

DESCRIPTION

Title of Invention: METHOD FOR ACTIVATION/PROLIFERATION OF T
CELLS

[Technical Field]

5 [0001]

The present invention relates to a nucleic acid delivery carrier with a T cell activating ligand added to the surface, a method for activating and/or proliferating T cells by using the nucleic acid delivery carrier, a method for delivering a
10 nucleic acid into T cells, and the like. The present invention also relates to a method for activating and/or proliferating T cells and a method for delivering a nucleic acid into T cells, each characteristically including bringing a T cell activating ligand and a nucleic acid delivery carrier into simultaneous
15 contact with T cells.

[0002]

(Background of the Invention)

The research and development of cancer immunotherapy using CAR-T cells or TCR-T cells introduced with a gene of
20 chimeric antigen receptor (CAR) or T-cell receptor (TCR) derived from cancer antigen-specific killer T cell is progressing rapidly. Current CAR-T cell therapy, such as Kymriah (trade name) and Yescarta (trade name), which were approved in the U.S., generally includes producing CAR-T cells
25 by introducing CAR genes into T cells collected from patients ex vivo using virus vectors such as lentivirus vector, and administering the CAR-T cells to the patients. However, this method has the problem that the production cost becomes high due to the cost of cell culture and preparation of virus
30 vectors because multiple steps are necessary over a long period of time such as activation/proliferation of T cells, preparation of virus vectors, gene transfer into T cells, and the like.

[0003]

35 As a method for introducing CAR into T cells without

using a virus vector, ex vivo or in vivo transfection of CAR into T cells has been reported which uses nanoparticles containing aggregates of CAR-encoding plasmid DNA and a cationic polymer that are coated with a non-cationic polymer conjugated with anti-CD3 antibody fragments (patent document 1, non-patent document 1), or nanocarrier containing mesoporous silica encapsulating CAR-encoding DNA in the pores and coated with a lipid having a surface modified with an anti-CD3 antibody (patent document 2).

10 Apart therefrom, techniques have been reported for delivering siRNA to a target cell by encapsulating the siRNA of interest in "lipid nanoparticles (LNP)", which do not have an internal pore structure and are composed of a cationic lipid, a non-cationic helper lipid, and a ligand for delivery to the target cell. For example, ex vivo or in vivo transfection of siRNA for CD45 into T cells by using an anti-CD4 antibody fragment as a targeted ligand has been reported (patent document 3, non-patent document 2).

 In addition, patent document 4 describes a cationic lipid for introducing an active ingredient such as a nucleic acid or the like into various cells including T cell, tissues and organs.

[0004]

 On the other hand, as a method for activating/proliferating T cells, a method for activating and/or proliferating T cells using beads on which anti-CD3/CD28 antibody is immobilized or nano-sized matrix beads has been reported (patent documents 5 and 6).

[0005]

30 However, no technique has been reported heretofore in which the step of activating/proliferating T cells and the step of introducing a gene into T cells can be performed simultaneously in one pod.

[Document List]

35 [Patent documents]

[0006]

patent document 1: US 2017/0296676

patent document 2: US 2016/0145348

patent document 3: WO 2016/189532

5 patent document 4: WO 2016/021683

patent document 5: US 6,352,694

patent document 6: US 2014/0087462

[Non-patent documents]

[0007]

10 non-patent document 1: Nature Nanotechnology 12, 813-820 (2017)

non-patent document 2: ACS Nano, 2015, 9(7), 6706-6716

[Summary of Invention]

[Technical Problem]

[0008]

15 An object of the present invention is to shorten and
simplify the production process of an agent for immune cell
therapy such as CAR-T therapy and the like, to provide an agent
for immune cell therapy in a short period of time with a low
production cost, and to provide a safer production process of
20 an agent for immune cell therapy that eliminates a potential
risk of carcinogenicity due to a virus vector.

[Solution to Problem]

[0009]

 The present inventors have conducted intensive studies in
25 an attempt to achieve the above-mentioned object and succeeded
in simultaneously performing a step of activating/proliferating
T cells and a step of introducing a gene into T cells in one
pod by using a nucleic acid delivery carrier having a T cell
activating ligand added to its surface. Furthermore, the
30 present inventors have surprisingly found that
activation/proliferation of T cells and introduction of nucleic
acid into T cells can be efficiently achieved by simply
bringing the T cell activating ligand and the nucleic acid
delivery carrier into contact with the T cells at the same time,
35 and completed the present invention.

[0010]

Accordingly, the present invention provides the following.

[1] A method for activating/proliferating T cells, comprising a step of contacting a cell population containing T cells with a nucleic acid delivery carrier having at least one kind of T cell activating ligand added to its surface.

[2] The method of [1], wherein the aforementioned T cell activating ligand includes an antibody to CD3 and/or an antibody to CD28.

10 [3] The method of [1] or [2], wherein the aforementioned nucleic acid delivery carrier has two or more kinds of T cell activating ligands added to a surface thereof.

[4] The method of any of [1] to [3], wherein the aforementioned nucleic acid delivery carrier is a lipid nanoparticle or a liposome.

15 [5] The method of any of [1] to [4], wherein the aforementioned nucleic acid delivery carrier comprises, in its inside, a nucleic acid that suppresses expression of a T cell activation inhibitory factor and/or a nucleic acid encoding a T cell activation promoting factor.

20 [6] The method of any of [1] to [5], wherein the aforementioned nucleic acid delivery carrier comprises a nucleic acid encoding CAR or TCR.

[7] The method of any of [1] to [6], wherein the method is performed ex vivo.

25 [8] A method for delivering a nucleic acid into T cells, comprising a step of contacting a cell population containing T cells with a nucleic acid delivery carrier having at least one kind of T cell activating ligand added to its surface and containing a nucleic acid inside.

30 [9] The method of [8], wherein the aforementioned T cell activating ligand includes an antibody to CD3 and/or an antibody to CD28.

[10] The method of [8] or [9], wherein the aforementioned nucleic acid delivery carrier has two or more kinds of T cell

activating ligands added to a surface thereof.

[11] The method of any of [8] to [10], wherein the aforementioned nucleic acid delivery carrier is a lipid nanoparticle or a liposome.

5 [12] The method of any of [8] to [11], wherein the aforementioned nucleic acid comprises a nucleic acid suppressing expression of a T cell activation inhibitory factor and/or a nucleic acid encoding a T cell activation promoting factor.

10 [13] The method of any of [8] to [12], wherein the aforementioned nucleic acid comprises a nucleic acid encoding CAR or TCR.

[14] The method of any of [8] to [13], wherein the method is performed ex vivo.

15 [15] A method for delivering a nucleic acid into T cells, comprising a step of contacting a cell population containing T cells simultaneously with at least one kind of T cell activating ligand, and a nucleic acid delivery carrier containing a nucleic acid inside and free of a T cell
20 activating ligand added to its surface.

[16] The method of [15], wherein the aforementioned T cell activating ligand includes an antibody to CD3 and/or an antibody to CD28.

[17] The method of [15] or [16], wherein two or more kinds of T
25 cell activating ligands are contacted.

[18] The method of any of [15] to [17], wherein the aforementioned nucleic acid delivery carrier is a lipid nanoparticle or a liposome.

[19] The method of any of [15] to [18], wherein the
30 aforementioned nucleic acid includes a nucleic acid suppressing expression of a T cell activation inhibitory factor and/or a nucleic acid encoding a T cell activation promoting factor.

[20] The method of any of [15] to [19], wherein the aforementioned nucleic acid comprises a nucleic acid encoding
35 CAR or TCR.

[21] The method of any of [15] to [20], wherein the method is performed *ex vivo*.

[22] A method for producing a medicament comprising T cells, comprising a step of contacting a cell population containing T
5 cells with a nucleic acid delivery carrier having at least one kind of T cell activating ligand added to its surface and containing a nucleic acid inside.

[23] The method of [22], wherein the aforementioned T cell activating ligand includes an antibody to CD3 and/or an
10 antibody to CD28.

[24] The method of [22] or [23], wherein the aforementioned nucleic acid delivery carrier has two or more kinds of T cell activating ligands added to a surface thereof.

[25] The method of any of [22] to [24], wherein the
15 aforementioned nucleic acid delivery carrier is a lipid nanoparticle or a liposome.

[26] The method of any of [22] to [25], wherein the aforementioned nucleic acid delivery carrier comprises a nucleic acid suppressing expression of a T cell activation
20 inhibitory factor and/or a nucleic acid encoding a T cell activation promoting factor.

[27] The method of any of [22] to [26], wherein the aforementioned nucleic acid comprises a nucleic acid encoding CAR or TCR.

[28] The method of any of [22] to [27], wherein the method is
25 performed *ex vivo*.

[29] A method for producing a medicament comprising T cells, comprising a step of contacting a cell population containing T cells simultaneously with at least one kind of T cell
30 activating ligand, and a nucleic acid delivery carrier containing a nucleic acid inside and free of a T cell activating ligand added to its surface.

[30] The method of [29], wherein the aforementioned T cell activating ligand includes an antibody to CD3 and/or an
35 antibody to CD28.

[31] The method of [29] or [30], wherein two or more kinds of T cell activating ligands are contacted.

[32] The method of any of [29] to [31], wherein the aforementioned nucleic acid delivery carrier is a lipid nanoparticle or a liposome.

[33] The method of any of [29] to [32], wherein the aforementioned nucleic acid delivery carrier comprises a nucleic acid suppressing expression of a T cell activation inhibitory factor and/or a nucleic acid encoding a T cell activation promoting factor.

[35] The method of any of [29] to [34], wherein the method is performed ex vivo.

[36] A nucleic acid delivery carrier having at least one kind of T cell activating ligand added to its surface.

[37] The nucleic acid delivery carrier of [36], wherein the aforementioned T cell activating ligand includes an antibody to CD3 and/or an antibody to CD28.

[38] The nucleic acid delivery carrier of [36] or [37], wherein the aforementioned nucleic acid delivery carrier has two or more kinds of T cell activating ligands added to a surface thereof.

[39] The nucleic acid delivery carrier of any of [36] to [38], wherein the aforementioned nucleic acid delivery carrier is a lipid nanoparticle or a liposome.

[40] The nucleic acid delivery carrier of any of [36] to [39], comprising, in the inside, a nucleic acid suppressing expression of a T cell activation inhibitory factor and/or a nucleic acid encoding a T cell activation promoting factor.

[41] The nucleic acid delivery carrier of any of [36] to [40], comprising, in the inside, a nucleic acid encoding CAR or TCR.

[42] A medicament comprising the nucleic acid delivery carrier of any of [36] to [41].

[43] A T cell into which a nucleic acid has been delivered by the method of any of [15] to [21].

[44] A medicament comprising the T cell of [43].

[45] A cell culture comprising a cell population containing T cells, at least one kind of T cell activating ligand, a nucleic acid delivery carrier without a T cell activating ligand added to the surface, and a medium.

5 [46] A composition for delivering a nucleic acid to T cells, comprising at least one kind of T cell activating ligand, and a nucleic acid delivery carrier without a T cell activating ligand added to the surface.

[47] A kit for delivering a nucleic acid into T cells,
10 comprising at least one kind of T cell activating ligand, and a nucleic acid delivery carrier without a T cell activating ligand added to the surface.

[48] A T cell into which a nucleic acid has been delivered by the method of any of [8] to [14].

15 [49] A medicament comprising the T cell of [48].

[50] A cell culture comprising a cell population containing T cells, a nucleic acid delivery carrier having at least one kind of T cell activating ligand added to its surface and containing a nucleic acid inside, and a medium.

20 [51] A composition for delivering a nucleic acid into T cells, comprising a nucleic acid delivery carrier having at least one kind of T cell activating ligand added to its surface and containing a nucleic acid inside.

[52] A kit for delivering a nucleic acid into T cells,
25 comprising a nucleic acid delivery carrier having at least one kind of T cell activating ligand added to its surface and containing a nucleic acid inside.

[Advantageous Effects of Invention]

[0011]

30 According to the present invention, a step of activating/proliferating T cells and a step of introducing a gene into T cells can be performed simultaneously in one pod without using a virus vector. As a result, an agent for immune cell therapy can be provided in a short period of time with a
35 low production cost.

[Brief Description of Drawings]

[0012]

Fig. 1 shows a comparison of efficiency of gene transfer into T cells by lipid nanoparticles having an anti-CD3 antibody added to surface thereof, and containing various cationic lipids (compounds 7, 11, 12, 21, 31 and 35).

Fig. 2 shows a comparison of efficiency of gene transfer into T cells by lipid nanoparticles having an anti-CD3 antibody and/or an anti-CD28 antibody added to surface thereof.

Fig. 3 shows that gene transfer (I) into T cells and activation (II) of T cells are simultaneously achieved by lipid nanoparticles having an anti-CD3 antibody and an anti-CD28 antibody added to surface thereof. In (I) and (II), the numerical value in the upper panel shows a concentration ($\mu\text{g/ml}$) of encapsulated mRNA, and the numerical value in the lower panel shows a concentration ($\mu\text{g/ml}$) of the antibody. For comparison, (III) shows efficiency of T cell activation by beads having conventionally-known anti-CD3 antibody and anti-CD28 antibody added to surface thereof.

Fig. 4 shows that luc mRNA is efficiently introduced into human peripheral blood CD3-positive pan-T cells by co-addition of an activation stimulant and lipid nanoparticles.

Fig. 5 shows the survival and proliferation rate of T cells transfected with luc mRNA by lipid nanoparticles.

Fig. 6 shows that luc mRNA is efficiently introduced into human CD4/CD8-positive T cells by co-addition of an activation stimulant and lipid nanoparticles (left), and that the survival and proliferation rate of T cells is maintained at a high level (right).

[0013]

(Detailed Description of the Invention)

1. Nucleic acid delivery carrier of the present invention

The present invention provides a nucleic acid delivery carrier having at least one kind of T cell activating ligand added to its surface (hereinafter to be also referred to as

"the nucleic acid delivery carrier of the present invention").

As used herein, the "nucleic acid delivery carrier" means a carrier capable of supporting a nucleic acid and delivering the nucleic acid into a cell. Being "capable of delivering the
5 nucleic acid into a cell" means that a nucleic acid being carried can be delivered at least into the cytoplasm of a cell.
[0014]

1-1. Nucleic acid delivery carrier

The nucleic acid delivery carrier to be used in the
10 present invention is not particularly limited in terms of the structure thereof, component molecules, and nucleic acid carrying form as long as it can support a nucleic acid and can deliver the nucleic acid into a cell, as described above. A representative drug delivery system (DDS) of nucleic acid is,
15 for example, a complex using positively-charged cationic liposomes, cationic polymers, and the like as carriers, and formed based on the electrostatic interaction between them and nucleic acid. The complex binds to a negatively-charged cell membrane and is then incorporated into the cell by adsorptive
20 endocytosis.

[0015]

More specifically, examples of the nucleic acid delivery carrier to be used in the present invention include, but are not limited to, lipid nanoparticles (LNP), liposomes (e.g.,
25 cationic liposome, PEG-modified liposome, etc.), and cationic polymers (e.g., polyethyleneimine, polylysine, polyornithine, chitosan, atelocollagen, protamine etc.), those in which a cationic polymer is encapsulated in liposomes, and the like. Alternatively, exosome, which is a component derived from
30 living organisms, can also be used. Preferred is lipid nanoparticle or liposome, more preferred is lipid nanoparticle.
[0016]

1-1-1. Lipid nanoparticle (LNP)

In the present specification, the "lipid nanoparticle
35 (LNP)" means a particle with an average diameter of less than 1

µm and free of a large pore structure (e.g., liposome) or a small pore structure (e.g., mesoporous material) inside the outer shell of a lipid aggregate containing cationic lipid and non-cationic lipid.

5 The components of the lipid nanoparticle are described below.

[0017]

(a) Cationic lipid

 In the present specification, the "cationic lipid" means
10 a lipid that has a net positive charge in a low pH environment such as in physiological pH, endosome and the like. The cationic lipids used in the lipid nanoparticle used in the present invention are not particularly limited. For example, cationic lipids and the like described in WO 2016/021683, WO
15 2015/011633, WO 2011/153493, WO 2013/126803, WO 2010/054401, WO 2010/042877, WO 2016/104580, WO 2015/005253, WO 2014/007398, WO 2017/117528, WO 2017/075531, WO 2017/00414, WO 2015/199952, US 2015/0239834, WO2019/131839, and the like can be mentioned.

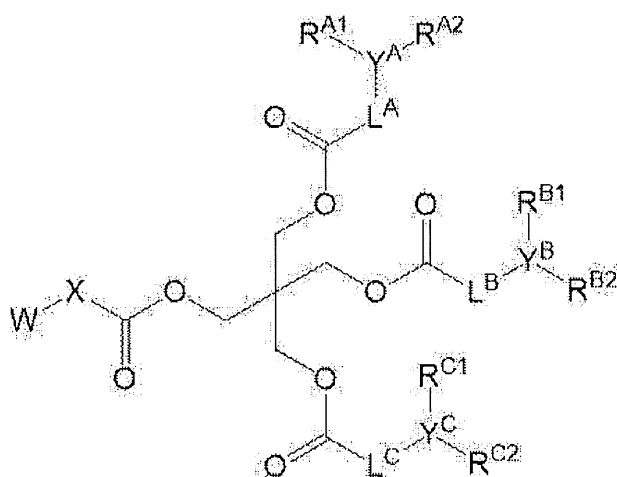
 Alternatively, the synthetic cationic lipids (e.g., K-E12,
20 H-A12, Y-E12, G-O12, K-A12, R-A12, cKK-E12, cPK-E12, PK1K-E12, PK500-E12, cQK-E12, cKK-A12, KK-A12, PK-4K-E12, cWK-E12, PK500-O12, PK1K-O12, cYK-E12, cDK-E12, cSK-E12, cEK-E12, cMK-E12, cKK-O12, cIK-E12, cKK-E10, cKK-E14, and cKK-E16, preferably, cKK-E12, cKK-E14) described in Dong et al. (Proc Natl Acad Sci
25 U S A. 2014 Apr 15; 111(15):5753), and the synthetic cationic lipids (e.g., C14-98, C18-96, C14-113, C14-120, C14-120, C14-110, C16-96 and C12-200, preferably C14-110, C16-96 and C12-200) described in Love KT et al. (Proc Natl Acad Sci U S A. 2010 May 25; 107(21):9915) can be mentioned.

30 [0018]

 In one preferred embodiment, a cationic lipid represented by the following general formula and described in WO 2016/021683 can be mentioned.

[0019]

35



[0020]

wherein

W is the formula $-NR^1R^2$ or the formula $-N^+R^3R^4R^5(Z^-)$,

5 R^1 and R^2 are each independently a C_{1-4} alkyl group or a hydrogen atom,

R^3 , R^4 and R^5 are each independently a C_{1-4} alkyl group,

Z^- is an anion,

X is an optionally substituted C_{1-6} alkylene group,

10 Y^A , Y^B and Y^C are each independently an optionally substituted methine group,

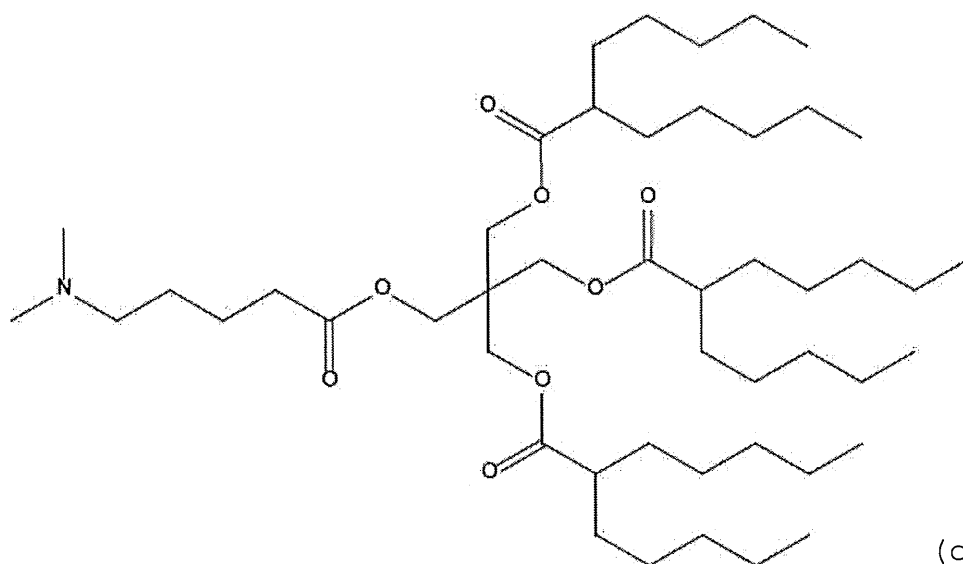
L^A , L^B and L^C are each independently an optionally substituted methylene group or a bond, and

RA^1 , RA^2 , RB^1 , RB^2 , RC^1 and RC^2 are each independently an
15 optionally substituted C_{4-10} alkyl group,
or a salt thereof.

[0021]

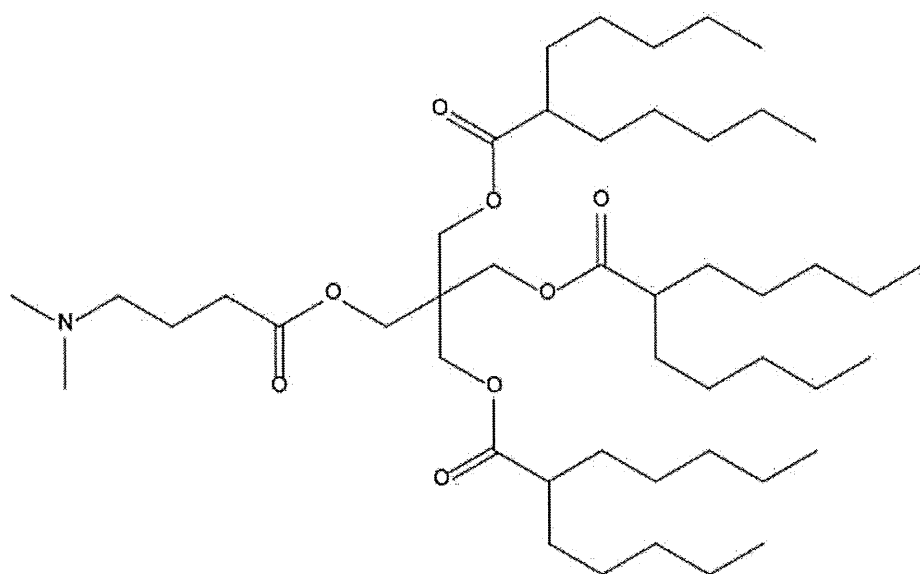
More preferably, cationic lipids represented by the following structural formulas can be mentioned.

20 [0022]



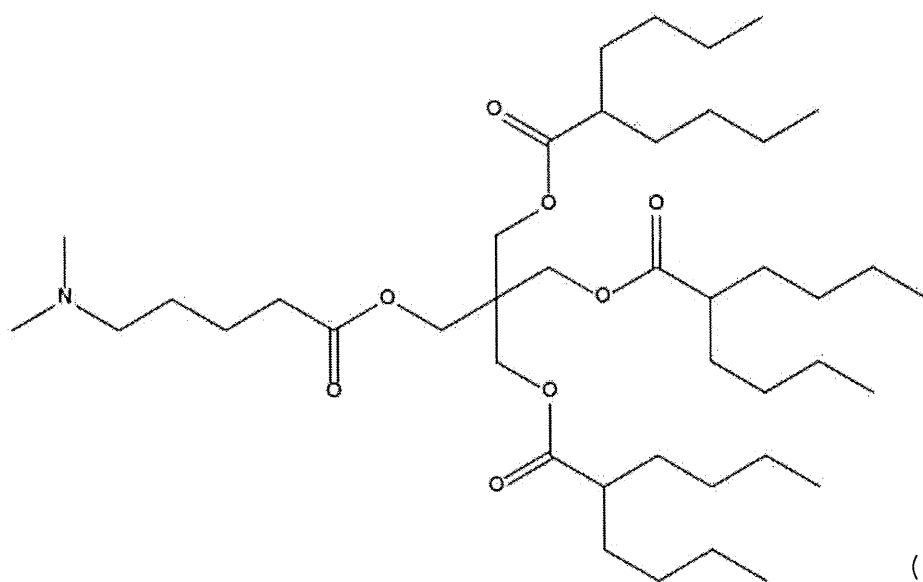
(compound 1)

[0023]



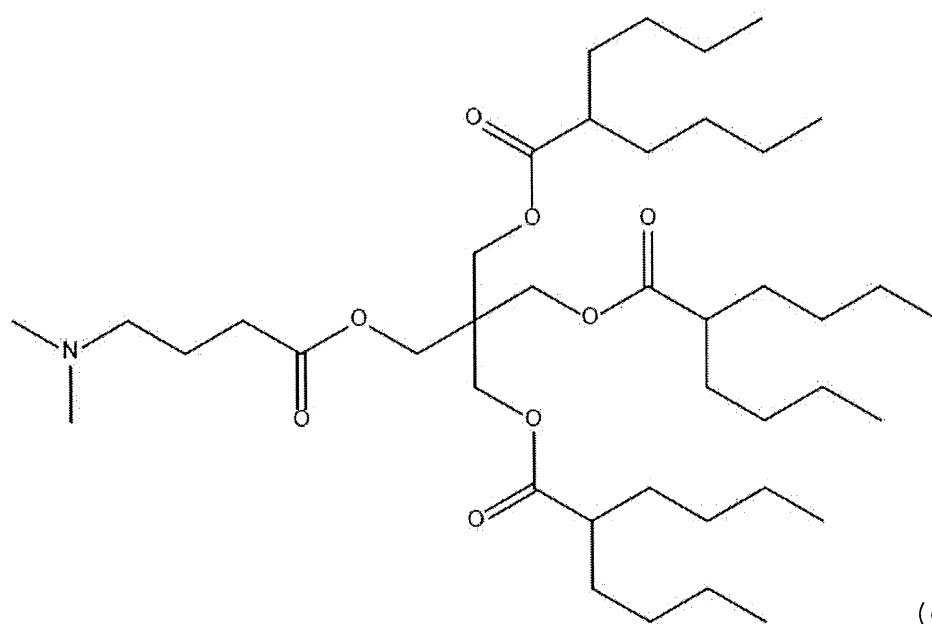
(compound 2)

[0024]



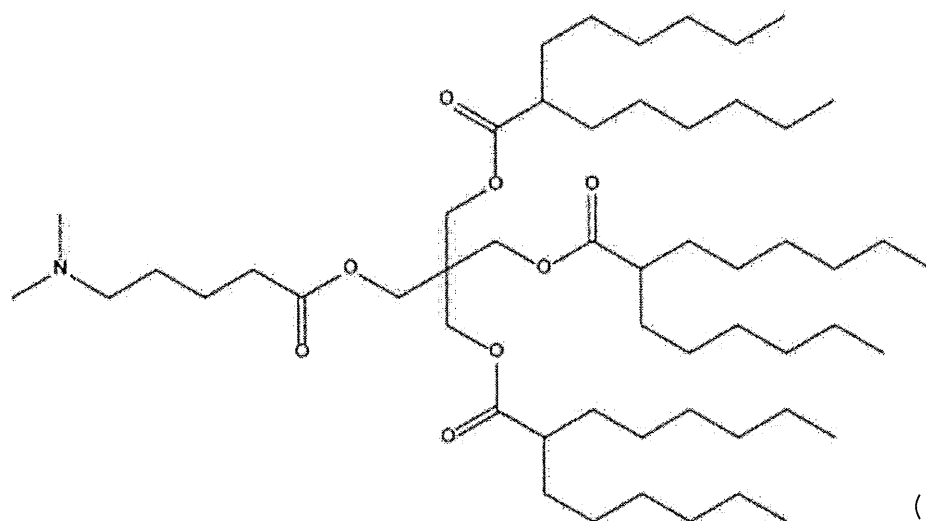
(compound 3)

[0025]



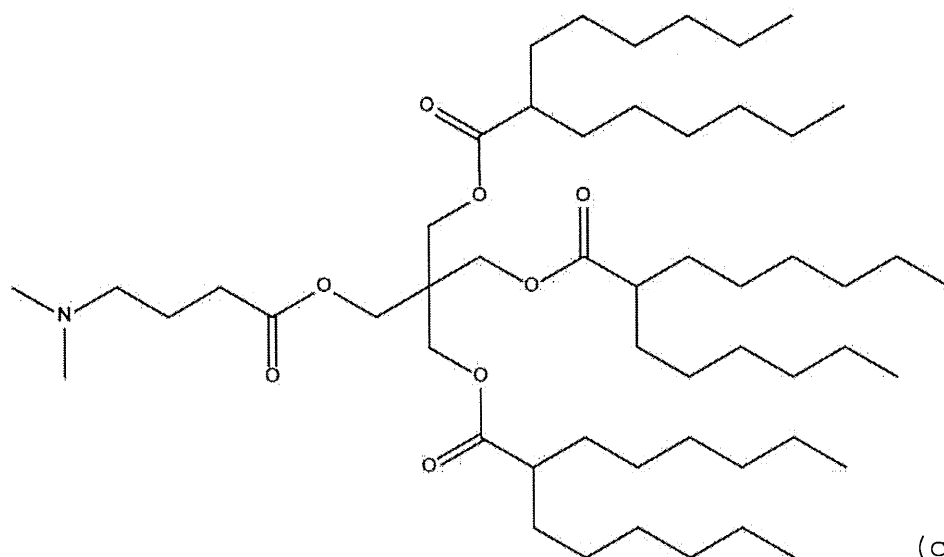
(compound 4)

[0026]



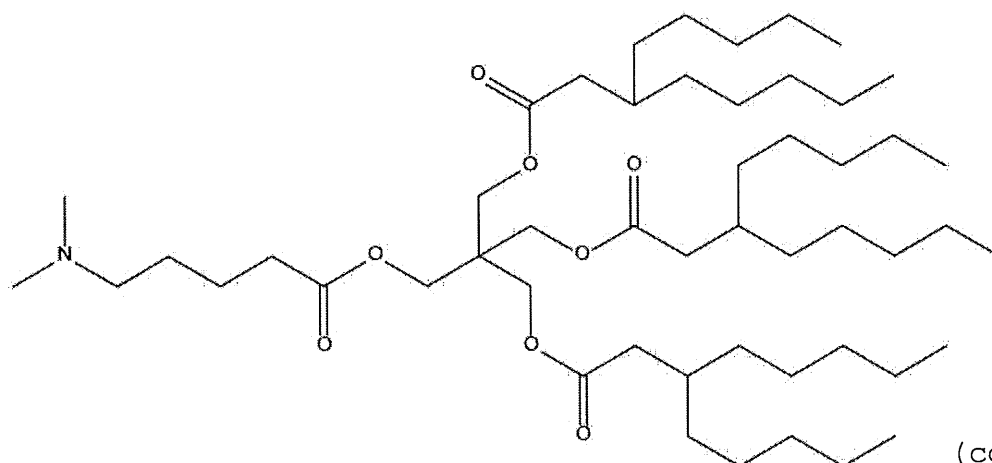
(compound 5)

[0027]



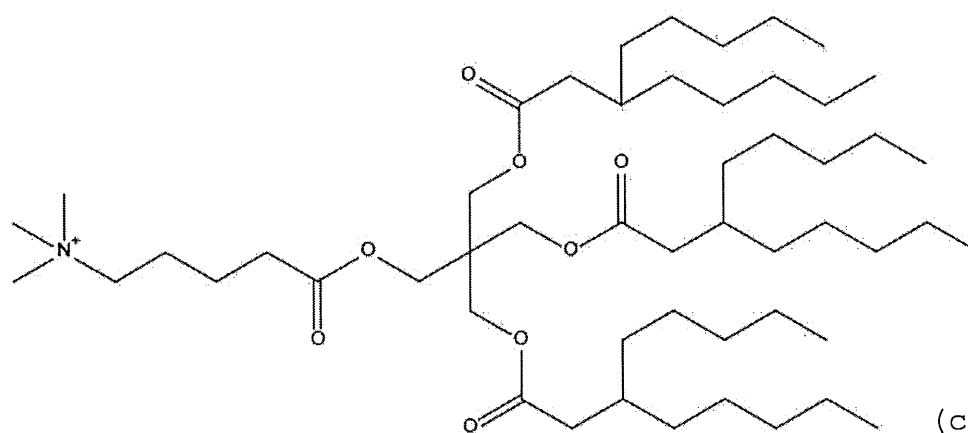
(compound 6)

[0028]



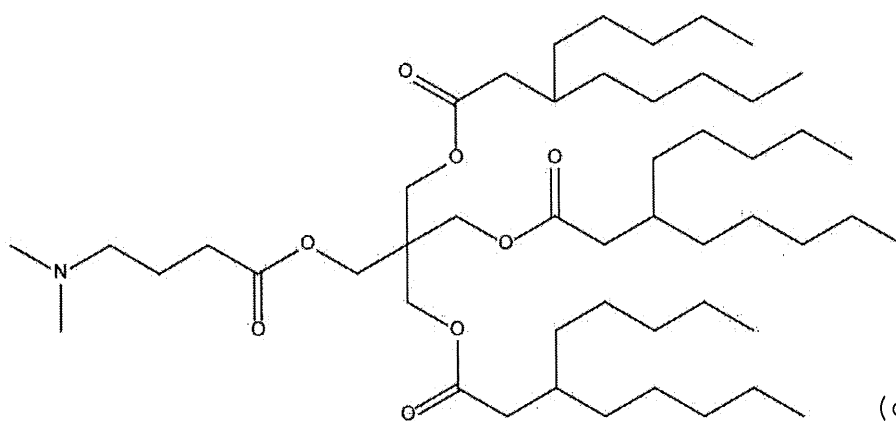
(compound 7)

5 [0029]



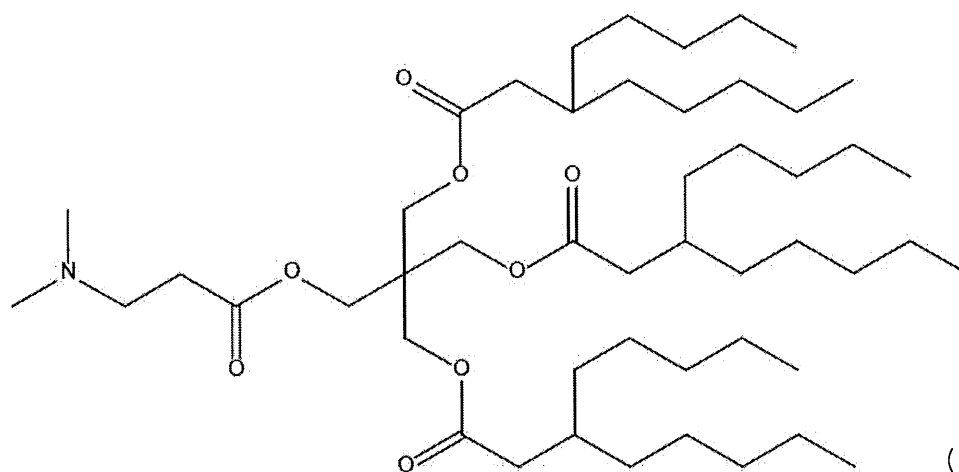
(compound 8)

[0030]



(compound 9) and

[0031]



(compound 10)

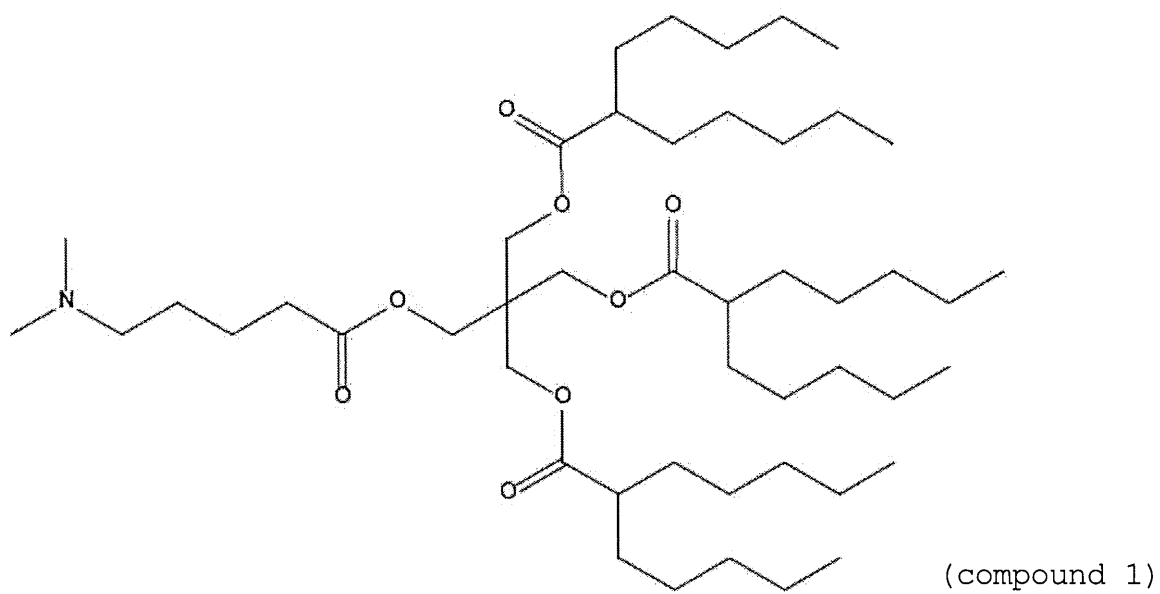
[0032]

5 and salts thereof.

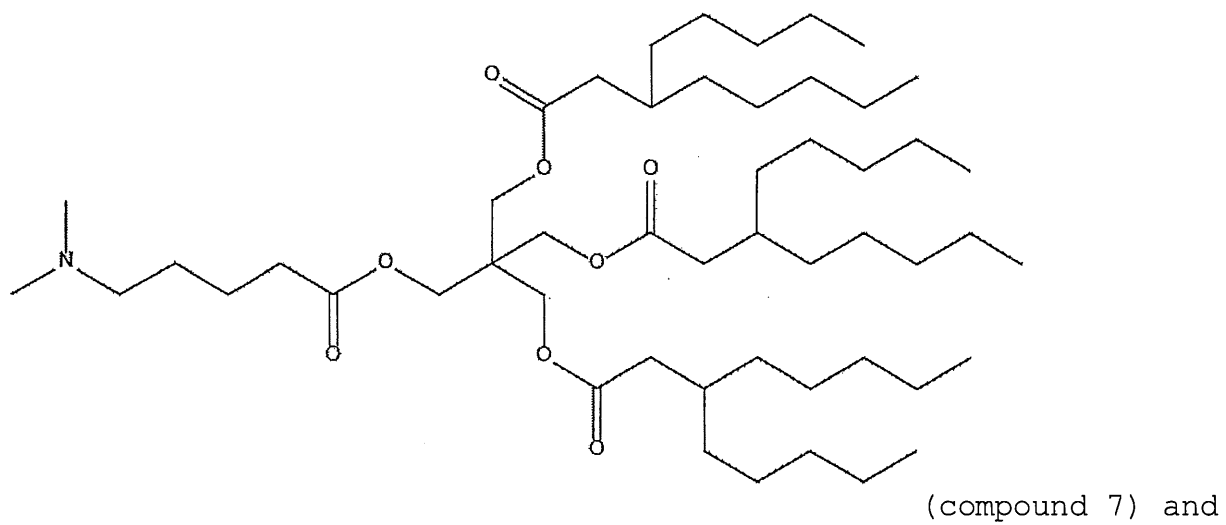
[0033]

Among the above-mentioned cationic lipids, more preferred cationic lipids are represented by the following structural formulas.

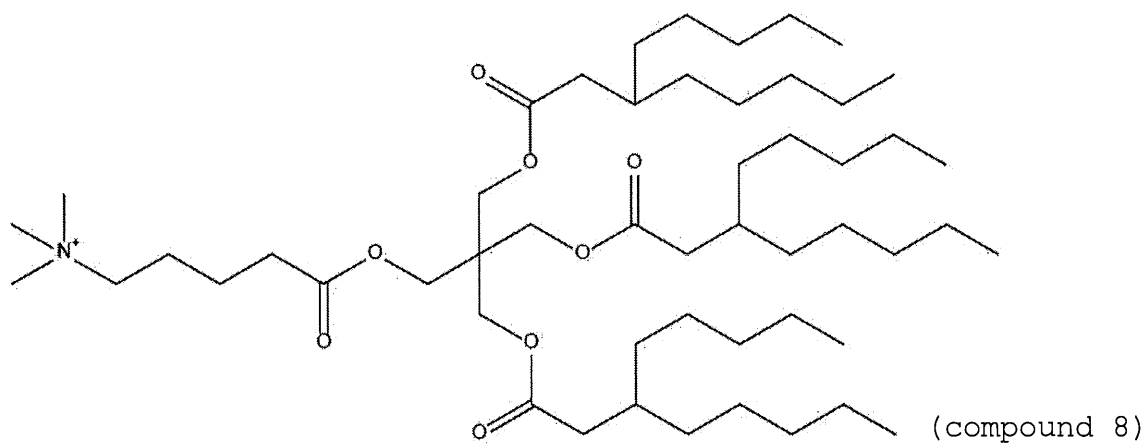
10 [0034]



[0035]



[0036]



5

[0037]

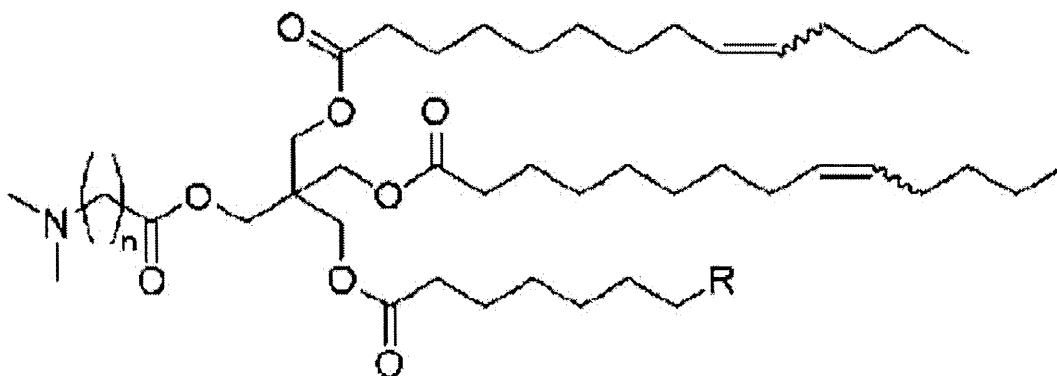
and salts thereof.

[0038]

In another preferred embodiment, a cationic lipid represented by the following structural formula and described
5 in WO 2019/131839 can be mentioned.

[0039]

A compound represented by



[0040]

10 wherein

n is an integer of 2 to 5,

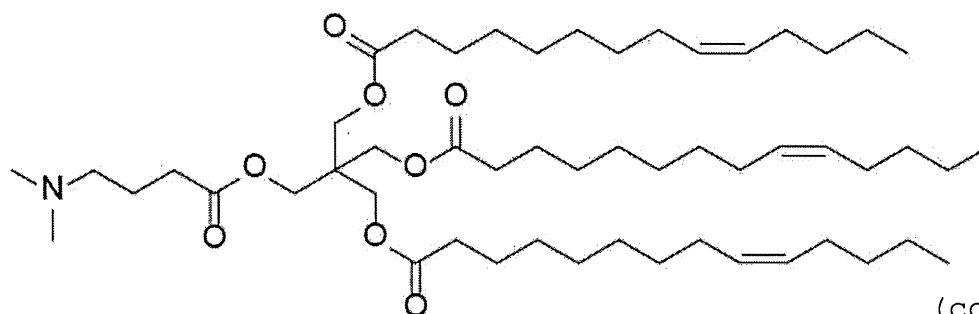
R is a linear C₁₋₅ alkyl group, a linear C₇₋₁₁ alkenyl group or a linear C₁₁ alkadienyl group, and

wavy lines are each independently shows a cis-type or
15 trans-type bond,
or a salt thereof.

[0041]

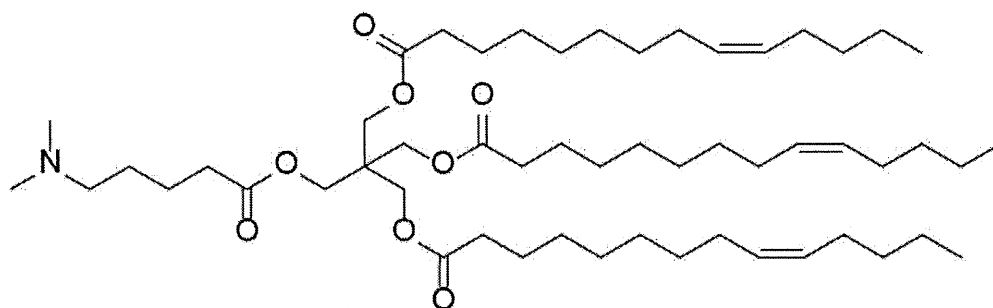
More preferably, cationic lipids represented by the following structural formulas can be mentioned.

20 [0042]



(compound 11)

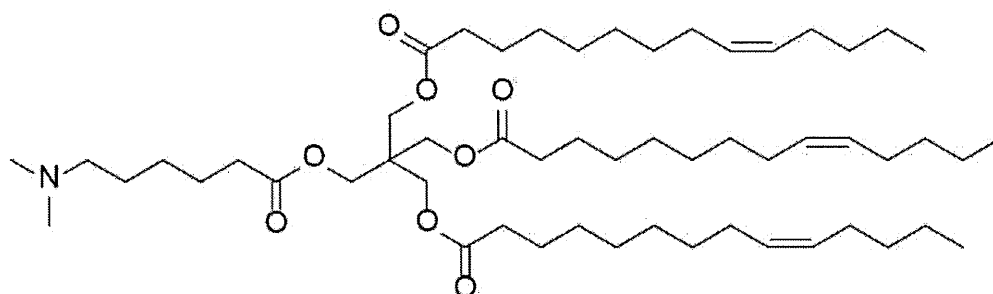
[0043]



(compound 12)

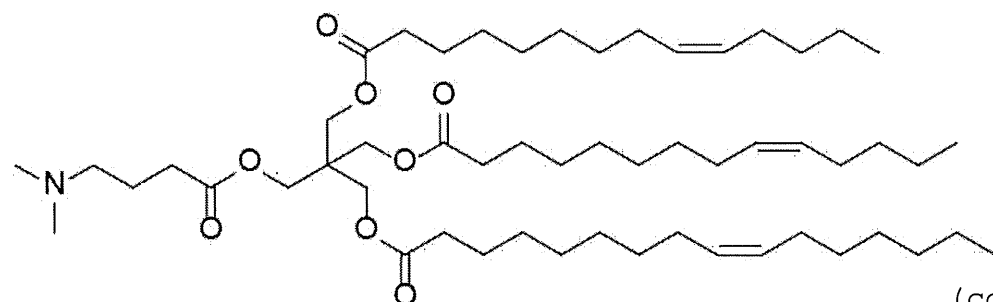
[0044]

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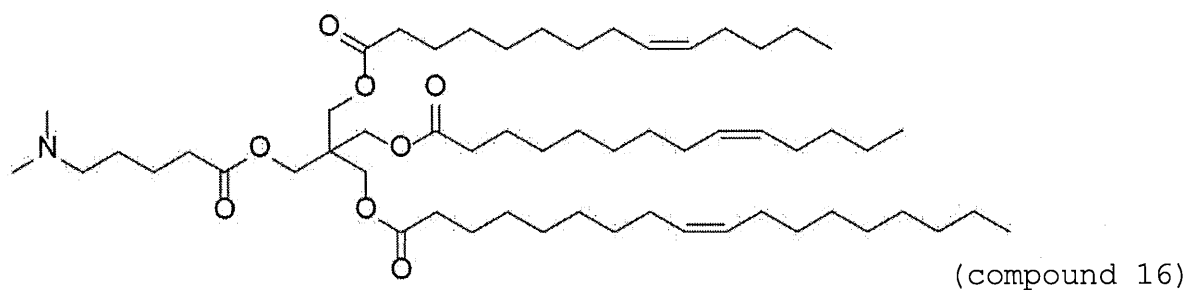
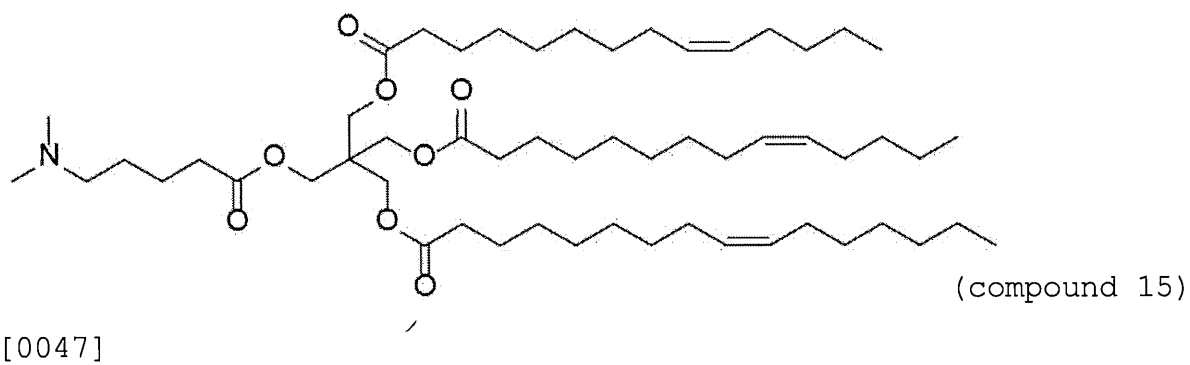
(compound 13)

[0045]

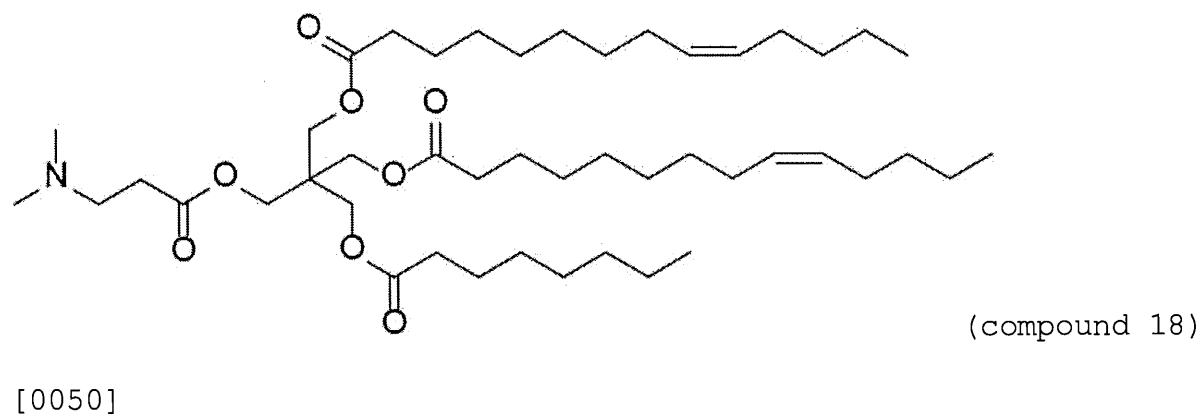
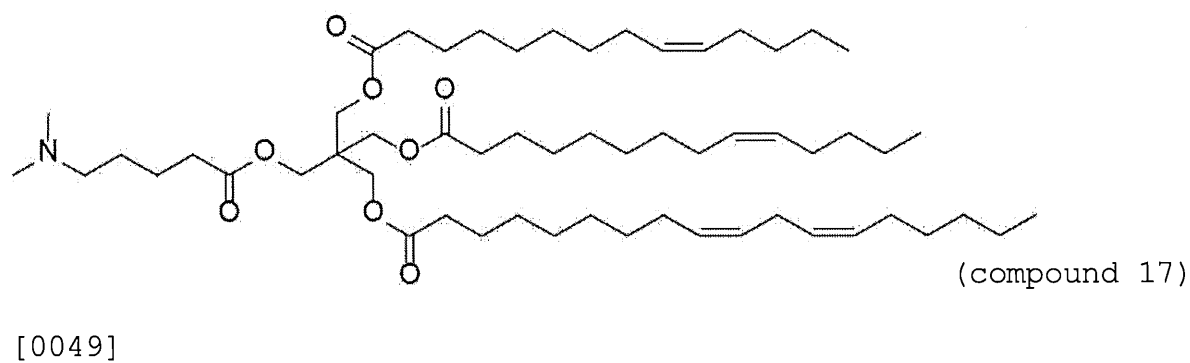


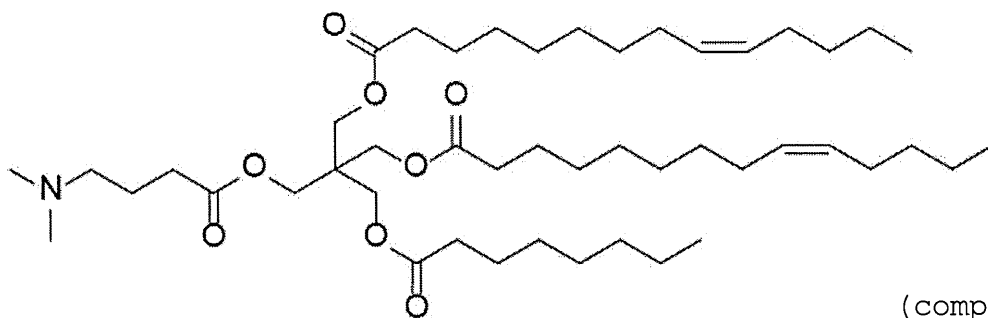
(compound 14)

10 [0046]



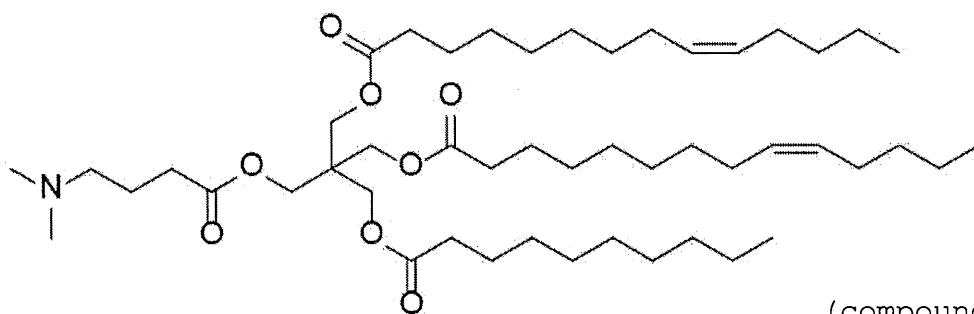
5 [0048]





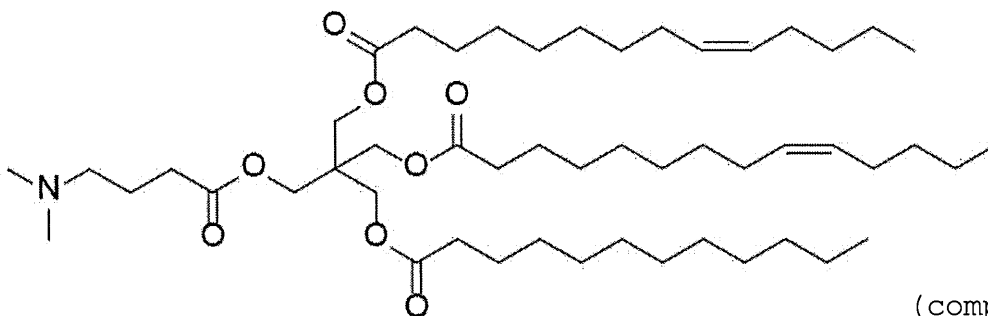
(compound 19)

[0051]



(compound 20) and

5 [0052]



(compound 21)

[0053]

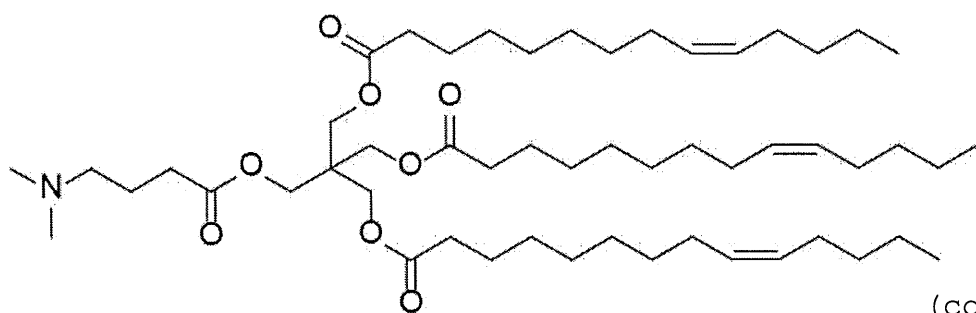
and salts thereof.

10 [0054]

Among the above-mentioned cationic lipids, more preferred cationic lipids are represented by the following structural formulas.

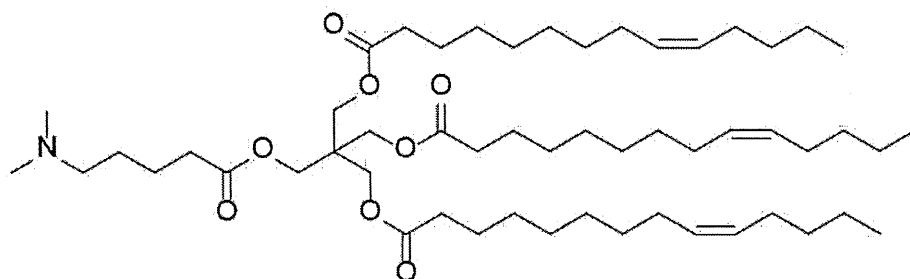
[0055]

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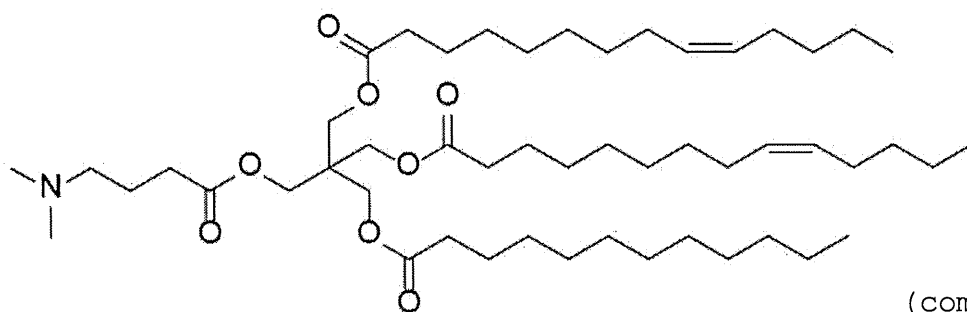
(compound 11)

[0056]



(compound 12) and

5 [0057]



(compound 21)

[0058]

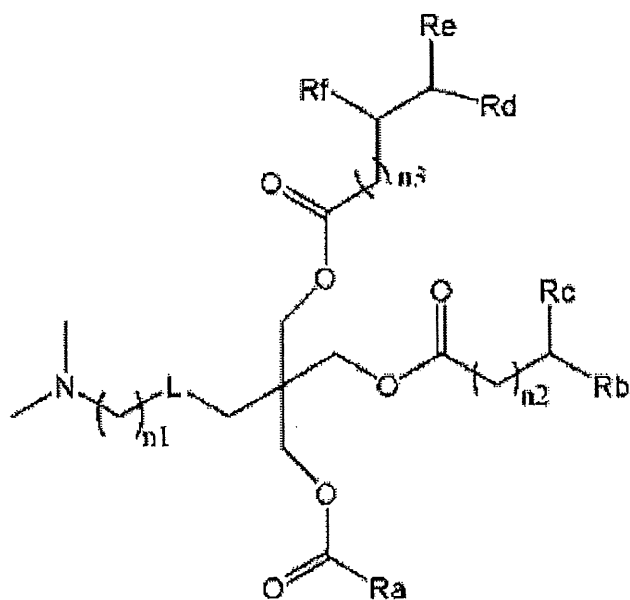
and salts thereof.

[0059]

10 In another preferred embodiment, a cationic lipid represented by the following general formula (III) can be mentioned.

[0060]

A compound represented by



(III)

[0061]

wherein

n_1 is an integer of 2 - 6,

5 n_2 is an integer of 0 - 2,

n_3 is an integer of 0 - 2,

L is $-\text{C}(\text{O})\text{O}-$ or $-\text{NHC}(\text{O})\text{O}-$,

Ra is a linear C_{5-13} alkyl group, a linear C_{13-17} alkenyl group or a linear C_{17} alkadienyl group,

10 Rb is a linear C_{2-9} alkyl group,

Rc is a hydrogen atom or a linear C_{2-9} alkyl group,

Rd is a hydrogen atom or a linear C_{2-9} alkyl group,

Re is a linear C_{2-9} alkyl group, and

Rf is a linear C_{2-9} alkyl group,

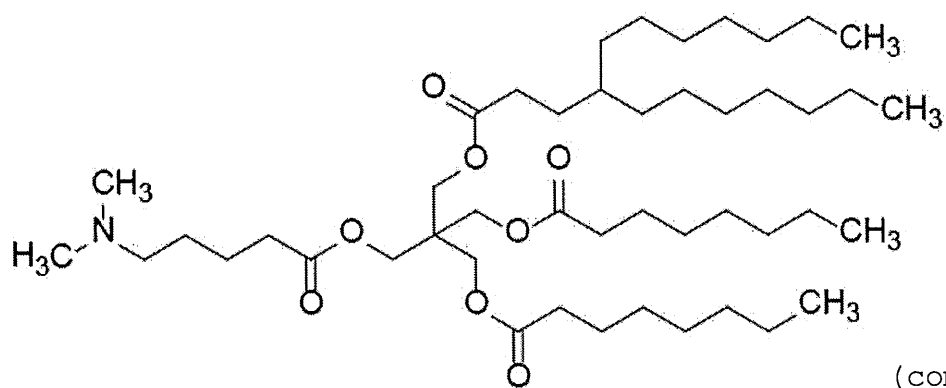
15 or a salt thereof.

[0062]

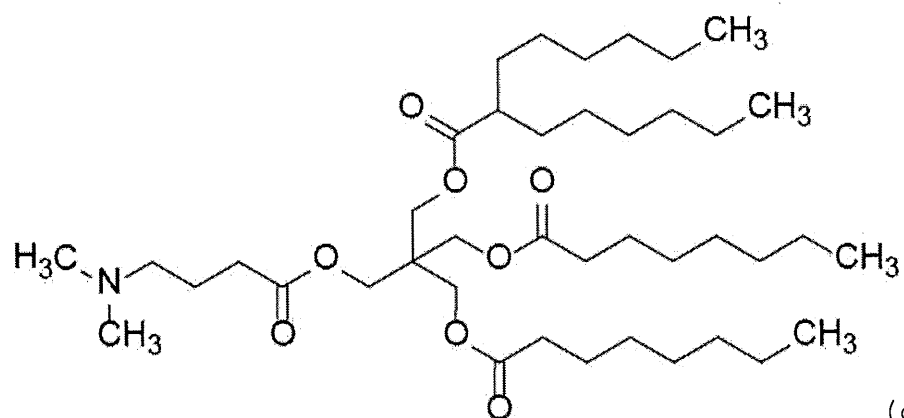
More preferably, cationic lipids represented by the following structural formulas can be mentioned.

[0063]

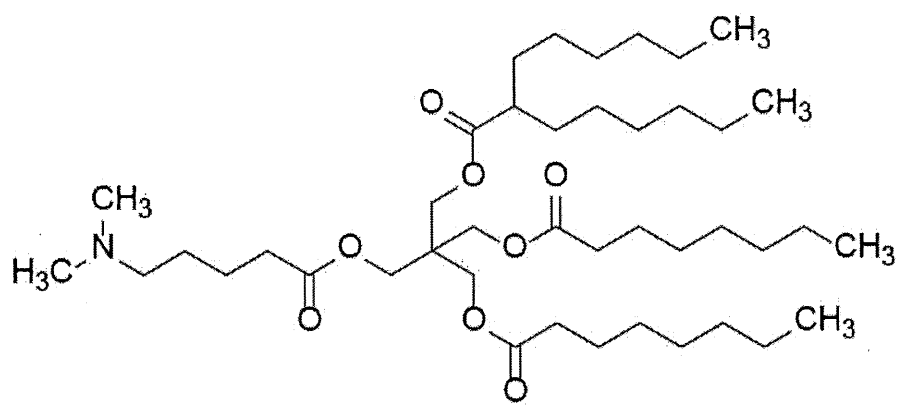
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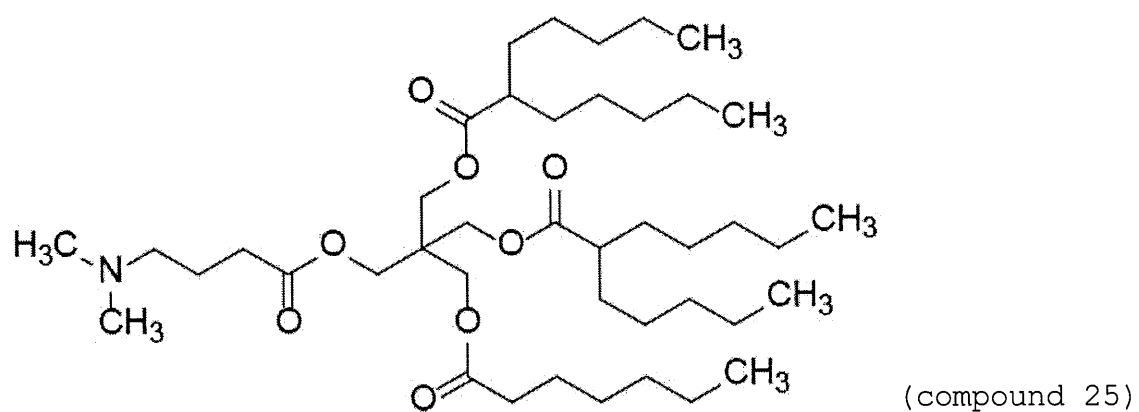
[0064]



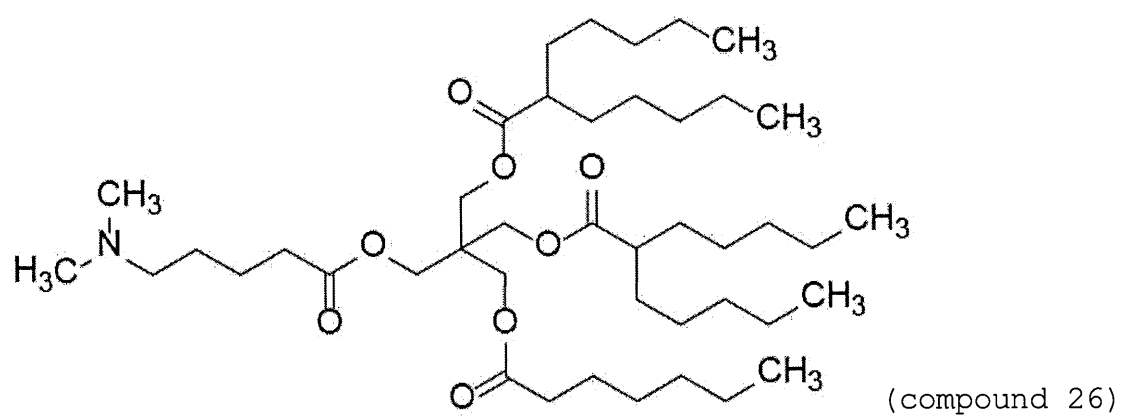
5 [0065]



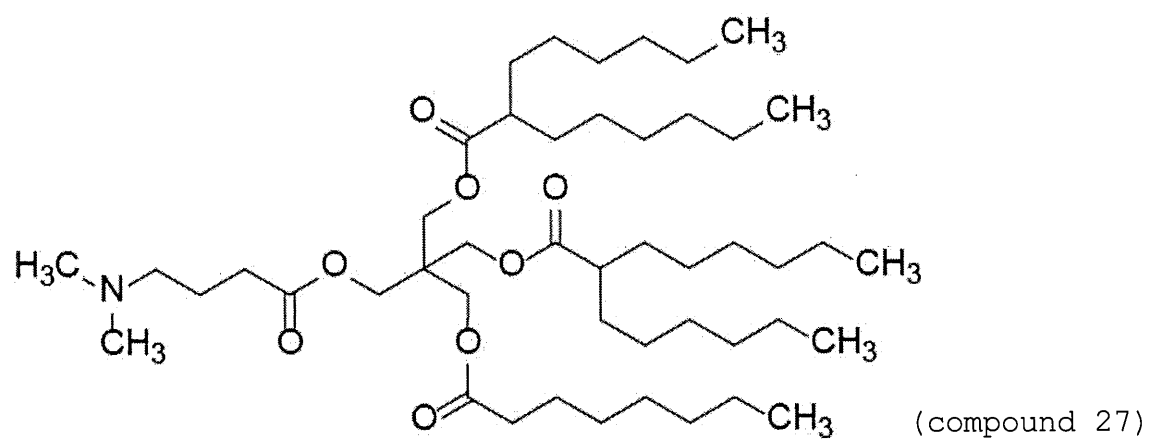
[0066]



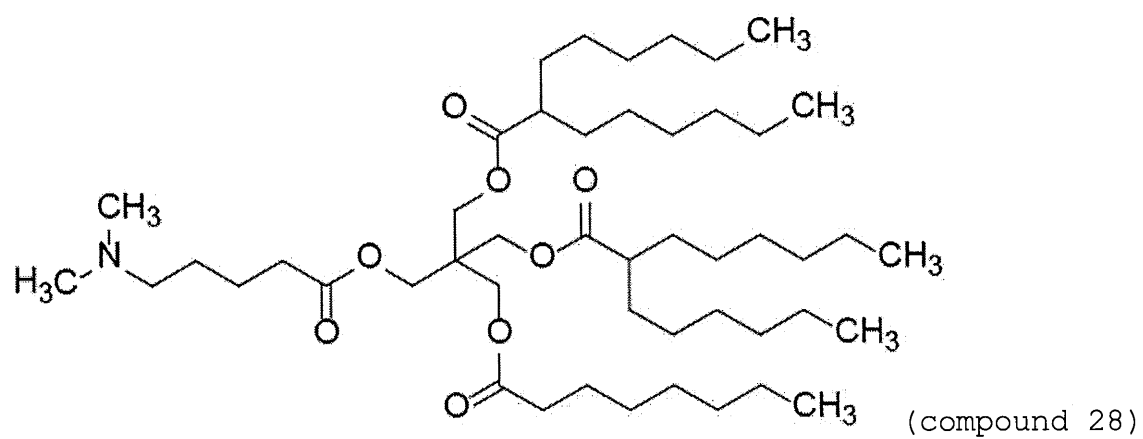
[0067]



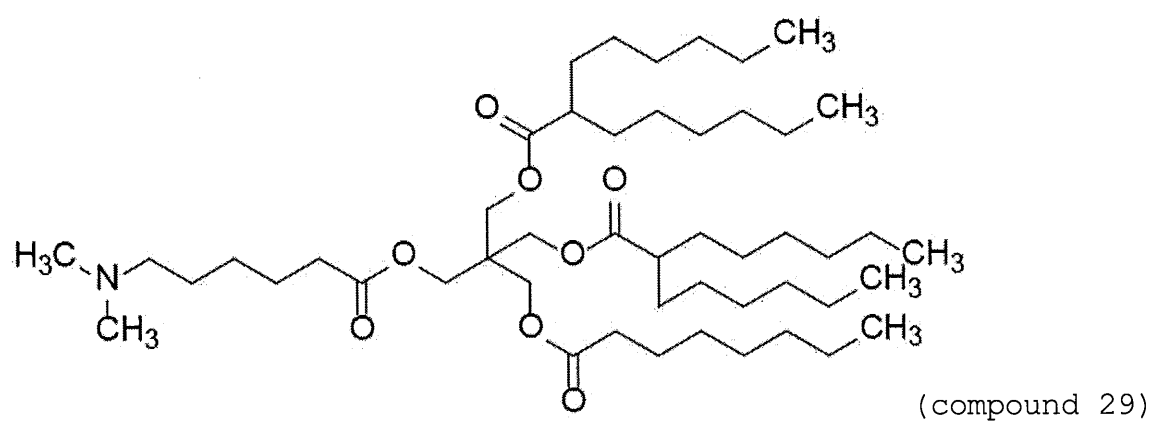
5 [0068]



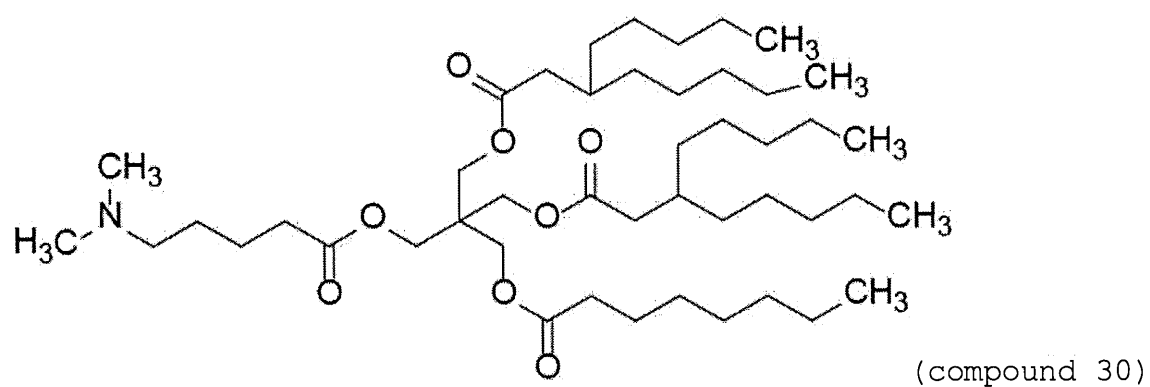
[0069]



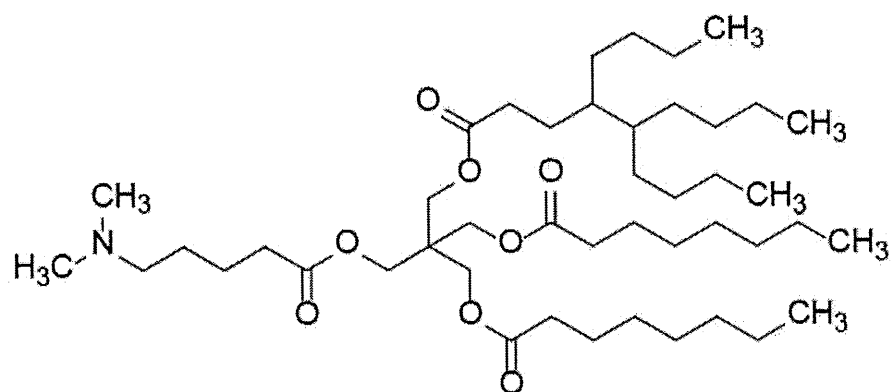
[0070]



5 [0071]

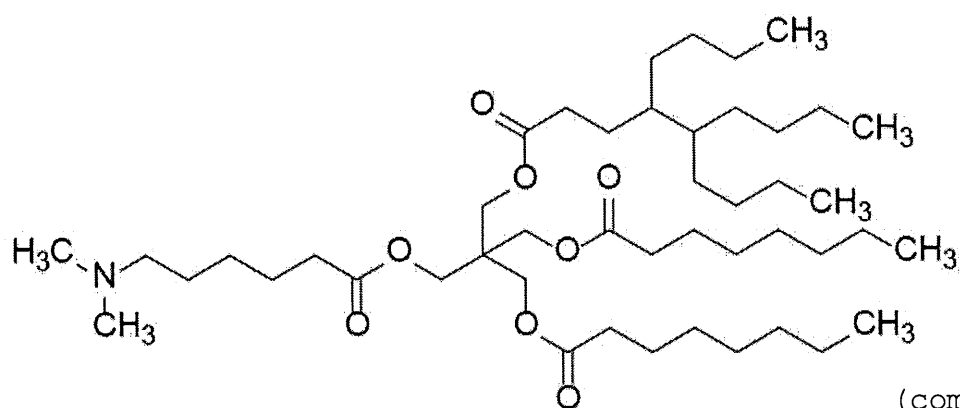


[0072]



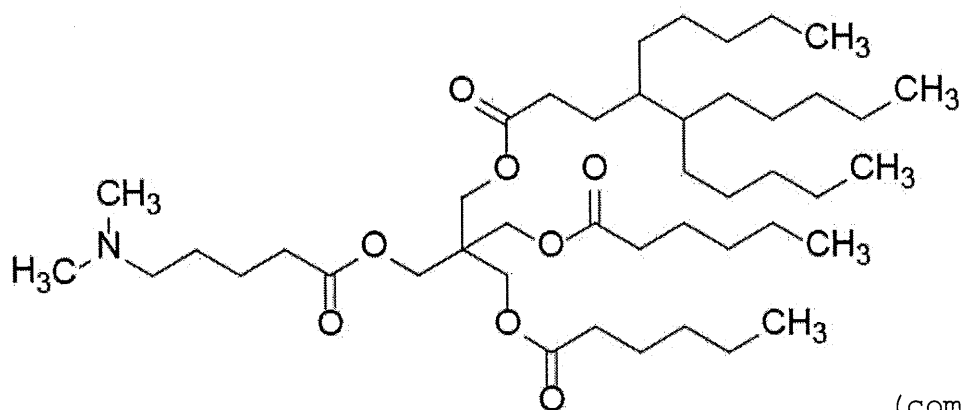
(compound 31)

[0073]



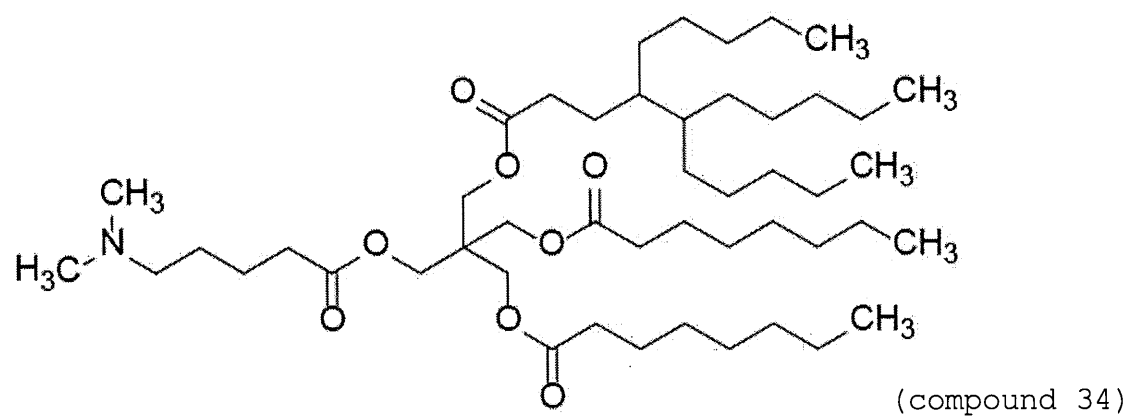
(compound 32)

5 [0074]

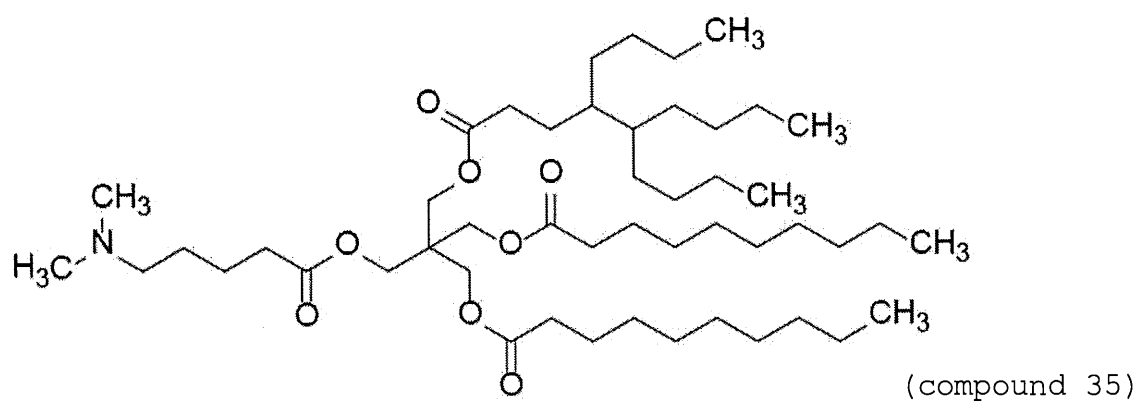


(compound 33)

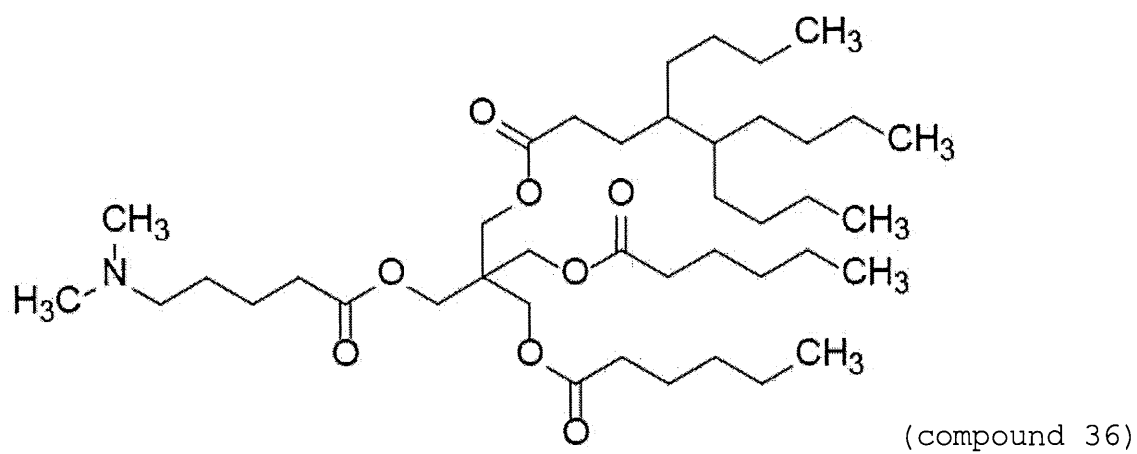
[0075]



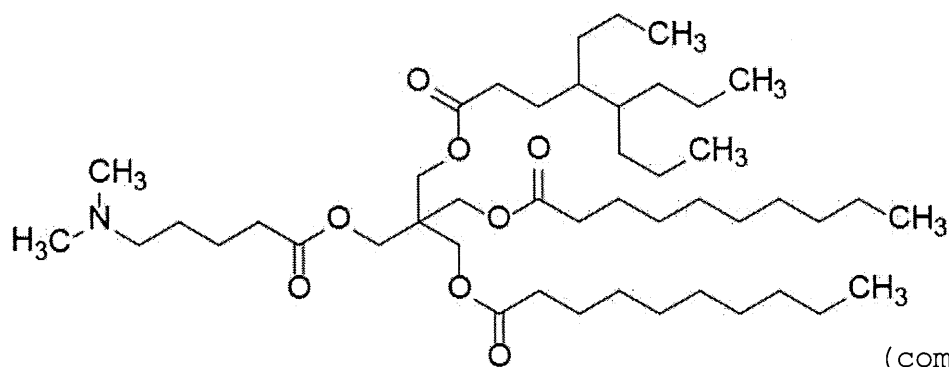
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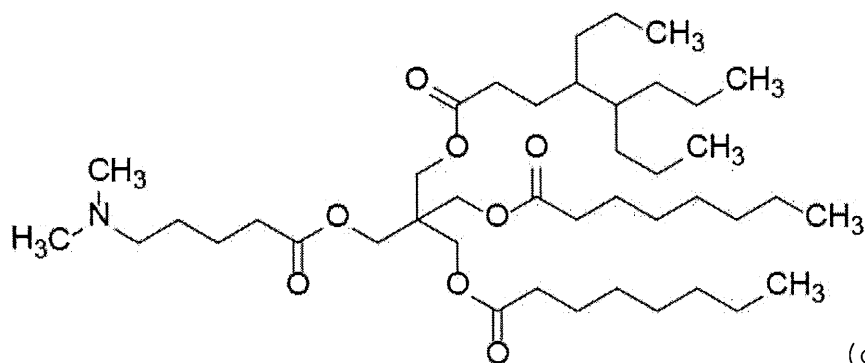
5 [0077]



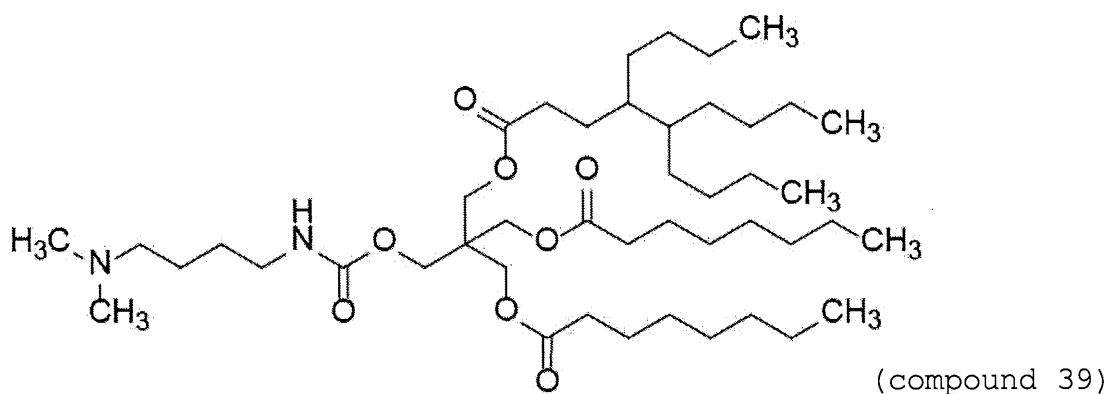
[0078]



[0079]



5 [0080]



[0081]

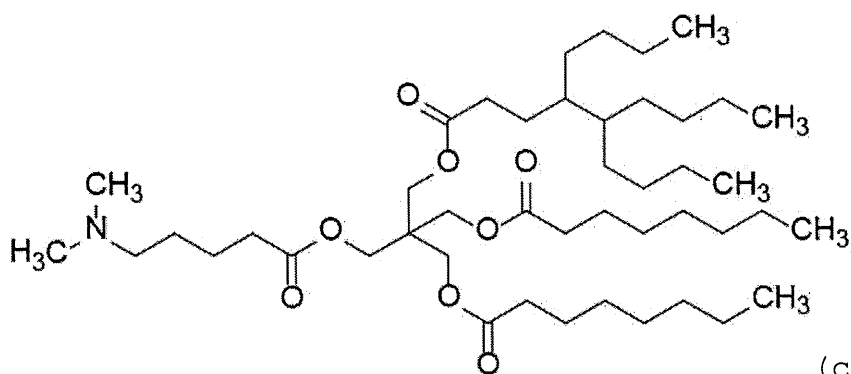
and salts thereof.

10 [0082]

Among the above-mentioned cationic lipids, more preferred are cationic lipids represented by the following structural formulas.

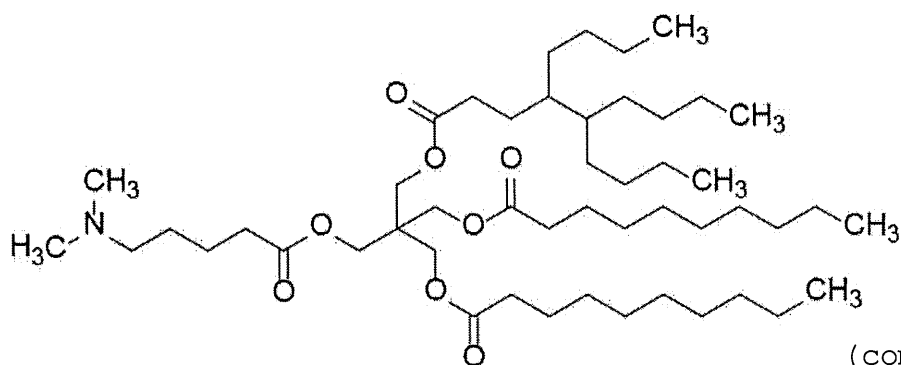
[0083]

15



(compound 31) and

[0084]



(compound 35)

5 [0085]

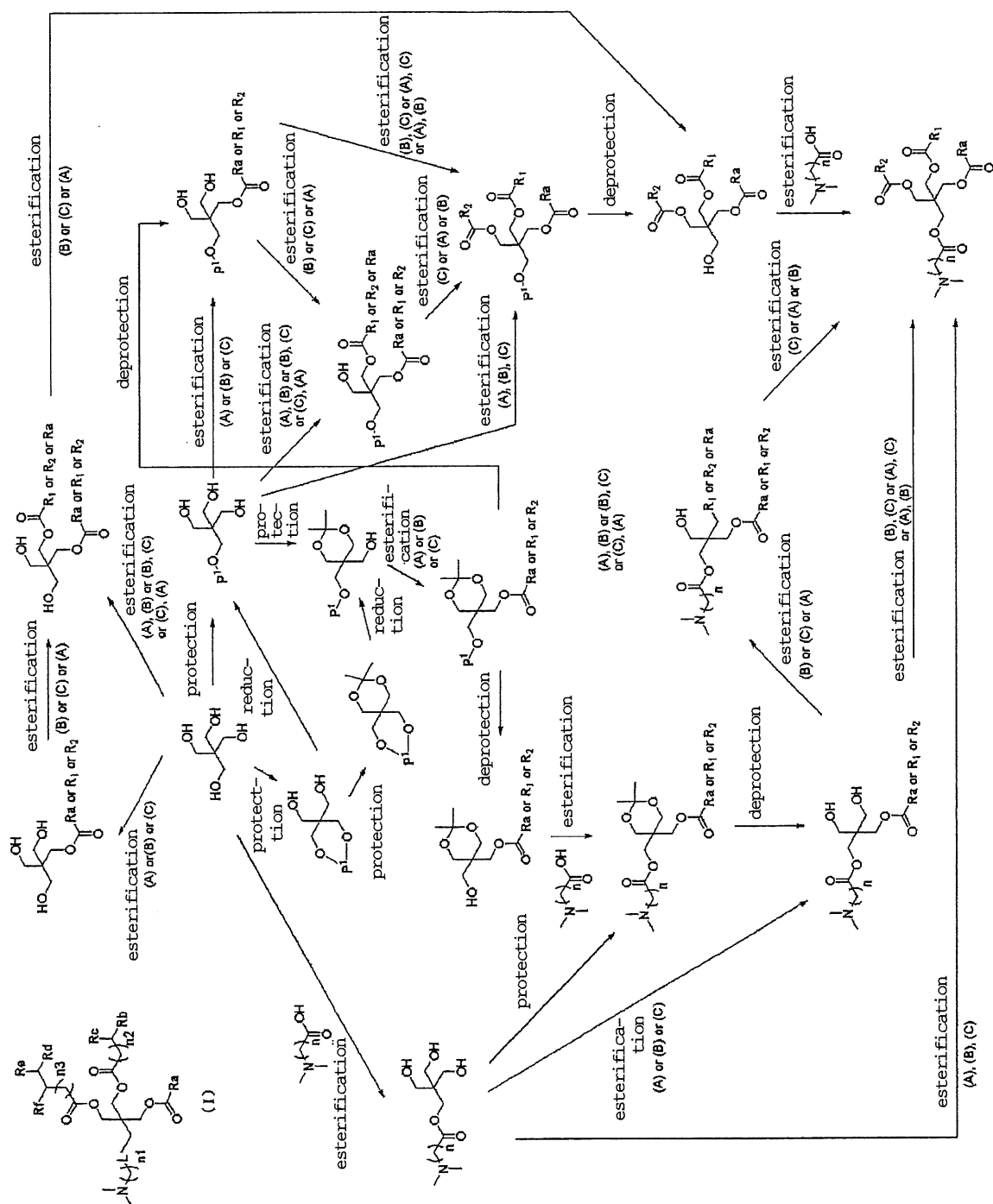
and salts thereof.

[0086]

The compound (III) can be produced, for example, by the following production method. In particular, compound (I) with
 10 a desired structure can be synthesized using appropriate starting materials according to the structure of the desired compound (III) in the esterification process. The salt of compound (III) can be obtained by appropriately mixing with an inorganic base, an organic base, an organic acid, a basic or an
 15 acidic amino acid.

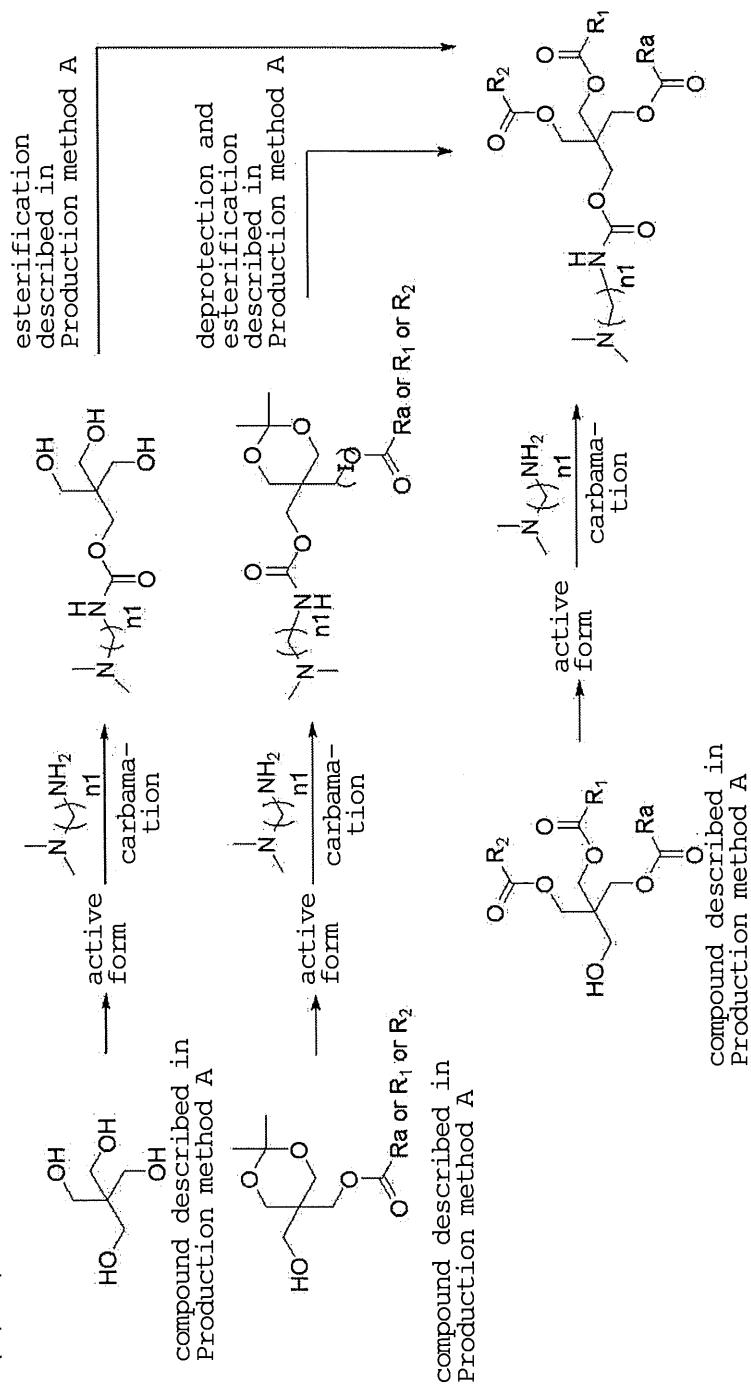
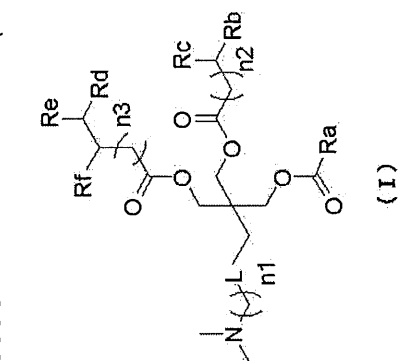
[0087]

Production method A (L is $-C(O)O-$)



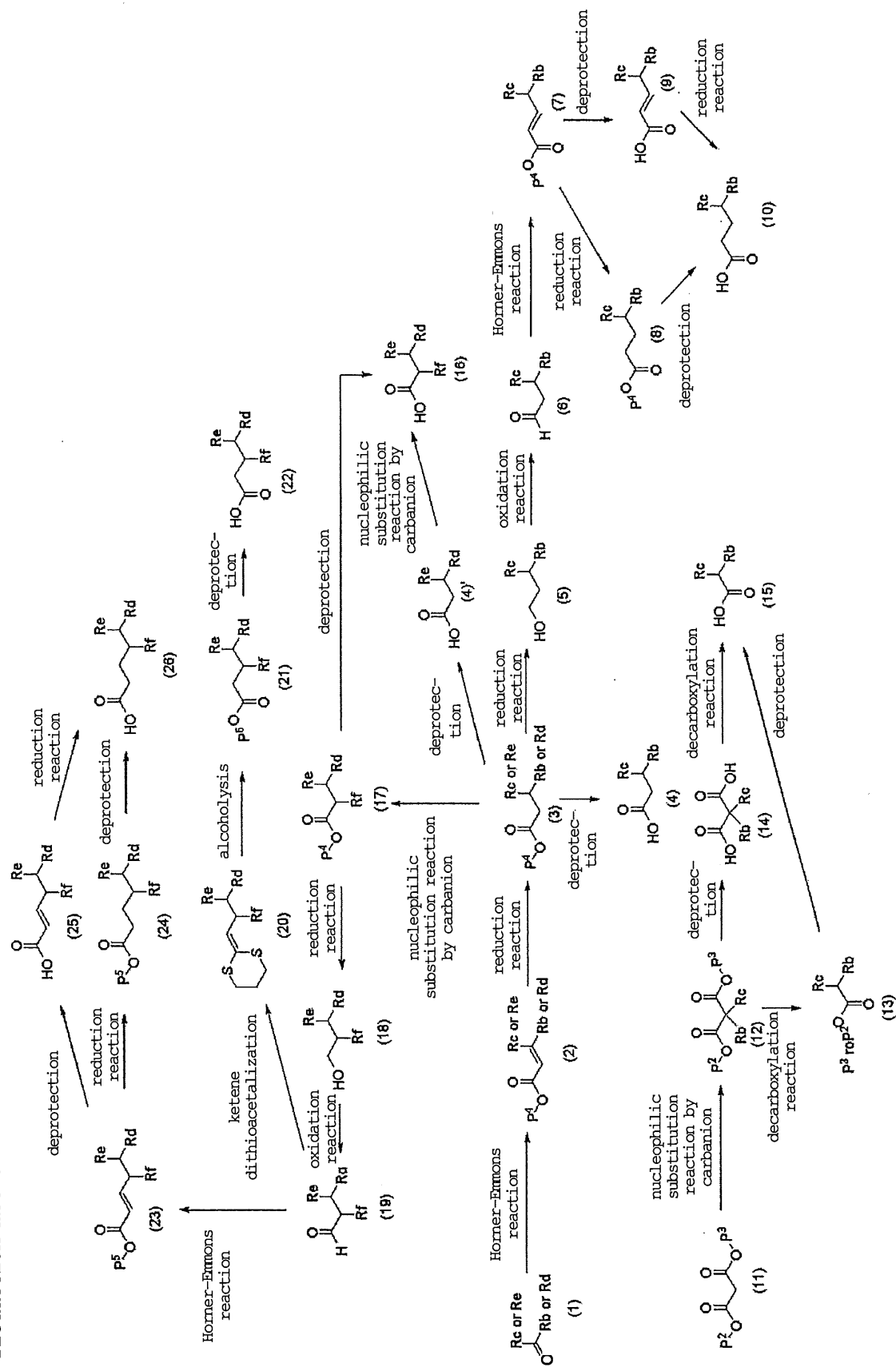
[0088]

Production method B (L is -NHC(O)O-)



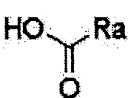
[0089]

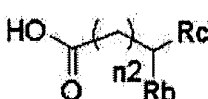
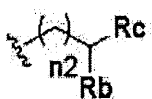
Production method C

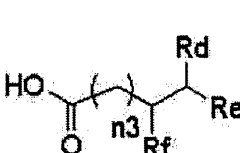
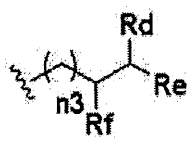


[0090]

In the above formulas, P^1 , P^2 , P^3 , P^4 , P^5 and P^6 are each independently protecting groups, compound (A) is

the formula:  , compound (B) is

the formula:  , R^1 is  , compound (C) is

the formula:  and R^2 is  .

[0091]

5 A starting material or a reagent used in each step in the above-mentioned production method, as well as the obtained compound, may each form a salt.

[0092]

When the compound obtained in each step is a free
10 compound, this compound can be converted to a salt of interest by a method known per se in the art. On the contrary, when the compound obtained in each step is a salt, this salt can be converted to a free form or another type of salt of interest by a method known per se in the art.

15 [0093]

The compound obtained in each step may be used in the next reaction directly in the form of its reaction solution or after being obtained as a crude product. Alternatively, the compound obtained in each step can be isolated and/or purified from the
20 reaction mixture by a separation approach such as concentration, crystallization, recrystallization, distillation, solvent extraction, fractionation, or chromatography according to a routine method.

[0094]

25 If a starting material or a reagent compound for each step is commercially available, the commercially available product

can be used directly.

[0095]

In the reaction of each step, the reaction time can differ depending on the reagent or the solvent used and is
5 usually 1 min to 48 hr, preferably 10 min to 8 hr, unless otherwise specified.

[0096]

In the reaction of each step, the reaction temperature can differ depending on the reagent or the solvent used and is
10 usually -78°C to 300°C, preferably -78°C to 150°C, unless otherwise specified.

[0097]

In the reaction of each step, the pressure can differ depending on the reagent or the solvent used and is usually 1
15 atm to 20 atm, preferably 1 atm to 3 atm, unless otherwise specified.

[0098]

In the reaction of each step, for example, a microwave synthesis apparatus such as a Biotage Initiator may be used.
20 The reaction temperature can differ depending on the reagent or the solvent used and is usually room temperature to 300°C, preferably room temperature to 250°C, more preferably 50°C to 250°C, unless otherwise specified. The reaction time can differ depending on the reagent or the solvent used and is usually 1
25 min to 48 hr, preferably 1 min to 8 hr, unless otherwise specified.

[0099]

In the reaction of each step, the reagent is used at 0.5 equivalents to 20 equivalents, preferably 0.8 equivalents to 5
30 equivalents, with respect to the substrate, unless otherwise specified. In the case of using the reagent as a catalyst, the reagent is used at 0.001 equivalents to 1 equivalent, preferably 0.01 equivalents to 0.2 equivalents, with respect to the substrate. When the reagent also serves as a reaction
35 solvent, the reagent is used in the amount for the solvent.

[0100]

In each step of a reaction, the reaction is carried out without a solvent or by dissolution or suspension in an appropriate solvent, unless otherwise specified. Specific
5 examples of the solvent include the following.

[0101]

alcohols: methanol, ethanol, isopropanol, isobutanol, tert-butyl alcohol, 2-methoxyethanol and the like;

ethers: diethyl ether, diisopropyl ether, diphenyl ether,
10 tetrahydrofuran, 1,2-dimethoxyethane and the like;

aromatic hydrocarbons: chlorobenzene, toluene, xylene and the like;

saturated hydrocarbons: cyclohexane, hexane, heptane and the like;

15 amides: N,N-dimethylformamide, N-methylpyrrolidone and the like;

halogenated hydrocarbon s: dichloromethane, carbon tetrachloride and the like;

nitriles: acetonitrile and the like;

20 sulfoxide: dimethyl sulfoxide and the like;

aromatic organic bases: pyridine and the like;

acid anhydrides: acetic anhydride and the like;

organic acids: formic acid, acetic acid, trifluoroacetic acid and the like;

25 inorganic acids: hydrochloric acid, sulfuric acid and the like;

esters: ethyl acetate, isopropyl acetate ester and the like;

ketones: acetone, methyl ethyl ketone and the like;

30 water.

Two or more of these solvents may be used as a mixture at an appropriate ratio.

[0102]

In each reaction step making use of a base, examples of
35 bases that may be used are those listed below.

[0103]

inorganic bases: sodium hydroxide, potassium hydroxide, magnesium hydroxide and the like;

basic salts: sodium carbonate, calcium carbonate, sodium
5 hydrogen carbonate and the like;

organic bases: triethylamine, diethylamine, pyridine, 4-dimethylaminopyridine, N,N-dimethylaniline, 1,4-diazabicyclo[2.2.2]octane, 1,8-diazabicyclo[5.4.0]-7-undecene, imidazole, piperidine and the like;

10 metal alkoxides: sodium ethoxide, potassium tert-butoxide, sodium tert-butoxide and the like;

alkali metal hydrides: sodium hydride and the like;

metal amides: sodium amide, lithium diisopropylamide, lithium hexamethyldisilazide and the like;

15 organic lithiums: n-butyllithium, sec-butyllithium and the like.

[0104]

In each reaction step making use of an acid or acid catalyst, the following acids or acid catalysts are used.

20 [0105]

inorganic acids: hydrochloric acid, sulfuric acid, nitric acid, hydrobromic acid, phosphoric acid and the like;

organic acids: acetic acid, trifluoroacetic acid, citric acid, p-toluenesulfonic acid, 10-camphor sulfonic acid and the
25 like;

Lewis acid: boron trifluoride diethyl ether complex, zinc iodide, anhydrous aluminum chloride, anhydrous zinc chloride, anhydrous iron chloride and the like.

[0106]

30 Unless stated otherwise, each reaction step may be carried out according to a method known per se in the art, such as those described in Jikken Kagaku Koza (Encyclopedia of Experimental Chemistry in English), 5th Ed., Vol. 13 to Vol. 19 (edited by the Chemical Society of Japan); Shin Jikken Kagaku
35 Koza (New Encyclopedia of Experimental Chemistry in English),

Vol. 14 to Vol. 15 (edited by the Chemical Society of Japan);
Fine Organic Chemistry, 2nd Ed. Revised (L. F. Tietze, Th.
Eicher, Nankodo); Organic Name Reactions; The Reaction
Mechanism and Essence, Revised (Hideo Togo, Kodansha); Organic
5 Syntheses Collective Volume I-VII (John Wiley & Sons, Inc.);
Modern Organic Synthesis in the Laboratory: A Collection of
Standard Experimental Procedures (Jie Jack Li, Oxford
University Press); Comprehensive Heterocyclic Chemistry III,
Vol. 1 to Vol. 14 (Elsevier Japan KK); Strategic Applications
10 of Named Reactions in Organic Synthesis (translated by Kiyoshi
Tomioka, Kagaku-Dojin Publishing); Comprehensive Organic
Transformations (VCH Publishers, Inc.), 1989; etc.

[0107]

In each step, the protection or deprotection reaction of
15 a functional group may be carried out according to a method
known per se in the art, for example, a method described in
"Protective Groups in Organic Synthesis, 4th Ed." (Theodora W.
Greene, Peter G. M. Wuts), Wiley-Interscience, 2007;
"Protecting Groups, 3rd Ed." (P.J. Kocienski) Thieme, 2004);
20 etc.

[0108]

Examples of a protective group for a hydroxy group or a
phenolic hydroxy group in alcohols or the like include: ether-
type protective groups such as methoxymethyl ether, benzyl
25 ether, p-methoxybenzyl ether, t-butyldimethylsilyl ether, t-
butyldiphenylsilyl ether, and tetrahydropyranyl ether;
carboxylic acid ester-type protective groups such as acetic
acid ester; sulfonic acid ester-type protective groups such as
methanesulfonic acid ester; and carbonic acid ester-type
30 protective groups such as t-butyl carbonate.

[0109]

Examples of a protective group for a carbonyl group in
aldehydes include: acetal-type protective groups such as
dimethylacetal; and cyclic acetal-type protective groups such
35 as cyclic 1,3-dioxane.

[0110]

Examples of a protective group for a carbonyl group in ketones include: ketal-type protective groups such as dimethylketal; cyclic ketal-type protective groups such as
5 cyclic 1,3-dioxane; oxime-type protective groups such as O-methyloxime; and hydrazone-type protective groups such as N,N-dimethylhydrazone.

[0111]

Examples of a protective group for a carboxyl group
10 include: ester-type protective groups such as methyl ester; and amide-type protective groups such as N,N-dimethylamide.

[0112]

Examples of a protective group for thiol include: ether-type protective groups such as benzyl thioether; and ester-type
15 protective groups such as thioacetic acid ester, thiocarbonate and thiocarbamate.

[0113]

Examples of a protective group for an amino group or aromatic heterocycle such as imidazole, pyrrole or indole
20 include: carbamate-type protective groups such as benzyl carbamate; amide-type protective groups such as acetamide; alkylamine-type protective groups such as N-triphenylmethylaniline; and sulfonamide-type protective groups such as methanesulfonamide.

25 [0114]

The protective groups can be removed by use of a method known per se in the art, for example, a method using an acid, a base, ultraviolet light, hydrazine, phenylhydrazine, sodium N-methyldithiocarbamate, tetrabutylammonium fluoride, palladium
30 acetate or trialkylsilyl halide (e.g., trimethylsilyl iodide or trimethylsilyl bromide), or a reduction method.

[0115]

In each step making use of a reduction reaction, examples of reducing agents that may be used include: metal hydrides
35 such as lithium aluminum hydride, sodium triacetoxyborohydride,

sodium cyanoborohydride, diisobutyl aluminum hydride (DIBAL-H), sodium borohydride and tetramethylammonium triacetoxymethylborohydride; boranes such as borane-tetrahydrofuran complex; Raney nickel; Raney cobalt; hydrogen; and formic acid.

5 For example, Raney-nickel or Raney cobalt can be used in the presence of hydrogen or formic acid. In the case of reducing a carbon-carbon double bond or triple bond, a method using a catalyst such as palladium-carbon or Lindlar's catalyst may be used.

10 [0116]

In each step making use of an oxidation reaction, examples of oxidizing agents that may be used include: peracids such as m-chloroperoxybenzoic acid (MCPBA), hydrogen peroxide and t-butyl hydroperoxide; perchlorates such as tetrabutylammonium perchlorate; chlorates such as sodium chlorate; chlorites such as sodium chlorite; periodates such as sodium periodate; high-valent iodine reagents such as iodosylbenzene; manganese reagents, such as manganese dioxide and potassium permanganate; lead reagents such as lead tetraacetate; chromium reagents, such as pyridinium chlorochromate (PCC), pyridinium dichromate (PDC) and Jones' reagent; halogen compounds such as N-bromosuccinimide (NBS); oxygen; ozone; sulfur trioxide-pyridine complex; osmium tetroxide; selenium dioxide; and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).

25 [0117]

In each step making use of a radical cyclization reaction, examples of radical initiators that may be used include: azo compounds such as azobisisobutyronitrile (AIBN); water-soluble radical initiators such as 4-4'-azobis-4-cyanopentanoic acid (ACPA); triethylboron in the presence of air or oxygen; and benzoyl peroxide. Examples of radical initiators to be used include tributylstannane, tris(trimethylsilyl)silane, 1,1,2,2-tetraphenyldisilane, diphenylsilane and samarium iodide.

[0118]

35 In each step making use of a Wittig reaction, examples of

Wittig reagents that may be used include alkylidenephosphoranes. The alkylidenephosphoranes can be prepared by a method known per se in the art, for example, the reaction between a phosphonium salt and a strong base.

5 [0119]

In each step making use of a Horner-Emmons reaction, examples of reagents that may be used include phosphonoacetic acid esters such as methyl dimethylphosphonoacetate and ethyl diethylphosphonoacetate, and bases such as alkali metal hydrides
10 and organic lithiums.

[0120]

In each step making use of a Friedel-Crafts reaction, examples of reagents that may be used include a Lewis acid and an acid chloride or alkylating agent (e.g. alkyl halides,
15 alcohols and olefins). Alternatively, an organic or inorganic acid may be used instead of the Lewis acid, and acid anhydrides such as acetic anhydride may be used instead of the acid chloride.

[0121]

20 In each step making use of an aromatic nucleophilic substitution reaction, a nucleophile (e.g., amine or imidazole) and a base (e.g., basic salts or organic bases) may be used as reagents.

[0122]

25 In each step making use of a nucleophilic addition reaction using a carbanion, nucleophilic 1,4-addition reaction (Michael addition reaction) using a carbanion, or nucleophilic substitution reaction using a carbanion, examples of bases that may be used for generating the carbanion include organolithium
30 reagents, metal alkoxides, inorganic bases and organic bases.

[0123]

In each step making use of a Grignard reaction, examples of Grignard reagents include aryl magnesium halides such as phenyl magnesium bromide, and alkyl magnesium halides such as
35 methyl magnesium bromide, isopropyl magnesium bromide. The

Grignard reagent can be prepared by a method known per se in the art, for example, the reaction between an alkyl halide or aryl halide and magnesium metal in ether or tetrahydrofuran as a solvent.

5 [0124]

In each step making use of a Knoevenagel condensation reaction, an active methylene compound flanked by two electron-attracting groups (e.g., malonic acid, diethyl malonate or malononitrile) and a base (e.g., organic bases, metal alkoxides
10 or inorganic bases) may be used as reagents.

[0125]

In each step making use of a Vilsmeier-Haack reaction, phosphoryl chloride and an amide derivative (e.g. N,N-dimethylformamide) may be used as reagents.

15 [0126]

In each step making use of an azidation reaction of alcohols, alkyl halides or sulfonic acid esters, examples of azidating agents that may be used include diphenylphosphorylazide (DPPA), trimethylsilylazide and sodium
20 azide. In the case of azidating, for example, alcohols, a method using diphenylphosphorylazide and 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU), a method using trimethylsilylazide and Lewis acid, or the like can be used.

[0127]

25 In each step making use of a reductive amination reaction, examples of reducing agents that may be used include sodium triacetoxyborohydride, sodium cyanoborohydride, hydrogen and formic acid. When the substrate is an amine compound, examples of carbonyl compounds that may be used include p-formaldehyde
30 as well as aldehydes such as acetaldehyde and ketones such as cyclohexanone. When the substrate is a carbonyl compound, examples of amines that may be used include primary amines such as ammonia and methylamine, and secondary amines such as dimethylamine.

35 [0128]

In each step making use of a Mitsunobu reaction, azodicarboxylic acid esters (e.g. diethyl azodicarboxylate (DEAD) and diisopropyl azodicarboxylate (DIAD)) and triphenylphosphine may be used as reagents.

5 [0129]

In each step making use of an esterification, amidation or ureation reaction, examples of reagents that may be used include acyl halides such as acid chlorides or acid bromides, and activated carboxylic acids such as acid anhydrides, active
10 esters or sulfate esters. Examples of the activating agents for carboxylic acids include: carbodiimide condensing agents such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSCD); triazine condensing agents such as 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium
15 chloride-n-hydrate (DMT-MM); carbonic acid ester condensing agents such as 1,1-carbonyldiimidazole (CDI); diphenylphosphorylazide (DPPA); benzotriazol-1-yloxy-trisdimethylaminophosphonium salt (BOP reagent); 2-chloro-1-methyl-pyridinium iodide (Mukaiyama reagent); thionyl chloride;
20 lower alkyl haloformate such as ethyl chloroformate; O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU); sulfuric acid; and combinations thereof. In the case of using a carbodiimide condensing agent, the addition of an additive such as 1-hydroxybenzotriazole
25 (HOBt), N-hydroxysuccinimide (HOSu) or dimethylaminopyridine (DMAP) to the reaction may be beneficial.

[0130]

In each step making use of a coupling reaction, examples of metal catalysts that may be used include palladium compounds
30 such as palladium(II) acetate, tetrakis(triphenylphosphine)palladium(0), dichlorobis(triphenylphosphine)palladium(II), dichlorobis(triethylphosphine)palladium(II), tris(dibenzylideneacetone)dipalladium(0), 1,1'-
35 bis(diphenylphosphino)ferrocene palladium(II) chloride and

palladium(II) acetate; nickel compounds such as tetrakis(triphenylphosphine)nickel(0); rhodium compounds such as tris(triphenylphosphine)rhodium(III) chloride; cobalt compounds; copper compounds such as copper oxide and copper(I) iodide; and platinum compounds. Addition of a base to the reaction may also be beneficial. Examples of such bases include inorganic bases and basic salts.

[0131]

In each step making use of a thiocarbonylation reaction, diphosphorus pentasulfide is typically used as a thiocarbonylating agent. A reagent having a 1,3,2,4-dithiadiphosphetane-2,4-disulfide structure such as 2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-disulfide (Lawesson reagent) may be used instead of diphosphorus pentasulfide.

[0132]

In each step making use of a Wohl-Ziegler reaction, examples of halogenating agents that may be used include N-iodosuccinimide, N-bromosuccinimide (NBS), N-chlorosuccinimide (NCS), bromine and sulfuryl chloride. The reaction can be accelerated by the further addition of a radical initiator such as heat, light, benzoyl peroxide or azobisisobutyronitrile.

[0133]

In each step making use of a halogenation reaction of a hydroxy group, examples of halogenating agents that may be used include a hydrohalic acid or the acid halide of an inorganic acid; examples include hydrochloric acid, thionyl chloride, and phosphorus oxychloride for chlorination and 48% hydrobromic acid for bromination. In addition, a method for obtaining an alkyl halide from an alcohol by the action of triphenylphosphine and carbon tetrachloride or carbon tetrabromide, etc., may also be used. Alternatively, a method for synthesizing an alkyl halide through a 2-step reaction involving the conversion of an alcohol to a sulfonic acid ester and subsequent reaction with lithium bromide, lithium chloride

or sodium iodide may also be used.

[0134]

In each step making use of an Arbuzov reaction, examples of reagents that may be used include alkyl halides such as
5 ethyl bromoacetate, and phosphites such as triethylphosphite and tri(isopropyl)phosphite.

[0135]

In each step making use of a sulfone-esterification reaction, examples of the sulfonylating agent used include
10 methanesulfonyl chloride, p-toluenesulfonyl chloride, methanesulfonic anhydride and p-toluenesulfonic anhydride and trifluoromethanesulfonic anhydride.

[0136]

In each step making use of a hydrolysis reaction, an acid
15 or a base may be used as a reagent. In the case of carrying out the acid hydrolysis reaction of a t-butyl ester, reagents such as formic acid, triethylsilane or the like may be added to reductively trap the by-product t-butyl cation.

[0137]

20 In each step making use of a dehydration reaction, examples of dehydrating agents that may be used include sulfuric acid, diphosphorus pentoxide, phosphorus oxychloride, N,N'-dicyclohexylcarbodiimide, alumina and polyphosphoric acid.

[0138]

25 A salt of the compound represented by the above-mentioned each structural formula is preferably a pharmacologically acceptable salt. Examples thereof include salts with inorganic bases (e.g., alkali metal salts such as sodium salt, potassium salt and the like; alkaline earth metal salts such as calcium
30 salt, magnesium salt and the like; aluminum salt, ammonium salt), salts with organic bases (e.g., salts with trimethylamine, triethylamine, pyridine, picoline, ethanolamine, diethanolamine, triethanolamine, tromethamine[tris(hydroxymethyl)methylamine], tert-butylamine,
35 cyclohexylamine, benzylamine, dicyclohexylamine, N,N-dibenzyl

ethylenediamine), salts with inorganic acids (e.g., salts with hydrofluoric acid, hydrochloric acid, hydrobromic acid, hydroiodic acid, nitric acid, sulfuric acid, phosphoric acid), salts with organic acids (salts with formic acid, acetic acid, 5 trifluoroacetic acid, phthalic acid, fumaric acid, oxalic acid, tartaric acid, maleic acid, citric acid, succinic acid, malic acid, methanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid), salts with basic amino acids (salts with arginine, lysine, ornithine) or salts with acidic amino acids 10 (salts with aspartic acid, glutamic acid).

[0139]

The ratio (mol%) of the cationic lipid to the total lipids present in the lipid nanoparticle is, for example, about 10% to about 80%, preferably about 20% to about 70%, more 15 preferably about 40% to about 60%; however, the ratio is not limited to these.

Only one kind of the above-mentioned cationic lipid may also be used or two or more kinds thereof may be used in combination. When multiple cationic lipids are used, the ratio 20 of the whole cationic lipid is preferably as mentioned above.

[0140]

(b) Non-cationic lipid

In the present specification, the "non-cationic lipid" means a lipid other than the cationic lipid, and is a lipid 25 that does not have a net positive electric charge at a selected pH such as physiological pH and the like. Examples of the non-cationic lipid used in the lipid nanoparticle of the present invention include phospholipid, steroids, PEG lipid and the like.

30 [0141]

To enhance the delivery of nucleic acid into, for example, T cell, the phospholipid is not particularly limited as long as it stably maintains nucleic acid and does not inhibit fusion with cellular membranes (plasma membrane and organelle 35 membrane). For example, phosphatidyl choline, phosphatidyl

ethanolamine, phosphatidyl serine, phosphatidyl inositol, phosphatidic acid, palmitoyloleoylphosphatidyl choline, lysophosphatidyl choline, lysophosphatidyl ethanolamine, dipalmitoylphosphatidyl choline, dioleoylphosphatidyl choline, 5 distearoylphosphatidyl choline, dilinolenoylphosphatidyl choline and the like can be mentioned.

[0142]

Preferred phospholipids include distearoylphosphatidyl choline (DSPC), dioleoylphosphatidyl choline (DOPC), 10 dipalmitoylphosphatidyl choline (DPPC), dioleoylphosphatidyl glycerol (DOPG), palmitoyloleoylphosphatidyl glycerol (POPG), dipalmitoylphosphatidyl glycerol (DPPG), dioleoyl-phosphatidyl ethanolamine (DOPE), palmitoyloleoylphosphatidyl choline (POPC), palmitoyloleoyl-phosphatidyl ethanolamine (POPE), and 15 dioleoylphosphatidyl ethanolamine 4-(N-maleimide methyl)-cyclohexane-1-carboxylate (DOPE-mal), more preferably DOPC, DPPC, POPC, and DOPE.

[0143]

The ratio (mol%) of the phospholipid to the total lipids 20 present in the lipid nanoparticle may be, for example, about 0% to about 90%, preferably about 5% to about 30%, more preferably about 8% to about 15%.

Only one kind of the above-mentioned phospholipid may be used or two or more kinds thereof may be used in combination. 25 When multiple phospholipids are used, the ratio of the whole phospholipid is preferably as mentioned above.

[0144]

As the steroids, cholesterol, 5 α -cholestanol, 5 β -coprostanol, cholesteryl-(2'-hydroxy)-ethylether, cholesteryl- 30 (4'-hydroxy)-butylether, 6-ketcholestanol, 5 α -cholestane, cholestenone, 5 α -cholestanone, 5 β -cholestanone, and cholesteryl decanoate can be mentioned, preferably cholesterol.

[0145]

The ratio (mol%) of the steroids to the total lipids 35 present in the lipid nanoparticle when steroids are present may

be, for example, about 10% to about 60%, preferably about 12% to about 58%, more preferably about 20% to about 55%.

Only one kind of the above-mentioned steroid may be used or two or more kinds thereof may be used in combination. When
5 multiple steroids are used, the ratio of the whole steroid is preferably as mentioned above.

[0146]

In the present specification, the "PEG lipid" means any complex of polyethylene glycol (PEG) and lipid. PEG lipid is
10 not particularly limited as long as it has an effect of suppressing aggregation of the lipid nanoparticle. For example, PEG conjugated with dialkylxypropyl (PEG-DAA), PEG conjugated with diacylglycerol (PEG-DAG) (e.g., SUNBRIGHT GM-020 (NOF CORPORATION)), PEG conjugated with phospholipids such as
15 phosphatidylethanolamine (PEG-PE), PEG conjugated with ceramide (PEG-Cer), PEG conjugated with cholesterol (PEG-cholesterol), or derivatives thereof, or mixtures thereof, mPEG2000-1,2-Di-O-alkyl-sn3-carbomoylglyceride (PEG-C-DOMG), 1-[8'-(1,2-dimyristoyl-3-propanoxy)-carboxamide-3',6-
20 dioxaoctanyl]carbamoyle- ω -methyl-poly(ethylene glycol) (2KPEG-DMG) and the like can be mentioned. Preferred PEG lipid includes PEG-DGA, PEG-DAA, PEG-PE, PEG-Cer, and a mixture of these, more preferably, a PEG-DAA conjugate selected from the group consisting of a PEG-didecyl oxypropyl conjugate, a PEG-
25 dilauryl oxypropyl conjugate, a PEG-dimyristyl oxypropyl conjugate, a PEG-dipalmityl oxypropyl conjugate, a PEG-distearyl oxypropyl conjugate, and mixtures thereof.

In addition to the methoxy group, the maleimide group, N-hydroxysuccinimidyl group and the like for binding the T cell
30 activating ligand can be used as the free end of PEG. For example, SUNBRIGHT DSPE-0201MA (NOF) can be used as a PEG lipid having a functional group for binding a T cell-activating ligand (sometimes to be referred to as "terminal reactive PEG lipid" in the present specification).

35 [0147]

The ratio (mol%) of the PEG lipid to the total lipids present in the lipid nanoparticle of the present invention may be, for example, about 0% to about 20%, preferably about 0.1% to about 5%, more preferably about 0.7% to about 2%.

5 The ratio (mol%) of the terminal reactive PEG lipid in the above-mentioned total PEG lipids may be, for example, about 10% to about 100%, preferably about 30% to about 100%, more preferably about 40% to about 100%.

Only one kind of the above-mentioned PEG lipid may be
10 used or two or more kinds thereof may be used in combination. When multiple PEG lipids are used, the ratio of the whole PEG lipid is preferably as mentioned above.

[0148]

1-1-2. Liposome

15 As another preferable nucleic acid delivery carrier to be used in the present invention, a liposome can be mentioned. As the liposome, those conventionally used in DDS of nucleic acids to cells can be similarly used. For example, liposomes prepared by mixing various cationic lipids (e.g., DOTMA, DOTAP,
20 DDAB, DMRIE etc.) developed as transfection reagents, and membrane-fused neutral lipids (e.g., DOPE, cholesterol etc.) that promote release from endosome are widely used. Liposomes in which functional molecules such as PEG, pH-responsive membrane fusion peptide, membrane permeation promoting peptide
25 and the like are added to the surface of the liposome can also be used.

[0149]

1-2. T cell activating ligand

In the nucleic acid delivery carrier of the present
30 invention, a T cell activating ligand is added to the surface of the above-mentioned nucleic acid delivery carrier.

The T cell activating ligand to be used in the present invention is not particularly limited as long as it is a molecule that interacts with the surface molecules of T cells
35 to promote activation and/or proliferation of the T cells. For

example, molecules having a function of specifically binding to CD3, which is coupled with TCR and responsible for signal transduction via TCR, and surface molecules CD28, ICOS, CD137, OX40, CD27, GITR, BAFFR, TACI, BMCA, CD40L and the like, which
5 are known as co-stimulation factors of T cell activation, and transducing activation/proliferation signals and co-signals in T cells or antigen-presenting cells can be mentioned. Such molecule may be a physiological ligand (or receptor) for the above-mentioned T cell surface molecule, or may be a non-
10 physiological ligand (or receptor) having an agonist activity. As the non-physiological ligand, an agonist antibody can be preferably mentioned.

[0150]

More preferably, the T cell activating ligand to be used
15 in the present invention includes an antibody against CD3 and/or an antibody against CD28. The antibody against CD3 and the antibody against CD28 each specifically bind to CD3 and CD28 expressed on target T cells to be induced to activate and/or proliferate (for example, when target T cell is derived
20 from human, the antibody against CD3 and the antibody against CD28 are desirably anti-human CD3 antibody and anti-human CD28 antibody, respectively), and may be a complete antibody or a fragment thereof (e.g., Fab, F(ab')₂, Fab', scFv, Fv, reduced antibody (rIgG), dsFv, sFv, diabody, triabody, etc.) as long as
25 they have the ability to stimulate these surface molecules of T cells and transduce signals in the T cells. The subclass of the antibody is also not particularly limited, but is preferably an IgG antibody.

[0151]

30 When an agonist antibody such as an antibody against CD3 or an antibody against CD28 is used as the T cell activating ligand, a commercially available anti-CD3 antibody, anti-CD28 antibody, or the like can also be used as long as it is a complete antibody molecule, or the antibody can also be
35 isolated from the culture medium of the cells that produce the

antibody. On the other hand, when the ligand is any of the
aforementioned antibody fragments, by treating a complete
antibody with a reducing agent (e.g., 2-mercaptoethanol,
dithiothreitol) or peptidase (e.g., papain, pepsin, ficin), or
5 by isolating a nucleic acid encoding fragments of anti-CD3
antibody, anti-CD28 antibody and the like in the same manner as
in obtaining a nucleic acid to be encapsulated in a nucleic
acid delivery carrier to be described later, the antibody
fragment can be recombinantly produced using the same.

10 [0152]

As the T cell activating ligand, only one kind may be
used, or two or more kinds may be used in combination. It is
preferable to combine two or more kinds. When two or more
kinds of T cell activating ligands are used in combination, at
15 least one kind is preferably an antibody against CD3 or an
antibody against CD28, more preferably an antibody against CD3.
Particularly preferably, both an antibody against CD3 and an
antibody against CD28 can be used as T cell activating ligands.
[0153]

20 When at least an antibody against CD3 and an antibody
against CD28 are used in combination as the T cell activating
ligands, the molar ratio of the two added to the surface of the
nucleic acid delivery carrier of the present invention is 1:4 -
4:1, preferably 1:2 - 2:1.

25 [0154]

When two or more kinds of T cell activating ligands are
used in combination, the T cell activating ligands may be
separately added to the surface of the nucleic acid delivery
carrier, or they may be complexed and added to the surface of
30 the nucleic acid delivery carrier as long as the T cell
activation activity of each is maintained. For example, when
the two kinds of the T cell activating ligands are antibodies
(e.g., antibody against CD3 and antibody against CD28), they
can be provided as bispecific antibodies known per se.

35 [0155]

In the nucleic acid delivery carrier of the present invention, the T cell activating ligand may bind to the outer shell in any manner as long as it is present on the surface of the nucleic acid delivery carrier. For example, when a lipid nanoparticle containing a terminally reactive PEG lipid as a non-cationic lipid is used as a nucleic acid delivery carrier, the ligand can be added to the terminal of PEG. For example, lipid nanoparticles labeled with a ligand (antibody) can be prepared by reacting a PEG lipid with a maleimide group introduced into the terminus (e.g., SUNBRIGHT DSPE-0200MA) with the thiol group of the above-mentioned reduced antibody. Even when a liposome modified with PEG is used as a nucleic acid delivery carrier, the T cell activating ligand can be added to the surface of the liposome surface in the same manner.

[0156]

1-3. Nucleic acid contained in the nucleic acid delivery carrier of the present invention

The nucleic acid delivery carrier of the present invention in a form free of a nucleic acid can also be used to induce activation and/or proliferation of T cells. In one preferred embodiment, activation and/or proliferation of T cells and delivery of the nucleic acid into T cells can be performed simultaneously in one step with the encapsulated nucleic acid. Therefore, in one preferred embodiment, the nucleic acid delivery carrier of the present invention further contains a nucleic acid to be delivered into T cells.

[0157]

When the nucleic acid delivery carrier of the present invention contains a nucleic acid inside, the nucleic acid is not particularly limited as long as the nucleic acid itself or a transcript or translation product thereof has a function of changing T cells into a desired state within the T cells.

[0158]

1-3-1. Nucleic acid suppressing expression of T cell activation inhibitory factor

In one preferred embodiment, the nucleic acid delivery carrier of the present invention contains inside a nucleic acid suppressing expression of a T cell activation inhibitory factor. The T cell activation inhibitory factor to be the target is not particularly limited as long as it suppresses activation of T cells. For example, immune checkpoint factors (e.g., CTLA-4, PD-1, TIM-3, LAG-3, TGIT, BTLA, VISTA(PD-1H) etc.) which are cell surface molecules that transmit negative signals to activation and/or proliferation of T cells upon receipt of stimulation from antigen-presenting cells or tumor cells, CD160, Cbl-b, endogenous TCR and the like can be mentioned.
[0159]

A nucleic acid suppressing expression of a T cell activation inhibitory factor may act at any level from transcription level of gene encoding the factor, post-transcriptional regulation level, protein translation level, post-translational modification level, and the like. Therefore, examples of the nucleic acid suppressing expression of a T cell activation inhibitory factor include a nucleic acid (e.g., antigene) inhibiting transcription of a gene encoding the factor, a nucleic acid inhibiting processing from initial transcripts to mRNA, a nucleic acid inhibiting translation from mRNA to protein (e.g., antisense nucleic acid, miRNA) or degrading mRNA (e.g., siRNA, ribozyme, miRNA) and the like. While the substances acting at any of those levels are preferably used, a substance that complementarily binds to mRNA to inhibit translation into protein or degrades mRNA is preferable. As the nucleic acid,
(a) nucleic acid having RNAi activity against mRNA encoding the factor, or a precursor thereof,
(b) antisense nucleic acid against mRNA encoding the factor,
(c) ribozyme nucleic acid against mRNA encoding the factor, and the like can be mentioned.

The nucleotide sequence of mRNA (cDNA) encoding each T cell activation inhibitory factor is known, and sequence

information can be obtained, for example, from public databases (e.g., NCBI, EMBL, DDBJ etc.).

[0160]

(a) Nucleic acid having RNAi activity against mRNA encoding T
5 cell activation inhibitory factor, or precursor thereof

As the nucleic acid having RNAi activity against mRNA encoding a T cell activation inhibitory factor, double-stranded RNA consisting of an oligo RNA complementary to the target mRNA and a complementary strand thereof, i.e., siRNA can be
10 mentioned. The siRNA can be designed based on the cDNA sequence information of the target gene, for example, according to the rules proposed by Elbashir et al. (Genes Dev., 15, 188-200 (2001)). The short hairpin RNA (shRNA), which is a precursor of siRNA, can be designed by appropriately selecting
15 any linker sequence (for example, about 5-25 bases) capable of forming a loop structure, and linking the sense strand and antisense strand of siRNA via the linker sequence.

[0161]

The siRNA and/or shRNA sequences can be searched using
20 search software provided free of charge on various websites. Examples of such site include, but are not limited to, siDESIGN Center provided by Dharmacon (<http://dharmacon.horizondiscovery.com/jp/design-center/?rdr=true&LangType=1041&pageid=17179928204>), siRNA
25 Target Finder provided by GenScript (<https://www.genscript.com/tools/sirna-target-finder>) and the like.

[0162]

In the present specification, microRNA (miRNA) that
30 targets mRNA encoding a T cell activation inhibitory factor is also defined as being included in the nucleic acid having RNAi activity against the mRNA. For miRNA, primary-microRNA (pri-miRNA), which is the primary transcript, is first transcribed from a gene encoding the miRNA, then processed by Drosha into
35 precursor-microRNA (pre-miRNA) of about 70 bases in length

having a characteristic hairpin structure, transported from the nucleus to the cytoplasm, and further processed by mediation of Dicer to become mature miRNA, which is taken up by RISC and acts on the target mRNA. Therefore, pre-miRNA or pri-miRNA, preferably pre-miRNA, can also be used as a precursor of miRNA. [0163]

miRNA can be searched using target prediction software provided free of charge on various websites. Examples of such site include, but are not limited to, TargetScan (http://www.targetscan.org/vert_72/) published by Whitehead Institute, USA, DIANA-micro-T-CDS (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=microT_CDS/index) published by Alexander Fleming Biomedical Sciences Research Centre, Greece, and the like. Alternatively, miRNA targeting mRNA encoding a T cell activation inhibitory factor can also be searched using TarBase (http://carolina.imis.athena-innovation.gr/diana_tools/web/index.php?r=tarbasev8/index) which is a database relating to miRNA that has been experimentally proven to act on the target mRNA, and published by the University of Thessaly, Pasteur Institute and the like. [0164]

The nucleotide molecules that constitute siRNA and/or shRNA, or miRNA and/or pre-miRNA may be native RNA or DNA. In order to improve stability (chemical and/or against enzyme) and specific activity (affinity for RNA), various chemical modifications known per se can be included. [0165]

siRNA can be prepared according a process comprising synthesizing a sense strand and an antisense strand of target sequence on mRNA each with the DNA/RNA automatic synthesizer, denaturing in a suitable annealing-buffer solution at about 90 to 95°C for about 1 minute, and annealing at about 30 to 70°C for about 1 to 8 hours. In addition, siRNA can also be prepared by synthesizing shRNA which is the precursor of siRNA and cleaving the shRNA with the use of a dicer. miRNA and pre-

miRNA can be synthesized by a DNA/RNA automatic synthesizer based on the sequence information thereof.

[0166]

In the present specification, a nucleic acid designed to
5 be able to produce siRNA or miRNA against mRNA encoding a T
cell activation inhibitor in vivo is also defined as being
included in the nucleic acid having RNAi activity against the
mRNA. Examples of such nucleic acid include expression vectors
constructed to express the above-mentioned shRNA or siRNA or
10 miRNA or pre-miRNA, and the like. As the promoter, a polII
promoter (e.g., CMV early-immediate promoter) can be used. It
is a general practice to use a polIII promoter to ensure
accurate transcription of short RNA. Examples of the polIII
promoter include mouse and human U6-snRNA promoters, human H1-
15 RNase P RNA promoter, human valine-tRNA promoter and the like.
In addition, a sequence in which four or more Ts are continuous
is used as the transcription termination signal. An expression
cassette of miRNA and pre-miRNA can also be produced in the
same manner as in shRNA.

20 [0167]

(b) Antisense nucleic acid against mRNA encoding T cell
activation inhibitory factor

The antisense nucleic acid against mRNA encoding a T cell
activation inhibitory factor is a nucleic acid comprising a
25 nucleotide sequence complimentary to the nucleotide sequence of
mRNA or a part thereof, which has a function of inhibiting the
protein synthesis by binding specifically with the target mRNA
to form a stable duplex. The antisense nucleic acid may be DNA
or RNA, or DNA/RNA chimera. When the antisense nucleic acid is
30 DNA, the RNA:DNA hybrid formed by the target RNA and the
antisense DNA is recognized by endogenous RNase H, thereby
undergoing the selective degradation of the target RNA. The
length of the target region of the antisense nucleic acid is
not particularly limited as long as the hybridization of the
35 antisense nucleic acid eventually inhibits the translation into

protein, and may be the entire sequence of mRNA encoding the protein or a partial sequence thereof. A short one may be about 10 bases, and a long one may be the entire sequence of mRNA or initial transcript. In addition, the antisense nucleic acid may be a nucleic acid that inhibits the translation into a protein by hybridizing with target mRNA or initial transcript, and it may as well as be the nucleic acid capable of forming a triplex by binding with these genes which are the double-stranded DNAs and inhibiting the transcription into RNA (anti-gene).

[0168]

The nucleotide molecule constituting the antisense nucleic acid may also be modified in the same manner as in the cases of the above-mentioned siRNA and the like in order to improve stability, specific activity and the like.

The antisense oligonucleotide can be prepared by determining a target sequence of mRNA or initial transcript based on the cDNA sequence or genomic DNA sequence of the target gene, and synthesizing its complementary sequence with the use of a commercially available DNA/RNA automatic synthesizer.

[0169]

In the present specification, a nucleic acid designed to be able to generate an antisense RNA for mRNA encoding a T cell activation inhibitory factor in vivo is also defined to be included in an antisense nucleic acid for the mRNA. Such nucleic acid can be exemplified by an expression vector so constructed as to express the above-mentioned antisense RNA, or the like. As the promoter, a polII promoter or a polIII promoter can be appropriately selected and used according to the length of the antisense RNA to be transcribed.

[0170]

(c) Ribozyme nucleic acid for mRNA encoding T cell activation inhibitory factor

A ribozyme nucleic acid capable of specifically cleaving

the internal coding region of the mRNA encoding a T cell activation inhibitory factor can also be used as a nucleic acid suppressing the expression of the factor. The "ribozyme" is narrowly-defined as RNA having enzymatic activity for cleaving nucleic acid, but the present specification also includes DNA as long as there is a sequence specific enzymatic activity for cleaving nucleic acid. Ribozyme nucleic acid with the broadest utility includes self-splicing RNA which can be found in infectious RNA such as viroid, a virusoid, etc., and hammer-head type or hairpin type are known. When ribozyme is used in the form of an expression vector having DNA which encodes the ribozyme, the ribozyme can be hybrid ribozyme further coupled with the sequence of modified tRNA so as to promote transport to cytoplasm of a transcript.

[0171]

(d) Nucleic acid suppressing expression of T cell activation inhibitory factor by genome editing

In a preferred embodiment different from the above-mentioned (a) - (c), the nucleic acid suppressing expression of a T cell activation inhibitory factor can be a nucleic acid that can inactivate (knock out) a gene encoding the factor. As such nucleic acid, a nucleic acid encoding an artificial nuclease composed of a nucleic acid sequence recognition module (e.g., CRISPR/Cas9, ZFmotif, TAL effector etc.) capable of specifically recognizing a partial nucleotide sequence in the gene as a target, and a nuclease that introduces double-strand break (DSB) into the gene in the inside of or near the target sequence can be mentioned. After DSB introduction, the gene can be knocked out by insertion or deletion mutation due to a non-homologous end joining (NHEJ) repair error. Alternatively, gene knockout by homologous recombination (HR) repair can also be performed by combining with a targeting vector in which a marker gene (e.g., reporter gene such as fluorescent protein gene and the like, selection marker gene such as drug resistance gene and the like) is inserted in the gene sequence.

In addition, the endogenous TCR gene can also be knocked in by HR repair with an exogenous TCR gene.

[0172]

1-3-2. Nucleic acid encoding T cell activation promoting factor

5 In another preferred embodiment, the nucleic acid delivery carrier of the present invention contains inside a nucleic acid encoding a T cell activation promoting factor. The T cell activation promoting factor of interest includes, for example, T cell surface molecules (e.g., CD28, ICOS, CD137, 10 OX40, CD27, GITR, BAFFR, TACI, BMCA, CD40L etc.) to which the aforementioned T cell activating ligand binds to transduce activation and/or proliferation signals in T cells, and the like.

The nucleotide sequence of mRNA (cDNA) encoding each T 15 cell activation promoting factor is known, and sequence information can be obtained, for example, from public databases (e.g., NCBI, EMBL, DDBJ etc.).

[0173]

The nucleic acid encoding the T cell activation promoting 20 factor can be encapsulated, in the form of an expression vector containing mRNA or DNA encoding the factor, in the nucleic acid delivery carrier of the present invention. The mRNA encoding the T cell activation promoting factor can be isolated by a method known per se, using RNA extracted from T cells as a 25 template, and using a probe or primer prepared based on the sequence information thereof. The obtained mRNA may be encapsulated as it is in the nucleic acid delivery carrier of the present invention, or can be converted into cDNA and amplified by RT-PCR.

30 [0174]

The obtained DNA encoding a T cell activation promoting factor can be inserted into an expression vector, preferably a plasmid vector, containing a functional promoter in T cells, either as is or after adding a suitable linker and/or nuclear 35 translocation signal and the like. Examples of the functional

promoter in T cells include, but are not limited to, constitutive SR α promoter, SV40 promoter, LTR promoter, CMV (cytomegalovirus) promoter, RSV (Rous sarcoma virus) promoter, MoMuLV (Moloney mouse leukemia virus) LTR, HSV-TK (herpes simplex virus thymidine kinase) promoter and the like in mammalian cells. In addition, gene promoters such as CD3, CD4, and CD8, which direct specific expression in T cells, can also be used.

[0175]

10 The mRNA encoding a T cell activation promoting factor can be prepared by transcription into mRNA in an in vitro transcription system known per se using an expression vector containing DNA encoding the factor as a template.

[0176]

15 1-3-3. Nucleic acid encoding chimeric antigen receptor (CAR) or exogenous T cell receptor (TCR)

As mentioned above, the nucleic acid delivery carrier of the present invention encapsulating the nucleic acid makes it possible to perform activation and/or proliferation of T cells, and delivery of the nucleic acid into T cells simultaneously in one step. Therefore, by encapsulating a nucleic acid encoding CAR or TCR in the nucleic acid delivery carrier of the present invention, a step of activation/proliferation of T cells and a step of gene transfer into T cells can be performed simultaneously in one pod.

[0177]

That is, in one preferred embodiment, the nucleic acid delivery carrier of the present invention contains inside a nucleic acid encoding CAR or TCR.

30 [0178]

(a) Nucleic acid encoding CAR

CAR is an artificially constructed hybrid protein containing the antigen-binding domain (e.g., scFv) of an antibody coupled to a T cell signal transduction domain. CAR is characterized by the ability to utilize the antigen-binding

property of the monoclonal antibody to redirect the specificity and responsiveness of T cells to a selected target in a non-MHC-restricted manner. Non-MHC-restricted antigen recognition confers on CAR-expressing T cells the ability to recognize
5 antigens independently of antigen processing, thereby bypassing the major mechanism of tumor escape. Furthermore, when expressed in T cells, CAR advantageously does not dimerize with the endogenous TCR α chain and β chain.

[0179]

10 CAR to be encapsulated in the nucleic acid delivery carrier of the present invention includes an antigen-binding domain of an antibody that can specifically recognize surface antigens (e.g., cancer antigen peptide, surface receptor showing promoted expression in cancer cells, etc.) that the
15 target T cell should recognize, an extracellular hinge domain, a transmembrane domain, and an intracellular T cell signal transduction domain.

[0180]

Examples of the surface antigens specifically recognized
20 by antigen-binding domains include, but are not limited to, surface receptors showing promoted expression in various cancers (e.g., acute lymphocytic cancer, alveolar rhabdomyosarcoma, bladder cancer, bone cancer, brain cancer (e.g., medulloblastoma), breast cancer, anus, anal canal or
25 anorectal cancer, cancer of the eye, cancer of the interhepatic bile duct, joint cancer, cervical, gallbladder or pleural cancer, nose, nasal cavity or middle ear cancer, oral cancer, vulvar cancer, chronic myelogenous cancer, colorectal cancer, esophageal cancer, cervical cancer, fibrosarcoma,
30 gastrointestinal carcinoid tumor, head and neck cancer (e.g., head and neck squamous cell carcinoma), hypopharyngeal cancer, kidney cancer, laryngeal cancer, leukemia (e.g., acute lymphoblastic leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myelogenous leukemia), liquid tumor,
35 liver cancer, lung cancer (e.g., non-small cell lung cancer),

lymphoma (e.g., Hodgkin lymphoma, non-Hodgkin lymphoma, diffuse large B cell lymphoma, follicular lymphoma), malignant mesothelioma, mastocytoma, melanoma, multiple myeloma, nasopharyngeal cancer, ovarian cancer, pancreatic cancer; 5 peritoneal, omentum and mesenteric cancer; pharyngeal cancer, prostate cancer, rectal cancer, kidney cancer, skin cancer, small intestinal cancer, soft tissue cancer, solid tumor, gastric cancer, testicular cancer, thyroid cancer, ureteral cancer and the like, for example, CD19, EGF receptor, BCMA, 10 CD30, Her2, ROR1, MUC16, CD20, mesothelin, B-cell mutation antigen, CD123, CD3, prostate specific membrane antigen (PSMA), CD33, MUC-1, CD138, CD22, GD2, PD-L1, CEA, chondroitin sulfate proteoglycan-4, IL-13 receptor α chain, IgG κ light chain, and cancer antigen peptides (e.g., peptides derived from WT1, GPC3, 15 MART-1, gp100, NY-ESO-1, MAGE-A4, etc.).

[0181]

The antigen-binding domain used in the present invention is not particularly limited as long as it is an antibody fragment that can specifically recognize the target antigen. 20 Considering the ease of preparation of CAR, a single-chain antibody (scFv) in which a light chain variable region and a heavy chain variable region are linked via a linker peptide is desirable. The configuration of the light chain variable region and heavy chain variable region in single-chain antibody 25 is not particularly limited as long as they can reconstitute a functional antigen-binding domain. They can generally be designed in the order of light chain variable region, linker peptide, and heavy chain variable region from the N-terminal side. As the linker peptide, a known linker peptide typically 30 used for the production of single-chain antibodies can be used. For example, DNA encoding light chain variable region and DNA encoding heavy chain variable region can be prepared by cloning light chain gene and heavy chain gene respectively from antibody-producing cells and performing PCR using them as 35 templates, or the like, or by chemically synthesizing them from

the sequence information of existing antibodies. DNA encoding a single-chain antibody can be obtained by ligating each obtained DNA fragment with a DNA encoding linker peptide by an appropriate method. The N-terminal side of the antigen-binding domain is preferably further added with a leader sequence to present CAR to the surface of T cell.

[0182]

As the extracellular hinge domain and transmembrane domain, T cell surface molecule-derived domains generally used in the relevant technical field can be used as appropriate. For example, they include, but are not limited to, domains derived from CD8 α and CD28.

[0183]

Examples of the intracellular signal transduction domain include, but not limited to, those having a CD3 ζ chain, those further having a co-stimulatory motif such as CD28, CD134, CD137, Lck, DAP10, ICOS, 4-1BB, and the like between the transmembrane domain and the CD3 ζ chain, those having two or more co-stimulatory motifs and the like. Any domains normally used in the relevant technical field can be used in combination.

[0184]

Sequence information of nucleic acids encoding extracellular hinge domain, transmembrane domain, and intracellular signaling domain is well known in the relevant technical field. Those of ordinary skill in the art can easily obtain DNA fragments encoding each domain from T cells based on such information.

DNA encoding CAR can be obtained by linking DNA fragments respectively encoding the thus-obtained antigen binding domain, extracellular hinge domain, transmembrane domain, and intracellular signal transduction domain, by a conventional method.

[0185]

The obtained DNA encoding CAR can be inserted into an expression vector, preferably a plasmid vector, containing a

functional promoter in T cells, either as is or after adding a suitable linker and/or nuclear localization signal and the like. Examples of the functional promoter in T cells include, but are not limited to, constitutive SR α promoter, SV40 promoter, LTR
5 promoter, CMV (cytomegalovirus) promoter, RSV (Rous sarcoma virus) promoter, MoMuLV (Moloney mouse leukemia virus) LTR, HSV-TK (herpes simplex virus thymidine kinase) promoter and the like in mammalian cells. In addition, gene promoters such as CD3, CD4, and CD8, which direct specific expression in T cells,
10 can also be used.

[0186]

RNA encoding a CAR, preferably mRNA, can be prepared by transcription into mRNA in an in vitro transcription system known per se using an expression vector containing DNA encoding
15 the above-mentioned CAR as a template.

[0187]

(b) Nucleic acid encoding exogenous TCR

In the present specification, the "T cell receptor (TCR)" means a receptor that consists of dimers of the TCR chain (α -
20 chain, β -chain) and recognizes an antigen or the antigen-HLA (human leukocyte type antigen) (MHC; major histocompatibility complex) complex and transduces a stimulatory signal to T cells. Each TCR chain consists of a variable region and a constant region, and the variable region contains three complementarity
25 determining regions (CDR1, CDR2, CDR3). The TCR used in the present invention includes not only those in which the α and β chains of the TCR constitute a heterodimer but also those in which they constitute a homodimer. Furthermore, the TCR includes those with a part or all of the constant regions
30 deleted, those with recombined amino acid sequence, and those with soluble TCR, and the like.

The "exogenous TCR" means being exogenous to T cell, which is the target cell of the nucleic acid delivery carrier of the present invention. The amino acid sequence of the
35 exogenous TCR may be the same as or different from that of the

endogenous TCR expressed by T cell, which is the target cell of the nucleic acid delivery carrier of the present invention.

[0188]

The nucleic acid encoding TCR encapsulate in the nucleic acid delivery carrier of the invention is a nucleic acid encoding the α chain and β chain of TCR that can specifically recognize surface antigens (e.g., cancer antigen peptide etc.) to be recognized by the target T cell.

The nucleic acid can be prepared by a method known per se. When the amino acid sequence or nucleic acid sequence of the desired TCR is known, a DNA encoding the full-length or a part of the TCR of the present invention can be constructed based on the sequence by, for example, chemically synthesizing a DNA strand or an RNA strand, or connecting a synthesized, partially overlapping oligo-DNA short strand by the PCR method or the Gibson assembly method.

[0189]

When the sequence of the desired TCR is not known, for example, T cell of interest is isolated from a population of cells containing the T cell expressing a TCR of interest, and a nucleic acid encoding the TCR can be obtained from the T cell. Specifically, a cell population (e.g., PBMC) containing T cells is collected from an living organism (e.g., human), the cell population is cultured in the presence of epitopes of cell surface antigens recognized by the TCR of interest while stimulating the cell population, and T cell that specifically recognizes cells expressing the cell surface antigen can be selected from the cell population by a known method and using, as indices, specificity for cells expressing the cell surface antigen and cell surface antigens such as CD8 and CD4. The specificity for cells expressing the cell surface antigen of T cells can be measured, for example, by dextramer assay, ELISPOT assay, cytotoxic assay, or the like. The aforementioned cell population containing T cells is preferably collected from, for example, an organism having a large number of cells expressing

a cell surface antigen recognized by the TCR of interest (e.g., patient with a disease such as cancer, or T cell-containing population contacted with an epitope of the antigen or dendritic cells pulsed with the epitope).

5 [0190]

The nucleic acid of the present invention can be obtained by extracting DNA from the aforementioned isolated T cell by a conventional method, amplifying and cloning the TCR gene based on the nucleic acid sequence of the constant region of the TCR
10 by using the DNA as a template. It can also be prepared by extracting RNA from a cell and synthesizing cDNA by a conventional method, and performing 5'-RACE (rapid amplification of cDNA ends) with the cDNA as templates using antisense primers complementary to the nucleic acids
15 respectively encoding the constant regions of the TCR α chain and β chain. 5'-RACE may be performed by a known method and can be performed, for example, using a commercially available kit such as SMART PCR cDNA Synthesis Kit (manufactured by clontech). The DNA encoding the α chain and β chain of the
20 obtained TCR can be inserted into an appropriate expression vector in the same way as the DNA encoding the above-mentioned CAR. The DNA encoding α chain and the DNA encoding β chain may be inserted into the same vector or separate vectors. When inserted into the same vector, the expression vector may
25 express both strands in a polycistronic or monocistronic manner. In the former case, an intervening sequence that permits polycistronic expression, such as IRES or FMV 2A, is inserted between the DNA encoding both strands.

In addition, RNA encoding each strand of the TCR,
30 preferably mRNA, can be prepared in the same way as the above-mentioned RNA encoding CAR, for example, by using the expression vector as a template.

[0191]

1-4. T cell-targeting ligand

35 The nucleic acid delivery carrier of the present

invention is desirably used to activate and/or proliferate T cells preferably ex vivo. It also includes an embodiment of in vivo administration to a subject. In this case, a ligand capable of targeting the nucleic acid delivery carrier to T
5 cells (hereinafter to be also referred to as "T cell targeting ligand") is further added to the surface of the nucleic acid delivery carrier of the present invention, whereby the targeting efficiency to T cells can be enhanced.

[0192]

10 The T cell-targeting ligand is not particularly limited as long as it can specifically recognize surface molecules that are specifically or highly expressed in T cells. Preferably, it includes one containing an antigen binding domain of an antibody against CD3, CD4 or CD8, more preferably, an anti-CD3
15 antibody. Here, the "antigen-binding domain" is synonymous with the antigen-binding domain that constitutes the above-mentioned CAR. However, since CAR needs to be prepared as a nucleic acid encoding same, restrictions occur and single-chain antibodies are generally used in many cases. Since the
20 antigen-binding domain as a T cell targeting ligand is contained in a protein state in the lipid nanoparticle of the present invention, not only single-chain antibodies, but also any other antibody fragments, such as complete antibody molecules, Fab, F(ab')₂, Fab', Fv, reduced antibody (rIgG),
25 dsFv, sFv, diabody, triabody, and the like, can also be used preferably. These antibody fragments can be prepared by treating the complete antibody (e.g., IgG) with a reduced agent (e.g., 2-mercaptoethanol, dithiothreitol) or peptidase (e.g., papain, pepsin, ficin), or by using a genetic recombination
30 operation.

[0193]

When the T-cell targeting ligand is a complete antibody molecule, commercially available anti-CD3, CD4, CD8 antibodies, etc. can be used, or the ligand can be isolated from the
35 culture of the cells producing the antibody. On the other hand,

when the ligand is any one of the aforementioned antigen-binding domain (antibody fragment), the nucleic acid encoding the antigen-binding domain, such as anti-CD3, CD4, CD8 antibodies, etc., is isolated in the same way as in the nucleic acid encoding the antigen-binding domain constituting the said CAR is obtained, and the antigen-binding domain can be recombinantly produced using the same.

[0194]

2. Production of the nucleic acid delivery carrier of the present invention

As a representative example of the nucleic acid delivery carrier of the present invention, a production example of the nucleic acid delivery carrier of the present invention using lipid nanoparticles as a carrier (hereinafter to be also referred to as "the lipid nanoparticle of the present invention") is explained in the following. Even when other carriers such as liposomes and the like are used, the nucleic acid delivery carrier of the present invention can be obtained in the same manner by appropriately making changes according to the carrier used.

[0195]

The lipid nanoparticle of the present invention can be produced, for example, by forming lipid nanoparticles by the method described in US9,404,127, and chemically binding the T cell-activating ligand. Alternatively, as described in WO 2016/021683, for example, an organic solvent solution dissolving cationic lipid and non-cationic lipid is prepared, the organic solvent solution is mixed with water or a buffer solution dissolving the nucleic acid to be encapsulated in the lipid nanoparticles to prepare lipid nanoparticles, and T cell activating ligand (further, T cell-targeting ligand as necessary when the lipid nanoparticle of the present invention is used in vivo) is chemically bound, whereby the lipid nanoparticle can be produced. The mixing ratio (molar ratio) of cationic lipid, phospholipid, cholesterol, and PEG lipid is,

for example, 40 to 60:0 to 20:0 to 50:0 to 5, but the ratio is not limited thereto. When PEG lipid is blended as a non-cationic lipid and a T cell-activating ligand is added to the terminal of PEG, the mixing ratio (molar ratio) of the PEG lipid and the ligand may be, for example, 20:1 to 1:20. The above-mentioned PEG lipid may contain terminal reactive PEG at a ratio (mol%) of about 10% to about 100%. The above-mentioned mixing can be conducted using a pipette, a micro fluid mixing system (e.g., Asia microfluidic system (Syrris)). The obtained lipid particles may be subject to purification by gel filtration, dialysis or sterile filtration.

The concentration of the total lipid component in the organic solvent solution is preferably 0.5 to 100 mg/mL.

[0196]

As the organic solvent, for example, methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, tert-butanol, acetone, acetonitrile, N,N-dimethylformamide, dimethyl sulfoxide, or a mixture thereof can be recited. The organic solvent may contain 0 to 20% of water or a buffer solution. As the buffer solution, acidic buffer solutions (e.g., acetate buffer solution, citrate buffer solution) or neutral buffer solutions (e.g., 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, (HEPE) buffer solution, tris(hydroxymethyl)aminomethane (Tris) buffer solution, a phosphate buffer solution, phosphate buffered saline (PBS)) can be recited.

[0197]

In the case where a micro fluid mixing system is used for mixing, preference is given to mixing 1 part by volume of an organic solvent solution with 1 to 5 parts by volume of water or a buffer solution. In addition, in said system, the flow rate of the mixture (a mixture solution of an organic solvent solution and water or a buffer solution) is preferably 0.1 to 10 mL/min, and the temperature preferably is 15 to 45°C.

[0198]

When a lipid particle dispersion is produced as described

above, the dispersion containing cationic lipid, non-cationic lipid, nucleic acid and T cell activating ligand can be produced by adding a nucleic acid to be encapsulated in lipid nanoparticles to water or buffer. Addition of the nucleic acid
5 in a manner to render the concentration thereof in water or a buffer solution 0.05 to 2.0 mg/mL is preferable.

In addition, the lipid nanoparticle can also be produced by admixing a lipid particle dispersion with the nucleic acid by a method known per se.

10 In the lipid nanoparticle of the present invention, the content of the nucleic acid is preferably 1 - 20 wt%. The content can be measured using Quant-iTTMRibogreen® (Invitrogen). In the lipid nanoparticle of the present invention, the encapsulation ratio of the nucleic acid can be calculated based
15 on the difference in fluorescence intensity in the presence or absence of the addition of a surfactant (e.g., Triton-X100).
[0199]

A dispersion medium can be substituted with water or a buffer solution by dialysis. For the dialysis, ultrafiltration
20 membrane of molecular weight cutoff 10 to 20K is used to carry out at 4°C to room temperature. The dialysis may repeatedly be carried out. For the dialysis, tangential flow filtration may be used.
[0200]

The ratio (weight ratio) of the nucleic acid and the
25 lipid in the lipid nanoparticle of the present invention obtained as mentioned above is about 0.01 to about 0.2.
[0201]

The average particle size of the lipid nanoparticle of the present invention is preferably 10 to 200 nm. The average
30 particle size of the lipid particles can be calculated using, for example, Zetasizer Nano ZS (Malvern Instruments) on cumulant analysis of an autocorrelation function.
[0202]

3. Activation/proliferation method of T cells, delivery method
35 of nucleic acid into T cells, and production method of

medicament containing T cells, using T cell activating ligand and nucleic acid delivery carrier

The present invention also provides an activation and/or proliferation method of T cells using the nucleic acid delivery carrier of the present invention obtained as mentioned above (hereinafter to be also referred to as "the activation/proliferation method of the present invention"). The method includes a step of contacting a cell population containing T cells with the nucleic acid delivery carrier of the present invention. As used herein, the T cell may be a T cell collected from a living organism (to be also referred to as "ex vivo T cell" in the present specification), or T cell in a living organism (to be also referred to as "in vivo T cell" in the present specification), with preference given to ex vivo T cell.

[0203]

In a different aspect, when the nucleic acid delivery carrier of the present invention contains a nucleic acid in the inside, the activation/proliferation method of the present invention can simultaneously deliver the nucleic acid into T cells. Therefore, the present invention also provides a delivery method of nucleic acid into T cells, including a step of contacting a cell population containing T cells with the nucleic acid delivery carrier of the present invention. In another embodiment, the present invention provides a delivery method of nucleic acid into T cells, including a step of contacting a cell population containing T cells simultaneously with at least one kind of T cell activating ligand, and any of the above-mentioned nucleic acid delivery carriers without a T cell activating ligand added to the surface (hereinafter the above-mentioned two embodiments are also to be collectively referred to as "the nucleic acid delivery method of the present invention"). In the present specification, when a free T cell activating ligand that is not bound to a nucleic acid delivery carrier is used, the ligand may be used alone, or as a complex

in which the ligand is bound to a carrier (e.g., Dynabeads(R) (Thermo Fisher Scientific company)). As such complex, a commercially available product (e.g., TransAct (Milteny Biotech company), Dynabeads Human T-Activator CD3/CD28 (ThermoFisher Scientific company)) may also be used.

[0204]

In another different aspect, T cells activated and/or proliferated using the nucleic acid delivery carrier of the present invention, or T cells into which a nucleic acid is delivered can be used as an agent for immune cell therapy. Therefore, the present invention also provides a production method of a medicament containing T cells, including a step of contacting a cell population containing T cells with the nucleic acid delivery carrier of the present invention. In another embodiment, the present invention provides a production method of a medicament containing T cells, including a step of contacting a cell population containing T cells simultaneously with at least one kind of T cell activating ligand, and any of the above-mentioned nucleic acid delivery carriers without a T cell activating ligand added to the surface.

[0205]

A cell population containing T cells to be brought into contact with the nucleic acid delivery carrier of the present invention, or a T cell activating ligand and a nucleic acid delivery carrier without a T cell activating ligand added to the surface may be an isolated T cell or, for example, a non-uniform cell population such as progenitor cells of lymphocytes including lymphocytes and pluripotent cells as long as it is a cell population containing T cell or a progenitor cell thereof. In the present invention, the "lymphocyte" means one of the subtypes of leukocyte in the immune system of vertebrates. Examples of the lymphocyte include T cell, B cell, and natural killer cell (NK cell), preferably, isolated and purified T cell. In the present invention, the "T cell" is one type of leukocyte found in lymphatic organs, peripheral blood, and the like, and

refers to one category of lymphocyte characterized by differentiation and maturation mainly in the thymus gland and expression of TCR. Examples of the T cell that can be used in the present invention include cytotoxic T cell (CTL), which is
5 a CD8-positive cell, helper T cell, which is a CD4-positive cell, regulatory T cell, and effector T cell, and preferably, cytotoxic T cell.

[0206]

The aforementioned lymphocyte can be collected from, for
10 example, peripheral blood, bone marrow, and umbilical cord blood of a human or non-human mammal. When ex vivo T cell contacted with the nucleic acid delivery carrier of the present invention is used for the treatment of diseases such as cancer, the cell population is preferably collected from the person to
15 be treated or a donor with the HLA type matching with that of the subject to be treated.

[0207]

A cell population containing T cells to be brought into contact with the nucleic acid delivery carrier of the present
20 invention, or a T cell activating ligand and a nucleic acid delivery carrier without a T cell activating ligand added to the surface may be a cell population of T cells obtained by differentiation induction from progenitor cells of lymphocytes containing pluripotent cells. Examples of the lymphocyte
25 progenitor cell, including pluripotent cell, include embryonic stem cell (ES cell), induced pluripotent stem cell (iPS cell), embryonic cancer cell (EC cell), embryonic germ cell (EG cell), hematopoietic stem cell, pluripotent progenitor cell that has lost self-renewal potential (multipotent progenitor: MMP),
30 common myelo-lymphoid progenitor cell (MLP), myeloid progenitor cell (MP), granulocyte mononuclear progenitor cell (GMP), macrophage-dendritic cell progenitor cell (MDP), dendritic cell progenitor cell (DCP) and the like. Undifferentiated cells such as pluripotent cell and the like can be differentiated
35 into T cell by a method known per se.

[0208]

There is no particular limitation on the method of contacting the nucleic acid delivery carrier of the present invention, or T cell activating ligand and a nucleic acid
5 delivery carrier without a T cell activating ligand added to the surface with ex vivo T cells, and, for example, the nucleic acid delivery carrier of the present invention, or T cell activating ligand and a nucleic acid delivery carrier without a T cell activating ligand added to the surface may be added to a
10 typical medium for T cells. Therefore, in another aspect, the present invention also provides a cell culture containing a cell population containing T cells, the nucleic acid delivery carrier of the present invention, or at least one kind of T cell activating ligand and a nucleic acid delivery carrier
15 without a T cell activating ligand added to the surface, and a medium.

In the nucleic acid delivery method of the present invention, to increase the efficiency of the delivery nucleic acid, for example, the calcium phosphate co-precipitation
20 method, PEG method, electroporation method, microinjection method, lipofection method, and the like may be used in combination.

[0209]

In the nucleic acid delivery method of the present
25 invention, when the nucleic acid delivery carrier of the present invention, or a nucleic acid delivery carrier without a T cell activating ligand added to the surface particularly contains a nucleic acid encoding exogenous TCR in the inside, the expression of endogenous TCR α chain and TCR β chain that
30 are inherently expressed by the T cell may be suppressed by siRNA from the viewpoint of an increase in the expression of exogenous TCR, inhibition of the appearance of mispaired TCR, or inhibition of self-reactivity. When the above-mentioned nucleic acid is applied to the method, to avoid the effect of
35 siRNA on exogenous TCR, the base sequence of a nucleic acid

encoding the TCR is preferably a sequence (codon conversion type sequence) different from the base sequence corresponding to RNA on which siRNA, which suppresses the expression of endogenous TCR α and TCR β chains, acts. The method therefor is
5 described, for example, in WO 2008/153029. The aforementioned base sequence can be produced by introducing a silent mutation into a naturally acquired nucleic acid encoding TCR or chemically synthesizing an artificially designed nucleic acid. Alternatively, to avoid mispair with the endogenous TCR chain,
10 a part or all of the constant regions of the nucleic acid encoding the exogenous TCR may be replaced with a constant region derived from an animal other than human, for example, a mouse.

Alternatively, as mentioned above, endogenous TCR gene
15 may also be knocked out using a genome editing technique.

[0210]

4. Nucleic acid delivery system containing at least one kind of T cell activating ligand, a nucleic acid delivery carrier without a T cell activating ligand added to the surface in
20 combination

As mentioned above, a nucleic acid can be delivered into T cells by contacting a cell population contacting T cells simultaneously with at least one kind of T cell activating ligand, and any of the above-mentioned nucleic acid delivery
25 carriers without a T cell activating ligand added to the surface. Therefore, the present invention also provides a nucleic acid delivery system containing at least one kind of T cell activating ligand, and a nucleic acid delivery carrier without a T cell activating ligand added to the surface in
30 combination. In the nucleic acid delivery system, the T cell activating ligand and the nucleic acid delivery carrier without a T cell activating ligand added to the surface may be provided as a composition containing the both, or in the form of a kit containing the both as separate components. The nucleic acid
35 delivery kit may further contain, in addition to the above-

mentioned both components, for example, a medium to be used in contacting a cell population containing T cells with such components, and the like, though not limited to these.

[0211]

- 5 5. Medicament containing ex vivo T cells activated and/or proliferated by T cell activating ligand and nucleic acid delivery carrier, or delivered with nucleic acid

The present invention also provides ex vivo T cells activated and/or proliferated by the activation/proliferation method of the present invention, ex vivo T cells having a
10 nucleic acid delivered by the nucleic acid delivery method of the present invention (including cell culture containing medium), and a medicament containing them.

[0212]

- 15 The ex vivo T cells activated and/or proliferated by the activation/proliferation method of the present invention specifically recognize cells expressing surface antigen specifically recognized by TCR expressed in the T cells and kill them (e.g., induction of apoptosis). In addition, the ex
20 vivo T cells into which a nucleic acid encoding CAR or exogenous TCR is delivered by the nucleic acid delivery method of the present invention express the CAR or exogenous TCR, specifically recognize cells expressing surface antigen specifically recognized by the CAR or exogenous TCR and can
25 kill them (e.g., induction of apoptosis). Therefore, ex vivo T cells in which T cells that express TCR recognizes a surface molecule that is specifically expressed as a surface antigen or showing enhanced expression in a disease cells, such as a cancer cell and the like, are activated and/or proliferated,
30 and ex vivo T cells introduced with a nucleic acid encoding CAR or exogenous TCR that recognizes the surface molecule can be used for the prophylaxis or treatment of diseases such as cancer and the like, and can be safely administered to mammals (human or other mammal (e.g., mouse, rat, hamster, rabbit, cat,
35 dog, bovine, sheep, monkey, preferably human)).

[0213]

In a medicament containing, as an active ingredient, ex vivo T cells contacted with the nucleic acid delivery carrier of the present invention, or T cell activating ligand and a
5 nucleic acid delivery carrier without a T cell activating ligand added to the surface, the T cells may be cultured using an appropriate medium before administration to a subject. In addition, the activation and/or proliferation of T cells can also be maintained or extended by adding a stimulation molecule
10 to the medium. Furthermore, serum or plasma may be added to the medium. While the amount of addition to these media is not particularly limited, 0% by volume - 20% by volume can be mentioned. Moreover, the amount of serum or plasma to be used can be changed according to the culturing stage. For example,
15 serum or plasma concentration can be reduced stepwise. The origin of serum or plasma may be either autologous or non-autologous, and autologous one is preferable from the aspect of safety.

[0214]

20 The medicament containing, as an active ingredient, ex vivo T cells contacted with the nucleic acid delivery carrier of the present invention, or T cell activating ligand and a nucleic acid delivery carrier without a T cell activating ligand added to the surface is preferably used by parenteral
25 administration to the subject. Examples of the method for parenteral administration include intravenous, intraarterial, intramuscular, intraperitoneal, and subcutaneous administration and the like. While the dose is appropriately selected according to the condition, body weight, age and the like of
30 the subject, the medicament is generally administered such that the cell number is generally 1×10^6 - 1×10^{10} cells, preferably 1×10^7 - 1×10^9 cells, more preferably 5×10^7 - 5×10^8 cells, per dose to a subject with body weight 60 kg. The medicament may be administered once, or in multiple divided portions. The
35 medicament containing, as an active ingredient, ex vivo T cells

contacted with the nucleic acid delivery carrier of the present invention can be formulated into a known form suitable for parenteral administration, for example, agent for injection or infusion. The medicament of the present invention may contain
5 pharmacologically acceptable excipients as appropriate. The pharmacologically acceptable excipient includes those described above. The medicament may contain saline, phosphate buffered saline (PBS), medium and the like to maintain the cells stably. Examples of the medium include, but are not limited to, media
10 such as RPMI, AIM-V, X-VIVO10 and the like. The medicament may be supplemented with a pharmaceutically acceptable carrier (e.g., human serum albumin), preservative and the like for stabilizing purposes.

[0215]

15 The medicament containing, as an active ingredient, ex vivo T cells contacted with the nucleic acid delivery carrier of the present invention, or T cell activating ligand and a nucleic acid delivery carrier without a T cell activating ligand added to the surface can be a prophylactic or
20 therapeutic drug for cancer. The cancer to be the application target for the medicament of the present invention is not particularly limited. Examples thereof include, but are not limited to, acute lymphocytic cancer, alveolar rhabdomyosarcoma, bladder cancer, bone cancer, brain cancer (e.g.,
25 medulloblastoma), breast cancer, anus, anal canal or anorectal cancer, cancer of the eye, cancer of the interhepatic bile duct, joint cancer, cervical, gallbladder or pleural cancer, nose, nasal cavity or middle ear cancer, oral cancer, vulvar cancer, chronic myelogenous cancer, colon cancer, esophageal cancer,
30 cervical cancer, fibrosarcoma, gastrointestinal carcinoid tumor, head and neck cancer (e.g., head and neck squamous cell carcinoma), hypopharyngeal cancer, kidney cancer, laryngeal cancer, leukemia (e.g., acute lymphoblastic leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, acute
35 myeloid leukemia), liquid tumor, liver cancer, lung cancer

(e.g., non-small cell lung cancer), lymphoma (e.g., Hodgkin lymphoma, non-Hodgkin lymphoma, diffuse large B cell lymphoma, follicular lymphoma), malignant mesothelioma, mastocytoma, melanoma, multiple myeloma, nasopharyngeal cancer, ovarian
5 cancer, pancreatic cancer; peritoneal, omentum and mesenteric cancer; pharyngeal cancer, prostate cancer, rectal cancer, renal cancer, skin cancer, small intestine cancer, soft tissue cancer, solid tumor, gastric cancer, testicular cancer, thyroid cancer, ureteral cancer and the like.

10 [0216]

6. Medicament containing the nucleic acid delivery carrier of the present invention

The nucleic acid delivery carrier of the present invention can induce activation and/or proliferation of T cells
15 in living organisms by in vivo administration to mammals such as human and the like. In addition, by in vivo administration of the nucleic acid delivery carrier of the present invention containing inside a nucleic acid encoding CAR or exogenous TCR to mammals such as human and the like, the nucleic acid is
20 delivered and expressed in T cells in the living organism, which in turn can impart the T cells with an ability to specifically recognize cells (e.g., cancer cells) expressing surface antigen (e.g., cancer antigen) specifically recognized by CAR or exogenous TCR and kill them (e.g., induction of
25 apoptosis). Therefore, the present invention also provides a medicament containing the nucleic acid delivery carrier of the present invention.

[0217]

A medicament containing the nucleic acid delivery carrier
30 of the present invention is preferably prepared as a pharmaceutical composition by mixing the nucleic acid delivery carrier with known pharmaceutically acceptable carriers (including excipient, diluent, bulking agent, binder, lubricant, flow aid, disintegrant, surfactant, and the like) and
35 conventional additives, and the like. The excipients are well

known to those of ordinary skill in the art and include, for example, phosphate-buffered saline (e.g., 0.01M phosphate, 0.138M NaCl, 0.0027M KCl, pH 7.4), aqueous solutions containing mineral acid salts such as hydrochloride, hydrobromate, 5 phosphate, sulfate, and the like, saline solutions, solutions of glycol, ethanol, and the like, and salts of organic acids such as acetate, propionate, malonate, benzoate, and the like. In addition, adjuvants such as wetting agent or emulsifier, and pH buffering agents can also be used. In addition, preparation 10 adjuvants such as suspension agent, preservative, stabilizer and dispersing agent may also be used. Alternatively, the above-mentioned pharmaceutical composition may be in a dry form which is reconstituted with a suitable sterile liquid prior to use. The pharmaceutical composition may be orally or 15 parenterally administered systemically or topically, depending on the form in which it is prepared (oral agents such as tablet, pill, capsule, powder, granule, syrup, emulsion, suspension and the like; parenteral agents such as injection, drip transfusion, external preparation, suppository and the like). For 20 parenteral administration, intravenous administration, intradermal administration, subcutaneous administration, rectal administration, transdermal administration and the like are available. When used in an injectable form, acceptable buffering agent, solubilizing agent, isotonic agent and the 25 like can also be added.

[0218]

The dosage of the medicament of the present invention containing the nucleic acid delivery carrier of the present invention is, for example, in the range of 0.001 mg to 10 mg as 30 the amount of a nucleic acid encoding CAR or exogenous TCR, per 1 kg body weight per dose. For example, when administered to a human patient, the dosage is in the range of 0.0001 to 50 mg for a patient weighing 60 kg. The above-mentioned dosage is an example, and the dosage can be appropriately selected according 35 to the type of nucleic acid to be used, administration route,

age, weight, symptoms, etc. of the subject of administration or patient.

[0219]

By administration to a mammal (e.g., human or other
5 mammal (e.g., mouse, rat, hamster, rabbit, cat, dog, bovine,
sheep, monkey), preferably, human), a medicament containing the
nucleic acid delivery carrier of the present invention can
induce the expression of CAR or exogenous TCR in T cell (in
vivo T cell) in the body of the animal. The in vivo T cells
10 kill diseased cells such as cancer cells and the like
expressing surface antigen targeted by CAR or exogenous TCR,
thereby demonstrating a prophylactic or therapeutic effect
against the disease.

[0220]

15 A medicament containing the nucleic acid delivery carrier
of the present invention may be a prophylactic or therapeutic
drug for cancer. The cancer to be the application target of
the medicament of the present invention is not particularly
limited. Examples thereof include, but are not limited to,
20 acute lymphocytic cancer, alveolar rhabdomyosarcoma, bladder
cancer, bone cancer, brain cancer (e.g., medulloblastoma),
breast cancer, anus, anal canal or anorectal cancer, cancer of
the eye, cancer of the interhepatic bile duct, joint cancer,
cervical, gallbladder or pleural cancer, nose, nasal cavity or
25 middle ear cancer, oral cancer, vulvar cancer, chronic
myelogenous cancer, colorectal cancer, esophageal cancer,
cervical cancer, fibrosarcoma, gastrointestinal carcinoid tumor,
head and neck cancer (e.g., head and neck squamous cell
carcinoma), hypopharyngeal cancer, kidney cancer, laryngeal
30 cancer, leukemia (e.g., acute lymphoblastic leukemia, acute
lymphocytic leukemia, chronic lymphocytic leukemia, acute
myelogenous leukemia), liquid tumor, liver cancer, lung cancer
(e.g., non-small cell lung cancer), lymphoma (e.g., Hodgkin
lymphoma, non-Hodgkin lymphoma, diffuse large B cell lymphoma,
35 follicular lymphoma), malignant mesothelioma, mastocytoma,

melanoma, multiple myeloma, nasopharyngeal cancer, ovarian cancer, pancreatic cancer; peritoneal, omentum and mesenteric cancer; pharyngeal cancer, prostate cancer, rectal cancer, kidney cancer, skin cancer, small intestinal cancer, soft
5 tissue cancer, solid tumor, gastric cancer, testicular tumor, thyroid cancer, ureteral cancer and the like.

[0221]

The present invention is explained in more detail in the following by referring to Examples which are mere
10 exemplifications and do not limit the present invention.

[Example]

[0222]

1. Reduction treatment of antibody

9.21 mg/ml anti-CD3 antibody and anti-CD28 antibody (Bio
15 X Cell company) solutions (each 111 μ l) were mixed with 10 mM DTT aqueous solution (12.3 μ l). The mixture of each antibody and DTT was mixed by vortex to carry out reaction at room temperature for 30 min. The reaction mixture was fractionated by HPLC (column: TSKgel G2000SWXL 7.8 mm $\times$ 30 cm, TOSOH, mobile
20 phase: PBS) to give a fraction solution containing the reduced antibody. The fraction solution was concentrated by ultrafiltration using Amicon 0.5 ml-10K. The concentrations of the antibody protein and thiol group in the concentrates were measured by absorbance at 230 nm and a fluorescence
25 colorimetric reaction with N-(7-dimethylamino-4-methylcoumarin-3-yl)maleimide (DACM), respectively.

[0223]

2. Preparation of maleimide-lipid nanoparticles

A lipid mixture containing compound 7, 11, 12, 21, 31 or
30 35 as cationic lipid (cationic lipid:DPPC:Cholesterol:SUNBRIGHT GM-020:SUNBRIGHT DSPE-020MA=60:10.6:28:1.4:1, molar ratio) was dissolved in 90% EtOH, 10% water to give a 7.0 mg/ml lipid solution. On the other hand, luciferase mRNA (TriLink company) was dissolved in 2-morpholinoethanesulfonic acid (MES) buffer
35 (pH 5.0) to give 0.2 mg/ml mRNA solution. The obtained lipid

solution and mRNA solution were mixed at room temperature by a Nanoassemblr apparatus (Precision Nanosystems) at a flow rate ratio of 3 ml/min:6 ml/min to give a dispersion containing the composition. The obtained dispersion was dialyzed using Slyde-
5 A-Lyzer (20k fraction molecular weight, Thermo Scientific) against water at room temperature for 1 hr, and against PBS at 4°C for 48 hr. Successively, the dialysate was filtered through a 0.2 µm syringe filter (Iwaki) and stored at 4°C.
[0224]

10 3. Binding reaction of reduced antibody and Maleimide-lipid nanoparticles

Maleimide-lipid nanoparticle dispersion was mixed with reduced antibody solution such that the molar concentration of reduced antibody was 1/20 of that of maleimide, and allowed to
15 stand at room temperature for 4 hr. Thereafter, the mixture was stored at 4°C until the purification step.
[0225]

4. Gel filtration purification of antibody-lipid nanoparticles

A reaction mixture of a reduced antibody and Maleimide-
20 lipid nanoparticles was loaded on a gel filtration column Sepharose CL-4B (Cat No. 17-0150-01/GE Healthcare), and fractionated with D-PBS(-) as a mobile phase. Successively, the protein concentration of each fraction was measured to identify the fraction containing the antibody-lipid
25 nanoparticles of interest, whereby antibody-lipid nanoparticles stock solution was obtained. The antibody-lipid nanoparticles were filtered through a 0.2 µm syringe filter and stored at 4°C.
[0226]

5. Particle size measurement of antibody-lipid nanoparticles

30 To 1 µl of an antibody-lipid nanoparticle stock solution was added 99 µl of phosphate buffered saline (137 mM NaCl, 7.99 mM Na₂HPO₄, 2.7 mM KCl, 1.47 mM KH₂PO₄, pH 7.4). The obtained dispersion was subjected to dynamic light scattering measurement using Zetasizer Nano ZS (Malvern instruments), and
35 the cumulant analysis of the autocorrelation function was

performed to measure the Z average particle size and the polydispersity index (PDI).

[0227]

6. ζ electric potential measurement of antibody-lipid

5 nanoparticles

To 50 μ l of an antibody-lipid nanoparticle stock solution was added 700 μ l of HEPES buffer (10 mM HEPES-NaOH, pH 7.3). The obtained dispersion was subjected to electrophoretic light scattering measurement using Zetasizer Nano ZS (Malvern
10 instruments) to measure the ζ electric potential.

[0228]

7. Measurement of mRNA encapsulation rate and concentration of antibody-lipid nanoparticles

An antibody-lipid nanoparticle stock solution was diluted
15 with TE buffer to adjust the mRNA concentration to about 4 μ g/ml. As an mRNA concentration standard solution, naked mRNA was diluted with TE buffer to 4 μ g/ml. The diluted antibody-lipid nanoparticles and naked mRNA concentration standard solution (each 60 μ l) were each mixed with 60 μ l of TE buffer
20 or TE buffer containing 2% Triton-X100. The mixture was allowed to stand at room temperature for 5 min, mixed with 120 μ l of Quant-iTTM RiboGreen (registered trade mark), and the mixture was further allowed to stand for 5 min. The fluorescence intensity of the mixture was measured using
25 Envision microplate reader (Perkin-Elmer company). The mRNA encapsulation rate and mRNA concentration were calculated by the following formulas.

[0229]

$$\% \text{ mRNA encapsulation rate} = (1 - F_{\text{TE}} / F_{\text{Triton}}) \times 100$$

$$30 \quad \text{mRNA concentration} = (F_{\text{Triton}} - b) \times d / m$$

(wherein F_{TE} shows RiboGreen fluorescence intensity of lipid nanoparticles mixed with TE buffer, F_{Triton} shows RiboGreen fluorescence intensity of lipid nanoparticles mixed with TE buffer containing 2% Triton-X100, b and m show y-intercept and
35 slope obtained from the calibration curve of the concentration

standard siRNA, and d is the dilution rate of lipid nanoparticles)

[0230]

A list of the analysis results of the prepared antibody-lipid nanoparticles is shown in the following Table.

[0231]

[Table 1]

	particle size (nm)	poly-dispersity index	mRNA encapsulation rate (%)	mRNA concentration (μg/ml)	antibody concentration (μg/ml)
anti-CD3-compound 7	78	0.114	95	94.1	20.4
anti-CD3-compound 11	78	0.071	97	87.0	29.4
anti-CD3-compound 12	99	0.134	96	93.9	27.6
anti-CD3-compound 31	135	0.121	90	92.3	49.6
anti-CD3-compound 21	89	0.095	96	73.1	72.7
anti-CD3-compound 35	108	0.129	97	72.1	65.3
anti-CD28-compound 12	93	0.137	94	128	10.1
anti-CD3/anti-CD28-compound 12	97	0.068	98	30.7	55.1

[0232]

8. mRNA transfection into human primary cultured T cells with lipid nanoparticles bound to anti-CD3 antibody

Human peripheral blood CD3 positive pan T cells (Precision Bioservices) were seeded on a round-bottomed 96-well

plate (Corning) at a cell density of 1×10^5 cells/well. A serum-free hematopoietic cell medium X-VIVO10 (Lonza) supplemented with 30 ng/ml recombinant IL-2 (Thermo Fisher Scientific) was used as the medium. Subsequently, anti-CD3 antibody-bound lipid nanoparticles encapsulating luciferase mRNA (TriLink) were added to the medium such that the concentration of luciferase mRNA in the medium was 1 μ g/ml, and the mixture was stood in a 5% CO₂ incubator at 37°C for 72 hr. Luciferase expressed in T cells was measured using Bright-Glo Luciferase Assay System Kit (Promega). The relative luciferase luminescence intensity of T cells supplemented with each anti-CD3 antibody-lipid nanoparticle is shown in Fig. 1.

[0233]

9. mRNA transfection into human primary cultured T cells with lipid nanoparticles bound to anti-CD3 antibody and/or anti-CD28 antibody

Human peripheral blood CD3 positive pan T cells (Precision Bioservices) were seeded on a round-bottomed 96-well plate (Corning) at a cell density of 1×10^5 cells/well. A serum-free hematopoietic cell medium X-VIVO10 (Lonza) supplemented with 30 ng/ml recombinant IL-2 (Thermo Fisher Scientific) was used as the medium. Subsequently, anti-CD3 antibody-bound lipid nanoparticles encapsulating luciferase mRNA (TriLink), anti-CD28 antibody-bound lipid nanoparticles, a mixture of anti-CD3 antibody-bound lipid nanoparticles and anti-CD28 antibody-bound lipid nanoparticles, and lipid nanoparticles bound with a mixture of anti-CD3 antibody and anti-CD28 antibody were added to the medium such that the concentration of luciferase mRNA in the medium was 1 μ g/ml, and the mixture was stood in a 5% CO₂ incubator at 37°C for 72 hr. Luciferase expressed in T cells was measured using Bright-Glo Luciferase Assay System Kit (Promega). The relative luciferase luminescence intensity of T cells supplemented with each antibody-lipid nanoparticle is shown in Fig. 2.

[0234]

10. mRNA transfection into human primary cultured T cells with lipid nanoparticles bound to anti-CD3/anti-CD28 antibody and T cell activation stimulation

Human peripheral blood CD3 positive pan T cells
5 (Precision Bioservices) were seeded on a round-bottomed 96-well plate (Corning) at a cell density of 1×10^5 cells/well. A serum-free hematopoietic cell medium X-VIVO10 (Lonza) supplemented with 30 ng/ml recombinant IL-2 (Thermo Fisher Scientific) was used as the medium. Subsequently, lipid
10 nanoparticles encapsulating luciferase mRNA (TriLink) and bound to anti-CD3 antibody and anti-CD28 antibody were added to the medium such that the concentration of luciferase mRNA in the medium was 0.3 or 1 $\mu\text{g/ml}$, and the mixture was stood in a 5% CO_2 incubator at 37°C for 72 hr. As a control sample of T cell
15 activation stimulation, T cells supplemented with TransAct (Miltenyi Biotec company) and Dynabeads (Thermo Fisher Scientific company) were also prepared. Luciferase expressed in T cells was measured using Bright-Glo Luciferase Assay System Kit (Promega). The number of viable T cells was
20 measured using CellTiter-Glo kit (Promega). The expression level of Luciferase is shown in Fig. 3(I), and the survival number of T cells is shown in Fig. 3(II) and (III).

[0235]

11. Preparation of Luc mRNA-encapsulating lipid nanoparticles

25 A lipid mixture containing compound 35 as cationic lipid (compound 35:DPPC:Cholesterol:SUNBRIGHT GM-020:SUNBRIGHT = 60:10.6:28:1.4 molar ratio) was dissolved in 90% EtOH, 10% water to give a 8.1 mg/ml lipid solution. On the other hand, luciferase mRNA (Luc mRNA) (TriLink company) was dissolved in
30 2-morpholinoethanesulfonic acid (MES) buffer (pH 5.0) to give 0.18 mg/ml mRNA solution. The obtained lipid solution and mRNA solution were mixed at room temperature by a Nanoassembler apparatus (Precision Nanosystems) at a flow rate ratio of 3 ml/min:6 ml/min to give a dispersion of Luc mRNA-encapsulating
35 lipid nanoparticles. The obtained dispersion was dialyzed

using Slyde-A-Lyzer (molecular weight 20k for fraction, Thermo Scientific) against water at room temperature for 1 hr, and against PBS at 4°C for 48 hr. Successively, the dialysate was filtered through a 0.2 µm syringe filter (Iwaki) and stored at 4°C.

[0236]

12. Particle size measurement of Luc mRNA-encapsulating lipid nanoparticles

To 1 µl of a Luc mRNA-encapsulating lipid nanoparticles stock solution was added 99 µl of phosphate buffered saline (137 mM NaCl, 7.99 mM Na₂HPO₄, 2.7 mM KCl, 1.47 mM KH₂PO₄, pH 7.4). The obtained dispersion was subjected to dynamic light scattering measurement using Zetasizer Nano ZS (Malvern instruments), and the cumulant analysis of the autocorrelation function was performed to measure the Z average particle size and the polydispersity index.

[0237]

13. ζ potential measurement of Luc mRNA-encapsulating lipid nanoparticles

To 50 µl of a Luc mRNA-encapsulating nanoparticle stock solution was added 700 µl of HEPES buffer (10 mM HEPES-NaOH, pH 7.3). The obtained dispersion was subjected to electrophoretic light scattering measurement using Zetasizer Nano ZS (Malvern instruments) to measure the ζ potential.

[0238]

14. Measurement of encapsulation rate and concentration of mRNA for Luc mRNA-encapsulating lipid nanoparticles

A Luc mRNA-encapsulating nanoparticle stock solution was diluted with TE buffer to adjust the mRNA concentration to about 4 µg/ml. As an mRNA concentration standard solution, naked mRNA was diluted with TE buffer to 4 µg/ml. The diluted Luc mRNA-encapsulating nanoparticles and naked mRNA concentration standard solution (each 60 µl) were each mixed with 60 µl of TE buffer or TE buffer containing 2% Triton-X100. The mixture was allowed to stand at room temperature for 5 min,

mixed with 120 μ l of Quant-iT™ RiboGreen (registered trade mark), and the mixture was further allowed to stand for 5 min. The fluorescence intensity of the mixture was measured using Envision microplate reader (Perkin-Elmer company). The mRNA
 5 encapsulation rate and mRNA concentration were calculated by the following formulas.

[0239]

$$\% \text{ mRNA encapsulation rate} = (1 - F_{TE} / F_{Triton}) \times 100$$

$$\text{mRNA concentration} = (F_{Triton} - b) \times d / m$$

10 (wherein F_{TE} shows RiboGreen fluorescence intensity of lipid nanoparticles mixed with TE buffer, F_{Triton} shows RiboGreen fluorescence intensity of lipid nanoparticles mixed with TE buffer containing 2% Triton-X100, b and m show y-intercept and slope obtained from the calibration curve of the concentration
 15 standard siRNA, and d is the dilution rate of lipid nanoparticles)

[0240]

The analysis results of the Luc mRNA-encapsulating lipid nanoparticles are shown in Table 2.

20 [0241]

[Table 2]

composition	Z-average particle size	polydispersity index	zeta potential	mRNA encapsulation rate
compound 35-luc mRNA	88 nm	0.029	-0.2 mV	96%

[0242]

15. Highly efficient mRNA transfection into human peripheral
 25 blood CD3 positive pan T cells by co-addition of activation
stimulant and lipid nanoparticles

Human peripheral blood CD3 positive pan T cells (Precision Bioservices) were seeded on a round-bottomed 96-well plate (Corning) at a cell density of 1×10^5 cells/well. A
 30 serum-free hematopoietic cell medium X-VIVO10 (Lonza) supplemented with 30 ng/ml recombinant IL-2 (Thermo Fisher

Scientific), and with TransAct (Milteny Biotech) or Dynabeads Human T-Activator CD3/CD28 (ThermoFisher Scientific), which stimulates activation of T cells, according to the protocol recommended by each manufacturer, was used as the medium.

5 Subsequently, lipid nanoparticle compound 35-luc mRNA encapsulating luciferase mRNA (TriLink) were added to the medium such that the concentration of luciferase mRNA in the medium was 0.1, 0.3 or 1 µg/ml, and the mixture was stood in a 5% CO₂ incubator at 37°C for 72 hr. Luciferase expressed in T
10 cells was measured using Bright-Glo Luciferase Assay System Kit (Promega). The survival and proliferation rate of T cells was measured using CellTiter-Glo Luminescent Cell Viability Assay kit (Promega KK). The obtained results are shown in Figs. 4 and 5. It was shown that addition of lipid nanoparticles
15 encapsulating Luc mRNA to T cells under activation stimulation dramatically improves transfection activity (Fig. 4). In addition, the survival and proliferation rate of T cells was maintained at a high level (Fig. 5).

[0243]

20 16. Highly efficient luc mRNA transfection into human CD4/CD8 positive T cells by co-addition of activation stimulant and lipid nanoparticles

Human peripheral blood leukocyte fraction Leukopak (HemaCare) was washed with LOVO Cell Processing System
25 (Fresenius), and CD4 and CD8 positive cells were collected with cell processing system CliniMACS (Milteny Biotec). The obtained CD4/CD8 positive cells were seeded on a flat-bottomed 96-well plate (Corning) at a cell density of 1×10^5 cells/well. For cell culture, a medium supplemented with 26 mL of OPTmizer
30 CTS T-cell Expansion Supplement, 20 mL of CTS Immune SR, 10 mL of L-Glutamine 200 mM (all ThermoFischer Scientific), 10 mL of Streptomycin Sulfate 10 mg/ml (MEIJI company), and 4.2 ng/ml MACS GMP Recombinant Human IL-2 (Milteny Biotec), per 1 L of OPTmizer CTS T-Cell Expansion Basal medium, was used. In
35 addition, as a T cell activation stimulant, TransAct (Milteny

Biotech) was added according to the protocol recommended by the manufacturer. Subsequently, lipid nanoparticle compound 35-luc mRNA encapsulating luciferase mRNA (TriLink) were added to the medium such that the concentration of luciferase mRNA in the
5 medium was 1, 3 or 10 µg/ml, and the mixture was stood in a 5% CO₂ incubator at 37°C for 72 hr. Luciferase expressed in T cells was measured using Bright-Glo Luciferase Assay System Kit (Promega). The survival and proliferation rate of T cells was measured using CellTiter-Glo Luminescent Cell Viability Assay
10 kit (Promega KK). The obtained results are shown in Fig. 6. It was shown that addition of lipid nanoparticles encapsulating Luc mRNA to T cells under activation stimulation dramatically improves transfection activity. In addition, the survival and proliferation rate of T cells was maintained at a high level.

15 [Industrial Applicability]

[0244]

Using the nucleic acid delivery carrier of the present invention or the nucleic acid delivery method of the present invention, a step of activating/proliferating T cells and a
20 step of introducing a gene into T cells can be performed simultaneously in one pod. As a result, an agent for immune cell therapy can be provided in a short period of time at a low production cost, and the present invention is extremely useful since an immunocyte therapy can be provided at a lower cost.

25 [0245]

This application is based on a patent application No. 2018-197069 filed in October 18, 2018 and a patent application No. 2019-124629 filed in July 3, 2019, the contents of which are hereby incorporated by reference in full herein.

CLAIMS

1. A method for activating/proliferating T cells, comprising a step of contacting a cell population containing T cells with a
5 nucleic acid delivery carrier having at least one kind of T cell activating ligand added to its surface.

2. The method according to claim 1, wherein the aforementioned T cell activating ligand includes an antibody to CD3 and/or an
10 antibody to CD28.

3. The method according to claim 1, wherein the aforementioned nucleic acid delivery carrier has two or more kinds of T cell activating ligands added to a surface thereof.

15 4. The method according to claim 1, wherein the aforementioned nucleic acid delivery carrier is a lipid nanoparticle or a liposome.

20 5. The method according to claim 1, wherein the aforementioned nucleic acid delivery carrier comprises, in its inside, a nucleic acid that suppresses expression of a T cell activation inhibitory factor and/or a nucleic acid encoding a T cell activation promoting factor.

25 6. The method according to claim 1, wherein the aforementioned nucleic acid delivery carrier comprises a nucleic acid encoding CAR or TCR.

30 7. The method according to claim 1, wherein the method is performed ex vivo.

8. A method for delivering a nucleic acid into T cells, comprising a step of contacting a cell population containing T
35 cells with a nucleic acid delivery carrier having at least one

kind of T cell activating ligand added to its surface and containing a nucleic acid inside, wherein the nucleic acid does not comprise a nucleic acid encoding CAR or TCR.

5 9. The method according to claim 8, wherein the aforementioned T cell activating ligand includes an antibody to CD3 and/or an antibody to CD28.

10 10. The method according to claim 8, wherein the aforementioned nucleic acid delivery carrier has two or more kinds of T cell activating ligands added to a surface thereof.

11. The method according to claim 8, wherein the aforementioned nucleic acid delivery carrier is a lipid nanoparticle or a
15 liposome.

12. The method according to claim 8, wherein the aforementioned nucleic acid comprises a nucleic acid suppressing expression of a T cell activation inhibitory factor and/or a nucleic acid
20 encoding a T cell activation promoting factor.

13. The method according to claim 8, wherein the method is performed ex vivo.

25 14. A method for delivering a nucleic acid into T cells, comprising a step of contacting a cell population containing T cells simultaneously with at least one kind of T cell activating ligand, and a nucleic acid delivery carrier containing a nucleic acid inside and free of a T cell
30 activating ligand added to its surface.

15. The method according to claim 14, wherein the aforementioned T cell activating ligand includes an antibody to CD3 and/or an antibody to CD28.

16. The method according to claim 14, wherein two or more kinds of T cell activating ligands are contacted.

17. The method according to claim 14, wherein the
5 aforementioned nucleic acid delivery carrier is a lipid nanoparticle or a liposome.

18. The method according to claim 14, wherein the
aforementioned nucleic acid includes a nucleic acid suppressing
10 expression of a T cell activation inhibitory factor and/or a nucleic acid encoding a T cell activation promoting factor.

19. The method according to claim 14, wherein the
aforementioned nucleic acid comprises a nucleic acid encoding
15 CAR or TCR.

20. The method according to claim 14, wherein the method is performed ex vivo.

21. A method for producing a medicament comprising T cells,
comprising a step of contacting a cell population containing T
cells simultaneously with at least one kind of T cell
activating ligand, and a nucleic acid delivery carrier
containing a nucleic acid inside and free of a T cell
25 activating ligand added to its surface.

22. The method according to claim 21, wherein the
aforementioned T cell activating ligand includes an antibody to
CD3 and/or an antibody to CD28.

30

23. The method according to claim 21, wherein two or more kinds of T cell activating ligands are contacted.

24. The method according to claim 21, wherein the
35 aforementioned nucleic acid delivery carrier is a lipid

nanoparticle or a liposome.

25. The method according to claim 21, wherein the
aforementioned nucleic acid delivery carrier comprises a
5 nucleic acid suppressing expression of a T cell activation
inhibitory factor and/or a nucleic acid encoding a T cell
activation promoting factor.

26. The method according to claim 21, wherein the
10 aforementioned nucleic acid comprises a nucleic acid encoding
CAR or TCR.

27. The method according to claim 21, wherein the method is
performed ex vivo.

15

28. A T cell into which a nucleic acid has been delivered by
the method according to claim 14.

29. A medicament comprising the T cell according to claim 28.

20

30. A cell culture comprising a cell population containing T
cells, at least one kind of T cell activating ligand, a nucleic
acid delivery carrier without a T cell activating ligand added
to the surface, and a medium.

25

31. A composition for delivering a nucleic acid to T cells,
comprising at least one kind of T cell activating ligand, and a
nucleic acid delivery carrier without a T cell activating
ligand added to the surface.

30

32. A kit for delivering a nucleic acid into T cells,
comprising at least one kind of T cell activating ligand, and a
nucleic acid delivery carrier without a T cell activating
ligand added to the surface.