

ANTIMICROBIAL SURFACE TREATMENT

TECHNICAL FIELD

The invention relates to antimicrobial surface treatment methods and constructs for use in such methods. In particular, the invention relates to
5 antibacterial surface treatment methods for surgical dressings and implants.

BACKGROUND ART

As stated in the publication of Gallo *et al* (2014) it is expected that the projected increased usage of implantable devices in medicine will result in a natural rise in the number of infections related to these cases. The current
10 knowledge of antimicrobial surface treatments suitable for prevention of infection is reviewed. Surface treatment modalities include minimizing bacterial adhesion, biofilm formation inhibition and bactericidal action.

The publications of Reid and others disclose biocidal formulations including a selenium (Se) compound. The selenium compounds may be deposited on a
15 surface and covalently or non-covalently associated with it. A broad range of selenium compounds are proposed, including compounds of the formula $RSeX$ where R is an aliphatic or phenolic group and X is a protecting group.

Cationic lipids have primarily been developed for use in liposomal gene delivery as an alternative to viral-based gene delivery, but have also been
20 identified as having bactericidal activity. Common cationic lipid classes include N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) and 3β [N-(N',N'-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol). At least partly because of the low efficiency of lipofection the vast majority of clinical trials of gene therapy have used alternative means of gene
25 delivery. The further development of cationic lipids has sought to improve the efficiency of lipofection.

The publications of Behr *et al* (1989) and Remy *et al* (1994) disclose spermine-lipid conjugates where the lipid is a phosphatidylethanolamine (DOPES and DPPES). Conjugation is via the carboxyl function of a
30 functionalised L-5-carboxyspermine derivative. The conjugates are used in the preparation of compacted lipopolyamine-coated plasmids. The coated plasmids are used in a transfection procedure.

The publication of Byk *et al* (1989) discloses structure-activity relationships amongst a series of cationic-lipids developed for use in DNA
35 transfer. Amongst the lipoamines evaluated in these studies, the polyamine geometry was shown to have an influence on transfection efficiency.

The publication of Randazzo *et al* (2009) discloses an exploration of the dual functionality of cationic lipids in the context of gene transfer and bactericidal activity. The cationic lipids demonstrated to possess these activities comprised a sterol moiety as the lipid component.

5 It is an object of the present invention to provide a method of treating the surface of surgical dressings and implants using water dispersible antimicrobial-lipid constructs that is effective to reduce the incidence of postoperative infections. It is an object of the present invention to provide antimicrobial-lipid constructs for use in this method. These objects are to
10 be read in the alternative with the object at least to provide a useful choice in the selection of such treatments and constructs.

DISCLOSURE OF INVENTION

In a **first** aspect the invention provides an antimicrobial surface treatment method comprising the step of contacting the surface of an object with an
15 aqueous dispersion of at least one functional-lipid construct where the lipid is a di-acyl, di-alkenyl or di-alkyl glycerophospholipid and the functional moiety of the construct confers the antimicrobial activity.

Preferably, the object is a surgical dressing or implant. More preferably, the object is a surgical implant. Most preferably, the surface is stainless
20 steel.

Preferably, the aqueous dispersion is devoid of detergents and organic solvents. More preferably, the aqueous dispersion consists of saline or water and the at least one functional-lipid construct.

Preferably, the lipid is a di-acyl glycerophospholipid. More preferably, the
25 lipid is a phosphatidylethanolamine. Most preferably, the lipid is a di-oleoyl phosphatidylethanolamine.

Preferably, the functional moiety is selected from the group consisting of: selenide and polycations. More preferably, the functional moiety is selected from the group consisting of: cyanoselenide and polyamines. Most preferably,
30 the functional moiety is cyanoselenide.

Preferably, the antimicrobial surface treatment is an antibacterial surface treatment. More preferably, the antimicrobial surface treatment is a bactericidal surface treatment.

Preferably, the contacting the surface is by immersing the object in the
35 dispersion for a time sufficient to provide the antimicrobial surface treatment. More preferably, the time is less than 60 seconds. Yet more

preferably, the time is less than 30 seconds. Most preferably, the time is less than 10 seconds.

Preferably, the dispersion is sonicated whilst the object is immersed.

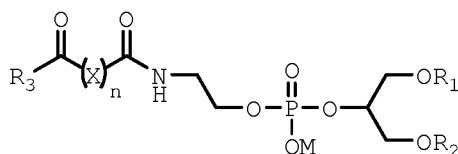
Preferably, the concentration of the construct in the dispersion is sufficient to provide the antimicrobial surface treatment. More preferably, the concentration is less than 1 mg/mL of construct.

In a first embodiment of the **first** aspect the invention provides an antimicrobial surface treatment method comprising the step of contacting the surface with an aqueous dispersion of a selenide-lipid construct where the lipid is a di-acyl, di-alkenyl or di-alkyl glycerophospholipid.

In a second embodiment of the **first** aspect the invention provides a method of treating the surface of a surgical implant comprising the step of contacting the surface with an aqueous dispersion of a cationic lipid construct of the structure F-S-L where F is an N¹-acylated polyamine, S is a spacer selected to provide a construct that is dispersible in water and L is a diacyl- or dialkylglycerolipid.

Preferably, L is a diacylglycerolipid. More preferably, L is a diacylglycerophospholipid. Most preferably, L is phosphatidylethanolamine.

Preferably, the cationic lipid construct is of the structure:

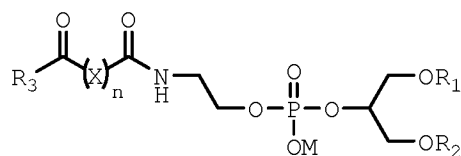


where M is a monovalent cation, n is the integer 3, 4 or 5 when X is the divalent radical methylene (-CH₂-), R₁ and R₂ are independently selected from the group consisting of C₁₄₋₂₀ saturated, mono- or di- unsaturated, unbranched acyl groups, and R₃ is an N¹-acylated polyamine.

Preferably, the aqueous dispersion is not saline in this second embodiment of the first aspect of the invention.

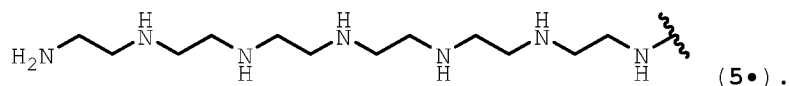
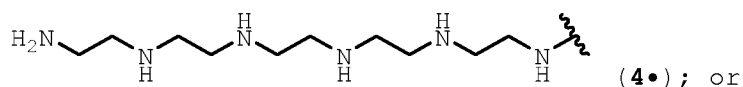
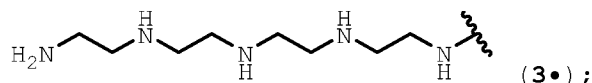
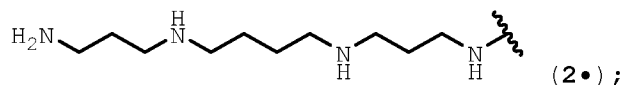
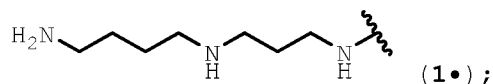
$$\text{Se} \equiv \text{C}-\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\text{CH}_2-\text{C}(=\text{O})-\text{N}(\text{CH}_2\text{C}(=\text{O})\text{OM})-\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\left(\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\text{CH}_2-\text{C}(=\text{O})-\text{N}(\text{CH}_2\text{C}(=\text{O})\text{OM})-\text{CH}_2-\text{C}(=\text{O})-\text{NH}\right)_m-\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\left(\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\text{CH}_2-\text{C}(=\text{O})-\text{N}(\text{CH}_2\text{C}(=\text{O})\text{OM})-\text{CH}_2-\text{C}(=\text{O})-\text{NH}\right)_m-\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\left(\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\text{CH}_2-\text{C}(=\text{O})-\text{N}(\text{CH}_2\text{C}(=\text{O})\text{OM})-\text{CH}_2-\text{C}(=\text{O})-\text{NH}\right)_n-\text{CH}_2-\text{C}(=\text{O})-\text{NH}-\text{CH}_2-\text{CH}_2-\text{O}-\text{P}(\text{OM}')(\text{OR}_1)(\text{OR}_2)$$

20 In a **third** aspect the invention provides a cationic-lipid construct of the structure:



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Preferably, R₃ is of the structure:



A **fourth** aspect the invention provides a bactericidal surface treatment preparation consisting essentially of a dispersion in water of at least one construct of the second or third aspect of the invention.

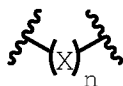
In the description and claims of this specification the following acronyms, terms and phrases have the meaning provided: "alicyclic" means cyclic aliphatic; "aliphatic" means alkanes, alkenes or alkynes or their derivatives and is used as a descriptor for compounds that do not have the special stability of aromatics; "alkanes" means a saturated hydrocarbon of the general formula C_nH_{2n+2}; "alkenes" means unsaturated hydrocarbons that contain one or more double carbon-carbon bonds; "alkynes" means unsaturated hydrocarbons that contain one or more triple carbon-carbon bonds; "aromatic" means containing a benzene ring or having similar chemical properties; "Boc" means *tert*-butoxycarbonyl; "Boc₃Spm" means (N¹,N⁴,N⁹-tri-*tert*-butoxycarbonyl)-1,12-diamino-4,9-diazadodecane; "comprising" means "including", "containing" or "characterized by" and does not exclude any additional element, ingredient or step; "consisting essentially of" means excluding any element, ingredient or step that is a material limitation; "consisting of" means excluding any element, ingredient or step not specified except for impurities and other incidentals; "dispersible in water" means dispersible in pure, deionised

water at 25 °C in the absence of organic solvents or surfactants to provide a dispersion at a concentration of at least 1 µmol/mL and "water dispersible" has a corresponding meaning; "DOPE" means 1,2-O-dioleoyl-sn-glycero-3-phosphatidylethanolamine; "DSPE" means 1,2-O-distereoyl-sn-glycero-3-phosphatidylethanolamine; "hydrophilic" means having a tendency to mix with, dissolve in, or be wetted by water and "hydrophilicity" has a corresponding meaning; "hydrophobic" means having a tendency to repel or fail to mix with water and "hydrophobicity" has a corresponding meaning; "monovalent cation" means an ion having a single positive charge and includes the monovalent cations H⁺, Na⁺, K⁺ or (CH₃CH₂)₃N⁺; "N¹-acylation" means the attachment of an acyl group (RCO-) at a terminal, primary amine of the longest chain of the molecule and "N¹-acylated" has a corresponding meaning; "polyamine" means an unbranched organic compound comprising three or more amine functions including at least two primary amino (-NH₂) functions; and "Spm" (or "spm") means spermine.

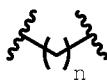
The terms "first", "second", "third", etc. used with reference to elements, features or integers of the subject matter defined in the Statement of Invention and Claims, or when used with reference to alternative embodiments of the invention are not intended to imply an order of preference.

Where concentrations or ratios of reagents are specified the concentration or ratio specified is the initial concentration or ratio of the reagents. Where values are expressed to one or more decimal places standard rounding applies. For example, 1.7 encompasses the range 1.650 recurring to 1.749 recurring.

In the absence of further limitation the use of plain bonds in the representations of the structures of compounds encompasses the diastereomers, enantiomers and mixtures thereof of the compounds. In the representations of the structures or substructures of compounds the repeat of a divalent radical is represented by:



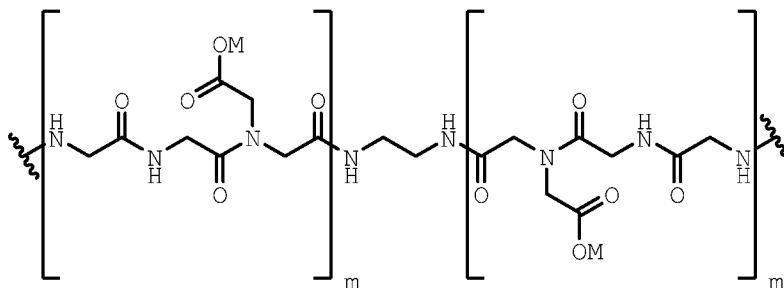
where -X- is the divalent radical repeated n times. Where the divalent radical is methylene (-CH₂-) the repeat of this divalent radical is represented by:



In the absence of further limitation the use of plain bonds in the representations of the structures of compounds encompasses the diastereomers, enantiomers and mixtures thereof of the compounds.

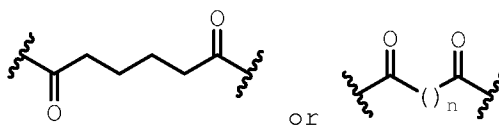
To facilitate the description of the preparation and use of the constructs
5 the following designations are used:

"-CMG(m) -" designates the substructure:



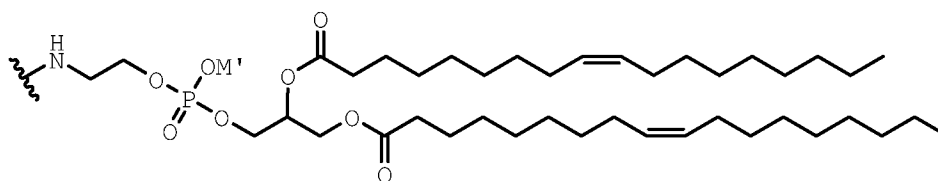
where m is the integer 1, 2, 3 or 4 and M is a monovalent substituent;

"-Ad-" designates the substructure:

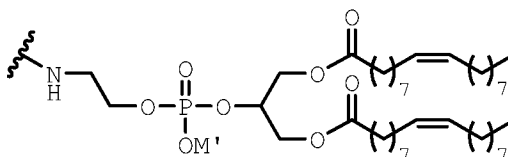


where n is the integer 4; and

"-DOPE" designates the substituent of the structure:



or



where M' is a monovalent cation (typically H^+).

The invention will now be described with reference to embodiments or examples and the figures of the accompanying drawings pages.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1. ^1H NMR spectrum of the cyanoselenide-lipid construct designated NCSeCH₂CO-CMG(2)-Ad-DOPE

Figure 2. Fluorescence microscopy of the surface of untreated (**A**) and treated (**B**) coupons following incubation in the presence of viable cultures of *Staphylococcus aureus*.

Figure 3. Fluorescence microscopy of the surface of untreated (**A**) and treated (**B**) coupons following incubation in the presence of viable cultures of *Staphylococcus epidermis*.

Figure 4. Photographs of incubated blood agar plates following inoculation with cultures of *Staphylococcus aureus* exposed to untreated (**A**) and treated (**B**) coupons.

Figure 5. Photographs of incubated blood agar plates following inoculation with cultures of *Staphylococcus epidermis* exposed to untreated (**A**) and treated (**B**) coupons.

Figure 6. Scanning electron micrographs (350x) of samples of untreated (**A**) and treated (**B**) surgical dressing using the construct designated NCSeCH₂CO-CMG(2)-Ad-DOPE in the treatment.

Figure 7. Scanning electron micrographs (3,500x) of samples of untreated (**A**) and treated (**B**) surgical dressing using the construct designated NCSeCH₂CO-CMG(2)-Ad-DOPE in the treatment.

DESCRIPTION OF EMBODIMENTS

The method of the invention provides a convenient biocompatible means of treating surgical dressings and implants at the location and time of use by clinicians and surgeons.

Cyanoselenide as the functional moiety

The preparation of the constructs designated Mal-(CH₂)₂CO-CMG(2)-Ad-DOPE and H-CMG(2)-Ad-DOPE is disclosed in the publication of Bovin *et al* (2008) and restated here for the sake of completeness. Acetone, benzene, chloroform, ethylacetate, methanol, toluene and o-xylene were from Chimmed (Russian Federation). Acetonitrile was from Cryochrom (Russian Federation). DMSO, DMF, CF₃COOH, Et₃N, N,N'-dicyclohexylcarbodiimide and N-hydroxysuccinimide were from Merck (Germany). Iminodiacetic acid dimethyl ester hydrochloride was from Reakhim (Russian Federation). Dowex 50X4-400 and Sephadex LH-20 were from Amersham Biosciences AB (Sweden). Silica gel 60 was from Merck

(Germany). Tetraamine $(\text{H}_2\text{N}-\text{CH}_2)_4\text{C} \times 2\text{H}_2\text{SO}_4$ was synthesized as described by Litherland et al. (1938). Thin-layer chromatography was performed using silica gel 60 F₂₅₄ aluminium sheets (Merck, 1.05554) with detection by charring after 7% H_3PO_4 soaking.

5 *Preparation of {[2-(2-tert-butoxycarbonylamino-acetylamino)-acetyl]-methoxycarbonylmethyl-amino}-acetic acid methyl ester*

To a stirred solution of (methoxycarbonylmethyl-amino)-acetic acid methyl ester hydrochloride (988 mg, 5 mmol) in DMF (15 ml) were added Boc-GlyGlyNos (3293 mg, 10 mmol) and $(\text{CH}_3\text{CH}_2)_3\text{N}$ (3475 μL , 25 mmol) were added. The mixture
10 was stirred overnight at room temperature and then diluted with o-xylene (70 ml) and evaporated. Flash column chromatography on silica gel (packed in toluene, and eluted with ethyl acetate) resulted in a crude product. The crude product was dissolved in chloroform and washed sequentially with water, 0.5 M NaHCO_3 and saturated KCl. The chloroform extract was evaporated and the
15 product purified on a silica gel column (packed in chloroform and eluted with 15:1 (v/v) chloroform/methanol). Evaporation of the fractions and drying under vacuum of the residue provided a colourless thick syrup. Yield 1785 mg, (95%). TLC: $R_f=0.49$ (7:1 (v/v) chloroform/methanol).

^1H NMR (500 MHz, $[\text{D}_6]\text{DMSO}$, 30 °C) δ , ppm: 7.826 (t, $J=5.1$ Hz, 1H; NHCO),
20 6.979 (t, $J=5.9$ Hz, 1H; NHCOO), 4.348 and 4.095 (s, 2H; NCH_2COO), 3.969 (d, $J=5.1$ Hz, 2H; COCH_2NH), 3.689 and 3.621 (s, 3H; OCH_3), 3.559 (d, $J=5.9$ Hz, 2H; $\text{COCH}_2\text{NHCOO}$), 1.380 (s, 9H; $\text{C}(\text{CH}_3)_3$).

Preparation of {[2-(2-tert-butoxycarbonylamino-acetylamino)-acetyl]-methoxycarbonylmethyl-amino}-acetic acid

25 To a stirred solution of {[2-(2-tert-butoxycarbonylamino-acetylamino)-acetyl]-methoxycarbonylmethyl-amino}-acetic acid methyl ester (1760 mg, 4.69 mmol) in methanol (25 ml) 0.2 M aqueous NaOH (23.5 ml) was added and the solution kept for 5 min at room temperature. The solution was then acidified with acetic acid (0.6 ml) and evaporated to dryness. Column chromatography of
30 the residue on silica gel (packed in ethyl acetate and eluted with 2:3:1 (v/v/v) i-PrOH/ethyl acetate/water) resulted in a recovered {[2-(2-tert-butoxycarbonylamino-acetylamino)-acetyl]-methoxycarbonylmethyl-amino}-acetic acid methyl ester (63 mg, 3.4%) and target compound (1320 mg). The intermediate product was then dissolved in methanol/water/pyridine mixture
35 (20:10:1, 30 ml) and passed through an ion exchange column (Dowex 50X4-400, pyridine form, 5 ml) to remove residual sodium cations. The column was then washed with the same solvent mixture, the eluant evaporated, the residue dissolved in chloroform/benzene mixture (1:1, 50 ml) and then evaporated and

dried under vacuum. Yield of **10** was 1250 mg (74%), white solid. TLC: $R_f=0.47$ (4:3:1 (v/v/v) *i*-PrOH/ethyl acetate/water).

^1H NMR (500 MHz, $[\text{D}_6]\text{DMSO}$, 30 °C), mixture of *cis*- and *trans*- conformers of *N*-carboxymethylglycine unit *c*.3:1. Major conformer; δ , ppm: 7.717 (t, $J=5$ Hz, 1H; NHCO), 7.024 (t, $J=5.9$ Hz, 1H; NHCOO), 4.051 (s, 2H; $\text{NCH}_2\text{COOCH}_3$), 3.928 (d, $J=5$ Hz, 2H; COCH_2NH), 3.786 (s, 2H; NCH_2COOH), 3.616 (s, 3H; OCH_3), 3.563 (d, $J=5.9$ Hz, 2H; $\text{COCH}_2\text{NHCOO}$), 1.381 (s, 9H; $\text{C}(\text{CH}_3)_3$) ppm; minor conformer, δ = 7.766 (t, $J=5$ Hz, 1H; NHCO), 7.015 (t, $J=5.9$ Hz, 1H; NHCOO), 4.288 (s, 2H; $\text{NCH}_2\text{COOCH}_3$), 3.928 (d, $J=5$ Hz, 2H; COCH_2NH), 3.858 (s, 2H; NCH_2COOH), 3.676 (s, 3H; OCH_3), 3.563 (d, $J=5.9$ Hz, 2H; $\text{COCH}_2\text{NHCOO}$), 1.381 (s, 9H; $\text{C}(\text{CH}_3)_3$).

*Preparation of {[2-(2-tert-butoxycarbonylamino-acetyl)-methoxycarbonylmethyl-amino}-acetic acid *N*-oxysuccinimide ester (Boc-Gly₂(MCMGly)Nos)*

To an ice-cooled stirred solution of {[2-(2-tert-butoxycarbonylamino-acetyl)-methoxycarbonylmethyl-amino}-acetic acid (1200 mg, 3.32 mmol) and *N*-hydroxysuccinimide (420 mg, 3.65 mmol) in DMF (10 ml) was added *N,N'*-dicyclohexylcarbodiimide (754 mg, 3.65 mmol). The mixture was stirred at 0°C for 30 min, then for 2 hours at room temperature. The precipitate of *N,N'*-dicyclohexylurea was filtered off, washed with DMF (5 ml), and filtrates evaporated to a minimal volume. The residue was then agitated with $(\text{CH}_3\text{CH}_2)_2\text{O}$ (50 ml) for 1 hour and an ether extract removed by decantation. The residue was dried under vacuum providing the active ester (1400 mg, 92%) as a white foam. TLC: $R_f=0.71$ (40:1 (v/v) acetone/acetic acid).

^1H NMR (500 MHz, $[\text{D}_6]\text{DMSO}$, 30 °C), mixture of *cis*- and *trans*- conformers of *N*-carboxymethylglycine unit *c*. 3:2.

Major conformer; δ , ppm: 7.896 (t, $J=5.1$ Hz, 1H; NHCO), 6.972 (t, $J=5.9$ Hz, 1H; NHCOO), 4.533 (s, 2H; NCH_2COON), 4.399 (s, 2H; $\text{NCH}_2\text{COOCH}_3$), 3.997 (d, $J=5.1$ Hz, 2H; COCH_2NH), 3.695 (s, 3H; OCH_3), 3.566 (d, $J=5.9$ Hz, 2H; $\text{COCH}_2\text{NHCOO}$), 1.380 (s, 9H; $\text{C}(\text{CH}_3)_3$).

Minor conformer; δ , ppm: 7.882 (t, $J=5.1$ Hz, 1H; NHCO), 6.963 (t, $J=5.9$ Hz, 1H; NHCOO), 4.924 (s, 2H; NCH_2COON), 4.133 (s, 2H; $\text{NCH}_2\text{COOCH}_3$), 4.034 (d, $J=5.1$ Hz, 2H; COCH_2NH), 3.632 (s, 3H; OCH_3), 3.572 (d, $J=5.9$ Hz, 2H; $\text{COCH}_2\text{NHCOO}$), 1.380 (s, 9H; $\text{C}(\text{CH}_3)_3$).

The active ester (1380 mg) was dissolved in DMSO to provide a volume of 6 ml and used as a 0.5 M solution (stored at -18 °C).

Preparation of {[2-(2-tert-butoxycarbonylamino-acetylamino)-acetyl]-methoxycarbonylmethyl-amino}-acetic acid methyl ester

To the stirred solution of (methoxycarbonylmethyl-amino)-acetic acid methyl ester hydrochloride (988 mg, 5 mmol) in DMF (15 ml) Boc-GlyGlyNos (3293 mg, 10 mmol) and Et₃N (3475 µl, 25 mmol) were added. The mixture was stirred overnight at room temperature (r.t.), then diluted with o-xylene (70 ml) and evaporated. Flash column chromatography on silica gel (packed in toluene and eluted with ethyl acetate) resulted in crude product. The crude product was dissolved in chloroform and washed sequentially with water, 0.5 M NaHCO₃ and saturated KCl. The chloroform extract was evaporated, and the product was purified on a silica gel column (packed in chloroform and eluted with chloroform/methanol 15:1). Evaporation of fractions and vacuum drying of residue resulted in a colorless thick syrup of (**3**) (1785 mg, 95%). TLC: R_f = 0.49 (chloroform/methanol 7:1).

¹H NMR (500 MHz, [D₆]DMSO, 30 °C) δ = 7.826 (t, J = 5.1 Hz, 1H; NHCO), 6.979 (t, J = 5.9 Hz, 1H; NHCOO), 4.348 and 4.095 (s, 2H; NCH₂COO), 3.969 (d, J = 5.1 Hz, 2H; COCH₂NH), 3.689 and 3.621 (s, 3H; OCH₃), 3.559 (d, J = 5.9 Hz, 2H; COCH₂NHCOO), 1.380 (s, 9H; CMe₃) ppm.

Preparation of {[2-(2-tert-butoxycarbonylamino-acetylamino)-acetyl]-methoxycarbonylmethyl-amino}-acetic acid

To the stirred solution of {[2-(2-tert-butoxycarbonylamino-acetylamino)-acetyl]-methoxycarbonylmethyl-amino}-acetic acid methyl ester (1760 mg, 4.69 mmol) in methanol (25 ml) 0.2 M aqueous NaOH (23.5 ml) was added. The solution was kept for 5 min at r.t., then acidified with acetic acid (0.6 ml) and evaporated to dryness. Column chromatography of the residue on silica gel (packed in ethyl acetate and eluted with iPrOH/ethyl acetate/water (2:3:1)) resulted in recovered (**3**) (63 mg, 3.4%) and crude target compound (1320 mg). The crude target compound was dissolved in methanol/water/pyridine mixture (20:10:1, 30 ml) and passed through an ion-exchange column (Dowex 50X4-400, pyridine form, 5 ml) to remove residual Na cations. The column was washed with the same mixture, eluant evaporated, dissolved in chloroform/benzene mixture (1:1, 50 ml) then evaporated and dried in vacuum to provide a yield of pure (**10**) was 1250 mg (74%), white solid. TLC: R_f = 0.47 (iPrOH/ethyl acetate/water (4:3:1)).

¹H NMR (500 MHz, [D₆]DMSO, 30 °C) of mixture of *cis*- and *trans*- conformers of N-carboxymethyl-glycine unit c.3:1.

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2H; NCH_2COOH), 3.616 (s, 3H; OCH_3), 3.563 (d, $J = 5.9$ Hz, 2H; $\text{COCH}_2\text{NHCOO}$), 1.381 (s, 9H; CMe_3) ppm.

Minor conformer: $\delta = 7.766$ (t, $J = 5$ Hz, 1H; NHCO), 7.015 (t, $J = 5.9$ Hz, 1H; NHCOO), 4.288 (s, 2H; NCH_2COOMe), 3.928 (d, $J = 5$ Hz, 2H; COCH_2NH), 3.858 (s, 2H; NCH_2COOH), 3.676 (s, 3H; OCH_3), 3.563 (d, $J = 5.9$ Hz, 2H; $\text{COCH}_2\text{NHCOO}$), 1.381 (s, 9H; CMe_3) ppm.

Preparation of {[2-(2-tert-butoxycarbonylamino-acetylamino)-acetyl]-methoxycarbonylmethyl-amino}-acetic acid N-oxysuccinimide ester Boc-Gly2 (MCMGly) Nos

10 To an ice-cooled stirred solution of {[2-(2-tert-butoxycarbonylamino-acetylamino)-acetyl]-methoxycarbonylmethyl-amino}-acetic acid (1200 mg, 3.32 mmol) and N-hydroxysuccinimide (420 mg, 3.65 mmol) in DMF (10 ml) N,N'-dicyclohexylcarbodiimide (754 mg, 3.65 mmol) was added. The mixture was stirred at 0 °C for 30 min, then for 2 h at r.t. The precipitate of N,N'-
15 dicyclohexylurea was filtered off, washed with DMF (5 ml) and the filtrates evaporated to a minimal volume. The residue was agitated with Et_2O (50 ml) for 1 h. An ether extract was removed by decantation, and the residue dried in vacuum to yield the target compound (1400 mg, 92%) as a white foam. TLC: $R_f = 0.71$ (acetone/acetic acid 40:1).

20 ^1H NMR (500 MHz, $[\text{D}_6]\text{DMSO}$, 30 °C), mixture of *cis*- and *trans*- conformers of N-carboxymethyl-glycine unit c. 3:2.

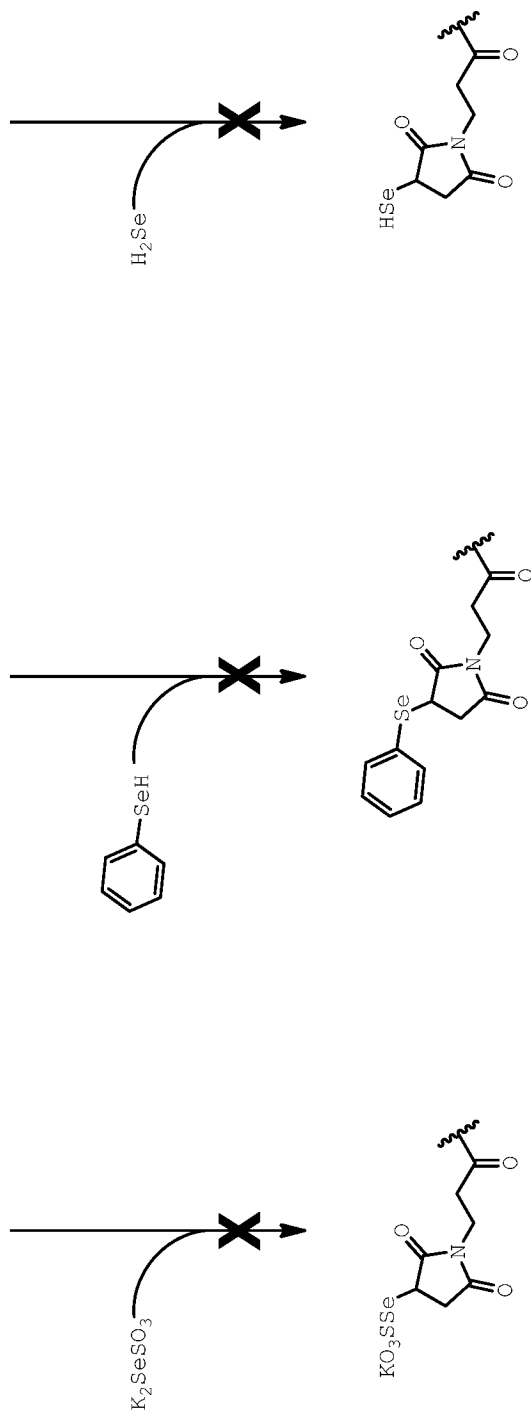
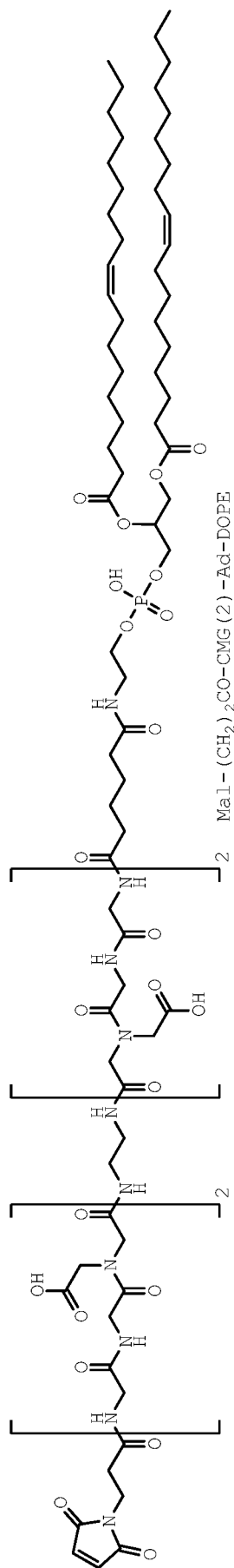
Major conformer: $\delta = 7.896$ (t, $J = 5.1$ Hz, 1H; NHCO), 6.972 (t, $J = 5.9$ Hz, 1H; NHCOO), 4.533 (s, 2H; NCH_2COON), 4.399 (s, 2H; NCH_2COOMe), 3.997 (d, $J = 5.1$ Hz, 2H; COCH_2NH), 3.695 (s, 3H; OCH_3), 3.566 (d, $J = 5.9$ Hz, 2H; $\text{COCH}_2\text{NHCOO}$), 1.380 (s, 9H; CMe_3) ppm.

Minor conformer: $\delta = 7.882$ (t, $J = 5.1$ Hz, 1H; NHCO), 6.963 (t, $J = 5.9$ Hz, 1H; NHCOO), 4.924 (s, 2H; NCH_2COON), 4.133 (s, 2H; NCH_2COOMe), 4.034 (d, $J = 5.1$ Hz, 2H; COCH_2NH), 3.632 (s, 3H; OCH_3), 3.572 (d, $J = 5.9$ Hz, 2H; $\text{COCH}_2\text{NHCOO}$), 1.380 (s, 9H; CMe_3) ppm.

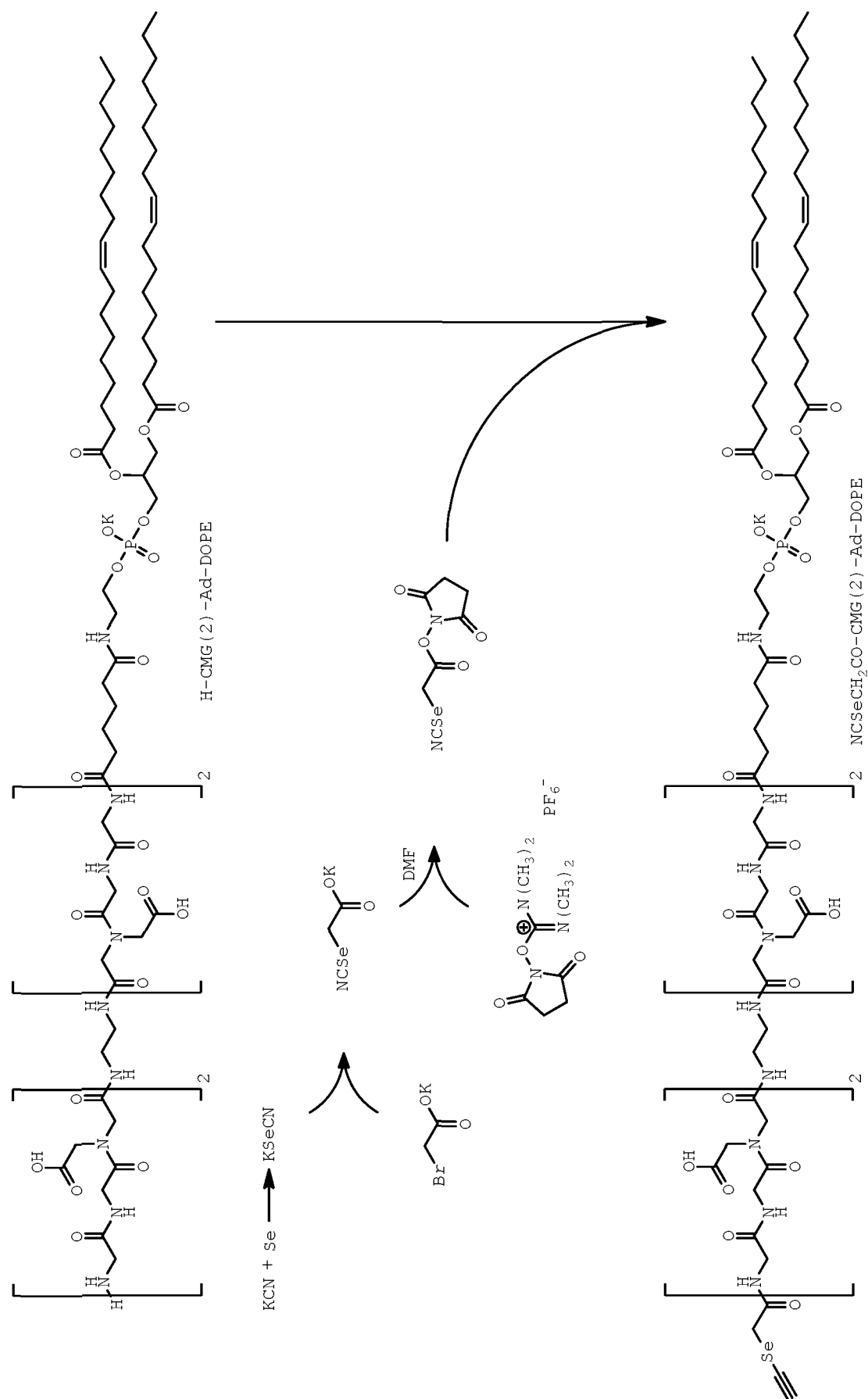
SCHEME C

SCHEME B

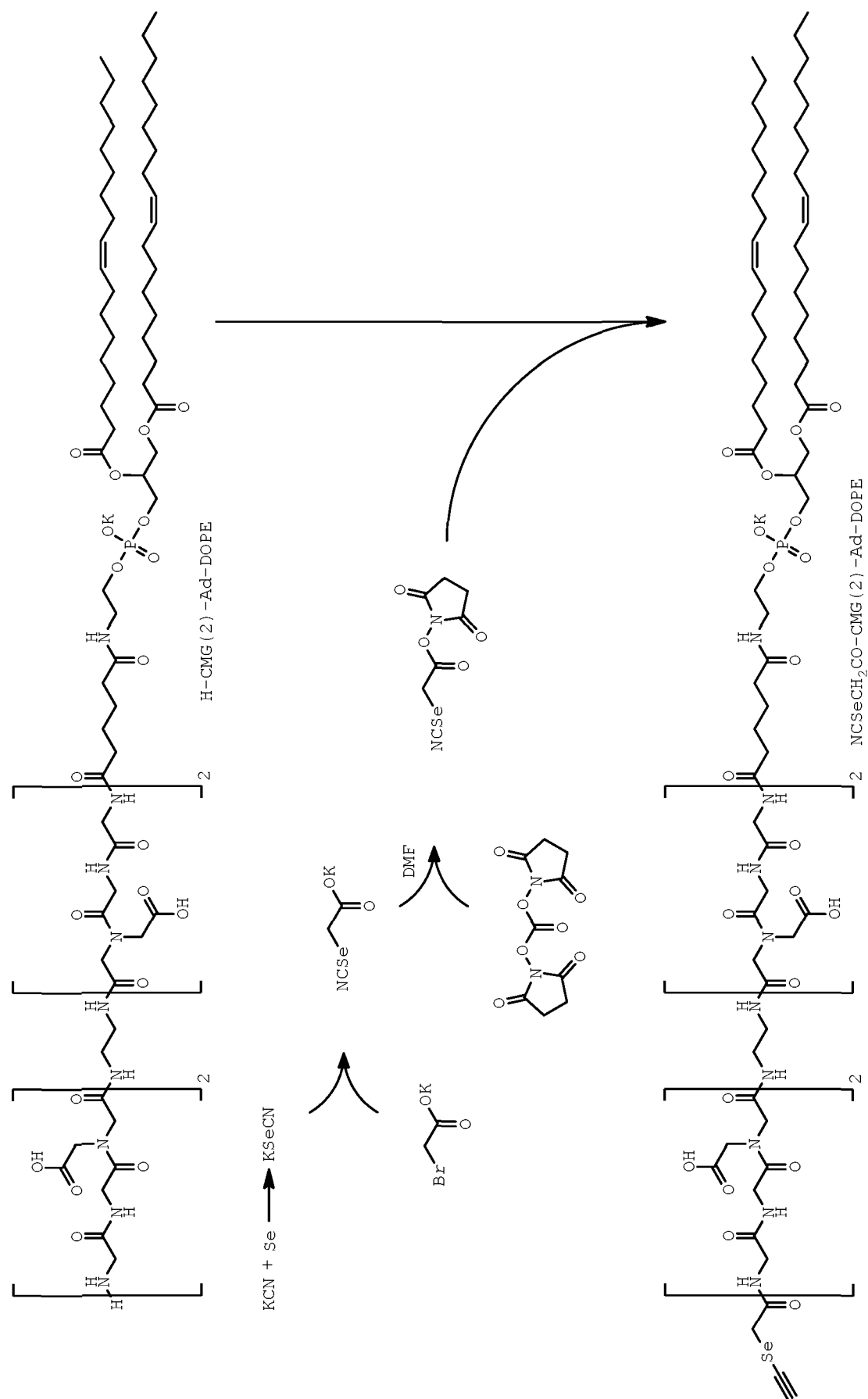
SCHEME A



SCHEME D (a)



SCHEME D (b)



Preparation of the constructs designated Mal-(CH₂)₂CO-CMG(2)-Ad-DOPE and H-CMG(2)-Ad-DOPE

The construct designated H-CMG(2)-Ad-DOPE was prepared from {[2-(2-tert-butoxycarbonylamino-acetyl-amino)-acetyl]-methoxycarbonylmethyl-amino}-acetic acid N-oxysuccinimide ester Boc-Gly₂(MCMGly)Nos according to Scheme III of the publication of Bovin et al (2008). The construct designated Mal-(CH₂)₂CO-CMG(2)-Ad-DOPE was prepared according to the first step of Scheme IV of the publication of Bovin et al (2008). Briefly, the construct designated H-CMG(2)-Ad-DOPE was treated with a 5-fold excess of 3-maleimidopropionic acid oxybenztriazol ester in i-PrOH-water. The maleimide-lipid construct was isolated in 40% yield after gel-permeation chromatography on Sephadex LH-20 (i-PrOH-water, 1:2).

Preparation of NCSeCH₂CO-CMG(2)-Ad-DOPE

Attempts to prepare a cyanoselenide-lipid construct via an addition reaction between the maleimide-lipid construct designated Mal-(CH₂)₂CO-CMG(2)-Ad-DOPE and potassium selenosulfite (K₂SeSO₃) [SCHEME A], selenophenol (PhSeH) [SCHEME B] and hydrogen selenide (H₂Se) [SCHEME C] were unsuccessful. With hindsight the failure to obtain a stable seleno-Bunte salt according to SCHEME A is at least in part predictable from the disclosure of the chemical behaviour of their sulfur analogues in the publication of Distler (1967). Both the attempted Michael additions of phenylselenide and hydrogen selenide in protic media according to SCHEME B and SCHEME C, respectively, yielded a product with a reduced maleimide double bond, as opposed to the desired selenylsuccinimides. Formation of selenylsuccinimides in quantitative yield has been disclosed in the publication of Numeo et al (1981). However, the disclosed use of anhydrous ether is incompatible with the use of the polyanionic maleimide-lipid construct designated Mal-(CH₂)₂CO-CMG(2)-Ad-DOPE.

It was subsequently discovered that the cyanoselenide-lipid construct designated NCSeCH₂CO-CMG(2)-Ad-DOPE could be successfully prepared via an activated 2-selenocyanatoacetic acid (NC-Se-CH₂COOH). The activated NC-Se-CH₂COOH was reacted with the lipid construct H-CMG(2)-Ad-DOPE according to SCHEME D(a) or SCHEME D(b). The prepared construct was stored in the dark under an inert atmosphere. Potassium selenocyanate was selected as the reagent of choice as it could readily be activated as an N-hydroxysuccinimide (NHS) ester according to SCHEME D(a) or (b) or mixed anhydride according to SCHEME D(c). Potassium selenocynoacetate (NCSeCH₂COOK) was synthesized from freshly prepared solutions of potassium selenocyanate (KSeCN) and potassium bromoacetate (BrCH₂COOK) according to the procedures disclosed in the publication of Klauss (1970). The synthesized NCSeCH₂COOK was stored in a

vacuum desiccator over potassium hydroxide (KOH) pellets in the dark prior to activation. For activation the potassium selenocynoacetate (156 mg, 0.77 mmol) was added in one portion to a solution of N,N,N',N'-tetramethyl-O-(N-succinimidyl)uraniumhexafluorophosphate (HSTU) (IRIS, Germany) (212 mg, 0.59 mmol) in 1 mL DMF while a gentle flow of dry argon via a PTFE capillary was bubbling through. The slurry thus obtained was stirred in this way for 30 minutes during which the initial solid changed to a more dense crystalline precipitate (KPF₆). The reaction mixture was sonicated for 1 to 2 minutes and combined with the construct designated H-CMG(2)-Ad-DOPE (110 mg, 0.06 mmol) dissolved in 1 mL of 20% IPA followed by 100 µL 1N KHCO₃. A sticky solid (presumably NCSCH₂COOSu) that precipitated immediately, was dissolved by dropwise addition of 30% IPA (circa 1.6 mL) with sonication and the reaction mixture was magnetically stirred for 3 hours at room temperature keeping pH in the range 8.0 to 8.5 (TLC control: Solvents were evaporated in vacuum and dry residue was triturated with 3 mL of acetonitrile with sonication until fine slurry formed and then transferred into Eppendorf tubes (2 x 2.2 mL), centrifuged and the solids washed 4 times consecutively with neat IPA and MeCN (2 mL of each, brief sonication followed by centrifugation). The wet solids were dissolved in 3.5 mL of 30% IPA-water and lyophilized to constant weight. 111 mg (92%) of the cyanoselenide-lipid construct designated NCSCH₂CO-CMG(2)-Ad-DOPE were obtained as a reddish amorphous powder. R_f~0.5, CHCl₃/methanol/water 2:6:1 (v/v); TLC aluminium sheets Silica gel 60 F₂₅₄ (Merck 1.05554). It is noted that mass spectroscopy did not appear suitable for the characterization of this construct. Only peaks of Se-free fragments could be detected. The ¹H NMR spectrum determined for the construct is provided in Figure 1.

Cations as the functional moiety

The cationic lipid construct **9a** was prepared and isolated as its trifluoroacetic acid (TFA) salt (SCHEME E). Briefly, desymmetritisation of the polyamine spermine [CAS# 71-44-3] (**2**) was performed according to a modified version of the method disclosed in the publication of Geall and Blagbrough (2000) employing Boc as the protecting group. It will be recognised that the method is also applicable to the desymmetritisation of other unbranched polyamines such as spermidine [CAS# 124-20-9] (**1**), tetraethylenepentamine [CAS# 112-57-2] (**3**); pentaethylenehexamine [CAS# 4067-16-7] (**4**) and hexaethyleneheptamine [4403-32-1] (**5**). Accordingly, a series of cationic lipid constructs may be accessed according to SCHEME E.

According to SCHEME E the Boc protected, desymmetritised intermediate N¹,N⁴,N⁹-tri-*tert*-butoxycarbonyl)-1,12-diamino-4,9-diazadodecane (**6**) is

conjugated to the diacylglycerophospholipid 1,2-O-dioleoyl-sn-glycero-3-phosphatidyl-ethanolamine [CAS# 4004-05-1] (DOPE) using the homobifunctional crosslinker disuccinimidyl adipate. It will be recognised that other disuccinimidyl compounds may be employed as the homobifunctional crosslinker.

5 These include

The activated lipid (**7a**) acylates the terminal, primary amino group of N¹,N⁴,N⁹-tri-*tert*-butoxycarbonyl)-1,12-diamino-4,9-diazadodecane (**6**) to provide a lipidated Boc protected polyamine intermediate (**8a**). Again, it will be recognised that according to Scheme I other

10 diacylglycerophospholipids, such as 1,2-O-distereoyl-sn-glycero-3-phosphatidylethanolamine [CAS#] (DSPE) may be substituted for DOPE.

In the final step of SCHEME E the lipidated polyamine intermediate (**8a**) is deprotected and the cationic lipid construct (**9a**) isolated as its trifluoroacetic acid salt.

15 *Materials and methods*

Chloroform, dichloroethane, dichloromethane, methanol and toluene were obtained from Chimmed (Russian Federation). Trifluoroacetic acid, triethylamine, di-*tert*-butyldicarbonate methyl trifluoroacetate were obtained from Merck (Germany). Spermine was obtained from Sigma-Aldrich (USA).

20 Sephadex LH-20 was obtained from Amersham Biosciences AB (Sweden). Silica gel 60 was obtained from Merck (Germany). Thin layer chromatographic (TLC) analysis was performed on silica gel 60 F₂₅₄ plates (Merck). Amino containing compounds were detected using ninhydrin reagent. DOPE containing compounds were detected using an aqueous solution of potassium permanganate (KMnO₄) or
25 by soaking in 8% (w/v) phosphoric acid in water followed by heating at over 200 °C. ¹H NMR spectra were recorded at 30 °C with a Bruker BioSpin GmbH 700 MHz instrument using the signal of the solvent's residual protons as reference ([D]CHCl₃, 7.270 ppm; [D₂]H₂O, 4.750 ppm). Mass spectra were recorded with an Agilent ESI-TOF 6224 LC/MS spectrometer.

30 *Preparation of Boc₃Spm (6)*

To a stirred solution of spermine (**2**) (1 equivalent, 1.34 g, 6.6 mmol) in methanol (90 mL) at -80 °C under nitrogen, a solution of methyl trifluoroacetate (1.1 equivalents, 0.730 mL, 7.26 mmol) in methanol (1.5 mL) was added drop-wise over a period of 30 min. Stirring was continued at -80°C
35 for a further period of 30 min and then the temperature increased to 0 °C. The reaction afforded predominantly the mono-trifluoroacetamide. Without isolation, the remaining amino functional groups were quantitatively protected by drop-wise addition of an excess of di-*tert*-butyldicarbonate (4

equivalents, 5.76 g, 26.4 mmol) in methanol over a period of 3 min. The reaction was then warmed to 25 °C and stirred for a further 15 hr to afford the fully protected spermine (R_f 0.33 (95:5 (v/v) CHCl_3 -i-PrOH)). The trifluoroacetate protecting group was then removed *in situ* by increasing the pH of the solution to greater than 11 pH units with concentrated aqueous ammonia (conc. aq. NH_3) and then stirred at 25 °C for a period of 15 hr. The solution was concentrated *in vacuo* and the residue purified over silica gel (95:5:1 to 90:10:1 (v/v/v) CHCl_3 -MeOH-conc. aq. NH_3) to afford the title compound (**6**) as a colourless homogeneous oil (1.5 g, 45%), R_f 0.32 (83:16:1 (v/v/v) CHCl_3 -MeOH-conc. aq. NH_3). MS, m/z : found 502.3725 ($M^+ + 1$), $\text{C}_{25}\text{H}_{50}\text{N}_4\text{O}_6$ required M^+ 501.3652.

^1H -NMR (700 MHz, CDCl_3 , 303 °K), δ , ppm: 3.4 (m, 2H, 1- CH_2), 3.05-3.30 (m, 8H, 3,4,7,8- CH_2), 3.01 (m, 2H, 10- CH_2), 2.03 (m, 2H, 9- CH_2), 1.67 (m, 2H, 2- CH_2), 1.50 (m, 4H, 5,6- CH_2), 1.44, 1.45, 1.46 (3 s, overlapping, 27 H, 3 O- $\text{C}(\text{CH}_3)_3$).

Preparation of SuO-Ad-DOPE (**7a**) and SuO-Ad-DSPE (**7b**)

To a solution of disuccinimidyl adipate (70 mg, 205 μmol) in dry N,N-dimethylformamide (1.5 ml) were added DOPE or DSPE (40 μmol) in chloroform (1.5 ml) followed by triethylamine (7 μl). The mixture was kept for 2 h at room temperature, then neutralized with acetic acid and partially concentrated *in vacuo*. Column chromatography (Sephadex LH-20, 1:1 (v/v) chloroform-methanol, 0.2% (w/v) aqueous acetic acid) of the residue yielded SuO-Ad-DOPE (**7a**) (37 mg, 95%) as a colourless syrup. TLC (6:3:0.5 (v/v/v) chloroform-methanol-water) R_f 0.5 (SuO-Ad-DOPE (**7a**)) and R_f 0.55 (SuO-Ad-DOPE (**7b**)).

^1H NMR (2:1 (v/v) $\text{CDCl}_3/\text{CD}_3\text{OD}$) δ :

SuO-Ad-DOPE (**7a**) - 5.5 (m, 4H, $2 \times (-\text{CH}=\text{CH}-)$), 5.39 (m, 1H, $-\text{OCH}_2-\text{CHO}-\text{CH}_2\text{O}-$), 4.58 (dd, 1H, $J=3.67$, $J=11.98$, $-\text{CCOOHCH}-\text{CHO}-\text{CH}_2\text{O}-$), 4.34 (dd, 1H, $J=6.61$, $J=11.98$, $-\text{CCOOHCH}-\text{CHO}-\text{CH}_2\text{O}-$), 4.26 (m, 2H, $\text{PO}-\text{CH}_2-\text{CH}_2-\text{NH}_2$), 4.18 (m, 2H, $-\text{CH}_2-\text{OP}$), 3.62 (m, 2H, $\text{PO}-\text{CH}_2-\text{CH}_2-\text{NH}_2$), 3.00 (s, 4H, ONSuc), 2.8 (m, 2H, $-\text{CH}_2-\text{CO}$ (Ad)), 2.50 (m, 4H, $2 \times (-\text{CH}_2-\text{CO})$), 2.42 (m, 2H, $-\text{CH}_2-\text{CO}$ (Ad)), 2.17 (m, 8H, $2 \times (-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2-)$), 1.93 (m, 4H, $\text{COCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}$), 1.78 (m, 4H, $2 \times (\text{COCH}_2\text{CH}_2-)$), 1.43, 1.47 (2 bs, 40H, 20 CH_2), 1.04 (m, 6H, 2 CH_3).

SuO-Ad-DSPE (**7b**) - 5.39 (m, 1H, $-\text{OCH}_2-\text{CHO}-\text{CH}_2\text{O}-$), 4.53 (dd, 1H, $J=3.42$, $J=11.98$, $-\text{CCOOHCH}-\text{CHO}-\text{CH}_2\text{O}-$), 4.33 (dd, 1H, $J=6.87$, $J=11.98$, $-\text{CCOOHCH}-\text{CHO}-\text{CH}_2\text{O}-$), 4.23 (m, 2H, $\text{PO}-\text{CH}_2-\text{CH}_2-\text{NH}_2$), 4.15 (m, 2H, $-\text{CH}_2-\text{OP}$), 3.61 (m, 2H, $\text{PO}-\text{CH}_2-\text{CH}_2-\text{NH}_2$), 3.00 (s, 4H, ONSuc), 2.81 (m, 2H, $-\text{CH}_2-\text{CO}$ (Ad)), 2.48 (m, 4H, $2 \times (-$

$\text{CH}_2\text{-CO}$), 2.42 (m, 2H, $-\text{CH}_2\text{-CO}$ (Ad)), 1.93 (m, 4H, $\text{COCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}$), 1.78 (m, 4H, $2\times(\text{COCH}_2\text{CH}_2-)$), 1.43, 1.47 (2 bs, 40H, 20 CH_2), 1.04 (m, 6H, 2 CH_3).

Preparation of Boc₃Spm-Ad-DOPE (8a)

To a stirred solution of Boc₃Spm (**6**) (552 mg, 1.1 mmol) in dichloroethane (25 ml) was added trimethylamine (1 ml, 7.2 mmol) followed by a solution of SuO-Ad-DOPE (1066 mg, 1.1 mmol) in dichloroethane (25 ml). The reaction mixture was stirred for a period of 2 hr and then the solvent was removed under reduced pressure at 37 °C. The crude product was purified by chromatography on silica gel by elution with 97:3 to 85:15 (v/v) CHCl_3 -MeOH to afford the title compound (**8a**) (1.16 g, 78%) as a viscous oil. TLC (10:6:0.8 (v/v/v) CH_2Cl_2 -EtOH- H_2O) R_f 0.36.

^1H NMR (700 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$ 1:1, 10 mg/mL, 303 °K) δ , ppm: 5.34 (m, 4H; 2 $\text{CH}=\text{CH}$), 5.19 (m, 1H; $\text{OCH}_2\text{CHCH}_2\text{O}$), 4.37 (dd, $J_{\text{gem}}\sim 11.1$ Hz, 1H, $\text{POCH}_2\text{-CH-CH}^{\text{a}}\text{-O(CO)}$), 4.13 (dd, $J\sim 7.2$ Hz, 1H, $\text{POCH}_2\text{-CH-CH}^{\text{b}}\text{-O(CO)}$), 3.94 (m, 4H), 3.48 (m, 2H), 3.05-3.30 (m, 12H, 1,3,4,7,8,10- CH_2), 2.71 (m, 2H), 2.20-2.42 (m, 8H), 1.98-2.04 (m, 8H), 1.64 (m, 8H), 1.58 (m, 4H), 1.49 (m, 4H, 5,6- CH_2), 1.44, 1.45, 1.46 (3s, 27H, 3 $\text{O-C(CH}_3)_3$), 1.22-1.37 (m, 40H, 20 CH_2), 0.88 and 0.89 (2d, $J\sim 7$ Hz, 6H, 2 CH_3).

Preparation of Spm-Ad-DOPE (9a)

To a stirred solution of **8a** (1.16 g, 0.85 mmol) in CHCl_3 (10 ml) at 25 °C TFA (5 ml, 95%) was added. After a period of 20 min the solution was concentrated *in vacuo* at 35 °C and the residue was co-evaporated with toluene (5 times 10 mL) to remove trace amounts of TFA. To remove any low molecular weight impurities the residue was dissolved in 1:1 (v/v) CHCl_3 -MeOH (2 mL) and passed in two portions through a Sephadex LH-20 column (volume 330 mL, eluent 1:1 (v/v) CHCl_3 -MeOH). Fractions containing pure **9a** (di-TFA salt) were combined and evaporated to dryness and the residue dissolved in water (~100 mL) and freeze-dried. A yield of was 975 mg (89%) was obtained. MS, m/z : found 1056.8063 (M^++1), $\text{C}_{57}\text{H}_{110}\text{N}_5\text{O}_{10}\text{P}$ required M^+ 1055.779.

^1H NMR (700 MHz, 1:1 (v/v) CDCl_3 - CD_3OD , 10 mg/mL, 303 °K) δ , ppm: 5.51 (m, 4H; 2 $\text{CH}=\text{CH}$), 5.42 (m, 1H; $\text{OCH}_2\text{CHCH}_2\text{O}$), 4.6 (dd, $J_{\text{gem}}=12.1$ Hz, $J=2.81$ Hz, 1H, $\text{POCH}_2\text{-CH-CH}^{\text{a}}\text{-O(CO)}$), 4.34 (dd, $J=7.09$ Hz, 1H, $\text{POCH}_2\text{-CH-CH}^{\text{b}}\text{-O(CO)}$), 4.14 (m, 2H, $\text{POCH}_2\text{CH}_2\text{N}$), 4.06 (m, 2H, $\text{POCH}_2\text{-CH-CH}_2$), 3.59 (m, 2H, $\text{OCH}_2\text{CH}_2\text{N}$), 3.49 (m, 2H, 1- CH_2), 3.11-3.28 (m, 10H, 3,4,7,8,10- CH_2), 2.42 and 2.51 (2m, 8H, 4 COCH_2), 2.26 (m, 2H, 2- CH_2), 2.19 (m, 8H, 2 $\text{CH}_2\text{CH}=\text{CHCH}_2$), 2.07 (m, 2H, 9- CH_2), 1.99 (m, 4H, 5,6- CH_2), 1.79 (m, 8H, 4 COCH_2CH_2), 1.40-1.54 (m, 40H, 20 CH_2), 1.05 and 1.06 (2t, $J\sim 7$ Hz, 6H, 2 CH_3).

SCHEME E



Antimicrobial surface treatments

The ability of the cyanoselenide-lipid construct designated NCSeCH₂CO-CMG(2)-Ad-DOPE to prevent the growth of bacteria on the surface of stainless steel was evaluated. Used stainless steel (316 SS) coupons (catalogue no. RD123-316, Biosurface Technologies) were soaked in a 1% (v/v) aqueous solution of commercially available disinfectant cleaner (TRIGENE™) followed by soaking in a 0.1% (v/v) aqueous solution of commercially available alkaline cleaning agent (PYRONEG™) before rinsing with deionised water. Organic residues and metal dust were removed from the rinsed coupons by soaking in 95% (v/v) ethanol followed by rinsing in the same solvent and then sonicating for 30 minutes in methanol. Finally the coupons were immersed in boiling methanol for 10 minutes before being dried at 90°C, wrapped and autoclaved at 121°C for 20 minutes. Treated coupons were prepared by immersion of the sterilised coupons in a degassed 50 µg/mL aqueous dispersion of the cyanoselenide-lipid construct designated NCSeCH₂CO-CMG(2)-Ad-DOPE. The aqueous dispersion was prepared from a degassed stock solution of the construct prepared at a concentration of 1 mg/mL in sterile distilled water. Untreated coupons were prepared as controls by immersion of the sterilised coupons in sterile distilled water. Treated and untreated coupons were dried in a laminar flow cabinet. Frozen stock solutions of *Staphylococcus aureus* and *Staphylococcus epidermis* were thawed and used to streak inoculate blood agar plates before incubation at 37°C overnight. Isolated colonies were suspended in 10 mL sterile water to provide an approximate cell density in suspension of 1 x 10⁸ c.f.u./mL and confirmed by viability counts for each suspension on blood agar plates (*S. aureus*, 1.15 x 10⁸ c.f.u./mL; *S. epidermis*, 1.27 x 10⁷ c.f.u./mL). Individual dried coupons were transferred to the wells of a sterile microplate and the surface of each coupon contacted with 10 µL of a suspension of cells of *Staphylococcus sp.* and the suspension allowed to dry (circa 20 minutes). A volume of 1 mL of 3 g/L tryptic soy broth was then introduced into the well so as to cover the coupon and the microplate covered and incubated at 37°C for 21 hours with agitation at 150 rpm. Following incubation coupons were removed, washed with water and dried.

The surface of the dried coupons was then stained with acridine orange by placing three drops of the stain on the surface of each of the coupons for two minutes before rinsing with sterile water and air drying. The observations from fluorescence microscopy at 1,000x magnification are presented in Figure 2 (*S. aureus*) and Figure 3 (*S. epidermis*). Both species of *Staphylococcus* were able to establish biofilms on the surface of untreated coupons. Neither of the species was able to establish a biofilm on the treated coupons. To assess viability of bacteria following exposure to the

coupons a 100 μ L volume of the broth following incubation was spread on the surface of blood agar plates. The plates were then incubated at 37°C overnight. Photographs of the incubated plates are presented in Figure 4 (*S. aureus*) and Figure 5 (*S. epidermis*). Exposure to the surface of the treated coupons significantly inhibited bacterial cell growth.

The ability of the cationic-lipid construct designated Spm-Ad-DOPE (**9a**) to prevent the growth of bacteria on the surface of stainless steel was evaluated. A dispersion of the construct was prepared at a concentration of 1 mg/mL in sterile deionised water. (It is noted that attempts to disperse the construct in saline will result in precipitation of the construct.) A volume of 100 μ L of the dispersion was dispensed onto the surface of a 1 x 1 cm stainless steel (SS 304) square. A control was prepared by dispensing the same volume of sterile deionised water onto the surface of a second stainless steel square. Both samples (test and control) were then dried at 60°C for a period of two hours. The samples were stored at room temperature prior to use. A volume of 1 mL of an actively growing (log phase) culture of *Escherichia coli* (ATCC 25922) in 21 g/L Mueller-Hinton broth (MHB) was serially diluted (10^{-6}) to provide 8 to 10 colony forming units (CFUs) per 100 μ L. Individual samples of the stainless steel squares were placed in each well of a sterile 12-well culture plate and 100 μ L of the serially diluted culture dispensed onto the surface of each sample. The culture was allowed to contact the surface for a period of 20 minutes at room temperature before washing each sample once with phosphate buffered saline (PBS) to remove non-adherent cells of the bacterium. Each washed sample was then immersed in a volume of 10 mL of MHB and incubated overnight at 37°C. Following overnight incubation each sample was washed as before and immersed in a volume of 9 mL of MHB. Alternate vortexing and sonicating was employed to remove bacteria from the sample surface. A volume of a serial dilution (10^{-4}) of the resulting broth was then spread on blood agar plates, incubated at 37°C overnight and colonies counted. Cell densities of the overnight cultures were calculated and are presented in Table 1.

Sample	CFU/mL
Control	No growth
Test	3×10^7

Table 1. Growth of overnight cultures of *Escherichia coli* (ATCC 25922) following contact with surface treated (Test) and untreated (Control) of 1 x 1 cm stainless steel samples.

The tabulated results indicate a biocidal action of the samples treated with the cationic-lipid construct designated Spm-Ad-DOPE (**9a**).

The ability of the cyanoselenide-lipid construct designated NCS₂CH₂CO-CMG(2)-Ad-DOPE to prevent the growth of bacteria on surgical dressings was also evaluated. Sterile surgical dressing (Propax®) was immersed for one second in an aqueous dispersion of the construct, dried and then contaminated with bacteria (clinical isolate of *S. epidermidis*). After 30 minutes the bacterially contaminated dressing was then placed in growth media for 24 hours, and growth in the media (as determined by counting colony forming units) and growth on the dressing was observed (to find bacteria in 10 random 1000x scanning electron microscope fields). Growth of bacteria in media was equivalent to 2.6 to 3.0 x 10⁷ colony forming units (cfus) per mL for untreated samples. Growth of bacteria in media was equivalent to 5 to 1.3 x 10⁴ colony forming units (cfus) per mL for treated samples. For untreated samples bacterial growth was observed in 100% (10 of 10) fields. For treated samples bacterial growth was observed in 10% (1 of 10) fields. Electron micrographs of treated and untreated samples of the surgical dressing are provided in Figures 6 and 7. Replicates performed on multiple occasions gave reproducible results.

Although the invention has been described with reference to embodiments or examples it should be appreciated that variations and modifications may be made to these embodiments or examples without departing from the scope of the invention. Where known equivalents exist to specific elements, features or integers, such equivalents are incorporated as if specifically referred to in this specification. In particular, variations and modifications to the embodiments or examples that include elements, features or integers disclosed in and selected from the referenced publications are within the scope of the invention unless specifically disclaimed. The advantages provided by the invention and discussed in the description may be provided in the alternative or in combination in these different embodiments of the invention.

INDUSTRIAL APPLICABILITY

The method of surface treating surgical treatments is performed ex vivo using synthetic, water dispersible cationic-lipid constructs.

PUBLICATIONS

Behr et al (1989) *Efficient gene transfer into mammalian primary endocrine cells with lipopolyamine-coated DNA* Proc. Natl. Acad. Sci. USA, 86, 6982-6986

Blagbrough et al (1997) *Polyamines and polyamine amides as potent selective receptor probes, novel therapeutic lead compounds and synthetic vectors in gene therapy* Pharmaceutical Sciences, 3, 223-233

Bovin et al (2008) *Functional Lipid Constructs* international PCT application no. PCT/NZ2008/000266 (publ. no. WO 2009/048343 A1)

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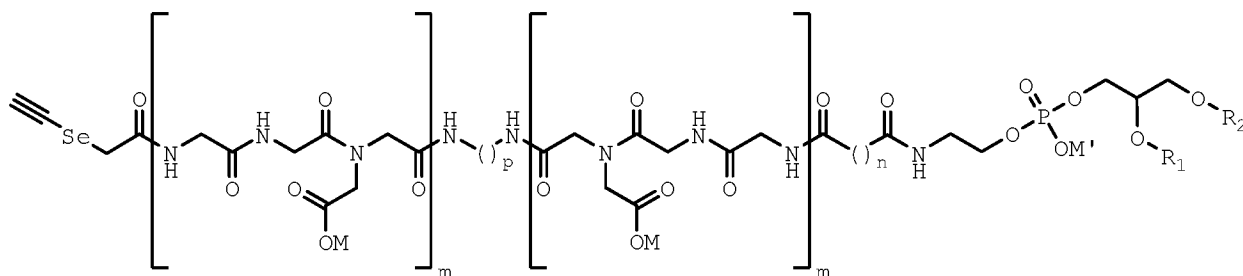
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CLAIMS

- [1] An antimicrobial surface treatment **method** comprising the step of contacting the surface of an object with an aqueous dispersion of at least one functional-lipid construct where the lipid is a di-acyl, di-alkenyl or di-alkyl glycerophospholipid and the functional moiety of the construct confers the antimicrobial activity.
- [2] The method of claim 1 where the object is a surgical dressing or implant.
- [3] The method of claim 2 where the object is a surgical implant.
- [4] The method of claim 3 where the surface is stainless steel.
- [5] The method of claim 1 where the aqueous dispersion is devoid of detergents and organic solvents.
- [6] The method of claim 5 where the aqueous dispersion consists of saline or water and the at least one functional-lipid construct.
- [7] The method of claim 1 where the lipid is a di-acyl glycerophospholipid.
- [8] The method of claim 7 where the lipid is a phosphatidylethanolamine.
- [9] The method of claim 8 where the lipid is a di-oleoyl phosphatidyl-ethanolamine.
- [10] The method of claim 1 where the functional moiety is selected from the group consisting of: selenide and polycations.
- [11] The method of claim 10 where the functional moiety is selected from the group consisting of: cyanoselenide and polyamines.
- [12] The method of claim 11 where the functional moiety is cyanoselenide.
- [13] The method of claim 1 where the antimicrobial surface treatment is an antibacterial surface treatment. More preferably, the antimicrobial surface treatment is a bactericidal surface treatment.
- [14] The method of claim 1 where the contacting the surface is by immersing the object in the dispersion for a time sufficient to provide the antimicrobial surface treatment.
- [15] The method of claim 14 where the time is less than 60 seconds.
- [16] The method of claim 15 where the time is less than 30 seconds.
- [17] The method of claim 16 where the time is less than 10 seconds.

[18] The method of claim 17 where the dispersion is sonicated whilst the object is immersed.

[19] A selenide-lipid **construct** of the structure:



where:

m is the integer 1, 2, 3 or 4; preferably the integer 1, 2 or 4; most preferably the integer 2;

n is the integer 3, 4 or 5; most preferably the integer 4;

p is the integer 1, 2 or 3; most preferably the integer 2;

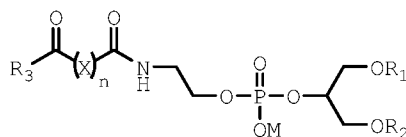
q is the integer 1, 2 or 3; most preferably the integer 1;

M is a monovalent substituent; preferably the monovalent substituent CH_3 or H; most preferably the monovalent substituent H;

M' is a monovalent cation or substituent; preferably the monovalent cation H^+ , K^+ or Na^+ ; most preferably the monovalent cation H^+ ; and

R_1 and R_2 are independently an aliphatic C_{14-20} acyl, aliphatic C_{14-20} alkenyl or aliphatic C_{14-20} alkyl substituent; preferably a substituent selected from the group consisting of myristyl, palmityl, stearyl, arachidyl, palmitoleyl, petroselenyl, oleoyl, elaidyl, vaccenyl and gondoyl; most preferably the aliphatic C_{18} alkenyl substituent oleoyl.

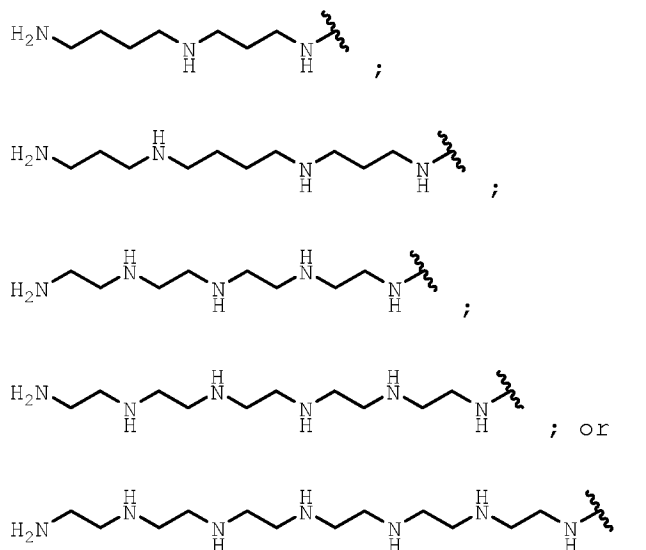
[20] A cationic-lipid **construct** of the structure:



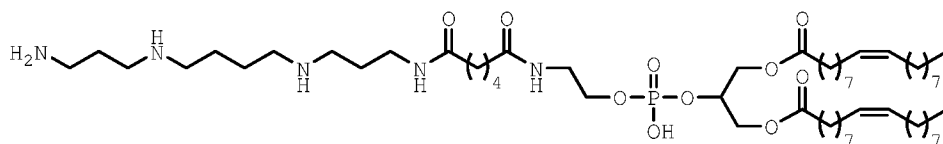
where X is $-\text{CH}_2-$ and n is the integer 3, 4 or 5; R_1 and R_2 are independently selected from the group consisting of C_{14-20} acyl groups that

are unbranched and saturated or mono-unsaturated; and R₃ is an N¹-acylated polyamine.

[21] The construct of claim 20 where R₃ is of the structure:



[22] A **cationic-lipid construct** of the structure:



designated Spm-Ad-DOPE.

[23] A **bactericidal surface treatment preparation** consisting essentially of a dispersion in water of a cationic-lipid construct of any one of claims 19 to 22.