DESCRIPTION

Title of Invention: LIPID NANOPARTICLE

Technical Field

[0001]

The present invention relates to a lipid nanoparticle which can introduce a drug into a cell efficiently and a constituent lipid of the lipid nanoparticle.

Background Art

[0002]

Lipid nanoparticles (LNPs) are used as carriers for encapsulating a lipophilic drug or a nucleic acid such as siRNA (small interfering RNA) and mRNA and delivering to target cells. For example, as lipid nanoparticles serving as carriers for delivering a nucleic acid such as siRNA into target cells efficiently, lipid nanoparticles containing a pH-sensitive cationic lipid which is electrically neutral at physiological pH and which turns into cationic in a weakly acidic pH environment such as in endosomes as a constituent lipid have been reported.

[0003]

The present applicant and the like have disclosed lipid nanoparticles containing a pH-sensitive cationic lipid which show a higher *in vivo* gene expression activity than that of a case using CL4F6 and CL15F6 as lipids (PTL 1). Moreover, the present applicant uses a retinoid conjugate in which a constituent lipid is bonded to retinoid through a linker for forming an siRNA-

containing liposome containing an ionizable cationic lipid (PTL 2). However, lipids which can be used for lipid nanoparticles for drug delivery are limited, and development of novel lipids which can introduce a drug into cells efficiently has been required.

Citation List

Patent Literature

[0004]

PTL 1: WO2022/071582

PTL 2: JP2015-523990A

Summary of Invention

Technical Problem

[0005]

An object of the present invention is to provide a lipid nanoparticle which can introduce a drug into a cell efficiently and a constituent lipid of the lipid nanoparticle.

Solution to Problem

[0006]

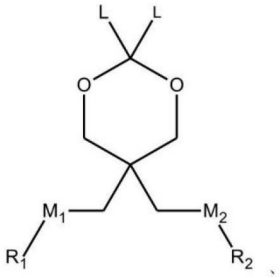
The present inventors have found that lipid nanoparticles containing a pH-sensitive cationic lipid having a dioxane structure such as 1,3-dioxane or a dioxolane structure such as 1,3-dioxolane as a constituent lipid can introduce a drug into cells efficiently and thus completed the present invention.

[0007]

That is, the present invention provides the following lipid nanoparticle and the constituent lipid thereof.

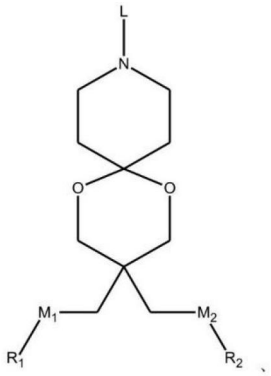
[1] A compound represented by a general formula I:

[Chem. 1]



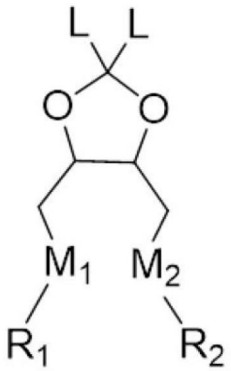
a general formula II:

[Chem. 2]



or a general formula III:

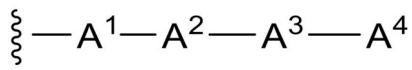
[Chem. 3]



[in the formulae,

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

[Chem. 4]



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

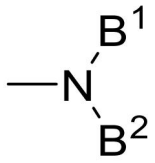
A^2 is -OC(=O)-, -OC(=O)-O-, or -OC(=O)-NH- or does not exist,

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

A^4 is a 4- to 15-membered nitrogen-containing heterocyclyl, where 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]



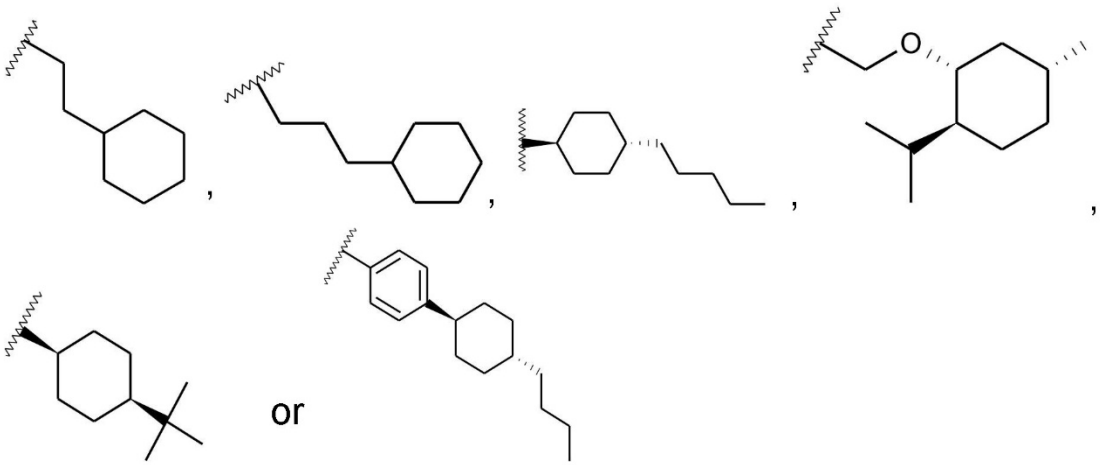
in which B^1 and B^2 are each independently a C_{1-4} alkyl, a hydroxy C_{1-4} alkyl, a C_{1-4} alkoxy C_{1-4} alkyl, or a 4- to 6-membered heterocyclyl, where the 4- to 6-membered heterocyclyl is unsubstituted or substituted with at least one selected from the group consisting of a C_{1-4} alkyl, hydroxy, 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

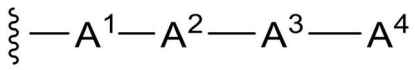
R_1 and R_2 are independently a linear C_{1-30} alkyl, a branched C_{3-30} alkyl, a linear C_{2-30} alkenyl, a branched C_{3-30} alkenyl, adamantyl, a sterol group,

[Chem. 6]



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

[Chem. 7]



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or a salt or a stereoisomer thereof.

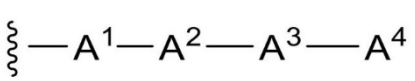
[0008]

[2] The compound, the salt, or the stereoisomer thereof according to [1]

in which the compound is represented by the general formula I

in which one of L's is H, and the other L is

[Chem. 8]

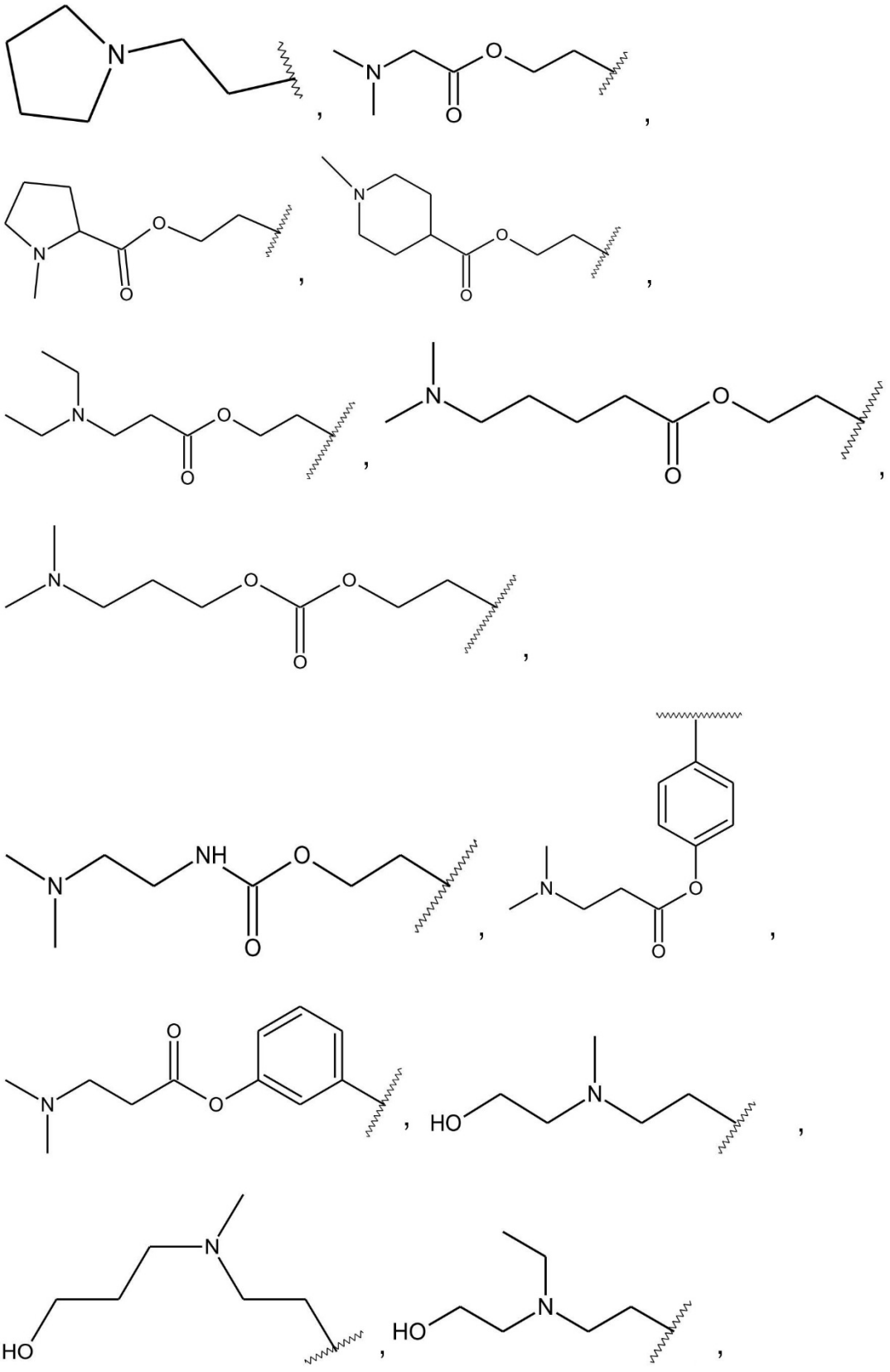


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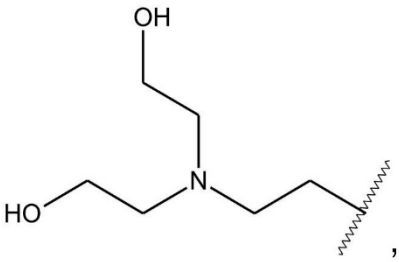
[3] The compound, the salt, or the stereoisomer thereof according to [1] or [2]

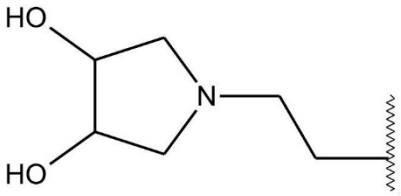
in which one of L's is H, and the other L is

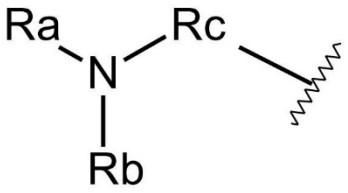
[Chem. 9-1]

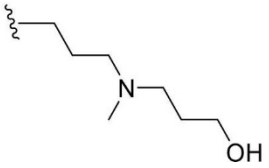


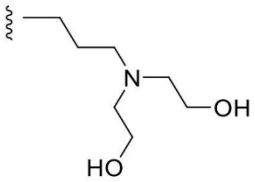
[Chem. 9-2]

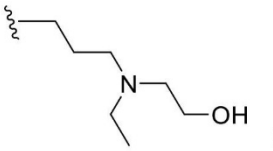


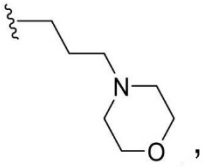
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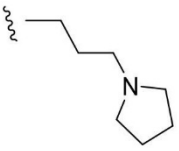
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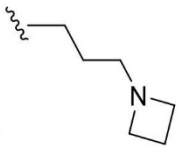
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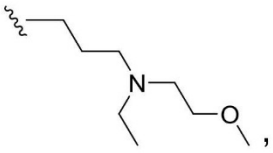
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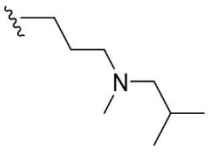
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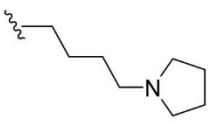
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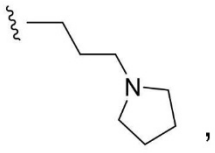
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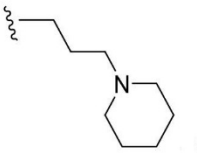
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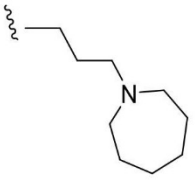
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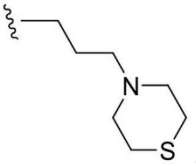
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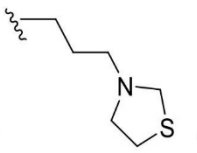
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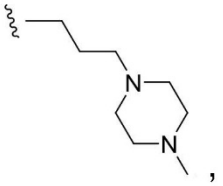
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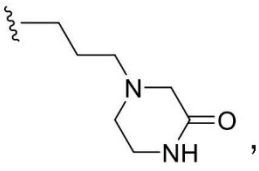
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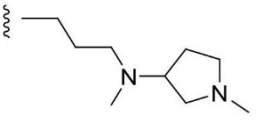
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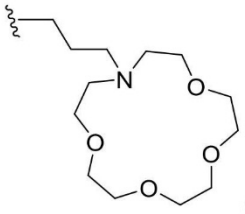
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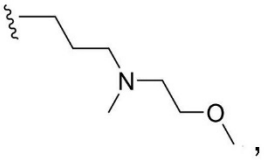
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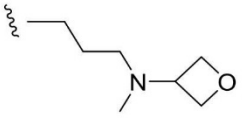
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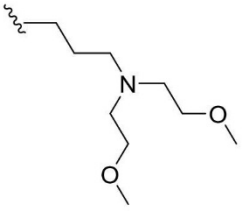
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or


(in the formula, R_a and R_b are independently methyl, ethyl, n-propyl, or

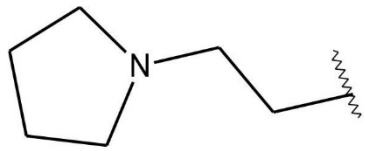
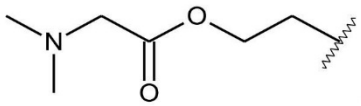
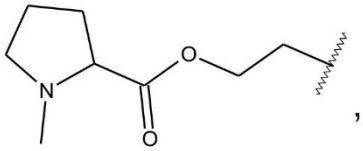
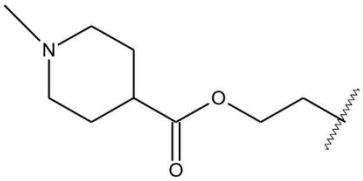
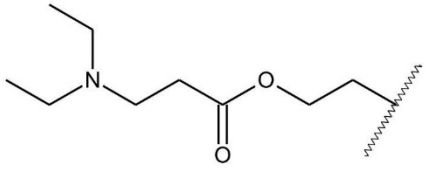
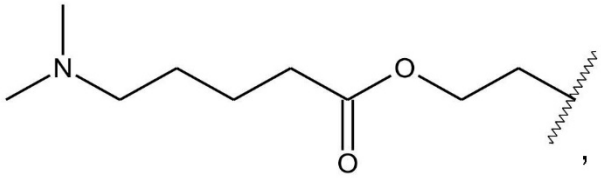
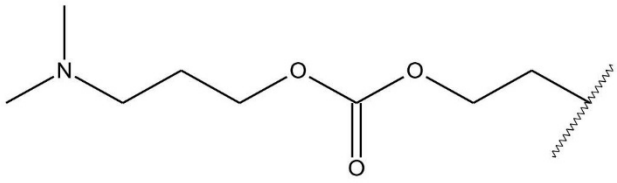
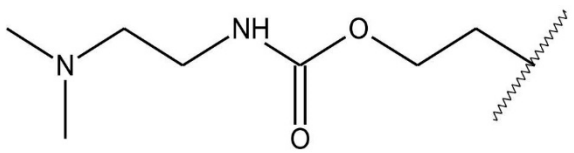
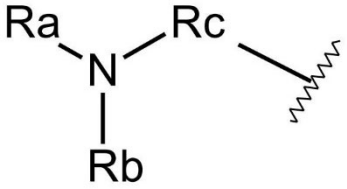
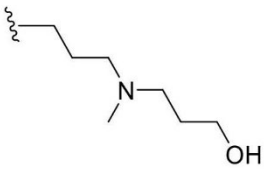
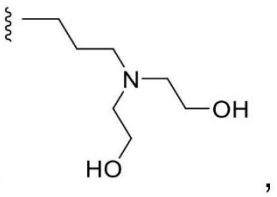
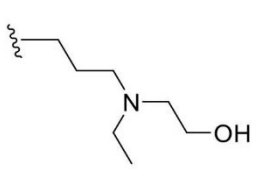
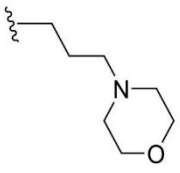
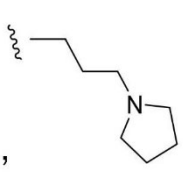
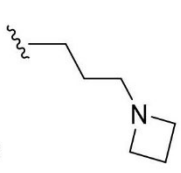
isopropyl, and R_c is a linear C_{1-6} alkylene.)

[0009]

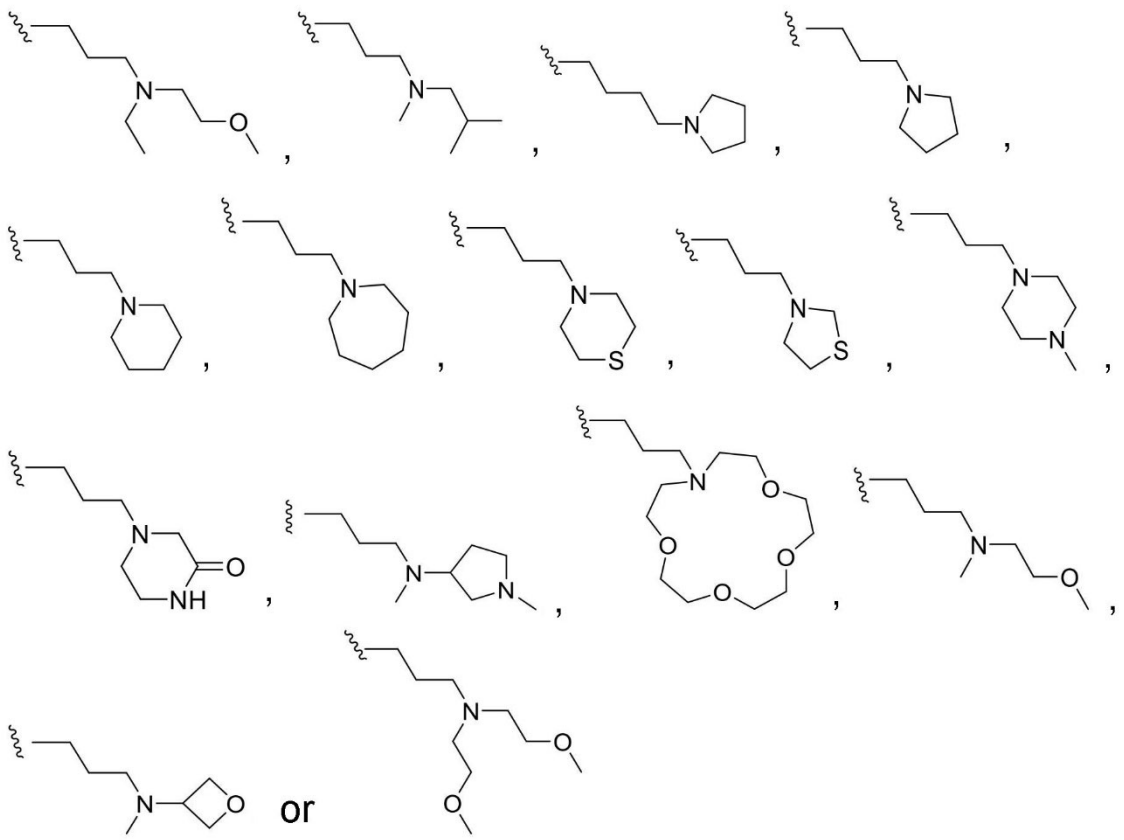
[4] The compound, the salt, or the stereoisomer thereof according to any one of [1] to [3]

in which one of L's is H, and the other L is

[Chem. 10-1]


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[Chem. 10-2]



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

[0010]

[5] The compound, the salt, or the stereoisomer thereof according to [1] to [4]

in which M_1 and M_2 are the same.

[6] The compound, the salt, or the stereoisomer thereof according to [5]

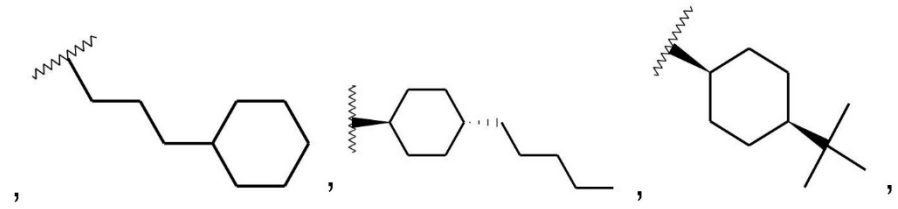
in which M_1 and M_2 are

-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)-.

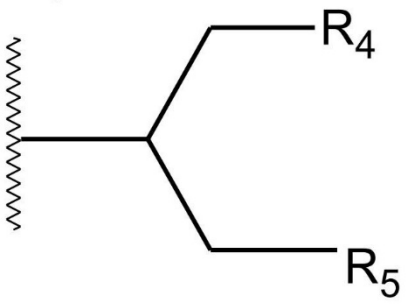
[7] The compound, the salt, or the stereoisomer thereof according to [1] to [6]

in which 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]



or,



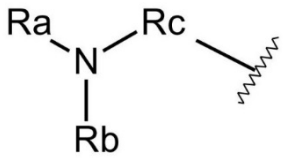
in which R_4 and R_5 are independently a linear C_{1-16} alkyl.

[0011]

[8] The compound, the salt, or the stereoisomer thereof according to [1] to [7]

in which one of L's is H, and the other L is

[Chem. 12]



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

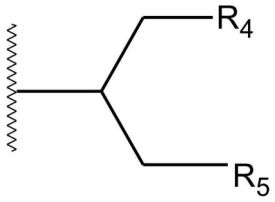
R_c is a linear C_{2-6} alkylene.

[9] The compound, the salt, or the stereoisomer thereof according to [1]

to [7]

in which R_1 and R_2 are independently

[Chem. 13]



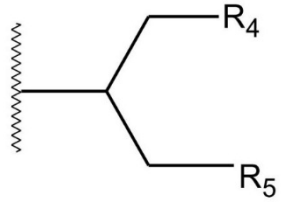
in which R_4 and R_5 are independently a linear C_{1-11} alkyl,

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

[10] The compound, the salt, or the stereoisomer thereof according to [1] to [7]

in which R_1 and R_2 are independently

[Chem. 14]



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

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

[0012]

[11] The compound, the salt, or the stereoisomer thereof according to [1]

in which the compound is represented by the general formula II

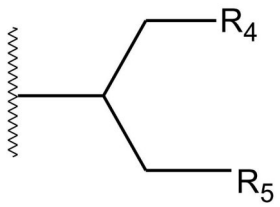
in which L is a linear C_{1-5} alkyl,

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. 15]



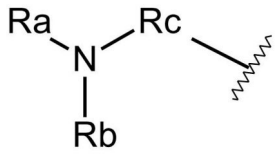
in which R_4 and R_5 are independently a linear C_{1-16} alkyl.

[12] The compound, the salt, or the stereoisomer thereof according to [1]

in which the compound is represented by the general formula III

in which one of L's is H, and the other L is

[Chem. 16]

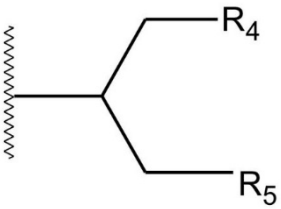


(in the formula, R_a and R_b are independently methyl, ethyl, n-propyl, or isopropyl, and R_c is a linear C_{1-6} alkylene),

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]



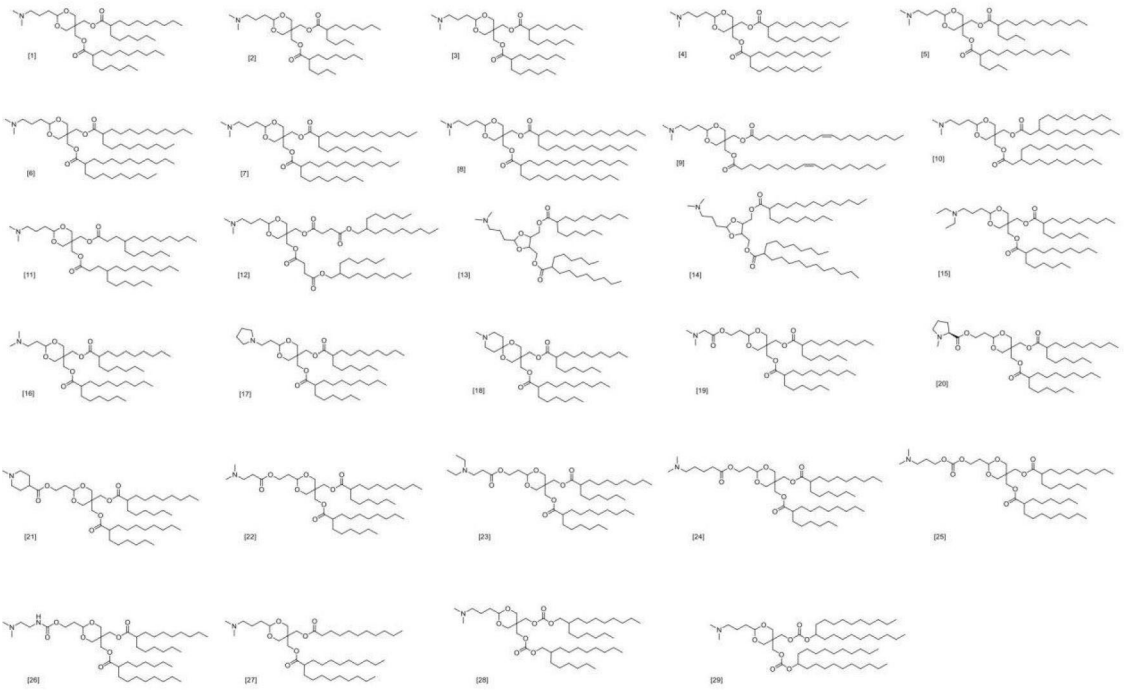
in which R_4 and R_5 are independently a linear C_{1-16} alkyl.

[0013]

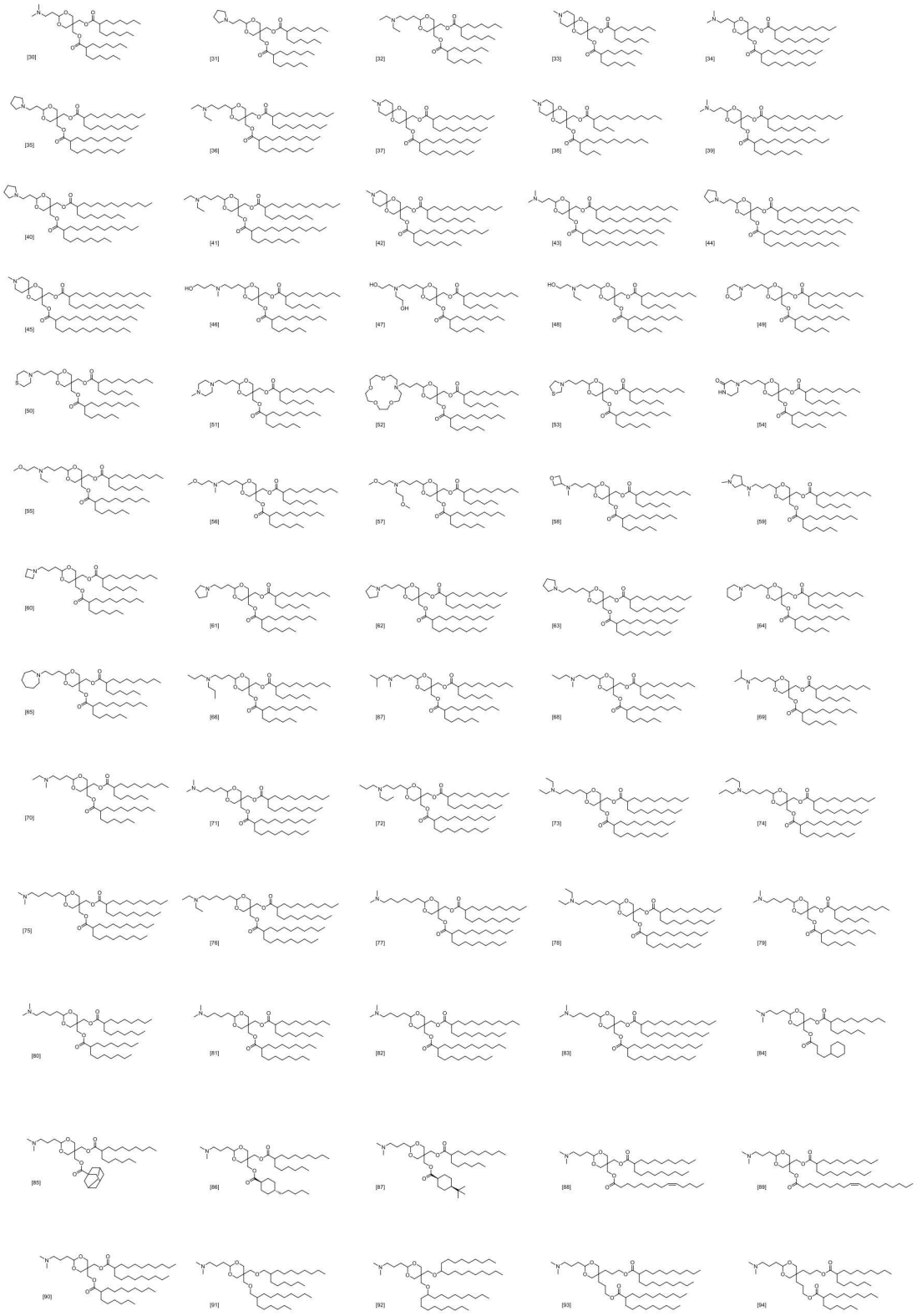
[13] The compound, the salt, or the stereoisomer thereof according to [1]

in which the compound is represented by any of the following formulae

[Chem. 18-1]



[Chem. 18-2]



[0014]

[14] A lipid nanoparticle containing the compound, the salt, or the stereoisomer thereof according to [1] to [13].

[15] The lipid nanoparticle according to [14] further containing a sterol and a polyalkylene glycol-modified lipid.

[16] The lipid nanoparticle according to [14] or [15] containing a nucleic acid.

[17] The lipid nanoparticle according to [16] in which the nucleic acid is mRNA or siRNA.

[18] A pharmaceutical composition containing the lipid nanoparticle according to [14] to [17].

[19] The pharmaceutical composition according to [18] which is used for gene therapy.

[20] A method for expressing an exogenous gene, including administering the lipid nanoparticle according to [14] to [17] which encapsulates a target exogenous gene to be expressed in a cell to a test animal (excluding a human) and expressing the exogenous gene in the cell of the test animal.

Advantageous Effects of Invention

[0015]

The lipid nanoparticle according to the present invention can introduce a drug into a cell efficiently. Accordingly, the lipid nanoparticle is useful as a gene delivery carrier used for gene therapy.

Description of Embodiments

[0016]

Embodiments of the present invention are specifically explained below.

[0017]

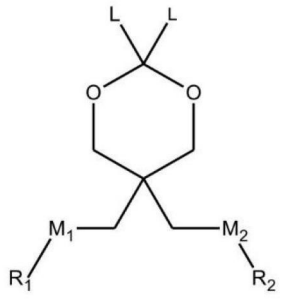
The lipid nanoparticle according to the present invention is a lipid nanoparticle containing a pH-sensitive cationic lipid having a dioxane structure or a dioxolane structure represented by the general formula (I), (II), or (III) below (sometimes referred to as "the pH-sensitive cationic lipid of the present invention" below).

Because the lipid nanoparticle has a pH-sensitive cationic lipid which is a compound represented by the general formula (I), (II), or (III), a salt, or a stereoisomer thereof as a constituent lipid of the lipid nanoparticle, the lipid nanoparticle according to the present invention can introduce a drug into a cell efficiently.

[0018]

General formula (I)

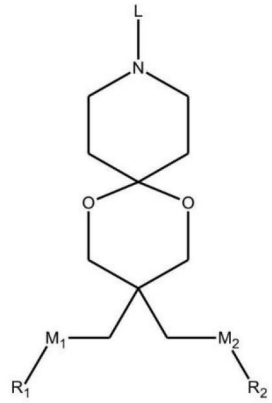
[Chem. 19]



[0019]

General formula (II)

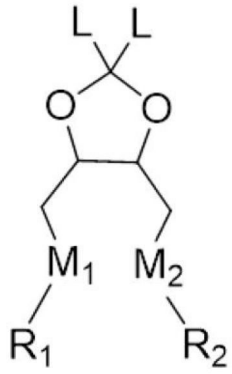
[Chem. 20]



[0020]

General formula (III)

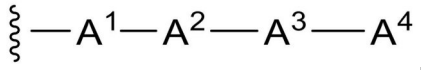
[Chem. 21]



[0021]

In the general formulae (I) to (III), L's are independently H, a linear $C_{1\text{-}10}$ alkyl, a branched $C_{3\text{-}10}$ alkyl, or

[Chem. 22]



.

A^1 is a $C_{1\text{-}6}$ alkylene or phenylene.

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

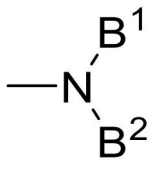
A^3 is a $C_{1\text{-}6}$ alkylene or does not exist.

A^4 is a 4- to 15-membered nitrogen-containing heterocyclyl, and the

nitrogen-containing heterocyclyl here is unsubstituted or substituted with at least one selected from the group consisting of a C_{1-4} alkyl, hydroxy, and oxo.

Alternatively, A^4 is

[Chem. 23]



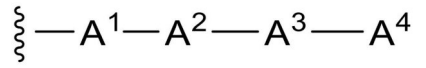
.

Here, 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, and the 4- to 6-membered heterocyclyl here is unsubstituted or substituted with at least one selected from the group consisting of a C_{1-4} alkyl, hydroxy, and oxo.

[0022]

In the general formulae (I) and (III), at least one of L's is

[Chem. 24]



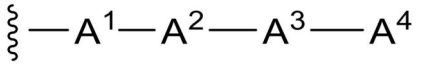
.

[0023]

When the compound is represented by the general formula (I),

it is preferable that one of L's is H and that the other L is

[Chem. 25]

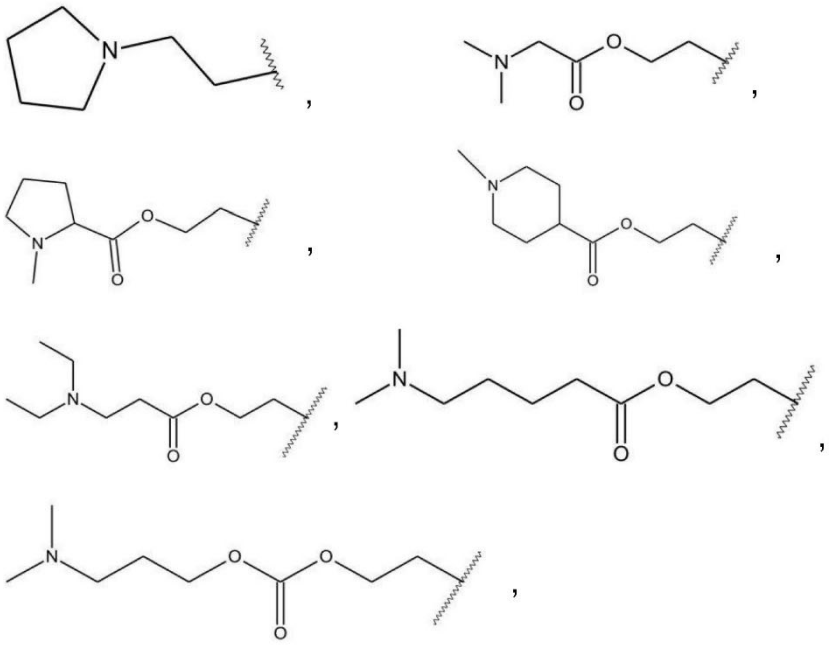


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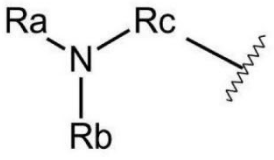
[0024]

In an aspect of the general formulae (I) to (III), L's are independently H, a linear or branched C_{1-10} alkyl,

[Chem. 26]



or,



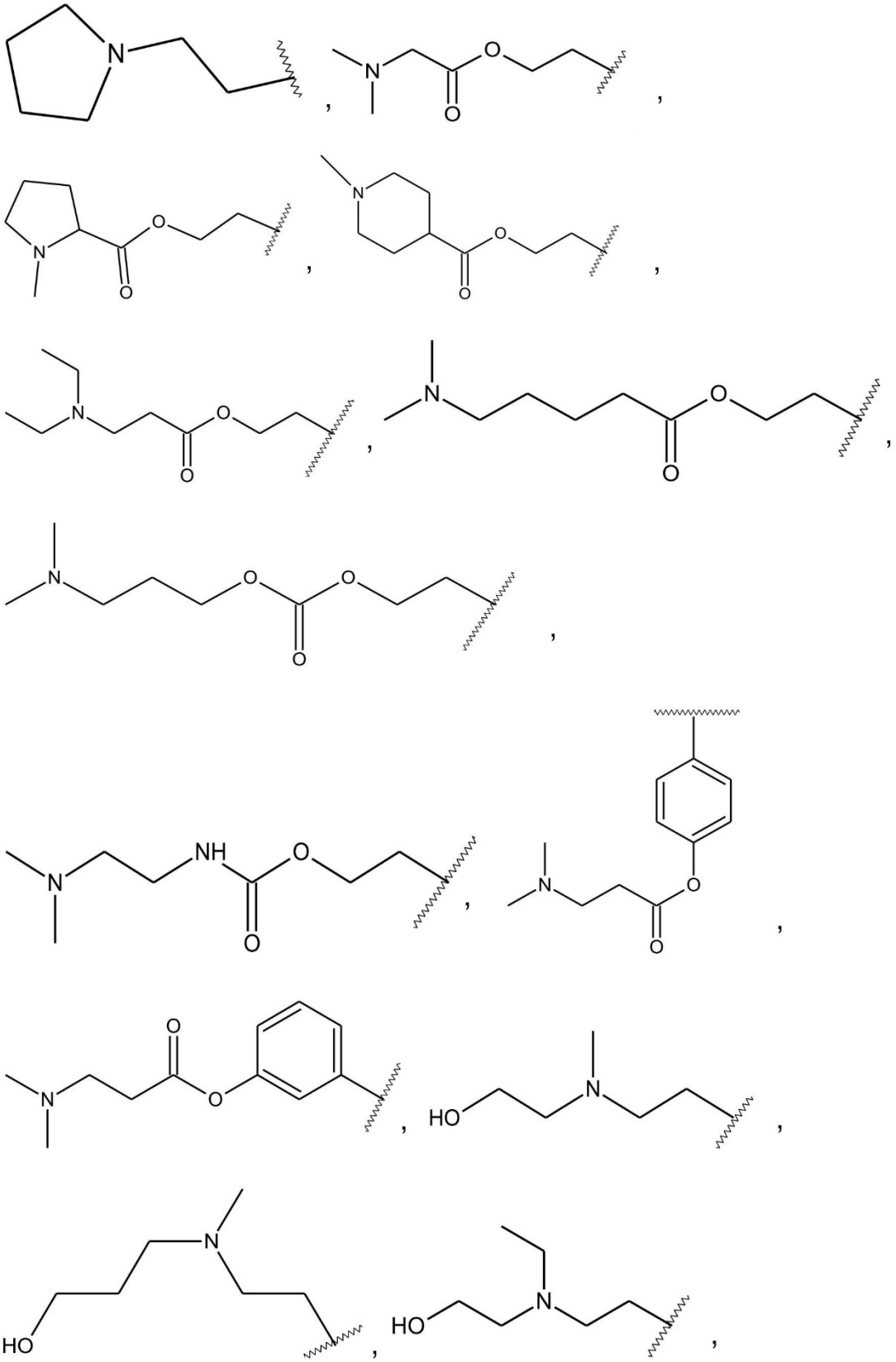
(in the formula, R_a and R_b are independently a linear or branched C_{1-3} alkyl, and R_c is a linear or branched C_{1-6} alkylene.)

[0025]

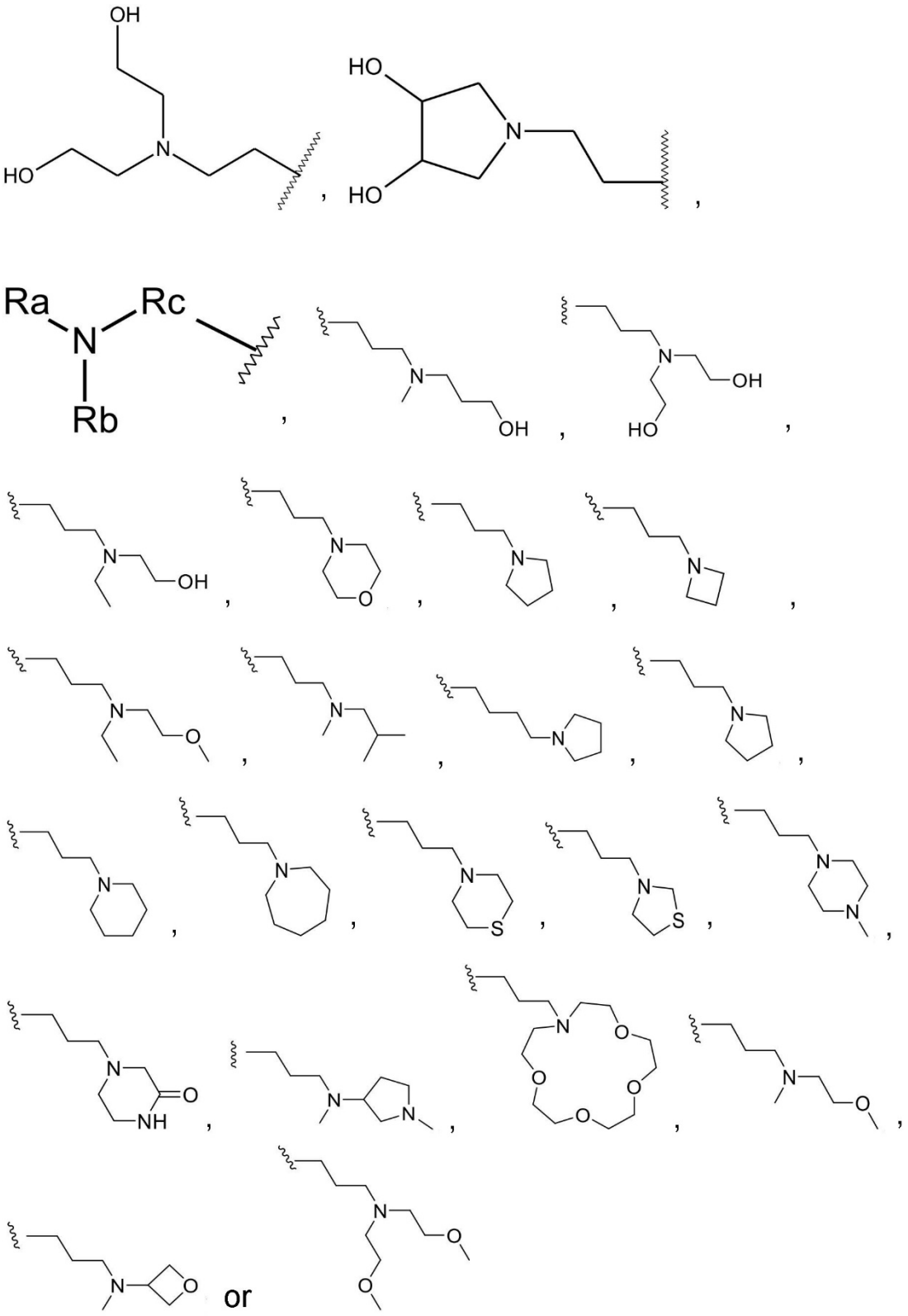
When the compound is represented by the general formula (I),

it is more preferable that one of L's is H and that the other L is

[Chem. 27-1]



[Chem. 27-2]

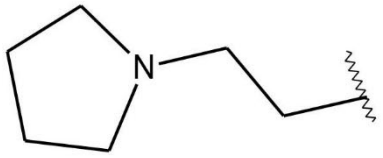
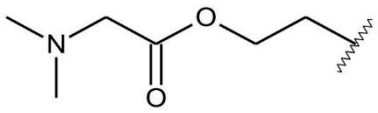
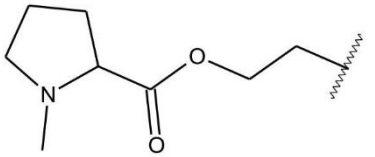
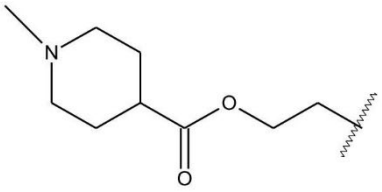
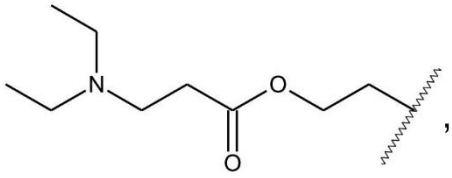
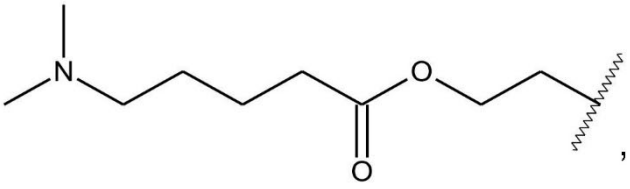
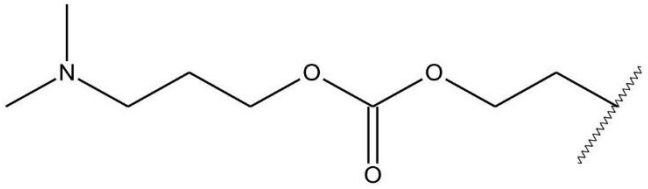
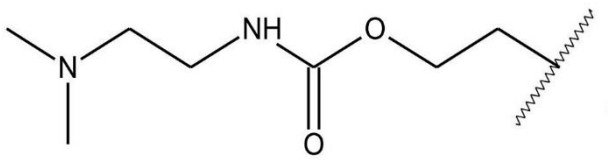
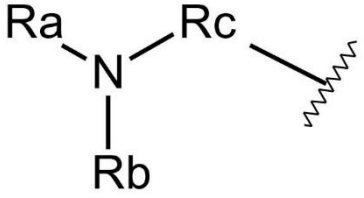
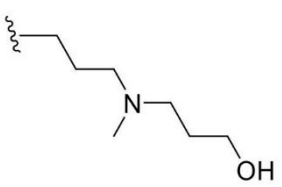
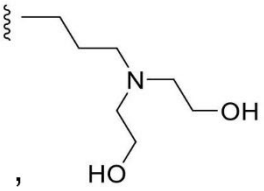
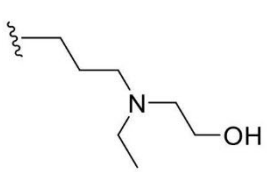
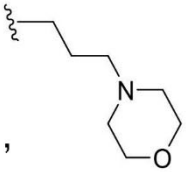
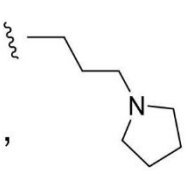
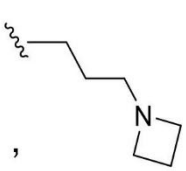
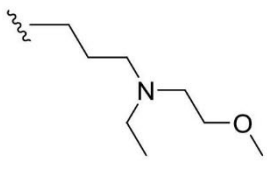
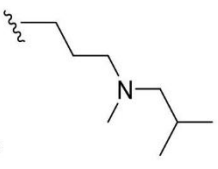
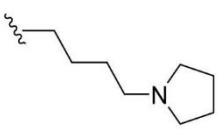
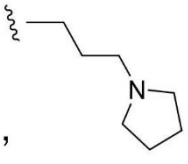


(in the formula, R_a and R_b are independently methyl, ethyl, n-propyl, or isopropyl, and R_c is a linear C_{1-6} alkylene.)

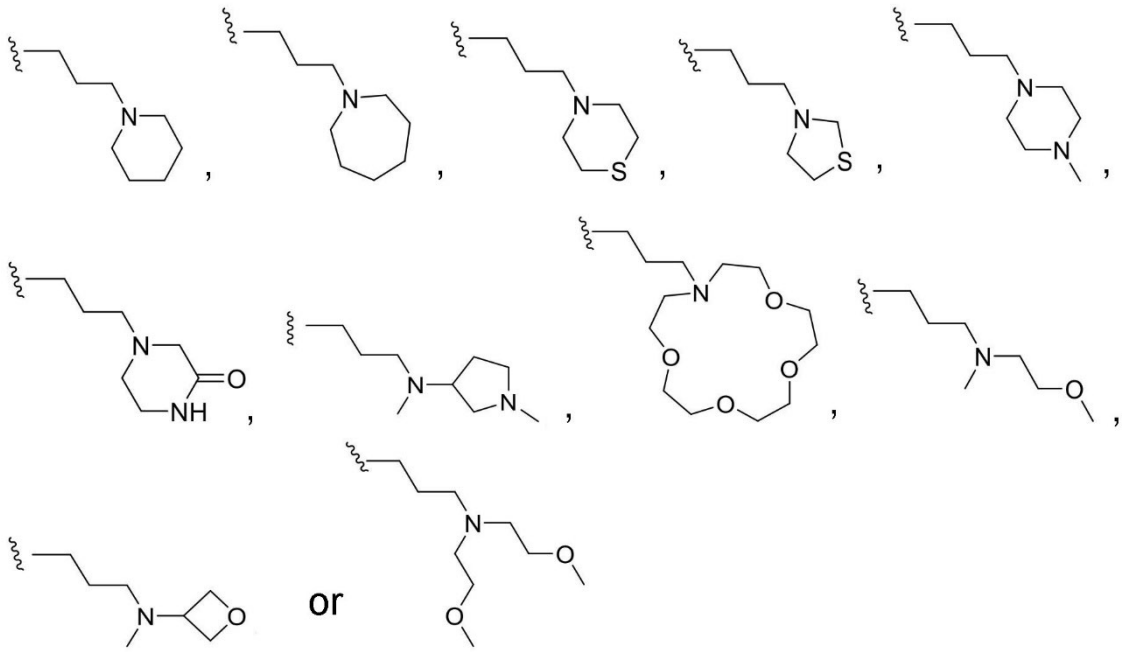
[0026]

When the compound is represented by the general formula (I), it is further preferable that one of L's is H and that the other L is

[Chem. 28-1]


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[Chem. 28-2]



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

[0027]

In the general formulae (I) to (III), 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-. In an aspect of the present invention, M_1 and M_2 are the same.

[0028]

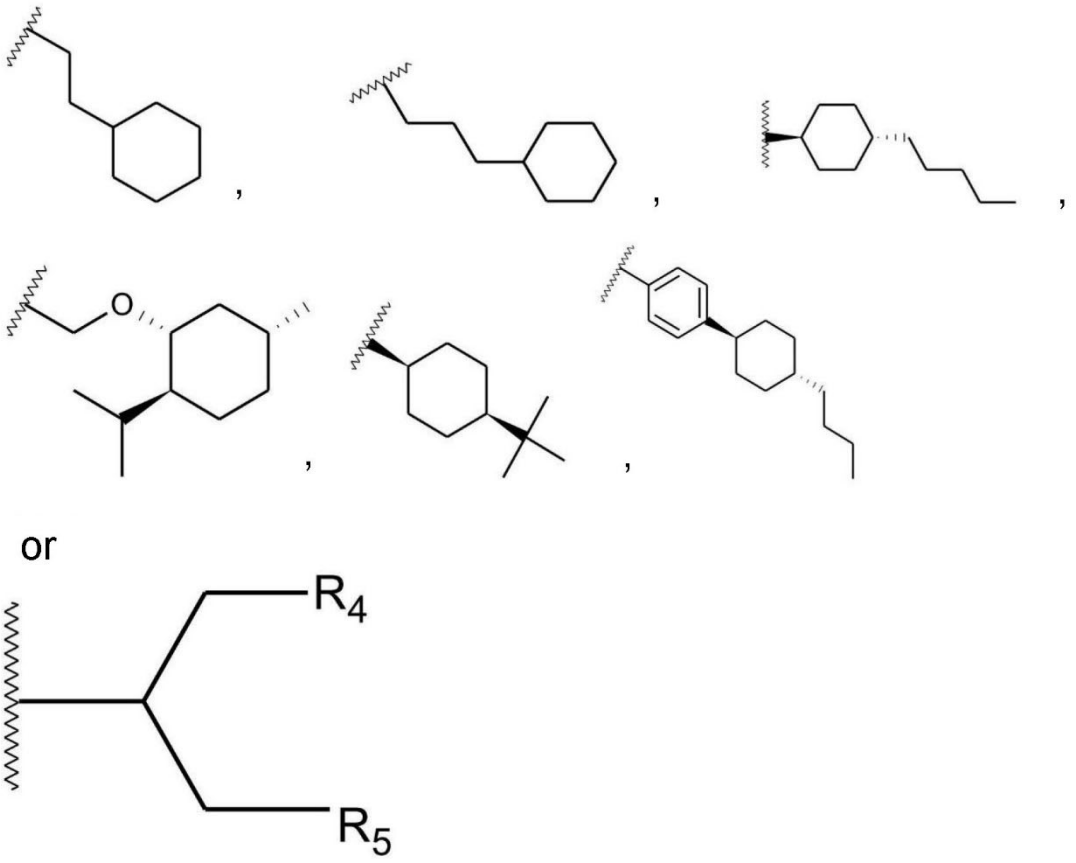
In the general formula (I), M_1 and M_2 are preferably the same. When M_1 and M_2 are the same, M_1 and M_2 are -O-, -O-CH_2-, -O-C(O)-, -O-C(O)-O-, -

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)-.

[0029]

In the general formulae (I) to (III), R_1 and R_2 are independently a linear or branched C_{1-30} alkyl, a linear or branched C_{2-30} alkenyl, adamantyl, a sterol group,

[Chem. 29]



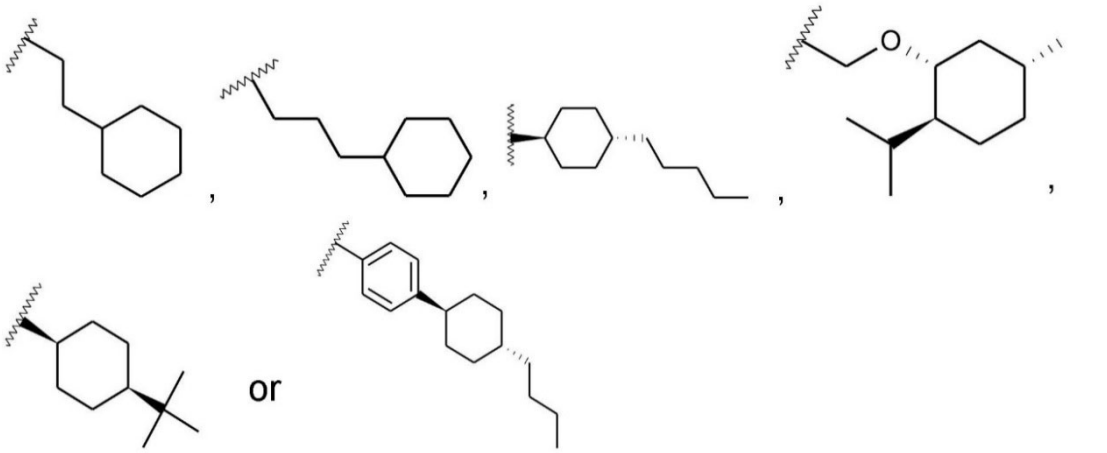
(in the formula, R_4 and R_5 are independently a linear or branched C_{1-16} alkyl.) In an aspect of the present invention, R_1 and R_2 are the same.

[0030]

In another aspect of the general formulae (I) to (III), 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,

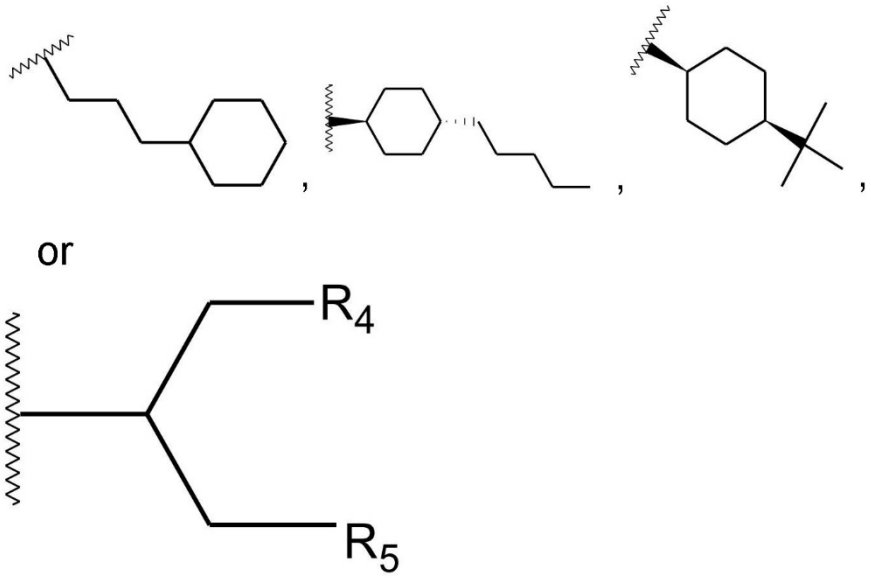
[Chem. 30]



[0031]

In the general formula (I), R_1 and R_2 are preferably independently a linear C_{1-30} alkyl, a linear C_{2-30} alkenyl, a branched C_{3-30} alkenyl, adamantyl,

[Chem. 31]



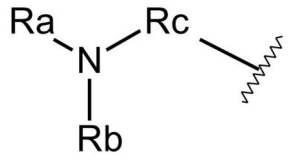
and, R_4 and R_5 are independently a linear C_{1-16} alkyl.

[0032]

In an aspect of the general formula (I), one of L's is H, and the other L

is

[Chem. 32]

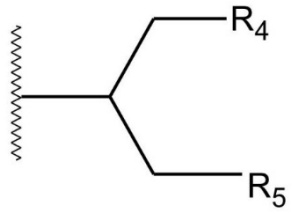


and, R_a and R_b are independently methyl, ethyl, n-propyl, or isopropyl. Here, the total number of carbon atoms of R_a and R_b is 4 or less.

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

Here, R_1 and R_2 are preferably independently

[Chem. 33]

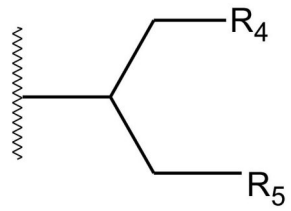


and, R_4 and R_5 are independently a linear C_{1-11} alkyl.

Here, the total number of carbon atoms of R_4 and R_5 is preferably 10 to 22.

Further preferably, R_1 and R_2 are independently

[Chem. 34]



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

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

[0033]

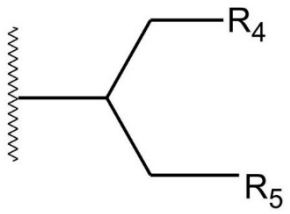
In an aspect of the general formula (II),

L's are a linear C_{1-5} alkyl.

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

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

[Chem. 35]



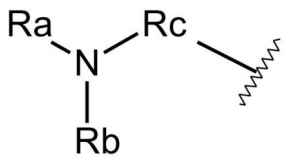
and, R_4 and R_5 are independently a linear C_{1-16} alkyl.

[0034]

In an aspect of the general formula (III),

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

[Chem. 36]

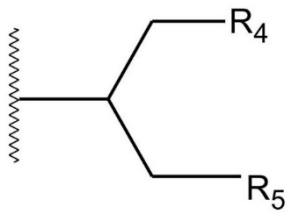


(in the formula, R_a and R_b are independently methyl, ethyl, n-propyl, or isopropyl, and R_c is a linear C_{1-6} alkylene.)

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

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

[Chem. 37]



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

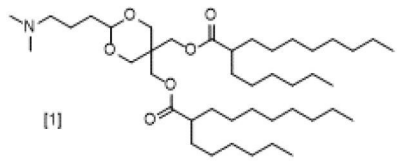
[0035]

Examples of the pH-sensitive cationic lipid of the present invention are pH-sensitive cationic lipids having a dioxane structure or a dioxolane structure and having any of the structures below, salts, or stereoisomers thereof.

[0036]

Compound 1: (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

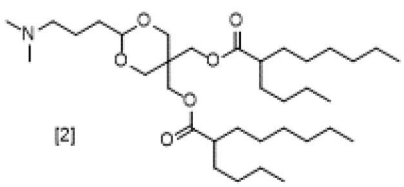
[Chem. 38]



[0037]

Compound 2: (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-butyloctanoate)

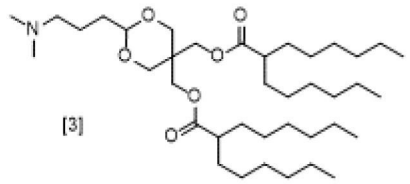
[Chem. 39]



[0038]

Compound 3: (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyloctanoate)

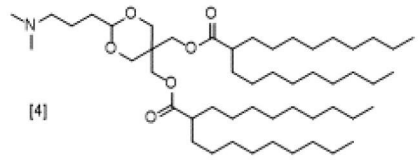
[Chem. 40]



[0039]

Compound 4: (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)

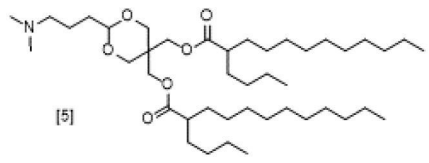
[Chem. 41]



[0040]

Compound 5: (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-butyldodecanoate)

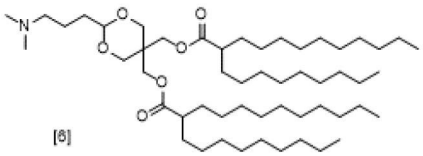
[Chem. 42]



[0041]

Compound 6: (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonyldodecanoate)

[Chem. 43]

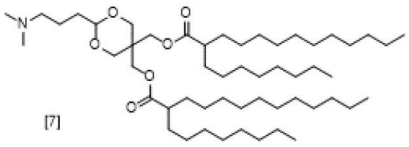


[6]

[0042]

Compound 7: (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-octyltridecanoate)

[Chem. 44]

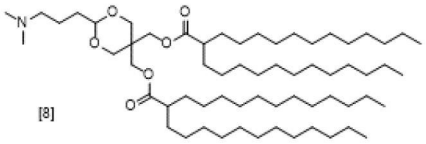


[7]

[0043]

Compound 8: (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-dodecyltetradecanoate)

[Chem. 45]

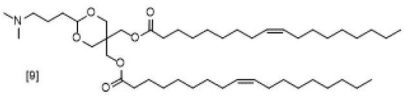


[8]

[0044]

Compound 9: (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) dioleate

[Chem. 46]



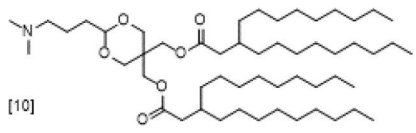
[9]

[0045]

Compound 10: (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-

diyl)bis(methylene) bis(3-nonyldodecanoate)

[Chem. 47]

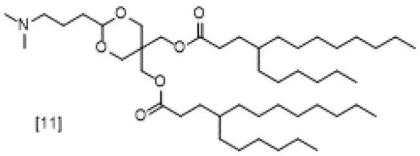


[10]

[0046]

Compound 11: (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(4-hexyldodecanoate)

[Chem. 48]

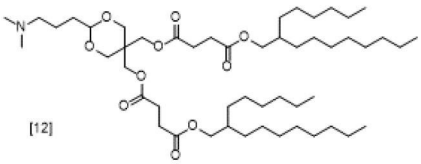


[11]

[0047]

Compound 12: O,O'-((2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene)) bis(2-hexyldecyl) disuccinate

[Chem. 49]

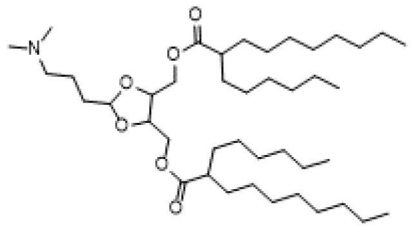


[12]

[0048]

Compound 13: (2-(3-(dimethylamino)propyl)-1,3-dioxolane-4,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 50]

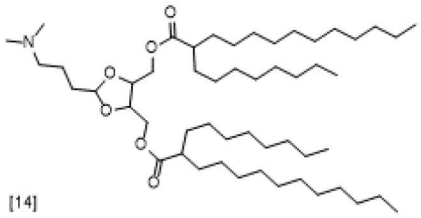


[13]

[0049]

Compound 14: (2-(3-(dimethylamino)propyl)-1,3-dioxolane-4,5-diyl)bis(methylene) bis(2-octyltridecanoate)

[Chem. 51]

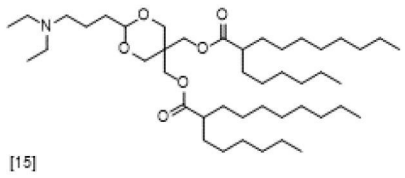


[14]

[0050]

Compound 15: (2-(3-(diethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 52]

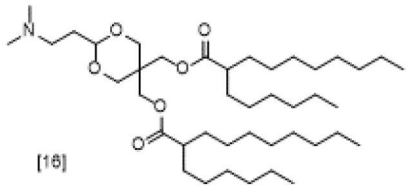


[15]

[0051]

Compound 16: (2-(2-(dimethylamino)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 53]

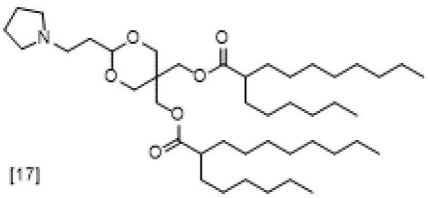


[16]

[0052]

Compound 17: (2-(2-(pyrrolidin-1-yl)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 54]

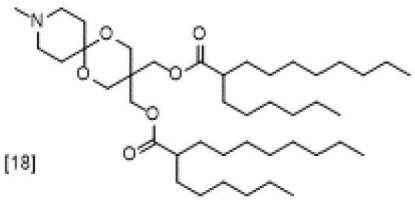


[17]

[0053]

Compound 18: (9-methyl-1,5-dioxa-9-azaspiro[5.5]undecane-3,3-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 55]

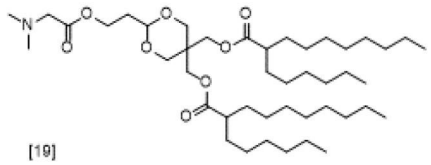


[18]

[0054]

Compound 19: (2-(2-((dimethylglycyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 56]

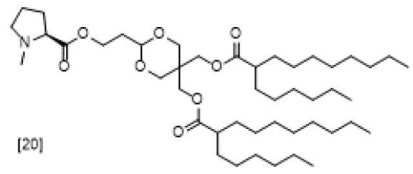


[19]

[0055]

Compound 20: (2-(2-((methyl-L-prolyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 57]

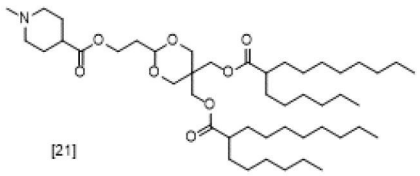


[20]

[0056]

Compound 21: (2-(2-((1-methylpiperidine-4-carbonyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 58]

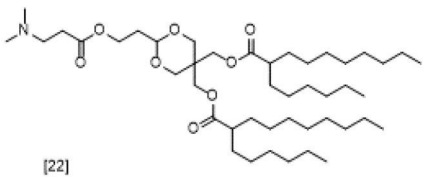


[21]

[0057]

Compound 22: (2-(2-((3-(dimethylamino)propanoyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 59]

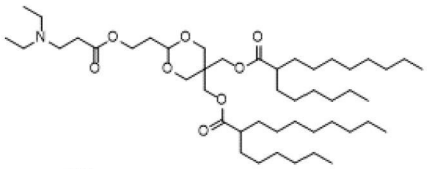


[22]

[0058]

Compound 23: (2-(2-((3-(diethylamino)propanoyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 60]

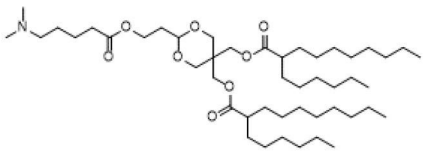


[23]

[0059]

Compound 24: (2-(2-((5-(dimethylamino)pentanoyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 61]

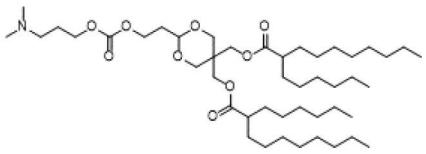


[24]

[0060]

Compound 25: (2-(2-(((3-(dimethylamino)propoxy)carbonyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 62]

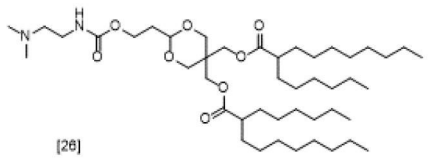


[25]

[0061]

Compound 26: (2-(2-(((3-(dimethylamino)propoxy)carbonyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 63]

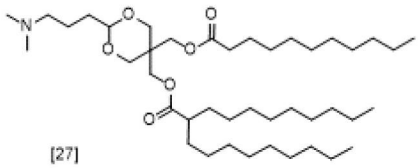


[26]

[0062]

Compound 27: (2-(3-(dimethylamino)propyl)-5-((undecanoyloxy)methyl)-1,3-dioxan-5-yl)methyl 2-nonylundecanoate

[Chem. 64]

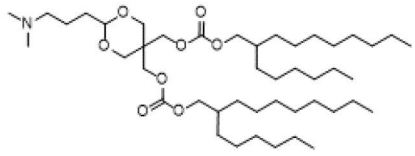


[27]

[0063]

Compound 28: (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecyl) bis(carbonate)

[Chem. 65]

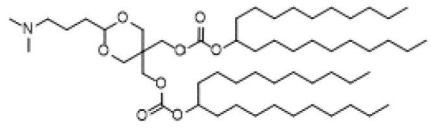


[28]

[0064]

Compound 29: (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) di(henicosan-11-yl) bis(carbonate)

[Chem. 66]

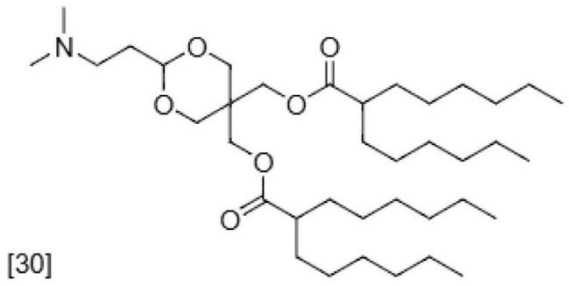


[29]

[0065]

Compound 30: (2-(2-(dimethylamino)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyloctanoate)

[Chem. 67]

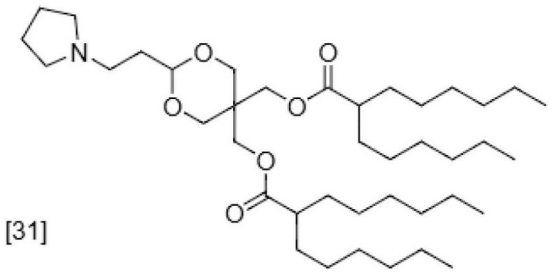


[30]

[0066]

Compound 31: (2-(2-(pyrrolidin-1-yl)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyloctanoate)

[Chem. 68]

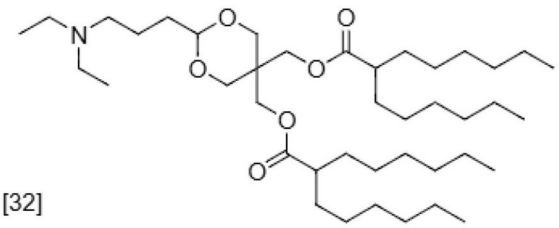


[31]

[0067]

Compound 32: (2-(3-(diethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyloctanoate)

[Chem. 69]

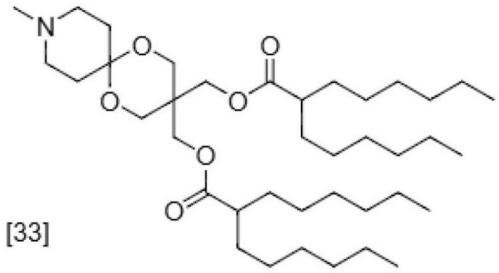


[32]

[0068]

Compound 33: (9-methyl-1,5-dioxa-9-azaspiro[5.5]undecane-3,3-diyl)bis(methylene) bis(2-hexyloctanoate)

[Chem. 70]

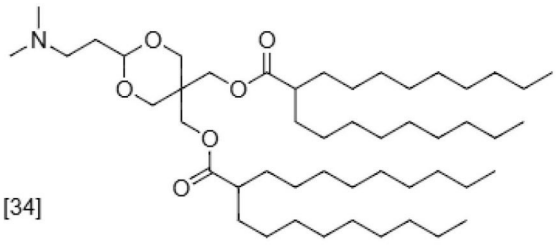


[33]

[0069]

Compound 34: (2-(2-(dimethylamino)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)

[Chem. 71]

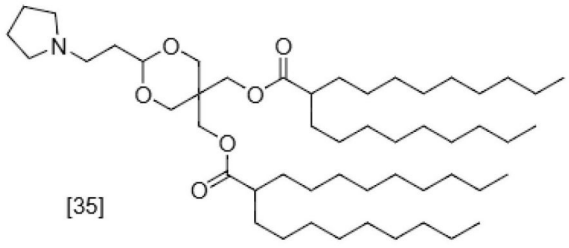


[34]

[0070]

Compound 35: (2-(2-(pyrrolidin-1-yl)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)

[Chem. 72]

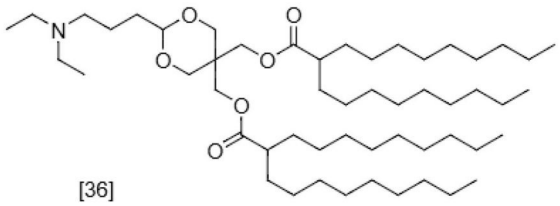


[35]

[0071]

Compound 36: (2-(3-(diethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)

[Chem. 73]

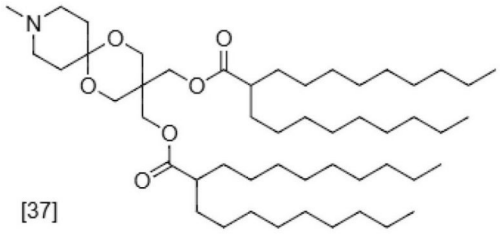


[36]

[0072]

Compound 37: (9-methyl-1,5-dioxa-9-azaspiro[5.5]undecane-3,3-diyl)bis(methylene) bis(2-nonylundecanoate)

[Chem. 74]

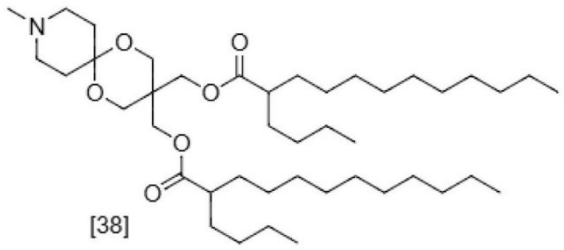


[37]

[0073]

Compound 38: (9-methyl-1,5-dioxa-9-azaspiro[5.5]undecane-3,3-diyl)bis(methylene) bis(2-butyldodecanoate)

[Chem. 75]

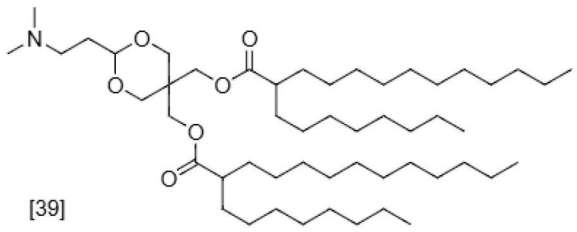


[38]

[0074]

Compound 39: (2-(2-(dimethylamino)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-octyltridecanoate)

[Chem. 76]

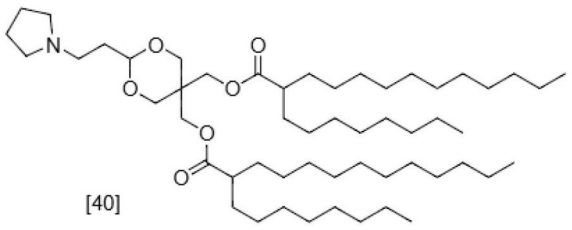


[39]

[0075]

Compound 40: (2-(2-(pyrrolidin-1-yl)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-octyltridecanoate)

[Chem. 77]

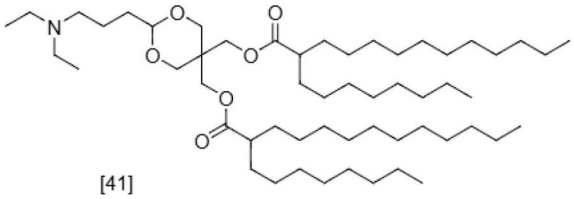


[40]

[0076]

Compound 41: (2-(3-(diethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-octyltridecanoate)

[Chem. 78]

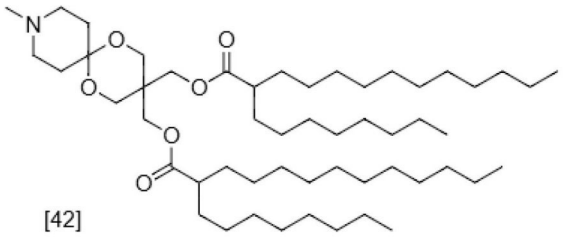


[41]

[0077]

Compound 42: (9-methyl-1,5-dioxa-9-azaspiro[5.5]undecane-3,3-diyl)bis(methylene) bis(2-octyltridecanoate)

[Chem. 79]

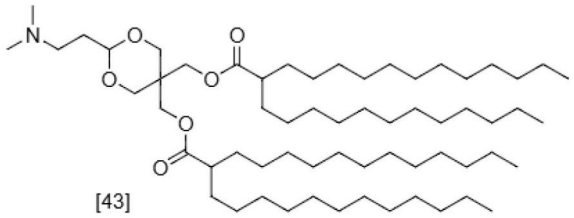


[42]

[0078]

Compound 43: (2-(2-(dimethylamino)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-dodecyltetradecanoate)

[Chem. 80]

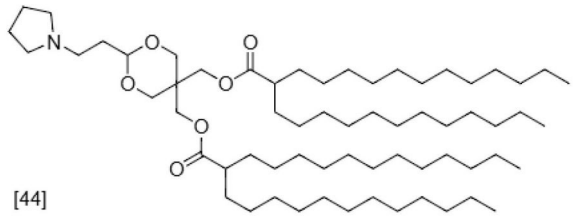


[43]

[0079]

Compound 44: (2-(2-(pyrrolidin-1-yl)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-dodecyltetradecanoate)

[Chem. 81]

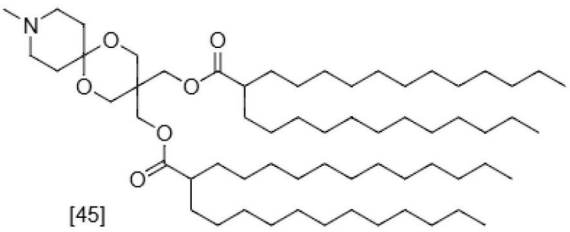


[44]

[0080]

Compound 45: (9-methyl-1,5-dioxa-9-azaspiro[5.5]undecane-3,3-diyl)bis(methylene) bis(2-dodecyltetradecanoate)

[Chem. 82]

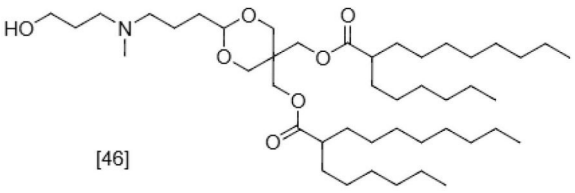


[45]

[0081]

Compound 46: (2-(3-((3-hydroxypropyl)(methyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 83]

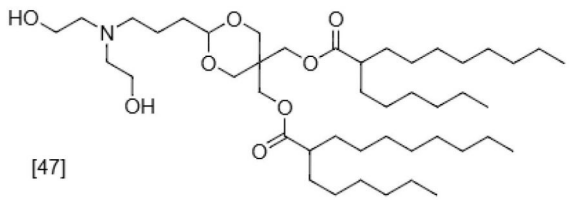


[46]

[0082]

Compound 47: (2-(3-(bis(2-hydroxyethyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 84]

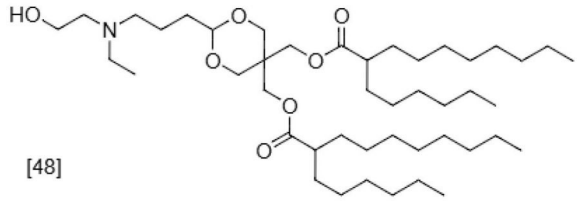


[47]

[0083]

Compound 48: (2-(3-(ethyl(2-hydroxyethyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 85]

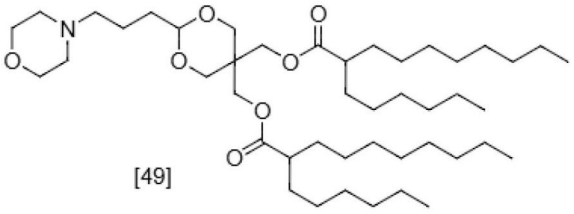


[48]

[0084]

Compound 49: (2-(3-morpholinopropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 86]

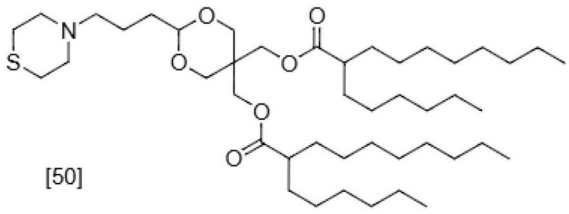


[49]

[0085]

Compound 50: (2-(3-thiomorpholinopropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 87]

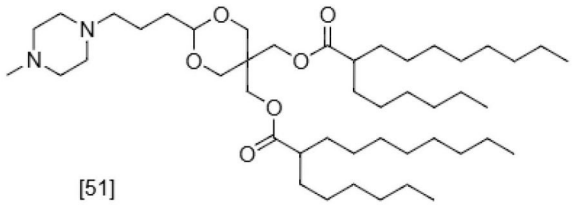


[50]

[0086]

Compound 51: (2-(3-(4-methylpilerazin-1-yl)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 88]

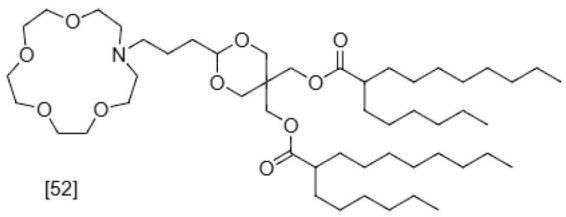


[51]

[0087]

Compound 52: (2-(3-(1,4,7,10-tetraoxa-13-azacyclopentadecan-13-yl)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 89]

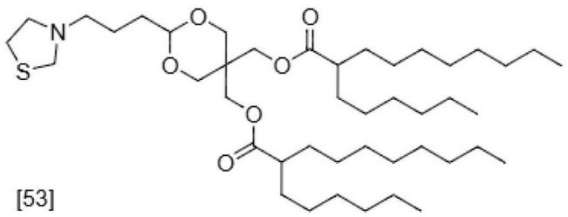


[52]

[0088]

Compound 53: (2-(3-(thiazolidin-3-yl)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 90]

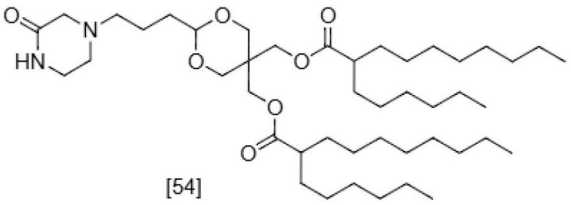


[53]

[0089]

Compound 54: (2-(3-(3-oxopiperazin-1-yl)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 91]

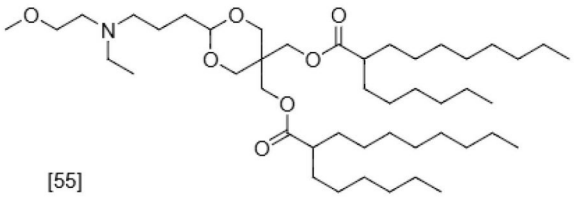


[54]

[0090]

Compound 55: (2-(3-(ethyl(2-methoxyethyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 92]

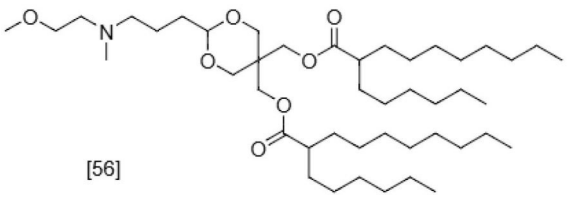


[55]

[0091]

Compound 56: (2-(3-((2-methoxyethyl)(methyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 93]

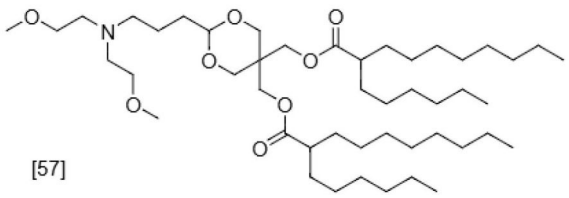


[56]

[0092]

Compound 57: (2-(3-(bis(2-methoxyethyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 94]

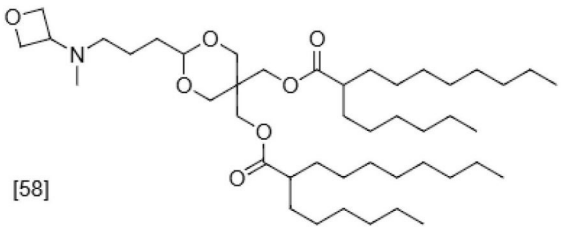


[57]

[0093]

Compound 58: (2-(3-(methyl(oxetan-3-yl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 95]

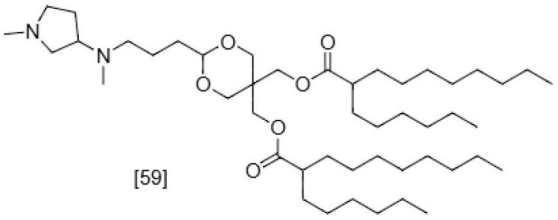


[58]

[0094]

Compound 59: (2-(3-(methyl(1-methylpyrrolidin-3-yl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 96]

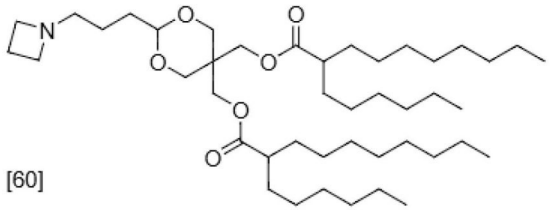


[59]

[0095]

Compound 60: (2-(3-(azetidin-1-yl)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 97]

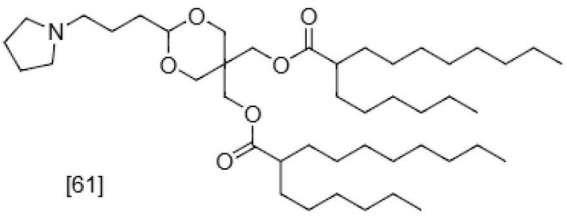


[60]

[0096]

Compound 61: (2-(3-(pyrrolidine-1-yl)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 98]

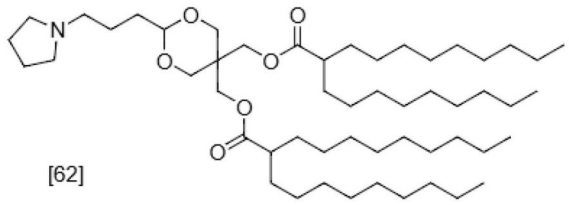


[61]

[0097]

Compound 62: (2-(3-(pyrrolidine-1-yl)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)

[Chem. 99]

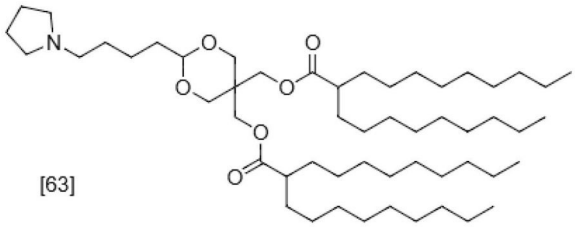


[62]

[0098]

Compound 63: (2-(4-(pyrrolidine-1-yl)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)

[Chem. 100]

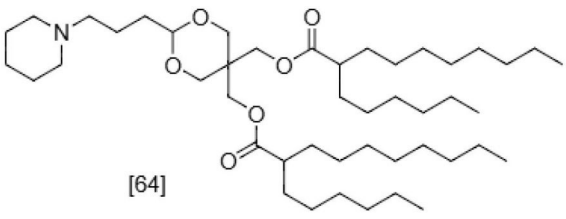


[63]

[0099]

Compound 64: (2-(3-(piperidin-1-yl)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 101]

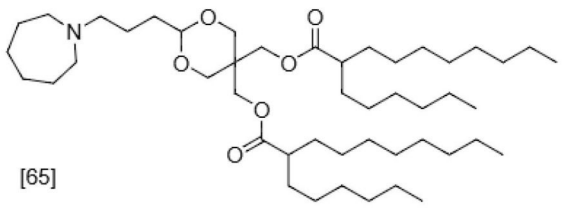


[64]

[0100]

Compound 65: (2-(3-(azepan-1-yl)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 102]

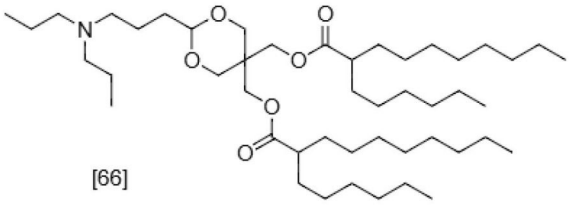


[65]

[0101]

Compound 66: (2-(3-(dipropylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 103]

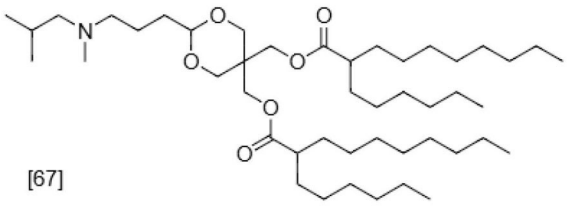


[66]

[0102]

Compound 67: (2-(3-(isobutyl(methyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 104]

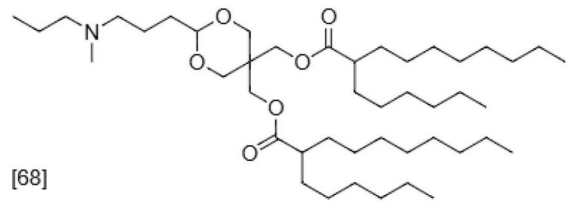


[67]

[0103]

Compound 68: (2-(3-(methyl(propyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 105]

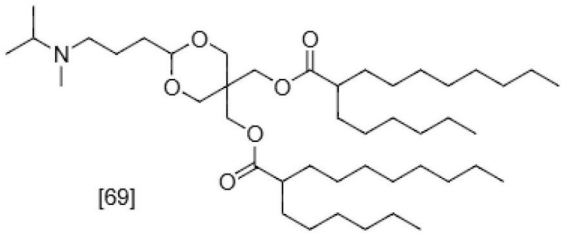


[68]

[0104]

Compound 69: (2-(3-(isopropyl(methyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 106]

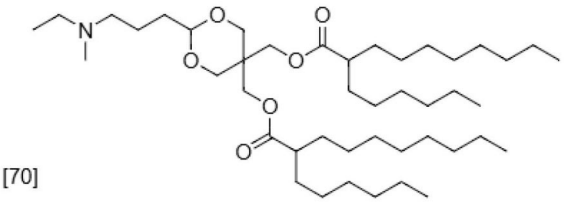


[69]

[0105]

Compound 70: (2-(3-(ethyl(methyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 107]

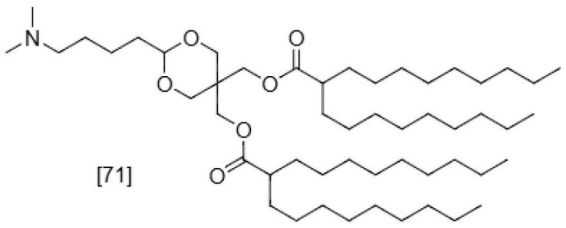


[70]

[0106]

Compound 71: (2-(4-(dimethylamino)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)

[Chem. 108]

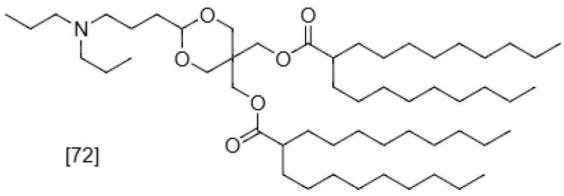


[71]

[0107]

Compound 72: (2-(3-(dipropylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)

[Chem. 109]

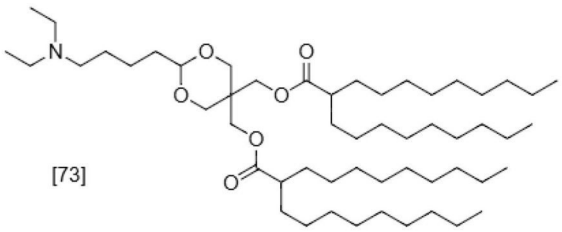


[72]

[0108]

Compound 73: (2-(4-(diethylamino)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)

[Chem. 110]

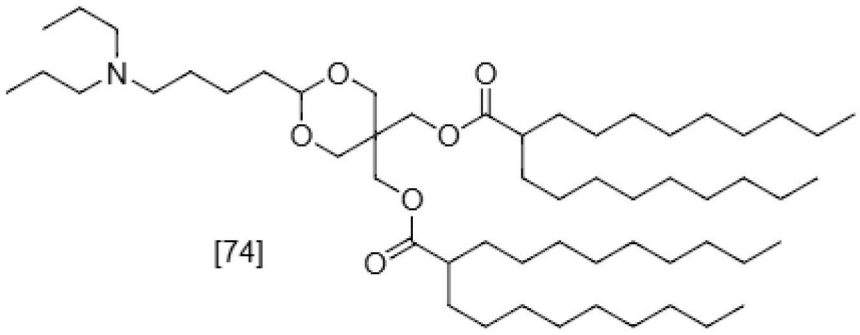


[73]

[0109]

Compound 74: (2-(4-(dipropylamino)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)

[Chem. 111]

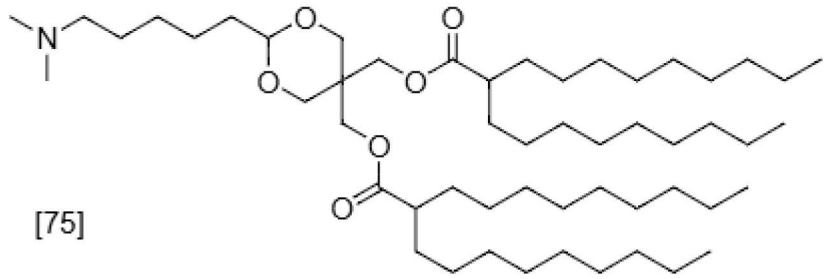


[74]

[0110]

Compound 75: (2-(5-(dimethylamino)pentyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)

[Chem. 112]

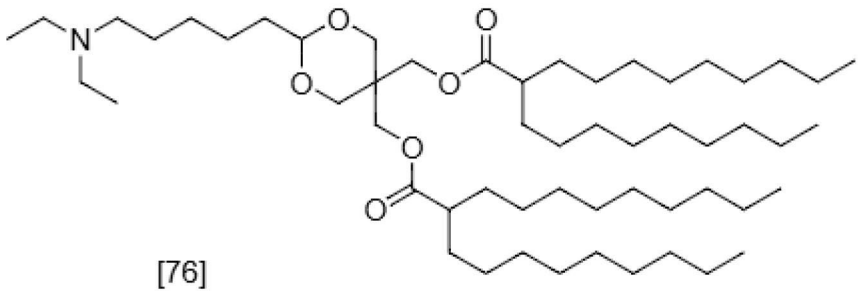


[75]

[0111]

Compound 76: (2-(5-(diethylamino)pentyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)

[Chem. 113]

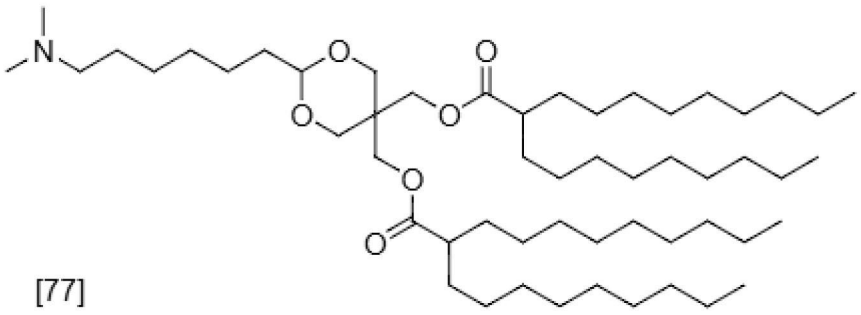


[76]

[0112]

Compound 77: (2-(6-(dimethylamino)hexyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)

[Chem. 114]

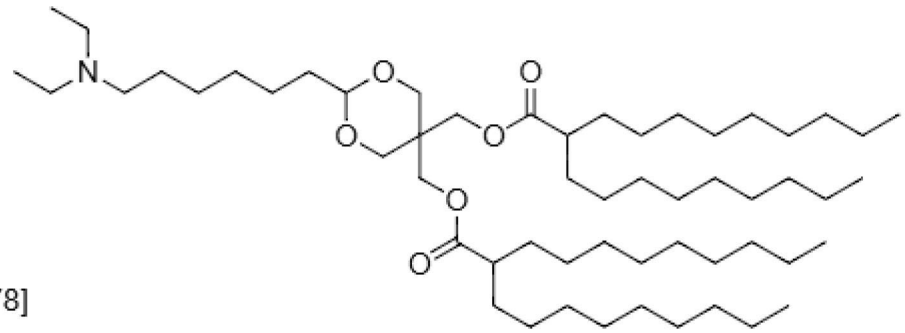


[77]

[0113]

Compound 78: (2-(6-(dimethylamino)hexyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)

[Chem. 115]

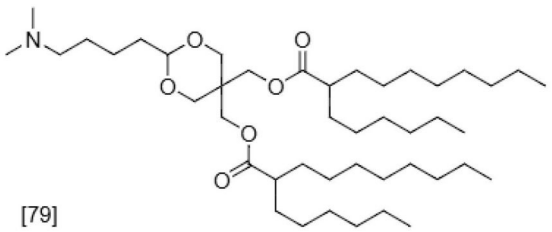


[78]

[0114]

Compound 79: (2-(4-(dimethylamino)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 116]

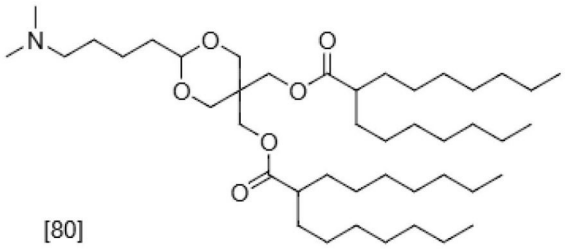


[79]

[0115]

Compound 80: (2-(4-(dimethylamino)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-heptylnonanoate)

[Chem. 117]

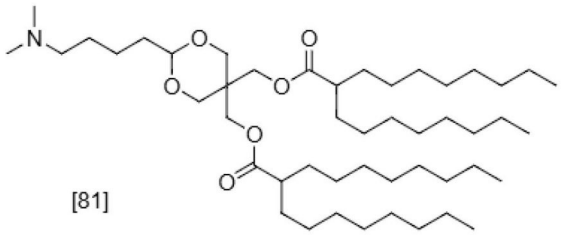


[80]

[0116]

Compound 81: (2-(4-(dimethylamino)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-octyldecanoate)

[Chem. 118]

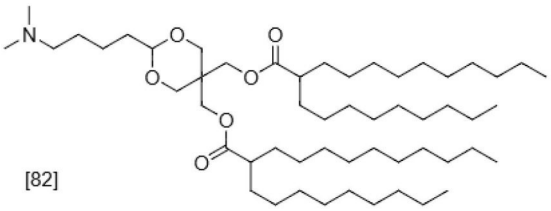


[81]

[0117]

Compound 82: (2-(4-(dimethylamino)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonyldodecanoate)

[Chem. 119]

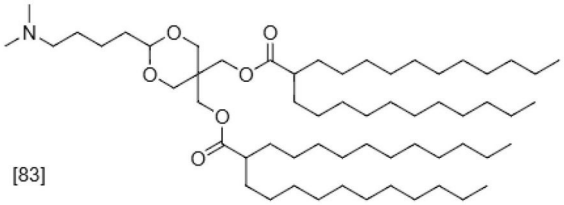


[82]

[0118]

Compound 83: (2-(4-(dimethylamino)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-undecyltridecanoate)

[Chem. 120]

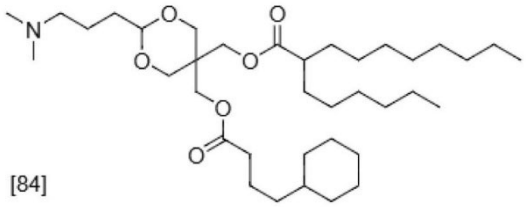


[83]

[0119]

Compound 84: (5-(((4-cyclohexylbutanoyl)oxy)methyl)-2-(3-(dimethylamino)propyl)-1,3-dioxan-5-yl)methyl 2-hexyldecanoate

[Chem. 121]

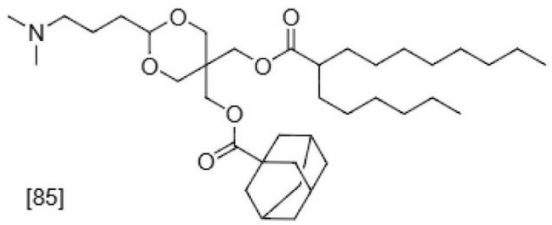


[84]

[0120]

Compound 85: (2-(3-(dimethylamino)propyl)-5-(((2-hexyldecanoyl)oxy)methyl)-1,3-dioxan-5-yl)methyl adamantane-1-carboxylate

[Chem. 122]

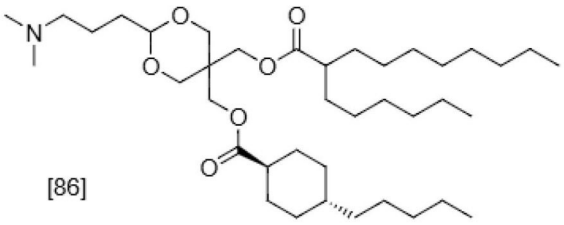


[85]

[0121]

Compound 86: (2-(3-(dimethylamino)propyl)-5-(((2-hexyldecanoyl)oxy)methyl)-1,3-dioxan-5-yl)methyl (1s,4r)-4-pentylcyclohexane-1-carboxylate

[Chem. 123]

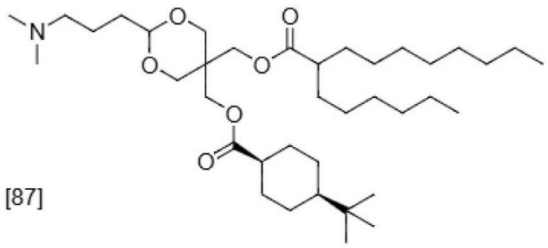


[86]

[0122]

Compound 87: (2-(3-(dimethylamino)propyl)-5-(((2-hexyldecanoyl)oxy)methyl)-1,3-dioxan-5-yl)methyl (1s,4s)-4-(tert-butyl)cyclohexane-1-carboxylate

[Chem. 124]

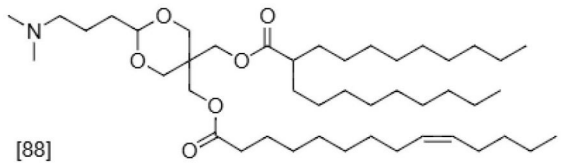


[87]

[0123]

Compound 88: (2-(3-(dimethylamino)propyl)-5-(((2-nonylundecanoyl)oxy)methyl)-1,3-dioxan-5-yl)methyl (Z)-tetradec-9-enoate

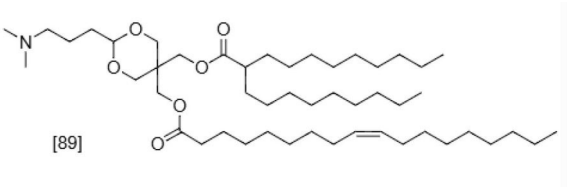
[Chem. 125]



[0124]

Compound 89: (2-(3-(dimethylamino)propyl)-5-(((2-nonylundecanoyl)oxy)methyl)-1,3-dioxan-5-yl)methyl oleate

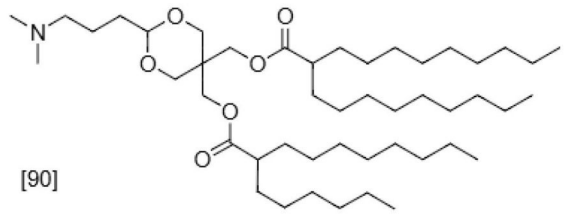
[Chem. 126]



[0125]

Compound 90: (2-(3-(dimethylamino)propyl)-5-(((2-hexyldecanoyl)oxy)methyl)-1,3-dioxan-5-yl)methyl 2-nonylundecanoate

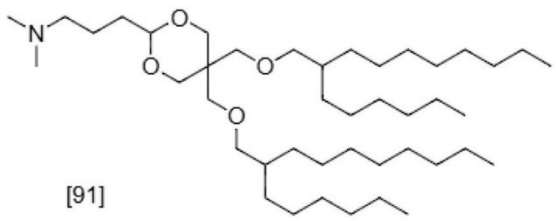
[Chem. 127]



[0126]

Compound 91: 3-(5,5-bis(((2-hexyldecyl)oxy)methyl)-1,3-dioxan-2-yl)-N,N-dimethylpropan-1-amine

[Chem. 128]

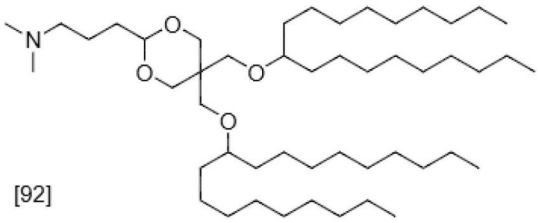


[91]

[0127]

Compound 92: 3-(5,5-bis((nonadecan-10-yloxy)methyl)-1,3-dioxan-2-yl)-N,N-dimethylpropan-1-amine

[Chem. 129]

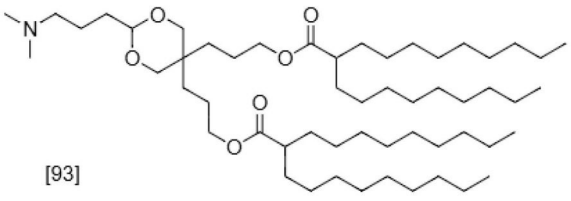


[92]

[0128]

Compound 93: (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(propane-3,1-diyl) bis(2-nonylundecanoate)

[Chem. 130]

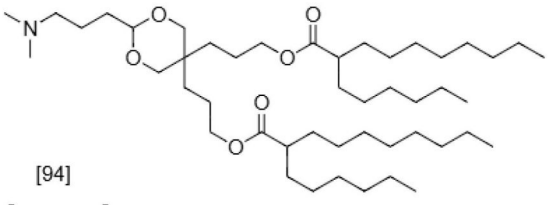


[93]

[0129]

Compound 94: (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(propane-3,1-diyl) bis(2-hexyldecanoate)

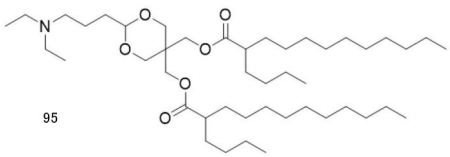
[Chem. 131]



[0130]

Compound 95: (2-(3-(diethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-butyldodecanoate)

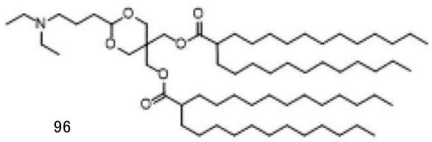
[Chem. 132]



[0131]

Compound 96: (2-(3-(diethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-dodecyltetradecanoate)

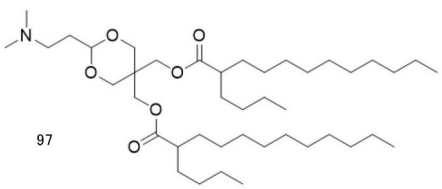
[Chem. 133]



[0132]

Compound 97: (2-(2-(dimethylamino)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-butyldodecanoate)

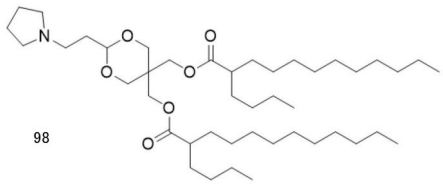
[Chem. 134]



[0133]

Compound 98: (2-(2-(pyrrolidin-1-yl)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-butyldodecanoate)

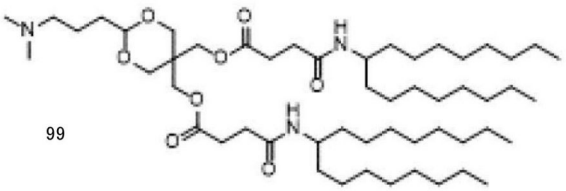
[Chem. 135]



[0134]

Compound 99: (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(4-(heptadecan-9-ylamino)-4-oxobutanoate)

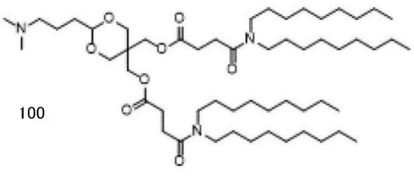
[Chem. 136]



[0135]

Compound 100: (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(4-(dinonylamino)-4-oxobutanoate)

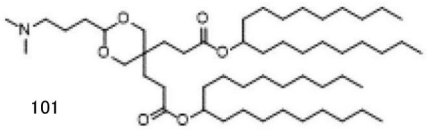
[Chem. 137]



[0136]

Compound 101: di(nonadecan-10-yl) 3,3'-(2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)dipropionate

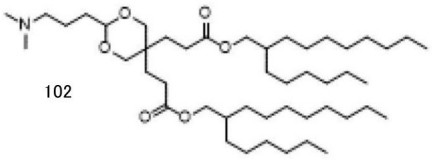
[Chem. 138]



[0137]

Compound 102: bis(2-hexyldecyl) 3,3'-(2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)dipropionate

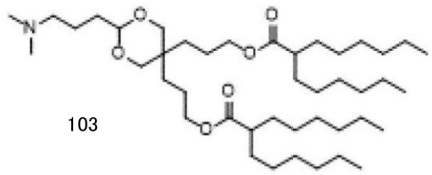
[Chem. 139]



[0138]

Compound 103: (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(propane-3,1-diyl) bis(2-hexyloctanoate)

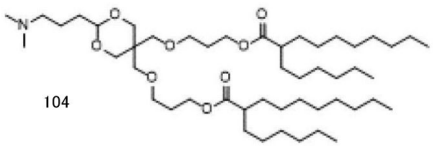
[Chem. 140]



[0139]

Compound 104: (((2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene))bis(oxy))bis(propane-3,1-diyl) bis(2-hexyldecanoate)

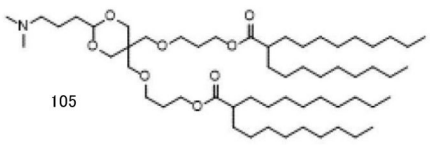
[Chem. 141]



[0140]

Compound 105: (((2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene))bis(oxy))bis(propane-3,1-diyl) bis(2-nonylundecanoate)

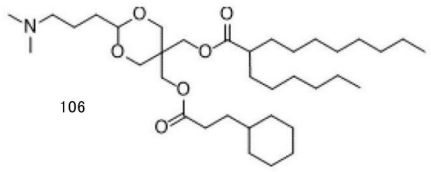
[Chem. 142]



[0141]

Compound 106: (5-(((3-cyclohexylpropanoyl)oxy)methyl)-2-(3-(dimethylamino)propyl)-1,3-dioxan-5-yl)methyl 2-hexyldecanoate

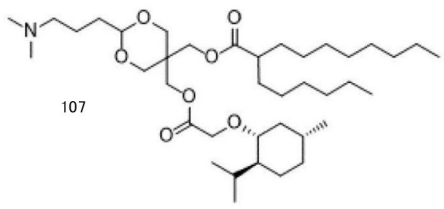
[Chem. 143]



[0142]

Compound 107: (2-(3-(dimethylamino)propyl)-5-((2-(((1R,2S,5R)-2-isopropyl-5-methylcyclohexyl)oxy)acetoxy)methyl)-1,3-dioxan-5-yl)methyl 2-hexyldecanoate

[Chem. 144]

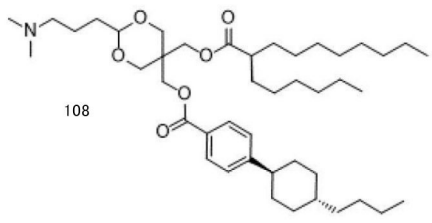


[0143]

Compound 108: (2-(3-(dimethylamino)propyl)-5-(((2-

hexyldecanoyl)oxy)methyl)-1,3-dioxan-5-yl)methyl 4-((1s,4r)-4-butylcyclohexyl)benzoate

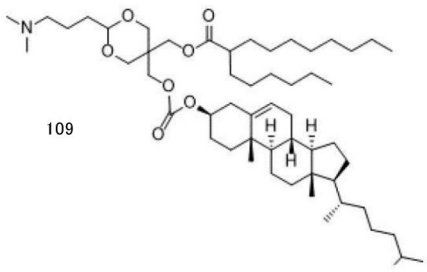
[Chem. 145]



[0144]

Compound 109: (5-((((((3R,8R,9R,10S,13S,14R,17S)-10,13-dimethyl-17-((S)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)carbonyl)oxy)methyl)-2-(3-(dimethylamino)propyl)-1,3-dioxan-5-yl)methyl 2-hexyldecanoate

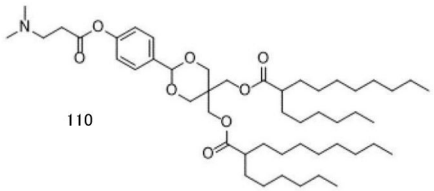
[Chem. 146]



[0145]

Compound 110: (2-(4-((3-(dimethylamino)propanoyl)oxy)phenyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 147]

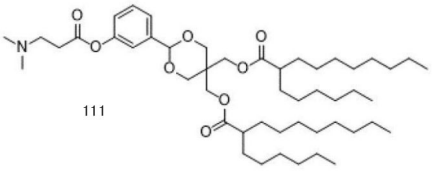


110

[0146]

Compound 111: (2-(3-((3-(dimethylamino)propanoyl)oxy)phenyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 148]

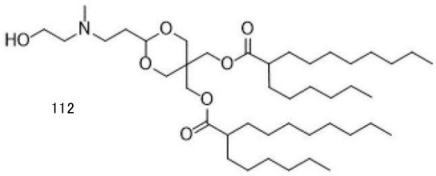


111

[0147]

Compound 112: (2-(2-((2-hydroxyethyl)(methyl)amino)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

[Chem. 149]

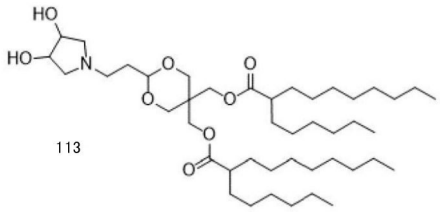


112

[0148]

Compound 113: (2-(2-(3,4-dihydroxypyrrolidin-1-yl)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

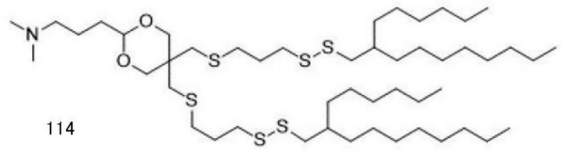
[Chem. 150]



[0149]

Compound 114: 3-(5,5-bis(((3-((2-hexyldecyl)disulfaneyl)propyl)thio)methyl)-1,3-dioxan-2-yl)-N,N-dimethylpropan-1-amine

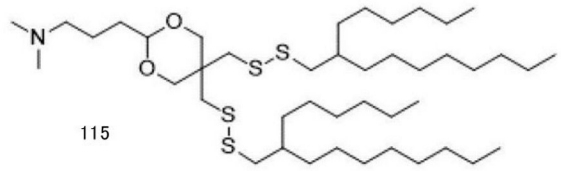
[Chem. 151]



[0150]

Compound 115: 3-(5,5-bis(((2-hexyldecyl)disulfaneyl)methyl)-1,3-dioxan-2-yl)-N,N-dimethylpropan-1-amine

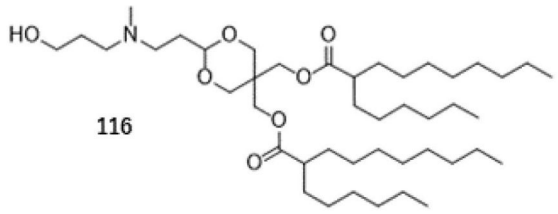
[Chem. 152]



[0151]

Compound 116: (2-(2-((3-hydroxypropyl)(methyl)amino)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

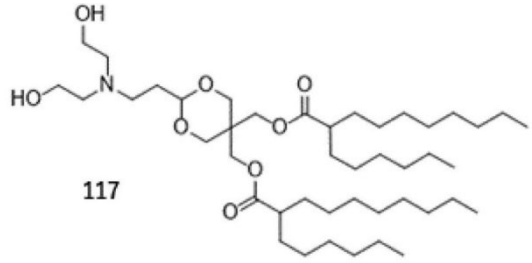
[Chem. 153]



[0152]

Compound 117: (2-(2-(bis(2-hydroxyethyl)amino)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

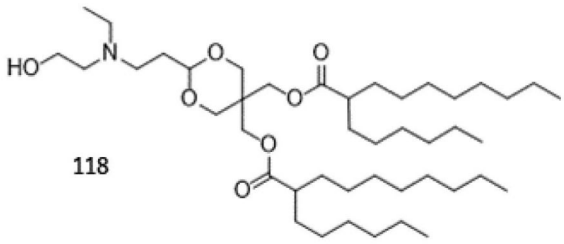
[Chem. 154]



[0153]

Compound 118: (2-(2-(ethyl(2-hydroxyethyl)amino)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

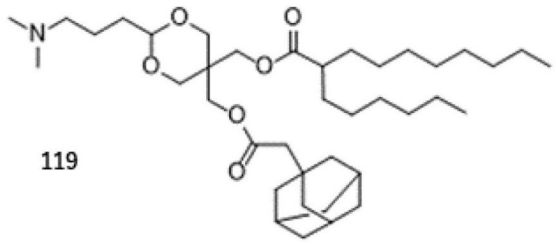
[Chem. 155]



[0154]

Compound 119: (5-((2-(adamantan-1-yl)acetoxy)methyl)-2-(3-(dimethylamino)propyl)-1,3-dioxan-5-yl)methyl 2-hexyldecanoate

[Chem. 156]



119

[0155]

In an aspect, the present invention relates to a pH-sensitive cationic lipid.

[0156]

The pH-sensitive cationic lipid represented by the general formula (I) can be easily produced, for example, by the method specifically shown in an Example of the present specification. Referring to the production method, one skilled in the art can easily produce any lipid falling within the range of the general formulae (I) to (III) by appropriately selecting a raw material compound, a regaent, reaction conditions, and the like.

[0157]

One kind alone or two or more kinds of the pH-sensitive cationic lipid of the present invention may form the lipid nanoparticle according to the present invention. When two or more kinds of the pH-sensitive cationic lipid of the present invention form the lipid nanoparticle according to the present invention, the amount of the pH-sensitive cationic lipid of the present invention means the total amount of the lipid molecules corresponding to the pH-sensitive cationic lipid of the present invention, of the lipid molecules forming the lipid nanoparticle.

[0158]

As the proportion of the pH-sensitive cationic lipid of the present invention in the lipid molecules forming the lipid nanoparticle is higher, the efficiency of incorporation of the lipid nanoparticle into the target cell becomes

higher. Accordingly, in the lipid nanoparticle according to the present invention, the proportion of the amount of the pH-sensitive cationic lipid of the present invention in the total amount of the lipids forming the lipid nanoparticle ([the amount of the pH-sensitive cationic lipid of the present invention (mol)]/([the amount of all the lipids forming the lipid nanoparticle (mol)])×100%) is preferably 20 mol% or more. On the other hand, when the proportion of the pH-sensitive cationic lipid in the lipid molecules forming the lipid nanoparticle is too high, it is sometimes difficult to make the particle size sufficiently small. Because the efficiency of incorporation of the lipid nanoparticle into the target cell is sufficient and because a lipid nanoparticle having a sufficiently small particle size is obtained, the proportion of the amount of the pH-sensitive cationic lipid of the present invention in the total amount of the lipids forming the lipid nanoparticle in the lipid nanoparticle according to the present invention is more preferably 30 mol% or more, further preferably 30 to 70 mol%, and still further preferably 40 to 60 mol%.

[0159]

Of the constituent lipids of the lipid nanoparticle according to the present invention, a lipid which is generally used for forming a liposome can be used as a lipid other than the pH-sensitive cationic lipid of the present invention. Examples of such a lipid include a phospholipid, a sterol, a sterol derivative, a glycolipid, and a saturated or unsaturated fatty acid. One kind thereof or a combination of two or more kinds thereof can be used.

[0160]

Examples of the phospholipid include glycerophospholipids such as phosphatidylserine, phosphatidylinositol, phosphatidylglycerol,

phosphatidylethanolamine, phosphatidylcholine, cardiolipin, plasmalogens, ceramide phosphorylglycerol phosphate, and phosphatidic acids and sphingophospholipids such as sphingomyelin, ceramide phosphorylglycerol, and ceramide phosphorylethanolamine. Moreover, naturally derived phospholipids such as egg yolk lecithin and soy lecithin can also be used. The fatty acid residues in the glycerophospholipid and the sphingophospholipid are not particularly limited. An example thereof is a saturated or unsaturated fatty acid residue having 12 to 24 carbon atoms, and a saturated or unsaturated fatty acid residue having 14 to 20 carbon atoms is preferable. Specific examples thereof include acyl groups derived from fatty acids such as lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, arachidonic acid, behenic acid, and lignoceric acid. When such a glycerolipid or a sphingolipid has two or more fatty acid residues, all the fatty acid residues may be the same groups or may be different groups.

[0161]

Examples of the phospholipid include diphytanoyl phosphatidyl ethanolamine (DPhPE), 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE), 1,2-distearoyl-sn-glycero-3-phosphorylethanolamine (DSPE), and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE).

[0162]

Examples of the sterol or the sterol derivative include animal-derived sterols such as cholesterol, cholesterol succinate, lanosterol, dihydrolanosterol,

desmosterol, and dihydrocholesterol, plant-derived sterols (phytosterols) such as stigmasterol, sitosterol, β-sitosterol, campesterol, and brassicasterol, and microorganism-derived sterols such as zymosterol and ergosterol. Examples of the glycolipid include glyceroglycolipids such as sulfoxyribosyl glyceride, diglycosyl diglyceride, digalactosyl diglyceride, galactosyl diglyceride, and glycosyl diglyceride and sphingoglycolipids such as galactosyl cerebroside, lactosyl cerebroside, and ganglioside. Examples of the saturated or unsaturated fatty acid include saturated or unsaturated fatty acids having 12 to 20 carbon atoms such as palmitic acid, oleic acid, stearic acid, arachidonic acid, and myristic acid.

[0163]

The constituent lipids of the lipid nanoparticle according to the present invention preferably contain a neutral lipid, more preferably contain a phospholipid or a sterol, further preferably contain a sterol and still further preferably contain cholesterol, in addition to the pH-sensitive cationic lipid of the present invention.

[0164]

The lipid nanoparticle according to the present invention preferably contains a polyalkylene glycol-modified lipid as a lipid component. Polyalkylene glycol is a hydrophilic polymer, and the surface of a lipid nanoparticle can be modified with polyalkylene glycol by forming the lipid nanoparticle using a polyalkylene glycol-modified lipid as a lipid film-forming lipid. By modifying the surface with polyalkylene glycol, the stability of the lipid nanoparticle, such as the blood retention property, can be sometimes improved.

[0165]

As the polyalkylene glycol, for example, polyethylene glycol, polypropylene glycol, polytetramethylene glycol, or polyhexamethylene glycol can be used. The molecular weight of the polyalkylene glycol is, for example, around 200 to 10,000, for example around 300 to 10,000, preferably around 500 to 10,000, and further preferably around 1,000 to 5,000. In an aspect of the present invention, the molecular weight of the polyalkylene glycol is about 200, about 300, about 350, about 400, about 500, about 550, about 750, about 1000, about 1500, about 2000, about 3000, about 3500, about 4000, about 5000, or about 10,000 Da.

[0166]

For example, for modification of the lipid with polyethylene glycol, stearylated polyethylene glycol (for example, PEG45 stearate (STR-PEG45)) can be used. In addition, polyethylene glycol derivatives such as N-[carbonyl-methoxypolyethylene glycol]-1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE-PEG), N-[carbonyl-methoxypolyethylene glycol]-1,2-distearoyl-sn-glycero-3-phosphoethanolamine) (DSPE-PEG), 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol (DMG-PEG), and N-[carbonyl-methoxypolyethylene glycol]-1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE-PEG) can be used. For example, polyethylene glycol derivatives such as N-[carbonyl-methoxypolyethylene glycol-2000]-1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE-PEG2000), n-[carbonyl-methoxypolyethylene glycol-5000]-1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE-PEG5000), N-[carbonyl-methoxypolyethylene glycol-750]-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG750), N-[carbonyl-methoxypolyethylene glycol-2000]-1,2-distearoyl-sn-glycero-3-

phosphoethanolamine (DSPE-PEG2000), N-[carbonyl-methoxypolyethylene glycol-5000]-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG5000), 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-750 (DMG-PEG750), 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (DMG-PEG2000), 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-5000 (DMG-PEG5000), N-[carbonyl-methoxypolyethylene glycol-750]-1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE-PEG750), N-[carbonyl-methoxypolyethylene glycol-2000]-1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE-PEG2000), and N-[carbonyl-methoxypolyethylene glycol-5000]-1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE-PEG5000) can be used, but the polyalkylene glycol lipid is not limited thereto.

[0167]

The proportion of the polyalkylene glycol-modified lipid in the total amount of the lipids forming the lipid nanoparticle according to the present invention is not particularly limited as long as the amount does not impair the gene expression activity when the lipid nanoparticle according to the present invention is used as a gene carrier. For example, the proportion of the polyalkylene glycol-modified lipid in the total amount of the lipids forming the lipid nanoparticle is preferably 0.5 to 3 mol%.

[0168]

The lipid nanoparticle according to the present invention may contain any one of DSPC, DOPC, and DOPE as the phospholipid and contain DMG-PEG as the polyalkylene glycol-modified lipid. For example, the lipid nanoparticle according to the present invention may contain 40 to 60 (for

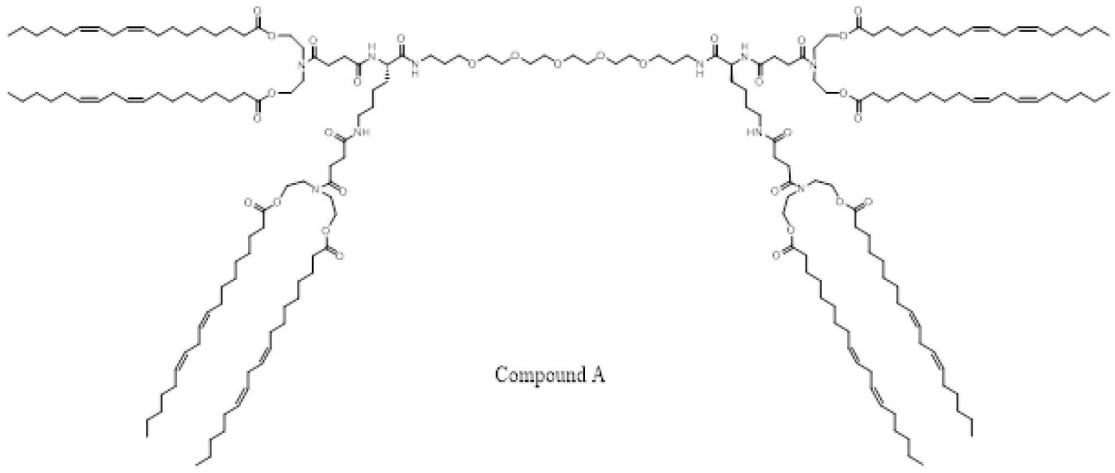
example, 40, 40.5, 41, 41.5, 42, 42.5, 43, 43.5, 44, 44.5, 45, 45.5, 46, 46.5, 47, 47.5, 48, 48.5, 49, 49.5, 50, 50.5, 51, 51.5, 52, 52.5, 53, 53.5, 54, 54.5, 55, 55.5, 56, 56.5, 57, 57.5, 58, 58.5, 59, 59.5, or 60) mol% of the pH-sensitive cationic lipid based on the total lipid amount of the lipid nanoparticle, 30-50 (for example, 30, 30.5, 31, 31.5, 32, 32.5, 33, 33.5, 34, 34.5, 35, 35.5, 36, 36.5, 37, 37.5, 38, 38.5, 39, 39.5, 40, 40.5, 41, 41.5, 42, 42.5, 43, 43.5, 44, 44.5, 45, 45.5, 46, 46.5, 47, 47.5, 48, 48.5, 49, 49.5, or 50) mol% of the sterol (for example, cholesterol) based on the total lipid amount of the lipid nanoparticle, 5-15 (for example, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, or 15) mol% of any one phospholipid of DSPC, DOPC, and DOPE based on the total lipid amount of the lipid nanoparticle, and 1-5 (for example, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or 5) mol% of DMG-PEG (for example, DMG-PEG2000) based on the total lipid amount of the lipid nanoparticle. In the lipid nanoparticle according to the present invention, the mole ratio of the pH-sensitive cationic lipid, the sterol, the one phospholipid of DSPC, DOPC, and DOPE, and DMG-PEG (pH-sensitive cationic lipid/sterol/phospholipid/DMG-PEG) is not particularly limited, and examples thereof include (60/31/8/1), (60/31/7.5/1.5), (60/31/7/2), (60/31/6.5/2.5), (60/31/6/3), (60/31/5.5/3.5), (60/31/5/4), (60/31/4.5/4.5), (60/31/4/5), (60/31.5/7.5/1), (60/30.5/7.5/2), (60/30/7.5/2.5), (60/29.5/7.5/3), (60/29/7.5/3.5), (60/28.5/7.5/4), (60/28/7.5/4.5), (60/27.5/7.5/5), (59/32/7.5/1.5), (58/33/7.5/1.5), (57/34/7.5/1.5), (56/35/7.5/1.5), (55/36/7.5/1.5), (60/29/10/1), (60/28.5/10/1.5), (60/28/10/2), (60/27.5/10/2.5), (60/27/10/3), (60/26.5/10/3.5), (60/26/10/4), (60/25.5/10/4.5), (60/25/10/5), (60/27/12/1), (60/27/11.5/1.5), (60/27/11/2), (60/27/10.5/2.5), (60/27/9.5/3.5), (60/27/9/4), (60/27/8.5/4.5), (60/27/8/5), (59/28/10/3), (58/29/10/3), (57/30/10/3),

(56/31/10/3), (55/32/10/3), (50/39/10/1), (50/38.5/10/1.5), (50/38/10/2), (50/37.5/10/2.5), (50/37/10/3), (50/36.5/10/3.5), (50/36/10/4), (50/35.5/10/4.5), (50/35/10/5), (50/38.5/10.5/1), (50/38.5/9.5/2), (50/38.5/9/2.5), (50/38.5/8.5/3), (50/38.5/8/3.5), (50/38.5/7.5/4), (50/38.5/7/4.5), (50/38.5/6.5/5), (51/37.5/10/1.5), (52/36.5/10/1.5), (53/35.5/10/1.5), (54/34.5/10/1.5), (55/33.5/10/1.5), (49/39.5/10/1.5), (48/40.5/10/1.5), (47/41.5/10/1.5), (46/42.5/10/1.5), (45/43.5/10/1.5), (51/34/10/5), (52/33/10/5), (53/32/10/5), (54/31/10/5), (55/30/10/5), (40/44/15/1), (40/43.5/15/1.5), (40/43/15/2), (40/42.5/15/2.5), (40/42/15/3), (40/41.5/15/3.5), (40/41/15/4), (40/40.5/15/4.5), (40/40/15/5), (41/39/15/5), (42/38/15/5), (43/37/15/5), (44/36/15/5), (45/35/15/5), (41/41/14/4), (42/42/13/3), (43/43/12/2), and (44/44/11/1).

[0169]

The lipid nanoparticle according to the present invention may contain compound A below. The compound A corresponds to the compound T3 in WO2019090359. All the contents disclosed in the document are incorporated in the present specification by reference.

[Chem. 157]



Compound A

[0170]

The lipid nanoparticle according to the present invention may contain any one of DOPE, DSPE, and DPPE as the phospholipid, contain any one of DPPE-PEG, DMPE-PEG, and DSPE-PEG as the polyalkylene glycol-modified lipid and contain the compound A. For example, the lipid nanoparticle according to the present invention may contain 20-60 (for example, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29, 29.5, 30, 30.5, 31, 31.5, 32, 32.5, 33, 33.5, 34, 34.5, 35, 35.5, 36, 36.5, 37, 37.5, 38, 38.5, 39, 39.5, 40, 40.5, 41, 41.5, 42, 42.5, 43, 43.5, 44, 44.5, 45, 45.5, 46, 46.5, 47, 47.5, 48, 48.5, 49, 49.5, 50, 50.5, 51, 51.5, 52, 52.5, 53, 53.5, 54, 54.5, 55, 55.5, 56, 56.5, 57, 57.5, 58, 58.5, 59, 59.5, or 60) mol% of the pH-sensitive cationic lipid based on the total lipid amount of the lipid nanoparticle, 15-50 (for example, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29, 29.5, 30, 30.5, 31, 31.5, 32, 32.5, 33, 33.5, 34, 34.5, 35, 35.5, 36, 36.5, 37, 37.5, 38, 38.5, 39, 39.5, 40, 40.5, 41, 41.5, 42, 42.5, 43, 43.5, 44, 44.5, 45, 45.5, 46, 46.5, 47, 47.5, 48, 48.5, 49, 49.5, or 50) mol% of the sterol (for example, cholesterol) based on the total lipid amount of the lipid nanoparticle, 5-40 (for example, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, 20, 20.5, 21, 21.5, 22, 22.5, 23, 23.5, 24, 24.5, 25, 25.5, 26, 26.5, 27, 27.5, 28, 28.5, 29, 29.5, 30, 30.5, 31, 31.5, 32, 32.5, 33, 33.5, 34, 34.5, 35, 35.5, 36, 36.5, 37, 37.5, 38, 38.5, 39, 39.5, or 40) mol% of any one phospholipid of DOPE, DSPE, and DPPE based on the total lipid amount of the lipid nanoparticle, 0.5-10 (for example, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10) mol% of any one polyalkylene glycol-modified lipid of DPPE-PEG (for example, DPPE-PEG2000), DMPE-PEG

(for example, DMPE-PEG2000), and DSPE-PEG (for example, DSPE-PEG2000) based on the total lipid amount of the lipid nanoparticle, and 0.5-10 (for example, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10) mol% of the compound A based on the total lipid amount of the lipid nanoparticle. In the lipid nanoparticle according to the present invention, the mole ratio of the pH-sensitive cationic lipid, the sterol, the one phospholipid of DOPE, DSPE, and DPPE, the one polyalkylene glycol-modified lipid of DPPE-PEG, DMPE-PEG, and DSPE-PEG, and the compound A (pH-sensitive cationic lipid/sterol/phospholipid/polyalkylene glycol-modified lipid/compound A) is not particularly limited, and examples thereof include (30/28/30/6/6), (30/29/30/5.5/5.5), (30/30/30/5/5), (30/31/30/4.5/4.5), (30/32/30/4/4), (30/33/30/3.5/3.5), (30/34/30/3/3), (30/35/30/2.5/2.5), (30/36/30/2/2), (30/37/30/1.5/1.5), (30/38/30/1/1), (30/30/31/4.5/4.5), (30/30/32/4/4), (30/30/33/3.5/3.5), (30/30/34/3/3), (30/30/35/2.5/2.5), (30/30/36/2/2), (30/30/37/1.5/1.5), (30/30/38/1/1), (35/25/30/5/5), (34/26/30/5/5), (33/27/30/5/5), (32/28/30/5/5), (31/29/30/5/5), (40/25/25/5/5), (38/26/26/5/5), (36/27/27/5/5), (34/28/28/5/5), (32/29/29/5/5), (50/20/20/5/5), (45/20/25/5/5), (40/25/25/5/5), (35/30/25/5/5), (30/35/25/5/5), (50/21/20/4.5/4.5), (50/22/20/4/4), (50/23/20/3.5/3.5), (50/24/20/3/3), (50/25/20/2.5/2.5), (50/26/20/2/2), (50/27/20/1.5/1.5), (50/28/20/1/1), (30/50/20/1/1), (30/48/20/2/2), (30/46/20/3/3), (30/44/20/4/4), (30/42/20/5/5), (25/40/25/5/5), (25/40/30/3/2), (25/35/35/3/2), (25/45/25/4/1), and (23/35/35/5/2).

[0171]

Regarding the size of the lipid nanoparticle according to the present invention, because high delivery efficiency into cells of a living body is easily

obtained, the average particle size is preferably 400 nm or less, the average particle size is more preferably 300 nm or less, and the average particle size is further preferably 200 nm or less, and still further preferably 150 nm or less. Here, the average particle size of the lipid nanoparticle means the Z average particle size measured by dynamic light scattering (DLS). The measurement by dynamic light scattering can be made by a normal method using a commercial DLS apparatus or the like.

[0172]

The form of the lipid nanoparticle according to the present invention is not particularly limited, and examples thereof include a unilamellar liposome, a multilamellar liposome, a spherical micelle, and an irregular layered structure as a form dispersed in an aqueous solvent. The lipid nanoparticle according to the present invention is preferably a unilamellar liposome or a multilamellar liposome.

[0173]

The lipid nanoparticle according to the present invention preferably includes a target component to be delivered into the target cell in the particle covered with a lipid membrane. The component that the lipid nanoparticle according to the present invention includes in the particle is not particularly limited as long as the component has a size which can be included. The lipid nanoparticle according to the present invention can encapsulate any substance such as a nucleic acid, a saccharide, a peptide, a small molecule, and a metal compound.

[0174]

The component included in the lipid nanoparticle according to the

present invention is preferably a nucleic acid. The nucleic acid may be DNA, may be RNA or may be an analog or a derivative thereof (for example, a peptide nucleic acid (PNA) or phosphorothioate DNA). The nucleic acid included in the lipid nanoparticle according to the present invention may be a single-strand nucleic acid or may be a double-strand nucleic acid and may be linear or may be cyclic.

In an aspect of the present invention, the lipid nanoparticle according to the present invention contains the pH-sensitive cationic lipid of the present invention, a salt, or a stereoisomer thereof and a nucleic acid.

[0175]

The nucleic acid included in the lipid nanoparticle according to the present invention preferably contains an exogenous gene to be expressed in the target cell, and a nucleic acid which functions to express an exogenous gene in a cell when the nucleic acid is incorporated in the cell is more preferable. The exogenous gene may be a gene which is originally included in the genome DNA of the target cell or may be a gene which is not included in the genome DNA. Such a nucleic acid may be a gene expression vector containing a nucleic acid consisting of a nucleotide sequence encoding the target gene to be expressed. The gene expression vector may exist as an extrachromosomal gene in the cell into which the gene expression vector is introduced or may be incorporated into the genome DNA by homologous recombination.

[0176]

The gene expression vector included in the lipid nanoparticle according to the present invention is not particularly limited, and a vector which is generally used for gene therapy or the like can be used. The gene expression vector

included in the lipid nanoparticle according to the present invention is preferably a nucleic acid vector such as a plasmid vector. The plasmid vector may be cyclic as it is, or may be encapsulated in the lipid nanoparticle according to the present invention in a linear state by being cleaved in advance. The gene expression vector can be designed by a normal method based on the nucleotide sequence information of the target gene to be expressed using a generally used molecular biological tool and can be produced by various known methods.

[0177]

The nucleic acid included in the lipid nanoparticle according to the present invention is also preferably a functional nucleic acid for regulating the expression of a target gene found in the target cell. Examples of the functional nucleic acid include an antisense oligonucleotide, antisense DNA, antisense RNA, siRNA, microRNA, and mRNA. Moreover, plasmid DNA (pDNA) which is an siRNA expression vector for expressing siRNA in a cell may also be used. The siRNA expression vector can be prepared from a commercial siRNA expression vector, or a commercial siRNA expression vector may be appropriately modified.

In an aspect of the present invention, the lipid nanoparticle according to the present invention contains the pH-sensitive cationic lipid of the present invention, a salt, or a stereoisomer thereof and a nucleic acid, and the nucleic acid here is siRNA, mRNA, or plasmid DNA.

[0178]

The method for producing the lipid nanoparticle according to the present invention is not particularly limited, and any method which one skilled in the art can use can be applied. For example, the lipid nanoparticle according to the

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 stereoisomer, 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.

Examples of the buffer include HEPES buffer, a phosphate buffer, and a tris buffer.

[0180]

In the present invention, the lipid nanoparticle preparation may be prepared by suspending lipid nanoparticles in an aqueous solution.

The pH of the lipid nanoparticle preparation of the present invention is, for example, 5.5 to 8.5, and preferably 6.8 to 8.0 at 25°C.

In an aspect, the present invention relates to a resuspended preparation obtained by resuspending a lipid nanoparticle preparation through addition of water or an aqueous solution.

[0181]

The animal to which the lipid nanoparticle according to the present invention is administered is not particularly limited and may be a human or a non-human animal. Examples of the non-human animal include mammals such as a cow, a pig, a horse, a sheep, a goat, a monkey, a dog, a cat, a rabbit, a mouse, a rat, a hamster, and a guinea pig and birds such as a fowl, a quail, and a duck.

Examples

[0182]

Next, the present invention is explained further in detail with Examples, but the present invention is not limited to the Examples below.

[0183]

In the Examples, the following abbreviations were used.

THF: tetrahydrofuran (super dehydrated) (containing no stabilizer)

LDA: lithium diisopropylamide solution (Sigma-Aldrich, 774766-4X25ML)

DMPU: 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone

DMAP: 4-dimethylaminopyridine

DCM: dichloromethane

EDCI: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (TCI, D1601)

TBDPSCl: tert-butyldiphenylchlorosilane

DMSO: dimethyl sulfoxide

TBAF: tetrabutylammonium fluoride

Pd/C 10%: palladium 10% on carbon (TCI, P1785)

DIPEA: N,N-diisopropylethylamine

DMF: N,N-dimethylformamide

[0184]

I. Synthesis of Lipids and Raw Materials

Raw Material Synthesis Example 1 (synthesis of α-alkyl branched carboxylic acids)

[Chem. 158]



2-hexyloctanoic acid

To THF (50 ml) which was cooled to -25°C, 23 ml of LDA (1 mol/L) was added, and the mixture was stirred for 60 min. Octanoic acid (1.42 g) dissolved in THF (20 ml) was added, and subsequently, DMPU (1.2 ml) was added. The temperature of the reaction solution was increased to -5°C, and the reaction

solution was further stirred for an hour. 1-Iodohexane (4.88 g) was added, and the mixture was stirred at room temperature overnight. An aqueous saturated sodium hydrogen sulfate solution and ethyl acetate were added to the reaction solution, 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 2-hexyloctanoic acid (1.75 g) of interest was obtained.

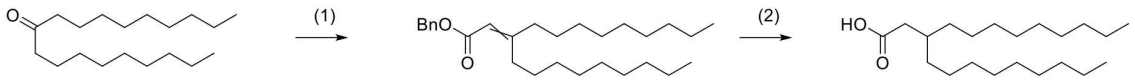
^{1}H NMR (300 MHz, $CDCl_3$) δ 2.41-2.30 (m, 1H), 1.66-1.56 (m, 2H), 1.51-1.42 (m, 2H), 1.28 (brs, 16H), 0.88 (t, J = 6.9 Hz, 6H).

2-Nonylundecanoic acid, 2-butyldodecanoic acid, 2-nonyldodecanoic acid, 2-octyltridecanoic acid, and 2-dodecyltetradecanoic acid were synthesized by the same method.

[0185]

Raw Material Synthesis Example 2 (synthesis of β-alkyl branched carboxylic acid)

[Chem. 159]



benzyl 3-nonyldodec-2-enoate　　3-nonyldodecanoic acid

(1) NaH (60%, dispersion in paraffin liquid) (216 mg) was dissolved in THF (15 ml), and the solution was stirred under ice-cooling for 10 minutes. Benzyl 2-(dimethoxyphosphoryl)acetate (1.13 ml) was added, and the mixture was further stirred for 30 minutes under ice-cooling. 10-Nonadecanone (1.27 g) was dissolved in THF (15 ml), and the solution was added dropwise to the

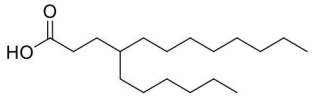
reaction liquid. The reaction liquid was heated to room temperature and stirred overnight. An aqueous saturated ammonium chloride solution was added to the reaction liquid, and extraction with ethyl acetate was conducted three times. After the organic layer was washed with saturated brine, sodium sulfate 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 1-2, except that 2-hexyldecanal was used

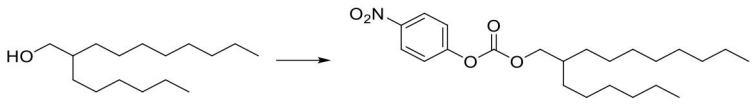
instead of 10-nonadecanone of Raw Material Synthesis Example 2(1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 2.35-2.30 (m, 2H), 1.64-1.56 (m, 2H), 1.33-1.26 (m, 25H), 0.90-0.86 (m, 6H).

[0187]

Raw Material Synthesis Example 4 (synthesis of 2-hexyldecyl (4-nitrophenyl) carbonate)

[Chem. 161]



2-hexyldecan-1-ol　　2-hexyldecyl (4-nitrophenyl) carbonate

2-Hexyldecan-1-ol (2.42 g) and 4-nitrophenyl carbonochloridate (3.02 g) were dissolved in DCM (10 ml). Pyridine (2.42 ml) was added dropwise, and the reaction solution was stirred at room temperature overnight. An aqueous saturated sodium hydrogen carbonate solution was added to the reaction solution, and extraction with DCM 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 2-hexyldecyl (4-nitrophenyl) carbonate (3.37 g) of interest was obtained.

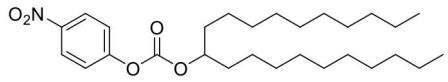
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31-8.26 (m, 2H), 7.41-7.36 (m, 2H), 4.19 (d, J = 5.8 Hz 2H), 1.76-1.71 (m, 1H), 1.33-1.29 (m, 24H), 0.91-0.86 (m, 6H).

[0188]

Raw Material Synthesis Example 5 (synthesis of henicosan-11-yl (4-nitrophenyl)

carbonate)

[Chem. 162]



henicosan-11-yl (4-nitrophenyl) carbonate

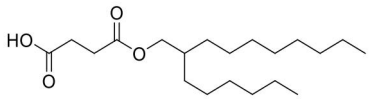
Henicosan-11-yl (4-nitrophenyl) carbonate was obtained in the same manner as in Raw Material Synthesis Example 4, except that henicosan-11-ol was used instead of 2-hexyldecan-1-ol of Raw Material Synthesis Example 4.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30-8.25 (m, 2H), 7.41-7.36 (m, 2H), 4.82 (quin, J = 5.6 Hz, 1H), 1.71-1.59 (m, 4H), 1.39-1.26 (m, 32H), 0.88 (t, J = 7.0 Hz, 6H).

[0189]

Raw Material Synthesis Example 6 (synthesis of 4-((2-hexyldecyl)oxy)-4-oxobutanoic acid)

[Chem. 163]



4-((2-hexyldecyl)oxy)-4-oxobutanoic acid

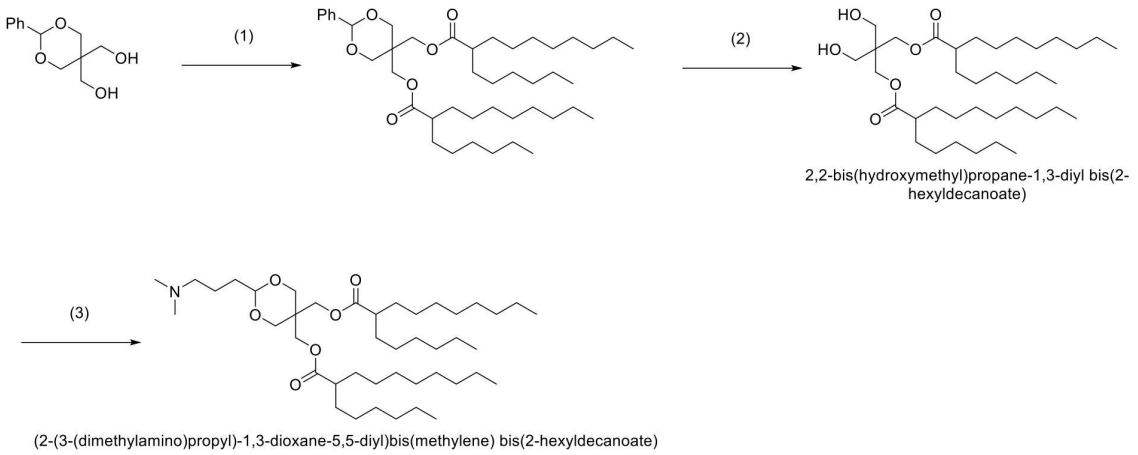
Succinic anhydride (304 mg) and 2-hexyldecan-1-ol (740 mg) were dissolved in toluene (2 ml), and the solution was stirred for six hours at 100°C. The solvent was evaporated under reduced pressure from the reaction liquid, and a crude product was obtained. The crude product was purified by silica gel chromatography [elution solvent; hexane/ethyl acetate], and 4-((2-hexyldecyl)oxy)-4-oxobutanoic acid (931 mg) of interest was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.99 (d, J = 5.8 Hz, 2H), 2.72-2.60 (m, 4H), 1.26 (brs, 24H), 0.90-0.86 (m, 6H).

[0190]

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

[Chem. 164]



(1) (2-Phenyl-1,3-dioxane-5,5-diyl)dimethanol (1.12 g), 2-hexyldecanoic acid (2.69 g), and DMAP (61.1 mg) were dissolved in DCM (6.0 ml), and EDCI (2.11 g) was added. The mixture was stirred at room temperature overnight. An aqueous saturated ammonium chloride solution was added to the reaction solution, and extraction with DCM 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.

(2) Methanol (32 ml), DCM (8.0 ml), and HCl (4 mol/L in 1,4-dioxane, 4.0 ml) were added to the obtained crude product (2.10 g), and the mixture was stirred at room temperature for four hours. An excess amount of sodium hydrogen carbonate was added to the reaction solution, and the mixture was concentrated under reduced pressure. Water and ethyl acetate were added to the residue, 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 2,2-bis(hydroxymethyl)propane-1,3-diyl bis(2-hexyldecanoate) (1.46 g) of interest was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.15 (s, 4H), 3.55 (d, J = 6.1 Hz, 4H), 2.87-2.83 (m, 2H), 2.43-2.33 (m, 2H), 1.61-1.43 (m, 8H), 1.26 (brs, 40H), 0.88 (t, J = 7.0 Hz, 12H)

(3) To a container in which a calcium chloride tube was placed, 2,2-bis(hydroxymethyl)propane-1,3-diyl bis(2-hexyldecanoate) (175 mg), 4,4-dimethoxy-N,N-dimethylbutan-1-amine (62.0 mg), p-toluenesulfonic acid monohydrate (65.6 mg), and toluene (1.0 ml) were added, and the mixture was stirred at 85°C overnight. An excess amount of an aqueous saturated sodium hydrogen carbonate solution was added to the reaction solution, 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; DCM/methanol], and (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) (131 mg) of interest was obtained.

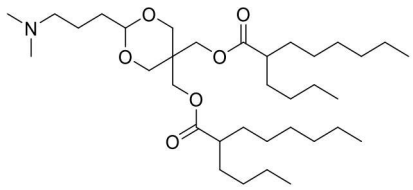
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.48 (t, J = 4.6 Hz, 1H), 4.36 (s, 2H), 3.92 (d, J = 11.9 Hz, 2H), 3.83 (s, 2H), 3.60 (d, J = 11.9 Hz, 2H), 2.37-2.25 (m, 4H), 2.22 (s, 6H), 1.72-1.40 (m, 12H), 1.25 (brs, 40H), 0.90-0.85 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 710.6, measured value m/z = 710.6.

[0191]

Lipid Synthesis Example 2 (synthesis of (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-butyloctanoate))

[Chem. 165]



(2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-butyloctanoate)

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

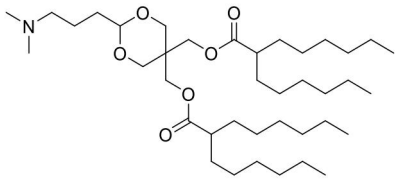
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.49 (t, J = 4.5 Hz, 1H), 4.36 (s 2H), 3.92 (d, J = 11.7 Hz, 2H), 3.84 (s, 2H), 3.60 (d, J = 11.7 Hz, 2H), 2.36-2.25 (m, 4H), 2.22 (s, 6H), 1.67-1.42 (m, 12H), 1.25 (brs, 24H), 0.85-0.90 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 598.5, measured value m/z = 598.5.

[0192]

Lipid Synthesis Example 3 (synthesis of (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyloctanoate))

[Chem. 166]



(2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyloctanoate)

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

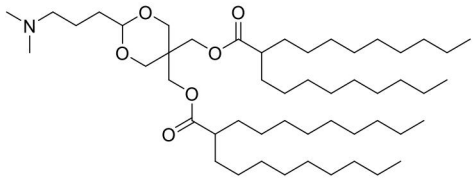
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.48 (t, J = 4.5 Hz, 1H), 4.36 (s, 2H), 3.92 (d, J = 11.7 Hz, 2H), 3.84 (s, 2H), 3.60 (d, J = 11.7 Hz, 2H), 2.38-2.24 (m, 4H), 2.21 (s, 6H), 1.62-1.45 (m, 12H), 1.25 (brs, 32H) 0.90-0.87 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 654.6, measured value m/z = 654.6.

[0193]

Lipid Synthesis Example 4 (synthesis of (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate))

[Chem. 167]



(2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)

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

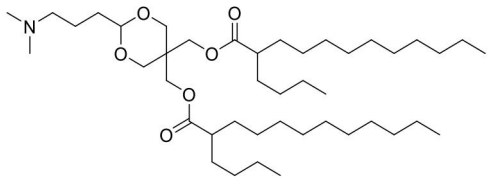
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.48 (t, J = 4.4 Hz, 1H), 4.36 (s, 2H), 3.92 (d, J = 11.8 Hz, 2H), 3.83 (s, 2H), 3.59 (d, J = 11.8 Hz, 2H), 2.37-2.24 (m, 4H), 2.21 (s, 6H), 1.65-1.40 (m, 12H), 1.25 (brs, 56H), 0.88 (t, J = 6.45 Hz, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 822.8, measured value m/z = 822.8.

[0194]

Lipid Synthesis Example 5 (synthesis of (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-butyldodecanoate))

[Chem. 168]



(2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-butyldodecanoate)

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

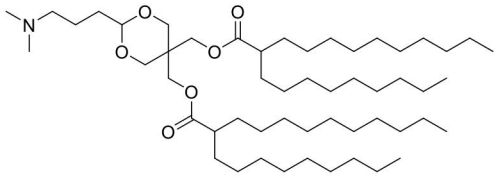
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.48 (t, J = 4.7 Hz, 1H), 4.36 (s, 2H), 3.92 (d, J = 11.7 Hz, 2H), 3.83 (s, 2H), 3.60 (d, J = 11.7 Hz, 2H), 2.37-2.24 (m, 4H), 2.21 (s, 6H), 1.66-1.40 (m, 12H), 1.30-1.25 (m, 40H), 0.90-0.86 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 710.6, measured value m/z = 710.6.

[0195]

Lipid Synthesis Example 6 (synthesis of (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonyldodecanoate))

[Chem. 169]



(2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonyldodecanoate)

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

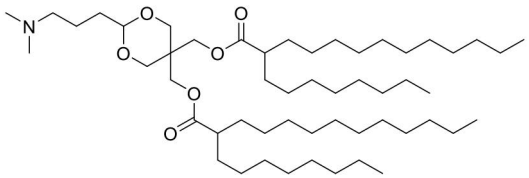
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.48 (t, J = 4.8 Hz, 1H), 4.36 (s, 2H), 3.92 (d, J = 11.7 Hz, 2H), 3.83 (s, 2H), 3.59 (d, J = 11.7 Hz, 2H), 2.35-2.24 (m, 4H), 2.22 (s, 6H), 1.57-1.45 (m, 12H), 1.25 (brs, 60H), 0.90-0.87 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 850.8, measured value m/z = 850.8.

[0196]

Lipid Synthesis Example 7 (synthesis of (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-octyltridecanoate))

[Chem. 170]



(2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-octyltridecanoate)

(2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-octyltridecanoate) of interest was obtained in the same manner as in Lipid Synthesis Example 1, except that 2-octyltridecanoic acid was used instead of 2-

hexyldecanoic acid in Lipid Synthesis Example 1(1).

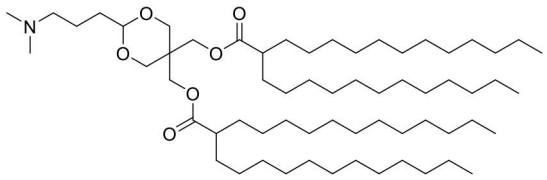
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.48 (t, J = 4.5Hz, 1H), 4.36 (s, 2H), 3.92 (d, J = 11.8 Hz, 2H), 3.83 (s, 2H), 3.59 (d, J = 11.8 Hz, 2H), 2.37-2.25 (m, 4H), 2.22 (s, 6H), 1.67-1.43 (m, 12H), 1.25 (brs, 60H), 0.90-0.87 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 850.8, measured value m/z = 850.8.

[0197]

Lipid Synthesis Example 8 (synthesis of (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-dodecyltetradecanoate))

[Chem. 171]



(2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-dodecyltetradecanoate)

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

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.48 (t, J = 4.5Hz, 1H), 4.36 (s, 2H), 3.92 (d, J = 11.7 Hz, 2H), 3.83 (s, 2H), 3.59 (d, J = 11.7 Hz, 2H), 2.39-2.24 (m, 4H), 2.22 (s, 6H), 1.62-1.43 (m, 12H), 1.25 (brs, 80H), 0.90-0.86 (m, 12H).

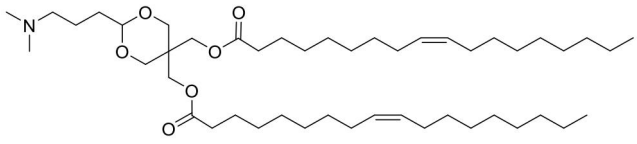
Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 990.9, measured value m/z = 990.9.

[0198]

Lipid Synthesis Example 9 (synthesis of (2-(3-(dimethylamino)propyl)-1,3-

dioxane-5,5-diyl)bis(methylene) dioleate)

[Chem. 172]



(2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) dioleate

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

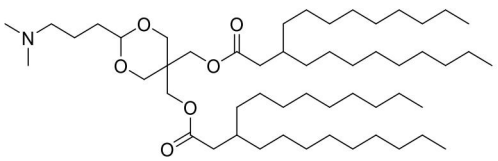
^{1}H NMR (300 MHz, $CDCl_3$) δ 5.36-5.32 (m, 4H), 4.56 (t, J = 4.0 Hz, 1H), 4.34 (s, 2H), 3.88 (d, J = 12.0 Hz, 2H), 3.85 (s, 2H), 3.60 (d, J = 12.0 Hz, 2H), 3.10-3.05 (m, 24H), 2.82 (s, 6H), 2.31 (td, J = 7.3 Hz, 5.9 Hz, 4H), 2.02-1.98 (m, 8H), 1.94-1.86 (m, 2H), 1.72-1.62 (m, 4H), 1.30-1.27 (m, 42H), 0.88 (t, J = 6.5 Hz, 6H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 762.7, measured value m/z = 762.7.

[0199]

Lipid Synthesis Example 10 (synthesis of (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(3-nonyldodecanoate))

[Chem. 173]



(2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(3-nonyldodecanoate)

(2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(3-nonyldodecanoate) was obtained in the same manner as in Lipid Synthesis

Example 1, except that 3-nonyldodecanoic acid was used instead of 2-hexyldecanoic acid in Lipid Synthesis Example 1(1).

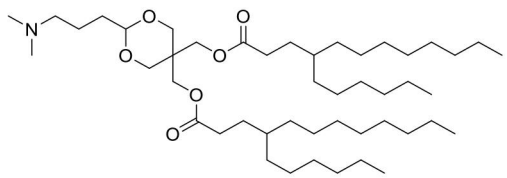
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.49 (t, J = 4.2 Hz, 1H), 4.35 (s, 2H), 3.92 (d, J = 11.8 Hz, 2H), 3.82 (s, 2H), 3.59 (d, J = 11.8 Hz, 2H), 2.31-2.22 (m, 12H), 1.81-1.63 (m, 6H), 1.25 (brs, 64H), 0.88 (t, J = 7.0 Hz, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 850.8, measured value m/z = 850.8.

[0200]

Lipid Synthesis Example 11 (synthesis of (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(4-hexyldodecanoate))

[Chem. 174]



(2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(4-hexyldodecanoate)

(2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(4-hexyldodecanoate) of interest was obtained in the same manner as in Lipid Synthesis Example 1, except that 4-hexyldodecanoic acid was used instead of 2-hexyldecanoic acid in Lipid Synthesis Example 1(1).

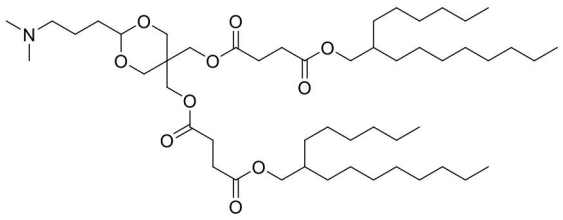
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.50 (t, J = 4.3 Hz, 1H), 4.35 (s, 2H), 3.93 (d, J = 12.0 Hz, 2H), 3.84 (s, 2H), 3.60 (d, J = 12.0 Hz, 2H), 2.36-2.25 (m, 12H), 1.65-1.53 (m, 8H), 1.33-1.24 (m, 50H), 0.88 (t, J = 6.9 Hz, 12H).

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

[0201]

Lipid Synthesis Example 12 (synthesis of O,O'-((2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene)) bis(2-hexyldecyl) disuccinate)

[Chem. 175]



O,*O*'-((2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene)) bis(2-hexyldecyl) disuccinate

O,O'-((2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene)) bis(2-hexyldecyl) disuccinate of interest was obtained in the same manner as in Lipid Synthesis Example 1, except that 4-((2-hexyldecyl)oxy)-4-oxobutanoic acid was used instead of 2-hexyldecanoic acid in Lipid Synthesis Example 1(1).

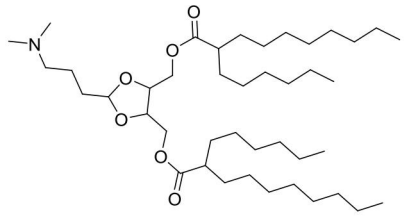
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.51 (t, J = 4.2 Hz, 1H), 4.39 (s, 2H), 3.98 (dd, J = 5.9 Hz, 2.0 Hz, 4H), 3.91 (d, J = 11.8 Hz, 2H), 3.87 (s, 2H), 3.59 (d, J = 11.8 Hz, 2H), 2.65-2.63 (m, 8H),2.55-2.50 (m, 2H), 2.41 (s, 6H), 1.67-1.62 (m, 6H), 1.27 (brs, 48H), 0.88 (t, J = 6.7 Hz, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 882.7, measured value m/z = 882.7.

[0202]

Lipid Synthesis Example 13 (synthesis of (2-(3-(dimethylamino)propyl)-1,3-dioxolane-4,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 176]



(2-(3-(dimethylamino)propyl)-1,3-dioxolane-4,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(3-(dimethylamino)propyl)-1,3-dioxolane-4,5-diyl)bis(methylene) bis(2-hexyldecanoate) of interest was obtained in the same manner as in Lipid Synthesis Example 1, except that ((4R,5R)-2-phenyl-1,3-dioxolane-4,5-diyl)dimethanol was used instead of (2-phenyl-1,3-dioxane-5,5-diyl)dimethanol in Lipid Synthesis Example 1(1).

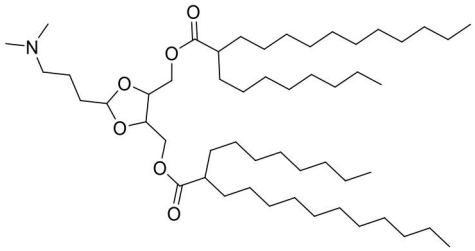
^{1}H NMR (300 MHz, $CDCl_3$) δ 5.19-5.14 (m, 0.3H), 5.10 (t, J = 4.7 Hz, 0.4H), 5.00 (t, J = 4.7 Hz, 0.15H), 4.90 (t, J = 4.7 Hz, 0.15H), 4.67-4.61 (m, 0.65H), 4.41-4.34 (m, 0.35H), 4.26-4.03 (m, 4H), 3.86-3.61 (m, 1H), 2.49-2.27 (m, 4H), 2.23-2.22 (m, 6H), 1.66-1.42 (m, 12H), 1.25 (brs, 40H), 0.88 (t, J = 7.0 Hz, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 696.6, measured value m/z = 696.6.

[0203]

Lipid Synthesis Example 14 (synthesis of (2-(3-(dimethylamino)propyl)-1,3-dioxolane-4,5-diyl)bis(methylene) bis(2-octyltridecanoate))

[Chem. 177]



(2-(3-(dimethylamino)propyl)-1,3-dioxolane-4,5-diyl)bis(methylene) bis(2-octyltridecanoate)

(2-(3-(dimethylamino)propyl)-1,3-dioxolane-4,5-diyl)bis(methylene) bis(2-octyltridecanoate) of interest was obtained in the same manner as in Lipid Synthesis Example 1, except that ((4R,5R)-2-phenyl-1,3-dioxolane-4,5-diyl)dimethanol was used instead of (2-phenyl-1,3-dioxane-5,5-diyl)dimethanol and that 2-octyltridecanoic acid was used instead of 2-hexyldecanoic acid in Lipid Synthesis Example 1(1).

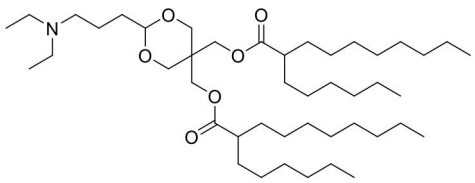
^{1}H NMR (300 MHz, $CDCl_3$) δ 5.18-5.14 (m, 0.3H), 5.10 (t, J = 4.6 Hz, 0.4H), 5.02-4.88 (m, 0.3H), 4.67-4.61 (m, 0.65H), 4.41-4.34 (m, 0.35H), 4.26-4.03 (m, 4H), 3.86-3.61 (m, 1H), 2.49-2.27 (m, 4H), 2.23-2.22 (m, 6H), 1.66-1.42 (m, 12H), 1.25 (brs, 60H), 0.88 (t, J = 7.2 Hz, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 836.8, measured value m/z = 836.8.

[0204]

Lipid Synthesis Example 15 (synthesis of (2-(3-(diethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 178]



(2-(3-(diethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(3-(diethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) of interest was obtained in the same manner as in Lipid Synthesis Example 1, except that 4,4-diethoxy-N,N-diethylbutan-1-amine was used instead of 4,4-dimethoxy-N,N-dimethylbutan-1-amine in Lipid Synthesis Example 1(3).

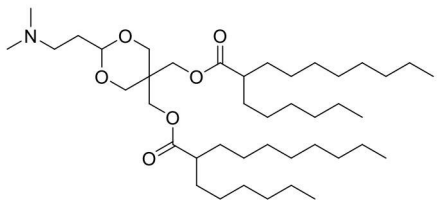
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.48 (t, J = 5.1 Hz, 1H), 4.36 (s, 2H), 3.92 (d, J = 11.8 Hz, 2H), 3.83 (s, 2H), 3.60 (d, J = 11.8 Hz, 2H), 2.51 (q, J = 7.0 Hz, 4H), 2.44 (t, J = 6.9 Hz, 2H), 2.37-2.28 (m, 2H), 1.61-1.43 (m, 12H), 1.25 (brs, 40H), 1.04 (t, J = 7.1 Hz, 6H), 0.90-0.85 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 738.7, measured value m/z = 738.7.

[0205]

Lipid Synthesis Example 16 (synthesis of (2-(2-(dimethylamino)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 179]



(2-(2-(dimethylamino)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(2-(dimethylamino)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) of interest was obtained in the same manner as in Lipid Synthesis Example 1, except that 3,3-diethoxy-N,N-dimethylpropan-1-amine was used instead of 4,4-dimethoxy-N,N-dimethylbutan-1-amine in Lipid Synthesis Example 1(3).

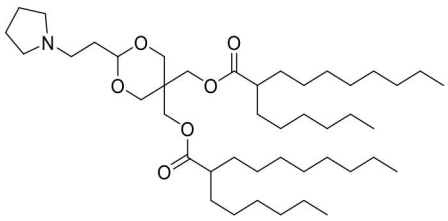
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.56 (t, J = 4.7 Hz, 1H), 4.35 (s, 2H), 3.91 (d, J = 11.5 Hz, 2H), 3.83 (s, 2H), 3.62 (d, J = 11.5 Hz, 2H), 2.43-2.30 (m, 4H), 2.25 (s, 6H), 1.85-1.79 (m, 2H), 1.63-1.53 (m, 4H), 1.50-1.40 (m, 4H), 1.25 (brs, 40H), 0.90-0.85 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 696.6, measured value m/z = 696.6.

[0206]

Lipid Synthesis Example 17 (synthesis of (2-(2-(pyrrolidin-1-yl)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 180]



(2-(2-(pyrrolidin-1-yl)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(2-(pyrrolidin-1-yl)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) of interest was obtained in the same manner as in Lipid Synthesis Example 1, except that 1-(3,3-diethoxypropyl)pyrrolidine was used instead of 4,4-dimethoxy-N,N-dimethylbutan-1-amine in Lipid Synthesis Example 1(3).

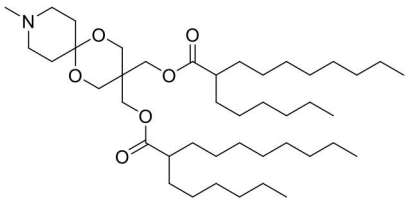
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.57 (t, J = 4.9 Hz, 1H), 4.35 (s, 2H), 3.91 (d, J = 11.6 Hz, 2H), 3.83 (s, 2H), 3.61 (d, J = 11.6 Hz, 2H), 2.61-2.54 (m, 6H), 2.39-2.28 (m, 2H), 1.92-1.85 (m, 2H), 1.82-1.77 (m, 4H), 1.63-1.53 (m, 4H), 1.50-1.40 (m, 4H), 1.25 (brs, 40H), 0.90-0.85 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 722.6, measured value m/z = 722.6.

[0207]

Lipid Synthesis Example 18 (synthesis of (9-methyl-1,5-dioxa-9-azaspiro[5.5]undecane-3,3-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 181]



(9-methyl-1,5-dioxa-9-azaspiro[5.5]undecane-3,3-diyl)bis(methylene) bis(2-hexyldecanoate)

(9-methyl-1,5-dioxa-9-azaspiro[5.5]undecane-3,3-diyl)bis(methylene) bis(2-hexyldecanoate) of interest was obtained in the same manner as in Lipid Synthesis Example 1, except that 1-methylpiperidin-4-one was used instead of 4,4-dimethoxy-N,N-dimethylbutan-1-amine in Lipid Synthesis Example 1(3).

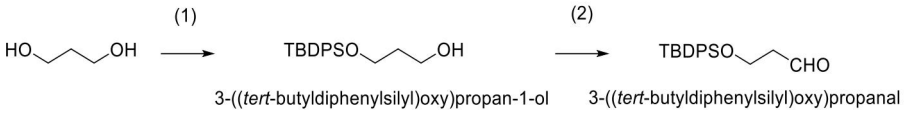
^{1}H NMR (300 MHz, $CDCl_3$) δ4.10 (s, 4H), 3.74 (s, 4H), 2.44-2.41 (m, 4H), 2.37-2.31 (m, 2H), 2.29 (s, 3H), 1.93-1.89 (m, 4H), 1.59-1.41 (m, 8H), 1.25 (brs, 40H), 0.90-0.85 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 708.6, measured value m/z = 708.6.

[0208]

Raw Material Synthesis Example 7 (synthesis of 3-((tert-butyldiphenylsilyl)oxy)propanal)

[Chem. 182]



(1) 1,3-Propanediol (2.28 g) and pyridine (3.22 ml) were dissolved in DCM (20 ml), and TBDPSCl (5.50 g) was added. The mixture was stirred at room temperature overnight. An aqueous saturated ammonium chloride solution was added to the reaction solution, and extraction with DCM 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 3-((tert-butyldiphenylsilyl)oxy)propan-1-ol (6.65 g) was obtained.

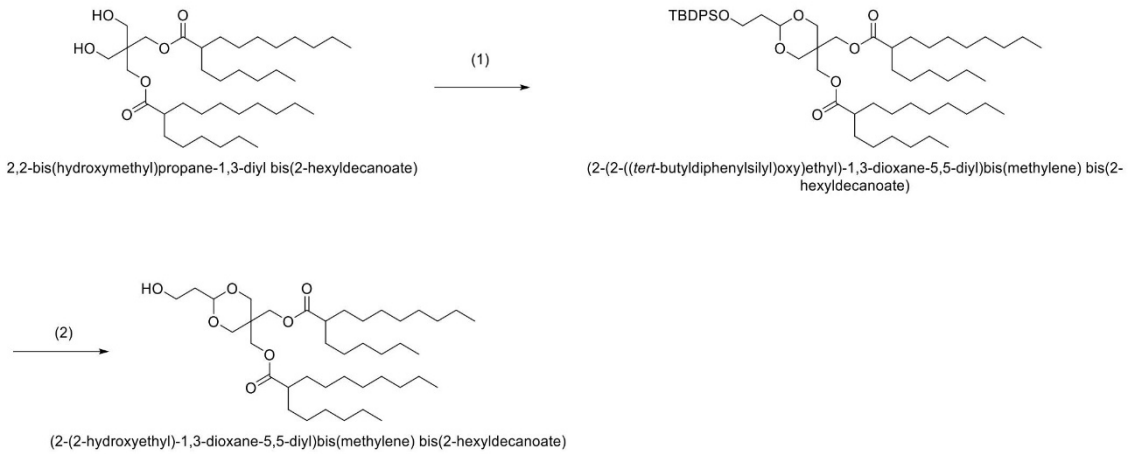
(2) 3-((tert-Butyldiphenylsilyl)oxy)propan-1-ol (3.15 g), DMSO (7.10 ml), and triethylamine (6.93 ml) were dissolved in DCM (30 ml), and the solution was stirred under ice-cooling. After 10 minutes, sulfur trioxide pyridine complex (4.77 g) was added, and the reaction solution was heated to room temperature and stirred overnight. An aqueous saturated ammonium chloride solution was added to the reaction solution, and extraction with DCM 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 3-((tert-butyldiphenylsilyl)oxy)propanal (2.37 g) was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.82 (t, J = 2.2 Hz, 1H), 7.77-7.64 (m, 4H), 7.44-7.36 (m, 6H), 4.02 (t, J = 6.0 Hz, 2H), 2.60 (td, J = 6.0 Hz, 2.2 Hz, 2H), 1.04 (s, 9H).

[0209]

Raw Material Synthesis Example 8 (synthesis of ((2-(2-hydroxyethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 183]



2,2-bis(hydroxymethyl)propane-1,3-diyl bis(2-hexyldecanoate)

(2-(2-((*tert*-butyldiphenylsilyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(2-hydroxyethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(1) To a container in which a calcium chloride tube was placed, 2,2-bis(hydroxymethyl)propane-1,3-diyl bis(2-hexyldecanoate) (2.76 g), 3-((tert-butyldiphenylsilyl)oxy)propanal (1.69 g), p-toluenesulfonic acid monohydrate (85.6 mg), and toluene (30 ml) were added, and the mixture was stirred at 80°C overnight. An excess amount of an aqueous saturated sodium hydrogen carbonate solution was added to the reaction solution, 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 (2-(2-((tert-butyldiphenylsilyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) (3.02 g) was obtained.

(2) TBAF (1.0 mol/L in THF, 30 ml) was added to (2-(2-((tert-butyldiphenylsilyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) (1.36 g) and acetic acid (2.57 ml), and the mixture was stirred at room temperature for three hours. An excess amount of an aqueous saturated sodium hydrogen carbonate solution was added to the reaction

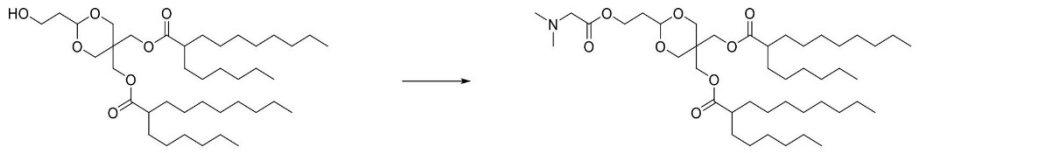
solution, and extraction with ethyl acetate was conducted three times. After the organic layer was washed with saturated brine, sodium sulfate was added, and the mixture was dehydrated. After filtration thereof, the solvent was evaporated under reduced pressure, and a crude product was obtained. The crude product was purified by silica gel chromatography [elution solvent; hexane/ethyl acetate], and (2-(2-hydroxyethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) (692 mg) was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.70 (t, J = 4.7 Hz, 1H), 4.38 (s, 2H), 3.94 (d, J = 11.8 Hz, 2H), 3.84 (s, 2H), 3.78 (q, J = 5.6 Hz, 2H), 3.64 (d, J = 11.8 Hz, 2H), 2.40-2.29 (m, 2H), 2.19 (t, J = 5.6 Hz, 1H), 1.91 (q, J = 4.9 Hz, 2H), 1.65-1.41 (m, 8H), 1.25 (brs, 40H), 0.89-0.86 (m, 12H).

[0210]

Lipid Synthesis Example 19 (synthesis of (2-(2-((dimethylglycyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 184]



(2-(2-hydroxyethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) (2-(2-((dimethylglycyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(2-Hydroxyethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) (134 mg), N,N-dimethylglycine (41.3 mg), and DMAP (2.44 mg) were dissolved in DCM (2 ml), and EDCI (76.7 mg) was added. The mixture was stirred at room temperature overnight. An aqueous saturated sodium hydrogen carbonate solution was added to the reaction solution, and extraction with DCM 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; DCM/methanol], and (2-(2-((dimethylglycyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) (92.0 mg) was obtained.

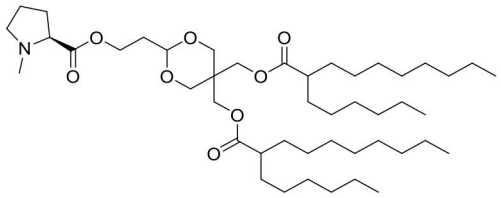
^{1}H NMR (300 MHz, $CDCl_3$) δ4.58 (t, J = 5.2 Hz, 1H), 4.35 (s, 2H), 4.24 (t, J = 6.5 Hz, 2H), 3.92 (d, J = 11.9 Hz, 2H), 3.83 (s, 2H), 3.60 (d, J = 11.9 Hz, 2H), 3.16 (s, 2H), 2.42-2.28 (m, 8H), 1.98 (q, J = 6.5 Hz, 2H), 1.63-1.40 (m, 8H), 1.25 (brs, 40H), 0.90-0.85 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 754.6, measured value m/z = 754.6.

[0211]

Lipid Synthesis Example 20 (synthesis of (2-(2-((methyl-L-prolyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 185]



(2-(2-((methyl-*L*-prolyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(2-((methyl-L-prolyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) of interest was obtained in the same manner as in Lipid Synthesis Example 19, except that N-methyl-L-proline monohydrate was used instead of N,N-dimethylglycine in Lipid Synthesis Example 19.

^{1}H NMR (300 MHz, $CDCl_3$) δ4.58 (t, J = 5.0 Hz, 1H), 4.35 (s, 2H), 4.24

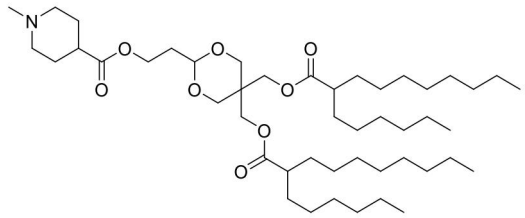
(td, J = 6.5 Hz, 1.8 Hz, 2H), 3.92 (d, J = 11.7 Hz, 2H), 3.83 (s, 2H), 3.59 (d, J = 11.7 Hz, 2H), 3.16-3.10 (m, 1H),2.97-2.92 (m, 1H), 2.40 (s, 3H), 2.37-2.27 (m, 3H), 2.20-2.10 (m, 1H), 2.02-1.86 (m, 4H), 1.84-1.72 (m, 1H), 1.63-1.40 (m, 8H), 1.25 (brs, 40H), 0.90-0.85 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 780.6, measured value m/z = 780.6.

[0212]

Lipid Synthesis Example 21 (synthesis of (2-(2-((1-methylpiperidine-4-carbonyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 186]



(2-(2-((1-methylpiperidine-4-carbonyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(2-((1-methylpiperidine-4-carbonyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) of interest was obtained in the same manner as in Lipid Synthesis Example 19, except that 1-methylpiperidine-4-carboxylic acid hydrochloride was used instead of N,N-dimethylglycine in Lipid Synthesis Example 19.

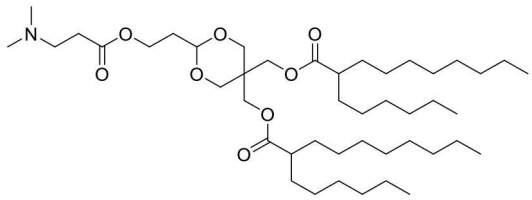
^{1}H NMR (300 MHz, $CDCl_3$) δ4.57 (t, J = 5.3 Hz, 1H), 4.35 (s, 2H), 4.18 (t, J = 6.5 Hz, 2H), 3.92 (d, J = 11.7 Hz, 2H), 3.83 (s, 2H), 3.59 (d, J = 11.7 Hz, 2H), 2.85-2.81 (m, 2H), 2.39-2.24 (m, 6H), 2.06-1.93 (m, 6H), 1.85-1.41 (m, 10H), 1.25 (brs, 40H), 0.90-0.85 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 794.7, measured value m/z = 794.7.

[0213]

Lipid Synthesis Example 22 (synthesis of (2-(2-((3-(dimethylamino)propanoyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 187]



(2-(2-((3-(dimethylamino)propanoyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(2-((3-(dimethylamino)propanoyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) of interest was obtained in the same manner as in Lipid Synthesis Example 19, except that 3-(dimethylamino)propanoic acid hydrochloride was used instead of N,N-dimethylglycine in Lipid Synthesis Example 19.

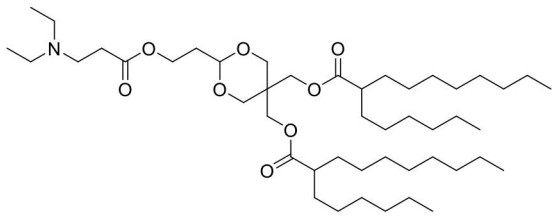
^{1}H NMR (300 MHz, $CDCl_3$) δ4.58 (t, J = 5.1 Hz, 1H), 4.35 (s, 2H), 4.19 (t, J = 6.4 Hz, 2H), 3.92 (d, J = 11.8 Hz, 2H), 3.83 (s, 2H), 3.60 (d, J = 11.8 Hz, 2H), 2.64-2.59 (m, 2H), 2.50-2.45 (m, 2H), 2.39-2.28 (m, 2H), 2.24 (s, 6H), 1.95 (q, J = 5.4 Hz, 2H), 1.66-1.40 (m, 8H), 1.25 (brs, 40H), 0.90-0.85 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 768.6, measured value m/z = 768.6.

[0214]

Lipid Synthesis Example 23 (synthesis of (2-(2-((3-(diethylamino)propanoyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 188]



(2-(2-((3-(diethylamino)propanoyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(2-((3-(diethylamino)propanoyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) of interest was obtained in the same manner as in Lipid Synthesis Example 19, except that 3-(diethylamino)propionic acid hydrochloride was used instead of N,N-dimethylglycine in Lipid Synthesis Example 19.

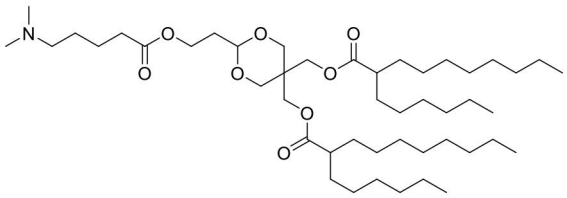
^{1}H NMR (300 MHz, $CDCl_3$) δ4.58 (t, J = 5.2 Hz, 1H), 4.35 (s, 2H), 4.18 (t, J = 6.5 Hz, 2H), 3.92 (d, J = 12.0 Hz, 2H), 3.83 (s, 2H), 3.60 (d, J = 12.0 Hz, 2H), 2.80 (t, J = 7.7 Hz, 2H), 2.52 (q, J = 7.2 Hz, 4H), 2.45 (t, J = 7.7 Hz, 2H), 2.36-2.30 (m, 2H), 1.95 (q, J = 6.6 Hz, 2H), 1.66-1.40 (m, 8H), 1.25 (brs, 40H), 1.03 (t, J = 7.2 Hz, 6H), 0.90-0.85 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 796.7, measured value m/z = 796.7.

[0215]

Lipid Synthesis Example 24 (synthesis of (2-(2-((5-(dimethylamino)pentanoyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 189]



(2-(2-((5-(dimethylamino)pentanoyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(2-((5-(dimethylamino)pentanoyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) of interest was obtained in the same manner as in Lipid Synthesis Example 19, except that 5-(dimethylamino)pentanoic acid hydrochloride was used instead of N,N-dimethylglycine in Lipid Synthesis Example 19.

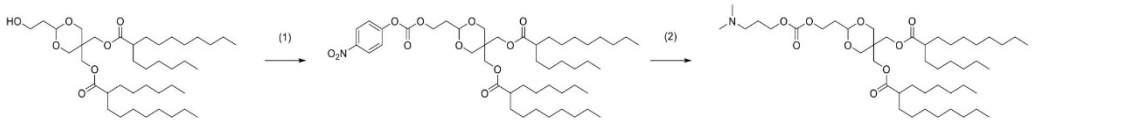
^{1}H NMR (300 MHz, $CDCl_3$) δ4.58 (t, J = 5.3 Hz, 1H), 4.35 (s, 2H), 4.17 (t, J = 6.4 Hz, 2H), 3.92 (d, J = 12.0 Hz, 2H), 3.83 (s, 2H), 3.61 (d, J = 12.0 Hz, 2H), 2.51-2.46 (m, 2H), 2.39 (s, 6H), 2.37-2.32 (m, 4H), 1.95 (q, J = 6.5 Hz, 2H), 1.66-1.40 (m, 12H), 1.25 (brs, 40H), 0.90-0.85 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 796.7, measured value m/z = 796.7.

[0216]

Lipid Synthesis Example 25 (synthesis of (2-(2-(((3-(dimethylamino)propoxy)carbonyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 190]



(1) 4-Nitrophenyl carbonochloridate (55.6 mg) and triethylamine (41.5 μl) were dissolved in DCM (2 ml). (2-(2-Hydroxyethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) (154 mg) was dissolved in DCM (1 ml), and the solution was added dropwise to the reaction liquid. The mixture was stirred at room temperature for four hours. Then, an aqueous saturated ammonium chloride solution was added to the reaction solution, 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 (2-(2-(((4-nitrophenoxy)carbonyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) (87.1 mg) was obtained.

(2) (2-(2-(((4-Nitrophenoxy)carbonyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) (41.7 mg) and triethylamine (15.3 μl) were dissolved in DCM (1 ml), and 3-(dimethylamino)propan-1-ol (18.8 μl) was added. The mixture was stirred at room temperature overnight. Then, an aqueous saturated sodium hydrogen carbonate solution was added to the reaction solution, and extraction with DCM 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; DCM/methanol], and (2-(2-(((3-(dimethylamino)propoxy)carbonyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) (16.4 mg) was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ4.63 (t, J = 5.2 Hz, 1H), 4.35 (s, 2H), 4.26-4.17 (m, 4H), 3.92 (d, J = 11.7 Hz, 2H), 3.83 (s, 2H), 3.61 (d, J = 11.7 Hz, 2H), 2.43-2.29 (m, 4H), 2.39 (s, 6H), 1.99 (q, J = 6.5 Hz, 2H), 1.87 (quin, J = 7.2 Hz, 2H), 1.65-1.40 (m, 8H), 1.25 (brs, 40H), 0.90-0.85 (m, 12H).

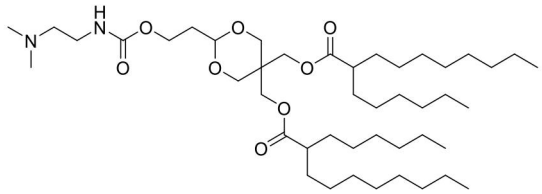
Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 798.6,

measured value m/z = 798.6.

[0217]

Lipid Synthesis Example 26 (synthesis of (2-(2-(((2-(dimethylamino)ethyl)carbamoyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 191]



(2-(2-(((2-(dimethylamino)ethyl)carbamoyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(2-(((4-Nitrophenoxy)carbonyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) (41.7 mg) and triethylamine (15.3 μl) were dissolved in DCM (1 ml), and N1,N1-dimethylethane-1,2-diamine (6.50 μl) was added. The mixture was stirred at room temperature overnight. Then, an aqueous saturated sodium hydrogen carbonate solution was added to the reaction solution, and extraction with DCM 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; DCM/methanol], and (2-(2-(((2-(dimethylamino)ethyl)carbamoyl)oxy)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) (22.6 mg) was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ5.21 (brs, 1H), 4.59 (t, J = 5.2 Hz, 1H), 4.36 (s, 2H), 4.15 (t, J = 6.2 Hz, 2H), 3.92 (d, J = 11.9 Hz, 2H), 3.83 (s, 2H), 3.60 (d, J = 11.9 Hz, 2H), 3.26 (q, J = 5.6 Hz, 2H), 2.47-2.29 (m, 4H), 2.25 (s, 6H), 1.99

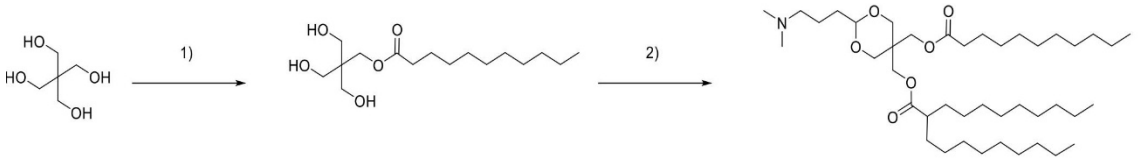
(q, J = 5.8 Hz, 2H), 1.63-1.40 (m, 8H), 1.25 (brs, 40H), 0.90-0.85 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 783.6, measured value m/z = 783.6.

[0218]

Lipid Synthesis Example 27 (synthesis of (2-(3-(dimethylamino)propyl)-5-((undecanoyloxy)methyl)-1,3-dioxan-5-yl)methyl 2-nonylundecanoate)

[Chem. 192]



(1) Pentaerythritol (1.36 g), undecanoic acid (931 mg), and DMAP (30.5 mg) were added to DMF (20 ml), and the reaction liquid was heated to 80°C to completely dissolve pentaerythritol. After decreasing the temperature to room temperature, EDCI (1.15 g) was added, and the mixture was stirred overnight. An aqueous saturated ammonium chloride solution was added to the reaction solution, and extraction with a mixed solvent of hexane:ethyl acetate (50%, 50%) 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 3-hydroxy-2,2-bis(hydroxymethyl)propyl undecanoate was obtained.

(2) To a container in which a calcium chloride tube was placed, 3-hydroxy-2,2-bis(hydroxymethyl)propyl undecanoate (183 mg), 4,4-dimethoxy-

N,N-dimethylbutan-1-amine (96.8 mg), p-toluenesulfonic acid monohydrate (1.14 mg), HCl (4.0 mol/L in dioxan, 150 μl), and DMF (2 ml) were added, and the mixture was stirred at 80°C overnight. After the reaction liquid was cooled to room temperature, 2-nonylundecanoic acid (188 mg), DMAP (7.33 mg), and EDCI (138 mg) were added, and the mixture was further stirred overnight. An aqueous sodium hydroxide solution (5.0 mol/L) was added to the reaction solution, and extraction with a mixed solvent of hexane:ethyl acetate (50%, 50%) 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; DCM/methanol], and (2-(3-(dimethylamino)propyl)-5-((undecanoyloxy)methyl)-1,3-dioxan-5-yl)methyl 2-nonylundecanoate (74.4 mg) was obtained.

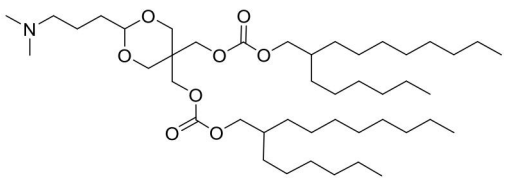
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.56 (t, J = 3.8 Hz, 1H), 4.34 (s, 2H), 3.93-3.85 (m, 4H), 3.60 (d, J = 11.8 Hz, 2H), 3.12-3.06 (m, 2H), 2.82 (s, 6H), 2.83-2.28 (m, 3H), 1.95-1.87 (m, 2H), 1.75-1.41 (m, 8H), 1.25 (brs, 42H), 0.88 (t, J = 6.9 Hz, 9H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 696.6, measured value m/z = 696.6.

[0219]

Lipid Synthesis Example 28 (synthesis of (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecyl) bis(carbonate))

[Chem. 193]



(2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecyl) bis(carbonate)

Pentaerythritol (136 mg) was added to DMF (5 ml), and the temperature was increased to 80°C to completely dissolve pentaerythritol. HCl (4.0 mol/L in dioxan, 100 µl), p-toluenesulfonic acid monohydrate (1.14 mg), and 4,4-dimethoxy-N,N-dimethylbutan-1-amine (64.5 mg) were added to the reaction solution, and the mixture was stirred at 80°C overnight. After the reaction liquid was cooled to room temperature, pyridine (484 µl), DMAP (48.9 mg), and 2-hexyldecyl (4-nitrophenyl) carbonate (1.63 g) were added, and the mixture was stirred at room temperature overnight. An aqueous sodium hydroxide solution (5.0 mol/L) was added to the reaction solution, and extraction with a mixed solvent of hexane:ethyl acetate (50%, 50%) 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; DCM/methanol], and (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecyl) bis(carbonate) (116 mg) was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.55 (t, J = 4.3 Hz, 1H), 4.42 (s, 2H), 4.02 (d, J = 5.7 Hz, 4H), 3.98-3.95 (m, 4H), 3.64 (d, J = 11.9 Hz, 2H),2.81-2.76 (m, 2H), 2.59 (s, 6H), 1.91-1.81 (m, 4H), 1.73-1.64 (m, 2H), 1.27 (brs, 48H), 0.88 (t, J = 6.8 Hz, 12H).

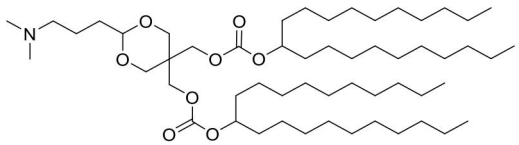
Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 770.7,

measured value m/z = 770.7.

[0220]

Lipid Synthesis Example 29 (synthesis of (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) di(henicosan-11-yl) bis(carbonate))

[Chem. 194]



(2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) di(henicosan-11-yl) bis(carbonate)

(2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) di(henicosan-11-yl) bis(carbonate) of interest was obtained in the same manner as in Lipid Synthesis Example 28, except that henicosan-11-yl (4-nitrophenyl) carbonate was used instead of 2-hexyldecyl (4-nitrophenyl) carbonate in Lipid Synthesis Example 28.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.68-4.64 (m, 2H), 4.51 (brs, 1H), 4.42 (s, 2H), 3.99-3.95 (m, 4H), 3.65 (d, J = 11.8 Hz, 2H), 2.43 (brs, 2H), 2.34 (s, 6H), 1.66-1.55 (m, 12H), 1.25 (brs, 64H), 0.88 (t, J = 6.9 Hz, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 910.8, measured value m/z = 910.8.

[0221]

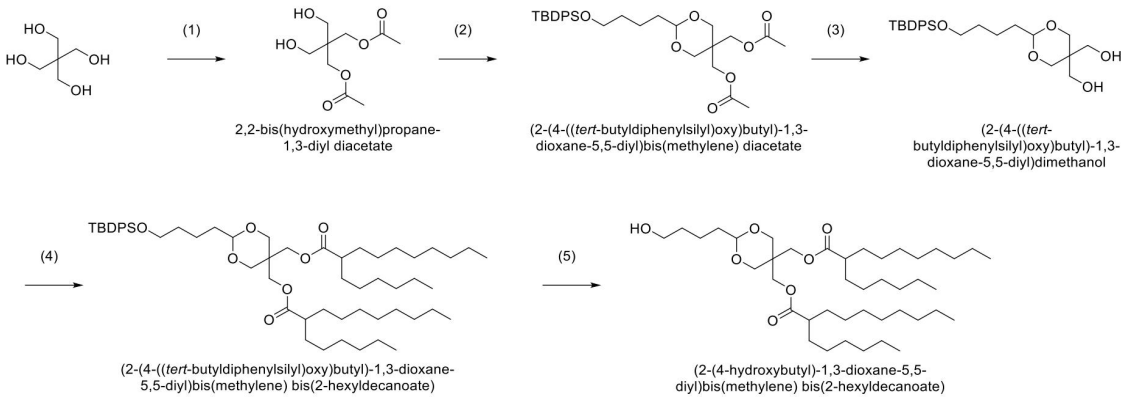
2-Heptylnonanoic acid, 2-octyldecanoic acid, and 2-undecyltridecanoic acid were synthesized in the same manner as in Raw Material Synthesis Example 1. Moreover, 4-((tert-butyldiphenylsilyl)oxy)butanal, 5-((tert-butyldiphenylsilyl)oxy)pentanal, 6-((tert-butyldiphenylsilyl)oxy)hexanal, and 7-((tert-butyldiphenylsilyl)oxy)heptanal were synthesized in the same manner as in Raw Material Synthesis Example 7. In addition, (2-(3-hydroxypropyl)-1,3-

dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate), (2-(3-hydroxypropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate), (2-(4-hydroxybutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate), (2-(5-hydroxypentyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate), and (2-(6-hydroxyhexyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate) were synthesized in the same manner as in Raw Material Synthesis Example 8.

[0222]

Raw Material Synthesis Example 9 (synthesis of (2-(4-hydroxybutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 195]



(1) Pentaerythritol (1.04 g), pyridine (16 ml), and DMAP (910 mg) were added to DMF (600 ml), and the mixture was heated in a water bath at 70°C to completely dissolve pentaerythritol. Acetic anhydride (14 ml) was added dropwise to the reaction solution, and the mixture was stirred overnight at room temperature. The solvent was evaporated under reduced pressure from the reaction solution, and a crude product was obtained. The crude product was purified by silica gel chromatography [elution solvent; DCM/methanol], and a mixture of 2,2-bis(hydroxymethyl)propane-1,3-diyl diacetate and DMF at about

1:1 was obtained.

(2) The 2,2-bis(hydroxymethyl)propane-1,3-diyl diacetate mixture (1.17 g), 5-((tert-butyldiphenylsilyl)oxy)pentanal (1.36 g), and p-toluenesulfonic acid monohydrate (38.0 mg) were added to toluene (20 ml), and the mixture was stirred at 85°C overnight. An excess amount of an aqueous saturated sodium hydrogen carbonate solution was added to the reaction solution, 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 (2-(4-((tert-butyldiphenylsilyl)oxy)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) diacetate (1.32 g) was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ7.67-7.64 (m, 4H), 7.45-7.33 (m, 6H), 4.43 (t, J = 5.1 Hz, 1H), 4.36 (s, 2H), 3.93 (d, J = 11.7 Hz, 2H), 3.83 (s, 2H), 3.67-3.58 (m, 4H), 2.07 (d, J = 1.2 Hz, 6H), 1.64-1.53 (m, 4H), 1.50-1.41 (m, 2H), 1.04 (s, 9H).

(3) (2-(4-((tert-Butyldiphenylsilyl)oxy)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) diacetate (1.32 g) and potassium carbonate (553 mg) were added to methanol (10 ml), and the mixture was stirred at room temperature for five hours. An excess amount of an aqueous saturated ammonium chloride solution was added to the reaction solution, 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 (2-(4-((tert-butyldiphenylsilyl)oxy)butyl)-1,3-dioxane-5,5-diyl)dimethanol (1.17 g) was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ7.67-7.64 (m, 4H), 7.45-7.33 (m, 6H), 4.43 (t, J = 5.1 Hz, 1H), 4.03-3.98 (m, 4H), 3.65 (t, J = 6.6 Hz, 2H), 3.54-3.48 (m, 4H), 2.12 (brs, 1H), 2.02 (brs, 1H), 1.63-1.53 (m, 4H), 1.50-1.41 (m, 2H), 1.04 (s, 9H).

(4) (2-(4-((tert-Butyldiphenylsilyl)oxy)butyl)-1,3-dioxane-5,5-diyl)dimethanol (233 mg), 2-hexyldecanoic acid (356 mg), triethylamine (169 μL), and DMAP (6.22 mg) were dissolved in DCM (2 ml), and EDCI (293 mg) was added. The mixture was stirred at room temperature overnight. An aqueous saturated ammonium chloride solution was added to the reaction solution, and extraction with DCM 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 (2-(4-((tert-butyldiphenylsilyl)oxy)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) (337 mg) was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ7.67-7.64 (m, 4H), 7.42-7.35 (m, 6H), 4.42 (t, J = 4.8 Hz, 1H), 4.35 (s, 2H), 3.91 (d, J = 11.7 Hz, 2H), 3.83 (s, 2H), 3.66-3.58 (m, 4H), 2.38-2.28 (m, 2H), 1.63-1.40 (m, 14H), 1.25 (brs, 40H), 1.04 (s, 9H), 0.98-0.85 (m, 12H).

(5) (2-(4-((tert-Butyldiphenylsilyl)oxy)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) (337 mg) was dissolved in THF (1 ml),

and TBAF (1.0 mol/L in THF, 1.5 ml) was added. The mixture was stirred at room temperature for four hours. An excess amount of an aqueous saturated sodium hydrogen carbonate solution was added to the reaction solution, 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 (2-(4-hydroxybutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) (226 mg) of interest was obtained.

[0223]

(2-(4-Hydroxybutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-heptylnonanoate),

(2-(4-hydroxybutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-octyldecanoate),

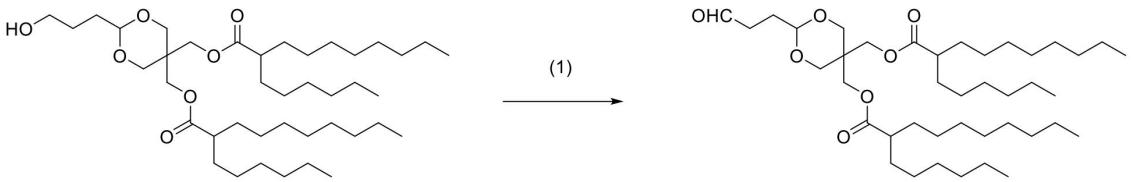
(2-(4-hydroxybutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonyldodecanoate), and

(2-(4-hydroxybutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-undecyltridecanoate) were also synthesized by the same method.

[0224]

Raw Material Synthesis Example 10 (synthesis of (2-(3-oxopropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 196]



(2-(3-hydroxypropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(3-oxopropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(3-Hydroxypropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) (3.48 g), DMSO (3.62 ml), and triethylamine (3.53 ml) were dissolved in DCM (25 ml), and the solution was stirred under ice-cooling. After 10 minutes, sulfur trioxide pyridine complex (2.44 g) was added, and the reaction solution was heated to room temperature and stirred for four hours. An aqueous saturated ammonium chloride solution was added to the reaction solution, and extraction with DCM 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 (2-(3-oxopropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) (2.83 g) of interest was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.76 (t, J = 1.2 Hz, 1H), 4.56 (t, J = 4.5 Hz, 1H), 4.34 (s, 2H), 3.91 (d, J = 11.7 Hz, 2H), 3.83 (s, 2H), 3.60 (d, J = 11.7 Hz, 2H), 2.58 (dt, J = 9.0 Hz, 1.2 Hz, 2H), 2.37-2.30 (m, 2H), 1.97 (dt, J = 7.2 Hz, 4.5 Hz, 2H), 1.74-1.39 (m, 8H), 1.25 (brs, 40H), 0.90-0.85 (m, 12H).

[0225]

(2-(3-Oxopropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate), (2-(4-oxobutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate), (2-(5-oxopentyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-

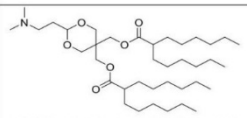
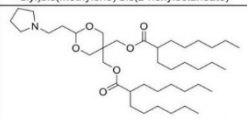
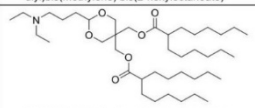
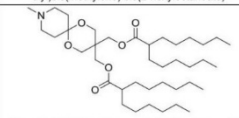
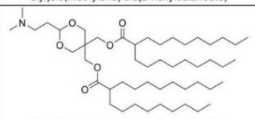
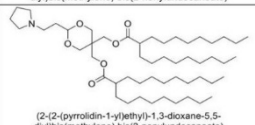
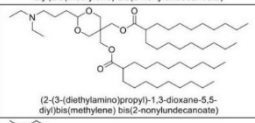
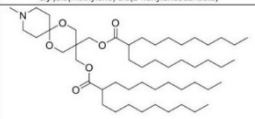
nonylundecanoate), (2-(6-oxohexyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate), (2-(4-oxobutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate), (2-(4-oxobutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-heptylnonanoate), (2-(4-oxobutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-octyldecanoate), (2-(4-oxobutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonyldodecanoate), and (2-(4-oxobutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-undecyltridecanoate) were also synthesized by the same method.

[0226]

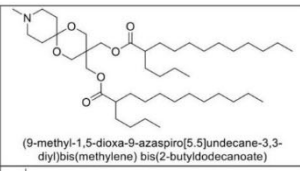
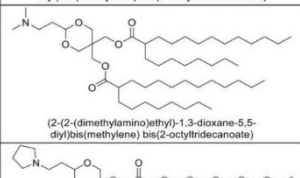
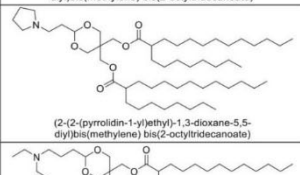
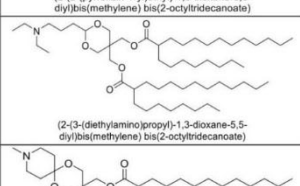
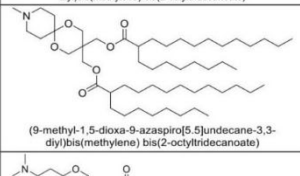
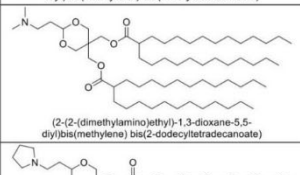
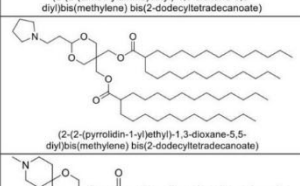
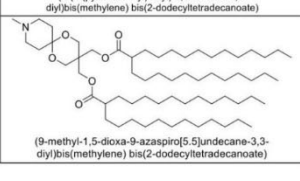
Lipid Synthesis Examples 30 to 45

The lipids in Table 1 below were synthesized by appropriately replacing 2-hexyldecanoic acid in Lipid Synthesis Example 1(1) and 4,4-dimethoxy-N,N-dimethylbutan-1-amine in Lipid Synthesis Example 1(3) with other raw materials.

[Table 1-1]

Lipid Synthesis Example	Molecular Structure	Mass Spectrometry Results (m/z)
[30]	 (2-(2-(dimethylamino)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyloctanoate)	640.6
[31]	 (2-(2-(pyrrolidin-1-yl)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyloctanoate)	666.6
[32]	 (2-(3-(diethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyloctanoate)	682.6
[33]	 (9-methyl-1,5-dioxa-9-azaspiro[5.5]undecane-3,3-diyl)bis(methylene) bis(2-hexyloctanoate)	652.6
[34]	 (2-(2-(dimethylamino)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	808.7
[35]	 (2-(2-(pyrrolidin-1-yl)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	834.8
[36]	 (2-(3-(diethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	850.8
[37]	 (9-methyl-1,5-dioxa-9-azaspiro[5.5]undecane-3,3-diyl)bis(methylene) bis(2-nonylundecanoate)	820.7

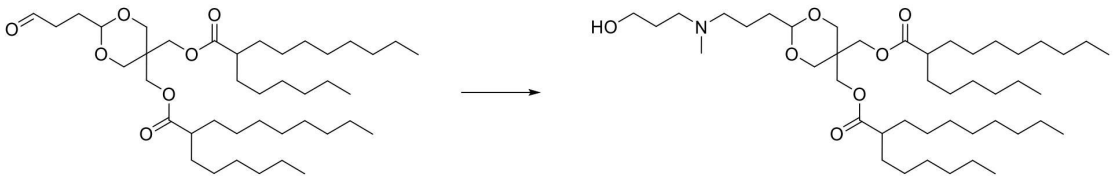
[Table 1-2]

[38]	 (9-methyl-1,5-dioxa-9-azaspiro[5.5]undecane-3,3-diyl)bis(methylene) bis(2-butyldodecanoate)	708.6
[39]	 (2-(2-(dimethylamino)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-octyltridecanoate)	836.8
[40]	 (2-(2-(pyrrolidin-1-yl)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-octyltridecanoate)	861.8
[41]	 (2-(3-(diethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-octyltridecanoate)	878.8
[42]	 (9-methyl-1,5-dioxa-9-azaspiro[5.5]undecane-3,3-diyl)bis(methylene) bis(2-octyltridecanoate)	848.8
[43]	 (2-(2-(dimethylamino)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-dodecyltetradecanoate)	976.9
[44]	 (2-(2-(pyrrolidin-1-yl)ethyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-dodecyltetradecanoate)	1002.9
[45]	 (9-methyl-1,5-dioxa-9-azaspiro[5.5]undecane-3,3-diyl)bis(methylene) bis(2-dodecyltetradecanoate)	988.9

[0227]

Lipid Synthesis Example 46 (synthesis of (2-(3-((3-hydroxypropyl)(methyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 197]



(2-(3-oxopropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(3-((3-hydroxypropyl)(methyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(3-Oxopropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) (102 mg) and 3-(methylamino)propan-1-ol (14.7 mg) were dissolved in THF (1 ml), and sodium triacetoxyborohydride (44.5 mg) was added subsequently. The mixture was stirred for four hours at room temperature. An aqueous saturated sodium hydrogen carbonate solution was added to the reaction solution, and extraction with DCM 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; DCM/methanol], and (2-(3-((3-hydroxypropyl)(methyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) (68.7 mg) of interest was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.48 (t, J = 4.2Hz, 1H), 4.35 (s, 2H), 3.91 (d, J = 11.7 Hz, 2H), 3.83 (s, 2H), 3.80 (t, J = 5.1 Hz, 2H), 3.60 (d, J = 11.7 Hz, 2H), 2.61 (t, J = 5.1 Hz, 2H), 2,42-2.30 (m, 4H), 2.26 (s, 3H), 1.72-1.43 (m, 14H), 1.25 (brs, 40H), 0.90-0.85 (m, 12H).

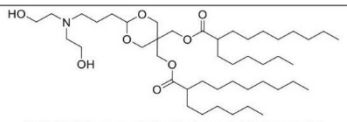
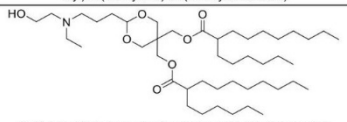
Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 754.7, measured value m/z = 754.7.

[0228]

Lipid Synthesis Examples 47 and 48

The lipids in Table 2 below were synthesized by replacing 3-(methylamino)propan-1-ol in Lipid Synthesis Example 46 with the amino alcohols in Table 2 below.

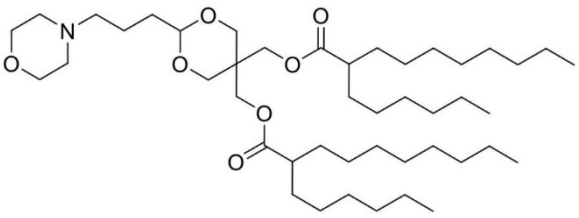
[Table 2]

Lipid Synthesis Example	Raw Material Amino Alcohol	Molecular Structure	Mass Spectrometry Results (m/z)
[47]	2,2'-Azanediylbis(ethan-1-ol)	 (2-(3-(bis(2-hydroxyethyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	770.7
[48]	2-(Ethylamino)ethan-1-ol	 (2-(3-(ethyl(2-hydroxyethyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	754.7

[0229]

Lipid Synthesis Example 49 (synthesis of (2-(3-morpholinopropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 198]



(2-(3-morpholinopropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(3-morpholinopropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) of interest was obtained in the same manner as in Lipid Synthesis Example 46, except that morpholine was used instead of 3-(methylamino)propan-1-ol in Lipid Synthesis Example 46.

^{1}H NMR (300 MHz, $CDCl_3$) δ4.48 (t, J = 4.5 Hz, 1H), 4.35 (s, 2H), 3.92 (d, J = 11.7 Hz, 2H), 3.84 (s, 2H), 3.70 (t, J = 4.5 Hz, 4H), 3.61 (d, J = 11.7 Hz, 2H), 2.42 (t, J = 4.5 Hz, 4H), 2.38-2.31 (m, 4H), 1.68-1.39 (m, 12H), 1.25 (brs, 40H), 0.90-0.85 (m, 12H).

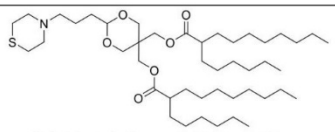
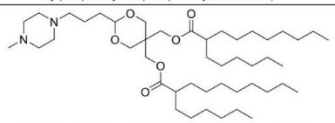
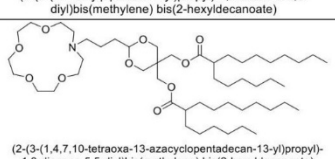
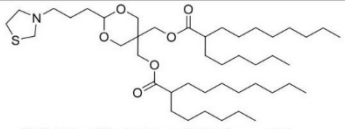
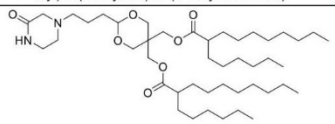
Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 752.6, measured value m/z = 752.6.

[0230]

Lipid Synthesis Examples 50 to 52

The lipids in Table 3 below were synthesized by replacing 3-(methylamino)propan-1-ol in Lipid Synthesis Example 46 with the cyclic amines in Table 3 below.

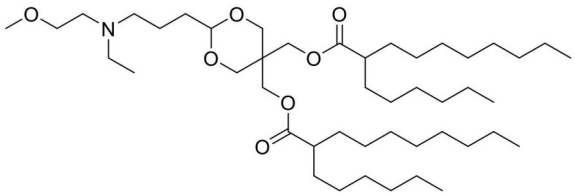
[Table 3]

Lipid Synthesis Example	Raw Material Cyclic Amine	Molecular Structure	Mass Spectrometry Results (m/z)
[50]	Thiomorpholine	 (2-(3-thiomorpholinopropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	768.6
[51]	1-Methylpiperazine	 (2-(3-(4-methylpiperazin-1-yl)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	765.7
[52]	1,4,7,10-Tetraoxa-13-azacyclopentadecane	 (2-(3-(1,4,7,10-tetraoxa-13-azacyclopentadecan-13-yl)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	884.7
[53]	Thiazolidine	 (2-(3-(thiazolidin-3-yl)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	754.6
[54]	Piperazin-2-one	 (2-(3-(3-oxopiperazin-1-yl)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	765.6

[0231]

Lipid Synthesis Example 55 (synthesis of (2-(3-(ethyl(2-methoxyethyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 199]



(2-(3-(ethyl(2-methoxyethyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(3-(ethyl(2-methoxyethyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) of interest was obtained in the same manner as in Lipid Synthesis Example 46, except that N-ethyl-2-methoxyethan-1-amine was used instead of 3-(methylamino)propan-1-ol in Lipid Synthesis Example 46.

^{1}H NMR (300 MHz, $CDCl_3$) δ4.47 (t, J = 4.5 Hz, 1H), 4.36 (s, 2H), 3.92 (d, J = 11.7 Hz, 2H), 3.83 (s, 2H), 3.59 (d, J = 11.7 Hz, 2H), 3.44 (t, J = 6.0 Hz, 2H), 3.43 (s, 3H), 2.63 (t, J = 6.0 Hz, 2H), 2.55 (q, J = 6.9 Hz, 2H), 2.47 (t, J = 7.2 Hz, 2H), 2.36-2.30 (m, 2H), 1.62-1.38 (m. 12H), 1.25 (brs, 40H), 1.00 (t, J = 6.9 Hz, 3H), 0.90-0.85 (m, 12H).

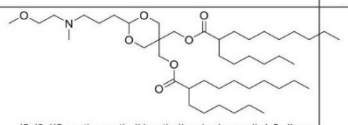
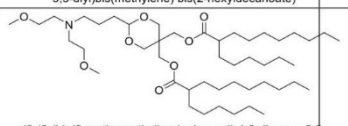
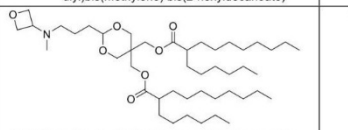
Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 768.7, measured value m/z = 768.7.

[0232]

Lipid Synthesis Examples 56 to 58

The lipids in Table 4 below were synthesized by replacing 3-(methylamino)propan-1-ol in Lipid Synthesis Example 46 with the ether amines in Table 4 below.

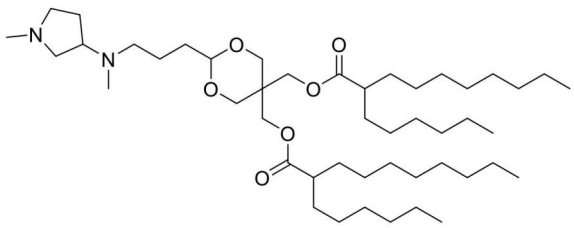
[Table 4]

Synthesis Example Number	Raw Material Ether Amine	Molecular Structure	Mass Spectrometry Results (m/z)
[56]	2-Methoxy-N-methylethan-1-amine	 (2-(3-((2-methoxyethyl)(methyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	754.7
[57]	bis(2-Methoxyethyl)amine	 (2-(3-(bis(2-methoxyethyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	798.7
[58]	N-methyloxetan-3-amine	 (2-(3-(methyl(oxetan-3-yl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	752.6

[0233]

Lipid Synthesis Example 59 (synthesis of (2-(3-(methyl(1-methylpyrrolidin-3-yl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 200]



(2-(3-(methyl(1-methylpyrrolidin-3-yl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(3-(methyl(1-methylpyrrolidin-3-yl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) of interest was obtained in the same manner as in Lipid Synthesis Example 46, except that N,1-dimethylpyrrolidin-3-amine was used instead of 3-(methylamino)propan-1-ol in Lipid Synthesis Example 46.

^{1}H NMR (300 MHz, $CDCl_3$) δ4.47 (t, J = 4.2 Hz, 1H), 4.36 (s, 2H), 3.92 (d, J = 11.7 Hz, 2H), 3.83 (s, 2H), 3.59 (d, J = 11.7 Hz, 2H), 3.08-2.98 (m, 1H), 2.79-2.74 (m, 1H), 2.68-2.52 (m, 1H), 2.48-2.27 (m, 9H), 2.18 (s, 3H), 2.02-1.90

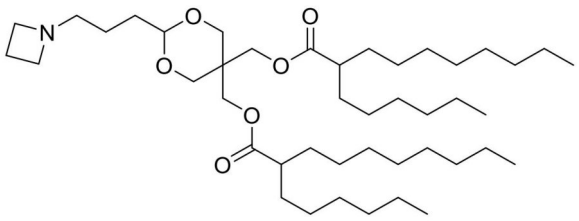
(m, 1H), 1.76-1.40 (m, 13H), 1.25 (brs, 40H), 0.90-0.85 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 779.7, measured value m/z = 779.7.

[0234]

Lipid Synthesis Example 60 (synthesis of (2-(3-(azetidin-1-yl)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 201]



(2-(3-(azetidin-1-yl)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(3-(azetidin-1-yl)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) of interest was obtained in the same manner as in Lipid Synthesis Example 46, except that azetidine was used instead of 3-(methylamino)propan-1-ol in Lipid Synthesis Example 46.

^{1}H NMR (300 MHz, $CDCl_3$) δ4.45 (t, J = 4.8 Hz, 1H), 4.35 (s, 2H), 3.91 (d, J = 11.7 Hz, 2H), 3.83 (s, 2H), 3.59 (d, J = 11.7 Hz. 2H), 3.16 (t, J = 6.9 Hz, 4H), 2.40-2.30 (m, 4H), 2.05 (quin, J = 6.9 Hz, 2H), 1.65-1.40 (m,12H), 1.25 (brs, 40H), 0.90-0.85 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 723.2, measured value m/z = 723.2.

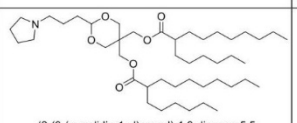
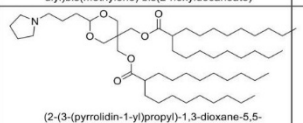
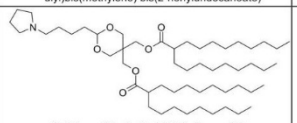
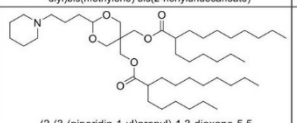
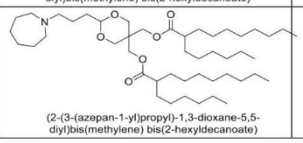
[0235]

Lipid Synthesis Examples 61 to 65

The lipids in Table 5 below were synthesized by replacing (2-(3-

oxopropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) and 3-(methylamino)propan-1-ol in Lipid Synthesis Example 46 with the aldehydes and the cyclic amines in Table 5 below.

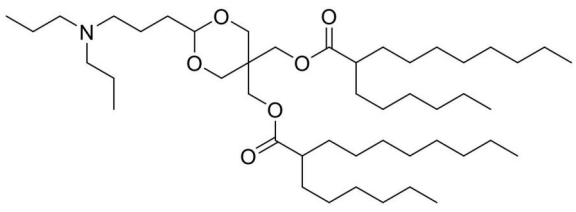
[Table 5]

Lipid Synthesis Example	Raw Material Aldehyde	Raw Material Cyclic Amine	Molecular Structure	Mass Spectrometry Results (m/z)
[61]	(2-(3-Oxopropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	Pyrrolidine	 (2-(3-(pyrrolidin-1-yl)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	736.7
[62]	(2-(3-Oxopropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	Pyrrolidine	 (2-(3-(pyrrolidin-1-yl)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	848.8
[63]	(2-(4-Oxobutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	Pyrrolidine	 (2-(4-(pyrrolidin-1-yl)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	862.8
[64]	(2-(3-Oxopropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	Piperidine	 (2-(3-(piperidin-1-yl)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	750.7
[65]	(2-(3-Oxopropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	Azepane	 (2-(3-(azepan-1-yl)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	764.7

[0236]

Lipid Synthesis Example 66 (synthesis of (2-(3-(dipropylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate))

[Chem. 202]



(2-(3-(dipropylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)

(2-(3-(dipropylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) of interest was obtained in the same manner as in Lipid

Synthesis Example 46, except that dipropylamine was used instead of 3-(methylamino)propan-1-ol in Lipid Synthesis Example 46.

^{1}H NMR (300 MHz, $CDCl_3$) δ4.47 (t, J = 4.8 Hz, 1H), 4.36 (s, 2H), 3.92 (d, J = 11.7 Hz, 2H), 3.83 (s, 2H), 3.60 (d, J = 11.7 Hz. 2H),2.43-2.30 (m, 8H), 1.64-1.39 (m, 16H), 1.25 (brs, 40H), 0.90-0.85 (m, 18H).

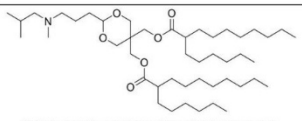
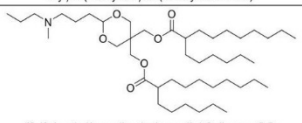
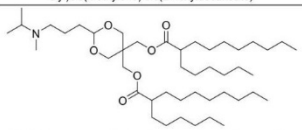
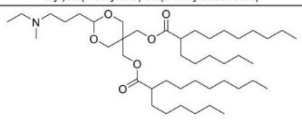
Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 766.7, measured value m/z = 766.7.

[0237]

Lipid Synthesis Examples 67 to 70

The lipids in Table 6 below were synthesized by replacing 3-(methylamino)propan-1-ol in Lipid Synthesis Example 46 with the secondary amines in Table 6 below.

[Table 6]

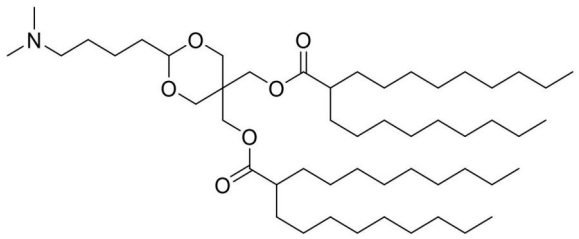
Lipid Synthesis Example	Raw Material Secondary Amine	Molecular Structure	Mass Spectrometry Results (m/z)
[67]	N,2-Dimethylpropan-1-amine	 (2-(3-(isobutyl(methyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	752.7
[68]	N-methylpropan-1-amine	 (2-(3-(methyl(propyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	738.7
[69]	N-Methylpropan-2-amine	 (2-(3-(isopropyl(methyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	738.7
[70]	N-Methylethanamine	 (2-(3-(ethyl(methyl)amino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	724.7

[0238]

Lipid Synthesis Example 71 (synthesis of (2-(4-(dimethylamino)butyl)-1,3-

dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate))

[Chem. 203]



(2-(4-(dimethylamino)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)

(2-(4-(dimethylamino)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate) of interest was obtained in the same manner as in Lipid Synthesis Example 46, except that dimethylamine 2 M solution in THF was used instead of 3-(methylamino)propan-1-ol and that (2-(4-oxobutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate) was used instead of (2-(3-oxopropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) in Lipid Synthesis Example 46.

^{1}H NMR (300 MHz, $CDCl_3$) δ4.45 (t, J = 4.8 Hz, 1H), 4.36 (s, 2H), 3.91 (d, J = 11.7 Hz, 2H), 3.83 (s, 2H), 3.60 (d, J = 11.7 Hz. 2H),2.37-2.25 (m, 10H), 1.68-1.40 (m, 14H), 1.25 (brs, 56H), 0.90-0.86 (m, 12H).

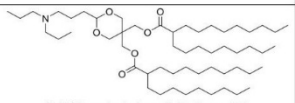
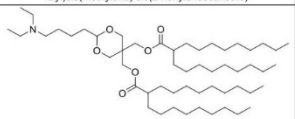
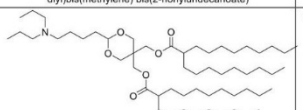
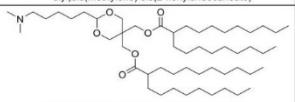
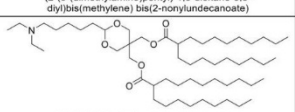
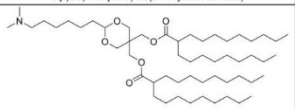
Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 836.8, measured value m/z = 836.8.

[0239]

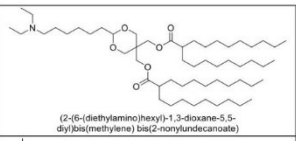
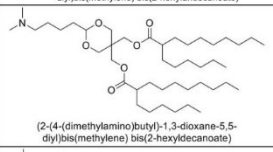
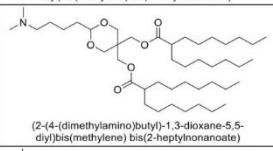
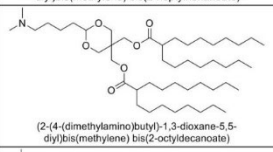
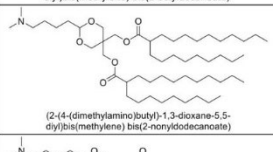
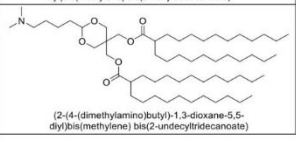
Lipid Synthesis Examples 72 to 83

The lipids in Table 7 below were synthesized by replacing (2-(3-oxopropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate) and 3-(methylamino)propan-1-ol in Lipid Synthesis Example 46 with the aldehydes and the secondary amines in Table 7 below.

[Table 7-1]

Lipid Synthesis Example	Raw Material Aldehyde	Raw Material Secondary	Molecular Structure	Mass Spectrometry Results (m/z)
[72]	(2-(3-Oxopropyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	Dipropylamine	 (2-(3-(dipropylamino)propyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	878.8
[73]	(2-(4-Oxobutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	Diethylamine	 (2-(4-(diethylamino)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	864.8
[74]	(2-(4-Oxobutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	Dipropylamine	 (2-(4-(dipropylamino)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	892.8
[75]	(2-(5-Oxopentyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	Dimethylamine THF solution	 (2-(5-(dimethylamino)pentyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	850.8
[76]	(2-(5-Oxopentyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	Diethylamine	 (2-(5-(diethylamino)pentyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	878.8
[77]	(2-(6-Oxohexyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	Dimethylamine THF solution	 (2-(6-(dimethylamino)hexyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	864.8

[Table 7-2]

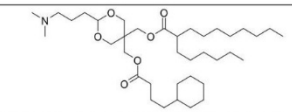
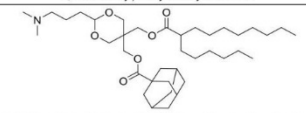
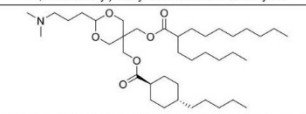
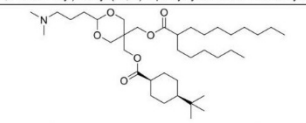
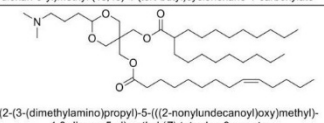
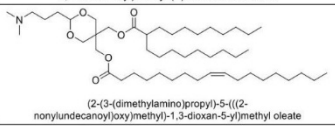
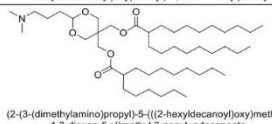
[78]	(2-(6-Oxohexyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	Diethylamine	 (2-(6-(diethylamino)hexyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonylundecanoate)	892.8
[79]	(2-(4-Oxobutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	Dimethylamine THF solution	 (2-(4-(dimethylamino)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-hexyldecanoate)	724.7
[80]	(2-(4-Oxobutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-heptylnonanoate)	Dimethylamine THF solution	 (2-(4-(dimethylamino)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-heptylnonanoate)	724.7
[81]	(2-(4-Oxobutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-octyldecanoate)	Dimethylamine THF solution	 (2-(4-(dimethylamino)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-octyldecanoate)	780.7
[82]	(2-(4-Oxobutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonyldodecanoate)	Dimethylamine THF solution	 (2-(4-(dimethylamino)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-nonyldodecanoate)	864.8
[83]	(2-(4-Oxobutyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-undecyltridecanoate)	Dimethylamine THF solution	 (2-(4-(dimethylamino)butyl)-1,3-dioxane-5,5-diyl)bis(methylene) bis(2-undecyltridecanoate)	948.9

[0240]

Lipid Synthesis Examples 84 to 90

The lipids in Table 8 below were synthesized by replacing undecanoic acid in Lipid Synthesis Example 27-(1) and 2-nonylundecanoic acid in (2) with the carboxylic acids in Table 8 below.

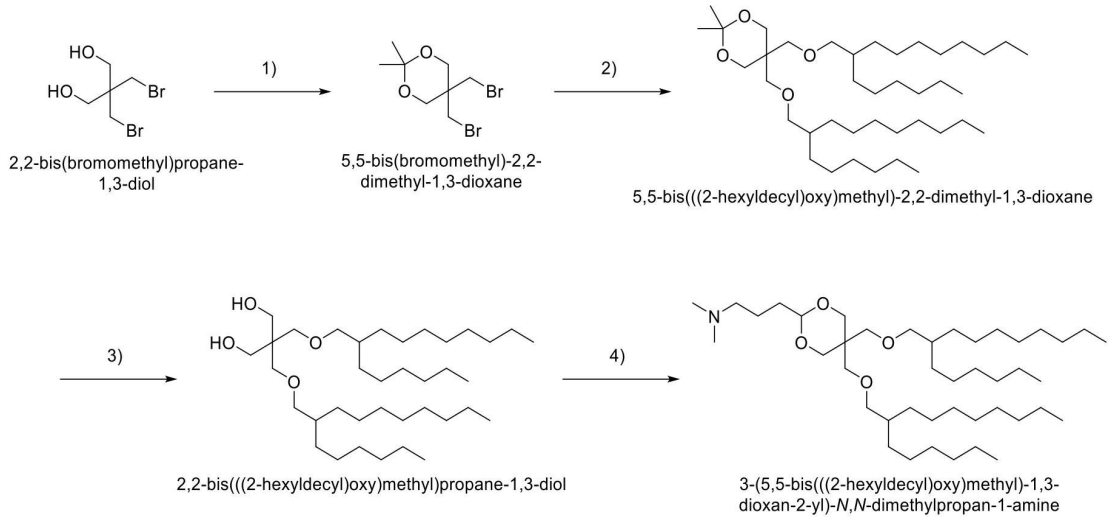
[Table 8]

Lipid Synthesis Example	Raw Material Carboxylic Acid 1	Raw Material Carboxylic Acid 2	Molecular Structure	Mass Spectrometry Results (m/z)
[84]	2-Hexyldecanoic acid	4-Cyclohexylbutanoic acid	 (5-(((4-cyclohexylbutanoyl)oxy)methyl)-2-(3-(dimethylamino)propyl)-1,3-dioxan-5-yl)methyl 2-hexyldecanoate	638.5
[85]	2-Hexyldecanoic acid	Adamantane-1-carboxylic acid	 (2-(3-(dimethylamino)propyl)-5-(((2-hexyldecanoyl)oxy)methyl)-1,3-dioxan-5-yl)methyl adamantane-1-carboxylate	634.5
[86]	2-Hexyldecanoic acid	(1s,4r)-4-Pentylcyclohexane-1-carboxylic acid	 (2-(3-(dimethylamino)propyl)-5-(((2-hexyldecanoyl)oxy)methyl)-1,3-dioxan-5-yl)methyl (1s,4r)-4-pentylcyclohexane-1-carboxylate	652.6
[87]	2-Hexyldecanoic acid	(1s,4s)-4-(tert-Butyl)cyclohexane-1-carboxylic	 (2-(3-(dimethylamino)propyl)-5-(((2-hexyldecanoyl)oxy)methyl)-1,3-dioxan-5-yl)methyl (1s,4s)-4-(*tert*-butyl)cyclohexane-1-carboxylate	638.5
[88]	2-Nonylundecanoic acid	Myristoleic acid	 (2-(3-(dimethylamino)propyl)-5-(((2-nonylundecanoyl)oxy)methyl)-1,3-dioxan-5-yl)methyl (Z)-tetradec-9-enoate	736.6
[89]	2-Nonylundecanoic acid	Oleic acid	 (2-(3-(dimethylamino)propyl)-5-(((2-nonylundecanoyl)oxy)methyl)-1,3-dioxan-5-yl)methyl oleate	792.7
[90]	2-Nonylundecanoic acid	2-Hexyldecanoic acid	 (2-(3-(dimethylamino)propyl)-5-(((2-hexyldecanoyl)oxy)methyl)-1,3-dioxan-5-yl)methyl 2-nonylundecanoate	766.7

[0241]

Lipid Synthesis Example 91 (synthesis of 3-(5,5-bis(((2-hexyldecyl)oxy)methyl)-1,3-dioxan-2-yl)-N,N-dimethylpropan-1-amine)

[Chem. 204]



(1) 2,2-Bis(bromomethyl)propane-1,3-diol (3.93 mg), 2,2-dimethoxypropane (1.84 ml), and p-toluenesulfonic acid monohydrate (28.5 mg) were dissolved in acetone (15 ml), and the solution was stirred overnight at room temperature. An aqueous saturated sodium hydrogen carbonate solution was added to the reaction solution, and acetone was evaporated under reduced pressure. The residue was subjected to extraction with ethyl acetate 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 5,5-bis(bromomethyl)-2,2-dimethyl-1,3-dioxane (3.51 g) was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.80 (s, 4H), 3.58 (s, 4H), 1.42 (s, 6H).

(2) 5,5-Bis(bromomethyl)-2,2-dimethyl-1,3-dioxane (227 mg), 2-hexyldecan-1-ol (476 µl), and NaH (60%, dispersion in paraffin liquid) (66 mg) were suspended in DMF (2 ml), and the suspension was stirred at 100°C for four

days. An aqueous saturated ammonium chloride solution was added to the reaction solution, 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. The residue was purified by silica gel chromatography [elution solvent; hexane/ethyl acetate], and a crude product containing 5,5-bis(((2-hexyldecyl)oxy)methyl)-2,2-dimethyl-1,3-dioxane was obtained.

(3) Methanol (8 ml), DCM (2.0 ml), and HCl (4 mol/L in 1,4-dioxane, 1.0 ml) were added to the crude product containing 5,5-bis(((2-hexyldecyl)oxy)methyl)-2,2-dimethyl-1,3-dioxane, and the mixture was stirred at room temperature for four hours. An excess amount of sodium hydrogen carbonate was added to the reaction solution, and the mixture was concentrated under reduced pressure. Water and ethyl acetate were added to the residue, 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 2,2-bis(((2-hexyldecyl)oxy)methyl)propane-1,3-diol (76.1 mg) was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.63 (d, J = 6.1 Hz, 4H), 3.49 (s, 4H), 3.30 (d, J = 5.6 Hz, 4H), 2.78 (t, J = 6.1 Hz, 2H), 1.58-1.51(m, 2H), 1.26 (brs, 48H), 0.90-0.86 (m, 12H).

(4) To a container in which a calcium chloride tube was placed, 2,2-bis(((2-hexyldecyl)oxy)methyl)propane-1,3-diol (76.1 mg), 4,4-dimethoxy-N,N-

dimethylbutan-1-amine (25.9 μL), p-toluenesulfonic acid monohydrate (29.7 mg), triethyl orthoformate (26.0 μL), and toluene (1.0 ml) were added, and the mixture was stirred at room temperature overnight. An excess amount of an aqueous saturated sodium hydrogen carbonate solution was added to the reaction solution, 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; DCM/methanol], and 3-(5,5-bis(((2-hexyldecyl)oxy)methyl)-1,3-dioxan-2-yl)-N,N-dimethylpropan-1-amine (62.6 mg) of interest was obtained.

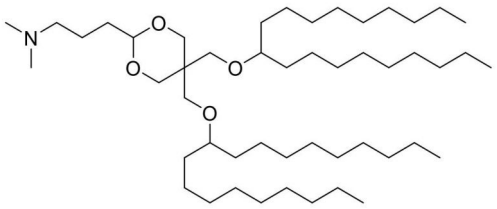
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.45 (t, J = 4.2 Hz, 1H), 3.90 (d, J = 11.6 Hz, 2H), 3.61 (d, J = 11.6Hz, 2H), 3.57 (s, 2H), 3.29 (d, J = 5.9 Hz, 2H), 3.17 (d, J = 5.73 Hz, 2H), 3.12 (s, 2H), 2.28 (t, J = 7.0 Hz, 2H), 2.22 (s, 6H), 1.68-1.51 (m, 6H), 1.26 (brs, 48H), 0.90-0.86 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 682.7, measured value m/z = 682.7.

[0242]

Lipid Synthesis Example 92 (synthesis of 3-(5,5-bis((nonadecan-10-yloxy)methyl)-1,3-dioxan-2-yl)-N,N-dimethylpropan-1-amine)

[Chem. 205]



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 86, except that nonadecan-10-ol was used instead of 2-hexyldecan-1-ol of Lipid Synthesis Example 91-(2).

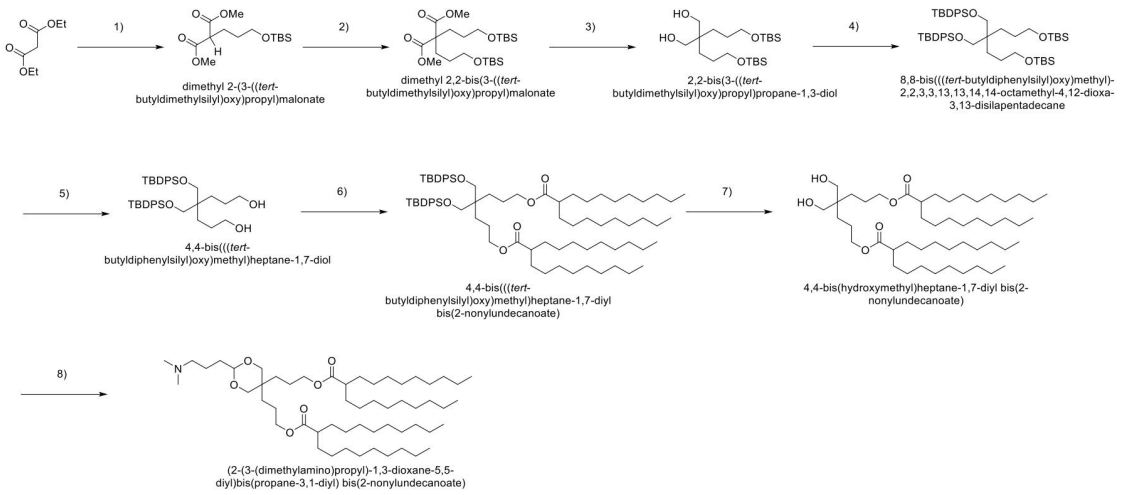
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.47 (t, J = 4.7 Hz, 1H), 3.90 (d, J = 11.5 Hz, 2H), 3.64-3.59 (m, 4H), 3.25-3.21 (m, 1H), 3.14 (s, 2H), 3.10-3.07 (m, 1H), 2.29 (t, J = :6.6 Hz, 2H), 2.23 (s, 6H), 1.61-1.33 (m, 12H), 1.26 (brs, 56H), 0.89-0.87 (m, 12H).

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]



(1) Dimethylmalonate (1.32 g), potassium carbonate (2.76 g), TBAB (322 mg), and (3-bromopropoxy)(tert-butyl)dimethylsilane (6.33 g) were added to toluene (30 ml), and the mixture was stirred at 85°C overnight. An aqueous saturated ammonium chloride solution was added to the reaction solution, 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 dimethyl 2-(3-((tert-butyldimethylsilyl)oxy)propyl)malonate (2.55 g) was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.74 (s, 6H), 3.63 (t, J = 6.2 Hz, 2H), 3.42 (t, J = 7.6 Hz, 1H), 2.00-1.93 (m, 2H), 1.60-1.49 (m, 2H), 0.89 (s, 9H), 0.04 (s, 6H).

(2) NaH (60%, dispersion in paraffin liquid) (600 mg) was suspended in THF (20 ml), and the suspension was stirred under ice-cooling for five minutes. Dimethyl 2-(3-((tert-butyldimethylsilyl)oxy)propyl)malonate (3.05 g) was added

to the reaction liquid, and the mixture was further stirred for five minutes. (3-Bromopropoxy)(tert-butyl)dimethylsilane (3.09 g) was added, and the mixture was stirred at room temperature overnight and then further stirred at 80°C for one day. An aqueous saturated ammonium chloride solution was added to the reaction solution, 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 dimethyl 2,2-bis(3-((tert-butyldimethylsilyl)oxy)propyl)malonate (939 mg) was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.71 (s, 6H), 3.59 (t, J = 6.3 Hz, 4H), 1.96-1.90 (m, 4H), 1.42-1.35 (m, 4H), 0.88 (s, 18H), 0.03 (s, 12H).

(3) Dimethyl 2,2-bis(3-((tert-butyldimethylsilyl)oxy)propyl)malonate (1.91 g) was dissolved in THF (10 mL), and the solution was stirred under ice-cooling for 10 minutes. A sodium bis(2-methoxyethoxy)aluminum hydride solution (≥60 wt. % in toluene) (4.0 ml) was added to the reaction solution, and the mixture was further stirred at room temperature overnight. An aqueous sodium hydroxide solution was added dropwise to the reaction solution under ice-cooling, and the reaction was quenched. 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 2,2-bis(3-((tert-

butyldimethylsilyl)oxy)propyl)propane-1,3-diol (607 mg) was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.60 (t, J = 6.1Hz, 4H), 3.56 (d, J = 5.8 Hz, 4H), 2.61 (t, J = 5.8 Hz, 2H), 1.52- 1.41 (m, 4H), 1.34-1.26 (m, 4H), 0.89 (s, 18H), 0.05 (s, 12H).

(4) 2,2-Bis(3-((tert-butyldimethylsilyl)oxy)propyl)propane-1,3-diol (512 mg) and imidazole (293 mg) were dissolved in DCM (4 ml), and TBDPSCl (835 mg) was added under ice-cooling. The mixture was further stirred at room temperature for three days. An aqueous ammonium chloride solution was added to the reaction solution, and extraction with DCM 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 8,8-bis(((tert-butyldiphenylsilyl)oxy)methyl)-2,2,3,3,13,13,14,14-octamethyl-4,12-dioxa-3,13-disilapentadecane (660 mg) was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64-7.62 (m, 8H), 7.42-7.28 (m, 12H), 3.51-3.46 (m, 8H), 1.28 (brs, 8H), 1.04 (s, 18H), 0.87 (s, 18H), 0.01 (s, 12H).

(5) 8,8-Bis(((tert-butyldiphenylsilyl)oxy)methyl)-2,2,3,3,13,13,14,14-octamethyl-4,12-dioxa-3,13-disilapentadecane (539 mg) was dissolved in THF (12 ml), and ultrapure water (1.92 ml) and 5 M hydrogen chloride in 1,4-dioxane (480 µl) were added. The mixture was stirred for 30 minutes. An aqueous sodium hydrogen carbonate solution was added to the reaction solution, 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 4,4-bis(((tert-butyldiphenylsilyl)oxy)methyl)heptane-1,7-diol (338 mg) was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66-7.62 (m, 8H), 7.44-7.31 (m ,12H), 3.52 (s, 4H), 3.46-3.41 (m, 4H), 1.28-1.18 (m, 8H), 1.06 (s, 18H).

(6) 4,4-Bis(((tert-butyldiphenylsilyl)oxy)methyl)heptane-1,7-diol (134 mg), 2-nonylundecanoic acid (138 mg), and DMAP (2.44 mg) were dissolved in DCM (1 ml), and EDCI (99.7 mg) was added. The mixture was stirred at room temperature overnight. An aqueous ammonium chloride solution was added to the reaction solution, and extraction with DCM 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 4,4-bis(((tert-butyldiphenylsilyl)oxy)methyl)heptane-1,7-diyl bis(2-nonylundecanoate) (229 mg) was obtained.

(7) THF (2 ml), acetic acid (343 µl), and TBAF (1.0 mol/L in THF, 4 ml) were added to 4,4-bis(((tert-butyldiphenylsilyl)oxy)methyl)heptane-1,7-diyl bis(2-nonylundecanoate) (229 mg), and the mixture was stirred at room temperature for two hours. An aqueous sodium hydrogen carbonate solution was added to the reaction solution, 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 4,4-bis(hydroxymethyl)heptane-1,7-diyl bis(2-nonylundecanoate) (51.3 mg) was obtained.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.06 (t, J = 6.4 Hz, 4H), 3.58 (s, 4H), 2.35-2.26 (m, 2H), 1.62-1.30 (m, 16H), 1.25 (s, 56H), 0.90-0.86 (m, 12H).

(8) 4,4-Dimethoxy-N,N-dimethylbutan-1-amine (10.2 μl), p-toluenesulfonic acid monohydrate (10.7 mg), and toluene (10 ml) were added to 4,4-bis(hydroxymethyl)heptane-1,7-diyl bis(2-nonylundecanoate) (34.1 mg), and the mixture was stirred at 85°C overnight. An excess amount of an aqueous saturated sodium hydrogen carbonate solution was added to the reaction solution, 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; DCM/methanol], and (2-(3-(dimethylamino)propyl)-1,3-dioxane-5,5-diyl)bis(propane-3,1-diyl) bis(2-nonylundecanoate) of interest was obtained.

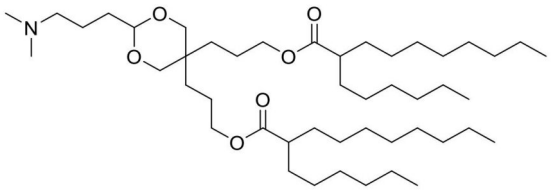
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.44 (t, J = 3.8 Hz, 1H), 4.09 (t, J = 6.5 Hz, 2H), 4.02 (t, J = 6.3 Hz, 2H), 3.75 (d, J = 11.4 Hz, 2H), 3.37 (t, J = 11.4 Hz, 2H), 2.34-2.27 (m, 10H), 1.75-1.40 (m, 16H), 1.25 (brs, 58H), 1.10-1.04 (m, 2H), 0.90-0.85 (m, 12H).

Mass spectrometry (ESI) calculated value $[M+H]^+$ m/z = 878.8, measured value m/z = 878.8.

[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 88, 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

(pH 3.5) was produced.

HEPES Buffer

Ultrapure water was added to 90.0 g of sucrose (FUJIFILM Wako Pure Chemical Corporation, product code: 196-00015) and 20 ml of HEPES buffer (1 mol/L, NACALAI TESQUE, INC., product code: 17557-94), and thus 1 L of a HEPES buffer (pH 7.45) was produced.

TE Buffer

TE buffer (20X), RNase-free (Invitrogen (trademark), product code: T11493) was diluted 20-fold with DEPC-Treated Water (NIPPON GENE CO., LTD., product code: 314-90205), and thus a TE buffer (pH 7.5) was adjusted.

PBS(-)

Dulbecco's phosphate buffered saline (NACALAI TESQUE, INC., product code: 14249-24) was used.

[0246]

<Preparation of Lipid Nanoparticles>

Lipid nanoparticles were prepared by an alcohol dilution method using a microfluidic device. As the microfluidic device, the NanoAssemblr (registered trademark) Benchtop (PRECISION NANOSYSTEMS) was used. First, a pH-sensitive cationic lipid, DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), cholesterol, and PEG-DMG 2000 (NOF CORPORATION, SUNBRIGHT GM-020) were mixed at a mole ratio of 50:10:38.5:1.5, and an ethanol solution having a total lipid concentration of 8 mM was adjusted. The nucleic acid solution was adjusted at 44.1 μg/ml in the case using CleanCap FLuc mRNA (TriLink BioTechnologies, product number L-7202-5) and at 128 μg/ml in the case using siRNA, by diluting with the citrate buffer. Using the microfluidic

device, 0.650 ml of the adjusted lipid solution and 1.95 ml of the nucleic acid solution were mixed in such a manner that the total flow rate became 12 ml/min, and 2.20 ml of the mixture solution was collected in a tube containing 2.00 ml of the HEPES buffer. The collected lipid nanoparticle solution was applied to a dialysis membrane having a molecular weight cut-off of 12-14 kD (REPLIGEN, Spectra/Por (registered trademark) 4), and dialysis was conducted using the HEPES buffer as the dialysate at 5°C overnight. The lipid nanoparticle solution after the dialysis was concentrated using an ultrafiltration unit (Amicon (registered trademark) Ultra-15), and the lipid nanoparticle solution was collected. The collected lipid nanoparticle solution was diluted with the HEPES buffer in such a manner that the nucleic acid concentration became 0.01 mg/ml and stored at -80°C until use.

[0247]

<Evaluation of Encapsulation Rate of Lipid Nanoparticles>

The nucleic acid encapsulation rate in the lipid nanoparticles was measured with Ribogreen reagent (Quant-it (trademark), product number: R114491). The lipid nanoparticle solution was diluted with the TE buffer in such a manner that the nucleic acid concentration became about 8 μg/ml, and thus a measurement solution A was adjusted. Moreover, the lipid nanoparticle solution was diluted with the TE buffer in such a manner that the nucleic acid concentration became about 1.2 μg/ml and that X-triton 100 (Sigma-Aldrich, T8787-50ML) became 1 %(w/w), and thus a measurement solution B was adjusted. On a 96-well microplate, 100 μl of the measurement solution and 100 μl of Ribogreen reagent were mixed and incubated at room temperature for five minutes, and the fluorescence intensity at an excitation wavelength of 485

nm and a measurement wavelength of 528 nm was measured. The nucleic acid concentration was calculated using a calibration curve created in the nucleic acid concentration range of 0 to 2.5 μg/ml. The encapsulation rate of the lipid nanoparticles was calculated by the calculation equation below.

Encapsulation rate % = (nucleic acid concentration (μg/ml) of measurement solution B - nucleic acid concentration (μg/ml) of measurement solution A) ÷ nucleic acid concentration (μg/ml) of measurement solution B × 100

[0248]

<Measurement of Average Particle Size of Lipid Nanoparticles>

The lipid nanoparticle solution was diluted 25-fold with PBS(-), and thus a measurement solution was adjusted. Using an analysis apparatus "Zetasizer Nano ZSP" (Malvern), the average particle size (z) and the polydispersity index (PDI) of the nanoparticles in the measurement solution were measured.

[0249]

<mRNA Introduction into *in vitro* Cultured Cells>

HepG2 cells or CHO-K1 cells were seeded in a 96-well plate (Corning, #3904) at 5,000 cells/well 24 hours before the transfection. The culture media used were culture media obtained by adjusting DMEM, low glucose, pyruvate (Gibco, #11885-084) and DMEM/F-12 (Gibco, 11320-033), respectively, at a final concentration of 10% FBS and a final concentration of 1% penicillin/streptomycin. The cells were transfected with LNPs encapsulating FLuc mRNA (TriLink Bio Technologies, L-7202-5) at 1 ng/well and incubated at 37°C in an environment of 5% CO2. The luciferase expression was quantified with ONE-Glo (trademark) + Tox Luciferase Reporter and Cell Viability Assay

(Promega, E7120) 24 hours after the transfection, and the drug introduction index was determined. 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 relative count of viable cells was estimated. Subsequently, the substrate for ONE-Glo (trademark) Luciferase Assay was added, and the luminescence intensity was measured after three minutes. The luminescence intensity was corrected with the viable cell count, and the drug introduction index was determined. The drug introduction index was the value obtained by multiplying the luminescence intensity corrected with the viable cell count by 10^5. The drug introduction indices are shown in Table 9. A higher drug introduction index indicates that a higher amount of mRNA as the drug was introduced into the cells.

[0250]

The nucleic acid encapsulation rates, the average particle sizes, the polydispersity indices (PDIs), and the *in vitro* drug introduction indices of the lipid nanoparticles using the lipids of pH-Sensitive Cationic Lipid Synthesis Examples 1 to 29 are summarized in Table 9-1 below. Moreover, the nucleic acid encapsulation rates, the average particle sizes, and the polydispersity indices (PDIs) of the lipid nanoparticles using the lipids of pH-Sensitive Cationic Lipid Synthesis Examples 30 to 89 are summarized in Table 9-2 and Table 9-3 below. The "HEPES buffer" in Table 9-1 is an experiment in which the HEPES buffer containing no lipid nanoparticles was used instead of the lipid nanoparticle-containing solution suspended in the HEPES buffer of each Synthesis Example. It was found that the lipid nanoparticles using the lipids of pH-Sensitive Cationic

Lipid Synthesis Examples 1 to 29 had high mRNA encapsulation rates, and an increase in the drug introduction indices was also observed. It is obvious that the lipid nanoparticles using the lipids of pH-Sensitive Cationic Lipid Synthesis Examples 1 to 29 could introduce the encapsulated mRNA into the cells. It was found that the lipid nanoparticles using the lipids of pH-Sensitive Cationic Lipid Synthesis Examples 30 to 89 had high mRNA encapsulation rates.

[0251]

[Table 9-1]

pH-Sensitive Cationic Lipid Synthesis Example	Nucleic Acid Encapsulation Rate (%)	Average Particle Size (nm)	Polydispersity Index	Drug Introduction Index	
				HepG2 Cells	CHO-KI Cells
[1]	99.53	95.18	0.060	268.807	227.059
[2]	98.92	143.10	0.128	5.166	2.535
[3]	100.00	122.50	0.100	45.634	14.145
[4]	99.84	80.44	0.064	172.742	80.346
[5]	99.37	84.13	0.102	52.496	57.309
[6]	99.75	77.19	0.080	69.429	31.639
[7]	99.89	80.45	0.049	177.030	51.397
[8]	99.99	89.57	0.066	34.395	12.181
[9]	99.68	101.60	0.229	428.155	60.926
[10]	100.00	84.81	0.023	442.501	154.324
[11]	100.00	85.18	0.093	260.508	268.419
[12]	100.00	108.60	0.098	411.766	222.161
[13]	99.93	77.69	0.088	85.658	47.090
[14]	99.88	75.83	0.089	18.042	11.273
[15]	99.82	87.66	0.076	151.393	81.440
[16]	99.94	75.77	0.059	57.510	34.329
[17]	99.97	74.05	0.079	44.172	77.425
[18]	99.77	73.98	0.077	13.751	6.999
[19]	97.62	69.12	0.115	3.948	2.367
[20]	97.86	68.36	0.097	1.260	1.476
[21]	99.77	82.88	0.067	163.285	78.676
[22]	99.71	73.16	0.109	22.875	9.624
[23]	99.06	74.10	0.115	2.610	1.339
[24]	100.00	86.56	0.089	258.440	185.008
[25]	99.92	86.68	0.087	71.899	48.106
[26]	100.00	84.99	0.068	331.264	177.904
[27]	99.98	73.12	0.092	349.985	245.928
[28]	100.00	80.02	0.099	346.436	231.826
[29]	99.96	78.52	0.041	131.171	35.029
HEPES buffer	-	-	-	1.107	0.669

[0252]

[Table 9-2]

pH-Sensitive Cationic Lipid Synthesis Example	Nucleic Acid Encapsulation Rate (%)	Average Particle Size (nm)	pH-Sensitive Cationic Lipid Synthesis Example
[30]	99.34	129.40	0.09
[31]	99.35	114.50	0.10
[32]	99.20	129.00	0.09
[33]	99.32	94.53	0.12
[34]	99.11	80.00	0.07
[35]	98.92	67.82	0.07
[36]	99.24	77.25	0.05
[37]	98.44	77.59	0.09
[38]	98.69	71.84	0.11
[39]	98.98	76.69	0.05
[40]	99.10	73.36	0.07
[41]	99.26	73.83	0.08
[42]	98.52	79.99	0.11
[43]	98.45	99.75	0.14
[44]	98.56	97.79	0.16
[45]	95.81	110.50	0.11
[46]	99.73	95.71	0.08
[47]	99.54	90.66	0.05
[48]	99.70	97.22	0.07
[49]	96.35	67.83	0.10
[50]	92.78	65.15	0.19
[51]	99.28	91.46	0.04
[52]	93.34	130.80	0.05
[53]	57.85	46.96	0.21
[54]	95.11	65.71	0.15
[55]	97.97	100.00	0.07
[56]	98.85	93.44	0.11
[57]	98.75	87.95	0.07
[58]	94.39	62.01	0.12
[59]	100.00	98.19	0.11
[60]	99.66	86.84	0.06
[61]	99.48	84.07	0.09

[Table 9-3]

[62]	99.53	82.64	0.04
[63]	99.46	76.98	0.06
[64]	98.76	82.10	0.08
[65]	98.77	65.93	0.27
[66]	98.84	86.48	0.08
[67]	98.01	83.13	0.09
[68]	98.85	89.30	0.06
[69]	99.25	92.55	0.07
[70]	99.42	89.97	0.07
[71]	99.55	79.32	0.04
[72]	98.45	65.25	0.14
[73]	99.36	77.25	0.08
[74]	98.40	72.50	0.07
[75]	99.59	78.19	0.06
[76]	99.41	74.10	0.06
[77]	99.54	76.48	0.07
[78]	99.13	72.52	0.07
[79]	99.63	81.87	0.05
[80]	99.58	85.33	0.05
[81]	99.61	78.03	0.02
[82]	99.63	79.60	0.02
[83]	99.64	81.73	0.03
[84]	99.77	112.80	0.10
[85]	99.08	151.20	0.12
[86]	99.70	86.67	0.11
[87]	99.07	129.30	0.11
[88]	99.51	82.42	0.06
[89]	99.49	81.88	0.09
[90]	99.46	77.89	0.06
[91]	99.90	74.67	0.05
[92]	99.64	78.35	0.10
[93]	99.66	94.27	0.03
[94]	99.71	101.10	0.06

[0253]

<siRNA Introduction into *in vitro* Cultured Cells>

HepG2 cells were seeded in a 96-well plate (Corning, #3904) at 2,500 cells/well 24 hours before the transfection. The culture medium used was a culture medium obtained by adjusting DMEM, low glucose, pyruvate (Gibco, #11885-084) at a final concentration of 10% FBS and a final concentration of 1% penicillin/streptomycin. LNPs encapsulating siRNA for human PLK1 (polo like kinase) or siRNA for luciferase were added in such a manner that the final

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 lipid containing siPLK1 and the lipid nanoparticle composition 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.

Comparative Example 1 was conducted in the same manner, except that the cells were transfected using siPLK1 according to the protocol of Lipofectamine (registered trademark) RNAiMAX Transfection Reagent (Thermo Fisher Scientific, product number 13778-150) instead of adding the lipid nanoparticles using the pH-sensitive cationic lipids. Moreover, Comparative Example 2 was conducted in the same manner, except that siRNA was added in such a manner that the final concentration in the culture solution became 1 nM without using lipid nanoparticles, regarding the lipid nanoparticles using the pH-sensitive cationic lipids.

[0254]

The nucleic acid encapsulation rates, the average particle sizes, the polydispersity indices (PDIs), and the viable cell indices of the lipid nanoparticles using the lipids of pH-Sensitive Cationic Lipid Synthesis Examples 1, 4, 10, 14, and 15 are shown in Table 10 below. Moreover, the sequences of the encapsulated siRNA are shown in Table 11.

It was found that the lipid nanoparticles using the pH-sensitive cationic lipids of the present invention had high siRNA encapsulation rates. Furthermore, it was found that the pH-sensitive cationic lipids of the present invention are useful for introducing siRNA into cells because the lipid nanoparticles using the pH-sensitive cationic lipids of the present invention all had low viable cell indices.

[0255]

[Table 10]

pH-Sensitive Cationic Lipid Synthesis Example	Encapsulated siRNA	Nucleic Acid Encapsulation Rate (%)	Average Particle Size (nm)	Polydispersity Index	Viable Cell Index (%)
					HepG2 Cells
[1]	siPLK1	99.74	81.72	0.075	46.96
	siLuc	99.71	76.74	0.055	
[4]	siPLK1	99.73	76.47	0.034	51.19
	siLuc	99.42	76.92	0.028	
[10]	siPLK1	100.00	81.78	0.042	42.95
	siLuc	99.89	97.72	0.032	
[14]	siPLK1	99.46	82.25	0.04	34.40
	siLuc	99.37	79.15	0.048	
[15]	siPLK1	99.57	80.77	0.083	42.95
	siLuc	99.50	77.69	0.07	
Comparative Example 1 (Lipofectamine™ RNAiMAX)	siPLK1	-	-	-	85.70
Comparative Example 2 (Naked)	siPLK1	-	-	-	100.8

[0256]

[Table 11]

Name		Sequence
siLuc	S	5'-CUU ACG CUG AGU ACU UCG AdTdT-3' (SEQ ID NO: 1)
	AS	5'-UCG AAG UAC UCA GCG UAA GdTdT-3' (SEQ ID NO: 2)
siPLK1	S	5' - AGAuCACCCuCCUuAAAuAUU-3' (SEQ ID NO: 3)
	AS	5' - UAUUUAAgGAGGGUGAuCUUU-3' (SEQ ID NO: 4)

In the above sequences, dT shows T of DNA, and small letters g and u show RNA with 2'-OMe.

[0257]

From the above results, the lipid nanoparticles using the pH-sensitive cationic lipids of the present invention had high mRNA encapsulation rates and increased drug introduction indices and thus can introduce encapsulated mRNA into the cells efficiently. Moreover, the pH-sensitive cationic lipids of the present invention are useful for introducing siRNA into cells.

[0258]

<Evaluation of mRNA Delivery Expression Ability on Laboratory Animals>

The produced lipid nanoparticle solutions were intravenously administered to six- to seven-week-old Balb/c mice (n=3) at such a dose that the encapsulated mRNA became at 0.1 mg kg-1. Six hours after the administration, 1.5 mg of VivoGlo Luciferin In Vivo Grade (Promega, US) dissolved in PBS(-) was injected into the tale vein, and the mice were sacrificed by bleeding after three minutes. The lungs, the livers, the spleens, the pancreas, and the kidneys were collected from the mice, and the luciferin luminescence was imaged using IVIS Imaging System. The region of interest of each organ was specified using ROI tool, and the Avg Radiance [p/s/cm2/sr] was calculated. The mRNA expression levels in the livers and the spleens of the lipid nanoparticles using the lipids of pH-Sensitive Cationic Lipid Synthesis Examples 1 to 89 are summarized in Table 12. A case in which HEPES was administered as a Comparative Example is also shown in Table 12. Luminescence which was the same or stronger than that of the HEPES administration was observed in the livers or the spleens for all the pH-sensitive cationic lipids of the present invention. It is obvious that they have mRNA delivery ability on laboratory animals.

[0259]

[Table 12-1]

pH-Sensitive Cationic Lipid Synthesis Example	Avg Radiance [p/s/cm²/sr]	
	Liver	Spleen
[1]	8.56.E+07	5.48.E+06
[2]	1.09.E+04	3.45.E+06
[3]	1.72.E+07	2.74.E+07
[4]	1.43.E+08	3.26.E+06
[5]	1.51.E+06	1.93.E+06
[6]	1.72.E+08	2.66.E+06
[7]	1.02.E+08	3.79.E+06
[8]	4.59.E+07	3.05.E+06
[9]	0.00.E+00	0.00.E+00
[10]	5.54.E+07	1.38.E+06
[11]	8.88.E+06	3.56.E+06
[12]	1.52.E+06	2.41.E+06
[13]	8.96.E+07	3.58.E+06
[14]	1.06.E+08	3.69.E+06
[15]	1.48.E+08	1.03.E+07
[16]	4.97.E+07	3.16.E+06
[17]	6.07.E+07	4.44.E+06
[18]	3.87.E+07	1.67.E+06
[21]	4.68.E+07	2.18.E+06
[22]	6.47.E+07	2.20.E+06
[23]	3.07.E+07	1.60.E+06
[24]	1.85.E+06	2.92.E+06
[25]	1.07.E+07	7.84.E+05
[26]	2.25.E+06	2.19.E+06
[28]	7.36.E+06	2.36.E+06
[29]	6.41.E+07	1.12.E+06
[30]	1.35.E+07	2.47.E+07
[31]	1.28.E+07	2.36.E+07
[32]	5.30.E+07	4.01.E+07
[33]	2.21.E+07	5.48.E+06
[34]	4.69.E+07	5.14.E+06
[35]	5.21.E+07	1.37.E+06

[Table 12-2]

[36]	1.22.E+08	4.14.E+06
[37]	6.76.E+06	7.53.E+05
[38]	4.89.E+06	2.31.E+06
[39]	6.56.E+07	3.91.E+06
[40]	6.37.E+07	1.48.E+06
[41]	5.49.E+07	2.88.E+06
[42]	8.71.E+06	9.35.E+05
[43]	5.61.E+06	4.58.E+05
[44]	3.45.E+06	3.21.E+05
[45]	2.55.E+05	9.12.E+04
[46]	4.53.E+06	8.99.E+06
[47]	3.69.E+06	9.99.E+05
[48]	1.31.E+07	2.36.E+07
[51]	1.27.E+07	1.70.E+06
[52]	7.10.E+05	1.04.E+07
[55]	5.40.E+07	5.41.E+06
[56]	4.95.E+07	6.33.E+06
[57]	1.84.E+05	2.32.E+04
[59]	1.01.E+05	3.59.E+06
[60]	2.23.E+07	2.02.E+07
[61]	4.15.E+07	1.21.E+07
[62]	1.80.E+08	1.05.E+07
[63]	1.31.E+08	4.81.E+06
[64]	4.12.E+07	6.46.E+06
[65]	4.21.E+06	9.36.E+05
[66]	4.80.E+05	1.71.E+05
[67]	1.11.E+05	1.15.E+04
[68]	3.03.E+07	4.58.E+06
[69]	1.08.E+08	1.75.E+07
[70]	1.05.E+08	1.07.E+07
[71]	2.39.E+08	3.09.E+06
[72]	3.05.E+04	8.09.E+03

[Table 12-3]

[73]	1.87.E+08	5.00.E+06
[74]	1.54.E+05	1.00.E+04
[75]	1.85.E+08	2.57.E+06
[76]	1.55.E+08	3.39.E+06
[77]	8.52.E+07	3.63.E+06
[78]	6.27.E+07	2.55.E+06
[79]	8.65.E+07	8.70.E+06
[80]	1.89.E+08	5.49.E+06
[81]	2.23.E+08	3.18.E+06
[82]	2.50.E+08	2.66.E+06
[83]	4.03.E+07	6.52.E+06
[84]	1.78.E+04	7.68.E+05
[85]	1.10.E+04	3.95.E+06
[88]	1.14.E+07	5.39.E+06
[89]	2.03.E+07	6.68.E+06
[90]	1.36.E+08	4.82.E+06
[91]	1.02.E+08	2.23.E+06
[92]	6.71.E+07	2.58.E+06
[93]	1.70.E+08	6.96.E+06
[94]	2.80.E+07	1.67.E+07
HEPES	5.93.E+02	3.18.E+02

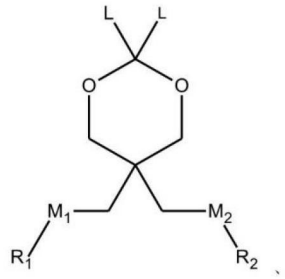
CLAIMS

[Claim 1]

A compound represented by

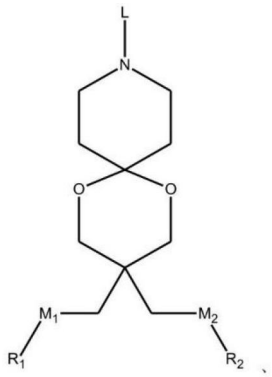
a general formula I:

[Chem. 1]



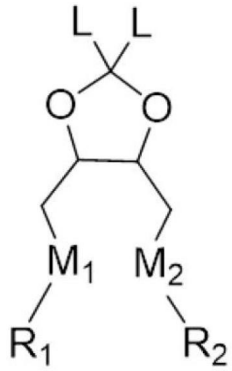
a general formula II:

[Chem. 2]



or a general formula III:

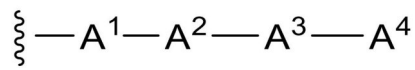
[Chem. 3]



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,

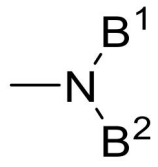
A^2 is -OC(=O)-, -OC(=O)-O-, or -OC(=O)-NH- or does not exist,

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]



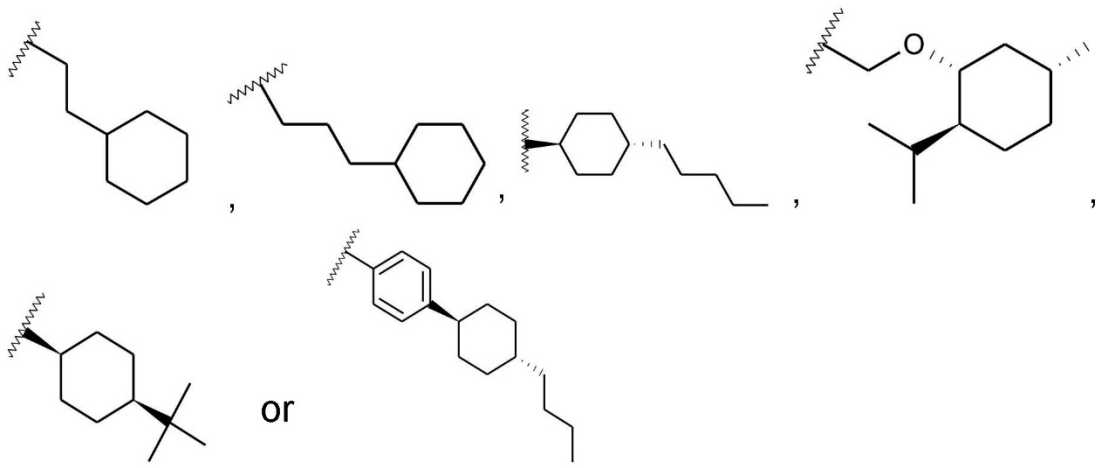
wherein B^1 and B^2 are each independently a C_{1-4} alkyl, a hydroxy C_{1-4} alkyl, a C_{1-4} alkoxy C_{1-4} alkyl, or a 4- to 6-membered heterocyclyl, wherein the 4- to 6-membered heterocyclyl is unsubstituted or substituted with at least one

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

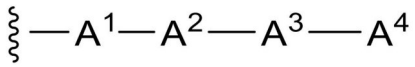
R_1 and R_2 are independently a linear C_{1-30} alkyl, a branched C_{3-30} alkyl, a linear C_{2-30} alkenyl, a branched C_{3-30} alkenyl, adamantyl, a sterol group, and

[Chem. 6]



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

[Chem. 7]



or a salt or a stereoisomer thereof.

[Claim 2]

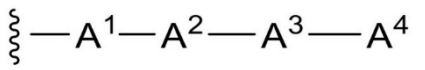
The compound, the salt, or the stereoisomer thereof according to claim

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]



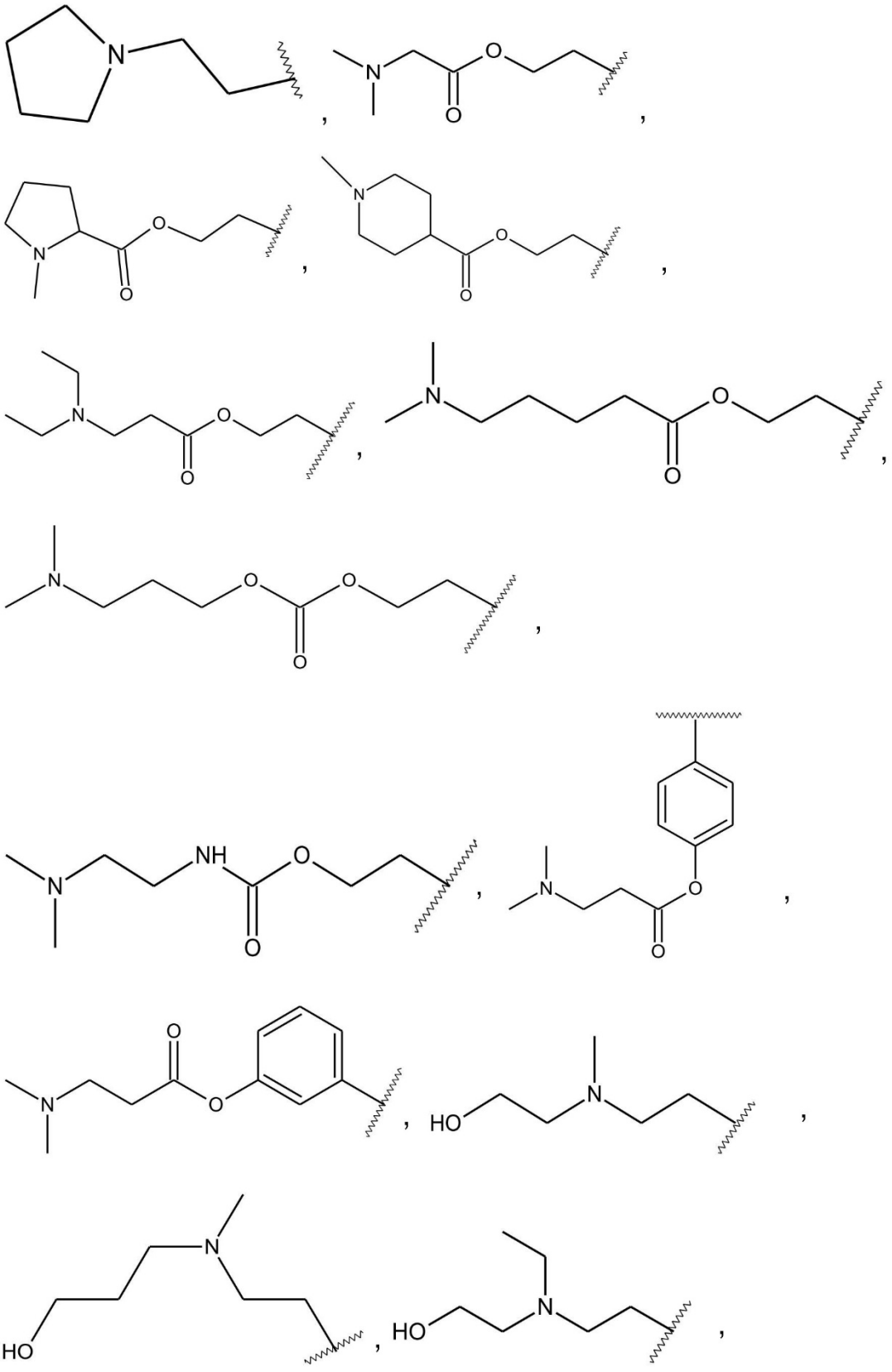
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[Claim 3]

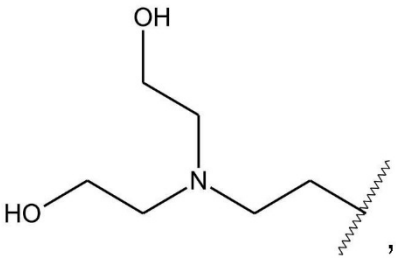
The compound, the salt, or the stereoisomer thereof according to claim 2,

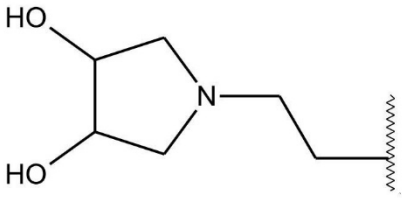
wherein one of L's is H, and the other L is

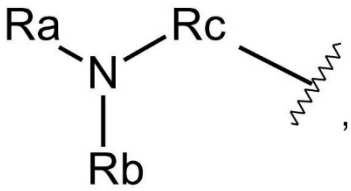
[Chem. 9-1]

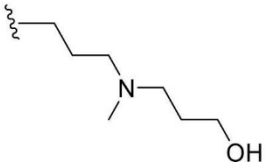


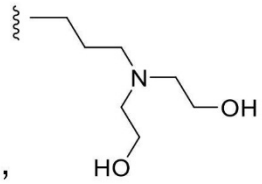
[Chem. 9-2]

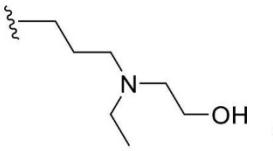


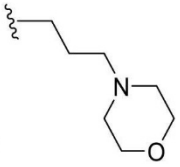
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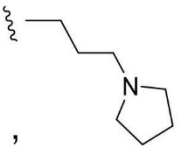
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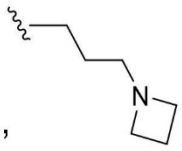
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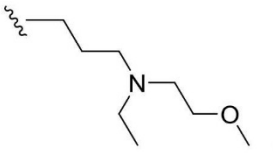
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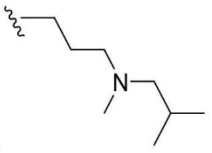
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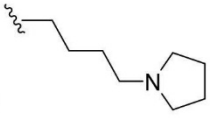
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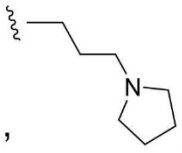
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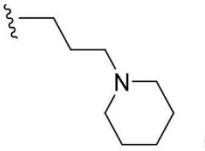
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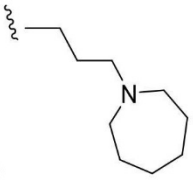
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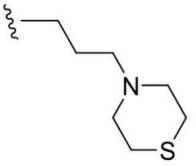
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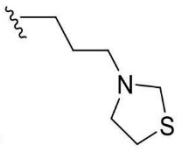
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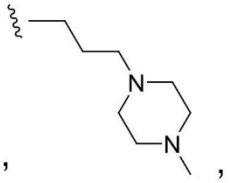
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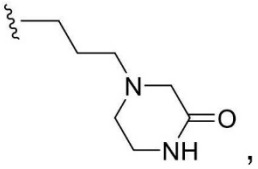
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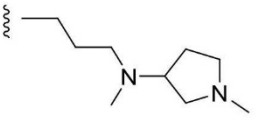
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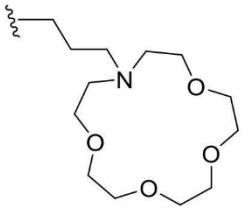
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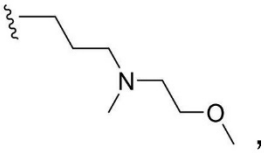
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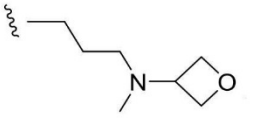
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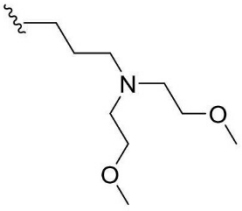
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or


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

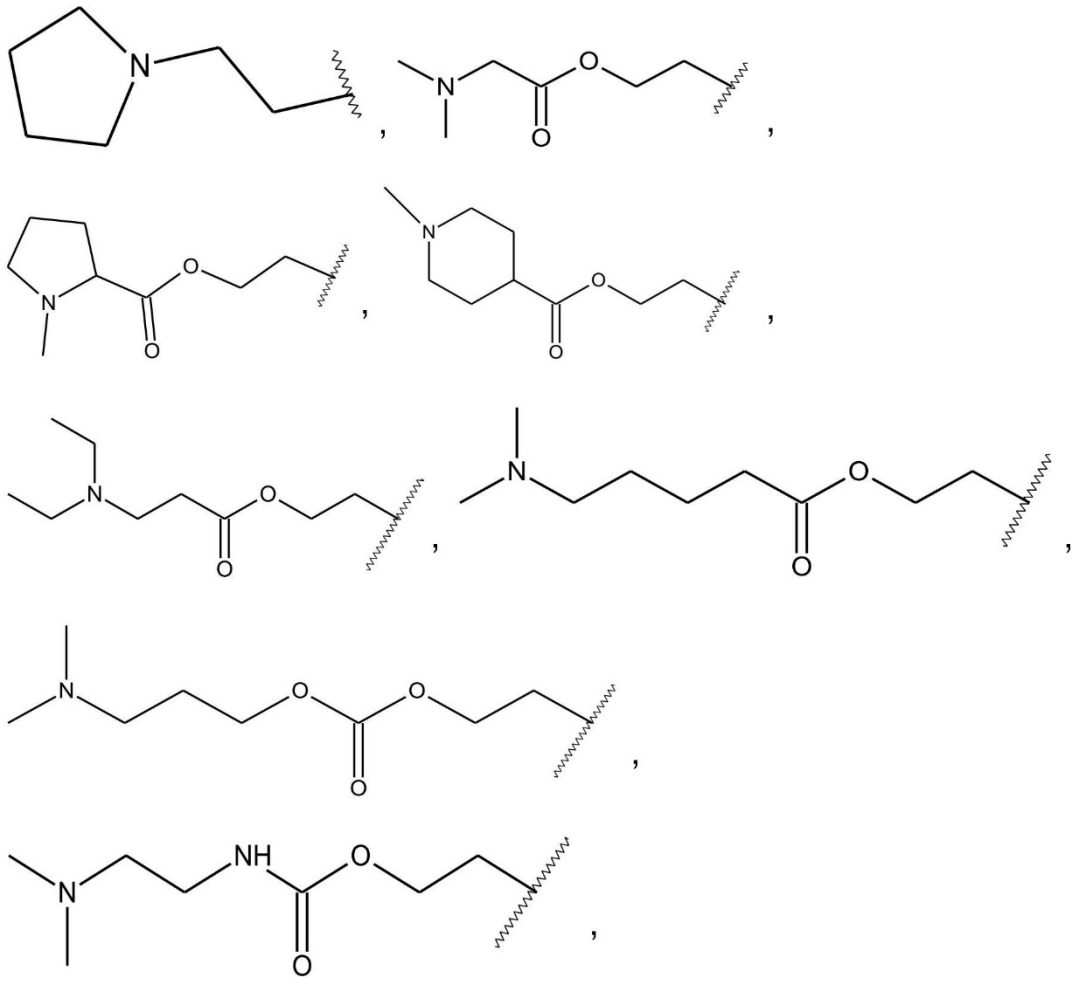
isopropyl, and R_c is a linear C_{1-6} alkylene.

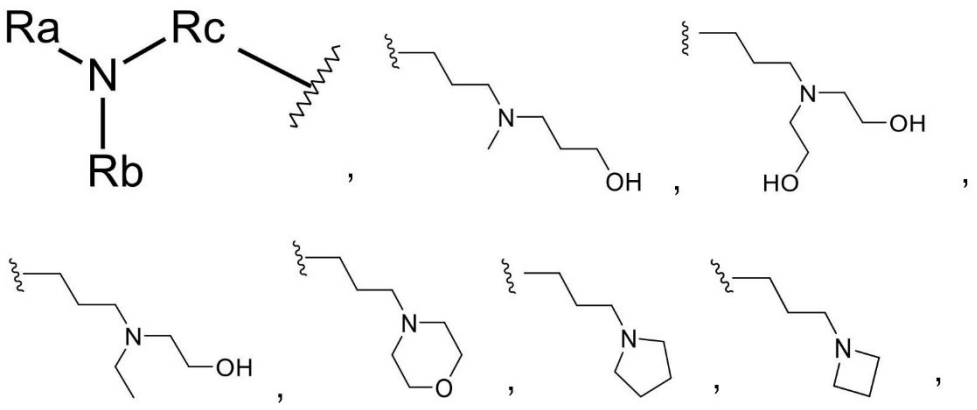
[Claim 4]

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

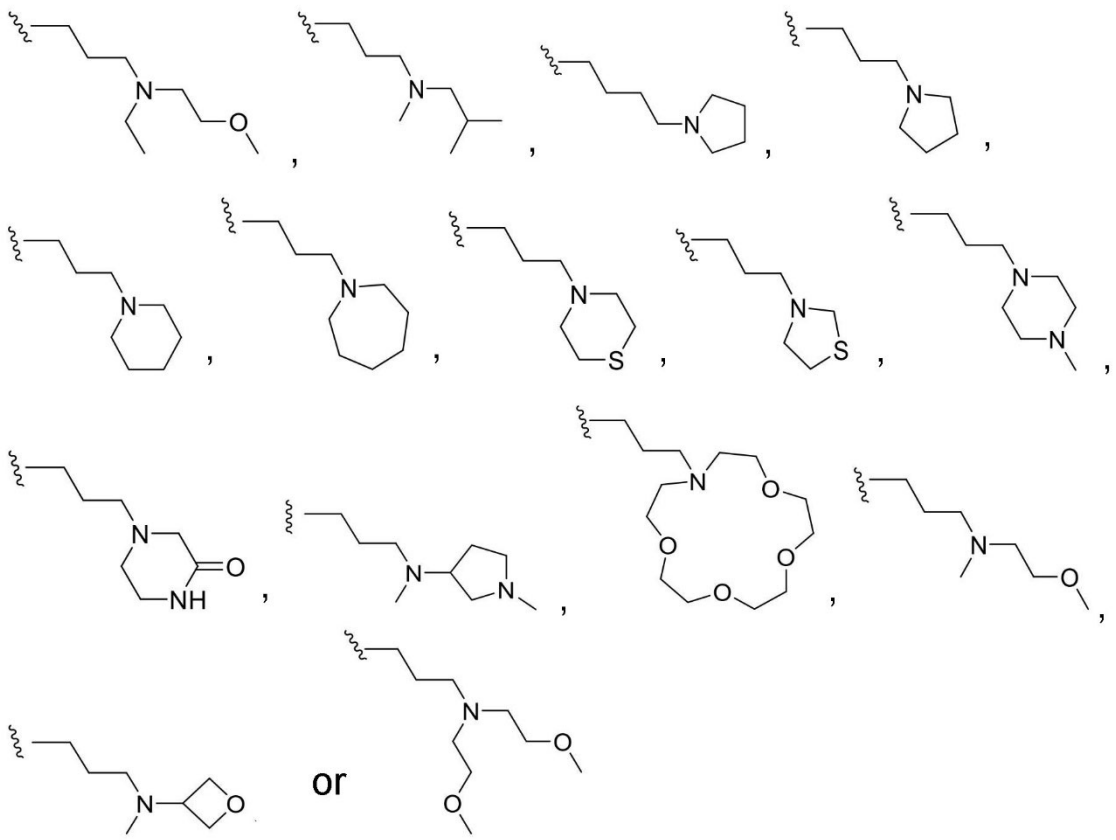
wherein one of L's is H, and the other L is

[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-

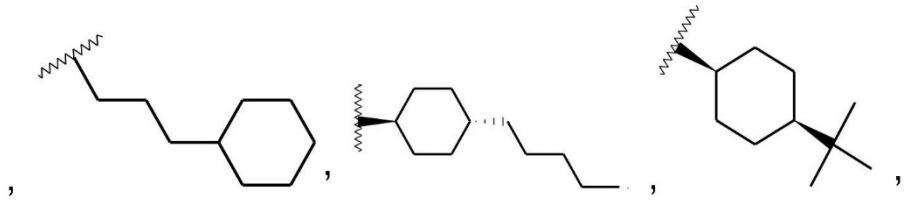
CH_2-, -O-C(O)-CH_2-, -O-C(O)-CH_2-CH_2-, -O-C(O)-CH_2-CH_2-C(O)-O-CH_2-, or -CH_2-CH_2-O-C(O)-.

[Claim 7]

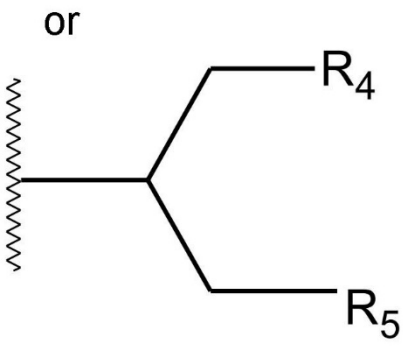
The compound, the salt, or the stereoisomer thereof according to claim 6,

wherein 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]



or



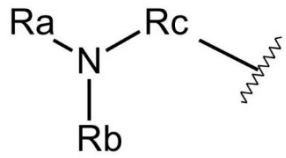
wherein R_4 and R_5 are independently a linear C_{1-16} alkyl.

[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

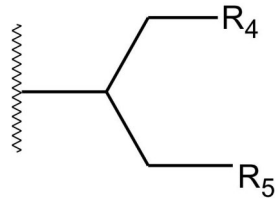
R_c is a linear C_{2-6} alkylene.

[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,

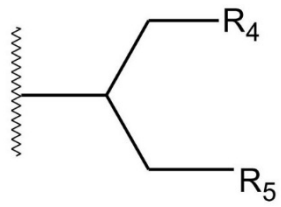
wherein the total number of carbon atoms of R_4 and R_5 is 10 to 22.

[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,

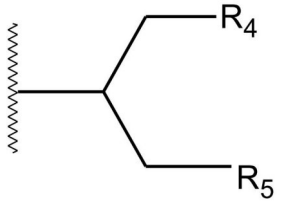
wherein the compound is represented by the general formula II,

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

M_1 and M_2 are -O-C(O)-, 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. 15]



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

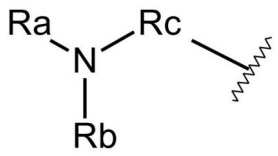
[Claim 12]

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]

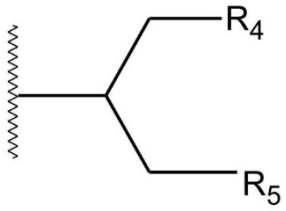


wherein R_a and R_b are independently methyl, ethyl, n-propyl, or isopropyl, and R_c is a linear C_{1-6} alkylene),

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]



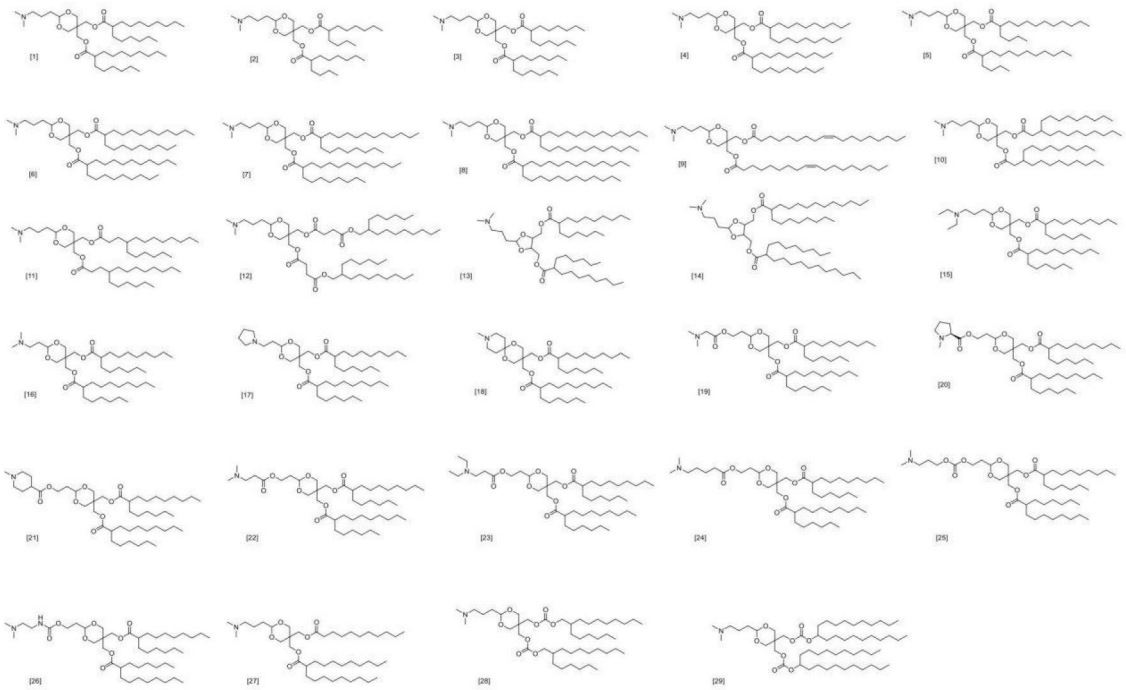
wherein R_4 and R_5 are independently a linear C_{1-16} alkyl.

[Claim 13]

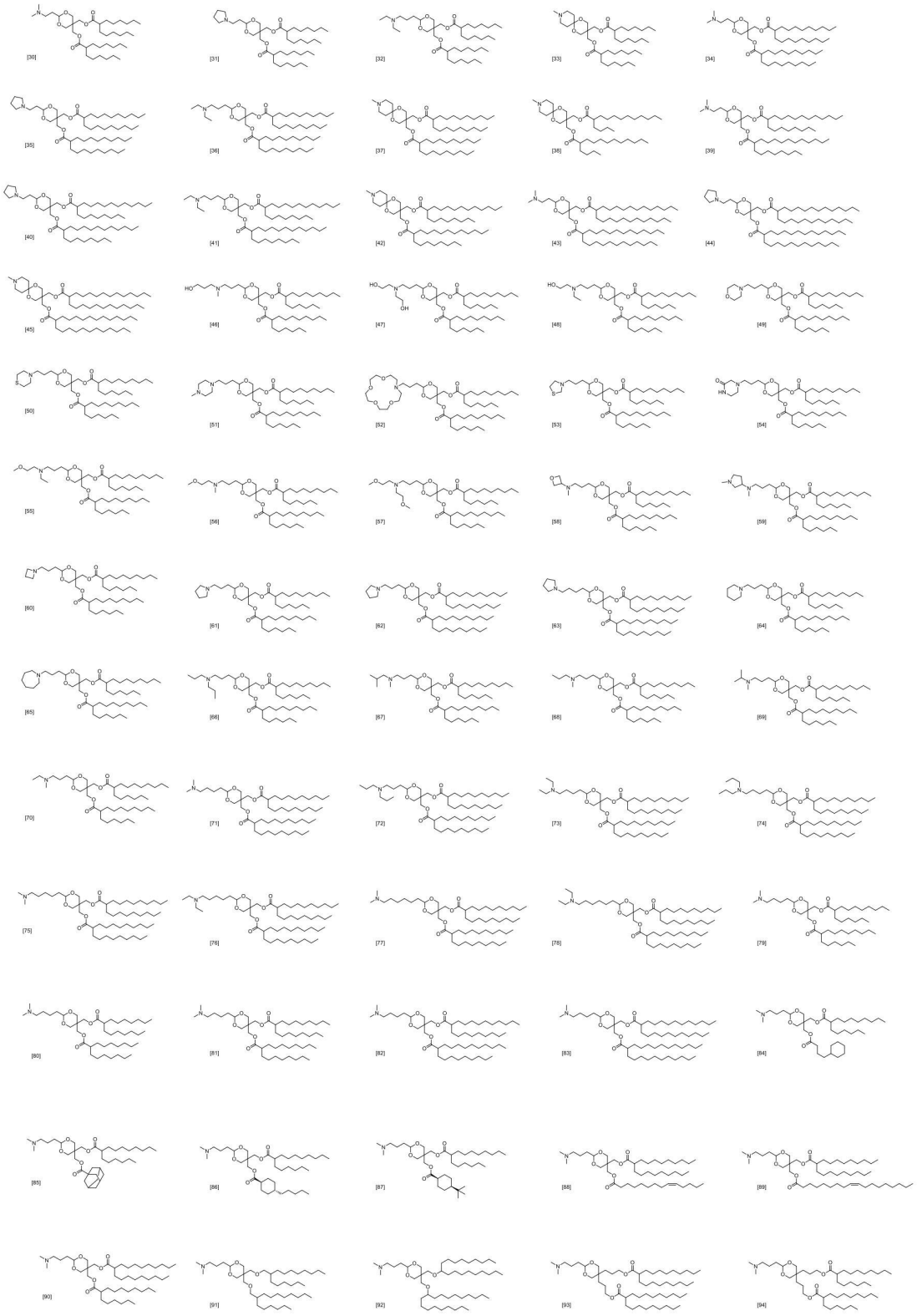
The compound, the salt, or the stereoisomer thereof according to claim 1,

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.

[Claim 20]

A method for expressing an exogenous gene, comprising administering

the lipid nanoparticle according to claim 14 which encapsulates a target exogenous gene to be expressed in a cell to a test animal (excluding a human) and expressing the exogenous gene in the cell of the test animal.