Supporting Information

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

Christopher A. Hurley,‡[a], John B. Wong,[a] Jimmy Ho,[a] Michele Writer,[b] Scott A. Irvine,[b] M. Jayne Lawrence,[c] Stephen L. Hart[b], Alethea B. Tabor[a] and Helen C. Hailes[a],*

[a] Department of Chemistry, University College London, 20 Gordon Street, London, WC1H 0AJ, UK. . Fax: +44 20 7679 7463; Tel: +44 20 7679 4654; E-mail: h.c.hailes@ucl.ac.uk

[b] Molecular Immunology Unit, Institute of Child Health, University College London, 30 Guilford Street, London WC1N 1EH, UK

[c] Pharmaceutical Science Research Division, King's College London, Stamford Street, London, SE1 9NH, UK

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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 N_2 atmosphere. CH_2Cl_2 was dried by distillation from CaH_2 under a dry N_2 atmosphere. Pyridine was dried by distillation over CaH_2 under a dry N_2 atmosphere. All moisture-sensitive reactions were performed under a nitrogen atmosphere using oven-dried glassware. Reactions were monitored by TLC on Kieselgel 60 F_{254} 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. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 solutions at the field indicated. Unless otherwise specified, NMR spectra were recorded at 298 K.

8-Bromooctan-1-ol,[1] 2-(8-bromooctyl-1-oxy)tetrahydro-2*H*-pyran[2] and 17-Bromo-3,6,9,12,15-pentaoxoheptadecan-1-ol[3] (**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-ol[4]

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-bromooctyl-1-oxy)tetrahydro-2*H*-pyran,[2] (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 ($MgSO_4$). The solvent was evaporated *in vacuo* and the crude product purified by flash silica chromatography (10% EtOAc in hexane) to yield 2-(tetradec-9-ynyl-1-oxy)tetrahydro-2*H*-pyran[5] (6.34 g, 81%), as a colourless oil. R_f = 0.25 (1:9, EtOAc: hexane); ν_{max} (film)/cm^{-1} 2932, 2855, 1119; δ_H (300 MHz; $CDCl_3$) 0.90 (3H, t, *J* 6.9, C*H*$_3$), 1.25–1.80 (22H, m), 2.13 (4H, m, C*H*$_2$C≡CC*H*$_2$), 3.36 (1H, m, THPOC*H*H), 3.49 (1H, m, OCHOC*H*H), 3.73 (1H, m, THPOC*H*H), 3.87 (1H, m, OCHOC*H*H), 4.57 (1H, m, OC*H*OCH$_2$); δ_C (75.4 MHz; $CDCl_3$) 13.6 (*C*H$_3$), 18.4, 18.7, 19.7, 25.5, 26.2, 28.8, 29.1, 29.2, 29.4 (signal overlap), 29.8, 30.8, 31.3, 62.3 (O*C*HOCH$_2$), 67.7 (THPO*C*H$_2$), 79.5 (*C*≡C), 80.2 (C≡*C*), 98.8 (O*C*HOCH$_2$); *m/z* (+ES) 317 (MNa$^+$, 100%).

To 2-(tetradec-9-ynyl-1-oxy)tetrahydro-2*H*-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 ($MgSO_4$). 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[4] (2.42 g, 82%) as a colourless oil. R_f = 0.45 (40% diethyl ether in hexane); ν_{max} (film)/cm^{-1} 3339, 2930, 2856, 1464, 1435; δ_H (300 MHz; $CDCl_3$) 0.89 (3H, t, *J* 7.1, C*H*$_3$), 1.31–1.58 (16H, m), 2.11 (4H, m, H_2CC≡CHCH_2), 3.62 (2H, t, *J* 6.6, CH_2OH); δ_C (75.4 MHz; $CDCl_3$) 13.6 (*C*H$_3$), 18.4, 18.7, 21.9, 25.7, 28.7, 29.1 (signal overlap), 29.3, 31.2, 32.7, 62.8 (*C*H$_2$OH), 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 ºC, 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 ($MgSO_4$), 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); ν_{max} (film)/cm^{-1} 2932, 2856, 1468, 1356; δ_H (300 MHz; $CDCl_3$) 0.88 (3H, t, *J* 7.1, C*H*$_3$CH$_2$), 1.30–1.44 (14H, m), 1.72 (2H, m, CH_2CH$_2$O), 2.11 (4H, m, H_2CC≡CCH_2), 2.98 (3H, s, C*H*$_3$SO$_2$), 4.19 (2H, t, *J* 6.6, CH_2O); δ_C (75.4 MHz; $CDCl_3$) 13.6 (*C*H$_3$CH$_2$), 18.4, 18.7, 21.9, 25.4, 28.7, 28.9, 29.1 (signals superimposed), 31.2, 37.3 (*C*H$_3$SO$_2$), 70.2 (CH_2O), 80.0 (C≡*C*), 80.3 (*C*≡C); *m/z* (+ES) 311 (MNa$^+$, 100%).

(*Z*)-9-Tetradecen-1-ol[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™ 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[4], as a pale yellow oil (2.39 g, 92%). R_f = 0.45 (40% diethyl ether in hexane); ν_{max} (film)/cm^{-1} 3333, 2934, 2855, 1458; δ_H (300 MHz; $CDCl_3$) 0.88 (3H, m, C*H*$_3$), 1.29 (14H, m), 1.54 (2H, m, CH_2CH$_2$OH), 2.00 (4H, m, H_2CC=CHCH_2), 3.62 (2H, t, *J* 6.6, CH_2OH), 5.33 (2H, m, CH_2=CH_2); δ_C (75.4 MHz; $CDCl_3$) 14.0 (*C*H$_3$), 22.4, 25.7, 26.9, 27.2, 29.2, 29.4, 29.5, 29.7, 32.0, 32.8, 63.0 (*C*H$_2$OH), 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 ºC, 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 ($MgSO_4$), 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. R_f = 0.60 (dichloromethane); δ_H (300 MHz; $CDCl_3$) 0.92 (3H, m, C*H*$_3$CH$_2$) 1.29 (14H, m), 1.72 (2H, m, OCH$_2$C*H*$_2$), 2.01 (4H, m, C*H*$_2$CH=CHC*H*$_2$), 2.99 (3H, s, C*H*$_3$SO$_2$), 4.21 (2H, m, C*H*$_2$O), 5.33 (2H, m, C*H*=C*H*); δ_C (75.4 MHz; $CDCl_3$) 14.0 (*C*H$_3$CH$_2$), 22.4, 25.4, 26.9, 27.2, 29.0 (signal overlap), 29.1, 29.3, 29.7, 32.0, 37.3 (*C*H$_3$SO$_2$), 70.2 (C*H*$_2$O), 129.7 (*C*=C), 130.0 (C=*C*); *m/z* (+ES) 313 (MNa^+, 100%).

2,3-Di-((9*Z*)-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 ºC 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 hydrogencarbonate solution (30 mL), saturated sodium chloride (30 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo* to give the crude product which was purified by silica gel flash chromatography (5% MeOH in CH_2Cl_2) to afford ***9*** (0.340 g, 68%) as a pale yellow oil. R_f = 0.36 (5% MeOH in CH_2Cl_2); ν_{max} (film)/cm^{-1} 2926, 2855, 1653, 1464; δ_H (300 MHz; $CDCl_3$) 0.86 (6H, t, *J* 6.5, 2 × CH$_2$C*H*$_3$), 1.17–1.42 (28H, m), 1.53 (4H, m, 2 × C*H*$_2$CH$_2$OH), 1.99 (8H, m, 2 × *H*$_2$CC=CHC*H*$_2$), 2.27 (6H, s, N(C*H*$_3$)$_2$), 2.40 (2H, m, NC*H*$_2$CH), 3.38–3.62 (7H, m, C*H*OC*H*$_2$, C*H*$_2$OC*H*$_2$), 5.32 (4H, m, 2 × C*H*=C*H*); δ_C (75.4 MHz; $CDCl_3$) 14.0 (2 × *C*H$_3$CH$_2$), 22.3, 26.2, 26.9, 27.2, 29.3, 29.5, 29.7 (signal overlap), 30.2, 32.0, 46.3 (N(*C*H$_3$)$_2$), 61.1 (N*C*H$_2$CH), 70.2 (CH*C*H$_2$O), 71.6 and 72.1 (2 × O*C*H$_2$CH$_2$), 76.6 (*C*HOCH$_2$), 129.9 (*C*=C), 130.3 (C=*C*); *m/z* (+ES) 509 (MH^+, 100%); Found (+HRES) MH^+ 508.50918. $C_{33}H_{66}NO_2$ 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 hydrogencarbonate 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 ($MgSO_4$) and concentrated *in vacuo*. Purification by flash silica chromatography (diethyl ether), afforded the titled compound as a colourless oil (0.92 g, 39%). R_f = 0.50 (diethyl ether); ν_{max} (film)/cm^{-1} 3385, 2876, 1121; δ_H (300 MHz; $CDCl_3$) 2.96 (1H, br s, O*H*), 3.41 (2H, t, *J* 6.0, C*H*$_2$Br), 3.53 (2H, m, OC*H*$_2$CH$_2$OH), 3.65 (2H, m, C*H*$_2$OH), 3.73 (2H, t, *J* 6.0, OC*H*$_2$CH$_2$Br); δ_H (75 MHz; $CDCl_3$) 30.6 (*C*H$_2$Br), 61.7 (*C*H$_2$OH), 70.9 (OCH$_2$CH$_2$Br), 72.1

(OCH_2CH_2OH); *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 hydrogencarbonate 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 ($MgSO_4$) and concentrated *in vacuo* to give the titled compound as a pale yellow oil (2.29 g, 36%). ν_{max} (film)/cm^{-1} 3416, 2920, 2872, 2339, 1454; δ_H (300 MHz; $CDCl_3$) 2.71 (1H, s, O*H*), 3.42 (2H, t, *J* 6.2, CH_2Br), 3.55 (2H, t, *J* 4.9, CH_2O), 3.61 (4H, m, 2 × CH_2O), 3.65 (2H, t, *J* 4.9, CH_2O), 3.76 (2H, t, *J* 6.2, OC$H_2$$CH_2$Br); δ_H (75 MHz; $CDCl_3$) 30.1 (*C*H_2Br), 61.5 (*C*H_2OH), 70.1, 70.3, 71.1, 72.4; *m/z* (+FAB) 215 (MH^+ (^{81}Br), 44%), 213 (MH^+ (^{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 hydrogencarbonate 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 ($MgSO_4$) 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). R_f = 0.46 (5% MeOH in diethyl ether), 0.77 (reverse phase, 50% acetonitrile in water); ν_{max} (film)/cm^{-1} 3415, 2920, 1120, 1070; δ_H (500 MHz; $CDCl_3$) 2.95 (1H, br s, O*H*), 3.40 (2H, t, *J* 6.3, CH_2Br), 3.52 (2H, t, *J* 4.6, C$H_2$$CH_2$OH), 3.58 (8H, m, OC$H_2$), 3.63 (2H, t, *J* 4.6, CH_2OH), 3.73 (2H, t, *J* 6.3, C$H_2$$CH_2$Br); δ_H (125 MHz; $CDCl_3$) 30.1 (*C*H_2Br), 61.4 (*C*H_2OH), 70.0, 70.2, 70.3, 70.4, 70.9 (*C*$H_2$$CH_2$Br), 72.3 (*C*$H_2$$CH_2$OH); *m/z* (+FAB) 281 ($MNa^+$ (^{81}Br), 95%), 279 (MNa^+(^{79}Br), 100).

2,3-Di-((9*Z*)-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%). ν_{max} (film)/cm^{-1} 3400, 2937, 2854, 1637, 1465; δ_H (300 MHz; $CDCl_3$) 0.87 (6H, t, *J* 6.6, 2 × CH_2C*H*$_3$), 1.25 (44H, m), 1.54 (4H, m, OCH_2C*H*$_2$), 2.01 (8H, m, CH_2CH=CHCH_2), 2.32 (1H, br s, O*H*), 3.40–4.10 (23H, m, 2 × CH_2O [PEG], C*H*OCH_2, CHOCH_2, C$H_2$$OC_{18}H_{35}$, $CH_2OCH_2CH_2$, 2 × N^+CH_2, 2 × N^+CH_3,

CH_2OH), 5.35 (4H, m, 2 × C*H*=C*H*); δ_C (75.4 MHz; $CDCl_3$) 14.0 (2 × CH_2CH_3), 22.6, 26.0, 26.2, 27.2, 29.1, 29.5 (signal overlap) 29.7, 30.0, 31.9, 32.6, 53.5 (2 × N^+CH_3), 61.2 (*C*H_2OH), 65.0, 65.1, 66.9, 68.8, 69.4, 72.5, 73.1, 73.4 (*C*HOCH_2), 129.7 (2 × CH=*C*H), 129.9 (2 × *C*H=CH); *m/z* (+ES) 709 ([M-Br]$^+$, 100%), 621 ([M-$(OCH_2CH_3)_2$]$^+$, 34); Found (+HRFAB) (M-Br)$^+$, 708.6857. $C_{45}H_{90}O_4N$ requires 708.6864; Found C, 67.08; H, 11.57; N, 1.92. $C_{45}H_{90}NO_4Br.H_2O$ requires C, 66.96; H, 11.49; N, 1.74%.

2,3-Di-((9*Z*)-octadecenyloxy)propyl-*N*-{2-[2-(2-hydroxyethoxy)ethoxy]ethyl}-*N*,*N*-dimethylammonium bromide (16).

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%). ν_{max} (film)/cm^{-1} 3371, 2917, 2854, 1642, 1465; δ_H (300 MHz; $CDCl_3$) 0.85 (6H, t, *J* 6.7, CH_2CH_3), 1.25 (44H, m), 1.54 (4H, m, 2 × OCH_2CH_2), 2.01 (8H, m, 2 × CH_2CH=$CHCH_2$), 2.88 (1H, br s, O*H*), 3.40–4.10 (27H, m, 4 × CH_2O (PEG), C*H*OCH_2, CHOCH_2, C$H_2OC_{18}H_{35}$, $CH_2OCH_2CH_2$, 2 × N^+CH_2, 2 × N^+CH_3, CH_2OH), 5.35 (4H, m, 2 × C*H*=C*H*); δ_C (75.4 MHz; $CDCl_3$) 14.0 (2 × CH_2CH_3), 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^+CH_3), 61.3 (*C*H_2OH), 65.2, 66.7, 68.7, 69.4, 70.2, 70.4 (signal overlap), 72.0, 72.5, 73.4 (*C*HOCH_2), 129.7 (2 × CH=*C*H), 129.9 (2 × *C*H=CH); *m/z* (+ES) 753 ([M-Br]$^+$, 100%), 621 ([M-$(OCH_2CH_3)_3$]$^+$, 32); Found (+HRFAB) (M-Br)$^+$ 752.7116. $C_{47}H_{94}O_5N$ requires 752.7126; Found C, 65.92; H, 11.70; N, 1.62. $C_{47}H_{94}NO_5Br.H_2O$ requires C, 66.32; H, 11.37; N, 1.65%.

2,3-Di-((9*Z*)-octadecenyloxy)propyl-*N*-{2-[2-(2-{2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}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 CH_2Cl_2) to yield ***18*** as a pale yellow oil (53 mg, 34%). R_f = 0.24 (10% MeOH in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3389, 2924, 2853, 1634; δ_H (300 MHz; $CDCl_3$) 0.84 (6H, t, *J* 6.6, 2 × CH_2CH_3) 1.22 (44H, m), 1.51 (4H, m, 2 × OCH_2CH_2), 1.98 (8H, m, 2 × CH_2CH=$CHCH_2$), 2.71 (1H, s, br, O*H*), 3.40 (6H, s, 2 × N^+CH_3), 3.50–4.10 (29H, m, 10 × CH_2O (PEG), C*H*OCH_2, CHOCH_2, C$H_2OC_{18}H_{35}$, $CH_2OCH_2CH_2$, 2 × N^+CH_2, CH_2OH), 5.30 (4H, m, 2 × C*H*=C*H*); δ_C (75.4 MHz; $CDCl_3$) 14.1 (2 × CH_2CH_3), 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^+CH_3), 61.3 (*C*H_2OH), 65.1, 66.7, 68.7, 69.2, 70.0–70.5 (signal overlap), 72.0, 72.8, 73.5, 129.8 (2 × CH=*C*H), 130.2 (2 × *C*H=CH); *m/z* (+ES) 885 ([M-Br]$^+$, 100%); Found (+HRFAB) (M-Br)$^+$ 884.7975. $C_{53}H_{106}NO_8$ requires 884.7913.

2,3-Di-((11*Z*)-hexadecenyloxy)propyl-*N*-(2-{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 CH_2Cl_2) to yield ***19*** as a pale yellow oil (64 mg, 44%). R_f = 0.24 (10% MeOH in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3385, 2924, 2853, 1634, 1466; δ_H (300 MHz; $CDCl_3$) 0.87 (6H, t, *J* 7.1, 2 × CH_2CH_3), 1.22 (36H, m), 1.53 (4H, m, 2 × OCH_2CH_2), 2.00 (8H, m, 2 × CH_2CH=$CHCH_2$), 2.29 (1H, s, br, O*H*), 3.43 (6H, s, 2 × N^+CH_3), 3.48–4.25 (25H, m, 6 × CH_2O (PEG), $CHOCH_2$, $CHOCH_2$, $CH_2OC_{18}H_{35}$, $CH_2OCH_2CH_2$, 2 × N^+CH_2, CH_2OH), 5.32 (4H, m, 2 × C*H*=C*H*); δ_C (75.4 MHz; $CDCl_3$) 14.0 (2 × CH_2CH_3), 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^+CH_3), 61.2, (CH_2OH), 65.2, 66.6, 68.6, 69.2, 70.1 (signal overlap), 70.5, 72.0, 72.6, 73.5, 129.9 (2 × CH=*C*H), 130.3 (2 × *C*H=CH); *m/z* (+FAB) 741 ([M-Br]$^+$, 100%); Found (+HRFAB) (M-Br)$^+$, 740.67748. $C_{45}H_{90}NO_6$ requires 740.67678; Found C, 62.01; H, 11.04; N, 1.53. $C_{45}H_{90}BrNO_6.3H_2O$ requires C, 61.76; H, 11.06; N, 1.60%.

2,3-Di-((11*Z*)-hexadecenyloxy)propyl-*N*-{2-[2-(2-{2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}ethoxy)ethoxy]ethyl}-*N*,*N*-dimethylammonium bromide (20). 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 CH_2Cl_2) to yield ***20*** as a pale yellow oil (73 mg, 45%). R_f = 0.24 (10% MeOH in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3400, 2924, 2853, 1634, 1466; δ_H (300 MHz; $CDCl_3$) 0.83 (6H, t, *J* 6.8, 2 × CH_2CH_3), 1.21 (36H, m), 1.49 (4H, m, 2 × OCH_2CH_2), 1.96 (8H, m, 2 × CH_2CH=$CHCH_2$), 2.87 (1H, s, br, O*H*), 3.46 (6H, s, 2 × N^+CH_3), 3.48–4.10 (33H, m, 10 × CH_2O (PEG), $CHOCH_2$, $CHOCH_2$, $CH_2OC_{18}H_{35}$, $CH_2OCH_2CH_2$, 2 × N^+CH_2, CH_2OH), 5.30 (4H, m, 2 × C*H*=C*H*); δ_C (75.4 MHz; $CDCl_3$) 14.0 (2 × CH_2CH_3), 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^+CH_3), 61.1 (CH_2OH), 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=*C*H), 130.3 (2 × *C*H=CH); *m/z* (+ES) 829 ([M-Br]$^+$, 100%); Found (+HRES) (M-Br)$^+$, 828.7266. $C_{49}H_{98}NO_8$ requires 828.7287.

2,3-Di-(tetradec-9-ynyloxy)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 CH_2Cl_2) to yield ***21*** as a pale yellow oil (0.710 g, 63%). R_f = 0.35 (15% MeOH in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3356, 2932, 2855, 2361, 1456; δ_H (300 MHz; $CDCl_3$) 0.88 (6H, t, *J* 7.1, 2 × CH_2CH_3), 1.15–1.53 (32H, m), 2.10 (8H, m, 2 × CH_2CH≡$CHCH_2$), 2.65 (1H, s, br, O*H*), 3.41 (4H, m, $CH_2OCH_2CH_2$, $CHOCH_2$), 3.47 (6H, s, 2 × N^+CH_3), 3.54–4.13 (13H, m, 2 × CH_2O (PEG), $CHOCH_2$, $CH_2OC_{14}H_{25}$, 2 × N^+CH_2, CH_2OH); δ_C (75.4 MHz; $CDCl_3$) 14.0 (2 ×

CH_2CH_3), 18.4 and 18.7 (2 × $CH_2CH{\equiv}CHCH_2$), 21.9, 25.9, 26.2, 28.2, 29.1, 29.3, 30.1, 31.3, 53.4 (2 × N^+CH_3), 61.3, (CH_2OH), 65.0, 67.0, 68.6, 69.4 ($CH_2OC_{14}H_{25}$), 72.0 ($OCH_2C_{13}H_{23}$), 72.9, 73.5 ($CHOCH_2$), 80.1 and 80.2 (2 × $C{\equiv}C$), *m/z* (+ES) 593 ([M-Br]$^+$, 100%); Found (+HRES) (M-Br)$^+$, 592.52942. $C_{37}H_{70}NO_4$ requires 592.52994.

2,3-Di-((9*Z*)-tetradecenyloxy)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; $CDCl_3$) 0.89 (6H, t, *J* 7.0, 2 × CH_2CH_3), 1.26 (28H, m), 1.53 (4H, m, 2 × OCH_2CH_2), 1.99 (8H, m, 2 × $CH_2CH{=}CHCH_2$), 2.83 (1H, s, br, O*H*), 3.44 (4H, m, $CH_2OCH_2CH_2$, $CHOCH_2$), 3.48 (6H, s, 2 × N^+CH_3), 3.50–4.12 (13H, m, 2 × CH_2O (PEG), $CHOCH_2$, $CH_2OC_{14}H_{27}$, 2 × N^+CH_2, CH_2OH), 5.31 (4H, m, 2 × C*H*=C*H*); δ_C (75.4 MHz; $CDCl_3$) 14.0 (2 × CH_2CH_3), 22.3, 26.0, 26.2, 26.9, 27.2, 29.3–30.0 (signal overlap), 31.9, 53.5 (2 × N^+CH_3), 61.3 (CH_2OH), 65.0, 66.9, 68.6, 69.4, 72.0 ($OCH_2C_{13}H_{25}$), 72.9, 73.4 ($CHOCH_2$), 129.7 (2 × CH=*C*H), 129.8 (2 × *C*H=CH); *m/z* (+ES) 597 ([M-Br]$^+$, 100%); Found (+HRES) (M-Br)$^+$, 596.5637. $C_{37}H_{74}NO_4$ requires 596.5612.

2,3-Di-((11*Z*)-tetradecenyloxy)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; $CDCl_3$) 0.92 (6H, t, *J* 7.5, 2 × CH_2CH_3), 1.26 (28H, m), 1.55 (4H, m, 2 × OCH_2CH_2), 1.78 (1H, s, br, O*H*), 2.02 (8H, m, 2 × $CH_2CH{=}CHCH_2$), 3.46 (4H, m, $CH_2OCH_2CH_2$, $CHOCH_2$), 3.49 (6H, s, 2 × N^+CH_3), 3.50–4.12 (13H, m, 2 × CH_2O (PEG), $CHOCH_2$, $CH_2OC_{14}H_{27}$, 2 × N^+CH_2, CH_2OH), 5.34 (4H, m, 2 × C*H*=C*H*); δ_C (75.4 MHz; $CDCl_3$) 14.4 (2 × CH_2CH_3), 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^+CH_3), 61.2 (CH_2OH), 65.0, 66.8, 68.7, 69.4, 72.1 ($OCH_2C_{13}H_{25}$), 72.9, 73.4 ($CHOCH_2$), 129.3 (2 × CH=*C*H), 131.5 (2 × *C*H=CH); *m/z* (+ES) 597 ([M-Br]$^+$, 100%); Found (+HRES) (M-Br)$^+$, 596.5621. $C_{37}H_{74}NO_4$ requires 596.5612.

2,3-Di-((11*Z*)-tetradecenyloxy)propyl-*N*-(2-{2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}ethyl)-*N*,*N*-dimethylammonium bromide (24).

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%). ν_{max} (film)/cm^{-1} 3364, 2926, 2855, 1631, 1464; δ_H (300 MHz; $CDCl_3$) 0.89 (6H, t, *J* 7.5, 2 × CH_2CH_3), 1.21 (28H, m), 1.48 (4H, m, 2 × OCH_2CH_2), 1.99 (8H, m, 2 × CH_2CH=$CHCH_2$), 2.59 (1H, s, br, O*H*), 3.37 (4H, m, $CH_2OCH_2CH_2$, $CHOCH_2$), 3.41 (6H, s, 2 × N^+CH_3), 3.47–4.12 (21H, m, 6 × CH_2O (PEG), $CHOCH_2$, $CH_2OC_{14}H_{27}$, 2 × N^+CH_2, CH_2OH), 5.30 (4H, m, 2 × C*H*=C*H*); δ_C (75.4 MHz; $CDCl_3$) 14.3 (2 × 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 × N^+CH_3), 61.1 (CH_2OH), 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 × CH=CH), 131.4 (2 × 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 for 2 h at rt. 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); ν_{max} (film)/cm^{-1} 3017, 2920, 2868, 1442, 1353; δ_H (300 MHz; $CDCl_3$) 3.44 (4H, t, *J* 6.2, 2 × CH_2CH_2Br), 3.63 (4H, s, 2 × CH_2O), 3.73 (4H, t, *J* 6.2, 2 × CH_2CH_2Br); δ_H (75 MHz; $CDCl_3$) 30.3 (2 × CH_2Br), 70.4 (2 × CH_2O), 71.1 (2 × CH_2O).

1,11-Dibromo-3,6,9-trioxoundecan-1-ol (27)[10,11].

This was prepared *via* modification of a previously reported route[11] using the Appel reaction[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); ν_{max} (film)/cm^{-1} 2920, 2870, 2739, 1454; δ_H (300 MHz; $CDCl_3$) 3.42 (4H, t, *J* 6.3, 2 × OCH_2CH_2Br), 3.64 (8H, m, 4 × CH_2O), 3.78 (4H, t, *J* 6.3, 2 × OCH_2CH_2Br); δ_H (75 MHz; $CDCl_3$) 30.2 (2 × CH_2Br), 70.4 (2 × CH_2O), 70.5 (2 × CH_2O), 71.1 (2 × CH_2O); *m/z* (+APCI) 323.1 (MH^+(^{81}Br and ^{81}Br), 12%), 321.1 (MH^+(^{79}Br and ^{81}Br), 24), 319.1 (MH^+(^{79}Br and ^{79}Br), 12), 151 (100).

2,3-Di-((9*Z*)-octadecenyloxy)propyl-*N*-{2-[2-(2-bromoethoxy)ethoxy]ethyl}-*N*,*N*-dimethylammonium bromide (32).

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: CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to yield ***32*** as a pale yellow oil (96 mg, 33%). ν_{max} (film)/cm^{-1} 2923, 2853, 1656, 1464; δ_H (300 MHz; $CDCl_3$) 0.83 (6H, t, *J* 6.7, 2 × CH_2CH_3), 1.22 (44H, m), 1.47 (4H, m, 2 × $OCH_2CH_2CH_2$), 1.96 (8H, m, 2 × $CH_2CH{=}CHCH_2$), 3.40 (4H, m, $CH_2OCH_2CH_2$, $CHOCH_2$), 3.43–4.10 (23H, m, 4 × CH_2O (PEG), $CHOCH_2$, $CH_2OC_{18}H_{35}$, 2 × N^+CH_2, CH_2Br, 2 × N^+CH_3), 5.32 (4H, m, 2 × $CH{=}CH$); δ_C (75 MHz; $CDCl_3$) 14.0 (2 × CH_2CH_3), 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^+CH_3), 65.2, 66.7, 68.5, 69.1, 70.0, 70.3, 70.8, 71.9 (signal overlap), 73.3 ($CHOCH_2$), 129.8 (2 × $CH{=}CH$), 130.0 (2 × $CH{=}CH$); *m/z* (+ES) 817 ([M-Br]$^+$ (^{81}Br), 68%), 815 ([M-Br]$^+$ (^{79}Br), 65), 157 (100); Found (+HRFAB) (M-^{79}Br)$^+$, 814.6289. $C_{47}H_{93}BrO_4N$ requires 814.6288.

2,3-Di-((9*Z*)-octadecenyloxy)propyl-*N*-(2-{2-[2-(2-bromoethoxy)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: $CHCl_3$ to 20% MeOH in $CHCl_3$) to yield **33** as a brown oil (80 mg, 22%). ν_{max} (film)/cm^{-1} 2927, 2853, 1656, 1464; δ_H (300 MHz; $CDCl_3$) 0.85 (6H, t, *J* 6.6, 2 × CH_2CH_3), 1.25 (44H, m), 1.54 (4H, m, 2 × $OCH_2CH_2CH_2$), 1.97 (8H, m, 2 × $CH_2CH{=}CHCH_2$), 3.41 (4H, m, $CH_2OCH_2CH_2$, $CHOCH_2$), 3.44–4.10 (27H, m, 6 × CH_2O (PEG), $CHOCH_2$, $CH_2OC_{18}H_{35}$, 2 × N^+CH_2, CH_2Br, 2 × N^+CH_3), 5.32 (4H, m, 2 × $CH{=}CH$); δ_C (75 MHz; $CDCl_3$) 14.1 (2 × CH_2CH_3), 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^+CH_3), 65.0, 65.2, 68.5, 69.3, 70.3–70.5 (signal overlap), 71.2, 72.0, 73.5 ($CHOCH_2$), 129.7 (2 × $CH{=}CH$), 130.0 (2 × $CH{=}CH$); *m/z* (+ES) 860.6 ([M-Br]$^+$ (^{81}Br), 100%), 858.6 ([M-Br]$^+$ (^{79}Br), 84), 157 (100);

2,3-Di-((9*Z*)-octadecenyloxy)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: CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to yield ***35*** as a colourless oil (120 mg, 87%). ν_{max} (film)/cm^{-1} 2924, 2853, 1634, 1464; δ_H (500 MHz; $CDCl_3$) 0.84 (6H, t, *J* 6.9, 2 × CH_2CH_3), 1.25 (48H, m), 1.49 (4H, m, 2 × $OCH_2CH_2CH_2$), 1.80 (2H, m), 1.91 (2H, m), 1.96 (8H, m, 2 × $CH_2CH{=}CHCH_2$), 3.40–3.90 (18H, m, $CH_2OC_{18}H_{35}$, $CH_2OCH_2CH_2$, $CHOCH_2$, 2 × N^+CH_2, CH_2Br, 2 × N^+CH_3), 4.01 (1H, m, $CHOCH_2$), 5.30 (4H, m, 2 × $CH{=}CH$); δ_C (75 MHz; $CDCl_3$) 13.9 (2 × CH_2CH_3), 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^+CH_3), 64.8, 65.8, 68.1, 69.1, 71.8, 73.2 ($CHOCH_2$), 129.4 (2 × $CH{=}CH$), 129.5 (2 × $CH{=}CH$); *m/z* (+ES) 784.7 ([M-Br]$^+$ (^{81}Br), 100%), 782.7 ([M-Br]$^+$ (^{79}Br), 98); Found

(+HRFAB) (M-^{79}Br)$^+$, 782.6338. $C_{47}H_{93}BrO_2N$ requires 782.6389.

2,3-Di-((9*Z*)-octadecenyloxy)propyl-*N*-(3-bromodecyl)-*N*,*N*-dimethylammonium bromide (36). 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: CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to yield ***36*** as a pale yellow oil (133 mg, 46%). ν_{max} (film)/cm^{-1} 3005, 2924, 2853, 1466; δ_H (300 MHz; $CDCl_3$) 0.86 (6H, t, *J* 6.7, 2 × CH_2CH_3), 1.27 (54H, m), 1.38 (4H, m), 1.54 (4H, m, 2 × $OCH_2CH_2CH_2$), 1.83 (2H, m), 1.98 (8H, m, 2 × CH_2CH=$CHCH_2$), 3.36–3.94 (18H, m, $CH_2OC_{18}H_{35}$, $CH_2OCH_2CH_2$, $CHOCH_2$, 2 × N^+CH_2, CH_2Br, 2 × N^+CH_3), 4.02 (1H, m, $CHOCH_2$), 5.32 (4H, m, 2 × C*H*=C*H*); δ_C (75 MHz; $CDCl_3$) 13.9 (2 × CH_2CH_3), 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^+CH_3), 64.5, 66.0, 68.2, 69.1, 71.8, 73.2 (*C*HOCH$_2$), 129.4 (2 × CH=*C*H), 129.6 (2 × *C*H=CH); *m/z* (+ES) 841 ([M-Br]$^+$ (^{81}Br), 100%), 839 ([M-Br]$^+$ (^{79}Br), 88); Found (+HRFAB) (M-^{79}Br)$^+$, 838.7010. $C_{51}H_{101}O_2NBr$ requires 838.7010.

2,3-Di-((9*Z*)-octadecenyloxy)propyl-*N*-{2-[2-(2-(*N*,*N*,*N*-trimethylammonium)ethoxy)ethoxy]ethyl}-*N*,*N*-dimethylammonium dibromide (38). Bromide **32** (50 mg, 0.056 mmol) and trimethylamine (45 wt% in H_2O; 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%). ν_{max} ($CHCl_3$)/cm^{-1} 2934, 2856, 1466; δ_H (500 MHz; $CDCl_3$) 0.86 (6H, t, *J* 6.9, 2 × CH_2CH_3), 1.25 (44H, m), 1.52 (4H, m, 2 × $OCH_2CH_2CH_2$), 1.99 (8H, m, 2 × CH_2CH=$CHCH_2$), 3.41 (4H, m, $CH_2OCH_2CH_2$, $CHOCH_2$), 3.44–4.07 (32H, m, 4 × CH_2O (PEG), $CH_2OC_{18}H_{35}$, $CHOCH_2$, 3 × N^+CH_2, 5 × N^+CH_3), 5.32 (4H, m, 2 × C*H*=C*H*); δ_C (125 MHz; $CDCl_3$) 14.1 (2 × CH_2CH_3), 22.6, 26.0, 26.2, 27.2 (2 × CH_2C=C), 29.3–29.7 (signal overlap), 30.0, 31.9, 32.6 (2 × C=CCH_2), 53.4 (N^+CH_3), 53.5 (N^+CH_3), 54.8 (3 × N^+CH_3), 65.0, 65.1, 65.2, 65.7, 66.5, 68.6, 69.4, 70.4, 70.5, 72.0, 73.3 (*C*HOCH$_2$), 129.8 (2 × CH=*C*H), 130.0 (2 × *C*H=CH); *m/z* (+ES) 397 (½[M-2Br]$^+$, 100%); Found (+HRFAB) ½(M-2Br)$^+$, 397.3917. ½($C_{50}H_{102}BrN_2O_4$) requires 397.3919.

2,3-Di-((9*Z*)-octadecenyloxy)propyl-*N*-(2-{2-[2-(2-(*N*,*N*,*N*-trimethylammonium)ethoxy)ethoxy]ethoxy}ethyl)-*N*,*N*-dimethylammonium dibromide (39). Bromide 33 (30 mg, 0.032 mmol) and trimethylamine (45 wt% in H_2O; 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%). ν_{max} ($CHCl_3$)/cm^{-1} 2934, 2856, 1466; δ_H (500 MHz; $CDCl_3$) 0.85 (6H, t, *J* 6.9, 2 × CH_2CH_3), 1.24 (44H, m), 1.52 (4H, m, 2 × $OCH_2CH_2CH_2$), 1.97 (8H, m, 2 × CH_2CH=$CHCH_2$), 3.40 (4H, m, $CH_2OCH_2CH_2$, $CHOCH_2$), 3.47 (3H, s, N^+CH_3), 3.50 (3H, s, N^+CH_3), 3.52 (9H, s, 3 × N^+CH_3), 3.53–4.05 (21H, m, 6 × CH_2O (PEG), $CH_2OC_{18}H_{35}$, $CHOCH_2$, 3 × N^+CH_2), 5.32 (4H, m, 2 × C*H*=C*H*); δ_C (125 MHz; $CDCl_3$)

14.1 (2 × CH_2CH_3), 22.7, 26.0, 26.2, 27.2 (2 × CH_2C=C), 29.2, 29.3–29.8 (signal overlap), 30.0, 31.9, 32.6 (2 × C=CCH_2), 53.2 (N^+CH_3), 53.4 (N^+CH_3), 54.7 (3 × N^+CH_3), 64.9, 65.1, 65.2, 65.4, 66.5, 68.6, 69.3, 70.2, 70.4, 70.7, 72.0, 73.3 ($CHOCH_2$), 129.7 (2 × CH=CH), 130.0 (2 × CH=CH); *m/z* (+ES) 419.6 (½[M-2Br]$^+$, 100%).

2,3-Di-((9*Z*)-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 H_2O; 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%). ν_{max} ($CHCl_3$)/cm^{-1} 2934, 2856, 1466; δ_H (300 MHz; $CDCl_3$) 0.84 (6H, t, *J* 6.7, 2 × CH_2CH_3), 1.26 (48H, m), 1.56 (4H, m, 2 × OCH_2CH_2), 1.68 (4H, m, 2 × $N^+CH_2CH_2$), 2.00 (8H, m, 2 × CH_2CH=CHCH_2), 3.39–4.02 (27H, m, $CH_2OC_{18}H_{35}$, $CH_2OCH_2CH_2$, $CHOCH_2$, 3 × N^+CH_2, 5 × N^+CH_3), 4.11 (1H, m, $CHOCH_2$), 5.33 (4H, m, 2 × CH=CH); δ_C (75 MHz; $CDCl_3$) 14.1 (2 × CH_2CH_3), 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^+CH_3), 52.5 (N^+CH_3), 53.7 (3 × N^+CH_3), 65.1 (signal overlap), 66.5, 68.6, 69.3, 72.0, 73.3 ($CHOCH_2$), 129.7 (2 × CH=CH), 129.8 (2 × CH=CH); *m/z* (+ES) 381.6 (½[M-2Br]$^+$, 100%).

2,3-Di-((9*Z*)-octadecenyloxy)propyl-*N*-3-(*N*,*N*,*N*-trimethylammoniumdecyl)-*N*,*N*-dimethylammonium dibromide (42).

Bromide **36** (66 mg, 0.072 mmol) and trimethylamine (45 wt% in H_2O; 0.22 mL, 1.44 mmol) were stirred in methanol (2 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 ***42*** (54 mg, 77%). ν_{max} ($CHCl_3$)/cm^{-1} 2921, 2853, 1462; δ_H (300 MHz; $CDCl_3$) 0.85 (6H, t, *J* 6.9, 2 × CH_2CH_3), 1.28 (52H, m), 1.40 (4H, m), 1.55 (4H, m, 2 × OCH_2CH_2), 1.78 (4H, m, 2 × $N^+CH_2CH_2$), 2.01 (8H, m, 2 × CH_2CH=CHCH_2), 3.40 (3H, s, N^+CH_3), 3.44 (3H, s, N^+CH_3), 3.46–4.02 (21H, m, $CH_2OC_{18}H_{35}$, $CH_2OCH_2CH_2$, $CHOCH_2$, 3 × N^+CH_2, 3 × N^+CH_3), 4.10 (1H, m, $CHOCH_2$), 5.33 (4H, m, 2 × CH=CH); δ_C (75 MHz; $CDCl_3$) 14.1 (2 × CH_2CH_3), 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^+CH_3), 52.5 (N^+CH_3), 53.5 (3 × N^+CH_3), 65.1, 66.3, 66.5, 68.6, 69.3, 72.0, 73.3 ($CHOCH_2$), 129.7 (2 × CH=CH), 129.9 (2 × CH=CH); Found C, 63.58; H, 11.07; N, 2.75. $C_{54}H_{110}N_2O_2$.2Br.2H_2O 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 ºC, 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).[12] 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 1×10^4 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 1: Transfection Data for Compounds 37–42 compared to Lipofectin (best formulation ratio 4:1 data presented)

Compound	Transfection relative to Lipofectin™
†Lipofectin	100%
†**37** + DOPE	7%
†**38** + DOPE	3%
†**39** + DOPE	2%
*__40__ + DOPE	46%
*__41__ + DOPE	3%
*__42__+ DOPE	2%

†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 2: Transfection Data for Compounds 15, 16, 17a (+ DOPE), Lipofectin (1HAEo-cells, 2:1 lipid:DNA ratio)

Compound	Transfection relative to 17a + DOPE
†Lipofectin	16%
†**15** + DOPE	41%
†**16** + DOPE	46%

†**17a** + DOPE	100%

†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)

Compound	Transfection relative to 17a	
	Peptide 3 (Peptide:DNA wt ratio, 1:1)	**Peptide 3 (Peptide:DNA wt ratio, 4:1)**
†DOTMA (L:D, 2:1)	16%	22%
DOTMA (L:D, 4:1)	86%	98%
Lipofectin (L:D, 2:1)	11%	5%
†Lipofectin (L:D, 4:1)	54%	66%
†**17a** + DOPE (L:D, 2:1)	41%	28%
†**17a** + DOPE (L:D, 4:1)	79%	100%

†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)

Compound	Transfection relative to Lipofectin (2:1, lipid/DNA)
†Lipofectin (2:1)	100%
†**23** + DOPE (2:1)	25%

†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

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*[12].

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

Compound	Transfection relative to 23 + DOPE (2:1, lipid/DNA)
†**21** + DOPE (2:1)	28%
†**22** + DOPE (2:1)	97%
†**23** + DOPE (2:1)	100%
†**24** + DOPE (2:1)	88%

†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.

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