4H), 2.50(br, 4H), 2.28 (m, 2H), 2.05(m, 4H), 1.61(br, 4H), 1.57(m, 4H), 1.54 - 1.24(br. m, 54H), 0.88(m, 12H).

Example 17 - Synthesis of A22

[0095] A22 was synthesized employing reaction steps similar to Example 1, 0.43 g oily product was obtained. ^1H NMR (400 MHz, CDCl_3) δ : ppm. 4.03(s, 2H), 3.98(t, 2H), 3.72(t, 2H), 3.28(m, 12H), 2.65(t, 2H), 2.45(t, 4H), 2.25-2.20(m, 4H), 1.66-1.19(m, 60H), 0.83(m, 12H), MS(ES): m/z ($M+H$) $^+$ 855.75.

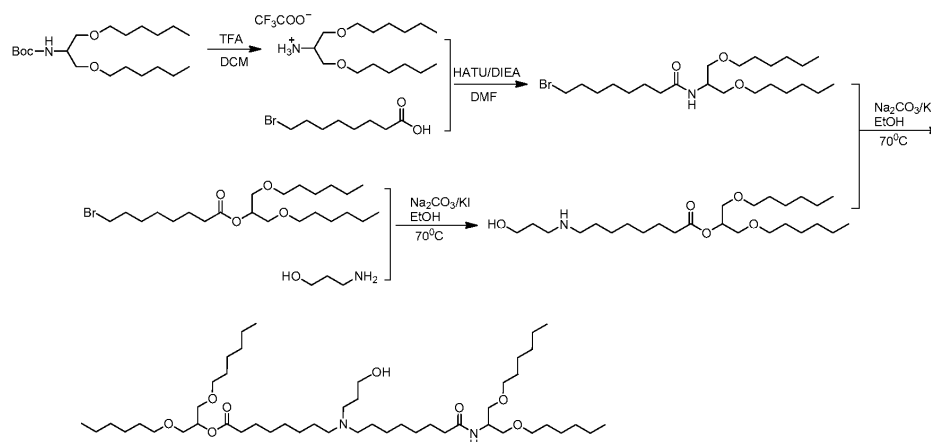
Example 18 - Synthesis of A23

[0096] A23 was synthesized employing reaction steps similar to Example 1, 1.8 g oily product was obtained. ^1H NMR (400 MHz, CDCl_3) δ : ppm. 4.10(s, 2H), 4.06(t, 2H), 3.56(t, 2H), 3.36-3.34(m, 12H), 2.61(t, 2H), 2.49(t, 4H), 2.30(m, 4H), 1.67-1.26(m, 58H), 0.88(m, 12H), MS(ES): m/z ($M+H$) $^+$ 841.74.

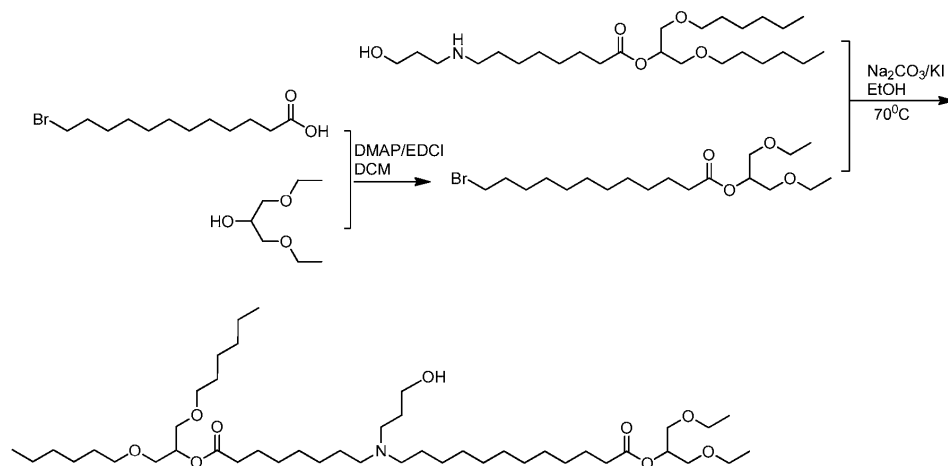
Example 19 - Synthesis of A24

[0097] A24 was synthesized employing reaction steps similar to Example 1, 0.72 g oily product was obtained. ^1H NMR (400 MHz, CDCl_3) δ : ppm. 4.10(s, 4H), 3.67(br, 2H), 3.35(m, 24H), 2.80-2.50(br, 6H), 2.28(m, 4H), 1.67-1.23(m, 68H), 0.89(m, 18H), MS(ES): m/z ($\text{M}+\text{H}$) $^+$ 1085.94.

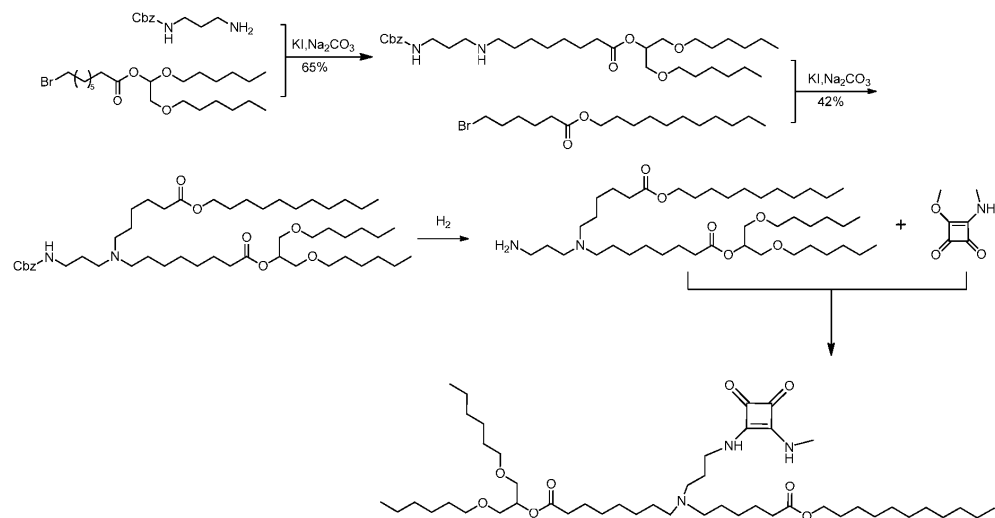
Example 20 - Synthesis of A25



[0098] A25 was synthesized employing reaction steps similar to Example 1, 0.3 g oily product was obtained. ^1H NMR (400 MHz, CDCl_3) δ : ppm. 5.23(m, 1H), 5.05(t, 1H), 4.11(br, 4H), 3.78(t, 2H), 3.49-3.34(br, m, 16H), 3.15(t, 2H), 3.01(t, 4H), 2.26(m, 2H), 2.10(m, 2H), 2.01(m, 2H), 1.79-1.18(br, m, 52H), 0.83(br, m, 12H), MS(ES): m/z ($\text{M}+\text{H}$) $^+$ 842.73.

Example 21 - Synthesis of A26

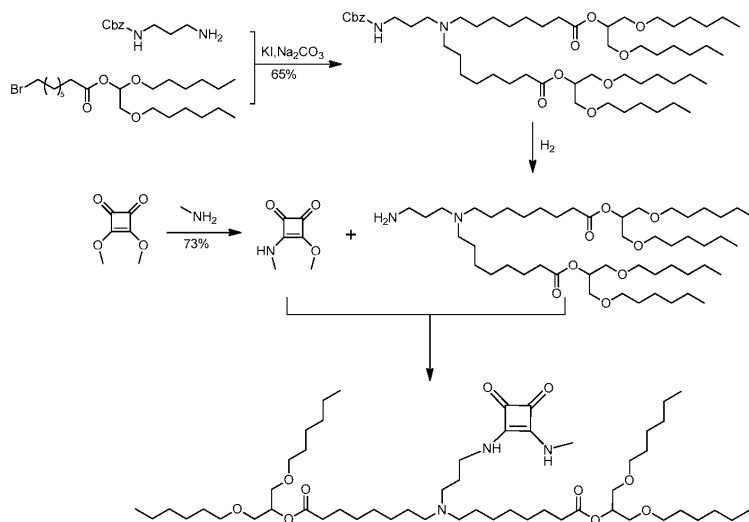
[0099] A26 was synthesized employing reaction steps similar to Example 1, 0.46 g oily product was obtained. ^1H NMR (400 MHz, CDCl_3) δ : ppm. 5.03(m, 2H), 3.75(t, 2H), 3.48-3.34(br, m, 16H), 2.91(br, 2H), 2.72(br, 4H), 2.27(t, 4H), 1.85(m, 2H), 1.58-1.10(br, m, 48H), 0.81(br, m, 6H), MS(ES): m/z ($\text{M}+\text{H}$) $^+$ 787.65.

Example 22 - Synthesis of A27

[0100] Cbz-1, 3- propylenediamineoctanoate was synthesized employing reaction steps similar to Example 1. Cbz-1, 3- propylenediamineoctanoate (3.5g, 5.9mmol), sodium

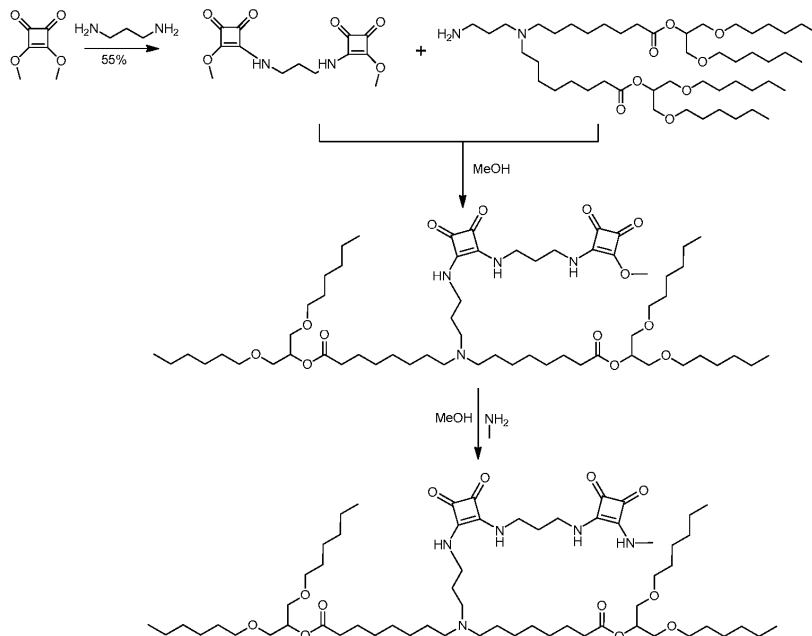
carbonate anhydrous (0.94g, 8.8mmol), KI (0.19g, 1.18mmol) were soluted in 30 mL absolute ethanol and 30 mL absolute acetonitrile, then bromide was added and reacted together at 75 °C for 24 h. After the reaction was completed, the solvent was removed by concentrating, the reaction was diluted with 200 mL dichloromethane, washed with 200 mL water, extracted, the organic layer was dried over, concentrated, and passed through a silica gel column for purification (DCM:MeOH=3%--10%), oily Cbz-amine product was obtained. Cbz-amine (2.1g, 2.43mmol) was soluted in 20 mL absolute methanol and 20 mL ethyl acetate, then palladium (0.35g, 10%) was added, after hydrogen was replaced for three times, hydrogenation occurred at room temperature for 20 h. After the reaction was completed, the palladium was removed by filtration, the solvent was concentrated and removed, extracted and the amine product was obtained. The obtained amine product (1.1g, 1.51mmol) was soluted in 20 mL absolute ethanol, Ketone-methylamine (0.22g, 1.51mmol) was added, solution was stirred and reacted at room temperature for 20 h. After the reaction was completed, the solvent was concentrated and removed, the filtrate was dried over, concentrated and passed through a silica gel column for purification (DCM:MeOH=3%--10%), A27 was obtained (0.6 g oily product). ¹HNMR (d-DMSO) δ : ppm. 4.99(p, 1H), 3.98(t, 2H), 3.50-3.31(m, 8H), 3.10(d, 3H), 2.49(dt, 8H), 2.26(m, 4H), 1.52(dd, 6H), 1.43(m, 6H), 1.26(m, 40H), 0.84(m, 9H), MS(E S):m/z (M+H)⁺ 835.66.

Example 23 - Synthesis of A28

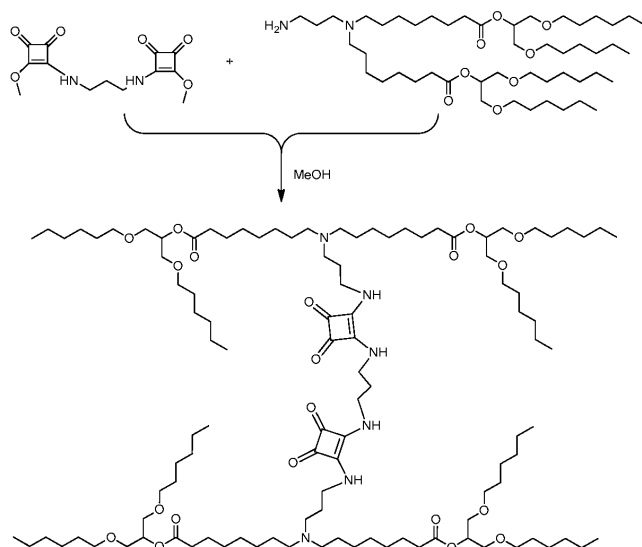


[0101] A28 was synthesized employing reaction steps similar to Example 22, 1.3 g oily product was obtained. ^1H NMR (CDCl_3) δ : ppm. 5.04(t, 2H), 3.61(t, 2H), 3.50-3.31(m, 16H), 3.21(s, 3H), 2.71(t, 2H), 2.55(t, 4H), 2.28-2.24(m, 4H), 1.86-1.19(m, 54H), 0.82(m, 12H), MS(ES): m/z ($M+H$) $^+$ 951.75.

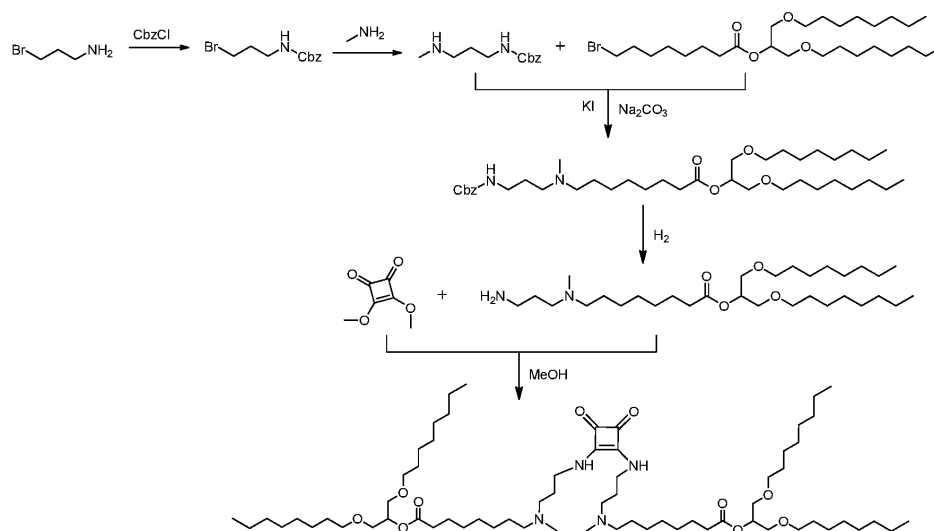
Example 24 - Synthesis of A29



[0102] A29 was synthesized employing reaction steps similar to Example 22, 0.11 g oily product was obtained. ^1H NMR ($d\text{-DMSO}$) δ : ppm. 5.00(m, 2H), 3.60-3.30(m, 16H), 3.11(s, 3H), 2.63-2.49(m, 10H), 2.36(m, 2H), 2.26(m, 4H), 1.80 (m, 2H), 1.46-1.26(m, 54H), 0.85(t, 12H), MS(ES): m/z ($M+H$) $^+$ 1103.81.

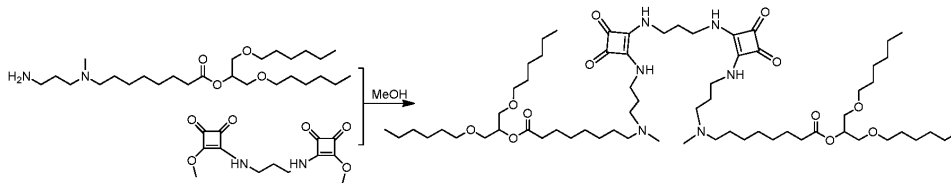


Example 26 - Synthesis of A31



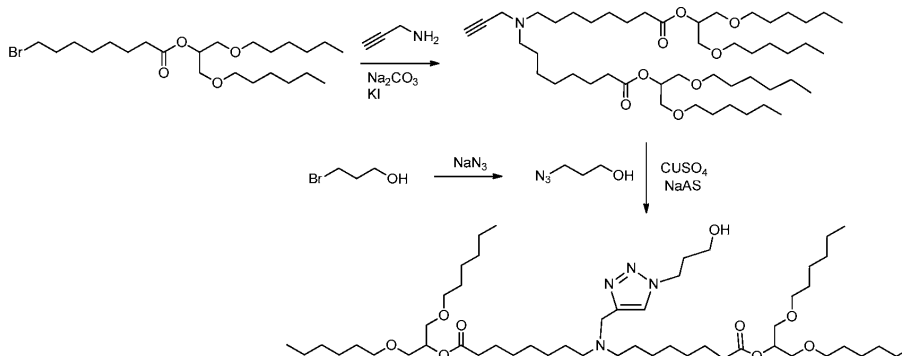
[0104] A31 was synthesized employing reaction steps similar to Example 22, 0.7 g oily product was obtained. ^1H NMR (d-DMSO) δ : ppm. 5.05(m, 2H), 3.60-3.30(m, 20H), 2.63-2.40(m, 12H), 2.2(m, 6H), 1.80-1.20(m, 64H), 0.85(m, 12H), MS(ES): m/z (M+H) $^+$ 1134.95.

Example 27 - Synthesis of A32



[0105] A32 was synthesized employing reaction steps similar to Example 22, 1.5 g oily product was obtained. ^1H NMR (d-DMSO) δ : ppm. 5.1(m, 2H), 3.60-3.30(m, 24H), 2.5(m, 4H), 2.4(m, 4H), 2.3(m, 4H), 2.2(m, 6H), 1.95(m, 2H), 1.8(m, 2H), 1.5-1.6(m, 8H), 1.2-1.4(m, 48H), 0.9(m, 8H), MS(ES): m/z (M+H) $^+$ 1174.88.

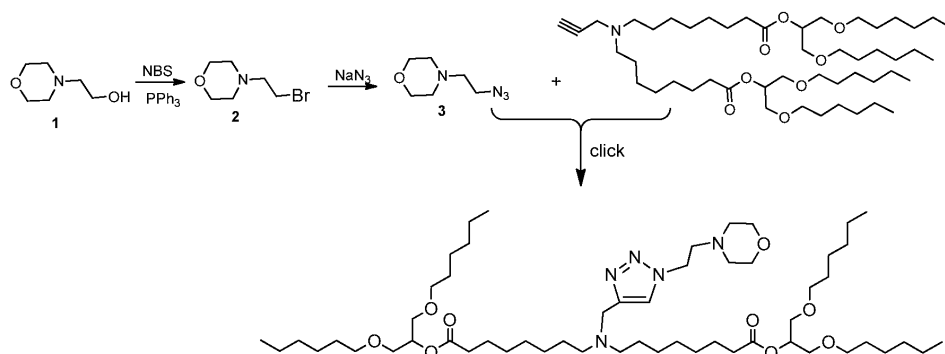
Example 28 - Synthesis of A34



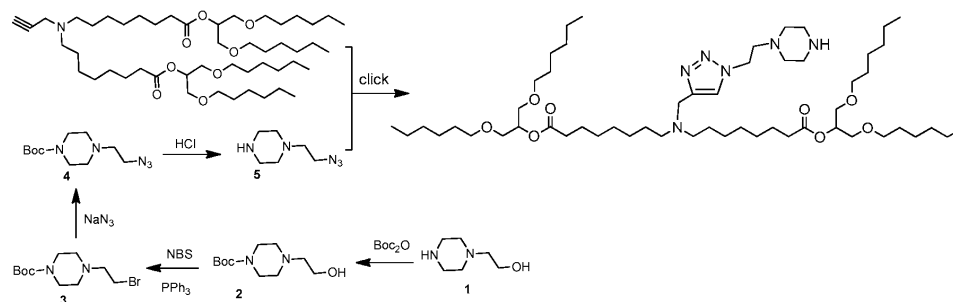
[0106] Alkynyl lipid intermediate was synthesized employing reaction steps similar to Example 1. Bromoalkyl ester (11 g), sodium carbonate (2.5 g), KI (0.4 g) were dissolved in 50 mL acetonitrile, alkynamine (0.65 g) was added. After the reaction was completed, it was concentrated to remove acetonitrile, stirred and extracted with 150 mL of ethyl acetate and water, the organic layer was dried over, concentrated, and passed through a silica gel column for purification (PE:EA=10:1-5:1), for obtaining the alkynyl lipid intermediate.

[0107] Steps to prepare A34: 3-azidopropanol-1 compound (0.5 g), anhydrous copper sulfate (0.15 g), sodium ascorbate (0.24 g) and alkynyl compound (0.08 g) were dissolved in 10 mL THF and 10 mL water, after the reaction occurred at room temperature, it was concentrated to remove THF, diluted with 100 mL dichloromethane, filtered to remove unsolved material, filtrate was stirred and extracted with water, the organic layer was dried over, concentrated, and passed through a silica gel column for purification (DCM: MeOH=1%-2%), 0.39 g A34 was obtained. ^1H NMR (CDCl_3) δ : ppm. 7.56(s, 1H), 5.12(p, 2H), 4.50(m, 2H), 3.77(s, 2H), 3.62-3.43(m, 18H), 2.44(s, 4H), 2.32(t, 4H), 2.13(tt, 2H), 1.70-1.50 (m, 16H), 1.26(m, 36H), 0.87(m, 12H); MS(ES): m/z ($\text{M}+\text{H}$) $^+$ 925.37.

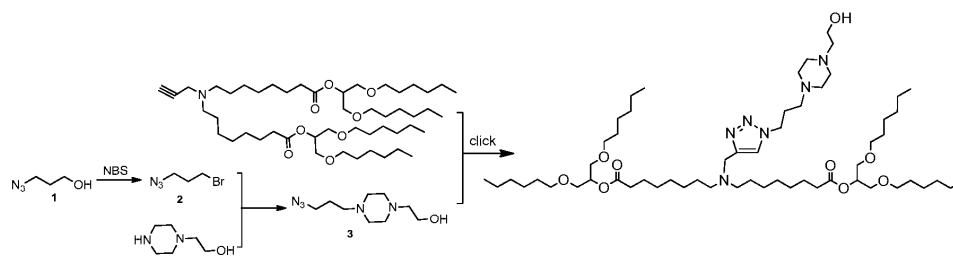
Example 29 - Synthesis of A35



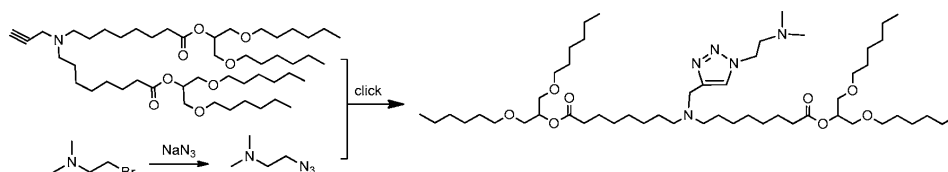
[0108] A35 was synthesized employing reaction steps similar to Example 28, 2.2 g product was obtained. ^1H NMR (CDCl_3) δ : ppm. 7.50(d, 1H), 5.11(p, 2H), 4.45(t, 2H), 3.77(s, 2H), 3.68-3.43(m, 20H), 2.82(t, 2H), 2.49(m, 4H), 2.41(s, 4H), 2.32(t, 4H), 1.70-1.50 (m, 20H), 1.26(m, 32H), 0.88(m, 12H); MS(ES): m/z ($\text{M}+\text{H}$) $^+$ 980.45.

Example 30 - Synthesis of A36

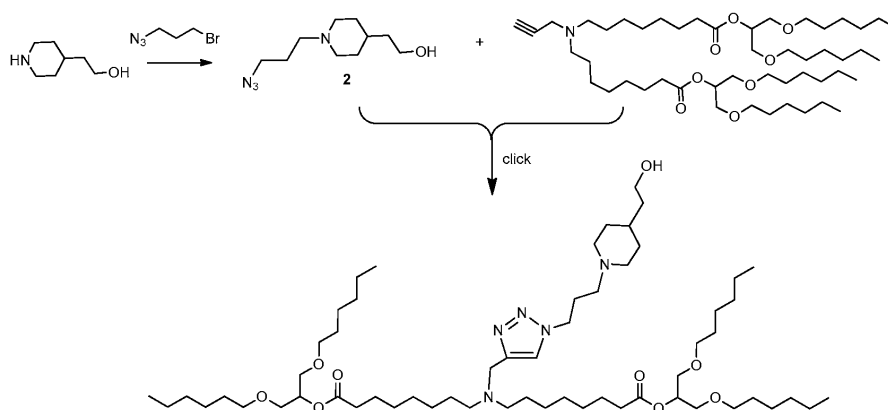
[0109] A36 was synthesized employing reaction steps similar to Example 28, 2.4 g product was obtained. ^1H NMR (500 MHz, DMSO) δ : ppm 7.86 (s, 1H), 5.00 (p, $J = 5.2$ Hz, 2H), 4.41 (t, $J = 6.3$ Hz, 2H), 3.62 (s, 2H), 3.55 – 3.41 (m, 10H), 3.42 – 3.35 (m, 8H), 2.68 (d, $J = 5.9$ Hz, 4H), 2.36 (s, 4H), 2.32 – 2.27 (m, 4H), 2.25 (t, $J = 7.3$ Hz, 4H), 1.55 – 1.48 (m, 4H), 1.46 – 1.43 (m, 6H), 1.41 – 1.36 (m, 4H), 1.25 (dd, $J = 16.2, 4.5$ Hz, 38H), 1.10 (s, 1H), 0.84 (t, $J = 6.9$ Hz, 12H). MS(ES): m/z ($M+H$) $^+$ 979.47.

Example 31 - Synthesis of A37

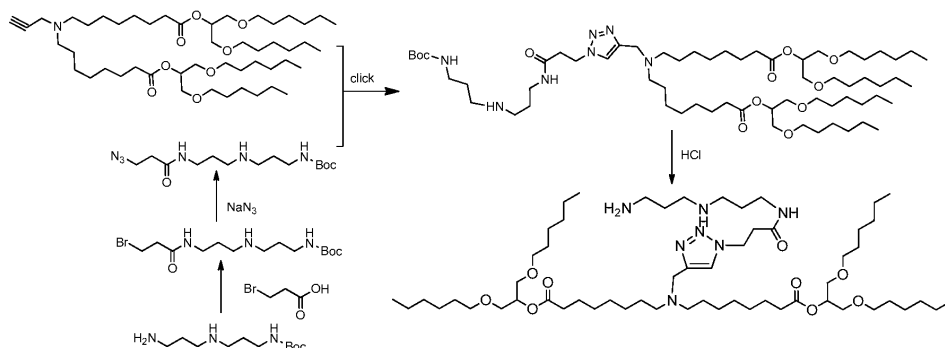
[0110] A37 was synthesized employing reaction steps similar to Example 28, 1.7 g product was obtained. ^1H NMR (CDCl₃) δ : ppm. 7.59(d, 1H), 5.10(p, 2H), 4.46(t, 2H), 3.75(s, 2H), 3.75-3.43(m, 20H), 2.94-2.87(t, 4H), 2.69-2.43(m, 4H), 2.41(s, 4H), 2.33(t, 4H), 1.70-1.50 (m, 22H), 1.26(m, 32H), 0.88(m, 12H); MS(ES): m/z ($M+H$) $^+$ 1037.54.

Example 32 - Synthesis of A38

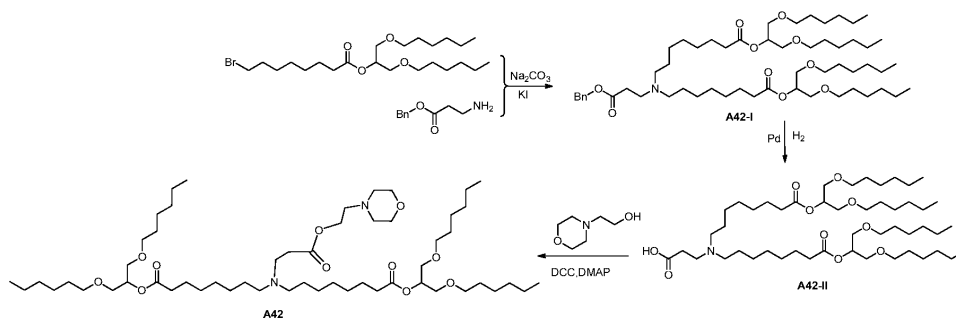
[0111] A38 was synthesized employing reaction steps similar to Example 28, 2.5 g product was obtained. ¹HNMR (CDCl₃) δ: ppm. 7.53(d, 1H), 5.11(p, 2H), 4.42(t, 2H), 3.76(s, 2H), 3.68-3.41(m, 20H), 2.83(t, 2H), 2.49(s, 6H), 2.32(t, 4H), 1.70-1.50 (m, 20H), 1.26(m, 32H), 0.87(m, 12H); MS(ES): m/z (M+H)⁺ 953.45.

Example 33 - Synthesis of A39

[0112] A39 was synthesized employing reaction steps similar to Example 28, 1.7 g product was obtained. ¹HNMR (CDCl₃) δ: ppm. 7.59(d, 1H), 5.11(p, 2H), 4.47(t, 2H), 3.73(s, 2H), 3.70-3.41(m, 24H), 2.78(t, 2H), 2.51(4, 4H), 2.42(m, 2H), 2.32(t, 4H), 1.59-1.25 (m, 59H), 0.87(m, 12H); MS(ES): m/z (M+H)⁺ 1036.56.

Example 34 - Synthesis of A40

[0113] A40 was synthesized employing reaction steps similar to Example 28, 1.4 g product was obtained. ^1H NMR (CDCl_3) δ : ppm. 7.53(d, 1H), 5.11(p, 2H), 4.47(t, 2H), 3.72(s, 2H), 3.70-3.40(m, 22H), 2.66(t, 2H), 2.65-2.55(m, 6H), 2.32(t, 4H), 1.59-1.25 (m, 44H), 0.87(m, 12H); MS(ES): m/z ($\text{M}+\text{H}$) $^+$ 1052.54.

Example 35 - Synthesis of A42

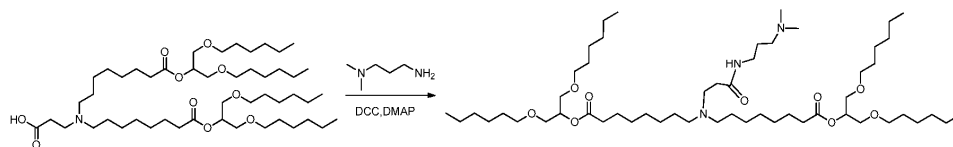
[0114] Alkynyl lipid intermediate was synthesized employing reaction steps similar to Example 1. Bromo-ether ester (10.6 g), sodium carbonate (2.42 g), KI (0.4 g) were dissolved in 50 mL acetonitrile, benzyl-alanine (2.04 g) was added, after reflux reaction was completed, it was concentrated to remove acetonitrile, stirred and extracted with ethyl acetate and water, the organic layer was dried over, concentrated, and passed through a silica gel column for purification (DCM:MeOH=2%-3%), 7.5 g A42-I was obtained.

[0115] A42-I intermediate (2 g), palladium on carbon (0.5 g) were dissolved in 50 mL methanol, after the hydrogenation room temperature reaction was completed, it was

filtered to remove the palladium on carbon, the filtrate was dried over, concentrated to obtain 1.7 g A42-II.

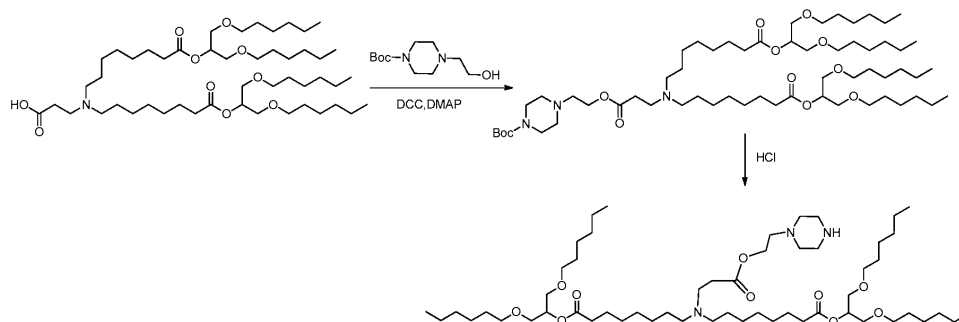
[0116] Steps to prepare A42: A42-II intermediate (1.5 g), DCC (0.54 g), DMAP (0.21 g) were dissolved in 50 mL dichloromethane, morpholine ethanol (0.23 g) was added, after room temperature reaction was completed, it was stirred and extracted with dichloromethane and water, the organic layer was dried over, concentrated, and passed through a silica gel column for purification (DCM:MeOH=1%-3%), 1.1 g A42 was obtained. $^1\text{H NMR}$ (CDCl_3) δ : ppm. 5.12(p, 2H), 4.41(t, 2H), 3.76(t, 2H), 3.55-3.40(m, 20H), 3.01-2.76(m, 10H), 2.49(t, 6H), 2.32(t, 4H), 1.59-1.25 (m, 52H), 0.87(m, 12H); MS(ES): m/z ($\text{M}+\text{H}$) $^+$ 971.44.

Example 36 - Synthesis of A43



[0117] A43 was synthesized employing reaction steps similar to Example 35, 0.5 g product was obtained. $^1\text{H NMR}$ (CDCl_3) δ : ppm. 5.12(p, 2H), 4.41(t, 2H), 3.76(t, 2H), 3.55-3.40(m, 20H), 3.01-2.76(m, 10H), 2.49(t, 2H), 2.32(t, 4H), 1.59-1.25 (m, 54H), 0.87(m, 12H); MS(ES): m/z ($\text{M}+\text{H}$) $^+$ 942.44.

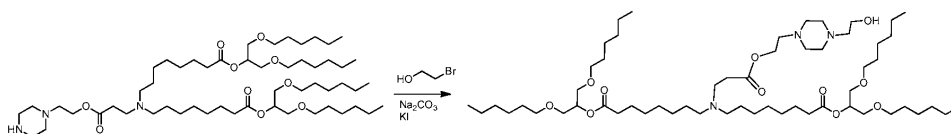
Example 37 - Synthesis of A44



[0118] Boc protected piperazine ethanol ester intermediate was synthesized referred to Example 35.

- [0119] Steps to prepare A44: Boc protected piperazine ethanol ester intermediate (1.1 g) was dissolved in 200 mL 2mol/L hydrogen ethanol, after the room temperature reaction was completed, it was dried over, concentrated to obtain 0.8 g A44. ^1H NMR (CDCl_3) δ : ppm. 5.10(p, 2H), 4.35(t, 2H), 3.60-3.40(m, 18H), 3.01-2.85(m, 6H), 2.65-2.50(t, 10H), 2.32(t, 4H), 1.59-1.25 (m, 52H), 0.87(m, 12H); MS(ES): m/z ($\text{M}+\text{H}$) $^+$ 970.45.

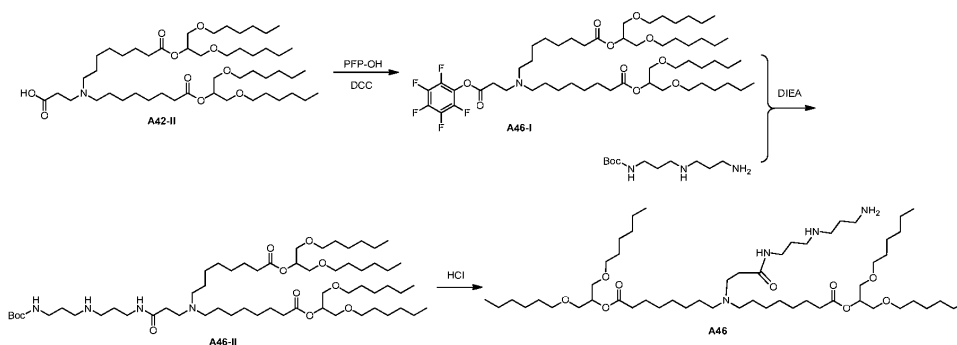
Example 38 - Synthesis of A45



- [0120] A44 was synthesized employing reaction steps similar to Example 37.

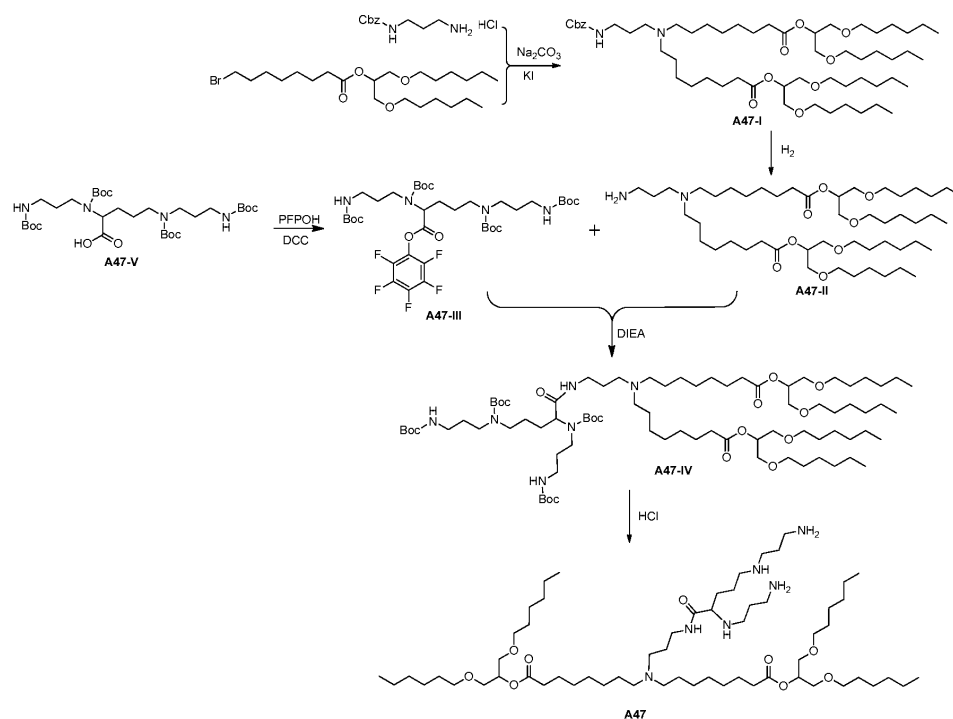
- [0121] Steps to prepare A45: A44 (1.6 g), sodium carbonate (0.17 g), KI (0.054 g) were dissolved in 50 mL acetonitrile, bromoethanol (0.2 g) was added, after reflux reaction was completed, it was concentrated to remove acetonitrile, stirred and extracted with ethyl acetate and water, the organic layer was dried over, concentrated, and passed through a silica gel column for purification (DCM:MeOH=3%-5%), 1.1 g A45 was obtained. ^1H NMR (CDCl_3) δ : ppm. 5.10(p, 2H), 4.35(t, 2H), 3.60-3.40(m, 18H), 3.32(t, 2H), 3.01-2.90(m, 6H), 2.53(t, 2H), 2.49-2.35(t, 10H), 2.32(t, 4H), 1.59-1.25 (m, 52H), 0.87(m, 12H); MS(ES): m/z ($\text{M}+\text{H}$) $^+$ 1014.50.

Example 39 - Synthesis of A46



- [0122] A42-II was synthesized employing reaction steps similar to Example 35.

- [0123]** Steps to prepare A46-I: A42-II (1.5 g), DCC (0.39 g), PFP-OH (0.35 g) were dissolved in 50 mL dichloromethane, after the room temperature reaction was completed, it was concentrated to remove DCM, diluted with ethyl acetate, filtered to remove white undissolved material, stirred and extracted with 10% sodium carbonate solution, the organic layer was dried over, concentrated, and passed through a silica gel column for purification (DCM:MeOH=1%-3%), 1.2g A46-I was obtained.
- [0124]** Steps to prepare A46-II: mono Boc-diaminodipropylamine (0.23 g), DIEA (0.19 g), A46-I (1 g) were dissolved in 20 mL dichloromethane, after room temperature reaction was completed, dichloromethane and sodium carbonate solution were added to stir and extract, the organic layer was dried over, concentrated, and passed through a silica gel column for purification (DCM:MeOH=1%-3%), 0.8g A46-II was obtained.
- [0125]** Steps to prepare A46: A46 was synthesized referred to steps to prepare A44 in Example 37. ¹HNMR (CDCl₃) δ: ppm. 5.10(p, 2H), 3.60-3.40(m, 18H), 3.37(t, 2H), 2.70-2.55(m, 10H), 2.50(t, 2H), 2.32(t, 4H), 1.72 (m, 4H), 1.59-1.25 (m, 52H), 0.87(m, 12H); MS(ES): m/z (M+H)⁺ 971.48.

Example 40 - Synthesis of A47

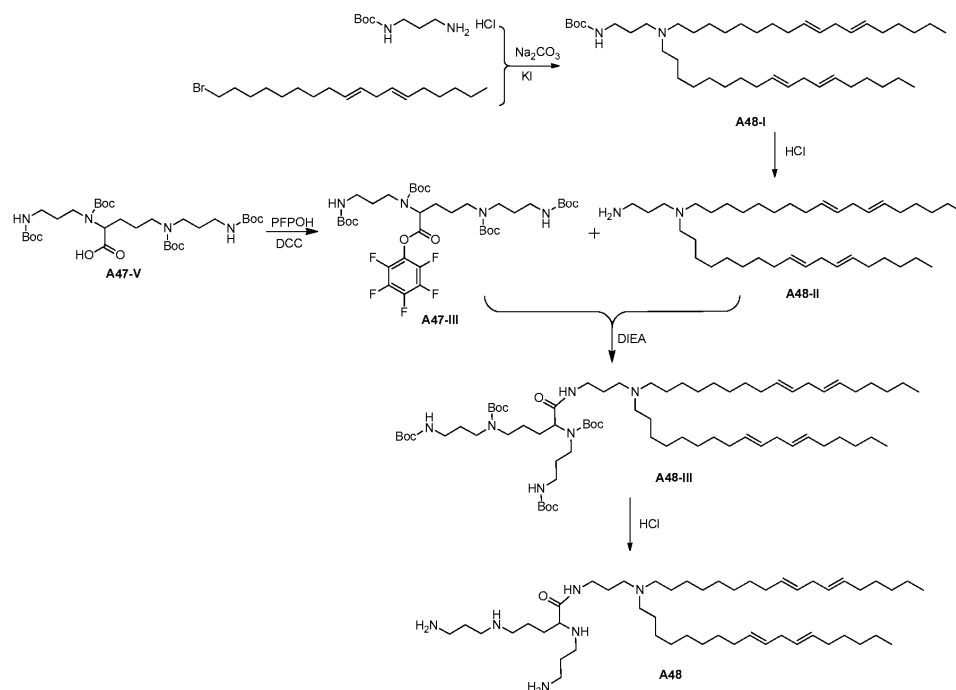
[0126] Steps to prepare A47-I: A47-I was synthesized according to steps used to prepare A42-I in Example 35.

[0127] Steps to prepare A47-II: A47-II was synthesized according to steps used to prepare A42-II in Example 35.

[0128] Steps to prepare A47-III: A47-III was synthesized according to steps used to prepare A46-I in Example 39.

[0129] Steps to prepare A47-IV: A47-IV was synthesized according to steps used to prepare A46-II in Example 39.

[0130] Steps to prepare A47: 1.1 g A47 was synthesized according to steps used to prepare A44 in Example 37. ^1H NMR (400 MHz, CD_3OD) δ : ppm. 5.10(m, 2H), 3.53(m, 8H), 3.44(m, 9H), 3.20-3.0 (m, 16H), 2.34(t, 4H), 2.09(m, 4H), 1.98(dt, 4H), 1.84 (dd, 4H), 1.71(s, 4H), 1.63(dd, 4H), 1.53(m, 8H), 1.40-1.20(m, 40H), 0.88(m, 12H); MS(ES): m/z ($\text{M}+\text{H}$) $^+$ 1071.65.

Example 41 - Synthesis of A48

[0131] Steps to prepare A48-I: A48-I was synthesized according to steps used to prepare A42-I in Example 35.

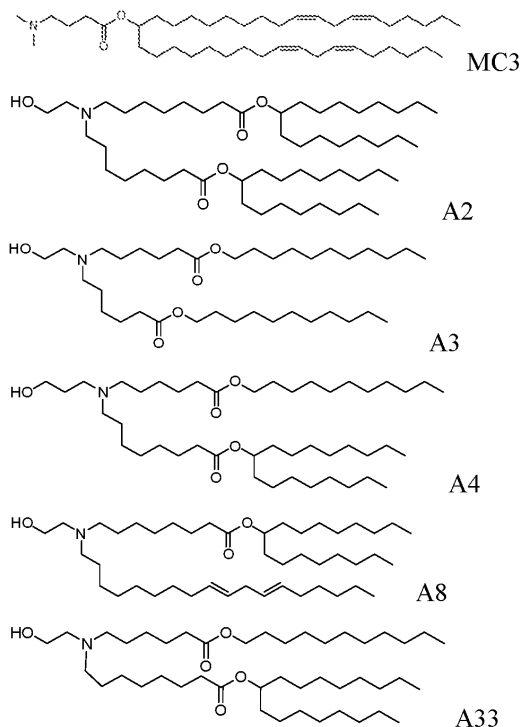
[0132] Steps to prepare A48-II: A48-II was synthesized according to steps used to prepare A42-II in Example 35.

[0133] Steps to prepare A48-III: A48-III was synthesized according to steps used to prepare A46-II in Example 39.

[0134] Steps to prepare A48: 0.5 g A48 was synthesized according to steps used to prepare A44 in Example 37. ^1H NMR (400 MHz, CD_3OD) δ : ppm. 7.78(m, 7H), 5.08(m, 8H), 3.87(m, 1H), 3.0-2.91 (m, 2H), 2.71 (m, 4H), 2.50 (m, 12H), 1.84(dd, 4H), 1.71(m, 4H), 1.63-1.53(m, 12H), 1.40-1.20(m, 36H), 0.88(m, 6H); MS(ES): m/z ($\text{M}+\text{H}$) $^+$ 799.35

Comparative Examples - MC3, A2, A3, A4, A8 and A33

[0135] MC3, A2, A3, A4, A8 and A33 are used for comparative example. Because MC3, A2, A3, A4, A8 and A33 are known cationic liposomes, their synthesis methods are not described specifically here. MC3, A2, A3, A4, A8 and A33 are shown as follows:



Example 42 – Production of lipid nanoparticles

[0136] Lipid nanoparticles were prepared using the following ingredients: (1) an ionizable lipid compound which were commercially available or synthesized, e.g., MC3 (purchased from Avanti), A1-A33 were synthesized in-house; (2) phospholipid (e.g., DOPE or DSPC, purchased from Avanti); (3) PEG lipid (e.g., PEG-DMG, purchased from Avanti or synthesized in-house); (4) structural lipid (e.g., cholesterol, purchased from Sigma-Aldrich); (5) effective composition/active ingredient (e.g., Luciferase mRNA, siRNA, SARS-CoV-2 S protein mRNA, Cas 9 mRNA, etc.)

[0137] Preparation and encapsulation method: (1) ionizable lipid, phospholipid, pegylated lipid and structural lipid were dissolved and mixed in ethanol at 50%, 10%, 1.5%, and 38.5% successively and respectively; (2) a microfluidic chip or T-type mixer were employed to mix lipid mixture and active ingredient (mRNA) evenly at a ratio of 1:3 to obtain lipid nanoparticles.

[0138] Encapsulation percentage reflects the encapsulation extent of the encapsulated substance. The higher the encapsulation percentage, the decomposition possibility of the encapsulated substance is less during the delivery in vivo.

Table 1. Performance of ionizable lipid and its lipid nanoparticle

Ionizable lipid	Size (nm)	PDI	Encapsulated percentage (%)
A1	127.7	0.090	88.79
A2	145.5	0.100	87.86
A3	140.6	0.120	81.47
A4	131.1	0.110	79.24
A5	135.0	0.090	90.11
A6	171.5	0.190	87.09
A7	105.1	0.175	94.63
A8	142.2	0.120	76.10
A9	129.4	0.120	93.35
A10	121.6	0.104	82.65
A12	119.3	0.083	86.53
A13	96.87	0.146	95.51
A17	109.5	0.1037	92.99
A18	104.8	0.0835	93.58
A19	110.1	0.0923	88.00
A20	77.17	0.121	97.09
A21	78.3	0.098	97.20
A22	86.19	0.049	96.85
A23	74.63	0.054	97.41
A24	78.69	0.78	96.77
A25	105.3	0.094	83.60
A26	120.3	0.064	83.56
A27	94.16	0.13	96.69
A28	91.19	0.13	89.72
A29	126.4	0.05	89.98
A30	110.1	0.28	98.14
A32	128.7	0.07	94.19
MC3	86.7	0.10	92.00

Example 43 – Experiments for proving transfection efficiency

- [0139] Encapsulated the nanoparticle of various cationic lipid compounds and luciferase mRNA according to the method in Example 42, tested the fluorescence intensity or total number of photons of luciferase mRNA encapsulated by different LNP.
- [0140] Experimental animal: SPF grade BALB/c mice, female, 6-8 week old, body weight ranged 18-22 g, were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd, with production license no.: SXCK (JING) 2016-0006. All animals were kept in adaptive feeding for more than 7 days before experiments, free to uptake food and drink water during experimental period, illumination 12/12 h alternating light and dark, room temperature was 20-26°C, humidity was 40-70%.
- [0141] Experimental method: female BALB/c mice, encapsulated luciferase mRNA with different LNP by four different administrations which included subcutaneous injection (under the armpit), tail vein injection, intraperitoneal injection, intramuscular injection (the tibialis anterior muscle of mouse hind leg); 3, 6, 24, 48h after administration, applied in vivo fluorescence imaging system (brand: Bruker, model: XTREME) for bioluminescence detection, the specific steps were as follows: substrate preparation: took appropriate amount of substrate Luciferin (brand: Promega) and added saline to make a 10mg/ml solution, avoided light, injected 100µl solution into each mouse intraperitoneally. Mouse moved freely for 5-10 min after being administrated substrate, then put them into anesthesia box and used 2.5% isoflurane for anesthesia. Put the anesthetized mouse into machine, set the bioluminescence setting and took the photos, captured the photograph and then adjusted the upper and lower values of photograph, then collected data on the concentrated distribution of fluorescence (e.g., fluorescence intensity, average photons number, and total photons number) and processed data. Statistical analysis: the in vivo imaging outcome were shown in the fluorescence intensity or average of total photons number of different animal in the same tested group, which were used to judge whether the fluorescence intensity or total photon number of luciferase mRNA encapsulated by different LNP were high or low.
- [0142] The fluorescence intensity and total photons number reflects the transfection efficiency of LNP, the value is higher, referring to the efficiency of delivering encapsulated substance into cell by LNP is higher.

Table 2. The expression of induced luciferase of A1-A9 ionizable lipid nanoparticle

Cationic lipid	Average fluorescence intensity					
	3 h		6 h		24 h	
	Leg (intramuscular injection)	Liver (tail vein injection)	Leg (intramuscular injection)	Liver (tail vein injection)	Leg (intramuscular injection)	Liver (tail vein injection)
A1	61.3	20.4	80.6	27.80	38.9	15.9
A2	1146	5325.2	1208.2	5934.7	377.7	162.6
A3	60.3	12.1	69.8	11.9	35.9	13.5
A4	1847.5	7962.6	1956.2	7745.9	1009.7	326.4
A5	675.7	2231.2	1566.4	3738.2	892.2	73.5
A6	60.3	11.9	40.9	9.0	19.1	12.2
A7	1683.2	21013.1	2990.1	21315.5	1436.4	444.2
A8	1111	6850.3	2161.8	10174.4	1294.9	220.8
A9	461.4	198.9	432.0	252.7	178.3	14.6
A33	1065.5	2841.8	1117.9	3144.6	418.8	100.0

Note: administration route: (1) intramuscular injection; (2) tail vein injection; dose: 10 μ g/mice; detection time: 3, 6, and 24 h after administration.

Table 3. The expression of induced luciferase of A18-A33 ionizable lipid nanoparticle

Cationic lipid	Total photons number		
	3 h	6 h	24 h
	Leg	Leg	Leg
A10	NA	3.17E+09	NA
A11	NA	1.33E+09	NA
A12	NA	6.91E+08	NA
A13	NA	3.66E+09	NA
A15	NA	8.78E+08	NA
A18	3.91E+09	4.16E+09	2.90E+08
A19	3.69E+09	4.77E+09	7.33E+08
A27	1.34E+09	1.04E+09	3.41E+08
A28	6.50E+08	4.26E+08	1.83E+08
A31	1.58E+08	2.65E+08	8.43E+07
A33	1.41E+08	2.35E+08	5.84E+07
MC3	3.43E+08	7.41E+08	2.86E+08

Note: administration route: intramuscular injection; dose: 1µg/ mice; detection time: 3, 6, and 24 h after administration.

Table 4. The expression of induced luciferase of A20-A25, A33 ionizable lipid nanoparticle

Cationic lipid	Total photons number		
	3 h	6 h	24 h
	Leg	Leg	Leg
A20	5.99E+08	5.65E+08	2.03E+08
A21	9.07E+08	7.11E+08	1.62E+08
A22	4.16E+09	5.74E+09	1.24E+09
A23	4.39E+09	2.68E+09	8.00E+08
A24	1.94E+08	1.56E+08	6.31E+07
A25	4.23E+08	2.49E+08	9.50E+07
A33	5.20E+08	4.62E+08	1.63E+08

Note: administration route: intramuscular injection; dose: 5µg/mice; detection time: 3, 6, and 24 h after administration.

Example 44 – Evaluate the Safety of LNP

[0143] Wistar rats including 4 males and 4 females, with a weight difference of no more than 10%, were selected and randomly divided into two groups: solvent control group and tested substance group. A18 was used in the tested substance group, and the LNP preparation's encapsulation condition and size were shown in table 5. The concentration used in the measurement was 2 mg/mL. Each animal was administered 3 times a day with the volume of each injection as 250 µl. The administration interval was 4 hours. Alternate administration to the left and right legs were performed. The total dosage level was 1.50 mg per rat, which was equivalent to 1200 times the maximum unit weight dosage level for human (assuming the maximum dosage level for human is 0.25 mg). Clinical observation was recorded as follows. Within 24 hours after administration, clinical observation was carried out every hour. Within 24-72 hours after administration, clinical observation was carried out once every 6 hours. Within 4-14 days after administration, clinical observation was carried out once a day. The symptoms of toxicity, the time when the symptoms appeared and disappeared, and the time of death (if occurred) were recorded. Body weight was recorded once a day after administration, and food intake was recorded every 2 days after administration. 14 days after the administration, all the

remaining animals were weighed, then euthanized, and major organs were obtained by anatomy. Heart, liver, spleen, lung, kidney, thymus, lymph node, were weighted and relative organ weight (the organ weight / the subject weight $\times$ 100%; also known as organ coefficient) was calculated. After anatomy, the heart, liver, spleen, lung, kidney, intestine, thymus, lymph node, muscle tissue at the injection site and other organs with pathological changes observed were stored in a fixation solution, and the pathology of each organ was examined by H&E staining. Lesion severity scores were given according to the table below.

Table 5. The LNP preparation's encapsulation condition and size

	Ionizable lipid	Size (nm)	PDI
Solvent control group	-	-	-
Tested substance group (LNP empty vector control group)	A18	147	0.088

[0144] The weight change results are shown in **FIGS. 1-2**. Specifically, the body weight of male rats in the solvent control group continued to increase. The body weight of rats in the tested substance group dropped initially, then recovered to the pre-administration level on Day 4- Day 6, and continued to increase subsequently. The results of changes in food intake are shown in **FIGS. 3-4**. The food intake per rat in the solvent control group within 24 hours was stable, within a range of 18-35 grams. The initial food intake of the rats in the tested substance group decreased initially, and returned to normal levels on Day 4- Day 7. The relative organ weight results are shown in **Table 6**. Compared with the solvent control group, the heart, liver, kidney, spleen, thymus and lymph node coefficients of rats in the tested substance group did not show significant difference. The results of histological changes are shown in **Table 8**. Compared with the rats in the solvent control group (one male and one female), the heart, liver, kidney, spleen, thymus, and lymph nodes of the rats in the tested substance group had no pathological changes. For the tested substance group, there were no other abnormal pathological changes except for the proliferation of partial interstitial cells in the lung and a small amount of inflammatory cell infiltration in the muscle tissue. Based on the above results, the rats showed only slight pathological changes in the lungs and legs after the 1200 times higher dosage level of the LNP injection.

		Heart	Liver	Spleen	Lung	Kidney	Kidney	Thymus	Lymph nodes	Brain
						left	right			
Solvent control group	Total	0.302±0.004	4.188±0.144	0.257±0.045	0.376±0.029	0.373±0.010	0.391±0.006	0.186±0.038	0.005±0.001	0.700±0.127
Tested substance group	Total	0.308 ± 0.013	3.773 ± 0.212	0.250 ± 0.029	0.413 ± 0.048	0.351 ± 0.015	0.356 ± 0.025	0.149 ± 0.024	0.006 ± 0.006	0.687 ± 0.142

Table 7. Lesion severity score standard

Score = 0 (does not exist)	Under the conditions of the experiment, taking into account the age, sex and strain of the animal, it can be considered that the tissue is within the normal range and there is no pathological change.
Score = 1 (minimal)	The first (lowest) grade of lesions in the 5 grades of minimal, mild, moderate, severe and serious.
Score = 2 (mild)	The second grade of lesion degree in the 5 grades of minimal, mild, moderate, severe and serious.
Score = 3 (moderate)	The third grade of lesion degree in the 5 grades of minimal, mild, moderate, severe and serious.
Score = 4 (severe)	The fourth grade of lesion degree in the 5 grades of minimal, mild, moderate, severe and serious.
Score = 5 (serious)	The fifth (highest) grade of lesion degree in the 5 grades of minimal, mild, moderate, severe and serious.

[illegible]

	1101	—	—	—	—	—	—	—	Partial stromal cell hyperplasia	—
	Score	0	0	0	0	0	0	0	1	0

Note: “—” means no abnormality.

Example 45 – Immunity study of mRNA encapsulated by LNP

[0145] SARS-CoV-2 S protein mRNA was produced by T7 in vitro transcription method, used ionizable lipid A7, A18 and A33 encapsulated lipid nanoparticle according to synthesis method in Example 42, and their encapsulation condition, encapsulated percentage and size were shown in Table 9.

Table 9 Encapsulated outcome

Ionizable lipid	Encapsulated percentage (%)	Size (nm)	PI
A33	95	76	0.104
A7	97	79	0.060
A18	94	86	0.075

Immunization program:

Ionizable lipid	Group	Dose	Species	Administration	Numbers of animal	Animal number
A33	1	4μg	BALB/c	intramuscular	9	8001-8009
	2	50μg	BALB/c	intramuscular	9	9001-9009
A7	1	4μg	BALB/c	intramuscular	9	16001-16009
	2	50μg	BALB/c	intramuscular	9	17001-17009
A18	1	5μg	BALB/c	intramuscular	9	3001-3009
	2	20μg	BALB/c	intramuscular	9	4001-4009

[0146] The 3 obtained LNP preparations were used in BALB/c mouse immunization test. The experiment was performed as follows. 6-8 week old female BALB/c mice (9 mice in each group) were administered twice with the LNP preparations on Day 0 and Day 14 by intramuscular injection. The injection volume was 50 μL. After 7 days, the mouse spleens were isolated to separate splenic lymphocytes. The T lymphocytes secreting INF γ were detected by the ELISPOT (Enzyme-linked immune absorbent spot) method and the outcome was shown in table 10, illustrating that mRNA induced stronger cellular immune

response in BALB/c mice. 14 days after the second immunization, the S protein specific IgG antibody was detected by indirect ELISA. The IgG antibody EC₅₀ was calculated by fitting the antibody titer curves, as shown in the figure 5. The results showed that A7, A18 and A33 were all induced higher titers of IgG antibodies in BALB/c mice

[0147] Specific operation of ELISPOT was carried according to Mouse IFN- γ precoated ELISPOT kit instruction.

Table 10. Elispot counted T lymphocytes secreting INF γ after administrating different LNP preparations

	A33		A7		A18	
	4 μ g	50 μ g	4 μ g	50 μ g	5 μ g	20 μ g
Blank control	0	2	12	29	1	0
S protein (1 μ g)	32	310	294	805	161	241
	42	338	811	700	153	251
Positive control	679	868	124	N/A	344	474

[0148] Specific operation of indirect ELISA to detect S protein specific IgG antibody titer:

1. coated antigen: S protein was diluted to 2 ng/uL with coating buffer, 100 uL/well, coated overnight at 4 °C;
2. washed plate 3 times with 1X PBST, washed 5 min each time;
3. blocked with 1% BSA blocking solution, 200 uL/well, and left to stand 1 h at 37 °C;
4. washed plate 3 times with 1X PBST, washed 5 min each time;
5. serum to be tested was diluted with dilution buffer by doubling dilution, 100 uL/well, incubated for 1 h at 37 °C, and set negative serum control group and blank control group without serum;
6. washed plate 3 times with 1X PBST, washed 5 min each time;
7. anti IgG secondary antibody was diluted by 1:1000, 100 uL/well, incubated for 1 h at 37 °C;
8. washed plate 3 times with 1X PBST, washed 5 min each time;
9. added fresh TMB chromogen solution, 100 uL/well, incubated for an appropriate time at 37 °C;
10. added 2 mol/L termination solution of sulfuric acid, 50 uL/well.

11. used enzyme-labeled instrument to measure OD450 nm absorbance.

ELISA to detect antibody

[0149] First immune 14 days antibody detection

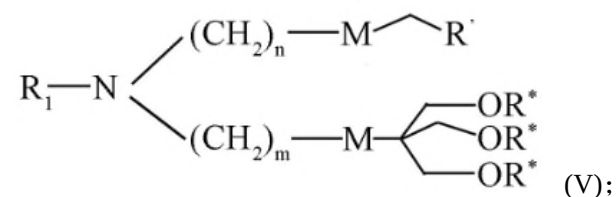
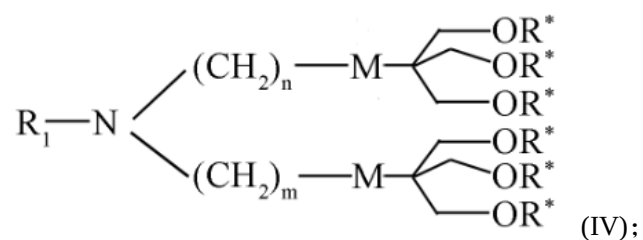
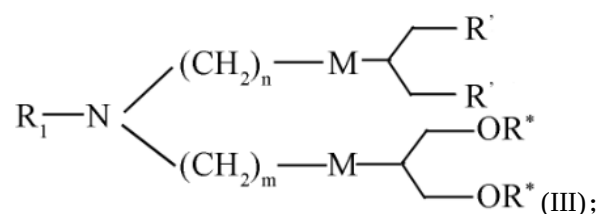
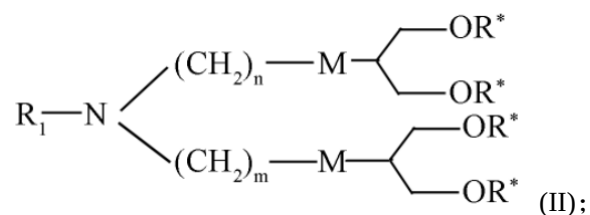
1. sample: first immunized 14 days mouse serum of immune group, solvent control group, mixed 6 mouse's serum in each group
2. antigen protein: SARS-CoV-2 (COVID-19) S protein (R683A, R685A), His Tag (SPN-C52H4)
3. coated antigen protein: 2 ng/ μ L, 100 μ L/well
4. second immune: Goat anti-mouse IgG (H+L), HRP conjugate 1:1000 dilution

[0150] Second immune 14 days antibody detection

1. sample: second immunized 14 days mouse serum of immune group, solvent control group, mixed 6 mouse's serum in each group
2. antigen protein: SARS-CoV-2 (COVID-19) S protein (R683A, R685A), His Tag (SPN-C52H4)
3. coated antigen protein: 2 ng/ μ L, 100 μ L/well
4. second immune: Goat anti-mouse IgG (H+L), HRP conjugate 1:1000 dilution
5. outcome shown in Figure 5.

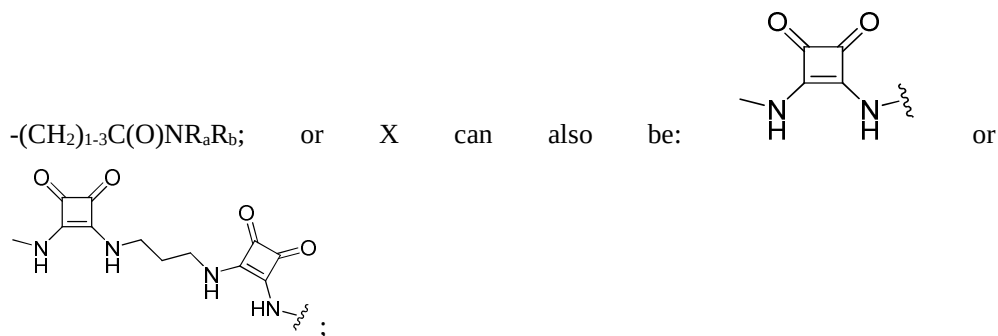
CLAIMS

1. A compound which has formula (II), formula (III), formula (IV) or formula (V):



wherein R_1 is selected from $-\text{R}_1'\text{---X}$,

R_1' is $-(\text{CH}_2)_{1-6}$, X is hydroxyl, $-\text{C}(\text{O})(\text{CH}_2)_{1-3}\text{NR}_a\text{R}_b$, $-\text{C}(\text{O})\text{O}(\text{CH}_2)_{1-3}\text{NR}_a\text{R}_b$, $-\text{OC}(\text{O})(\text{CH}_2)_{1-3}\text{NR}_a\text{R}_b$, $-\text{C}(\text{O})\text{NH}(\text{CH}_2)_{1-3}\text{NR}_a\text{R}_b$, $-\text{NHC}(\text{O})(\text{CH}_2)_{1-3}\text{NR}_a\text{R}_b$, $-\text{NHC}(\text{O})\text{CH}(\text{NR}_a\text{R}_b)(\text{CH}_2)_{1-3}\text{NR}_a\text{R}_b$, or 5-10 membered heteroaryl, the said heteroaryl is optionally substituted by the following groups: $-(\text{CH}_2)_{1-3}\text{OH}$, $-(\text{CH}_2)_{1-3}\text{NR}_a\text{R}_b$, and



R_a , and R_b are independently selected from H, C_{1-3} alkyl, $-(\text{CH}_2)_{1-3}\text{NH}_2$, and $-(\text{CH}_2)_{1-3}\text{NH}(\text{CH}_2)_{1-3}\text{NH}_2$; or R_a , and R_b together with the nitrogen to which they are attached form a 5-10 membered heterocycle including 1-3 heteroatoms selected from N, O or S, by said heterocycle is optionally substituted by one or more of the following groups: C_{1-6} alkyl, C_{1-6} alkyl halides, C_{1-6} alkyl hydroxyl and C_{1-6} alkyl amino;

each M is independently selected from $-\text{CH}_2-$, $-\text{CH}=\text{CH}-$, $-\text{NH}-$, $-\text{C}(\text{O})-$, $-\text{O}-$, $-\text{C}(\text{O})\text{O}-$, $-\text{OC}(\text{O})-$, $-\text{C}(\text{O})\text{NH}-$, and $-\text{NHC}(\text{O})-$;

each R' is independently selected from C_{1-10} alkyl or C_{3-12} alkenyl;

each R^* is independently selected from C_{1-10} alkyl or C_{3-12} alkenyl;

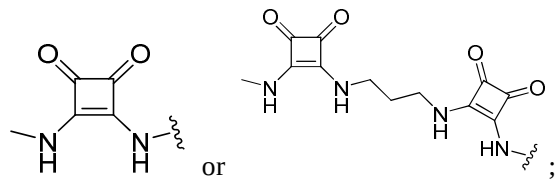
n , m are independently selected from the integer range from 1-9;

or a salt;

in the formula (III): each R' is independently selected from C_{2-8} alkyl; each M is independently selected from $-\text{C}(\text{O})\text{O}-$ or $-\text{OC}(\text{O})-$; or a salt thereof.

2. The compound of claim 1, wherein:

R_1' is $-(\text{CH}_2)_{2-3}$, X is hydroxyl, $-\text{C}(\text{O})(\text{CH}_2)_{2-3}\text{NR}_a\text{R}_b$, $-\text{C}(\text{O})\text{O}(\text{CH}_2)_{2-3}\text{NR}_a\text{R}_b$, $-\text{C}(\text{O})\text{NH}(\text{CH}_2)_{2-3}\text{NR}_a\text{R}_b$, or 5-10 heteroaryl which is optionally substituted by the following groups: $-(\text{CH}_2)_{2-3}\text{OH}$, $-(\text{CH}_2)_{2-3}\text{NR}_a\text{R}_b$, $-(\text{CH}_2)_{2-3}\text{C}(\text{O})\text{NR}_a\text{R}_b$; or X can also be:



R_a , and R_b are independently selected from H, C_{1-3} alkyl, $-(\text{CH}_2)_{2-3}\text{NH}_2$, or $-(\text{CH}_2)_{2-3}\text{NH}(\text{CH}_2)_{2-3}\text{NH}_2$; or R_a , and R_b together with the nitrogen to which they are attached form a 5-10 membered heterocycle including 1-3 heteroatoms selected from

N or O, said heterocycle is optionally substituted by the following groups: C₁₋₆ alkyl, C₁₋₆ alkyl halides, C₁₋₆ alkyl hydroxyl groups and C₁₋₆ alkyl amino groups;

or a salt thereof.

3. The compound of any one of claims 1-2, wherein:

each M is independently selected from -CH₂-, -CH=CH-, -C(O)O-, -OC(O)-, -C(O)NH-, -NHC(O)-;

or a salt thereof.

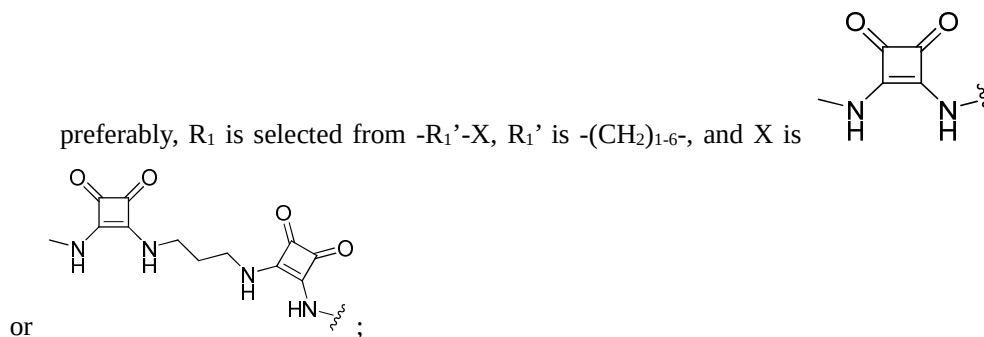
4. The compound of any one of claims 1-3, wherein in the formula (II):

each R* is independently selected from C₂₋₁₀ alkyl, preferably C₆₋₁₀ alkyl, preferably C₆ alkyl;

preferably, each M is independently selected from -C(O)O- or -OC(O)-, preferably -C(O)O-;

preferably, R₁ is selected from -R₁'-X, R₁' is -(CH₂)₁₋₆-, and X is hydroxyl;

preferably, R₁ is selected from R₁'-X, R₁' is -(CH₂)₁₋₆-, X is 5-6 membered heteroaromatic group, preferably triazolyl, the said heteroaromatic group is optionally substituted with the following groups: -(CH₂)₂₋₃OH, -(CH₂)₂₋₃NR_aR_b, -(CH₂)₂₋₃C(O)NR_aR_b; R_a, and R_b are independently selected from H, C₁₋₃ alkyl, -(CH₂)₂₋₃NH₂, -(CH₂)₂₋₃NH(CH₂)₂₋₃NH₂, or 5-10 membered heterocycle containing 1-3 heteroatoms selected from N or O, which is formed together by R_a, R_b and their connected nitrogen atom, preferably morpholinyl, piperazinyl or piperidinyl, the said heterocycle is optionally substituted by the following groups: C₁₋₆ alkyl hydroxyl;



or a salt thereof.

5. The compound of claim 4, wherein:

each n is 7, and m is 7;

preferably, each M is independently selected from -C(O)O- or -OC(O)-, preferably -C(O)O-;

or a salt thereof.

6. The compound of any one of claims 1-3, wherein in the formula (III):

each M is independently selected from -C(O)O-;

or a salt thereof.

7. The compound of any one of claims 1-3, wherein in the formula (IV):

each R* is independently selected from C₂₋₁₀ alkyl, preferably C₆₋₁₀ alkyl, preferably C₆ alkyl;

preferably, each M is independently selected from -C(O)O- or -OC(O)-, preferably -C(O)O-;

or a salt thereof.

8. The compound of any one of claims 1-3, wherein in the formula (V):

each R* is independently selected from C₂₋₁₀ alkyl, preferably C₆₋₁₀ alkyl, preferably C₆ alkyl;

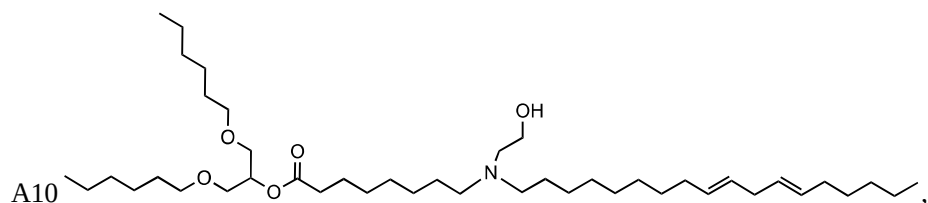
preferably, each M is independently selected from -CH=CH-, -C(O)O- or -OC(O)-, preferably -CH=CH- or -C(O)O-;

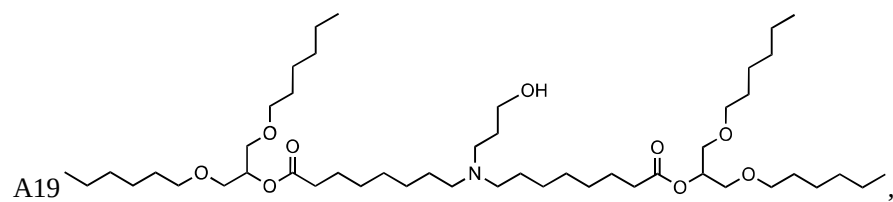
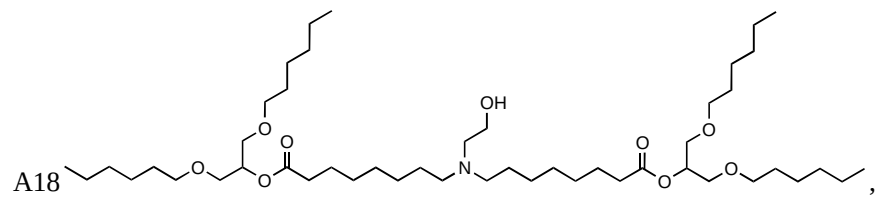
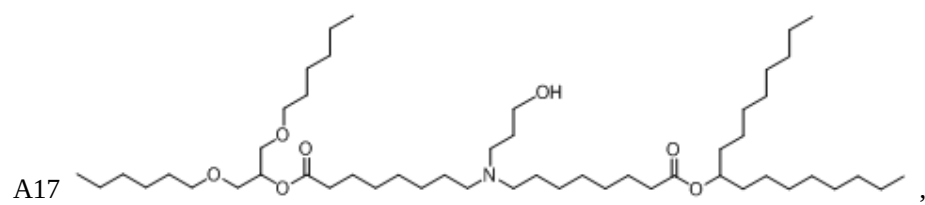
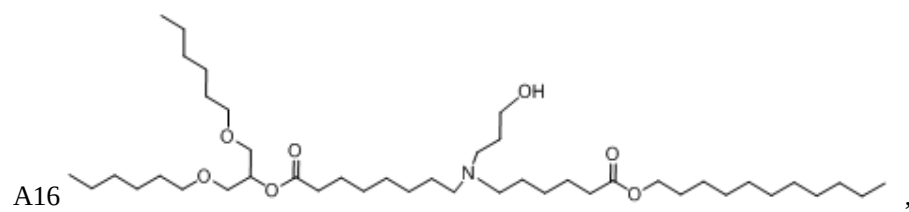
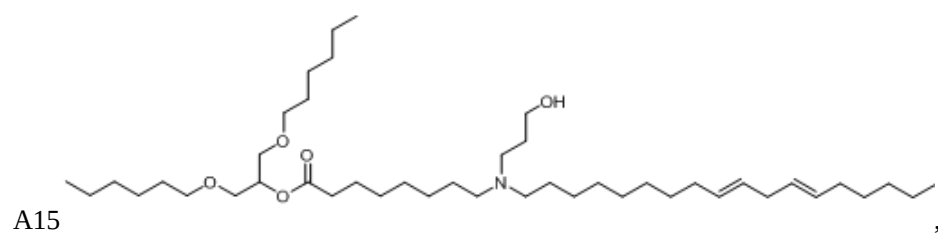
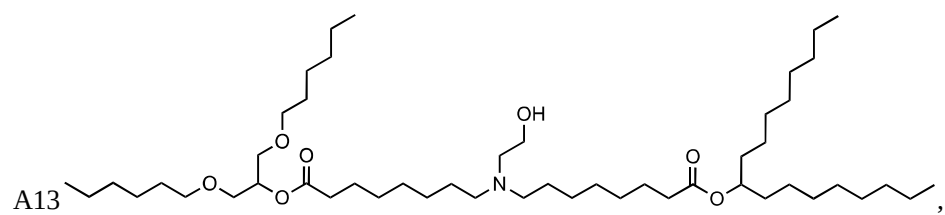
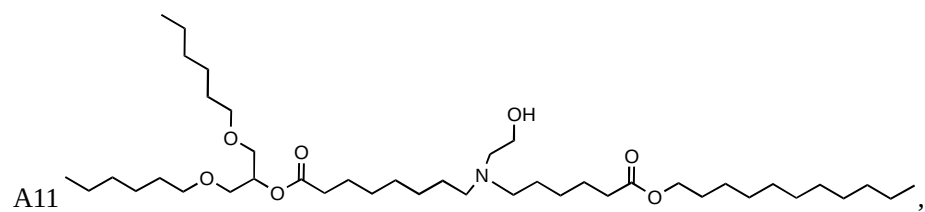
preferably, each R' is independently selected from C₁₋₁₀ alkyl or C₃₋₁₂ alkenyl, preferably C₁₀ alkyl or C₈ alkenyl;

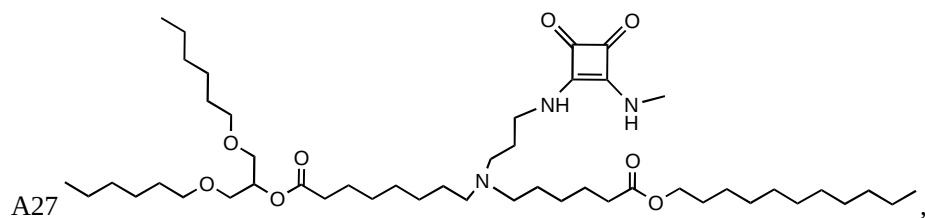
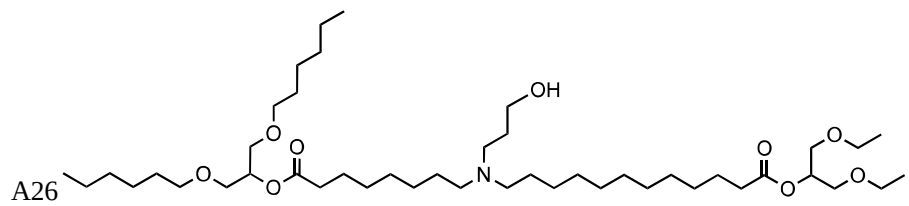
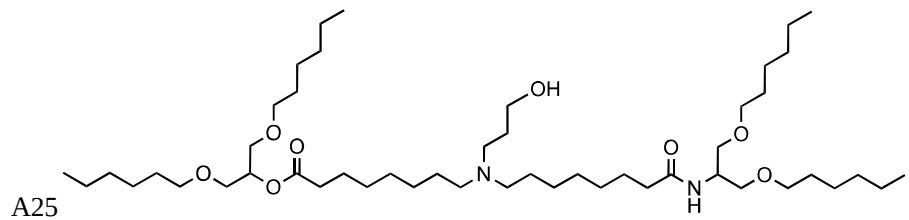
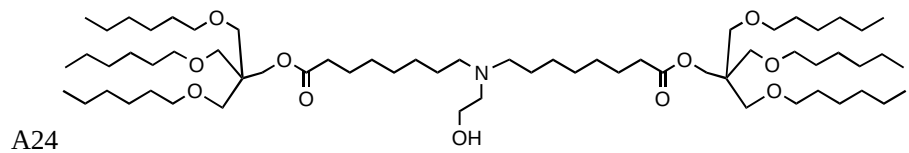
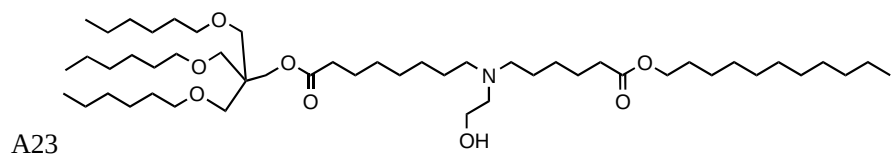
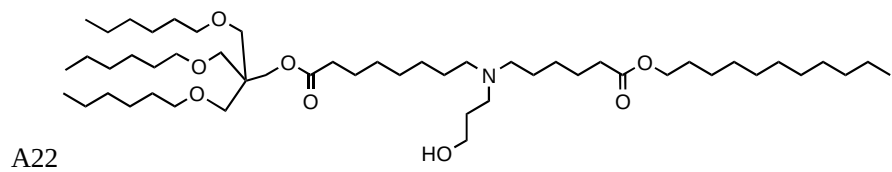
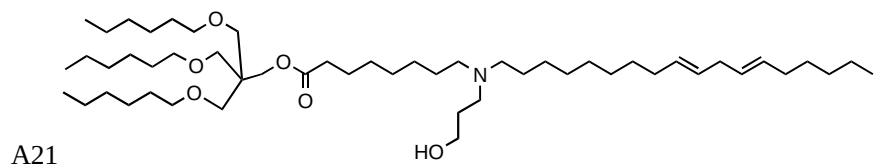
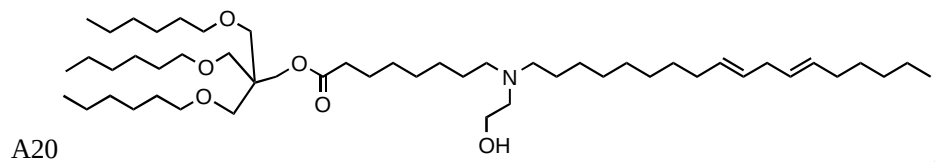
or a salt thereof.

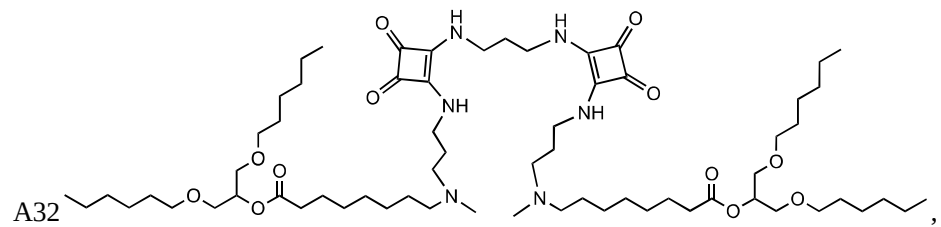
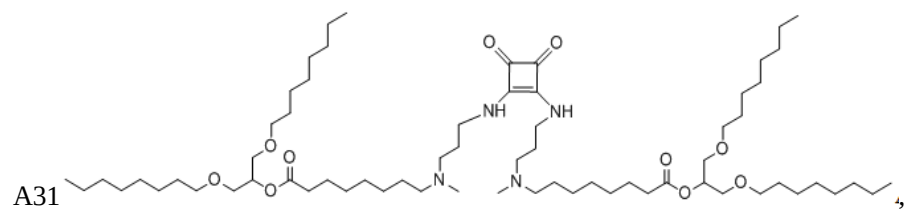
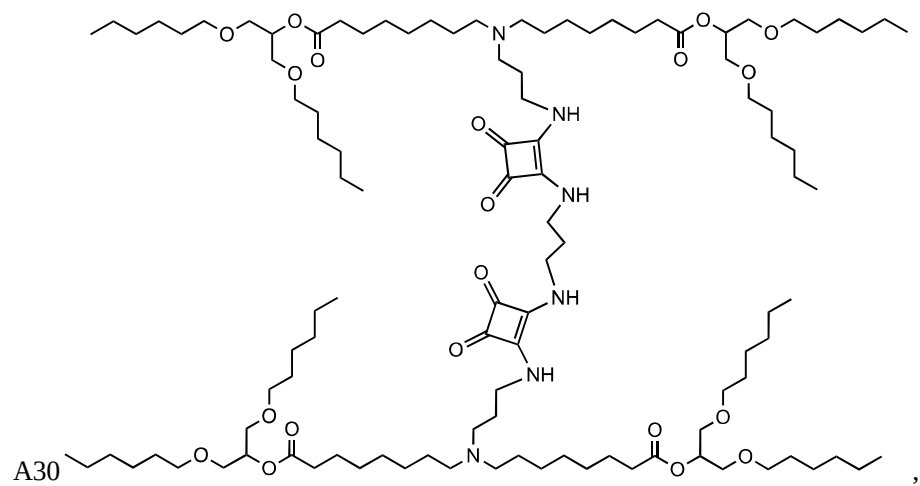
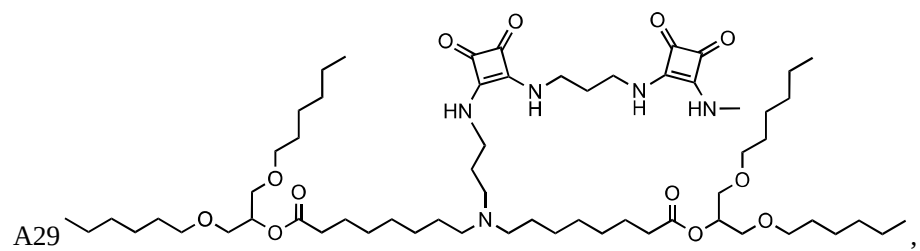
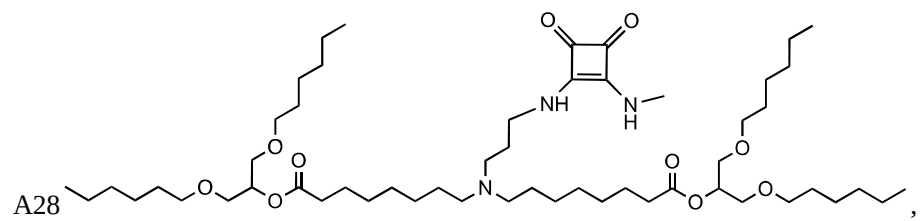
9. A compound or salts thereof, wherein:

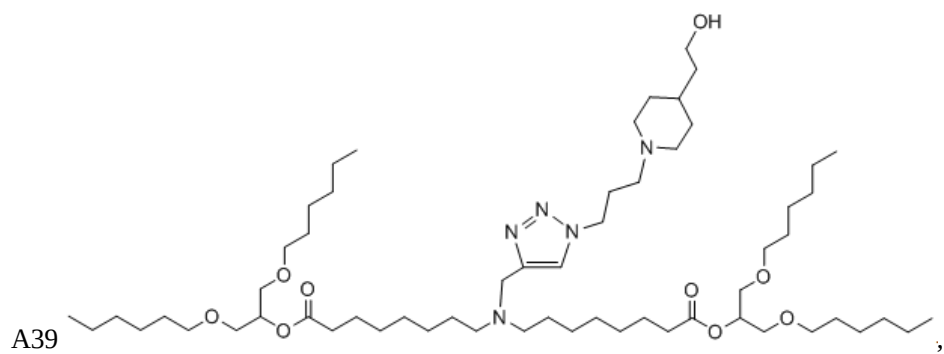
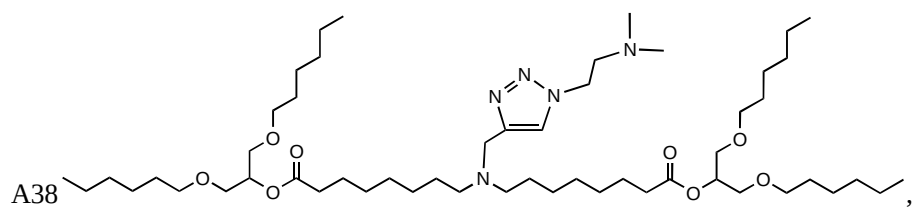
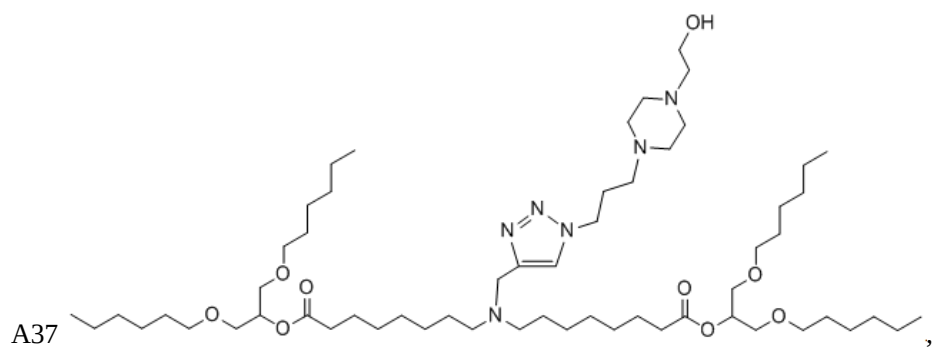
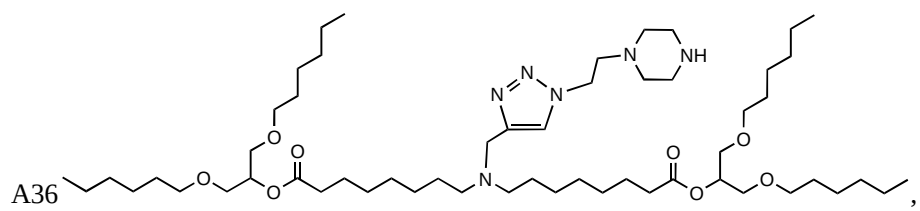
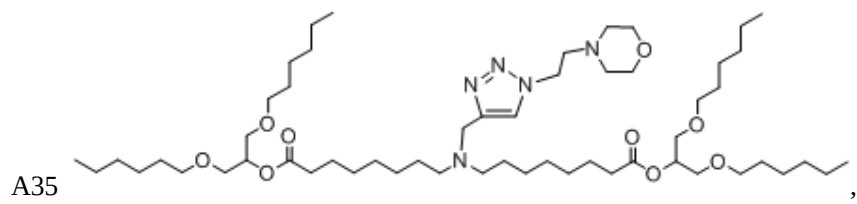
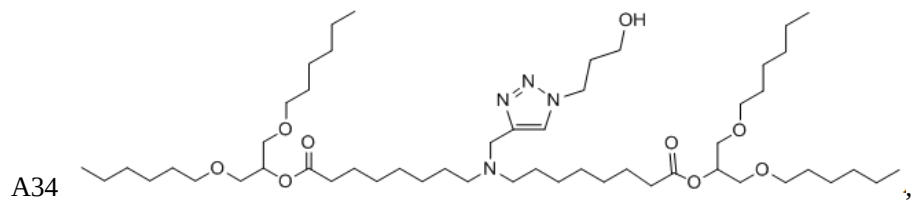
the compound is one of

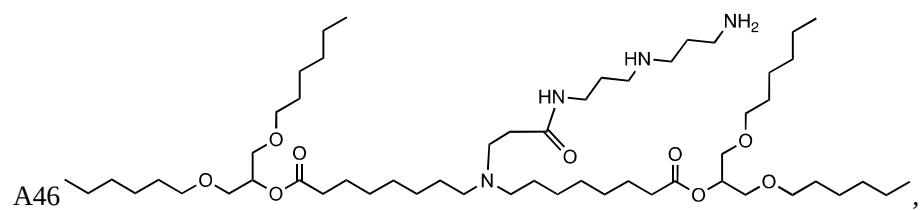
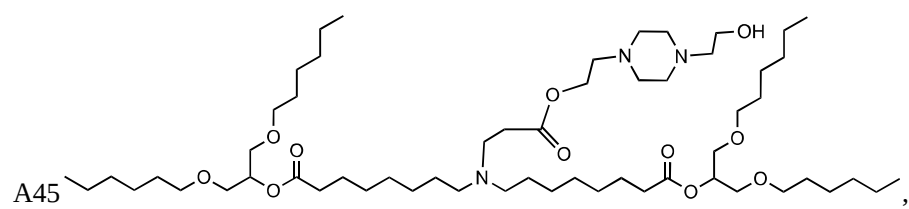
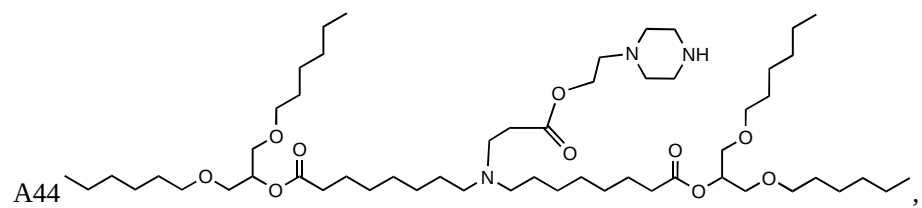
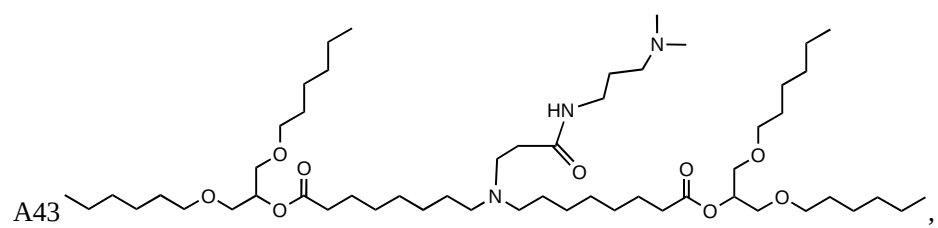
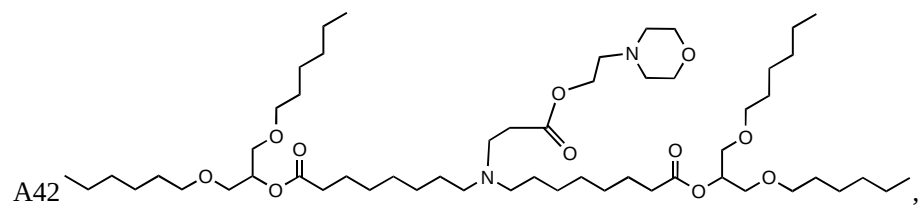
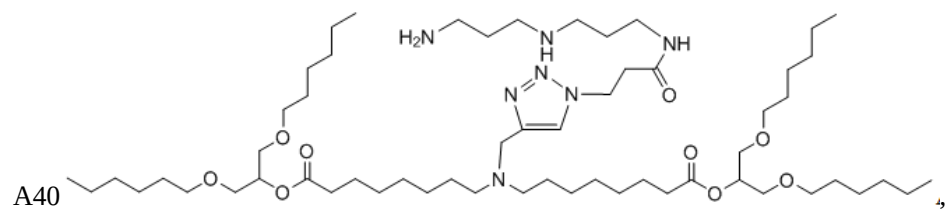


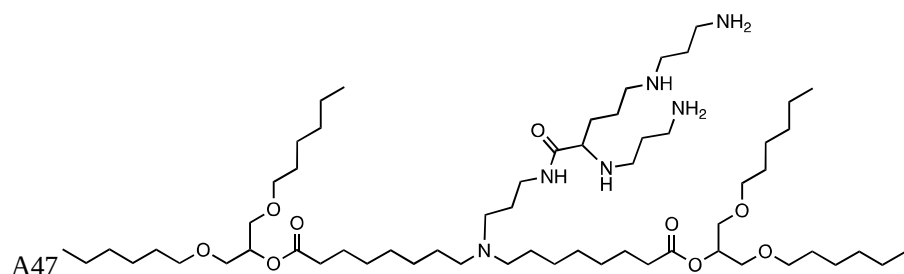












10. A composition comprising a compound according to any one of the claim 1-8 as an ionizable lipid compound.
11. The composition of claim 10, further comprising a phospholipid, preferably, the phospholipid is selected from 1,2-dilinoyleyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-5'-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-5'-glycero-3-phosphocholine (OChemPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinenoyl-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), dipalmitoylphosphatidylglycerol (DPPG), palmitoyl-oleoylphosphatidylethanolamine (POPE), distearoyl-phosphatidyl-ethanolamine (DSPE), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), 1-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE), 1-stearoyl-2-oleoyl-phosphatidylcholine (SOPC), sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyl-oleoyl phosphatidylcholine, lysophosphatidylcholine, lysophosphatidylethanolamine (LPE), or a combination thereof.

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12. The composition of claim 10 or 11, further comprising a PEG lipid, preferably, the PEG lipid is selected from PEG-modified phosphatidylethanolamine, PEG- modified phosphatidic acid, PEG-modified ceramide, PEG-modified dialkylamine, PEG- modified diacylglycerol, PEG-modified dialkylglycerol, or a combination thereof.
13. The composition of any one of claims 10-12, further comprising a structural lipid, preferably, the structural lipid is selected from: cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, ursolic acid, alpha-tocopherol, or a combination thereof.
14. The composition of any one of claims 10-13, further comprising an active ingredient, which is selected from at least any one of: DNA, RNA, protein, or an active pharmaceutical molecule,
- preferably, the ionizable lipid compound is from 20% to 80%, the PEG lipid is from 1% to 10%, the structural lipid is from 10% to 50% and the phospholipid is from 5% to 30%, each of these percentages being calculated based on mole percentage of all lipids in the composition;
- preferably, the ionizable lipid compound is from 20% to 80%, the PEG lipid is from 1% to 5%, the structural lipid is from 10% to 50% and the phospholipid is from 5% to 30%, each of these percentages being calculated based on mole percentage of all lipids in the composition;
- preferably, the active agent is RNA and the RNA is selected from at least any one of: mRNA, siRNA, aiRNA, miRNA, dsRNA, aRNA, or lncRNA;
- preferably, the active agent is a protein which is selected from at least any one of: antibody, enzyme, recombinant protein, polypeptide and short chain polypeptide.
15. The composition of any one of claims 10-14, wherein:
- the composition is in the form of a lipid nanoparticle.
16. A method of producing lipid nanoparticle, comprising:
- mixing an ionizable compound of claim 1 with a PEG lipid, a structural lipid and a phospholipid to form a lipid mixture.
17. The method of claim 16, further comprising:
- mixing an active ingredient with the lipid mixture to form lipid nanoparticle by mixer.

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18. The compound of any one of claims 1-8, for use in the production of lipid nanoparticle.
19. The compound of claim 18, wherein:
- the said lipid nanoparticle is neutral and uncharged in a neutral medium, and is positively charged after being protonated in an acidic medium;
- preferably, the compound is dissolved and mixed with a PEG lipid, a structural lipid and a phospholipid to form a lipid mixture, then mixing an active ingredient with the lipid mixture by mixer to form a lipid nanoparticle.
20. A pharmaceutical composition comprising the lipid nanoparticle of claim 15 and pharmaceutically acceptable carrier.
21. The lipid nanoparticle of claim 15 or pharmaceutical composition of claim 20, for use in the production of medicine;
- preferably, the medicine further comprises an active ingredient, the active ingredient selected from at least any one of DNA, RNA, protein, or an active pharmaceutical molecule;
- preferably, the active ingredient is RNA that is selected from at least any one of: mRNA, siRNA, aiRNA, miRNA, dsRNA, aRNA, or lncRNA;
- preferably, the active ingredient is a protein is selected from at least any one of: antibody, enzyme, recombinant protein, polypeptide and short chain polypeptide;
- preferably, the said medicine can be administered to a human by intravenous injection, intramuscular injection, subcutaneous injection, microneedle patch, oral administration, or oral and nasal spray.