

NOVEL STRUCTURAL LIPIDS FOR LIPID NANOPARTICLE FORMULATION

5 The present invention relates to the use of compounds in lipid micro- and nanoparticle compositions and novel lipid micro- and nanoparticle compositions per se. The compositions are suitable for therapeutic or prophylactic delivery, with improved stability and enhanced control over bio-distribution. In particular, the lipid micro- and nanoparticle compositions could be used to deliver (modified) RNA or DNA. The invention further relates to medical and research uses of the compositions.

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Nanomedicine involves the use of nanoscale materials, such as biocompatible nanoparticles, for diagnosis, delivery, sensing or actuation purposes. This is an increasingly important field, promising to provide novel diagnostic, therapeutic and/or prophylactic options. In particular, micro- and nanoparticle formulations can enable the use of molecular entities that are otherwise (a) unstable, (b) non-conforming to druglikeness rules, such as the Lipinski's rule of five, and/or (c) have unfavourable pharmacokinetics, including unsuitable bio-distribution behaviour, thus expanding the use of these molecular entities in terms of dosing scheme, concentration and thereby therapeutic efficacy, and/or reaching of specific target cells. In these formulations, the micro- or nanoparticles encapsulate, absorb, conjugate or otherwise associate with an active pharmaceutical ingredient, thereby allowing the separation of pharmacodynamic and pharmacokinetic properties between independent molecular entities. This greatly facilitates drug design, fine-tuning of pharmacological properties and improves both the efficacy and safety of the resultant medicines.

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The use of micro- and nanoparticle formulations has been particularly beneficial for the field of DNA and RNA-based medicines. This is because of the uniquely intracellular activity of such molecules in combination of their large size and negative charge, hampering uptake in most cells. The central role of both DNA and RNA in the biology of all life forms and the high level of specificity obtainable with these molecules provides unprecedented opportunities to find high-value treatments and cures for a large number of diseases in both humans and other species.

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Today, several examples are known of successful nanomedicines, with a variety of types of nanoparticle formulation. Examples from the field of vaccines include the lipid-

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nanoparticle SARS-CoV-2 directed vaccines Comirnaty, marketed by Pfizer/BioNTech, and SpikeVax, marketed by Moderna, that have been a tremendous commercial and humanitarian success. Another example is the Influenza-targeted oil-in-water emulsion of inactivated viruses Pandemrix (H1N1 Influenza) and Prepandrix (pre-pandemic Influenza), marketed by GlaxoSmithKline. Examples from the field of siRNA include, lipid-nanoparticles Onpattro (patisiran) for hereditary transthyretin-mediated amyloidosis, Givlaari (givosiran) for acute hepatic porphyria, and Oxlumo (lumasiran), all developed by Alnylam, for treatment of primary hyperoxaluria type 1. Other examples include liposomes such as Ambisome (liposomal amphotericin) for fungal infections, Visudyne (liposomal verteporfin) for macular degeneration, Abelcet (liposomal morphine) for pain treatment, Caelyx (liposomal doxorubicin) for cancer, Cimzia (Rha/b Fab against TNF- α), nanocrystals Lipidic (fenofibrate) for hypercholesterolaemia, Rapamune (liposomal sirolimus) against organ-rejection, nanosuspension Risperadol (risperidone nanosuspension) for Schizophrenia, and polymeric nanoparticles Renagel (sevelamer) for hyperphosphatemia and Copaxone (glatiramer acetate) for relapsing multiple sclerosis.

Despite the growing number of successful commercial applications of nanomedicines, several challenges remain. This is particularly true for the more advanced applications, such as the delivery of RNA or DNA. In such applications, there is a need for stable encapsulation or association of the cargo with the delivery vehicle in order to protect the cargo and shield its negative charge to allow entry over the negatively charged plasma- or endosomal membrane. Such encapsulation and/or association needs to be completely reversed upon the intended moment of release, but not before and also not later. As a consequence, stable, yet efficient delivery vehicles often have a very narrow range of compositions that function (sufficiently for therapeutic use).

Thus far, lipid nanoparticles with a solid lipid core, often referred to as LNPs or SNALPs (stable nucleic acid lipid particles), have shown a particularly high delivery efficiency in comparison to other nucleic acid delivery technologies and thus have been advanced furthest in clinical practise and commercial use. It is this technology which is used in the SARS-CoV-2 directed vaccines Comirnaty and SpikeVax. Such LNPs differ from liposomes due to the fact that only a small amount, if any, water is present in the core. Additionally, the LNPs possess a mostly solid lipid structure instead of the lipid bilayer that defines a liposome. Also for LNPs the lipids themselves complex and neutralize the negative charge of the RNA/DNA whereas for liposomes, the RNA/DNA has to be

complexed with a charge-neutralizing agent such as a positively charged polymer before encapsulation into the liposome.

For the solid lipid nanoparticles in clinical or commercial use, the lipid composition is remarkably similar. For instance, one of the most frequently used formulations for the delivery of mRNA has 50 mol% ionizable lipid (*e.g.* DLin-MC3-DMA), 38.5 mol% structural lipid (also referred to as core lipid, *e.g.* cholesterol), 10 mol% phospholipid or helper lipid (*e.g.* DSPC or DSPE), and 1.5 mol% shielding lipid (*e.g.* DMG-PEG2k), and slight variations thereof. In a large majority of cases, cholesterol, a cholesterol derivative or a structurally similar sterol is used as the core lipid. The cholesterol in the formulation is thought to contribute structural integrity, endosomal uptake through binding of ApoE and the ApoE receptors on the cell surface, to enhance membrane fusion (*e.g.* Tenchov B.G., MacDonald R.C., and Siegel D.P. *Biophysical J.* **91**, 2508-2515 (2006)), and thereby also endosomal escape, mostly through its crystalline form on the LNP surface. The importance of the role of cholesterol in the delivery of nucleic acids is underscored by the role of cholesterol and cholesterol transport proteins in the cytosolic delivery of viruses, as well as the enhanced gene delivery that can be achieved by using specific structural variants of cholesterol (Paunovska K et al. *ACS Nano* (2018)), such as the naturally occurring β -sitosterol, a C-24 alkyl cholesterol analogue (Patel S, et al. *Nat Commun* **11**, 983 (2020)).

Formulations and naturally occurring nanoparticles (*e.g.*, LDL particles) using significant amounts of cholesterol as structural lipid have been shown to bind the naturally occurring serum-protein, ApoE, in the bloodstream and possibly also other tissues. The natural role of ApoE is to bind low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) particles in the bloodstream and mediate binding to the LDL receptor(s), which are highly expressed on hepatocytes. Binding to the LDL receptor(s) results in clathrin-mediated endocytosis of the LDL particles, and endosomal cholesterol processing through NPC2 and NPC1, both of which have been shown to be involved in LNP processing (Akinc A. et al. *Mol. Ther.* **18**, 1357-1364 (2010) and Sahay G et al. *Nat. Biotechnol.* **31**, 653-658 (2013)).

Because of the very high density of LDL-receptors present on hepatocytes, in combination with the large volume of venous blood that passes through the liver, ApoE binding of cholesterol-based LNPs significantly affects their biodistribution, creating a strong bias in their biodistribution towards the liver. In fact, to this point, achieving

maximal ApoE binding and liver targeting was a major rationale in the further development of LNPs following the argument that if it cannot be prevented, then it should at least be maximized (Jayaraman M, et al. *Angew Chem Int Ed Engl.* 2012 Aug 20;51(34):8529-33.doi: 10.1002/anie.201203263). Intravenously administered LNPs, which interact first with blood components before reaching any tissue, may be significantly biased to the liver. For therapies relying on the release of the produced protein in the blood or bile, such as antibody treatments, the liver may be a suitable target organ with a high protein production capacity, making ApoE binding a convenient method of uptake. However, for any treatment, diagnostic or prophylactic that requires a high local concentration, intracellular delivery in a particular cell-type, or is toxic to the liver, a liver-biased biodistribution will decrease efficacy and/or safety. In such cases, to achieve the desired local concentration of the LNP and/or or expressed protein, a higher dose is required, increasing the chance and magnitude of side-effects, as well as elevating cost and potential bio-accumulation effects.

The conjugation of a drug formulation with Active active targeting moieties, such as antibodies, antibody fragments, DARPin, small molecules, peptides, and others, are a well-known method to induce preferential uptake in target cells that present a protein or other chemical entity on their surface that is specifically bound by the targeting moiety. The addition of an active targeting moiety to the outside of the delivery vehicle often results in an improved biodistribution towards the target cell. However, the magnitude of such improvements depends highly on the relative strength of the active targeting moiety to the relative strength of all other properties, including ApoE binding, that induce or facilitate uptake in other organs. Importantly, as long as the LNP formulation contains cholesterol, ApoE binding is difficult to prevent. For that very reason, shielding lipids, such as PEGylated DSPE are often included in the LNP formulation to prevent or reduce unwanted cellular and molecular interactions, and thus an improper biodistribution. However, the use of high amounts of PEG-lipid results in effective shielding, at the cost of delivery efficiency. Therefore, for the majority of LNP formulations currently in use, a balance is struck between shielding potency and effective delivery, resulting in partial availability of the cholesterol containing LNP surface for binding by ApoE and a liver-dominant bio-distribution upon intravenous administration. In certain LNP compositions, DMG-PEG2000 is used, which by virtue of its short singular lipid tail desorbs from the surface of the LNP and makes over time the surface available for adsorption of serum proteins, resulting in organ-specific uptake based on the properties of the surface. As so far, cholesterol (or a derivative) is always

included in the LNP formulation because of structural stability and delivery efficiency, cholesterol is an important determinant of the surface properties, and thus, cholesterol or cholesterol-derivative based LNPs are best suited for local application (e.g. intramuscular injection) and liver-targeted applications, whereas the majority of other
5 applications would benefit from a different core lipid with no ApoE binding propensity in combination with an active targeting moiety. Exceptions may be surface charge-based methods of biodistribution, targeting LNPs towards cells of the reticulo-endothelial system (RES) and uptake through scavenger receptors, as these interfere with/modify ApoE/serum protein binding while also having extensive and early interaction with
10 receptors after intravenous injection.

Recently, chemical adduct formation and breakdown of the RNA while packaged in the LNP, especially during prolonged storage, has come into view as a factor influencing the efficacy and safety of oligo- and poly-nucleotide therapies and prophylactics (Packer M,
15 et al. *Nat Commun* 12, 6777 (2021)). Besides reactive impurities arising from lipid synthesis, the pure, intended ingredients of the LNP can be a major contributor to adduct formation and degradative processes. Cholesterol is prone to oxidation due to its unsaturated bond at the D 5-6 position of the sterol nucleus (Maerker G, *Current status. J Am Oil Chem Soc* 64: 387-392 (1987)). During autoxidation, hydroperoxides and
20 oxidized cholesterol products, so-called oxysterols, are formed. The hydroperoxides may subsequently trigger the oxidation of nearby cholesterol or polyunsaturated fatty acids, and thus propagate or even accelerate the oxidation of the LNP. This process does not only impact the lipid properties of the LNP and by extension the endosomal escape efficiency, it also may affect the mRNA directly through hydrolysis. Multiple oxysterol
25 products with hydroxyl, ketone or epoxide groups at positions 4, 5, 6 or 7 of rings A and B of the cholesterol, are known to be formed through cholesterol oxidation. Especially the epoxide species are highly reactive towards a broad range of nucleophiles, including the 2-amino group of guanine in RNA and DNA.

30 In addition to direct effects on the LNP and mRNA, oxidation products from the lipid constituents of the LNP may directly or indirectly affect the target cell. Many LNP formulations cause a transitory increase in pro-inflammatory cytokines within a few hours after injection, which is due to its timeframe of this reaction attributed to the lipid components, rather than to the nucleic acid cargo of the LNPs (Lutz J et al. *npj Vaccines*
35 2 (2017)). In addition, the lipid components of LNPs were found to enhance pre-existing inflammatory or immune-challenged conditions through inflammation exacerbation

(IE) (Parhiz H et al. J. of Controlled Release 344: 50-61 (2021)). The ionizable lipid component was identified as the main driver of the pro-inflammatory response (Ndeupen S et al. iScience 24 (2021)), however, a contribution of oxidized cholesterol can not be excluded based on these findings. Importantly, oxidized cholesterol is a well-known pro-inflammatory signal, for example contributing to inflammation and subsequent plaque erosion in arteries in atherosclerosis. It is therefore, conceivable that oxidized cholesterol and fatty acids oxidized by the hydroperoxides originating from cholesterol autooxidation contribute to the pro-inflammatory effect by LNPs. Metabolization of oxidized cholesterol by monocytes may also induce long-term inflammatory effects via epigenetic reprogramming of monocytes into foam cells (Bekkering S. et al. Arterioscler Thromb Vasc Biol. 2014 Aug;34(8):1731-8). In addition, the formed epoxides may form carcinogenic adducts with endogenous RNA, DNA and proteins, affecting the viability and genomic integrity of the cell.

Solutions from the prior art to prevent oxidation, including protection of the drug substance/drug product from light, storage at low temperatures, and under a protective atmosphere (*e.g.* nitrogen or argon). These may prevent cholesterol from oxidation, especially when only low levels of oxidized lipids are present at the start. However, achieving such low levels of oxidized lipids through preventing oxidation might be extremely difficult, as LNPs are typically formed by mixing an organic solvent (such as ethanol) containing the lipid components with an excess of aqueous solution containing the oligo- or poly-nucleotide, and large volume dialysis by tangential flow filtration (TFF) is required to remove or dilute the organic solvent. Said large volumes may contain significant amounts of dissolved oxygen, and removing the oxygen poses a technical challenge.

Addition of antioxidants may be another effective strategy to prevent oxidation. However, addition of water-soluble antioxidants has limited effect on oxidation inside the lipid core of the LNP. Addition of hydrophobic antioxidants, including vitamin E may affect LNP functioning, and introduces additional complexity to the formulation and its quality control.

Solutions from the prior art to reduce the pro-inflammatory effect of LNPs rely on the use of corticosteroids (*e.g.* dexamethasone), either as a supplemental treatment or by incorporating the corticosteroid in the LNP itself, have shown to significantly reduce the inflammation and simultaneously increase expression of the nucleic acid encoded

protein (Zhang H et al. J Biomedical Materials Res 2022:1-8). However, dexamethasone and other corticosteroids display significant adverse effects including abdominal discomfort, skin rash, swelling, and hot flushes (Min KH et al. Korean J Audiol. 16:65-70 (2012)). By incorporating the dexamethasone into the LNP, a much lower dose can be
5 used and side-effects are reduced. The downside, however, is the addition of another component in the LNP composition, complicating manufacture, quality control and prescription.

Furthermore, cholesterol plays a major role in the stability, organization (*e.g.* through
10 lipid rafts) and functioning of plasma-membranes. If a substantial volume of LNPs is targeted at a given cell, as might be necessary for a given therapeutic or prophylactic effect, the cell may experience a transient increase in cholesterol, and plasma-membrane function may be altered. For example, it is known that in neuronal cells where plasma-membrane fluidity and lipid raft organization controls receptor density and location,
15 changes in cholesterol levels affect cognitive functioning (Egawa et al. J Physiol. 594(16): 4565-4579 (2016)).

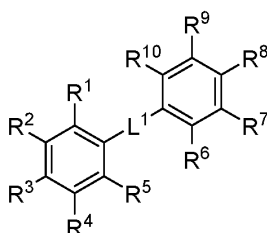
Similarly, cholesterol plays a role in the structural integrity of LNPs, by changing the fluidity of the LNP surface (Cheng X and Lee RJ. Adv. Drug Deliv. Rev. 99:129-137
20 (2016)). Cholesterol-free and cholesterol reduced LNPs were shown to be less stable and have a lower potency (Rodriguez et al. Biochemistry 34: 6208-6217 (1995) and Sato Y et al. Acta Biomater. 102: 341-350 (2020)). A higher physical stability of the nanoparticle is required to maintain performance-relevant formulation characteristics such as particle size, shape and low polydispersity, during post-processing steps (*e.g.* freeze drying and
25 concentration), storage and application (*e.g.* nebulization and spraying). However, even with the inclusion of cholesterol, nebulization resulted in increased nanoparticle sizes and decreased encapsulation efficiencies of the nucleic acid cargo (Zhang H et al. Pharmaceutics). No obvious trend was observed between particle stability in the context of nebulization and relative cholesterol content.

30 Therefore, to advance nucleic acid-based therapy, prophylaxis and diagnostics there is an urgent need for a novel core/structural lipid that can be substituted for cholesterol and has a combination of the following characteristics: maintains or enhances endosomal escape relative to cholesterol, offers a better control over biodistribution to
35 organs other than the liver, reduces pro-inflammatory signalling upon contact of cells with the LNP and imparts a better chemical and/or physical stability to the LNP. The

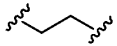
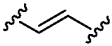
present invention arose from the inventors work in attempting to identify a novel core/structural lipid.

In accordance with a first aspect of the invention, there is provided the use of a

5 compound of formula (I):



(I)

, wherein L¹ is ,  or  ;

- 10 R¹ to R¹⁰ are each independently H, OR¹¹, OCOR¹¹, COOR¹¹, NR¹¹R¹², N⁺R¹¹R¹²R¹³, an optionally substituted C₁-₃₀ alkyl, an optionally substituted C₂-₃₀ alkenyl, an optionally substituted C₂-₃₀ alkynyl, an optionally substituted C₃-₁₀ cycloalkyl, an optionally substituted C₃-₁₀ cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle, an optionally substituted C₆-₁₂ aryl, an optionally substituted 5 to 10 membered
- 15 heteroaryl or a sugar moiety; and
- R¹¹ to R¹³ are each independently H, an optionally substituted C₁-₃₀ alkyl, an optionally substituted C₂-₃₀ alkenyl, an optionally substituted C₂-₃₀ alkynyl, an optionally substituted C₃-₁₀ cycloalkyl, an optionally substituted C₃-₁₀ cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle, an optionally substituted C₆-₁₂ aryl or an optionally
- 20 substituted 5 to 10 membered heteroaryl,
- or a pharmaceutically acceptable complex, salt, solvate, tautomeric form or polymorphic form thereof,
- in a micro- or nanoparticle formulation.

- 25 Advantageously, a compound of formula (I) may be used in an improved lipid nanoparticle composition, which exhibit one or more of the following characteristics; enhanced delivery of agent(s) to a target cell, increased physical stability, increased chemical stability, enhanced biodistribution when using an active targeting agent, and/or display less release of pro-inflammatory cytokines when on contact with a cell.

The lipid structures may be beneficially used in lipid-based nano- or micro-particle compositions for the delivery of (modified) RNA, DNA, peptides, or small molecules into mammalian cells.

- 5 Preferably, the compound of formula (I) is used as a core lipid in the micro- or nanoparticle formulation. It may be appreciated that a core lipid can also be referred to as a structural lipid.

10 The term “alkyl” as used herein, unless otherwise specified, refers to a saturated straight or branched hydrocarbon.

“Alkenyl” refers to olefinically unsaturated hydrocarbon groups which can be unbranched or branched, i.e. the hydrocarbon group contains one or more carbon-carbon double bonds. It may be appreciated that an alkenyl group may be partially
15 saturated. For instance, an alkenyl group could contain one or more double bonds in addition to a number of saturated single bonds.

“Alkynyl” refers to acetylenically unsaturated hydrocarbon groups which can be unbranched or branched, i.e. the hydrocarbon group contains one or more carbon-carbon triple bonds. In addition to containing one or more carbon-carbon triple bonds,
20 an alkynyl group may also contain one or more carbon-carbon double bond. It may be appreciated that an alkynyl group may be partially saturated. For instance, an alkynyl group could contain one or more triple bonds in addition to a number of saturated single bonds.

25 An alkyl, alkenyl and/or alkynyl, can be unsubstituted or substituted with one or more of halogen, $-NR^{14}R^{15}$, $-N^+R^{14}R^{15}R^{16}$, $-SR^{14}$, $-OR^{14}$, $-CN$, $-COR^{14}$, $-COOR^{14}$, $-OCOR^{14}$, $-CONR^{14}R^{15}$, $-NR^{14}SO_2R^{15}$, $-SO_2NR^{14}R^{15}$, $-NR^{14}COR^{15}$, $-OP(O)(OH)OR^{14}$, oxo, optionally substituted C_{3-6} cycloalkyl, optionally substituted C_{3-6} cycloalkenyl, optionally
30 substituted C_{6-12} aryl, optionally substituted 3 to 10 membered heterocycle or optionally substituted 5 to 10 membered heteroaryl, and R^{14} to R^{16} are each independently H, an optionally substituted C_{1-30} alkyl, an optionally substituted C_{2-30} alkenyl, an optionally substituted C_{2-30} alkynyl, an optionally substituted C_{3-10} cycloalkyl, an optionally substituted C_{3-10} cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle,
35 an optionally substituted C_{6-12} aryl or an optionally substituted 5 to 10 membered heteroaryl. R^{14} to R^{16} may each independently be selected from the group consisting of

H, C₁₋₁₂ alkyl, C₂₋₁₂ alkenyl, C₂₋₁₂ alkynyl, C₃₋₆ cycloalkyl, C₃₋₆ cycloalkenyl, C₆₋₁₂ aryl, 3 to 10 membered heterocycle or 5 to 10 membered heteroaryl.

“Cycloalkyl” refers to a non-aromatic, saturated, hydrocarbon ring system.
5 Representative examples of a C₃-C₆ cycloalkyl include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl. Similarly, “cycloalkenyl” refers to a non-aromatic, unsaturated, hydrocarbon 3 to 6 membered ring system.

“Aryl” refers to an aromatic hydrocarbon group. An aryl may be monocyclic, bicyclic or
10 multicyclic. The term “aryl” may be understood to encompass bicyclic ring systems where one of the rings is aromatic and one of the rings is unsaturated or partially saturated and multicyclic ring systems where at least one of the rings is aromatic and at least one of the rings is unsaturated or partially saturated. Examples of a C₆-C₁₂ aryl group include, but are not limited to, phenyl, α-naphthyl, β-naphthyl, biphenyl,
15 tetrahydronaphthyl and indanyl.

“Heterocycle” or “heterocyclyl” refers to 3 to 10 membered ring system in which at least one ring atom is a heteroatom. The or each heteroatom may be independently selected from the group consisting of oxygen, sulfur and nitrogen. A heterocycle may be saturated
20 or partially saturated. Exemplary heterocyclyl groups include but are not limited to aziridine, oxirane, oxirene, thiirane, pyrroline, pyrrolidine, dihydrofuran, tetrahydrofuran, dihydrothiophene, tetrahydrothiophene, dithiolane, piperidine, 1,2,3,6-tetrahydropyridine-1-yl, tetrahydropyran, pyran, morpholine, piperazine, thiane, thiine, piperazine, azepane, diazepane, oxazine.

25 “Heteroaryl” refers to an aromatic 5 to 10 membered ring system in which at least one ring atom is a heteroatom. A heteroaryl may be monocyclic, bicyclic or multicyclic. In bicyclic and multicyclic structures, a group may be understood to be a heteroaryl if at least one of the rings comprises a heteroatom. The term “heteroaryl” may be understood
30 to encompass bicyclic ring systems where one of the rings is aromatic and one of the rings is unsaturated or partially saturated and multicyclic ring systems where at least one of the rings is aromatic and at least one of the rings is unsaturated or partially saturated. The or each heteroatom may be independently selected from the group consisting of oxygen, sulfur and nitrogen. Examples of 5 to 10 membered heteroaryl groups include
35 furan, thiophene, indole, azaindole, oxazole, thiazole, isoxazole, isothiazole, imidazole, N-methylimidazole, pyridine, pyrimidine, pyrazine, pyrrole, N-methylpyrrole, pyrazole,

N-methylpyrazole, 1,3,4-oxadiazole, 1,2,4-triazole, 1-methyl-1,2,4-triazole, 1H-tetrazole, 1-methyltetrazole, benzoxazole, benzothiazole, benzofuran, benzisoxazole, benzimidazole, N-methylbenzimidazole, azabenzimidazole, indazole, quinazoline, quinoline, and isoquinoline. Bicyclic 5 to 10 membered heteroaryl groups include those
5 where a phenyl, pyridine, pyrimidine, pyrazine or pyridazine ring is fused to a 5 or 6-membered monocyclic heteroaryl ring.

Any cycloalkyl, cycloalkenyl, aryl, heterocycle and/or heteroaryl group can be unsubstituted or substituted with one or more of optionally substituted C₁-C₆ alkyl, optionally substituted C₁-C₆ alkenyl, optionally substituted C₁-C₆ alkynyl, halogen, -
10 NR¹⁴R¹⁵, -N⁺R¹⁴R¹⁵R¹⁶, -SR¹⁴, -OR¹⁴, -CN, -COR¹⁴, -COOR¹⁴, -OCOR¹⁴, -CONR¹⁴R¹⁵, -NR¹⁴SO₂R¹⁵, -SO₂NR¹⁴R¹⁵, -NR¹⁴COR¹⁵, -OP(O)(OH)OR¹⁴, oxo, optionally substituted C₃₋₆ cycloalkyl, optionally substituted C₃₋₆ cycloalkenyl, optionally substituted C₆₋₁₂ aryl, optionally substituted 3 to 10 membered heterocycle or optionally substituted 5 to 10
15 membered heteroaryl, and R¹⁴ to R¹⁶ are each independently H, an optionally substituted C₁₋₃₀ alkyl, an optionally substituted C₂₋₃₀ alkenyl, an optionally substituted C₂₋₃₀ alkynyl, an optionally substituted C₃₋₁₀ cycloalkyl, an optionally substituted C₃₋₁₀ cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle, an optionally substituted C₆₋₁₂ aryl or an optionally substituted 5 to 10 membered heteroaryl. R¹⁴ to R¹⁶ may each
20 independently be selected from the group consisting of H, C₁₋₁₂ alkyl, C₂₋₁₂ alkenyl, C₂₋₁₂ alkynyl, C₃₋₆ cycloalkyl, C₃₋₆ cycloalkenyl, C₆₋₁₂ aryl, 3 to 10 membered heterocycle or 5 to 10 membered heteroaryl.

A "sugar moiety" may be a monosaccharide, a disaccharide, a polysaccharide or a
25 derivative thereof.

The term "pharmaceutically acceptable salt" may be understood to refer to any salt of a compound provided herein which retains its biological properties and which is not toxic or otherwise undesirable for pharmaceutical use. Such salts may be derived from a
30 variety of organic and inorganic counter-ions well known in the art. Such salts include, but are not limited to: (1) acid addition salts formed with organic or inorganic acids such as hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, sulfamic, acetic, adipic, aspartic, trifluoroacetic, trichloroacetic, propionic, hexanoic, cyclopentylpropionic, glycolic, glutaric, pyruvic, lactic, malonic, succinic, sorbic, ascorbic, malic, maleic,
35 fumaric, tartaric, citric, benzoic, 3-(4-hydroxybenzoyl)benzoic, picric, cinnamic, mandelic, phthalic, lauric, methanesulfonic, ethanesulfonic, 1,2-ethane-disulfonic, 2-

hydroxyethanesulfonic, benzenesulfonic, 4-chlorobenzenesulfonic, 2-naphthalenesulfonic, 4-toluenesulfonic, camphoric, camphorsulfonic, 4-methylbicyclo[2.2.2]-oct-2-ene-1-carboxylic, glucoheptonic, 3-phenylpropionic, trimethylacetic, tert-butylacetic, lauryl sulfuric, gluconic, benzoic, glutamic, hydroxynaphthoic, salicylic, stearic, cyclohexylsulfamic, quinic, muconic acid and the like acids; or (2) base addition salts formed when an acidic proton present in the parent compound either (a) is replaced by a metal ion, e.g., an alkali metal ion, an alkaline earth ion or an aluminium ion, or alkali metal or alkaline earth metal hydroxides, such as sodium, potassium, calcium, magnesium, aluminium, lithium, zinc, and barium hydroxide, ammonia or (b) coordinates with an organic base, such as aliphatic, alicyclic, or aromatic organic amines, such as ammonia, methylamine, dimethylamine, diethylamine, picoline, ethanolamine, diethanolamine, triethanolamine, ethylenediamine, lysine, arginine, ornithine, choline, N,N'-dibenzylethylene-diamine, chlorprocaine, diethanolamine, procaine, N-benzylphenethylamine, N-methylglucamine piperazine, tris(hydroxymethyl)-aminomethane, tetramethylammonium hydroxide, and the like.

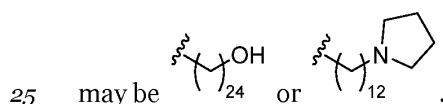
Pharmaceutically acceptable salts may include, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium and the like, and when the compound contains a basic functionality, salts of non-toxic organic or inorganic acids, such as hydrohalides, e.g. hydrochloride, hydrobromide and hydroiodide, carbonate or bicarbonate, sulfate or bisulfate, borate, phosphate, hydrogen phosphate, dihydrogen phosphate, pyroglutamate, saccharate, stearate, sulfamate, nitrate, orotate, oxalate, palmitate, pamoate, acetate, trifluoroacetate, trichloroacetate, propionate, hexanoate, cyclopentylpropionate, glycolate, glutarate, pyruvate, lactate, malonate, succinate, tannate, tartrate, tosylate, sorbate, ascorbate, malate, maleate, fumarate, tartarate, camsylate, citrate, cyclamate, benzoate, isethionate, esylate, formate, 3-(4-hydroxybenzoyl)benzoate, picrate, cinnamate, mandelate, phthalate, laurate, methanesulfonate (mesylate), methylsulphate, naphthylate, 2-napsylate, nicotinate, ethanesulfonate, 1,2-ethane-disulfonate, 2-hydroxyethanesulfonate, benzenesulfonate (besylate), 4-chlorobenzenesulfonate, 2-naphthalenesulfonate, 4-toluenesulfonate, camphorate, camphorsulfonate, 4-methylbicyclo[2.2.2]-oct-2-ene-1-carboxylate, glucoheptonate, 3-phenylpropionate, trimethylacetate, tert-butylacetate, lauryl sulfate, gluceptate, gluconate, glucoronate, hexafluorophosphate, hibenzate, benzoate, glutamate, hydroxynaphthoate, salicylate, stearate, cyclohexylsulfamate, quinate, muconate, xinofoate and the like.

R¹ may be H.

In some embodiments, R³ may be H.

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In alternative embodiments, R³ may be an optionally substituted C₁₋₃₀ alkyl, an optionally substituted C₂₋₃₀ alkenyl, an optionally substituted C₂₋₃₀ alkynyl, an optionally substituted C₃₋₁₀ cycloalkyl, an optionally substituted C₃₋₁₀ cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle, an optionally substituted C₆₋₁₂ aryl or an optionally substituted 5 to 10 membered heteroaryl. More preferably, R³ may be an optionally substituted C₆₋₂₆ alkyl, an optionally substituted C₆₋₂₆ alkenyl, an optionally substituted C₆₋₂₆ alkynyl, an optionally substituted C₃₋₆ cycloalkyl, an optionally substituted C₃₋₆ cycloalkenyl, an optionally substituted 3 to 6 membered heterocycle, an optionally substituted phenyl or an optionally substituted 5 or 6 membered heteroaryl. More preferably, R³ may be an optionally substituted C₁₂₋₂₄ alkyl, an optionally substituted C₁₂₋₂₄ alkenyl, an optionally substituted C₁₂₋₂₄ alkynyl, a C₃₋₆ cycloalkyl, a C₃₋₆ cycloalkenyl, a 3 to 6 membered heterocycle, a phenyl or a 5 or 6 membered heteroaryl. The alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heterocycle, aryl or heteroaryl may be unsubstituted or substituted with one or more substituents selected from OH, SH, NH₂, CN, oxo, C₃₋₆ cycloalkyl, C₃₋₆ cycloalkenyl, C₆₋₁₂ aryl, 3 to 10 membered heterocycle or 5 to 10 membered heteroaryl. Preferably, the alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heterocycle, aryl or heteroaryl is unsubstituted or substituted with one or more substituents selected from OH, SH, NH₂, CN, oxo, C₃₋₆ cycloalkyl, C₃₋₆ cycloalkenyl, phenyl, 5 or 6 membered heterocycle or 5 or 6 membered heteroaryl. Accordingly, R³



R⁵ may be H.

In some embodiments, R⁶ may be H.

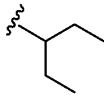
30

In alternative embodiments, R⁶ may be OR¹¹, OCOR¹¹, COOR¹¹, NR¹¹R¹² or N⁺R¹¹R¹²R¹³. More preferably, R⁶ is OR¹¹ or NR¹¹R¹². Most preferably, R⁶ is OR¹¹. R¹¹ to R¹³ may independently be H, an optionally substituted C₁₋₁₂ alkyl, an optionally substituted C₂₋₁₂ alkenyl, an optionally substituted C₂₋₁₂ alkynyl, an optionally substituted C₃₋₁₀ cycloalkyl,

an optionally substituted C₃₋₁₀ cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle, an optionally substituted C₆₋₁₂ aryl or an optionally substituted 5 to 10 membered heteroaryl. More preferably, R¹¹ to R¹³ may independently be H, a C₁₋₆ alkyl, a C₂₋₆ alkenyl or a C₂₋₆ alkynyl. Most preferably, R¹¹ to R¹³ are H. Accordingly, R⁶ may be OH.

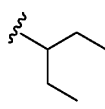
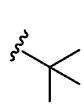
In some embodiments, R⁷ may be H.

In alternative embodiments, R⁷ may be OR¹¹, OCOR¹¹, COOR¹¹, NR¹¹R¹², N⁺R¹¹R¹²R¹³, an optionally substituted C₁₋₃₀ alkyl, an optionally substituted C₂₋₃₀ alkenyl, an optionally substituted C₂₋₃₀ alkynyl, an optionally substituted C₃₋₁₀ cycloalkyl, an optionally substituted C₃₋₁₀ cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle, an optionally substituted C₆₋₁₂ aryl or an optionally substituted 5 to 10 membered heteroaryl. More preferably, R⁷ may be OR¹¹, NR¹¹R¹², an optionally substituted C₁₋₁₂ alkyl, an optionally substituted C₂₋₁₂ alkenyl, an optionally substituted C₂₋₁₂ alkynyl, an optionally substituted C₃₋₆ cycloalkyl, an optionally substituted C₃₋₆ cycloalkenyl, an optionally substituted 3 to 6 membered heterocycle, an optionally substituted phenyl or an optionally substituted 5 or 6 membered heteroaryl. More preferably, R⁷ may be OR¹¹, a C₃₋₆ alkyl, a C₃₋₆ alkenyl or a C₃₋₆ alkynyl. R¹¹ to R¹³ may independently be H, an optionally substituted C₁₋₁₂ alkyl, an optionally substituted C₂₋₁₂ alkenyl, an optionally substituted C₂₋₁₂ alkynyl, an optionally substituted C₃₋₁₀ cycloalkyl, an optionally substituted C₃₋₁₀ cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle, an optionally substituted C₆₋₁₂ aryl or an optionally substituted 5 to 10 membered heteroaryl. More preferably, R¹¹ to R¹³ may independently be H, a C₁₋₆ alkyl, a C₂₋₆ alkenyl or a C₂₋₆ alkynyl. More preferably, R¹¹ to R¹³ are H or methyl, and most preferably methyl.

Accordingly, R⁷ may be OMe,  or .

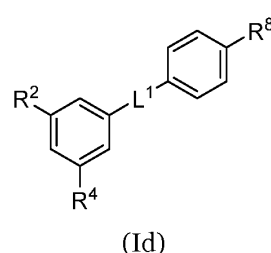
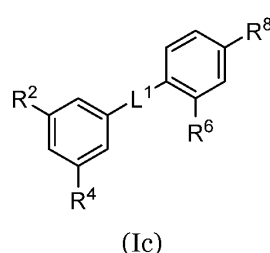
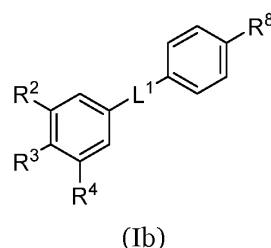
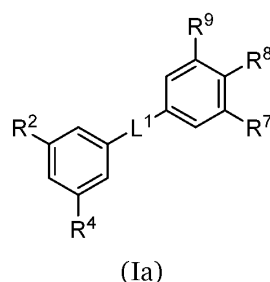
In some embodiments, R⁹ may be H. In alternative embodiments, R⁹ may be OR¹¹, OCOR¹¹, COOR¹¹, NR¹¹R¹², N⁺R¹¹R¹²R¹³, an optionally substituted C₁₋₃₀ alkyl, an optionally substituted C₂₋₃₀ alkenyl, an optionally substituted C₂₋₃₀ alkynyl, an optionally substituted C₃₋₁₀ cycloalkyl, an optionally substituted C₃₋₁₀ cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle, an optionally substituted C₆₋₁₂ aryl or an optionally substituted 5 to 10 membered heteroaryl. More preferably, R⁹ may be OR¹¹, NR¹¹R¹², an optionally substituted C₁₋₁₂ alkyl, an optionally substituted C₂₋₁₂ alkenyl, an optionally

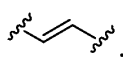
substituted C₂₋₁₂ alkynyl, an optionally substituted C₃₋₆ cycloalkyl, an optionally substituted C₃₋₆ cycloalkenyl, an optionally substituted 3 to 6 membered heterocycle, an optionally substituted phenyl or an optionally substituted 5 or 6 membered heteroaryl. More preferably, R⁹ may be OR¹¹, a C₃₋₆ alkyl, a C₃₋₆ alkenyl or a C₃₋₆ alkynyl. R¹¹ to R¹³ may independently be H, an optionally substituted C₁₋₁₂ alkyl, an optionally substituted C₂₋₁₂ alkenyl, an optionally substituted C₂₋₁₂ alkynyl, an optionally substituted C₃₋₁₀ cycloalkyl, an optionally substituted C₃₋₁₀ cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle, an optionally substituted C₆₋₁₂ aryl or an optionally substituted 5 to 10 membered heteroaryl. More preferably, R¹¹ to R¹³ may independently be H, a C₁₋₆ alkyl, a C₂₋₆ alkenyl or a C₂₋₆ alkynyl. More preferably, R¹¹ to R¹³ are H or methyl, and

most preferably methyl. Accordingly, R⁹ may be OMe,  or .

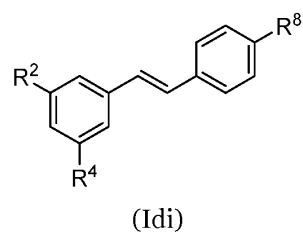
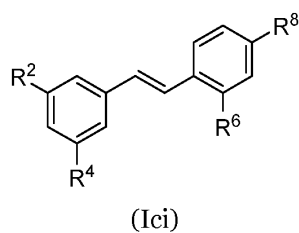
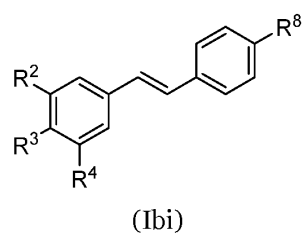
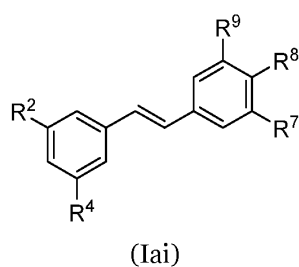
R¹⁰ may be H.

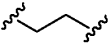
Accordingly, the compound may be a compound of formula (Ia), (Ib), (Ic) or (Id):



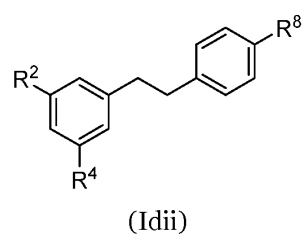
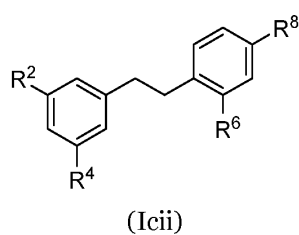
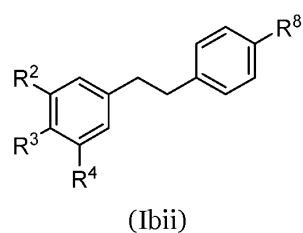
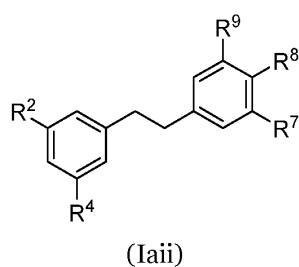
L¹ may be .

Accordingly, in some embodiments, the compound is a compound of formula (Iai), (Ibi), (Ici) or (Idi):



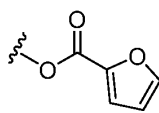
Alternatively, L¹ may be .

Accordingly, in alternative embodiments, the compound is a compound of formula (Iai),
 5 (Ibi), (Ici) or (Idi):

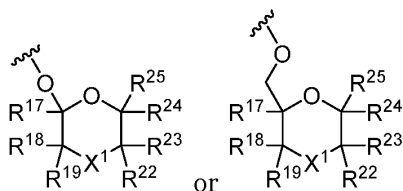


R², R⁴ and R⁸ may independently be H, OR¹¹, OCOR¹¹, COOR¹¹, NR¹¹R¹², N⁺R¹¹R¹²R¹³ or a sugar moiety. More preferably, R², R⁴ and R⁸ are independently H, OR¹¹, OCOR¹¹ or a sugar moiety.

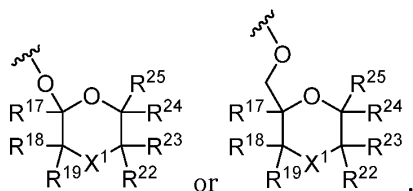
R¹¹ to R¹³ may each independently be H, an optionally substituted C₁₋₂₀ alkyl, an optionally substituted C₂₋₂₀ alkenyl, an optionally substituted C₂₋₂₀ alkynyl, an optionally substituted C₃₋₁₀ cycloalkyl, an optionally substituted C₃₋₁₀ cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle, an optionally substituted C₆₋₁₂ aryl or an optionally substituted 5 to 10 membered heteroaryl. More preferably, R¹¹ to R¹³ are independently H, a C₁₋₁₂ alkyl, a C₂₋₁₂ alkenyl, a C₂₋₁₂ alkynyl, a C₃₋₆ cycloalkyl, a C₃₋₆ cycloalkenyl, a 3 to 6 membered heterocycle, a phenyl or a 5 or 6 membered heteroaryl. More preferably, R¹¹ to R¹³ are independently H, a C₁₋₆ alkyl, a C₂₋₆ alkenyl, a C₂₋₆ alkynyl, a C₃₋₆ cycloalkyl, a C₃₋₆ cycloalkenyl, a 3 to 6 membered heterocycle, a phenyl or a 5 or 6 membered heteroaryl. More preferably, R¹¹ to R¹³ are independently H, a C₁₋₃ alkyl, a C₂₋₃ alkenyl, a C₂₋₃ alkynyl or a 5 or 6 membered heteroaryl. Most preferably, R¹¹ to R¹³ are independently H, methyl or a 5 membered heteroaryl.

Accordingly, R², R⁴ and R⁸ may independently be H, OH, OCH₃,  or a sugar moiety.

A sugar moiety may be understood to have general formula:



, wherein X¹ is a bond of CR²⁰R²¹; and R¹⁷ to R²⁵ are each independently H, OH, CH₂OH, COOH, NH₂, C₁₋₆ alkyl, halo,



In some embodiments, X¹ is a bond. Preferably, X¹ is CR²⁰R²¹.

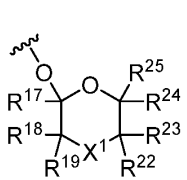
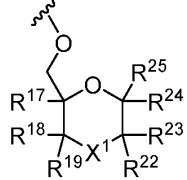
In some embodiments, R^{17} is H. Alternatively, R^{17} may be OH, CH_2OH , COOH or NH_2 .
 R^{17} may be OH or CH_2OH .

Preferably, R^{18} , R^{22} and R^{24} are H. Preferably, in embodiments where it is present, R^{20} is
 5 H.

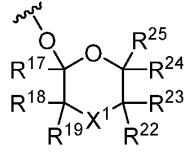
Preferably, R^{19} is H, OH or NH_2 , and more preferably are each OH.

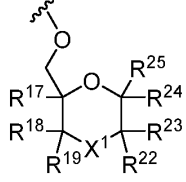
Preferably, in embodiments where it is present, R^{21} is H, OH or NH_2 , and more preferably
 10 is OH.

Preferably, R^{23} is , OH or NH_2 , and more preferably are each OH. Alternatively, R^{23} may

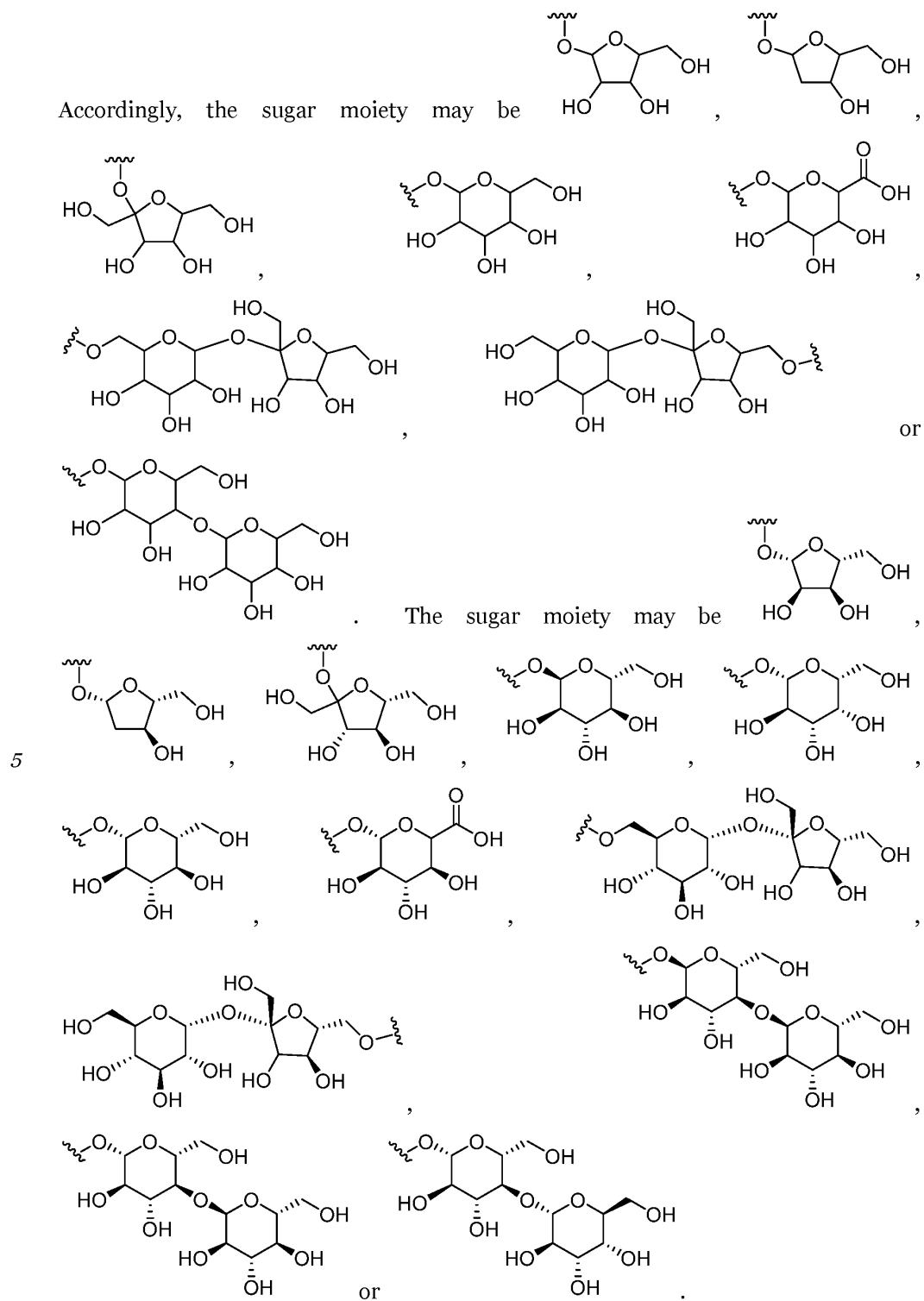
be  or . R^{17} to R^{25} in the additional sugar ring may be as
 defined herein.

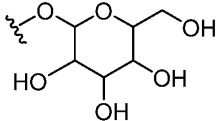
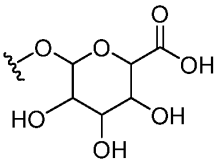
15

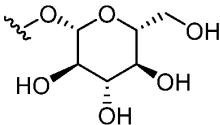
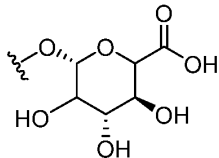
Preferably, R^{25} is CH_2OH or COOH. Alternatively, R^{25} may be  or

. R^{17} to R^{25} in the additional sugar ring may be as defined herein.

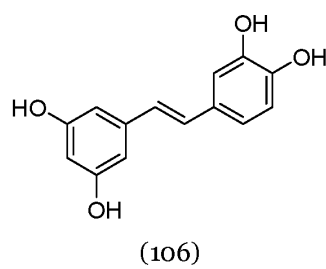
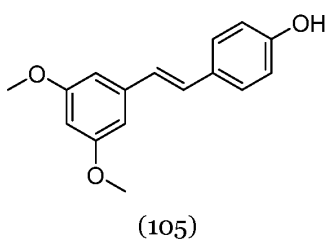
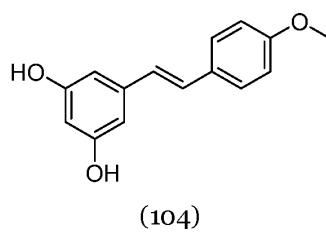
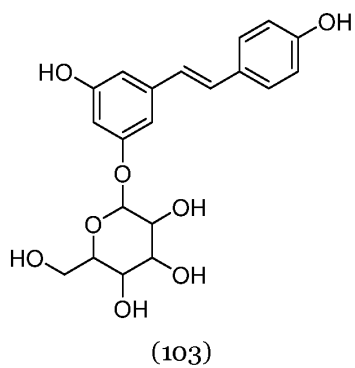
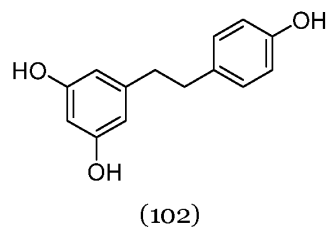
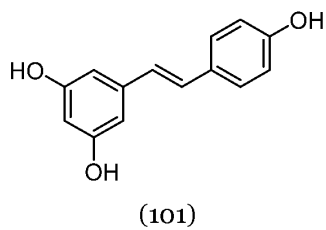
The sugar moiety may be a ribose moiety, a deoxyribose moiety, a fructose moiety, a
 20 glucose moiety, a galactose moiety, a sucrose moiety, a maltose moiety or a lactose
 moiety.

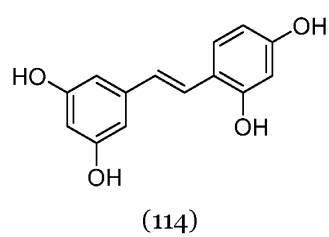
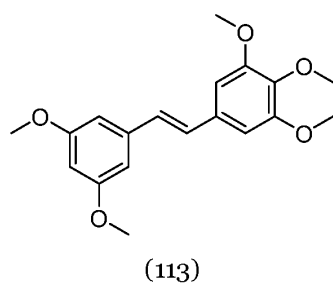
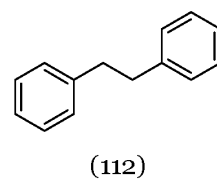
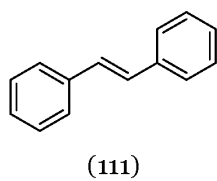
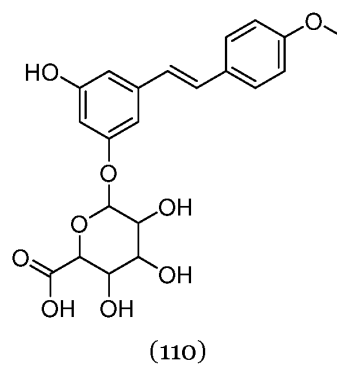
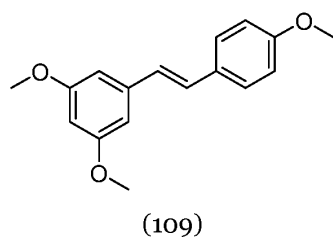
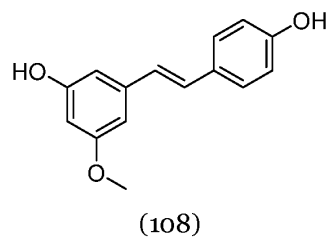
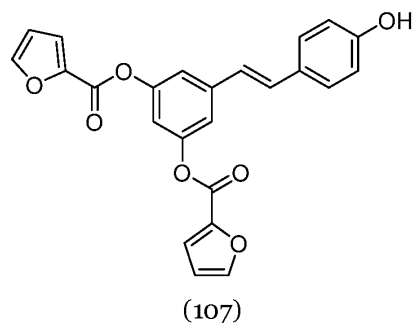


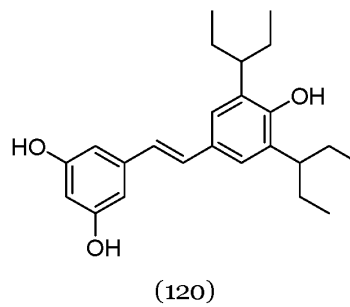
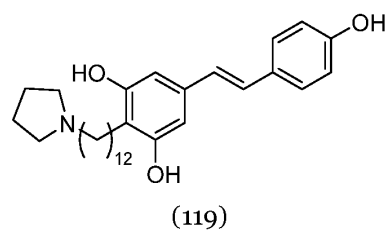
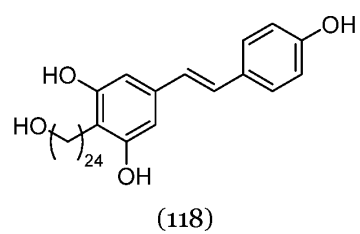
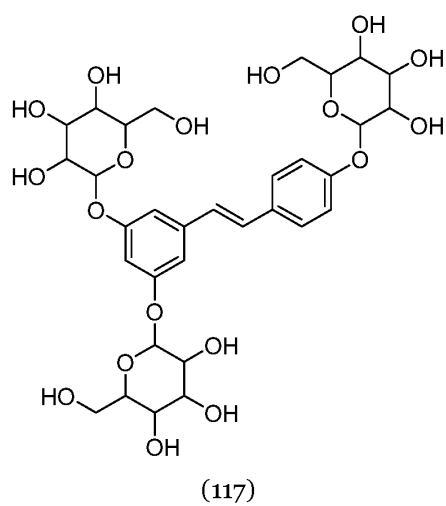
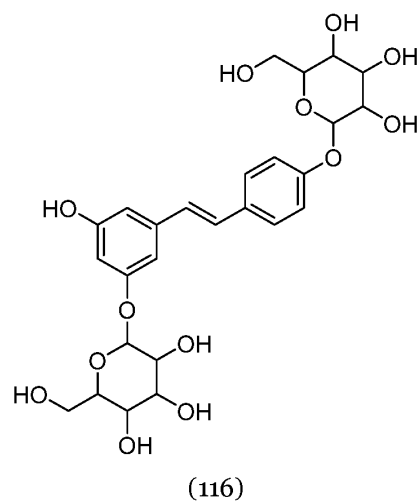
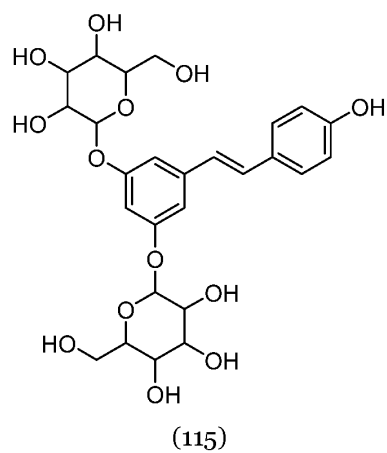
In some embodiments, the sugar moiety is  or .

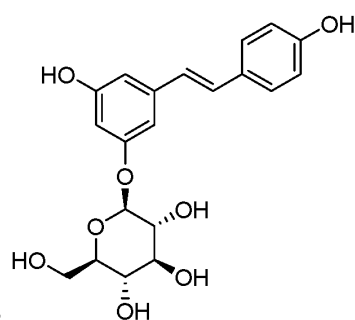
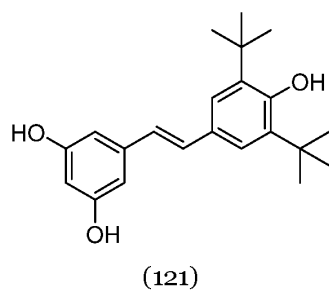
More preferably, the sugar moiety is  or .

The compound of formula (I) may be:

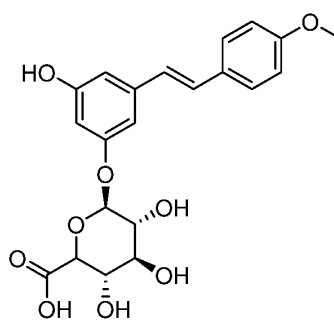






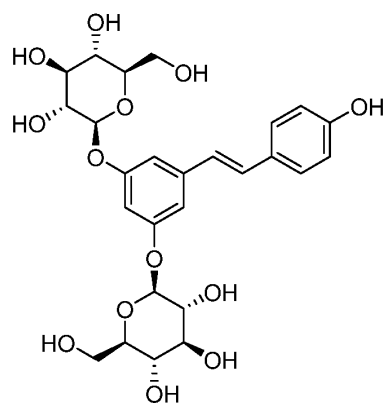


The compound of formula (103) may be .

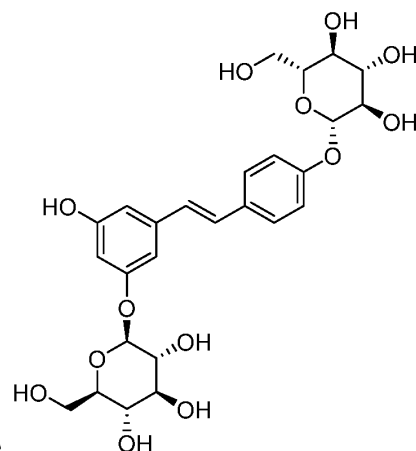


The compound of formula (110) may be .

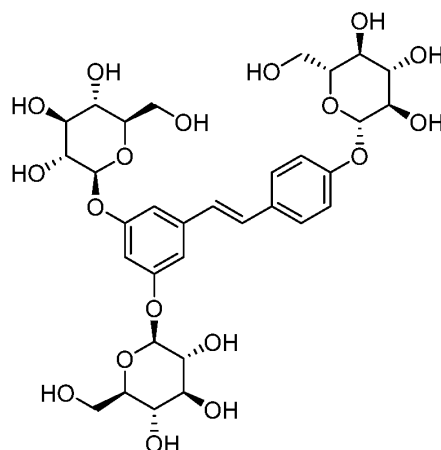
5



The compound of formula (115) may be .



The compound of formula (116) may be .



The compound of formula (117) may be .

- 5 In accordance with a second aspect of the invention, there is provided a micro- or nanoparticle comprising a compound of formula (I), as defined by the first aspect, or a pharmaceutically acceptable complex, salt, solvate, tautomeric form or polymorphic form thereof, and an optional payload molecule.
- 10 The micro- or nanoparticle may define a compact or hollow structure. The micro- or nanoparticle may define a solid structure. The micro- or nanoparticle may optionally consist of one or more lipid bilayers, which may be optionally crosslinked to each other, encapsulating a void. The micro- or nanoparticle may be a lipid nanoparticle (LNP), a liposome, a lipoplex, a micelle or a lipid vesicle. In some preferred embodiments, the
- 15 micro- or nanoparticle is an LNP.

The micro- or nanoparticle may have a diameter less than 10 μm , less than 1 μm , less than 500 nm or less than 250 nm. More preferably, the micro- or nanoparticle may have a diameter of less than 200 nm, less than 175 nm, less than 150 nm, less than 125 nm, less than 100 nm, less than 90 nm or less than 80 nm. The micro- or nanoparticle may
5 have a diameter between 25 nm and 1 μm , between 30 and 500 nm, between 35 and 250 nm, between 40 and 200 nm, between 45 and 150 nm, between 50 and 125 nm, between 55 and 100 nm, between 60 and 90 nm or between 65 and 80 nm. The diameter of the micro- or nanoparticle may be measured using dynamic light scattering.

10 In addition to the compound of formula (I), or a pharmaceutically acceptable complex, salt, solvate, tautomeric form or polymorphic form thereof, the micro- or nanoparticle may comprise one or more further lipids. Accordingly, the micro- or nanoparticle may comprise a lipid component comprising the compound of formula (I), or a pharmaceutically acceptable complex, salt, solvate, tautomeric form or polymorphic
15 form thereof, and one or more further lipids. The one or more further lipids may be selected from the group consisting of a phospholipid, a permanent cationic lipid, an ionisable cationic lipid, a permanent anionic lipid, an ionisable anionic lipid, a structural lipid, a shield lipid, a functionalised lipid, a further core lipid and combinations thereof.

20 In some embodiments, the lipid component comprises a compound of formula (I), or a pharmaceutically acceptable complex, salt, solvate, tautomeric form or polymorphic form thereof and a permanent or ionisable cationic lipid or a mixture thereof. In some embodiments, the lipid component of the micro- or nanoparticle formulation comprises a compound of formula (I), or a pharmaceutically acceptable complex, salt, solvate,
25 tautomeric form or polymorphic form thereof, a permanent or ionisable lipid and a shield lipid. In some embodiments, the lipid component of the micro- or nanoparticle formulation further comprises a phospholipid or helper lipid. In some embodiments, the lipid component of the micro- or nanoparticle formulation further comprises a functionalised lipid. In some embodiments, the lipid component of the micro- or
30 nanoparticle formulation further comprises a further core lipid.

The lipid component of the micro- or nanoparticle preferably comprises a core lipid component which comprises the compound of formula (I). In some embodiments, the core lipid component consists of the compound of formula (I). In alternative
35 embodiments, the core lipid component comprises a compound of formula (I) and a further core lipid. The core lipid component may comprise at least 5 mol% of the

compound of formula (I). The core lipid component may comprise at least 10 mol%, at least 20 mol %, at least 30 mol%, at least 40 mol% or at least 50 mol% of the compound of formula (I). The core lipid component may comprise at least 60 mol%, at least 70 mol %, at least 80 mol%, at least 90 mol% or 100 mol% of the compound of formula (I). The
5 core lipid component may comprise less than 100 mol% of the compound of formula (I). The core lipid component may comprise less than 95 mol%, less than 90 mol%, less than 80 mol%, less than 70 mol%, less than 60 mol% or less than 50 mol% of the compound of formula (I). The core lipid component may comprise less than 40 mol%, less than 30 mol%, less than 20 mol% or less than 10 mol% of the compound of formula (I). By
10 varying the amounts of the compound of formula (I) and the further core lipid, properties of the micro- or nanoparticle may be fine-tuned.

In some embodiments, the lipid component of the micro- or nanoparticle comprises between 5 to 80 mol%, between 10 to 60 mol%, between 20 to 50 mol%, between 30 and
15 45 mol% or between 35 to 40 mol% of the core lipid component.

Accordingly, in some embodiments, the lipid component of the micro- or nanoparticle comprises between 5 to 80 mol%, between 10 to 60 mol%, between 20 to 50 mol%, between 30 and 45 mol% or between 35 to 40 mol% of the compound of Formula (I), or
20 a pharmaceutically acceptable complex, salt, solvate, tautomeric form or polymorphic form thereof.

In some embodiments, the lipid component of the micro- or nanoparticle formulation comprises between 10 and 90 mol%, between 20 and 80 mol%, between 30 and 70 mol%,
25 between 40 and 60 mol% or between 45 and 55 mol% of the permanent cationic lipid, the ionisable cationic lipid, or a mixture thereof.

In some embodiments, the lipid component of the micro- or nanoparticle formulation comprises between 0 and 30 mol%, between 2.5 and 20 mol%, between 5 and 15 mol%
30 or between 7.5 and 12.5 mol% of the phospholipid.

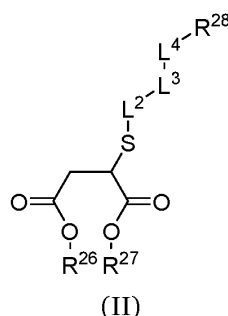
In some embodiments, the lipid component of the micro- or nanoparticle formulation comprises between 0 and 15 mol%, between 0.1 and 10 mol%, between 0.5 and 5 mol%, between 0.75 and 3 mol% or between 1 and 2 mol% of the shield lipid.

The further core lipid may be a sterol. The sterol may be cholesterol or a cholesterol derivative. The cholesterol derivative may be beta-sitosterol, Vitamin D2, Vitamin D3, Calcipotriol, Stigmasterol, Campesterol, Fucosterol, Brassicasterol, Ergosterol, 9,11-dehydroergosterol, Daucosterol, beta-Sitosterol-Acetate, Betutin, Lupeol, Ursolic acid,
5 or Oleanotic acid.

The phospholipid may be di-oleoyl-phosphatidylethanolamine (DOPE), di-oleoyl-phosphatidylcholine (DOPC), Di-oleoyl-phosphatidylserine (DOPS), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine
10 (DSPE) or any naturally occurring or synthetically derived phospholipid.

A shielding lipid may be understood to be a functionalized lipid that prevents the unwanted interaction of the lipid nanoparticle with other nanoparticles, extracellular compounds and/or cellular surfaces. The shielding lipid may prevent the unwanted
15 interaction of the lipid nanoparticle with other nanoparticles, extracellular compounds and/or cellular surfaces through steric hindrance or a similar mechanism. The shield lipid may be a lipid which has been modified to comprise a shielding polymer. The shielding polymer may be a polyethylene glycol (PEG) group, a poly-sarcosine group, an oligopeptide or a polypeptide, a hydroxyl-containing non-ionic water-soluble polymer,
20 polyvinylpyrrolidone (PVP), a poly(2-alkyl-2-oxazoline) or a zwitterionic polymer or any other suitable shielding polymer. The oligopeptide or polypeptide may be PAS. PAS may be understood to be an oligopeptide or polypeptide consisting or comprising proline, alanine and serine residues. The hydroxyl-containing non-ionic water-soluble polymer may be poly(glycerol) (PG), poly(N (2 hydroxypropyl)methacrylamide) (pHPMA),
25 polysarcosine, or poly(vinyl alcohol). The zwitterionic polymer may be a polybetaine. In some embodiments, the shield lipid may be a phospholipid which has been modified to comprise a shielding polymer. In embodiments where the shield lipid is a phospholipid which has been modified to comprise a shielding polymer, the phospholipid which is modified may be a phospholipid as defined above. In some embodiments the shield lipid
30 may be a DSPE, DMG or DPPC lipid which has been modified to comprise a shielding polymer. The shield lipid may comprise a shielding polymer with an average molecular weight of between 500 and 5000, between 1000 and 3000, between 1250 and 1750, between 1500 and 2500, between 1750 and 2250 Da or between 1900 and 2100 Da. The shield lipid may be di-myristoyl-glycerol-PEG (DMG-PEG), PEG-DSPE, PEG-di-palmitoyl-phosphatidyl-choline (DPPC), pSar-DMG, pSar-DSPE or pSar-DPPC. In some
35 embodiments, the shield lipid is PEG-DSPE or pSar-DSPE.

The permanent or ionisable cationic lipid may be 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLin-DMA), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (DLin-MC3-DMA), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), 1,2-Dioleoyl-3-trimethylammonium propane (DODAP), or a compound of formula (II):



wherein:

R²⁶ and R²⁷ are independently selected from the group consisting of optionally substituted C₁₋₅₀ alkyl, optionally substituted C₂₋₅₀ alkenyl or optionally substituted C₂₋₅₀ alkynyl;

L² and L⁴ are independently absent or is an optionally substituted C₁₋₁₀ alkylene, an optionally substituted C₂₋₁₀ alkenylene or an optionally substituted C₂₋₁₀ alkynylene;

L³ is absent or is NH, S or O;

R²⁸ is selected from the group consisting of -NR²⁹R³⁰, -N⁺R²⁹R³⁰R³¹, -H, -SR²⁹, -OR²⁹, -CN, -COR²⁹, -COOR²⁹, -OCOR²⁹, -CONR²⁹R³⁰, -NR²⁹SO₂R³⁰, -SO₂NR²⁹R³⁰, -NR²⁹COR³⁰, -OP(O)(OH)OR²⁹, optionally substituted C₃₋₆ cycloalkyl, optionally substituted C₃₋₆ cycloalkenyl, optionally substituted C₆₋₁₂ aryl, optionally substituted 3 to 10 membered heterocycle or optionally substituted 5 to 10 membered heteroaryl; and

R²⁹ to R³⁰ are independently selected from the group consisting of H, optionally substituted C₁₋₃₀ alkyl, optionally substituted C₂₋₃₀ alkenyl, optionally substituted C₂₋₃₀ alkynyl, optionally substituted C₃₋₆ cycloalkyl, optionally substituted C₃₋₆ cycloalkenyl, optionally substituted C₆₋₁₂ aryl, optionally substituted 3 to 10 membered heterocycle or optionally substituted 5 to 10 membered heteroaryl;

or a pharmaceutically acceptable complex, salt, solvate, tautomeric form or polymorphic form thereof.

Compounds of formula (II) are described in British patent application no. 2215200.3, which is incorporated herein by reference. Specific compounds of formula (II) disclosed in British patent application no. 2215200.3 are di((9Z,12Z)-octadeca-9,12-dien-1-yl) 2-
 5 ((2-(dimethylamino)ethyl)thio)succinate maleate, di((9Z,12Z)-octadeca-9,12-dien-1-yl) 2-((2-(dimethylamino)methyl)thio)succinate maleate, di((9Z,12Z)-octadeca-9,12-dien-1-yl) 2-((2-(dimethylamino)propyl)thio)succinate maleate, di((9Z,12Z)-octadeca-9,12-dien-1-yl) 2-((2-(methylethylamino)ethyl)thio)succinate maleate, di((9Z,12Z)-octadeca-9,12-dien-1-yl) 2-((2-(diethylamino)ethyl)thio)succinate maleate and dioleyl 2-((2-
 10 (diethylamino)ethyl)thio)succinate maleate.

Accordingly, the permanent or ionisable cationic lipid may be 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLin-DMA), 2,2-dilinoley-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl 4-
 15 (dimethylamino)butanoate (DLin-MC3-DMA), 2,2-dilinoley-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), 1,2-Dioleoyl-3-trimethylammonium propane (DODAP), di((9Z,12Z)-octadeca-9,12-dien-1-yl) 2-((2-(dimethylamino)ethyl)thio)succinate maleate, di((9Z,12Z)-octadeca-9,12-dien-1-yl) 2-
 20 ((2-(dimethylamino)methyl)thio)succinate maleate, di((9Z,12Z)-octadeca-9,12-dien-1-yl) 2-((2-(dimethylamino)propyl)thio)succinate maleate, di((9Z,12Z)-octadeca-9,12-dien-1-yl) 2-((2-(methylethylamino)ethyl)thio)succinate maleate, di((9Z,12Z)-octadeca-9,12-dien-1-yl) 2-((2-(diethylamino)ethyl)thio)succinate maleate or dioleyl 2-((2-(diethylamino)ethyl)thio)succinate maleate.

25

The permanent or ionisable anionic lipid may be cholesteryl hemisuccinate, a phosphatidylinositol phosphate (PIP, also known as phosphoinositide), phosphatidylserine (PS) or phosphatidic acid (PA).

30 The functionalised lipid may comprise one or more moieties which allow conjunction thereto. The one or more moieties may independently be selected from the group consisting of azide, alkyne, tetrazine, dibenzocyclooctyne (DBCO), maleimide, trans-cyclooctene (TCO), vinyl, methylcyclopropene and succinimidyl-ester.

In a preferred embodiment, the micro- or nanoparticle comprises a payload molecule. The payload molecule may be a biomolecule, and/or an active pharmaceutical ingredient (API), and/or a diagnostic compound.

- 5 The API may be a hydrophobic or hydrophilic API. The API may be a macromolecule or a small molecule. It may be appreciated that a small molecule could be considered to be a molecule with a molecular weight of less than 900 daltons. In some embodiments, a small molecule may have a molecular weight of less than 800 daltons, less than 700 daltons, less than 600 daltons, less than 500 daltons or less than 400 daltons. Similarly,
10 a macromolecule may be considered to be a molecule with a molecular weight of at least 900 daltons.

Examples of an API include an anti-inflammatory compound (e.g. non-steroidal anti-inflammatory drugs such as aspirin, ibuprofen, naproxen, celecoxib, diclofenac,
15 indomethacin, oxaprozin and/or piroxicam, more preferably steroidal inflammatory drugs such as prednisone, cortisone and methylprednisone, or anti-rejection drugs such as tacrolimus, cyclosporine, mycophenolate mofetil, azathioprine, rapamycin, sirolimus), an anti-cancer agent (e.g. chemotherapy such as alkylating agents (examples include Altretamine, Bendamustine, Busulfan, Carboplatin, Carmustine, Chlorambucil,
20 Cisplatin, Cyclophosphamide, Dacarbazine, Ifosfamide, Lomustine, Mechlorethamine, Melphalan, Oxaliplatin, Temozolomide, Thiotepe, Trabectedin), nitrosoureas (examples include carmustine, lomustine, streptozocin), antimetabolites (examples include Azacitidine, 5-fluorouracil (5-FU), 6-mercaptopurine (6-MP), Capecitabine, Cladribine, Clofarabine, Cytarabine (Ara-C), Decitabine, Floxuridine, Fludarabine, Gemcitabine,
25 Hydroxyurea, Methotrexate, Nelarabine, Pemetrexed, Pentostatin, Pralatrexate, Thioguanine, Trifluridine/tipiracil combination), anti-tumor antibiotics (examples include the anthracyclines Daunorubicin, Doxorubicin (Adriamycin), Doxorubicin liposomal, Epirubicin, Idarubicin, Valrubicin, and the anti-tumor antibiotics Bleomycin, Dactinomycin, Mitomycin-C, Mitoxantrone), topoisomerase inhibitors Irinotecan,
30 Irinotecan liposomal, Topotecan, Etoposide (VP-16), Mitoxantrone, Teniposide), mitotic inhibitors (examples include the Taxanes Cabazitaxel, Docetaxel, Nab-paclitaxel and Paclitaxel, and the Vinca alkaloids Vinblastine, Vincristine, Vincristine liposomal, Vinorelbine), and other chemotherapy drugs (examples include All-trans-retinoic acid, Arsenic trioxide, Asparaginase, Eribulin, Hydroxyurea, Ixabepilone, Mitotane,
35 Omacetaxine, Pegaspargase, Procarbazine, Romidepsin, Vorinostat)), cytokine drugs (including cytokines (examples include (recombinant versions of) IL-1, IL-2, TNF-alpha,

IL-6, IL-7, IL-10, IL-12, IL-17, IL-21, IL-22, IL-23, IFN-alpha, IFN-beta, IFN-gamma, IFN-lambda1, IFN-lambda2, IFN-lambda3, IFN-omega, IP-10, MIP-1alpha, TGF-beta(1-3)) and anti-cytokines (e.g. cytokine-binding antibodies, decoy receptors, IL-1R antagonist)), growth-factors (examples include bone morphogenetic proteins (BMPs),
5 vascular endothelial growth factor (VEGF), Granulocyte-macrophage colony-stimulating factor (GM-CSF), epidermal growth factor (EGF), erythropoietin (EPO), insulin-like growth factor (IGF), fiboblast growth factor (FGF), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF), thrombopoietin (TPO)), hormones (examples include aldosterone, vasopressin,
10 adrenocorticotrophic hormone (ACTH), luteinizing hormone(LH), follicle-stimulating hormone (FSH), oxytocin, prolactin, thyroid-stimulating hormone (TSH), renin, angiotensin, glucagon, insulin, estrogen, progesterone, parathyroid hormone (PTH), Thyroid hormone, Epinephrine, Norepinephrine, testosterone, melatonin, growth hormone releasing hormone (GHRH), Thyrotropic releasing hormone (TRH),
15 Gonadotropic releasing hormone (GnRH), corticotropin releasing hormone (CRH), humoral factors), cardiovascular medication (e.g. anti-coagulants (examples include Apixaban, Dabigatran, Edoxaban, Heaprin, Rivaroxaban and Warfarin), anti-platelet agents (examples include Aspirin, Clopidogrel, Dipyridamole, Prasugrel and Ticagrelor), Angiotensin-Converting Enzyme (ACE)-inhibitors (examples include Benazepril,
20 Captopril, Enalapril, Fosinopril, Lisinopril, Moexipril, Perindopril, Quinapril, Ramipril and Trandolapril), Angiotensin II receptor blockers (Azilsartan, Candesartan, Eprosartan, Irbesartan, Losartan, Olmesartan, Telmisartan, and Valsartan), beta-adrenergic blockers (Acebutolol, Atenolol, Betaxolol, Bisoprolol, METoprolol, Nadolol, Propranolol, and Sotalol), Calcium channel blockers (examples include Amlodipine,
25 Diltiazem, Felodipine, Nifedipine, Nimodipine, Nisoldipine, and Verapamil), cholesterol-lowering medication (examples include Statins Atorvastatin, Fluvastatin, Lovastatin, Pitavastatin, Pravastatin, Rosuvastatin and Simvastatin, Nitotinic acid Niacin, and cholesterol absorption inhibitor Ezetimibe), digitalis preparations (Digoxin), diuretics (examples include Acetazolamide, Amiloride, Bumetanide, Chlorothiazide,
30 Chlorthalidone, Furosemide, Hydro-chlorothiazide, Indapamide, Metalozone, Spironolactone, and Torsemide), and vasodilators (Isosorbide dinitrate, Isosorbide mononitrate, Hydralazine, Nitroglycerin and Minoxidil)), intestinal medication (e.g. Proton pump inhibitors (examples include Omeprazole, Lansoprazole, Rabeprazole, Esomeprazole, and Pantoprazole), Histamine2 blockers (examples include Cimetidine, Ranitidine, Famotidine, and Nizatidine), Promotility agents and laxatives (Metoclopramide)), eye medication (e.g. ocular allergy medicines (examples include

Ketorolac, Ketotifen, loteprednol, Bepotastine, Epinastine, Emedastine, Alcaftadine, Azelastine, Olopatadine, Nedocromil, Iodoxamide, and Cromolyn), topical antibiotics (examples include Besifloxacin, Ciprofloxacin, Moxifloxacin, Ofloxacin, Gatifloxacin, Tobramycin, Gentamycin, Polymyxin D, Neomycin, Bacitracin, Azithromycin, and
5 Erythromycin), lipid-based artificial tears (examples include castor oil, glycerol, and mineral oil), NSAIDS and corticosteroids, glaucoma drugs (examples include Levobunolol, Timolol, Betaxolol, Bimatoprost, Travoprost, Latanoprost, Tafluprost, Brimonidine, Brinzolamide, and Dorzolamide), and antiviral treatment (examples include Acyclovir, Valacyclovir, and Fanciclovir)), lung medication (e.g. anti-asthmatics
10 (examples include dyphylline, guaifenesin, Albuterol, Levalbuterol), anti-histamines (examples include Brompheniramine, Carbinoxamine, Chlorpheniramine, Clemastine, Diphenhydramine, Hydroxyzine, Tripolidine, Azelastine, Cetirizine, Desloratadine, Fexofenadine, Levocetirizine, Loratadine, Olopatadine), Antitussives (examples include Dextromethorphan and Benzonatate), bronchodilators (Ipratropium, Theophylline,
15 Albuterol, EpiNephine, Levalbuterol, Arformoterol, Formoterol, Olodaterol, Terbutaline, Pirbuterol, Metaproterenol, Salmeterol, Isoproterenol, Indacaterol, Tiotropium, Umeclidinium, Aclidinium, Ipratropium, Revafenacin, Glycopyrrolate, Ipratropium, Theophylline, Aminopylline and Dyphylline), decongestants (examples include Levmetamfetamine, Naphazoline, Oxymetazoline, Phenylephrine,
20 Propylhexedrine, Pseudoephedrine, and Xylometazoline), expectorants (Guaifenesin), leukotriene modifiers (examples include Montelukast, Zafirlukast, Zileuton), lung surfactants (examples include Beractant, Lucinactant, Calfactant and Poractant), mucolytics (acetylcysteine), anti-infectives (examples include Zanamivir, Ribavirin, Tobramycin, Pentamidine, and Colistimethate), inhaled corticosteroids (examples
25 include Fluticasone, Budesone, Mometasone, Beclomethasone, and Ciclesonide), mast-cell stabilizers (examples include Cromolyn and Nedocormil), and phosphodiesterase-4 inhibitors(including Roflumilast)), , anti-microbial medication (including antibiotics (e.g. aminoglycosides (examples including Amikacin, Gentamycin, Kanamycin, Neomycin, Netilmicin, Tobramycin, Paromomycin, Streptomycin and Spectinomycin),
30 Ansamycins (examples include Geldanamycin, Herbimycin, and Rifaximin), Carbacephem (for example Laracarbef), Carbapenems (examples include Ertapenem, Doripenem, Imipenem, and Meropenem), Cephalosporins (examples include Cefadroxil, Cefazolin, Cephadrine, Cephapirin, Cephalothin, Cefalexin, Cefaclor, Cefoxitin, Cefotetan, Cefamandole, Cefmetazole, Cefonicid, Cefprozil, Cefuroxime, Cefixime,
35 Cefdinir, Cefditoren, Cefoperazone, Cefotaxime, Cefpodoxime, Ceftazidime, Ceftibuten, Ceftizoxime, Maxalactam, Ceftriaxone, Cefepime, Ceftaroline fosamil, and Ceftobiprole),

Glycopeptides (examples include Teicoplanin, Vancomycin, Telavancin, Dalbavancin, and Oritavancin), Lincosamides (Clindamycin and Lincomycin), Lipopeptide (for example Daptomycin), Macrolides (Azithromycin, Clarithromycin, Erythromycin, Roxithromycin, Telithromycin, Spiramycin, and Fidaxomicin), Monobactams (for
5 example Aztreonam), Nitrofurans (for example Furazolidone and Nitrofurantoin), Oxazolidinones (examples include Linezolid, Posizolid, Radezolid, and Torezolid), Penicillins (examples include Amoxicillin, Ampicillin, Azlocillin, Dicloxacillin, Flucloxacillin, Mezlocillin, Methicillin, Nafcillin, Oxacillin, Penicillin G, Penicillin V, Piperacillin, Temocillin, and Ticarcillin), Polypeptides (examples include Bacitracin,
10 Cilistin and Polymyxin B), Quinolones (examples include Ciprofloxacin, Enoxacin, Gatifloxacin, Gemifloxacin, Levofloxacin, Lomefloxacin, Moxifloxacin, Nadifloxacin, Nalidixic acid, Norfloxacin, Ofloxacin, Trovafloxacin, Grepafloxacin, Sparfloxacin, and Temafloxacin), Sulfonamides (examples include Mafenide, Sulfacetamide, Sulfadiazine, Sulfadimethoxine, Sulfamethizole, Sulfamethoxazole, Sulfanilimide, Sulfasalazine,
15 Sulfisoxazole, and Sulfonamidochrysoidine), Tetracyclines (examples include Demeclocycline, Doxycycline, Metacycline, Minocycline, Oxytetracycline, and Tetracycline), mycobacterium-specific antibiotics (examples include Clofazimine, Dapsone, Capreomycin, Cycloserine, Ethambutol, Ethionamide, Isoniazid, Pyrazinamide, Rifampicin, Rifabutin, Rifapentine and Streptomycin)), anti-fungals (e.g.
20 Polyene antimycotics (examples include Amphotericin B, Candicidin, Filipin, Hamycin, Natamycin, Nystatin, and Rimocidin), Azoles (examples include Imidazoles Bifonazole, Butoconazole, Clotrimazole, Econazole, Fenticonazole, Isoconazole, Ketoconazole, Luliconazole, Miconazole, Omoconazole, Oxiconazole, Sertaconazole, Sulconazole and Tioconazole, Triazoles Albaconazole, Efinaconazole, Epoxiconazole, Fluconazole,
25 Isavuconazole, Itraconazole, Posaconazole, Propiconazole, Ravuconazole, Terconazole, and Voriconazole, and Thiazole (for example Abafungin), Allylamines (examples include Butenafine, naftifine and terbinafine), Echinocandins (Anidulafungin, Caspofungin, and Micafungin), and Triterpenoids (for example Ibrexafungerp)), and anti-parasite drugs (e.g. the broad-spectrum Nitazoxanide, antiprotozoals (examples include Melarsoprol,
30 Eflornithine, Metronidazole, Tinidazole, and Miltefosine), Antinematodes (examples include Mebendazole, Pyrantel pamoate, Thiabendazole, Diethylcarbamazine and Ivermectin), Anticestodes (examples include Niclosamide, Praziquantel, and Albendazole), antitrepatodes (for example Praziquantel), and Antiamoebics (for example Rifampicin and Amphotericin B))), anti-diabetic medication (e.g.
35 Insulin(analogues), Amylinomimetic drugs (for example Pramlintide), Alpha-glucosidase inhibitors (examples include Acarbose, and miglitol), Biguanides (e.g.

- metformin(analogues and combinations), Dopamine agonists (for example Bromocriptine), Dipeptidyl peptidase-4 (DDP-4) inhibitors (examples include Alogliptin, Linagliptin, Saxagliptin and Sitagliptin), Glucagon-like peptide-1 receptor agonists(examples include Albiglutide, Dualglutide, Exenatide, Liraglutide, and
- 5 Semaglutide), Meglitinides (examples include Nateglinide and Repaglinide), Sodium-glucose transporter (SGLT-2) inhibitors (examples include Dapagliflozine, Canagliflozine, Ertugliflozine and Empagliflozine), Sulfonylureas (examples include Glimepiride, Gliclazide, Glipizide, Glyburide, Chlorpropamide, Tolazamide and Tolbutamide), Thiazolidinediones (for example Rosiglitazone and Pioglitazone)), anti-
- 10 viral medication (examples include Abacavir, Acyclovir, Adefovir, Amntadine, Ampligen, Amprenavir, Umifenovir, Atazanavir, Atripla, Oseltamivir, Zanamivir, Peramivir, Baloxavir, Biktegravir, Emtricitabine, Tenofovir, Boceprevir, Bulevirtide, Cidofovir, Cobicistat, Daclatasvir, Darunavir, Delavirdine, Didanosine, Docosanol, Dolutegravir, Doravirine, Edoxudine, Efavirenz, Emtricitabine, Enfuvirtide, Ensivirtide, Ensitrelvir,
- 15 Entecavir, Entravirine, Famciclovir, Fomivirsen, Fosamprenavir, Foscarnet, Ganaciclovir, Ibacitabine, Ibalizumab, Idoxuridine, Imiquimod, Insoine pranobex, Indinavir, Lamivudine, Letemovir, Lopinavir, Loviride, Maraviroc, Methisazone, Moroxydine, Nelfinavir, Nevirapine, Nitazoxanide, Norvir, Penciclovir, Pleconaril, Podophyllotoxin, Raltegravir, Remdesivir, Ribavirin, Rilpivirine, Rimantadine,
- 20 Ritonavir, Saquinavir, Simeprevir, Sofosbuvir, Stavudine, Taribavirin, Telaprevir, Telbivudine, Tenofovir, Tiprenavir, Trifluridine, Trizivir, Tromantadine, Truvada, Umifenovir, Valaciclovir, Valganciclovir, Vicriviroc, Vidarabine, Zalcitabine, Zanamivir, and Zidovudine), or structural or functional analogues thereof.
- 25 In a preferred embodiment, the payload molecule is a biomolecule. For instance, the biomolecule may be or comprise an amino acid, a peptide, an affimer, a polypeptide or protein, a glycoprotein, a saccharide, a lipid, a lipopolysaccharide, an antibody or a fragment thereof, a polymer, or a nucleic acid, or a combination thereof.
- 30 The nucleic acid may be DNA, RNA, XNA (xeno nucleic acid, including 1,5-anhydrohexitol nucleic acid (HNA)), cyclohexene nucleic acid (CeNA), threose nucleic acid (TNA), glycol nucleic acid (GNA), locked nucleic acid (LNA), peptide nucleic acid (PNA), FANA (Fluoro Arabino nucleic acid) and unlocked nucleic acid (UNA), or a DNA/RNA hybrid sequence. Preferably, the nucleic acid is DNA or RNA.

Most preferably, the nucleic acid is RNA. The RNA may be single stranded or double stranded. The RNA may be selected from the group consisting of: messenger RNA (mRNA); circular RNA (circRNA or oRNA); self-amplifying RNA (saRNA); transamplifying RNA (taRNA), long non-coding RNA, split-replicon RNA, viral RNA,
5 antisense RNA (AON or asRNA); RNA aptamers; interference RNA; micro-RNA (miRNA); short interfering RNA (siRNA); short hairpin RNA (shRNA); and small RNA.

Preferably, the RNA is a messenger RNA (mRNA).

10 The nucleic acid sequence, preferably RNA, may be at least 10 bases in length, at least 20 bases in length, at least 50 bases in length, at least 100 bases in length, at least 200 bases in length, at least 300 bases in length, at least 400 bases in length, at least 500 bases in length, at least 600 bases in length at least 700 bases in length, at least 800 bases in length or at least 900 bases in length. In one preferred embodiment, the RNA is saRNA
15 or mRNA.

The nucleic acid sequence, preferably RNA, and most preferably mRNA, may be at least 100 bases in length, at least 500 bases in length, at least 1000 bases in length, at least 2000 bases in length, at least 3000 bases in length, at least 4000 bases in length, at least
20 5000 bases in length, at least 6000 bases in length, at least 7000 bases in length, at least 8000 bases in length, at least 9000 bases in length at least 10000 bases in length, at least 11000 bases in length or at least 12000 bases in length.

In one embodiment, the nucleic acid sequence is at least 6000 bases in length. In one
25 embodiment, the RNA is at least 6000 bases in length. In a preferred embodiment, the saRNA is at least 6000 bases in length.

In an alternative embodiment, the nucleic acid sequence is at least 900 bases in length. In one embodiment, the RNA is at least 900 bases in length. In a preferred embodiment,
30 the mRNA is at least 900 bases in length.

Alternatively, the nucleic acid sequence, preferably RNA, and most preferably mRNA, may be between 50 and 10000 bases in length, between 100 and 9000 bases in length, between 200 and 8000 bases in length, between 300 and 7000 bases in length, between
35 400 and 6000 bases in length, between 500 and 6000 bases in length, between 600 and

5000 bases in length, between 700 and 4000 bases in length, between 800 and 3000 bases in length or between 900 and 2000 bases in length.

5 In one embodiment, the nucleic acid sequence is between 6000 and 15000 bases in length. The nucleic acid sequence may be between 8000 and 12000 bases in length. The RNA may be between 6000 and 15000 bases in length. The RNA may be between 8000 and 12000 bases in length. Preferably, the saRNA is between 6000 and 15000 bases in length. Preferably the saRNA is between 8000 and 12000 bases in length.

10 In an alternative embodiment, the nucleic acid sequence is between 100 and 14000, between 500 and 10000, between 600 and 7500, between 700 and 5000, between 800 and 4000 or between 900 and 2000 bases in length. The RNA may be between 400 and 14000, between 500 and 10000, between 600 and 7500, between 700 and 5000, between 800 and 4000 or between 900 and 2000 bases in length. Preferably, the mRNA
15 is between 100 and 14000, between 500 and 10000, between 600 and 7500, between 700 and 5000, between 800 and 4000 or between 900 and 2000 bases in length.

The skilled person would appreciate that when the nucleic acid is double stranded, for example double stranded RNA, "bases in length" will refer to the length of base pairs.

20

The weight ratio of the lipid component to the payload molecule may be between 1:1 and 100:1, between 2:1 and 80:1, between 3:1 and 70:1, between 4:1 and 60:1 or between 5:1 and 50:1.

25 In embodiments where the payload molecule is a biomolecule, the weight ratio of the lipid component to the payload molecule may be between 6:1 and 45:1, between 8:1 and 40:1, between 10:1 and 35:1 or between 12:1 and 30:1. In some embodiments, the weight ratio of the lipid component to the payload molecule may be between 13:1 and 25:1 between 14:1 and 20:1 or between 15:1 and 17:1. In some embodiments, the weight ratio
30 of the lipid component to the payload molecule may be between 15:1 and 27.5:1, between 20:1 and 25:1 or between 22:1 and 23:1.

In embodiments where the payload molecule is a biomolecule, the N:P ratio may be between 1:2 and 50:1, between 1:1 and 30:1, between 2:1 and 20:1 between 3:1 and 15:1
35 or between 5:1 and 12:1. In some embodiments, the N:P ratio may be between 3:1 and 10:1 or between 4:1 and 6:1. In alternative embodiments, the N:P ratio may be between

4:1 and 10:1, between 5:1 and 9:1 or between 6:1 and 8:1. It may be understood that the N:P ratio is the ratio of positively-chargeable polymer amine (N) groups to negatively-charged nucleic acid phosphate (P) groups.

- 5 Preferably, the micro- or nanoparticle has an encapsulation efficiency of at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94% or at least 95%. It may be appreciated that encapsulation efficiency is determined by the amount of the payload molecule that is encapsulated in the micro- or nanoparticle (*i.e.*, not available and/or accessible for the aqueous environment outside
10 the micro- or nanoparticle) relative to the total amount of the payload molecule that was initially provided. The encapsulation efficiency may be determined using a RiboGreen-assay, which detects solvent accessible RNA by the increase in fluorescence upon intercalation of the water-soluble Ribogreen reagent with the RNA.
- 15 The micro- or nanoparticle may have a zeta-potential at physiological pH between -50 and +50 mV, between -40 and +40 mV, between -30 and +30 mV or between -20 and +20 mV, more preferably between -10 and +10 mV or between -5 and +5 mV. It may be appreciated that the zeta-potential may be measured by suspending the LNPs in an electrically conducting buffer with defined pH. The electrically conducting buffer may
20 be PBS (phosphate buffered saline pH7.2).

The micro- or nanoparticle may further comprise one or more adjuvants. The or each adjuvant may be selected from the group consisting of aluminium hydroxide, Pam2CSK4, Pam3CSK4, Glucopyranosyl Lipid adjuvant (GLA), LPS and analogues
25 thereof, CpG oligodeoxynucleotides and other TLR-agonists such as Poly I:C and dsRNA.

The micro- or nanoparticle may further comprise one or more additional compounds. The one or more additional compounds may be selected from the group consisting of a hydrophobic compound, a polymer, a permeability enhancer molecule, a carbohydrate,
30 a surface modifier, an excipient and combinations thereof. The polymer may be polylactic glycolic acid (PLGA). It may be appreciated that the excipient may change the pharmacokinetic properties of the composition but not the pharmacodynamic properties of the payload.

- 35 In accordance with a third aspect, there is provided a composition comprising a plurality of micro- or nanoparticles of the second aspect.

The micro- or nanoparticles may have an average diameter less than 10µm, less than 1 µm, less than 500 nm or less than 250 nm. More preferably, the micro- or nanoparticles may have an average diameter of less than 200 nm, less than 175 nm, less than 150 nm, less than 125 nm, less than 100 nm, less than 90 nm or less than 80 nm. The micro- or nanoparticles may have an average diameter between 25 nm and 1 µm, between 30 and 500 nm, between 35 and 250 nm, between 40 and 200 nm, between 45 and 150 nm, between 50 and 125 nm, between 55 and 100 nm, between 60 and 90nm or between 65 and 80 nm. The average diameter of the micro- or nanoparticles may be measured using dynamic light scattering.

The micro- or nanoparticles may have a polydispersity index (PDI) of less than 0.5, less than 0.4 or less than 0.3, and more preferably have a PDI of less than 0.25, less than 0.2, less than 0.15, less than 0.13 or less than 0.11. The micro- or nanoparticles may have a PDI of between 0.001 and 0.05, between 0.005 and 0.4, between 0.01 and 0.3, between 0.02 and 0.025, between 0.04 and 0.2, between 0.06 and 0.15, between 0.08 and 0.13 or between 0.09 and 0.11.

The composition may comprise a pharmaceutically acceptable carrier. The pharmaceutical acceptable carrier may improve colloidal stability, especially under concentrated and/or refrigerated conditions (*e.g.*, storage and/or shipment at a temperature between 4°C and -80°C, for example at 4°C, -20°C, or -70°C or -80°C). Such low temperature conditions may be used to extend the shelf life of the composition and/or more specifically the payload.

The composition may further comprise one or more solvents, a buffer, a suspension aid, a filler, a glidant, a binder, a salt, an isotonic agent, a thickening agent, an emulsifying agent and/or a preservative.

In a fourth aspect, there is provided the micro- or nanoparticle of the second aspect, or the composition of the third aspect, for use as a medicament.

In a fifth aspect, there is provided the micro- or nanoparticle of the second aspect, or the composition of the third aspect, for use in the treatment and/or prevention and/or prophylaxis of a disease or disorder.

In a sixth aspect, there is provided a method of treating and/or preventing a disease or disorder, the method comprising administering, or having administered, to a subject in need thereof, a prophylactic and/or therapeutic amount of the micro- or nanoparticle of the second aspect, or the composition of the third aspect.

5

The disease or disorder may be selected from the group consisting of inflammatory diseases, infectious diseases, proliferative diseases (*e.g.*, cancer), auto-immune diseases, eye diseases, lung diseases, skin diseases, intestinal diseases, metabolic diseases (*e.g.*, diabetes), vascular diseases (including cardiovascular and renovascular diseases),
10 neurological diseases (*e.g.*, neurodegenerative diseases), disorders of the endocrine system (including disorders related to hormones, growth factors, and/or cytokines), disorders of the reproductive system, and rare diseases.

In a seventh aspect, there is provided a vaccine composition comprising the micro- or
15 nanoparticle of the second aspect, or the composition of the third aspect.

The vaccine may comprise a suitable adjuvant.

In an eighth aspect, there is provided the micro- or nanoparticle of the second aspect, or
20 the composition of the third aspect or the vaccine of the seventh aspect, for use in stimulating an immune response in a subject.

The immune response may be stimulated against a protozoa, bacterium, virus, fungus, multicellular parasite, or cancer, or parts thereof.

25

In a ninth aspect, there is provided a method of vaccinating a subject, the method comprising administering, or having administered, to a subject in need thereof, a prophylactic and/or therapeutic amount of the micro- or nanoparticle of the second aspect, or the composition of the third aspect or the vaccine of the seventh aspect.

30

The micro- or nanoparticle, the composition or the vaccine of the invention may be combined in compositions having a number of different forms depending, in particular, on the manner in which the composition is to be used. Thus, for example, the composition may be in the form of a powder, tablet, capsule, liquid, ointment, cream,
35 shampoo, gel, hydrogel, aerosol, spray, micellar solution, transdermal patch (including microneedles), drug-depot/slow-release formulations, (liposome) suspension,

wash/instillation, incorporated in a biomaterial for regenerative medicine, a coating of (implantable) medical devices, or any other suitable form that may be administered to a person or animal in need of treatment. It will be appreciated that the vehicle of medicaments according to the invention should be one which is well-tolerated by the
5 subject to whom it is given.

The micro- or nanoparticle, the composition or the vaccine of the invention may also be incorporated within a slow- or delayed-release device. Such devices may, for example, be inserted on or under the skin, and the medicament may be released over weeks or
10 even months. The device may be located at least adjacent to, or upstream of the treatment site.

In a preferred embodiment, however, medicaments according to the invention may be administered to a subject by injection into the blood stream, muscle, skin or directly into
15 a site requiring treatment. Injections may be intravenous (bolus or infusion), subcutaneous (bolus or infusion), intradermal (bolus or infusion), intramuscular (bolus or infusion), intrathecal (bolus or infusion), intravitreal (bolus), epidural (bolus or infusion) or intraperitoneal (bolus or infusion).

It will be appreciated that the amount of micro- or nanoparticle, the composition or the vaccine that is required is determined by its intended therapeutic or prophylactic use and its biological activity and bioavailability, which in turn depends on the mode of administration, the physiochemical properties of the payload, the micro- or nanoparticle, the composition or the vaccine and whether it is being used as a monotherapy or in a
20 combined therapy.
25

The frequency of administration will also be influenced by the half-life of the active agent and/or the half-life of the therapeutic effect (e.g., the half-life of the therapeutic protein translated from the therapeutic mRNA transfected with the micro- or nanoparticle
30 composition, or e.g. the half-life of RNA-based CRISPR gene-therapy) within the subject being treated. Optimal dosages to be administered may be determined by those skilled in the art, and will vary with the micro- or nanoparticle, the composition or the vaccine in use, the strength of the pharmaceutical composition, the mode of administration, and the type of treatment. Additional factors depending on the particular subject being
35 treated will result in a need to adjust dosages, including subject age, weight, gender, diet, and time of administration.

The required dose may depend upon a number of factors including, but not limited to, the active agent being administered, the disease being treated and/or vaccinated against, the subject being treated, etc.

5

Generally, a dose of between 0.001 µg/kg of body weight and 10 mg/kg of body weight, or between 0.01 µg/kg of body weight and 1 mg/kg of body weight, of the micro- or nanoparticle, the composition or the vaccine of the invention may be used, depending upon the active agent used. A dose may be understood to relate to the quantity of the payload molecule which is delivered.

10

Doses may be given as a single administration (e.g., a single injection). Alternatively, the micro- or nanoparticle, the composition or the vaccine may require more than one administration. As an example, the micro- or nanoparticle, the composition or the vaccine may be administered as two or more doses of between 0.07 µg and 700 mg (i.e., assuming a body weight of 70 kg). Alternatively, a slow-release device may be used to provide optimal doses of the micro- or nanoparticle, the composition or the vaccine according to the invention to a patient without the need to administer repeated doses. Routes of administration may incorporate intravenous, intradermal subcutaneous, intramuscular, intrathecal, epidural, intravitreal, or intraperitoneal routes of injection.

15

20

Known procedures, such as those conventionally employed by the pharmaceutical industry (e.g., *in vivo* experimentation, clinical trials, etc.), may be used to form specific formulations of the micro- or nanoparticle, the composition or the vaccine according to the invention and precise therapeutic regimes (such as doses of the agents and the frequency of administration).

25

A “subject” may be a vertebrate, mammal, or domestic animal. Hence, compositions and medicaments according to the invention may be used to treat any mammal, for example livestock (e.g., a horse), pets, or may be used in other veterinary and agricultural applications. Most preferably, however, the subject is a human being.

30

A “therapeutically effective amount” of the micro- or nanoparticle, the composition or the vaccine is any amount which, when administered to a subject, is the amount of the aforementioned that is needed to produce a therapeutic effect, whether partial or full.

35

For example, a therapeutically effective amount of the micro- or nanoparticle, the composition or the vaccine of the invention may comprise from about 0.001 µg to about 800 mg of the payload molecule, and preferably from about 0.01 mg to about 500 mg of the payload molecule.

5

A “pharmaceutically acceptable vehicle” as referred to herein, is any known compound or combination of known compounds that are known to those skilled in the art to be useful in formulating pharmaceutical compositions.

10 In one embodiment, the pharmaceutically acceptable vehicle may be a solid, and the composition may be in the form of a powder, a capsule or tablet. A solid pharmaceutically acceptable vehicle may include one or more substances which may also act as flavouring agents, lubricants, solubilisers, suspending agents, dyes, fillers, glidants, compression
15 aids, inert binders, sweeteners, preservatives, dyes, coatings, or tablet-disintegrating agents. The vehicle may also be an encapsulating material. In powders, the vehicle is a finely divided solid that is in admixture with the finely divided active agents according to the invention. In tablets, the active agent (e.g., micro- or nanoparticle of the invention) may be mixed with a vehicle having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The pharmaceutical vehicle
20 may be a gel and the composition may be in the form of a cream or the like.

Alternatively, the pharmaceutical vehicle may be a liquid, and the pharmaceutical composition is in the form of a solution. Liquid vehicles are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The micro- or
25 nanoparticle according to the invention may be dissolved or suspended in a pharmaceutically acceptable liquid vehicle such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid vehicle can contain other suitable pharmaceutical additives such as solubilisers, emulsifiers, buffers, preservatives, sweeteners, flavouring agents, suspending agents, thickening agents,
30 colours, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid vehicles for oral and parenteral administration include water (partially containing additives as above, e.g., cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g., glycols) and their derivatives, and oils (e.g., fractionated coconut oil and arachis oil). For
35 parenteral administration, the vehicle can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid vehicles are useful in sterile liquid form compositions

for parenteral administration. The liquid vehicle for pressurized compositions can be a halogenated hydrocarbon or other pharmaceutically acceptable propellant.

Liquid pharmaceutical compositions, which are sterile solutions or suspensions, can be utilized by, for example, intramuscular, intrathecal, intravitreal, epidural, intraperitoneal, intravenous and subcutaneous injection. The micro- or nanoparticle of the invention may be prepared as any appropriate sterile injectable medium.

The micro- or nanoparticle may be administered by inhalation. For instance, the micro- or nanoparticle may be provided in the form of an aerosol.

The micro or nanoparticle and/or the composition of the invention may be administered orally in the form of a sterile solution or suspension containing other solutes or suspending agents (for example, enough saline or glucose to make the solution isotonic), bile salts, acacia, gelatin, sorbitan monoleate, polysorbate 80 (oleate esters of sorbitol and its anhydrides copolymerized with ethylene oxide) and the like. The micro or nanoparticle and/or the composition according to the invention can also be administered orally either in liquid or solid composition form. Compositions suitable for oral administration include solid forms, such as pills, capsules, granules, tablets, and powders, and liquid forms, such as solutions, syrups, elixirs, and suspensions. Forms useful for parenteral administration include sterile solutions, emulsions, and suspensions.

All of the features described herein (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined with any of the above aspects in any combination, except combinations where at least some of such features and/or steps are mutually exclusive.

For a better understanding of the invention, and to show how embodiments of the same may be carried into effect, reference will now be made, by way of example, to the accompanying Figures, in which:-

Figure 1 shows the average diameter and polydispersity index (PDI) of lipid nanoparticle (LNP) formulations measured with dynamic light scattering (DLS), where the lipid component of the LNP comprised 50 mol% ionizable lipid, 38.5 mol% structural lipid, 10 mol% phospholipid and 1.5 mol% PEG-modified lipid, and the structural lipid

was either resveratrol (RSV), cholesterol (chol), or a combination thereof as indicated in Figure 1, where the ratios of combinations are molar ratios;

Figure 2 shows the average diameter and PDI of LNP formulations with 5 mol% DOTAP included as uptake enhancer measured with dynamic light scattering, such that the lipid component of the LNP comprised 45 mol% ionizable lipid, 5 mol% DOTAP, 38.5 mol% structural lipid, 10 mol% phospholipid and 1.5 mol% PEG-modified lipid, and the structural lipid was either resveratrol (RSV), cholesterol (chol), or a combination thereof as indicated in Figure 1, where the ratios of combinations are molar ratios;

Figure 3 shows the pKa of various LNP formulations determined by a 2-(p-toluidino) naphthalene-6-sulfonic acid (TNS)-assay at 20°C over a pH range of 3-10 in 0.5 pH increments, where the LNP formulations contained different structural lipids or mixtures thereof, all percentages are molar percentages;

Figure 4 shows the encapsulation efficiency of LNP compositions prepared with various core lipids and core lipid mixtures as determined by RiboGreen assay; the core lipids are either resveratrol (RSV), cholesterol or a mixture thereof, or a resveratrol derivative or related structure, all percentages are molar percentages;

Figure 5 shows toxicity testing of LNP formulations containing different core lipids and core lipid mixtures as determined by resazurin assay. (A) shows the effect of resveratrol and cholesterol separately, and in a mixture, on the metabolic activity of HeLa cells, all ratios are molar ratios; and (B) shows the effect of resveratrol derivatives and related structures on the toxicity of LNP formulations containing said structures as a core lipid;

Figure 6 provides *in vitro* activity of LNP compositions containing different core lipids and core lipid mixtures as measured by the secreted nano-luciferase assay. (A) shows the effect of resveratrol and cholesterol separately, and in a mixture, on the transfection efficiency of LNP formulations containing said structures as core lipid, all ratios are molar ratios; and (B) shows the effect of resveratrol derivatives and related structures on the transfection efficiency of LNP formulations containing said structures as the core lipid; and

Figure 7 demonstrates the lack of ApoE-mediated uptake and functional delivery in HeLa cells, transfection was performed in the presence of various amounts of ApoE, freshly added to the medium, transfection efficiency was subsequently determined by secreted nano-luciferase assay.

Examples

Example 1: Production of Lipid Nano Particles (LNPs)

To determine the safety and efficacy of the lipid compositions of the invention for the delivery of a therapeutic and/or prophylactic molecule to cells, a range of formulations
5 were prepared and tested.

Uniformly sized nanoparticles were produced reproducibly with an inverted herringbone microfluidic mixer. Said mixer rapidly (in the order of milliseconds) mixes the aqueous fluid generally containing the water soluble therapeutic and/or prophylactic molecule
10 with an organic solvent containing the pre-mixed lipid components. Other types of microfluidic mixers (*e.g.* Y-junction, T-junction or direct high-speed injection) produced similar results, as long as a similar mixing ratio between fluid streams and mixing speed was obtained.

15 Lipid compositions were prepared by combining core/structural lipids (each obtained from Sigma-Aldrich) with ionizable lipid L1 (di((9Z,12Z)-octadeca-9,12-dien-1-yl) 2-((2-(dimethylamino)ethyl)thio)succinate maleate, disclosed in British patent application GB2215200.3), a phospholipid (such as DOPE, obtained from Avanti Polar Lipids), optionally a cationic lipid (such as DOTAP, obtained from Avanti Polar Lipids), a PEG-
20 modified lipid (such as 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (polyethyleneglycol)-2000 (also known as PEG-DSPE), obtained from Avanti Polar Lipids). Control formulations contained cholesterol (obtained from Sigma-Aldrich) as the structural lipid. The lipids were typically combined in a ratio of 45 mol% ionizable lipid, 5 mol% DOTAP, 38.5 mol% structural lipid, 10 mol% phospholipid and 1.5 mol%
25 PEG-modified lipid. For formulations not containing DOTAP, the lipids were combined in a ratio of 50 mol% ionizable lipid, 38.5 mol% structural lipid, 10 mol% phospholipid and 1.5 mol% PEG-modified lipid.

Certain formulations display low generic cellular uptake *in vitro* and/or *in vivo*, which
30 can be overcome by the incorporation of cell-receptor or cell-membrane targeting ligands, such as natural ligands or antibodies directed against cell-surface receptors. Alternatively, increasing the zeta-potential of the nanoparticle composition to at least +1mV, but preferably +10mV, results in generic interaction with the plasma-membrane, resulting in uptake of the nanoparticle and/or cargo. In such cases, the following lipid
35 ratio was used: 40-50 mol% ionizable lipid, 0-10 mol% cationic lipid, 38.5 mol% structural lipid, 10 mol% phospholipid and 1.5 mol% PEG-modified lipid.

The lipid mixture was diluted with ethanol to a final concentration of between 12.5mM total lipid and 50mM total lipid. Lipids dissolved in ethanol were stored at -20°C, under argon, protected from light.

5

Nanoparticle compositions were made by combining a therapeutic and/or prophylactic molecule in acidic (pH 4 or 5) or neutral (pH 7.4) aqueous solution with the lipid mix in ethanol at a lipid to therapeutic molecule weight ratio between 5:1 and 50:1. To produce a well-defined nanoparticle population, the aqueous solution comprising the therapeutic and/or prophylactic molecule ("the aqueous solution") and the lipid solution were rapidly mixed at a volumetric ratio of about 2:1 (aqueous solution:lipid solution) to about 5:1 (aqueous solution:lipid solution) in an inverted herringbone microfluidic mixer at total flow rates in between 10ml/min and 18 ml/min.

15 To make nanoparticle compositions containing RNA, the RNA was diluted to around between 0.1mg/ml and 3mg/ml, preferably 0.15mg/ml in 100mM sodium citrate buffer at pH 4 to 5 and subsequently mixed with the lipid mixture. The RNA to lipid weight was typically between 1:10 and 1:30, resulting in an N:P ratio of between 3 and 12, preferably about 5.

20

Subsequent to mixing the aqueous and lipid solutions, the nanoparticle composition was dialyzed to remove ethanol to below 0.1 vol%, optionally concentrate or dilute the solution, and exchange the buffer for a buffer of physiological pH (*e.g.* pH 7.4, for example phosphate buffered saline (PBS)). Formulations were dialyzed three times against at least a 100-fold excess of PBS using 100kDa MWCO dialysis tubing (such as Spectrum™ Spectra/Por™ Biotech Cellulose Ester (CE) Dialysis Membrane Tubing, obtained from Fisher Scientific). The first dialysis step was performed for at least 2h at room temperature, subsequent dialysis rounds were performed for at least 8h at room temperature, or overnight at 4°C.

30

Size distribution of the LNPs was determined by dynamic light scattering (DLS) using a Zetasizer Pro (Red label, Malvern) with standard settings for LNPs (NIBS, adaptive correlation).

35 LNPs were formed using secNLuc (incl. PolyA ~1000nt) mRNA and their size was measured using DLS in 1x PBS (10mM phosphate buffer, 150mM NaCl) pH 7.4. Multiple

LNP formulations (50 mol% ionizable lipid (L1; di((9Z,12Z)-octadeca-9,12-dien-1-yl) 2-((2-(dimethylamino)ethyl)thio)succinate maleate), 38.5 mol% structural lipid (resveratrol (RSV), cholesterol or a mixture thereof), 10 mol% phospholipid (DOPE) and 1.5 mol% DSPE-PEG(2000), at N/P 5 corresponding to a lipid to Oligo weight (LOW) of around 16), were produced. As shown in Figure 1, these formulations revealed an average size of 70 nm (65-90 nm) with an average polydispersity index (PDI) of 0.1.

Next, formulations including 5% DOTAP were produced; (45 mol% ionizable lipid (L1; di((9Z,12Z)-octadeca-9,12-dien-1-yl) 2-((2-(dimethylamino)ethyl)thio)succinate maleate), 5 mol% DOTAP, 38.5 mol% structural lipid (resveratrol, cholesterol or a mixture thereof), 10 mol% phospholipid (DOPE) and 1.5 mol% DSPE-PEG(2000), at N/P 5 corresponding to a lipid to Oligo weight (LOW) of around 16). Interestingly, the inclusion of DOTAP did not significantly alter the size or PDI of the LNPs, as shown in Figure 2.

15

Example 2: pKa determination of lipids

The pKa of the ionizable lipid is known to be a major determinant for the endosomal escape of the contents of the LNP when exposed to cells. During endosomal uptake and passage through endosomal compartments, the LNP experiences a gradual decrease of the pH from the physiological pH (pH 7.4, as is present outside the cell) to around pH 4.5 – 5.0 in the lysosome, as the end-stage of most endocytosis vesicles. Trafficking towards the lysosome is generally to be avoided as breakdown of cargo (*e.g.*, mRNA) may occur due to the degradative environment, thus endosomal escape ideally happens at a pH above 5.0, thus before the endosome has matured into a lysosome. A rapid increase in cationic charge of the ionizable lipid during this acidification process is thought to facilitate interaction with the inner endosomal membrane. Therefore, lipids which carry a neutral or near neutral charge at pH 7.4 and which are fully ionized at pH 5.5 are considered ideal; this corresponds to a pKa of around 6.4-6.5, similar to what was found to be optimal by Jayaraman et al. (2012 Angewandte Chemie, DOI: 10.1002/ang.201203263).

30

The local environment of the ionizable lipid (relating to the incorporation in an LNP) may influence the acquisition of charge and thus influences the pKa. It is well conceivable that core lipids residing directly under the surface of the LNP may alter the surface properties and thus the pKa. Therefore, the experimentally determined pKa of the ionizable lipid L1 was measured by the addition of 2-(p-toluidino) naphthalene-6-

35

sulfonic acid (TNS; obtained from Sigma-Aldrich) to nanoparticle compositions containing a core lipid of the invention, or mixtures thereof, in the presence of 20mM phosphate-citrate-ammonium citrate (pH 3-10 in 0.5 increments, all obtained from Sigma Aldrich). TNS is a compound that electrostatically interacts with the cationic lipid, resulting in fluorescence. Briefly, a mixture of 50µl of 5µM TNS, 25µM LNPs (containing 12.5µM ionizable lipid) and 20mM buffer were added subsequently to the phosphate-citrate-ammonium citrate buffer samples in a 384-well plate, and measured on a plate reader (iD3, Molecular Devices) at 325 nm excitation and 435 nm emission. Samples containing only LNPs and buffer were used for background subtraction for each increment in pH. The background-corrected measured fluorescence was then normalized against the difference between maximum and minimum fluorescence obtained during the assay, and a curve fit was performed to obtain an S-shaped curve for each lipid composition. The pKa of each ionizable lipid was determined as the pH value at which half of the maximum fluorescence was reached, and the results are given in Figure 3 for each of the LNP compositions containing a core lipid of the invention, cholesterol or a mixture thereof.

It is noted that none of the core lipids significantly altered the pKa of the LNP formulations based on the L1 ionizable lipid. It is therefore concluded that the core lipid does not influence the ionization of the ionizable lipid.

Example 3: Encapsulation efficiency of LNPs

A high encapsulation efficiency of oligo- and poly-nucleotide cargo is important to prevent exposure of the cargo to degradative enzymes, immune-stimulatory cells and receptors, and achieving a high transfection efficiency. Therefore, the encapsulation efficiency was determined by means of a RiboGreen-assay.

Secreted NanoLuc (m)RNA-containing nanoparticle compositions (N/P 5:1, LOW ~16) were mixed 1:1 with QUANT-IT RIBOGREEN RNA assay (Invitrogen, obtained from Thermo Fisher Scientific) at a concentration of around 5µg/ml in TE-buffer (10mM Tris, HCl pH7.5, 1mM EDTA, obtained from Sigma-Aldrich) or 2% Triton X-100 (Sigma-Aldrich) in TE-buffer) with an equal volume of 1:100 RiboGreen reagent. Samples were thoroughly mixed and incubated for 5 minutes at room temperature. Next, the fluorescence intensity was measured on a plate reader (iD3, Molecular Devices), at 480 nm excitation and 520 nm emission. Blanks containing TE-buffer with 1:200 RiboGreen reagent or 2%-TritonX-100 buffer with 1:200 RiboGreen reagent, were used as

fluorescence background control. A standard curve of naked (m)RNA was used to quantify the absolute amount of non-encapsulated RNA. The signal obtained with 2% Titron was used to normalize all samples against and was set at 100%. The results are provided in Table 1 and Figure 4.

5

Table 1: Details of LNP compositions and encapsulation efficiency of mRNA

Formulation	Composition	Encapsulation efficiency
1	50 mol% L1, 38.5 mol% cholesterol, 10 mol% DOPE, 1.5 mol% DSPE-PEG	97%
2	50 mol% L1, 28.9 mol% cholesterol, 9.6 mol% resveratrol, 10 mol% DOPE, 1.5 mol% DSPE-PEG	94%
3	50 mol% L1, 19.25 mol% cholesterol, 19.25 mol% resveratrol, 10 mol% DOPE, 1.5 mol% DSPE-PEG	94%
4	50 mol% L1, 9.6 mol% cholesterol, 28.9 mol% resveratrol, 10 mol% DOPE, 1.5 mol% DSPE-PEG	94%
5	50 mol% L1, 4.8 mol% cholesterol, 33.7 mol% resveratrol, 10 mol% DOPE, 1.5 mol% DSPE-PEG	93%
6	50 mol% L1, 2.4 mol% cholesterol, 36.1 mol% resveratrol, 10 mol% DOPE, 1.5 mol% DSPE-PEG	96%
7	50 mol% L1, 38.5 mol% resveratrol, 10 mol% DOPE, 1.5 mol% DSPE-PEG	96%

Formulation	Composition	Encapsulation efficiency
8	50 mol% L1, 38.5 mol% Dihydroresveratrol, 10 mol% DOPE, 1.5 mol% DSPE-PEG	96%
9	50 mol% L1, 38.5 mol% Deoxyrhaptogenin, 10 mol% DOPE, 1.5 mol% DSPE-PEG	88%
10	50 mol% L1, 38.5 mol% Isorhaptogenin, 10 mol% DOPE, 1.5 mol% DSPE-PEG	92%
11	50 mol% L1, 38.5 mol% Piceatannol, 10 mol% DOPE, 1.5 mol% DSPE-PEG	93%
12	50 mol% L1, 38.5 mol% Pterostilbene, 10 mol% DOPE, 1.5 mol% DSPE-PEG	95%
13	50 mol% L1, 38.5 mol% Polydatin, 10 mol% DOPE, 1.5 mol% DSPE-PEG	97%

All LNP compositions of the invention displayed high encapsulation efficiency and are therefore suitable to protect oligo- and poly-nucleotides from degradative enzymes and to prevent any other effects of exposed oligo- and poly-nucleotides.

5

Example 4: Toxicity of LNP formulations on cells

Each of the tested core lipids of the invention is considered a GRAS (generally recognized as safe) compound, that is present in various food products. Therefore, the inventors investigated the effect of the core lipids on the toxicity of the complete LNP formulation.

10

Given the well-known anti-inflammatory, anti-oxidative properties of the core lipids, both toxic and protective effects were of interest. To measure toxic effects of the core lipids, the inventors added a concentration range of the LNPs to cells, defined by the mRNA content added to the cells.

Briefly, a concentration range of 10-100 ng of formulated mRNA, corresponding to 160-1600 ng of total lipids were added to HeLa cells, pre-mixed with cell-culture medium in a total volume of 100 µl per well of a 96-well plate. After 24h incubation, metabolic activity was tested by the resazurin assay. For this purpose, culture medium was replaced
5 with medium containing 0.1 mg/ml resazurin and incubated for 1-4 hours at 37 °C and 5% CO₂. Subsequently, fluorescence was determined in the supernatant (excitation 540/25 nm, emission 620/40 nm).

Increasing concentrations of the lipids did not reveal any significant toxic effect on HeLa
10 cells, as shown in Figure 5.

Example 5: Activity of core-lipid LNPs *in vitro*

To determine if the alternative core lipid-containing LNP formulations can effectively deliver mRNA into cells, the inventors incubated cells with luciferase mRNA-containing
15 LNPs, containing the core lipid of interest and 5% DOTAP as an uptake enhancer.

Core-lipid-containing LNPs were formulated with 5 mol% DOTAP (45 mol% L1, 5 mol% DOTAP, 10 mol% DOPE, 38.5 mol% resveratrol, resveratrol-derivative, and/or cholesterol, and 1.5 mol% DSPE-PEG(2000)) as described in example 1, containing
20 secreted nano-luciferase mRNA at an N/P of 5:1 (corresponding to a LOW of around 16). A dose range of LNPs, corresponding to 100, 50 and 10 ng per well containing a volume of 100 µl was added to HeLa cells in a 96-well plate. After 24 hours, medium was collected, and secreted nano-luciferase activity was determined using the Nano-Glo Luciferase Assay System (Promega).

25 Incubation of HeLa cells with resveratrol-lipid-containing LNPs demonstrated a dose-dependent induction of luciferase activity compared to LNPs containing only cholesterol as core lipid. Especially with higher ratios of resveratrol over cholesterol an increase up to 300% of control (containing only cholesterol as core lipid) was observed. Interestingly,
30 several structural variants of resveratrol performed similarly to each other, with the exception of deoxyrhaptoegenin being a worse performer and polydatin outperforming the structural variants.

As shown in Figure 6A (activity of LNPs with cholesterol, resveratrol or a mixture thereof) and Figure 6B (activity of LNPs with resveratrol-derivatives, with cholesterol and resveratrol as controls).
35

Example 6: Resveratrol-containing LNPs do not bind ApoE *in vitro*

To determine the absence of ApoE-dependent uptake, the cellular uptake of resveratrol-containing LNPs was tested with a concentration range of ApoE .

5

L1-containing LNPs were formulated with resveratrol or cholesterol as described in example 1, containing secreted nano-luciferase mRNA. In addition, resveratrol- and cholesterol-containing LNPs were formulated with 5% DOTAP. LNPs, corresponding to 100 ng mRNA per well containing a volume of 100 µl, were added in the absence or
10 presence of a concentration of 1 to 20 µg/ml ApoE (R&D Systems), to HeLa cells in a 96-well plate. After 24 hours, medium was collected, and secreted nano-luciferase activity was measured using the Nano-Glo Luciferase Assay System (Promega).

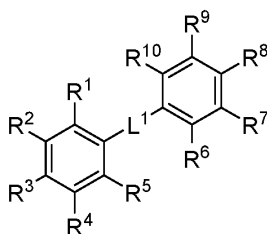
Incubation of HeLa cells with resveratrol-containing LNPs demonstrated no induction
15 of luciferase activity without or with increasing concentrations of ApoE. In contrast, cholesterol-containing LNPs showed a dose-dependent increase in luciferase activity. Resveratrol-containing LNPs with DOTAP showed similar, or even an increased, luciferase activity compared to cholesterol-containing LNPs with DOTAP, proving that the resveratrol-LNPs are not inherently inactive, as shown in Figure 7.

20

Therefore, resveratrol-containing LNPs demonstrate an ApoE-independent uptake mechanism that distinguishes them from cholesterol-containing LNPs and enables bio-distribution not influenced by ApoE.

Claims

1. Use of a compound of formula (I):



(I)

5

, wherein L¹ is ;

R¹ to R¹⁰ are each independently H, OR¹¹, OCOR¹¹, COOR¹¹, NR¹¹R¹², N⁺R¹¹R¹²R¹³, an optionally substituted C₁₋₃₀ alkyl, an optionally substituted C₂₋₃₀ alkenyl, an optionally substituted C₂₋₃₀ alkynyl, an optionally substituted C₃₋₁₀ cycloalkyl, an optionally substituted C₃₋₁₀ cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle, an optionally substituted C₆₋₁₂ aryl, an optionally substituted 5 to 10 membered heteroaryl or a sugar moiety; and

R¹¹ to R¹³ are each independently H, an optionally substituted C₁₋₃₀ alkyl, an optionally substituted C₂₋₃₀ alkenyl, an optionally substituted C₂₋₃₀ alkynyl, an optionally substituted C₃₋₁₀ cycloalkyl, an optionally substituted C₃₋₁₀ cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle, an optionally substituted C₆₋₁₂ aryl or an optionally substituted 5 to 10 membered heteroaryl;

or a pharmaceutically acceptable complex, salt, solvate, tautomeric form or polymorphic form thereof;

in a micro- or nanoparticle formulation.

2. The use of claim 1, wherein the compound of formula (I) is used as a core lipid in the micro- or nanoparticle formulation.

25

3. The use according to any preceding claim, wherein:

- R¹ is H;
- R³ is H, an optionally substituted C₁₋₃₀ alkyl, an optionally substituted C₂₋₃₀ alkenyl, an optionally substituted C₂₋₃₀ alkynyl, an optionally substituted C₃₋₁₀ cycloalkyl,

an optionally substituted C₃₋₁₀ cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle, an optionally substituted C₆₋₁₂ aryl or an optionally substituted 5 to 10 membered heteroaryl;

- R⁵ is H;

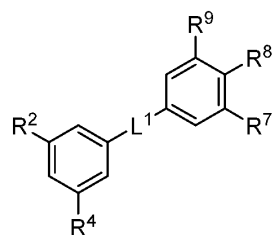
5 - R⁶ is H, OR¹¹, OCOR¹¹, COOR¹¹, NR¹¹R¹² or N⁺R¹¹R¹²R¹³ and R¹¹ to R¹³ are independently H, an optionally substituted C₁₋₁₂ alkyl, an optionally substituted C₂₋₁₂ alkenyl, an optionally substituted C₂₋₁₂ alkynyl, an optionally substituted C₃₋₁₀ cycloalkyl, an optionally substituted C₃₋₁₀ cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle, an optionally substituted C₆₋₁₂ aryl or an optionally substituted 5 to 10 membered heteroaryl;

10 - R⁷ is H, OR¹¹, OCOR¹¹, COOR¹¹, NR¹¹R¹², N⁺R¹¹R¹²R¹³, an optionally substituted C₁₋₃₀ alkyl, an optionally substituted C₂₋₃₀ alkenyl, an optionally substituted C₂₋₃₀ alkynyl, an optionally substituted C₃₋₁₀ cycloalkyl, an optionally substituted C₃₋₁₀ cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle, an optionally substituted C₆₋₁₂ aryl or an optionally substituted 5 to 10 membered heteroaryl and R¹¹ to R¹³ are independently H, an optionally substituted C₁₋₁₂ alkyl, an optionally substituted C₂₋₁₂ alkenyl, an optionally substituted C₂₋₁₂ alkynyl, an optionally substituted C₃₋₁₀ cycloalkyl, an optionally substituted C₃₋₁₀ cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle, an optionally substituted C₆₋₁₂ aryl or an optionally substituted 5 to 10 membered heteroaryl;

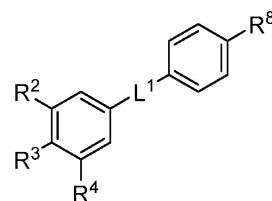
20 - R⁹ is H, OR¹¹, OCOR¹¹, COOR¹¹, NR¹¹R¹², N⁺R¹¹R¹²R¹³, an optionally substituted C₁₋₃₀ alkyl, an optionally substituted C₂₋₃₀ alkenyl, an optionally substituted C₂₋₃₀ alkynyl, an optionally substituted C₃₋₁₀ cycloalkyl, an optionally substituted C₃₋₁₀ cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle, an optionally substituted C₆₋₁₂ aryl or an optionally substituted 5 to 10 membered heteroaryl and R¹¹ to R¹³ is independently H, an optionally substituted C₁₋₁₂ alkyl, an optionally substituted C₂₋₁₂ alkenyl, an optionally substituted C₂₋₁₂ alkynyl, an optionally substituted C₃₋₁₀ cycloalkyl, an optionally substituted C₃₋₁₀ cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle, an optionally substituted C₆₋₁₂ aryl or an optionally substituted 5 to 10 membered heteroaryl; and/or

30 - R¹⁰ is H.

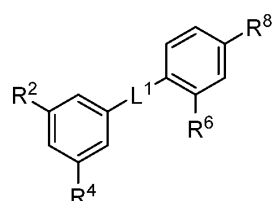
4. The use according to any preceding claim, wherein the compound is a compound of formula (Ia), (Ib), (Ic) or (Id):



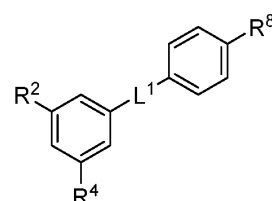
(Ia)



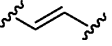
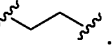
(Ib)



(Ic)

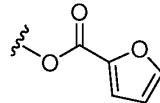


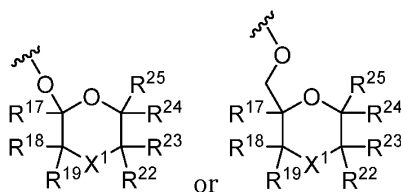
(Id)

5. The use according to any preceding claim, wherein L¹ is  or .

6. The use according to any preceding claim, wherein R², R⁴ and R⁸ are
 5 independently H, OR¹¹, OCOR¹¹, COOR¹¹, NR¹¹R¹², N⁺R¹¹R¹²R¹³ or a sugar moiety, and R¹¹
 to R¹³ are each independently H, an optionally substituted C₁₋₂₀ alkyl, an optionally
 substituted C₂₋₂₀ alkenyl, an optionally substituted C₂₋₂₀ alkynyl, an optionally substituted
 C₃₋₁₀ cycloalkyl, an optionally substituted C₃₋₁₀ cycloalkenyl, an optionally substituted 3
 to 10 membered heterocycle, an optionally substituted C₆₋₁₂ aryl or an optionally
 10 substituted 5 to 10 membered heteroaryl.

7. The use according to claim 6, wherein R², R⁴ and R⁸ are independently H, OH,

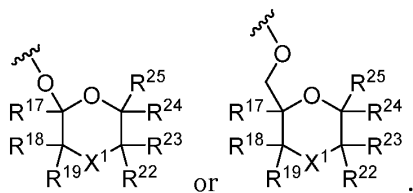
OCH₃,  or a sugar moiety or general formula:



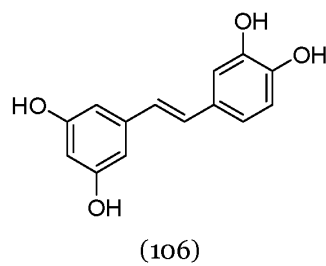
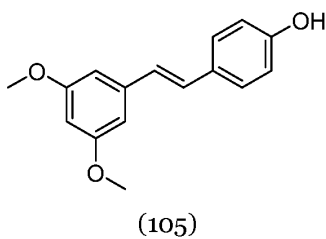
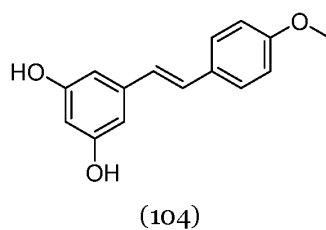
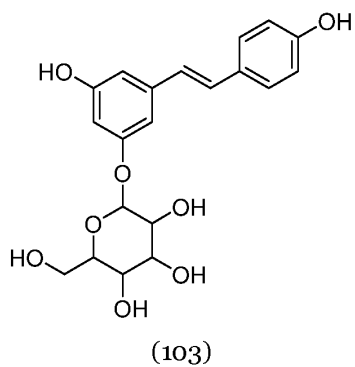
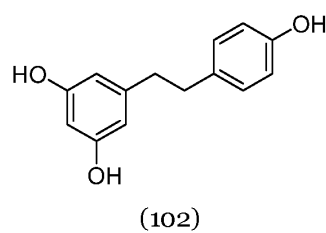
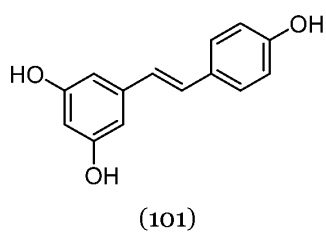
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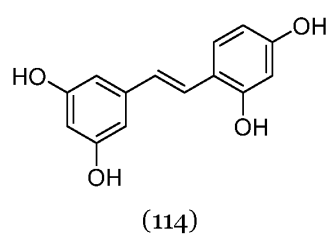
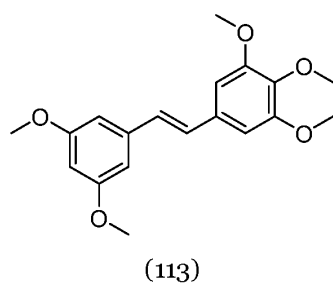
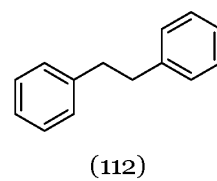
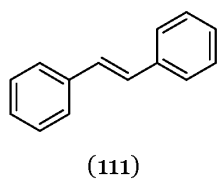
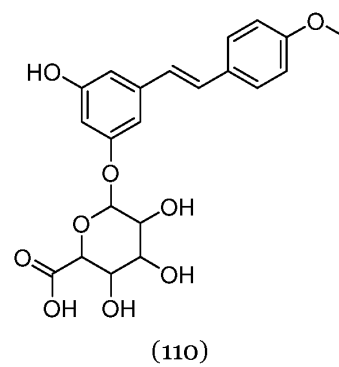
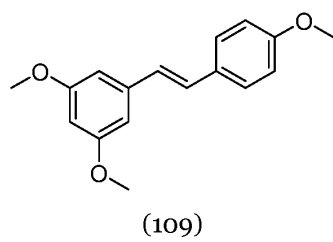
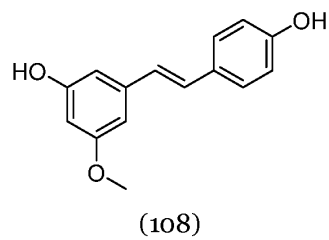
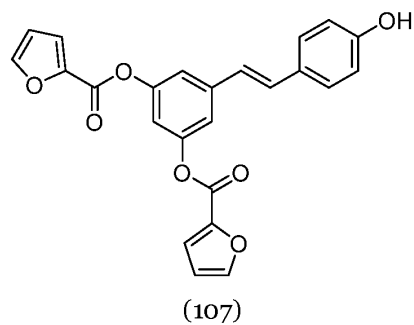
, wherein X¹ is a bond of CR²⁰R²¹; and

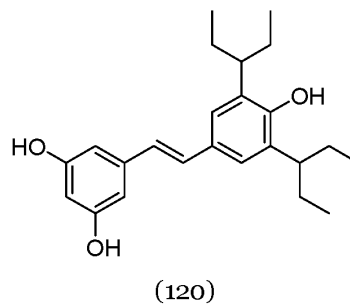
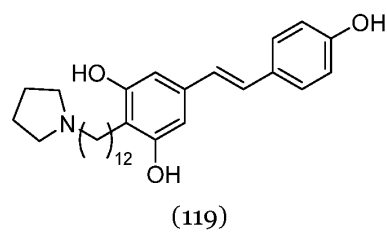
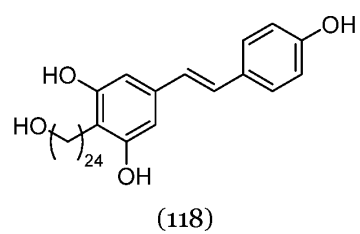
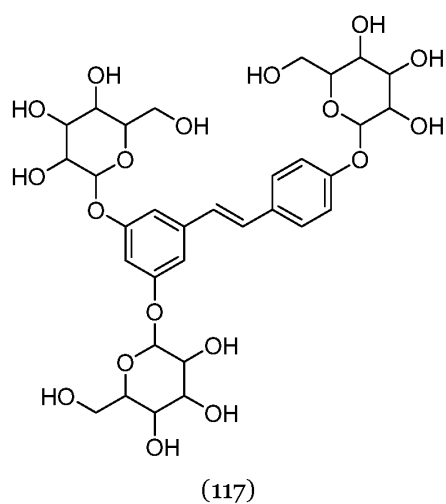
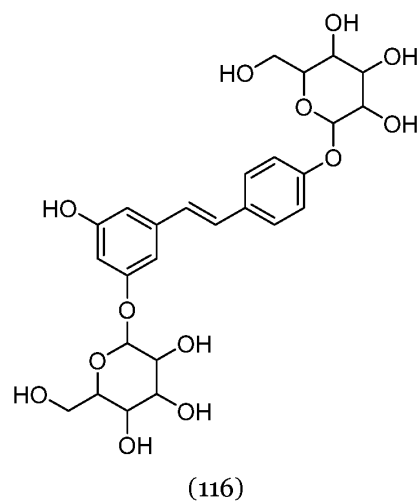
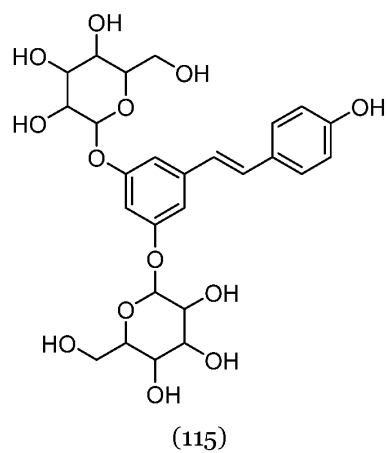
R¹⁷ to R²⁵ are each independently H, OH, CH₂OH, COOH, NH₂, C₁₋₆ alkyl, halo,

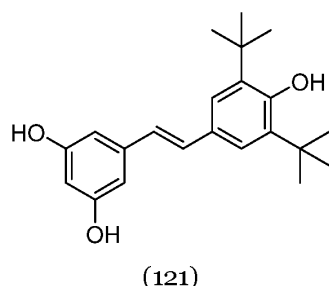


8. The use according to any preceding claim, wherein the compound of formula (I)
 5 is:

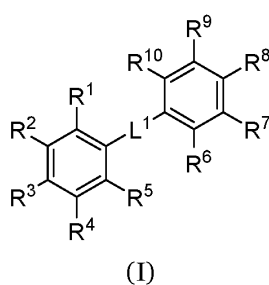








9. A micro- or nanoparticle comprising a compound of formula (I):



5

, wherein L¹ is ;

R¹ to R¹⁰ are each independently H, OR¹¹, OCOR¹¹, COOR¹¹, NR¹¹R¹², N⁺R¹¹R¹²R¹³, an optionally substituted C₁-₃₀ alkyl, an optionally substituted C₂-₃₀ alkenyl, an optionally substituted C₂-₃₀ alkynyl, an optionally substituted C₃-₁₀ cycloalkyl, an optionally substituted C₃-₁₀ cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle, an optionally substituted C₆-₁₂ aryl, an optionally substituted 5 to 10 membered heteroaryl or a sugar moiety; and

R¹¹ to R¹³ are each independently H, an optionally substituted C₁-₃₀ alkyl, an optionally substituted C₂-₃₀ alkenyl, an optionally substituted C₂-₃₀ alkynyl, an optionally substituted C₃-₁₀ cycloalkyl, an optionally substituted C₃-₁₀ cycloalkenyl, an optionally substituted 3 to 10 membered heterocycle, an optionally substituted C₆-₁₂ aryl or an optionally substituted 5 to 10 membered heteroaryl;

or a pharmaceutically acceptable complex, salt, solvate, tautomeric form or polymorphic form thereof;

20 and an optional payload molecule.

10. The micro- or nanoparticle of claim 9, wherein the micro- or nanoparticle is a lipid nanoparticle (LNP), a liposome, a lipoplex, a micelle or a lipid vesicle, and preferably is an LNP.
- 5 11. The micro- or nanoparticle of claim 9 or 10, wherein the micro- or nanoparticle comprises a lipid component which comprises the compound of formula (I), or a pharmaceutically acceptable complex, salt, solvate, tautomeric form or polymorphic form thereof, and one or more further lipids selected from the group consisting of a phospholipid, a permanent cationic lipid, an ionisable cationic lipid, a permanent
10 anionic lipid, an ionisable anionic lipid, a structural lipid, a shield lipid, a functionalised lipid, a further core lipid and combinations thereof.
12. The micro- or nanoparticle of any one of claims 9 to 12, wherein the lipid component of the micro- or nanoparticle comprises between 5 to 80 mol%, between 10
15 to 60 mol%, between 20 to 50 mol%, between 30 and 45 mol% or between 35 to 40 mol% of the compound of Formula (I), or a pharmaceutically acceptable complex, salt, solvate, tautomeric form or polymorphic form thereof.
13. The micro- or nanoparticle of any one of claims 9 to 12, wherein the micro- or
20 nanoparticle comprises a payload molecule, which is a biomolecule, an active pharmaceutical ingredient (API) and/or a diagnostic compound.
14. The micro- or nanoparticle of any one of claims 9 to 13, wherein the payload molecule is a biomolecule, and is or comprises an amino acid, a peptide, an affimer, a
25 polypeptide or protein, a glycoprotein, a saccharide, a lipid, a lipopolysaccharide, an antibody or a fragment thereof, a polymer, or a nucleic acid, or a combination thereof, preferably wherein the biomolecule is or comprises a nucleic acid.
15. The micro- or nanoparticle of claim 14, wherein the nucleic acid is DNA, RNA,
30 xeno nucleic acid (XNA), cyclohexene nucleic acid (CeNA), threose nucleic acid (TNA), glycol nucleic acid (GNA), locked nucleic acid (LNA), peptide nucleic acid (PNA), fluoroarabinonucleic acid (FANA), unlocked nucleic acid (UNA), or a DNA/RNA hybrid sequence.
- 35 16. The micro- or nanoparticle of claim 15, wherein the nucleic acid is RNA, and the RNA is single stranded or double stranded and is selected from the group consisting of:

messenger RNA (mRNA); circular RNA (circRNA or oRNA); self-amplifying RNA (saRNA); transamplifying RNA (taRNA), long non-coding RNA, split-replicon RNA, viral RNA, antisense RNA (AON or asRNA); RNA aptamers; interference RNA; micro-RNA (miRNA); short interfering RNA (siRNA); short hairpin RNA (shRNA); and small RNA.

5

17. A composition comprising a plurality of micro- or nanoparticles as defined in any one of claims 9 to 16.

18. The micro- or nanoparticle of any one of claims 9 to 16, or the composition of claim 17, for use as a medicament.

10

19. The micro- or nanoparticle of any one of claims 9 to 16, or the composition of claim 17, for use in the treatment and/or prevention and/or prophylaxis of a disease or disorder.

15

20. A vaccine composition comprising the micro- or nanoparticle of any one of claims 9 to 16, or the composition of claim 17.

21. The micro- or nanoparticle of any one of claims 9 to 16, or the composition of claim 17 or the vaccine of claim 20, for use in stimulating an immune response in a subject.

20

25