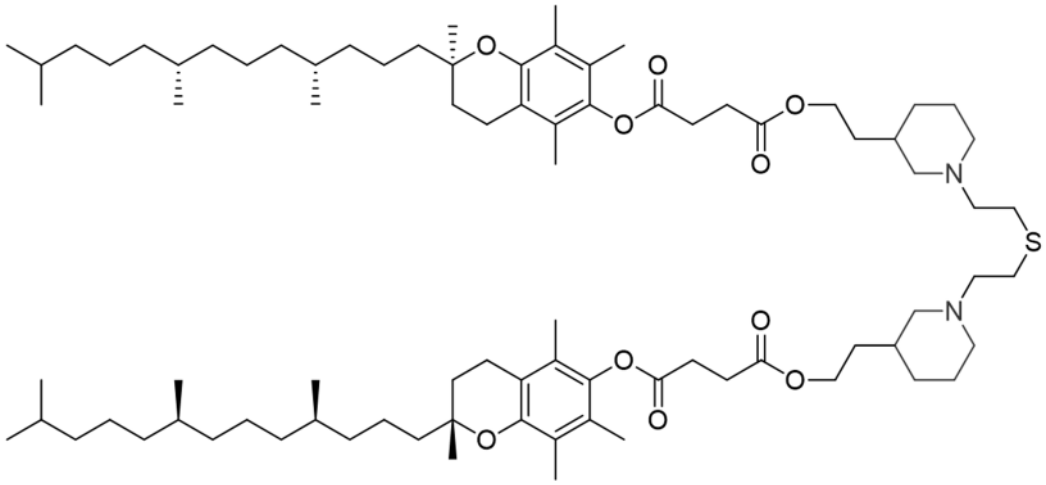
SUMMARY

In an aspect, there is provided a lipid nanoparticle comprising:

- about 49 mol% of an ionizable lipid,



("CVL1-meta" or C28)

or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof,

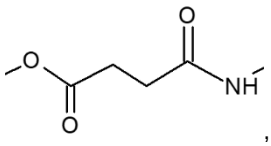
- about 7.5 mol% of the phospholipid DPhyPE,
- about 2.5 mol% of the phosphatidylserine DPhyPS;
- about 40 mol% of cholesterol;
- about 1 mol% of a polymer conjugated lipid according to formula (I):

[P]-[linker]-[L] formula (I),

wherein

[P] is a homopolymer moiety, comprising poly(2-methyl-2-oxazoline) with 50 repeats (PMeOz50),

[linker] has the structure of



, and

[L] is a lipid moiety comprising ditetradecylamin, wherein the linker moiety [linker],

- (—NHC(O)CH_2CH_2C(O)—), is forming an amide connection by connection to the N-atom of ditetradecylamin; and
- one or more nucleic acid, preferably an mRNA.

In another aspect, there is provided a pharmaceutical composition comprising one or more lipid nanoparticles as defined above and an acceptable pharmaceutical carrier.

In another aspect, there is provided a pharmaceutical composition as defined above or the lipid nanoparticle as defined above for use as a medicament, wherein the medicament is a prophylactic or therapeutic vaccine.

In another aspect, there is provided the use of the pharmaceutical composition as defined above or the lipid nanoparticle as defined above in the manufacture of a medicament for prophylaxis or treatment of cancer or

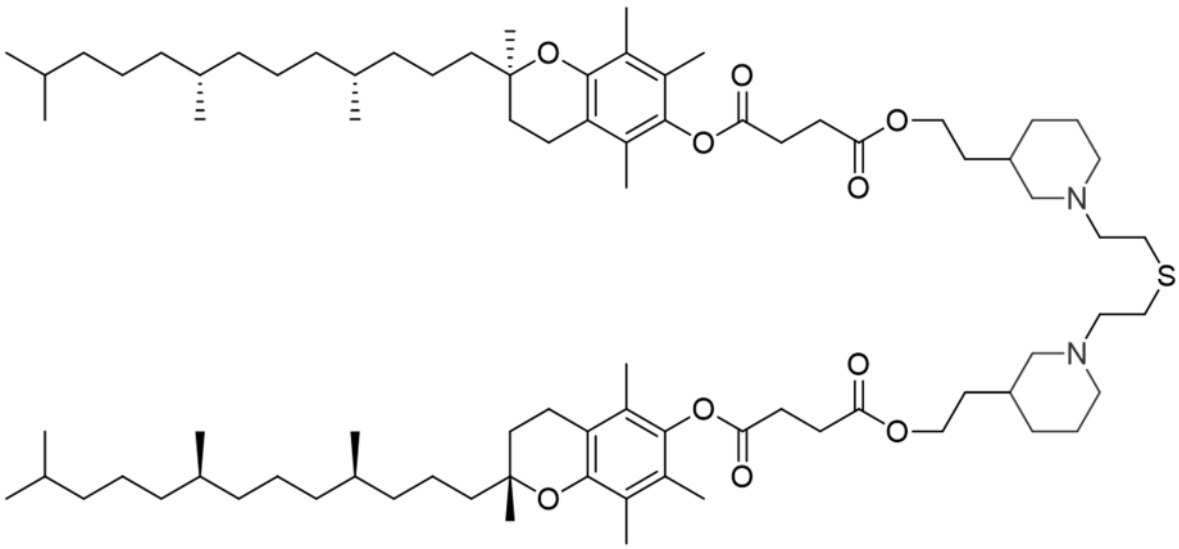
infectious diseases in a subject, most preferably for the prophylaxis or treatment of cancer diseases in a subject, preferably for use in human or veterinary medicine.

In another aspect, there is provided a kit or kit of parts, comprising any one of the lipid nanoparticle as defined above, or the pharmaceutical composition as defined above, optionally comprising a liquid vehicle for solubilizing, and, optionally, technical instructions providing information on administration and dosage of the components.

In another aspect, there is provided the use of t the lipid nanoparticle as defined above, the pharmaceutical composition as defined above, or the kit or kit of parts as defined above, in the manufacture of a medicament for inducing an antigen specific immune response in a subject.

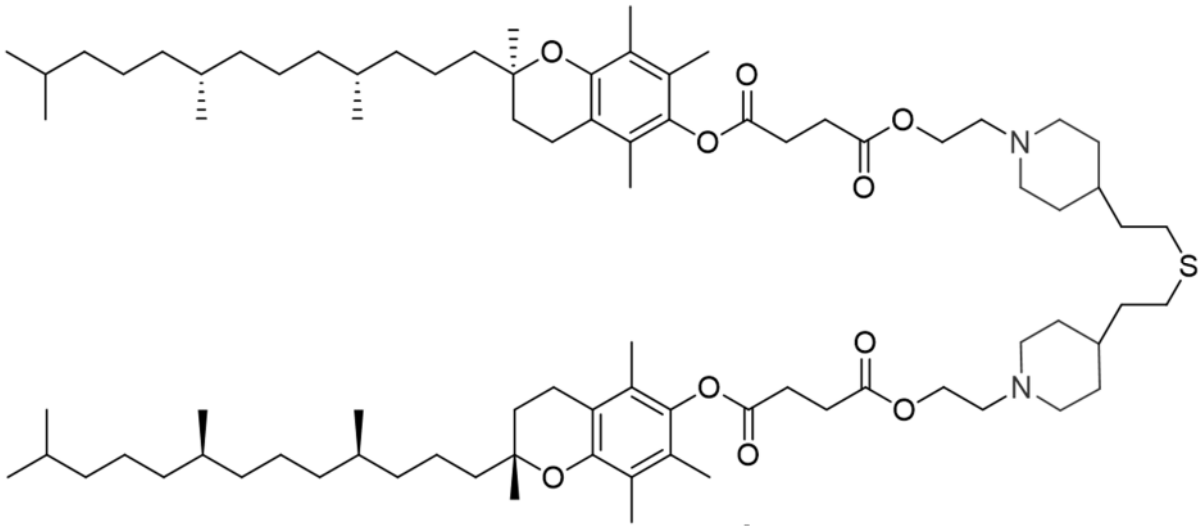
In another aspect, there is provided the use of the lipid nanoparticle as defined above, the pharmaceutical composition as defined above, or the kit or kit of parts as defined above, in the manufacture of a medicament for treating a disease or condition in a subject in need, preferably a cancer disease in a subject in need.

In another aspect, there is provide an ionizable lipid according to the following structure



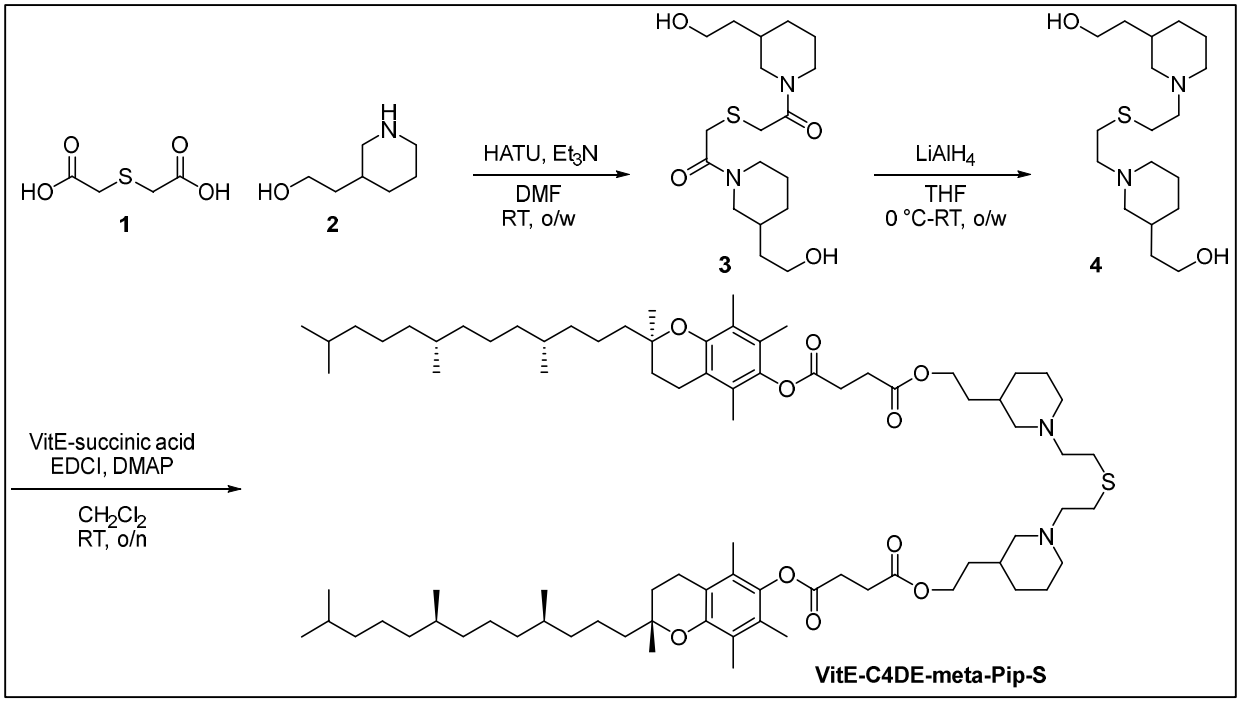
(“CVL1-meta“ or C28).

or according to the following structure



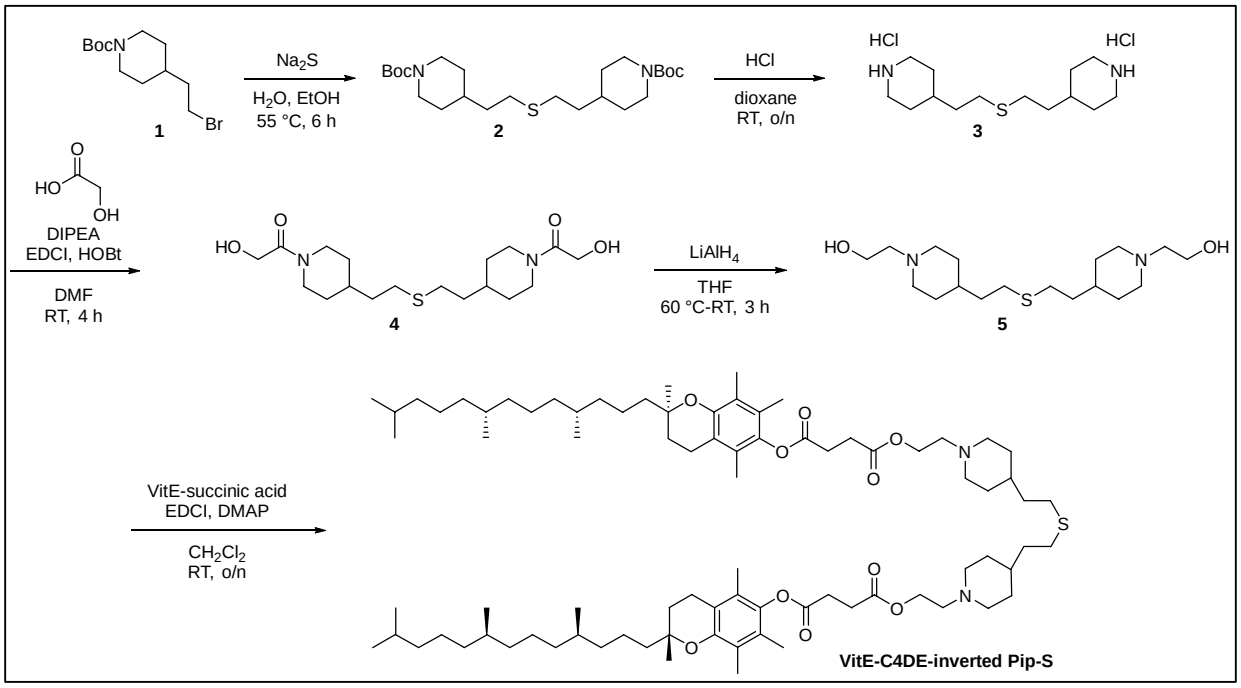
("CVL1-inverted" or C29).

In another aspect, there is provided a method of synthesizing the ionizable lipid C28 or the ionizable lipid C29, according to scheme 1 for C28



(scheme 1 – C28)

or according to scheme 2 for C29



(scheme 2 – C29).

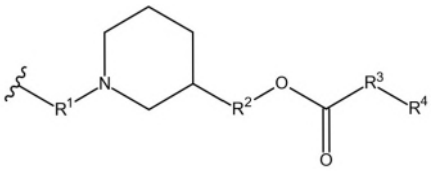
In a first example, the present disclosure related to a lipid nanoparticle comprising:

- about 45 mol% to about 65 mol% of an ionizable lipid, preferably an ionizable lipid according to formula (II)

  $R^a–A–R^b$ formula (II)

  or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof, wherein

  R^a is selected from: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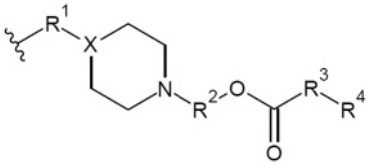
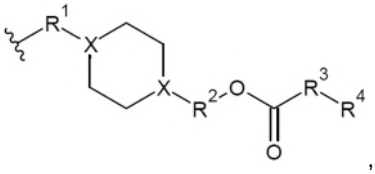

, or

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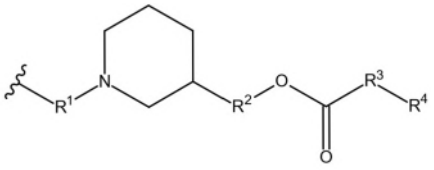
  R^b is selected from: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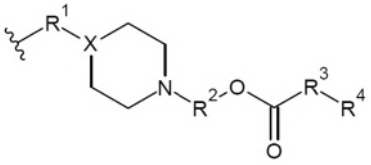
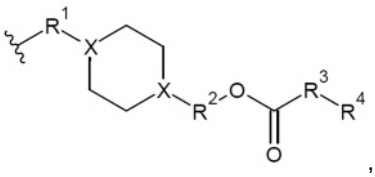

, or

,

  A is –S–;

R^1 is an ethanediyl or linear or unbranched alkanediyl having 2 to 3 carbon atoms;

R^2 is an alkanediyl having 2 to 8 carbon atoms;

R^3 is optional, and if present, is $–R^5–C(O)–O–$, $–R^5–O–C(O)–$, $–R^5–C(O)–NH–$, $–R^5–OC(O)–NH–$, or $R^5–NH–C(O)O–$;

R^4 is a lipophilic substituent with 12 to 36 carbon atoms, wherein the lipophilic substituent with 12 to 36 carbon atoms is derived from tocopherol or tocotreinol;

R^5 is an alkanediyl having 1 to 6 carbon atoms;

X is a carbon atom bonded to a hydrogen atom (CH) or a nitrogen atom, preferably a carbon atom bonded to a hydrogen atom (CH);

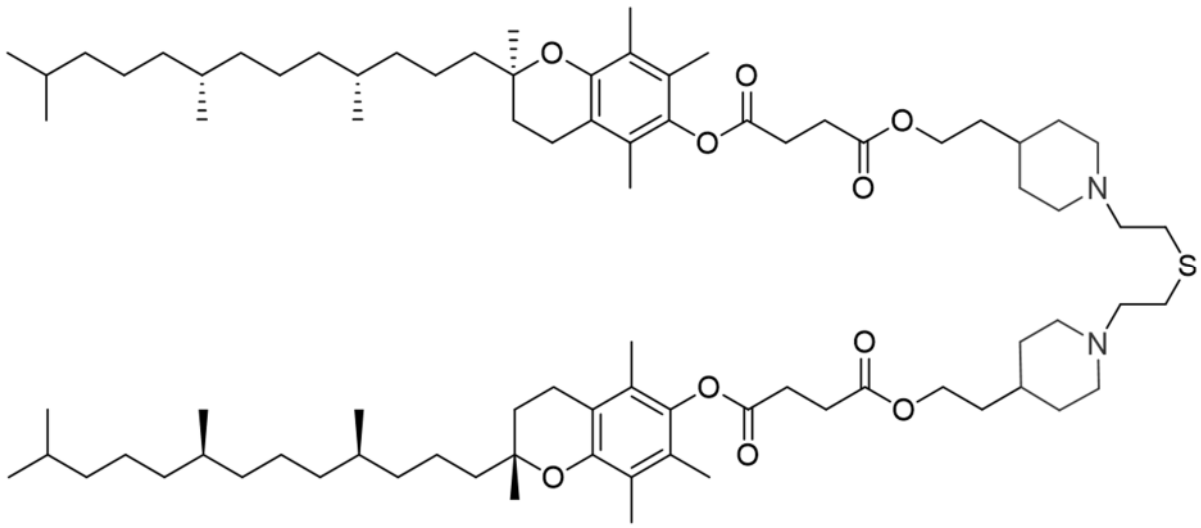
- about 4 mol% to about 15 mol%, preferably about 4 mol% to about 9 mol% of a phospholipid, preferably a phospholipid selected from DSPC and DPhyPE, more preferably wherein the phospholipid is DPhyPE;
- about 1 mol% to about 6 mol%, preferably about 2.5 mol% to about 5 mol%, more preferably about 2.5 mol% of a phosphatidylserine, preferably DPhyPS;
- about 25 mol% to about 45 mol% of a sterol, preferably cholesterol;
- less than about 1.5 mol%, preferably about 1 mol% of a polymer conjugated lipid, preferably of a PMOZ-lipid, more preferably a PMOZ-lipid not comprising a sulphur group (–S–); and
- one or more nucleic acid, preferably an mRNA.

In an example, the present disclosure relates to a lipid nanoparticle composition comprising

a) at least one nucleic acid encoding at least one antigen or fragment or variant thereof; and

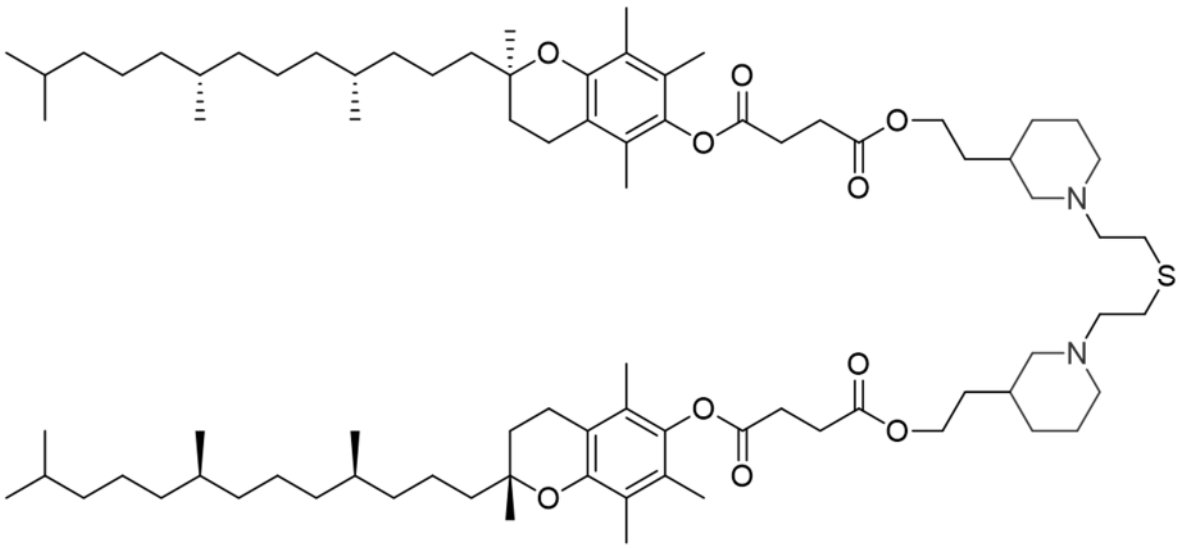
b) a carrier composition, wherein the carrier composition comprises the phospholipid phosphatidylserine, preferably about 2.5 mol% or 5 mol% phosphatidylserine, more preferably about 2.5 mol% or 5 mol% DPhyPS, a polymer conjugated lipid according to formula (I), and an ionizable lipid according to formula (II) as described herein below.

In another example, the lipid nanoparticle preferably comprises the ionizable lipid



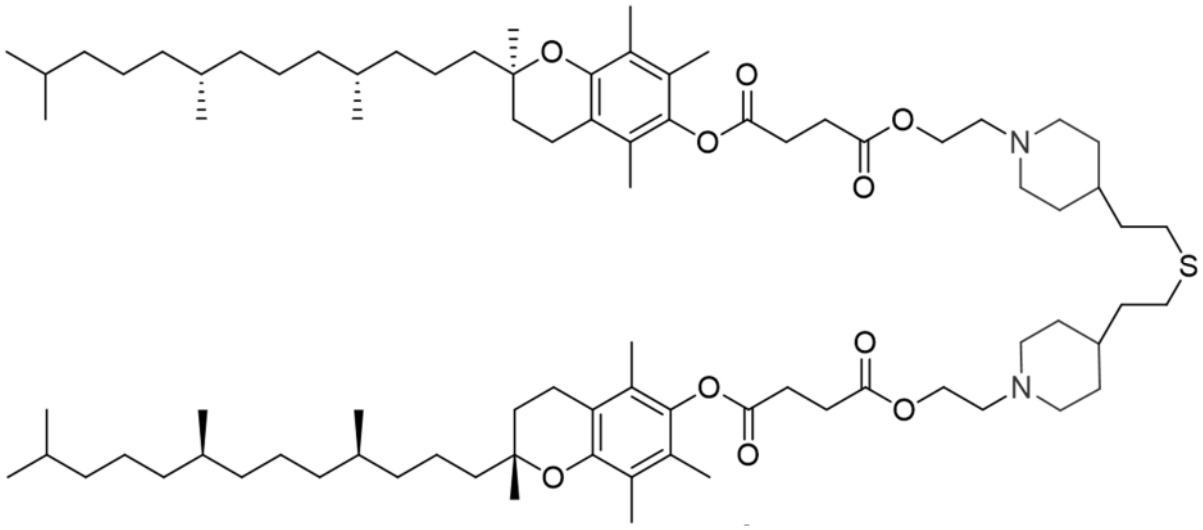
("CVL1" or C24).

In another example, the lipid nanoparticle preferably comprises the ionizable lipid



("CVL1-meta" or C28).

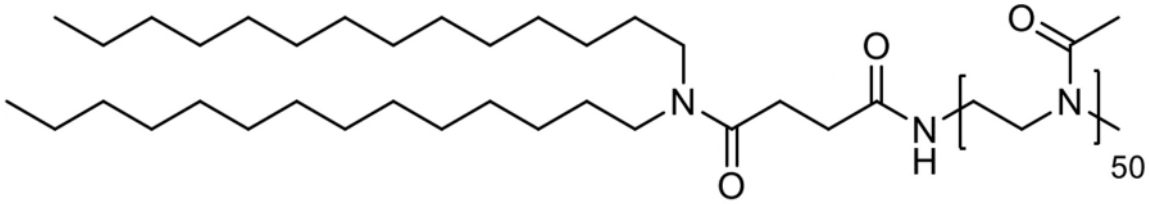
In another example, the lipid nanoparticle preferably comprises the ionizable lipid



(“CVL1-inverted” or C29).

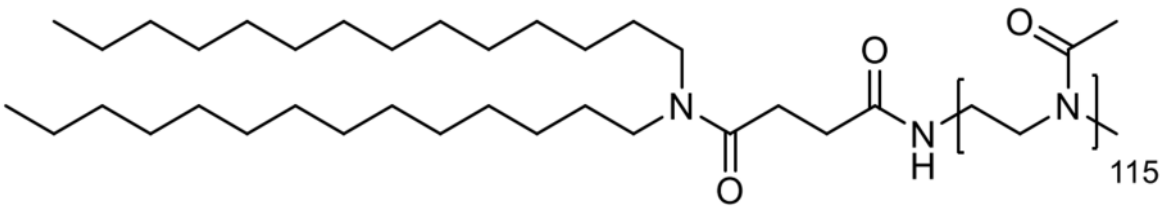
In another example, the lipid nanoparticle preferably comprises:

- about 45 mol% to about 65 mol% of the ionizable lipid, preferably about 49 mol% or about 59 mol% of the ionizable lipid;
- about 4 mol% to about 15 mol%, preferably about 4 mol% to about 9 mol% of the phospholipid, preferably about 5 mol% or about 7.5 mol% of the phospholipid, more preferably about 7.5 mol% DPhyPE;
- about 2.5 mol% phosphatidylserine, preferably DPhyPS;
- about 0.5 mol% to about 1 mol%, preferably about 1 mol% of the PMOZ-lipid, preferably a PMOZ-lipid not comprising a sulphur group (–S–), more preferably a PMOZ-lipid comprising a linker group [linker] being ($C(O)CH_2CH_2C(O)NH$), even more preferably being (i) “PMOZ 4” (“N-methyl-2-(N4', N4'-di(tetradecyl)succinamide)-poly[(N-acetyl)ethylamine” or abbreviated “PMOz-DM-amide”) with n = 50 i.e. having 50 monomer repeats



(“PMOZ 4” with n = 50 i.e. having 50 monomer repeats); or

(ii) “PMOZ 4” with n = 115 i.e. having 115 monomer repeats

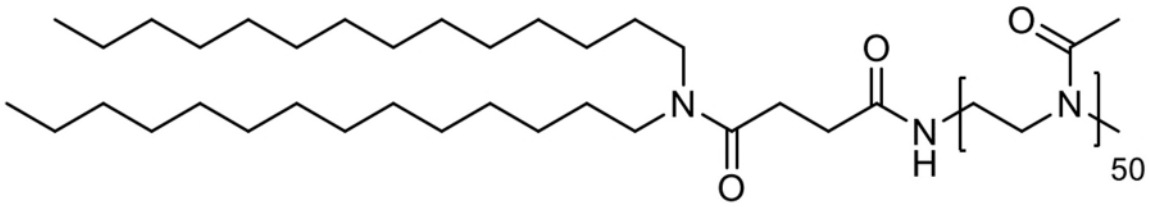


(“PMOZ 4” with n = 115 i.e. having 115 monomer repeats);

- about 29 mol% to about 41 mol% sterol, preferably cholesterol; and
- one or more nucleic acid, preferably an mRNA.

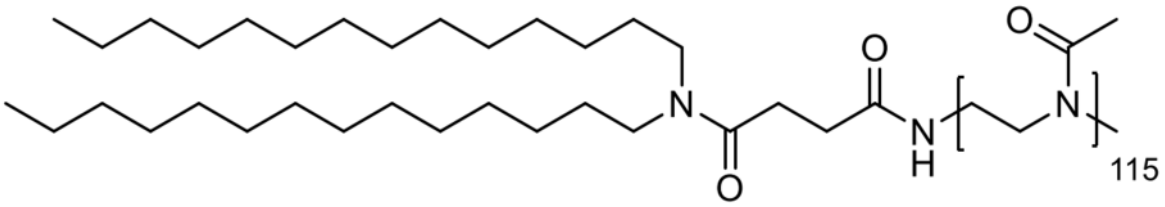
In another example, the lipid nanoparticle preferably comprises:

- about 49 mol% or about 59 mol% of the ionizable lipid;
- about 5 mol% or about 7.5. mol% of DPhyPE;
- about 2.5 mol% or about 5 mol% phosphatidylserine, preferably DPhyPS;
- about 0.5 mol% to about 1 mol%, preferably about 1 mol% of a PMOZ-lipid, preferably a PMOZ-lipid not comprising a sulphur group (–S–), more preferably a PMOZ-lipid comprising a linker group [linker] being ($C(O)CH_2CH_2C(O)NH$), even more preferably being (i) "PMOZ 4" with n = 50 i.e. having 50 monomer repeats



("PMOZ 4" with n = 50 i.e. having 50 monomer repeats); or

(ii) "PMOZ 4" with n = 115 i.e. having 115 monomer repeats



("PMOZ 4" with n = 115 i.e. having 115 monomer repeats);

- about 29 mol% to about 41 mol% cholesterol; and
- one or more nucleic acid, preferably an mRNA.

In another example, the lipid nanoparticle preferably comprises one or more nucleic acid, preferably an mRNA, and the lipid composition of the lipid nanoparticle is selected from the group consisting of

(i) about 49 mol% C24, about 40 mol% cholesterol, about 7.5 mol% DPhyPE, about 2.5 mol% DPhyPS, and about 1 mol% PMOZ 4 with n = 50 i.e. having 50 monomer repeats;

(ii) about 59 mol% C24, about 30 mol% cholesterol, about 7.5 mol% DPhyPE, about 2.5 mol% DPhyPS, and about 1 mol% PMOZ 4 with n = 50 i.e. having 50 monomer repeats;

(iii) about 49 mol% C24, about 40.5 mol% cholesterol, about 7.5 mol% DPhyPE, about 2.5 mol% DPhyPS, and about 0.5 mol% PMOZ 4 with n = 115 i.e. having 115 monomer repeats; and

(iv) about 49 mol% C24, about 40 mol% cholesterol, about 7.5 mol% DPhyPE, about 2.5 mol% DPhyPS, and about 1 mol% PMOZ 4 with n = 115 i.e. having 115 monomer repeats.

In another example, the lipid nanoparticle preferably comprises one or more nucleic acid, preferably an mRNA, and the lipid composition of the lipid nanoparticle is selected from the group consisting of

(i) about 59 mol% C28, about 30 mol% cholesterol, about 7.5 mol% DPhyPE, about 2.5 mol% DPhyPS, and about 1 mol% PMOZ 4 with n = 50 i.e. having 50 monomer repeats; and

(ii) about 49 mol% C28, about 40 mol% cholesterol, about 7.5 mol% DPhyPE, about 2.5 mol% DPhyPS, and about 1 mol% PMOZ 4 with n = 50 i.e. having 50 monomer repeats.

In another example, the lipid nanoparticle preferably comprises one or more nucleic acid, preferably an mRNA, and about 49 mol% C29, about 40 mol% cholesterol, about 7.5 mol% DPhyPE, about 2.5 mol% DPhyPS, and about 1 mol% PMOZ 4 with n = 50 i.e. having 50 monomer repeats.

In another example, the lipid nanoparticle preferably comprises:

- about 45 mol% to about 65 mol% of an ionizable lipid, preferably an ionizable lipid according to formula (II) as shown herein below and above, more preferably the ionizable lipid C24, the ionizable lipid C28 or the ionizable lipid C29;
- about 4 mol% to about 15 mol%, preferably about 4 mol% to about 9 mol% DPhyPE;
- about 2.5 mol% to about 5 mol% of a phosphatidylserine, preferably DPhyPS;
- about 25 mol% to about 45 mol% of a sterol, preferably cholesterol;
- about 1 mol% to about 2 mol% of a PEG-lipid, preferably DMG-PEG2000; and
- one or more nucleic acid, preferably an mRNA.

In another example, the antigen is derived from a tumor antigen, a pathogenic antigen, an allergenic antigen or an autoimmune self-antigen, preferably derived from a tumor antigen.

In another example, the amount of the phosphatidylserine is not more than 9 mol%, preferably not more than 5 mol%, of the total molar amount of all lipidic excipients in the composition.

In another example, the carrier composition is a lipid nanoparticle composition. In yet another example, the lipid nanoparticle composition further comprises

(i) an ionizable lipid, preferably according to formula (II), more preferably C24, C28 or C29, preferably C24;
(ii) a steroid;
(iii) a further phospholipid in addition to a phosphatidylserine which is preferably DPhyPS, preferably wherein the further phospholipid is DPhyPE; and
(iv) a polymer conjugated lipid, preferably according to formula (I), more preferably “PMOZ 4”.

In another example, the present disclosure relates to a method of delivering a vaccine composition comprising at least one nucleic acid encoding at least one antigen or fragment or variant thereof to the spleen or lymph nodes, wherein the carrier composition comprises the phospholipid phosphatidylserine, as when compared to vaccine compositions not comprising phosphatidylserine. Generally, in certain examples, this disclosure involves directing LNPs comprising mRNA to the lymphatic system, specifically focusing on secondary lymphoid organs, notably the spleen. In various examples, the targeted cells reside within lymph nodes or spleen cells themselves. Additionally, the target may be an antigen-presenting cell, such as a professional antigen-presenting cell, or a dendritic cell within the spleen. Consequently, the RNA compositions or formulations outlined herein could be utilized for conveying RNA to these specified target cells. The lymphatic system, an integral part of both the circulatory and immune systems, comprises a network of vessels transporting lymph. It encompasses lymphatic organs, a network of vessels, and circulating lymph. Primary lymphoid organs, like the thymus and bone marrow, produce lymphocytes from immature progenitor cells, while secondary lymphoid organs, including lymph nodes and the spleen, sustain mature naïve lymphocytes and trigger adaptive immune responses. Lipid-based RNA delivery systems naturally

tend to accumulate in the liver due to the hepatic vasculature's discontinuous nature or lipid metabolism. In specific examples, the intended site for RNA expression is the liver and its corresponding tissue. In further specific preferred examples, the intended site for RNA expression is the spleen. In further specific preferred examples, the intended site for RNA expression are the lymph nodes.

In the second example, the present disclosure is concerned with a pharmaceutical composition comprising the vaccine composition according to the first example and a pharmaceutically acceptable carrier, diluent or excipient, preferably wherein the pharmaceutical 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.

In the third example, the present disclosure relates to the vaccine composition according to the first example or the pharmaceutical composition according to the second example for use in the treatment or prophylaxis of infectious diseases; cancer or tumor 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; in a subject. In a very preferred example of the third example of the disclosure, the present disclosure relates to a vaccine composition for use in the treatment or prophylaxis of a cancer or tumor disease.

In the fourth example, the present disclosure is concerned with a kit or kit of parts, comprising the vaccine composition according to the first example or the pharmaceutical composition according to the second example, optionally comprising a liquid vehicle for solubilizing, and, optionally, technical instructions providing information on administration and dosage of the components.

In the fifth example, the present disclosure relates to a method of treatment or prophylaxis of cancer or tumor diseases, disorders or conditions; infectious diseases; liver diseases selected from the group consisting of liver fibrosis, liver cirrhosis and liver cancer; allergies; or autoimmune disease, disorder or condition; in a subject comprising the steps:

a) providing the vaccine composition of the first example or the pharmaceutical composition according to the second example or the kit or kit of parts according to the fourth example; and
b) applying or administering the vaccine composition or the pharmaceutical composition or the kit or kit of parts to a tissue or an organism of the subject.

In the sixth example, the present disclosure relates to a method of inducing an immune response in a subject, the method comprising administering to the subject the vaccine composition of the first example or the pharmaceutical composition of second example in an amount effective to produce an antigen-specific immune response in the subject.

In the seventh example, the present disclosure is concerned with a use of a vaccine composition of the first example or the pharmaceutical composition according to the second example or the kit or kit of parts according to the fourth example for (i) inducing an immune response, for (ii) inducing an antigen specific T-cell response or preferably for (iii) inducing CD8+ T cells responses, in a subject.

In other examples, the present disclosure is concerned with a method of inducing interferon (IFN) production, the method comprising administering to a subject in need the lipid nanoparticle, the pharmaceutical composition or the

kit or kit of parts of the disclosure, whereby IFN production is increased following administration of aforementioned lipid nanoparticle, the pharmaceutical composition or the kit or kit of parts of the disclosure.

In other examples, the present disclosure is concerned with a method comprising: administering to a subject in need the lipid nanoparticle, the pharmaceutical composition or the kit or kit of parts of the disclosure, in an amount sufficient to induce an immune response in the subject, preferably wherein the immune response involves the production of cytokines, more preferably wherein the immune response involves modulation of a type I IFN, type II IFN, and/or type III IFN.

In other examples, the present disclosure is concerned with a method comprising: administering to a subject in need the lipid nanoparticle, the pharmaceutical composition or the kit or kit of parts of the disclosure, in an amount sufficient to induce an immune response in the subject, preferably wherein the immune response involves the production of type I IFN, more preferably wherein the immune response involves modulation of IFN is IFNa, IFNb, IFNe, IFNk or IFN, most preferably IFNa and/or IFNb.

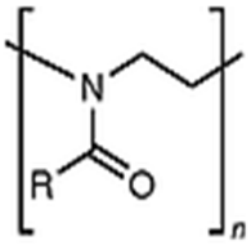
In another example, the present disclosure relates to novel polymer conjugated lipids which are useful for the delivery of nucleic acids into living cells.

In a specific example, the polymer conjugated lipids are compounds according to formula (I):

[P]-[linker]-[L] formula (I)

or a pharmaceutically acceptable salt, prodrug, tautomer or stereoisomer thereof, wherein

[P] is a homopolymer moiety comprising at least one polyoxazoline (POZ) monomer unit



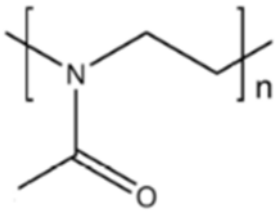
,

wherein R is C1-9 alkyl or C2-9 alkenyl, preferably C1 or C2 alkyl, and n has a mean value ranging from about 45 to about 55, preferably n is about 50 or wherein n is selected such that the [P] moiety has an average molecular weight of about 4.2 kDa to about 4.4 kDa, or most preferably about 4.3 kDa

[linker] is an optional linker group, and

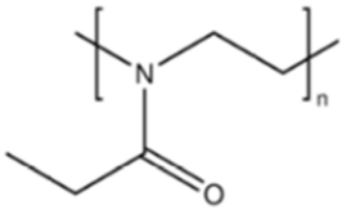
[L] is a lipid moiety.

In another example, the polymer conjugated lipid comprises as [P] a heteropolymer moiety or homopolymer moiety comprising multiple monomer units selected from the group consisting of
poly(2-methyl-2-oxazoline) (PMOZ)



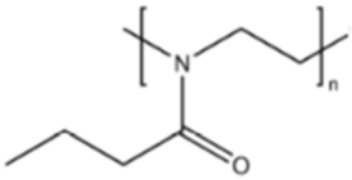
,

poly(2-ethyl-2-oxazoline) (PEOZ)



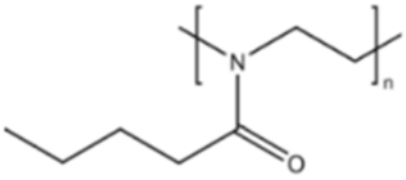
,

poly(2-propyl-2-oxazoline) (PPOZ)



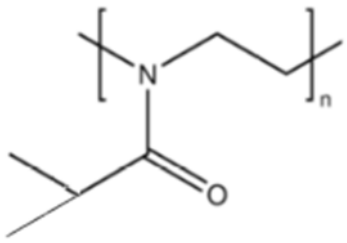
,

poly(2-butyl-2-oxazoline) (PBOZ)



,

poly(2-isopropyl-2-oxazoline) (PIPOZ)



,

poly(2-methoxymethyl-2-oxazoline) (PMeOMeOx), and
poly(2-dimethylamino-2-oxazoline) (PDMAOx),
preferably wherein [P] is a homopolymer moiety comprising multiple PMOZ or PEOZ monomer units, more preferably wherein [P] comprises or preferably consists of multiple PMOZ monomer units,
wherein
(i) n has a mean value ranging from about 45 to about 55, preferably n is about 50 or wherein
(ii) n is selected such that the [P] moiety has an average molecular weight of about 4.2 kDa to about 4.4 kDa, or most preferably about 4.3 kDa.

In very preferred examples, the homopolymer moieties [P] are selected from the group consisting of PMeOz50 (polymethyloxazoline or poly(2-methyl-2-oxazoline) with 50 repeats), PEtOz50 (polyethyloxazoline with 50 repeats),

PMeOz25 (polymethyloxazoline with 25 repeats) and PEtOz25 (polyethyloxazoline with 25 repeats), preferably PMeOz50 (polymethyloxazoline or poly(2-methyl-2-oxazoline) with 50 repeats).

In another example, the polymer conjugated lipid is selected from the group consisting of a POZ-monoacylglycerol conjugate, POZ-diacylglycerol conjugate, a POZ-dialkyloxypropyl conjugate, a POZ-steroid or POZ-sterol conjugate, a POZ-phospholipid conjugate, a POZ-ceramide conjugate, and a mixture thereof. In a further example, the lipid moiety [L] comprises at least one straight or branched, saturated or unsaturated alkyl chain containing from 6 to 30 carbon atoms, preferably wherein the lipid moiety [L] comprises at least one straight or branched saturated alkyl chain, wherein the alkyl chain is optionally interrupted by one or more biodegradable group(s) and/or optionally comprises one terminal biodegradable group, wherein the biodegradable group is selected from the group consisting of but not limited to a pH-sensitive moiety, an alkyl or alkenyl moiety (C_{1-9} alkyl or C_{2-9} alkenyl), a zwitterionic linker, non-ester containing linker moieties and ester-containing linker moieties (—C(O)O— or —OC(O)—), amido (—C(O)NH—), disulfide (—S—S—), carbonyl (—C(O)—), ether (—O—), thioether (—S—), oxime (e.g., —C(H)=N—O— or —O— N=C(H)—), carbamate (—NHC(O)O—), urea (—NHC(O)NH—), succinyl (—(O)CCH_2CH_2C(O)—), succinamidyl (—NHC(O)CH_2CH_2C(O)NH—), (—NHC(O)CH_2CH_2C(O)—), —C(R5)=N—, —N=C(R^5)—, —C(R^5)=N—O—, —O—N=C(R^5)—, —O—C(O)O—, —C(O)N(R^5), —N(R^5)C(O)—, —C(S)N(R^5)—, (NR^5)C(S)—, —N(R^5)C(O)N(R^5)—, —C(O)S—, —SC(O)—, —C(S)O—, —OC(S)—, —OSi(R^5)$_2$O—, —C(O)(CR^3R^4)C(O)O—, or —OC(O)(CR^3R^4)C(O)—, carbonate (—OC(O)O—), nitrogen (N), succinoyl, succinate, phosphate esters (—O—(O)POH—O—), cyclic compound, heterocyclic compound, piperidine, pyrazine, pyridine, piperazine, and sulfonate esters, as well as combinations thereof, wherein R^3, R^4 and R^5 are, independently H or alkyl (e.g. C_1-C_4 alkyl).

In a further example, the lipid moiety [L] comprises two straight unsaturated alkyl chain containing from 6 to 30 carbon atoms, preferably wherein the lipid moiety [L] comprises at least one straight or branched saturated alkyl chain, wherein the alkyl chain is optionally interrupted by one or more biodegradable group(s) and/or optionally comprises one terminal biodegradable group, wherein the biodegradable group is selected from the group consisting of but not limited to a pH-sensitive moiety, an alkyl or alkenyl moiety (C_{1-9} alkyl or C_{2-9} alkenyl), a zwitterionic linker, non-ester containing linker moieties and ester-containing linker moieties (—C(O)O— or —OC(O)—), amido (—C(O)NH—), disulfide (—S—S—), carbonyl (—C(O)—), ether (—O—), thioether (—S—), oxime (e.g., —C(H)=N—O— or —O— N=C(H)—), carbamate (—NHC(O)O—), urea (—NHC(O)NH—), succinyl (—(O)CCH_2CH_2C(O)—), succinamidyl (—NHC(O)CH_2CH_2C(O)NH—), (—NHC(O)CH_2CH_2C(O)—), —C(R5)=N—, —N=C(R^5)—, —C(R^5)=N—O—, —O—N=C(R^5)—, —O—C(O)O—, —C(O)N(R^5), —N(R^5)C(O)—, —C(S)N(R^5)—, (NR^5)C(S)—, —N(R^5)C(O)N(R^5)—, —C(O)S—, —SC(O)—, —C(S)O—, —OC(S)—, —OSi(R^5)$_2$O—, —C(O)(CR^3R^4)C(O)O—, or —OC(O)(CR^3R^4)C(O)—, carbonate (—OC(O)O—), nitrogen (N), succinoyl, succinate, phosphate esters (—O—(O)POH—O—), cyclic compound, heterocyclic compound, piperidine, pyrazine, pyridine, piperazine, and sulfonate esters, as well as combinations thereof, wherein R^3, R^4 and R^5 are, independently H or alkyl (e.g. C_1-C_4 alkyl).

In a further example, the lipid moiety [L] comprises two straight unsaturated alkyl chain each containing 14 carbon atoms.

In a further most preferred example, the polymer conjugated lipid comprises a lipid moiety [L] comprising ditetradecylamin and a linker group [linker], preferably wherein the linker group [linker] is (—NHC(O)CH_2CH_2C(O)—). In a further preferred example, the lipid moiety [L] comprises ditetradecylamin, wherein the linker moiety [linker], preferably (—NHC(O)CH_2CH_2C(O)—), is forming an amide connection by connection to the N-atom of

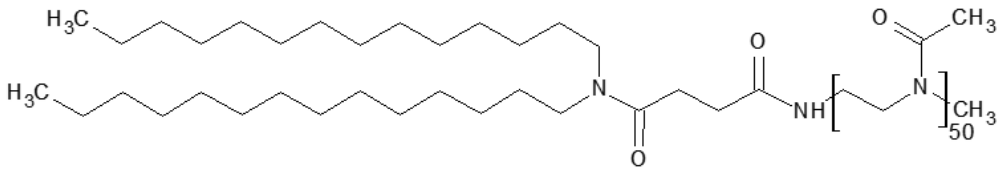
ditetradecylamin. In most preferred examples, the polymer conjugated lipid comprises a linker (—NHC(O)CH_2CH_2C(O)—), wherein the linker is orientated in such way, that an carboxamide connection is formed through connection to the N-atom of ditetradecylamin.

In a further most preferred example, the polymer conjugated lipid comprises a lipid moiety [L] comprising ditetradecylamin and a linker group [linker], preferably wherein the linker group [linker] is (C(O)CH_2CH_2C(O)NH). In a further preferred example, the lipid moiety [L] comprises ditetradecylamin, wherein the linker moiety [linker], preferably (—NHC(O)CH_2CH_2C(O)—), is forming an amide connection by connection to the N-atom of ditetradecylamin.

In a very preferred example, the lipid moiety [L] is the lipid moiety as used in “PMOZ 4”.

In a very preferred example, the linker moiety [linker] is the linker moiety as used in “PMOZ 4”.

In further most preferred example, the disclosure relates to a polymer conjugated lipid having a lipid moiety [L], being the lipid moiety as used in “PMOZ 4”. In another most preferred example, the disclosure relates to a polymer conjugated lipid having a linker moiety [linker], being the linker as used in “PMOZ 4”.



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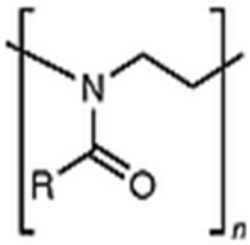
In another example, the disclosure provides novel lipid nanoparticles comprising a homopolymer moiety comprising at least one polyoxazoline (POZ) monomer unit



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wherein R is C1-9 alkyl or C2-9 alkenyl, preferably C1 or C2 alkyl, and n has a mean value ranging from about 45 to about 55, preferably n is about 50 or wherein n is selected such that the [P] moiety has an average molecular weight of about 4.2 kDa to about 4.4 kDa, or most preferably about 4.3 kDa,

preferably, wherein the homopolymer moiety comprising multiple monomer units comprises poly(2-methyl-2-oxazoline) (PMOZ), poly(2-ethyl-2-oxazoline) (PEOZ), poly(2-propyl-2-oxazoline) (PPOZ), poly(2-butyl-2-oxazoline) (PBOZ), poly(2-isopropyl-2-oxazoline) (PIPOZ), poly(2-methoxymethyl-2-oxazoline) (PMeOMeOx), or poly(2-dimethylamino-2-oxazoline) (PDMAOx).

Most preferably, R is C_1 (i.e. CH_3 or methyl), yielding in polymethyloxazoline or poly(2-methyl-2-oxazoline) i.e. “PMOZ”.

In further examples, the disclosure provides vaccine compositions comprising the lipid nanoparticle of the disclosure, or a kit or kit of parts comprising the inventive ionizable and polymer conjugated lipids for use as a medicament,

and/or for prevention, prophylaxis, treatment and/or amelioration of a disease selected from infectious diseases including viral, bacterial or protozoological infectious diseases, cancer or tumor diseases.

In a further example, the disclosure provides methods of treatment or prophylaxis of infectious diseases; cancer or tumor 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 comprising the steps:

a) providing a lipid nanoparticle, comprising a homopolymer moiety comprising at least one polyoxazoline (POZ) monomer, preferably the polymer conjugated lipid of the disclosure, the vaccine composition, or the kit or kit of parts of the disclosure; and

b) applying or administering the mRNA, the lipid nanoparticle, the vaccine composition or the kit or kit of parts to a tissue or an organism.

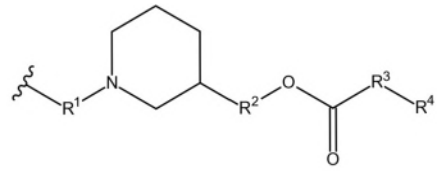
In another specific example, the present disclosure relates to lipid nanoparticles comprising novel polymer conjugated lipids according to formula (I), novel ionizable lipids according to formula (II), phosphatidylserine and DPhyPE, which are useful for the delivery of nucleic acids into living cells.

In one example, the present disclosure relates to novel cationic lipids which are useful for the delivery of nucleic acids into living cells. The cationic lipids are compounds according to formula (II):

R^a–A–R^b formula (II)

wherein

R^a is selected from: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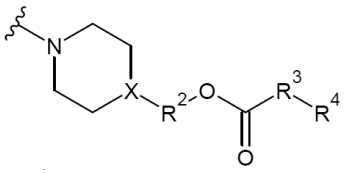
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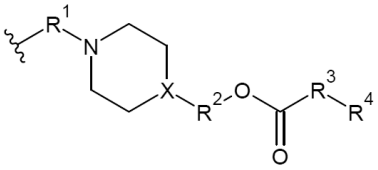
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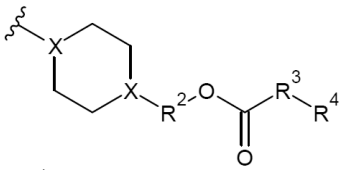
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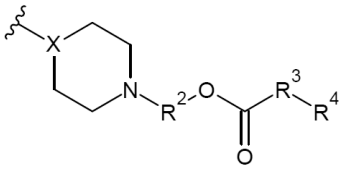
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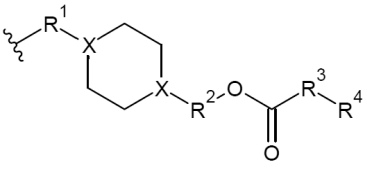
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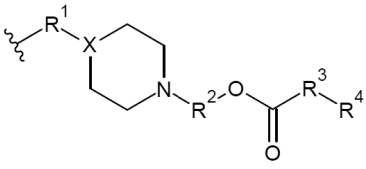
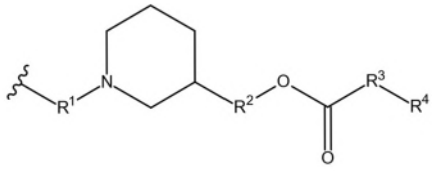

, or

–R^1–N(H)–C(O)–R^3–R^4;

R^b is selected from: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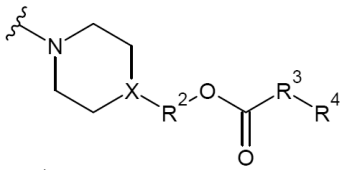
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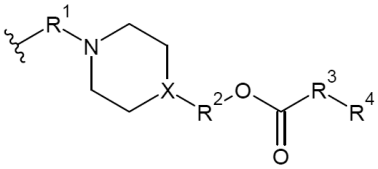
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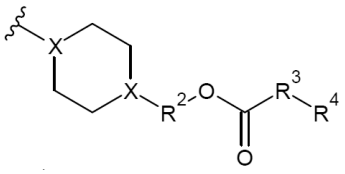
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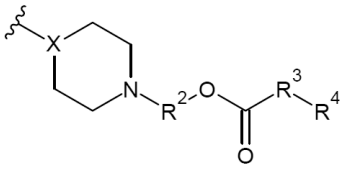
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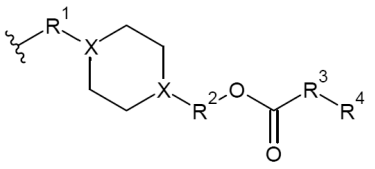
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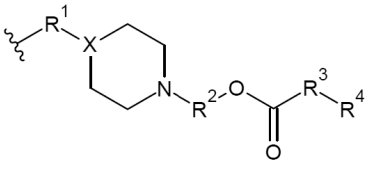

, or $–R^1–N(H)–C(O)–R^3–R^4$, or $–R^1–N(CH_3)_2$;

A is –S–, –S–S–, –S–C(O)– –NH–C(O)–, –NH–C(O)O–, –NH–C(O)–NH–, –S–C(O)–N(H)–, –C(O)O–, or –O–P(O)(OH)–O–;

R^1 is an optionally substituted ethanediyl, propanediyl, butanediyl, or linear or unbranched alkanediyl having 2 to 8 carbon atoms;

R^2 is an alkanediyl having 2 to 8 carbon atoms;

R^3 is optional, and if present, is $–R^5–C(O)–O–$, or $–R^5–O–C(O)–$, $–R^5–C(O)–NH–$, $–R^5–OC(O)–NH–$, or $R^5–NH–C(O)O–$;

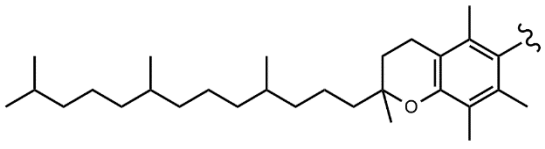
R^4 is a lipophilic substituent with 12 to 36 carbon atoms;

R^5 is an alkanediyl having 1 to 6 carbon atoms;

X is a carbon or nitrogen atom;

wherein all selections are independent of one another,

optionally provided that if 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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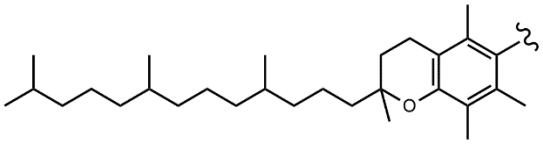
In this regard, an alkanediyl is a term for a ($–C_nH_{2n}–$) group; f.e. an “alkanediyl having 2 to 8 carbon atoms” accordingly equals an alkanediyl group having the formula $–C_2H_4–$, $–C_3H_6–$, $–C_4H_8–$, $–C_5H_{10}–$, $–C_6H_{12}–$, $–C_7H_{14}–$, or respectively $–C_8H_{16}–$. In other words, 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 (II) 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 (II)).

In another example, the disclosure provides 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 tumors.

In further examples, the disclosure provides 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 another example of the present disclosure, the present disclosure also provides 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 another example of the present disclosure, the present disclosure also provides a pharmaceutical composition comprising a lipid nanoparticle of the disclosure, a kit or kit of parts of the disclosure, or the vaccine composition of the disclosure for use in vaccination and/or treatment of a subject comprising an effective dose of mRNA encoding a cancer antigen or virus antigen, preferably a cancer antigen.

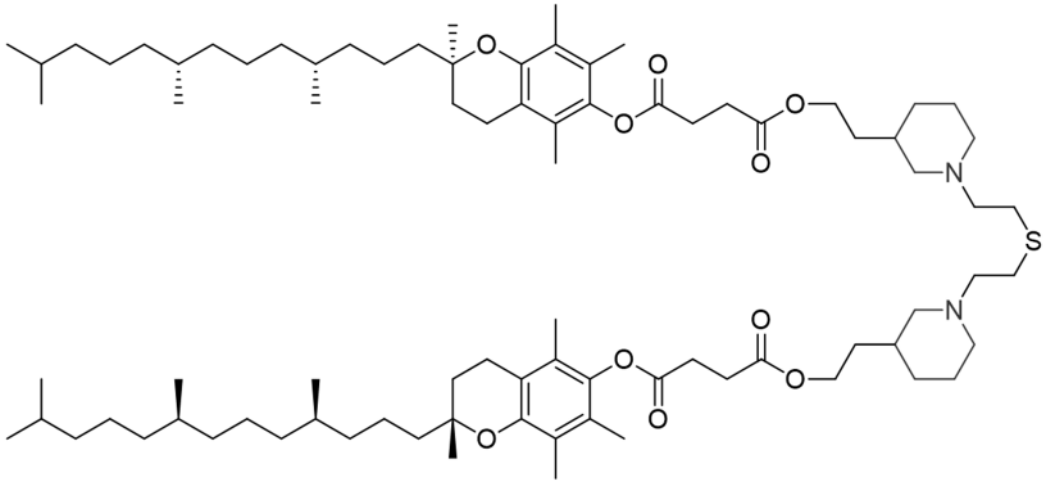
In yet another example of the disclosure, the present disclosure provides improved lyophilizable lipid nanoparticles, which have advantageous physiochemical properties after being lyophilized and reconstituted.

In yet another example of the disclosure, the present disclosure provides improved lipid nanoparticles, which have advantageous physiochemical properties after being frozen and thawed.

CLAIMS

1. A lipid nanoparticle comprising:

- about 49 mol% of an ionizable lipid,



("CVL1-meta" or C28)

or a pharmaceutically acceptable salt, prodrug or stereoisomer thereof,

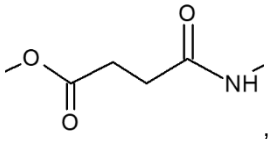
- about 7.5 mol% of the phospholipid DPhyPE,
- about 2.5 mol% of the phosphatidylserine DPhyPS;
- about 40 mol% of cholesterol;
- about 1 mol% of a polymer conjugated lipid according to formula (I):

[P]-[linker]-[L] formula (I),

wherein

[P] is a homopolymer moiety, comprising poly(2-methyl-2-oxazoline) with 50 repeats (PMeOz50),

[linker] has the structure of

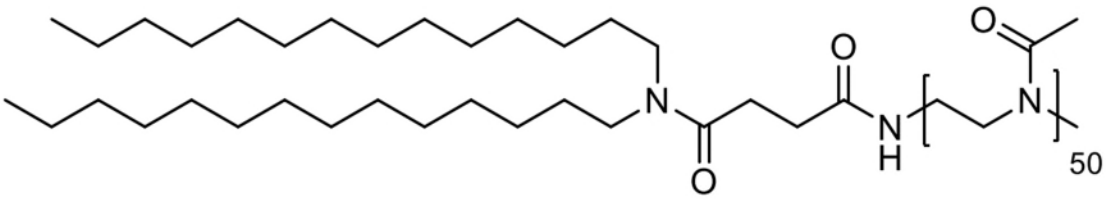


, and

[L] is a lipid moiety comprising ditetradecylamin, wherein the linker moiety [linker], (—NHC(O)CH_2CH_2C(O)—), is forming an amide connection by connection to the N-atom of ditetradecylamin; and

- one or more nucleic acid, preferably an mRNA.

2. The lipid nanoparticle according to **claim 1**, wherein the polymer conjugated lipid is "PMOZ 4":



("PMOZ 4" with n = 50 i.e. having 50 monomer repeats).

3. The lipid nanoparticle according to **claim 1 or claim 2**, wherein the lipid nanoparticles comprise the nucleic acid, preferably mRNA at an amount such as to achieve an N/P ratio in the range of about 5 to about 15, more preferably about 8 to about 12, even more preferably about 9 to about 11, most preferably about 10.

4. The lipid nanoparticle according to any one of **claim 1 to claim 3**, wherein the nucleic acid is
(a) an mRNA comprising at least one coding sequence encoding a peptide or protein, or a fragment or variant thereof, wherein the peptide or protein is an antigen, wherein the antigen preferably is derived from a tumor antigen, or a fragment or variant thereof; or the mRNA comprises at least one coding sequence encoding a therapeutic protein, or a fragment or variant thereof, wherein the therapeutic protein is a therapeutic protein for use in the treatment of cancer or tumor diseases.

5. The lipid nanoparticle according to any one of **claim 1 to claim 4**, wherein the lipid nanoparticle is a sterile solid composition for reconstitution with a sterile liquid carrier, and wherein the lipid nanoparticle 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.

6. The lipid nanoparticle according to any one of **claim 1 to claim 5**, wherein the lipid nanoparticle is a sterile liquid composition, and wherein the lipid nanoparticles have a mean hydrodynamic diameter as determined by dynamic laser scattering from about 50 nm to about 300 nm, or from about 60 nm to about 250 nm, or from about 60 nm to about 200 nm, or from about 70 to 200 nm, or from about 75 nm to about 160, or from about 85 nm to about 140 nm, or from about 90 nm to about 130 nm, or from about 50 nm to about 120 nm.

7. The lipid nanoparticle according to any one of **claim 1 to claim 6**, wherein the mRNA compound is a mono-, bi-, or multicistronic mRNA.

8. The lipid nanoparticle of **claim 7**, wherein the mRNA comprises a coding region encoding a peptide or protein, wherein the coding region exhibits a sequence modification, wherein the sequence modification is selected from a G/C content modification, a codon modification, a codon optimization or a C-optimization of the sequence; preferably wherein, compared with the coding region of the corresponding wild-type mRNA, the
- G/C content of the coding region is increased;
- C content of the coding region is increased;
- codon usage in the coding region is adapted to the human codon usage; and/or
- codon adaptation index (CAI) is increased or maximized in the coding region.

9. The lipid nanoparticle according to any one of **claim 1 to claim 8**, wherein the mRNA comprises a 5'-CAP structure, preferably m7G, CAP0, CAP1, CAP2, a modified CAP0 or a modified CAP1 structure.

10. The lipid nanoparticle according to any one of **claim 1 to claim 9**, wherein the mRNA 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, a fragment or variant 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, a fragment, or a variant of any one of these genes.

11. The lipid nanoparticle according to any one of **claim 1 to claim 10**, wherein the mRNA comprises a (i) HSD17B4 5'-UTR and a PSMB3 3'-UTR, more preferably a HSD17B4 5'-UTR (SEQ ID NO:12, SEQ ID NO:13) and a PSMB3 3'-UTR (SEQ ID NO: 46, SEQ ID NO:47).

12. The lipid nanoparticle according to any one **claim 1 to claim 11**, wherein the mRNA comprises no chemical modification, preferably no base modification, more preferably no base modification selected from the group consisting of pseudouridine (psi or ψ), N1-methylpseudouridine (N1MPU, N1Mpsi or N1Mψ), 1-ethylpseudouracil, 2-thiouracil (s2U), 4-thiouracil, 5-methylcytosine, 5-methyluracil, 5-methoxyuracil, most preferably no N1-methylpseudouridine (N1MPU, N1Mpsi or N1Mψ) modification.

13. The lipid nanoparticle of any one **claim 1 to claim 12**, wherein the mRNA comprises at least one chemical modification.

14. A pharmaceutical composition comprising one or more lipid nanoparticles as defined in any one of **claim 1 to claim 13** and an acceptable pharmaceutical carrier.

15. A pharmaceutical composition of **claim 14** or the lipid nanoparticle according to any one of **claim 1 to claim 13** for use as a medicament, wherein the medicament is a prophylactic or therapeutic vaccine.

16. Use of the pharmaceutical composition of **claim 14** or the lipid nanoparticle according to any one of **claim 1 to claim 13** in the manufacture of a medicament for prophylaxis or treatment of cancer or infectious diseases in a subject, most preferably for the prophylaxis or treatment of cancer diseases in a subject, preferably for use in human or veterinary medicine.

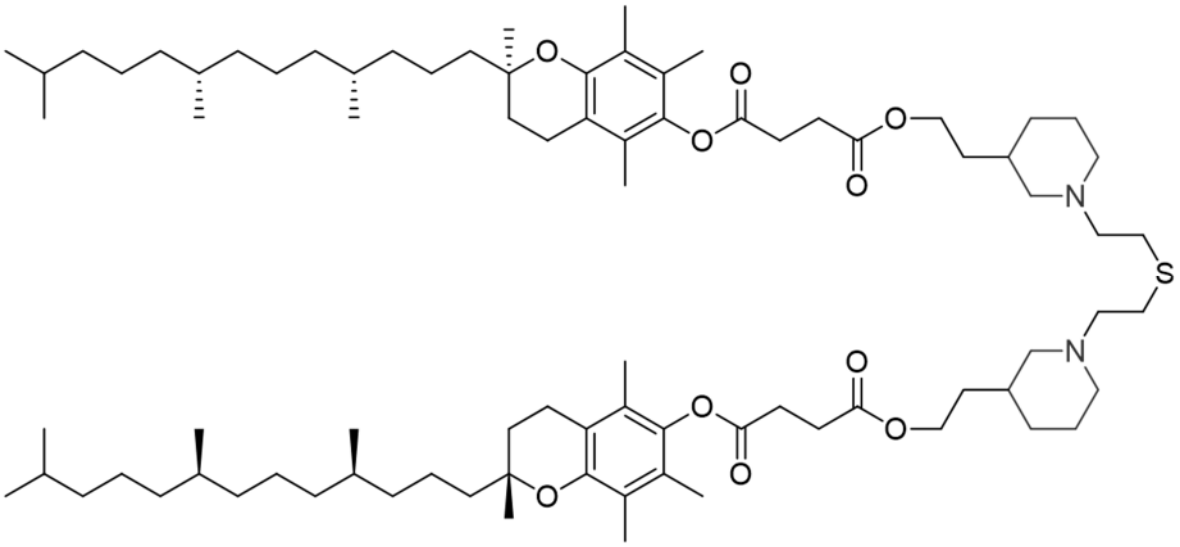
17. A kit or kit of parts, comprising any one of the lipid nanoparticle according to **claim 1 to claim 13**, or the pharmaceutical composition according to **claim 14,** optionally comprising a liquid vehicle for solubilizing, and, optionally, technical instructions providing information on administration and dosage of the components.

18. Use of t the lipid nanoparticle according to any one of **claim 1 to claim 13**, the pharmaceutical composition according to **claim 14**, or the kit or kit of parts of **claim 17** in the manufacture of a medicament for inducing an antigen specific immune response in a subject.

19. Use of the lipid nanoparticle according to any one of **claim 1 to claim 13**, the pharmaceutical composition according to **claim 14**, or the kit or kit of parts of **claim 17**, in the manufacture of a medicament for treating a disease or condition in a subject in need, preferably a cancer disease in a subject in need.

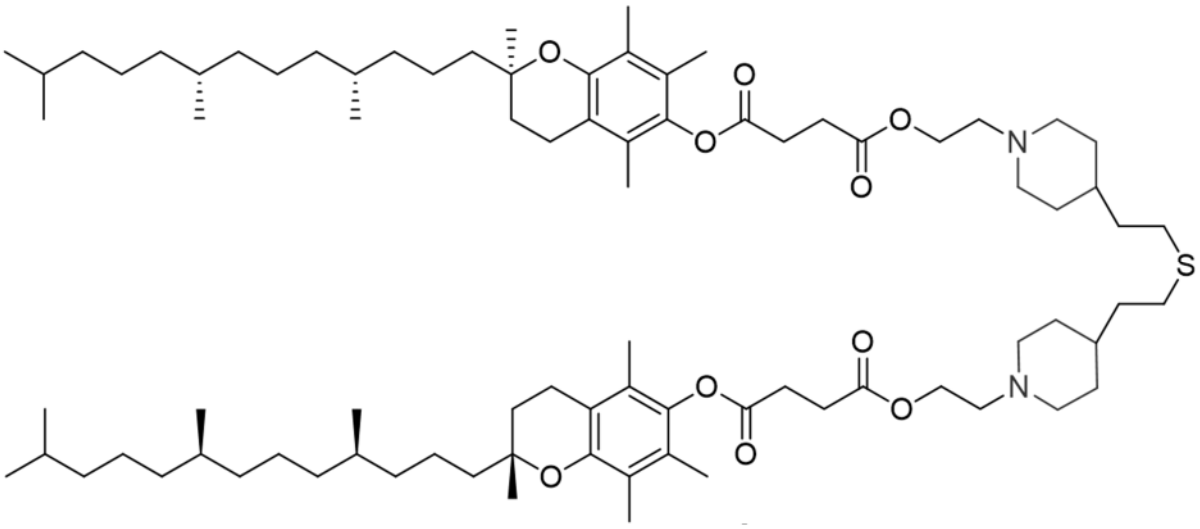
20. The use according to any one of **claim 18 to claim 19**, wherein administration of the lipid nanoparticle, the pharmaceutical composition or the kit or kit of parts results in expression of the antigen encoded by mRNA in the lymphocytes of the subject, or in the spleen and/or lymph nodes.

21. An ionizable lipid according to the following structure



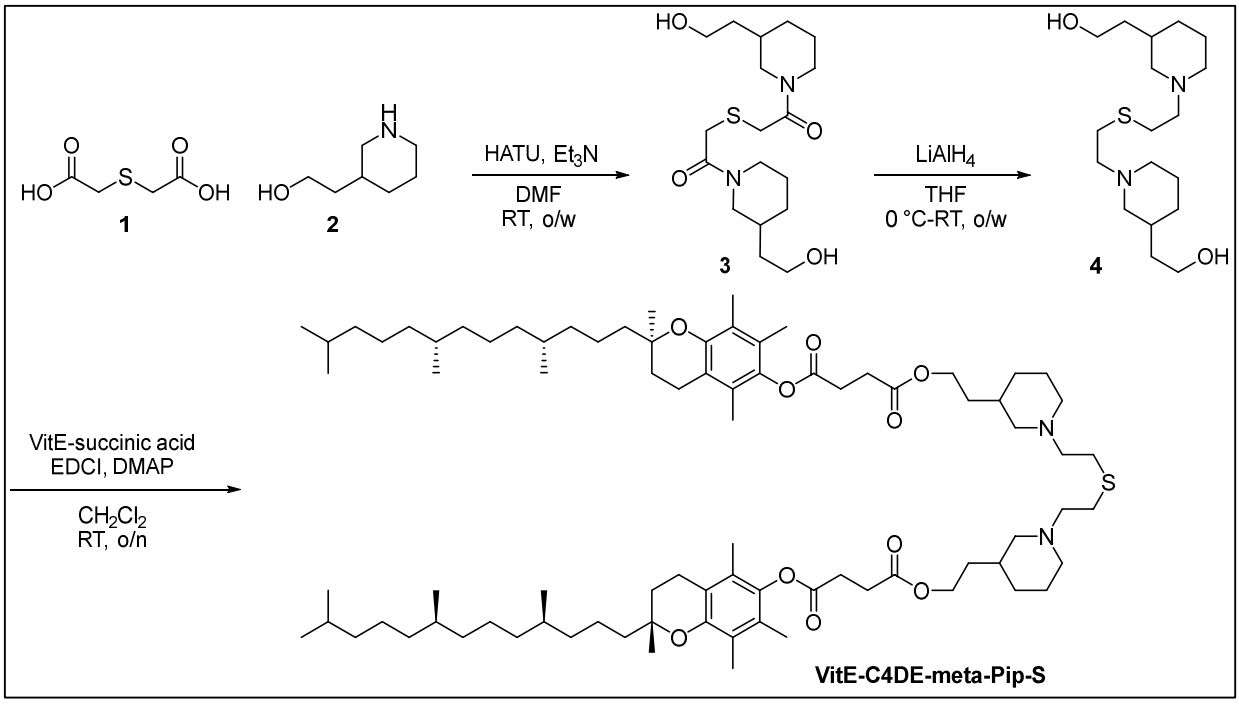
(“CVL1-meta“ or C28).

or according to the following structure



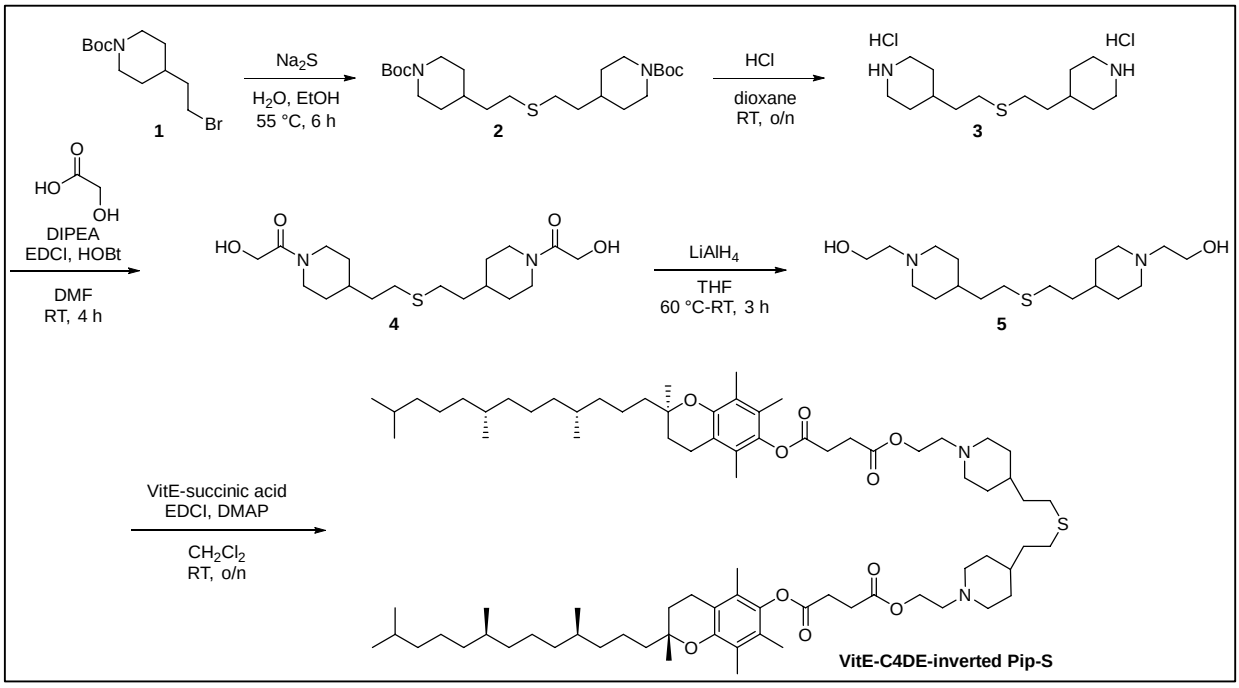
(“CVL1-inverted” or C29).

22. A method of synthesizing the ionizable lipid C28 or the ionizable lipid C29, according to scheme 1 for C28



(scheme 1 – C28)

or according to scheme 2 for C29



(scheme 2 – C29).