

CALIXARENE COMPOUNDS, THEIR SYNTHESIS AND USE IN PHARMACEUTICAL COMPOSITIONS

FIELD OF THE INVENTION

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The present invention relates to a compound of Formula (I) or a pharmaceutically acceptable salt thereof. The present invention further relates to a pharmaceutical composition and use thereof.

10 BACKGROUND

The ability to safely and efficiently transfer foreign DNA into cells is a fundamental goal in biotechnology. Toward this end, rapid advances have recently been made in our understanding of mechanisms for DNA stability and transport within cells.

15 Current synthetic DNA delivery systems are versatile and safe, but substantially less efficient than viruses. Indeed, most current systems address only one of the obstacles to DNA delivery by enhancing DNA uptake. In fact, the effectiveness of gene expression is also dependent on several additional factors, including the release of intracellular DNA, stability of DNA in the cytoplasm, unpackaging of the DNA-

20 vector complex, and the targeting of DNA to the nucleus. Furthermore, recent advances in the generation, purification and cellular delivery of RNA have enabled development of RNA-based therapeutics for a broad array of applications. RNA therapeutics comprise a rapidly expanding category of drugs that will change the standard of care for many diseases and actualize personalized medicine. These drugs

25 are cost effective, relatively simple to manufacture, and can target previously undruggable pathways. It is a disruptive therapeutic technology, as small biotech startups, as well as academic groups, can rapidly develop new and personalized RNA constructs. However, employing nucleic acids as therapeutics is challenging because they are susceptible to degradation by nucleases, contribute to immune activation

30 and have unfavorable physicochemical characteristics that prevent facile transfection into cells. Safe and effective nucleic acid therapeutics therefore require sophisticated delivery platform technologies.

A big challenge in the field of mRNA vaccines for instance relies on the development of efficient delivery vectors that can (i) compact mRNA, (ii) protect it from nuclease

35 degradation, (iii) target the adequate organs and cells, and (iv) ensure its intracellular delivery. Appropriate carriers can help to avoid degradation and enhance immune responses, effector presentation, biocompatibility and biosafety. Among all

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non-viral carriers, lipid nanoparticles (LNPs) are by far the most studied and advanced into clinical development. However, these systems remain perfectible, particularly in terms of biodistribution (off-target effects), (thermo)stability and reactogenicity. Therefore, next-generation compounds and systems for RNA delivery are still under development.

The invention thereto aims to provide compounds having improved characteristics for use in nucleotide delivery systems.

SUMMARY OF THE INVENTION

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The present invention and embodiments thereof serve to provide a solution to one or more of above-mentioned disadvantages. To this end, the present invention relates to a compound of Formula (I) or a pharmaceutically acceptable salt thereof according to claim 1. Preferred embodiments of this compound are shown in any of the claims 2 to 22.

In a second aspect, the present invention relates to a composition comprising above mentioned compound according to claim 23.

20 In a further aspect, the invention relates to a pharmaceutical composition comprising above mentioned compound or salt, or above mentioned composition, and one or more nucleotides according to claim 24.

In a further aspect, the invention relates to above mentioned compound or salt, for use as a delivery system according to claim 25.

DESCRIPTION OF FIGURES

30 **Figure 1** shows a synthesis scheme of compounds according to an embodiment of the present invention.

Figure 2 shows a visualization of the estimated model (average + 95% confidence interval) of *in vivo* luminescence as a function of the (lipid+CX)/RNA mass ratio, the helper mass ratio and the PEG lipid mass ratio in a delivery system according to an embodiment of the current invention.

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Figure 3 shows the *in vivo* protein expression at 3h, 6h and 9h after the intramuscular injection of 1 µg of Fluc mRNA into both left and right hindlimb of mice (geometric mean signal with 95% confidence intervals) using various delivery systems according to embodiments of the current invention.

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Figure 4 shows saRNA-FLuc encapsulation in nanoparticles according to an embodiment of the invention having a different CX/RNA mass ratio and the resulting *in vitro* protein expression after transfection of these nanoparticles.

10 **Figure 5** shows *in vivo* saRNA-Fluc delivery efficiency of a delivery system according to an embodiment of the current invention compared to a reference delivery system without a calixarene.

Figure 6 shows virus neutralizing titers (VNT) in serum measured after 15, 35 and 15 64/65 days in mice which were vaccinated using a delivery system according to an embodiment of the invention comprising an ionizable calixarene and loaded with mRNA encoding for the G-protein of the Rabies virus. A 21 days prime-boost regimen using either 0.6 µg or 2.5 µg of mRNA per dose was used.

20 **Figure 7** shows a cryo-TEM image of a delivery system of the current invention encapsulating mRNA.

Figure 8 shows virus neutralizing titers (VNT) in serum measured after 15 and 35 25 days in mice which were vaccinated using a delivery system according to an embodiment of the invention comprising a cationic calixarene (e.g., CX4), a PEGylated lipid (either DMG-PEG2000 or DSG-PEG2000), DOPE and cholesterol. The delivery system was loaded with mRNA encoding for the G-protein of the Rabies virus. A 21 days prime-boost regimen using either 0.6 µg or 2.5 µg of mRNA per dose was used.

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Figure 9A shows VNTs serum levels measured after 15 (after prime), 35 and 64/65 (after boost) days for each LNP, including LNPs according to an embodiment of the current invention.

35 **Figure 9B** shows the virus neutralizing titers (VTNs) in serum measured after 15 (after prime), 35 and 65 days (after boost) days in mice which were vaccinated using SM-102 LNPs (control), DLin-DMA LNPs and DLin-DMA/CX4 LNPs, the latter being a delivery system according to an embodiment of the current invention. A 21 days

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prime-boost regimen using 2.5 µg of mRNA per dose was used (RNA encoding for the glycoprotein G from the Rabies virus. The results are shown as a comparison with the control delivery system (SM-102 LNPs). UDL = upper decision limit, LDL = lower decision limit. All points crossing the limits indicate a significant difference as compared to the control.

Figure 10 shows VNTs serum levels measured after 15 (after prime), 35 and 65 (after boost) days for each nanoparticle (A: 0.6 µg doses, B: 2.5 µg doses IM administration in BALB/c), including an LNP where a cationizable calixarene was used as an adjuvant according to an embodiment of the current invention.

Figure 11 shows the intensity distribution of an LNP according to an embodiment of the invention comprising DLin-DMA and a cationic calixarene (DLin-DMA:DOPE:cholesterol:DMG-PEG2000:CX12 LNP in 20 mM TRIS buffer) encapsulating saRNA encoding for the spike protein of SARS-CoV-2 (FIG. 11A) and of a reference LNP not comprising a cationic calixarene (SM-102:DSPC:cholesterol:DMG-PEG2000 (50:10:38.5:1.5 molar ratio)) encapsulating saRNA encoding for an undisclosed gene of similar length (FIG. 11B).

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DETAILED DESCRIPTION OF THE INVENTION

Definitions

Unless otherwise defined, all terms used in disclosing the invention, including technical and scientific terms, have the meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. By means of further guidance, term definitions are included to better appreciate the teaching of the present invention.

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As used herein, the following terms have the following meanings:

"A", "an", and "the" as used herein refers to both singular and plural referents unless the context clearly dictates otherwise. By way of example, "a compartment" refers to one or more than one compartment.

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"About" as used herein referring to a measurable value such as a parameter, an amount, a temporal duration, and the like, is meant to encompass variations of +/-

20% or less, preferably +/-10% or less, more preferably +/-5% or less, even more preferably +/-1% or less, and still more preferably +/-0.1% or less of and from the specified value, in so far such variations are appropriate to perform in the disclosed invention. However, it is to be understood that the value to which the modifier
5 "about" refers is itself also specifically disclosed.

"Comprise", "comprising", and "comprises" and "comprised of" as used herein are synonymous with "include", "including", "includes" or "contain", "containing",
10 "contains" and are inclusive or open-ended terms that specifies the presence of what follows e.g. component and do not exclude or preclude the presence of additional, non-recited components, features, element, members, steps, known in the art or disclosed therein.

Furthermore, the terms first, second, third and the like in the description and in the
15 claims, are used for distinguishing between similar elements and not necessarily for describing a sequential or chronological order, unless specified. It is to be understood that the terms so used are interchangeable under appropriate circumstances and that the embodiments of the invention described herein are capable of operation in other sequences than described or illustrated herein.

20 The recitation of numerical ranges by endpoints includes all numbers and fractions subsumed within that range, as well as the recited endpoints.

The expression "% by weight", "weight percent", "%wt" or "wt%", here and
25 throughout the description unless otherwise defined, refers to the relative weight of the respective component based on the overall weight of the formulation.

Whereas the terms "one or more" or "at least one", such as one or more or at least one member(s) of a group of members, is clear *per se*, by means of further
30 exemplification, the term encompasses *inter alia* a reference to any one of said members, or to any two or more of said members, such as, e.g., any ≥ 3 , ≥ 4 , ≥ 5 , ≥ 6 or ≥ 7 etc. of said members, and up to all said members.

Unless otherwise defined, all terms used in disclosing the invention, including
35 technical and scientific terms, have the meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. By means of further guidance, definitions for the terms used in the description are included to better appreciate the

teaching of the present invention. The terms or definitions used herein are provided solely to aid in the understanding of the invention.

Reference throughout this specification to "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, appearances of the phrases "in one embodiment" or "in an embodiment" in various places throughout this specification are not necessarily all referring to the same embodiment, but may. Furthermore, the particular features, structures or characteristics may be combined in any suitable manner, as would be apparent to a person skilled in the art from this disclosure, in one or more embodiments. Furthermore, while some embodiments described herein include some but not other features included in other embodiments, combinations of features of different embodiments are meant to be within the scope of the invention, and form different embodiments, as would be understood by those in the art. For example, in the following claims, any of the claimed embodiments can be used in any combination. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. All patents and publications referred to herein are incorporated by reference.

"Alkyl" refers to a straight or branched hydrocarbon chain radical consisting solely of carbon and hydrogen atoms, containing no unsaturation, and preferably having from one to fifteen carbon atoms (*i.e.*, C₁-C₁₅ alkyl). In certain embodiments, an alkyl comprises one to thirteen carbon atoms (*i.e.*, C₁-C₁₃ alkyl). In certain embodiments, an alkyl comprises one to eight carbon atoms (*i.e.*, C₁-C₈ alkyl). In other embodiments, an alkyl comprises one to five carbon atoms (*i.e.*, C₁-C₅ alkyl). In other embodiments, an alkyl comprises one to four carbon atoms (*i.e.*, C₁-C₄ alkyl). In other embodiments, an alkyl comprises one to three carbon atoms (*i.e.*, C₁-C₃ alkyl). In other embodiments, an alkyl comprises one to two carbon atoms (*i.e.*, C₁-C₂ alkyl). In other embodiments, an alkyl comprises one carbon atom (*i.e.*, C₁ alkyl). In other embodiments, an alkyl comprises five to fifteen carbon atoms (*i.e.*, C₅-C₁₅ alkyl). In other embodiments, an alkyl comprises five to eight carbon atoms (*i.e.*, C₅-C₈ alkyl). In other embodiments, an alkyl comprises two to five carbon atoms (*i.e.*, C₂-C₅ alkyl). In other embodiments, an alkyl comprises three to five carbon atoms (*i.e.*, C₃-C₅ alkyl). In certain embodiments, the alkyl group is selected from methyl, ethyl, 1-propyl (*n*-propyl), 1-methylethyl (*iso*-propyl), 1-butyl (*n*-butyl), 1-methylpropyl (*sec*-butyl), 2-methylpropyl (*iso*-butyl), 1,1-dimethylethyl

(*tert*-butyl), 1-pentyl (*n*-pentyl). The alkyl is attached to the rest of the molecule by a single bond.

5 The term "C_{x-y}" when used in conjunction with a chemical moiety, such as alkyl, alkenyl, or alkynyl is meant to include groups that contain from x to y carbons in the chain. For example, the term "C₁₋₆alkyl" refers to substituted or unsubstituted saturated hydrocarbon groups, including straight-chain alkyl and branched-chain alkyl groups that contain from 1 to 6 carbons. The term -C_{x-y}alkylene- refers to a substituted or unsubstituted alkylene chain with from x to y carbons in the alkylene
10 chain. For example -C₁₋₆alkylene- may be selected from methylene, ethylene, propylene, butylene, pentylene, and hexylene, any one of which is optionally substituted.

"Alkoxy" refers to a radical bonded through an oxygen atom of the formula -O-alkyl,
15 where alkyl is an alkyl chain as defined above.

"Alkenyl" refers to a straight or branched hydrocarbon chain radical group consisting solely of carbon and hydrogen atoms, containing at least one carbon-carbon double bond, and preferably having from two to twelve carbon atoms (*i.e.*, C₂-C₁₂ alkenyl).
20 In certain embodiments, an alkenyl comprises two to eight carbon atoms (*i.e.*, C₂-C₈ alkenyl). In certain embodiments, an alkenyl comprises two to six carbon atoms (*i.e.*, C₂-C₆ alkenyl). In other embodiments, an alkenyl comprises two to four carbon atoms (*i.e.*, C₂-C₄ alkenyl). The alkenyl is attached to the rest of the molecule by a single bond, for example, ethenyl (*i.e.*, vinyl), prop-1-enyl (*i.e.*, allyl), but-1-enyl,
25 pent-1-enyl, penta-1,4-dienyl, and the like.

"Alkynyl" refers to a straight or branched hydrocarbon chain radical group consisting solely of carbon and hydrogen atoms, containing at least one carbon-carbon triple bond, and preferably having from two to twelve carbon atoms (*i.e.*, C₂-C₁₂ alkynyl).
30 In certain embodiments, an alkynyl comprises two to eight carbon atoms (*i.e.*, C₂-C₈ alkynyl). In other embodiments, an alkynyl comprises two to six carbon atoms (*i.e.*, C₂-C₆ alkynyl). In other embodiments, an alkynyl comprises two to four carbon atoms (*i.e.*, C₂-C₄ alkynyl). The alkynyl is attached to the rest of the molecule by a single bond, for example, ethynyl, propynyl, butynyl, pentynyl, hexynyl, and the
35 like.

The terms "C_{x-y}alkenyl" and "C_{x-y}alkynyl" refer to substituted or unsubstituted unsaturated aliphatic groups analogous in length and possible substitution to the

alkyls described above, but that contain at least one double or triple bond, respectively. The term $-C_{x-y}$ alkenylene- refers to a substituted or unsubstituted alkenylene chain with from x to y carbons in the alkenylene chain. For example, $-C_{2-6}$ alkenylene- may be selected from ethenylene, propenylene, butenylene, pentenylene, and hexenylene, any one of which is optionally substituted. An alkenylene chain may have one double bond or more than one double bond in the alkenylene chain. The term $-C_{x-y}$ alkynylene- refers to a substituted or unsubstituted alkynylene chain with from x to y carbons in the alkenylene chain. For example, $-C_{2-6}$ alkynylene- may be selected from ethynylene, propynylene, butynylene, pentynylene, and hexynylene, any one of which is optionally substituted. An alkynylene chain may have one triple bond or more than one triple bond in the alkynylene chain.

"Alkylene" or "alkylene chain" refers to a straight or branched divalent hydrocarbon chain linking the rest of the molecule to a radical group, consisting solely of carbon and hydrogen, containing no unsaturation, and preferably having from one to twelve carbon atoms, for example, methylene, ethylene, propylene, *n*-butylene, and the like. The alkylene chain is attached to the rest of the molecule through a single bond and to the radical group through a single bond. The points of attachment of the alkylene chain to the rest of the molecule and to the radical group may be through any two carbons within the chain. In certain embodiments, an alkylene comprises one to ten carbon atoms (*i.e.*, C_1-C_{10} alkylene). In certain embodiments, an alkylene comprises one to eight carbon atoms (*i.e.*, C_1-C_8 alkylene). In other embodiments, an alkylene comprises one to five carbon atoms (*i.e.*, C_1-C_5 alkylene). In other embodiments, an alkylene comprises one to four carbon atoms (*i.e.*, C_1-C_4 alkylene). In other embodiments, an alkylene comprises one to three carbon atoms (*i.e.*, C_1-C_3 alkylene). In other embodiments, an alkylene comprises one to two carbon atoms (*i.e.*, C_1-C_2 alkylene). In other embodiments, an alkylene comprises one carbon atom (*i.e.*, C_1 alkylene). In other embodiments, an alkylene comprises five to eight carbon atoms (*i.e.*, C_5-C_8 alkylene). In other embodiments, an alkylene comprises two to five carbon atoms (*i.e.*, C_2-C_5 alkylene). In other embodiments, an alkylene comprises three to five carbon atoms (*i.e.*, C_3-C_5 alkylene).

"Alkenylene" or "alkenylene chain" refers to a straight or branched divalent hydrocarbon chain linking the rest of the molecule to a radical group, consisting solely of carbon and hydrogen, containing at least one carbon-carbon double bond, and preferably having from two to twelve carbon atoms. The alkenylene chain is attached to the rest of the molecule through a single bond and to the radical group

through a single bond. The points of attachment of the alkenylene chain to the rest of the molecule and to the radical group may be through any two carbons within the chain. In certain embodiments, an alkenylene comprises two to ten carbon atoms (*i.e.*, C₂-C₁₀ alkenylene). In certain embodiments, an alkenylene comprises two to eight carbon atoms (*i.e.*, C₂-C₈ alkenylene). In other embodiments, an alkenylene comprises two to five carbon atoms (*i.e.*, C₂-C₅ alkenylene). In other embodiments, an alkenylene comprises two to four carbon atoms (*i.e.*, C₂-C₄ alkenylene). In other embodiments, an alkenylene comprises two to three carbon atoms (*i.e.*, C₂-C₃ alkenylene). In other embodiments, an alkenylene comprises two carbon atom (*i.e.*, C₂ alkenylene). In other embodiments, an alkenylene comprises five to eight carbon atoms (*i.e.*, C₅-C₈ alkenylene). In other embodiments, an alkenylene comprises three to five carbon atoms (*i.e.*, C₃-C₅ alkenylene).

"Alkynylene" or "alkynylene chain" refers to a straight or branched divalent hydrocarbon chain linking the rest of the molecule to a radical group, consisting solely of carbon and hydrogen, containing at least one carbon-carbon triple bond, and preferably having from two to twelve carbon atoms. The alkynylene chain is attached to the rest of the molecule through a single bond and to the radical group through a single bond. The points of attachment of the alkynylene chain to the rest of the molecule and to the radical group may be through any two carbons within the chain. In certain embodiments, an alkynylene comprises two to ten carbon atoms (*i.e.*, C₂-C₁₀ alkynylene). In certain embodiments, an alkynylene comprises two to eight carbon atoms (*i.e.*, C₂-C₈ alkynylene). In other embodiments, an alkynylene comprises two to five carbon atoms (*i.e.*, C₂-C₅ alkynylene). In other embodiments, an alkynylene comprises two to four carbon atoms (*i.e.*, C₂-C₄ alkynylene). In other embodiments, an alkynylene comprises two to three carbon atoms (*i.e.*, C₂-C₃ alkynylene). In other embodiments, an alkynylene comprises two carbon atom (*i.e.*, C₂ alkynylene). In other embodiments, an alkynylene comprises five to eight carbon atoms (*i.e.*, C₅-C₈ alkynylene). In other embodiments, an alkynylene comprises three to five carbon atoms (*i.e.*, C₃-C₅ alkynylene).

"Aryl" refers to a radical derived from an aromatic monocyclic or aromatic polycyclic hydrocarbon ring system by removing a hydrogen atom from a ring carbon atom. The aromatic monocyclic or aromatic polycyclic hydrocarbon ring system contains only hydrogen and carbon and from five to eighteen carbon atoms, where at least one of the rings in the ring system is aromatic, *i.e.*, it contains a cyclic, delocalized (4n+2) π -electron system in accordance with the Hückel theory. The ring system

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from which aryl groups are derived include, but are not limited to, groups such as benzene, fluorene, indane, indene, tetralin and naphthalene.

5 "Aralkyl" refers to a radical of the formula $-R^c$ -aryl where R^c is an alkylene chain as defined above, for example, methylene, ethylene, and the like.

"Aralkenyl" refers to a radical of the formula $-R^d$ -aryl where R^d is an alkenylene chain as defined above. "Aralkynyl" refers to a radical of the formula $-R^e$ -aryl, where R^e is an alkynylene chain as defined above.

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"Carbocycle" refers to a saturated, unsaturated or aromatic rings in which each atom of the ring is carbon. Carbocycle may include 3- to 10-membered monocyclic rings, 6- to 12-membered bicyclic rings, and 6- to 12-membered bridged rings. Each ring of a bicyclic carbocycle may be selected from saturated, unsaturated, and aromatic rings. An aromatic ring, e.g., phenyl, may be fused to a saturated or unsaturated ring, e.g., cyclohexane, cyclopentane, or cyclohexene. Any combination of saturated, unsaturated and aromatic bicyclic rings, as valence permits, are included in the definition of carbocyclic. Exemplary carbocycles include cyclopentyl, cyclohexyl, cyclohexenyl, adamantyl, phenyl, indanyl, and naphthyl. Bicyclic carbocycles may be fused, bridged or spiro-ring systems. In some cases, spiro-ring carbocycles have at least two molecular rings with only one common atom.

25 The term "unsaturated carbocycle" refers to carbocycles with at least one degree of unsaturation and excluding aromatic carbocycles. Examples of unsaturated carbocycles include cyclohexadiene, cyclohexene, and cyclopentene.

"Cycloalkyl" refers to a fully saturated monocyclic or polycyclic hydrocarbon radical consisting solely of carbon and hydrogen atoms, which includes fused or bridged ring systems, and preferably having from three to twelve carbon atoms. In certain embodiments, a cycloalkyl comprises three to ten carbon atoms. In other embodiments, a cycloalkyl comprises five to seven carbon atoms. The cycloalkyl may be attached to the rest of the molecule by a single bond. Examples of monocyclic cycloalkyls include, e.g., cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. Polycyclic cycloalkyl radicals include, for example, adamantyl, norbornyl (*i.e.*, bicyclo[2.2.1]heptanyl), norbornenyl, decalanyl, 7,7-dimethyl-bicyclo[2.2.1]heptanyl, and the like.

"Cycloalkenyl" refers to an unsaturated non-aromatic monocyclic or polycyclic hydrocarbon radical consisting solely of carbon and hydrogen atoms, which includes fused or bridged ring systems, preferably having from three to twelve carbon atoms and comprising at least one double bond. In certain embodiments, a cycloalkenyl comprises three to ten carbon atoms. In other embodiments, a cycloalkenyl comprises five to seven carbon atoms. The cycloalkenyl may be attached to the rest of the molecule by a single bond. Examples of monocyclic cycloalkenyls includes, e.g., cyclopentenyl, cyclohexenyl, cycloheptenyl, and cyclooctenyl.

10 "Cycloalkylalkyl" refers to a radical of the formula $-R^c$ -cycloalkyl where R^c is an alkylene chain as described above.

"Cycloalkylalkoxy" refers to a radical bonded through an oxygen atom of the formula $-O-R^c$ -cycloalkyl where R^c is an alkylene chain as described above.

15 "Halo" or "halogen" refers to halogen substituents such as bromo, chloro, fluoro and iodo substituents.

As used herein, the term "haloalkyl" or "haloalkane" refers to an alkyl radical, as defined above, that is substituted by one or more halogen radicals, for example, trifluoromethyl, dichloromethyl, bromomethyl, 2,2,2-trifluoroethyl, 1-fluoromethyl-2-fluoroethyl, and the like. In some embodiments, the alkyl part of the fluoroalkyl radical is optionally further substituted. Examples of halogen substituted alkanes ("haloalkanes") include halomethane (e.g., chloromethane, bromomethane, fluoromethane, iodomethane), di-and trihalomethane (e.g., trichloromethane, tribromomethane, trifluoromethane, triiodomethane), 1-haloethane, 2-haloethane, 1,2-dihaloethane, 1-halopropane, 2-halopropane, 3-halopropane, 1,2-dihalopropane, 1,3-dihalopropane, 2,3-dihalopropane, 1,2,3-trihalopropane, and any other suitable combinations of alkanes (or substituted alkanes) and halogens (e.g., Cl, Br, F, I, etc.). When an alkyl group is substituted with more than one halogen radicals, each halogen may be independently selected e.g., 1-chloro,2-fluoroethane.

35 "Fluoroalkyl" refers to an alkyl radical, as defined above, that is substituted by one or more fluoro radicals, for example, trifluoromethyl, difluoromethyl, fluoromethyl, 2,2,2-trifluoroethyl, 1-fluoromethyl-2-fluoroethyl, and the like.

"Hydroxyalkyl" refers to an alkyl radical, as defined above, that is substituted by one or more hydroxy radicals, for example, propan-1-ol, butane-1,4-diol, pentane-1,2,4-triol, and the like.

- 5 "Alkoxyalkyl" refers to an alkyl radical, as defined above, that is substituted by one or more alkoxy radicals, for example, methoxymethane, 1,3-dimethoxybutane, 1-methoxypropane, 2-ethoxypentane, and the like.

- 10 "Cyanoalkyl" as used herein refers to an alkyl radical, as defined above, that is substituted by one or more cyano radicals, for example, acetonitrile, 2-ethyl-3-methylsuccinonitrile, butyronitrile, and the like.

- "Heterocycle" refers to a saturated or unsaturated or aromatic ring comprising one or more heteroatoms. Exemplary heteroatoms include N, O, Si, P, B, and S atoms.
- 15 Heterocycles include 3- to 10-membered monocyclic rings, 6- to 12-membered bicyclic rings, and 6- to 12-membered bridged rings. Each ring of a bicyclic heterocycle may be selected from saturated, unsaturated, and aromatic rings. Bicyclic heterocycles may be fused, bridged or spiro-ring systems. In some cases, spiro-ring heterocycles have at least two molecular rings with only one common
- 20 atom. The spiro-ring heterocycle includes at least one heteroatom.

"Heterocyclene" refers to a divalent heterocycle linking the rest of the molecule to a radical group.

- 25 "Heteroaryl" or "aromatic heterocycle" refers to a radical derived from a heteroaromatic ring radical that comprises one to eleven carbon atoms and at least one heteroatom wherein each heteroatom may be selected from N, O, and S. As used herein, the heteroaryl ring may be selected from monocyclic or bicyclic and fused or bridged ring systems rings wherein at least one of the rings in the ring
- 30 system is aromatic, *i.e.*, it contains a cyclic, delocalized $(4n+2)$ π -electron system in accordance with the Hückel theory. The heteroatom(s) in the heteroaryl radical may be optionally oxidized. One or more nitrogen atoms, if present, are optionally quaternized. The heteroaryl may be attached to the rest of the molecule through any atom of the heteroaryl, valence permitting, such as a carbon or nitrogen atom of the
- 35 heteroaryl. Examples of heteroaryls include, but are not limited to, pyridine, pyrimidine, oxazole, furan, pyran, thiophene, isoxazole, benzimidazole, benzthiazole, and imidazopyridine.

An "X-membered heteroaryl" refers to the number of endocyclic atoms, i.e., X, in the ring. For example, a 5-membered heteroaryl ring or 5-membered aromatic heterocycle has 5 endocyclic atoms, e.g., triazole, oxazole, thiophene, etc.

- 5 The term "unsaturated heterocycle" refers to heterocycles with at least one degree of unsaturation and excluding aromatic heterocycles. Examples of unsaturated heterocycles include dihydropyrrole, dihydrofuran, oxazoline, pyrazoline, and dihydropyridine. Heterocycles may be optionally substituted by one or more substituents such as those substituents described herein.

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- The term "substituted" refers to moieties having substituents replacing a hydrogen on one or more carbons or substitutable heteroatoms, e.g., NH, of the structure. It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, i.e., a compound which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. In certain embodiments, substituted refers to moieties having substituents replacing two hydrogen atoms on the same carbon atom, such as substituting the two hydrogen atoms on a single carbon with an oxo, imino or thioxo group.
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- As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and non-aromatic substituents of organic compounds. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this disclosure, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. In some
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- embodiments, substituents may include any substituents described herein, for example: halogen, hydroxy, oxo (=O), thioxo (=S), cyano (-CN), nitro (-NO₂), imino (=N-H), oximo (=N-OH), hydrazino (=N-NH₂), -R^b-OR^a, -R^b-OC(O)-R^a, -R^b-OC(O)-OR^a, -R^b-OC(O)-N(R^a)₂, -R^b-N(R^a)₂, -R^b-C(O)R^a, -R^b-C(O)OR^a, -R^b-C(O)N(R^a)₂, -R^b-O-R^c-C(O)N(R^a)₂, -R^b-N(R^a)C(O)OR^a, -R^b-N(R^a)C(O)R^a, -R^b-N(R^a)S(O)_tR^a (where t is 1 or 2), -R^b-S(O)_tR^a (where t is 1 or 2), -R^b-S(O)_tOR^a (where t is 1 or 2), and -R^b-S(O)_tN(R^a)₂ (where t is 1 or 2); and alkyl, alkenyl, alkynyl, aryl, aralkyl, aralkenyl, aralkynyl, cycloalkyl, cycloalkylalkyl, and heterocycle, any of which may be optionally substituted by alkyl, alkenyl,
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- alkynyl, halogen, haloalkyl, haloalkenyl, haloalkynyl, oxo (=O), thioxo (=S), cyano (-CN), nitro (-NO₂), imino (=N-H), oximo (=N-OH), hydrazine (=N-NH₂), -R^b-OR^a, -R^b-OC(O)-R^a, -R^b-OC(O)-OR^a, -R^b-OC(O)-N(R^a)₂, -R^b-N(R^a)₂, -R^b-C(O)R^a, -R^b-C(O)OR^a, -R^b-C(O)N(R^a)₂, -R^b-O-R^c-C(O)N(R^a)₂, -R^b-N(R^a)C(O)OR^a, -R^b-N(R^a)C(O)R^a, -R^b-N(R^a)S(O)_tR^a (where t is 1 or 2), -R^b-S(O)_tR^a (where t is 1 or 2), -R^b-S(O)_tOR^a (where t is 1 or 2) and -R^b-S(O)_tN(R^a)₂ (where t is 1 or 2); wherein each R^a is independently selected from hydrogen, alkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heterocycloalkyl, heterocycloalkylalkyl, heteroaryl, or heteroarylalkyl, wherein each R^a, valence permitting, may be optionally substituted with alkyl, alkenyl, alkynyl, halogen, haloalkyl, haloalkenyl, haloalkynyl, oxo (=O), thioxo (=S), cyano (-CN), nitro (-NO₂), imino (=N-H), oximo (=N-OH), hydrazine (=N-NH₂), -R^b-OR^a, -R^b-OC(O)-R^a, -R^b-OC(O)-OR^a, -R^b-OC(O)-N(R^a)₂, -R^b-N(R^a)₂, -R^b-C(O)R^a, -R^b-C(O)OR^a, -R^b-C(O)N(R^a)₂, -R^b-O-R^c-C(O)N(R^a)₂, -R^b-N(R^a)C(O)OR^a, -R^b-N(R^a)C(O)R^a, -R^b-N(R^a)S(O)_tR^a (where t is 1 or 2), -R^b-S(O)_tR^a (where t is 1 or 2), -R^b-S(O)_tOR^a (where t is 1 or 2) and -R^b-S(O)_tN(R^a)₂ (where t is 1 or 2); and wherein each R^b is independently selected from a direct bond or a straight or branched alkylene, alkenylene, or alkynylene chain, and each R^c is a straight or branched alkylene, alkenylene or alkynylene chain.
- As used herein, the term "optional" or "optionally" means that the subsequently described event or circumstances may or may not occur, and that the description includes instances where the event or circumstance occurs and instances in which it does not. For example, "optionally substituted aryl" means that the aryl group may or may not be substituted and that the description includes both substituted aryl groups and aryl groups having no substitution.
- The term "salt" or "pharmaceutically acceptable salt" refers to salts derived from a variety of organic and inorganic counter ions well known in the art. Pharmaceutically acceptable acid addition salts can be formed with inorganic acids and organic acids. Inorganic acids from which salts can be derived include, for example, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Organic acids from which salts can be derived include, for example, acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluenesulfonic acid, salicylic acid, and the like. Pharmaceutically acceptable base addition salts can be formed with inorganic and organic bases. Inorganic bases from which salts can be derived include, for example, sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum, and the like. Organic bases

from which salts can be derived include, for example, primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, basic ion exchange resins, and the like, specifically such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine. In some embodiments, the pharmaceutically acceptable base addition salt is chosen from ammonium, potassium, sodium, calcium, and magnesium salts.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically acceptable excipient" or "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

In certain embodiments, the term "prevent" or "preventing" as related to a disease or disorder may refer to a compound that, in a statistical sample, reduces the occurrence of the disorder or condition in the treated sample relative to an untreated control sample, or delays the onset or reduces the severity of one or more symptoms of the disorder or condition relative to the untreated control sample.

The terms "treat," "treating" or "treatment," as used herein, may include alleviating, abating or ameliorating a disease or condition symptoms, preventing additional symptoms, ameliorating or preventing the underlying causes of symptoms, inhibiting the disease or condition, e.g., arresting the development of the disease or condition, relieving the disease or condition, causing regression of the disease or condition, relieving a condition caused by the disease or condition, or stopping the symptoms of the disease or condition either prophylactically and/or therapeutically.

The term "lipid" refers to a group of organic compounds that comprise, but are not limited to, esters of branched or unbranched fatty acids and are generally characterized by being poorly soluble in water, but soluble in many organic solvents. Lipids are usually divided into at least three classes: (1) "simple lipids," which include fats and oils as well as waxes; (2) "compound lipids," which include phospholipids or glycolipids; and (3) "derived lipids" such as steroids.

In the context of the present invention, the term "sterol", also known as steroid alcohol, is a subgroup of steroids that occurs naturally in plants, animal and fungi, or can be produced by some bacteria.

The term "neutral lipid" refers to any of a number of lipid species that exist either in an uncharged or neutral zwitterionic form at a selected pH. At physiological pH, such lipids include, but are not limited to, phosphatidylcholines such as 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), phosphatidylethanolamines such as 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), sphingomyelins (SM), ceramides, steroids such as sterols and their derivatives. Neutral lipids may be synthetic or naturally derived.

In the context of the present disclosure the term "ionizable" in the context of a compound or lipid means the presence of any uncharged group in said compound or lipid which is capable of associating with an ion (usually an H⁺ ion) and thus itself

becoming positively charged (also referred to as "cationisable"). Alternatively, any uncharged group in said compound or lipid may yield an ion (usually an H⁺ ion) and thus becoming negatively charged. In the context of the present disclosure any type of ionizable lipid can suitably be used.

5

The term "lipid nanoparticle" refers to a particle having at least one dimension in the order of nanometers (e.g., 1-1,000 nm) and comprises a plurality of lipid molecules physically associated with each other by intermolecular forces. The lipid nanoparticles may be, e.g., microspheres (including unilamellar and multilamellar vesicles, e.g. liposomes), a dispersed phase in an emulsion, micelles or an internal phase in a suspension. An active agent or therapeutic agent, such as a nucleic acid, is encapsulated in the lipid portion of the lipid nanoparticle or an aqueous space enveloped by some or all of the lipid portion of the lipid nanoparticle, thereby protecting it from enzymatic degradation or other undesirable effects induced by the mechanisms of the host organism or cells e.g. an adverse immune response.

The term "oligonucleotide" or "polynucleotide" as used herein refers to a polymer containing at least two deoxyribonucleotides or ribonucleotides in either single- or double-stranded form and includes DNA, RNA, and hybrids thereof. DNA may be in the form of antisense molecules, plasmid DNA, cDNA, PCR products, or vectors. RNA may be in the form of self-amplifying RNA (saRNA), small hairpin RNA (shRNA), messenger RNA (mRNA), antisense RNA, miRNA, micRNA, multivalent RNA, dicer substrate RNA or viral RNA (vRNA), guide RNA (gRNA), and combinations thereof. Nucleic acids include nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, and which have similar binding properties as the reference nucleic acid. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2'-O-methyl ribonucleotides, and peptide-nucleic acids (PNAs). Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), alleles, orthologs, single nucleotide polymorphisms, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues. "Nucleotides" contain a sugar deoxyribose (DNA)

or ribose (RNA), a base, and a phosphate group. Nucleotides are linked together through the phosphate groups. "Bases" include purines and pyrimidines, which further include natural compounds adenine, thymine, guanine, cytosine, uracil, inosine, and natural analogs, and synthetic derivatives of purines and pyrimidines, which include, but are not limited to, modifications which place new reactive groups such as, but not limited to, amines, alcohols, thiols, carboxylates, and alkylhalides.

As used herein, buffering agents include, but are not limited to, citrate buffer solutions, acetate buffer solutions, phosphate buffer solutions, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium gluconate, calcium gluceptate, calcium gluconate, d-gluconic acid, calcium glycerophosphate, calcium lactate, calcium lactobionate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, amino-sulfonate buffers (e.g. HEPES), magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, and/or combinations thereof.

As used herein, the term N/P ratio, or N:P ratio is the molar ratio of nitrogen atoms in a complexing lipid (or other chemical structure comprising at least one amino group, used for encapsulation) to phosphate groups in an RNA. This ratio describes the interaction between the cationic charge of the amino (N^+) group in amino-lipid (or other chemical structure) to the anionic charge of the phosphate (PO_4^-) groups in the backbone of nucleic acids and is the basis of the complexation of RNA with the amino-lipid (or other chemical structure comprising at least one amino group). The N/P ratio of a lipid/nucleic acid complex can potentially influence other properties such as its net surface charge, size, and stability.

"Effective amount" or "therapeutically effective amount" refers to that amount of a pharmaceutical composition, which, when administered to an animal, preferably a mammal, more preferably a human, is sufficient to effect treatment in the mammal, preferably a human. The amount of the pharmaceutical composition of the invention which constitutes a "therapeutically effective amount" will vary depending on the compound, the condition and its severity, the manner of administration, and the age

of the animal to be treated, but can be determined routinely by one of ordinary skill in the art having regard to his own knowledge and to this disclosure.

5 The term "self-replicating and "self-amplifying" as used herein are used interchangeably and relate to molecules such as RNA comprising within their sequence specific signals or signature sequences that allow the self-replication or self-amplification of said molecule.

10 "Encapsulation efficiency" as used herein refers to the ratio of encapsulated RNA to total RNA in the sample. While there are a handful of methods to characterize encapsulation efficiency of an LNP formulation for its cargo, modified RiboGreen assay is by far the most common. RiboGreen is a dye developed for quantitation of RNA in which fluorescence is produced upon nucleic acid binding. For determination of encapsulation efficiency in LNPs, RiboGreen is first added to the sample with LNPs
15 intact to measure the unencapsulated RNA concentration. A detergent solution (e.g. Triton X-100) is then added to disrupt the nanoparticles, releasing encapsulated RNA, and the total amount of RNA in the sample is calculated from RiboGreen fluorescence. Encapsulated RNA is calculated by subtracting unencapsulated RNA from total RNA and encapsulation efficiency is then taken as the ratio of encapsulated
20 RNA to total RNA in the sample.

"Adjuvants" are usually defined as compounds that can increase and/or modulate the intrinsic immunogenicity of an antigen. To reduce negative side effects, new vaccines have a more defined composition that often leads to lower immunogenicity
25 compared with previous whole- cell or virus-based vaccines. Adjuvants are therefore required to assist new vaccines to induce potent and persistent immune responses, with the additional benefit that less antigen and fewer injections are needed.

"Adjuvant/adjuvant component": An adjuvant or an adjuvant component in the broadest sense is typically a (e.g. pharmacological or immunological) agent or
30 composition that may modify, e.g. enhance, the efficacy of other agents, such as a drug or vaccine. Conventionally the term refers in the context of the invention to a compound or composition that serves as a carrier or auxiliary substance for immunogens and/or other pharmaceutically active compounds. In the context of the
35 present invention an adjuvant will preferably enhance the specific immunogenic effect of the active agents of the present invention. Typically, "adjuvant" or "adjuvant component" has the same meaning and can be used mutually. Adjuvants may be divided, e.g., into immuno potentiators, antigenic delivery systems or even

combinations thereof. The term "adjuvant" is typically understood not to comprise agents which confer immunity by themselves. An adjuvant assists the immune system unspecifically to enhance the antigen-specific immune response by e.g. promoting presentation of an antigen to the immune system or induction of an unspecific innate immune response. Furthermore, an adjuvant may preferably e.g. modulate the antigen-specific immune response by e.g. shifting the dominating Th2-based antigen specific response to a more Th1 -based antigen specific response or vice versa and/or by inducing of mucosal immune responses and/or increased IgA titers. Accordingly, an adjuvant may favourably modulate cytokine expression/secretion, antigen presentation, type of immune response etc.

Advantages of adjuvants include the enhancement of the immunogenicity of antigens, modification of the nature of the immune response, the reduction of the antigen amount needed for a successful immunization, the reduction of the frequency of booster immunizations needed and an improved immune response in elderly and immunocompromised vaccinees. These may be co-administered by any route, e.g., intramuscular/, subcutaneous, IV or intradermal injections.

The term "antigen" as used herein refers to a substance which may be recognized by the immune system and may be capable of triggering an antigen-specific immune response, e.g. by formation of antibodies or antigen-specific T-cells as part of an adaptive immune response.

"Epitope" (also called "antigen determinant") as used herein refers to T cell epitopes that may comprise fragments preferably having a length of about 6 to about 20 or even more amino acids, e.g. fragments as processed and presented by MHC class I molecules, preferably having a length of about 8 to about 10 amino acids, e.g. 8, 9, or 10, (or even 11, or 12 amino acids), or fragments as processed and presented by MHC class II molecules, preferably having a length of about 13 or more amino acids, e.g. 13, 14, 15, 16, 17, 18, 19, 20 or even more amino acids, wherein these fragments may be selected from any part of the amino acid sequence. These fragments are typically recognized by T cells in form of a complex consisting of the peptide fragment and an MHC molecule. B cell epitopes are typically fragments located on the outer surface of (native) protein or peptide antigens.

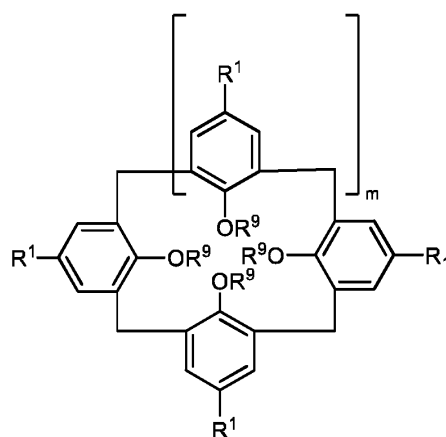
"A vaccine" as used herein refers to a prophylactic or therapeutic material providing at least one antigen or antigenic function. The antigen or antigenic function may stimulate the body's adaptive immune system to provide an adaptive immune response.

A "stereoisomer" refers to a compound made up of the same atoms bonded by the same bonds but having different three-dimensional structures, which are not interchangeable. Embodiments of the present invention contemplates various stereoisomers and mixtures thereof and includes "enantiomers", which refers to two stereoisomers whose molecules are non-superimposable mirror images of one another.

A "tautomer" refers to a proton shift from one atom of a molecule to another atom of the same molecule. Embodiments of the present invention include tautomers of any said compounds.

Compounds

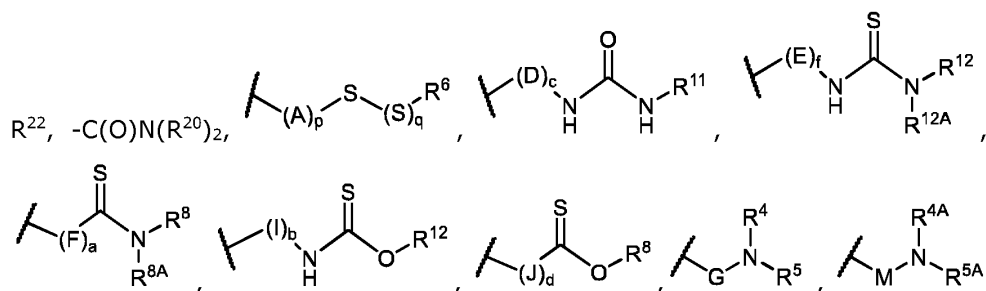
One aspect of the present invention is directed to a compound of Formula (I):

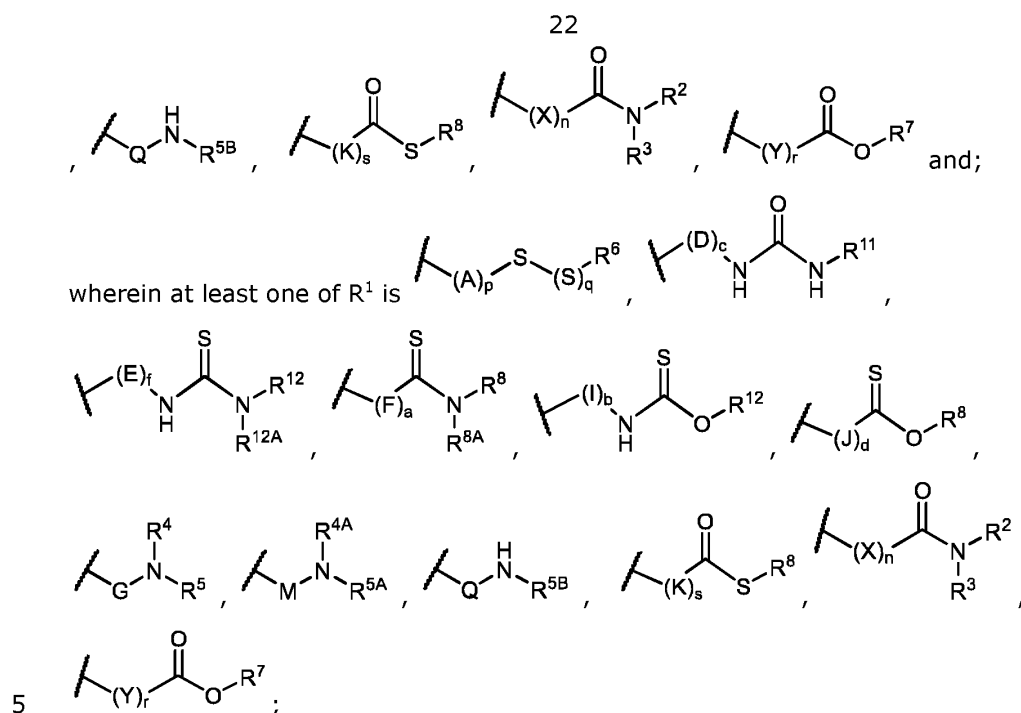


Formula (I)

or a pharmaceutically acceptable salt thereof wherein:

R¹ is selected from hydrogen, halogen, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₁₀ haloalkyl, -CN, -OR²⁰, -SR²⁰, -SF₅, -NO₂, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -O-L-





- A, D, E, F, G, I, J, K, M, Q, X, and Y are each independently selected from C_{1-10} alkylene, C_{2-10} alkenylene, C_{2-10} alkynylene, wherein the C_{1-10} alkylene, C_{2-10} alkenylene, C_{2-10} alkynylene are optionally substituted with one or more substituents independently selected from halogen, C_{1-10} haloalkyl, -CN, -NO₂, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);
- 10 each R², R⁶, R⁸, is independently selected from C_{3-10} alkyl, wherein the C_{3-10} alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);
- 15 each R⁷ is selected from:

- C_{1-2} alkyl, wherein the C_{1-2} alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰); and
- 20

- C_{3-10} alkyl, wherein the C_{3-10} alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted
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with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

5 R^{8A} is independently selected from hydrogen and C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

10 each R¹¹ is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²¹)₂, -N(R²¹)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

15 each R¹² is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)C₁₋₁₀ alkyl, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

20 each R^{12A} is independently selected from hydrogen and C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)C₁₋₁₀ alkyl, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

25 each R³ is independently selected from hydrogen and C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is optionally substituted with one or more substituents independently selected from halogen, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

30 each R⁴ is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is optionally substituted with one or more substituents selected from 3- to 12-membered saturated heterocycle, wherein the 3- to 12-membered saturated heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

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each R^{4A} is independently selected from C_{1-10} alkyl, wherein the C_{1-10} alkyl is substituted with one or more substituents selected from -OH and $-OC_{1-10}$ alkyl; wherein when each R^1 is $-CH_2-N(CH_2CH_2OH)_2$, each R^9 is independently selected from R^{9A} ;

5 each R^5 , R^{5A} is independently selected from C_{1-10} alkyl, wherein the C_{1-10} alkyl is substituted with one substituent selected from $-OR^{20}$ and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, -CN, $-NO_2$, =O, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, -
10 $C(O)N(R^{20})_2$, and $-S(O)_2(R^{20})$;

each R^{5B} is independently selected from C_{1-10} alkyl, wherein the C_{1-10} alkyl is optionally substituted with one substituent selected from $-OR^{20}$ and 3- to 12-membered saturated heterocycle, wherein the 3- to 12-membered saturated heterocycle is optionally substituted with one or more substituents independently
15 selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, -CN, $-NO_2$, =O, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, and $-S(O)_2(R^{20})$;

wherein when R^{5B} is unsubstituted C_{1-10} alkyl, each R^9 is independently selected from R^{9B} ;

each R^9 is independently selected from hydrogen, $-(R^{10})_j-C(O)OR^{20}$, $-(R^{10})_j-C(O)N(R^{20})_2$, $-(R^{10})_j-NHC(S)NHR^{20}$, $-(R^{10})_j-SR^{20}$, $-(R^{10})_j-S-S-R^{20}$, PEG₁₋₂₀₀,
20 mannose, an adjuvant, a carbohydrate, an antibody, C_{1-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl, wherein the C_{1-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl are each optionally substituted with one or more substituents independently selected from halogen, -CN, $-NO_2$, =O, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, -
25 $S(O)_2(R^{20})$, C_{3-12} carbocycle, and 3- to 12-membered heterocycle;

each R^{9A} is independently selected from $-(R^{10})_j-C(O)OR^{20}$, $-(R^{10})_j-C(O)N(R^{20})_2$, $-(R^{10})_j-NHC(S)NHR^{20}$, $-(R^{10})_j-SR^{20}$, $-(R^{10})_j-S-S-R^{20}$, PEG₁₋₂₀₀,
mannose, an adjuvant, a carbohydrate, an antibody, C_{12-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl, wherein the C_{12-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl are each
30 optionally substituted with one or more substituents independently selected from halogen, -CN, $-NO_2$, =O, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, -
 $S(O)_2(R^{20})$, C_{3-12} carbocycle, and 3- to 12-membered heterocycle;

each R^{9B} is independently selected from $-(R^{10})_j-C(O)OR^{20}$, $-(R^{10})_j-C(O)N(R^{20})_2$, $-(R^{10})_j-NHC(S)NHR^{20}$, $-(R^{10})_j-SR^{20}$, $-(R^{10})_j-S-S-R^{20}$, PEG₃₋₂₀₀, mannose,
35 an adjuvant, a carbohydrate, an antibody, C_{5-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl, wherein the C_{5-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl are each optionally substituted with one or more substituents independently selected from halogen, -CN, $-NO_2$, =O,

25

-OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, -S(O)₂(R²⁰), C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle;

each R¹⁰ is independently selected from C₁₋₂₀ alkylene, C₂₋₂₀ alkenylene, and C₂₋₂₀ alkynylene;

5 each R²⁰ is independently selected from hydrogen; and C₁₋₂₀ alkyl, C₂₋₂₀ alkenyl, C₂₋₂₀ alkynyl, C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle, each of which is optionally substituted with one or more substituents independently selected from halogen, -OH, -CN, -NO₂, -NH₂, -N(C₁₋₆ alkyl)₂, -NHC₁₋₆ alkyl, -NHC₁₋₆ hydroxyalkyl, -N(C₁₋₆ alkyl)C₁₋₆ hydroxyalkyl, C₁₋₁₀ alkyl, -C₁₋₁₀ haloalkyl, -O-C₁₋₁₀ alkyl, oxo, =NH, C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle;

10 each R²¹ is independently selected from hydrogen; and C₁₋₂₀ alkyl, C₂₋₂₀ alkenyl, C₂₋₂₀ alkynyl, C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle, each of which is optionally substituted with one or more substituents independently selected from halogen, -OH, -CN, -NO₂, -NH₂, -N(C₁₋₆ alkyl)₂, -NHC₁₋₆ alkyl, -NHC₁₋₆ hydroxyalkyl, C₁₋₁₀ alkyl, -C₁₋₁₀ haloalkyl, -O-C₁₋₁₀ alkyl, =NH, C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle;

each R²² is selected from mannose, an adjuvant, a carbohydrate, and an antibody;

L is selected from a covalent linker;

20 a, b, c, d, f, j, n, p, q, r, and s, are each independently selected from 0, 1, 2, and 3; and
m is selected from 1, 3, and 5.

In a preferred embodiment, m is 1 and the compound is hence a calix[4]arene. In
25 another embodiment, m is 3 and the compound is hence a calix[6]arene. In another embodiment, m is 5 and the compound is hence a calix[8]arene.

In a preferred embodiment, a, b, c, d, f, j, n, p, q, r, and s, are each independently selected from 0 and 1. In another embodiment, a, b, c, d, f, j, n, p, q, r, and s, are
30 each independently selected from 0. In another embodiment, a is 0. In another embodiment, b is 0. In another embodiment, c is 0. In another embodiment, d is 0. In another embodiment, f is 0. In another embodiment, j is 0. In another embodiment, n is 0. In another embodiment, p is 0. In another embodiment, q is 0. In another embodiment, r is 0. In another embodiment, s is 0. In another
35 embodiment, a is 1. In another embodiment, b is 1. In another embodiment, c is 1. In another embodiment, d is 1. In another embodiment, f is 1. In another embodiment, j is 1. In another embodiment, n is 1. In another embodiment, p is 1.

In another embodiment, q is 1. In another embodiment, r is 1. In another embodiment, s is 1.

5 The inventors found that the compounds according to the current invention are especially useful for use in *in vivo* delivery systems for nucleotides. In a preferred embodiment, the compounds according to the current invention are used in delivery systems for mRNA vaccines. The encapsulation of negatively charged nucleotides largely depends on the interaction with positively charged compounds. The selection of such positively charged compounds is of major importance as they aid in the
10 entrapment of RNA molecules and in facilitating endosomal escape. The compounds according to the current invention provide for certain advantages.

The compounds according to the current invention have a controllable size, rigidity and geometry. Compounds with a fixed number (4, 6, 8) of phenolic units may be
15 obtained. In addition, the compounds adopt a conical conformation easily. This conformation is a key property to facilitate nucleotide (RNA) release through membrane destabilization and endosomal escape.

Furthermore, the calixarene compounds according to the current invention allow
20 easy derivatization (on the lower and upper rim) to yield amphiphilic compounds that self-assemble into micelles in water, and can contain a payload, either in their cavity or through formation of ionic pairs between their functional groups and the charged payload (e.g., nucleotides).

25 In addition, the compounds according to the current invention allow to modulate the number of cationic head groups (the R¹ group) onto the same molecule. Increasing the number of cationic heads improves the encapsulation efficiency and the nucleotide (e.g. mRNA) release by increasing the N/P ratio without modifying mass ratio between mRNA and the cationic component.

30 Furthermore, calixarenes constitute a unique opportunity to design very modulable ionizable components for RNA delivery. Indeed, as the different phenolic units of calixarenes can be derivatized separately (particularly true for calix[4]arenes), molecules with a variable number of ionizable heads and hydrophobic tails can be
35 designed. Several chemically different heads or tails could even be combined to precisely control the encapsulation/delivery properties or to improve immunogenicity of the resulting delivery particles by introducing adjuvant patterns.

Furthermore, the inventors unexpectedly discovered that calixarenes in itself have an adjuvant effect and that calixarenes can be used in an immunogenic composition, wherein said composition comprises an immunogenic component encapsulated in a lipid nanoparticle comprising said calixarene and wherein said LNP is an adjuvant for said immunogenic composition. Examples below describe this adjuvant effect of the calixarenes.

In an embodiment, said lipid nanoparticle further comprising a PEGylated lipid. In a further embodiment, said lipid nanoparticle further comprises a sterol and/or a phospholipid. In an embodiment, said lipid nanoparticle comprises an ionizable calixarene as adjuvant and further comprises a PEGylated lipid, a sterol and a phospholipid. In an embodiment, said lipid nanoparticle comprises a cationic calixarene as adjuvant and further comprises a PEGylated lipid, a sterol, an ionizable lipid and a phospholipid.

In a preferred embodiment, said lipid nanoparticle comprises at least one calixarene which is a cationic calixarene, comprising at least one moiety that is positively charged, and/or at least one calixarene which is an ionizable calixarene, comprising at least one moiety that is capable of associating with an ion and becoming positively charged. In a further embodiment, said positively charged moiety is an amine-bearing group comprising a secondary, ternary and/or quaternary amine. In an embodiment, said calixarene is an ionizable calix[4]arene having 4 head groups, wherein at least one of said head groups comprises at least one secondary or ternary amine (examples include for instance CX5 having 4 identical head groups having one ternary amine group, depicted in figure 16). In a further embodiment, said calixarene is a cationic calix[4]arene having 4 head groups, wherein at least one of said head groups comprises at least one quaternary amine. In an alternative further embodiment, said calixarene is a cationic calix[4]arene having 4 head groups, wherein at least one of said head groups comprises at least one quaternary amine (examples include for instance CX4, having 4 identical head groups having one quaternary amine group).

When referring to the "mass fraction" or the "concentration expressed as (w/w)" of the calixarene component or the lipid component relative to the overall weight of the formulation, the weight of the calixarene component or the lipid component is relative to the weight of the total sum of the calixarenes and lipids (hence excluding the cargo (e.g. RNA) and other excipients (e.g. sucrose, TRIS, etc.)) present in the delivery system. Hence, the mass fraction or concentration expressed as (w/w)

refers to the mass of the lipid or calixarene with respect to the total mass of "lipid plus calixarene".

When used as an adjuvant in an immunogenic composition, said calixarene is preferably present in said lipid nanoparticle at a concentration of 0.1- 60% (w/w). In an embodiment, when the calixarene is ionizable, it is present in said lipid nanoparticle at a concentration of 10-60 % (w/w). In an embodiment, when used as an adjuvant in an immunogenic composition, said ionizable calixarene is present in said lipid nanoparticle at a concentration of 10-60 % (w/w), such as 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59% or 60% (w/w) or any value in between. In an embodiment, when used as an adjuvant in an immunogenic composition, said ionizable calixarene is present in said lipid nanoparticle at a concentration between 10-15%, 15-20%, 20-25%, 25-30%, 30-35%, 35-40%, 40-45%, 45-50%, 50-55% or 55-60% (w/w). In an embodiment, when used as an adjuvant in an immunogenic composition, said ionizable calixarene is present in said lipid nanoparticle at a concentration between 10-20%, 20-30%, 30-40%, 40-50% or 50-60% (w/w).

In an embodiment, when the calixarene is cationic, it is present in said lipid nanoparticle at a concentration of 0.1-50 % (w/w). In an embodiment, when used as an adjuvant in an immunogenic composition, said cationic calixarene is present in said lipid nanoparticle at a concentration of 0.1-50 % (w/w), preferably 0.2-10 % (w/w), such as 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 5.5%, 6%, 6.5%, 7%, 7.5, 8%, 8.5%, 9%, 9.5%, 10% (w/w) or any value in between. In an embodiment, when used as an adjuvant in an immunogenic composition, said cationic calixarene is present in said lipid nanoparticle at a concentration between 0.1-10%, 10-20%, 20-30%, 30-40% or 40-50% (w/w). In an embodiment, when used as an adjuvant in an immunogenic composition, said cationic calixarene is present in said lipid nanoparticle at a concentration between 0.1-1%, 1-2%, 2-3%, 3-4%, 4-5%, 5-6%, 6-7%, 7-8%, 8-9%, 9-10%, 10-11%, 11-12%, 12-13%, 13-14% or 14-15% (w/w).

When used as an adjuvant, the calixarene mass comprised in said lipid nanoparticle is preferably less than 10 times, more preferably less than 5 times, more preferably lower than the nucleic acid mass encapsulated in said lipid nanoparticle.

As such, the calixarene is comprised in the delivery system of the current invention, providing the advantages as described above related to the efficient delivery (efficiently encapsulating nucleic acids, facilitating the encapsulation of very long RNA and/or minimizing the inherent toxicity of cationic components and/or reducing the non-specific adsorption of proteins that usually limits their efficacy), but also functioning on the level of immunogenic response when used as an adjuvant in an immunogenic composition comprising an immunogenic component encapsulated in a lipid nanoparticle comprising said calixarene.

Similarly to delivery system as described above, said lipid nanoparticle can further comprise an ionizable lipid. In an embodiment, said ionizable lipid is present in said immunogenic composition at a concentration of at most 60 % (w/w).

When the calixarene is used as an adjuvant in an immunogenic composition, said immunogenic component preferably comprises at least one nucleic acid molecule encoding at least one epitope of at least one antigen. In a preferred embodiment, said encoding nucleic acid molecule is a mRNA or an saRNA molecule.

Preferably, said immunogenic composition is intramuscularly administered to a subject.

In a further aspect, the disclosure relates to a vaccine, wherein said vaccine comprises an immunogenic component encapsulated in a lipid nanoparticle, wherein said lipid nanoparticle comprises at least one calixarene molecule acting as an adjuvant in said vaccine.

In an embodiment, said vaccine is a DNA vaccine, said immunogenic component comprising DNA. In an embodiment, said vaccine is a RNA vaccine, said immunogenic component comprising RNA. In an embodiment, said vaccine is a cancer vaccine. In a preferred embodiment, said calixarene increases the immunogenicity of the immunogenic component after *in vivo* administration to a subject as measured by an elevated virus neutralizing titer (VNT) as compared to the same immunogenic composition not comprising a calixarene.

In a further aspect, the invention relates to a method of preparing an immunogenic preparation such as a vaccine, comprising a step of encapsulating an immunogenic component into a lipid nanoparticle, said lipid nanoparticle comprises at least one calixarene molecule acting as an adjuvant in said lipid nanoparticle.

30

As described above, when used as an adjuvant, said calixarene is preferably an ionizable or a cationic calixarene and said immunogenic component is preferably a nucleotide, such as a mRNA or an saRNA molecule.

5

As a comparison, the flexibility exhibited by traditional lipid or lipid platform is by far lower. Traditional ionizable lipids usually bear only one ionizable head and introduction of supplementary hydrophobic tails to enhance their cone-shaped conformation usually requires multi-step synthesis, while this last is a natural
10 property of calixarenes. Concerning lipids, their derivatization usually leads to symmetric compounds, without any possibility to modulate the nature of the ionizable heads and hydrophobic tails between them.

As recently demonstrated for several lipid structures, increasing the number of
15 ionizable head groups usually improves the encapsulation efficiency and thermostability and the RNA release by increasing the N/P ratio (charge density) without modifying mass ratio between RNA and the ionizable component. This allows to increase the stability and safety of the delivery system. With calixarenes, this number of ionizable heads may reach 8, allowing us to consider the delivery of very
20 long RNA molecules, such as saRNA, which remain very challenging with the current LNP technology based on ionizable lipids. In the same time, for traditional non-replicating mRNA, increasing the number of ionizable functions will allow us to reduce the quantity of ionizable component needed for good encapsulation and release, thereby diminishing the overall cost of the delivery system.

25

In addition, the compounds according to the current invention allow to modulate the nature of the hydrophobic tail (the R⁹ group) and to combine different hydrophobic motifs on the same molecule. These motifs can for instance provide adjuvanticity to the molecule, help with the endosomal escape or help target tissues or cells.

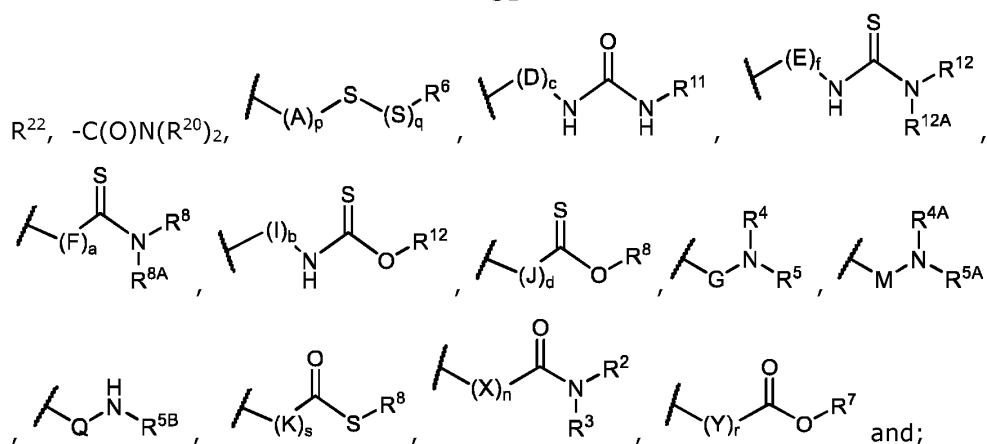
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The compounds according to the current invention allow for easy incorporation of biodegradable functionalities (ester, amide, disulfide bridge ...) to link the hydrophobic tails and the cationic heads on the macrocyclic core.

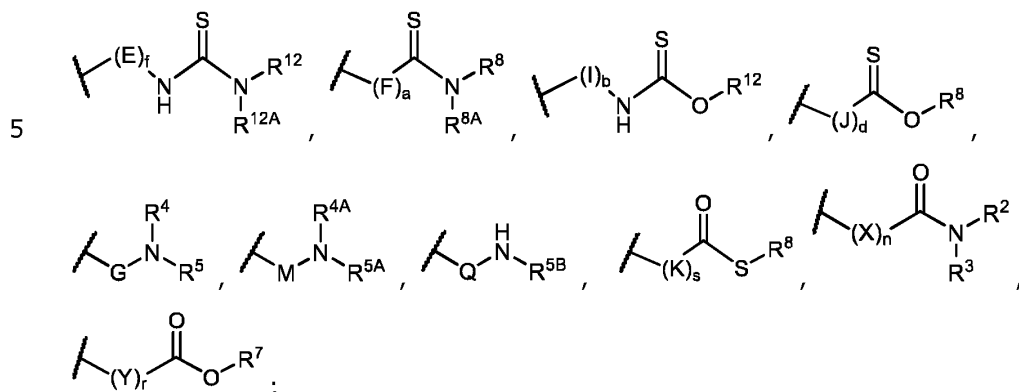
35 In an embodiment, the invention relates to a compound or salt as described above,

R¹ is selected from hydrogen, halogen, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₁₀ haloalkyl, -CN, -OR²⁰, -SR²⁰, -SF₅, -NO₂, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -O-L-

31



wherein at least one of R^1 is $\text{---}(A)_p-S-(S)_q-R^6$, $\text{---}(D)_c-NH-C(=O)-NH-R^{11}$,



A, D, E, F, G, I, J, K, M, Q, X, and Y are each independently selected from C_{1-10} alkylene, C_{2-10} alkenylene, C_{2-10} alkynylene, wherein the C_{1-10} alkylene, C_{2-10} alkenylene, C_{2-10} alkynylene are optionally substituted with one or more substituents independently selected from halogen, C_{1-10} haloalkyl, -CN, -NO₂, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

each R², R⁶, R⁷, R⁸, is independently selected from C_{3-10} alkyl, wherein the C_{3-10} alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

R^{8A} is independently selected from hydrogen and C_{1-10} alkyl, wherein the C_{1-10} alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered

heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

each R¹¹ is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²¹)₂, -N(R²¹)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

each R¹² is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)C₁₋₁₀ alkyl, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

each R^{12A} is independently selected from hydrogen and C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)C₁₋₁₀ alkyl, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

each R³ is independently selected from hydrogen and C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is optionally substituted with one or more substituents independently selected from halogen, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

each R⁴ is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is optionally substituted with one or more substituents selected from 3- to 12-membered saturated heterocycle, wherein the 3- to 12-membered saturated heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

each R^{4A} is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with one or more substituents selected from -OH and -OC₁₋₁₀ alkyl; wherein when each R¹ is -CH₂-N(CH₂CH₂OH)₂, each R⁹ is independently selected from R^{9A};

each R⁵, R^{5A} is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with one substituent selected from -OR²⁰ and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally

substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

each R^{5B} is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is optionally substituted with one substituent selected from -OR²⁰ and 3- to 12-membered saturated heterocycle, wherein the 3- to 12-membered saturated heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

wherein when R^{5B} is unsubstituted C₁₋₁₀ alkyl, each R⁹ is independently selected from R^{9B};

each R⁹ is independently selected from hydrogen, -(R¹⁰)_j-C(O)OR²⁰, -(R¹⁰)_j-C(O)N(R²⁰)₂, -(R¹⁰)_j-NHC(S)NHR²⁰, -(R¹⁰)_j-SR²⁰, -(R¹⁰)_j-S-S-R²⁰, PEG₁₋₂₀₀, mannose, an adjuvant, a carbohydrate, an antibody, C₁₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl, wherein the C₁₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl are each optionally substituted with one or more substituents independently selected from halogen, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, -S(O)₂(R²⁰), C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle;

each R^{9A} is independently selected from -(R¹⁰)_j-C(O)OR²⁰, -(R¹⁰)_j-C(O)N(R²⁰)₂, -(R¹⁰)_j-NHC(S)NHR²⁰, -(R¹⁰)_j-SR²⁰, -(R¹⁰)_j-S-S-R²⁰, PEG₁₋₂₀₀, mannose, an adjuvant, a carbohydrate, an antibody, C₁₂₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl, wherein the C₁₂₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl are each optionally substituted with one or more substituents independently selected from halogen, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, -S(O)₂(R²⁰), C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle;

each R^{9B} is independently selected from -(R¹⁰)_j-C(O)OR²⁰, -(R¹⁰)_j-C(O)N(R²⁰)₂, -(R¹⁰)_j-NHC(S)NHR²⁰, -(R¹⁰)_j-SR²⁰, -(R¹⁰)_j-S-S-R²⁰, PEG₃₋₂₀₀, mannose, an adjuvant, a carbohydrate, an antibody, C₅₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl, wherein the C₅₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl are each optionally substituted with one or more substituents independently selected from halogen, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, -S(O)₂(R²⁰), C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle;

each R¹⁰ is independently selected from C₁₋₂₀ alkylene, C₂₋₂₀ alkenylene, and C₂₋₂₀ alkynylene;

each R²⁰ is independently selected from hydrogen; and C₁₋₂₀ alkyl, C₂₋₂₀ alkenyl, C₂₋₂₀ alkynyl, C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle, each of which is optionally substituted with one or more substituents independently selected from halogen, -OH, -CN, -NO₂, -NH₂, -N(C₁₋₆ alkyl)₂, -NHC₁₋₆ alkyl, -NHC₁₋₆

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hydroxyalkyl, -N(C₁₋₆ alkyl)C₁₋₆ hydroxyalkyl, C₁₋₁₀ alkyl, -C₁₋₁₀ haloalkyl, -O-C₁₋₁₀ alkyl, oxo, =NH, C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle;

each R²¹ is independently selected from hydrogen; and C₁₋₂₀ alkyl, C₂₋₂₀ alkenyl, C₂₋₂₀ alkynyl, C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle, each of which is optionally substituted with one or more substituents independently selected from halogen, -OH, -CN, -NO₂, -NH₂, -N(C₁₋₆ alkyl)₂, -NHC₁₋₆ alkyl, -NHC₁₋₆ hydroxyalkyl, C₁₋₁₀ alkyl, -C₁₋₁₀ haloalkyl, -O-C₁₋₁₀ alkyl, =NH, C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle;

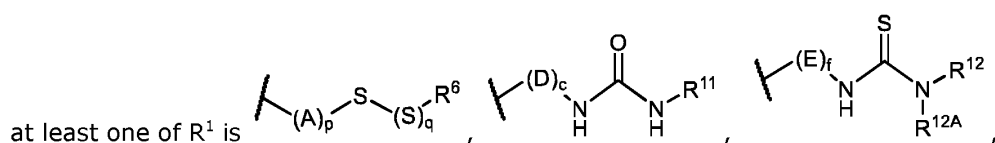
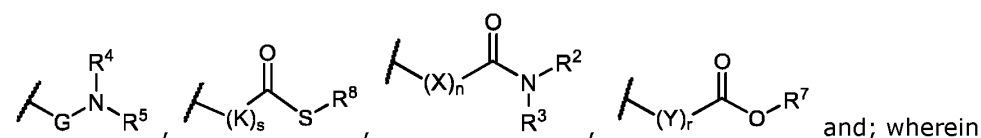
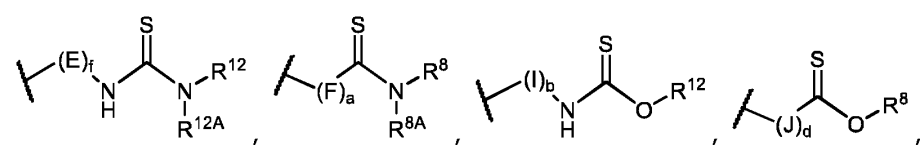
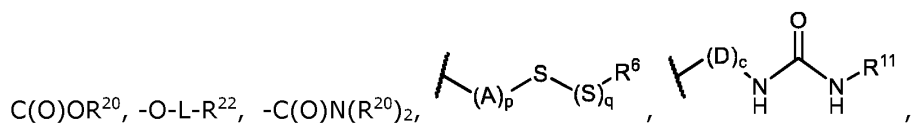
each R²² is selected from mannose, an adjuvant, a carbohydrate, and an antibody;

L is selected from a covalent linker;

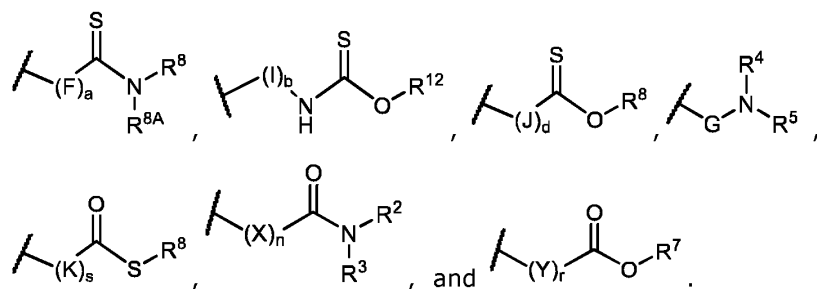
a, b, c, d, f, j, n, p, q, r, and s, are each independently selected from 0, 1, 2, and 3; and

m is selected from 1, 3, and 5.

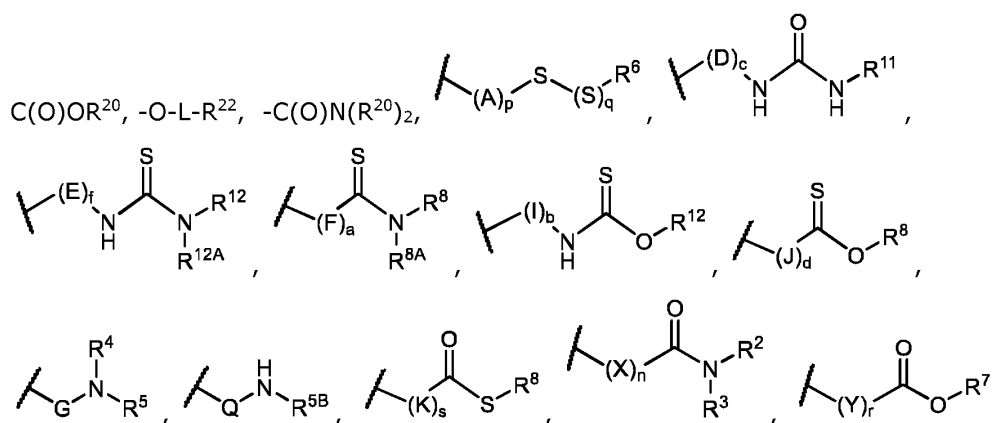
In an embodiment, the invention relates to a compound or salt as described above, wherein each R¹ is selected from hydrogen, halogen, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₁₀ haloalkyl, -CN, -OR²⁰, -SR²⁰, -SF₅, -NO₂, -N(R²⁰)₂, -C(O)R²⁰, -



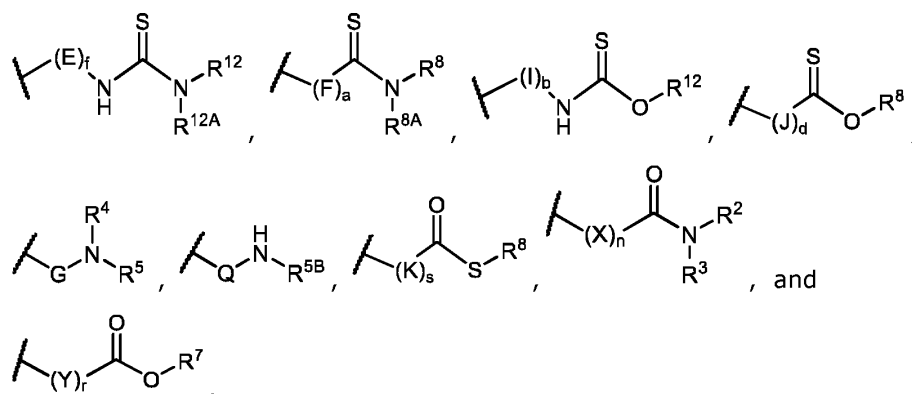
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- 5 In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is selected from hydrogen, halogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-10} haloalkyl, $-\text{CN}$, $-\text{OR}^{20}$, $-\text{SR}^{20}$, $-\text{SF}_5$, $-\text{NO}_2$, $-\text{N(R}^{20})_2$, $-\text{C(O)R}^{20}$, $-\text{C(O)OR}^{20}$, $-\text{O-L-R}^{22}$, $-\text{C(O)N(R}^{20})_2$,

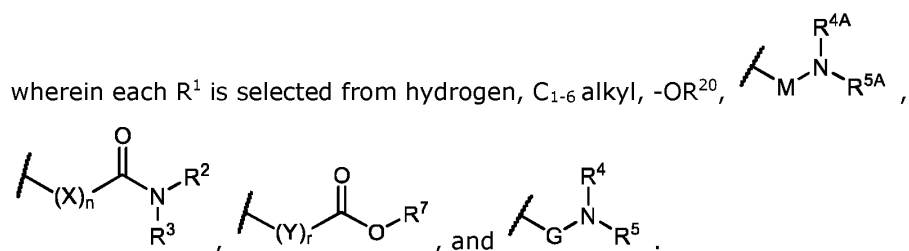


- 10 and; wherein at least one of R^1 is $\text{---(A)}_p\text{---S---(S)}_q\text{---R}^6$, $\text{---(D)}_c\text{---NH-C(=O)N(R}^{11}\text{)}$,

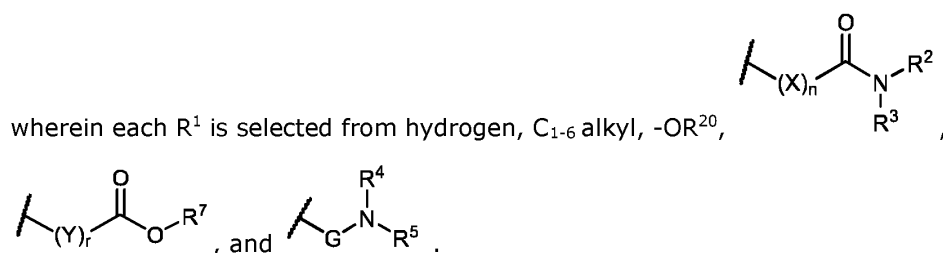


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In an embodiment, the invention relates to a compound or salt as described above,



5 In an embodiment, the invention relates to a compound or salt as described above,



In an embodiment, the invention relates to a compound or salt as described above,

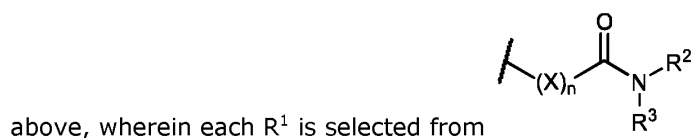
10 wherein each R^1 is selected from $\text{---}(X)_n\text{---}C(=O)N(R^2)(R^3)$. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is

selected from $\text{---}(Y)_r\text{---}C(=O)OR^7$. In another embodiment, the invention relates to a

compound or salt as described above, wherein each R^1 is selected from $\text{---}G\text{---}N(R^4)(R^5)$.

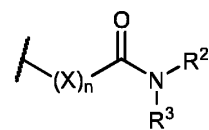
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As described above, the invention may relate to a compound or salt as described

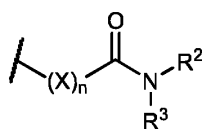


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In an embodiment, the invention relates to a compound or salt as described above,

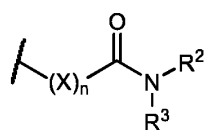


wherein each R^1 is selected from hydrogen, -OH, and R^3 . In another embodiment, the invention relates to a compound or salt as described above, each



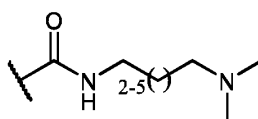
R^1 is selected from hydrogen and R^3 . In another embodiment, the

5 invention relates to a compound or salt as described above, each R^1 is selected from

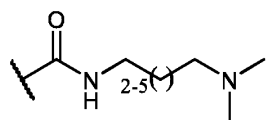


-OH and R^3 . In another embodiment, the invention relates to a compound or salt as described above, wherein n is 0. In another embodiment, the invention relates to a compound or salt as described above, wherein n is 1.

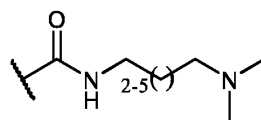
In an embodiment, the invention relates to a compound or salt as described above,



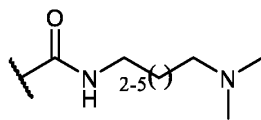
10 wherein each R^1 is selected from R^3 . In an embodiment, the invention relates to a compound or salt as described above, wherein at least one R^1



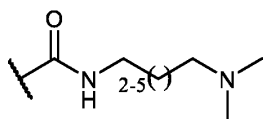
is R^3 . In an embodiment, the invention relates to a compound



or salt as described above, wherein one R^1 is R^3 . In an embodiment, the invention relates to a compound or salt as described above,

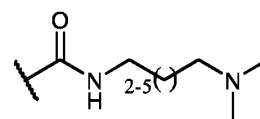


15 wherein at least two R^1 are R^3 . In an embodiment, the invention relates to a compound or salt as described above, wherein two R^1 are

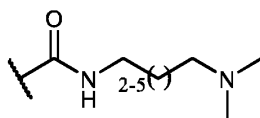


R^3 . In an embodiment, the invention relates to a compound or

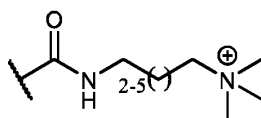
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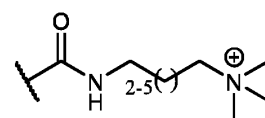
salt as described above, wherein at least three R^1 are . In an embodiment, the invention relates to a compound or salt as described above,



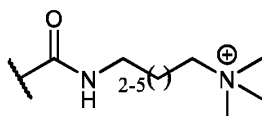
wherein three R^1 are . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is selected from



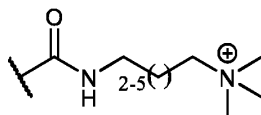
5 . In an embodiment, the invention relates to a compound or



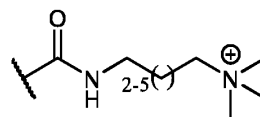
salt as described above, wherein at least one R^1 is . In an embodiment, the invention relates to a compound or salt as described above,



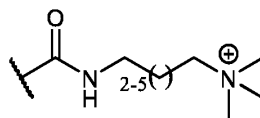
wherein one R^1 is . In an embodiment, the invention relates to a compound or salt as described above, wherein at least two R^1 are



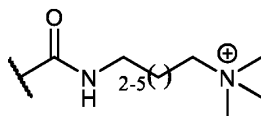
10 . In an embodiment, the invention relates to a compound or



salt as described above, wherein two R^1 are . In an embodiment, the invention relates to a compound or salt as described above,

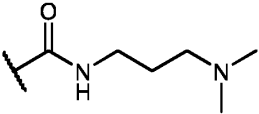


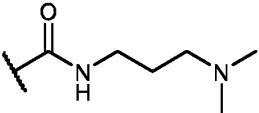
wherein at least three R^1 are . In an embodiment, the invention relates to a compound or salt as described above, wherein three R^1 are

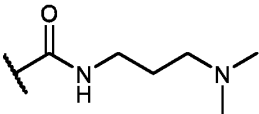


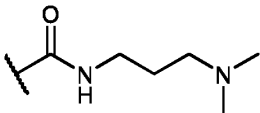
15 . In an embodiment, the invention relates to a compound or

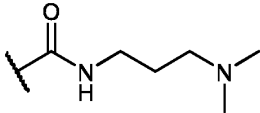
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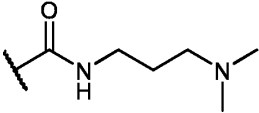
salt as described above, wherein each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above,

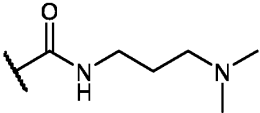
wherein at least one R^1 is . In an embodiment, the invention relates to a compound or salt as described above, wherein one R^1 is

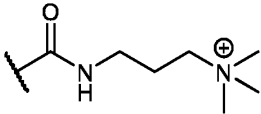
5 . In an embodiment, the invention relates to a compound or

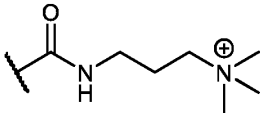
salt as described above, wherein at least two R^1 are . In an embodiment, the invention relates to a compound or salt as described above,

wherein two R^1 are . In an embodiment, the invention relates to a compound or salt as described above, wherein at least three R^1 are

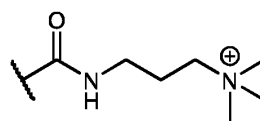
10 . In an embodiment, the invention relates to a compound or

salt as described above, wherein three R^1 are . In an embodiment, the invention relates to a compound or salt as described above,

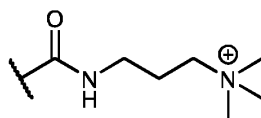
wherein each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein at least one R^1

15 is . In an embodiment, the invention relates to a compound

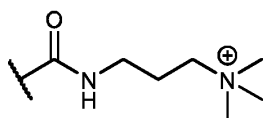
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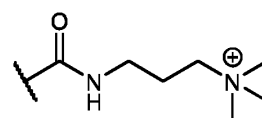
or salt as described above, wherein one R^1 is . In an embodiment, the invention relates to a compound or salt as described above,



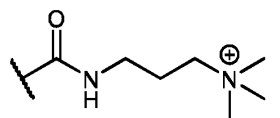
wherein at least two R^1 are . In an embodiment, the invention relates to a compound or salt as described above, wherein two R^1 are



5 . In an embodiment, the invention relates to a compound or



salt as described above, wherein at least three R^1 are . In an embodiment, the invention relates to a compound or salt as described above,



wherein three R^1 are

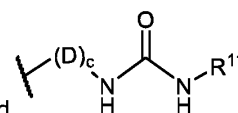
10

In an embodiment, the invention relates to a compound or salt as described above,

wherein each R^1 is selected from hydrogen, -OH, and $\text{---}(\text{A})_p\text{---}(\text{S})_q\text{---}R^6$. In another embodiment, the invention relates to a compound or salt as described above, wherein p is 0. In another embodiment, the invention relates to a compound or salt as described above, wherein p is 1. In another embodiment, the invention relates to a compound or salt as described above, wherein q is 0. In another embodiment, the invention relates to a compound or salt as described above, wherein q is 1.

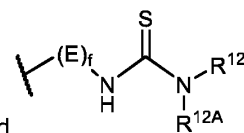
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In an embodiment, the invention relates to a compound or salt as described above,



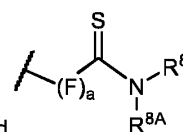
20 wherein each R^1 is selected from hydrogen, -OH, and . In another embodiment, the invention relates to a compound or salt as described above, wherein c is 0. In another embodiment, the invention relates to a compound or salt as described above, wherein c is 1.

In an embodiment, the invention relates to a compound or salt as described above,



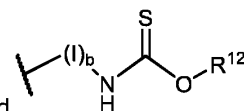
wherein each R^1 is selected from hydrogen, -OH, and R^{12A} . In another embodiment, the invention relates to a compound or salt as described above, wherein f is 0. In another embodiment, the invention relates to a compound or salt as described above, wherein f is 1.

In an embodiment, the invention relates to a compound or salt as described above,



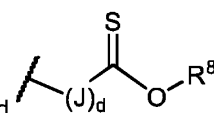
wherein each R^1 is selected from hydrogen, -OH, and R^{8A} . In another embodiment, the invention relates to a compound or salt as described above, wherein a is 0. In another embodiment, the invention relates to a compound or salt as described above, wherein a is 1.

In an embodiment, the invention relates to a compound or salt as described above,



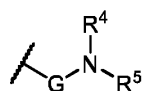
wherein each R^1 is selected from hydrogen, -OH, and R^{12A} . In another embodiment, the invention relates to a compound or salt as described above, wherein b is 0. In another embodiment, the invention relates to a compound or salt as described above, wherein b is 1.

In an embodiment, the invention relates to a compound or salt as described above,



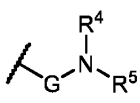
wherein each R^1 is selected from hydrogen, -OH, and R^{8A} . In another embodiment, the invention relates to a compound or salt as described above, wherein d is 0. In another embodiment, the invention relates to a compound or salt as described above, wherein d is 1.

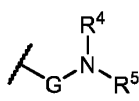
In an embodiment, the invention relates to a compound or salt as described above,

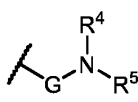


wherein each R^1 is selected from hydrogen, -OH, and R^5 . In another embodiment, the invention relates to a compound or salt as described above,

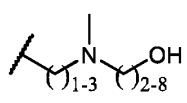
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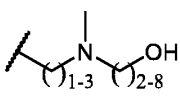
wherein each R^1 is selected from hydrogen and . In another embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is

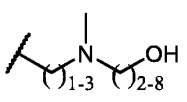
selected from -OH and . In another embodiment, the invention relates

to a compound or salt as described above, wherein each R^1 is selected .

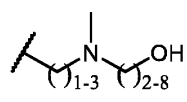
5 In an embodiment, the invention relates to a compound or salt as described above,

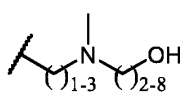
wherein each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein at least one R^1 is

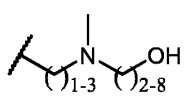
. In an embodiment, the invention relates to a compound or salt as

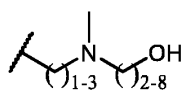
described above, wherein one R^1 is . In an embodiment, the

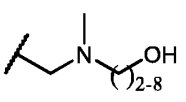
10 invention relates to a compound or salt as described above, wherein at least two R^1

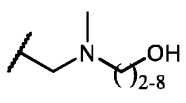
are . In an embodiment, the invention relates to a compound or salt

as described above, wherein two R^1 are . In an embodiment, the invention relates to a compound or salt as described above, wherein at least three

R^1 are . In an embodiment, the invention relates to a compound or

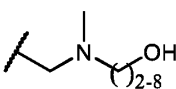
15 salt as described above, wherein three R^1 are . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is

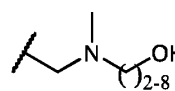
selected from . In an embodiment, the invention relates to a

compound or salt as described above, wherein at least one R^1 is . In

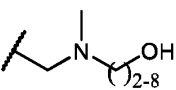
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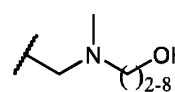
an embodiment, the invention relates to a compound or salt as described above,

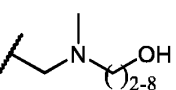
wherein one R^1 is . In an embodiment, the invention relates to a

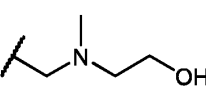
compound or salt as described above, wherein at least two R^1 are .

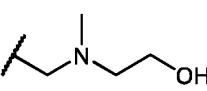
In an embodiment, the invention relates to a compound or salt as described above,

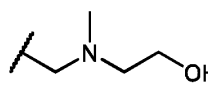
5 wherein two R^1 are . In an embodiment, the invention relates to a

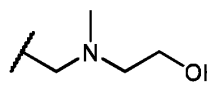
compound or salt as described above, wherein at least three R^1 are . In an embodiment, the invention relates to a compound or salt as described above,

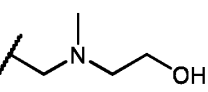
wherein three R^1 are . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is selected from

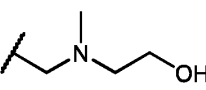
10 . In an embodiment, the invention relates to a compound or salt

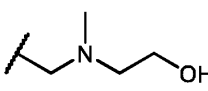
as described above, wherein at least one R^1 is . In an embodiment, the invention relates to a compound or salt as described above,

wherein one R^1 is . In an embodiment, the invention relates to a

15 compound or salt as described above, wherein at least two R^1 are . In an embodiment, the invention relates to a compound or salt as described above,

wherein two R^1 are . In an embodiment, the invention relates to a compound or salt as described above, wherein at least three R^1 are

. In an embodiment, the invention relates to a compound or salt

as described above, wherein three R^1 are .

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In an embodiment, the invention relates to a compound or salt as described above,

wherein each R^1 is selected from hydrogen, -OH, and $\text{---}(K)_s\text{---C(=O)S-R}^8$. In another embodiment, the invention relates to a compound or salt as described above, wherein s is 0. In another embodiment, the invention relates to a compound or salt as described above, wherein s is 1.

In an embodiment, the invention relates to a compound or salt as described above,

wherein each R^1 is selected from hydrogen, -OH, and $\text{---}(X)_n\text{---C(=O)N(R}^2\text{)(R}^3\text{)}$. In another embodiment, the invention relates to a compound or salt as described above,

10 wherein each R^1 is selected from hydrogen and $\text{---}(X)_n\text{---C(=O)N(R}^2\text{)(R}^3\text{)}$. In another embodiment, the invention relates to a compound or salt as described above,

wherein each R^1 is selected from -OH and $\text{---}(X)_n\text{---C(=O)N(R}^2\text{)(R}^3\text{)}$. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is

selected from $\text{---}(X)_n\text{---C(=O)N(R}^2\text{)(R}^3\text{)}$.

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In another embodiment, the invention relates to a compound or salt as described above, wherein n is 0. In another embodiment, the invention relates to a compound or salt as described above, wherein n is 1.

20 In an embodiment, the invention relates to a compound or salt as described above,

wherein each R^1 is selected from hydrogen, -OH, and $\text{---}(Y)_r\text{---C(=O)O-R}^7$. In another embodiment, the invention relates to a compound or salt as described above,

wherein each R^1 is selected from hydrogen and $\text{---}(Y)_r\text{---C(=O)O-R}^7$. In another

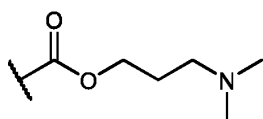
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embodiment, the invention relates to a compound or salt as described above,

wherein each R^1 is selected from $-OH$, and $\text{---}(\text{Y})_r\text{---C(=O)OR}^7$. In another embodiment, the invention relates to a compound or salt as described above,

wherein each R^1 is selected from $\text{---}(\text{Y})_r\text{---C(=O)OR}^7$. In an embodiment, the invention

5 relates to a compound or salt as described above, wherein each R^1 is selected from



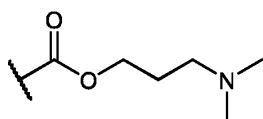
. In an embodiment, the invention relates to a compound or

salt as described above, wherein at least one R^1 is . In an embodiment, the invention relates to a compound or salt as described above,

wherein one R^1 is . In an embodiment, the invention relates to a

10 compound or salt as described above, wherein at least two R^1 are . In an embodiment, the invention relates to a compound or salt as described above,

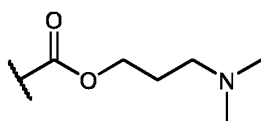
wherein two R^1 are . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is selected from



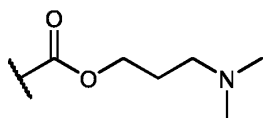
. In an embodiment, the invention relates to a compound or

15 salt as described above, wherein at least one R^1 is . In an embodiment, the invention relates to a compound or salt as described above,

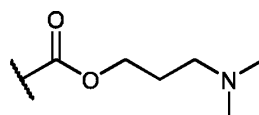
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wherein one R^1 is . In an embodiment, the invention relates to a compound or salt as described above, wherein at least two R^1 are

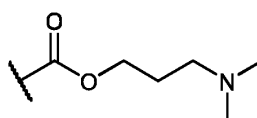


. In an embodiment, the invention relates to a compound or

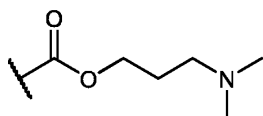


salt as described above, wherein two R^1 are . In an

5 embodiment, the invention relates to a compound or salt as described above,

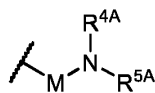


wherein at least three R^1 are . In an embodiment, the invention relates to a compound or salt as described above, wherein three R^1 are

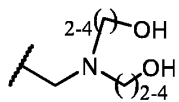


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In an embodiment, the invention relates to a compound or salt as described above,

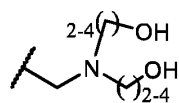


wherein each R^1 is selected from . In another embodiment, the invention relates to a compound or salt as described above, M is independently selected from C_{1-10} alkylene. In another embodiment, the invention relates to a compound or salt as described above, M is independently selected from C_2 alkylene. In another embodiment, the invention relates to a compound or salt as described above, M is independently selected from C_1 alkylene. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is

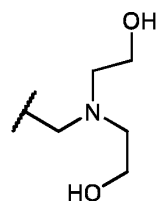


selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein at least one R^1 is selected from

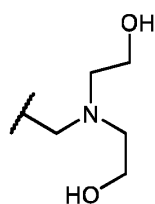
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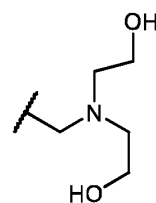
. In an embodiment, the invention relates to a compound or salt as



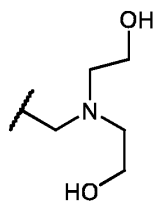
described above, wherein each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein at least one



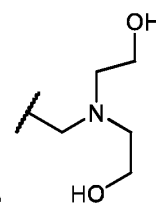
R^1 is selected from . In an embodiment, the invention relates to a



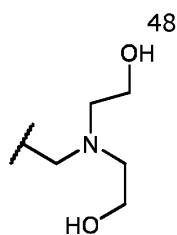
5 compound or salt as described above, wherein one R^1 is . In an embodiment, the invention relates to a compound or salt as described above,



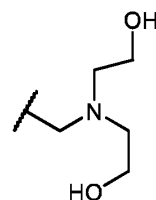
wherein at least two R^1 are . In an embodiment, the invention relates



to a compound or salt as described above, wherein two R^1 are . In an embodiment, the invention relates to a compound or salt as described above,

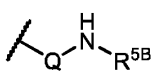


wherein at least three R^1 are . In an embodiment, the invention relates

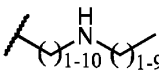


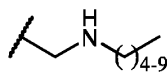
to a compound or salt as described above, wherein three R^1 are .

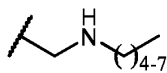
In an embodiment, the invention relates to a compound or salt as described above,

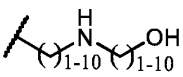
- 5 wherein each R^1 is selected from  . In another embodiment, the invention relates to a compound or salt as described above, Q is independently selected from C_{1-10} alkylene. In another embodiment, the invention relates to a compound or salt as described above, Q is independently selected from C_3 alkylene. In another embodiment, the invention relates to a compound or salt as described above, Q is independently selected from C_2 alkylene. In another embodiment, the invention relates to a compound or salt as described above, Q is independently selected from C_1 alkylene. In another embodiment, the invention relates to a compound or salt as described above, each R^{5B} is independently selected from C_{1-10} alkyl. In another embodiment, the invention relates to a compound or salt as described above, each R^{5B} is independently selected from C_{1-10} alkyl, wherein the C_{1-10} alkyl is unsubstituted. In another embodiment, the invention relates to a compound or salt as described above, each R^{5B} is independently selected from C_{3-10} alkyl, wherein the C_{3-10} alkyl is unsubstituted. In another embodiment, the invention relates to a compound or salt as described above, each R^{5B} is independently selected from C_{5-10} alkyl, wherein the C_{5-10} alkyl is unsubstituted. In another embodiment, the invention relates to a compound or salt as described above, each R^{5B} is independently selected from C_{5-8} alkyl, wherein the C_{5-8} alkyl is unsubstituted. In another embodiment, the invention relates to a compound or salt as described above, each R^{5B} is independently selected from C_{1-10} alkyl, wherein the C_{1-10} alkyl is optionally substituted with one substituent selected from $-OR^{20}$ and 3- to 12-membered saturated heterocycle. In another embodiment, the invention relates to a compound or salt as described above, each R^{5B} is independently selected from C_{3-10} alkyl, wherein the C_{3-10} alkyl is optionally substituted with one substituent selected from -
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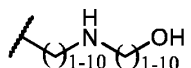
OR²⁰ and saturated 3- to 12-membered heterocycle. In another embodiment, the invention relates to a compound or salt as described above, each R^{5B} is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is optionally substituted with one substituent selected from -OH and saturated 3- to 6-membered heterocycle. In another embodiment, the invention relates to a compound or salt as described above, each R^{5B} is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with one substituent selected from -OR²⁰ and saturated 3- to 12-membered heterocycle. In another embodiment, the invention relates to a compound or salt as described above, each R^{5B} is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with one substituent selected from -OH and saturated 3- to 6-membered heterocycle. In another embodiment, the invention relates to a compound or salt as described above, each R^{5B} is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with one substituent selected from -OH. In another embodiment, the invention relates to a compound or salt as described above, each R^{5B} is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with one substituent selected from saturated 3- to 6-membered heterocycle. In another embodiment, the invention relates to a compound or salt as described above, each R^{5B} is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with one substituent selected from saturated 3- to 6-membered heterocycle, and wherein the substitution is at the terminal end of the C₁₋₁₀ alkyl. In an embodiment, the invention relates to a compound or salt as described above,

wherein each R¹ is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein each R¹ is selected from

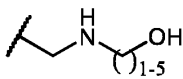
. In an embodiment, the invention relates to a compound or salt as

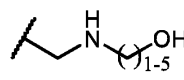
described above, wherein each R¹ is selected from . In an embodiment, the invention relates to a compound or salt as described above,

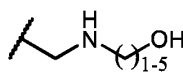
wherein each R¹ is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein at least one R¹ is selected

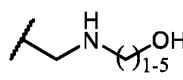
from . In an embodiment, the invention relates to a compound or salt

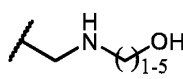
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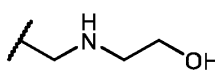
as described above, wherein each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above,

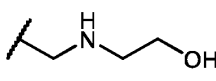
wherein at least one R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein two R^1 are

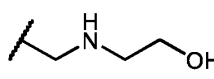
5 . In an embodiment, the invention relates to a compound or salt as

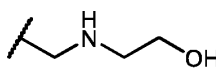
described above, wherein at least three R^1 are . In an embodiment, the invention relates to a compound or salt as described above, wherein three R^1 are

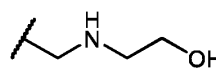
. In an embodiment, the invention relates to a compound or salt as

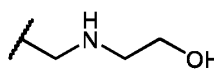
described above, wherein each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above,

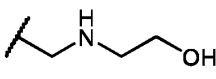
10 wherein at least one R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein one R^1 is

. In an embodiment, the invention relates to a compound or salt

as described above, wherein at least two R^1 are . In an embodiment, the invention relates to a compound or salt as described above,

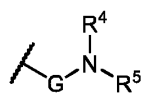
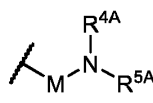
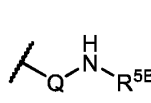
15 wherein two R^1 are . In an embodiment, the invention relates to a compound or salt as described above, wherein at least three R^1 are

. In an embodiment, the invention relates to a compound or salt

as described above, wherein three R^1 are .

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In an embodiment, the invention relates to a compound or salt as described above,

wherein each R^1 is selected from , , and . In

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an embodiment, the invention relates to a compound or salt as described above,

wherein each R^1 is selected from $\text{---G---N}^{\text{R}^4}\text{---R}^5$, and $\text{---Q---N}^{\text{H}}\text{---R}^{5\text{B}}$. In an embodiment, the invention relates to a compound or salt as described above,

wherein each R^1 is selected from $\text{---N}^{\text{H}}\text{---CH}_2\text{CH}_2\text{OH}$, $\text{---N}^{\text{H}}\text{---CH}_2\text{CH}_2\text{CH}_2\text{OH}$, and $\text{---N}^{\text{H}}\text{---CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$.

In an embodiment, the invention relates to a compound or salt as described above,

wherein each R^1 is selected from hydrogen, -OH, $\text{---(X)}_n\text{---C(=O)---N}^{\text{R}^2}\text{---R}^3$, $\text{---(Y)}_r\text{---C(=O)---O---R}^7$, $\text{---G---N}^{\text{R}^4}\text{---R}^5$, $\text{---M---N}^{\text{R}^{4\text{A}}}\text{---R}^{5\text{A}}$, and $\text{---Q---N}^{\text{H}}\text{---R}^{5\text{B}}$. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is selected from

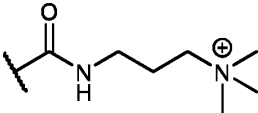
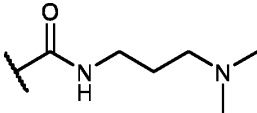
$\text{---(X)}_n\text{---C(=O)---N}^{\text{R}^2}\text{---R}^3$, $\text{---(Y)}_r\text{---C(=O)---O---R}^7$, $\text{---G---N}^{\text{R}^4}\text{---R}^5$, $\text{---M---N}^{\text{R}^{4\text{A}}}\text{---R}^{5\text{A}}$, and $\text{---Q---N}^{\text{H}}\text{---R}^{5\text{B}}$. In an embodiment, the invention relates to a compound or salt as described above,

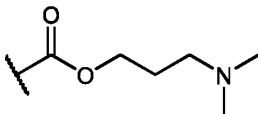
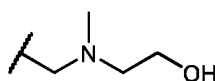
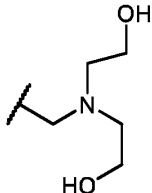
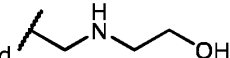
wherein each R^1 is selected from hydrogen, C_{1-6} alkyl, -OH, $\text{---G---N}^{\text{R}^4}\text{---R}^5$, $\text{---M---N}^{\text{R}^{4\text{A}}}\text{---R}^{5\text{A}}$, and $\text{---Q---N}^{\text{H}}\text{---R}^{5\text{B}}$. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is selected from hydrogen, -

OH, $\text{---G---N}^{\text{R}^4}\text{---R}^5$, $\text{---M---N}^{\text{R}^{4\text{A}}}\text{---R}^{5\text{A}}$, and $\text{---Q---N}^{\text{H}}\text{---R}^{5\text{B}}$. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is selected from

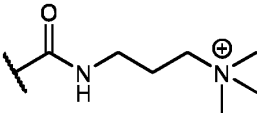
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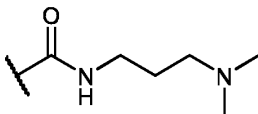
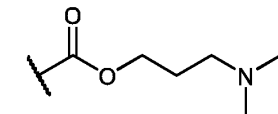
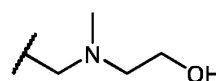
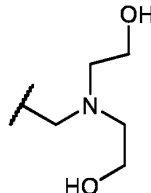
$\text{---G---N}^{\text{R}^4}\text{---R}^{\text{R}^5}$, $\text{---M---N}^{\text{R}^{4\text{A}}}\text{---R}^{\text{R}^{5\text{A}}}$, and $\text{---Q---N}^{\text{H}}\text{---R}^{\text{R}^{5\text{B}}}$. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is selected from

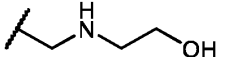
hydrogen, ---OH , C_{1-6} alkyl, , ,

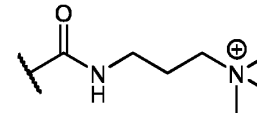
, , , and . In

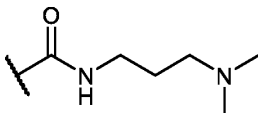
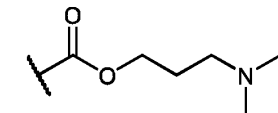
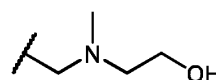
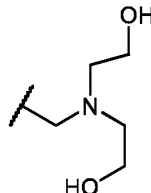
5 an embodiment, the invention relates to a compound or salt as described above,

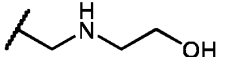
wherein each R^1 is selected from hydrogen, ---OH , ,

, , , ,

and . In an embodiment, the invention relates to a compound or

salt as described above, wherein each R^1 is selected from ,

10 , , , ,

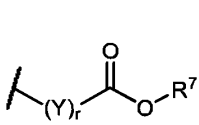
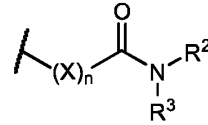
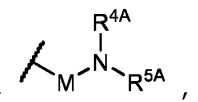
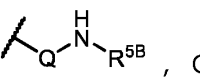
and .

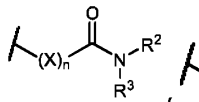
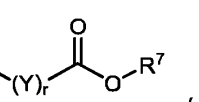
In an embodiment, the invention relates to a compound or salt as described above, each R^2 , R^6 , R^7 , R^8 , is independently selected from C_{3-10} alkyl, wherein the C_{3-10} alkyl

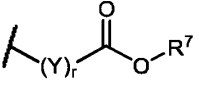
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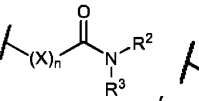
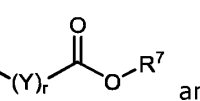
is substituted with one or more substituents selected from $-N(R^{20})_2$, $-N(R^{20})_3^+$, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, $-CN$, $-NO_2$, $=O$, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, and $-S(O)_2(R^{20})$; or R^7 is selected from C_{1-2} alkyl, wherein the C_{1-2} alkyl is substituted with one or more substituents selected from $-N(R^{20})_2$, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, $-CN$, $-NO_2$, $=O$, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, and $-S(O)_2(R^{20})$. In an embodiment, the invention relates to a compound or salt as described above, each R^2 , R^6 , R^7 , R^8 , is independently selected from C_{3-10} alkyl, wherein the C_{3-10} alkyl is substituted with one or more substituents selected from $-N(R^{20})_2$, $-N(R^{20})_3^+$, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, $-CN$, $-NO_2$, $=O$, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, and $-S(O)_2(R^{20})$.

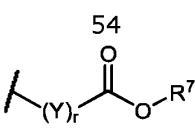
In an embodiment, the invention relates to a compound or salt as described above,

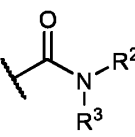
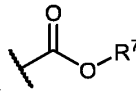
wherein each R^1 is selected from , , , , C_{1-6} alkyl, and hydrogen. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is

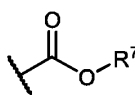
selected from , , C_{1-6} alkyl, and hydrogen. In an embodiment, the invention relates to a compound or salt as described above,

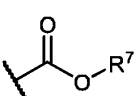
wherein each R^1 is selected from , C_{1-6} alkyl, and hydrogen. In an embodiment, the invention relates to a compound or salt as described above,

wherein each R^1 is selected from ,  and hydrogen. In an embodiment, the invention relates to a compound or salt as described above,

wherein each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is selected from

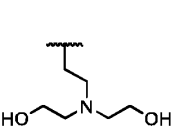
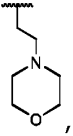
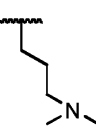
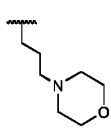
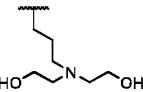
,  and hydrogen. In an embodiment, the invention relates to

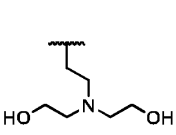
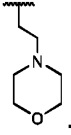
a compound or salt as described above, wherein each R^1 is selected from  and hydrogen. In an embodiment, the invention relates to a compound or salt as

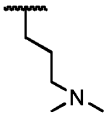
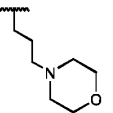
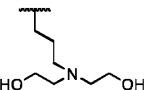
described above, wherein each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^7 is selected from substituted C_{3-6} alkyl, and substituted C_{1-2} alkyl. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^7 is selected from substituted C_{3-5} alkyl, and substituted C_2 alkyl. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^7 is selected from substituted C_{3-5} alkyl. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^7 is selected from substituted C_2 alkyl. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^7 is selected from substituted C_{3-5} alkyl substituted with one or more substituents selected from $-N(R^{20})_2$, and 4- to 6-membered heterocycle; and C_2 alkyl, which is substituted with one or more substituents selected from $-N(R^{20})_2$, and 4- to 6-membered heterocycle. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^7 is selected from C_{3-5} alkyl substituted with one or more substituents selected from $-N(R^{20})_2$, and 4- to 6-membered heterocycle. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^7 is selected from C_{3-5} alkyl substituted with one or more substituents selected from $-N(R^{20})_2$. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^7 is selected from C_{3-5} alkyl substituted with one or more substituents selected from 4- to 6-membered heterocycle. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^7 is selected from C_{3-5} alkyl substituted with one or more substituents selected from 4- to 6-membered heterocycle. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^7 is selected from C_2 alkyl, which is substituted with one or more substituents selected from $-N(R^{20})_2$. In an embodiment,

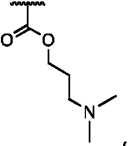
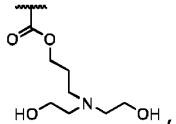
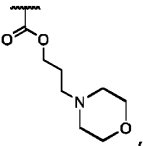
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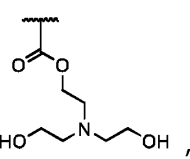
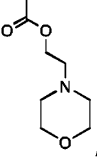
the invention relates to a compound or salt as described above, wherein each R^7 is selected from C_2 alkyl, which is substituted with one or more substituents selected from 4- to 6-membered heterocycle. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{20} is independently selected from C_{1-4} alkyl, which is optionally substituted with one or more -OH. In an embodiment, the invention relates to a compound or salt as described above,

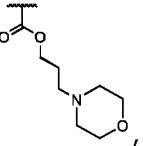
wherein each R^7 is selected from , , , and , and . In an embodiment, the invention relates to a compound or salt as

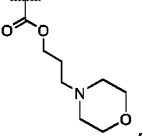
described above, wherein each R^7 is selected from , and . In an embodiment, the invention relates to a compound or salt as described above,

wherein each R^7 is selected from , , and . In an embodiment, the invention relates to a compound or salt as described above,

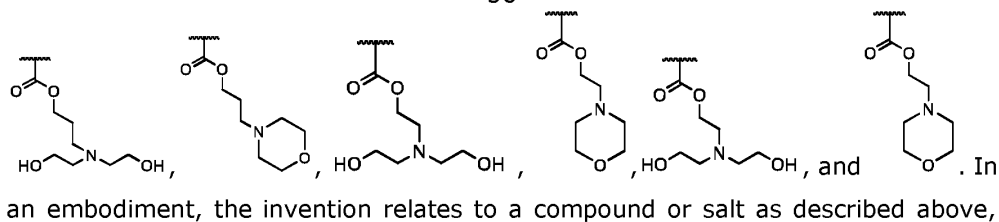
wherein each R^1 is selected from , , and ,

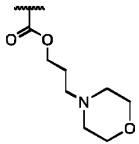
, , and hydrogen. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is selected from

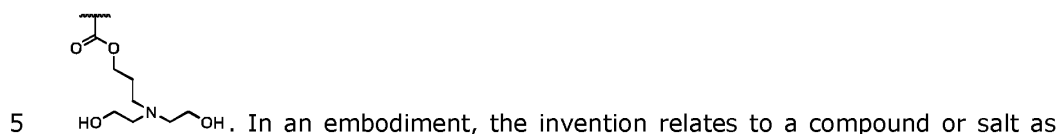
, and hydrogen. In an embodiment, the invention relates to a compound

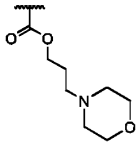
or salt as described above, wherein each R^1 is selected from ,

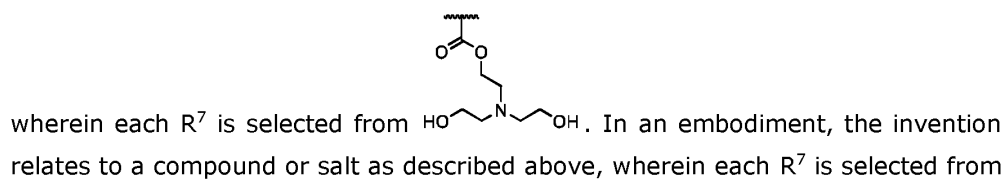
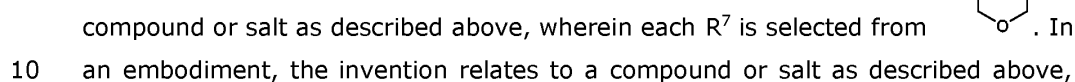
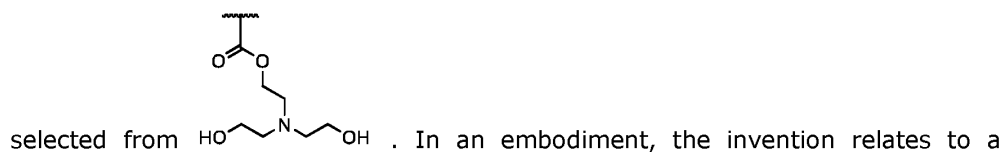
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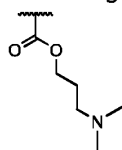
wherein each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^7 is selected from

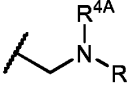


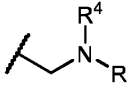
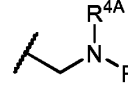
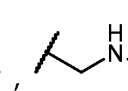
described above, wherein each R^7 is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^7 is

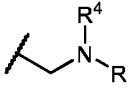


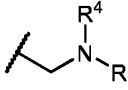
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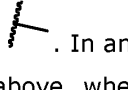
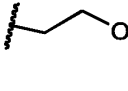


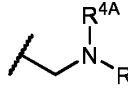
above, each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is selected from

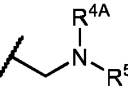
, , , and hydrogen. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is

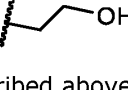
5 selected from , and hydrogen. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is selected from

. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^4 is selected from C_{1-4} alkyl. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^4 is

10 selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^5 is selected from C_{1-4} alkyl, wherein the C_{1-10} alkyl is substituted with one substituent selected from -OH. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^5 is selected from . In an embodiment, the invention relates to a compound

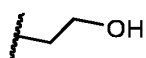
15 or salt as described above, wherein each R^1 is selected from , and hydrogen. In an embodiment, the invention relates to a compound or salt as

described above, wherein each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{4A} is selected from C_{2-4} alkyl, wherein the C_{2-4} alkyl is substituted with one or more substituents selected from -OH. In an embodiment, the invention relates to a

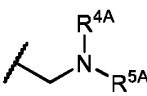
20 compound or salt as described above, wherein each R^{4A} is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{5A} is selected from C_{2-4} alkyl, wherein the C_{2-4} alkyl is substituted with one or more substituents selected from -OH. In an embodiment, the invention relates

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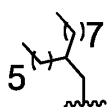
to a compound or salt as described above, wherein each R^{5A} is selected from

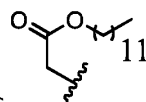


. In an embodiment, the invention relates to a compound or salt as

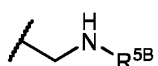
described above, wherein each R^1 is selected from , and each R^9 is independently selected from R^{9A} . In an embodiment, the invention relates to a

- 5 compound or salt as described above, wherein each R^{9A} is C_{12-16} alkyl. In an embodiment, the invention relates to a compound or salt as described above,

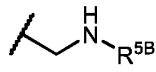
wherein each R^{9A} is . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{9A} is $-(C_{1-20} \text{ alkylene})-C(O)OR^{20}$. In an embodiment, the invention relates to a compound or salt as



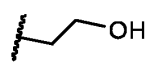
- 10 described above, wherein each R^{9A} is . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is selected from



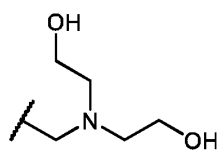
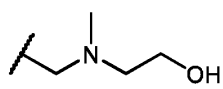
, and hydrogen. In an embodiment, the invention relates to a

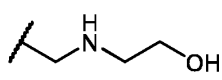
compound or salt as described above, wherein each R^1 is selected from .

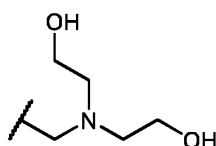
- 15 In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{5B} is selected from C_{2-4} alkyl, wherein the C_{2-4} alkyl is substituted with one substituent selected from $-OH$. In an embodiment, the invention relates to a

compound or salt as described above, wherein each R^{5B} is selected from .

. In an embodiment, the invention relates to a compound or salt as described above,

- 20 wherein each R^1 is selected from , ,

, and hydrogen. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is selected from



. In an embodiment, the invention relates to a compound or salt

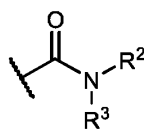
as described above, wherein each R^1 is selected from and hydrogen. In an embodiment, the invention relates to a compound or salt as

described above, wherein each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above,

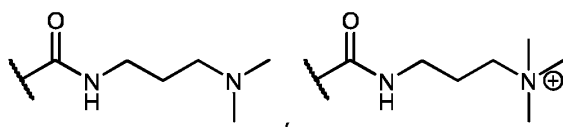
wherein each R^1 is selected from and hydrogen. In an embodiment, the invention relates to a compound or salt as described above,

wherein each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is

selected from and hydrogen. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is selected from

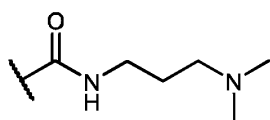


wherein each R^1 is selected from and hydrogen. In an embodiment, the invention relates to a compound or salt as described above, each R^1 is selected from



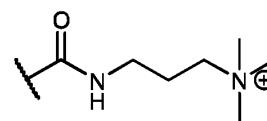
the invention relates to a compound or salt as described above, each R^1 is selected

from and hydrogen. In an embodiment, the invention relates to a compound or salt as described above, each R^1 is selected from

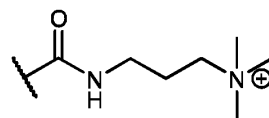


. In an embodiment, the invention relates to a compound or

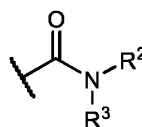
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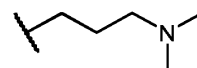
salt as described above, each R^1 is selected from , and hydrogen. In an embodiment, the invention relates to a compound or salt as



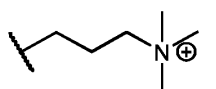
described above, each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above,



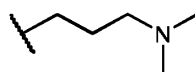
- 5 wherein each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^3 is selected from hydrogen. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^2 is selected from C_{3-10} alkyl, wherein the C_{3-10} alkyl is substituted with one or more substituents selected from $-N(R^{20})_2$, and $-N(R^{20})_3^+$.
- 10 In an embodiment, the invention relates to a compound or salt as described above, wherein each R^2 is selected from C_{3-5} alkyl, wherein the C_{3-5} alkyl is substituted with one or more substituents selected from $-N(R^{20})_2$. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^2 is selected from C_{3-5} alkyl, wherein the C_{3-5} alkyl is substituted with one or more substituents selected
- 15 from $-N(R^{20})_3^+$. In an embodiment, the invention relates to a compound or salt as



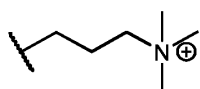
described above, wherein each R^2 is selected from , and



In an embodiment, the invention relates to a compound or salt as described above,

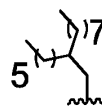


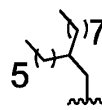
- wherein each R^2 is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^2 is selected from
- 20

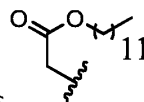


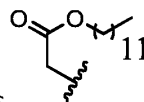
- . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^9 is C_{12-16} alkyl. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^9 is C_{12} alkyl. In an embodiment, the invention relates to a compound or salt as described above,
- 25 wherein each R^9 is C_{16} alkyl. In an embodiment, the invention relates to a compound

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or salt as described above, wherein each R^9 is . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^9 is $-(C_{1-20} \text{ alkylene})-C(O)OR^{20}$. In an embodiment, the invention relates to a compound or



salt as described above, wherein each R^9 is .

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In another embodiment, the invention relates to a compound or salt as described above, wherein r is 0. In another embodiment, the invention relates to a compound or salt as described above, wherein r is 1.

In an embodiment, the invention relates to a compound or salt as described above, wherein at least one R^1 is selected from hydrogen, halogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-10} haloalkyl, and $-OR^{20}$. In another embodiment, the invention relates to a compound or salt as described above, wherein at least one R^1 is selected from hydrogen, C_{1-6} alkyl, and $-OR^{20}$. In another embodiment, the invention relates to a compound or salt as described above, wherein at least one R^1 is selected from hydrogen, and $-OR^{20}$. In another embodiment, the invention relates to a compound or salt as described above, wherein at least one R^1 is selected from hydrogen, and $-OH$. In another embodiment, the invention relates to a compound or salt as described above, wherein one R^1 is $-OH$. In another embodiment, the invention relates to a compound or salt as described above, wherein two R^1 s are $-OH$. In another embodiment, the invention relates to a compound or salt as described above, wherein three R^1 s are $-OH$. In another embodiment, the invention relates to a compound or salt as described above, wherein one R^1 is H. In another embodiment, the invention relates to a compound or salt as described above, wherein two R^1 s are H. In another embodiment, the invention relates to a compound or salt as described above, wherein three R^1 s are H.

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In an embodiment, the invention relates to a compound or salt as described above, wherein A, D, E, F, G, I, J, K, X, and Y are each independently selected from C_{1-10} alkylene, wherein the C_{1-10} alkylene are optionally substituted with one or more substituents independently selected from halogen, C_{1-10} haloalkyl, $-CN$, $-NO_2$, $-OR^{20}$,

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$-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, and $-S(O)_2(R^{20})$. In another embodiment, the invention relates to a compound or salt as described above, wherein A, D, E, F, G, I, J, K, X, and Y are each independently selected from C_{1-10} alkylene. In another embodiment, the invention relates to a compound or salt as described above, wherein A is selected from C_{1-10} alkylene. In another embodiment, the invention relates to a compound or salt as described above, wherein D is selected from C_{1-10} alkylene. In another embodiment, the invention relates to a compound or salt as described above, wherein E is selected from C_{1-10} alkylene. In another embodiment, the invention relates to a compound or salt as described above, wherein F is selected from C_{1-10} alkylene. In another embodiment, the invention relates to a compound or salt as described above, wherein I is selected from C_{1-10} alkylene. In another embodiment, the invention relates to a compound or salt as described above, wherein J is selected from C_{1-10} alkylene. In another embodiment, the invention relates to a compound or salt as described above, wherein K is selected from C_{1-10} alkylene. In another embodiment, the invention relates to a compound or salt as described above, wherein X is selected from C_{1-10} alkylene. In another embodiment, the invention relates to a compound or salt as described above, wherein Y is selected from C_{1-10} alkylene. In another embodiment, the invention relates to a compound or salt as described above, wherein G is selected from C_{1-10} alkylene. In another embodiment, the invention relates to a compound or salt as described above, wherein G is selected from C_{1-5} alkylene. In another embodiment, the invention relates to a compound or salt as described above, wherein G is selected from C_1 alkylene. In another embodiment, the invention relates to a compound or salt as described above, wherein G is selected from C_2 alkylene. In another embodiment, the invention relates to a compound or salt as described above, wherein G is selected from C_3 alkylene. In another embodiment, the invention relates to a compound or salt as described above, wherein G is selected from C_4 alkylene. In another embodiment, the invention relates to a compound or salt as described above, wherein G is selected from C_5 alkylene. In another embodiment, the invention relates to a compound or salt as described above, wherein G is selected from C_6 alkylene.

In an embodiment, the invention relates to a compound or salt as described above, wherein each R^2 , R^6 , R^7 , and R^8 , is independently selected from C_{3-10} alkyl, wherein the C_{3-10} alkyl is substituted with one or more substituents selected from $-N(R^{20})_2$, $-N(R^{20})_3^+$, and 3- to 12-membered heterocycle; wherein at least one of the substitution is at the terminal end of the C_{3-10} alkyl; and wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents

independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰). In an embodiment, the invention relates to a compound or salt as described above, wherein each R², R⁶, R⁷, and R⁸, is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one substituent selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle; wherein the substitution is at the terminal end of the C₃₋₁₀ alkyl; and wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰). In an embodiment, the invention relates to a compound or salt as described above, wherein each R², R⁶, R⁷, and R⁸, is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰). In another embodiment, the invention relates to a compound or salt as described above, wherein each R², R⁶, R⁷, and R⁸, independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, and -N(R²⁰)₂. In another embodiment, the invention relates to a compound or salt as described above, wherein each R², R⁶, R⁷, and R⁸, independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, =O, -OH, -O-C₁₋₆ alkyl, -NHC₁₋₆ alkyl, -NH₂, and -N(C₁₋₆ alkyl)₂. In another embodiment, the invention relates to a compound or salt as described above, wherein each R², R⁶, R⁷, and R⁸, independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, =O, -OH, -O-C₁₋₆ alkyl, -NHC₁₋₆ alkyl, -NH₂, and -N(C₁₋₆ alkyl)₂. In another embodiment, the invention relates to a compound or salt as described above, wherein each R², R⁶, R⁷, and R⁸, is selected from C₃₋₆ alkyl, wherein the C₃₋₆ alkyl is substituted with one or more substituents

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selected from $-N(R^{20})_2$, $-N(R^{20})_3^+$, and 5- to 6-membered heterocycle, wherein the 5- to 6-membered heterocycle has at least one nitrogen atom, wherein the 5- to 6-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, $-CN$, $=O$, $-OH$, $-O-$

5 C_{1-6} alkyl, $-NHC_{1-6}$ alkyl, $-NH_2$, and $-N(C_{1-6} \text{ alkyl})_2$. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^2 , R^6 , R^7 , and R^8 , independently selected from C_{3-10} alkyl, wherein the C_{3-10} alkyl is substituted with one or more substituents selected from $-N(R^{20})_2$. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^2 , R^6 , R^7 ,

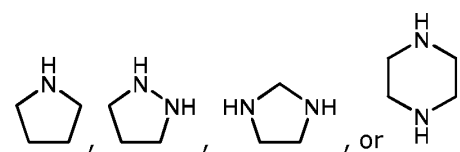
10 and R^8 , is independently selected from C_{3-10} alkyl, wherein the C_{3-10} alkyl is substituted with one or more substituents selected from $-N(R^{20})_3^+$. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^2 , R^6 , R^7 , and R^8 , is independently selected from C_{3-10} alkyl, wherein the C_{3-10} alkyl is substituted with one or more substituents selected from 5- to 6-

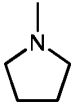
15 membered heterocycle, wherein the 5- to 6-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, $-CN$, $=O$, $-OH$, $-O-C_{1-6}$ alkyl, $-NHC_{1-6}$ alkyl, $-NH_2$, and $-N(C_{1-6} \text{ alkyl})_2$. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^2 , R^6 , R^7 , and R^8 , is independently selected from C_{3-10}

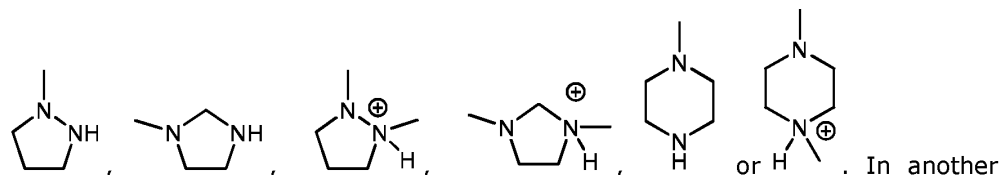
20 C_{10} alkyl, wherein the C_{3-10} alkyl is substituted with a 5- to 6-membered heterocycle, wherein the 5- to 6-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, $-CN$, $=O$, $-OH$, $-O-C_{1-6}$ alkyl, $-NHC_{1-6}$ alkyl, $-NH_2$, and $-N(C_{1-6} \text{ alkyl})_2$. In another embodiment, the invention relates to a compound or salt as described above,

25 wherein the heterocycle has at least one nitrogen atom. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle has at least two nitrogen. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle is a saturated heterocycle. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle is a heteroaryl. In another embodiment,

30 the invention relates to a compound or salt as described above, wherein the heterocycle has only 1 nitrogen atom and no other heteroatoms. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle has only 2 nitrogen atoms and no other heteroatoms.

35 Examples of such heterocycles include: , each

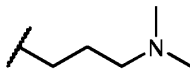
of which is optionally substituted. Examples of such heterocycles include: ,

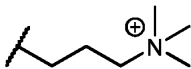


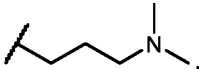
. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^2 , R^6 , R^7 , and R^8 , is independently selected from C_{3-10} alkyl, wherein

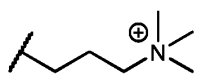
- 5 the C_{3-10} alkyl is substituted with . In another embodiment, the invention relates to a compound or salt as described above, wherein each R^2 , R^6 , R^7 , and R^8 , is independently selected from C_{3-10} alkyl, wherein the C_{3-10} alkyl is substituted with

. In another embodiment, the invention relates to a compound or salt as

described above, wherein each R^2 , R^6 , R^7 , and R^8 , is selected from 

- 10 and . In another embodiment, the invention relates to a compound or salt as described above, wherein each R^2 , R^6 , R^7 , and R^8 , is selected from

. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^2 , R^6 , R^7 , and R^8 , is selected from



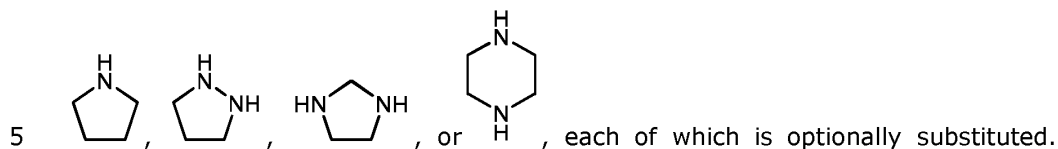
- 15 In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{8A} , is independently selected from hydrogen and C_{1-10} alkyl, wherein the C_{1-10} alkyl is substituted with one or more substituents selected from $-N(R^{20})_2$, $-N(R^{20})_3^+$, and 3- to 12-membered heterocycle; wherein at least one of the
- 20 substitution is at the terminal end of the C_{1-10} alkyl; and wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, $-CN$, $-NO_2$, $=O$, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, and $-S(O)_2(R^{20})$. In an embodiment, the invention relates to a compound or salt as described above,
- 25 wherein each R^{8A} , is independently selected from hydrogen and C_{1-10} alkyl, wherein

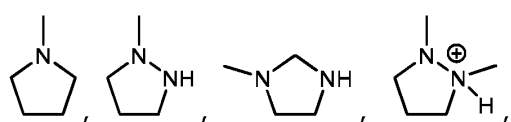
the C₁₋₁₀ alkyl is substituted with one substituent selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle; wherein the substitution is at the terminal end of the C₁₋₁₀ alkyl; and wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰). In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{8A}, is independently selected from hydrogen and C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰). In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{8A}, is independently selected from hydrogen and C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with one substituent selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰). In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{8A} is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰). In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{8A} is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, and -N(R²⁰)₂. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{8A} is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, =O, -OH, -O-C₁₋₆ alkyl, -NHC₁₋₆ alkyl, -NH₂, and -N(C₁₋₆ alkyl)₂. In another embodiment, the invention relates to a compound or

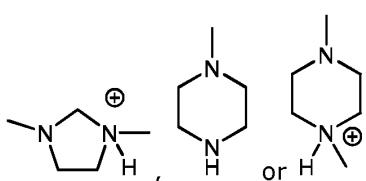
salt as described above, wherein each R^{8A} is independently selected from C_{3-10} alkyl, wherein the C_{3-10} alkyl is substituted with one or more substituents selected from $-N(R^{20})_2$, $-N(R^{20})_3^+$, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, $-CN$, $=O$, $-OH$, $-O-C_{1-6}$ alkyl, $-NHC_{1-6}$ alkyl, $-NH_2$, and $-N(C_{1-6} \text{ alkyl})_2$. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{8A} is selected from C_{1-2} alkyl, wherein the C_{1-2} alkyl is substituted with one or more substituents selected from $-N(R^{20})_2$, $-N(R^{20})_3^+$, and 5- to 6-membered heterocycle, wherein the 5- to 6-membered heterocycle has at least one nitrogen atom, wherein the 5- to 6-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, $-CN$, $=O$, $-OH$, $-O-C_{1-6}$ alkyl, $-NHC_{1-6}$ alkyl, $-NH_2$, and $-N(C_{1-6} \text{ alkyl})_2$. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{8A} is independently selected from C_{1-10} alkyl, wherein the C_{1-10} alkyl is substituted with one or more substituents selected from $-N(R^{20})_2$. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{8A} is independently selected from C_{1-10} alkyl, wherein the C_{1-10} alkyl is substituted with one or more substituents selected from $-N(R^{20})_3^+$. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{8A} is independently selected from C_{1-10} alkyl, wherein the C_{1-10} alkyl is substituted with one or more substituents selected from 5- to 6-membered heterocycle, wherein the 5- to 6-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, $-CN$, $=O$, $-OH$, $-O-C_{1-6}$ alkyl, $-NHC_{1-6}$ alkyl, $-NH_2$, and $-N(C_{1-6} \text{ alkyl})_2$. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{8A} is independently selected from C_{1-10} alkyl, wherein the C_{1-10} alkyl is substituted with a 5- to 6-membered heterocycle, wherein the 5- to 6-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, $-CN$, $=O$, $-OH$, $-O-C_{1-6}$ alkyl, $-NHC_{1-6}$ alkyl, $-NH_2$, and $-N(C_{1-6} \text{ alkyl})_2$. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle has at least one nitrogen atom. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle has at least two nitrogen. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle has only 1 nitrogen atom and no other heteroatoms. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle has only 2 nitrogen atoms and no other

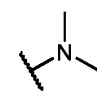
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heteroatoms. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle is a saturated heterocycle. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle is a heteroaryl. Examples of such heterocycles include:

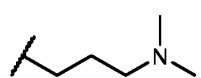
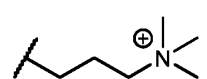


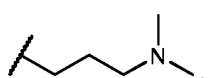
Examples of such heterocycles include:  ,

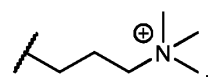
 . In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{8A} is independently selected

10 from C_{1-10} alkyl, wherein the C_{1-10} alkyl is substituted with  . In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{8A} is independently selected from C_{1-10} alkyl, wherein the C_{1-10} alkyl is

substituted with  . In another embodiment, the invention relates to a

compound or salt as described above, each R^{8A} is selected from  and  . In another embodiment, the invention relates to a compound

15 or salt as described above, each R^{8A} is selected from  . In another embodiment, the invention relates to a compound or salt as described above, each

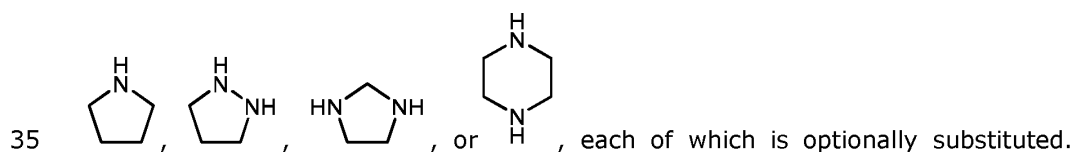
R^{8A} is selected from  . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{8A} , hydrogen.

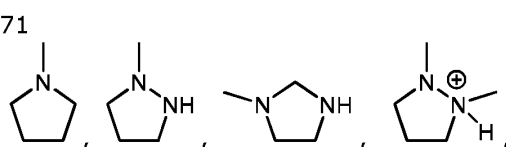
20 In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{11} is independently selected from C_{3-10} alkyl, wherein the C_{3-10} alkyl is substituted with one or more substituents selected from $-N(R^{21})_2$, $-N(R^{21})_3^+$, and 3- to 12-membered heterocycle; wherein at least one of the substitution is at the

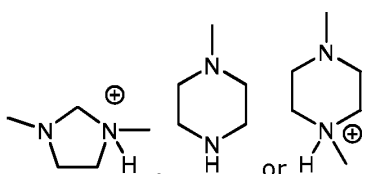
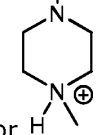
terminal end of the C₃₋₁₀ alkyl; and wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰). In an embodiment, the invention relates to a compound or salt as described above, wherein each R¹¹ is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one substituent selected from -N(R²¹)₂, -N(R²¹)₃⁺, and 3- to 12-membered heterocycle; wherein the substitution is at the terminal end of the C₃₋₁₀ alkyl; and wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰). In an embodiment, the invention relates to a compound or salt as described above, wherein each R¹¹ is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²¹)₂, -N(R²¹)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰). In another embodiment, the invention relates to a compound or salt as described above, wherein each R¹¹ is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²¹)₂, -N(R²¹)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, and -N(R²⁰)₂. In another embodiment, the invention relates to a compound or salt as described above, wherein each R¹¹ is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²¹)₂, -N(R²¹)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, =O, -OH, -O-C₁₋₆ alkyl, -NHC₁₋₆ alkyl, -NH₂, and -N(C₁₋₆ alkyl)₂. In another embodiment, the invention relates to a compound or salt as described above, wherein each R¹¹ is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²¹)₂, -N(R²¹)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, =O, -OH, -O-C₁₋₆ alkyl, -NHC₁₋₆ alkyl, -NH₂, and -N(C₁₋₆ alkyl)₂. In another embodiment, the invention relates to a compound or salt as described above, wherein each R¹¹ is selected from C₃₋₆ alkyl, wherein the C₃₋

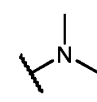
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₆ alkyl is substituted with one or more substituents selected from $-N(R^{21})_2$, $-N(R^{21})_3^+$, and 5- to 6-membered heterocycle, wherein the 5- to 6-membered heterocycle has at least one nitrogen atom, wherein the 5- to 6-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, =O, -OH, -O-C₁₋₆ alkyl, -NHC₁₋₆ alkyl, -NH₂, and -N(C₁₋₆ alkyl)₂. In another embodiment, the invention relates to a compound or salt as described above, wherein each R¹¹ is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from $-N(R^{21})_2$. In another embodiment, the invention relates to a compound or salt as described above, wherein each R¹¹ is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from $-N(R^{21})_3^+$. In another embodiment, the invention relates to a compound or salt as described above, wherein each R¹¹ is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from 5- to 6-membered heterocycle, wherein the 5- to 6-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, =O, -OH, -O-C₁₋₆ alkyl, -NHC₁₋₆ alkyl, -NH₂, and -N(C₁₋₆ alkyl)₂. In another embodiment, the invention relates to a compound or salt as described above, wherein each R¹¹ is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with a 5- to 6-membered heterocycle, wherein the 5- to 6-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, =O, -OH, -O-C₁₋₆ alkyl, -NHC₁₋₆ alkyl, -NH₂, and -N(C₁₋₆ alkyl)₂. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle has at least one nitrogen atom. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle has at least two nitrogen. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle has only 1 nitrogen atom and no other heteroatoms. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle has only 2 nitrogen atoms and no other heteroatoms. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle is a saturated heterocycle. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle is a heteroaryl. Examples of such heterocycles include:

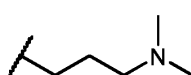
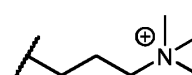
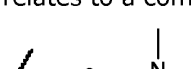


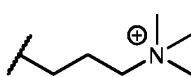
Examples of such heterocycles include:  ,

 , or  . In another embodiment, the invention relates to a compound or salt as described above, wherein each R¹¹ is independently selected

from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with  . In another embodiment, the invention relates to a compound or salt as described above, wherein each R¹¹ is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is

substituted with  . In another embodiment, the invention relates to a compound or salt as described above, wherein each R¹¹ is selected from

 and  . In another embodiment, the invention relates to a compound or salt as described above, wherein each R¹¹ is selected from  . In another embodiment, the invention relates to a compound or

salt as described above, wherein each R¹¹ is selected from  .

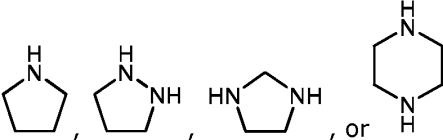
In an embodiment, the invention relates to a compound or salt as described above, wherein each R¹² is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)C₁₋₁₀ alkyl, -N(R²⁰)₃⁺, and 3- to 6-membered heterocycle; wherein at least one of the substitution is at the terminal end of the C₃₋₁₀ alkyl; wherein the 3- to 6-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰). In an embodiment, the invention relates to a compound or salt as described above, wherein each R¹² is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one substituent selected from -N(R²⁰)C₁₋₁₀ alkyl, -N(R²⁰)₃⁺, and 3- to 6-membered heterocycle; wherein the substitution is at the terminal end of the C₃₋₁₀ alkyl; wherein the 3- to 6-membered heterocycle is optionally substituted with one or more substituents independently

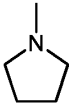
selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰). In an embodiment, the invention relates to a compound or salt as described above, wherein each R¹² is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)C₁₋₁₀ alkyl, -N(R²⁰)₃⁺, and 3- to 6-membered heterocycle, wherein the 3- to 6-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰). In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle is a saturated heterocycle. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle is a heteroaryl.

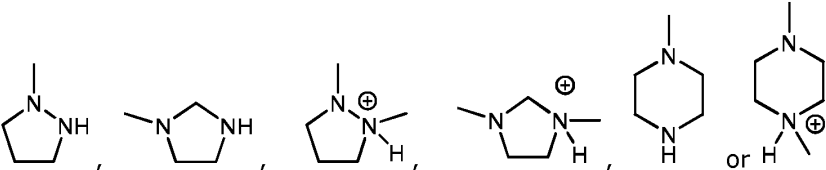
In an embodiment, the invention relates to a compound or salt as described above, wherein each R³ is independently selected from hydrogen and C₁₋₁₀ alkyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R³ is independently selected from hydrogen and C₁₋₃ alkyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R³ is hydrogen. In another embodiment, the invention relates to a compound or salt as described above, wherein each R³ is C₁₋₁₀ alkyl.

In an embodiment, the invention relates to a compound or salt as described above, wherein each R⁴ is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is optionally substituted with one or more substituents selected from 3- to 12-membered saturated heterocycle, wherein the substitution is at the terminal end of the C₁₋₁₀ alkyl; and wherein the 3- to 12-membered saturated heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰). In an embodiment, the invention relates to a compound or salt as described above, wherein each R⁴ is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is optionally substituted with one or more substituents selected from 3- to 12-membered saturated heterocycle, wherein the 3- to 12-membered saturated heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰). In another embodiment, the invention relates to a compound or salt as described above, wherein each R⁴ is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is optionally substituted with one or more substituents selected from 3- to 12-

- membered saturated heterocycle, wherein the 3- to 12-membered saturated heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, =O, -OH, -O-C₁₋₆ alkyl, and -N(R²⁰)₂.
- In another embodiment, the invention relates to a compound or salt as described above, wherein each R⁴ is independently selected from C₁₋₅ alkyl, wherein the C₁₋₅ alkyl is optionally substituted with at least one substituent selected from 3- to 6-membered saturated heterocycle, wherein the 3- to 6-membered saturated heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, =O, -OH, -O-C₁₋₆ alkyl, and -N(R²⁰)₂.
- In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle has at least one nitrogen atom. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle has at least two nitrogen. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle has only 1 nitrogen atom and no other heteroatoms. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle has only 2 nitrogen atoms and no other heteroatoms. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle is a saturated heterocycle. In another embodiment, the invention relates to a compound or salt as described above, wherein the heterocycle is a heteroaryl.

Examples of such heterocycles include: , each

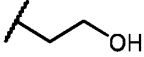
of which is optionally substituted. Examples of such heterocycles include: ,

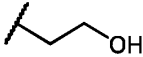
. In another

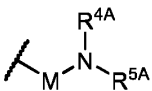
- embodiment, the invention relates to a compound or salt as described above, wherein each R⁴ is independently selected from C₁₋₁₀ alkyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R⁴ is independently selected from C₁ alkyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R⁴ is independently selected from C₂ alkyl. In another embodiment, the invention relates to a compound or salt

as described above, wherein each R^4 is independently selected from C_3 alkyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^4 is independently selected from C_4 alkyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^4 is independently selected from C_5 alkyl. In another embodiment, the invention relates to a compound or salt as described above, wherein the alkyl of each R^4 is unsubstituted. In another embodiment, the invention relates to a compound or salt as described above, R^4 and R^5 are different.

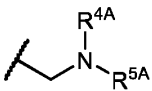
In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{4A} is independently selected from C_{1-10} alkyl, wherein the C_{1-10} alkyl is substituted with one or more substituents selected from -OH and -OC₁₋₁₀ alkyl, and wherein the substitution is at the terminal end of the C_{1-10} alkyl. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{4A} is independently selected from C_{1-10} alkyl, wherein the C_{1-10} alkyl is substituted with one or more substituents selected from -OH and -OC₁₋₁₀ alkyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{4A} is independently selected from C_{2-5} alkyl, wherein the C_{2-5} alkyl is substituted with one or more substituents selected from -OH and -OC₁₋₁₀ alkyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{4A} is independently selected from C_2 alkyl, wherein the C_2 alkyl is substituted with one or more substituents selected from -OH and -OC₁₋₁₀ alkyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{4A} is independently selected from C_2 alkyl, wherein the C_2 alkyl is substituted with one or more substituents selected from -OH. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{4A} is independently

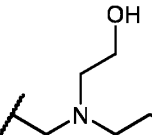
selected from  . In another embodiment, the invention relates to a

compound or salt as described above, wherein at least one R^{4A} is  . In an embodiment, the invention relates to a compound or salt as described above,

wherein when each R^1 is  , each R^9 is independently selected from R^{9A} .

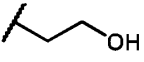
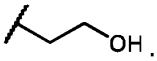
In an embodiment, the invention relates to a compound or salt as described above,

wherein when each R^1 is  , each R^9 is independently selected from R^{9A} . In an embodiment, the invention relates to a compound or salt as described above,

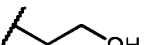
wherein when each R^1 is  , each R^9 is independently selected from R^{9A} . In an embodiment, the invention relates to a compound or salt as described above, wherein when each R^1 is -M-N(CH₂CH₂OH)₂, each R^9 is independently selected

from R^{9A}. In an embodiment, the invention relates to a compound or salt as described above, wherein when each R¹ is -CH₂-N(CH₂CH₂OH)₂, each R⁹ is independently selected from R^{9A}. In an embodiment, the invention relates to a compound or salt as described above, wherein when all four R¹ are -CH₂-N(CH₂CH₂OH)₂, each R⁹ is independently selected from R^{9A}. In an embodiment, the invention relates to a compound or salt as described above, wherein when each R¹ is -CH₂-N(CH₂CH₂OH)₂, each R^{9A} is selected from C₁₂₋₂₀ alkyl.

In an embodiment, the invention relates to a compound or salt as described above, wherein each R⁵ is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with -OR²⁰, and wherein the substitution is at the terminal end of the C₁₋₁₀ alkyl. In an embodiment, the invention relates to a compound or salt as described above, wherein each R⁵ is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with saturated 3- to 12-membered heterocycle, wherein the saturated 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, and -N(R²⁰)₂. In an embodiment, the invention relates to a compound or salt as described above, wherein each R⁵ is independently selected from C₂₋₁₀ alkyl, wherein the C₂₋₁₀ alkyl is substituted with saturated 3- to 12-membered heterocycle, wherein the saturated 3- to 12-membered saturated heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, and -N(R²⁰)₂. In an embodiment, the invention relates to a compound or salt as described above, wherein each R⁵ is independently selected from C₂₋₁₀ alkyl, wherein the C₂₋₁₀ alkyl is substituted with saturated 3- to 6-membered heterocycle, wherein the saturated 3- to 6-membered saturated heterocycle is optionally substituted with one or more substituents independently selected from C₁₋₆ alkyl. In an embodiment, the invention relates to a compound or salt as described above, wherein each R⁵ is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with -OR²⁰. In another embodiment, the invention relates to a compound or salt as described above, wherein each R⁵ is independently selected from C₁₋₅ alkyl, wherein the C₁₋₅ alkyl is substituted with -OR²⁰. In another embodiment, the invention relates to a compound or salt as described above, wherein each R⁵ is independently selected from C₂₋₅ alkyl, wherein the C₂₋₅ alkyl is substituted with -OR²⁰. In another embodiment, the invention relates to a compound or salt as described above, wherein each R⁵ is independently selected from C₁₋₃ alkyl, wherein the C₁₋₃ alkyl is substituted with -OR²⁰. In another embodiment, the invention relates to a compound or salt as described above, wherein each R⁵ is independently selected from C₂ alkyl, wherein

the C₂ alkyl is substituted with -OR²⁰. In another embodiment, the invention relates to a compound or salt as described above, wherein each R⁵ is independently selected from C₄ alkyl, wherein the C₄ alkyl is substituted with -OR²⁰. In another embodiment, the invention relates to a compound or salt as described above, wherein each R⁵ is independently selected from C₃ alkyl, wherein the C₃ alkyl is substituted with -OR²⁰. In another embodiment, the invention relates to a compound or salt as described above, wherein each R⁵ is independently selected from C₂ alkyl, wherein the C₂ alkyl is substituted with -OR²⁰. In another embodiment, the invention relates to a compound or salt as described above, R²⁰ is hydrogen. In another embodiment, the invention relates to a compound or salt as described above, R²⁰ is selected from C₁₋₅ alkyl. In another embodiment, each R⁵ is selected from C₂₋₅ alkyl, wherein the C₂₋₅ alkyl is substituted with one -OR²⁰. In another embodiment, each R⁵ is selected from C₂₋₅ alkyl, wherein the C₂₋₅ alkyl is substituted with one -OH. In another embodiment, the invention relates to a compound or salt as described above, wherein each R⁵ is selected from  OH. In another embodiment, the invention relates to a compound or salt as described above, wherein at least one R⁵ is selected from .

In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{5A} is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with -OR²⁰, wherein the substitution is at the terminal end of the C₁₋₁₀ alkyl. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{5A} is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with saturated 3- to 12-membered heterocycle, wherein the saturated 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, and -N(R²⁰)₂. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{5A} is independently selected from C₂₋₁₀ alkyl, wherein the C₂₋₁₀ alkyl is substituted with saturated 3- to 12-membered heterocycle, wherein the saturated 3- to 12-membered saturated heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, and -N(R²⁰)₂. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{5A} is independently selected from C₂₋₁₀ alkyl, wherein the C₂₋₁₀ alkyl is substituted with saturated 3- to 6-membered heterocycle, wherein the saturated 3- to 6-membered saturated heterocycle is optionally substituted with one or more

substituents independently selected from C₁₋₆ alkyl. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{5A} is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with -OR²⁰. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{5A} is independently selected from C₁₋₅ alkyl, wherein the C₁₋₅ alkyl is substituted with -OR²⁰. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{5A} is independently selected from C₂₋₅ alkyl, wherein the C₂₋₅ alkyl is substituted with -OR²⁰. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{5A} is independently selected from C₁₋₃ alkyl, wherein the C₁₋₃ alkyl is substituted with -OR²⁰. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{5A} is independently selected from C₁ alkyl, wherein the C₁ alkyl is substituted with -OR²⁰. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{5A} is independently selected from C₄ alkyl, wherein the C₄ alkyl is substituted with -OR²⁰. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{5A} is independently selected from C₃ alkyl, wherein the C₃ alkyl is substituted with -OR²⁰. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{5A} is independently selected from C₂ alkyl, wherein the C₂ alkyl is substituted with -OR²⁰. In another embodiment, the invention relates to a compound or salt as described above, R²⁰ is hydrogen. In another embodiment, the invention relates to a compound or salt as described above, R²⁰ is selected from C₁₋₅ alkyl. In another embodiment, each R^{5A} is selected from C₂₋₅ alkyl, wherein the C₂₋₅ alkyl is substituted with one -OR²⁰. In another embodiment, each R^{5A} is selected from C₂₋₅ alkyl, wherein the C₂₋₅ alkyl is substituted with one -OH. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{5A} is selected from  OH.

In an embodiment, the invention relates to a compound or salt as described above, wherein each R⁹ is independently selected from hydrogen, -(R¹⁰)_j-C(O)OR²⁰, -(R¹⁰)_j-C(O)N(R²⁰)₂, -(R¹⁰)_j-NHC(S)NHR²⁰, -(R¹⁰)_j-SR²⁰, -(R¹⁰)_j-S-S-R²⁰, C₁₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl, wherein the C₁₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl are each optionally substituted with one or more substituents independently selected from halogen, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, -S(O)₂(R²⁰), C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle. In another embodiment, the invention relates to a compound or salt as described above, wherein each R⁹ is independently selected from hydrogen, -(R¹⁰)_j-C(O)OR²⁰, -(R¹⁰)_j-

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$C(O)N(R^{20})_2$, $-(R^{10})_j-NHC(S)NHR^{20}$, $-(R^{10})_j-SR^{20}$, $-(R^{10})_j-S-S-R^{20}$, C_{1-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl, wherein the C_{1-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^9 is independently selected from hydrogen, $-(R^{10})_j-C(O)OR^{20}$, $-(R^{10})_j-C(O)N(R^{20})_2$, C_{1-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl, wherein the C_{1-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^9 is independently selected from hydrogen, C_{1-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl, wherein the C_{1-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^9 is independently selected from hydrogen and C_{1-20} alkyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^9 is independently selected from hydrogen and C_{10-15} alkyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^9 is independently selected from hydrogen. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^9 is independently selected from C_{1-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^9 is independently selected from C_{1-10} alkyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^9 is independently selected from C_{10-15} alkyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^9 is independently selected from C_{12-14} alkyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^9 is independently selected from C_{12} alkyl.

In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{9A} is independently selected from $-(R^{10})_j-C(O)OR^{20}$, $-(R^{10})_j-C(O)N(R^{20})_2$, $-(R^{10})_j-NHC(S)NHR^{20}$, $-(R^{10})_j-SR^{20}$, $-(R^{10})_j-S-S-R^{20}$, PEG_{1-200} , mannose, an adjuvant, a carbohydrate, an antibody, C_{12-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl, wherein the C_{12-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl are each optionally substituted with one or more substituents independently selected from halogen, $-CN$, $-NO_2$, $=O$, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, $-S(O)_2(R^{20})$, C_{3-12} carbocycle, and 3- to 12-membered heterocycle. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{9A} is independently selected from C_{12-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl, wherein the C_{12-20} alkyl, C_{2-20} alkenyl, and C_{2-20} alkynyl are each optionally substituted with one or more substituents independently selected from halogen, $-CN$, $-NO_2$, $=O$, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, $-S(O)_2(R^{20})$, C_{3-12} carbocycle, and 3- to 12-

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membered heterocycle. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{9A} is independently selected from C₁₂₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{9A} is independently selected from C₁₂₋₂₀ alkyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R^{9A} is independently selected from C₁₂ alkyl.

In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{9B} is independently selected from PEG₃₋₂₀₀, and C₅₋₂₀ alkyl, wherein the C₅₋₂₀ alkyl, is optionally substituted with one or more substituents independently selected from halogen, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, -S(O)₂(R²⁰), C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle. In another embodiment, the invention relates to a compound or salt as described above, each R^{9B} is independently selected from PEG₃₋₂₀₀, and C₅₋₂₀ alkyl. In another embodiment, the invention relates to a compound or salt as described above, each R^{9B} is independently selected from PEG₃₋₂₀₀. In another embodiment, the invention relates to a compound or salt as described above, each R^{9B} is independently selected from PEG₆₋₂₀. In another embodiment, the invention relates to a compound or salt as described above, each R^{9B} is independently selected from C₅₋₂₀ alkyl. In another embodiment, the invention relates to a compound or salt as described above, each R^{9B} is independently selected from C₈₋₂₀ alkyl. In another embodiment, the invention relates to a compound or salt as described above, each R^{9B} is independently selected from C₁₂₋₂₀ alkyl. In another embodiment, the invention relates to a compound or salt as described above, each R^{9B} is independently selected from C₈₋₁₆ alkyl. In another embodiment, the invention relates to a compound or salt as described above, each R^{9B} is independently selected from C₈₋₁₂ alkyl. In another embodiment, the invention relates to a compound or salt as described above, each R^{9B} is independently selected from C₈₋₁₂ alkyl. In another embodiment, the invention relates to a compound or salt as described above, each R^{9B} is independently selected from C₁₂ alkyl.

In an embodiment, the invention relates to a compound or salt as described above, wherein each R¹⁰ is independently selected from C₁₋₂₀ alkylene. In another embodiment, the invention relates to a compound or salt as described above, wherein each R¹⁰ is independently selected from C₂₋₂₀ alkenylene. In another

embodiment, the invention relates to a compound or salt as described above, wherein each R¹⁰ is independently selected from C₂₋₂₀ alkynylene.

In an embodiment, the invention relates to a compound or salt as described above, wherein each R²⁰ is independently selected from hydrogen. In another embodiment, the invention relates to a compound or salt as described above, wherein each R²⁰ is independently selected from C₁₋₂₀ alkyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R²⁰ is independently selected from C₂₋₂₀ alkenyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R²⁰ is independently selected from C₂₋₂₀ alkynyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R²⁰ is independently selected from C₃₋₁₂ carbocycle. In another embodiment, the invention relates to a compound or salt as described above, wherein each R²⁰ is independently selected from 3- to 12-membered heterocycle.

In an embodiment, the invention relates to a compound or salt as described above, wherein each R²¹ is independently selected from hydrogen; and C₁₋₂₀ alkyl which is optionally substituted with one or more substituents independently selected from halogen, -OH, -CN, -NO₂, -NH₂, -N(C₁₋₆ alkyl)₂, -NHC₁₋₆ alkyl, -NHC₁₋₆ hydroxyalkyl, C₁₋₁₀ alkyl, -C₁₋₁₀ haloalkyl, -O-C₁₋₁₀ alkyl, =NH, C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle. In another embodiment, the invention relates to a compound or salt as described above, wherein each R²¹ is independently selected from hydrogen; and C₁₋₂₀ alkyl which is optionally substituted with one or more substituents independently selected from halogen, -OH, and -O-C₁₋₁₀ alkyl. In another embodiment, the invention relates to a compound or salt as described above, wherein each R²¹ is independently selected from hydrogen. In an embodiment, the invention relates to a compound or salt as described above, wherein each R²¹ is independently selected from C₁₋₂₀ alkyl. In an embodiment, the invention relates to a compound or salt as described above, wherein each R²¹ is independently selected from C₂₋₂₀ alkenyl. In an embodiment, the invention relates to a compound or salt as described above, wherein each R²¹ is independently selected from C₂₋₂₀ alkynyl. In an embodiment, the invention relates to a compound or salt as described above, wherein each R²¹ is independently selected from C₃₋₁₂ carbocycle. In an embodiment, the invention relates to a compound or salt as described above, wherein each R²¹ is independently selected from 3- to 12-membered heterocycle.

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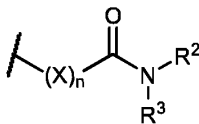
In an embodiment, the invention relates to a compound or salt as described above, wherein a, b, c, d, f, j, n, p, q, r, and s, are each independently selected from 0, 1, and 2. In another embodiment, the invention relates to a compound or salt as described above, wherein a, b, c, d, f, j, n, p, q, r, and s, are each independently selected from 0 and 1. In another embodiment, the invention relates to a compound or salt as described above, wherein a, b, c, d, f, j, n, p, q, r, and s, are each independently selected from 0. In another embodiment, the invention relates to a compound or salt as described above, wherein a, b, c, d, f, j, n, p, q, r, and s, are each independently selected from 1.

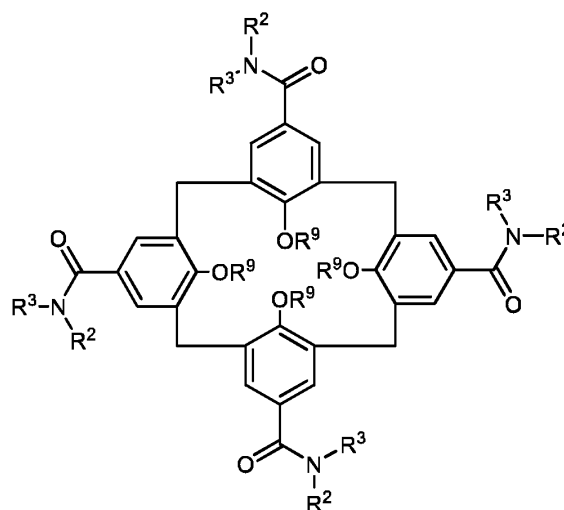
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In an embodiment, the invention relates to a compound or salt as described above, wherein m is 1. In another embodiment, the invention relates to a compound or salt as described above, wherein m is 3. In another embodiment, the invention relates to a compound or salt as described above, wherein m is 5.

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In a further embodiment, the invention relates to a compound or salt as described

above, wherein each R^1 is selected from  and Formula (I) is represented by Formula (II):



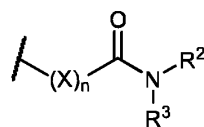
Formula (II)

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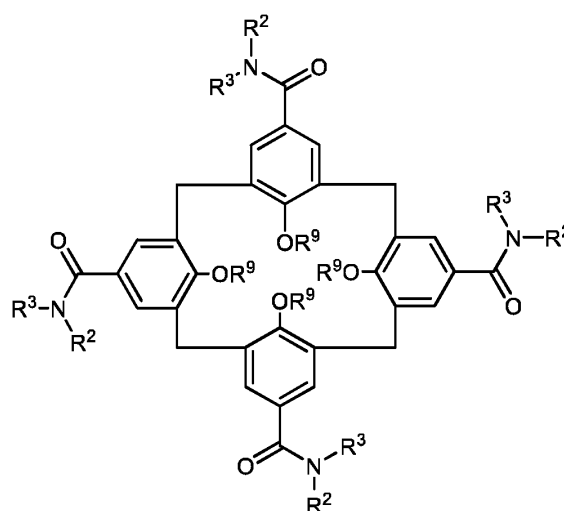
or a pharmaceutically acceptable salt thereof.

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In a further embodiment, the invention relates to a compound or salt as described



above, wherein each R^1 is selected from and Formula (I) is represented by Formula (II):



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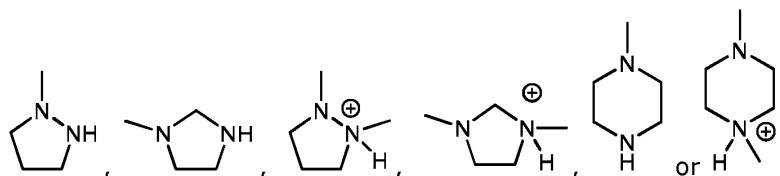
Formula (II)

or a pharmaceutically acceptable salt thereof and R^2 is selected from C_{3-6} alkyl, wherein the C_{3-6} alkyl is substituted with one or more substituents selected from - $N(R^{20})_2$, - $N(R^{20})_3^+$, and 5- to 6-membered heterocycle, wherein the 5- to 6-membered heterocycle has at least one nitrogen atom. Examples of such heterocycles include:

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Examples of such heterocycles include: , each

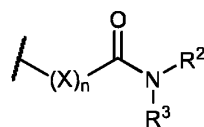
of which is optionally substituted. Examples of such heterocycles include:



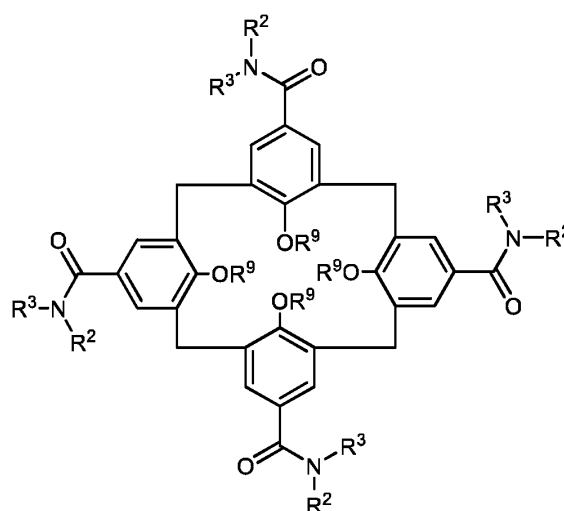
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In a further embodiment, the invention relates to a compound or salt as described

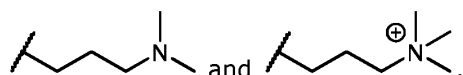


above, wherein each R^1 is selected from and Formula (I) is represented by Formula (II):

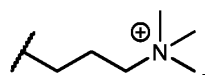


Formula (II)

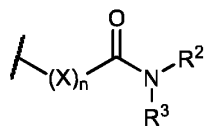
or a pharmaceutically acceptable salt thereof and each R^2 is selected from



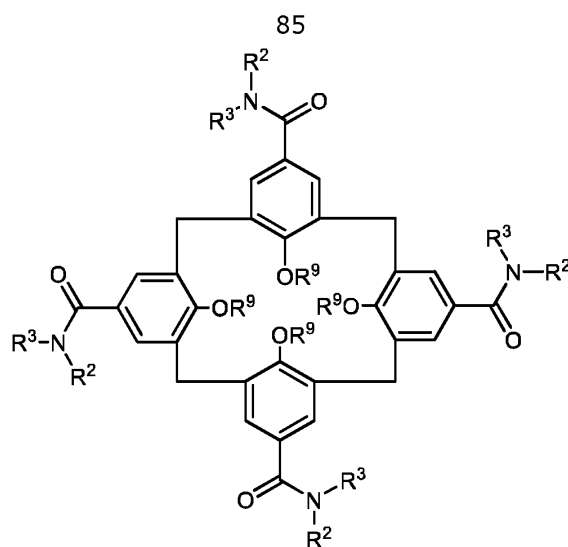
In an embodiment, each R^2 is . In another embodiment, each R^2 is



In an embodiment, the invention relates to a compound or salt as described above,



wherein each R^1 is selected from and Formula (I) is represented by Formula (II):

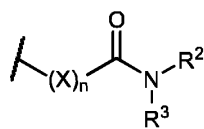


Formula (II)

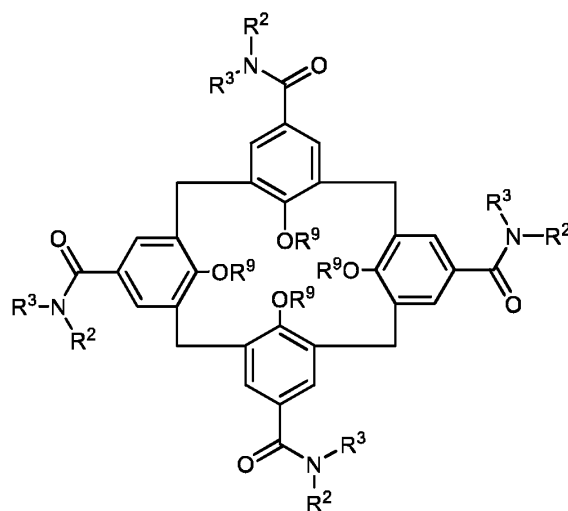
or a pharmaceutically acceptable salt thereof and each R³ is selected from hydrogen and C₁₋₁₀ alkyl. In an embodiment, each R³ is hydrogen.

5

In an embodiment, the invention relates to a compound or salt as described above,



wherein each R¹ is selected from and Formula (I) is represented by Formula (II):



Formula (II)

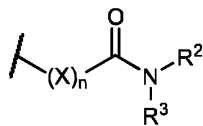
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or a pharmaceutically acceptable salt thereof and R² is selected from C₃₋₆ alkyl, wherein the C₃₋₆ alkyl is substituted with one or more substituents selected from -

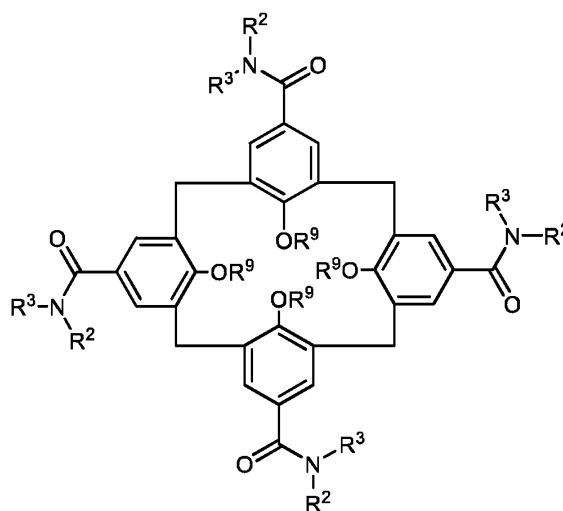
86

$N(R^{20})_2$, $-N(R^{20})_3^+$, and 5- to 6-membered heterocycle, wherein the 5- to 6-membered heterocycle has at least one nitrogen atom and each R^3 is selected from hydrogen and C_{1-10} alkyl. In an embodiment, each R^3 is hydrogen.

- 5 In an embodiment, the invention relates to a compound or salt as described above,



wherein each R^1 is selected from $\text{---}(X)_n\text{---}C(=O)\text{---}N(R^2)(R^3)$ and Formula (I) is represented by Formula (II):



Formula (II)

- 10 or a pharmaceutically acceptable salt thereof and each R^2 is selected from

$\text{---}CH_2CH_2CH_2CH_2N(R^3)_2$ and $\text{---}CH_2CH_2CH_2CH_2N^+(R^3)_2$ and each R^3 is selected from hydrogen and C_{1-10} alkyl. In an embodiment, each R^2 is $\text{---}CH_2CH_2CH_2CH_2N(R^3)_2$. In another embodiment, each R^2 is $\text{---}CH_2CH_2CH_2CH_2N^+(R^3)_2$. In a further embodiment, each R^3 is hydrogen.

- 15

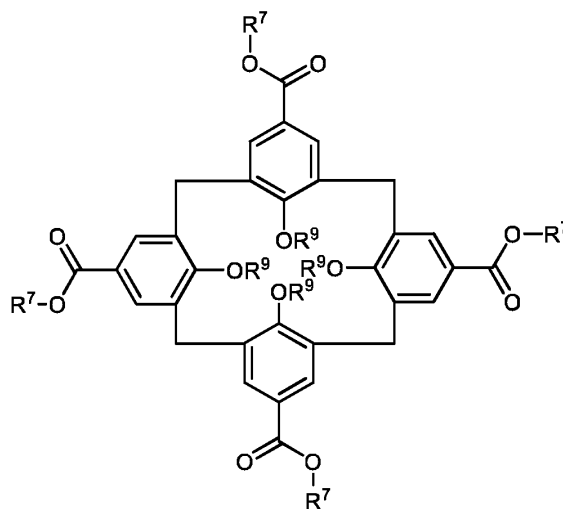
As described above, the invention may relate to a compound or salt as described

above, wherein each R^1 is selected from $\text{---}(Y)_r\text{---}C(=O)\text{---}OR^7$.

87

In a further embodiment, the invention relates to a compound or salt as described

above, wherein each R^1 is selected from $\text{---}(\text{Y})_r\text{---C(=O)OR}^7$ and wherein Formula (I) is represented by Formula (III):



5

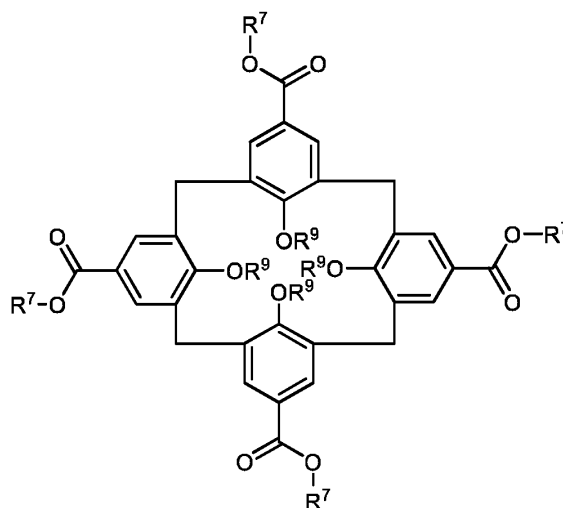
Formula (III)

or a pharmaceutically acceptable salt thereof.

In a further embodiment, the invention relates to a compound or salt as described

above, wherein each R^1 is selected from $\text{---}(\text{Y})_r\text{---C(=O)OR}^7$ and wherein Formula (I) is represented by Formula (III):

10

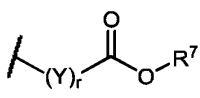


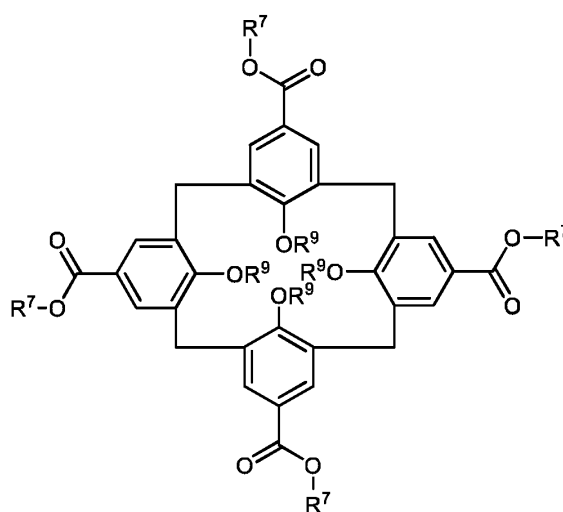
Formula (III)

88

or a pharmaceutically acceptable salt thereof and wherein R^7 is selected from C_{3-6} alkyl, wherein the C_{3-6} alkyl is substituted with one or more substituents selected from $-N(R^{20})_2$, $-N(R^{20})_3^+$, and 5- to 6-membered heterocycle, wherein the 5- to 6-membered heterocycle has at least one nitrogen atom. Examples of such heterocycles are described above.

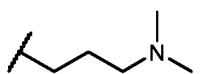
In a further embodiment, the invention relates to a compound or salt as described

above, wherein each R^1 is selected from  and wherein Formula (I) is represented by Formula (III):

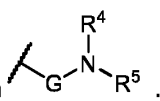


Formula (III)

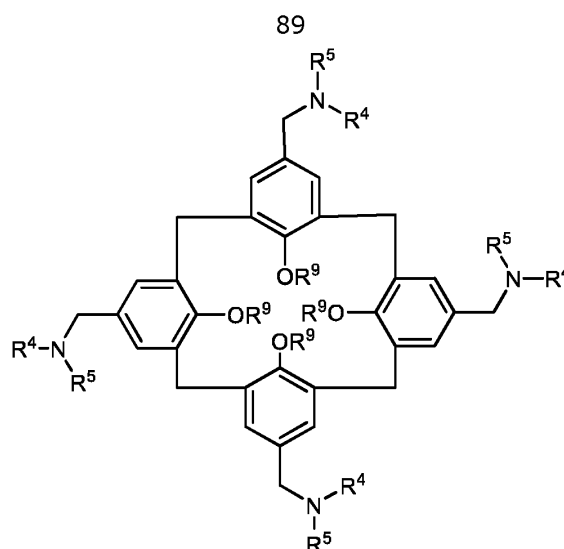
or a pharmaceutically acceptable salt thereof and wherein each R^7 is



As described above, the invention may relate to a compound or salt as described

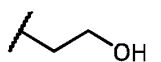
above, wherein each R^1 is selected from .

In an embodiment, Formula (I) is represented by Formula (IV):

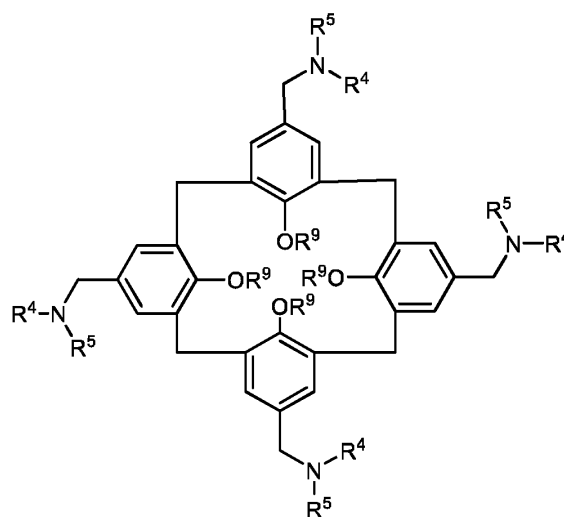


Formula (IV)

or a pharmaceutically acceptable salt thereof. In a further embodiment, each R^5 is selected from C_{2-5} alkyl, wherein the C_{2-5} alkyl is substituted with one $-OR^{20}$. In a further embodiment, each R^5 is selected from C_{2-5} alkyl, wherein the C_{2-5} alkyl is substituted with one $-OH$. In a further embodiment, each R^5 is selected from



In a further embodiment, Formula (I) is represented by Formula (IV):

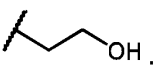


Formula (IV)

or a pharmaceutically acceptable salt thereof and each R^4 is selected from C_{1-2} alkyl. In a further embodiment, each R^5 is selected from C_{2-5} alkyl, wherein the C_{2-5} alkyl is substituted with one $-OR^{20}$. In a further embodiment, each R^5 is selected from C_{2-5}

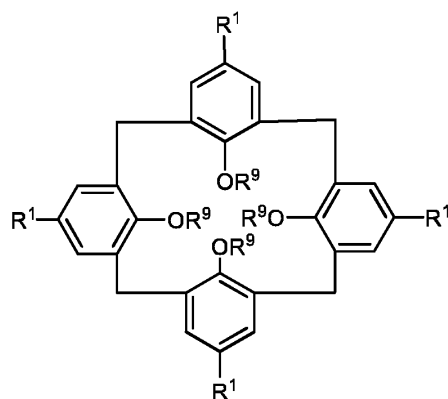
90

alkyl, wherein the C₂₋₅ alkyl is substituted with one -OH. In a further embodiment,

each R⁵ is selected from .

In an embodiment, the invention relates to a compound or salt as described above,
5 wherein each R⁹ is selected from unsubstituted C₁₋₁₂ alkyl.

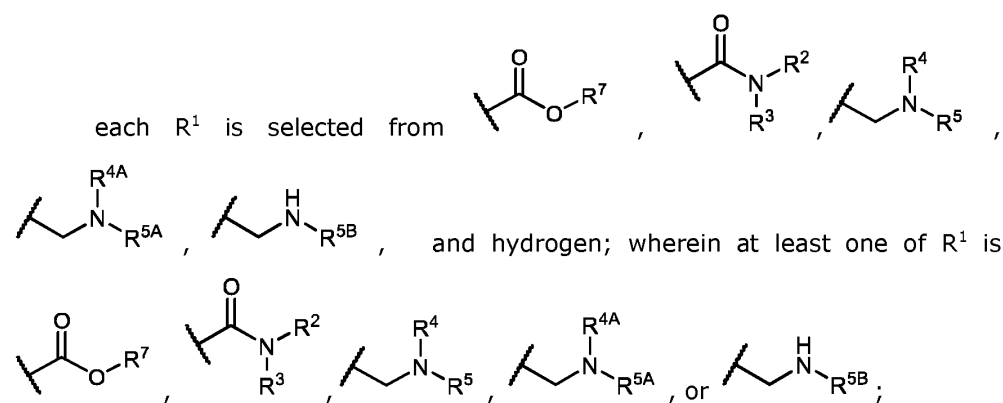
In an embodiment, Formula (V) is provided, wherein Formula (V) is represented
by:



10

Formula (V)

or a pharmaceutically acceptable salt thereof wherein;



15

each R², independently selected from C₃₋₅ alkyl, wherein the C₃₋₅ alkyl
is substituted with one or more substituents selected from -N(R²⁰)₂, and -
N(R²⁰)₃⁺;

each R³ is selected from hydrogen and C₁₋₁₀ alkyl;

each R⁴ is independently selected from C₁₋₁₀ alkyl;

20

each R^{4A} is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀
alkyl is substituted with one or more substituents selected from -OH and -

91

OC₁₋₁₀ alkyl; wherein when each R¹ is -CH₂-N(CH₂CH₂OH)₂, each R⁹ is independently selected from R^{9A};

each R⁵ is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with one substituent selected from -OH;

5 each R^{5A} is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with one substituent selected from -OH;

each R^{5B} is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with one substituent selected from -OH;

R⁷ is selected from:

10 C₁₋₂ alkyl, wherein the C₁₋₂ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, and 4- to 6-membered heterocycle; and

C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 4- to 6-membered heterocycle;

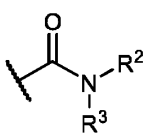
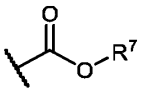
15 each R⁹ is independently selected from C₁₋₂₀ alkyl, wherein the C₁₋₂₀ alkyl is optionally substituted with one or more substituents independently selected from =O, -OR²⁰, and -C(O)OR²⁰;

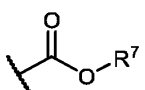
20 each R^{9A} is independently selected from C₁₂₋₂₀ alkyl, wherein the C₁₂₋₂₀ alkyl, is optionally substituted with one or more substituents independently selected from =O, -OR²⁰, and -C(O)OR²⁰;

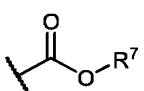
each R²⁰ is independently selected from hydrogen; and C₁₋₂₀ alkyl, which is optionally substituted with one or more substituents independently selected from -OH.

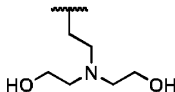
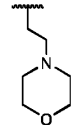
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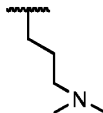
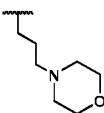
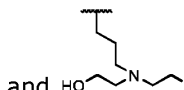
In an embodiment, the invention relates to a compound or salt as described above,

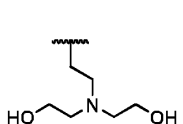
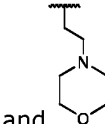
wherein each R¹ is selected from ,  and hydrogen. In an embodiment, the invention relates to a compound or salt as described above,

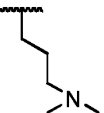
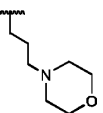
wherein each R¹ is selected from  and hydrogen. In an embodiment, the invention relates to a compound or salt as described above, wherein each R¹ is

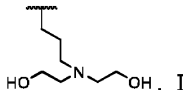
30 selected from . In an embodiment, the invention relates to a compound

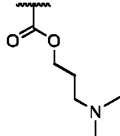
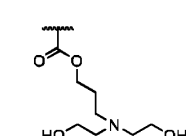
or salt as described above, wherein each R^7 is selected from , ,

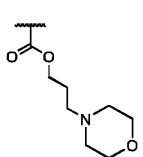
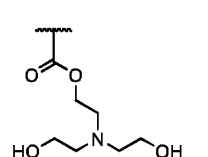
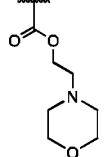
, , and . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^7 is selected from

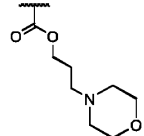
, and . In an embodiment, the invention relates to a compound

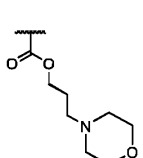
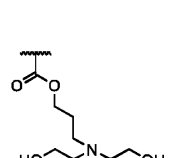
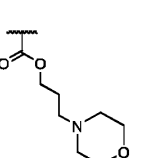
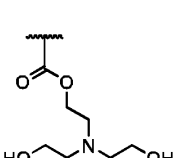
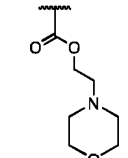
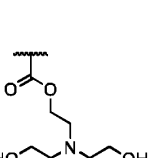
5 or salt as described above, wherein each R^7 is selected from , , and

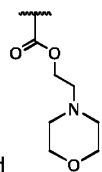
. In an embodiment, the invention relates to a compound or salt as

described above, wherein each R^1 is selected from , ,

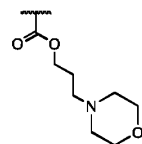
, , , and hydrogen. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is

10 selected from , and hydrogen. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is selected from

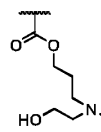
, , , , , ,



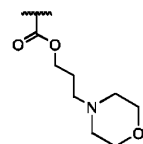
and . In an embodiment, the invention relates to a compound or salt as



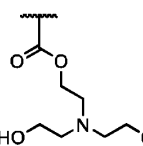
described above, wherein each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^7 is



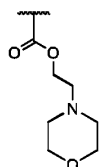
selected from HOCCNCCO. In an embodiment, the invention relates to a compound



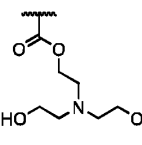
5 or salt as described above, wherein each R^7 is selected from . In an embodiment, the invention relates to a compound or salt as described above,



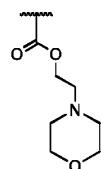
wherein each R^7 is selected from HOCCNCCO. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^7 is selected from



. In an embodiment, the invention relates to a compound or salt as described

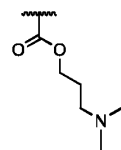


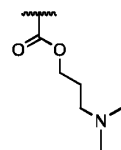
10 above, wherein each R^7 is selected from HOCCNCCO. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^7 is

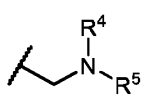
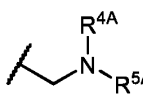
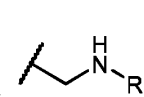


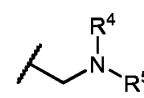
selected from . In an embodiment, the invention relates to a compound or

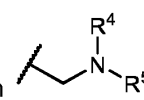
94



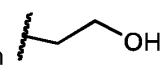
salt as described above, each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is

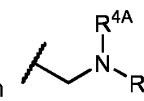
selected from , , , and hydrogen. In an embodiment, the invention relates to a compound or salt as described above,

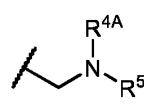
5 wherein each R^1 is selected from , and hydrogen. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is

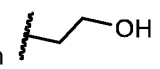
selected from . In an embodiment, the invention relates to a compound

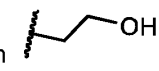
or salt as described above, wherein each R^4 is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^5 is

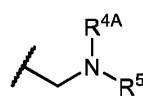
10 selected from . In an embodiment, the invention relates to a compound

or salt as described above, wherein each R^1 is selected from , and hydrogen. In an embodiment, the invention relates to a compound or salt as

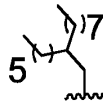
described above, wherein each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{4A} is

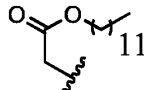
15 selected from . In an embodiment, the invention relates to a compound

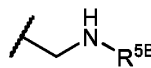
or salt as described above, wherein each R^{5A} is selected from . In an embodiment, the invention relates to a compound or salt as described above,

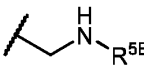
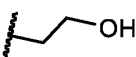
wherein each R^1 is selected from , and each R^9 is independently selected from R^{9A} . In an embodiment, the invention relates to a compound or salt as

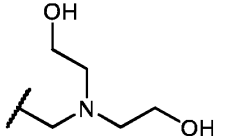
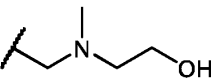
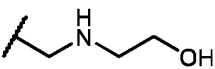
20 described above, wherein each R^{9A} is C_{12-16} alkyl. In an embodiment, the invention

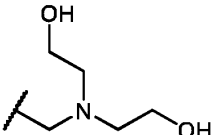
relates to a compound or salt as described above, wherein each R^{9A} is . In an embodiment, the invention relates to a compound or salt as described above,

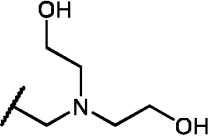
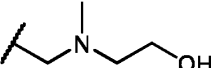
wherein each R^{9A} is . In an embodiment, the invention relates to a

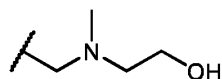
compound or salt as described above, wherein each R^1 is selected from , and hydrogen. In an embodiment, the invention relates to a compound or salt as

described above, wherein each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^{5B} is selected from . In an embodiment, the invention relates to a compound

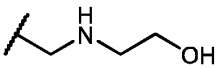
or salt as described above, wherein each R^1 is selected from ,
, , and hydrogen. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is

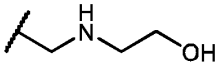
selected from . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is selected from

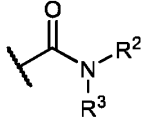
 and hydrogen. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^1 is selected from . In an embodiment, the invention relates to a compound or salt

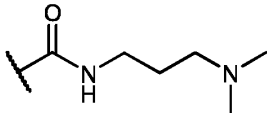
as described above, wherein each R^1 is selected from  and hydrogen. In an embodiment, the invention relates to a compound or salt as

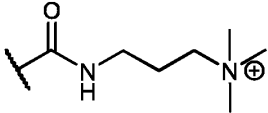
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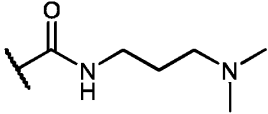
described above, wherein each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above,

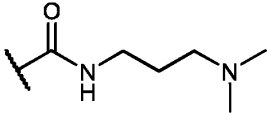
wherein each R^1 is selected from  and hydrogen. In an embodiment, the invention relates to a compound or salt as described above,

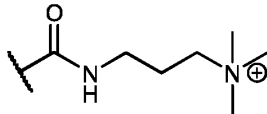
- 5 wherein each R^1 is selected from wherein each R^1 is selected from  and hydrogen. In an embodiment, the invention relates to a compound or salt as

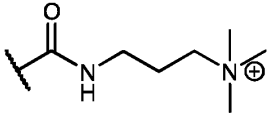
described above, each R^1 is selected from ,

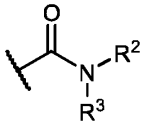
, and hydrogen. In an embodiment, the invention relates to a compound or salt as described above, each R^1 is selected from

- 10  and hydrogen. In an embodiment, the invention relates to a compound or salt as described above, each R^1 is selected from

. In an embodiment, the invention relates to a compound or

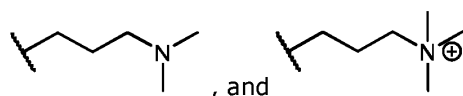
salt as described above, each R^1 is selected from , and hydrogen. In an embodiment, the invention relates to a compound or salt as

- 15 described above, each R^1 is selected from . In an embodiment, the invention relates to a compound or salt as described above,

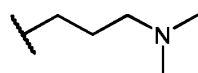
wherein each R^1 is selected from . In an embodiment, the invention

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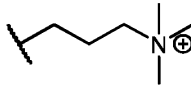
relates to a compound or salt as described above, wherein each R^2 is selected from

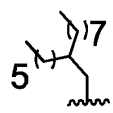


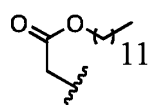
, and . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^2 is selected from



. In an embodiment, the invention relates to a compound or salt

- 5 as described above, wherein each R^2 is selected from  . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^9 is C_{12-16} alkyl. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^9 is C_{12} alkyl. In an embodiment, the invention relates to a compound or salt as described above, wherein each R^9 is C_{16} alkyl. In an embodiment, the invention relates to a compound

or salt as described above, wherein each R^9 is  . In an embodiment, the invention relates to a compound or salt as described above, wherein each R^9 is



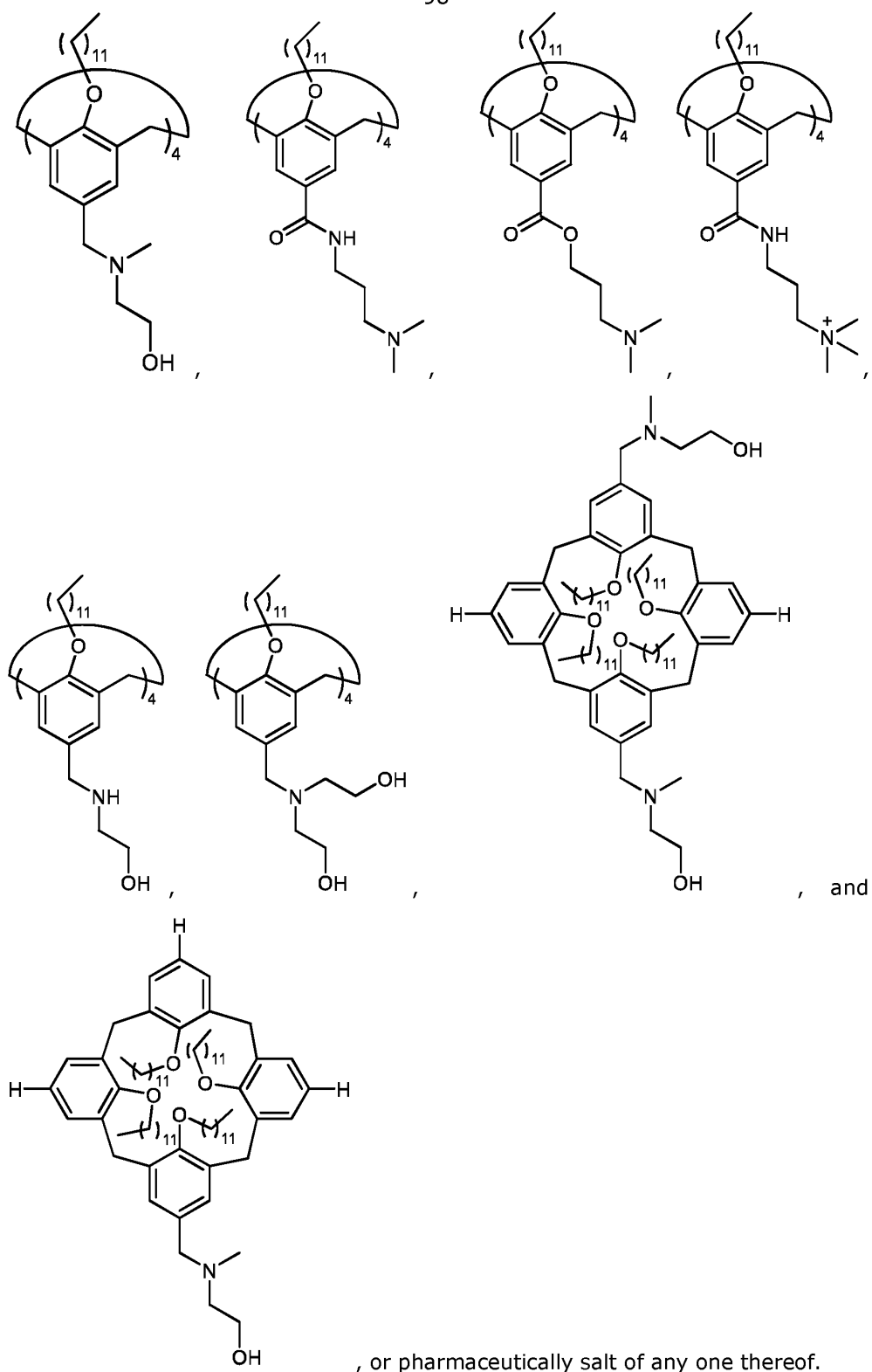
- 15 In an embodiment, the invention relates to a compound or salt as described above, wherein at least two R^1 s are different substituents. In an embodiment, the invention relates to a compound or salt as described above, wherein two R^1 s are the same substituent. In an embodiment, the invention relates to a compound or salt as described above, wherein three R^1 s are the same substituent. In an embodiment, the invention relates to a compound or salt as described above, wherein four R^1 s are the same substituent.
- 20

In an embodiment, the invention relates to a compound or salt as described above, further comprising functionalizing R^1 with one or more functional groups.

25

In an embodiment, the compound of Formula (I) is selected from:

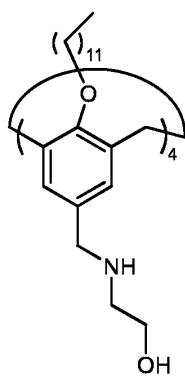
98



, or pharmaceutically salt of any one thereof.

In an embodiment, the compound of Formula (I) is selected from:

100
pharmaceutically salt of any one thereof. In an embodiment, the compound of



Formula (I) is selected from: (CX6).

5

In an embodiment, the compound of Formula (I) is present as a stereoisomer, tautomer, racemic, metabolite, prodrug, salt, hydrate, or solvate thereof. In an embodiment, the compound of Formula (I) is present as a pharmaceutically acceptable salt, stereoisomer or tautomer thereof. The inventors have found that compounds according to Formula (I) are very effective for use as a delivery system for nucleotides, for instance for the *in vivo* delivery of mRNA vaccines.

15 In an embodiment, the compound of Formula (V) is present as a stereoisomer, tautomer, racemic, metabolite, prodrug, salt, hydrate, or solvate thereof. In an embodiment, the compound of Formula (V) is present as a pharmaceutically acceptable salt, stereoisomer or tautomer thereof. The inventors have found that compounds according to Formula (V) are very effective for use as a delivery system for nucleotides, for instance for the *in vivo* delivery of mRNA vaccines.

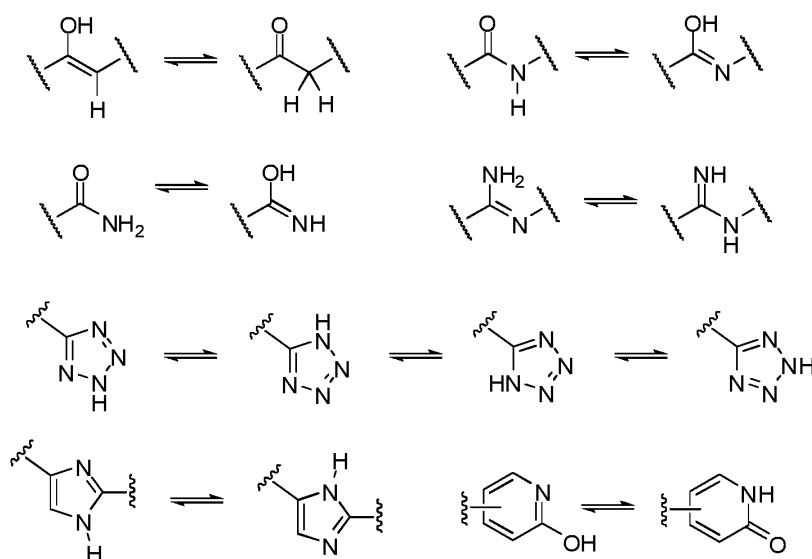
The compounds depicted herein may be present as salts even if salts are not depicted and it is understood that the present disclosure embraces all salts and solvates of the compounds depicted here, as well as the non-solvate form of the compound, as is well understood by the skilled artisan. In some embodiments, the salts of the compounds provided herein are pharmaceutically acceptable salts.

25

Included in the present disclosure are salts, particularly pharmaceutically acceptable salts, of the compounds described herein. The compounds of the present invention that possess a sufficiently acidic, a sufficiently basic, or both functional groups, can react with any of a number of inorganic bases, and inorganic and organic acids, to form a salt. Alternatively, compounds that are inherently charged, such as those with a quaternary nitrogen, can form a salt with an appropriate counterion, e.g., a halide such as bromide, chloride, or fluoride, particularly bromide.

Chemical entities having carbon-carbon double bonds or carbon-nitrogen double bonds may exist in Z- or E- form (or cis- or trans- form). Furthermore, some chemical entities may exist in various tautomeric forms. Unless otherwise specified, compounds described herein are intended to include all Z-, E- and tautomeric forms as well.

A "tautomer" refers to a molecule wherein a proton shift from one atom of a molecule to another atom of the same molecule is possible. The compounds presented herein, in certain embodiments, exist as tautomers. In circumstances where tautomerization is possible, a chemical equilibrium of the tautomers will exist. The exact ratio of the tautomers depends on several factors, including physical state, temperature, solvent, and pH. Some examples of tautomeric equilibrium include:



The compounds disclosed herein, in some embodiments, are used in different enriched isotopic forms, e.g., enriched in the content of ^2H , ^3H , ^{11}C , ^{13}C and/or ^{14}C . In one particular embodiment, the compound is deuterated in at least one position. Such deuterated forms can be made by the procedure described in U.S. Patent Nos. 5,846,514 and 6,334,997. As described in U.S. Patent Nos. 5,846,514 and

6,334,997, deuteration can improve the metabolic stability and or efficacy, thus increasing the duration of action of drugs.

Unless otherwise stated, compounds described herein are intended to include compounds which differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of a hydrogen by a deuterium or tritium, or the replacement of a carbon by ^{13}C - or ^{14}C -enriched carbon are within the scope of the present disclosure.

The compounds of the present disclosure optionally contain unnatural proportions of atomic isotopes at one or more atoms that constitute such compounds. For example, the compounds may be labeled with isotopes, such as for example, deuterium (^2H), tritium (^3H), iodine-125 (^{125}I) or carbon 14 (^{14}C). Isotopic substitution with ^2H , ^{11}C , ^{13}C , ^{14}C , ^{15}C , ^{12}N , ^{13}N , ^{15}N , ^{16}N , ^{16}O , ^{17}O , ^{14}F , ^{15}F , ^{16}F , ^{17}F , ^{18}F , ^{33}S , ^{34}S , ^{35}S , ^{36}S , ^{35}Cl , ^{37}Cl , ^{79}Br , ^{81}Br , and ^{125}I are all contemplated. All isotopic variations of the compounds of the present invention, whether radioactive or not, are encompassed within the scope of the present invention.

In certain embodiments, the compounds disclosed herein have some or all of the ^1H atoms replaced with ^2H atoms. The methods of synthesis for deuterium-containing compounds are known in the art and include, by way of non-limiting example only, the following synthetic methods.

Deuterium substituted compounds are synthesized using various methods such as described in: Dean, Dennis C.; Editor. Recent Advances in the Synthesis and Applications of Radiolabeled Compounds for Drug Discovery and Development. [In: Curr., Pharm. Des., 2000; 6(10)] 2000, 110 pp; George W.; Varma, Rajender S. The Synthesis of Radiolabeled Compounds via Organometallic Intermediates, Tetrahedron, 1989, 45(21), 6601-21; and Evans, E. Anthony. Synthesis of radiolabeled compounds, J. Radioanal. Chem., 1981, 64(1-2), 9-32.

Deuterated starting materials are readily available and are subjected to the synthetic methods described herein to provide for the synthesis of deuterium-containing compounds. Large numbers of deuterium-containing reagents and building blocks are available commercially from chemical vendors, such as Aldrich Chemical Co.

Compounds of the present invention also include crystalline and amorphous forms of those compounds, pharmaceutically acceptable salts, and active metabolites of these compounds having the same type of activity, including, for example, polymorphs, pseudopolymorphs, solvates, hydrates, unsolvated polymorphs (including anhydrides), conformational polymorphs, and amorphous forms of the compounds, as well as mixtures thereof.

The compounds described herein may in some cases exist as diastereomers, enantiomers, or other stereoisomeric forms. Where absolute stereochemistry is not

specified, the compounds presented herein include all diastereomeric, enantiomeric, and epimeric forms as well as the appropriate mixtures thereof. Separation of stereoisomers may be performed by chromatography or by forming diastereomers and separating by recrystallization, or chromatography, or any combination thereof.

5 (Jean Jacques, Andre Collet, Samuel H. Wilen, "Enantiomers, Racemates and Resolutions", John Wiley And Sons, Inc., 1981, herein incorporated by reference for this disclosure). Stereoisomers may also be obtained by stereoselective synthesis. The methods and compositions described herein include the use of amorphous forms as well as crystalline forms (also known as polymorphs). The compounds described

10 herein may be in the form of pharmaceutically acceptable salts. As well, in some embodiments, active metabolites of these compounds having the same type of activity are included in the scope of the present disclosure. In addition, the compounds described herein can exist in unsolvated as well as solvated forms with pharmaceutically acceptable solvents such as water, ethanol, and the like. The

15 solvated forms of the compounds presented herein are also considered to be disclosed herein.

Synthetic chemistry transformations and methodologies useful in synthesizing the compounds described herein are known in the art and include, for example, those described in R. Larock, Comprehensive Organic Transformations (1989); T. W. Greene and P. G. M. Wuts, Protective Groups in Organic Synthesis, 2d. Ed. (1991);

20 L. Fieser and M. Fieser, Fieser and Fieser's Reagents for Organic Synthesis (1994); and L. Paquette, ed., Encyclopedia of Reagents for Organic Synthesis (1995).

Compositions, pharmaceutical Compositions and Formulations

25 In a further aspect, the invention relates to a (pharmaceutical) composition that serves as a delivery system to deliver one or more cargo to one or more cells, wherein the delivery system comprises at least a calixarene (e.g., a compound or salt of Formula (I), Formula (II), Formula (III), Formula (IV), or Formula (V)), a

30 phospholipid, an additional lipid such as a sterol, and optionally a PEGylated lipid.

In a preferred embodiment, the delivery system is a lipid nanoparticle (LNP) comprising at least a calixarene (e.g., a compound or salt of Formula (I), Formula (II), Formula (III), Formula (IV), or Formula (V)), a phospholipid, a sterol, and a

35 PEGylated lipid.

Lipid nanoparticles are the leading technology for nonviral nucleic acid delivery. Naked RNA is quickly degraded after administration by cellular ribonucleases

(RNases). LNPs slow down the degradation process to ensure RNA stability while also promoting cellular internalization via endocytosis and allowing intracellular release of RNA into the cytoplasm for translation by cellular machinery.

- 5 The composition of LNPs known from the art typically includes an ionizable cationic lipid and three neutral helper lipids: phospholipid, cholesterol, and lipid-anchored polyethylene glycol (PEGylated lipid). The ionizable cationic lipids are complexed with polyanionic RNA through ion pairing interactions to enable its encapsulation by the neutral lipids and facilitate cellular uptake and endosomal escape.

10

- Cationic or ionizable lipids (under acidic pH) promote ion pairing interaction with the negatively charged backbone of nucleic acids. This ion pairing interaction facilitates encapsulation of nucleic acid cargo within the electron dense LNP core. Permanently cationic lipids can cause unwanted toxicity and immune response issues, resulting in the increasing adoption of ionizable lipids. Ionizable lipids are positively charged during LNP formation, while mostly neutral at physiological pH. Maintaining neutral pH during circulation helps prevent adsorption of negatively charged biological molecules, thereby preventing rapid clearance by immune cells and increasing circulation time. The ionizable lipid also facilitates nucleic acid cargo release due to electrostatic interactions with the anionic endosomal membrane, which occurs because of the protonated state of the ionizable lipid under acidic pH within the acidic microenvironment of the endosome.
- 15
- 20

- LNPs were initially optimized for formulating siRNA (~23 nt) and have recently evolved to encapsulate larger RNA-agents, including mRNA (~1000 nt). Recently, there has been increased activity for even larger RNA payloads, such as self-amplifying mRNA (saRNA). saRNA is a promising alternative to mRNA as it has been shown to induce immune responses with up to 100-fold lower doses and extended protein expression in vivo compared to mRNA. However, saRNA (~10 000 nt) is larger than mRNA (~1000 nt) and has more secondary structure, making it more difficult to encapsulate and deliver. The inherent chemical and structural differences between mRNA and saRNA in terms of length, stability, and charge density suggests that LNP delivery formulations for saRNA may require conditions significantly different from those developed for mRNA delivery.
- 25
- 30

35

The ionizable lipid is considered to be the most important factor for improving encapsulation efficiency, as this is the component responsible for complexing with RNA cargo. However, in order to obtain a sufficient encapsulation efficiency for

105

saRNA, high amounts of ionizable lipids is necessary (for instance 20 times the amount of ionizable lipid compared to the amount of saRNA).

5 Besides influencing encapsulation efficiency, cellular uptake and promotion of endosomal escape of nucleic acid cargo, the various constituents of the LNP are also important to facilitate monodisperse nanoparticle formation and improve nanoparticle stability. Polydispersity index (PDI) is a normalized value that indicates nanoparticle size range in a sample, and is a useful indicator of sample quality. In
10 samples with high dispersity, larger particles in the distribution will tend to aggregate and sediment, which leads to diminished effective RNA concentration and inconsistent dosing. Typically, LNP formulations developed for biological application should have a PDI below 0.2, which indicates the colloid is acceptably monodisperse. Monodispersity of nanoparticle drugs is crucial to ensure the consistent behavior of the intended drug, as size influences how particles interact with the body.

15 However, in order to obtain a monodisperse population of LNPs having an saRNA cargo, high amounts of ionizable lipids are necessary.

20 In the current invention, the size of the calixarene can vary depending on the plurality of desired features, however, the calixarene is preferably a calix[4]arene.

Calix[4]arenes display several key features that makes them suitable for nucleic acid delivery. The most important is their natural cone-shaped conformation that was found crucial for lipid nanoparticles/ionizable lipids to achieve high endosomal escape
25 and favor the release of RNA in the cytosol.

As described above, a permanently charged cationic component can cause unwanted toxicity and immune response issues, which resulted in the incorporation of ionizable lipids in the traditional LNPs known from the state of the art.

30 In an embodiment, the current invention now provides a delivery system wherein an ionizable calixarene is used in RNA lipid nanoparticles (RNA-LNPs) to replace the traditional ionizable lipid. The resulting delivery system hence comprises 4 components: an ionizable calixarene, a helper lipid, a sterol, and a PEG lipid.

35 Calixarenes are platforms which facilitate the synthesis of ionizable compounds with multiple amine heads, meaning that the charge density (number of amines/molecule) could be increased easily. Besides reducing the amount of

ionizable component necessary for efficiently encapsulating nucleic acids, this property also facilitates the encapsulation of very long RNA, such as self-amplifying RNA (saRNA), by increasing the number of amines without changing the mass ratio between the ionizable component and RNA, while this task remains challenging with the current LNP technology having a formulation that does not comprise calixarenes.

In an alternative embodiment, the current invention provides a delivery system wherein a cationic calixarene is incorporated as a fifth component into lipid nanoparticles (LNPs) made of an ionizable lipid, a helper lipid, a sterol, and a PEG lipid. As described above and similar to the ionizable calixarenes, the charge density (number of amines/molecule) could be increased easily in cationic calixarenes, facilitating the encapsulation of nucleic acids, especially that of very long RNA, such as self-amplifying RNA (saRNA). Furthermore, by incorporating a cationic calixarene into a traditional LNP ("5 component delivery system") the current invention also allows (i) to minimize the inherent toxicity of these cationic components by combining them with non-toxic biocompatible lipids, and (ii) to reduce the non-specific adsorption of proteins that usually limits their efficacy.

In a preferred embodiment, the delivery system of the current invention comprises a nucleic acid cargo, such as mRNA or saRNA. In an embodiment, the mass of the calixarene is at most 25 times higher than the mass of the nucleic acid cargo.

4-component system

As described above, in an embodiment, the current invention also provides a delivery system wherein an ionizable calixarene is used in RNA lipid nanoparticles (RNA-LNPs) to replace the traditional ionizable lipid (4 components: ionizable calixarene, a helper lipid, a sterol, and a PEG lipid).

It is worth noting that the PEGylated lipid is required to obtain stable monodisperse nanoparticles that do not aggregate as the PEGylated lipid ensures the shielding of particles and stabilizes their lipid-water interface. As a result, the PEGylate lipid mass fraction must be precisely controlled; a too low amount in PEG induces the aggregation of the particles, while a too high amount of PEGylated lipid limits their transfection capabilities (see *in vivo* protein expression discussed in example 2 below). The presence of PEG is crucial for improved physicochemical properties of LNPs; however, the level has to be minimized as there have been recorded cases of anaphylactic shock due to PEG-induced hypersensitivity reactions (HRs); in fact, PEG

is considered one of the possible causes of anaphylaxis associated with COVID-19 vaccines like the Pfizer-BioNTech and Moderna mRNA vaccines.

5 A library of ionizable calixarenes was synthesized to better understand the structure-activity relationship. Calixarenes bearing one (CX14, CX16, CX24) or four (CX1, CX2, CX3, CX5, CX6, CX29) ionizable head groups (i-head) were synthesized. Ionizable heads were selected from the group of secondary amines (CX6, CX14) and ternary amines (CX1, CX2, CX3, CX5, CX16, CX24, CX29), cyclic or substituted with methyl or hydroxyethyl groups. Two biodegradable groups were also explored to link the
10 ionizable head to the macrocyclic core and facilitate the metabolic degradation of the resulting compounds and avoid bioaccumulation (amide and ester links in CX2 and CX3/CX29 respectively). All these calixarenes were self-assembled into nanoparticles with a helper lipid (phospholipid), a sterol and a PEGylated lipid. Several helper (DOPE, DSPC) and PEG lipids (DMG-PEG2000, DSG-PEG2000) were
15 explored. Examples of nanoparticles made with ionizable calixarenes and encapsulating a Fluc-mRNA (if not precised) or another RNA are presented in Table 1 of example 2.

20 Ratios between these components were defined to produce stable monodisperse nanoparticles. Interestingly, it was discovered that the N/P and molar ratios that are usually used in the field to define the resulting delivery system are not convenient for this kind of system. This is especially true when moving from 1-headed to 4-headed calixarenes, because their higher charge density (number of amines/molecule) completely changes the mass ratios between components and
25 these parameters are of crucial importance for the stability of particles.

The inventors have observed that calix[4]arenes bearing 4 ionizable head groups (for instance depicted as R^1 in Formula I) show especially favorable characteristics for the incorporation of nucleic acids compared to calix[4]arenes bearing only 1
30 ionizable head group (see examples described below).

Hence, in a preferred embodiment, the delivery system according to the current invention comprises a calix[4]arene bearing 4 ionizable head groups.

35 Said ionizable head groups could for instance comprise secondary amines (see for instance CX6 or CX14) or ternary amines (see for instance CX1, CX2, CX3, CX5, CX16, CX24, CX29). The inventors performed in vitro potency assays in cells using LNPs comprising various ionizable calixarenes and discovered that calixarene

compounds with ternary amines are more potent than calixarene compounds with secondary amines (see results in Table 2 of Example 4). In a preferred embodiment, one or more of said ionizable head groups of said calixarenes hence comprise one or more ternary amines.

5

As described above, said ionizable head groups of the calixarene could be different or the same. In an embodiment, said calixarene comprises 4 identical ionizable head groups.

- 10 The inventors discovered that a minimum mass ratio of [Calixarenes+Lipids]/RNA is needed to obtain stable and monodisperse particles of LNPs and optimize the *in vivo* protein expression of an RNA encapsulated in the LNP after administration (see Example 4). This minimum ratio RNA is ca. 20 for the 1-headed calixarenes tested, while it may be decreased to 10 with the 4-headed calixarenes tested. This
- 15 interesting result shows that less material is needed to achieve similar encapsulation efficiency and monodispersity as compared to commonly used ionizable lipids (e.g., SM-102). The difference may be due to the increased charge density on the 4-headed ionizable calixarenes. *In vitro* potency assays of LNPs comprising ionizable calixarenes in Jurkat cells using Fluc mRNA as a reporter gene were also performed.
- 20 The results (see Table 2 of Example 4) provided further evidence of the superiority of the 4-headed calixarenes over the 1-headed calixarene.

- Likewise, experiments were also performed investigating the *in vivo* protein expression at 3h, 6h and 9h after the intramuscular injection of 1 µg of Fluc mRNA
- 25 encapsulated in LNPs comprising either 1-headed or 4-headed calixarenes (see Example 2, Figure 3). Overall, this experiment indicated that the kinetic of protein expression is different between 1-headed and 4-headed calixarenes. While the signal of CX16 (1-headed) rapidly decreased, the protein expression induced by the 4-headed calixarenes (CX1, CX2, CX3 and CX5) remained stable over the different
- 30 timepoints (from 3 to 9h after injection). This result shows again the superiority of the 4-headed calixarenes over the 1-headed for achieving a stable and high protein expression. This observation is strengthened by the lower amount of 4-headed calixarenes needed to reach this performance (ca. 5µg 4-headed CX/µg RNA vs. ca. 10µg 1-headed CX/µgRNA).

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[Calixarenes+Lipids] refers to the sum of the masses of the calixarene component and the lipid components in the LNP. In an embodiment, this mass equals the sum

of the masses of the calixarene component, the helper lipid component, the sterol component and the PEGylated lipid component.

5 In an embodiment, when using a calixarene bearing one ionizable head group (for instance CX14, CX16, CX24) this mass ratio is below 50, more preferably below 40, more preferably below 30, more preferably below 29, 28, 27, 26, 25, 24, 23, 22, 21, such as 20. In this case, the total mass of the calixarene component plus the lipid component can be as low as only 20 times the mass of the RNA cargo.

10 In an embodiment, when using a calixarene bearing 4 ionizable head groups (for instance CX1, CX2, CX3, CX5, CX6, CX29 as depicted in figure 1) this mass ratio is below 50, more preferably below 40, more preferably below 30, more preferably 20, more preferable below 19, 18, 17, 16, 15, 14, 13, 12, 11 such as 10. In this case, the total mass of the calixarene component plus the lipid component can be as low
15 as only 10 times the mass of the RNA cargo.

This interesting result shows that less material is needed to achieve similar encapsulation efficiency and monodispersity as compared to commonly used ionizable lipids (e.g., SM-102). The difference may be due to the increased charge
20 density on the 4-headed ionizable calixarenes.

With respect to the helper lipid, the sterol and the PEGylated lipid, their mass fractions are conserved when moving from 1-headed to 4-headed systems. These lipid mass fractions (expressed in percent, wherein the total mass of the delivery
25 system without cargo represent 100%) are preferably:

Ionizable calixarene: from 10 to 60%

Helper lipid: from 5 to 35%

Sterol: from 15 to 50%

PEGylated lipid: from 4 to 24%

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As such, in an embodiment, when an ionizable calixarene is used in RNA lipid nanoparticles (RNA-LNPs) to replace the traditional ionizable lipid (4 components: ionizable calixarene, a helper lipid, a sterol, and a PEG lipid), the mass of said ionizable calixarene is 10-60% of the total mass of the delivery system.

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In an embodiment of the 4 component-system, said ionizable calixarene is present in said delivery system at a concentration of 10-60 % (w/w), such as 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%,

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26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59% or 60% (w/w) or any value in between. In an embodiment, said ionizable calixarene is present in delivery system at a concentration between 10-15%, 15-20%, 20-25%, 25-30%, 30-35%, 35-40%, 40-45%, 45-50%, 50-55% or 55-60% (w/w). In an embodiment, said ionizable calixarene is present in said delivery system at a concentration between 10-20%, 20-30%, 30-40%, 40-50% or 50-60% (w/w).

10 In an embodiment, when an ionizable calixarene is used in RNA lipid nanoparticles (RNA-LNPs) to replace the traditional ionizable lipid (4 components: ionizable calixarene, a helper lipid, a sterol, and a PEG lipid), the mass of said phospholipid is 5-35%, more preferably 10-30% of the total mass of the delivery system.

15 In an embodiment of the 4-component system, said phospholipid is present in said delivery system at a concentration of 5-35% (w/w), more preferably 10-30% (w/w), such as 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29% or 30% (w/w) or any value in between.

20 In an embodiment, when an ionizable calixarene is used in RNA lipid nanoparticles (RNA-LNPs) to replace the traditional ionizable lipid (4 components: ionizable calixarene, a helper lipid, a sterol, and a PEG lipid), the mass of said sterol is 15-50% of the total mass of the delivery system.

25 In an embodiment of the 4-component system, said sterol is present in said delivery system at a concentration of 15-50% (w/w), such as 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49% or 50% (w/w) or any value in between.

30 In an embodiment, when an ionizable calixarene is used in RNA lipid nanoparticles (RNA-LNPs) to replace the traditional ionizable lipid (4 components: ionizable calixarene, a helper lipid, a sterol, and a PEG lipid), the mass of said PEGylated lipid is 2-24%, more preferably 10-24% of the total mass of the delivery system.

In an embodiment of the 4-component system, said PEGylated lipid is present in said delivery system at a concentration of 4-24 % (w/w), more preferably 10-24 %

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(w/w), such as 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23% or 24% (w/w) or any value in between.

5 The inventors further performed experiments to investigate the optimal ratio between the mass of the calixarene and the mass of the cargo to optimize encapsulation efficiency (see Example 4).

10 In a preferred embodiment, the delivery system of the current invention comprises a nucleic acid cargo, such as mRNA or saRNA.

The inventors discovered an optimized mass ratio (Calixarene/saRNA) (see Example 4) that allowed to obtain stable nanoparticles with high encapsulation efficiency (> 80%) (see Figure 4) and adequate protein expression *in vivo* (see Figure 5).

15 In an embodiment, said delivery system comprising an ionizable calixarene, a phospholipid, a sterol and a PEGylated lipid and wherein said cargo comprises a nucleic acid, wherein the mass of the ionizable calixarene is at most 15 times higher than the mass of the nucleic acid cargo.

20 In an embodiment, the delivery system comprises an ionizable calixarene, a phospholipid, a sterol and a PEGylated lipid and comprises a nucleic acid cargo, wherein the mass of the ionizable calixarene is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 times higher than the mass of the nucleic acid cargo.

25 In an embodiment, the delivery system comprises an ionizable calixarene, a phospholipid, a sterol and a PEGylated lipid and comprises a nucleic acid cargo, wherein the mass of the ionizable calixarene is between 5-6, 6-7, 7-8, 8-9, 9-10, 10-11, 11-12, 12-13, 13-14 or 14-15 times higher than the mass of the nucleic acid cargo.

30 As described above, saRNA (~10 000 nt) is larger than mRNA (~1000 nt) and has more secondary structure, making it more difficult to encapsulate and deliver. The inherent chemical and structural differences between mRNA and saRNA in terms of length, stability, and charge density suggests that LNP delivery formulations for
35 saRNA may require conditions significantly different from those developed for mRNA delivery.

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In a preferred embodiment, the delivery system comprises an ionizable calixarene, a phospholipid, a sterol and a PEGylated lipid and comprises an saRNA cargo, wherein the mass of the ionizable calixarene is at most 15 times higher, more preferably at most 10 times higher than the mass of the saRNA cargo.

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In an embodiment, the delivery system comprises an ionizable calixarene, a phospholipid, a sterol and a PEGylated lipid and further comprises an saRNA cargo, wherein the mass of the ionizable calixarene is 5 to 15 times higher than the mass of the saRNA cargo.

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In an embodiment, the delivery system comprises an ionizable calixarene, a phospholipid, a sterol and a PEGylated lipid and comprises an saRNA cargo, wherein the mass of the ionizable calixarene is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 times higher than the mass of the saRNA cargo.

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In an embodiment, the delivery system comprises an ionizable calixarene, a phospholipid, a sterol and a PEGylated lipid and comprises an saRNA cargo, wherein the mass of the ionizable calixarene is between 5-6, 6-7, 7-8, 8-9, 9-10, 10-11, 11-12, 12-13, 13-14 or 14-15 times higher than the mass of the saRNA cargo.

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It is worth mentioning that this optimized mass ratio is smaller in comparison to those needed (ca. 20 μg i-lipid/ μg RNA) to reach similar properties with saRNA (encapsulation efficiency and in vitro protein expression) with well-known ionizable lipids used in the field (SM-102, ALC-0315).

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The inventors further preclinically validated a delivery system of the current invention comprising an ionizable calixarene (CX5), a phospholipid, a sterol and a PEGylated lipid. Mice were vaccinated following a 21 days prime-boost regimen using 0.6 μg or 2.5 μg of mRNA per dose. As shown in Figure 6, the delivery system yielded strong VNTs (virus neutralization titers), well above the correlate of protection (0.5 IU/mL), using the two dosing regimens (0.6 or 2.5 μg). These results were accompanied without any adverse effect (loss of weight, impact on spleen, liver and kidneys weight, inflammation or an excessive reactogenic response) on the animals.

35 **5-component system**

In an alternative embodiment, the current invention provides a delivery system wherein a cationic calixarene is incorporated as a fifth component into lipid

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nanoparticles (LNPs) made of an ionizable lipid, a helper lipid, a sterol, and a PEG lipid (5 components).

In a preferred embodiment, said cationic calixarene is a calix[4]arene.

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In an embodiment of the 5-component system, said calixarene is a calix[4]arene bearing 4 cationic head groups. In a further embodiment, said calixarene comprises 4 identical cationic head groups.

10 In an embodiment, one or more of said cationic head groups comprises at least one quaternary amine group. In an embodiment, each of said cationic head groups comprises at least one quaternary amine group. In an embodiment, one or more of said cationic head groups comprises more than one quaternary amine group.

15 The inventors demonstrated that these cationic calixarenes can be embedded into lipid nanoparticles encapsulating RNA (Table 3) and are made with:

- Ionizable lipids: for instance DODAP, DLin-DMA, DLin-MC3-DMA, ALC-0315, SM-102;
- Helper lipids: for instance DOPE, DOPC;
- 20 - Sterol: for instance cholesterol;
- and PEG lipids: for instance DMG-PEG2000, DSG-PEG2000.

As such, in an embodiment, when a cationic calixarene is incorporated as a fifth component into lipid nanoparticles (LNPs) made of an ionizable lipid, a helper lipid,
25 a sterol, and a PEG lipid (5 components), the mass of said cationic calixarene is 0.1-50% (w/w) of the total mass of the delivery system.

In an embodiment of the 5 component-system, said cationic calixarene is present in said delivery system at a concentration of 0.2-60 % (w/w), such as 0.2%, 0.3%,
30 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 5.5%, 6%, 6.5%, 7%, 7.5, 8%, 8.5%, 9%, 9.5%, 10% (w/w) or any value in between. In an embodiment, said cationic calixarene is present in said delivery system at a concentration between 0.1-10%, 10-20%, 20-30%, 30-40% or 40-50% (w/w). In an embodiment, said cationic calixarene is present in said lipid
35 nanoparticle at a concentration between 0.1-1%, 1-2%, 2-3%, 3-4%, 4-5%, 5-6%, 6-7%, 7-8%, 8-9%, 9-10%, 10-11%, 11-12%, 12-13%, 13-14% or 14-15% (w/w).

In an embodiment, when a cationic calixarene is incorporated as a fifth component into lipid nanoparticles (LNPs) made of an ionizable lipid, a helper lipid, a sterol, and a PEG lipid (5 components), the mass of said phospholipid is 5-35%, more preferably 10-30% of the total mass of the delivery system.

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In an embodiment of the 5-component system, said phospholipid is present in said delivery system at a concentration of 5-35% (w/w), more preferably 10-30% (w/w), such as 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29% or 30% (w/w) or any value in between.

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In an embodiment, when a cationic calixarene is incorporated as a fifth component into lipid nanoparticles (LNPs) made of an ionizable lipid, a helper lipid, a sterol, and a PEG lipid (5 components), the mass of said sterol is 15-50% of the total mass of the delivery system.

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In an embodiment of the 5-component system, said sterol is present in said delivery system at a concentration of 15-50% (w/w), such as 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49% or 50% (w/w) or any value in between.

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In an embodiment, when a cationic calixarene is incorporated as a fifth component into lipid nanoparticles (LNPs) made of an ionizable lipid, a helper lipid, a sterol, and a PEG lipid (5 components), the mass of said PEGylated lipid is 2-24%, more preferably 10-24% of the total mass of the delivery system.

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In an embodiment of the 5-component system, said PEGylated lipid is present in said delivery system at a concentration of 2-24 % (w/w), more preferably 10-24 % (w/w), such as 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23% or 24% (w/w) or any value in between.

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In an embodiment, when a cationic calixarene is incorporated as a fifth component into lipid nanoparticles (LNPs) made of an ionizable lipid, a helper lipid, a sterol, and a PEG lipid (5 components), the mass of said ionizable lipid is 15-50% of the total mass of the delivery system.

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In an embodiment of the 5-component system, said ionizable lipid is present in said delivery system at a concentration of 15-50 % (w/w), such as 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%,
5 46%, 47%, 48%, 49% or 50% (w/w) or any value in between.

The inventors further performed experiments and showed that LNPs made with between 0.1 and 20.7 times more cationic calixarene than mRNA (mass ratio) were all found to be monodisperse and encapsulate more than 95% of the RNA. The addition of the cationic calixarene was found to increase both size (from 77 to 129
10 nm) and charge (from 5.8 to 11.0 mV) (see table 4 and Figure 9, Example 3).

The inventors further demonstrated that cationic calixarenes combined with DLin-DMA as the ionizable lipid yields potent LNPs (Example 3) . These results also suggest that the PEGylated lipid choice impacts the immune response: a difference as small
15 as 4 additional carbons on the two alkyl chains of the PEGylated lipid can significantly decrease the immune potency of an otherwise identical LNP (see Figure 10, Example 3).

The inventors further demonstrated that the 5-component system can be used to
20 efficiently encapsulate saRNAs. The resulting LNPs was found monodisperse with a Z-average of 120 nm (Figure 11A) and encapsulated 76.6% of the saRNA. This result is particularly interesting as common ionizable lipids are difficult to use to encapsulate saRNA. This was demonstrated with an saRNA of similar length and encoding for an undisclosed gene of interest. The saRNA was formulated using a SM-
25 102:DSPC:cholesterol:DMG-PEG2000 (50:10:38.5:1.5 molar ratio). The resulting LNP was found polydisperse (Figure 11B).

In a further aspect, the invention relates to a composition comprising the compound
30 or salt as described above and an excipient.

In an embodiment, the compound or salt as described above is by preference present in the composition in a concentration of about 0.1-60 mol%, preferably 0.1-40 mol% and more preferably 0.1-25 mol% of said composition, depending on the desired
35 characteristics of the composition. Such as, the concentration of the compound of the invention in the composition can range from 0.1-50 mol%, from 0.1-44% mol%, from 0.1-40 mol%, from 0.1-30 mol%, from 0.1-20 mol%, from 0.1-10 mol%, from

0.1-5 mol% or from 1-60 mol%, from 10-60 mol%, from 20-60 mol%, from 30-60 mol%, from 35-60 mol% or any ranges and subranges therein between.

5 In an embodiment, the compound or salt as described above is a cationic calixarene, said cationic calixarene is by preference present in the composition in a concentration of about 0.1-10 mol% of said composition. For example, the concentration of cationic calixarene can be between 0.1-10 mol%, 0.5-9 mol%, 1-8 mol%, 2-7 mol%, 3- 6 mol%, 4-5 mol% or 0.1-9 mol%, 0.1-8 mol%, 0.1-7 mol%, 0.1-6 mol%, 0.1-5 mol%, 0.1-4 mol%, 0.1-3 mol%, 0.1-2 mol%, 0.1-1 mol%, or 0.5-10 mol%, 1- 10 mol%, 2-10 mol%, 3-10 mol%, 4-10 mol%, 5-10 mol%, 6-10 mol%, 7-10 mol%, 8-10 mol%, 9-10 mol% and all ranges and subranges therein between.

15 In an embodiment, the compound or salt as described above is an ionizable calixarene, said ionizable calixarene is by preference present in the composition in a concentration of about 10-60 mol% of said composition. For example, the concentration of ionizable calixarene can be between is 10-60 mol%, 10-50 mol%, 10-45 mol%, 10-40 mol%, 10-35 mol%, 10-30 mol%, 10-25 mol% 10-20 mol%, 10-15 mol% or 15-60 mol%, 20-60 mol%, 25-60 mol%, 30-60 mol%, 35-60 mol% or 15-55 mol%, 15-45 mol%, 15-40 mol%, 20-35 mol%, 20-30 mol%, 20-25 mol%, 20 25-30 mol%, or any ranges and subranges therein between.

In an embodiment, the excipient is selected from sugars, starches, cellulose, oils, glycols, polyols, esters, buffering agents, ethyl alcohol, phosphate buffer solutions, and lipids or any combination thereof.

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In an embodiment, the excipient is selected from an ionizable lipid, a cationic lipid, a helper lipid, an additional lipid (for instance a sterol), and a polyethylene glycol (PEG) lipid or PEG conjugate, or any combination of any of the foregoing. In a preferred embodiment, the additional lipid confers rigidity to the delivery system.

30 In an embodiment, the additional lipid can be a sterol, a fatty acid, a glycerol monooleate or a trioleate or any short molecule saturated (chosen for their rigidity property). In embodiments, the short saturated molecule is less than 17 carbon (C) atoms long e.g., at most 16 C, at most 15 C, at most 14 C, at most 13 C, at most 12 C, at most 11 C, at most 10 C, at most 9 C, at most 8 C, at most 7 C, at most 6 C, at most 5 C, at most 4 C, at most 3 C atoms long, preferably between 10 C and 35 3 C atoms long.

In an embodiment, the excipient does not comprise a PEG lipid or PEG conjugate. In an embodiment, the excipient comprises a polypeptide such as polysarcosine instead of a PEG lipid or PEG conjugate. Polysarcosine (pSar) is a polypeptoid based on the endogenous amino acid sarcosine (N-methylated glycine).

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In an embodiment, the excipient is selected from an ionizable lipid, a helper lipid, an additional lipid (for instance a sterol) and a PEG lipid or PEG conjugate, or any combination of any of the foregoing. In an embodiment, the excipient consists of an ionizable lipid, a helper lipid, an additional lipid (for instance a sterol) and a PEG lipid or PEG conjugate. In an embodiment, the excipient consists of an ionizable lipid, a helper lipid and an additional lipid (for instance a sterol). In an embodiment, the additional lipid can be a sterol, a fatty acid, a glycerol monooleate or a trioleate or any short molecule saturated (chosen for their rigidity property).

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In an embodiment, the excipient is selected from a helper lipid, an additional lipid (for instance a sterol), and a PEG lipid or PEG conjugate, or any combination of any of the foregoing. In an embodiment, the excipient consists of a helper lipid, an additional lipid (for instance a sterol), and a PEG lipid or PEG conjugate. In an embodiment, the excipient consists of a helper lipid and an additional lipid (for instance a sterol). In an embodiment, the additional lipid can be a sterol, a fatty acid, a glycerol monooleate or a trioleate or any short molecule saturated (chosen for their rigidity property).

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In an embodiment, when the PEG lipid or PEG conjugate is present in the composition, it is present in a concentration of 0.2-10 mol%, preferably 0.5-5 mol%. The PEG compound is preferably selected from PEG-ceramide, PEG-DMG, PEG-PE, poloxamer, and DSPE carboxy PEG. For instance, in certain embodiments, the PEG compound is C14 PEG2000 DMG, C15 PEG2000 DMG, C16 PEG2000 DMG, C18 PEG2000 DMG, C14 PEG2000 ceramide, C15 PEG2000 ceramide, C16 PEG2000 ceramide, C18 PEG2000 ceramide, C14 PEG2000 PE, C15 PEG2000 PE, C16 PEG2000 PE, C18 PEG2000 PE, C14 PEG350 PE, C14 PEG5000 PE, poloxamer F-127, poloxamer F-68, poloxamer L-64, or DSPE carboxy PEG. In a particularly preferred embodiment, said PEG conjugate is DMG-PEG.

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PEG is often used for its stealth functions in nanoparticle formulations because it is a hydrophilic and flexible polymer. The conjugation of PEG to the composition (for instance used as a delivery system) reduces the interaction of the composition with plasma proteins. As a result, this prevents plasma proteins from adsorbing to the

surface of the composition and uptake of the composition by the reticuloendothelial system (RES). The conjugation of PEG or PEGylation allows delivery systems to circulate within the body for a longer period of time, extending their circulation half-life and, consequently, increasing the accumulation of delivery systems within the target tissues, cells such as tumors and tumor cells.

In a specific embodiment, a combination of a PEGylated lipid, or PEG conjugate and polysarcosine is used.

In an embodiment, the additional lipid (for instance a sterol compound) is present in the composition according to the current invention in a concentration of 20-70 mol%, preferably 35-60 mol%, more preferably 42-57 mol% of said composition. In embodiments, the additional lipid concentration can range between 20-60 mol%, 20-50 mol%, 20-40 mol%, 20-30 mol%, 20-25 mol% or 30-70 mol%, 40-70 mol%, 50-70 mol%, 60-70 mol%, 65-70 mol%, and all the ranges and subranges therein between, preferably 35-60 mol%, preferably 40-60 mol%, more preferably 42-57 mol%.

In an embodiment, when the additional lipid is a sterol, said sterol is preferably selected from the group of cholesterol, sitosterol, sitosterol-amino acid conjugates, stigmasterol, campesterol, fucosterol, brassicasterol, ergosterol, 9, 11-dehydroergosterol, and hydroxycholesterol. In a preferred embodiment, said sterol is cholesterol.

In an embodiment, the composition according to the current invention comprises at least a helper lipid, such as a phospholipid. These helper lipids are neutral lipids. Possible non-limiting examples of phospholipids are distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylethanolamine (DOPE), palmitoyl-oleoylphosphatidylcholine (POPC), palmitoyl-oleoylphosphatidylethanolamine (POPE), dioleoylphosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), DLPE (1,2-dilauroyl-sn-glycero-3-phosphoethanolamine), DPPS (1,2-dipalmitoyl-sn-glycero-3-phospho-L-serine), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE) or 1,2-dioleoyl-sn-glycero-3-

phosphoethanolamine (DOPE) or any combination thereof. The phospholipid fortifies the delivery system and also aids in endosomal escape. In an embodiment, the phospholipid in the composition is DOPE. In an embodiment, the composition comprises a phospholipid, wherein said phospholipid is present in a concentration of 1-45 mol%, preferably 1-35 mol% in said composition, said phospholipid is preferably DOPE. For example, the phospholipid concentration in the composition can range from 1-40 mol%, from 1-30 mol%, from 1-20 mol%, from 1-10 mol%, from 1-5 mol%, or from 5-45 mol%, from 10-45 mol%, from 20-45 mol%, from 30-45 mol%, from 35-45 mol%, or from 5 to 40 mol%, from 10 to 35 mol%, from 15 to 30 mol%, from 20 to 30 mol% and any ranges and subranges therein between.

In an embodiment, the composition according to the current invention comprises an ionizable lipid. Possible non-limiting examples for this ionizable lipid are dioleoyl-3-trimethylammonium propane (DODAP), 1,2-dioleoyloxy-3-dimethylaminopropane (DODMA), ICE (imidazole cholesterol ester), 1,2-dilinoleoyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-dilinoleoyloxy-3-(dimethylamino)acetoxyp propane (DLin-DAC), 1,2-dilinoleoyloxy-3-morpholinopropane (DLin-MA), 1,2-dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-linoleoyl-2-linoleoyloxy-3dimethylaminopropane (DLin-2-DMA), 1,2-dilinoleoyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), 1,2-dilinoleoyloxy-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminebutyrate (DLin-MC3-DMA), di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319), 1,1'-((2-(4-(2-((2-bis(2-hydroxydodecyl)amino)ethyl) (2-hydroxydodecyl)amino)ethyl) piperazin-1-yl)ethyl)azanediyl) bis(dodecan-2-ol) (C12-200), 3,6-bis({4-[bis(2-hydroxydodecyl)amino]butyl})piperazine-2,5-dione (cKK-E12) or any combination thereof. In an embodiment, said ionizable lipid is present in the composition according to the current invention in a concentration of 0.5-40 mol%, preferably 0.5-30 mol%. In a preferred embodiment, the ionizable lipid is dioleoyl-3-trimethylammonium propane (DODAP) or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof.

In an embodiment, said cationic lipids are selected from the group consisting of DOTAP (1,2-dioleoyl-3-trimethylammonium-propane), DC-cholesterol (3 β -[N-(N',N'-dimethylaminoethane)-carbamoyle]cholesterol), DORI (N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(oleoyloxy)propan-1-aminiumbromide), DOSPA (2,3-

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dioleyloxy-N-(2-(sperminecarboxamido)ethyl)-N,N-dimethyl-1-propanaminium Trifluoroacetate), ICE (imidazole cholesterol ester), DOTMA (1,2-di-O- octadecenyl-3-trimethylammonium propane), EPC (1,2-dioleoyl-sn-glycero-3-ethylphosphocholine) or any combination thereof.

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In an embodiment, the composition comprises the compound according to the current invention or a pharmaceutically acceptable salt thereof in a concentration of 0.1-40 mol%; a phospholipid in a concentration of 1-45 mol%; an additional lipid such as a sterol in a concentration of 20-70 mol%, wherein the sum of the concentrations does not exceed 100%.

In an embodiment, the composition comprises the compound according to the current invention (e.g. a cationic calixarene) or a pharmaceutically acceptable salt thereof and an ionizable lipid. In an embodiment, the sum of the concentrations of the compound according to the current invention and the ionizable lipid is comprised between 10 and 60 mol% in said composition. In embodiments, the combined concentration of ionizable lipid and cationic calixarene is 10-60 mol%, 10-55 mol%, 10-50 mol%, 10-45 mol%, 10-40 mol%, 10-35 mol%, 10-30 mol%, 10-25 mol%, 10-20 mol%, 10-15 mol% or 15-60 mol%, 20-60 mol%, 25-60 mol%, 30-60 mol%, 35-60 mol% or 15-55 mol%, 15-45 mol%, 15-40 mol%, 20-35 mol%, 20-30 mol%, 20-25 mol%, 25-30 mol%, or any ranges and subranges therein between.

In an embodiment, the composition (delivery system) can be tailored to include specific compositions of one or more calixarenes, phospholipid, additional lipid (preferably sterol), ionizable lipid, and optionally a PEGylated lipid.

In an embodiment, the composition (delivery system) comprises at least an ionizable calixarene, a phospholipid, a sterol and optionally a PEGylated lipid.

In an embodiment, the composition (delivery system) comprises an ionizable calixarene, DOPE, cholesterol and PEGylated lipid.

In an embodiment, the composition (delivery system) comprises at least a cationic calixarene, a phospholipid lipid, a sterol, an ionizable lipid, and optionally a PEGylated lipid.

In an embodiment, the composition (delivery system) comprises a cationic calixarene, DODAP, DOPE, cholesterol and PEGylated lipid.

In an embodiment, the composition further comprises a targeting agent. Carbohydrates, including various structures/forms of mannose, have been broadly utilized to target carbohydrate binding receptors on Antigen Presenting Cells (APCs).

5 As such, in an embodiment, the targeting agent is selected from a carbohydrate (for instance mannose). In another embodiment, the targeting agent is selected from or an antibody or a derivative thereof. The composition of the current invention, once administered to the patient, can localize to specific organs or cells based on the specificity of the targeting agent.

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In an embodiment, the composition further comprises an adjuvant. An adjuvant or an adjuvant component in the broadest sense is typically a (e.g. pharmacological or immunological) agent or composition that may modify, e.g. enhance, the efficacy of other agents, such as a drug or vaccine. It is to be interpreted in a broad sense and refers to a broad spectrum of substances that are able to increase the immunogenicity of antigens incorporated into or co-administered with the adjuvant in question. Typically, "adjuvant" or "adjuvant component" has the same meaning and can be used mutually. Adjuvants may be divided, e.g., into immuno potentiators, antigenic delivery systems or even combinations thereof. The term "adjuvant" is typically understood not to comprise agents which confer immunity by themselves. An adjuvant assists the immune system unspecifically to enhance the antigen-specific immune response by e.g. promoting presentation of an antigen to the immune system or induction of an unspecific innate immune response. Furthermore, an adjuvant may preferably e.g. modulate the antigen-specific immune response by e.g. shifting the dominating Th2-based antigen specific response to a more Th1 - based antigen specific response or vice versa and/or by inducing of mucosal immune responses and/or increased IgA titers. Accordingly, an adjuvant may favourably modulate cytokine expression/secretion, antigen presentation, type of immune response etc.

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35 Advantages of adjuvants include the enhancement of the immunogenicity of antigens, modification of the nature of the immune response, the reduction of the antigen amount needed for a successful immunization, the reduction of the frequency of booster immunizations needed and an improved immune response in elderly and immunocompromised vaccinees. Adjuvants are known from the prior art and include, but are not limited to, lipopolysaccharide (LPS), hydrocarbon-based or carbohydrate-based compounds, such as imidazoquinoline compounds (preferably Imiquimods compounds, e.g. R-837 (Imiquimod-1-(2-methylpropyl)-1H-imidazol[4,5-c]quinoline-4-amine), or R-848 (Resiquimod)). Adjuvants can also be

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nucleotide-based or nucleoside-based compounds (e.g. an oligodeoxynucleotide containing unmethylated CpG motifs compound (CpG-ODN) or double-stranded nucleic acid compounds, such as double-stranded RNA (dsRNA) compounds, e.g. synthetic dsRNA (for instance polyionisinic:polycytidylic acid (Poly(I:C) - which may act as an agonist for TLR3).

In an embodiment, the composition may include one or more small molecule immunopotentiators. For example, the composition may include a TLR2 agonist (e.g. Pam3CSK4), a TLR4 agonist (e.g. an aminoalkyl glucosaminide phosphate, such as E6020), a TLR7 agonist (e.g. imiquimod), a TLR8 agonist (e.g. resiquimod) and/or a TLR9 agonist (e.g. IC31). Any such agonist ideally has a molecular weight of <2000Da.

In a further aspect, the invention relates to a pharmaceutical composition comprising the compound or salt as described above, or the composition described above, and a cargo. The cargo to be delivered using the calixarene-based delivery system of the invention can be selected from the group consisting of a nucleic acid, a protein, a chemical substance, a polysaccharide, and combinations thereof. Preferably, the cargo is a nucleic acid, more preferably RNA or DNA. The cargo may include therapeutic agents, such as gene therapies, siRNA, mRNA, CRISPR/Cas9 components, proteins, enzymes, antibodies, small molecules, or other chemical substances, as well as imaging agents or contrast agents for diagnostic applications. The cargo can be encapsulated within the delivery system or be associated with the system through electrostatic interactions, covalent bonding, or other means of attachment.

In a preferred embodiment, the invention relates to a pharmaceutical composition comprising the compound or salt as described above, or the composition described above, and one or more nucleotides.

In an embodiment, the invention relates to a pharmaceutical composition comprising the compound or salt as described above, or the composition described above, and one or more polynucleotides. In an embodiment, the pharmaceutical composition comprises one polynucleotide. In an alternative embodiment, the pharmaceutical composition comprises more than one polynucleotide.

In some embodiments, said polynucleotides are selected from long-chain RNA, coding RNA, non-coding RNA, long non-coding RNA, single stranded RNA (ssRNA),

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double stranded RNA (dsRNA), linear RNA (linRNA), circular RNA (circRNA), messenger RNA (mRNA), Trans amplifying mRNA, RNA oligonucleotides, antisense oligonucleotides, small interfering RNA (siRNA), small hairpin RNA (shRNA), antisense RNA (asRNA), CRISPR/Cas9 guide RNAs (gRNA), riboswitches, immunostimulating RNA (isRNA), ribozymes, aptamers, ribosomal RNA (rRNA), transfer RNA (tRNA), viral RNA (vRNA), retroviral RNA or replicon RNA, small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), microRNA (miRNA), and a Piwi-interacting RNA (piRNA).

- 10 In an embodiment, the invention relates to a pharmaceutical composition comprising the compound or salt as described above, or the composition described above, and micro-RNA, saRNA, circRNA or mRNA.

In some embodiments, the pharmaceutical composition comprises modified RNA molecules. In some embodiments, the modification of RNA molecule comprises chemical modifications comprising backbone modifications as well as sugar modifications or base modifications. In this context, a modified RNA molecule as defined herein comprises nucleotide analogues/modifications, e.g. backbone modifications, sugar modifications or base modifications. A backbone modification in connection with the present disclosure is a modification, in which phosphates of the backbone of the nucleotides contained in an RNA molecule are chemically modified. A sugar modification in connection with the present disclosure is a chemical modification of the sugar of the nucleotides of the RNA molecule. Furthermore, a base modification in connection with the present disclosure is a chemical modification of the base moiety of the nucleotides of the RNA molecule. In this context, nucleotide analogues or modifications are selected from nucleotide analogues, which are applicable for transcription and/or translation. In further embodiments, the modified RNA comprises nucleoside modifications selected from 6-aza-cytidine, 2-thio-cytidine, α -thio-cytidine, pseudo-iso-cytidine, 5-aminoallyl-uridine, 5-iodo-uridine, N1-methyl-pseudouridine, 5,6-dihydrouridine, α -thio-uridine, 4-thio-uridine, 6-aza-uridine, 5-hydroxy-uridine, deoxy-thymidine, 5-methyl-uridine, pyrrolo-cytidine, inosine, α -thio-guanosine, 6-methyl-guanosine, 5-methyl-cytidine, 8-oxo-guanosine, 7-deaza-guanosine, N1-methyl-adenosine, 2-amino-6-chloro-purine, N6-methyl-2-amino-purine, pseudo-iso-cytidine, 6-chloro-purine, N6-methyl-adenosine, α -thio-adenosine, 8-azido-adenosine, 7-deaza-adenosine.

In an embodiment, the invention relates to a pharmaceutical composition comprising the compound or salt as described above, or the composition described above, and DNA.

- 5 In an embodiment, the invention relates to a pharmaceutical composition comprising the compound or salt as described above, or the composition described above, and self-amplifying RNA (saRNA).

10 It was found that the pharmaceutical composition is also suited to be used in combination with RNA of a large size, such as self-amplifying RNA (saRNA). Since saRNA is larger than conventional mRNA, lipid nanoparticles described in the state of the art often function improperly as they result in poor encapsulation, or sub-optimal *in vivo* delivery. This is solved by the pharmaceutical compositions as currently defined.

15 By preference, the size of the (sa)RNA can be between 500 and 50 000 nucleotides (nt), preferably between 1000 and 40 000 nt, more preferably between 5000 and 30 000, or between 8000 and 16 000 nt.

20 The self-replicative nature of the mRNA constructs is based on the genomic RNA of RNA viruses, but lack the genes encoding one or more structural proteins. The self-replicating RNA molecules are capable of being translated to produce non-structural proteins of the RNA virus and heterologous proteins encoded by the self-replicating RNA. Self-replicating RNA molecules are designed so that the self-replicating RNA molecule cannot induce production of infectious viral particles. One suitable system for achieving self-replication is to use an alphavirus-based RNA replicon. These +-stranded replicons are translated after delivery to a cell to give of a replicase (or replicase-transcriptase). The replicase is translated as a polyprotein which auto-cleaves to provide a replication complex which creates genomic --strand copies of the +-strand delivered RNA. These --strand transcripts can themselves be transcribed to give further copies of the +-stranded parent RNA and also to give a subgenomic transcript which encodes the desired gene product. Translation of the subgenomic transcript thus leads to in situ expression of the desired gene product by the cell. Suitable alphavirus replicons can use a replicase from a Sindbis virus, a Semliki Forest Virus, an eastern equine encephalitis virus, a Venezuelan Equine Encephalitis Virus, etc. A preferred self-replicating RNA molecule encodes (i) a RNA-dependent RNA polymerase which can transcribe RNA from the self-replicating RNA

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molecule and (ii) protein/peptide of interest. The polymerase can be an alphavirus replicase e.g., comprising alphavirus protein nsP4.

5 In a further aspect, the invention relates to the compound or salt as described above, for use as a delivery system.

10 The (mean) diameter of the delivery system can be quantified by any means known from the state of the art, such as quasi-electric light scattering (QELS), dynamic light scattering (DLS), Nanoparticle Tracking Analysis (NTA) and by imaging methods (such as, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and cryo-(TEM)). In an embodiment, DLS allows to determine the average particle size and polydispersity index (PDI, a measure of particle size distribution), while NTA allows to determine the mean, mode, and span of the particle population. In an embodiment, the delivery system comprises a monodispersed population. As
15 such, in a preferred embodiment, the delivery system has a PDI below 0.25, preferably below 0.2.

In an embodiment, the delivery system comprises a monodispersed population. As such, in a preferred embodiment, the delivery system has a PDI below 0.25,
20 preferably below 0.2.

Figure 7 shows a delivery system according to the current invention, more specifically an LNP comprising an ionizable calixarene, a phospholipid, a sterol and a PEGylated lipid (CX5:DOPE:cholesterol:DMG-PEG2000 nanoparticles encapsulating
25 mRNA-FLuc) which were imaged using cryo-TEM. The LNPs as imaged are monodisperse, with the smallest and largest LNPs around 40 nm and 80 nm respectively, which is in good agreement with DLS measurements (Z-average of ca. 50 nm).

30 In an embodiment, advanced mathematical analyses (e.g. CUMULANT analysis) can be used to estimate the mean size and PDI of the delivery system of the current disclosure. Besides influencing the biodistribution, the diameter also influences the loading capacity of the delivery system.

35 In an embodiment, the mean diameter of the delivery system is between 10 nm and 10 000 nm, more preferably between 10 nm and 9000 nm, more preferably between 10 nm and 8000 nm, more preferably between 10 nm and 7000 nm, more preferably between 10 nm and 6000 nm, more preferably between 10 nm and 5000 nm, more

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preferably between 10 nm and 4000 nm, more preferably between 10 nm and 3000 nm, more preferably between 10 nm and 2000 nm, more preferably 10 and 1000 nm, more preferably between 50 nm and 200 nm. In an embodiment, the mean diameter of the delivery system is at least 10 nm, preferably at least 20 nm, more preferably at least 25 nm, more preferably at least 30 nm, more preferably at least 35 nm, more preferably at least 40 nm, more preferably at least 45 nm, more preferably at least 50 nm. In an embodiment, the mean diameter of the delivery system is at most 900 nm, more preferably at most 800 nm, more preferably at most 700 nm, more preferably at most 600 nm, more preferably at most 500 nm, more preferably at most 400 nm, more preferably at most 300 nm, more preferably at most 200 nm.

In an embodiment, the mean diameter of the delivery system is between 10 nm and 200 nm, between 10 nm and 190 nm, between 10 nm and 180 nm, between 10 nm and 170 nm, between 10 nm and 160 nm, between 10 nm and 150 nm, between 10 nm and 140 nm, between 10 nm and 130 nm, between 10 nm and 120 nm, between 10 nm and 110 nm, between 10 nm and 100 nm, between 10 nm and 90 nm, between 10 nm and 80 nm, between 10 nm and 70 nm, between 10 nm and 60 nm, between 10 nm and 50 nm, between 10 nm and 40 nm, between 10 nm and 30 nm, between 10 nm and 20 nm, between 20 nm and 200 nm, between 20 nm and 190 nm, between 20 nm and 180 nm, between 20 nm and 170 nm, between 20 nm and 160 nm, between 20 nm and 150 nm, between 20 nm and 140 nm, between 20 nm and 130 nm, between 20 nm and 120 nm, between 20 nm and 110 nm, between 20 nm and 100 nm, between 20 nm and 90 nm, between 20 nm and 80 nm, between 20 nm and 70 nm, between 20 nm and 60 nm, between 20 nm and 50 nm, between 20 nm and 40 nm, between 20 nm and 30 nm, between 30 nm and 200 nm, between 30 nm and 190 nm, between 30 nm and 180 nm, between 30 nm and 170 nm, between 30 nm and 160 nm, between 30 nm and 150 nm, between 30 nm and 140 nm, between 30 nm and 130 nm, between 30 nm and 120 nm, between 30 nm and 110 nm, between 30 nm and 100 nm, between 30 nm and 90 nm, between 30 nm and 80 nm, between 30 nm and 70 nm, between 30 nm and 60 nm, between 30 nm and 50 nm, between 30 nm and 40 nm, between 40 nm and 200 nm, between 40 nm and 190 nm, between 40 nm and 180 nm, between 40 nm and 170 nm, between 40 nm and 160 nm, between 40 nm and 150 nm, between 40 nm and 140 nm, between 40 nm and 130 nm, between 40 nm and 120 nm, between 40 nm and 110 nm, between 40 nm and 100 nm, between 40 nm and 90 nm, between 40 nm and 80 nm, between 40 nm and 70 nm, between 40 nm and 60 nm, between 40 nm and 50 nm, between 50 nm and 200 nm, between 50 nm and 190 nm, between 50 nm

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and 180 nm, between 50 nm and 170 nm, between 50 nm and 160 nm, between 50 nm and 150 nm, between 50 nm and 140 nm, between 50 nm and 130 nm, between 50 nm and 120 nm, between 50 nm and 110 nm, between 50 nm and 100 nm, between 50 nm and 90 nm, between 50 nm and 80 nm, between 50 nm and 70 nm, between 50 nm and 60 nm, between 60 nm and 200 nm, between 60 nm and 190 nm, between 60 nm and 180 nm, between 60 nm and 170 nm, between 60 nm and 160 nm, between 60 nm and 150 nm, between 60 nm and 140 nm, between 60 nm and 130 nm, between 60 nm and 120 nm, between 60 nm and 110 nm, between 60 nm and 100 nm, between 60 nm and 90 nm, between 60 nm and 80 nm, between 60 nm and 70 nm, between 70 nm and 200 nm, between 70 nm and 190 nm, between 70 nm and 180 nm, between 70 nm and 170 nm, between 70 nm and 160 nm, between 70 nm and 150 nm, between 70 nm and 140 nm, between 70 nm and 130 nm, between 70 nm and 120 nm, between 70 nm and 110 nm, between 70 nm and 100 nm, between 70 nm and 90 nm, between 70 nm and 80 nm, between 80 nm and 200 nm, between 80 nm and 190 nm, between 80 nm and 180 nm, between 80 nm and 170 nm, between 80 nm and 160 nm, between 80 nm and 150 nm, between 80 nm and 140 nm, between 80 nm and 130 nm, between 80 nm and 120 nm, between 80 nm and 110 nm, between 80 nm and 100 nm, between 80 nm and 90 nm, between 90 nm and 200 nm, between 90 nm and 190 nm, between 90 nm and 180 nm, between 90 nm and 170 nm, between 90 nm and 160 nm, between 90 nm and 150 nm, between 90 nm and 140 nm, between 90 nm and 130 nm, between 90 nm and 120 nm, between 90 nm and 110 nm, between 90 nm and 100 nm, between 100 nm and 200 nm, between 100 nm and 190 nm, between 100 nm and 180 nm, between 100 nm and 170 nm, between 100 nm and 160 nm, between 100 nm and 150 nm, between 100 nm and 140 nm, between 100 nm and 130 nm, between 100 nm and 120 nm, between 100 nm and 110 nm, between 110 nm and 200 nm, between 110 nm and 190 nm, between 110 nm and 180 nm, between 110 nm and 170 nm, between 110 nm and 160 nm, between 110 nm and 150 nm, between 110 nm and 140 nm, between 110 nm and 130 nm, between 110 nm and 120 nm, between 120 nm and 200 nm, between 120 nm and 190 nm, between 120 nm and 180 nm, between 120 nm and 170 nm, between 120 nm and 160 nm, between 120 nm and 150 nm, between 120 nm and 140 nm, between 120 nm and 130 nm, between 130 nm and 200 nm, between 130 nm and 190 nm, between 130 nm and 180 nm, between 130 nm and 170 nm, between 130 nm and 160 nm, between 130 nm and 150 nm, between 130 nm and 140 nm, between 140 nm and 200 nm, between 140 nm and 190 nm, between 140 nm and 180 nm, between 140 nm and 170 nm, between 140 nm and 160 nm, between 140 nm and 150 nm, between 150 nm and 200 nm, between 150 nm and 190 nm, between 150 nm and 180 nm, between 150 nm and

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170 nm, between 150 nm and 160 nm, between 160 nm and 200 nm, between 160 nm and 190 nm, between 160 nm and 180 nm, between 160 nm and 170 nm, between 170 nm and 200 nm, between 170 nm and 190 nm, between 170 nm and 180 nm, between 170 nm and 180 nm, between 180 nm and 190 nm, between 190 nm and 200 nm.

In an embodiment, the mean diameter of the delivery system is between 200 nm and 1000 nm, between 200 nm and 950 nm, between 200 nm and 900 nm, between 200 nm and 850 nm, between 200 nm and 800 nm, between 200 nm and 750 nm, between 200 nm and 700 nm, between 200 nm and 650 nm, between 200 nm and 600 nm, between 200 nm and 550 nm, between 200 nm and 500 nm, between 200 nm and 450 nm, between 200 nm and 400 nm, between 200 nm and 350 nm, between 200 nm and 300 nm, between 200 nm and 250 nm, between 250 nm and 1000 nm, between 250 nm and 950 nm, between 250 nm and 900 nm, between 250 nm and 850 nm, between 250 nm and 800 nm, between 250 nm and 750 nm, between 250 nm and 700 nm, between 250 nm and 650 nm, between 250 nm and 600 nm, between 250 nm and 550 nm, between 250 nm and 500 nm, between 250 nm and 450 nm, between 250 nm and 400 nm, between 250 nm and 350 nm, between 250 nm and 300 nm, between 300 nm and 1000 nm, between 300 nm and 950 nm, between 300 nm and 900 nm, between 300 nm and 850 nm, between 300 nm and 800 nm, between 300 nm and 750 nm, between 300 nm and 700 nm, between 300 nm and 650 nm, between 300 nm and 600 nm, between 300 nm and 550 nm, between 300 nm and 500 nm, between 300 nm and 450 nm, between 300 nm and 400 nm, between 300 nm and 350 nm, between 350 nm and 1000 nm, between 350 nm and 950 nm, between 350 nm and 900 nm, between 350 nm and 850 nm, between 350 nm and 800 nm, between 350 nm and 750 nm, between 350 nm and 700 nm, between 350 nm and 650 nm, between 350 nm and 600 nm, between 350 nm and 550 nm, between 350 nm and 500 nm, between 350 nm and 450 nm, between 350 nm and 400 nm, between 400 nm and 1000 nm, between 400 nm and 950 nm, between 400 nm and 900 nm, between 400 nm and 850 nm, between 400 nm and 800 nm, between 400 nm and 750 nm, between 400 nm and 700 nm, between 400 nm and 650 nm, between 400 nm and 600 nm, between 400 nm and 550 nm, between 400 nm and 500 nm, between 400 nm and 450 nm, between 450 nm and 1000 nm, between 450 nm and 950 nm, between 450 nm and 900 nm, between 450 nm and 850 nm, between 450 nm and 800 nm, between 450 nm and 750 nm, between 450 nm and 700 nm, between 450 nm and 650 nm, between 450 nm and 600 nm, between 450 nm and 550 nm, between 450 nm and 500 nm, between 500 nm and 1000 nm, between 500 nm and 950 nm, between 500 nm and

900 nm, between 500 nm and 850 nm, between 500 nm and 800 nm, between 500 nm and 750 nm, between 500 nm and 700 nm, between 500 nm and 650 nm, between 500 nm and 600 nm, between 500 nm and 550 nm, between 550 nm and 1000 nm, between 550 nm and 950 nm, between 550 nm and 900 nm, between 550 nm and 850 nm, between 550 nm and 800 nm, between 550 nm and 750 nm, between 550 nm and 700 nm, between 550 nm and 650 nm, between 550 nm and 600 nm, between 600 nm and 1000 nm, between 600 nm and 950 nm, between 600 nm and 900 nm, between 600 nm and 850 nm, between 600 nm and 800 nm, between 600 nm and 750 nm, between 600 nm and 700 nm, between 600 nm and 650 nm, between 650 nm and 1000 nm, between 650 nm and 950 nm, between 650 nm and 900 nm, between 650 nm and 850 nm, between 650 nm and 800 nm, between 650 nm and 750 nm, between 650 nm and 700 nm, between 700 nm and 1000 nm, between 700 nm and 950 nm, between 700 nm and 900 nm, between 700 nm and 850 nm, between 700 nm and 800 nm, between 700 nm and 750 nm, between 750 nm and 1000 nm, between 750 nm and 950 nm, between 750 nm and 900 nm, between 750 nm and 850 nm, between 750 nm and 800 nm, between 800 nm and 1000 nm, between 800 nm and 950 nm, between 800 nm and 900 nm, between 800 nm and 850 nm, between 850 nm and 950 nm, between 850 nm and 900 nm, between 900 nm and 950 nm, between 950 nm and 1000 nm.

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Preparation of the delivery system: The delivery system components, including calixarene, phospholipid, additional lipid such as sterol, optionally a PEGylated lipid, and optionally ionizable and/or cationic lipid, are combined and formulated into a delivery system. The nucleotides (further also referred to as "cargo") to be delivered are incorporated into the delivery system, either by encapsulation, association through electrostatic interactions, or other means of attachment. The cargo can be incorporated during or post-combination of the components of the delivery system.

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The cargo can be encapsulated within the delivery system or be associated with the system through ion pairing interactions, covalent bonding, or other means of attachment.

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According to the invention, inclusion of buffered agents and solvents can be further present in the delivery system and/or aid with the production thereof. For example, for the fabrication of the delivery system, preferred solvent is ethanol for the lipid phase and acidic aqueous buffer for the aqueous phase. In embodiments, the preferred buffers are citrate and acetate buffers with a ranging pH and concentrations. In specific embodiments the pH of the buffers are between pH 3-5.5

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and the concentration is ranging between 10-50 mM. However, none of the example buffers given above are limiting a various buffers known in the field that are used for the formation of the delivery systems can be used and that is obvious to a skilled person. Not limiting but preferred method of fabrication is microfluidic mixing.

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In embodiments, the preferred buffers are citrate and acetate buffers with a ranging pH and concentrations. In specific embodiments the pH of the buffers are between pH 3-6 and the concentration is ranging between 1 and 100 mM.

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Delivery of cargo to target cells: Once the delivery system is administered, it can facilitate the transport of the cargo to the target cells. The calixarene-based delivery system may enhance cellular uptake, endosomal escape, and release of the cargo into the cytoplasm or other intracellular compartments, depending on the cargo and the target cells.

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Evaluation of therapeutic or diagnostic effects: After the cargo has been delivered to the target cells, the therapeutic or diagnostic effects can be assessed. This may include monitoring changes in gene expression, protein levels, cellular function, disease progression, or other relevant outcomes. In the case of diagnostic applications, imaging or other techniques may be employed to visualize the distribution and effects of the cargo.

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In another aspect of the invention, a pharmaceutical composition for use as a vaccine is disclosed. Said pharmaceutical compositions or vaccines are particularly useful for veterinary and human use.

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The pharmaceutical composition or vaccine may be formulated in a aqueous liquid, comprising one of more buffering agents.

The pharmaceutical composition detailed herein may be formulated for any available delivery route, including an oral, mucosal (e.g., nasal, sublingual, vaginal, buccal, or rectal), parenteral (e.g., intraarticular, intravenous, intraperitoneal, intramuscular, intradermal or subcutaneous injection), topical or transdermal delivery form. Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions or suspensions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents,

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stabilizers, and preservatives. In the practice of this invention, compositions are preferably administered, for example, by intravenous infusion, orally, topically, intraperitoneally, intravesically, or intrathecally.

- 5 The pharmaceutical composition may be prepared in unit dose form. In some embodiments a unit dose may have a volume of between about 0.1-1.0ml, e.g. about 0.5ml. The pharmaceutical compositions or vaccines can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials. Injection solutions and suspensions can be prepared from sterile powders, granules, and tablets. Cells
10 transfected with the pharmaceutical composition of the invention can also be administered intravenously or parenterally.

- Said pharmaceutical compositions or vaccines can be administered as a single dose or as a multi-dose, requiring a series of two or more doses, administered within a
15 pre-defined timespan. Such timespan may be a week, two weeks, three weeks, four weeks, five weeks, six weeks, seven weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks up until one year.

- In an embodiment, the pharmaceutical compositions or vaccines are administered
20 periodically, such as annually or bi-annually. A suited dose may be between 0.05 and 1 ml, more preferably between 0.25 and 0.75 ml, such as 0.5 ml.

- The pharmaceutical composition or the vaccines are preferably sterile and may be sterilized by conventional sterilization techniques. The vaccines or compositions may
25 contain pharmaceutically acceptable auxiliary substances, to approximate physiological conditions, such as pH adjusting and/or buffering agents and tonicity adjusting agents, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like.

- 30 The tonicity of the pharmaceutical compositions or vaccines may have to be adjusted with sodium salts, for example, sodium chloride. The tonicity of a pharmaceutical composition for parenteral administration is typically 0,9% or 9mg/ml NaCl. The vaccines of the current invention may have an osmolality of between 200 mOsm/kg and 400 mOsm/kg, e.g. between 240-360 mOsm/kg or between 290-310 mOsm/kg.

- 35 Preservative-free vaccines are preferred. However, if desired, the vaccine of the invention may include one or more preservatives, such as phenol and 2-

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phenoxyethanol. Thiomersal, a mercury containing preservative, should be avoided as mercury-free compositions are preferred.

5 The vaccines of the invention is preferably non-pyrogenic e.g. containing <1 EU (endotoxin unit, a standard measure) per dose and preferably <0.1 EU per dose. The vaccine is preferably gluten free.

10 The pharmaceutical composition may contain preservatives, solubilizers, stabilizers, re-wetting agents, emulators, sweeteners, dyes, adjusters, and salts for the adjustment of osmotic pressure, buffers, coating agents, or antioxidants. The pharmaceutical composition may also contain other substances which have valuable therapeutic properties. The pharmaceutical composition may be prepared by known pharmaceutical methods.

15 The pharmaceutical compositions as disclosed herein will typically include a pharmaceutically acceptable carrier in addition to the delivery system comprising a compound according to the current invention. The pharmaceutical composition may include the delivery system in plain water (e.g. w.f.i.) or in a buffer e.g. a phosphate buffer, a Tris buffer, a borate buffer, a succinate buffer, a histidine buffer, or a citrate
20 buffer. Buffer salts will typically be included in the 5-20 mM range.

The pharmaceutical composition may have a pH between about 5.0 and 9.5 e.g. between about 6.0 and 8.0.

25 Pharmaceutical compositions described herein may include sodium salts (e.g. sodium chloride) to give tonicity. A concentration of 10 ± 2 mg/ml NaCl is typical, e.g. about 9 mg/ml.

Pharmaceutical compositions described herein may include metal ion chelators.
30 These can prolong RNA stability by removing ions which can accelerate phosphodiester hydrolysis. Thus a pharmaceutical composition may include one or more of EDTA, EGTA, BAPTA, pentetic acid, etc.. Such chelators are typically present at between 10-500 μ M, e.g. 0.1 mM. A citrate salt, such as sodium citrate, can also act as a chelator, while advantageously also providing buffering activity.

35 The pharmaceutical composition may have an osmolality of between about 200 mOsm/kg and 400 mOsm/kg, e.g. between 240-360 mOsm/kg, or between 290-310 mOsm/kg.

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The pharmaceutical composition may include one or more preservatives, such as thiomersal or 2-phenoxyethanol. Mercury-free compositions are preferred, and preservative-free vaccines can be prepared.

- 5 The pharmaceutical compositions may be prepared as injectables, either as solutions or suspensions. The pharmaceutical composition may be prepared for pulmonary administration e.g. by an inhaler, using a fine spray. The pharmaceutical composition may be prepared for nasal, aural or ocular administration e.g. as spray or drops. Injectables for intramuscular administration are typical.

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The pharmaceutical compositions may be lyophilized or stabilized in a dry form. "Lyophilizing" in this document refers to freeze-drying a liquid or pre-lyophilization formulation. Freeze-drying is performed by freezing the formulation and then subliming ice from the frozen content at a temperature suitable for primary drying.

- 15 Under this condition the product temperature is below the collapse temperature of the formulation. A secondary drying stage may then be carried out, which produces a suitable lyophilized cake. Lyophilization is commonly used in the production of pharmaceutical compounds to increase the stability of the Active Pharmaceutical Ingredient (API) by removing solvents. Lyophilization offers many advantages as it
20 allows the processing and development of pharmaceutical compounds, otherwise unstable in solution, hence improving their shelf life. This technique can facilitate development, usage, distribution and commercialization of new drugs.

Methods of treatment and use of compounds or compositions

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In a further aspect, the invention relates to a method of treating or preventing a disease or disorder using a pharmaceutical composition as described above. In some embodiments, the pharmaceutical composition is for use as a human or veterinary medicament.

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The present invention has broad applicability in various therapeutic and diagnostic settings, including gene therapy, RNA interference, genome editing, protein replacement therapy, drug delivery, and imaging. The improved cargo delivery system may enhance the efficacy and safety of these interventions, ultimately
35 benefiting patients and advancing the field of medicine.

In an embodiment, said pharmaceutical composition is used in gene therapy. Gene therapy can include protein replacement strategies (for instance by means of DNA

or mRNA delivery) or gene silencing (for instance by means of siRNA or miRNA delivery). In a preferred embodiment, said pharmaceutical composition is a vaccine or can be used to immunize or vaccinate subjects.

- 5 In an embodiment, the present disclosure provides RNA (e.g., mRNA) vaccines that include at least one RNA (e.g., mRNA) polynucleotide having an open reading frame encoding at least one antigenic polypeptide or an immunogenic fragment thereof (e.g., an immunogenic fragment capable of inducing an immune response to the antigenic polypeptide). While not wishing to be bound by theory, it is believed that
10 the RNA (e.g., mRNA) vaccines, for example, as mRNA polynucleotides, are better designed to produce the appropriate protein conformation upon translation, as the RNA (e.g., mRNA) vaccines co-opt natural cellular machinery. Unlike traditional vaccines, which are manufactured *ex vivo* and may trigger unwanted cellular responses, RNA (e.g., mRNA) vaccines are presented to the cellular system in a more
15 native fashion.

- Pharmaceutical compositions comprise an immunologically effective amount of polynucleotides, as well as any other components, as needed. By 'immunologically effective amount', it is meant that the administration of that amount to a subject,
20 either in a single dose or as part of a series, is effective for treatment or prophylaxis. This amount varies depending upon the health and physical condition of the subject to be treated, age, the taxonomic group of subject to be treated (e.g. non-human primate, primate, cattle etc.), the capacity of the subject's immune system to synthesise antibodies, the degree of protection desired, the formulation of the
25 vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials. The RNA content of the pharmaceutical compositions described herein will generally be expressed in terms of the amount of RNA per dose. In an embodiment, said dose has $\leq$ about 100 μ g RNA (e.g. from 10-
30 100 μ g, such as about 10 μ g, 25 μ g, 50 μ g, 75 μ g or 100 μ g), but expression can be seen at much lower levels, e.g. $\leq$ 1 μ g/dose, $\leq$ 100ng/dose, $\leq$ 10ng/dose, $\leq$ 1ng/dose, etc.

- The disclosure also provides a delivery device (e.g. syringe, nebuliser, sprayer,
35 inhaler, dermal patch, etc.) containing a pharmaceutical composition as disclosed herein. This device can be used to administer the pharmaceutical composition to a subject.

Also disclosed herein are methods of treatment. More particularly, disclosed herein are pharmaceutical compositions as described herein for use as a human or veterinary medicament. Even more particularly, the above pharmaceutical compositions of the disclosure can be used in a method of treating or prophylactically
5 treating disorders in a human or non-human animal comprising administering to a human or non-human animal, a therapeutically effective amount of a pharmaceutical composition according to the current invention.

The animal may be a land animal, an aquatic animal, an avian, or an amphibian. The
10 animal may be a mammal, or a non-mammal. In a preferred embodiment, the animal is a human. In another embodiment, the animal is a non-human animal. The non-human animal can be an animal raised for human consumption or a domesticated animal. Examples of animals that can be administered the disclosed composition for use include, but are not limited to, ruminant species, such as a sheep, goat, cow,
15 deer, bison, buffalo, elk, alpaca, camel or llama; ungulates, such as a horse, donkey, or pig; avians, such as chickens, including laying hens and broilers, turkey, goose, duck, Cornish game hen, quail, partridge, pheasant, guinea-fowl, ostrich, emu, swan, or pigeon; aquatic animals, such as an aquaculture species, such as fish (e.g., salmon, trout, tilapia, sea bream, carp, cod, halibut, snapper, herring, catfish,
20 flounder, hake, smelt, anchovy, lingcod, moi, perch, orange roughy, bass, tuna, mahi mahi, mackerel, eel, barracuda, marlin, Atlantic ocean perch, Nile perch, Arctic char, haddock, hoki, Alaskan Pollock, turbot, freshwater drum, walleye, skate, sturgeon, Dover sole, common sole, wolfish, sablefish, American shad, John Dory, grouper, monkfish, pompano, lake whitefish, tilefish, wahoo, cusk, bowfin, kingklip,
25 opah, mako shark, swordfish, cobia, croaker, or hybrids thereof, and the like), crustaceans (e.g., lobster, shrimp, prawns, crab, krill, crayfish, barnacles, copepods, and the like), or molluscs (e.g., squid, octopus, abalone, conchs, rock snails, whelk, clams, oysters, mussels, cockles, and the like). Additionally, or alternatively, the animal may be a companion animal, such as canines; felines; rabbits; rodents, such
30 as a rat, mouse, hamster, gerbil, guinea pig or chinchilla; birds, such as parrots, canaries, parakeets, finches, cockatoos, macaws, parakeets or cockatiel; reptiles, such as snakes, lizards, tortoises or turtles; fish; crustaceans; and amphibians, such as frogs, toads and newts.

35 In some embodiments, the individual is an animal, preferably a mammal. In some embodiments, the individual is a primate, bovine, ovine, porcine, equine, canine, feline, or rodent. In some embodiments, the individual is a human. In some embodiments, the individual has any of the diseases or disorders disclosed herein.

In some embodiments, the individual is at risk of developing any of the diseases or disorders disclosed herein. In some embodiments, the individual is human. In some embodiments, the human is at least about or is about any of 21, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, or 85 years old. In some embodiments, the human is a child. In some embodiments, the human is less than about or about an 20, 18, 15, 12, 10, 8, 6, 5, 4, 3, 2, or 1 year.

In a preferred embodiment, the polynucleotide in the pharmaceutical composition encodes an antigen, preferably an antigen linked to an infectious disease or agent.

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In a specific embodiment, the antigen is a target-specific antigen which can be a tumor antigen, or a bacterial, viral or fungal antigen. Said target-specific antigen can be derived from either one of: total mRNA isolated from (a) target cell(s), one or more specific target mRNA molecules, protein lysates of (a) target cell(s), specific proteins from (a) target cell(s), or a synthetic target-specific peptide or protein and synthetic mRNA or DNA encoding a target-specific antigen or its derived peptides. To avoid any misunderstanding, the pharmaceutical compositions as disclosed herein may comprise a single mRNA molecule, or they may comprise multiple mRNA molecules, such as a combination of one or more mRNA molecules encoding immune modulating proteins and/or one or more mRNA molecules encoding antigen- and/or disease-specific proteins.

In an embodiment, said polynucleotides encode an immunogen. The immunogen may elicit an immune response against a bacterium, a virus, a fungus, a parasite, an allergen, or a tumor antigen. The immune response may comprise an antibody response (usually including IgG) and/or a cell-mediated immune response. The immunogen can for instance be a surface polypeptide (e.g. an adhesin, a hemagglutinin, an envelope glycoprotein, a spike glycoprotein, etc), an internal protein (e.g. a nucleoprotein) or any combination of any of the foregoing.

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In some examples the immunogen elicits an immune response against one of these bacteria:

- *Neisseria meningitidis*: useful immunogens include, but are not limited to, membrane proteins such as adhesins, autotransporters, toxins, iron acquisition proteins, and factor H binding protein.
- *Streptococcus pneumoniae*: useful immunogens include, but are not limited to, the RrgB pilus subunit, the beta-N-acetyl-hexosaminidase precursor

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(spr0057), spr0096, General stress protein GSP-781 (spr2021, SP2216), serine/threonine kinase StkP (SP 1732), and pneumococcal surface adhesin PsaA.

- *Streptococcus pyogenes*.
- 5 • *Moraxella catarrhalis*.
- *Bordetella pertussis*: Useful pertussis immunogens include, but are not limited to, pertussis toxin or toxoid (PT), filamentous haemagglutinin (FHA), pertactin, and agglutinogens 2 and 3.
- *Staphylococcus aureus*: Useful immunogens include, but are not limited to a hemolysin, esxA, esxB, ferrichrome-binding protein (sta006) and/or the sta011 lipoprotein.
- 10 • *Clostridium tetani*: the typical immunogen is tetanus toxoid.
- *Corynebacterium diphtheria*: the typical immunogen is diphtheria toxoid.
- *Haemophilus influenzae*
- 15 • *Pseudomonas aeruginosa*
- *Streptococcus agalactiae*
- *Chlamydia trachomatis*: Useful immunogens include, but are not limited to, PepA, LcrE, ArtJ, DnaK, CT398, OmpH-like, L7/L12, OmcA, AtoS, CT547, Eno, HtrA and MurG.
- 20 • *Chlamydia pneumonia*
- *Helicobacter pylori*: Useful immunogens include, but are not limited to, CagA, VacA, NAP, and/or urease.
- *Escherichia coli*: Useful immunogens include, but are not limited to, immunogens derived from enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAggEC), diffusely adhering *E. coli* (DAEC), enteropathogenic *E. coli* (EPEC), extraintestinal pathogenic *E. coli* (ExPEC) and/or enterohemorrhagic *E. coli* (EHEC). ExPEC strains include uropathogenic *E. coli* (UPEC) and meningitis/sepsis-associated *E. coli* (MNEC). A useful immunogen for several *E. coli* types is AcfD.
- 25 • *Bacillus anthracis*
- *Yersinia pestis*
- *Staphylococcus epidermis*
- *Clostridium perfringens* or *Clostridium botulinum*
- *Legionella pneumophila*
- 30 • *Coxiella burnetii*
- *Brucella*, such as *B. abortus*, *B. canis*, *B. melitensis*, *B. neotomae*, *B. ovis*, *B. suis*, *B. pinnipediae*.
- *Francisella*, such as *F. novicida*, *F. philomiragia*, *F. tularensis*.

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- *Neisseria gonorrhoea*
- *Treponema pallidum*
- *Haemophilus ducreyi*
- *Enterococcus faecalis* or *Enterococcus faecium*
- 5 • *Staphylococcus saprophyticus*
- *Yersinia enterocolitica*
- *Mycobacterium tuberculosis*
- *Rickettsia*
- *Listeria monocytogenes*
- 10 • *Vibrio cholera*
- *Salmonella typhi*
- *Borrelia burgdorferi*
- *Porphyromonas gingivalis*
- *Klebsiella*

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In some examples the immunogen elicits an immune response against one of these viruses:

- 20 • *Orthomyxovirus*: Useful immunogens can be from an influenza A, B or C virus, such as the hemagglutinin, neuraminidase or matrix M2 proteins. Where the immunogen is an influenza A virus hemagglutinin it may be from any subtype e.g. H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 or H16.
- 25 • *Paramyxoviridae* viruses: Viral immunogens include, but are not limited to, those derived from Pneumoviruses (e.g. respiratory syncytial virus, RSV), Rubulaviruses (e.g. mumps virus), Paramyxoviruses (e.g. parainfluenza virus), Metapneumoviruses and Morbilliviruses (e.g. measles).
- 30 • *Poxviridae*: Viral immunogens include, but are not limited to, those derived from Orthopoxvirus such as Variola vera, including but not limited to, Variola major and Variola minor.
- 35 • *Picornavirus*: Viral immunogens include, but are not limited to, those derived from Picornaviruses, such as Enteroviruses, Rhinoviruses, Heparnavirus, Cardioviruses and Aphthoviruses. In one embodiment, the enterovirus is a poliovirus e.g. a type 1, type 2 and/or type 3 poliovirus. In another embodiment, the enterovirus is an EV71 enterovirus. In another embodiment, the enterovirus is a coxsackie A or B virus.
- *Bunyavirus*: Viral immunogens include, but are not limited to, those derived from an Orthobunyavirus, such as California encephalitis virus, a Phlebovirus,

such as Rift Valley Fever virus, or a Nairovirus, such as Crimean-Congo hemorrhagic fever virus.

- *Heparnavirus*: Viral immunogens include, but are not limited to, those derived from a Heparnavirus, such as hepatitis A virus (HAV).
- 5 • *Filovirus*: Viral immunogens include, but are not limited to, those derived from a filovirus, such as an Ebola virus (including a Zaire, Ivory Coast, Reston or Sudan ebolavirus) or a Marburg virus.
- *Togavirus*: Viral immunogens include, but are not limited to, those derived from a Togavirus, such as a Rubivirus, an Alphavirus, or an Arterivirus. This includes rubella virus.
- 10 • *Flavivirus*: Viral immunogens include, but are not limited to, those derived from a Flavivirus, such as Tick-borne encephalitis (TBE) virus, Dengue (types 1, 2, 3 or 4) virus, Yellow Fever virus, Japanese encephalitis virus, Kyasanur Forest Virus, West Nile encephalitis virus, St. Louis encephalitis virus, Russian spring-summer encephalitis virus, Powassan encephalitis virus.
- 15 • *Pestivirus*: Viral immunogens include, but are not limited to, those derived from a Pestivirus, such as Bovine viral diarrhea (BVDV), Classical swine fever (CSFV) or Border disease (BDV).
- *Hepadnavirus*: Viral immunogens include, but are not limited to, those derived from a Hepadnavirus, such as Hepatitis B virus. A composition can include hepatitis B virus surface antigen (HBsAg).
- 20 • Other *hepatitis* viruses: A composition can include an immunogen from a hepatitis C virus, delta hepatitis virus, hepatitis E virus, or hepatitis G virus.
- *Rhabdovirus*: Viral immunogens include, but are not limited to, those derived from a Rhabdovirus, such as a Lyssavirus (e.g. a Rabies virus) and Vesiculovirus (VSV).
- 25 • *Caliciviridae*: Viral immunogens include, but are not limited to, those derived from Caliciviridae, such as Norwalk virus (Norovirus), and Norwalk-like Viruses, such as Hawaii Virus and Snow Mountain Virus.
- 30 • *Coronavirus*: Viral immunogens include, but are not limited to, those derived from a SARS coronavirus, avian infectious bronchitis (IBV), Mouse hepatitis virus (MHV), and Porcine transmissible gastroenteritis virus (TGEV). The coronavirus immunogen may be a spike polypeptide.
- *Retrovirus*: Viral immunogens include, but are not limited to, those derived from an Oncovirus, a Lentivirus (e.g. HIV-1 or HIV-2) or a Spumavirus.
- 35 • *Reovirus*: Viral immunogens include, but are not limited to, those derived from an Orthoreovirus, a Rotavirus, an Orbivirus, or a Coltivirus.

- *Parvovirus*: Viral immunogens include, but are not limited to, those derived from Parvovirus B19.
- *Herpesvirus*: Viral immunogens include, but are not limited to, those derived from a human herpesvirus, such as, by way of example only, Herpes Simplex Viruses (HSV) (e.g. HSV types 1 and 2), Varicella-zoster virus (VZV), Epstein-Barr virus (EBV), Cytomegalovirus (CMV), Human Herpesvirus 6 (HHV6), Human Herpesvirus 7 (HHV7), and Human Herpesvirus 8 (HHV8).
- *Papovaviruses*: Viral immunogens include, but are not limited to, those derived from Papillomaviruses and Polyomaviruses. The (human) papillomavirus may be of serotype 1, 2, 4, 5, 6, 8, 11, 13, 16, 18, 31, 33, 35, 39, 41, 42, 47, 51, 57, 58, 63 or 65 e.g. from one or more of serotypes 6, 11, 16 and/or 18.
- *Adenovirus*: Viral immunogens include those derived from adenovirus serotype 36 (Ad-36).

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Fungal immunogens may be derived from *Dermatophytes*, including: *Epidermophyton floccosum*, *Microsporum audouini*, *Microsporum canis*, *Microsporum distortum*, *Microsporum equinum*, *Microsporum gypsum*, *Microsporum nanum*, *Trichophyton concentricum*, *Trichophyton equinum*, *Trichophyton gallinae*, *Trichophyton gypseum*, *Trichophyton megnini*, *Trichophyton mentagrophytes*, *Trichophyton quinckeanum*, *Trichophyton rubrum*, *Trichophyton schoenleinii*, *Trichophyton tonsurans*, *Trichophyton verrucosum*, *T. verrucosum* var. *album*, var. *discoides*, var. *ochraceum*, *Trichophyton violaceum*, and/or *Trichophyton faviforme*; or from *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus nidulans*, *Aspergillus terreus*, *Aspergillus sydowi*, *Aspergillus flavatus*, *Aspergillus glaucus*, *Blastoschizomyces capitatus*, *Candida albicans*, *Candida enolase*, *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida stellatoidea*, *Candida kusei*, *Candida parakwsei*, *Candida lusitanae*, *Candida pseudotropicalis*, *Candida guilliermondi*, *Cladosporium carrionii*, *Coccidioides immitis*, *Blastomyces dermatidis*, *Cryptococcus neoformans*, *Geotrichum clavatum*, *Histoplasma capsulatum*, *Klebsiella pneumonia*, *Microsporidia*, *Encephalitozoon spp.*, *Septata intestinalis* and *Enterocytozoon bieneusi*; the less common are *Brachiola spp.*, *Microsporidium spp.*, *Nosema spp.*, *Pleistophora spp.*, *Trachipleistophora spp.*, *Vittaforma spp.*, *Paracoccidioides brasiliensis*, *Pneumocystis carinii*, *Pythium insidiosum*, *Pityrosporum ovale*, *Sacharomyces cerevisiae*, *Saccharomyces boulardii*, *Saccharomyces pombe*, *Scedosporium apiospermum*, *Sporothrix schenckii*, *Trichosporon beigeli*, *Toxoplasma gondii*, *Penicillium marneffe*, *Malassezia spp.*, *Fonsecaea spp.*, *Wangiella spp.*, *Sporothrix spp.*, *Basidiobolus spp.*, *Conidiobolus*

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spp., *Rhizopus spp.*, *Mucor spp.*, *Absidia spp.*, *Mortierella spp.*, *Cunninghamiella spp.*, *Saksenaea spp.*, *Alternaria spp.*, *Curvularia spp.*, *Helminthosporium spp.*, *Fusarium spp.*, *Aspergillus spp.*, *Penicillium spp.*, *Monolinia spp.*, *Rhizoctonia spp.*, *Paecilomyces spp.*, *Pithomyces spp.*, and *Cladosporium spp.*

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In some examples the immunogen elicits an immune response against a parasite from the *Plasmodium* genus, such as *P.falciparum*, *P.vivax*, *P.malariae* or *P.ovale*. Thus the disclosure may be used for immunising against malaria. In some examples the immunogen elicits an immune response against a parasite from the *Caligidae* family, particularly those from the *Lepeophtheirus* and *Caligus* genera e.g. sea lice such as *Lepeophtheirus salmonis* or *Caligus rogercresseyi*.

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In some examples the immunogen elicits an immune response against: pollen allergens (tree-, herb, weed-, and grass pollen allergens); insect or arachnid allergens (inhalant, saliva and venom allergens ,e.g. mite allergens, cockroach and midges allergens, hymenoptera venom allergens); animal hair and dandruff allergens (from e.g. dog, cat, horse, rat, mouse,etc .); and food allergens (e.g. a gliadin). Important pollen allergens from trees, grasses and herbs are such originating from the taxonomic orders of Fagales, Oleales, Pinales and platanaceae including, but not limited to, birch (*Betula*), alder (*Alnus*), hazel (*Corylus*), hornbeam (*Carpinus*) and olive (*Olea*), cedar (*Cryptomeria* and *Juniperus*), plane tree (*Platanus*), the order of Poales including grasses of the genera *Lolium*, *Phleum*, *Poa*, *Cynodon*, *Dactylis*, *Holcus*, *Phalaris*, *Secale*, and *Sorghum*, the orders of Asterales and Urticales including herbs of the genera *Ambrosia*, *Artemisia*, and *Parietaria*. Other important inhalation allergens are those from house dust mites of the genus *Dermatophagoides* and *Euroglyphus*, storage mite e.g. *Lepidoglyphus*, *Glycyphagus* and *Tyrophagus*, those from cockroaches, midges and flea se.g. *Blatella*, *Periplaneta*, *Chironomus* and *Ctenocephalides*, and those from mammals such as cat, dog and horse, venom allergens including such originating from stinging or biting insects such as those from the taxonomic order of Hymenoptera including bees (*Apidae*), wasps (*Vespidae*), and ants (*Formicoidae*).

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In some examples the immunogen is a tumor antigen selected from: (a) cancer-testis antigens such as NY-ESO-1, SSX2, SCP1 as well as RAGE, BAGE, GAGE and MAGE family polypeptides, for example, GAGE-1, GAGE-2, MAGE-1, MAGE-2, MAGE-3, MAGE-4, MAGE-5, MAGE-6, and MAGE-12 (which can be used, for example, to address melanoma, lung, head and neck, NSCLC, breast, gastrointestinal, and bladder tumors; (b) mutated antigens, for example, p53 (associated with various solid tumors, e.g., colorectal, lung, head and neck cancer), p21/Ras (associated

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with, e.g., melanoma, pancreatic cancer and colorectal cancer), CDK4 (associated with, e.g., melanoma), MUM1 (associated with, e.g., melanoma), caspase-8 (associated with, e.g., head and neck cancer), CIA 0205 (associated with, e.g., bladder cancer), HLA-A2-R1701, beta catenin (associated with, e.g., melanoma),

5 TCR (associated with, e.g., T-cell non-Hodgkins lymphoma), BCR-abl (associated with, e.g., chronic myelogenous leukemia), triosephosphate isomerase, KIA 0205, CDC-27, and LDLR-FUT; (c) over-expressed antigens, for example, Galectin 4 (associated with, e.g., colorectal cancer), Galectin 9 (associated with, e.g., Hodgkin's disease), proteinase 3 (associated with, e.g., chronic myelogenous leukemia), WT 1

10 (associated with, e.g., various leukemias), carbonic anhydrase (associated with, e.g., renal cancer), aldolase A (associated with, e.g., lung cancer), PRAME (associated with, e.g., melanoma), HER-2/neu (associated with, e.g., breast, colon, lung and ovarian cancer), mammaglobin, alpha-fetoprotein (associated with, e.g., hepatoma), KSA (associated with, e.g., colorectal cancer), gastrin (associated with,

15 e.g., pancreatic and gastric cancer), telomerase catalytic protein, MUC-1 (associated with, e.g., breast and ovarian cancer), G-250 (associated with, e.g., renal cell carcinoma), p53 (associated with, e.g., breast, colon cancer), and carcinoembryonic antigen (associated with, e.g., breast cancer, lung cancer, and cancers of the gastrointestinal tract such as colorectal cancer); (d) shared antigens, for example,

20 melanoma-melanocyte differentiation antigens such as MART-1/Melan A, gp100, MC1R, melanocyte-stimulating hormone receptor, tyrosinase, tyrosinase related protein-1/TRP1 and tyrosinase related protein-2/TRP2 (associated with, e.g., melanoma); (e) prostate associated antigens such as PAP, PSA, PSMA, PSH-P1, PSM-P1, PSM-P2, associated with, e.g., prostate cancer; (f) immunoglobulin idiotypes

25 (associated with myeloma and B cell lymphomas, for example). In certain embodiments, tumor immunogens include, but are not limited to, p15, Hom/Mel-40, H-Ras, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR, Epstein Barr virus antigens, EBNA, human papillomavirus (HPV) antigens, including E6 and E7, hepatitis B and C virus antigens, human T-cell lymphotropic virus antigens, TSP-180, p185erbB2,

30 p180erbB-3, c-met, mn-23H1, TAG-72-4, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, p16, TAGE, PSCA, CT7, 43-9F, 5T4, 791 Tgp72, beta-HCG, BCA225, BTAA, CA 125, CA 15-3 (CA 27.29\BCAA), CA 195, CA 242, CA-50, CAM43, CD68\KP1, CO-029, FGF-5, Ga733 (EpCAM), HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB/70K, NY-CO-1, RCAS1, SDCCAG16, TA-90 (Mac-2 binding protein/cyclophilin C-associated

35 protein), TAAL6, TAG72, TLP, TPS, and the like.

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In an embodiment, the pharmaceutical composition comprises one polynucleotide. In an alternative embodiment, the pharmaceutical composition comprises more than one polynucleotide.

- 5 In a preferred embodiment, said composition is a vaccine or can be used to immunize or vaccinate subjects.

In an embodiment, the present disclosure provides RNA (e.g., mRNA) vaccines that include at least one RNA (e.g., mRNA) polynucleotide having an open reading frame
10 encoding at least one antigenic polypeptide or an immunogenic fragment thereof (e.g., an immunogenic fragment capable of inducing an immune response to the antigenic polypeptide). While not wishing to be bound by theory, it is believed that the RNA (e.g., mRNA) vaccines, for example, as mRNA polynucleotides, are better designed to produce the appropriate protein conformation upon translation, as the
15 RNA (e.g., mRNA) vaccines co-opt natural cellular machinery. Unlike traditional vaccines, which are manufactured *ex vivo* and may trigger unwanted cellular responses, RNA (e.g., mRNA) vaccines are presented to the cellular system in a more native fashion.

20 The invention also provides methods for the prevention and/or treatment of disorder; said method comprises administering to a subject in need thereof a pharmaceutical composition according to this invention. In some embodiments, said compound is administered orally or parenterally. In some embodiments, said compound is administered topically.

25 The compounds and compositions may also be used in *in vitro* methods, such as in vitro methods of administering a compound or composition to cells for screening purposes and/or for conducting quality control assays.

30 The compounds and compositions may also be used for *ex-vivo* treatment (e.g. Chimeric antigen receptor (CAR) T-cell therapy).

In certain embodiments, pharmaceutical compositions of the invention may be used alone or conjointly administered with another type of therapeutic agent. As used
35 herein, the phrase "conjoint administration" refers to any form of administration of two or more different therapeutic compounds such that the second compound is administered while the previously administered therapeutic compound is still effective in the body (e.g., the two compounds are simultaneously effective in the

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patient, which may include synergistic effects of the two compounds). For example, the different therapeutic compounds can be administered either in the same formulation or in a separate formulation, either concomitantly or sequentially. In certain embodiments, the different therapeutic compounds can be administered within one hour, 12 hours, 24 hours, 36 hours, 48 hours, 72 hours, or a week of one another. Thus, an individual who receives such treatment can benefit from a combined effect of different therapeutic compounds.

Articles of Manufacture and Kits

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The present disclosure further provides articles of manufacture comprising a compound described herein or a salt thereof, a (pharmaceutical) composition described herein, or one or more-unit dosages described herein in suitable packaging. In certain embodiments, the article of manufacture is for use in any of the methods described herein. Suitable packaging is known in the art and includes, for example, vials, vessels, ampules, bottles, jars, flexible packaging, and the like. An article of manufacture may further be sterilized and/or sealed.

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The present disclosure further provides kits for carrying out the methods of the invention, which comprises one or more compounds described herein or a (pharmaceutical) composition described herein. The kits may employ any of the compounds disclosed herein. In one embodiment, the kit employs a compound described herein or a salt thereof. The kits may be used for any one or more of the uses described herein. Kits generally comprise suitable packaging. The kits may comprise one or more containers comprising any compound described herein. Each component (if there is more than one component) can be packaged in separate containers or some components can be combined in one container where cross-reactivity and shelf-life permit. The kits may be in unit dosage forms, bulk packages (e.g., multi-dose packages) or sub-unit doses. For example, kits may be provided that contain sufficient dosages of a compound as disclosed herein and/or an additional pharmaceutically active compound useful for a disease detailed herein to provide effective treatment of an individual for an extended period, such as any of a week, 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks, 3 months, 4 months, 5 months, 7 months, 8 months, 9 months, or more. Kits may also include multiple unit doses of the compounds and instructions for use and be packaged in quantities sufficient for storage and use in pharmacies (e.g., hospital pharmacies and compounding pharmacies).

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The kits may optionally include a set of instructions, generally written instructions, although electronic storage media (e.g., magnetic diskette or optical disk) containing instructions are also acceptable, relating to the use of component(s) of the methods of the present invention. The instructions included with the kit generally include
5 information as to the components and their administration to an individual.

Synthesis

The compounds of this invention may be prepared by a variety of procedures and
10 synthetic routes. In a further aspect, the invention relates to a method to synthesize the compounds of the current invention.

In an embodiment, the compounds of the invention are prepared by base-catalyzed condensation, the most common method for synthesizing calixarenes. It involves the
15 reaction of phenols (or substituted phenols) with formaldehyde in the presence of a strong base, typically an alkali metal hydroxide (e.g., sodium hydroxide or potassium hydroxide). The reaction typically takes place in a polar aprotic solvent, such as dimethyl sulfoxide (DMSO) or dimethylformamide (DMF), under reflux conditions. Two different synthetic processes are commonly used. The first one is a one-step
20 process, all the components (phenol/formaldehyde/base) being refluxed in a high boiling solvent (ex. xylene). The second one is a two-steps process, wherein a solid phenol/formaldehyde condensation product is obtained during a first step (the so-called precursor). This solid precursor is subsequently refluxed in a high boiling solvent during a second step, leading to the formation of calixarenes.

25 In an embodiment, the compounds of the invention are prepared by template-directed synthesis. Template-directed synthesis is an alternative approach to synthesizing calixarenes with predefined cavity sizes and functional groups. It involves the use of a template molecule that guides the formation of the macrocycle.
30 After the macrocycle is formed, the template molecule can be removed through various methods, such as extraction or cleavage.

In an embodiment, the compounds of the invention are prepared by acid-catalyzed condensation. In some cases, calixarenes can be synthesized using an acid catalyst,
35 such as sulfuric acid, hydrochloric acid, or p-toluenesulfonic acid. The reaction typically occurs at lower temperatures compared to the base-catalyzed condensation.

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In an embodiment, the compounds of the invention are prepared by click chemistry-based methods. This approach involves the use of click chemistry reactions, such as the copper-catalyzed azide-alkyne cycloaddition (CuAAC) or the thiol-ene reaction, to synthesize calixarenes. This can offer precise control over the functional groups and substitution patterns in the macrocycle.

In an embodiment, functional groups can be introduced to preformed calixarenes using various post-functionalization techniques. These include electrophilic aromatic substitution, nucleophilic aromatic substitution, and transition metal-catalyzed cross-coupling reactions, such as the Suzuki-Miyaura, Stille, or Heck reactions.

In an embodiment, reaction conditions, such as temperature, catalyst, and solvent, are optimized to achieve the best yield and selectivity.

In an embodiment, protecting groups or post-functionalization strategies to introduce additional functional groups or to control the substitution pattern in the macrocycle are used.

In an embodiment, the synthesized calixarenes are characterized using techniques such as NMR spectroscopy, mass spectrometry, and X-ray crystallography to confirm its structure and purity.

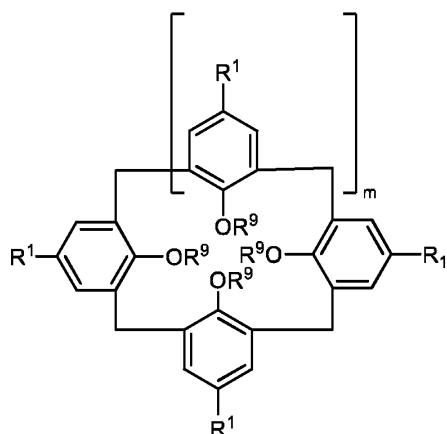
Representative, non-limitative, procedures and synthetic routes are shown in Figure 1.

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The invention comprises following embodiments:

1. A compound of Formula (I):

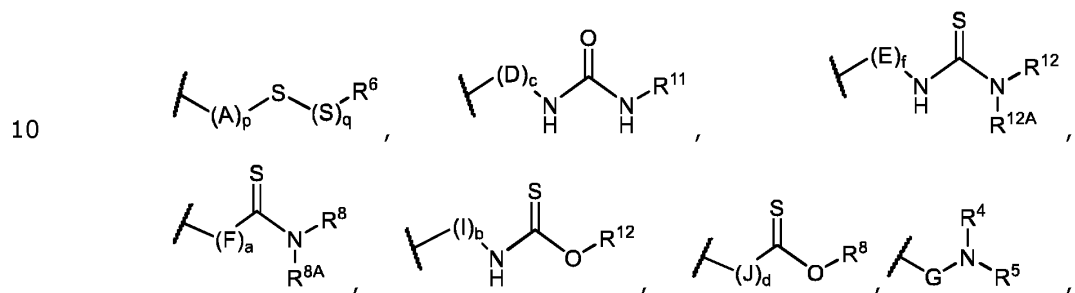
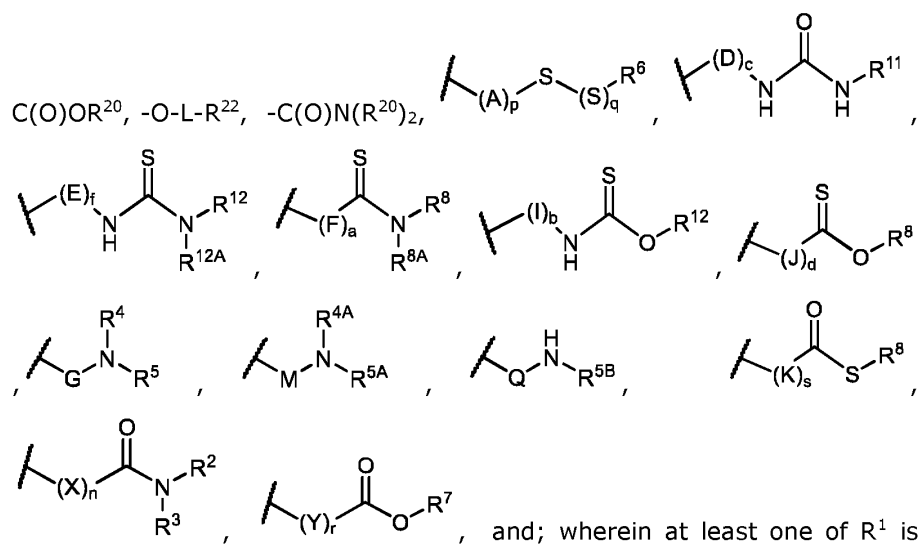
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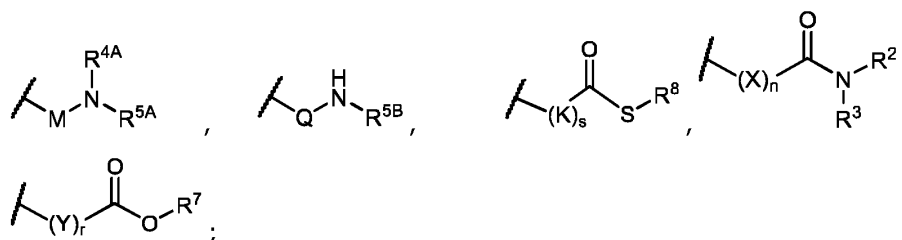
Formula (I)

or a pharmaceutically acceptable salt thereof wherein:

5 R^1 is selected from hydrogen, halogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-10} haloalkyl, $-CN$, $-OR^{20}$, $-SR^{20}$, $-SF_5$, $-NO_2$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-$



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A, D, E, F, G, I, J, K, M, Q, X, and Y are each independently selected from C₁₋₁₀ alkylene, C₂₋₁₀ alkenylene, C₂₋₁₀ alkynylene, wherein the C₁₋₁₀ alkylene, C₂₋₁₀ alkenylene, C₂₋₁₀ alkynylene are optionally substituted with one or more substituents independently selected from halogen, C₁₋₁₀ haloalkyl, -CN, -NO₂, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

each R², R⁶, R⁸, is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

each R⁷ is selected from:

C₁₋₂ alkyl, wherein the C₁₋₂ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰); and

C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

R^{8A} is independently selected from hydrogen and C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

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each R^{11} is independently selected from C_{3-10} alkyl, wherein the C_{3-10} alkyl is substituted with one or more substituents selected from $-N(R^{21})_2$, $-N(R^{21})_3^+$, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, $-CN$, $-NO_2$, $=O$, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, and $-S(O)_2(R^{20})$;

each R^{12} is independently selected from C_{3-10} alkyl, wherein the C_{3-10} alkyl is substituted with one or more substituents selected from $-N(R^{20})C_{1-10}$ alkyl, $-N(R^{20})_3^+$, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, $-CN$, $-NO_2$, $=O$, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, and $-S(O)_2(R^{20})$;

each R^{12A} is independently selected from hydrogen and C_{1-10} alkyl, wherein the C_{1-10} alkyl is substituted with one or more substituents selected from $-N(R^{20})C_{1-10}$ alkyl, $-N(R^{20})_3^+$, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, $-CN$, $-NO_2$, $=O$, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, and $-S(O)_2(R^{20})$;

each R^3 is independently selected from hydrogen and C_{1-10} alkyl, wherein the C_{1-10} alkyl is optionally substituted with one or more substituents independently selected from halogen, $-CN$, $-NO_2$, $=O$, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, and $-S(O)_2(R^{20})$;

each R^4 is independently selected from C_{1-10} alkyl, wherein the C_{1-10} alkyl is optionally substituted with one or more substituents selected from 3- to 12-membered saturated heterocycle, wherein the 3- to 12-membered saturated heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, $-CN$, $-NO_2$, $=O$, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, and $-S(O)_2(R^{20})$;

each R^{4A} is independently selected from C_{1-10} alkyl, wherein the C_{1-10} alkyl is substituted with one or more substituents selected from $-OH$ and $-OC_{1-10}$ alkyl; wherein when each R^1 is $-CH_2-N(CH_2CH_2OH)_2$, each R^9 is independently selected from R^{9A} ;

each R^5 , R^{5A} is independently selected from C_{1-10} alkyl, wherein the C_{1-10} alkyl is substituted with one substituent selected from $-OR^{20}$ and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected

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from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

5 each R^{5B} is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is optionally substituted with one substituent selected from -OR²⁰ and 3- to 12-membered saturated heterocycle, wherein the 3- to 12-membered saturated heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

10 wherein when R^{5B} is unsubstituted C₁₋₁₀ alkyl, each R⁹ is independently selected from R^{9B};

15 each R⁹ is independently selected from hydrogen, -(R¹⁰)_j-C(O)OR²⁰, -(R¹⁰)_j-C(O)N(R²⁰)₂, -(R¹⁰)_j-NHC(S)NHR²⁰, -(R¹⁰)_j-SR²⁰, -(R¹⁰)_j-S-S-R²⁰, PEG₁₋₂₀₀, mannose, an adjuvant, a carbohydrate, an antibody, C₁₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl, wherein the C₁₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl are each optionally substituted with one or more substituents independently selected from halogen, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, -S(O)₂(R²⁰), C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle;

20 each R^{9A} is independently selected from -(R¹⁰)_j-C(O)OR²⁰, -(R¹⁰)_j-C(O)N(R²⁰)₂, -(R¹⁰)_j-NHC(S)NHR²⁰, -(R¹⁰)_j-SR²⁰, -(R¹⁰)_j-S-S-R²⁰, PEG₁₋₂₀₀, mannose, an adjuvant, a carbohydrate, an antibody, C₁₂₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl, wherein the C₁₂₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl are each optionally substituted with one or more substituents independently selected from halogen, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, -S(O)₂(R²⁰), C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle;

25 each R^{9B} is independently selected from -(R¹⁰)_j-C(O)OR²⁰, -(R¹⁰)_j-C(O)N(R²⁰)₂, -(R¹⁰)_j-NHC(S)NHR²⁰, -(R¹⁰)_j-SR²⁰, -(R¹⁰)_j-S-S-R²⁰, PEG₃₋₂₀₀, mannose, an adjuvant, a carbohydrate, an antibody, C₅₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl, wherein the C₅₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl are each optionally substituted with one or more substituents independently selected from halogen, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, -S(O)₂(R²⁰), C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle;

35 each R¹⁰ is independently selected from C₁₋₂₀ alkylene, C₂₋₂₀ alkenylene, and C₂₋₂₀ alkynylene;

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each R^{20} is independently selected from hydrogen; and C_{1-20} alkyl, C_{2-20} alkenyl, C_{2-20} alkynyl, C_{3-12} carbocycle, and 3- to 12-membered heterocycle, each of which is optionally substituted with one or more substituents independently selected from halogen, -OH, -CN, -NO₂, -NH₂, -N(C_{1-6} alkyl)₂, -NHC₁₋₆ alkyl, -NHC₁₋₆ hydroxyalkyl, -N(C_{1-6} alkyl)C₁₋₆ hydroxyalkyl, C_{1-10} alkyl, - C_{1-10} haloalkyl, -O- C_{1-10} alkyl, oxo, =NH, C_{3-12} carbocycle, and 3- to 12-membered heterocycle;

each R^{21} is independently selected from hydrogen; and C_{1-20} alkyl, C_{2-20} alkenyl, C_{2-20} alkynyl, C_{3-12} carbocycle, and 3- to 12-membered heterocycle, each of which is optionally substituted with one or more substituents independently selected from halogen, -OH, -CN, -NO₂, -NH₂, -N(C_{1-6} alkyl)₂, -NHC₁₋₆ alkyl, -NHC₁₋₆ hydroxyalkyl, C_{1-10} alkyl, - C_{1-10} haloalkyl, -O- C_{1-10} alkyl, =NH, C_{3-12} carbocycle, and 3- to 12-membered heterocycle;

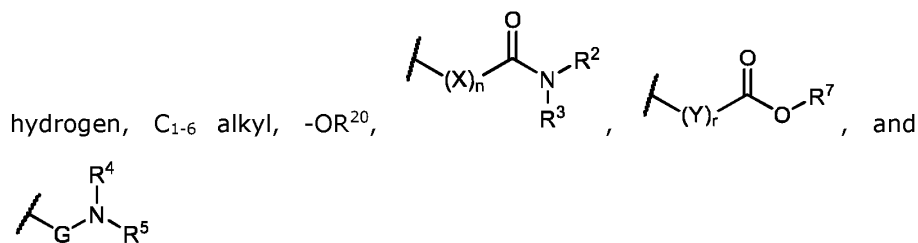
each R^{22} is selected from mannose, an adjuvant, a carbohydrate, and an antibody;

L is selected from a covalent linker;

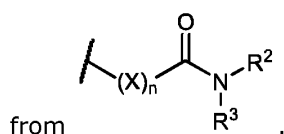
a, b, c, d, f, j, n, p, q, r, and s, are each independently selected from 0, 1, 2, and 3; and

m is selected from 1, 3, and 5.

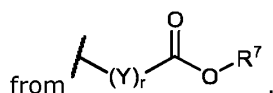
2. The compound or salt of embodiment 1, wherein each R^1 is selected from



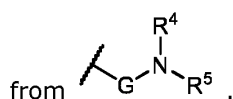
3. The compound or salt of embodiments 1 or 2, wherein each R^1 is selected



4. The compound or salt of embodiments 1 or 2, wherein each R^1 is selected

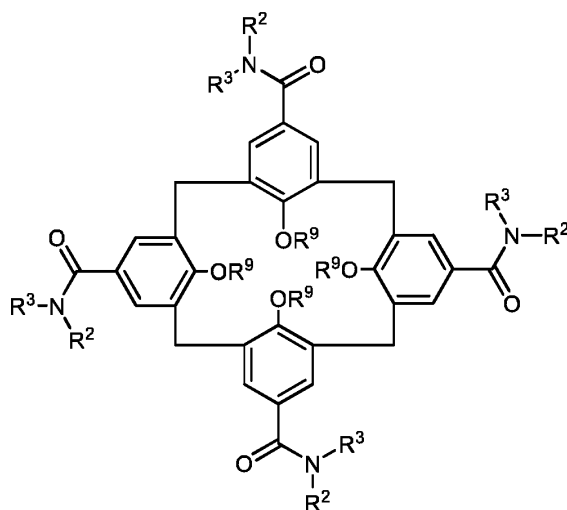


5. The compound or salt of embodiments 1 or 2, wherein each R^1 is selected



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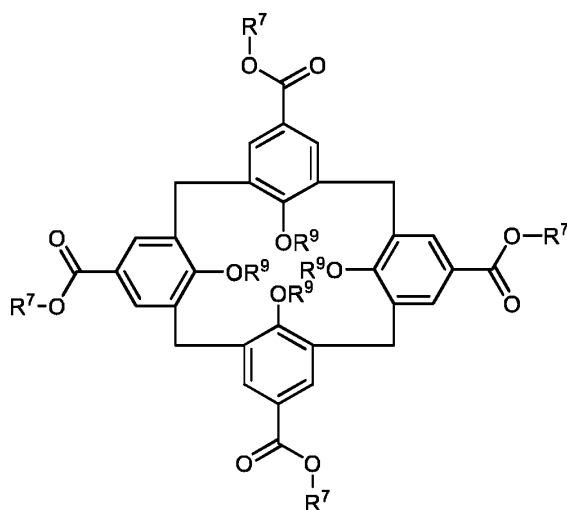
6. The compound or salt of embodiment 3, wherein Formula (I) is represented by Formula (II):



Formula (II)

- 5 or a pharmaceutically acceptable salt thereof.
7. The compound or salt of embodiments 1, 2, 3, or 6, wherein R² is selected from C₃₋₆ alkyl, wherein the C₃₋₆ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 5- to 6-membered heterocycle, wherein the 5- to 6-membered heterocycle has at least one nitrogen atom.
- 10 8. The compound or salt of embodiment 7, wherein each R² is selected from
- The structures show a 4-ethylpiperazine ring and a 4-ethylpiperazine ring with a positive charge on the nitrogen atom.
9. The compound or salt of embodiment 8, wherein each R² is selected from
- The structure shows a 4-ethylpiperazine ring.
- 15 10. The compound or salt of embodiment 8, wherein each R² is selected from
- The structure shows a 4-ethylpiperazine ring with a positive charge on the nitrogen atom.
11. The compound or salt of any one of embodiments 6 to 10, wherein each R³ is selected from hydrogen and C₁₋₁₀ alkyl.
12. The compound or salt of embodiment 11, wherein each R³ is hydrogen.
- 20 13. The compound or salt of embodiment 4, wherein Formula (I) is represented by Formula (III):

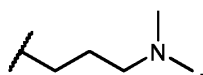
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Formula (III)

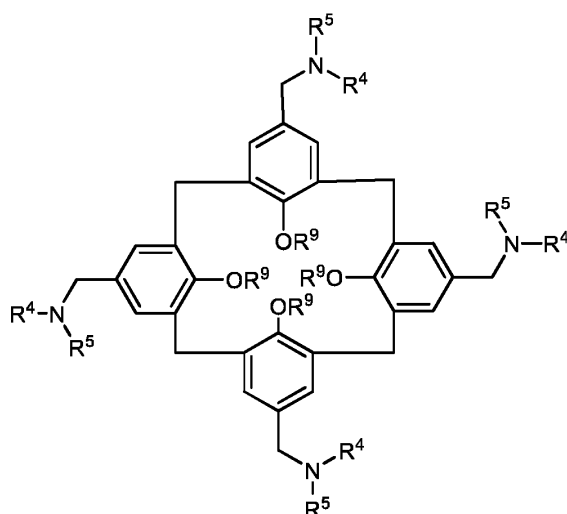
or a pharmaceutically acceptable salt thereof.

14. The compound or salt of embodiments 1, 2, 4, or 13, wherein R⁷ is selected from C₃₋₆ alkyl, wherein the C₃₋₆ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 5- to 6-membered heterocycle, wherein the 5- to 6-membered heterocycle has at least one nitrogen atom.
15. The compound or salt of embodiment 14, wherein R⁷ is selected from C₃₋₆ alkyl, wherein the C₃₋₆ alkyl is substituted with one -N(R²⁰)₂.
16. The compound or salt of embodiment 15, wherein each R⁷ is selected from



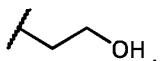
17. The compound or salt of embodiment 1, wherein Formula (I) is represented by Formula (IV):

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Formula (IV)

or a pharmaceutically acceptable salt thereof.

18. The compound or salt of embodiments 1, 2, 5, 17, wherein each R⁴ is selected from C₁₋₂ alkyl.
19. The compound or salt of embodiments 17 or 18, wherein each R⁵ is selected from C₂₋₅ alkyl, wherein the C₂₋₅ alkyl is substituted with one -OR²⁰.
20. The compound or salt of embodiment 19, wherein each R⁵ is selected from C₂₋₅ alkyl, wherein the C₂₋₅ alkyl is substituted with one -OH.
21. The compound or salt of embodiment 20, wherein each R⁵ is selected from .
22. The compound or salt of any one of embodiments 1 to 21, wherein each R⁹ is selected from unsubstituted C₁₋₁₂ alkyl.
23. The compound or salt of any one of embodiments 1 to 22, wherein at least two R¹s are different substituents.
24. The compound or salt of any one of embodiments 1 to 22, wherein two R¹s are the same substituent.
25. The compound or salt of any one of embodiments 1 to 22, wherein three R¹s are the same substituent.
26. The compound or salt of any one of embodiments 1 to 22, wherein four R¹s are the same substituent.
27. The compound or salt of embodiment 1, further comprising functionalizing R¹ with one or more functional groups.
28. A composition comprising the compound or salt of any one of embodiments 1 to 27 and an excipient.

29. The composition of embodiment 28, wherein the excipient is selected from sugars, starches, cellulose, oils, glycols, polyols, esters, buffering agents, ethyl alcohol, phosphate buffer solutions, and lipids.
- 5 30. The composition of embodiment 29, wherein the excipient is selected from an ionizable lipid, a cationic lipid, a helper lipid, an additional lipid wherein the additional lipid is selected from a sterol, a fatty acid, a glycerol monooleate, a trioleate or a short saturated molecule, and a PEG lipid or any combination of any of the foregoing.
- 10 31. The composition of embodiments 29 or 30, wherein the excipient is selected from an ionizable lipid, a helper lipid, an additional lipid wherein the additional lipid is selected from a sterol, a fatty acid, a glycerol monooleate, a trioleate or a short saturated molecule, and a PEG lipid or any combination of any of the foregoing.
- 15 32. The composition of embodiments 29 or 30, wherein the excipient is selected from a helper lipid, an additional lipid wherein the additional lipid is selected from a sterol, a fatty acid, a glycerol monooleate, a trioleate or a short saturated molecule and a PEG lipid or any combination of any of the foregoing.
- 20 33. The composition of any one of embodiments 28 to 32, further comprising a targeting agent and/or an adjuvant.
34. The composition of embodiment 33, wherein the targeting agent is selected from a carbohydrate or an antibody.
- 25 35. A pharmaceutical composition comprising the compound or salt of any one of embodiments 1 to 27, or the composition of any one of embodiments 28 to 34, and one or more nucleotides.
36. A pharmaceutical composition comprising the compound or salt of any one of embodiments 1 to 27, or the composition of any one of embodiments 28 to 34, and one or more polynucleotides.
- 30 37. A pharmaceutical composition comprising the compound or salt of any one of embodiments 1 to 27, or the composition of any one of embodiments 28 to 34, and micro-RNA, saRNA, circRNA or mRNA.
38. A pharmaceutical composition comprising the compound or salt of any one of embodiments 1 to 27, or the composition of any one of embodiments 28 to 34, and DNA.
- 35 39. A pharmaceutical composition comprising the compound or salt of any one of embodiments 1 to 27, or the composition of any one of embodiments 28 to 34, and saRNA.

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40. The compound or salt of any one of embodiments 1 to 27, for use as a delivery system.

41. A method of treating or preventing a disease or disorder using a pharmaceutical composition of any one of embodiments 35 to 39.

The present invention will be now described in more details, referring to examples that are not limitative.

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EXAMPLES AND/OR DESCRIPTION OF FIGURES

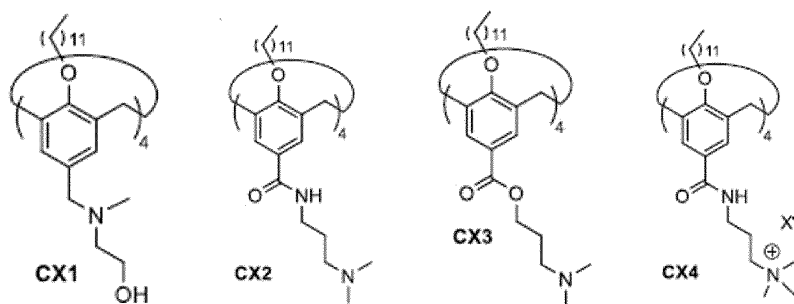
The present invention is in no way limited to the embodiments described in the examples and/or shown in the figures. On the contrary, methods according to the present invention may be realized in many different ways without departing from the scope of the invention.

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Example 1: synthesis of four compounds according to an embodiment of the invention.

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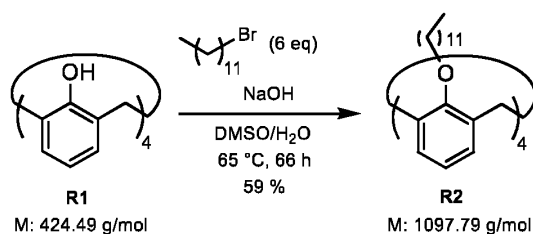
Figure 1 depicts the synthesis, including the solvents and the specific reactions, of CX1, CX2, CX3 and CX4, four compounds according to an embodiment of the invention (see below).



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Example 2: synthesis of compounds according to an embodiment of the invention.

Intermediate R2



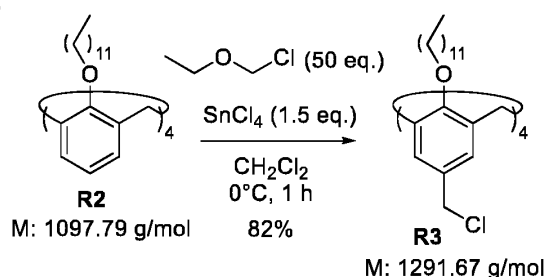
To a solution of calix(4)arene **R1** (2.32 g, 5.47 mmol, 1 eq) in DMSO (25 mL) was added 50% NaOH_{aq} (2.9 mL). The solution was heated at 65 °C and 1-

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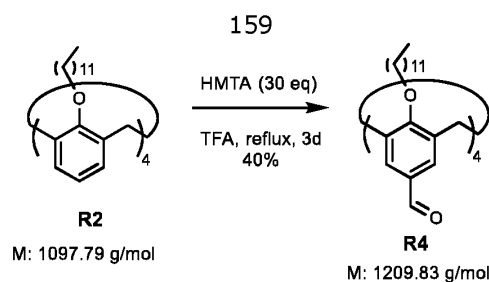
bromododecane (8.1 g, 32.5 mmol, 6 eq) was added in 3 portions every 5 minutes. The solution was kept under heating and stirring for 66 hours. After completion of the reaction, the solution was cooled to room temperature, then HCl_{aq} (50 mL, 1 M) was added slowly with stirring. The solution was then extracted with DCM (3 x 30 mL), and the organic layer was dried by filtrating it through sand. The solvent was removed under reduced pressure to afford a yellow paste and liquid (possibly remaining DMSO). The crude product was dissolved in a mixture of DCM/MeCN (120 mL + 40 mL) and the solution was left to crystallize under the fume hood by slow evaporation of the DCM. The resulting crystals were collected by filtration, washed with MeCN, and dried to afford the product **R2** as a white crystalline solid (3.57 g, 3.25 mmol). Yield: 59%. ¹H NMR (400 MHz, CDCl₃, 298 K): δ (ppm) 6.58 (m, 8H), 6.56 (m, 4H), 4.43 (d, 4H), 3.86 (t, 8H), 3.12 (d, 4H), 1.89 (p, 8H), 1.35 (s, 8H), 1.26 (s, 64H).

15 **Intermediate R3**



To a stirred solution of calix[4]arene **R2** (500 mg, 0.455 mmol) in CH₂Cl₂ (20 mL) in an oven dried flask under argon at 0 °C were successively added dropwise chloromethyl ethyl ether (2.11 mL, 22.8 mmol, d: 1.02 g/mL) and anhydrous SnCl₄ (0.080 mL, 0.68 mmol, d: 2.226 g/mL). The mixture was then stirred at 0 °C for 1 h. 10 mL of water were added to the mixture which was then strongly stirred at r.t. for 30 min until the magenta color faded to yellow. 10 mL of HCl_{aq}, 1 M were added to the mixture which was further stirred 30 min. The aqueous layer was extracted twice with 5 mL of DCM. The combined organic layers were washed with 20 mL of water then concentrated with a rotary evaporator. 3 mL of MeCN were added to the residue and the resulting white solid was collected by filtration to afford the product **R3** as a white powder (480 mg, 0.372 mmol). Yield: 82%. ¹H NMR (300 MHz, CDCl₃, 298 K): δ (ppm) 6.65 (s, 8H), 4.40 (d, 4H), 4.30 (s, 8H), 3.86 (t, 8H), 3.13 (d, 4H), 1.89 (bs, 8H), 1.17–1.45 (m, 72H), 0.88 (t, 12H).

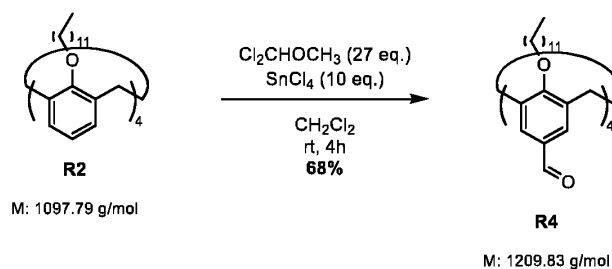
30 **Intermediate R4**



To a solution of calix(4)arene **R2** (1 g, 0.911 mmol, 1 eq) in TFA (35 mL), hexamethylenetetramine (3.84 g, 27.4 mmol, 30 eq) was added. The solution was sparged with argon and heated at reflux at 80°C under argon and stirring for 3 days.

5 After completion of the reaction, the solution was cooled down to room temperature and an aqueous solution of HCl (30 ml, 1 M) and DCM (30 mL) were added. The organic layer was separated, and the aqueous layer was extracted with DCM (2 x 30 mL). The organic layers were combined and extracted with a saturated solution of Na₂CO₃ (50 mL) to remove any residual TFA. The organic layer was concentrated
10 under reduced pressure to obtain a yellow sticky paste. The residue was purified by flash chromatography using silica gel with a DCM/Acetone gradient (100/0, 99/1 then 98/2) and afforded **R4** as a white powder in a yield of 40% (442 mg, 0.365 mmol). ¹H NMR (300 MHz, CDCl₃, 298 K): δ (ppm) 9.58 (s, 4H), 7.15 (s, 8H), 4.49 (d, 4H), 3.96 (t, 9H), 3.34 (d, 4H), 2.04– 1.77 (m, 8H), 1.49 – 1.14 (m, 72H), 0.88
15 (t, 12H).

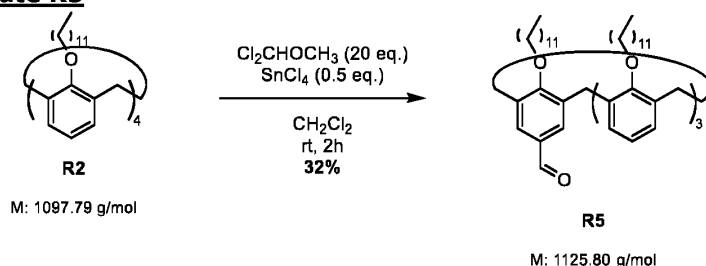
Intermediate R4 (Method 2)



In an oven dried flask, under argon, the calix[4]arene **R2** (2 g, 1.825 mmol, 1 eq.) was dissolved in CH₂Cl₂ (60 mL). Then, under stirring, the dichloro(methoxy)methane (4 mL, 1.43 g/mL, 5.72 g, 49.75 mmol, 27 eq.) was added, followed by the addition of SnCl₄ (2.20 mL, 2.23 g/mL, 4.91 g, 18.83 mmol, 10 eq.). The magenta mixture was stirred under argon at room temperature for 4h. After completion, 100 mL of HCl_{aq} 1M was added and the mixture was stirred vigorously at room temperature until the magenta color faded to yellow (at least 30 min). The organic phase was washed twice with milliQ water (2x 30 mL) and the combined aqueous phases were then extracted with CH₂Cl₂ (1x50 mL). The organic

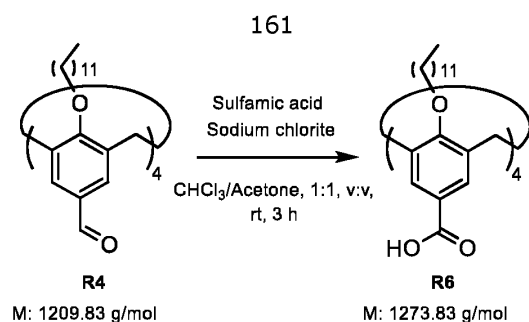
160

phases were combined and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (eluent: CH₂Cl₂/Acetone gradient from 100:0 to 95:5) to afford **R4** as a slightly yellow solid (1.50 g, 1.24 mmol). Yield: 68 %. ¹H NMR (300 MHz, CDCl₃, 298 K): δ (ppm) 9.58 (s, 4H), 7.15 (s, 8H), 4.49 (d, 4H), 3.96 (t, 9H), 3.34 (d, 4H), 2.04–1.77 (m, 8H), 1.49–1.14 (m, 72H), 0.88 (t, 12H).

Intermediate R5

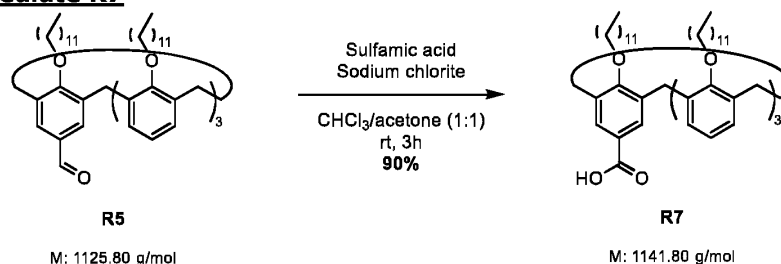
In an oven dried flask, under argon, the calix[4]arene **R2** (2 g, 1.825 mmol, 1 eq.) was dissolved in CH₂Cl₂ (120 mL). Then, under stirring, the dichloro(methoxy)methane (3.3 mL, 1.43 g/mL, 36 mmol, 20 eq.) was added, followed by the addition of SnCl₄ (101.4 μL, 2.23 g/mL, 0.91 mmol, 0.5 eq.). The magenta mixture was stirred under argon at room temperature for 2h. After completion, 200 mL of HCl_{aq} 1M was added and the mixture was stirred vigorously at room temperature until the magenta color faded to yellow (at least 30 min). The organic phase was washed twice with milliQ water (2x 75 mL) and the combined aqueous phases were then extracted with CH₂Cl₂ (1x200 mL). The organic phases were combined and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (eluent: cyclohexane/CH₂Cl₂ gradient from 100:0 to 50:50) to afford **R5** as a white solid (647 mg, 0.57 mmol). Yield: 32 %. ¹H NMR (300 MHz, CDCl₃, 298 K): δ (ppm) 9.55 (s, 1H), 6.99 (s, 2H), 6.75-6.64 (m, 6H), 6.43-6.39 (m, 3H), 4.44 (t, 4H), 3.94-3.79 (m, 8H), 3.21 (d, 2H), 3.14 (d, 2H) 1.88 (m, 8H), 1.49–1.14 (m, 72H), 0.88 (t, 12H).

Intermediate R6



To a solution of calix(4)arene **R4** (0.494 g, 0.409 mmol, 1 eq) in CHCl_3 /Acetone 50/50 v:v (60 mL) in an ice bath is added sulfamic acid (0.465 g, 4.79 mmol, 11.7 eq) and sodium chlorite (0.363 g, 4.01 mmol, 9.8 eq) solubilized in 2 mL of distilled water. The reaction mixture is stirred at room temperature for 3 hours. Every hour extra sulfamic acid (0.465 g, 4.79 mmol, 11.7 eq) and sodium chlorite (0.363 g, 4.01 mmol, 9.8 eq) solubilized in 2 mL of distilled water were added until completion of the reaction. After completion of the reaction, the solution was concentrated under reduced pressure, and HCl_{aq} (40 mL, 6 M) was added to the yellow residue. The mixture was filtered and the retained solid was washed with distilled water (20 mL), then methanol (20 mL). The residue was dried under reduced pressure to afford the product **R6** as a white, slightly yellow powder in a yield of 84% (439 mg, 0.345 mmol). ^1H NMR (300 MHz, CDCl_3 /Acetone- d_6 50/50 v:v, 298 K, calibrated on CDCl_3): δ (ppm) 6.93 (s, 8H), 4.05 (d, 4H), 3.54 (t, 8H) 2.87 (d, 4H), 1.48 (q, 8H), 1.17 – 0.70 (m, 72H), 0.44 (t, 12H).

Intermediate R7

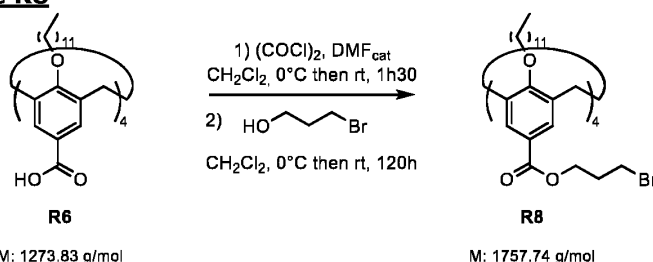


The calixarene **R5** (252 mg, 0.224 mmol, 1 eq.) was dissolved in CHCl_3 /Acetone 50/50 (30 mL) and the mixture was cooled down at 0°C . Under argon, the sulfamic acid (65.2 mg, 0.671 mmol, 3 eq.) was added, followed by the addition of a 2 mL aqueous solution of sodium chlorite (55.2 mg, 0.671 mmol, 3 eq.). The reaction mixture was stirred at room temperature for 3h. After 1h, extra sulfamic acid (0.465 g, 4.79 mmol, 11.7 eq.) and sodium chlorite solution (2 mL) (0.363 g, 4.01 mmol, 9.8 eq.) were added. After completion, the reaction mixture was concentrated under reduced pressure, and HCl_{aq} (20 mL, 6 M) was added to the yellow residue. The mixture was filtered and the residue was washed with MeOH (2 mL). The residue

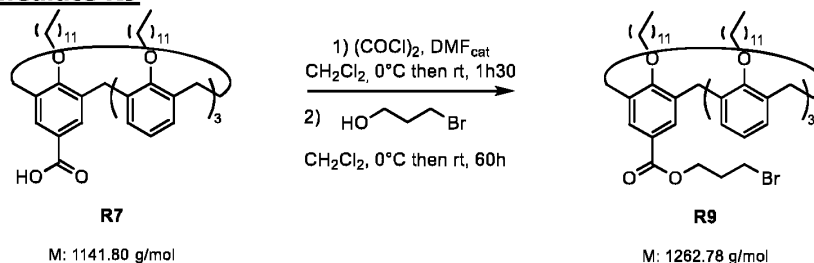
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was dried under reduced pressure to afford R7 as an orange oil (230 mg, 0.201 mmol). Yield: 90%. ^1H NMR (300 MHz, CDCl_3 , 298 K): δ (ppm) 7.30 (s, 2H), 6.70-6.33 (m, 9H), 4.44 (d, 2H), 4.42 (d, 2H), 3.97-3.78 (m, 8H), 3.20 (d, 2H), 3.14 (d, 2H), 1.88 (m, 8H), 1.50 – 1.10 (m, 72H), 0.88 (t, 12H).

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Intermediate R8

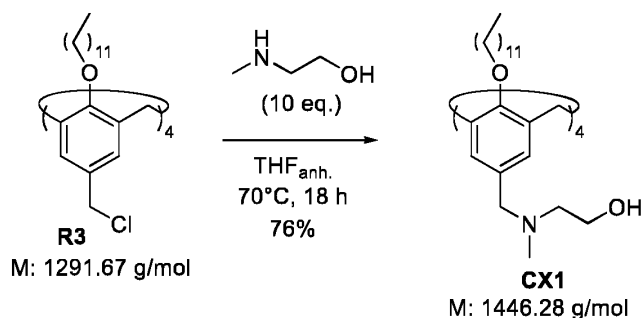
The calixarene R6 (0.200 g, 0.157 mmol, 1 eq) was dissolved in anhydrous CH_2Cl_2 (8 mL). The solution was sparged with argon gas and then cooled down to 0°C . (COCl) $_2$ (0.239 g, d = 1.615 g/mL, 0.148 mL, 1.883 mmol, 12 eq) was added dropwise, followed by the addition of DMF (10 μL). The solution was stirred under argon atmosphere at 0°C for 30 minutes, and then at room temperature for 1 hour. The solution was thoroughly concentrated under reduced pressure to afford a yellow residue. Anhydrous CH_2Cl_2 (8 mL) was added, and the solution was cooled down to 0°C . 3-bromo-1-propanol (0.548 g, d = 1.566 g/mL, 0.350 mL, 3.943 mmol, 25 eq) was added and the solution was stirred under argon atmosphere at 0°C for 30 minutes, and then at room temperature for 120 hours. After 24h, 3-bromo-1-propanol (1.566 g, d = 1.566 g/mL, 1.0 mL, 72 eq) was added again. After completion, the solution was concentrated under reduced pressure. Acetonitrile (5 mL) was added to the yellow oil to precipitate the product. The white precipitate was washed twice with acetonitrile (2 mL) and purified by flash chromatography on silica gel (eluent: $\text{CH}_2\text{Cl}_2/\text{AcOEt}$ gradient from 99:1 to 95:5) to afford R8 as a white powder (52 mg, 0.0296 mmol). Yield: 19 %. ^1H NMR (300 MHz, CDCl_3 , 298 K): δ (ppm) 7.32 (s, 8H), 4.43 (d, 4H), 4.32 (t, 8H), 3.92 (t, 8H), 3.49 (t, 8H), 3.27 (d, 4H), 2.27 (q, 8H), 1.93-1.80 (m, 8H), 1.43-1.21 (m, 72H), 0.88 (t, 12H).

Intermediate R9

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The calixarene **R7** (0.100 g, 0.0876 mmol, 1 eq) was dissolved in anhydrous CH_2Cl_2 (5 mL). The solution was sparged with argon and then cooled down to 0°C . $(\text{COCl})_2$ (0.0468 g, $d = 1.615 \text{ g/mL}$, 0.029 mL, 0.369 mmol, 4 eq) was added dropwise, followed by the addition of DMF (10 μL). The solution was stirred under argon atmosphere at 0°C for 30 minutes, and then at room temperature for 1 hour. The solution was thoroughly concentrated under reduced pressure to afford a yellow residue. Anhydrous CH_2Cl_2 (5 mL) was added, and the solution was cooled to 0°C . 3-bromo-1-propanol (0.304 g, $d = 1.566 \text{ g/mL}$, 0.194 mL, 2.186 mmol, 25 eq) was added and the solution was stirred under argon atmosphere at 0°C for 30 minutes, and then at room temperature for 64 hours. After completion, the reaction mixture was concentrated under reduced pressure. Acetonitrile (2 mL) was added, and the mixture was centrifugated, and the sticky brown residue isolated. The process was repeated once again. The residue was purified by flash chromatography on silica gel (eluent: cyclohexane/ CH_2Cl_2 gradient from 75:25 to 50:50) to afford **R9** as a sticky yellow oil (29 mg, 0.0232 mmol). Yield: 35 %. ^1H NMR (300 MHz, CDCl_3 , 298 K): δ (ppm) 7.18 (s, 2H), 6.71-6.59 (m, 6H), 6.49 (d, 2H), 6.41 (t, 1H), 4.45 (d, 2H), 4.41 (d, 2H), 4.31 (t, 2H), 3.94-3.81 (m, 8H), 3.47 (t, 2H), 3.18 (d, 2H), 3.14 (d, 2H), 2.23 (q, 2H), 1.95-1.80 (m, 8H), 1.40-1.18 (m, 72H), 0.88 (t, 12H).

20 **Product CX1**

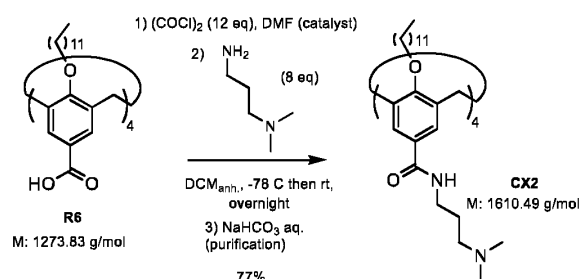


To a stirred solution of calix[4]arene **R3** (79.7 mg, 61.7 μmol) in $\text{THF}_{\text{anh.}}$ (1.5 mL) in a vial at r.t. was added 2-(methanolamino)ethanol (50 μL , 0.62 mmol, $d: 0.935 \text{ g/mL}$). The mixture was stirred at 70°C for 18 h. An insoluble oil decanted over the course of the reaction. The THF layer was collected and the vial was rinsed with 1.5 mL of CH_2Cl_2 . To the combined THF and CH_2Cl_2 fractions were added 6 mL of MeCN. The resulting milky suspension was centrifugated (5 min. at 2500 g). The milky supernatant (fraction A) was separated from the solid. The process was repeated 4 more times resulting in supernatant fractions B, C, D, E and the solid F. Fraction A was evaporated to dryness under vacuum, dissolved in 2 mL of CH_2Cl_2 and 1 mL of MeOH, then precipitated with 6 mL of MeCN and the solid was collected by centrifugation (5 min, 2500 g.) affording fraction G. Fractions B, C, and G contained

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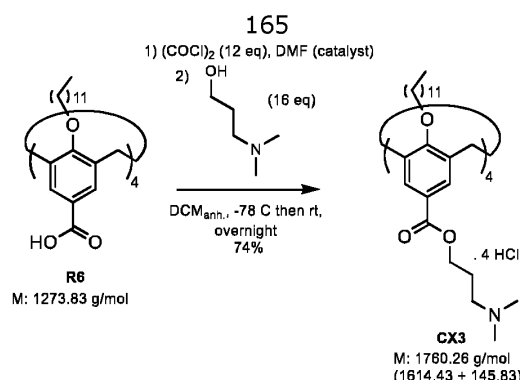
pure product **CX1** according to ^1H NMR analysis. These combined fractions afforded **CX1** as a white solid (67.6 mg, 46.7 μmol). Yield: 76%. ^1H NMR (400 MHz, CDCl_3 , 298 K): δ (ppm) 6.60 (s, 8H), 4.41 (d, 4H), 3.86 (t, 8H), 3.56 (t, 8H), 3.19 (s, 8H), 3.11 (d, 4H), 2.47 (t, 8H), 2.07 (s, 12H), 1.93 (bs, 8H), 1.20–1.45 (m, 72H), 0.88 (t, 12H).

Product CX2



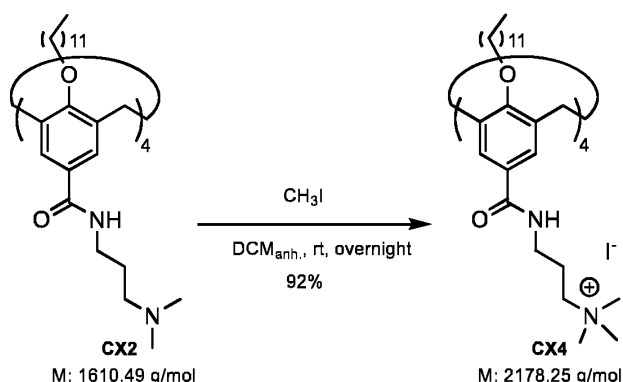
Calix(4)arene **R6** (0.144 g, 0.113 mmol, 1 eq) was added to a flask with anhydrous DCM (5 mL). The solution was sparged with argon gas and then cooled to -78°C . $(\text{COCl})_2$ (0.117 mL, 1.36 mmol, 12 eq) was added dropwise over a period of 3 minutes, and then DMF (10 μL) was added. The solution was stirred under argon gas at -78°C for 30 minutes, and then at room temperature for 1 hour. The solution was thoroughly concentrated under reduced pressure to afford a yellow oil, and anhydrous DCM was added. The solution was cooled at -78°C , then the diamine (0.114 mL, 0.906 mmol, 8 eq) was added dropwise over a period of 3 minutes. The solution was stirred under argon gas at -78°C for 30 minutes, and then at room temperature overnight. After the reaction was complete, the solution was concentrated under reduced pressure, and the oily residue was extracted with a solution of NaHCO_3 (2 mL, prepared from a saturated solution diluted x2). The solution was extensively sonicated then centrifugated (2500 g, 10 minutes). To the isolated precipitate was then added water (2 mL), and the solution was centrifugated (4000 rpm, 10 minutes). The precipitate was isolated and dried to afford the product **CX2** as a white powder in a yield of 77% (140 mg, 0.0869 mmol). ^1H NMR (300 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$ 9/1 v:v, 298 K, calibrated on CDCl_3): δ (ppm) 7.18 (s, 8H), 4.36 (d, 4H), 3.81 (d, 8H) 3.21 (t, 8H), 3.17 (d, 4H), 2.70 (s, 8H), 2.47 (s, 24H), 1.81 (s, 8H), 1.81 (s, 8H), 1.40 – 1.10 (m, 72H), 0.77 (t, 12H).

Product CX3



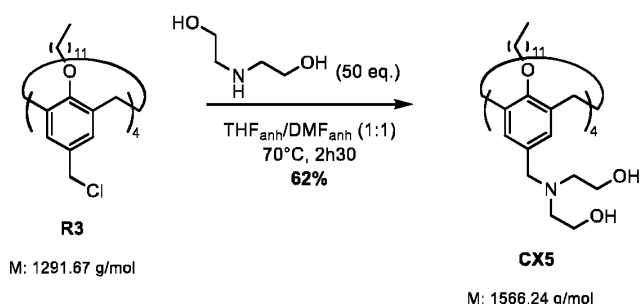
- Calix(4)arene **R6** (0.050 g, 0.0392 mmol, 1 eq) was added to a flask with anhydrous DCM (2 mL). The solution was sparged with argon gas and then cooled to -78°C . $(\text{COCl})_2$ (0.0405 mL, 0.472 mmol, 12 eq) was added dropwise over a period of 3 minutes, and then DMF (10 μL) was added. The solution was stirred under argon gas at -78°C for 30 minutes, and then at room temperature for 1 hour. The solution was thoroughly concentrated under reduced pressure to afford a yellow oil, and anhydrous DCM was added. The solution was cooled at -78°C , then the alcohol (0.0736 mL, 0.628 mmol, 16 eq) was added dropwise over a period of 3 minutes.
- The solution was stirred under argon gas at -78°C for 30 minutes, and then at room temperature for one hour. After completion, the solution was concentrated under reduced pressure to afford a yellow oil. Acetonitrile (3 mL) was added, and the mixture was centrifugated, and the precipitate isolated. The process was repeated once again, and the precipitate was then thoroughly dried to afford the product **CX3** as a white powder with a yield of 74% (51 mg, 0.029 mmol). ^1H NMR (300 MHz, CDCl_3 , 298 K): δ (ppm) 12.13 (s, 4H), 7.29 (s, 8H), 4.44 (d, 4H), 4.32 (s, 8H), 3.93 (t, 8H), 3.29 (d, 4H), 3.25 (s, 8H), 2.89 (d, 24H), 2.32 (d, 8H), 1.87 (s, 8H), 1.36 (s, 8H), 1.27 (s, 64H), 0.88 (t, 12H).

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Product CX4

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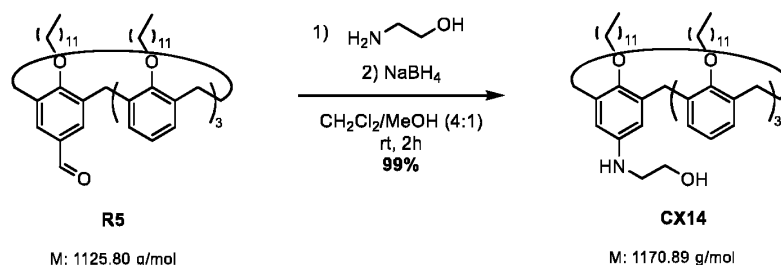
Calix(4)arene **CX2** (0.060 g, 0.0373 mmol, 1 eq) was added to a flask with anhydrous DCM (5 mL). CH₃I (0.0635 g, 0.447 mmol, 12 eq) was added and the solution was stirred overnight under argon. After completion, the solution was concentrated to afford the product **CX4** as a white, slightly beige powder with a yield of 92% (75 mg, 0.0344 mmol). ¹H NMR (300 MHz, CDCl₃, 298 K): δ (ppm) 7.82 (s, 4H), 7.42 (s, 8H), 4.40 (s, 4H), 3.89 (s, 8H), 3.77 (s, 8H), 3.48 (s, 8H), 3.44 (s, 4H), 3.37 (s, 36H), 2.26 (s, 8H), 1.88 (m, 8H), 1.27 (m, 72H), 0.88 (t, 12H).

Product CX5

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The calixarene **R3** (197 mg, 0.152 mmol, 1 eq) was solubilized in 3 mL anhydrous THF/DMF (1:1, v:v). Then, diethanolamine (731 μL, 7.62 mmol, 50 eq) was added and the reaction medium was stirred vigorously at 70 °C for 2h30. The medium was brought to room temperature, CH₃CN (5 mL) was added, and the resulting white precipitate was isolated by centrifugation. The solid was redissolved in a minimum of CH₂Cl₂, precipitated with CH₃CN (5 mL) and isolated by centrifugation. The solid was finally washed with MeOH (2 x 5 mL), leading to **CX5** as a white solid (146.5 mg, 0.0935 mmol). Yield: 62%. ¹H NMR (300 MHz, CDCl₃, 298 K): δ (ppm) 6.71 (s, 8H), 4.42 (d, 4H), 3.87 (t, 8H), 3.49 (t, 16H), 3.38 (s, 8H), 3.15 (d, 4H), 2.59 (t, 16H), 1.95 (bs, 8H), 1.20–1.45 (m, 72H), 0.88 (t, 12H).

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Product CX14

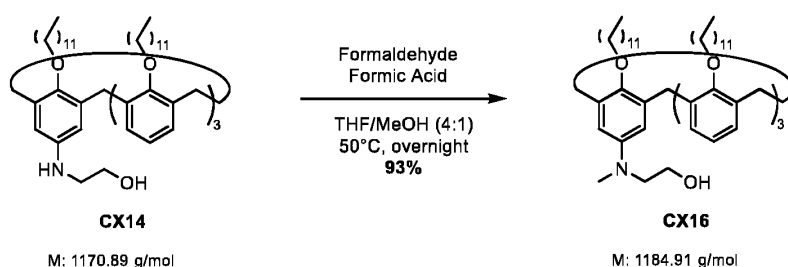
The calixarene **R5** (155 mg, 0.138 mmol, 1 eq.) was solubilized in 5 mL CH₂Cl₂/MeOH (4:1). The aminoethanol (16.5 μL, 0.28 mmol, 2 eq.) was then added, followed by the addition of NaBH₄ (10.4 mg, 0.28 mmol, 2 eq.) 1h later. After 1 extra hour of

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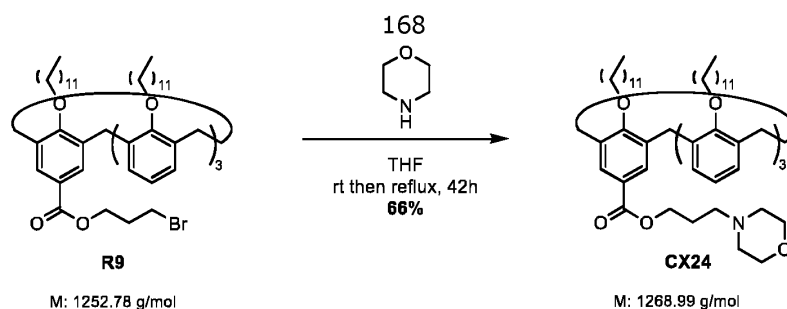
reaction, the volatiles were evaporated under reduced pressure. The residue was then solubilized in 10 mL CH₂Cl₂ and washed with milliQ water (2x 5 mL). The combined aqueous phase were extracted with 10 mL CH₂Cl₂. The organic layers were combined and evaporated under reduced pressure to afford **CX14** as a pale-yellow oil (161 mg, 0.137 mmol). Yield: 99%. ¹H NMR (300 MHz, CDCl₃, 298 K): δ (ppm) 6.83-6.44 (m, 11H), 4.48 (d, 2H), 4.47 (d, 2H), 3.97 (t, 4H), 3.85 (t, 4H), 3.61 (t, 2H), 3.43 (s, 2H), 3.18 (d, 2H), 3.16 (d, 2H), 2.63 (t, 2H), 2.05-1.82 (m, 8H), 1.49 – 1.14 (m, 72H), 0.93 (t, 12H).

10 Product CX16



To a stirred solution of calixarene **CX14** (201 mg, 0.172 mmol, 1 eq) in 10 mL THF/MeOH (4:1) at *rt* were added a solution of 35% formaldehyde in MeOH (40.8 μL, 0.687 mmol, 4 eq) and formic acid (19.4 μL, 0.687 mmol, 4 eq). The reaction mixture was stirred at 50°C overnight. Volatiles were then evaporated under reduced pressure. The residue was solubilized in 10 mL CH₂Cl₂ and washed with milliQ water (2x 5 mL) then the collected aqueous phases were washed with CH₂Cl₂ (1x 15 mL). The combined organic layers were evaporated under reduced pressure to afford **CX16** as an orange powder (197 mg, 0.160 mmol). Yield: 93%. Two ¹H NMR spectra are shown below. From top to bottom: (i) the product as the formate salt form, and (ii) the deprotonated product. ¹H NMR (300 MHz, CDCl₃, 298 K): δ (ppm) 6.97-6.87 (m, 4H), 6.78 (t, 2H), 6.51-6.28 (m, 5H, *ArH*), 4.45 (d, 2H), 4.44 (d, 2H), 4.10-3.90 (m, 4H), 3.84-3.67 (m, 4H), 3.59-3.48 (m, 2H), 3.53 (s, 2H), 3.16 (d, 4H), 1.55 – 1.05 (m, 72H), 0.99-0.76 (m, 12H). ¹H NMR (300 MHz, CDCl₃, 298 K): δ (ppm) 6.73-6.41 (m, 11H), 4.44 (d, 2H), 4.42 (d, 2H), 4.00-3.76 (m, 8H), 3.52 (t, 2H), 3.21 (s, 2H), 3.14 (d, 2H), 3.12 (d, 2H), 2.35 (t, 2H), 2.05-1.82 (m, 8H), 1.93 (s, 3H), 1.54 – 1.14 (m, 72H), 0.99-0.79 (m, 12H).

Product CX24

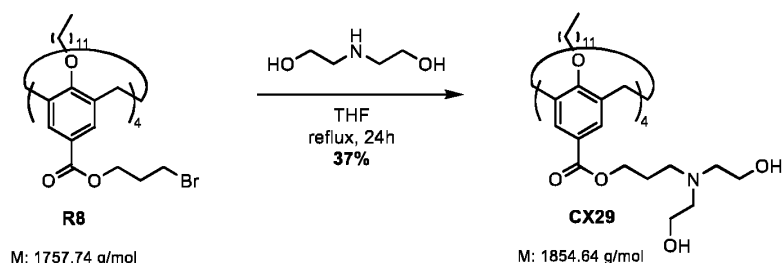


To a solution of **R9** (25.84 mg, 0.0205 mmol, 1 eq) in anhydrous THF (0.8 mL) was added morpholine (2.14 mg, d = 1.0007 g/mL, 0.002 mL, 0.0246 mmol, 1.2 eq).

- 5 The reaction was stirred in a sealed tube under argon atmosphere at room temperature for 1h30, then at reflux for 41h. After 16h, morpholine (17.83 mg, d = 1.0007 g/mL, 0.018 mL, 0.2046 mmol, 10 eq) was added. After completion, the solvent was evaporated under reduced pressure. Then, pentane (1 mL) was added to the crude. The mixture was centrifugated and this operation was repeated once.
- 10 The supernatant was isolated and evaporated under reduced pressure to afford **CX24** as a yellow sticky oil (17 mg, 0.0136 mmol). Yield: 66 %. ^1H NMR (300 MHz, CDCl_3 , 298 K): δ (ppm) 6.67-6.41 (m, 11H), 4.45 (d, 2H), 4.41 (d, 2H), 4.25 (t, 4H), 3.95-3.79 (m, 8H), 3.74 (t, 4H), 3.16 (t, 4H), 2.52-2.40 (s, 6H), 1.95-1.79 (m, 10H), 1.44-1.16 (m, 72H), 0.88 (t, 12H).

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Product CX29



To a solution of **R8** (50.92 mg, 0.0290 mmol, 1 eq) in anhydrous THF (1 mL) was added diethanolamine (0.149 g, d = 1.0966 g/mL, 0.136 mL, 1.4185 mmol, 50 eq).

- 20 The reaction was stirred in a sealed tube at reflux for 24 h under argon atmosphere. After completion, the solvent was evaporated under reduced pressure. The residue was dissolved in a minimum of MeOH (1mL), then acetonitrile was added until **CX29** precipitated. The mixture was centrifugated, then the white powder was dried under high vacuum to afford **CX29** as a white solid (20 mg, 0.0106 mmol). Yield: 37 %.
- 25 ^1H NMR (300 MHz, CDCl_3 , 298 K): δ (ppm) 7.33 (s, 8H), 4.44 (d, 4H), 4.30 (t, 8H),

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3.92 (t, 8H), 3.61 (t, 16H), 3.28 (d, 4H), 2.61-2.68 (m, 24H), 1.94-1.70 (m, 16H), 1.42-1.20 (m, 72H), 0.88 (t, 12H).

5 Example 3 - Example of a possible delivery system according to an embodiment of the current invention: use of cationic calixarene in a delivery system.

Molar ratios of components used for the formation of the delivery system:

- Ionizable + cationic components = 25 to 40 %
 - Part of ionizable = 80 to 95 %
 - Calixarene = 1.25 to 8 %
- 10 • Helper lipid (phospholipids) = 25 to 35 %
- Additional lipid (Sterol) = 25 to 50 %

The delivery systems are prepared according to the molar ratios using cationic calixarene. Specifically, DODAP is used as an ionizable component, Calixarene is used as a cationic component, DOPE is used as a phospholipid, Cholesterol is used as an additional lipid.

Key physico-chemical properties of the prepared delivery systems were assessed and the results are given below.

- 20 • Particle Size = 75-150 nm (PDI (polydispersity index) < 0.3)
- Encapsulation efficiency % > 85 %
- Protein expression is increased when calixarene is combined with DODAP

25 Example 4 – Development and characterization of lipid nanoparticles according to an embodiment of the invention comprising ionizable calix[4]arenes

Introduction

This example focuses on the design of ionizable calix[4]arenes and their use in RNA lipid nanoparticles (RNA-LNPs) to replace the traditional ionizable lipid. This modification is expected to change completely the behavior of the resulting delivery systems, especially their encapsulation and releasing capabilities. Calix[4]arenes display several key features that makes them suitable for nucleic acid delivery. The most important is their natural cone-shaped conformation that was found crucial for lipid nanoparticles/ionizable lipids to achieve high endosomal escape and favor the release of RNA in the cytosol. In addition, calix[4]arenes are platforms, which facilitates the synthesis of ionizable calix[4]arenes with 1, 2, 3, 4 or more amine

heads, meaning that the charge density (number of amines/molecule) could be increased easily. This property helps the encapsulation of very long RNA, such as self-amplifying RNA (saRNA), by increasing the number of amines without changing the mass ratio between the ionizable component and RNA, while this task remains
5 challenging with the current LNP technology not comprising calixarenes.

Ionizable calix[4]arenes synthesis and self-assembly into monodisperse nanoparticles according to an embodiment of the invention encapsulating mRNA-FLuc.

10 A library of ionizable calixarenes was synthesized to better understand the structure-activity relationship. Calixarenes bearing one (CX14, CX16, CX24) or four (CX1, CX2, CX3, CX5, CX6, CX29) ionizable head groups (i-head) were synthesized. Ionizable heads were selected from the group of secondary amines (CX6, CX14) and ternary amines (CX1, CX2, CX3, CX5, CX16, CX24, CX29), cyclic or substituted with methyl
15 or hydroxyethyl groups. Two biodegradable groups were also explored to link the ionizable head to the macrocyclic core and facilitate the metabolic degradation of the resulting compounds and avoid bioaccumulation (amide and ester links in CX2 and CX3/CX29 respectively).

20 All these calixarenes were self-assembled into nanoparticles according to an embodiment of the invention with a helper lipid (phospholipid), a sterol and a PEGylated lipid. Several helper (DOPE, DSPC) and PEG lipids (DMG-PEG2000, DSG-PEG2000) were explored. Ratios between these components were defined to produce stable monodisperse nanoparticles.

25 A minimum mass ratio of [Calixarenes+Lipids]/RNA is needed to obtain stable and monodisperse particles. This minimum ratio is ca. 20 for the 1-headed calixarene, while it may be decreased to 10 with the 4-headed calixarenes. This interesting result suggests that less material is needed to achieve similar encapsulation efficiency and monodispersity as compared to commonly used ionizable lipids (e.g., SM-102). The
30 difference may be due to the increased charge density on the 4-headed ionizable calixarenes.

With respect to the helper lipid, the sterol and the PEGylated lipid, their mass fractions are conserved when moving from 1-headed to 4-headed systems. These lipid mass fractions (expressed in percent, wherein the mass of the delivery system without cargo represent 100%) are preferentially:

Ionizable calixarene: from 10 to 60%

Helper lipid: from 5 to 35%
Sterol: from 15 to 50%
PEGylated lipid: from 4 to 24%

5 It is worth noting that the PEGylated lipid is required to obtain stable monodisperse nanoparticles that do not aggregate as the PEGylated lipid ensures the shielding of particles and stabilizes their lipid-water interface. As a result, the PEGylate lipid mass fraction must be precisely controlled; a too low amount in PEG induces the aggregation of the particles, while a too high amount of PEGylated lipid limits their
10 transfection capabilities (see *in vivo* protein expression results below). Examples of nanoparticles made with ionizable calixarenes according to an embodiment of the invention are given in Table 1 below.

15 Table 1. Examples of nanoparticles made with ionizable calixarenes and encapsulating Fluc-mRNA (if not precised) or another RNA.

CX (mass ratio)	Helper (mass ratio)	Sterol (mass ratio)	PEGylated lipid (mass ratio)	(Lipids+CX)/RNA mass ratio	Size (nm) [PDI]	Encapsulation efficiency (%)
CX1 (45%)	DOPE (35%)	Cholesterol (15%)	DMG- PEG2000 (5%)	10	84 [0.08]	97
CX1 (10%)	DOPE (35%)	Cholesterol (40%)	DMG- PEG2000 (15%)	10	70 [0.12]	80
CX1 (60%)	DOPE (10%)	Cholesterol (25%)	DMG- PEG2000 (5%)	10	125 [0.10]	96
CX1 (10%)	DOPE (25%)	Cholesterol (50%)	DMG- PEG2000 (15%)	30	56 [0.20]	99
CX1 (60%)	DOPE (10%)	Cholesterol (20%)	DMG- PEG2000 (10%)	30	51 [0.08]	99
CX1 (36.5%)	DOPE (22.5%)	Cholesterol (36%)	DMG- PEG2000 (5%)	20	73 [0.04]	99
CX1 (22%)	DOPE (29%)	Cholesterol (37%)	DMG- PEG2000 (12%)	19	53 [0.17]	97
CX2 (24%)	DOPE (28%)	Cholesterol (36%)	DMG- PEG2000 (11%)	20	54 [0.15]	98

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CX3 (26%)	DOPE (27%)	Cholesterol (35%)	DMG- PEG2000 (11%)	20	56 [0.14]	98
CX5 (24%)	DOPE (28%)	Cholesterol (36%)	DMG- PEG2000 (11%)	20	55 [0.19]	97
CX5 (25%)	DSPC (19%)	Cholesterol (44%)	DMG- PEG2000 (12%)	19	67 [0.11]	90
CX5 (23%)	DOPE (11%)	Cholesterol (43%)	DMG- PEG2000 (23%)	32	47 [0.13]	98
CX5 (25%)	DOPE (12%)	Cholesterol (46%)	DMG- PEG2000 (18%)	19	47 [0.13]	98
CX5 (28%)	DOPE (27%)	Cholesterol (34%)	DMG- PEG2000 (11%)	25	63 [0.04]	99 ¹
CX5 (28%)	DOPE (27%)	Cholesterol (34%)	DMG- PEG2000 (11%)	25	63 [0.07]	98 ²
CX5 (28%)	DOPE (27%)	Cholesterol (34%)	DMG- PEG2000 (11%)	39	65 [0.15]	91 ³
CX16 (37%)	DOPE (28%)	Cholesterol (31%)	DMG- PEG2000 (4%)	29	113 [0.05]	96
CX16 (49%)	DOPE (24%)	Cholesterol (22%)	DMG- PEG2000 (5%)	22	61 [0.13]	99
CX16 (54%)	DSPC (6%)	Cholesterol (36%)	DMG- PEG2000 (4%)	20	81 [0.10]	98
CX16 (29%)	DSPC (31%)	Cholesterol (36%)	DMG- PEG2000 (4%)	37	58 [0.18]	99
CX29 (27%)	DOPE (27%)	Cholesterol (35%)	DMG- PEG2000 (11%)	20	51 [0.11]	98
CX29 (69%)	DOPE (7%)	Cholesterol (17%)	DMG- PEG2000 (7%)	20	98 [0.20]	92

¹mRNA encoding for the spike protein from SARS-CoV2²mRNA encoding for the glycoprotein G from Rabies virus³saRNA encoding for FLuc

In vitro transfection of mRNA-FLuc using delivery systems according to an embodiment of the invention

In vitro potency assays in Jurkat cells using Fluc mRNA as a reporter gene were performed. Average luminescence was measured 24h after transfection of 100 ng mRNA in Jurkat cells. Cells were seeded at day 1 (10000 cells/well in a 96-well plate) and cells were transfected at day 2. The luminescence was read at day 3 after luciferin addition. The first results (**Table 2**) demonstrated that CX compounds with ternary amines are more potent. At the same time, these assays provided evidence of the superiority of the 4-headed calixarenes over the 1-headed calixarenes. For the specific group of 4-headed calixarenes, we further demonstrated that increasing the number of hydroxyethyl functions improves the potency.

Table 2: Average luminescence measured 24h after transfection of 100 ng mRNA in Jurkat cells using a delivery system according to an embodiment of the invention.

CX (mass ratio)	Helper (mass ratio)	Sterol (mass ratio)	PEGylated lipid (mass ratio)	(Lipids+CX)/RNA mass ratio	Average luminescence (a.u.)
CX1 (22%)	DOPE (29%)	Cholesterol (37%)	DMG-PEG2000 (12%)	19	21196
CX2 (24%)	DOPE (28%)	Cholesterol (36%)	DMG-PEG2000 (11%)	20	723
CX3 (26%)	DOPE (27%)	Cholesterol (35%)	DMG-PEG2000 (11%)	20	2324
CX5 (24%)	DOPE (28%)	Cholesterol (36%)	DMG-PEG2000 (11%)	20	21368
CX14 (37%)	DOPE (21%)	Cholesterol (37%)	DMG-PEG2000 (5%)	27	104
CX16 (37%)	DOPE (28%)	Cholesterol (31%)	DMG-PEG2000 (4%)	29	754
CX24 (38%)	DOPE (28%)	Cholesterol (31%)	DMG-PEG2000 (4%)	30	205
CX29 (27%)	DOPE (27%)	Cholesterol (35%)	DMG-PEG2000 (11%)	20	1492

CX: calixarene
a.u.: arbitrary unit

mRNA-FLuc in vivo protein expression is modulated by the mass ratios

- Within the range of optimal mass ratios defined above to obtain stable monodisperse particles, an experiment was designed to further refine these ratios and maximize the protein expression *in vivo*. The best *in vitro* performer (CX5) was selected, and nanoparticles, where the total (lipid+CX)/RNA mass ratio was varied between 15 and 45, the helper content between 10% and 30%, and the PEGylated lipid between 10% and 24%, were tested.
- 1 μ g of Fluc mRNA was administrated via an intramuscular route and the luminescence at the injection site was used to estimate the effect of the above-mentioned factors. Visualization of the estimated model (average + 95% confidence intervals) is given in **Figure 2** and clearly indicates favorable helper and PEG lipid mass ratios to increase the *in vivo* protein expression. As mentioned above, a minimal PEG amount is preferred, but a lower limit is needed to achieve colloidal stability. **Figure 2** shows visualization of the estimated model (average + 95% confidence interval) of *in vivo* luminescence as a function of the (lipid+CX)/RNA mass ratio, the helper mass ratio and the PEG lipid mass ratio.
- After having optimized the molar ratios using CX5 *in vivo*, different calixarenes were compared. 4-headed CX1, CX2, CX3, CX5 were formulated at the optimal ratio defined for CX5. The 1-headed CX16 was also included in the study at its own optimal ratios defined *in vitro*. Results of protein expression per calixarene delivery system (geometric mean signal with 95% confidence intervals) at 3h, 6h and 9h after the intramuscular injection of 1 μ g of Fluc mRNA are shown in **Figure 3**. 1 μ g of RNA was administrated to both left and right hindlimb of mice. A DoE was used to randomize samples and avoid interactions between delivery systems and luminescence was estimated by integrating the radiance at the injection site. These data showed that CX5, the 4-headed calixarene bearing the most hydroxyethyl groups, performs best. Overall, this experiment indicated that the kinetic of protein expression is different between 1-headed and 4-headed calixarenes. While the signal of CX16 (1-headed) rapidly decreased, the protein expression induced by the 4-headed calixarenes (CX1, CX2, CX3 and CX5) remained stable over the different timepoints (from 3 to 9h after injection). This result shows again the superiority of the 4-headed calixarenes over the 1-headed for achieving a stable and high protein expression. This observation is strengthened by the lower amount of 4-headed calixarenes needed to reach this performance (ca. 5 μ g 4-headed CX/ μ g RNA vs. ca. 10 μ g 1-headed CX/ μ gRNA).

saRNA-FLuc encapsulation in nanoparticles according to an embodiment of the invention

While keeping the optimized mass ratio between the four components (CX, helper, cholesterol, PEG lipid), saRNA encoding for FLuc was then encapsulated using CX5 by varying the CX/saRNA mass ratio. Over the whole screening space (CX/saRNA mass ratio from 2.3 to 9.2), stable nanoparticles with high encapsulation efficiency (> 80%) were obtained (see **Figure 4**). **Figure 4** shows the encapsulation efficiency and *in vitro* protein expression as a function of the CX5/saRNA mass ratio of various delivery systems according to an embodiment of the invention. Two independent samples were produced per condition. Interestingly, as shown in **Figure 4**, the encapsulation efficiency increased when the CX/saRNA mass ratio was increased from 2.3 to 5.8, but then remained stable at a mass ratio of 9.2, showing a saturation effect of the CX quantity needed to reach a maximal encapsulation efficiency. However, further increasing the amount of CX5 allowed us to increase the protein expression. It is worth mentioning that this optimized mass ratio (9.2) remains small in comparison to those needed (ca. 20 µg i-lipid/µg RNA) to reach similar properties with saRNA (encapsulation efficiency and *in vitro* protein expression) with well-known ionizable lipids used in the field (SM-102, ALC-0315).

In vivo saRNA-Fluc delivery efficiency of a delivery system according to an embodiment of the current invention compared to a reference delivery system without a calixarene.

The great performance demonstrated *in vitro* was also confirmed *in vivo*. The intramuscular injection of 0.2 µg saRNA encoding for FLuc induced a strong production of luciferase that increased over time until at least 1 week (**Figure 5**). Similar performance was obtained with a reference system (SM-102 based LNP) but with approximatively a two-fold higher quantity in ionizable component (20.9 vs. 9.2), showing again the superiority of using 4-headed calixarenes with high charge density. **Figure 5** shows the geometric mean signal (+ signal from each mouse) per delivery system (delivery system comprising CX5 in blue, reference delivery system in red) over time. 0.2 µg of saRNA was administrated to the right hindlimb of each mice. Luminescence was estimated by integrating the radiance at the injection site.

Pre-clinical evaluation of a delivery system according to an embodiment of the invention comprising mRNA encoding for the G-protein of the Rabies virus

A delivery system comprising CX5 was tested in an immunogenicity study using the glycoprotein G from Rabies as a model antigen. Mice were vaccinated following a 21

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days prime-boost regimen using 0.6 µg or 2.5 µg of mRNA per dose. As shown in **Figure 6**, CX5 yielded strong VNTs (virus neutralization titers), well above the correlate of protection (0.5 IU/mL), using the two dosing regimens (0.6 or 2.5 µg), without inducing any adverse effect (loss of weight, impact on spleen, liver and kidneys weight, inflammation or an excessive reactogenic response) on the animals. **Figure 6** shows VNTs serum levels measured after 15 (after prime), 35 and 65 (after boost) days (left: 0.6 µg doses, right: 2.5 µg doses).

Cryo-TEM imaging of nanoparticles made with Ionizable calix[4]arenes according to an embodiment of the invention

CX5:DOPE:cholesterol:DMG-PEG2000 nanoparticles encapsulating mRNA-FLuc were imaged using cryo-TEM (Error! Reference source not found.). Lacey Formvar/Si monoxide grids, 300 mesh Cu (Ted Pella Inc, 01887-F) were glow discharged using an ELMO glow discharger for 20 s at 4.5 mA. 3.5 µl of sample was applied to the grid plunged in liquid ethane at 95% humidity and 20 degrees Celsius in the chamber on a Gatan Cp-3 using 4s double sided blotting. The cryo grids were imaged using a JEOL1400 microscope equipped with a TVIPS F416 at 60 000x and Gatan 626 side entry holder at 60 k magnification. 60 k Magnification images are 4kx4k, with a pixel size of 0.194 nm/px. Sample was applied undiluted. LNPs are monodisperse, with the smallest and largest LNPs around 40 nm and 80 nm respectively, which is in good agreement with DLS measurements (Z-average of *ca.* 50 nm).

Example 5 - Development and characterization of lipid nanoparticles according to an embodiment of the invention comprising cationic calix[4]arenes

Introduction

This example focuses on cationic calixarenes and their incorporation as a fifth component into lipid nanoparticles (LNPs) made of an ionizable lipid, a helper lipid, a sterol, and a PEG lipid.

By adding cationic calixarenes the behaviour of the resulting delivery systems is completely changed, especially their encapsulation and releasing capabilities. As described above, calix[4]arenes actually display several key features that makes them suitable for nucleic acid delivery. The most important is their natural cone-shaped conformation that was deemed crucial for lipid nanoparticles/ionizable lipids to achieve high endosomal escape and favor the release of RNA in the cytosol. In addition, as a platform, the calix[4]arenes facilitate the access to cationic compounds with 1, 2, 3, 4 or more quaternary amine heads, meaning that the charge density

(number of amines/molecule) could be increased easily. This property should help us to consider the encapsulation of very long RNA, such as self-amplifying RNA (saRNA) by adding a small amount of cationic calixarene into an LNP, while this task remains challenging with the current LNP technology. Furthermore, incorporation of the cationic calixarene into an LNP allows (i) to minimize the inherent toxicity of these cationic components by combining them with non-toxic biocompatible lipids, and (ii) to reduce the non-specific adsorption of proteins that usually limits their efficacy.

10 *Cationic calix[4]arenes synthesis and addition into monodisperse lipid nanoparticles according to an embodiment of the invention encapsulating mRNA-FLuc*

The cationic calixarene (CX4) was synthesized and tested, CX4.
Experiments demonstrated that these cationic calixarenes can be embedded into lipid nanoparticles encapsulating RNA (**Table 3**) and made with:

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- Ionizable lipids: DODAP, DLin-DMA, DLin-MC3-DMA, ALC-0315, SM-102
 - Helper lipids: DOPE, DOPC
 - Sterol: cholesterol
 - PEG lipids: DMG-PEG2000, DSG-PEG2000

20 Table 3. Examples of nanoparticles made with cationic calixarenes and encapsulating an Fluc-mRNA (if not precised) or another RNA.

i-lipid (mass fraction)	Helper (mass fraction)	Sterol (mass fraction)	PEGylated lipid (mass fraction)	CX (mass fraction)	(Lipids+CX)/RNA (mass ratio)	Size (nm) [PDI]	Encapsulation efficiency (%)
DLin-DMA (39.1%)	DOPE (16.6%)	Cholesterol (37.2%)	DSG-PEG2000 (6.3%)	CX4 (0.8%)	27.1	143 [0.19]	97
DLin-DMA (39.7%)	DOPE (16.9%)	Cholesterol (37.9%)	DMG-PEG2000 (4.7%)	CX4 (0.8%)	26.8	154 [0.19]	98
DLin-DMA (39.3%)	DOPE (16.7%)	Cholesterol (37.5%)	DMG-PEG2000 (5.6%)	CX4 (0.8%)	27.0	154 [0.25]	98 ¹

¹mRNA encoding for the glycoprotein G from Rabies virus

²mRNA encoding for the spike protein from SARS-CoV2

³saRNA encoding for the spike protein from SARS-CoV2

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Pre-clinical evaluation of candidate LNPs according to an embodiment of the invention comprising a cationic calixarene and DLin-DMA

2 different LNPs were fabricated using CX4, and DMG-PEG2000 or DSG-PEG2000. In all cases, DLin-DMA was used as the ionizable lipid. mRNA encoding for G-protein of the Rabies virus was used. Physicochemical characteristics of the LNPs produced are shown in **Table 5**.

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Table 5. Physicochemical characteristics of 2 LNPs according to an embodiment of the invention comprising a cationic calixarene

LNP	Mass fraction (%)	Zeta-Potential (mV)	Z-Average (nm)	PDI	EE (%)
DLin-DMA:DOPE:cholesterol:DMG-PEG2000:CX4	39.3:16.7:37.5:5.6:0.8	1.9	195.7	0.36	98.0
DLin-DMA:DOPE:cholesterol:DSG-PEG2000:CX4	39.3:16.7:37.5:5.9:0.7	2.8	153.9	0.25	97.6

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BALB/c mice were vaccinated following a prime-boost regimen 21 days apart (prime at day zero and boost at 21 days) with DLin-DMA:CX4 LNPs with two different PEGylated lipids. VNTs serum levels were measured after 15 or 35 days for 0.6 or 2.5 µg RNA doses. LNPs were frozen at -80°C for shipment and storage. The same batches were used to prime and boost the mice. Geometric mean titers are shown in **Figure 8**. VNTs produced using all LNPs yield strong VNTs after 35 days. The raw data shows a dose response effect, with doses of 2.5 µg on average higher than the 0.6 µg after both 15 and 35 days. For all LNPs and doses, the VNTs are well above the correlate of protection (shown as the dashed black line at 0.5 a.u.). These results demonstrate that cationic calixarenes combined with DLin-DMA as the ionizable lipid yields potent LNPs. These results also suggest that the PEGylated lipid choice impacts the immune response: a difference as small as 4 additional carbons on the two alkyl chains of the PEGylated lipid can significantly decrease the immune potency of an otherwise identical LNP.

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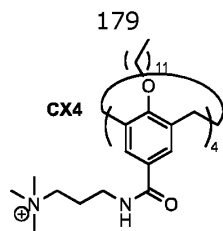
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Example 6: Cationic calix[4]arenes in LNPs for use as an adjuvant according to an embodiment of the invention

The use of cationic calix[4]arenes as adjuvant in an LNP was observed. LNPs made with N,N-dimethyl-2,3-bis[(9Z,12Z)-9,12-octadecadien-1-yloxy]-1-propanamine (DLin-DMA), a helper, cholesterol and a PEGylated lipid were fabricated with or without integrating a cationic calix[4]arenes as an adjuvant. CX4 was tested.

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All LNPs were found monodisperse and encapsulated mRNA (see Table 7). All LNPs encapsulated mRNA encoding for the G-protein of the Rabies virus. 0.6 or 2.5 µg mRNA was administrated in mice hind leg and a 21 days prime-boost regimen was followed. Virus neutralizing titers (VNTs) were measured in blood serum after 15 and 35 days after the prime. Results are shown in Figure 9A and Figure 9B.

Table 7: Physicochemical characteristics of LNPs fabricated

LNP	Zeta-Potential (mV)	Z-Average (nm)	PDI	EE (%)
DLin-DMA	2.1	201.1	0.12	97.9
DLin-DMA: CX4	1.9	195.7	0.36	98.0

As shown in Figure 9A, VNTs obtained using DLin-DMA LNPs boosted with CX4, well above the correlate of protection (0.5 IU/mL).

Figure 9B shows the virus neutralizing titers (VTNs) in serum measured after 15 (after prime), 35 and 65 days (after boost) days in mice which were vaccinated using SM-102 LNPs (control), DLin-DMA LNPs and DLin-DMA/CX4 LNPs. A 21 days prime-boost regimen using 2.5 µg of mRNA per dose was used (RNA encoding for the glycoprotein G from the Rabies virus). The results are shown as a comparison with the control delivery system (SM-102 LNPs). UDL = upper decision limit, LDL = lower decision limit. All points crossing the limits indicate a significant difference as compared to the control.

This result shows again the potent adjuvant and synergetic effect of adding a cationic calixarene into a lipid nanoparticle.

Example 7: Cationizable calix[4]arenes for use as an adjuvant

The present discovery is not limited to cationic calix[4]arenes and the same adjuvanticity was observed for cationizable calix[4]arenes, i.e., calix[4]arenes bearing secondary or ternary amines (preferably ternary amines). CX5 was synthesized, and self-assembled into hybrid nanoparticles with a helper lipid (phospholipid), a sterol and a PEGylated lipid. Unlike cationic calixarene that were

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self-assembled alongside an i-lipid, the cationizable calixarene was used instead of the i-lipid in these nanoparticles. The molar ratios were again optimized to produce monodisperse nanoparticles encapsulating mRNA. For the best performing nanoparticles produced with CX5 and tested as part of the optimization study, the protein expression reached only 5% of an LNP made with SM-102 or ALC-0315 as the i-lipid (1 µg of Fluc mRNA, IM administration in BALB/c mice, estimated radiance at 6 hours post-administration – data not shown).

CX5 was tested in an immunogenicity study with mRNA encoding for the glycoprotein G from Rabies as a model antigen. BALB/c Mice were vaccinated following a 21 days prime-boost regimen using 0.6 µg or 2.5 µg of RNA per dose. As shown in Figure 10, CX5 yielded strong Virus neutralizing titers (VNTs), well above the correlate of protection (0.5 IU/mL), using the two dosing regimens (0.6 or 2.5 µg). After 15 days (after priming), the response induced by CX5 was comparable to the one obtained with two comparators (SM-102 LNPs and ALC-0315 LNPs), whatever the dosing regimen (0.6 or 2.5 µg). At 0.6 µg, this response remained comparable to the SM-102 and ALC-0315 LNPs after the boost (35 days) and at the end study (65 days). On the contrary, at 2.5 µg, the two comparators were shown to slightly outperform CX5 after the boost (35 and 65 days). The observation that protein expression is 20 times lower for CX5 as compared to other LNPs, but still reaches similar or only slightly lower VNTs, is another demonstration that calix[4]arenes, in this case cationizable calix[4]arenes, are potent adjuvants for nucleic acid-based vaccines.

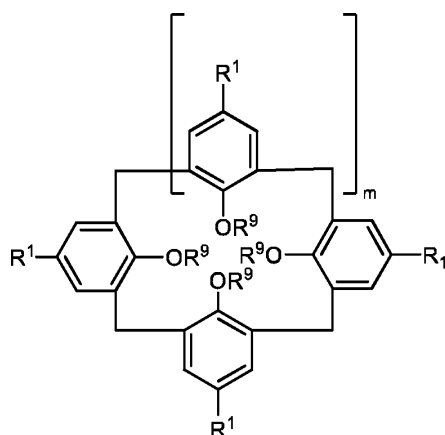
It is worth mentioning that the nanoparticle made with ionizable calixarenes were shown to induce no adverse effect (loss of weight, impact on spleen, liver and kidneys weight, inflammation or an excessive reactogenic response) on the animals that were used during the *in vivo* studies.

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CLAIMS

1. A compound of Formula (I):

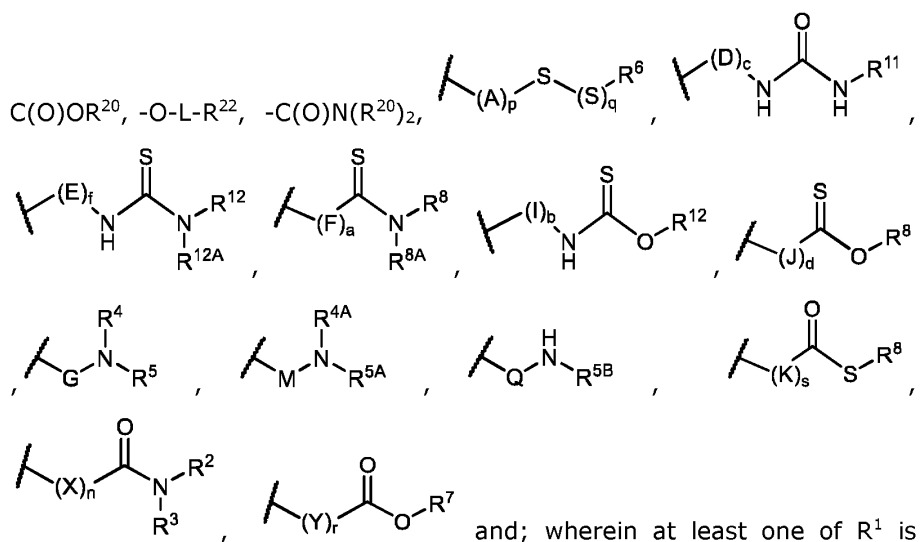


Formula (I)

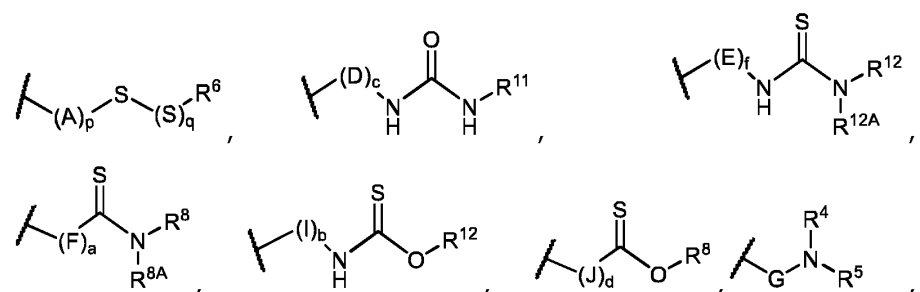
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or a pharmaceutically acceptable salt thereof wherein:

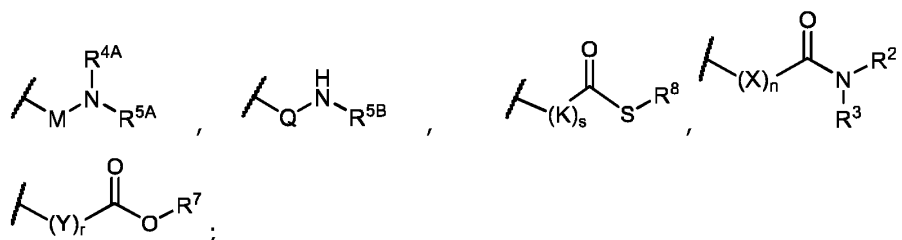
R¹ is selected from hydrogen, halogen, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₁₀ haloalkyl, -CN, -OR²⁰, -SR²⁰, -SF₅, -NO₂, -N(R²⁰)₂, -C(O)R²⁰, -



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A, D, E, F, G, I, J, K, M, Q, X, and Y are each independently selected from C₁₋₁₀ alkylene, C₂₋₁₀ alkenylene, C₂₋₁₀ alkynylene, wherein the C₁₋₁₀ alkylene, C₂₋₁₀ alkenylene, C₂₋₁₀ alkynylene are optionally substituted with one or more substituents independently selected from halogen, C₁₋₁₀ haloalkyl, -CN, -NO₂, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

each R², R⁶, R⁸, is independently selected from C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

each R⁷ is selected from:

C₁₋₂ alkyl, wherein the C₁₋₂ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰); and

C₃₋₁₀ alkyl, wherein the C₃₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

R^{8A} is independently selected from hydrogen and C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

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each R^{11} is independently selected from C_{3-10} alkyl, wherein the C_{3-10} alkyl is substituted with one or more substituents selected from $-N(R^{21})_2$, $-N(R^{21})_3^+$, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, $-CN$, $-NO_2$, $=O$, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, and $-S(O)_2(R^{20})$;

each R^{12} is independently selected from C_{3-10} alkyl, wherein the C_{3-10} alkyl is substituted with one or more substituents selected from $-N(R^{20})C_{1-10}$ alkyl, $-N(R^{20})_3^+$, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, $-CN$, $-NO_2$, $=O$, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, and $-S(O)_2(R^{20})$;

each R^{12A} is independently selected from hydrogen and C_{1-10} alkyl, wherein the C_{1-10} alkyl is substituted with one or more substituents selected from $-N(R^{20})C_{1-10}$ alkyl, $-N(R^{20})_3^+$, and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, $-CN$, $-NO_2$, $=O$, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, and $-S(O)_2(R^{20})$;

each R^3 is independently selected from hydrogen and C_{1-10} alkyl, wherein the C_{1-10} alkyl is optionally substituted with one or more substituents independently selected from halogen, $-CN$, $-NO_2$, $=O$, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, and $-S(O)_2(R^{20})$;

each R^4 is independently selected from C_{1-10} alkyl, wherein the C_{1-10} alkyl is optionally substituted with one or more substituents selected from 3- to 12-membered saturated heterocycle, wherein the 3- to 12-membered saturated heterocycle is optionally substituted with one or more substituents independently selected from halogen, C_{1-6} alkyl, C_{1-10} haloalkyl, $-CN$, $-NO_2$, $=O$, $-OR^{20}$, $-N(R^{20})_2$, $-C(O)R^{20}$, $-C(O)OR^{20}$, $-C(O)N(R^{20})_2$, and $-S(O)_2(R^{20})$;

each R^{4A} is independently selected from C_{1-10} alkyl, wherein the C_{1-10} alkyl is substituted with one or more substituents selected from $-OH$ and $-OC_{1-10}$ alkyl; wherein when each R^1 is $-CH_2-N(CH_2CH_2OH)_2$, each R^9 is independently selected from R^{9A} ;

each R^5 , R^{5A} is independently selected from C_{1-10} alkyl, wherein the C_{1-10} alkyl is substituted with one substituent selected from $-OR^{20}$ and 3- to 12-membered heterocycle, wherein the 3- to 12-membered heterocycle is optionally substituted with one or more substituents independently selected

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from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

5 each R^{5B} is independently selected from C₁₋₁₀ alkyl, wherein the C₁₋₁₀ alkyl is optionally substituted with one substituent selected from -OR²⁰ and 3- to 12-membered saturated heterocycle, wherein the 3- to 12-membered saturated heterocycle is optionally substituted with one or more substituents independently selected from halogen, C₁₋₆ alkyl, C₁₋₁₀ haloalkyl, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, and -S(O)₂(R²⁰);

10 wherein when R^{5B} is unsubstituted C₁₋₁₀ alkyl, each R⁹ is independently selected from R^{9B};

15 each R⁹ is independently selected from hydrogen, -(R¹⁰)_j-C(O)OR²⁰, -(R¹⁰)_j-C(O)N(R²⁰)₂, -(R¹⁰)_j-NHC(S)NHR²⁰, -(R¹⁰)_j-SR²⁰, -(R¹⁰)_j-S-S-R²⁰, PEG₁₋₂₀₀, mannose, an adjuvant, a carbohydrate, an antibody, C₁₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl, wherein the C₁₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl are each optionally substituted with one or more substituents independently selected from halogen, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, -S(O)₂(R²⁰), C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle;

20 each R^{9A} is independently selected from -(R¹⁰)_j-C(O)OR²⁰, -(R¹⁰)_j-C(O)N(R²⁰)₂, -(R¹⁰)_j-NHC(S)NHR²⁰, -(R¹⁰)_j-SR²⁰, -(R¹⁰)_j-S-S-R²⁰, PEG₁₋₂₀₀, mannose, an adjuvant, a carbohydrate, an antibody, C₁₂₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl, wherein the C₁₂₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl are each optionally substituted with one or more substituents independently selected from halogen, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, -S(O)₂(R²⁰), C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle;

25 each R^{9B} is independently selected from -(R¹⁰)_j-C(O)OR²⁰, -(R¹⁰)_j-C(O)N(R²⁰)₂, -(R¹⁰)_j-NHC(S)NHR²⁰, -(R¹⁰)_j-SR²⁰, -(R¹⁰)_j-S-S-R²⁰, PEG₃₋₂₀₀, mannose, an adjuvant, a carbohydrate, an antibody, C₅₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl, wherein the C₅₋₂₀ alkyl, C₂₋₂₀ alkenyl, and C₂₋₂₀ alkynyl are each optionally substituted with one or more substituents independently selected from halogen, -CN, -NO₂, =O, -OR²⁰, -N(R²⁰)₂, -C(O)R²⁰, -C(O)OR²⁰, -C(O)N(R²⁰)₂, -S(O)₂(R²⁰), C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle;

30 each R¹⁰ is independently selected from C₁₋₂₀ alkylene, C₂₋₂₀ alkenylene, and C₂₋₂₀ alkynylene;

each R²⁰ is independently selected from hydrogen; and C₁₋₂₀ alkyl, C₂₋₂₀ alkenyl, C₂₋₂₀ alkynyl, C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle,

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each of which is optionally substituted with one or more substituents independently selected from halogen, -OH, -CN, -NO₂, -NH₂, -N(C₁₋₆ alkyl)₂, -NHC₁₋₆ alkyl, -NHC₁₋₆ hydroxyalkyl, -N(C₁₋₆ alkyl)C₁₋₆ hydroxyalkyl, C₁₋₁₀ alkyl, -C₁₋₁₀ haloalkyl, -O-C₁₋₁₀ alkyl, oxo, =NH, C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle;

each R²¹ is independently selected from hydrogen; and C₁₋₂₀ alkyl, C₂₋₂₀ alkenyl, C₂₋₂₀ alkynyl, C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle, each of which is optionally substituted with one or more substituents independently selected from halogen, -OH, -CN, -NO₂, -NH₂, -N(C₁₋₆ alkyl)₂, -NHC₁₋₆ alkyl, -NHC₁₋₆ hydroxyalkyl, C₁₋₁₀ alkyl, -C₁₋₁₀ haloalkyl, -O-C₁₋₁₀ alkyl, =NH, C₃₋₁₂ carbocycle, and 3- to 12-membered heterocycle;

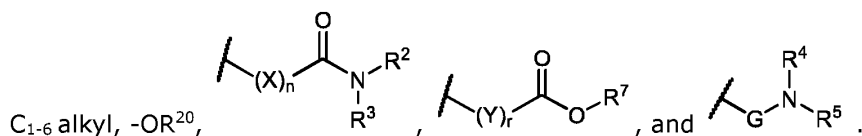
each R²² is selected from mannose, an adjuvant, a carbohydrate, and an antibody;

L is selected from a covalent linker;

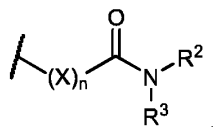
a, b, c, d, f, j, n, p, q, r, and s, are each independently selected from 0, 1, 2, and 3; and

m is selected from 1, 3, and 5.

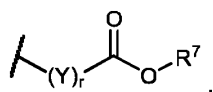
2. The compound or salt of claim 1, wherein each R¹ is selected from hydrogen,



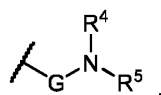
3. The compound or salt of claims 1 or 2, wherein each R¹ is selected from



4. The compound or salt of claims 1 or 2, wherein each R¹ is selected from

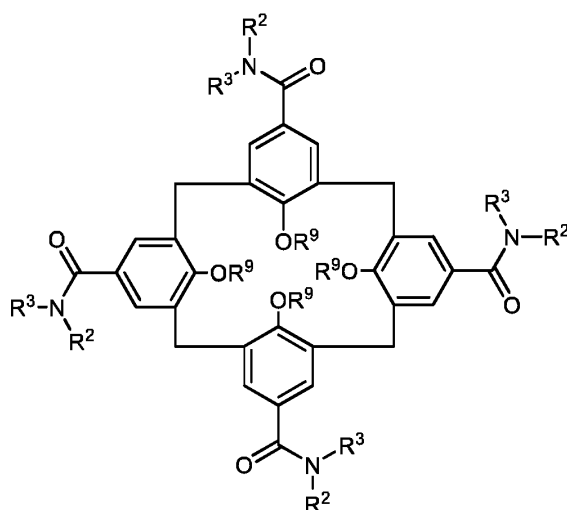


5. The compound or salt of claims 1 or 2, wherein each R¹ is selected from



6. The compound or salt of claim 3, wherein Formula (I) is represented by Formula (II):

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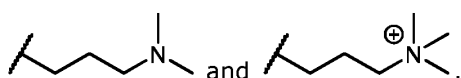


Formula (II)

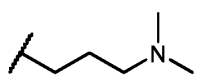
or a pharmaceutically acceptable salt thereof.

7. The compound or salt of claims 1, 2, 3, or 6, wherein R^2 is selected from C_{3-6} alkyl, wherein the C_{3-6} alkyl is substituted with one or more substituents selected from $-N(R^{20})_2$, $-N(R^{20})_3^+$, and 5- to 6-membered heterocycle, wherein the 5- to 6-membered heterocycle has at least one nitrogen atom.

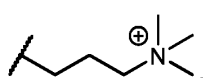
8. The compound or salt of claim 7, wherein each R^2 is selected from



9. The compound or salt of claim 8, wherein each R^2 is selected from



10. The compound or salt of claim 8, wherein each R^2 is selected from

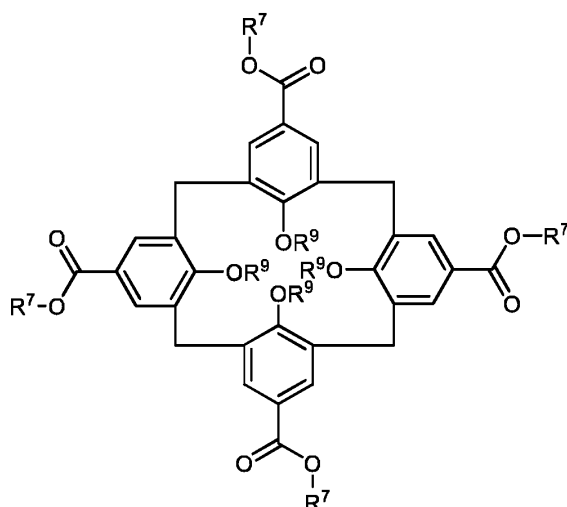


11. The compound or salt of any one of claims 6 to 10, wherein each R^3 is selected from hydrogen and C_{1-10} alkyl.

12. The compound or salt of claim 11, wherein each R^3 is hydrogen.

13. The compound or salt of claim 4, wherein Formula (I) is represented by Formula (III):

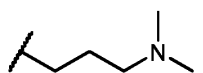
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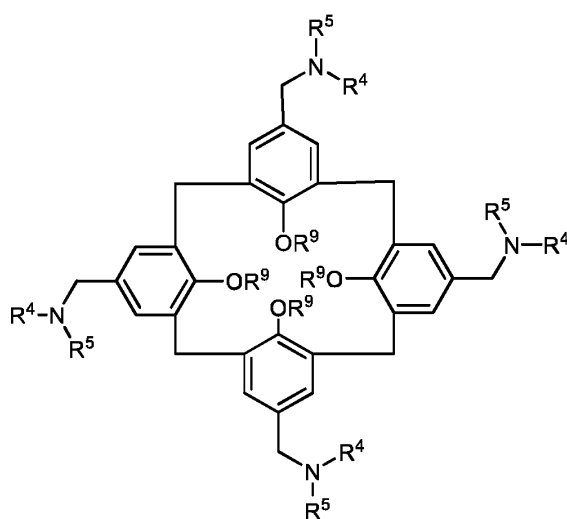
Formula (III)

or a pharmaceutically acceptable salt thereof.

14. The compound or salt of claims 1, 2, 4, or 13, wherein R⁷ is selected from C₃₋₆ alkyl, wherein the C₃₋₆ alkyl is substituted with one or more substituents selected from -N(R²⁰)₂, -N(R²⁰)₃⁺, and 5- to 6-membered heterocycle, wherein the 5- to 6-membered heterocycle has at least one nitrogen atom.
15. The compound or salt of claim 14, wherein R⁷ is selected from C₃₋₆ alkyl, wherein the C₃₋₆ alkyl is substituted with one -N(R²⁰)₂.
16. The compound or salt of claim 15, wherein each R⁷ is selected from



17. The compound or salt of claim 1, wherein Formula (I) is represented by Formula (IV):



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Formula (IV)

or a pharmaceutically acceptable salt thereof.

18. The compound or salt of claims 1, 2, 5, or 17, wherein each R^4 is selected from C_{1-2} alkyl.

5 19. The compound or salt of claims 17 or 18, wherein each R^5 is selected from C_{2-5} alkyl, wherein the C_{2-5} alkyl is substituted with one $-OR^{20}$.

20. The compound or salt of claim 19, wherein each R^5 is selected from C_{2-5} alkyl, wherein the C_{2-5} alkyl is substituted with one $-OH$.

21. The compound or salt of claim 20, wherein each R^5 is selected from



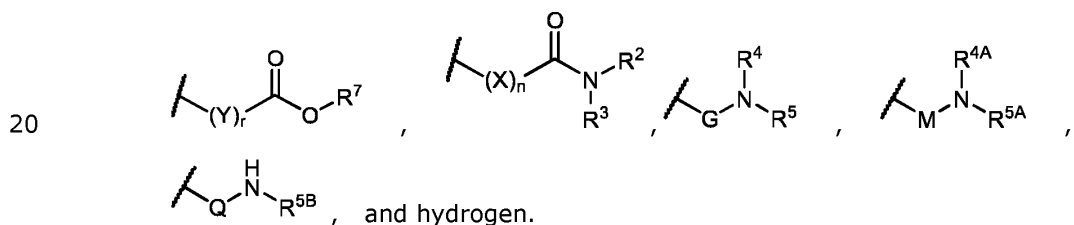
22. The compound or salt of any one of claims 1 to 21, wherein each R^9 is selected from unsubstituted C_{1-12} alkyl.

23. A composition comprising the compound or salt of any one of claims 1 to 22 and an excipient.

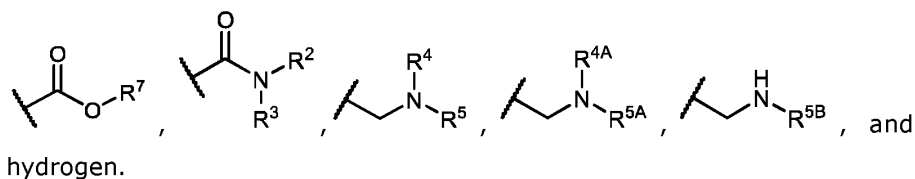
15 24. A pharmaceutical composition comprising the compound or salt of any one of claims 1 to 22, or the composition of claim 23, and one or more nucleotides.

25. The compound or salt of any one of claims 1 to 22, for use as a delivery system.

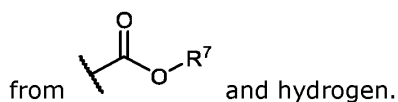
26. The compound or salt of claim 1, wherein each R^1 is selected from



27. The compound or salt of claims 1 or 26, wherein each R^1 is selected from



25 28. The compound or salt of claims 1 or 26 to 27, wherein each R^1 is selected

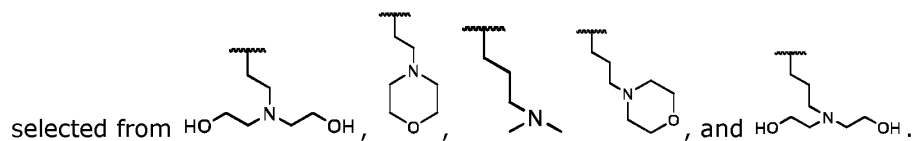


29. The compound or salt of claims 1, 2, 4, 13 or 26 to 28, wherein each R^7 is selected from substituted C_{3-5} alkyl, and substituted C_2 alkyl.

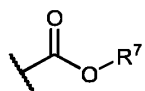
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30. The compound or salt of claims 1, 2, 4, 13, or 26 to 29, wherein each R^7 is selected from C_{3-5} alkyl substituted with one or more substituents selected from $-N(R^{20})_2$, and 4- to 6-membered heterocycle; and C_2 alkyl, which is substituted with one or more substituents selected from $-N(R^{20})_2$, and 4- to 6-membered heterocycle.
31. The compound or salt of claims 1, 2, 4, 13 or 26 to 30, wherein each R^{20} is independently selected from C_{1-4} alkyl, which is optionally substituted with one or more $-OH$,
32. The compound or salt of claims 1, 2, 4, 13 or 26 to 31, wherein each R^7 is

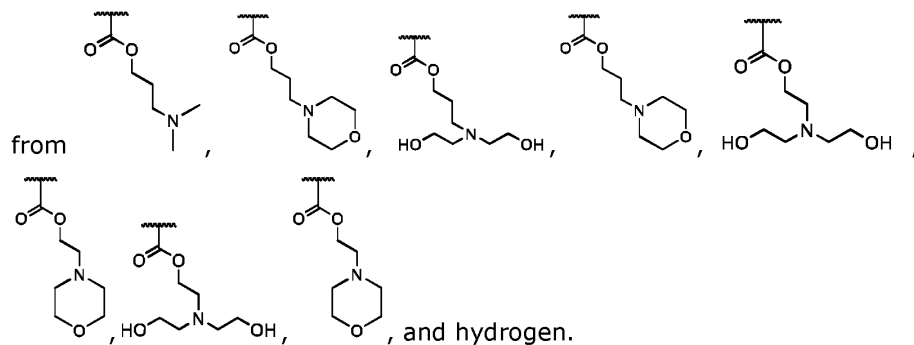
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33. The compound or salt of claims 1 or 26-28, wherein each R^1 is selected from

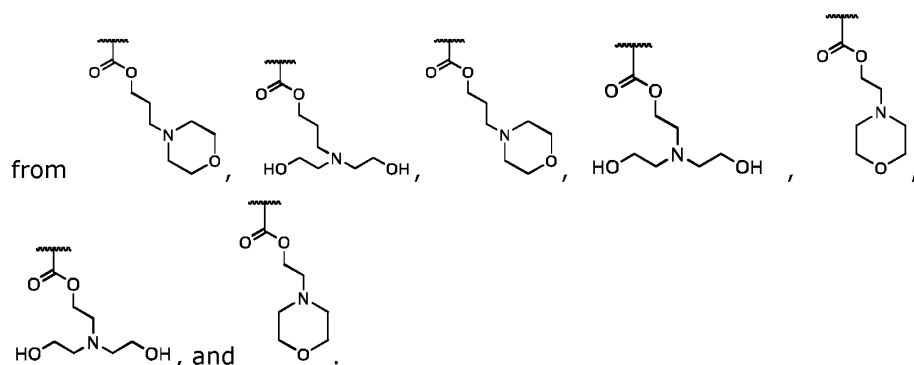


34. The compound or salt of claims 1, 2, 4, or 33, wherein each R^1 is selected



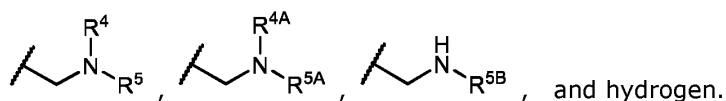
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35. The compound or salt of claims 1, 2, 4, or 33-34, wherein each R^1 is selected

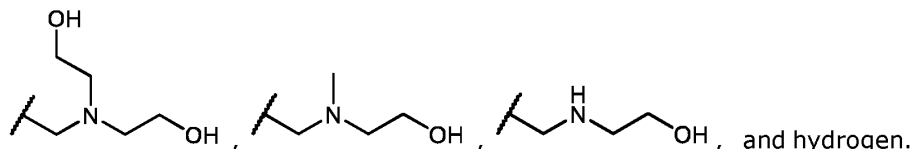


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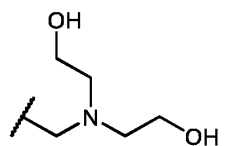
36. The compound or salt of claims 1 or 26, wherein each R^1 is selected from



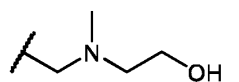
37. The compound or salt of claims 1, or 36, wherein each R^1 is selected from



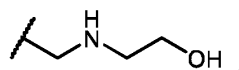
5 38. The compound or salt of claims 1 or 37, wherein each R^1 is selected from



39. The compound or salt of claims 1 or 37, wherein each R^1 is selected from

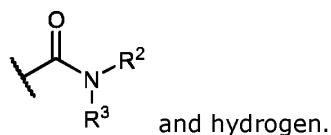


40. The compound or salt of claims 1 or 37, wherein each R^1 is selected from

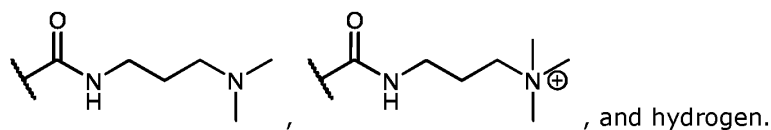


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41. The compound or salt of claim 1 or 26, wherein each R^1 is selected from

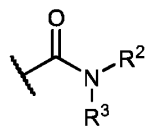


42. The compound or salt of claim 1, wherein each R^1 is selected from



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43. The compound or salt of claims 1 or 26, wherein each R^1 is selected from

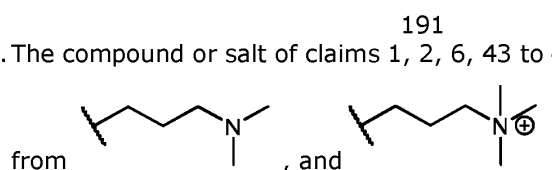


44. The compound or salt of claims 1, 2, 6, or 43, wherein each R^3 is selected from hydrogen.

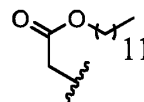
45. The compound or salt of claims 1, 2, 6, 43 or 44, wherein each R^2 is selected from C_{3-10} alkyl, wherein the C_{3-10} alkyl is substituted with one or more substituents selected from $-N(R^{20})_2$, and $-N(R^{20})_3^+$.

20

46. The compound or salt of claims 1, 2, 6, 43 to 45, wherein each R² is selected



47. The compound or salt of any one of claims 1 to 21, or 26 to 46, wherein each



R⁹ and R^{9A} is selected from C₁₂₋₁₆ alkyl, and .

5 48. The compound or salt of claims 26 to 47, wherein each R⁹ is selected from C₁₂₋₁₆ alkyl.

49. The compound or salt of claims 26 to 47, wherein each R^{9A} is selected from C₁₂₋₁₆ alkyl.

10 50. A composition comprising the compound or salt of any one of claims 26 to 49 and an excipient.

51. A pharmaceutical composition comprising the compound or salt of any one of claims 26 to 49, or the composition of claim 50, and one or more nucleotides.

52. The compound or salt of any one of claims 26-49, for use as a delivery system.

15 53. Use of a compound or salt in an immunogenic composition, wherein said composition comprises an immunogenic component encapsulated in a lipid nanoparticle (LNP) comprising said compound or salt of any one of claims 1 to 22 or 26 to 49 and wherein said LNP is an adjuvant for said immunogenic composition

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