Rapid Access to Synthetic Lysobisphosphatidic Acids Using P^{III} Chemistry

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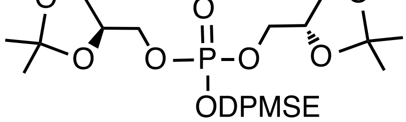
Supporting Information

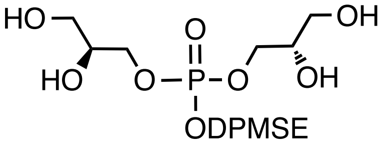
General. Reagents for synthesis were purchased from Aldrich or Fluka. Solvents were dried and distilled before use. All reactions were performed under nitrogen atmosphere. Column chromatography was carried out on silica gel 60 (Fluka, 40-63 µm). Analytical thin layer chromatography TLC was performed on silica gel 60 (Fluka, 0.2 mm). DEAE-Sephadex® A-25 was from Pharmacia Biotech. IR spectra were recorded on a Perkin Elmer Paragon 500 FT-IR Spectrometer using NaCl cells and reported in cm^{-1}. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a Brucker 400 MHz or 500 MHz Spectrometer and reported as chemical shifts (δ) in ppm relative to TMS (δ = 0). Spin multiplicities are reported as singlet (s), doublet (d), triplet (t), quartet (q) or multiplet (m); coupling constants (*J*) are given in Hz. ESI-MS were performed on a Finnigan MAT SSQ 7000. Optical Rotations were measured on Perkin Elmer 241 Polarimeter.

2-(Diphenymethylsilyl)ethyl bis(2,3-*O*-isopropylidene-*sn*-glycero 1-)phosphate (*S,S*-8)



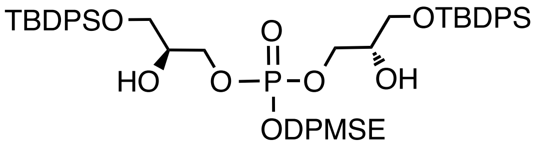
To a solution of phosphorus trichloride (0.8 mmol) in methylenechloride (0.4ml) and diethyl ether (1 ml) at 0˚C a solution of 2-(diphenymethyllsilyl)ethanol (**4**, 0.18 ml, 0.79 mmol) in ether (1 ml) was slowly added. The reaction mixture was stirred for 3 h at rt. Then it was cooled to 0˚C whereupon pyridine (0.2 ml, 2.5 mmol) and a solution of L-α,β-isopropylideneglycerol (*R*-**6**, 0.2 ml, 1.6 mmol) in ether (1 ml) were successively added. After 1h of stirring at rt, a solution of 3-chloroperbenzoic acid (200 mg, 0.81 mmol) in methylenechloride (2 ml) was added to the reaction mixture and it was further stirred for 30 min. Ethylacetate was added to the reaction mixture and the solution was washed successively with 1M HCl aqueous solution, brine, saturated $NaHCO_3$ aqueous solution, and brine. After drying with Na_2SO_4 the organic layer was concentrated *in vacuo*. The residue was chromatographed on silica gel (CH_2Cl_2 : acetone 9 : 1) to give pure title compound (274 mg, 63 %) as a colorless oil : R_f 0.36 (CH_2Cl_2/Acetone 7 / 1); $[\alpha]^{20}_{D}$ –4.0 (*c* 0.58, $CHCl_3$) ; ^{1}H NMR ($CDCl_3$) δ 7.51 (m, 4 H), 7.40 (m, 6 H), 4.27 (m, 2 H), 4.22 (m, 2 H), 4.04 (m, 2 H), ~4.0 (m, 4 H), 3.79 (dd, 2 H, *J* = 5.4, 8.5 Hz), 1.68 (m, 2 H), 1.40 (s, 3 H), 1.39 (s, 3 H), 1.35 (s, 6 H), 0.61 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 135.48 (2 x s), 134.32 (4 x d), 129.62 (2 x d), 128.05 (4 x d), 109.85 (2 x s), 73.98 (d, J_{PC} 8.0 Hz), 73.97 (d, J_{PC} 8.0 Hz), 67.50 (t, J_{PC} 5.7 Hz), 67.45 (t, J_{PC} 6.9 Hz), 66.37 (t, J_{PC} 5.7 Hz), 66.10 (2 x t), 26.71 (2 x q), 25.24 (2 x q), 17.52 (t, J_{PC} 5.7 Hz), -4.10 (q); ^{31}P NMR ($CDCl_3$) δ -3.58; IR (film, cm^{-1}) 2986, 2896, 1372, 1260, 1216, 1156, 1113, 1003, 930, 793, 735, 700; MS (ESI) *m/z* 573 [M+Na$^+$]; Anal. Calcd for $C_{27}H_{39}O_8PSi$: C, 58.89; H, 7.14. Found: C, 56.67; H, 7.00.

2-(Diphenymethylsilyl)ethyl bis(*sn*-glycero 1-)phosphate (*S,S*-9)



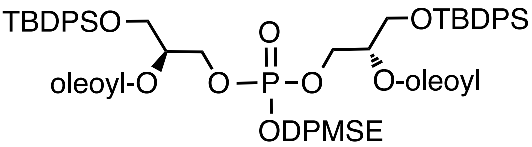
A solution of 2-(diphenymethylsilyl)ethyl bis(2,3-*O*-isopropylidene-*sn*-glycero-1-) phosphate (*S,S*-**8**, 120 mg, 0.22 mmol) in aqueous acetic acid (80 %, 5 ml) was heated at 60˚C for 45 min. The reaction mixture was concentrated in vacuo, and the residue was chromatographed on silica gel (CH_2Cl_2 : MeOH 85 : 15) to give pure title compound (86 mg, 84 %) as a colorless oil : R_f 0.35 (CH_2Cl_2/MeOH 4 / 1); $[\alpha]^{20}_{D}$ 0 (*c* 0.99, $CHCl_3$) ; ^{1}H NMR ($CDCl_3$) δ 7.49 (m, 4 H), 7.37 (m, 6 H), 4.19 (m, 2 H), 4.05 (m, 2 H), 3.97 (m, 2 H), 3.85 (m, 2 H), 3.58 (m, 4 H), 1.65 (m, 2 H), 0.59 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 135.42 (2 x s), 134.30 (4 x d), 129.62 (2 x d), 128.06 (4 x d), 70.60 (d, J_{PC} 5.7 Hz), 70.49 (d, J_{PC} 5.7 Hz), 68.77 (t, J_{PC} 6.9 Hz), 68.60 (t, J_{PC} 6.9 Hz), 66.63 (t, J_{PC} 5.7 Hz), 62.62 (t), 62.56 (t), 17.41 (t, J_{PC} 5.7 Hz), -4.13 (q); IR (film, cm^{-1}) 3367, 2957, 1428, 1254, 1113, 1012, 794, 734, 699; MS (ESI) *m/z* 258 [M+2Na$^+$]; Anal. Calcd for $C_{21}H_{31}O_8PSi$: C, 53.61; H, 6.64. Found: C, 51.88; H, 6.82.

2-(Diphenymethylsilyl)ethyl bis(3-*tert*-butyldiphenylsilyl-*sn*-glycero-1-) phosphate (*S,S*-10)



To a solution of 2-(diphenylmethylsilyl)ethyl bis(*sn*-glycero-1-)phosphate (*S,S*-**9**, 59 mg, 0.13 mmol) in DMF (2 ml) at 0˚C imidazole (52 mg, 0.76 mmol) and *tert*-butyldiphenylchlorosilane (105 mg, 0.38 mmol) were added. After 16 h (over night) of stirring at 4˚C, ethylacetate was added to the reaction mixture and the solution was washed successively with saturated NH_4Cl aqueous solution, and brine. After drying with Na_2SO_4 the organic layer was concentrated *in vacuo*. The residue was chromatographed on silica gel (CH_2Cl_2 : MeOH 30 : 1) to give pure title compound (97 mg, 82 %) as a colorless oil : R_f 0.33 (CH_2Cl_2/MeOH 20 / 1); $[\alpha]^{20}_D$ +5.7 (*c* 0.68, $CHCl_3$) ; ^1H NMR ($CDCl_3$) δ 7.64 (m, 8 H), 7.50 (m, 4 H), 7.38 (m, 18 H), 4.21 (m, 2 H), 4.12 (m, 2 H), 4.07 (m, 2 H), 3.90 (m, 2 H), 3.66 (m, 4 H), 1.66 (m, 2 H), 1.06 (s, 9 H), 1.05 (s, 9 H), 0.58 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 135.50 (8 x d & 2 x s), 134.32 (4 x d), 132.86 (4 x s), 129.89 (4 x d), 129.59 (2 x d), 128.03 (4 x d), 127.81 (8 x d), 70.72 (d, J_{PC} 5.7 Hz), 70.68 (d, J_{PC} 5.0 Hz), 68.97 (t, J_{PC} 6.9 Hz), 68.92 (t, J_{PC} 6.9 Hz), 66.45 (t, J_{PC} 6.9 Hz), 63.86 (2 x t), 26.80 (6 x q), 19.21 (2 x s), 17.50 (t, J_{PC} 5.7 Hz), -4.10 (q); IR (film, cm^{-1}) 3394, 2931, 2858, 1255, 1113, 1008, 939, 794, 739, 701; MS (ESI) *m/z* 969 $[M+Na^+]$

2-(Diphenylmethylsilyl)ethyl bis(3-*tert*-butyldiphenylsilyl-2-oleoyl-*sn*-glycero-1-) phosphate (*S,S*-3)



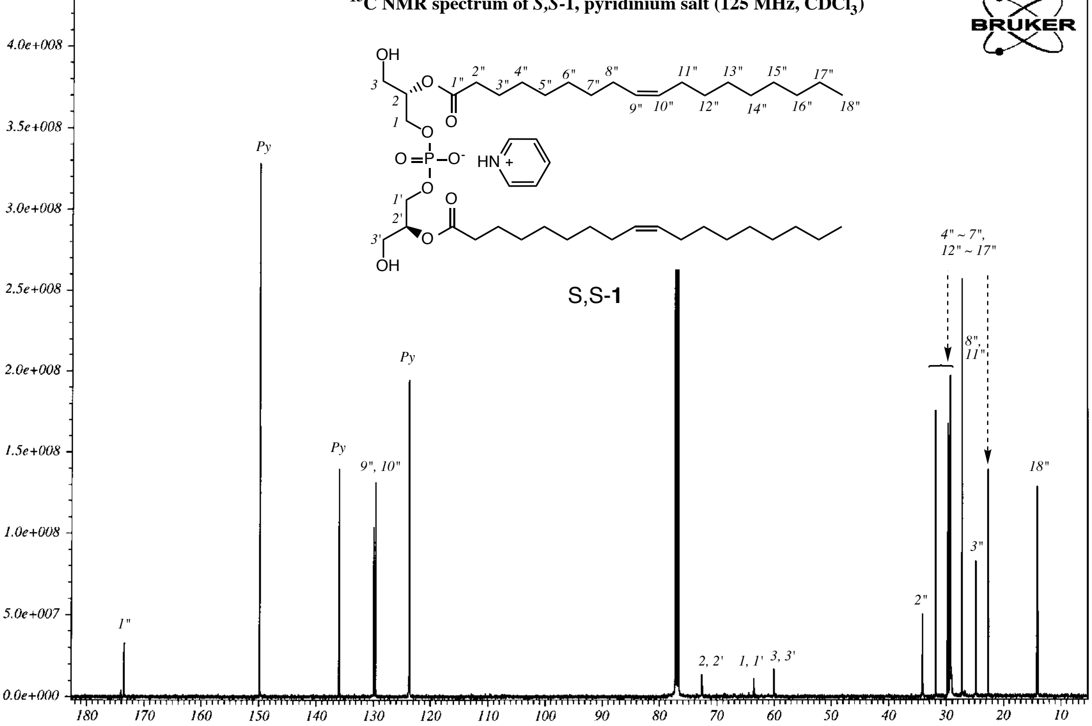
To a solution of 2-(diphenylmethylsilyl)ethyl bis(3-*tert*-butyldiphenylsilyl-*sn*-glycero-1-)phosphate (*S,S*-**10**, 69 mg, 0.074 mmol) in pyridine (2 ml) at rt was added DMAP (18 mg, 0.15 mmol) and oleic anhydride (160 mg, 0.29 mmol). The reaction mixture was stirred for 16 h (overnight). Ether was added to the reaction mixture and the solution was washed successively with 1M HCl aqueous solution, brine, saturated $NaHCO_3$ aqueous solution, and brine. After drying with Na_2SO_4 the organic layer was concentrated *in vacuo*. The residue was chromatographed on silica gel (CH_2Cl_2 : acetone 100 : 1) to give pure title compound (85 mg, 79 %) as a colorless oil : R_f 0.36 (CH_2Cl_2/Acetone 50 / 1); $[\alpha]^{20}_D$ + 6.48 (*c* 1.05, $CHCl_3$) ; ^1H NMR ($CDCl_3$) δ 7.64 (m, 8 H), 7.50 (m, 4 H), 7.38 (m, 18 H), 5.35 (m, 4 H) 5.10(m, 2 H), ~4.20 (m, 6 H), 3.74 (m, 4 H), 2.22 (m, 4 H), 2.02 (m, 8 H), 1.63 (m, 4 H), 1.55 (m, 8 H), 1.28 (m, 48 H), 1.04 (s, 9 H), 1.03 (s, 9 H), 0.89 (t, 6 H, J = 7.0 Hz), 0.56 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 172.81 (2 x s), 135.52 (4 x d), 135.47 (4 x d & 2 x s), 134.29 (4 x d), 132.95 (2 x s), 132.91 (2 x s), 129.97 (2 x d), 129.81 (4 x d), 129.73 (2 x d), 129.57 (2 x d), 128.03 (4 x d), 127.76 (4 x d), 127.74 (4 x d), 71.97 (2 x d, J_{PC} 6.9 Hz), 66.21 (t, J_{PC} 5.8 Hz), 65.57 (t, J_{PC} 5.8 Hz), 65.50 (t, J_{PC} 5.8 Hz), 61.86 (t), 61.83 (t), 34.15 (2 x t), 31.90 (2 x t), 29.77 (2 x t), 29.73 (2 x t), 29.52 (2 x t), 29.32 (4 x t), 29.23 (2 x t), 29.13 (4 x t), 27.22 (2 x t), 27.20 (2 x t), 26.70 (6 x q), 24.77 (2 x t), 22.67 (2 x t), 19.19 (2 x s), 17.51 (t, J_{PC} 5.7 Hz), 14.12 (2 x q), -4.11 (q) ; IR (film, cm^{-1}) 2928, 2856, 1743, 1464, 1428, 1274, 1114, 1007, 794, 738, 701; MS (ESI) *m/z* 1497 $[M + Na^+]$: Anal. Calcd for $C_{89}H_{131}O_{10}PSi$: C, 72.41; H, 8.94. Found: C, 70.76; H, 8.80.

_S,S_-LBPA (_S,S_-1)

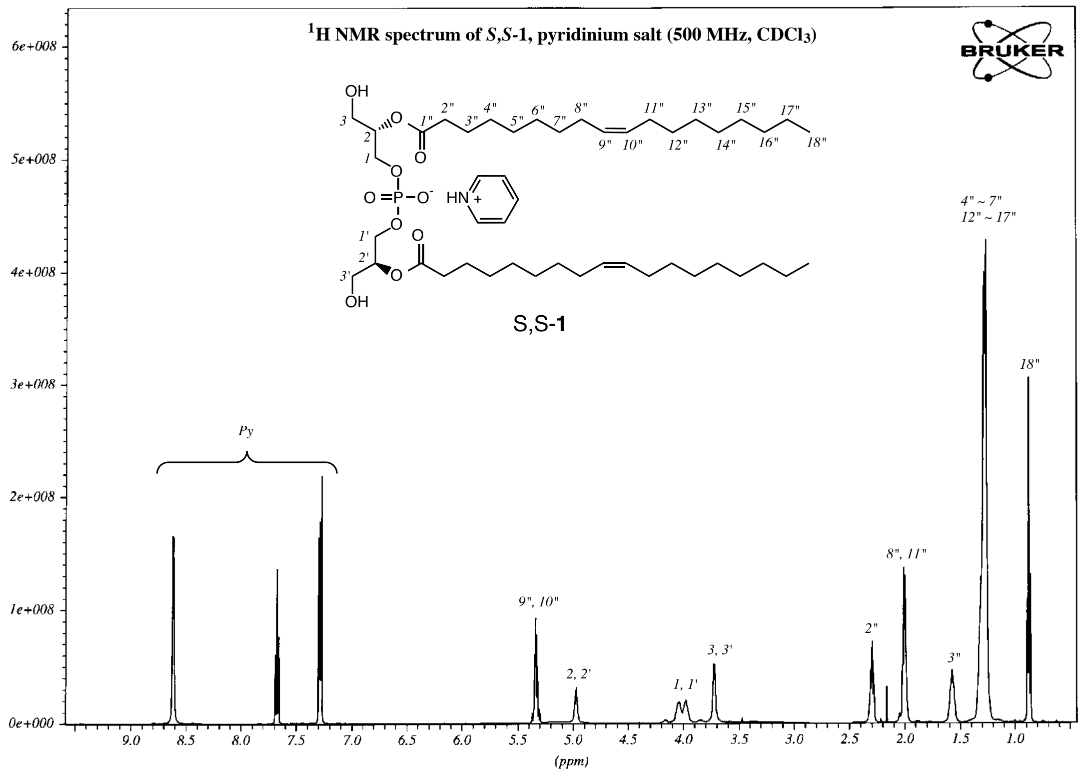
A solution of 2-(Diphenylmethylsilyl)ethyl bis(3-*tert*-butyldiphenylsilyl-2-oleoyl-*sn*-glycero-1-)phosphate (*S,S*-**3**) (28 mg, 19 μmol) in THF (4 ml) was treated successively with acetic acid (21.7 μl, 0.38 mmol) and tetrabutylammoniumfluoride trihydrate (120 mg, 0.38 mmol) at rt. After stirring for 16 h (overnight) the reaction mixture was transferred to separatory funnel and $CHCl_3$ (30 ml), MeOH (30 ml) and water (30 ml) were added. After mixing, the lower layer was concentrated *in vacuo*. The residue was dissolved in $CHCl_3$ / MeOH / H_2O 30 / 60 / 8 and applied to a DEAE-Sephadex column (A-25, acetate form, 2g) equilibrated with the same mixture of solvents ($CHCl_3$ / MeOH / H_2O 30 / 60 / 8). The column was washed with 50 ml of the same solvent mixture, and then the product was eluted with $CHCl_3$ / MeOH / 0.8 M NaOAc (pH 7.0) 30 / 60 / 8. The fractions containing the product were transferred to separatory funnel and $CHCl_3$ and water were added to give the ratio of $CHCl_3$: MeOH : H_2O 1 : 1 : 1. After mixing the lower layer was separated and concentrated *in vacuo* to give pure *S,S*-LBPA as a colorless liquid (13 mg, 89 %) : R_f 0.64 (CH_2Cl_2/MeOH/H_2O 65/25/4); $[\alpha]^{20}_D$ –3.60 (*c* 0.50, $CHCl_3$) ; ^1H NMR ($CDCl_3$ containing ~ 2 eq of pyridine) δ 5.34 (m, 4 H), 4.98 (m, 2H), 4.04 (m, 2H), 3.98 (m, 2H), 3.72 (m, 4 H), 2.30 (br.t, 4 H, J = 7.5 Hz), 2.00 (m, 8 H), 1.57 (m, 4 H), 1.27 (m, 40 H), 0.88 (t, 6 H, J = 7.0 Hz) ; ^{13}C NMR ($CDCl_3$ containing ~ 2 eq of pyridine) δ 173.50 (2 x s), 129.95 (2 x d), 129.60 (2 x d), 72.66 (2 x d, J_{PC} 6.9 Hz), 63.53 (2 x t, br), 60.05 (2 x t), 34.17 (2 x t), 31.87 (2 x t), 29.76 (2 x t), 29.73 (2 x t), 29.50 (2 x t), 29.30 (6 x t), 29.21 (2 x t), 29.17 (2 x t), 27.19 (4 x t), 24.83 (2 x t), 22.65 (2 x t), 14.08 (2 x q); ^{31}P NMR ($CDCl_3$ containing ~ 2 eq of pyridine) δ 0.00; IR (film, cm^{-1}) 3394, 2925, 2854, 1735, 1457, 1226, 1051 ; MS (ESI) *m/z* 773.5 $[M^-]$.

R,R-LBPA (*R,R*-1)

R,R-LBPA (*R,R*-**1**) was prepared following the same procedure but starting with D-α,β-isopropylideneglycerol (*S*-**6**), instead of L-α,β-isopropylideneglycerol (*R*-**6**): $[\alpha]^{20}_D$ +3.27 (*c* 0.49, $CHCl_3$); all the other spectral data were identical to those of *S,S*-LBPA (*S,S*-**1**).



^{13}C NMR spectrum of *S,S*-1, pyridinium salt (125 MHz, $CDCl_3$)


^{1}H NMR spectrum of *S*,*S*-1, pyridinium salt (500 MHz, $CDCl_3$)
BRUKER
S,S-1
Py
9", 10"
2, 2'
1, 1'
3, 3'
2"
8", 11"
3"
4" ~ 7"
12" ~ 17"
18"
6e+008
5e+008
4e+008
3e+008
2e+008
1e+008
0e+000
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
(ppm)