CLAIMS

1. A lyophilized formulation of lipid nanoparticles comprising, relative to the total weight of the lyophilized formulation,

(A) 0.2-20 wt.% of lipid nanoparticles comprising a cationic lipid; and

(B) a cryoprotectant combination comprising

(i) 75-99 wt.% of sucrose; and

(ii) 0.1-10 wt.% of a non-polar amino acid.

2. The lyophilized formulation of claim 1, wherein

the particle size of the lipid nanoparticles is from 80 nm to 100 nm, preferably from 80 nm to 95 nm, or from 82 nm to 95 nm; and/or

the lipid nanoparticle comprises, relative to the total mole number of all lipids present in the nanoparticle:

(a) from about 30 mol % to about 55 mol % of a cationic lipid;

(b) from about 20 mol % to about 50 mol % of a structural lipid;

(c) from about 5 mol % to about 40 mol % of a phospholipid; and

(d) from about 0.5 mol % to about 5 mol % of a polymer conjugated lipid;

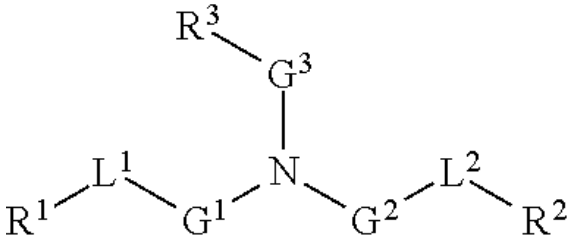
preferably

30-55 mol% of a cationic lipid, 5-40 mol% of DSPC, 20-50 mol% of cholesterol, and 0.5-3 mol% of PEG-lipid, relative to the total mole number of all lipids in the nanoparticle.

3. The lyophilized formulation of claim 1 or 2, wherein the lyophilized formulation comprises

0.1-10 wt.%, preferably 0.1-1 wt.%, more preferably 0.2-0.7 wt.%, relative to the total weight of the lyophilized LNP formulation, of a cationic lipid selected from

compounds of Formula 06-I:



(06-I)

wherein

L^1 and L^2 is -O(C═O)-;

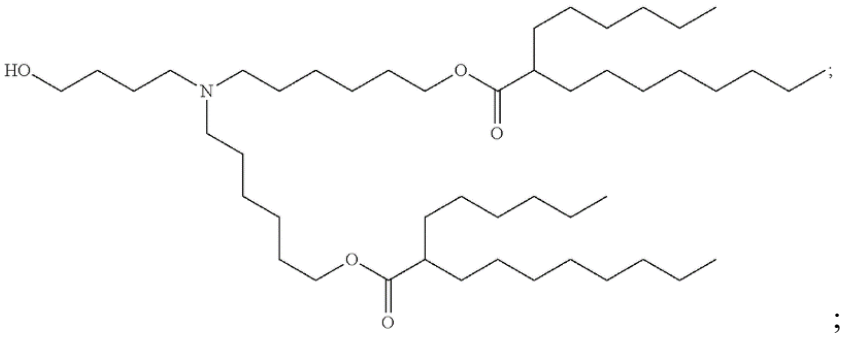
G^1 and G^2 are each independently unsubstituted C_4-C_8 alkylene;

G^3 is C_3-C_8 alkylene;

R^1 and R^2 are each independently C_{12}-C_{22} alkyl;

R^3 is H or OH,

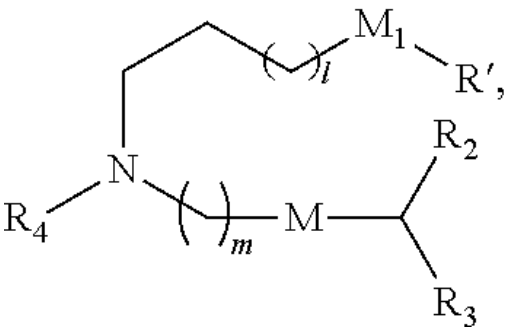
preferably the cationic lipid is compound of the following formula:



;

and/or

compounds of Formula 05-I:



(05-I)

wherein

l is selected from 1, 2, 3, 4, and 5;

m is selected from 5, 6, 7, 8, and 9;

M_1 is -C(O)O-;

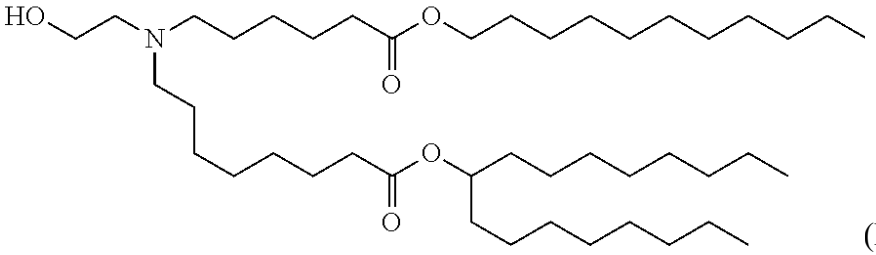
R_4 is $-(CH_2)_nOH$, and n is selected from 1, 2, 3, 4, or 5;

M is -OC(O)-;

R_2 and R_3 are both C_{6-10} alkyl; and

R' is a linear alkyl;

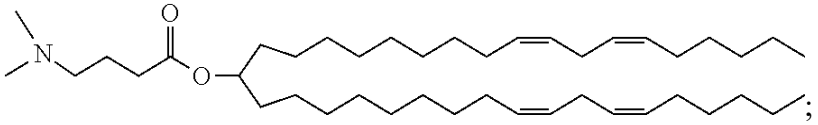
preferably the cationic lipid is compound of the following formula B:



(Formula B);

and/or

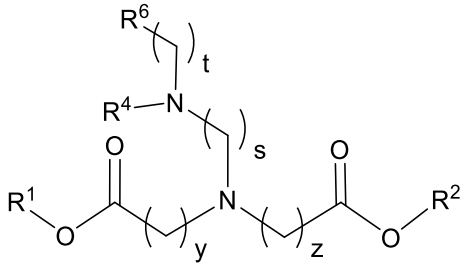
the following formula:



;

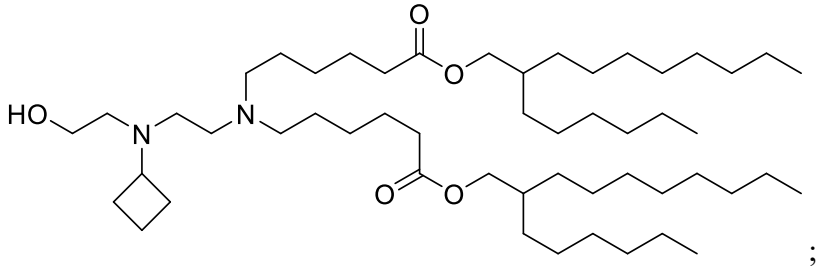
and/or

compounds of Formula (01-I-O):



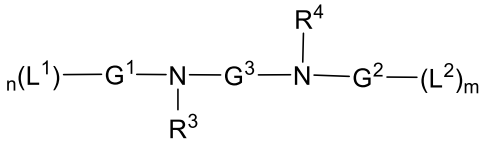
(01-I-O),

wherein y and z are each independently an integer from 4 to 6,
s is an integer from 2 to 4,
t is an integer from 1 to 3, and
R^1 and R^2 are each independently C_{12}-C_{22} alkyl;
R^4 is C_3-C_8 cycloalkyl;
R^6 is hydrogen or hydroxyl,
preferably the cationic lipid is compound of the following formula:



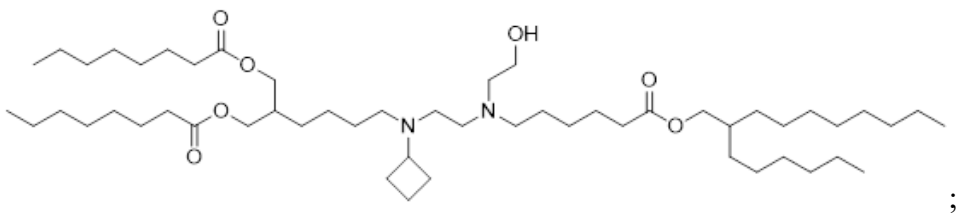
;

and/or
compounds of Formula (03-I):



(03-I),

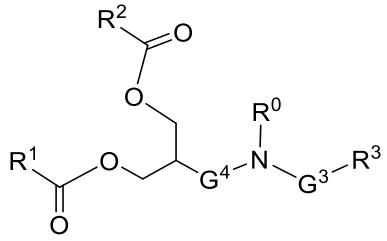
wherein
G^1 and G^2 are each independently C_3-C_8 alkylene;
each L^1 is independently -OC(=O)R^1;
each L^2 is independently -C(=O)OR^2;
R^1 is independently C_6-C_{10} alkyl;
R^2 is independently C_{12}-C_{22} alkyl;
G^3 is C_2-C_{12} alkylene;
R^3 is C_3-C_8 cycloalkyl;
R^4 is C_1-C_4 hydroxylalkyl;
n is 2;
m is 1,
preferably the cationic lipid is compound of the following formula:



;

and/or

compounds of Formula (07-III):



(07-III),

wherein

R^1 and R^2 are each independently C_6-C_{22} alkyl;

R^0 is C_3-C_8 cycloalkyl;

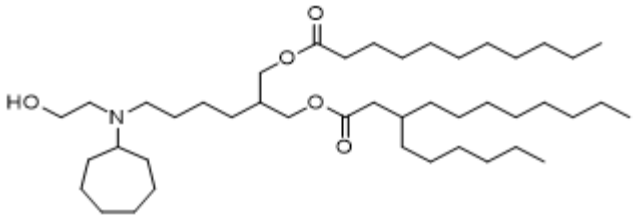
G^3 is C_2-C_6 alkylene;

G^4 is C_2-C_6 alkylene;

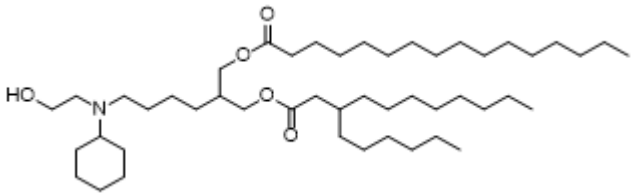
R^3 is -OR^6;

R^6 is hydrogen;

preferably the cationic lipid is compound of the following formula:



or



; and/or

78-99 wt.%, for example, 80-98.5 wt.%, 82-98 wt.%, 84-97.5 wt.%, 85-97.5 wt.%, 87-97.5 wt.%, 89-97.5 wt.%, 91-97.5 wt.%, or 93-97.5 wt.% of sucrose, relative to the total weight of the lyophilized formulation; and/or

0.2-9 wt.%, for example, 0.4-9 wt.%, 0.6-9 wt.%, 0.8-8 wt.%, 1-7 wt.%, 1.5-6 wt.%, 1.8-5 wt.%, or 2-4.8 wt.% of the non-polar amino acid, relative to the total weight of the lyophilized formulation; and/or

wherein the non-polar amino acid is selected from alanine, isoleucine, leucine, valine, proline, glycine, and a combination thereof;

for example,

the lyophilized formulation comprises

0.4-6 wt.%, for example, 1-4 wt.% or 1-3 wt.% of alanine, relative to the total weight of the lyophilized formulation;

and/or

0.6-9 wt.%, preferably 1-5 wt.% of isoleucine, relative to the total weight of the lyophilized formulation;

and/or

0.6-9 wt.%, preferably 1-5 wt.% of leucine, relative to the total weight of the lyophilized LNP formulation;

and/or

0.2-9 wt.%, for example, 0.5-8 wt. or 1.5-6 wt.% of valine, relative to the total weight of the lyophilized formulation;

and/or

0.5-8 wt.%, preferably 1-5 wt.% of proline, relative to the total weight of the lyophilized formulation;

and/or

0.3-5 wt.%, preferably 0.8-3 wt.% of glycine, relative to the total weight of the lyophilized formulation;

and/or

1.5-4 wt.% of valine and 1-3 wt.% of alanine, relative to the total weight of the lyophilized formulation;

and/or

1.5-4 wt.% of valine and 1.5-4 wt.% of leucine, relative to the total weight of the lyophilized formulation;

and/or

1.5-4 wt.% of valine and 1.5-4 wt.% of isoleucine, relative to the total weight of the lyophilized formulation.

4. The lyophilized formulation of any one of claims 1 to 3, wherein the lyophilized formulation comprises 0.01-3 wt.%, 0.1-3 wt.%, 0.15-2 wt.%, 0.2-1.5 wt.%, or 0.2-1.3 wt.% of a salt or Tris, by weight relative to the total weight of the lyophilized formulation; and/or

wherein the salt is selected from tris-HCl, KCl, NaCl, K_2HPO_4, KH_2PO_4, sodium citrate, sodium acetate, and a combination thereof.

5. The lyophilized formulation of any one of claims 1 to 4, wherein the lyophilized formulation comprises

80-98.5 wt.% of sucrose, 0.2-9 wt.% of valine and 0.01-3 wt.% of a salt or Tris, by weight relative to the total weight of the lyophilized formulation;

for example,

93.39 wt.% of sucrose, 4.38 wt.% of valine and 1.58 wt.% of Tris, by weight relative to the total weight of the lyophilized formulation; or

95.14 wt.% of sucrose, 2.97 wt.% of valine, 0.03 wt.% of K_2HPO_4, 0.66 wt.% of KH_2PO_4 and 0.74 wt.% of NaCl, by weight relative to the total weight of the lyophilized formulation; or

96.58 wt.% of sucrose, 1.51 wt.% of valine, 0.03 wt.% of K_2HPO_4, 0.67 wt.% of KH_2PO_4 and 0.75 wt.% of NaCl, by weight relative to the total weight of the lyophilized formulation; or

97.31 wt.% of sucrose, 0.76 wt.% of valine, 0.03 wt.% of K_2HPO_4, 0.68 wt.% of KH_2PO_4 and 0.76 wt.% of NaCl, by weight relative to the total weight of the lyophilized formulation; or

97.68 wt.% of sucrose, 0.38 wt.% of valine, 0.03 wt.% of K_2HPO_4, 0.68 wt.% of KH_2PO_4 and 0.76 wt.% of NaCl, by weight relative to the total weight of the lyophilized formulation.

6. The lyophilized formulation of any one of claims 1 to 5, wherein the lyophilized formulation further comprises a therapeutic and/or prophylactic agent in the lipid nanoparticles; and/or

wherein

the weight ratio of the lipid to the therapeutic and/or prophylactic agent is from 10: 1 to 60: 1, or 2: 1 to 30: 1, or 20:1 to 30:1; and/or

the therapeutic and/or prophylactic agent is a nucleic acid,

for example,

a ribonucleic acid,

preferably

a messenger RNA,

for example,

rabies mRNA, respiratory syncytial virus mRNA, human erythropoietin mRNA, varicella zoster virus mRNA, all optionally modified with 1-N-Methyl-Pseudouridine, and a combination thereof.

7. The lyophilized formulation of any one of claims 1 to 6, wherein the encapsulation efficiency of the lyophilized formulation is at least 85%, at least 87%, at least 90%, at least 92%, or at least 94%.

8. A liquid formulation of lipid nanoparticles comprising, by weight relative to the total volume of the liquid formulation:

(A) 0.001-0.2% w/v of lipid nanoparticles comprising a cationic lipid; and

(B) a cryoprotectant combination comprising

(i) 0.001-40% w/v of sucrose, and

(ii) 0.01-10% w/v of a non-polar amino acid.

9. The liquid formulation of claim 8, wherein

the particle size of the lipid nanoparticles is about 50 nm to about 140 nm, preferably from about 60 nm to about 120 nm; and/or

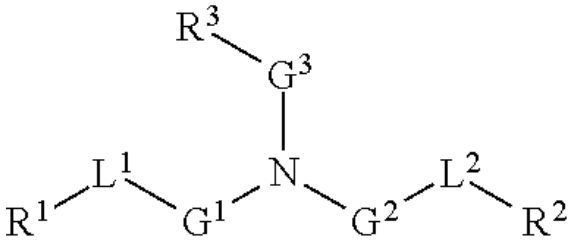
the liquid formulation comprises 0.001-0.2% w/v, 0.001-0.1% w/v, 0.1-0.2 % w/v, 0.1-0.15 % w/v, 0.001-0.05% w/v, 0.001-0.03% w/v, 0.001-0.01% w/v, 0.01-0.1% w/v, or 0.05-0.1% w/v of the lipid nanoparticle, by weight relative to the total volume of the LNP formulation; and/or

wherein the lipid nanoparticle comprises 30-55 mol % of a cationic lipid, 20-50 mol % of a structural lipid; 5-40 mol % of a phospholipid; and from 0.5-5 mol % of a polymer conjugated lipid, preferably 30- 55 mol% of a cationic lipid, 5-40 mol% of DSPC, 20-50 mol% of cholesterol, and 0.5 -3 mol% of PEG-lipid, relative to the total mole number of all lipids in the nanoparticle.

10. The liquid formulation of claim 8 or 9, wherein the liquid formulation comprises

0.01-1 % w/v, preferably 0.01-0.1 % w/v, more preferably 0.02-0.07% w/v, by weight relative to the total volume of the liquid LNP formulation, of a cationic lipid selected from

compounds of Formula 06-I :



(06-I)

wherein

L^1 and L^2 is -O(C=O)-;

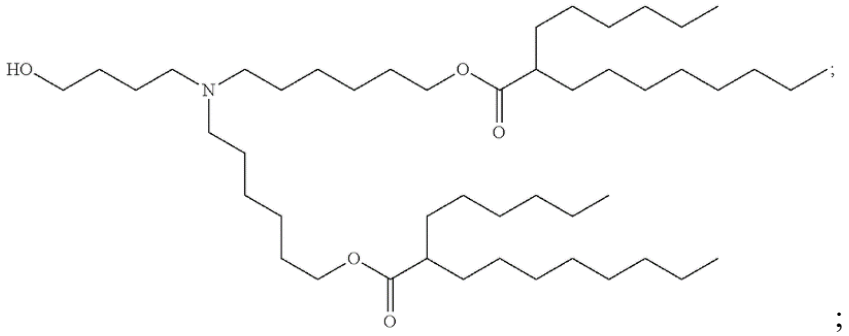
G^1 and G^2 are each independently unsubstituted C_4-C_8 alkylene;

G^3 is C_3-C_8 alkylene;

R^1 and R^2 are each independently C_{12}-C_{22} alkyl;

R^3 is H or OH,

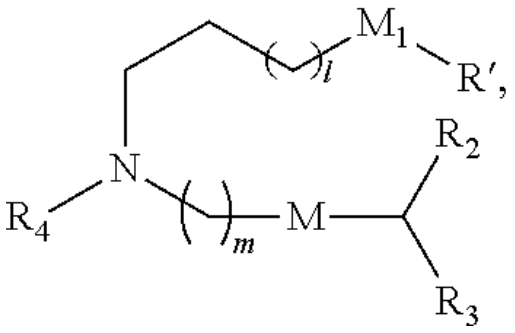
preferably the cationic lipid is compound of the following formula:



;

and/or

compounds of Formula 05-I:



(05-I)

wherein

l is selected from 1, 2, 3, 4, and 5;

m is selected from 5, 6, 7, 8, and 9;

M_1 is -C(O)O-;

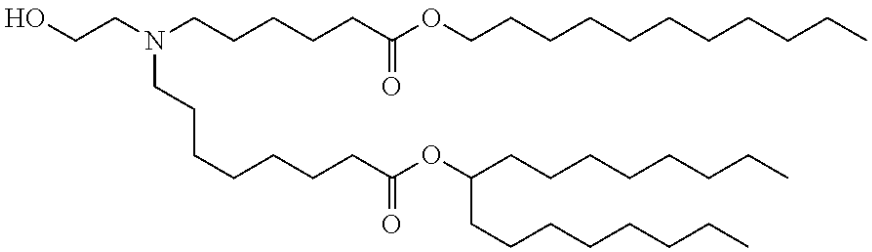
R_4 is $-(CH_2)_nOH$, and n is selected from 1, 2, 3, 4, or 5;

M is -OC(O)-;

R_2 and R_3 are both C_{6-10} alkyl; and

R' is a linear alkyl;

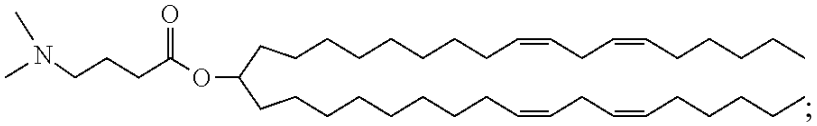
preferably the cationic lipid is compound of the following formula B:



(Formula B);

and/or

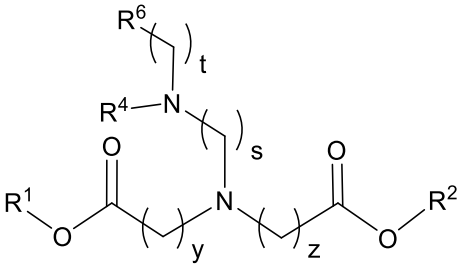
the following formula:



;

and/or

compounds of Formula (01-I-O):



(01-I-O),

wherein y and z are each independently an integer from 4 to 6,

s is an integer from 2 to 4,

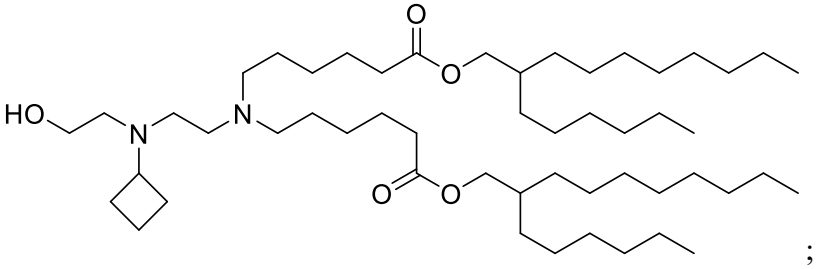
t is an integer from 1 to 3, and

R^1 and R^2 are each independently C_{12}-C_{22} alkyl;

R^4 is C_3-C_8 cycloalkyl;

R^6 is hydrogen or hydroxyl,

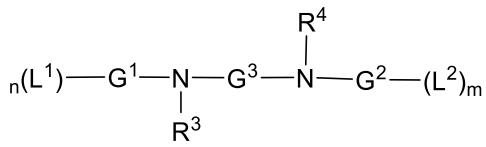
preferably the cationic lipid is compound of the following formula:



;

and/or

compounds of Formula (03-I):



(03-I),

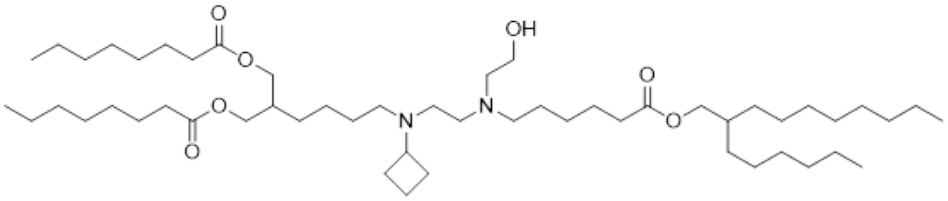
wherein

G^1 and G^2 are each independently C_3-C_8 alkylene;

each L^1 is independently –OC(=O)R^1;

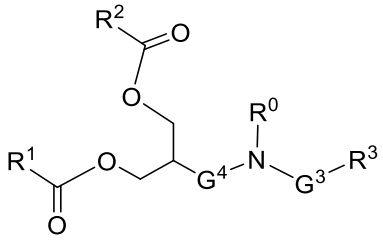
each L^2 is independently -C(=O)OR^2;

R^1 is independently C_6-C_{10} alkyl;
R^2 is independently C_{12}-C_{22} alkyl;
G^3 is C_2-C_{12} alkylene;
R^3 is C_3-C_8 cycloalkyl;
R^4 is C_1-C_4 hydroxylalkyl;
n is 2;
m is 1,
preferably the cationic lipid is compound of the following formula:



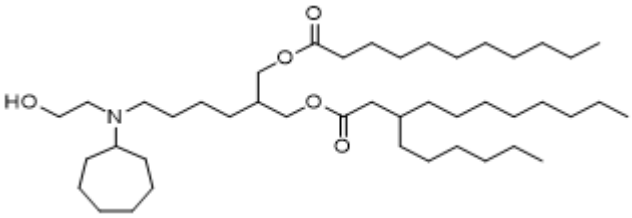
;

and/or
compounds of Formula (07-III):

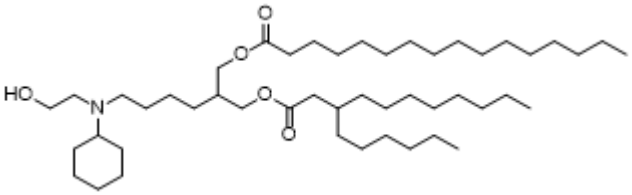


(07-III),

wherein
R^1 and R^2 are each independently C_6-C_{22} alkyl;
R^0 is C_3-C_8 cycloalkyl;
G^3 is C_2-C_6 alkylene;
G^4 is C_2-C_6 alkylene;
R^3 is -OR^6;
R^6 is hydrogen;
preferably the cationic lipid is compound of the following formula:



or



; and/or

0.01-35% w/v, for example, 0.1-30% w/v, 1-30% w/v, 2-25% w/v, 3-20% w/v, 4-15% w/v, 5-14% w/v, 6-13% w/v, 7-12% w/v, 8-11% w/v, 9-10% w/v, or 9.3-10 % w/v of sucrose, by weight relative to the total volume of the liquid LNP formulation; and/or

0.04-1% w/v, for example, 0.06-0.9% w/v, 0.08-0.8% w/v, 0.1-0.7% w/v, 0.15-0.6% w/v, 0.18-0.5% w/v, or 0.2-0.5% w/v of a non-polar amino acid, by weight relative to the total volume of the liquid LNP formulation.

11. The liquid formulation of any one of claims 8 to 10, wherein the non-polar amino acid is selected from alanine, isoleucine, leucine, valine, proline, glycine, and a combination thereof;

for example,

the liquid formulation comprises

0.04-0.6 % w/v, for example, 0.1-0.4% w/v or 0.1-0.3% w/v of alanine, by weight relative to the total volume of the liquid formulation;

and/or

0.06-0.9 % w/v, preferably 0.1-0.5% w/v of isoleucine, by weight relative to the total volume of the liquid formulation;

and/or

0.06-0.9 % w/v, for example, 0.1-0.6% w/v or 0.1-0.5% w/v of leucine, by weight relative to the total volume of the liquid formulation;

and/or

0.05-0.8 % w/v, preferably 0.15-0.6% w/v of valine, by weight relative to the total volume of the liquid formulation;

and/or

0.05-0.8 % w/v, preferably 0.1-0.5% w/v of proline, by weight relative to the total volume of the liquid formulation;

and/or

0.03-0.5 % w/v, preferably 0.08-0.3% w/v of glycine, by weight relative to the total volume of the liquid formulation;

and/or

0.15-0.4 % w/v of valine and 0.1-0.3 % w/v of alanine, by weight relative to the total volume of the liquid formulation;

and/or

0.15-0.4 % w/v of valine and 0.15-0.4 % w/v of leucine, by weight relative to the total volume of the liquid formulation;

and/or

0.15-0.4 % w/v of valine and 0.15-0.4 % w/v of isoleucine, by weight relative to the total volume of the liquid formulation.

12. The liquid formulation of any one of claims 8 to 11, wherein the liquid formulation comprises 0.001-0.3%, 0.01-0.3% w/v, 0.015-0.2 % w/v, 0.02-0.15 % w/v, or 0.02-0.13 % w/v of a salt or Tris, by weight relative to the total weight of the liquid formulation; and/or

wherein the salt is selected from tris-HCl, KCl, NaCl, K_2HPO_4, KH_2PO_4, sodium citrate, sodium acetate, and a combination thereof.

13. The liquid formulation of any one of claims 8 to 12, wherein the liquid formulation comprises

4-15% w/v of sucrose, 0.05-0.8 % w/v of valine and 0.001-0.3% w/v of a salt or Tris, by weight relative to the total weight of the liquid formulation;

for example,

10% w/v of sucrose, 0.47% w/v of valine and 0.17% w/v of Tris, by weight relative to the total weight of the liquid formulation; or

10% w/v of sucrose, 0.47% w/v of valine, 0.005% w/v of K_2HPO_4, 0.10% w/v of KH_2PO_4 and 0.117% w/v of NaCl, by weight relative to the total weight of the liquid formulation; or

10% w/v of sucrose, 0.23% w/v of valine, 0.005% w/v of K_2HPO_4, 0.10% w/v of KH_2PO_4 and 0.117% w/v of NaCl, by weight relative to the total weight of the liquid formulation; or

10% w/v of sucrose, 0.12% w/v of valine, 0.005% w/v of K_2HPO_4, 0.10% w/v of KH_2PO_4 and 0.117% w/v of NaCl, by weight relative to the total weight of the liquid formulation; or

10% w/v of sucrose, 0.06% w/v of valine, 0.005% w/v of K_2HPO_4, 0.10% w/v of KH_2PO_4 and 0.117% w/v of NaCl, by weight relative to the total weight of the liquid formulation.

14. The liquid formulation of any one of claims 8 to 13, wherein the lipid formulation further comprises a therapeutic and/or prophylactic agent in the lipid nanoparticles; and/or

wherein

the weight ratio of the lipid to the therapeutic and/or prophylactic agent is from 10: 1 to 60: 1, or 2: 1 to 30: 1, or 20:1 to 30:1; and/or

the therapeutic and/or prophylactic agent is a nucleic acid,

for example,

a ribonucleic acid,

preferably

a messenger RNA,

for example,

rabies mRNA, respiratory syncytial virus mRNA, human erythropoietin mRNA, varicella zoster virus mRNA, all optionally modified with 1-N-Methyl-Pseudouridine, and a combination thereof.

15. The liquid formulation of any one of claims 8 to 14, wherein the encapsulation efficiency of the liquid formulation is at least 75%, at least 77%, at least 79%, at least 81%, or at least 83%.