

A Dialkynoyl-Analogue of DOPE Improves Gene Transfer of Lower-Charged Cationic Lipoplexes

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Supporting Information

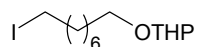
General Information

All reactions were carried out under an atmosphere of nitrogen or argon, in oven-dried glassware. Flash column chromatography was performed as described by Still¹⁶⁵ with Merck silica gel 60 (230 – 240 mesh). TLC refers to thin layer chromatography performed on pre-coated Merck silica gel 60 F₂₅₄ aluminium-backed plates, and visualised with a UV lamp (254 and 366 nm) and / or stained with acidic ammonium molybdate (IV), basic potassium manganate (VII; KmnO₄), iodine, or ninhydrin. CH₂Cl₂ was distilled over P₂O₅, and other solvents were bought and pre-dried as required. All chemicals were purchased from Sigma-Aldrich, Lancaster and Merck Biosciences. Special solvent mixtures: solvent A – CH₂Cl₂:MeOH:H₂O, 77.54:23.23:2.23; solvent B – CH₂Cl₂:MeOH:H₂O, 65:25:4; solvent C – CH₂Cl₂:MeOH:NH₄OH, 25:7:1, solvent D – CH₂Cl₂:MeOH:AcOH, 92:7:1, solvent E – CH₂Cl₂:MeOH:NH₄OH, 65:25:4. ¹H and ¹³C NMR spectra were recorded on either Bruker DRX300, Joel GX-270Q or Bruker Avance 400, using residual isotopic solvent (CDCl₃, δ_H = 7.27 ppm, δ_C = 77.00 ppm; CD₃OD, δ_H = 3.84 ppm, δ_C = 49.05 ppm; (CD₃)₂SO, δ_H = 2.50 ppm, δ_C = 39.43 ppm; D₂O, δ_H = 4.82 ppm) as an internal reference. Data are reported as follows: (integration; br = broad; s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, sex = sextet, m = multiplet; coupling constant(s) in Hz; assignment). Mass spectra were recorded using VG-070B, Joel SX-102 or Bruker Esquire 3000 ESI instruments. IRs were obtained on a JASCO FT/IR-620 infra-red spectrometer. Analytical HPLC (Hitachi-LaChrom L-7150 pump system equipped with a Polymer Laboratories PL-ELS 1000 evaporative light scattering detector) was conducted on a HICHROM KR60-5-2493 silica column, with mobile

phase A (CHCl₃:MeOH:NH₄OH, 80:19.5:0.5) and mobile phase B (CHCl₃:MeOH:H₂O:NH₄OH, 60:34:5.5:0.5), A/B, 0 – 6 min [100/0], gradient 6 – 28 min [0/100], 28 – 38 [0/100], gradient 38 – 40 min [100/0], 40 – 60 min [100/0].

Synthesis of Lipids

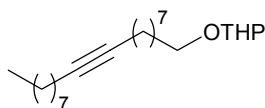
2-(8-Iodooctyl-1-oxy)tetrahydropyran (**6**).



To a stirred solution of 8-bromooctan-1-ol (10.0 g, 47.8 mmol) in anhydrous CH₂Cl₂ (230 cm³) at 0°C, under N₂ was added DHP (5.45 cm³, 59.8 mmol, 1.25) and PPTS (1.80 g, 15 mol%). The reaction mixture was allowed to stir for 16 h in the water bath, under a N₂ atmosphere, by which time the solution had turned slightly brown in colour. The reaction mixture was neutralised with aqueous NaHCO₃ (150 cm³), the organic phase was collected, and the aqueous phase was re-extracted with CH₂Cl₂ (2 x 100 cm³). The organic fractions were combined, washed with water (130 cm³), brine (130 cm³), dried (Na₂SO₄), filtered, and concentrated. The crude residue was purified by SiO₂ flash chromatography (hexane:EtOAc, 9:1), to furnish the bromo THP ether as a bright yellow oil (13.0 g, 93%): R_f 0.60 [hexane:EtOAc, 1:1]; δ_H (300 MHz; CDCl₃) 1.28 – 1.49 (8 H, m, 4 x CH₂), 1.50 – 1.67 (6 H, m, 3 x CH₂), 1.68 – 1.92 (4 H, 2 m, 2 x CH₂), 3.36 – 3.43 (1 H, m, CHO), 3.42 (2 H, t, *J* 6.8, CH₂Br), 3.48 – 3.55 (1 H, m, CHO), 3.71 – 3.79 (1 H, m, CHO), 3.85 – 3.92 (1 H, m, CHO), 4.59 (1 H, t, *J* 3.6, OCHO); δ_C (100 MHz; CDCl₃) 20.06, 25.88, 26.50, 28.47, 29.06, 29.80, 30.05 (7 x CH₂), 31.14 (CH₂CHO), 33.16 (CH₂CH₂Br), 34.29 (CH₂Br), 62.67, 67.92 (2 x CH₂O) 99.18 (OCHO); *m/z* (EI) 293 ([M – H]⁺, 15), 291 ([M – H]⁺, 15), 221 (6), 219 (6), 193 (5), 191 (6), 164 (5), 162 (5), 101 (55), 85 (100), 69 (100), 58 (80), 57 (100), 55 (100), 49 (58), 44 (87), 41 (100); found [M – H]⁺, 293.094513, C₁₃H₂₄O₂⁸¹Br requires [M – H]⁺, 293.093920, found [M – H]⁺, 291.096336, C₁₃H₂₄O₂⁷⁹Br requires [M – H]⁺, 291.095966. NaI (8.59 g, 57.3 mmol) was

dissolved in a solution of the bromo THP ether (8.40 g, 28.6 mmol) in acetone (160 cm³). The stirred, slightly cloudy solution was then placed under reflux, under N₂ for 15 min, during which period a solid precipitated out of solution. The reaction mixture was filtered, and concentrated. The residue was dissolved in water (150 cm³) and CH₂Cl₂ (200 cm³). Then, the organic layer was separated, and the aqueous layer was extracted with CH₂Cl₂ (2 x 200 cm³). The organic layers were combined, washed with water (100 cm³), brine (100 cm³), dried (Na₂SO₄), filtered, and concentrated to afford crude **6** as a bright yellow, non-viscous oil. This residue was purified by silica gel column chromatography (hexane:EtOAc, 13:1), giving **6** as a pale yellow, non-viscous oil (8.66 g, 89%): R_f 0.62 [hexane:EtOAc, 1:1]; δ_H (270 MHz; CDCl₃) 1.25 – 1.44 (8 H, m, 4 x CH₂), 1.45 – 1.73 (6 H, m, 3 x CH₂), 1.74 – 1.90 (4 H, 2 m, 2 x CH₂), 3.17 (2 H, t, *J* 7.0, CH₂), 3.32 – 3.41 (1 H, m, CHO), 3.34 – 3.43 (1 H, m, CHO), 3.67 – 3.76 (1 H, m, CHO), 3.81 – 3.90 (1 H, m, CHO), 4.56 (1 H, t, *J* 3.6, OCHO); δ_C (100 MHz; CDCl₃), 7.62 (CH₂), 20.09, 25.89, 26.53, 28.85, 29.62, 30.07 (6 x CH₂), 30.82 (CH₂CH₂CH₂), 62.72, 67.96 (2 x CH₂O), 99.22 (OCHO); *m/z* (EI) 339 ([M – H]⁺, 10), 101 (46), 86 (79), 85 (100), 69 (96), 67 (44), 58 (53), 57 (89), 55 (100), 49 (100), 47 (53), 44 (73); found [M – H]⁺, 339.082839, C₁₃H₂₄O₂ requires [M – H]⁺, 339.082107.

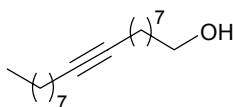
2-(Octadec-9-ynyl-1-oxy)tetrahydropyran (7).



To a stirred solution of 1-decyne (2.40 cm³, 13.5 mmol) in anhydrous THF (23 cm³) at 0°C, under a N₂ atmosphere, was added *n*-BuLi (2.17 M in hexanes; 8.71 cm³, 18.9 mmol) dropwise. After 15 min, the solution had become orange-red in colour. HMPA (23 cm³) was added to the solution, turning the reaction mixture blood red in colour. After a further 15 min, **6** (5.50 g, 16.2 mmol) was added with anhydrous THF (11 cm³), turning the solution yellow-orange. Within 1.5 h, the

solution had turned darker in colour. The reaction mixture was allowed to stir at RT for a further 48 h, then poured onto an ice / water mixture (150 cm³). Crude **7** was extracted with diethyl ether (3 x 150 cm³), and the combined organic layers were combined, washed with water (75 cm³), brine (75 cm³), dried (Na₂SO₄), and concentrated. The crude residue was purified by flash chromatography residue (hexane:EtOAc, 30:1) to yield **7** as a pale-yellow oil (3.42 g, 72%): R_f 0.58 [hexane:EtOAc, 2:1]; δ_H (400 MHz; CDCl₃) 0.99 (3 H, t, *J* 6.8, CH₃), 1.33 – 1.98 (30 H, 4 m, 15 x CH₂), 2.24 (4 H, 2 t, *J* 7.2, CH₂C≡CCH₂), 3.49 (1 H, m, CHO), 3.44 – 3.61 (1 H, m, CHO), 3.84 (1 H, m, CHO), 3.98 (1 H, m, CHO), 4.68 (1 H, t, *J* 3.2, OCHO); δ_C (100 MHz; CDCl₃) 13.86 (CH₃), 18.11, 19.01, 21.32, 22.48, 25.78, 26.35, 29.69, 29.92, 29.95, 29.97, 29.99, 30.09 (15 CH₂), 31.39 (CH₂CHO), 62.49, 67.89 (2 x CH₂O), 80.21, 80.30 (C≡C), 99.57 (OCHO); *m/z* (FAB⁺) 351 ([M + H]⁺, 4), 349 ([M – H]⁺, 5), 85 (100); found [M – H]⁺, 349.310951, C₂₃H₄₁O₂ requires [M – H]⁺, 349.310656.

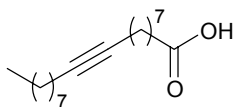
Octadec-9-yn-1-ol (**8**).



Compound **7** (3.31 g, 9.44 mmol) was dissolved in methanol (150 cm³), and *p*-TsOH.H₂O (180 mg, 10 mol%) was added. The reaction mixture was allowed to stir at RT for 20 h, after which the mixture was diluted with diethyl ether (300 cm³), then quenched with aqueous NaHCO₃ (150 cm³). The organic ether layer was separated, and the aqueous/methanol layer was re-extracted with further diethyl ether (2 x 200 cm³). The combined organic layers were washed with water (100 cm³), brine (100 cm³), dried (MgSO₄), filtered, and concentrated *in vacuo* to give a yellow oil. The crude material was dry-loaded onto silica gel and purified by flash column chromatography (hexane:EtOAc, 4:1), yielding the title compound as a white powder (2.32 g, 92%): R_f 0.37 [hexane:EtOAc, 2:1]; ν_{max} (nujol mull)/cm⁻¹ 3399 (br), 2243; δ_H (400 MHz; CDCl₃) 0.65 (3 H, t, *J* 6.8, CH₃),

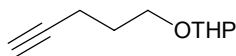
1.00 – 1.42 (24 H, 3 m, 12 x CH₂), 1.91 (4 H, 2 t, *J* 6.8, CH₂C≡CCH₂), 3.41 (2 H, t, *J* 6.6, CH₂OH); δ_C (100 MHz; CDCl₃) 14.51 (CH₃), 19.14, 19.17, 23.08, 25.04, 29.02, 29.18, 29.23, 29.37, 29.47, 29.54, 29.58, 29.64, 32.26, 32.74 (14 x CH₂), 63.82 (CH₂OH), 80.48, 80.78 (C≡C); *m/z* (Cl⁺) 284 ([M + NH₄]⁺, 100); found [M + NH₄]⁺, 284.294269, C₁₈H₃₈NO requires [M + NH₄]⁺, 284.295340.

Octadec-9-ynoic acid (9).



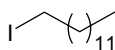
To a stirred solution of **8** (2.21 g, 8.29 mmol) in acetone (56 cm³) at 0°C was added Jones's reagent (4.48 cm³), prepared from CrO₃ (1.21 g, 12.1 mmol), concentrated H₂SO₄ (1.03 cm³) and water (3.45 cm³). After 2 h oxidation, excess reagent was destroyed with propan-2-ol. The solution was neutralised with aqueous NaHCO₃, and the solvent was removed *in vacuo*. The residue was dissolved in diethyl ether (250 cm³), and washed with a 2 M solution of HCl. The organic layer was collected, and the aqueous phase was extracted further with diethyl ether (3 x 150 cm³). The combined organic layers were washed with water (150 cm³), brine (150 cm³), dried (MgSO₄), and concentrated. The residue was crystallised from hexane, to give **9** as a white powder (1.59 g, 69%): R_f 0.21 [hexane:EtOAc, 2:1]; ν_{max} (nujol mull)/cm⁻¹ 2263, 1724; δ_H (400 MHz, CDCl₃) 0.74 (3 H, t, *J* 6.8, CH₃), 1.10 – 1.57 (22 H, 2 m, 11 x CH₂), 1.61 (2 H, quin *J* 7.2, CH₂CH₂CO₂H), 2.02 (4 H, t, *J* 6.7, CH₂C≡CCH₂), 2.21 (2 H, t, *J* 7.4, CH₂CO₂H); δ_C (100 MHz; CDCl₃) 14.51 (CH₃), 19.14, 19.17, 23.08, 25.04, 29.02, 29.18, 29.23, 29.37, 29.47, 29.54, 29.58, 29.64, 32.26 (13 x CH₂), 34.40 (CH₂CO₂H), 80.48, 80.78 (C≡C), 180.33 (CO₂H); *m/z* 298 ([M + NH₄]⁺, 100); found [M + NH₄]⁺, 298.273873, C₁₈H₃₆NO₂ requires [M + NH₄]⁺, 298.274605.

2-(Pent-4-ynyl-1-oxy)tetrahydropyran (**13**).



To a stirred solution of 4-pentyn-1-ol (6 cm³, 64.5 mmol), in CH₂Cl₂ (150 cm³) at 0°C, under Ar, was added DHP (7.35 cm³, 80.6 mmol) and PPTS (2.43 g, 15 mol%). The reaction mixture was stirred for 30 min at 0°C, then allowed to warm to room temperature, and left to stir for 15 h, after which time the reaction mixture was quenched with NaHCO₃ (150 cm³). The aqueous phase was extracted with CH₂Cl₂ (3 x 50 cm³), and the combined organic layers were washed with water (80 cm³), brine (80 cm³), dried (Na₂SO₄), and concentrated. The residue was purified by silica gel column chromatography (hexane:EtOAc, 9:1) to afford **13** as a colourless oil (6.02 g, 95%): R_f 0.26 [hexane:EtOAc, 9:1]; ν_{max} (nujol mull)/cm⁻¹ 3304, 2118; δ_H (300 MHz; CDCl₃) 1.51 - 1.65 (4 H, m, 2 x CH₂), 1.66 - 1.78 (2 H, m, CH₂), 1.83 (2 H, quin, *J* 6.6, HC≡CCH₂CH₂CH₂O), 1.96 (1 H, t, *J* 2.8, C≡CH), 2.33 (2 H, dt, *J* 7.0, ⁴*J* 2.6, CH₂C≡CH), 3.46 - 3.56 (2 H, 2 m, 2 x CHO), 3.81 - 3.92 (2 H, 2 m, 2 x CHO), 4.62 (1 H, t, *J* 3.4, OCHO); δ_C (100 MHz; CDCl₃) 15.51, 19.77, 25.91, 29.04, 31.03 (5 x CH₂), 62.52, 66.11 (2 x CH₂O), 68.81 (CH₂C≡CH), 84.75 (CH₂C≡CH), 99.13 (OCHO); *m/z* (CI) 186 ([M + NH₄]⁺, 100), 168 (27); found [M + NH₄]⁺, 186.149475, C₁₀H₂₀NO₂ requires [M + NH₄]⁺, 186.149404.

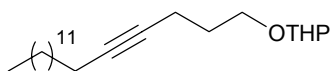
1-Iodotridecane (**14**).



NaI (13.7 g, 91.2 mmol) was added to a stirred solution of 1-bromotridecane (12.0 g, 45.6 mmol) in acetone (250 cm³) at RT. The reaction system was flushed with N₂, and placed under reflux. After 16 h, the white precipitate was filtered off, and the filtrate was concentrated *in vacuo*. The residue was

dissolved in CH₂Cl₂ (150 cm³), and washed with water (100 cm³), and the organic layer was separated. The aqueous layer was extracted further with CH₂Cl₂ (2 x 150 cm³). All organic layers were combined, washed with water (100 cm³), brine (100 cm³), dried (MgSO₄), and concentrated *in vacuo* to give crude **14** as a pale-yellow oil in >95% purity (11.9 g, 84%): R_f 0.74 [EtOAc]; δ_H (270 MHz; CDCl₃) 0.87 (3 H, t, *J* 6.6, CH₃), 1.13 - 1.44 (20 H, m, 10 x CH₂), 1.81 (2 H, quin, *J* 7.1, CH₂CH₂l), 3.17 (2H, t, *J* 7.0, CH₂l); δ_C (100 MHz; CDCl₃) 7.32 (CH₂l), 14.57 (CH₃), 23.14, 29.03, 29.83, 29.91, 30.03, 30.10, 30.11, 30.14, 30.99 (9 x CH₂), 32.38 (CH₂CH₂CH₂l), 34.05 (CH₂CH₂l); *m/z* (EI) 310 (M⁺, 21), 183 (14), 99 (14), 85 (47), 71 (71), 57 (100), 55 (28), 43 (78), 41 (38); found [M]⁺, 310.115178, C₁₃H₂₇I requires [M]⁺, 310.115753.

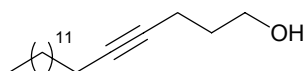
2-(Octadec-4-ynyl-1-oxy)tetrahydropyran (**15**).



To a stirred solution of **13** (5 g, 29.7 mmol) in anhydrous THF (60 cm³) at 0°C, under a flow of N₂, was added n-BuLi (2.27 M in hexanes; 17.7 cm³, 40.2 mmol) dropwise. After 15 min, HMPA (60 cm³) was added to the red solution, turning the reaction mixture a darker red in colour. After a further 15 min, **14** (11.1 g, 35.7 mmol) was added with anhydrous THF (30 cm³), turning the solution a caramel colour. The reaction mixture was allowed to stir in the water bath for 20 h, under a N₂ atmosphere, then poured onto an ice / water mixture (200 cm³). Crude **15** was extracted with diethyl ether (3 x 200 cm³), and the combined organic layers were combined, washed with water (100 cm³), brine (100 cm³), dried (Na₂SO₄), and concentrated. The residue was subjected to flash column chromatography (petroleum ether:ether, 15:1) to afford the title compound as a pale-yellow oil (5.11 g, 49%): R_f 0.48 [hexane:EtOAc, 9:2]; ν_{max} (nujol mull)/cm⁻¹ 2273; δ_H (270 MHz; CDCl₃) 0.86 (3 H, t, *J* 6.4, CH₃), 1.13 - 1.37 (20 H, m, 10 x CH₂), 1.38 - 1.60 (6 H, m, 3 x CH₂), 1.61 - 1.89 (2 H, m, C≡C(CH₂)₂CH₂O), 1.75

(2 H, quin, J 6.7, $\text{CH}_2(\text{CH}_2)_{10}\text{CH}_3$), 2.06 – 2.16 (2 H, m, $\text{C}\equiv\text{CCH}_2(\text{CH}_2)_{11}\text{CH}_3$), 2.19 – 2.31 (2 H, m, $\text{C}\equiv\text{CCH}_2(\text{CH}_2)_2\text{O}$), 3.41 - 3.52 (2 H, 2 m, 2 x CHO), 3.75 - 3.89 (2 H, 2 m, 2 x CHO), 4.58 (1 H, t, J 3.3, OCHO); δ_{C} (100 MHz; CDCl_3) 14.48 (CH_3), 16.01, 19.11, 19.83, 23.06, 26.87, 29.26, 29.51, 29.56, 29.66, 29.74, 29.94, 30.03, 30.05, 30.07, 31.03, 32.30 (17 x CH_2), 62.42, 66.42 (2 x CH_2O), 79.70, 80.95 ($\text{C}\equiv\text{C}$), 99.07 (OCHO); m/z (FAB⁺) 351 ($[\text{M} + \text{H}]^+$, 9), 349 (9), 279 (1), 238 (1), 167 (3), 85 (100), 67 (10), 57 (9), 55 (12), 43 (9), 41 (11); found $[\text{M} + \text{H}]^+$, 349.312202, $\text{C}_{23}\text{H}_{41}\text{O}_2$ requires $[\text{M} + \text{H}]^+$, 349.310656.

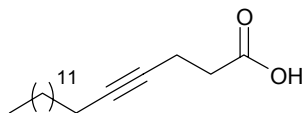
Octadec-4-yn-1-ol (**16**).



Compound **15** (4.93 g, 14.1 mmol) was dissolved in methanol (230 cm^3), and *p*-TsOH.H₂O (270 mg, 10 mol%) was added. The reaction mixture was allowed to stir at RT for 16 h, after which the mixture was diluted with diethyl ether (300 cm^3), then quenched with aqueous NaHCO₃ (150 cm^3). The organic ether layer was separated, and the aqueous/methanol layer was re-extracted with further diethyl ether (2 x 200 cm^3). The combined organic layers were washed with water (100 cm^3), brine (100 cm^3), dried (MgSO₄), filtered, and concentrated *in vacuo* to yield **16** (>95% purity) as an off-white powder (3.27 g, 87%): R_f 0.39 [hexane:EtOAc, 2:1]; ν_{max} (nujol mull)/ cm^{-1} 3434 (br), 2259; δ_{H} (270 MHz; CDCl_3) 0.86 (3 H, t, J 6.7, CH_3), 1.17 - 1.39 (20 H, m, 10 x CH_2), 1.46 (2 H, quin, J 6.9, $\text{CH}_2(\text{CH}_2)_{10}\text{CH}_3$), 1.55 (s, OH), 1.72 (2 H, quin, J 6.4, $\text{CH}_2\text{CH}_2\text{OH}$), 2.12 (2 H, tt, J 6.9, 5J 2.3, $\text{CH}_2\text{C}\equiv\text{CCH}_2(\text{CH}_2)_2\text{OH}$), 2.27 (2 H, tt, J 6.8, 5J 2.3, $\text{CH}_2\text{C}\equiv\text{CCH}_2(\text{CH}_2)_2\text{OH}$), 3.75 (2 H, t, J 6.1, CH_2OH); δ_{C} (100 MHz; CDCl_3) 14.49 (CH_3), 15.80, 19.10, 23.07, 29.29, 29.47, 29.55, 29.75, 29.94, 30.04, 30.05, 30.08, 32.00, 32.31 (14 x CH_2), 62.34 (CH_2OH), 79.62, 81.48 ($\text{C}\equiv\text{C}$); m/z (FAB⁺) 267 ($[\text{M} + \text{H}]^+$, 100), 265 (20), 239 (20), 120 (28), 95 (43), 91 (45), 81 (53), 77

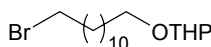
(55), 69 (52), 67 (48), 57 (50), 55 (78), 43 (76), 41 (79); found $[M + H]^+$, 267.268707, $C_{18}H_{35}O$ requires $[M + H]^+$, 267.268791.

Octadec-4-ynoic acid (17).



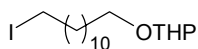
To a stirred solution of **16** (2.95 g, 11.1 mmol) in acetone (75 cm³) at 0°C was added Jones's reagent (6.0 cm³), prepared from CrO₃ (1.62 g, 16.2 mmol), concentrated H₂SO₄ (1.38 cm³) and water (4.62 cm³). After 2 h oxidation, excess reagent was destroyed with propan-2-ol. The solution was neutralised with aqueous NaHCO₃, and the solvent was removed *in vacuo*. The residue was dissolved in diethyl ether (300 cm³), and washed with a 2 M solution of HCl. The organic layer was collected, and the aqueous phase was extracted further with diethyl ether (3 x 200 cm³). The combined organic layers were washed with water (150 cm³), brine (150 cm³), dried (MgSO₄), and concentrated. The residue was crystallised from hexane, to give the title compound as a white powder (2.17 g, 67%): R_f 0.19 [hexane:EtOAc, 2:1]; ν_{\max} (nujol mull)/cm⁻¹ 2237, 1712; δ_H (270 MHz; CDCl₃) 0.86 (3 H, t, *J* 6.6, CH₃), 1.17 - 1.38 (20 H, m, 10 x CH₂), 1.44 (2 H, quin, *J* 6.9, CH₂(CH₂)₁₀CH₃), 2.08 - 2.13 (2 H, m, CH₂(CH₂)₁₁CH₃), 2.42 - 2.50 (2 H, m, CH₂CH₂CO₂H), 2.51 - 2.56 (2 H, m, CH₂CO₂H); δ_C (67.5 MHz; CDCl₃) 14.17 (CH₃), 14.53, 18.73, 22.77, 28.90, 28.99, 29.24, 29.45, 29.62, 29.74 (13 x CH₂), 34.04 (CH₂CO₂H), 77.69, 81.45 (C≡C), 178.83 (CO₂H); *m/z* (CI) 298 ([M + NH₄]⁺, 94), 188 (12), 53 (41), 51 (7); found $[M + NH_4]^+$, 298.275103, $C_{18}H_{36}NO_2$ requires $[M + NH_4]^+$, 298.274605.

2-(12-Bromododecyl-1-oxy)tetrahydropyran (18).



12-Bromododecan-1-ol (10.9 g, 41.1 mmol) was dissolved in anhydrous CH₂Cl₂ (200 cm³) at 0°C, under Ar. To the stirred solution was added DHP (4.70 cm³, 51.4 mmol) and PPTS (1.33 g, 15 mol%). After 30 min, the reaction vessel was removed from the ice bath, and allowed to stir for 16 h at RT. The reaction mixture was neutralised with aqueous NaHCO₃ (150 cm³), and the organic phase was separated. The aqueous phase was re-extracted with CH₂Cl₂ (2 x 100 cm³), and the combined organic layers were washed with water (100 cm³), brine (100 cm³), dried (Na₂SO₄), filtered, and concentrated *in vacuo*. The residue was purified by silica gel flash chromatography (hexane:EtOAc, 13:1) to furnish **18** as a pale-yellow oil (14.1 g, 98%): R_f 0.34 [hexane:EtOAc, 9:1]; δ_H (270 MHz; CDCl₃) 1.20 - 1.44 (16 H, m, 8 x CH₂), 1.45 - 1.60 (6 H, m, 3 x CH₂), 1.61 - 1.89 (4 H, m, 2 x CH₂), 3.32 - 3.41 (1 H, m, CH₂O), 3.39 (2 H, t, *J* 6.8, CH₂Br), 3.44 - 3.52 (1H, m, CH₂O), 3.67- 3.76 (1 H, m, CH₂O), 3.82 - 3.90 (1 H, m, CH₂O), 4.56 (1 H, t, *J* 3.3, OCHO); δ_C (100 MHz; CDCl₃) 20.07, 25.90, 26.61 (3 x CH₂), 28.55 (CH₂CH₂CH₂Br), 29.14, 29.80, 29.85, 29.89, 29.92, 29.94, 30.13 (7 x CH₂), 31.16 (CH₂CHO), 34.34 (CH₂Br), 62.67, 68.03 (2 x CH₂O), 99.37 (OCHO); *m/z* (FAB⁺) 351 ([M + H]⁺, 5) 349 ([M + H]⁺, 8), 85 (100), 69 (22), 57 (22), 55 (29); found [M + H]⁺, 351.174248, C₁₇H₃₄O₂⁸¹Br requires [M + H]⁺, 351.172171; found [M – H]⁺, 347.159813, C₁₇H₃₂O₂⁷⁹Br requires [M – H]⁺, 347.158567.

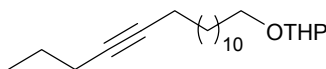
2-(12-Iodododecyl-1-oxy)tetrahydropyran (**19**).



NaI (7.29 g, 48.6 mmol) was dissolved in a stirred solution of **18** (8.50 g, 24.3 mmol) in acetone (250 cm³) at RT. The reaction system was flushed with N₂, and left under reflux for 20 h. The white precipitate was filtered off, and the reaction mixture was concentrated *in vacuo*. The resultant residue was dissolved in CH₂Cl₂ (150 cm³), and washed with water (100 cm³). The organic layer was separated, and the aqueous layer was extracted further with CH₂Cl₂ (2 x 150

cm³). The combined organic layers were washed with water (100 cm³), brine (100 cm³), dried (MgSO₄), and concentrated *in vacuo*. The residue was subjected to silica gel flash column chromatography (hexane:EtOAc, 13:1) to afford the title compound (**19**) as a pale-yellow oil (7.75 g, 80%): R_f 0.34 [hexane:EtOAc, 9:1]; δ_H (270 MHz; CDCl₃) 1.20 - 1.43 (16 H, m, 8 x CH₂), 1.45 - 1.63 (6 H, m, 3 x CH₂), 1.64 - 1.88 (4 H, m, 2 x CH₂), 3.17 (2H, t, *J* 7.0, CH₂l), 3.32 - 3.40 (1 H, m, CH₂O), 3.45 - 3.52 (1 H, m, CH₂O), 3.67 - 3.76 (1 H, m, CH₂O), 3.81 - 3.90 (1 H, m, CH₂O), 4.56 (1 H, t, *J* 3.5, OCHO); δ_C (400 MHz; CDCl₃) 7.66 (CH₂l), 20.08, 25.91, 26.62, 28.93, 29.79, 29.86, 29.90, 29.92, 29.94, 30.13 (10 x CH₂), 30.89 (CH₂CH₂CH₂l), 31.17 (CH₂CHO), 33.95 (CH₂CH₂l), 62.67, 68.03 (CH₂O), 99.17 (OCHO); *m/z* (FAB⁺) 397 ([M + H]⁺, 6), 395 ([M - H]⁺, 4), 85 (100), 69 (30), 67 (19), 57 (28), 55 (39); found [M + H]⁺, 397.160049, C₁₇H₃₂O₂l requires [M + H]⁺, 397.160358; found [M - H]⁺, 395.144836, C₁₇H₃₂O₂l requires [M - H]⁺, 395.144708.

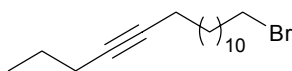
2-(Heptadec-13-ynyl-1-oxy)tetrahydropyran (**20**).



To a stirred solution of 1-pentyne (2.25 cm³, 22.9 mmol) in anhydrous THF (40 cm³) at 0°C, under a N₂ atmosphere, was added *n*-BuLi (1.80 M in hexanes; 14.3 cm³, 25.7 mmol) dropwise. After 15 min, HMPA (40 cm³) was added to the bright yellow solution, turning the reaction mixture caramel in colour. After a further 15 min, **19** (7.55 g, 19.0 mmol) was added with anhydrous THF (20 cm³), turning the solution yellow-orange, through blue-green. Within 1.5 h, the solution had turned darker in colour. The reaction mixture was allowed to stir at RT for a further 48 h, then poured onto an ice / water mixture (200 cm³). Crude **20** was extracted with diethyl ether (3 x 200 cm³), and the combined organic layers were combined, washed with water (100 cm³), brine (100 cm³), dried (Na₂SO₄), and concentrated. The crude residue was purified by flash column chromatography of the residue

(hexane:EtOAc, 20:1) to yield **20** as a pale-yellow oil (3.76 g, 59%): R_f 0.19 [hexane:EtOAc, 30:1]; δ_H (270 MHz; $CDCl_3$) 0.95 (3 H, t, J 7.3, CH_3), 1.21 - 1.40 (16 H, m, 8 x CH_2), 1.41 - 1.64 (10 H, m, 5 x CH_2), 1.65 - 1.89 (2 H, 2 m, CH_2), 2.06 - 2.16 (4 H, m, $CH_2C\equiv CCH_2$), 3.32 - 3.41 (1 H, m, CH_2O), 3.44 - 3.52 (1 H, m, CH_2O), 3.67 - 3.76 (1 H, m, CH_2O), 3.82 - 3.90 (1 H, m, CH_2O), 4.56 (1 H, t, J 3.6, OCHO); δ_C (100 MHz; $CDCl_3$) 13.17 (CH_3), 18.02, 19.15, 20.00, 22.83, 25.91, 26.66, 29.23, 29.54, 29.87, 29.89, 29.96, 30.11 (15 CH_2), 31.22 (CH_2CHO), 62.59, 68.12 (2 x CH_2O), 80.01, 80.33 ($C\equiv C$), 99.23 (OCHO); m/z (FAB⁺) 337 ([$M + H$]⁺, 4), 335 (3), 238 (4), 85 (100), 81 (10), 69 (9), 67 (11), 57 (8), 55 (15); found [$M - H$]⁺, 335.295944, $C_{22}H_{39}O_2$ requires [$M - H$]⁺, 335.295006.

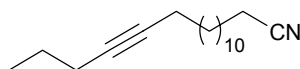
1-Bromoheptadec-13-yne (21).



To a stirred solution of PPh_3Br_2 (5.87 g, 13.9 mmol) and PPh_3 (1.41 g, 5.36 mmol) in anhydrous CH_2Cl_2 (150 cm^3) at 0°C, under N_2 was added **20** (3.61 g, 10.7 mmol). After 15 min, excess PPh_3Br_2 was destroyed with 10% K_2CO_3 solution. The organic phase was separated, and the aqueous phase was re-extracted with CH_2Cl_2 (3 x 150 cm^3). The combined organic layers were washed with water (150 cm^3), brine (150 cm^3), dried ($MgSO_4$), filtered, and concentrated. Crude **21** was subjected to silica gel column chromatography (100% hexane → 95% hexane:EtOAc → 90% hexane:EtOAc) to furnish the title compound as an 84% pure pale-yellow oil (3.02 g, 90%): R_f 0.61 [hexane:EtOAc, 9:2]; ν_{max} (nujol mull)/ cm^{-1} 2199; δ_H (300 MHz; $CDCl_3$) 0.99 (3 H, t, J 7.4, CH_3), 1.24 - 1.58 (18 H, 2 m, 9 x CH_2), 1.50 (2 H, sextet, J 7.1, CH_2CH_3), 1.87 (2 H, quin, J 7.2, CH_2CH_2Br), 2.11 - 2.21 (4 H, m, $CH_2C\equiv CCH_2$), 3.43 (2 H, t, J 6.9, CH_2Br); δ_C (400 MHz, $CDCl_3$) 13.37 (CH_3), 18.69, 20.88, 22.61, 25.01 ($CH_3CH_2CH_2C\equiv CCH_2$, CH_2), 28.59, 29.17, 29.31, 29.55, 29.87, 29.91, 29.98,

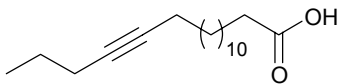
30.19 (8 x CH₂), 33.23 (CH₂CH₂Br), 34.22 (CH₂Br), 80.01, 80.42 (C≡C); *m/z* (EI) 316 (M⁺, 0.33), 314 (M⁺, 0.45), 109 (38), 95 (77), 82 (43), 81 (100), 67 (100), 56 (47), 55 (54), 49 (58), 41 (78).

Octadec-14-yne nitrile (**22**).



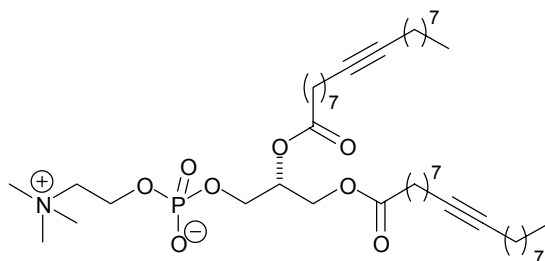
NaCN (0.61 g, 12.5 mmol) was dissolved in a stirred solution of **21** (3.02 g, 9.58 mmol) in anhydrous DMF (150 cm³), under Ar. The reaction mixture was allowed to stir for 16 h at 60°C, then poured into water (300 cm³). Crude **22** was extracted into benzene (500 cm³, then 4 x 100 cm³). The combined organic layers were washed with water (100 cm³), brine (100 cm³), dried (MgSO₄), filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane:EtOAc, 9:1) to afford the title nitrile as a pale-yellow oil (1.99 g, 95%): R_f 0.44 [hexane:EtOAc, 3:1]; ν_{max} (nujol mull)/cm⁻¹ 2359, 2246; δ_H (300 MHz; CDCl₃) 0.98 (3 H, t, *J* 7.4, CH₃), 1.24 - 1.58 (18 H, m, 9 x CH₂), 1.49 (2 H, sextet, *J* 7.1, CH₂CH₃), 1.67 (2 H, quin, *J* 7.3, CH₂CH₂CH₃), 2.10 - 2.21 (4 H, m, CH₂C≡CCH₂), 2.35 (2 H, t, *J* 7.1, CH₂CN); δ_C (100 MHz; CDCl₃), 12.47 (CH₃), 16.10, 17.75, 19.77, 21.57, 24.39 (CH₃CH₂CH₂C≡CCH₂, CH₂CH₂CN), 27.66, 27.78, 27.84, 28.15, 28.18, 28.31, 28.49, 28.51, 28.53 (9 x CH₂), 78.97, 79.32 (C≡C), 118.82 (CN); *m/z* (FAB⁺) 262 ([M + H]⁺, 100), 95 (30), 81 (43), 77 (33), 67 (39), 55 (55), 41 (40); found [M + H]⁺, 262.254333, C₁₈H₃₂N requires [M + H]⁺, 262.253475.

Octadec-14-ynoic acid (**23**).



To a stirred solution of nitrile **21** (1.83 g, 7.00 mmol) in ethanol (2 cm³) was added 25 N NaOH solution (1 cm³). The reaction mixture was stirred under reflux until no further evolution of ammonia gas could be detected (3 h), then poured onto an ice-water mixture (200 cm³), and acidified to pH 2 with concentrated aqueous HCl. The crude title acid was extracted into diethyl ether (3 x 200 cm³), and the combined organic layers were washed with water (100 cm³), brine (100 cm³), dried (MgSO₄), filtered, and concentrated, affording **23** in >95% purity. Crystallisation from hexane gave pure **23** as a white powder (1.85 g, 94%): R_f 0.38 [hexane:EtOAc, 2:1]; ν_{max} (nujol mull)/cm⁻¹ 2273, 1706; δ_H (270 MHz; CDCl₃) 0.95 (3 H, t, *J* 7.3, CH₃), 1.17 - 1.55 (18 H, 2 m, 9 x CH₂), 1.47 (2 H, sex, *J* 7.2, CH₂CH₃), 1.61 (2 H, quin, *J* 7.0, CH₂CH₂CO₂H), 2.06 - 2.20 (4 H, m, CH₂C≡CCH₂), 2.33 (2 H, t, *J* 7.4, CH₂CO₂H); δ_C (67.5 MHz; CDCl₃) 13.51 (CH₂), 18.80, 20.82, 22.62, 24.72 (CH₃CH₂CH₂C≡CCH₂, CH₂CH₂CO₂H), 28.91, 29.12, 29.22, 29.30, 29.49, 29.63 (9 x CH₂), 34.19 (CH₂CO₂H), 80.04, 80.42 (C≡C), 180.67 (CO₂H); *m/z* (CI) 298 ([M + NH₄]⁺, 100), 297 (48), 242 (19), 83 (14), 81 (23), 58 (13); found [M + NH₄]⁺, 298.275163, C₁₈H₃₆NO₂ requires [M + NH₄]⁺, 298.274605.

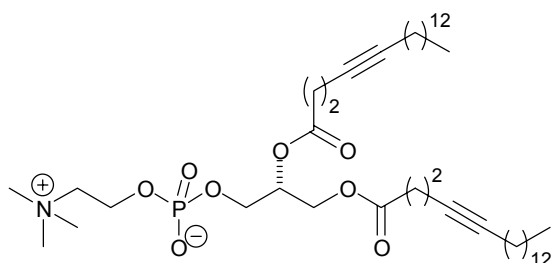
Di(octadec-9-ynoyl)phosphatidylcholine (DS(9-yne)PC, **11**).



To a stirred solution of octadec-9-ynoic acid (**9**; 680 mg, 2.43 mmol) in anhydrous CHCl_3 (10 cm^3) was added CDI (491 mg, 3.03 mmol) under a N_2 atmosphere at RT. Meanwhile, GPC (**10**; 401 mg, 0.91 mmol) was dissolved in DMSO (10 cm^3), with a little heating. DBU (363 μl , 2.43 mmol) was then added. The CHCl_3 solution was transferred to the DMSO solution *via* a cannular with further CHCl_3 (2 cm^3). Periodically, the reaction mixture was warmed in a water bath to approximately 40°C , for about 20 min. After stirring for 24 h at RT, the crude reaction mixture was neutralised with 0.1 M acetic acid (30 cm^3), then extracted into a 2:1 mixture of CHCl_3 :MeOH (total volume 150 cm^3), and washed with a 1:1 mixture of H_2O :MeOH (100 $\text{cm}^3 \rightarrow$ 250 cm^3 stepwise, over five washes), back-extracting each time. The subsequent organic fractions were combined, and concentrated *in vacuo*, azeotroping the water and methanol with chloroform. The residual orange-brown, viscous oil was purified by silica gel flash column chromatography (solvent A), to give DS(9-yne)PC (**9**) as an off-white, waxy solid (473 mg, 67%): R_f 0.47 [solvent B]; ν_{max} (nujol mull)/ cm^{-1} 2237, 1709, 1626, 1451, 1279, 1246; δ_{H} (270 MHz; CDCl_3) 0.81 (6 H, t, J 6.8, 2 x CH_2CH_3), 1.17 – 1.58 (44 H, 2 m, 20 x CH_2 , 2 x $\text{CH}_2\text{CH}_2\text{CO}_2$), 2.04 – 2.11 (8 H, m, 2 x $\text{CH}_2\text{C}\equiv\text{CCH}_2\text{CH}_2\text{CO}_2$), 2.36 – 2.57 (8 H, m, 2 x $\text{CH}_2\text{CH}_2\text{CO}_2$), 3.33 (9 H, s, $\text{N}(\text{CH}_3)_3$), 3.74 (2 H, m, $\text{CH}_2\text{N}(\text{CH}_3)_3$), 3.87 (2 H, m, glycerol-C3- $\text{H}_{\text{a,b}}$), 4.05 (1 H, dd, 2J 12.0, 3J 7.2, glycerol-C1- H_{b}), 4.24 (2 H, m, $\text{CH}_2\text{CH}_2\text{N}(\text{CH}_3)_3$), 4.33 (1 H, dd, 2J 12.0, 3J 2.4, glycerol-C1- H_{a}), 5.13 (1 H, m, glycerol-C2-H); δ_{C} (100 MHz; CDCl_3) 14.49 (2 x CH_2CH_3), 19.13, 23.04, 25.21, 25.29, 29.10, 29.27, 29.40, 29.44, 29.51, 29.55, 29.60, 32.22 (26 x CH_2), 34.45, 34.65 (2 x CH_2CO_2), 54.75

(N(CH₃)₃), 59.68 (d, *J* 4.4, POCH₂CH₂N(CH₃)₃), 63.38 (POCH₂CHCH₂), 63.70 (d, *J*_{CP} 4.9, POCH₂CH₂N(CH₃)₃), 66.70 (d, *J*_{CP} 5.9, POCH₂CHCH₂), 70.90 (d, *J*_{CP} 7.6, POCH₂CHCH₂), 80.39, 80.69 (2 x C≡C), 173.51, 173.87 (2 x CO₂); *m/z* (FAB⁺) 782 ([M + H]⁺, 35), 184 ([C(CH₃)₃NCH₂CH₂OPO₃H + H]⁺, 100); found [M + H]⁺, 782.571487, C₄₄H₈₁NO₈P requires [M + H]⁺, 782.569983; HPLC *t*_R = 11.5 min.

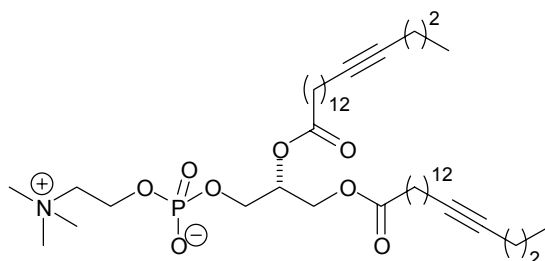
Di(octadec-4-ynoyl)phosphatidylcholine (DS(4-yne)PC, **24**).



To a stirred solution of octadec-4-ynoic acid (**17**; 562 mg, 2.00 mmol) in anhydrous CHCl₃ (13 cm³) was added CDI (405 mg, 2.49 mmol) under N₂ at RT. Meanwhile GPC (**10**; 330 mg, 0.75 mmol) was dissolved in DMSO (13 cm³), with a little heating. To this solution was added DBU (300 μl, 2.00 mmol). The CHCl₃ solution was transferred to the DMSO solution *via* a cannular with further CHCl₃ (2 cm³). After 7 h, the crude reaction mixture was neutralised with 0.1 M acetic acid (30 cm³), then extracted into a 2:1 mixture of CHCl₃:MeOH (total volume 150 cm³), and washed with a 1:1 mixture of H₂O:MeOH (100 cm³ → 250 cm³ stepwise, over five washes), back-extracting each time. The subsequent organic fractions were combined, and concentrated *in vacuo*, azeotroping the water and methanol with benzene. The residual orange-brown, viscous oil was purified by silica gel flash column chromatography (solvent A), to afford DS(4-yne)PC (**24**) as a pale yellow, waxy solid (335 mg, 57%): *R*_f 0.45 [solvent B]; *v*_{max} (nujol mull)/cm⁻¹ 2235, 1723, 1683, 1424, 1373, 1235; δ_H (270 MHz; CDCl₃) 0.86 (6 H, t, *J* 6.5, 2 x CH₂CH₃), 1.15 – 1.49 (44 H, 2 m, 20 x CH₂, 2 x (CH₂)₁₀CH₂CH₂C≡C), 2.05 – 2.14 (4 H, m, 2 x C≡CCH₂CH₂CO₂), 2.36 – 2.57 (8 H, m, 2 x CH₂CH₂CO₂),

3.33 (9 H, s, N(CH₃)₃) 3.77 (2 H, m, CH₂N(CH₃)₃), 3.92 (2 H, m, glycerol-C3-H_{a,b}), 4.14 (1 H, dd, ²J 11.9, ³J 7.2, glycerol-C1-H_b), 4.34 – 4.42 (2 H, m, CH₂CH₂N(CH₃)₃), 4.38 (1 H, dd, ²J 12.1, ³J 2.7, glycerol-C1-H_a), 5.20 (1 H, m, glycerol-C2-H); δ_C (100 MHz; CDCl₃) 14.50 (2 x CH₂CH₃), 15.00, 15.07, 19.11, 23.07, 29.35, 29.37, 29.43, 29.45, 29.61, 29.75, 29.98, 30.05, 30.08, 32.30 (26 x CH₂), 34.43, 34.26 (2 x CH₂CO₂), 54.68 (N(CH₃)₃), 59.70 (d, J 3.9, POCH₂CH₂N(CH₃)₃), 63.50 (POCH₂CHCH₂), 63.65 (d, J_{CP} 4.7, POCH₂CH₂N(CH₃)₃), 66.64 (d, J_{CP} 6.5, POCH₂CHCH₂), 71.17 (d, J_{CP} 7.0, POCH₂CHCH₂), 78.18, 81.61, 81.64 (2 x C≡C), 171.99, 172.32 (2 x CO₂); m/z (FAB⁺) 782 ([M + H]⁺, 52), 184 ([C(CH₃)₃NCH₂CH₂OPO₃H + H]⁺, 100), 86 (47), 58 (35); found [M + H]⁺, 782.572556, C₄₄H₈₁NO₈P requires [M + H]⁺, 782.569983; HPLC t_R = 11.5 min.

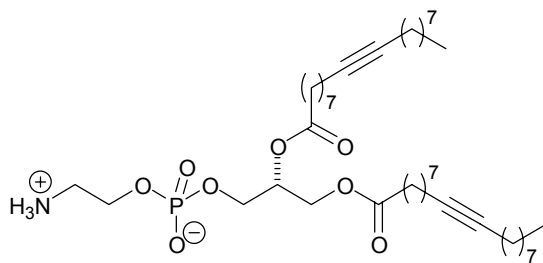
Di(octadec-14-ynoyl)phosphatidylcholine (DS(14-yne)PC, **25**).



To a stirred solution of octadec-14-ynoic acid (**23**; 680 mg, 2.43 mmol) in anhydrous CHCl₃ (10 cm³) was added CDI (491 mg, 3.03 mmol) under a N₂ atmosphere at RT. Meanwhile, GPC (**10**; 401 mg, 0.91 mmol) was dissolved in DMSO (10 cm³), with a little heating. DBU (363 μl, 2.43 mmol) was then added. The CHCl₃ solution was transferred to the DMSO solution *via* a cannular with further CHCl₃ (2 cm³). Periodically, the reaction mixture was warmed in a water bath to approximately 40°C, for about 20 min. After stirring for 24 h at RT, the crude reaction mixture was worked up as for **11**. The residual orange-brown, viscous oil was purified by silica gel flash column chromatography (solvent A), to yield DO(14-yne)PC (**25**) as a pale yellow, waxy solid (420 mg, 59%): R_f 0.44

[solvent B]; ν_{\max} (nujol mull)/ cm^{-1} 2269, 1701, 1672, 1497, 1322; δ_{H} (270 MHz; CDCl_3) 0.94 (6 H, t, J 7.4, 2 x CH_2CH_3), 1.17 – 1.62 (44 H, 2 m, 18 x CH_2 , 2 x CH_2CH_3 , 2 x $\text{CH}_2\text{CH}_2\text{CO}_2$), 2.05 – 2.18 (8 H, m, 2 x $\text{CH}_2\text{C}\equiv\text{CCH}_2$), 2.25 – 2.31 (4 H, m, 2 x CH_2CO_2), 3.33 (9 H, s, $\text{N}(\text{CH}_3)_3$), 3.81 (2 H, m, $\text{CH}_2\text{N}(\text{CH}_3)_3$), 3.93 (2 H, m, glycerol-C3- $\text{H}_{\text{a,b}}$), 4.10 (1 H, dd, 2J 11.9, 3J 7.4, glycerol-C1- H_{b}), 4.30 (2 H, m, $\text{CH}_2\text{CH}_2\text{N}(\text{CH}_3)_3$), 4.37 (1 H, dd, 2J 12.1, 3J 2.7, glycerol-C1- H_{a}), 5.18 (1 H, m, glycerol-C2- H); δ_{C} (100 MHz; CDCl_3) 13.85 (2 x CH_2CH_3), 19.12, 21.14, 22.92, 25.27, 25.35, 29.26, 29.57, 29.68, 29.73, 29.76, 29.87, 29.96, 30.04 (26 x CH_2), 34.50, 34.69 (2 x CH_2CO_2), 54.67 ($\text{N}(\text{CH}_3)_3$), 59.71 (d, J 4.6, $\text{POCH}_2\text{CH}_2\text{N}(\text{CH}_3)_3$), 63.36 ($\text{POCH}_2\text{CHCH}_2$), 63.71 (d, J_{CP} 4.8, $\text{POCH}_2\text{CH}_2\text{N}(\text{CH}_3)_3$), 66.62 (d, J_{CP} 6.5, $\text{POCH}_2\text{CHCH}_2$), 70.86 (d, J_{CP} 7.5, $\text{POCH}_2\text{CHCH}_2$), 80.37, 80.73 (2 x $\text{C}\equiv\text{C}$), 173.54, 173.90 (2 x CO_2); m/z (FAB^+) 782 ($[\text{M} + \text{H}]^+$, 40), 184 ($[(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{OPO}_3\text{H} + \text{H}]^+$, 100), 86 (38), 58 (37); found $[\text{M} + \text{H}]^+$, 782.568970, $\text{C}_{44}\text{H}_{81}\text{NO}_8\text{P}$ requires $[\text{M} + \text{H}]^+$, 782.569983; HPLC $t_{\text{R}} = 11.5$ min.

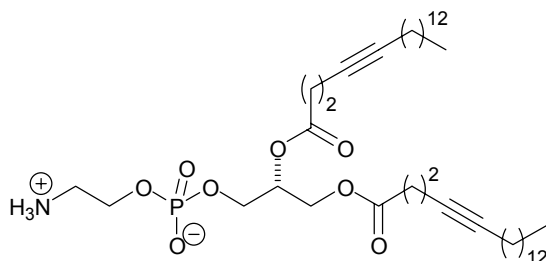
Di(octadec-9-ynoyl)phosphatidylethanolamine (DS(9-yne)PE, **3**).



A solution of ethanolamine (110 μl , 1.50 mmol) in a 100 mM NaOAc / 50 mM CaCl_2 buffer (0.795 cm^3) at pH 6.5 (pH adjusted with acetic acid), was added to a stirred solution of **11** (237 mg, 0.303 mmol) in CHCl_3 (6 cm^3) at 30°C. To this biphasic system was added PLD (175 units in 700 μl of the aforementioned buffer, pH 6.5), and the reaction mixture was allowed to stir at 30°C for 3 h. The crude organic material was extracted by washing with CHCl_3 :MeOH, 2:1 (30 cm^3 x 3). The organic layers were combined and washed with H_2O (15 cm^3), then

concentrated *in vacuo*, and subjected to silica gel flash column chromatography (solvent A) to purify. This afforded the title compound, **3**, as a very pale-yellow, waxy solid (211 mg, 94%): R_f 0.58 [solvent B]; ν_{\max} (nujol mull)/ cm^{-1} 2219, 1709, 1431, 1274, 1072; δ_{H} (270 MHz; CDCl_3) 0.81 (6 H, t, J 6.8, 2 x CH_2CH_3), 1.15 – 1.59 (44 H, 2 m, 22 x CH_2), 2.03 – 2.13 (8 H, m, 2 x $\text{CH}_2\text{C}\equiv\text{CCH}_2$), 2.19 – 2.30 (4 H, m, 2 x CH_2CO_2), 3.09 (2 H, m, CH_2NH_3), 3.88 (2 H, m, glycerol-C3- $\text{H}_{\text{a,b}}$), 4.02 (2 H, m, $\text{CH}_2\text{CH}_2\text{NH}_3$), 4.07 (1 H, dd, 2J 12.0, 3J 6.8, glycerol-C1- H_{b}), 4.32 (1 H, dd, 2J 11.6, 3J 2.4, glycerol-C1- H_{a}), 5.15 (1 H, m, glycerol-C2-H), 8.20 – 8.45 (br s, NH_3); δ_{C} (100 MHz; CDCl_3) 14.49 (2 x CH_2CH_3), 19.14, 23.05, 25.20, 25.27, 29.15, 29.28, 29.43, 29.47, 29.52, 29.57, 29.61, 32.23 (26 x CH_2), 34.40, 34.60 (2 x CH_2CO_2), 40.80 (CH_2NH_3), 62.59 (m, $\text{POCH}_2\text{CH}_2\text{NH}_3$) 62.97 (m, $\text{POCH}_2\text{CHCH}_2$), 64.28 (d, J_{CP} 4.0, $\text{POCH}_2\text{CHCH}_2$), 70.67 (d, J_{CP} 7.4, $\text{POCH}_2\text{CHCH}_2$), 80.37, 80.66 (2 x $\text{C}\equiv\text{C}$), 173.73, 173.45 (2 x CO_2); m/z (FAB^+) 740 ($[\text{M} + \text{H}]^+$, 9), 600 (8), 599 (28), 44 (100); found $[\text{M} + \text{H}]^+$, 740.523073, $\text{C}_{41}\text{H}_{75}\text{NO}_8\text{P}$ requires $[\text{M} + \text{H}]^+$, 740.523033; HPLC $t_{\text{R}} = 4.3$ min.

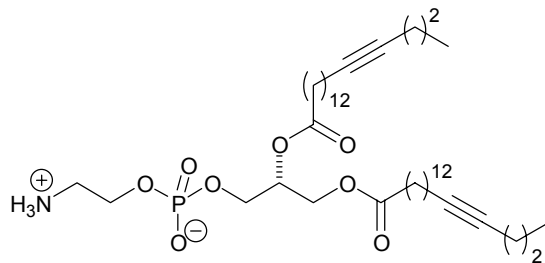
Di(octadec-4-ynoyl)phosphatidylethanolamine (DS(4-yne)PE, **2**).



A solution of ethanolamine (91 μl , 1.50 mmol) in a 100 mM NaOAc / 50 mM CaCl_2 buffer (0.625 cm^3) at pH 6.5 (pH adjusted with acetic acid), was added to a stirred solution of **24** (193 mg, 0.25 mmol) in CHCl_3 (5 cm^3) at 30°C. To this biphasic system was added PLD (110 units in 440 μl of the aforementioned buffer, pH 6.5), and the reaction mixture was allowed to stir at 30°C for 3 h. Further PLD (35 units) was added. After 10 h, the aqueous phase was diluted to 15 cm^3 , and the crude organic material was extracted by washing with

CHCl₃:MeOH, 2:1 (30 cm³ x 3). The organic layers were combined and washed with H₂O (15 cm³), then concentrated *in vacuo*, and subjected to silica gel flash column chromatography (solvent A) to purify. This furnished **2** as a very pale-yellow, waxy solid (170 mg, 92%): R_f 0.56 [solvent B]; ν_{max} (nujol mull)/cm⁻¹ 2238, 1714, 1424, 1291, 1101; δ_H (270 MHz; CDCl₃) 0.81 (6 H, t, *J* 6.7, 2 x CH₂CH₃), 1.15 – 1.31 (40 H, 2 m, 20 x CH₂), 1.35 – 1.43 (4 H, m, 2 x (CH₂)₁₀CH₂CH₂C≡C), 2.01 – 2.06 (4 H, m, 2 x (CH₂)₁₁CH₂C≡C), 2.34 – 2.41 (4 H, m, 2 x CH₂CH₂CO₂), 2.42 – 2.49 (4 H, m, 2 x CH₂CO₂), 3.05 – 3.13 (2 H, m, CH₂NH₃), 3.85 – 3.91 (2 H, m, glycerol-C3-H_{a,b}), 3.97 – 4.05 (2 H, m, CH₂CH₂NH₃), 4.12 (1 H, dd, ²*J* 12.0, ³*J* 6.4, glycerol-C1-H_b), 4.33 (1 H, dd, ²*J* 11.8, ³*J* 2.6, glycerol-C1-H_a), 5.17 (1 H, m, glycerol-C2-H), 8.20 – 8.60 (br s, NH₃); δ_C (100 MHz; CDCl₃) 14.51 (2 x CH₂CH₃), 15.00, 15.06, 19.13, 23.09, 29.39, 29.42, 29.46, 29.49, 29.63, 29.65, 29.77, 30.00, 30.02, 30.07, 30.12, 32.33 (26 x CH₂), 34.25, 34.39 (2 x CH₂CO₂), 40.84 (CH₂NH₃), 62.63 (m, POCH₂CH₂NH₃) 63.11 (m, POCH₂CHCH₂), 64.17 (d, *J*_{CP} 5.5, POCH₂CHCH₂), 70.93 (d, *J*_{CP} 6.9, POCH₂CHCH₂), 78.13, 78.19, 81.61, 81.66 (2 x C≡C), 171.96, 172.21 (2 x CO₂); *m/z* (FAB⁺) 740 ([M + H]⁺, 7), 600 (10), 599 (24), 44 (100); found [M + H]⁺, 740.523087, C₄₁H₇₅NO₈P requires [M + H]⁺, 740.523033; HPLC *t*_R = 4.3 min.

Di(octadec-14-ynoyl)phosphatidylethanolamine (DS(14-yne)PE, **4**).



A solution of ethanolamine (102 μl, 1.69 mmol) in a 100 mM NaOAc / 50 mM CaCl₂ buffer (0.64 cm³) at pH 6.5 (pH adjusted with acetic acid), was added to a stirred solution of **25** (220 mg, 0.28 mmol) in CHCl₃ (5 cm³) at 30°C. To this biphasic system was added PLD (160 units in 640 μl of the aforementioned

buffer, pH 6.5), and the reaction mixture was allowed to stir at 30°C for 4 h. The aqueous phase was diluted to 15 cm³, and the crude organic material was extracted by washing with CHCl₃:MeOH, 2:1 (30 cm³ x 3). The organic layers were combined and washed with H₂O (15 cm³). All organic material was concentrated *in vacuo*, and purified by silica gel flash column chromatography (solvent A), giving the DOPE-analogue, **4**, as an almost colourless, waxy solid (207 mg, 93%): R_f 0.56 [solvent B]; ν_{\max} (nujol mull)/cm⁻¹ 2291, 1728, 1461, 1238, 1075; δ_{H} (270 MHz; CDCl₃) 0.90 (6 H, t, *J* 7.4, 2 x CH₂CH₃), 1.17 – 1.34 (34 H, 2 m, 17 x CH₂), 1.36 – 1.48 (6 H, m, 3 CH₂), 1.48 – 1.56 (4 H, m, 2 x CH₂CH₂CO₂), 2.02 – 2.10 (8 H, m, 2 x CH₂C≡CCH₂), 2.18 – 2.28 (4 H, m, 2 x CH₂CO₂), 3.03 – 3.15 (2 H, m, CH₂NH₃), 3.82 – 3.93 (2 H, m, glycerol-C3-H_{a,b}), 3.97 – 4.05 (2 H, m, CH₂CH₂NH₃), 4.08 (1 H, dd, ²*J* 12.0, ³*J* 6.8, glycerol-C1-H_b), 4.31 (1 H, dd, ²*J* 11.8, ³*J* 2.6, glycerol-C1-H_a), 5.14 (1 H, m, glycerol-C2-H), 8.20 – 8.60 (br s, NH₃); δ_{C} (100 MHz; CDCl₃) 13.85 (2 x CH₂CH₃), 19.14, 22.93, 25.26, 25.33, 29.29, 29.59, 29.77, 29.82, 29.92, 30.00, 30.08, 30.10 (26 x CH₂), 34.45, 34.64 (2 x CH₂CO₂), 40.77 (CH₂NH₃), 62.63 (m, POCH₂CH₂NH₃) 62.95 (m, POCH₂CHCH₂), 64.29 (m, POCH₂CHCH₂), 70.63 (d, *J*_{CP} 8.0, POCH₂CHCH₂), 80.37, 80.73, (2 x C≡C), 173.48, 173.76 (2 x CO₂); *m/z* (FAB⁺) 740 ([M + H]⁺, 19), 600 (14), 599 (30), 81 (40), 69 (63), 55 (90), 44 (100); found [M + H]⁺, 740.522568, C₄₁H₇₅NO₈P requires [M + H]⁺, 740.523033; HPLC *t*_R = 4.3 min.