Supporting Information

Mono- and Dicationic short PEG and Methylene Dioxyalkylglycerols for use in Synthetic Gene Delivery Systems

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General Experimental Methods. Unless otherwise noted, solvents and reagents were reagent grade from commercial suppliers and used without further purification. THF was dried by distillation from a sodium/benzophenone suspension under a dry N₂ atmosphere. CH₂Cl₂ was dried by distillation from CaH₂ under a dry N₂ atmosphere. Pyridine was dried by distillation over CaH₂ under a dry N₂ atmosphere. All moisture-sensitive reactions were performed under a nitrogen atmosphere using oven-dried glassware. Reactions were monitored by TLC on Kieselgel 60 F₂₅₄ plates with detection by UV, or permanganate, ninhydrin (for ureas) and phosphomolybdic acid stains. Flash column chromatography was carried out using silica gel (particle size 40-63 µm). Melting points are uncorrected.

1H NMR and 13C NMR spectra were recorded in CDCl₃ and DMSO-d₆ solutions at the field indicated. Unless otherwise specified, NMR spectra were recorded at 298 K.

8-Bromo-1-octanol, 1-(8-bromo-1-oxy)tetrahydro-2H-pyran² and 17-Bromo-3,6,9,12,15-pentaoxoheptadecan-1-ol³ (14) were prepared as previously described. 2-Bromoethyl ether (25), 1,3-dibromopropane (28), 1,6-dibromohexane (29) and 1,10-dibromodecane (30) were commercially available (Aldrich) and used as supplied.

9-Tetradecyn-1-ol⁴

This was prepared via modification of previously reported routes as described below. To a stirred solution of 1-hexyne (4.57 mL, 39.8 mmol) in anhydrous THF (20 mL) and hexamethylphosphoramide (HMPA) (10 mL) at −10 ºC, was added n-butyllithium (2.5 M solution in hexane; 16.0 ml, 40.0 mmol) by dropwise addition. The mixture was stirred for 2 h at −10 ºC, then a solution of 2-(8-bromo-1-oxy)tetrahydro-2H-pyran² (7.77 g, 26.5 mmol) in anhydrous THF (30 mL) was added. The reaction mixture was warmed to room temperature and stirred for 48 h. Saturated ammonium chloride solution (150 mL) and dichloromethane (300 mL) were added and organic layer extracted and dried (MgSO₄).

To 2-(tetradec-9-ynyl-1-oxy)tetrahydro-2H-pyran (4.16 g, 14.1 mmol) was added a solution of concentrated HCl/water/methanol (1:1:5; 175 mL). The reaction was stirred for 18 h at rt, then neutralised with 10 M NaOH solution. The mixture was concentrated in vacuo, extracted with diethyl ether (3 × 70 mL), and the combined organic extracts dried (MgSO₄). The solvent was removed in
vacuo and the product purified by flash silica chromatography (40% diethyl ether in hexane) to yield 9-tetradecyn-1-ol\textsuperscript{4} (2.42 g, 82%) as a colourless oil. \( R_f = 0.45 \) (40% diethyl ether in hexane); \( \nu_{max} \) (film)/cm\textsuperscript{-1} 3339, 2930, 2856, 1464, 1435; \( \delta_h \) (300 MHz; CDCl\textsubscript{3}) 0.89 (3H, t, \( J \ 7.1 \), \( CH_3 \)), 1.31–1.58 (16H, m), 2.11 (4H, m, \( H_2CC\equivCHCH_2 \)), 3.62 (2H, t, \( J \ 6.6 \), \( CH_2OH \)); \( \delta_C \) (75.4 MHz; CDCl\textsubscript{3}) 13.6 (\( CH_3 \)), 18.4, 18.7, 21.9, 25.7, 28.7, 29.1 (signal overlap), 29.3, 31.2, 32.7, 62.8 (\( CH_2OH \)), 80.1 (C≡C), 80.2 (C≡C).

**9-Tetradecyn-1-yloxy mesylate**

To a stirred solution of 9-tetradecyn-1-ol (1.60 g, 7.62 mmol) and methanesulfonyl chloride (1.18 mL, 15.2 mmol) in anhydrous dichloromethane (15 mL) at 0 °C, was added triethylamine (2.11 mL, 15.2 mmol). The reaction mixture was warmed to rt and stirred for 24 h. Dichloromethane (100 mL) was added and the organic phase washed with saturated sodium hydrogencarbonate solution (100 mL) and saturated sodium chloride solution (100 mL). The organic layer was dried (MgSO\textsubscript{4}), evaporated in vacuo and the crude product purified by flash silica chromatography (dichloromethane) to give the titled compound (1.98 g, 91%) as a pale yellow oil. \( R_f = 0.65 \) (dichloromethane); \( \nu_{max} \) (film)/cm\textsuperscript{-1} 2932, 2856, 1468, 1356; \( \delta_h \) (300 MHz; CDCl\textsubscript{3}) 0.88 (3H, t, \( J \ 7.1 \), \( CH_2CH_2 \)), 1.30–1.44 (14H, m), 1.72 (2H, m, \( CH_2CH_2O \)), 2.11 (4H, m, \( H_2CC\equivCHCH_2 \)), 2.98 (3H, s, \( CH_3SO_2 \)), 4.19 (2H, t, \( J \ 6.6 \), \( CH_2O \)); \( \delta_C \) (75.4 MHz; CDCl\textsubscript{3}) 13.6 (\( CH_3CH_2 \)), 18.4, 18.7, 21.9, 25.4, 28.7, 28.9, 29.1 (signals superimposed), 31.2, 37.3 (\( CH_3SO_2 \)), 70.2 (\( CH_2O \)), 80.0 (C≡C), 80.3 (C≡C); m/z (+ES) 311 (MNa\textsuperscript{+}, 100%).

**\( Z \)-9-Tetradecen-1-ol\textsuperscript{4}**

To a solution of 9-tetradecyn-1-ol (2.57 g, 12.2 mmol) in ethanol (30 mL) was added quinoline (0.26 g, 2.45 mmol) and Lindlar catalyst (20 mol%; 0.26 g, 2.45 mmol). The flask was deoxygenated under vacuum, and the suspension stirred vigorously under a hydrogen atmosphere at rt for 24 h. The mixture was filtered through a pad of Celite\textsuperscript{TM} and the filtrate concentrated in vacuo. The crude product was purified by flash silica chromatography (40% diethyl ether in hexane) to yield the titled compound\textsuperscript{4}, as a pale yellow oil (2.39 g, 92%). \( R_f = 0.45 \) (40% diethyl ether in hexane); \( \nu_{max} \) (film)/cm\textsuperscript{-1} 3333, 2934, 2855, 1458; \( \delta_h \) (300 MHz; CDCl\textsubscript{3}) 0.88 (3H, m, \( CH_3 \)), 1.29 (14H, m), 1.54 (2H, m, \( CH_2CH_2OH \)), 2.00 (4H, m, \( H_2CC\equivCHCH_2 \)), 3.62 (2H, t, \( J \ 6.6 \), \( CH_2OH \)), 5.33 (2H, m, \( CH_2=CH_2 \)); \( \delta_C \) (75.4 MHz; CDCl\textsubscript{3}) 14.0 (\( CH_3 \)), 22.4, 25.7, 26.9, 27.2, 29.2, 29.4, 29.5, 29.7, 32.0, 32.8, 63.0 (\( CH_2OH \)), 129.8 (C≡C), 129.9 (C≡C).

**\( Z \)-9-Tetradecen-1-yloxy mesylate**

To a stirred solution of \( Z \)-9-tetradecen-1-ol (1.28 g, 6.04 mmol) and methanesulfonyl chloride (0.56 mL, 7.25 mmol) in anhydrous dichloromethane (30 mL) at 0 °C, was added triethylamine (1.00 mL, 7.25 mmol). The reaction mixture was warmed to rt and stirred for 24 h. Dichloromethane was added (50 mL) and washed with saturated sodium hydrogencarbonate solution (50 mL) and saturated sodium
chloride solution (50 mL). The organic layer was dried (MgSO₄), evaporated in vacuo and the crude product purified by flash silica chromatography (dichloromethane) to give the titled compound (1.61 g, 92%) as a pale yellow oil. Rₜ = 0.60 (dichloromethane); δ₁ (300 MHz; CDCl₃) 0.92 (3H, m, CH₃CH₂) 1.29 (14H, m), 1.72 (2H, m, OCH₂CH₂), 2.01 (4H, m, CH₂CH⁻CHCH₂), 2.99 (3H, s, CH₃SO₂), 4.21 (2H, m, CH₂O), 5.33 (2H, m, CH=CH); δC (75.4 MHz; CDCl₃) 14.0 (CH₂CH₂), 22.4, 25.4, 26.9, 27.2, 29.0 (signal overlap), 29.1, 29.3, 29.7, 32.0, 37.3 (CH₃SO₂), 70.2 (CH₂O), 129.7 (C=C), 130.0 (C=C); m/z (+ES) 313 (MNa⁺, 100%).

2,3-Di-((9Z)-tetradecenyloxy)propyl-N,N-dimethylamine (9).

To a stirring solution of sodium hydride (60% in mineral oil; 0.117 g, 2.93 mmol) in anhydrous toluene (15 mL) at rt was added 3-dimethylaminopropane-1,2-diol (0.12 mL, 1.00 mmol). The mixture was heated at 50 °C for 20 min, and (Z)-tetradec-9-enyl mesylate (0.850 g, 2.93 mmol) was added. The reaction was then heated at reflux for 72 h. On cooling, water (50 mL) was added and the product extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were washed with saturated sodium hydrogen carbonate solution (30 mL), saturated sodium chloride (30 mL) and dried (MgSO₄). The solvent was removed in vacuo to give the crude product which was purified by silica gel flash chromatography (5% MeOH in CH₂Cl₂) to afford 9 (0.340 g, 68%) as a pale yellow oil. Rₜ = 0.36 (5% MeOH in CH₂Cl₂); νmax (film)/cm⁻¹ 2926, 2855, 1653, 1464; δ₁ (300 MHz; CDCl₃) 0.86 (6H, t, J 6.5, 2 × CH₂CH₃), 1.17–1.42 (28H, m), 1.53 (4H, m, 2 × CH₂CH₂OH), 1.99 (8H, m, 2 × H₂CC=CHCH₂), 2.27 (6H, s, N(CH₃)₂), 2.40 (2H, m, NCH₂CH), 3.38–3.62 (7H, m, CHOCH₂, CH₂OCH₂), 5.32 (4H, m, 2 × CH=CH); δC (75.4 MHz; CDCl₃) 14.0 (2 × CH₂CH₂), 22.3, 26.2, 26.9, 27.2, 29.3, 29.5, 29.7 (signal overlap), 30.2, 32.0, 46.3 (N(CH₃)₂), 61.1 (NCH₂CH), 70.2 (CHCH₂O), 71.6 and 72.1 (2 × OCH₂CH₂), 76.6 (CHOCH₂), 129.9 (C=C), 130.3 (C=C); m/z (+ES) 509 (MH⁺, 100%); Found (+HRES) MH⁺ 508.50918. C₃₃H₆₆NO₂ requires 508.50936.

5-Bromo-3-oxopentan-1-ol (11)⁶.

To a stirred solution of diethylene glycol (3.00 g, 28.3 mmol) in anhydrous dichloromethane (200 mL) at 0 °C, thionyl bromide (1.10 ml, 14.1 mmol) was added dropwise. The solution was warmed to rt., stirred for 18 h, then water (40 mL) was cautiously added followed by saturated sodium hydrogen carbonate solution until effervescence ceased. Water (100 mL) was added, the phases partitioned and the aqueous layer further extracted with dichloromethane (2 × 70 mL). The combined organic extracts were dried (MgSO₄) and concentrated in vacuo. Purification by flash silica chromatography (diethyl ether), afforded the titled compound as a colourless oil (0.92 g, 39%). Rₜ = 0.50 (diethyl ether); νmax (film)/cm⁻¹ 3385, 2876, 1121; δ₁ (300 MHz; CDCl₃) 2.96 (1H, br s, OH), 3.41 (2H, t, J 6.0, CH₂Br), 3.53 (2H, m, OCH₂CH₂OH), 3.65 (2H, m, CH₂OH), 3.73 (2H, t, J 6.0, OCH₂CH₂Br); δ₁ (75 MHz; CDCl₃) 30.6 (CH₂Br), 61.7 (CH₂OH), 70.9 (OCH₂CH₂Br), 72.1
(OCH₂CH₂OH); m/z (+ES) 191 (MNa⁺(81)Br), 95%), 193 (MNa⁺ (79)Br), 100).

8-Bromo-3,6-dioxooctan-1-ol (12)⁷.

Triethylene glycol (4.50 g, 30.0 mmol) and hydrobromic acid solution (48% in water; 5.13 mL, 45.0 mmol) were stirred vigorously in toluene (70 mL) with heating at reflux for 72 h. After cooling, the solution was neutralized by the addition of saturated sodium hydrogen carbonate solution until effervescence ceased. Water (50 mL) was added and the resulting mixture was extracted with dichloromethane (3 × 50 mL). The organic extract was dried (MgSO₄) and concentrated in vacuo to give the titled compound as a pale yellow oil (2.29 g, 36%). νmax (film)/cm⁻¹ 3416, 2920, 2872, 2339, 1454; δH (300 MHz; CDCl₃) 2.71 (1H, s, OCH₂Br), 3.42 (2H, t, J 6.2, CH₂Br), 3.55 (2H, t, J 4.9, CH₂O), 3.61 (4H, m, 2 × CH₂O), 3.65 (2H, t, J 4.9, CH₂O), 3.76 (2H, t, J 6.2, OCH₂CH₂Br); δH (75 MHz; CDCl₃) 30.1 (CH₂Br), 61.5 (CH₂OH), 70.1, 70.3, 71.1, 72.4; m/z (+FAB) 215 (M⁺ (81)Br), 44%), 213 (M⁺ (79)Br), 48), 153 (20), 151 (24), 110 (32), 109 (31), 89 (100).

11-Bromo-3,6,9-trioxoundecan-1-ol (13)⁸.

To a stirred solution of tetraethylene glycol (5.00 g, 25.8 mmol) in anhydrous dichloromethane (180 mL) at 0 °C, thionyl bromide (1.00 mL, 12.9 mmol) was added dropwise. The solution was warmed to rt, stirred for 18 h, then water (30 mL) was cautiously added followed by saturated sodium hydrogen carbonate solution until effervescence ceased. Water (90 mL) was added, the phases partitioned and the aqueous layer further extracted with dichloromethane (2 × 60 mL). The combined organic extracts were dried (MgSO₄) and concentrated in vacuo. Purification by flash silica chromatography (5% methanol in diethyl ether), afforded the titled compound as a colourless oil (1.93 g, 58%). The product could also be purified on reverse phase silica (gradient: water to 50% acetonitrile in water). Rf = 0.46 (5% MeOH in diethyl ether), 0.77 (reverse phase, 50% acetonitrile in water); νmax (film)/cm⁻¹ 3415, 2920, 1120, 1070; δH (500 MHz; CDCl₃) 2.95 (1H, br s, OH), 3.40 (2H, t, J 6.3, CH₂Br), 3.52 (2H, t, J 4.6, CH₂CH₂OH), 3.58 (8H, m, OCH₂), 3.63 (2H, t, J 4.6, CH₂OH), 3.73 (2H, t, J 6.3, CH₂CH₂Br); δH (125 MHz; CDCl₃) 30.1 (CH₂Br), 61.4 (CH₂OH), 70.0, 70.2, 70.3, 70.4, 70.9 (CH₂CH₂Br), 72.3 (CH₂CH₂OH); m/z (+FAB) 281 (MNa⁺ (81)Br), 95%), 279 (MNa⁺ (79)Br), 100).

2,3-Di-(9Z-octadecenyloxy)propyl-N-[2-(2-hydroxyethoxy)ethyl]-N,N-dimethylammonium bromide (15).

Compound 11 (162 mg, 0.959 mmol) and the amine 6 (300 mg, 0.485 mmol) in methanol (2 mL) were stirred at 90 °C in a sealed tube for 24 h. The solvent was removed in vacuo and the product purified by low temperature recrystallization (ethyl acetate) to yield 15 as a pale yellow oil (278 mg, 73%). νmax (film)/cm⁻¹ 3400, 2937, 2854, 1637, 1465; δH (300 MHz; CDCl₃) 0.87 (6H, t, J 6.6, 2 × CH₂CH₃), 1.25 (44H, m), 1.54 (4H, m, OCH₂CH₂), 2.01 (8H, m, CH₂CH=CHCH₂), 2.32 (1H, br s, OH), 3.40–4.10 (23H, m, 2 × CH₂O [PEG], CHOCH₂, CHOCH₂, CH₂OC₁₈H₃₅, CH₃OCH₂CH₂, 2 × N⁺CH₂, 2 × N⁺CH₃,

S6
Compound 12 (223 mg, 1.04 mmol) and the amine 6 (300 mg, 0.485 mmol) in methanol (2 mL) were stirred at 90 °C in a sealed tube for 24 h. The solvent was removed in vacuo and the product purified by low temperature recrystallization (ethyl acetate) to yield 16 as a pale yellow oil (247 mg, 62%). νmax (film)/cm⁻¹ 3371, 2917, 2854, 1642, 1465; δt (300 MHz; CDCl₃) 0.85 (6H, t, J 6.7, CH₂CH₃), 1.25 (44H, m), 1.54 (4H, m, 2 × CH₂CH₂), 2.01 (8H, m, 2 × CH₂CH=CHCH₂), 2.88 (1H, br s, OH), 3.40–4.10 (27H, m, 4 × CH₂O (PEG), CHOCH₂, CHOCH₂, CH₂OC₁₈H₃₅, CH₂OCH₂CH₂, 2 × N⁺CH₂, 2 × N⁺CH₃, CH₂OH), 5.35 (4H, m, 2 × CH=CH); δc (75.4 MHz; CDCl₃) 14.0 (2 × CH₂CH₃), 22.6, 26.0, 26.2, 27.2, 29.2, 29.3, 29.4, 29.7 (signal overlap), 30.0, 31.9, 32.6, 53.5 (2 × N⁺CH₃), 61.3 (CH₂OH), 65.2, 66.7, 68.7, 69.4, 70.2, 70.4 (signal overlap), 72.0, 72.5, 73.4 (CHOCH₂), 129.7 (2 × CH=CH), 129.9 (2 × CH=CH); m/z (+ES) 753 ([M-Br]⁺, 100%), 621 ([M-(OCH₂CH₃)]⁺, 32); Found (+HRFAB) (M-Br)⁺ 752.7116. C₄₇H₉₄O₅N requires 752.7126; Found C, 65.92; H, 11.70; N, 1.62. C₄₇H₉₄NO₅Br.H₂O requires C, 66.32; H, 11.37; N, 1.65%.

2,3-Di-((9Z)-octadecenyloxy)propyl-N-[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]ethyl]-N,N-dimethylammonium bromide (18). Compound 14 (60 mg, 0.17 mmol) and the amine 6 (100 mg, 0.161 mmol) in methanol (1 mL) were stirred at 90 °C in a sealed tube for 24 h. The solvent was removed in vacuo and the product purified by flash silica gel chromatography (10% MeOH in CH₂Cl₂) to yield 18 as a pale yellow oil (53 mg, 34%). Rf = 0.24 (10% MeOH in CH₂Cl₂); νmax (film)/cm⁻¹ 3389, 2924, 2853, 1634; δt (300 MHz; CDCl₃) 0.84 (6H, t, J 6.6, 2 × CH₂CH₃) 1.22 (44H, m), 1.51 (4H, m, 2 × OCH₂CH₂), 1.98 (8H, m, 2 × CH₂CH=CHCH₂), 2.71 (1H, s, br, OH), 3.40 (6H, s, 2 × N⁺CH₃), 3.50–4.10 (29H, m, 10 × CH₂O (PEG), CHOCH₂, CHOCH₂, CH₂OC₁₈H₃₅, CH₂OCH₂CH₂, 2 × N⁺CH₂, CH₂OH), 5.30 (4H, m, 2 × CH=CH); δc (75.4 MHz; CDCl₃) 14.1 (2 × CH₂CH₃), 22.7, 26.0, 26.2, 27.2, 29.2, 29.3, 29.5 (signal overlap), 29.7, 29.8, 30.0, 31.9, 32.6, 53.1 and 53.3 (2 × N⁺CH₃), 61.3 (CH₂OH), 65.1, 66.7, 68.7, 69.2, 70.0–70.5 (signal overlap), 72.0, 72.8, 73.5, 129.8 (2 × CH=CH), 130.2 (2 × CH=CH); m/z (+ES) 885 ([M-Br]⁺, 100%); Found (+HRFAB) (M-Br)⁺ 884.7975. C₅₃H₁₀₆N₄O₈ requires 884.7913.

2,3-Di-((11Z)-hexadecenyloxy)propyl-N-[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]ethyl]-N,N-dimethylammonium bromide (19). Compound 13 (58 mg, 0.23 mmol) and the amine 7 (100 mg,
0.177 mmol) in methanol (1 mL) were stirred at 90 °C in a sealed tube for 24 h. The solvent was removed in vacuo and the product purified by flash silica chromatography (10% MeOH in CH2Cl2) to yield 19 as a pale yellow oil (64 mg, 44%). Rf = 0.24 (10% MeOH in CH2Cl2); νmax (film)/cm⁻¹ 3385, 2924, 2853, 1634, 1466; δH (300 MHz; CDCl3) 0.87 (6H, t, J 7.1, 2 × CH2CH3), 1.22 (36H, m), 1.53 (4H, m, 2 × OCH2CH2), 2.00 (8H, m, 2 × CH2CH=CHCH2), 2.29 (1H, s, br, OCH3), 2.43 (6H, s, 2 × N⁺CH3), 3.48–4.25 (25H, m, 6 × CH2O (PEG), CHOCH2, CHOCH2, CH2OC18H35, CH2OCH2CH2, 2 × N⁺CH2, CH2OH), 5.32 (4H, m, 2 × CH=CH); δC (75.4 MHz; CDCl3) 14.0 (2 × CH2CH3), 22.3, 26.1, 26.2, 26.9, 27.2, 29.3, 29.5 (signal overlap), 29.8, 30.0, 32.0, 53.0 and 53.6 (2 × N⁺CH3), 61.2, (CH2OH), 65.2, 66.6, 68.6, 69.2, 70.1 (signal overlap), 70.5, 72.0, 72.6, 73.5, 129.9 (2 × CH=CH), 130.3 (2 × CH=CH); m/z (+FAB) 741 ([M-Br]+, 100%); Found (+HRFAB) (M-Br)+, 740.67748. C45H90BrNO6.3H2O requires C, 61.76; H, 11.06; N, 1.60%.


Compound 14 (75 mg, 0.22 mmol) and the amine 7 (100 mg, 0.177 mmol) in methanol (1 mL) were stirred at 90 °C in a sealed tube for 24 h. The solvent was removed in vacuo and the product purified by flash silica gel chromatography (10% MeOH in CH2Cl2) to yield 20 as a pale yellow oil (73 mg, 45%). Rf = 0.24 (10% MeOH in CH2Cl2); νmax (film)/cm⁻¹ 3400, 2924, 2853, 1634, 1466; δH (300 MHz; CDCl3) 0.83 (6H, t, J 6.8, 2 × CH2CH3), 1.21 (36H, m), 1.49 (4H, m, 2 × OCH2CH2), 1.96 (8H, m, 2 × CH2CH=CHCH2), 2.87 (1H, s, br, OH), 3.46 (6H, s, 2 × N⁺CH3), 3.48–4.10 (33H, m, 10 × CH2O (PEG), CHOCH2, CHOCH2, CH2OC18H35, CH2OCH2CH2, 2 × N⁺CH2, CH2OH), 5.30 (4H, m, 2 × CH=CH); δC (75.4 MHz; CDCl3) 14.0 (2 × CH2CH3), 22.3, 26.0, 26.2, 26.9, 27.2, 29.2, 29.3, 29.5 (signal overlap), 29.8, 30.0, 31.9, 53.2 (2 × N⁺CH3), 61.1 (CH2OH), 65.0, 66.6, 68.7, 69.2, 69.9, 70.1, 70.3 (signal overlap), 71.9, 72.6, 73.4, 129.8 (2 × CH=CH), 130.3 (2 × CH=CH); m/z (+ES) 829 ([M-Br]+, 100%); Found (+HRES) (M-Br)+, 828.7266. C49H98BrNO6 requires 828.7287.

2,3-Di-(tetradec-9-ynlyloxy)propyl-N-(2-[2-hydroxyethoxy]ethyl)-N,N-dimethylammonium bromide (21).

Compound 11 (0.420 g, 2.49 mmol) and the amine 8 (0.840 g, 1.67 mmol) in methanol (4 mL) were stirred at 90 °C in a sealed tube for 48 h. The solvent was removed in vacuo and the product purified by flash silica gel chromatography (15% MeOH in CH2Cl2) to yield 21 as a pale yellow oil (0.710 g, 63%). Rf = 0.35 (15% MeOH in CH2Cl2); νmax (film)/cm⁻¹ 3356, 2932, 2855, 2361, 1456; δH (300 MHz; CDCl3) 0.88 (6H, t, J 7.1, 2 × CH2CH3), 1.15–1.53 (32H, m), 2.10 (8H, m, 2 × CH2CH=CHCH2), 2.65 (1H, s, br, OH), 3.41 (4H, m, CH2OCH2CH2, CHOCH2), 3.47 (6H, s, 2 × N⁺CH3), 3.54–4.13 (13H, m, 2 × CH2O (PEG), CHOCH2, CH2OC18H35, 2 × N⁺CH2, CH2OH); δC (75.4 MHz; CDCl3) 14.0 (2 ×
CH₂CH₃), 18.4 and 18.7 (2 × CH₂CH≡CHCH₂), 21.9, 25.9, 26.2, 28.2, 29.1, 29.3, 30.1, 31.3, 53.4 (2 × N⁺CH₂), 61.3, (CH₂OH), 65.0, 67.0, 68.6, 69.4 (CH₂OC₁₃H₂₅), 72.0 (OCH₂C₁₃H₂₃), 72.9, 73.5 (CHOCH₂), 80.1 and 80.2 (2 × C≡C), m/z (+ES) 593 ([M-Br]⁺, 100%); Found (+HRES) (M-Br)⁺, 592.52942. C₃₇H₇₀NO₄ requires 592.52994.

2,3-Di-((9Z)-tetradecenlyloxy)propyl-N-(2-[2-hydroxyethoxy]ethyl)-N,N-dimethylammonium bromide (22).

Compound 11 (0.210 g, 1.24 mmol) and the amine 9 (0.420 g, 0.827 mmol) in methanol (2 mL) were stirred at 90 °C in a sealed tube for 48 h. The solvent was removed in vacuo and the product purified by low temperature recrystallisation (ethyl acetate) to yield 22 as a pale yellow oil (0.271 g, 48%). ν_max (film)/cm⁻¹ 3356, 2924, 2855, 1635, 1458; δ_H (300 MHz; CDCl₃) 0.89 (6H, t, J 7.0, 2 × CH₂CH₃), 1.26 (28H, m), 1.53 (4H, m, 2 × OCH₂CH₂), 1.99 (8H, m, 2 × CH₂CH=CHCH₂), 2.83 (1H, s, br, OH), 3.44 (4H, m, CH₂OCH₂CH₂, CHOCH₂), 3.48 (6H, s, 2 × N⁺CH₃), 3.50–4.12 (13H, m, 2 × CH₂O (PEG), CHOCH₂, CH₂OC₁₄H₂₇, 2 × N⁺CH₂, CH₂OH), 5.31 (4H, m, 2 × CH=CH); δ_C (75.4 MHz; CDCl₃) 14.0 (2 × CH₂CH₃), 22.3, 26.0, 26.2, 26.9, 27.2, 29.3–30.0 (signal overlap), 31.9, 53.5 (2 × N⁺CH₃), 61.3 (CH₂OH), 65.0, 66.9, 68.6, 69.4, 72.0 (OCH₂C₁₃H₂₅), 72.9, 73.4 (CHOCH₂), 129.7 (2 × CH=CH), 129.8 (2 × CH=CH); m/z (+ES) 597 ([M-Br]⁺, 100%); Found (+HRES) (M-Br)⁺, 596.5637. C₃₇H₇₄NO₄ requires 596.5612.

2,3-Di-((11Z)-tetradecenlyloxy)propyl-N-(2-[2-hydroxyethoxy]ethyl)-N,N-dimethylammonium bromide (23).

Compound 11 (0.180 g, 1.06 mmol) and the amine 10 (0.370 g, 0.729 mmol) in methanol (2 mL) were stirred at 90 °C in a sealed tube for 48 h. The solvent was removed in vacuo and the product purified by low temperature recrystallisation (ethyl acetate) to yield 23 as a pale yellow oil (0.210 g, 43%). ν_max (film)/cm⁻¹ 3356, 2920, 2855, 1674, 1458; δ_H (300 MHz; CDCl₃) 0.92 (6H, t, J 7.5, 2 × CH₂CH₃), 1.26 (28H, m), 1.55 (4H, m, 2 × OCH₂CH₂), 1.78 (1H, s, br, OH), 2.02 (8H, m, 2 × CH₂CH=CHCH₂), 3.46 (4H, m, CH₂OCH₂CH₂, CHOCH₂), 3.49 (6H, s, 2 × N⁺CH₃), 3.50–4.12 (13H, m, 2 × CH₂O (PEG), CHOCH₂, CH₂OC₁₄H₂₇, 2 × N⁺CH₂, CH₂OH), 5.34 (4H, m, 2 × CH=CH); δ_C (75.4 MHz; CDCl₃) 14.4 (2 × CH₂CH₃), 20.5, 26.0, 26.2, 27.1, 27.2, 29.3, 29.4–29.6 (signal overlap), 29.8, 30.0, 53.5 (2 × N⁺CH₃), 61.2 (CH₂OH), 65.0, 66.8, 68.7, 69.4, 72.1 (OCH₂C₁₃H₂₅), 72.9, 73.4 (CHOCH₂), 129.3 (2 × CH=CH), 131.5 (2 × CH=CH); m/z (+ES) 597 ([M-Br]⁺, 100%); Found (+HRES) (M-Br)⁺, 596.5621. C₃₇H₇₄NO₄ requires 596.5612.


Compound 13 (0.450 g, 1.75 mmol) and the amine 10 (0.600 g, 1.18 mmol) in methanol (3 mL) were stirred at 90 °C in a sealed tube for 48 h. The solvent was removed in vacuo and the product purified by
low temperature recrystallisation (ethyl acetate) to yield 24 as a pale yellow oil (0.470 g, 52%). \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 3364, 2926, 2855, 1631, 1464; \(\delta_{\text{H}}\) (300 MHz; CDCl\(_3\)) 0.89 (6H, \(t, J\) 7.5, 2 \(\times CH_2CH_2\)), 1.21 (28H, m), 1.48 (4H, m, 2 \(\times\) OCH\(_2\)CH\(_2\)), 1.99 (8H, m, 2 \(\times CH_2CH=CHCH_2\)), 2.59 (1H, s, br, OH), 3.37 (4H, m, CH\(_2\)OCH\(_2\)CH\(_2\), CHOCH\(_2\), CH\(_2\)OC\(_14\)H\(_27\), 2 \(\times N^+\)CH\(_2\), CH\(_2\)OH), 5.30 (4H, m, 2 \(\times CH=CH\)); \(\delta_{\text{C}}\) (75.4 MHz; CDCl\(_3\)) 14.3 (2 \(\times CH_2CH_3\)), 20.4, 25.9, 26.1, 27.0, 29.1, 29.3–29.6 (signal overlap), 29.9, 52.9 and 53.5 (2 \(\times N^+\)CH\(_3\)), 61.1 (CH\(_2\)OH), 65.1, 66.4, 68.6, 69.1, 69.9–70.2 (signal overlap), 71.8, 72.5, 73.3 (CHOCH\(_2\)), 129.1 (2 \(\times CH=CH\)), 131.4 (2 \(\times CH=CH\)); \(m/z\) (+ES) 685 ([M-Br]\(^+\), 100%); Found (+HRES) (M-Br\(^+\), 684.61641. \(C_{41}H_{82}NO_6\) requires 684.61367.

1,8-Dibromo-3,6-dioxooctan-1-ol (26).\(^9\)

This was prepared using the Appel reaction.\(^10\) Triethylene glycol (1.50 g, 10.0 mmol) and carbon tetrabromide (8.30 g, 25.0 mmol) were stirred in anhydrous dichloromethane (50 mL) for 15 min. Triphenylphosphine (6.56 g, 25.0 mmol) was added in portions and stirring was continued at rt for 2 h. The mixture was concentrated in vacuo and the resulting residue stirred in dichloromethane/ether (1:1, 50 mL). The triphenylphosphine oxide by-product was removed by filtration and the filtrate concentrated in vacuo. The product was purified by flash silica chromatography (15% hexane in ether) to give 25 as a yellow oil (2.47 g, 90%).\(^9\) \(R_f = 0.76\) (15% hexane in ether); \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 3017, 2920, 2868, 1442, 1353; \(\delta_{\text{H}}\) (300 MHz; CDCl\(_3\)) 3.44 (4H, \(t, J\) 6.2, 2 \(\times CH_2CH_2Br\)), 3.63 (4H, s, 2 \(\times CH_2O\)), 3.73 (4H, \(t, J\) 6.2, 2 \(\times CH_2CH_2Br\)); \(\delta_{\text{C}}\) (75 MHz; CDCl\(_3\)) 30.3 (2 \(\times CH_2Br\), 70.4 (2 \(\times CH_2O\)), 71.1 (2 \(\times CH_2O\)).

1,11-Dibromo-3,6,9-trioxoundecan-1-ol (27).\(^10,11\)

This was prepared via modification of a previously reported route\(^10\). Tetraethylene glycol (1.94 g, 10.0 mmol) and carbon tetrabromide (8.30 g, 25.0 mmol) were stirred in anhydrous dichloromethane (50 mL) for 15 min. Triphenylphosphine (6.56 g, 25.0 mmol) was added in portions and stirring continued at rt for 2 h. The mixture was concentrated in vacuo, the resulting residue stirred in dichloromethane/ether (1:1, 50 mL) and triphenylphosphine oxide by-product removed by filtration. The filtrate was concentrated in vacuo and the resulting product purified by flash silica chromatography (10% hexane in ether) to give 26 as a yellow oil (2.18 g, 68%).\(^11\) \(R_f = 0.55\) (10% hexane in ether); \(\nu_{\text{max}}\) (film)/cm\(^{-1}\) 2920, 2870, 2739, 1454; \(\delta_{\text{H}}\) (300 MHz; CDCl\(_3\)) 3.42 (4H, \(t, J\) 6.3, 2 \(\times OCH_2CH_2Br\)), 3.64 (8H, m, 4 \(\times CH_2O\)), 3.78 (4H, \(t, J\) 6.3, 2 \(\times OCH_2CH_2Br\)); \(\delta_{\text{C}}\) (75 MHz; CDCl\(_3\)) 30.2 (2 \(\times CH_2Br\), 70.4 (2 \(\times CH_2O\)), 70.5 (2 \(\times CH_2O\)), 71.1 (2 \(\times CH_2O\)); \(m/z\) (+APCI) 323.1 (MH\(^{+}\)(81Br and 81Br), 12%), 321.1 (MH\(^{+}\)(79Br and 81Br), 24), 319.1 (MH\(^{+}\)(79Br and 79Br), 12), 151 (100).

Compound 26 (356 mg, 1.29 mmol) and amine 6 (200 mg, 0.322 mmol) were stirred in methanol (3 mL) at 90 °C in a sealed tube for 18 h. The solvent was removed in vacuo and the product purified by flash silica gel chromatography (gradient: CH₂Cl₂ to 10% MeOH in CH₂Cl₂) to yield 32 as a pale yellow oil (96 mg, 33%). v_max (film)/cm⁻¹ 2923, 2853, 1656, 1464; δ_H (300 MHz; CDCl₃) 0.83 (6H, t, J 6.7, 2 × CH₂CH₃), 1.22 (44H, m), 1.47 (4H, m, 2 × OCH₂CH₂CH₂), 1.96 (8H, m, 2 × CH₂CH=CHCH₂), 3.40 (4H, m, CH₂OCH₂CH₂, CHOCH₂), 3.43–4.10 (23H, m, 4 × CH₂O (PEG), CHOCH₂, CH₂OCH₁₈H₃₅, 2 × N⁺CH₂, CH₂Br, 2 × N⁺CH₃), 5.32 (4H, m, 2 × CH=CH); δ_C (75 MHz; CDCl₃) 14.0 (2 × CH₂CH₃), 22.5, 25.9, 26.2, 27.2, 29.1, 29.3–29.7 (signal overlap), 29.9, 30.0, 30.6, 31.8, 32.6, 53.3 (2 × N⁺CH₃), 65.2, 66.7, 68.5, 69.1, 70.0, 70.3, 70.8, 71.9 (signal overlap), 73.3 (CHOCH₂), 129.8 (2 × CH=CH), 130.0 (2 × CH=CH); m/z (+ES) 817 ([M-Br]⁺ (⁸¹Br), 68%), 815 ([M-Br]⁺ (⁷⁹Br), 65), 157 (100); Found (+HRFAB) (M⁺⁻⁷⁹Br)⁺, 814.6289. C₄₇H₉₃BrO₄N requires 814.6288.

2,3-Di-(9Z-octadecenlyoxy)propyl-N-(2-[2-(2-bromoethoxyethoxy)ethoxy]ethoxy)ethyl)-N,N-dimethylammonium bromide (33).

Compound 27 (645 mg, 2.02 mmol) and amine 6 (250 mg, 0.403 mmol) were stirred in acetone (2 mL) at 90 °C in a sealed tube for 24 h. The solvent was removed in vacuo and the product purified by flash silica gel chromatography (gradient: CHCl₃ to 20% MeOH in CHCl₃) to yield 33 as a brown oil (80 mg, 22%). v_max (film)/cm⁻¹ 2927, 2853, 1656, 1464; δ_H (300 MHz; CDCl₃) 0.85 (6H, t, J 6.6, 2 × CH₂CH₃), 1.25 (44H, m), 1.54 (4H, m, 2 × OCH₂CH₂CH₂), 1.97 (8H, m, 2 × CH₂CH=CHCH₂), 3.41 (4H, m, CH₂OCH₂CH₂, CHOCH₂), 3.44–4.10 (27H, m, 6 × CH₂O (PEG), CHOCH₂, CH₂OCH₁₈H₃₅, 2 × N⁺CH₂, CH₂Br, 2 × N⁺CH₃), 5.32 (4H, m, 2 × CH=CH); δ_C (75 MHz; CDCl₃) 14.1 (2 × CH₂CH₃), 22.6, 26.0, 26.2, 27.2, 29.2, 29.3–29.7 (signal overlap), 30.0, 30.5, 31.7, 32.6, 53.2 and 53.4 (2 × N⁺CH₃), 65.0, 65.2, 68.5, 69.3, 70.3–70.5 (signal overlap), 71.2, 72.0, 73.5 (CHOCH₂), 129.7 (2 × CH=CH), 130.0 (2 × CH=CH); m/z (+ES) 860.6 ([M-Br]⁺ (⁸¹Br), 100%), 858.6 ([M-Br]⁺ (⁷⁹Br), 84), 157 (100);

2,3-Di-(9Z-octadecenlyoxy)propyl-N-(3-bromohexyl)-N,N-dimethylammonium bromide (35).

Amine 6 (100 mg, 0.161 mmol) and 1,6-dibromohexane (29) (300 mg, 1.23 mmol) were stirred in methanol (2 mL) in a sealed tube at 90 °C for 24 h. The solvent was removed in vacuo and the product purified by flash silica gel chromatography (gradient: CH₂Cl₂ to 10% MeOH in CH₂Cl₂) to yield 35 as a colourless oil (120 mg, 87%). v_max (film)/cm⁻¹ 2924, 2853, 1634, 1464; δ_H (500 MHz; CDCl₃) 0.84 (6H, t, J 6.9, 2 × CH₂CH₃), 1.25 (48H, m), 1.49 (4H, m, 2 × OCH₂CH₂CH₂), 1.80 (2H, m), 1.91 (2H, m), 1.96 (8H, m, 2 × CH₂CH=CHCH₂), 3.40–3.90 (18H, m, CH₂OCH₁₈H₃₅, CH₂OCH₂CH₂, CHOHCH₂, 2 × N⁺CH₂, CH₂Br, 2 × N⁺CH₃), 4.01 (1H, m, CHOHCH₂), 5.30 (4H, m, 2 × CH=CH); δ_C (75 MHz; CDCl₃) 13.9 (2 × CH₂CH₃), 22.4, 25.2, 25.9, 26.0, 27.0, 27.4, 29.0–29.5 (signal overlap), 29.8, 31.7, 32.0, 32.4, 33.2, 52.0 and 52.2 (2 × N⁺CH₃), 64.8, 65.8, 68.1, 69.1, 71.8, 73.2 (CHOHCH₂), 129.4 (2 × CH=CH), 129.5 (2 × CH=CH); m/z (+ES) 784.7 ([M-Br]⁺ (⁸¹Br), 100%), 782.7 ([M-Br]⁺ (⁷⁹Br), 98); Found
(CHCl product purified by low temperature recrystallization (ethyl acetate) to give 

Amine 6 (200 mg, 0.322 mmol) and 1,10-dibromodecane (30) (0.48 mL, 1.60 mmol) were stirred in methanol (3 mL) in a sealed tube at 90 °C for 24 h. The solvent was removed in vacuo and the product purified by flash silica gel chromatography (gradient: CH2Cl2 to 10% MeOH in CH2Cl2) to yield 36 as a pale yellow oil (133 mg, 46%). νmax (film) cm⁻¹ 3005, 2924, 2853, 1466; δH (300 MHz; CDCl3) 0.86 (6H, t, J 6.7, 2 × CH2CH3), 1.27 (54H, m), 1.38 (4H, m), 1.54 (4H, m, 2 × OCH2CH2CH2), 1.83 (2H, m), 1.98 (8H, m, 2 × CH2CH=CHCH2), 3.36–3.94 (18H, m, CH2OC18H35, CH2OCH2CH2, CHOCH2, 2 × N⁺CH2, CH2Br, 2 × N⁺CH3), 4.02 (1H, m, CHOCH2), 5.32 (4H, m, 2 × CH=CH); δC (75 MHz; CDCl3) 13.9 (2 × CH2CH3), 22.4, 25.9, 26.0, 27.0, 27.9, 28.6, 29.0–29.5 (signal overlap), 31.7, 32.5, 33.6, 52.0 and 52.3 (2 × N⁺CH3), 64.5, 66.0, 68.2, 69.1, 71.8, 73.2 (CHOCH2), 129.4 (2 × CH=CH), 129.6 (2 × CH=CH); m/z (+ES) 841 ([M-Br]+ (81Br), 100%), 839 ([M-Br]+ (79Br), 88); Found (+HRFAB) (M-79Br)+, 838.7010. C51H102O2NBr requires 838.7010.

2,3-Di-((9Z)-octadecenyloxy)propyl-N-(3-bromodecyl)-N,N-dimethylammonium bromide (36). Bromide 32 (50 mg, 0.056 mmol) and trimethylamine (45 wt% in H2O; 0.17 mL, 1.12 mmol) were stirred in methanol (1.5 mL) in a sealed tube at 90 °C for 18 h. The solvent was removed in vacuo and the product purified by low temperature recrystallization (ethyl acetate) to give 38 (17 mg, 32%). νmax (CHCl3/cm⁻¹ 2934, 2856, 1466; δH (500 MHz; CDCl3) 0.86 (6H, t, J 6.9, 2 × CH2CH3), 1.25 (44H, m), 1.52 (4H, m, 2 × OCH2CH2CH2), 1.99 (8H, m, 2 × CH2CH=CHCH2), 3.41 (4H, m, CH2OCH2CH2, CHOCH2), 3.44–4.07 (32H, m, 4 × CH2O (PEG), CH2OC18H35, CHOCH2, 3 × N⁺CH2, 5 × N⁺CH3), 5.32 (4H, m, 2 × CH=CH); δC (125 MHz; CDCl3) 14.1 (2 × CH2CH3), 22.6, 26.0, 26.2, 27.2 (2 × CH2C=C), 29.3–29.7 (signal overlap), 30.0, 31.9, 32.6 (2 × C=CCH2), 53.4 (N⁺CH3), 53.5 (N⁺CH3), 54.8 (3 × N⁺CH3), 65.0, 65.1, 65.2, 65.7, 66.5, 68.6, 69.4, 70.4, 70.5, 72.0, 73.3 (CHOCH2), 129.8 (2 × CH=CH), 130.0 (2 × CH=CH); m/z (+ES) 397 (½[M-2Br]+, 100%); Found (+HRFAB) ½(M-2Br)+, 397.3917. ½(C50H102BrN2O4) requires 397.3919.

2,3-Di-((9Z)-octadecenyloxy)propyl-N-2-[2-(2-(N,N,N-trimethylammonium)ethoxy)ethoxy]ethyl]-N,N-dimethylammonium dibromide (38). Bromide 33 (30 mg, 0.032 mmol) and trimethylamine (45 wt% in H2O; 0.098 mL, 0.64 mmol) were stirred in methanol (1 mL) in a sealed tube at 90 °C for 18 h. The solvent was removed in vacuo and the product purified by low temperature recrystallization (ethyl acetate) to give 39 (24 mg, 73%). νmax (CHCl3/cm⁻¹ 2934, 2856, 1466; δH (500 MHz; CDCl3) 0.85 (6H, t, J 6.9, 2 × CH2CH3), 1.24 (44H, m), 1.52 (4H, m, 2 × OCH2CH2CH2), 1.97 (8H, m, 2 × CH2CH=CHCH2), 3.40 (4H, m, CH2OCH2CH2, CHOCH2), 3.47 (3H, s, N⁺CH3), 3.50 (3H, s, N⁺CH3), 3.52 (9H, s, 3 × N⁺CH3), 3.53–4.05 (21H, m, 6 × CH2O (PEG), CH2OC18H35, CHOCH2, 3 × N⁺CH2), 5.32 (4H, m, 2 × CH=CH); δC (125 MHz; CDCl3)
14.1 (2 × CH₂CH₃), 22.7, 26.0, 26.2, 27.2 (2 × CH₂C=C), 29.2, 29.3–29.8 (signal overlap), 30.0, 31.9, 32.6 (2 × C=CCH₂), 53.2 (N⁺CH₃), 53.4 (N⁺CH₃), 54.7 (3 × N⁺CH₃), 64.9, 65.1, 65.2, 65.4, 66.5, 68.6, 69.3, 70.2, 70.4, 70.7, 72.0, 73.3 (CHOCH₂), 129.7 (2 × CH=CH), 130.0 (2 × CH=CH); m/z (+ES) 419.6 (½[M-2Br]^+, 100%).

2,3-Di-((9Z)-octadecenyloxy)propyl-N-3-(N,N,N-trimethylammoniumhexyl)-N,N-dimethylammonium dibromide (41).

Bromide 35 (30 mg, 0.035 mmol) and trimethylamine (45 wt% in H₂O; 0.054 mL, 0.35 mmol) were stirred in methanol (1 mL) in a sealed tube at 90 °C for 24 h. The solvent was removed in vacuo and the product purified by low temperature recrystallization (ethyl acetate) to give 41 as a waxy solid/oil (30 mg, 93%). νₘₐₓ (CHCl₃)/cm⁻¹ 2934, 2856, 1466; δ₁ (300 MHz; CDCl₃) 0.84 (6H, t, J 6.7, 2 × CH₂CH₃), 1.26 (48H, m), 1.56 (4H, m, 2 × OCH₂CH₂), 1.68 (4H, m, 2 × N⁺CH₂CH₂), 2.00 (8H, m, 2 × CH₂CH=CHCH₂), 3.39–4.02 (27H, m, CH₂OC₁₈H₃₅, CH₂OCH₂CH₂, CHOCH₂, 3 × N⁺CH₂, 5 × N⁺CH₃), 4.11 (1H, m, CHOCH₂), 5.33 (4H, m, 2 × CH=CH); δ_C (75 MHz; CDCl₃) 14.1 (2 × CH₂CH₃), 22.6, 25.7, 26.0, 27.2, 28.0, 28.1, 28.3, 29.1–29.7 (signal overlap), 30.0, 31.9, 32.6, 52.2 (N⁺CH₃), 52.5 (N⁺CH₃), 53.7 (3 × N⁺CH₃), 65.1 (signal overlap), 66.5, 68.6, 69.3, 72.0, 73.3 (CHOCH₂), 129.7 (2 × CH=CH), 129.8 (2 × CH=CH); m/z (+ES) 381.6 (½[M-2Br]^+, 100%), νₘₐₓ (CHCl₃)/cm⁻¹ 2921, 2853, 1462; δ₁ (300 MHz; CDCl₃) 0.85 (6H, t, J 6.9, 2 × CH₂CH₃), 1.28 (52H, m), 1.40 (4H, m), 1.55 (4H, m, 2 × OCH₂CH₂), 1.78 (4H, m, 2 × N⁺CH₂CH₂), 2.01 (8H, m, 2 × CH₂CH=CHCH₂), 3.40 (3H, s, N⁺CH₃), 3.44 (3H, s, N⁺CH₃), 3.46–4.02 (21H, m, CH₂OC₁₈H₃₅, CH₂OCH₂CH₂, CHOCH₂, 3 × N⁺CH₃, 3 × N⁺CH₃), 4.10 (1H, m, CHOCH₂), 5.33 (4H, m, 2 × CH=CH); δ_C (75 MHz; CDCl₃) 14.1 (2 × CH₂CH₃), 22.6, 25.7, 26.0, 26.2, 27.2, 28.0, 28.1, 28.3, 29.1, 29.2–29.7 (signal overlap), 30.0, 31.9, 32.6, 52.0 (N⁺CH₃), 52.5 (N⁺CH₃), 53.5 (3 × N⁺CH₃), 65.1, 66.3, 66.5, 68.6, 69.3, 72.0, 73.3 (CHOCH₂), 129.7 (2 × CH=CH), 129.9 (2 × CH=CH); Found C, 63.58; H, 11.07; N, 2.75. C₅₄H₁₁₀N₂O₂.2Br.2H₂O requires C, 63.88; H, 11.32; N, 2.76%.

**Lipid formulation**

Cationic lipids were either formulated alone or with DOPE (weight ratio, 1:1). Lipid (10 mg/mL; 100 µL [1 mg of lipid]), either lipid alone or formulated with DOPE 2, in chloroform was placed in a sterile glass vial. The solvents were removed in vacuo and further traces of chloroform removed on the high vacuum for 24 h. Sterile water (1 mL) was added to the lipid film, to generate a 1 mg/mL (total lipid)
lipid suspension in water. The suspension was allowed to hydrate at 4 °C for 24 h. After warming to 40 °C the mixture was sonicated (bath or probe sonication [see below]- sonication method was not observed to influence transfection results) for approximately 5 min to generate a clear solution. The resulting liposome formulations were stable for up to 6 months.

**Sizing protocol**
The lipid was formulated as described above, hydrated and warmed to 35–40 °C and left to stand for 10 min to equilibrate. The formulated lipid solution was then cooled to 0 °C, and sonicated using a titanium-probe sonicator (Soniprobe 7535A, Lucas Dawe Ultrasonics) for 2–4 min. The resulting clear liposome solution is transferred from the vial and into eppendorfs (0.5 mL), and centrifuged for 1 min at 10,000 rpm. The liposome solution was then carefully removed from the eppendorf via pipette, whilst leaving the titanium precipitate, and transferred to a 1 cm cuvette. The cuvette was placed into a light scattering machine (ZetaPlus, Brookhaven Instruments Corporation), and the mean vesicle size and polydispersity of the vesicles, was measured, as well as their zeta-potential. The final measurements are obtained from three concurrent measurements using three different samples of the same lipid that were prepared in parallel.

**Transfection of Cells** The complete growth medium was removed from cells plated at 2 x 10^4 cells/well overnight in 96-well plates and 200 µL of complex (0.25 µg of plasmid DNA) added to each well, leaving minimal time between preparing the complex and adding to the cells. All transfections were carried out in 6 wells each. The cells were incubated with the complexes for 4 h before replacing with normal media for 24 to 48 h, after which reporter gene expression was analysed by luciferase assay (Promega, Southampton, U. K.).

The components of the LID vector complex were mixed in lipid/peptide/DNA weight ratios of, 2:4:1 or 4:4:1. In a typical procedure lipopolyplex formulations were prepared by mixing the components in the following order: lipid then peptide 3, then the luciferase reporter plasmid pCILux in OptiMEM). Plasmid pCILux was prepared by subcloning a luciferase gene from pGL3 Control (Life Technologies, Paisley, UK) into the eukaryotic expression vector pCI (Promega, Southampton, U. K.). The complex was mixed by pipetting briefly before diluting in OptiMEM to a final volume of 400 µL.

**Luciferase assays.** Cells were washed twice with PBS before the addition of 100 µL of 1 x Reporter Lysis Buffer (Promega, Southampton, U. K.) to the cells for 20 min at 4 °C before freezing at -20 °C for at least 30 min followed by thawing at room temperature (± 20°C). 20 µL of the lysate at room temperature was transferred to a white polystyrene 96-well plate (Porvair Sciences Ltd., Shepperton, U. K.) and the luciferase activity was measured using the Luciferase Assay System (Promega) and a Lucy-1 Luminometer (Anthos Ltd., Saltzburg, Austria). The amount of protein present in each transfection lysate was determined with the Bio-Rad protein assay reagent by the manufacturer’s instructions, adding
20 µL from the luciferase test to 200 µL of the reagent diluted 1 in 5 and incubating at room temperature for 10 min before comparing the OD_{590} to a range of BSA standards. Luciferase activity was expressed as Relative Light Units (RLU) per mg of protein (RLU/mg).

**Transfection protocol of 1HAEo- cells in the presence of serum**

Cells were seeded at 1 × 10⁴ cells per well in a 96 well plate overnight at 37 °C. Medium was removed from the cells and replaced with 100 µL per well of Eagles Minimal Essential Medium (MEM) HEPES modification (Sigma, Poole, UK) containing Foetal Bovine Serum (FBS) (Sigma, Poole, UK) at concentrations of 0, 1, 2.5, 5, 7.5, and 10%. The components of the LID vector complex were mixed in lipid/peptide/DNA weight ratios of 2:4:1 by adding the transfection components in the following order; lipid, peptide then plasmid, all diluted in OptiMEM. The complexes were mixed by pipetting briefly before diluting in OptiMEM to give a final volume of 400 µL. 100 µL of freshly prepared complexes was added to wells containing serum in triplicate, mixed and incubated at 37 °C for 4 h to allow transfection. Complexes and serum medium was removed and replaced with growth medium for 37 °C for 24 h before measuring reporter gene expression by luciferase assay.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Transfection relative to Lipofectin™</th>
</tr>
</thead>
<tbody>
<tr>
<td>†Lipofectin</td>
<td>100%</td>
</tr>
<tr>
<td>‡37 + DOPE</td>
<td>7%</td>
</tr>
<tr>
<td>‡38 + DOPE</td>
<td>3%</td>
</tr>
<tr>
<td>‡39 + DOPE</td>
<td>2%</td>
</tr>
<tr>
<td>*40 + DOPE</td>
<td>46%</td>
</tr>
<tr>
<td>*41 + DOPE</td>
<td>3%</td>
</tr>
<tr>
<td>*42 + DOPE</td>
<td>2%</td>
</tr>
</tbody>
</table>

The lipid + peptide (3) + DNA complexes (4 µg of lipid+ DOPE (1:1), 4 µg 3 and 1 µg plasmid pCILux in 200 µl of OptiMEM) and 1HAEo-cells were incubated for 4 h at 37 °C. *The lipid + peptide (3) + DNA complexes (2 µg of lipid+ DOPE (1:1), 4 µg 3 and 1 µg plasmid pCILux in 200 µl of OptiMEM) and 1HAEo-cells were incubated for 4 h at 37 °C.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Transfection relative to 17a + DOPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>†Lipofectin</td>
<td>16%</td>
</tr>
<tr>
<td>†15 + DOPE</td>
<td>41%</td>
</tr>
<tr>
<td>†16 + DOPE</td>
<td>46%</td>
</tr>
</tbody>
</table>
The lipid + peptide (3)+DNA complexes (2 µg of lipid + DOPE (1:1), 4 µg 3 and 1 µg plasmid pCILux in 200 µl of OptiMEM) and 1HAEo-cells were incubated for 4 h at 37 °C

Table 3: Transfection Data for Compounds DOTMA, 17a and Lipofectin (1HAEo-cells)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Transfection relative to 17a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peptide 3 (Peptide:DNA wt ratio, 1:1)</td>
</tr>
<tr>
<td>†DOTMA (L:D, 2:1)</td>
<td>16%</td>
</tr>
<tr>
<td>DOTMA (L:D, 4:1)</td>
<td>86%</td>
</tr>
<tr>
<td>Lipofectin (L:D, 2:1)</td>
<td>11%</td>
</tr>
<tr>
<td>†Lipofectin (L:D, 4:1)</td>
<td>54%</td>
</tr>
<tr>
<td>†17a + DOPE (L:D, 2:1)</td>
<td>41%</td>
</tr>
<tr>
<td>†17a + DOPE (L:D, 4:1)</td>
<td>79%</td>
</tr>
</tbody>
</table>

The lipid+peptide (3)+DNA complexes (2 or 4 µg of lipid+ DOPE (1:1), 4 µg 3 and 1 µg plasmid pCILux in 200 µl of OptiMEM) and 1HAEo-cells were incubated for 4 h at 37 °C

Table 4: Transfection Data for Compound 23 (+ DOPE), Lipofectin (1HAEo-cells)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Transfection relative to Lipofectin (2:1, lipid/DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>†Lipofectin (2:1)</td>
<td>100%</td>
</tr>
<tr>
<td>†23 + DOPE (2:1)</td>
<td>25%</td>
</tr>
</tbody>
</table>

The C-14 lipids generally preformed poorly for HAEo- cell delivery but were much better for smooth muscle cell applications (see below, Table 5 for transfection to porcine smooth muscle cells), which is consistent with other data for C-14 lipids we have observed in vitro\textsuperscript{12}.

Table 5: Relative Transfection Data for Compounds 21-24 (+ DOPE) (PVSMC-cells)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Transfection relative to 23 + DOPE (2:1, lipid/DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>†21 + DOPE (2:1)</td>
<td>28%</td>
</tr>
<tr>
<td>†22 + DOPE (2:1)</td>
<td>97%</td>
</tr>
<tr>
<td>†23 + DOPE (2:1)</td>
<td>100%</td>
</tr>
<tr>
<td>†24 + DOPE (2:1)</td>
<td>88%</td>
</tr>
</tbody>
</table>

The lipid+peptide (3)+DNA complexes (2 µg of lipid+ DOPE (1:1), 4 µg 3 and 1 µg plasmid pCILux in 200 µl of OptiMEM) and 1HAEo-cells were incubated for 4 h at 37 °C

When comparing across the C-14 PEG lipid series, the dialkyne was less effective in transfections and the remaining lipids similar in their efficacy.
References