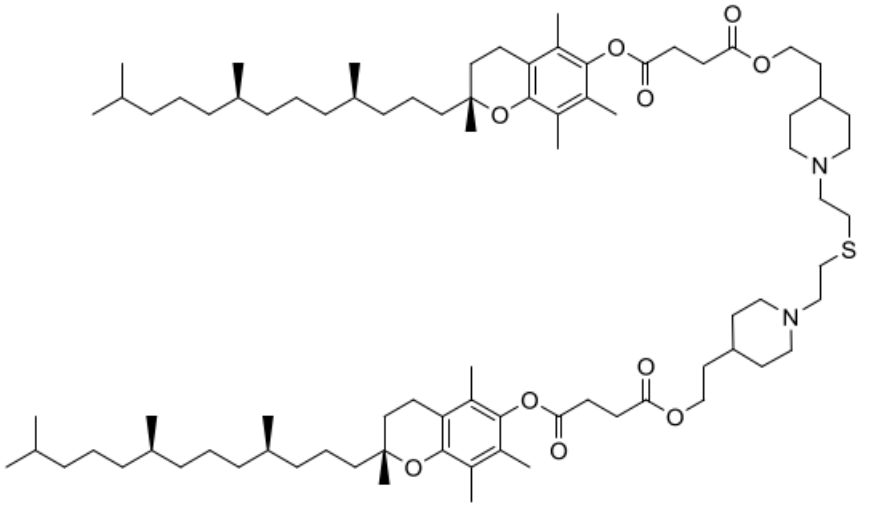
In an aspect, there is provided a cationic lipid

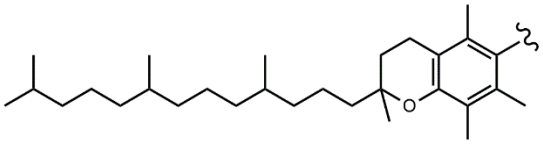


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In another aspect, there is provided a composition comprising the cationic lipid as defined above.

In this regard, an alkanediyl is a term for a (–C_nH_{2n}–) group; f.e. an "alkanediyl having 2 to 8 carbon atoms" accordingly equals an alkanediyl group having the formula –C_2H_4–, –C_3H_6–, –C_4H_8–, –C_5H_{10}–, –C_6H_{12}–, –C_7H_{14}–, or respectively –C_8H_{16}–. In other words, an alkanediyl is a series of divalent radicals of the general formula C_nH_{2n} derived from aliphatic hydrocarbons. Unless specified otherwise, such alkanediyls include substituted alkanediyls.

In another example, in case R^1, R^2 and R^5 are all ethanediyl, A is –S–S–, and R^a and R^b are identical, then R^4 is not



, or respectively in one example, a lipid according to formula (I) is not lipid C23 as disclosed in **Table 1** herein or respectively lipid SS-EC as described herein below (for the avoidance of doubt i.e. in some selected examples, cationic lipid COATSOME® SS-EC is disclaimed from examples which are related to cationic lipids according to formula (I)).

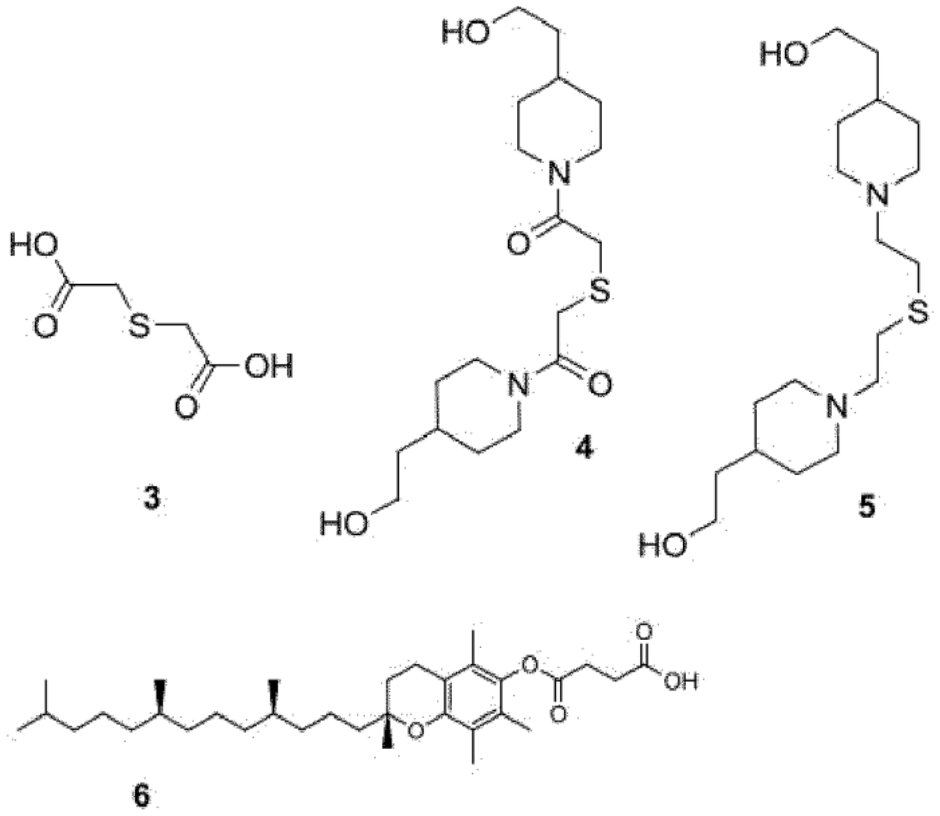
In another example, the disclosure describes novel compositions incorporating cationic lipids such as the novel cationic lipids defined above.

The cationic lipids and the compositions have been found to be particularly effective in introducing nucleic acids into living cells. For example, they enable improved RNA (e.g. mRNA) vaccines i.e. mRNA-based vaccines against certain infectious diseases or tumours.

In further examples, the disclosure describes the use of the compositions incorporating a cationic lipid and a nucleic acid compound as medicines, and in particular as vaccines, as well as vaccination methods based on these vaccines. In one aspect, the present disclosure refers to use of the composition as described herein in the manufacture of a medicament (i) in the treatment or prophylaxis of infectious diseases; cancer or tumour diseases, disorders or conditions; liver diseases selected from the group consisting of liver fibrosis, liver cirrhosis and liver cancer; allergies; or autoimmune disease; disorder or condition; and/or (ii) for enzyme replacement therapy in treating metabolic or endocrine disorders; the composition preferably comprising at least one coding RNA, wherein said at least one coding RNA comprises at least one coding sequence encoding at least one peptide or protein for use in treatment or prevention of a disease, disorder or condition, wherein said composition is to be administered via intramuscular or intradermal injection a subject in need thereof.

In another aspect of the present disclosure, the present disclosure also describes a kit, in particular a kit of parts, comprising the mRNA compound comprising mRNA sequence as defined herein and at least one lipid according to formula (I) or formula (II) as defined herein. In yet another aspect, the present disclosure refers to a kit or kit of parts, comprising any one of the compositions as disclosed herein, optionally comprising a liquid vehicle for solubilizing, and, optionally, technical instructions providing information on administration and dosage of the components. In a further aspect, the present disclosure describes a use of the composition or a kit or kit of parts as described herein in the manufacture of a vaccine for prevention, prophylaxis, treatment and/or amelioration of a disease selected from infectious diseases including viral, bacterial or protozoological infectious diseases, cancer or tumour diseases. In another aspect, the present disclosure refers to a pharmaceutical composition comprising a composition or a kit or kit of parts as described herein for use as a medicament in vaccination of a subject comprising an effective dose of mRNA encoding a virus antigen.

In another aspect, there is provided a method of making the cationic lipid as defined above, the method comprising coupling dicarboxylic acid derivative 3 and 4-piperidineethanol to give bisamide 4, subsequent reduction with $LiAlH_4$ to give molecule 5, and coupling with Vitamin E derivative 6 to give the compound as defined above:



In another aspect, there is provided a method of making a lipid nanoparticle composition containing mRNA as defined above, compositing the steps of:

(i) providing;

a) the cationic lipid as defined above;

b) a polymer conjugated lipid;

c) at least one mRNA compound comprising an mRNA sequence encoding at least one antigenic peptide or protein,

d) optionally, a steroid; and

e) optionally a neutral lipid;

(ii) solubilizing the cationic lipid and/or the polymer conjugated lipid and optionally the neutral lipid and/or the steroid or a steroid derivative in an alcohol;

(iii) mixing the alcoholic lipid solution with an aqueous solution comprising the mRNA; (iv) removing the alcohol to form lipid nanoparticle encapsulating or associating with the mRNA;

and optionally

(v) separating or purifying the lipid nanoparticles.

DEFINITIONS

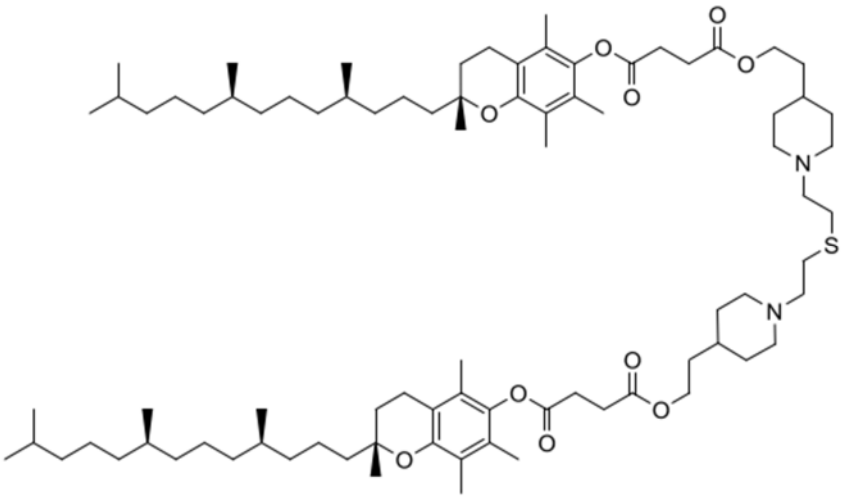
For the sake of clarity and readability, the following scientific background information and definitions are provided. Any technical features mentioned herein or disclosed thereby can be part of or may be read on each and every embodiment of the invention. Additional definitions and explanations can be provided in the context of this disclosure.

Unless defined otherwise, or unless the specific context requires otherwise, all technical terms used herein have the same meaning as is commonly understood by a person skilled in the relevant technical field.
Unless the context indicates or requires otherwise, the words “comprise”, “comprises” and “comprising” and similar expressions are to be construed in an open and inclusive sense, as “including, but not limited to” in this description and in the claims.

The expressions, “one embodiment”, “an embodiment”, “a specific embodiment” and the like mean that a particular feature, property or characteristic, or a particular group or combination of features, properties or characteristics, as referred to in combination with the respective expression, is present in at least one of the embodiments of the invention. The occurrence of these expressions in various places throughout this description do not necessarily

CLAIMS

1. A cationic lipid



2. A composition comprising

the cationic lipid of **claim 1**.

3. The composition of claim 2, further comprising one or more of the following excipients:

(i) a steroid, preferably cholesterol;

(ii) a neutral lipid;

wherein said neutral lipid preferably is 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (DPhyPE), optionally in combination with the neutral lipid 1,2-diheptanoyl-sn-glycero-3-phosphocholine (DHPC); or wherein said neutral lipid is a zwitterionic compound, optionally a zwitterionic compound having two fatty acid moieties selected from myristoyl, palmitoyl, stearoyl and oleyol, in combination with a zwitterionic compound having two fatty acid moieties selected from pentanoyl, hexanoyl, heptanoyl, octanoyl, nonaoyl and decanoyl;

and/or

(iii) a polymer conjugated lipid;

wherein said polymer conjugated lipid is a compound according to formula (II):

P-A-L formula (II);

wherein P is a hydrophilic polymer moiety, A is an optional linker, and L is a lipid moiety.

4. The composition of claim 3, comprising the following excipients:

(i) a steroid, which is cholesterol;

(ii) a neutral lipid, which is 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (DPhyPE),

and

(iii) a polymer conjugated lipid, which is a compound according to formula (II):

P-A-L formula (II);

wherein P is a hydrophilic polymer moiety, A is an optional linker, and L is a lipid moiety.

5. The composition of any one of **claims 2 to 4** wherein

the composition comprises excipients in a ratio selected from the group consisting of

(a-i) the cationic lipid at an amount of 30-70 mol%; the steroid at an amount of 20-50 mol%; the neutral lipid at an amount of 5-25 mol%; and the polymer conjugated lipid at an amount of 0.5-5 mol%;

(a-ii) the cationic lipid at an amount of 40-70 mol%; the steroid at an amount of 20-50 mol%; the neutral lipid at an amount of 5-15 mol%; and the polymer conjugated lipid at an amount of 0.5-5 mol%;

(a-iii) the cationic lipid at an amount of 20-60 mol%; the steroid at an amount of 25-55 mol%; the neutral lipid, which is a phospholipid at an amount of 5-25 mol%; and the polymer conjugated lipid at an amount of 0.5-15 mol%;

(a-iv) the cationic lipid at an amount of 45-65 mol%; the steroid at an amount of 25-45 mol%; the neutral lipid, which is a phospholipid at an amount of 8-12 mol%; and the polymer conjugated lipid at an amount of 1-3 mol%;

(a-v) the cationic lipid at an amount of 45-65 mol%; cholesterol at an amount of 25-45 mol%; the neutral lipid at an amount of 8-12 mol%; and the polymer conjugated lipid at an amount of 1-3 mol%(a-vi) the cationic lipid at an amount of 45-65 mol%; cholesterol at an amount of 25-45 mol%; DPhyPE at an amount of 8-12 mol% and optionally DHPC at an amount of 1 to 10 mol%; and the polymer conjugated lipid at an amount of 1-3 mol%.

6. The composition of any one of **claims 2 to 5**, further comprising a biologically active ingredient;

wherein the biologically active ingredient is preferably a nucleic acid compound selected from the group consisting of an artificial mRNA, chemically modified or unmodified messenger RNA (mRNA) comprising at least one coding sequence, self-replicating RNA, circular RNA, viral RNA, and replicon RNA; or any combination thereof, more preferably wherein the biologically active ingredient is an mRNA or an mRNA compound.

7. The composition of **claim 6**, wherein the composition comprises the mRNA

(i) at an amount such as to achieve an N/P ratio in the range of 10 to 20; or

(ii) at an amount such as to achieve a lipid: mRNA weight ratio in the range of 20 to 60, or in the range of 10 to 50; and/or wherein the composition is a sterile solid composition for reconstitution with a sterile liquid carrier, and wherein the composition further comprises one or more inactive ingredients selected from pH-modifying agents, bulking agents, stabilizers, non-ionic surfactants and antioxidants, and wherein the sterile liquid carrier is an aqueous carrier; and/or wherein the composition is a sterile liquid composition, and wherein the lipid nanoparticles have a mean hydrodynamic diameter as determined by dynamic laser scattering from 50 nm to 300 nm, or from 60 nm to 250 nm, or from 60 nm to 200 nm, or from 70 to 200 nm, or from 75 nm to 160, or from 90 nm to 140 nm, or from 100 nm to 140 nm; and/or wherein the lipid nanoparticles exhibit a zeta potential in the range of -50 mV to +50 mV.

8. The composition of **claim 6 or 7**, wherein the mRNA compound is a mono-, bi-, or multicistronic mRNA; or

wherein the mRNA compound comprises at least one chemical modification;
wherein the chemical modification is preferably selected from the group consisting of base modifications, sugar modifications, backbone modifications and lipid modifications, more preferably wherein the chemical modification is a base modification, most preferably wherein the base modification is selected from the group consisting of pseudouracil (ψ), N1-methylpseudouracil (N1Mψ), 1-ethylpseudouracil, 2-thiouracil (s2U), 4-thiouracil, 5-methylcytosine, 5-methyluracil, 5-methoxyuracil, and any combination thereof.

9. The composition of any one of **claims 6 to 8**, wherein the mRNA compound comprises a coding region encoding a peptide or protein, wherein the coding region exhibits a sequence modification;
wherein the sequence modification is preferably selected from a G/C content modification, a codon modification, a codon optimization or a C-optimization of the sequence; more preferably wherein
- the G/C content of the coding region is increased;
- the C content of the coding region is increased;
- the codon usage in the coding region is adapted to the human codon usage; and/or the codon adaptation index (CAI) is increased or maximised in the coding region
compared with the coding region of the corresponding wild-type mRNA.

10. The composition of any one of **claims 6 to 9**, wherein the mRNA compound further comprises
a) a 5'-CAP structure;

b) at least one miRNA sequence, preferably wherein the microRNA binding site is for a microRNA selected from the group consisting of miR-126, miR-142, miR-144, miR-146, miR-150, miR-155, miR-16, miR-21, miR-223, miR-24, miR-27, miR-26a, or any combination thereof;

c) at least one 5'-UTR element;

d) at least one poly(A) sequence;

e) at least one poly(C) sequence;

f) at least one 3'-UTR element;

or any combinations of these; and/or

wherein the least one coding RNA comprises a 5'-CAP structure, preferably m7G, CAP0, CAP1, CAP2, a modified CAP0 or a modified CAP1 structure; and/or

wherein the at least one coding RNA comprises at least one heterologous 5'-UTR and/or at least one heterologous 3'-UTR, preferably wherein the at least one heterologous 5'-UTR comprises a nucleic acid sequence derived from a 5'-UTR of a gene selected from HSD17B4, RPL32, ASAH1, ATP5A1, MP68, NDUFA4, NOSIP, RPL31, SLC7A3, TUBB4B and UBQLN2, or from a homolog of any one of these genes; and/or

preferably wherein the at least one heterologous 3'-UTR comprises a nucleic acid sequence derived from a 3'-UTR of a gene selected from PSMB3, ALB7, alpha-globin, CASP1, COX6B1, GNAS, NDUFA1 and RPS9, or from a homolog of any one of these genes; and/or

wherein the at least one coding RNA comprises a (i) HSD17B4 5'-UTR and a PSMB3 3'-UTR or (ii) a RPL32 5'-UTR and an ALB7 3'-UTR; and/or

comprising the following elements in the 5' to 3' direction:

a) a 5'-CAP structure, preferably selected from the group consisting of m7G(5'), m7G(5')ppp(5')(2'OMeA) and m7G(5')ppp(5')(2'OMeG);

b) a 5'-UTR element comprising a nucleic acid sequence derived from the 5'-UTR of a TOP gene, said nucleic acid sequence preferably comprising an RNA sequence that corresponds to the nucleic acid sequence according to SEQ ID NO:22, 24, 26, or a homolog thereof;

c) at least one coding sequence;

d) a 3'-UTR element comprising a nucleic acid sequence derived from an α-globin gene, said nucleic acid sequence preferably comprising an RNA sequence that corresponds to the nucleic acid sequence according to SEQ ID NO:6, 8, 10, 12, 14, 16, 18, 20, or a homolog thereof; and/or a 3'-UTR element comprising a nucleic acid sequence derived from an albumin gene, said nucleic acid sequence preferably comprising an RNA sequence that corresponds to the nucleic acid sequence according to SEQ ID NO:18, or a homolog thereof;

e) optionally, at least one poly(A) sequence, preferably consisting of 10 to 200, 10 to 100, 40 to 80, or 50 to 70 adenosine nucleotides;

f) optionally, at least one poly(C) sequence, preferably consisting of 10 to 200, 10 to 100, 20 to 70, 20 to 60 or 10 to 40 cytosine nucleotides; and

g) optionally, at least one histone stem-loop, preferably comprising the RNA sequence according to SEQ ID NO:4; and/or

wherein the biologically active ingredient is

(a) an mRNA comprising at least one coding sequence encoding a peptide or protein, wherein the peptide or protein is an antigen, wherein the antigen preferably is derived from pathogenic antigens, tumour antigens, allergenic antigens or autoimmune self-antigens; or

(b) an mRNA comprising at least one coding sequence encoding a therapeutic protein, wherein the therapeutic protein is selected from the group consisting of

(i) therapeutic proteins for use in enzyme replacement therapy for the treatment of metabolic, endocrine or amino acid disorders or for use in replacing an absent, deficient or mutated protein;

(ii) therapeutic proteins for use in the treatment of blood disorders, diseases of the circulatory system, diseases of the respiratory system, infectious diseases or immune deficiencies;

(iii) therapeutic proteins for use in the treatment of cancer or tumour diseases;

(iv) therapeutic proteins for use in hormone replacement therapy;

(v) therapeutic proteins for use in reprogramming somatic cells into pluri- or omnipotent stem cells;

(vi) therapeutic proteins for use as adjuvant or immunostimulation;

(vii) therapeutic proteins being a therapeutic antibody;

(viii) therapeutic proteins being a gene editing agent; and

(ix) therapeutic proteins for use in treating or preventing a liver disease selected from the group consisting of liver fibrosis, liver cirrhosis and liver cancer;

wherein the antigen of subitem (a) encodes a pathogenic antigen selected from the group consisting of a bacterial, viral, fungal and protozoal antigen;

wherein the pathogenic antigen is preferably derived from a SARS coronavirus 2 (SARS-CoV-2), nCoV-2019 coronavirus, SARS coronavirus (SARS-CoV), Bunyavirales virus, Cytomegalovirus (CMV), Dengue viruses (DEN-1, DEN-2, DEN-3 and DEN-4), Ebola virus, Flavivirus, Hepatitis B virus (HBV), Herpes simplex virus (HSV), Human immunodeficiency virus (HIV), Human metapneumovirus (hMPV), Human Papilloma virus (HPV), Human parainfluenza viruses (HPIV), Influenza virus, extraintestinal pathogenic E. coli, Lassa mammarenavirus (LASV), MERS coronavirus, Mycobacterium tuberculosis, Nipah virus, Norovirus, Rabies virus, Respiratory Syncytial Virus (RSV), Rhinovirus, Rota

virus, Vaccinia virus, Yellow Fever Virus, Zika virus, Chlamydia trachomatis (i.e. bacterium chlamydia causing chlamydia), or Malaria parasite (e.g. Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, or Plasmodium ovale).

11. Use of the composition of any one of **claims 6 to 10** in the manufacture of a medicament
(i) in the treatment or prophylaxis of infectious diseases; cancer or tumour diseases, disorders or conditions; liver diseases selected from the group consisting of liver fibrosis, liver cirrhosis and liver cancer; allergies; or autoimmune disease. disorder or condition; and/or
(ii) for enzyme replacement therapy in treating metabolic or endocrine disorders;

the composition preferably comprising at least one coding RNA, wherein said at least one coding RNA comprises at least one coding sequence encoding at least one peptide or protein for use in treatment or prevention of a disease, disorder or condition, wherein said composition is to be administered via intramuscular or intradermal injection a subject in need thereof.

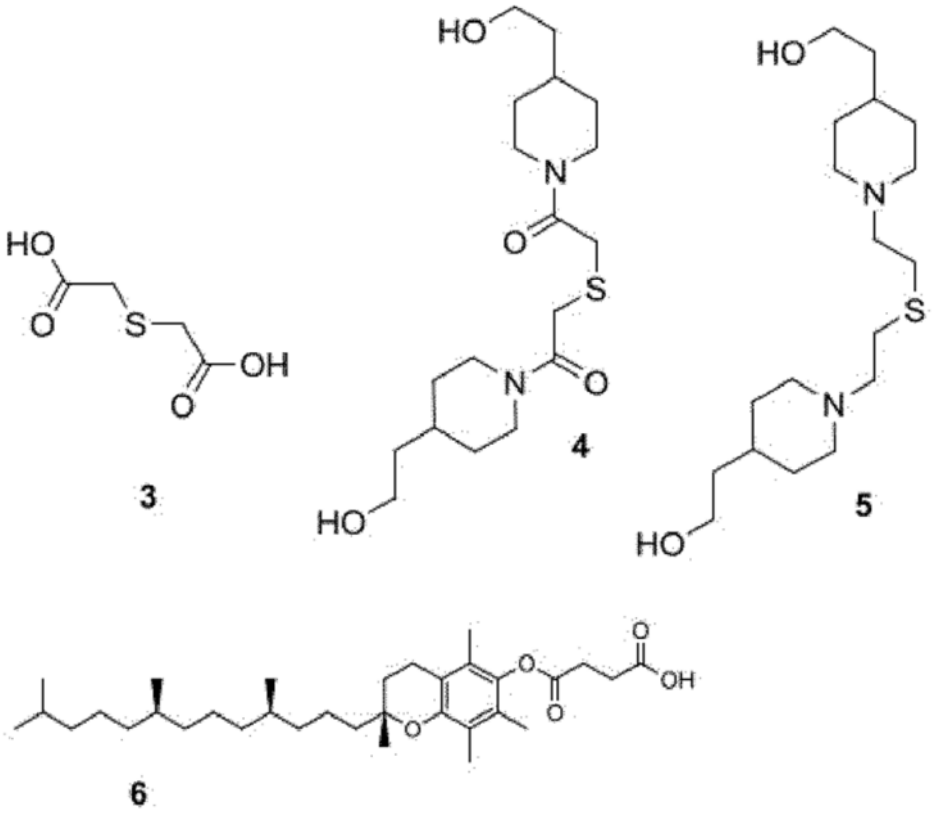
12. A kit or kit of parts, comprising any one of the compositions of **claims 6 to 10**, optionally comprising a liquid vehicle for solubilizing, and, optionally, technical instructions providing information on administration and dosage of the components.

13. The composition of any one of **claims 6 to 10** or the kit or kit of parts of **claim 12** for use as a medicament.

14. Use of the composition of any one of **claims 6 to 10** or a kit or kit of parts of **claim 12** in the manufacture of a vaccine for prevention, prophylaxis, treatment and/or amelioration of a disease selected from infectious diseases including viral, bacterial or protozoological infectious diseases, cancer or tumour diseases.

15. A pharmaceutical composition comprising a composition of any one of **claims 6 to 10** or a kit or kit of parts of **claim 12** for use as a medicament in vaccination of a subject comprising an effective dose of mRNA encoding a virus antigen.

16. A method of making the cationic lipid of claim 1, the method comprising coupling dicarboxylic acid derivative 3 and 4-piperidineethanol to give bisamide 4, subsequent reduction with $LiAlH_4$ to give molecule 5, and coupling with Vitamin E derivative 6 to give the compound of claim 1:



17. A method of making a lipid nanoparticle composition containing mRNA, of any one of claims 6 to 10, compositing the steps of:

(i) providing;

a) the cationic lipid of claim 1;

b) a polymer conjugated lipid;

c) at least one mRNA compound comprising an mRNA sequence encoding at least one antigenic peptide or protein,

d) optionally, a steroid; and

e) optionally a neutral lipid;

(ii) solubilizing the cationic lipid and/or the polymer conjugated lipid and optionally the neutral lipid and/or the steroid or a steroid derivative in an alcohol;

(iii) mixing the alcoholic lipid solution with an aqueous solution comprising the mRNA; (iv) removing the alcohol to form lipid nanoparticle encapsulating or associating with the mRNA;

and optionally

(v) separating or purifying the lipid nanoparticles.