(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
08 May 2025 (08.05.2025)

WIPO | PCT

(10) International Publication Number
WO 2025/092756 A1

(51) International Patent Classification:
C07C 229/16 (2006.01) *C07D 295/13* (2006.01)
A61K 9/127 (2025.01)

(21) International Application Number:
PCT/CN2024/128274

(22) International Filing Date:
29 October 2024 (29.10.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
202311424873.0 30 October 2023 (30.10.2023) CN

(71) Applicants: **GUANGZHOU RIBOBIO CO., LTD.** [CN/CN]; No.7 Suida Street, Huangpu District, Guangzhou, Guangdong 510530 (CN). **HK RIBO BIOLOGY LIMITED** [CN/CN]; Suite 2702, 27/f., Shui On Center, NOS. 6-8 Harbour Road, Wanchai, Hong Kong (CN).

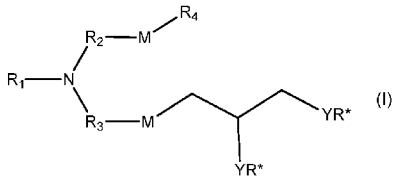
(72) Inventors: **ZHANG, Bill Biliang**; No.7 Suida Street, Huangpu District, Guangzhou, Guangdong 510530 (CN). **ZHAO, Haoting**; No.7 Suida Street, Huangpu District, Guangzhou, Guangdong 510530 (CN).

(74) Agent: **KANGXIN PARTNERS , P.C.**; Floor 16, Tower A, Indo Building A48 Zhichun Road, Haidian District, Beijing 100098 (CN).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE,

(54) Title: LIPID COMPOUND AND COMPOSITION THEREOF



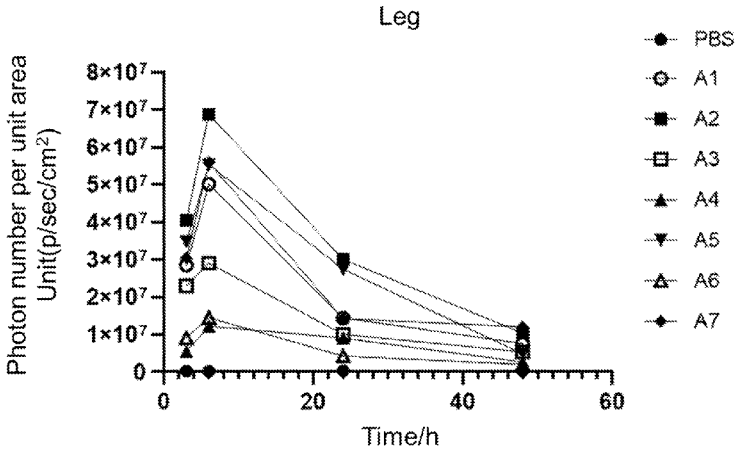


Figure 1A

(57) Abstract: The application relates to a lipid compound in Formula (I), a lipid nanoparticle containing it, a preparation method thereof, and a use in drug delivery.

[Continued on next page]

WO 2025/092756 A1

SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

Lipid Compound and Composition Thereof

Cross-Reference to Related Application

The application is based upon and claims priority to Chinese application No. 202311424873.0 filed on October 30, 2023, the disclosure of which is hereby incorporated by reference in its entirety.

Technical Field

The application belongs to the field of biomedicine and biotechnology, and relates to an active ingredient delivery system for a new-type lipid compound.

Background

Nucleic acid drugs are various oligoribonucleotides (RNA) or oligodeoxyribonucleotides (DNA) with different functions, which may regulate genes expressing related proteins based on the principle of base complementary, and may directly act on pathogenic target genes or target mRNA, to play a role in the treatment of diseases at the genetic level. The sequence design of the nucleic acid drugs is very easy, and the nucleic acid drugs may avoid the limitation issues of undruggable targets faced by traditional small molecule drugs and antibody drugs. Moreover, it may regulate both intracellular and extracellular proteins as well as cell membrane proteins. However, the development of the nucleic acid drugs mainly faces three major problems: 1. the instability of nucleic acid molecules, especially RNA, in vivo; 2. potential side effects; and 3. difficulties in drug delivery systems and the like. With the development of new technologies, there are already good solution methods to some difficulties, herein breakthroughs in chemical modifications and delivery system technologies play a crucial role in the development of the nucleic acid drugs. Although the chemical modifications may improve the stability and immunogenicity of the nucleic acid drugs, the nucleic acid drugs need to enter cells to take effect. Since the nucleic acid drugs are relatively large in molecular weight and usually negatively charged, the efficiency of uptake by the cells and the efficiency of endosome escape are relatively low, thus the power of delivery systems is required.

Lipid nanoparticles (LNP) are a new-type nucleic acid biomolecular delivery technology, and LNP is typically composed of four components: (1) an ionizable lipid that may be self-assembled with mRNA into virus-sized particles and release mRNA from endosomes into cytoplasms; (2) a pegylated lipid that may increase the half-life of LNP in blood; (3) a cholesterol that may increase the stability of the nanoparticles; and (4) a phospholipid lipid that is helpful to the formation of lipid bilayer structures. LNP may protect mRNA from being digested by RNA enzymes, and prevent mRNA molecules from being recognized by toll-like receptors (TLRs), thus the excessive activation of innate immune systems is avoided; and the ionizable lipid not only promotes the cellular uptake, but also help drug molecules escape from the endosomes, as to achieve therapeutic effects.

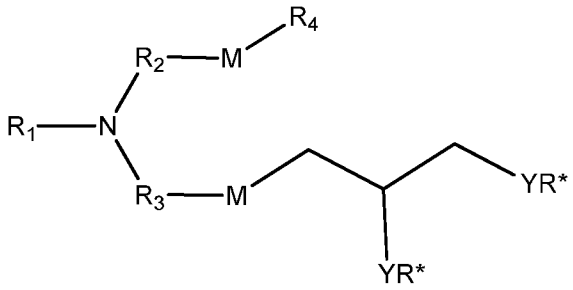
At present, the LNP delivery system is still regarded as one of key technologies to promote the entry of the nucleic acid drug molecules into therapeutic applications, and lipid compounds with novel structures, high efficiency, and stable delivery performance still need to be developed.

Summary

Based on this, it is necessary to discover a novel ionized lipid compound to improve delivery efficiency and stability in allusion to existing technical problems.

The application provides a novel lipid compound, a synthesis method thereof, and a nanoparticle delivery system formed by mixing and encapsulating drug molecules by it with a pegylated lipid compound, a structural lipid cholesterol, and a natural phospholipid and the like, which may be used for in vitro cell delivery and in vivo organ-targeted cell delivery, it is specifically as follows.

In an implementation scheme, the application discloses a lipid compound in Formula (I) below, or a salt or isomer thereof:

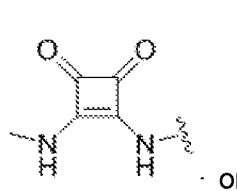
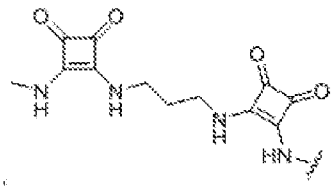


(I)

herein R_1 is -C_{1-6} alkylene-X,

herein X is selected from amino, hydroxyl, acetylenyl, 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, and H atoms on the C_{3-7} cycloalkyl, the 4-7-membered heterocyclic group, the C_{6-10} aryl, and the 5-10-membered heteroaryl groups are optionally substituted by the following groups: -$(CH_2)_{1-3}OH$, -$(CH_2)_{1-3}NR_aR_b$ or -$(CH_2)_{1-3}C(O)NR_aR_b$;

or X is:

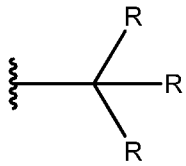



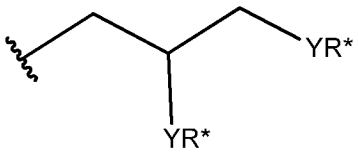
or ;

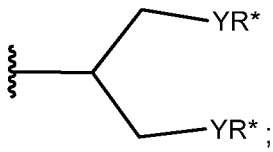
R_2 is C_{3-20} alkylene, herein one or more methylenes are each independently substituted by the following groups: -CH=CH-, -C≡C -, or -O-;

R_3 is C_{5-20} alkylene, herein one or more methylenes are each independently substituted by the following groups: -CH=CH-, -C≡C-, or -O-;

M is independently selected from -CH_2-, -CH=CH-, -C≡C-, -NH-, -C(O)-, -C(S)-, -O-, -S-, -C(O)O-, -OC(O)-, -C(O)NH-, -NHC(O)-, -C(O)-S-, -S-C(O)-, -C(S)-O-, -O-C(S)-, -C(S)-S-, -S-C(S)-, -S-S-, -C(S)NH-, -NHC(S)- or -O-P(O)(OH)-O-;

R_4 is 

, 

, or 

;

herein R is selected from H, R* or -$(CH_2)_{1-5}$-YR*;

Y is selected from -NH-, -C(O)-, -C(S)-, -O-, -S-, -C(O)O-, -OC(O)-, -C(O)NH-, -NHC(O)-, -C(O)-S-, -S-C(O)-, -C(S)-O-, -O-C(S)-, -C(S)-S-, -S-C(S)-, -S-S-, -C(S)NH-, -NHC(S)-, or -O-P(O)(OH)-O-;

R* is independently selected from C_{1-12} alkyl, C_{2-12} alkenyl, or C_{2-12} alkyne; and

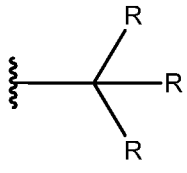
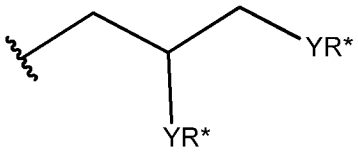
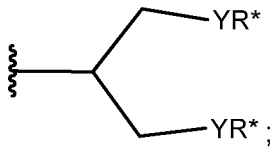
R_a and R_b are independently selected from H, C_{1-6} alkyl or C_{1-6} halogenated alkyl.

In an implementation scheme, R_1 in Formula (I) is -C_{1-6} alkylene-X, herein X is selected from amino, hydroxyl, acetylenyl or cyano;

R_2 is C_{3-20} alkylene;

R_3 is a C_{5-20} alkylene;

M is independently selected from -CH_2-, -CH=CH-, -C≡C-, -NH-, -C(O)-, -C(S)-, -O-, -S-, -C(O)O-, -OC(O)-, -C(O)NH-, -NHC(O)-, -C(O)-S-, -S-C(O)-, -C(S)-O-, -O-C(S)-, -C(S)-S-, -S-C(S)-, -S-S-, -C(S)NH-, or -NHC(S)-;

R_4 is  ,  , or  ;

herein R is selected from H, R* or -$(CH_2)_{1-5}$-YR*;

Y is selected from -NH-, -C(O)-, -C(S)-, -O-, or -S-; and

R* is independently selected from C_{1-12} alkyl, C_{2-12} alkenyl, or C_{2-12} alkynyl.

In an implementation scheme of Formula (I), R_1 is -C_{1-6} alkylene-OH, preferably -C_{2-6} alkylene-OH, preferably -C_{2-4} alkylene-OH, and preferably -C_2 alkylene-OH.

In an implementation scheme of Formula (I), R_2 is C_{3-20} alkylene, preferably C_{3-11} alkylene, preferably C_{7-11} alkylene, and preferably C_{5-11} alkylene.

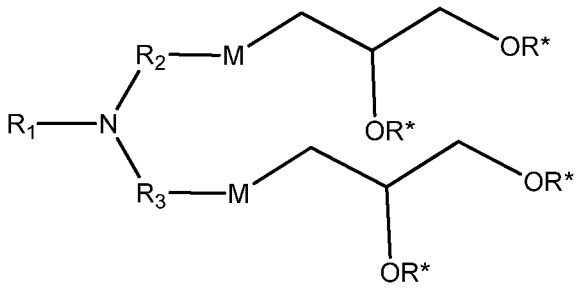
In an implementation scheme of Formula (I), R_3 is C_{5-20} alkylene, preferably C_{7-20} alkylene, preferably C_{7-11} alkylene, preferably C_{5-11} alkylene, preferably C_{7-9} alkylene, and preferably C_7 alkylene.

In an implementation scheme of Formula (I), each M is independently -C(O)O-, -OC(O)-, -C(O)NH- or -NHC(O)-, preferably -C(O)O- or -OC(O)-, and preferably -C(O)O-.

In an implementation scheme of Formula (I), each Y is independently selected from -O-.

In an implementation scheme of Formula (I), each R* is independently selected from C_{1-11} alkyl, herein one or more methylenes are each independently substituted by the following groups: -CH=CH-, and -C≡C-, preferably C_{1-8} alkyl, preferably C_{2-8} alkyl, preferably C_{1-6} alkyl, preferably C_{2-6} alkyl, and preferably C_6 alkyl.

In an implementation scheme, the application discloses a lipid compound in Formula (II) below, or a salt or isomer thereof:



(II)

herein, the definitions of R_1, R_2, R_3, M, and R* are the same as in Formula (I).

In an implementation scheme of Formula (II), R_1 is -C_{2-6} alkylene-OH;

M is selected from -CH_2-, -CH=CH-, -C≡C-, -NH-, -C(O)-, -C(S)-, -O-, -S-, -C(O)O-, -OC(O)-, -C(O)NH-, -NHC(O)-, -C(O)-S-, -S-C(O)-, -C(S)-O-, -O-C(S)-, -C(S)-S-, -S-C(S)-, -S-S-, -C(S)NH-, -NHC(S)-, or -O-P(O)(OH)-O-;

R_2 is C_{3-20} alkylene;

R_3 is C_{7-20} alkylene;

R* is independently selected from C_{1-11} alkyl, herein one or more methylenes are each independently substituted by the following groups: -CH=CH- or -C≡C-, preferably C_{1-8} alkyl, preferably C_{2-8} alkyl, preferably C_{1-6} alkyl, preferably C_{2-6} alkyl, and preferably C_6 alkyl.

In an implementation scheme of Formula (II), R_1 is -C_{2-6} alkylene-OH; M is selected from

-C(O)O-, -OC(O)-, -C(O)NH-, or -NHC(O)-; R_2 is C_{3-11} alkylene; R_3 is C_{7-11} alkylene; and R* is independently selected from C_{1-6} alkyl.

In an implementation scheme of Formula (II), R_1 is -C_{2-4} alkylene-OH; M is selected from -C(O)O-, -OC(O)-, -C(O)NH-, or -NHC(O)-; R_2 is C_{7-11} alkylene; R_3 is C_{7-9} alkylene; and R* is independently selected from C_{1-6} alkyl.

In an implementation scheme of Formula (II), R_1 is -C2 alkylene-OH; M is -C(O)O- or -OC(O)-; R_2 is C_{7-11} alkylene; R_3 is C_{7-9} alkylene; and R* is independently selected from C_{1-6} alkyl.

In an implementation scheme of Formula (II), R_1 is -C_2 alkylene-OH; M is -C(O)O- or -OC(O)-; R_2 is C_{7-11} alkylene; R_3 is C_7 alkylene; and R* is independently selected from C_6 alkyl.

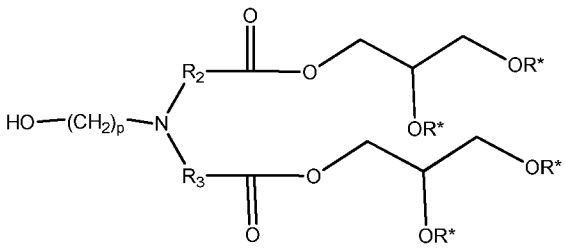
In an implementation scheme of Formula (II), R_1 is -C_2 alkylene-OH. In an implementation scheme, R_1 is -C_3 alkylene-OH. In an implementation scheme, R_1 is -C_4 alkylene-OH. In an implementation scheme, R_1 is -C_5 alkylene-OH. In an implementation scheme, R_1 is -C_6 alkylene-OH.

In an implementation scheme of Formula (II), M is independently selected from -C(O)O-.

In an implementation scheme of Formula (II), R_2 is independently selected from C_{3-11} alkylene, and R_3 is independently selected from C_{7-11} alkylene. In an implementation scheme, R_2 is independently C_3 alkylene. In an implementation scheme, R_2 is independently C_4 alkylene. In an implementation scheme, R_2 is independently C_5 alkylene. In an implementation scheme, R_2 is independently C_6 alkylene. In an implementation scheme, R_2 is independently C_7 alkylene. In an implementation scheme, R_2 is independently C_8 alkylene. In an implementation scheme, R_2 is independently C_9 alkylene. In an implementation scheme, R_2 is independently C_{10} alkylene. In an implementation scheme, R_2 is independently C_{11} alkylene. In an implementation scheme, R_3 is independently C_7 alkylene. In an implementation scheme, R_3 is independently C_8 alkylene. In an implementation scheme, R_3 is independently C_9 alkylene. In an implementation scheme, R_3 is independently C_{10} alkylene. In an implementation scheme, R_3 is independently C_{11} alkylene.

In an implementation scheme of Formula (II), R* is independently selected from C_{1-6} alkyl. In an implementation scheme, R* is independently selected from C_{4-6} alkyl. In an implementation scheme, R* is all C_6 alkyl.

In an implementation scheme, the application discloses a lipid compound in Formula (III) below, or a salt or isomer thereof:



(III)

herein, the definitions of R_2, R_3, and R* are the same as in Formula (I), and p is an integer of 2-6.

In an implementation scheme of Formula (III), p is 2; R_2 is C_{7-11} alkylene; R_3 is C_{7-9} alkylene; and R* is independently selected from C_{1-6} alkyl.

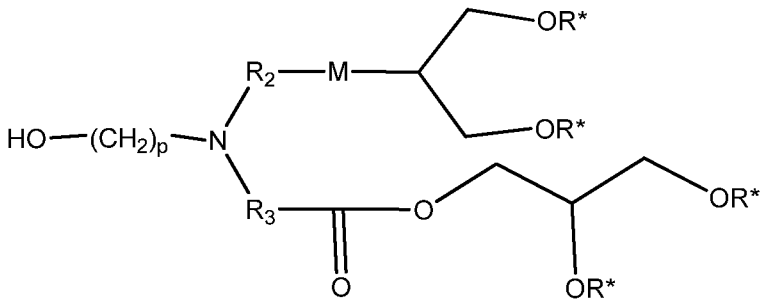
In an implementation scheme of Formula (III), p is 2; R_2 is C_{7-11} alkylene; R_3 is C_7 alkylene; and R* is independently C_6 alkyl.

In an implementation scheme of Formula (III), R_2 is independently selected from C_{7-11} alkylene, and R_3 is independently selected from C_{7-9} alkylene. In an implementation scheme, R_2 is independently C_7 alkylene. In an implementation scheme, R_2 is independently C_8 alkylene. In an

implementation scheme, R_2 is independently C_9 alkylene. In an implementation scheme, R_2 is independently C_{10} alkylene. In an implementation scheme, R_2 is independently C_{11} alkylene. In an implementation scheme, R_3 is independently C_7 alkylene. In an implementation scheme, R_3 is independently C_8 alkylene. In an implementation scheme, R_3 is independently C_9 alkylene.

In an implementation scheme of Formula (III), R* is independently selected from C_{1-6} alkyl. In an implementation scheme, R* is independently selected from C_{4-6} alkyl. In an implementation scheme, R* is all C_6 alkyl.

In an implementation scheme, the application discloses a lipid compound in Formula (IV) below, or a salt or isomer thereof:



(IV)

herein, the definitions of R_2, R_3, M, and R* are the same as in Formula (I), and p is an integer of 2-6.

In an implementation scheme of Formula (IV), p is 2-4;

M is selected from $-CH_2-$, -CH=CH-, -C≡C-, -NH-, -C(O)-, -C(S)-, -O-, -S-, -C(O)O-, -OC(O)-, -C(O)NH-, -NHC(O)-, -C(O)-S-, -S-C(O)-, -C(S)-O-, -O-C(S)-, -C(S)-S-, -S-C(S)-, -S-S-, -C(S)NH-, -NHC(S)-, or -O-P(O)(OH)-O-;

R_2 is C_{3-20} alkylene;

R_3 is a C_{7-20} alkylene;

R* is independently selected from C_{1-11} alkyl, herein one or more methylenes are each independently substituted by the following groups: -CH=CH- or -C≡C-, preferably C_{1-8} alkyl, preferably C_{2-8} alkyl, preferably C_{1-6} alkyl, preferably C_{2-6} alkyl, and preferably C_6 alkyl.

In an implementation scheme of Formula (IV), p is 2-4; M is selected from -C(O)O-, -OC(O)-, -C(O)NH-, or -NHC(O)-; R_2 and R_3 are independently selected from C_{5-11} alkylene; and R* is independently selected from C_{1-6} alkyl.

In an implementation scheme of Formula (IV), p is 2-4; M is -C(O)O- or -OC(O)-; R_2 is C_{7-11} alkylene; R_3 is C_{7-9} alkylene; and R* is independently selected from C_{1-6} alkyl.

In an implementation scheme, the application discloses a composition, and it includes the ionizable lipid compound of the application. In an implementation scheme, the composition further includes a phospholipid. In an implementation scheme, the phospholipid is selected from at least one of 1,2-dilinoleoyl-sn-glycerol-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycerol-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycerol-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycerol-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycerol-3-phosphocholine (DSPC), 1,2-diundecanoyl-sn-glycerol-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycerol -3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterol semisuccinyl-sn-glycerol -3-phosphocholine (OChemsPC), 1-hexadecyl-sn-glycerol-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenyl-sn-glycerol-3-phosphocholine, 1,2-diarachidonoyl-sn-glycerol-3-phosphocholine,

1,2-didocosahexaenoyl-sn-glycerol-3-phosphocholine, 1,2-dioleoyl-sn-glycerol-3-phosphate ethanolamine (DOPE), 1,2-diphytanyl-sn-glycerol-3-phosphate ethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycerol-3-phosphate ethanolamine, 1,2-dilinoleoyl-sn-glycerol-3-phosphate ethanolamine, 1,2-dilinolenyl-sn-glycerol-3-phosphate ethanolamine, 1,2-diarachidonoyl-sn-glycerol-3-phosphate ethanolamine, 1,2-didocosahexaenoyl-sn-glycerol-3-phosphate ethanolamine, 1,2-dioleoyl-sn-glycerol-3-phosphate-rac-(1-glycerol) sodium salt (DOPG), dipalmitoyl phosphatidyl glycerol (DPPG), palmitoyl oleoyl phosphatidyl ethanolamine (POPE), distearoyl-phosphatidyl-ethanolamine (DSPE), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanol amine (DMPE), 1-stearoyl-2-oleoyl-phosphatidyl ethanolamine (SOPE), 1-stearoyl-2-oleoyl-phosphatidyl choline (SOPC), sphingomyelin, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, phosphatidic acid, palmitoyl oleoyl phosphatidyl choline, lysophosphatidyl choline, and lysophosphatidyl ethanolamine (LPE).

In an implementation scheme, the composition further includes a pegylated lipid compound. In an implementation scheme, the pegylated lipid compound is selected from at least one of a PEG modified phosphatidyl ethanolamine, a PEG modified phosphatidic acid, a PEG modified ceramide, a PEG modified dialkylamine, a PEG modified diacylglycerol, and a PEG modified dialkyglycerol.

In an implementation scheme, the composition further includes a structural lipid. In an implementation scheme, the structural lipid is selected from at least one of cholesterol, coprosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, ursolic acid, and α-tocopherol.

In an implementation scheme, the composition further includes an active ingredient, and the active ingredient is selected from at least one of DNA, RNA, protein, and drug active molecules. In an implementation scheme, the RNA is selected from at least one of mRNA, siRNA, aiRNA, miRNA, dsRNA, aRNA, and lncRNA. In an implementation scheme, the protein is selected from at least one of an antibody, an enzyme, a recombinant protein, a polypeptide, and an oligopeptide.

In an implementation scheme of the composition of the application, calculated by molar unit, the ionizable lipid compound of the application is 20%-80%, the pegylated lipid compound is 1% -10%, the structural lipid is 10% -50%, and the phospholipid is 5-30%.

In an implementation scheme, the application discloses a lipid nanoparticle, and it contains the composition of the application.

In an implementation scheme, the application discloses a method for preparing the lipid nanoparticle, and it includes Step (1): the ionizable lipid compound of the application is optionally dissolved and mixed with the pegylated lipid compound, the structural lipid, and the phospholipid. Optionally, the method also includes Step (2): the lipid nanoparticle is prepared by mixing with the active ingredient through a mixer.

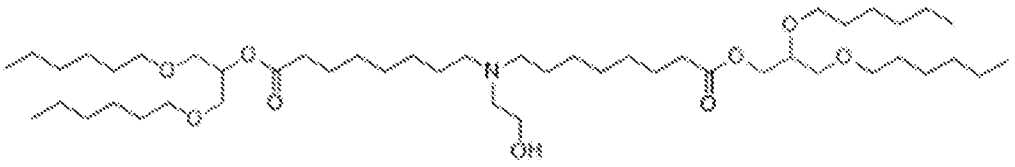
In an implementation scheme, the application discloses a use of the ionizable lipid compound of the application in preparation of the lipid nanoparticle. Optionally, the lipid nanoparticle is neutral and uncharged in a neutral medium, and positively charged after protonation in an acidic medium.

In an implementation scheme, the application discloses a pharmaceutical composition, and it includes the lipid nanoparticle of the application and a pharmaceutically acceptable carrier.

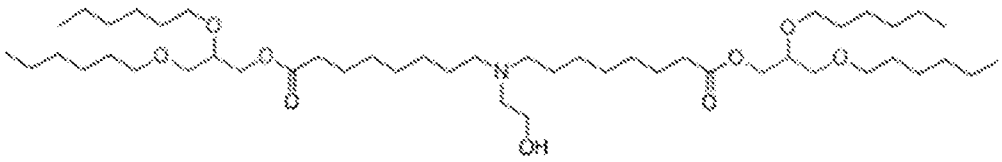
In an implementation scheme, the application discloses a use of the lipid nanoparticle or the pharmaceutical composition of the application in preparation of a drug. In an implementation scheme, the drug contains an active ingredient, and the active ingredient is selected from at least one of DNA, RNA, protein, or drug active molecules. In an implementation scheme, the RNA is

selected from at least one of mRNA, siRNA, aiRNA, miRNA, dsRNA, aRNA, and lncRNA. In an implementation scheme, the protein is selected from at least one of an antibody, an enzyme, a recombinant protein, a polypeptide, and an oligopeptide. In an implementation scheme, the drug is used for a person by intravenous injection, intramuscular injection, subcutaneous injection, microneedle patch, oral administration, oral and nasal spray, and daubing.

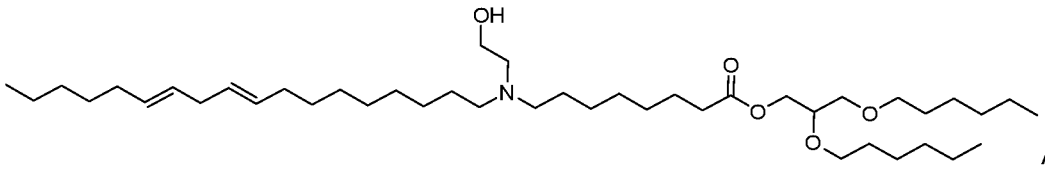
In an implementation scheme, the chemical formula of the ionizable lipid compound of the application is as follows:



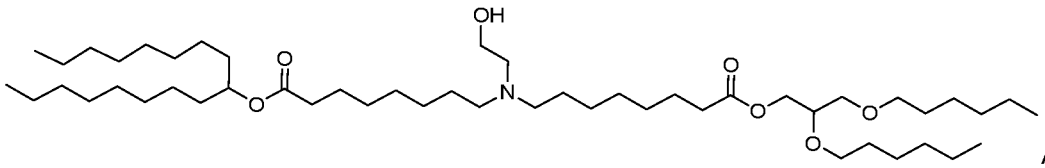
A1,



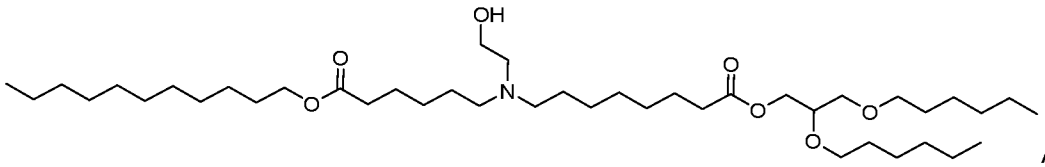
A2,



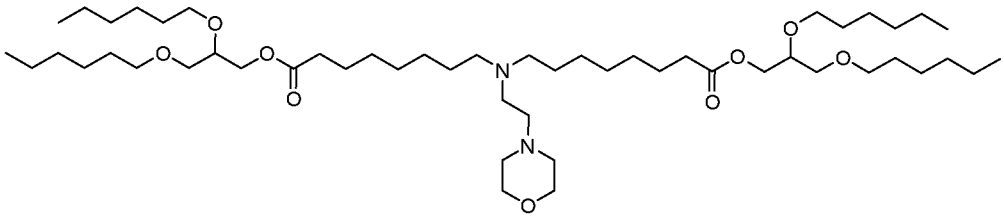
A3,



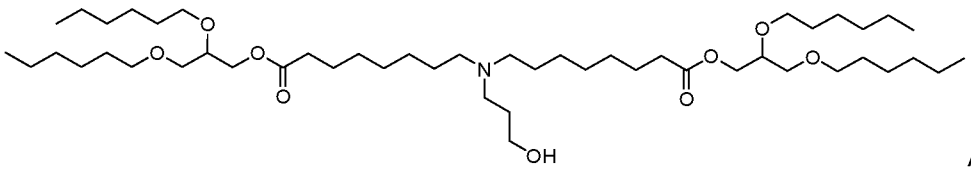
A4,



A5,



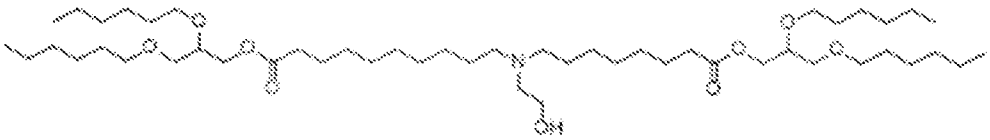
A6,



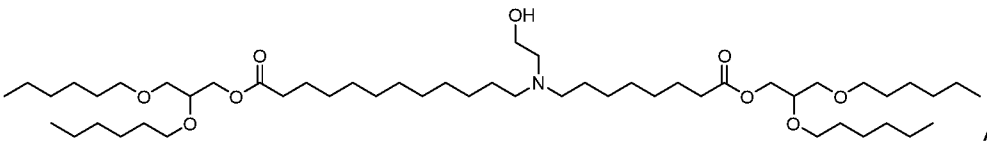
A7,

A8,

A9,

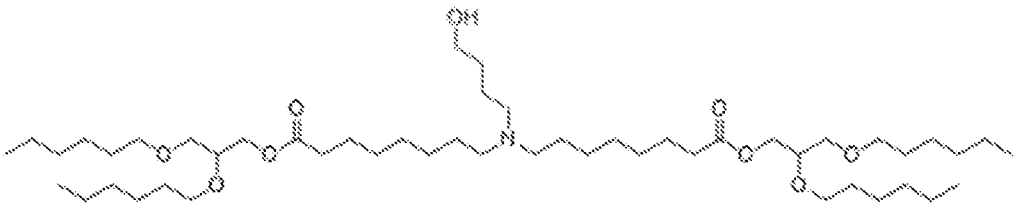


A10,

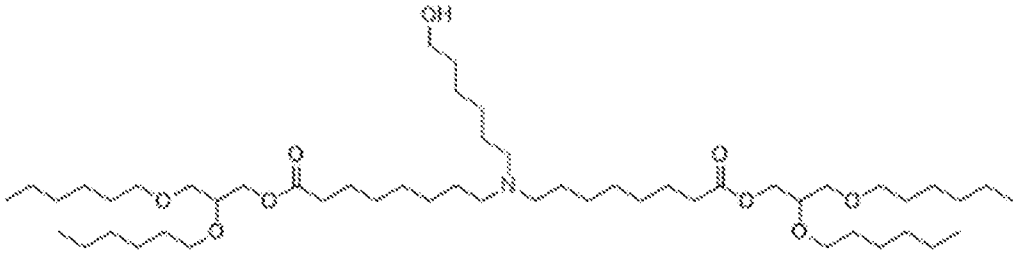


A11,

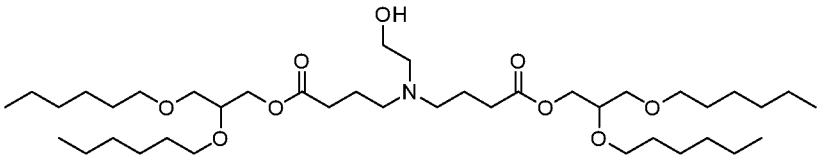
A12,



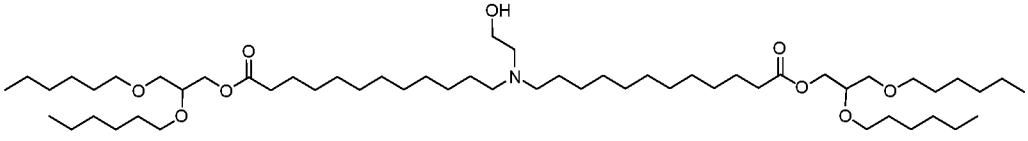
A13,



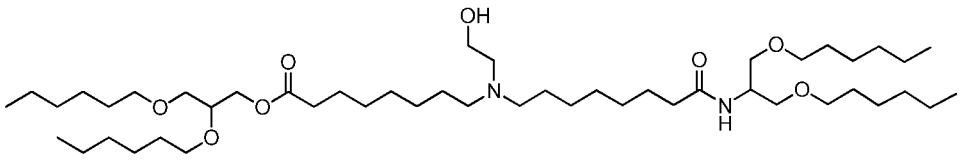
A14,



A15,



A16,



A17.

Compared with the prior art, the difference from the ionizable lipid in the present application is as follows.

1. Different chemical structures: Two or one of the fatty chains containing an ester group are connected to a nitrogen atom (N) of a tertiary amine, respectively, through the hydroxyl group at position 1 or 3 of glycerol, to achieve connection with saturated or unsaturated fatty chains, thereby forming a novel fatty chain containing an ether bond. And a result shows the better efficiency in ionizable lipid transfection; and

2. Simple synthesis and easy availability of raw materials: the starting raw materials are glycerol, short fatty alcohol, and fatty acid, the raw materials are cheap and easy to obtain, and the synthesis is simple.

Brief Description of the Drawings

Figs. 1A-1D are schematic diagrams of the photon number per unit area (p/sec/cm^2) of legs and livers at different time points after exemplary compounds of the application are administered to each group of mice;

Fig. 2 is a schematic diagram of an IgG antibody in each group of the mice after the first immunization for 14 days (ns: $p>0.05$; and ****: $p<0.0001$); and

Fig. 3 is a schematic diagram of the IgG antibody in each group of the mice after the second immunization for 14 days (ns: $p>0.05$; and ***: $p<0.001$).

Fig. 4 is a schematic diagram of the secretion of T lymphocytes in the mice after vaccination with the vaccine formulations in each group (ns: $p>0.05$; *: $p<0.1$; **: $p<0.01$; ***: $p<0.001$).

Definition:

When a numerical range is listed, it is set to include each value and a sub-range within the range. For example, "C_{1-6}" includes C_1, C_2, C_3, C_4, C_5, C_6, C_{1-6}, C_{1-5}, C_{1-4}, C_{1-3}, C_{1-2}, C_{2-6}, C_{2-5}, C_{2-4}, C_{2-3}, C_{3-6}, C_{3-5}, C_{3-4}, C_{4-6}, C_{4-5}, and C_{5-6} carbon atoms.

"X-Y-membered" represents X-Y cyclic atoms contained. For example, "4-7-membered" represent 4-7 cyclic atoms, such as 4 cyclic atoms, 5 cyclic atoms, 6 cyclic atoms, or 7 cyclic atoms, the cyclic atom may be a carbon atom or a heterocyclic atom, such as nitrogen, oxygen, sulfur, boron, phosphorus, and silicon.

The term "alkyl" refers to a linear-chain or branched-chain saturated hydrocarbyl that includes one or more 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, "C_{1-12} alkyl" refers to a linear-chain or branched-chain saturated hydrocarbyl that includes 1-12 carbon atoms. "C_{2-18} alkyl" refers to an optionally substituted linear-chain or branched-chain saturated hydrocarbyl that includes 2-18 carbon atoms. Unless otherwise specified, the alkyl referred in this article refers to unsubstituted and substituted alkyls.

The term "alkenyl" or "chain alkenyl" refers to a linear-chain or branched-chain hydrocarbyl that contains two or more carbon atoms and at least one carbon-carbon double bond (for example, 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 may include one, two, three, four, or more carbon-carbon double bonds. Specifically, "C_{3-12} alkenyl" refers to a linear-chain or branched-chain hydrocarbyl that includes 3-12 carbon atoms and at least one carbon-carbon double bond. "C_{4-18} alkenyl" refers to a linear-chain or branched-chain hydrocarbyl that includes 4-18 carbon atoms and at least one carbon-carbon double bond. Unless

otherwise specified, the alkenyl referred in this article refers to unsubstituted and substituted alkenyls.

The term "halogenated" or "halogen" refers to fluorine (F), chlorine (Cl), bromine (Br), and iodine (I).

The term "halogenated alkyl" means that the above "alkyl" is substituted by one or more halogen groups. The exemplary halogenated alkyl includes but not limited to: $-CF_3$, $-CH_2F$, $-CHF_2$, $-CHFCH_2F$, $-CH_2CHF_2$, $-CF_2CF_3$, $-CCl_3$, $-CH_2Cl$, $-CHCl_2$, 2,2,2-trifluoro-1,1-dimethyl-ethyl, and the like.

The term "cycloalkyl" refers to a non-aromatic cyclic hydrocarbon group that is composed of cyclic carbon atoms and does not contain cyclic heteroatoms. The exemplary cycloalkyl includes but not limited to: cyclopropyl (C3), cyclopropanyl (C3), cyclobutyl (C4), cyclobutenyl (C4), cyclopentyl (C5), cyclopentenyl (C5), cyclohexyl (C6), cyclohexenyl (C6), cyclohexadienyl (C6), cycloheptyl (C7), cycloheptenyl (C7), cycloheptadienyl (C7), cycloheptrienyl (C7), and the like. The cycloalkyl group may be optionally substituted by one or more substituents, for example, it is substituted by 1 to 5 substituents, 1 to 3 substituents, or 1 substituent.

The term "heterocyclic group" refers to a non-aromatic cyclic group with heterocyclic atoms, herein each heteroatom is independently selected from nitrogen, oxygen, sulfur, boron, phosphorus, and silicon. In the heterocyclic group containing one or more nitrogen atoms, as long as the valence allows, the linkage point may be a carbon or nitrogen atom. The exemplary 3-membered heterocyclic group containing one heteroatom includes but not limited to: nitrogen heterocyclic propyl, oxygen heterocyclic propyl, and sulfur heterocyclic propyl (thiorenyl). The exemplary 4-membered heterocyclic group containing one heteroatom includes but not limited to: nitrogen heterocyclic butyl, oxygen heterocyclic butyl, and sulfur heterocyclic butyl. The exemplary 5-membered heterocyclic group containing one heteroatom includes but not limited to: tetrahydrofuran group, dihydrofuran group, tetrahydrothiophene group, dihydrothiophene group, pyrrolidyl group, dihydropyrrole group, and pyrrole-2,5-dione. The exemplary 5-membered heterocyclic group containing two heteroatoms includes but not limited to: dioxacyclopentyl, oxasulfuranyl, disulfuranyl, and oxazolidine-2-one. The exemplary 5-membered heterocyclic group containing three heteroatoms includes but not limited to: triazolinyl, oxadiazolinyl, and thiadiazolinyl. The exemplary 6-membered heterocyclic group containing one heteroatom includes but not limited to: piperidyl, tetrahydropyran group, dihydropyridine group, and thiacyclohexyl (thianyl). The exemplary 6-membered heterocyclic group containing two heteroatoms includes but not limited to: piperazine group, morpholine group, dithiacyclohexyl, and dioxanyl. The exemplary 6-membered heterocyclic group containing three heteroatoms includes but not limited to: hexahydrotriazinyl (triazinanyl). The exemplary 7-membered heterocyclic group containing one heteroatom includes but not limited to: azacycloheptyl, oxacycloheptyl, and thiacycloheptyl.

The term "aryl" refers to a mono-cyclic or multi-cyclic (for example, bicyclic) 4n+2 aromatic cyclic system (for example, with 6 or 10 π electrons shared in cyclic arrangement) group composed of cyclic carbon atoms and free of cyclic heteroatoms. In some implementation schemes, the aryl has six cyclic carbon atoms ("C_6 aryl"; for example, phenyl). In some implementation schemes, the aryl has ten cyclic carbon atoms ("C_{10} aryl"; for example, naphthyl, such as 1-naphthyl and 2-naphthyl).

The term "heteroaryl" refers to a mono-cyclic or multi-cyclic 4n+2 aromatic cyclic system (for example, with 6 or 10 π electrons shared in cyclic arrangement) group with heteroatoms, herein each heteroatom is independently selected from nitrogen, oxygen, and sulfur. In the heteroaryl

containing one or more nitrogen atoms, as long as the valence allows, the linkage point may be a carbon or nitrogen atom. The heteroaryl bicyclic system may include one or more heteroatoms in one or two cycles. The heteroaryl also includes a cyclic system in which the above heteroaryl cycle is fused with one or more cycloalkyls or heterocyclic groups, and the linkage point is on the heteroaryl cycle. In this case, the number of carbon atoms continues to represent the number of carbon atoms in the heteroaryl cyclic system. The exemplary 5-membered heteroaryl containing one heteroatom includes but not limited to: pyrrolyl, furanyl, and thienyl. The exemplary 5-membered heteroaryl containing two heteroatoms includes but not limited to: imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, and isothiazolyl. The exemplary 5-membered heteroaryl containing three heteroatoms includes but not limited to: triazolyl, oxadiazolyl (for example, 1,2,4-oxadiazolyl), and thiadiazolyl. The exemplary 5-membered heteroaryl containing four heteroatoms includes but not limited to: tetrazolyl. The exemplary 6-membered heteroaryl containing one heteroatom includes but not limited to: pyridyl. The exemplary 6-membered heteroaryl containing two heteroatoms includes but not limited to: pyridazinyl, pyrimidyl, and pyrazinyl. The exemplary 6-membered heteroaryl containing three or four heteroatoms includes but not limited to: triazinyl and tetraazinyl. The exemplary 7-membered heteroaryl containing one heteroatom includes but not limited to: azacycloheptatrienyl, oxacycloheptaenyl, and thiacycloheptatrienyl. The exemplary 5,6-bicyclic heteroaryl includes but not limited to: indolyl, isoindolyl, indazolyl, benzotriazolyl, benzothiophenyl, isobenzothiophenyl, benzofuryl, benzoisofuranyl, benzimidazolyl, benzoxazolyl, benzoisoxazolyl, benzoxadiazolyl, benzothiazolyl, benzoisothiazolyl, benzothiadiazolyl, indanyl, and purinyl. The exemplary 6,6-dicyclic heteroaryl includes but not limited to: nalididyl, petrinyl, quinolyl, isoquinolyl, azolinyl, quinoxalinyl, phthalazinyl, and quinazolinyl.

The term "isomer" refers to a compound with the same molecular formula, including an enantiomer, a diastereomer, a stereoisomer, and the like. The application particularly prefers the stereoisomer, and the term "stereoisomer" refers to an isomer with only different atomic spatial arrangements.

In some cases, the compound of the application may form salts, and these salts are also within the scope of the application. The term "salt (one or more)" refers to acidic and/or basic salts formed with inorganic and/or organic acids and bases. The application particularly prefers pharmaceutically acceptable salts.

The term "pharmaceutically acceptable salts" refers to carboxylate and amino acid addition salts of the compound of the application, which are suitable for contact with patient tissues within reliable medical judgment and do not produce inappropriate toxicity, irritant effects, allergic reactions, and the like, and are effective for their intended applications, including (if possible) the zwitterionic form of the compound of the application, commensurate with a reasonable benefit/risk ratio.

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

Alkali addition salts of acidic compounds may be prepared by contacting the free acid form with a sufficient amount of alkalis required in a conventional manner to generate the salts. By contacting the salt form with acids in the conventional manner and then separating the free acid, the free acid may be regenerated. The free acid forms differ to some extent from their respective salt forms in

certain physical properties, such as solubility in polar solvents, but for the purpose of the application, the salts are still equivalent to their respective free acids.

The salt may be sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, nitrate, phosphate, monohydrogen phosphate, dihydrogen phosphate, metaphosphate, pyrophosphate, chloride, bromide, and iodide prepared from inorganic acids, such as hydrochloric acid, nitric acid, sulfuric acid, hydrobromic acid, hydroiodic acid, and phosphoric acid. The representative salts include: hydrobromate, hydrochloride, sulfate, bisulfate, nitrate, acetate, oxalate, valerate, oleate, palmitate, stearate, laurate, borate, benzoate, lactate, phosphate, toluenesulfonate, citrate, maleate, fumarate, succinate, tartrate, naphthalate, methanesulfonate, gluceptate, lactonate, laurylsulfonate, and isethionate, and the like. The salt may also be prepared from organic acids, such as aliphatic mono and dicarboxylic acid, phenyl-substituted alkanoic acid, hydroxyalkanoic acid, alkanedioic acid, aromatic acid, and aliphatic and aromatic sulfonic acid. The representative salts include acetate, propionate, octanoate, isobutyrate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, mandelate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, naphthalate, benzenesulfonate, tosylate, phenylacetate, citrate, lactate, maleate, tartrate, methanesulfonate, and the like. The pharmaceutically acceptable salts may include cations based on alkali metals and alkaline earth metals, such as sodium, lithium, potassium, calcium, and magnesium, 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. It also covers salts of amino acids, such as arginine salt, gluconate salt, and galacturonic acid salt. (for example, Berge S. M. et al.， "Pharmaceutical Salts," J. Pharm. Sci.， 1977; 66: 1-19, introduced as a reference).

The term "acceptable carrier" refers to a suitable carrier that may be used to utilize existing substances for the purpose of the application, without excessive toxicity, irritation, allergic reactions and the like, equivalent to having a reasonable benefit/risk ratio.

The following abbreviations may be used in embodiments and entire description:

AOP: 7-azobenzotriazole-1-yl-oxy-tris(dimethylamino)phosphine hexafluorophosphate,

BOP: Benzotriazole-1-yl-oxy-tris(dimethylamino)phosphine hexafluorophosphate,

BOP-Cl: Bis(2-oxo-3-oxazolidinyl)hypophosphoryl chloride,

BDMP: 1-N-methylpyrroliolinium-3-oxy-benzotriazole hexachloroantimony salt,

BMMP: 1-N-ethylpyrrolidonium-3-oxy-benzotriazole hexachloroantimony salt,

BOMI: 1-tert-butoxycarbonyl-5-methoxy-indole-2-yl-boronic acid,

CDI: 1,1'-carbonyldiimidazole,

CDMT: 2-chloro-4,6-dimethoxy-1,3,5-triazine,

DCC: Dicyclohexylcarbodiimide,

DCMT: 4,5-dichloro-2- (methylthio)pyrimidine,

DECP: Diethyl cyanophosphonate,

DIC: N,N'-diisopropylcarbodiimide,

DPPA: Diphenylphosphoryl azide,

DPP-Cl: Diphenylphosphoryl chloride,

EDC: 1-ethyl-(3-dimethylaminopropyl)carbodiimide,

EDCI: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride,

EEDQ: 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline,

EMPA: Ethylmethylphosphinic anhydride,

HATU: O-(7-azobenzotriazole-1-yl)-di(dimethylamino) carbonium hexafluorophosphate,

HBTU: O-(benzotriazole-1-yl)-di(dimethylamino)carbonium hexafluorophosphate,

HCTU: O-(5-chlorobenzotriazole-1-yl)-di(dimethylamino)carbonium hexafluorophosphate,

HAPyU: O-(7-azobenzotriazole-1-yl)-di(tetrahydropyrrolyl)carbonium hexafluorophosphate,

HBPyU: O-(benzotriazole-1-yl)-di(tetrahydropyrrolyl)carbonium hexafluorophosphate,

MPTA: Thiodimethylphosphoryl azide,

PyAOP: 7-azobenzotriazole-1-yl-oxy-tris(tetrahydropyrrolyl) phosphonium hexafluorophosphate,

PyBOP: Benzotriazole-1-yl-oxy-tris(tetrahydropyrrolyl) phosphonium hexafluorophosphate,

T3P: n-propyl phosphonic anhydride,

TBTU: O-(benzotriazole-1-yl)-di(dimethylamino)carbonium tetrafluoroborate,

TNTU: O-(N-endo-5-norcamphene-2,3-dicarbodiimide)-di(dimethylamino)carbonium tetrafluoroborate,

TSTU: O-(N-succinimide)-di(dimethylamino)carbonium tetrafluoroborate,

DMAP: 4-dimethylaminopyridine,

HOAt: 1-hydroxy-7-azobenzotriazole,

HOBt: 1-hydroxybenzotriazole,

HOSu: N-hydroxysuccinimide,

4-PPY: 4-pyrrolidylpyridine,

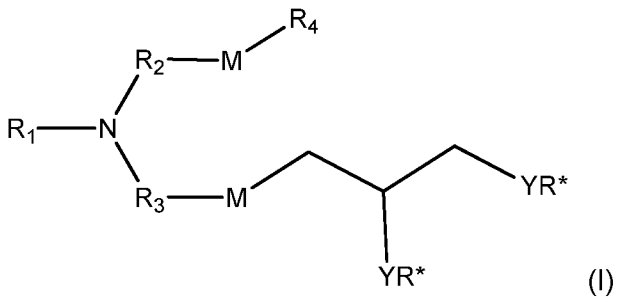
9-AJ: 9-azajulolidine,

TMAJ: 1,1,7,7-tetramethyl-9-azajulolidine,

NHPI: N-hydroxyphthalimide.

Specific Implementation Scheme

Scheme 1. A compound in Formula (I), or a salt or isomer thereof:



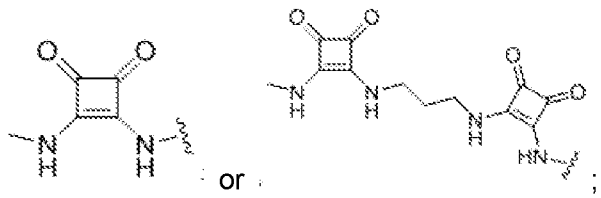
(I)

herein,

R_1 is -C_{1-6} alkylene-X,

herein X is selected from amino, hydroxyl, acetylenyl, 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, and H atoms on the C_{3-7} cycloalkyl, the 4-7-membered heterocyclic group, the C_{6-10} aryl, and the 5-10-membered heteroaryl groups are optionally substituted by the following groups: -$(CH_2)_{1-3}OH$, -$(CH_2)_{1-3}NR_aR_b$ or -$(CH_2)_{1-3}C(O)NR_aR_b$;

or X is:

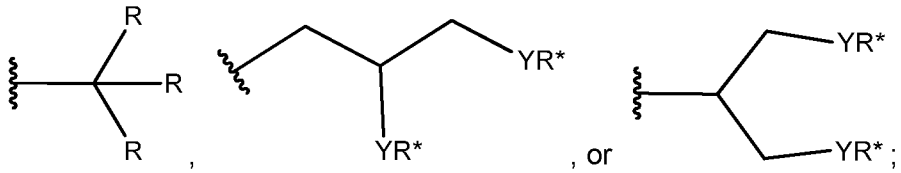


;

R_2 is C_{3-20} alkylene, herein one or more methylenes in R_2 are each independently substituted by the following groups: -CH=CH-, -C≡C -, or -O-;

R_3 is C_{5-20} alkylene, herein one or more methylenes in R_3 are each independently substituted by the following groups: -CH=CH-, -C≡C-, or -O-;

M is independently selected from -CH_2-, -CH=CH-, -C≡C-, -NH-, -C(O)-, -C(S)-, -O-, -S-, -C(O)O-, -OC(O)-, -C(O)NH-, -NHC(O)-, -C(O)-S-, -S-C(O)-, -C(S)-O-, -O-C(S)-, -C(S)-S-, -S-C(S)-, -S-S-, -C(S)NH-, -NHC(S)- or -O-P(O)(OH)-O-;

R_4 is 

;

herein R is selected from H, R* or -$(CH_2)_{1-5}$-YR*;

Y is selected from -NH-, -C(O)-, -C(S)-, -O-, -S-, -C(O)O-, -OC(O)-, -C(O)NH-, -NHC(O)-, -C(O)-S-, -S-C(O)-, -C(S)-O-, -O-C(S)-, -C(S)-S-, -S-C(S)-, -S-S-, -C(S)NH-, -NHC(S)-, or -O-P(O)(OH)-O-;

R* is independently selected from C_{1-12} alkyl, C_{2-12} alkenyl, or C_{2-12} alkyne; and

R_a and R_b are independently selected from H, C_{1-6} alkyl or C_{1-6} halogenated alkyl.

Scheme 2. The compound in Formula (I) or the salt or isomer thereof according to Scheme 1, herein,

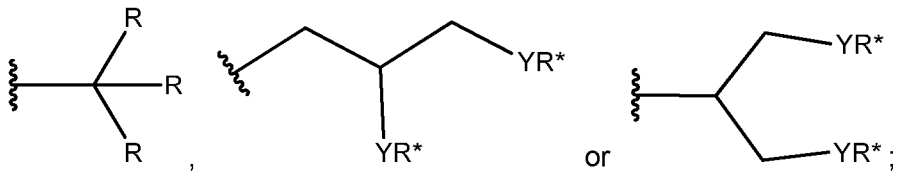
R_1 is -C_{1-6} alkylene-X,

herein X is selected from amino, hydroxyl, acetylenyl or cyano;

R_2 is C_{3-20} alkylene;

R_3 is a C_{5-20} alkylene;

M is independently selected from -CH_2-, -CH=CH-, -C≡C-, -NH-, -C(O)-, -C(S)-, -O-, -S-, -C(O)O-, -OC(O)-, -C(O)NH-, -NHC(O)-, -C(O)-S-, -S-C(O)-, -C(S)-O-, -O-C(S)-, -C(S)-S-, -S-C(S)-, -S-S-, -C(S)NH-, or -NHC(S)-;

R_4 is 

;

herein R is selected from H, R* or -$(CH_2)_{1-5}$-YR*;

Y is selected from -NH-, -C(O)-, -C(S)-, -O-, or -S-; and

R* is independently selected from C_{1-12} alkyl, C_{2-12} alkenyl, or C_{2-12} alkynyl.

Scheme 3. The compound or the salt or isomer thereof according to any one of Schemes 1-2, herein R_1 is -C_{1-6} alkylene-OH, preferably -C_{2-6} alkylene-OH, preferably -C_{2-4} alkylene-OH, and preferably -C_2 alkylene-OH.

Scheme 4. The compound or the salt or isomer thereof according to any one of Schemes 1-3, herein R_2 is C_{3-20} alkylene, preferably C_{3-11} alkylene, preferably C_{5-11} alkylene, and preferably C_{7-11} alkylene.

Scheme 5. The compound or the salt or isomer thereof according to any one of Schemes 1-4, herein R_3 is C_{5-20} alkylene, preferably C_{7-20} alkylene, preferably C_{7-11} alkylene, preferably C_{5-11} alkylene, preferably C_{7-9} alkylene, and preferably C_7 alkylene.

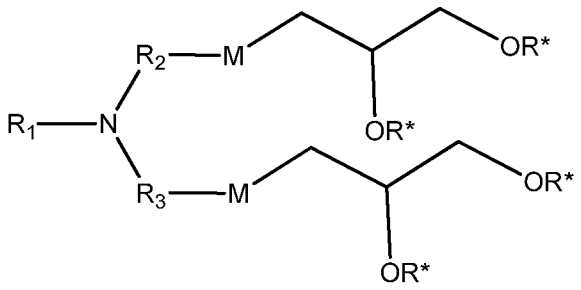
Scheme 6. The compound or the salt or isomer thereof according to any one of Schemes 1-5,

herein each M is independently -C(O)O-, -OC(O)-, -C(O)NH- or -NHC(O)-, preferably -C(O)O- or -OC(O)-, and preferably -C(O)O-.

Scheme 7. The compound or the salt or isomer thereof according to any one of Schemes 1-6, herein each Y is independently selected from -O-.

Scheme 8. The compound or the salt or isomer thereof according to any one of Schemes 1-7, herein each R* is independently selected from C_{1-11} alkyl, herein one or more methylenes are each independently substituted by the following groups: -CH=CH-, and -C≡C-, preferably C_{1-8} alkyl, preferably C_{2-8} alkyl, preferably C_{1-6} alkyl, preferably C_{2-6} alkyl, and preferably C_6 alkyl.

Scheme 9. The compound or the salt or isomer thereof according to Scheme 1, herein the compound has a Formula (II):



(II)

herein,

R_1 is -C_{2-6} alkylene-X;

M is selected from -CH_2-, -CH=CH-, -C≡C-, -NH-, -C(O)-, -C(S)-, -O-, -S-, -C(O)O-, -OC(O)-, -C(O)NH-, -NHC(O)-, -C(O)-S-, -S-C(O)-, -C(S)-O-, -O-C(S)-, -C(S)-S-, -S-C(S)-, -S-S-, -C(S)NH-, -NHC(S)-, or -O-P(O)(OH)-O-;

R_2 is C_{3-20} alkylene;

R_3 is C_{7-20} alkylene;

R* is independently selected from C_{1-11} alkyl, herein one or more methylenes in R* are each independently substituted by the following groups: -CH=CH-, and -C≡C-, preferably C_{1-8} alkyl, preferably C_{2-8} alkyl, preferably C_{1-6} alkyl, preferably C_{2-6} alkyl, and preferably C_6 alkyl.

Scheme 10. The compound in Formula (II) or the salt or isomer thereof according to Scheme 9, herein,

R_1 is -C_{2-6} alkylene-OH;

M is selected from -C(O)O-, -OC(O)-, -C(O)NH-, or -NHC(O)-;

R_2 is C_{3-11} alkylene;

R_3 is C_{7-11} alkylene; and

R* is independently selected from C_{1-6} alkyl.

Scheme 11. The compound in Formula (II) or the salt or isomer thereof according to Scheme 9, herein,

R_1 is -C_{2-4} alkylene-OH;

M is selected from -C(O)O-, -OC(O)-, -C(O)NH-, or -NHC(O)-;

R_2 is C_{7-11} alkylene;

R_3 is C_{7-9} alkylene; and

R* is independently selected from C_{1-6} alkyl.

Scheme 12. The compound in Formula (II) or the salt or isomer thereof according to Scheme 9, herein,

R_1 is -C_2 alkylene-OH;

M is -C(O)O- or -OC(O)-;

R_2 is C_{7-11} alkylene;

R_3 is C_{7-9} alkylene; and

R* is independently selected from C_{1-6} alkyl.

Scheme 13. The compound in Formula (II) or the salt or isomer thereof according to Scheme 9, herein,

R_1 is -C_2 alkylene-OH;

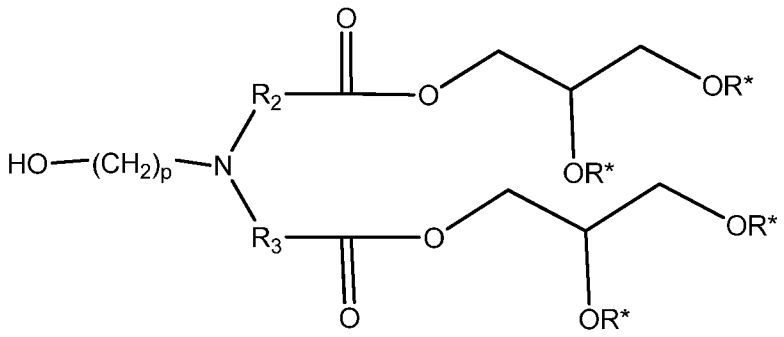
M is -C(O)O- or -OC(O)-;

R_2 is C_{7-11} alkylene;

R_3 is C_7 alkylene; and

R* is independently selected from C_6 alkyl.

Scheme 14. The compound or the salt or isomer thereof according to Scheme 1, herein the compound has a Formula (III):



(III)

herein,

p is 2;

R_2 is C_{7-11} alkylene;

R_3 is C_{7-9} alkylene; and

R* is independently selected from C_{1-6} alkyl.

Scheme 15. The compound in Formula (III) or the salt or isomer thereof according to Scheme 14, herein,

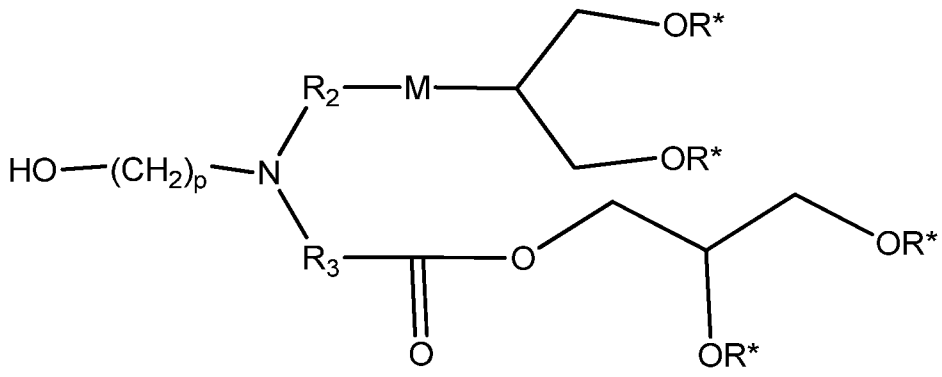
p is 2;

R_2 is C_{7-11} alkylene;

R_3 is C_7 alkylene; and

R* is independently selected from C_6 alkyl.

Scheme 16. The compound or the salt or isomer thereof according to Scheme 1, herein the compound has a Formula (IV):



(IV)

herein,

p is 2-4;

M is selected from -CH_2-, -CH=CH-, -C≡C-, -NH-, -C(O)-, -C(S)-, -O-, -S-, -C(O)O-, -OC(O)-, -C(O)NH-, -NHC(O)-, -C(O)-S-, -S-C(O)-, -C(S)-O-, -O-C(S)-, -C(S)-S-, -S-C(S)-, -S-S-, -C(S)NH-, -NHC(S)-, or -O-P(O)(OH)-O-;

R_2 is C_{3-20} alkylene;

R_3 is a C_{7-20} alkylene; and

R* is independently selected from C_{1-11} alkyl, herein one or more methylenes are each independently substituted by the following groups: -CH=CH- or -C≡C-, preferably C_{1-8} alkyl, preferably C_{2-8} alkyl, preferably C_{1-6} alkyl, preferably C_{2-6} alkyl, and preferably C_6 alkyl.

Scheme 17. The compound in Formula (IV) or the salt or isomer thereof according to Scheme 16, herein,

p is 2-4;

M is selected from -C(O)O-, -OC(O)-, -C(O)NH-, or -NHC(O)-;

R_2 and R_3 are independently selected from C_{5-11} alkylene; and

R* is independently selected from C_{1-6} alkyl.

Scheme 18. The compound in Formula (IV) or the salt or isomer thereof according to Scheme 16, herein,

p is 2-4;

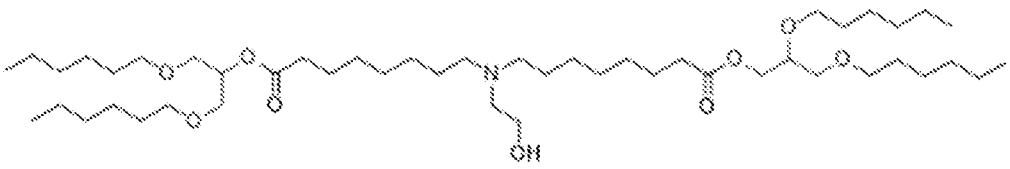
M is -C(O)O- or -OC(O)-;

R_2 is C_{7-11} alkylene;

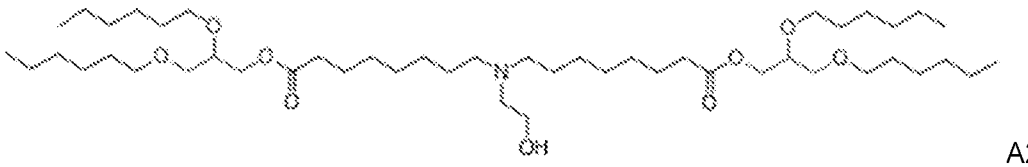
R_3 is C_{7-9} alkylene; and

R* is independently selected from C_{1-6} alkyl.

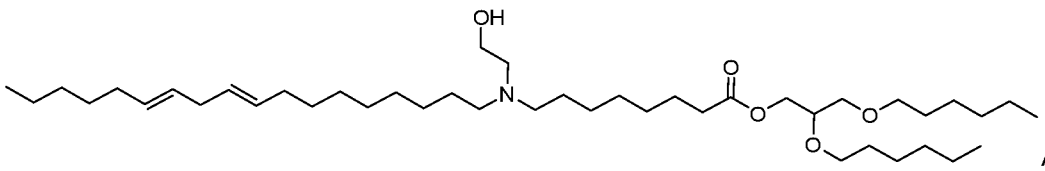
Scheme 19. A compound or a salt or isomer thereof, herein the compound is selected from any one of A1-A17:



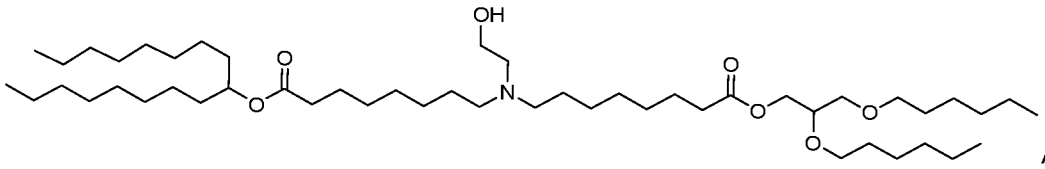
A1,



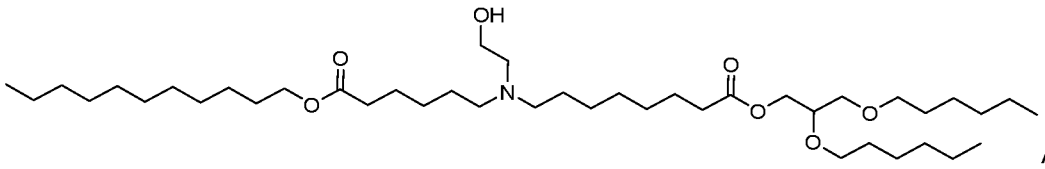
A2,



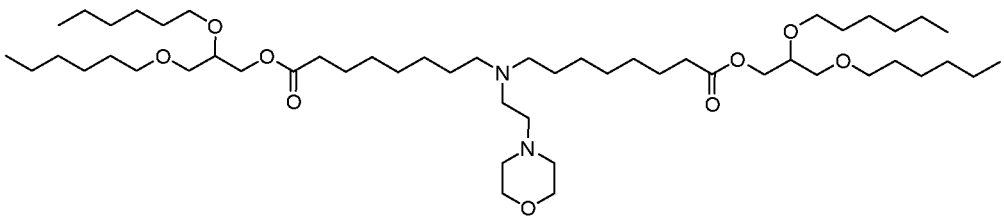
A3,



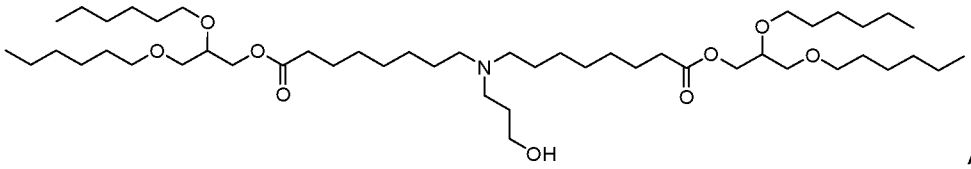
A4,



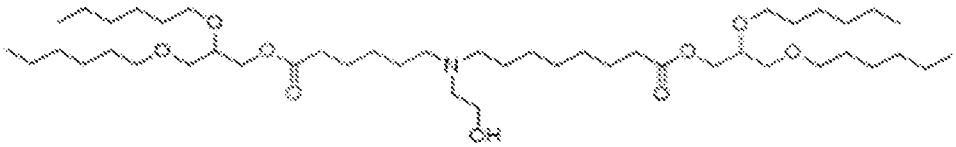
A5,



A6,

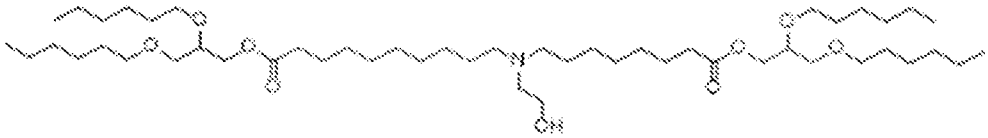


A7,

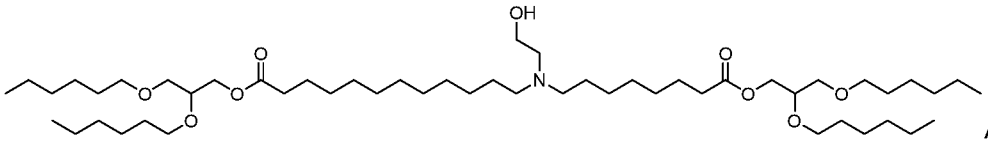


A8,

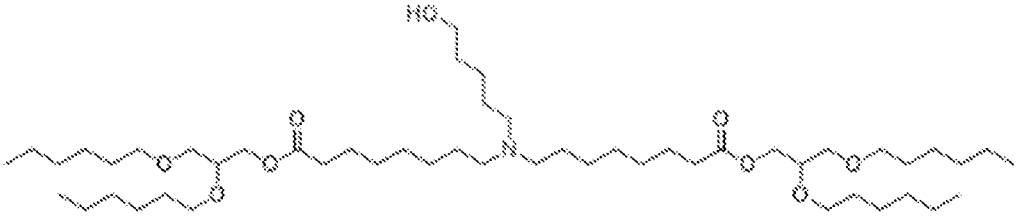
A9,



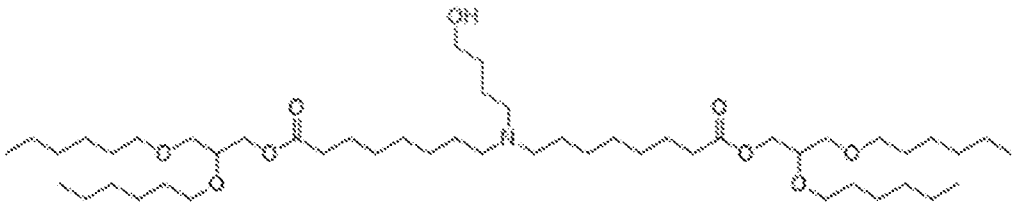
A10,



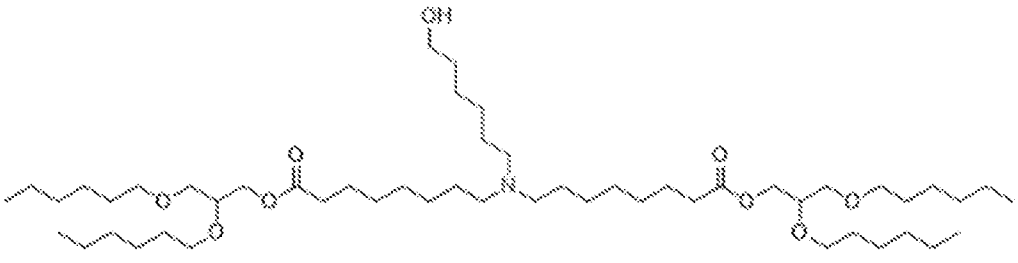
A11,

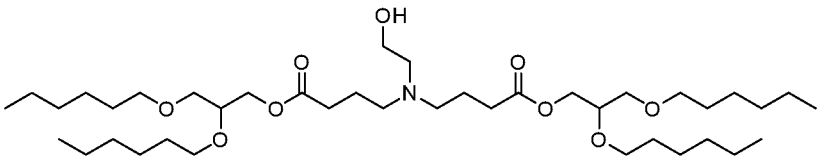


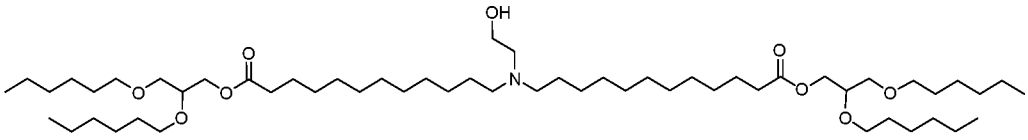
A12,

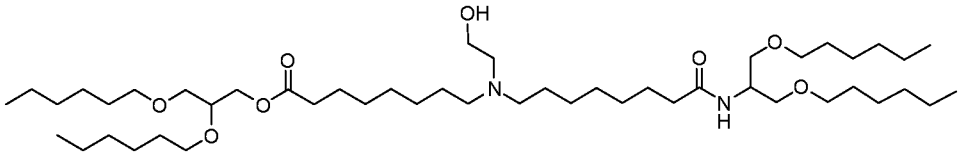


A13,


A14,


A15,


A16,


A17.

Scheme 20. A composition, wherein it includes the compound according to any one of Schemes 1-19.

Scheme 21. The composition according to Scheme 20, wherein it further includes a phospholipid.

Scheme 22. The composition according to Scheme 21, wherein the phospholipid is selected from at least one of 1,2-dilinoleoyl-sn-glycerol-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycerol-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycerol-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycerol-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycerol-3-phosphocholine (DSPC), 1,2-diundecanoyl-sn-glycerol-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycerol -3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterol semisuccinyl-sn-glycerol -3-phosphocholine (OChemsPC), 1-hexadecyl-sn-glycerol-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenyl-sn-glycerol-3-phosphocholine, 1,2-diarachidonoyl-sn-glycerol-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycerol-3-phosphocholine, 1,2-dioleoyl-sn-glycerol-3-phosphate ethanolamine (DOPE), 1,2-diphytanyl-sn-glycerol-3-phosphate ethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycerol-3-phosphate ethanolamine, 1,2-dilinoleoyl-sn-glycerol-3-phosphate ethanolamine, 1,2-dilinolenyl-sn-glycerol-3-phosphate ethanolamine, 1,2-diarachidonoyl-sn-glycerol-3-phosphate ethanolamine, 1,2-didocosahexaenoyl-sn-glycerol-3-phosphate ethanolamine, 1,2-dioleoyl-sn-glycerol-3-phosphate-rac-(1-glycerol) sodium salt (DOPG), dipalmitoyl phosphatidyl glycerol (DPPG), palmitoyl oleoyl phosphatidyl ethanolamine (POPE), distearoyl-phosphatidyl-ethanolamine (DSPE), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanol amine (DMPE), 1-stearoyl-2-oleoyl-phosphatidyl ethanolamine (SOPE), 1-stearoyl-2-oleoyl-phosphatidyl choline (SOPC), sphingomyelin, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, phosphatidic acid, palmitoyl

oleoyl phosphatidyl choline, lysophosphatidyl choline, and lysophosphatidyl ethanolamine (LPE).

Scheme 23. The composition according to any one of Schemes 20-22, wherein it further includes a pegylated lipid compound.

Scheme 24. The composition according to Scheme 23, wherein the pegylated lipid compound is selected from at least one of a PEG modified phosphatidyl ethanolamine, a PEG modified phosphatidic acid, a PEG modified ceramide, a PEG modified dialkylamine, a PEG modified diacylglycerol, and a PEG modified dialkyglycerol.

Scheme 25. The composition according to any one of Schemes 20-24, wherein it further includes a structural lipid.

Scheme 26. The composition according to Scheme 25, wherein the structural lipid is selected from at least one of cholesterol, coprosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, ursolic acid, and α-tocopherol.

Scheme 27. The composition according to any one of Schemes 20-26, wherein calculated by molar unit, the composition contains 20%-80% of the compound according to any one of Schemes 1-19, 1% -10% of the pegylated lipid compound, 10% -50% of the structural lipid, and 5-30% of the phospholipid.

Scheme 28. The composition according to Scheme 27, wherein the composition further includes an active ingredient, and the active ingredient is selected from at least one of DNA, RNA, protein, and drug active molecules.

Scheme 29. The composition according to Scheme 28, wherein the RNA is selected from at least one of mRNA, siRNA, aiRNA, miRNA, dsRNA, aRNA, and lncRNA.

Scheme 30. The composition according to Scheme 28, the protein is selected from at least one of an antibody, an enzyme, a recombinant protein, a polypeptide, and an oligopeptide.

Scheme 31. A lipid nanoparticle, it contains the composition according to any one of Schemes 20-30.

Scheme 32. A method for preparing the lipid nanoparticle according to Scheme 31, it includes Step (1): the compound according to Schemes 1-19 is optionally dissolved and mixed with the pegylated lipid compound, the structural lipid, and the phospholipid.

Scheme 33. The method according to Scheme 32, wherein it also includes Step (2): the lipid nanoparticle is formed by mixing with the active ingredient through a mixer.

Scheme 34. A use of the compound according to any one of Schemes 1-19 in preparation of the lipid nanoparticle.

Scheme 35. The use according to Scheme 34, wherein the lipid nanoparticle is neutral and uncharged in a neutral medium, and positively charged after protonation in an acidic medium.

Scheme 36. A pharmaceutical composition, it includes the lipid nanoparticle according to Scheme 31 and a pharmaceutically acceptable carrier.

Scheme 37. A use of the lipid nanoparticle according to Scheme 31 or the pharmaceutical composition according to Scheme 36 in preparation of a drug.

Scheme 38. The use according to Scheme 37, wherein the drug contains an active ingredient, and the active ingredient is selected from at least one of DNA, RNA, protein, or drug active molecules.

Scheme 39. The use according to Scheme 38, wherein the RNA is selected from at least one of mRNA, siRNA, aiRNA, miRNA, dsRNA, aRNA, and lncRNA.

Scheme 40. The use according to Scheme 38, wherein the protein is selected from at least one of an antibody, an enzyme, a recombinant protein, a polypeptide, and an oligopeptide.

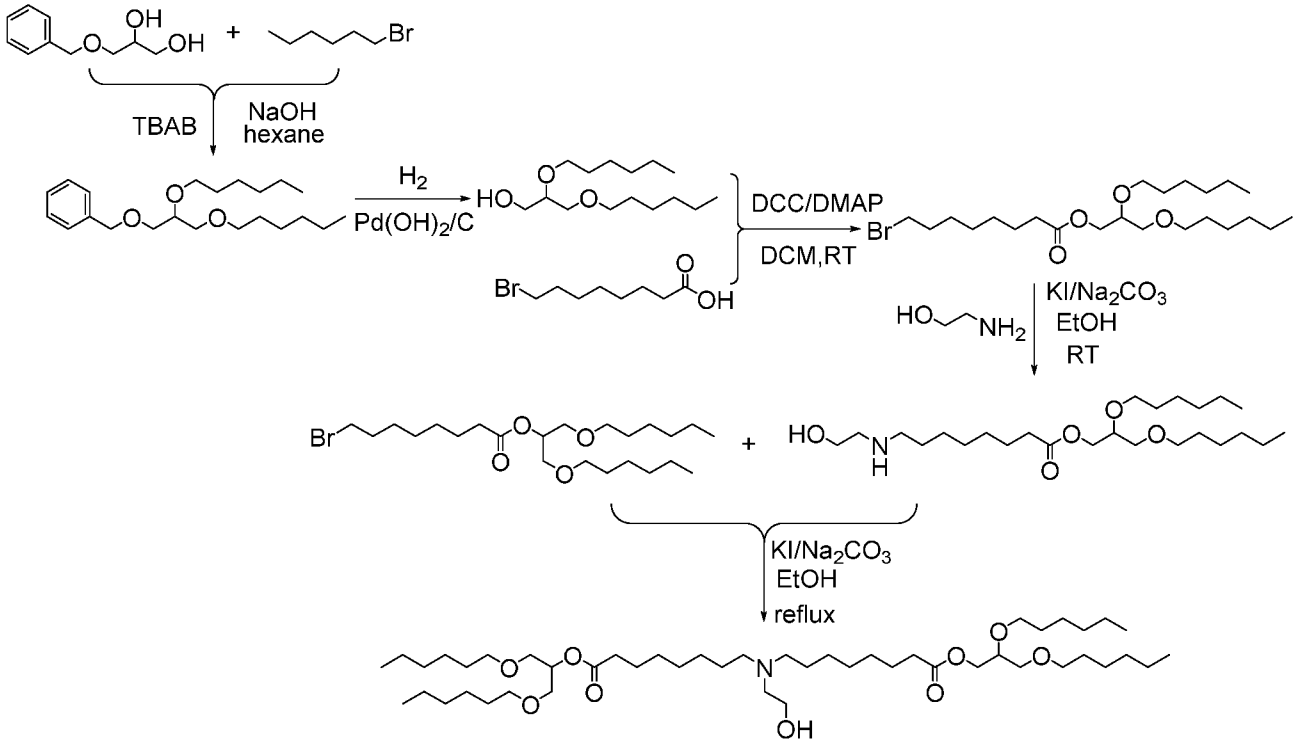
Scheme 41. The use according to any one of Schemes 37-40, wherein the drug is used for a person by intravenous injection, intramuscular injection, subcutaneous injection, microneedle patch, oral administration, oral and nasal spray, and daubing.

Detailed Description of the Embodiments

In order to make purposes, technical schemes, and advantages of the application clearer, the application is further described in detail below by embodiments and in combination with drawings. The following embodiments are only intended to describe the application and should not be considered as limiting the scope of the application. If specific conditions are not specified in the embodiments, it is implemented according to conventional conditions or conditions recommended by manufacturers. Reagents or instruments used without specifying the manufacturers are conventional products that may be obtained by market purchase.

Embodiment 1-Synthesis of A1

Synthesis route:



Synthesis step:

1. Synthesis of ((2,3-bis(hexoxy)propoxy)methyl)benzene

50 mL of n-hexane, 1-benzyloxy-2,3-propanediol (10.0 g), and tetrabutylammonium bromide (0.88 g) were added, and stirred for 10 min, then sodium hydroxide solution (18 mol/L) and bromohexane (36.2 g) were added, heated and stirred for a reaction. After the reaction was completed, it was cooled to a room temperature, and stilly placed for layering, an organic layer was collected and washed for extraction, and the organic layer was separated and purified through a column (n-heptane: ethyl acetate=5%), to obtain ((2,3-bis(hexoxy)propoxy)methyl)benzene.

2. Synthesis of 2,3-bis(hexoxy)-1-propanol

((2,3-bis(hexoxy)propoxy)methyl)benzene (15.0 g), ethyl acetate (50 mL), glacial acetic acid (3.75 mL), and carbon attached palladium hydroxide (20%, and 1.0 g) were added, and stirred at the room temperature for a hydrogenation reaction. After the reaction was completed, it was filtered, a filtrate was collected, and the filtrate was washed and extracted for liquid separation. An organic

phase was collected. It was concentrated under a reduced pressure until dryness, to obtain compound 2,3-bis(hexoxy)-1-propanol.

3. Synthesis of 2,3-bis(hexoxy)propyl-8-bromooctanoic acid ester

8-bromooctanoic acid (10.3 g) was dissolved in dichloromethane (100 mL), DCC (10.3 g), DMAP (6.1 g), and 2,3-bis(hexoxy)-1-propanol (10 g) were added, and stirred at the room temperature for a reaction. After the reaction was completed, it was concentrated to remove the dichloromethane solvent, and extracted for liquid separation. An organic phase was dried, and concentrated until dryness. A crude product was separated and purified through a column (petroleum ether (PE): ethyl acetate (EA)=20:1), to obtain 2,3-bis(hexoxy)propyl-8-bromooctanoic acid ester.

4. Synthesis of 2,3-bis(hexoxy)propyl-8-((2-hydroxyethyl)amino)octanoate

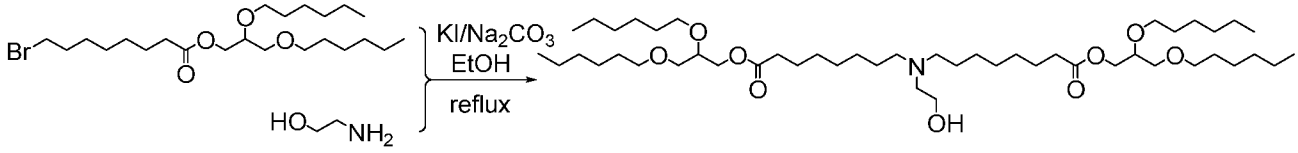
2,3-bis(hexoxy)propyl-8-bromooctanoic acid ester (5.0 g) was dissolved in anhydrous ethanol (50 mL), sodium carbonate (2.2 g), potassium iodide (KI) (0.17 g), and ethanolamine (20 g) were added, and stirred at the room temperature for a reaction. After the reaction was completed, it was concentrated to remove the solvent, it was washed and extracted for liquid separation. An organic phase was dried, and it was filtered and concentrated until dryness. A crude product was separated and purified through a column (MeOH: DCM=3%), to obtain 2,3-bis(hexoxy)propyl-8-(2-hydroxyethyl)amino)octanoate.

5. Synthesis of A1

1,3-bis(hexoxy)-2-propanol-8-bromooctanoic acid ester (2.08 g) and 2,3-dis(hexoxy)propyl-8-((2-hydroxyethyl)amino)octanoate (2.0 g) were dissolved in anhydrous ethanol (20 mL), and sodium carbonate (0.95 g) and KI (0.15 g) were added for a reflux reaction. After the reaction was completed, it was concentrated to remove the solvent, washed and extracted for liquid separation. An organic phase was dried, and it was filtered and concentrated until dryness. A crude product was separated and purified through a column (MeOH: DCM=3%), to obtain an oily product A1 (2.1 g). ^{1}H NMR (500MHz, $CDCl_3$) δ(ppm)= 5.05 (p, J = 5.1 Hz, 1H), 4.15 (dd, J = 11.6, 4.1Hz, 1H), 4.03 (dd, J = 11.6, 5.8Hz, 1H), 3.55 (dt,J = 10.4,5.4 Hz, 1H), 3.49 (m, 8H), 3.43 – 3.30 (m,8H), 2.53 (t, J = 5.1 Hz, 2H), 2.43-2.35 (m, 4H), 2.25 (t, J = 7.5 Hz,4H), 1.60-1.52 (m,4H), 1.48 (m, 8H), 1.38 (m, 4H), 1.31- 1.15 (m, 36H), 0.82 (t, J = 6.8 Hz, 12H).MS(ESI): m/z $(M+H)^+$ 832.10,$(M+Na)^+$ 853.2.

Embodiment 2-Synthesis of A2

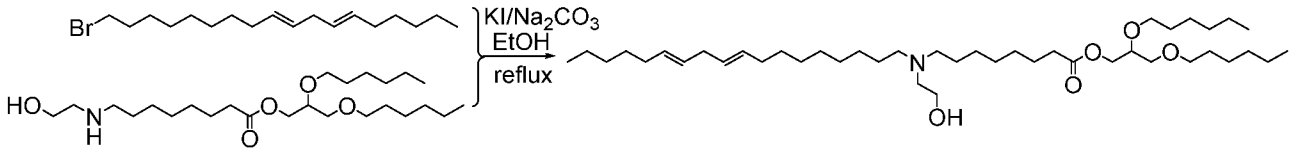
Synthesis route:



A2 was prepared by referring to the method in Embodiment 1, and an oily product (4.01 g) was obtained. ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm)=4.15 (dd, J = 11.6, 4.1 Hz, 2H), 4.02 (dd, J = 11.6, 5.8 Hz, 2H), 3.54 (m, 2H), 3.48 (t, J= 6.6 Hz, 6H), 3.42–3.34 (m, 8H), 2.54 (t, J = 5.0 Hz, 2H), 2.44 – 2.36 (t, 4H), 2.25 (t, J = 7.5 Hz, 4H), 1.59–1.52 (m, 4H), 1.52–1.44 (m, 8H), 1.39 (dd, J = 14.0, 7.3 Hz, 4H), 1.32 –1.17 (m, 36H), 0.82 (t, J = 6.8 Hz, 12H). MS(ESI): m/z $(M+H)^+$ 831.92.

Embodiment 3-Synthesis of A3

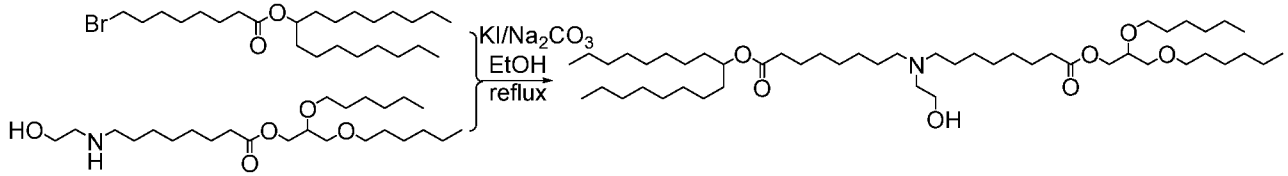
Synthesis route:



A3 was prepared by referring to the method in Embodiment 1, and an oily product (0.3 g) was obtained. 1H NMR (500 MHz, $CDCl_3$) δ(ppm)= 5.36–5.21 (m, 4H), 4.15 (dd, J = 11.6, 4.1 Hz, 1H), 4.03 (dd, J = 11.6, 5.8 Hz, 1H), 3.76–3.65 (m, 2H), 3.58 – 3.52 (m, 1H), 3.52 –3.31 (m,8H), 2.81 (s, 2H), 2.73 –2.65 (m, 4H), 2.25 (t, J = 7.5 Hz, 2H), 1.98 (m, 4H), 1.61–1.44 (m, 10H), 1.33– 1.16 (m, 34H), 0.82 (t, J = 8.0Hz, 9H).MS(ESI): m/z $(M+H)^+$ 695.40.

Embodiment 4-Synthesis of A4

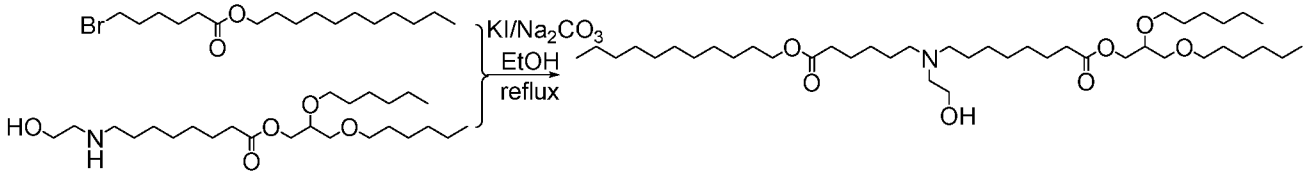
Synthesis route:



A4 was prepared by referring to the method in Embodiment 1, and an oily product (1.65 g) was obtained. 1H NMR (500 MHz, $CDCl_3$) δ(ppm)= 4.28–4.18 (dd, 1H), 4.14–4.00 (dd, 1H), 3.75 (m 1H), 3.62 (dd, J = 11.3, 6.7 Hz, 1H), 3.58 – 3.49 (m, 4H), 3.49 – 3.40 (m, 4H), 2.61 (s, 2H), 2.52 – 2.44 (m, 4H), 2.28 (t, J = 14.3Hz,4H), 1.60-1.45 (m, 16H), 1.28 (m, 48H), 0.87 (t, J = 6.4 Hz, 16H).MS(ESI): m/z $(M+H)^+$ 827.10.

Embodiment 5-Synthesis of A5

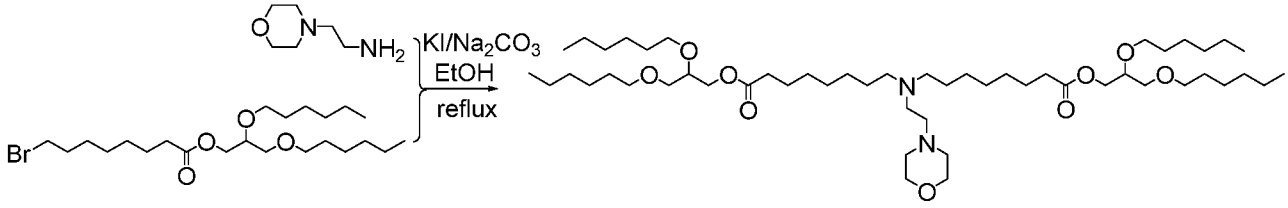
Synthesis route:



A5 was prepared by referring to the method in Embodiment 1, and an oily product (2.15 g) was obtained. ^1H NMR (500 MHz, $CDCl_3$)δ(ppm)=4.22 (dd, J = 11.6, 4.1 Hz, 1H), 4.13 – 4.02 (m, 3H), 3.64 – 3.58 (m, 1H), 3.57 – 3.50 (m, 4H), 3.45 (dt, J = 13.3, 5.6 Hz, 4H), 2.58 (t, J = 5.2 Hz, 2H), 2.45 (dd, J = 14.5, 7.0 Hz, 4H), 2.30 (t, 4H), 1.68 – 1.51 (m, 10H), 1.45 (dt, J = 15.1, 7.6 Hz, 4H), 1.37 – 1.20 (m, 36H), 0.88 (t, J = 6.2 Hz, 9H).MS(ESI): m/z $(M+H)^+$ 714.9,$(M+Na)^+$736.8.

Embodiment 6-Synthesis of A6

Synthesis route:

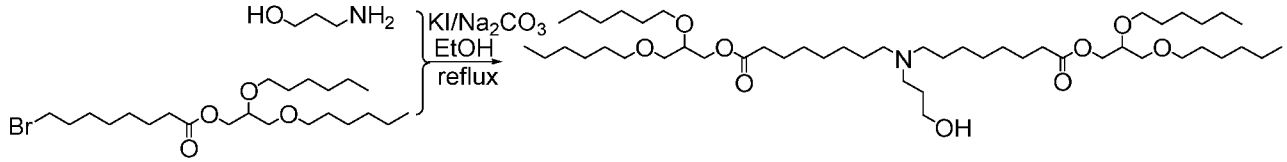


A6 was prepared by referring to the method in Embodiment 1, and an oily product (1.3 g) was

obtained. ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm)=4.21 (dd, J = 11.6, 4.0 Hz, 2H), 4.09 (dd, J = 11.5, 5.8 Hz, 2H), 3.73 – 3.66 (t, 4H), 3.61 (dd, J = 9.9, 5.1 Hz, 2H), 3.54 (t, J = 6.6 Hz, 4H), 3.50 – 3.39 (m, 8H), 2.60 (s, 2H), 2.46 (s, 10H), 2.31 (t, J = 7.5 Hz, 4H), 1.66 – 1.58 (m, 4H), 1.58 – 1.50 (m, 8H), 1.43 (s,4H), 1.28 (m, 36H), 0.88 (t, J = 6.7 Hz, 12H). MS(ESI): m/z $(M+H)^+$ 901.4.

Embodiment 7-Synthesis of A7

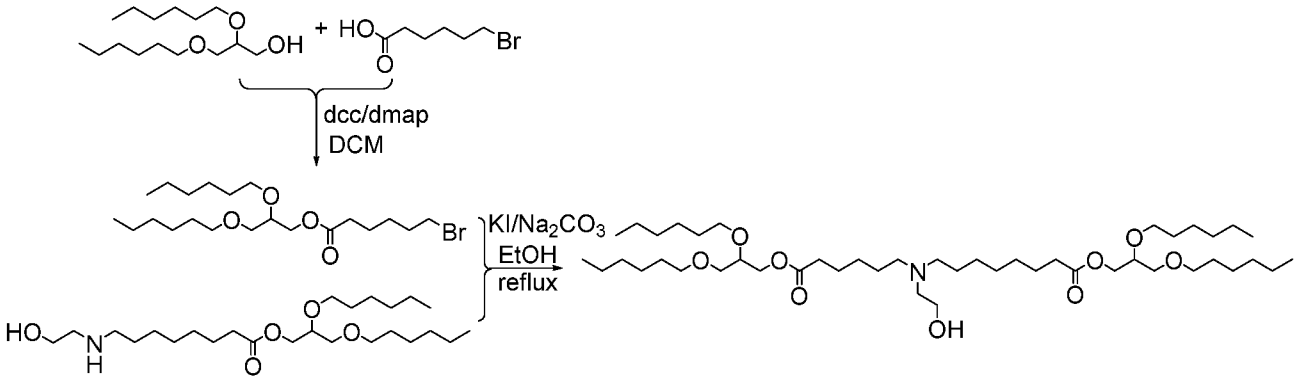
Synthesis route:



A7 was prepared by referring to the method in Embodiment 1, and an oily product (1.05 g) was obtained. ^{1}H NMR (500 MHz, $CDCl_3$)δ(ppm)= 4.22 (dd, *J* = 11.6, 4.1 Hz, 2H), 4.09 (dd, *J* = 11.6, 5.8 Hz, 2H), 3.78 (t, *J* = 5.0 Hz, 2H), 3.64 – 3.57 (m, 2H), 3.55 (t, *J* = 6.7 Hz, 4H), 3.46 (m, 8H), 2.67–2.61 (t, 2H), 2.46–2.37 (t, 4H), 2.31 (t, *J* = 7.5 Hz, 4H), 1.71 –1.58 (m, 6H), 1.52 (dq, *J* = 33.4, 6.8 Hz, 12H), 1.28 (m, 36H), 0.88 (t, *J* = 6.8 Hz, 12H). MS(ESI): m/z $(M+H)^+$ 846.0, $(M+Na)^+$868.0.

Embodiment 8-Synthesis of A8

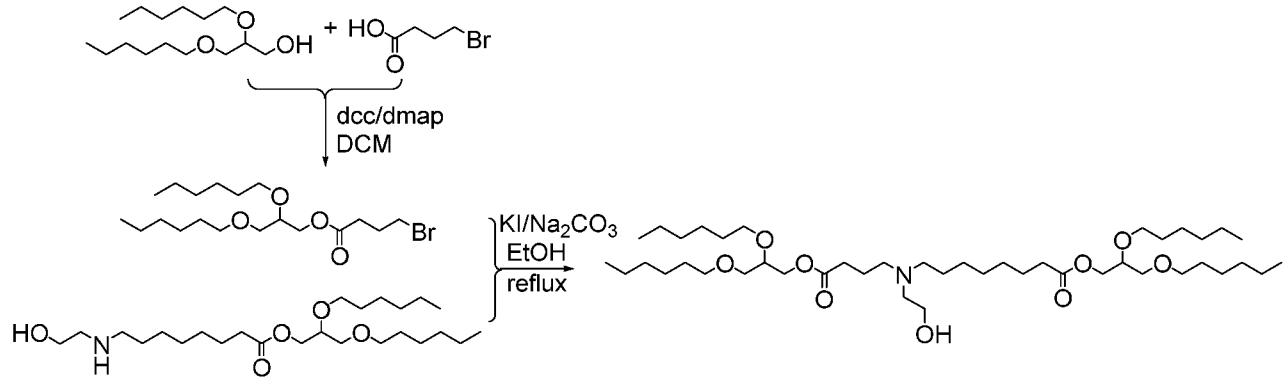
Synthesis route:



A8 was prepared by referring to the method in Embodiment 1, and an oily product (1.19 g) was obtained. ^{1}HNMR (500 MHz, $CDCl_3$) δ(ppm) = 4.22 (dd, J_1=11.5Hz,J_2=2.1Hz, 2H), 4.09 (dd, J_1= 11.6 Hz, J_2= 5.8 Hz, 2H), 3.64 – 3.58 (m, 2H), 3.55 (m, 6H), 3.49 – 3.41 (m, 8H), 2.60 (t, J = 5.0 Hz, 2H), 2.47 (dd, J = 14.3,6.8 Hz, 4H), 2.32 (dd, J = 13.4,7.2 Hz, 4H), 1.64 -1.46 (m,16H), 1.30 (m, 32H), 0.88 (t, J = 6.8 Hz, 12H). MS(ESI): m/z $(M+H)^+$ 803.11.

Embodiment 9-Synthesis of A9

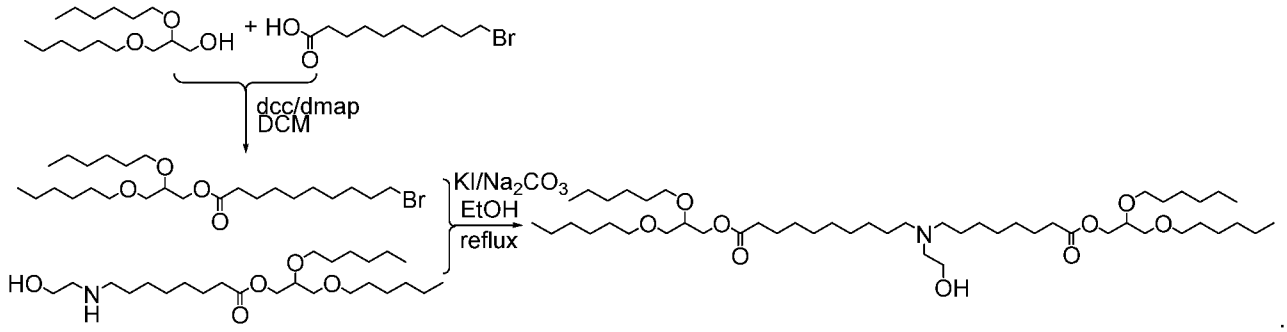
Synthesis route:



A9 was prepared by referring to the method in Embodiment 1, and an oily product (1.5 g) was obtained. ^{1}HNMR (500MHz, $CDCl_3$) δ(ppm) = 4.23(dt, J=9.2, 6.2Hz,2H), 4.13–4.05(m, 2H),3.65 – 3.58(m,2H), 3.57–3.50 (m,6H), 3.45(dt, J = 13.2, 5.5 Hz,8H), 2.58 (t, J=5.0 Hz,2H), 2.46 (dt,J=26.2,7.2 Hz, 4H), 2.38 –2.28 (t, 4H), 1.77-1.47 (m, 14H), 1.30 (m , 30H), 0.88 (t, J = 6.6 Hz, 12H). MS(ESI): m/z $(M+H)^+$ 775.44.

Embodiment 10-Synthesis of A10

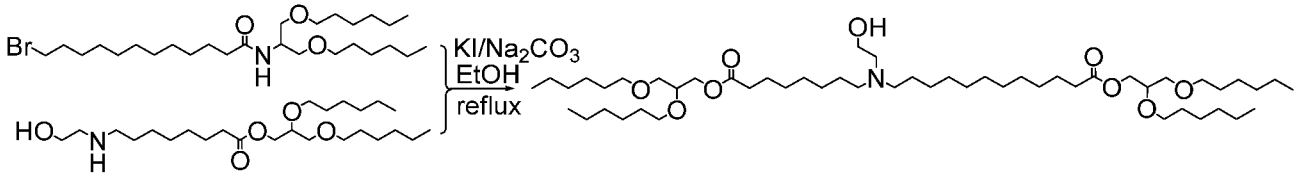
Synthesis route:



A10 was prepared by referring to the method in Embodiment 1, and an oily product (0.8 g) was obtained. ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) = 4.22 (dd, *J* = 11.6, 4.0 Hz, 2H), 4.09 (dd, *J*= 11.6, 5.8 Hz, 2H),3.64–3.57 (m, 4H), 3.57–3.50 (t, *J* = 6.6 Hz, 4H), 3.50–3.40 (m, 8H), 2.66 (s, 2H), 2.58–2.48 (m, 4H), 2.31 (t, J = 7.4 Hz, 4H),1.65–1.47 (m, 16H), 1.31 (m, 40H), 0.88 (t, *J* = 6.7 Hz, 12H). MS(ESI): m/z $(M+H)^+$ 859.30.

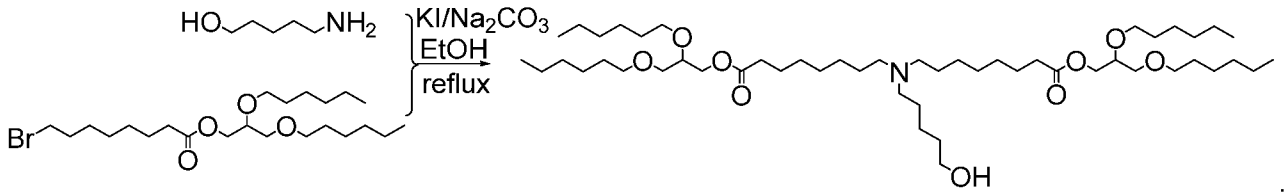
Embodiment 11-Synthesis of A11

Synthesis route:



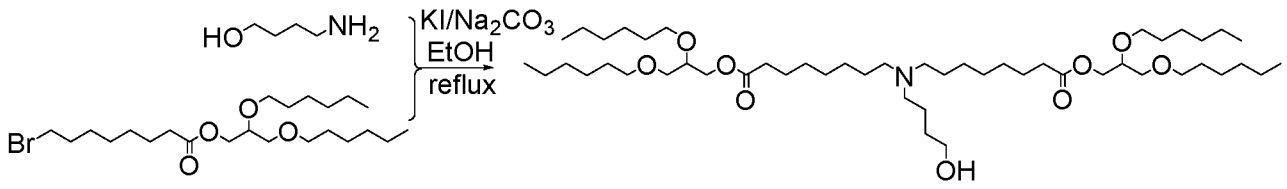
A11 was prepared by referring to the method in Embodiment 1, and an oily product (0.9 g) was obtained. ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) = 4.22 (dd, J = 11.6, 4.0 Hz, 2H), 4.09 (dd, J= 11.6, 5.9 Hz, 2H), 3.64–3.58 (m, 2H), 3.57–3.50 (t, J = 6.6 Hz, 6H), 3.49–3.40 (m, 8H), 2.60(t, *J* = 4.8 Hz, 2H), 2.51–2.43 (t, 4H), 2.31 (t, J= 7.5 Hz, 4H), 1.57–1.45 (m, 16H), 1.31 (m, 44H), 0.88 (t, J =6.8 Hz, 12H). MS(ESI): m/z $(M+H)^+$ 887.62.

Embodiment 12-Synthesis of A12



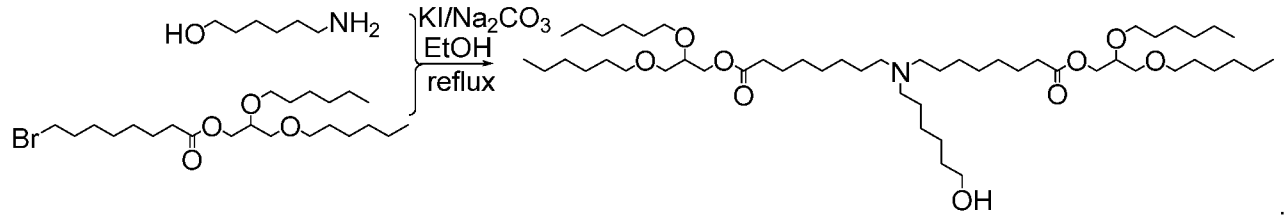
A12 was prepared by referring to the method in Embodiment 1, and an oily product (0.3 g) was obtained. ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) = 4.22 (dd, *J* = 11.6, 4.0 Hz, 2H), 4.09 (dd, *J*= 11.5, 5.8 Hz, 2H), 3.62 (m, 4H), 3.55 (t, *J* = 6.6 Hz, 4H), 3.50–3.40 (m, 8H), 2.41 (s, 6H), 2.31 (t, *J* = 7.5 Hz, 4H), 1.65–1.40 (m, 20H), 1.31 (m, 38H), 0.88 (t, *J* = 6.6 Hz, 12H).MS(ESI): m/z $(M+H)^+$ 873.30.

Embodiment 13-Synthesis of A13



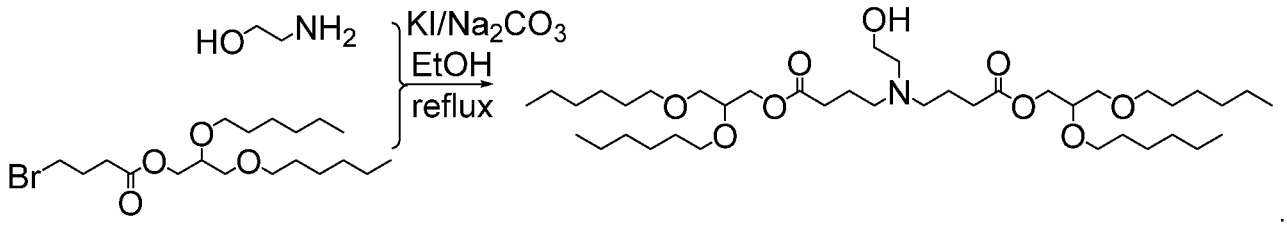
A13 was prepared by referring to the method in Embodiment 1, and an oily product (0.73 g) was obtained. ^{1}HNMR (500MHz, $CDCl_3$) δ(ppm) = 4.22 (dd, J=11.6, 4.1 Hz,2H), 4.09 (dd, J=11.6, 5.8 Hz, 2H), 3.62 (m, 2H), 3.55 (t, J = 6.7 Hz, 4H), 3.45 (dt, J = 13.3, 5.7 Hz, 8H), 3.38 (m, 4H), 2.53 (s, 4H), 2.31 (t, J = 7.5 Hz, 4H), 1.69 -1.50 (m, 20H), 1.37–1.23 (m, 36H), 0.88 (t, J= 6.8 Hz, 12H). MS(ESI): m/z $(M+H)^+$ 859.33.

Embodiment 14-Synthesis of A14



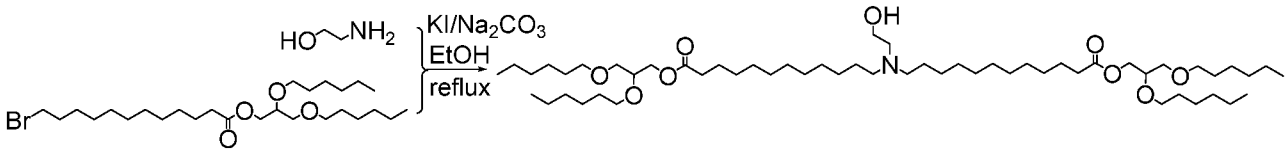
A14 was prepared by referring to the method in Embodiment 1, and an oily product (0.70 g) was obtained. ^{1}HNMR (500MHz, $CDCl_3$) δ(ppm) = 4.22 (dd, J_1=11.6Hz, J_2=4.1Hz, 2H), 4.09 (dd, J_1=11.6Hz, J_2=5.8Hz, 2H), 3.62 (m, 4H), 3.55 (t, J=6.7Hz, 4H), 3.51 – 3.39 (m, 8H), 2.46 (s,6H), 2.32 (t, J=7.5Hz, 4H), 1.58 (m,20H), 1.32 (m, 40H), 0.88 (t, J=6.7Hz, 12H). MS(ESI): m/z $(M+H)^+$ 887.57.

Embodiment 15-Synthesis of A15



A15 was prepared by referring to the method in Embodiment 1, and an oily product (0.35 g) was obtained. ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) = 4.22 (dd, J = 11.6, 4.1 Hz, 2H), 4.10 (dd, J = 11.6, 5.8 Hz, 2H), 3.61 (m, 2H), 3.55 (m, 6H), 3.45 (dt, J= 13.3, 5.2 Hz,8H), 2.60 (t, J = 5.1Hz, 2H), 2.51 (t, J =7.2Hz, 4H), 2.34 (t, J = 7.2Hz, 4H), 1.78 (m, 4H), 1.59–1.50 (m, 8H), 1.38–1.22 (m, 24H), 0.88 (t, J = 6.8Hz, 12H). MS(ESI): m/z $(M+H)^+$ 718.70.

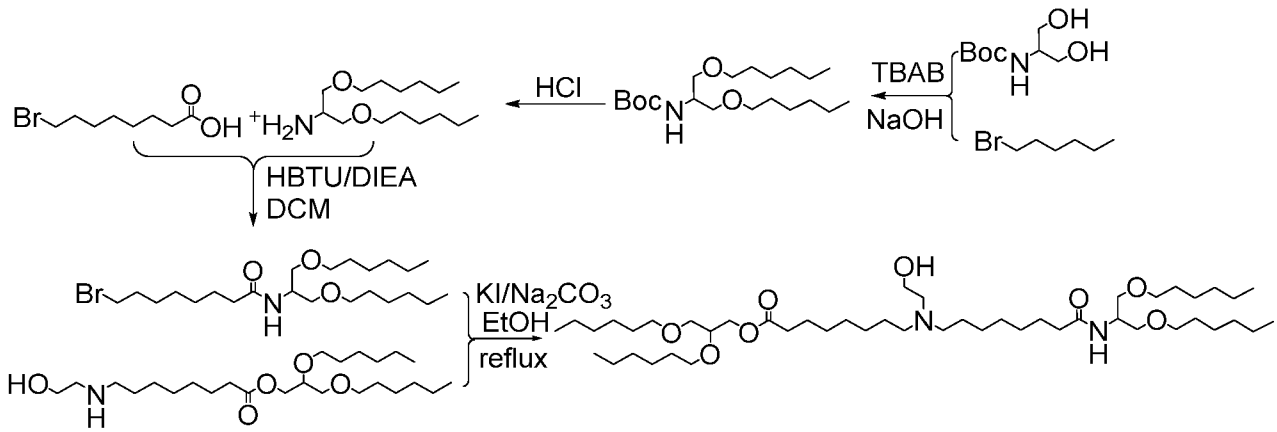
Embodiment 16-Synthesis of A16



A16 was prepared by referring to the method in Embodiment 1, and an oily product (0.23 g) was obtained. ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) = 4.22 (dd, J =11.6, 4.1 Hz, 2H), 4.09 (dd, J = 11.6, 5.9 Hz, 2H), 3.63–3.59 (m, 2H), 3.55 (t, J = 6.7 Hz, 4H), 3.49–3.41 (m, 8H), 2.73 (t, 2H), 2.61 (t, 4H), 2.31 (t, J = 7.5 Hz, 4H), 1.65 –1.59 (m, 4H), 1.59 –1.50 (m, 12H), 1.30 (dd, J = 21.2, 8.1 Hz, 52H),

0.88 (t, *J* = 6.8 Hz, 12H). MS(ESI): m/z $(M+H)^+$ 943.15.

Embodiment 17-Synthesis of A17



A17 was prepared by referring to the method in Embodiment 1, and an oily product (0.2 g) was obtained. ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) = 5.80 (d, *J* = 8.2 Hz, 1H), 4.25 – 4.15 (m, 2H), 4.13 – 4.06 (m, 1H), 3.68–3.58 (m, 3H), 3.57–3.49 (m, 4H), 3.45 (m, 10H), 2.72 (t, 2H), 2.60 (t, 4H), 2.31 (t, *J* = 7.5 Hz, 2H), 2.16 (t, *J* = 7.5 Hz, 2H), 1.58 (m, 16H), 1.30 (m, 36H), 0.88 (t, *J* = 6.3Hz, 12H). MS(ESI): m/z $(M+H)^+$ 830.07.

Embodiment 18- Encapsulation rate

A lipid nanoparticle included (1) an ionizable lipid compound, the ionizable lipid compound might be commercially purchased or self-made, such as MC3 (purchased from Avanti), A1-A17 were self-made; (2) a phospholipid (such as DOPE or DSPC, purchased from Avanti); (3) a pegylated lipid compound (such as PEG-DMG, purchased from Avanti or self-made); (4) a structural lipid (such as cholesterol, purchased from Sigma-Aldrich); and (5) an active ingredient (such as Luciferase mRNA, siRNA, SARS-CoV-2S protein mRNA, and Cas9 mRNA).

Preparation and encapsulation method: (1) the ionizable lipid, the phospholipid, the pegylated lipid, and the structural lipid were sequentially dissolved and mixed in ethanol at a ratio (calculated by molar unit) of 50%, 10%, 1.5%, and 38.5% respectively; and (2) a lipid mixture was uniformly mixed with the active ingredient (mRNA) at a ratio of 1:3 with a microfluidic chip or T-mixer to obtain the lipid nanoparticle.

The encapsulation rate reflected a degree of encapsulation of a material encapsulated. The encapsulation rate was higher, it was indicated that the material encapsulated was less likely to be decomposed during the delivery process in vivo.

Table 1: Performance of ionizable lipid and lipid nanoparticle thereof

Ionizable lipid	Particle size (nm)	Polydispersity index (PDI)	Encapsulation rate (%)
A1	100.4	0.084	98.38%
A2	87.94	0.12	98.77%
A3	85.5	0.20	95.93%
A4	69.23	0.17	97.41%
A5	111.1	0.16	97.86%
A7	107.8	0.06	97.34%
A8	103.8	0.08383	94.55%
A9	96.77	0.09479	91.01%
A10	78.78	0.2276	89.91%

A11	77.82	0.2294	88.65%
A12	129.2	0.09332	82.68%
A13	81.4	0.212	89.86%
A14	127.8	0.1126	82.95%
A15	91.43	0.21	86.91%
A16	66.62	0.2399	89.57%
A17	135.5	0.2592	88.50%

Embodiment 19-Transfection efficiency

According to the method in Embodiment 18, various cationic lipid compounds and luciferase mRNA nanoparticles were encapsulated, and the fluorescence intensity or total photon number of luciferase mRNA encapsulated with different LNPs was tested.

Experimental animal: SPF-grade BALB/c mice that passed quarantine, female, 7-9 weeks old, purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., randomly grouped by animal weight, with 3 mice in each group. All animals were adaptively raised for more than 7 days before an experiment. During the experiment, they were free to eat and drink water, with 12/12 h of alternating light and dark. The indoor temperature was 20~26°C and the humidity was 40-70%.

Experimental method: the female BALB/c mice were injected intramuscularly (tibialis anterior muscles in right legs of the mice) with luciferase mRNA encapsulated with different LNPs; and at 3, 6, 24, 48, and 72 h after the administration, a small animal live imaging system (Brand: Bruker, and Model: XTREME) was used for bioluminescence detection. Specific operating steps were as follows: substrate preparation: an appropriate amount of a Luciferin (Brand: Promega) substrate was taken and physiological saline was added to prepare 10 mg/mL of solution, it was kept in dark and used for standby, and the dosage of the test substance was 5 μg/mouse, and 50 μL/leg. The mice were administered with the substrate and allowed to move freely for 5-10 min. Then, the mice were placed in an anesthesia box and anesthetized with isoflurane with a concentration of 2.5%. The mice anesthetized were put into the small animal live imaging system, a bioluminescence parameter was set and an image was taken. After the image was collected, numerical values of upper and lower limits of the image were adjusted according to the fluorescence intensity of different groups, and data collection (such as: fluorescence intensity, average photon number, and total photon number) and data processing were performed on areas in which fluorescence is concentrated and distributed. Statistical analysis: a living imaging result was expressed as a mean value of the fluorescence intensity or total photon number of different animals in the same test substance group, in order to determine the fluorescence intensity or total photon number of luciferase mRNA encapsulated with the different LNPs. Data of luciferase expression induced by the ionizable lipid nanoparticle preparation in the mice after 6 h of the injection were shown in Tables 2-1 and 2-2.

The fluorescence intensity and total photon number reflected the transfection efficiency of LNP, and the numerical value was higher, it was indicated that the efficiency of LNP in delivering the substances encapsulated into cells was higher.

Table 2-1: Expression of luciferase induced by ionizable lipid nanoparticle preparation after 6 h of injection

Test substance	Average fluorescence intensity		Total photon number (p/sec)		Photon number per unit area (p/sec/cm^2)	
	Leg	Liver	Leg	Liver	Leg	Liver

PBS	22.5	21.3	1.04E+09	8.86E+08	3.31E+05	3.14E+05
A1	3411.3	828.8	4.14E+11	1.21E+11	5.02E+07	1.22E+07
A2	4669.8	3880.4	7.69E+11	9.56E+11	6.88E+07	5.71E+07
A3	1972.8	680.8	1.75E+11	7.68E+10	2.91E+07	1.00E+07
A4	834.3	776.5	6.29E+10	8.95E+10	1.23E+07	1.14E+07
A5	3763.3	124.2	4.27E+11	5.66E+09	5.54E+07	1.83E+06
A6	990.8	16.8	5.66E+10	1.04E+09	1.46E+07	2.47E+05
A7	3805.9	1416.3	5.42E+11	1.95E+11	5.60E+07	2.09E+07

Table 2-2: Expression of luciferase induced by ionizable lipid nanoparticle preparation after 6 h of injection

Test substance	Average fluorescence intensity		Total photon number (p/sec)		Photon number per unit area (p/sec/cm^2)	
	Leg	Liver	Leg	Liver	Leg	Liver
PBS	16.9	14.9	7.79E+08	1.02E+09	2.49E+05	2.20E+05
A8	2510.5	747.0	2.76E+11	6.97E+10	3.70E+07	1.10E+07
A9	661.2	549.1	6.28E+10	4.21E+10	9.74E+06	8.09E+06
A10	1801.0	1656.0	1.69E+11	2.80E+11	2.65E+07	2.44E+07
A11	3077.8	3463.2	4.76E+11	9.37E+11	4.53E+07	5.10E+07
A12	3544.5	657.0	5.39E+11	7.74E+10	5.22E+07	9.68E+06
A13	3160.1	1322.4	4.06E+11	2.25E+11	4.65E+07	1.95E+07
A14	2651.5	401.3	3.43E+11	2.29E+10	3.90E+07	5.91E+06
A16	1102.8	581.4	7.25E+10	6.11E+10	1.62E+07	8.56E+06
A17	1338.2	107.4	1.15E+11	9.83E+09	1.97E+07	1.58E+06

Embodiment 20--Immunogenicity research of LNP encapsulated mRNA

S protein mRNA (the sequence was found in SEQ ID NO. 17 in CN202310812939.7) of a SARS-CoV-2 BA.2.75.2 mutant strain was prepared by a T7 in vitro transcription method, and the encapsulation method in Embodiment 18 was referred. Ionizable lipids A18 (self-made, referring to A18 in CN202110617445.4), A2 and A11 were respectively used for encapsulation, and mRNA vaccines were obtained.

Table 3: mRNA encapsulation result

mRNA vaccine	Ionizable lipid	Encapsulation rate (%)	Particle size (nm)	Polydispersity index (PDI)
L1	A18	97	92	0.03
L2	A2	98	87	0.05
L3	A11	95	82	0.15

Table 4: Immunization regimen

Test sample	Group	mRNA	Dose	Species	Administration mode	Number of animals (mouse)	Animal number
PBS	Negative control	/	/	/	Intramuscular injection	10	A001-A010

	group						
L1	Low-dose group	BA.2.75.2 S protein	1 μg	BALB/c	Intramuscular injection	10	B001-B010
	High-dose group		20 μg	BALB/c	Intramuscular injection	10	C001-C010
L2	Low-dose group	BA.2.75.2 S protein	1 μg	BALB/c	Intramuscular injection	10	D001-D010
	High-dose group		20 μg	BALB/c	Intramuscular injection	10	E001-E010
L3	Low-dose group	BA.2.75.2 S protein	1 μg	BALB/c	Intramuscular injection	10	F001-F010
	High-dose group		20 μg	BALB/c	Intramuscular injection	10	G001-G010

3 mRNA vaccine preparations obtained were used for immunity testing in BALB/c mice. Specific operations were as follows: 6-8 week old BALB/c mice (female) were selected, and there were 10 mice in each group. The vaccine preparations were respectively administered twice by intramuscular injection on Day 0 and Day 21, the injection volume was 100 μL, and the negative control group was administered with 100 μL of phosphate buffer solution (PBS).

On Day 14 after the second immunization, spleens of 4 mice were taken and spleen lymphocytes were separated, and T lymphocytes secreting IFN-γ and IL-4 in the mice were detected with an ELISPOT method. Specific operations of the ELISPOT test were performed according to instructions of Mouse IL-4 precoated ELISPOT kit (Dakewe, 2210402) and Mouse IFN-γ precoated ELISPOT kit (Dakewe, 2210005).

On Day 14 and Day 35 after the first immunization (namely Day 14 after the second immunization), blood was collected from orbits of the remaining 6 mice (hemolysis was avoided), serum was collected, and a BA.2.75.2-type Spike (S) protein specific IgG antibody was detected by an indirect ELISA method. Specific operations for detecting the titer of the BA.2.75.2-type S protein specific IgG antibody using the indirect ELISA method were as follows.

Coating antigen: a BA.2.75.2 S protein (ACROBiosystems, Cat#SPN-C522r) was diluted to 2 ng/μL with a coating buffer at 100 μL/well, and it was coated overnight at 4°C.

The plate was washed with 1×PBST for 3 times, and 5 min each time.

It was blocked with 1% BSA blocking solution at 200 μL/well, and stilly placed at 37°C for 1 h.

The plate was washed with 1×PBST for 3 times, and 5 min each time.

The serum was doubling-diluted with a dilution buffer, and added at 100 μL/well, it was incubated at 37°C for 1 h, and negative serum control and blank control wells without the serum were set.

The plate was washed with 1×PBST for 3 times, and 5 min each time.

The anti-IgG secondary antibody was diluted at 1:1000, and added at 100 μL/well, and it was incubated at 37°C for 1 h.

The plate was washed with 1×PBST for 3 times, and 5 min each time.

TMB substrate developing solution prepared freshly was added at 100 μL/well, and it was incubated at 37°C for an appropriate time.

2 mol/L of sulfuric acid stopping solution was added at 50 μL/well.

The absorbance value of OD450 nm was measured with an enzyme-linked immunosorbent assay (ELISA) reader.

Results of IgG antibody titers were shown in Figs. 2 and 3, the 3 mRNA vaccine preparations might induce humoral immune responses and generate the S protein specific IgG antibody in the BALB/c mice. In both low and high doses, regardless of the first or second immunization, the antibody IgG level produced by L2 was basically the same as L1. After the first immunization, the IgG antibody titer reached 4Log10 or above, and after the second immunization, the IgG antibody titer reached 6Log10~7Log10. The antibody IgG level produced by L3 was relatively low, but after the second immunization, the IgG antibody titers all reached 6Log10 or above. The ELISPOT results were shown in Fig. 4. After being inoculated with low and high-dose vaccine preparations, the T lymphocytes secreting INF-γ in groups L1, L2, and L3 were significantly increased, the T lymphocytes secreting INF-γ in the group L3 were highest, the second was the group L2, and the worst was the group L1. After being inoculated with the low and high-dose vaccine preparations, T lymphocytes secreting IL-4 in groups L1, L2, and L3 were significantly increased, and in the low dose, the T lymphocytes secreting IL-4 in the group L3 were highest, the second was the group L2, and the worst was the group L1; and in the high dose, there was no significant difference between the groups. In conclusion, it might be concluded that, compared to L1, L2, and L3, L3 might induce the strongest cellular immune response. The humoral immune response induced by L2 was equivalent to L1, but the cellular immune response produced was stronger.

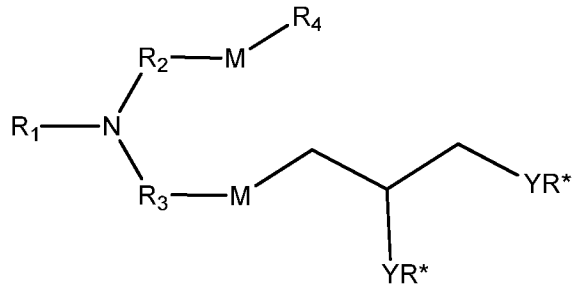
The content of all reference documents (including documents referred by the reference documents, published patents, published patent applications, and pending patent applications) cited in the full text is introduced by entirety here as a reference. Unless otherwise defined, all technical and scientific terms used in this article are consistent with meanings commonly known to those of ordinary skill in the art.

All features disclosed in the description may be combined in any combinations. Each feature disclosed in the description may be replaced by alternative features with the same, equivalent, or similar purpose. Therefore, unless otherwise explicitly stated, each disclosed feature is only an example of a series of equivalent or similar features.

Based on the above description, those skilled in the art may easily determine the basic features of the application, and without departing from the spirit and scope of the application, make various changes and modifications to the application so that it adapts to various uses and conditions. Therefore, other embodiments are also within the scope of the appended claims.

What is claimed is:

1. A compound in Formula (I), or a salt or isomer thereof:



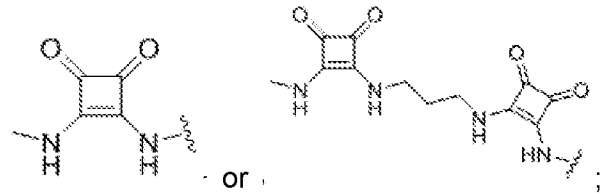
(I)

wherein,

R_1 is -C_{1-6} alkylene-X,

wherein X is selected from amino, hydroxyl, acetylenyl, 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, and H atoms on the C_{3-7} cycloalkyl, the 4-7-membered heterocyclic group, the C_{6-10} aryl, and the 5-10-membered heteroaryl groups are optionally substituted by the following groups: -$(CH_2)_{1-3}OH$, -$(CH_2)_{1-3}NR_aR_b$ or -$(CH_2)_{1-3}C(O)NR_aR_b$;

or X is:



or

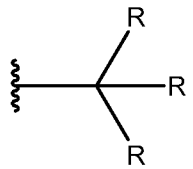
;

R_2 is C_{3-20} alkylene, wherein one or more methylenes in R_2 are each independently substituted by the following groups: -CH=CH-, -C≡C -, or -O-;

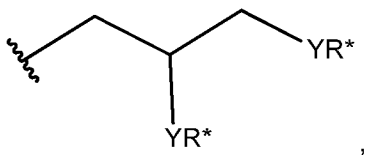
R_3 is C_{5-20} alkylene, wherein one or more methylenes in R_3 are each independently substituted by the following groups: -CH=CH-, -C≡C-, or -O-;

M is independently selected from -CH_2-, -CH=CH-, -C≡C-, -NH-, -C(O)-, -C(S)-, -O-, -S-, -C(O)O-, -OC(O)-, -C(O)NH-, -NHC(O)-, -C(O)-S-, -S-C(O)-, -C(S)-O-, -O-C(S)-, -C(S)-S-, -S-C(S)-, -S-S-, -C(S)NH-, -NHC(S)- or -O-P(O)(OH)-O-;

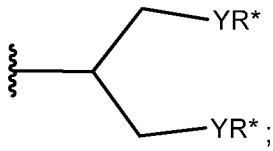
R_4 is



,



, or



;

wherein R is selected from H, R* or -$(CH_2)_{1-5}$-YR*;

Y is selected from -NH-, -C(O)-, -C(S)-, -O-, -S-, -C(O)O-, -OC(O)-, -C(O)NH-, -NHC(O)-, -C(O)-S-, -S-C(O)-, -C(S)-O-, -O-C(S)-, -C(S)-S-, -S-C(S)-, -S-S-, -C(S)NH-, -NHC(S)-, or -O-P(O)(OH)-O-;

R* is independently selected from C_{1-12} alkyl, C_{2-12} alkenyl, or C_{2-12} alkyne; and

R_a and R_b are independently selected from H, C_{1-6} alkyl or C_{1-6} halogenated alkyl.

2. The compound in Formula (I) or the salt or isomer thereof according to claim 1, wherein,

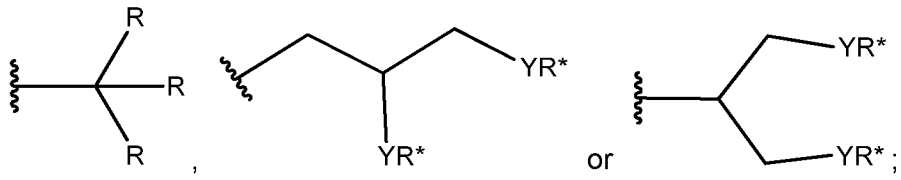
R_1 is -C_{1-6} alkylene-X,

wherein X is selected from amino, hydroxyl, acetylenyl or cyano;

R_2 is C_{3-20} alkylene;

R_3 is a C_{5-20} alkylene;

M is independently selected from $-CH_2-$, -CH=CH-, -C≡C-, -NH-, -C(O)-, -C(S)-, -O-, -S-, -C(O)O-, -OC(O)-, -C(O)NH-, -NHC(O)-, -C(O)-S-, -S-C(O)-, -C(S)-O-, -O-C(S)-, -C(S)-S-, -S-C(S)-, -S-S-, -C(S)NH-, or -NHC(S)-;

R_4 is 

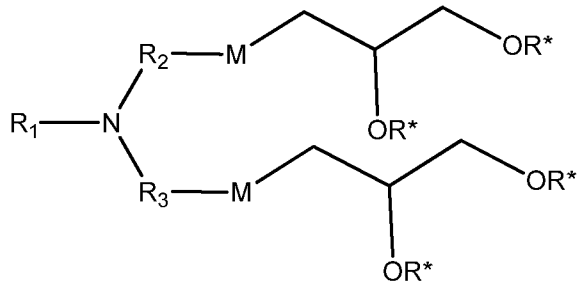
;

wherein R is selected from H, R* or $-(CH_2)_{1-5}$-YR*;

Y is selected from -NH-, -C(O)-, -C(S)-, -O-, or -S-; and

R* is independently selected from C_{1-12} alkyl, C_{2-12} alkenyl, or C_{2-12} alkynyl.

3. The compound or the salt or isomer thereof according to any one of claims 1-2, wherein R_1 is $-C_{1-6}$ alkylene-OH, preferably $-C_{2-6}$ alkylene-OH, preferably $-C_{2-4}$ alkylene-OH, and preferably $-C_2$ alkylene-OH.
4. The compound or the salt or isomer thereof according to any one of claims 1-3, wherein R_2 is C_{3-20} alkylene, preferably C_{3-11} alkylene, preferably C_{5-11} alkylene, and preferably C_{7-11} alkylene.
5. The compound or the salt or isomer thereof according to any one of claims 1-4, wherein R_3 is C_{5-20} alkylene, preferably C_{7-20} alkylene, preferably C_{7-11} alkylene, preferably C_{5-11} alkylene, preferably C_{7-9} alkylene, and preferably C_7 alkylene.
6. The compound or the salt or isomer thereof according to any one of claims 1-5, wherein each M is independently -C(O)O-, -OC(O)-, -C(O)NH- or -NHC(O)-, preferably -C(O)O- or -OC(O)-, and preferably -C(O)O-.
7. The compound or the salt or isomer thereof according to any one of claims 1-6, wherein each Y is independently selected from -O-.
8. The compound or the salt or isomer thereof according to any one of claims 1-7, wherein each R* is independently selected from C_{1-11} alkyl, wherein one or more methylenes are each independently substituted by the following groups: -CH=CH-, and -C≡C-, preferably C_{1-8} alkyl, preferably C_{1-6} alkyl, and preferably C_6 alkyl.
9. The compound or the salt or isomer thereof according to claim 1, wherein the compound has a formula (II):



(II)

wherein,

R_1 is $-C_{2-6}$ alkylene-OH;

M is selected from $-CH_2-$, -CH=CH-, -C≡C-, -NH-, -C(O)-, -C(S)-, -O-, -S-, -C(O)O-, -OC(O)-, -C(O)NH-, -NHC(O)-, -C(O)-S-, -S-C(O)-, -C(S)-O-, -O-C(S)-, -C(S)-S-, -S-C(S)-, -S-S-, -C(S)NH-, -NHC(S)-, or -O-P(O)(OH)-O-;

R_2 is C_{3-20} alkylene;

R_3 is C_{7-20} alkylene;

R* is independently selected from C_{1-11} alkyl, wherein one or more methylenes in R* are each independently substituted by the following groups: -CH=CH- or -C≡C-.

10. The compound in Formula (II) or the salt or isomer thereof according to claim 9, wherein,

R_1 is -C_{2-6} alkylene-OH;

M is selected from -C(O)O-, -OC(O)-, -C(O)NH-, or -NHC(O)-;

R_2 is C_{3-11} alkylene;

R_3 is C_{7-11} alkylene; and

R* is independently selected from C_{1-6} alkyl.

11. The compound in Formula (II) or the salt or isomer thereof according to claim 9, wherein,

R_1 is -C_{2-4} alkylene-OH;

M is selected from -C(O)O-, -OC(O)-, -C(O)NH-, or -NHC(O)-;

R_2 is C_{7-11} alkylene;

R_3 is C_{7-9} alkylene; and

R* is independently selected from C_{1-6} alkyl.

12. The compound in Formula (II) or the salt or isomer thereof according to claim 9, wherein,

R_1 is -C_2 alkylene-OH;

M is -C(O)O- or -OC(O)-;

R_2 is C_{7-11} alkylene;

R_3 is C_{7-9} alkylene; and

R* is independently selected from C_{1-6} alkyl.

13. The compound in Formula (II) or the salt or isomer thereof according to claim 9, wherein,

R_1 is -C_2 alkylene-OH;

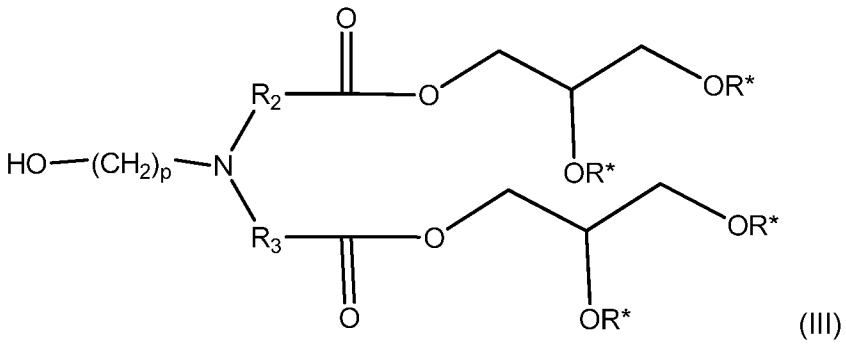
M is -C(O)O- or -OC(O)-;

R_2 is C_{7-11} alkylene;

R_3 is C_7 alkylene; and

R* is independently selected from C_6 alkyl.

14. The compound in Formula (II) or the salt or isomer thereof according to claim 1, wherein the compound has a Formula (III):



(III)

wherein,

p is 2;

R_2 is C_{7-11} alkylene;

R_3 is C_{7-9} alkylene; and

R* is independently selected from C_{1-6} alkyl.

15. The compound in Formula (II) or the salt or isomer thereof according to claim 14, wherein,

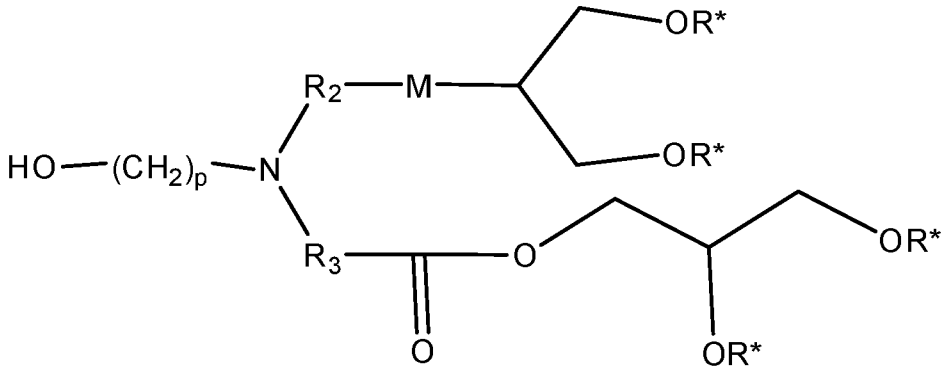
p is 2;

R_2 is C_{7-11} alkylene;

R_3 is C_7 alkylene; and

R* is independently selected from C_6 alkyl.

16. The compound in Formula (II) or the salt or isomer thereof according to claim 1, wherein the compound has a Formula (IV):



(IV)

wherein,

p is 2-4;

M is selected from -CH_2-, -CH=CH-, -C≡C-, -NH-, -C(O)-, -C(S)-, -O-, -S-, -C(O)O-, -OC(O)-, -C(O)NH-, -NHC(O)-, -C(O)-S-, -S-C(O)-, -C(S)-O-, -O-C(S)-, -C(S)-S-, -S-C(S)-, -S-S-, -C(S)NH-, -NHC(S)-, or -O-P(O)(OH)-O-;

R_2 is C_{3-20} alkylene;

R_3 is a C_{7-20} alkylene; and

R* is independently selected from C_{1-11} alkyl, wherein one or more methylenes are each independently substituted by the following groups: -CH=CH- or -C≡C-.

17. The compound in Formula (II) or the salt or isomer thereof according to claim 16, wherein,

p is 2-4;

M is selected from -C(O)O-, -OC(O)-, -C(O)NH-, or -NHC(O)-;

R_2 and R_3 are independently selected from C_{5-11} alkylene; and

R* is independently selected from C_{1-6} alkyl.

18. The compound in Formula (II) or the salt or isomer thereof according to claim 16, wherein,

p is 2-4;

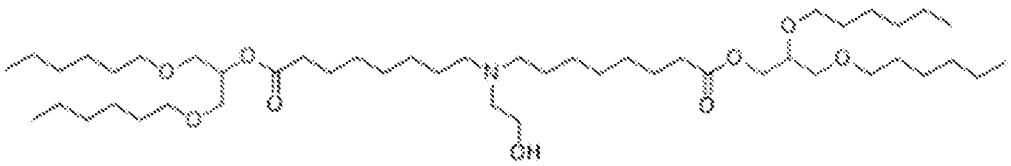
M is -C(O)O- or -OC(O)-;

R_2 is C_{7-11} alkylene;

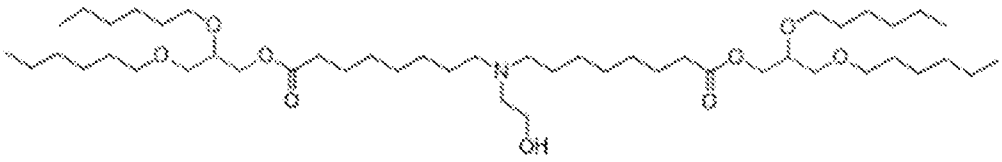
R_3 is C_{7-9} alkylene; and

R* is independently selected from C_{1-6} alkyl.

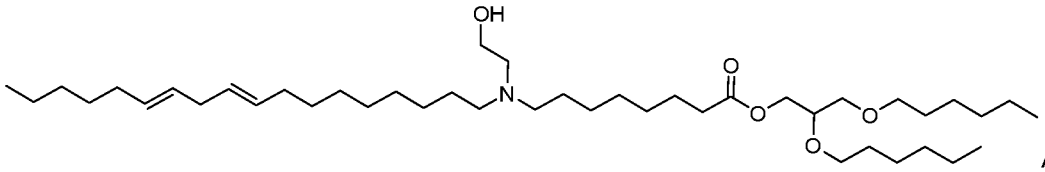
19. A compound or a salt or isomer thereof, wherein the compound is selected from any one of A1-A17:



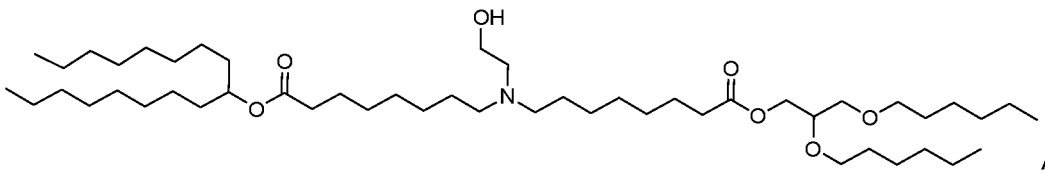
A1,



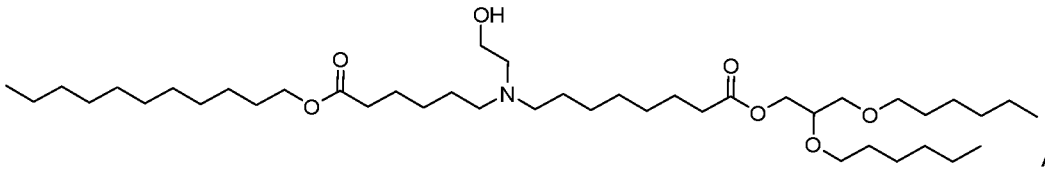
A2,



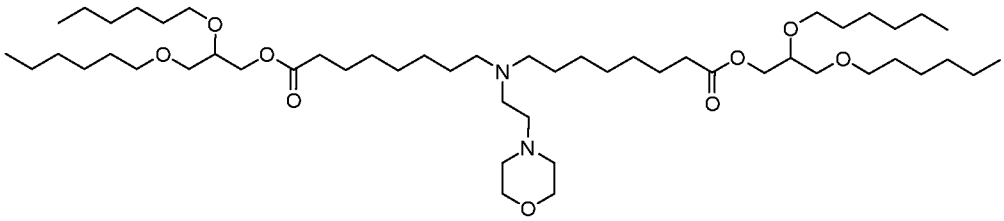
A3,



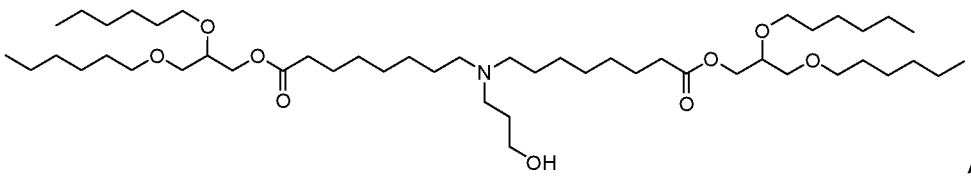
A4,



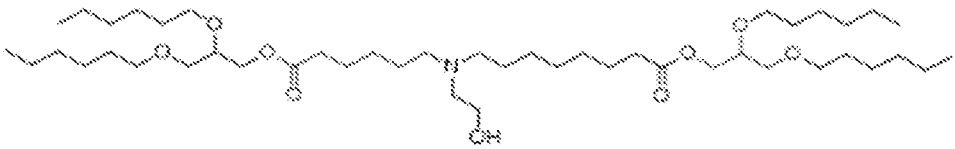
A5,



A6,

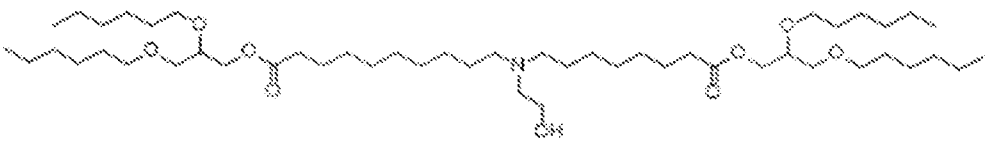


A7,

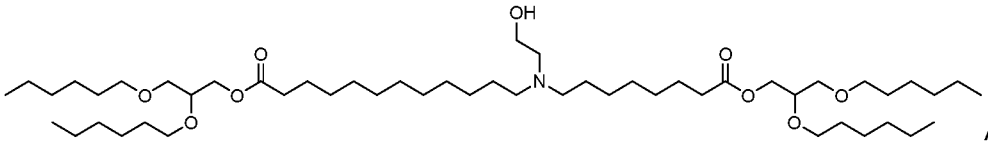


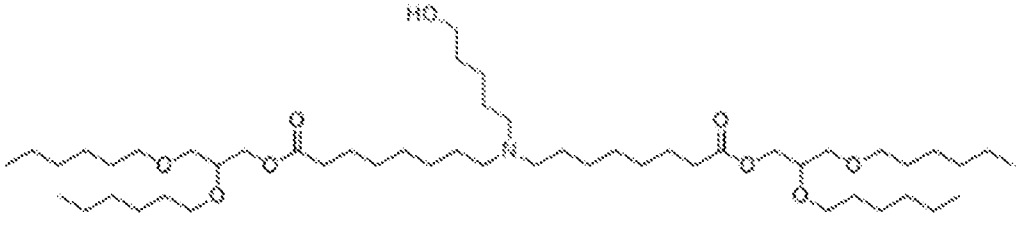
A8,

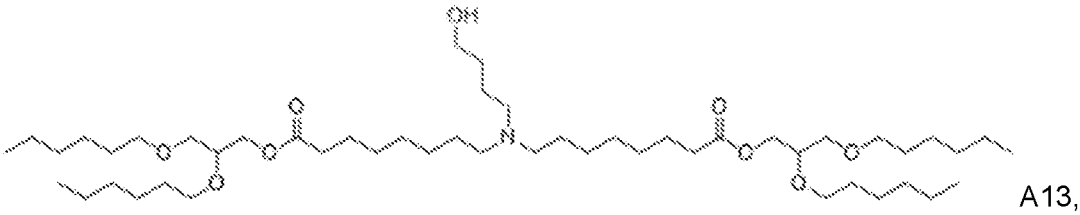
A9,

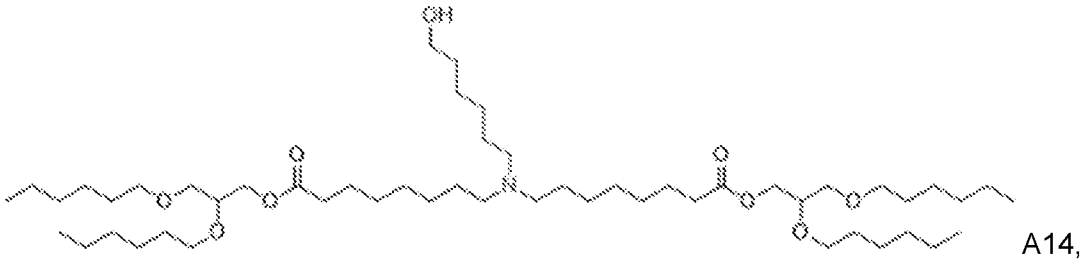


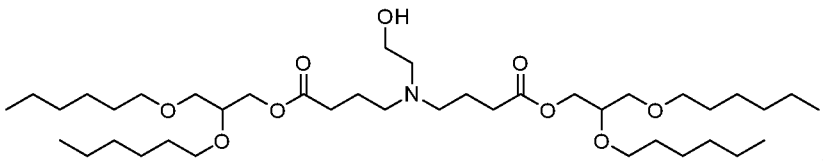
A10,

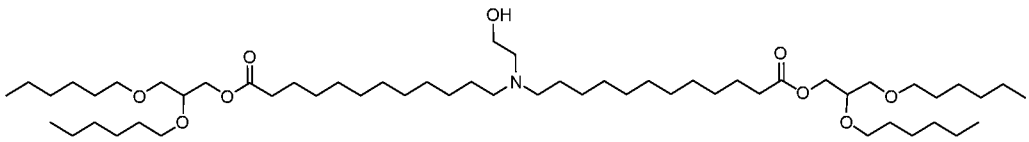

A11,

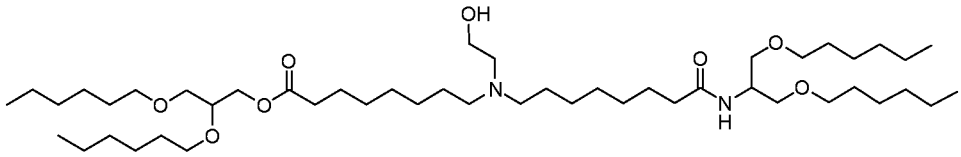

A12,


A13,


A14,


A15,


A16,


A17.

20. A composition, wherein the composition comprises the compound according to any one of claims 1-19.
21. The composition according to claim 20, wherein the composition further comprises a phospholipid.
22. The composition according to claim 21, wherein the phospholipid is selected from at least one of 1,2-dilinoleoyl-sn-glycerol-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycerol-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycerol-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycerol-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycerol-3-phosphocholine (DSPC), 1,2-diundecanoyl-sn-glycerol-phosphocholine (DUPC),

1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycerol -3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterol semisuccinyl-sn-glycerol -3-phosphocholine (OChemsPC), 1-hexadecyl-sn-glycerol-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenyl-sn-glycerol-3-phosphocholine, 1,2-diarachidonoyl-sn-glycerol-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycerol-3-phosphocholine, 1,2-dioleoyl-sn-glycerol-3-phosphate ethanolamine (DOPE), 1,2-diphytanyl-sn-glycerol-3-phosphate ethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycerol-3-phosphate ethanolamine, 1,2-dilinoleoyl-sn-glycerol-3-phosphate ethanolamine, 1,2-dilinolenyl-sn-glycerol-3-phosphate ethanolamine, 1,2-diarachidonoyl-sn-glycerol-3-phosphate ethanolamine, 1,2-didocosahexaenoyl-sn-glycerol-3-phosphate ethanolamine, 1,2-dioleoyl-sn-glycerol-3-phosphate-rac-(1-glycerol) sodium salt (DOPG), dipalmitoyl phosphatidyl glycerol (DPPG), palmitoyl oleoyl phosphatidyl ethanolamine (POPE), distearoyl-phosphatidyl-ethanolamine (DSPE), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanol amine (DMPE), 1-stearoyl-2-oleoyl-phosphatidyl ethanolamine (SOPE), 1-stearoyl-2-oleoyl-phosphatidyl choline (SOPC), sphingomyelin, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, phosphatidic acid, palmitoyl oleoyl phosphatidyl choline, lysophosphatidyl choline, and lysophosphatidyl ethanolamine (LPE).

23. The composition according to any one of claims 20-22, wherein the composition further comprises a pegylated lipid compound.
24. The composition according to claim 23, wherein the pegylated lipid compound is selected from at least one of a PEG modified phosphatidyl ethanolamine, a PEG modified phosphatidic acid, a PEG modified ceramide, a PEG modified dialkylamine, a PEG modified diacylglycerol, and a PEG modified dialkyglycerol.
25. The composition according to any one of claims 20-24, wherein the composition further comprises a structural lipid.
26. The composition according to claim 25, wherein the structural lipid is selected from at least one of cholesterol, coprosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, ursolic acid, and α-tocopherol.
27. The composition according to any one of claims 20-26, wherein calculated by molar unit, the composition comprises 20%-80% of the compound according to any one of claims 1-19, 1% -10% of the pegylated lipid compound, 10% -50% of the structural lipid, and 5-30% of the phospholipid.
28. The composition according to claim 27, wherein the composition further comprises an active ingredient, and the active ingredient is selected from at least one of DNA, RNA, protein, and drug active molecules.
29. The composition according to claim 28, wherein the RNA is selected from at least one of mRNA, siRNA, aiRNA, miRNA, dsRNA, aRNA, and lncRNA.
30. The composition according to claim 28, the protein is selected from at least one of an antibody, an enzyme, a recombinant protein, a polypeptide, and an oligopeptide.
31. A lipid nanoparticle, wherein the lipid nanoparticle comprises the composition according to any one of claims 20-30.
32. A method for preparing the lipid nanoparticle according to claim 31, wherein the method comprises Step (1): dissolving and mixing of the compound according to any one of claims 1-19 with a pegylated lipid compound, a structural lipid, and a phospholipid.

33. The method according to claim 32, wherein the method also comprises Step (2): forming the lipid nanoparticle by mixing a mixture obtained in the Step (1) with an active ingredient through a mixer.
34. A use of the compound according to any one of claims 1-19 in the preparation of a lipid nanoparticle.
35. The use according to claim 34, wherein the lipid nanoparticle is neutral and uncharged in a neutral medium, and is positively charged upon protonation in an acidic medium.
36. A pharmaceutical composition, wherein the pharmaceutical composition comprises the lipid nanoparticle according to claim 31 and a pharmaceutically acceptable carrier.
37. A use of the lipid nanoparticle according to claim 31 or the pharmaceutical composition according to claim 36 in preparation of a drug.
38. The use according to claim 37, wherein the drug comprises an active ingredient, and the active ingredient is selected from at least one of DNA, RNA, protein, or drug active molecules.
39. The use according to claim 38, wherein the RNA is selected from at least one of mRNA, siRNA, aiRNA, miRNA, dsRNA, aRNA, and lncRNA.
40. The use according to claim 38, wherein the protein is selected from at least one of an antibody, an enzyme, a recombinant protein, a polypeptide, and an oligopeptide.
41. The use according to any one of claims 37-40, wherein the drug is used for a person by intravenous injection, intramuscular injection, subcutaneous injection, microneedle patch, oral administration, oral and nasal spray, and daubing.
42. The lipid nanoparticle according to claim 31 or the pharmaceutical composition according to claim 36 for use as a medicament;

optionally, the medicament comprises an active ingredient, and the active ingredient is selected from at least one of DNA, RNA, protein, or drug active molecules;

optionally, the RNA is selected from at least one of mRNA, siRNA, aiRNA, miRNA, dsRNA, aRNA, and lncRNA;

optionally, the protein is selected from at least one of an antibody, an enzyme, a recombinant protein, a polypeptide, and an oligopeptide;

optionally, the medicament is used for a person by intravenous injection, intramuscular injection, subcutaneous injection, microneedle patch, oral administration, oral and nasal spray, and daubing.
43. A method for treating a disease, wherein the method comprises administering a therapeutically effective amount of the lipid nanoparticle according to claim 31 or the pharmaceutical composition according to claim 36 to a subject suffering from the disease;

optionally, the lipid nanoparticle or the pharmaceutical composition comprises an active ingredient, and the active ingredient is selected from at least one of DNA, RNA, protein, or drug active molecules;

optionally, the RNA is selected from at least one of mRNA, siRNA, aiRNA, miRNA, dsRNA, aRNA, and lncRNA;

optionally, the protein is selected from at least one of an antibody, an enzyme, a recombinant protein, a polypeptide, and an oligopeptide;

optionally, the lipid nanoparticle or the pharmaceutical composition is used for the subject by intravenous injection, intramuscular injection, subcutaneous injection, microneedle patch, oral administration, oral and nasal spray, and daubing.

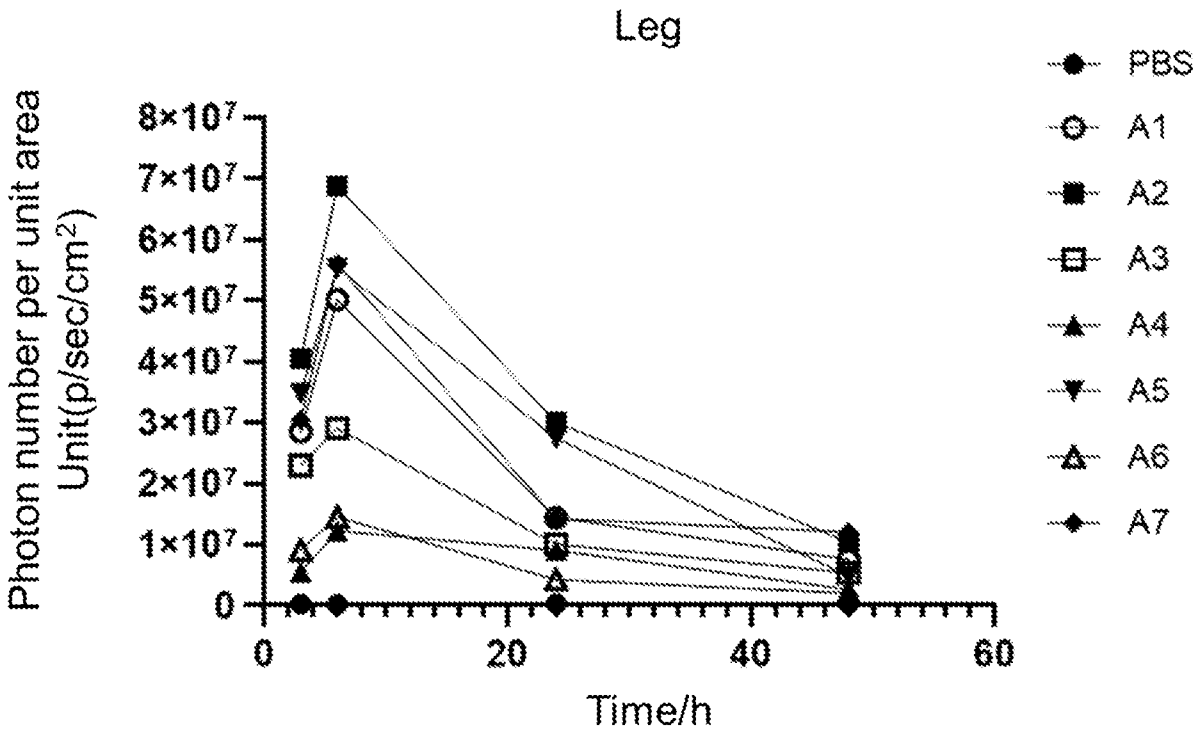


Figure 1A

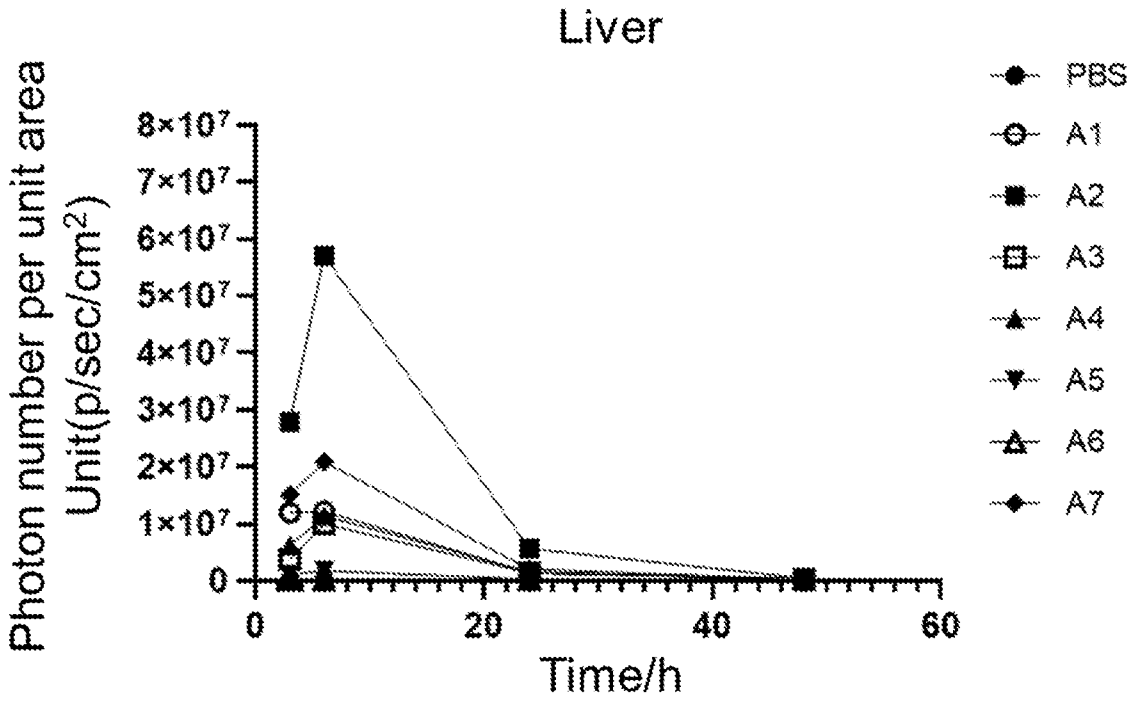


Figure 1B

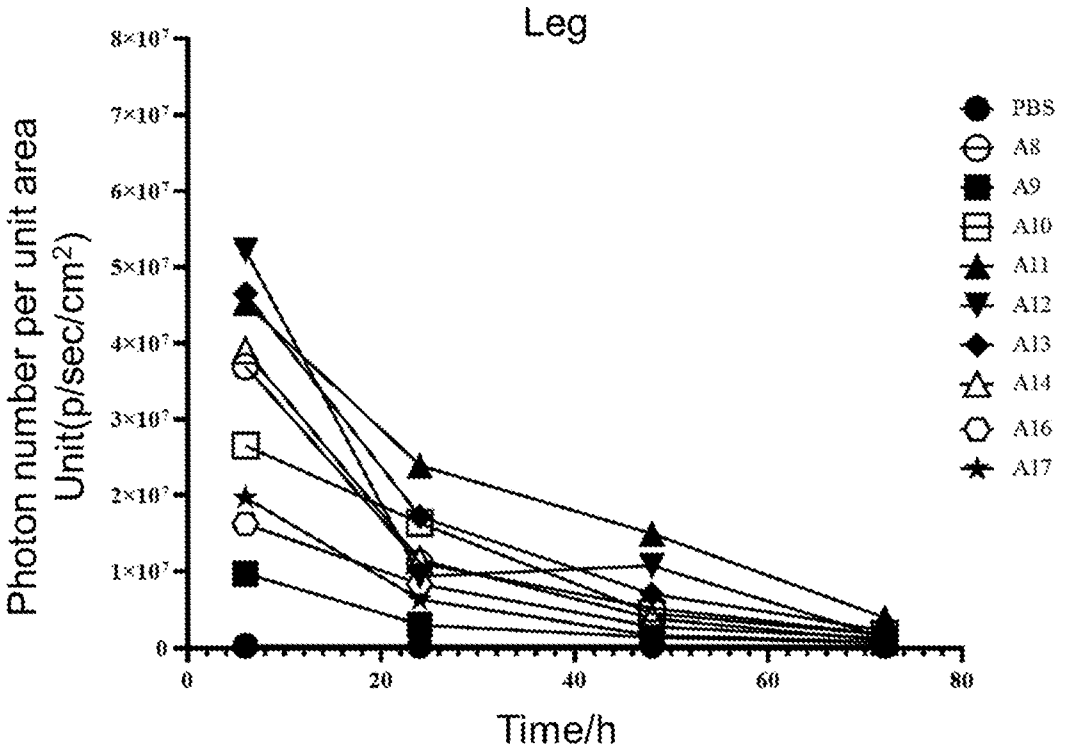


Figure 1C

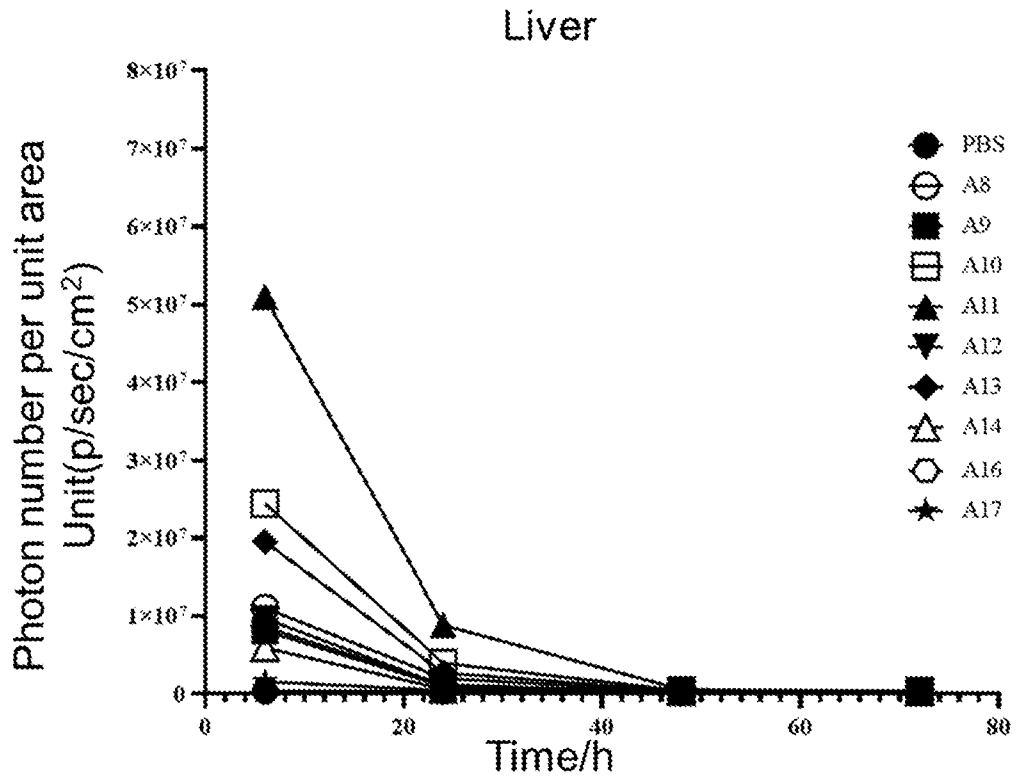


Figure 1D

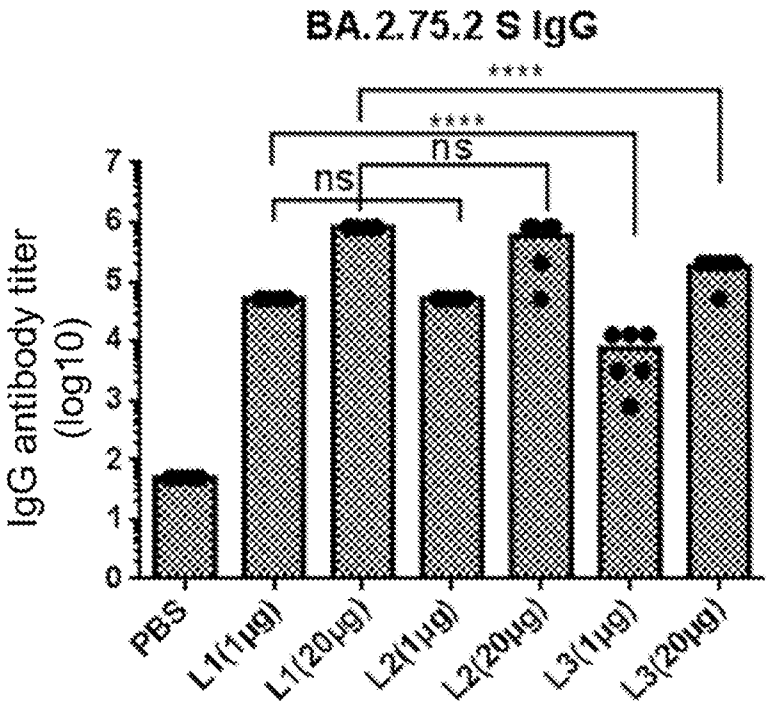


Figure 2

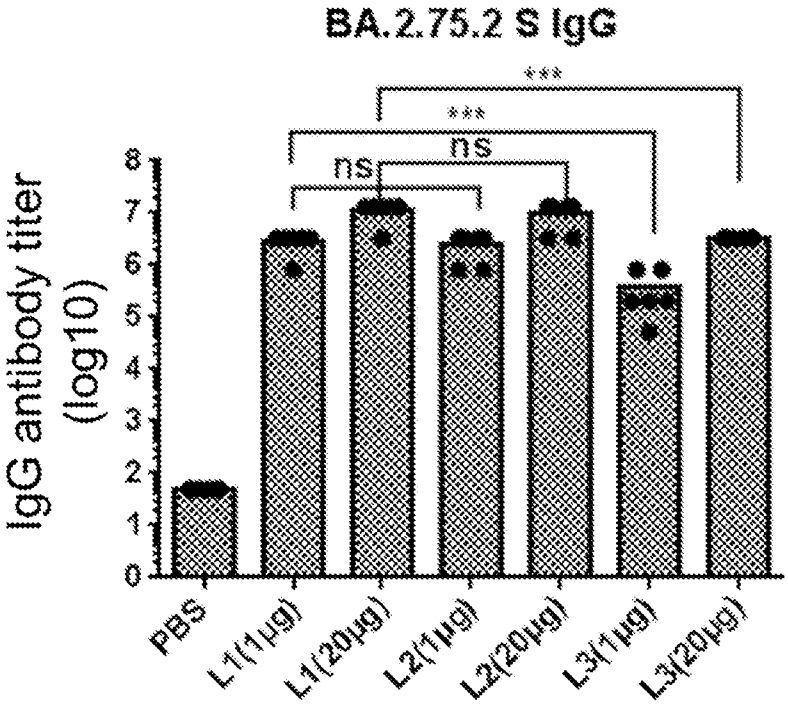


Figure 3

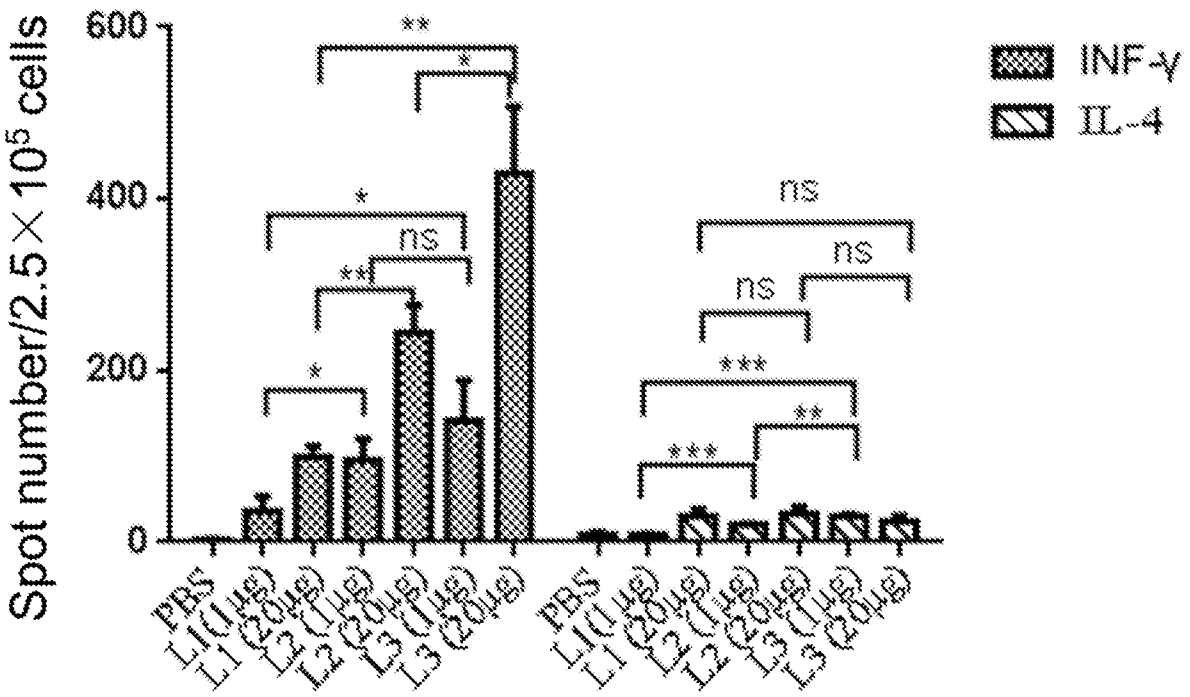

Spot number/2.5×10^5 cells
600
400
200
0
INF-γ
IL-4
**
*
*
ns
**
*
ns
ns
ns
***
**
***
PBS
L1(0μg)
L1(20μg)
L2(0μg)
L2(20μg)
L3(0μg)
L3(20μg)

Figure 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/CN2024/128274

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07C229/16 A61K9/127 C07D295/13
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07C A61K C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 113 185 421 B (GUANGZHOU RIBOBIO CO LTD) 25 January 2022 (2022-01-25) cited in the application	1-6,8, 20-43
A	page 15; compound A18 claim 33 page 19, paragraph 82	7,9-19

Y	WO 2017/049245 A2 (MODERNATX INC [US]) 23 March 2017 (2017-03-23)	1-6,8, 20-43
A	pages 26-34 claims 132, 150, 163, 164, 169	7,9-19

Y	WO 2022/152109 A2 (SUZHOU ABOGEN BIOSCIENCES CO LTD [CN]) 21 July 2022 (2022-07-21)	1-6,8, 20-43
A	page 157; example 12; compound 20 page 296; example 81 page 297; table 2	7,9-19

	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 February 2025	13/03/2025

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Watchorn, Peter

2

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/CN2024/128274

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6 696 424 B1 (WHEELER CARL [US]) 24 February 2004 (2004-02-24)	1-6,8, 20-43
A	abstract figure 1A page 22, paragraph 5-6	7,9-19

Y	PLYAVNIK N. V. ET AL: "Synthesis of Alkyl Glycerolipids with Functional Groups in Their Polar Heads", RUSSIAN JOURNAL OF BIOORGANIC CHEMISTRY, vol. 30, no. 5, 1 September 2004 (2004-09-01), pages 454-458, XP093246302, Moscow ISSN: 1068-1620, DOI: 10.1023/B:RUBI.0000043789.84688.c7	1-6,8, 20-43
A	page 455; compounds IIIa, IIIb page 454, column 1, paragraph 1	7,9-19

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/CN2024/128274

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CN 113185421 B	25-01-2022	CN 113185421 A	30-07-2021
		TW 202248190 A	16-12-2022
WO 2017049245 A2	23-03-2017	AU 2016324310 A1	12-04-2018
		AU 2021204763 A1	05-08-2021
		CA 2998810 A1	23-03-2017
		CY 1125154 T1	09-06-2023
		DK 3350157 T3	14-02-2022
		EP 3350157 A2	25-07-2018
		EP 3736261 A1	11-11-2020
		EP 4286012 A2	06-12-2023
		ES 2910425 T3	12-05-2022
		ES 2969082 T3	16-05-2024
		HR P20220156 T1	15-04-2022
		HU E057613 T2	28-05-2022
		JP 6948313 B2	13-10-2021
		JP 6948313 B6	10-11-2021
		JP 6948313 B6	14-01-2022
		JP 6948313 B6	08-02-2022
		JP 7326395 B2	15-08-2023
		JP 2018532721 A	08-11-2018
		JP 2022008431 A	13-01-2022
		JP 2023164798 A	14-11-2023
		LT 3350157 T	25-02-2022
		PL 3350157 T3	16-05-2022
		PT 3350157 T	18-03-2022
		RS 63030 B1	29-04-2022
		SI 3350157 T1	29-04-2022
		SM T202200178 T1	12-05-2022
		TW 201718017 A	01-06-2017
		TW 202241844 A	01-11-2022
		US 2017210697 A1	27-07-2017
		US 2017210698 A1	27-07-2017
		US 2017224844 A1	10-08-2017
		US 2017233332 A1	17-08-2017
		US 2018201572 A1	19-07-2018
		US 2018273467 A1	27-09-2018
		US 2019016669 A1	17-01-2019
		US 2020123100 A1	23-04-2020
		US 2022380299 A1	01-12-2022
		US 2024124388 A1	18-04-2024
		WO 2017049245 A2	23-03-2017
WO 2022152109 A2	21-07-2022	TW 202229227 A	01-08-2022
		WO 2022152109 A2	21-07-2022
US 6696424 B1	24-02-2004	NONE	