present invention can be produced by an alcohol dilution method using flow paths. The method is a method for producing lipid nanoparticles by introducing a solution obtained by dissolving a lipid component in an alcohol solvent and a solution obtained by dissolving a water-soluble component to be encapsulated in the lipid nanoparticles in an aqueous solvent from separate flow paths and joining the solutions. Examples of the aqueous solvent used in the alcohol dilution method include buffers such as a phosphate buffer, a citrate buffer, and phosphate-buffered saline, physiological saline, and a culture medium for culturing cells.

[0179]

In an aspect, the present invention relates to a lipid nanoparticle preparation containing a mixture of the pH-sensitive cationic lipid of the present invention, a salt, or a stereoisomer thereof. In another aspect, the present invention relates to a lipid nanoparticle preparation containing (i) a sterol or a sterol derivative, (ii) a polyalkylene glycol-modified lipid, (iii) a nucleic acid, (iv) a buffer, (v) DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), and (vi) the pH-sensitive cationic lipid of the present invention, a salt, or a stereoisomer thereof.

Examples of the sterol or the sterol derivative include cholesterol and sitosterol, and cholesterol is preferable.

Examples of the polyalkylene glycol-modified lipid include a polyethylene glycol-modified lipid and a polypropylene glycol-modified lipid, and a polyethylene glycol-modified lipid is preferable.

Examples of the nucleic acid include siRNA, pDNA, and mRNA, and siRNA and mRNA are preferable.

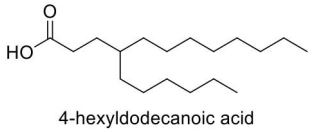
reaction liquid. The reaction liquid was heated to room temperature and stirred overnight. An aqueous saturated ammonium chloride solution was added to the reaction liquid, and extraction with ethyl acetate was conducted three times. After the organic layer was washed with saturated brine, sodium sulfate was added, and the mixture was dehydrated. After filtration thereof, the solvent was evaporated under reduced pressure, and a crude product was obtained. The crude product was purified by silica gel chromatography [elution solvent; hexane/ethyl acetate], and a mixture (928 mg) of benzyl 3-nonyldodec-2-enoate of interest and raw material 10-nonadecanone was obtained.

(2) The mixture obtained in (1) and Pd/C 10% (130 mg) were added to a mixture solution of THF (4 ml)/MeOH (8 ml). Subsequently, triethylsilane (796 μl) was added dropwise, and the mixture was stirred for four hours. After filtration of the reaction liquid, the solvent was evaporated under reduced pressure, and a crude product was obtained. The crude product was purified by silica gel chromatography [elution solvent; hexane/ethyl acetate], and 3-nonyldodecanoic acid of interest was obtained.

[0186]

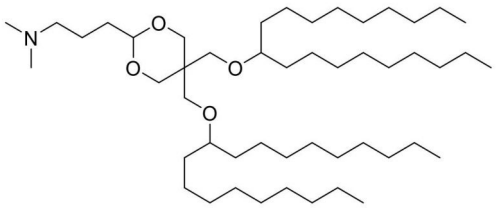
Raw Material Synthesis Example 3 (synthesis of γ-alkyl branched carboxylic acid)

[Chem. 160]



4-hexyldodecanoic acid

4-hexyldodecanoic acid of interest was obtained in the same manner as in Raw Material Synthesis Example 2, except that 2-hexyldecanal was used



3-(5,5-bis((nonadecan-10-yloxy)methyl)-1,3-dioxan-2-yl)-*N*,*N*-dimethylpropan-1-amine

3-(5,5-bis((nonadecan-10-yloxy)methyl)-1,3-dioxan-2-yl)-N,N-dimethylpropan-1-amine of interest was obtained in the same manner as in Lipid Synthesis Example 91, except that nonadecan-10-ol was used instead of 2-hexyldecan-1-ol of Lipid Synthesis Example 91-(2).

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.47 (t, J = 4.7 Hz, 1H), 3.90 (d, J = 11.5 Hz, 2H), 3.64-3.59 (m, 4H), 3.25-3.21 (m, 1H), 3.14 (s, 2H), 3.10-3.07 (m, 1H), 2.29 (t, J = :6.6 Hz, 2H), 2.23 (s, 6H), 1.61-1.33 (m, 12H), 1.26 (brs, 56H), 0.89-0.87 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 766.8, measured value m/z = 766.8.

[0243]

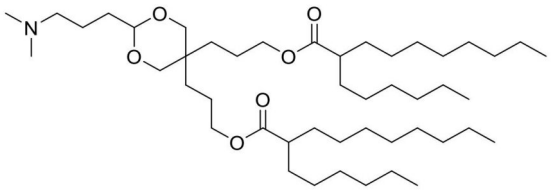
Lipid Synthesis Example 93 (synthesis of (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(propane-3,1-diyl) bis(2-nonylundecanoate))

[Chem. 206]

[0244]

Lipid Synthesis Example 94 (synthesis of (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(propane-3,1-diyl) bis(2-hexyldecanoate))

[Chem. 207]



(2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(propane-3,1-diyl) bis(2-hexyldecanoate)

(2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(propane-3,1-diyl) bis(2-hexyldecanoate) of interest was obtained in the same manner as in Lipid Synthesis Example 93, except that 2-hexyldecanoic acid was used instead of 2-nonylundecanoic acid in Lipid Synthesis Example 93.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.44 (t, J = 4.1 Hz, 1H), 4.10 (t, J = 6.5 Hz, 2H), 4.02 (t, J = 6.3 Hz, 2H), 3.75 (d, J = 11.4 Hz, 2H), 3.38 (d, J = 11.4 Hz, 2H), 2.36-2.26 (m, 10H), 1.73-1.40 (m, 16H), 1.26 (brs, 42H), 1.10-1.04 (m, 2H), 0.90-0.85 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 766.7, measured value m/z = 766.7.

[0245]

II. Production and Evaluation of Lipid Nanoparticles

<Adjustment of Buffers>

Citrate Buffer

Ultrapure water was added to 6.90 g of citric acid (FUJIFILM Wako Pure Chemical Corporation, product code: 030-05525) and 4.15 g of sodium citrate dihydrate (Aldrich, catalog number: W302600), and thus 1 L of a citrate buffer

concentration in the culture solution became 1 nM for transfection, and the cells were incubated at 37°C in an environment of 5% CO2. PLK1 is a kinase regulating the progress of the M phase of the cell cycle, and it is known that the gene knockdown thereof suppresses the cell growth. In this evaluation, to evaluate the degree of gene knockdown using the viable cell proportion as an indicator, the viable cell proportion was measured with CellTiter-Fluor (trademark) Cell Viability Assay (Promega, G6080) 72 hours after the transfection. The assay conditions were in accordance with the manufacturer's protocol. GF-AFC was added, and the cells were incubated at 37°C in an environment of 5% CO2 for two hours. Then, the fluorescence intensity was measured, and the viable cell count was measured. The viable cell index, which was the proportion of the viable cell count after treating with siRNA for human PLK1 (siPLK1) to the viable cell count after treating with siRNA for luciferase (siLuc), was calculated with the calculation equation below.

Calculation equation: viable cell index (%) = viable cell count after treating with siPLK1/viable cell count after treating with siLuc × 100

The lipid compositions of the lipid nanoparticle containing siPLK1 and the lipid nanoparticle containing siLuc were the same, and correction with the viable cell count after treating with siLuc was conducted to exclude the influence of the contact of the LNPs containing siRNA to the cells.

The cell growth is inhibited when siPLK1 is introduced into cells. Accordingly, when the siRNA introduction ability of the lipid nanoparticles containing siPLK1 is high, the viable cell index becomes a low value. Therefore, a lower viable cell index (%) indicates that the introduction efficiency of siPLK1 into cells is higher.

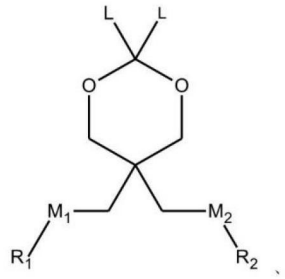
CLAIMS

[Claim 1]

A compound represented by

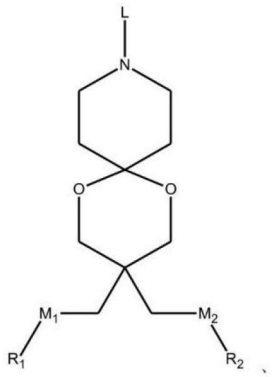
a general formula I:

[Chem. 1]



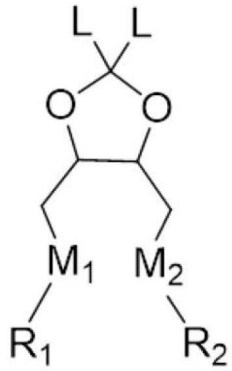
a general formula II:

[Chem. 2]



or a general formula III:

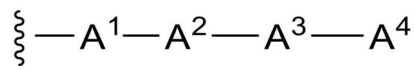
[Chem. 3]



wherein in the formulae,

L's are independently H, a linear C_{1-10} alkyl, a branched C_{3-10} alkyl, or

[Chem. 4]



wherein A^1 is a C_{1-6} alkylene or phenylene,

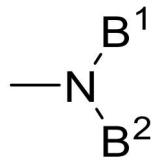
A^2 is -OC(=O)-, -OC(=O)-O-, or -OC(=O)-NH- or does not exist,

A^3 is a C_{1-6} alkylene or does not exist, and

A^4 is a 4- to 15-membered nitrogen-containing heterocyclyl, wherein the nitrogen-containing heterocyclyl is unsubstituted or substituted with at least one selected from the group consisting of a C_{1-4} alkyl, hydroxy, and oxo, or

A^4 is

[Chem. 5]



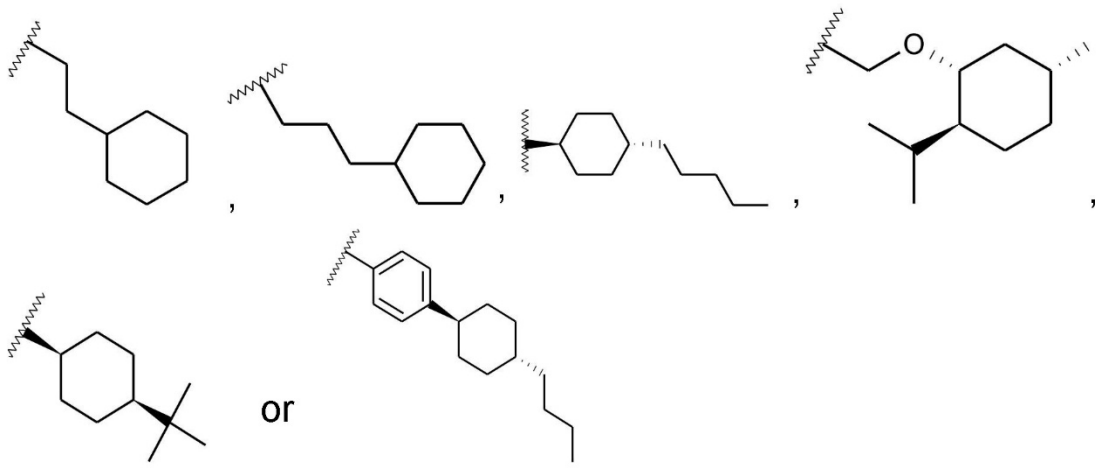
wherein B^1 and B^2 are each independently a C_{1-4} alkyl, a hydroxy C_{1-4} alkyl, a C_{1-4} alkoxy C_{1-4} alkyl, or a 4- to 6-membered heterocyclyl, wherein the 4- to 6-membered heterocyclyl is unsubstituted or substituted with at least one

selected from the group consisting of a C_{1-4} alkyl, hydroxy, and oxo,

M_1 and M_2 are independently -O-, -O-CH_2-, -O-C(O)-, -O-C(O)-O-, -O-C(O)-O-CH_2-, -O-C(O)-CH_2-, -O-C(O)-CH_2-CH_2-, -O-C(O)-CH_2-CH_2-CH_2-, -O-C(O)-CH_2-CH_2-CH_2-CH_2-, -O-C(O)-C-C-C(O)-, -O-C(O)-CH_2-CH_2-C(O)-O-, -O-C(O)-CH_2-CH_2-C(O)-O-CH_2-, -O-C(O)-CH_2-CH_2-C(O)-NH-, -O-CH_2-CH_2-CH_2-O-C(O)-, -C-C(O)-O-, -CH_2-C(O)-O-CH_2-, -C-CH_2-O-C(O)-, -S-S-CH_2-, or -S-CH_2-C-C-S-S-CH_2-, and

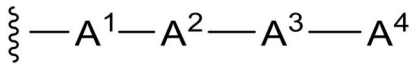
R_1 and R_2 are independently a linear C_{1-30} alkyl, a branched C_{3-30} alkyl, a linear C_{2-30} alkenyl, a branched C_{3-30} alkenyl, adamantyl, a sterol group, and

[Chem. 6]



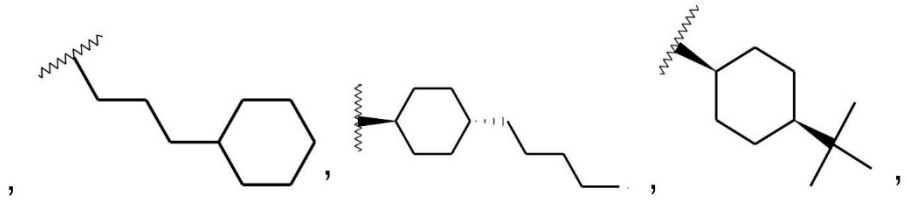
wherein in the general formulae I and III, at least one of L's is

[Chem. 7]

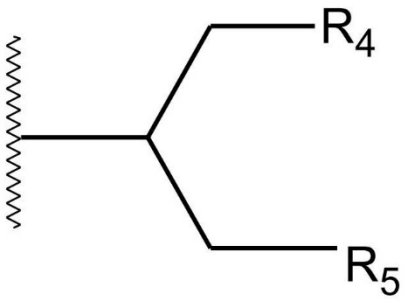


and, wherein in the general formulae II, R_1 and R_2 are independently a linear C_{1-30} alkyl, adamantyl,

[Chem. 11]



or



wherein R_4 and R_5 are independently a linear $C_{1\text{-}16}$ alkyl,

or a salt or a stereoisomer thereof.

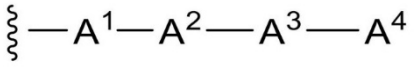
[Claim 2]

The compound, the salt, or the stereoisomer thereof according to claim 1,

wherein the compound is represented by the general formula I,

wherein one of L's is H, and the other L is

[Chem. 8]



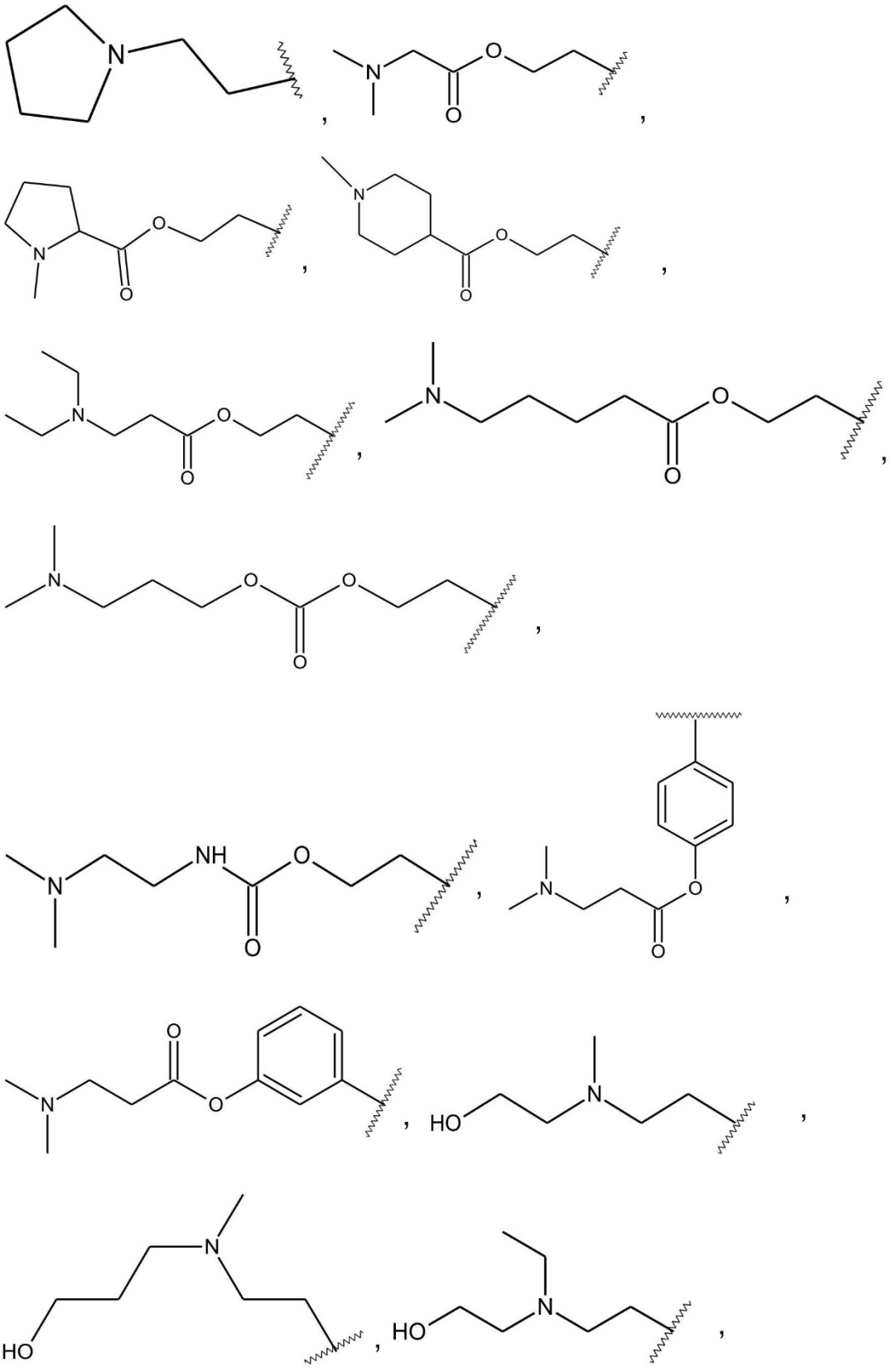
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[Claim 3]

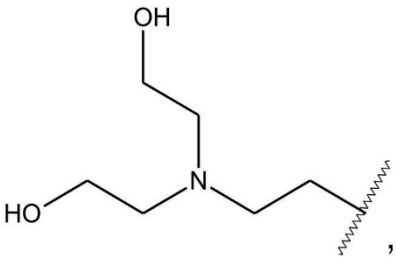
The compound, the salt, or the stereoisomer thereof according to claim 2,

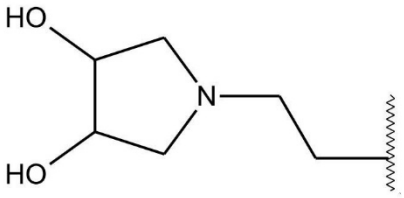
wherein one of L's is H, and the other L is

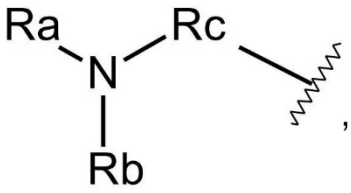
[Chem. 9-1]

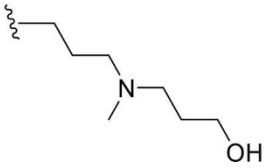


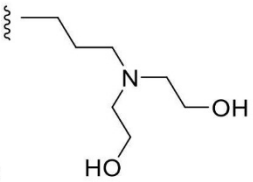
[Chem. 9-2]

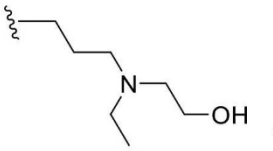


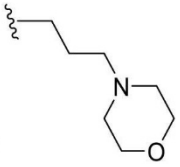
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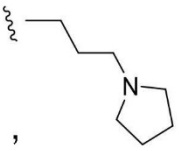
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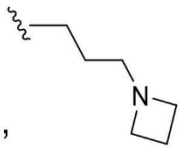
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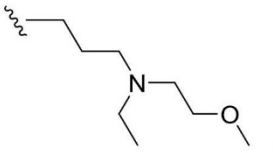
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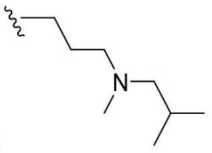
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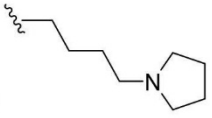
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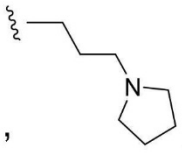
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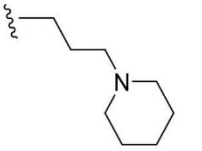
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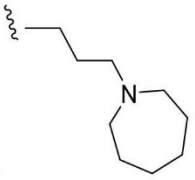
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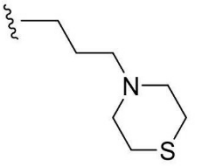
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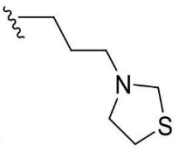
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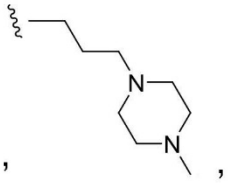
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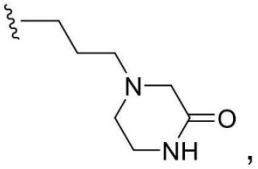
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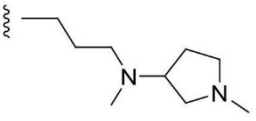
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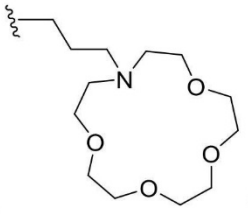
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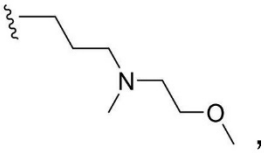
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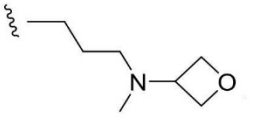
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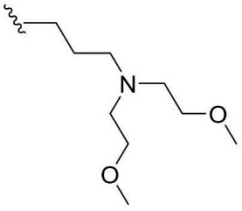
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or


wherein R_a and R_b are independently methyl, ethyl, n-propyl, or

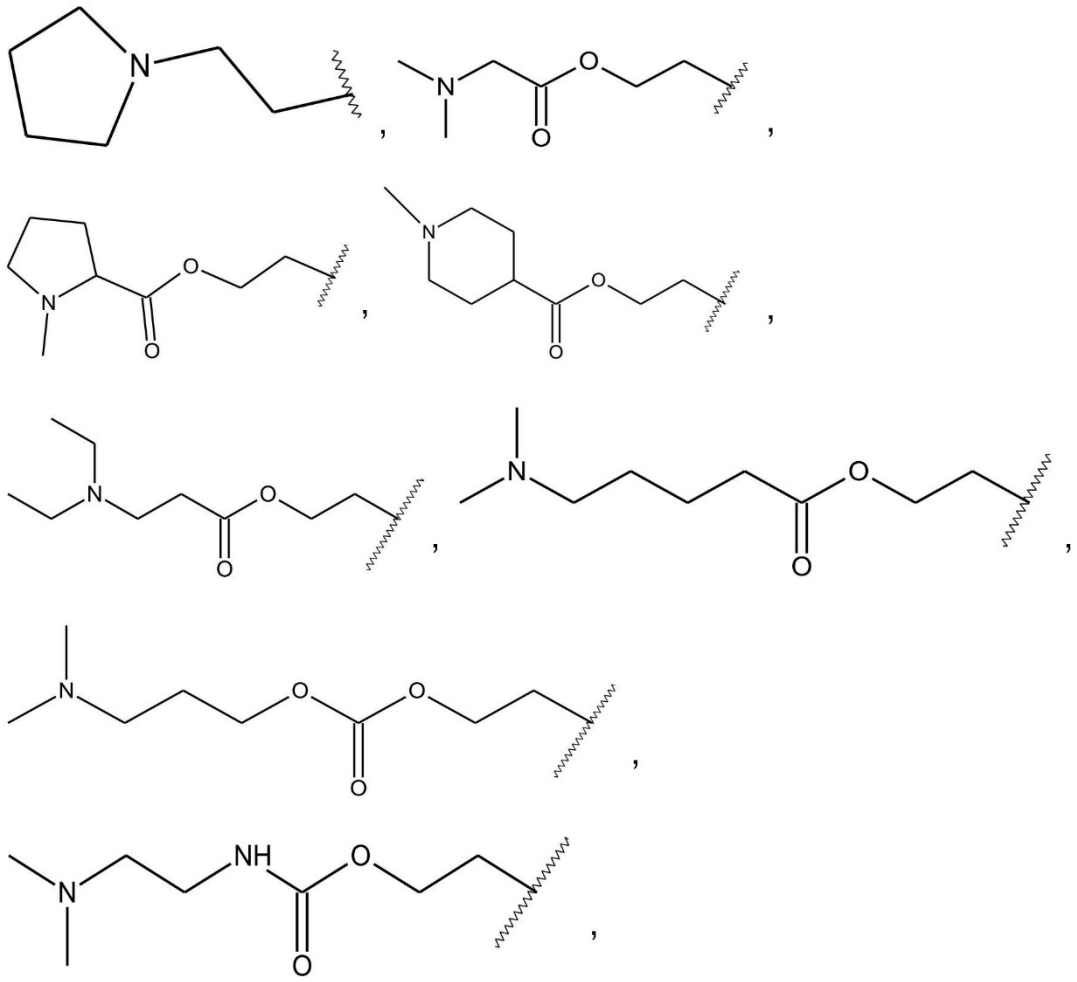
isopropyl, and R_c is a linear C_{1-6} alkylene.

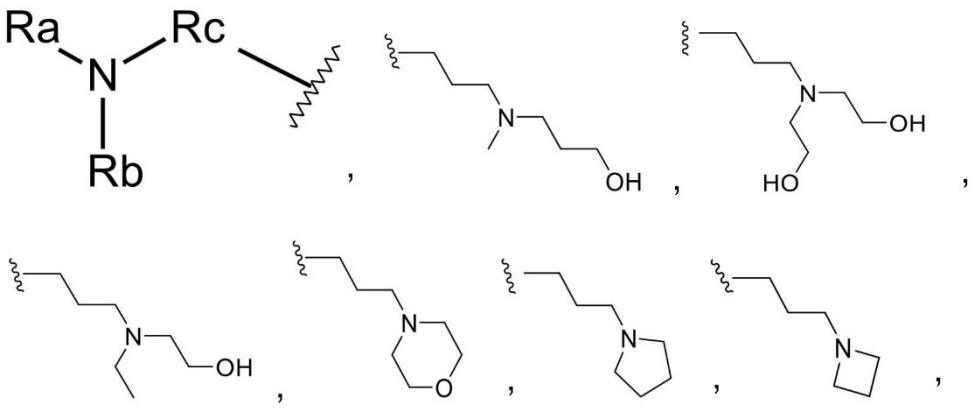
[Claim 4]

The compound, the salt, or the stereoisomer thereof according to claim 3,

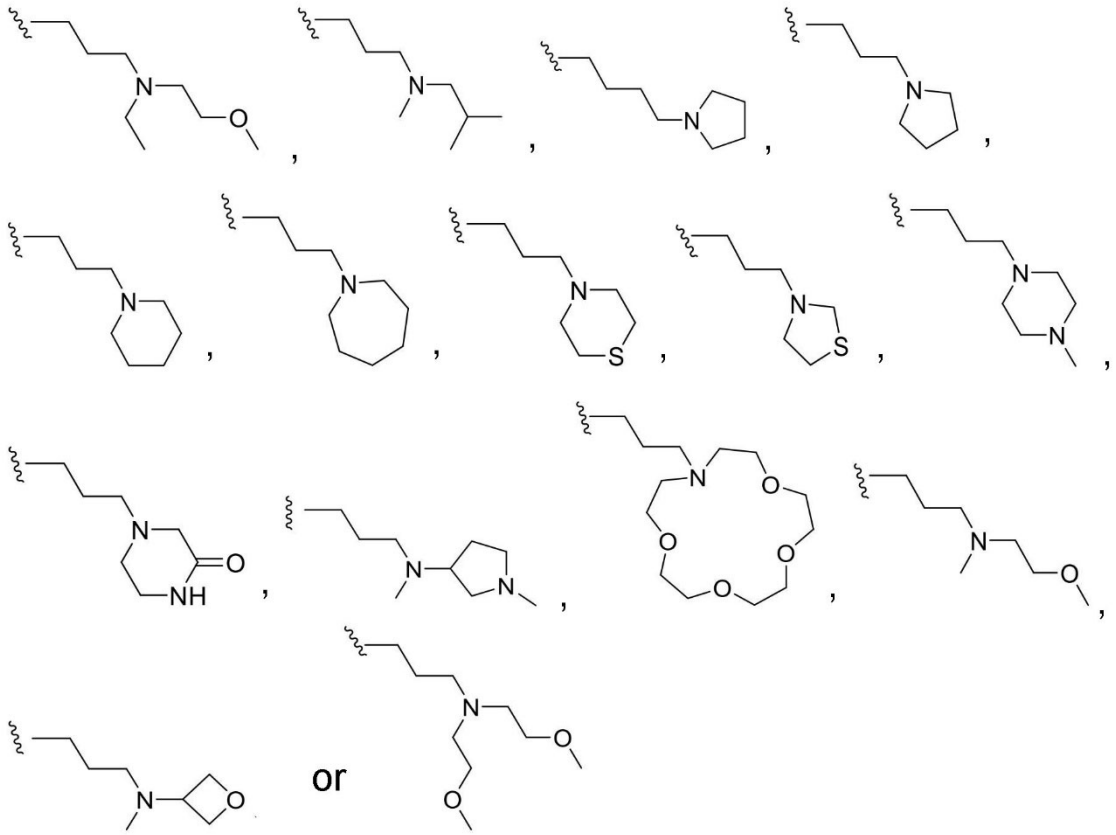
wherein one of L's is H, and the other L is

[Chem. 10-1]





[Chem. 10-2]



wherein R_a and R_b are independently methyl, ethyl, n-propyl, or isopropyl, and R_c is a linear C_{1-6} alkylene.

[Claim 5]

The compound, the salt, or the stereoisomer thereof according to claim 4,

wherein M_1 and M_2 are the same.

[Claim 6]

The compound, the salt, or the stereoisomer thereof according to claim 5,

wherein M_1 and M_2 are -O-, -O-CH_2-, -O-C(O)-, -O-C(O)-O-, -O-C(O)-O-

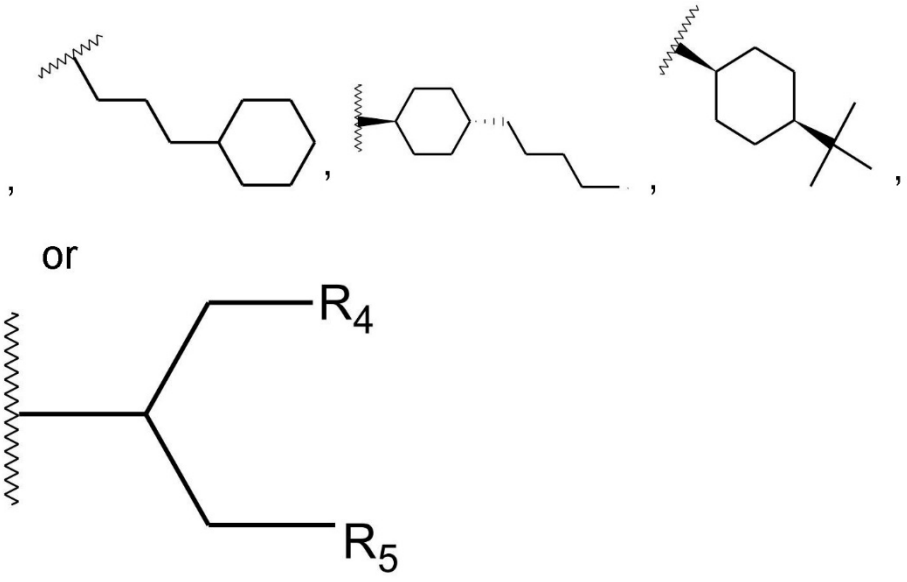
CH_2-, -O-C(O)-CH_2-, -O-C(O)-CH_2-CH_2-, -O-C(O)-CH_2-CH_2-C(O)-O-CH_2-, or -CH_2-CH_2-O-C(O)-.

[Claim 7]

The compound, the salt, or the stereoisomer thereof according to claim 6,

wherein in the general formulae I and III, R_1 and R_2 are independently a linear C_{1-30} alkyl, a linear C_{2-30} alkenyl, a branched C_{3-30} alkenyl, adamantyl,

[Chem. 11]



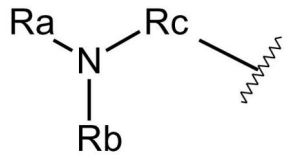
wherein R_4 and R_5 are independently a linear C_{1-16} alkyl.

[Claim 8]

The compound, the salt, or the stereoisomer thereof according to claim 7,

wherein one of L's is H, and the other L is

[Chem. 12]



wherein R_a and R_b are independently methyl, ethyl, n-propyl, or isopropyl, wherein the total number of carbon atoms of R_a and R_b is 4 or less, and

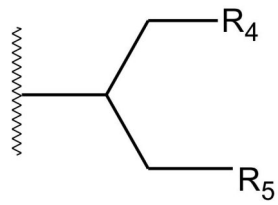
R_c is a linear C_{2-6} alkylene.

[Claim 9]

The compound, the salt, or the stereoisomer thereof according to claim 7,

wherein R_1 and R_2 are independently

[Chem. 13]



wherein R_4 and R_5 are independently a linear C_{1-11} alkyl,

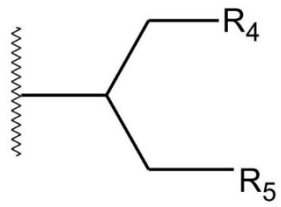
wherein the total number of carbon atoms of R_4 and R_5 is 10 to 22.

[Claim 10]

The compound, the salt, or the stereoisomer thereof according to claim 7,

wherein R_1 and R_2 are independently

[Chem. 14]



wherein R_4 and R_5 are independently a linear C_{5-11} alkyl,

wherein the difference of the carbon atom numbers of R_4 and R_5 is 0 to 3.

[Claim 11]

The compound, the salt, or the stereoisomer thereof according to claim 1,

wherein the compound is represented by the general formula II,

wherein L is a linear C_{1-5} alkyl,

M_1 and M_2 are -O-C(O)-.

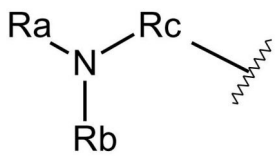
[Claim 12]

The compound, the salt, or the stereoisomer thereof according to claim 1,

wherein the compound is represented by the general formula III,

wherein one of L's is H, and the other L is

[Chem. 16]

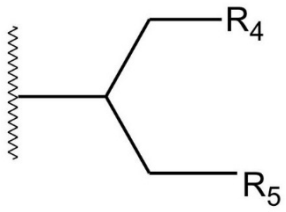


wherein R_a and R_b are independently methyl, ethyl, n-propyl, or isopropyl, and R_c is a linear C_{1-6} alkylene),

M_1 and M_2 are -O-C(O)-, and

R_1 and R_2 are the same and are a linear C_{1-30} alkyl, a linear C_{2-30} alkenyl, or

[Chem. 17]



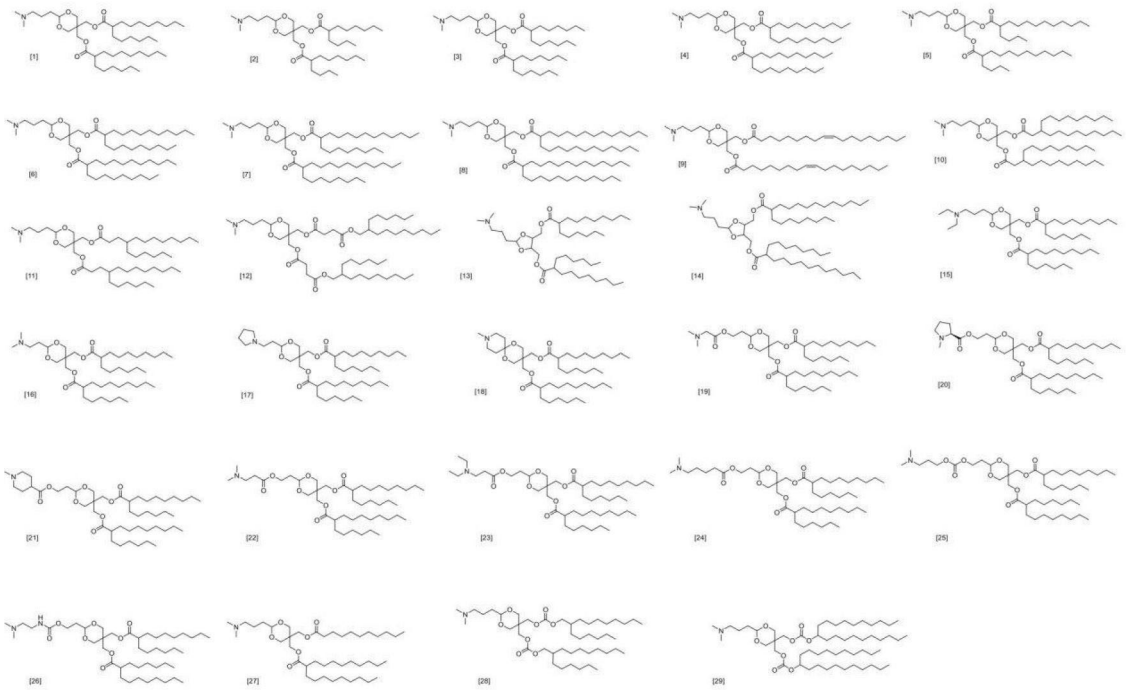
wherein R_4 and R_5 are independently a linear C_{1-16} alkyl.

[Claim 13]

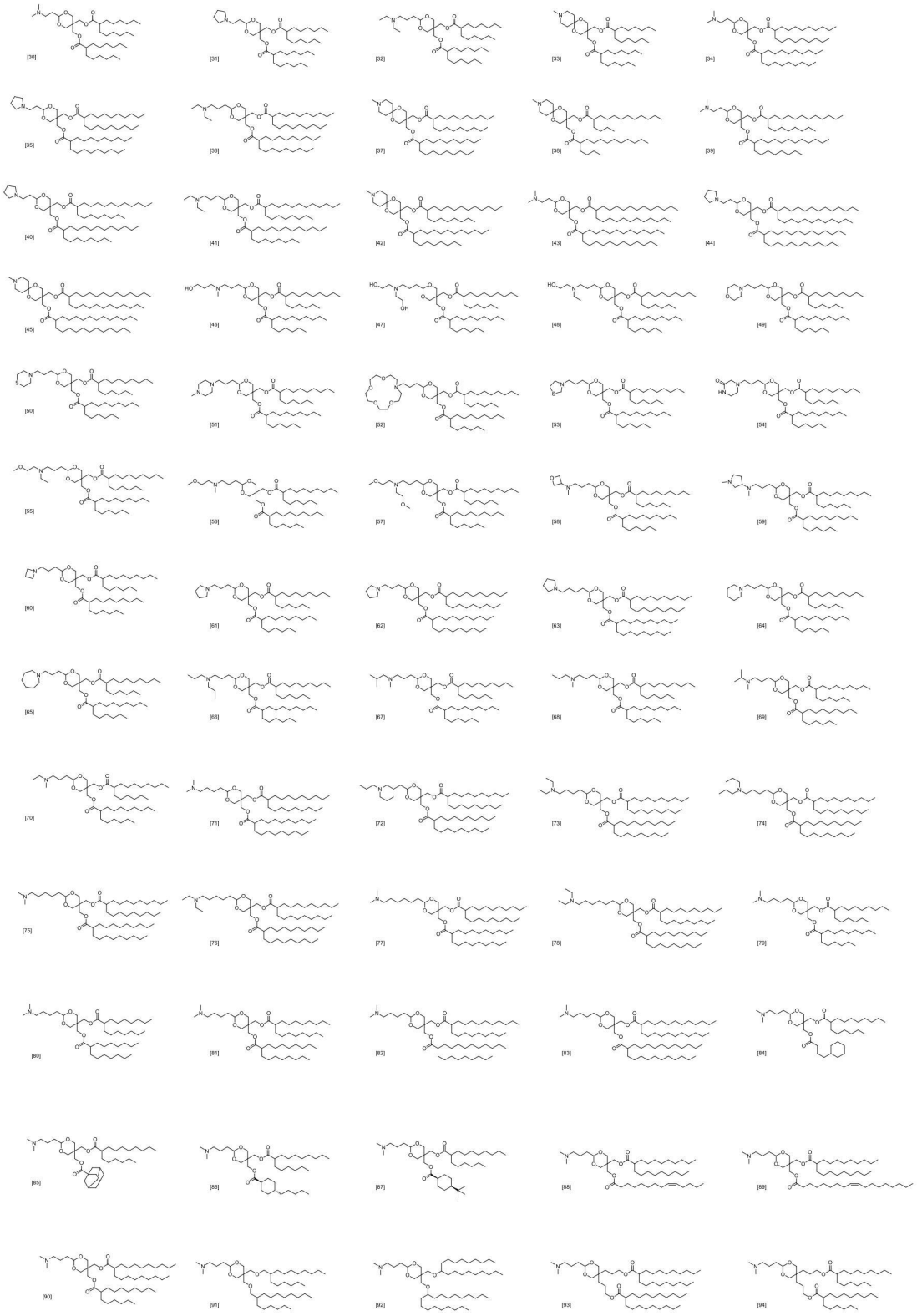
The compound, the salt, or the stereoisomer thereof according to claim 1,

wherein the compound is represented by any of the following formulae

[Chem. 18-1]



[Chem. 18-2]



[Claim 14]

A lipid nanoparticle comprising the compound, the salt, or the stereoisomer thereof according to claim 1.

[Claim 15]

The lipid nanoparticle according to claim 14 further comprising a sterol and a polyalkylene glycol-modified lipid.

[Claim 16]

The lipid nanoparticle according to claim 14 comprising a nucleic acid.

[Claim 17]

The lipid nanoparticle according to claim 16, wherein the nucleic acid is mRNA or siRNA.

[Claim 18]

A pharmaceutical composition comprising the lipid nanoparticle according to claim 14.

[Claim 19]

The pharmaceutical composition according to claim 18 which is used for gene therapy.