

**A COMPOUND FOR PREPARING LIPID NANOPARTICLES
ENCAPSULATING AN AGENT, NANOPARTICLE COMPOSITION
COMPRISING SAID COMPOUND AND RELATED METHODS THEREOF**

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TECHNICAL FIELD

The present disclosure relates broadly to a compound for preparing lipid nanoparticles encapsulating an agent and a method of preparing said compound.

10 The present disclosure also relates to a nanoparticle composition comprising said compound and related methods and uses.

BACKGROUND

15 Lipid nanoparticles are widely used in the delivery of therapeutic, prophylactic and/or biological agents (e.g., polynucleotides such as mRNA). However, a safe, stable and efficacious delivery system remains a challenge. Particularly, there have been reports of adverse health effects and cytotoxicity associated with the use of lipid nanoparticles for delivery.

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Currently, lipid nanoparticles have seen successful use in delivering Moderna's and Pfizer-BioNtech's mRNA Covid-19 vaccines, which have been approved by the United States Food and Drug Administration (US FDA) for human use. Both vaccines utilize SARS-CoV-2 mRNA as the antigen and lipid nanoparticles (LNPs) as the carrier. The LNPs consist of 4 different types of lipids (ionizable lipid, PEG-lipid conjugate, helper lipid and cholesterol). The lipids assemble with the mRNA to form nanoparticles that stimulate the immune cells for prophylactic response against the SARS-CoV-2 virus.

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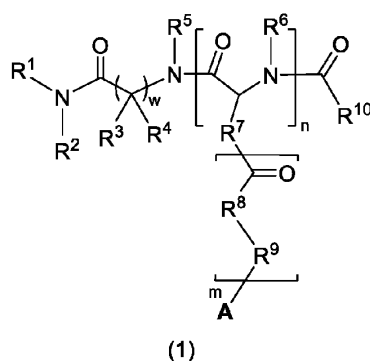
However, the currently available formulations have several disadvantages and drawbacks, and are far from desirable. Firstly, the production of anti-lipid and

anti-PEG antibodies was evident with such formulations (due to use/presence of PEG-lipid conjugate), which can result in hypersensitivity and anaphylaxis in some subjects. Polyethylene glycol (PEG) has been reported to be a high-risk allergen found hidden in drug/food items. Binding of PEG to basophils through IgE can cause release of compounds that induce allergies and individuals may develop anaphylactic conditions from PEG present in medications. PEG has also been identified as the cause of accelerated blood clearance (ABC) phenomenon. In addition, the presence of anti-PEG antibodies in the body may reduce plasma half-life of mRNA LNPs and their vaccination efficacy. Furthermore, currently available formulations lack immune cell-targeting ability.

In view of the above, there is a need to address or at least ameliorate the above-mentioned problems. In particular, there is a need to provide a compound and/or nanoparticle composition for a cost efficient, substantially safe and stable, and/or efficacious delivery of therapeutic, prophylactic and/or biological agents.

SUMMARY

In one aspect, there is provided a compound represented by general formula (1) for preparing lipid nanoparticles encapsulating a therapeutic, prophylactic and/or biological agent:



wherein

A comprises a carbohydrate and/or a derivative thereof;

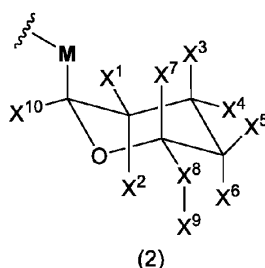
- R¹ and R² are each independently a hydrophobic group;
 R³, R⁴, R⁵, and R⁶ are each independently H, optionally substituted alkyl,
 optionally substituted alkenyl or optionally substituted alkynyl;
 R⁷, R⁹ and R¹⁰ are each independently optionally substituted alkyl, optionally
 5 substituted alkenyl or optionally substituted alkynyl;
 R⁸ is -O-, -S-, or -NR^a-, where R^a is selected from the group consisting of H,
 optionally substituted alkyl, optionally substituted alkenyl or optionally substituted
 alkynyl;
 m is 0 or 1;
 10 n ≥ 1; and
 w ≥ 1.

In one embodiment, the compound is capable of targeting carbohydrate
 receptors on a cell surface.

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In one embodiment, **A** comprises a moiety selected from the group
 consisting of monosaccharide, disaccharide, oligosaccharide, polysaccharide
 and derivatives thereof.

20 In one embodiment, **A** is represented by general formula (2) having a 6-
 membered ring structure:

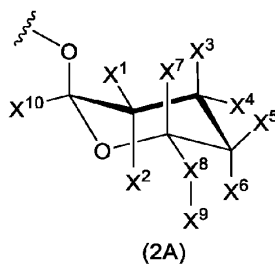


wherein

- X¹ to X⁷ and X⁹ to X¹⁰ are each independently selected from -H or -OH;
 25 X⁸ is alkyl/alkylene; and
M is -O- or -S-.

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In one embodiment, **A** is represented by general formula (2A) having a 6-membered ring structure:



wherein

- 5 X¹ to X⁷ and X⁹ to X¹⁰ are each independently selected from -H or -OH; and
X⁸ is alkyl/alkylene.

In one embodiment, R¹ and R² contains at least linear aliphatic, branched aliphatic and/or cyclic hydrocarbons.

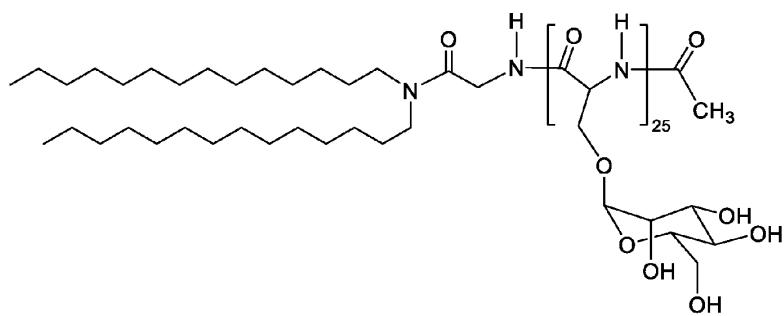
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In one embodiment, m = 1 and R⁷ is -CH₂CH₂-.

In one embodiment, m = 0 and R⁷ is -CH₂-.

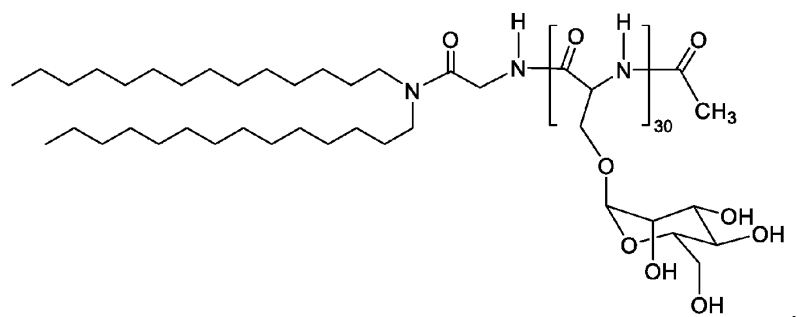
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In one embodiment, the compound comprises a structure selected from one or more of the following:

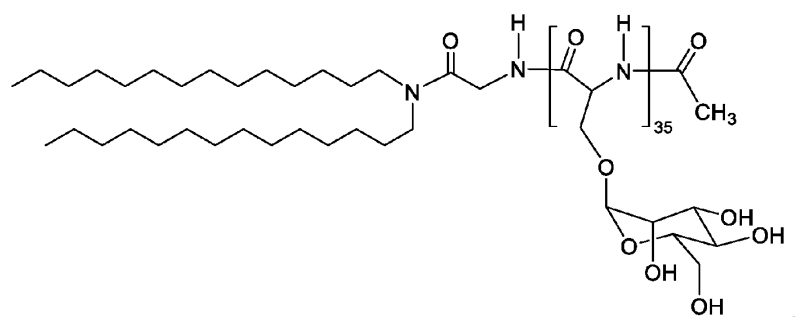


LPSM01 (n = 25)

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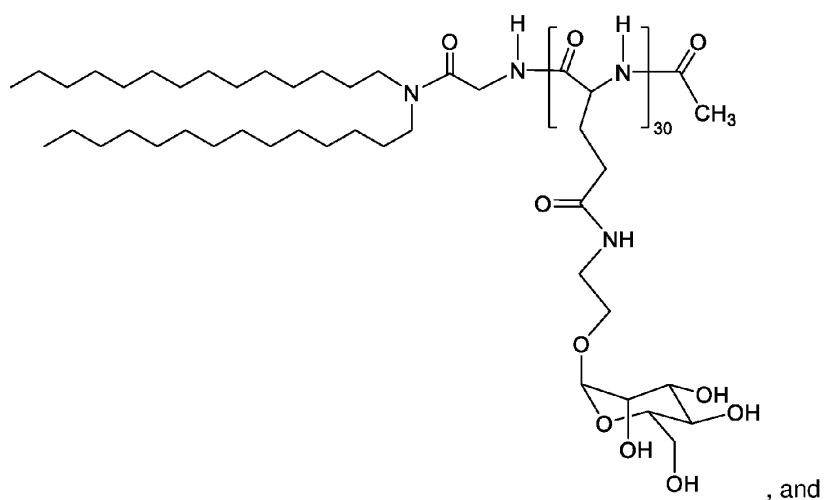


LPSM02 (n = 30)



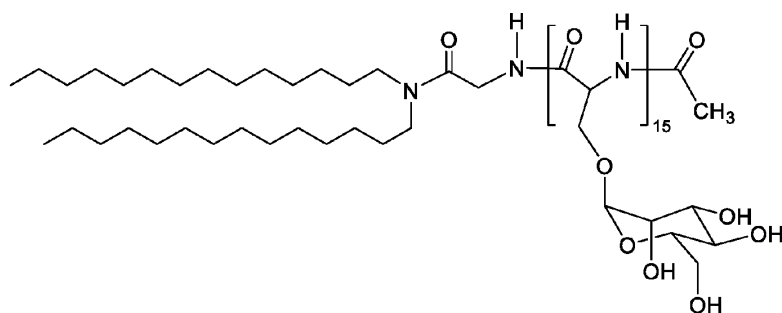
LPSM03 (n = 35)

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LPGM01 (n = 30)

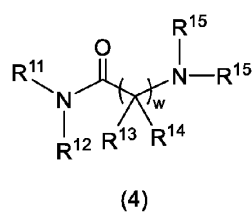
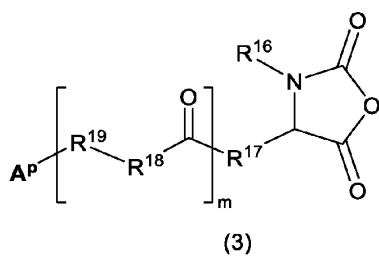
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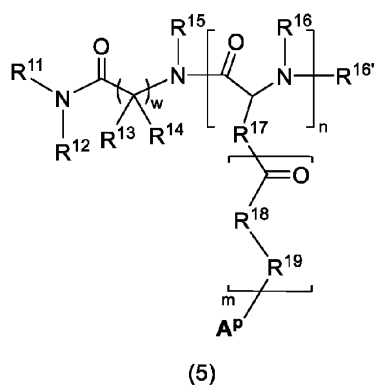
LPSM04 (n = 15)

In another aspect, there is provided a method of preparing a compound as
 5 claimed in any one of the preceding claims, the method comprising:

- (i) polymerizing/reacting one or more N-carboxyanhydride (NCA) monomers represented by general formula (3) with a lipid initiator/molecule represented by general formula (4) to obtain a first intermediate compound represented by general formula (5):



10



wherein

A^P represents **A** protected with one or more protecting groups, where **A** comprises a carbohydrate and/or a derivative thereof;

R^{11} and R^{12} are each independently a hydrophobic group;

R^{13} , R^{14} , R^{15} , and R^{16} are each independently H, optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

R^{17} and R^{19} are each independently optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

R^{18} is $-O-$, $-S-$, or $-NR^{a1}-$, where R^{a1} is selected from the group consisting of H, optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

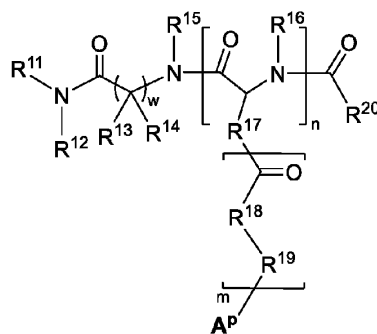
$R^{15'}$ and $R^{16'}$ are each independently H, optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl; and

m is 0 or 1;

$n \geq 1$; and

$w \geq 1$;

- (ii) reacting the first intermediate compound represented by general formula (5) with an acylating agent to obtain a second intermediate compound represented by general formula (7):



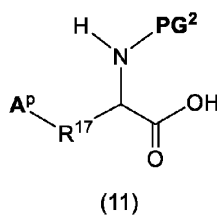
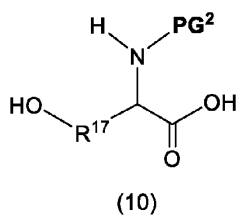
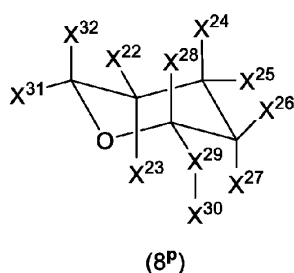
(7)

wherein R^{20} is optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

- (iii) deprotecting the second intermediate compound represented by general formula (7) to obtain the compound represented by general formula (1).

In one embodiment, the method further comprises, prior to step (i):

- 5 (a-i) reacting a protected monosaccharide represented by general formula (8^P) with a protected compound represented by general formula (10) in the presence of a Lewis acid to obtain a first intermediate compound represented by general formula (11):



10

wherein

X^{22} to X^{28} and X^{30} to X^{32} are each independently selected from $-H$ or $-OPG^1$;

X^{29} is alkyl;

15 PG^1 is $-C(=O)-R^{22}$;

R^{22} is optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

A^P represents **A** protected with one or more protecting groups, where **A** comprises a carbohydrate and/or a derivative thereof;

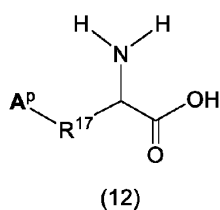
20 R^{17} is independently optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

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PG² is a protecting group selected from 9-Fluorenylmethoxycarbonyl (Fmoc), 2-(4-Nitrophenylsulfonyl)ethoxycarbonyl (Nsc), 2-Fluoro-Fmoc (Fmoc(2F)), 2-Monoisooctyl-Fmoc (mio-Fmoc), 2,7-Diisooctyl-Fmoc (dio-Fmoc), N-carboxybenzyl (Cbz), or combinations thereof;

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- (a-ii) deprotecting the first intermediate compound represented by general formula (11) to obtain a second intermediate compound represented by general formula (12):



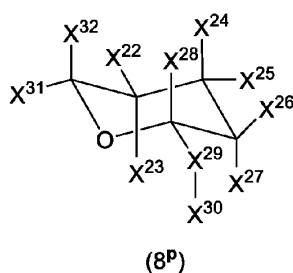
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- (a-iii) reacting the second intermediate compound represented by general formula (12) with a carbonylating agent to obtain the N-carboxyanhydride (NCA) monomer represented by general formula (3).

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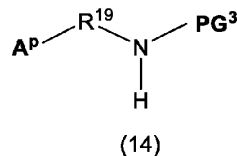
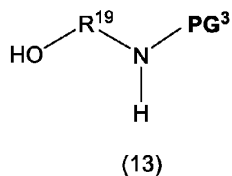
In one embodiment, the method further comprises, prior to step (i):

- (b-i) optionally reacting a protected monosaccharide represented by general formula (8^p) with a protected alkanolamine compound represented by general formula (13) in the presence of a Lewis acid to obtain a first intermediate compound represented by general formula (14):



20

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wherein

X^{22} to X^{28} and X^{30} to X^{32} are each independently selected from $-\text{H}$ or $-\text{OPG}^1$;

5 X^{29} is alkyl;

PG^1 is $-\text{C}(=\text{O})-\text{R}^{22}$;

R^{22} is optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

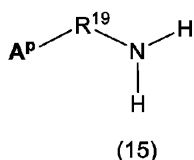
10 A^p represents **A** protected with one or more protecting groups, where **A** comprises a carbohydrate and/or a derivative thereof;

R^{19} is independently optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

PG^3 is a protecting group selected from N-carboxybenzyl, benzyloxycarbonyl (Cbz), or combinations thereof;

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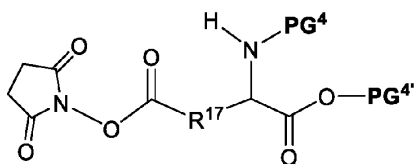
(b-ii) optionally deprotecting the first intermediate compound represented by general formula (14) to obtain a second intermediate compound represented by general formula (15):



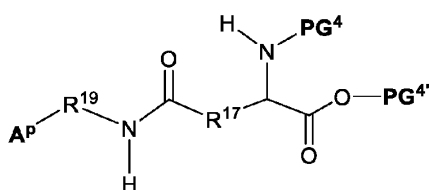
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(b-iii) reacting the second intermediate compound represented by general formula (15) with a protected compound represented by general formula (16) in the presence of a base to obtain a third intermediate compound represented by general formula (17):

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(16)



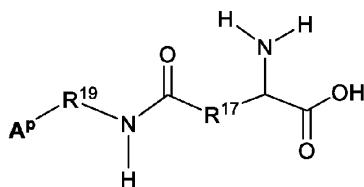
(17)

wherein

R¹⁷ is independently optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl; and

PG⁴ and PG^{4'} are each independently a protecting group selected from *tert*-butoxycarbonyl (Boc), *tert*-butyl, benzyloxycarbonyl protecting group (Cbz), or combinations thereof;

- 10 (b-iv) deprotecting the third intermediate compound represented by general formula (17) to obtain a fourth intermediate compound represented by general formula (18):



(18)

- 15 (b-v) reacting the fourth intermediate compound represented by general formula (18) with a carbonylating agent to obtain the N-carboxyanhydride (NCA) monomer represented by general formula (3).

In another aspect, there is provided a nanoparticle composition for delivery of a therapeutic, prophylactic and/or biological agent, the nanoparticle composition comprising:

- a compound as disclosed herein; and
- 5 a therapeutic, prophylactic and/or biological agent.

In one embodiment, the composition further comprises:

- (a) ionizable lipid;
- (b) helper lipid; and
- 10 (c) cholesterol and/or derivatives thereof.

In one embodiment, the ionizable lipid, helper lipid, cholesterol and/or derivatives thereof, and compound represented by general formula (1) are mixed at a mole ratio of 20 – 50 : 4 – 20 : 25 – 50 : 0.5 – 20.

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In one embodiment, the ionizable lipid is selected from ALC-0315, SM-102, Lipid 5, DLinDMA, D-Lin-MC2-DMA, DLin-MC3-DMA, D-Lin-MC4-DMA, Dlin-KC2-DMA, YSK05, AA3-Dlin, SSPalmM, SSPalmO-Phe, Lipid A9, L319, DODMA, CL1, BP Lipid 310, ATX-001, ATX-100, Lipid 2, 80-O16B, BP Lipid 309,
20 BP Lipid 307, 93-O17S, 93-O17O, NT1-O14B, 306-O12B-3, 306-O12B, 113-O16B, 306Oi10, 306Oi9-cis2, BAMEA-O16B, AI-28, 113-O12B, 98N12-5, Ckk-E12, OF-02, C12-200, BP Lipid 311, BP Lipid 308, BP Lipid 314, BP Lipid 312, LP01, TCL053, Lipid C24, BP Lipid 315, Lipid 29, 9A1P9, C13-112-tri-tail, C13-113-tri-tail, C13-112-tetra-tail, or C13-113-tetra-tail, C12-200 or combinations
25 thereof.

In one embodiment, the helper lipid is selected from 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dilinoleoyl-sn-glycero-3-phosphocholine
30 (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-

oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 5 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 10 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), sphingomyelin or combinations thereof.

In one embodiment, the cholesterol and/or derivatives thereof is selected from cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, 15 brassicasterol, avenasterol or combinations thereof.

In one embodiment, the nanoparticle composition comprises nanoparticles having a N/P ratio from 1:1 to 20:1.

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In one embodiment, the nanoparticle composition comprises nanoparticles having an average particle size of from 40 nm to 500 nm.

In one embodiment, the nanoparticle composition comprises nanoparticles having a zeta potential of from -20 mV to +20 mV.

25

In another aspect, there is provided a nanoparticle composition as disclosed herein for use in medicine.

In another aspect, there is provided a nanoparticle composition as disclosed herein for use in the treatment or prophylaxis of a disease, disorder or condition in a subject in need thereof.

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In another aspect, there is provided use of a nanoparticle composition as disclosed herein in the manufacture of a medicament for treatment or prophylaxis of a disease, disorder or condition in a subject in need thereof.

5 In another aspect, there is provided a method of treating or preventing a disease, disorder or condition in a subject in need thereof, the method comprising administering a therapeutically effective amount of the nanoparticle composition as disclosed herein to the subject.

10 In one embodiment, an immune response in the subject is to be induced through the administration of the nanoparticle composition thereto.

In one embodiment, the disease, disorder or condition is mediated by a coronavirus.

15

In one embodiment, the coronavirus is a SARS-CoV-2 coronavirus.

DEFINITIONS

20 The term "particle" as used herein broadly refers to a discrete entity or a discrete body. The particle described herein can include an organic, an inorganic, a composite particle or a biological particle. The particle used described herein may also be a macro-particle that is formed by an aggregate of a plurality of sub-particles or a fragment of a small object. The particle of the present disclosure
25 may be spherical, substantially spherical, or non-spherical, such as irregularly shaped particles or ellipsoidally shaped particles. The term "size" when used to refer to the particle broadly refers to the largest dimension of the particle. For example, the term "size" when used in the context of nanoparticle can refer to the diameter of the nanoparticle although it is not limited as such. In various
30 embodiments, when the particle is substantially spherical, the term "size" can refer to the diameter of the particle; or when the particle is substantially non-spherical, the term "size" can refer to the largest length of the particle.

The term "nano" as used herein is to be interpreted broadly to include dimensions in a nanoscale, *i.e.*, less than about 1000 nm, about 1 nm to less than about 1000 nm, about 1 nm to about 900 nm, about 1 nm to about 800 nm, about 1 nm to about 700 nm, about 1 nm to about 600 nm, about 1 nm to about 500 nm, about 1 nm to about 400 nm, about 1 nm to about 300 nm, about 1 nm to about 200 nm, or from about 1 nm to about 100 nm. Accordingly, the term "nanostructures", "nanoparticles", "nanomaterials" and the like as used herein may include structures that have at least one dimension in the range of no more than said range. The term "nanostructures", "nanoparticles", "nanomaterials" and the like as used herein may include structures that have at least one dimension that is no more than about 1,000 nm, no more than about 950 nm, no more than about 900 nm, no more than about 850 nm, no more than about 800 nm, no more than about 750 nm, no more than about 700 nm, no more than about 650 nm, no more than about 600 nm, no more than about 550 nm, no more than about 500 nm, no more than about 450 nm, no more than about 400 nm, no more than about 350 nm, no more than about 300 nm, no more than about 250 nm, no more than about 200 nm, no more than about 150 nm, no more than about 100 nm, no more than about 90 nm, no more than about 80 nm, no more than about 70 nm, no more than about 60 nm, no more than about 50 nm, no more than about 40 nm, no more than about 30 nm, no more than about 20 nm, or no more than about 10 nm.

The term "micro" as used herein is to be interpreted broadly to include dimensions from about 1 micron to about 1000 microns, from about 1 micron to less than about 1000 microns, from about 1 micron to about 900 microns, from about 1 micron to about 800 microns, from about 1 micron to about 700 microns, from about 1 micron to about 600 microns, from about 1 micron to about 500 microns, from about 1 micron to about 400 microns, from about 1 micron to about 300 microns, from about 1 micron to about 200 microns, from about 1 micron to about 100 microns, or from about 1 micron to about 5 microns.

The term "treatment", "treat" and "therapy", and synonyms thereof as used herein refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) a medical condition, which includes but is not limited to diseases, symptoms and disorders.

5 A medical condition also includes a body's response to a disease or disorder, e.g., inflammation. Those in need of such treatment include those already with a medical condition as well as those prone to getting the medical condition or those in whom a medical condition is to be prevented.

10 As used herein, the term "therapeutically effective amount" of a compound is intended to refer to an amount that is sufficient or capable of preventing or at least slowing down (lessening) a medical condition, such as infectious diseases (e.g., dengue disease caused by dengue virus), respiratory illnesses (e.g., coronavirus caused by the SARS-CoV-2 virus or flu caused by influenza virus),
15 cancer, autoimmune diseases and cardiovascular diseases etc. Dosages and administration of compounds, compositions and formulations of the present disclosure may be determined by one of ordinary skill in the art of clinical pharmacology or pharmacokinetics. An effective amount of the active agent of the present disclosure to be employed therapeutically will depend, for example,
20 upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it may be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect.

25 The term "subject" is intended to broadly refer to any animal, such as a mammal, and including humans. Exemplary subjects include but are not limited to humans and non-human primates. The term "subject" as used herein also includes patients and non-patients. The term "patient" refers to individuals suffering or are likely to suffer from a medical condition such as infectious
30 diseases (e.g., coronavirus caused by the SARS-CoV-2 virus, dengue disease caused by dengue virus etc), while "non-patients" refer to individuals not suffering and are likely to not suffer from the medical condition. "Non-patients" include

healthy individuals, non-diseased individuals and/or an individual free from the medical condition. As used herein, the term "mammal" includes vertebrate such as a human or a large veterinary mammal (e.g., horses, cattle, deer, sheep, llamas, goats, pigs).

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The term "bond" refers to a linkage between atoms in a compound or molecule. The bond may be a single bond, a double bond, or a triple bond.

10 In the definitions of a number of substituents below, it is stated that "the group may be a terminal group or a bridging group". This is intended to signify that the use of the term is intended to encompass the situation where the group is a terminal group/moiety as well as the situation where the group is a linker between two other portions of the molecule. Using the term "alkyl" having 1 carbon atom as an example, it will be appreciated that when existing as a terminal
15 group, the term "alkyl" having 1 carbon atom may mean -CH_3 and when existing as a bridging group, the term "alkyl" having 1 carbon atom may mean $\text{-CH}_2\text{-}$ or the like.

The term "alkyl" as a group or part of a group refers to a straight or
20 branched aliphatic hydrocarbon group having 1 to 20 carbon atoms, 1 to 10 carbon atoms, 1 to 6 carbon atoms, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 carbon atoms. Examples of suitable straight and branched alkyl substituents include methyl, ethyl, n-propyl, 2-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, t-butyl, hexyl, amyl, 1,2-dimethylpropyl, 1,1-
25 dimethylpropyl, pentyl, isopentyl, hexyl, 4-methylpentyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 1,2,2-trimethylpropyl, 1,1,2-trimethylpropyl, 2-ethylpentyl, 3-ethylpentyl, heptyl, 1-methylhexyl, 2,2-dimethylpentyl, 3,3-dimethylpentyl, 4,4-dimethylpentyl, 1,2-dimethylpentyl, 1,3-dimethylpentyl, 1,4-
30 dimethylpentyl, 1,2,3-trimethylbutyl, 1,1,2-trimethylbutyl, 1,1,3-trimethylbutyl, 5-methylheptyl, 1-methylheptyl, octyl, nonyl, decyl and the like. The group may be a terminal group or a bridging group.

The term "alkenyl" as a group or part of a group denotes an aliphatic hydrocarbon group containing at least one carbon-carbon double bond and which may be straight or branched having 2 to 20 carbon atoms, 2 to 10 carbon atoms, 2 to 6 carbon atoms, or 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 carbon atoms in the chain. The group may contain a plurality of double bonds and the orientation about each double bond is independently E or Z. Exemplary alkenyl groups include, but are not limited to, ethenyl, vinyl, allyl, 1-methylvinyl, 1-propenyl, 2-propenyl, 2-methyl-1-propenyl, 2-methyl-1-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, 1,3-butadienyl, 1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 1,3-pentadienyl, 2,4-pentadienyl, 1,4-pentadienyl, 3-methyl-2-butenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 1,3-hexadienyl, 1,4-hexadienyl, 2-methylpentenyl, 1-heptenyl, 2-heptenyl, 3-heptenyl, 1-octenyl, 2-octenyl, 3-octenyl, 1-nonenyl, 2-nonenyl, 3-nonenyl, 1-decenyl, 2-decenyl, 3-decenyl and the like. The group may be a terminal group or a bridging group.

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The term "alkynyl" as a group or part of a group denotes an aliphatic hydrocarbon group containing at least one carbon-carbon triple bond and which may be straight or branched having 2 to 20 carbon atoms, 2 to 10 carbon atoms, 2 to 6 carbon atoms, or 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 carbon atoms in the chain. The group may contain a plurality of triple bonds. Exemplary alkynyl groups include, but are not limited to, acetylenyl, propynyl, 1-butyne, 2-butyne, 3-butyne, 1-pentyne, 2-pentyne, 3-methyl-1-butyne, 4-pentyne, 1-hexyne, 2-hexyne, 5-hexyne, 1-heptyne, 2-heptyne, 6-heptyne, 1-octyne, 2-octyne, 7-octyne, 1-nonyne, 2-nonyne, 8-nonyne, 1-decynyl, 2-decynyl, 9-decynyl and the like. The group may be a terminal group or a bridging group.

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The term "cyclic" as used herein broadly refers to a structure where one or more series of atoms are connected to form at least one ring. The term includes, but is not limited to, both saturated and unsaturated 5-membered and saturated and unsaturated 6-membered rings. Examples of groups having a cyclic structure include, but are not limited to, cyclopentane, cyclopentene,

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cyclohexane, cyclohexene, benzene and the like. The term "cyclic" as used herein includes "heterocyclic".

The term "heterocyclic" as used herein broadly refers to a structure where
5 two or more different kinds of atoms are connected to form at least one ring. For example, a heterocyclic ring may be formed by carbon atoms and at least another atom (i.e. heteroatom) selected from oxygen (O), nitrogen (N) or (NR) and sulfur (S), where R is independently a hydrogen or an organic group. The term also includes, but is not limited to, saturated and unsaturated 5-membered, and
10 saturated and unsaturated 6-membered rings. Examples of groups having a heterocyclic structure include, but are not limited to furan, thiophene, 1H-pyrrole, 2H-pyrrole, 1-pyrroline, 2-pyrroline, 3-pyrroline, 1-pyrazoline, 2-pyrazoline, 3-pyrazoline, 2-imidazoline, 3-imidazoline, 4-imidazoline, pyrazole, imidazole, oxazole, isoxazole, thiazole, isothiazole, 1,2,3-triazole, 1,2,4-triazole, 1,2,3-
15 oxadiazole, disubstituted 1,2,4-oxadiazole, 1,2,5-oxadiazole, 1,3,4-oxadiazole, 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, 1,3,4-thiadiazole, tetrahydrofuran, tetrahydrothiophene, pyrrolidine, 1,3-dioxolane, 1,2-oxathiolane, 1,3-oxathiolane, pyrazolidine, imidazolidine, pyridine, pyridazine, pyrimidine, pyrazine, 1,2-oxazine, 1,3-oxazine, 1,4-oxazine, thiazine, 1,2,3-triazine, 1,2,4-
20 triazine, 1,3,5-triazine, 2H-pyran, 4H-pyran, 2-pyrone, 4-pyrone, 1,4-dioxin, 2H-thiopyran, 4H-thiopyran, tetrahydropyran, thiane, piperidine, 1,4-dioxane, 1,2-dithiane, 1,3-dithiane, 1,4-dithiane, 1,3,5-trithiane, piperazine, morpholine, thiomorpholine and the like.

25 The term "amine group" or the like is intended to broadly refer to a group containing -NR_2 , where R is independently a hydrogen or an organic group. The group may be a terminal group or a bridging group.

The term "amide group" or the like is intended to broadly refer to a group
30 containing -C(=O)NR_2 , where R is independently a hydrogen or an organic group. The group may be a terminal group or a bridging group.

The term "aryl" as a group or part of a group denotes (i) an optionally substituted monocyclic, or fused polycyclic, aromatic carbocycle (ring structure having ring atoms that are all carbon) preferably having from 5 to 20, or 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 carbon atoms per ring. Examples of aryl groups include but are not limited to phenyl, tolyl, xylyl, naphthyl, anthracenyl, phenanthrenyl, fluorenyl, indenyl or indanyl and the like.

The term "heteroaryl" as a group or part of a group refers to groups containing an aromatic ring (preferably a 5- or 6- membered aromatic ring) having one or more carbon atoms (for example 1 to 6 carbon atoms) in the ring replaced by a heteroatom. Suitable heteroatoms may include nitrogen (N) or (NH), oxygen (O) and sulfur (S). Examples of heteroaryl include but are not limited to thiophene, benzothiophene, benzofuran, benzimidazole, benzoxazole, benzothiazole, benzisothiazole, naphtha[2,3-b]thiophene, furan, isoindolizine, xantholene, phenoxatine, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, tetrazole, indole, isoindole, 1H-indazole, purine, quinoline, isoquinoline, phthalazine, naphthyridine, quinoxaline, cinnoline, carbazole, phenantridine, acridine, phenazine, thiazole, isothiazole, phenothiazine, oxazole, isooxazole, furazane, phenoxazine, 2-, 3- or 4-pyridyl, 2-, 3-, 4-, 5-, or 8-quinolyl, 1-, 3-, 4-, or 5-isoquinolyl, 1-, 2-, or 3-indolyl, and 2-, or 3-thienyl and the like. The group may be a terminal group or a bridging group.

The term "halogen" represents chlorine, fluorine, bromine or iodine. The term "halide" represents chloride, fluoride, bromide or iodide.

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The term "optionally substituted," when used to describe a chemical structure or moiety, refers to the chemical structure or moiety wherein one or more of its hydrogen atoms is optionally substituted with a chemical moiety or functional group such as alcohol, alkoxy, alkanoyloxy, alkoxycarbonyl, alkenyl, alkyl (e.g., methyl, ethyl, propyl, t-butyl), alkynyl, alkylcarbonyloxy (–OC(O)alkyl), amide (–C(O)NH–alkyl– or –alkylNHC(O)alkyl), amine (such as alkylamino, arylamino, arylalkylamino), aryl, aryloxy, azo, carbamoyl

(-NHC(O)O-alkyl- or -OC(O)NH-alkyl), carbamyl (e.g., CONH₂, as well as CONH-alkyl, CONH-aryl, and CONH-arylalkyl), carboxyl, carboxylic acid, cyano, ester, ether (e.g., methoxy, ethoxy), halo, haloalkyl (e.g., -CCl₃, -CF₃, -C(CF₃)₃), heteroalkyl, isocyanate, isothiocyanate, nitrile, nitro, phosphodiester, sulfide, sulfonamido (e.g., SO₂NH₂), sulfone, sulfonyl (including alkylsulfonyl, arylsulfonyl and arylalkylsulfonyl), sulfoxide, thiol (e.g., sulfhydryl, thioether) or urea (-NHCONH-alkyl-).

The terms "coupled" or "connected" as used in this description are intended to cover both directly connected or connected through one or more intermediate means, unless otherwise stated.

The term "associated with", used herein when referring to two elements refers to a broad relationship between the two elements. The relationship includes, but is not limited to a physical, a chemical or a biological relationship. For example, when element A is associated with element B, elements A and B may be directly or indirectly attached to each other or element A may contain element B or vice versa.

The term "adjacent" used herein when referring to two elements refers to one element being in close proximity to another element and may be but is not limited to the elements contacting each other or may further include the elements being separated by one or more further elements disposed therebetween.

The term "and/or", e.g., "X and/or Y" is understood to mean either "X and Y" or "X or Y" and should be taken to provide explicit support for both meanings or for either meaning.

Further, in the description herein, the word "substantially" whenever used is understood to include, but not restricted to, "entirely" or "completely" and the like. In addition, terms such as "comprising", "comprise", and the like whenever used, are intended to be non-restricting descriptive language in that they broadly

include elements/components recited after such terms, in addition to other components not explicitly recited. For example, when "comprising" is used, reference to a "one" feature is also intended to be a reference to "at least one" of that feature. Terms such as "consisting", "consist", and the like, may in the appropriate context, be considered as a subset of terms such as "comprising", "comprise", and the like. Therefore, in embodiments disclosed herein using the terms such as "comprising", "comprise", and the like, it will be appreciated that these embodiments provide teaching for corresponding embodiments using terms such as "consisting", "consist", and the like. Further, terms such as "about", "approximately" and the like whenever used, typically means a reasonable variation, for example a variation of +/- 5% of the disclosed value, or a variance of 4% of the disclosed value, or a variance of 3% of the disclosed value, a variance of 2% of the disclosed value or a variance of 1% of the disclosed value.

Furthermore, in the description herein, certain values may be disclosed in a range. The values showing the end points of a range are intended to illustrate a preferred range. Whenever a range has been described, it is intended that the range covers and teaches all possible sub-ranges as well as individual numerical values within that range. That is, the end points of a range should not be interpreted as inflexible limitations. For example, a description of a range of 1% to 5% is intended to have specifically disclosed sub-ranges 1% to 2%, 1% to 3%, 1% to 4%, 2% to 3% etc., as well as individually, values within that range such as 1%, 2%, 3%, 4% and 5%. The intention of the above specific disclosure is applicable to any depth/breadth of a range.

Additionally, when describing some embodiments, the disclosure may have disclosed a method and/or process as a particular sequence of steps. However, unless otherwise required, it will be appreciated that the method or process should not be limited to the particular sequence of steps disclosed. Other sequences of steps may be possible. The particular order of the steps disclosed herein should not be construed as undue limitations. Unless otherwise required, a method and/or process disclosed herein should not be limited to the steps being

carried out in the order written. The sequence of steps may be varied and still remain within the scope of the disclosure.

Furthermore, it will be appreciated that while the present disclosure provides embodiments having one or more of the features/characteristics discussed herein, one or more of these features/characteristics may also be disclaimed in other alternative embodiments and the present disclosure provides support for such disclaimers and these associated alternative embodiments.

It will also be appreciated that where priority is claimed to an earlier application, the full contents of the earlier application is also taken to form part of the present disclosure and may serve as support for embodiments disclosed herein.

DESCRIPTION OF EMBODIMENTS

Exemplary, non-limiting embodiments of a compound for preparing lipid nanoparticles encapsulating an agent, a method of preparing said compound, a nanoparticle composition comprising said compound and related methods/uses thereto are disclosed hereinafter.

COMPOUND

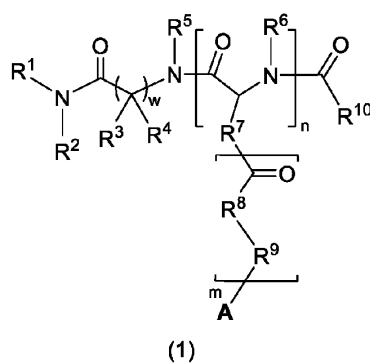
There is provided a compound for preparing lipid nanoparticles. In various embodiments, the compound comprises one or more peptide units/blocks and/or derivative(s) thereof. For example, the compound may comprise one or more oligopeptides, polypeptides/poly(amino acids) and/or derivative(s) thereof. In various embodiments, the total number of peptide units/blocks and/or derivative(s) thereof (or total length of oligopeptides, polypeptides/poly(amino acids) and/or derivative(s) thereof) in the compound is adjustable as desired. Advantageously, in various embodiments, the compound is designed/configured to allow the hydrophilicity/hydrophobicity balance of said compound to be

customizable by the adjustment of the number of peptide units/blocks and/or derivative(s) thereof (or length of the oligopeptides, polypeptides/poly(amino acids) and/or derivative(s) thereof and length of lipid attached to oligopeptides, polypeptides/poly(amino acids) and/or derivative(s) thereof). In various
5 embodiments, the peptide unit (e.g., oligopeptide or polypeptide) and/or derivative(s) thereof is further functionalized with/conjugated with one or more sugar/carbohydrate/saccharide unit(s)/group(s) and/or derivative(s) thereof. Advantageously, the presence of sugar/carbohydrate/saccharide unit(s)/group(s) and/or derivative(s) thereof equips the compound with or imparts the ability of
10 targeting sugar/carbohydrate/saccharide receptors found in/on cell surfaces (e.g., surfaces of immune cells such as macrophages and dendritic cells, surfaces of fibroblasts and keratinocytes, and liver sinusoidal endothelial cells). Advantageously, in various embodiments therefore, the compound comprises cell-targeting ability, e.g., immune cell-targeting ability. In various embodiments,
15 the sugar/carbohydrate/saccharide and/or derivatives thereof is/are hydrophilic. Advantageously, the presence of sugar/carbohydrate/saccharide and/or derivatives thereof increases the hydrophilicity of the compound, and consequently solubility of the compound. Even more advantageously, the structure of the compound allows for embodiments of the compound to be
20 used/formulated into nanoparticles in a composition that may be used as an encapsulation/loading agent, delivery vehicle/system and/or transfection vehicle/system. In various embodiments, the design of the compound helps prevent non-specific protein absorption, particle aggregation and controls the size of the nanoparticles formed. In various embodiments, embodiments of the
25 compound help maintain colloidal stability (of the nanoparticles), and facilitate the condensation and encapsulating/loading of molecules/cargoes into the nanoparticle composition. In various embodiments, the compound is designed/configured to allow loading/encapsulation of one or more types of molecules or cargoes. In various embodiments, the compound is also
30 designed/configured to allow the loaded/encapsulated agent to be released from a composition containing said compound and/or subsequently delivered to a desired target (e.g., cell, cytosol, tissue or organ). The molecules/cargoes to be

loaded/encapsulated onto/into/within a composition containing the compound may include but is not limited to a therapeutic agent, a prophylactic agent, a biological agent or the like. In various embodiments, the molecules/cargoes to be loaded/encapsulated comprises a nucleic acid. For example, the molecules/cargoes to be loaded/encapsulated may be a nucleic acid selected from ribonucleic acid (RNA), messenger ribonucleic acid (mRNA), microRNA (miRNA), small interfering ribonucleic acid (siRNA), deoxyribonucleic acid (DNA), plasmid deoxyribonucleic acid (pDNA), oligonucleotides such as antisense oligonucleotide (ASO) or the like or combinations thereof. In various embodiments, the molecules/cargoes to be loaded/encapsulated comprises therapeutics, e.g., negatively-charged therapeutics. For example, the molecules/cargoes to be loaded/encapsulated may be therapeutics selected from drug molecule, vaccine (e.g., dengue vaccine, Covid-19 vaccine), or the like or combinations thereof. Advantageously, the compound is suitable for use in formulating into nanoparticles for encapsulating and/or delivering one or more therapeutic agent, prophylactic agent and/or biological agent to a desired target (e.g., subject, cell, cytosol, tissue or organ).

Accordingly, in various embodiments, there is also provided a carrier,
20 nanocarrier or delivery system/vehicle comprising the compound.

In various embodiments, the compound comprises a structure that is represented by general formula (1):



(1)

wherein

A comprises a hydrophilic moiety or component selected from carbohydrate/sugar/saccharide and derivatives thereof;

R¹ and R² are each independently a hydrophobic tail/chain/group;

5 R³, R⁴, R⁵, and R⁶ are each independently H, optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

R⁷ and R⁹ are each independently optionally substituted alkyl/alkylene, optionally substituted alkenyl/alkenylene or optionally substituted alkynyl/alkynylene;

R⁸ is -O-, -S-, or -NR^a-, where R^a is selected from the group consisting of H,
10 optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

R¹⁰ is optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

m is 0 or 1;

15 n ≥ 1; and

w ≥ 1.

In various embodiments, the compound comprises a
polypeptide/poly(amino acid) and/or derivative(s) thereof, e.g., a block
20 polypeptide and/or derivative(s) thereof.

In various embodiments, the peptide units/blocks and/or derivative(s)
thereof are functionalized with/conjugated to a moiety **A**. **A** may be a hydrophilic
moiety. In various embodiments, **A** is selected from
25 carbohydrate/sugar/saccharide and derivatives thereof. In various embodiments,
the carbohydrate/sugar/saccharide is selected from the group consisting of
monosaccharide, disaccharide, oligosaccharide, polysaccharide and derivatives
thereof. In various embodiments, the carbohydrate/saccharide is in a cyclic form,
for example as a 5-membered ring (e.g., fructose or ribose) or 6-membered ring
30 (e.g., mannose or glucose). Thus, in various embodiments, the
carbohydrate/saccharide is a furanose or pyranose. In various embodiments, the
carbohydrate/saccharide is in a linear form, for example as a linear-chain

monosaccharide. In various embodiments, the compound comprises a carbohydrate-functionalized polypeptide or carbohydrate-functionalized poly(amino acid). In various embodiments, the compound comprises a carbohydrate-functionalized polypeptide-*b*-lipid or carbohydrate-functionalized poly(amino acid)-*b*-lipid where a carbohydrate is attached onto each unit of the polypeptide. In various embodiments, the compound comprises a monosaccharide-functionalized polypeptide such as a mannose-functionalized polypeptide where a mannose is attached onto each unit of the polypeptide. In various embodiments, the compound comprises a monosaccharide-functionalized polypeptide-*b*-lipid such as a mannose-functionalized polypeptide-*b*-lipid where a mannose is attached onto each unit of the polypeptide. For example, the compound may comprise a mannose-functionalized polyserine/polyglutamic acid-*b*-lipid wherein the mannose is attached onto each unit of the polyserine/polyglutamic acid.

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Advantageously, the presence of carbohydrate/sugar/saccharide and/or derivatives thereof allows embodiments of the compound to be capable of targeting sugar/carbohydrate/saccharide receptors found in/on cell surfaces (e.g., immune cell surfaces, fibroblast and keratinocyte surfaces, and liver sinusoidal endothelial cell surfaces). In various embodiments, the design of the compound allows easy access of the carbohydrate to the cell (e.g., immune cell) in order to target carbohydrate receptors on the cell surface (e.g., immune cell surface), thereby reducing risk of allergic reactions, prolonging the plasma half-life of nucleic acid and enhancing vaccination or treatment efficiency. In particular, in various embodiments, the design of installing/attaching a carbohydrate/sugar/saccharide group and/or derivatives thereof onto each unit of the hydrophilic peptide/oligopeptide/polypeptide allows said carbohydrate/sugar/saccharide group and/or derivatives thereof to be easily accessible to cells (e.g., immune cells). In various embodiments, the carbohydrate/sugar/saccharide group and/or derivatives thereof is not installed/attached to cholesterol and/or derivatives thereof which may otherwise prevent access of the carbohydrate/sugar/saccharide group and/or derivatives

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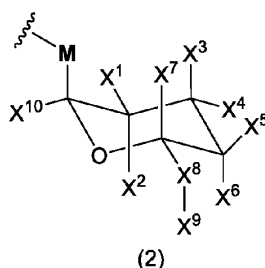
thereof to cells (e.g., making it inaccessible to immune cells), e.g., when used in PEGylated LNPs or LNPs coated with another hydrophilic polymer.

Advantageously, the presence of sugar/carbohydrate/saccharide and/or derivatives thereof increases the hydrophilicity of the compound, and consequently solubility of the compound. Advantageously, in various embodiments, the presence of carbohydrate/sugar/saccharide and/or derivatives thereof in the compound eliminates the requirement of a hydrophilic polyethylene glycol (PEG) which is otherwise necessary in a conventional PEG-lipid conjugate for LNP formulations. In various embodiments, by eliminating the presence of polyethylene glycol (PEG) in the compound, embodiments of the carbohydrate/sugar/saccharide group and/or derivatives thereof and lipid nanoparticles formed therefrom are not and/or avoid the possibility of being shielded by the long PEG chain. In various embodiments, the compound is substantially devoid of polyethylene glycol (PEG). In various embodiments, the compound is substantially devoid of polyethylene glycol (PEG)-modified lipid conjugates, polyethylene glycol (PEG)-modified lipid, PEGylated lipid, PEG-conjugated lipid, PEG-lipid conjugate, and/or lipid modified with PEG. Advantageously, in various embodiments, the design of the structure of the compound allows said compound to be used, in lieu of or as a substitute/replacement for a conventional PEG-lipid conjugate (e.g., ALC-0159).

It will be appreciated that various sugar molecules may be used as **A** in embodiments of the compound represented as general formula (1) as long as the sugar molecule is capable of providing a targeting ability and/or imparting hydrophilicity as a form of replacement of PEG. For example, **A** may be mannose (e.g., for targeting immune cells) or glucose (e.g., for imparting/increasing hydrophilicity or to be used as a hydrophilic component).

In various embodiments, **A** is represented by general formula (2) having a 6-membered ring structure:

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wherein

X^1 to X^7 and X^9 to X^{10} are each independently selected from $-H$ or $-OH$;

X^8 is alkyl/alkylene; and

5 M is $-O-$ or $-S-$.

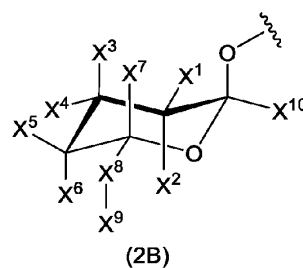
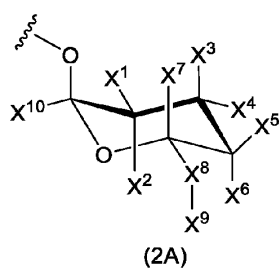
In various embodiments, X^8 is optionally substituted $-C_aH_{2a}-$, where a is from about 1 to about 20. For example, X^8 may be $-CH_2-$, $-C_2H_4-$, $-C_3H_6-$, $-C_4H_8-$, $-C_5H_{10}-$, $-C_6H_{12}-$, $-C_7H_{14}-$, $-C_8H_{16}-$, $-C_9H_{18}-$, or $-C_{10}H_{20}-$. In various

10 embodiments, X^8 is methylene (i.e. $-CH_2-$).

In various embodiments, M is $-O-$. In various embodiments, A is chemically coupled/bonded to the rest of general formula (1) *via* its hydroxy group. For example, A may be connected to R^7 or R^9 *via* a glycosidic/ether

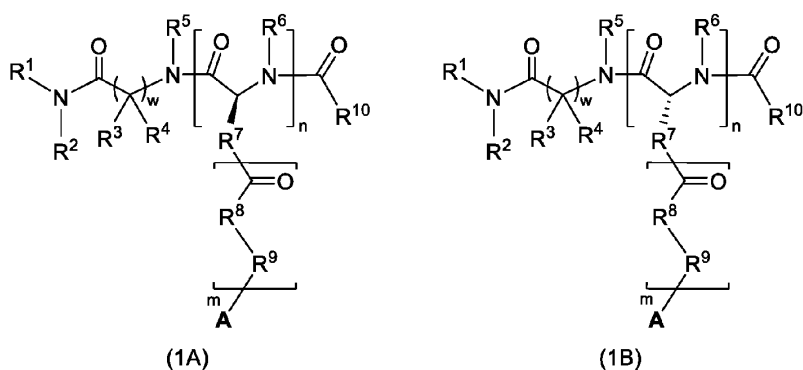
15 bond/linkage.

In various embodiments, A is represented by general formula (2A) and/or (2B) having a 6-membered ring structure:



wherein X^1 to X^{10} contain one or more features and/or share one or more properties that are similar to those described above (e.g., as defined in general formula (2)). In various embodiments, **A** comprises D-sugar and/or derivatives thereof (e.g., represented by general formula (2A)). In various embodiments, **A** comprises L-sugar and/or derivatives thereof (e.g., represented by general formula (2B)). In various embodiments, **A** comprises a mixture of two enantiomers, namely D-sugar and/or derivatives thereof (e.g., represented by general formula (2A); and L-sugar and/or derivatives thereof (e.g., represented by general formula (2B)). In various embodiments, **A** comprises a mixture (e.g., racemic mixture of enantiomers) containing both D-sugar and L-sugar. For example, **A** may comprise D-mannose, L-mannose and/or derivatives thereof.

In various embodiments, the compound comprises one or more structure(s) that is/are represented by general formulae (1A) and (1B):



wherein **A**, R¹ to R¹⁰, w, m, n, contain one or more features and/or share one or more properties that are similar to those described above (e.g., as defined in general formula (1)). In various embodiments, the compound comprises polypeptide/poly(amino acid) having repeating unit(s) or block(s) of L-amino acid (e.g., L-serine or L-glutamic acid) and/or derivatives thereof. In such embodiments, the compound comprises a structure that is represented by general formula (1A). In various embodiments, the compound comprises polypeptide/poly(amino acid) having repeating unit(s) or block(s) of D-amino acid

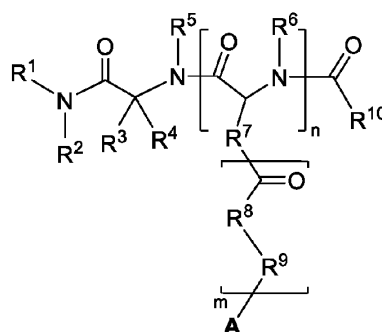
(e.g., D-serine or D-glutamic acid) and/or derivatives thereof. In such embodiments, the compound comprises a structure that is represented by general formula (1B). In various embodiments, the compound comprises a mixture of two enantiomers, namely polypeptide/poly(amino acid) having repeating unit(s) or block(s) of L-amino acid (e.g., L-serine or L-glutamic acid) and/or derivatives thereof; and polypeptide/poly(amino acid) having repeating unit(s) or block(s) of D-amino acid (e.g., D-serine or D-glutamic acid) and/or derivatives thereof. In various embodiments, the compound comprises a mixture (e.g., racemic mixture of enantiomers) containing both L-amino acid and D-amino acid.

In various embodiments, n is an integer ≥ 1 . In various embodiments, $n \geq 1, n \geq 2, n \geq 3, n \geq 4, n \geq 5, n \geq 6, n \geq 7, n \geq 8, n \geq 9, n \geq 10, n \geq 11, n \geq 12, n \geq 13, n \geq 14, n \geq 15, n \geq 16, n \geq 17, n \geq 18, n \geq 19, n \geq 20, n \geq 21, n \geq 22, n \geq 23, n \geq 24, n \geq 25, n \geq 26, n \geq 27, n \geq 28, n \geq 29, n \geq 30, n \geq 31, n \geq 32, n \geq 33, n \geq 34, n \geq 35, n \geq 36, n \geq 37, n \geq 38, n \geq 39, n \geq 40, n \geq 41, n \geq 42, n \geq 43, n \geq 44, n \geq 45, n \geq 46, n \geq 47, n \geq 48, n \geq 49, n \geq 50, n \geq 51, n \geq 52, n \geq 53, n \geq 54, n \geq 55, n \geq 56, n \geq 57, n \geq 58, n \geq 59, n \geq 60, n \geq 61, n \geq 62, n \geq 63, n \geq 64, n \geq 65, n \geq 66, n \geq 67, n \geq 68, n \geq 69, n \geq 70, n \geq 71, n \geq 72, n \geq 73, n \geq 74, n \geq 75, n \geq 76, n \geq 77, n \geq 78, n \geq 79, n \geq 80, n \geq 81, n \geq 82, n \geq 83, n \geq 84, n \geq 85, n \geq 86, n \geq 87, n \geq 88, n \geq 89, n \geq 90, n \geq 91, n \geq 92, n \geq 93, n \geq 94, n \geq 95, n \geq 96, n \geq 97, n \geq 98, n \geq 99, \text{ or } n \geq 100$. In various embodiments, n is from about 1 to about 100, from about 10 to about 90, from about 20 to about 80, from about 30 to about 70, from about 40 to about 60, or about 50.

Advantageously, in various embodiments, the compound is designed/configured to allow the hydrophilicity/hydrophobicity balance of said compound to be customizable by adjusting the length or hydrophobicity of R^1 and R^2 , and/or adjusting the number of peptide units/blocks and/or derivative(s) thereof (or length of the oligopeptides or polypeptides/poly(amino acids) and/or derivative(s) thereof), that is, by adjusting/altering/tuning the value of n and/or controlling the degree of polymerization.

In various embodiments, w is an integer ≥ 1 . In various embodiments, $w \geq 1$, $w \geq 2$, $w \geq 3$, $w \geq 4$, $w \geq 5$, $w \geq 6$, $w \geq 7$, $w \geq 8$, $w \geq 9$, $w \geq 10$, $w \geq 11$, $w \geq 12$, $w \geq 13$, $w \geq 14$, $w \geq 15$, $w \geq 16$, $w \geq 17$, $w \geq 18$, $w \geq 19$, $w \geq 20$, $w \geq 21$, $w \geq 22$, $w \geq 23$, $w \geq 24$, $w \geq 25$, $w \geq 26$, $w \geq 27$, $w \geq 28$, $w \geq 29$, $w \geq 30$, $w \geq 31$, $w \geq 32$, $w \geq 33$,
 5 $w \geq 34$, $w \geq 35$, $w \geq 36$, $w \geq 37$, $w \geq 38$, $w \geq 39$, $w \geq 40$, $w \geq 41$, $w \geq 42$, $w \geq 43$, $w \geq 44$, $w \geq 45$, $w \geq 46$, $w \geq 47$, $w \geq 48$, $w \geq 49$, or $w \geq 50$.

In various embodiments, $w = 1$. In such embodiments, the compound comprises a structure that is represented by general formula (1C):



(1C)

10

In various embodiments, $m = 0$. In such embodiments, the compound comprises polypeptide/poly(amino acid) having repeating unit(s) or block(s) of serine and/or derivatives thereof. For example, when $m = 0$, the compound
 15 comprises polyserine and/or derivatives thereof. In various embodiments, $m = 0$ and R^7 is $-\text{CH}_2-$.

In various embodiments, $m = 1$. In such embodiments, the compound comprises polypeptide/poly(amino acid) having repeating unit(s) or block(s) of glutamic acid and/or derivatives thereof. For example, when $m = 1$, the compound
 20 comprises polyglutamic acid and/or derivatives thereof. In various embodiments, $m = 1$ and R^7 is $-\text{CH}_2\text{CH}_2-$.

In various embodiments, the compound comprises a lipid compound. Accordingly, in various embodiments therefore, the term “compound” may comprise and/or may be used interchangeably with the terms “lipid polypeptide”, “lipid-polypeptide”, “polypeptide lipid”, “polypeptide-lipid”, “lipid-*block*-polypeptide”, “carbohydrate-functionalized polypeptide-lipid”, “sugar-functionalized polypeptide-lipid”, “mannose-functionalized polypeptide-lipid”, “mannose-functionalized polypeptide-*block*-lipid”, “mannose-functionalized polypeptide-*b*-lipid”, or the like.

10 In various embodiments, R^1 and R^2 are each independently hydrophobic tail/chain/group, or contains at least linear aliphatic, branched aliphatic and/or cyclic hydrocarbons

In various embodiments, the compound comprises hydrophobic
15 parts/tails/chains/groups at both R^1 and R^2 .

In various embodiments, R^1 and R^2 each independently contains at least linear aliphatic, branched aliphatic and/or cyclic hydrocarbons. In various embodiments, the hydrophobic tail/chain/group at R^1 and R^2 each independently
20 comprises optionally substituted alkyl. In various embodiments, the hydrophobic tail/chain/group at R^1 and R^2 each independently comprises unsaturated hydrocarbons such as an optionally substituted alkenyl. For example, R^1 and R^2 may contain one or more C=C double bond(s). The alkyl or alkenyl may have at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least
25 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 carbon atoms. For example, R^1 and R^2 may be each independently C_xH_{2x+1} or C_xH_{2x} , where x is an integer $\geq$ 5, $x \geq 6$, $x \geq 7$, $x \geq 8$, $x \geq 9$, $x \geq 10$, $x \geq 11$, $x \geq 12$, $x \geq 13$, $x \geq 14$, $x \geq 15$, $x \geq 16$, x
30 ≥ 17 , $x \geq 18$, $x \geq 19$, $x \geq 20$, $x \geq 21$, $x \geq 22$, $x \geq 23$, $x \geq 24$, $x \geq 25$, $x \geq 26$, $x \geq 27$, $x \geq 28$, $x \geq 29$, or $x \geq 30$. Advantageously, in various embodiments, the presence of hydrophobic parts/tails/chains/groups in the compound allows for ease of

integration of the compound into the lipid domain of lipid nanoparticles (LNPs), presenting the sugar/carbohydrate/saccharide-functionalized polypeptide on the surface of the LNPs for stability and cell-targeting ability (e.g., immune cells-targeting ability).

5

In various embodiments, R^3 , R^4 , R^5 and R^6 are each independently selected from H, optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl. In various embodiments, R^3 , R^4 , R^5 and R^6 are each independently optionally substituted alkyl, optionally substituted alkenyl or
 10 optionally substituted alkynyl. For example, R^3 , R^4 , R^5 , R^6 and R^8 may be selected from methyl, ethyl, *n*-propyl, 2-propyl, isopropyl, *n*-butyl, isobutyl, *sec*-butyl, *t*-butyl, hexyl, amyl, 1,2-dimethylpropyl, 1,1-dimethylpropyl, pentyl, isopentyl, hexyl, 4-methylpentyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 1,2,2-
 15 trimethylpropyl, 1,1,2-trimethylpropyl, 2-ethylpentyl, 3-ethylpentyl, heptyl, 1-methylhexyl, 2,2-dimethylpentyl, 3,3-dimethylpentyl, 4,4-dimethylpentyl, 1,2-dimethylpentyl, 1,3-dimethylpentyl, 1,4-dimethylpentyl, 1,2,3-trimethylbutyl, 1,1,2-trimethylbutyl, 1,1,3-trimethylbutyl, 5-methylheptyl, 1-methylheptyl, octyl, nonyl, decyl, or the like or combinations thereof. In various embodiments, R^3 and
 20 R^4 are H. In various embodiments, R^5 is H. In various embodiments, R^6 is H.

In various embodiments, R^7 is optionally substituted $-C_yH_{2y}-$, where y is an integer ≥ 1 . In various embodiments, $y \geq 1$, $y \geq 2$, $y \geq 3$, $y \geq 4$, $y \geq 5$, $y \geq 6$, $y \geq 7$, $y \geq 8$, $y \geq 9$, $y \geq 10$, $y \geq 11$, $y \geq 12$, $y \geq 13$, $y \geq 14$, $y \geq 15$, $y \geq 16$, $y \geq 17$, $y \geq 18$,
 25 $y \geq 19$, $y \geq 20$, $y \geq 21$, $y \geq 22$, $y \geq 23$, $y \geq 24$, $y \geq 25$, $y \geq 26$, $y \geq 27$, $y \geq 28$, $y \geq 29$, $y \geq 30$, $y \geq 31$, $y \geq 32$, $y \geq 33$, $y \geq 34$, $y \geq 35$, $y \geq 36$, $y \geq 37$, $y \geq 38$, $y \geq 39$, $y \geq 40$, $y \geq 41$, $y \geq 42$, $y \geq 43$, $y \geq 44$, $y \geq 45$, $y \geq 46$, $y \geq 47$, $y \geq 48$, $y \geq 49$, or $y \geq 50$. In various embodiments, y is from about 1 to about 50, from about 10 to about 40, from about 20 to about 30, or about 25. For example, R^7 may be $-CH_2-$, $-C_2H_4-$,
 30 $-C_3H_6-$, $-C_4H_8-$ or $-C_5H_{10}-$.

In various embodiments, when m is 1, R^8 is present. In various embodiments, R^8 is $-O-$, $-S-$, or $-NR^a-$, where R^a is selected from the group consisting of H, optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl. In various embodiments, R^a is $-H$ or optionally substituted $-C_bH_{2b+1}$, where b is an integer ≥ 1 . In various embodiments, $b \geq 1$, $b \geq 2$, $b \geq 3$, $b \geq 4$, $b \geq 5$, $b \geq 6$, $b \geq 7$, $b \geq 8$, $b \geq 9$, $b \geq 10$, $b \geq 11$, $b \geq 12$, $b \geq 13$, $b \geq 14$, $b \geq 15$, $b \geq 16$, $b \geq 17$, $b \geq 18$, $b \geq 19$, $b \geq 20$, $b \geq 21$, $b \geq 22$, $b \geq 23$, $b \geq 24$, $b \geq 25$, $b \geq 26$, $b \geq 27$, $b \geq 28$, $b \geq 29$, $b \geq 30$, $b \geq 31$, $b \geq 32$, $b \geq 33$, $b \geq 34$, $b \geq 35$, $b \geq 36$, $b \geq 37$, $b \geq 38$, $b \geq 39$, $b \geq 40$, $b \geq 41$, $b \geq 42$, $b \geq 43$, $b \geq 44$, $b \geq 45$, $b \geq 46$, $b \geq 47$, $b \geq 48$, $b \geq 49$, or $b \geq 50$. For example, R^8 may be $-NH-$, $-NCH_3-$, or $-NC_2H_5-$. In various embodiments when m is 0, R^8 is absent from the compound.

In various embodiments, when m is 1, R^9 is present. In various embodiments, R^9 is optionally substituted $-C_zH_{2z}-$, where z is an integer ≥ 1 . In various embodiments, $z \geq 1$, $z \geq 2$, $z \geq 3$, $z \geq 4$, $z \geq 5$, $z \geq 6$, $z \geq 7$, $z \geq 8$, $z \geq 9$, $z \geq 10$, $z \geq 11$, $z \geq 12$, $z \geq 13$, $z \geq 14$, $z \geq 15$, $z \geq 16$, $z \geq 17$, $z \geq 18$, $z \geq 19$, $z \geq 20$, $z \geq 21$, $z \geq 22$, $z \geq 23$, $z \geq 24$, $z \geq 25$, $z \geq 26$, $z \geq 27$, $z \geq 28$, $z \geq 29$, $z \geq 30$, $z \geq 31$, $z \geq 32$, $z \geq 33$, $z \geq 34$, $z \geq 35$, $z \geq 36$, $z \geq 37$, $z \geq 38$, $z \geq 39$, $z \geq 40$, $z \geq 41$, $z \geq 42$, $z \geq 43$, $z \geq 44$, $z \geq 45$, $z \geq 46$, $z \geq 47$, $z \geq 48$, $z \geq 49$, or $z \geq 50$. In various embodiments, z is from about 1 to about 50, from about 10 to about 40, from about 20 to about 30, or about 25. For example, R^9 may be $-CH_2-$, $-C_2H_4-$, $-C_3H_6-$, $-C_4H_8-$ or $-C_5H_{10}-$. In various embodiments when m is 0, R^9 is absent from the compound.

In various embodiments, R^{10} is selected from optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl. For example, R^{10} may be selected from methyl, ethyl, *n*-propyl, 2-propyl, isopropyl, *n*-butyl, isobutyl, *sec*-butyl, *t*-butyl, hexyl, amyl, 1,2-dimethylpropyl, 1,1-dimethylpropyl, pentyl, isopentyl, hexyl, 4-methylpentyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 1,2,2-trimethylpropyl, 1,1,2-trimethylpropyl, 2-ethylpentyl, 3-ethylpentyl, heptyl, 1-methylhexyl, 2,2-dimethylpentyl, 3,3-dimethylpentyl, 4,4-dimethylpentyl, 1,2-

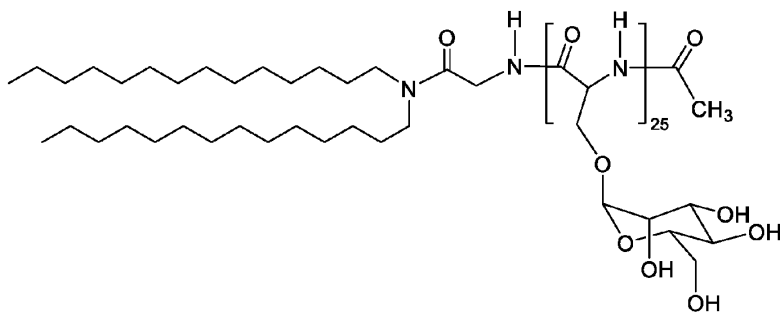
dimethylpentyl, 1,3-dimethylpentyl, 1,4-dimethylpentyl, 1,2,3-trimethylbutyl, 1,1,2-trimethylbutyl, 1,1,3-trimethylbutyl, 5-methylheptyl, 1-methylheptyl, octyl, nonyl, decyl, or the like or combinations thereof.

5 In various embodiments, the compound has an average molecular weight or a number average molecular weight (M_n) of from about 700 g/mol to about 50,000 g/mol, In various embodiments, the compound has a short peptide length (e.g., an average molecular weight or a number average molecular weight (M_n) of from about 700 g/mol to about 3,000 g/mol, from about 800 g/mol to about
10 2,900 g/mol, from about 900 g/mol to about 2,800 g/mol, from about 1,000 g/mol to about 2,700 g/mol, from about 1,100 g/mol to about 2,600 g/mol, from about 1,200 g/mol to about 2,500 g/mol, from about 1,300 g/mol to about 2,400 g/mol, from about 1,400 g/mol to about 2,300 g/mol, from about 1,500 g/mol to about 2,200 g/mol, from about 1,600 g/mol to about 2,100 g/mol, from about 1,700 g/mol
15 to about 2,000 g/mol, or from about 1,800 g/mol to about 1,900 g/mol). In various embodiments, the compound has an average molecular weight or a number average molecular weight (M_n) of from 3,000 g/mol to about 50,000 g/mol, from about 4,000 g/mol to about 49,000 g/mol, from about 5,000 g/mol to about 48,000 g/mol, from about 6,000 g/mol to about 47,000 g/mol, from about 7,000 g/mol to
20 about 46,000 g/mol, from about 8,000 g/mol to about 45,000 g/mol, from about 9,000 g/mol to about 44,000 g/mol, from about 10,000 g/mol to about 43,000 g/mol, from about 11,000 g/mol to about 42,000 g/mol, from about 12,000 g/mol to about 41,000 g/mol, from about 13,000 g/mol to about 40,000 g/mol, from about 14,000 g/mol to about 39,000 g/mol, from about 15,000 g/mol to about
25 38,000 g/mol, from about 16,000 g/mol to about 37,000 g/mol, from about 17,000 g/mol to about 36,000 g/mol, from about 18,000 g/mol to about 35,000 g/mol, from about 19,000 g/mol to about 34,000 g/mol, from about 20,000 g/mol to about 33,000 g/mol, from about 21,000 g/mol to about 32,000 g/mol, from about 22,000 g/mol to about 31,000 g/mol, from about 23,000 g/mol to about 30,000 g/mol,
30 from about 24,000 g/mol to about 29,000 g/mol, from about 25,000 g/mol to about 28,000 g/mol, from about 26,000 g/mol to about 27,000 g/mol, about 20,000

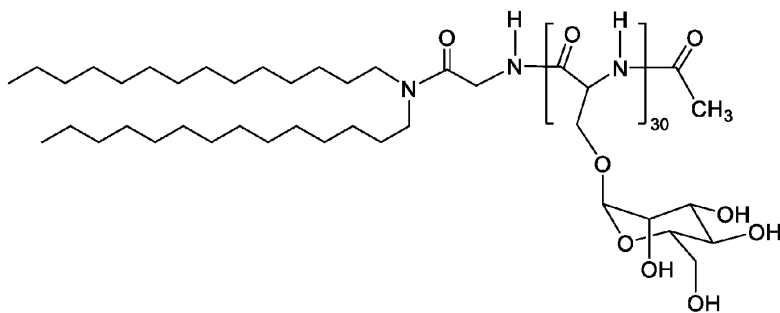
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g/mol, about 25,000 g/mol, about 30,000 g/mol, about 35,000 g/mol, about 40,000 g/mol, or about 45,000 g/mol.

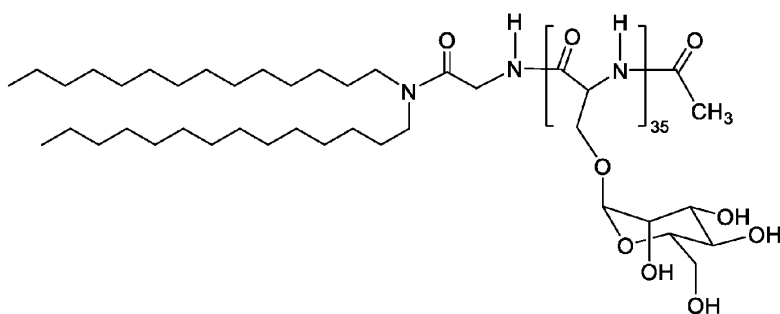
In various embodiments, the compound comprises a structure selected from one or more of the following:



LPSM01 (n = 25)



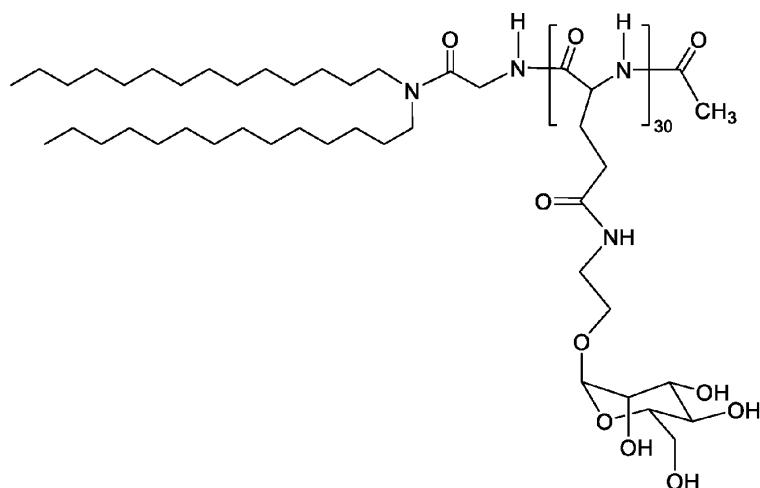
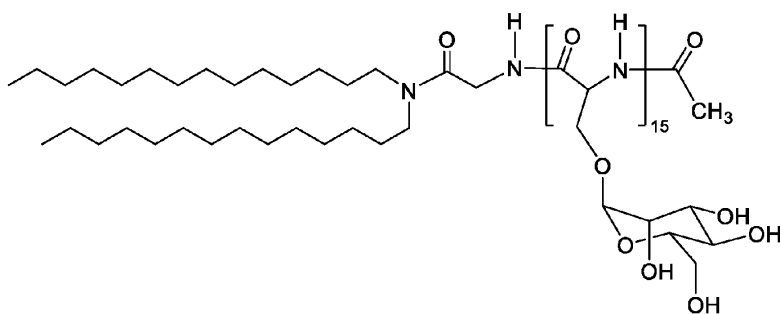
LPSM02 (n = 30)



LPSM03 (n = 35)

10

38

**LPGM01 (n = 30)****LPSM04 (n = 15)**

5

In various embodiments, D-mannose in LPSM01, LPSM02, LPSM03, LPSM04 and LPGM01 may be replaced with L-mannose. In various embodiments, both D-mannose and L-mannose are present in LPSM01, LPSM02, LPSM03, LPSM04 and LPGM01.

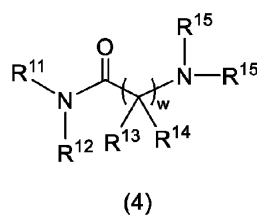
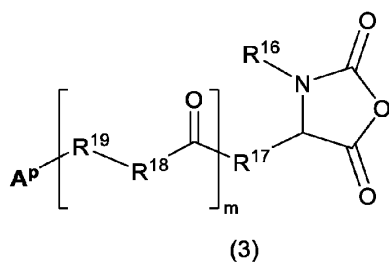
In various embodiments, L-serine in LPSM01, LPSM02, LPSM03 and LPSM04 may be replaced with D-serine. In various embodiments, both L-serine and D-serine are present in LPSM01, LPSM02, LPSM03 and LPSM04. In various

embodiments, L-glutamic acid in LPGM01 may be replaced with D-glutamic acid. In various embodiments, both L-glutamic acid and D-glutamic acid are present in LPGM01.

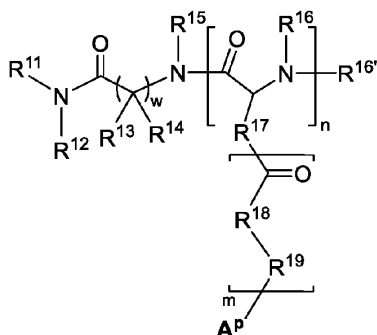
- 5 In various embodiments, the compound comprises a structure selected from lipid-*block*-poly(L-Ser-D-Mannose), lipid-*block*-poly(L-Ser-L-Mannose), lipid-*block*-poly(D-Ser-L-Mannose), lipid-*block*-poly(D-Ser-D-Mannose), lipid-*block*-poly(D,L-Ser-D-Mannose), lipid-*block*-poly(D,L-Ser-L-Mannose), lipid-*block*-poly(L-Glu-D-Mannose), lipid-*block*-poly(L-Glu-L-Mannose), lipid-*block*-poly(D-Glu-L-Mannose), lipid-*block*-poly(D-Glu-D-Mannose), lipid-*block*-poly(D,L-Glu-D-Mannose), lipid-*block*-poly(D,L-Glu-L-Mannose), lipid-*block*-poly(L-Ser-D,L-Mannose), lipid-*block*-poly(D-Ser-D,L-Mannose), lipid-*block*-poly(D,L-Ser-D,L-Mannose), lipid-*block*-poly(L-Glu-D,L-Mannose), lipid-*block*-poly(D-Glu-D,L-Mannose), lipid-*block*-poly(D,L-Glu-D,L-Mannose), the like, or combinations thereof.

METHOD OF PREPARING COMPOUND

- 20 There is provided a method of preparing a compound represented by general formula (1) as disclosed herein, the method comprising:
- (i) polymerizing/reacting one or more N-carboxyanhydride (NCA) monomers represented by general formula (3) with a lipid initiator/molecule represented by general formula (4) to obtain a first intermediate compound represented by general formula (5):



40



(5)

wherein

A^p represents **A** functionalized/protected with one or more protecting groups, where **A** comprises a hydrophilic moiety or component selected from carbohydrate/sugar/saccharide and derivatives thereof;

R¹¹ and R¹² are each independently a hydrophobic tail/chain/group;

R¹³, R¹⁴, R¹⁵, and R¹⁶ are each independently H, optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

R¹⁷ and R¹⁹ are each independently optionally substituted alkyl/alkylene, optionally substituted alkenyl/alkenylene or optionally substituted alkynyl/alkynylene;

R¹⁸ is -O-, -S-, or -NR^{a1}-, where R^{a1} is selected from the group consisting of H, optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

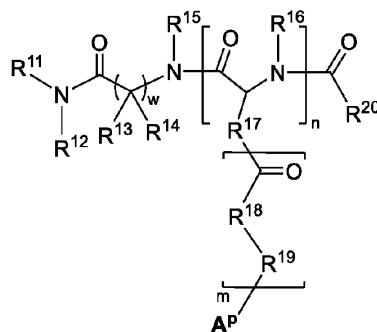
R^{15'} and R^{16'} are each independently H, optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl; and

m is 0 or 1;

$$n \geq 1; \text{ and}$$
 $w \geq 1;$

(ii) reacting the first intermediate compound represented by general formula (5) with an acylating agent to obtain a second intermediate compound represented by general formula (7):

41



(7)

wherein R²⁰ is optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

- 5 (iii) deprotecting the second intermediate compound represented by general formula (7) to obtain the compound represented by general formula (1).

In various embodiments, the polymerizing/reacting step (i) comprises ring opening polymerization of the NCA ring/group in the compound represented by
 10 general formula (3). Advantageously, the NCA undergoes ring-opening and polymerizes despite the bulky structure of the protected mannose group. Without being bound by theory, it is believed that such a successful cyclization (NCA formation) and polymerization is due to the specific combination of L-amino acids (e.g., L-serine) and D-sugars (e.g., D-mannose), which avoids steric hindrance.
 15 It will be appreciated that in step (i), the one or more NCA monomers (i.e. represented by general formula (3)) may be added sequentially to the lipid initiator to polymerize and form lipid-block polypeptides or lipid-random polypeptides or lipid-polypeptides formed from both L-serine-NCA and D-serine-NCA, or L-glutamic acid-NCA and D-glutamic acid-NCA.

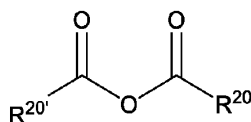
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In various embodiments, A^P represents A being functionalized/protected with one or more protecting groups. For example, one or more of X¹ to X⁷ and X⁹ to X¹⁰ in A (as defined in general formula (2)) may be functionalized/protected

with a protecting group to obtain **A^P**. In various embodiments, the protecting group comprises $-\text{C}(=\text{O})-\text{R}$, where R is optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl. For example, one or more of hydroxyl group(s) (i.e. $-\text{OH}$) in **A** may be functionalized/protected with a protecting group and converted into acetyloxy group(s) (i.e. $-\text{O}-\text{C}(=\text{O})-\text{R}$) in **A^P**. In various embodiments, the protecting group comprises an acetyl group and **A^P** comprises one or more acetoxy or acetyloxy group(s).

In various embodiments, R^{11} to R^{19} contain one or more features and/or share one or more properties that are similar to those described respectively above for R^1 to R^9 . In various embodiments, w, m and n contain one or more features and/or share one or more properties that are similar to those described above.

In various embodiments, the acylating agent (in step (ii)) is selected from acid anhydride (e.g., acetic anhydride (Ac_2O)), acid halide (e.g., acyl halide), N-hydroxysuccinimide (NHS) esters, imidoesters or the like or combinations thereof. In various embodiments, the acylating agent is represented by general formula (6):



(6)

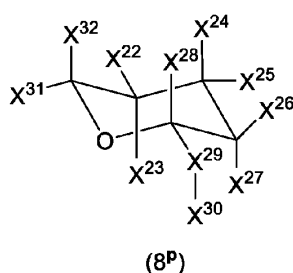
wherein R^{20} and $\text{R}^{20'}$ are each independently optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl. In various embodiments, R^{20} and $\text{R}^{20'}$ are each independently selected from methyl, ethyl, n-propyl, 2-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, t-butyl, hexyl, amyl, 1,2-dimethylpropyl, 1,1-dimethylpropyl, pentyl, isopentyl, hexyl, 4-methylpentyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 1,2,2-trimethylpropyl, 1,1,2-trimethylpropyl, 2-ethylpentyl, 3-ethylpentyl, heptyl, 1-methylhexyl, 2,2-

dimethylpentyl, 3,3-dimethylpentyl, 4,4-dimethylpentyl, 1,2-dimethylpentyl, 1,3-dimethylpentyl, 1,4-dimethylpentyl, 1,2,3-trimethylbutyl, 1,1,2-trimethylbutyl, 1,1,3-trimethylbutyl, 5-methylheptyl, 1-methylheptyl, octyl, nonyl, decyl, or the like or combinations thereof. In various embodiments, R²⁰ to R^{20'} contain one or more
 5 features and/or share one or more properties that are similar to those described respectively above for R¹⁰.

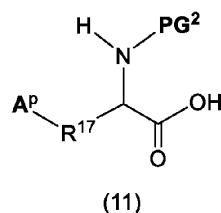
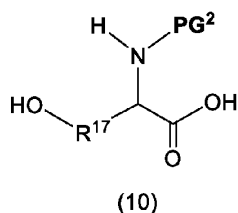
In various embodiments, the deprotecting/deprotection step (iii) of the second intermediate compound represented by general formula (7) comprises
 10 subjecting the compound represented by general formula (7) to basic conditions. For example, the deprotection step (iii) may be carried out in the presence of one or more base(s). The base may be anhydrous base such as sodium methoxide (MeONa) in methanol (MeOH). In various embodiments, the deprotection step (iii) is carried out in the presence of one or more of the following: a nucleophilic
 15 compound comprising hydroxyl group, a metal alkoxide (e.g., sodium methoxide), and an alcohol (e.g., methanol).

In various embodiments, the method further comprises, prior to step (i):

20 (a-i) reacting a protected monosaccharide represented by general formula (8^P) (e.g., obtained from step (c-i)) with a protected compound represented by general formula (10) in the presence of a Lewis acid to obtain a first intermediate compound represented by general formula (11):



44



wherein

X^{22} to X^{28} and X^{30} to X^{32} are each independently selected from $-H$ or $-OPG^1$;

5 X^{29} is alkyl;

PG^1 is $-C(=O)-R^{22}$;

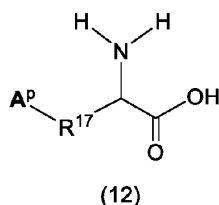
R^{22} is optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

10 R^{17} and A^p contain one or more features and/or share one or more properties that are similar to those described above;

PG^2 is a protecting group selected from 9-Fluorenylmethoxycarbonyl (Fmoc), 2-(4-Nitrophenylsulfonyl)ethoxycarbonyl (Nsc), 2-Fluoro-Fmoc (Fmoc(2F)), 2-Monoisooctyl-Fmoc (mio-Fmoc), 2,7-Diisooctyl-Fmoc (dio-Fmoc), N-carboxybenzyl (Cbz), the like, or combinations thereof;

15

(a-ii) deprotecting the first intermediate compound represented by general formula (11) to obtain a second intermediate compound represented by general formula (12):



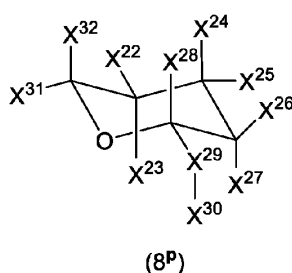
20 (a-iii) reacting the second intermediate compound represented by general formula (12) with a carbonylating agent (e.g., triphosgene) to obtain the N-carboxyanhydride (NCA) monomer represented by general formula (3).

In various embodiments, the deprotecting/deprotection step (a-ii) of the first intermediate compound represented by general formula (11) comprises selectively deprotecting/removing the protecting group **PG²** from the first intermediate compound represented by general formula (11). In various
 5 embodiments, the deprotecting/deprotection step (a-ii) of the compound represented by general formula (11) comprises subjecting the compound represented by general formula (11) to basic conditions. For example, the deprotection step (a-ii) may be carried out in the presence of one or more base(s). The base may be piperidine. It will be appreciated that any suitable base that
 10 effectively forms an adduct (e.g., stable adduct) with **PG²** and/or byproduct(s) of **PG²** may be used in for the deprotection step (a-ii).

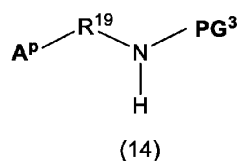
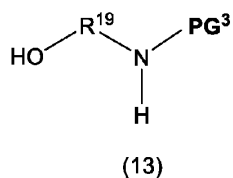
It will be appreciated that, in various embodiments, the second intermediate compound represented by general formula (12) comprises -NH_2
 15 and -COOH in order for ring cyclization to occur to obtain/make NCA prior to ring-opening polymerization.

In various embodiments, the method further comprises, prior to step (i):

- 20 (b-i) reacting a protected monosaccharide represented by general formula (8^P) (e.g., obtained from step (c-i)) with a protected alkanolamine compound represented by general formula (13) in the presence of a Lewis acid to obtain a first intermediate compound represented by general formula (14):



46



wherein

X^{22} to X^{28} and X^{30} to X^{32} are each independently selected from $-\text{H}$ or $-\text{OPG}^1$;

5 X^{29} is alkyl;

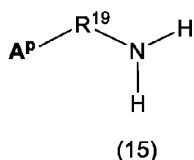
PG^1 is $-\text{C}(=\text{O})-\text{R}^{22}$;

R^{22} is optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

10 R^{19} and A^p contain one or more features and/or share one or more properties that are similar to those described above; and

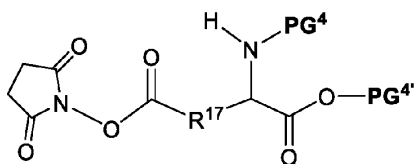
PG^3 is a protecting group selected from N-carboxybenzyl, benzyloxycarbonyl (Cbz), the like, or combinations thereof;

15 (b-ii) deprotecting the first intermediate compound represented by general formula (14) to obtain a second intermediate compound represented by general formula (15):

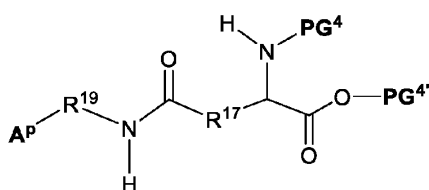


20 (b-iii) reacting the second intermediate compound represented by general formula (15) with a protected compound represented by general formula (16) in the presence of a base (e.g., non-nucleophilic base such as N,N-diisopropylethylamine (DIPEA) or the like) to obtain a third intermediate compound represented by general formula (17):

47



(16)



(17)

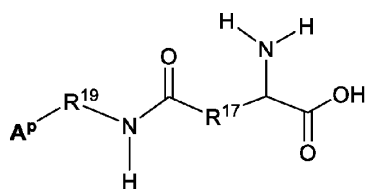
wherein

R¹⁷ contains one or more features and/or share one or more properties that are similar to those described above; and

5

PG⁴ and PG^{4'} are each independently a protecting group selected from *tert*-butoxycarbonyl (Boc), *tert*-butyl, benzyloxycarbonyl protecting group (Cbz), the like, or combinations thereof;

- 10 (b-iv) deprotecting the third intermediate compound represented by general formula (17) to obtain a fourth intermediate compound represented by general formula (18):



(18)

- 15 (b-v) reacting the fourth intermediate compound represented by general formula (18) with a carbonylating agent (e.g., triphosgene) to obtain the N-carboxyanhydride (NCA) monomer represented by general formula (3).

In various embodiments, the protected alkanolamine compound represented by general formula (13) is obtained by reacting an unprotected alkanolamine compound with $\text{PG}^3\text{-LG}$ with a Lewis base, where **LG** is a leaving group such as a halide (i.e., F, Cl, Br, or I). In various embodiments, the
5 unprotected alkanolamine compound may be selected from ethanolamine, diglycoamine, diethanolamine, triethanolamine, diisopropanolamine, N-methylethanolamine, N-methyldiethanolamine, aminomethyl propanol, or the like or combinations thereof. In various embodiments, the Lewis base is selected from amines such as primary amines (e.g., ammonia (NH_3)), tertiary amines (e.g.,
10 triethylamine (TEA)), aromatic amines (e.g., pyridine), the like or combinations thereof. In various embodiments, $\text{PG}^3\text{-LG}$ is benzyl haloformate or halides of benzyloxycarbonyl (e.g., benzyl chloroformate or Cbz-Cl).

In various embodiments, the deprotecting/deprotection step (b-ii) of the
15 first intermediate compound represented by general formula (14) comprises selectively deprotecting/removing the protecting group PG^3 from the first intermediate compound represented by general formula (14). In various embodiments, the deprotecting/deprotection step (b-ii) of the compound represented by general formula (14) is carried out in the presence of a metal
20 catalyst. For example, the deprotection step (b-ii) may be carried out in the presence of one or more metal(s). The metal catalyst may be palladium.

In various embodiments, the deprotecting/deprotection step (b-iv) of the third intermediate compound represented by general formula (17) comprises
25 selectively deprotecting/removing the protecting group PG^4 from the third intermediate compound. In various embodiments, the deprotecting/deprotection step (b-iv) comprises subjecting the third intermediate compound represented by general formula (17) to acidic conditions. For example, the deprotection step (b-iv) may be carried out in the presence of one or more acids (e.g., trifluoroacetic
30 acid (TFA), hydrobromic acid (HBr) and acetic acid (AcOH)).

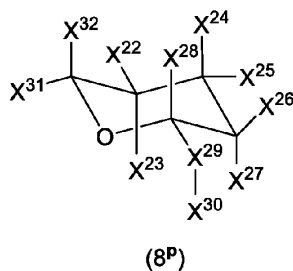
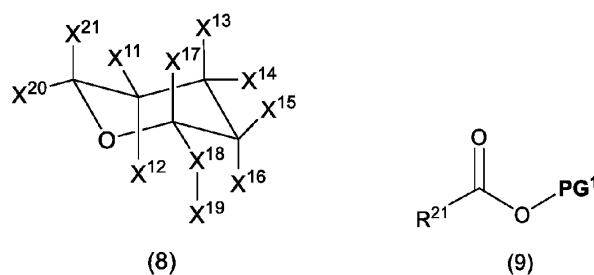
It will be appreciated that, in various embodiments, the fourth intermediate compound represented by general formula (18) comprises -NH_2 and -COOH in order for ring cyclization to occur to obtain/form NCA prior to ring-opening polymerization to yield the final product.

5

In various embodiments, the method further comprises, prior to step (a-i) and/or (b-i):

- (c-i) reacting a monosaccharide represented by general formula (8) with an acid anhydride represented by general formula (9) to obtain a protected monosaccharide represented by general formula (8^P), wherein one or more -OH group(s) in the monosaccharide represented by general formula (8) are converted into -OPG^1 :

10



15

wherein

X^{11} to X^{17} and X^{19} to X^{21} are each independently selected from -H or -OH ;
 X^{22} to X^{28} and X^{30} to X^{32} are each independently selected from -H or -OPG^1 ;

20

X^{18} and X^{29} are each alkyl;

PG¹ is $-\text{C}(=\text{O})-\text{R}^{22}$; and

R^{21} and R^{22} are each independently optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

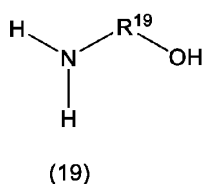
5 In various embodiments, X^{18} and X^{29} are each independently optionally substituted $-\text{C}_p\text{H}_{2p}-$, where p is from about 1 to about 20. For example, X^{18} and/or X^{29} maybe $-\text{CH}_2-$, $-\text{C}_2\text{H}_4-$, $-\text{C}_3\text{H}_6-$, $-\text{C}_4\text{H}_8-$, $-\text{C}_5\text{H}_{10}-$, $-\text{C}_6\text{H}_{12}-$, $-\text{C}_7\text{H}_{14}-$, $-\text{C}_8\text{H}_{16}-$, $-\text{C}_9\text{H}_{18}-$, or $-\text{C}_{10}\text{H}_{20}-$. In various embodiments, X^{18} is methylene (i.e. $-\text{CH}_2-$).

10

In various embodiments, the method further comprises, prior to step (b-i):

(d-i) reacting an alkanolamine compound represented by general formula (19) with a compound comprising protecting group (**PG³**) in the presence of a Lewis base to obtain a protected alkanolamine compound represented by general formula (13):

15



20 In various embodiments, step (d-i) comprises protecting the alkanolamine compound represented by general formula (19). In various embodiments, the Lewis base is selected from amines such as primary amines (e.g., ammonia (NH_3)), tertiary amines (e.g., triethylamine (TEA)), aromatic amines (e.g., pyridine), the like or combinations thereof. In various embodiments, the compound comprising protecting group (**PG³**) is benzyl haloformate or halides of benzyloxycarbonyl (e.g., benzyl chloroformate or Cbz-Cl).

25

In various embodiments, the reacting step (c-i) comprises adding the acid anhydride represented by general formula (9) in a dropwise manner to the monosaccharide represented by general formula (8). In various embodiments, step (c-i) comprises protecting the monosaccharide represented by general formula (8). In various embodiments, the step (c-i) of protecting the monosaccharide represented by general formula (8) is performed in the presence of a base. In various embodiments, the base is selected from amines such as primary amines (e.g., ammonia (NH₃)), tertiary amines (e.g., triethylamine (TEA)), aromatic amines (e.g., pyridine), the like or combinations thereof.

10

In various embodiments, the reacting step (a-i) and/or (b-i) comprises adding the Lewis acid in a dropwise manner to the protected monosaccharide represented by general formula (8^P) (e.g., obtained from step (c-i)). Advantageously, such dropwise addition(s) ensures good control over the reaction and reduces risk of rapid, sudden and/or exothermic reactions.

15

In various embodiments, the Lewis acid (e.g., used in step (a-i) and/or (b-i)) is selected from boron trifluoride diethyl etherate (BF₃•OEt₂), boron trifluoride, the like or combinations thereof.

20

In various embodiments, the reacting step (a-iii) and/or (b-v) comprises cyclization of the second intermediate compound represented by general formula (12) and/or the fourth intermediate compound represented by general formula (18). In various embodiments, the carbonylating agent (e.g., used in step (a-iii) and/or (b-v)) is selected from phosgene, diphosgene, triphosgene, the like, or combinations thereof.

25

In various embodiments, the polymerizing/reacting/deprotecting step (i), (ii), (iii), (a-i), (a-ii), (a-iii), (b-i), (b-ii), (b-iii), (b-iv), (b-v), (c-i) and/or (d-i) comprises one or more of the following steps: suspending, dispersing, mixing, stirring, dissolving, sonicating and/or ultrasonication.

30

In various embodiments, the polymerizing/reacting/deprotecting step (i), (ii), (iii), (a-i), (a-ii), (a-iii), (b-i), (b-ii), (b-iii), (b-iv), (b-v), (c-i) and/or (d-i) is/are performed in the presence of an organic solvent. In various embodiments, any organic solvent that effectively serves as a medium to contain the components of the reaction mixture (e.g., reactants/substrates) may be used in embodiments of the reaction mixture disclosed herein. In various embodiments, the organic solvent is capable of substantially dissolving the components present in the reaction mixture. The organic solvent may be an organic solvent such as dichloromethane (DCM), tetrahydrofuran (THF), dimethylsulfoxide (DMSO), acetonitrile, ethyl acetate, dimethylformamide (DMF) or the like or combinations thereof. In various embodiments, the organic solvent may be provided in a dry or anhydrous form. For example, polymerizing/reacting step (i) may comprise suspending, dispersing, mixing, stirring, dissolving, sonicating and/or ultrasonication the one or more N-carboxyanhydride (NCA) monomers represented by general formula (3) with a lipid initiator represented by general formula (4) in the presence of dry/anhydrous organic solvent. For example, reacting step (a-iii) may comprise suspending, dispersing, mixing, stirring, dissolving, sonicating and/or ultrasonication the compound represented by general formula (12) with a carbonylating agent in the presence of dry/anhydrous organic solvent. For example, reacting step (b-v) may comprise suspending, dispersing, mixing, stirring, dissolving, sonicating and/or ultrasonication the compound represented by general formula (18) with a carbonylating agent in the presence of dry/anhydrous organic solvent.

In various embodiments, the polymerizing/reacting/deprotecting step (i), (ii), (iii), (a-i), (a-ii), (a-iii), (b-i), (b-ii), (b-iii), (b-iv), (b-v), (c-i) and/or (d-i) is/are carried out under vacuum or in an inert atmosphere. For example, the step(s) of suspending, dispersing, mixing, stirring, dissolving, sonicating and/or ultrasonication may be performed in a glove box, in the presence of an inert gas such as argon or nitrogen, or in the absence of reactive gases such as oxygen (e.g., dissolved oxygen).

In various embodiments, the polymerizing/reacting/deprotecting step (i), (ii), (iii), (a-i), (a-ii), (a-iii), (b-i), (b-ii), (b-iii), (b-iv), (b-v), (c-i) and/or (d-i) is/are performed over a time duration of from about 20 minutes to about 200 hours, from about 1 hour to about 190 hours, from about 10 hours to about 180 hours, from about 20 hours to about 170 hours, from about 30 hours to about 160 hours, from about 40 hours to about 150 hours, from about 50 hours to about 140 hours, from about 60 hours to about 130 hours, from about 70 hours to about 120 hours, from about 80 hours to about 110 hours, from about 90 hours to about 100 hours, or about 95 hours. The polymerizing/reacting/deprotecting step (i), (ii), (iii), (a-i), (a-ii), (a-iii), (b-i), (b-ii), (b-iii), (b-iv), (b-v), (c-i) and/or (d-i) may also be performed over a time duration of from about 1 hour to about 72 hours, from about 2 hours to about 60 hours, from about 3 hours to about 48 hours, from about 4 hours to about 36 hours, from about 5 hours to about 24 hours, or from about 6 hours to about 12 hours.

In various embodiments, the polymerizing/reacting/deprotecting step (i), (ii), (iii), (a-i), (a-ii), (a-iii), (b-i), (b-ii), (b-iii), (b-iv), (b-v), (c-i) and/or (d-i) is/are performed at a temperature that is from about 10.0°C to about 100.0°C, from about 20.0°C to about 90.0°C, from about 30.0°C to about 80.0°C, from about 40.0°C to about 70.0°C, from about 50.0°C to about 60.0°C, or about 55.0°C. In various embodiments, the polymerizing/reacting/deprotecting step (i), (ii), (iii), (a-i), (a-ii), (a-iii), (b-i), (b-ii), (b-iii), (b-iv), (b-v), (c-i) and/or (d-i) is/are performed at room temperature e.g., that is from about 20°C to about 30°C, about 21°C, about 22°C, about 23°C, about 24°C, about 25°C, about 26°C, about 27°C, about 28°C, about 29°C, or about 30°C.

In various embodiments, the polymerizing/reacting/deprotecting step (i), (ii), (iii), (a-i), (a-ii), (a-iii), (b-i), (b-ii), (b-iii), (b-iv), (b-v), (c-i) and/or (d-i) is/are optionally performed at a temperature that is from about 40.0 °C to about 100.0 °C, from about 45.0 °C to about 95.0 °C, from about 50.0 °C to about 90.0 °C, from about 55.0 °C to about 85.0 °C, from about 60.0 °C to about 80.0 °C, from about 65.0 °C to about 75.0 °C, or about 70.0 °C.

In various embodiments, the polymerizing/reacting/deprotecting step (i), (ii), (iii), (a-i), (a-ii), (a-iii), (b-i), (b-ii), (b-iii), (b-iv), (b-v), (c-i) and/or (d-i) is/are optionally performed at a temperature that is from about -10°C to about 10°C, from about -9°C to about 9°C, from about -8°C to about 8°C, from about -7°C to about 7°C, from about -6°C to about 6°C, from about -5°C to about 5°C, from about -4°C to about 4°C, from about -3°C to about 3°C, from about -2°C to about 2°C, from about -1°C to about 1°C, or 0°C, e.g., to control reaction kinetics. For example, the reacting step may be performed in an ice bath.

- 10 In various embodiments, the method further comprises:
- (e-i) a step of isolating the first intermediate compound represented by general formula (5) after step (i);
 - (e-ii) a step of isolating the second intermediate compound represented by general formula (7) after step (ii);
 - 15 (e-iii) a step of isolating the compound represented by general formula (1) after step (iii);
 - (e-iv) a step of isolating the first intermediate compound represented by general formula (11) after step (a-i);
 - (e-v) a step of isolating the second intermediate compound represented by general formula (12) after step (a-ii);
 - 20 (e-vi) a step of isolating the N-carboxyanhydride (NCA) monomer represented by general formula (3) after step (a-iii);
 - (e-vii) a step of isolating the first intermediate compound represented by general formula (14) after step (b-i);
 - 25 (e-viii) a step of isolating the second intermediate compound represented by general formula (15) after step (b-ii);
 - (e-ix) a step of isolating the third intermediate compound represented by general formula (17) after step (b-iii);
 - (e-x) a step of isolating the fourth intermediate compound represented by general formula (18) after step (b-iv);
 - 30 (e-xi) a step of isolating the N-carboxyanhydride (NCA) monomer represented by general formula (3) after step (b-v);

- (e-xii) a step of isolating the protected monosaccharide represented by general formula (8^P) after step (c-i); and/or
- (e-xiii) a step of isolating the protected alkanolamine compound represented by general formula (13) after step (d-i).

5

In various embodiments, the isolating step(s) comprises one or more of the following steps: re-dissolving, purifying, centrifuging, washing, precipitating and/or recrystallizing the first intermediate compound represented by general formula (5), the second intermediate compound represented by general formula (7), the compound represented by general formula (1), the protected monosaccharide, the first intermediate compound represented by general formula (11), the second intermediate compound represented by general formula (12), the N-carboxyanhydride (NCA) monomer represented by general formula (3), the first intermediate compound represented by general formula (14), the second intermediate compound represented by general formula (15) and/or the third intermediate compound represented by general formula (17). In various embodiments, the isolating step is performed to remove by-products from the first intermediate compound represented by general formula (5), the second intermediate compound represented by general formula (7), the compound represented by general formula (1), the protected monosaccharide represented by general formula (8^P), the first intermediate compound represented by general formula (11), the second intermediate compound represented by general formula (12), the N-carboxyanhydride (NCA) monomer represented by general formula (3), the first intermediate compound represented by general formula (14), the second intermediate compound represented by general formula (15), the third intermediate compound represented by general formula (17), and/or the alkanolamine compound represented by general formula (13).

In various embodiments, the step(s) of purifying, centrifuging, recrystallizing and/or washing is/are repeated at least 1 time, 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, at least 10 times, at least 15 times, at least 20

times with a washing medium. In various embodiments, the step(s) of purifying, centrifuging, recrystallizing and/or washing is/are typically/usually repeated at least 3 times.

5 In various embodiments, the washing medium comprises aqueous medium/solutions such as salt solution, deionized water, acid or the like, or combinations thereof. The salt solution may be bicarbonate salts such as sodium bicarbonate, chloride salts such as saturated sodium chloride (brine). The acid may be hydrochloric acid. In various embodiments, the salt solution comprises
10 highly concentrated/saturated salt solution.

In various embodiments, the method further comprises one or more of the following post reaction steps: drying optionally under low temperature (e.g., freeze drying), under vacuum, and/or in an inert atmosphere.

15

In various embodiments, the step(s) of drying is performed in the presence of a drying agent such as anhydrous sodium sulfate, anhydrous magnesium sulfate, anhydrous calcium sulfate, and anhydrous calcium chloride, the like or combinations thereof.

20

In various embodiments, the yield of the compound represented by general formula (1) is from about 50.0% to about 100.0%, from about 51.0% to about 99.0%, from about 52.0% to about 98.0%, from about 53.0% to about 97.0%, from about 54.0% to about 96.0%, from about 55.0% to about 95.0%,
25 from about 60.0% to about 90.0%, from about 65.0% to about 85.0%, from about 70.0% to about 80.0%, or about 75.0%. The yield of the compound represented by general formula (1) may be from about 50.0% to about 98.0%.

Advantageously, embodiments of the method are straightforward to
30 perform and/or have a low production/manufacturing cost (i.e. cost effective) as the reaction conditions are mild and do not require harsh and/or tedious step(s). Advantageously, embodiments of the method comprise simple purification steps

(e.g., ease of isolation from by-products etc) and products are synthesized with high yields. Advantageously, embodiments of the method are scalable and/or have substantially high scalability.

5 NANOPARTICLE COMPOSITION

Advantageously, in various embodiments, the design of the structure of the compound represented by general formula (1) allows said compound to be used, in lieu or in replacement/substitute of a conventional lipid-PEG conjugate (e.g., ALC-0159), in the formulation of nanoparticles in a composition. In various
10 embodiments, embodiments of the compound are capable of being formulated into nanoparticles in a composition. Advantageously, in various embodiments, the design of the compound represented by general formula (1) helps prevent non-specific protein absorption, particle aggregation and controls the size of the
15 nanoparticles formed. In various embodiments, embodiments of the compound represented by general formula (1) helps maintain colloidal stability (of the lipid nanoparticles), and facilitate the condensation and encapsulating/loading of molecules/cargoes into the nanoparticle composition.

20 The term “nanoparticles” may comprise and/or may be used interchangeably with the terms “lipid nanoparticles”, “encapsulated lipid nanoparticles”, “loaded lipid nanoparticles”, “LNPs”, “cell-targeting lipid nanoparticles”, “immune cells-targeting lipid nanoparticles”, or the like.

25 There is provided a nanoparticle composition comprising:

- (i) a compound represented by general formula (1) as disclosed herein; and
- (ii) a therapeutic agent, prophylactic agent and/or biological agent that is encapsulated/loaded in said composition.

30 Advantageously, the composition is suitable for use in the encapsulation, delivery and/or transfection of one or more therapeutic agent, prophylactic agent

and/or biological agent e.g., to a desired target (such as subject, cell, cytosol, tissue or organ).

In various embodiments, the composition further comprises:

- 5 (a) ionizable lipid;
- (b) neutral/helper lipid; and
- (c) cholesterol and/or derivatives thereof.

In various embodiments, the composition is substantially devoid of
10 polyethylene glycol (PEG). In various embodiments, the composition is substantially devoid of polyethylene glycol (PEG)-modified lipid conjugates, polyethylene glycol (PEG)-modified lipid, PEGylated lipid, PEG-conjugated lipid, PEG-lipid conjugate and/or lipid modified with PEG. In various embodiments, the compound is a substitute/replacement for PEG-lipid conjugate.

15

In various embodiments, the compound, ionizable lipid, neutral/helper lipid, and cholesterol and/or derivatives thereof are mixed/dissolved in an organic solvent. In various embodiments, the formation of lipid nanoparticle comprises self-assembly of the lipid components and one or more types of molecules or
20 cargoes. In various embodiments, any organic solvent that effectively serves as a medium to contain the lipid components may be used in embodiments of the lipid materials disclosed herein. In various embodiments, the organic solvent is capable of substantially dissolving the components present in the mixture. The organic solvent may comprise ethanol, isopropanol, acetonitrile, ethyl acetate,
25 methanol, tetrahydrofuran, dimethyl sulfoxide, dimethylformamide or the like or combinations thereof.

In various embodiments, the ionizable lipid, helper lipid, cholesterol and/or derivatives thereof, and compound represented by general formula (1) are mixed
30 at a mole ratio of about 20 – 50 : about 4 – 20 : about 25 – 50 : about 0.5 – 20, at a mole ratio of about 24 – 46 : about 6 – 18 : about 28 – 47 : about 1.0 – 15, at a mole ratio of about 28 – 42 : about 8 – 16 : about 31 – 44 : about 1.5 – 10, at a

mole ratio of about 30 – 38 : about 10 – 14 : about 34 – 41 : about 2.0 – 5, at a mole ratio of about 32 – 34 : about 12 – 13 : about 37 – 38 : about 2.5 – 3.

In various embodiments, the ionizable lipid includes, but is not limited to
5 ALC-0315, SM-102, Lipid 5, DLinDMA, D-Lin-MC2-DMA, DLin-MC3-DMA, D-Lin-MC4-DMA, Dlin-KC2-DMA, YSK05, AA3-Dlin, SSPalmM, SSPalmO-Phe, Lipid A9, L319, DODMA, CL1, BP Lipid 310, ATX-001, ATX-100, Lipid 2, 80-O16B, BP Lipid 309, BP Lipid 307, 93-O17S, 93-O17O, NT1-O14B, 306-O12B-3, 306-O12B, 113-O16B, 306Oi10, 306Oi9-cis2, BAMEA-O16B, AI-28, 113-O12B,
10 98N12-5, Ckk-E12, OF-02, C12-200, BP Lipid 311, BP Lipid 308, BP Lipid 314, BP Lipid 312, LP01, TCL053, Lipid C24, BP Lipid 315, Lipid 29, 9A1P9, C13-112-tri-tail, C13-113-tri-tail, C13-112-tetra-tail, or C13-113-tetra-tail, C12-200 and combinations thereof. It will be appreciated that any suitable ionizable lipid (e.g., commercially available ionizable lipids) that effectively
15 modulates/adjusts/changes its charge depending on the environmental pH may be used in embodiments of the composition disclosed herein.

In various embodiments, the composition comprises from about 20.0 mol% to about 60.0 mol%, from about 25.0 mol% to about 55.0 mol%, from about
20 30.0 mol% to about 50.0 mol%, from about 35.0 mol% to about 45.0 mol%, or about 40.0 mol% of ionizable lipid. In various embodiments, the ionizable lipid is present in an amount of about 20 mol%, about 21 mol%, about 22 mol%, about 23 mol%, about 24 mol%, about 25 mol%, about 26 mol%, about 27 mol%, about 28 mol%, about 29 mol%, about 30 mol%, about 31 mol%, about 32 mol%, about
25 33 mol%, about 34 mol%, about 35 mol%, about 36 mol%, about 37 mol%, about 38 mol%, about 39 mol%, about 40 mol%, about 41 mol%, about 42 mol%, about 43 mol%, about 44 mol%, about 45 mol%, about 46 mol%, about 47 mol%, about 48 mol%, about 49 mol%, about 50 mol%, about 51 mol%, about 52 mol%, about 53 mol%, about 54 mol%, about 55 mol%, about 56 mol%, about 57 mol%, about
30 58 mol%, about 59 mol%, about 60 mol% of the composition.

In various embodiments, the neutral/helper lipid comprises a phospholipid such as an unsaturated lipid. Examples of phospholipid includes, but are not limited to, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), sphingomyelin, and the like and combinations thereof.

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In various embodiments, the composition comprises from about 1.0 mol% to about 20.0 mol%, from about 1.5 mol% to about 19.5 mol%, from about 2.0 mol% to about 19.0 mol%, from about 2.5 mol% to about 18.5 mol%, from about 3.0 mol% to about 18.0 mol%, from about 3.5 mol% to about 17.5 mol%, from about 4.0 mol% to about 17.0 mol%, from about 4.5 mol% to about 16.5 mol%, from about 5.0 mol% to about 16.0 mol%, from about 5.5 mol% to about 15.5 mol%, from about 6.0 mol% to about 15.0 mol%, from about 6.5 mol% to about 14.5 mol%, from about 7.0 mol% to about 14.0 mol%, from about 7.5 mol% to about 13.5 mol%, from about 8.0 mol% to about 13.0 mol%, from about 8.5 mol% to about 12.5 mol%, from about 9.0 mol% to about 12.0 mol%, from about 9.5 mol% to about 11.5 mol%, from about 10.0 mol% to about 11.0 mol%, about 10.5 of neutral/helper lipid. In various embodiments, the neutral/helper lipid is present

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in an amount of about 1 mol%, about 2 mol%, about 3 mol%, about 4 mol%, about 5 mol%, about 6 mol%, about 7 mol%, about 8 mol%, about 9 mol%, about 10 mol%, about 11 mol%, about 12 mol%, about 13 mol%, about 14 mol%, about 15 mol%, %, about 16 mol%, about 17 mol%, about 18 mol%, about 19 mol%, or
5 about 20 mol% of the composition.

In various embodiments, the cholesterol and/or derivatives thereof includes, but is not limited to cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, avenasterol, or the like or
10 combinations thereof.

In various embodiments, the composition comprises from about 25.0 mol% to about 60.0 mol%, from about 30.0 mol% to about 55.0 mol%, from about 35.0 mol% to about 50.0 mol%, or from about 40.0 mol% to about 45.0 mol% of
15 cholesterol and/or derivatives thereof. In various embodiments, the cholesterol and/or derivatives thereof is/are present in an amount of about 25 mol%, about 26 mol%, about 27 mol%, about 28 mol%, about 29 mol%, about 30 mol%, about 31 mol%, about 32 mol%, about 33 mol%, about 34 mol%, about 35 mol%, about 36 mol%, about 37 mol%, about 38 mol%, about 39 mol%, about 40 mol%, about
20 41 mol%, about 42 mol%, about 43 mol%, about 44 mol%, about 45 mol%, about 46 mol%, about 47 mol%, about 48 mol%, about 49 mol%, about 50 mol%, about 51 mol%, about 52 mol%, about 53 mol%, about 54 mol%, about 55 mol%, about 56 mol%, about 57 mol%, about 58 mol%, about 59 mol%, about 60 mol% of the composition.

25

In various embodiments, the composition comprises from about 0.5 mol% to about 20.0 mol%, from about 1.0 mol% to about 19.5 mol%, from about 1.5 mol% to about 19.0 mol%, from about 2.0 mol% to about 18.5 mol%, from about 2.5 mol% to about 18.0 mol%, from about 3.0 mol% to about 17.5 mol%, from
30 about 3.5 mol% to about 17.0 mol%, from about 4.0 mol% to about 16.5 mol%, from about 4.5 mol% to about 16.0 mol%, from about 5.0 mol% to about 15.5 mol%, from about 5.5 mol% to about 15.0 mol%, from about 6.0 mol% to about

14.5 mol%, from about 6.5 mol% to about 14.0 mol%, from about 7.0 mol% to about 13.5 mol%, from about 7.5 mol% to about 13.0 mol%, from about 8.0 mol% to about 12.5 mol%, from about 8.5 mol% to about 12.0 mol%, from about 9.0 mol% to about 11.5 mol%, from about 9.5 mol% to about 11.0 mol%, from about 10.0 mol% to about 10.5 mol%, from about 0.5 mol% to about 5.0 mol%, from about 0.6 mol% to about 4.9 mol%, from about 0.7 mol% to about 4.8 mol%, from about 0.8 mol% to about 4.7 mol%, from about 0.9 mol% to about 4.6 mol%, from about 1.0 mol% to about 4.5 mol%, from about 1.1 mol% to about 4.4 mol%, from about 1.2 mol% to about 4.3 mol%, from about 1.3 mol% to about 4.2 mol%, from about 1.4 mol% to about 4.1 mol%, from about 1.5 mol% to about 4.0 mol%, from about 1.6 mol% to about 3.9 mol%, from about 1.7 mol% to about 3.8 mol%, from about 1.8 mol% to about 3.7 mol%, from about 1.9 mol% to about 3.6 mol%, from about 2.0 mol% to about 3.5 mol%, from about 2.1 mol% to about 3.4 mol%, from about 2.2 mol% to about 3.3 mol%, from about 2.3 mol% to about 3.2 mol%, from about 2.4 mol% to about 3.1 mol%, from about 2.5 mol% to about 3.0 mol%, from about 2.6 mol% to about 2.9 mol%, from about 2.7 mol% to about 2.8 mol%, or about 2.75 mol% of compound represented by general formula (1).

In various embodiments, the compound represented by general formula (1) is the major component of the composition and is present in an amount of about 0.5 mol%, about 0.6 mol%, about 0.7 mol%, about 0.8 mol%, about 0.9 mol%, about 1.0 mol%, about 1.1 mol%, about 1.2 mol%, about 1.3 mol%, about 1.4 mol%, about 1.5%, about 1.6 mol%, about 1.7 mol%, about 1.8 mol%, about 1.9 mol%, about 2.0 mol%, about 2.1 mol%, about 2.2 mol%, about 2.3 mol%, about 2.4 mol%, about 2.5 mol%, about 2.6 mol%, about 2.7 mol%, about 2.8 mol%, about 2.9 mol%, about 3.0 mol%, about 3.1 mol%, about 3.2 mol%, about 3.3 mol%, about 3.4 mol%, about 3.5 mol%, about 3.6 mol%, about 3.7 mol%, about 3.8 mol%, about 3.9 mol%, about 4.0 mol%, about 4.1 mol%, about 4.2 mol%, about 4.3 mol%, about 4.4 mol%, about 4.5 mol%, about 4.6 mol%, about 4.7 mol%, about 4.8 mol%, about 4.9 mol%, or about 5.0 mol% of the composition.

In various embodiments, the therapeutic agent, prophylactic agent and/or biological agent is provided in an aqueous buffer. The aqueous buffer may be sodium acetate.

- 5 In various embodiments, the nanoparticle composition comprises nanoparticles formed from the compound represented by general formula (1).

NANOPARTICLES

- 10 There is provided nanoparticles (e.g., lipid nanoparticles) comprising:
- (i) the compound represented by general formula (1) as disclosed herein; and
 - (ii) a therapeutic and/or prophylactic agent and/or biological agent that is encapsulated/loaded/coupled/bonded/linked/bound in/to said nanoparticles.

- 15 In various embodiments, the nanoparticles have a N:P or N/P ratio (i.e. molar ratio of ionizable nitrogen atoms (in the physiological pH range) in the ionizable lipid to phosphate groups in the therapeutic agent, prophylactic agent and/or biological agent (e.g., nucleic acid) is from about 1:1 to about 20:1. The
- 20 nanoparticles may have a N:P or N/P ratio that is from about 1:1 to about 20:1, from about 2:1 to about 19:1, from about 3:1 to about 18:1, from about 4:1 to about 17:1, from about 5:1 to about 16:1, from about 6:1 to about 15:1, from about 7:1 to about 14:1, from about 8:1 to about 13:1, from about 9:1 to about 12:1, or from about 10:1 to about 11:1.

- 25 It will be appreciated that in various embodiments, the optimal N/P ratio is dependent on the type of therapeutic and/or prophylactic agent and/or biological agent (e.g., a nucleic acid such as mRNA, siRNA, miRNA, pDNA and oligonucleotides). For example, the optimal N/P ratio may be different for siRNA,
- 30 pDNA and oligonucleotides. In various embodiments, it will be appreciated that shorter nucleic acid therapeutics (e.g. siRNA) or prophylactic agents (e.g., mRNA) require more (i.e. a larger amount/concentration/volume of) ionizable

lipids to encapsulate them into lipid nanoparticles. In various embodiments therefore, a N/P ratio of up to about 20:1 is used to encapsulate and deliver nucleic acid therapeutics (e.g. shorter nucleic acid therapeutics siRNA).

5 In various embodiments, the encapsulation/loading/binding efficiency of the therapeutic agent, prophylactic agent and/or biological agent in the composition/nanoparticles is at least about 5.0%, at least about 10.0%, at least about 15.0%, at least about 20.0%, at least about 25.0%, at least about 30.0%, at least about 35.0%, at least about 40.0%, at least about 45.0%, at least about
10 50.0%, at least about 55.0%, at least about 60.0%, at least about 65.0%, at least about 70.0%, at least about 75.0%, at least about 80.0%, at least about 85.0%, at least about 90.0%, at least about 95.0%, at least about 96.0%, at least about 97.0%, at least about 98.0%, at least about 99.0%, at least about 99.5%, at least about 99.9%, or about 100%.

15 In various embodiments, the nanoparticles have an encapsulation efficiency that is slightly lower, comparable to, no less or is higher than that of corresponding nanoparticles using ALC-0159 as the PEG-lipid conjugate under similar conditions. For example, the encapsulation efficiency may be at least
20 about 50% of that of a corresponding nanoparticle using ALC-0159 as the PEG-lipid conjugate under similar conditions. In another example, the encapsulation efficiency may be at least about 0.1% to at least about 10% higher than that of corresponding nanoparticles using ALC-0159 as the PEG-lipid conjugate under similar conditions.

25 In various embodiments, the nanoparticles comprising polyserine (e.g., poly-L-serine) have an encapsulation efficiency that is slightly lower, comparable to, no less or is higher than that of corresponding nanoparticles using poly(glutamic acid) under similar conditions. For example, the encapsulation
30 efficiency may be at least about 5% to at least about 30% higher than that of corresponding nanoparticles comprising poly(glutamic acid) under similar conditions.

In various embodiments, the cell/nucleic acid transfection efficiency (% of the cells/nucleic acids that are transfected with the gene) of the composition/nanoparticles is at least about 1.0%, at least about 5.0%, at least about 10.0%, at least about 15.0%, at least about 20.0%, at least about 25.0%,
5 at least about 30.0%, at least about 35.0%, at least about 40.0%, at least about 45.0%, at least about 50.0%, at least about 55.0%, at least about 60.0%, at least about 65.0%, at least about 70.0%, at least about 75.0%, at least about 80.0%, at least about 85.0%, at least about 90.0%, at least about 95.0%, at least about 96.0%, at least about 97.0%, at least about 98.0%, at least about 99.0%, at least
10 about 99.5%, at least about 99.9%, or about 100%. In various embodiments, the cell transfection efficiency is not required to be 100%. For example, it will be appreciated that vaccine applications may not need/require to transfect 100% cells in order to mediate an immune response, unlike in the case of cancer therapy applications.

15

In various embodiments, the nucleic acid transfection efficiency of the therapeutic and/or prophylactic agent and/or biological agent in the composition/nanoparticle is greater than those using PEG-conjugated lipids (e.g., ALC-0159).

20

In various embodiments, the nanoparticles have a nucleic acid translation efficiency that is no less or higher than that of corresponding nanoparticles using ALC-0159 PEG-modified lipid under similar conditions. For example, the nucleic acid (e.g., mRNA) transfection efficiency may be at least 2 times, at least 3 times,
25 at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times, at least 9 times, at least 10 times, at least 15 times, at least about 20 times, at least 25 times, at least 30 times, at least 35 times, at least 40 times, at least 45 times, at least 50 times, at least 55 times, at least 60 times, at least 65 times, at least 70 times, at least 75 times, at least 80 times, at least 85 times, at least 90
30 times, at least 95 times, or at least 100 times higher than that of corresponding nanoparticles using ALC-0159 PEG-modified lipid under similar conditions.

In various embodiments, the nanoparticles comprising polyserine (e.g., poly-L-serine) have a nucleic acid translation efficiency that is no less or higher than that of a corresponding nanoparticle using poly(glutamic acid) under similar conditions. For example, the nucleic acid (e.g., mRNA) translation efficiency may be 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, at least 10 times, at least 15 times, at least about 20 times, at least 25 times, at least 30 times, at least 35 times, at least 40 times, at least 45 times, at least 50 times, at least 55 times, at least 60 times, at least 65 times, at least 70 times, at least 75 times, at least 80 times, at least 85 times, at least 90 times, at least 95 times, or at least 100 times higher than that of a corresponding nanoparticle comprising poly(glutamic acid) under similar conditions.

In various embodiments, the nanoparticles have a cell translation efficiency that is comparable to, no less or is higher than that of corresponding nanoparticles using ALC-0159 as the PEG-lipid conjugate under similar conditions. For example, the cell translation efficiency may be at least about 30% of that of a corresponding nanoparticle using ALC-0159 as the PEG-lipid conjugate under similar conditions. In another example, the cell translation efficiency may be at least about 50% to at least about 1,000% higher than that of corresponding nanoparticles using ALC-0159 as the PEG-lipid conjugate under similar conditions.

In various embodiments, the translation efficiency in certain cell lines is lower than that of corresponding nanoparticles using ALC-0159 as the PEG-lipid conjugate under similar conditions but still at the same order of magnitude. It will be appreciated that such level of gene transfection may still be applicable for gene therapy.

Advantageously, in various embodiments, the average or mean particle size (or diameter) nanoparticles are designed to be customizable/adjustable to suit a desired application.

In various embodiments, the nanoparticles have an average or mean particle size (or diameter) in the micron meter range. The average or mean particle size of the nanoparticles may be from about 0.10 μm to about 10.0 μm , from about 0.15 μm to about 9.5 μm , from about 0.20 μm to about 9.0 μm , from about 0.25 μm to about 8.5 μm , from about 0.50 μm to about 8.0 μm , from about 0.75 μm to about 7.5 μm , from about 1.0 μm to about 7.0 μm , from about 1.5 μm to about 6.5 μm , from about 2.0 μm to about 6.0 μm , from about 2.5 μm to about 5.5 μm , from about 3.0 μm to about 5.0 μm , from about 3.5 μm to about 4.5 μm , or about 4.0 μm . In various embodiments, the nanoparticles have an average or mean particle size (or diameter) in the micron meter range, that are suitable for use in nasal spray applications.

In various embodiments, the nanoparticles have an average or mean particle size (or diameter) in the nano meter range. In various embodiments, the nanoparticles have an average or mean particle size (or diameter) of no more than about 800 nm, no more than about 750 nm, no more than about 700 nm, no more than about 650 nm, no more than about 600 nm, no more than about 550 nm, no more than about 500 nm, no more than about 450 nm, no more than about 400 nm, no more than about 350 nm, no more than about 300 nm, no more than about 250 nm, no more than about 200 nm, no more than about 150 nm, no more than about 100 nm, no more than about 90 nm, no more than about 80 nm, no more than about 70 nm, no more than about 60 nm, no more than about 50 nm, no more than about 40 nm, no more than about 30 nm, no more than about 20 nm, or no more than about 10 nm. In various embodiments, the nanoparticles have an average or mean particle size (or diameter) of from about 40.0 nm to about 500.0 nm, from about 50.0 nm to about 490.0 nm, from about 60.0 nm to about 480.0 nm, from about 70.0 nm to about 470.0 nm, from about 80.0 nm to about 460.0 nm, from about 90.0 nm to about 450.0 nm, from about 100.0 nm to about 440.0 nm, from about 110.0 nm to about 430.0 nm, from about 120.0 nm to about 420.0 nm, from about 130.0 nm to about 410.0 nm, from about 140.0 nm to about 400.0 nm, from about 150.0 nm to about 390.0 nm, from about 160.0 nm to about 380.0 nm, from about 170.0 nm to about 370.0 nm, from about 180.0 nm to about

360.0 nm, from about 190.0 nm to about 350.0 nm, from about 200.0 nm to about 340.0 nm, from about 210.0 nm to about 330.0 nm, from about 220.0 nm to about 320.0 nm, from about 230.0 nm to about 310.0 nm, from about 240.0 nm to about 300.0 nm, from about 250.0 nm to about 290.0 nm, from about 260.0 nm to about 280.0 nm, about 270.0 nm, about 200.0 nm, about 250.0 nm, about 300.0 nm, about 350.0 nm, about 400.0 nm, or about 450.0 nm.

In various embodiments, the composition comprising the nanoparticles has a polydispersity index (PDI) of from about 0.050 to about 0.300, from about 0.060 to about 0.290, from about 0.070 to about 0.280, from about 0.080 to about 0.270, from about 0.090 to about 0.260, from about 0.100 to about 0.250, from about 0.110 to about 0.240, from about 0.120 to about 0.230, from about 0.130 to about 0.220, from about 0.140 to about 0.210, from about 0.150 to about 0.200, from about 0.160 to about 0.190, from about 0.170 to about 0.180, or about 0.175.

In various embodiments, the composition comprising the nanoparticles has a polydispersity index (PDI) of from about 0.01 to about 0.50, from about 0.0125 to about 0.45, from about 0.015 to about 0.40, from about 0.020 to about 0.35, from about 0.025 to about 0.30, from about 0.030 to about 0.25, from about 0.035 to about 0.20, from about 0.040 to about 0.15, from about 0.045 to about 0.10, from about 0.050 to about 0.095, from about 0.055 to about 0.090, from about 0.060 to about 0.085, from about 0.065 to about 0.080, or from about 0.070 to about 0.075. Advantageously, in various embodiments, the nanoparticles have a narrow particle size distribution (e.g., not more than about 0.2) and/or the nanoparticles or nanoparticle composition is relatively/substantially homogenous.

25

In various embodiments, the nanoparticles have a zeta potential of from about -50.0 mV to about +30.0 mV, from about -40.0 mV to about +25.0 mV, from about -30.0 mV to about +20.0 mV, from about -20.0 mV to about +20.0 mV, from about -15.0 mV to about +15.0 mV, from about -14.0 mV to about +14.0 mV, from about -13.0 mV to about +13.0 mV, from about -12.0 mV to about +12.0 mV, from about -11.0 mV to about +11.0 mV, from about -10.0 mV to about +10.0 mV, from about -9.0 mV to about +9.0 mV, from about -8.0 mV to about +8.0 mV, from

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about -7.0 mV to about +7.0 mV, from about -6.0 mV to about +6.0 mV, from about -5.0 mV to about +5.0 mV, from about -4.0 mV to about +4.0 mV, from about -3.0 mV to about +3.0 mV, from about -2.0 mV to about +2.0 mV, from about -1.0 mV to about +1.0 mV, or about 0 mV in saline (e.g., phosphate-buffered saline (PBS)) or in a physiological environment. In various embodiments, the nanoparticles have a zeta potential of from about -10.0 mV to about +20.0 mV, from about -9.0 mV to about +19.0 mV, from about -8.0 mV to about +18.0 mV, from about -7.0 mV to about +17.0 mV, from about -6.0 mV to about +16.0 mV, from about -5.0 mV to about +15.0 mV, from about -4.0 mV to about +14.0 mV, from about -3.0 mV to about +13.0 mV, from about -2.0 mV to about +12.0 mV, from about -1.0 mV to about +11.0 mV, from about 0.0 mV to about +10.0 mV, from about +1.0 mV to about +9.0 mV, from about +2.0 mV to about +8.0 mV, from about +3.0 mV to about +7.0 mV, from about +4.0 mV to about +6.0 mV, or about +5.0 mV. In various embodiments, a zeta potential of about ± 10 mV is desirable for *in vivo* applications. Advantageously, in various embodiments, the nanoparticles have a substantially neutral surface charge or a negative charge or a positive charge, making the nanoparticles suitable/desirable for *in vivo* applications. In various embodiments, the nanoparticles with a negative charge may be used to target spleen and lymph nodes. In various embodiments, the nanoparticles with a positive charge may be used for local delivery of cargos across the mucus layer, e.g. nasal delivery. In various embodiments, the nanoparticles with a negative charge may be used to target the spleens or lymph nodes.

In various embodiments, the cell viability of the composition/nanoparticle is at least about 50.0%, at least about 60.0%, at least about 70.0%, at least about 80.0%, at least about 90.0%, at least about 95.0%, at least about 96.0%, at least about 97.0%, at least about 98.0%, at least about 99.0%, at least about 99.5%, or at least about 99.9%.

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In various embodiments, the nanoparticle has a cell viability that is comparable or is no less or is higher than that of a corresponding nanoparticle

using ALC-0519 as the PEG-lipid conjugate under similar conditions. For example, the cell viability may be at least comparable to that of a corresponding nanoparticle using ALC-0519 as the PEG-lipid conjugate under similar conditions. Accordingly, in various embodiments, the nanoparticle
5 comprises/possesses high cytocompatibility and/or negligible cytotoxicity.

In various embodiments, the composition/compound/nanoparticles is/are biocompatible, i.e. the composition/compound/nanoparticle is compatible with biological systems or parts of the biological systems without substantially or
10 significantly eliciting an adverse physiological response such as a toxic reaction/response (e.g., cytotoxicity), an immune reaction/response, an injury or the like when used on the human or animal body. In various embodiments, the composition/compound/nanoparticle is substantially devoid of substances that elicit an adverse physiological response. It will be appreciated that the
15 composition/compound/nanoparticle may trigger/elicit an immune response (e.g., to enhance vaccination efficacy), and in such embodiments, the composition/compound/nanoparticle is still considered to be biocompatible. Advantageously, the nanoparticles (e.g., lipid nanoparticles) are capable of binding therapeutic agent, prophylactic agent and/or biological agent (e.g., RNA)
20 effectively and/or providing high transfection efficiency without causing/inducing substantial or any cytotoxicity.

METHOD OF PREPARING NANOPARTICLES

25 There is provided a method of preparing nanoparticles as disclosed herein, the method comprising:
(f-i) preparing an aqueous composition comprising therapeutic and/or prophylactic agent and/or biological agent;
(f-ii) mixing the aqueous composition obtained from (f-i) with the composition
30 as disclosed herein to obtain nanoparticles.

In various embodiments, the step (f-i) comprises mixing therapeutic and/or prophylactic agent and/or biological agent in an aqueous buffer. The aqueous buffer may be sodium acetate, citrate buffer, phosphate buffer, glycine buffer solution, or the like or combinations thereof.

5

In various embodiments, the mixing step (f-i) is performed at a pH value of from about 2.5 to about 6.5, from about 2.6 to about 6.4, from about 2.7 to about 6.3, from about 2.8 to about 6.2, from about 2.9 to about 6.1, from about 3.0 to about 6.0, from about 3.1 to about 5.9, from about 3.2 to about 5.8, from about 3.3 to about 5.7, from about 3.4 to about 5.6, from about 3.5 to about 5.5, from about 3.6 to about 5.4, from about 3.7 to about 5.3, from about 3.8 to about 5.2, from about 3.9 to about 5.1, from about 4.0 to about 5.0, from about 4.1 to about 4.9, from about 4.2 to about 4.8, from about 4.3 to about 4.7, from about 4.4 to about 4.6, or about 4.5.

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In various embodiments, the composition as disclosed herein comprises organic phase (e.g., ethanol). In various embodiments, the aqueous composition comprises aqueous phase. In various embodiments, the step (f-ii) comprises mixing the aqueous composition with the organic composition as disclosed herein at a volume ratio of the aqueous phase to organic phase from about 10:1 to about 1:1. For example, the aqueous phase may be mixed with the organic phase at a volume ratio of from about 10:1 to about 1:1, at about 9:1, at about 8:1, at about 7:1, at about 6:1, at about 5:1, at about 4:1, at about 3:1, or at about 2:1.

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In various embodiments, the step (f-ii) of mixing the aqueous composition with the organic composition comprises injecting (e.g., direct injecting) the organic composition into the aqueous composition. In various embodiments, the step (f-ii) of mixing the aqueous composition with the organic composition comprises pipetting (e.g., rapid pipetting) the organic composition into the aqueous composition.

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In various embodiments, the step (f-ii) of mixing the aqueous composition with the composition comprises micro-mixing, e.g., microfluidic mixing using a microfluidic device. The micro-mixing may be performed *via* passive mixing using passive micromixers such as T-shaped or Y-shaped microfluidic mixers parallel
5 lamination, sequential, focusing enhanced mixers or droplet micromixers. The micro-mixing may also be performed *via* active mixing using external forces such as pressure field, electrokinetic, dielectrophoretic, electrowetting, magneto-hydrodynamic or ultrasound. Advantageously, as microfluidic mixing comprises mixing the two compositions (i.e. aqueous composition and composition
10 disclosed herein) in a controlled manner and/or with a specified/fixed/controlled/precise mixing ratio, the interaction between the two compositions (e.g., between ionizable lipid and therapeutic, prophylactic and/or biological agent) is regulated, thereby producing nanoparticles with a smaller particle size and/or with a narrow size distribution or homogeneity (e.g, smaller
15 PDI).

In various embodiments, the method further comprises removing the organic phase (e.g. ethanol). For example, removing the organic phase may include dialysis or filtration. Advantageously, removal of the organic phase
20 through dialysis or filtration may improve the encapsulation efficiency of the therapeutic and/or prophylactic agent and/or biological agent.

In various embodiments, there is also provided a carrier, nanocarrier or delivery system/vehicle comprising the composition/compound/nanoparticles as
25 disclosed herein.

In various embodiments, there is also provided a vaccine composition comprising the composition/compound/nanoparticles as disclosed herein.

30 In various embodiments, there is also provided a carrier, a nanocarrier, a delivery system/vehicle, a compound, a nanoparticle composition, nanoparticles (or lipid nanoparticles) disclosed herein for use in medicine (e.g., for the treatment

or prophylaxis of one or more of the diseases, disorders or conditions mentioned herein).

In various embodiments, there is also provided a carrier, a nanocarrier, a
5 delivery system/vehicle, a compound, a nanoparticle composition, nanoparticles
(or lipid nanoparticles) disclosed herein for use in the treatment or prophylaxis of
a disease, disorder or condition, the use of said carrier, a nanocarrier, a delivery
system/vehicle, a compound, a nanoparticle composition, nanoparticles (or lipid
nanoparticles) in the manufacture of a medicament for the treatment or
10 prophylaxis of a disease, disorder or condition and/or a method of treatment or
prophylaxis of a disease, disorder or condition, comprising a step of administering
(e.g. in a therapeutically effective amount of) said carrier, a nanocarrier, a delivery
system/vehicle, a compound, a nanoparticle composition, nanoparticles (or lipid
nanoparticles) to a subject (e.g., vertebrate such as a human or a large veterinary
15 mammal (e.g., horses, cattle, deer, sheep, llamas, goats, pigs) in need thereof.

The disease, disorder or condition may be selected from the group
consisting of infectious/contagious diseases, viral infections (i.e. diseases caused
by virus), bacterial infections (i.e. diseases caused by bacteria), fungal infections
20 (i.e. diseases caused by fungi), respiratory diseases or the like, cancer,
cardiovascular diseases, skin disease or the like, or combinations thereof. In
various embodiments, the disease, disorder or condition is mediated by an
influenza virus (e.g., influenza A, B, C and/or D virus). For example, the disease
may be influenza A, B, C or D such as H1N1, H3N2). In various embodiments,
25 the disease, disorder or condition is mediated by a coronavirus (e.g., severe
acute respiratory syndrome coronavirus such as SARS-CoV-2 or SARS-CoV-1).
For example, the disease, disorder or condition may be SARS-CoV-2 coronavirus
disease. In various embodiments, the disease, disorder or condition is mediated
by a dengue virus (e.g., DEN-1, DEN-2, DEN-3, and/or DEN-4 virus). For
30 example, the disease may be dengue disease.

In various embodiments, there is also provided a carrier, a nanocarrier, a delivery system/vehicle, a compound, a nanoparticle composition, nanoparticles (or lipid nanoparticles) disclosed herein for use in encapsulating and/or delivering a therapeutic, prophylactic and/or biological agent to a subject, cell, cytosol, tissue or organ (e.g., a mammalian cell, cytosol, tissue or organ), the use of said carrier, a nanocarrier, a delivery system/vehicle, a compound, a nanoparticle composition, nanoparticles (or lipid nanoparticles) in the manufacture of a medicament for encapsulating and/or delivering a therapeutic, prophylactic and/or biological agent to a subject, cell, cytosol, tissue or organ (e.g., a mammalian cell, cytosol, tissue or organ), and/or a method of delivering a therapeutic, prophylactic and/or biological agent to a subject, cell, cytosol, tissue or organ (e.g., a mammalian cell, cytosol, tissue or organ), comprising a step of administering (e.g. in a therapeutically effective amount of) said carrier, a nanocarrier, a delivery system/vehicle, a compound, a nanoparticle composition, nanoparticles (or lipid nanoparticles) to a subject (e.g., vertebrate such as a human or a large veterinary mammal (e.g., horses, cattle, deer, sheep, llamas, goats, pigs)) in need thereof.

In various embodiments, there is also provided a carrier, a nanocarrier, a delivery system/vehicle, a compound, a nanoparticle composition, nanoparticles (or lipid nanoparticles) disclosed herein for use in inducing an immune response in a subject (e.g., vertebrate such as a human or a large veterinary mammal (e.g., horses, cattle, deer, sheep, llamas, goats, pigs)), the use of said carrier, a nanocarrier, a delivery system/vehicle, a compound, a nanoparticle composition, nanoparticles (or lipid nanoparticles) in the manufacture of a medicament for inducing an immune response in a subject, and/or a method of inducing an immune response in a subject, comprising a step of administering (e.g. in a therapeutically effective amount of) said carrier, a nanocarrier, a delivery system/vehicle, a compound, a nanoparticle composition, nanoparticles (or lipid nanoparticles) to a subject in need thereof. In various embodiments, an immune response in the subject is to be induced through the administration of the compound, a nanoparticle composition, nanoparticles (or lipid nanoparticles)

thereto. In various embodiments, by inducing an immune response in the subject, the subject is protected against various diseases, disorders or conditions e.g., infectious/contagious diseases, viral infections (i.e. diseases caused by virus), bacterial infections (i.e. diseases caused by bacteria), fungal infections (i.e. diseases caused by fungi), respiratory diseases or the like, or combinations thereof as mentioned herein. The carrier, nanocarrier, delivery system/vehicle, compound, nanoparticle composition, nanoparticles may be delivered to a subject in the form of or as a component of a vaccine.

10 In various embodiments, the disease, disorder or condition is mediated by an influenza virus (e.g., influenza A, B, C and/or D virus). For example, the disease may be influenza A, B, C or D such as H1N1, H3N2). In various embodiments, the disease, disorder or condition is mediated by a coronavirus (e.g., severe acute respiratory syndrome coronavirus such as SARS-CoV-2 or
15 SARS-CoV-1). For example, the disease, disorder or condition may be SARS-CoV-2 coronavirus disease.

In various embodiments, the carrier, nanocarrier, delivery system/vehicle, compound, nanoparticle composition, nanoparticles prepared from embodiments
20 of the method disclosed herein comprises one or more of the following characteristics or properties: broad applicability (e.g., can be used to encapsulate, deliver and/or transfect a wide range of therapeutic, prophylactic and/or biological reagents), nanosized, substantially neutral surface charge or negative surface charge, high encapsulation efficiency (e.g., > 80%), high
25 transfection efficiency (e.g., > 80%), high stability, low toxicity (e.g., low cytotoxicity), low production/synthesis cost, therefore making them suitable for *in vivo* applications that require efficient cellular uptake and/or gene transfection.

In various embodiments, a mannose group is installed onto each unit of
30 the hydrophilic polypeptide, easily accessible to immune cells. In various embodiments, the mannose group is not attached to cholesterol while a PEGylated lipid is used, as this may make it inaccessible to immune cells.

Furthermore, the compound is also devoid of a PEG with a longer chain than mannose, which can shield the surface of LNP and the mannose group. Instead, embodiments of the present technology involve replacement of typically used PEG with mannopolypeptide.

5

In various embodiments, the lipid composition of the present technology is different from the lipid composition in the art that contains mannose group attached to PEG-modified lipid. In various embodiments, the lipid composition in the present technology is substantially devoid of PEG.

10

In various embodiments, the lipid composition of the present technology is different from the lipid composition in the art that contains mannose group attached to cholesterol. In various embodiments, the lipid composition in the present technology has mannose group installed onto each unit of the hydrophilic polypeptide. Advantageously, in various embodiments, the mannose group is easily accessible to immune cells and hence targets mannose receptors on immune cell surface to reduce the risk of allergic reaction by the PEG-conjugated lipids, prolonging the plasma half-life of nucleic acid and enhancing vaccination efficiency. In various embodiments, the encapsulation efficiency and transfection efficiency of the presently disclosed lipid composition are higher than or comparable to those of a lipid composition containing PEG-lipid conjugate.

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In various embodiments, the lipid composition that contains lipid-*block*-poly(L-Ser-D-Mannose) (LPSM) is chemically and structurally different from those of the art which contain phosphatidylserine. Accordingly, in various embodiments, the compound of the present technology does not contain phosphatidylserine.

25

In various embodiments, the lipid composition of the present technology involves direct replacement of PEG-lipid conjugate with lipid-*block*-mannopolypeptide in a lipid composition to achieve enhanced encapsulation

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efficiencies and mRNA transfection efficiencies. Such replacement and technical effects are not taught or expected from the prior art.

In various embodiments, the lipid composition of the present technology is
5 different from lipid compositions in the art that do not contain an amino acid group such as a serine group or glutamic acid group.

BRIEF DESCRIPTION OF FIGURES

10 FIG. 1 shows ^1H NMR of 1,2,3,4,6-penta-O-acetyl-manno-D-pyranose (solvent, CDCl_3) in accordance with various embodiments disclosed herein.

FIG. 2 shows ^1H NMR of Fmoc-L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-OH (solvent, CDCl_3) in accordance with various embodiments
15 disclosed herein.

FIG. 3 shows ^1H NMR of L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA (solvent, CDCl_3) in accordance with various embodiments disclosed herein.

20 FIG. 4 shows ^{13}C NMR of L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA (solvent, CDCl_3) in accordance with various embodiments disclosed herein.

25 FIG. 5 shows ^1H NMR of lipid-*block*-poly(L-Ser-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) with DP 25 (solvent, CDCl_3) in accordance with various embodiments disclosed herein.

FIG. 6 shows ^1H NMR of lipid-*block*-poly(L-Ser-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) with DP 30 (solvent, CDCl_3) in accordance with various
30 embodiments disclosed herein.

FIG. 7 shows ^1H NMR of lipid-*block*-poly(L-Ser-D-Mannose) with DP 25 (solvent, DMSO-d_6) in accordance with various embodiments disclosed herein.

FIG. 8 shows ^1H NMR of lipid-*block*-poly(L-Ser-D-Mannose) with DP 30
5 (solvent, DMSO-d_6) in accordance with various embodiments disclosed herein.

FIG. 9 shows ^1H NMR of Cbz-ethanolamine (solvent, CDCl_3) in accordance with various embodiments disclosed herein.

10 FIG. 10 shows ^1H NMR of Cbz-ethanolamine-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose (solvent, CDCl_3) in accordance with various embodiments disclosed herein.

FIG. 11 shows ^1H NMR of ethanolamine-1,2,3,4,6-penta-O-acetyl-manno-D-
15 D-pyranose (solvent, CD_2Cl_2) in accordance with various embodiments disclosed herein.

FIG. 12 shows ^1H NMR of Boc-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-otBu (solvent, CDCl_3) in accordance with various embodiments
20 disclosed herein.

FIG. 13 shows ^1H NMR of L-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA (solvent, CDCl_3) in accordance with various embodiments
25 disclosed herein.

FIG. 14 shows ^{13}C NMR of L-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA (solvent, CDCl_3) in accordance with various embodiments disclosed herein.

30 FIG. 15 shows ^1H NMR of lipid-*block*-poly(L-Glu-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) with DP 30 (solvent, CDCl_3) in accordance with various embodiments disclosed herein.

FIG. 16 shows ^1H NMR of lipid-*block*-poly(L-Glu-D-Mannose) with DP 30 (solvent, DMSO- d_6) in accordance with various embodiments disclosed herein.

FIG. 17 shows ^1H NMR of lipid-*block*-poly(D-Ser-D-Mannose) with DP 15 (solvent, DMSO- d_6) in accordance with various embodiments disclosed herein.

FIG. 18 shows ^1H NMR of lipid-*block*-poly(D, L-Ser-D-Mannose) with DP 15 (solvent, DMSO- d_6) in accordance with various embodiments disclosed herein.

FIG. 19 shows cell viability and mRNA translation efficiency of mRNA LNPs in HeLa cells after 48 h of incubation with mRNA LNPs designed in accordance with various embodiments disclosed herein. Statistical significance was determined using the Mann – Whitney test and comparing ALC-0159 to the other LPSM or LPGM formulations at mole ratio of 1.2% (* $p < 0.05$, ** $p < 0.01$).

FIG. 20 shows cell viability and mRNA translation efficiency of mRNA LNPs in HeLa cells after 48 h of incubation with formulated mRNA LNPs designed in accordance with various embodiments disclosed herein. Statistical significance was determined using the Mann – Whitney test and comparing ALC-0159 to the other LPSM or LPGM formulations at mole ratio of 1.4% (* $p < 0.05$, ** $p < 0.01$).

FIG. 21 shows cell viability and mRNA translation efficiency of mRNA LNPs in HeLa cells after 48 h of incubation with formulated mRNA LNPs designed in accordance with various embodiments disclosed herein. Statistical significance was determined using the Mann – Whitney test and comparing ALC-0159 to the other LPSM or LPGM formulations at mole ratio of 1.6% (** $p < 0.01$).

FIG. 22 shows cell viability and mRNA translation efficiency of mRNA LNPs in RAW264.7 cells after 48 h of incubation with formulated mRNA LNPs designed in accordance with various embodiments disclosed herein. Statistical significance was determined using the Mann – Whitney test and comparing ALC-

0159 to the other LPSM or LPGM formulations at mole ratio of 1.2% (* $p < 0.05$, ** $p < 0.01$).

FIG. 23 shows cell viability and mRNA translation efficiency of mRNA LNP
5 LNP in RAW264.7 cells after 48 h of incubation with formulated mRNA LNPs designed in accordance with various embodiments disclosed herein. Statistical significance was determined using the Mann – Whitney test and comparing ALC-0159 to the other LPSM or LPGM formulations at mole ratio of 1.4% (* $p < 0.05$, ** $p < 0.01$).

10

FIG. 24 shows cell viability and mRNA translation efficiency of mRNA LNP
LNPs in RAW264.7 cells after 48 h of incubation with formulated mRNA LNPs designed in accordance with various embodiments disclosed herein. Statistical significance was determined using the Mann – Whitney test and comparing ALC-
15 0159 to the other LPSM or LPGM formulations at mole ratio of 1.6% (* $p < 0.05$, ** $p < 0.01$).

EXAMPLES

20 Example embodiments of the disclosure will be better understood and readily apparent to one of ordinary skill in the art from the following examples, tables and if applicable, in conjunction with the figures. It should be appreciated that other modifications related to structural, and/or chemical changes may be made without deviating from the scope of the invention. Example embodiments
25 are not necessarily mutually exclusive as some may be combined with one or more embodiments to form new example embodiments. The example embodiments should not be construed as limiting the scope of the disclosure.

The following examples describe the development of a series of lipid-
30 *block*-carbohydrate functionalized polypeptides that are useful for delivery of a therapeutic and/or prophylactic agent and/or biological agent (e.g., a nucleic acid such as mRNA, siRNA, miRNA, ASOs, pDNA etc). Advantageously,

embodiments of the lipid-*block*-carbohydrate functionalized polypeptides disclosed herein are designed to replace/substitute conventional PEG-lipid conjugate such as ALC-0159. In the following examples, the polypeptide block in the lipid-*block*-carbohydrate functionalized polypeptides is functionalized with mannose groups, forming a series of lipid-*block*-mannopolypeptides. Mannose groups are designed for targeting mannose receptors on immune cell surface to enhance vaccination efficacy. Advantageously, mRNA LNPs formulated from lipid-*block*-mannopolyserine (LPSM) and lipid-*block*-mannopolyglutamic acid (LPGM) designed in accordance with various embodiments disclosed herein are nanosized with a narrow size distribution ($PDI < 0.2$) and high encapsulation efficiencies. Even more advantageously, translation efficiencies of mRNA LNPs formulated from LPSMs and LPGMs designed in accordance with various embodiments disclosed herein are significantly higher than those of mRNA LNPs of the art that are formulated with a conventional PEG-lipid conjugate ALC-0159 (e.g., such as that currently used in Pfizer/BioNTech's mRNA LNP covid19 vaccine) especially in immune cells, without causing cytotoxicity. The present application also demonstrated that the LPSM LNPs mediated more than about 15 times higher mRNA expression than conventional ALC-0159 LNPs. As shown in the following examples, the LPSMs and LPGMs of the present application can serve as a viable replacement for ALC-0159 in the delivery of mRNA, RNA and other genes, potentially reducing the risk of allergic reaction by the PEG-conjugated lipids, prolonging the plasma half-life of mRNA LNPs and enhancing vaccination efficacy.

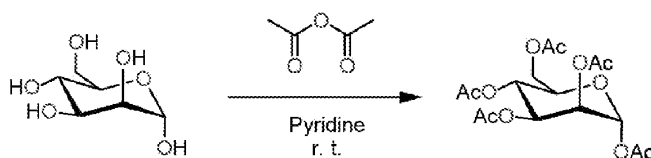
Advantageously, the present application has also surprisingly demonstrated that it is possible to cyclize protected mannose-functionalized serine to form NCA (N-carboxy anhydride) and to further ring-open and polymerize protected mannose-functionalized NCA due to the bulky structure of the protected mannose group. Without being bound by theory, it is believed that the successful cyclization (NCA formation) and polymerization is due to the choice of L-serine and D-mannose, which avoids steric hindrance.

Example 1: Materials and Methods**1.1. Materials**

Chemical reagents for the synthesis of the PEG-free lipids were purchased from Sigma-Aldrich and used as received unless otherwise noted. 1,2-Distearoyl-sn-glycerol-3-phosphocholine (DSPC), cholesterol and ALC-0315 were purchased from MedChemExpress (Monmouth Junction, NJ, USA). Sodium acetate was purchased from Sigma-Aldrich (St. Louis, MO, USA). Triton®-X100, Tris-EDTA, and VivoGlo Luciferin (In Vivo Grade) were purchased from Promega (Madison, WI, USA). Alamar Blue and Pierce Firefly Luciferase Glow assay kit were purchased from Invitrogen (Waltham, MA, USA). Other reagents used were of analytical grade.

1.2. Synthesis of 1,2,3,4,6-penta-O-acetyl-manno-D-pyranose

Synthesis strategy for 1,2,3,4,6-penta-O-acetyl-manno-D-pyranose was showed in **Scheme 1**. D-Mannose (30.6 g, 170 mmol) was dissolved in 150 mL of pyridine under N₂. To the solution was added dropwise acetic anhydride (160 mL, 1.7 mol) at 0°C. The reaction mixture was stirred for 24 h at room temperature. The reaction solution was slowly poured into 1000 mL of ice water and then extracted with 300 mL of ethyl acetate for 3 times. The organic phase was washed with 300 mL of saturated NaHCO₃ for 2 times, and then washed with 1.0 M HCl, saturated brine. The organic phase was dried over anhydrous Na₂SO₄ and the organic solvent was evaporated in vacuo. The resulting crude product was purified by flash silica gel column chromatography (Hexane/ ethyl acetate 8:2, v/v). The yield of the compound was 90.3%. The structure of the product was verified by ¹H NMR spectrum (FIG. 1).



Scheme 1. Synthesis strategy for 1,2,3,4,6-penta-O-acetyl-manno-D-pyranose

1.3. Synthesis of L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA

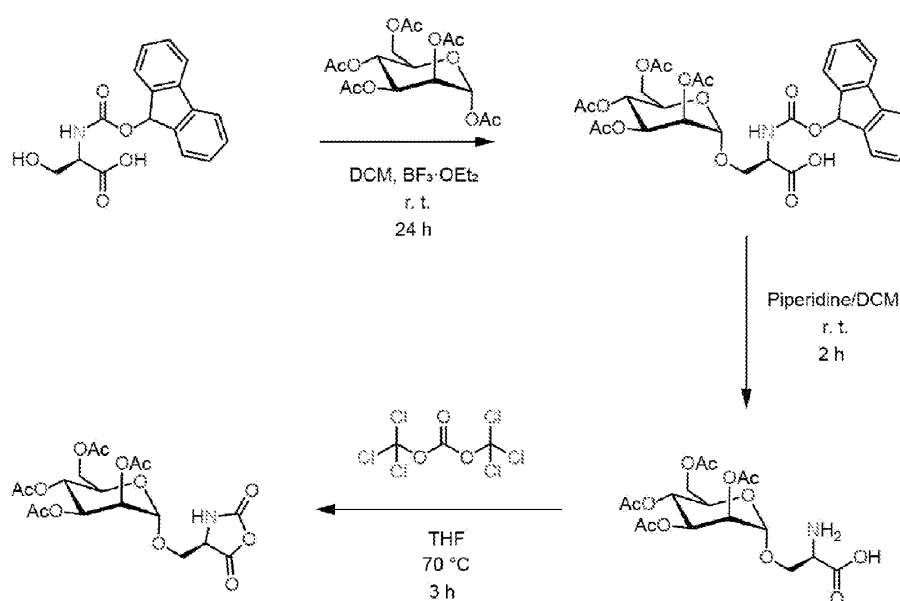
Synthesis strategy for the L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) N-carboxy anhydride (NCA) was showed in **Scheme 2**. Fmoc-L-Ser(1,2,3,4,6-Penta-O-acetyl-manno-D-pyranose)-OH was first synthesized.

- 5 General procedure for Fmoc-L-Ser(1,2,3,4,6-Penta-O-acetyl-manno-D-pyranose)-OH: To a mixture of Fmoc-L-Serine (9.0 g, 27.5 mmol) and 1,2,3,4,6-Penta-O-acetyl-manno-D-pyranose (14.2 g, 36.4 mmol) in 250 mL of CH₂Cl₂ (DCM) was dropwise added 26.5 mL of BF₃·OEt₂, and the reaction mixture was stirred under N₂ for 24 h at room temperature. The solvent was evaporated in
10 vacuo. The resulting residue was dissolved in 400 mL of ethyl acetate, washed with saturated brine, dried over anhydrous Na₂SO₄, and concentrated in vacuo. The obtained crude product was purified by flash silica gel column chromatography (DCM/MeOH 30:1, v/v) to give Fmoc-L-Ser(1,2,3,4,6-Penta-O-acetyl-manno-D-pyranose)-OH (11.2 g, 63%). The structure of product was
15 verified by ¹H NMR spectrum (FIG. 2).

The Fmoc group deprotection of Fmoc-L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-OH was then performed to yield L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-OH. General procedure for L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-OH: To Fmoc-L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-OH (6.43 g, 10 mmol) was added 20% piperidine /DCM (100
20 mL), and the mixture was stirred for 2 h at room temperature. The solvent was evaporated in vacuo. The resulting crude product was purified by flash silica gel column chromatography (DCM/MeOH 20:1, v/v) to give L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-OH (4.0 g, 92%).

- 25 L-Ser(1,2,3,4,6-Penta-O-acetyl-manno-D-pyranose)-NCA was finally synthesized. General procedure for L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA: L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-OH (4.0 g, 9.2 mmol) was suspended in 80 mL of dry tetrahydrofuran (THF) and then triphosgene (2.0 g) was added under N₂. The mixture was stirred at 70 °C under
30 a flow of N₂ for 3 h. After the reaction mixture was cooled down to room temperature, the crude product was precipitated by pouring the mixture solution

into iced hexane (600 ml), collected by filtration. The resulting crude product was purified by recrystallizing with THF/hexane mixture for three times. The yield of L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA was 58%. The structure of L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA was verified by ^1H NMR and ^{13}C NMR spectra (FIG. 3 and FIG. 4).



Scheme 2. Synthesis strategy for the L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA

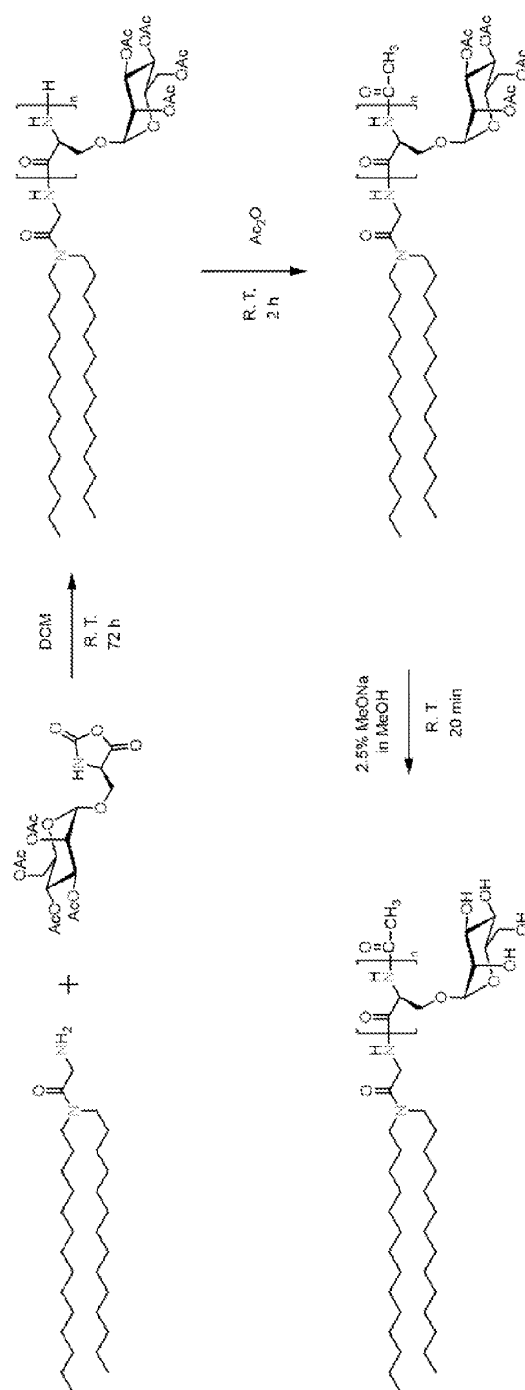
1.4. Synthesis of lipid-*block*-poly(L-Ser-D-Mannose)

Synthesis strategy for lipid-*block*-poly(L-Ser-D-Mannose) was showed in **Scheme 3**. Lipid-*block*-poly(L-Ser-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) was first synthesized. General synthetic method for lipid-*block*-poly(L-Ser-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose): In the glove box, 2-amino-N,N-ditetradecylacetamide (23.3 mg, 0.05 mmol) and L-Ser(1,2,3,4,6-Penta-O-acetyl-manno-D-pyranose)-NCA (922 mg, 2.0 mmol) were dissolved in 40 mL of anhydrous DCM. The mixture was stirred for 72 h at room temperature in the

glove box. Then, 3.0 mL of acetic anhydride was added and the reaction was continued for 2 h. The crude product was precipitated by pouring the mixture solution into glacial ether (300 mL), collected by centrifugation. The resulting crude product was purified by dissolving with DCM and precipitated by pouring
5 the solution into glacial ether. The resulting product was dried under vacuum. The number of polymerization unit for lipid-*block*-poly(L-Ser-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) can be adjusted by varying the amount of L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA. The yield of the protected lipid-polypeptide was 70%. The typical structure of lipid-*block*-poly(L-Ser-1,2,3,4,6-
10 penta-O-acetyl-manno-D-pyranose) was verified by ¹H NMR (FIG. 5 and FIG. 6).

The deprotection of lipid-*block*-poly(L-Ser-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) was then performed to yield lipid-*block*-poly(L-Ser-D-mannose) (LPSM). General procedure for lipid-*block*-poly(L-Ser-D-mannose):
15 300 mg of lipid-*block*-poly(L-Ser-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) was dissolved in 30 mL of MeOH, to which was added 3 mL of 25% MeONa in MeOH. The mixture was stirred in room temperature for 20 min. The crude product was purified by dialysis with deionized water for removing MeONa and MeOH. The product was obtained by freeze-drying under vacuum. The yield of
20 the deprotected lipid-*block*-poly(L-Ser-D-mannose) was 98%. The typical structure of lipid-*block*-poly(L-Ser-D-mannose) was verified by ¹H NMR (FIG. 7 and FIG. 8).

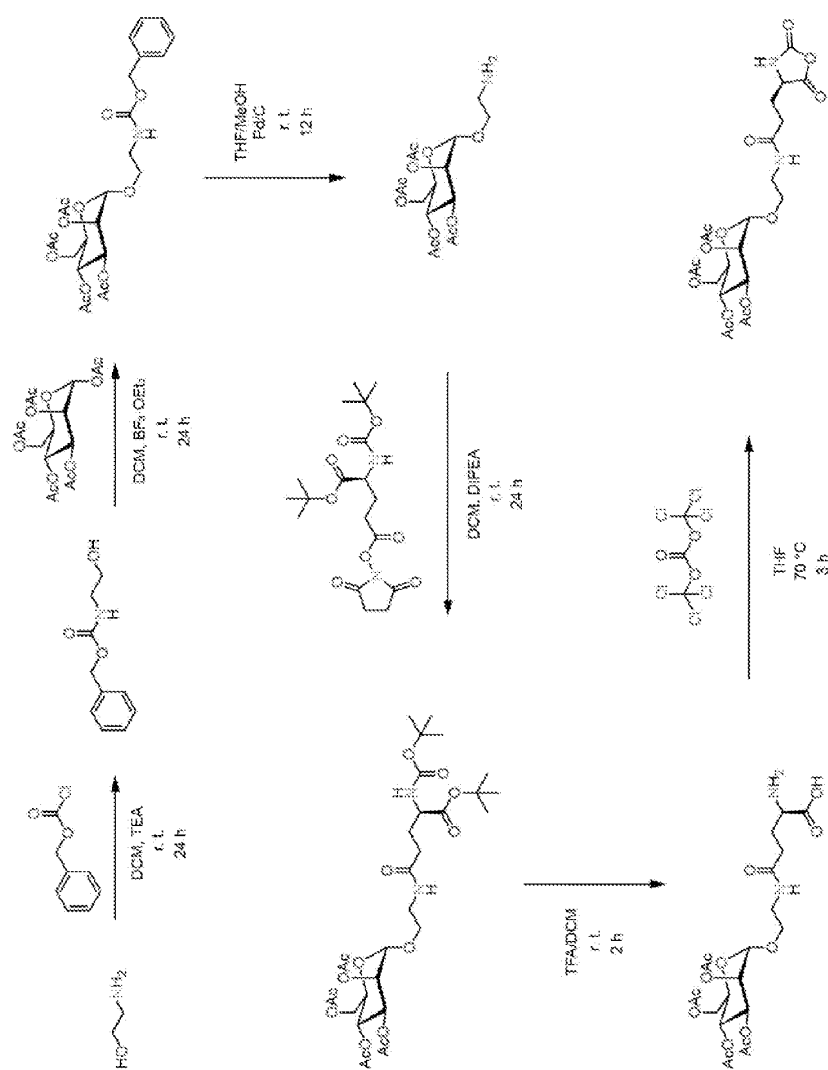
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**Scheme 3.** Synthesis strategy for lipid-block-poly(L-Ser-D-Mannose).

1.5. Synthesis of L-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA

Synthesis strategy for L-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA was showed in **Scheme 4**. Benzyl (2-hydroxyethyl)carbamate (Cbz-ethanolamine) was first synthesized. General procedure for Cbz-ethanolamine: To a mixture of ethanolamine (10.0 g, 163.5 mmol) and triethylamine (TEA, 27.5 mL, 197.5 mmol) in 250 mL of dry DCM was added 28 mL of benzyl carbonochloridate in ice bath, and the reaction mixture was stirred for 24 h at room temperature. The solvent was evaporated in vacuo. The resulting residue was dissolved in 400 mL of DCM, washed with saturated NaHCO₃, saturated brine, dried over anhydrous Na₂SO₄, and concentrated in vacuo. The obtained crude product was purified by flash silica gel column chromatography (DCM/MeOH 30:1, v/v) to give Cbz-ethanolamine (30.3 g, 95%). The structure of product was verified by ¹H NMR spectrum (FIG. 9).

Cbz-ethanolamine-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose was then synthesized. General procedure for Cbz-ethanolamine-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose: To a mixture of Cbz-ethanolamine (5.36 g, 27.5 mmol) and 1,2,3,4,6-penta-O-acetyl-manno-D-pyranose (14.2 g, 36.4 mmol) in 200 mL of dry DCM was dropwise added 26.5 mL of BF₃·OEt₂, and the reaction mixture was stirred under N₂ for 24 h at room temperature. The solvent was evaporated in vacuo. The resulting residue was dissolved in 400 mL of ethyl acetate, washed with saturated brine, dried over anhydrous Na₂SO₄, and concentrated in vacuo. The obtained crude product was purified by flash silica gel column chromatography (DCM/MeOH 30:1, v/v) to give Cbz-ethanolamine-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose (9.4 g, 65%). The structure of product was verified by ¹H NMR spectrum (FIG. 10).



Scheme 4. Synthesis strategy for L-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA.

The Cbz group deprotection of Cbz-ethanolamine-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose was then performed to yield ethanolamine-1,2,3,4,6-Penta-O-acetyl-manno-D-pyranose (this compound may also be purchased from a commercial company). General procedure for ethanolamine-1,2,3,4,6-Penta-O-acetyl-manno-D-pyranose: Cbz-ethanolamine-1,2,3,4,6-Penta-O-acetyl-manno-D-pyranose (7.9 g, 15 mmol) was dissolved in 40 mL of a 1:1 mixture of THF and MeOH. Pd/C 10w% (790 mg) was added into mixture under rigorous stirring. The reaction mixture was stirred under 7 mbar hydrogen atmosphere for 12 h. The resulting crude product was purified by flash silica gel column chromatography (DCM/MeOH 30:1, v/v) to give ethanolamine-1,2,3,4,6-Penta-O-acetyl-manno-D-pyranose (5.5 g, 95%). The structure of product was verified by ¹H NMR spectrum (FIG. 11).

Boc-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-otBu was further synthesized. General procedure for Boc-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-otBu: To a mixture of ethanolamine-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose (4.7 g, 12 mmol) and Boc-Glu(oSu)-otBu (4.8 g, 12 mmol) in 150 mL of dry DCM was added 5.0 mL of N,N-diisopropylethylamine (DIPEA), and the reaction mixture was stirred under N₂ for 24 h at room temperature. The solvent was evaporated in vacuo. The resulting crude product was purified by flash silica gel column chromatography (DCM/MeOH 30:1, v/v) to give Boc-Glu(1,2,3,4,6-Penta-O-acetyl-manno-D-pyranose)-otBu (7.3 g, 90%). The structure of product was verified by ¹H NMR spectrum (FIG. 12).

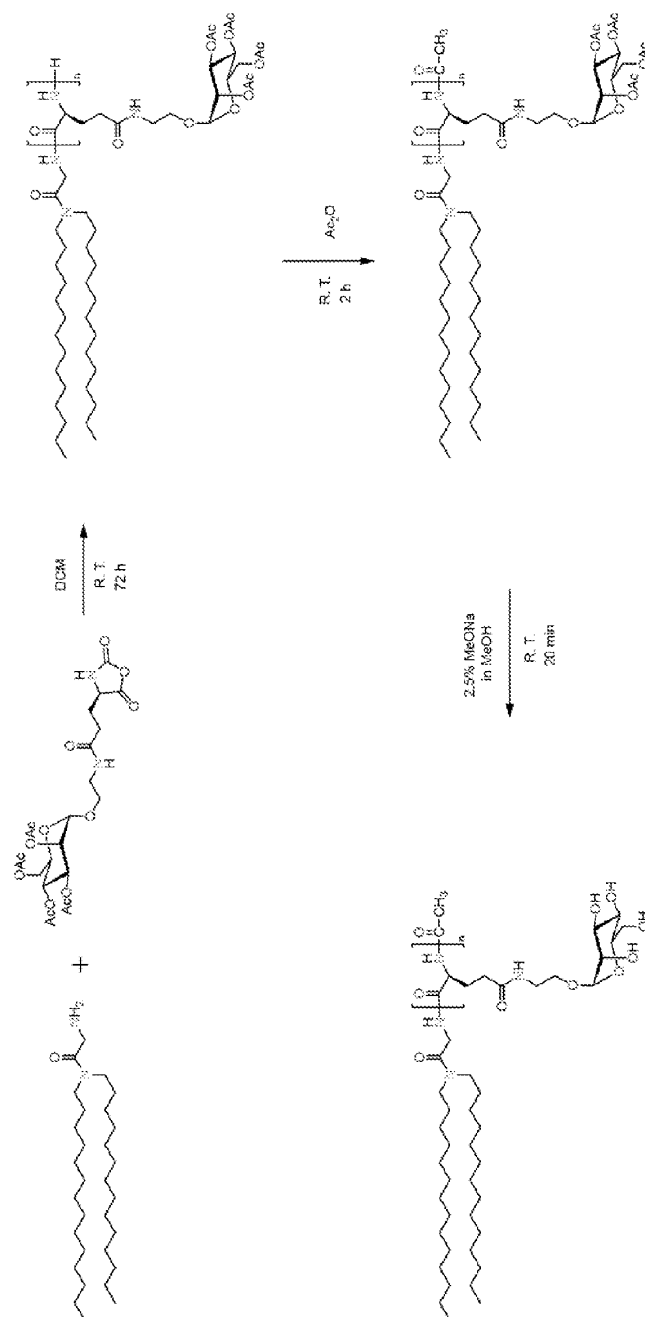
The deprotection of Boc-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-otBu for Boc and tBu groups was performed to yield L-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-OH. General procedure for L-Glu(1,2,3,4,6-Penta-O-acetyl-manno-D-pyranose)-OH: To Boc-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-otBu (6.76 g, 10 mmol) was added 30% TFA/DCM (100 mL), and the mixture was stirred for 2 h at room temperature. The solvent was evaporated in vacuo. The resulting crude product was purified by flash silica gel

column chromatography (DCM/MeOH 20:1, v/v) to give L-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-OH (4.9 g, 95%).

L-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA was finally
5 synthesized. General procedure for L-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA: L-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-OH (4.16 g, 8.0 mmol) was suspended in 80 mL of dry tetrahydrofuran (THF) and then triphosgene (2.0 g) was added under N₂. The mixture was stirred at 70 °C under a flow of N₂ for 3 h. After the reaction mixture was cooled down to room
10 temperature, the crude product was precipitated by pouring the mixture solution into iced hexane (600 ml), collected by filtration. The resulting crude product was purified by recrystallizing with THF/hexane mixture for three times. The yield of L-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA was 55%. The structure of L-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA was
15 verified by ¹H NMR and ¹³C NMR spectra (FIG. 13 and FIG. 14).

1.6. Synthesis of lipid-*block*-poly(L-Glu-D-Mannose)

Synthesis strategy for lipid-*block*-poly(L-Glu-D-Mannose) was showed in
Scheme 5. Lipid- *block*-poly(L-Glu-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)
20 was first synthesized. General synthetic method for lipid-*block*-poly(L-Glu-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose): In the glove box, 2-amino-N, N-ditetradecylacetamide (23.3 mg, 0.05 mmol) and L-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA (1365 mg, 2.5 mmol) were dissolved in 40 mL of anhydrous DCM. The mixture was stirred for 72 h at room temperature in the
25 glove box. Then, 3.0 mL of acetic anhydride was added and the reaction was continued for 2 h. The crude product was precipitated by pouring the mixture solution into glacial ether (300 mL), collected by centrifugation. The resulting crude product was purified by dissolving with DCM and precipitated by pouring the solution into glacial ether. The resulting product was dried under vacuum. The
30 yield of the protected polymer was 65%. The typical structure of lipid-*block*-poly(L-Glu-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) was verified by ¹H NMR (FIG. 15).

**Scheme 5.** Synthesis strategy for lipid-block-poly(L-Glu-D-Mannose).

The deprotection of lipid-*block*-poly(L-Glu-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) was then performed to yield lipid-*block*-poly(L-Glu-D-mannose) (LPGM). General synthetic method for LPGM: 250 mg of lipid-*block*-poly(L-Glu-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) was dissolved in 25 mL of MeOH, to which was added 2.8 mL of 25% MeONa in MeOH. The mixture was stirred in room temperature for 20 min. The crude product was purified by dialysis with deionized water for removing MeONa and MeOH. The product was obtained by freeze-drying under vacuum. The yield of the deprotected product (LPGM) was 98%. The typical structure of LPGM was verified by ¹H NMR (FIG. 16).

1.7. Synthesis of lipid-*block*-poly(D-Ser-D-Mannose)

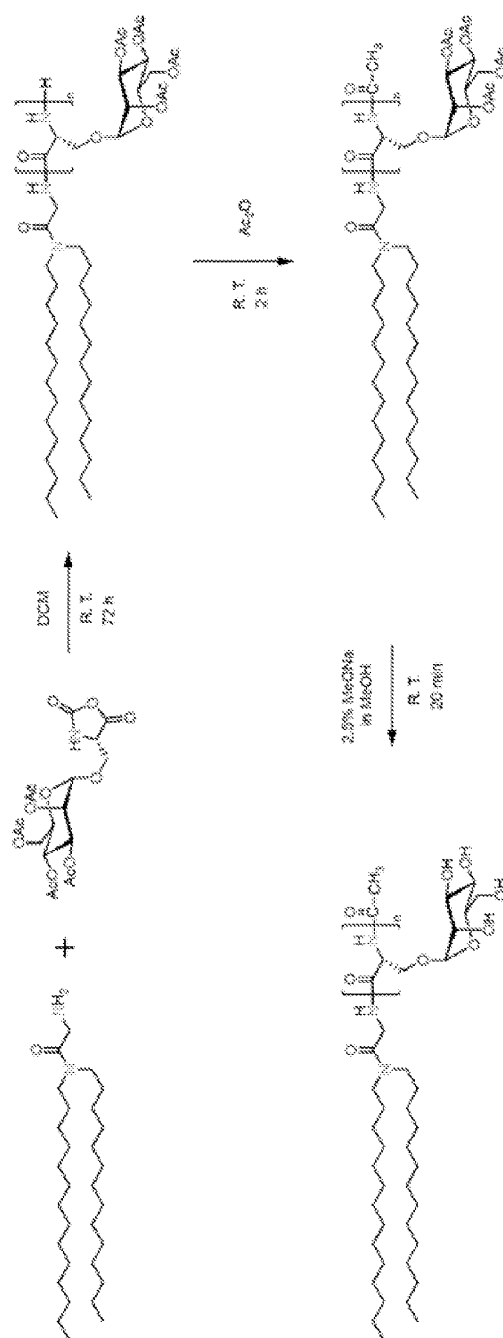
Synthesis strategy for lipid-*block*-poly(D-Ser-D-Mannose) is shown in **Scheme 6**. In the glove box, 2-amino-N, N-ditetradecylacetamide (46.6 mg, 0.1 mmol) and D-Ser(1,2,3,4,6-Penta-O-acetyl-manno-D-pyranose)-NCA (922 mg, 2.0 mmol) were dissolved in 20 mL of anhydrous DCM. The mixture was stirred for 72 h at room temperature in the glove box. Then, 3.0 mL of acetic anhydride was added and the reaction was continued for 2 h. The crude product was precipitated by pouring the mixture solution into glacial ether (300 mL), collected by centrifugation. The resulting crude product was purified by dissolving with DCM and precipitated by pouring the solution into glacial ether. The resulting product was dried under vacuum. The number of polymerization unit for lipid-*block*-poly(D-Ser-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) can be adjusted by varying the amount of D-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA. The yield of the protected lipid-polypeptide was 62%. The deprotection of lipid-*block*-poly(D-Ser-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) was then performed to yield lipid-*block*-poly(D-Ser-D-mannose) (LPSM-D). General procedure for lipid-*block*-poly(D-Ser-D-mannose): 300 mg of lipid-*block*-poly(D-Ser-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) was dissolved in 30 mL of MeOH, to which was added 3 mL of 25% MeONa in MeOH. The mixture was stirred in room temperature for 20 min. The crude product was purified by dialysis with deionized water for removing MeONa and MeOH. The product was obtained by freeze-drying under vacuum. The yield of the deprotected lipid-*block*-poly(D-

Ser-D-mannose) was 95%. The typical structure of lipid-*block*-poly(D-Ser-D-mannose) was verified by ^1H NMR (FIG. 17).

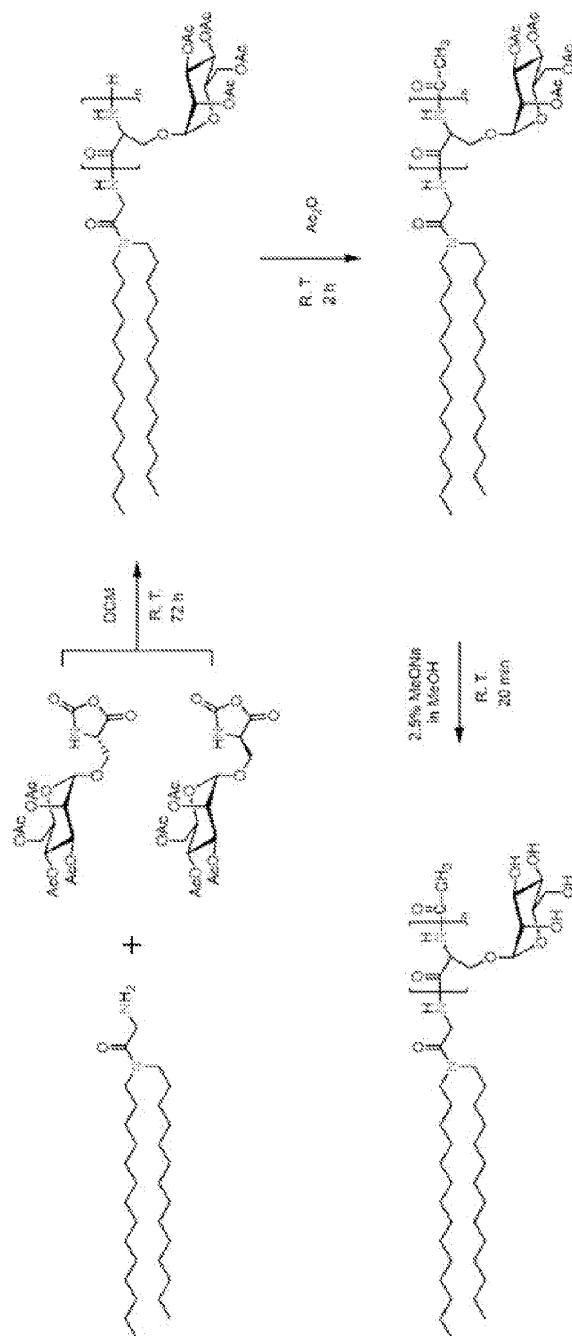
1.8. Synthesis of lipid-*block*-poly(D, L-Ser-D-Mannose)

5 Synthesis strategy for lipid-*block*-poly(D, L-Ser-D-Mannose) is shown in **Scheme 7**. In the glove box, 2-amino-N, N-ditetradecylacetamide (46.6 mg, 0.1 mmol), D-Ser(1,2,3,4,6-Penta-O-acetyl-manno-D-pyranose)-NCA (466 mg, 1.0 mmol) and L-Ser(1,2,3,4,6-Penta-O-acetyl-manno-D-pyranose)-NCA (466 mg, 1.0 mmol) were dissolved in 20 mL of anhydrous DCM. The mixture was stirred
10 for 72 h at room temperature in the glove box. Then, 3.0 mL of acetic anhydride was added and the reaction was continued for 2 h. The crude product was precipitated by pouring the mixture solution into glacial ether (300 mL), collected by centrifugation. The resulting crude product was purified by dissolving with DCM and precipitated by pouring the solution into glacial ether. The resulting
15 product was dried under vacuum. The number of polymerization unit for lipid-*block*-poly(D, L-Ser-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) can be adjusted by varying the amount of D, L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA. The yield of the protected lipid-polypeptide was 72%. The deprotection of lipid-*block*-poly(D, L-Ser-1,2,3,4,6-penta-O-acetyl-manno-D-
20 pyranose) was then performed to yield lipid-*block*-poly(L-Ser-D-mannose) (LPSM-DL). General procedure for lipid-*block*-poly(D, L-Ser-D-mannose): 300 mg of lipid-*block*-poly(D, L-Ser-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) was dissolved in 30 mL of MeOH, to which was added 3 mL of 25% MeONa in MeOH. The mixture was stirred in room temperature for 20 min. The crude
25 product was purified by dialysis with deionized water for removing MeONa and MeOH. The product was obtained by freeze-drying under vacuum. The yield of the deprotected lipid-*block*-poly(D, L-Ser-D-mannose) was 92%. The typical structure of lipid-*block*-poly(D, L-Ser-D-mannose) was verified by ^1H NMR (FIG. 18).

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**Scheme 6.** Synthesis strategy for lipid-block-poly(D-Ser-D-Mannose).

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**Scheme 7.** Synthesis strategy for lipid-block-poly(D, L-Ser-D-Mannose).

1.9. Formulation of mRNA-loaded lipid nanoparticles (mRNA LNPs)

To conduct a high-throughput screening of the varying LPSM compounds at different mole ratios, LNPs were manually formulated as according to **Tables 1, 2**. For each formulation, the amount of mRNA used was standardized at a dose of 5 µg. Furthermore, the N/P ratio between the ionizable lipids and mRNA content was standardized at a ratio of 6:1.

To replace the lipid-PEG conjugate (ALC-0159) present in mRNA LNPs, LPSM were synthesized with different LPSM subunit lengths. LPSM was mixed with ALC-0315, a helper lipid (DSPC), and cholesterol at different mole contents to optimize mRNA LNP formulation. The difference between the mole ratio of LPSM and ALC-0159 (1.6%) was distributed relative to the mole contents of the other lipids, respectively.

The formulation of mRNA LNPs involved two distinct phases, the organic phase and the aqueous phase. The organic phase contained the mixture of lipids dissolved in ethanol to reach a final volume of 25 µL. The aqueous phase contained 5 µL of 1 mg/mL firefly luciferase mRNA (TriLink Biotechnologies) diluted in 70 µL of 10 mM sodium acetate solution at pH 4 to reach a final volume of 75 µL. The organic phase was added to the aqueous phase and mixed thoroughly through rapid pipetting. The mRNA-lipid mixture was then allowed to incubate at room temperature for at least 30 min to provide time for encapsulation and self-assembly of LNPs.

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Table 1. Mole ratio (content) of lipids used in the formulations.

Lipid	ALC-0315			ALC-0159	LPSM/LPGM
	/LPSM /LPGM	DSPC	Cholesterol		
Molecular weight (g/mol)	766.3	790.2	386.7	2481.0	Refer to Table 2
Mole ratio – ALC-0159 (%)	46.3	9.4	42.7	1.6	–
Mole ratio – LPSM/LPGM (%)	46.5	9.4	42.9		1.2%
	46.4	9.4	42.8	-	1.4%
	46.3	9.4	42.7		1.6%
Stock concentration (mg/mL)	20.0	10.0	10.0	3.0	10.0

Table 2. Characteristics of lipid-*block*-poly(L-Ser-D-Mannose) (LPSM) and lipid-*block*-poly(L-Glu-D-Mannose) (LPGM).

Lipid- <i>block</i> -polypeptide(D-mannose)	Number of repeat units (n)	Lipid-polypeptide end-group	M_n^{NMR} [g mol ⁻¹]
LPSM01	25	-CO-CH ₃	6739.4
LPSM02	30	-CO-CH ₃	7985.5
LPSM03	35	-CO-CH ₃	9231.5
LPGM01	30	-CO-CH ₃	10538.6

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1.10. Assessing encapsulation efficiency of mRNA in LNPs

To determine the encapsulation efficiency of the mRNA LNPs after formulation, Quant-it™ RiboGreen RNA Assay Kit (Invitrogen, Waltham, MA, USA) was used to elucidate the mRNA concentration of the mRNA LNP mixture in solutions with or without Triton-X100. The Ribogreen reagent was diluted 200 times with either Tris-EDTA buffer containing 5% Triton-X100 or only Tris-EDTA buffer. The buffer solution containing the diluted Ribogreen reagent was aliquoted at 90 μL into each well of a black 96-well plate and mixed with 10 μL of mRNA

LNP sample. The mixtures were then incubated at 37°C for 20 min to provide time for the emulsification of mRNA LNPs and the stabilization of the signal. After incubation, the fluorescence intensity of the samples were determined using a microplate reader (Tecan, Männedorf, Switzerland) at an excitation wavelength of 485 nm and an emission wavelength of 520 nm. The values obtained were then used to determine the encapsulation efficiency of the various mRNA LNP formulations through the following equation.

$$\text{Encapsulation Efficiency} = \frac{\text{Conc}_{\text{Trit}} - \text{Conc}_{\text{TE}}}{\text{Conc}_{\text{Trit}}} \times 100$$

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where $\text{Conc}_{\text{Trit}}$ is the concentration of mRNA obtained by adding the mRNA LNPs to 5% Triton-X100 diluted in Tris-EDTA buffer, while Conc_{TE} is the concentration of the respective mRNA obtained by adding the mRNA LNPs to Tris-EDTA buffer without Triton-X100.

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1.11. Characterization of mRNA LNPs

The size, polydispersity index (PDI), and zeta potential of the mRNA LNPs were also characterized using a Zetasizer (Malvern, UK). The size and PDI were obtained through dynamic light scattering (DLS) by diluting 25 µL of the mRNA LNPs sample with saline solution to achieve a final volume of 500 µL. The sample was measured three times at 25°C with 20 runs each time. The sample was also measured at 1.68 s per run. The surface zeta potential of the mRNA LNPs was measured by diluting 25 µL of the sample with saline solution to reach a final volume of 1 mL. The samples were also measured three times at 25°C with 20 runs each time when the samples were measured.

1.12. Cell culturing and dosing of HeLa and RAW264.7 cells with mRNA LNPs

To test the cytotoxicity and transfection efficiency of the mRNA LNPs, HeLa and RAW264.7 cells were cultured and dosed with the mRNA LNPs. The HeLa cell line was cultured in DMEM media containing 10% Fetal Bovine Serum (FBS) (v/v) and 1% Penicillin/Streptomycin (v/v). The cells were allowed to

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incubate at 37°C with 5% CO₂ in an incubator (Thermo Fisher, Waltham, MA, USA). The cells were then seeded at 10,000 cells per well in a white 96-well plate for testing mRNA transfection efficiency and in a black 96-well plate for cytotoxicity evaluation of mRNA LNPs. The amount of mRNA LNPs added to each well was standardized to a dose of 100 ng of mRNA per well. After dosing, the 96-well plates were incubated for 48 hours before assessing cell viability and transfection efficiency.

1.13. In vitro viability of HeLa and RAW264.7 cells after incubation with mRNA LNPs

After the 48 hours of incubation, old media consisting of the mRNA LNPs was removed from the wells of the black 96-well plate. Alamar Blue reagent was then diluted 10x with fresh DMEM media and 100 µL of the diluted Alamar Blue reagent was added to each well of the plate. The samples were then incubated at 37°C for 2 hours to allow reduction of the Alamar Blue compound by live cells and stabilization of the signal. Fluorescence intensity was measured using the microplate reader (Tecan, Männedorf, Switzerland) at an excitation wavelength of 570 nm and an emission wavelength of 600 nm. The *in vitro* cell viability was then taken as a percentage relative to the negative control wells that did not receive any treatment.

1.14. In vitro transfection efficiency of mRNA LNPs in HeLa and RAW264.7 cells after incubation with mRNA LNPs

The transfection efficiency of mRNA LNPs in HeLa and RAW264.7 cells was measured after 48 hours using the Firefly Assay kit. Old media was removed from the white 96-well plates and replaced with 100 µL of Firefly Assay mixture. The mixture contained 50 µL of 2x cell lysis buffer diluted in phosphate-buffered saline (PBS) and 50 µL of 100x D-luciferin diluted in Firefly Assay Buffer. The samples were then incubated at 37°C for 10 min to allow for cell lysis and signal stabilization. Luminescence intensity was read using the microplate reader (Tecan, Männedorf, Switzerland) at an exposure time of 1000 ms.

Example 2: Synthesis and characterization of LPSM and LPGM

LPSM was synthesized *via* the ring-opening polymerization (ROP) of L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-N-carboxyanhydride (**Scheme 2**) using the lipid of 2-amino-N, N-ditetradecylacetamide as the initiator, followed by the deprotection of lipid-*block*-poly(L-Ser-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) in MeOH containing 2.5% MeONa (**Scheme 3**). The structures of lipid-*block*-poly(L-Ser-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) were confirmed by ¹H NMR spectroscopy. FIG. 5 and FIG. 6 clearly showed the peak of **n** from amide groups in polypeptide backbone at 8.33 ppm, the peaks of **f-m** and **p** from L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) units and the peak of **a** (-CH₃) from lipid at 0.85 ppm, suggested successful block of lipid and poly(L-Ser-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose). The successful synthesis of lipid-*block*-poly(L-Ser-D-Mannose) was verified by ¹H NMR spectroscopy. As shown in FIG. 7 and FIG. 8, the disappearance of peaks **p1-4** (-CO-CH₃) at δ 2.15, 2.11, 2.06 and 1.99 ppm, respectively, indicated successful deprotection of lipid-poly(L-Ser-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose). The degrees of polymerization (DPs) of L-Ser-D-Mannose in PLSM can be adjusted by varying the amount of L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-NCA. The DPs of L-Ser(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) in lipid-poly(L-Ser-1,2,3,4,6-Penta-O-acetyl-manno-D-pyranose) were determined by the integration area (peak **I** of the polypeptide; peak **a** of the lipid) (FIG. 5 and FIG. 6). The typical lipid-poly(L-Ser-D-Mannose) with different DPs including 25, 30, and 35, were synthesized and denoted as LPSM1, LPSM2 and LPSM3, respectively.

Furthermore, the lipid-*block*-poly(L-Glu-D-Mannose) (LPGM) was synthesized *via* the ROP of L-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose)-N-carboxyanhydride (**Scheme 4**) using the lipid of 2-amino-N, N-ditetradecylacetamide as the initiator, followed by the deprotection of lipid-*block*-poly(L-Glu-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) in 2.5% MeONa in MeOH (**Scheme 5**). The structure of lipid-*block*-poly(L-Glu-1,2,3,4,6-penta-O-

acetyl-manno-D-pyranose) was verified by ^1H NMR spectroscopy. FIG. 15 clearly showed the peak of **r** from amide groups in the polypeptide backbone at 8.41 ppm, the peaks of **k-p** and **t** from L-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) units and the peak of **a** ($-\text{CH}_3$) from the lipid at 0.87 ppm, suggested
5 successful synthesis of lipid-*block*-poly(L-Glu-1,2,3,4,6-Penta-O-acetyl-manno-D-pyranose). The successful synthesis of lipid-*block*-poly(L-Glu-D-Mannose) (LPGM) was also confirmed by ^1H NMR spectroscopy. As shown in FIG. 16, the disappearance of peaks **t1-4** ($-\text{CO}-\text{CH}_3$) at δ 2.16, 2.11, 2.06 and 1.99 ppm, respectively, indicated successful deprotection of lipid-*block*-poly(L-Glu-
10 1,2,3,4,6-Penta-O-acetyl-manno-D-pyranose). The DP of L-Glu(1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) in lipid-*block*-poly(L-Glu-1,2,3,4,6-penta-O-acetyl-manno-D-pyranose) was determined by the integration area (peak **o** of the polypeptide; peak **a** of the lipid) (FIG. 15). The typical lipid-*block*-poly(L-Glu-D-Mannose) with DP 30 was denoted as LPGM1.

15

Example 3: Size, size distribution (PDI), and zeta potential of mRNA LNPs

The characterization of the mRNA LNPs was performed using a Zetasizer (Malvern, UK), which would elucidate the size, PDI, and zeta potential of the
20 respective mRNA LNPs. The values are displayed in **Tables 3-5**. As observed from the results, LNPs formulated with LPSM or LPGM showed nanosize (< 200 nm) with a small polydispersity index (< 0.2) and near neutral zeta potentials ($< \pm 10$ mV). The small size and low PDI of the formulated mRNA LNPs indicate a narrow size distribution and homogenous particle population, indicating the LNPs
25 are viable for efficient cellular uptake and intracellular delivery of the mRNA payload. Furthermore, the near neutral zeta potential reduces undesirable non-specific interactions with proteins in the physiological environment and provides *in vivo* stability.

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Table 3. Characteristics of manually formulated mRNA LNPs at LPSM/LPGM mole ratio of 1.2%.

Formulation	Mole ratio (%)	Diameter (nm)	PDI	Zeta potential (mV)
LPSM01	1.2	149 ± 3	0.129 ± 0.004	1.00 ± 1.74
LPSM02		183 ± 2	0.074 ± 0.041	1.75 ± 1.21
LPSM03		155 ± 1	0.137 ± 0.010	9.32 ± 1.19
LPGM01		155 ± 2	0.158 ± 0.028	-0.32 ± 1.64

Table 4. Characteristics of manually formulated mRNA LNPs at LPSM/LPGM mole ratio of 1.4%.

Formulation	Mole ratio (%)	Diameter (nm)	PDI	Zeta potential (mV)
LPSM01	1.4	160 ± 2	0.130 ± 0.028	2.24 ± 0.83
LPSM02		182 ± 1	0.063 ± 0.020	1.97 ± 0.96
LPSM03		159 ± 6	0.183 ± 0.021	2.29 ± 2.09
LPGM01		153 ± 1	0.145 ± 0.015	-1.22 ± 1.58

Table 5. Characteristics of manually formulated mRNA LNPs at AL-0159/LPSM/LPGM mole ratio of 1.6%.

Formulation	Mole ratio (%)	Diameter (nm)	PDI	Zeta potential (mV)
ALC-0159	1.6	115 ± 1	0.143 ± 0.007	-2.39 ± 2.22
LPSM01		175 ± 3	0.151 ± 0.045	1.23 ± 0.37
LPSM02		181 ± 0	0.098 ± 0.029	2.76 ± 2.27
LPSM03		153 ± 1	0.120 ± 0.005	4.41 ± 0.42
LPGM01		153 ± 3	0.164 ± 0.016	-0.55 ± 0.53

3.3 Encapsulation efficiency of formulated mRNA LNPs

The encapsulation efficiency of the mRNA LNPs was determined through the Ribogreen Assay Kit and the values are displayed in **Tables 6-8**. With the

exception of LNPs formulated using LPGM01, the remaining LNPs demonstrated an encapsulation efficiency comparable or greater than the encapsulation efficiency of LNPs formulated using ALC-0159 that is used in Pfizer/BioNTech's mRNA Covid19 vaccine. High encapsulation efficiency of mRNA in LNPs is critical for effective mRNA delivery as it ascertains a sufficient therapeutic payload that is delivered intracellularly for mRNA expression. Taken together with the characterization of the mRNA LNPs, these results suggest that the mRNA LNPs formulated with LPSM or LPGM polypeptides are viable for *in vivo* applications.

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Table 6. Encapsulation efficiency of mRNA LNPs at mole ratio of 1.2%.

Lipid	Mole ratio (%)	Encapsulation efficiency (%)
LPSM01	1.2	75.2 ± 0.2
LPSM02		75.3 ± 0.1
LPSM03		73.5 ± 1.0
LPGM01		61.6 ± 0.4

Table 7. Encapsulation efficiency of mRNA LNPs at mole ratio of 1.4%.

Lipid	Mole ratio (%)	Encapsulation efficiency (%)
LPSM01	1.4	74.0 ± 0.0
LPSM02		73.5 ± 0.6
LPSM03		69.0 ± 0.2
LPGM01		59.6 ± 0.3

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Table 8. Encapsulation efficiencies of mRNA LNPs at mole ratio of 1.6%.

Lipid	Mole ratio (%)	Encapsulation efficiency (%)
ALC-0159	1.6	69.5 ± 0.3
LPSM01		72.6 ± 0.5
LPSM02		69.6 ± 0.8
LPSM03		75.5 ± 0.5
LPGM01		55.4 ± 0.5

Example 4: *In vitro* cytocompatibility and transfection efficiency of mRNA LNPs in HELA cells

The transfection efficiency and cytotoxicity of the mRNA LNPs formed from LPSM and LPGM were determined by dosing the formulated LNPs in HeLa cells, a cell line commonly used for *in vitro* testing. The results obtained are displayed in FIG. 19, FIG. 20 and FIG. 21. From the cytocompatibility tests using the AlamarBlue assay, all formulations show negligible cytotoxicity. Further inspection of the luminescence intensity of HeLa cells from the luciferase assay demonstrates that the formulations formed from LPSM are capable of significantly improving transfection efficiency of Fluc mRNA over ALC-0159, especially formulations consisting of LPSM01 or LPSM03. This suggests that LPSM compounds not only are capable of replacing PEG-conjugated lipid as a viable compound in producing nanosized LNPs, but also enable greater transfection efficiency of mRNA as compared to the baseline of the current commercially available mRNA LNP vaccines in the market. Interestingly, formulations containing LPGM01 did not outperform ALC-0159, indicating the possibility of glutamic acid subunits providing lower intracellular delivery of mRNA compared to serine subunits. Variations in the transfection efficiency of the varying LPSM and LPGM at different mole ratios suggest that there is a hydrophobicity – hydrophilicity balance that enables an optimal level of transfection efficiency.

Example 5: *In vitro* cytocompatibility and transfection efficiency of mRNA LNPs in RAW264.7 cells

As LPSM and LPGM are functionalized with mannose to serine or glutamic acid, the efficacy of the compounds in inducing transfection of mRNA in RAW264.7 cells was determined in order to elucidate if the LNPs formed from LPSM or LPGM would be able to mRNA transfection in RAW264.7 cells that over express mannose receptors on the surface. The cytocompatibility and transfection efficiency results of the dosing of mRNA LNPs in RAW264.7 cells

are detailed in FIG. 22, FIG. 23 and FIG. 24. Similar to HeLa cells, analysis of the AlamarBlue assay results in RAW264.7 cells reveal that the mRNA LNPs tested have negligible cytotoxicity. The Luciferase assay however shows a different trend. While the LNPs made from LPSM1-LPSM3 outperforms ALC-0159 as observed in HeLa cells, the LPSM1-3 LNPs surpass ALC-0159 to a much greater degree in RAW264.7 cells, highlighting the efficiency of mannose in the LNPs targeting and binding to mannose receptors. Interestingly, LPSM02 induces lower mRNA transfection efficiency as compared to its counterparts (LPSM01 and LPSM03) in HeLa cells, but performs much better in RAW264.7 cells. Likely the length of LPSM02 (30 subunits) better facilitates the binding of mannose to the mannose receptors on RAW264.7 cells. Similar to HeLa cells, mRNA LNPs formulated with LPSM01 show a smaller degree of transfection efficiency compared to mRNA LNPs formed from ALC-0159 even with its mannose-functionalized subunits. Glutamic acid subunits may have some influence in interfering with intracellular delivery of mRNA, which impedes mRNA transfection. The mRNA LNPs formulated with LPSM or LPSM showing varying transfection efficiency at different mole ratios may be explained with the aforementioned hydrophobicity and hydrophilicity balance.

Example 6: Summary

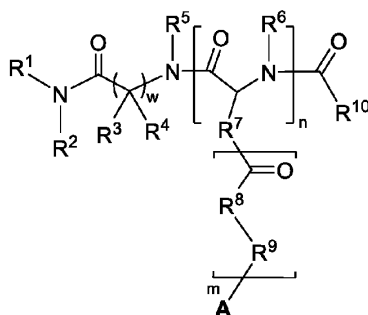
Lipid-block-mannopolypeptides LPSM and LPSM are successfully made. mRNA LNPs formulated from LPSM or LPSM have nanosize (< 200 nm), homogeneous particle population ($PDI < 0.2$) and neutral surface (zeta potential: $< \pm 10$ mV), ideal for *in vivo* applications. The mRNA LNPs formed from LPSM01, LPSM02, or LPSM03 provide greater mRNA transfection efficiency than the mRNA LNPs made from ALC-0159 in HeLa cells, and to an even greater extent in RAW264.7 cells. All mRNA LNPs formulations tested show negligible cytotoxicity. LPSM1-3 are thus a viable replacement for ALC-0159 and other PEG-conjugated lipids as they not only are capable of fulfilling the function of PEG in maintaining nanosize particles, but also provide a greater mRNA transfection efficiency especially in immune cells. These PEG-free LNPs are

promising nanocarriers for targeted delivery of mRNA and other genes as vaccine and treatment options.

It will be appreciated by a person skilled in the art that other variations
5 and/or modifications may be made to the embodiments disclosed herein without
departing from the spirit or scope of the disclosure as broadly described. For
example, in the description herein, features of different exemplary embodiments
may be mixed, combined, interchanged, incorporated, adopted, modified,
included etc. or the like across different exemplary embodiments. The present
10 embodiments are, therefore, to be considered in all respects to be illustrative and
not restrictive.

CLAIMS

1. A compound represented by general formula (1) for preparing lipid nanoparticles encapsulating a therapeutic, prophylactic and/or biological agent:



(1)

wherein

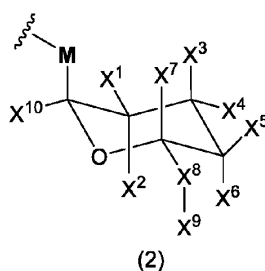
- A** comprises a carbohydrate and/or a derivative thereof;
 R¹ and R² are each independently a hydrophobic group;
 R³, R⁴, R⁵, and R⁶ are each independently H, optionally substituted alkyl,
 optionally substituted alkenyl or optionally substituted alkynyl;
 R⁷, R⁹ and R¹⁰ are each independently optionally substituted alkyl,
 optionally substituted alkenyl or optionally substituted alkynyl;
 R⁸ is -O-, -S-, or -NR^a-, where R^a is selected from the group consisting
 of H, optionally substituted alkyl, optionally substituted alkenyl or optionally
 substituted alkynyl;
 m is 0 or 1;
 n ≥ 1; and
 w ≥ 1.

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2. The compound of claim 1, wherein the compound is capable of targeting carbohydrate receptors on a cell surface.

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3. The compound of any one of the preceding claims, wherein **A** comprises a moiety selected from the group consisting of monosaccharide, disaccharide, oligosaccharide, polysaccharide and derivatives thereof.
- 5 4. The compound of any one of the preceding claims, wherein **A** is represented by general formula (2) having a 6-membered ring structure:



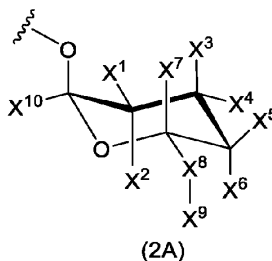
wherein

X¹ to X⁷ and X⁹ to X¹⁰ are each independently selected from –H or –OH;

10 X⁸ is alkyl/alkylene; and

M is –O– or –S–.

5. The compound of any one of the preceding claims, wherein **A** is represented by general formula (2A) having a 6-membered ring structure:



wherein

X¹ to X⁷ and X⁹ to X¹⁰ are each independently selected from –H or –OH;

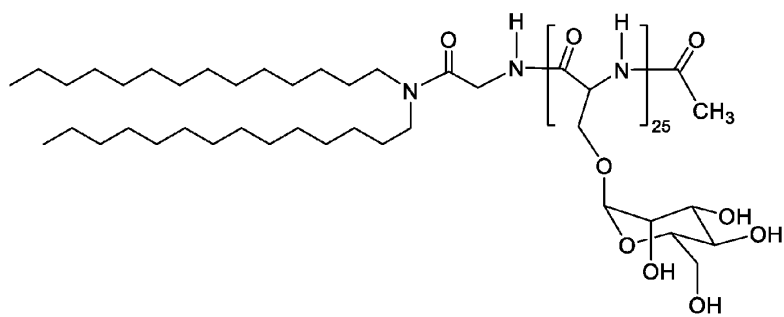
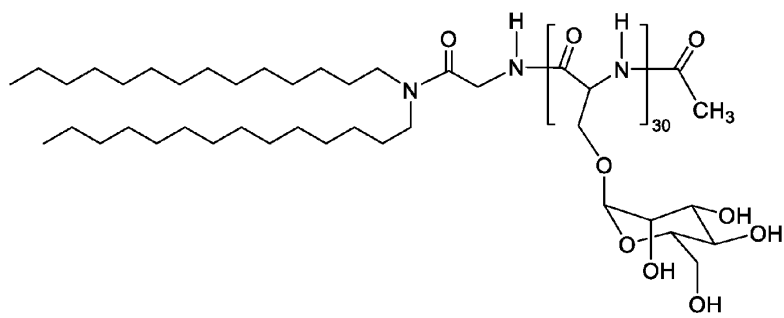
and

X⁸ is alkyl/alkylene.

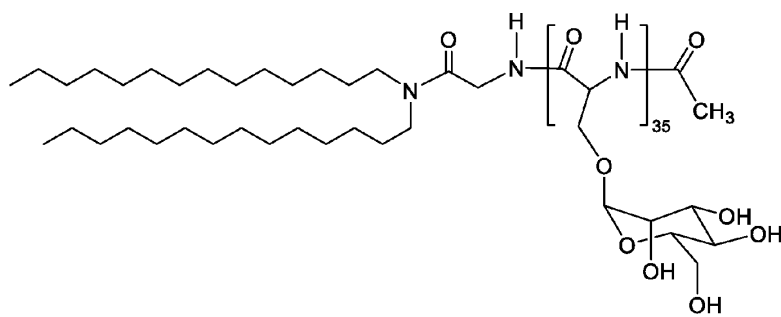
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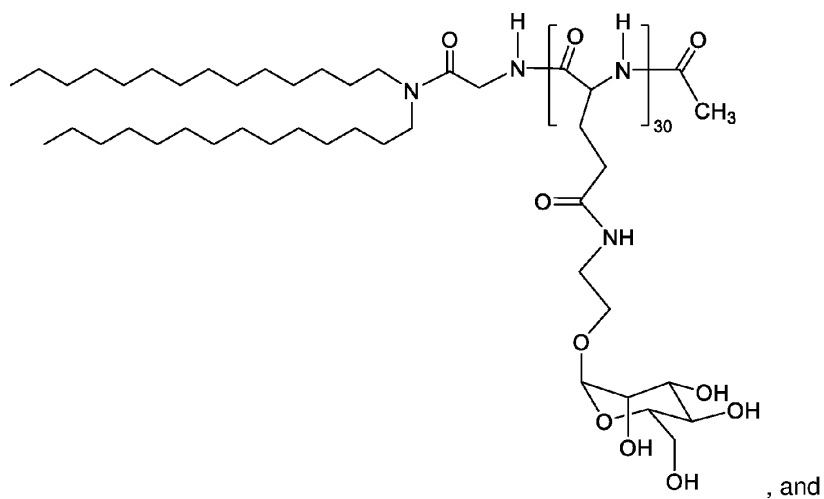
6. The compound of any one of the preceding claims, wherein $m = 1$ and R^7 is $-\text{CH}_2\text{CH}_2-$.
7. The compound of any one of the preceding claims, wherein $m = 0$ and R^7 is $-\text{CH}_2-$.
8. The compound of any one of the preceding claims, wherein the compound comprises a structure selected from one or more of the following:

LPSM01 ($n = 25$)LPSM02 ($n = 30$)

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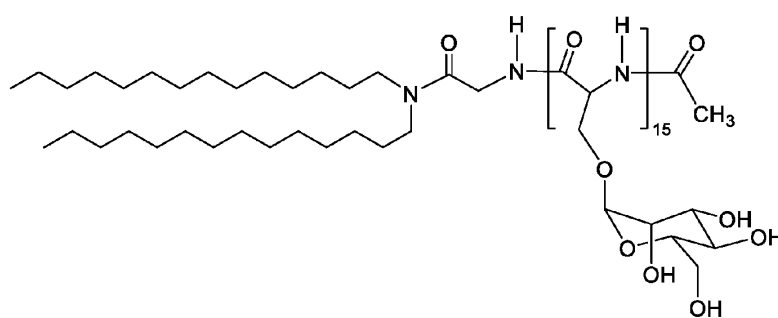


LPSM03 (n = 35)



LPGM01 (n = 30)

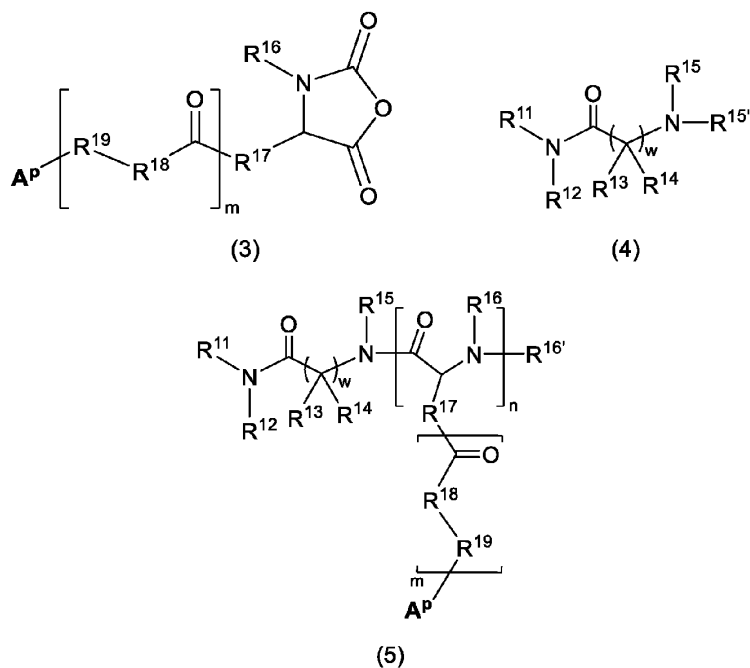
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LPSM04 (n = 15)

9. A method of preparing a compound as claimed in any one of the preceding claims, the method comprising:

- (i) polymerizing/reacting one or more N-carboxyanhydride (NCA) monomers represented by general formula (3) with a lipid initiator/molecule represented by general formula (4) to obtain a first intermediate compound represented by general formula (5):



wherein

- 10 **A^P** represents **A** protected with one or more protecting groups, where **A** comprises a carbohydrate and/or a derivative thereof;
- R¹¹ and R¹² are each independently a hydrophobic group;
- R¹³, R¹⁴, R¹⁵, and R¹⁶ are each independently H, optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;
- 15 R¹⁷ and R¹⁹ are each independently optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

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R^{18} is $-O-$, $-S-$, or $-NR^{a1}-$, where R^{a1} is selected from the group consisting of H, optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

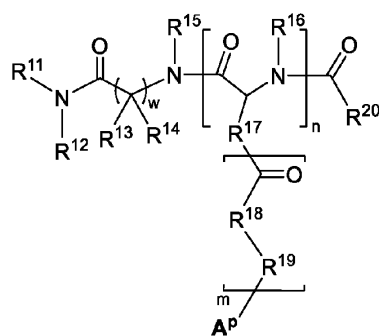
$R^{15'}$ and $R^{16'}$ are each independently H, optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl; and

m is 0 or 1;

$n \geq 1$; and

$w \geq 1$;

- 10 (ii) reacting the first intermediate compound represented by general formula (5) with an acylating agent to obtain a second intermediate compound represented by general formula (7):



(7)

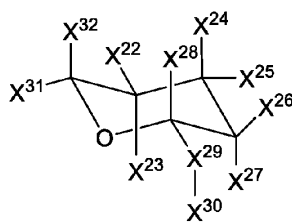
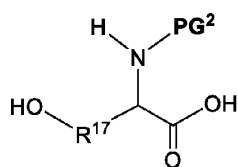
wherein R^{20} is optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

- (iii) deprotecting the second intermediate compound represented by general formula (7) to obtain the compound represented by general formula (1).

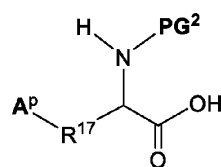
10. The method of claim 9, wherein the method further comprises, prior to step (i):

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(a-i) reacting a protected monosaccharide represented by general formula (8^p) with a protected compound represented by general formula (10) in the presence of a Lewis acid to obtain a first intermediate compound represented by general formula (11):

(8^p)

(10)



(11)

wherein

X²² to X²⁸ and X³⁰ to X³² are each independently selected from -H or -OPG¹;

X²⁹ is alkyl;

PG¹ is -C(=O)-R²²;

R²² is optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

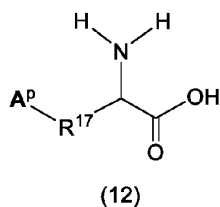
A^p represents A protected with one or more protecting groups, where A comprises a carbohydrate and/or a derivative thereof;

R¹⁷ is independently optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

PG² is a protecting group selected from 9-Fluorenylmethoxycarbonyl (Fmoc), 2-(4-Nitrophenylsulfonyl)ethoxycarbonyl (Nsc), 2-Fluoro-Fmoc (Fmoc(2F)), 2-Monoisooctyl-Fmoc (mio-Fmoc), 2,7-Diisooctyl-Fmoc (dio-Fmoc), N-carboxybenzyl (Cbz), or combinations thereof;

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- (a-ii) deprotecting the first intermediate compound represented by general formula (11) to obtain a second intermediate compound represented by general formula (12):



5

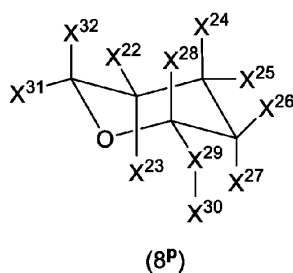
- (a-iii) reacting the second intermediate compound represented by general formula (12) with a carbonylating agent to obtain the N-carboxyanhydride (NCA) monomer represented by general formula (3).

10

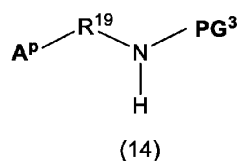
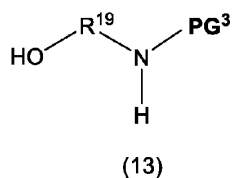
11. The method of claim 9, wherein the method further comprises, prior to step (i):

- (b-i) optionally reacting a protected monosaccharide represented by general formula (8^P) with a protected alkanolamine compound represented by general formula (13) in the presence of a Lewis acid to obtain a first intermediate compound represented by general formula (14):

15



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wherein

X^{22} to X^{28} and X^{30} to X^{32} are each independently selected from $-\text{H}$ or $-\text{OPG}^1$;

5 X^{29} is alkyl;

PG^1 is $-\text{C}(=\text{O})-\text{R}^{22}$;

R^{22} is optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

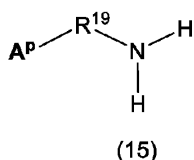
10 A^p represents **A** protected with one or more protecting groups, where **A** comprises a carbohydrate and/or a derivative thereof;

R^{19} is independently optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl;

PG^3 is a protecting group selected from N-carboxybenzyl, benzyloxycarbonyl (Cbz), or combinations thereof;

15

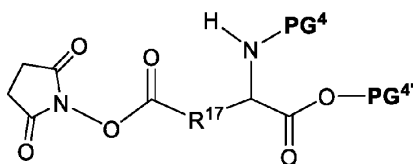
(b-ii) optionally deprotecting the first intermediate compound represented by general formula (14) to obtain a second intermediate compound represented by general formula (15):



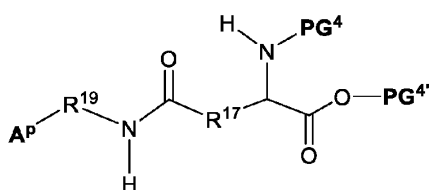
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(b-iii) reacting the second intermediate compound represented by general formula (15) with a protected compound represented by general formula (16) in the presence of a base to obtain a third intermediate compound represented by general formula (17):

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(16)



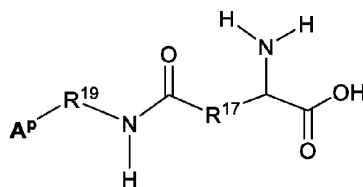
(17)

wherein

R^{17} is independently optionally substituted alkyl, optionally substituted alkenyl or optionally substituted alkynyl; and

PG^4 and $\text{PG}^{4'}$ are each independently a protecting group selected from *tert*-butoxycarbonyl (Boc), *tert*-butyl, benzyloxycarbonyl protecting group (Cbz), or combinations thereof;

(b-iv) deprotecting the third intermediate compound represented by general formula (17) to obtain a fourth intermediate compound represented by general formula (18):



(18)

(b-v) reacting the fourth intermediate compound represented by general formula (18) with a carbonylating agent to obtain the N-carboxyanhydride (NCA) monomer represented by general formula (3).

12. A nanoparticle composition for delivery of a therapeutic, prophylactic and/or biological agent, the nanoparticle composition comprising:
a compound as claimed in any one of claims 1 to 8; and
a therapeutic, prophylactic and/or biological agent.
- 5
13. The nanoparticle composition of claim 12, wherein the composition further comprises:
(a) ionizable lipid;
(b) helper lipid; and
10 (c) cholesterol and/or derivatives thereof.
14. The nanoparticle composition of claim 13, wherein the ionizable lipid, helper lipid, cholesterol and/or derivatives thereof, and compound represented by general formula (1) are mixed at a mole ratio of 20 – 50 :
15 4 – 20 : 25 – 50 : 0.5 – 20.
15. The nanoparticle composition of any one of claims 13 to 14, wherein the ionizable lipid is selected from ALC-0315, SM-102, Lipid 5, DLinDMA, D-Lin-MC2-DMA, DLin-MC3-DMA, D-Lin-MC4-DMA, Dlin-KC2-DMA,
20 YSK05, AA3-Dlin, SSPalmM, SSPalmO-Phe, Lipid A9, L319, DODMA, CL1, BP Lipid 310, ATX-001, ATX-100, Lipid 2, 80-O16B, BP Lipid 309, BP Lipid 307, 93-O17S, 93-O17O, NT1-O14B, 306-O12B-3, 306-O12B, 113-O16B, 306Oi10, 306Oi9-cis2, BAMEA-O16B, AI-28, 113-O12B, 98N12-5, Ckk-E12, OF-02, C12-200, BP Lipid 311, BP Lipid 308, BP Lipid
25 314, BP Lipid 312, LP01, TCL053, Lipid C24, BP Lipid 315, Lipid 29, 9A1P9, C13-112-tri-tail, C13-113-tri-tail, C13-112-tetra-tail, or C13-113-tetra-tail, C12-200 or combinations thereof.
16. The nanoparticle composition of any one of claims 13 to 15, wherein the helper lipid is selected from 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-
- 30

- dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), sphingomyelin or combinations thereof.
17. The nanoparticle composition of any one of claims 13 to 16, wherein the cholesterol and/or derivatives thereof is selected from cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, avenasterol or combinations thereof.
18. The nanoparticle composition of any one of claims 12 to 17, wherein the nanoparticle composition comprises nanoparticles having a N/P ratio from 1:1 to 20:1.
19. The nanoparticle composition of any one of claims 12 to 18, wherein the nanoparticle composition comprises nanoparticles having an average particle size of from 40 nm to 500 nm.

20. The nanoparticle composition of any one of claims 12 to 19, wherein the nanoparticle composition comprises nanoparticles having a zeta potential of from -20 mV to +20 mV.
- 5 21. The nanoparticle composition as claimed in any one of claims 12 to 20 for use in medicine.
22. The nanoparticle composition as claimed in any one of claims 12 to 20 for use in the treatment or prophylaxis of a disease, disorder or condition in a
10 subject in need thereof.
23. Use of a nanoparticle composition as claimed in any one of claims 12 to 20 in the manufacture of a medicament for treatment or prophylaxis of a disease, disorder or condition in a subject in need thereof.
15
24. A method of treating or preventing a disease, disorder or condition in a subject in need thereof, the method comprising administering a therapeutically effective amount of the nanoparticle composition as claimed in any one of claims 12 to 20 to the subject.
20
25. The nanoparticle composition of claim 22, the use of claim 23 or the method of claim 24, wherein an immune response in the subject is to be induced through the administration of the nanoparticle composition thereto.
25
26. The nanoparticle composition of claim 22, the use of claim 23 or the method of claim 24, wherein the disease, disorder or condition is mediated by a coronavirus.
- 30 27. The nanoparticle composition, the use or the method of claim 26, wherein the coronavirus is a SARS-CoV-2 coronavirus.