Electronic Supporting Information

Synthesis and Biological Evaluation of Phosphatidylinositol Phosphate Affinity Probes

Stuart J. Conway,[a] James Gardiner,*[b] Simon J. A. Grove,[a] Melloney K. Johns,[a] Ze-Yi Lim,[a] Gavin F. Painter,[a] Diane E. J. E. Robinson,*[b] Christine Schieber,[b] Jan W. Thuring,[a] Leon S.-M. Wong,[b] Meng-Xin Yin,[b] Antony W. Burgess,[c] Bruno Catimel,[c] Phillip T. Hawkins,[d] Nicholas T. Ktistakis,[d] Leonard R. Stephens[d] and Andrew B. Holmes*[b]

[a] *Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, UK.*

[b] *School of Chemistry, Bio21 Institute, University of Melbourne, Building 102, 30 Flemington Road, Parkville, Victoria 3010, Australia*

[c] *Ludwig Institute for Cancer Research, Melbourne Tumour Biology Branch, Royal Melbourne Hospital, Parkville, Victoria, 3052, Australia*

[d] *Babraham Institute, Department of Signalling, Inositide Laboratory, Cambridge, CB2 4AT, UK*

CONTENTS

Experimental Section

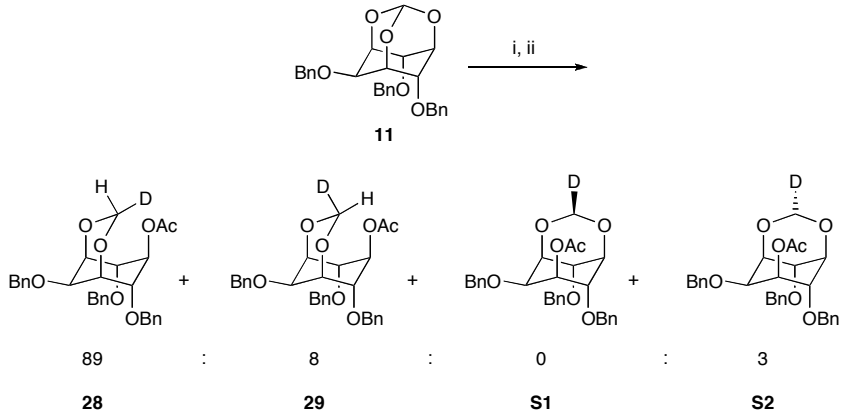
1.1 General Methods

Unless otherwise specified, all *^{1}H*, *^{13}C*, and *^{31}P* NMR spectra were recorded on a Bruker AV400 spectrometer at 400 MHz, 100 MHz, and 162 MHz respectively, or a Bruker DRX500 spectrometer at 500 MHz, 125 MHz, or 202 MHz respectively, using $CDCl_3$ (or other indicated solvents) as reference (δ = 7.26 ppm and 77.0 ppm for ^{1}H and ^{13}C NMR respectively). Chemical shifts (δ) are measured in ppm. Chemical shift data for ^{31}P signals are reported in ppm relative to an external standard of 85% H_3PO_4 (δ = 0.00 ppm). Coupling constants (*J*) are quoted in Hz. *Infrared spectra* were recorded on Perkin-Elmer 1600 series FTIR and Perkin-Elmer Spectrum One ATR-FTIR spectrometers. *Mass spectra* were recorded at the EPSRC National Mass Spectroscopy Centre, University of Wales, Swansea, the Mass Spectrometry Service at the University of Cambridge, and at the University of Melbourne Mass Spectrometry Service. *Melting points* were determined using a Reichert-Koeffler hot stage or Büchi melting point apparatus and are uncorrected. *Specific optical rotations* were measured using a Jasco Dip-1000 digital polarimeter or a Perkin-Elmer 241 polarimeter, in a microcell of 1 dm path length for 1 mL of solution, units are 10^{-1} deg cm^2 g^{-1} and concentration is expressed in g/100 mL. Solvents and reagents were purified and dried where necessary by standard techniques. Where appropriate, and if not stated otherwise, all reactions were performed in flame-dried apparatus under an inert atmosphere of nitrogen or argon.

The following compounds were synthesised following literature procedures: Cores **A**,[1,2] **B**,[2] **C**,[3] **D**,[2] **E**,[1,2] **11**,[4] **34**,[5] **35**,[3] **38**,[3] **47**,[2] **73**,[2,6] **79**,[2] **89**,[3] **96**,[2,6] **103**,[7] **S7**,[8] (benzyloxy)bis(*N,N*-diisopropylamino)phosphine[9] and bis(benzyloxy)(*N,N*-diisopropylamino)phosphine.[10]

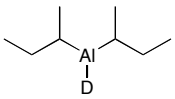
1.2 Deuterium-labelling Investigations into the Reductive Cleavage of *myo*-Inositol Orthoformates

Reductive Cleavage of Orthoformate 11 with DIBAL-D



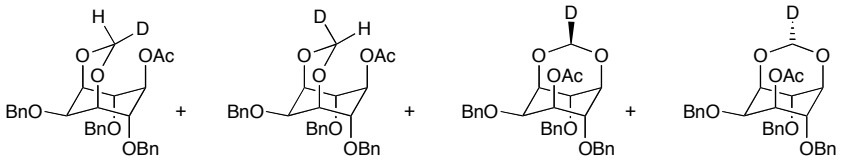
Reagents and conditions: i. DIBAL-D, CH_2Cl_2, 0 ºC to rt; ii. Ac_2O, Et_3N, DMAP, CH_2Cl_2, rt

Diisobutylaluminium deuteride (DIBAL-D)[11]



To a suspension of lithium deuteride (1.15 g, 128 mmol, 1.28 equiv) in dry ether (40 mL) at 0 °C was added diisobutylaluminium chloride (19.5 mL, 100 mmol, 1.0 equiv). The suspension was refluxed for 48 h then cooled to room temperature. A small aliquot of the supernatant was removed and quenched with dilute nitric acid and tested with silver nitrate solution, which revealed the presence of chloride. Further lithium deuteride (0.175 g, 19.5 mmol, 0.195 equiv) was added and the suspension was refluxed for a further 17 h. The suspension was cooled to room temperature and tested for chloride, as before. The test revealed the absence of chloride, indicating that the reaction had gone to completion (no diisobutylaluminium chloride remained). The supernatant was transferred *via* a cannular to a coarse frit filter and filtered into a very dry Claisen flask, under argon, and the ether removed by distillation. The residual liquid was stirred at a temperature of 90 °C and pressure of 3 mmHg for 2 h. Distillation of the liquid afforded diisobutylaluminium deuteride (11.88 g, 83%) as a clear colourless liquid: bp 85-92 °C at 0.05 mmHg (lit.[11] 112-118 °C at 0.5 mmHg); δ_H (90 MHz; $CDCl_3$) 1.85 (2 H, septet, *J* 6.0, C*H*CH_3), 1.05 (12 H, d, *J* 6.0, CH_3), 0.30 (4 H, d, *J* 7.5, CH_2).

Reduction of orthoformate 11[4] with DIBAL-D to give a mixture of acetates comprising: (1*R*,3*r*,5*S*,6*R*,7*s*,8*S*,9*s*)-6,8,9-Tris(benzyloxy)-[3-$^2H_1$]-2,4-dioxabicyclo[3.3.1]non-7-yl ethanoate 28, (1*R*,3*s*,5*S*,6*R*,7*s*,8*S*,9*s*)-6,8,9-Tris(benzyloxy)-[3-$^2H_1$]-2,4-dioxabicyclo-[3.3.1]non-7-yl ethanoate 29, (1*R*,3*R*,5*S*,6*R*,7*R*,8*R*,9*R*)- and (1*R*,3*R*,5*S*,6*S*,7*R*,8*S*,9*R*)-6,8,9-Tris(benzyloxy)-[3-$^2H_1$]-2,4-dioxabicyclo[3.3.1] non-3-yl ethanoate S1 and (1*R*,3*S*,5*S*,6*R*,7*R*,8*R*,9*R*)- and (1*R*,3*S*,5*S*,6*S*,7*R*,8*S*,9*R*)-6,8,9-Tris(benzyloxy)-[3-$^2H_1$]-2,4-dioxabicyclo[3.3.1]-non-3-yl ethanoate S2

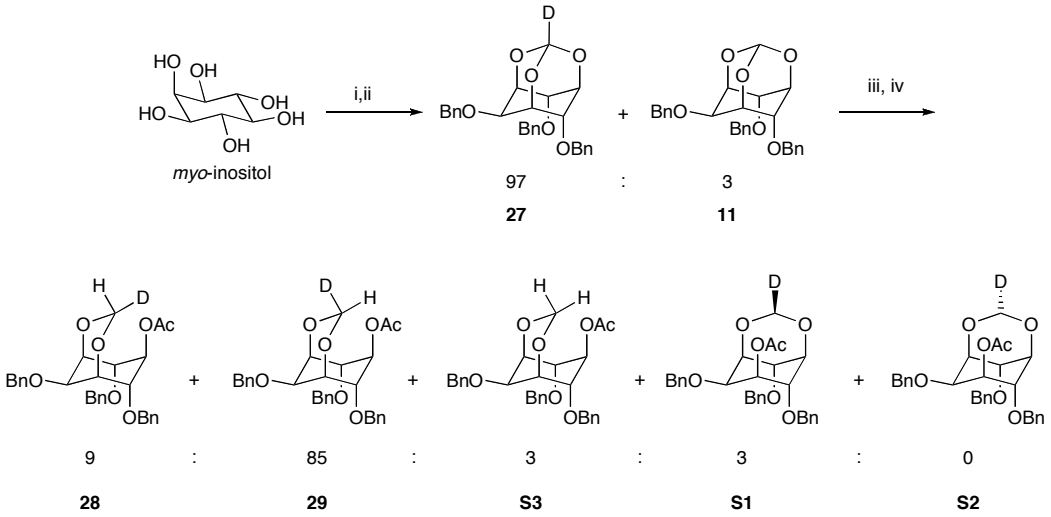


To a stirred solution of the orthoformate **11**[4] (5 mL of a 0.17M solution in dichloromethane, 0.85 mmol, 1.0 equiv) at 0 °C was added DIBAL-D (2.125 mL of a 1M solution in hexane, 2.125 mmol, 2.5 equiv) dropwise with stirring. The solution was stirred at 0 °C for 15 min then at rt for 24 h. The reaction was poured into a vigorously stirred 1.5M aqueous solution of sodium potassium tartrate (50 mL) and saturated ammonium chloride (50 mL) at 0 °C and ethyl acetate (200 mL) was added. The mixture was stirred at rt for 2 h. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried ($MgSO_4$) and evaporated. Flash chromatography (20% ethyl acetate/hexane) gave the mixture of acetal products as an oil (R_f 0.47, 50% ethyl acetate/hexane). To a portion of the acetal mixture (0.077 g, 0.17 mmol, 1.0 equiv) in dry dichloromethane (1 mL) was added triethylamine (0.15 mL, 1.1 mmol, 6.47 equiv), acetic anhydride (0.032 mL, 0.34 mmol, 2.0 equiv) and *N*, *N*-dimethylaminopyridine (cat.). The solution was stirred at rt for 6 h, diluted with dichloromethane (25 mL), and extracted with 2M sodium hydroxide solution (2 × 25 mL), 2M hydrochloric acid (2 × 25 mL) and brine (25 mL). The organic layer was dried ($MgSO_4$) and evaporated. Flash chromatography (20% ethyl acetate/hexane) gave the acetates **28**, **29**, and **S2** (Scheme 1) as a gum that slowly solidified to a white solid. The solid was dissolved in benzene-d_6 and the isomer ratio determined as 89:8:0:3 (**28**:**29**:**S1**:**S2**) by ^{1}H NMR. Purification of the mixture by HPLC (50% ethyl acetate/hexane, 12 mL/min) gave an inseparable mixture of *acetates* **28** and **29** as a white solid and **S2** as an oil. Compound **S1** was not observed.

Data for **28** and **29**: δ_H (400 MHz; C_6D_6) 7.28-7.07 (15 H, m, Ph), 5.82 (0.56 H, s, 3-H of **28**), 5.55 (1 H, br s, 7-H), 4.85 (0.46 H, s, 3-H of **29**), 4.62 (2 H, d, *J* 12.0, CH_AH$_B$Ph), 4.58 (2 H, br d, *J* 1.2, 1,5-H), 4.47 (1 H, t, *J* 1.3, 9-H), 4.42 (2 H, d, *J* 12.0, CH_AH$_B$Ph), 4.34 (2 H, s, CH_2Ph), 4.12 (2 H, dd, *J* 3.1, 2.3, 6,8-H), 1.80 (3 H, s, CH_3).

Data for **S2**: δ_H (400 MHz; C_6D_6) 7.32-7.05 (15 H, m, Ph), 5.87 (1 H, t, *J* 7.7, 7-H), 5.14 (1 H, s, 3-H), 4.62-4.53 (4 H, m, 3 × CH_2Ph and 8-H), 4.45 (1 H, dd, *J* 3.7, 1.4, 5-H), 4.41-4.38 (2 H, m, OCH_2Ph and 9-H), 4.23 (1 H, d, *J* 10.9, CH_AH_BPh), 4.19 (1 H, d, *J* 7.5, 6-H), 4.08 (1 H, d, *J* 11.5, CH_AH_BPh), 4.03 (1 H, td, *J* 3.6, 0.9, 9-H), 1.81 (3 H, s, CH_3); δ_C (100 MHz; C_6D_6) 170.0, 138.8, 138.6, 138.1, 128.6, 128.5, 128.5, 128.2, 128.2, 128.0, 128.0, 127.9, 127.8, 127.8, 85.6 (t, *J* 25.2, O_2CHD), 79.5, 73.0, 72.9, 72.5, 72.2, 71.9, 71.3, 70.9, 68.4, 20.7; HRMS Calcd. for $C_{30}H_{31}DO_7$ ($M+H^+$) 506.229. Found: 506.228.

Reductive Cleavage of Deuterated Orthoformate 27 with DIBAL-H

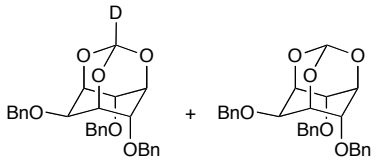


Reagents and Conditions. i. *p*-TSA.H_2O, $CD(OEt)_3$, DMSO, 100 ºC; ii. NaH, DMF, BnBr, 0 ºC to rt; iii. DIBAL-H, CH_2Cl_2, 0 ºC to rt; iv. Ac_2O, Et_3N, DMAP, CH_2Cl_2, rt

[1-$^2H_1$]-Tris(ethyloxy)methane

To a solution of ethanol-d_1 (193.25 mL, 154.8 g, 3.29 mol, 12.65 equiv) (freshly distilled from sodium) and chloroform-d_1 (21.0 mL, 31.55 g, 0.26 mol, 1.0 equiv) (freshly distilled from calcium chloride) was added sodium (13.34 g, 0.58 mol, 2.23 equiv) in small pieces over a period of 60 min with the suspension cooled by immersion in a stirred ice bath. The resulting suspension was left overnight then filtered and distilled through a *Vigreux* column to remove most of the unreacted chloroform-d_1 and ethanol-d_1, bp 61-78 °C. The resulting clear brown liquid was separated from the residual salt and distilled through a *Vigreux* column to give *deuterated triethyl orthoformate* (4.42 g, 11%) as a colourless liquid: bp 141-146°C; ν_{max} (CCl_4)/cm^{-1} 2980, 2950, 2920, 2870, 2150, 2100; δ_H (250 MHz; $CDCl_3$) 3.45 (2 H, q, *J* 7.1, CH_2CH$_3$), 1.27 (3 H, t, *J* 7.1, CH$_2$CH_3); δ_C (100 MHz; $CDCl_3$) 112.1 (t, *J* 28.4, CDO_3), 59.3 (CH_2), 14. 9 (CH_3).

(1*R*,3*s*,5*S*,6*R*,7*s*,8*S*,9*s*)-3-[$^2H_1$]-2,4,10-Trioxatricyclo[3.3.1.1^{3,7}]decane-6,8,9-triol 27 and (1*R*,3*s*,5*S*,6*R*,7*s*,8*S*,9*s*)-2,4,10-Trioxatricyclo[3.3.1.1^{3,7}]decane-6,8,9-triol 11



97 : 3

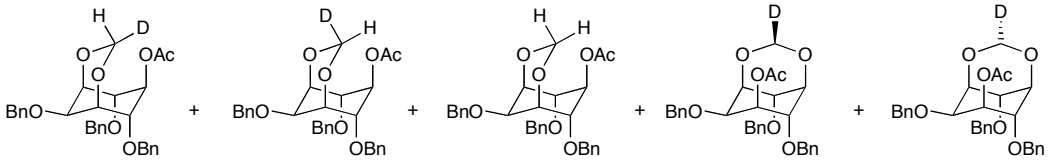
27 **11**

To a solution of *myo*-inositol (1.08 g, 6.0 mmol, 1.0 equiv) and *p*-toluenesulfonic acid monohydrate (0.059 g, 0.31 mmol, 0.052 equiv) in dry DMSO (10 mL) was added deuterated triethyl orthoformate (1.1 mL, 6.7 mmol, 1.12 equiv) and the solution was stirred at 100 °C for 17 h. The resulting brown solution was cooled to rt and quenched by the addition of $NaHCO_3$ (0.6 g) in water (5 mL). Removal of the solvent under reduced pressure gave a brown gum. Flash chromatography (5% methanol/ethyl acetate) gave an inseparable mixture of the *deuterated* and *non-deuterated* (trace) orthoformates (0.497 g, 43%) as white cubes: δ_H (250 MHz; D_2O) 4.55 (2 H, t, *J* 3.9), 4.36-4.28 (1 H, m), 4.28-4.18 (3 H, m); δ_C (100 MHz; D_2O) 104.4 (t, *J* 31.0), 76.4, 71.9, 69.3, 62.1.

A portion of this mixture (0.411 g, 2.6 mmol, 1.0 equiv) was dissolved in dry DMF (5 mL) and added to a 0 °C suspension of sodium hydride (0.414 g, 60% dispersion in mineral oil, 10.3 mmol, 3.96 equiv) in dry DMF (10 mL). The suspension was stirred at rt for 2 h after which benzyl bromide (1.3 mL, 10.9 mmol, 4.19 equiv) was added slowly and the suspension was stirred at rt for 17 h until TLC analysis indicated the presence of a single product of R_f 0.55 (50% ethyl acetate/hexane). The reaction was quenched by the addition of water (1 mL) and the solvent removed under reduced pressure. The solid residue was partitioned between dichloromethane (100 mL) and water (100 mL) and the organic layer separated. The aqueous layer was extracted with dichloromethane (2 × 50 mL) and the combined organic layers washed with brine (100 mL) and dried over $MgSO_4$. Flash chromatography (20% ethyl acetate/hexane), followed by recrystallisation from petroleum ether gave the *deuterated orthoformate* **27** and orthoformate **11**, in a 97:3 ratio by ^1H NMR, (0.879 g, 89%) as a white solid (Found: C, 73.00; H, 6.18. $C_{28}H_{27}DO_6$ requires C, 72.86; H, 6.13%): mp 101.5-102 °C (from petroleum ether); δ_H (400 MHz; $CDCl_3$) 7.36-7.19 (15 H, m), 5.53 (0.03 H, d, *J* 1.5), 4.64 (2 H, s), 4.61 (2 H, d, *J* 11.6), 4.47 (2 H, d, *J* 11.6) 4.43 (1 H, tt, *J* 3.4, 1.7), 4.38-4.36 (2 H, m), 4.33 (2 H, t, *J* 3.8), 4.04 (1 H, t, *J* 1.7); δ_C (100 MHz; $CDCl_3$) 137.7, 137.5, 128.4, 128.3, 128.1, 127.8, 127.5, 102.83 (t, *J* 31.0), 74.0, 71.5, 70.5, 68.0, 67.2; *m/z* (CI, NH_3) 462 (95, M+H$^+$), 372 (14), 356 (6), 282 (6), 266 (7), 250 (3), 198 (5), 181 (5), 160 (8), 106 (100); HRMS (CI) Calcd. for $C_{28}H_{28}DO_6$ (M+H$^+$) 462.2027. Found: 462.2027.

Reduction of deuterated orthoformate 27 with DIBAL-H to give a mixture of acetates comprising: (1*R*,3*r*,5*S*,6*R*,7*s*,8*S*,9*s*)-6,8,9-Tris(benzyloxy)-[3-$^2H_1$]-2,4-dioxabicyclo[3.3.1]non-7-yl ethanoate 28, (1*R*,3*s*,5*S*,6*R*,7*s*,8*S*,9*s*)-6,8,9-Tris(benzyloxy)-[3-$^2H_1$]-2,4-dioxabicyclo-[3.3.1]non-7-yl ethanoate 29, (1*R*,5*S*,6*R*,7*s*,8*S*,9*s*)-6,8,9-Tris(benzyloxy)-2,4-dioxabicyclo[3.3.1]non-7-yl ethanoate S3, (1*R*,3*R*,5*S*,6*R*,7*R*,8*R*,9*R*)- and (1*R*,3*R*,5*S*,6*S*,7*R*,8*S*,9*R*)-6,8,9-Tris(benzyloxy)-[3-$^2H_1$]-2,4-dioxabicyclo[3.3.1] non-3-yl ethanoate S1 and (1*R*,3*S*,5*S*,6*R*,7*R*,8*R*,9*R*)- and (1*R*,3*S*,5*S*,6*S*,7*R*,8*S*,9*R*)-6,8,9-Tris(benzyloxy)-[3-$^2H_1$]-2,4-dioxabicyclo[3.3.1]-non-3-yl ethanoate S2

Reductive Cleavage of Deuterated Orthoformate 27 with DIBAL-H

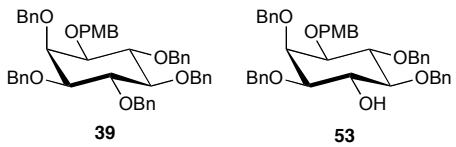


This reaction was carried out in a manner identical to that described for the reductive cleavage of **11**, except that DIBAL-H was used. The resulting mixture of acetals was acetylated to give acetates **28**, **29**, **S3**, and **S1** (Scheme 2) in a ratio of 9:85:3:3:0 (**28**:**29**:**S3**:**S1**:**S2**) by ^{1}H NMR with data of **28**, **29** matching above. Compound **S1** was not isolated. Compound **S2** was not observed.

Acetate **S3**: white solid, mp 91-92 °C (from ethyl acetate-hexane); R_f 0.56 (50% ethyl acetate in hexane); $\nu_{max}(CCl_4)/cm^{-1}$ 3105, 3085, 3050, 1740 (CO ester), 1500; 1460, 1376, 1240, 1186, 1160, 1100, 1035, 900; δ_H (250 MHz; C_6D_6) 7.24-7.03 (15 H, m, Ph), 5.50 (1 H, br s, 7-H), 5.47 (1 H, d, *J* 4.3, 3-$H_{pro\text{-}S}$; irradiation results in a nuclear Overhauser enhancement of 4.30 ppm singlet), 4.82 (1 H, d, *J* 4.3, 3-$H_{pro\text{-}R}$; irradiation results in a nuclear Overhauser enhancement of 4.07 ppm triplet), 4.58 (2 H, d, *J* 12.0, OCH_AC$_B$Ph), 4.53 (2 H, br d, *J* 1.2, 1,5-H), 4.42 (1 H, br s, 9-H), 4.38 (2 H, d, *J* 12.0, OCH$_A$$H_B$Ph), 4.30 (2 H, s, 9-OC$H_2$Ph), 4.07 (2 H, t, *J* 2.8, 6,8-H), 1.76 (3 H, s, CH_3); δ_C (100 MHz; $CDCl_3$) 170.3, 137.7, 137.7, 128.5, 128.4, 127.9, 127.8, 127.6, 85.4, 79.5, 71.8, 71.1, 70.7, 70.1, 69.7.

1.3 Synthesis of NH_2-PI 42 and affinity probe 43

(+)-1D-2,3,4,5,6-Penta-*O*-benzyl-1-*O*-(4'-methoxybenzyl)-*myo*-inositol 39[12] and (+)-1D-2,3,5,6-tetra-*O*-benzyl-1-*O*-(4'-methoxybenzyl)-*myo*-inositol 53[13]

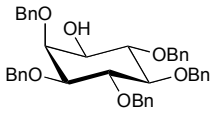


Sodium hydride (0.012 g, 60% in mineral oil, 0.289 mmol, 3.0 equiv) was added to a solution of core **A** (0.055 g, 0.096 mmol, 1.0 equiv) in dry dimethylformamide (10 mL) at 0 °C and stirred for

30 min, before being warmed to rt and stirred for a further 1 h. The mixture was then cooled to 0 °C and tetrabutylammonium iodide (0.021 g, 0.0058 mmol, 0.6 equiv) and benzyl bromide (0.030 mL, 0.0289 mmol, 3.0 equiv) were added. The solution was then allowed to warm to rt and stirred overnight. The reaction was quenched with water (5 mL), the solvent removed under reduced pressure, and the resulting residue partitioned between ethyl acetate (20 mL) and water (20 mL). The aqueous layer was separated, back-extracted with ethyl acetate (3 × 20 mL) and the combined organic layers were washed with brine (20 mL), dried ($MgSO_4$) and the solvent removed under reduced pressure. Flash chromatography (10-15% ethyl acetate/petroleum ether) gave (+)-**39** (0.035 g, 49%) as a white solid: mp 89-91 °C; $[\alpha]_D^{21}$ +5.5 (*c* 1.3 in $CHCl_3$) (lit.[12] $[\alpha]_D^{25}$ +0.6 (*c* 0.6 in $CHCl_3$)); δ_H (500 MHz; $CDCl_3$) 7.44 (2 H, d, *J* 8.4), 7.35-7.25 (25 H, m), 6.88 (2 H, d, *J* 8.4), 4.94 (1 H, d, *J* 10.7), 4.90 (4 H, s), 4.86 (1 H, d, *J* 10.7), 4.85 (1 H, d, *J* 10.7), 4.68 (1 H, d, *J* 11.7), 4.63 (1 H, d, *J* 11.7), 4.61 (1 H, d, *J* 12.0), 4.57 (1 H, d, *J* 12.0), 4.11 (1 H, t, *J* 9.5), 4.10 (1 H, t, *J* 9.5), 4.04 (1 H, t, *J* 2.2), 3.84 (3 H, s), 3.50 (1 H, t, *J* 9.3), 3.38 (1 H, dd, *J* 9.8, 2.2), 3.37 (1 H, dd, *J* 9.8, 2.2).

Tetrabenzyl inositol (+)-**53** (0.025 g, 39%) was also isolated as a white solid: mp 63-65 °C; $[\alpha]_D^{25}$ +8.0 (*c* 1.3 in $CHCl_3$); (lit.[13] $[\alpha]_D^{25}$ + 8.5 (*c* 1 in $CHCl_3$)); δ_H (500 MHz; $CDCl_3$) 7.40-7.23 (22 H, m), 6.86 (2 H, d, *J* 8.7), 4.92 (1 H, d, *J* 10.6), 4.90 (1 H, d, *J* 11.1), 4.87 (1 H, d, *J* 12.0), 4.85 (1 H, d, *J* 11.1), 4.82 (1 H, d, *J* 10.6), 4.79 (1 H, d, *J* 12.0), 4.60 (1 H, d, *J* 11.5), 4.59 (1 H, d, *J* 11.8), 4.57 (1 H, d, 11.5), 4.54 (1 H, d, *J* 11.8), 4.17 (1 H, t, *J* 9.5), 4.04 (1 H, t, *J* 9.5), 4.02 (1 H, t, *J* 2.3), 3.82 (3 H, s), 3.35 (1 H, t, *J* 9.5), 3.34 (1 H, dd, *J* 9.5, 2.3), 3.19 (1 H, dd, *J* 9.5, 2.3), 2.49 (1 H, br s).

(–)-1D-2,3,4,5,6-Penta-*O*-benzyl-*myo*-inositol 40[14]

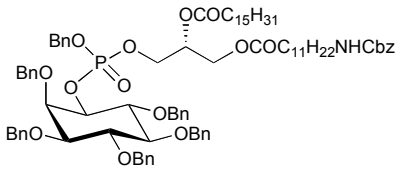


40

To a stirred solution of (+)-**39** (0.100 g, 0.13 mmol, 1.0 equiv) in acetonitrile (4 mL) and distilled water (1 mL) under air, was added ceric ammonium nitrate (0.439 g, 0.80 mmol, 6.15 equiv). After stirring for 45 min, the solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate (20 mL) and water (20 mL). The aqueous phase was separated, back-extracted with ethyl acetate (5 × 20 mL), and the combined organic layers were washed with sat. aq. sodium hydrogen carbonate (20 mL), brine (20 mL), dried ($MgSO_4$) and the solvent was removed under reduced pressure. Purification by flash chromatography (15-25% ethyl acetate/petroleum ether) gave (–)-**40** (0.070 g, 85%) as a colourless oil: $[\alpha]_D^{25}$ –9.0 (*c* 0.3 in $CHCl_3$) (lit.[14] $[\alpha]_D^{18}$ -10.0

(*c* 2.3 in $CHCl_3$)); δ_H (500 MHz; $CDCl_3$) 7.39-7.29 (25 H, m), 5.03-4.70 (10 H, m), 4.10 (1 H, t, *J* 9.5), 4.06 (1 H, t, *J* 2.8), 3.84 (1 H, t, *J* 9.5), 3.54-3.48 (3 H, m), 2.23 (1 H, d, *J* 6.3).

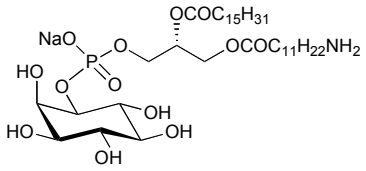
(+)-1D-2,3,4,5,6-Penta-*O*-benzyl-*myo*-inositol-1-{1'-*O*-[12-*N*-(benzyloxycarbonyl) aminododecanoyl]-2'-*O*-hexadecanoyl-*sn*-glycer-3'-yl benzyl phosphate} 41



41

A solution of the phosphoramidite (+)-**38** (0.043 g, 0.048 mmol, 2.53 equiv), and 1*H*-tetrazole (0.004 g, 0.057 mmol, 3.0 equiv) in dry dichloromethane (1 mL) was stirred under argon for 1 h. A solution of alcohol (–)-**40** (0.012 g, 0.019 mmol, 1.0 equiv) in dry dichloromethane (1 mL) was then added and the resulting mixture was stirred overnight at room temperature. The reaction was then cooled to –78 °C and *m*CPBA (0.016 g, 0.088 mmol, 4.63 equiv) was added. After 20 min at room temperature, the reaction was quenched by addition of saturated aqueous sodium hydrogen sulfite solution (5 mL) and diluted with dichloromethane (5 mL). The aqueous phase was separated and extracted with dichloromethane (3 × 5 mL), and the combined organic layers were washed successively with saturated aqueous sodium hydrogen carbonate solution (5 mL) and brine (5 mL), dried ($MgSO_4$) and the solvent was removed under reduced pressure. Purification by flash chromatography (20% ethyl acetate/petroleum ether) gave (+)-**41** (0.020 g, 73%) as a colourless oil: $[\alpha]_D^{25}$ +3.0 (*c* 0.3 in $CHCl_3$); ν_{max} (neat)/cm^{-1} 2924, 2854, 1739, 1498, 1455, 1360, 1260, 1071, 1026, 923, 805, 734, 696; δ_H (500 MHz; $CDCl_3$) 7.41-7.22 (35 H, m), 5.13-4.60 (15 H, m), 4.35 (1 H, q, *J* 2.5), 4.29-3.94 (6 H, m), 3.91 (1 H, dd, *J* 12.0, 5.9), 3.53-3.45 (2 H, m), 3.20 (2 H, q, *J* 6.5), 2.26-2.16 (4 H, m), 1.60-1.48 (6 H, m), 1.34-1.22 (38 H, m), 0.90 (3 H, t, *J* 6.9); δ_C (125 MHz; $CDCl_3$) 173.1, 172.7, 156.3, 138.7, 138.6, 138.4, 138.1, 136.6, 135.6, 135.5, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 127.5, 127.4, 83.1, 81.3, 81.2, 80.6, 80.5, 80.0, 79.9, 78.8, 78.7, 78.6, 76.4, 76.0, 75.9, 75.6, 75.5, 75.0, 72.8, 72.7, 69.6, 69.5, 69.4, 69.2, 66.5, 65.7, 65.3, 61.5, 60.4, 34.1, 34.0, 33.9, 31.9, 29.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 26.7, 24.8, 22.7, 14.1; δ_P (202 MHz; $CDCl_3$) –1.05, –1.08; HRMS (ES^+) Calcd. for $C_{87}H_{114}NNaO_{15}P$ ($M+Na^+$) 1466.7740. Found: 1466.7818.

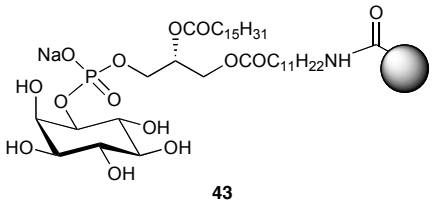
(–)-1D-*myo*-Inositol-1-(1'-*O*-12-aminododecanoyl-2'-*O*-hexadecanoyl-*sn*-glycer-3'-yl sodium phosphate) 42



42

To a solution (+)-**41** (0.016 g, 0.0111 mmol, 1.0 equiv) in *tert*-butanol (6 mL) and water (1 mL) was added palladium black (0.022 g, 0.20 mmol, 18.0 equiv) and sodium hydrogen carbonate (0.0015 g, 0.0179 mmol, 1.61 equiv). The resulting suspension was placed under hydrogen (4.1 bar) and stirred at rt for 48 h. The reaction vessel was then flushed with argon and the suspension was centrifuged (3000 rpm, 45 min). The supernatant was removed and the residue was washed with ethyl acetate (20 mL) and the suspension was centrifuged as before. The pellet was then washed and centrifuged successively with water (20 mL) and chloroform/methanol/water (3:6:1, 3 × 20 mL). The combined chloroform/methanol/water layers were filtered through *Celite* and the filtrate was centrifuged. The organic solvent was removed under reduced pressure and the remaining aqueous solution was lyophilised to give (–)-**42** (0.0067 g, 76%) as a white solid: mp 105-106 °C (lyophilised from water): $[\alpha]_D^{26}$ –26.8 (*c* 0.2 in H_2O); ν_{max} (neat)/cm^{-1} 3318, 2919, 2850, 1746, 1732, 1643, 1568, 1512, 1464, 1411, 1375, 1262, 1219, 1166, 1105, 1043, 942, 899, 807, 777, 720; δ_H (500 MHz; $CDCl_3/CD_3OD$) (some peaks obscured by solvent) 5.29-5.27 (1 H, m), 4.24-4.06 (4 H, m), 3.91 (1 H, dt, *J* 9.7, 2.7), 3.77 (1 H, t, *J* 9.6), 3.65 (1 H, t, *J* 9.6), 3.47 (1 H, dd, *J* 9.9, 2.8), 3.28 (1 H, t, *J* 9.3), 2.95 (2 H, t, *J* 7.8), 2.37 (2 H, t, *J* 7.3), 2.34 (2 H, t, *J* 7.3), 1.70-1.62 (6 H, m), 1.32-1.28 (38 H, m), 0.89 (3 H, t, *J* 6.9); δ_C (125 MHz; $CDCl_3/CD_3OD$) 175.3, 175.0, 77.5 (d), 75.4, 73.4, 72.6 (d), 72.4, 72.1, 71.6 (d), 64.5 (d), 63.8, 40.5, 35.0, 34.8, 32.7, 30.4, 30.3, 30.1, 30.0, 29.8, 29.7, 29.6, 29.5, 28.3, 27.1, 25.7, 25.6, 25.5, 23.4, 14.6; δ_P (202 MHz; $CDCl_3/CD_3OD$) 0.52; HRMS (EI^+) Calcd. for $C_{37}H_{72}NNaO_{13}P$ ($M+H^+$) 792.4633. Found: 792.4649.

1D-*myo*-Inositol-1-{1'-*O*-[12-*N*-(Affi-Gel 10)aminododecanoyl]-2'-*O*-hexadecanoyl-*sn*-glycer-3'-yl sodium phosphate} 43

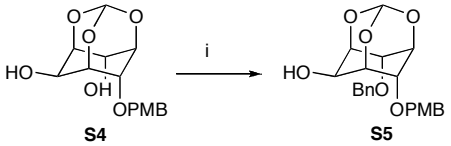


43

Affi-Gel® 10 beads (2 mL slurry, 0.030 mmol, 5.0 equiv), were washed with chloroform/methanol/water (3:6:1, 20 mL), and then suspended in chloroform/methanol/water

(3:6:1, 2 mL), and added to a stirred solution of (–)-**42** (0.0086 g, 0.006 mmol, 1.0 equiv) and sodium hydrogen carbonate (0.010 g, 0.12 mmol, 20.0 equiv) in chloroform/methanol/water (3:6:1, 2 mL) at 0 °C. After stirring at 0 °C for 1 h, the reaction mixture was warmed to rt and stirred overnight. The suspension was filtered and the beads were washed with chloroform/methanol/water (3:6:1, 30 mL) and water (20 mL), and then resuspended in water (2 mL) and stored at 0 °C. The washings were combined and evaporated and the amount of the lipid (–)-**42** present was quantified using ^{1}H NMR with addition of inositol **S5** (0.0015 g, 0.00375 mmol, 0.625 equiv) as an internal standard. Integration of the methine signal of the phosphatidyl side-chain of (–)-**42** (δ_H 5.28 ppm, 1 H, br s) with respect to the signal of the orthoformate proton of the internal standard **S5** (δ_H 5.50 ppm, 1 H, s) indicated that 0.00282 mmol of the amine (–)-**42** had reacted with the beads, giving a loading of 9%.

Preparation of NMR Standard (1*R*,3*S*,5*R*,7*S*,8*R*,9*R*)-8-Benzyloxy-9-[(4'-methoxyphenyl) methoxy-2,4,10-trioxatricyclo[3.3.1.1^{3,7}]decane-6-ol S5



S4 **S5**

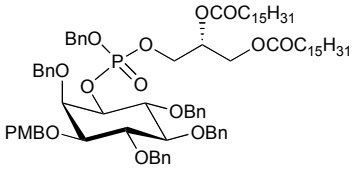
Reagents and Conditions: i. NaH, BnBr, DMF, -5 ºC to rt.

Sodium hydride (0.0645 g, 60% dispersion in mineral oil, 1.6 mmol, 1.0 equiv) was added portionwise to a –5 °C stirred solution of diol **S4**[2] (0.500 g, 1.6 mmol, 1.0 equiv) in dry *N*,*N*-dimethylformamide (10 mL) under argon. The mixture was stirred at –5 °C for 30 min and then at rt for 1h. The solution was re-cooled to –5 °C and benzyl bromide (0.211 mL, 1.77 mmol, 1.1 equiv) was added dropwise and the solution was slowly warmed to rt. After 72 h stirring the reaction was quenched by the addition of ethanol (1 mL) followed by water (1 mL). The solvent was removed under reduced pressure and the resulting oil was partitioned between ethyl acetate (10 mL) and water (10 mL). The ethyl acetate layer was removed and the water layer was washed with ethyl acetate (4 × 10 mL). The combined organic extracts were washed with saturated brine (10 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification by flash chromatography (30% ethyl acetate/hexane gave **S5** (0.298 g, 46%) as a white solid (Found: C, 65.94; H, 5.96. $C_{22}H_{24}O_7$ requires C, 65.99; H, 6.04%): mp 90-92 °C (ether/hexane); ν_{max} (film)/ cm^{-1} 3474, 2961.18, 2878, 1613, 1586, 1514, 1247, 1162, 1101, 992; δ_H (500 MHz; $CDCl_3$) 7.31-7.26 (6 H, m), 7.19 (2 H, d, *J* 8.6), 6.82 (2 H, d, *J* 8.6), 5.47 (1 H, s), 4.65 (1 H, d, *J* 11.3), 4.59 (1 H, d, *J* 7.1), 4.57 (1 H, d, *J* 7.1), 4.51 (1 H, d, *J* 11.3), 4.44-4.43 (1 H, m), 4.37-4.35 (2 H, m), 4.24-4.18 (3 H, m), 3.80 (3 H, s), 3.12 (1 H, d, *J* 11.6); δ_C (63 MHz; $CHCl_3$) 159.4, 137.5, 129.6, 129.3, 128.4, 127.9, 127.7, 113.9, 103.4, 73.8, 73.5, 73.0, 73.0, 71.6, 71.4, 67.8, 61.4, 55.3; m/z (CI$^+$

(NH_3)) 401 (30, M+H^+), 121.1 (100); m/z (EI^+) 400.2 (100, M), 337.1 (75), 121.1 (100), 91.0 (90); HRMS (ES^+) Calcd. for $C_{22}H_{25}O_7$ (M+H^+) 401.1595. Found: 401.1604.

1.4 Synthesis of dipalmitoyl-PI 45

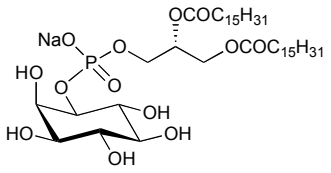
(+)-1D-2,4,5,6-Tetra-*O*-benzyl-3-*O*-(4'-methoxybenzyl)-*myo*-inositol-1-(1',2'-di-*O*-hexadecanoyl-*sn*-glycer-3'-yl benzyl phosphate) 44



44

To a solution of phosphoramidite **34**[5] (0.248 g, 0.308 mmol, 3.2 equiv) in dry dichloromethane (1.5 mL) was added 1*H*-tetrazole (0.7 mL of a 0.4 M solution in acetonitrile, 0.315 mmol, 3.3 equiv). The resulting mixture was stirred at rt under nitrogen for 5 minutes, after which a white precipitate formed. To the resulting mixture was added a solution of *ent*-core **B** (0.0634 g, 0.0959 mmol, 1.0 equiv) in dry dichloromethane (2 mL) dropwise *via* cannula. The resulting mixture was stirred at rt for 17 h, then cooled to –30 ºC, and 3-chloroperbenzoic acid (0.175 g, 0.710 mmol, 7.4 equiv) added in one portion. The mixture was then warmed to rt and 10% aq. sodium hydrogen sulfite (10 mL) and dichloromethane (5 mL) added. After stirring for 30 min the mixture was extracted with dichloromethane (3 × 10 mL) and the organic extracts combined, washed with sodium hydrogen carbonate (50 mL), dried ($MgSO_4$) and the solvent removed under reduced pressure. Purification by flash chromatography (20-35% ethyl acetate/petroleum ether, then 10% ethyl acetate/dichloromethane) gave (+)-**44** (0.073 g, 55%) as a colourless oil: $[\alpha]_D^{19}$ +4.7 (*c* 1.00 in $CHCl_3$); υ_{max} (neat)/cm^{-1} 3063, 3032, 2923, 2853, 1741, 1514, 1455, 1359, 1248, 1212, 1163, 1070, 1025, 893, 822, 732, 696; δ_H (500 MHz; $CDCl_3$) 7.41-7.20 (27 H, m), 6.85 (2 H, d, *J* 9.0), 5.13-4.71(10 H, m), 4.62-4.53 (2 H, m), 4.34-3.87 (9 H, m), 3.81 (3 H, s), 3.52-3.42 (2 H, m), 2.26-2.16 (4 H, m), 1.61-1.49 (4 H, m), 1.34-1.21 (48 H, m), 0.89 (6 H, t, *J* 7.0); δ_C (125 MHz; $CDCl_3$) 173.2, 173.2, 172.8, 172.8, 159.3, 138.9, 138.9, 138.6, 138.6, 135.8, 135.8, 135.7, 135.7, 130.4, 130.4, 129.3, 129.3, 128.7, 128.7, 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 128.1, 127.9, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 113.9, 83.3, 81.4, 81.4, 80.5, 80.4, 80.2, 80.2, 80.1, 80.1, 79.0, 78.9, 78.8, 78.8, 76.6, 76.5, 76.1, 75.9, 75.7, 75.6, 75.2, 72.7, 72.6, 69.7, 69.7, 69.6, 69.5, 69.4, 69.4, 65.8, 65.8, 65.5, 65.5, 61.7, 61.7, 55.4, 34.2, 34.1, 32.1, 29.8, 29.8, 29.8, 29.8, 29.6, 29.5, 29.4, 29.3, 29.2, 25.0, 24.9, 22.8, 14.2; δ_P (200 MHz; $CDCl_3$) –1.15, –1.17; *m/z* (ESI^+) 1403.7 (100, M+Na^+), 1381.2 (9, M+H^+), 1259.2 (4); HRMS (ESI^+) Calcd for $C_{84}H_{117}NaO_{14}P$ (M+Na^+) 1403.8073. Found: 1403.8073.

1D-*myo*-Inositol-1-(1',2'-di-*O*-hexadecanoyl-*sn*-glycer-3'-yl sodium phosphate) 45[15]

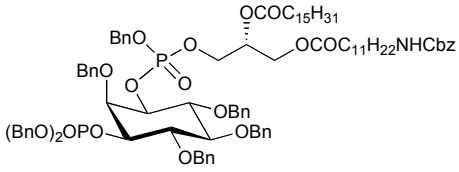


45

Lipid (+)-**44** (0.071 g, 0.0514 mmol, 1.0 equiv) was treated with palladium black (0.0512 g, 0.481 mmol, 9.4 equiv) and sodium hydrogen carbonate (0.0071 g, 0.0845 mmol, 1.6 equiv) under hydrogen (10 bar), according to the procedure described for the preparation of **42**, to give **45** as a white powder (0.044 g, >99%) with data in agreement with the literature.[15]

1.5 Synthesis of NH_2-PI(3)P 51 and affinity probe 52

(+)-1D-2,4,5,6-Tetra-*O*-benzyl-*myo*-inositol-1-{1'-*O*-[12-*N*-(benzyloxycarbonyl) aminododecanoyl]-2'-*O*-hexadecanoyl-*sn*-glycer-3'-yl benzyl phosphate}-3-(dibenzyl phosphate) 49

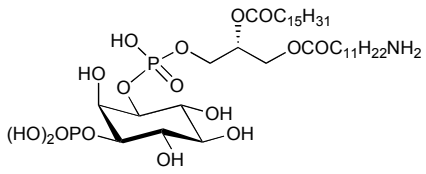


49

Alcohol (–)-**47**[2] (0.225 g, 0.281 mmol, 1.0 equiv) was treated with phosphoramidite **38**, (0.625 g, 0.700 mmol, 2.49 equiv), 1*H*-tetrazole (0.056 g, 0.799 mmol, 2.84 equiv), and 3-chloroperbenzoic acid (0.275 g, 1.59 mmol, 5.66 equiv) according to the procedure described for the preparation of **41**. Purification by flash chromatography (30-40% ethyl acetate/hexane) gave (+)-**49** (0.245 g, 84%) as a colourless gum (Found: C, 70.1; H, 7.65; N, 0.8; P, 3.8. Calcd. for $C_{94}H_{121}O_{18}NP_2$: C, 69.1; H, 7.55; N, 0.9; P, 3.8%): $[\alpha]_D$ +1.28 (c 0.78 in $CHCl_3$); ν_{max} (neat)/cm^{-1} 3063, 3033, 2924, 2853, 1739, 1455, 1259, 1215, 1157, 1120, 1071, 1009, 733, 695; δ_H (500 MHz; $CDCl_3$) 7.40-7.15 (40 H, m), 5.09 (2 H, s), 5.09-4.69 (16 H, m), 4.47 (1 H, dt, *J* 7.5, 2.6), 4.36-4.27 (2 H, m), 4.26-4.21 (1 H, m), 4.12 (1 H, ddd, *J* 19.9, 11.9, 4.1), 4.06-3.95 (2 H, m), 3.93-3.89 (1 H, m), 3.85 (1 H, dd, *J* 11.7, 5.7), 3.47 (1 H, dt, *J* 14.0, 9.2), 3.21-3.15 (2 H, m), 2.24-2.13 (4 H, m), 1.57-1.45 (6 H, m), 1.32-1.19 (38 H, m), 0.88 (3 H, t, *J* 7.0); δ_C (125 MHz; $CDCl_3$) 173.2, 173.2, 172.9, 172.8, 156.5, 138.6, 138.4, 138.4, 138.4, 138.2, 138.2, 136.8, 135.8, 135.8, 135.7, 135.7, 135.6, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.6, 127.4, 127.3, 82.8, 80.0, 80.0, 79.9, 79.9, 79.9, 79.8, 79.8, 78.4, 78.4, 78.2, 78.2, 78.1, 78.0, 78.0, 77.9, 76.2, 76.2, 75.8, 75.8, 75.7, 75.6, 69.8, 69.8, 69.6, 69.6, 69.4, 69.4, 66.7, 65.9, 65.8, 65.6, 65.6, 61.7, 61.7, 41.2, 34.2, 34.2, 34.1, 32.0, 30.1, 29.8, 29.8, 29.8, 29.8, 29.6, 29.6, 29.5, 29.4, 29.4, 29.2, 29.2, 26.9, 24.9, 22.8, 14.2; δ_P (202

MHz, $CDCl_3$) -0.97, -1.02, -1.12; *m/z* (ESI^+) 1638 ($M+Na^+$, 100); HRMS (ESI^+) Calcd for $C_{94}H_{121}NNaO_{18}P_2$ ($M+Na^+$) 1637.7985. Found: 1637.7995.

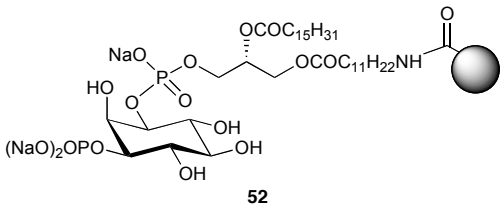
(+)-1D-*myo*-Inositol-1-(1'-*O*-12-aminododecanoyl-2'-*O*-hexadecanoyl-*sn*-glycer-3'-yl sodium phosphate)-3-phosphate 51



51

To a solution of (+)-**49** (0.080 g, 0.050 mmol, 1.0 equiv) dissolved in *tert*-butanol (12 mL), was added palladium black (0.104 g, 0.977 mmol, 19.5 equiv) and the reaction vessel was purged with hydrogen gas (× 10) and pressurized with hydrogen to 3.8 bar. The reaction vessel was then heated to 30 °C and rocked for 48 h. The reaction was vented and the combined contents were centrifuged to remove the catalyst. The catalyst was then stirred with 1:1 $CHCl_3$-MeOH (20 mL) and re-centrifuged. The supernatants were then passed through *Celite* and re-centrifuged. Solvent was removed under reduced pressure to give (+)-**51** (0.026 g, 62%) as a thin film: mp 177-181 °C (decomp.); $[\alpha]_D^{19}$ +1.7 (*c* 1.09 in D_2O); ν_{max} (neat)/cm^{-1} 3266, 2919, 2857, 1741, 1205, 1159, 1090, 1043, 1013, 864, 722; δ_H (500 MHz; D_2O/CD_3OD) 5.35 (1 H, m), 4.54 (1 H, m), 4.46 (1 H, m), 4.27 (1 H, m), 4.13 (2 H, m), 4.03 (2 H, m), 3.82 (2 H, m), 3.44 (1 H, t, *J* 9.2 Hz), 3.04 (2 H, t, *J* 7.2), 2.50-2.31 (4 H, m), 1.80-1.59 (6 H, m), 1.48-1.25 (38 H, m), 0.90 (3 H, m); δC (125 MHz; D_2O/CD_3OD) 175.8, 175.4, 77.4, 77.3, 76.3, 75.3, 73.1, 72.8, 72.7, 72.3, 72.0, 65.3, 64.7, 40.9, 35.6, 35.3, 33.4, 31.4, 31.4, 31.3, 30.9, 30.7, 30.5, 30.4, 30.2, 30.2, 30.1, 28.5, 27.7, 26.5, 25.9, 24.1, 15.3; δ_P (202 MHz; D_2O) 2.04, -0.06; m/z (ESI^+) 894 (100), 850 (40, $M+H^+$), 510 (31); HRMS (ESI^+) Calcd. for $C_{37}H_{73}NNaO_{16}P_2$ ($M+Na^+$) 872.4297. Found: 872.4300.

1D-*myo*-Inositol-1-{1'-*O*-[12-*N*-(Affi-Gel 10)aminododecanoyl]-2'-*O*-hexadecanoyl-*sn*-glycer-3'-yl sodium phosphate}-3-(disodium phosphate) 52



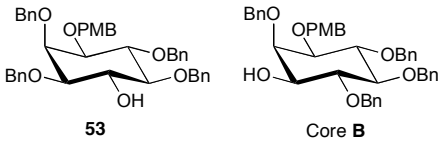
52

Affi-Gel® 10 (4 mL slurry, 0.060 mmol, 2.14 equiv) was filtered and washed with ice cold water (2 × 5 mL) to remove the storage solvent, isopropyl alcohol. Before drying out, the gel was transferred to a stirred solution containing the amine (+)-**51** (24 mg, 0.028 mmol, 1.0 equiv) and sodium hydrogen carbonate (0.025 g, 0.30 mmol, 10.7 equiv), in DMF (5 ml). The reaction mixture was stirred at 0 °C for 2 h and then at rt for a further 8 h, after which the solution was filtered and

washed with ice cold water (15-20 mL). The gel was stored as a slurry in water (2 mL) at 4-6 °C. The loading on the beads was determined to be 2% by ^{1}H NMR analysis.

1.6 Synthesis of dipalmitoyl-PI(4)P 58

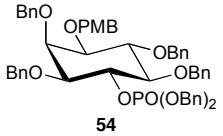
(+)-1D-2,3,5,6-Tetra-*O*-benzyl-1-*O*-(4'-methoxybenzyl)-*myo*-inositol 53[13] and (+)-1D-2,4,5,6-tetra-*O*-benzyl-1-*O*-(4'-methoxybenzyl)-*myo*-inositol Core B[2,16]



To a stirred solution of core **A** (0.240 g, 0.42 mmol, 1.0 equiv) in dry acetonitrile (50 mL) under argon, was added dibutyltin oxide (0.115 g, 0.46 mmol, 1.1 equiv), tetrabutylammonium iodide (0.155 g, 0.42 mmol, 1.0 equiv) and benzyl bromide (0.24 mL, 2.02 mmol, 4.8 equiv). The resulting suspension was heated at reflux overnight with a Soxhlet extractor containing 3Å molecular sieves. The volatiles were removed under reduced pressure and the residue was taken up in ether (20 mL) and water (20 mL). The organic phase was separated and washed with sat. aq. sodium hydrogen carbonate (10 mL), brine (10 mL), dried ($MgSO_4$) and filtered through *Celite*. The solvent was removed under reduced pressure and the resulting residue was purified by flash chromatography (20% ethyl acetate/petroleum ether) to give (+)-**53** (0.210 g, 76%) as a white solid. Spectroscopic data were identical to those reported for **53** above.

Also isolated was the 4-benzyl ether, core **B** (0.017 g, 6%) as a white solid: mp 88-89 °C; $[\alpha]_D^{26}$ +9.8 (c 0.7 $CHCl_3$) (lit.[16] $[\alpha]_D^{23}$ +7.5 (0.2 $CHCl_3$)); δ_H (500 MHz; $CDCl_3$) 7.38-7.23 (22 H, m), 6.84 (2 H, d, *J* 8.7), 4.98 (1 H, d, *J* 11.6), 4.92 (1 H, d, *J* 10.9), 4.90 (1 H, d, *J* 10.7), 4.87 (1 H, d, *J* 11.1), 4.82 (1 H, d, *J* 10.9), 4.81 (1 H, d, *J* 10.7), 4.75 (1 H, d, *J* 11.1), 4.69 (1 H, d, *J* 11.6), 4.63 (1 H, d, *J* 11.4), 4.60 (1 H, d, *J* 11.4), 4.02 (1 H, t, *J* 9.5), 3.98 (1 H, t, *J* 2.5), 3.82 (3 H, s), 3.79 (1 H, t, *J* 9.5), 3.48-3.44 (1 H, m), 3.46 (1 H, t, *J* 9.5), 3.43 (1 H, dd, *J* 9.5, 2.5), 2.19 (1 H, d, *J* 6.4); δ_C (125 MHz, $CDCl_3$) 159.2, 138.8, 138.7, 138.6, 138.6, 138.6, 130.3, 129.3, 128.5, 128.3, 128.3, 128.0, 128.0, 127.8, 127.8, 127.7, 127.6, 127.5, 127.5, 113.8, 107.6, 83.6, 82.1, 81.9, 80.8, 77.2, 75.8, 75.7, 75.5, 74.7, 72.6, 72.4, 67.4, 55.3.

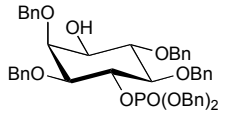
(+)-1D-2,3,5,6-Tetra-*O*-benzyl-1-*O*-(4'-methoxybenzyl)-*myo*-inositol-4-(dibenzyl phosphate) 54



Bis(benzyloxy)(*N,N*-diisopropylamino) phosphine[10] (0.061 g, 0.18 mmol, 2.05 equiv) was added to a stirred solution of (+)-**53** (0.058 g, 0.088 mmol, 1.0 equiv) and 1*H*-tetrazole (0.012 g, 0.18 mmol,

2.05 equiv) in dry dichloromethane (3 mL) under argon. After stirring at rt overnight, the reaction mixture was cooled to -78 °C and 3-chloroperbenzoic acid (0.036 g, 0.20 mmol, 2.25 equiv) was added. The solution was stirred for 20 min at rt, quenched by addition of sat. aq. sodium hydrogen sulfite (5 mL), and then diluted with dichloromethane (5 mL). The aqueous layer was separated, back-extracted with dichloromethane (3 × 5 mL), and the combined organic phases were washed with sat. aq. sodium hydrogen carbonate (5 mL), brine (5 mL), dried ($MgSO_4$), and the solvent was removed under reduced pressure. Purification by flash chromatography (60% ethyl acetate/petroleum ether) gave (+)-**54** (0.0751 g, 93%) as a white solid: mp 86-89 °C (from ethyl acetate and petroleum ether); $[\alpha]_D^{25}$ +0.8 (*c* 1.3 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3042, 2976, 2894, 1514, 1453, 1390, 1250, 1045; δ_H (500 MHz; $CDCl_3$) 7.65-7.10 (32 H, m), 6.88-6.86 (2 H, m), 5.02-4.77 (11 H, m), 4.63 (1 H, d, *J* 11.7), 4.58-4.52 (3 H, m), 4.10 (1 H, t, *J* 9.5), 3.99 (1 H, br s), 3.82 (3 H, s), 3.54 (1 H, t, *J* 9.2), 3.38 (2 H, t, *J* 9.1); δ_C (62.5 MHz; $CDCl_3$) 159.3, 138.7, 138.6, 137.8, 136.3, 130.3, 129.3, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.2, 113.8, 81.9, 81.3, 80.2, 80.1, 78.9, 75.8, 75.0, 74.2, 74.0, 72.5, 72.4 69.2, 68.9, 55.3; δ_P (162 MHz; $CDCl_3$) -1.04; HRMS (ES^+) Calcd. for $C_{56}H_{61}NO_{10}P$ ($M+NH_4^+$) 938.4033. Found: 938.4014.

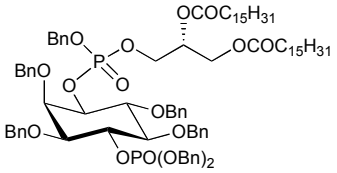
(–)-1D-2,3,5,6-Tetra-*O*-benzyl-*myo*-inositol-4-(dibenzyl phosphate) 55



55

The PMB ether of inositol (+)-**54** (0.075 g, 0.081 mmol, 1.0 equiv) was removed using ceric ammonium nitrate (0.268 g, 0.49 mmol, 6.05 equiv) according to the procedure described for the preparation of **40**. Purification by flash chromatography (40% ethyl acetate/petroleum ether) gave (–)-**55** (0.148 g, 84%) as a white solid: mp 109-111 °C (from ethyl acetate and petroleum ether); $[\alpha]_D^{17}$ –3.8 (*c* 0.4 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3023, 3001, 2966, 2877, 1602, 1497, 1454, 1362, 1268, 1199, 1129, 1071, 1020; δ_H (500 MHz; $CDCl_3$) 7.40-7.10 (30 H, m), 4.99-4.63 (13 H, m), 4.03 (1 H, s), 3.84 (1 H, t, *J* 9.4), 3.56-3.49 (3 H, m), 2.19 (1 H, d, *J* 6.6); δ_C (62.5 MHz; $CDCl_3$) 138.4, 138.3, 137.6, 136.3, 136.2, 136.1, 128.5, 128.4, 128.2, 127.9, 127.8, 127.7, 127.6, 127.3, 81.7, 80.1, 79.2, 79.1, 76.4, 75.5, 75.0, 74.8, 72.8, 72.3, 69.3, 69.1; δ_P (202 MHz; $CDCl_3$) -0.86. HRMS (ES^+) Calcd. for $C_{48}H_{53}NO_9P$ ($M+NH_4^+$) 818.3458. Found: 818.3457.

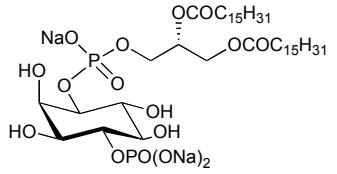
(+)-1D-2,3,5,6-Tetra-*O*-benzyl-*myo*-inositol-1-(1',2'-di-*O*-hexadecanoyl-*sn*-glycer-3'-yl benzyl phosphate)-4-(dibenzyl phosphate) 56



56

Alcohol (–)-**55** (0.06 g, 0.07 mmol, 1.0 equiv) was treated with phosphoramidite **34** (0.14 g, 0.174 mmol, 2.5 equiv), 1*H*-tetrazole (0.014 g, 0.21 mmol, 3.0 equiv) and 3-chloroperbenzoic acid (0.06 g, 0.35 mmol, 5.0 equiv) according to the procedure described for the preparation of **41**. Purification by flash chromatography (40-60% ethyl acetate/hexane) gave (+)-**56** (0.08 g, 78%) as a colourless oil: $[\alpha]_D^{25}$ +5.0 (*c* 0.3 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3034, 2999, 2927, 2854, 1737, 1601, 1455, 1362, 1272, 1219, 1198, 1126, 1018; δ_H (500 MHz; $CDCl_3$) 7.40-7.03 (35 H, m), 5.14-4.59 (15 H, m), 4.34 - 3.90 (7 H, m), 3.55-3.44 (3 H, m), 2.24-2.17 (4 H, m), 1.57 (8 H, br s), 1.31-1.22 (44 H, m), 0.87 (6 H, t, *J* 6.7); δ_C (125 MHz; $CDCl_3$) 173.1, 172.7, 138.3, 138.2, 138.1, 137.5, 136.2, 136.0, 135.5, 130.5, 128.7, 128.6, 128.1, 128.0, 127.8, 127.7, 127.5, 127.4, 81.5, 79.7, 78.8, 75.3, 75.1, 72.4, 69.6, 69.4, 69.3, 69.2, 69.1, 69.0, 65.4, 60.5, 34.1, 33.9, 32.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 29.0, 24.8, 22.7, 14.1; δ_P (162 MHz; $CDCl_3$) –0.95, –1.08; *m/z* (FAB) 1521 (29, $M+H^+$), 1543 (100, $M+Na^+$).

1D-*myo*-Inositol-1-(1',2'-di-*O*-hexadecanoyl-*sn*-glycer-3'-yl sodium phosphate)-4-phosphate 58[17]

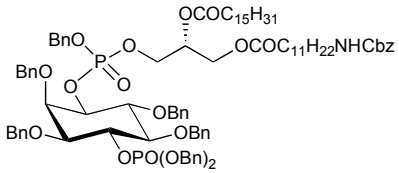


58

Protected lipid (+)-**56** (0.04 g, 0.026 mmol, 1.0 equiv) was treated with palladium black (0.05 g, 0.55 mmol, 21.1 equiv) and sodium hydrogen carbonate (0.007 g, 0.078 mmol, 3.0 equiv) under hydrogen (15 bar) according to the procedure described for the preparation of **42**, to give **58** (0.013 g, 59%) as a white solid: δ_H (400 MHz; $CDCl_3/CD_3OD/D_2O$) 5.21-5.20 (1 H, m), 4.39-3.87 (8 H, m), 2.30-2.14 (4 H, m), 1.55-1.53 (4 H, m) 1.21 (48 H, m), 0.84 (6 H, t, *J* 7.1). δ_P (162 MHz; $CDCl_3/CD_3OD/D_2O$) 4.7, 0.7.

1.7 Synthesis of NH_2-PI(4)P 59 and affinity probe 60

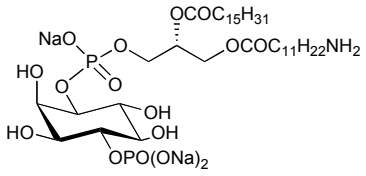
(+)-1D-2,3,5,6-Tetra-*O*-benzyl-*myo*-inositol-1-{1'-*O*-[12-*N*-(benzyloxycarbonyl) aminododecanoyl]-2'-*O*-hexadecanoyl-*sn*-glycer-3'-yl benzyl phosphate}-4-(dibenzyl phosphate) 57



57

Alcohol (–)-**55** (0.036 g, 0.044 mmol, 1.0 equiv) was treated with phosphoramidite (+)-**38** (0.100 g, 0.11 mmol, 2.5 equiv), 1*H*-tetrazole (0.009 g, 0.13 mmol, 2.95 equiv), and 3-chloroperbenzoic acid (0.045 g, 0.26 mmol, 5.91 equiv) according to the procedure described for the preparation of **41**. Purification by flash chromatography (40-60% ethyl acetate/petroleum ether) gave (+)-**57** (0.055 g, 77%) as a colourless oil: $[\alpha]_D^{29}$ +3.4 (*c* 0.40 in $CHCl_3$); ν_{max} (neat)/cm^{-1} 2924, 2854, 1741, 1498, 1456, 1365, 1260, 1012, 925, 803, 734, 696; δ_H (500 MHz; $CDCl_3$) 7.35-7.02 (40 H, m), 5.08 (2 H, s), 4.99-4.66 (14 H, m), 4.59 (2 H, d, *J* 5.8), 4.33 (1 H, q, *J* 2.9), 4.26-3.87 (7 H, m), 3.54-3.43 (2 H, m), 3.17 (2 H, q, *J* 6.7), 2.23-2.12 (4 H, m), 1.56-1.47 (6 H, m), 1.24-1.21 (38 H, m), 0.87 (3 H, t, *J* 6.5); δ_C (125 MHz; $CDCl_3$) 173.1, 172.7, 156.3, 138.3, 138.1, 137.5, 137.4, 136.6, 136.2, 136.1, 136.0, 135.5, 135.4, 128.6, 128.5, 128.3, 128.2, 128.0, 127.8, 127.7, 127.6, 127.5, 81.4, 79.6-79.5 (m), 78.6-78.2 (m), 75.6, 75.5, 75.3-75.2 (m), 72.5, 72.4, 69.6, 69.5, 69.2, 69.0, 66.5, 65.7, 65.4, 61.5, 41.1, 34.1, 34.0, 31.9, 29.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 26.7, 24.8, 22.7, 14.1; δ_P (202 MHz; $CDCl_3$) -0.96, -1.02; HRMS (ESI^+) Calcd. for $C_{94}H_{121}NNaO_{18}P_2$ ($M+Na^+$) 1636.8088. Found: 1636.7951.

(+)-1D-*myo*-Inositol-1-(1'-*O*-12-aminododecanoyl-2'-*O*-hexadecanoyl-*sn*-glycer-3'-yl sodium phosphate)-4-(disodium phosphate) 59

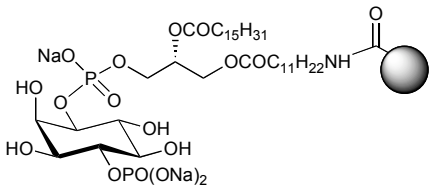


59

Lipid (+)-**57** (0.054 g, 0.0334 mmol, 1.0 equiv) was treated with palladium black (0.085 g, 0.80 mmol, 24.0 equiv) and sodium hydrogen carbonate (0.008 g, 0.10 mmol, 3.0 equiv) under hydrogen (4.1 bar) according to the procedure described for the preparation of (–)-**42**, to give (+)-**59** (0.023 g, 75%) as a white solid: mp 159-161 °C (lyophilised from water); $[\alpha]_D^{26}$ +8.9 (*c* 0.10 in MeOH); ν_{max} (neat)/cm^{-1} 3341, 2918, 2851, 1742, 1638, 1467, 1371, 1223, 1168, 1090, 1044, 1009, 943, 915, 900, 875, 722, 690; δ_H (500 MHz; CD_3OD) 5.26-5.24 (1 H, m), 4.58 (2 H, br s), 4.51 (1 H, dd, *J* =

12.0, 3.4), 4.26 (1 H, q, *J* 8.0), 4.24 (1 H, t, *J* 2.7), 4.19 (1 H, dd, *J* 12.0, 6.0), 4.11-3.99 (3 H, m), 3.87 (1 H, t, *J* 9.4), 3.58 (1 H, dd, *J* 9.6, 2.7), 3.43 (1 H, t, *J* 9.1), 2.95 (2 H, t, *J* 7.7), 2.38-2.33 (4 H, m), 1.70-1.60 (6 H, m), 1.45-1.22 (38 H, m), 0.89 (3 H, t, *J* 6.9); δ_C (125 MHz; CD_3OD) 175.0, 174.7, 79.2, 78.1, 75.9, 73.1, 72.9, 72.5, 72.1, 69.1, 64.9, 63.5, 40.8, 35.1, 34.9, 33.1, 30.8, 30.7, 30.6, 30.5, 30.2, 30.0, 29.9, 29.7, 28.5, 27.3, 26.9, 26.0, 25.8, 23.7, 14.5; δ_P (202 MHz; CD_3OD) 3.12, 0.89; HRMS Calcd. for $C_{37}H_{71}NNa_3O_{16}P_2$ ($M+Na^+$) 916.3941. Found: 916.3936.

1D-*myo*-Inositol-1-{1'-*O*-[12-*N*-(Affi-Gel 10)aminododecanoyl]-2'-*O*-hexadecanoyl-*sn*-glycer-3'-yl sodium phosphate}-4-(disodium phosphate) 60

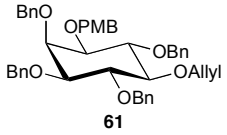


60

Affi-Gel® 10 (2 mL slurry, 0.030 mmol, 4.55 equiv) was treated with (+)-**59** (0.006 g, 0.0066 mmol, 1.0 equiv) and sodium hydrogen carbonate (0.011 g, 0.13 mmol, 19.7 equiv) according to the procedure described for the preparation of **43**, to give **60** (loading 0.0048 mmol, 16%).

1.8 Synthesis of dipalmitoyl-PI(5)P 67

(–)-5-*O*-Allyl-1-*O*-(4'-methoxybenzyl)-2,3,4,6-*O*-tetrabenzyl-*myo*-inositol 61

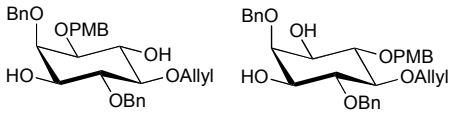


61

Sodium hydride (0.092 g, 60% in mineral oil, 2.3 mmol, 3.0 equiv) was washed with dry hexane under argon. After removal of the solvent the residual solid was suspended in dimethylformamide (3 mL) and cooled to 0 ºC. A solution of core **C** (0.398 g, 0.764 mmol, 1.0 equiv) in dimethylformamide (6 mL) was added *via* a cannula with stirring. The suspension was stirred for 15 min and then warmed to rt, after which benzyl bromide (0.236 mL, 1.99 mmol, 2.6 equiv) was added dropwise and the suspension was stirred at rt for 20 h. The reaction was quenched by the addition of methanol (0.5 mL) and the solvent was removed under reduced pressure. The residue was partitioned between ethyl acetate (20 mL) and water (15 mL) and the organic layer separated. The aqueous layer was then back-extracted with ethyl acetate (20 mL) and the combined organic extracts were washed with brine (10 mL), dried (Na_2SO_4), and the solvent removed under reduced pressure. Purification by flash chromatography (25-40% ether/hexane) gave (–)-**61** (501 mg, 94%) as a white solid (Found: C, 77.05; H, 6.92. $C_{45}H_{48}O_7$ requires C, 77.12; H, 6.90%): mp 91-93 ºC; $[\alpha]_D^{22}$ –0.3 (c 1.1 in $CHCl_3$); ν_{max} (CCl_4)/cm^{-1} 3065, 3032, 2865, 1612, 1586, 1513, 1497, 1451,

1360, 1302, 1249, 1208, 1172, 1131, 1073, 734, 696; δ_H (500 MHz; $CHCl_3$) 7.43-7.27 (20 H, m), 7.24 (2 H, d, *J* 8.6), 6.86 (2 H, d, *J* 8.6), 5.99 (1 H, ddt, *J* 17.2, 10.4, 5.7), 5.29 (1 H, ddd, *J* 17.2, 3.3, 1.7), 5.16 (1 H, dd, *J* 10.4, 1.7), 4.90-4.80 (6 H, m), 4.67 (1 H, d, *J* 11.8), 4.61 (1 H, d, *J* 11.8), 4.59 (1 H, d, *J* 11.8), 4.54 (1 H, d, *J* 11.8), 4.36 (2 H, dt, *J* 5.7, 1.3), 4.05-4.00 (3 H, m), 3.83 (3 H, s), 3.45-3.29 (3 H, m); δ_C (100 MHz; $CHCl_3$) 159.1, 139.0, 138.9, 138.8, 138.4, 135.3, 130.5, 129.2, 128.3, 128.3, 128.2, 128.1, 128.1, 127.8, 127.5, 127.5, 127.3, 116.5, 113.7, 83.4, 81.6, 80.8, 80.5, 75.9, 75.8, 74.6, 74.4, 74.0, 72.8, 72.4, 55.3; *m/z* (ES^+) 723.3 ($M+Na^+$); HRMS (EI^+) Calcd. for $C_{45}H_{52}NO_7$ ($M+NH_4^+$) 718.3744. Found: 718.3740.

(+)-5-Allyl-2,4-bisbenzyl-1-(4'-methoxybenzyl)-*myo*-inositol 71 and (–)-5-allyl-2,4-bisbenzyl-6-(4'-methoxybenzyl)-*myo*-inositol S6

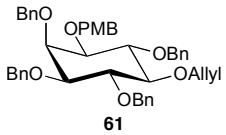


71 **S6**

ent-Core **A** (0.87 g, 2.17 mmol, 1.0 equiv), dibutyltin oxide (0.614 g, 2.47 mmol, 1.14 equiv) and tetrabutylammonium bromide (0.707 g, 2.19 mmol, 1.01 equiv) were suspended in acetonitrile (100 mL) and toluene (50 mL) and heated at reflux using a Soxhlet apparatus containing freshly activated 3Å molecular sieves. After 2 h, the reaction mixture was cooled to rt and *p*-methoxybenzyl chloride (0.40 mL, 2.95 mmol, 1.36 equiv) and sodium bromide (0.274 g, 2.66 mmol, 1.23 equiv) were added. The resulting mixture was heated at reflux overnight, cooled to room temperature and quenched with water (100 mL), and diluted with ether (50 mL). The aqueous layer was separated, extracted with dichloromethane (3 × 50 mL) and the organic layers combined, dried ($MgSO_4$) and the solvent removed under reduced pressure. Purification by flash chromatography (30-60% ethyl acetate/petroleum spirits) gave (+)-**71** (0.818 g, 72%) as a white solid: mp 66-68 ºC; $[\alpha]_D^{22}$ +1.76 (*c* 1.00 in $CHCl_3$); υ_{max} (film)/cm^{-1} 3457, 3030, 2914, 2873, 1513, 1454, 1247, 1174, 1112, 1060, 1028, 927, 821, 734, 697; δ_H (500 MHz; $CDCl_3$) 7.40-7.27 (10 H, m), 7.26 (2 H, d, *J* 9.0), 6.89 (2 H, d, *J* 9.0), 5.99 (1 H, ddt, *J* 17.0, 10.5, 5.7), 5.30 (1 H, dq, *J* 17.0, 1.7), 5.18 (1 H, dq, *J* 10.5, 1.7), 4.92 (1 H, d, *J* 11.5), 4.91 (1 H, d, *J* 11.0), 4.76 (1 H, d, *J* 11.5), 4.71 (1 H, d, *J* 11.5), 4.62 (1 H, d, *J* 11.5), 4.53 (1 H, d, *J* 11.5), 4.39 (1 H, ddt, *J* 12.5, 6.0, 1.5), 4.34 (1 H, ddt, *J* 12.5, 6.0, 1.5), 4.08 (1 H, t, *J* 9.5), 4.03 (1 H, t, *J* 2.5), 3.81 (3 H, s), 3.74 (1 H, t, *J* 9.5), 3.49 (1 H, ddd, *J* 9.3, 6.0, 3.0), 3.26 (1 H, dd, *J* 9.3, 9.3), 3.25 (1 H, d, *J* 10.0), 2.58 (1 H, br s), 2.30 (1 H, brd, *J* 6.0); δ_C (125 MHz; $CDCl_3$) 159.5, 138.8, 138.7, 135.4, 130.0, 129.6, 128.6, 128.5, 128.2, 127.9, 127.9, 127.7, 116.9, 114.1, 83.0, 82.0, 80.1, 76.3, 75.6, 74.7, 74.2, 73.1, 72.7, 72.3, 55.4; *m/z* (ESI^+) 559 (26, $M + K^+$), 543 (100, $M+Na^+$) and 121 (25); HRMS (ESI^+) Calcd for $C_{31}H_{36}NaO_7$ ($M+Na^+$) 543.2353. Found: 543.2354.

Further elution gave (–)-**S6** (0.258 g, 23%) as a colourless oil: $[\alpha]_D^{22}$ –10.1 (*c* 1.00 in $CHCl_3$); υ_{max}(film)/cm^{-1} 3456, 3028, 2918, 2877, 1613, 1514, 1455, 1358, 1302, 1249, 1125, 1065, 1037, 932, 821, 698; δ_H (500 MHz; $CDCl_3$) 7.40-7.28 (10 H, m), 7.31 (2 H, d, *J* 9.0), 6.89 (2 H, d, *J* 9.0), 6.00 (1 H, ddt, *J* 17.5, 10.5, 5.6), 5.32 (1 H, dq, *J* 17.5, 1.6), 5.19 (1 H, dq, *J* 10.5, 1.6), 4.91 (1 H, d, *J* 11.0), 4.85 (1 H, d, *J* 11.0), 4.80 (2 H, d, *J* 1.5), 4.76 (1 H, d, *J* 11.0), 4.68 (1 H, d, *J* 11.0), 4.37 (1 H, dd, *J* 2.4, 1.2), 4.36 (1 H, dd, *J* 2.4, 1.2), 3.99 (1 H, t, *J* 2.8), 3.80 (3 H, s), 3.74 (1 H, dd, *J* 9.3, 0.8), 3.72 (1 H, dd, *J* 9.5, 1.0), 3.51 (2 H, m), 3.32 (1 H, t, *J* 9.3), 2.26 (1 H, brd, *J* 6.0), 2.23 (1 H, brd, *J* 5.5); δ_C (125 MHz, $CDCl_3$) 159.6, 138.8, 138.7, 135.2, 130.8, 130.0, 128.7, 128.6, 128.3, 128.0, 127.9, 127.9, 116.9, 114.2, 83.5, 82.4, 81.9, 79.1, 75.7, 75.4, 75.3, 74.4, 72.7, 72.7, 55.4; *m*/*z* (ESI^+) 543 (100, $M+Na^+$), 121 (72); HRMS Calcd for $C_{31}H_{36}NaO_7$ ($M+Na^+$) 543.2353. Found: 543.2352.

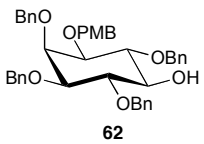
(–)-5-*O*-Allyl-1-*O*-(4'-methoxybenzyl)-2,3,4,6-*O*-tetrabenzyl-*myo*-inositol 61



61

Sodium hydride (0.316 g, 7.90 mmol, 3.01 equiv) was washed with dry hexane (2 × 5 mL) before suspending in dry DMF (12 mL). The resulting suspension was cooled to 0 °C before addition of the diol (+)-**71** (1.37 g, 2.17 mmol, 1.0 equiv) as a solution in DMF (6 mL). The reaction was stirred for 10 min before warming to room temperature for 1 h, followed by addition of benzyl bromide (0.90 mL, 7.58 mmol, 2.9 equiv) and stirring for 17 h. The reaction was quenched by careful addition of water (1 mL), with cooling on ice. DMF was removed on the rotary evaporator and the crude product was separated between water (50 mL) and ethyl acetate (50 mL). The aqueous layer was extracted with ethyl acetate (2 × 50 mL) and the organic layers were combined, dried with magnesium sulfate and reduced *in vacuo*. The crude product was purified by flash chromatography using a gradient elution (10 to 25% ethyl acetate / petroleum spirits) to give (–)-**61** (1.21 g, 66%) as a white solid with data matching that above ($[\alpha]_D^{21}$ –0.92 (*c* 1.00 in $CHCl_3$)).

(+)-1-*O*-(4'-Methoxybenzyl)-2,3,4,6-*O*-tetrabenzyl-*myo*-inositol 62

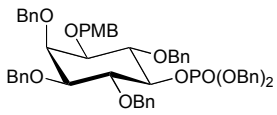


62

To a solution of (–)-**61** (0.474 g, 0.676 mmol, 1.0 equiv) and *N*,*N*-diisopropylethylamine (0.035 mL, 0.20 mmol, 0.3 equiv) in ethanol-toluene-water 7:3:1 (25 mL) was added $(Ph_3P)_3RhCl$ (0.094 g, 0.10 mmol, 0.15 equiv). The solution was then refluxed for 2 h, cooled to rt, and filtered over

hyflo washing with ethyl acetate. The solvent was removed under reduced pressure and the residue suspended in water (15 mL) and extracted with ether (3 × 20 mL). The combined organic layers were washed with brine (25 mL), dried ($MgSO_4$) and the solvent removed to give the crude propenyl ether (a mixture of geometric isomers) as brown oil. This was then dissolved in dichloromethane/methanol (5:3, 32 mL), and acetyl chloride (0.030 mL, 0.42 mmol, 0.62 equiv) was added. The solution was stirred at rt for 75 min, quenched with triethylamine (1 mL), and the volatiles removed under reduced pressure. Purification by flash chromatography (20% ethyl acetate/hexane) gave (+)-**62** (0.372 g, 83%) as a white solid (Found: C, 75.55; H, 6.58. $C_{42}H_{44}O_7$ requires C, 76.34; H, 6.71%): mp 162-163 ºC; $[\alpha]_D^{20}$ +0.6 (c 0.8 $CHCl_3$); ν_{max} (neat)/cm^{-1} 3588, 3060, 3028, 2922, 1614, 1586, 1514, 1496, 1454, 1360, 1304, 1251, 1209, 1160, 1111, 1092, 1045, 825, 734, 697; δ_H (500 MHz; $CDCl_3$) 7.44-7.28 (20 H, m), 7.25 (2 H, d, *J* 8.6), 6.87 (2 H, d, *J* 8.6), 4.95 (2 H, d, *J* 11.2), 4.89 (2 H, s), 4.82 (1 H, d, *J* 5.8), 4.80 (1 H, d, *J* 5.8), 4.66 (1 H, d, *J* 11.8), 4.61 (1 H, d, *J* 11.8), 4.58 (1 H, d, *J* 11.4), 4.55 (1 H, d, *J* 11.4), 4.04 (1 H, br s), 3.95 (2 H, q, *J* 9.1), 3.83 (3 H, s), 3.53 (1 H, td, *J* 9.1, 1.9), 3.37-3.33 (2 H, m), 2.48 (1 H, d, *J* 1.9); δ_C (100 MHz; $CHCl_3$) 159.2, 139.0, 138.9, 138.8, 138.3, 130.4, 129.2, 128.4, 128.3, 128.2, 128.0, 127.9, 127.7, 127.6, 127.5, 127.3, 113.8, 81.1, 81.1, 80.7, 80.4, 75.4, 75.3, 75.1, 74.5, 74.1, 72.6, 72.3, 55.3; *m/z* (ES^+) 683.4 ($M+Na^+$); HRMS (EI^+) Calcd. for $C_{42}H_{48}NO_7$ ($M+NH_4^+$) 678.3431. Found: 678.3440.

1-*O*-(4'-Methoxybenzyl)-2,3,4,6-tetra-*O*-benzyl-*myo*-inositol-5-(dibenzyl phosphate) 63

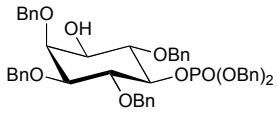


63

Bis(benzyloxy)(*N,N*-diisopropylamino) phosphine[10] (0.0973 g, 0.28 mmol, 3.0 equiv) and recrystallised 1*H*-tetrazole (0.0197 mg, 0.28 mmol, 3.0 equiv) were combined and placed under high vacuum for 10 min. The vacuum was quenched with argon and dry dichloromethane (2 mL) was added. The mixture was cooled to –10 ºC and a solution of (+)-**62** (0.062 g, 0.09 mmol, 1.0 equiv) in dry dichloromethane (4 mL) was added *via* cannula and washed in with further dry dichloromethane (4 mL). The solution was stirred for 30 min at –10 ºC and then warmed to rt and stirred under argon for 12-48 hours or until TLC analysis indicated that all the starting alcohol had been consumed and that a less polar product had formed. The solution was cooled to –78 ºC and 3-chloroperbenzoic acid (48.5 mg, 0.28 mmol, 3.0 equiv) was added in one portion. The resulting mixture was stirred at –78 ºC for 30 min and then at rt for 3 h. The solution was washed with 10% aq. sodium hydrogen sulfite (10 mL), which was back extracted with dichloromethane (3 × 10 mL). The organic fractions were combined, washed with sat. aq. sodium hydrogen carbonate (10 mL), brine (10 mL), dried ($MgSO_4$), and the solvent removed under reduced pressure. Purification by

flash chromatography (30% ethyl acetate/hexane gave **63** (0.079 g, 91%) as a white solid (Found: C, 72.6; H, 6.5. Calcd. for $C_{56}H_{57}O_{10}P$: C, 73.0; H, 6.2%): mp 104-105 ºC; $[\alpha]_D^{23}$ 0.0 (*c* 0.775 in $CHCl_3$); ν_{max} (film)/cm^{-1} 3010, 2935, 1612, 1514, 1497, 1455, 1365, 1249, 1090, 1020, 735, 696; δ_H (500 MHz; $CHCl_3$) 7.41-7.17 (26 H, m), 7.13 (2 H, d, *J* 8.7), 7.01 (4 H, d, *J* 7.9), 6.78 (2 H, d, *J* 8.7), 5.00 (2 H, d, *J* 8.1), 4.92-4.85 (5 H, m), 4.80-4.70 (3 H, m), 4.56 (1 H, d, *J* 11.7), 4.53 (1 H, d, *J* 11.7), 4.48 (2 H, s), 4.45 (1 H, q, *J* 9.0), 4.06 (2 H, q, *J* 9.5), 3.95 (1 H, t, *J* 2.2), 3.80 (3 H, s), 3.35 (1 H, dd, *J* , 5.7, 2.3), 3.33 (1 H, dd, *J* , 5.7, 2.3); δ_C (125 MHz; $CHCl_3$) 159.2, 138.8, 138.7, 137.9, 136.1, 135.8, 130.0, 129.4, 128.5, 128.5, 128.4, 128.2, 128.1, 128.0, 127.0, 127.9, 127.9, 127.8, 127.7, 127.6, 127.5, 127.5, 127.4, 127.1, 113.7, 81.1, 80.2, 80.0, 79.4, 79.3, 74.7, 74.6, 74.1, 74.0, 72.7, 72.4, 69.3, 68.9, 55.3; δ_P (162 MHz; $CDCl_3$): –0.64; *m/z* (ES^+) 921.5 ($M+H^+$), 943.4 ($M+Na^+$); HRMS (ES^+) Calcd. for $C_{56}H_{58}O_{10}P$ ($M+H^+$) 921.3762. Found: 921.3736.

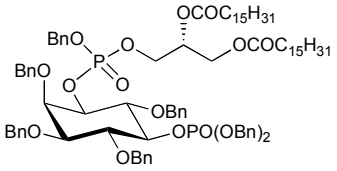
(–)-2,3,4,6-Tetra-*O*-benzyl-*myo*-inositol-5-(dibenzyl phosphate) 64



64

The PMB ether of inositol **63** (0.322 g, 0.350 mmol, 1.0 equiv) was removed using ceric ammonium nitrate (1.17 g, 2.13 mmol, 6.1 equiv) according to the procedure described for the preparation of (–)-**40**. Purification by flash chromatography (33-40% ethyl acetate/hexane) gave (–)-**64** (0.222 g, 79%) as a colourless oil: $[\alpha]_D^{20}$ –6.0 (c 0.7 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3568, 3067, 3020, 2944, 2873, 1497, 1435, 1364, 1267, 1220, 1212, 1130, 1074, 1018, 728; δ_H (400 MHz; $CDCl_3$) 7.38-7.11 (30 H, m), 5.04-4.66 (12 H, m), 4.47 (1 H, q, *J* 9.1), 4.07 (1 H, t, *J* 9.5), 4.03 (1 H, t, *J* 2.5), 3.86 (1 H, t, *J* 9.5), 3.57-3.52 (1 H, m), 3.49 (1 H, dd, *J* 2.3, 9.6), 2.19 (1 H, d, *J* 6.6); δ_C (100 MHz; $CDCl_3$) 138.6, 138.6, 138.4, 137.8, 136.1, 136.0, 128.4, 128.4, 128.3, 128.1, 128.0, 127.8, 127.7, 127.7, 127.6, 127.6, 127.2, 80.8, 80.7, 80.5, 79.4, 76.8, 74.8, 74.7, 74.4, 73.0, 71.8, 69.1, 69.1; δ_P (162 MHz; $CDCl_3$) –0.64; *m/z* (ES^+) 801.3 (60, $M+H^+$), 823.3 (21, $M+Na^+$), 121.9 (34), 119.9 (38), 92.2 (38), 91.2 (100); HRMS (EI^+) Calcd. for $C_{48}H_{53}NO_9P$ ($M+NH_4^+$) 818.3458. Found: 818.3448.

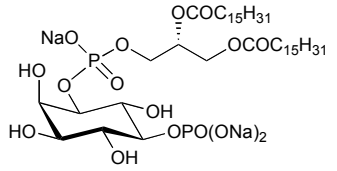
(+)-1D-2,3,4,6-Tetra-*O*-benzyl-*myo*-inositol-1-(1',2'-di-*O*-hexadecanoyl-*sn*-glycer-3'-yl benzyl phosphate)-5-(dibenzyl phosphate) 65



65

Alcohol (–)-**64** (0.073 g, 0.091 mmol, 1.0 equiv) was treated with phosphoramidite **34** (0.184 g, 0.228 mmol, 2.5 equiv), 1*H*-tetrazole (0.019 g, 0.27 mmol, 3.0 equiv), and 3-chloroperbenzoic acid (0.072 g, 0.41 mmol, 4.5 equiv) according to the procedure described for the preparation of (+)-**41**. Purification by flash chromatography (25-33% ethyl acetate/hexane) gave (+)-**65** (0.086 g, 62%) as a colourless oil: $[\alpha]_D^{20}$ +2.5 (c 1.3 $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 2926, 2854, 1738, 1602, 1456, 1265, 1009, 733, 696; δ_H (400 MHz; $CDCl_3$) 7.38-7.15 (31 H, m), 7.00 (4 H, m), 5.07-3.77 (24 H, m), 3.46 (1 H, dd, *J* 2.2, 9.8), 2.23-2.14 (4 H, m), 1.53 (4 H, m), 1.25 (48 H, m), 0.88 (6 H, t, *J* 6.8); δ_C (100 MHz; $CDCl_3$) 173.1, 172.7, 138.5, 138.2, 137.7, 136.0, 135.4, 128.6, 128.3, 128.3, 128.1, 128.1, 127.8, 127.6, 127.5, 127.4, 127.3, 127.2, 80.4, 79.9, 79.0, 78.2, 76.1, 76.0, 75.1, 74.7, 74.6, 72.8, 72.7, 69.6, 69.5, 69.2, 69.1, 65.8, 65.5, 61.5, 34.1, 33.9, 31.9, 29.7, 29.5, 29.3, 29.3, 29.1, 29.1, 24.8, 22.7, 14.1; δ_P (162 MHz; $CDCl_3$) –0.71, –1.20; *m/z* (FAB^+) 1522.4 (28, $M+H^+$), 1544.4 (100, $M+Na^+$); HRMS (ESI^+) Calcd. for $C_{90}H_{122}NaO_{16}P_2$ ($M+Na^+$) 1543.8100. Found: 1543.8091.

(–)-1D-*myo*-Inositol-1-(1',2'-di-*O*-hexadecanoyl-*sn*-glycer-3'-yl sodium phosphate)-5-phosphate 67



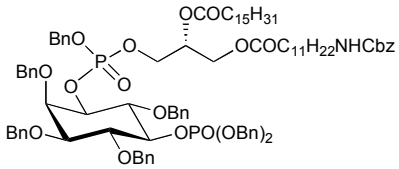
67

Lipid (+)-**65** (0.038 g, 0.025 mmol, 1.0 equiv) was treated with palladium black (40 mg, 0.37 mmol, 15.0 equiv) and sodium bicarbonate (6.3 mg, 0.075 mmol, 3.0 equiv) under hydrogen (3.5 bar), according to the procedure described for the preparation of (–)-**42**, to give (–)-**67** (0.021 g, 86%) as a fluffy white solid. For characterisation purposes a sample of the product was converted into the tetrabutyl ammonium salt by stirring with Bu_4NCl (3.0 equiv) in $CHCl_3$/MeOH/H_2O 4:5:2. All analytical data are from this salt: $[\alpha]_D^{28}$ –4.7 (*c* 0.58, CH_3Cl); ν_{max} (neat)/cm^{-1} 3303, 2917, 2850, 1740, 1467, 1162, 1110, 1020; δ_H (500 MHz; $CDCl_3$) 5.30 (1H, br s), 4.43 (1H, d, *J* 9.0), 4.31 (1H, br s), 4.24 (1H, br,s), 4.17 (1H, dd, *J* 12.3, 6.5), 4.11-4.07 (1H, m), 4.02-3.99 (1H, m), 3.93-3.82 (2H, m), 3.73 (2H, q, *J* 7.0), 2.26 (4H, br s), 1.67 (24H, br s), 1.58 (24H, br s), 1.35 (24H, br s), 1.25 (44H, s), 1.01 (36H, br s), 0.88 (6H, t, *J* 6.7); δ_P (162 MHz; $CDCl_3$) –2.40, 1.03; *m/z* (ESI^+)

913.7 (70, M+Na$^+$); HRMS (ESI$^+$) Calcd. for $C_{41}H_{80}NNaO_{16}P_2$ (M+Na$^+$) 913.4814). Found: 913.4773.

1.9 Synthesis of NH_2-PI(5)P 68 and affinity probe 69

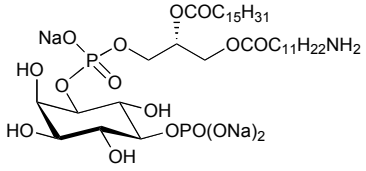
(+)-1D-2,3,4,6-Tetra-*O*-benzyl-*myo*-Inositol-1-{1'-*O*-[12-*N*-(benzyloxycarbonyl) aminododecanoyl]-2'-*O*-hexadecanoyl-*sn*-glycer-3'-yl benzyl phosphate}-5-(dibenzyl phosphate) 66



66

Alcohol (–)-**64** (0.063 g, 0.079 mmol, 1.0 equiv) was treated with phosphoramidite **38** (0.177 g, 0.20 mmol, 2.5 equiv), 1*H*-tetrazole (0.017 g, 0.24 mmol, 3.0 equiv) and 3-chloroperbenzoic acid (0.062 g, 0.36 mmol, 4.5 equiv) according to the procedure described for the preparation of (+)-**41**. Purification by flash chromatography (6-10% ethyl acetate/dichloromethane) gave (+)-**66** (0.081 g, 64%) as a colourless oil: $[\alpha]_D^{20}$ +3.1 (c 1.5 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3452, 2929, 2855, 1736, 1603, 1518, 1456, 1363, 1266; δ_H (400 MHz; $CDCl_3$) 7.38-7.15 (36 H, m), 7.00 (4 H, m), 5.09-3.77 (27 H, m), 3.47 (1 H, dd, *J* 2.1, 9.8), 3.17 (2 H, q, *J* 6.6), 2.23-2.14 (4 H, m), 1.51 (4 H, m), 1.25 (41 H, m), 0.88 (3 H, t, *J* 6.8). δ_C (100 MHz; $CDCl_3$) 173.0, 172.7, 156.4, 138.5, 138.2, 137.7, 137.7, 136.7, 136.0, 128.6, 128.5, 128.4, 128.3, 128.1, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 80.0, 79.9, 79.0, 78.2, 76.1, 76.0, 75.1, 74.7, 74.6, 72.8, 72.7, 69.7, 69.6, 69.2, 69.1, 66.5, 61.5, 41.1, 34.1, 33.9, 31.9, 30.0, 29.7, 29.5, 29.3, 29.2, 29.1, 26.7, 24.8, 22.7, 14.1; δ_P (162 MHz; $CDCl_3$) –0.72, –1.19, –1.21; *m/z* (FAB$^+$) 1614.5 (M+H$^+$), 1637.4 (M+Na$^+$).

(+)-1D-*myo*-Inositol-1-(1'-*O*-12-aminododecanoyl-2'-*O*-hexadecanoyl-*sn*-glycer-3'-yl sodium phosphate)-5-(disodium phosphate) 68

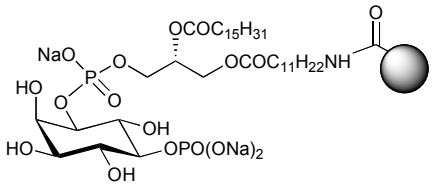


68

Lipid (+)-**66** (0.081 g, 0.0502 mmol, 1.0 equiv) was treated with palladium-black (0.090 g, 0.99 mmol, 19.7 equiv) and sodium hydrogen carbonate (0.0126 g, 0.150 mmol, 3.0 equiv) under hydrogen (35 bar) according to the procedure described for the preparation of (–)-**42**, to give (+)-**68** (0.038 g, 83%) as a fluffy white solid: $[\alpha]_D^{20}$ +1.1 (c 0.2 in H_2O); ν_{max} (neat)/cm^{-1} 3207, 2920, 2851, 1741, 1639, 1540, 1467, 1223, 1064, 1041; δ_H (400 MHz; D_2O) 5.23 (1 H, m), 4.41 (1 H, m),

4.17-4.14 (2 H, m), 4.00-3.94 (3 H, m), 3.79-3.67 (3 H, m), 3.54 (1 H, dd, *J* 2.3, 9.8), 2.93-2.89 (2 H, m), 2.37-2.21 (4 H, m), 1.63-1.53 (6 H, m), 1.21-1.19 (38 H, m), 0.79 (3 H, br t); δ_P (162 MHz; D_2O) 4.65, 0.10; *m/z* (ES^-) 847.6 ($M–2H^-$); HRMS (ES^-) Calcd. for $C_{37}H_{71}NO_{16}P_2$ ($M–2H^-$) 847.4248. Found: 847.4240.

1D-*myo*-Inositol-1-{1'-*O*-[12-*N*-(Affi-Gel 10)aminododecanoyl]-2'-*O*-hexadecanoyl-*sn*-glycer-3'-yl sodium phosphate}-5-bis(disodium phosphate) 69

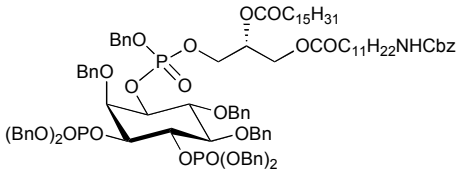


69

Affi-Gel® 10 (3.1 mL slurry, 0.047 mmol, 4.3 equiv) was treated with amine (+)-**68** (0.010 g, 0.0109 mmol, 1.0 equiv) and sodium hydrogen carbonate (0.0092 g, 0.11 mmol, 10.0 equiv) according to the procedure described for the preparation of **43**, to give **69** (loading 0.0035 mmol, 8%).

1.10 Synthesis of NH_2-PI(3,4)P2 77 and affinity probe 84

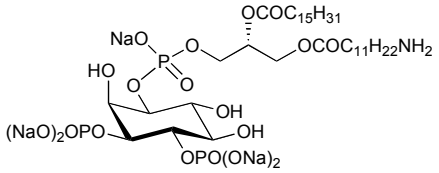
(+)-1D-2,5,6-Tri-*O*-benzyl-*myo*-inositol-1-{1'-*O*-[12-*N*-(benzyloxycarbonyl)aminododecanoyl]-2'-*O*-hexadecanoyl-*sn*-glycer-3'-yl benzyl phosphate}-3,4-bis(dibenzyl phosphate) 75



75

Alcohol (+)-**73**[2] (0.222 g, 0.229 mmol, 1.0 equiv) was treated with phosphoramidite **38**, (0.550 g, 0.612 mmol, 2.67 equiv), 1*H*-tetrazole (0.055 g, 0.714 mmol, 3.12 equiv), and 3-chloroperbenzoic acid (0.220 g, 1.27 mmol, 5.55 equiv) according to the procedure described for the preparation of (+)-**41**. Purification by flash chromatography (20-50% ethyl acetate/hexane) gave (+)-**75** (0.339 g, 83%) as a colourless gum (Found: C, 67.96; H, 7.28; N, 0.82; P, 5.24. Calcd. for $C_{101}H_{128}NO_{21}P_3$: C, 67.96; H, 7.23; N, 0.78, P, 5.21%): $[\alpha]_D^{25}$ +3.67 (*c* 1.58 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3453, 3067, 2927, 2855, 1736, 1514, 1455, 1267, 1017; δ_H (250 MHz; $CDCl_3$): 7.36-7.02 (45 H, m), 5.05-4.60 (22 H, m), 4.38-3.80 (7 H, m), 3.46 (1 H, q, *J* 8.4), 3.17 (2 H, q, *J* 6.6), 2.28-2.12 (4 H, m,), 1.60-1.40 (6 H, m), 1.30-1.18 (38 H, m), 0.87 (3 H, t, *J* 6.9); δ_P (101 MHz; $CDCl_3$) -0.94, -1.19, -1.23, -1.56, -1.59; *m/z* (ESI^+) 804 (60), 1163 (20), 1807 ($M+Na^+$, 100); δ_P (101.25 MHz; $CDCl_3$), -0.94, -1.19, -1.23, -1.56, -1.59; *m/z* (ESI) 1807 ($M+Na^+$, 100), 1163 (20), 804 (60); HRMS Calcd. for $C_{101}H_{128}NNaO_{21}P_3$ ($M+Na^+$) 1806.8089. Found: 1806.8133.

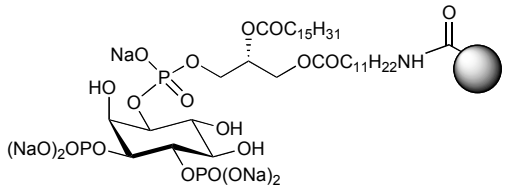
(+)-1D-*myo*-Inositol-1-(1'-*O*-12-aminododecanoyl-2'-*O*-hexadecanoyl-*sn*-glycer-3'-yl sodium phosphate)-3,4-bis(disodium phosphate) 77



77

Lipid (+)-**75** (0.243 g, 0.136 mmol, 1.0 equiv) was treated with palladium-black (0.348 g, 3.27 mmol, 24.0 equiv) and sodium hydrogen carbonate (0.057 g, 0.678 mmol, 3.0 equiv) under hydrogen (3.6 bar) according to the procedure described for the preparation of (+)-**51**, to give (+)-**77** (0.113 g, 80%) as a white solid: $[\alpha]_D^{22}$ +9.3 (*c* 0.28 in H_2O); ν_{max} (KBr)/cm^{-1} 3404, 2920, 2850, 1742, 1498, 1466, 1374, 1260, 1238, 1094, 1012, 925, 803, 734, 696; δ_H (250 MHz; D_2O) 5.25 (1 H, br s), 4.50-3.91 (8 H, m), 3.85-3.70 (1 H, m), 3.55-3.45 (1 H, m), 3.00-2.89 (2 H, m), 2.40-2.20 (4 H, m), 1.70-1.50 (6 H, m), 1.40-1.15 (38 H, m), 0.89-0.75 (3 H, m); δ_P (101.25 MHz; D_2O) 4.20, 3.52, 2.15; *m/z* (FAB$^-$) 1017 (15, M–Na$^+$), 995, (80), 973 (100), 950 (15).

1D-*myo*-Inositol-1-{1'-*O*-[12-*N*-(Affi-Gel 10)aminododecanoyl]-2'-*O*-hexadecanoyl-*sn*-glycer-3'-yl sodium phosphate}-3,4-bis(disodium phosphate) 84

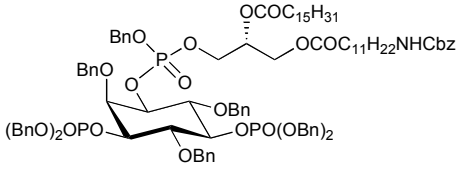


84

Affi-Gel® 10 beads (4 mL slurry, 0.060 mmol) were washed with Milli-Q water and centrifuged (2 × 5 mL, 2000 rpm, 3 min) and the excess supernatant removed. A solution of amine (+)-**77** (0.002 g, 0.00192 mmol, 0.032 equiv) in Milli-Q water (4 mL) was then added and the mixture incubated overnight at 4 °C. The beads were then capped by treatment with ethanolamine (5 mL) for 2 h. The beads were then washed with Milli-Q water (5 × 5mL) to give **84**. Loading was determined by Biacore analysis[18] (0.0019 mmol, 3%).

1.11 Synthesis of NH_2-PI(3,5)P2 83 and affinity probe 85

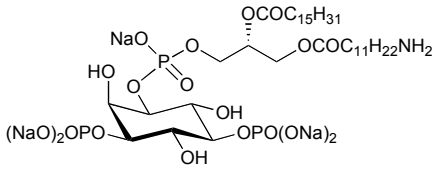
(–)-1D-2,4,6-Tri-*O*-benzyl-*myo*-inositol-1-{1'-*O*-[12-*N*-(benzyloxycarbonyl)aminododecanoyl]-2'-*O*-hexadecanoyl-*sn*-glycer-3'-yl benzyl phosphate}-3,5-bis(dibenzyl phosphate) 81



81

Alcohol (–)-**79**[2] (0.275 g, 0.283 mmol, 1.0 equiv) was treated with phosphoramidite **38**, (0.630 g, 0.701 mmol, 2.48 equiv), 1*H*-tetrazole (0.057 g, 0.814 mmol, 2.88 equiv), and 3-chloroperbenzoic acid (0.220 g, 1.27 mmol, 4.49 equiv) according to the procedure described for the preparation of **41**. Purification by flash chromatography (30-60% ethyl acetate/hexane) gave (–)-**81** (0.439 g, 88%) as a colourless gum (Found: C, 67.73; H, 7.22; N, 0.76; P, 5.27. Calcd. for $C_{101}H_{128}NO_{21}P_3$: C, 67.96; H, 7.23; N, 0.78; P, 5.21%): $[\alpha]_D^{22}$ –0.6 (*c* 1.68 in $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3322, 3032, 2925, 2852, 1741, 1529, 1498, 1456, 1378, 1365, 1270, 1260, 1012, 925, 803, 734, 696; δ_H (250 MHz; $CDCl_3$), 7.45-7.00 (41 H, m), 6.98-6.92 (4 H, m), 5.15-3.68 (30 H, m), 3.17 (2H, q, *J* 6.9), 2.25-2.10 (4 H, m), 1.60-1.40 (6 H, m), 1.40-1.10 (38 H, m), 0.90-0.80 (3 H, m); δ_P (101.25 MHz; $CDCl_3$), –0.76, –0.77, –0.99, –1.03, –1.06, –1.09.

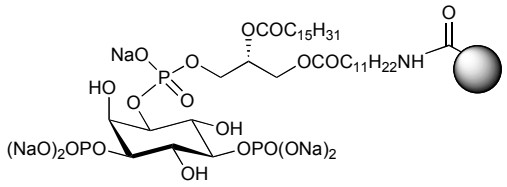
(+)-1D-*myo*-Inositol-1-(1'-*O*-12-aminododecanoyl-2'-*O*-hexadecanoyl-*sn*-glycer-3'-yl sodium phosphate)-3,4-phosphate 83



83

Lipid (–)-**81** (0.140 g, 0.0796 mmol, 1.0 equiv) was treated with palladium-black (0.348 g, 3.27 mmol, 41.0 equiv) and sodium hydrogen carbonate (0.033g, 0.393 mmol, 4.93 equiv) under hydrogen (3.6 bar) according to the procedure described for the preparation of (+)-**51**, to give (+)-**83** (0.073 g, 88%) as a white solid: $[\alpha]_D$ +1.25 (*c* 0.32 in D_2O); ν_{max} (neat)/cm^{-1} 3422, 2921, 2851, 1744, 1617, 1459, 1228, 1100, 970, 811; δ_H (250 MHz; D_2O) 5.24 (1 H, br s), 4.45-3.75 (10 H, m), 2.98-2.85 (2 H, m), 2.40-2.20 (4 H, m), 1.70-1.45 (6 H, m), 1.40-1.12 (38 H, m), 0.89-0.75 (3 H, m); δ_P (101.25 MHz; D_2O) 5.73, 4.97, 0.61; *m/z* (FAB^-) 1016 (30, M-Na^+), 996, (82, M-2Na^+), 974 (100, M-3Na^+), 952 (30).

1D-*myo*-Inositol-1-{1'-*O*-[12-*N*-(Affi-Gel 10)aminododecanoyl]-2'-*O*-hexadecanoyl-*sn*-glycer-3'-yl sodium phosphate}-3,5-bis(disodium phosphate) 85

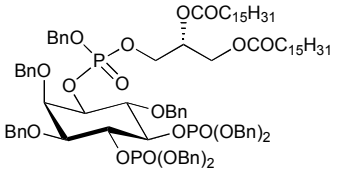


85

Affi-Gel® 10 beads (4 mL slurry, 0.060 mmol) were treated with amine (+)-**83** (0.002 g, 0.00192 mmol, 0.032 equiv) according to the procedure described for the preparation of **84**, to give **85** (loading 0.0019 mmol, 3%).

1.12 Synthesis of dipalmitoyl-PI(4,5)P2 92

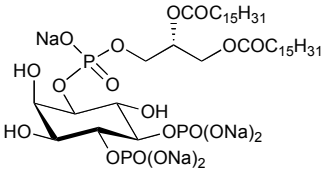
(–)-1D-2,3,6-Tri-*O*-benzyl-*myo*-inositol-1-(1',2'-di-*O*-hexadecanoyl-*sn*-glycer-3'-yl benzyl phosphate)-4,5-(dibenzyl phosphate) 90



90

Alcohol (–)-**89**[3] (0.083 g, 0.085 mmol, 1.0 equiv), dissolved in dichloromethane (3 mL), was added *via* cannula to a mixture of the phosphoramidite **34** (0.19 g, 0.231 mmol, 2.72 equiv) and 1*H*-tetrazole (0.023 g, 0.333 mmol, 3.92 equiv) in dichloromethane (3 mL). After stirring at rt for 2 h, the reaction mixture was cooled to –78 °C and 3-chloroperbenzoic acid (0.11 g, 0.64 mmol, 7.53 equiv) was added in a single portion and the mixture stirred at –78 °C for 45 min. The solution was then warmed to rt and stirred for a further 4 h after which the reaction mixture was diluted with dichloromethane (10 mL) and washed with 10% sodium hydrogen sulfite solution (10 mL). The aqueous phase was extracted with dichloromethane (3 × 10 mL) and the combined dichloromethane extracts were washed with sat. aq. sodium hydrogen carbonate solution (10 mL), brine (10 mL), dried ($MgSO_4$) and the solvent removed under reduced pressure. Purification of the residue by flash chromatography (30-60% ethyl acetate/hexane) gave (–)-**90** (0.12 g, 83%) as a colourless gum: $[\alpha]_D^{20}$ –4.3 (*c* 0.53 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 7.38-7.06 (38 H, m), 6.95 (2 H, d, *J* 8.0), 5.07-4.51 (18 H, m), 4.34-4.20 (3 H, m), 4.11-3.74 (6 H, m), 3.55 (1 H, dd, *J* 9.9, 1.9), 3.49 (1 H, dd, *J* 9.9, 1.9), 2.23-2.14 (4 H, m), 1.54 (4 H, br s), 0.87 (6 H, t, *J* 6.8); δ_P (162 MHz; $CDCl_3$) –1.66, –1.86, –1.89, –2.01, –2.05.

(+)-1D-*myo*-Inositol-1-(1',2'-di-*O*-hexadecanoyl-*sn*-glycer-3'-yl sodium phosphate)-4,5-phosphate 92

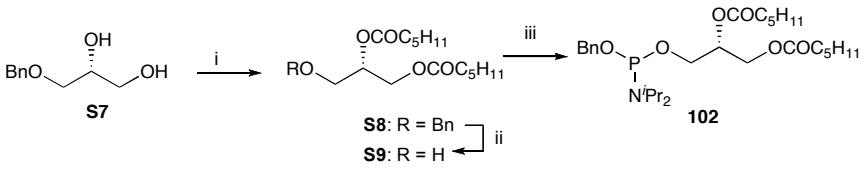


92

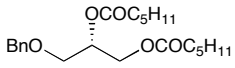
Lipid (–)-**90** (0.12 g, 0.071 mmol, 1.0 equiv) was treated with palladium black (0.12 g, 1.128 mmol, 15.9 equiv) and sodium hydrogen carbonate (0.030 g, 0.35 mmol, 4.93 equiv) under hydrogen (25 bar) according to the procedure described for the preparation of **42**, to give (+)-**92** (0.060 g, 78%) as a fluffy white solid: $[\alpha]_D^{20}$ +0.3 (*c* 0.2 in H_2O); δ_H (500 MHz; D_2O) 5.24 (1 H, br s), 4.34 (1 H, br d, *J* 10.9), 4.14 (3 H, m), 3.99 (3 H, m), 3.86 (2 H, m), 3.65 (1 H, br d, *J* 9.7), 2.35-2.26 (4 H, m), 1.53 (4 H, m), 1.21 (48 H, br s), 0.78 (6 H, br s); δ_P (162 MHz; D_2O) 4.9, 4.5, 0.6.

1.13 Synthesis of dihexanoyl-PI(3,4,5)P3 108

Synthesis of phosphoramidite 102



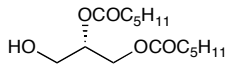
(+)-3-*O*-Benzyl-1,2-di-*O*-hexanoyl-*sn*-glycerol S8[19]



S8

To a stirred solution of **S7**[8] (1.80 g, 9.90 mmol, 1.0 equiv), pyridine (2.00 mL, 24.7 mmol, 2.5 equiv) and *N*,*N*-dimethylaminopyridine (0.035 g, 0.286 mmol, 0.03 equiv) in dichloromethane (100 mL), cooled to 0 °C under nitrogen, was added hexanoyl chloride (3.06 mL, 21.9 mmol, 2.21 equiv). After stirring at rt for 24 h, the reaction was quenched with the addition of 2M HCl (100 mL) and extracted with ether (2 × 200 mL). The combined organic extract was washed with brine (200 mL), dried ($MgSO_4$) and the solvent removed under reduced pressure. Purification of the residue by silica chromatography (10-20% ethyl acetate/hexane) gave (+)-**S8** (3.25 g, 87%) as a colourless oil: $[\alpha]_D$ +11.1 (*c* 6.3 in $CHCl_3$); δ_H (250 MHz; $CDCl_3$) 7.40-7.21 (5 H, m), 5.30-5.18 (1 H, m), 4.54 (2 H, d, *J* 12.2), 4.35 (1 H, dd, *J* 11.9, 3.8), 4.19 (1 H, dd, *J* 11.9, 6.4), 3.59 (2 H, d, *J* 5.2), 2.30 (2 H, t, *J* 7.5), 2.27 (2 H, t, *J* 7.6), 1.69-1.55 (4 H, m), 1.35-1.20 (8 H, m), 0.93-0.82 (6 H, m); δ_C (62.5 MHz; $CDCl_3$), 173.3, 173.0, 137.7, 128.4, 127.8, 127.6, 73.3, 70.0, 68.3, 62.7, 34.3, 34.1, 31.2, 24.6, 24.5, 22.3, 22.3, 13.9; HRMS (FIB) Calcd. for $C_{22}H_{35}O_5$ ($M+H^+$) 379.2484. Found: 379.2509.

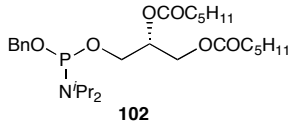
(–)-1,2-Di-*O*-hexanoyl-*sn*-glycerol S9[19]



S9

To a solution of (+)-**S8** (3.25 g, 8.59 mmol, 1.0 equiv) in dichloromethane (60 mL) was added 10% palladium-on-carbon (0.540 g, 17% w/w) and the suspension was degassed with hydrogen (× 3). The mixture was then stirred under hydrogen for 24 h and filtered through *Celite*, washing with dichloromethane (150 mL). The solvent was removed under reduced pressure and the residue purified by silica chromatography (20-50% ethyl acetate/hexane) to give (–)-**S9** (1.89 g, 76%) as a colourless oil: $[\alpha]_D$ –7.3 (*c* 1.32 in $CHCl_3$); δ_H (250 MHz; $CDCl_3$) 5.06 (1 H, q, *J* 5.1), 4.31 (1 H, dd, *J* 11.9, 4.3), 4.19 (1 H, dd, *J* 11.9, 5.9), 3.71 (2 H, t, *J* 5.4), 2.41 (1 H, bt, *J* 5.9), 2.31 (2 H, t, *J* 7.3), 2.27 (2 H, t, *J* 7.7), 1.68-1.52 (4 H, m), 1.35-1.24 (8 H, m), 0.87 (6 H, t, *J* 6.8); δ_C (62.5 MHz; $CDCl_3$) 173.7, 173.4, 72.1, 62.1, 61.4, 34.2, 34.0, 31.2, 31.1, 24.5, 22.2, 13.8; HRMS (FIB) Calcd. for $C_{15}H_{29}O_5$ ($M+H^+$) 289.2015. Found: 289.2032.

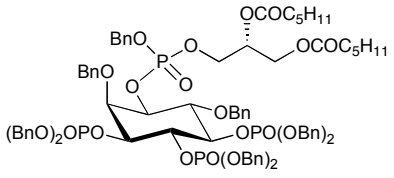
(+)-Benzyloxy(*N*,*N*-diisopropylamino)(1,2-di-*O*-hexanoyl-*sn*-glycer-3-yl)phosphine 102



102

Dry dichloromethane (20 mL) was added *via* cannula to a mixture of (–)-**S9** (0.546 g, 1.89 mmol, 1.0 equiv), (benzyloxy)bis(*N,N*-diisopropylamino)phosphine[9] (0.762 g, 2.25 mmol, 1.2 equiv) and 1*H*-tetrazole (0.087 g, 1.24 mmol, 0.66 equiv) under nitrogen. After stirring at rt for 3 h the solution was diluted with dichloromethane (150 mL) and washed with water (100 mL). The aqueous phase was extracted with dichloromethane (100 mL) and the combined organic extracts washed with sat. aq. sodium hydrogen carbonate (120 mL), brine (150 mL), dried ($NaSO_4$), and the solvent removed under reduced pressure. Purification by flash chromatography (triethylamine/ethyl acetate/hexane, 5:15:80) gave (+)-**102** (0.592 g, 60%) as a colourless oil: $[\alpha]_D$ +11.3 (*c* 0.48 in $CHCl_3$); δ_H (250 MHz; $CDCl_3$) 7.37-7.20 (5 H, m), 5.25-5.15 (1 H, m), 4.80-4.60 (2 H, m), 4.40-4.31 (1 H, m), 4.25-4.12 (1 H, m), 3.85-3.55 (3 H, m), 2.29 (4 H, t, *J* 7.4), 1.68-1.55 (4 H, m), 1.35-1.25 (8 H, m), 1.18 (6 H, t, *J* 6.8), 1.16 (6 H, t, *J* 6.8), 0.89 (6 H, t, *J* 6.4); δ_C (62.5 MHz; $CDCl_3$) 173.4, 173.0, 139.3, 128.3, 127.3, 127.0, 70.9, 70.8, 70.7, 65.5, 65.2, 62.5, 62.5, 61.9, 61.7, 61.5, 61.4, 43.2, 43.0, 34.3, 34.1, 31.3, 24.7, 24.6, 24.5, 22.3, 13.9; δ_P (101.25 MHz; $CDCl_3$) 149.3, 149.1; HRMS (FIB) Calcd. for $C_{28}H_{49}NO_6P$ ($M+H^+$) 526.3298. Found: 526.3304.

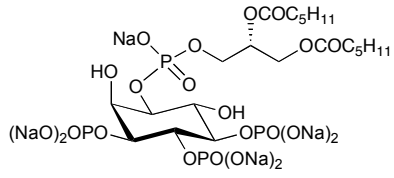
(–)-1D-2,6-Di-*O*-benzyl-*myo*-inositol-1-(1',2'-di-*O*-hexanoyl-*sn*-glycer-3'-yl benzyl phosphate)-3,4,5-tris(dibenzyl phosphate) 105



105

Alcohol (–)-**96**[2] (0.192 g, 0.168 mmol, 1.0 equiv) was treated with phosphoramidite **102**, (0.244 g, 0.464 mmol, 2.76 equiv), 1*H*-tetrazole (0.045 g, 0.642 mmol, 3.82 equiv) and 3-chloroperbenzoic acid (0.285 g, 1.65 mmol, 9.82 equiv) according to the procedure described for the preparation of (+)-**41**. Purification by flash chromatography (30-70% ethyl acetate/hexane) gave (–)-**105** (0.178 g, 0.113 mmol, 67%) as a colourless gum: $[\alpha]_D$ –4.3 (*c* 2.4 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3049, 2957, 1741, 1498, 1456, 1276, 1016; δ_H (250 MHz; $CDCl_3$) 7.42-7.09 (43 H, m), 6.98-6.90 (2 H, m), 5.10-3.70 (29 H, m), 2.28-2.10 (4 H, m), 1.65-1.48 (4 H, m), 1.35-1.20 (8 H, m), 0.95-0.82 (6 H, m,); δ_P (101.25 MHz; $CDCl_3$) –0.93, –0.94, –1.15, –1.33, –1.64, –1.67; *m/z* (FIB^+) 1221.6 (30), 1311.7 (40), 1492.1 (50), 1582.2 (100, $M+H^+$); HRMS (FIB^+) Calcd. for $C_{84}H_{97}O_{22}P_4$ ($M+H^+$) 1581.5422. Found: 1581.5389.

(+)-1D-*myo*-Inositol-1-(1',2'-di-*O*-hexanoyl-*sn*-glycer-3'-yl sodium phosphate)-3,4,5-tris(disodium phosphate) 108

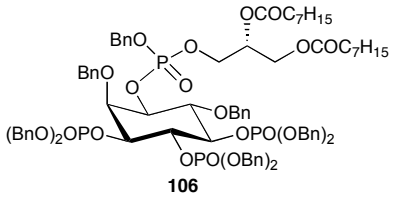


108

Lipid (–)-**105** (0.042 g, 0.027 mmol, 1.0 equiv) was treated with palladium-black (0.069 g, 0.65 mmol, 24.1 equiv) and sodium hydrogen carbonate (0.016 g, 0.19 mmol, 7.03 equiv) under hydrogen (3.5 bar) according to the procedure described for the preparation of (+)-**51**, to give (+)-**108** (0.026 g, 96%) as a white solid: $[\alpha]_D$ 0.0, $[\alpha]_{546}$ +2.5, $[\alpha]_{365}$ –3.5 (*c* 0.2 in H_2O); υ_{max} (KBr)/cm^{-1} 3396, 2958, 1734, 1654, 1412, 1246, 1103, 1202, 971, 877, 802; δ_H (500 MHz, D_2O), 5.24 (1H, br s), 4.39-4.20 (4H, m), 4.05-3.62 (6H, m), 2.36-2.29 (4H, m), 1.56-1.48 (4H, m), 1.25-1.17 (8H, m), 0.80-0.76 (6H, m); δ_P (101.25 MHz, D_2O), 5.74, 4.55, 3.04, 0.50; *m/z* (FAB^-) 856 (100, $M–3Na+H^+$).

1.14 Synthesis of dioctanoyl-PI(3,4,5)P3 109

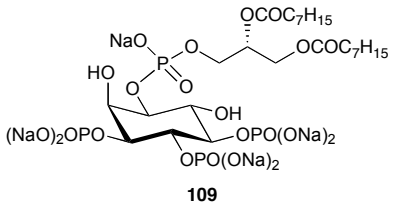
(–)-1D-2,6-Di-*O*-benzyl-*myo*-inositol-1-(1',2'-di-*O*-octanoyl-*sn*-glycer-3'-yl benzyl phosphate)-3,4,5-tris(dibenzyl phosphate) 106



106

Alcohol (–)-**96**[2] (0.200 g, 0.18 mmol, 1.0 equiv) was treated with phosphoramidite **103**[7] (0.3059 g, 0.53 mmol, 3.0 equiv), 1*H*-tetrazole (0.0369 g, 0.53 mmol, 3.0 equiv), and 3-choroperbenzoic acid (0.0906 g, 0.53 mmol, 3.0 equiv) according to the procedure described for the preparation of (+)-**41**. Purification by silica chromatography (50% ethyl acetate/hexane) gave (–)-**106** (0.215 g, 75%) as a clear oil (Found: C, 64.48; H, 6.39. Calcd. for $C_{88}H_{104}O_{22}P_4$: C, 64.54; H, 6.40%): $[\alpha]_D^{26}$ –3.5 (*c* 0.9 in $CHCl_3$); ν_{max} (film)/cm^{-1} 2955, 2927, 2856, 1741, 1498, 1456, 1274, 998, 735, 696; δ_H (500 MHz; $CHCl_3$) 7.41 (2 H, t, *J* 7.5), 7.36-7.12 (41 H, m), 7.01-6.98 (2 H, m), 5.10-4.67 (21 H, m), 4.55-4.28 (4 H, m), 4.15-3.76 (4 H, m), 2.27-2.17 (4 H, m), 1.61-1.53 (4 H, m), 1.33-1.22 (16 H, m), 0.88 (6 H, t, *J* 6.9); δ_C (125 MHz; $CHCl_3$) 173.1 (d), 172.7 (d), 138.1 (d), 138.0 (d), 136.1-135.9 (m), 135.7-135.3 (m), 128.6-128.5 (m), 128.4-127.9 (m), 127.9, 127.6, 127.4 (d), 127.3-127.2 (m), 127.1, 78.1, 77.7 (d), 77.4-77.2 (m), 76.0, 75.4, 69.2 (d), 65.8 (d), 65.6 (d), 61.5, 34.0 (d), 31.6 (d), 29.0, 29.0, 28.9, 24.8, 24.7, 22.5; δ_P (202 MHz; $CHCl_3$) –0.63, –0.85, –1.03, –1.36, –1.39; *m/z* (ESI^+) 1660 (25, $M+Na^+$) 1638 (10, $M+H^+$), 1312 (30), 372 (100), 181 (10), 127 (15), 91 (70).

1D-*myo*-Inositol-1-(1',2'-di-*O*-octanoyl-*sn*-glycer-3'-yl sodium phosphate)-3,4,5-tris(disodium phosphate) 109

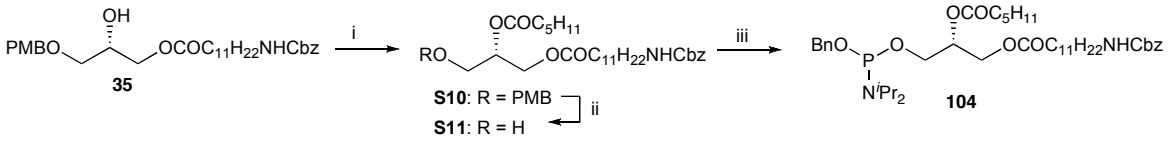


109

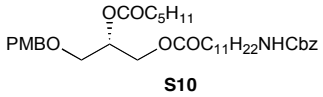
Lipid (–)-**106** (0.200 g, 0.12 mmol, 1.0 equiv) was treated with palladium black (0.351 g, 3.30 mmol, 27.0 equiv) and sodium hydrogen carbonate (0.0718 g, 0.85 mmol, 7.0 equiv) under hydrogen (3.1 bar), according to the procedure described for the preparation of (–)-**42**, to give (–)-**109** (0.1186 g, 80%) as a white solid: δ_H (500 MHz; D_2O) 5.28-5.24 (1H, m), 4.37 (2H, m), 4.31-4.22 (2H, m), 4.07-4.04 (2H, m), 4.01-3.94 (2H, m), 3.89-3.86 (2H, m), 2.38 (2H, t, *J* 7.4), 2.34 (2H, td, *J* 7.4, 1.7), 1.56-1.54 (4H, m), 1.26-1.18 (16H, m), 0.81 (6H, t, *J* 6.7); δ_P (202 MHz; D_2O) 4.96, 3.75, 1.85, -0.13; *m/z* (ESI^+) 981 (10, $M+H^+$), 959 (15), 914 (15), 882 (20), 854 (15), 826 (15), 718 (30), 648 (100), 620 (20), 553 (25).

1.15 Synthesis of NH_2-hexanoyl-PI(3,4,5)P_3 110

Synthesis of phosphoramidite 104

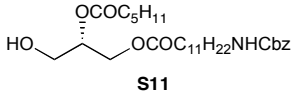


(+)-1-*O*-[12-*N*-(Benzyloxycarbonyl)aminododecanoyl]-2-*O*-hexanoyl-3-*O*-(4'-methoxybenzyl)-*sn*-glycerol S10



To a solution of **35**[3] (0.498 g, 0.917 mmol, 1.0 equiv) and *N*,*N*-dimethylaminopyridine (0.0112 g, 0.0917 mmol, 0.1 equiv) in pyridine (7 mL) at 0 ºC, was added hexanoyl chloride (0.256 mL, 1.83 mmol, 2.0 equiv) dropwise. The solution was stirred at rt for 5 h and the volatiles removed under reduced pressure. The residue was partitioned between sat. aq. potassium hydrogen sulfate (10 mL) and ether (10 mL). The aqueous phase was extracted with ether (2 × 10 mL) and the combined organic extracts were washed with brine (10 mL), dried ($MgSO_4$), and the solvent removed under reduced pressure. Purification by flash chromatography (20% ethyl acetate/hexane) gave (+)-**S10** (0.384 g, 71%) as a colourless oil: $[\alpha]_D^{20}$ +6.5 (c 1.3 in $CHCl_3$); ν_{max} (neat)/cm^{-1} 3374, 2928, 2855, 1732, 1613, 1514, 1455, 1248, 1172, 1104, 1035; δ_H (500 MHz; $CDCl_3$) 7.36-7.22 (7 H, m), 6.87 (2 H, d, *J* 8.5), 5.24-5.20 (1 H, m), 5.09 (2 H, br s), 4.71 (1 H, br s), 4.46 (2 H, d, *J* 11.7), 4.32 (1 H, dd, *J* 3.7, 11.8), 4.17 (1 H, dd, *J* 6.5, 11.8), 3.80 (3 H, s), 3.58-3.53 (2 H, m), 3.20-3.16 (2 H, m), 2.31 (2 H, t, *J* 7.5 Hz), 2.27 (2 H, t, *J* 7.5), 1.65-1.25 (24 H, m), 0.89 (3 H, t, *J* 6.8); δ_C (62.5 MHz; $CDCl_3$) 173.4, 173.1, 159.3, 136.7, 129.8, 129.3, 128.5, 128.0, 113.8, 73.0, 70.0, 66.6, 62.7, 55.3, 41.4, 34.3, 34.1, 31.2, 30.0, 29.5, 29.4, 29.2, 29.1, 26.7, 24.9, 24.6, 22.3, 13.9; *m/z* (ES^+) 664.5 ($M+Na^+$), 642.5 ($M+H^+$); HRMS (ES^+) Calcd. for $C_{37}H_{56}NO_8$ ($M+H^+$) 642.4006. Found: 642.4013.

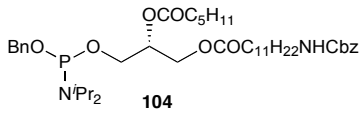
(–)-1-*O*-[12-*N*-(benzyloxycarbonyl)aminododecanoyl]-2-*O*-hexanoyl-*sn*-glycerol S11



To a solution of (+)-**S10** (0.210 g, 0.327 mmol, 1.0 equiv) in dichloromethane (10 mL) and water (1 mL) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.149 g, 0.654 mmol, 2.0 equiv), and the mixture was stirred at rt in air for 15 h. The solution was diluted with dichloromethane and washed with sat. aq. sodium hydrogen carbonate (10 mL), water (10 mL) and brine (10 mL). The aqueous phases were filtered over *Celite* under vacuum and extracted with dichloromethane (20 mL). The combined organic extracts were dried (Na_2SO_4) and the solvent removed under reduced

pressure. Purification by flash chromatography (25-40% ethyl acetate/hexane) gave (–)-**S11** (0.130 g, 76%) as a colourless oil that solidified upon standing: $[\alpha]_D^{18}$ –3.0 (c 0.5 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3683, 3619, 3451, 2974, 2931, 2857, 1724, 1518, 1476, 1422; δ_H (250 MHz; $CDCl_3$) 7.35 (5 H, m), 5.09 (3 H, m), 4.76 (1 H, br s), 4.32 (1 H, dd, *J* 4.5, 11), 4.22 (1 H, dd, *J* 5.7, 11.9), 3.72 (2 H, t, *J* 5.7), 3.18 (2 H, q, *J* 6.5), 2.37-2.29 (4 H, m), 2.21 (1 H, t, *J* 6.2), 1.68-1.27 (24 H, m), 0.90 (3 H, t, *J* 6.7); δ_C (62.5 MHz; $CDCl_3$) 173.7, 173.4, 156.4, 136.7, 128.5, 128.1, 72.1, 66.6, 62.1, 61.5, 41.1, 34.3, 34.1, 31.2, 30.0, 29.4, 29.4, 29.2, 29.1, 26.7, 24.9, 24.6, 22.3, 13.9; *m/z* (ES^+) 544.6 ($M+Na^+$); HRMS (ES^+) Calcd. for $C_{29}H_{48}NO_7$ ($M+H^+$) 522.3431. Found: 522.3434.

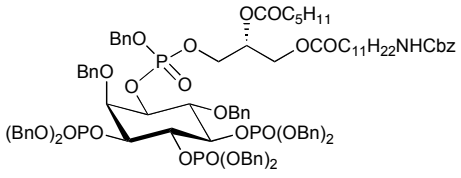
(+)-Benzyloxy(*N*,*N*-diisopropylamino)-1-*O*-[12-*N*-(benzyloxycarbonyl)aminododecanoyl]-2-*O*-hexanoyl-*sn*-glycer-3-yl)phosphine 104



104

A solution of (benzyloxy)bis(*N,N*-diisopropylamino)phosphine[9] (0.105 g, 0.311 mmol, 1.25 equiv) in dichloromethane (3 mL) was added *via* cannula to a stirred mixture of (–)-**S11** (0.130 g, 0.249 mmol, 1.0 equiv) and 1*H*-tetrazole (0.012 g, 0.164 mmol, 0.66 equiv) in dichloromethane (2 mL). The reaction mixture was stirred at rt for 2 h, diluted with dichloromethane (7 mL), and then washed with water (10 mL). The aqueous phase was extracted with dichloromethane (10 mL) and the combined organic extracts were washed with sat. aq. sodium hydrogen carbonate (5 mL), brine (5 mL), dried (Na_2SO_4), and the solvent removed under reduced pressure. Purification by flash chromatography (triethylamine/ethyl acetate/hexane, 5:15:80) gave (+)-**104** (0.156 g, 83%) as a colourless oil: $[\alpha]_D^{20}$ +7.5 (c 1.9 in $CHCl_3$); ν_{max} (CCl_4)/cm^{-1} 3459, 2965, 2929, 2856, 1740, 1551, 1508, 1456, 1364, 1218, 1183, 1102, 1012, 978; δ_H (500 MHz; $CDCl_3$) 7.36-7.26 (10 H, m), 5.19 (1 H, m), 5.09 (2 H, br s), 4.75-4.63 (3 H, m), 4.37-4.31 (1 H, m), 4.20-4.15 (1 H, m), 3.81-3.59 (4 H, m), 3.18 (2 H, q, *J* 6.6), 2.31-2.27 (4 H, m), 1.64-1.58 (4 H, m), 1.48 (2 H, m), 1.30-1.25 (18 H, m), 1.19-1.17 (12 H, m), 0.89 (3 H, t, *J* 6.5); δ_C (100 MHz; $CDCl_3$) 70.9, 70.9, 70.8, 70.8, 66.6, 65.5, 65.5, 65.3, 65.3, 62.5, 62.5, 61.8, 61.7, 61.5, 43.2, 43.0, 41.1, 34.3, 34.1, 31.2, 30.0, 29.5, 29.4, 29.3, 29.1, 26.7, 24.9, 24.7, 24.6, 24.6, 24.5, 24.5, 22.3, 13.9; δ_P (162 MHz; $CDCl_3$) 149.4, 149.5; *m/z* (ES^+) 759.5 ($M+H^+$); HRMS (ES^+) Calcd. for $C_{42}H_{68}N_2O_8P$ 759.4713 ($M+H^+$). Found: 759.4711.

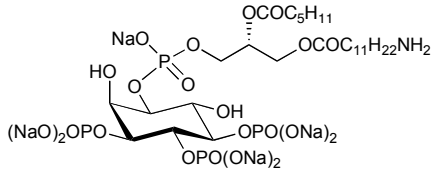
(–)-1D-2,6-Di-*O*-benzyl-*myo*-inositol-1-[1'-*O*-dodecanoyl-12-(benzyloxycarbonyl)-amino)-2'-hexanoyl-*sn*-glycer-3'-ylbenzyl phosphate) 3,4,5-tris(dibenzyl phosphate) 107



107

Alcohol (–)-**96**[2] (0.046 g, 0.0403 mmol, 1.0 equiv) was treated with phosphoramidite (+)-**104** (0.073 g, 0.0968 mmol, 2.4 equiv), 1*H*-tetrazole (0.0085 g, 0.121 mmol, 3.0 equiv) and 3-chloroperbenzoic acid (0.032 g, 0.185 mmol, 4.6 equiv) according to the procedure described for the preparation of (+)-**41**. Purification by flash chromatography (40-50% ethyl acetate/hexane) gave (–)-**107** (0.058 mg, 79%) as a colourless oil: $[\alpha]_D^{20}$ –2.2 (c 0.9 in $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3450, 2930, 2856, 1723, 1576, 1456, 1269, 1017; δ_H (250 MHz; $CDCl_3$) 8.07 (2 H, m), 7.97 (2 H, d, *J* 7.8), 7.58-7.53 (2 H, m), 7.41-7.11 (32 H, m), 6.98-6.95 (2 H, m), 5.10-4.29 (26 H, m), 4.11-3.71 (6 H, m), 3.18 (2 H, q, *J* 6.5), 2.23-2.12 (4 H, m), 1.51 (6 H, m), 1.25 (18 H, m), 0.85 (3 H, t, *J* 6.7); δ_C (62.5 MHz; $CDCl_3$) 173.1, 172.7, 168.7, 138.0, 135.3, 133.4, 130.2, 129.7, 128.6, 128.6, 128.5, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 128.0, 128.0, 127.9, 127.4, 78.0, 77.3, 76.2, 75.9, 75.5, 74.6, 69.9, 69.8, 69.7, 69.7, 69.6, 69.4, 69.4, 69.3, 69.2, 66.6, 65.8, 65.6, 61.5, 41.1, 34.0, 33.9, 31.1, 30.0, 29.4, 29.3, 29.1, 26.7, 24.8, 24.4, 22.2, 13.9; δ_P (162 MHz; $CDCl_3$) –1.49, –1.47, –1.15, –0.97, –0.75; *m/z* (FAB^+) 1813.5 (M), 1836.5 ($M+Na^+$).

(–)-1D-*myo*-Inositol-1-(1'-*O*-12-aminododecanoyl-2'-*O*-hexadecanoyl-*sn*-glycer-3'-yl sodium phosphate)- 3,4,5-tris(phosphate) 110



110

Lipid (+)-**109** (0.058 g, 0.032 mmol, 1.0 equiv) was treated with palladium-black (60 mg, 0.564 mmol, 17.6 equiv) and sodium hydrogen carbonate (0.0188 g, 0.224 mmol, 7.0 equiv) under hydrogen (25 bar) according to the procedure described for the preparation of (–)-**42**, to give (–)-**110** (0.0255 g, 78%) as a fluffy white solid: $[\alpha]_D^{20}$ –5.0 (c 0.12 in H_2O); ν_{max} (neat)/cm^{-1} 3209, 2926, 2852, 1740, 1647, 1556, 1467, 1381, 1212, 1093, 1050; δ_H (400 MHz; D_2O) 5.14-5.09 (1 H, m), 4.34 (1 H, dd, *J* 2.8, 12.3), 4.26 (1 H, t, *J* 2.5), 4.18 (1 H, q, *J* 9.2), 4.10 (1 H, dd, *J* 6.6, 12.3), 3.97-3.72 (6 H, m), 2.80 (2 H, t, *J* 7.6), 2.27-2.21 (4 H, m), 1.50-1.42 (6 H, m), 1.13 (18 H, m), 0.70 (3 H, t, *J* 6.9); δ_P (162 MHz; D_2O) –0.10, 1.98, 3.81, 5.03; *m/z* (ES^-) 867.2 ($M–2H^-$); HRMS (ES^-) Calcd. for $C_{27}H_{53}NO_{22}P_4$ ($M–2H^-$) 867.2010. Found: 867.2010.

1.16 Affinity Capture Techniques[18]

Briefly, affinity capture experiments were performed using two techniques. For further details see reference 18.

Affinity Supports

Crude LIM1215 cytosolic extracts were first incubated with ethanolamine derivatized Affi-Gel® 10 beads to remove any proteins that bound non-specially to the beads. These 'blank beads' were then removed using centrifugation and this precleared cytosolic fraction was incubated overnight with the immobilised affinity probes **85**, **94** or **101**. After the beads were washed, the bound proteins were desorbed using SDS-PAGE buffer and detected using SDS-PAGE, sensitive Coomassie staining and MS/MS analysis.

Liposomal Capture

Affinity capture was also performed by incubating PC/PE/phosphoinositide liposomes[18] overnight with LIM1215 cytosolic extracts. Liposomes and liposomal bound proteins were separated from unbound proteins by size exclusion chromatography and then analyzed using SDS-PAGE, sensitive Coomassie staining and MS/MS analysis.

1.17 References

1. S. J. A. Grove, I. H. Gilbert, A. B. Holmes, G. F. Painter and M. L. Hill, *Chem. Commun.*, 1997, 1633.
2. G. F. Painter, S. J. A. Grove, I. H. Gilbert, A. B. Holmes, P. R. Raithby, M. L. Hill, P. T. Hawkins and L. R. Stephens, *J. Chem. Soc., Perkin Trans. 1*, 1999, 923.
3. Z.-Y. Lim, J. W. Thuring, A. B. Holmes, M. Manifava and N. T. Ktistakis, *J. Chem. Soc., Perkin Trans. 1*, 2002, 1067.
4. H. W. Lee and Y. Kishi, *J. Org. Chem.*, 1985, **50**, 4402; D. C. Billington, R. Baker, J. J. Kulagowski, I. M. Mawer, J. P. Vacca, S. J. Desolms and J. R. Huff, *J. Chem. Soc., Perkin Trans. 1*, 1989, 1423.
5. M. K. Johns, M.-X. Yin, S. J. Conway, D. E. J. E. Robinson, L. S.-M. Wong, R. Bamert, R. E. H. Wettenhall and A. B. Holmes, *Org. Biomol. Chem.*, 2009, 3691.
6. S. J. A. Grove, A. B. Holmes, G. F. Painter, P. T. Hawkins and L. R. Stephens, *Chem. Commun.*, 1997, 1635.
7. K. K. Reddy, M. Saady, J. R. Falck and G. Whited, *J. Org. Chem.*, 1995, **60**, 3385.
8. J.-D. Lee, M. Ueno, Y. Miyajima and N. Nakamura, *Org. Lett.*, 2007, **9**, 323; K. Yamauchi, F. Une, S. Tabata and M. Kinoshita, *J. Chem. Soc., Perkin Trans. 1*, 1986, 765.
9. K. L. Yu and B. Fraser-Reid, *Tetrahedron Lett.*, 1988, **29**, 979; W. Bannwarth and A. Trzeciak, *Helv. Chim. Acta*, 1987, **70**, 175.
10. J. W. Perich and R. B. Johns, *Tetrahedron Lett.*, 1987, **28**, 101.
11. J. J. Eisch and S. G. Rhee, *J. Am. Chem. Soc.*, 1974, **96**, 7276; *Organometallic Syntheses*, ed. J. J. Eisch and R. B. King, Academic Press, New York, Vol. 2, p. 137.
12. C. Murakata and T. Ogawa, *Carbohydr. Res.*, 1992, **234**, 75.
13. T. Desai, J. Gigg, R. Gigg and S. Payne, *Carbohydr. Res.*, 1992, **228**, 209.
14. R. Aneja, S. G. Aneja, V. P. Pathak and P. T. Ivanova, *Tetrahedron Lett.*, 1994, **35**, 6061; J. Gigg, S. Payne and R. Conant, *J. Chem. Soc., Perkin Trans. 1*, 1987, 1757.
15. R. J. Kubiak and K. S. Bruzik, *J. Org. Chem.*, 2003, **68**, 960.
16. D. S. Wang and C. S. Chen, *J. Org. Chem.*, 1996, **61**, 5905.
17. J. Chen, L. Feng and G. D. Prestwich, *J. Org. Chem.*, 1998, **63**, 6511.
18. B. Catimel, M.-X. Yin, C. Schieber, M. Condron, H. Patsiouras, J. Catimel, D. E. J. E. Robinson, L. S.-M. Wong, E. C. Nice, A. B. Holmes and A. W. Burgess, *J. Proteome Res.*, 2009, **8**, 3712; B. Catimel, C. Schieber, M. Condron, H. Patsiouras, L. Connolly, J. Catimel, E. C. Nice, A. W. Burgess and A. B. Holmes, *J. Proteome Res.*, 2008, **7**, 5295.
19. S. F. Matrin, J. A. Josey, Y.-L. Wong and D. W. Dean, *J. Org. Chem.*, 1994, **59**, 4805.