The synthesis of novel asymmetrical single-chain phosphoglycol-based bolaamphiphiles

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Materials and Methods

All chemicals were purchased from Sigma Aldrich Co. and were used without further purification. All solvents for synthetic purpose were dried and distilled before use. The purity of all compounds was checked by thin-layer chromatography (TLC) using silica gel 60 F₂₅₄ plates. Silica gel (0.063–0.200 mm) was used for column chromatography of the products. Melting points were determined with a Boetius apparatus. ¹H and ¹³C NMR spectra were recorded on a VNMRS 400 MHz spectrometer or an INOVA 500 MHz spectrometer with the use of CDCl₃ or CD₃OD as internal standard. Mass spectrometric data were obtained with a Finnigan MAT SSQ 710 C (ESI–MS) or were recorded on an AMD 402 (70 eV) spectrometer (EI–MS). High-resolution mass spectra (HRMS) were recorded on a Thermo Fisher Scientific LTQ-Orbitrap mass spectrometer with static nano-electrospray ionization. Elemental analyses were conducted using a Leco CHNS-932.

Synthetic Procedures

2-[(16-Benzyloxyhexadec-1-yl)oxy]tetrahydro-2H-pyran (THPO-C16-OBn, 4):

A solution of 3.2 g (80 mmol, 60% in paraffin oil) NaH in dry toluene (100 ml) was stirred under argon atmosphere. Within 30 min, a solution of 8.3 ml benzyl alcohol (8.65 g, 80 mmol, freshly distilled) in toluene (30 ml) was added while stirring, which was continued for 18 h at room temperature. Afterwards, 13.0 g of 2-[(16-bromohexadec-1-yl)oxy]tetrahydro-2H-pyran^[1] (THPO-C16-Br, **3**, 32 mmol) dissolved in dry toluene (75 ml) and catalytic amounts of *n*-tetrabutylammonium iodide (TBAI) were added and the mixture was headed to reflux for 20 h. For work-up the mixture was poured into a cold saturated solution of NH₄Cl (150 ml) and was extracted several times with Et₂O (100 ml). The combined organic phases were washed with brine, dried over Na₂SO₄ and the solvent was evaporated under reduced pressure. The purification was carried out by chromatography using a heptane/CHCl₃ gradient (+0.5% triethylamine, TEA) as eluent to obtain 9.7 g (70% yield) of THPO-C16-OBn (**4**) as white waxy solid.

(16-Benzyloxyhexadec-1-yl)bromide (Br-C16-OBn, 5):

Triphenylphosphane (15.7 g, 60 mmol) was dissolved in dry CH₂Cl₂ (150 ml) and 9.6 g bromine (3.1 ml, 60 mmol), diluted in CH₂Cl₂ (50 ml), was added slowly into the solution whilst stirring at 0 °C. THPO-C16-OBn (**4**, 11.6 g, 26.8 mmol), dissolved in CH₂Cl₂ (50 ml), was added and the mixture was stirred overnight at room temperature. Afterwards, the organic layer was washed with H₂O (2× 200 ml), dried over Na₂SO₄, evaporated, and the crude bromide was purified by column chromatography with heptane as eluent yielding 10.0 g (91%) of Br-C16-OBn (**5**) as white solid.

2-[(32-Benzyloxydotriacont-1-yl)oxy]tetrahydro-2H-pyran (THPO-C32-OBn, 6):

A solution of 12.7 g 2-[(16-bromohexadec-1-yl)oxy]tetrahydro-2H-pyran^[1] (THPO-C16-Br, **3**, 31.2 mmol) in dry THF (75 ml) was added dropwise with stirring to magnesium turnings (1.22 g, 50 mmol) under argon atmosphere. After the exothermic reaction had subsided, the mixture was stirred at 45 °C for 3 h. The excess magnesium was removed under argon and the Grignard solution was cooled to –5 °C. A freshly prepared solution of Li₂CuCl₄ (0.1M in THF, 3.0 ml) was added with stirring. After a solution of 9.94 g Br-C16-OBn (**5**, 24.2 mmol) in dry THF (70 ml) was added in one portion, stirring was continued for further 3 h at about 0 °C. For work-up Et₂O (150 ml) was added and the resulting mixture was poured into a cold saturated solution of NH₄Cl (150 ml). The organic layer was separated and the aqueous phase was extracted with Et₂O (2× 50 ml). The combined organic phases were washed with H₂O and brine, dried over Na₂SO₄ and concentrated to dryness under reduced pressure. The crude product was recrystallised from acetone in order to remove the short-chain by-products. The final purification was carried out by chromatography using a heptane/CHCl₃ gradient (+0.5% TEA) as eluent to obtain 8.7 g (55%) of THPO-C32-OBn (**6**) as white solid.

32-Benzoyloxydotriacontan-1-ol (HO-C32-OBn, 7):

For cleavage of the THP protecting group 8.7 g of the THPO-C32-OBn (**6**, 13.24 mmol) was dissolved in dry MeOH (120 ml) and heated under reflux for 3 h with catalytic amounts of pyridinium *p*-toluenesulfonate (PPTS). The hot suspension was filtered and the white residue was recrystallised from heptane to give the pure HO-C32-OBn (**7**) as white crystals in 91% yield (7.13 g).

32-Benzoyloxydotriacontane-1-yl-{2-[N-(3-dimethylaminopropyl)-N,N-dimethylammonio]ethylphosphate} (DMAPPC-C32-OBn, 8):

The reaction procedure is based on the general synthesis of headgroup modified dotriacontane-1,32-diylbis(phosphocholines) described previously.^[1-2] The crude product was used without further purification.

32-Hydroxydotriacontane-1-yl-{2-[N-(3-dimethylaminopropyl)-N,N-dimethylammonio]ethylphosphate} (DMAPPC-C32-OH, 9):

The benzyl protection group of DMAPPC-C32-OBn (**8**) was cleaved by hydrogenation in EtOH (HPLC-grade) with catalytic amounts of Pd on carbon (10%) and H₂ (5 bar). After 1 h, the catalyst was removed by filtration and the crude product was purified by column chromatography using CHCl₃/MeOH/H₂O as eluent and gradient technique to give DMAPPC-C32-OH (**9**) as white substance (40% with respect to compound **7**).

32-[[Hydroxy(2-hydroxyethoxy)phosphoryl]oxy]dotriacontane-1-yl-{2-[N-(3-dimethylaminopropyl)-N,N-dimethylammonio]ethylphosphate} (DMAPPC-C32-POH, 2a):

This compound can either be obtained as by-product during the synthesis and chromatographic purification of the symmetrical DMAPPC-C32-DMAPPC described previously^[2] or it can be synthesized from the DMAPPC-C32-OH (**9**) using the phosphorylation reaction^[1] with 2-bromoethylphosphoric acid dichloride. For the second pathway, 0.48 g 2-bromoethylphosphoric acid dichloride (2 mmol) was poured into dry CHCl₃ (10 ml) under cooling with ice-water. A solution of 0.5 ml TEA in 10 ml CHCl₃ was added slowly and the resulting mixture was stirred for 30 min at 0 °C. Subsequent, the DMAPPC-C32-OH (**9**) was added in one portion and the mixture was gently heated until the alcohol has completely dissolved. The stirring was continued for 24 h at 25 °C. Afterwards, crushed ice was added to the solution and the mixture was stirred vigorously for another 2 h. The organic layer was separated and the aqueous phase was extracted with CHCl₃ (3 × 15 ml). The combined organic layers were evaporated and the crude bromoethyl ester was dissolved in 10 ml THF/H₂O (9/1, v/v) with K₂CO₃ and stirred for 10 h at room temperature. For workup, the complete mixture was concentrated under reduced pressure and the product was purified by column chromatography using CHCl₃/MeOH/H₂O as eluent and gradient technique to give DMAPPC-C32-POH (**2a**) as white substance.

Characterization of Products

32-[[Hydroxy(2-hydroxyethoxy)phosphoryl]oxy]dotriacontane-1-yl-{2-[N-(3-dimethylaminopropyl)-N,N-dimethylammonio]ethylphosphate} (DMAPPC-C32-POH, 2a).

R_f = 0.54 ($\text{CHCl}_3/\text{MeOH}/\text{NH}_3$, 5/5/1, v/v/v); ^1H NMR (500 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$): δ = 1.15-1.28 (m, 56H, $-(\text{CH}_2)_{28}-$), 1.48-1.54 (m, 4H, $-\text{OCH}_2\text{CH}_2(\text{CH}_2)_{28}\text{CH}_2\text{CH}_2\text{O}-$), 2.09-2.16 (m, 2H, $-\text{NCH}_2\text{CH}_2\text{CH}_2\text{N}-$), 2.62 (s, 6H, $(\text{H}_3\text{C})_2\text{NCH}_2-$), 2.83-2.91 (m, 2H, $(\text{H}_3\text{C})_2\text{NCH}_2-$), 3.08 (s, 6H, $-\text{CH}_2\text{N}(\text{CH}_3)_2\text{CH}_2-$), 3.29-3.43 (m, 2H, $(\text{H}_3\text{C})_2\text{NCH}_2\text{CH}_2\text{CH}_2\text{N}-$), 3.49-3.51 (m, 2H, $-\text{NCH}_2\text{CH}_2\text{O}-$), 3.60-3.62 (m, 2H, $-\text{OCH}_2\text{CH}_2\text{OH}$), 3.72-3.77 (m, 4H, $-\text{OCH}_2(\text{CH}_2)_{30}\text{CH}_2\text{O}-$), 3.80-3.85 (m, 2H, $-\text{OCH}_2\text{CH}_2\text{OH}$), 4.08-4.12 ppm (m, 2H, $-\text{NCH}_2\text{CH}_2\text{O}-$); ^{31}P NMR (161.9 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$): δ = 1.455, 0.012 ppm; ESI-MS: m/z = 842.1 $[\text{M} - \text{H}]^-$, 844.8 $[\text{M} + \text{H}]^+$, 866.1 $[\text{M} + \text{Na}]^+$; anal. calcd (%) for $\text{C}_{43}\text{H}_{92}\text{N}_2\text{O}_9\text{P}_2 \times 2\text{H}_2\text{O}$: C 58.74, H 11.00, N 3.19; found: C 58.45, H 10.83, N 3.01. HRMS: calcd for $\text{C}_{43}\text{H}_{93}\text{N}_2\text{O}_9\text{P}_2$ $[\text{M} + \text{H}]^+$ 843.6351, found 843.6356; $\text{C}_{43}\text{H}_{92}\text{N}_2\text{O}_9\text{NaP}_2$ $[\text{M} + \text{Na}]^+$ 865.6170, found 865.6176.

32-[[Hydroxy(2-hydroxyethoxy)phosphoryl]oxy]dotriacontane-1-yl-{2-[N-(2-dimethylaminoethyl)-N,N-dimethylammonio]ethylphosphate} (DMAEPC-C32-POH, 2b).

R_f = 0.54 ($\text{CHCl}_3/\text{MeOH}/\text{NH}_3$, 5/5/1, v/v/v); ^1H NMR (500 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$): δ = 1.14-1.26 (m, 56H, $-(\text{CH}_2)_{28}-$), 1.47-1.53 (m, 4H, $-\text{OCH}_2\text{CH}_2(\text{CH}_2)_{28}\text{CH}_2\text{CH}_2\text{O}-$), 2.47 (s, 6H, $(\text{H}_3\text{C})_2\text{NCH}_2-$), 3.04-3.12 (bm, 2H, $(\text{H}_3\text{C})_2\text{NCH}_2-$), 3.13 (s, 6H, $-\text{CH}_2\text{N}(\text{CH}_3)_2\text{CH}_2-$), 3.50-3.52 (m, 2H, $-\text{NCH}_2\text{CH}_2\text{O}-$), 3.58-3.63 (m, 4H, $-\text{OCH}_2\text{CH}_2\text{OH}$, $(\text{H}_3\text{C})_2\text{NCH}_2\text{CH}_2\text{N}-$), 3.71-3.76 (m, 4H, $-\text{OCH}_2(\text{CH}_2)_{30}\text{CH}_2\text{O}-$), 3.79-3.82 (m, 2H, $-\text{OCH}_2\text{CH}_2\text{OH}$), 4.09-4.13 ppm (m, 2H, $-\text{NCH}_2\text{CH}_2\text{O}-$); ^{31}P NMR (161.9 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$): δ = -0.315, -0.562 ppm; anal. calcd (%) for $\text{C}_{42}\text{H}_{90}\text{N}_2\text{O}_9\text{P}_2 \times 2\text{H}_2\text{O}$: C 58.31, H 10.95, N 3.24; found: C 58.37, H 10.56, N 3.41. ESI-MS: m/z = 828.0 $[\text{M} - \text{H}]^-$, 830.0 $[\text{M} + \text{H}]^+$, 852.0 $[\text{M} + \text{Na}]^+$. HRMS: calcd for $\text{C}_{42}\text{H}_{91}\text{N}_2\text{O}_9\text{P}_2$ $[\text{M} + \text{H}]^+$ 829.6194, found 829.6208.

32-[[Hydroxy(2-hydroxyethoxy)phosphoryl]oxy]dotriacontane-1-yl-[2-(N,N-dimethyl-N-prop-2-yn-1-yl-ammonio)ethylphosphate] (PPC-C32-POH, 2c).

R_f = 0.59 ($\text{CHCl}_3/\text{MeOH}/\text{NH}_3$, 5/5/1, v/v/v); ^1H NMR (500 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$): δ = 1.08-1.17 (m, 56H, $-(\text{CH}_2)_{28}-$), 1.40-1.47 (m, 4H, $-\text{OCH}_2\text{CH}_2(\text{CH}_2)_{28}\text{CH}_2\text{CH}_2\text{O}-$), 2.92 (t, 4J = 2.5 Hz, 1H, $\text{HC}\equiv\text{CCH}_2\text{N}$), 3.09 (s,

6H, 2× -CH₃), 3.49-3.54 (m, 4H, NCH₂CH₂O-, -OCH₂CH₂OH), 3.64-3.70 (m, 4H, -OCH₂(CH₂)₃₀CH₂O-), 3.72-3.76 (m, 2H, -OCH₂CH₂OH), 4.04-4.08 (m, 2H, -NCH₂CH₂O-), 4.21 ppm (d, ⁴J = 2.5 Hz, 2H, HC≡CCH₂N); ¹³C NMR (100 MHz, CDCl₃/CD₃OD): δ = 24.99, 25.20, 28.67, 28.80, 28.97, 29.02, 29.05, 29.06, 29.07, 29.84 (d, ³J_{C,P} = 7.2 Hz, POCH₂CH₂CH₂), 30.17 (d, ³J_{C,P} = 7.2 Hz, POCH₂CH₂CH₂), 50.61 (CH₃), 54.80 (b, HC≡CCH₂), 58.20 (d, ²J_{C,P} = 4.6 Hz, NCH₂CH₂O), 60.88 (d, ³J_{C,P} = 7.2 Hz, OCH₂CH₂OH), 63.69 (d, ²J_{C,P} = 6.5 Hz, OCH₂CH₂OH), 65.49 (d, ²J_{C,P} = 6.1 Hz, POCH₂(CH₂)₃₁O), 66.44 (d, ²J_{C,P} = 6.1 Hz, POCH₂(CH₂)₃₁O), 67.40 (d, ³J_{C,P} = 5.7 Hz, NCH₂CH₂O), 70.41 (HC≡C), 81.41 ppm (HC≡C); ³¹P NMR (161.9 MHz, CDCl₃/CD₃OD): δ = 0.127, -0.156 ppm; ³¹P NMR (161.9 MHz, CDCl₃/CD₃OD): δ = 0.127, -0.156 ppm; ESI-MS: *m/z* = 794.5 [M - H]⁻, 818.6 [M + Na]⁺; anal. calcd (%) for C₄₁H₈₃NO₉P₂×2H₂O: C 59.18, H 10.58, N 1.68; found: C 59.35, H 10.71, N 1.87. HRMS: calcd for C₄₁H₈₄NO₉P₂Na [M + H + Na]²⁺ 409.7754, found 409.7754; C₄₁H₈₄NO₉P₂ [M + H]⁺ 796.5616, found 796.5619.

32-[[Hydroxy(2-hydroxyethoxy)phosphoryl]oxy]dotriacontane-1-yl-[2-(N-allyl-N,N-dimethylammonio)-ethylphosphate] (APC-C32-POH, 2d).

R_f = 0.61 (CHCl₃/MeOH/NH₃, 5/5/1, v/v/v); ¹H NMR (500 MHz, CDCl₃/CD₃OD): δ = 1.15-1.27 (m, 56H, -(CH₂)₂₈-), 1.48-1.58 (m, 4H, -OCH₂CH₂(CH₂)₂₈CH₂CH₂O-), 3.03 (s, 6H, 2× -CH₃), 3.45-3.46 (m, 2H, -OCH₂CH₂OH), 3.62-3.65 (m, 2H, NCH₂CH₂O-), 3.75-3.80 and 3.83-3.88 (2m, 4H, -OCH₂(CH₂)₃₀CH₂O-), 3.91 (d, ²J = 7.3 Hz, 2H, NCH₂CH=CH₂), 3.92-3.94 (m, 2H, -OCH₂CH₂OH), 4.13-4.19 ppm (m, 2H, -NCH₂CH₂O-), 5.60-5.67 (m, 2H, NCH₂CH=CH₂), 5.89 (ddt, ³J[E] = 17.3 Hz, ³J[Z] = 10.3 Hz, ³J = 7.3 Hz, 1H, NCH₂CH=CH₂); ¹³C NMR (100 MHz, CDCl₃/CD₃OD): δ = 25.01, 25.24, 28.70, 28.85, 29.02, 29.06, 29.08, 29.10, 29.11, 29.13, 29.15, 29.85 (d, ³J_{C,P} = 7.2 Hz, POCH₂CH₂CH₂), 30.21 (d, ³J_{C,P} = 7.2 Hz, POCH₂CH₂CH₂), 50.30 (CH₃), 58.23 (d, ²J_{C,P} = 7.2 Hz, NCH₂CH₂O), 60.86 (d, ³J_{C,P} = 7.2 Hz, OCH₂CH₂OH), 63.52 (b, OCH₂CH₂OH), 65.60 (d, ²J_{C,P} = 6.1 Hz, POCH₂(CH₂)₃₁O), 66.69 (d, ²J_{C,P} = 5.7 Hz, POCH₂(CH₂)₃₁O), 67.42 (b, NCH₂CH=CH₂), 67.63 (d, ³J_{C,P} = 5.7 Hz, NCH₂CH₂O), 123.85 (CH=CH₂), 129.12 ppm (CH=CH₂); ³¹P NMR (161.9 MHz, CDCl₃/CD₃OD): δ = -0.181, -0.199 ppm; ESI-MS: *m/z* = 796.9 [M - H]⁻, 799.6 [M + H]⁺, 821.2 [M + Na]⁺; anal. calcd (%) for C₄₁H₈₅NO₉P₂×2H₂O: C 59.04, H 10.76, N 1.68; found: C 59.40, H 10.90, N 1.96. HRMS: calcd for C₄₁H₈₆NO₉P₂Na [M + H + Na]²⁺ 410.7832, found 410.7831; C₄₁H₈₆NO₉P₂ [M + H]⁺ 798.5772, found 798.5773.

2-[(16-Benzyloxyhexadec-1-yl)oxy]tetrahydro-2H-pyran (THPO-C16-OBn, 4).

M.p.: 31 °C; *R_f* = 0.25 (CHCl₃/heptane, 3/2, v/v); ¹H NMR (400 MHz, CDCl₃): δ = 1.29-1.40 (m, 24H, -(CH₂)₁₂-), 1.53-1.67 (m, 8H, 4× -CH₂CH₂O-), 1.71-1.77 (m, 1H, -CHOCH₂CH₂(CH₂)₁₄O-), 1.83-1.91 (m, 1H, -CHOCH₂CH₂(CH₂)₁₄O-), 3.41 (dt, ²J = 9.6 Hz, ³J = 6.7 Hz, 1H, -CHOCH₂(CH₂)₁₅O-), 3.49 (t, ³J = 6.7

Hz, 2H, -CHO(CH₂)₁₅CH₂O-), 3.51-3.55 (m, 1H, -OCH₂(CH₂)₃CH-), 3.76 (dt, ²*J* = 9.6 Hz, ³*J* = 6.8 Hz, 1H, -CHOCH₂(CH₂)₁₅O-), 3.88-3.93 (m, 1H, -OCH₂(CH₂)₃CH-), 4.53 (s, 2H, -CH₂C₆H₅), 4.60-4.61 (m, 1H, -OCHO-), 7.28-7.38 ppm (m, 5H, -C₆H₅); ¹³C NMR (100 MHz, CDCl₃): δ = 19.66, 25.50, 26.17, 26.22, 29.47, 29.57, 29.59, 29.64, 29.65, 29.74, 29.75, 30.77 (-CH₂CH-), 62.26 (-OCH₂(CH₂)₃CH-), 67.65 (-CHOCH₂(CH₂)₁₅-), 70.50 (-OCH₂C₆H₅), 72.81 (-CH₂OCH₂C₆H₅), 98.78 (-CH-), 127.38, 127.54, 128.26, 138.71 ppm; EI-MS (70 eV): *m/z* (%) = 432 (5) [M]⁺; anal. calcd (%) for C₂₈H₄₈O₃ (432.68): C 77.72, H 11.18; found: C 77.37, H 11.31.

(16-Benzyloxyhexadec-1-yl)bromide (Br-C16-OBn, 5).

M.p.: 36 °C; *R_f* = 0.55 (CHCl₃/heptane, 3/2, v/v); ¹H NMR (400 MHz, CDCl₃): δ = 1.26-1.46 (m, 24H, -(CH₂)₁₂-), 1.58-1.65 (m, 2H, -CH₂CH₂O-), 1.82-1.89 (m, 2H, BrCH₂CH₂-), 3.41 (t, ³*J* = 6.8 Hz, 2H, BrCH₂-), 3.47 (t, ³*J* = 6.7 Hz, 2H, -CH₂OCH₂C₆H₅), 4.51 (s, 2H, -CH₂C₆H₅), 7.25-7.35 ppm (m, 5H, -C₆H₅); ¹³C NMR (100 MHz, CDCl₃): δ = 26.20, 28.18, 28.77, 29.44, 29.49, 29.54, 29.60, 29.64, 29.78, 32.85, 34.00, 70.54 (-OCH₂C₆H₅), 72.84 (-CH₂OCH₂C₆H₅), 127.42, 127.58, 128.31, 138.74 ppm; EI-MS (70 eV): *m/z* (%) = 410/412 (2) [M]⁺; anal. calcd (%) for C₂₃H₃₉OBr (411.46): C 67.14, H 9.55; found: C 66.93, H 9.78.^[3-5]

2-[(32-Benzyloxydotriacont-1-yl)oxy]tetrahydro-2H-pyran (THPO-C32-OBn, 6).

M.p.: 62-64 °C; *R_f* = 0.22 (CHCl₃/heptane, 3/2, v/v); ¹H NMR (400 MHz, CDCl₃): δ = 1.24-1.37 (m, 56H, -(CH₂)₂₈-), 1.47-1.63 (m, 8H, 4× -CH₂CH₂O-), 1.66-1.73 (m, 1H, -CHOCH₂CH₂(CH₂)₃₀O-), 1.77-1.85 (m, 1H, -CHOCH₂CH₂(CH₂)₃₀O-), 3.36 (dt, ²*J* = 9.6 Hz, ³*J* = 6.7 Hz, 1H, -CHOCH₂(CH₂)₃₁O-), 3.44 (t, ³*J* = 6.7 Hz, 2H, -CHO(CH₂)₃₁CH₂O-), 3.45-3.51 (m, 1H, -OCH₂(CH₂)₃CH-), 3.71 (dt, ²*J* = 9.6 Hz, ³*J* = 6.8 Hz, 1H, -CHOCH₂(CH₂)₃₁O-), 3.83-3.88 (m, 1H, -OCH₂(CH₂)₃CH-), 4.48 (s, 2H, -CH₂C₆H₅), 4.55-4.57 (m, 1H, -OCHO-), 7.23-7.33 ppm (m, 5H, -C₆H₅); ¹³C NMR (100 MHz, CDCl₃): δ = 19.71, 25.53, 26.21, 26.26, 29.50, 29.61, 29.62, 29.67, 29.71, 29.78, 29.79, 30.80 (-CH₂CH-), 62.32 (-OCH₂(CH₂)₃CH-), 67.70 (-CHOCH₂(CH₂)₁₅-), 70.55 (-OCH₂C₆H₅), 72.85 (-CH₂OCH₂C₆H₅), 98.83 (-CH-), 127.42, 127.59, 128.31, 138.75 ppm; EI-MS (70 eV): *m/z* (%) = 657 (12) [M]⁺; anal. calcd (%) for C₄₄H₈₀O₃ (657.10): C 80.42, H 12.27; found: C 80.81, H 12.15.

32-Benzyloxydotriacontan-1-ol (HO-C32-OBn, 7).

M.p.: 86-88 °C; *R_f* = 0.48 (CHCl₃/Et₂O, 4/1, v/v); ¹H NMR (500 MHz, CDCl₃): δ = 1.24-1.35 (m, 56H, -(CH₂)₂₈-), 1.52-1.62 (m, 4H, 2× -CH₂CH₂O-), 3.44 (t, ³*J* = 6.6 Hz, 2H, HOCH₂-), 3.62 (t, ³*J* = 6.6 Hz, 2H,

-CH₂OCH₂C₆H₅), 4.48 (s, 2H, -CH₂C₆H₅), 7.23-7.32 ppm (m, 5H, -C₆H₅); ¹³C NMR (125 MHz, CDCl₃): δ = 25.75, 26.21, 29.45, 29.50, 29.62, 29.67, 29.71, 29.79, 32.84 (HOCH₂CH₂-), 63.12 (HOCH₂-), 70.55 (-OCH₂C₆H₅), 72.86 (-CH₂OCH₂C₆H₅), 127.44, 127.61, 128.32, 138.75 ppm; EI-MS (70 eV): *m/z* (%) = 572 (23) [M]⁺; anal. calcd (%) for C₃₉H₇₂O₂ (572.99): C 81.75, H 12.67; found: C 81.37, H 12.49.

32-Hydroxydotriacontane-1-yl-{2-[N-(3-dimethylaminopropyl)-N,N-dimethylammonio]ethylphosphate} (DMAPPC-C32-OH, 9).

M.p.: 115-118 °C; *R_f* = 0.25 (CHCl₃/MeOH/NH₃, 5/5/1, v/v/v); ¹H NMR (400 MHz, CDCl₃/CD₃OD): δ = 1.23-1.35 (m, 56H, -(CH₂)₂₈-), 1.47-1.54 (m, 2H, -CH₂CH₂CH₂O-), 1.56-1.64 (m, 2H, -CH₂CH₂CH₂O-), 2.13-2.21 (m, 2H, -NCH₂CH₂CH₂N-), 2.70 (s, 6H, (H₃C)₂N-), 2.93 (t, *J* = 7.4 Hz, 2H, (H₃C)₂NCH₂-), 3.17 (s, 6H, -CH₂N(CH₃)₂CH₂-), 3.46-3.51 (m, 2H, (H₃C)₂NCH₂CH₂CH₂N-), 3.52 (t, *J* = 6.8 Hz, 2H, -CH₂OH), 3.59-3.62 (m, 2H, -NCH₂CH₂O-), 3.83 ("quar", *J* = 6.6 Hz, 2H, -OCH₂(CH₂)₃₁OH), 4.17-4.22 ppm (m, 2H, -NCH₂CH₂O-); ¹³C NMR (100 MHz, CDCl₃/CD₃OD): δ = 18.90, 25.30 (d, -POCH₂CH₂CH₂-), 28.89, 29.00, 29.12, 29.14, 29.15, 29.15, 29.17, 29.18, 29.19, 30.26 (d, -POCH₂CH₂(CH₂)₃₀OH), 32.02 (-CH₂CH₂OH), 36.56, 42.94 ((H₃C)₂NCH₂CH₂CH₂-), 51.74 (t, -CH₂N(CH₃)₂CH₂-), 53.86 (b, (H₃C)₂NCH₂CH₂CH₂-), 58.41 (d, NCH₂CH₂O-), 61.35 (b, NCH₂CH₂O-), 61.75 ((H₃C)₂NCH₂CH₂CH₂-), 62.80 (b, -CH₂OH), 65.61 ppm (d, -POCH₂(CH₂)₃₁OH); ³¹P NMR (161.9 MHz, CDCl₃/CD₃OD): δ = -0.233 ppm; ESI-MS: *m/z* = 719.53 [M + H]⁺, 742.57 [M + Na]⁺, 1438.07 [2M + H]⁺; anal. calcd (%) for C₄₁H₈₇N₂O₅P×2H₂O: C 65.21, H 12.15, N 3.71; found: C 65.46, H 12.31, N 3.85. HRMS: calcd for C₄₁H₈₈N₂O₅P [M + H]⁺ 719.6425, found 719.6411.

^1H , ^{13}C and ^{31}P NMR Spectra, MS and HRMS of Products

32-[[Hydroxy(2-hydroxyethoxy)phosphoryl]oxy]dotriacontane-1-yl-{2-[N-(3-dimethylaminopropyl)-N,N-dimethylammonio]ethylphosphate} (DMAPPC-C32-POH, 2a).

ESI-MS, positive mode

ESI-MS, negative mode

D:\Data\Service\lsd-16-a-4
Drescher

09/05/06 09:51:42 AM

LSD-16/a - 955,3

S#: 1-5 RT: 0.01-0.15 AV: 5 NL: 1.57E7
T: - c Full ms [50.00 - 2000.00]

E:\Data\...DMAPPC-C32-POH4_120720145659

7/31/2012 10:10:51 AM

DMAPPC-C32-POH4

T: FTMS + p NSI Full ms [50.00-2000.00]

RT: 5.47 AV: 1 NL: 5.07E5

¹H NMR

H,H-COSY NMR

Drescher_8931-32_LSD-16/a/OH

Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory: auto_130ct2006

Pulse Sequence: COSY

Solvent: cdc13
Temp: 27.0 C / 300.1 K
Sample #3, user vnmr1
File: OH03
INOVA-550 "max2"

Relax. delay 1.000 sec
Acq. time 0.216 sec
Width 4743.8 Hz
2D Width 4743.8 Hz
8 repetitions
200 increments
OBSERVE H1, 499.7768284 MHz
DATA PROCESSING
Sq. sine bell 0.108 sec
F1 DATA PROCESSING
Sq. sine bell 0.042 sec
FT size 2048 x 2048

<p>PULSE SEQUENCE</p> <p>Relax. delay 1.000 sec Pulse 45.0 degrees Acq. time 1.363 sec Width 48076.9 Hz 1024 repetitions</p>	<p>OBSERVE F31, 161.9073137 DECOUPLE H1, 399.9633287 Power 39 dB on during acquisition off during delay WALTZ-16 modulated</p>	<p>DATA PROCESSING</p> <p>Line broadening 0.5 Hz FT size 262144 Total time 40 minutes</p>	<p>Drescher_5583-84 SD-16-a-OH</p>	<p>Solvent: cd3od Temp. 27.0 C / 300.1 K Sample #27, Operator: vmmr1 File: Drescher_5583-84_PHOSPHORUS_09Jul1 VNMR-400 "lampe"</p>
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32-[[Hydroxy(2-hydroxyethoxy)phosphoryl]oxy]dotriacontane-1-yl-{2-[N-(2-dimethylaminoethyl)-N,N-dimethylammonio]ethylphosphate} (DMAEPC-C32-POH, 2b).

ESI-MS, positive mode

ESI-MS, negative mode

¹H NMR

32-[[Hydroxy(2-hydroxyethoxy)phosphoryl]oxy]dotriacontane-1-yl-[2-(N,N-dimethyl-N-propinyl-ammonio)ethylphosphate] (PPC-C32-POH, 2c).

ESI-MS, positive mode

ESI-MS, negative mode

Drescher_5585
SD-30-a-OH

Sample Name:
Drescher_5585
Data Collected on:
lamps-vnmrs400
Archive directory:
/home/vnmr1/data/07_2013
Sample directory:
Drescher_5585
FidFile: Drescher_5585_CARBON_09Jul2013_01

Pulse Sequence: CARBON (s2pul)
Solvent: cd3od
Data collected on: Jul 9 2013

Temp. 27.0 C / 300.1 K
Sample #28, Operator: vnmr1

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.311 sec
Width 25000.0 Hz
5000 repetitions
OBSERVE C13, 100.5703561 MHz
DECOUPLE H1, 399.9633287 MHz
Power 39 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 131072
Total time 3 hr, 12 min

<p>PULSE SEQUENCE</p> <p>Relax. delay 1.000 sec</p> <p>Pulse 45.0 degrees</p> <p>Acq. time 1.363 sec</p> <p>Width 48076.9 Hz</p> <p>512 repetitions</p>	<p>OBSERVE P31, 161.9073137</p> <p>DECOUPLE H1, 399.9633287</p> <p>Power 39 dB</p> <p>on during acquisition</p> <p>off during delay</p> <p>WALTZ-16 modulated</p>	<p>DATA PROCESSING</p> <p>Line broadening 0.5 Hz</p> <p>FT size 262144</p> <p>Total time 20 minutes</p>	<p>Drescher 5480</p> <p>SD-30-a-OH</p> <p>Solvent: cd3od</p> <p>Temp. 27.0 C / 300.1 K</p> <p>Sample #32, Operator: vmmr1</p> <p>File: Drescher_5480_PHOSPHORUS_05Jul201</p> <p>VNMR-400 "lampe"</p>
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32-[[Hydroxy(2-hydroxyethoxy)phosphoryl]oxy]dotriacontane-1-yl-[2-(N-allyl-N,N-dimethylammonio)-ethylphosphate] (APC-C32-POH, 2d).

ESI-MS, positive mode

ESI-MS, negative mode

Drescher_5581
SD-14-a-OH

Sample Name:
Drescher_5581
Data Collected on:
lamps-vnmrs400
Archive directory:
/home/vnmr1/data/07_2013
Sample directory:
Drescher_5581
FidFile: Drescher_5581_CARBON_08Jul2013_01

Pulse Sequence: CARBON (s2pul)
Solvent: cd3od
Data collected on: Jul 8 2013

Temp. 27.0 C / 300.1 K
Sample #25, Operator: vnmr1

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.311 sec
Width 25000.0 Hz
2000 repetitions
OBSERVE C13, 100.5703561 MHz
DECOUPLE H1, 399.9633287 MHz
Power 39 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 131072
Total time 1 hr, 17 min

2-[(16-Benzyloxyhexadec-1-yl)oxy]tetrahydro-2H-pyran (THPO-C16-OBn, 4)

¹H NMR

¹³C NMR

PULSE SEQUENCE Relax. delay 1.000 sec Pulse 45.0 degrees Acq. time 1.042 sec Width 31446.5 Hz 504 repetitions	OBSERVE C13, 125.6649878 DECOUPLE H1, 499.7634368 Power 41 dB continuously on WALTZ-16 modulated	DATA PROCESSING Line broadening 1.0 Hz FT size 131072 Total time 17 minutes	Drescher Bz-C16-THP Nr. 655 Solvent: cdcl3 Temp. 27.0 C / 300.1 K Sample #3, Operator: vnmr1 INOVA-500 "max2"
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(16-Benzyloxyhexadec-1-yl)bromide (Br-C16-OBn, 5).

^1H NMR

^{13}C NMR

2-[(32-Benzyloxydotriacont-1-yl)oxy]tetrahydro-2H-pyran (THPO-C32-OBn, 6).

¹H NMR

¹³C NMR

32-Benzyloxydotriacontan-1-ol (HO-C32-OBn, 7).

¹H NMR

¹³C NMR

32-Hydroxydotriacontane-1-yl-{2-[N-(3-dimethylaminopropyl)-N,N-dimethylammonio]ethylphosphate}
(DMAPPC-C32-OH, 9).

ESI-MS, positive mode

Drescher_1910-11
LSD-50-b

Sample Name:
Drescher_1910-11
Data Collected on:
lamps-vmrz400
Archive directory:
/home/vmrz1/data/03_2013
Sample directory:
Drescher_1910-11
FidFile: Drescher_1910-11_PROTON_20Mar2013_01

Pulse Sequence: PROTON (s2pul)
Solvent: cd3od
Data collected on: Mar 20 2013

Temp. 27.0 C / 300.1 K
Sample #25, Operator: vmr1

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 5.112 sec
Width 6410.3 Hz
16 repetitions
OBSERVE H1, 399.9613289 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 131072
Total time 1 min 38 sec

Drescher_1910-11
LSD-50-b

Sample Name:

Drescher_1910-11
Data Collected on:
lampe-vnmrs400
Archive directory:
/home/vnmr1/data/03_2013

Sample directory:

Drescher_1910-11
FidFile: Drescher_1910-11_CARBON_20Mar2013_01

Pulse Sequence: CARBON (a2pul)

Solvent: cd3od

Data collected on: Mar 20 2013

Temp. 27.0 C / 300.1 K

Sample #25, Operator: vnmr1

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.311 sec

Width 25000.0 Hz

2000 repetitions

OBSERVE C13, 100.5703561 MHz

DECOUPLE H1, 399.9633287 MHz

Power 39 dB

continuously on

WALTZ-16 modulated

DATA PROCESSING

Line broadening 0.5 Hz

FT size 131072

Total time 1 hr, 17 min

References

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- [4] M. Hanbali, M. Vela-Ruiz, D. Bagnard, B. Luu, *Bioorg. Med. Chem. Letters* **2006**, *16*, 2637-2640.
- [5] B. Mahipal, K. Mallikarjun, S. Chandrasekhar, *Tetrah. Letters* **2012**, *53*, 45-47.