

Lipidoids for nucleic acid transfection and use thereofField of Art

The invention relates to novel ionizable lipidoids and to the use of these compounds for transfection and administration of nucleotides and nucleic acids and their synthetic analogues into cells and tissues.

Background Art

The development of nucleic acid (NA)-based therapies has experienced an unprecedented renaissance in recent years. Due to the high efficacy and at the same time the low risk of adverse side effects compared to previously tested therapeutic deoxyribonucleic acids (DNA), ribonucleic acid (RNA) therapies are now gaining ground. Several such drugs have already reached clinical use, for example patisiran for hereditary transthyretin amyloidosis, eteplirsen for certain types of Duchenne muscular dystrophy, or nusinersen for the treatment of spinal muscular atrophy. All of these diseases are life-threatening and there is no alternative treatment for them. Potential drugs targeting ribonucleic acid (RNA) or its use can be divided into three categories according to whether they target NA or proteins or encode proteins. The first category includes single-stranded antisense oligonucleotides of 13-25 nucleotides (nt) that block the translation of messenger RNA (mRNA) or RNA splicing (nusinersen, eteplirsen); and small interfering RNAs (siRNA, 21-23nt) that degrade mRNA (patisiran). Therapeutic RNA molecules targeting proteins use a type of molecule known as an RNA aptamer. It is designed to modulate the function of a particular protein. An example of such a drug is pegaptanib, used to treat neovascular age-related macular degeneration, which was the first approved drug of its kind in 2004. Therapies using mRNA are mainly used for the preparation of so-called personalized vaccines against cancer or vaccines against infectious diseases (eg. Zika virus, SARS-CoV-2). It is in viral diseases that candidates for a prophylactic vaccine based on mRNA against rabies and pandemic influenza have been shown to induce safe antibody production in healthy volunteers. Enormous efforts have been put into vaccine development against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the virus responsible for the COVID-19 pandemic. These efforts led to safe and highly effective mRNA vaccine candidates that are now being distributed for widespread use. Protein-replacing mRNA therapies are also in the preclinical stages of development, for example for treating hemophilia.

Molecular technologies enabling direct genome editing, especially those based on the CRISPR-Cas9 system, are now booming. CRISPR technology is a tool that allows you to change DNA sequences and modify gene function. Potential applications include the repair of genetic defects, the treatment and prevention of the spread of diseases or the improvement of agricultural crops. The CRISPR-Cas9 system has been tested in a number of preclinical and clinical studies including HIV treatment, the treatment of hematological malignancies and genetic disorders, including sickle cell disease and β -thalassemia. RNA editing is then enabled by the system of ADARs (adenosine deaminase acting on RNA), which so far

seems to be safer from a clinical point of view. Molecular technologies for direct genome editing can be delivered to the site of action in the form of mRNA that encodes the appropriate enzyme responsible for editing.

A key factor enabling the safe use of all the above technologies (or others, based on NA) is their safe and efficient transport to the site of action. The critical step is the penetration of negatively charged NAs through the phospholipid membrane of the cell; the process of deliberately introducing NAs into eukaryotic cells is called transfection. In recent decades, there has been an intensive development of carriers (so-called vectors) to efficiently transport NA across the cell membrane while protecting NA from degradation *in vivo* (Stewart, M. P.: *Chem. Rev.* **2018**, *118*, 7409–7531).

Both viral and non-viral (physical, chemical) vectors are used for NA transfection. Although approximately 70 % of clinical trials in the field of gene therapy have so far been performed using viral vectors, this approach carries numerous risks (carcinogenicity, induction of an immune response, tissue non-specificity, limited NA incorporation capacity and manufacturing complexity). Physical methods (eg. electroporation) are difficult to use systemically in human medicine.

In contrast, synthetic chemical vectors usually have lower immunogenicity, are able to transport larger amounts of genetic material, and because they are composed of well-defined molecules, their structure can be influenced as needed to increase their efficiency and suppress toxicity. Cationic polymers or cationic lipids are used as chemical vectors, which form a complex with negatively charged NA. This complex is able to penetrate the cell membrane and at the same time protects NA from degradation in the extracellular environment.

From a structural point of view, so-called lipid nanoparticles (LNPs) are currently the most promising and clinically advanced form of these complexes. In them, cationic lipids are usually formulated with a PEGylated lipid that prevents aggregation, affects particle size and transfection efficiency, with helper lipid and cholesterol, which are necessary for stable NK encapsulation, as shown, for example, in an siRNA transfection system (Kulkarni, J.: *Nanoscale*, **2019**, *11*, 21733–21739). LNPs can accommodate NA molecules ranging in size from a few nucleotide units to millions.

Synthetic cationic lipids and lipidoids (synthetic molecules similar to lipids differing in a large number of hydrophobic chains) are formed by a cationic and a hydrophobic domain. To date, a large number of these substances have been developed with high structural variability in both domains, both by targeted design and by testing combinatorially generated libraries.

Lipids and lipidoids such as D-Lin-MC3-DMA, C12-200, cKK-E12, SA2-SC8 and others have been specially developed for siRNA transfection (Dong, Y.: *Adv. Drug Deliv. Rev.* **2019**, *144*, 133–147). A formulation containing D-Lin-MC3-DMA was recently (August 2018) introduced into clinical practice under the name Onpattro (formerly Patisiran), making it the first approved siRNA drug in history (Zhang, X.: *J. Clin. Pharmacol.* **2020**, *60* (1), 37-49). However, formulations developed for siRNA may

not be effective for mRNA, and targeted optimization is therefore necessary (Cullis, P.: *Mol. Ther.* **2017**, 25 (7), 1467–1475).

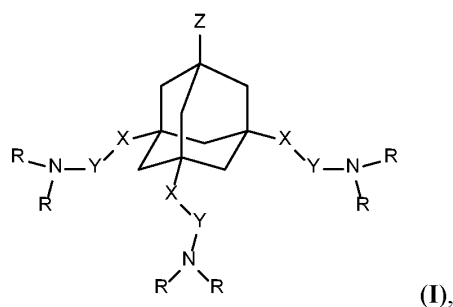
Ionizable lipids and lipidoids such as D-Lin-MC3-DMA, C12-200, cKK-E12, and TT3 are used to transfect mRNA (Zhong, Z.: *Nano Today* **2018**, 23, 16–39; Kowalski, P.: *Mol. Ther.* **2019**, 27 (4), 1-19; Li, B.: *Nano Lett.* **2015**, 15, 8099–8107). Ionizable lipids are also used for DNA transfection. Again, it should be emphasized that transfection systems optimized for small molecule (siRNA) transfection are not always suitable for DNA transfection, and even formulations developed for mRNA may not be effective for DNA (Buck, J.: *ACS Nano* **2019**, 13, 3754–3782).

Due to the fact that despite their enormous therapeutic potential, very few synthetic vectors based on ionizable lipids have been brought to the stage of clinical use so far, it is necessary to develop new systems with higher efficiency, which would also have very low *in vivo* toxicity.

Disclosure of the Invention

The present invention provides a solution to the problem of the efficiency of transfection and targeted delivery of nucleotides and nucleic acids and their synthetic analogs using ionizable (cationic) lipids and the problem of the toxicity of these lipids to the target organism or cell. We have surprisingly found that if adamantane is used as the central core of the ionizable lipidoid, the transfection efficiency of such lipidoids is significantly increased compared to previously known solutions, especially in the transfection of mRNA, cyclic dinucleotides, siRNA and DNA. At the same time, these adamantane-containing lipidoids exhibit extremely low cytotoxicity at relevant doses. They show no signs of toxicity in mice after intravenous or intraperitoneal administration. A specific property of the adamantane core, used as the central structural motif of the new ionizable lipidoids, is steric complexity in its vicinity and a high rigidity in comparison with the ionizable lipids and lipidoids known so far. A further advantage of bioactive substances structurally derived from adamantane is generally good biocompatibility, and thus suitability for pharmaceutical use, in particular in human medicine.

The invention relates to ionizable lipidoids of general formula I

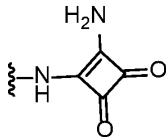
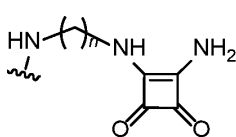


wherein X is selected from a group consisting of -C(=O)NH-, -C(=O)O-, -C(=S)O-, -C(=O)S-,
-C(=S)S-, -C(=O)NHNH-, -CH₂-, -O-, -OC(=O)-, -S-, -SC(=O)-, -NH-, -NHNH-, -NHC(=O)-,

-NHNHC(=O)-, $-C\equiv C-$, $-CH=CH-$, a five-membered heterocycle containing at least 2 nitrogen atoms, $-CH_2C(=O)NH-$, $-CH_2C(=O)O-$, $-CH_2C(=S)O-$, $-CH_2C(=S)S-$, $-CH_2C(=O)NHNH-$, $-N=CH-$, and $-CH=N-$;

Y is independently selected from a group consisting of alkylene chains C_2-C_{10} , wherein in the said alkylene chain, one or more $-CH_2-$ groups may optionally be replaced with one or more O or S atoms;

Z is selected from the group consisting of hydrogen, $-OH$, $-CH_2OH$, $-NH_2$, $-N^+(CH_3)_2-(CH_2)_3-SO_3^-$, $-N^+(CH_3)_2-(CH_2)_2-COO^-$, and $-NHCH_3$, $-N(CH_3)_2$, $-N^+(CH_3)_3$, $-OCH_3$, $-OCH_2CH_3$, $-C(=O)R^1$, wherein R^1 is selected from $-NH_2$, $-NH(CH_2)_nOH$, $-N[(CH_2)_nOH]_2$, $-NHCH(CH_2OH)_2$, $-NHCH_2CH(OH)CH_2OH$, $-NH(CH_2)_nC(=O)NH_2$, $-N[CH_2C(=O)NH_2]_2$,

$-NHCH[C(=O)NH_2]_2$, $-NH(CH_2)_2NHC(=O)NH_2$, , and , wherein n is an integer within the range from 2 to 5;

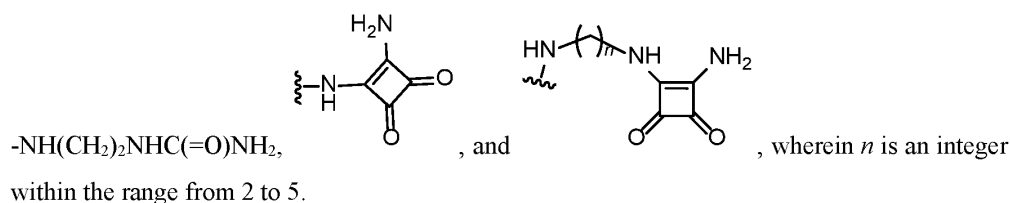
and R are the same or different from each other, each R being independently selected from the group consisting of alkyl C_8-C_{20} , alkenyl C_8-C_{20} , and alkynyl C_8-C_{20} , wherein in the said alkyl, alkenyl or alkynyl, one or more $-CH_2-$ groups may optionally be replaced with one or more groups selected from $-CH(OH)-$, $-OC(=O)-$, $-C(=O)O-$, $-S-S-$, $-C(=O)NH-$, $-NHC(=O)-$, $-O-$, and $-S-$; and pharmaceutically acceptable salts, addition salts and solvates thereof.

In some embodiments, X is selected from a group consisting of $-C(=O)NH-$, $-C(=O)O-$, $-C(=S)O-$, $-C(=O)S-$, $-C(=S)S-$, $-C(=O)NHNH-$, $-CH_2-$, $-O-$, $-S-$, $-NH-$, $-NHNH-$, $-NHC(=O)-$, $-NHNHC(=O)-$, $-C\equiv C-$, $-CH=CH-$, five-membered heterocycle comprising at least two nitrogen atoms, $-CH_2C(=O)NH-$, $-CH_2C(=O)O-$, $-CH_2C(=S)O-$, $-CH_2C(=S)S-$, $-CH_2C(=O)NHNH-$, $-N=CH-$, $-CH=N-$.

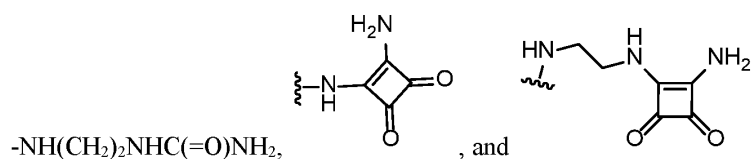
In some embodiments, Y is selected from a group consisting of alkylene chains C_2-C_{10} .

In some embodiments, Z is selected from a group consisting of hydrogen atom, $-OH$, $-NH_2$, $-NHCH_3$, $-N(CH_3)_2$, $-N^+(CH_3)_3$, $-OCH_3$, $-OCH_2CH_3$, $-N^+(CH_3)_2-(CH_2)_3-SO_3^-$, $-N^+(CH_3)_2-(CH_2)_2-COO^-$.

In some embodiments, Z is selected from a group consisting of hydrogen, $-OH$, $-CH_2OH$, $-NH_2$, $-N^+(CH_3)_2-(CH_2)_3-SO_3^-$, $-N^+(CH_3)_2-(CH_2)_2-COO^-$, and $-C(=O)R^1$, wherein R^1 is selected from $-NH_2$, $-NH(CH_2)_nOH$, $-N[(CH_2)_nOH]_2$, $-NHCH(CH_2OH)_2$, $-NHCH_2CH(OH)CH_2OH$, $-NH(CH_2)_nC(=O)NH_2$, $-N[CH_2C(=O)NH_2]_2$, $-NHCH[C(=O)NH_2]_2$,



- In some embodiments, Z is selected from a group consisting of hydrogen, -OH, -CH₂OH, -NH₂,
 5 $\text{-N}^+(\text{CH}_3)_2\text{-(CH}_2\text{)}_3\text{-SO}_3^-$, $\text{-N}^+(\text{CH}_3)_2\text{-(CH}_2\text{)}_2\text{-COO}^-$, and -C(=O)R^1 ,
 wherein R¹ is selected from -NH₂, -NH(CH₂)₂OH, -N[(CH₂)₂OH]₂, -NHCH(CH₂OH)₂,
 -NHCH₂CH(-OH)CH₂OH, -NH(CH₂)₂C(=O)NH₂, -N[CH₂C(=O)NH₂]₂, -NHCH[C(=O)NH₂]₂,



- 10 The term "alkyl" means a saturated hydrocarbon chain, which may be straight, branched or cyclic or cycle-containing.

The term "alkenyl" means a hydrocarbon chain containing at least one double bond between carbon atoms. The hydrocarbon chain may be straight, branched or cyclic or cycle-containing.

- The term "alkynyl" means a hydrocarbon chain containing at least one triple bond between carbon atoms, and optionally further one or more double bonds between carbon atoms. The hydrocarbon chain
 15 may be straight, branched or cyclic or cycle-containing.

The term "alkylene chain" means a saturated hydrocarbon chain, which may be straight, branched or cyclic or cycle-containing, but is preferably straight. This chain has two valencies, *i.e.* it binds as a linker or bridge via two bonds.

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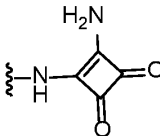
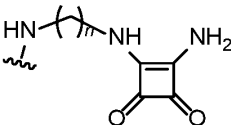
When the molecule of general formula I has a positive charge, the compound includes a counterion, which may be a pharmaceutically acceptable anion of an organic or inorganic acid, to form a pharmaceutically acceptable salt. Such an anion may be selected, for example, from the group consisting of acetate, aspartate, benzenesulphonate, benzoate, besylate, bicarbonate, bitartrate, bromide, camsylate,
 25 carbonate, chloride, citrate, decanoate, edetate, esylate, fumarate, gluceptate, gluconate, glutamate, glycolate, hexanoate, iodide, lactate, malate, maleate, mandelate, mesylate, methanesulphate, napsylate, nitrate, octanoate, oleate, palmoate, pantothenate, phosphate, polygalacturonate, propionate, salicylate, stearate, succinate, sulphate, tartrate, and tosylate.

- 30 When the compound of formula I contains chiral centers, then the formula I includes pure enantiomers as well as mixtures of enantiomers, including the racemate.

Formula I includes compounds of formula I in free form, as well as in the form of salts, addition salts (with acids or bases) and/or solvates, including hydrates or alcohol solvates.

- 5 The linker X in the formula I is formed by the reaction of attachment of the amine moieties of the molecule to the central adamantane core. These may therefore be different linkers, depending on the reaction chosen, *e.g.* the formation of ester, amide and their analogues, click reaction (*e.g.* azido-alkyne cycloaddition), etc. X is thus selected from the group consisting of -C(=O)NH-, -C(=O)O-, -C(=S)O-, -C(=O)S-, -C(=S)S-, -C(=O)NHNH-, -CH₂-, -O-, -OC(=O)-, -S-, -SC(=O)-, -NH-,
- 10 -NHNH-, -NHC(=O)-, -NHNHC(=O)-, -C≡C-, -CH=CH-, a five-membered heterocycle containing at least 2 nitrogen atoms, -CH₂C(=O)NH-, -CH₂C(=O)O-, -CH₂C(=S)O-, -CH₂C(=S)S-, -CH₂C(=O)NHNH-, -N=CH-, and -CH=N-. Preferably, X is selected from -C(=O)NH-, a five membered heterocycle containing at least 2 nitrogen atoms, and -C(=O)O-.
- 15 The linker Y is an alkylene chain providing at least a minimum distance of the amine from the linker X and the adamantane core. Y is a C₂-C₁₀ alkylene chain, preferably C₂-C₈ alkylene chain, wherein in the said alkylene chain, one or more -CH₂- groups may optionally be replaced with one or more O or S atoms.
- 20 The substituent Z may further modify the properties of the compound of formula I to a small extent.

- Preferably, Z is a hydrogen atom or -C(=O)R¹. The moiety -C(=O)R¹ is typically formed by a reaction of an amine-terminated molecule with the central adamantane core bearing a carboxylic acid group. R¹ is thus selected from the group consisting of -NH₂, -NH(CH₂)_nOH, -N[(CH₂)_nOH]₂, -NHCH(CH₂OH)₂,
- 25 -NHCH₂CH(-OH)CH₂OH, -NH(CH₂)_nC(=O)NH₂, -N[CH₂C(=O)NH₂]₂, -NHCH[C(=O)NH₂]₂,

-NH(CH₂)₂NHC(=O)NH₂, , and , wherein n is an integer within the range from 2 to 5.

- Preferably, R¹ is selected from -NH₂, -NH(CH₂)₂OH, -NHCH(CH₂OH)₂.
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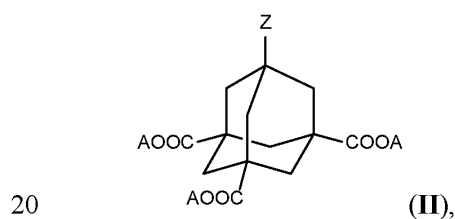
The R chains may be the same or different from each other, preferably they are the same, or all nitrogen atoms are substituted identically (with two identical Rs or two different Rs), for synthetic simplicity. R substituents are fatty chains, and they are selected from the group consisting of C₈-C₂₀ alkyl, C₈-C₂₀

alkenyl, and C₈-C₂₀ alkynyl, wherein in the said alkyl, alkenyl or alkynyl, one or more -CH₂- groups may optionally be replaced by one or more groups selected from -CH(OH)-, -OC(=O)-, -C(=O)O-, -SS-, -C(=O)NH-, -NHC(=O)-, -O-, and -S-. Preferably, substituents R are selected from a group consisting of C₈-C₂₀ alkyl, C₈-C₂₀ alkenyl, and C₈-C₂₀ alkynyl, wherein in the said alkyl, alkenyl or alkynyl, one or more -CH₂- groups may optionally be replaced by one or more groups selected from -CH(OH)-, -OC(=O)-, and -C(=O)O-.

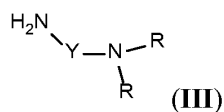
Preferably, R is selected from C₁₀-C₁₆ alkyl, C₁₀-C₁₆ alkyl in which -CH₂- group is replaced with -CH(OH)- or -C(=O)O-, C₁₄-C₂₀ alkenyl with one or two or three double bonds, C₁₄-C₂₀ alkenyl with one or two or three double bonds in which -CH₂- group is replaced by -C(=O)O- or -CH(OH)-.

Compounds of formula I are prepared by the corresponding reaction of an adamantane precursor substituted with group Z in position 7 and precursor groups of linker X in positions 1, 3, 5 with an amine of general formula X'-Y-NR₂, wherein X' is a precursor group of the linker X. The amine can be prepared by reactions and procedures known to those skilled in the art, and suitable amines are also commercially available.

In a more specific example, the compounds of formula I are preferably prepared by reacting a compound of formula II



wherein A is hydrogen, with a diamine of formula III



in the presence of a condensing agent and a base, or by reacting a compound of general formula II wherein A is a halogen, with a diamine of general formula III in the presence of a base.

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In formulas II and III, Z, Y and R are as described above.

The present invention also relates to a transfection agent comprising at least one lipidoid of general formula I, and at least one helper lipid. The transfection agent can be prepared by combining the components. Transfection agent in the form of a solution may be prepared by dissolving and mixing the

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components. Transfection agent in solid form (particulate form, preferably nanoparticulate form) can be prepared by means of techniques used in conventional nanoparticle technology, for example, by microfluidic mixing. The particles of the transfection agent are usually nanoparticles, which is generally understood to mean particles with dimensions in the range of 1 to 500 nm. Typically, the dimensions of the nanoparticles are in the range of 30 to 250 nm, more preferably 40 to 150 nm.

Preferably, the transfection agent contains at least one lipidoid of general formula I in an amount of 10 to 50 mol. %, and at least one helper lipid in an amount of 50 to 90 mol. %. Preferably, the transfection agent contains at least one lipidoid of general formula I in an amount of 15 to 30 mol. %, and at least one helper lipid in an amount of 70 to 85 mol. %. In some preferred embodiments, the transfection agent comprises at least one lipidoid of general formula I in an amount of 15 to 30 mol. %, cholesterol in an amount of 30 to 55 mol. %, and at least one other helper lipid in an amount of 20 to 50 mol. %.

In a particularly preferred embodiment, the transfection agent contains at least one lipidoid of general formula I in an amount of 15 to 30 mol. %, cholesterol in an amount of 30 to 55 mol. %, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine in an amount of 20 to 45 mol. % and 1,2-dimyristoyl-*rac*-glycero-3-methoxypolyethyleneglycol-2000 in an amount of 0.5 to 5 mol. %.

The present invention also relates to a transfection particle comprising at least one lipidoid of formula I, at least one nucleic acid and/or a part thereof and/or nucleic acid derivative, and preferably also at least one helper lipid. The transfection particles can be prepared, for example, by mixing a solution of a lipidoid of general formula I, optionally containing helper lipids, with a solution of the nucleic acid and/or a part thereof and/or nucleic acid derivative. Mixing can be performed by means of techniques used in conventional nanoparticle technology, for example, by microfluidic mixing.

The weight ratio of the total amount of nucleic acid and/or a part thereof and/or nucleic acid derivative to the total amount of lipidoid of general formula I and helper lipids in the transfection particle is preferably in the range of 1:2 to 1: 500, more preferably 1:5 to 1:100. Specifically and for illustration: in the particles which are prepared in the examples herein below, this ratio was around 1:9 for mRNA and around 1:68 for siRNA.

Transfection particles are usually nanoparticles, which is generally understood to mean particles with dimensions in the range of 1 to 500 nm. Typically, the dimensions of the transfection nanoparticles are in the range of 30 to 250 nm, preferably 40 to 200 nm, more preferably 40 to 150 nm.

The structure of the transfection particles was observed by cryogenic transmission electron microscopy, and the observation showed that the transfection particles were compact layered lipid nanoparticles containing nucleic acid (or a part or derivative thereof) inside.

Helper lipids in transfection reagents and transfection particles are mainly neutral lipids, sterols or lipid conjugates with hydrophilic polymers.

- Neutral lipids have a zero net charge at physiological pH and they can exist in an uncharged form or electroneutral zwitterionic form at physiological pH, such as 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(Cyanine 5).
- Sterols may, for example, be selected from cholesterol, β -sitosterol, stigmasterol, campesterol, fucosterol, avenasterol, fecosterol, brassicasterol, ergosterol, and 9,11-dehydroergosterol. Preferably, sterol is cholesterol.
- Lipid conjugates with hydrophilic polymers comprise a lipid portion and a polymer portion such as poly(ethyleneglycol), poly(2-ethyl-2-oxazoline), poly(2-methyl-2-oxazoline), poly(glycerol), poly(*N*-(2-hydroxypropyl) methacrylamide), poly(sarcosine) or glycol chitosan. Preferably, the polymer portion consists of poly(ethylene glycol) of molecular weight which may range from about 500 to about 10,000 Da, more preferably from about 1,000 to about 5,000 Da. Lipid conjugates with hydrophilic polymers may, for example, be selected from 1,2-dimyristoyl-*rac*-glycero-3-methoxy poly(ethyleneglycol)-2000, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-poly(ethylene glycol)-2000, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-poly(ethylene glycol)-2000, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-poly(ethylene glycol)-2000, and the like. Lipid conjugate with hydrophilic polymers may be preferably 1,2-dimyristoyl-*rac*-glycero-3-methoxy poly(ethyleneglycol)-2000.
- The nucleic acid or a part thereof are moieties containing one or more nucleotides and/or deoxynucleotides. The nucleic acid or the part thereof may be a therapeutic, diagnostic or prophylactic agent or may provide labeling for the cells or tissues into which they are transfected. The compounds of formula I thus have predominantly therapeutic or biotechnological uses.
- The term "nucleic acid or a part thereof" is understood to mean nucleic acids or their segments selected preferably from oligonucleotides (1-100 nucleotides, *e.g.* aptamers), cyclic dinucleotides (*e.g.* 2',3'-cGAMP), antisense oligonucleotides, deoxyribonucleic acid (single-stranded DNA, double-stranded DNA, cDNA, plasmid DNA encoding a gene or genes), ribonucleic acid, typically messenger RNA (mRNA), transfer RNA (tRNA), small interfering RNA (siRNA), double-stranded RNA, micro-RNA (miRNA), piwi-RNA (piRNA), antisense RNA (asRNA), guide RNA (gRNA) for the CRISPR system and their combinations (typically *e.g.* gRNA and mRNA encoding Cas9 nuclease, Cas13a/C2c2 and

Cas13b, or analogous nucleases, suitable for use in CRISPR, CRISPRi and other variations and subsequent modification of the host cell or tissue genome or modification of the host cell or tissue transcriptome). Furthermore, all nucleic acids (NA) disclosed herein may be formed or modified with synthetic base analogs, for example, to increase their stability in biological systems. Synthetic NA
5 analogs involve, in particular, the following substitutions: phosphorylation at the 5' and/or 3' end of the strand, 5-methylcytidine-5'-triphosphate, N¹-methylpseudouridine-5'-triphosphate, P¹-(5'-(3'-O-methyl)-7-methyl-guanosyl)-P³-(5'-(guanosyl))triphosphate, P¹-(guanosyl) P³-(5'-(guanosyl))triphosphate, P¹-(5'-7-methyl-guanosyl) P³-(5'-(guanosyl))triphosphate, P¹-(5'-2,2,7-trimethyl-guanosyl) P³-(5'-(guanosyl))triphosphate, N⁶-methyladenosine-5'-triphosphate, 2-thiouridine-5'-triphosphate,
10 pseudouridine-5'-triphosphate, 5-methoxyuridine-5'-triphosphate, N¹-methyladenosine-5'-triphosphate, N⁴-acetylcytidine-5'-triphosphate, 2'-O-methyl, 2'-O-methoxyethyl, 2'-fluoro, a methylene bridge between the 2'-oxygen and the 4'-carbon of the pentose ring (a so-called locked nucleic acid), boranophosphonates, or phosphorothioates.

15 The present invention further includes the use of lipidoids of formula I or transfection agents or transfection particles for transfecting cells or tissues with nucleic acid and/or a part thereof and/or nucleic acid derivative *in vitro*. In addition, the invention includes lipidoids of formula I or transfection particles for use in transfecting cells or tissues with nucleic acid and/or a part thereof and/or nucleic acid derivative *in vivo* (excluding the transfection of human embryos for industrial or commercial use, and
20 excluding the modification of human germ line).

Transfection particles containing lipidoids of general formula I are useful in a number of biological applications in basic research, especially for transfection of cell cultures or animals to deliver active nucleic acid and subsequent silencing or activation of a chromosomal gene or genes, genome editing
25 (gene excision, gene insertion or mutation introduction) or transcriptome editing, or enabling the expression of a given protein encoded by the nucleic acid inserted by means of a transfection particle, so-called "*in trans*".

In veterinary and human medicine, transfection particles containing lipidoids of the general formula I
30 can preferably be used for therapeutic or prophylactic purposes. Particles containing therapeutic nucleic acid can be administered to an animal or human to silence or activate chromosomal gene(s), to silence or activate immunogens, to inhibit or activate signaling pathways, to edit the genome (gene excision, gene insertion or mutation introduction) or the transcriptome, or enable the expression of protein(s) encoded by the nucleic acid.

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The present invention also provides lipidoids of general formula I or transfection agents or transfection particles for use as medicaments, in particular for gene therapy. In particular, they are suitable for use in the treatment of malignancies and/or genetic disorders.

- 5 The lipidoids of general formula I or transfection particles can be formulated for therapeutic, cosmetic or biotechnological use in the form of preparations with pharmaceutically acceptable excipients. The formulations may be in liquid or solid form, or in other forms, such as an aerosol. Liquid forms include solutions, suspensions, dispersions, adapted *e.g.* for injection or oral administration. Solid forms include, for example, capsules, tablets, coated tablets, powders, suppositories, and other forms.
- 10 The liquid formulations can be nebulized. Nebulized suspensions may be breathed in directly from the nebulizing device or the nebulizing device can be attached to face masks tent, or intermittent positive pressure breathing machine.
- The solid dosage forms may also be administered via inhalation using dry-powder inhalers. Suspension or dry powder formulations can be administered orally or nasally from devices which deliver the
- 15 pharmaceutical composition in an appropriate manner.
- In order to deliver an active substance on the skin or mucous membranes, the transfection particles with the active substance can be also prepared in the form of a cream, gel, ointment, paste, balm, liquid. These topical forms may be applied directly on the site of action.
- 20 Pharmaceutically acceptable excipients include solvents, solubility control agents, pH adjusting agents, carriers, fillers, binders, glidants, disintegrants, preservatives, sorbents, viscosity control agents, agents that affect sensory properties such as taste, odor or the color of the formulation.

Furthermore, lipidoids of general formula I or the transfection agents or transfection particles can

25 preferably be used for the purposes of the cosmetics industry in order to deliver an active substance to the site of action. The transfection particles with the active substance can be prepared in the form of a cream, gel, ointment, paste, balm, liquid and the like, and used as make-up, hair cosmetics or a personal hygiene product.

30 Brief Description of Drawings

- Figure 1. Synthetic scheme of lipidoids **4a-g**.
- Figure 2. Synthetic scheme of lipidoid **9**.
- Figure 3. Synthetic scheme of lipidoid **13**.
- Figure 4. Synthetic scheme of compound **19**, precursor of lipidoid **21**.
- 35 Figure 5. Synthetic scheme of lipidoid **21**.
- Figure 6. Synthetic scheme of compound **22** and its acylchloride, precursors of lipidoids **23-25**.

Figure 7. Synthetic scheme of lipidoids **23-25**.

Figure 8. Synthetic scheme of lipidoids **29a-f**

Figure 9. Synthetic scheme of lipidoids **31a-d**.

Figure 10. Test of functional delivery of mRNA-LNP (**B39**) to murine liver.

5

Examples

List of abbreviations:

	eq.	equivalent
10	R_f	retention factor
	TLC	thin-layer chromatography
	RVE	rotary vacuum evaporator
	rt	room temperature
	br s	broad signal
15	s	singlet
	d	doublet
	m	multiplet
	dd	doublet of doublets
	J	interaction constant
20	δ	chemical shift
	HRMS	high-resolution mass spectrometry
	ESI	electrospray ionization
	MALDI	matrix-assisted laser desorption/ionisation
	IR	infrared spectroscopy
25	NMR	nuclear magnetic resonance
	CE5	cyclohexane-ethylacetate mixture 95:5 (v/v)
	CE20	cyclohexane-ethylacetate mixture 80:20 (v/v)
	CE50	cyclohexane-ethylacetate mixture 50:50 (v/v)
	D1	dichloromethane-methanol-25% aqueous NH_3 mixture 75::3 (v/v/v)
30	D2	dichloromethane-methanol-25% aqueous NH_3 mixture 175:22:3 (v/v/v)
	D3	dichloromethane-methanol-25% aqueous NH_3 mixture 275:22:3 (v/v/v)
	D4	dichloromethane-methanol-25% aqueous NH_3 mixture 375:22:3 (v/v/v)
	TFA	trifluoroacetic acid
	HCTU	<i>O</i> -(1 <i>H</i> -6- chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium
35		hexafluorophosphate
	DIPEA	<i>N,N</i> - diisopropylethylamine

	DMF	<i>N,N</i> -dimethylformamide
	DCM	dichloromethane
	ACN	acetonitrile
	TBDPSCI	<i>tert</i> -butyldiphenylchlorosilane
5	DIC	diisopropylcarbodiimide
	DMAP	4-dimethylaminopyridine
	LNP	lipid nanoparticles
	NA	nucleic acid
	DNA	deoxyribonucleic acid
10	RNA	ribonucleic acid
	mRNA	messenger RNA
	siRNA	small interfering RNA
	tRNA	transfer RNA
	miRNA	micro RNA
15	ssDNA/RNA	single-stranded DNA/RNA
	dsDNA/RNA	double-stranded DNA/RNA
	DMG-PEG ₂₀₀₀	1,2-dimyristoyl- <i>rac</i> -glycero-3-methoxypolyethyleneglycol-2000
	DOPE	1,2-dioleoyl- <i>sn</i> -glycero-3-phosphoethanolamine
	DOPC	1,2-dioleoyl- <i>sn</i> -glycero-3-phosphocholine
20	DSPC	1,2-distearoyl- <i>sn</i> -glycero-3-phosphocholine
	DOPE-Cy5	1,2-dioleoyl- <i>sn</i> -glycero-3-phosphoethanolamine- <i>N</i> -(Cyanine 5)
	Lip2000	Lipofectamine [®] 2000 (Invitrogen)

Example 1

25

***N*¹,*N*¹-Didodecylethan-1,2-diamine 3a**

A 500 ml round-bottom flask equipped with a chlorocalcium cap and magnetic stirrer was filled with a solution of amine **1a** (5.00 g, 31.2 mmol) in DCM (100 ml) and cooled to 0 °C in an ice bath. With intensive stirring, n-dodecylaldehyde (20.8 ml, 93.6 mmol, 3 eq.) was added, followed by sodium triacetoxyborohydride (19.8 g, 93.6 mmol, 3 eq.) in three portions over 10 minutes. The cooling bath was removed, and the reaction mixture was stirred at room temperature for 2 h. The progress of the reaction was monitored by TLC using an 80:20 (v/v) hexane-ethylacetate mobile phase on a TLC plate pre-saturated with ammonia (detection with ninhydrin). After completion of the reaction, aqueous NaOH solution (1 M, 200 ml) was added, the reaction mixture was stirred for 15 min, then poured into a separatory funnel and diluted with water (300 ml). The product was extracted with DCM (300 ml, 2x50 ml), the combined organic phase was washed with brine (50 ml), dried over anhydrous sodium

sulphate, filtered through an S2 frit, and the solvents were evaporated in an RVE. The dark oily residue was purified by silica gel column chromatography using a linear gradient of ethyl acetate in hexane (10-30 %). Amine **2a** (5.12 g, 33.0 %) was obtained as a yellowish oil.

Trifluoroacetic acid (10 ml) was added to a solution of compound **2a** (5.12 g) in DCM (10 ml), cooled to 0 °C with stirring in an ice bath, and the reaction mixture was left at 0 °C for 3 h. The solution was then poured into a 1 l separatory flask, diluted with 20% aqueous Na₂CO₃ (300 ml), and the product was extracted with DCM (250 ml, 2 x 50 ml). The combined organic phase was washed with brine (100 ml), dried over anhydrous sodium sulphate, filtered through an S2 frit, and the solvents were evaporated in an RVE. The crude product was purified by silica gel column chromatography using a linear gradient of D1 in DCM (0-70 %). The diamine **3a** (2.55 g, 62.4 %; *R_f* 0.46 in mobile phase D2 on a TLC plate pre-saturated with ammonia, detection with ninhydrin) was obtained in the form of a yellowish oil. ¹H NMR (600 MHz, CDCl₃): δ = 2.895, 2.64, 2.48, 1.45, 1.28, 1.26, 1.24–1.28, 1.24, 0.87 ppm. ¹³C NMR (150.9 MHz, CDCl₃): δ = 54.31, 53.85, 38.33, 31.90, 29.65, 29.62, 29.61, 29.52, 29.34, 26.36, 23.88, 22.67, 14.10 ppm. IR (film): ν_{max}/cm⁻¹ = 3371 w and 3315 w (ν NH₂), 2801 m (ν_s N-CH₂), 2953 s (ν_{as} CH₃), 2924 vs (ν_{as} CH₂), 2853 s (ν_s CH₂), 1467 m and 1457 m, sh (β_s CH₂ and δ_{as} CH₃), 1378 w and 1367 w (δ_s CH₃), 721 m (β_{as} CH₂). HRMS (ESI): *m/z* calculated for C₂₆H₅₇N₂ [M + H]⁺ 397.45163; found 397.45093.

N*¹,*N*³,*N*⁵-Tris(2-(didodecylamino)ethyl) adamantane-1,3,5-tricarboxamide **4a*

O-(1*H*-6-chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU, 256 mg, 0.596 mmol, 4 eq.) and *N,N*-diisopropylethylamine (DIPEA, 0.415 ml, 2.39 mmol, 16 eq.) were added to a solution of adamantane-1,3,5-tricarboxylic acid (40 mg, 0.149 mmol) in anhydrous DMF (1.5 ml), and the solution was stirred for 15 min at room temperature. Then a solution of *N*¹,*N*¹-didodecylethane-1,2-diamine **3a** (237 mg, 0.149 mmol, 4 eq.) in DCM (1.0 ml) was added, and the reaction mixture was stirred for 12 h. The solution was poured into a 250 ml separatory flask, diluted with saturated aqueous NaHCO₃ (50 ml), and the product was extracted with DCM (50 ml, 2 x 20 ml). The combined organic phase was washed with brine (20 ml), dried over anhydrous sodium sulphate, filtered through an S2 frit, and the solvents were evaporated in an RVE. The crude product was purified by silica gel column chromatography using a linear gradient of D1 in DCM (20-50%). Lipidoid **4a** (71 mg, 33.9 %; *R_f* 0.73 in D2 in mobile phase D2 on a TLC plate pre-saturated with ammonia, detection with ninhydrin) was obtained in the form of a viscous yellowish oil. ¹H NMR (600 MHz, CDCl₃): δ = 7.33, 3.56, 3.32, 3.125, 3.075, 2.34, 2.01, 1.91, 1.79, 1.67, 1.36, 1.285, 1.26–1.32, 1.25, 0.88 ppm. ¹³C NMR (150.9 MHz, CDCl₃): δ = 180.48, 56.72, 53.78, 41.38, 39.04, 36.86, 36.29, 31.90, 29.64, 29.62, 29.50, 29.40, 29.35, 29.04, 27.79, 26.36, 23.88, 22.68, 14.10 ppm. IR (CCl₄): ν_{max}/cm⁻¹ = 3440 w and 3322 w (ν NH), 1653 w (amide I) and 1623 w (amide I bound), 1535 w (amide II), 2956 m (ν_{as} CH₃), 2927 vs (ν_{as} CH₂), 2855

m (ν_s CH₂), 1467 w and 1457 w (β_s CH₂ and δ_{as} CH₃), 1378 w (δ_s CH₃). **HRMS** (MALDI): m/z calculated for C₉₁H₁₇₉N₆O₃ [M + H]⁺ 1404.4033; found 1404.4012.

Example 2

5 *N*¹,*N*¹-Didodecylpropane-1,3-diamine **3b**

Amine **2b** was prepared from amine **1b** (6.0 g, 34.43 mmol), n-dodecylaldehyde (22.91 ml, 103.30 mmol, 3 eq.) and sodium triacetoxyborohydride (21.89 g, 103.30 mmol, 3 eq.) according to the procedure described for compound **2a** in Example 1. Amine **2b** was obtained as a yellowish oil (7.72 g, 43.9 %).

- 10 The deprotection of amine **2b** was performed according to the procedure described for compound **2a** in Example 1; diamine **3b** (4.26 g, 68.6 %; *R_f* 0.35 in mobile phase D2 on a TLC plate pre-saturated with ammonia, detection with ninhydrin) was obtained in the form of a yellowish oil. **¹H NMR** (600 MHz, CDCl₃): δ = 3.07, 2.70, 2.50, 1.81, 1.46, 1.28, 1.26, 1.25–1.29, 1.24, 0.87 ppm. **¹³C NMR** (150.9 MHz, CDCl₃): δ = 53.70, 53.30, 41.18, 31.90, 29.64, 29.62, 29.60, 29.58, 29.48, 29.33, 27.42, 25.71, 23.87, 22.67, 14.10 ppm. **IR** (film): ν_{max}/cm^{-1} = 3361 w and 3274 w (ν NH₂), 2803 m (ν_s N-CH₂), 2954 s (ν_{as} CH₃), 2924 vs (ν_{as} CH₂), 2853 s (ν_s CH₂), 1467 m and 1456 m, sh (β_s CH₂ and δ_{as} CH₃), 1378 w and 1364 w (δ_s CH₃), 720 m (β_{as} CH₂). **HRMS** (ESI): m/z calculated for C₂₇H₅₉N₂ [M + H]⁺ 411.46728; found 411.46652.

20 *N*¹,*N*³,*N*⁵-tris(3-(didodecylamino)propyl)adamantane-1,3,5-tricarboxamide **4b**

Lipidoid **4b** was prepared from adamantane-1,3,5-tricarboxylic acid (20 mg, 0.075 mmol), HCTU (128 mg, 0.298 mmol, 4 eq.), DIPEA (0.208 ml, 1.19 mmol, 16 eq.) and diamine **3b** (123 mg, 0.298 mmol, 4 eq.) according to the procedure described for compound **4a** in Example 1; lipidoid **4b** (64 mg, 59.3 %; *R_f* 0.51 in mobile phase D2 on a TLC plate pre-saturated with ammonia, detection with ninhydrin) was obtained as a viscous yellow oil. **¹H NMR** (600 MHz, CDCl₃): δ = 7.59, 3.36, 2.99, 2.88, 2.31, 2.13, 2.00, 1.96, 1.81, 1.67, 1.31, 1.28, 1.25–1.30, 1.24, 0.87 ppm. **¹³C NMR** (150.9 MHz, CDCl₃): δ = 177.39, 55.22, 50.70, 41.78, 40.38, 37.20, 35.67, 31.89, 29.60, 29.51, 29.48, 29.32, 29.19, 28.39, 26.94, 24.10, 23.68, 22.67, 14.10 ppm. **IR** (CCl₄): ν_{max}/cm^{-1} = 3466 w and 3287 w (ν NH), 1656 m (amide I) and 1511 m (amide II), 2814 w (ν_s CH₂NR₂), 2954 s (ν_{as} CH₃), 2927 vs (ν_{as} CH₂), 2871 m (ν_s CH₃), 2855 s (ν_s CH₂), 1468 m and 1456 m (β_s CH₂ and δ_{as} CH₃), 1378 w (δ_s CH₃), 721 w (β_{as} CH₂). **HRMS** (MALDI): m/z calculated for C₉₄H₁₈₅O₃N₆ [M + H]⁺ 1446.45027; found 1446.44896.

Example 3

*N*¹,*N*¹-Didodecylbutane-1,4-diamine **3c**

Amine **2c** was prepared from amine **1c** (5.0 g, 26.56 mmol), n-dodecylaldehyde (17.67 ml, 79.67 mmol, 3 eq.) and sodium triacetoxyborohydride (16.89 g, 79.67 mmol, 3 eq.) according to the procedure described for compound **2a** in Example 1. Amine **2c** was obtained as a yellowish oil (4.15 g, 29.8 %).

The deprotection of amine **2c** was performed according to the procedure described for compound **2a** in Example 1; diamine **3c** (2.36 g, 70.3 %; R_f 0.29 in mobile phase D2 on a TLC plate pre-saturated with ammonia, detection with ninhydrin) was obtained in the form of a yellowish oil. $^1\text{H NMR}$ (600 MHz, CDCl_3): δ = 2.85, 2.81, 2.59, 1.725, 1.68, 1.51, 1.28, 1.265, 1.25–1.30, 1.24, 0.87 ppm. $^{13}\text{C NMR}$ (150.9 MHz, CDCl_3): δ = 53.33, 52.74, 40.44, 31.90, 29.64, 29.60, 29.60, 29.40, 29.33, 28.65, 27.46, 24.83, 24.40, 22.67, 14.10 ppm. IR (film): $\nu_{\text{max}}/\text{cm}^{-1}$ = 3370 w and 3274 w ($\nu \text{ NH}_2$), 2798 m ($\nu_s \text{ N-CH}_2$), 2957 s ($\nu_{\text{as}} \text{ CH}_3$), 2924 vs ($\nu_{\text{as}} \text{ CH}_2$), 2853 s ($\nu_s \text{ CH}_2$), 1467 m and 1456 m, sh ($\beta_s \text{ CH}_2$ and $\delta_{\text{as}} \text{ CH}_3$), 1378 w and 1367 w ($\delta_s \text{ CH}_3$), 720 m ($\beta_{\text{as}} \text{ CH}_2$). HRMS (ESI): m/z calculated for $\text{C}_{28}\text{H}_{61}\text{N}_2$ $[\text{M} + \text{H}]^+$ 425.48293; found 425.48227.

*N*¹,*N*³,*N*⁵-tris(4-(didodecylamino)butyl)adamantane-1,3,5-tricarboxamide **4c**

Lipidoid **4c** was prepared from adamantane-1,3,5-tricarboxylic acid (40 mg, 0.149 mmol), HCTU (256 mg, 0.596 mmol, 4 eq.), DIPEA (0.416 ml, 2.39 mmol, 16 eq.) and diamine **3c** (253 mg, 0.596 mmol, 4 eq.) according to the procedure described for compound **4a** in Example 1; lipidoid **4c** (64 mg, 28.8 %; R_f 0.48 in mobile phase D2 on a TLC plate pre-saturated with ammonia, detection with ninhydrin) was obtained in the form of a viscous yellowish oil. $^1\text{H NMR}$ (600 MHz, CDCl_3): δ = 7.27, 3.335, 3.03, 2.98, 2.28, 2.12, 2.06, 1.875, 1.81, 1.74, 1.64, 1.33, 1.28, 1.25–1.29, 1.24, 0.87 ppm. $^{13}\text{C NMR}$ (150.9 MHz, CDCl_3): δ = 177.27, 52.78, 52.27, 41.83, 40.26, 37.36, 37.20, 31.88, 29.58, 29.49, 29.43, 29.31, 29.10, 28.37, 26.82, 26.56, 22.94, 22.66, 20.79, 14.10 ppm. IR (CCl_4): $\nu_{\text{max}}/\text{cm}^{-1}$ = 3441 w and 3329 w ($\nu \text{ NH}$), 1641 m (amide I), 1534 w (amide II), 2956 m ($\nu_{\text{as}} \text{ CH}_3$), 2927 vs ($\nu_{\text{as}} \text{ CH}_2$), 2855 m ($\nu_s \text{ CH}_2$), 1466 m and 1458 m ($\beta_s \text{ CH}_2$ and $\delta_{\text{as}} \text{ CH}_3$), 1378 w ($\delta_s \text{ CH}_3$). HRMS (MALDI): m/z calculated for $\text{C}_{97}\text{H}_{191}\text{N}_6\text{O}_3$ $[\text{M} + \text{H}]^+$ 1488.4972; found 1488.4956.

Example 4

*N*¹,*N*¹-Didodecylpentane-1,5-diamine **3d**

Amine **2d** was prepared from amine **1d** (5.0 g, 24.72 mmol), n-dodecylaldehyde (16.45 ml, 74.15 mmol, 3 eq.) and sodium triacetoxyborohydride (15.71 g, 74.15 mmol, 3 eq.) According to the procedure described for compound **2a** in Example 1. Amine **2d** was obtained as a yellowish oil (6.01 g, 45.1 %).

The deprotection of amine **2d** was performed according to the procedure described for compound **2a** in Example 1; diamine **3d** (4.32 g, 88.3 %; R_f 0.28 in mobile phase D2 on a TLC plate pre-saturated with ammonia, detection with ninhydrin) was obtained as a yellowish oil. $^1\text{H NMR}$ (600 MHz, CDCl_3): δ = 2.75, 2.68, 2.55, 1.565, 1.53, 1.50, 1.35, 1.28, 1.27, 1.24–1.28, 1.24, 0.87 ppm. $^{13}\text{C NMR}$ (150.9 MHz, CDCl_3): δ = 53.61, 53.55, 41.48, 32.10, 31.89, 29.63, 29.61, 29.58, 29.45, 29.32, 27.41, 25.74, 24.90,

24.55, 22.66, 14.09 ppm. **IR** (film): $\nu_{\max}/\text{cm}^{-1} = 3367$ w and 3284 w (ν NH₂), 2797 m (ν N-CH₂), 2956 s (ν_{as} CH₃), 2924 vs (ν_{as} CH₂), 2853 s (ν_{s} CH₂), 1467 m and 1456 m, sh (β_{s} CH₂ and δ_{as} CH₃), 1378 w and 1367 w (δ_{s} CH₃), 720 m (β_{as} CH₂). **HRMS** (ESI): m/z calculated for C₂₉H₆₃N₂ [M + H]⁺ 439.49858; found 439.49783.

5

***N*¹,*N*³,*N*⁵-Tris(5-(didodecylamino)pentyl)adamantane-1,3,5-tricarboxamide 4d**

Lipidoid **4d** was prepared from adamantane-1,3,5-tricarboxylic acid (40 mg, 0.149 mmol), HCTU (256 mg, 0.596 mmol, 4 eq.), DIPEA (0.416 ml, 2.39 mmol, 16 eq.) and diamine **3d** (262 mg, 0.596 mmol, 4 eq.) according to the procedure described for compound **4a** in Example 1; lipidoid **4d** (74 mg, 32.4 %; *R*_f 0.49 in mobile phase D2 on a TLC plate pre-saturated with ammonia, detection with ninhydrin) was obtained as a viscous yellowish oil. **¹H NMR** (600 MHz, CDCl₃): $\delta = 6.91, 3.27, 2.99, 2.29, 2.09, 1.97, 1.82, 1.81, 1.76, 1.59, 1.45, 1.34, 1.28, 1.25\text{--}1.30, 1.24, 0.87$ ppm. **¹³C NMR** (150.9 MHz, CDCl₃): $\delta = 177.01, 52.77, 52.30, 41.82, 40.10, 38.33, 37.59, 31.88, 29.58, 29.48, 29.43, 29.30, 29.09, 28.53, 28.39, 26.84, 23.83, 23.09, 23.02, 22.66, 14.10$ ppm. **IR** (CCl₄): $\nu_{\max}/\text{cm}^{-1} = 3322$ w (ν NH), 1640 m (amide I), 1535 w (amide II), 2956 m (ν_{as} CH₃), 2927 vs (ν_{as} CH₂), 2855 m (ν_{s} CH₂), 1467 m and 1457 m (β_{s} CH₂ and δ_{as} CH₃), 1378 w (δ_{s} CH₃), 722 m (β_{as} CH₂). **HRMS** (MALDI): m/z calculated for C₁₀₀H₁₉₇N₆O₃ [M + H]⁺ 1530.5442; found 1530.5478.

Example 5

***N*¹,*N*¹-Didodecylhexane-1,6-diamine 3e**

Amine **2e** was prepared from amine **1e** (5.0 g, 23.11 mmol), n-dodecylaldehyde (15.38 ml, 69.34 mmol, 3 eq.) and sodium triacetoxyborohydride (14.70 g, 69.34 mmol, 3 eq.) according to the procedure described for compound **2a** in Example 1. Amine **2e** was obtained as a yellowish oil (3.67 g, 28.7 %). The deprotection of amine **2e** was performed according to the procedure described for compound **2a** in Example 1; diamine **3e** (2.17 g, 72.2 %; *R*_f 0.31 in mobile phase D2 on a TLC plate pre-saturated with ammonia, detection with ninhydrin) was obtained as a yellowish oil. **¹H NMR** (600 MHz, CDCl₃): $\delta = 2.73, 2.65, 2.57, 1.56, 1.52, 1.51, 1.36, 1.31, 1.28, 1.25\text{--}1.29, 1.24, 0.87$ ppm. **¹³C NMR** (150.9 MHz, CDCl₃): $\delta = 53.51, 53.20, 41.62, 32.40, 31.89, 29.63, 29.61, 29.57, 29.44, 29.32, 27.39, 27.09, 26.53, 25.65, 25.02, 22.66, 14.10$ ppm. **IR** (film): $\nu_{\max}/\text{cm}^{-1} = 3374$ w and 3294 w (ν NH₂), 2797 m (ν N-CH₂), 2956 s (ν_{as} CH₃), 2924 vs (ν_{as} CH₂), 2853 s (ν_{s} CH₂), 1467 m and 1455 m, sh (β_{s} CH₂ and δ_{as} CH₃), 1378 w and 1367 w (δ_{s} CH₃), 721 m (β_{as} CH₂). **HRMS** (ESI): m/z calculated for C₃₀H₆₅N₂ [M + H]⁺ 453.51423; found 453.51340.

***N*¹,*N*³,*N*⁵-Tris(6-(didodecylamino)hexyl)adamantane-1,3,5-tricarboxamide 4e**

Lipidoid **4e** was prepared from adamantane-1,3,5-tricarboxylic acid (40 mg, 0.149 mmol), HCTU (256 mg, 0.596 mmol, 4 eq.), DIPEA (0.416 ml, 2.39 mmol, 16 eq.) and diamine **3e** (270 mg, 0.596 mmol, 4

eq.) according to the procedure described for compound **4a** in Example 1; lipidoid **4e** (91 mg, 38.8 %; R_f 0.52 in mobile phase D2 on a TLC plate pre-saturated with ammonia, detection with ninhydrin) was obtained as a viscous yellowish oil. $^1\text{H NMR}$ (600 MHz, CDCl_3): δ = 7.80, 3.33, 3.03, 2.99, 2.37, 2.27, 2.02, 1.92, 1.81, 1.76, 1.62, 1.43, 1.41, 1.34, 1.285, 1.26–1.30, 1.24, 0.88 ppm. $^{13}\text{C NMR}$ (150.9 MHz, CDCl_3): δ = 177.87, 52.87, 52.26, 41.74, 39.31, 36.86, 31.90, 29.60, 29.50, 29.44, 29.32, 29.10, 28.26, 26.82, 25.77, 25.52, 23.44, 23.14, 22.68, 14.12 ppm. **IR** (CCl_4): $\nu_{\text{max}}/\text{cm}^{-1}$ = 3463 w and 3327 w (ν NH), 1641 m (amide I), 1535 m (amide II), 2958 s (ν_{as} CH_3), 2871 s (ν_{s} CH_3), 1467 m and 1457 m (β_{s} CH_2 and δ_{as} CH_3), 2927 s (ν_{as} CH_2), 2855 s (ν_{s} CH_2), 1378 w (δ_{s} CH_3), 721 w (β_{as} CH_2), 2799 w (ν_{s} CH_2NR_2). **HRMS** (MALDI): m/z calculated for $\text{C}_{103}\text{H}_{203}\text{N}_6\text{O}_3$ [$\text{M} + \text{H}$] $^+$ 1572.5911; found 1572.5881.

10

Example 6

N^1,N^1 -Didodecylheptane-1,7-diamine **3f**

Amine **2f** was prepared from amine **1f** (1.0 g, 4.34 mmol), *n*-dodecylaldehyde (3.14 ml, 13.02 mmol, 3 eq.) and sodium triacetoxyborohydride (2.76 g, 13.02 mmol, 3 eq.) according to the procedure described for compound **2a** in Example 1. Amine **2f** was obtained as a yellowish oil (1.74 g, 70.6 %).

The deprotection of amine **2f** was performed in a mixture of TFA (4 ml) and DCM (4 ml) according to the procedure described for **2a** in Example 1; diamine **3f** (0.842 g, 59.1 %; R_f 0.38 in mobile phase D2 on a TLC plate pre-saturated with ammonia, detection with ninhydrin) was obtained as a yellowish oil. $^1\text{H NMR}$ (600 MHz, CDCl_3): δ = 2.73, 2.69, 2.64, 1.56, 1.50, 1.30, 1.28, 1.25–1.31, 1.25, 0.87 ppm. $^{13}\text{C NMR}$ (150.9 MHz, CDCl_3): δ = 53.37, 53.20, 41.62, 32.22, 31.89, 27.10–29.60, 26.57, 25.14, 22.66, 14.10 ppm. **IR** (CCl_4): $\nu_{\text{max}}/\text{cm}^{-1}$ = 3391 vw (ν_{as} NH_2); 2960 s, sh (ν_{as} CH_3); 2927 vs (ν_{as} CH_2); 2872 s, sh (ν_{s} CH_3); 2855 vs (ν_{s} CH_2); 2798 m (ν_{s} N-CH_2); 1467 s and 1458 m (β_{s} CH_2 and δ_{as} CH_3); 1378 w (δ_{s} CH_3); 1302 w (γ_{s} CH_2); 721 w (β_{as} and γ_{as} CH_2). **HRMS** (ESI): m/z calculated for $\text{C}_{31}\text{H}_{67}\text{N}_2$ [$\text{M} + \text{H}$] $^+$ 467.52988; found 467.52974.

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N^1,N^3,N^5 -Tris(7-(didodecylamino)heptyl)adamantane-1,3,5-tricarboxamide **4f**

Lipidoid **4f** was prepared from adamantane-1,3,5-tricarboxylic acid (40 mg, 0.149 mmol), HCTU (256 mg, 0.596 mmol, 4 eq.), DIPEA (0.416 ml, 2.39 mmol, 16 eq.) and diamine **3f** (278 mg, 0.596 mmol, 4 eq.) according to the procedure described for compound **4a** in Example 1; lipidoid **4f** (199 mg, 82.6 %; R_f 0.55 in mobile phase D2 on a TLC plate pre-saturated with ammonia, detection with ninhydrin) was obtained as a viscous yellowish oil. $^1\text{H NMR}$ (600 MHz, CDCl_3): δ = 7.97, 3.22, 3.09, 2.34, 1.92, 1.83, 1.82, 1.70, 1.58, 1.52, 1.36, 1.30, 1.285, 1.28, 1.25–1.32, 1.25, 0.88 ppm. $^{13}\text{C NMR}$ (150.9 MHz, CDCl_3): δ = 177.27, 54.00, 53.14, 41.59, 39.48, 39.30, 36.92, 31.89, 29.60, 29.48, 29.39, 29.32, 29.06, 28.22, 26.50, 25.58, 23.63, 23.38, 22.67, 14.10 ppm. **IR** (CCl_4): $\nu_{\text{max}}/\text{cm}^{-1}$ = 3438 w (free) and 3339, 3196 w (bound) (ν NH); 1627 m (amide I); 1535 m (amide II); 2956 s, sh (ν_{as} CH_3); 2873 m, sh (ν_{s} CH_3); 1468 m and 1457 m, sh (β_{s} CH_2 and δ_{as} CH_3); 2927 vs (ν_{as} CH_2); 2856 s (ν_{s} CH_2); 1378 w (δ_{s} CH_3); 722

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w (β_{as} CH₂); 2805 w (ν_{s} CH₂NR₂). **HRMS** (MALDI): m/z calculated for C₁₀₆H₂₀₉N₆O₃ [M + H]⁺ 1614.6381; found 1614.6414.

Example 7

5 *N*¹,*N*¹-Didodecyloctane-1,8-diamine **3g**

Amine **2g** was prepared from amine **1g** (1.0 g, 4.09 mmol), n-dodecylaldehyde (2.96 ml, 12.28 mmol, 3 eq.) and sodium triacetoxyborohydride (2.60 g, 12.28 mmol, 3 eq.) according to the procedure described for compound **2a** in Example 1. The amine **2g** was obtained as a yellowish oil (1.98 g, 83.1 %).

- 10 The deprotection of amine **2g** was performed in a mixture of TFA (4 ml) and DCM (4 ml) according to the procedure described for **2a** in Example 1; diamine **3g** (0.848 g, 52.0 %; *R_f* 0.35 in mobile phase D2 on a TLC plate pre-saturated with ammonia, detection with ninhydrin) was obtained in the form of a yellowish oil. **¹H NMR** (600 MHz, CDCl₃): δ = 2.70, 2.63, 2.53, 1.51, 1.47, 1.32, 1.28, 1.25–1.32, 1.25, 0.87 ppm. **¹³C NMR** (150.9 MHz, CDCl₃): δ = 53.61, 53.20, 41.92, 33.00, 31.90, 27.30–29.60, 26.70, 25.83, 22.67, 14.10 ppm. **IR** (CCl₄): $\nu_{\text{max}}/\text{cm}^{-1}$ = 3391 vw (ν_{as} NH₂); 2960 s, sh (ν_{as} CH₃); 2927 vs (ν_{as} CH₂); 2872 s, sh (ν_{s} CH₃); 2855 vs (ν_{s} CH₂); 2799 m (ν_{s} N-CH₂); 1467 s and 1458 m (β_{s} CH₂ and δ_{as} CH₃); 1378 w (δ_{s} CH₃); 1302 w (γ_{s} CH₂); 721 w (β_{as} and γ_{as} CH₂). **HRMS** (ESI): m/z calculated for C₃₂H₆₉N₂ [M + H]⁺ 481.54553; found 481.54507.

20 *N*¹,*N*³,*N*⁵-Tris(8-(didodecylamino)octyl)adamantane-1,3,5-tricarboxamide **4g**

Lipidoid **4g** was prepared from adamantane-1,3,5-tricarboxylic acid (40 mg, 0.149 mmol), HCTU (256 mg, 0.596 mmol, 4 eq.), DIPEA (0.416 ml, 2.39 mmol, 16 eq.) and diamine **3g** (286 mg, 0.596 mmol, 4 eq.) according to the procedure described for compound **4a** in Example 1; lipidoid **4g** (211 mg, 85.4 %; *R_f* 0.60 in mobile phase D2 on a TLC plate pre-saturated with ammonia, detection with ninhydrin) was obtained as a viscous yellowish oil. **¹H NMR** (600 MHz, CDCl₃): δ = 8.03, 3.21, 3.09, 3.07, 2.35, 1.985, 1.82, 1.815, 1.71, 1.51, 1.335, 1.33, 1.29, 1.28, 1.25–1.33, 1.25, 0.88 ppm. **¹³C NMR** (150.9 MHz, CDCl₃): δ = 177.22, 54.04, 53.07, 41.56, 40.01, 39.08, 36.95, 31.89, 29.60, 29.49, 29.40, 29.32, 29.06, 28.45, 28.42, 28.30, 26.53, 26.13, 26.01, 23.88, 23.45, 22.67, 14.10 ppm. **IR** (CCl₄): $\nu_{\text{max}}/\text{cm}^{-1}$ = 3439 w (free) and 3341, 3196 w (bound) (ν NH); 1635, 1627 w (amide I); 1533 w (amide II); 2954 m, sh (ν_{as} CH₃); 2873 m, sh (ν_{s} CH₃); 1467 m and 1457 w, sh (β_{s} CH₂ and δ_{as} CH₃); 2927 vs (ν_{as} CH₂); 2856 s (ν_{s} CH₂); 1378 w (δ_{s} CH₃); 2810 vw, sh (ν_{s} CH₂NR₂). **HRMS**: m/z calculated for C₁₀₉H₂₁₅N₆O₃ [M + H]⁺ 1656.6856; found 1656.6882.

Example 8

35 **1,2-Epoxydodecane 6**

N-chlorosuccinimide (NCS, 3.44 g, 25.77 mmol, 0.95 eq.) and L-proline (0.937 g, 8.14 mmol, 0.30 eq.) were added to a solution of n-dodecylaldehyde (6.0 ml, 27.13 mmol) in acetonitrile (70 ml), cooled to 0 °C in an ice bath, and the mixture was stirred at 0 °C for 2 h. Then the solution was diluted with ethanol (40 ml), NaBH₄ (2.57 g, 67.82 mmol, 2.5 eq.) was added, and the reaction mixture was stirred at 0 °C for 3.5 h. The solution was poured into a 1000 ml separatory flask, diluted with water (100 ml) and brine (100 ml), and the product was extracted with ethyl acetate (300 ml, 50 ml). The combined organic phase was washed with brine (100 ml), dried over anhydrous sodium sulphate, filtered through an S2 frit, and the solvents were evaporated in an RVE. The crude product was purified by silica gel column chromatography using a linear gradient of ethyl acetate in cyclohexane (0-20 %). Chloroalcohol **5** (2.79 g, 46.6 %; *R_f* 0.42 in mobile phase CE20, detection with KMnO₄) was obtained as a colorless oil.

A solution of NaOH (11.37 g, 0.284 mmol, 22.5 eq.) in water (49 ml) was added to a solution of chloroalcohol **5** (2.79 g, 12.64 mmol) in dioxane (38 ml), and the mixture was stirred for 30 h at 35 °C. The solution was then poured into a 500 ml separatory flask, diluted with water (100 ml), and the product was extracted with DCM (100 ml, 50 ml). The combined organic phase was washed with brine (50 ml), dried over anhydrous sodium sulphate, filtered through an S2 frit, and the solvents were evaporated in an RVE. The crude product was purified by silica gel column chromatography using a linear gradient of ethyl acetate in cyclohexane (0-5 %). Epoxide **6** (1.797 g, 77.2 %; *R_f* 0.38 in mobile phase CE5, detection with phosphomolybdic acid/Ce⁴⁺) was obtained as a colorless oil. ¹H NMR (400 MHz, CDCl₃): δ = 0.92 (t, *J* = 6 Hz, 3H), 1.29-1.60 (m, 18H), 2.47-2.49 (m, 1H), 2.76-2.78 (m, 1H), 2.90-2.95 (m, 1H) ppm. ¹³C NMR (101 MHz, CDCl₃) δ = 14.11, 22.68, 25.97, 29.33, 29.45, 29.56, 29.59, 31.90, 32.50, 47.14, 52.42 ppm. IR (CCl₄): ν_{max}/cm⁻¹ = epoxid: 2997 w, sh (ν_{as} CH₂); 1482 w, 1410 w, 1130 w (δ_s O-CH₂); 1259 w (ν_s skeleton, respiratory); 917 w (δ_{as} circle); 896 vw (δ_{as} COC); alif. chain: 2957 s (ν_{as} CH₃); 2928 vs (ν_{as} CH₂); 2872 m (ν_s CH₃); 2856 s (ν_s CH₂); 1467 m and 1458 m (β_s CH₂ and δ_{as} CH₃); 1379 w (δ_s CH₃), HRMS (EI): *m/z* calculated for C₁₂H₂₄O [M]⁺ 184.1827; found 184.1832.

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N,N'-Bis(2-hydroxydodecyl)hexan-1,6-diamine **8**

Amine **1e** (0.86 g, 3.98 mmol) and epoxide **6** (1.76 g, 9.54 mmol, 2.4 eq.) were mixed in a 4 ml glass vial, and the mixture was heated in the absence of solvent to 80 °C under an argon atmosphere for 24 h. The resulting yellowish liquid was purified by silica gel column chromatography using a linear gradient of D1 in DCM (0-30 %). Amine **7** (1.98 g, 85.1 %; *R_f* 0.51 in mobile phase D3, detection with ninhydrin) was obtained as a yellowish oil.

The deprotection of amine **7** was performed in a mixture of TFA (4 ml) and DCM (6 ml) according to the procedure described for compound **2a** in Example 1; diamine **8** (1.271 g, 77.4 %; *R_f* 0.20 in mobile phase D2, detection with ninhydrin) was obtained as a viscous yellowish oil. ¹H NMR (600 MHz, CDCl₃): δ = 3.65, 3.63, 2.84, 2.82, 2.565, 2.55, 2.41, 2.325, 1.60, 1.59, 1.41, 1.38, 1.35, 1.30-1.48, 1.28, 1.25-1.29, 1.25, 0.87 ppm. ¹³C NMR (150.9 MHz, CDCl₃): δ = 69.39, 67.71, 62.72, 61.05, 55.78, 54.77,

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40.86, 40.56, 35.22, 35.08, 31.90, 30.45, 29.6–29.9, 29.33, 25.65–26.77, 22.67, 14.10 ppm. **IR** (CCl₄): $\nu_{\max}/\text{cm}^{-1}$ = 3412 w, vbr (ν OH); 1077 w, vbr (ν C-OH); 1621 vw, vbr (β_s NH₂); 1090 w (ν C-NH₂); 2956 m, sh (ν_{as} CH₃); 2928 vs (ν_{as} CH₂); 2871 m (ν_s CH₃); 2855 s (ν_s CH₂); 2810 w, sh (ν_s N-CH₂); 1467 w and 1457 w (β_s CH₂ and δ_{as} CH₃); 1378 vw (δ_s CH₃); 722 vw (β_{as} and γ_{as} CH₂). **HRMS** (ESI): m/z calculated for C₃₀H₆₅N₂O₂ [M + H]⁺ 485.50406; found 485.50461.

***N*¹,*N*³,*N*⁵-Tris(6-(bis(2-hydroxydodecyl)amino)hexyl)adamantane-1,3,5-tricarboxamide 9**

Lipidoid **9** was prepared from adamantane-1,3,5-tricarboxylic acid (40 mg, 0.149 mmol), HCTU (256 mg, 0.596 mmol, 4 eq.), DIPEA (0.416 ml, 2.39 mmol, 16 eq.) and diamine **8** (289 mg, 0.596 mmol, 4 eq.) according to the procedure described for compound **4a** in Example 1; lipidoid **9** (188 mg, 75.5 %; *R*_f 0.43 in mobile phase D2, detection with ninhydrin) was obtained as a viscous yellowish oil. **¹H NMR** (600 MHz, CDCl₃): δ = 6.97, 4.10, 4.075, 4.04, 4.00, 3.38, 3.31, 3.28, 3.24, 3.21, 3.19, 3.16, 3.11, 2.34, 2.14, 1.93, 1.87, 1.28, 1.25–1.31, 1.25, 0.88 ppm. **¹³C NMR** (150.9 MHz, CDCl₃): δ = 177.50, 66.38, 65.92, 65.10, 64.73, 61.43, 61.23, 60.55, 59.64, 57.63, 54.48, 53.38, 41.70, 39.42, 39.23, 37.16, 31.90, 29.63, 29.56, 29.52, 29.34, 28.39, 22.68, 14.11 ppm. **IR** (CCl₄): $\nu_{\max}/\text{cm}^{-1}$ = 3300–3500 m, br (ν OH); 1090 w (ν C-OH); 3439 w (free) and 3344 w (bound) (ν NH); 1635 m (amide I); 1536 m (amide II); 2956 m, sh (ν_{as} CH₃); 2873 m, sh (ν_s CH₃); 2927 vs (ν_{as} CH₂); 2855 s (ν_s CH₂); 1378 w (δ_s CH₃); 2808 w, sh (ν_s CH₂NR₂); 721 w (β_s CH₂). **HRMS** (MALDI): m/z calculated for C₁₀₃H₂₀₃N₆O₉ [M + H]⁺ 1668.5612; found 1668.5628.

Example 9

Linoleylaldehyde 10

Dess-Martin periodinane (4.45 g, 10.49 mmol, 1.3 eq.) was added to a solution of linoleyl alcohol (2.50 ml, 8.07 mmol) in DCM (120 ml), cooled to 0 °C in an ice bath, and the mixture was stirred at 0 °C for 4 h. The reaction was then quenched by the addition of sodium thiosulphate solution (20 g Na₂S₂O₃·5H₂O/100 ml H₂O) and saturated aqueous sodium bicarbonate solution (50 ml), and stirred for 1 h at rt until the initially milky solution turned clear. The solution was poured into a 1000 ml separatory flask, diluted with water (150 ml), and the product was extracted with DCM (150 ml, 2x50 ml). The combined organic phase was washed with brine (150 ml), dried over anhydrous sodium sulphate, filtered through an S2 frit, and the solvents were evaporated in an RVE. The crude material was purified by silica gel column chromatography (isocratic conditions, 5% ethyl acetate in cyclohexane). Aldehyde **10** (1.271 g, 59.6 %; *R*_f 0.36 in mobile phase CE5, detection with KMnO₄) was obtained as a colorless oil.

***N*¹,*N*¹-Di((9*Z*,12*Z*)-octadeca-9,12-dien-1-yl)hexane-1,6-diamine 12**

Amine **11** was prepared from amine **1e** (0.345 g, 1.59 mmol), aldehyde **10** (1.27 g, 4.78 mmol, 3 eq.) and sodium triacetoxymethylborohydride (1.01 g, 4.78 mmol, 3 eq.) according to the procedure described for

compound **2a** in Example 1. Amine **11** was obtained as a yellowish oil (1.08 g, 94.9 %; R_f 0.18 in mobile phase CE20, detection with ninhydrin).

The deprotection of amine **11** was performed in a mixture of TFA (4 ml) and DCM (5 ml) according to the procedure described for **2a** in Example 1; diamine **12** (0.594 g, 64.0 %; R_f 0.13 in mobile phase D2, detection with ninhydrin) was obtained as a yellowish oil. $^1\text{H NMR}$ (600 MHz, CDCl_3): δ = 5.30–5.40, 2.765, 2.73, 2.67, 2.59, 2.04, 1.51, 1.385, 1.37, 1.34, 1.295, 1.29, 1.28–1.34, 1.28, 0.88 ppm. $^{13}\text{C NMR}$ (150.9 MHz, CDCl_3): δ = 130.19, 130.06, 127.99, 127.89, 53.49, 53.45, 41.67, 32.52, 31.50, 29.62, 29.46, 29.20–29.48, 27.37, 27.20, 27.18, 26.52, 25.61, 22.56, 14.06 ppm. **IR** (CCl_4): $\nu_{\text{max}}/\text{cm}^{-1}$ = 3011 s ($\nu_{\text{as}}=\text{CH}$); 1646–1673 m ($\nu \text{ C}=\text{C}$); 3455 w ($\nu_{\text{as}} \text{ NH}_2$); 3394 ($\nu_{\text{s}} \text{ NH}_2$); 1620 w ($\beta_{\text{s}} \text{ NH}_2$); 1087 m ($\nu \text{ C-NH}_2$); 2957 s, sh ($\nu_{\text{as}} \text{ CH}_3$); 2928 vs ($\nu_{\text{as}} \text{ CH}_2$); 2873 s, sh ($\nu_{\text{s}} \text{ CH}_3$); 2856 vs ($\nu_{\text{s}} \text{ CH}_2$); 2801 m ($\nu_{\text{s}} \text{ N-CH}_2$); 1467 m and 1457 m, sh ($\beta_{\text{s}} \text{ CH}_2$ and $\delta_{\text{as}} \text{ CH}_3$); 1378 m ($\delta_{\text{s}} \text{ CH}_3$); 721 m (β_{as} and $\gamma_{\text{as}} \text{ CH}_2$). **HRMS**: m/z calculated for $\text{C}_{42}\text{H}_{81}\text{N}_2$ $[\text{M} + \text{H}]^+$ 613.63943; found 613.63899.

15 N^1, N^3, N^5 -Tris(6-(bis((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)hexyl)adamantane-1,3,5-tricarboxamide **13**

Lipidoid **13** was prepared from adamantane-1,3,5-tricarboxylic acid (30 mg, 0.112 mmol), HCTU (192 mg, 0.447 mmol, 4 eq.), DIPEA (0.312 ml, 2.39 mmol, 16 eq.) and diamine **12** (274 mg, 0.447 mmol, 4 eq.) according to the procedure described for compound **4a** in Example 1; lipidoid **13** (154 mg, 67.1 %; R_f 0.48 in mobile phase D2, detection with ninhydrin) was obtained as a viscous yellowish oil. $^1\text{H NMR}$ (600 MHz, CDCl_3): δ = 6.32, 5.37, 5.32, 3.23, 2.76, 2.31, 2.04, 1.90, 1.81, 1.52, 1.35, 1.305, 1.295, 1.29, 1.28–1.35, 1.28, 0.88 ppm. $^{13}\text{C NMR}$ (150.9 MHz, CDCl_3): δ = 176.42, 130.21, 129.99, 128.04, 127.87, 52.94, 41.72, 39.98, 38.99, 37.77, 31.50, 29.60, 29.18, 29.08, 29.0–29.7, 28.57, 27.18, 26.26, 25.61, 22.55, 14.07 ppm. **IR** (CCl_4): $\nu_{\text{max}}/\text{cm}^{-1}$ = 3011 m ($\nu_{\text{as}}=\text{CH}$); 1661 m ($\nu \text{ C}=\text{C}$); 3464 w (free) and 3347 w (bound) ($\nu \text{ NH}$); 1645 m, sh (amide I); 1534 w (amide II); 2957 m, sh ($\nu_{\text{as}} \text{ CH}_3$); 2873 m, sh ($\nu_{\text{s}} \text{ CH}_3$); 2929 vs ($\nu_{\text{as}} \text{ CH}_2$); 2856 s ($\nu_{\text{s}} \text{ CH}_2$); 1378 and 1366 w ($\delta_{\text{s}} \text{ CH}_3$); 1086 w, sh ($\nu \text{ C-N}$); 722 w (β_{as} and $\gamma_{\text{as}} \text{ CH}_2$). **HRMS** (MALDI): m/z calculated for $\text{C}_{139}\text{H}_{251}\text{N}_6\text{O}_3$ $[\text{M} + \text{H}]^+$ 2052.9667; found 2052.9672.

Example 10

8-((*tert*-Butyldiphenylsilyl)oxy)octane-1-ol **14**

30 *Tert*-butyldiphenylchlorosilane (17.50 ml, 68.39 mmol, 1 eq.) was added to a solution of 1,8-octanediol (10.0 g, 68.39 mmol) and imidazole (5.59 g, 82.06 mmol, 1.2 eq.) in DCM (250 ml), and the reaction mixture was stirred for 24 h at rt. The solution was poured into a 1000 ml separatory flask, diluted with water (400 ml) and brine (100 ml), and the product was extracted with DCM (2 x 100 ml). The combined organic phase was washed with brine (100 ml), dried over anhydrous sodium sulphate, filtered through
35 an S2 frit, and the solvents were evaporated in an RVE. The crude product was purified by silica gel column chromatography using a linear gradient of ethyl acetate in cyclohexane (0–30 %). Alcohol **14**

(13.65 g, 51.9 %; R_f 0.35 in mobile phase CE20, detection with KMnO_4) was obtained as a colorless oil. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ = 1.08 (s, 9H), 1.31-1.41 (m, 8H), 1.55-1.62 (m, 4H), 3.64-3.70 (m, 4H), 7.38-7.47 (m, 6H), 7.69-7.71 (m, 4H) ppm. $^{13}\text{C NMR}$ (101 MHz, CDCl_3): δ = 19.24, 25.68, 25.72, 26.89, 29.33, 29.38, 32.56, 32.79, 63.09, 63.99, 127.57, 129.48, 134.19, 135.58 ppm. **IR** (CCl_4): $\nu_{\text{max}}/\text{cm}^{-1}$ = 3072 m (20a); 3053 m (20b); 2529 s, sh (tBu, $\nu_{\text{as}} \text{CH}_3$); 2898 s, sh (tBu, $\nu_{\text{s}} \text{CH}_3$); 1590, 1568 (8a, 8b); 1487 m (19a); 1463 m, 1473 s (tBu, $\delta_{\text{as}} \text{CH}_3$); 1428 s (19b); 1390 m, 1362 m (tBu, $\delta_{\text{s}} \text{CH}_3$); 1189 m; 1112 vs, 1094 vs ($\nu_{\text{as}} \text{Si-Ph}$); 1030 m (18a); 1008 m ($\delta \text{Ph-Si}$); 939 m ($\nu_{\text{s}} \text{CH}_3$); 701 vs ($\nu_{\text{s}} \text{COSi}$); 688 m (4); 622 m (6b); 614 s (6a); 505 s (16b); 489 m ($\delta \text{Si-Ph}$); 2932 vs ($\nu_{\text{as}} \text{CH}_2$); 2858 vs ($\nu_{\text{s}} \text{CH}_2$); 3636 m, 3341 m, br ($\nu_{\text{s}} \text{OH}$); 1057 m ($\nu_{\text{s}} \text{C-OH}$). **HRMS** (ESI): m/z calculated for $\text{C}_{24}\text{H}_{36}\text{O}_2\text{NaSi}$ [$\text{M} + \text{Na}$] $^+$ 407.23768; found 407.23742.

8-((*tert*-Butyldiphenylsilyl)oxy)octanoic acid 15

Dess-Martin periodinane (19.54 g, 46.07 mmol, 1.3 eq.) was added to a solution of alcohol **14** (13.63 g, 35.44 mmol) in DCM (250 ml), cooled to 0 °C in an ice bath, and the mixture was stirred at 0 °C for 4 h. The reaction was then quenched by the addition of sodium thiosulphate solution (50 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ /150 ml H_2O) and saturated aqueous sodium bicarbonate solution (100 ml), and stirred for 1 h at rt until the initially milky solution turned clear. The solution was poured into a 1000 ml separatory flask, diluted with water (200 ml), and the product was extracted with DCM (200 ml, 2x50 ml). The combined organic phase was washed with brine (200 ml), dried over anhydrous sodium sulphate, filtered through an S2 frit, and the solvents were evaporated in an RVE. 13.56 g of a colourless oil were obtained.

The obtained residue was dissolved in a 2 l flask in a mixture of acetone (450 ml) and water (90 ml); 2-methyl-2-butene (15.02 ml, 141.8 mmol, 4 eq.) and $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (11.06 g, 70.88 mmol, 2 eq.) were added to the solution, and the suspension was cooled to 0 °C in an ice bath. A solution of sodium chlorite (9.62 g, 106.32 mmol, 3 eq.) in water (60 ml) was then added gradually from a dropping funnel over 30 min, the reaction mixture was removed from the cooling bath, and stirred vigorously at rt for 12 h. The solution was poured into a 1000 ml separatory flask, diluted with a solution of citric acid (70 g) in water (300 ml), and the product was extracted with diethyl ether (300 ml, 50 ml). The combined organic phase was washed with brine (300 ml), dried over anhydrous sodium sulphate, filtered through an S2 frit, and the solvents were evaporated in an RVE. The crude product was purified by silica gel column chromatography (isocratic conditions, 5% methanol in chloroform). Acid **15** (12.92 g, 91.5 %; R_f 0.35 in mobile phase MeOH- CHCl_3 5:95 (v/v), detection with KMnO_4) was obtained as a viscous colourless oil. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ = 1.05 (s, 9H), 1.27-1.39 (m, 6H), 1.51-1.66 (m, 4H), 2.34 (t, J = 7.5 Hz, 2H), 3.65 (t, J = 6.5 Hz, 2H), 7.36-7.44 (m, 6H), 7.66-7.68 (m, 4H) ppm. $^{13}\text{C NMR}$ (101 MHz, CDCl_3): δ = 19.23, 24.63, 25.59, 26.89, 28.97, 29.02, 32.48, 33.95, 63.91, 127.57, 129.49, 134.15, 135.58, 179.60 ppm. **IR** (CCl_4): $\nu_{\text{max}}/\text{cm}^{-1}$ = 3534 w (νOH , monomer); 3100 w, 2740 w, 2674 w (νOH ,

dimer); 1711 vs (ν C=O); 1413 w, 1289 w (ν CO and δ COH); 2561 m, sh (tBu, ν_{as} CH₃); 2898 m, sh (tBu, ν_s CH₃); 1590 w; 1487 w (19a); 1463 m, 1472 m (tBu, δ_{as} CH₃); 1429 m (19b); 1390 w, 1362 w (tBu, δ_s CH₃); 1112 s, (ν_{as} COSi); 1093 m (18b); 1030 w; 1008 w (δ Ph-Si); 940 w (ν CH₃); 701 s (4); 688 w (SiOC); 622 w (6b); 614 m (6a); 505 w (16b); 2932 s (ν_{as} CH₂); 2858 m (ν_s CH₂). **HRMS** (ESI): m/z calculated for C₂₄H₃₃O₃Si [M + H]⁺ 397.22044; found 397.22018.

(Z)-Non-2-en-1-yl-8-hydroxyoctanoate 17

Diisopropylcarbodiimide (1.08 ml, 6.90 mmol, 1.1 eq.) and 4-dimethylaminopyridine (23.0 mg, 0.188 mmol, 0.03 eq.) were added to a solution of acid **15** (2.50 g, 6.27 mmol) in DCM (100 ml), cooled to 0 °C in an ice bath, and the mixture was stirred at 0 °C for 30 min. Then *trans*-2-nonen-1-ol (1.37 ml, 8.15 mmol, 1.3 eq.) was added, and the reaction mixture was stirred for 12 h at rt. The solvent was evaporated in an RVE, and the residue was purified by silica gel column chromatography (isocratic conditions, 5% ethyl acetate in cyclohexane). Ester **16** (2.784 g, 84.9 %; *R_f* 0.61 in mobile phase CE5, detection with KMnO₄) was obtained as a colorless oil.

A solution of tetrabutylammonium fluoride monohydrate (2.95 g, 10.56 mmol, 2 eq.) in tetrahydrofuran (10 ml), was added to a solution of ester **16** (2.76 g, 5.28 mmol) in tetrahydrofuran (40 ml), and the reaction the mixture was stirred for 20 h at rt. The solution was poured into a 500 ml separatory flask, diluted with 10% aqueous ammonium chloride solution (150 ml), and the product was extracted with diethyl ether (150 ml, 50 ml). The combined organic phase was washed with brine (100 ml), dried over anhydrous sodium sulfate, filtered through an S2 frit, and the solvents were evaporated in an RVE. The crude product was purified by silica gel column chromatography using a linear gradient of ethyl acetate in cyclohexane (0-45 %). Alcohol **17** (1.311 g, 87.3 %; *R_f* 0.61 in mobile phase CE50, detection with KMnO₄) was obtained as a slightly yellowish oil. ¹H NMR (400 MHz, CDCl₃): δ = 0.87 (t, *J* = 6 Hz, 3H), 1.26-1.38 (m, 14H), 1.51-1.66 (m, 4H), 2.07-2.12 (m, 2H), 2.30 (t, *J* = 7.5 Hz, 2H), 3.63 (t, *J* = 6.6 Hz, 2H), 4.61-4.62 (m, 2H), 5.48-5.55 (m, 1H), 5.60-5.67 (m, 1H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ = 14.07, 22.60, 24.88, 25.54, 27.54, 28.86, 29.02, 29.07, 29.39, 31.68, 32.68, 34.30, 60.22, 62.97, 123.34, 135.45, 173.75 ppm. **IR** (CCl₄): ν_{max}/cm^{-1} = 3637 w, 3453 w (ν OH); 1056 m (ν C-OH); 1736 vs (ν C=O); 1238 m, 1170 s (ν C-O); 3025 w (ν_{as} =CH); 1659 w (ν C=C); 1419 w (ρ =C-H); 2955 s (ν_{as} CH₃); 2931 vs (ν_{as} CH₂); 2858 s (ν_s CH₂); 2872 s, sh (ν_s CH₃); 1466 m and 1457 m (β_s CH₂ and δ_{as} CH₃); 1378 m (δ_s CH₃); 722 m (β_{as} and γ_{as} CH₂). **HRMS** (EI): m/z calculated for C₁₇H₃₂O₃ [M]⁺ 284.2351; found 284.2355.

(Z)-Non-2-en-1-yl-8-oxooctanoate 18

Dess-Martin periodinane (2.46 g, 5.80 mmol, 1.3 eq.) was added to a solution of alcohol **17** (1.27 ml, 4.46 mmol) in DCM (100 ml), cooled to 0 °C in an ice bath, and the mixture was stirred at 0 °C for 4 h. The reaction was then quenched by the addition of sodium thiosulfate solution (10 g Na₂S₂O₃·5H₂O/50

ml H₂O) and saturated aqueous sodium bicarbonate solution (50 ml), and stirred for 1 h at rt until the initially milky solution turned clear. The solution was poured into a 500 ml separatory flask, diluted with water (100 ml), and the product was extracted with DCM (100 ml, 50 ml). The combined organic phase was washed with brine (150 ml), dried over anhydrous sodium sulfate, filtered through an S2 frit, and the solvents were evaporated in an RVE. The crude product was purified by silica gel column chromatography using a linear gradient of ethyl acetate in cyclohexane (0-30 %). Aldehyde **18** (0.573 g, 45.4 %; *R_f* 0.49 in mobile phase CE20, detection with KMnO₄) was obtained as a colorless oil. ¹H NMR (400 MHz, CDCl₃): δ = 0.88 (t, *J* = 7 Hz, 3H), 1.26-1.38 (m, 14H), 1.59-1.67 (m, 4H), 2.07-2.12 (m, 2H), 2.30 (t, *J* = 7.5 Hz, 2H), 2.40-2.44 (m, 2H), 4.61-4.63 (m, 2H), 5.48-5.55 (m, 1H), 5.61-5.68 (m, 1H), 9.76 (t, *J* = 1.8 Hz, 1H) ppm. ¹³C NMR (101 MHz, CDCl₃): δ = 14.07, 21.86, 22.60, 24.71, 27.54, 28.77, 28.83, 28.86, 29.39, 31.68, 34.19, 43.79, 60.25, 123.31, 135.49, 173.59, 202.62 ppm. IR (CCl₄): ν_{max}/cm⁻¹ = 2818 m, 2716 m (aldehyd, ν CH); 1733 vs (aldehyd, ν C=O); 1395 m, sh (aldehyd, δ OCH); 1733 vs (ν C=O); 1244 m, 1172 s (ν C-O); 3026 m (ν_{as} =CH); 1659 w (ν C=C); 2956 s (ν_{as} CH₃); 2930 vs (ν_{as} CH₂); 2858 s (ν_s CH₂); 2873 s, sh (ν_s CH₃); 1466 m a 1462 m (β_s CH₂ a δ_{as} CH₃); 1378 m a 1373 m (δ_s CH₃). HRMS (ESI): *m/z* calculated for C₁₇H₂₉O₄ [M - H]⁻ 297.20713; found 297.20722.

*N*¹,*N*¹-Bis(8-((*Z*)-non-2-en-1-yl)oxy-8-oxooctyl)hexane-1,6-diamine **20**

Amine **19** was prepared from amine **1e** (140 mg, 0.647 mmol), aldehyde **18** (0.548 g, 1.94 mmol, 3 eq.) and sodium triacetoxymethylborohydride (0.411 g, 1.94 mmol, 3 eq.) according to the procedure described for **2a** in Example 1, except that the reaction mixture was evaporated without prior extraction and the residue was directly purified by chromatography. Amine **19** was obtained as a slightly yellowish oil (0.446 g, 92.0%).

The deprotection of amine **19** was performed in a mixture of TFA (4 ml) and DCM (4 ml) according to the procedure described for **2a** in Example 1; diamine **20** (0.333 g, 86.2 %; *R_f* 0.16 in mobile phase D2, detection with ninhydrin) was obtained as a yellowish oil. ¹H NMR (600 MHz, CDCl₃): δ = 5.63, 5.50, 4.61, 3.06, 3.02, 2.99, 2.29, 2.08, 1.82, 1.74, 1.60, 1.57, 1.42, 1.34, 1.28, 1.27, 0.87 ppm. ¹³C NMR (150.9 MHz, CDCl₃): δ = 173.59, 135.39, 129.29, 60.24, 52.64, 52.19, 39.50, 34.12, 31.64, 29.34, 28.84, 28.82, 28.73, 27.51, 26.60, 25.76, 25.36, 24.69, 23.11, 22.57, 14.05 ppm. IR (CCl₄): ν_{max}/cm⁻¹ = 3446 w (ν_{as} NH₂); 1612 w (β_s NH₂); 2800 m, sh (ν_s N-CH₂); 1736 vs (ν C=O); 1236 m, 1168 s (ν C-O); 3024 m (ν_{as} =CH); 1679 w (ν C=C); 1419 w (ρ =CH); 2957 s (ν_{as} CH₃); 2931 vs (ν_{as} CH₂); 2858 s (ν_s CH₂); 2873 s, sh (ν_s CH₃); 1467 m and 1457 m (β_s CH₂ and δ_{as} CH₃); 1378 m (δ_s CH₃); 721 w (β_{as} and γ_{as} CH₂). HRMS (ESI): *m/z* calculated for C₄₀H₇₇O₄N₂ [M + H]⁺ 649.58779; found 649.58767.

*N*¹,*N*³,*N*⁵-Tris(6-(bis(8-((*Z*)-non-2-en-1-yl)oxy-8-oxooctyl)amino)hexyl)adamantane-1,3,5-tricarboxamide **21**

Lipidoid **21** was prepared from adamantane-1,3,5-tricarboxylic acid (30 mg, 0.112 mmol), HCTU (192 mg, 0.447 mmol, 4 eq.), DIPEA (0.312 ml, 1.79 mmol, 16 eq.) and diamine **20** (290 mg, 0.447 mmol, 4 eq.) according to the procedure described for compound **4a** in Example 1; lipidoid **21** (181 mg, 74.9 %; R_f 0.29 in mobile phase D2, detection with ninhydrin) was obtained as a viscous yellowish oil. $^1\text{H NMR}$ (600 MHz, CDCl_3): δ = 7.54, 5.63, 5.50, 4.61, 3.29, 3.00, 2.98, 2.34, 2.29, 2.23, 2.08, 1.98, 1.89, 1.76, 1.60, 1.585, 1.42, 1.38, 1.35, 1.28, 1.26, 0.87 ppm. $^{13}\text{C NMR}$ (150.9 MHz, CDCl_3): δ = 177.29, 173.53, 135.41, 123.27, 60.25, 52.62, 52.27, 41.72, 39.57, 39.20, 37.12, 34.09, 31.64, 29.34, 28.85, 28.82, 28.74, 28.44, 28.37, 27.51, 26.63, 25.83, 25.56, 24.67, 23.11, 22.57, 14.06 ppm. **IR** (CCl_4): $\nu_{\text{max}}/\text{cm}^{-1}$ = 3321 w (v NH); 1644 m (amide I); 1535 w-m (amide II); 1736 s (v C=O); 1276 w-m, 1166 m (v C-O); 3025 w (v_{as} =CH); 1419 w (ρ =CH); 2956 s, sh (v_{as} CH₃); 2930 vs (v_{as} CH₂); 2858 s (v_s CH₂); 2873 m, sh (v_s CH₃); 1467 m and 1457 m (β_s CH₂ and δ_{as} CH₃); 1378 w-m (δ_s CH₃). **HRMS** (MALDI): m/z calculated for $\text{C}_{133}\text{H}_{239}\text{N}_6\text{O}_{15}$ [$\text{M} + \text{H}$]⁺ 2160.8118; found 2160.8164.

Example 11

15

3,5,7-Tris((6-(didodecylamino)hexyl)carbamoyl)adamantane-1-carboxylic acid **22**

Thionylchloride (300 μl) and DMF (2 μl) was added to adamantane-1,3,5,7-tetracarboxylic acid (21 mg, 0.067 mmol), and the suspension was stirred for 2 h at 70 °C in a closed vial; during this time the suspension turned into a clear homogeneous solution. Excessive SOCl_2 was blown out with a stream of dry nitrogen, the residue was dried in vacuo (10 min), and after cooling down to rt was dissolved in 0.5 ml of anhydrous DMF to form a clear solution. Then, a solution of N^1, N^1 -didodecylhexane-1,6-diamine (76 mg, 0.168 mmol, 2.5 eq.) and DIPEA (117 μl, 0.672 mmol, 10 eq.) in a mixture of DCM (1.5 ml) and DMF (0.5 ml), and the reaction mixture was stirred for 10 min at rt. The reaction mixture was then adsorbed onto silica (10 g), and the solvents were evaporated in vacuo. The crude product was purified by flash chromatography on silica (elution with a linear gradient of D1 in DCM, 35-85 %) to yield the target compound **22** (45 mg, 41.4 %; R_f 0.50 in D2/3, visualization by ninhydrin) as a thick pale yellow oil. $^1\text{H NMR}$ (600 MHz, CDCl_3): δ = 7.32, 3.24, 3.05-2.95, 2.16, 1.97, 1.92, 1.80-1.72, 1.55, 1.38-1.23, 0.87 ppm. $^{13}\text{C NMR}$ (150.9 MHz, CDCl_3): δ = 176.52, 52.89, 52.20, 42.28, 39.39, 38.96, 31.89, 29.60, 29.50, 29.44, 29.32, 29.10, 28.72, 26.83, 26.23, 25.99, 23.47, 23.14, 22.66, 14.10 ppm. **IR** (CCl_4): $\nu_{\text{max}}/\text{cm}^{-1}$ = 3314 w, br (v NH), 1643 m (amide I) and 1638 w (amide II), 2954 s (v_{as} CH₃), 2927 vs (v_{as} CH₂), 2855 s (v_s CH₂), 1467 m, sh and 1457 m, sh (β_s CH₂ and δ_{as} CH₃), 1378 w (δ_s CH₃); -COOH dimer: 2591 w, vbr (v OH), 1715 w (v C=O), 1411 vw and 1283 w, br (δ COH and v CCO). **HRMS** (MALDI): m/z calcd. for $\text{C}_{104}\text{H}_{203}\text{N}_6\text{O}_5$ [$\text{M} + \text{H}$]⁺ 1616.5810; found 1616.58014.

35 N^1, N^3, N^5 -Tris(6-(didodecylamino)hexyl)adamantane-1,3,5,7-tetracarboxamide **23**

DMF (2 μ l) and thionylchloride (24 μ l, 0.349 mmol, 14 eq.) were added to a solution of acid **22** (27 mg, 0.017 mmol) in DCM (3.5 ml), and the suspension was stirred for 2 h at rt in a closed vial. The solution was then bubbled with a gentle stream of anhydrous NH_3 for 5 min; a white precipitate formed immediately. After 10 min, the reaction mixture was filtered through a celite pad, the filtrate was adsorbed onto silica (10 g), and the solvents were evaporated in vacuo. The crude product was purified by flash chromatography on silica (elution with a linear gradient of D1 in DCM, 30-70 %) to yield target compound **23** (15 mg, 55.6 %; R_f 0.57 in D2/3, visualization by ninhydrin) as a thick pale yellow oil. ^1H NMR (600 MHz, CDCl_3): δ = 6.69, 6.49, 3.21, 2.67, 1.99, 1.96, 1.57, 1.51, 1.33, 1.285, 1.245, 0.87 ppm. ^{13}C NMR (150.9 MHz, CDCl_3): δ = 178.41, 175.71, 53.42, 53.07, 42.38, 39.52, 39.40, 39.18, 31.88, 29.62, 29.60, 29.58, 29.55, 29.37, 29.32, 29.02, 27.24, 26.56, 26.21, 22.66, 14.09 ppm. IR (CCl_4): $\nu_{\text{max}}/\text{cm}^{-1}$ = 3330 w, br (ν NH), 1649 m (amide I) and 1536 w (amide II), 2953 m (ν_{as} CH_3), 2927 vs (ν_{as} CH_2), 2875 m, sh (ν_{s} CH_3), 2855 s and 2805 vw (ν_{s} CH_2), 1467 m, and 1457 w, 1438 w, sh (β_{s} CH_2 and δ_{as} CH_3), 1378 w (δ_{s} CH_3); prim. amide: 3199 w, br (ν NH_2 v $\acute{a}z.$), 1697 vw, sh (ν C=O), 1605 w, sh (β_{s} NH_2). HRMS (MALDI): m/z calcd. for $\text{C}_{104}\text{H}_{204}\text{N}_7\text{O}_4$ $[\text{M} + \text{H}]^+$ 1615.5969; found 1615.5998.

15

Example 12

N^1, N^3, N^5 -Tris(6-(didodecylamino)hexyl)- N^7 -(2-hydroxyethyl)adamantane-1,3,5,7-tetracarboxamide **24**

DMF (2 μ l) and thionylchloride (20 μ l, 0.286 mmol, 14 eq.) were added to a solution of acid **22** (33 mg, 0.020 mmol) in DCM (1.5 ml), and the suspension was stirred for 1 h at rt in a closed vial. Ethanolamine (50 μ l, 0.816 mmol, 40 eq.) was then added, and a white precipitate formed immediately. After 20 min, the reaction mixture was filtered through a celite pad, the filtrate was adsorbed onto silica (10 g), and the solvents were evaporated in vacuo. The crude product was purified by flash chromatography on silica (elution with a linear gradient of D1 in DCM, 35-85 %) to yield the target compound **24** (31 mg, 91.5 %; R_f 0.59 in D2/3, visualization by ninhydrin) as a thick pale yellow oil. ^1H NMR (600 MHz, CDCl_3): δ = 7.39, 6.92, 3.69, 3.37, 3.22, 3.00, 2.02, 1.96, 1.76, 1.53, 1.40-1.22, 0.86 ppm. ^{13}C NMR (150.9 MHz, CDCl_3): δ = 176.76, 176.16, 61.73, 61.38, 52.73, 52.16, 43.13, 42.55, 42.41, 39.54, 39.43, 38.74, 31.86, 29.56, 29.45, 29.40, 29.28, 29.06, 28.64, 26.78, 25.92, 25.64, 23.38, 23.08, 22.64, 14.07 ppm. IR (CCl_4): $\nu_{\text{max}}/\text{cm}^{-1}$ = 3324 w, br (ν NH), 1646 m (amide I) and 1538 w (amide II), 2955 s (ν_{as} CH_3), 2927 vs (ν_{as} CH_2), 2855 s and 2876 m, sh (ν_{s} CH_3), 1467 m, 1457 m and 1435 w, sh (β_{s} CH_2 and δ_{as} CH_3), 1378 w (δ_{s} CH_3), 1060 w, vbr, 1036 w, br (ν C-OH). HRMS (MALDI): m/z calcd. for $\text{C}_{106}\text{H}_{208}\text{N}_7\text{O}_5$ $[\text{M} + \text{H}]^+$ 1659.6231; found 1659.62718.

30

Example 13

N^1, N^3, N^5 -Tris(6-(didodecylamino)hexyl)- N^7 -(1,3-dihydroxypropan-2-yl)adamantane-1,3,5,7-tetracarboxamide **25**

35

Following the procedure outlined for **24**, the target compound **25** was prepared from acid **22** (51 mg, 0.031 mmol), and serinol (115 mg, 1.26 mmol, 40 eq.) to yield lipidoid **25** as a thick pale yellow oil (19 mg, 35.6%; R_f 0.59 in D2/3). $^1\text{H NMR}$ (600 MHz, CDCl_3): δ = 7.79, 7.21, 7.17, 7.08, 3.26, 3.01, 2.03, 1.92, 1.775, 1.57, 1.385, 1.33, 1.28-1.24 ppm. $^{13}\text{C NMR}$ (150.9 MHz, CDCl_3): δ = 176.14, 175.04, 52.74, 52.66, 52.32, 52.17, 39.28-38.65, 31.89, 29.68, 29.59, 29.49, 29.43, 29.32, 29.09, 28.47, 26.83, 26.80, 25.98-25.40, 23.05, 22.67, 14.10 ppm. IR (CCl_4): $\nu_{\text{max}}/\text{cm}^{-1}$ = 3324 w, br (ν NH), 1646 m (amide I) and 1538 w (amide II), 2955 s (ν_{as} CH_3), 2927 vs (ν_{as} CH_2), 2855 s and 2876 m, sh (ν_{s} CH_3), 1467 m, 1457 m and 1435 w, sh (β_{s} CH_2 and δ_{as} CH_3), 1378 w (δ_{s} CH_3), 1060 w, vbr, 1036 w, br (ν C-OH). HRMS (MALDI): m/z calcd. for $\text{C}_{107}\text{H}_{210}\text{N}_7\text{O}_6$ [$\text{M} + \text{H}$] $^+$ 1689.6337; found 1616.63235.

Example 14

*N*¹,*N*¹-Di((heptyloxycarbonyl)propyl)hexane-1,6-diamine **28a**

Diisopropylcarbodiimide (3.17 ml, 20.24 mmol, 1.3 eq.) and DMAP (57.1 mg, 0.467 mmol, 0.03 eq.) were added to a solution of 4-bromobutyric acid (2.60 g, 15.57 mmol) and 1-heptanol (2.64 ml, 18.68 mmol, 1.2 eq.) in DCM (60 ml), and the mixture was stirred at rt for 1 h. The reaction mixture was then adsorbed onto silica (16 g), and the solvents were evaporated in vacuo. The crude product was purified by flash chromatography on silica (80 g, elution with a linear gradient of ethylacetate in cyclohexane, 0-10 %) to yield the target compound **26a** (3.916 g, 94.8 %; R_f 0.36 in CE5, visualization by KMnO_4) as a colorless oil.

Bromoester **26a** (1.53 g, 5.78 mmol, 2.5 eq.) and potassium carbonate (3.19 g, 23.11 mmol, 10 eq.) were added to a solution of *N*-Boc-1,6-diaminohexane (0.50 g, 2.31 mmol) in ACN (10 ml), and the mixture was stirred at 35 °C for 3 days. The reaction mixture was then adsorbed onto silica (16 g), and the solvents were evaporated in vacuo. The crude product was purified by flash chromatography on silica (40 g, elution with a linear gradient of ethylacetate in cyclohexane, 0-100 %) to yield the target compound **27a** (1.093 g, 80.9 %; R_f 0.35 in CE50 on an NH_3 -pretreated TLC plate, visualization by ninhydrin) as a pale yellow oil.

The deprotection of amine **27a** was performed according to the procedure described for compound **2a** in Example 1; diamine **28a** (0.817 g, 90.2 %; R_f 0.27 in mobile phase D2, detection with ninhydrin) was obtained in the form of a yellowish oil. $^1\text{H NMR}$ (600 MHz, CDCl_3): δ = 4.05, 3.63, 3.21, 3.15, 2.91, 2.85, 2.67, 2.53-2.41, 2.32, 1.86, 1.76, 1.61, 1.46, 1.35-1.25, 0.88 ppm. $^{13}\text{C NMR}$ (150.9 MHz, CDCl_3): δ = 173.66, 64.58, 53.66, 52.98, 52.87, 40.78, 40.34, 31.87, 31.70, 30.10, 28.90, 28.62, 26.85, 26.45, 25.87, 22.56, 22.02, 14.04 ppm. HRMS (ESI): m/z calcd. for $\text{C}_{28}\text{H}_{57}\text{N}_2\text{O}_4$ [$\text{M} + \text{H}$] $^+$ 485.43128; found 485.43039.

*N*¹,*N*³,*N*⁵-Tris(6-(bis((heptyloxycarbonyl)propyl)amino)hexyl)adamantane-1,3,5-tricarboxamide **29a**

Thionylchloride (300 µl) and DMF (2 µl) was added to adamantane-1,3,5-tricarboxylic acid (60 mg, 0.224 mmol), and the suspension was stirred for 1 h at 70 °C in a closed vial; during this time the suspension turned into a clear homogeneous solution. Excessive SOCl₂ was blown out with a stream of dry nitrogen, the residue was dried in vacuo (10 min), and after cooling down to rt was dissolved in 0.5 ml of anhydrous DMF to form a clear solution. Then, a solution of amine **28a** (434 mg, 0.895 mmol, 4 eq.) and DIPEA (390 µl, 2.24 mmol, 10 eq.) in a mixture of DCM (1.5 ml) and DMF (0.5 ml), and the reaction mixture was stirred for 10 min at rt. The reaction mixture was then adsorbed onto silica (16 g), and the solvents were evaporated in vacuo. The crude product was purified by flash chromatography on silica (40 g, elution with a linear gradient of D1 in DCM, 20-60 %) to yield the target compound **29a** (115 mg, 30.8 %; *R_f* 0.51 in D2, visualization by ninhydrin) as a thick pale yellow oil. ¹H NMR (600 MHz, CDCl₃): δ = 5.76, 4.05, 3.21, 2.42, 2.32, 2.01, 1.84, 1.80, 1.74, 1.61, 1.47, 1.41, 1.34–1.25, 0.88 ppm. ¹³C NMR (150.9 MHz, CDCl₃): δ = 176.01, 173.73, 64.53, 53.72, 53.00, 41.65, 39.77, 39.48, 37.88, 31.96, 31.70, 29.51, 28.90, 28.63, 27.02, 26.76, 25.87, 22.56, 14.05 ppm. HRMS (MALDI): *m/z* calcd. for C₉₇H₁₇₉N₆O₁₅ [M + H]⁺ 1668.3423; found 1668.3398.

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Example 15

*N*¹,*N*¹-Di((hexyloxycarbonyl)butyl)hexane-1,6-diamine **28b**

Following the procedure outlined for **26a**, bromoester **26b** was prepared from 5-bromopentanoic acid (3.0 g, 16.57 mmol), 1-hexanol (2.48 ml, 19.89 mmol, 1.2 eq.), DIC (3.37 ml, 21.54 mmol, 1.3 eq.) and DMAP (61 mg, 0.497 mmol, 0.03 eq.) to yield **26b** as a colorless oil (3.938 g, 89.6 %, *R_f* 0.28 in CE5, visualization by KMnO₄).

Following the procedure outlined for **27a**, Boc-derivative **27b** was prepared from bromoester **26b** (1.53 g, 5.78 mmol, 2.5 eq.), *N*-Boc-1,6-diaminohexane (0.50 g, 2.31 mmol) and potassium carbonate (3.19 g, 23.11 mmol, 10 eq.) to yield **27b** as a pale yellow oil (1.080 g, 79.9 %, *R_f* 0.30 in CE50 on an NH₃-pretreated TLC plate, visualization by ninhydrin).

The deprotection of amine **27b** was performed according to the procedure described for compound **2a** in Example 1; diamine **28b** (0.788 g, 86.9 %; *R_f* 0.13 in mobile phase D2, detection with ninhydrin) was obtained in the form of a yellowish oil. ¹H NMR (600 MHz, CDCl₃): δ = 4.05, 3.63, 3.21, 3.15, 2.79, 2.68, 2.60, 2.51, 2.42, 2.32, 1.61, 1.50, 1.36–1.28, 0.88 ppm. ¹³C NMR (150.9 MHz, CDCl₃): δ 173.62, 64.52, 53.68, 53.29, 41.22, 40.26, 34.03, 31.41, 28.58, 25.75, 25.57, 22.80, 22.51, 13.98 ppm. HRMS (ESI): *m/z* calcd. for C₂₈H₅₇N₂O₄ [M + H]⁺ 485.43128; found 485.43052.

*N*¹,*N*³,*N*⁵-Tris(6-(bis((hexyloxycarbonyl)butyl)amino)hexyl)adamantane-1,3,5-tricarboxamide **29b**

Following the procedure outlined for **29a** in Example 14, lipidoid **29b** was prepared from adamantane-1,3,5-tricarboxylic acid (53 mg, 0.198 mmol), amine **28b** (383 mg, 0.790 mmol, 4 eq.) and DIPEA

(344 μ l, 1.98 mmol, 10 eq.) to yield **29b** as a thick pale yellow oil (191 mg, 57.9 %, R_f 0.48 in D2, visualization by ninhydrin). ^1H NMR (600 MHz, CDCl_3): δ = 5.72, 4.05, 3.21, 2.38, 2.35, 2.30, 2.01, 1.83, 1.80, 1.61, 1.44, 1.40, 1.35–1.27, 0.88 ppm. ^{13}C NMR (150.9 MHz, CDCl_3): δ = 175.96, 173.77, 64.44, 53.98, 53.63, 41.64, 39.73, 39.55, 37.92, 34.27, 31.42, 29.59, 28.60, 27.22, 26.89, 26.63, 25.58, 23.01, 22.52, 13.99 ppm. HRMS (MALDI): m/z calcd. for $\text{C}_{97}\text{H}_{179}\text{N}_6\text{O}_{15}$ $[\text{M} + \text{H}]^+$ 1668.3428; found 1668.3418.

Example 16

*N*¹,*N*¹-Di((nonyloxycarbonyl)propyl)hexane-1,6-diamine **28c**

Following the procedure outlined for **26a**, bromoester **26c** was prepared from 4-bromobutyric acid (2.60 g, 15.57 mmol), 1-nonanol (3.26 ml, 18.68 mmol, 1.2 eq.), DIC (3.17 ml, 20.24 mmol, 1.3 eq.) and DMAP (57 mg, 0.467 mmol, 0.03 eq.) to yield **26c** as a colorless oil (4.025 g, 88.2 %, R_f 0.32 in CE5, visualization by KMnO_4).

Following the procedure outlined for **27a**, Boc-derivative **27c** was prepared from bromoester **26c** (1.25 g, 4.28 mmol, 2.5 eq.), *N*-Boc-1,6-diaminohexane (0.370 g, 1.71 mmol) and potassium carbonate (2.36 g, 17.10 mmol, 10 eq.) to yield **27c** as a pale yellow oil (0.848 g, 77.3 %, R_f 0.45 in CE50 on an NH_3 -pretreated TLC plate, visualization by ninhydrin).

The deprotection of amine **27c** was performed according to the procedure described for compound **2a** in Example 1; diamine **28c** (0.631 g, 88.2 %, R_f 0.29 in mobile phase D2, detection with ninhydrin) was obtained in the form of a yellowish oil. ^1H NMR (600 MHz, CDCl_3): δ = 4.66, 4.05, 3.16, 2.91, 2.63, 2.59, 2.53, 2.48, 2.34, 1.81, 1.70, 1.61, 1.54, 1.47, 1.42, 1.35–1.24, 0.87 ppm. ^{13}C NMR (150.9 MHz, CDCl_3): δ = 173.39, 64.72, 64.66, 52.53, 31.84, 31.59, 29.46, 29.25, 29.22, 28.61, 28.60, 25.91, 25.90, 22.64, 14.08 ppm. HRMS (ESI): m/z calcd. for $\text{C}_{32}\text{H}_{65}\text{N}_2\text{O}_4$ $[\text{M} + \text{H}]^+$ 541.49388; found 541.49303.

*N*¹,*N*³,*N*⁵-Tris(6-(bis((nonyloxycarbonyl)propyl)amino)hexyl)adamantane-1,3,5-tricarboxamide **29c**

Following the procedure outlined for **29a** in Example 14, lipidoid **29c** was prepared from adamantane-1,3,5-tricarboxylic acid (42 mg, 0.157 mmol), amine **28c** (339 mg, 0.626 mmol, 4 eq.) and DIPEA (273 μ l, 1.57 mmol, 10 eq.) to yield **29c** as a thick pale yellow oil (134 mg, 46.6 %, R_f 0.56 in D2, visualization by ninhydrin). ^1H NMR (600 MHz, CDCl_3): δ = 5.72, 4.05, 3.21, 2.40, 2.36, 2.31, 2.01, 1.83, 1.80, 1.72, 1.61, 1.47, 1.38, 1.33–1.24, 0.87 ppm. ^{13}C NMR (150.9 MHz, CDCl_3): δ = 175.96, 173.84, 64.48, 53.80, 53.12, 41.65, 39.74, 39.54, 37.92, 32.06, 31.84, 29.47, 29.26, 29.22, 28.64, 25.92, 22.64, 14.09 ppm. HRMS (MALDI): m/z calcd. for $\text{C}_{109}\text{H}_{203}\text{N}_6\text{O}_{15}$ $[\text{M} + \text{H}]^+$ 1836.5306; found 836.5319.

Example 17

***N*¹,*N*¹-Di((octyloxycarbonyl)butyl)hexane-1,6-diamine 28d**

Following the procedure outlined for **26a**, bromoester **26d** was prepared from 5-bromopentanoic acid (3.0 g, 16.57 mmol), 1-octanol (3.13 ml, 19.89 mmol, 1.2 eq.), DIC (3.37 ml, 21.54 mmol, 1.3 eq.) and DMAP (61 mg, 0.497 mmol, 0.03 eq.) to yield **26d** as a colorless oil (4.327 g, 89.0 %, *R*_f 0.32 in CE5, visualization by KMnO₄).

Following the procedure outlined for **27a**, Boc-derivative **27d** was prepared from bromoester **26d** (1.73 g, 5.89 mmol, 2.5 eq.), *N*-Boc-1,6-diaminohexane (0.510 g, 2.36 mmol) and potassium carbonate (3.26 g, 23.58 mmol, 10 eq.) to yield **27d** as a pale yellow oil (1.241 g, 82.1 %, *R*_f 0.18 in CE50 on an NH₃-pretreated TLC plate, visualization by ninhydrin).

The deprotection of amine **27d** was performed according to the procedure described for compound **2a** in Example 1; diamine **28d** (1.09 g, quantitative; *R*_f 0.22 in mobile phase D2, detection with ninhydrin) was obtained in the form of a yellowish oil. ¹H NMR (600 MHz, CDCl₃): δ = 8.22, 4.86, 4.05, 3.04, 2.37, 1.75, 1.67, 1.61, 1.48, 1.40, 1.32–1.25, 0.88 ppm. ¹³C NMR (150.9 MHz, CDCl₃): δ = 172.99, 64.85, 52.44, 51.90, 39.41, 33.13, 31.75, 29.18, 29.14, 28.54, 26.54, 25.86, 25.49, 24.88, 22.60, 21.83, 14.05 ppm. HRMS (ESI): *m/z* calcd. for C₃₂H₆₅N₂O₄ [M + H]⁺ 541.49388; found 541.49297.

***N*¹,*N*³,*N*⁵-Tris(6-(bis((octyloxycarbonyl)butyl)amino)hexyl)adamantane-1,3,5-tricarboxamide 29d**

Following the procedure outlined for **29a** in Example 14, lipidoid **29d** was prepared from adamantane-1,3,5-tricarboxylic acid (70 mg, 0.261 mmol), amine **28d** (565 mg, 1.04 mmol, 4 eq.) and DIPEA (455 μl, 2.61 mmol, 10 eq.) to yield **29d** as a thick pale yellow oil (229 mg, 47.8 %, *R*_f 0.58 in D2, visualization by ninhydrin). ¹H NMR (600 MHz, CDCl₃): δ = 5.79, 4.05, 3.21, 2.44, 2.31, 2.01, 1.84, 1.80, 1.61, 1.47, 1.34–1.24, 0.88 ppm. ¹³C NMR (150.9 MHz, CDCl₃): δ = 176.03, 173.63, 64.50, 53.54, 41.65, 39.77, 39.45, 37.88, 34.12, 31.76, 29.48, 29.20, 29.16, 28.63, 25.91, 22.87, 22.61, 14.07 ppm. HRMS (ESI): *m/z* calcd. for C₁₀₉H₂₀₃N₆O₁₅ [M + H]⁺ 1836.53010; found 1836.52959.

Example 18***N*¹,*N*¹-Di((heptyloxycarbonyl)pentyl)hexane-1,6-diamine 28e**

Following the procedure outlined for **26a**, bromoester **26e** was prepared from 6-bromohexanoic acid (3.0 g, 15.38 mmol), 1-heptanol (2.61 ml, 18.46 mmol, 1.2 eq.), DIC (3.13 ml, 19.99 mmol, 1.3 eq.) and DMAP (56 mg, 0.461 mmol, 0.03 eq.) to yield **26e** as a colorless oil (4.177 g, 92.6 %, *R*_f 0.27 in CE5, visualization by KMnO₄).

Following the procedure outlined for **27a**, Boc-derivative **27e** was prepared from bromoester **26e** (1.49 g, 5.08 mmol, 2.5 eq.), *N*-Boc-1,6-diaminohexane (0.440 g, 2.03 mmol) and potassium carbonate (2.81 g, 20.34 mmol, 10 eq.) to yield **27e** as a pale yellow oil (1.076 g, 82.5 %, *R*_f 0.15 in CE50 on an NH₃-pretreated TLC plate, visualization by ninhydrin).

The deprotection of amine **27e** was performed according to the procedure described for compound **2a** in Example 1; diamine **28e** (0.895 g, 98.6 %; R_f 0.17 in mobile phase D2, detection with ninhydrin) was obtained in the form of a yellowish oil. $^1\text{H NMR}$ (600 MHz, CDCl_3): δ = 8.40, 4.04, 3.04, 2.99, 2.31, 2.05, 1.72, 1.66, 1.61, 1.48, 1.39, 1.33–1.26, 0.88 ppm. $^{13}\text{C NMR}$ (150.9 MHz, CDCl_3): δ = 173.38, 64.64, 52.02, 33.78, 31.68, 28.88, 28.58, 26.70, 26.25, 26.13, 25.83, 25.68, 24.93, 24.20, 22.99, 22.55, 22.48, 14.03 ppm. **HRMS** (ESI): m/z calcd. for $\text{C}_{32}\text{H}_{65}\text{N}_2\text{O}_4$ $[\text{M} + \text{H}]^+$ 541.49388; found 541.49303.

N*¹,*N*³,*N*⁵-Tris(6-(bis((heptyloxycarbonyl)pentyl)amino)hexyl)adamantane-1,3,5-tricarboxamide **29e*

Following the procedure outlined for **29a** in Example 14, lipidoid **29e** was prepared from adamantane-1,3,5-tricarboxylic acid (55 mg, 0.205 mmol), amine **28e** (444 mg, 0.820 mmol, 4 eq.) and DIPEA (357 μl , 2.05 mmol, 10 eq.) to yield **29e** as a thick pale yellow oil (150 mg, 39.8 %, R_f 0.37 in D2, visualization by ninhydrin). $^1\text{H NMR}$ (600 MHz, CDCl_3): δ = 5.72, 4.05, 3.21, 2.37, 2.29, 2.01, 1.83, 1.80, 1.62, 1.47, 1.42, 1.33–1.25, 0.88 ppm. $^{13}\text{C NMR}$ (150.9 MHz, CDCl_3): δ = 175.97, 173.84, 64.42, 53.96, 41.64, 39.74, 39.53, 37.91, 34.35, 31.70, 29.59, 28.89, 28.63, 27.14, 25.87, 24.97, 22.56, 14.05 ppm. **HRMS** (ESI): m/z calcd. for $\text{C}_{109}\text{H}_{203}\text{N}_6\text{O}_{15}$ $[\text{M} + \text{H}]^+$ 1836.53010; found 1836.52930.

Example 19

N*¹,*N*¹-Di(((nonan-3-yl)oxycarbonyl)propyl)hexane-1,6-diamine **28f*

Following the procedure outlined for **26a**, bromoester **26f** was prepared from 6-bromohexanoic acid (3.2 g, 16.41 mmol), 3-nonanol (2.60 g, 18.05 mmol, 1.1 eq.), DIC (3.27 ml, 21.33 mmol, 1.3 eq.) and DMAP (60 mg, 0.492 mmol, 0.03 eq.) to yield **26f** as a colorless oil (2.88 g, 54.5 %, R_f 0.42 in CE5, visualization by KMnO_4).

Following the procedure outlined for **27a**, Boc-derivative **27f** was prepared from bromoester **26f** (2.82 g, 8.78 mmol, 2.5 eq.), *N*-Boc-1,6-diaminohexane (0.760 g, 3.51 mmol) and potassium carbonate (4.86 g, 35.13 mmol, 10 eq.) to yield **27f** as a pale yellow oil (1.72 g, 70.19 %, R_f 0.56 in CE50 on an NH_3 -pretreated TLC plate, visualization by ninhydrin).

The deprotection of amine **27f** was performed according to the procedure described for compound **2a** in Example 1; diamine **28f** (1.84 g, quantitative; R_f 0.25 in mobile phase D2, detection with ninhydrin) was obtained in the form of a yellowish oil. $^1\text{H NMR}$ (600 MHz, CDCl_3): δ = 8.29, 4.80, 2.99, 2.305, 1.69–1.66, 1.55–1.51, 1.445, 1.38, 1.28–1.24, 0.87, 0.86 ppm. $^{13}\text{C NMR}$ (150.9 MHz, CDCl_3): δ = 173.14, 75.61, 52.10, 39.27, 34.08, 33.56, 31.71, 29.15, 26.90, 26.74, 26.14, 25.69, 25.27, 24.86, 24.33, 22.97, 22.56, 22.51, 14.03, 9.57 ppm. **HRMS** (ESI): m/z calcd. for $\text{C}_{36}\text{H}_{73}\text{N}_2\text{O}_4$ $[\text{M} + \text{H}]^+$ 597.55649; found 597.55631.

***N*¹,*N*³,*N*⁵-Tris(6-(bis(((nonan-3-yl)oxycarbonyl)propyl)amino)hexyl)adamantane-1,3,5-tricarboxamide 29f**

Following the procedure outlined for **29a** in Example 14, lipidoid **29f** was prepared from adamantane-1,3,5-tricarboxylic acid (50 mg, 0.186 mmol), amine **28f** (334 mg, 746 mmol, 4 eq.) and DIPEA (325 µl, 1.86 mmol, 10 eq.) to yield **29f** as a thick pale yellow oil (237 mg, 63.4 %, *R*_f 0.44 in D2, visualization by ninhydrin). ¹H NMR (600 MHz, CDCl₃): δ = 6.385, 4.79, 3.21, 2.99, 2.305, 2.015, 1.88, 1.80, 1.73, 1.66, 1.55–1.50, 1.38–1.35, 1.27–1.24, 0.865, 0.855 ppm. ¹³C NMR (150.9 MHz, CDCl₃): δ = 176.57, 173.02, 76.58, 52.43, 51.98, 41.69, 39.89, 38.89, 37.64, 34.09, 33.55, 31.70, 29.14, 28.84, 26.90, 26.28, 26.01, 25.78, 24.39, 23.13, 22.96, 22.55, 14.04, 9.59 ppm. HRMS (MALDI): *m/z* calcd. for C₁₂₁H₂₂₇N₆O₁₅ [M + H]⁺ 2004.7179; found 2004.7187.

Example 20

6-(Di((heptyloxycarbonyl)propyl)amino)hexan-1-ol 30a

Bromoester **26a** (1.13 g, 4.27 mmol, 2.5 eq.) and potassium carbonate (2.36 g, 17.07 mmol, 10 eq.) were added to a solution of 6-aminohexan-1-ol (0.20 g, 1.71 mmol) in ACN (10 ml), and the mixture was stirred at 50 °C for 20 h. The reaction mixture was then adsorbed onto silica (16 g), and the solvents were evaporated in vacuo. The crude product was purified by flash chromatography on silica (40 g, elution with a linear gradient of D1 in DCM, 0-50 %) to yield the target compound **30a** (0.552 g, 63.0 %, *R*_f 0.56 in D2, visualization by KMnO₄) as a pale yellow oil.

¹H NMR (600 MHz, CDCl₃): δ = 4.05, 3.63, 2.52, 2.33, 1.80, 1.61, 1.565, 1.50, 1.375, 1.32–1.27, 0.88 ppm. ¹³C NMR (150.9 MHz, CDCl₃): δ = 173.54, 64.63, 53.45, 52.82, 32.59, 31.78, 31.69, 28.89, 28.60, 26.95, 25.86, 25.45, 22.55, 21.78, 14.03 ppm. HRMS (ESI): *m/z* calcd. for C₂₈H₅₆NO₅ [M + H]⁺ 486.41530; found 486.41467.

tris(6-(Di((heptyloxycarbonyl)propyl))hexyl)adamantane-1,3,5-tricarboxylate 31a

Tetramethylfluoroformamidinium hexafluorophosphate (TFFH, 169 mg, 0.640 mmol, 3.3 eq.) and DIPEA (0.506 ml, 2.91 mmol, 15 eq.) were added to a solution of adamantane-1,3,5-tricarboxylic acid (52 mg, 0.194 mmol) in anhydrous DMF (5 ml), and the solution was stirred for 30 min at 0 °C. Then a solution of alcohol **30a** (311 mg, 0.640 mmol, 3.3 eq.) and DMAP (7 mg, 0.058 mmol, 0.3 eq.) in DMF (2.0 ml) was added, and the reaction mixture was stirred for 12 h at rt. The solution was poured into a 250 ml separatory flask, diluted with saturated aqueous NaHCO₃ (50 ml), and the product was extracted with diethyl ether (100 ml, 2 x 50 ml). The combined organic phase was washed with brine (50 ml), dried over anhydrous sodium sulphate, filtered through an S2 frit, and the solvents were evaporated in an RVE. The crude product was purified by silica gel column chromatography using a linear gradient of D1 in DCM (0-65%). Lipidoid **31a** (33 mg, 10.3 %, *R*_f 0.57 in mobile phase D4, detection with ninhydrin) was obtained in the form of a viscous yellowish oil. ¹H NMR (600 MHz, CDCl₃): δ = 4.05,

2.40, 2.05–1.94, 1.83, 1.61, 1.35–1.25, 0.886 ppm. ^{13}C NMR (150.9 MHz, CDCl_3): δ = 176.02, 64.95, 64.45, 41.35, 39.14, 37.14, 31.67, 28.87, 28.55, 25.83, 22.54, 14.03 ppm. HRMS (MALDI): m/z calcd. for $\text{C}_{97}\text{H}_{176}\text{N}_3\text{O}_{18}$ $[\text{M} + \text{H}]^+$ 1671.2943; found 1671.2973.

5 Example 21

6-(Di((hexyloxycarbonyl)butyl)amino)hexan-1-ol 30b

Following the procedure outlined for **30a**, alcohol **30b** was prepared from 6-aminohexan-1-ol (0.20 g, 1.71 mmol), bromoester **26b** (1.13 g, 4.27 mmol, 2.5 eq.) and K_2CO_3 (2.36 g, 17.07 mmol, 10 eq.) to yield **30b** as a pale yellow oil (0.599 g, 72.3 %, R_f 0.55 in D2, visualization by KMnO_4).

- 10 ^1H NMR (600 MHz, CDCl_3): δ = 4.04, 3.63, 2.46, 2.32, 1.76, 1.60, 1.56, 1.45, 1.37–1.25, 0.87 ppm. ^{13}C NMR (150.9 MHz, CDCl_3): δ = 173.69, 64.56, 62.79, 53.57, 52.96, 32.64, 31.90, 31.82, 29.45, 29.24, 29.21, 28.61, 27.02, 25.91, 25.51, 22.63, 14.07 ppm.

Tris(6-(di((hexyloxycarbonyl)butyl))hexyl)adamantane-1,3,5-tricarboxylate 31b

- 15 Lipidoid **31b** was prepared from adamantane-1,3,5-tricarboxylic acid (84 mg, 0.313 mmol), TFFH (273 mg, 1.03 mmol, 3.3 eq.), DIPEA (0.818 ml, 4.179 mmol, 15 eq.), DMAP (11 mg, 0.093 mmol, 0.3 eq.) and alcohol **30b** (608 mg, 1.25 mmol, 4 eq.) according to the procedure described for compound **31a** in Example 20; lipidoid **31b** (55 mg, 10.4 %; R_f 0.74 in mobile phase D2, detection with ninhydrin) was obtained as a viscous yellow oil. ^1H NMR (600 MHz, CDCl_3): δ = 4.05, 2.96, 2.37, 2.33, 2.01, 1.94, 20 1.85–1.78, 1.66, 1.60, 1.38, 1.34–1.28, 0.88 ppm. ^{13}C NMR (150.9 MHz, CDCl_3): δ = 176.01, 172.90, 64.76 – 64.34, 52.16, 47.81, 46.61, 41.35, 39.14, 37.12, 33.22, 31.38, 28.53, 25.54, 22.50, 22.09, 13.97 ppm. HRMS (MALDI): m/z calcd. for $\text{C}_{97}\text{H}_{176}\text{N}_3\text{O}_{18}$ $[\text{M} + \text{H}]^+$ 1671.2943; found 1671.2910.

Example 22

25 **6-(Di((nonyloxycarbonyl)propyl)amino)hexan-1-ol 30c**

Following the procedure outlined for **30a**, alcohol **30c** was prepared from 6-aminohexan-1-ol (0.20 g, 1.71 mmol), bromoester **26c** (1.25 g, 4.27 mmol, 2.5 eq.) and K_2CO_3 (2.36 g, 17.07 mmol, 10 eq.) to yield **30c** as a pale yellow oil (0.651 g, 70.4 %, R_f 0.59 in D2, visualization by KMnO_4).

- ^1H NMR (600 MHz, CDCl_3): δ = 4.07, 3.65, 3.09, 3.03, 2.45, 2.16, 1.89, 1.61, 1.56, 1.45, 1.34–1.25, 30 0.88 ppm. ^{13}C NMR (150.9 MHz, CDCl_3): δ = 172.24, 65.25, 62.39, 52.41, 51.55, 32.11, 31.84, 30.65, 29.45, 29.22, 28.54, 26.29, 25.87, 22.65, 18.39, 14.09 ppm.

Tris(6-(di((nonyloxycarbonyl)propyl))hexyl)adamantane-1,3,5-tricarboxylate 31c

- Lipidoid **31c** was prepared from adamantane-1,3,5-tricarboxylic acid (86 mg, 0.320 mmol), TFFH (279 mg, 1.06 mmol, 3.3 eq.), DIPEA (0.838 ml, 4.81 mmol, 15 eq.), DMAP (12 mg, 0.096 mmol, 0.3 eq.) and alcohol **30c** (695 mg, 1.28 mmol, 4 eq.) according to the procedure described for compound **31a** in
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Example 20; lipidoid **31c** (23 mg, 3.9 %; R_f 0.91 in mobile phase D2, detection with ninhydrin) was obtained as a viscous yellow oil. ^1H NMR (600 MHz, CDCl_3): δ = 4.05, 2.37, 2.01, 1.95, 1.83, 1.61, 1.35–1.24, 0.87 ppm. ^{13}C NMR (150.9 MHz, CDCl_3): δ = 176.02, 64.70, 41.35, 39.15, 37.15, 31.82, 29.44, 29.23, 29.20, 28.58, 28.47, 25.88, 22.63, 14.07 ppm. HRMS (MALDI): m/z calcd. for $\text{C}_{109}\text{H}_{200}\text{N}_3\text{O}_{18}$ $[\text{M}]^+$ 1839.4821; found 1839.4799.

Example 23

6-(Di((octyloxycarbonyl)butyl)amino)hexan-1-ol **30d**

Following the procedure outlined for **30a**, alcohol **30d** was prepared from 6-aminohexan-1-ol (0.20 g, 1.71 mmol), bromoester **26d** (1.25 g, 4.27 mmol, 2.5 eq.) and K_2CO_3 (2.36 g, 17.07 mmol, 10 eq.) to yield **30d** as a pale yellow oil (0.623 g, 67.4 %, R_f 0.50 in D2, visualization by KMnO_4). ^1H NMR (600 MHz, CDCl_3): δ = 4.05, 3.63, 2.47, 2.31, 1.61, 1.56, 1.50, 1.37–1.24, 0.87 ppm. ^{13}C NMR (150.9 MHz, CDCl_3): δ = 173.65, 64.52, 62.78, 53.69, 53.42, 34.07, 32.60, 31.75, 29.19, 29.15, 28.61, 27.05, 25.90, 25.49, 22.85, 22.61, 14.06 ppm.

Tris(6-(di((octyloxycarbonyl)butyl)hexyl)adamantane-1,3,5-tricarboxylate **31d**

Lipidoid **31d** was prepared from adamantane-1,3,5-tricarboxylic acid (83 mg, 0.309 mmol), TFFH (270 mg, 1.02 mmol, 3.3 eq.), DIPEA (0.808 ml, 4.64 mmol, 15 eq.), DMAP (11 mg, 0.093 mmol, 0.3 eq.) and alcohol **30d** (671 mg, 1.24 mmol, 4 eq.) according to the procedure described for compound **31a** in Example 20; lipidoid **31d** (147 mg, 25.8 %; R_f 0.82 in mobile phase D2, detection with ninhydrin) was obtained as a viscous yellow oil. ^1H NMR (600 MHz, CDCl_3): δ = 4.05, 2.34, 2.01, 1.95, 1.83, 1.78–1.56, 1.36–1.24, 0.87 ppm. ^{13}C NMR (150.9 MHz, CDCl_3): δ = 176.04, 64.76–64.46, 41.36, 39.16, 37.15, 31.75, 29.19, 29.15, 28.60, 25.90, 22.61, 14.06 ppm. HRMS (MALDI): m/z calcd. for $\text{C}_{109}\text{H}_{200}\text{N}_3\text{O}_{18}$ $[\text{M}]^+$ 1839.4821; found 1839.4792.

Example 24

Preparation of transfection reagents

Reagents were generated by mixing the individual components listed in Table 1 to Table 4. All tables contain the final molar concentrations in the transfection reagent. Stock 5 mM solutions of the individual components in 99.7% ethanol were used for the preparation. Only the DOPE-Cy5 stock solution had a concentration of 0.79 mM and was prepared in chloroform.

Table 1. Composition of transfection reagents A01-A10.

[illegible]

Table 2. Composition of transfection reagents A11-A13.

compound	Concentration of individual components in transfection reagents (mM)		
	A11	A12	A13
23	1.1	—	—
24	—	1.1	—
25	—	—	1.1
cholesterol	2.18	2.18	2.18
DOPE	1.65	1.65	1.65
DMG-PEG ₂₀₀₀	0.075	0.075	0.075

Table 3. Composition of transfection reagents A14-A23.

[illegible]

Table 4. Composition of transfection reagents **A24-A29** (A26 and A27 are comparative examples).

compound	Concentration of individual components in transfection reagents (mM)					
	A24	A25	A26	A27	A28	A29
4e	1.1	1.1	—	—	1.1	—
4d	—	—	—	—	—	1.1
D-Lin-MC3-DMA	—	—	2.5	—	—	—
TT3	—	—	—	1.1	—	—
cholesterol	2.18	2.18	1.93	2.18	2.18	2.18
DOPE	—	—	—	1.64	1.65	1.65
DOPC	1.64	—	—	—	—	—
DSPC	—	1.64	0.49	—	—	—
DMG-PEG ₂₀₀₀	0.075	0.075	0.075	0.075	0.075	0.075
DOPE-Cy5	1.19×10^{-3}	1.19×10^{-3}	1.19×10^{-3}	1.19×10^{-3}	—	—

Example 25**Preparation of lipid nanoparticles (LNP) containing mRNA**

5 DNA encoding the fluorescent protein mKate2 was amplified from the plasmid pmKate2-C (Evrogen) using the primers (5'-CGCCACCATGGTGAGCGAGCTG-3' (SEQ ID NO. 1); 5'-CCTCCTCCACCTCTGTGCCCCAG-3' (SEQ ID NO. 2)) and cloned into the pET24a vector (Invitrogen) under the T7 promoter. Messenger RNA (mRNA) encoding mKate2 was transcribed *in vitro* using the Ampliscribe T7-Flash transcription kit (Lucigen) according to the manufacturer's protocol. The RNA cap analog ARCA (Jena Bioscience) was added to the *in vitro* transcription reaction, and the poly(A) terminus was synthesized using poly(A) polymerase (New England Biolabs) according to the standard protocol.

The mRNA-containing LNPs (mRNA-LNPs) were prepared as follows: 300 μ l of a solution of each of the **A01-A27** transfection reagents prepared in Example 24 was mixed with a solution of 120 μ g of mRNA in 300 μ l of 10 mM citrate buffer (pH 3.0) using a "Y" microfluidic device with two inputs and one output for sampling. The lipid mixture and the mRNA solution were injected separately into each inlet by a linear pump at a constant flow rate of 300 μ l/min. The resulting 600 μ l nanoparticle solution was collected and immediately diluted by the addition of 600 μ l PBS; the corresponding nanoparticle samples designated **B01-B27** were thus formed from the transfection reagents **A01-A27**. Each of the mRNA-LNP samples (**B01-B27**) was prepared in triplicate. The hydrodynamic diameter of freshly formed mRNA-LNPs was measured using dynamic light scattering (NanoZS Zetasizer, Malvern, Worcestershire, UK) at a scattering angle of 173 ° at 25 ° C. The hydrodynamic diameter of mRNA-LNPs ranged from 72 to 135 nm with the exception of B18 with diameter of 265 nm (**Table 5**). In this form, the particles were used for subsequent biological tests.

Table 5. Hydrodynamic diameter of mRNA-LNPs including standard deviation measured by dynamic light scattering.

LNP	Diameter (nm)			LNP	Diameter (nm)			LNP	Diameter (nm)		
B01	81.5	±	8.0	B09	90.8	±	5.5	B17	72.2	±	4.0
B02	99.3	±	2.7	B10	89.5	±	5.0	B18	265.7	±	19.9
B03	85.1	±	4.1	B11	82.5	±	0.2	B19	71.8	±	4.2
B04	107.7	±	4.2	B12	85.5	±	1.1	B20	85.7	±	6.4
B05	76.1	±	1.9	B13	102.1	±	10.7	B21	135.1	±	1.1
B06	72.9	±	8.6	B14	92.6	±	5.1	B22	80.3	±	6.7
B07	73.6	±	3.9	B15	118.0	±	5.5	B23	80.1	±	5.4
B08	108.0	±	18.3	B16	79.4	±	2.7				

Example 26**5 Comparison of mRNA transfection using new LNPs in vitro using various helper lipids in a lipid mixture**

LNPs **B05**, **B24** and **B25** containing mRNA encoding the fluorescent protein mKate2 prepared in triplicate in Example 25 were tested on cells of the human cell line HEK293. Cells were cultured in 96-well plates (5×10^4 cells in 100 μ l culture medium per well) in IMDM medium supplemented with 10% FBS at 37 °C in 5% CO₂. Cells were transfected with 2 μ l of mRNA-LNP (with a final total concentration of all lipid components of 20 μ M) and subsequently incubated for 24 hours. Transfections were performed in triplicates. The intensity of Cy5 fluorescence, indicating LNP entry into cells, and mKate2 fluorescence, indicating the translation of mRNA released from LNP after cell transfection, were analysed in a BD LSR Fortessa cytometer.

15 The novel mRNA-LNPs, whose lipid mixture contained DOPE, DOPC or DSPC helper lipid, were able to efficiently transfect mRNA into the HEK293 cell line, with the most efficient transfection being achieved with mRNA-LNP containing DOPE helper lipid (**Table 6**).

Table 6. Transfection efficiency of new mRNA-LNPs containing helper lipids DOPE (**B05**), DOPC (**B24**) or DSPC (**B25**). The indicated values of the fluorescence intensity of Cy5 and mKate2 are normalized to the control variant of transfection with LNPs containing DOPE (**B05**).

LNP	Fluorescence Cy5			Fluorescence mKate2		
B05	1.00	±	0.04	1.00	±	0.19
B24	0.52	±	0.01	0.18	±	0.01
B25	0.42	±	0.03	0.12	±	0.02

Example 27

25 Comparison of mRNA transfection using new LNPs and mRNA-LNPs formed by known transfection reagents

The transfection efficiency of mRNA-LNP **B05** was compared with selected known, highly efficient transfection reagents, namely D-Lin-MC3-DMA (MedChemExpress Europe) and ionisable lipidoid TT3 (Li, B.: *Nano Lett.* **2015**, *15*, 8099–8107) formulated in Example 25 as **B26-B27**, and also with Lipofectamine® 2000 reagent (Invitrogen, used according to the standard protocol provided by the manufacturer, designated as Lip2000). Cells of the human cell line HEK293T were cultured in 96-well plates (5×10^4 in 100 μ l of culture medium per well) in DMEM medium supplemented with 10% FBS at 37 °C in 5% CO₂. Cells were transfected with 2 μ l of mRNA-LNP (with a final total concentration of all lipid components of 20 μ M) and subsequently incubated for 24 hours. Transfections were performed in triplicates. The fluorescence intensity of mKate2 and the percentage of cells expressing mKate2 were analysed in a BD LSR Fortessa cytometer.

The new mRNA-LNPs (labelled **B05**) exhibited significantly higher transfection efficiency than known transfection reagents (**Table 7**).

Table 7. Comparison of new mRNA-LNPs with mRNA-LNPs formed by commercial ionisable lipids D-Lin-MC3-DMA (**B26**), TT3 (**B27**) and Lipofectamine® 2000 (Lip2000). Transfection efficiency is expressed as the percentage of cells expressing the mKate2 fluorescent protein and as the mKate2 fluorescence intensity normalized to the value from the control transfection with Lipofectamine® 2000.

LNP	% cells expressing mKate2			Fluorescence mKate2		
B05	95.20	±	0.84	13.29	±	0.02
B26	84.22	±	1.18	0.76	±	0.03
B27	90.04	±	2.43	1.96	±	0.06
Lip2000	40.70	±	1.13	1.00	±	0.03

Example 28

Efficiency of mRNA incorporation into lipid nanoparticles

The packaging efficiency of mRNA encoding the mKate2 fluorescent protein into mRNA-LNPs **B01-B27** prepared in Example 25 was determined using a Qubit 4 RNA HS Assay Kit (Life Technologies) according to the manufacturer's protocol. The efficiency of incorporation was determined by comparing the concentration of mRNA freely available in the nanoparticle solution and the concentration of mRNA released from the nanoparticles after their decomposition. mRNA-LNPs were decomposed with buffer containing Triton X-100 (10 mM Tris-HCl, pH 8.0; 0.1 mM EDTA, 2% Triton X-100). The mRNA packaging efficiency was demonstrated, ranging from 43 to 93 % (**Table 8**).

Table 8. Efficiency of packaging mRNA encoding fluorescent protein mKate2 into mRNA-LNP, including standard deviations from triplicates.

LNP	mRNA packaging (%)	LNP	mRNA packaging (%)	LNP	mRNA packaging (%)
B01	92.59 ± 2.79	B09	67.51 ± 6.27	B17	65.9 ± 7.3
B02	82.30 ± 2.20	B10	78.33 ± 0.79	B18	65.3 ± 3.8
B03	90.94 ± 0.55	B11	92.3 ± 4.4	B19	83.6 ± 1.8
B04	93.18 ± 0.77	B12	88.3 ± 1.0	B20	43.4 ± 5.8
B05	80.86 ± 2.31	B13	78.3 ± 7.0	B21	54.8 ± 2.8
B06	83.74 ± 0.24	B14	60.8 ± 2.8	B22	66.3 ± 9.8
B07	76.49 ± 6.16	B15	62.2 ± 4.2	B23	64.2 ± 1.7
B08	81.22 ± 1.33	B16	64.3 ± 1.4		

Example 29

5 Cellular toxicity of mRNA-LNP

A human cell line derived from embryonic kidney cells (HEK293), the same line expressing SV40 large T antigen (HEK293T), human osteosarcoma-derived cell line (U2OS), and human hepatocyte carcinoma cell line (HepG2) were cultured in 96-well plates (5×10^4 cells in 100 μ l of culture medium per well) in Dulbecco's modified medium (DMEM) or in IMDM medium (Iscove's Modified Dulbecco's medium) supplemented with 10% foetal bovine serum (FBS) at 37 °C in 5% CO₂. Cells were transfected with 2 μ l of mRNA-LNPs generated in triplicates in Example 25 (the final total concentration of all lipid components in the well was 20 μ M) or 10 μ l of mRNA-LNPs (the final total concentration of all lipid components in the well was 100 μ M) and subsequently incubated for 24 hours. The cytotoxicity of LNPs was analysed in a CellTiterGlo 2.0 cell viability assay (Promega, USA). Cell viability was normalized to non-transfected cells (control). The results are summarized in **Tables 9A,B** and **10A,B**.

When using mRNA-LNPs with a total concentration of all lipid components of 20 μ M, the maximum cytotoxicity measured in the HEK293 cell line was 16 %, measured for **B02** and **B03**. No significant toxicity was demonstrated for particles **B01** and **B05-B23**. With a 5 times higher total concentration of all lipid components of 100 μ M, the trend was analogous, with the highest toxicity of 38 % in **B03**; particles **B04-B08** exhibited a cytotoxicity of approximately 20 %, **B09** and **B10** exhibited no toxicity. Particles **B11-B23** also exhibited no toxicity. In the HEK293T line, the highest cytotoxicity was measured again in **B02** and **B03**; in other particles, the toxicity was very low. Particles with total concentrations of all lipid components of 20 μ M exhibited almost no toxicity on the U2OS cell line, with a concentration of 100 μ M, the highest toxicity was 35 % for **B10** particles. Particles formed by lipidoids **4a**, **4b**, **4d** and **4e** were non-toxic, particles formed by lipidoids **4f**, **4g**, **9** and **13** were very slightly toxic (**Tab. 9A**, **Tab. 10B**).

Table 9A. Cytotoxicity of mRNA-LNPs expressed as cell viability (%) after the addition of 20 μ M transfection mixture with mRNA for individual cell line types.

LNP	HEK293			HEK293T			U2OS		
Control	100.00	$\pm$	6.75	100.00	$\pm$	2.34	100.00	$\pm$	2.65
B01	98.15	$\pm$	1.00	95.54	$\pm$	0.29	96.51	$\pm$	0.47
B02	84.02	$\pm$	1.98	80.75	$\pm$	2.61	98.08	$\pm$	1.38
B03	84.62	$\pm$	0.32	75.04	$\pm$	2.58	90.09	$\pm$	1.64
B04	91.76	$\pm$	0.45	89.54	$\pm$	1.13	98.59	$\pm$	0.88
B05	98.98	$\pm$	1.73	93.75	$\pm$	2.51	96.45	$\pm$	2.97
B06	96.89	$\pm$	4.71	92.70	$\pm$	2.59	92.75	$\pm$	2.53
B07	96.91	$\pm$	5.64	93.45	$\pm$	0.94	92.53	$\pm$	2.70
B08	97.70	$\pm$	5.13	95.16	$\pm$	4.00	92.98	$\pm$	4.65
B09	107.46	$\pm$	6.15	84.34	$\pm$	3.78	95.89	$\pm$	1.87
B10	97.13	$\pm$	3.47	81.06	$\pm$	2.69	98.92	$\pm$	1.11

Table 9B. Cytotoxicity of mRNA-LNPs expressed as cell viability (%) after the addition of 20 μ M transfection mixture with mRNA for individual cell line types.

LNP	HEK293T			HepG2		
B11	103.8	$\pm$	6.8	104.2	$\pm$	8.4
B12	99.3	$\pm$	6.5	105.7	$\pm$	4.5
B13	101.5	$\pm$	5.6	104.0	$\pm$	7.6
B14	102.3	$\pm$	3.7	106.6	$\pm$	4.8
B15	98.9	$\pm$	1.4	99.7	$\pm$	1.9
B16	100.7	$\pm$	1.7	107.4	$\pm$	3.3
B17	100.2	$\pm$	4.5	108.7	$\pm$	4.9
B18	100.2	$\pm$	3.8	104.2	$\pm$	1.3
B19	96.6	$\pm$	1.6	79.6	$\pm$	4.2
B20	101.7	$\pm$	1.8	106.3	$\pm$	2.4
B21	105.6	$\pm$	1.9	106.4	$\pm$	1.2
B22	104.3	$\pm$	1.6	106.6	$\pm$	3.0
B23	102.9	$\pm$	1.1	104.7	$\pm$	5.0

Table 10A. Cytotoxicity of mRNA-LNPs expressed as cell viability (%) after the addition of 100 μ M transfection mixture with mRNA for individual cell line types.

LNP	HEK293			HEK293T			U2OS		
Control	100.00	$\pm$	6.75	100.00	$\pm$	2.34	100.00	$\pm$	2.65
B01	95.98	$\pm$	0.88	90.01	$\pm$	0.24	94.63	$\pm$	0.58
B02	70.24	$\pm$	2.90	65.65	$\pm$	2.32	91.32	$\pm$	2.40
B03	62.00	$\pm$	2.89	57.21	$\pm$	2.18	75.64	$\pm$	4.92
B04	77.44	$\pm$	1.35	78.14	$\pm$	1.30	97.02	$\pm$	0.73
B05	88.00	$\pm$	4.82	88.92	$\pm$	1.39	96.51	$\pm$	1.87
B06	81.58	$\pm$	3.97	83.86	$\pm$	0.98	83.31	$\pm$	1.74
B07	87.19	$\pm$	1.71	91.09	$\pm$	1.98	85.02	$\pm$	1.37

LNP	HEK293			HEK293T			U2OS	
B08	77.93	±	14.22	88.46	±	2.81	84.76	± 2.16
B09	100.44	±	12.84	82.46	±	5.08	84.88	± 5.59
B10	94.09	±	5.58	86.73	±	8.29	65.30	± 7.71

Table 10B. Cytotoxicity of mRNA-LNPs expressed as cell viability (%) after the addition of 100 μ M transfection mixture with mRNA for individual cell line types.

LNP	HEK293T			HepG2		
B11	98.2	±	3.3	99.7	±	3.8
B12	95.9	±	3.8	101.6	±	3.1
B13	90.9	±	4.3	96.3	±	3.0
B14	98.3	±	0.7	103.8	±	4.5
B15	97.5	±	0.6	109.9	±	9.6
B16	96.8	±	3.3	108.5	±	5.1
B17	96.7	±	2.9	105.1	±	6.6
B18	101.5	±	5.9	108.2	±	4.6
B19	82.2	±	1.8	84.4	±	4.2
B20	96.9	±	4.2	104.5	±	3.8
B21	96.7	±	1.7	102.3	±	6.7
B22	96.1	±	3.1	103.1	±	4.3
B23	100.1	±	4.4	103.6	±	3.9

5 Example 30

Transfection of mRNA using new LNPs *in vitro*

A human cell line derived from embryonic kidney cells (HEK293), the same line expressing SV40 large T antigen (HEK293T), liver carcinoma cells (HepG2, Huh7) and a human osteosarcoma-derived cell line (U2OS), were cultured in 96-well plates (5×10^4 cells in 100 μ l culture medium per well) in Dulbecco's modified medium (DMEM) or in IMDM medium supplemented with 10% fetal bovine serum (FBS) at 37 °C in 5% CO₂. Cells were transfected with 2 μ l of mRNA-LNP **B01** to **B05** prepared in Example 25 (final total concentration of all lipid components in the well was 20 μ M) carrying mRNA encoding the fluorescent protein mKate2 and subsequently incubated for 24 hours. Lipofectamine® 2000 was used as a control transfection reagent. Transfections were performed in three biological replicates, with each biological replicate having three technical replicates. The percentage of cells expressing the mKate2 fluorescent protein and the fluorescence intensity of the mKate2 were analyzed in a BD LSR Fortessa cytometer. For fluorescence intensity, data are normalized to the commercial transfection reagent Lipofectamine® 2000.

For the HEK293 line, the percentage of cells expressing the mKate2 protein was more than 2-fold higher for all the lipidoids used than for a commercially available transfection reagent. The fluorescence intensity was more than 2x higher for all lipidoids used, with the **B02** particles formed by lipidoid **4d** it

was 3x higher than for commercially available Lipofectamine® 2000. mRNA-LNPs formed with the new lipidoids transfected the HepG2 cell line in all cases at least 2.5 times better than commercial Lipofectamine® 2000, the fluorescence intensity for the mRNA-LNPs **B03** formed with lipidoid **4c** was 4.5-fold that for Lipofectamine® 2000. A similar improvement in transfection with new mRNA-LNPs compared to commercial Lipofectamine® 2000 was achieved in other cell lines (**Tab. 11**, **Tab. 12**).

Table 11. Transfection efficiency of new mRNA-LNPs for different cell lines expressed as a percentage of cells expressing fluorescent mKate2 protein from mRNA transfected with particles for HEK293, HepG2, HEK293T, Huh7 and U2OS lines. Statistics were evaluated by Student's unpaired t-test. The p values are always related to the control variant of Lip2000 transfection in the respective cell line; p values <0.001 are indicated with the letter "a".

LNP	HEK293	p	HepG2	p	HEK293T	p	Huh7	p	U2OS	p
B01	91.24 ± 4.25	a	94.57 ± 0.38	a	—		97.08 ± 0.40	a	—	
B02	96.39 ± 0.72	a	97.10 ± 0.70	a	93.10 ± 1.63	a	—		—	
B03	95.63 ± 0.79	a	96.93 ± 0.39	a	92.31 ± 1.56	a	88.23 ± 2.61	a	82.37 ± 1.68	a
B04	90.57 ± 1.54	a	97.56 ± 0.36	a	93.53 ± 3.58	a	90.66 ± 0.79	a	—	
B05	91.56 ± 3.02	a	96.42 ± 0.75	a	93.53 ± 3.58	a	96.26 ± 0.37	a	—	
Lip2000	42.63 ± 17.56	a	38.10 ± 1.54	a	46.03 ± 1.88	a	64.67 ± 1.40	a	53.93 ± 1.93	a

Table 12. Transfection efficiency of new mRNA-LNPs for different cell lines expressed as relative fluorescence intensity of mKate2 from mRNA transfected with particles for HEK293, HepG2, HEK293T, Huh7 and U2OS lines. For fluorescence intensity, data are normalized to the commercial transfection reagent Lipofectamine® 2000. Statistics were evaluated by Student's unpaired t-test. The p values are always relative to the control variant of Lip2000 transfection in the respective cell line; values of p <0.001 are marked with the letter "a", p <0.01 are marked with "b".

LNP	HEK293	p	HepG2	p	HEK293T	p	Huh7	p	U2OS	p
B01	2.33 ± 0.17	a	3.05 ± 0.22	a	—		2.04 ± 0.09	a	—	
B02	2.74 ± 0.37	a	2.87 ± 0.17	a	3.00 ± 0.47	a	—		—	
B03	2.92 ± 0.38	a	4.54 ± 0.22	a	3.93 ± 0.57	a	1.19 ± 0.08	b	2.07 ± 0.27	a
B04	3.08 ± 0.21	a	3.93 ± 0.24	a	3.50 ± 0.55	a	1.26 ± 0.08	a	—	
B05	2.61 ± 0.11	a	3.48 ± 0.32	a	2.87 ± 0.31	a	1.96 ± 0.13	a	—	
Lip2000	1.00 ± 0.30	a	1.00 ± 0.11	a	1.00 ± 0.05	a	1.00 ± 0.03	ab	1.00 ± 0.03	a

Example 31

Transfection of mRNA using new LNPs formed by lipidoids modified in "Z"

Human cell line HepG2 was cultured in 96-well plates (5 × 10⁴ cells in 100 µl culture medium per well) in DMEM medium supplemented with 10% FBS at 37 °C in 5% CO₂. Cells were transfected with 2 µl of mRNA-LNP **B11-B13** prepared in Example 25 (final total concentration of all lipid components in

the well was 20 μ M) and subsequently incubated for 24 hours. Lipofectamine[®] 2000 was used as a control transfection reagent. Transfections were performed in three biological replicates, with each biological replicate having three technical replicates. The percentage of cells expressing the mKate2 fluorescent protein and the fluorescence intensity of the mKate2 were analyzed in a BD LSR Fortessa cytometer. New mRNA-LNP with Z modified substituents of lipidoids were able to efficiently transfect the mRNA into HepG2 cell line (**Tab. 13**) in all cases better than control transfection reagent Lipofectamine[®] 2000.

Table 13. Transfection efficiency of new mRNA-LNPs expressed as a percentage of cells expressing fluorescent mKate2 protein and as relative fluorescence intensity of mKate2 from mRNA transfected with particles for HepG2 cell line. For fluorescence intensity, data are normalized to the commercial transfection reagent Lipofectamine[®] 2000 (Lip2000). Statistics were evaluated by Student's unpaired t-test. The p values are always related to the control variant of Lip2000 transfection in the respective cell line; p values <0.001 are indicated with the letter "a".

LNP	% cells expressing mKate2			p	Fluorescence intensity			p
B11	97.92	±	0.62	a	3.81	±	0.51	a
B12	95.92	±	2.55	a	1.62	±	0.34	a
B13	95.31	±	0.53	a	2.23	±	0.26	a
Lip2000	40.70	±	1.13	a	1.00	±	0.03	a

Example 32

Transfection of mRNA using new LNPs formed by biodegradable lipidoids

Human cell lines HEK293, HEK293T and U2OS were cultured in 96-well plates (5×10^4 cells in 100 μ l culture medium per well) in DMEM or IMDM medium supplemented with 10% FBS at 37 °C in 5% CO₂. Cells were transfected with 2 μ l of mRNA-LNP **B05**, **B08–B10** and **B14–B23** prepared in Example 25 (final total concentration of all lipid components in the well was 20 μ M) and subsequently incubated for 24 hours. Lipofectamine[®] 2000 was used as a control transfection reagent. Transfections were performed in three biological replicates, with each biological replicate having three technical replicates. The percentage of cells expressing the mKate2 fluorescent protein and the fluorescence intensity of the mKate2 were analyzed in a BD LSR Fortessa cytometer.

Cell lines were transfected significantly better with the new mRNA-LNPs compared to commercial Lipofectamine[®] 2000. Lipidoids **4e**, **9**, **13** and **21** had comparable effects on the percentage of cells transfected. The fluorescence intensity of the translated mRNA encoding the mKate2 protein was then always significantly increased in the HEK293 and HEK293T lines compared to commercial Lipofectamine[®] 2000. In the U2OS line, lipidoid **13** transfected similarly to the lipidoid **4e** (**Tab. 14**, **Tab. 15**).

Table 14. Transfection efficiency of new mRNA-LNPs for various cell lines expressed as the percentage of cells expressing the mKate2 fluorescent protein from the mRNA transfected with the particles. HEK293, HEK293T and U2OS lines are listed. Statistics were evaluated by Student's unpaired t-test.

5 The p values are always related to the control variant of Lip2000 transfection in the respective cell line; values of $p < 0.001$ are marked with the letter "a".

LNP	HEK293	p	HEK293T	p	U2OS	p
B08	86.50 ± 0.71	a	95.63 ± 4.50	a	86.87 ± 4.72	a
B09	87.50 ± 2.15	a	96.03 ± 4.79	a	88.65 ± 0.93	a
B10	86.50 ± 0.71	a	96.83 ± 3.52	a	86.85 ± 2.32	a
B14			92.94 ± 0.90	a		
B15			87.30 ± 1.44	a		
B16			92.81 ± 0.95	a		
B17			92.94 ± 2.50	a		
B18			92.16 ± 2.02	a		
B19			99.67 ± 0.12	a		
B20			90.23 ± 0.38	a		
B21			91.68 ± 0.53	a		
B05	89.20 ± 0.34	a	98.57 ± 0.23	a	90.15 ± 2.10	a
Lip2000	36.73 ± 1.86	a	59.40 ± 2.78	a	38.87 ± 3.18	a

Table 15. Transfection efficiency of new mRNA-LNPs for various cell lines expressed as relative fluorescence intensity of mKate2 related to the Lip2000 transfection control variant. HEK293, HEK293T and U2OS lines are listed. Statistics were evaluated by Student's unpaired t-test. The p values are always related the control variant of Lip2000 transfection in the respective cell line; values of $p < 0.001$ are marked with the letter "a", $p < 0.01$ are marked with "b".

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LNP	HEK293	p	HEK293T	p	U2OS	p
B08	3.11 ± 0.49	a	1.37 ± 0.08	b	1.08 ± 0.12	b
B09	4.31 ± 0.62	a	1.49 ± 0.13	a	1.93 ± 0.05	a
B10	2.96 ± 0.19	a	1.77 ± 0.26	b	1.06 ± 0.11	b
B14			1.41 ± 0.04	a		
B15			2.51 ± 0.21	a		
B16			1.03 ± 0.14			
B17			1.74 ± 0.29	b		
B18			1.94 ± 0.08	a		
B19			7.47 ± 0.90	a		
B05	8.54 ± 0.44	a	2.93 ± 0.15	a	2.14 ± 0.10	a
Lip2000	1.00 ± 0.09	a	1.00 ± 0.11	ab	1.00 ± 0.15	ab

Example 33**Transfection of siRNA using new LNPs *in vitro***

LNPs containing small interfering RNA (siRNA, catalog number AM4626, Ambion) causing degradation of mRNA encoding green fluorescent protein (GFP) were prepared as follows: 300 µl of **A28** transfection reagent solution prepared in Example 24 was mixed with a solution of 1.20 nmol siRNA in 300 µl 10 mM citrate buffer (pH 3.0) using a microfluidic device analogously to Example 25. The resulting siRNA-LNPs were immediately diluted in 600 µl PBS; the corresponding nanoparticles labeled **B28** were thus formed from transfection reagent **A28**. LNPs carrying control (scrambled; 4390843, Ambion) siRNAs that do not target any endogenously cell-transcribed mRNA (**B29**) were prepared analogously. Lipofectamine RNAiMax (Invitrogen) was used as a control transfection reagent specifically for siRNA transfection according to the manufacturer's standard protocol. A human cell line U2OS stably expressing the green fluorescent protein (GFP) was used for siRNA-LNP knockdown. Cells were cultured in 96-well plates (5×10^4 cells in 100 µl culture medium per well) in DMEM medium supplemented with 10% FBS at 37 °C in 5% CO₂. Cells were transfected with 2 µl of siRNA-LNP, the final total concentration of all lipid components in the well was 20 µM; final siRNA concentration was 16 nM and subsequently incubated for 24 hours. Transfections were performed in three biological replicates. The percentage of cells expressing the green fluorescent protein GFP and the fluorescence intensity of the GFP were analyzed in a BD LSR Fortessa cytometer.

With the new siRNA-LNPs (**B28**), a decrease in GFP expression was observed down to 1 % of GFP-expressing cells; with commercial RNAiMax, GFP expression was still detected in 13.6 % of cells. The fluorescence intensity of GFP when using the new siRNA-LNPs (**B28**) decreased by 1.25-fold compared to the commercial RNAiMax reagent (**Tab. 16**).

Table 16. Reduction in GFP-encoding mRNA in the U2OS cell line by siRNA-LNP (**B28**) expressed as a percentage of GFP-expressing cells and GFP fluorescence intensity compared to the commercial transfection reagent RNAiMax and siRNA-LNP carrying control siRNA (**B29**). Statistics were evaluated by Student's unpaired t-test. The p values are related to the **B28** transfection mixture tested; values of $p < 0.001$ are marked with the letter "a", $p < 0.01$ are marked with "b".

LNP	% cells expressing GFP			p	GFP fluorescence intensity			p
Control	82.30	±	1.60		0.86	±	0.02	
B28	1.00	±	0.30	a	0.26	±	0.03	ab
B29	83.60	±	0.90	a	0.92	±	0.06	a
RNAiMax	13.60	±	1.20	a	0.32	±	0.03	b

siRNA-LNPs containing small interfering RNA (siRNA, catalog number 4392420, Ambion) causing the degradation of mRNA encoding tyrosyl-DNA phosphodiesterase 2 (TDP2) were prepared in the

same manner as **B28** particles in this example. The corresponding nanoparticles designated **B30** were thus formed from the transfection reagent **A28**. LNPs carrying control (scrambled; 4390843, Ambion) siRNAs that do not target any endogenously cell-transcribed mRNA (**B29**) were also used as a control. Lipofectamine RNAiMax (Invitrogen) was used as a control transfection reagent specifically for siRNA transfection. The human cell line HEK293 and two lines derived from human multiple myeloma, which are very difficult to transfect with available transfection reagents, were used for siRNA-LNP knockdown (Brito J.L.R., Brown N., Morgan G.J. (2010) Transfection of siRNAs in Multiple Myeloma Cell Lines. In: Min WP., Ichim T. (eds) RNA Interference. Methods in Molecular Biology (Methods and Protocols), vol 623. Humana Press). Cells were cultured in 96-well plates (5×10^4 cells in 100 μ l culture medium per well) in DMEM medium supplemented with 10% FBS at 37 °C in 5% CO₂. Cells were transfected with 2 μ l siRNA-LNP (with a final total concentration of all lipid components of 20 μ M and a final concentration of siRNA of 16 nM) and subsequently incubated for 24 hours. Transfections were performed in three biological replicates. RNA was isolated with an RNAeasy Plus Micro Kit (Qiagen). The cDNA was prepared with TATAA GrandScript cDNA Supermix (TATAAbiocenter) according to the manufacturer's recommendations. Quantitative RT-PCR was performed in a LightCycler 480 (Roche Life Science). The primers for amplifying mRNA encoding TDP2 were as follows: 5'-CGAGAGGAGGGTCTCAAAGAG-3' (SEQ ID NO. 3) and 5'-ATTTCGGAAGGCTGCTGTC-3' (SEQ ID NO. 4). mRNA encoding GAPDH was used to normalize the data (Primers: 5'-AATCCCATCACCATCTTCCA-3' (SEQ ID NO. 5) and 5'-TGGACTCCACGACGTACTCA-3' (SEQ ID NO. 6)).

In all these cases, the new siRNA-LNPs significantly reduced the level of TDP2 mRNA in cells compared to the commercial transfection reagent RNAiMax. In the HEK293 cell line it was 2.86-fold, in the OPM-2 myeloma line 4.3-fold, and in the RPMI8226 myeloma line 6.7-fold compared to a commercial reagent for siRNA transfection (**Tab. 17**).

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Table 17. Reduction of endogenously expressed TDP2 mRNA levels in cells by new siRNA-LNPs (**B30**) compared to the commercial transfection reagent RNAiMax and compared to control siRNA-LNPs (**B29**) in the HEK293, OPM-2 and RPMI8226 cell lines.

Statistics were evaluated by Student's unpaired t-test. The p values are related to the **B30** transfection mixture tested; p values <0.001 are indicated with the letter "a".

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LNP	HEK293	p	OPM-2	p	RPMI8226	p
Control	1.00 ± 0.10		1.00 ± 0.01		1.00 ± 0.03	
B30	0.03 ± 0.01	a	0.14 ± 0.02	a	0.13 ± 0.00	a
B29	0.99 ± 0.17	a	1.09 ± 0.01	a	0.78 ± 0.01	a
RNAiMax	0.09 ± 0.01	a	0.58 ± 0.02	a	0.87 ± 0.11	a

Example 34**Transfection of plasmid DNA using new LNPs *in vitro***

LNPs containing 4706 bp plasmid DNA (Evrogen, Cat. No. FP181) encoding the mKate2 fluorescent protein were prepared as follows: 300 µl of the **A28** transfection reagent solution prepared in Example 24 was mixed with a solution of 120 µg of plasmid DNA in 300 µl of 10 mM citrate buffer (pH 3.0) using a microfluidic device analogously to Example 25. The resulting DNA-LNPs were immediately diluted in 600 µl PBS; the corresponding nanoparticles designated **B31** were thus formed from transfection reagent **A28**. Transfections were performed on the human cell line HEK293T. Cells were cultured in 96-well plates (5 × 10⁴ cells in 100 µl culture medium per well) in DMEM medium supplemented with 10% FBS at 37 °C in 5% CO₂. Cells were transfected with 2 µl of DNA-LNP (with a final total concentration of all lipid components of 20 µM) and subsequently incubated for 24 hours. Lipofectamine® 2000 (Lip2000, Invitrogen) was used as a control transfection reagent. Transfections were performed in three biological replicates, with each biological replicate having three technical replicates. The percentage of cells expressing the mKate2 fluorescent protein and the fluorescence intensity of the mKate2 were analyzed in a BD LSR Fortessa cytometer.

The percentage of cells expressing the fluorescent protein mKate2 was 3.8-fold higher with the new DNA-LNPs compared to the commercial transfection reagent. The fluorescence intensity was 2.9 times higher than with the commercial reagent (**Tab. 18**).

Table 18. Transfection efficiency of new DNA-LNPs (**B31**) compared to commercial Lipofectamine® 2000 (Lip2000) in the HEK293T cell line expressed as % of mKate2-expressing cells and as mKate2 fluorescence intensity. For fluorescence intensity, data are normalized to the commercial transfection reagent Lipofectamine® 2000. Statistics were evaluated by Student's unpaired t-test. Values of p < 0.001 are indicated with the letter "a".

LNP	% cells expressing mKate2			p	mKate2 fluorescence intensity			p
B31	91.16	±	0.98	a	2.93	±	0.36	a
Lip2000	24.30	±	1.00	a	1.00	±	0.07	a

Example 35**Transfection of mRNA with new LNPs into human primary hepatocytes**

The gene encoding the NanoLuc bioluminescent protein was amplified from plasmid pET51b(+)_S-Luc_CLIP (Addgene, Cat. No. 113923; obtained as a gift from Kai Johnsson) using the primers: (5'-TAATACGACTCACTATAGGG-3' (SEQ ID NO. 7); 5'-GCTAGTTATTGCTCAGCGG-3' (SEQ ID NO. 8)). Messenger RNA (mRNA) was prepared *in vitro* analogously to Example 25 and packaged into LNP as follows: A 300 µl sample of transfection reagent **A28**, and 120 µg of mRNA in 300 µl of 10 mM citrate buffer (pH 3.0) were assembled into LNP using a microfluidic device analogously to Example

25. The resulting mRNA-LNPs were immediately diluted in 600 μ l PBS; the corresponding nanoparticles labeled **B32** were thus formed from transfection reagent **A28**. Primary hepatocytes were isolated from a human donor according to a published protocol (LeCluyse, E.L.: *Methods in Molecular Biology* 2005; 290, 207-230). Cells were transfected with 2 μ l of mRNA-LNP (with a final total concentration of all lipid components of 20 μ M) and incubated for 24 hours. Lipofectamine[®] 2000 transfection reagent was used as a control. Substrate in the Nano-Glo[®] Luciferase Assay System kit (Promega) was then added to the cells and the luminescence intensity was analyzed in a Microplate Reader: Infinite[®] M1000 PRO (Tecan).

The new mRNA-LNPs efficiently transfected human primary hepatocytes, with a significant 2.0-fold increase in luminescence compared to the non-transfected control (**Tab. 19**).

Table 19. Transfection efficiency of new mRNA-LNPs (**B32**) in the primary line of human hepatocytes compared to the commercial transfection reagent Lipofectamine[®] 2000 expressed as bioluminescence intensity normalized to the value from control transfection. Statistics were evaluated by Student's unpaired t-test. Values of $p < 0.05$ are indicated with the letter "c".

LNP	bioluminescence intensity			p
B32	1.97	$\pm$	0.60	c
Lip2000	1.00	$\pm$	0.12	c

Example 36

Transfection of cyclic dinucleotides with new LNPs in vitro

The cyclic dinucleotide (2',3'-cGAMP) (Sigma, cat. No. SML1229-.5UMO) was packaged in LNP as follows: 300 μ l samples of **A01-A06** transfection reagents, and 120 nmol of cGAMP in 300 μ l of 10 mM citrate buffer (pH 3.0) were added to the LNP using a microfluidic device analogous to Example 25. The resulting cGAMP-LNPs were immediately diluted in 600 μ l PBS; samples of nanoparticles designated **B33-B38** were thus formed from the corresponding transfection reagents **A01-A06**. A reporter assay showing the degree of induction of the interferon response by the cyclic dinucleotide cGAMP depending on the STING pathway was used to analyse the efficiency of cGAMP-LNP particle transfection. For this purpose, a cell reporter line HEK293T expressing the common type of STING protein and a luciferase reporter gene under the IRF3 interferon-stimulated (ISRE) promoter were used according to Novotná *et al.* (Novotná, B.: *J. Med. Chem.* **2019**, 62 (23), 10676-10690). Cells were transferred to poly-D-lysine (Sigma-Aldrich)-coated 96-well plates (Greiner Bio-One) at a density of 2.5×10^4 in DMEM medium containing glucose (containing L-glutamine; Biowest) supplemented with 10% FBS (Capricorn Scientific) and 1% penicillin-streptomycin (Biowest). After incubation at 37 °C in 5% CO₂ overnight, serially diluted compounds were added to the cells for 7 hours. In parallel, HEK293T cells were incubated with the test compounds alone for 30 minutes, washed twice with fresh medium,

and then cultured for an additional 6.5 hours. Finally, 50 μ l of cell culture medium was mixed with 30 μ l of Bright-Glo Luciferase Assay System reagent (Promega) in white 96-well plates and luminescence was read in a Spark[®] spectrophotometer (TECAN, Grödig). Values of 50% effective concentration (EC_{50}) were calculated using GraphPad Prism (La Jolla) as described in Novotná *et al.* (Novotná, B.: *J. Med. Chem.* **2019**, 62 (23), 10676-10690) (Tab. 20).

The cyclic dinucleotide 2',3'-cGAMP exhibited EC_{50} values of STING activation $\pm$ 30 μ M. All new cGAMP-LNPs formed by ionizable lipidoids **4a-4f** efficiently transfected HEK293T cells and activated STING in the nanomolar region, increasing transfection efficiency approximately 2000-15000x. The most effective was lipidoid **4d**, exhibiting an EC_{50} of 2.00 ± 0.36 nM.

Table 20. EC_{50} values of STING activation for 2',3'-cGAMP transfected with new cGAMP-LNPs (**B33-B38**) formed by ionizable lipidoids **4a-4f**.

LNP	EC_{50} STING activation (nM)		
B33	9.00	$\pm$	1.75
B34	12.00	$\pm$	1.80
B35	10.00	$\pm$	6.42
B36	2.00	$\pm$	0.36
B37	4.00	$\pm$	1.12
B38	14.00	$\pm$	2.61

Example 37

Toxicity of new mRNA-LNPs *in vivo*

Messenger RNA (mRNA) encoding NanoLuc protein was prepared and packaged into particles analogously to Example 35. The resulting mRNA-LNPs (**B32**) were administered intraperitoneally to three C57B1/6 mice (BIOCEV, Vestec) at a concentration of 0.5 mg mRNA/kg, wherein three control C57B1/6 mice were administered PBS intraperitoneally. Another three C57B1/6 mice were administered mRNA-LNP at a 5-fold higher concentration (2.5 mg mRNA/kg) in the same manner. Similarly, identical doses of mRNA-LNPs were again administered to 3 C57B1/6 mice (0.5 mg mRNA/kg) and 3 C57B1/6 mice (2.5 mg mRNA/kg) intravenously, and the animals were anesthetized prior to the administration (2.5 mg/mouse ketaminum, 0.4 mg/mouse xylazinum, Bioveta). Mice were then observed for 48 hours and none showed any signs of toxicity or phenotypic changes compared to control animals.

Example 38

Biodistribution of new mRNA-LNPs *in vivo*

The gene encoding the CRE recombinase protein was amplified from the plasmid pCAG-Cre-IRES2-GFP (Addgene, catalog number 26646, donated by Anjen Chenn) using the primers: (5'-TAATACGACTCACTATAGAAATTTACT-3' (SEQ ID NO. 9); 5'-CTAATCGCCATCTTCCAGCA-3' (SEQ ID NO. 10)). Messenger RNA (mRNA) was prepared *in vitro* analogously to Example 25 and packaged into LNP as follows: A 300 µl sample of transfection reagent **A28**, and 120 µg of mRNA in 300 µl of 10 mM citrate buffer (pH 3.0) were assembled into LNP using a microfluidic device analogously to Example 25. The resulting mRNA-LNPs were immediately diluted in 600 µl PBS; the corresponding nanoparticles labeled **B39** were thus formed from transfection reagent **A28**. mRNA-LNPs (**B39**) were administered intravenously at a concentration of 0.5 mg mRNA/kg to 2.5 mg mRNA/kg in each case to 3 mice with a global dual Cre reporter (Mazumdar, M.D.: *Genesis* **2007**, 45:593-605) (breeding BIOCEV, Vestec) enabling the analysis of successful recombination. In the cells to which mRNA-LNPs carrying Cre recombinase mRNA were successfully delivered, chromosomal recombination and subsequent excision of the membrane red protein gene (so-called red tomato) and "turning on" the transcription of membrane green protein (GFP) gene occurred. Mice, including non-particulate control mice, were sacrificed 3 days after particulate application, and all organs were subjected to histological analysis according to a standardized protocol. Histological images show a complete distribution of particles into the liver, which led to a 30-50% conversion of cells expressing the red membrane protein to cells expressing the green membrane protein 3 days after application (**Fig. 7**). In **Fig. 7**, histological images of liver show the conversion of cells expressing the red membrane protein to cells expressing the green membrane protein 3 days after application of the mRNA-LNPs [2.5 mg/kg of mRNA] encoding Cre recombinase (labeled as "3 dpi mRNA-LNP"). "PBS control" – injection of PBS buffer only.

Example 39

25 Stability of new mRNA-LNPs and siRNA-LNPs at 4 °C

Human cell line HEK293T was cultured in 96-well plates (5 x 10⁴ cells in 100 µl culture medium per well) in DMEM medium supplemented with 10% FBS at 37 °C in 5% CO₂. Cells were transfected with 2 µl of mRNA-LNP **B05**, prepared in Example 25 (final total concentration of all lipid components in the well was 20 µM) and subsequently incubated for 24 hours. The **B05** mRNA-LNPs were stored at 4 °C and transfections were analogously repeated one, three and five weeks after assembly. Transfections were performed in triplicates. The fluorescence intensity of the mKate2 was analyzed in a BD LSR Fortessa cytometer.

Transfection efficiency of the new mRNA-LNPs (labelled **B05**) remained unchanged for at least three weeks and decreased to 81% compared to Day 1, while stored at 4°C (**Tab. 21**).

Table 21. Transfection efficiency of **B05** mRNA-LNPs stored at 4 °C for HEK293 cells expressed as relative fluorescence intensity of mKate2. Data normalized to fluorescence intensity measured at Day 1 after assembly. Five weeks later, the **B05** mRNA-LNPs still kept 81% of transfection efficiency while stored at 4 °C.

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Time	Fluorescence of mKate2		
1 day	1.00	±	0.17
1 week	1.14	±	0.26
3 weeks	1.04	±	0.16
5 weeks	0.81	±	0.11

siRNA-LNPs (labelled **B30** and **B40**) containing small interfering RNA (siRNA, catalog number 4392420, Ambion) causing the degradation of mRNA encoding tyrosyl-DNA phosphodiesterase 2 (TDP2) were prepared in the same manner as described in Example 33 and were stored at 4°C. siRNA-LNPs **B40** were formed from transfection reagent A29 prepared in Example 24. HEK293T cells were cultured in 96-well plates (5 x 10⁴ cells in 100 µl culture medium per well) in DMEM medium supplemented with 10% FBS at 37 °C in 5% CO₂. Cells were transfected with 2 µl siRNA-LNPs (with a final total concentration of all lipid components of 20 µM and a final concentration of siRNA of 16 nM) and subsequently incubated for 24 hours. Transfections were performed in triplicates. RNA isolation, cDNA preparation and qRT-PCR were performed as described in Example 33. The whole procedure was repeated one, two and three months after the LNP assembly.

The transfection efficacy of the new siRNA-LNPs (labelled **B30** and **B40**) expressed as their ability to reduce the level of TDP2 mRNA in cells remained practically unchanged while stored at 4 °C for three months (**Tab. 22**).

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Table 22. Relative level of TDP2 mRNA in HEK293T cells after transfection of siRNA-LNPs stored at 4 °C at respective time points.

Time	B30		B40	
1 day	0.0380	± 0.0107	0.0336	± 0.0079
1 month	0.0700	± 0.0036	0.0817	± 0.0100
2 months	0.0423	± 0.0026	0.0492	± 0.0037
3 months	0.0387	± 0.0067	0.0646	± 0.0164

25 Example 40

Transfection of siRNA by preformed empty LNPs

Transfection reagents **A28** and **A29** from Example 24 were used to assemble empty nanoparticles **B41** and **B42** in citrate buffer excluding nucleic acid analogously to Example 25, with no post-dilution. One µl of LNPs was mixed with 1 pmol of siRNA targeting TDP2 10 minutes prior to transfection, analogously as recommended for Lipofectamine RNAiMax, and incubated at room temperature.

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HEK293T and HepG2 cells (5×10^4 cells in 100 μ l culture medium per well) were transfected with the mixture of preassembled LNPs and siRNA and incubated for 24 hours. Transfections were performed in triplicates. Lipofectamine RNAiMax was used as a control transfection reagent. Preassembled LNPs subsequently mixed with 1 pmol of siRNA were able to knock down TDP2 mRNA expression by 90-97%, both significantly better than commercial transfection reagent Lipofectamine RNAiMax (Tab. 23).

Table 23. Reduction of TDP2 mRNA levels in cells by preassembled empty LNPs mixed with siRNA targeting TDP2 compared to commercial transfection reagent RNAiMax. Statistics were determined by Student's unpaired t-test. P values are related to control transfection with Lipofectamine RNAiMax, values $p < 0.001$ are indicated by letter "a", values $p < 0.01$ are indicated by letter "b".

LNP	HEK293T	p	HepG2	p
Control	1.00 $\pm$ 0.25		1.00 $\pm$ 0.08	
B41	0.03 $\pm$ 0.01	a	0.10 $\pm$ 0.03	b
B42	0.07 $\pm$ 0.01	b	0.08 $\pm$ 0.01	a
RNAiMax	0.12 $\pm$ 0.03	ab	0.18 $\pm$ 0.01	ab

Example 41

Transfection of peripheral blood mononuclear cells (PBMC) by siRNA-LNPs and evaluation of cytokine response

Transfection reagent **A28** from Example 24 was used to form siRNA-LNP designated **B43** containing siRNA causing the degradation of mRNA encoding Poly(U)-binding-splicing factor (PUF60). The siRNA-LNPs were prepared analogously as in Example 33. Lipofectamine RNAiMax was used as a control transfection reagent. Peripheral blood mononuclear cells (PBMC) from 3 anonymous blood donors (with the agreement number 13/06/2012 of ethical committee of the Institute of Hematology and Blood Transfusion, Prague, Czech Republic) were isolated by Ficoll (Ficoll® Paque Plus, 17-1440-02, GE Healthcare) gradient and cultured in RPMI medium supplemented with 10% of FBS and 50U/ml of penicillin/streptomycin. Cells were seeded at 10^5 cells/100ul (200ul in total) in 96-well plates and incubated at 37°C. The ON-target plus SMARTpool siRNA targeting PUF60 were purchased from Dharmacon (catalogue number L-012505-01-0005; Ilkirch, France) and resuspended in water at 20 pmol/ul. The final concentrations of 1 pmol or 10 pmol were used. Each condition was performed in triplicate. 24 hours after transfection the cells were harvested including the supernatant. Total RNA was extracted using Nucleospin RNA extraction kit (Macherey Nagel) following manufacturer's instruction. Reverse transcription was performed with 500 ng of RNA. Quantitative RT-PCR was performed in a LightCycler 480 (Roche Life Science) in duplicate using the SYBERGREEN mix. The primers for amplifying PUF60 were as follows: 5-CCTTCAACCGCATCTACGTG-3 (SEQ ID NO. 7) and 5-CTGGGCCTTCTCGTACTCAA-3 (SEQ ID NO. 8). RPLP0 was used to normalize the data (Primers:

5-CACCATTGAAATCCTGAGTGATG-3 (SEQ NO. 9) and 5-TGACCAGCCCAAAGGAGAAG-3 (SEQ NO. 10)). The quantities of total IFN- α and IFN- γ produced by PBMC after transfections were measured in cell-free supernatants using human ELISA kits (Human IFN- α ELISABASIC kit (HRP), 3425-1H-20, Mabtech and Human IFN- λ 1 ELISABASIC kit, 3570-1H-20, Mabtech). 24 hours treatment of PBMC with 1 μ M CpG ODN 2216 Class A (Invivogen) was used as a positive control. In all cases, the siRNA-LNPs reduced the level of PUF60 mRNA in PBMC cells (**Tab. 24**) without upregulating the cytokine response as opposed to RNAiMax (**Tab. 25 and Tab. 26**).

Table 24. Reduction of endogenously expressed PUF60 mRNA levels in PBMC by new siRNA-LNPs (**B43**) compared to the transfection reagent RNAiMax and compared to non-treated cells.

LNP	Donor 1	Donor 2	Donor 3
Non treated	1.00 $\pm$ 0.23	1.00 $\pm$ 0.34	1.00 $\pm$ 0.18
B43 [1 pmol of siRNA]	0.55 $\pm$ 0.04	0.12 $\pm$ 0.20	0.65 $\pm$ 0.32
B43 [10 pmol of siRNA]	0.40 $\pm$ 0.14	0.50 $\pm$ 0.02	0.07 $\pm$ 0.03
RNAiMax [1 pmol of siRNA]	0.51 $\pm$ 0.08	1.15 $\pm$ 0.01	0.62 $\pm$ 0.40
RNAiMax [10 pmol of siRNA]	0.21 $\pm$ 0.20	0.29 $\pm$ 0.30	0.22 $\pm$ 0.10

Table 25. IFN- α response of PBMC after treatment of siRNA-LNPs compared to RNAiMax.

LNP	Donor 1	Donor 2	Donor 3
Non treated	2.38 $\pm$ 2.06	0.44 $\pm$ 0.56	0.00 $\pm$ 0.00
Positive control	533 $\pm$ 13	661 $\pm$ 58	2265 $\pm$ 29
B43 [1 pmol of siRNA]	0.00 $\pm$ 0.00	1.52 $\pm$ 1.54	0.00 $\pm$ 0.00
B43 [10 pmol of siRNA]	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	16.8 $\pm$ 20.1
RNAiMax [1 pmol of siRNA]	0.00 $\pm$ 0.00	6.05 $\pm$ 8.58	0.65 $\pm$ 1.12
RNAiMax [10 pmol of siRNA]	433 $\pm$ 251	1814 $\pm$ 243	1951 $\pm$ 31

Table 26. IFN- λ response of PBMC after treatment of siRNA-LNPs compared to RNAiMax.

LNP	Donor 1			Donor 2			Donor 3		
Non treated	2.49	±	2.34	2.99	±	2.75	37.9	±	7.4
Positive control									
	713	±	67	1765	±	180	1000	±	52
B43 [1 pmol of siRNA]									
	0.00	±	0.00	0.00	±	0.00	20.4	±	2.7
B43 [10 pmol of siRNA]									
	11.7	±	20.2	0.00	±	0.00	40.7	±	4.2
RNAiMax [1 pmol of siRNA]									
	0.00	±	0.00	0.00	±	0.00	44.5	±	13.2
RNAiMax [10 pmol of siRNA]									
	28.7	±	49.8	100	±	59	706	±	188

Example 42**Effectivity of siRNA-LNPs *in vivo***

- 5 Transfection reagent **A29** from Example 24 was used to form siRNA-LNPs designated as **B44** with siRNA targeting mouse apolipoprotein B (ApoB) gene, a hepatocyte-expressed gene involved in cholesterol transport (ApoB) (catalogue number 238055 Apob mouse siPOOL-40 kit, siTOOLS Biotech GmbH) and alternatively siRNA-LNPs (**B45**) with control non-targeted siRNA-LNPs (enclosed in 238055 Apob mouse siPOOL-40 kit, siTOOLS Biotech GmbH), assembled as described in Example 33.
- 10 The siRNA-LNPs were dialyzed to PBS. The endotoxin levels were < 2 EU/ml. Mice were fasted for 4 hours before plasma collection by retroorbital bleed. The siRNA-LNPs targeting ApoB were administered intravenously to 5 C57B1/6 mice (BIOCEV, Czech Center of Phenogenomics, Vestec) at a concentration of 32 µg of siRNA and 16 µg of siRNA, respectively, wherein the control 5 mice were administered with 32 µg of non-targeting siRNA-LNPs and another 5 mice were administered PBS
- 15 control. All mice were sacrificed 2 days after LNP application. Plasma levels of cholesterol, triglycerides and LDL-C were measured by using automated systems at the Czech Center of Phenogenomics according to standardized protocol.
- Clinical biochemistry of plasma markers such as total cholesterol, triglycerides and LDL-C, affected by ApoB knock down, were significantly decreased compared to control animals, demonstrating thus the
- 20 efficient delivery of ApoB siRNA by novel LNPs into the liver (**Tab. 27**).

Table 27. Clinical biochemistry of plasma markers indicating efficient ApoB knockdown in the liver. Statistics were evaluated by Student's unpaired t-test. The p1 values are always relative to the control mice injected with PBS; the p2 values are always relative to the mice injected with **B45** LNP with control non-targeted siRNA; values of $p < 0.001$ are marked with the letter "a", $p < 0.01$ are marked with "b".

LNP	Total cholesterol		p1	p2	Triglycerides		p1	p2	LDL-C		p1	p2
Control	1.99	± 0.34	a		0.46	± 0.12	ab		0.39	± 0.04	a	
B44 [16 µg]	0.43	± 0.18	a	a	0.18	± 0.03	a	a	0.15	± 0.05	a	a
B44 [32 µg]	0.35	± 0.13	a	a	0.19	± 0.03	b	a	0.13	± 0.03	a	a
B45 [32 µg]	2.58	± 0.61		a	0.53	± 0.04		a	0.84	± 0.12		a

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Example 43

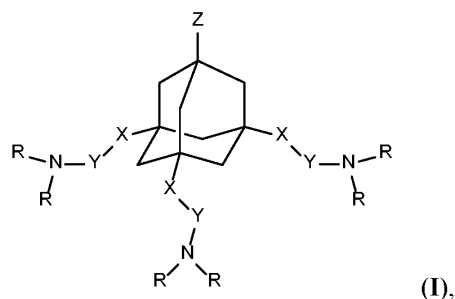
Effectivity of nucleoside-modified mRNA-LNPs *in vivo*

The gene encoding the CRE recombinase protein was amplified from the plasmid pCAG-Cre-IRES2-GFP (Addgene, catalog number 26646, donated by Anjen Chenn) using the primers: (5'-TAATACGACTCACTATAGAATTTACT-3' (SEQ ID NO. 9); 5'-CTAATCGCCATCTTCCAGCA-3' (SEQ ID NO. 10)). Messenger RNA (mRNA) was prepared *in vitro* analogously to Example 25, with the exception that CTP was 100% exchanged for 5-Methyl-CTP (NU-1138L, Biogen Praha s.r.o.) and UTP was 100% exchanged for N1-Methylpseudo-UTP (NU-890L, Biogen Praha s.r.o.), and packaged into LNP as follows: A 300 µl sample of transfection reagent **A28**, and 120 µg of mRNA in 300 µl of 10 mM citrate buffer (pH 3.0) were assembled into LNP using a microfluidic device analogously to Example 25. The resulting mRNA-LNPs were immediately diluted in 600 µl PBS; the corresponding nanoparticles labeled **B46** were thus formed from transfection reagent **A28**. mRNA-LNPs (**B46**) were administered intravenously at a concentration of 0.5 mg mRNA/kg to 2.5 mg mRNA/kg in each case to 3 mice with a global dual Cre reporter (Mazumdar, M.D.: *Genesis* **2007**, 45:593-605) (breeding BIOCEV, Vestec) enabling the analysis of successful recombination. In the cells to which mRNA-LNPs carrying Cre recombinase mRNA were successfully delivered, chromosomal recombination and subsequent excision of the membrane red protein gene (so-called red tomato) and "turning on" the transcription of membrane green protein (GFP) gene occurred. Mice, including non-particulate control mice, were sacrificed 3 days after particulate application, and all organs were subjected to histological analysis according to a standardized protocol. Histological images show a complete distribution of particles into the liver, which led to a 35-75% conversion of cells expressing the red membrane protein to cells expressing the green membrane protein 3 days after application.

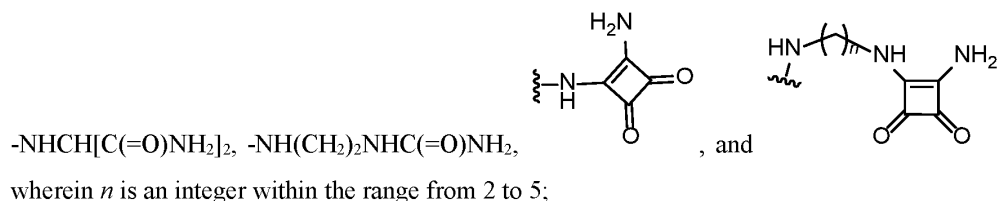
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C L A I M S

1. Lipidoids of general formula I

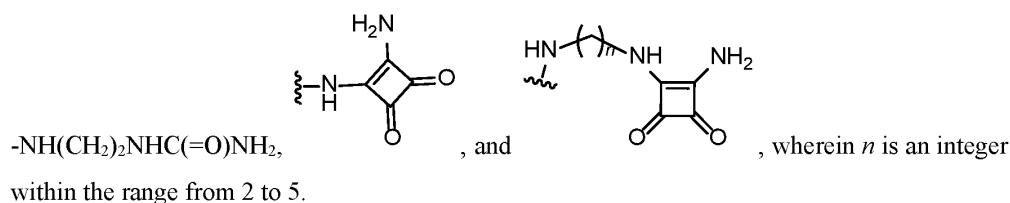


- 5 wherein X is selected from a group consisting of -C(=O)NH-, -C(=O)O-, -C(=S)O-, -C(=O)S-,
 -C(=S)S-, -C(=O)NHNH-, -CH₂-, -O-, -OC(=O)-, -S-, -SC(=O)-, -NH-, -NHNH-, -NHC(=O)-,
 -NHNHC(=O)-, -C≡C-, -CH=CH-, a five-membered heterocycle containing at least 2 nitrogen
 atoms, -CH₂C(=O)NH-, -CH₂C(=O)O-, -CH₂C(=S)O-, -CH₂C(=S)S-, -CH₂C(=O)NHNH-,
 -N=CH-, and -CH=N-;
- 10 Y is independently selected from a group consisting of alkylene chains C₂-C₁₀, wherein in the said
 alkylene chain, one or more -CH₂- groups may optionally be replaced with one or more O or S atoms;
- Z is selected from the group consisting of hydrogen, -OH, -CH₂OH, -NH₂, -N⁺(CH₃)₂-(CH₂)₃-SO₃⁻,
 -N⁺(CH₃)₂-(CH₂)₂-COO⁻, and -NHCH₃, -N(CH₃)₂, -N⁺(CH₃)₃, -OCH₃, -OCH₂CH₃, -C(=O)R¹,
 wherein R¹ is selected from -NH₂, -NH(CH₂)_nOH, -N[(CH₂)_nOH]₂, -NHCH(CH₂OH)₂,
 15 -NHCH₂CH(OH)CH₂OH, -NH(CH₂)_nC(=O)NH₂, -N[CH₂C(=O)NH₂]₂,



- and R are the same or different from each other, each R being independently selected from the group
 consisting of alkyl C₈-C₂₀, alkenyl C₈-C₂₀, and alkynyl C₈-C₂₀, wherein in the said alkyl, alkenyl or
 20 alkynyl, one or more -CH₂- groups may optionally be replaced with one or more groups selected
 from -CH(OH)-, -OC(=O)-, -C(=O)O-, -S-S-, -C(=O)NH-, -NHC(=O)-, -O-, and -S-;
 and pharmaceutically acceptable salts, addition salts and solvates thereof.

2. The lipidoid according to claim 1, wherein Z is selected from a group consisting of
 25 hydrogen, -OH, -CH₂OH, -NH₂, -N⁺(CH₃)₂-(CH₂)₃-SO₃⁻, -N⁺(CH₃)₂-(CH₂)₂-COO⁻, and -C(=O)R¹,
 wherein R¹ is selected from -NH₂, -NH(CH₂)_nOH, -N[(CH₂)_nOH]₂, -NHCH(CH₂OH)₂,
 -NHCH₂CH(OH)CH₂OH, -NH(CH₂)_nC(=O)NH₂, -N[CH₂C(=O)NH₂]₂, -NHCH[C(=O)NH₂]₂,



3. The lipidoid according to claim 1 or 2, wherein X is selected from -C(=O)NH- , a five membered heterocycle containing at least 2 nitrogen atoms, or -C(=O)O- .
4. The lipidoid according to any one of the preceding claims, wherein R is independently selected from the group consisting of $\text{C}_8\text{-C}_{20}$ alkyl, $\text{C}_8\text{-C}_{20}$ alkenyl, and $\text{C}_8\text{-C}_{20}$ alkynyl, wherein in the said alkyl, alkenyl or alkynyl, one or more $\text{-CH}_2\text{-}$ groups may optionally be replaced by one or more groups selected from -CH(OH)- , -OC(=O)- , and -C(=O)O- .
5. A lipidoid according to any one of the preceding claims, wherein all R in the molecule are the same, or all nitrogen atoms in the molecule are substituted identically by two identical R or two different R.
6. A transfection agent comprising at least one lipidoid of general formula I according to any one of claims 1 to 5 in an amount of 10 to 50 mol. %, and at least one helper lipid in a total amount of 50 to 90 mol. %.
7. A transfection agent according to claim 6, comprising at least one lipidoid of general formula I according to any one of claims 1 to 5 in an amount of 15 to 30 mol. %, cholesterol in an amount of 30 to 55 mol. %, and at least one further helper lipid in an amount of 20 to 50 mol. %.
8. A transfection particle comprising at least one lipidoid of general formula I according to any one of claims 1 to 5, at least one nucleic acid and/or a part thereof and/or nucleic acid derivative, and preferably also at least one helper lipid.
9. Use of the lipidoid of general formula I according to any one of claims 1 to 5 or the transfection agent according to claim 6 or 7, or the transfection particle according to claim 8 for *in vitro* transfection of cells or tissues with nucleic acid and/or a part thereof and/or nucleic acid derivative.
10. Use according to claim 9 for silencing or activating chromosomal gene(s), silencing or activating immunogens, inhibiting or activating signaling pathways, editing genome or transcriptome, or enabling the expression of the protein(s) encoded by the nucleic acid.

11. The lipidoid of formula I according to any one of claims 1 to 5 or the transfection agent according to claim 6 or 7, or the transfection particle according to claim 8 for use in transfecting cells or tissues with nucleic acid and/or a part thereof and/or nucleic acid derivative *in vivo*, preferably except for the transfection of human embryos for use in industrial or commercial purposes and except for the modification of a human germ line.
12. The lipidoid of formula I according to any one of claims 1 to 5 or the transfection agent according to claim 6 or 7, or the transfection particle according to claim 8 for use according to claim 11 for silencing or activating chromosomal genes(s), silencing or activating immunogens, inhibiting or activating signaling pathways, editing genome or transcriptome, or enabling the expression of the protein(s) encoded by the nucleic acid.
13. The lipidoid of formula I according to any one of claims 1 to 5 or the transfection agent according to claim 6 or 7, or the transfection particle according to claim 8 for use as a medicament, in particular for gene therapy, preferably for the treatment of malignancies and/or genetic disorders.
14. Use of the lipidoid of general formula I according to any one of claims 1 to 5 or the transfection agent according to claim 6 or 7, or the transfection particle according to claim 8 in cosmetic preparations for delivering the active ingredient to the site of action.