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A synthesis of dioctanoyl phosphatidylinositol

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ABSTRACT

A synthesis of the naturally occurring enantiomer of phosphatidylinositol is reported. A resolution strategy, using camphor as a chiral auxiliary is employed to obtain the desired, enantiomerically pure, inositol derivative. Dioctanoyl lipid chains are appended to the molecule, which are shorter than the naturally occurring lipid chains, providing the molecule with enhanced water solubility.

1. Introduction

Phosphatidylinositol phosphates (PtdInsP_ns) are important phospholipid components of plant, animal and bacterial cell membranes. These molecules play a central role in cellular signalling[1–3] and act by recruiting their effector proteins to the cell membrane through interaction with selective inositol phosphate-binding domains.[4] The central intracellular role of the PtdInsP_ns is responsible for their involvement in a number of disease related processes, including insulin signalling and pathways involved in several types of cancers.[5,6]

Phosphatidylinositol (PtdIns, **1**, Fig. 1) is one of the main acidic phospholipid components of (for example) erythrocyte cells, comprising approximately 1% of all lipids, by weight.[4] It was first isolated from the brain[7] and subsequently, in a more pure form, from soy beans.[8] The structure of PtdIns was unambiguously assigned by degradation and synthetic studies by Ballou and Pizer.[9] However, it is only more recently that the true significance of the PtdInsP_ns in cellular signalling has been appreciated. PtdIns is biosynthesised predominantly in the endoplasmic reticulum and is then delivered to other membranes.[10] Perhaps the most significant role of PtdIns is as the biosynthetic precursor to monophosphorylated phospholipids, phosphatidylinositol 3-phosphate (PtdIns3*P*), phosphatidylinositol 4-phosphate (PtdIns4*P*) and phosphatidylinositol 5-phosphate (PtdIns5*P*),[10–12] which are in turn transformed into the multiply phosphorylated phosphatidylinositols. PtdIns itself has been implicated as playing a role in bipolar disorder,[10] and is indirectly involved in many other aspects of cellular function and dysfunction through its biochemical relationship with the other PtdInsP_ns.

The important biological action of the PtdInsP_ns, coupled with the densely functionalised nature of their structure has led to significant interest in their synthesis.[12,13] In addition to the naturally occurring compounds, a number of unnatural derivatives, which are useful chemical probes, have been developed.[12–14] The important biological activity of PtdIns, coupled with our interest in the synthesis of inositol-based molecular probes,[15–17] phospholipid analogues[18] and PtdInsP_ns derivatives[19] has prompted us to develop a synthesis of PtdIns.[19–26] We have a particular interest in obtaining the dioctanoyl PtdIns derivative that has enhanced water solubility, which is advantageous for use in biological assays and crystallisation studies for X-ray analysis.[27]

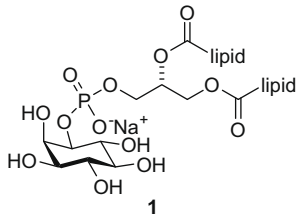

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Figure 1. The general structure of phosphatidylinositol (PtdIns, **1**). The constitution of the lipid chains varies depending on the source of the lipid.

2. Results and discussion

The synthesis commenced from *myo*-inositol, which is a cheap and readily available starting material. Using a well-documented series of transformations, we obtained the chiral, non-racemic camphor derivative **2** in seven steps.[19,28–31] The hydroxyl group at the D-1-position of the ring was protected as the TIPS ether (**3**, Scheme 1). Other syntheses have employed PMB ethers as the protecting group at this position, but we have found that the TIPS group, which has been relatively under utilised in inositol chemistry, can be added and deprotected in high yields, often in the presence of protected phosphate groups. Removal of the camphor chiral auxiliary and the allyl group was effected simultaneously

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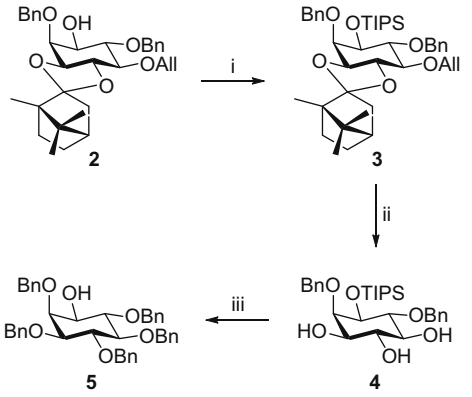
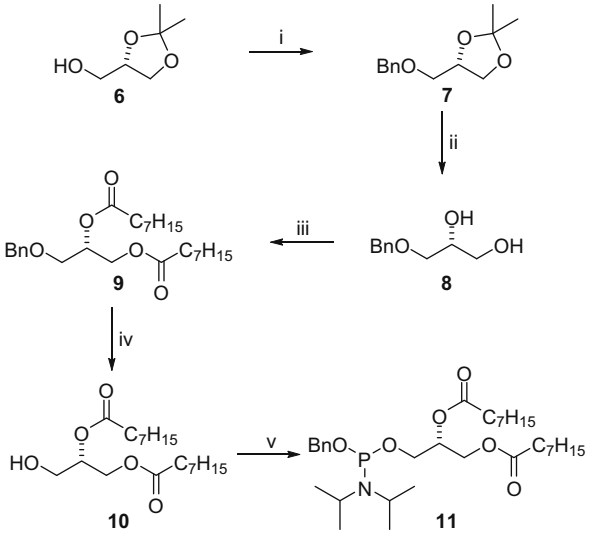


Scheme 1. The synthesis of the inositol fragment **5**. Reagents and conditions: (i) TIPSOTf, Et_3N, CH_2Cl_2, rt, 87%; (ii) $PdCl_2$, MeOH, rt, 81%; (iii) (a) BnBr, NaH, DMF, 0 °C→rt, 68%; (b) TBAF, THF, rt, 88%.

by treatment with $PdCl_2$ in methanol, affording **4**. There are several possible mechanisms for the allyl group deprotection. Some reports suggest that $PdCl_2$ will undergo an anti-Markovnikov methoxypalladation followed by β-alkoxy cleavage, furnishing the desired hydroxyl group.[32] Alternatively, it is reasonable to suggest that the $PdCl_2$ isomerises the allyl group to the corresponding enol ether and that a low concentration of HCl is generated, which catalyses methanolysis of the enol ether. The camphor acetal could be cleaved by either the Lewis acid action of $PdCl_2$, or the low concentration of HCl in methanol, or both. We had previously employed Wilkinson's catalyst to isomerise the allyl group to the corresponding enol ether, followed by treatment with acetyl chloride in methanol, to cleave the enol ether and the camphor acetal. The latter conditions provided a yield of only 37%, compared to that of 81% when using $PdCl_2$ in methanol. Benzylation of the 3-, 4- and 5-position hydroxyl groups was achieved using standard benzylation conditions, in a 68% yield. This moderate yield can likely be attributed to the unfavourable steric demands of benzylating on three contiguous hydroxyl groups. In addition, it is unlikely that three alkoxide anions will form simultaneously on the same molecule, due to the disfavoured anionic interactions. The TIPS ether was removed by treatment with TBAF in THF at room temperature, to give an 88% yield of the inositol fragment **5**.



Scheme 2. The synthesis of the lipid amidite fragment **x**. Reagents and conditions: (i) NaH, BnBr, DMF, rt, 92%; (ii) concd HCl, MeOH, reflux, 96%; (iii) DMAP, pyridine, octanoyl chloride, rt, 92%; (iv) $Pd(OH)_2$, H_2, THF, rt, 87%; (v) benzyloxybis(*N*,*N*-diisopropylamino)phosphine, 1*H*-tetrazole, CH_2Cl_2, rt, 71%.

The lipid fragment **11** was synthesised in a manner similar to that reported previously.[18,19,28–30,33–35] The synthesis commenced with benzyl protection of (+)-1,2-*O*-isopropylidene glycerol **6**, furnishing **7**, followed by an acid-catalysed methanolysis of the isopropylidene acetal (Scheme 2), to give the diol **8**. DMAP-catalysed coupling of the free hydroxyl groups with octanoyl chloride installed the lipid chains of **9**. Hydrogenolysis, mediated by Pearlman's catalyst, effected debenzylation of the lipid **9**, yielding **10**. The amidite intermediate **11** was obtained by treatment of the alcohol **10** with benzyloxybis(*N*,*N*-diisopropylamino)phosphine. The amidite **11** was used immediately after preparation, in order to minimise degradation.

With both fragment **5** and fragment **11** in hand, we proceeded to couple them (Scheme 3). Treatment of the inositol fragment **5** with the lipid phosphoramidite **11**, afforded a presumed P(III) intermediate, which was not isolated, but oxidised directly using *m*CPBA to afford the fully protected PtdIns precursor **12**. Palladium black-catalysed hydrogenolysis of **12** afforded PtdIns in moderate yield. The inclusion of $NaHCO_3$ led to PtdIns **1** being isolated as its sodium salt, which exists as a colourless solid after lyophilisation.

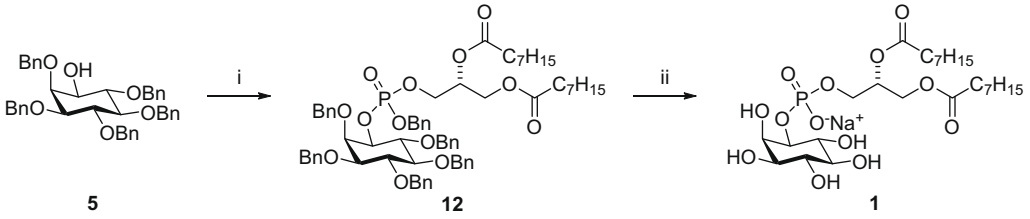
3. Conclusion

In summary, we report a concise and robust synthesis of the dioctanoyl derivative of PtdIns. We have demonstrated that the use of a TIPS ether is a viable protecting group strategy in the synthesis of inositol derivatives.

4. Experimental

4.1. General experimental details

^{1}H NMR spectra were recorded on Bruker DPX250 (250 MHz); Bruker Avance 300 (300 MHz); Bruker Avance 400 (400 MHz); Bruker Avance II 400 (400 MHz); Bruker DRX500 (500 MHz); Bruker Avance 500 (500 MHz); or Bruker Avance III (500 MHz) using deuterochloroform (unless indicated otherwise) as a reference for internal deuterium lock. The chemical shift data for each signal are given as δ in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ_{TMS} = 0.00 ppm. The multiplicity of each signal is indicated by: s (singlet); d (doublet); dd (doublet of doublets); dt (doublet of triplets); ddd (doublet of doublet of doublets); dddd (doublet of doublet of doublet of doublets); and m (multiplet). The number of protons (*n*) for a given resonance signal is indicated by *n*H. Coupling constants (*J*) are quoted in hertz and are recorded to the nearest 0.1 Hz. Identical proton coupling constants (*J*) are averaged in each spectrum and reported to the nearest 0.1 Hz. The coupling constants are determined by analysis using Bruker TopSpin software. ^{13}C NMR spectra were recorded on Bruker Avance 300 (75 MHz) or Bruker Avance III (125 MHz) spectrometers using the PENDANT or DEPT Q pulse sequences with broadband proton decoupling and internal deuterium lock. The chemical shift data for each signal are given as δ in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ_{TMS} = 0.00 ppm. Where appropriate, coupling constants (*J*) are quoted in hertz and are recorded to the nearest 0.1 Hz. ^{1}H and ^{13}C spectra were assigned using 2D NMR experiments including COSY, HSQC, HMBC, DEPT-135, HMQC and DEPT Q. ^{31}P NMR spectra were recorded on a Bruker Avance 300 (121 MHz) using broadband proton decoupling pulse sequences and deuterium internal lock. The chemical shift data for each signal are given as δ in units of parts per million (ppm) relative to 85% phosphoric acid as an external reference. Mass spectra were acquired using electrospray ionisation on Micromass LCT; Micromass LCT Premier; and Bruker MicroTOF spectrometers, operating in positive or negative mode,



Scheme 3. The synthesis of PtdIns **1** by coupling of fragment **5** with fragment **11** and subsequent hydrogenolysis. Reagents and conditions: (i) (a) **11**, 1*H*-tetrazole, CH_2Cl_2, rt; (b) *m*CPBA, −78 °C→rt, 52%; (ii) Pd black, H_2, $NaHCO_3$, ^tBuOH, H_2O, rt, 64%.

from solutions of methanol, acetonitrile or water. Certain samples were submitted to the National Mass Spectrometry Centre, Swansea. *m/z* Values are reported in Daltons and followed by their percentage abundance in parentheses. Microanalyses were obtained on a Carlo Erber EA1110 analyser by the St Andrews University microanalysis service. Certain samples were submitted to the Elemental Analysis Service, London Metropolitan University, London. Melting points were determined using an Electrothermal 9100 melting point apparatus or Kofler hot stage microscope and are uncorrected. Infrared Spectra were obtained from a thin film on sodium chloride plates. The spectra were recorded on Perkin Elmer GX FT-IR or Bruker Tensor 27 spectrometers. Absorption maxima are reported in wavenumbers (cm^{-1}). Specific Optical Rotations were measured using Perkin Elmer Model 241 and 341 polarimeters, in cells with a path length of 1 dm. The light source was maintained at 589 nm. The concentration (*c*) is expressed in g/100 mL (equivalent to g/0.1 dm^3). Specific rotations are denoted $[\alpha]_D^T$ and are given in implied units of 10^{-1} deg cm^2 g^{-1} (*T* = ambient temperature in °C). Analytical thin layer chromatography (TLC) was carried out on Merck Silica Gel 60 F_{254} aluminium-supported thin layer chromatography sheets. Visualisation was by absorption of UV light (λ_{max} 254 or 365 nm), or thermal development after dipping in one of: (a) ethanolic solution of phosphomolybdic acid (PMA); or (b) aqueous solution of potassium permanganate. Flash Column chromatography was carried out on Apollo Scientific Ltd Silica Gel 40–63 micron or Merck Silica Gel 60 (240–400 mesh), eluting with solvents as supplied, under a positive pressure of compressed air (unless otherwise stated). Anhydrous solvents were obtained under the following conditions: dry acetonitrile was distiled from calcium hydride in a recycling still; dry *N*,*N*-dimethylformamide was purchased from SigmaAldrich UK in a SureSeal™ bottle and used without further purification or was distiled under reduced pressure from activated 4 Å molecular sieves and stored over 4 Å molecular sieves under an N_2 atmosphere; dry dimethyl sulfoxide was pre-dried over activated alumina, then distiled from calcium hydride and stored over activated 4 Å molecular sieves under an N_2 atmosphere; anhydrous 1,4-dioxane was distiled from sodium and benzophenone in a recycling still and stored over activated 3 Å molecular sieves under an Ar atmosphere. Anhydrous dichloromethane, diethyl ether, toluene, hexane and tetrahydrofuran were obtained using a MBRAUN GmbH MB SPS-800 solvent purification system, where solvent was dried by passage through filter columns and dispensed under an atmosphere of N_2 or Ar gas. Chemicals were purchased from Acros UK, Sigma Aldrich UK, Alfa Aesar UK, Fisher UK, Fluka UK, Fluorochem, Merck or TCI-Europe. Compound **11**[35] was synthesised using methods similar to those previously reported.[18,19,28–30,33–35] All solvents and reagents were purified, when necessary, by standard techniques. Where appropriate and if not stated otherwise, all non aqueous reactions were performed in a flame-dried flask under an inert atmosphere of nitrogen or argon, using a double vacuum manifold with the inert gas passing through a bed of activated 4 Å molecular sieves and self indicating silica gel. Brine refers to a saturated aqueous solution of sodium chloride. Hexane refers to a mixture of hexane isomers and petroleum ether to the fraction boiling between 40 and 60 °C.

4.2. (−)-1D-5-*O*-Allyl-2,6-di-*O*-benzyl-1-*O*-triisopropylsilyl-3-*O*-*exo*-(l-1′,7′,7′-trimethylbicyclo[2.2.1]hept-2′-ylidine)-*myo*-inositol 3

To a stirred solution of triisopropylsilane trifluoromethanesulfonate (2.26 g, 1.66 mL, 7.98 mmol) in dry CH_2Cl_2 (3 mL) under an atmosphere of nitrogen, was added triethylamine (943.8 mg, 1.3 mL, 9.35 mmol). The solution was allowed to stir for 1 h at rt, turning to a dark orange colour. To this solution was added a solution of (−)-1D-5-*O*-allyl-2,6-di-*O*-benzyl-3-*O*-*exo*-(l-1′,7′,7′-trimethylbicyclo[2.2.1]hept-2′-ylidine)-*myo*-inositol **2** (3.0 g, 5.61 mmol) in dry CH_2Cl_2 (30 mL). The reaction mixture was stirred at rt overnight. After this time TLC analysis indicated that the reaction was complete, so the reaction mixture was diluted with CH_2Cl_2 (20 mL) and water (50 mL) was added. The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were dried over magnesium sulfate, filtered and the filtrate was concentrated under vacuum to yield a dark brown oil. The oil was adsorbed onto silica gel and purified by silica gel column chromatography eluting with diethyl ether and petroleum ether (2:98) to afford (−)-*1D-5-O-allyl-2,6-di-O-benzyl-1-O-triisopropylsilyl-3-O-exo-(l-1′,7′,7′-trimethylbicyclo[2.2.1]hept-2′-ylidine)-myo-inositol* **3** (1.12 g, 87%) as a colourless oil: R_f 0.51 (ethyl acetate/hexane 10:90); $[\alpha]_D^{25} = -1.5$ (*c* 1.23, $CHCl_3$); ν_{max} (NaCl plates)/cm^{-1} 2944 (s), 2867 (s), 2363 (w), 1493 (w), 1454 (m), 1390 (m), 1369 (w), 1309 (w), 1246 (w), 1202 (w), 1182 (m), 1114 (s), 1068 (s), 922 (w), 883 (m), 820 (m), 730 (m), 696 (m), 681 (w); ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.38–7.12 (10H, m, Ar*H*), 5.81 (1H, dddd, *J* 17.2, 10.4, 5.5, 5.5, CH_X=CH_YH$_Z$), 5.17 (1H, dd, *J* 17.2, 1.5, CH$_X$=CH_YH$_Z$), 5.03 (1H, d, *J* 10.4, CH$_X$=CH$_Y$*H*$_Z$), 4.92 (1H, d, *J* 11.5, OCH_AH$_B$-Ph), 4.85 (1H, d, *J* 11.1, OC$H_{A'}$H$_{B'}$-Ph), 4.68 (1H, d, *J* 11.1, OCH$_{A'}$*H*$_{B'}$-Ph), 4.63 (1H, d, *J* 11.5, OCH$_A$*H*$_B$-Ph), 4.27 (1H, dd, *J* 12.7, 5.5, OCH_V*H*$_W$–CH=CH$_2$), 4.10 (1H, dd, *J* 2.7, 1.5, H-2) 4.03 (1H, dd, *J* 12.7, 5.5, OCH_V*H*$_W$–CH=CH$_2$), 3.94 (1H, dd, *J* 9.7, 9.7, H-6), 3.83 (1H, dd, *J* 9.0, 2.7, H-3), 3.67 (1H, dd, *J* 9.0, 9.0, H-4), 3.44 (1H, dd, *J* 9.7, 9.0, H-5), 3.21 (1H, dd, *J* 9.7, 1.5, H-1), 2.07 (1H, dt, *J* 13.4, 3.8, camphor ring), 1.89–1.77 (1H, m, camphor ring), 1.72–1.58 (2H, m, camphor ring), 1.43–1.36 (1H, m, camphor ring), 1.36–1.25 (1H, m, camphor ring), 1.18–1.09 (1H, m, camphor ring), 0.95–0.93 (21H, m, Si-[C*H*(CH_3)$_2$]$_3$), 0.93 (3H, s, C*H*-camphor ring), 0.77 (3H, s, CH_3-camphor bridge), 0.73 (3H, s, CH_3-camphor bridge); ^{13}C NMR (75 MHz; $CDCl_3$) δ 139.9, 139.3, 135.8 (*C*H=CH$_2$), 128.5, 128.4, 127.9, 127.8, 127.7, 127.4, 120.7 (ketal carbon), 116.9 (CH=*C*H$_2$), 83.7 (inositol ring), 81.9 (inositol ring), 77.9 (inositol ring), 77.2 (inositol ring), 76.4 (inositol ring), 76.3 (*C*H$_2$), 75.3 (inositol ring), 74.2 (*C*H$_2$), 72.1 (*C*H$_2$), 53.3 (*C*$_q$-camphor ring), 48.7 (*C*$_q$-camphor ring), 46.7 (*C*H$_2$), 45.3 (*C*H), 29.3 (*C*H$_2$), 27.1 (*C*H$_2$), 20.7 (*C*H$_3$), 20.5 (*C*H$_3$), 18.6 (*C*H$_3$), 13.3 (*C*H$_3$), 10.0 (*C*H); HRMS *m/z* (ES$^+$) [Found: (M+Na)$^+$ 713.4209. $C_{42}H_{62}O_6SiNa^+$ requires M$^+$, 713.4213]; *m/z* (ES$^+$) 713 ([M+Na]$^+$, 100%); Anal. Calcd for $C_{42}H_{62}O_6Si$: C, 73.0; H, 9.0. Found: C, 72.7; H, 9.3.

4.3. (+)-2,6-Bis-*O*-benzyl-1-*O*-triisopropylsilyl-*myo*-inositol 4

(−)-1D-5-*O*-Allyl-2,6-di-*O*-benzyl-1-*O*-triisopropylsilyl-3-*O*-*exo*-(l-1',7',7'-trimethylbicyclo[2.2.1]hept-2'-ylidine)-*myo*-inositol **3** (700 mg, 1.01 mmol) was dissolved in methanol (70 mL), to this (under an atmosphere of nitrogen) was added $PdCl_2$ (179 mg, 1.01 mmol). The reaction was left to stir at rt for 2 h before the palladium residues were removed by filtration through Celite®. The filtrate was concentrated under vacuum and the residue partitioned between CH_2Cl_2 (50 mL) and a solution of H_2O_2 (15% w/w, 60 mL). The layers were stirred vigorously together for 30 min before being separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$, brine, then dried over magnesium sulfate and filtered. The filtrate was concentrated under vacuum and the residue purified by silica gel column chromatography, eluting with methanol and chloroform (1:99, 2:98 then 3:97), affording (−)-*2,6-bis-O-benzyl-1-O-triisopropylsilyl myo-inositol* **4** (426 mg, 81% yield) as a colourless gum: R_f 0.30 (ethyl acetate); $[\alpha]_D^{25} = +14.8$ (*c* 1.36, $CHCl_3$); ν_{max} (NaCl plates)/cm^{-1} 3405 (s), 2943 (s), 1455 (s), 1150 (s), 1061 (s); ^1H NMR (400 MHz; $CDCl_3$) δ 7.31–7.17 (10H, m, Ar*H*), 5.01 (1H, d, *J* 11.1, OCH_AH_B-Ph), 4.88 (1H, d, *J* 11.9, OCH_A·H_B-Ph), 4.67 (1H, d, *J* 11.1, OCH$_A$$H_B$-Ph), 4.66 (1H, d, *J* 11.9, OCH$_A$·H_B-Ph), 3.83 (1H, dd, *J* 2.4, 2.4, H-2), 3.83 (1H, dd, *J* 9.1, 2.4, H-1), 3.71 (1H, dd, *J* 9.1, 9.1, H-6), 3.68 (1H, ddd, *J* 9.1, 9.1, 2.4, H-4), 3.42 (1H, ddd, *J* 9.1, 7.8, 2.4, H-3), 3.30 (1H, ddd, *J* 9.1, 9.1, 2.4, H-5), 2.49 (1H, d, *J* 2.4, 4-O*H*), 2.30 (1H, d, *J* 2.4, 5-O*H*), 2.26 (1H, d, *J* 7.8, 3-O*H*), 1.20–1.06 (21H, m, Si–[C*H*(CH_3)$_2$]$_3$); ^{13}C NMR (75 MHz; $CDCl_3$) δ 139.3, 139.2, 128.82, 128.8, 128.1, 128.0, 127.8, 82.2, 81.7, 75.8, 75.5, 75.3, 74.9, 74.4, 72.7, 18.7, 13.2; HRMS *m/z* (ES^+) [Found: $(M+H)^+$ 517.2978. $C_{29}H_{45}O_6Si^+$ requires M^+, 517.2980]; *m/z* (ES^+) 575 ([M+NH_4·MeCN]$^+$, 100%); *m/z* (ES^-) 515 ([M−H]$^-$, 100%), 575 ([M+OAc]$^-$, 80%); Anal. Calcd for $C_{29}H_{44}O_6Si$: C, 67.4; H, 8.6. Found: C, 67.3; H, 8.5.

4.4. (+)-2,3,4,5,6-Penta-*O*-benzyl-1-*O*-triisopropylsilyl *myo*-inositol

(+)-2,6-Bis-*O*-benzyl-1-*O*-triisopropylsilyl *myo*-inositol **4** (128 mg, 0.25 mmol) was dissolved in dry DMF (10 mL) under an atmosphere of nitrogen and then cooled to 0 °C. With vigorous stirring, sodium hydride (60% dispersion w/w in mineral oil, 40 mg, 0.99 mmol) was added. The resulting slurry was allowed to warm to rt and stirred for a further 2 h. The slurry was cooled to 0 °C and benzyl bromide (170 mg, 117 μL, 0.99 mmol) was added with vigorous stirring. The reaction mixture was warmed to rt and stirred overnight. The reaction was judged to be incomplete by TLC analysis. Therefore, the reaction mixture was cooled to 0 °C and an additional sodium hydride (60% dispersion w/w in mineral oil, 40 mg, 0.99 mmol) and benzyl bromide (170 mg, 117 μL, 0.99 mmol) were added and stirred at rt for 5 h. The sodium hydride was quenched with the addition of water (2 mL). The volatile components were removed under vacuum and the residue was partitioned between ethyl acetate (20 mL) and water (20 mL). The layers were separated and the aqueous phase was extracted with ethyl acetate (3 × 15 mL). The combined organic components were dried over magnesium sulfate, filtered and the filtrate concentrated under vacuum to yield an orange oil. The oil was adsorbed onto silica gel and purified by silica gel column chromatography, eluting with ethyl acetate and hexane (1:99, 2:98 then 3:97) giving (−)-*2,3,4,5,6-penta-O-benzyl-1-O-triisopropylsilyl myo-inositol* (77 mg, 68% yield) as a colourless oil: R_f 0.40 (petroleum ether/diethyl ether 90:10); $[\alpha]_D^{25} = +1.8$ (*c* 1.00, $CHCl_3$); ν_{max} (NaCl plates)/cm^{-1} 3089 (w), 3064 (w), 3030 (m), 2925 (s), 2866 (s), 2361 (m), 2342 (m), 1947 (w), 1869 (w), 1808 (w), 1734 (w); ^1H NMR (300 MHz; $CDCl_3$) δ 7.38–7.05 (25H, m, Ar*H*), 4.91–4.57 (10H, m, 5 × OCH_AH_B-Ph), 3.99 (1H, dd, *J* 9.5, 9.5, H-4), 3.87 (1H, dd, *J* 9.5, 9.5, H-6), 3.83 (1H, dd, *J* 2.0, 2.0, H-2), 3.64 (1H, dd, *J* 9.5, 2.0, H-1), 3.38 (1H, dd, *J* 9.5, 9.5, H-5), 3.34 (1H, dd, *J* 9.5, 2.0, H-3), 0.99–0.93 (21H, m, Si–[C*H*(CH_3)$_2$]$_3$); ^{13}C NMR (100 MHz; $CDCl_3$) δ 139.4, 139.3, 139.0, 138.7, 138.4, 128.4, 128.3, 128.2, 128.1, 128.0, 127.97, 127.8, 127.79, 127.7, 127.5, 127.4, 127.3, 127.2, 127.1, 126.9, 84.2, 82.1, 81.9, 80.9, 79.9, 75.9 (*C*H_2), 75.7 (*C*H_2), 75.4 (*C*H_2), 74.6 (*C*H_2), 74.0, 72.9 (*C*H_2), 18.2 (Si–[*C*H(CH_3)$_2$]$_3$), 18.18 (Si–[*C*H(CH_3)$_2$]$_3$), 12.8 (Si–[CH(*C*H_3)$_2$]$_3$); HRMS *m/z* (ES^+) [Found: $(M+Na)^+$ 809.4204. $C_{50}H_{62}O_6SiNa$ requires M^+, 809.4208]; *m/z* (ES^+) 804 ([M+NH_4]$^+$, 45%), 809 ([M+Na]$^+$, 50%), 845 ([M+NH_4·MeCN]$^+$, 100%); Anal. Calcd for $C_{50}H_{62}O_6Si$: C, 76.3, H, 7.9; Found: C, 76.4, H, 7.9.

4.5. (−)-2,3,4,5,6-Penta-*O*-benzyl *myo*-inositol 5

(−)-2,3,4,5,6-Penta-*O*-benzyl-1-*O*-triisopropylsilyl *myo*-inositol (136 mg, 0.17 mmol) was dissolved in THF (15 mL). To this solution was added TBAF (1 M solution in THF, 190 μL, 0.19 mmol), the mixture was stirred at rt and monitored by TLC analysis. After 3 h the reaction was adjudged to be complete by TLC analysis and was diluted with diethyl ether (40 mL) and water (40 mL). The layers were separated and the aqueous phase was further extracted with diethyl ether (3 × 20 mL). The combined organic layers were dried over magnesium sulfate, filtered and the filtrate concentrated under vacuum. The residue was purified by silica gel column chromatography eluting with ethyl acetate and petroleum ether (20:80 then 30:70) furnishing (−)-2,3,4,5,6-penta-*O*-benzyl *myo*-inositol **5** (96 mg, 88% yield) as a colourless gum: R_f 0.46 (petroleum ether/ethyl acetate 70:30); $[\alpha]_D^{25} = -10.9$ (*c* 0.5, $CHCl_3$) [lit.[29] $[\alpha]_D^{18} = -10.0$ (*c* 2.3, $CHCl_3$)]; ^1H NMR (300 MHz; $CDCl_3$) δ 7.42–7.27 (25H, m, Ar*H*), 5.06–4.70 (10H, m, 5 × OCH_AH_B-Ph), 4.09 (1H, dd, *J* 9.5, 9.5, inositol ring), 4.06 (1H, dd, *J* 2.5, 2.5, inositol ring), 3.84 (1H, dd, *J* 9.5, 9.5, inositol ring), 3.56–3.46 (3H, m, inositol ring), 2.22 (1H, d, *J* 6.3, OH); *m/z* (ES^+) 648 ([M+NH_4]$^+$, 30%), 653 ([M+Na]$^+$, 100%), 732 ([M+$HNEt_3$]$^+$, 25%). These data are in agreement with the literature values.[29,36]

4.6. (+)-1D-1-(1,2-Dioctanoyl-*sn*-glycerol-3-phospho)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol 12

To a stirred solution of phosphoramidite **11** (120 mg, 0.206 mmol) in dry CH_2Cl_2 (5 mL) under an atmosphere of nitrogen was added 1*H*-tetrazole (0.43 M solution in acetonitrile, 479 μL, 206 μmol). This mixture was stirred at rt for 10 min before the addition of (−)-2,3,4,5,6-penta-*O*-benzyl *myo*-inositol **5** (65 mg, 103 μmol) as a solution in CH_2Cl_2 (2 mL). The reaction solution was stirred at rt overnight. TLC analysis indicated that further equivalents of phosphoramidite **11** (120 mg, 0.206 mmol) were required and the reaction was stirred at rt for another 3 h before the addition of more phosphoramidite **11** (120 mg, 0.206 mmol). After stirring for a further 2 h the reaction mixture was cooled to −78 °C and *m*CPBA (60% purity, 178 mg, 618 μmol) added. The reaction solution was stirred at rt for 20 min before being partitioned between sodium hydrogen sulfite (10% aq solution, 15 mL) and an additional CH_2Cl_2 (5 mL), the layers were separated and the aqueous layer was further extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with a saturated solution of $NaHCO_3$, and then dried over magnesium sulfate and filtered. The filtrate was concentrated under vacuum and the residue purified by silica gel column chromatography eluting with ethyl acetate and hexane (20:80 then 40:60) to give (+)-*1D-1-(1,2-dioctanoyl-sn-glycerol-3-phospho)-2,3,4,5,6-penta-O-benzyl-myo-inositol* **12** (60 mg, 52% yield) as a colourless oil: R_f 0.66 (ethyl acetate/petroleum ether 60:40); $[\alpha]_D^{20} = +3.2$ (*c* 0.79, $CHCl_3$) as a colourless oil: ν_{max} (NaCl plates)/cm^{-1} 3090 (s), 3064 (s), 2954 (s), 2894 (s), 1956

(w), 1885 (w), 1721 (s), 1313 (s), 1008 (s); ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.34–7.11 (30H, m, Ar*H*), 5.06–4.54 (13H, m, 6 × OCH_AH_B-Ph + glycerol C*H*), 4.28–4.24 (1H, m, inositol ring), 4.22–3.77 (7H, m, 3 × inositol ring + 4 × glycerol CH_2), 3.47–3.34 (2H, m, 2 × inositol ring), 2.26–2.06 (4H, m, 2 × αCOCH_2), 1.58–1.39 (4H, m, 2 × βCOCH$_2$CH_2), 1.27–1.10 (16H, m, octanoyl CH_2), 0.85–0.75 (6H, m, 2 × CH_3); ^{13}C NMR (125 Hz; $CDCl_3$) δ 128.6, 128.5, 128.34, 128.3, 128.27, 128.2, 128.19, 128.15, 128.0, 127.8, 127.77, 127.66, 127.6, 127.57, 127.5, 127.47, 127.4, 83.1, 81.2, 80.4, 79.9, 78.6, 76.3, 76.0 (*C*H$_2$), 75.9 (*C*H$_2$), 75.5 (*C*H$_2$), 75.0 (*C*H$_2$), 72.7 (*C*H$_2$), 69.4 (*C*H$_2$), 69.0 (*C*H glycerol), 65.5 (CH$_2$ glycerol), 61.5 (*C*H$_2$ glycerol), 34.1 (αCOCH$_2$), 33.9 (αCOCH_2), 31.6 (*C*H$_2$), 29.0 (*C*H$_2$), 29.0 (*C*H$_2$), 28.9 (*C*H$_2$), 24.7 βCOCH$_2$CH_2), 22.5 (*C*H$_2$), 14.0 (*C*H$_3$); ^{31}P NMR (121 MHz; $CDCl_3$) δ 1.72, 1.69; HRMS *m/z* (ES$^+$) [Found: (M+NH$_4$)$^+$ 1144.5918. $C_{67}H_{87}O_{13}NP^+$ requires M$^+$, 1144.5910]; *m/z* (ES$^+$) 1149 ([M+Na]$^+$, 100%).

4.7. (+)-1D-Dioctanoyl phosphatidylinositol 1

To a stirred solution of (+)-1D-1-(1,2-dioctanoyl-*sn*-glycerol-3-phospho)-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol **12** (23 mg, 20 μmol) in ^{t}BuOH (8 mL) and H_2O (1.5 mL) was added Pd black (39 mg, 367 μmol) and $NaHCO_3$ (2.5 mg, 33 μmol). The mixture was stirred under H_2 (1 atm) at rt for 24 h. The mixture was then filtered through Celite® and the solid was washed with H_2O. The filtrate was lyophilised to afford (+)-1D-dioctanoyl phosphatidylinositol **1** (8 mg, 64% yield) as a colourless solid: $[\alpha]_D^{25} = +2.25$ (*c* 0.20, H_2O, pH 10) [lit.[20] $[\alpha]_D$ = +2.8 (*c* 1.0, H_2O, pH 9]; ^{1}H NMR (500 MHz; D_2O) δ 5.29–5.20 (1H, m, glycerol C*H*), 4.37 (1H, d, *J* 11.7, glycerol CH_XH_Y-Oct), 4.24–4.12 (2H, m, glycerol CH$_X$*H*$_Y$-Oct + inositol ring, H-2), 4.06–3.95 (2H, m, glycerol POC$H_{X'}H_{Y'}$), 3.89 (1H, ddd, *J* 9.6, 1.9, $^3J_{HP}$ 9.6, inositol ring, H-1), 3.68 (1H, dd, *J* 9.6, 9.6, inositol ring, H-6), 3.58 (1H, dd, *J* 9.6, 9.6, inositol ring, H-4), 3.48 (1H, dd, *J* 9.6, 1.9, inositol ring, H-3), 3.27 (1H, dd, *J* 9.6, 9.6, inositol ring, H-5), 2.42–2.18 (4H, m, 2 × αCOCH_2), 1.64–1.44 (4H, m, 2 × βCOCH$_2$CH_2), 1.30–1.07 (16H, m, octanoyl chain, CH_2), 0.83–0.76 (6H, m, 2 × octanoyl CH_3); ^{13}C NMR (125 MHz; D_2O) δ 174.6 (*C*=O), 174.5 (*C*=O), 76.2 (d, $^2J_{CP}$ 5.6, inositol ring, C-1), 73.9 (inositol ring, C-5), 72.2 (inositol ring, C-4), 71.4 (inositol ring, C-6), 71.2 (inositol ring, C-2), 70.8–70.6 (m, glycerol POCH$_2$ + inositol ring), 63.8 (d, $^2J_{CP}$ 3.8, glycerol POCH_2), 63.2 (glycerol OCH$_2$-Oct), 34.1 (αCOCH$_2$) 34.0 (αCOCH_2), 31.8 (octanoyl chain, CH_2), 29.1 (octanoyl chain, CH_2), 29.09 (octanoyl chain, CH_2), 29.0 (octanoyl chain, CH_2), 28.9 (octanoyl chain, CH_2), 24.8 (βCOCH$_2$CH$_2$), 24.7 (βCOCH$_2$CH$_2$) 22.6 (octanoyl chain, CH_2), 22.5 (octanoyl chain, CH_2), 13.8 (octanoyl chain, CH_3), 13.7 (octanoyl chain, CH_3); ^{31}P NMR (121 MHz; D_2O) δ −0.84; HRMS *m/z* (ES$^-$) [Found: (M−Na)$^-$ 585.2690. $C_{25}H_{46}O_{13}P_1$ requires M$^-$, 585.2682]; *m/z* (ES$^-$) 585 ([M−Na]$^-$, 100%). These data are in good agreement with the literature values.[20,24]

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