Synthesis and biological evaluation of a PtdIns(4,5)P_2 and a phosphatidic acid affinity matrix †

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Analogues of dipalmitoyl phosphatidic acid (PA), dilauroyl PA and phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] were synthesised and immobilised onto a solid support, Affi-Gel 10. Using them as affinity matrices, a number of known proteins as well as a set of novel proteins were found to bind specifically to PA.

Introduction

Polyphosphoinositides (PIP$_n$'s) (Fig. 1) represent a class of

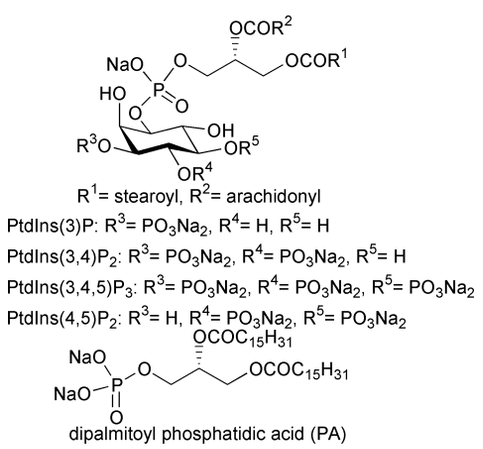

Fig. 1 Phosphoinositides and dipalmitoyl PA as their sodium salts.

membrane phospholipids, which exhibit a wide range of activities in cell signalling cascades.[1] The 3-phosphorylated lipid products of phosphoinositide 3-kinase (PI3K), *viz.* PtdIns(3)P, PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3, mediate cell functions as diverse as cell movement, division, survival, as well as glucose transport and many others, upon cell surface receptor stimulation by hormones and growth factors.[2–4] The last mentioned phosphoinositide is the product of 3-phosphorylation of the relatively abundant PtdIns(4,5)P_2.

Another well established function of PtdIns(4,5)P_2 is the phospholipase C (PLC) promoted hydrolysis to give diacylglycerol and inositol(1,4,5)P_3, which activates protein kinase C (PKC) and releases Ca^{2+} from internal stores.[5] Recent work has shown that PtdIns(4,5)P_2 is a highly versatile signalling molecule in its own right, and is involved in fundamental processes in membrane trafficking and plasma membrane–cytoskeleton linkages.[6] As such, PtdIns(4,5)P_2 serves as an effector of numerous multiple downstream proteins, many of which remain to be identified. The biosynthesis of PtdIns(4,5)P_2 involves mainly 5-phosphorylation of PtdIns(4)P by phosphatidylinositol 4-phosphate (PI4P) 5-kinase,[5] which is activated by phosphatidic acid (PA), a product of phospholipase D (PLD) catalysed hydrolysis of phosphatidyl choline.[7] It is suspected that PA, besides being an important intermediate in the biosynthesis of glycerophospholipids, fulfils crucial roles in lipid based signalling and intracellular trafficking, because PLD activation is associated with the regulation of these processes.[8,9] This would imply the existence of PA-interacting proteins that operate downstream of PLD activation.

To identify the direct downstream effectors of PtdIns(4,5)P_2 and PA, and hence to gain more insight in their roles in signalling and housekeeping, we embarked on the synthesis of immobilised PAs **1a** and **b** and PtdIns(4,5)P_2 **2**, all of which have saturated fatty acid chains, as is illustrated in Fig. 2.[10]

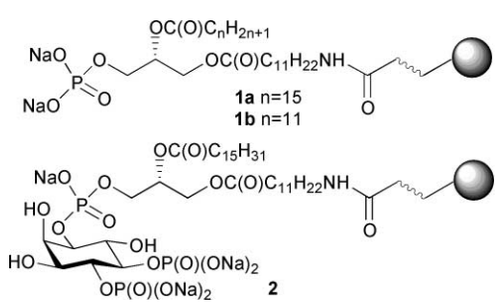

Fig. 2 Immobilised PAs and PtdIns(4,5)P_2.

Results and discussion

Synthesis of immobilised PAs

Immobilised PAs **1a**, **b** and immobilised PtdIns(4,5)P_2 **2** were prepared by attaching a terminal fatty acid amino function at the *sn*-1 position of the glycerol moiety, as in **3a**, **b** and **4**, to an agarose solid support.

The soluble PA analogues **3a** and **3b** were prepared by extension of methodology previously developed by Prestwich[11] and by us (Scheme 1).[12] The commercially available (*S*)-(+)-1,2-*O*-isopropylidene glycerol **5** was converted to both alcohols (−)-**10a** and (−)-**10b** by first protecting the free hydroxy group with a PMB (4-methoxybenzyl) group. The isopropylidene moiety

† Dedicated to the memory of the late Professor Roy Gigg.

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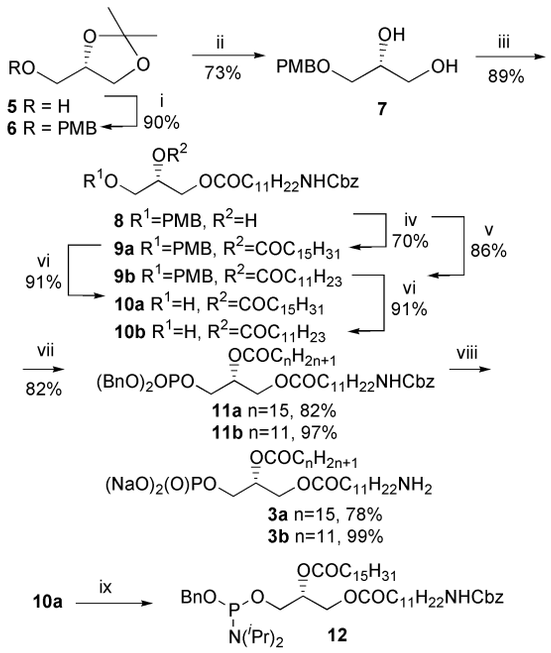


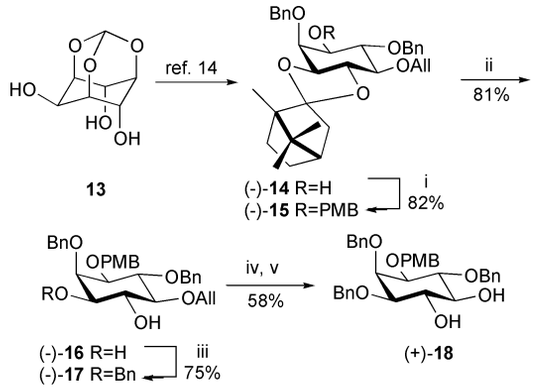
Scheme 1 *Reagents and conditions*: i, PMBCl, NaH, DMF, 0 °C–rt; ii, TsOH, MeOH; iii, $HOOCC_{11}H_{22}NHCbz$, DMAP, DCC, CH_2Cl_2, 0 °C–rt; iv, palmitoyl chloride, pyr., DMAP, CH_2Cl_2, 0 °C–rt; v, lauroyl chloride, pyr., DMAP, CH_2Cl_2, 0 °C–rt; vi, DDQ, 'wet' CH_2Cl_2; vii, $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, CH_2Cl_2, rt, then MCPBA, −78 °C–rt; viii, H_2, Pd black, Bu^tOH–H_2O, $NaHCO_3$, 15 bar; ix, $BnOP(NPr^i_2)_2$, 1*H*-tetrazole, CH_2Cl_2, rt [PMB = 4-methoxybenzyl; Cbz = *N*-benzyloxycarbonyl].

was removed by acidic hydrolysis to afford the diol (−)-**7** in good yield. DCC coupling of the primary hydroxy group over the secondary hydroxy group with 12-*N*-(benzyloxycarbonyl)aminododecanoic acid gave the mono lipid (+)-**8** which was acylated with palmitoyl chloride or lauroyl chloride to afford the PMB protected lipids (+)-**9a** and (+)-**9b** respectively, in good yield. Alcohols (−)-**10a** and (−)-**10b** were obtained by deprotection of the PMB group with DDQ oxidation. Subsequently, phosphitylation of alcohols (−)-**10a** and (−)-**10b** with $(BnO)_2P(NPr^i_2)$ followed by *in situ* oxidation with MCPBA gave phosphates (+)-**11a** and (+)-**11b**. Reductive debenzylation was readily effected using H_2 (15 bar) in the presence of Pd-black and $NaHCO_3$ in *tert*-butyl alcohol–H_2O (6 : 1) as the solvent, to afford amines **3a** and **3b** isolated as sodium salts, in good yield. Alternatively, (−)-**10a** was phosphitylated with $BnOP(NPr^i_2)_2$ to give the phosphoramidite (+)-**12**, which is the lipid synthon in the preparation of the ω-amino PtdIns(4,5)P_2 **4** (*vide infra*).[11,13]

Synthesis of PtdIns(4,5)P_2

The synthesis of **4**[14] (Scheme 2) started from the readily available *myo*-inositol orthoformate **13**, which was converted in 6 steps to the optically pure camphor acetal (−)-**14**, derived from (−)-camphor. The intermediate (−)-**14**, in which the resolving camphor fragment served to protect the 3- and 4-positions of the *myo*-inositol ring, has previously been used by us in the synthesis of dipalmitoyl PtdIns(3,4,5)P_3.[15,16]

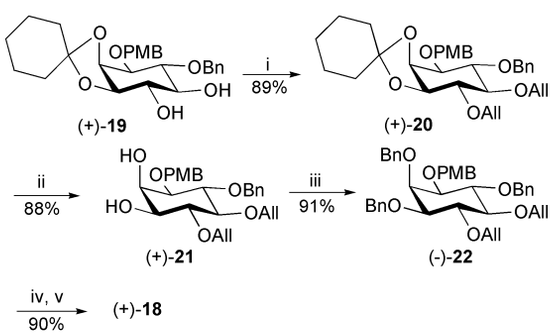
Subsequent *p*-methoxybenzylation followed by acetal deprotection afforded the 3,4-diol (−)-**16**. Chemoselective benzylation of the 3-position was readily effected *via* the *in situ* generated stannane acetal in the presence of tetrabutylammonium bromide and benzyl bromide in refluxing acetonitrile. Using these conditions developed by Gigg *et al.*,[17] the ratio of 3-benzyl : 4-benzyl was approximately 4 : 1 as judged from ^{1}H NMR analysis. It should be noted that preformation of the cyclic stannane acetal, followed by benzylation in the presence of CsF in DMF,[18] resulted in a lower yield and selectivity. The required 3-benzylated product (−)-**17** was purified by flash



Scheme 2 *Reagents and conditions*: i, PMBCl, NaH, DMF, 0 °C–rt; ii, AcCl, MeOH–CH_2Cl_2; iii, Bu_2SnO, 3 Å MS, BnBr, Bu_4NBr, CH_3CN, reflux; iv, $Rh(PPh_3)_3Cl$, $EtNPr^i_2$, toluene–H_2O–EtOH, reflux; v, AcCl, MeOH–CH_2Cl_2, 0 °C–rt.

chromatography and trituration. Deprotection of the allyloxy position using Wilkinson's catalyst, followed by acid treatment, gave the known diol (+)-**18**.

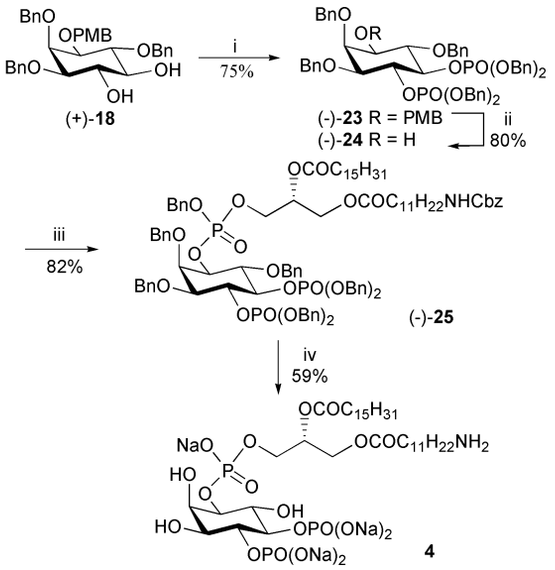
Definite proof for the observed regioselectivity of the stannylation–benzylation process was obtained by an independent synthesis of the diol (+)-**18**, starting from the known enantiomerically pure diol (+)-**19**.[19] A four step sequence (Scheme 3) yielded (+)-**18**, which has identical optical rotatory and ^{1}H NMR data to the material prepared *via* Scheme 2.



Scheme 3 *Reagents and conditions*: i, allyl Br, NaH, DMF, 0 °C–rt; ii, AcCl, MeOH, CH_2Cl_2; iii, BnBr, NaH, DMF, 0 °C–rt; iv, $Rh(PPh_3)_3Cl$, $EtNPr^i_2$, toluene–H_2O–EtOH, reflux; v, AcCl, MeOH–CH_2Cl_2, 0 °C–rt.

The diol (+)-**18** obtained was phosphorylated and PMB-deprotected under standard conditions to afford the alcohol (−)-**24** (Scheme 4),[19] which was then coupled with the phosphoramidite **12** in the presence of 1*H*-tetrazole, followed by *in situ* oxidation with MCPBA to give the fully protected phosphoinositide (−)-**25** (Scheme 4). Global deprotection was carried out using similar conditions as mentioned above to give the ω-amino PtdIns(4,5)P_2 analogue **4**.

Finally, the PA amines **3a** and **3b** and PtdIns(4,5)P_2 amine **4** were coupled to the *N*-hydroxysuccinimide (NHS) activated ester resin, Affi-Gel 10, to afford the corresponding affinity matrices **1a**, **1b** and **2**. The PtdIns(4,5)P_2 modified matrix **2** was prepared in water using excess $NaHCO_3$, by reacting 60 μmol (4 cm^3) of the activated Affi-Gel 10 resin with 14.3 μmole of the ω-amino-substituted phospholipid **4** to give a loading of 4.5 μmol as judged from the recovery of the amino phospholipid **4**, which was quantified by 500 MHz ^{1}H NMR spectroscopy in the presence of *myo*-inositol orthoformate **13** as the internal standard. The poor solubility of the amino terminated PAs **3a** and **3b** necessitated the use of the solvent combination chloroform–methanol–water, 4 : 5 : 1 to yield material with a loading of 16–17% of PAs **3a** and **3b** onto 30 μmol of activated ester resin respectively, as estimated by ^{1}H NMR analysis of the remaining lipid **3a** or **3b**.



Scheme 4 *Reagents and conditions*: i, $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, CH_2Cl_2, rt, then MCPBA, −78 °C–rt; ii, CAN, CH_3CN–H_2O; iii, lipid **12**, 1*H*-tetrazole, CH_2Cl_2, rt, then MCPBA, −78 °C–rt; iv, H_2, Pd black, 15 bar, $NaHCO_3$, Bu^tOH–H_2O.

Biological evaluation

By incubating the PA-modified affinity resin **1a** with a brain cytosol extract in the presence of a non-ionic detergent, we found that a number of proteins bind specifically to those beads, *i.e.* their binding was inhibited by soluble dilauroyl PA.[20] Among the PA-binding proteins identified are the β-cop subunit of coatomer, ADP ribosylation factor (Arf), *N′*-ethylmaleimide-sensitive factor (NSF) and kinesin, all of which are involved in intracellular traffic.[20] In addition, a set of five novel proteins was found. None of the above mentioned proteins showed any binding to the uncoupled Affi-Gel 10 beads nor to the PtdIns(4,5)P_2 beads **2**. An example of the binding of β-cop to PA beads is shown in Fig. 3 We incubated in parallel brain

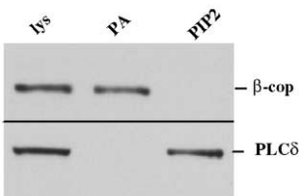


Fig. 3 Specificity of binding to lipid-coupled beads from sheep brain cytosol. Cytosol was made 0.5% in the ionic detergent NP-40, and lysates were mixed with PA- or PI(4,5)P2-coupled beads as indicated. Following binding and SDS-PAGE, the gels were blotted and probed with antibodies indicated. Lysate in each case represents 1% of total used during binding.

cytosol with PA-resin **1a** and immobilised PtdIns(4,5)P_2 **2**. After SDS-PAGE analysis of the bound material and probing with antibodies to β-cop, we found that β-cop bound strongly to the PA-beads **1a**, and not at all to PtdIns(4,5)P_2 beads **2**. The use of PtdIns(4,5)P_2 beads **2** as a control was verified with PLCδ [a known effector of PtdIns(4,5)P_2, *vide supra*]. Using the same conditions as above, PLCδ had a strong interaction with the PtdIns(4,5)P_2 beads **2**, whereas its binding to PA-resin **1a** was undetectable. The binding properties of immobilised PA **1b** was found to be very similar to that of immobilised PA **1a**.

The binding specificities of the lipid beads for potential partners were also characterised in lysates of tissue culture cells. COS-7 cells were lysed with the three types of detergents, ionic (deoxycholate), non-ionic (NP-40) and zwitterionic (CHAPS) at 0.5% each, and lysates were mixed with PA or PI(4,5)P_2 beads as indicated (Fig. 4). After binding and extensive washes,

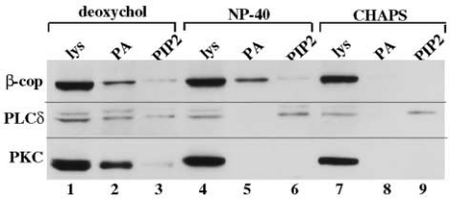


Fig. 4 The specificity of binding to lipid-coupled beads from tissue culture lysates depends on lysis conditions. Lysates in each case represents 5% of the total used in binding.

the bound fraction was analysed by SDS-PAGE and immunoblotting. We found that non-ionic detergents (in this case NP-40 but also others used) maintain the specificity of PA for β-cop and PI(4,5)P_2 for PLCδ. Importantly, protein kinase C (PKC, an abundant cytosolic protein that binds acidic phospholipids and diacylglycerol) did not bind to PA beads **1a** and PtdIns(4,5)P_2 beads **2** in the presence of NP-40. This further exemplified the fact that the binding specificity of PA and PtdIns(4,5)P_2 beads is maintained even in cell lysate provided a non-ionic detergent is used for lysis. Although we do not understand why other detergents show less specificity, we speculate that it may be related to their possible effects (such as denaturation or charge modification) on the target proteins.

In conclusion, we have reported the full synthesis of PA- and PtdIns(4,5)P_2-coupled beads and have successfully used them as affinity matrices to identify PA-interacting proteins. A number of known proteins, Arf, kinesin, β-cop and NSF, all intracellular trafficking proteins, as well as several novel proteins were identified to bind to PA, specifically with reference to PtdIns(4,5)P_2. The observed binding profiles with these proteins and the high degrees of specificity warrant the further use of these materials in cellular biology as has been demonstrated by the general interest in the biological studies, full details of which can be found elsewhere.[20]

Experimental

^{1}H NMR spectra were recorded on Bruker WM-250 (250 MHz), WM-400 (400 MHz) and WM-500 (500 MHz) instruments, using deuterochloroform (or other indicated solvents) as reference or internal deuterium lock. The chemical shift data for each signal are given in units of δ relative to tetramethylsilane where δ (tetramethylsilane) = 0. The multiplicity of each signal is indicated by: s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), qn (quintet), dd (doublet of doublets), ddd (doublet of doublet of doublets), dt (doublet of triplets) and m (multiplets). The number of protons (*n*) for a given resonance is indicated by *n*H. ^{13}C NMR spectra were recorded on Bruker WM-250 (63.5 MHz), WM-400 (100 MHz) and WM-500 (125 MHz) instruments using an internal deuterium lock and proton decoupling. The chemical shift data for each signal are given as δ in units of parts per million (ppm) relative to tetramethylsilane where δ (tetramethylsilane) = 0. The multiplicity of each signal was determined by an applied proton test experiment. ^{31}P NMR spectra were recorded on Bruker WM-250 (101 MHz) and WM-400 (162 MHz) instruments with proton decoupling. The chemical shift data for each signal are given as δ in units of parts per million (ppm) relative to external 85% H_3PO_4. Infrared spectra were recorded on a Perkin Elmer 1310 spectrometer. The sample was prepared as a solution in the indicated solvent. Calibration in each case was made relative to polystyrene at 1603 cm^{-1}. Mass spectra were recorded at both the EPSRC Mass Spectrometry Centre, University of Wales, Swansea and the University Chemical Laboratory. Microanalyses were carried out by the staff of the University Chemical Laboratory, Cambridge, Microanalytical Department. Melting points were determined using a Büchi 510 melting point apparatus and are uncorrected. Optical rotations

were measured using a Perkin Elmer 241 polarimeter, in a cell of 1 dm path length. The concentration (*c*) is expressed in g 100 cm^{-3} (equivalent to g 0.1 dm^{-3}) and values are given in 10^{-1} deg cm^2 g^{-1}.

(+)-1,2-*O*-Isopropylidene-3-*O*-(4-methoxybenzyl)-*sn*-glycerol 6

Sodium hydride (0.48 g, 60% dispersion in mineral oil, 12.00 mmol) was suspended in a solution of dry DMF (5 cm^3) and cooled to 0 °C. (*S*)-(−)-2,2-Dimethyl-1,3-dioxolane-4-methanol **5** (1.00 g, 7.56 mmol) was added dropwise and the solution was warmed to rt and stirred for 1 h. The reaction mixture was cooled to 0 °C and PMBCl (1.77 g, 1.53 cm^3, 11.00 mmol) was added dropwise. The reaction mixture was stirred at rt overnight. The reaction was quenched by careful addition of water (15 cm^3) and the aqueous phase was extracted with diethyl ether (3 × 15 cm^3). The combined ethereal layers were washed with water (5 × 10 cm^3), brine (10 cm^3), dried over $MgSO_4$ and solvent removed *in vacuo*. Flash chromatography (20–40% ethyl acetate in hexane) gave the PMB ether (+)-**6** (1.71 g, 90%) as a colourless oil: $[\alpha]_D^{20}$ = +23.7 (*c* 1.45 in $CHCl_3$) [lit.,[21] $[\alpha]_D^{21}$ = +21.8 (*c* 1 in $CHCl_3$)]; δ_H (250 MHz; $CDCl_3$) 7.27–7.24 (2 H, m, C_6H_4OMe), 6.89–6.85 (2 H, m, C_6H_4OMe), 4.53 (1 H, dd, J_{AB} 11.7, CH_AH_B), 4.47 (1 H, dd, J_{AB} 11.7, CH_AH_B), 4.28 (1 H, qn, *J* 6.3, CH_2CHCH_2), 4.04 (1 H, dd, *J* 8.2, 6.3, CH_2CHCH_2), 3.79 (3 H, s, OCH_3), 3.72 (1 H, dd, *J* 8.2, 6.3, CH_2CHCH_2), 3.53 (1 H, dd, *J* 9.7, 6.3, CH_2CHCH_2), 3.43 (1 H, dd, *J* 9.7, 6.3, CH_2CHCH_2), 1.41 (3 H, s, CH_3), 1.36 (3 H, s, CH_3).

(*R*)-3-(4-Methoxybenzyloxy)propane-1,2-diol 7

The PMB ether (+)-**6** (7.07 g, 28.0 mmol) was hydrolysed with TsOH (0.28 g, 1.47 mmol) in methanol (10 cm^3) to give the diol (−)-**7**. Flash chromatography eluting with ethyl acetate gave the diol (−)-**7** (0.23 g, 73%) as a pale yellow solid: mp 41–43 °C (from ethyl acetate) [lit.,[22] 43.5–45.1 °C (from toluene–light petroleum)]; $[\alpha]_D^{22}$ = −1.12 (*c* 3.04 in $CHCl_3$) [lit.,[22] $[\alpha]_D$ = −1.54 (*c* 3.7 in $CHCl_3$)]; δ_H (250 MHz; $CDCl_3$) 7.24–7.21 (2 H, m, $C_6H_4OCH_3$), 6.87–6.84 (2 H, m, $C_6H_4OCH_3$), 4.44 (2 H, s, OCH_2), 3.85–3.80 (1 H, m, CH_2CHCH_2), 3.78 (3 H, s, OCH_3), 3.68–3.42 (3 H, m, CH_2CHCH_2), 3.53 (1 H, dd, *J* 9.7, 6.3, CH_2CHCH_2), 3.31 (1 H, d, *J* 4.5, OH), 2.96 (1 H, t, *J* 5.5, OH).

(+)-1-*O*-[12-*N*-(Benzyloxycarbonyl)aminododecanoyl]-3-*O*-(4-methoxybenzyl)-*sn*-glycerol 8

The diol (−)-**7** (2.47 g, 12 mmol), DCC (2.64 g, 13 mmol) and DMAP (1.56 g, 13 mmol) were added to dry dichloromethane (100 cm^3) under nitrogen and the reaction was stirred for 30 min at 0 °C. 12-*N*-(Benzyloxycarbonyl)aminododecanoic acid (2.27 g, 6.5 mmol) in dry dichloromethane (5 cm^3) was transferred *via* cannula into the reaction mixture under nitrogen and stirred at rt overnight. The reaction was quenched by addition of water (50 cm^3) and the aqueous phase was extracted with dichloromethane (3 × 40 cm^3). The combined organic extracts were washed with brine (20 cm^3), dried over $MgSO_4$ and the solvent was removed *in vacuo*. Flash chromatography (30–60% ethyl acetate in hexane) of the residue gave alcohol (+)-**8** (3.15 g, 89%) as a white solid: mp 50–51 °C (from ethyl acetate); $[\alpha]_D^{20}$ = +1.7 (*c* 0.52 in $CHCl_3$); R_f = 0.48 (50% ethyl acetate in hexane); ν_{max} ($CHCl_3$)/cm^{-1} 3689, 3585, 3540, 3004, 2931, 2856, 1720, 1655, 1612, 1514, 1463, 1454, 1252, 1234, 1174, 1132, 1101 and 1035; δ_H (400 MHz; $CDCl_3$) 7.35–7.28 (5 H, m, C_6H_5), 7.25–7.22 (2 H, m, C_6H_4OMe), 6.88–6.86 (2 H, m, C_6H_4OMe), 5.08 (2 H, s, OCH_2), 4.84 (1 H, br s, NH), 4.50–4.42 (2 H, m, OCH_2), 4.18–4.00 (2 H, m, CH_2CHCH_2), 3.99–3.97 (1 H, m, CH_2CHCH_2), 3.79 (3 H, s, OCH_3), 3.51 (1 H, dd, *J* 9.6, 4.4, CH_2CHCH_2), 3.44 (1 H, *J* 9.6, 6.0, CH_2CHCH_2), 3.18–3.13 (2 H, m, CH_2NH), 2.70 (1 H, br s, OH), 2.35–2.28 (2 H, m, $OCOCH_2$), 1.61–1.56 (2 H, m, CH_2CH_2NH), 1.47–1.37 (2 H, m, $OCOCH_2CH_2$), 1.25 (16 H, br s, $C_{11}H_{22}NH$); δ_C (100 MHz; $CDCl_3$) 173.9 (OCO), 159.4 (NHCO), 156.4 (CH_3OC), 136.7, 129.8, 129.4, 129.2, 128.4, 128.1, 113.9 (7 × C_6H_5 and C_6H_4), 73.1 (CH), 70.6, 68.9, 66.5, 65.4 (4 × CH_2), 55.2 (OCH_3), 41.1 (CH_2NH), 34.1, 29.9, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 26.7, 24.5 (10 × CH_2); *m*/*z* (CI, NH_3) [Found $(M + H)^+$ 544.3302. $C_{31}H_{46}O_7N$ requires *M*, 544.3274].

(+)-1-*O*-[12-*N*-(Benzyloxycarbonyl)aminododecanoyl]-2-*O*-hexadecanoyl-3-*O*-(4-methoxybenzyl)-*sn*-glycerol 9a

To a solution of alcohol (+)-**8** (3.14 g, 5.7 mmol) in dry dichloromethane (20 cm^3) under nitrogen was added DMAP (0.035 g, 0.29 mmol). The resulting solution was cooled to 0 °C and dry pyridine (0.68 g, 0.70 mL, 8.68 mmol) was added dropwise. After stirring for 30 min, palmitoyl chloride (1.75 g, 1.93 cm^3, 6.37 mmol) was added dropwise under nitrogen and the reaction mixture was stirred overnight at rt. Water (50 cm^3) was added to quench the reaction. The aqueous phase was extracted with diethyl ether (3 × 50 cm^3) and the ethereal layers were washed with 2 M hydrochloric acid (20 cm^3). The acid phase was back-extracted with diethyl ether (50 cm^3) and the combined ethereal layers were washed with brine (20 cm^3), dried over $MgSO_4$ and the solvent was removed *in vacuo*. Flash chromatography eluting with 50% ethyl acetate in hexane gave the ester (+)-**9a** (3.17 g, 70%) as a white solid (Found: C, 72.6; H, 9.85; N, 1.8. $C_{47}H_{75}O_8N$ requires C, 72.2; H, 9.7; N, 1.8%); mp 44–47 °C (from ethyl acetate); $[\alpha]_D^{20}$ = +5.5 (*c* 0.59 in $CHCl_3$); R_f = 0.20 (20% ethyl acetate in hexane); ν_{max} ($CHCl_3$)/cm^{-1} 3451, 2926, 2854, 1728, 1612, 1514, 1405, 1366, 1302, 1252, 1175 and 1110; δ_H (400 MHz; $CDCl_3$) 7.35–7.30 (5 H, m, C_6H_5), 7.24–7.22 (2 H, m, C_6H_4OMe), 6.88–6.86 (2 H, m, C_6H_4OMe), 5.23–5.21 (1 H, m, CH_2CHCH_2), 5.09 (2 H, s, OCH_2), 4.74 (1 H, br s, NH), 4.48 (1 H, dd, J_{AB} 12.0, OCH_AH_B), 4.44 (1 H, dd, J_{AB} 12.0, OCH_AH_B), 4.32 (1 H, dd, *J* 11.8, 3.8, CH_2CHCH_2), 4.17 (1 H, dd, *J* 11.8, 6.4, CH_2CHCH_2), 3.80 (3 H, s, OCH_3), 3.55 (2 H, dd, *J* 5.1, 1.1, CH_2CHCH_2), 3.18 (2 H, q, *J* 6.6, CH_2NH), 2.33–2.25 (4 H, m, 2 × $OCOCH_2$), 1.66–1.56 (6 H, m, 2 × $OCOCH_2CH_2$, CH_2CH_2NH), 1.55–1.47 (2 H, m, $CH_2CH_2CH_2NH$), 1.25 (36 H, br s, $COC_{15}H_{31}$, $C_{11}H_{22}NH$), 0.88 (3 H, t, *J* 6.6, CH_3); δ_C (100 MHz; $CDCl_3$) 173.4, 173.1 (2 × OCO), 159.3 (NHCO), 136.7, 129.8, 129.3, 129.0, 128.5, 128.06, 113.81 (7 × C_6H_5 and C_6H_4), 72.9 (CH_2), 70.1 (CH), 70.0, 67.9, 66.5, 62.7 (4 × CH_2), 55.8 (OCH_3), 41.1 (CH_2NH), 34.3, 34.1, 31.9, 29.9, 29.7, 29.5, 29.4, 29.3, 29.2, 29.1, 24.9, 24.8, 22.6 (13 × CH_2), 14.1 (CH_3); *m*/*z* (CI, NH_3) [Found $(M + H)^+$ 782.56140. $C_{47}H_{76}O_8N$ requires *M*, 782.55707].

(+)-1-*O*-[12-*N*-(Benzyloxycarbonyl)aminododecanoyl]-2-*O*-dodecanoyl-3-*O*-(4-methoxybenzyl)-*sn*-glycerol 9b

The alcohol (+)-**8** (0.40 g, 0.70 mmol) was lauroylated according to procedure as described above for lipid (+)-**9a** affording the lipid (+)-**9b** (0.44 g, 86%) as a white solid (Found: C, 71.3; H, 9.2; N, 2.1. $C_{43}H_{67}O_8N$ requires C, 71.1; H, 9.3; N, 1.9%); mp 41–43 °C (from ethyl acetate); $[\alpha]_D^{20}$ = +6.6 (*c* 0.8 in $CHCl_3$); R_f = 0.26 (30% ethyl acetate in hexane); ν_{max} ($CHCl_3$)/cm^{-1} 3040, 2927, 2855, 1727, 1513, 1612, 1466, 1255, 1244, 1235, 1174, 1104 and 1035; δ_H (250 MHz; $CDCl_3$) 7.35 (5 H, s, C_6H_5), 7.23 (2 H, d, *J* 8.6, C_6H_4OMe), 6.87 (2 H, d, *J* 8.6, C_6H_4OMe), 5.26–5.18 (1 H, m, CH_2CHCH_2), 5.10 (2 H, s, OCH_2), 4.49 (1 H, dd, J_{AB} 11.7, OCH_AH_B), 4.44 (1 H, dd, J_{AB} 11.7, OCH_AH_B), 4.33 (1 H, dd, *J* 11.9, 3.8, CH_2CHCH_2), 4.16 (1 H, dd, *J* 11.9, 6.4, CH_2CHCH_2), 3.80 (3 H, s, OCH_3), 3.52 (2 H, d, *J* 5.1, CH_2CHCH_2), 3.16 (2 H, q, *J* 6.7, CH_2NH), 2.31 (2 H, t, *J* 7.5, $OCOCH_2$), 2.27 (2 H, t, *J* 7.5, $OCOCH_2$), 1.61–1.46 (6 H, m, 2 × $OCOCH_2CH_2$, CH_2CH_2NH), 1.55–1.47 (2 H, m, $CH_2CH_2CH_2NH$), 1.26 (28 H, br s, $COC_{11}H_{23}$, $C_{11}H_{22}NH$), 0.88 (3 H, t, *J* 6.2, CH_3); δ_C (100 MHz; $CDCl_3$) 173.4, 173.1 (2 × OCO), 159.3 (NHCO), 136.7, 129.8, 129.3, 128.5, 128.1, 113.8 (6 × C_6H_5 and C_6H_5), 72.9 (CH_2), 70.4 (CH), 67.9, 66.5, 62.7 (4 ×

CH_2), 55.8 (OCH_3), 41.1 (CH_2NH), 34.3, 34.1, 31.9, 29.9, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 26.7, 24.9, 24.8, 22.6 (15 × CH_2), 14.1 (CH_3); *m*/*z* (CI, NH_3) [Found $(M + H)^+$ 726.4947. $C_{43}H_{68}O_8N$ requires *M*, 726.4945].

(−)-1-*O*-[12-*N*-(Benzyloxycarbonyl)amino dodecanoyl]-2-*O*-hexadecanoyl-*sn*-glycerol 10a

To a solution of dichloromethane (50 cm^3) and water (5 cm^3) under air was added ester (+)-**9a** (3.12 g, 4.00 mmol). Dichloro (dicyano)quinone (1.81 g, 8.00 mmol) was added and the reaction was stirred overnight at rt. The reaction was diluted with dichloromethane (50 cm^3) and washed with saturated $NaHCO_3$ solution (20 cm^3), brine (20 cm^3), dried over $MgSO_4$ and the solvent removed *in vacuo*. Flash chromatography eluting with 40–50% ethyl acetate in hexane gave the alcohol (−)-**10a** (2.40 g, 91%) as an off-white solid (Found: C, 70.7; H, 10.1; N, 2.1. $C_{39}H_{67}O_7N$ requires C, 70.7; H, 10.2; N, 2.1%); mp 56–58 °C; $[\alpha]_D^{20} = -3.2$ (*c* 1.02 in $CHCl_3$); $R_f = 0.20$ (30% ethyl acetate in hexane); ν_{max} ($CHCl_3$)/cm^{-1} 3450, 2924, 2854, 1724, 1602, 1517, 1465, 1413, 1372, 1251 and 1164; δ_H (400 MHz; $CDCl_3$) 7.34–7.25 (5 H, m, C_6H_5), 5.09–5.06 (3 H, m, CH_2CHCH_2 and OCH_2), 4.81 (1 H, br s, N*H*), 4.31 (1 H, dd, *J* 11.9, 3.8, CH_2CHCH_2), 4.17 (1 H, dd, *J* 11.9, 6.4, CH_2CHCH_2), 3.55 (2 H, dd, *J* 5.2, 1.2, CH_2CHCH_2), 3.18 (2 H, q, *J* 6.7, CH_2NH), 2.33–2.25 (4 H, m, 2 × $OCOCH_2$), 1.66–1.56 (6 H, m, 2 × $OCOCH_2CH_2$, CH_2CH_2NH), 1.56–1.47 (2 H, m, $CH_2CH_2CH_2NH$), 1.25 (36 H, br s, $COC_{15}H_{31}$, $C_{11}H_{22}NH$), 0.87 (3 H, t, *J* 6.7, CH_3); δ_C (63 MHz; $CDCl_3$) 173.7, 173.4 (*O*CO), 156.4 (NH*C*O), 136.7, 128.5, 128.4, 128.1 (4 × C_6H_5), 72.1 (*C*H), 66.5, 62.4, 61.1 (3 × CH_2), 41.1 ($NHCH_2$), 34.1, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 24.9, 24.8, 22.7 (12 × CH_2), 14.1 (CH_3); *m*/*z* (CI, NH_3) [Found $(M + Na)^+$ 684.4828. $C_{39}H_{67}O_7NNa$ requires *M*, 684.4816].

(−)-1-*O*-[12-*N*-(Benzyloxycarbonyl)amino dodecanoyl]-2-*O*-dodecanoyl-*sn*-glycerol 10b

The lipid (+)-**9b** (0.40 g, 0.55 mmol) was oxidised with DDQ (0.24 g, 0.11 mmol) as described above for alcohol (−)-**10a** to give alcohol (−)-**10b** (0.27 g, 91%) as a white solid (Found: C, 69.5; H, 9.8; N, 2.3. $C_{35}H_{59}O_7N$ requires C, 69.4; H, 9.8; N, 2.3%); mp 55–58 °C (ethyl acetate–hexane); $[\alpha]_D^{22} = -2.8$ (*c* 0.6 in $CHCl_3$); $R_f = 0.38$ (30% ethyl acetate in hexane); ν_{max} ($CHCl_3$)/cm^{-1} 3451, 3049, 3007, 2928, 2855, 1724, 1514, 1456, 1413, 1372, 1259, 1155 and 1111; δ_H (250 MHz; $CDCl_3$) 7.34 (5 H, s, C_6H_5), 5.12–5.03 (3 H, m, CH_2CHCH_2 and OCH_2), 4.79 (1 H, br s, N*H*), 4.32 (1 H, dd, *J* 11.9, 4.5, CH_2CHCH_2), 4.25–4.13 (1 H, m, CH_2CHCH_2), 3.72 (2 H, br s, CH_2CHCH_2), 3.16 (2 H, q, *J* 6.4, CH_2NH), 2.37–2.28 (4 H, m, 2 × $OCOCH_2$), 1.62–1.48 (6 H, m, 2 × $OCOCH_2CH_2$, CH_2CH_2NH), 1.26 (30 H, br s, $COC_{11}H_{23}$, $C_{11}H_{32}NH$), 0.87 (3 H, t, *J* 6.9, CH_3); δ_C (100 MHz; $CDCl_3$) 173.7, 173.4 (*O*CO), 156.3 (NH*C*O), 136.6, 128.4, 128.1 (3 × C_6H_5), 72.1 (*C*H), 66.6, 62.0, 61.5 (3 × CH_2), 41.1 ($NHCH_2$), 34.3, 34.1, 31.9, 29.9, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 24.9, 24.8, 22.6 (14 × CH_2), 14.1 (CH_3); *m*/*z* (+ES) [Found $(M + H)^+$ 606.4367. $C_{35}H_{68}O_7N$ requires *M*, 606.4370].

(+)-1-*O*-[12-*N*-(Benzyloxycarbonyl)amino dodecanoyl]-2-*O*-hexadecanoyl-*sn*-glycerol 3-(dibenzyl phosphate) 11a

Dry dichloromethane (10 cm^3) was added to a mixture of the alcohol (−)-**10a** (1.00 g, 1.51 mmol), 1*H*-tetrazole (1.04 g, 3.02 mmol) and $(BnO)_2PNPr^i_2$ (0.31 g, 4.54 mmol). The reaction mixture was stirred at rt for 2 h. The reaction mixture was cooled to −78 °C and MCPBA (1.43 g, 8.3 mmol) was added in one portion. The reaction mixture was warmed to rt over 2 h and stirred overnight. The reaction mixture was diluted with dichloromethane (30 cm^3) and washed with 10% $NaHSO_3$ solution (50 cm^3). The aqueous phase was back-extracted with dichloromethane (2 × 50 cm^3). The combined organic extracts were washed with saturated $NaHCO_3$ solution (20 cm^3), brine (20 cm^3), dried over $MgSO_4$ and concentrated. The residue was chromatographed (40% ethyl acetate in hexane) affording phosphate **11a** (1.14 g, 82%) as a white solid (Found: C, 69.2; H, 8.8; N, 1.5; P, 3.4. $C_{53}H_{80}O_{10}NP$ requires C, 69.0; H, 8.75; N, 1.5; P, 3.4%); mp 40–42 °C (from ethyl acetate–hexane); $[\alpha]_D^{25} = +1.75$ (*c* 0.23 in $CHCl_3$); $R_f = 0.11$ (30% ethyl acetate in hexane); ν_{max} ($CHCl_3$)/cm^{-1} 3542, 3022, 2853, 1733, 1601, 1512, 1433, 1222, 1217 and 1032; δ_P (101 MHz; $CDCl_3$) −0.64; δ_H (400 MHz; $CDCl_3$) 7.37–7.27 (15 H, m, C_6H_5), 5.16–5.13 (1 H, m, CH_2CHCH_2), 5.08–5.00 (6 H, m, 3 × OCH_2), 4.85 (1 H, br s, N*H*), 4.25 (1 H, dd, *J* 11.9, 4.4, CH_2CHCH_2), 4.13–4.05 (3 H, m, CH_2CHCH_2), 3.19–3.14 (2 H, m, CH_2NH), 2.28–2.23 (4 H, m, 2 × $OCOCH_2$), 1.58–1.57 (4 H, m, 2 × $OCOCH_2CH_2$), 1.48–1.46 (2 H, m, CH_2CH_2NH), 1.25 (38 H, br s, $COC_{15}H_{31}$, $C_{11}H_{22}NH$), 0.87 (3 H, t, *J* 6.6, CH_3); δ_C (63 MHz; $CDCl_3$) 173.1, 172.7 (2 × *O*CO), 156.4 (NH*C*O), 136.7, 135.6, 135.5 (3 × CH_2C), 128.6, 128.5, 128.1, 128.0, 127.9, 127.8 (6 × C_6H_5), 69.5 (d, J_{C-P} 5.5, CH_2), 69.4 (d, J_{C-P} 10.1, *C*H), 69.3, 66.5 (2 × CH_2), 65.4 (d, J_{C-P} 5.4, CH_2), 61.6 (CH_2), 41.10 (CH_2NH), 34.1, 33.9, 31.9, 29.68, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 26.7, 24.8, 22.7 (15 × CH_2), 14.11 (CH_3); *m*/*z* (FAB) [Found $(M + Na)^+$ 944.5417. $C_{53}H_{80}O_{10}NPNa$ requires *M*, 944.5427].

(+)-1-*O*-[12-*N*-(Benzyloxycarbonyl)amino dodecanoyl]-2-*O*-dodecanoyl-*sn*-glycerol 3-(dibenzyl phosphate) 11b

The alcohol (−)-**10b** (0.23 g, 0.33 mmol) was phosphorylated, according to the procedure described above for phosphate (+)-**11a**, to give the phosphate (+)-**11b** (0.25 g, 97%) as a colourless gum (Found: C, 67.9; H, 8.4; N, 1.7; P, 3.8. $C_{49}H_{72}O_{10}NP$ requires C, 67.9; H, 8.4; N, 1.6; P, 3.6%); $[\alpha]_D^{23} = +4.0$ (*c* 0.2 in $CHCl_3$); $R_f = 0.5$ (50% ethyl acetate in hexane); ν_{max} ($CDCl_3$)/cm^{-1} 2928, 2855, 1735, 1515, 1464, 1456, 1415, 1377, 1264, 1166, 1102 and 1019; δ_P (101 MHz; $CDCl_3$) −0.36; δ_H (500 MHz; $CDCl_3$) 7.49–7.28 (15 H, m, 3 × C_6H_5), 5.16 (1 H, qn, *J* 4.9, CH_2CHCH_2), 5.09 (2 H, s, OCH_2), 5.05–5.99 (4 H, m, 2 × OCH_2), 4.76 (1 H, br s, N*H*), 4.25 (1 H, dd, *J* 11.9, 4.9, CH_2CHCH_2), 4.14–4.05 (3 H, m, CH_2CHCH_2), 3.18 (2 H, q, *J* 6.5, CH_2NH), 2.26 (4 H, q, *J* 7.5, 2 × $OCOCH_2$), 1.58–1.57 (4 H, m, 2 × $OCOCH_2CH_2$), 1.48–1.47 (2 H, m, CH_2CH_2NH), 1.14 (30 H, br s, $COC_{11}H_{23}$, $C_{11}H_{22}NH$), 0.88 (3 H, t, *J* 6.7, CH_3); δ_C (63 MHz; $CDCl_3$) 173.2, 172.8 (*O*CO), 156.4 (NH*C*O), 136.7, 135.6 (2 × CH_2C), 128.6, 128.5, 128.0 (3 × C_6H_5), 69.5 (d, J_{C-P} 5.6, CH_2), 69.4 (d, J_{C-P} 4.3, *C*H), 66.9 (CH_2), 65.4 (d, J_{C-P} 5.4, CH_2), 61.6 (CH_2), 41.1 (CH_2NH), 34.1, 34.0, 31.9, 30.0, 29.6, 29.5, 29.4, 29.3, 29.1, 26.7, 24.8, 22.7 (12 × CH_2), 14.1 (CH_3); *m*/*z* (+ES) [Found $(M + H)^+$ 866.4971. $C_{49}H_{73}O_{10}NP$ requires *M*, 866.4971].

(−)-1-*O*-[12-*N*-(Benzyloxycarbonyl)amino dodecanoyl]-2-*O*-hexadecanoyl-*sn*-glycerol 3-(disodium phosphate) 3a

To a solution of *tert*-butyl alcohol (18 cm^3) and water (3 cm^3) in a glass tube was added the benzyl phosphate (+)-**11a** (0.1 g, 0.11 mmol). The reaction mixture was placed in an autoclave and the glass tube and autoclave were vented with hydrogen four and five times respectively. The autoclave was finally pressurised to 15 bar and stirred for 18 h. The pressure was slowly released and the reaction mixture was centrifuged. The *tert*-butyl alcohol–water layer was discarded and the residue was washed with methanol–chloroform (1 : 1 v/v, 20 cm^3). The suspension was centrifuged and the organic layer was collected and passed through a pad of Celite. The filtrate was centrifuged to remove traces of Celite and the organic layer was collected and concentrated *in vacuo* affording phosphate **3a** (0.052 g, 78%) as a white solid: $[\alpha]_D^{20} = -8.9$ [*c* 0.09 in CH_3OH–$CHCl_3$ (1 : 1)]; ν_{max} (KBr)/cm^{-1} 3838, 2918, 2849, 1732, 1684, 1469, 1417, 1382, 1242, 1166, 1044 and 937; δ_P [101 MHz; CD_3OD–$CDCl_3$ (1 : 1)] 1.31; δ_H [500 MHz; CD_3OD–$CDCl_3$ (1 : 1)] 4.88

(1 H, br s, CH_2CHCH_2), 4.13 (1 H, dd, *J* 12.0, 3.0, CH_2-$CHCH_2$), 3.83 (1 H, dd, *J* 12.0, 3.0, CH_2CHCH_2), 3.66–3.64 (2 H, m, CH_2CHCH_2), 2.55 (2 H, t, *J* 7.6, CH_2NH_2), 2.01–1.98 (4 H, m, 2 × $COCH_2$), 1.33–1.28 (6 H, m, 2 × $COCH_2CH_2$, $CH_2CH_2NH_2$), 0.98–0.92 (38 H, m, $COC_{15}H_{31}$, $C_{11}H_{22}NH$), 0.54 (3 H, t, *J* 6.5, CH_3); δ_C [500 MHz; CD_3OD–$CDCl_3$ (1 : 1)] 173.5, 173.1 (2 × q), 70.2, 62.0 (2 × glycerol carbons), 39.0, 33.6, 33.6, 33.3, 31.3, 29.0, 28.8, 28.7, 28.5, 27.7, 27.6, 27.4, 27.2, 26.7, 25.3, 24.2, 23.8, 22.0, 13.1 (19 × d); *m*/*z* (+ES) [Found (M + H)$^+$ 608.4291. $C_{31}H_{63}O_8NP$ requires *M*, 608.4292].

(−)-1-*O*-[12-*N*-(Benzyloxycarbonyl)aminododecanoyl]-2-*O*-dodecanoyl-*sn*-glycerol 3-(disodium phosphate) 3b

To a solution of (−)-**11b** (0.069 g, 0.07 mmol) in *tert*-butyl alcohol–water (6 : 1 v/v, 18 cm^3) at rt were added Pd black (0.026 g, 0.15 mmol) and $NaHCO_3$ (0.013 g, 0.15 mmol). The reaction mixture was stirred under an atmosphere of hydrogen for 18 h. The reaction mixture was centrifuged and the *tert*-butyl alcohol–water layer was discarded. The residue was washed with methanol–chloroform (1 : 1 v/v, 20 cm^3). The suspension was centrifuged and the organic layer was collected and passed through a pad of Celite. The filtrate was centrifuged to remove traces of Celite and the organic layer was collected and concentrated *in vacuo* affording phosphate (−)-**3b** (0.043 g, 99%) as a white solid: $[\alpha]_D^{18}$ = −7.3 [*c* 0.055 in CH_3OH–$CHCl_3$ (1 : 1)]; ν_{max} (Nujol)/cm^{-1} 3384, 1718, 1169, 1042 and 935.6; δ_P [162 MHz; CD_3OD–$CDCl_3$ (1 : 1)] 1.37; δ_H [500 MHz; CD_3OD–$CDCl_3$ (1 : 1)] 4.91–4.89 (1 H, m, CH_2CHCH_2), 4.17 (1 H, dd, *J* 12.0, 3.0, CH_2CHCH_2), 3.84 (1 H, dd, *J* 12.0, 6.6, CH_2CHCH_2), 3.65–3.59 (2 H, m, CH_2CHCH_2), 2.55 (2 H, t, *J* 7.6, CH_2NH_2), 2.01–1.98 (4 H, m, 2 × $COCH_2$), 1.34–1.27 (6 H, m, 2 × $COCH_2CH_2$, $CH_2CH_2NH_2$), 0.99–0.94 (30 H, m, $COC_{11}H_{23}$, $C_{11}H_{22}NH$), 0.55 (3 H, t, *J* 6.6, CH_3); δ_C [100 MHz; CD_3OD–$CDCl_3$ (1 : 1)] 173.0, 173.7 (2 × *C*O), 69.8 (d, *J* 8.2, CH_2), 62.2, 61.6 (2 × glycerol carbons), 38.6 (CH_2NH_2), 33.1, 32.9, 30.9, 28.6, 28.5, 28.4, 28.3, 28.0, 27.6, 27.4, 27.3, 26.3, 25.1, 23.8, 23.5, 21.6 (16 × CH_2), 12.7 (CH_3); *m*/*z* (−ES) [Found (M − H)$^-$ 550.3508. $C_{31}H_{63}O_8NP$ requires *M*, 550.3509].

(−)-5-*O*-Allyl-2,6-di-*O*-benzyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol 16

A solution of the camphor acetal (−)-**15** (0.062 g, 0.950 mmol) in methanol–dichloromethane (v/v 3 : 5, 16 cm^3) was stirred with acetyl chloride (41 μL, 0.57 mmol) at rt for 1 h. Triethylamine (0.5 cm^3) was added and the solution was concentrated. Flash chromatography (50% ethyl acetate in hexane) of the residue yielded (−)-**16** (0.041 g, 82%) as a white solid: mp 125–126 °C (from ethyl acetate–hexane) [lit.,[15] mp 125–126 °C (ethyl acetate–light petroleum)]; $[\alpha]_D^{20}$ = −26.6 (*c* 1.0 in $CHCl_3$) [lit.,[15] $[\alpha]_D^{28}$ = −26.4 (*c* 1.2 in $CHCl_3$)]; δ_H (250 MHz; $CDCl_3$) 7.35–7.24 (12 H, m, 2 × C_6H_5, C_6H_4OMe), 6.85 (2 H, d, *J* 8.0, C_6H_4OMe), 5.99–5.92 (1 H, m, $CHCH_2$), 5.27 (1 H, dd, *J* 17.2, 1.6, $CHCH_2$), 5.16 (1 H, d, *J* 10.4, $CHCH_2$), 5.04 (1H, dd, J_{AB} 11.5, OCH_AH_B), 4.89 (1 H, dd, J_{AB} 10.7, OCH_AH_B), 4.85 (1 H, dd, J_{AB} 10.7, OCH_AH_B), 4.66 (2 H, dd, J_{AB} 11.5, 2 × OCH_AH_B), 4.61 (1 H, dd, J_{AB} 11.5, OCH_AH_B), 4.40 (1 H, dd, *J* 12.5, 5.5, OCH_2), 4.26 (1 H, dd, *J* 12.5, 5.9, OCH_2), 3.98 (1 H, t, *J* 2.5, 2-H), 3.93 (1 H, t, *J* 9.5, 6-H), 3.81 (3 H, s, OCH_3), 3.78 (1 H, dd, *J* 9.5, 2.5, 3-H), 3.43 (1 H, dd, *J* 9.5, 2.5, 1-H), 3.36 (1 H, dt, *J* 9.5, 2.0, 4-H), 3.19 (1 H, t, *J* 9.5, 5-H), 2.51 (1 H, d, *J* 2.0, O*H*), 2.25 (1 H, d, *J* 8.0, O*H*).

(−)-5-*O*-Allyl-1-*O*-(4-methoxybenzyl)-2,3,6-tri-*O*-benzyl-*myo*-inositol 17

A mixture of the diol (−)-**16** (0.16 g, 0.307 mmol), Bu_2SnO (0.084 g, 0.34 mmol), tetrabutylammonium bromide (0.099 g; 0.307 mmol) and benzyl bromide (0.25 g, 1.47 mmol) in acetonitrile (20 cm^3) was stirred under reflux with removal of water using a Soxhlet apparatus filled with 3 Å molecular sieves. After 21 h the solution was concentrated to dryness. The residue was suspended in water (15 cm^3) and extracted with ethyl acetate (2 × 20 cm^3). The organic fractions were combined and stirred with saturated $NaHCO_3$ solution (15 cm^3), filtered over Hyflo, and the layers were separated. The organic phase was washed with brine (10 cm^3) and dried over Na_2SO_4. Evaporation of the solvent and purification by flash chromatography (30–40% diethyl ether in hexane) afforded the 3-benzyl ether (−)-**17** (0.14 g, 75%) as a colourless oil that solidified upon standing. Further purification was achieved by stirring in hexane–ethyl acetate to give the pure product (−)-**17** as a white powder (Found: C, 74.7; H, 7.0. $C_{39}H_{42}O_7$ requires C, 74.7; H, 6.9%); mp 60–61 °C (from ethyl acetate–hexane); $[\alpha]_D^{20}$ = −0.6 (*c* 0.4 in $CHCl_3$); R_f 0.16 (40% diethyl ether in hexane); ν_{max} ($CHCl_3$)/cm^{-1} 3462, 3030, 2873, 1612, 1586, 1513, 1454, 1360, 1302, 1248, 1052, 925, 821 and 735; δ_H (250 MHz; $CDCl_3$) 7.40–7.21 (17 H, m, 3 × C_6H_5, C_6H_4OMe), 6.85 (2 H, d, *J* 8.6, C_6H_4OMe), 6.05–5.89 (1 H, m, $CHCH_2$), 5.27 (1 H, d, *J* 17.2, 1.5, $CHCH_2$), 5.15 (1 H, d, *J* 10.4, 1.5, $CHCH_2$), 4.90–4.75 (4 H, m, OCH_2), 4.63–4.51 (4 H, m, OCH_2), 4.36–4.33 (2 H, m, OCH_2), 4.10 (1 H, dt, *J* 9.5, 1.8, inositol H), 4.01–3.94 (2 H, m, inositol H), 3.82 (3 H, s, OCH_3), 3.32 (1 H, dd, *J* 9.5, 2.2, inositol H), 3.23 (1 H, t, *J* 9.2, inositol H), 3.16 (1 H, dd, *J* 9.8, 2.1, inositol H), 2.50 (1 H, d, *J* 1.8, O*H*); δ_C (100 MHz; $CDCl_3$) 159.2 (*C*OMe), 138.9, 138.0, 135.3, 130.5 (4 × *C*), 129.2, 128.5, 128.3, 128.1, 128.1, 127.8, 127.7, 127.5, 127.3 (9 × C_6H_5, C_6H_4), 116.7 (*C*H), 113.8 (CH_2), 83.0, 81.4, 80.8, 80.0, 75.7, 74.2, 74.0, 73.8, 72.7, 72.5, 72.3 (6 × inositol C, 5 × CH_2), 55.3 (OCH_3); *m*/*z* (+ES) [Found (M + NH_4)$^+$ 628.3275. $C_{38}H_{46}O_7N$ requires *M*, 628.3274].

(+)-1-*O*-(4-Methoxybenzyl)-2,3,6-tri-*O*-benzyl-*myo*-inositol 18

To a solution of the allyl ether (−)-**17** (0.024 g, 0.039 mmol) and diisopropylethylamine (2.0 μL, 0.012 mmol) in ethanol–toluene–water (7 : 3 : 1, 5 cm^3) was added $(Ph_3P)_3RhCl$ (5.5 μg, 5.9 μmol) and the solution refluxed for 2 h, then cooled to rt. The mixture was filtered over Hyflo and washed with ethyl acetate. The filtrate was concentrated to dryness, suspended in water (5 cm^3) and extracted with ethyl acetate (3 × 5 cm^3). The combined organic layers were washed with brine (5 cm^3) and dried over $MgSO_4$ to give the crude propenyl ether as a brown oil (mixture of geometric isomers). The brown oil was dissolved in dichloromethane–methanol (5 : 3, 3 cm^3) and acetyl chloride (2 μL, 24 μmol) was added. The solution was stirred at rt for 30 mins, followed by quenching with three drops of triethylamine and concentrated. Flash chromatography (30–50% ethyl acetate in hexane) afforded the diol (+)-**18** (0.013 g, 58%) as a white solid: mp 104–106 °C (from ethyl acetate–hexane); $[\alpha]_D^{20}$ = +16.9 (*c* 0.7 in $CHCl_3$) [lit.,[19] $[\alpha]_D^{20}$ = +12.8 (*c* 1 in $CHCl_3$)]; δ_H (250 MHz; $CDCl_3$) 7.42–7.22 (17 H, m, 3 × C_6H_5, C_6H_4OMe), 6.87 (2 H, d, *J* 8.7, C_6H_4OMe), 5.00–4.75 (4 H, m, 2 × OCH_2), 4.63–4.51 (4 H, m, 2 × OCH_2), 4.10–4.01 (2 H, m, 2 × inositol H), 3.91 (1 H, t, *J* 9.3, inositol H), 3.82 (3 H, s, OCH_3), 3.43 (1 H, t, *J* 9.3, inositol H), 3.37 (1 H, dd, *J* 9.7, 2.3, inositol H), 3.19 (1 H, dd, *J* 9.7, 2.3, inositol H); 2.55 (2 H, br s, 2 × O*H*).

(+)-6-*O*-Benzyl-2,3-*O*-cyclohexylidene-4,5-di-*O*-allyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol 20

Sodium hydride (0.096 mg, 60% dispersion in mineral oil, 2.4 mmol) was washed with dry hexane under argon. After removal of the solvent, the residual solid was suspended in DMF (3 cm^3) and cooled to 0 °C. A solution of the alcohol (+)-**19** (0.047 g, 1.00 mmol) in DMF (7 cm^3) was added *via* cannula with stirring. The suspenson was stirred for 15 mins at 0 °C and allyl bromide (190 μL, 2.20 mmol) was added dropwise. The temperature was slowly increased to rt and the suspension was

stirred for 14 h. The reaction was quenched by the addition of methanol (0.5 cm^3) and the solvent was removed *in vacuo*. The residue was partitioned between ethyl acetate (30 cm^3) and water (15 cm^3). The organic layer was separated and the aqueous layer extracted with ethyl acetate (20 cm^3) and the combined organic extracts were washed with water (15 cm^3), brine (15 cm^3) and dried over Na_2SO_4. Evaporation of the solvent and flash chromatography eluting with 30% diethyl ether in hexane afforded the diallyl ether (+)-**20** (0.049 g, 89%) as a colourless oil: $[\alpha]_D^{20} = +28.8$ (*c* 0.9 in $CHCl_3$); R_f 0.18 (30% ether–hexane); ν_{max} (CCl_4)/cm^{-1} 2936, 2864, 1612, 1463, 1354, 1249, 1163 and 1072; δ_H (250 MHz; $CDCl_3$) 7.38–7.26 (7 H, m, C_6H_5, C_6H_4OMe), 6.85 (2 H, d, *J* 8.7, C_6H_4OMe), 6.01–5.90 (2 H, m, $CHCH_2$), 5.32–5.13 (4 H, m, $CHCH_2$), 4.84 (1 H, dd, J_{AB} 10.8, OCH_AH_B), 4.79 (1 H, dd, J_{AB} 10.8, OCH_AH_B), 4.75 (1 H, dd, J_{AB} 11.9, OCH_AH_B), 4.69 (2 H, dd, *J* 11.9, OCH_AH_B), 4.34–4.18 (5 H, m, OCH_2, inositol H), 3.97 (1 H, t, *J* 5.8, inositol H), 3.85 (1 H, t, *J* 8.6, inositol H), 3.80 (3 H, s, OCH_3), 3.63–3.57 (2 H, m, inositol H), 3.19 (1 H, t, *J* 8.6, inositol H), 1.76–1.44 (10 H, m, 10 × cyclohexyl H); δ_C (63 MHz; $CDCl_3$) 159.4 (*C*OMe), 138.8, 135.5, 135.4, 130.4, 129.6, 128.4, 128.1, 127.6 (8 × C_6H_5, C_6H_4), 116.7 (*C*H), 113.8 (CH_2), 110.3 (CO_2), 82.5, 81.9, 80.9, 78.7, 77.7, 75.3, 74.2, 74.1, 73.0, 72.7 (6 × inositol C, 4 × CH_2), 55.3 (OCH_3), 35.2, 25.1, 24.0, 23.7 (4 × CH_2); *m*/*z* (CI, NH_3) [Found $(M + NH_4)^+$ 568.3274. $C_{33}H_{46}O_7N$ requires *M*, 568.3274].

(+)-6-*O*-Benzyl-4,5-di-*O*-allyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol 21

A solution of the acetal (+)-**20** (0.048 g; 0.88 mmol) in methanol–dichloromethane (1 : 3, 16 cm^3) was stirred with acetyl chloride (70 μL; 0.98 mmol) at rt for 3 h. Triethylamine (300 μL, 2.15 mmol) was added and the solution was concentrated. Flash chromatography (50% ethyl acetate in hexane) of the residue yielded (+)-**21** (0.037 g, 88%) as a white solid (Found: C, 68.4; H, 7.2. $C_{27}H_{34}O_7$ requires C, 68.9; H, 7.3%); mp 108–110 °C (from hexane–ethyl acetate); $[\alpha]_D^{20} = +12.5$ (*c* 0.4 in $CHCl_3$); R_f 0.14 (50% ethyl acetate in hexane); ν_{max} ($CHCl_3$)/cm^{-1} 3582, 3012, 1612, 1456, 1359, 1303, 1125 and 1070; δ_H (250 MHz; $CDCl_3$) 7.35–7.24 (7 H, m, C_6H_5, C_6H_4OMe), 6.85 (2 H, d, *J* 8.5, C_6H_4OMe), 6.05–5.90 (2 H, m, $CHCH_2$), 5.33–5.14 (4 H, m, $CHCH_2$), 4.86 (1 H, dd, J_{AB} 10.8, OCH_AH_B), 4.80 (1 H, dd, J_{AB} 10.8, OCH_AH_B), 4.66 (1 H, dd, J_{AB} 12.1, OCH_AH_B), 4.60 (1 H, dd, J_{AB} 12.1, OCH_AH_B), 4.46–4.22 (4 H, m, OCH_2), 4.15 (1 H, br s, inositol H), 3.85 (1 H, t, *J* 9.5, inositol H), 3.80 (3 H, s, OCH_3), 3.65 (1 H, t, *J* 9.5, inositol H), 3.36–3.41 (2 H, m, inositol H), 3.26 (1 H, t, *J* 9.5, inositol H), 2.66–2.62 (2 H, m, O*H*); δ_C (63 MHz; $CDCl_3$) 159.4 (*C*OMe), 138.8, 135.2, 135.1, 130.0, 129.5, 128.4, 128.0, 127.6 (8 × C_6H_5, C_6H_4), 117.1, 116.7 (2 × *C*H), 113.9 (CH_2), 82.9, 81.6, 80.8, 79.6, 75.9, 74.4, 74.3, 72.5, 71.6, 69.3 (10 × inositol C, 4 × CH_2), 55.3 (OCH_3); *m*/*z* (CI, NH_3) [Found $(M + NH_4)^+$ 488.2650. $C_{27}H_{38}O_7N$ requires *M*, 488.2648].

(−)-2,3,6-Tri-*O*-benzyl-4,5-di-*O*-allyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol 22

Sodium hydride (0.074 g, 60% dispersion in mineral oil, 1.85 mmol) was washed with dry hexane under argon. After removal of the solvent, the residual solid was suspended in DMF (3 cm^3) and cooled to 0 °C. A solution of the diol (+)-**21** (0.036 g, 0.77 mmol) in DMF (5 cm^3) was added *via* a cannula with stirring. The suspenson was stirred for 15 mins at the same temperature, and benzyl bromide (201 μL, 1.69 mmol) was added dropwise. The temperature was slowly increased to rt and the suspension was stirred for 14 h. The reaction was quenched by the addition of methanol (0.5 cm^3) and the solvent was removed *in vacuo*. The residue was partitioned between ethyl acetate (25 cm^3) and water (15 cm^3). The organic layer was separated and the aqueous layer extracted with ethyl acetate (25 cm^3) and the combined organic extracts were washed with water (20 cm^3), brine (15 cm^3) and dried over $MgSO_4$. Evaporation of the solvent and flash chromatography eluting with 5–30% diethyl ether in hexane afforded the tribenzyl ether (−)-**22** (0.045 g, 91%) as a colourless oil which solidified upon standing (Found: C, 75.55; H, 7.2. $C_{41}H_{46}O_7$ requires C, 75.7; H, 7.1%); mp 57–60 °C (from diethyl ether–hexane); $[\alpha]_D^{14} = -8.2$ (*c* 0.5 in $CHCl_3$); R_f 0.12 (30% diethyl ether in hexane); ν_{max} (CCl_4)/cm^{-1} 3032, 2879, 1514, 1454, 1358, 1249, 1132 and 1075; δ_H (250 MHz; $CDCl_3$) 7.44–7.23 (17 H, m, 3 × C_6H_5, C_6H_4OMe), 6.87 (2 H, d, *J* 8.6, C_6H_4OMe), 6.09–5.93 (2 H, m, $CHCH_2$), 5.34–5.26 (2 H, m, $CHCH_2$), 5.17 (2 H, d, *J* 10.3, $CHCH_2$), 4.92–4.80 (4 H, m, 2 × OCH_2), 4.71–4.51 (4 H, m, 2 × OCH_2), 4.43–4.29 (4 H, m, 2 × OCH_2), 4.02–3.94 (2 H, m, inositol H), 3.90 (1 H, t, *J* 9.6, inositol H), 3.83 (3 H, s, OCH_3), 3.55–3.24 (3 H, m, inositol H); δ_C (63 MHz; $CDCl_3$) 159.2 (*C*OMe), 139.1, 138.6, 153.5, 130.6 (4 × *C*), 129.2, 128.4, 128.1, 127.8, 127.5 (5 × C_6H_5, C_6H_4), 116.5 (*C*H), 113.8 (CH_2), 83.4, 81.6, 81.4, 80.7, 80.5, 75.8, 74.7, 74.5, 74.1, 72.9, 72.5 (6 × inositol C, 5 × CH_2), 55.3 (OCH_3); *m*/*z* (CI, NH_3) [Found $(M + NH_4)^+$ 668.3576. $C_{41}H_{50}O_7N_2$ requires *M*, 668.3587].

(+)-1D-1-*O*-(4-Methoxybenzyl)-2,3,6-tri-*O*-benzyl-*myo*-inositol 18

To a solution of the diallyl ether (−)-**22** (0.27 g, 0.42 mmol) and DIPEA (44 μL, 0.25 mmol) in ethanol–toluene–water (7 : 3 : 1, 20 cm^3) was added $(Ph_3P)_3RhCl$ (0.12 g, 0.13 mmol) and the solution refluxed for 2 h. The reaction mixture was then cooled to rt and diluted with water (15 cm^3) and diethyl ether (30 cm^3) and any insoluble material was removed by filtration under vacuum. The layers were separated and the aqueous phase was extracted with diethyl ether (2 × 20 cm^3). The combined organic layers were washed with brine (2 × 15 cm^3), dried over Na_2SO_4 and evaporated. Flash chromatography eluting with 15% diethyl ether in hexane afforded the diprop-2-enyl ether as a mixture of geometric isomers (0.24 g, 87%) as a brown oil. The brown oil was dissolved in dichloromethane–methanol (2 : 1, 21 cm^3) and acetyl chloride (13 μL, 0.17 mmol) was added. The solution was stirred at rt for 30 min, followed by quenching with triethylamine (69 μL, 0.50 mmol) and concentrated. Flash chromatography (30–60% ethyl acetate in hexane) afforded the diol (+)-**18** (0.19 g, 90%) as a white solid: $[\alpha]_D^{20} = +17.4$ (*c* 0.9 in $CHCl_3$); ^1H NMR data and mp were identical to those of the material prepared above.

(+)-1D-2,3,6-Tri-*O*-benzyl-1-*O*-(4-methoxybenzyl)-*myo*-inositol 4,5-bis(dibenzyl phosphate) 23

A solution of the alcohol (+)-**18** (0.23g, 0.39 mmol) in dichloromethane (5 cm^3) was added *via* cannula to a mixture of $(BnO)_2PNPr^i_2$ (0.55 g, 1.58 mmol) and 1*H*-tetrazole (0.22 g, 3.15 mmol) in dichloromethane (10 cm^3). After stirring at rt for 5 h, the reaction mixture was cooled to −78 °C and MCPBA (0.57 g, 3.31 mmol) was added in a single portion. After 1 h, the temperature was increased to rt and stirring was continued for 1 h. The reaction mixture was diluted with dichloromethane (10 cm^3) and washed with 10% $NaHSO_3$ solution (15 cm^3). The aqueous phase was extracted with dichloromethane (2 × 15 cm^3) and the combined dichloromethane extracts were washed with water (10 cm^3), brine (10 cm^3) and dried over Na_2SO_4. The residue obtained after evaporation was purified by flash chromatography (30–50% ethyl acetate in hexane) to afford the fully protected phospholipid (−)-**23** (0.32 g, 75%) as a colourless oil; $[\alpha]_D^{23} = -11.1$ (*c* 1 in $CHCl_3$) [lit.,[19] $[\alpha]_D^{27} = -10.2$ (*c* 1 in $CHCl_3$)]; δ_P (162 MHz; $CDCl_3$) −1.71, −2.11 [lit.,[19] δ_P (162 MHz; $CDCl_3$) −1.75, −2.15]; δ_H (250 MHz; $CDCl_3$) 7.41–6.99 (37 H, m, 7 × C_6H_5, C_6H_4OMe), 6.80 (2 H, d, *J* 8.7, C_6H_4OMe), 5.13–4.81 (12 H, m, 6 × CH_2), 4.75–4.45 (6 H,

2 × CH_2, 2 × inositol H), 4.10 (1 H, t, *J* 9.5, inositol H), 3.91 (1 H, br s, inositol H), 3.81 (3 H, s, OCH_3), 3.46–3.36 (2 H, m, inositol H).

(−)-1D-2,3,6-Tri-*O*-benzyl-*myo*-inositol 4,5-bis(dibenzyl phosphate) 24

A solution of ceric ammonium nitrate (0.22 g, 0.39 mmol) in acetonitrile–water (4 : 1, 3.75 cm^3) was added to the bis-(dibenzyl phosphate) (−)-**23** (0.070 g, 0.064 mmol) at 0 °C and stirred for 1 h. Water was added (10 cm^3) and the reaction mixture was extracted with ethyl acetate (3 × 10 cm^3). The combined organic layers were washed with saturated $NaHCO_3$ solution (10 cm^3), brine (10 cm^3), dried over $MgSO_4$ and concentrated. The residue was chromatographed with 50–80% ethyl acetate in hexane to afford the bis(dibenzyl phosphate) (−)-**24** (0.023 g, 80%) as a colourless oil: $[\alpha]_D^{20} = -17.8$ (*c* 1.66 in $CHCl_3$) [lit.,[19] $[\alpha]_D^{25} = -15.6$ (*c* 1 in $CHCl_3$)]; δ_P (162 MHz; $CDCl_3$) −0.80, −1.12 [lit.,[19] δ_P (162 MHz; $CDCl_3$) −1.55, −1.82]; δ_H (250 MHz; $CDCl_3$) 7.36–7.08 (35 H, m, 7 × C_6H_5), 5.12–4.90 (11 H, m, 5 × CH_2, inositol H), 4.86–4.48 (5 H, 2 × CH_2, inositol H), 3.94 (1 H, t, *J* 2.3, inositol H), 3.86 (1 H, br s, inositol H), 3.59–3.50 (2 H, m, inositol H), 2.14 (1 H, s, O*H*).

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

A solution of $(BnO)P(NPr^i_2)_2$ (0.022 g, 0.66 mmol) in dichloromethane (3 cm^3) was added *via* cannula to a stirred mixture of the glycerol **10a** (0.035 g, 0.53 mmol) and 1*H*-tetrazole (0.025 g, 0.35 mmol) in CH_2Cl_2 (7 cm^3). After stirring at rt for 2 h, the reaction mixture was diluted with dichloromethane (50 cm^3) and washed with water (30 cm^3). The aqueous phase was extracted with dichloromethane (2 × 25 cm^3) and the combined dichloromethane extracts were washed with saturated $NaHCO_3$ solution (30 cm^3), brine (30 cm^3) and dried over Na_2SO_4. The residue obtained after evaporation was purified by flash chromatography (hexane–ethyl acetate–triethylamine 80 : 15 : 5) to afford the phosphoramidite **12** (0.042 g, 88%) as a colourless oil: $[\alpha]_D^{20} = +7.0$ (*c* 1.9 in $CHCl_3$); δ_P (162 MHz; $CDCl_3$) 149.4, 149.5; δ_H (250 MHz; $CDCl_3$) 7.34–7.22 (10 H, m, 5 × C_6H_5), 5.23–5.15 (1 H, m, CH_2CHCH_2), 5.10 (2 H, s, OCH_2), 4.89–4.61 (3 H, m, OCH_2, N*H*), 4.28–4.39 (1 H, m, CH_2CHCH_2), 4.21–4.13 (1 H, m, CH_2CHCH_2), 3.84–3.56 (4 H, m, CH_2CHCH_2, $CH(CH_3)_2$), 3.18 (2 H, q, *J* 6.7, CH_2NH), 2.29 (4 H, t, *J* 7.3, 2 × $COCH_2$), 1.63–1.46 (6 H, m, 2 × $OCOCH_2CH_2$, CH_2CH_2NH), 1.26 (40 H, br s, $COC_{15}H_{31}$, $C_{11}H_{22}NH$), 1.19 (6 H, t, *J* 6.8, $CH(CH_3)_2$), 1.18 (6 H, t, *J* 6.8, $CH(CH_3)_2$), 0.88 (3 H, t, *J* 6.6, CH_3); *m*/*z* (FAB) [Found $(M + Na)^+$ 921.6022. $C_{52}H_{87}N_2O_8PNa$ requires *M*, 921.6098].

(−)-1D-2,3,6-Tri-*O*-benzyl-*myo*-inositol 1-{1′-*O*-[12-*N*-(benzyloxycarbonyl)aminododecanoyl]-2′-*O*-hexadecanoyl-*sn*-3′-deoxyglycer-3′-yl benzyl phosphate} 4,5-bis(dibenzyl phosphate) 25

A solution of the alcohol (−)-**24** (0.070 g, 0.0721 mmol) in dichloromethane (2 cm^3) was added *via* cannula to a mixture of the phosphoramidite **12** (0.016 g, 0.18 mmol) and 1*H*-tetrazole (0.015 g, 0.22 mmol) in dichloromethane (1.5 cm^3). After stirring at rt for 2 h, the reaction mixture was cooled to −78 °C and MCPBA (0.057 g, 0.33 mmol) was added in a single portion. After 30 min the temperature was increased to rt and stirring was continued for 45 min. The reaction mixture was diluted with dichloromethane (10 cm^3) and washed with 10% $NaHSO_3$ solution (15 cm^3). The aqueous phase was extracted with dichloromethane (2 × 15 cm^3) and the combined dichloromethane extracts were washed with water (10 cm^3), brine (10 cm^3) and dried over Na_2SO_4. The residue obtained after evaporation was purified by flash chromatography (25–75% ethyl acetate in hexane) to afford the fully protected phospholipid (−)-**25** (0.11 g, 82%) as a colourless oil (Found: C, 68.0; H, 7.2; N, 1.0; P, 5.2. $C_{101}H_{128}NO_{21}P_3$ requires C, 68.0; H, 7.2; N, 0.8; P, 5.2%); $[\alpha]_D^{20} = -3.5$ (*c* 2.0 in $CHCl_3$); R_f 0.23 (60% ethyl acetate in hexane); ν_{max} (CCl_4)/cm^{-1} 2932, 2856, 1741, 1496, 1453, 1278, 1131 and 1022; δ_P (162 MHz; $CDCl_3$) −0.10, −1.20, −1.35, −1.39; δ_H (500 MHz; $CDCl_3$) 7.36–7.07 (43 H, m, 9 × C_6H_5), 6.95 (2 H, d, *J* 7.6, C_6H_5), 5.09–4.52 (22 H, m, 9 × OCH_2, CH_2CHCH_2, N*H*, 2 × inositol H), 4.35–4.22 (2 H, m, 2 × inositol H), 4.11–3.75 (5 H, m, CH_2CHCH_2, inositol H), 3.56–3.48 (1 H, m, inositol H), 3.18 (2 H, q, *J* 6.4, CH_2NH), 2.24–2.15 (4 H, m, 2 × $COCH_2$), 1.55–1.48 (6 H, m, 2 × $OCOCH_2CH_2$, CH_2CH_2NH), 1.24 (38 H s, $COC_{15}H_{31}$, $C_{11}H_{22}NH$), 0.88 (3 H, t, *J* 6.9, CH_3); *m*/*z* (FAB) 1806.4 (100%), 1784.3 (39%), 644.5 (47%), 536.5 (26%).

(+)-1D-*myo*-Inositol 1-(1′-*O*-12-aminododecanoyl-2′-*O*-hexadecanoyl-*sn*-3′-deoxyglycer-3′-yl sodium phosphate) 4,5-bis-(disodium phosphate) 4

To a solution of the protected lipid (−)-**24** (0.095 g; 0.053 mmol) in *tert*-butyl alcohol (18 cm^3) and water (3 cm^3) was added $NaHCO_3$ (0.022 g; 0.27 mmol) followed by Pd black (0.10 g, catalytic). The reaction vessel was then pressurised with hydrogen (27 bar) and stirred for 21 h at rt. The mixture was centrifuged and the supernatant was removed. The residue was washed with ethyl acetate and centrifuged again. The solid residue was stirred with water (10 cm^3) and centrifuged and the aqueous supernatant decanted. This procedure was repeated thrice (3 × 8 cm^3 water). The combined aqueous extracts were passed through a plug of cotton wool. The filtrate was centrifuged and the supernatant freeze-dried to afford (+)-**4** (0.032 g; 59%) as a fluffy white solid: $[\alpha]_D^{20} = +3.8$ (*c* 0.2 in H_2O); ν_{max} (neat)/cm^{-1} 3158, 2918, 2850, 1741, 1639, 1564, 1467, 1238, 1091 and 1047; δ_P (162 MHz; D_2O) 5.48, 5.31, 0.08; δ_H (250 MHz; D_2O) 5.23 (1 H, br s, CH_2CHCH_2), 4.37 (1 H, br s, inositol H), 4.13 (3 H, br s, CH_2CHCH_2), 4.00–3.96 (3 H, br m, CH_2NH, CH_2CHCH_2), 3.87 (1 H, t, *J* 9.4, inositol H), 3.81 (1 H, br s, inositol H), 3.63 (1 H, d, *J* 9.2, inositol H), 2.90 (2 H, br s, inositol H), 2.34–2.25 (4 H, m, 2 × $COCH_2$), 1.62–1.53 (6 H, m, 2 × $OCOCH_2CH_2$, CH_2CH_2NH), 1.24–1.20 (38 H, m, $COC_{15}H_{31}$, $C_{11}H_{22}NH$), 0.81 (3 H, br s, CH_3); *m*/*z* (−ES) [Found $(M - 2H)^-$ 927.3918. $C_{37}H_{67}NO_{19}P_3$ requires *M*, 927.3912].

1-*O*-[12-*N*-(Affi-Gel 10)Aminododecanoyl]-2-*O*-hexadecanoyl-*sn*-glycerol 3-phosphate 1a

Affi-Gel 10 (2 cm^3 slurry, 30.00 μmol) was filtered and washed with chloroform–methanol–water (0.8 : 1 : 0.2, 15 cm^3). It was transferred to a stirred solution of the acid **3a** (4.0 mg, 6.14 μmol) and $NaHCO_3$ (0.025 g, 0.30 mmol) in chloroform–methanol–water (0.8 : 1 : 0.2, 2 cm^3). The reaction mixture was stirred at rt for 18 h. The reaction mixture was filtered and washed with water (5 cm^3), chloroform–methanol–water (0.8 : 1 : 0.2, 10 cm^3) and water (5 cm^3) again. The gel was stored in water at 0 °C. The filtrates were combined and 4.83 μmol of (1*R**,3*S**,5*R**,6*R**,7*S**,8*R**,9*R**)-6-[(4′-methoxy)phenylmethyl oxy]-2,4,10-trioxatricyclo[3.3.1.1]decane-8,9-diol[14] was added. The solvent was removed *in vacuo* and the white residue was taken up in $CDCl_3$–CD_3OD (1 : 1 v/v) for proton NMR. The orthoformate : amine ratio was estimated to be 1 : 0.22 which implied that 5.33 μmol of the amine were loaded onto the beads.

1-*O*-[12-*N*-(Affi-Gel 10)Aminododecanoyl]-2-*O*-dodecanoyl-*sn*-glycerol 3-phosphate 1b

Phosphatidic acid **3b** (3.57 mg, 6.00 μmol) was coupled onto Affi-Gel 10 (2 cm^3 slurry, 30.00 μmol), according to the procedure described above for immobilised PA **1a**, to give immobilised PA **1b** with 17% loading onto the beads.

1D-*myo*-Inositol 1-{1′-*O*-[12-*N*-(Affi-Gel 10)aminododecanoyl]-2′-*O*-hexadecanoyl-*sn*-3′-deoxyglycer-3′-yl sodium phosphate} 4,5-bis(disodium phosphate) 2

Affi-Gel 10 (4 cm^3 slurry, 60 μmol) was filtered and washed with ice-cold water (10 cm^3). The wet gel slurry was transferred to a stirred solution containing the amine (+)-**4** (10.5 mg, 10.1 μmol) and $NaHCO_3$ (8.5 mg, 0.10 mmol) in water (15 cm^3). The reaction mixture was stirred for 18 h at 0 °C. The reaction mixture was filtered and washed with ice-cold water (15 cm^3). The gel was stored as a slurry in water (3 cm^3) at 4–6 °C. All the washings were combined and *myo*-inositol orthoformate **13** (1.9 mg, 9.9 μmol) was added. The solvent was removed by lyophilisation to give a solid mixture which was taken up in D_2O and analysed by 500 MHz NMR analysis. This revealed that 3.1 μmol of the amine (+)-**4** went onto the gel, affording a loading of 7.1%.

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