LIPID NANOPARTICLES AND LIPID NANOPARTICLE COMPOSITION

FIELD OF TECHNOLOGY

[0001] The present application belongs to the field of biotechnology, and specifically relates to a lipid nanoparticle and a lipid nanoparticle composition.

BACKGROUND

[0002] The effective targeted delivery of bioactive substances such as small molecule drugs, proteins, and nucleic acids remains an ongoing medical challenge. The key to successful gene therapy lies in whether therapeutic drugs can be safely and effectively delivered into target cells *in vivo* via carriers. Due to relative instability of nucleic acids and low cellular permeability of such substances, the delivery of nucleic acids into cells becomes difficult. Therefore, there is a need to develop methods and compositions to facilitate the delivery of therapeutic and/or prophylactic drugs such as nucleic acids into cells. Gene therapy vectors are classified into viral vectors and non-viral vectors. Although the viral vectors serve as efficient delivery systems to achieve the purposes of target gene transfection and therapy, the viral vectors have problems such as viral proteins with immunogenicity, limited loading capacities for target genes, and high costs, so that lipid nanoparticles (LNPs) as the non-viral vectors have been widely concerned due to their advantages such as good stability *in vitro*, degradability *in vivo*, safety, and reliability. Moreover, the lipid nanoparticles have been extensively applied in gene therapy research for both congenital and acquired genetic defects.

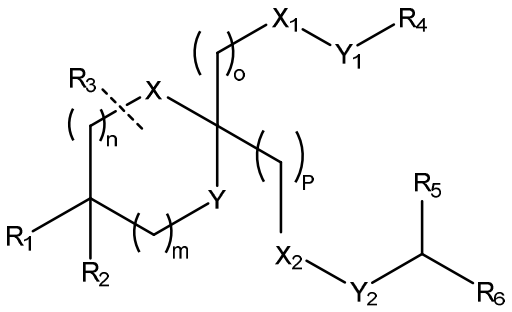
[0003] Lipid-containing nanoparticles or lipid nanoparticles, liposomes, and lipid complexes have been proven to be effective transport carriers for bioactive substances such as small molecule drugs, proteins, and nucleic acids to enter cells and/or within cells. LNPs refer to small vesicles formed from one or more lipid components that can efficiently condense and deliver various nucleic acid molecules, ranging from DNA and RNA to chromosomes and even cells. LNPs are conducive to large-scale production due to their characteristics such as defined construction schemes and ease of modification with targeting ligands.

SUMMARY

[0004] The objective of the present application is to provide a lipid nanoparticle and a lipid nanoparticle composition.

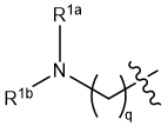
[0005] Specifically, the present application relates to the following aspects:

[0006] 1. A lipid nanoparticle, comprising an ionizable lipid, a phospholipid, a structural lipid, and a PEG lipid in a molar ratio of the ionizable lipid, a sum of the phospholipid and the structural lipid, and the PEG lipid of (35-65):(35-65):(0.5-5), wherein the ionizable lipid is a compound of formula (I), or a salt or isomer thereof,



(I)

[0007] wherein



[0008] R_1 is , wherein R^{1a} and R^{1b} are independently selected from H and C1-C6 alkyl; and R_2 and R_3 are independently selected from H or C1-C6 alkyl;

[0009] R_4, R_5, and R_6 are independently selected from C1-C14 alkyl;

[0010] X and Y are independently selected from O or S;

[0011] X_1 and X_2 are independently selected from C=O or O, and Y_1 and Y_2 are independently selected from C=O or O, provided that X_1 and Y_1 as well as X_2 and Y_2 are not simultaneously C=O or O;

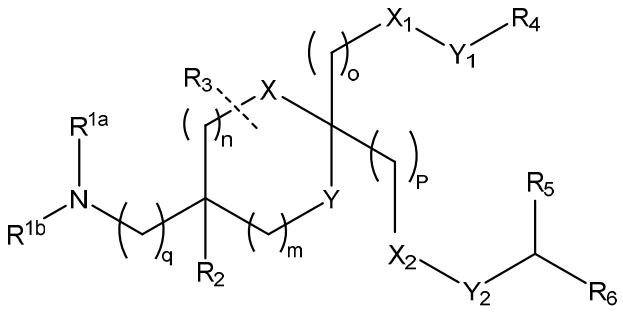
[0012] m and n are independently selected from 0, 1, and 2, provided that m and n are not simultaneously 0;

[0013] o and p are independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10; and

[0014] q is selected from 0, 1, 2, 3, 4, 5, and 6.

[0015] 2. The lipid nanoparticle according to item 1, wherein the compound of formula (I) is shown as formula (IA),

[0016]



(IA)

[0017] wherein q is selected from 0, 1, 2, 3, and 4.

[0018] 3. The lipid nanoparticle according to item 1 or 2, wherein R_4 is C10-C11 alkyl.

[0019] 4. The lipid nanoparticle according to item 3, wherein R_4 is C10-C11 linear alkyl.

[0020] 5. The lipid nanoparticle according to any one of items 1-4, wherein R_5 and R_6 are C8 alkyl.

[0021] 6. The lipid nanoparticle according to item 5, wherein R_5 and R_6 are C8 linear alkyl.

[0022] 7. The lipid nanoparticle according to any one of items 1-6, wherein o is 5 or 6.

[0023] 8. The lipid nanoparticle according to any one of items 1-7, wherein p is 7 or 8.

[0024] 9. The lipid nanoparticle according to any one of items 1-8, wherein

[0025] o is 5, R_4 is C11 linear alkyl, p is 7, and R_5 and R_6 are C8 linear alkyl; or

[0026] o is 6, R_4 is C10 linear alkyl, p is 7, and R_5 and R_6 are C8 linear alkyl.

[0027] 10. The lipid nanoparticle according to any one of items 1-9, wherein X_1 and X_2 are C=O, and Y_1 and Y_2 are O.

[0028] 11. The lipid nanoparticle according to any one of items 1-9, wherein X_1 is O, Y_1 is C=O, X_2 is C=O, and Y_2 is O.

[0029] 12. The lipid nanoparticle according to any one of items 1-9, wherein X_1 is C=O, Y_1 is O, X_2 is O, and Y_2 is C=O.

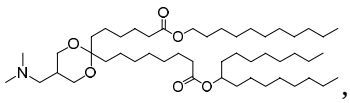
[0030] 13. The lipid nanoparticle according to any one of items 1-9, wherein X_1 and X_2 are O, and Y_1 and Y_2 are C=O.

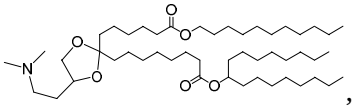
[0031] 14. The lipid nanoparticle according to any one of items 1-9, wherein both X and Y are O.

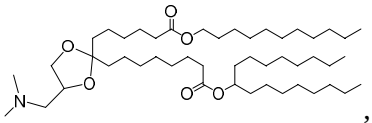
[0032] 15. The lipid nanoparticle according to any one of items 1-9, wherein n is 0, and m is 1; or n is 1, and m is 0; or n is 1, and m is 1.

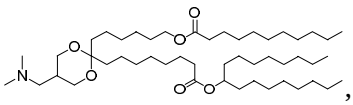
[0033] 16. The lipid nanoparticle according to any one of items 1-9, wherein the C1-C6 alkyl is selected from $-CH_3$, $-CH_2CH_3$, or $-CH(CH_3)_2$.

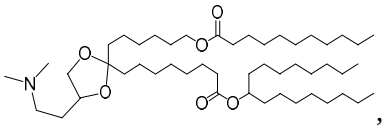
[0034] 17. The lipid nanoparticle according to item 1, wherein the compound of formula (I) and a stereoisomer thereof are selected from:

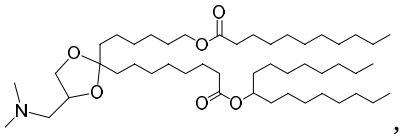
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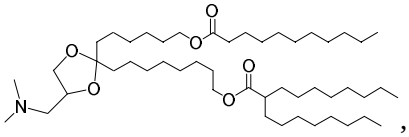
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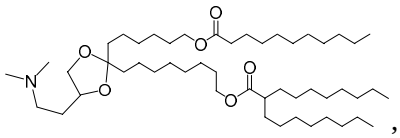
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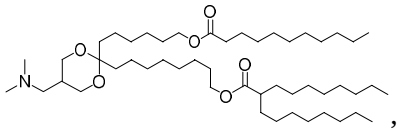
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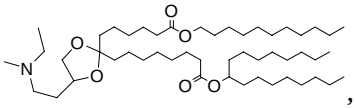
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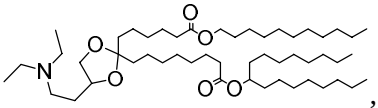
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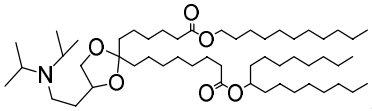
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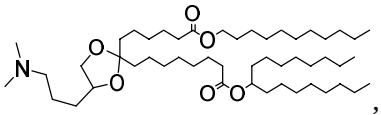
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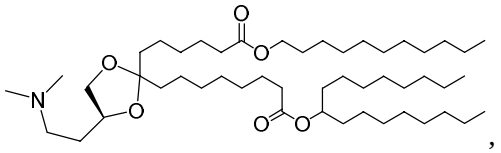
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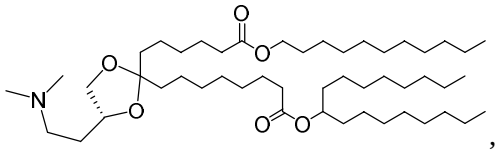
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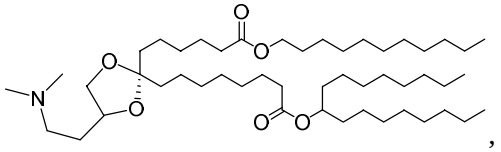
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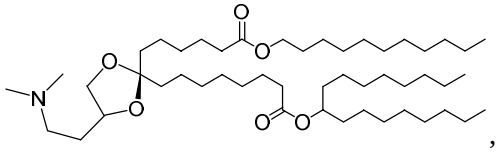
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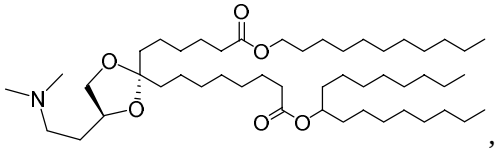
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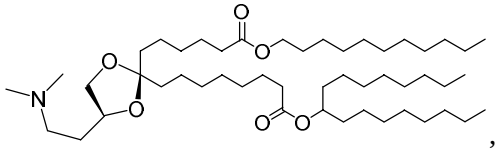
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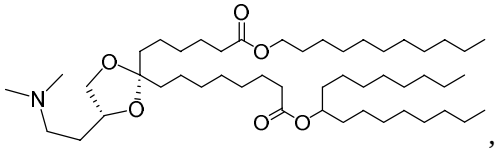
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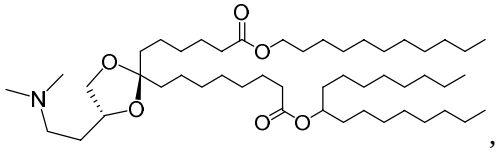
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compound 2D ,

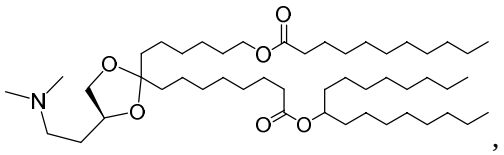
compound 2E ,

compound 2F ,

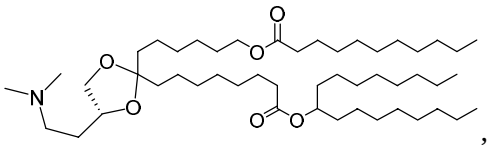
compound 2G ,

compound 2H ,

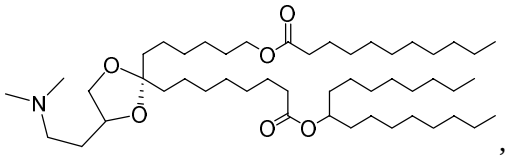
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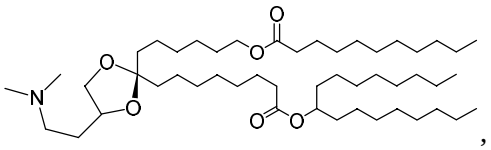
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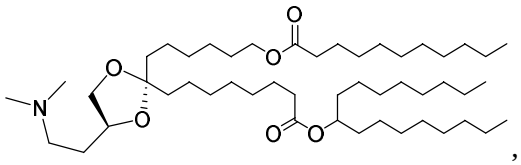
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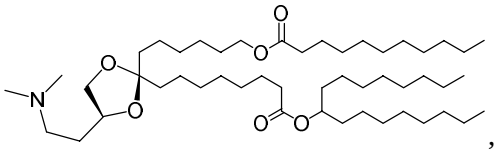
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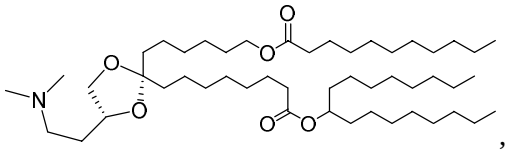
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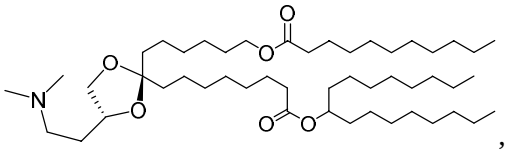
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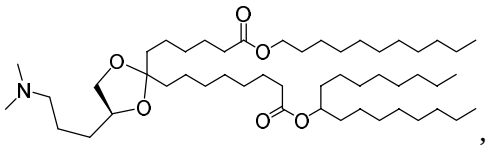
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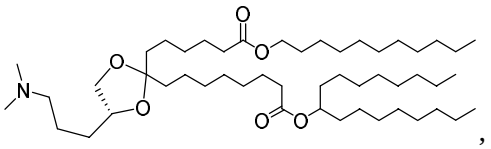
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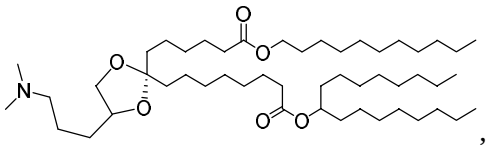
compound 13A



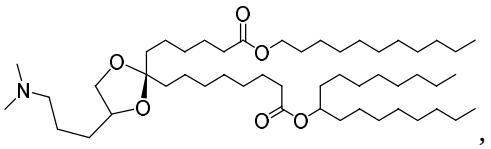
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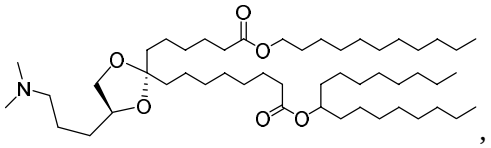
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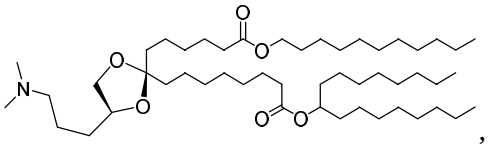
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compound 13E

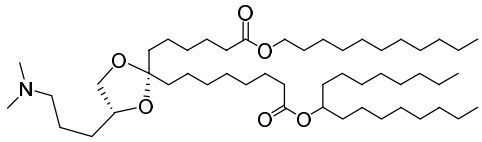
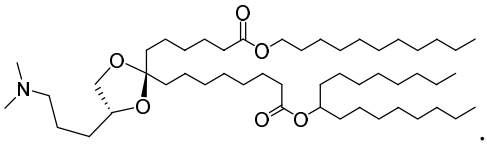


compound 13F



compound 13G

compound 13H


, or 
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[0035] 18. The lipid nanoparticle according to any one of items 1-17, wherein the phospholipid is selected from one or two or more of the following compounds:

[0036] dilauroylphosphatidylcholine (DLPC),

[0037] dimyristoylphosphatidylcholine (DMPC),

[0038] dioleoylphosphatidylcholine (DOPC),

[0039] dipalmitoylphosphatidylcholine (DPPC),

[0040] distearoylphosphatidylcholine (DSPC),

[0041] dioleoylphosphatidylcholine (DUPC),

[0042] palmitoyloleoylphosphatidylcholine (POPC),

[0043] 1,2-di-O-octadecyl-sn-glycero-3-phosphocholine (18:0 Diether PC),

[0044] 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC),

[0045] 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC),

[0046] 1,2-divinyl-sn-glycero-3-phosphocholine,

[0047] 1,2-diarylacyl-sn-glycero-3-phosphocholine,

[0048] 1,2-dioleoyl-SN-glycero-3-phosphoethanolamine (DOPE),

[0049] 1,2-dihydroxystannyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE),

[0050] 1,2-distearoyl-sn-glycero-3-phosphoethanolamine,

[0051] 1,2-divinylol-sn-glycero-3-phosphoethanolamine,

[0052] 1,2-divinyl-sn-glycero-3-phosphoethanolamine,

[0053] 1,2-diarylyl-sn-glycero-3-phosphoethanolamine,

[0054] 1,2-dithiohexaenoate-sn-glycero-3-phosphoethanolamine,

[0055] 1,2-diol-sn-glycero-3-phospho-(1-glycerol) sodium salt (DOPG), or sphingomyelin.

[0056] 19. The lipid nanoparticle according to item 18, wherein the phospholipid is DSPC.

[0057] 20. The lipid nanoparticle according to items 1-19, wherein the structural lipid

is selected from one or two or more of cholesterol, coprostanol, sitosterol, ergosterol, and stigmasterol.

[0058] 21. The lipid nanoparticle according to item 20, wherein the structural lipid is cholesterol.

[0059] 22. The lipid nanoparticle according to any one of items 1-21, wherein the PEG lipid is selected from one or two or more of PEG-modified phosphatidylethanolamine, PEG-modified phosphatidic acid, PEG-modified ceramide, PEG-modified dialkylamine, PEG-modified diacylglycerol, or PEG-modified dialkylglycerol.

[0060] 23. The lipid nanoparticle according to item 22, wherein the PEG lipid is DMG-PEG2000.

[0061] 24. The lipid nanoparticle according to any one of items 1-23, wherein the molar ratio of the ionizable lipid, the sum of the phospholipid and the structural lipid, and the PEG lipid is (40-50):(35-65):(1-3).

[0062] 25. The lipid nanoparticle according to any one of items 1-23, wherein the molar ratio of the ionizable lipid, the sum of the phospholipid and the structural lipid, and the PEG lipid is (50-65):(35-65):(1-3).

[0063] 26. The lipid nanoparticle according to item 24, wherein a molar ratio of the ionizable lipid, the phospholipid, the structural lipid, and the PEG lipid is (40-50):(5-15):(30-50):(1-3).

[0064] 27. The lipid nanoparticle according to item 26, wherein the molar ratio of the ionizable lipid, the phospholipid, the structural lipid, and the PEG lipid is (40-50):(10-15):(35-45):(1.5-2.5).

[0065] 28. The lipid nanoparticle according to item 25, wherein the molar ratio of the ionizable lipid, the phospholipid, the structural lipid, and the PEG lipid is (50-65):(5-15):(30-50):(1-3).

[0066] 29. The lipid nanoparticle according to item 28, wherein the molar ratio of the ionizable lipid, the phospholipid, the structural lipid, and the PEG lipid is (50-65):(10-15):(35-45):(1.5-2.5).

[0067] 30. The lipid nanoparticle according to items 1-29, wherein

[0068] a content of the ionizable lipid is 35 mol% to 65 mol%,

[0069] a content of the sum of the phospholipid and the structural lipid is 35 mol% to 65 mol%, and

[0070] a content of the PEG lipid is 0.5 mol% to 5 mol%,

[0071] wherein mol% is calculated based on a mole number of total lipids in a lipid nanoparticle composition.

[0072] 31. The lipid nanoparticle according to any one of items 1-30, wherein

[0073] the content of the ionizable lipid is 40 mol% to 50 mol%, preferably 45 mol% to 49 mol%, and further preferably 48 mol% to 49 mol%.

[0074] 32. The lipid nanoparticle according to any one of items 1-30, wherein

[0075] the content of the ionizable lipid is 50 mol% to 65 mol%, preferably 51 mol% to 55 mol%, and further preferably 52 mol% to 53 mol%.

[0076] 33. The lipid nanoparticle according to any one of items 1-32, wherein

[0077] the content of the sum of the phospholipid and the structural lipid is 35 mol% to 65 mol%, preferably 35 mol% to 55 mol%, and further preferably 45 mol% to 50 mol%.

[0078] 34. The lipid nanoparticle according to any one of items 1-33, wherein

[0079] a content of the phospholipid is 5 mol% to 15 mol%, preferably 10 mol% to 15 mol%.

[0080] 35. The lipid nanoparticle according to any one of items 1-34, wherein

[0081] a content of the structural lipid is 30 mol% to 50 mol%, preferably 35 mol% to 45 mol%.

[0082] 36. A lipid nanoparticle composition, comprising the lipid nanoparticle according to any one of items 1-35 and a therapeutic agent and/or a prophylactic agent, wherein the therapeutic agent and/or the prophylactic agent is selected from a vaccine or a compound or nucleic acid capable of eliciting an immune response.

[0083] 37. The lipid nanoparticle composition according to item 36, wherein the nucleic acid is RNA, and the RNA is selected from one or two or more of siRNA, aiRNA, miRNA, dsRNA, shRNA, or mRNA.

[0084] 38. The lipid nanoparticle composition according to item 37, wherein the nucleic acid is mRNA.

[0085] 39. The lipid nanoparticle composition according to item 37, wherein a coding

sequence of the mRNA is as shown in SEQ ID NO: 12.

[0086] 40. The lipid nanoparticle composition according to any one of items 36-39, wherein the therapeutic agent and/or the prophylactic agent is mRNA, and an N/P ratio of the ionizable lipid to the mRNA is 3.5-5.5.

[0087] 41. The lipid nanoparticle composition according to any one of items 36-40, wherein an encapsulation efficiency of the therapeutic agent and/or the prophylactic agent is ≥ 50%, ≥ 80%, or ≥ 90%.

[0088] 42. The lipid nanoparticle composition according to any one of items 36-41, wherein an average particle size of the nanoparticle is 50 nm to 150 nm, preferably 50 nm to 80 nm.

[0089] 43. The lipid nanoparticle composition according to any one of items 36-42, wherein a dispersity index of the nanoparticle is 0.001-0.16.

[0090] 44. A pharmaceutical composition, comprising the lipid nanoparticle composition according to any one of items 36-43 and a pharmaceutically acceptable excipient or auxiliary ingredient.

[0091] 45. A method for delivering a therapeutic agent and/or a prophylactic agent to a mammalian cell, comprising administering to a subject the lipid nanoparticle composition according to any one of items 36-43 or the pharmaceutical composition according to item 44, wherein the administering comprises allowing the cell to get in contact with the nanoparticle composition or the pharmaceutical composition to deliver the therapeutic agent and/or the prophylactic agent to the cell.

[0092] 46. The method according to item 45, wherein the mammalian cell is in a mammal.

[0093] 47. The method according to item 45 or 46, wherein the mammal is a human.

[0094] 48. The method according to any one of items 45-47, wherein the lipid nanoparticle is administered intravenously, intramuscularly, intradermally, subcutaneously, or intranasally, or administered via inhalation.

[0095] 49. A method for producing a target polypeptide in a mammalian cell, comprising allowing the cell to get in contact with the lipid nanoparticle composition according to any one of items 36-43 or the pharmaceutical composition according to item 44

to deliver a therapeutic agent and/or a prophylactic agent to the cell, wherein the therapeutic agent and/or the prophylactic agent is mRNA, and the mRNA encodes the target polypeptide, thereby enabling the mRNA to be translated in the cell to produce the target polypeptide.

[0096] 50. The method according to item 49, wherein the mammalian cell is in a mammal.

[0097] 51. The method according to item 49 or 50, wherein the mammal is a human.

[0098] 52. The method according to any one of items 49-51, wherein the lipid nanoparticle composition or the pharmaceutical composition is administered intravenously, intramuscularly, intradermally, subcutaneously, or intranasally, or administered via inhalation.

[0099] 53. A method for treating a disease or disorder in a mammal, comprising administering to the mammal a therapeutically effective amount of the lipid nanoparticle composition according to any one of items 36-43 or the pharmaceutical composition according to item 44.

[0100] 54. The method according to item 53, wherein the disease or disorder is characterized by dysfunctional or abnormal protein or polypeptide activity.

[0101] 55. The method according to item 53 or 54, wherein the disease or disorder is selected from an infectious disease, a cancer and proliferative disease, a genetic disease, an autoimmune disease, diabetes mellitus, a neurodegenerative disease, a cardiovascular disease, a renovascular disease, or a metabolic disease.

[0102] 56. The method according to any one of items 53-55, wherein the mammal is a human.

[0103] 57. The method according to any one of items 53-56, wherein the lipid nanoparticle composition or the pharmaceutical composition is administered intravenously, intramuscularly, intradermally, subcutaneously, or intranasally, or administered via inhalation.

[0104] 58. A method for specifically delivering a therapeutic agent and/or a prophylactic agent to a mammalian organ, comprising administering to a mammal the lipid nanoparticle composition according to any one of items 36-43 or the pharmaceutical composition according to item 44, wherein the administering comprises allowing the mammalian organ to get in contact with the nanoparticle, thereby delivering the therapeutic agent and/or the prophylactic agent to the organ.

[0105] 59. The method according to item 58, wherein the mammal is a human.

[0106] 60. The method according to item 58 or 59, wherein the lipid nanoparticle composition or the pharmaceutical composition is administered intravenously, intramuscularly, intradermally, subcutaneously, or intranasally, or administered via inhalation.

[0107] 61. The method according to any one of items 58-60, wherein the mammal is pretreated at 24 hours or less hours before a contacting or administering step.

[0108] 62. The method according to any one of items 58-61, wherein the mammal is pretreated at approximately one hour before the contacting or administering step.

[0109] 63. The lipid nanoparticle according to any one of items 1-35, the lipid nanoparticle composition according to any one of items 36-43, or the pharmaceutical composition according to item 44, further comprising a divalent metal cation, wherein the divalent metal cation is preferably Ca^{2+}, Mg^{2+}, Zn^{2+}, or Mn^{2+}.

[0110] 64. The lipid nanoparticle, the lipid nanoparticle composition, or the pharmaceutical composition according to item 63, wherein a molar ratio of the divalent metal cation to the total lipids in the lipid nanoparticle composition is (0.5-400):100, preferably (10-50):100, (30-60):100, (100-200):100, or (300-400):100.

[0111] 65. The lipid nanoparticle composition according to any one of items 63-64, wherein a method for preparing the lipid nanoparticle composition comprises:

[0112] formulating a divalent metal cation solution at a certain concentration with an acidic buffer, and then diluting mRNA with the divalent metal cation solution to obtain an mRNA working solution, wherein a final concentration of mRNA in the working solution is 100-300 μg/ml, and a final concentration of the divalent metal cation solution is 0.8-20 mM; and mixing the mRNA working solution with a hybrid total lipid-ethanol phase in a volume ratio of (1-5):1 to prepare LNP, subjecting a sample to dilution, concentration, and replacement after the mixing to remove the ethanol, then adjusting the sample to a constant volume, and adjusting a pH value to 7-8.

[0113] 66. The method according to item 65, wherein the acidic buffer is an acetate buffer.

[0114] 67. The method according to items 65-66, wherein a pH of the acidic buffer is approximately 4.0-6.0, preferably approximately 5.0-5.5.

[0115] 68. The method according to items 65-67, wherein the final concentration of mRNA in the mRNA working solution is 200 μg/ml.

[0116] 69. The method according to items 65-68, wherein the volume ratio of the mRNA working solution to the hybrid total lipid-ethanol phase is 2.5:1 to 4:1.

[0117] 70. The method according to items 65-69, wherein a mixing manner of the mRNA working solution and the hybrid total lipid-ethanol phase is mixing by a T-shaped flow mixing device.

[0118] 71. The method according to items 65-70, wherein the concentration of the divalent metal cation solution in the mRNA working solution is capable of being selected from 0.8 mM, 1.6 mM, 3.2 mM, 6.4 mM, 8 mM, 12.8 mM, 16 mM, and 20 mM.

[0119] 72. The method for preparing the lipid nanoparticle composition according to items 65-71.

[0120] The lipid nanoparticle composition of the present application can achieve encapsulation and delivery of a therapeutic/prophylactic agent, safely deliver the therapeutic/prophylactic agent to a target site, achieve high expression, and exert an effect of the therapeutic/prophylactic agent. The lipid nanoparticle of the present application has advantages such as small average particle size, good dispersity, high encapsulation efficiency, and low toxicity.

BRIEF DESCRIPTION OF THE DRAWINGS

[0121] FIG. 1 shows flow cytometry analysis results in Example 2.

[0122] FIGs. 2-3 show Elispot analysis results in Example 3.

DESCRIPTION OF THE EMBODIMENTS

[0123] The present application is further described below in conjunction with examples. It should be understood that the examples are provided solely for further explanation and illustration of the present application and are not intended to limit the present application.

[0124] Unless otherwise defined, technical and scientific terms used in the specification have the same meanings as commonly understood by those skilled in the art.

Although methods and materials similar or identical to those described herein can be applied in experiments or practical applications, the materials and methods are described below. In the case of a conflict, definitions included in the specification shall prevail. Additionally, the materials, methods, and examples are for illustrative purposes only and are not limiting. The present application is further described below in conjunction with specific examples, but the examples are not intended to limit the scope of the present application.

[0125] Terms and definitions

[0126] As used herein, the term "alkyl" refers to a group containing one or more carbon atoms (for example, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, or more carbon atoms), which is optionally substituted. The term "C1-C14 alkyl" refers to a linear or branched saturated hydrocarbon containing 1-14 carbon atoms, which is optionally substituted. Unless otherwise specified, the alkyl described herein refers to both unsubstituted and substituted alkyl.

[0127] Unless otherwise specified, the alkyl may be optionally substituted. An optional substituent may be selected from, but is not limited to, a halogen atom (for example, chloro, bromo, fluoro, or iodo), carboxylic acid (for example, -C(O)OH), alcohol (for example, hydroxyl, -OH), ester (for example, -C(O)OR or -OC(O)R), aldehyde (for example, -C(O)H), carbonyl (for example, -C(O)R, or represented by C=O), acyl halide (for example, -C(O)X, wherein X is a halide selected from a bromide, a fluoride, a chloride, and an iodide), carbonate (for example, -OC(O)OR), alkoxy (for example, -OR), acetal, phosphate, thiol (for example, -SH), sulfoxide (for example, -S(O)R), sulfurous acid (for example, -S(O)OH), sulfonic acid (for example, $-S(O)_2OH$), thione (for example, -C(S)H), sulfate, sulfonyl (for example, $-S(O)_2-$), amide (for example, $-C(O)NR_2$ or -N(R)C(O)R), azido (for example, $-N_3$), nitro (for example, $-NO_2$), cyano (for example, -CN), isocyano (for example, -NC), acyloxy (for example, -OC(O)R), amino (for example, $-NR_2$, -NRH, or $-NH_2$), carbamoyl (for example, $-OC(O)NR_2$, -OC(O)NRH, or $-OC(O)NH_2$), sulfonamide, alkyl, alkenyl, and cyclyl (for example, carbocyclyl or heterocyclyl). In any one of the foregoing descriptions, R is an alkyl or alkenyl as defined herein. In some embodiments, the substituent itself may be further substituted with, for example, one, two, three, four, five, or six substituents as defined herein.

For example, a C_{1-6} alkyl may be further substituted with 1, 2, 3, 4, 5, or 6 substituents as described herein.

[0128] As used herein, the term "compound" refers to all isomers and isotopes that include a described structure. The "isotopes" refer to atoms having the same atomic number but different mass numbers due to different numbers of neutrons in atomic nuclei. For example, isotopes of hydrogen include tritium and deuterium. In addition, compounds, salts, or complexes of the present application may be prepared by conventional methods in combination with solvents or water molecules to form sols and hydrates.

[0129] As used herein, the term "contact" refers to establishing a physical connection between two or more entities. For example, allowing a mammalian cell to get in contact with a nanoparticle composition means that the mammalian cell and a nanoparticle share a physical connection. Methods for allowing cells to get in contact with external entities *in vivo* and *ex vivo* are well known in the field of biology. For example, a nanoparticle composition can be brought into contact with a mammalian cell within a mammal by various routes of administration (for example, intravenous, intramuscular, intradermal, and subcutaneous), and various amounts of the nanoparticle composition may be involved. In addition, the nanoparticle composition may get in contact with more than one mammalian cell.

[0130] As used herein, the term "specific delivery" or "specific transport" refers to delivering more (for example, at least 1.5 times, at least 2 times, at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times, at least 9 times, and at least 10 times) therapeutic and/or prophylactic drugs to a target tissue (for example, a mammalian liver) via a nanoparticle compared with a non-target tissue. The level of the nanoparticle delivered to the specific tissue can be measured by comparing a weight of a protein produced in the tissue with a weight of the tissue, comparing a therapeutic and/or prophylactic amount in the tissue with the weight of the tissue, comparing the weight of the protein produced in the tissue with a weight of total proteins in the tissue, or comparing the therapeutic and/or prophylactic amount in the tissue with a total therapeutic and/or prophylactic amount in the tissue.

[0131] As used herein, "encapsulation efficiency" refers to an amount of a therapeutic agent and/or a prophylactic agent that becomes a part of a nanoparticle composition, relative

to a total amount of the therapeutic agent or the prophylactic agent used in preparation of the nanoparticle composition. For example, if 97 mg of a therapeutic agent and/or a prophylactic agent in a total of 100 mg of the therapeutic agent and/or the prophylactic agent initially provided to a composition is encapsulated in the nanoparticle composition, an encapsulation efficiency may be 97%. As used herein, "encapsulation" may refer to complete, substantial, or partial encapsulation, enclosure, surrounding, or containment.

[0132] As used herein, "expression" of a nucleic acid sequence refers to translation of mRNA into a polypeptide or a protein and/or post-translational modification of a polypeptide or a protein.

[0133] As used herein, the term "*in vitro*" refers to an event occurring in an artificial environment, for example, an event occurring in a test tube or a reaction vessel, in cell culture, in a culture dish, and the like, rather than within an organism (for example, an animal, a plant, or a microorganism).

[0134] As used herein, the term "*in vivo*" refers to an event occurring within an organism (for example, an animal, a plant, or a microorganism or a cell or tissue thereof).

[0135] As used herein, the term "*ex vivo*" refers to an event occurring outside an organism (for example, an animal, a plant, or a microorganism or a cell or tissue thereof). An *ex vivo* event may occur in an environment with minimal changes relative to a natural (for example, *in vivo*) environment.

[0136] As used herein, the term "isomer" refers to any geometric isomer, tautomer, zwitterion, stereoisomer, enantiomer, or diastereomer of a compound. The compound may include one or more chiral centers and/or double bonds and thus may exist as a stereoisomer, for example, a double bond isomer (that is, geometric E/Z isomer) or a diastereomer (for example, an enantiomer (that is, (+) or (-)) or a cis/trans isomer). The present application covers any and all isomers of the compound described herein. Mixtures of enantiomers and stereoisomers of the compound and methods for resolving them into their components of enantiomers or stereoisomers are well known.

[0137] As used herein, a "lipid component" refers to a component of a nanoparticle composition that contains one or more lipids. For example, the lipid component may include one or more cationic/ionizable lipids, PEGylated lipids, structural lipids, or other lipids such

as phospholipids.

[0138] As used herein, an "ionizable lipid" or a "cationic lipid" refers to any one of a number of lipid species that carry a net positive charge at a selected pH value, such as a physiological pH value.

[0139] As used herein, a "PEG lipid" or a "PEGylated lipid" refers to a lipid containing a polyethylene glycol ingredient, and for example, it may be a PEG-conjugated lipid, a PEG-modified lipid, or a PEG-derivatized lipid.

[0140] As used herein, a "phospholipid" refers to a lipid that includes a phosphate ester moiety and one or more carbon chains, such as unsaturated fatty acid chains. The phospholipid may contain one or more (for example, double or triple) bonds (for example, one or more unsaturated bonds). A specific phospholipid may facilitate fusion with a membrane. For example, a cationic phospholipid may interact with one or more negatively charged phospholipids of a membrane (for example, a cell membrane or an intracellular membrane). The fusion of a phospholipid with a membrane may allow one or more elements of a lipid-containing composition to pass through the membrane, thereby allowing, for example, delivery of one or more elements to a cell.

[0141] As used herein, a "lipid nanoparticle" refers to a composition containing one or more lipids, including an ionizable lipid, a phospholipid, a structural lipid, and a PEG lipid. The particle size of the lipid nanoparticle is typically on the order of microns, nanometers, or less.

[0142] As used herein, a "lipid nanoparticle composition" includes a lipid nanoparticle (LNP) and a therapeutic agent and/or a prophylactic agent, with the therapeutic agent and/or the prophylactic agent encapsulated by the lipid nanoparticle (LNP), wherein the therapeutic agent and/or the prophylactic agent is selected from a vaccine or a compound, nucleic acid, or protein capable of eliciting an immune response.

[0143] As used herein, "administration method" may include an intravenous method, an intramuscular method, an intradermal method, a subcutaneous method, or other methods for delivering a composition to a subject. Any one administration method may be selected for targeted delivery (for example, specific delivery) to a particular region or system of the body.

[0144] As used herein, a "patient" refers to a subject who may seek or require

treatment, requires treatment, is receiving treatment, or will receive treatment, or a subject who is cared for by a trained professional for a specific disease. The term "pharmaceutically acceptable" used herein refers to those compounds, materials, compositions, and/or dosage forms that are suitable for contact with human and animal tissues within the scope of reasonable medical judgment without undue toxicity, irritation, allergic response, or other problems or complications, commensurate with a reasonable benefit/risk ratio.

[0145] As used herein, the phrase "pharmaceutically acceptable excipient" refers to any ingredient other than the compound described herein (for example, a medium capable of suspending, complexing, or dissolving the active compound), and is substantially non-toxic and non-inflammatory to a patient. The excipient may include, for example, an anti-adherent, an antioxidant, a binder, a coating, an anti-compression aid, a disintegrant, a dye (colorant), an emollient, an emulsifier, a filler (diluent), a film-forming agent or a coating, a flavoring agent, a fragrance, a glidant (flow enhancer), a lubricant, a preservative, a printing ink, an adsorbent, a suspending agent or a dispersant, a sweetener, and water of hydration. Exemplary excipients include, but are not limited to, butylated hydroxytoluene (BHT), calcium carbonate, calcium phosphate (binary acid), calcium stearate, cross-linked carboxymethyl cellulose, cross-linked polyvinylpyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine, methylcellulose, methyl p-hydroxybenzoate, microcrystalline cellulose, polyethylene glycol, polyvinylpyrrolidone, povidone, pregelatinized starch, propyl p-hydroxybenzoate, retinyl palmitate, shellac, silica, sodium carboxymethylcellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, sucrose, talc powder, titanium dioxide, vitamin A, vitamin E (α-tocopherol), vitamin C, xylitol, and other substances disclosed herein.

[0146] In the present application, for convenience, a structural formula of the compound described represents a certain isomer. However, the present application includes all isomers, for example, geometric isomers, optical isomers based on asymmetric carbon, stereoisomers, tautomers, and the like. Additionally, the compound represented by the structural formula of the compound in the present application may exhibit crystalline polymorphism. It is noted that any crystal form, a mixture of crystal forms, or an anhydride

or hydrate thereof is included within the scope of the present application.

[0147] The term "crystalline polymorph", "polymorph", or "crystal form" refers to a crystal structure in which the compound (or a salt or solvate thereof) may crystallize in different crystal packing arrangements, and all of these crystals have the same elemental composition. Different crystal forms typically have different X-ray diffraction patterns, infrared spectra, melting points, densities, hardnesses, crystal shapes, optical and electrical properties, stabilities, and solubilities. A recrystallization solvent, a crystallization rate, a storage temperature, and other factors may result in one predominant crystal form. The crystalline polymorph of the compound may be prepared by crystallization under different conditions.

[0148] The nanoparticle composition of the present application may further include one or more salts of the compound. The salts may be pharmaceutically acceptable salts. As used herein, a "pharmaceutically acceptable salt" refers to a derivative of the disclosed compound in which an existing acid or base moiety is converted to its salt form (for example, by allowing a free base to react with a suitable organic acid) to change a parent compound. Examples of the pharmaceutically acceptable salt include, but are not limited to, inorganic or organic acid salts of basic residues such as amines, basic or organic salts of acidic residues such as carboxylic acids, and the like. Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentylpropionate, digluconate, dodecyl sulfate, ethanesulfonate, fumarate, gluceptate, glucoheptonate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pectinate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate, and the like.

[0149] Representative alkali metal or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. The pharmaceutically acceptable salt of the present

application includes, for example, a conventional nontoxic salt of a parent compound formed by a nontoxic inorganic or organic acid. The pharmaceutically acceptable salt of the present application may be synthesized from a parent compound containing a basic or acidic moiety by a conventional chemical method. Typically, these salts may be prepared by allowing free acid or base forms of these compounds to react with a stoichiometric amount of a suitable base or acid in water, in an organic solvent, or in a mixture of both. Typically, a non-aqueous medium, such as ether, ethyl acetate, ethanol, isopropanol, or acetonitrile, is preferred.

[0150] As used herein, a "polydispersity index" is a ratio that describes the uniformity of particle size distribution of a system. A smaller value, for example, less than 0.3, indicates a narrow particle size distribution.

[0151] As used herein, the term "polypeptide" or "target polypeptide" refers to a polymer of amino acid residues typically connected by peptide bonds, which may be produced naturally (for example, by isolation or purification) or synthetically.

[0152] As used herein, "RNA" refers to ribonucleic acid that may exist naturally or non-naturally. For example, RNA may include a modified and/or non-naturally occurring component, such as one or more nucleobases, nucleosides, nucleotides, or linkers. RNA may include a cap structure, a chain-terminating nucleoside, a stem-loop, a polyA sequence, and/or a polyadenylation signal. RNA may have a nucleotide sequence encoding the target polypeptide. For example, RNA may be messenger RNA (mRNA). Translation of mRNA encoding a specific polypeptide, for example, *in vivo* translation of mRNA inside a mammalian cell, may produce the encoded polypeptide. RNA may be selected from a non-limiting group, including small interfering RNA (siRNA), asymmetric interfering RNA (aiRNA), microRNA (miRNA), double-stranded RNA (dsRNA), short hairpin RNA (shRNA), mRNA, and mixtures thereof.

[0153] As used herein, a "single unit dose" is the dose of any therapeutic agent administered as a single dose/per occasion/single route/single point of contact, that is, a single administration.

[0154] As used herein, a "divided dose" is a single unit dose or a total daily dose divided into two or more doses.

[0155] As used herein, a "total daily dose" is an amount given or prescribed within 24

hours. It may be administered as a single unit dose.

[0156] As used herein, in the context of the nanoparticle composition, a "particle size" or an "average particle size" refers to an average diameter of the nanoparticle composition.

[0157] As used herein, the term "subject" or "patient" refers to any organism to which the composition of the present application may be administered, for example, for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include animals (for example, mammals such as mice, rats, rabbits, non-human primates, and humans) and/or plants.

[0158] As used herein, a "target cell" refers to any one or more target cells. The cells may be found *in vitro*, *in vivo*, *in situ*, or in tissues or organs of an organism. The organism may be an animal, preferably a mammal, more preferably a human, and most preferably a patient.

[0159] As used herein, a "target tissue" refers to any one or more types of target tissues in which therapeutic and/or prophylactic delivery will result in a desired biological and/or pharmacological effect. Examples of the target tissue include specific tissues, organs, and systems or groups thereof. In certain applications, the target tissue may be the kidney, the lung, the spleen, and vascular endothelium of the kidney (for example, intracoronary or intrafemoral) or blood vessels (for example, by intratumoral injection). A "non-target tissue" refers to any one or more types of tissues in which expression of an encoded protein does not result in a desired biological and/or pharmacological effect. In certain applications, the non-target tissue may include the liver and the spleen.

[0160] The term "therapeutic agent" or "prophylactic agent" refers to any agent that, when administered to a subject, has a therapeutic, diagnostic, and/or prophylactic effect and/or induces a desired biological and/or pharmacological effect. The therapeutic agent is also referred to as an "active agent" or an "active component". Such substances include, but are not limited to, cytotoxins, radioactive ions, chemotherapeutic agents, small molecule drugs, proteins, and nucleic acids.

[0161] As used herein, the term "therapeutically effective amount" refers to a sufficient amount of an agent to be delivered (for example, a nucleic acid, a drug, a composition, a therapeutic agent, a diagnostic agent, a prophylactic agent, and the like) in a

current case, when administered to a subject suffering from or susceptible to an infection, a disease, a disorder, and/or a condition to treat and ameliorate symptoms, and to diagnose, prevent, and/or delay the onset of the infection, the disease, the disorder, and/or the condition.

[0162] As used herein, "transfection" refers to introduction of a species (for example., RNA) into a cell. The transfection may be performed, for example, *in vitro*, *ex vivo*, or *in vivo*.

[0163] As used herein, the term "treatment" refers to partial or complete remission, alleviation, amelioration, mitigation, delay of onset, inhibition of progression, reduction in severity, and/or reduction in the incidence of one or more symptoms or features. Specific infections, diseases, disorders, and/or conditions are involved. For example, "treatment" of cancer may refer to inhibition of the survival, growth, and/or metastasis of tumors. To reduce risks, subjects who do not exhibit a disease, a disorder, and/or a condition, and/or subjects who only exhibit early signs of the disease, the disorder, and/or the condition may be treated. Pathological developmental states are associated with the disease, the disorder, and/or the condition.

[0164] As used herein, "mol%" is calculated based on a mole number of total lipids in the lipid nanoparticle, that is, a molar percentage of a lipid ingredient in the lipid nanoparticle relative to the total lipids present in the lipid nanoparticle.

[0165] As used herein, the total lipids refer to all the lipids in the lipid nanoparticle. Specifically, the total lipids may consist of an ionizable lipid, a phospholipid, a structural lipid, and a PEG lipid.

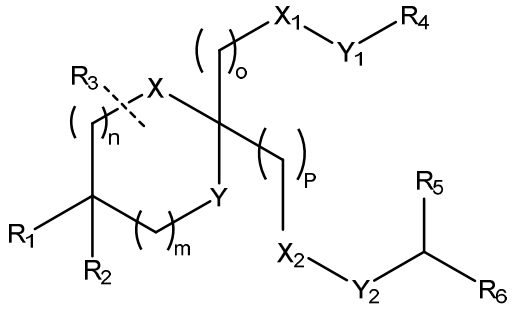
[0166] As used herein, a "coding sequence" may refer to a ribonucleotide sequence in mature mRNA that can be translated into a protein, or may refer to a complementary sequence of a deoxyribonucleotide (DNA) sequence that serves as a template for transcribing the ribonucleotide (RNA) sequence. In addition, the "coding sequence" of the present application may further include a polynucleotide sequence encoding a functional nucleic acid, such as miRNA, shRNA, dsRNA, and the like.

[0167] As used herein, an "N:P ratio" or an "N/P ratio" is a molar ratio of an ionizable nitrogen atom in a lipid to a phosphate group in RNA.

[0168] The present invention provides a lipid nanoparticle, which includes an

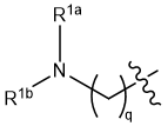
ionizable lipid, a phospholipid, a structural lipid, and a PEG lipid.

[0169] The ionizable lipid described herein may be a compound of formula (I), or a salt or isomer thereof,



(I)

[0170] wherein



[0171] R_1 is , wherein R^{1a} and R^{1b} are independently selected from H and C1-C6 alkyl; and R_2 and R_3 are independently selected from H or C1-C6 alkyl;

[0172] R_4, R_5, and R_6 are independently selected from C1-C14 alkyl;

[0173] X and Y are independently selected from O or S;

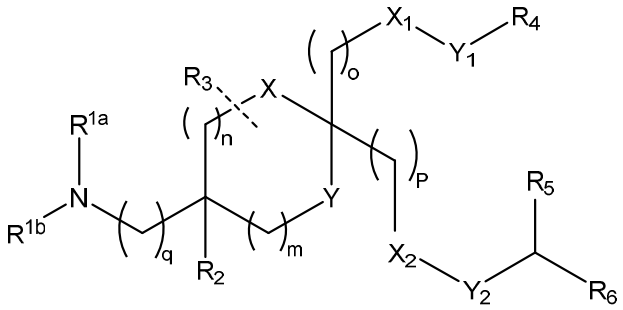
[0174] X_1 and X_2 are independently selected from C=O or O, and Y_1 and Y_2 are independently selected from C=O or O, provided that X_1 and Y_1 as well as X_2 and Y_2 are not simultaneously C=O or O;

[0175] m and n are independently selected from 0, 1, and 2, provided that m and n are not simultaneously 0;

[0176] o and p are independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10; and

[0177] q is selected from 0, 1, 2, 3, 4, 5, and 6.

[0178] In a specific embodiment, the compound of formula (I) is shown as formula (IA),



(IA)

[0179] wherein q is selected from 0, 1, 2, 3, and 4.

[0180] In a specific embodiment, in the above formula (I) or (IA), R_4 is C10-C11 alkyl. For example, R_4 is C10 alkyl or C11 alkyl. In a specific embodiment, in the above formula (I) or (IA), R4 is C10-C11 linear alkyl. For example, R4 is C10 linear alkyl or C11 linear alkyl.

[0181] In a specific embodiment, in the above formula (I) or (IA), R_5 and R_6 are C8 alkyl. In a specific embodiment, in the above formula (I) or (IA), R_5 and R_6 are C8 linear alkyl.

[0182] In a specific embodiment, in the above formula (I) or (IA), o is 5 or 6.

[0183] In a specific embodiment, in the above formula (I) or (IA), p is 7 or 8.

[0184] In a specific embodiment, in the above formula (I) or (IA), o is 5, R_4 is C11 linear alkyl, p is 7, and R_5 and R_6 are C8 linear alkyl.

[0185] In a specific embodiment, in the above formula (I) or (IA), o is 6, R_4 is C10 linear alkyl, p is 7, and R_5 and R_6 are C8 linear alkyl.

[0186] In a specific embodiment, in the above formula (I) or (IA), X_1 and X_2 are C=O, and Y_1 and Y_2 are O.

[0187] In a specific embodiment, in the above formula (I) or (IA), X_1 is O, Y_1 is C=O, X_2 is C=O, and Y_2 is O.

[0188] In a specific embodiment, in the above formula (I) or (IA), X_1 is C=O, Y_1 is O, X_2 is O, and Y_2 is C=O.

[0189] In a specific embodiment, in the above formula (I) or (IA), X_1 and X_2 are O, and Y_1 and Y_2 are C=O.

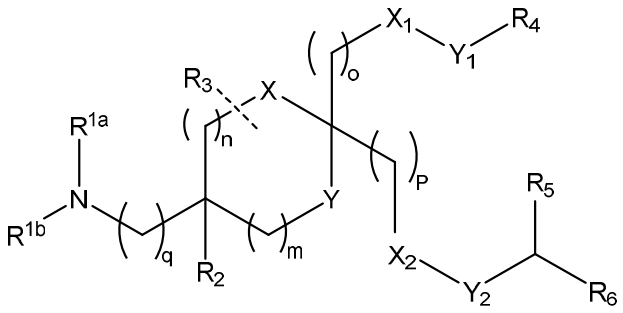
[0190] In a specific embodiment, in the above formula (I) or (IA), X and Y are both

O.

[0191] In a specific embodiment, in the above formula (I) or (IA), n is 0, and m is 1. In a specific embodiment, in the above formula (I) or (IA), n is 1, and m is 0. In a specific embodiment, in the above formula (I) or (IA), n is 1, and m is 1.

[0192] In a specific embodiment, R^{1a} and R^{1b} are selected from $-CH_3$, $-CH_2CH_3$, or $-CH(CH_3)_2$.

[0193] In a specific embodiment, the compound of formula (I) is shown as formula (IA),



(IA)

[0194] wherein q is selected from 2 and 3, and R^{1a} and R^{1b} are selected from $-CH_3$,

[0195] R_2 is H, and R_3 is H,

[0196] n is 1, and m is 0,

[0197] X and Y are both O,

[0198] o is 5 or 6,

[0199] p is 7,

[0200] X_1 and X_2 are C=O, and Y_1 and Y_2 are O, or X_1 is O, Y_1 is C=O, X_2 is C=O, and Y_2 is O,

[0201] R_4 is C10 linear alkyl or C11 linear alkyl, and

[0202] R_5 and R_6 are C8 linear alkyl.

[0203] In a specific embodiment, the C1-C6 alkyl is selected from $-CH_3$, $-CH_2CH_3$, or $-CH(CH_3)_2$.

[0204] In the lipid nanoparticle and lipid nanoparticle composition of the present application, the ionizable lipid may further include, for example, one or more of the following: 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLinDMA),

1,2-dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLin-K-C2-DMA; "XTC2"), 2,2-dilinoleyl-4-(3-dimethylaminopropyl)-[1,3]-dioxolane (DLin-K-C3-DMA), 2,2-dilinoleyl-4-(4-dimethylaminobutyl)-[1,3]-dioxolane (DLin-K-C4-DMA), 2,2-dilinoleyl-5-dimethylaminomethyl-[1,3]-dioxane (DLin-K6-DMA), 2,2-dilinoleyl-4-N-methylpepiazino-[1,3]-dioxolane (DLin-K-MPZ), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), 1,2-dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-dilinoleyloxy-3-(dimethylamino)acetoxypropane (DLin-DAC), 1,2-dilinoleyloxy-3-morpholinopropane (DLin-MA), 1,2-dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-linoleoyl-2-linoleyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-dilinoleyloxy-3-trimethylaminopropane chloride (DLin-TMA.Cl), 1,2-dilinoleoyl-3-trimethylaminopropane chloride (DLin-TAP.Cl), 1,2-dilinoleyloxy-3-(N-methylpiperazinyl)propane (DLin-MPZ), 3-(N,N-dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-dioleylamino)-1,2-propanediol (DOAP), 1,2-dilinoleyloxy-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), 1,2-dioleyloxy-N,N-dimethylaminopropane (DODMA), 1,2-distearyloxy-N,N-dimethylaminopropane (DSDMA), N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), 3-(N-(N',N'-dimethylaminoethane)-carbamoyl)cholesterol (DC-Chol), N-(1,2-dimyristoyloxyprop-3-yl)-N,N-dimethyl-N-carboxyethylammonium bromide (DMRIE),

2,3-dioleyloxy-N-[2(spermine-formamido)ethyl]-N,N-dimethyl-1-propylammonium trifluoroacetate (DOSPA), dioctadecylaminoglycyl spermine (DOGS), 3-dimethylamino-2-(cholest-5-en-3-β-oxybutan-4-oxy)-1-(cis,cis-9,12-octadecadienoxy)prop

ane (CLinDMA), 2-[5'-(cholest-5-en-3-β-oxy)-3'-oxapentoxy]-3-dimethyl-1-(cis,cis-9',1-2'-octadecadienoxy)propane (CpLinDMA), N,N-dimethyl-3,4-dioleyloxybenzylamine (DMOBA), 1,2-N,N'-dioleylcarbamoyl-3-dimethylaminopropane (DOcarbDAP), 1,2-N,N'-dilinoleylcarbamoyl-3-dimethylaminopropane (DLincarbDAP), MC3, SM-102, and ALC-0315.

[0205] In the present application, the phospholipid may be selected from one or two or more of the following compounds:

[0206] dilauroylphosphatidylcholine (DLPC),

[0207] dimyristoylphosphatidylcholine (DMPC),

[0208] dioleoylphosphatidylcholine (DOPC),

[0209] dipalmitoylphosphatidylcholine (DPPC),

[0210] distearoylphosphatidylcholine (DSPC),

[0211] dioleoylphosphatidylcholine (DUPC),

[0212] palmitoyloleoylphosphatidylcholine (POPC),

[0213] 1,2-di-O-octadecyl-sn-glycero-3-phosphocholine (18:0 Diether PC),

[0214] 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC),

[0215] 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC),

[0216] 1,2-divinyl-sn-glycero-3-phosphocholine,

[0217] 1,2-diarylacyl-sn-glycero-3-phosphocholine,

[0218] 1,2-dioleoyl-SN-glycero-3-phosphoethanolamine (DOPE),

[0219] 1,2-dihydroxystannyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE),

[0220] 1,2-distearoyl-sn-glycero-3-phosphoethanolamine,

[0221] 1,2-divinylol-sn-glycero-3-phosphoethanolamine,

[0222] 1,2-divinyl-sn-glycero-3-phosphoethanolamine,

[0223] 1,2-diarylyl-sn-glycero-3-phosphoethanolamine,

[0224] 1,2-dithiohexaenoate-sn-glycero-3-phosphoethanolamine,

[0225] 1,2-diol-sn-glycero-3-phospho-(1-glycerol) sodium salt (DOPG), or sphingomyelin.

[0226] In a specific embodiment, the phospholipid is DSPC.

[0227] In a specific embodiment, the phospholipid is DOPE.

[0228] In a specific embodiment, the phospholipid is a combination of DSPC and DOPE.

[0229] In the present application, the structural lipid may be selected from, but is not limited to, a group consisting of the following: cholesterol, coprosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, tomatine, ursolic acid, α-tocopherol, and a mixture thereof. In some embodiments, the structural lipid is cholesterol. In some embodiments, the structural lipid includes cholesterol and corticosteroid (for example, prednisolone, dexamethasone, prednisone, and hydrocortisone) or a combination thereof.

[0230] In the present application, the PEG lipid may alternatively be referred to as a polyethylene glycolated lipid. The PEG lipid is a lipid modified with polyethylene glycol. The PEG lipid may be selected from a non-limiting group consisting of PEG-modified phosphatidylethanolamine, PEG-modified phosphatidic acid, PEG-modified ceramide, PEG-modified dialkylamine, PEG-modified diacylglycerol, PEG-modified dialkylglycerol, and a mixture thereof. For example, the PEG lipid may be a PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, or PEG-DSPE lipid.

[0231] In a specific embodiment, the PEG lipid is DMG-PEG2000.

[0232] In some embodiments of the present application, in the lipid nanoparticle, a content of the ionizable lipid is 35 mol% to 65 mol% and may be, for example, 35 mol%, 35.5 mol%, 36 mol%, 36.5 mol%, 37 mol%, 37.5 mol%, 38 mol%, 38.5 mol%, 39 mol%, 39.5 mol%, 40 mol%, 40.5 mol%, 41 mol%, 41.5 mol%, 42 mol%, 42.5 mol%, 43 mol%, 43.5 mol%, 44 mol%, 44.5 mol%, 45 mol%, 45.5 mol%, 46 mol%, 46.5 mol%, 47 mol%, 47.5 mol%, 48 mol%, 48.1 mol%, 48.2 mol%, 48.3 mol%, 48.4 mol%, 48.5 mol%, 48.6 mol%, 48.7 mol%, 48.8 mol%, 48.9 mol%, 49 mol%, 49.1 mol%, 49.2 mol%, 49.3 mol%, 49.4 mol%, 49.5 mol%, 49.6 mol%, 49.7 mol%, 49.8 mol%, 49.9 mol%, 50 mol%, 50.5 mol%, 51 mol%, 51.5 mol%, 52 mol%, 52.5 mol%, 53 mol%, 53.5 mol%, 54 mol%, 54.5 mol%, 55 mol%, 55.5 mol%, 56 mol%, 56.5 mol%, 57 mol%, 57.5 mol%, 58 mol%, 58.5 mol%, 59 mol%, 59.5 mol%, 60 mol%, 60.5 mol%, 61 mol%, 61.5 mol%, 62 mol%, 62.5 mol%, 63 mol%, 63.5 mol%, 64 mol%, 64.5 mol%, or 65 mol%.

[0233] In some embodiments of the present application, in the lipid nanoparticle, a content of a sum of the phospholipid and the structural lipid may be 35 mol% to 65 mol% and may be, for example, 35 mol%, 35.5 mol%, 36 mol%, 36.5 mol%, 37 mol%, 37.5 mol%, 38 mol%, 38.5 mol%, 39 mol%, 39.5 mol%, 40 mol%, 40.5 mol%, 41 mol%, 41.5 mol%, 42 mol%, 42.5 mol%, 43 mol%, 43.5 mol%, 44 mol%, 44.5 mol%, 45 mol%, 45.5 mol%, 46 mol%, 46.5 mol%, 47 mol%, 47.5 mol%, 48 mol%, 48.1 mol%, 48.2 mol%, 48.3 mol%, 48.4 mol%, 48.5 mol%, 48.6 mol%, 48.7 mol%, 48.8 mol%, 48.9 mol%, 49 mol%, 49.1 mol%, 49.2 mol%, 49.3 mol%, 49.4 mol%, 49.5 mol%, 49.6 mol%, 49.7 mol%, 49.8 mol%, 49.9 mol%, 50 mol%, 50.5 mol%, 51 mol%, 51.5 mol%, 52 mol%, 52.5 mol%, 53 mol%, 53.5 mol%, 54 mol%, 54.5 mol%, 55 mol%, 55.5 mol%, 56 mol%, 56.5 mol%, 57 mol%, 57.5 mol%, 58 mol%, 58.5 mol%, 59 mol%, 59.5 mol%, 60 mol%, 60.5 mol%, 61 mol%, 61.5 mol%, 62 mol%, 62.5 mol%, 63 mol%, 63.5 mol%, 64 mol%, 64.5 mol%, or 65 mol%.

[0234] In some embodiments of the present application, in the lipid nanoparticle, a content of the phospholipid may be 5 mol% to 15 mol% and may be for example, 5 mol%, 5.5 mol%, 6 mol%, 6.5 mol%, 7 mol%, 7.5 mol%, 8 mol%, 8.5 mol%, 9 mol%, 9.5 mol%, 10 mol%, 10.5 mol%, 11 mol%, 11.5 mol%, 12 mol%, 12.5 mol%, 13 mol%, 13.5 mol%, 14 mol%, 14.5 mol%, or 15 mol%. For example, the content may be 10 mol% to 15 mol%.

[0235] In some embodiments of the present application, in the lipid nanoparticle, a content of the structural lipid may be 30 mol% to 50 mol% and may be, for example, 30 mol%, 30.5 mol%, 31 mol%, 31.5 mol%, 32 mol%, 32.5 mol%, 33 mol%, 33.5 mol%, 34 mol%, 34.5 mol%, 35 mol%, 35.5 mol%, 36 mol%, 36.5 mol%, 37 mol%, 37.5 mol%, 38 mol%, 38.5 mol%, 39 mol%, 39.5 mol%, 40 mol%, 40.5 mol%, 41 mol%, 41.5 mol%, 42 mol%, 42.5 mol%, 43 mol%, 43.5 mol%, 44 mol%, 44.5 mol%, 45 mol%, 45.5 mol%, 46 mol%, 46.5 mol%, 47 mol%, 47.5 mol%, 48 mol%, 48.1 mol%, 48.2 mol%, 48.3 mol%, 48.4 mol%, 48.5 mol%, 48.6 mol%, 48.7 mol%, 48.8 mol%, 48.9 mol%, 49 mol%, 49.1 mol%, 49.2 mol%, 49.3 mol%, 49.4 mol%, 49.5 mol%, 49.6 mol%, 49.7 mol%, 49.8 mol%, 49.9 mol%, or 50 mol%. For example, the content may be 35 mol% to 45 mol%.

[0236] In some embodiments of the present application, in the lipid nanoparticle, a content of the PEG lipid is 0.5 mol% to 5 mol% and may be, for example, 0.5 mol%, 0.6 mol%, 0.7 mol%, 0.8 mol%, 0.9 mol%, 1 mol%, 1.1 mol%, 1.2 mol%, 1.3 mol%, 1.4 mol%,

1.5 mol%, 1.6 mol%, 1.7 mol%, 1.8 mol%, 1.9 mol%, 2 mol%, 2.1 mol%, 2.2 mol%, 2.3 mol%, 2.4 mol%, 2.5 mol%, 2.6 mol%, 2.7 mol%, 2.8 mol%, 2.9 mol%, 3 mol%, 3.1 mol%, 3.2 mol%, 3.3 mol%, 3.4 mol%, 3.5 mol%, 3.6 mol%, 3.7 mol%, 3.8 mol%, 3.9 mol%, 4 mol%, 4.1 mol%, 4.2 mol%, 4.3 mol%, 4.4 mol%, 4.5 mol%, 4.6 mol%, 4.7 mol%, 4.8 mol%, 4.9 mol%, or 5 mol%.

[0237] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein a content of the ionizable lipid is 35 mol% to 65 mol%, a content of a sum of the phospholipid and the cholesterol is 35 mol% to 65 mol%, and a content of the PEG lipid is 0.5 mol% to 5 mol%.

[0238] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein a component ratio is not as follows: a content of the ionizable lipid is 50 mol%, a content of a sum of the phospholipid and the cholesterol is 48.5 mol%, and a content of the PEG lipid is 1.5 mol%.

[0239] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 40 mol% to 50 mol%, a content of the phospholipid is 10 mol% to 15 mol%, a content of the cholesterol is 35 mol% to 45 mol%, and a content of the PEG lipid is 1.5 mol% to 2.5 mol%.

[0240] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 48 mol% to 49 mol%, a content of the phospholipid is 10 mol% to 15 mol%, a content of the cholesterol is 35 mol% to 45 mol%, and a content of the PEG lipid is 1.5 mol% to 2.5 mol%.

[0241] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 40 mol%, a content of the phospholipid is 15 mol%, a content of the cholesterol is 42.5 mol%, and a content of the PEG lipid is 2.5 mol%.

[0242] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 40 mol%, a content of the phospholipid is 15 mol%, a content of the

cholesterol is 43.5 mol%, and a content of the PEG lipid is 1.5 mol%.

[0243] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 48.5 mol%, a content of the phospholipid is 5 mol%, a content of the cholesterol is 45 mol%, and a content of the PEG lipid is 1.5 mol%.

[0244] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 44.3 mol%, a content of the phospholipid is 9.3 mol%, a content of the cholesterol is 45 mol%, and a content of the PEG lipid is 1.5 mol%.

[0245] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 50 mol%, a content of the phospholipid is 5 mol%, a content of the cholesterol is 43 mol%, and a content of the PEG lipid is 2.1 mol%.

[0246] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 40 mol%, a content of the phospholipid is 12.5 mol%, a content of the cholesterol is 45 mol%, and a content of the PEG lipid is 2.5 mol%.

[0247] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 46 mol%, a content of the phospholipid is 11 mol%, a content of the cholesterol is 41 mol%, and a content of the PEG lipid is 2 mol%.

[0248] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 47.5 mol%, a content of the phospholipid is 5 mol%, a content of the cholesterol is 45 mol%, and a content of the PEG lipid is 2.5 mol%.

[0249] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 40 mol%, a content of the phospholipid is 13 mol%, a content of the cholesterol is 45 mol%, and a content of the PEG lipid is 2 mol%.

[0250] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a

phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 50 mol%, a content of the phospholipid is 12.5 mol%, a content of the cholesterol is 35 mol%, and a content of the PEG lipid is 2.5 mol%.

[0251] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 50 mol%, a content of the phospholipid is 8 mol%, a content of the cholesterol is 39.5 mol%, and a content of the PEG lipid is 2.5 mol%.

[0252] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 42.3 mol%, a content of the phospholipid is 15 mol%, a content of the cholesterol is 40.3 mol%, and a content of the PEG lipid is 2.5 mol%.

[0253] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 46 mol%, a content of the phospholipid is 11 mol%, a content of the cholesterol is 41 mol%, and a content of the PEG lipid is 2 mol%.

[0254] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 47.5 mol%, a content of the phospholipid is 15 mol%, a content of the cholesterol is 35 mol%, and a content of the PEG lipid is 2.5 mol%.

[0255] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 48.5 mol%, a content of the phospholipid is 15 mol%, a content of the cholesterol is 35 mol%, and a content of the PEG lipid is 1.5 mol%.

[0256] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 50 mol%, a content of the phospholipid is 5 mol%, a content of the cholesterol is 43.5 mol%, and a content of the PEG lipid is 1.5 mol%.

[0257] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 40 mol%, a content of the phospholipid is 15 mol%, a content of the

cholesterol is 43.5 mol%, and a content of the PEG lipid is 1.5 mol%.

[0258] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 48 mol%, a content of the phospholipid is 10.3 mol%, a content of the cholesterol is 39.6 mol%, and a content of the PEG lipid is 2.1 mol%.

[0259] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a component ratio is not as follows: a content of the ionizable lipid is 50 mol%, a content of the phospholipid is 10 mol%, a content of the cholesterol is 38.5 mol%, and a content of the PEG lipid is 1.5 mol%.

[0260] In some embodiments of the present application, in the lipid nanoparticle, a molar ratio of the ionizable lipid, a sum of the phospholipid and the structural lipid, and the PEG lipid is (35-65):(35-65):(0.5-5).

[0261] In the lipid nanoparticle of the present application, for the molar ratio of the ionizable lipid, the sum of the phospholipid and the structural lipid, and the PEG lipid of (35-65):(35-65):(0.5-5), 35-65 may be any numerical value between 35 and 65 and may be, for example, 35, 35.5, 36, 36.5, 37, 37.5, 38, 38.5, 39, 39.5, 40, 40.5, 41, 41.5, 42, 42.5, 43, 43.5, 44, 44.5, 45, 45.5, 46, 46.5, 47, 47.5, 48, 48.5, 49, 49.5, 50, 50.5, 51, 51.5, 52, 52.5, 53, 53.5, 54, 54.5, 55, 55.5, 56, 56.5, 57, 57.5, 58, 58.5, 59, 59.5, 60, 60.5, 61, 61.5, 62, 62.5, 63, 63.5, 64, 64.5, or 65; and 0.5-5 may be any numerical value between 0.5 and 5 and may be, for example, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, or 5.

[0262] In a specific embodiment, in the lipid nanoparticle, a molar ratio of the ionizable lipid, a sum of the phospholipid and the structural lipid, and the PEG lipid is (40-50):(35-65):(1-3).

[0263] In a specific embodiment, in the lipid nanoparticle, a molar ratio of the ionizable lipid, a sum of the phospholipid and the structural lipid, and the PEG lipid is (50-65):(35-65):(1-3).

[0264] In a specific embodiment, a molar ratio of the ionizable lipid, a sum of the

phospholipid and the structural lipid, and the PEG lipid is not 50: 48.5: 1.5.

[0265] In a specific embodiment, in the lipid nanoparticle, a molar ratio of the ionizable lipid, the phospholipid, the structural lipid, and the PEG lipid is (40-65):(5-15):(30-50):(1-3).

[0266] In a specific embodiment, in the lipid nanoparticle, a molar ratio of the ionizable lipid, the phospholipid, the structural lipid, and the PEG lipid is (40-65):(10-15):(35-45):(1.5-2.5).

[0267] In a specific embodiment, in the lipid nanoparticle, a molar ratio of the ionizable lipid, the phospholipid, the structural lipid, and the PEG lipid is (40-50):(5-15):(30-50):(1-3). For the molar ratio of (40-50):(5-15):(30-50):(1-3), 40-50 may be any numerical value between 40 and 50 and may be, for example, 40, 40.5, 41, 41.5, 42, 42.5, 43, 43.5, 44, 44.5, 45, 45.5, 46, 46.5, 47, 47.5, 48, 48.5, 49, 49.5, or 50; 5-15 may be any numerical value between 5 and 15 and may be, for example, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, or 15; 30-50 may be any numerical value between 30 and 50 and may be, for example, 30, 30.5, 31, 31.5, 32, 32.5, 33, 33.5, 34, 34.5, 35, 35.5, 36, 36.5, 37, 37.5, 38, 38.5, 39, 39.5, 40, 40.5, 41, 41.5, 42, 42.5, 43, 43.5, 44, 44.5, 45, 45.5, 46, 46.5, 47, 47.5, 48, 48.5, 49, 49.5, or 50; and 1-3 may be any numerical value between 1 and 3 and may be, for example, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, or 3.

[0268] In a specific embodiment, in the lipid nanoparticle, a molar ratio of the ionizable lipid, the phospholipid, the structural lipid, and the PEG lipid is (40-50):(5-15):(35-45):(1.5-2.5). For the molar ratio of (40-50):(5-15):(35-45):(1.5-2.5), 40-50 may be any numerical value between 40 and 50 and may be, for example, 40, 40.5, 41, 41.5, 42, 42.5, 43, 43.5, 44, 44.5, 45, 45.5, 46, 46.5, 47, 47.5, 48, 48.5, 49, 49.5, or 50; 5-15 may be any numerical value between 5 and 15 and may be, for example, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, or 15; 35-45 may be any numerical value between 35 and 45 and may be, for example, 35, 35.5, 36, 36.5, 37, 37.5, 38, 38.5, 39, 39.5, 40, 40.5, 41, 41.5, 42, 42.5, 43, 43.5, 44, 44.5, or 45; and 1.5-2.5 may be any numerical value between 1.5 and 2.5 and may be, for example, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, or 2.5.

[0269] In a specific embodiment, in the lipid nanoparticle, a molar ratio of the ionizable lipid, the phospholipid, the structural lipid, and the PEG lipid is (50-65):(5-15):(30-50):(1-3). For the molar ratio of (50-65):(5-15):(30-50):(1-3), 50-65 may be any numerical value between 50 and 65 and may be, for example, 51, 51.5, 52, 52.5, 53, 53.5, 54, 54.5, 55, 55.5, 56, 56.5, 57, 57.5, 58, 58.5, 59, 59.5, 60, 60.5, 61, 61.5, 62, 62.5, 63, 63.5, 64, 64.5, or 65; 5-15 may be any numerical value between 5 and 15 and may be, for example, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, or 15; 30-50 may be any numerical value between 30 and 50 and may be, for example, 30, 30.5, 31, 31.5, 32, 32.5, 33, 33.5, 34, 34.5, 35, 35.5, 36, 36.5, 37, 37.5, 38, 38.5, 39, 39.5, 40, 40.5, 41, 41.5, 42, 42.5, 43, 43.5, 44, 44.5, 45, 45.5, 46, 46.5, 47, 47.5, 48, 48.5, 49, 49.5, or 50; and 1-3 may be any numerical value between 1 and 3 and may be, for example, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, or 3.

[0270] In a specific embodiment, a molar ratio of the ionizable lipid, the phospholipid, the structural lipid, and the PEG lipid is not 50:10:38.5:1.5.

[0271] In a specific embodiment, in the lipid nanoparticle, a molar ratio of the ionizable lipid, the phospholipid, the structural lipid, and the PEG lipid is (50-65):(5-15):(35-45):(1.5-2.5). For the molar ratio of (50-65):(5-15):(35-45):(1.5-2.5), 50-65 may be any numerical value between 50 and 65 and may be, for example, 51, 51.5, 52, 52.5, 53, 53.5, 54, 54.5, 55, 55.5, 56, 56.5, 57, 57.5, 58, 58.5, 59, 59.5, 60, 60.5, 61, 61.5, 62, 62.5, 63, 63.5, 64, 64.5, or 65; 5-15 may be any numerical value between 5 and 15 and may be, for example, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, or 15; 35-45 may be any numerical value between 35 and 45 and may be, for example, 35, 35.5, 36, 36.5, 37, 37.5, 38, 38.5, 39, 39.5, 40, 40.5, 41, 41.5, 42, 42.5, 43, 43.5, 44, 44.5, or 45; and 1.5-2.5 may be any numerical value between 1.5 and 2.5 and may be, for example, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, or 2.5.

[0272] In a specific embodiment, the lipid nanoparticle includes compound 2, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 2, a sum of the DSPC and the cholesterol, and the DMG-PEG2000 is (35-65):(35-65):(0.5-5).

[0273] In a specific embodiment, the lipid nanoparticle includes compound 2, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 2, the DSPC, the

cholesterol, and the DMG-PEG2000 is (40-49):(5-15):(30-50):(1-3).

[0274] In a specific embodiment, the lipid nanoparticle includes compound 2, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 2, the DSPC, the cholesterol, and the DMG-PEG2000 is (40-49):(10-15):(35-45):(1.5-2.5).

[0275] In a specific embodiment, the lipid nanoparticle includes compound 2, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 2, the DSPC, the cholesterol, and the DMG-PEG2000 is (51-60):(5-15):(30-50):(1-3).

[0276] In a specific embodiment, the lipid nanoparticle includes compound 2, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 2, the DSPC, the cholesterol, and the DMG-PEG2000 is (51-60):(10-15):(35-45):(1.5-2.5).

[0277] In a specific embodiment, the lipid nanoparticle includes compound 2, DSPC, cholesterol, and DMG-PEG2000, wherein a content of the compound 2 is 35 mol% to 65 mol%, a content of a sum of the DSPC and the cholesterol is 35 mol% to 65 mol%, and a content of the DMG-PEG2000 is 0.5 mol% to 5 mol%.

[0278] In a specific embodiment, the lipid nanoparticle includes compound 2, DSPC, cholesterol, and DMG-PEG2000, wherein in the lipid nanoparticle, a content of the compound 2 is 40 mol% to 50 mol%, a content of the DSPC is 10 mol% to 15 mol%, a content of the cholesterol is 35 mol% to 45 mol%, and a content of the DMG-PEG2000 is 1.5 mol% to 2.5 mol%.

[0279] In a specific embodiment, the lipid nanoparticle includes compound 2, DSPC, cholesterol, and DMG-PEG2000, wherein in the lipid nanoparticle, a content of the compound 2 is 48 mol% to 49 mol%, a content of the DSPC is 10 mol% to 15 mol%, a content of the cholesterol is 35 mol% to 45 mol%, and a content of the DMG-PEG2000 is 1.5 mol% to 2.5 mol%.

[0280] In a specific embodiment, the lipid nanoparticle includes compound 4, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 4, a sum of the DSPC and the cholesterol, and the DMG-PEG2000 is (35-65):(35-65):(0.5-5).

[0281] In a specific embodiment, the lipid nanoparticle includes compound 4, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 4, the DSPC, the cholesterol, and the DMG-PEG2000 is (40-49):(5-15):(30-50):(1-3).

[0282] In a specific embodiment, the lipid nanoparticle includes compound 4, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 4, the DSPC, the cholesterol, and the DMG-PEG2000 is (40-49):(10-15):(35-45):(1.5-2.5).

[0283] In a specific embodiment, the lipid nanoparticle includes compound 4, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 4, the DSPC, the cholesterol, and the DMG-PEG2000 is (51-60):(5-15):(30-50):(1-3).

[0284] In a specific embodiment, the lipid nanoparticle includes compound 4, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 4, the DSPC, the cholesterol, and the DMG-PEG2000 is (51-60):(10-15):(35-45):(1.5-2.5).

[0285] In a specific embodiment, the lipid nanoparticle includes compound 4, DSPC, cholesterol, and DMG-PEG2000, wherein in the lipid nanoparticle composition, a content of the compound 4 is 35 mol% to 65 mol%, a content of a sum of the DSPC and the cholesterol is 35 mol% to 65 mol%, and a content of the DMG-PEG2000 is 0.5 mol% to 5 mol%.

[0286] In a specific embodiment, the lipid nanoparticle includes compound 4, DSPC, cholesterol, and DMG-PEG2000, wherein in the lipid nanoparticle composition, a content of the compound 4 is 40 mol% to 50 mol%, a content of the DSPC is 10 mol% to 15 mol%, a content of the cholesterol is 35 mol% to 45 mol%, and a content of the DMG-PEG2000 is 1.5 mol% to 2.5 mol%.

[0287] In a specific embodiment, the lipid nanoparticle includes compound 4, DSPC, cholesterol, and DMG-PEG2000, wherein in the lipid nanoparticle composition, a content of the compound 4 is 48 mol% to 49 mol%, a content of the DSPC is 10 mol% to 15 mol%, a content of the cholesterol is 35 mol% to 45 mol%, and a content of the DMG-PEG2000 is 1.5 mol% to 2.5 mol%.

[0288] In a specific embodiment, the lipid nanoparticle includes compound 5, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 5, a sum of the DSPC and the cholesterol, and the DMG-PEG2000 is (35-65):(35-65):(0.5-5).

[0289] In a specific embodiment, the lipid nanoparticle includes compound 5, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 5, the DSPC, the cholesterol, and the DMG-PEG2000 is (40-49):(5-15):(30-50):(1-3).

[0290] In a specific embodiment, the lipid nanoparticle includes compound 5, DSPC,

cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 5, the DSPC, the cholesterol, and the DMG-PEG2000 is (40-49):(10-15):(35-45):(1.5-2.5).

[0291] In a specific embodiment, the lipid nanoparticle includes compound 5, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 5, the DSPC, the cholesterol, and the DMG-PEG2000 is (51-60):(5-15):(30-50):(1-3).

[0292] In a specific embodiment, the lipid nanoparticle includes compound 5, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 5, the DSPC, the cholesterol, and the DMG-PEG2000 is (51-60):(10-15):(35-45):(1.5-2.5).

[0293] In a specific embodiment, the lipid nanoparticle includes compound 5, DSPC, cholesterol, and DMG-PEG2000, wherein in the lipid nanoparticle, a content of the compound 5 is 35 mol% to 65 mol%, a content of a sum of the DSPC and the cholesterol is 35 mol% to 65 mol%, and a content of the DMG-PEG2000 is 0.5 mol% to 5 mol%.

[0294] In a specific embodiment, the lipid nanoparticle includes compound 5, DSPC, cholesterol, and DMG-PEG2000, wherein in the lipid nanoparticle, a content of the compound 5 is 40 mol% to 50 mol%, a content of the DSPC is 10 mol% to 15 mol%, a content of the cholesterol is 35 mol% to 45 mol%, and a content of the DMG-PEG2000 is 1.5 mol% to 2.5 mol%.

[0295] In a specific embodiment, the lipid nanoparticle includes compound 5, DSPC, cholesterol, and DMG-PEG2000, wherein in the lipid nanoparticle, a content of the compound 5 is 48 mol% to 49 mol%, a content of the DSPC is 10 mol% to 15 mol%, a content of the cholesterol is 35 mol% to 45 mol%, and a content of the DMG-PEG2000 is 1.5 mol% to 2.5 mol%.

[0296] In a specific embodiment, the lipid nanoparticle includes compound 5A, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 5A, a sum of the DSPC and the cholesterol, and the DMG-PEG2000 is (35-65):(35-65):(0.5-5).

[0297] In a specific embodiment, the lipid nanoparticle includes compound 5A, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 5A, the DSPC, the cholesterol, and the DMG-PEG2000 is (40-49):(5-15):(30-50):(1-3).

[0298] In a specific embodiment, the lipid nanoparticle includes compound 5A, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 5A, the

DSPC, the cholesterol, and the DMG-PEG2000 is (40-49):(10-15):(35-45):(1.5-2.5).

[0299] In a specific embodiment, the lipid nanoparticle includes compound 5A, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 5A, the DSPC, the cholesterol, and the DMG-PEG2000 is (51-60):(5-15):(30-50):(1-3).

[0300] In a specific embodiment, the lipid nanoparticle includes compound 5A, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 5A, the DSPC, the cholesterol, and the DMG-PEG2000 is (51-60):(10-15):(35-45):(1.5-2.5).

[0301] In a specific embodiment, the lipid nanoparticle includes compound 5A, DSPC, cholesterol, and DMG-PEG2000, wherein in the lipid nanoparticle, a content of the compound 5A is 35 mol% to 65 mol%, a content of a sum of the DSPC and the cholesterol is 35 mol% to 65 mol%, and a content of the DMG-PEG2000 is 0.5 mol% to 5 mol%.

[0302] In a specific embodiment, the lipid nanoparticle includes compound 5A, DSPC, cholesterol, and DMG-PEG2000, wherein in the lipid nanoparticle, a content of the compound 5A is 40 mol% to 50 mol%, a content of the DSPC is 10 mol% to 15 mol%, a content of the cholesterol is 35 mol% to 45 mol%, and a content of the DMG-PEG2000 is 1.5 mol% to 2.5 mol%.

[0303] In a specific embodiment, the lipid nanoparticle includes compound 5A, DSPC, cholesterol, and DMG-PEG2000, wherein in the lipid nanoparticle, a content of the compound 5A is 48 mol% to 49 mol%, a content of the DSPC is 10 mol% to 15 mol%, a content of the cholesterol is 35 mol% to 45 mol%, and a content of the DMG-PEG2000 is 1.5 mol% to 2.5 mol%.

[0304] In a specific embodiment, the lipid nanoparticle includes compound 8, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 8, a sum of the DSPC and the cholesterol, and the DMG-PEG2000 is (35-65):(35-65):(0.5-5).

[0305] In a specific embodiment, the lipid nanoparticle includes compound 8, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 8, the DSPC, the cholesterol, and the DMG-PEG2000 is (40-49):(5-15):(30-50):(1-3).

[0306] In a specific embodiment, the lipid nanoparticle includes compound 8, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 8, the DSPC, the cholesterol, and the DMG-PEG2000 is (40-49):(10-15):(35-45):(1.5-2.5).

[0307] In a specific embodiment, the lipid nanoparticle includes compound 8, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 8, the DSPC, the cholesterol, and the DMG-PEG2000 is (51-60):(5-15):(30-50):(1-3).

[0308] In a specific embodiment, the lipid nanoparticle includes compound 8, DSPC, cholesterol, and DMG-PEG2000, wherein a molar ratio of the compound 8, the DSPC, the cholesterol, and the DMG-PEG2000 is (51-60):(10-15):(35-45):(1.5-2.5).

[0309] In a specific embodiment, in the lipid nanoparticle, the lipid nanoparticle composition includes compound 8, DSPC, cholesterol, and DMG-PEG2000, wherein a content of the compound 8 is 35 mol% to 65 mol%, a content of a sum of the DSPC and the cholesterol is 35 mol% to 65 mol%, and a content of the DMG-PEG2000 is 0.5 mol% to 5 mol%.

[0310] In a specific embodiment, in the lipid nanoparticle, the lipid nanoparticle includes compound 8, DSPC, cholesterol, and DMG-PEG2000, wherein in the lipid nanoparticle, a content of the compound 8 is 40 mol% to 50 mol%, a content of the DSPC is 10 mol% to 15 mol%, a content of the cholesterol is 35 mol% to 45 mol%, and a content of the DMG-PEG2000 is 1.5 mol% to 2.5 mol%.

[0311] In a specific embodiment, the lipid nanoparticle includes compound 8, DSPC, cholesterol, and DMG-PEG2000, wherein in the lipid nanoparticle, a content of the compound 8 is 48 mol% to 49 mol%, a content of the DSPC is 10 mol% to 15 mol%, a content of the cholesterol is 35 mol% to 45 mol%, and a content of the DMG-PEG2000 is 1.5 mol% to 2.5 mol%.

[0312] In a specific embodiment, in the lipid nanoparticle, the lipid nanoparticle composition includes compound 13, DSPC, cholesterol, and DMG-PEG2000, wherein a content of the compound 13 is 35 mol% to 65 mol%, a content of a sum of the DSPC and the cholesterol is 35 mol% to 65 mol%, and a content of the DMG-PEG2000 is 0.5 mol% to 5 mol%.

[0313] In a specific embodiment, in the lipid nanoparticle, the lipid nanoparticle includes compound 13, DSPC, cholesterol, and DMG-PEG2000, wherein in the lipid nanoparticle, a content of the compound 13 is 40 mol% to 50 mol%, a content of the DSPC is 10 mol% to 15 mol%, a content of the cholesterol is 35 mol% to 45 mol%, and a content of

the DMG-PEG2000 is 1.5 mol% to 2.5 mol%.

[0314] In a specific embodiment, the lipid nanoparticle includes compound 13, DSPC, cholesterol, and DMG-PEG2000, wherein in the lipid nanoparticle, a content of the compound 13 is 48 mol% to 49 mol%, a content of the DSPC is 10 mol% to 15 mol%, a content of the cholesterol is 35 mol% to 45 mol%, and a content of the DMG-PEG2000 is 1.5 mol% to 2.5 mol%.

[0315] Except for those described in the foregoing sections, the lipid nanoparticle or lipid nanoparticle composition of the present application may further include one or more components. For example, the lipid nanoparticle or lipid nanoparticle composition may include one or more small hydrophobic molecules, such as vitamins (for example, vitamin A or vitamin E) or sterols.

[0316] The lipid nanoparticle or lipid nanoparticle composition may further include one or more permeability enhancer molecules, carbohydrates, polymers, surface modifiers, or other components. The permeability enhancer molecules may be molecules described in U.S. Patent Application 2005/0222064. The carbohydrates may include monosaccharides (for example, glucose) and polysaccharides (for example, glycogen and derivatives and analogs thereof).

[0317] The lipid nanoparticle or lipid nanoparticle composition may further include one or more polymers, wherein the polymers may be biodegradable and/or biocompatible. The polymers may be selected from, but are not limited to, polyamine, polyether, polyamide, polyester, polycarbamate, polyurea, polycarbonate, polystyrene, polyimide, polysulfone, polyurethane, polyacetylene, polyethylene, polyethyleneimine, polyisocyanate, polyacrylate, polymethacrylate, polyacrylonitrile, and polyarylester. For example, the polymers may include polycaprolactone (PCL), an ethylene-vinyl acetate copolymer (EVA), polylactic acid (PLA), poly-L-lactic acid (PLLA), polyglycolic acid (PGA), poly(lactic-co-glycolic acid) (PLGA), poly(L-lactic-co-glycolic acid) (PLLGA), poly(D,L-lactide) (PDLA), poly(L-lactide) (PLLA), poly(D,L-lactide-co-caprolactone), poly(D,L-lactide-caprolactone-glycolide), poly(D,L-lactide-PEO-co-D,L-lactide), poly(D,L-lactide-co-PPO-co-D,L-lactide), poly(alkyl cyanoacrylate), polyurethane, poly-L-lysine (PLL), hydroxypropyl methacrylate (HPMA), polyethylene glycol, poly-L-glutamic acid, polyhydroxy acid, polyanhydride, polyorthoester,

poly(esteramide), polyamide, poly(esterether), polycarbonate, and polyalkylene such as polyethylene and polypropylene; polyalkylene glycol such as poly(ethylene glycol) (PEG); poly(ethylene oxide) (PEO); polyalkylene terephthalate such as polyethylene terephthalate; polyvinyl alcohol (PVA); polyvinyl ether, polyvinyl ester such as poly(vinyl acetate), polyvinyl halide such as polyvinyl chloride (PVC), polyvinylpyrrolidone (PVP), polysiloxane, polystyrene (PS), polyurethane, derivatized cellulose such as alkyl cellulose, hydroxyalkyl cellulose, cellulose ether, cellulose ester, nitrocellulose, hydroxypropyl cellulose, carboxymethyl cellulose, acrylic polymers such as poly(methyl(meth)acrylate) (PMMA), poly(methyl) ethylacrylate, poly(methyl) butylacrylate, poly(methyl) isobutylacrylate, poly(methyl) hexylacrylate, poly(methyl) isodecylacrylate, poly(methyl) laurylacrylate, poly(methyl) phenylacrylate, poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate), as well as copolymers and mixtures thereof, polydioxanone and copolymers thereof, polyhydroxyalkanoate, poly(propylene fumarate), polyformaldehyde, poloxamer, polyethylenediamine, poly(orthoester), poly(butyrate), poly(valerate), poly(lactide-caprolactone), trimethylene carbonate, poly(N-acryloylmorpholine) (PAcM), poly(2-methyl-2-oxazoline) (PMOX), poly(2-ethyl-2-oxazoline) (PEOZ), and polyglycerol.

[0318] The lipid nanoparticle or lipid nanoparticle composition may further include one or more surface modifiers. The surface modifiers may include, but are not limited to, anionic proteins (for example, bovine serum albumin), surfactants (for example, cationic surfactants such as dimethyldioctadecylammonium bromide), sugars or sugar derivatives (for example, cyclodextrin), nucleic acids, polymers (for example, heparin, polyethylene glycol, and poloxamer), mucolytics (for example, acetylcysteine, *Artemisia argyi*, bromelain, papain, *Cynanchum amplexicaule*, caprolactone bromide, carbocisteine, eprazinone, mesna, ambroxol, sobrerol, domiodol, letosteine, stepronin, tiopronin, gelsolin, thymosin β4, dornase alfa, neltenexine, and erdosteine), and DNase (for example, rhDNase). The surface modifier may be disposed within the nanoparticle and/or on a surface of the nanoparticle composition (for example, by coating, adsorption, covalent bonding, or other methods).

[0319] In addition to the above components, the lipid nanoparticle or lipid nanoparticle composition may include any substance useful in a pharmaceutical composition.

For example, the nanoparticle composition may include one or more pharmaceutically acceptable excipients or auxiliary ingredients, such as but not limited to one or more solvents, dispersion media, diluents, dispersing aids, suspending aids, granulation aids, disintegrants, fillers, flow aids, liquid carriers, binders, surfactants, isotonic agents, thickeners or emulsifiers, buffers, lubricants, oils, preservatives, and other substances. Excipients may also be included, such as wax, butter, colorants, coatings, flavoring agents, and fragrances. Pharmaceutically acceptable excipients are well known in the art.

[0320] Examples of the buffers include, but are not limited to, a citrate buffer solution, an acetate buffer solution, a phosphate buffer solution, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium gluconate, d-gluconic acid, calcium glycerophosphate, calcium lactate, calcium lactobionate, propionic acid, calcium levulinate, valeric acid, dicalcium hydrogen phosphate, phosphoric acid, tricalcium phosphate, calcium hydrogen phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium phosphate mixtures, tromethamine, an aminosulfonate buffer, magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethanol, and/or combinations thereof. The lubricants may be selected from magnesium stearate, calcium stearate, stearic acid, silica, talc powder, malt, hydrogenated vegetable oil, polyethylene glycol, sodium benzoate, sodium acetate, sodium chloride, leucine, magnesium laurate, sulfate, sodium dodecyl sulfate, and combinations thereof.

[0321] The present application further provides a lipid nanoparticle, which includes any one of the above lipid nanoparticle compositions and a therapeutic agent and/or a prophylactic agent, wherein the therapeutic agent and/or the prophylactic agent is selected from a vaccine or a compound, nucleic acid, or protein capable of eliciting an immune response.

[0322] The therapeutic agent and/or the prophylactic agent includes a bioactive substance and may alternatively be referred to as an "active agent". The therapeutic agent and/or the prophylactic agent may be a substance that, once delivered to a cell or organ,

produces a desired change in the cell, organ, or other body tissues or systems. Such a substance can be used for treating one or more diseases, disorders, or conditions. In some embodiments, the therapeutic agent and/or the prophylactic agent is a small molecule drug that can be used for treating a specific disease, disorder, or condition. Examples of drugs suitable for use in the nanoparticle composition include, but are not limited to, antitumor agents (for example, vincristine, doxorubicin, mitoxantrone, camptothecin, cisplatin, bleomycin, cyclophosphamide, methotrexate, and streptozotocin), antitumor drugs (for example, actinomycin D, and vincristine), vincaleukoblastinum, cystine arabinoside, anthracycline drugs, alkylating agents, platinum compounds, antimetabolites and nucleoside analogs (for example, methotrexate, purine, and pyrimidine analogs), anti-infective agents, local anesthetics (for example, dibucaine and chlorpromazine), β-adrenergic blockers (for example, propranolol, timolol, and labetalol), antihypertensive drugs (for example, clonidine and hydralazine), antidepressants (for example, imipramine, amitriptyline, and doxepin), anticonvulsants (for example, phenytoin), antihistamines (for example, diphenhydramine, chlorpheniramine, and promethazine), antibiotics/antibacterial agents (for example, gentamicin, ciprofloxacin, and cefoxitin), antifungal agents (for example, miconazole, terconazole, econazole, isoconazole, butoconazole, clotrimazole, itraconazole, nystatin, and naftifine), antiparasitic agents, hormones, hormone antagonists, immunomodulators, neurotransmitter antagonists, antiglaucoma agents, vitamins, anesthetics, and imaging agents.

[0323] In some embodiments, the therapeutic agent and/or the prophylactic agent is a protein. Therapeutic proteins suitable for use in the nanoparticle composition of the present application include, but are not limited to, gentamicin, amikacin, insulin, erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), Factor VIII, luteinizing hormone-releasing hormone (LHRH) analogs, interferons, heparin, hepatitis B surface antigen, typhoid vaccine, and cholera vaccine.

[0324] In some embodiments, the therapeutic agent is a polynucleotide or a nucleic acid (for example, ribonucleic acid or deoxyribonucleic acid). The term "polynucleotide", in its broadest sense, includes any compound and/or substance that is or may be incorporated into an oligonucleotide chain. Exemplary polynucleotides as used in the present application

include, but are not limited to, one or more of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) including messenger RNA (mRNA), hybrids thereof, RNAi inducers, RNAi agents, siRNA, shRNA, miRNA, antisense RNA, ribozymes, catalytic DNA, triple helix-forming RNA inducers, aptamers, vectors, and the like. In some embodiments, the therapeutic agent and/or the prophylactic agent is RNA. RNA suitable for use in the composition and method described herein may be selected from, but is not limited to, telomers, antigametocytes, antisenses, ribozymes, small interfering RNA (siRNA), asymmetric interfering RNA (aiRNA), microRNA (miRNA), double-stranded RNA (dsRNA), short hairpin RNA (shRNA), transfer RNA (tRNA), messenger RNA (mRNA), and mixtures thereof. In certain embodiments, the RNA is mRNA.

[0325] In certain embodiments, the therapeutic agent and/or the prophylactic agent is mRNA. The mRNA may encode any target polypeptide, including any naturally occurring, non-naturally occurring, or otherwise modified polypeptide. The polypeptide encoded by the mRNA may be of any size and may have any secondary structure or activity. In some embodiments, the polypeptide encoded by the mRNA may have a therapeutic effect when expressed in a cell.

[0326] In some embodiments, part or all of uridine in the mRNA molecule is 1-methyl-pseudouridine. In some embodiments, the mRNA further includes a 5’ cap structure. In some embodiments, the 5’ cap structure is type O, type I, or type II. In some embodiments, the 5’ cap structure is m7G(5’)ppp(5’)(2’-OMeA)pG. In some embodiments, the mRNA further includes a polyA tail. In some embodiments, the polyA tail sequence includes at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, or at least 100 adenosines. In some embodiments, the polyA tail sequence includes at most 500, at most 400, at most 300, at most 200, at most 150, at most 140, at most 130, at most 120, at most 110, at most 100, at most 90, at most 80, at most 70, or at most 60 adenosines (A), particularly about 120 A.

[0327] In a specific embodiment, a coding sequence of the mRNA of the present invention is as shown in SEQ ID NO: 12 or is a sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 12.

[0328] In a specific embodiment, a coding sequence of the mRNA of the present

invention includes a sequence as shown in SEQ ID NO: 10 or includes a sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 10.

[0329] In a specific embodiment, a coding sequence of the mRNA of the present invention is the sequence as shown in SEQ ID NO: 10 or is a sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 10.

[0330] In a specific embodiment, a coding sequence of the mRNA of the present invention includes a sequence as shown in SEQ ID NO: 11 or includes a sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 11.

[0331] In a specific embodiment, a coding sequence of the mRNA of the present invention is the sequence as shown in SEQ ID NO:11 or is a sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 11.

[0332] In a specific embodiment, the mRNA of the present invention encodes a protein including a sequence as shown in SEQ ID NO: 3 or encodes a protein including a sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 3.

[0333] In a specific embodiment, the mRNA of the present invention encodes a protein having the sequence as shown in SEQ ID NO: 3 or encodes a protein having a sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 3.

[0334] In a specific embodiment, the mRNA of the present invention encodes a protein including a sequence as shown in SEQ ID NO: 4 or encodes a protein including a sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 4.

[0335] In a specific embodiment, the mRNA of the present invention encodes a protein having the sequence as shown in SEQ ID NO: 4 or encodes a protein having a sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to SEQ ID NO: 4.

[0336] The present invention further provides an mRNA vaccine for treating and/or preventing an HPV virus infection-related disease, which includes mRNA encoding an HPV antigen, wherein the mRNA is encapsulated in the lipid nanoparticle (LNP) of the present

invention.

[0337] In a specific embodiment, a sequence of the encoded HPV antigen includes or is the sequence as shown in SEQ ID NO: 3 or SEQ ID NO: 4.

[0338] In a specific embodiment, all of uridine in the mRNA molecule is 1-methyl-pseudouridine.

[0339] In a specific embodiment, the mRNA includes a 5' cap structure, wherein the 5' cap structure is m7G(5')ppp(5')(2'-OMeA)pG.

[0340] The sequence of the mRNA molecule sequentially includes, from a 5' end to a 3' end, a 5' cap, 5'UTR, ORF, 3'UTR, and a polyA tail, wherein the 5' cap, the 5'UTR, the ORF, the 3'UTR, and the polyA tail are operably linked to each other.

[0341] In a specific embodiment, an ORF sequence of the mRNA encoding the HPV antigen is as shown in SEQ ID NO: 10 or SEQ ID NO: 11.

[0342] In a specific embodiment, a sequence of the encoded HPV antigen is as shown in SEQ ID NO: 3 or SEQ ID NO: 4.

[0343] In a specific embodiment, a sequence of the 5'UTR is as shown in SEQ ID NO: 5.

[0344] In a specific embodiment, a sequence of the 3'UTR is as shown in SEQ ID NO: 6.

[0345] In a specific embodiment, a sequence of the polyA tail is as shown in SEQ ID NO: 7.

[0346] In a specific embodiment, a full-length sequence of the mRNA encoding the HPV antigen is as shown in SEQ ID NO: 12.

[0347] In a specific embodiment, the lipid nanoparticle includes compound 5A, DSPC, cholesterol, and DMG-PEG2000, wherein in the lipid nanoparticle, a content of the compound 5A is 48 mol% to 49 mol%, a content of the DSPC is 10 mol% to 15 mol%, a content of the cholesterol is 35 mol% to 45 mol%, and a content of the DMG-PEG2000 is 1.5 mol% to 2.5 mol%.

[0348] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid 5A, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 48.5 mol%, a content of the phospholipid is 5 mol%, a content of the

cholesterol is 45 mol%, and a content of the PEG lipid is 1.5 mol%.

[0349] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid 5A, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 40 mol%, a content of the phospholipid is 15 mol%, a content of the cholesterol is 43.5 mol%, and a content of the PEG lipid is 1.5 mol%.

[0350] In a specific embodiment, the lipid nanoparticle includes an ionizable lipid 5A, a phospholipid, cholesterol, and a PEG lipid, wherein in the lipid nanoparticle, a content of the ionizable lipid is 48 mol%, a content of the phospholipid is 10.3 mol%, a content of the cholesterol is 39.6 mol%, and a content of the PEG lipid is 2.1 mol%.

[0351] In other embodiments, the therapeutic agent and/or the prophylactic agent is siRNA. The siRNA may selectively knock down or downregulate the expression of a target gene. For example, the siRNA may be selected to silence a gene associated with a specific disease, disorder, or condition when the nanoparticle composition including the siRNA is administered to a subject in need thereof. The siRNA may include a sequence complementary to an mRNA sequence encoding a gene or protein of interest. In some embodiments, the siRNA may be immunomodulatory siRNA.

[0352] In some embodiments, the therapeutic agent and/or the prophylactic agent is shRNA or a vector or plasmid encoding the shRNA. After delivery of a suitable construct to a cell nucleus, the shRNA may be produced within a target cell. Constructs and mechanisms related to the shRNA are well known in the relevant art.

[0353] The amount of the therapeutic agent and/or the prophylactic agent in the nanoparticle may depend on the size, composition, intended target and/or application, or other properties of the nanoparticle, as well as properties of the therapeutic agent and/or the prophylactic agent. For example, the amount of RNA suitable for use in the nanoparticle may depend on the size, sequence, and other features of the RNA.

[0354] The lipid nanoparticle or lipid nanoparticle composition of the present application or the pharmaceutical composition of the present application may further include a divalent metal cation. Specifically, the divalent metal cation may be Ca^{2+}, Mg^{2+}, Zn^{2+}, or Mn^{2+}.

[0355] In some embodiments of the present application, a molar ratio of the divalent

metal cation to the total lipids in the lipid nanoparticle composition, the lipid nanoparticle, or the pharmaceutical composition is (0.1-400):100 and may be (1-30):100, (10-50):100, (30-60):100, (30-35):100, (100-200):100, (150-180):100, (300-320):100, or (300-400):100.

[0356] In some embodiments of the present application, a divalent metal cation solution of a certain concentration is formulated with an acidic buffer, and then mRNA is diluted with the divalent metal cation solution to obtain an mRNA working solution, wherein a final concentration of mRNA in the mRNA working solution is 100-300 μg/ml, preferably 200 μg/ml, and a final concentration of the divalent metal cation solution is 0.8-20 mM. The mRNA working solution is mixed with a hybrid total lipid-ethanol phase in a volume ratio of (1-5):1, preferably 2.5:1 to 4:1, to prepare LNP, wherein a mixing is preferably performed by a T-shaped flow mixing device. A sample is subjected to dilution, concentration, and replacement after the mixing to remove the ethanol, then the sample is adjusted to a constant volume, and a pH value is adjusted to 7-8.

[0357] Preferably, the concentration of the divalent metal cation solution in the mRNA working solution may be 0.8 mM, 1.6 mM, 3.2 mM, 6.4 mM, 8 mM, 12.8 mM, 16 mM, or 20 mM.

[0358] Preferably, the acidic buffer is an acetate buffer.

[0359] In some embodiments of the present application, a pH of the acidic buffer is 5.0-6.0, preferably 5.0-5.5.

[0360] In some embodiments of the present application, a pH of the mRNA working solution is 5.0-6.0, preferably 5.0-5.5.

[0361] In some embodiments of the present application, a divalent metal cation solution of a concentration of 0.8-20 mM is formulated with an acidic buffer, and then mRNA is diluted with the divalent metal cation solution to obtain an mRNA aqueous phase, wherein a final concentration of the mRNA aqueous phase is 100-300 μg/ml, preferably 200 μg/ml. The mRNA aqueous phase is mixed with a hybrid total lipid-ethanol phase in a volume ratio of (1-5):1, preferably 2.5:1 to 4:1, wherein a mixing is preferably performed by a T-shaped flow mixing device. A sample is subjected to dilution, concentration, and replacement after the mixing to remove the ethanol, then the sample is adjusted to a constant volume, and a pH value is adjusted to 7-8.

[0362] A concentration of the divalent metal cation solution formulated with the acidic buffer may be 0.8 mM, 1.6 mM, 3.2 mM, 6.4 mM, 8 mM, 12.8 mM, 16 mM, or 20 mM.

[0363] Preferably, the acidic buffer is an acetate buffer.

[0364] In some embodiments of the present application, a pH of the acidic buffer is 5.0-6.0, preferably 5.0-5.5.

[0365] In some embodiments of the present application, a pH of the mRNA aqueous phase is 5.0-6.0, preferably 5.0-5.5.

[0366] In a specific embodiment, the therapeutic agent and/or the prophylactic agent is mRNA, wherein an N/P ratio of the ionizable lipid to the mRNA in the lipid nanoparticle is 3.5-5.5 and may be, for example, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, or 5.5.

[0367] The encapsulation efficiency of the therapeutic agent and/or the prophylactic agent describes an amount of the therapeutic agent and/or the prophylactic agent that is encapsulated or otherwise associated with the nanoparticle composition in another manner after preparation, relative to an initial amount provided. A higher encapsulation efficiency is expected to be better (for example, approaching 100%). The encapsulation efficiency may be measured, for example, by comparing the amounts of the therapeutic agent and/or the prophylactic agent in a solution containing the nanoparticle composition before and after the nanoparticle composition is disrupted with one or more organic solvents or detergents. Fluorescence can be used for measuring the amount of a free therapeutic agent and/or a prophylactic agent (for example, RNA) in the solution. For the nanoparticle composition described herein, the encapsulation efficiency of the therapeutic agent and/or the prophylactic agent may be at least 50%, for example, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%. In some embodiments, the encapsulation efficiency may be at least 80%. In certain embodiments, the encapsulation efficiency may be at least 90%.

[0368] The lipid nanoparticle may be characterized by various methods. For example, a microscope (for example, a transmission electron microscope or a scanning electron microscope) may be used for examining the morphology and size distribution of the

nanoparticle. Dynamic light scattering or potentiometry (for example, a potentiometric titration method) may be used for measuring a ζ potential. The dynamic light scattering may also be used for determining a particle size.

[0369] In a specific embodiment, the lipid nanoparticle has an average particle size of 50 nm to 150 nm.

[0370] In a specific embodiment, the lipid nanoparticle has an average particle size of 50 nm to 100 nm.

[0371] In a specific embodiment, the lipid nanoparticle has an average particle size of 50 nm to 80 nm.

[0372] In a specific embodiment, the lipid nanoparticle has an average particle size of 50 nm to 60 nm.

[0373] The lipid nanoparticle may be relatively uniform. The polydispersity index may be used for indicating the uniformity of the nanoparticle. A small (for example, less than 0.3) polydispersity index generally indicates a narrow particle size distribution.

[0374] In a specific embodiment, the lipid nanoparticle has a polydispersity index of 0.001-0.16. For example, the polydispersity index may be 0.001, 0.005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.11, 0.12, 0.13, 0.14, 0.15, or 0.16.

[0375] The present application further provides a pharmaceutical composition, which includes one or more pharmaceutically acceptable excipients or auxiliary ingredients, such as those described herein, except that any conventional excipient or auxiliary ingredient may be incompatible with one or more components of the nanoparticle. If the excipient or auxiliary ingredient is incompatible with a component of the nanoparticle, a combination of the excipient or auxiliary ingredient with the component may result in any undesired biological effect or other harmful effects.

[0376] According to one or more lipid nanoparticles in the pharmaceutical composition of the present application, the relative amounts of one or more pharmaceutically acceptable excipients and/or auxiliary ingredients will vary depending on an identity, a size, and/or disease conditions of a subject to be treated, and further depending on a route of administration of the composition. For example, the pharmaceutical composition may include 0.1% to 100% (wt/wt) of one or more lipid nanoparticles.

[0377] The lipid nanoparticle and/or the pharmaceutical composition may be administered to any patient or subject, including those patients or subjects who may benefit from a therapeutic effect provided by delivering the therapeutic agent and/or the prophylactic agent to one or more specific cells, tissues, organs, or their systems or tissues (for example, the renal system). Although the description of the lipid nanoparticle and the pharmaceutical composition provided herein is primarily directed to administration to humans, those skilled in the art will understand that such the lipid nanoparticle and the pharmaceutical composition are generally suitable for administration to any other mammals. To make the lipid nanoparticle and the pharmaceutical composition suitable for administration to various animals, it is well known to modify the lipid nanoparticle and the pharmaceutical composition suitable for administration to humans, and ordinarily skilled veterinary pharmacologists can make such modifications with only routine (if any) experimental design and/or procedures, including but not limited to humans, other primates, and other artificial animals, including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, dogs, mice, and/or rats.

[0378] The pharmaceutical composition of the present application may be prepared by any method known or hereafter developed in the field of pharmacology. Generally, such a preparation method includes combining an active ingredient with an excipient and/or one or more other auxiliary ingredients, and then, if desired or necessary, dividing, shaping, and/or packaging a product in a desired single-dose or multi-dose unit.

[0379] The pharmaceutical composition of the present application may be prepared, packaged, and/or sold in a single unit dose and/or multiple single unit doses in batches. As used herein, a "unit dose" refers to an exact amount of the pharmaceutical composition containing a predetermined amount of an active ingredient (for example, the nanoparticle composition). The amount of the active ingredient is generally equal to a dose of the active ingredient administered to a subject and/or a convenient fraction of the dose, for example, one-half or one-third of the dose.

[0380] The pharmaceutical composition may be prepared in various forms suitable for various routes and methods of administration. For example, the pharmaceutical composition may be prepared into a liquid, a powder, and a granule as a liquid dosage form (for example,

an emulsion, a microemulsion, a nanoemulsion, a solution, a suspension, a syrup, and an agent), an injectable form, and a solid dosage form (for example, a capsule, a tablet, a troche, and the like), a topical and/or transdermal administration dosage form (for example, an ointment, a paste, a cream, a lotion, a gel, a powder, a solution, a spray, an inhaler, and a patch), a suspension, a powder, and other forms. Liquid dosage forms for oral and parenteral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, nanoemulsions, solutions, suspensions, syrups, and/or agents.

[0381] In addition to the active ingredient, a liquid dosage form may include an inert diluent commonly used in the art, for example, water or other solvents, solubilizers, and emulsifiers, such as ethanol, isopropanol, ethylene carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butanediol, dimethylformamide, oils (especially cottonseed oil, peanut oil, corn oil, germ oil, olive oil, castor oil, and sesame oil), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycol, and sorbitan fatty acid esters and mixtures thereof. In addition to the inert diluent, an oral composition may further include other therapeutic agents and/or prophylactic agents, other reagents such as wetting agents, emulsifiers, suspending agents, sweeteners, flavoring agents, and/or fragrances. In certain embodiments for parenteral administration, the composition is mixed with a solubilizer (for example, an alcohol, an oil, a modified oil, a diol, polysorbate, cyclodextrin, a polymer, and/or a combination thereof).

[0382] An injectable preparation may be formulated using a suitable dispersant, a wetting agent, and/or a suspending aid according to known techniques, for example, as a sterile injectable aqueous or oily suspension. A sterile injectable preparation may be a sterile injectable solution, suspension, and/or emulsion in a non-toxic and parenterally acceptable diluent and/or solvent, for example, in a solution of 1,3-butanediol. Acceptable media and solvents that can be used include water. Sterile non-volatile oils are commonly used as solvents or suspension media. Thus, any mild and non-volatile oil can be used, including synthetic monoglyceride or diglyceride. Fatty acids such as oleic acid can be used in preparation of injections.

[0383] Injectable preparations may be sterilized, for example, by filtration through a bacterial-retentive filter and/or by incorporating a sterilizing agent in the form of a sterile

solid composition, wherein the sterile solid composition may be used before being dissolved or dispersed in sterile water or other sterile injectable media.

[0384] To prolong the effect of the active ingredient, it is generally expected to slow the absorption of the active ingredient from subcutaneous or intramuscular injection. This purpose can be achieved by using a liquid suspension of a crystal or an amorphous substance having poor water solubility. Then, an absorption rate of a drug depends on its dissolution rate, and the dissolution rate depends on a size and crystalline form of the crystal. Alternatively, delayed absorption of a drug form for parenteral administration may be achieved by dissolving or suspending a drug in an oil carrier. An injectable drug form is prepared by forming a microencapsulated matrix of a drug in a biodegradable polymer (for example, polylactide-polyethylene glycol). A drug release rate may be controlled according to a ratio of a drug to a polymer and properties of the specific polymer used. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Injectable stock preparations are prepared by embedding drugs in liposomes or microemulsions compatible with body tissues.

[0385] Compositions for rectal or vaginal administration are usually suppositories, which may be prepared by mixing the compositions with suitable non-irritating excipients such as cocoa butter, polyethylene glycol, or suppository wax, wherein the excipients are solids at ambient temperature but liquid at body temperature and are thus melted in the rectal or vaginal cavity to release the active ingredient.

[0386] Solid dosage forms for oral administration include capsules, tablets, pills, films, powders, and granules. In such solid dosage forms, the active ingredient is mixed with at least one pharmaceutically acceptable inert excipient such as sodium citrate or dicalcium phosphate and/or a filler or an extender (for example, starch, lactose, sucrose, glucose, mannitol, and silicic acid), a binder (for example, carboxymethyl cellulose, alginate, gelatin, polyvinylpyrrolidone, sucrose, and arabic gum), a moisturizer (for example, glycerol), a disintegrant (for example, agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicate, and sodium carbonate), a slow-release agent (for example, paraffin), an absorption enhancer (for example, a quaternary ammonium compound), a wetting agent (for example, cetyl alcohol and glyceryl monostearate), an absorbent (for example, kaolin,

bentonite, and silicate) and a lubricant (for example, talc powder, calcium stearate, magnesium stearate, solid polyethylene glycol, and sodium lauryl sulfate), and mixtures thereof. For capsules, tablets, and pills, the dosage forms may include buffers.

[0387] Similar types of solid compositions may be used as fillers in soft and hard-filled gelatin capsules using excipients such as lactose or lactose and high-molecular-weight polyethylene glycol. Solid dosage forms such as tablets, sugar-coated pills, capsules, pills, and granules may be prepared with coatings and shells such as enteric coatings and other coatings well known in the field of pharmaceutical formulation. They may optionally include opacifiers and may have compositions that release the active ingredient only, or preferably, in a part of the intestine, preferably, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. Similar types of solid compositions may be used as fillers in soft and hard-filled gelatin capsules using excipients such as lactose and lactose as well as high-molecular-weight polyethylene glycol.

[0388] Dosage forms for topical and/or transdermal administration of a composition may include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalers, and/or patches. Generally, the active ingredient is combined under sterile conditions with a pharmaceutically acceptable excipient and/or any required preservative and/or buffer. Additionally, the use of transdermal patches is taken into consideration in the present application. The transdermal patches typically have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms may be prepared, for example, by dissolving and/or dispersing the compound in an appropriate medium. Alternatively or additionally, a rate may be controlled by any of the following: providing a rate-controlling membrane and/or dispersing the compound in a polymer matrix and/or gel.

[0389] Preparations suitable for topical administration include, but are not limited to, liquid and/or semi-liquid preparations, for example, liniments, lotions, and oil-in-water and/or water-in-oil emulsions, such as creams, ointments, and/or pastes, and/or solutions, and/or suspensions, despite that a concentration of the active ingredient may be as high as a solubility limit of the active ingredient in a solvent. Preparations for topical administration may further include one or more other ingredients as described herein.

[0390] The pharmaceutical composition may be prepared, packaged, and/or sold as a preparation suitable for pulmonary administration via the oral cavity. Such a preparation may contain a dry particle of the active ingredient. Such a composition is conveniently in a dry powder form, for administration using a device including a dry powder reservoir and/or using a self-propelling solvent/powder dispensing container (for example, a device containing the active ingredient dissolved and/or suspended in a low-boiling-point propellant in a sealed container), and the powder composition may include a solid and fine-powder diluent such as a sugar and is conveniently provided in a unit dose form.

[0391] The low-boiling-point propellant generally includes a liquid propellant having a boiling point below 65°F at atmospheric pressure. Typically, the propellant may account for 50% to 99.9% (wt/wt) of the composition, while the active ingredient may account for 0.1% to 20% (wt/wt) of the composition. The propellant may further include other ingredients, for example, a liquid nonionic and/or solid anionic surfactant and/or a solid diluent (whose particle size may be of the same order of magnitude as the particle containing the active ingredient).

[0392] The pharmaceutical composition formulated for pulmonary delivery can provide the active ingredient in the form of droplets of a solution and/or a suspension. Such preparations may be prepared, packaged, and/or sold as aqueous and/or dilute alcoholic solutions and/or suspensions, optionally sterile, containing the active ingredient, and may be conveniently administered using any nebulization and/or atomization device. Such preparations may further include one or more other ingredients, including but not limited to flavoring agents such as sodium saccharin, volatile oils, buffers, surfactants, and/or preservatives such as methyl hydroxybenzoate. The droplets provided by this route of administration may have an average diameter within a range of 1 nm to 200 nm.

[0393] The preparations suitable for pulmonary delivery described herein may be used for intranasal delivery of the pharmaceutical composition. Another preparation suitable for intranasal administration is a coarse powder containing the active ingredient and having an average particle size of 0.2 μm to 500 μm. Such a preparation is administered in the manner of taking snuff, that is, by rapid inhalation through nasal passages from a powder container held near the nose.

[0394] A preparation suitable for intranasal administration may, for example, include 0.1% (wt/wt) to 100% (wt/wt) of the active ingredient and may include one or more other active ingredients described herein. The pharmaceutical composition may be prepared, packaged, and/or sold in a preparation suitable for buccal administration. Such a preparation may, for example, be in the form of a tablet and/or a lozenge prepared using a conventional method, and may include, for example, 0.1% to 20% (wt/wt) of the active ingredient, the balance of an orally dissolvable and/or degradable composition, and optionally, one or more other ingredients as described herein. Alternatively, preparations suitable for oral administration may include powders and/or atomized and/or spray solutions and/or suspensions containing the active ingredient. Such powdered, aerosolized, and/or atomized preparations may have an average particle and/or droplet size within a range of 0.1 nm to 200 nm upon dispersion, and may further include one or more other ingredients as described herein.

[0395] The pharmaceutical composition may be prepared, packaged, and/or sold in a preparation suitable for ophthalmic administration. Such a preparation may, for example, be in the form of a drop, including, for example, 0.1/1.0% (wt/wt) of a solution and/or a suspension of the active ingredient in an aqueous or oily liquid excipient. Such a drop may further include a buffer, a salt, and/or any one or more other additive ingredients as described herein. Other preparations suitable for ophthalmic administration include those containing the active ingredient in a microcrystalline form and/or in a liposomal preparation. Ear drops and/or eye drops are considered as methods for producing polypeptides in cells within the scope of the present disclosure.

[0396] The present application further provides a method for producing a target polypeptide in a mammalian cell. The method for producing a polypeptide includes allowing the cell to get in contact with a lipid nanoparticle including mRNA encoding the target polypeptide. After the cell is allowed to get in contact with the lipid nanoparticle, the mRNA can be absorbed and translated in the cell to produce the target polypeptide.

[0397] Typically, the step of allowing the mammalian cell to get in contact with the nanoparticle including mRNA encoding the target polypeptide can be performed *in vivo*, *ex vivo*, in a culture, or *in vitro*. The amount of the nanoparticle and/or the amount of the mRNA

therein that gets in contact with the cell may depend on a type of the cell or tissue being contacted, a route of administration, physicochemical properties of the nanoparticle composition and the mRNA (for example, size, charge, and chemical ingredients), as well as other factors. Typically, an effective amount of the nanoparticle allows for efficient production of the polypeptide in the cell. Efficiency measurement standards may include polypeptide translation (as indicated by polypeptide expression), an mRNA degradation level, and immune response indicators.

[0398] The step of allowing the nanoparticle including mRNA to get in contact with the cell may involve or result in transfection. A phospholipid included in a lipid component of the nanoparticle may, for example, facilitate transfection and/or improve transfection efficiency through interaction and/or fusion with the cell or an intracellular membrane. The transfection may allow for intracellular translation of the mRNA.

[0399] In certain embodiments, the mRNA included in the nanoparticle may encode a recombinant polypeptide, wherein the recombinant polypeptide may substitute for one or more polypeptides that may be substantially absent in the cell in contact with the nanoparticle. One or more polypeptides that are substantially absent may be lacking due to genetic mutations in a coding gene or its regulatory pathway. Alternatively, the recombinant polypeptide produced by the translation of the mRNA may antagonize the activity of an endogenous protein present in the cell, on a surface of the cell, or secreted from the cell. The antagonistic recombinant polypeptide may be desirable to counteract deleterious effects caused by the activity of the endogenous protein, such as localization caused by activity alteration or mutation. In another alternative scheme, the recombinant polypeptide produced by the translation of the mRNA may indirectly or directly antagonize the activity of a biological moiety present in the cell, on a surface of the cell, or secreted from the cell. The antagonized biological moiety may include, but is not limited to, a lipid (for example, cholesterol), a lipoprotein (for example, a low-density lipoprotein), a nucleic acid, a carbohydrate, and a small molecule toxin. The recombinant polypeptide produced by the translation of the mRNA may be engineered to be localized within the cell, for example, to a specific compartment such as a nucleus, or may be engineered to be secreted from the cell or transported to a plasma membrane of the cell.

[0400] In some embodiments, allowing the cell to get in contact with the nanoparticle including the mRNA may reduce an innate immune response of the cell to an exogenous nucleic acid. The cell may be allowed to get in contact with a first nanoparticle composition including a first amount of first exogenous mRNA, wherein the first exogenous mRNA includes a translatable region, and the level of an innate immune response of the cell to the first exogenous mRNA may be determined. Subsequently, the cell may be allowed to get in contact with a second composition including a second amount of the first exogenous mRNA, wherein the second amount is a lesser amount of the first exogenous mRNA compared with the first amount. Alternatively, the second composition may include a first amount of second exogenous mRNA different from the first exogenous mRNA. The step of allowing the cell to get in contact with the first and second compositions may be repeated one or more times. In addition, the production efficiency of the polypeptide (for example, by translation) in the cell may optionally be determined, and the cell may be repeatedly allowed to get in contact again with the first and/or second composition until a desired protein production efficiency is achieved.

[0401] The present application further provides a method for treating a disease or disorder in a mammal. The method includes administering to the mammal a therapeutically effective amount of any one lipid nanoparticle or pharmaceutical composition of the present application. In particular, the lipid nanoparticle or pharmaceutical composition can be used for treating a disease, disorder, or condition characterized by loss or abnormality of protein or polypeptide activity. For example, the lipid nanoparticle or pharmaceutical composition containing mRNA encoding a missing or abnormal polypeptide may be administered or delivered to a cell. Subsequent translation of the mRNA may produce a polypeptide, thereby reducing or eliminating problems caused by abnormal activity resulting from the missing polypeptide or the polypeptide. Because translation may occur rapidly, the method and composition may be used for treating an acute disease, disorder, or condition such as sepsis, stroke, and myocardial infarction. The therapeutic agent and/or the prophylactic agent included in the nanoparticle composition may also be capable of altering the transcription rate of a given species, thereby affecting gene expression.

[0402] Diseases, disorders, and/or conditions characterized by dysfunctional or

abnormal protein or polypeptide activity include, but are not limited to, rare diseases, infectious diseases (for example, vaccines and therapeutic agents), cancers and proliferative diseases, genetic diseases (for example, cystic fibrosis), autoimmune diseases, diabetes mellitus, neurodegenerative diseases, cardiovascular and renovascular diseases, and metabolic diseases.

[0403] Those skilled in the art will understand that a specific dosage for a particular subject may vary depending on the species, age, and general situations of the subject; administration purposes; specific ingredients; modes of administration; and the like. The lipid nanoparticle or pharmaceutical composition of the present application may be formulated in a unit dose form to facilitate administration and dose uniformity. However, it should be understood that the total daily dose of the lipid nanoparticle or pharmaceutical composition of the present application will be determined by the attending physician within the scope of reasonable medical judgment. A specific therapeutically effective, prophylactically effective, or other appropriate dose level (for example, for imaging) for any particular patient depends on a variety of factors, including the severity and identification (if any) of a disease being treated; one or more therapeutic agents and/or prophylactic agents used; specific ingredients used; the age, body weight, general health state, sex, and diet of the patient; an administration timing, a route of administration, and a rate of excretion of a particular pharmaceutical ingredient used; a duration of treatment; drugs used in combination with or simultaneously with the particular pharmaceutical ingredient used; and factors well known in the medical community.

[0404] The present application further provides a method for specifically delivering a therapeutic agent and/or a prophylactic agent to a mammalian organ. The method includes administering to a mammal any one lipid nanoparticle or pharmaceutical composition of the present application, wherein the administering includes allowing the mammalian organ to get in contact with the lipid nanoparticle or pharmaceutical composition, thereby delivering the therapeutic agent and/or the prophylactic agent to the organ. The therapeutic agent and/or the prophylactic agent, for example, a protein, a cytotoxic agent, a radioactive ion, a chemotherapeutic agent, or a nucleic acid (for example, RNA, such as mRNA), may be delivered to a cell or organ. In the case that the therapeutic agent and/or the prophylactic

agent is mRNA, when the cell gets in contact with the lipid nanoparticle or pharmaceutical composition, the translatable mRNA may be translated in the cell to produce a target polypeptide. However, essentially non-translatable mRNAs may also be delivered to the cell. The essentially non-translatable mRNAs may be used as vaccines and/or translational ingredients capable of isolating the cell to reduce the expression of other species in the cell.

[0405] In some embodiments, the lipid nanoparticle may target a specific type or class of cells (for example, cells of a particular organ or system). For example, the lipid nanoparticle containing a target therapeutic agent and/or a prophylactic agent may be specifically delivered to the liver, kidney, spleen, femur, or lung of a mammal. Specific delivery to a particular class of cells, organs, or its system or group means that, relative to other destinations, the lipid nanoparticle including a higher proportion of the therapeutic agent and/or the prophylactic agent, for therapy and/or prophylaxis, is delivered to a target destination (for example, a tissue), for example, when the nanoparticle is administered to the mammal. In some embodiments, the target tissue is selected from a liver, a kidney, a lung, a spleen, a femur, an ocular tissue (for example, by intraocular, subretinal, or intravitreal injection), vascular endothelium in a blood vessel (for example, intracoronary or intrafemoral) or a kidney, and a tumor tissue (for example, by intratumoral injection).

[0406] The lipid nanoparticle or pharmaceutical composition of the present application may be administered by any route. In some embodiments, administration is performed by one or more of multiple routes including oral administration, intravenous administration, intramuscular administration, intra-arterial administration, intramedullary administration, intrathecal administration, subcutaneous administration, intraventricular administration, transdermal or intradermal administration, intradermic administration, rectal administration, intravaginal administration, intraperitoneal administration, intraocular administration, subretinal administration, intravitreal administration, topical administration (for example, by a powder, an ointment, a cream, a gel, a lotion, and/or a drop), mucosal administration, nasal administration, buccal administration, enteric administration, vitreous administration, intratumoral administration, sublingual administration, and intranasal administration; by intratracheal instillation, bronchial instillation, and/or inhalation; as an oral spray and/or a powder, or a nasal spray and/or an aerosol; and/or via portal vein catheter. In

some embodiments, the lipid nanoparticle or pharmaceutical composition may be administered intravenously, intramuscularly, intradermically, intra-arterially, intratumorally, subcutaneously, intraocularly, subretinally, or intravitreally, or via inhalation. However, in view of possible advances in drug delivery science, the present application encompasses delivery or description of the lipid nanoparticle or pharmaceutical composition of the present application by any suitable route. Typically, the most appropriate route of administration depends on various factors, including properties of the lipid nanoparticle composition, including one or more therapeutic and/or prophylactic measures (for example, their stability in various body environments (for example, blood and the gastrointestinal tract)), situations of a patient (for example, whether the patient can tolerate a particular route of administration), and the like.

[0407] In certain embodiments, the therapeutic agent and/or the prophylactic agent is administered to the mammal at a dose of 0.0001 mg/kg to 10 mg/kg. For example, the dose may be 0.0001 mg/kg to 10 mg/kg, 0.001 mg/kg to 10 mg/kg, 0.005 mg/kg to 10 mg/kg, 0.01 mg/kg to 10 mg/kg, 0.05 mg/kg to 10 mg/kg, 0.1 mg/kg to 10 mg/kg, 1 mg/kg to 10 mg/kg, 2 mg/kg to 10 mg/kg, 5 mg/kg to 10 mg/kg, 0.0001 mg/kg to 5 mg/kg, 0.001 mg/kg to 5 mg/kg, 0.005 mg/kg to 5 mg/kg, 0.01 mg/kg to 5 mg/kg, 0.05 mg/kg to 5 mg/kg, 0.1 mg/kg to 5 mg/kg, 1 mg/kg to 5 mg/kg, 2 mg/kg to 5 mg/kg, 0.0001 mg/kg to 2.5 mg/kg, 0.001 mg/kg to 2.5 mg/kg, 0.005 mg/kg to 2.5 mg/kg, 0.01 mg/kg to 2.5 mg/kg, 0.05 mg/kg to 2.5 mg/kg, 0.1 mg/kg to 2.5 mg/kg, 1 mg/kg to 2.5 mg/kg, 2 mg/kg to 2.5 mg/kg, 0.0001 mg/kg to 1 mg/kg, 0.001 mg/kg to 1 mg/kg, 0.005 mg/kg to 1 mg/kg, 0.01 mg/kg to 1 mg/kg, 0.05 mg/kg to 1 mg/kg, 0.1 mg/kg to 1 mg/kg, 0.0001 mg/kg to 0.25 mg/kg, 0.001 mg/kg to 0.25 mg/kg, 0.005 mg/kg to 0.25 mg/kg, 0.01 mg/kg to 0.25 mg/kg, 0.05 mg/kg to 0.25 mg/kg, or 0.1 mg/kg to 0.25 mg/kg.

[0408] The dose may be administered once or multiple times per day in the same or different amounts to achieve a desired level of mRNA expression and/or a therapeutic, diagnostic, prophylactic, or imaging effect. The desired dose may, for example, be delivered three times daily, twice daily, once daily, every other day, every three days, weekly, every two weeks, every three weeks, or every four weeks. In certain embodiments, the desired dose may be delivered using multiple administrations (for example, two, three, four, five, six, seven,

eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations). In some embodiments, for example, before or after surgery, or in the case of an acute disease, disorder, or condition, a single dose may be administered.

[0409] The lipid nanoparticle of the present application may be used in combination with one or more other therapeutic, prophylactic, diagnostic, or imaging agents. "In combination with....." does not necessarily mean that reagents need to be administered and/or formulated simultaneously for co-delivery, although such delivery methods are within the scope of the present application. For example, one or more nanoparticles containing one or more different therapeutic agents and/or prophylactic agents may be administered in combination. The nanoparticles may be administered simultaneously, before, or after one or more other desired therapeutic or medical procedures. Typically, each agent is administered at a dose and/or schedule determined for the agent. In some embodiments, the present application includes combined delivery of the lipid nanoparticle of the present application with an agent that increases its bioavailability, reduces and/or alters its metabolism, inhibits its excretion, and/or modulates contraction *in vivo*.

[0410] In a specific combination of therapies (therapies or procedures) used in a combination scheme, the compatibility of the desired therapies and/or procedures and a desired therapeutic effect to be achieved are taken into account. It will also be recognized that the therapies employed may achieve the desired effect for the same disorder (for example, a composition for treating cancer may be administered simultaneously with a chemotherapeutic agent), or they may achieve different effects (for example, control of any side effect, such as an infusion-related response).

[0411] In some embodiments, a method for treating a subject in need or delivering a therapeutic and/or prophylactic drug to a subject (for example, a mammal) may include pretreating the subject with one or more agents before administration of a nanoparticle. For example, the subject may be pretreated with an effective amount (for example, 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, or any other effective amount) of dexamethasone, methotrexate, acetaminophen, an H1 receptor blocker, or an H2 receptor blocker. Pretreatment may occur 24 hours or fewer hours (for example, 24 hours, 20 hours, 16 hours, 12 hours, 8 hours, 4 hours, 2 hours, 1 hour, 50 minutes, 40 minutes, 30 minutes, 20

minutes, or 10 minutes) before the administration of the nanoparticle, and may occur once, twice, or more times, for example, in the case of an increased dose.

[0412] In another aspect of the present application, a vaccine preparation is provided, which includes an mRNA vaccine of the present invention and a pharmaceutically acceptable carrier.

[0413] In a specific embodiment, the pharmaceutically acceptable carrier includes a cryoprotectant, a pH buffer, a pH regulator, and water for injection.

[0414] In a specific embodiment, the cryoprotectant is sucrose.

[0415] In a specific embodiment, the pH buffer is glacial acetic acid and/or tromethamine.

[0416] In a specific embodiment, the pH regulator is sodium hydroxide and/or hydrochloric acid.

[0417] In a specific embodiment, the preparation has a pH of 7.2-8.

[0418] In another aspect of the present application, a method for treating or preventing an HPV infection-related disease is provided, which includes administering to an individual the lipid nanoparticle composition, the pharmaceutical composition, the mRNA vaccine, or the vaccine preparation of the present invention.

[0419] In some embodiments, the HPV infection-related disease is cervical cancer.

[0420] In some embodiments, the HPV infection-related disease is a cervical precancerous lesion.

[0421] In some embodiments, the HPV infection-related disease is a cervical high-grade intraepithelial lesion. In some embodiments, the HPV infection-related disease is a high-grade squamous intraepithelial lesion (HSIL).

[0422] In some embodiments, the HPV infection-related disease is cervical intraepithelial neoplasia (CIN) grade 2 or 3 (CIN2/3).

[0423] In some embodiments, the HPV infection-related disease is low-grade LSIL intraepithelial neoplasia (CIN 1).

[0424] In some embodiments, the HPV infection-related disease is adenocarcinoma in situ (AIS).

[0425] In some embodiments, the HPV infection-related disease is head and neck

squamous cell carcinoma (HNSCC). In some embodiments, the HPV infection-related disease is cervical cancer (CC).

[0426] In some embodiments, the HPV infection-related disease is oropharyngeal squamous cell carcinoma.

[0427] In some embodiments, the HPV infection-related disease is one of anal cancer, vaginal cancer, vulvar cancer, and penile cancer.

[0428] In the above embodiments, the HPV infection-related disease is a disease associated with human papillomavirus (HPV) type 16 and/or type 18.

[0429] Preferably, the HPV infection-related disease is cervical intraepithelial neoplasia grade 2 or 3 (CIN2/3) associated with human papillomavirus (HPV) type 16 and/or type 18.

[0430] Preferably, the HPV infection-related disease is cervical cancer associated with human papillomavirus (HPV) type 16 and/or HPV18.

[0431] Preferably, the HPV infection-related disease is head and neck squamous cell carcinoma associated with human papillomavirus (HPV) type 16 and/or type 18.

[0432] Preferably, the HPV infection-related disease is anal cancer associated with human papillomavirus (HPV) type 16 and/or HPV18.

[0433] Preferably, the HPV infection-related disease is vaginal cancer associated with human papillomavirus (HPV) type 16 and/or HPV18.

[0434] Preferably, the HPV infection-related disease is vulvar cancer associated with human papillomavirus (HPV) type 16 and/or HPV18.

[0435] Preferably, the HPV infection-related disease is penile cancer associated with human papillomavirus (HPV) type 16 and/or HPV18.

[0436] In some embodiments, the administering is intratumoral or perilymphatic injection or intramuscular injection. In some embodiments, the method further includes administering to the individual an immunostimulatory factor, chemotherapy, radiotherapy, and/or targeted therapy. In some embodiments, the targeted therapy is an antibody or a functional domain thereof against a cervical cancer-specific tumor target.

[0437] In some embodiments, the method further includes administering to the individual an immune checkpoint inhibitor. In some embodiments, the immune checkpoint

inhibitor is a PD-1 inhibitor or a PD-L1 inhibitor.

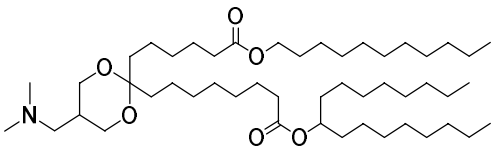
[0438] In some embodiments, the PD-1 or PD-L1 inhibitor is one or more of pembrolizumab, nivolumab, tislelizumab, toripalimab, sintilimab, camrelizumab, penpulimab, zimberelimab, envafolimab, sugemalimab, serplulimab, pucotenlimab, and adebrelimab. In some preferred embodiments, the PD-1 inhibitor is pembrolizumab.

[0439] It should be understood that the aspects and embodiments of the present application described herein include aspects and embodiments of "comprising", "consisting of", and "essentially consisting of". The above provides detailed descriptions of the preferred embodiments of the present application. However, the present application is not limited thereto. Within the scope of the technical concept of the present application, various simple variations may be made to the technical solutions of the present application, including combinations of respective technical features in any other suitable manner. Such simple variations and combinations shall also be regarded as the content disclosed by the present application and are all within the scope of protection of the present application.

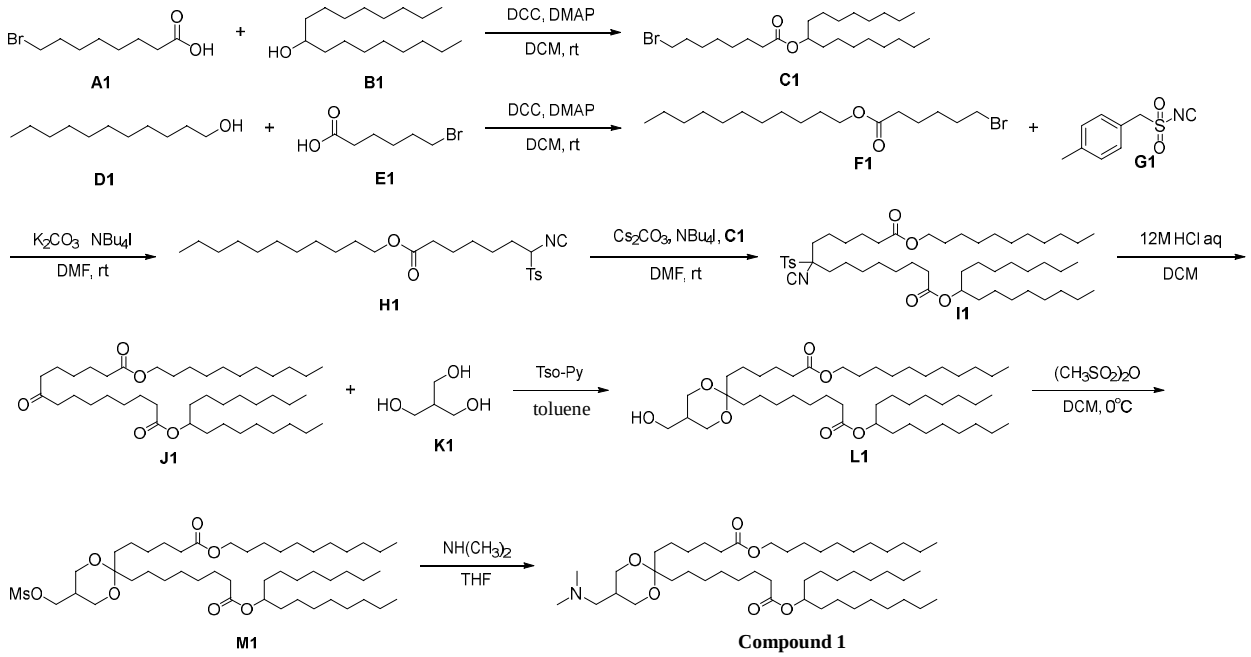
[0440] **Example 1 Synthesis of ionizable lipid compounds**

[0441] The following example is illustrative of the present application and is not intended to limit the present application in any manner. Unless otherwise specified, all temperatures are in degrees Celsius, and pressures are atmospheric pressure or approach atmospheric pressure. Except for synthetic intermediates or where otherwise indicated, all reagents and raw materials used in the present application are obtained through commercial channels.

[0442] Synthesis of compound 1

[0443] compound 1 

[0444] A synthetic route is shown below:



[0445] Specifically, to a solution of compound A1 (2.0 g, 8.96 mmol) in dichloromethane (30 ml), compound B1 (1.9 g, 7.47 mmol), DCC (1.8 g, 8.96 mmol), and DMAP (437 mg, 3.58 mmol) were added and stirred at room temperature for 7 hours, with monitoring of a reaction by thin-layer chromatography (TLC). After completion of the reaction, an equal volume of a saturated sodium bicarbonate solution was added to the reaction solution for dilution, followed by liquid separation. The organic layer was dried over anhydrous magnesium sulfate as added, and filtered. The filtrate was concentrated and treated by silica gel column chromatography (petroleum ether:ethyl acetate = 30:1) to obtain compound C1 (2.9 g, 85.00%).

[0446] To a solution of compound E1 (5.0 g, 25.63 mmol) in dichloromethane (60 ml), compound D1 (3.7 g, 21.36 mmol), DCC (5.29 g, 25.63 mmol), and DMAP (1.2 g, 10.25 mmol) were added and stirred at room temperature for 7 hours, with monitoring of a reaction by TLC. After completion of the reaction, an equal volume of a saturated sodium bicarbonate solution was added to the reaction solution for dilution, followed by liquid separation. The organic layer was dried over anhydrous magnesium sulfate as added, and filtered. The filtrate was concentrated and treated by silica gel column chromatography (petroleum ether:ethyl acetate = 30:1) to obtain compound F1 (6.5 g, 88.00%).

[0447] To a solution of the compound F1 (500 mg, 1.44 mmol) in DMF (15 ml), compound G (562 mg, 2.88 mmol), NBu_4I (795 mg, 2.15 mmol), and K_2CO_3 (597 mg, 4.32 mmol) were added and stirred at room temperature for 12 hours, with monitoring of a reaction by TLC. After completion of the reaction, an equal volume of water was added to the reaction solution for dilution, followed by extraction with ethyl acetate (10 ml × 3). The reaction solution was concentrated and treated by silica gel column chromatography (petroleum ether:ethyl acetate = 10:1) to obtain compound H1 (580 mg, yield 87%).

[0448] To a solution of the compound H1 (500 mg, 1.08 mmol) in DMF (15 ml), the compound C1 (745 mg, 1.62 mmol), NBu_4I (598 mg, 1.62 mmol), and Cs_2CO_3 (1.4 g, 4.32 mmol) were added and stirred at room temperature for 15 hours, with monitoring of a reaction by TLC. After completion of the reaction, an equal volume of water was added to the reaction solution for dilution, followed by extraction with ethyl acetate (10 ml × 3). The reaction solution was concentrated and treated by silica gel column chromatography (petroleum ether:ethyl acetate = 10:1) to obtain compound I1 (811 mg, yield 89%).

[0449] To a solution of the compound I1 (10 g, 11.85 mmol) in dichloromethane (100 ml), concentrated hydrochloric acid (60 ml) was added and stirred at room temperature for 3 hours, with monitoring of a reaction by TLC. After completion of the reaction, the reaction solution was subjected to liquid separation. The organic layer was washed with a saturated sodium bicarbonate solution, dried over anhydrous magnesium sulfate, concentrated, and treated by silica gel column chromatography (petroleum ether:ethyl acetate = 30:1) to obtain compound J1 (6.5 g, 81.00%).

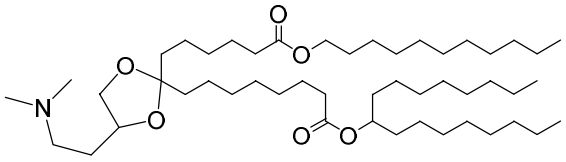
[0450] To a solution of the compound J1 (1 g, 1.48 mmol) in toluene (25 ml), compound K1 (470 mg, 4.44 mmol) and pyridinium 4-methylbenzenesulfonate (744 mg, 2.96 mmol) were added and heated under reflux for 20 hours using a Dean-Stark device, with monitoring of a reaction by TLC. After completion of the reaction, the reaction solution was cooled to room temperature, and an equal volume of water was added to the reaction solution for dilution, followed by extraction with ethyl acetate (10 ml × 3). The reaction solution was concentrated and treated by silica gel column chromatography (petroleum ether:ethyl acetate = 5:1) to obtain compound L1 (648 mg, yield 57%).

[0451] At 0°C, to a solution of the compound L1 (750 mg, 0.98 mmol) in ultra-dry

dichloromethane (10 ml), methanesulfonic anhydride (340 mg, 1.96 mmol) and anhydrous triethylamine (0.45 ml) were added and stirred for 12 hours, with monitoring of a reaction by TLC. After completion of the reaction, the reaction solution was placed to reach room temperature, and an equal volume of water was added to the reaction solution for dilution, followed by liquid separation. The organic layer was dried over anhydrous magnesium sulfate as added, and filtered. The filtrate was concentrated and treated by silica gel column chromatography (petroleum ether:ethyl acetate = 5:1) to obtain compound M1 (800 mg, yield 97%).

[0452] To the compound M1 (750 mg, 0.89 mmol), a dimethylamine (2 M in THF) solution (30 ml) was added. The mixture was sealed and stirred at room temperature for 6 days, with monitoring of a reaction by TLC. After completion of the reaction, the mixture was concentrated and treated by silica gel column chromatography (dichloromethane:methanol = 30:1) to obtain oily compound 1 (212 mg, yield 30%). 1H NMR (600 MHz, CDCl3) δ 4.87 - 4.82 (m, 1H), 4.05 - 4.02 (m, 2H), 3.90 - 3.85 (m, 2H), 3.59 - 3.56 (m, 2H), 2.31 - 2.23 (m, 4H), 2.21 - 2.19 (m, 8H), 1.94 -1.87 (brs, 1H), 1.68 - 1.56 (m, 10H), 1.52 - 1.42 (m, 4H), 1.36 - 1.21 (m, 52H), 0.87 - 0.84 (m, 9H); 13C NMR (151 MHz, CDCl3) δ 173.98, 173.77, 100.76, 74.22, 64.55, 62.87, 62.81, 59.34, 59.31, 45.97, 35.37, 35.28, 34.86, 34.49, 34.46, 34.28, 32.47, 32.39, 32.03, 31.99, 29.99, 29.72, 29.65, 29.46, 29.36, 28.80, 26.06, 25.44, 25.29, 25.16, 23.49, 23.26, 23.16, 22.92, 22.79, 14.22; MS-ESI (m/z): 794.6 (M+H)+.

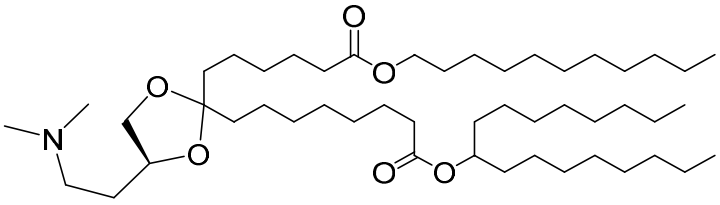
[0453] Synthesis of compound 2

[0454] compound 2 

[0455] A preparation method is the same as that of the compound 1, except that 1,2,4-butanetriol was used to replace the compound K1 as a raw material to prepare oily compound 2. 1H NMR (600 MHz, CDCl3) δ 4.82 - 4.78 (m, 1H), 4.04 - 3.96 (m, 4H), 3.42 - 3.39 (m, 1H), 2.39 - 2.34 (brs, 1H), 2.29 - 2.25 (brs, 1H), 2.23 - 2.18 (m, 10H), 1.77 - 1.71 (brs, 1H), 1.66 - 1.65 (brs, 1H), 1.58 - 1.39 (m, 13H), 1.33 - 1.16 (m, 53H), 0.82 - 0.80 (m,

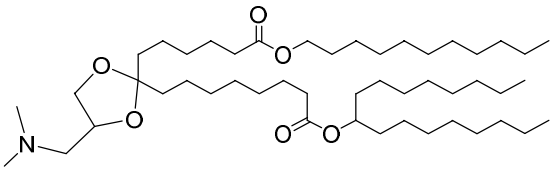
9H); 13C NMR (151 MHz, CDCl3) δ 173.62, 173.41, 111.82, 74.63, 74.61, 73.88, 69.89, 64.25, 56.18, 45.28, 37.73, 37.48, 37.40, 37.20, 34.59, 34.19, 34.18, 34.09, 31.83, 31.78, 31.60, 29.70, 29.68, 29.52, 29.50, 29.45, 29.34, 29.32, 29.25, 29.20, 29.15, 29.09, 29.07, 28.59, 25.86, 25.23, 25.07, 24.89, 23.88, 23.58, 23.56, 23.24, 22.60, 22.58, 14.01; MS-ESI (m/z): 794.6 (M+H)+.

[0456] Synthesis of compound 2A

[0457] compound 2A 

[0458] A preparation method is the same as that of the compound 1, except that (S)-1,2,4-butanetriol was used to replace the compound K1 as a raw material to prepare oily compound 2A. 1H NMR (400 MHz, CDCl3) δ 4.97 - 4.83 (m, 1H), 4.17 - 4.05 (m, 4H), 3.52 (t, J = 8.0 Hz, 1H), 2.58 - 2.37 (m, 2H), 2.35 - 2.23 (m, 10H), 1.91 - 1.70 (m, 2H), 1.69 - 1.50 (m, 14H), 1.48 - 1.24 (m, 52H), 0.92 (t, J = 8.0 Hz, 9H); 13C NMR (100 MHz, CDCl3) δ 174.04, 173.69, 112.10, 74.59, 74.12, 69.94, 64.34, 56.22, 45.21, 37.79, 37.70, 37.44, 37.38, 34.74, 34.42, 34.16, 31.91, 31.88, 31.43, 31.41, 29.80, 29.57, 29.55, 29.52, 29.49, 29.32, 29.28, 29.25, 29.19, 28.63, 28.61, 25.95, 25.33, 25.17, 25.00, 23.89, 23.71, 23.59, 22.68, 14.12; MS-ESI (m/z): 795.0 (M+H)+.

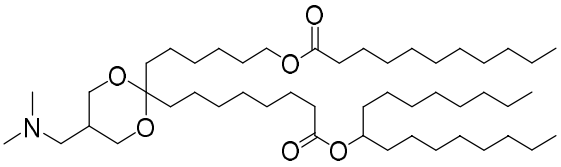
[0459] Synthesis of compound 3

[0460] compound 3 

[0461] A preparation method is the same as that of the compound 1, except that 3-dimethylamino-1,2-propanediol and p-toluenesulfonic acid monohydrate were used to replace the compound K1 and the pyridinium 4-methylbenzenesulfonate as raw materials to prepare oily compound 3. 1H NMR (600 MHz, CDCl3) δ 4.85 - 4.81 (m, 1H), 4.20 - 4.16 (m, 1H), 4.06 - 4.01 (m, 3H), 3.49 - 3.46 (m, 1H), 2.48 - 2.44 (m, 1H), 2.35 - 2.32 (m, 1H), 2.27 - 2.22 (m, 10H), 1.60 - 1.54 (m, 8H), 1.48 - 1.47 (m, 4H), 1.31 - 1.20 (m, 54H), 0.86 - 0.83 (m,

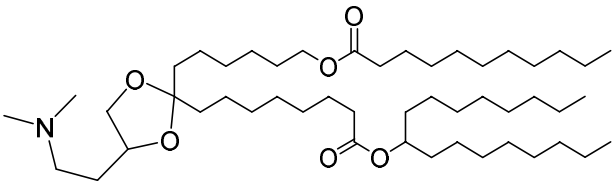
9H); 13C NMR (151 MHz, CDCl3) δ 173.90, 173.69, 112.58, 74.40, 74.36, 74.13, 69.06, 64.48, 62.62, 62.59, 46.22, 37.80, 37.55, 37.51, 37.30, 34.79, 34.40, 34.38, 34.24, 31.99, 31.94, 29.88, 29.83, 29.68, 29.66, 29.61, 29.58, 29.52, 29.47, 29.41, 29.38, 29.33, 29.31, 29.27, 29.25, 28.74, 26.01, 25.39, 25.24, 25.06, 25.06, 24.03, 23.70, 23.35, 22.74, 14.17; MS-ESI (m/z): 780.6 (M+H)+.

[0462] Synthesis of compound 4

[0463] compound 4 

[0464] A preparation method is the same as that of the compound 1, except that undecanoic acid and 6-bromo-1-hexanol were used to replace the compound D1 and the compound E1 as raw materials to prepare oily compound 4. 1H NMR (600 MHz, CDCl3) δ 4.86 - 4.81 (m, 1H), 4.04 - 4.01 (m, 2H), 3.90 - 3.84 (m, 2H), 3.59 - 3.55 (m, 2H), 2.27 - 2.22 (m, 4H), 2.20 - 2.17 (m, 8H), 1.94 - 1.85 (brs, 1H), 1.67 - 1.55 (m, 10H), 1.48 - 1.45 (m, 4H), 1.36 - 1.22 (m, 52H), 0.86 - 0.83 (m, 9H); 13C NMR (151 MHz, CDCl3) δ 174.01, 173.70, 100.73, 74.14, 64.40, 64.36, 62.83, 62.75, 59.31, 59.28, 45.98, 35.50, 35.25, 34.79, 34.47, 34.24, 32.45, 32.43, 32.22, 31.98, 31.95, 29.94, 29.70, 29.64, 29.61, 29.59, 29.55, 29.42, 29.39, 29.35, 29.32, 29.29, 29.25, 28.73, 26.05, 25.40, 25.24, 25.10, 23.45, 23.32, 23.21, 23.08, 22.75, 14.18; MS-ESI (m/z): 794.6 (M+H)+.

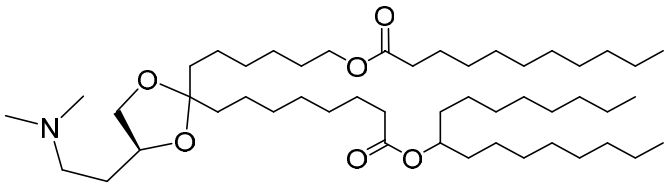
[0465] Synthesis of compound 5

[0466] compound 5 

[0467] A preparation method is the same as that of the compound 1, except that undecanoic acid, 6-bromo-1-hexanol, and 1,2,4-butanetriol were used to replace the compound D1, the compound E1, and the compound K1 as raw materials to prepare oily compound 5. 1H NMR (600 MHz, CDCl3) δ 4.81 (p, J = 6.2 Hz, 1H), 4.03 (t, J = 6.6 Hz, 1H), 3.99 (t, J = 6.6 Hz, 3H), 3.42 (t, J = 7.6 Hz, 1H), 2.41 - 2.36 (brs, 1H), 2.31 - 2.26 (brs, 1H),

2.23 - 2.20 (m, 10H), 1.78 - 1.73 (brs, 1H), 1.66 - 1.60 (brs, 1H), 1.58 - 1.43 (m, 12H), 1.31 - 1.20 (m, 54H), 0.82 (t, J = 7.0 Hz, 9H); 13C NMR (151 MHz, CDCl3) δ 173.92, 173.60, 112.00, 74.75, 74.73, 74.05, 70.00, 64.31, 56.29, 45.41, 37.83, 37.73, 37.50, 37.42, 34.73, 34.40, 34.20, 31.93, 31.90, 31.73, 31.71, 29.82, 29.80, 29.58, 29.56, 29.53, 29.50, 29.33, 29.30, 29.26, 29.20, 28.67, 28.65, 25.96, 25.35, 25.19, 25.05, 23.90, 23.71, 23.59, 22.69, 14.13; MS-ESI (m/z): 794.6 (M+H)+.

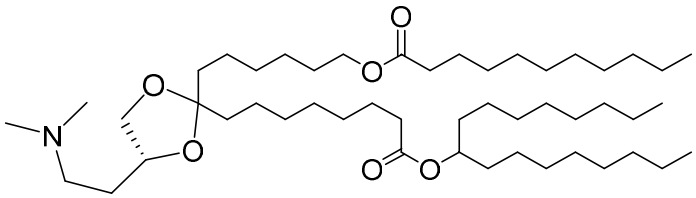
[0468] Synthesis of compound 5A



[0469]

[0470] A preparation method is the same as that of the compound 1, except that undecanoic acid, 6-bromo-1-hexanol, and (S)-1,2,4-butanetriol were used to replace the compound D1, the compound E1, and the compound K1 as raw materials to prepare oily compound 5A. 1H NMR (400 MHz, CDCl3) δ 4.97 - 4.83 (m, 1H), 4.17 - 4.06 (m, 4H), 3.51 (t, J = 4.0 Hz, 1H), 2.51 - 2.45 (m, 1H), 2.44 - 2.32 (m, 1H), 2.31 - 2.23 (m, 10H), 1.87 - 1.80 (m, 1H), 1.74 - 1.67 (m, 1H), 1.66 - 1.50 (m, 14H), 1.48 - 1.24 (m, 52H), 0.92 (t, J = 8.0 Hz, 9H); 13C NMR (100 Hz, CDCl3) δ 173.96, 173.62, 111.98, 74.71, 74.08, 69.96, 64.30, 56.24, 45.36, 37.60, 37.53, 37.50, 37.43, 34.71, 34.39, 34.13, 31.87, 31.84, 31.67, 31.61, 29.78, 29.70, 29.51, 29.48, 29.30, 29.28, 29.24, 29.18, 28.64, 28.61, 25.94, 25.32, 25.17, 25.03, 23.89, 23.70, 23.59, 22.67, 14.11; MS-ESI (m/z): 795.0 (M+H)+.

[0471] Synthesis of compound 5B

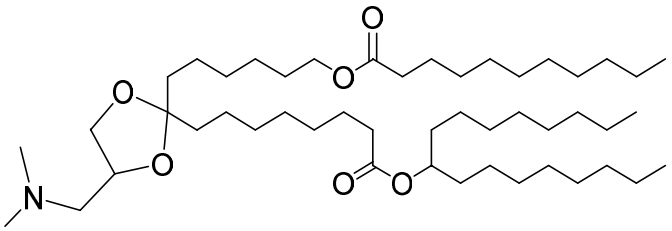


[0472]

[0473] A preparation method is the same as that of the compound 1, except that undecanoic acid, 6-bromo-1-hexanol, and (R)-1,2,4-butanetriol were used to replace the compound D1, the compound E1, and the compound K1 as raw materials to prepare oily compound 5B. 1H NMR (400 MHz, CDCl3) δ 4.97 - 4.83 (m, 1H), 4.17 - 4.05 (m, 4H), 3.51

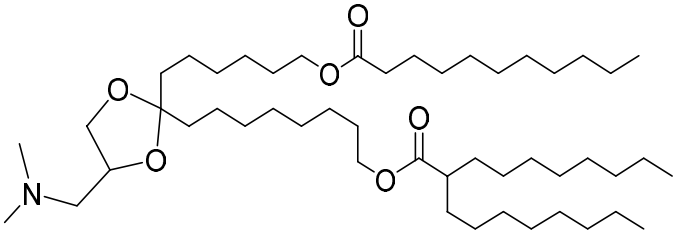
(t, J = 4.0 Hz, 1H), 2.51 - 2.45 (m, 1H), 2.44 - 2.32 (m, 1H), 2.31 - 2.23 (m, 10H), 1.86 - 1.80 (m, 1H), 1.74 - 1.67 (m, 1H), 1.66 - 1.51 (m, 14H), 1.48 - 1.24 (m, 52H), 0.92 (t, J = 8.0 Hz, 9H); 13C NMR (100 MHz, CDCl3) δ 173.97, 173.62, 111.98, 74.71, 74.08, 69.96, 64.30, 56.24, 45.36, 37.60, 37.43, 37.50, 37.44, 34.71, 34.39, 34.13, 31.87, 31.84, 31.67, 31.61, 29.79, 29.70, 29.51, 29.48, 29.30, 29.28, 29.24, 29.18, 28.64, 28.61, 25.94, 25.32, 25.18, 25.03, 23.89, 23.70, 23.59, 22.67, 14.11; MS-ESI (m/z): 795.0 (M+H)+.

[0474] Synthesis of compound 6

[0475] compound 6 

[0476] A preparation method is the same as that of the compound 1, except that undecanoic acid, 6-bromo-1-hexanol, and 3-dimethylamino-1,2-propanediol were used to replace the compound D1, the compound E1, and the compound K1 as raw materials to prepare oily compound 6. 1H NMR (500 MHz, CDCl3) δ 4.84 (p, J = 6.2 Hz, 1H), 4.19 (p, J = 6.5 Hz, 1H), 4.06 - 4.01 (m, 3H), 3.49 (t, J = 7.8 Hz, 1H), 2.48 (dd, J = 12.6, 6.8 Hz, 1H), 2.35 (dd, J = 12.6, 5.0 Hz, 1H), 2.27 - 2.23 (m, 10H), 1.62 - 1.53 (m, 10H), 1.49 - 1.46 (m, 4H), 1.33 - 1.24 (m, 52H), 0.85 (t, J = 6.8 Hz, 9H); 13C NMR (126 MHz, CDCl3) δ 174.05, 173.72, 112.66, 74.44, 74.18, 69.08, 64.40, 62.65, 46.25, 37.82, 37.71, 37.49, 37.44, 34.83, 34.50, 34.26, 32.00, 31.97, 29.91, 29.86, 29.63, 29.61, 29.40, 29.37, 29.33, 29.27, 28.73, 26.04, 25.42, 25.26, 25.12, 24.06, 23.95, 23.73, 23.61, 22.77, 14.19; MS-ESI (m/z): 780.6 (M+H)+.

[0477] Synthesis of compound 7

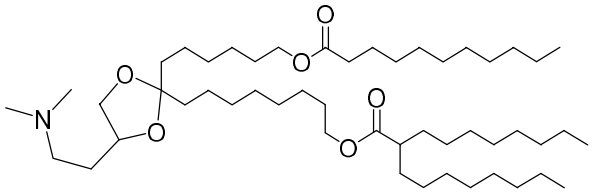
[0478] compound 7 

[0479] A preparation method is the same as that of the compound 1, except that

8-bromo-1-octanol, 2-octyl-decanoic acid, and 3-dimethylamino-1,2-propanediol were used to replace the compound A1, the compound B1, and the compound K1 as raw materials to prepare oily compound 7. 1H NMR (500 MHz, CDCl3) δ 4.22 - 4.17 (m, 1H), 4.06 - 4.01 (m, 5H), 3.48 (t, J = 7.8 Hz, 1H), 2.50 - 2.46 (m, 1H), 2.39 - 2.36 (m, 1H), 2.28 - 2.24 (m, 9H), 1.60 - 1.53 (m, 13H), 1.42 - 1.37 (m, 2H), 1.30 - 1.22 (m, 53H), 0.85 (t, J = 6.8 Hz, 9H); 13C NMR (126 MHz, CDCl3) δ 176.64, 173.92, 112.62, 74.26, 74.21, 68.96, 64.29, 64.10, 62.48, 46.07, 45.85, 37.75, 37.60, 37.42, 37.32, 34.40, 32.56, 31.90, 31.87, 29.91, 29.86, 29.57, 29.46, 29.31, 29.25, 29.18, 28.75, 28.64, 27.47, 25.99, 25.94, 25.03, 23.98, 23.85, 23.64, 23.51, 22.67, 14.10; MS-ESI (m/z): 794.6 (M+H)+.

[0480] Preparation of compound 8

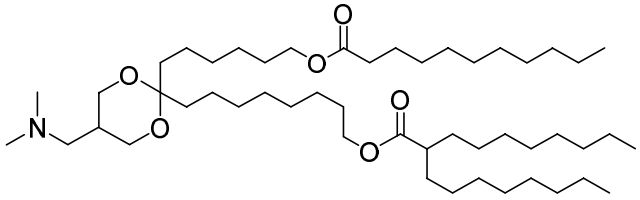
[0481] compound 8



[0482] A preparation method is the same as that of the compound 1, except that 8-bromo-1-octanol, 2-octyl-decanoic acid, and 1,2,4-butanetriol were used to replace the compound A1, the compound B1, and the compound K1 as raw materials to prepare oily compound 8. 1H NMR (500 MHz, CDCl3) δ 4.07 - 4.00 (m, 6H), 3.44 (t, J = 7.5 Hz, 1H), 2.42 - 2.36 (brs 1H), 2.32 - 2.21 (m, 10H), 1.81 - 1.74 (brs, 1H), 1.68 - 1.51 (m, 14H), 1.42 - 1.34 (brs, 2H), 1.26 - 1.22 (m, 53H), 0.84 (t, J = 6.9 Hz, 9H); 13C NMR (126 MHz, CDCl3) δ 176.69, 173.97, 112.05, 74.81, 70.06, 64.36, 64.16, 56.36, 45.92, 45.50, 37.93, 37.78, 37.59, 37.48, 34.46, 32.63, 31.97, 31.94, 31.82, 29.63, 29.53, 29.37, 29.32, 29.24, 28.81, 28.71, 27.54, 26.06, 26.02, 25.09, 24.07, 23.94, 23.77, 23.64, 22.73, 14.16; MS-ESI (m/z): 808.6 (M+H)+.

[0483] Preparation of compound 9

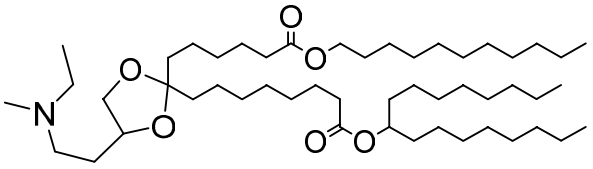
[0484] compound 9



[0485] A preparation method is the same as that of the compound 1, except that

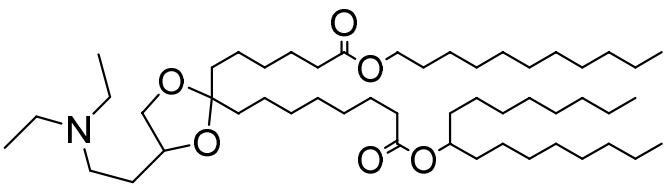
8-bromo-1-octanol, 2-octyl-decanoic acid, undecanoic acid, and 6-bromo-1-hexanol were used to replace the compound A1, the compound B1, the compound D1, and the compound E1 as raw materials to prepare oily compound 9. 1H NMR (500 MHz, CDCl3) δ 4.04 - 4.01 (m, 4H), 3.89 - 3.85 (m, 2H), 3.59 - 3.56 (m, 2H), 2.31 - 2.18 (m, 11H), 1.93 - 1.86 (brs, 1H), 1.67 - 1.52 (m, 12H), 1.43 - 1.23 (m, 56H), 0.85 (t, J = 6.9 Hz, 9H); 13C NMR (126 MHz, CDCl3) δ 176.71, 174.00, 100.75, 64.40, 64.36, 64.18, 62.83, 62.76, 59.34, 59.31F, 45.98, 45.94, 35.46, 35.25, 34.48, 32.64, 32.54, 32.46, 32.34, 31.98, 31.96, 30.06, 29.71, 29.65, 29.55, 29.39, 29.34, 29.26, 28.83, 28.74, 27.56, 26.08, 25.11, 23.46, 23.32, 23.23, 23.09, 22.75, 14.18; MS-ESI (m/z): 808.5 (M+H)+.

[0486] Preparation of compound 10

[0487] compound 10 

[0488] A preparation method is the same as that of the compound 1, except that 1,2,4-butanetriol and N-ethylmethylamine were used to replace the compound K1 and the dimethylamine as raw materials to prepare oily compound 10. 1H NMR (600 MHz, CDCl3) δ 4.83 - 4.75 (m, 1H), 4.05 - 3.93 (m, 4H), 3.42 - 3.35 (m, 1H), 2.46 - 2.30 (m, 4H), 2.23 - 2.15 (m, 7H), 1.79 - 1.70 (m, 1H), 1.67 - 1.59 (m, 1H), 1.56 - 1.51 (m, 6H), 1.43 (d, J = 7.2 Hz, 4H), 1.29 - 1.14 (m, 56H), 0.99 (t, J = 7.1 Hz, 3H), 0.80 (t, J = 7.0 Hz, 9H); 13C NMR (151 MHz, CDCl3) δ 173.72, 173.51, 111.83, 74.85, 74.81, 73.96, 69.99, 64.33, 53.70, 51.43, 41.49, 37.80, 37.56, 37.48, 37.28, 34.66, 34.26, 34.25, 34.15, 31.90, 31.85, 31.30, 29.77, 29.59, 29.57, 29.51, 29.49, 29.41, 29.38, 29.32, 29.29, 29.24, 29.22, 29.16, 29.13, 28.65, 25.92, 25.30, 25.13, 24.97, 23.96, 23.66, 23.31, 22.65, 14.08, 12.09; MS-ESI (m/z): 808.7 (M+H)+.

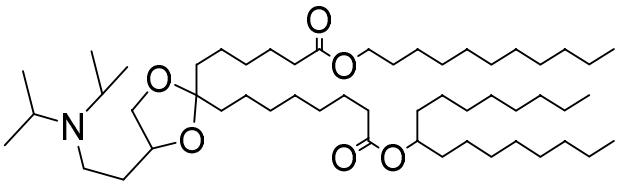
[0489] Preparation of compound 11

[0490] compound 11 

[0491] A preparation method is the same as that of the compound 1, except that 1,2,4-butanetriol and diethylamine were used to replace the compound K1 and the dimethylamine as raw materials to prepare oily compound 11. 1H NMR (600 MHz, CDCl3) δ 4.86 - 4.80 (m, 1H), 4.08 - 4.00 (m, 4H), 3.49 - 3.43 (m, 1H), 2.77 - 2.62 (m, 7H), 2.25 (dt, J = 10.5, 7.5 Hz, 4H), 1.85 - 1.75 (m, 2H), 1.63 - 1.56 (m, 6H), 1.50 - 1.44 (m, 4H), 1.37 - 1.19 (m, 56H), 1.12 (t, J = 7.2 Hz, 6H), 0.85 (td, J = 7.1, 2.4 Hz, 9H); 13C NMR (151 MHz, CDCl3) δ 173.95, 173.73, 112.22, 74.59, 74.55, 74.17, 69.94, 64.52, 49.36, 46.95, 37.84, 37.59, 37.42, 37.21, 34.80, 34.39, 34.24, 31.99, 31.95, 30.30, 29.87, 29.69, 29.67, 29.62, 29.59, 29.50, 29.47, 29.42, 29.35, 29.32, 29.29, 29.26, 28.74, 26.02, 25.40, 25.24, 25.07, 24.09, 23.82, 23.76, 23.47, 22.75, 14.20, 10.74; MS-ESI (m/z): 822.9 (M+H)+.

[0492] Preparation of compound 12

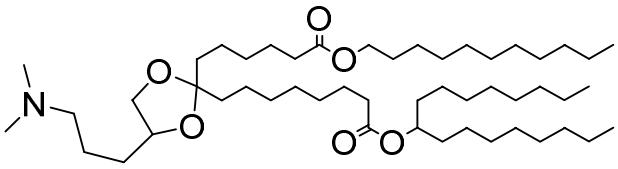
[0493] compound 12



[0494] Compound 12 is prepared with reference to the preparation of the compound 2.

[0495] Preparation of compound 13

[0496] compound 13



[0497] A preparation method is the same as that of the compound 1, except that 1,2,5-pentanetriol was used to replace the compound K1 as a raw material to prepare oily compound 13. 1H NMR (600 MHz, CDCl3) δ 4.81 - 4.74 (m, 1H), 4.00 - 3.92 (m, 4H), 3.38 - 3.33 (m, 1H), 2.25 - 2.17 (m, 6H), 2.15 (s, 6H), 1.59 - 1.46 (m, 15H), 1.46 - 1.37 (m, 5H), 1.29 - 1.12 (m, 50H), 0.79 (td, J = 7.1, 2.3 Hz, 9H); 13C NMR (151 MHz, CDCl3) δ 173.69, 173.47, 111.86, 76.08, 76.05, 73.92, 69.93, 69.91, F64.29, 59.48, 53.38, 45.24, 37.80, 37.56, 37.47, 37.25, 34.64, 34.24, 34.22, 34.13, 31.88, 31.83, 31.20, 29.76, 29.57, 29.55, 29.49, 29.47, 29.39, 29.37, 29.30, 29.27, 29.22, 29.20, 29.14, 29.11, 28.63, 25.90, 25.28, 25.12,

24.95, 23.95, 23.89, 23.64, 23.62, 23.29, 22.64, 22.62, 14.05; MS-ESI (m/z): 808.8 (M+H)+.

[0498] Example 2 Synthesis of mRNA

[0499] 2.1 Synthesis of an HPV vaccine sequence and construction of a recombinant vector

[0500] In this example, an antigen sequence of an HPV mRNA vaccine was a polynucleotide sequence obtained by connecting coding fragments of E6 and E7 proteins of HPV types 16 and 18 in series. The coded nucleotide sequence, from a 5' end, sequentially containing a T7 promoter with XbaI at the 5' end, 5' UTR, tPA-SP, Flt3L, HPV E2, an E6/E7 protein or a variant thereof, 3' UTR, and/or a poly A tail, was subjected to double digestion with XbaI and NotI and a ligation reaction with a pUC57-GW-Kan (Genewiz) vector backbone fragment digested with XbaI and NotI to construct a recombinant plasmid.

[0501] 2.2 Preparation of mRNA

[0502] 2.2.1 Plasmid linearization

[0503] The recombinant plasmid constructed in the step 1 contained a SapI digestion site after the last A of a polyA tail sequence. The plasmid containing a target gene was linearized using a restriction endonuclease SapI, with a reaction system as shown in Table 1, and digestion was performed at 37°C for 3 h.

Table 1. Plasmid linearization digestion system

10× Cutsmart buffer	5 μL
SapI enzyme (10000 U/mL)	1 μL
Plasmid	10 μg
ddH_2O	Supplemented up to 50 μL

[0504] 2 μL of a digestion product was taken for 1% agarose gel electrophoresis to detect linearization situations of the plasmid. The linearized plasmid was purified using a PCR product recovery kit (Cowin).

[0505] (2) *In vitro* transcription and purification

[0506] *In vitro* transcription was performed using a high yield T7 RNA transcription kit with the linearized recombinant plasmid obtained in the step (1) as a template. The high

yield T7 RNA transcription kit had a product name of High Yield T7 RNA Synthesis Kit, was purchased from Shanghai Zhaowei Technology Development Co., Ltd., and had a product catalog number of ON-040. A 5× Reaction Buffer, a 100 mM ATP Solution, a 100 mM CTP Solution, a 100 mM GTP Solution, an Enzyme mix, DNase I, an Ammonium Acetate Stop Solution, and a Lithium Chloride (LiCl) Precipitation Solution were all components in the high yield T7 RNA transcription kit. A 100 mM ΨUTP Solution (pseudouridine triphosphate) had a full name of N1-Me-pUTP, 100 mM, was purchased from Shanghai Zhaowei Technology Development Co., Ltd., and had a product catalog number of R5-027. Various components were added according to the following system (Table 2) (taking a 20 μL reaction system as an example) and mixed uniformly to perform a reaction at 37°C for 3 h.

Table 2. *In vitro* transcription system

5 × Reaction Buffer	4 μL
ATP (100 mM)	2 μL
ΨTP (100 mM)	2 μL
CTP (100 mM)	2 μL
GTP (100 mM)	2 μL
Enzyme mix	1 μL
Linearized DNA template	500 ng - 1 μg
CleanCap AG (100 mM)	1 μL
Nuclease-free H_2O	Supplemented up to 20 μL

[0507] CleanCap AG was m7G(5')ppp(5')(2'-OMeA)pG, with a catalog number of ON-134, and was purchased from Shanghai Zhaowei.

[0508] After completion of the transcription reaction, 1 μL of DNase I was added to perform a reaction at 37°C for 15 min, and 15 μL of the Ammonium Acetate Stop Solution was added and mixed uniformly. Then, 1/3 volume of the 7.5 M Lithium Chloride (LiCl) Precipitation Solution (to reach a final concentration of 2.5 M) was added, and the mixture was placed at -20°C for 30 min. Centrifugation was performed at 12,000 g for 15 min until an RNA precipitate was obtained at a bottom, and the supernatant was discarded. The RNA was

washed with 1 mL of 70% ethanol, centrifuged at 12,000 g for 5 min, and the supernatant was discarded. After air drying, 50 μL of RNA enzyme-free water was added to dissolve the precipitate, and mRNA quantification was performed using an ultraviolet spectrophotometer to obtain capped *in vitro* transcribed mRNA.

[0509] Example 3 Encapsulation and determination of a lipid nanoparticle (LNP)

[0510] HPV mRNA (as shown in SEQ ID NO: 12) was formulated into a solution of a concentration of 200 μg/ml with acetic acid having a pH of 4.0-6.0. According to a formulation amount, four lipids were formulated into a mixed lipid solution with anhydrous ethanol. An oil phase and an aqueous phase were mixed through a T-shaped flow mixing device at a flow rate of 1:3 using a plunger pump or a syringe pump to form LNP, which was then diluted 2-10 times with sucrose or an aqueous solution. The ethanol in a sample was removed in an ultrafiltration manner, and replacement was performed with an acetic acid solution at a replacement factor not lower than 3. After the replacement, a pH value of a product was adjusted to 7.0-8.0 with Tris, and an osmotic pressure was adjusted to approach a physiological value with sucrose. Sterilization and filtration were performed, and the sample was sealed and stored at -20°C. Finally, a total content and a free content of mRNA in the LNP were detected using a Ribogreen kit and 10% OTG as a demulsifier, and an encapsulation efficiency of the LNP was calculated. The final LNP product was diluted with a diluent. 1 mL of the product was added to a particle size cell and placed on a Malvern ZetaSizer instrument to detect a particle size of the LNP. Results are shown in Table 3.

Table 3. Experimental results of encapsulation efficiency of HPV mRNA using LNP formulations with different proportions of the compound 5A

Sample number	N/P	Compound 5A (×100 mol%)	DSPC (×100 mol%)	Cholesterol (×100 mol%)	DMG-PEG2K (×100 mol%)	Day 0		
						Encapsulation efficiency /%	Particle size /nm	PDI
1	4.50	0.443	0.093	0.450	0.015	97.68	72.3	0.05706
2	4.50	0.400	0.150	0.425	0.025	98.76	60.19	0.0751
3	4.50	0.500	0.050	0.430	0.021	98.53	72.95	0.01876

4	3.50	0.400	0.125	0.450	0.025	98.80	59.65	0.07077
5	3.50	0.460	0.110	0.410	0.020	98.75	67.24	0.03711
6	5.50	0.475	0.050	0.450	0.025	98.51	64.56	0.06519
7	3.50	0.475	0.050	0.450	0.025	98.06	67.57	0.03252
8	5.50	0.400	0.150	0.435	0.015	95.17	66.27	0.1762
9	3.50	0.485	0.050	0.450	0.015	97.79	83.23	0.006533
10	5.50	0.400	0.130	0.450	0.020	97.17	61.13	0.1007
11	5.50	0.500	0.125	0.350	0.025	99.14	66.89	0.03853
12	3.50	0.500	0.080	0.395	0.025	97.81	69.05	0.03225
13	5.50	0.423	0.150	0.403	0.025	97.50	61.27	0.06134
14	4.50	0.460	0.110	0.410	0.020	98.49	66.52	0.04339
15	3.50	0.500	0.135	0.350	0.015	97.89	79.23	0.03701
16	5.50	0.460	0.110	0.410	0.020	98.66	69.06	0.06607
17	3.50	0.475	0.150	0.350	0.025	100.00	63.25	0.06019
18	5.50	0.485	0.150	0.350	0.015	97.67	68.54	0.0823
19	5.50	0.500	0.050	0.435	0.015	96.46	76.89	0.01484
20	3.50	0.400	0.150	0.435	0.015	97.88	70.05	0.07959
21	5.2	0.480	0.103	0.396	0.021	95	72.9	0.08
Control group (SM-102)	5.2	0.5	0.1	0.385	0.015	98.39	68.76	0.04722

[0511] As can be seen from Table 3, the results show that the LNP formed by the compound 5A in the example exhibits excellent performance such as particle size uniformity and encapsulation efficiency, which is comparable to or superior to a currently known SM-102 standard formulation.

[0512] **Flow cytometry analysis**

[0513] Flow cytometry is used to analyze a cellular immune level in test animals, thereby providing a scientific basis for evaluating the effectiveness of candidate vaccines.

Cytotoxic T lymphocytes (CTL cells) are commonly referred to as CD8+ T cells and play an important role in the immune system to defend against pathogens (for example, viruses, bacteria, and tumors). IFN-γ, as a cytokine secreted by immunocompetent cells, plays an important immunomodulatory role in inducing cellular immunity, including activation of cytotoxic T lymphocytes (CTL), natural killer (NK) cells, and phagocytes. Currently, detection of an IFN-γ level has gradually replaced various traditional cellular immune detection methods and has become an important method for detecting a cellular immune effect.

[0514] **Experimental steps**

[0515] C57BL/6 mouse immunization step: An HPV mRNA sequence encapsulated with a formulation in Table 3 was injected intramuscularly into hind limbs of mice, at an immunization dose of 12.5 μg and an immunization volume of 50 μL. 7 days after immunization, a test endpoint was reached.

[0516] At the test endpoint, the C57BL/57 mice were sacrificed, and spleens were taken out in an aseptic environment and placed in a 6-well cell culture plate containing 2 mL of an RPMI 1640 complete culture medium. The spleens were ground to prepare a single-cell suspension and filtered through a cell strainer. The single-cell filtrate was transferred to a 50 mL sterile centrifuge tube and centrifuged at 300 g for 5 min at room temperature. The supernatant was discarded, 3 mL of a red blood cell lysis buffer was added to resuspend cells thoroughly, and the cells were placed in a cell incubator set at 37°C and providing 5% CO_2 for incubation for 3 min. After completion of the incubation, an equal volume of an RPMI 1640 complete culture medium was added and mixed uniformly and thoroughly to terminate a reaction. Then, the single-cell suspension was filtered again through a cell strainer. A single-cell filtrate was obtained and centrifuged at 300 g for 5 min, and the supernatant was discarded. 2 mL of an RPMI 1640 complete culture medium was added to resuspend the cells thoroughly, and the cells were then counted, with a cell concentration adjusted to 1 x 10^7 cells/mL. A 96-well U-bottom cell culture plate was taken, and the cells were seeded at a quantity of 1 x 10^6 cells per well, with an HPV vaccine peptide library as a stimulant, BFA + monensin as a blocker, a peptide library working concentration of 2 μg/ml/peptide, a BFA dilution factor of 0.1 μL/test (1000-fold dilution), and a monensin dilution factor of 0.1

μL/test (1000-fold dilution).

[0517] The 96-well U-bottom cell plate was placed in an incubator set at 37°C and providing 5% CO_2 for culture overnight. The stimulated 96-well U-bottom cell plate was taken out and centrifuged at 300 g for 5 min, and the supernatant was discarded.

[0518] Extracellular flow antibody staining

[0519] Formulation of extracellular staining antibodies (formulated with 1× PBS)

[0520] Anti-CD3-cy5.5 had a staining concentration of 1 μL/test.

[0521] Anti-CD4-APC (Biolegend) had a staining concentration of 0.5 μL/test.

[0522] Anti-CD8-FITC (MBL) had a staining concentration of 5 μL/test.

[0523] 100 μL of an extracellular antibody staining mixed solution was added to each well for incubation at 4°C in the dark for 30 min. 100 μL of PBS was added, centrifugation was performed at 300 g for 5 min, and the supernatant was discarded. 200 μL of a fixation solution was added to each well to resuspend the cells thoroughly, and the cells were incubated at 4°C in the dark for 30 min. Centrifugation was performed at 300 g for 5 min, and the supernatant was discarded. 100 μL of a 1× permeabilization buffer was added to each well to resuspend the cells thoroughly. Centrifugation was performed at 300 g for 5 min, and the supernatant was discarded.

[0524] Intracellular flow antibody staining

[0525] Formulation of an intracellular staining antibody (formulated with a 1× permeabilization buffer)

[0526] Anti-IFN-γ-PE (Biolegend) had a staining concentration of 0.5 μL/test. 100 μL of an intracellular antibody staining mixed solution was added to each well, and the cells were incubated under shaking at room temperature in the dark for more than 1 h.

[0527] After completion of the incubation, centrifugation was performed at 300 g for 5 min, and the supernatant was discarded. 200 μL of PBS was added to each well to resuspend the cells thoroughly, centrifugation was performed at 300 g for 5 min, and the supernatant was discarded. 4.9.4 200 μL of PBS was added to each well to resuspend the cells thoroughly, and the cells were analyzed on an instrument.

[0528] A detection result of CD8 + IFN-γ + double-positive cells in an SM-102 standard formulation sample of a control group was set as 1, and expression amounts of

different formulation samples of the compound 5A were compared with it. Results are shown in FIG. 1. The number of CD8 + IFN-γ + positive expressing cells using different formulation proportions of the compound 5A is significantly better than that of the SM-102 standard formulation sample of the control group, with the proportion of the CD8 + IFN-γ + positive cells increased by 1.2-1.7 times. It is indicated that formulations based on the compound 5A, compared with an SM-102 standard formulation, can induce a stronger cellular immune response in delivering an mRNA sequence.

[0529] **Example 4**

[0530] Calcium chloride was dissolved in an acetate buffer to obtain a Ca^{2+} solution of a certain concentration, which was then used to dilute HPV mRNA to obtain an mRNA working solution with a final mRNA concentration of 200 μg/ml and a final Ca^{2+} solution concentration (as shown in Table 4). An mRNA aqueous phase and a hybrid lipid-ethanol phase were mixed in a volume ratio of 3:1. A sample was diluted, concentrated, and replaced after the mixing to remove the ethanol, then the sample was adjusted to a constant volume, and a pH value was adjusted to 7-8 (for specific steps, refer to Example 2). An osmotic pressure was adjusted to approach a physiological value with sucrose. Sterilization and filtration were performed, and the sample was sealed and stored at -20°C.

[0531] 0.5 mL of an HPV mRNA injection was taken, and 0.5 mL of a 10% Triton solution was added for demulsification. Then, the mixture was diluted to 10 mL with a 2% aqueous solution of nitric acid and shaken uniformly. Detection was performed according to inductively coupled plasma atomic emission spectrometry in the Chinese Pharmacopoeia (2020 edition) 0411.

Table 4: Formulation composition

Sample number	N/P	Compound 5A (×100 mol%)	DSPC (×100 mol%)	Cholesterol (×100 mol%)	DMG-PEG2K (×100 mol%)	Ca^{2+} concentration mM
21	5.2	0.480	0.103	0.396	0.021	0
21-1	5.2	0.480	0.103	0.396	0.021	0.8
21-2	5.2	0.480	0.103	0.396	0.021	3.2

21-3	5.2	0.480	0.103	0.396	0.021	8
13	5.5	0.423	0.150	0.403	0.025	0
13-1	5.5	0.423	0.150	0.403	0.025	1.6

[0532] **Elispot analysis**

[0533] Elispot analysis is used to analyze a cellular immune level in test animals, thereby providing a scientific basis for evaluating the effectiveness of candidate vaccines. Cytotoxic T lymphocytes (CTL cells) are commonly referred to as $CD8^+$ T cells and play an important role in the immune system to defend against pathogens (for example, viruses, bacteria, and tumors). IFN-γ, as a cytokine secreted by immunocompetent cells, plays an important immunomodulatory role in inducing cellular immunity, including activation of cytotoxic T lymphocytes (CTL), natural killer (NK) cells, and phagocytes. Currently, detection of an IFN-γ level has gradually replaced various traditional cellular immune detection methods and has become an important method for detecting a cellular immune effect.

[0534] An HPV mRNA injection was injected intramuscularly into mice at a dose of 25 μg/mouse based on mRNA, with 5 mice per sample. 7 days after administration, the mice were sacrificed, and spleens were removed to obtain a single-cell suspension of a concentration of 1.5 x 10^6 cells/ml. The number of $CD8^+$ IFN-γ-positive secretory-expressing cells was detected using an IFN-γ Elispot KIT. According to the Elispot KIT, positive cells secreting the cytokine IFN-γ showed clear and distinct spots through a chromogenic reaction. The spots were then counted using an ELISPOT analysis system, with 1 spot representing 1 active cell. Based on this, a frequency of the cells secreting the cytokine was calculated and typically expressed as a number of active cells in 10^6 cells.

[0535] IFN-γ secretion and expression effects in the mouse spleens of each sample were detected using an IFN-γ Elispot KIT, with a result expressed as a number of positive cells in 10^6 cells. An IFN-γ positive cell detection result of a sample without the addition of Ca^{2+} was set as 1, and other samples were compared with it. As shown in FIG. 2 (Formulations 21-1, 21-2, and 21-3) and FIG. 3 (Formulation 13-1), compared with an expression effect of the sample without the addition of Ca^{2+}, within an investigated range, the

expression effects of the samples containing different concentrations of Ca^{2+} are increased by 1-5 times.

[0536] The sequences used in the above examples of the present application are listed in the following sequence list. It should be understood that the following sequences are merely exemplary sequences of the embodiments of the present application and are not intended to limit the embodiments of the present application in any way. Nucleic acid sequences in the following sequence list may represent DNA sequences or RNA sequences, and when they represent RNA sequences, "T" denotes uridine.

[0537] **Sequence List:**

SEQ ID NO.	Name	Sequence
Amino acid sequence		
1	Flt3L	ITQDCSFQHSPISSDFAVKIRELSDYLLQDYPVTVASNLQDEELCGGLWRL VLAQRWMERLKTVAGSKMQGLLERVNTEIHFVTKCAFQPPPSCLRFVQT NISRLLQETSEQLVALKPWITRQNFSRCLELQCQPDSSTLPPPWSPRPLEAT APTAPGGGSGD
2	tPA-SP	MDAMKRGLCCVLLLCGAVFVSPS
3	HPV antigen sequence	MNVCQDKILEHYENDSKDILEHYENDSKDLCDHICDHIDYWKHIRLECAI MYKARIRLECAIMYKAREMGFHQFDGDICNTMHYTNWIYICEDAQCTV VEGQVDKKWEVHAGGQVILCPESGQRRIKRPRSENCHPNKLLILKCLRY RFKKHCKLSSTWHWTCHDGKHKWHWTCHDGKHKNAIVTLTYDHIDYW KAIRQENAIFFAARHQVVPALNICKAKACKAIEWNTEPKHCFKKGGQHIE VWFDYVAWDSVYYCGDDGWCKTEAEKYGCKGTWEVHFGNSIDCNDS MCSTFDDNVSATELVKMARFEDPTRRPYKLPDLCTELNTSLQDIEITCVY CKTVLELTEVFEFAFKDLFVVYRDSIPHAACHKCIDFYSRIRELRYYSDSV MYGPKATLQDIVLHLEPQNEIPVDLLCHEQLSDSEEENDEIDGVNHQHLP ARRAEPQRHTMLCMCFYSRIRELRYYSDSVYGDTLEKLTNTGLYNLLIRC LRCQKPLNPAEKLRHLNEKRRFHKIAGHYRGQCHSCCNRARQERLQRRR

		ETQVARRAEPQRHTMLCMCCKCEARIELVVESSADDLRAFQQLFLSTLSF VCPWCASQQMHQKRTAMFQDPQERPRKLPHLCTELQTTIHDIILECVYCK QQLLRREVYDFAFRDLCIVYRDGNPYAVCDKCLKFYSKISEYRYMHGDTP TLHEYMLDLQPETTDLYCYEQLNDSSEEEDEIDGPAGQAEPDRAHYNIVT FCCKCDSTLDKCLKFYSKISEYRYYCYSVYGTTLEQQYNKPLCDLLIRCIN CQKPLCPEEKQRHLDKKQRFHNIRGRWTGRCMSCCRSSRTRRETQLHYN IVTFCCKCDSTLRLCVQSTHVDIRTLEDLLMGTLGIVCPICSQKP
4	tPA-SP + Flt3L + HPV-antigen sequence	MDAMKRGLCCVLLLCGAVFVSPSITQDCSFQHSPISSDFAVKIRELSDYLL QDYPVTVASNLQDEELCGGLWRLVLAQRWMERLKTVAGSKMQGLLERV NTEIHFVTKCAFQPPPSCLRFVQTNISRLLQETSEQLVALKPWITRQNFSRC LELQCQPDSSTLPPPWSPRPLEATAPTAPGGGSGDMNVCQDKILEHYEND SKDILEHYENDSKDLCDHICDHIDYWKHIRLECAIMYKARIRLECAIMYK AREMGFHQFDGDICNTMHYTNWIYICEDAQCTVVEGQVDKKWEVHAG GQVILCPESGQRRIKRPRSENCHPNKLLILKCLRYRFKKHCKLSSTWHWT CHDGKHKWHWTCHDGKHKNAIVTLTYDHIDYWKAIRQENAIFFAARHQ VVPALNICKAKACKAIEWNTEPKHCFKKGGQHIEVWFDYVAWDSVYYC GDDGWCKTEAEKYGCKGTWEVHFGNSIDCNDSMCSTFDDNVSATELVK MARFEDPTRRPYKLPDLCTELNTSLQDIEITCVYCKTVLELTEVFEFAFKD LFVVYRDSIPHAACHKCIDFYSRIRELRYYSDSVMYGPKATLQDIVLHLEP QNEIPVDLLCHEQLSDSEEENDEIDGVNHQHLPARRAEPQRHTMLCMCF YSRIRELRYYSDSVYGDTLEKLTNTGLYNLLIRCLRCQKPLNPAEKLRHLN EKRRFHKIAGHYRGQCHSCCNRARQERLQRRRETQVARRAEPQRHTML CMCCKCEARIELVVESSADDLRAFQQLFLSTLSFVCPWCASQQMHQKRT AMFQDPQERPRKLPHLCTELQTTIHDIILECVYCKQQLLRREVYDFAFRDL CIVYRDGNPYAVCDKCLKFYSKISEYRYMHGDTPTLHEYMLDLQPETTDL YCYEQLNDSSEEEDEIDGPAGQAEPDRAHYNIVTFCCKCDSTLDKCLKFY SKISEYRYYCYSVYGTTLEQQYNKPLCDLLIRCINCQKPLCPEEKQRHLD KKQRFHNIRGRWTGRCMSCCRSSRTRRETQLHYNIVTFCCKCDSTLRLCV QSTHVDIRTLEDLLMGTLGIVCPICSQKP

Nucleic acid sequence component		
5	5’UTR	ACTCTTCTGGTCCCCACAGACTCAGAGAGAACCCACC
6	3’UTR	GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCCCCAG CCCCTCCTCCCCTTCCTGCACCCGTACCCCCGTGGTCTTTGAATAAAGT CTGAGTGGGCGGC
7	PolyA tail	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAGATATCAAAAAAAAAAAAAAAAAAAGAAAAA AAAAAAAAAAAAAAAGAAAAAAAAAAAAAAAAA
ORF sequence		
8	Polynucleotide sequence of tPA-SP	ATGGACGCCATGAAGCGCGGTCTTTGCTGCGTCCTGCTGTTATGCGGC GCTGTGTTCGTCAGCCCTAGC
9	Polynucleotide sequence of Flt3L	ATAACGCAGGACTGCAGCTTCCAGCACAGCCCTATATCTTCGGACTTCG CGGTCAAGATTCGGGAGCTCTCCGACTATCTGCTCCAGGACTATCCCGT CACGGTCGCCAGCAACCTTCAGGACGAAGAGTTGTGTGGCGGCCTGT GGCGGTTAGTCCTGGCGCAGCGTTGGATGGAGCGCCTGAAGACCGTG GCTGGTTCGAAGATGCAGGGCTTGCTGGAGCGGGTGAACACGGAGAT TCACTTCGTGACCAAGTGCGCGTTTCAGCCTCCGCCGAGCTGTCTGAG GTTCGTGCAGACGAACATCAGTCGGCTCTTGCAGGAGACTAGCGAGC AGTTGGTCGCGTTGAAGCCGTGGATCACCCGCCAGAACTTCTCACGTT GCCTTGAGCTGCAGTGCCAGCCGGATTCCTCGACCCTCCCTCCGCCGT GGTCGCCGCGGCCGCTGGAGGCCACGGCGCCCACGGCGCCTGGCGGC GGGAGCGGAGAT
10	Polynucleotide sequenc	ATGAACGTGTGCCAGGATAAGATCTTGGAGCATTACGAGAATGACTCC AAGGATATCCTGGAGCACTATGAGAACGACTCCAAGGATCTATGTGATC ACATCTGTGATCACATAGATTATTGGAAGCACATCCGGCTGGAGTGCGC CATTATGTATAAGGCTCGGATACGGCTGGAATGTGCTATAATGTATAAAG

	e for coding an HPV antigen	CCCGTGAGATGGGCTTTCACCAGTTCGATGGTGACATTTGTAACACAAT GCACTATACGAACTGGATATACATCTGCGAGGACGCGCAGTGTACGGTA GTTGAGGGTCAGGTAGACAAGAAGTGGGAGGTTCACGCGGGTGGGCA GGTCATCCTCTGCCCGGAGTCCGGGCAGAGGAGGATCAAGCGTCCGC GGAGTGAAAACTGCCACCCTAACAAGCTACTGATCCTCAAGTGCCTTC GCTATCGCTTCAAGAAGCATTGCAAGTTGTCGTCCACGTGGCACTGGA CTTGCCACGACGGCAAGCACAAGTGGCACTGGACGTGCCACGACGGC AAGCACAAGAACGCGATAGTGACGCTTACCTATGATCACATAGATTATT GGAAAGCGATCCGGCAGGAGAATGCGATATTCTTCGCTGCTAGACACC AGGTGGTGCCCGCGTTGAATATCTGCAAGGCGAAGGCCTGCAAGGCCA TAGAGTGGAACACAGAGCCGAAGCACTGCTTTAAGAAAGGCGGTCAG CACATCGAGGTCTGGTTCGACTATGTGGCCTGGGACTCGGTCTACTATT GCGGCGACGACGGTTGGTGCAAGACCGAGGCCGAGAAATACGGCTGC AAAGGGACGTGGGAAGTTCACTTCGGGAACTCCATCGATTGCAACGA CTCCATGTGCAGCACGTTTGACGATAACGTGTCTGCCACGGAGTTGGT AAAGATGGCCCGATTCGAAGATCCCACAAGAAGACCCTACAAGCTGCC CGACCTGTGCACCGAGCTGAACACAAGCCTCCAAGACATCGAGATTAC CTGCGTGTACTGCAAGACCGTGCTGGAGCTGACCGAGGTGTTCGAGTT CGCCTTCAAGGACCTGTTCGTGGTGTACAGAGACAGCATCCCACACGC CGCCTGCCACAAGTGCATCGACTTCTACAGCCGGATTCGGGAACTGCG GTACTACAGCGACAGCGTGATGTATGGGCCCAAGGCCACCCTGCAAGA CATCGTGCTGCACCTGGAGCCTCAGAACGAAATACCCGTGGACCTGCT GTGCCATGAGCAACTGAGCGACAGCGAGGAAGAGAATGATGAGATCG ACGGCGTGAATCATCAGCACCTGCCCGCTAGAAGGGCCGAGCCACAA CGGCACACCATGCTGTGCATGTGCTTCTACAGCAGAATCAGAGAACTC CGGTATTACAGCGACTCCGTGTACGGAGATACCCTCGAGAAGCTGACC AACACCGGCCTGTACAATCTGCTGATCAGATGTCTGAGATGTCAGAAA CCCTTAAATCCCGCCGAGAAGCTGAGACACCTGAACGAGAAGAGAAG ATTCCACAAGATCGCCGGCCACTACAGAGGGCAGTGCCACAGCTGCTG

		CAACAGAGCTAGACAAGAGAGACTGCAGAGAAGACGGGAGACCCAA GTGGCTAGAAGGGCTGAGCCGCAAAGACATACCATGCTGTGTATGTGC TGCAAATGCGAGGCTAGAATCGAGCTGGTGGTGGAGAGCAGCGCCGA CGACCTGAGAGCCTTTCAGCAGCTGTTCCTGAGCACCCTGAGCTTCGT GTGTCCCTGGTGCGCTAGCCAACAGATGCATCAGAAGAGAACCGCCAT GTTCCAAGATCCGCAAGAGAGACCTAGAAAACTCCCTCATCTGTGCAC CGAACTGCAGACCACCATCCACGACATCATCCTGGAGTGCGTGTATTG CAAACAACAGCTGCTGAGAAGAGAGGTGTACGACTTCGCCTTCAGAG ACCTGTGCATCGTGTACCGGGACGGCAATCCCTACGCCGTGTGCGACA AGTGCCTCAAGTTCTATAGCAAGATCAGCGAGTATAGATACATGCACGG AGATACGCCGACCCTGCACGAGTACATGCTGGACCTGCAGCCCGAGAC CACCGACCTGTACTGCTACGAGCAGCTGAACGACAGCAGCGAAGAGG AGGACGAGATTGACGGCCCGGCCGGCCAAGCCGAGCCCGACAGAGCC CACTACAACATCGTGACCTTCTGCTGTAAATGCGATAGCACACTGGAC AAGTGCCTGAAGTTCTACAGCAAGATTAGCGAGTACAGATATTACTGCT ACTCCGTGTACGGGACAACCCTGGAGCAGCAGTACAACAAGCCTCTG TGCGACCTGCTGATCCGGTGTATTAACTGTCAGAAGCCCTTGTGTCCCG AGGAGAAGCAGAGACACCTGGACAAGAAGCAGAGATTCCACAACATC AGAGGCAGATGGACCGGCAGATGCATGAGCTGCTGCAGAAGCAGCAG AACAAGAAGAGAGACACAACTGCACTACAATATCGTCACCTTCTGCTG CAAGTGCGACTCCACACTGCGGCTCTGCGTGCAGAGCACCCACGTGG ACATCAGAACCCTGGAGGACCTGCTGATGGGCACCCTGGGCATCGTGT GTCCCATCTGCAGCCAAAAGCCCTGATGA
11	Polynucleotide sequence of ORF	ATGGACGCCATGAAGCGCGGTCTTTGCTGCGTCCTGCTGTTATGCGGC GCTGTGTTCGTCAGCCCTAGCATAACGCAGGACTGCAGCTTCCAGCAC AGCCCTATATCTTCGGACTTCGCGGTCAAGATTCGGGAGCTCTCCGACT ATCTGCTCCAGGACTATCCCGTCACGGTCGCCAGCAACCTTCAGGACG AAGAGTTGTGTGGCGGCCTGTGGCGGTTAGTCCTGGCGCAGCGTTGGA TGGAGCGCCTGAAGACCGTGGCTGGTTCGAAGATGCAGGGCTTGCTG

		GAGCGGGTGAACACGGAGATTCACTTCGTGACCAAGTGCGCGTTTCA GCCTCCGCCGAGCTGTCTGAGGTTCGTGCAGACGAACATCAGTCGGCT CTTGCAGGAGACTAGCGAGCAGTTGGTCGCGTTGAAGCCGTGGATCAC CCGCCAGAACTTCTCACGTTGCCTTGAGCTGCAGTGCCAGCCGGATTC CTCGACCCTCCCTCCGCCGTGGTCGCCGCGGCCGCTGGAGGCCACGGC GCCCACGGCGCCTGGCGGCGGGAGCGGAGATATGAACGTGTGCCAGG ATAAGATCTTGGAGCATTACGAGAATGACTCCAAGGATATCCTGGAGCA CTATGAGAACGACTCCAAGGATCTATGTGATCACATCTGTGATCACATA GATTATTGGAAGCACATCCGGCTGGAGTGCGCCATTATGTATAAGGCTC GGATACGGCTGGAATGTGCTATAATGTATAAAGCCCGTGAGATGGGCTT TCACCAGTTCGATGGTGACATTTGTAACACAATGCACTATACGAACTGG ATATACATCTGCGAGGACGCGCAGTGTACGGTAGTTGAGGGTCAGGTA GACAAGAAGTGGGAGGTTCACGCGGGTGGGCAGGTCATCCTCTGCCC GGAGTCCGGGCAGAGGAGGATCAAGCGTCCGCGGAGTGAAAACTGCC ACCCTAACAAGCTACTGATCCTCAAGTGCCTTCGCTATCGCTTCAAGAA GCATTGCAAGTTGTCGTCCACGTGGCACTGGACTTGCCACGACGGCAA GCACAAGTGGCACTGGACGTGCCACGACGGCAAGCACAAGAACGCGA TAGTGACGCTTACCTATGATCACATAGATTATTGGAAAGCGATCCGGCA GGAGAATGCGATATTCTTCGCTGCTAGACACCAGGTGGTGCCCGCGTT GAATATCTGCAAGGCGAAGGCCTGCAAGGCCATAGAGTGGAACACAG AGCCGAAGCACTGCTTTAAGAAAGGCGGTCAGCACATCGAGGTCTGG TTCGACTATGTGGCCTGGGACTCGGTCTACTATTGCGGCGACGACGGTT GGTGCAAGACCGAGGCCGAGAAATACGGCTGCAAAGGGACGTGGGA AGTTCACTTCGGGAACTCCATCGATTGCAACGACTCCATGTGCAGCAC GTTTGACGATAACGTGTCTGCCACGGAGTTGGTAAAGATGGCCCGATT CGAAGATCCCACAAGAAGACCCTACAAGCTGCCCGACCTGTGCACCG AGCTGAACACAAGCCTCCAAGACATCGAGATTACCTGCGTGTACTGCA AGACCGTGCTGGAGCTGACCGAGGTGTTCGAGTTCGCCTTCAAGGAC CTGTTCGTGGTGTACAGAGACAGCATCCCACACGCCGCCTGCCACAAG

		TGCATCGACTTCTACAGCCGGATTCGGGAACTGCGGTACTACAGCGAC AGCGTGATGTATGGGCCCAAGGCCACCCTGCAAGACATCGTGCTGCAC CTGGAGCCTCAGAACGAAATACCCGTGGACCTGCTGTGCCATGAGCAA CTGAGCGACAGCGAGGAAGAGAATGATGAGATCGACGGCGTGAATCA TCAGCACCTGCCCGCTAGAAGGGCCGAGCCACAACGGCACACCATGC TGTGCATGTGCTTCTACAGCAGAATCAGAGAACTCCGGTATTACAGCG ACTCCGTGTACGGAGATACCCTCGAGAAGCTGACCAACACCGGCCTGT ACAATCTGCTGATCAGATGTCTGAGATGTCAGAAACCCTTAAATCCCGC CGAGAAGCTGAGACACCTGAACGAGAAGAGAAGATTCCACAAGATCG CCGGCCACTACAGAGGGCAGTGCCACAGCTGCTGCAACAGAGCTAGA CAAGAGAGACTGCAGAGAAGACGGGAGACCCAAGTGGCTAGAAGGG CTGAGCCGCAAAGACATACCATGCTGTGTATGTGCTGCAAATGCGAGG CTAGAATCGAGCTGGTGGTGGAGAGCAGCGCCGACGACCTGAGAGCC TTTCAGCAGCTGTTCCTGAGCACCCTGAGCTTCGTGTGTCCCTGGTGC GCTAGCCAACAGATGCATCAGAAGAGAACCGCCATGTTCCAAGATCCG CAAGAGAGACCTAGAAAACTCCCTCATCTGTGCACCGAACTGCAGAC CACCATCCACGACATCATCCTGGAGTGCGTGTATTGCAAACAACAGCT GCTGAGAAGAGAGGTGTACGACTTCGCCTTCAGAGACCTGTGCATCGT GTACCGGGACGGCAATCCCTACGCCGTGTGCGACAAGTGCCTCAAGTT CTATAGCAAGATCAGCGAGTATAGATACATGCACGGAGATACGCCGACC CTGCACGAGTACATGCTGGACCTGCAGCCCGAGACCACCGACCTGTAC TGCTACGAGCAGCTGAACGACAGCAGCGAAGAGGAGGACGAGATTGA CGGCCCGGCCGGCCAAGCCGAGCCCGACAGAGCCCACTACAACATCG TGACCTTCTGCTGTAAATGCGATAGCACACTGGACAAGTGCCTGAAGT TCTACAGCAAGATTAGCGAGTACAGATATTACTGCTACTCCGTGTACGG GACAACCCTGGAGCAGCAGTACAACAAGCCTCTGTGCGACCTGCTGAT CCGGTGTATTAACTGTCAGAAGCCCTTGTGTCCCGAGGAGAAGCAGAG ACACCTGGACAAGAAGCAGAGATTCCACAACATCAGAGGCAGATGGA CCGGCAGATGCATGAGCTGCTGCAGAAGCAGCAGAACAAGAAGAGAG

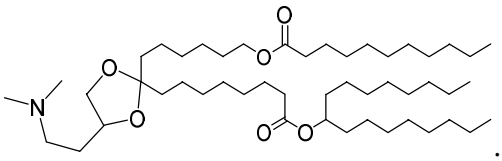
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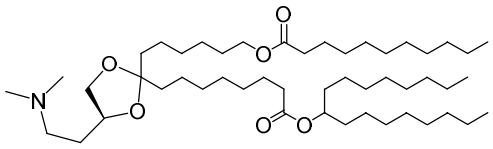
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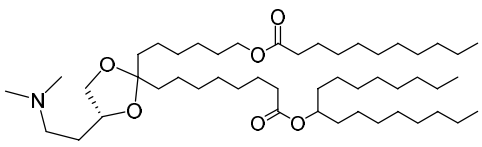
WHAT IS CLAIMED IS:

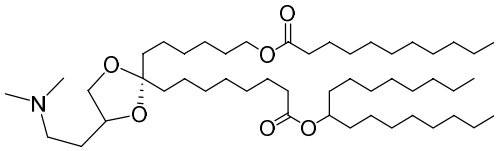
1. A lipid nanoparticle, comprising an ionizable lipid, a phospholipid, a structural lipid, and a PEG lipid in a molar ratio of the ionizable lipid, the phospholipid, the structural lipid, and the PEG lipid of (40-50):(5-15):(30-50):(1-3), wherein the ionizable lipid is compound 5, or a salt or stereoisomer thereof,

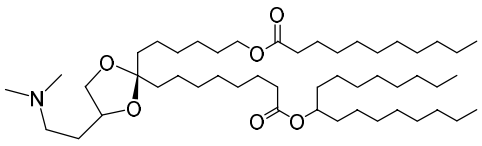
compound 5 .

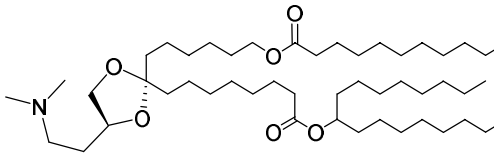
2. The lipid nanoparticle according to claim 1, wherein a structure of the stereoisomer of the ionizable lipid is selected from one or more of

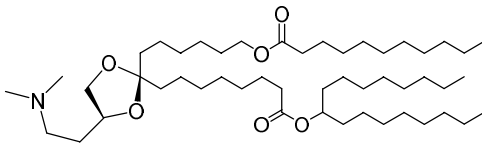
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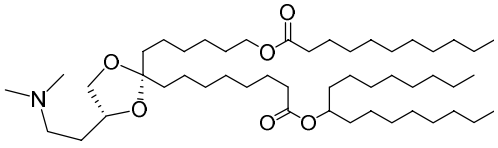
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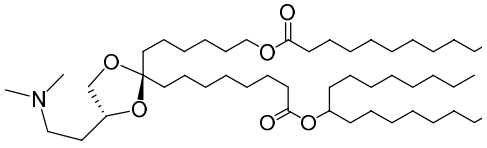
compound 5C 

compound 5D 

compound 5E 

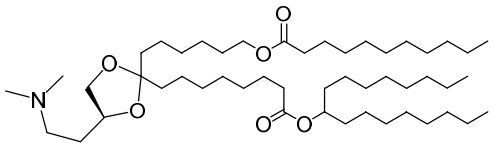
compound 5F 

compound 5G 

compound 5H 

.

3. The lipid nanoparticle according to claim 1, wherein a structure of the ionizable lipid is selected from



compound 5A.

4. The lipid nanoparticle according to any one of claims 1-3, wherein the phospholipid is selected from one or two or more of the following compounds:

dilauroylphosphatidylcholine (DLPC),

dimyristoylphosphatidylcholine (DMPC),

dioleoylphosphatidylcholine (DOPC),

dipalmitoylphosphatidylcholine (DPPC),

distearoylphosphatidylcholine (DSPC),

dioleoylphosphatidylcholine (DUPC),

palmitoyloleoylphosphatidylcholine (POPC),

1,2-di-O-octadecyl-sn-glycero-3-phosphocholine (18:0 Diether PC),

1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC),

1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC),

1,2-divinyl-sn-glycero-3-phosphocholine,

1,2-diarylacyl-sn-glycero-3-phosphocholine,

1,2-dioleoyl-SN-glycero-3-phosphoethanolamine (DOPE),

1,2-dihydroxystannyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE),

1,2-distearoyl-sn-glycero-3-phosphoethanolamine,

1,2-divinylol-sn-glycero-3-phosphoethanolamine,

1,2-divinyl-sn-glycero-3-phosphoethanolamine,

1,2-diarylyl-sn-glycero-3-phosphoethanolamine,

1,2-dithiohexaenoate-sn-glycero-3-phosphoethanolamine,

1,2-diol-sn-glycero-3-phospho-(1-glycerol) sodium salt (DOPG), or sphingomyelin.

5. The lipid nanoparticle according to any one of claims 1-3, wherein the phospholipid is DSPC.

6. The lipid nanoparticle according to any one of claims 1-3, wherein the structural lipid is selected from one or two or more of cholesterol, coprostanol, sitosterol, ergosterol, and stigmasterol.

7. The lipid nanoparticle according to any one of claims 1-3, wherein the structural lipid is cholesterol.

8. The lipid nanoparticle according to any one of claims 1-3, wherein the PEG lipid is selected from one or two or more of PEG-modified phosphatidylethanolamine, PEG-modified phosphatidic acid, PEG-modified ceramide, PEG-modified dialkylamine, PEG-modified diacylglycerol, or PEG-modified dialkylglycerol.

9. The lipid nanoparticle according to any one of claims 1-3, wherein the PEG lipid is DMG-PEG2000.

10. The lipid nanoparticle according to any one of claims 1-3, wherein the molar ratio of the ionizable lipid, the phospholipid, the structural lipid, and the PEG lipid is (40-50):(10-15):(35-45):(1.5-2.5), (45-49):(10-15):(35-45):(1.5-2.5), or (48-49):(10-15):(35-45):(1.5-2.5).

11. The lipid nanoparticle according to any one of claims 1-3, wherein

a content of the ionizable lipid is 40 mol% to 50 mol%, preferably 45 mol% to 49 mol%, and further preferably 48 mol% to 49 mol%,

a content of the phospholipid is 5 mol% to 15 mol%, preferably 10 mol% to 15 mol%,

a content of the structural lipid is 30 mol% to 50 mol%, preferably 35 mol% to 45 mol%, and

a content of the PEG lipid is 1 mol% to 3 mol%, preferably 1.5 mol% to 2.5 mol%,

wherein mol% is calculated based on a mole number of total lipids in a lipid nanoparticle composition.

12. The lipid nanoparticle according to any one of claims 1-3, wherein the molar ratio of the ionizable lipid, the phospholipid, the structural lipid, and the PEG lipid is not 50:10:38.5:1.5.

13. A lipid nanoparticle composition, comprising the lipid nanoparticle according to any one of claims 1-12 and a therapeutic agent and/or a prophylactic agent, wherein the therapeutic agent and/or the prophylactic agent is selected from a vaccine or a compound,

nucleic acid, or protein capable of eliciting an immune response, and the therapeutic agent and/or the prophylactic agent is encapsulated by the lipid nanoparticle.

14. The lipid nanoparticle composition according to claim 13, wherein the nucleic acid is RNA, and the RNA is selected from one or two or more of siRNA, aiRNA, miRNA, dsRNA, shRNA, or mRNA.

15. The lipid nanoparticle composition according to claim 14, wherein the nucleic acid is mRNA.

16. The lipid nanoparticle composition according to claim 15, wherein the mRNA encodes a protein comprising a sequence as shown in SEQ ID NO: 3 or SEQ ID NO: 4, or wherein the mRNA encodes a protein having a sequence as shown in SEQ ID NO: 3 or SEQ ID NO: 4.

17. The lipid nanoparticle composition according to claim 15, wherein a coding sequence of the mRNA comprises a sequence as shown in SEQ ID NO: 10 or SEQ ID NO: 11, or wherein a coding sequence of the mRNA is as shown in SEQ ID NO: 10 or SEQ ID NO: 11.

18. The lipid nanoparticle composition according to claim 15, wherein a coding sequence of the mRNA comprises a sequence as shown in SEQ ID NO: 12, or wherein a coding sequence of the mRNA is as shown in SEQ ID NO: 12.

19. The lipid nanoparticle composition according to any one of claims 15-18, wherein the coding sequence of the mRNA comprises a 5' UTR coding sequence, and preferably, the 5' UTR coding sequence is as shown in SEQ ID NO: 5.

20. The lipid nanoparticle composition according to any one of claims 15-18, wherein the coding sequence of the mRNA comprises a 3' UTR coding sequence, and preferably, the 3' UTR coding sequence is as shown in SEQ ID NO: 6.

21. The lipid nanoparticle composition according to any one of claims 15-18, wherein the coding sequence of the mRNA comprises a poly A tail coding sequence, and preferably, the poly A tail coding sequence is as shown in SEQ ID NO: 7.

22. The lipid nanoparticle composition according to any one of claims 15-18, wherein the coding sequence of the mRNA comprises a 5' cap, and preferably, the 5' cap has a structure of m7G(5')ppp(5')(2'-OMeA)pG.

23. The lipid nanoparticle composition according to any one of claims 15-18, wherein

part or all of uridine in the mRNA molecule is 1-methyl-pseudouridine.

24. The lipid nanoparticle composition according to any one of claims 15-18, wherein an N/P ratio of the ionizable lipid to the mRNA is 3.5-5.5, preferably 5.2.

25. The lipid nanoparticle composition according to any one of claims 15-18, further comprising a divalent metal cation, wherein the divalent metal cation is preferably Ca^{2+}, Mg^{2+}, Zn^{2+}, or Mn^{2+}.

26. A pharmaceutical composition, comprising the lipid nanoparticle according to any one of claims 1-12 or the lipid nanoparticle composition according to any one of claims 13-25 and a pharmaceutically acceptable excipient or auxiliary ingredient.

27. The pharmaceutical composition according to claim 26, further comprising a divalent metal cation, wherein the divalent metal cation is preferably Ca^{2+}, Mg^{2+}, Zn^{2+}, or Mn^{2+}.