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Synthesis of D- and L-*myo*-inositol 2,4,5-trisphosphate and trisphosphorothioate: structural analogues of D-*myo*-inositol 1,4,5-trisphosphate

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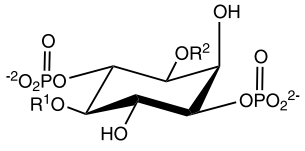
Abstract

The preparation of D- and L-*myo*-inositol 2,4,5-trisphosphate is described, together with the phosphorothioate counterparts. The known chiral diols D- and L-1,4-di-*O*-benzyl-5,6-bis-*O*-*p*-methoxybenzyl-*myo*-inositol were regioselectively protected at the 3-position using a benzyl group via a 2,3-*O*-stannylene acetal. Removal of the *p*-methoxybenzyl groups of each enantiomer gave D- and L-1,3,6-tri-*O*-benzyl-*myo*-inositol. Phosphitylation with bis(benzyloxy)diisoproplyaminophosphine and 1*H*-tetrazole gave the trisphosphite intermediate for each enantiomer. Oxidation with 3-chloroperoxybenzoic acid gave the fully protected D- and L-*myo*-inositol 2,4,5-trisphosphates. Sulphoxidation of the D- and L-2,4,5-trisphosphite intermediates gave the fully protected D- and L-*myo*-inositol 2,4,5-trisphosphorothioate compounds. The fully protected trisphosphates were deblocked using hydrogenolysis and the phosphorothioates were deprotected using sodium in liquid ammonia. The individual compounds were then purified using ion exchange chromatography to afford pure D- and L-*myo*-inositol 2,4,5-trisphosphates together with the corresponding phosphorothioates.

Keywords: Benzyl ether; D- and L-*myo*-Inositol 2,4,5-trisphosphate; D- and L-*myo*-Inositol 2,4,5-trisphosphorothioate; Chiral intermediates; Phosphitylation

1. Introduction

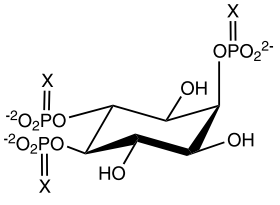
It is well established that D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **1**] is a second messenger.[1] This hydrophilic product interacts with specific receptors that are recognised as a family of tetrameric ligand-gated Ca^{2+} channels. When Ins(1,4,5)P_3, **1** interacts with a site on the N-terminal portion of its receptor, Ca^{2+} ions are released from intracellular stores. There are three fully characterised Ins(1,4,5)P_3 receptor subtypes (I, II and III) known to date. From the protein monomers, a tetrameric Ca^{2+} channel is assembled, to give homo- and heteromeric receptors. Most mammalian cells express a mixture of the Ins(1,4,5)P_3 receptor subtypes in a given cell line or tissue.[1,2]



$R^1 = PO_3^{2-}$; R^2 = H, Ins(1,4,5)P_3 **1**

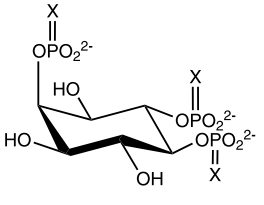
$R^1 = R^2$ = H; Ins(1,4)P_2 **2**

$R^1 = R^2 = PO_3^{2-}$; Ins(1,3,4,5)P_4 **3**



X = O; D-Ins(2,4,5)P_3 **4a**

X = S; D-Ins(2,4,5)PS_3 **5a**



X = O; L-Ins(2,4,5)P_3 **4b**

X = S; L-Ins(2,4,5)PS_3 **5b**

Racemic Ins(2,4,5)P_3 **4ab**

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Once Ca^{2+} ions have been released from the stores, the signal is then 'switched off' providing inactive or less active compounds. Deactivation of Ins(1,4,5)P_3 can occur by a specific 5-phosphatase enzyme, which removes the phosphate group at C-5 of Ins(1,4,5)P_3 to generate *myo*-inositol 1,4-bisphosphate [Ins(1,4)P_2, **2**] and turn the signal off. Second, Ins(1,4,5)P_3 may be phosphorylated by a specific 3-kinase, to afford *myo*-inositol 1,3,4,5-tetrakisphosphate, [Ins(1,3,4,5)P_4, **3**]. The role of the latter compound remains controversial; however, recent studies imply that it regulates the frequency of Ca^{2+} oscillations in vivo in conjunction with the 3-kinase.[3] Compound **3** is a potent bimodal regulator of cellular sensitivity[4] to Ins(1,4,5)P_3 and is the most potent 5-phosphatase inhibitor known, with an IC_{50} value of 0.15 μM.[4]

Since the first synthesis of D-Ins(1,4,5)P_3, numerous analogues have been synthesised and evaluated in structure–activity studies for their ability to mimic the action of the natural ligand.[5] One of our goals is to develop analogues based on Ins(1,4,5)P_3 to probe the polyphosphate pathway of signal transduction. We wanted to synthesise D-*myo*-inositol 2,4,5-trisphosphate [Ins(2,4,5)P_3, **4a**], a regioisomer of Ins(1,4,5)P_3, and its enantiomer, L-*myo*-inositol 2,4,5-trisphosphate [L-Ins(2,4,5)P_3, **4b**] together with their new phosphorothioate counterparts, D-*myo*-inositol 2,4,5-trisphosphorothioate [D-Ins(2,4,5)PS_3, **5a**] and L-*myo*-inositol 2,4,5-trisphosphorothioate [L-Ins(2,4,5)PS_3, **5b**]. Previously, when phosphorothioate groups replace phosphate moieties in inositol phosphate analogues,[5–8] they have provided compounds that are resistant to 5-phosphatase and 3-kinase and can display partial agonist activity when tested for Ca^{2+} release under specific experimental conditions.[6–8]

D-Ins(2,4,5)P_3 **4a** has been extracted from several tissues.[9–13] However, it is unclear whether **4a** is synthesised in vivo, or is due to migration of the phosphate group from position C-1-OH to C-2-OH under certain conditions. Ins(2,4,5)P_3 **4a** is an inositol polyphosphate regioisomer of Ins(1,4,5)P_3 **1** which has been the target of fewer synthetic approaches compared with the natural receptor ligand,[5] Ins(1,4,5)P_3. Racemic and chiral Ins(2,4,5)P_3, were made in the first few years of intense synthetic work which culminated in two syntheses[14,15] of chiral Ins(2,4,5)P_3. The synthesis by Tegge and co-workers[14] delivered both enantiomers. The D-enantiomer **4a** was derived from D-pinitol and the L-enantiomer **4b** from L-quebrachitol after several protecting and deprotecting steps. However, the starting materials required for the synthesis of **4a** and **4b** are expensive compared to *myo*-inositol. The first synthesis of D-Ins(2,4,5)P_3 **4a** was achieved by Watanabe and co-workers[15] who resolved a racemic *myo*-inositol derivative using a chiral auxillary then separated the two diastereoisomers using medium pressure silica gel chromatography. Gigg and co-workers[16] synthesised a fully protected D-*myo*-inositol 2,4,5-trisphosphate derivative that was only partially deblocked. Two syntheses of racemic Ins(2,4,5)P_3 have also appeared in the literature, using *myo*-inositol as the starting material,[17,18] and a novel route using benzene has also been published.[19]

Early biological studies by Putney Jr.[20] formally demonstrated that Ins(2,4,5)P_3 is a poor substrate for 3-kinase and a full agonist like Ins(1,4,5)P_3, albeit with a longer lifetime. Like Ins(1,4,5)P_3, **4a** can fully activate sustained Ca^{2+} entry as well as Ca^{2+} release in lacrimal acinar cells. Racemic *myo*-inositol 2,4,5-trisphosphate was evaluated for Ca^{2+} mobilisation and found to be 30-fold weaker than Ins(1,4,5)P_3 in some cell types.[11,13] Racemic Ins(2,4,5)P_3 has also been used as a metabolically resistant analogue[11] because it is only a weak substrate for Ins(1,4,5)P_3 5-phosphatase and a poor substrate for Ins(1,4,5)P_3 3-kinase.[11,21] In other studies, D-Ins(2,4,5)P_3 was 6-fold,[10] 13-fold[12] or 25-fold[14] less potent than Ins(1,4,5)P_3 for Ca^{2+} mobilisation. In experiments carried out by Tegge and co-workers, **4a** and **4b** were tested in saponin permeabilised rat basophilic leukemic (RBL) cells.[14] The RBL cells consist of type II and type III Ins(1,4,5)P_3 receptor subtypes and **4a** was found to be 25-fold less potent than Ins(1,4,5)P_3, while **4b** was 650-fold less active than Ins(1,4,5)P_3 for Ca^{2+} release. This is the only study where both D- and L-Ins(2,4,5)P_3 have been tested, albeit in one cell line. Racemic Ins(2,4,5)P_3 was tested for Ca^{2+} release in permeabilised rat hepatocytes[22] and found to be a partial agonist in these cells. Racemic Ins(2,4,5)P_3 released some 65% of the total Ca^{2+} compared to Ins(1,4,5)P_3. Chock and co-workers[3] used Ins(2,4,5)P_3 to stimulate Ca^{2+} release in HeLa cells. However, only Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 enhanced the frequency of Ca^{2+} oscillations, and this activity distinguished the natural ligand from the Ins(2,4,5)P_3 derivative. Ins(2,4,5)P_3 has also been used at submaximal levels in Ca^{2+} release experiments to show that pure synthetic Ins(1,3,4,5)P_4 does not release Ca^{2+} ions from intracellular stores.[4] These examples illustrate the importance of *myo*-inositol 2,4,5-trisphosphate as a powerful tool for investigating Ins(1,4,5)P_3 metabolism and Ca^{2+} signalling and our new route to both enantiomers of this compound and their phosphorothioate derivatives should enhance the potential of this important tool.

2. Results and discussion

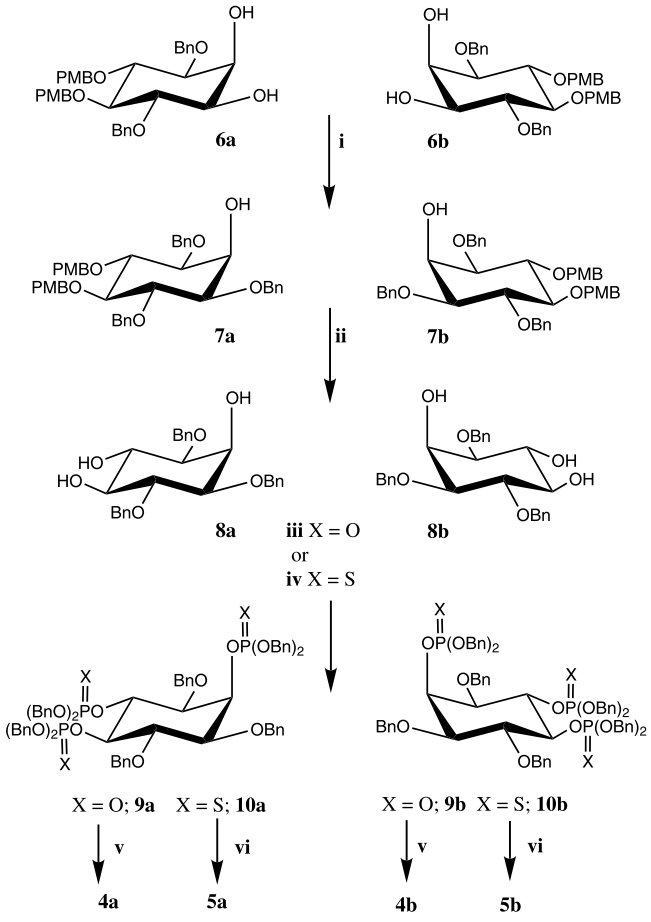
Previously,[23] we have described the synthesis of the chiral diols, D-1,4-di-*O*-benzyl-5,6-bis-*O*-*p*-methoxy-

benzyl-*myo*-inositol **6b**, and its L-enantiomer **6a**.[†] They were synthesised when the racemic 2,3-diol **6ab** was reacted with the chiral acid, (*S*)-(+)-*O*-acetylmandelic acid and the resulting diastereoisomers were separated by flash chromatography. The chiral auxillary was then removed by methanolysis to provide the individual diols **6a** and **6b** (Scheme 1). Selective tin-mediated benzylation[24] of **6a** and **6b** at the equatorial 3-hydroxy was accomplished via the *cis*-2,3-*O*-dibutylstannylene in the presence of caesium fluoride in *N*,*N*-dimethylformamide (DMF) to provide the intermediates **7a** and **7b** in 85 and 80% yield, respectively. The key 2,4,5-triol intermediates **8a** and **8b** were obtained by deprotection of the *p*-methoxybenzyl groups in a mixture of trifluoroacetic acid in CH_2Cl_2 (1:10) at room temperature for 30 min. Under these conditions, the benzyl groups remained intact to give a suitably protected 2,4,5-triol derivative. The two triols **8a** and **8b** have equal and opposite specific rotations (+20°) and (−20°), respectively, which were similar to the literature value[16] $[\alpha]_D$ +16.2° (for the D-enantiomer of 1,3,6-tri-*O*-benzyl-*myo*-inositol) and possessed the same liquid crystalline properties which have also been described by Gigg and co-workers.[16]

The phosphate groups were introduced by a P^{III} method followed by oxidation. Thus, a mixture of bis(benzyloxy)diisopropylaminophosphine[25] and 1*H*-tetrazole was stirred in CH_2Cl_2 at room temperature. A tetrazolide intermediate[23] (δ_P +127 ppm) was observed by ^{31}P NMR and the individual triols **8a** and **8b** were added in separate experiments. The intermediate trisphosphite was stirred for a short time and oxidised with *m*-chloroperoxybenzoic acid (MCPBA) at −78 °C to yield the fully protected phosphate triesters (**9a** and **9b**), after work-up and purification. Deprotection of the benzyl protective groups was accomplished by hydrogenolysis in the presence of 10% palladium on carbon as the catalyst, to afford the corresponding D- and L-2,4,5-trisphosphates (**4a** and **4b**), respectively. These compounds were further purified by ion exchange chromatography on Q Sepharose Fast Flow where the compounds eluted between 500 and 600 mmol dm^{-3} triethylammonium hydrogencarbonate (TEAB) buffer, and isolated as pure glassy triethylammonium salts and quantified by a modification of the Briggs phosphate analysis.[26]

The phosphorothioate counterparts **5a** and **5b** were synthesised in a similar way; however, the intermediate P^{III} trisphosphites were oxidised using sulphur in a mixed solvent of pyridine and DMF[7] to afford the fully blocked trisphosphorothioate triesters **10a** and **10b**. Treatment of **10a** or **10b** with sodium in liquid ammonia furnished the trisphosphorothioates **5a** and **5b**, which were then purified by ion exchange chromatography on Q Sepharose Fast Flow in a similar manner for **4a** and **4b**.

There is no doubt that the source and purity of D-Ins(2,4,5)P_3 plays a pivotal role in the quality of data arising from biological experiments. We have synthesised pure D-Ins(2,4,5)P_3 **4a** and the L-enantiomer **4b** from a racemic precursor, which was then resolved using a chiral auxillary and then transformed into the named target compounds. Furthermore, pure D-Ins(2,4,5)PS_3 **5a**, which has previously been unavailable, may release less Ca^{2+} than its phosphate counterpart, and behave as a low intrinsic activity partial agonist at the Ins(1,4,5)P_3 receptor[6–8] under specific experimental conditions. Synthetic Ins(2,4,5)P_3



Scheme 1. *Reagents and conditions*: (i) Dibutyltin oxide, $PhCH_3$, Dean–Stark apparatus; then CsF, BnBr, DMF (85% for **7a**, 80% for **7b**); (ii) CH_2Cl_2–trifluoroacetic acid (10:1), 30 min (85% for **8a**, 87% for **8b**); (iii) $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, CH_2Cl_2, rt, 15 min, then add triols **8a** or **8b** in separate experiments, 10 min, then MCPBA, 0 °C, 30 min (73% for **9a**, 83% for **9b**); (iv) $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, CH_2Cl_2, rt, triols **8a** or **8b** in separate experiments, then add S_8/DMF/pyridine, 15 min (90% for **10a**, 93% for **10b**); (v) H_2 10% Pd/C, 30 psi, (overnight), followed by purification by ion-exchange chromatography; (94% for **4a**, 51% for **4b**). (vi) Na/NH_3 (−78 °C), then purification by ion exchange chromatography (72% for **5a**, 70% for **5b**).

[†] After compounds **6a** and **6b** there is a switch of D- and L-nomenclature. Thus, D-→L- and L-→D-.

was tested in a preliminary fashion for its Ca^{2+} mobilising properties in a clam homogenate assay (A. Galione, S. J. Mills and B. V. L. Potter, unpublished data), where it was found to have potent activity, albeit around an order of magnitude weaker than Ins(1,4,5)P_3.

Recently, Ins(2,4,5)P_3 synthesised by the route described here, was used as a biological tool to investigate its effects in leech photoreceptors.[27] It was more effective than Ins(1,4,5)P_3 at Ca^{2+} release, since it is not immediately metabolised by deactivating enzymes. Further experimental findings also demonstrated that Ins(2,4,5)P_3 mimicked the effect of excitation and adaptation, produced in the same way as light. However, Ins(1,4,5)P_3 could not depolarise intact leech photoreceptors in the same way. Many biologists have used Ins(2,4,5)P_3 in their Ca^{2+} signalling experiments since it behaves as a long lived agonist in most cells and a surrogate for Ins(1,4,5)P_3 at its receptor. We have tested compound **4a** in competitive binding experiments using recombinant mammalian type I, II and III Ins(1,4,5)P_3 receptors expressed in a baculovirus/*Spodoptera frugiperda* 9 (*Sf*9) cell system.[28] It was found that Ins(2,4,5)P_3 bound tightest to type II and type III receptors; K_d of 131 nM for type II (*cf* Ins(1,4,5)P_3 is 11 nM), and type III; K_d, 194 nM (*cf* Ins(1,4,5)P_3 is 17 nM), which is around 11 fold weaker than Ins(1,4,5)P_3, for both type II and type III receptor. However, binding was weakest in the type I receptor, where K_d was 615 nM for Ins(2,4,5)P_3 and 24 nM for Ins(1,4,5)P_3, which is 24 fold weaker in this receptor subtype. We have thus achieved a practical route to biologically active D-Ins(2,4,5)P_3, its L-enantiomer and the corresponding phosphorothioate counterparts without contamination of other trisphosphates or phosphorothioates. The compounds will be of use for investigating signal transduction in the polyphosphoinositide pathway.

3. Experimental

General methods.—Chemicals were purchased from Aldrich, Fluka and Lancaster. Sodium hydride was used as a 60% dispersion in mineral oil. Thin-layer chromatography (TLC) was performed on precoated plates (Merck TLC aluminium sheets silica 60 F_{254}); products were visualised by spraying with phosphomolybdic acid in methanol, which was then heated for thirty seconds at high temperature. Flash chromatography refers to the procedure developed by Still and co-workers[29] and was carried out on Sorbsil C60 silica gel.

NMR spectra (proton frequency 270 or 400 MHz) were referenced to internal $SiMe_4$ or deuterium oxide. Samples recorded in D_2O were approximately pH 4–5. The ^{31}P NMR shifts were measured in ppm relative to external 85% phosphoric acid. Mps (uncorrected) were determined using a Reichert–Jung Thermo Galen Kofler block. Microanalysis was carried out by the University of Bath Microanalysis Service. Mass spectra were recorded by the University of Bath Mass Spectrometry Service using positive and negative ion fast atom bombardment (FAB) with 3-nitrobenzyl alcohol (NBA) as the matrix. Optical rotations were measured using an Optical Activity Ltd. AA-10 polarimeter, and $[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1} and were measured at ambient temperature. Ion exchange chromatography was performed on an LKB-Pharmacia medium-pressure ion exchange chromatograph using Q Sepharose Fast Flow with gradients of triethylammonium hydrogen carbonate (TEAB) as eluent. Column fractions containing inositol polyphosphates and phosphorothioates were assayed for total phosphate and phosphorothioate by a modification of the Briggs test.[26]

(Diisopropylamino)dichlorophosphine was prepared by the method of Tanaka and co-workers[30] by adding two mole equivalents of diisopropylamine to a solution of phosphorus trichloride in dry diethyl ether at −78 °C. The crude product was purified by distillation under reduced pressure (δ_P +169.4 ppm) and could be stored as a crystalline solid at −20 °C. Two equivalents of benzyl alcohol in the presence of triethylamine were then reacted with the purified product in CH_2Cl_2, to afford bis(benzyloxy)(diisopropylamino)phosphine[25] (δ_P +147.9 ppm) which was pure by ^{31}P NMR, (R_f 0.78, hexane–triethylamine 10:1).

D-1,3,6-Tri-O-benzyl-4,5-bis-O-(p-methoxybenzyl)-myo-inositol **7a**.—A mixture of compound[23] **6a** (1.88 g, 3.13 mmol) and dibutyltin oxide (0.85 g, 3.4 mmol) were heated at reflux in toluene (250 mL) using a Dean–Stark apparatus for 2.5 h. The reaction mixture was cooled and the toluene was evaporated to give a syrup that was dried under vacuum for a further 2 h. Caesium fluoride (1.89 g, 7.82 mmol) and dry DMF (30 mL) were added to the syrup under an atmosphere of nitrogen, together with benzyl bromide (1.07 g, 6.26 mmol), and the reaction was stirred overnight at room temperature. The reaction was complete by TLC (CH_2Cl_2–Et_2O, 15:1) and showed a single product (R_f 0.30). The solvents were evaporated under reduced pressure and the syrupy residue was extracted with CH_2Cl_2 (100 mL), and the extract was washed with water (100 mL) and stirred with sodium hydrogen carbonate solution (10% w/v) for 30 min, washed with water again, dried over $MgSO_4$, then filtered through a pad of Celite and the solvent was evaporated. The crude product was purified by flash chromatography (CH_2Cl_2–Et_2O, 15:1) to give the title compound **7a** as a solid. Yield (1.83 g, 85%); mp 106–108 °C (from EtOAc–hexane); $[\alpha]_D$ −9° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.20–7.34 (m, 19 H, 3 × CH_2*Ph* and 2 × 0.5

$CH_2C_6H_4OMe$), 6.84 (d, 2 H, J 8.8 Hz, 0.5 × $CH_2C_6H_4OMe$), 6.83 (d, 2 H, J 8.6 Hz, 0.5 × $CH_2C_6H_4OMe$), 4.69–4.92 (m, 10 H, 3 × CH_2Ph, and 2 × $CH_2C_6H_4OMe$), 4.21 (dd, 1 H, $J_{2,3}$ 2.25 Hz, H-2), 3.97 (2 × dd overlapping, 2 H, $J_{4,5}$ and $J_{1,6}$ 9.5 Hz, H-4 and H-6), 3.79 (s, 3 H, $CH_2C_6H_4OMe$), 3.78 (s, 3 H, $CH_2C_6H_4OMe$), 3.40 (m, 3 H, overlapping, H-1, H-3 and H-5), 2.48 (br s, 1 H, D_2O, exchangeable HO-2); ^{13}C NMR ($CDCl_3$): δ 158.96 (C_q, CH_2PhOMe), 138.69, 137.91, 137.86 (C_q, CH_2Ph), 132.53, 130.86, 130.78, 129.38, 128.23, 128.12, 127.71, 127.65, 127.58, 127.31 (CH_2Ph), 113.55 (CH_2PhOMe), 82.79, 81.05, 80.83, 79.70, 79.53, 67.16 (6 × *myo*-inositol ring carbons), 75.65, 75.36, 72.44, 72.41 (CH_2PhOMe and CH_2Ph), 55.02 (CH_2PhOMe); Anal. Calcd for $C_{43}H_{46}O_8$: C, 74.76; H 6.71. Found: C, 74.5; H, 6.72.

L-1,3,6-Tri-O-*benzyl*-4,5-*bis*-O-(p-*methoxybenzyl*)-myo-*inositol* **7b**.—A mixture of compound **6b** (1.42 g, 2.36 mmol) and dibutyltin oxide (0.65 g, 2.6 mmol) were heated at reflux in toluene (250 mL) using a Dean–Stark apparatus for 2.5 h. The reaction mixture was cooled and the toluene was evaporated to provide a syrup and dried under vacuum for 2 h. Caesium fluoride (1.075 g, 7.08 mmol) and dry DMF (30 mL) were added to the syrup under an atmosphere of nitrogen, together with benzyl bromide (0.81 g, 6.26 mmol), and the reaction was stirred overnight at room temperature. Work-up and purification, as for **7a**, furnished **7b**. Yield (1.30 g, 80%); mp 106–108 °C (from EtOAc–hexane); $[\alpha]_D$ +9° (*c* 1, $CHCl_3$); NMR data were identical with those of the D-enantiomer; Anal. Calcd for $C_{43}H_{46}O_8$: C, 74.76; H, 6.71. Found: C, 74.9; H, 6.70.

D-1,3,6-Tri-O-*benzyl*-myo-*inositol* **8a**.—Compound **7a** (1.52 g, 2.20 mmol) was stirred in a mixture of CH_2Cl_2–trifluoroacetic acid (55 mL, 10:1) for 30 min. TLC (ether) showed the product R_f 0.20 and the deprotected *p*-methoxybenzyl group (R_f 0.70). The reaction mixture was partitioned between H_2O and CH_2Cl_2 (100 mL of each), dried ($MgSO_4$), then evaporated to give the crude product. The title compound **8a** was purified by flash chromatography ($CHCl_3$–EtOAc, 1:1). Yield (0.84 g, 85%); mp 109–112 °C (liquid crystal), 126–127 °C (clear liquid, from EtOAc–hexane); $[\alpha]_D$ +20° (*c* 1, $CHCl_3$), Lit.[16] mp 112 °C (liquid crystal) 127 °C (clear liquid); $[\alpha]_D$ +16.2° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.24–7.36 (m, 15 H, 3 × CH_2Ph), 4.76, 5.00 (AB, 2 H, J_{AB} 11.2 Hz, CH_2Ph), 4.62, 4.70 (AB, 4 H, J_{AB} 11.35 Hz, 2 × CH_2Ph), 4.20 (dd, 1 H, $J_{2,3}$ 2.6 Hz, H-2), 3.94 (dd, 1 H, $J_{1,6}$ 9.5 Hz, H-6), 3.81 (dd, 1 H, $J_{4,5}$ 9.3 Hz, H-4), 3.34–3.41 (m, 2 H, overlapping, H-5, H-3), 3.21 (dd, 1 H, $J_{1,2}$ 2.75 Hz, H-1), 2.85 (br s, 3 H, exchangeable HO-2, HO-4 and HO-5); ^{13}C NMR ($CDCl_3$): δ 138.64, 137.73, 137.67 (C_q, CH_2Ph), 128.51, 128.44, 128.39, 127.91, 127.83, 127.66 (CH_2Ph), 80.41, 79.66, 78.96, 71.86, 66.91 (6 × *myo*-inositol ring carbons), 75.43, 74.19, 72.39 (CH_2Ph); Anal. Calcd for $C_{27}H_{30}O_6$: C, 71.98; H, 6.71. Found: C, 72.0; H, 6.70.

L-1,3,6-Tri-O-*benzyl*-myo-*inositol* **8b**.—Compound **7b** (1.05 g, 1.52 mmol) was stirred in a mixture of CH_2Cl_2–trifluoroacetic acid (55 mL, 10:1) for 30 min. After this time TLC showed a product R_f 0.20 (ether) and a deprotected *p*-methoxybenzyl derivative (R_f 0.70). Work-up and purification, as for the D-derivative **8a**, provided the title compound (0.59 g, 87%); mp 109–112 °C (liquid crystal), 126–127 °C, (clear liquid, from EtOAc–hexane); $[\alpha]_D$ −20° (*c* 1, $CHCl_3$). The NMR data were identical with those of the D-enantiomer; Anal. Calcd for $C_{27}H_{30}O_6$: C, 71.98; H, 6.71. Found: C, 71.7; H, 6.68.

D-1,3,6-Tri-O-*benzyl*-2,4,5-*tris*-O-*[di(benzyloxy)phosphoryl]*-myo-*inositol* **9a**.—A mixture of bis(benzyloxy)(diisopropylamino)phosphine[25] (0.69 g, 2 mmol) and 1*H*-tetrazole (0.42 g, 6 mmol) in dry CH_2Cl_2 (10 mL), was stirred for 15 min at rt. D-1,3,6-Tri-*O*-benzyl-*myo*-inositol **8a** (0.22 g, 0.49 mmol) was then added to the reaction mixture which was stirred for a further 15 min. The solution was cooled to −78 °C and MCPBA (1.00 g, 2.81 mmol) was added and the mixture was allowed to warm-up over 30 min. The reaction mixture was then partitioned between CH_2Cl_2 and a 10% aq solution of sodium metabisulfite (50 mL of each). The organic layer was then washed with brine and water (50 mL of each), dried ($MgSO_4$) and the solvent was evaporated to give the crude product, which was purified by flash chromatography ($CHCl_3$–acetone 10:1, R_f 0.40) to give the title compound **9a** as a syrup. Yield (0.44 g, 73%); $[\alpha]_D$ −9.2° (*c* 6.2, $CHCl_3$); Lit.[16] $[\alpha]_D$ −9.0° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.98–7.45 (m, 45 H, 9 × CH_2Ph), 5.38 (br, ddd, 1 H, $J_{P,H}$ 9.2 Hz, H-2), 4.52–5.15 (m, 20 H, H-4, H-5, 9 × CH_2Ph), 3.89 (dd, 1 H, $J_{1,6}$ 9.5 Hz, H-6), 3.52 (dd, 1 H, $J_{3,4}$ 9.8 Hz, H-3), 3.51 (dd, 1 H, $J_{1,2}$ 2.1 Hz, H-1); ^{13}C NMR ($CDCl_3$): δ 138.38, 136.79, 136.52, 136.04, 135.99, 135.91, 135.84, 135.75 (C_q, CH_2Ph), 128.66, 128.51, 128.34, 128.25, 128.07, 128.00, 127.94, 127.77, 127.72, 127.68, 127.61, 127.23, 127.16, 127.08 (CH_2Ph), 79.04, 78.27, 77.96, 77.50, 75.55, 72.46 (6 × *myo*-inositol ring carbons), 74.56, 72.51, 72.18, 69.44, 69.40, 69.35, 69.15, 69.09, 69.02 (CH_2Ph); ^{31}P NMR ($CDCl_3$): δ −1.57, −1.65, −1.87 (^{31}P–^1H decoupled).

L-1,3,6-Tri-O-*benzyl*-2,4,5-*tris*-O-*[di(benzyloxy)phosphoryl]*-myo-*inositol* **9b**.—A mixture of bis(benzyloxy)(diisopropylamino)phosphine[25] (0.69 g, 2 mmol) and 1*H*-tetrazole (0.42 g, 6 mmol) in dry CH_2Cl_2 (10 mL), was stirred for 15 min. L-1,3,6-Tri-*O*-benzyl-*myo*-inositol **8b** (0.22 g, 0.49 mmol), was then added to the reaction mixture which was stirred for a further 15 min. The solution was cooled to −78 °C and MCPBA (1.00 g, 2.81 mmol) was added and the mixture was allowed to warm-up over 30 min. Work-up and purification was carried out as for the D-enantiomer. Yield (0.50 g,

83%); $[\alpha]_D$ +9.3° (*c* 6.34, $CHCl_3$). The NMR data were identical with those of the D-enantiomer.

D-myo-*Inositol 2,4,5-trisphosphate* **4a**.—D-1,3,6-Tri-*O*-benzyl-2,4,5-tris-*O*-[di(benzyloxy)phosphoryl]-*myo*-inositol **9a** (0.115 g, 0.0935 mmol) was hydrogenolysed in a mixture of methanol–water (4:1, 50 mL) over palladium on carbon (10%, 0.20 g) at 30 psi overnight. The reaction mixture was then filtered through a bed of Celite to remove the solid components, and the remaining solvents were evaporated to give a syrup. The residue was then dissolved in MilliQ water (150 mL) and purified by ion exchange chromatography on Q Sepharose Fast flow, eluting with a gradient of TEAB buffer (0–1000 mmol) at pH 8.6. The triethylammonium salt of **4a** eluted at ca. 500 mmol buffer. Yield (0.090 mmol, 94%); $[\alpha]_D$ −7.9° (*c* 1.52, MeOH) (triethylammonium salt); Lit.[14] $[\alpha]_{546}$ −8.05° (H_2O) (hexakiscyclohexylammonium salt); ^{1}H NMR (D_2O): δ 4.53 (d, 1 H, $J_{P,H}$ 8.1 Hz, H-2), 4.26 (ddd, 1 H, $J_{4,5}$ 9.3 Hz, H-4), 3.95 (ddd, 1 H, $J_{5,6}$ 9.0 Hz, H-5), 3.81 (dd, 1 H, $J_{1,6}$ 9.7 Hz, H-6), 3.68 (br d, 1 H, $J_{3,4}$ 9.9 Hz, H-3), 3.57 (br d, 1 H, $J_{1,2}$ 9.9 Hz, H-1); ^{31}P NMR (D_2O): δ 2.18 (d, 1 P, $J_{P,H}$ 6.7 Hz), 2.08 (d, 1 P, $J_{P,H}$ 10.1 Hz), 1.96 (d, 1 P, $J_{P,H}$ 6.7 Hz); MS: (FAB) *m*/*z* Calcd for $C_6H_{14}O_{15}P_3$ $[M-H]^-$ 418.9545. Found 418.9542.

L-myo-*Inositol 2,4,5-trisphosphate* **4b**.—L-1,3,6-Tri-*O*-benzyl-2,4,5-tris-*O*-[di(benzyloxy)phosphoryl]-*myo*-inositol **9b** (0.215 g, 0.175 mmol) was hydrogenolysed in a mixture of methanol–water (4:1, 50 mL) over palladium on carbon (10%, 0.20 g) at 30 psi overnight. The reaction mixture was then filtered over a bed of Celite to remove the solid components, and the remaining solvents were evaporated to give a syrup. Work-up and purification was carried out in the same way as for the D-enantiomer. The title compound **4b** eluted at ca. 600 mmol of TEAB buffer. Yield (0.090 mmol, 51%); $[\alpha]_D$ +8° (*c* 1.5, MeOH) (triethylammonium salt); Lit.[14] $[\alpha]_{546}$ +9.5° (H_2O) (hexakiscyclohexylammonium) salt. The mass spectrum and NMR data were identical with those of the D-enantiomer; MS: (FAB) *m*/*z* Calcd for $C_6H_{14}O_{15}P_3$ $[M-H]^-$ 418.954. Found 418.953.

D-1,3,6-Tri-O-*benzyl-2,4,5-tris*-O-*[di(benzyloxy)thiophosphoryl]*-myo-*inositol* **10a**.—A mixture of D-1,3,6-tri-*O*-benzyl-*myo*-inositol (0.10 g, 0.22 mmol), bis(benzyloxy)diisopropylaminophosphine[25] (0.46 g, 1.3 mmol) and 1*H*-tetrazole (0.15 g, 2.14 mmol) in dry CH_2Cl_2 (10 mL) was stirred at room temperature for 30 min. The solvent was evaporated and dry DMF (3 mL), dry pyridine (1 mL), and sulphur (0.46 g) were added to the residue and the mixture was stirred for a further 15 min. After this time the solvents were evaporated in vacuo and the reaction mixture was dissolved in $CHCl_3$ (30 mL) then washed with brine and water (30 mL of each), dried ($MgSO_4$), and the solvent was evaporated to give the crude product. Purification was carried out by flash chromatography (light petroleum–EtOAc, 5:1) to furnish the title compound **9a** as a syrup. Yield (0.25 g, 90%); $[\alpha]_D$ −9.5° (*c* 1.7, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 6.88–7.40 (m, 45 H, 9 × CH_2*Ph*), 5.53 (br ddd, $J_{H,P}$ 12.6 Hz, H-2), 4.45–5.33 (m, 20 H, H-4 and H-5, 9 × CH_2Ph), 3.94 (dd, 1 H, $J_{1,6}$ 9.6 Hz, H-6), 3.60 (dd, 2 H, $J_{3,4}$ 9.5 Hz, H-1 and H-3); ^{13}C NMR ($CDCl_3$): δ 138.74, 136.81, 136.58, 136.06, 136.00, 135.92, 135.85, 135.61, 135.48 (C_q, CH_2*Ph*), 128.78, 128.31, 128.23, 127.99, 127.94, 127.87, 127.84, 127.81, 127.74, 127.19, 126.84, 126.30 (CH_2*Ph*), 79.89, 78.64, 78.51, 75.67, 73.97, 73.42 (6 × *myo*-inositol ring carbons), 73.98, 72.90, 71.97, 69.70, 69.64, 69.56, 69.43, 69.34 (*C*H_2Ph); ^{31}P NMR ($CDCl_3$): δ 67.51, 68.29, 68.82; MS: (FAB) *m*/*z* Calcd for $C_{69}H_{69}O_{12}P_3S_3$ $[M-H]^-$ 1279.324. Found 1279.326.

L-1,3,6-Tri-O-*benzyl-2,4,5-tris*-O-*[di(benzyloxy)thiophosphoryl]*-myo-*inositol* **10b**.—Compound **10b** was obtained in an identical fashion to that described for **10a**. Yield (0.26 g, 93%); $[\alpha]_D$ +10.0° (*c* 1.8, CH_2Cl_2); MS: (FAB) *m*/*z* Calcd for $C_{69}H_{69}O_{12}P_3S_3$ $[M-H]^-$ 1279.324. Found 1279.329.

D-myo-*Inositol 2,4,5-trisphosphorothioate* **5a**.—Ammonia was condensed into a three-neck flask at −78 °C. An excess of sodium was added to the liquid ammonia then distilled into a second three-neck flask and kept at −78 °C. Sodium was added until the solution remained blue. Compound **10a** (0.10 g, 0.08 mmol) was dissolved in dry 1,4-dioxane (2 mL) and added to the blue solution of sodium in liquid ammonia. The mixture was stirred for 10 min then quenched with ethanol. The ammonia and ethanol were evaporated off under a stream of nitrogen and the crude product was purified by ion-exchange chromatography on Q Sepharose Fast Flow with a gradient of TEAB buffer (0.1–1.0 mol dm^{-3}), pH 8.0, to give the triethylammonium salt of the trisphosphorothioate **5a**. Yield (0.062 g, 72%); $[\alpha]_D$ −5.2° (*c* 0.8, MeOH); ^{1}H NMR (D_2O): δ 4.69 (br s, 1 H, H-2), 4.52 (ddd, 1 H, $J_{4,5}$ 9.6 Hz, $J_{P,H}$ 11.0 Hz, H-4), 4.14 (ddd, 1 H, $J_{5,6}$ 8.9, 9.1 Hz, $J_{P,H}$ 10.9 Hz, H-5), 3.87 (dd, 1 H, $J_{1,6}$ 9.3 Hz, H-6), 3.68 (d, 1 H, $J_{3,4}$ 9.8 Hz, H-3), 3.51 (dd, 1 H, $J_{1,2}$ 3.4 Hz, H-1); ^{31}P NMR (D_2O): δ 44.33, 44.62, 44.88; MS: (FAB) *m*/*z* Calcd for $C_6H_{14}O_{12}P_3S_3$ $[M-H]^-$ 466.886. Found 466.885.

L-myo-*Inositol 2,4,5-trisphosphorothioate* **5b**.—Compound **5b** was obtained in an identical manner to that described for compound **5a**. Yield (0.06 g, 70%); $[\alpha]_D$ +5.9° (*c* 0.6, MeOH); MS: (FAB) *m*/*z* Calcd for $C_6H_{14}O_{12}P_3S_3$ $[M-H]^-$ 466.886. Found 466.885.

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