

CL4F 10-8

[0004] There is a continuing need for lipid molecules for efficient delivery of nucleic acids and other agents to target cells and organs.

### SUMMARY

[0005] The present disclosure relates to a pH-sensitive cationic lipid that is useful for preparing nanoparticles that have favorable transfection property to target cells.

[0006] Specifically, the present disclosure includes the following embodiments (1) to (15).

Embodiment (1). A compound represented by general formula (I) or pharmaceutically acceptable salt thereof:

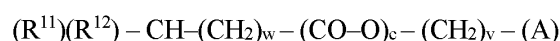


wherein:

a represents an integer of 3-5;

b represents 0 or 1;

$R^1$  and  $R^2$  each independently represent a group represented by general formula (A):



wherein:

$R^{11}$  and  $R^{12}$  each independently represent a  $C_{5-15}$  alkoxy group;

each c independently represents 0 or 1;

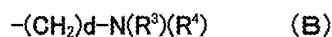
each v independently represents an integer of 4-12;

each w independently represents an integer of 0-3;

and

X represents a 5- to 7-membered non-aromatic heterocyclic group, wherein a carbon atom of said heterocyclic group is bound to  $(O-CO)_b-$  and one or two hydrogen atoms of said heterocyclic group may optionally be replaced with a  $C_{1-4}$  alkyl

group or C<sub>2-4</sub> alkenyl group, or  
 a group represented by general formula (B):



wherein:

d represents an integer of 0-3; and  
 R<sup>3</sup> and R<sup>4</sup> each independently represent a C<sub>1-4</sub> alkyl group or C<sub>2-4</sub> alkenyl group, or  
 R<sup>3</sup> and R<sup>4</sup> are bound to each other to form a 5- to 7-membered non-aromatic  
 heterocyclic group, wherein one or two hydrogen atoms of said  
 heterocyclic group may optionally be replaced with a C<sub>1-4</sub> alkyl group or  
 C<sub>2-4</sub> alkenyl group.

Embodiment (2). The compound of Embodiment (1) or pharmaceutically acceptable salt thereof,  
 wherein c is 1.

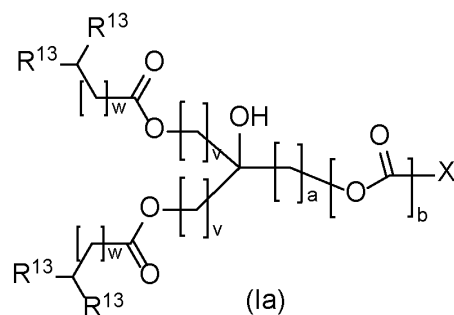
Embodiment (3). The compound of Embodiment (1) or (2), or pharmaceutically acceptable salt  
 thereof, wherein w is 1 or 2.

Embodiment (4). The compound of any one of Embodiments (1) to (3), or pharmaceutically  
 acceptable salt thereof, wherein b is 0 and X is the group represented by the general formula  
 (B).

Embodiment (5). The compound of any one of Embodiments (1) to (4), or pharmaceutically  
 acceptable salt thereof, wherein b is 0, X is the group represented by the general formula  
 (B), d is 0, and R<sup>3</sup> and R<sup>4</sup> are each independently a C<sub>1-4</sub> alkyl group.

Embodiment (6). The compound of any one of Embodiments (1) to (4), or pharmaceutically  
 acceptable salt thereof, wherein b is 1 and X is a 5- to 7-membered non-aromatic  
 heterocyclic group.

Embodiment (7). The compound of any one of Embodiments (1) to (6), or pharmaceutically  
 acceptable salt thereof, wherein the compound is represented by general formula (Ia):



wherein:

a represents an integer of 3-5;

each  $R^{13}$  independently represents a  $C_{5-15}$  alkoxy group;

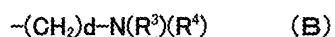
b represents 0 or 1;

each v independently represents an integer of 4-12;

each w independently represents an integer of 1-3;

X represents a 5- to 7-membered non-aromatic heterocyclic group, wherein a carbon atom of said heterocyclic group is bound to  $(O-CO)_b-$ , and one or two hydrogen atoms of said heterocyclic group may optionally be replaced with a  $C_{1-4}$  alkyl group or  $C_{2-4}$  alkenyl group, or

a group represented by general formula (B):



wherein:

d represents an integer of 0-3; and

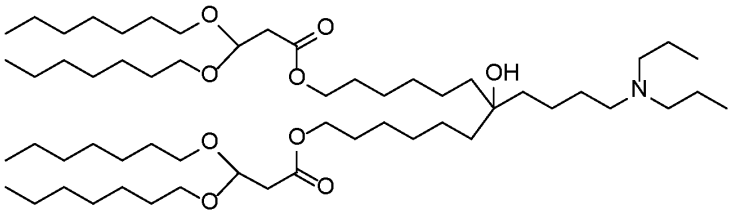
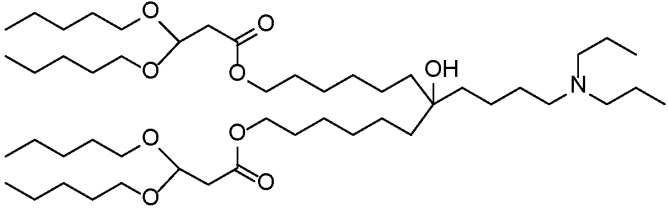
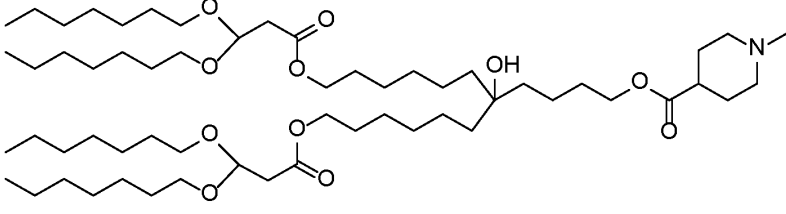
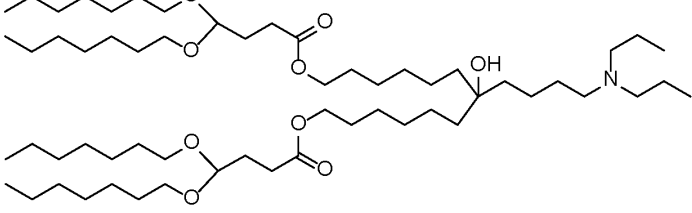
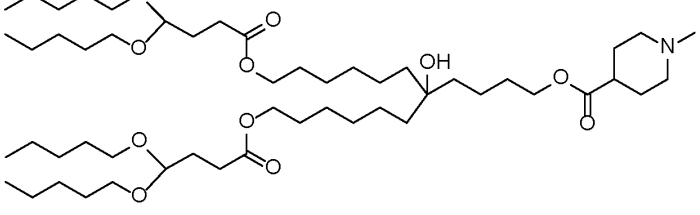
$\text{R}^3$  and  $\text{R}^4$  each independently represent a  $C_{1-4}$  alkyl group or  $C_{2-4}$  alkenyl group, or

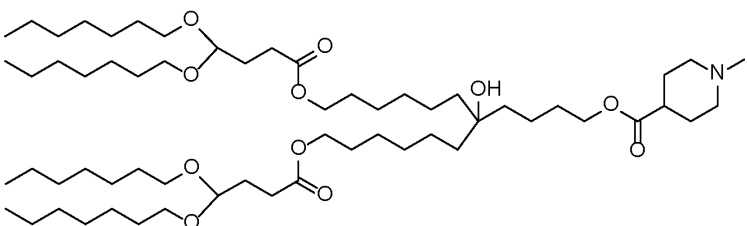
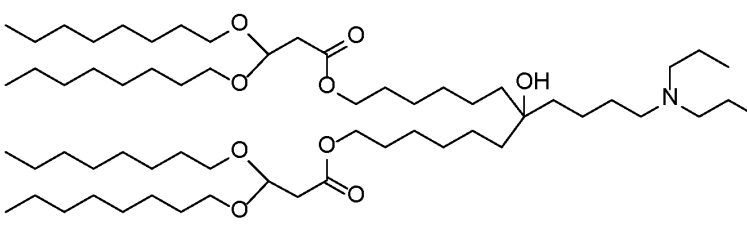
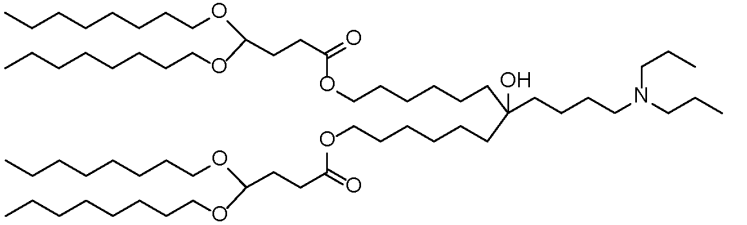
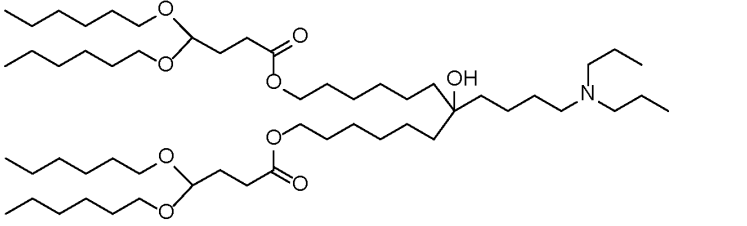
$\text{R}^3$  and  $\text{R}^4$  are bound to each other to form a 5- to 7-membered non-aromatic heterocyclic group, wherein one or two hydrogen atoms of said heterocyclic group may optionally be replaced with  $C_{1-4}$  alkyl group or  $C_{2-4}$  alkenyl group.

Embodiment (8). The compound of any one of Embodiments (1) to (7), or pharmaceutically acceptable salt thereof, wherein the compound is selected from the group consisting of Compounds Nos. 1 to 9 as shown in the following Table 1.

**Table 1**

| Compound | Structure |
|----------|-----------|
|----------|-----------|

| No. |                                                                                      |
|-----|--------------------------------------------------------------------------------------|
| 1   |    |
| 2   |    |
| 3   |   |
| 4   |  |
| 5   |  |

|   |                                                                                      |
|---|--------------------------------------------------------------------------------------|
| 6 |    |
| 7 |    |
| 8 |   |
| 9 |  |

Embodiment (9). A lipid nanoparticle comprising the compound of any one of Embodiments (1) to (8), or pharmaceutically acceptable salt thereof.

Embodiment (10). The lipid nanoparticle of Embodiment (9), further comprising a sterol, a phospholipid, and a polyalkylene glycol-modified lipid.

Embodiment (11). The lipid nanoparticle of Embodiment (10), wherein the phospholipid is selected from the group consisting of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), wherein the polyalkylene glycol-modified lipid is 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol (DMG-PEG).

Embodiment (12). The lipid nanoparticle of Embodiment (10) or (11), wherein the lipid

nanoparticle comprises:

40-60 mol% of the compound of any one of Embodiments (1) to (8), or pharmaceutically acceptable salt thereof, to the total lipid amount of the lipid nanoparticle;

30-50 mol% of the sterol to the total lipid amount of the lipid nanoparticle;

5-15 mol% of the phospholipid selected from the group consisting of DSPC, DOPC and DOPE to the total lipid amount of the lipid nanoparticle; and

1-5 mol% of the polyalkylene glycol-modified lipid that is DMG-PEG to the total lipid amount of the lipid nanoparticle.

Embodiment (13). The lipid nanoparticle of any one of Embodiments (9) to (12), further comprising a nucleic acid encapsulated in the lipid nanoparticle.

Embodiment (14). A method of delivering a nucleic acid to a cell, comprising contacting the cell with the lipid nanoparticle of Embodiment (13).

Embodiment (15). A method of delivering a nucleic acid to a subject in need thereof, comprising administering to the subject the lipid nanoparticle of Embodiment (13).

### DETAILED DESCRIPTION

[0007] Embodiments of the present disclosure are specifically described below. In this application, "X1-X2" (where X1 and X2 are real numbers satisfying  $X1 < X2$ ) means "X1 or more and X2 or less".

#### [0008] Compound

In one aspect, the present disclosure relates to a pH-sensitive cationic lipid that is a compound represented by the following general formula (I) or pharmaceutically acceptable salt thereof (hereinafter may be referred to as "pH-sensitive cationic lipids of the present disclosure"):

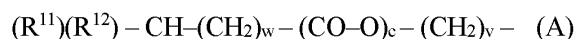


wherein:

a represents an integer of 3-5;

b represents 0 or 1;

$R^1$  and  $R^2$  each independently represent a group represented by general formula (A):



wherein:

$R^{11}$  and  $R^{12}$  each independently represent a  $C_{5-15}$  alkoxy group;

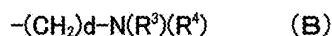
each  $c$  independently represents 0 or 1;

each  $v$  independently represents an integer of 4-12;

each  $w$  independently represents an integer of 0-3;

and

$X$  represents a 5- to 7-membered non-aromatic heterocyclic group, wherein a carbon atom of said heterocyclic group is bound to  $(O-CO)_b-$ , and one or two hydrogen atoms of said heterocyclic group may optionally be replaced with a  $C_{1-4}$  alkyl group or  $C_{2-4}$  alkenyl group, or a group represented by general formula (B):



wherein:

$d$  represents an integer of 0-3; and

$R^3$  and  $R^4$  each independently represent a  $C_{1-4}$  alkyl group or  $C_{2-4}$  alkenyl group, or

$R^3$  and  $R^4$  are bound to each other to form a 5- to 7-membered non-aromatic heterocyclic group, wherein one or two hydrogen atoms of said heterocyclic group may optionally be replaced with a  $C_{1-4}$  alkyl group or  $C_{2-4}$  alkenyl group.

**[0009]** As used herein, the term “alkyl” refers to a linear or a branched hydrocarbyl radical of a saturated aliphatic group, which can be of any length unless otherwise specified.

**[0010]** As used herein, the term “alkenyl” refers to a linear or a branched hydrocarbyl radical having at least one carbon-carbon double bond, which can be of any length unless otherwise specified.

**[0011]** As used herein, the term “alkoxy” refers to a linear or a branched alkyl bound through a single, terminal ether linkage.

**[0012]** In one embodiment,  $R^1$  is the same as  $R^2$ . In another embodiment,  $R^1$  is different from  $R^2$ .

**[0013]** In one embodiment,  $R^{11}$  is the same as  $R^{12}$ . In another embodiment,  $R^{11}$  is different from  $R^{12}$ .

**[0014]** In some embodiments,  $R^{11}$  and  $R^{12}$  are each independently a  $C_{5-14}$  alkoxy,  $C_{5-13}$  alkoxy,  $C_{5-12}$  alkoxy,  $C_{5-11}$  alkoxy,  $C_{5-10}$  alkoxy,  $C_{5-9}$  alkoxy,  $C_{5-8}$  alkoxy, or  $C_{5-7}$  alkoxy group.

[0015] In some embodiments, R<sup>11</sup> and R<sup>12</sup> are each independently:

n-pentyloxy group, 1-methylbutyloxy group, 2-methylbutyloxy group, 3-methylbutyloxy group, 1-ethylpropoxy group, 1,1-dimethylpropoxy group, or 2,2-dimethylpropoxy group;

n-hexyloxy group, 1-methylpentyloxy group, 2-methylpentyloxy group, 3-methylpentyloxy group, 4-methylpentyloxy group, 1-ethylbutyloxy group, 1,1-dimethylbutyloxy group, 2,2-dimethylbutyloxy group, 3,3-dimethylbutyloxy group, 1,2-dimethylbutyloxy group, or 1-methyl-2,2-dimethylbutyloxy group;

n-heptyloxy group, 1-methylhexyloxy group, 2-methylhexyloxy group, 3-methylhexyloxy group, 4-methylhexyloxy group, 5-methylhexyloxy group, 1-ethylpentyloxy group, 1,1-dimethylpentyloxy group, 2,2-dimethylpentyloxy group, 3,3-dimethylpentyloxy group, 4,4-dimethylpentyloxy group, 1-methyl-3,3-dimethylbutyloxy group, or 2-methyl-3,3-dimethylbutyloxy group;

n-octyloxy group, 1-methylheptyloxy group, 2-methylheptyloxy group, 3-methylheptyloxy group, 4-methylheptyloxy group, 5-methylheptyloxy group, 6-methylheptyloxy group, 1-ethylhexyloxy group, 1,1-dimethylhexyloxy group, 2,2-dimethylhexyloxy group, 3,3-dimethylhexyloxy group, 4,4-dimethylhexyloxy group, 5,5-dimethylhexyloxy group, 1-methyl-4,4-dimethylpentyloxy group, 2-methyl-4,4-dimethylpentyloxy group, or 3-methyl-4,4-dimethylpentyloxy group;

n-nonyloxy group, 1-methyloctyloxy group, 2-methyloctyloxy group, 3-methyloctyloxy group, 4-methyloctyloxy group, 5-methyloctyloxy group, 6-methyloctyloxy group, 7-methyloctyloxy group, 1-ethylheptyloxy group, 1,1-dimethylheptyloxy group, 2,2-dimethylheptyloxy group, 3,3-dimethylheptyloxy group, 4,4-dimethylheptyloxy group, 5,5-dimethylheptyloxy group, 6,6-dimethylheptyloxy group, 1-methyl-5,5-dimethylhexyloxy group, 2-methyl-5,5-dimethylhexyloxy group, 3-methyl-5,5-dimethylhexyloxy group, or 4-methyl-5,5-dimethylhexyloxy group;

n-decyloxy group, 1-methylnonyloxy group, 2-methylnonyloxy group, 3-methylnonyloxy group, 4-methylnonyloxy group, 5-methylnonyloxy group, 6-methylnonyloxy group, 7-methylnonyloxy group, 8-methylnonyloxy group, 1-ethyloctyloxy group, 1,1-dimethyloctyloxy group, 2,2-dimethyloctyloxy group, 3,3-dimethyloctyloxy group, 4,4-dimethyloctyloxy group, 5,5-dimethyloctyloxy group, 6,6-dimethyloctyloxy group, 7,7-dimethyloctyloxy group, 1-methyl-6,6-dimethylheptyloxy group, 2-methyl-6,6-dimethylheptyloxy group, 3-methyl-6,6-dimethylheptyloxy group, 4-methyl-6,6-dimethylheptyloxy group, or 5-

methyl-6,6-dimethylheptyloxy group;

n-undecyloxy group, 1-methyldecyloxy group, 2-methyldecyloxy group, 3-methyldecyloxy group, 4-methyldecyloxy group, 5-methyldecyloxy group, 6-methyldecyloxy group, 7-methyldecyloxy group, 8-methyldecyloxy group, 9-methyldecyloxy group, 1-ethylnonyloxy group, 1,1-dimethylnonyloxy group, 2,2-dimethylnonyloxy group, 3,3-dimethylnonyloxy group, 4,4-dimethylnonyloxy group, 5,5-dimethylnonyloxy group, 6,6-dimethylnonyloxy group, 7,7-dimethylnonyloxy group, 8,8-dimethylnonyloxy group, 1-methyl-7,7-dimethyloctyloxy group, 2-methyl-7,7-dimethyloctyloxy group, 3-methyl-7,7-dimethyloctyloxy group, 4-methyl-7,7-dimethyloctyloxy group, 5-methyl-7,7-dimethyloctyloxy group, or 6-methyl-7,7-dimethyloctyloxy group;

n-dodecyloxy group, 1-methylundecyloxy group, 2-methylundecyloxy group, 3-methylundecyloxy group, 4-methylundecyloxy group, 5-methylundecyloxy group, 6-methylundecyloxy group, 7-methylundecyloxy group, 8-methylundecyloxy group, 9-methylundecyloxy group, 10-methylundecyloxy group, 1-ethyldecyloxy group, 1,1-dimethyldecyloxy group, 2,2-dimethyldecyloxy group, 3,3-dimethyldecyloxy group, 4,4-dimethyldecyloxy group, 5,5-dimethyldecyloxy group, 6,6-dimethyldecyloxy group, 7,7-dimethyldecyloxy group, 8,8-dimethyldecyloxy group, 9,9-dimethyldecyloxy group, 1-methyl-8,8-dimethylnonyloxy group, 2-methyl-8,8-dimethylnonyloxy group, 3-methyl-8,8-dimethylnonyloxy group, 4-methyl-8,8-dimethylnonyloxy group, 5-methyl-8,8-dimethylnonyloxy group, 6-methyl-8,8-dimethylnonyloxy group, or 7-methyl-8,8-dimethylnonyloxy group;

n-tridecyloxy group, 1-methyldodecyloxy group, 2-methyldodecyloxy group, 3-methyldodecyloxy group, 4-methyldodecyloxy group, 5-methyldodecyloxy group, 6-methyldodecyloxy group, 7-methyldodecyloxy group, 8-methyldodecyloxy group, 9-methyldodecyloxy group, 10-methyldodecyloxy group, 11-methyldodecyloxy group, 1-ethylundecyloxy group, 1,1-dimethylundecyloxy group, 2,2-dimethylundecyloxy group, 3,3-dimethylundecyloxy group, 4,4-dimethylundecyloxy group, 5,5-dimethylundecyloxy group, 6,6-dimethylundecyloxy group, 7,7-dimethylundecyloxy group, 8,8-dimethylundecyloxy group, 9,9-dimethylundecyloxy group, 10,10-dimethylundecyloxy group, 1-methyl-9,9-dimethyldecyloxy group, 2-methyl-9,9-dimethyldecyloxy group, 3-methyl-9,9-dimethyldecyloxy group, 4-methyl-9,9-dimethyldecyloxy group, 5-methyl-9,9-dimethyldecyloxy group, 6-methyl-9,9-dimethyldecyloxy group, 7-methyl-9,9-dimethyldecyloxy group, or 8-methyl-9,9-dimethyldecyloxy group;

n-tetradecyloxy group, 1-methyltridecyloxy group, 2-methyltridecyloxy group, 3-

methyltridecyloxy group, 4-methyltridecyloxy group, 5-methyltridecyloxy group, 6-methyltridecyloxy group, 7-methyltridecyloxy group, 8-methyltridecyloxy group, 9-methyltridecyloxy group, 10-methyltridecyloxy group, 11-methyltridecyloxy group, 12-methyltridecyloxy group, 1-ethyl-dodecyloxy group, 1,1-dimethyldodecyloxy group, 2,2-dimethyldodecyloxy group, 3,3-dimethyldodecyloxy group, 4,4-dimethyldodecyloxy group, 5,5-dimethyldodecyloxy group, 6,6-dimethyldodecyloxy group, 7,7-dimethyldodecyloxy group, 8,8-dimethyldodecyloxy group, 9,9-dimethyldodecyloxy group, 10,10-dimethyldodecyloxy group, 11,11-dimethyldodecyloxy group, 1-methyl-10,10-dimethylundecyloxy group, 2-methyl-10,10-dimethylundecyloxy group, 3-methyl-10,10-dimethylundecyloxy group, 4-methyl-10,10-dimethylundecyloxy group, 5-methyl-10,10-dimethylundecyloxy group, 6-methyl-10,10-dimethylundecyloxy group, 7-methyl-10,10-dimethylundecyloxy group, 8-methyl-10,10-dimethylundecyloxy group, or 9-methyl-10,10-dimethylundecyloxy group;

n-pentadecyloxy group, 1-methyltetradecyloxy group, 2-methyltetradecyloxy group, 3-methyltetradecyloxy group, 4-methyltetradecyloxy group, 5-methyltetradecyloxy group, 6-methyltetradecyloxy group, 7-methyltetradecyloxy group, 8-methyltetradecyloxy group, 9-methyltetradecyloxy group, 10-methyltetradecyloxy group, 11-methyltetradecyloxy group, 12-methyltetradecyloxy group, 13-methyltetradecyloxy group, 1-ethyltridecyloxy group, 1,1-dimethyltridecyloxy group, 2,2-dimethyltridecyloxy group, 3,3-dimethyltridecyloxy group, 4,4-dimethyltridecyloxy group, 5,5-dimethyltridecyloxy group, 6,6-dimethyltridecyloxy group, 7,7-dimethyltridecyloxy group, 8,8-dimethyltridecyloxy group, 9,9-dimethyltridecyloxy group, 10,10-dimethyltridecyloxy group, 11,11-dimethyltridecyloxy group, 12,12-dimethyltridecyloxy group, 1-methyl-11,11-dimethyldodecyloxy group, 2-methyl-11,11-dimethyldodecyloxy group, 3-methyl-11,11-dimethyldodecyloxy group, 4-methyl-11,11-dimethyldodecyloxy group, 5-methyl-11,11-dimethyldodecyloxy group, 6-methyl-11,11-dimethyldodecyloxy group, 7-methyl-11,11-dimethyldodecyloxy group, 8-methyl-11,11-dimethyldodecyloxy group, 9-methyl-11,11-dimethyldodecyloxy group, or 10-methyl-11,11-dimethyldodecyloxy group.

**[0016]** In some embodiments,  $R^{11}$  and  $R^{12}$  are each independently n-pentyloxy, n-hexyloxy, n-heptyloxy, n-octyloxy, n-nonyloxy, n-decyloxy, n-undecyloxy, n-dodecyloxy, n-tridecyloxy, n-tetradecyloxy, or n-pentadecyloxy. In some embodiments, both of  $R^{11}$  and  $R^{12}$  are n-pentyloxy, n-hexyloxy, n-heptyloxy, n-octyloxy, n-nonyloxy, n-decyloxy, n-undecyloxy, n-dodecyloxy, n-tridecyloxy, n-tetradecyloxy, or n-pentadecyloxy.

**[0017]** In one embodiment, c is 0. In another embodiment, c is 1.

**[0018]** In some embodiments, v is an integer of 4-11, 4-10, 4-9, 4-8, 4-7, 4-6, 5-12, 5-11, 5-10,

5-9, 5-8, 5-7, 5-6, 6-12, 6-11, 6-10, 6-9, 6-8, or 6-7. In some embodiments, v is 6.

**[0019]** In some embodiments, w is 1, 2 or 3. In some embodiments, w is 1 or 2.

**[0020]** In some embodiments, a is 3 or 4. In some embodiments, a is 4 or 5. In some embodiments, a is 4.

**[0021]** In some embodiments, b is 0. In some embodiments, b is 0 and X is the group represented by general formula (B). In some embodiments, b is 0, X is the group represented by general formula (B), d is 0, and R<sup>3</sup> and R<sup>4</sup> each independently a C<sub>1-4</sub> alkyl group. In some embodiments, b is 0, X is the group represented by general formula (B), d is 0, and R<sup>3</sup> and R<sup>4</sup> are n-propyl group.

**[0022]** In some embodiments, d is 0, 1 or 2. In some embodiments, d is 0 or 1. In some embodiments, d is 0.

**[0023]** In some embodiments, b is 1. In some embodiments, b is 1 and X is a 5- to 7-membered non-aromatic heterocyclic group, wherein a carbon atom of the heterocyclic group is bound to (O-CO)- and one or two hydrogen atoms of the heterocyclic group may be replaced with a C<sub>1-4</sub> alkyl group or C<sub>2-4</sub> alkenyl group. In some embodiments, b is 1 and X is 1-methyl-4-piperidinyl group.

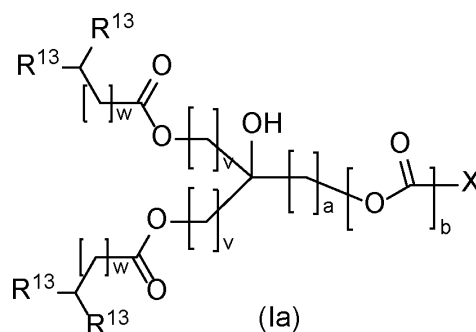
**[0024]** In some embodiments, R<sup>3</sup> and R<sup>4</sup> each independently represent a C<sub>1-4</sub> alkyl group or C<sub>2-4</sub> alkenyl group. In some embodiments, the C<sub>1-4</sub> alkyl group of R<sup>3</sup> and/or R<sup>4</sup> is methyl group, ethyl group, propyl group, isopropyl group, n-butyl group, isobutyl group, or tert-butyl group. In some embodiments, the C<sub>2-4</sub> alkenyl group of R<sup>3</sup> and/or R<sup>4</sup> is vinyl group, 1-propenyl group, 2-propenyl group, 1-methylvinyl group, 2-methyl-1-propenyl group, 1-butenyl group, 2-butenyl group, or 3-butenyl group.

**[0025]** In some embodiments, R<sup>3</sup> and R<sup>4</sup> are bound to each other to form a 5- to 7-membered non-aromatic heterocyclic group. In some embodiments, the 5- to 7-membered non-aromatic heterocyclic group formed by R<sup>3</sup> and R<sup>4</sup> bound to each other is, for example, 1-pyrrolidinyl group, 1-piperidinyl group, 1-morpholinyl group, or 1-piperazinyl group. In the 5- to 7-membered non-aromatic heterocyclic group formed by R<sup>3</sup> and R<sup>4</sup> bound to each other, one or two hydrogen atoms in the heterocyclic group may be replaced with a C<sub>1-4</sub> alkyl group or C<sub>2-4</sub> alkenyl group. When two hydrogen atoms in said heterocyclic group are replaced with a C<sub>1-4</sub> alkyl group or C<sub>2-4</sub> alkenyl group, they may be replaced with the same group or by different groups.

**[0026]** In some embodiments, when X is a 5- to 7-membered non-aromatic heterocyclic group, the hetero atom comprised in said heterocyclic group is nitrogen atom, oxygen atom, or sulfur atom. The hetero atom(s) constituting the heterocycle in said heterocyclic group may be one, two,

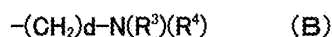
or more, and may be same or different. The 5- to 7-membered non-aromatic heterocyclic group may be a saturated heterocycle and may comprise one or more double bonds, but the heterocycle is never an aromatic ring.

**[0027]** In some embodiments, the pH-sensitive cationic lipid of the present disclosure is a compound represented by the following general formula (Ia) or pharmaceutically acceptable salt thereof:



wherein:

- a represents an integer of 3-5;
- each  $R^{13}$  independently represents a C<sub>5-15</sub> alkoxy group;
- b represents 0 or 1;
- each v independently represents an integer of 4-12;
- each w independently represents an integer of 1-3;
- X represents a 5- to 7-membered non-aromatic heterocyclic group, wherein a carbon atom of said heterocyclic group is bound to (O-CO)<sub>b</sub>-, and one or two hydrogen atoms of said heterocyclic group may optionally be replaced with a C<sub>1-4</sub> alkyl group or C<sub>2-4</sub> alkenyl group, or a group represented by general formula (B):



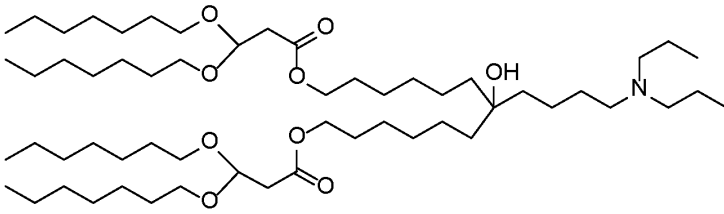
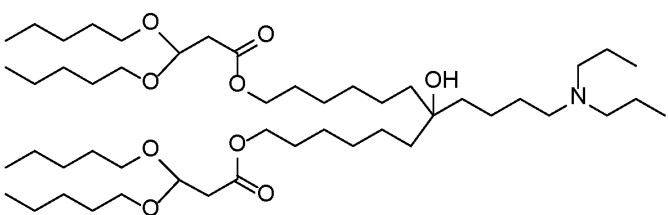
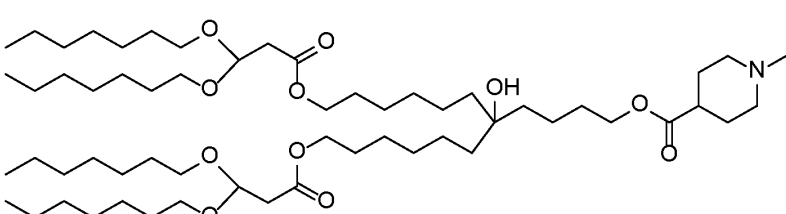
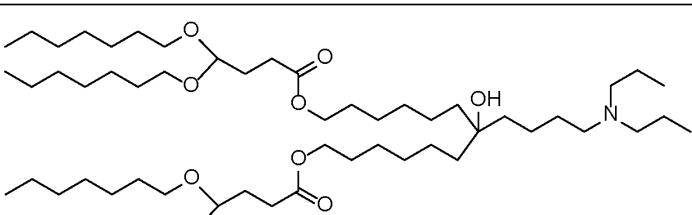
wherein:

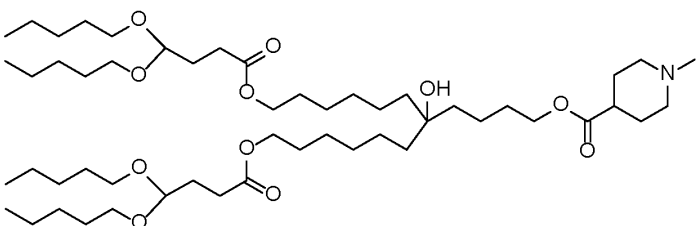
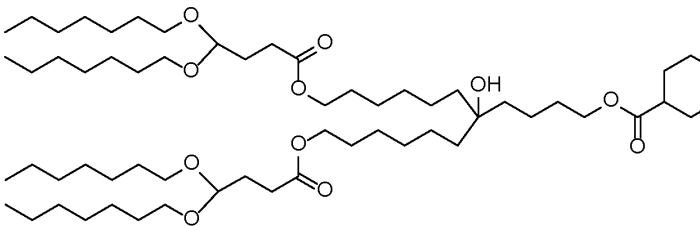
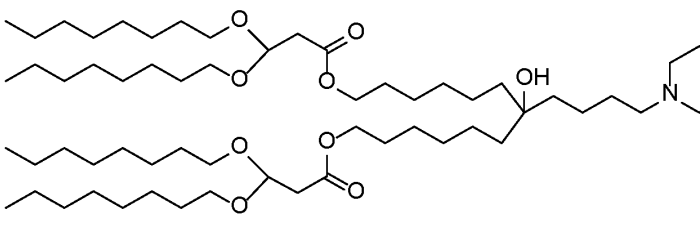
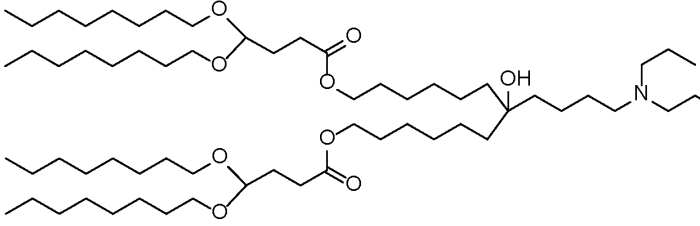
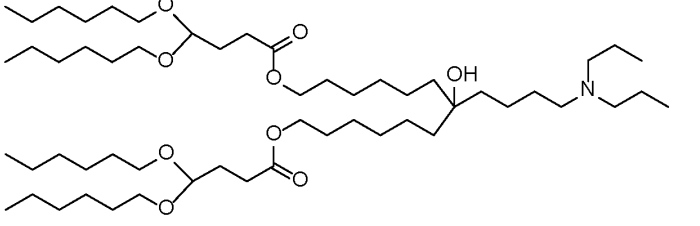
- d represents an integer of 0-3; and
- $R^3$  and  $R^4$  each independently represent a C<sub>1-4</sub> alkyl group or C<sub>2-4</sub> alkenyl group, or
- $R^3$  and  $R^4$  are bound to each other to form a 5- to 7-membered non-aromatic heterocyclic group, wherein one or two hydrogen atoms of said heterocyclic group may optionally be replaced with a C<sub>1-4</sub> alkyl group or

C<sub>2-4</sub> alkenyl group.

[0028] In some embodiments, the pH-sensitive cationic lipid of the present disclosure is selected from the group consisting of Compounds Nos. 1 to 9 as shown in the following Table 1, or pharmaceutically acceptable salt thereof:

Table 1

| Compound No | Structure                                                                            |
|-------------|--------------------------------------------------------------------------------------|
| 1           |    |
| 2           |   |
| 3           |  |
| 4           |  |

|   |                                                                                      |
|---|--------------------------------------------------------------------------------------|
| 5 |    |
| 6 |    |
| 7 |   |
| 8 |  |
| 9 |  |

**[0029]** As used herein, the term “pharmaceutically acceptable” refers to being compatible with use in subjects, for example, mammals such as human.

**[0030]** The pharmaceutically acceptable salt of the present disclosure includes, but is not limited to, salts containing a chloride, bromide, fluoride, iodide, nitrate, sulfate, methyl sulfate, phosphate, acetate, benzoate, citrate, glutamate and/or lactate. The pharmaceutically acceptable

salt of the compound of the general formula (I) of the present disclosure can be synthesized by conventional chemical methods. For example, the pharmaceutically acceptable salt of the compound of the general formula (I) of the present disclosure is prepared either by ion exchange chromatography or by reacting the free base in the compound with stoichiometric amounts or with an excess of the desired salt-forming inorganic or organic acid in a suitable solvent or various combinations of solvents.

**[0031]** In general, the compounds of the general formula (I) of the present disclosure may contain one or more chiral centers. The compounds containing one or more chiral centers may include those described as an “isomer”, a “stereoisomer”, a “diastereomer”, an “enantiomer”, an “optical isomer”, or a “racemic mixture”. Conventions for stereochemical nomenclature, for example, the stereoisomer naming rules of Cahn, Ingold, and Prelog, as well as methods for the determination of stereochemistry and the separation of stereoisomers known in the art, *see*, for example, Michael B. Smith and Jerry March, March's Advanced Organic Chemistry, 5th edition, 2001, can be applied to the compounds of the general formula (I) of the present disclosure. The compounds and structures of the present disclosure, including chemical drawings, are meant to encompass all possible isomers, chemically reasonable positional isomers, stereoisomers, diastereomers, enantiomers, and/or optical isomers that would be understood to exist for the specified compound or structure, including any mixture, racemic, *etc.* thereof.

**[0032]** The pH-sensitive cationic lipids represented by the general formula (I) can be easily produced, for example, by the methods specifically shown in the examples herein. By referring to these production methods and appropriately selecting raw material compounds, reagents, and reaction conditions, one skilled in the art can easily produce any lipids included in the range of the general formula (I).

#### **Lipid nanoparticle**

**[0033]** In one aspect, the present disclosure relates to a lipid nanoparticle comprising the pH-sensitive cationic lipids of the present disclosure (hereinafter may be referred to as “lipid nanoparticles according to the present disclosure”).

**[0034]** In the constituent lipids of the lipid nanoparticles according to the present disclosure, lipids which are generally used to form liposomes can generally be used as lipids other than the pH-sensitive cationic lipids of the present disclosure. Such lipids include, for example, phospholipid, sterol or sterol derivative, glycolipid, or saturated or unsaturated fatty acids, *etc.* These can be used in one type or a combination of two or more types. In some embodiments, the

lipid nanoparticle comprises the pH-sensitive cationic lipid of the present disclosure, a phospholipid, a sterol, and a polyalkylene glycol-modified lipid.

**[0035]** The phospholipids can include glycerophospholipids such as phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, phosphatidylethanolamine, phosphorylcholine, cardiolipin, plasmalogen, ceramide phosphorylglycerol phosphate, phosphatidic acid; and sphingophospholipids such as sphingomyelin, ceramide phosphorylglycerol, ceramide phosphoryl ethanolamine; *etc.* In addition, phospholipids derived from natural products such as egg yolk lecithin and soy lecithin can also be used. Fatty acid residues in glycerophospholipids and sphingophospholipids are not particularly limited, but can include, for example, saturated or unsaturated fatty acid residues having carbon number of 12-24, saturated or unsaturated fatty acid residues having carbon number of 14-20 are preferable. Specifically, acyl groups derived from fatty acids such as lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, arachidonic acid, behenic acid, and lignoceric acid can be included. When these glycerolipids or sphingolipids have two or more fatty acid residues, all fatty acid residues may be the same group or may be different group from each other. Examples of the phospholipids includes diphytanoyl phosphatidyl ethanolamine (DPhPE), 1,2-Diphytanoyl-*sn*-Glycero-3-Phosphocholine (DPhPC), 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2- dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE), and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE).

**[0036]** Sterols or sterol derivatives include, for example, animal-derived sterols such as cholesterol, cholesterol succinic acid, lanosterol, dihydrolanosterol, desmosterol, and dihydrocholesterol; plant-derived sterols (phytosterols) such as stigmasterol, sitosterol,  $\beta$ -sitosterol, campesterol, brassicasterol; and microorganism-derived sterols such as zymosterol and ergosterol, *etc.* Glycolipids include, for example, glyceroglycolipids such as sulfoxorybosylglyceride, diglycosyl diglyceride, digalactosyl diglyceride, galactosyl diglyceride, glycosyl diglyceride; sphingoglycolipids such as galactosylcerebroside, lactosylcerebroside, ganglioside; *etc.* Saturated or unsaturated fatty acids include, for example, saturated or unsaturated fatty acids having carbon number of 12-20 such as palmitic acid, oleic acid, stearic acid, arachidonic acid, and myristic acid.

**[0037]** The constituent lipids of the lipid nanoparticles according to the present disclosure, in

addition to the pH-sensitive cationic lipids of the present disclosure, preferably comprise neutral lipid, more preferably comprise phospholipid or sterol, further preferably comprise sterol, and more further preferably comprise cholesterol.

**[0038]** The lipid nanoparticles according to the present disclosure preferably comprise polyalkylene glycol-modified lipids as a lipid component. Polyalkylene glycol is a hydrophilic polymer, and, by constructing lipid nanoparticles using polyalkylene glycol-modified lipids as lipid membrane constituent lipids, surface of the lipid nanoparticles can be modified with polyalkylene glycol. Surface modification with polyalkylene glycol may be able to enhance the stability such as blood retention of lipid nanoparticles.

**[0039]** As polyalkylene glycol, for example, polyethylene glycol, polypropylene glycol, polytetramethylene glycol, polyhexamethylene glycol, *etc.* can be used. In some embodiments, the average molecular weight of polyalkylene glycol is, for example, approximately between 200 and 10,000, preferably approximately between 500 and 10,000, further preferably approximately between 1,000 and 5,000. In some embodiments, the molecular weight of polyalkylene glycol is about 200, 300, 350, 400, 500, 550, 750, 1000, 1500, 2000, 3000, 3500, 4000, 5000 or 10,000 Da.

**[0040]** For example, stearylated polyethylene glycol (*e.g.*, PEG-45 stearate (STR-PEG45), *etc.*) can be used for modification of lipids by polyethylene glycol. Additionally, polyethylene glycol derivatives such as N-[carbonyl-methoxypolyethylene glycol]-1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE-PEG), N-[carbonyl-methoxypolyethylene glycol]-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG), and 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol (DMG-PEG) can be used. For example, N-[carbonyl-methoxypolyethylene glycol-2000]-1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE-PEG2000), n-[carbonyl-methoxypolyethylene glycol-5000]-1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE-PEG5000), N-[carbonyl-methoxypolyethylene glycol-750]-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG750), N-[carbonyl-methoxypolyethylene glycol-2000]-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG2000), N-[carbonyl-methoxypolyethylene glycol-5000]-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG5000), and 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (DMG-PEG2000), *etc.* can be used.

**[0041]** In some embodiments, the lipid nanoparticles according to the present disclosure comprises any one of DSPC, DOPC and DOPE as the phospholipid and DMG-PEG as the polyalkylene glycol-modified lipid. For example, an embodiment of the lipid nanoparticles according to the present disclosure comprises 40-60 mol% of the pH-sensitive cationic lipids of

the present disclosure to the total lipid amount of the lipid nanoparticle; 30-50 mol% of the sterol (such as cholesterol) to the total lipid amount of the lipid nanoparticle; 5-15 mol% of any one of DSPC, DOPC and DOPE to the total lipid amount of the lipid nanoparticle; and 1-5 mol% of DMG-PEG (such as DMG-PEG2000) to the total lipid amount of the lipid nanoparticle. The molar ratio of the pH-sensitive cationic lipids of the present disclosure, the sterol, the phospholipid selected from the group consisting of DSPC, DOPC and DOPE, and DMG-PEG in the lipid nanoparticle according to the present disclosure (pH-sensitive cationic lipid/ sterol/ phospholipid/DMG-PEG) includes, but not limited to, (60/31/8/1), (60/31/7.5/1.5), (60/31/7/2), (60/31/6.5/2.5), (60/31/6/3), (60/31/5.5/3.5), (60/31/5/4), (60/31/4.5/4.5), (60/31/4/5), (60/31.5/7.5/1), (60/30.5/7.5/2), (60/30/7.5/2.5), (60/29.5/7.5/3), (60/29/7.5/3.5), (60/28.5/7.5/4), (60/28/7.5/4.5), (60/27.5/7.5/5), (59/32/7.5/1.5), (58/33/7.5/1.5), (57/34/7.5/1.5), (56/35/7.5/1.5), (55/36/7.5/1.5), (60/29/10/1), (60/28.5/10/1.5), (60/28/10/2), (60/27.5/10/2.5), (60/27/10/3), (60/26.5/10/3.5), (60/26/10/4), (60/25.5/10/4.5), (60/25/10/5), (60/27/12/1), (60/27/11.5/1.5), (60/27/11/2), (60/27/10.5/2.5), (60/27/9.5/3.5), (60/27/9/4), (60/27/8.5/4.5), (60/27/8/5), (59/28/10/3), (58/29/10/3), (57/30/10/3), (56/31/10/3), (55/32/10/3), (50/39/10/1), (50/38.5/10/1.5), (50/38/10/2), (50/37.5/10/2.5), (50/37/10/3), (50/36.5/10/3.5), (50/36/10/4), (50/35.5/10/4.5), (50/35/10/5), (50/38.5/10.5/1), (50/38.5/9.5/2), (50/38.5/9/2.5), (50/38.5/8.5/3), (50/38.5/8/3.5), (50/38.5/7.5/4), (50/38.5/7/4.5), (50/38.5/6.5/5), (51/37.5/10/1.5), (52/36.5/10/1.5), (53/35.5/10/1.5), (54/34.5/10/1.5), (55/33.5/10/1.5), (49/39.5/10/1.5), (48/40.5/10/1.5), (47/41.5/10/1.5), (46/42.5/10/1.5), (45/43.5/10/1.5), (51/34/10/5), (52/33/10/5), (53/32/10/5), (54/31/10/5), (55/30/10/5), (40/44/15/1), (40/43.5/15/1.5), (40/43/15/2), (40/42.5/15/2.5), (40/42/15/3), (40/41.5/15/3.5), (40/41/15/4), (40/40.5/15/4.5), (40/40/15/5), (41/39/15/5), (42/38/15/5), (43/37/15/5), (44/36/15/5), (45/35/15/5), (41/41/14/4), (42/42/13/3), (43/43/12/2) and (44/44/11/1). The lipid nanoparticle according to the present disclosure may comprise (1) Compound 1: cholesterol: DOPE: DMG-PEG, preferably in ratio 57.5:38.5:2.5:1.5 (mol%), or (2) Compound 1: cholesterol: DOPE: DMG-PEG, preferably in ratio 59:34:5.5:1.5 (mol%).

**[0042]** The lipid nanoparticles according to the present disclosure can be subjected to appropriate surface modification, as necessary. The lipid nanoparticles according to the present disclosure can be modified on the surface with hydrophilic polymers, *etc.* to enhance blood retention. Surface modification may be able to be achieved by using lipids modified with these modifying groups as constituent lipid of the lipid nanoparticles.

**[0043]** In the production of lipid nanoparticles according to the present disclosure, for

example, glycophorin, ganglioside GM1, phosphatidylinositol, ganglioside GM3, glucuronic acid derivatives, glutamic acid derivatives, and polyglycerol phospholipid derivatives, *etc.* can be used as lipid derivatives to enhance blood retention. In addition, dextran, pullulan, ficoll, polyvinyl alcohol, styrene-maleic anhydride alternating copolymer, divinyl ether-maleic anhydride alternating copolymer, amylose, amylopectin, chitosan, mannan, cyclodextrin, pectin and carrageenan, *etc.*, other than polyalkylene glycol, can be used for surface modification, as hydrophilic polymers to enhance blood retention.

**[0044]** In addition, in order to facilitate nuclear translocation of the lipid nanoparticles according to the present disclosure, for example, lipid nanoparticles can be surface-modified with oligosaccharide compounds with three or more saccharides. The type of oligosaccharide compounds with three or more saccharides is not particularly limited, but for example, oligosaccharide compounds in which approximately between 3 and 10 saccharide units are bound can be used, preferably oligosaccharide compounds in which approximately between 3 and 6 saccharide units are bound can be used. Among them, preferably, oligosaccharide compounds with trimer or hexamer of glucose can be used, and, further preferably, oligosaccharide compounds with trimer or tetramer of glucose can be used. More specifically, isomaltotriose, isopanose, maltotriose, maltotetraose, maltopentaose, or maltohexaose can be preferably used, among which maltotriose, maltotetraose, maltopentaose, or maltohexaose with  $\alpha$ 1-4 bound glucose are further preferable. Particularly preferred are maltotriose or maltotetraose, and most preferred is maltotriose. Surface modification amount of lipid nanoparticles by oligosaccharide compound is not particularly limited, but, for example, it is approximately between 1 and 30 mol%, preferably approximately between 2 and 20 mol%, and more preferably approximately between 5 and 10 mol% to the total lipid amount.

**[0045]** The method for surface modifying lipid nanoparticles with oligosaccharide compound is not particularly limited, but, for example, liposomes in which lipid nanoparticles are surface modified with monosaccharides such as galactose and mannose (PCT publication No. WO 2007/102481) are known, so the method for the surface modification described in the publication can be employed. The surface modification method described in this publication can be adopted to the present disclosure. All of the disclosures in above publication shall be included by reference as the disclosures in the present application.

**[0046]** In addition, the lipid nanoparticles according to the present disclosure can also be imparted any one or more functions such as temperature change sensitive function, membrane permeability function, gene expression function, and pH-sensitive function. Adding these

functions appropriately can improve the retention of lipid nanoparticles in the blood and allow the lipid nanoparticles to efficiently escape from endosomes after endocytosis in target cells.

**[0047]** The lipid nanoparticles according to the present disclosure may comprise one or more substances selected from the group consisting of anti-oxidizing agents such as tocopherol, propyl gallate, ascorbyl palmitate, butylated hydroxytoluene, charged substances, and membrane polypeptides, *etc.* Charged substances which impart positive charges can include, for example, saturated or unsaturated aliphatic amines such as stearylamine and oleylamine, and charged substances which impart negative charges can include, for example, dicetyl phosphate, cholesteryl hemisuccinate, phosphatidylserine, phosphatidylinositol, phosphatidic acid, *etc.* Membrane polypeptides include, for example, membrane extrinsic polypeptide or membrane intrinsic polypeptide, *etc.* The compounded amount of these substances is not particularly limited and can be appropriately selected according to the purpose.

**[0048]** The average particle size of the lipid nanoparticles according to the present disclosure is, for example, 400 nm or less, 300 nm or less, 200 nm or less, or 150 nm or less. As used herein, the “average particle size of the lipid nanoparticles” means the Z-average particle size measured by dynamic light scattering (DLS). Measurement by dynamic light scattering can be carried out by usual method using commercially available DLS equipment, *etc.*

**[0049]** The polydispersity index (PDI) of the lipid nanoparticles according to the present disclosure is, for example, approximately between 0.01 and 0.7, preferably approximately between 0.01 and 0.6, further preferably approximately between 0.03 and 0.3. The zeta potential at pH 7.4 can be in the range of -50 mV-5 mV, preferably -45 mV-5 mV.

**[0050]** The morphology of the lipid nanoparticles according to the present disclosure is not particularly limited, but can include, for example, unilamellar liposome, multilayer liposome, spherical micelle, or unshaped layered structure as morphology dispersed in aqueous solvent. The lipid nanoparticles according to the present disclosure are preferably unilamellar liposome or multilayer liposome.

**[0051]** The lipid nanoparticles according to the present disclosure preferably encapsulate components for the purpose of being delivered into the target cells inside the particle covered with lipid membranes. The components which the lipid nanoparticles according to the present disclosure encapsulate inside the particles are not limited as long as they are sized available to be encapsulated. The lipid nanoparticles according to the present disclosure can encapsulate any component such as nucleic acids, saccharides, peptides, low molecular weight compounds, and metallic compounds. In some embodiments, the component is an active pharmaceutical

ingredient.

**[0052]** The component encapsulated in the lipid nanoparticles according to the present disclosure is preferably nucleic acid. The nucleic acid may be DNA, or may be RNA, or also may be analogs or derivatives thereof (*e.g.*, peptide nucleic acid (PNA) or phosphorothioate DNA, *etc.*). The nucleic acids to be encapsulated in the lipid nanoparticles according to the present disclosure may be single-stranded nucleic acids, may be double-stranded nucleic acids, also may be linear, or cyclic.

**[0053]** In some embodiments, the nucleic acids to be encapsulated in the lipid nanoparticles according to the present disclosure comprise a foreign gene to be expressed in the target cell, preferably they are nucleic acids which function to express the foreign gene in the cell by being taken up into the cell. The foreign genes may be genes originally comprised in the genomic DNA of the target cells, or they may be genes not comprised in the genomic DNA. Such nucleic acids include gene expression vectors comprising nucleic acids consisting of base sequences encoding genes of interest to be expressed. The gene expression vectors may be present as extrachromosomal genes in the introduced cell, or it may be taken up into the genomic DNA by homologous recombination.

**[0054]** The gene expression vectors to be encapsulated in the lipid nanoparticles according to the present disclosure are not particularly limited, and vectors generally used in gene therapy, *etc.* can be used. The gene expression vectors to be encapsulated in the lipid nanoparticles according to the present disclosure are preferably nucleic acid vectors such as plasmid vectors. The plasmid vectors may remain in a circular form or may be encapsulated in the lipid nanoparticles according to the present disclosure in a pre-cut linear form. The gene expression vectors can be designed by usual method using commonly used molecular biological tools based on the base sequence information of the gene of the target to be expressed, and can be produced by various known methods.

**[0055]** The nucleic acids to be encapsulated in the lipid nanoparticles according to the present disclosure are also preferably functional nucleic acids which control the expression of target genes present in the target cells. The functional nucleic acids include antisense oligonucleotide, antisense oligonucleotide (including antisense DNA and antisense RNA), siRNA, microRNA(miRNA), and mRNA, *etc.* Also, they may be plasmid DNA (pDNA) becoming siRNA expression vectors which express siRNA in the cells. The siRNA expression vectors can be prepared from commercially available siRNA expression vectors, also which may be appropriately modified. In one embodiment of the present disclosure, the lipid nanoparticles according to the present disclosure

comprise pH-sensitive cationic lipids of the present disclosure and mRNA.

**[0056]** As used herein, the “N/P ratio” is the ratio of the number of cationic nitrogen atoms (N) of the pH-sensitive cationic lipids of the present disclosure to the number of phosphate residues (P) of the nucleic acids encapsulated in the lipid nanoparticles according to the present disclosure. When the nucleic acids are mRNAs, the N/P ratio may be, for example, in the range of 3.0 to 12.0.

**[0057]** The production method of lipid nanoparticles according to the present disclosure is not particularly limited, and any method available to those skilled in the art can be adopted. As an example, they can be produced by, after forming a lipid film by dissolving all lipid components in an organic solvent such as chloroform and then drying under reduced pressure by an evaporator or spray drying by a spray dryer, adding components to be encapsulated into the lipid nanoparticles (for example, aqueous solvent comprising nucleic acids, *etc.*) to dried above mixture, then emulsifying by emulsifier such as homogenizer, ultrasonic emulsifier, or high pressure jet spray emulsifier, *etc.* They can also be produced by a well-known method for producing liposomes, for example, reversed-phase evaporation method. If the size of the lipid nanoparticles is to be controlled, extrusion (extruding filtration) may be carried out under high pressure using membrane filter with uniform pore size, *etc.*

**[0058]** The composition of the aqueous solvents (dispersion media) is not particularly limited, but can include, for example, buffer solutions such as phosphate buffer solution, citrate buffer solution, and phosphate buffered physiological saline, physiological saline, and culture media for cell culture. These aqueous solvents (dispersion media) can stably disperse lipid nanoparticles, but they may furthermore be added saccharides (aqueous solution) such as: monosaccharides such as glucose, galactose, mannose, fructose, inositol, ribose, and xylose; disaccharides such as lactose, sucrose, cellobiose, trehalose, and maltose; trisaccharides such as raffinose and meredinoside; polysaccharides such as cyclodextrin; sugar alcohols such as erythritol, xylitol, sorbitol, mannitol, and maltitol; and polyalcohols (aqueous solution) such as glycerin, diglycerin, polyglycerin, propylene glycol, polypropylene glycol, ethylene glycol, diethylene glycol, triethylene glycol, polyethylene glycol, ethylene glycol monoalkyl ether, diethylene glycol monoalkyl ether, and 1,3-butylene glycol. In order to stably store the lipid nanoparticles dispersed in this aqueous solvent for a long time, it is desirable to eliminate electrolytes in the aqueous solvent as much as possible in terms of physical stability such as aggregation control, *etc.* In addition, in terms of chemical stability of the lipids, it is desirable to set the pH of the aqueous solvent between weak acidity and

near neutral (approximately between pH 3.0 and 8.0) and/or to remove dissolved oxygen by nitrogen bubbling, *etc.*

**[0059]** The lipid nanoparticles according to the present disclosure also can be produced by alcohol dilution method using flow channel. The method is a method for producing lipid nanoparticles by introducing a solution in which lipid components are dissolved in alcohol solvent and a solution in which water-soluble components to be included in lipid nanoparticles are dissolved in aqueous solvent from different flow channels and merging them together. By using microchannel with built-in three-dimensional micromixer which can achieve instantaneous mixing of two liquids, lipid nanoparticles with a diameter of about 30 nm can be produced at high reproducibility (*See*, Leung et al., Journal of Physical Chemistry C Nanomater Interfaces, 2012, vol.116(34), p.18440-18450).

**[0060]** When obtained aqueous dispersions of lipid nanoparticles is lyophilized or spray dried, the stability may be able to be improved using, for example, saccharide (aqueous solution) such as: monosaccharides such as glucose, galactose, mannose, fructose, inositol, ribose, and xylose; disaccharides such as lactose, sucrose, cellobiose, trehalose, and maltose; trisaccharides such as raffinose and meredinese; polysaccharides such as cyclodextrin; sugar alcohols such as erythritol, xylitol, sorbitol, mannitol, and maltitol. In addition, when freezing above aqueous dispersions, the stability may be able to be improved using, for example, aforementioned saccharides and polyalcohols (aqueous solutions) such as glycerin, diglycerin, polyglycerin, propylene glycol, polypropylene glycol, ethylene glycol, diethylene glycol, triethylene glycol, polyethylene glycol, ethylene glycol monoalkyl ether, diethylene glycol monoalkyl ether, and 1,3-butylene glycol.

**[0061]** In one embodiment of the present disclosure, the lipid nanoparticles according to the present disclosure are lyophilized.

**[0062]** In some embodiments, the lipid nanoparticles according to the present disclosure are synthesized by injecting ethanol solution of lipids into a buffer solution including a nucleic acid in the same manner as described in U.S. publication No. 2013-0022665, PCT publication No. WO2019/090359, and PCT publication No. WO2020/102668. In some embodiments, the lipid nanoparticles according to the present disclosure are synthesized by combining a lipid solution with a nucleic acid using a microfluidic mixing device such as NanoAssemblr™ (Precision Nano Systems).

**[0063]** In some embodiments, the lipid nanoparticles according to the present disclosure have excellent stability. The lipid nanoparticles of the disclosure are, for example, stable for at least 1 week when kept at -80°C.

[0064] An apparent pKa values of the lipid nanoparticles according to the present disclosure is not particularly limited, but can be selected, for example, in the range of approximately between 4.0 and 9.0, preferably approximately between 4.5 and 8.5. The pKa values can be determined by using 2-(p-toluidino)-6-naphthalene sulfonic acid (TNS) (for example, see PCT publication No. WO2022/071582)

#### **Method of delivering a nucleic acid to a cell or a subject**

[0065] In one aspect, the present disclosure relates to a method of delivering a nucleic acid to a cell, comprising contacting the lipid nanoparticle according to the present disclosure that encapsulates the nucleic acid with the cell. In certain embodiments, the cell is in vitro. In certain embodiments, the cell is in vivo. In certain embodiments, the cell is ex vivo.

[0066] In one aspect, the present disclosure relates to a method of delivering a nucleic acid to a subject in need thereof, comprising administering the lipid nanoparticle according to the present disclosure that encapsulates the nucleic acid to the subject. The subject may be human or non-human animals. The non-human animals include mammals such as cattle, pig, horse, sheep, goat, monkey, dog, cat, rabbit, mouse, rat, hamster, and guinea pig, and birds such as chicken, quail, and duck, *etc.* The lipid nanoparticle may be administered by any means known in the art including, but not limited to, oral or parenteral routes, including intravenous, intramuscular, subcutaneous, transdermal, or airway (aerosol) administration.

#### **EXAMPLES**

[0067] The present disclosure is described in more detail below with examples, but the present disclosure is not limited to the examples below.

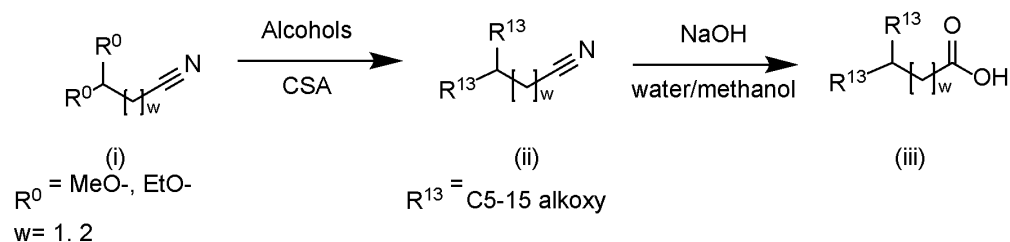
[0068] Abbreviations

EDC.HCl: N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride

DMAP: 4-Dimethylaminopyridine

DCM: Dichloromethane

CSA: DL-10-camphorsulfonic acid

**General procedure A: Synthesis of Compound (iii)**

**[0069]** To the mixture of Compound (i) (1.0 eq) and alcohol (3.0 eq) was added DL-10-camphorsulfonic acid (CSA; 0.05 eq). The reaction mixture was heated at 100 °C and stirred overnight. The reaction was cooled to ambient temperature and purified on a short pad of silica gel. The filtrate was concentrated in vacuo and dissolved in MeOH to give a 0.4 M of final concentration. To the mixture, 8.0 M sodium hydroxide aqueous solution (1.5 eq) was added. The mixture was stirred at 60 °C overnight. The reaction was diluted with ethyl acetate and brine. The aqueous layer was titrated to neutral pH with saturated aqueous solution of ammonium chloride and extracted with ethyl acetate twice. The combined organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography (ethyl acetate/hexane = 0-40%) to provide the desired Compound (iii).

**Synthesis of Compound (iii-b5) (w = 1, R<sup>13</sup> = n-pentyloxy)**

**[0070]** The compound was obtained by the general procedure A above, wherein 1-pentanol (Combi-Blocks) was used as the alcohol and 3,3-diethoxypropanenitrile (Combi-Blocks) was used as Compound (i) (w = 1, R<sup>0</sup> = ethoxy).

**Synthesis of Compound (iii-b7) (w = 1, R<sup>13</sup> = n-heptyloxy)**

**[0071]** The compound was obtained by the general procedure A above, wherein 1-heptanol (Combi-Blocks) was used as the alcohol and 3,3-diethoxypropanenitrile (Combi-Blocks) was used as Compound (i) (w = 1, R<sup>0</sup> = ethoxy).

**Synthesis of Compound (iii-b8) (w = 1, R<sup>13</sup> = n-octyloxy)**

**[0072]** The compound was obtained by the general procedure A above, wherein 1-octanol (Combi-Blocks) was used as the alcohol and 3,3-diethoxypropanenitrile (Combi-Blocks) was used as Compound (i) (w = 1, R<sup>0</sup> = ethoxy).

**Synthesis of Compound (iii-g5) (w = 2, R<sup>13</sup> = n-pentyloxy)**

**[0073]** The compound was obtained by the general procedure A above, wherein 1-pentanol (Combi-Blocks) was used as the alcohol and 4,4-dimethoxybutanenitrile (AK Scientific) was used as Compound (i) (w = 2, R<sup>0</sup> = methoxy).

**Synthesis of Compound (iii-g6) ( $w = 2$ ,  $R^{13} = n\text{-hexyloxy}$ )**

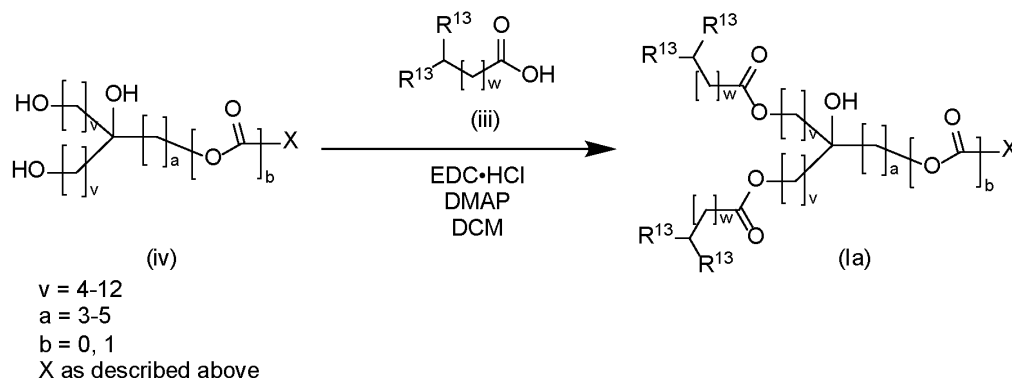
[0074] The compound was obtained by the general procedure A above, wherein 1-hexanol (Combi-Blocks) was used as the alcohol and 4,4-dimethoxybutanenitrile (AK Scientific) was used as Compound (i) ( $w = 2$ ,  $R^0 = \text{methoxy}$ ).

**Synthesis of Compound (iii-g7) ( $w = 2$ ,  $R^{13} = n\text{-heptyloxy}$ )**

[0075] The compound was obtained by the general procedure A above, wherein 1-heptanol (Combi-Blocks) was used as the alcohol and 4,4-dimethoxybutanenitrile (AK Scientific) was used as Compound (i) ( $w = 2$ ,  $R^0 = \text{methoxy}$ ).

**Synthesis of Compound (iii-g8) ( $w = 2$ ,  $R^{13} = n\text{-octyloxy}$ )**

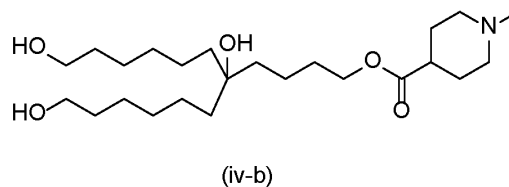
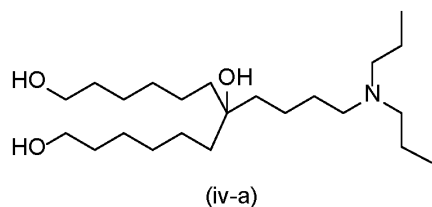
[0076] The compound was obtained by the general procedure A above, wherein 1-octanol (Combi-Blocks) was used as the alcohol and 4,4-dimethoxybutanenitrile (AK Scientific) was used as Compound (i) ( $w = 2$ ,  $R^0 = \text{methoxy}$ ).

**General procedure B: Synthesis of the compounds of Formula (Ia)**

[0077] In a round bottom flask, Compound (iv) (1.0 eq), Compound (iii) (2.3 eq), and 4-dimethylaminopyridine (Sigma-Aldrich, 0.2 eq) were dissolved in dichloromethane (containing 0.2 M of Compound (iv)), and 3-ethylcarbodiimide hydrochloride (TCI Chemicals, 2.8 eq) was added to the stirring solution. The resulting mixture was stirred at room temperature overnight. The reaction mixture was concentrated in reduced pressure, and saturated aqueous NaCl solution was added and extracted with dichloromethane three times. The combined organic layer was dried over anhydrous sodium sulfate, filtered, evaporated, and then the residue was purified by column chromatography to obtain the desired Compound (Ia).

[0078] Compound (iv) can be produced according to or in analogy to methods known in the art. For example, Compound (iv-a) ( $v = 6$ ,  $a = 4$ ,  $b = 0$ ,  $X = \text{di-}n\text{-propylamino}$ ) and Compound (iv-b) ( $v = 6$ ,  $a = 4$ ,  $b = 1$ ,  $X = 1\text{-methyl-4-piperidiny}$ ) were prepared according to the procedures described

in PCT publication No. WO2018/230710, which is hereby expressly incorporated by reference in its entirety.



### Synthesis of Compound No. 1

[0079] According to the general procedure B above, 1.1 g of the desired Compound No. 1 was obtained using Compound (iv-a) (2.9 mmol) as diol and Compound (iii-b7) as carboxylic acid. ESI+: detected  $m/z = 957.2$ .

### Synthesis of Compound No. 2

[0080] According to the general procedure B above, 201 mg of the desired Compound No. 2 was obtained using Compound (iv-a) (1.00 mmol) as diol and Compound (iii-b5) as carboxylic acid. ESI+: detected  $m/z = 844.8$ .

### Synthesis of Compound No. 3

[0081] According to the general procedure B above, 247 mg of the desired Compound No. 3 was obtained using Compound (iv-b) (1.60 mmol) as diol and Compound (iii-b7) as carboxylic acid. ESI+: detected  $m/z = 999.1$ .

### Synthesis of Compound No. 4

[0082] According to the general procedure B above, 182 mg of the desired Compound No. 4 was obtained using Compound (iv-a) (0.50 mmol) as diol and Compound (iii-g7) as carboxylic acid. ESI+: detected  $m/z = 985.2$ .

### Synthesis of Compound No. 5

[0083] According to the general procedure B above, 67 mg of the desired Compound No. 5 was obtained using Compound (iv-b) (1.00 mmol) as diol and Compound (iii-g5) as carboxylic acid. ESI+: detected  $m/z = 915.0$ .

### Synthesis of Compound No. 6

[0084] According to the general procedure B above, 167 mg of the desired Compound No. 6 was obtained using Compound (iv-b) (1.00 mmol) as diol and Compound (iii-g7) as carboxylic acid. ESI+: detected  $m/z = 1027.1$ .

### Synthesis of Compound No. 7

[0085] According to the general procedure B above, 143 mg of the desired Compound No. 7

was obtained using Compound (iv-a) (0.30 mmol) as diol and Compound (iii-b8) as carboxylic acid.  
ESI+: detected m/z = 1013.0.

#### Synthesis of Compound No. 8

[0086] According to the general procedure B above, 240 mg of the desired Compound No. 8 was obtained using Compound (iv-a) (0.55 mmol) as diol and Compound (iii-g6) as carboxylic acid.  
ESI+: detected m/z = 1040.9.

#### Synthesis of Compound No. 9

[0087] According to the general procedure B above, 91 mg of the desired Compound No. 9 was obtained using Compound (iv-a) (0.30 mmol) as diol and Compound (iii-g6) as carboxylic acid.  
ESI+: detected m/z = 928.8.

#### Example 1. In vitro expression of Fluc mRNA

[0088] LNP formulations with Fluc mRNA were prepared with the following compositions:  
(Test compound/ cholesterol/DSPC/DMG-PEG2000) = 50/38/10/2 (mol%)

[0089] The test compounds are Compounds Nos. 1 to 8, CL15F6, CL4F6, and CL4F 10-8. CL15F6, CL4F6, and CL4F 10-8 were prepared according to PCT publication No. WO2022/071582, which is hereby expressly incorporated by reference in its entirety. N/P ratio was fixed at 6.0 for these examples.

[0090] LNP formulations were prepared by injecting ethanol solution of lipids into a Fluc mRNA (TriLink, 5moU) buffer solution, in the same manner as described in U.S. publication No. 2013-0022665, PCT publication No. WO2019/090359, and PCT publication No. WO2020/102668, which are hereby expressly incorporated by reference in their entirety. The average particle size (PS), the polydispersity index (PDI), the encapsulation efficiency for mRNA (%EE), and the yield of each LNP formulation obtained is shown in the following Table 2. PS and PDI were obtained by using Malvern Zetasizer Nano-ZS ZEN 3600. %EE was obtained by the Ribogreen fluorescence assay following GenVoy-ILM™ User Guide by Precision NanoSystems.

**Table 2**

| LNP    | Test compound | PS (nm) | PDI   | %EE | yield |
|--------|---------------|---------|-------|-----|-------|
| LNP 1a | Compound 1    | 155     | 0.139 | 83  | 90    |
| LNP 2a | Compound 2    | 167     | 0.073 | 47  | 82    |
| LNP 3a | Compound 3    | 107     | 0.116 | 96  | 89    |
| LNP 4a | Compound 4    | 176     | 0.090 | 65  | 74    |
| LNP 5a | Compound 5    | 170     | 0.059 | 76  | 67    |

| LNP    | Test compound | PS (nm) | PDI   | %EE | yield |
|--------|---------------|---------|-------|-----|-------|
| LNP 6a | Compound 6    | 175     | 0.134 | 95  | 71    |
| LNP 7a | Compound 7    | 118     | 0.167 | 47  | 77    |
| LNP 8a | Compound 8    | 210     | 0.163 | 91  | 66    |
| LNP R1 | CL15F6        | 188     | 0.100 | 99  | 79    |
| LNP R2 | CL4F6         | 132     | 0.086 | 91  | 81    |
| LNP R3 | CL4F 10-8     | 189     | 0.050 | 75  | 76    |

**[0091]** In vitro expression of Fluc mRNA were measured according to the following protocol 1 or 2:

Protocol 1:

**[0092]** A549, Hep3B, and Panc-1 cell lines were cultured in media supplemented with 10% HI-FBS (Gibco Ref # 10082-147). F-12K media (ATCC Ref # 30-2004), EMEM media (ATCC Ref # 30-2003), and DMEM media (Gibco Ref # 11965-092) were used respectively. On day 0, cells were plated in white opaque 96-well TC-treated plates (Greiner Ref # 655083) at a density of 5000 cells/well using 90µL of cell mixture per well. The plates were placed in a 37°C incubator with 5% CO<sub>2</sub> overnight to allow cell attachment. On day 1, the mRNA/LNP complex was equilibrated to room temperature, then diluted with DPBS (Gibco Ref # 14190-144) to create a dose-response curve and added to plates at a volume of 10µL/well. The plates were placed back in the 37°C incubator with 5% CO<sub>2</sub> for 24 hours. On day 2, the Promega Luciferase Assay System (Ref # E1501) buffer and substrate were equilibrated to room temperature and combined, then added to the SpectraMax L Luminometer (Molecular Devices) injectors. The plates were prepared according to Promega kit guidelines by first removing the media in each well, then gently rinsing the well with DPBS, and finally adding 20µL of 1X reporter lysis buffer (Ref # E397A) to each well. The plates were placed in the Luminometer and injected with 100 µL of luciferase buffer/substrate mixture per well while luminescence values were obtained. EC<sub>50</sub> values were determined by fitting dose-response curve with 4-parameter logistic model using GraphPad/Prism. EC<sub>50</sub> values of the LNP formulation including any one of Compounds 1 to 3 were determined according to the protocol 1.

Protocol 2:

**[0093]** A549, Hep3B, and Panc-1 cell lines were cultured in media supplemented with 10% HI-FBS (Gibco Ref # 10082-147). F-12K media (ATCC Ref # 30-2004), EMEM media (ATCC Ref # 30-2003), and DMEM media (Gibco Ref # 11965-092) were used respectively. On day 0, cells were plated in white opaque 384-well TC-treated plates (USA Scientific Ref # 5678-1080) at

a density of 1500 cells/well using the Multidrop Combi+ (Thermo Scientific) by adding 30 $\mu$ L of cell mixture per well. The plates were placed in a 37°C incubator with 5% CO<sub>2</sub> overnight to allow cell attachment. On day 1, the mRNA/LNP complex was equilibrated to room temperature, then diluted with DPBS (Gibco Ref # 14190-144) to create a dose-response curve and added to plates at a volume of 3.3 $\mu$ L/well. The plates were placed back in the 37°C incubator with 5% CO<sub>2</sub> for 24 hours. On day 2, the ONE-Glo EX Luciferase Assay System (Promega Ref # E8130) was equilibrated to room temperature and combined. The plates were prepared according to Promega kit guidelines by adding 33.3 $\mu$ L of reagent mixture to each well. The plates were placed on a plate shaker for 3 minutes to assure cell lysis. Once lysis was complete, the plates were added to the Luminometer (Molecular Devices) and luminescence values were obtained. EC<sub>50</sub> values were determined by fitting dose-response curve with 4-parameter logistic model using GraphPad/Prism. EC<sub>50</sub> values of the LNP formulation (including one of Compounds 4 to 8, CL15F6, CL4F6, or CL4F 10-8) were determined according to the protocol 2. Experimental results are shown in Table 3.

**Table 3**

| <b>LNP Formulation</b> | <b>EC<sub>50</sub> (nM) in A549</b> | <b>EC<sub>50</sub> (nM) in HEP3B</b> | <b>EC<sub>50</sub> (nM) in Panc-1</b> |
|------------------------|-------------------------------------|--------------------------------------|---------------------------------------|
| LNP 1a                 | 0.05                                | 0.06                                 | 0.02                                  |
| LNP 2a                 | 0.30                                | 0.17                                 | 0.06                                  |
| LNP 3a                 | 0.01                                | 0.05                                 | 0.26                                  |
| LNP 4a                 | 0.26                                | 0.13                                 | 0.24                                  |
| LNP 5a                 | 0.18                                | 0.01                                 | 0.66                                  |
| LNP 6a                 | 0.06                                | 0.01                                 | 0.36                                  |
| LNP 7a                 | 0.33                                | 0.23                                 | 0.24                                  |
| LNP 8a                 | 0.07                                | 0.08                                 | 0.21                                  |
| LNP R1                 | 0.24                                | 0.12                                 | 0.69                                  |
| LNP R2                 | 0.36                                | 0.19                                 | 1.01                                  |
| LNP R3                 | 0.30                                | 0.18                                 | 0.44                                  |

**[0094]** LNP formulations containing the pH-sensitive cationic lipids of the present disclosure showed high transfection property to the target cells as seen in Table 3 above. For example, when LNP 1a was applied to Panc-1 cells, the EC<sub>50</sub> value was about 22 to 51-fold lower than when any one of the control LNPs (LNP R1, LNP R2, and LNP R3) was applied to the same cells. When LNP 2a was applied to Panc-1 cells, the EC<sub>50</sub> value was about 7.3 to 16.8-fold lower than when any one of the control LNPs was applied to the same cells. When LNP 3a was applied to A549 cells, the EC<sub>50</sub> value was about 24 to 36-fold lower than when any one of the control LNPs was

applied to the same cells. When LNP 4a was applied to Panc-1 cells, the EC<sub>50</sub> value was about 1.8 to 4.2-fold lower than when any one of the control LNPs was applied to the same cells. When LNP 5a or LNP 6a was applied to HEP3B cells, the EC<sub>50</sub> value was about 12 to 19-fold lower than when any one of the control LNPs was applied to the same cells. When LNP 7a was applied to Panc-1 cells, the EC<sub>50</sub> value was about 1.8 to 4.2-fold lower than when any one of the control LNPs was applied to the same cells. When LNP8a was applied to A549 cells, the EC<sub>50</sub> value was about 3.4 to 5.1-fold lower than when any one of the control LNPs was applied to the same cells.

#### Example 2. In vivo expression of Fluc mRNA

[0095] LNP formulations with Fluc mRNA were prepared according to the same procedure as explained in Example 1 except that N/P ratio varied. PS, PDI, %EE, and the yield of representative LNP formulations are shown in the following Table 4.

**Table 4**

| LNP | Lipid composition                                                    | N/P  | PS (nm) | PDI   | %EE | yield (%) |
|-----|----------------------------------------------------------------------|------|---------|-------|-----|-----------|
| 1b  | (Compound 1/<br>cholesterol/DSPC/DMG-PEG2000) =<br>50/38/10/2 (mol%) | 10.0 | 121     | 0.007 | 65  | 61        |
| 2b  | (Compound 3/<br>cholesterol/DSPC/DMG-PEG2000) =<br>50/38/10/2 (mol%) | 6.0  | 126     | 0.092 | 95  | 66        |

[0096] LNP with CleanCap® FLuc mRNA (5moU) (from TriLink) was delivered and transfected into Balb/c mice. The animals were intravenously given a single injection with one of LNP 1b and 2b at a dose of 0.5 mg/kg. Mice were anesthetized 6 hours after mRNA injection and then sacrificed immediately, and different organs were harvested and saved under -80° C until further analysis. The organs were homogenized in Reporter Lysis Buffer (Promega Ref # E3971) and the content of luciferase was quantified with the Promega Luciferase Assay System (Ref # E1501). The total luminescence value (RLU) per organ was determined by the SpectraMax L Luminometer (Molecular Devices). The experimental results obtained are shown in Table 5 below.

**Table 5**

| Injected drug            | Total RLU from Liver (average of n = 4) |
|--------------------------|-----------------------------------------|
| HEPES (negative control) | 2.35E+05                                |
| LNP 1b                   | 8.27E+09                                |

| Injected drug | Total RLU from Liver (average of n = 4) |
|---------------|-----------------------------------------|
| LNP 2b        | 7.09E+09                                |

[0097] The term “comprising” as used herein is synonymous with “including,” “containing,” or “characterized by,” and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps.

[0098] The above description discloses several methods and materials of the present invention. This invention is susceptible to modifications in the methods and materials, as well as alterations in the fabrication methods and equipment. Such modifications will become apparent to those skilled in the art from consideration of this disclosure or practice of the invention disclosed herein. Consequently, it is not intended that this invention be limited to the specific embodiments disclosed herein, but that it covers all modifications and alternatives coming within the true scope and spirit of the invention.

[0099] All references cited herein, including but not limited to published and unpublished applications, patents, and literature references, are incorporated herein by reference in their entirety and are hereby made a part of this specification. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

## WHAT IS CLAIMED IS:

1. A compound represented by general formula (I) or pharmaceutically acceptable salt thereof:

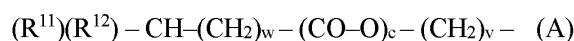


wherein:

a represents an integer of 3-5;

b represents 0 or 1;

$R^1$  and  $R^2$  each independently represent a group represented by general formula (A):



wherein:

$R^{11}$  and  $R^{12}$  each independently represent  $C_{5-15}$  alkoxy group;

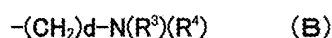
each c independently represents 0 or 1;

each v independently represents an integer of 4-12;

each w independently represents an integer of 0-3;

and

X represents a 5- to 7-membered non-aromatic heterocyclic group, wherein a carbon atom of said heterocyclic group is bound to  $(O-CO)_b-$  and one or two hydrogen atoms of said heterocyclic group may optionally be replaced with a  $C_{1-4}$  alkyl group or  $C_{2-4}$  alkenyl group, or a group represented by general formula (B):



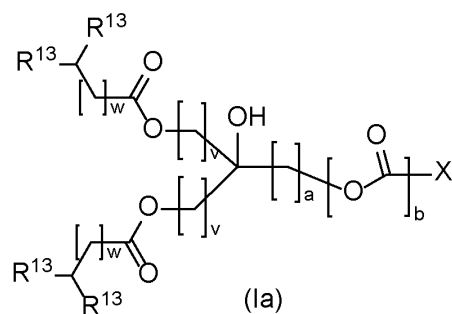
wherein:

d represents an integer of 0-3; and

$R^3$  and  $R^4$  each independently represent a  $C_{1-4}$  alkyl group or  $C_{2-4}$  alkenyl group, or

$R^3$  and  $R^4$  are bound to each other to form a 5- to 7-membered non-aromatic heterocyclic group, wherein one or two hydrogen atoms of said heterocyclic group may optionally be replaced with a  $C_{1-4}$  alkyl group or  $C_{2-4}$  alkenyl group.

2. The compound of claim 1 or pharmaceutically acceptable salt thereof, wherein c is 1.
3. The compound of claim 1 or pharmaceutically acceptable salt thereof, wherein w is 1 or 2.
4. The compound of claim 1 or pharmaceutically acceptable salt thereof, wherein b is 0 and X is the group represented by the general formula (B).
5. The compound of claim 1 or pharmaceutically acceptable salt thereof, wherein b is 0, X is the group represented by the general formula (B), d is 0, and R<sup>3</sup> and R<sup>4</sup> are each independently a C<sub>1-4</sub> alkyl group.
6. The compound of claim 1 or pharmaceutically acceptable salt thereof, wherein b is 1 and X is a 5- to 7-membered non-aromatic heterocyclic group.
7. The compound of claim 1 or pharmaceutically acceptable salt thereof, wherein the compound is represented by general formula (Ia):



wherein:

- a represents an integer of 3-5;
- each R<sup>13</sup> independently represents a C<sub>5-15</sub> alkoxy group;
- b represents 0 or 1;
- each v independently represents an integer of 4-12;
- each w independently represents an integer of 1-3;
- X represents a 5- to 7-membered non-aromatic heterocyclic group, wherein a carbon atom of said heterocyclic group is bound to (O-CO)<sub>b</sub>-, and one or two hydrogen atoms of said heterocyclic group may optionally be replaced with a C<sub>1-4</sub> alkyl group or C<sub>2-4</sub> alkenyl group, or a group represented by general formula (B):



wherein:

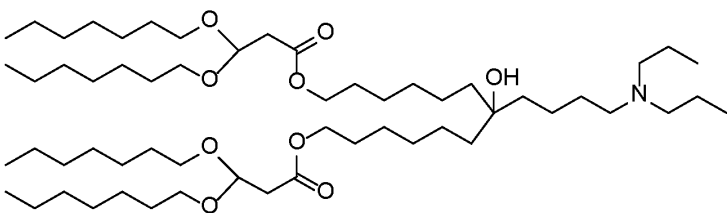
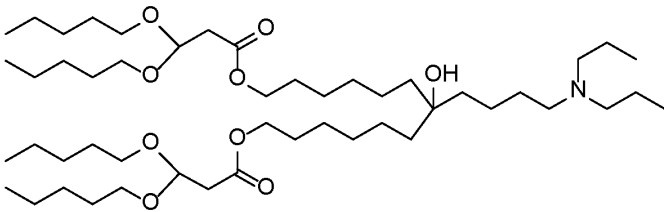
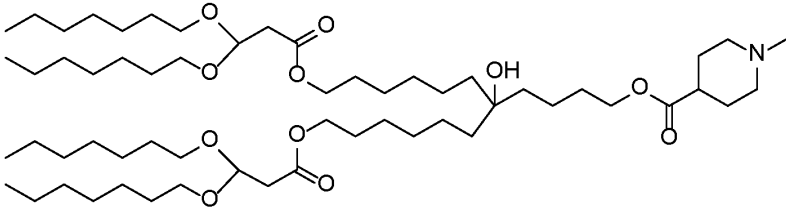
d represents an integer of 0-3; and

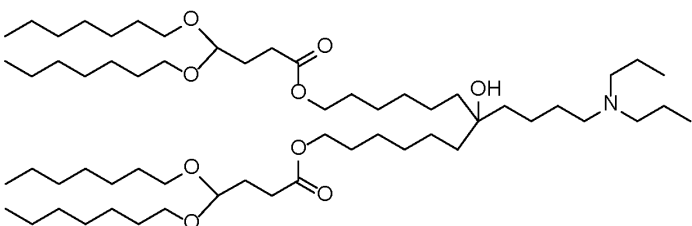
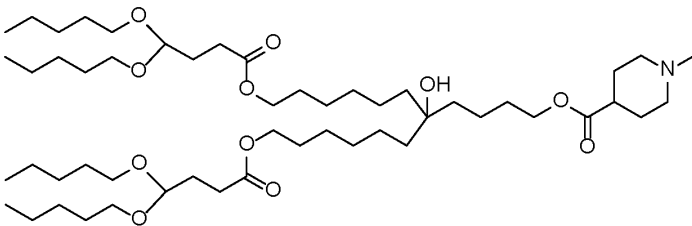
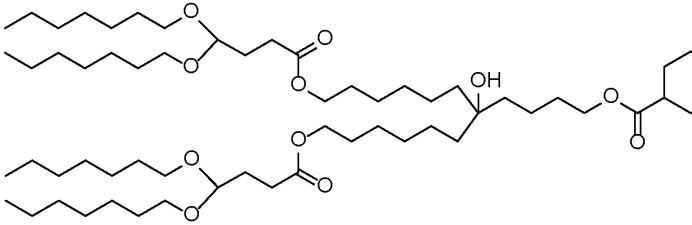
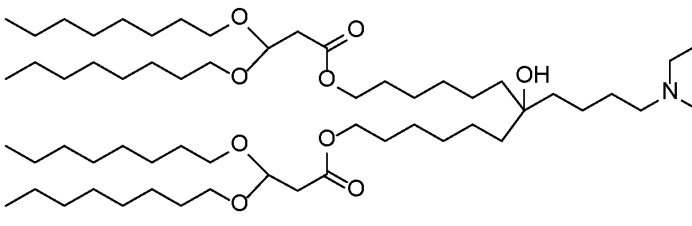
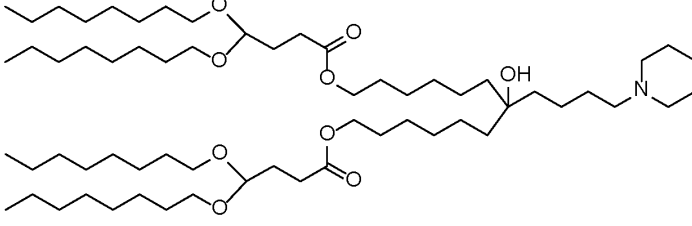
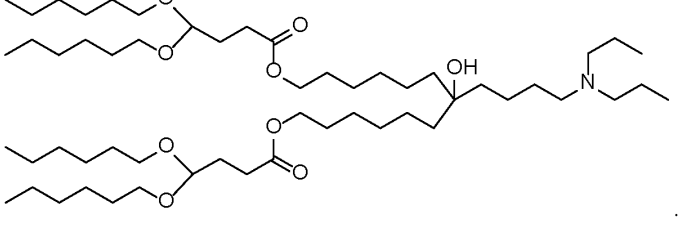
$R^3$  and  $R^4$  each independently represent a  $C_{1-4}$  alkyl group or  $C_{2-4}$  alkenyl group, or

$R^3$  and  $R^4$  are bound to each other to form a 5- to 7-membered non-aromatic heterocyclic group, wherein one or two hydrogen atoms of said heterocyclic group may optionally be replaced with a  $C_{1-4}$  alkyl group or  $C_{2-4}$  alkenyl group.

8. The compound of claim 1 or pharmaceutically acceptable salt thereof, wherein the compound is selected from the group consisting of Compounds Nos. 1 to 9 as shown in the following Table 1:

**Table 1**

| Compound No | Structure                                                                            |
|-------------|--------------------------------------------------------------------------------------|
| 1           |   |
| 2           |  |
| 3           |  |

|   |                                                                                      |
|---|--------------------------------------------------------------------------------------|
| 4 |    |
| 5 |    |
| 6 |   |
| 7 |  |
| 8 |  |
| 9 |  |

9. A lipid nanoparticle comprising the compound of claim 1 or pharmaceutically acceptable salt thereof.
10. The lipid nanoparticle of claim 9, further comprising a sterol, a phospholipid, and a polyalkylene glycol-modified lipid.
11. The lipid nanoparticle of claim 10, wherein the phospholipid is selected from the group consisting of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), wherein the polyalkylene glycol-modified lipid is 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol (DMG-PEG).
12. The lipid nanoparticle of claim 10, wherein the lipid nanoparticle comprises:  
40-60 mol% of the compound of claim 1 or pharmaceutically acceptable salt thereof, to total lipid amount of the lipid nanoparticle;  
30-50 mol% of the sterol to the total lipid amount of the lipid nanoparticle;  
5-15 mol% of the phospholipid selected from the group consisting of DSPC, DOPC and DOPE to the total lipid amount of the lipid nanoparticle; and  
1-5 mol% of the polyalkylene glycol-modified lipid that is DMG-PEG to the total lipid amount of the lipid nanoparticle.
13. The lipid nanoparticle of claim 9, further comprising a nucleic acid encapsulated in the lipid nanoparticle.
14. A method of delivering a nucleic acid to a cell, comprising contacting the cell with the lipid nanoparticle of claim 13.
15. A method of delivering a nucleic acid to a subject in need thereof, comprising administering to the subject the lipid nanoparticle of claim 13.