Supporting Information

The Importance of Lipid Geometry on Lipid Assembly in Lipoplexes

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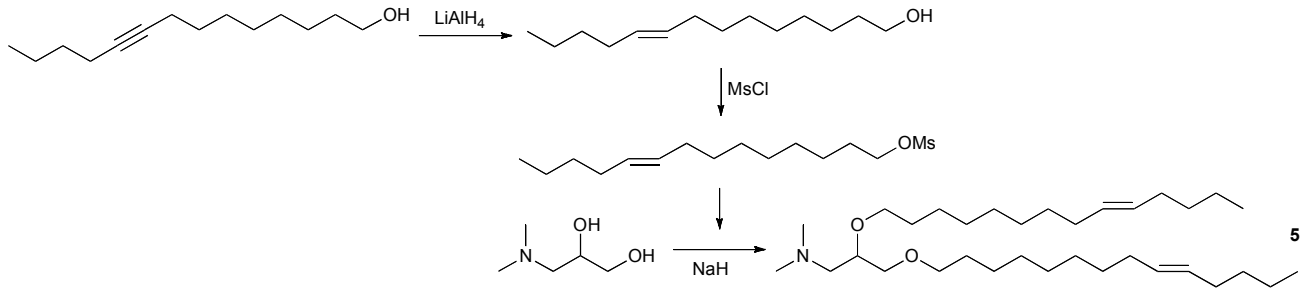
General Experimental Methods

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 for normal phase TLC, with detection by UV, or permanganate, and phosphomolybdic acid stains. Flash column chromatography was carried out using silica gel (particle size 40-63 μm) purchased from Sigma-Aldrich Co. Ltd.

Melting points are uncorrected. IR spectra were recorded using a FT-IR Shimidasu 8700 instrument. Only selected peaks were reported (cm^{-1}). ^1H NMR and ^{13}C spectra were recorded on Bruker® AMX300 MHz, AVANCE500 MHz machines in $CDCl_3$. Unless otherwise specified, NMR spectra were recorded at 298 K. Mass spectra (+ES) were recorded on a Micromass Quatto LC spectrospray (+ES), a UG70FE (FAB), and a MAT 900XP spectrometer (+HRFAB and +HRES).

Tertiary amines **3**, **4**, **6** were prepared as previously described.[1,2] The iodide salt of **11** was prepared as previously described.[2,3] 2-Dodec-11-ynyloxy-tetrahydro-2*H*-pyran was synthesised as reported in the literature.[4,5]



Scheme 1. Route to **5**

(*E*)-9-Tetradecen-1-ol

9-Tetradecyn-1-ol[1] (2.30 g, 11.0 mmol) in diglyme (5 mL) was added dropwise to a stirring solution of lithium aluminium hydride[6] (1.45 g, 38.3 mmol) in anhydrous diglyme (45 mL) at –10 °C. The mixture was warmed to rt and heated at reflux for 72 h. The reaction mixture was quenched by the addition of ice (5 g), and neutralised with 1 M HCl. Diethyl ether (20 mL) and water (40 mL) were added to the mixture, and the organic phase was extracted with diethyl ether (3 × 30 mL). The combined organic extracts were washed with brine (100 mL), dried ($MgSO_4$) and evaporated *in vacuo*. Purification by

flash silica chromatography (40% Et_2O in hexane) gave *(E)-9-tetradecen-1-ol* (1.39 g, 60%) as a pale yellow oil. R_f = 0.45 (40% Et_2O in hexane); ν_{max} (film)/cm^{-1} 3333, 2934, 1458; δ_H (300 MHz; $CDCl_3$) 0.86 (3H, m, C*H*$_3$), 1.29 (14H, m, 7 × CH_2), 1.60 (3H, m, C*H*$_2$$CH_2$O*H*), 1.96 (4H, m, *H*$_2$CC=CHC*H*$_2$), 3.61 (2H, t, *J* 6.6 Hz, C*H*$_2$OH), 5.37 (2H, m, C*H*$_2$=C*H*$_2$); δ_C (75 MHz; $CDCl_3$) 13.9 (*C*H_3), 22.2, 25.7, 29.1, 29.3, 29.4, 29.6, 31.8, 32.2, 32.6, 32.8, 63.0 (*C*H_2OH), 130.2 (*C*=C), 130.3 (C=*C*); *m/z* (+ES) 213 (MH^+, 100%).

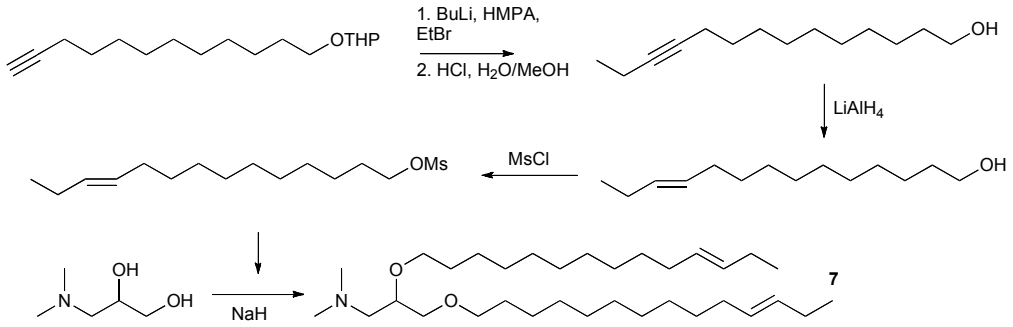
(*E*)-*9*-Tetradecen-1-yloxy mesylate

(*E*)-9-Tetradecen-1-ol (1.39 g, 6.56 mmol) and methanesulfonyl chloride (0.61 mL, 7.87 mmol) in anhydrous dichloromethane were stirred at rt for 15 min. The mixture was cooled to 0 °C and triethylamine (1.09 mL, 7.87 mmol) added dropwise. The reaction mixture was warmed to rt and stirred for 18 h, then the organic layer extracted with dichloromethane (50 mL) and washed with saturated sodium hydrogencarbonate solution (50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$) and the solvent removed *in vacuo* to yield the titled compound (1.81 g, 95%) as a pale yellow oil. R_f = 0.60 (CH_2Cl_2); δ_H (300 MHz; $CDCl_3$) 0.90 (3H, m, CH_2C*H*$_3$) 1.31 (14H, m, 7 × CH_2), 1.74 (2H, br quint *J* 5.6 Hz, OCH_2C*H*$_2$), 1.96 (4H, m, C*H*$_2$CH=CHC*H*$_2$), 2.99 (3H, s, C*H*$_3$S), 4.21 (2H, t, *J* 6.6 Hz, C*H*$_2$O), 5.38 (2H, m, C*H*=C*H*); δ_C (75 MHz; $CDCl_3$) 14.0 (CH_2*C*H_3), 22.2, 25.4, 29.0, 29.1, 29.3 (signal overlap), 29.6, 31.8, 32.3, 32.6, 37.4 (*C*H_3S), 70.2 (C*H*$_2$O), 130.2 (*C*=C), 130.4 (C=*C*); *m/z* (+ES) 329 (MK^+, 25%), 313 (MNa^+, 100).

2,3-Di-((9*E*)-tetradecenyloxy)propyl-*N,N*-dimethylamine (5)

To a stirring solution of sodium hydride (60% in mineral oil; 0.27 g, 6.72 mmol) in anhydrous toluene (35 mL) at rt was added 3-dimethylaminopropane-1,2-diol (0.27 mL, 2.24 mmol). The mixture was heated at 50 ºC for 20 min, and (*E*)-tetradec-9-enyl mesylate (1.95 g, 6.72 mmol) was added. The reaction was then heated at reflux for 72 h. On cooling, water (100 mL) was added and the product extracted with ethyl acetate (3 × 50 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 flash chromatography (5% MeOH in CH_2Cl_2) to afford *5* (0.710 g, 63%) as a pale yellow oil. R_f = 0.36 (5% MeOH in CH_2Cl_2); ν_{max} (film)/cm^{-1} 2926, 2854, 1660, 1460; δ_H (300 MHz; $CDCl_3$) 0.88 (6H, m, 2 × CH_2C*H*$_3$), 1.29 (28H, m), 1.54 (4H, m, 2 × C*H*$_2$$CH_2$OH), 1.95 (8H, m, 2 × *H*$_2$CC=CHC*H*$_2$), 2.28 (6H, s, N(C*H*$_3$)$_2$), 2.41 (2H, m, NC*H*$_2$CH), 3.39–3.61 (7H, m, C*H*OC*H*$_2$, C*H*$_2$OC*H*$_2$), 5.38 (4H, m, C*H*$_2$=C*H*$_2$); δ_H (75 MHz; $CDCl_3$) 14.0 (CH_2*C*H_3), 22.2, 26.1, 29.1, 29.5, 29.7 (signals superimposed), 30.2, 31.8, 32.3, 32.6, 46.3 (N(*C*H_3)$_2$), 61.1 (N*C*H_2CH), 70.2 (CH*C*H_2O), 71.6 and 72.0 (O*C*$H_2$$CH_2$), 73.5

(*C*HOCH$_2$), 130.3 (*C*=*C*); *m/z* (+ES) 509 (MH$^+$, 100%), 481 (13); Found (+HRES) MH$^+$ 508.50896. $C_{33}H_{66}NO_2$ requires 508.50936.



Scheme 2. Route to **7**

11-Tetradecyn-1-ol

This was prepared via modification of reported routes as described below. *n*-Butyllithium (2.5 M solution in hexane; 7.82 mL, 19.6 mmol) was added dropwise over 2 min to a stirred solution of 2-dodec-11-ynyloxy-tetrahydro-2*H*-pyran[4,5] (4.35 g, 16.3 mmol) at –10 °C in anhydrous THF (40 mL) and hexamethylphosphoramide (HMPA) (10 mL). The reaction was stirre at –10 °C and for 2 h, followed by the addition of bromoethane (1.83 mL, 24.5 mmol). The reaction mixture was warmed to rt and then stirred for a further 48 h. Water (150 mL) and saturated ammonium chloride solution (150 mL) was added to the mixture and the organic layer extracted with dichloromethane (3 × 150 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent evaporated *in vacuo*. The crude product was purified by flash silica chromatography (5% EtOAc in hexane) to yield 2-(tetradec-11-ynyl-1-oxy)tetrahydro-2*H*-pyran (3.89 g, 83%) as a clear yellow oil. R_f = 0.25 (1: 19 EtOAc in hexane); ν_{max} (film)/cm^{-1} 2932, 2855, 2365; δ_H (300 MHz; $CDCl_3$) 1.49 (3H, t, *J* 6.9 Hz, C*H*$_3$), 1.56–1.30 (22H, m), 2.13 (4H, m, C*H*$_2$C≡CC*H*$_2$), 3.35 (1H, m, THPOC*H*H), 3.47 (1H, m, OCHOC*H*H), 3.75 (1H, m, THPOC*H*H), 3.85 (1H, m, OCHOC*H*H), 4.56 (1H, m, OC*H*OCH$_2$); δ_H (75 MHz; $CDCl_3$) 14.4 (*C*H$_3$), 18.7, 19.7, 22.6, 25.5, 26.2, 28.8, 29.1, 29.4, 29.5, 29.7, 30.7, 30.8, 62.3 (O*C*HOCH$_2$), 67.7 (THPO*C*H$_2$), 79.5 (*C*≡C), 81.5 (C≡*C*), 98.8 (O*C*HOCH$_2$); *m/z* (+ES) 317 (MNa$^+$,100%).

2-(Tetradec-11-ynyl-1-oxy)tetrahydro-2*H*-pyran (1.85 g, 6.29 mmol) was added a solution of concentrated HCl/water/methanol (91 mL; 1:1:5). The reaction mixture was stirred for 18 h at rt, followed by neutralisation with 10 M NaOH aqueous solution. The mixture was concentrated *in vacuo*, and water (100 mL) was added. The product was extracted with diethyl ether (3 × 70 mL), the combined organic extracts were dried ($MgSO_4$) and the solvent was removed *in vacuo*. Purification by flash silica

chromatography (40% Et_2O in hexane) gave 11-tetradecyn-1-ol[7] (0.93 g, 70%) as a colourless oil. R_f = 0.45 (40% Et_2O in hexane); ν_{max} (film)/cm^{-1} 3333, 2924, 2855, 2361, 1458; δ_H (300 MHz; $CDCl_3$) 1.11 (3H, t, *J* 7.3 Hz, C*H*$_3$), 1.29–1.65 (16H, m), 2.15 (4H, m, *H*$_2$CC≡CHC*H*$_2$), 3.62 (2H, t, *J* 6.6 Hz, C*H*$_2$OH); δ_H (75 MHz; $CDCl_3$) 12.4, 14.4, 18.7, 25.7, 28.8, 29.1, 29.3 (signal overlap), 29.4, 29.5, 32.8, 63.1 (*C*H$_2$OH), 79.6 (*C*≡C), 81.6 (C≡*C*); *m/z* (+ES) 233 (MNa^+,10%).

(*E*)-11-Tetradecen-1-ol

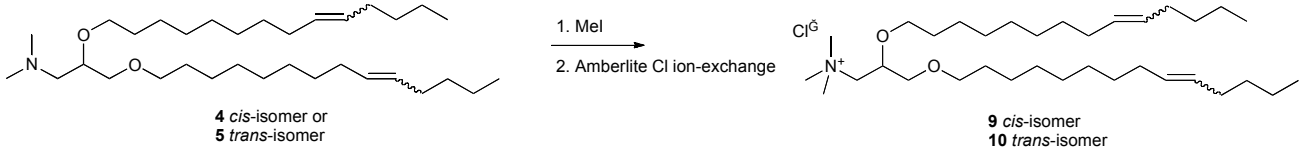
11-Tetradecyn-1-ol (0.85 g, 4.05 mmol) in diglyme (5 mL) was added dropwise to a stirring solution of lithium aluminium hydride (0.54 g, 14.2 mmol; 3.5 eq) in anhydrous diglyme (5 mL) at -10 °C. The mixture was warmed to rt and heated at reflux for 72 h. The reaction mixture was quenched by the addition of ice (5 g), and neutralised with 1 M HCl. Diethyl ether (20 mL) and water (40 mL) was added to the mixture, and the organic phase was extracted with diethyl ether (3 × 30 mL). The combined organic extracts were washed with brine (100 mL), dried ($MgSO_4$) and evaporated *in vacuo*. Purification by flash silica chromatography (40% Et_2O in hexane) (*E*)-11-tetradecen-1-ol (0.42 g, 49%) as a pale yellow oil. R_f = 0.45 (2:3, Et_2O: hexane); ν_{max} (film)/cm^{-1} 3333, 2934, 2855, 1458, 1057; δ_H (300 MHz; $CDCl_3$) 0.95 (3H, t, *J* 7.5 Hz, CH_3), 1.28–1.40 (14H, m), 1.56 (2H, m, CH_2CH_2OH), 1.97 (4H, m, *H*$_2$CC=CHC*H*$_2$), 3.62 (2H, t, *J* 6.6 Hz, C*H*$_2$OH), 5.39 (2H, m, C*H*=C*H*); δ_C (75 MHz; $CDCl_3$) 14.0 (*C*H_3), 25.6, 25.7, 26.1, 29.2, 29.4, 29.5, 29.6 (signals superimposed), 29.7, 32.6, 32.8, 63.0 (*C*H_2OH), 129.4 and 131.9 (*C*=*C*); *m/z* (+ES) ; *m/z* (+ES) 213 (MH^+, 100%)

(*E*)-11-Tetradecen-1-yloxy mesylate

(*E*)-11-Tetradecen-1-ol (2.35 g, 11.1 mmol) and methanesulfonyl chloride (1.02 mL, 13.3 mmol) in anhydrous dichloromethane (60 mL) were stirred at rt for 15 min. The mixture was cooled to 0 °C, and triethylamine (1.84 mL, 13.3 mmol) added dropwise. The reaction mixture was warmed to rt and stirred for 18 h. The organic layer was extracted with dichloromethane (50 mL) and washed with saturated sodium hydrogencarbonate solution (50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$) and the solvent removed *in vacuo*. Purification by flash silica chromatography (CH_2Cl_2) gave the titled compound (3.01 g, 94%) as a pale yellow oil. R_f = 0.60 (CH_2Cl_2); δ_H (300 MHz; $CDCl_3$) 0.95 (3H, t, *J* 7.5 Hz, CH_2CH_3), 1.26–1.43 (14H, m), 1.78 (2H, quin, *J* 6.6 Hz, OCH_2CH_2), 1.97 (4H, m, C*H*$_2$CH=CHC*H*$_2$), 2.99 (3H, s, C*H*$_3$SO$_2$), 4.21 (2H, t, *J* 6.6 Hz, C*H*$_2$O), 5.39 (2H, m, C*H*=C*H*); δ_C (75 MHz; $CDCl_3$) 14.0 (CH_2CH_3), 25.4, 25.6, 29.0, 29.1, 29.4, 29.5, 29.6 (signal overlap), 30.9, 32.6, 37.3 (*C*H$_3$SO$_2$), 70.2 (C*H*$_2$O), 129.3 (*C*=C), 131.9 (C=*C*); *m/z* (+ES) 313 (MNa^+, 100%).

2,3-Di-((11*E*)-tetradecenyloxy)propyl-*N*,*N*-dimethylamine (7)

To a stirring solution of sodium hydride (60% in mineral oil; 0.34 g, 8.41 mmol) in anhydrous toluene (40 mL) at rt was added 3-dimethylaminopropane-1,2-diol (0.33 mL, 2.80 mmol). The mixture was heated at 50 ºC for 20 min, and (*E*)-11-tetradecen-1-yloxy mesylate (2.44 g, 8.41 mmol) was added. The reaction was then heated at reflux for 72 h. On cooling, water (100 mL) was added and the product extracted with ethyl acetate (3 × 50 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 flash chromatography (5% MeOH in CH_2Cl_2) to afford ***7*** (0.720 g, 51%) as a pale yellow oil. R_f = 0.36 (5% MeOH: CH_2Cl_2); ν_{max} (film)/cm^{-1} 2923, 2853, 1645, 1460; δ_H (300 MHz; $CDCl_3$) 0.94 (6H, t, *J* 7.5 Hz, 2 × CH_2CH_3), 1.26 (28H, m), 1.57 (4H, quin, *J* 6.6 Hz, 2 × CH_2CH_2O), 2.00 (8H, m, 2 × $H_2CC{=}CHCH_2$), 2.28 (6H, s, $N(CH_3)_2$), 2.39 (2H, m, NCH_2CH), 3.40–3.61 (7H, m, $CHOCH_2$, CH_2OCH_2), 5.33 (4H, m, $CH_2{=}CH_2$); δ_H (75 MHz; $CDCl_3$) 14.0 (CH_2CH_3), 25.6, 26.2, 29.2, 29.5 (signals superimposed), 29.6, 29.7, 30.2, 32.6, 46.3 ($N(CH_3)_2$), 61.1 (NCH_2CH), 70.2 ($CHCH_2O$), 71.6 and 72.0 (OCH_2CH_2), 73.6 ($CHOCH_2$), 129.3 (*C*=C), 131.8 (C=*C*); *m/z* (+ES) 509 (MH^+, 100%), 483 (7); Found (+HRES) MH^+ 508.50938. $C_{33}H_{66}NO_2$ requires 508.50936.



Scheme 3. Routes to lipids **9** and **10**

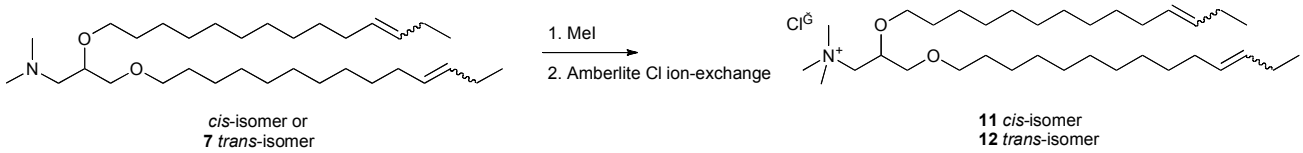
2,3-Di-((9*Z*)-tetradecenyloxy)propyl-*N,N,N*-trimethylammonium chloride (9)

2,3-Di-((9*Z*)-tetradecenyloxy)propyl-*N,N*-dimethylamine[1] (**4**) (0.560 g, 1.10 mmol) and iodomethane (0.69 mL, 11.1 mmol) were stirred in a sealed tube in the dark for 18 h at rt. Excess iodomethane was removed *in vacuo* and the product purified by flash silica chromatography (5% MeOH in CH_2Cl_2) to give the iodide salt (0.670 g, 93%) as a yellow solid. The iodide salt (131 mg, 0.20 mmol) was then dissolved in CH_2Cl_2 and MeOH (1:1, 10 mL) and the resulting solution was stirred with Amberlite® IRA-400 (Cl) ion-exchange resin (0.5 g) for 18 h at rt. The mixture was filtered, and the filtrate concentrated *in vacuo* to give **9** (105 mg, 94%) as a yellow oil. ν_{max} (film)/cm^{-1} 2926, 2855, 1643, 1466; δ_H (300 MHz; $CDCl_3$) 0.88 (6H, m, 2 × CH_2CH_3), 1.28 (28H, m), 1.53 (4H, m, 2 × CH_2CH_2O), 1.98 (8H, m, 2 x $H_2CC{=}CHCH_2$), 3.40 (4H, t, *J* 6.7 Hz, 2 x CH_2CH_2O), 3.46 (9H, s, $N^+(CH_3)_3$), 3.53–3.72 (3H, m), 4.06 (2H, m), 5.34 (4H, m, $CH_2{=}CH_2$); δ_C (75 MHz; $CDCl_3$) 13.9 (CH_2CH_3), 22.3, 26.0, 26.2, 26.9, 27.2, 29.3, 29.4 (signal overlap), 29.7, 30.0, 31.9, 54.9 ($N^+(CH_3)_3$), 68.4, 69.3, 72.0

($OCH_2C_{13}H_{25}$), 73.6 ($CHOCH_2$), 129.8 and 129.9 (C=C); *m/z* (+ES) 523 ($[MH\text{-}Cl]^+$, 100%); Found (+HREI) $[M\text{-}Cl]^+$ 522.52406. $C_{34}H_{68}NO_2$ requires 522.52446.

2,3-Di-((9*E*)-tetradecenyloxy)propyl-*N,N,N*-trimethylammonium chloride (10)

2,3-Di-((9*E*)-tetradecenyloxy)propyl-*N,N*-dimethylamine (**5**) (0.63 g, 1.24 mmol) and iodomethane (0.77 mL, 12.4 mmol) were stirred in a sealed tube in the dark for 18 h at rt. Excess iodomethane was removed *in vacuo* and the product purified by flash silica chromatography (5% MeOH: CH_2Cl_2) to give the iodide salt (0.76 g, 94%) as a yellow solid. The iodide salt (104 mg, 0.16 mmol) was dissolved in CH_2Cl_2 and MeOH (1:1, 10 mL) and the resulting solution stirred with Amberlite® IRA-400 (Cl) ion-exchange resin (0.5 g) for 18 h at rt. The mixture was filtered, and the filtrate concentrated *in vacuo* to give **10** (83 mg, 93%) as a yellow oil. R_f = 0.32 (5% MeOH: CH_2Cl_2); ν_{max} (film)/cm^{-1} 2924, 2852, 1634, 1466; δ_H (300 MHz; $CDCl_3$) 0.87 (6H, m, 2 × CH_2CH_3), 1.30 (28H, m), 1.54 (4H, m, 2 × CH_2CH_2OH), 1.96 (8H, m, 2 × H_2CC=$CHCH_2$), 3.42 (4H, t, *J* 6.7 Hz, 2 × CH_2CH_2O), 3.51 (9H, s, $N^+(CH_3)_3$), 3.52–3.72 (3H, m), 4.07 (2H, m), 5.37 (4H, m, CH_2=CH_2); δ_H (75 MHz; $CDCl_3$) 14.0 (CH_2CH_3), 22.2, 26.0, 26.2, 29.1, 29.4, 29.5 (signals overlap), 30.0, 31.8, 32.3, 32.6, 54.9 ($N^+(CH_3)_3$), 68.4, 69.3, 72.0 ($OCH_2C_{13}H_{25}$), 73.6 ($CHOCH_2$), 130.2, (C=C), 130.4 (C=C); *m/z* (+ES) 523 ($[M\text{-}Cl]^+$, 100%); Found (+HREI) $[M\text{-}Cl]^+$ 522.52404. $C_{34}H_{68}NO_2$ requires 522.52446.



cis-isomer or
7 *trans*-isomer

11 *cis*-isomer
12 *trans*-isomer

Scheme 4. Routes to lipids **11** and **12**

2,3-Di-((11*Z*)-tetradecenyloxy)propyl-*N,N,N*-trimethylammonium chloride (11)

2,3-Di-((11*Z*)-tetradecenyloxy)propyl-*N,N,N*-trimethylammonium iodide[2] (96 mg, 0.15 mmol) was dissolved in CH_2Cl_2 and MeOH (1:1, 10 mL) and stirred with Amberlite® IRA-400 (Cl) ion-exchange resin (0.5 g) for 18 h at rt. The mixture was filtered, and the filtrate concentrated *in vacuo* to give **11** (78 mg, 94%) as a yellow oil. ν_{max} (film)/cm^{-1} 2922, 2853, 1645, 1466; δ_H (300 MHz; $CDCl_3$) 0.95 (6H, t, *J* 7.5 Hz, 2 × CH_2CH_3), 1.26 (28H, m), 1.55 (4H, m, 2 × CH_2CH_2O), 2.02 (8H, m, 2 × H_2CC=$CHCH_2$), 3.42 (4H, t, *J* 6.6 Hz, 2 × CH_2CH_2O), 3.49 (9H, s, $N^+(CH_3)_3$), 3.54–3.71 (3H, m), 4.07 (2H, m), 5.32 (4H, m, CH_2=CH_2); δ_H (75 MHz; $CDCl_3$) 14.3 (CH_2CH_3), 20.4, 25.9, 26.1, 26.9, 29.2, 29.3, 29.5 (signals overlap), 29.9, 54.6 ($N^+(CH_3)_3$), 67.7, 68.4, 69.2, 71.9 ($OCH_2C_{13}H_{25}$), 73.5 ($CHOCH_2$), 129.2 (C=C), 131.4 (C=C); Found (+HREI) $[M\text{-}Cl]^+$ 522.52484. $C_{34}H_{68}NO_2$ requires 522.52446.

2,3-Di-((11*E*)-tetradecenyloxy)propyl-*N,N,N*-trimethylammonium chloride (12)

2,3-Di-((11*E*)-tetradecenyloxy)propyl-*N,N*-dimethylamine (**7**) (0.420 g, 0.826 mmol) and iodomethane (0.52 mL, 8.35 mmol) were stirred in a sealed tube in the dark for 18 h at rt. Excess iodomethane was removed *in vacuo* and the product purified by flash silica chromatography (5% MeOH in CH_2Cl_2) to give the iodide salt (0.500 g, 93%) as a yellow solid. The iodide salt (124 mg, 0.191 mmol) was then dissolved in CH_2Cl_2 and MeOH (1:1, 10 mL) and the resulting solution stirred with Amberlite® IRA-400 (Cl) ion-exchange resin (0.5 g) for 18 h at rt. The mixture was filtered, and the filtrate concentrated *in vacuo* to give **12** (100 mg, 94%) as a yellow oil. R_f = 0.32 (5% MeOH in CH_2Cl_2); ν_{max} (film)/cm^{-1} 2919, 2851, 1640, 1466; δ_H (300 MHz; $CDCl_3$) 0.92 (6H, t, *J* 7.4 Hz, 2 × CH_2CH_3), 1.22 (28H, m), 1.52 (4H, m, 2 × CH_2CH_2O), 1.96 (8H, m, 2 × H_2CC=$CHCH_2$), 3.38 (4H, t, *J* 6.7 Hz, 2 × CH_2CH_2O), 3.43 (9H, s, $N^+(CH_3)_3$), 3.46–3.65 (3H, m), 3.91–4.04 (2H, m), 5.37 (4H, m, CH_2=CH_2); δ_H (75 MHz; $CDCl_3$) 14.0 (CH_2CH_3), 25.6, 26.0, 26.2, 29.2, 29.4 (signal overlap), 29.6, 30.0, 32.6, 54.8 ($N^+(CH_3)_3$), 67.8, 68.5, 69.3, 72.0 ($OCH_2C_{13}H_{25}$), 73.6 ($CHOCH_2$), 129.3 (*C*=C), 131.8 (C=*C*); Found (+HREI) $[M-Cl]^+$ 522.52491. $C_{34}H_{68}NO_2$ requires 522.52446.

Tables

Table 1 The z-average diameter (nm), polydispersity index value and zeta potential (mV) measured at 298 K for the cationic vesicles formed by the C14 DOTMA analogues (chloride salt) after 2 minutes probe sonication. The standard deviation values from 10 repeat measurements on a single vesicle preparation are shown in brackets.

Lipids used for vesicle preparation	z-Average diameter in nm (± SD)	Polydispersity (± SD)	Zeta potential in mV (± SD)
1	135.3 (1.1)	0.258 (0.01)	21.6 (1.2)
8	71.0 (0.9)	0.286 (0.01)	39.7 (0.9)
9	101.0 (1.2)	0.268 (0.01)	41.8 (2.2)
10	63.3 (1.0)	0.235 (0.01)	52.1 (1.5)
11	104.7 (0.6)	0.299 (0.01)	55.0 (0.9)
12	94.0 (0.5)	0.275 (0.01)	37.7 (1.2)

Table 2 The z-average diameters (nm), polydispersity index values and zeta potential (mV) measured at 298 K for the cationic vesicles formed by the DOTMA analogues (iodide salt). The standard deviation values from 10 repeat measurements on a single vesicle preparation are shown in brackets.

Lipids used for vesicle preparation	z-Average diameter in nm (± SD)	Polydispersity (± SD)	Zeta potential in mV (± SD)
1	124.7 (0.9)	0.211 (0.01)	16.5 (2.6)
8	111.0 (1.0)	0.256 (0.01)	39.8 (1.6)
9	92.7 (1.3)	0.265 (0.01)	50.3 (1.0)
10	99.0 (0.7)	0.209 (0.01)	N/D
11	93.3 (0.8)	0.215 (0.01)	35.0 (1.3)
12	105.0 (1.2)	0.218 (0.01)	41.3 (1.5)

Table 3 The z-average diameters (nm) and zeta potential (mV) of the LD complexes formed from different weight ratios of cationic lipids **1** and **8** (iodide salt) and ctDNA measured at 298 K. The standard deviation values from 10 repeat measurements on a single vesicle preparation are shown in brackets

Composition of LD complex		Mean particle size (nm)	Zeta Potential (mV)
Lipid	**Lipid:DNA weight ratio**		
1	**16:1**	120.9 (2.0)	16.9 (1.2)
1	**8:1**	348.5 (4.7)	4.3 (0.5)
1	**4:1**	430.1 (6.2)	-5.1 (0.7)
8	**16:1**	115.4 (2.5)	34.5 (1.5)
8	**8:1**	260.7 (5.4)	6.3 (0.9)
8	**4:1**	359.3 (6.5)	-7.8 (0.9)

Table 4 The z-average diameters (nm), polydispersity index values and zeta potential (mV) of cationic vesicles formed by the DOTMA:DOPE analogues (iodide salt) after probe sonication for 5 minutes and measured at 298 K. The standard deviation values from 10 repeat measurements on a single vesicle preparation are shown in brackets.

Lipid	z-Average diameter in nm (± SD)	Polydispersity (± SD)	Zeta potential in mV (± SD)
1:2	173.3 (1.4)	0.241 (0.01)	29.8 (1.2)
8:2	152.3 (2.1)	0.208 (0.01)	26.1 (2.2)
9:2	N/D	N/D	N/D
10:2	131.3 (1.3)	0.295 (0.01)	N/D
11:2	114.7 (1.4)	0.288 (0.01)	N/D
12:2	155.3 (1.0)	0.277 (0.01)	N/D

Table 5 Effect of different weight ratio on particle size of lipid:DNA complexes prepared from vesicles containing 2 and pDNA. The standard deviation values from 5 repeat measurements on a single preparation are shown in brackets.

Lipids used to prepare the LD complexes	Mean particle size (nm)				
	Lipid:DNA Weight ratios (total lipid)				
	1:1	2:1	4:1	8:1	16:1
Lipofectamine	141.8 (2.4)	141.3 (2.8)	132.9 (8.8)	224.8 (4.1)	167.3 (9.0)
1:2	249.4 (23.1)	272.0 (17.8)	304.1 (16.0)	308.6 (9.7)	403.1 (12.9)
10:2	205.5 (1.1)	251.8 (2.8)	256.5 (18.8)	339.2 (6.9)	257.3 (9.5)
12:2	205.6 (4.2)	216.6 (4.5)	218.6 (3.9)	435.7 (25.7)	283.8 (11.1)
9:2	273.4 (8.8)	270.6 (8.4)	274.3 (7.3)	310.4 (8.6)	489.6 (10.5)
11:2	151.1 (8.7)	155.9 (15.1)	172.3 (2.5)	219.6 (3.9)	402.0 (4.0)
8:2	180.2 (15.8)	181.3 (7.3)	208.8 (15.5)	269.9 (31.4)	384.9 (7.9)

Table 6 Effect of different weight ratio on zeta potential of lipid:DNA complexes. prepared from vesicles containing 2 and pDNA The standard deviation values from 5 repeat measurements on a single preparation are shown in brackets.

Lipids used to prepare the LD complexes	Mean zeta potential (mV)				
	Lipid:DNA Weight ratios (total lipid)				
	1:1	2:1	4:1	8:1	16:1
Lipofectamine	-22.7 (3.0)	-10.4 (4.8)	-0.3 (0.2)	4.3 (1.5)	36.2 (4.2)
1:2	-22.0 (2.6)	-20.7 (2.1)	-16.2 (5.0)	-15.6 (3.9)	12.4 (2.3)
10:2	-16.3 (2.0)	-15.8 (1.5)	-12.5 (3.3)	-11.1 (1.3)	29.9 (2.0)
12:2	-16.0 (0.4)	-11.3 (1.9)	-10.5 (1.2)	-4.9 (1.6)	26.6 (3.4)
9:2	-18.8 (1.0)	-15.8 (1.9)	-8.5 (1.8)	-8.2 (5.1)	12.8 (1.3)
11:2	-15.1 (1.7)	-14.3 (1.1)	-9.1 (1.1)	-8.3 (1.2)	3.0 (2.0)
8:2	-16.6 (1.8)	-10.4 (1.9)	-8.5 (1.1)	-5.1 (2.6)	24.1 (2.0)

Table 7 Structural parameters obtained for LD complexes formed from cationic vesicles containing 2 and pDNA at a lipid:DNA weight ratio of 4:1 (derived from FISH modelling of their SANS data) measured at 298 K. *d*-spacing obtained from analysis of position of peak in SANS curve using the Bragg equation also shown. Figures in parenthesis indicate the standard errors on the fitted parameter values (derived from the least-squares variance-covariance matrix).

Lipids used to prepare the LD complexes	Thickness (nm)	Number of layers	*d*-spacing[1] (nm)	*d*-spacing[2] (nm)
1:2	4.54 (0.09)	56.0	6.18 (0.08)	6.2
8:2	4.12 (0.10)	56.0	5.71 (0.08)	5.7
9:2	4.33 (0.05)	56.0	5.91 (0.09)	5.9
10:2	4.47 (0.08)	56.0	6.06 (0.09)	6.1
11:2	4.39 (0.10)	56.0	6.04 (0.08)	6.0
12:2	4.71 (0.07)	56.0	6.38 (0.07)	6.4

[1] *d*-spacing obtained from FISH modelling

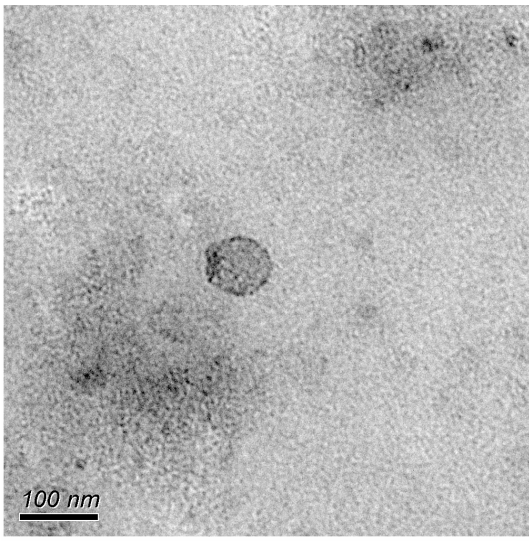
[2] *d*-spacing obtained using Bragg equation

Table 8 Structural parameters obtained for the lipoplexes formed from cationic vesicles of E11containing a 1:1 weight ratio of 2 and ctDNA prepared at a weight mixing ratio of 4:1 (derived from FISH modelling of their SANS data) measured at 298 ± 0.1 K. Figures in parenthesis indicate the standard errors on the fitted parameter values (derived from the least-squares variance-covariance matrix).

Concentration of 12:2	Thickness (nm)	Number of layers	*d*-spacing (nm)
1.0 mg/mL	4.68 (0.10)	55	6.37 (0.07)
0.5 mg/mL	4.88 (0.04)	55	6.35 (0.05)
0.25 mg/mL	4.57 (0.09)	55	6.28 (0.05)

Figures

Figure 1: Transmission electron micrograph of vesicles of **12** at a concentration of 1 mg/mL and a magnification of 150,000x.



Specimen : Sample 16
Operator : Laila
Voltage : 120 kV
Microscope Name : Tecnai 12 BioTWIN
Camera Name : MSC SI003 1
Indicated Magnification : X150000

Image Name : Frame
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Acquisition Time : 11:29:00 AM
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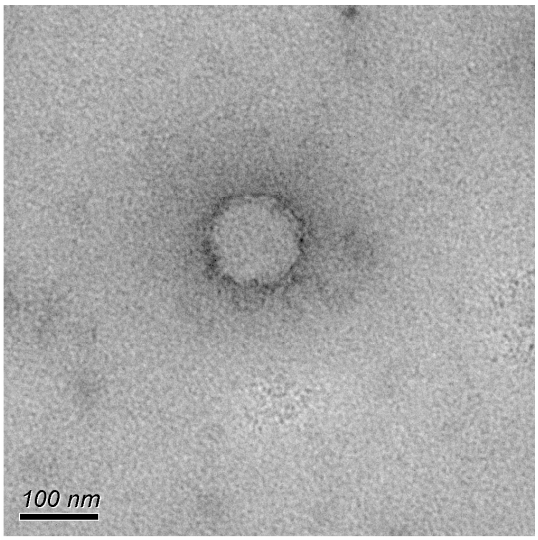

Specimen : Sample 16
Operator : Laila
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Indicated Magnification : X150000

Image Name : Frame
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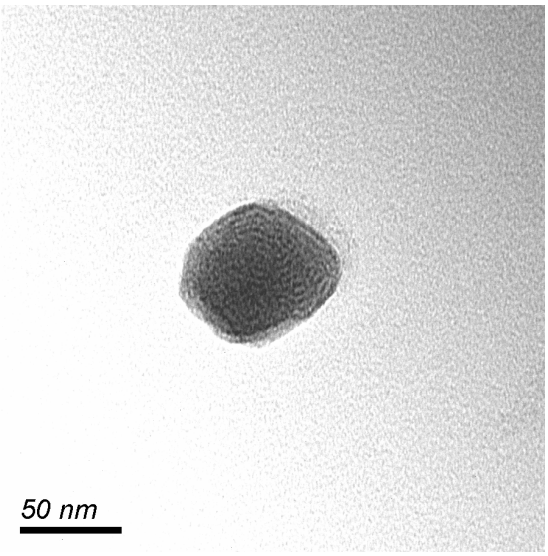
Figure 2: Transmission electron micrograph of LD complexes prepared from a **12:2** with ctDNA at a weight ratio of 16:1 and a magnification of 635,000 and 467,000x respectively.



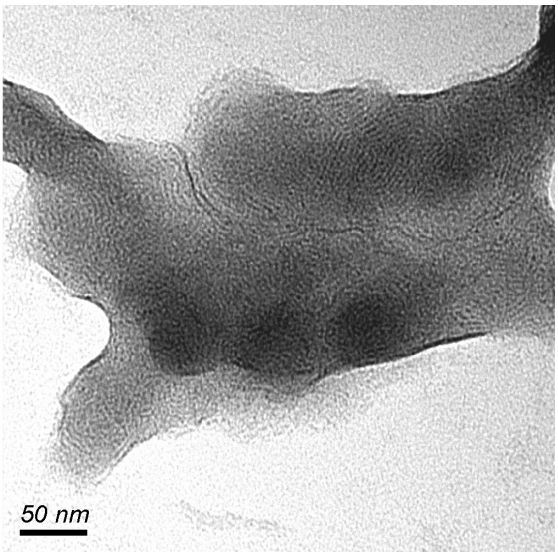


Figure 3 Gel electrophoresis of LD complexes containing a 4:1 and 8:1 weight ratio of lipid:pDNA prepared from vesicles of lipids **1** and **8** containing a 1:1 weight ratio of cationic lipid:**2**.

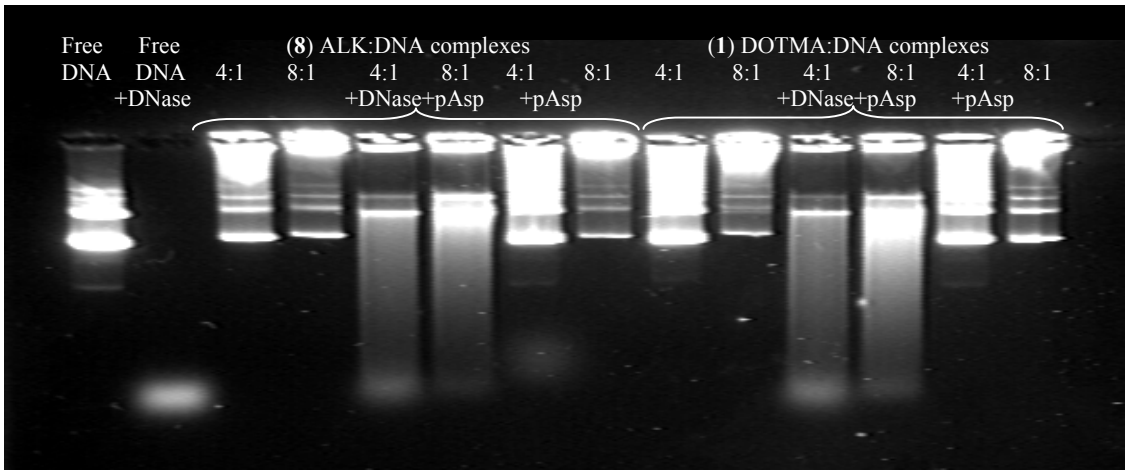
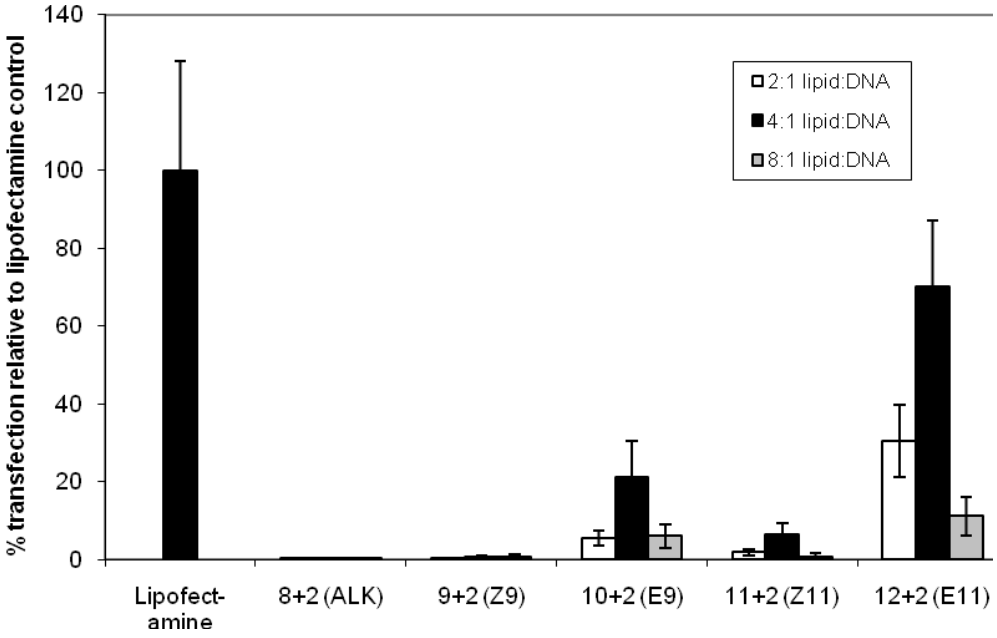


Figure 4 Luciferase expression in 1HAEo cells upon transfection with lipofectamine® as positive controls and the LD complexes using the cationic lipids **8-12** with a 1:1 weight ratio of DOPE at pDNA:lipid weight ratios of 1:2–1:16 (n=4 ± SD).



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