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# Synthesis of D- and L-*myo*-inositol 1,2,4,6-tetrakisphosphate, regioisomers of *myo*-inositol 1,3,4,5 tetrakisphosphate: activity against Ins(1,4,5)$P_3$ binding proteins

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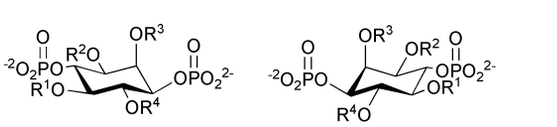
We report here the synthesis of D- and L-*myo*-inositol 1,2,4,6-tetrakisphosphate **3a** and **3b** and the racemic modification **3ab**. Racemic *myo*-inositol 1,2,4,6-tetrakisphosphate **3ab** was synthesised from DL-1,2,4,6-tetra-*O*-allyl-*myo*-inositol **9ab**. Benzylation and de-allylation provided the tetraol **11ab**, which was phosphitylated in the presence of bis(benzyloxy)diisopropylaminophosphine and 1*H*-tetrazole, then oxidised to give the fully protected 1,2,4,6-tetrakisphosphate **13ab**. Hydrogenolysis of **13ab** and purification of product by ion exchange chromatography gave racemic *myo*-inositol 1,2,4,6-tetrakisphosphate **3ab**, which showed no demonstrable agonism or antagonism for $Ca^{2+}$ release at 200 μM in permeabilised hepatocytes. The chiral derivatives, D-**3a** and L-*myo*-inositol 1,2,4,6-tetrakisphosphate **3b** were synthesised from 5-*O*-benzyl-1,4,6-tri-*O*-*p*-methoxybenzyl-*myo*-inositol **19ab**, which was resolved using *R*-(−)-*O*-acetylmandelic acid providing two diastereoisomers **21** and **22** which were separated and deacylated to give the corresponding enantiomers. Further transformations gave the corresponding chiral 1,2,4,6-tetraols which were phosphitylated, oxidised, deprotected and purified as for the racemic mixture. The enantiomeric tetrakisphosphates **3a** and **3b** were evaluated for inhibition of the metabolic enzymes inositol 1,4,5-trisphosphate 5-phosphatase and 3-kinase in comparison with the enantiomers of another synthetic regioisomer D- and L-*myo*-inositol 1,2,4,5-tetrakisphosphate. Both D- and L-*myo*-inositol 1,2,4,6-tetrakisphosphate inhibit 5-phosphatase with an $IC_{50}$ value of 3.8 μM and 14 μM, repectively. However, both enantiomers were poorly recognised by the 3-kinase enzyme, with $IC_{50}$ values greater than 100 μM. The enantiomers of the 1,2,4,5-tetrakisphosphate showed the same relative pattern of activity towards the two enzymes but were more potent against 5-phosphatase (0.47 μM and 3 μM respectively).

## Introduction

D-*myo*-Inositol 1,4,5-trisphosphate [Ins(1,4,5)$P_3$ **1a**] (Fig. 1) is a ubiquitous hydrophilic second messenger, which elevates the levels of cytostolic $Ca^{2+}$ ions.[1] After $Ca^{2+}$ release, Ins(1,4,5)$P_3$ is deactivated and the cell returns to a basal state. This is achieved in two ways: first, an Ins(1,4,5)$P_3$ 5-phosphatase removes the 5-phosphate group from Ins(1,4,5)$P_3$ to give *myo*-inositol 1,4-bisphosphate [Ins(1,4)$P_2$] which does not release $Ca^{2+}$. Ins(1,4)$P_2$ is an allosteric activator of 6-phosphofructo-1-kinase[2] and also activates DNA polymerase-α.[3] Second, Ins(1,4,5)$P_3$ can be phosphorylated by an Ins(1,4,5)$P_3$ 3-kinase to give *myo*-inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)$P_4$ **2a**] which acts as a potent bi-modal regulator of cellular sensitivity to Ins(1,4,5)$P_3$ and facilitates the regulation of $Ca^{2+}$ signalling.[4] Thus, both products may be regarded as 'off' signals. The function of Ins(1,3,4,5)$P_4$ has not been fully resolved; however, it could be the natural inhibitor of 5-phosphatase *in vivo*.[4] Studies have shown that D-Ins(1,3,4,5)$P_4$ is the most potent inhibitor of 5-phosphatase[4] ($IC_{50}$ of 0.15 μM). However, the enantiomer of **2a** L-Ins(1,3,4,5)$P_4$ **2b** is more than ten-fold weaker ($IC_{50}$ of 1.8 μM).[4] These data support the finding that 5-phosphatase is inhibited by a number of tetrakisphosphates.[5] However, there are no known inhibitors which are as potent as D-Ins(1,3,4,5)$P_4$.

We have previously described[6] how *myo*-inositol 1,4,6-trisphosphate, [Ins(1,4,6)$P_3$, **4a**] can mimic the $Ca^{2+}$ releasing activity of Ins(1,4,5)$P_3$, by spatial inversion and rotation of the former molecule, such that positions 4-, 1- and 6- of Ins(1,4,6)$P_3$ (Fig. 2) can be superimposed on positions 1-, 4- and 5- of Ins(1,4,5)$P_3$ respectively. The hydroxyl groups at positions 3- and 2- of Ins(1,4,6)$P_3$, can be superimposed on positions 2- and 3- of Ins(1,4,5)$P_3$ respectively, but the stereochemistry at these sites is inverted. Ins(1,4,6)$P_3$ can release $Ca^{2+}$ from intracellular stores, albeit with reduced potency compared to Ins(1,4,5)$P_3$. Since Ins(1,4,6)$P_3$ can be superimposed on



**1a** $R^1 = PO_3^{2-}$; $R^2 = R^3 = R^4 = H$; = D-Ins(1,4,5)$P_3$ — **1b** $R^1 = PO_3^{2-}$; $R^2 = R^3 = R^4 = H$; = L-Ins(1,4,5)$P_3$

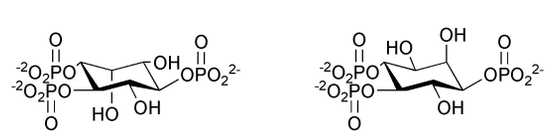
**2a** $R^1 = R^2 = PO_3^{2-}$; $R^3 = R^4 = H$; = D-Ins(1,3,4,5)$P_4$ — **2b** $R^1 = R^2 = PO_3^{2-}$; $R^3 = R^4 = H$ = L-Ins(1,3,4,5)$P_4$

**3a** $R^1 = R^2 = H$; $R^3 = R^4 = PO_3^{2-}$; = D-Ins(1,2,4,6)$P_4$ — **3b** $R^1 = R^2 = H$; $R^3 = R^4 = PO_3^{2-}$; = L-Ins(1,2,4,6)$P_4$

**4a** $R^1 = R^2 = R^3 = H$; $R^4 = PO_3^{2-}$; = D-Ins(1,4,6)$P_3$ — **4b** $R^1 = R^2 = R^3 = H$; $R^4 = PO_3^{2-}$; = L-Ins(1,4,6)$P_3$

**5a** $R^2 = R^4 = H$; $R^1 = R^3 = PO_3^{2-}$; = D-Ins(1,2,4,5)$P_4$ — **5b** $R^2 = R^4 = H$; $R^1 = R^3 = PO_3^{2-}$; = L-Ins(1,2,4,5)$P_4$

**Fig. 1**



**4a** D-Ins(1,4,6)$P_3$ — **1a** D-Ins(1,4,5)$P_3$

**Fig. 2** Orientation of Ins(1,4,6)$P_3$ to mimic Ins(1,4,5)$P_3$.

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Ins(1,4,5)$P_3$, the phosphates and hydroxyl groups of Ins-(1,2,4,6)$P_4$ **3a** can also, in principle, be arranged in a pattern which broadly overlaps with Ins(1,3,4,5)$P_4$ **2a**, by spatially inverting and rotating the molecule (Fig. 3). The tetrakisphosphate **3a** has a similar arrangement of phosphates as compound **2a**; however, where the 3-phosphate of Ins(1,3,4,5)$P_4$ **2a** is equatorial, the corresponding position of **3a** is axial and the equivalent position-2 is now equatorial. Similarly, if the enantiomer L-Ins(1,2,4,6)$P_4$ **3b** is inverted and rotated in the same way, the new 5-position (axial 2-phosphate) of **3b** corresponding to the phosphate of **2a** is axial and the 3-position is equatorial and **3b** can be superimposed on D-Ins(1,3,4,5)$P_4$ **2a**. The other three phosphates are equatorial at pseudo positions 1-, 3- and 4- (Fig. 3). Thus, both D-Ins(1,2,4,6)$P_4$ **3a** and L-Ins(1,2,4,6)$P_4$ **3b** can be superimposed on D-Ins(1,3,4,5)$P_4$ with an axial phosphate group at position-3 for **3a** or at position-5 for **3b**. We report here the synthesis of D- and L-*myo*-inositol 1,2,4,6-tetrakisphosphate and its racemic mixture, and the biological evaluation of compounds **3a** and **3b** and Ins(1,3,4,5)$P_4$ **2a**, together with another tetrakisphosphate enantiomeric pair D-Ins(1,2,4,5)$P_4$ **5a** and L-Ins(1,2,4,5)$P_4$ **5b**, with the metabolic enzymes, Ins(1,4,5)$P_3$ 3-kinase and 5-phosphatase.

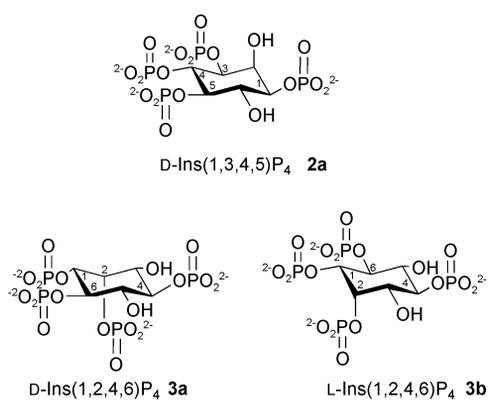
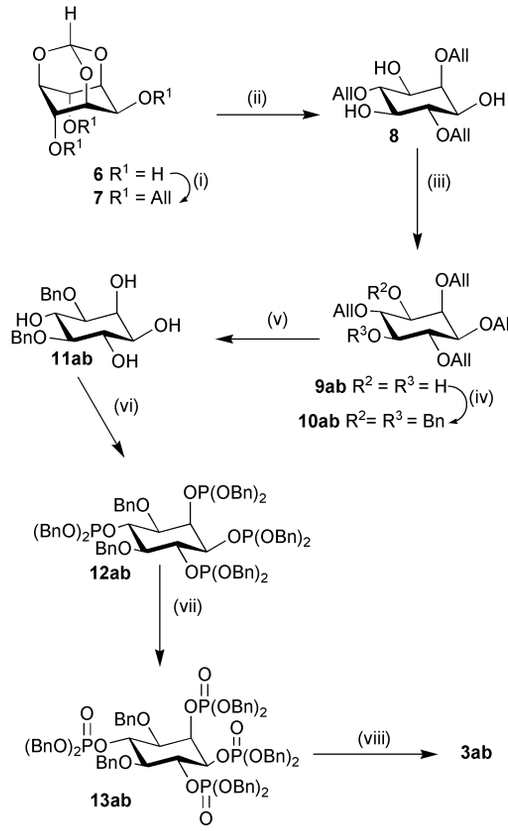



**Fig. 3** Orientation of D- and L-Ins(1,2,4,6)$P_4$ to mimic D-Ins(1,3,4,5)$P_4$.

## Results and discussion

*myo*-Inositol orthoformate **6** (Scheme 1) was prepared according to Vasella and co-workers[7] in 73% yield to give a highly crystalline compound. Allylation of the two axial and one equatorial hydroxyl groups was accomplished using sodium hydride and allyl bromide in dry DMF at room temperature. Purification of the crude product by flash chromatography provided the *meso*- compound 2,4,6-tri-*O*-allyl-*myo*-inositol orthoformate **7** as a syrup in 89% yield. The orthorformate protective group was then removed from compound **7** by acid hydrolysis using aq. HCl at reflux temperature to afford 2,4,6-tri-*O*-allyl-*myo*-inositol **8** in excellent yield. When the orthoformate protecting group was removed from compound **7**, the hydroxyl groups at positions 1-, 3- and 5- were exposed. Under these conditions it was possible to allylate selectively the more reactive 1- or 3-hydroxyl position and provide a tetra-*O*-allyl derivative that was used to prepare racemic Ins(1,2,4,6)$P_4$ **3ab**. Monoallylation of the triol **8** to give the tetra-*O*-allyl derivative **9ab** was envisaged because the starting material was water soluble, however, the product was soluble in the organic layer. Thus, monoallylation would release tetra-*O*-allyl-*myo*-inositol **9ab** into the organic layer and move the equilibrium in favour of the monoallylated product. Selective allylation of the triol **8** was achieved under phase-transfer conditions,[8] using tetrabutylammonium sulfate and allyl bromide in a 5% aqueous solution of sodium hydroxide and an equal volume of dichloromethane at reflux temperature for 24 h. Racemic 1,2,4,6-tetra-*O*-allyl-*myo*-inositol **9ab** was isolated in 74% yield



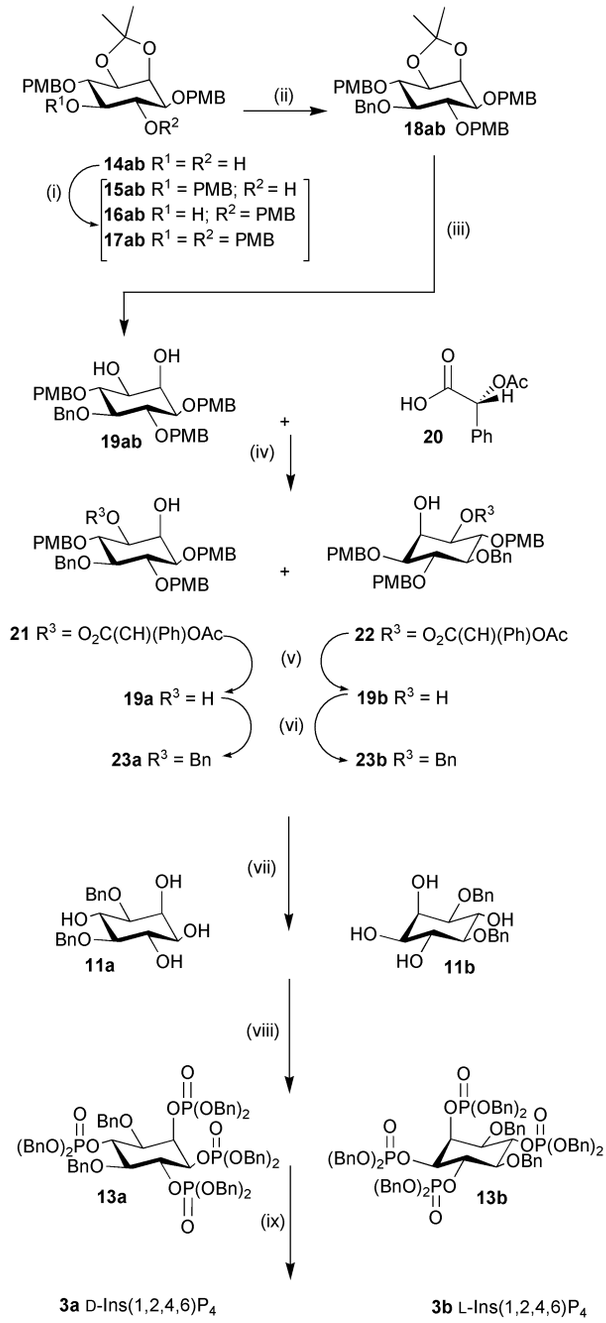

**Scheme 1** Synthesis of racemic Ins(1,2,4,6)$P_4$. *Reagents and conditions* (i) NaH, AllBr, DMF, rt, 3 h, (89%); (ii) ethanol–1 mol $dm^{-3}$ aq. HCl (2 : 1), reflux 3 h, (93%); (iii) AllBr, $(Bu_4N)_2SO_4$, (1 eq.), $CH_2Cl_2$–5% aq. NaOH, 1 : 1, reflux 24 h, (74%); (iv) NaH, BnBr, DMF, (88%); (v) 10% Pd/C, *p*-toluene sulfonic acid, MeOH–$H_2O$ (5 : 1), reflux, (49%); (vi) $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, $CH_2Cl_2$; (vii) MCPBA (50–60%), 0 °C, 30 min, (91%); (viii) $H_2$, Pd/C, MeOH–$H_2O$ (4 : 1), 20 h, then purification by ion exchange chromatography on Q-Sepharose Fast Flow, (33%).

as a syrup, since selective allylation at positions C-1-OH or C-3-OH leads to the same racemic product **9ab**. Penta-*O*-allyl-*myo*-inositol together with starting material were observed by TLC, but these compounds were not isolated after chromatography. Benzylation of diol **9ab** with benzyl bromide and sodium hydride in DMF provided the fully blocked compound DL-1,2,4,6-tetra-*O*-allyl-3,5-di-*O*-benzyl-*myo*-inositol **10ab**. Removal of all four allyl protective groups was achieved using 10% palladium on carbon in the presence of an acid to provide the racemic 1,2,4,6-tetraol **11ab**. Compound **11ab** was subjected to phosphitylation using the P(III) reagent bis-(benzyloxy)(diisopropylamino)phosphine[9] ($\delta_P$ = +147.9 ppm) in the presence of excess 1*H*-tetrazole. The reaction of the P(III) reagent in the presence of 1*H*-tetrazole provided a reactive tetrazolide intermediate ($\delta_P$ = +126.73 ppm).[10] The tetraol **11ab** was added to the tetrazolide derivative to provide a tetrakisphosphite intermediate **12ab**. The $^{31}$P NMR spectrum of the 1,2,4,6-tetrakisphosphite **12ab**, operating at 36.2 MHz, and a sweep width of 2500 KHz, showed eight assignable peaks derived from several $^5J_{pp}$ coupling systems.[10] There was a doublet at $\delta_P$ = 139.0 ppm, for 2-P, $^5J_{pp}$ = 2.4 Hz; a doublet of doublets at $\delta_P$ = 140 ppm for 1-P, $^5J_{pp}$ = 4.3 and 2.4 Hz; a doublet at 142.5 ppm for 6-P, $^5J_{pp}$ = 4.3 Hz, and a singlet at $\delta_P$ = 141.8 ppm for 4-P. This was definitive proof that phosphitylation of the three adjacent positions and the isolated C-4-OH had occurred. The reaction mixture was then cooled and oxidised using *m*-chloroperoxybenzoic acid (MCPBA) to give the P(v) fully protected phosphotriester **13ab** in excellent yield. After oxidation, there is no $^5J_{pp}$ coupling, thus, the pattern of the $^{31}$P NMR spectrum gives four separate phosphorus singlets at *ca.* −1 to −3 ppm for the $^{31}$P–$^1$H decoupled NMR spectrum. The decabenzyl derivative **13ab** was hydro-

genolysed under pressure, in the presence of 10% palladium on carbon. The solid components of the reaction mixture were filtered off over a bed of Celite and the methanolic-aqueous solution was evaporated to give a syrup. The crude compound was dissolved in MilliQ and purified by ion-exchange chromatography on Q-Sepharose Fast Flow using a gradient of triethylammonium hydrogen carbonate (TEAB) buffer. Pure Ins(1,2,4,6)$P_4$ **3ab** was obtained as the triethylammonium salt after ion exchange chromatography, and eluted at *ca*. 800 mmol $dm^{-3}$ TEAB buffer and was quantified by phosphate analysis.[11]

A different approach was used to synthesise the chiral antipodes, **3a** and **3b** (Scheme 2). Racemic 5-*O*-benzyl-1,4,6-tris-*O*-(*p*-methoxybenzyl)-*myo*-inositol **19ab** was resolved using the chiral auxillary (*R*)-(−)-*O*-acetylmandelic acid **20** in order to give suitable intermediates for the preparation of the tetrakisphosphates **4a** and **4b**. Intermediate **19ab** was prepared in three steps from the *trans* diol **14ab**,[12] which was selectively alkylated, at the more reactive C-6-OH.[13] Thus, treatment of diol **14ab** with dibutyltin oxide, tetrabutylammonium bromide/iodide and *p*-methoxybenzyl chloride in acetonitrile resulted in three products in an approximate ratio of (8 : 4 : 1) for compounds **16ab**, **15ab** and **17ab**. The $^1$H NMR spectrum of **16ab** showed a broad triplet at $\delta$ = 3.32 ppm for 5-H, which was the signal for the ring proton furthest upfield. Following $D_2O$ exchange, the broad triplet at $\delta$ = 3.32 ppm collapsed to give a sharp triplet, however, the $D_2O$ exchange had no effect on the signal for 6-H or 4-H. The other products, 2,3-*O*-isopropylidene-1,4,5-tris-*O*-*p*-methoxybenzyl-*myo*-inositol **15ab** and 2,3-*O*-isopropylidene-1,4,5,6-tetrakis-*O*-*p*-methoxybenzyl-*myo*-inositol **17ab** were isolated in 26% and 6% yield respectively. Racemic 5-*O*-benzyl-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol **19ab** was prepared by benzylation of compound **16ab** to give **18ab** and careful acid hydrolysis of the isopropylidene gave the *cis*-diol **19ab**.

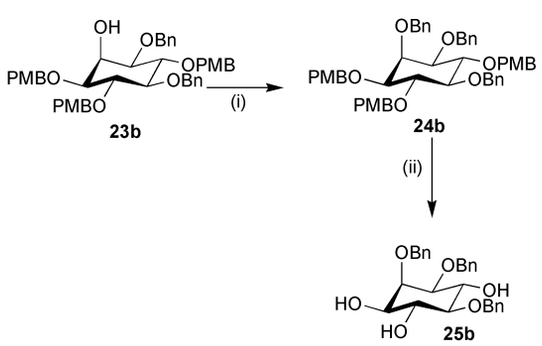
Numerous methods are available to resolve *myo*-inositol phosphate precursors;[14] most of these procedures use a chiral auxiliary such as an acid, that is coupled to one or more of the hydroxyl groups on the *myo*-inositol ring to form a pair of diastereoisomeric ester derivatives. The resulting two diastereoisomers can be separated by flash chromatography or crystallisation.[10,13,14] Ogawa and co-workers[15,16] have used (*S*)-(+)-*O*-acetylmandelic acid coupled to DL-1,4,5,6-tetra-*O*-benzyl-*myo*-inositol[15] to then provide precursors to previously inaccessible hexoses,[15] and β-glucosidase and α-mannosidase inhibitors (+)- and (−)-norjirimycin.[16] They also used (*R*)-(−)-*O*-acetylmandelic acid to acylate aminocyclopentane-1,2,3,4-tetraols[17] which were then used to synthesise (+) and (−)-mannostatin A. The previous success of this reagent for resolving racemic *myo*-inositol derivatives containing a free *cis*-diol, prompted us to use it as our reagent of choice. The *cis*-diol DL-5-*O*-benzyl-1,4,6-tris-*O*-(*p*-methoxybenzyl)-*myo*-inositol **19ab** was resolved by coupling to (*R*)-(−)-*O*-acetylmandelic acid **20**, in the presence of DCCI and DMAP at −20 °C in order to form two equatorial substituted diastereoisomers **21** and **22**. We used the *R*-enantiomer of acetylmandelic acid, because from previous resolutions[10,13] we predicted the less polar product (by TLC) would lead to the D-enantiomer **3a** and the more polar product should give the L-enantiomer **3b**. The critical resolution step in this synthesis gave diastereoisomers **21** and **22**, which had $R_f$ values of 0.34 and 0.24 respectively in our TLC system ($CHCl_3$–acetone 15 : 1). Separation and purification of the two diastereoisomers was then accomplished using flash chromatography and crystallisation. In the NMR spectrum, the 1-H proton from both diastereoisomers could not be identified due to the methylene AB coupling pattern of a benzyl or *p*-methoxybenzyl moiety which obscured the doublet of doublet signal for 1-H in both compounds. However, it was shifted downfield due to the carbonyl deshielding effect from the ester. The slower running diastereoisomer showed an unusual upfield shift of a methylene AB system from the adjacent *p*-methoxybenzyl moiety at $\delta$ = 4.05 and 4.36. The *myo*-inositol ring protons at position (2-H) for both diastereoisomers were identified, and their signal was indicative of unacylated products and the equatorial C-1-OH was acylated under our experimental conditions. The unique singlet at $\delta$ = 5.97 for diastereoisomer **21** and $\delta$ = 5.99 for diastereoisomer **22** [for $CH_3CO_2CH(Ph)CO_2$Ins] indicated the purity of these compounds. The individual diastereoisomers **21** and **22** were deacylated using methanolic sodium hydroxide to give the enantiomers **19a** and **19b** which




**Scheme 2** Synthesis of D- and L-Ins(1,2,4,6)$P_4$. *Reagents and conditions* (i) $Bu_2SnO$, $CH_3CN$, $(Bu)_4NBr$ and $(Bu)_4NI$, *p*-methoxybenzyl chloride, Soxhlet, reflux 40 h, (46% **16ab**, 26% **15ab**, 6% **17ab**); (ii) BnBr, NaH, DMF, 2 h, (86%); (iii) MeOH–1.0 mol $dm^{-3}$ aq. HCl (9 : 1), 50 °C, 30 min, (79%); (iv) (*R*)-(−)-*O*-acetylmandelic acid, DMAP, DCCI, −20 °C, overnight, (32% **21**, 26% **22**); (v) MeOH–NaOH, reflux, 30 min, (96% **19a**, 99% **19b**); (vi) $Bu_2SnO$, $PhCH_3$, Dean-and-Stark apparatus, 3 h. Then DMF, CsF, BnBr, rt, overnight, (83% **23a**, 95% **23b**); (vii) 1 Mol $dm^{-3}$ aq. HCl–ethanol (1 : 2) reflux, 4 h, (92% **11a**, 93% **11b**); (viii) $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, $CH_2Cl_2$, 10 min, then add tetraols, 10 min, then add (50–60%) MCPBA, 0 °C, 30 min, (79% **13a**, 86% **13b**); (ix) 10% Pd/C, $H_2$, MeOH–$H_2O$ (4 : 1), 20 h, then purification by ion exchange chromatography on Q-Sepharose Fast Flow, (65% **4a**, 36% **4b**).

had equal and opposite optical rotations. The equatorial hydroxyl group was then selectively benzylated over the axial hydroxyl moiety *via* the *cis*-1,2-*O*-dibutylstannylene derivative which was formed by refluxing the individual enantiomers **19a** and **19b** separately, in the presence of dibutyltin oxide and toluene, with continuous removal of water. The *cis*-1,2-*O*-dibutylstannylene derivatives were dried then dissolved in dry DMF followed by the addition of three equivalents of caesium fluoride and the mixtures stirred under nitrogen. Benzyl bromide was added dropwise and the reactions were stirred overnight, after which a single product was obtained after work up and chromatography in each case to give the individual enantiomers **23a** and **23b**. The three *p*-methoxybenzyl groups were then removed in the presence of the benzyl ethers under acidic conditions, to give D- and L-3,5-di-*O*-benzyl-*myo*-inositol **11a** and **11b** respectively, which had equal and opposite optical rotations.

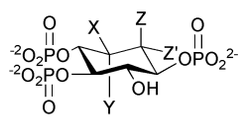
We firmly established the absolute configuration of D- and L-3,5-di-*O*-benzyl-*myo*-inositol **11a** and **11b** respectively, by transforming L-3,5-di-*O*-benzyl-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol **23b** to the known compound, L-2,3,5-tri-*O*-benzyl-*myo*-inositol[13] **25b** (Scheme 3). The 2-hydroxyl group of **23b** was benzylated to give the fully blocked intermediate L-2,3,5-tri-*O*-benzyl-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol **24b**. The three *p*-methoxybenzyl groups were removed from **24b** by acidic hydrolysis to give triol **25b** and the melting point and optical rotation agreed well with our literature value.[13] The faster running compound (higher $R_f$) by TLC led to the synthesis of D-3,5-di-*O*-benzyl-*myo*-inositol **11a** and the slower running compound provided the L-3,5-di-*O*-benzyl-*myo*-inositol **11b**. Phosphorylation of the individual chiral tetraols was carried out in the same way as for the racemic mixture, using the P(III) approach and oxidation to P(V) intermediate using MCPBA. The benzyl protective groups for both fully protected tetrakisphosphate enantiomers **13a** and **13b** were then removed by hydrogenolysis in the presence of 10% palladium on carbon. The product was then purified on Q-Sepharose Fast Flow using a gradient of TEAB as buffer to give the corresponding pure tetrakisphosphates D-Ins(1,2,4,6)$P_4$ **3a** and L-Ins(1,2,4,6)$P_4$ **3b** respectively. The specific rotations for the chiral antipodes **3a** and **3b** were determined in methanol and found to be −15.4 and +15 for **3a** and **3b** respectively. Recently, syntheses for compounds **3a** and **3b** have been reported by Chung *et al.*,[18] using a different route. The magnitude and sign of the optical rotation for each enantiomer, agree fully with our data.




**Scheme 3** Determination of the absolute configuration of compound **23b**. *Reagents and conditions* (i) NaH, BnBr, DMF, rt, 2 h, (82%); (ii) 1 Mol $dm^{-3}$ aq. HCl–ethanol (1 : 2), reflux 4 h, (88%).

Compounds D- and L-Ins(1,2,4,6)$P_4$ (**3a** and **3b**), which are structurally similar to *myo*-inositol 1,3,4,5-tetrakisphosphate **2a** (see Fig. 3), were evaluated in the presence of the Ins(1,4,5)$P_3$ metabolising enzymes, 5-phosphatase (type I from human brain)[19] and 3-kinase (type A, from rat brain, clone C5).[20] For a complete study, several other structurally related inositol tetrakisphosphates were evaluated under the same experimental conditions: these included the naturally occurring D-Ins(1,3,4,5)$P_4$ **2a** together with the synthetic regioisomers D-Ins(1,2,4,5)$P_4$ **5a** and L-Ins(1,2,4,5)$P_4$ **5b**, which differ in the position of one phosphate.[10] This study is the first evaluation of D- and L-Ins(1,2,4,6)$P_4$ and D- and L-Ins(1,2,4,5)$P_4$ as potential inhibitors of 3-kinase and 5-phosphatase.

Initial studies for synthesising new analogues derived from Ins(1,4,5)$P_3$ focused on the analogue[5] L-*chiro*-Ins(2,3,5)$P_3$ (**26** in Fig. 4) which interacts with the Ins(1,4,5)$P_3$ receptor and deactivating enzymes, 3-kinase and 5-phosphatase. This molecule is similar to Ins(1,4,5)$P_3$ but the position-3 of Ins(1,4,5)$P_3$ is now axial and the name and numbering of the resulting inositol phosphate derivative changes. L-*chiro*-Ins(2,3,5)$P_3$ **26** is a full agonist for $Ca^{2+}$ release and inhibits both 5-phosphatase ($K_i$ value of 7.7 μM), [$K_m$ for Ins(1,4,5)$P_3$ is 13.8 μM] and 3-kinase ($K_i$ value of 0.97 μM), [$K_m$ value for Ins(1,4,5)$P_3$ is 0.85 μM].[5] It was a direct lead to Ins(1,4,6)$P_3$ **4a** where the axial hydroxyl group of L-*chiro*-Ins(2,3,5)$P_3$ **26**, corresponding to position-2 of Ins(1,4,5)$P_3$, is now equatorial. Ins(1,4,6)$P_3$ **4a** is a full agonist for $Ca^{2+}$ release[6] and only 2-fold less potent than Ins(1,4,5)$P_3$ in permeabilised platelets. An enzyme study by Hirata *et al.*[21] demonstrated that Ins(1,4,6)$P_3$ **4a** is resistant to 5-phosphatase [$K_i$ value 9.2 μM, (*cf* Ins(1,4,5)$P_3$, $K_i$ value 15.9 μM)]† and a potent 3-kinase inhibitor: $IC_{50}$ value of 2 μM (approx.) in the presence of 30 μM $Ca^{2+}$ and 8 μM at < 0.01 μM $Ca^{2+}$. This work shows that the hydroxyl orientation at position-2 of Ins(1,4,5)$P_3$ is not significant for 3-kinase recognition. However, inverting the stereochemistry at position-3 of Ins(1,4,5)$P_3$ to give L-*chiro*-Ins(2,3,5)$P_3$, delivers a potent 3-kinase and 5-phosphatase inhibitor and a molecule which retains $Ca^{2+}$ releasing properties when it binds to the Ins(1,4,5)$P_3$ receptor.




**1a** Ins(1,4,5)$P_3$ X and Z = OH; Y and Z' = H

**2a** D-Ins(1,3,4,5)$P_4$ Z = OH; X = $OPO_3^{2-}$ Y and Z' = H

**3a** D-Ins(1,2,4,6)$P_4$ Z' = OH; Y = $OPO_3^{2-}$ X and Z = H

**4a** D-Ins(1,4,6)$P_3$ X and Z = H; Y and Z' = OH

**5a** D-Ins(1,2,4,5)$P_4$ X = OH; Z = $OPO_3^{2-}$ Y and Z' = H

**26** L-*chiro*-Ins(2,3,5)$P_3$ X and Z' = OH; Y and Z' = H

**27** L-*chiro*-Ins(1,2,3,5)$P_4$ Z = OH; Y = $OPO_3^{2-}$ X and Z' = H

**Fig. 4** Modifications of positions 2- and 3- around a D-1,4,5-trisphosphate environment.

The addition of a phosphate group at the equatorial position-3 of Ins(1,4,5)$P_3$ gives Ins(1,3,4,5)$P_4$ **2a**, the most potent 5-phosphatase inhibitor (albeit a co-substrate, $IC_{50}$ value of 0.15 μM).[4] Kozikowski *et al.*[23] synthesised L-*chiro*-Ins(1,2,3,5)$P_4$ **27** which is an analogue of Ins(1,3,4,5)$P_4$ in which a phosphate has been substituted at position-1 of **26** to give tetrakisphosphate **27**, resulting in a significant reduction in $Ca^{2+}$ release for **27**, which is 700-fold less potent than Ins(1,4,5)$P_3$ at binding to the Ins(1,4,5)$P_3$ receptor. In this respect, **27** is similar to Ins(1,3,4,5)$P_4$ **2a** in its $Ca^{2+}$ releasing properties.[4] Ins(1,3,4,5)$P_4$

† $IC_{50}$ is the concentration of agent inhibiting the enzyme activity by 50% at 1 μM substrate concentration. In these experiments, inhibiting the phosphorylation of [$^3$H] Ins(1,4,5)$P_3$. $K_i$ is the dissociation constant for an enzyme–inhibitor complex calculated from $IC_{50}$ values, using the Cheng and Prusoff equation.[22]

**Table 1** $IC_{50}$ values determined on recombinant enzymes and calculated by non linear regression (curve fit) at 1 μM $Ins(1,4,5)P_3$. Analogues were evaluated over a range of concentrations 0.1–100 μM. Typical data for 5-phosphatase are given

| Compound | Recombinant $Ins(1,4,5)P_3$ 5-phosphatase from human brain $IC_{50}$/μM | Recombinant 3-kinase-A from rat brain $IC_{50}$/μM |
|---|---|---|
| D-$Ins(1,3,4,5)P_4$ **2a** | 0.19 | 1.4 |
| D-$Ins(1,2,4,6)P_4$ **4a** | 3.8 | >100 |
| L-$Ins(1,2,4,6)P_4$ **4b** | 14 | >>100 |
| D-$Ins(1,2,4,5)P_4$ **5a** | 0.47 | >100 |
| L-$Ins(1,2,4,5)P_4$ **5b** | 3 | >>100 |

**2a** has been evaluated for $Ca^{2+}$ release and the inhibition of 3-kinase. Some literature (Pollokoff *et al.*[24] and Gawler *et al.*[25]) indicates that it releases $Ca^{2+}$ from intracellular stores, but Bird and Putney Jr.[26] demonstrated that $Ins(1,3,4,5)P_4$ was ineffective at mobilising $Ca^{2+}$: the older literature studies probably used $Ins(1,3,4,5)P_4$ contaminated with small quantities of $Ins(1,4,5)P_3$. Only a small number of groups have evaluated racemic $Ins(1,3,4,5)P_4$ against 3-kinase. They found it was a poor inhibitor of the enzyme with $IC_{50}$ values of 90 μM[24] and 220 μM.[27] However, in our experiments, (Table 1 and Fig. 5) D-$Ins(1,3,4,5)P_4$ **2a** was a potent 3-kinase inhibitor (type-A, from rat brain, clone C5), with an $IC_{50}$ value of 1.4 μM. This may result from the use of recombinant enzyme, different assay conditions or the use of other $Ins(1,4,5)P_3$ 3-kinase isoenzymes. Moreover, evaluating inositol phosphate analogues in crude lysates may lead to degradation of some compounds due to $Ins(1,4,5)P_3$ 5-phosphatase activity in the assay. The fact that $Ins(1,3,4,5)P_4$ is an inhibitor of $Ins(1,4,5)P_3$ 3-kinase is not surprising and probably results from product inhibition derived from our assay conditions.

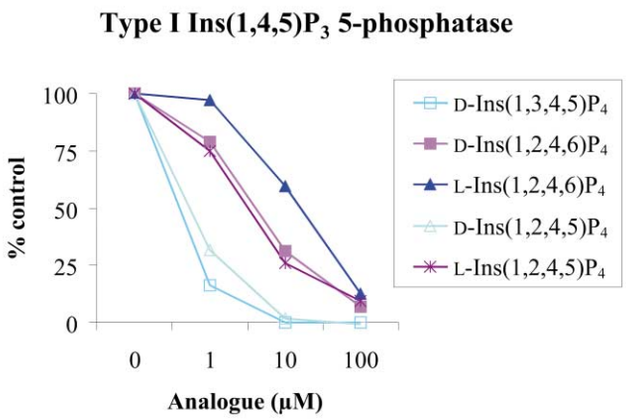



**Fig. 5** The curves illustrate the inhibition of recombinant $Ins(1,4,5)P_3$ 5-phosphatase from human brain by $Ins(1,3,4,5)P_4$, D- and L-$Ins(1,2,4,6)P_4$ and D- and L-$Ins(1,2,4,5)P_4$ in the presence of 1 μM $Ins(1,4,5)P_3$.

Using similar reasoning for the synthesis of $Ins(1,4,6)P_3$ derived from $Ins(1,4,5)P_3$ and L-*chiro*-$Ins(2,3,5)P_3$, addition of a phosphate at position-2 of $Ins(1,4,6)P_3$ would deliver $Ins(1,2,4,6)P_4$, a regioisomer of $Ins(1,3,4,5)P_4$. The axial 2-phosphate of $Ins(1,2,4,6)P_4$ can be superimposed on the 3-position of $Ins(1,3,4,5)P_4$ and the axial 2-hydroxyl of $Ins(1,3,4,5)P_4$ and the 3-hydroxyl in $Ins(1,2,4,6)P_4$ are equatorial (see Fig. 3): the L-enantiomer was also synthesised and evaluated. Examination of structures **1a**, **3a** and **3b** (Fig. 1) illustrates that, should DL-$Ins(1,2,4,6)P_4$ possess $Ins(1,4,5)P_3$-like $Ca^{2+}$ mobilising activity, we would expect activity to reside only in the D-enantiomer as for the $Ins(1,4,5)P_3$ enantiomers.[14] We found that at a concentration of 200 μM there was however, no demonstrable agonism or antagonism in permeabilised hepatocytes for $Ca^{2+}$ release for racemic $Ins(1,2,4,6)P_4$ **3ab** and in receptor binding it had an $IC_{50}$ value of 1.06 μM in cerebellum {*cf* $IC_{50}$ *circa* 50 nM for [$^3$H]$Ins(1,4,5)P_3$}. Previously, racemic $Ins(1,2,4,6)P_4$ **3ab** was synthesised by Chung and Chang[28] and was evaluated for $Ca^{2+}$ release in Chinese Hamster Ovary cells,[29] where it mobilised only 34.7% of the intracellular $Ca^{2+}$ stores at 100 μM concentration. Its affinity for the $Ins(1,4,5)P_3$ receptor of bovine adrenal cortical membranes was some 653-fold lower than $Ins(1,4,5)P_3$.[29] Fig. 6 shows the biologically active $Ins(1,4,5)P_3$ regioisomer $Ins(1,4,6)P_3$ superimposed upon $Ins(1,4,5)P_3$ and docked into the $Ins(1,4,5)P_3$ binding site of the recently published crystal structure of type-I $Ins(1,4,5)P_3$ receptor.[30] Using such docking studies we can predict that a phosphate group cannot be accommodated comfortably in the axial direction at position-3 of $Ins(1,4,5)P_3$, which corresponds to the 2-position of $Ins(1,4,6)P_3$ (Fig. 6). The resulting $Ins(1,2,4,6)P_4$ should therefore have low affinity for the $Ins(1,4,5)P_3$ receptor and be ineffective at $Ca^{2+}$ release, as demonstrated by our results above and those of others.

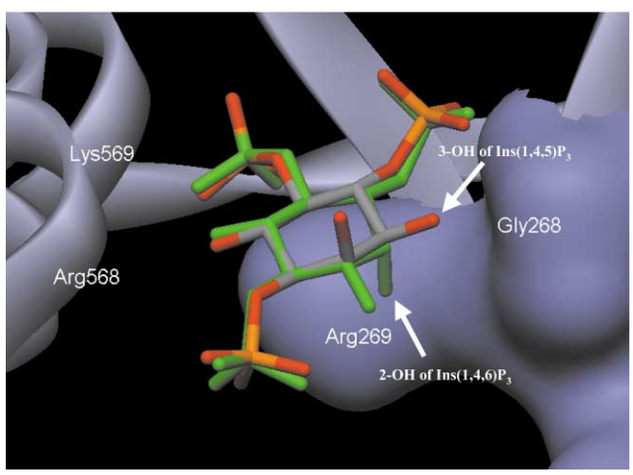



**Fig. 6** Three dimensional structure of the $Ins(1,4,5)P_3$ binding site of the type 1 receptor based on the X-ray crystal structure of the mouse receptor core binding domain in complex with $Ins(1,4,5)P_3$.[30] Molecular docking experiments suggest that $Ins(1,4,6)P_3$ (green) may be a relatively effective mimic of $Ins(1,4,5)P_3$ at the type 1 $Ins(1,4,5)P_3$ binding site because it can bind in an orientation that allows its phosphate groups to mimic the three phosphate groups of $Ins(1,4,5)P_3$ while its axial 2-hydroxyl group is accepted by an open region close to Gly-268. The region close to the 2-hydroxyl group of $Ins(1,4,6)P_3$ is sterically constrained to addition of another phosphate group as in $Ins(1,2,4,6)P_4$. For clarity, all hydrogen atoms and the six crystallographic observed water molecules have been omitted.

Racemic $Ins(1,2,4,6)P_4$ **3ab**, was tested for inhibition of 3-kinase from rat brain.[27] In their study, Choi and co-workers[27] reported that racemic $Ins(1,2,4,6)P_4$ showed greater inhibition of 3-kinase ($IC_{50}$ value of 42.1 μM) than $Ins(1,3,4,5)P_4$ ($IC_{50}$ value of 220 μM). Our study is the first in which both enantiomers, D- and L-$Ins(1,2,4,6)P_4$ (**3a** and **3b**), have been evaluated against both 3-kinase and 5-phosphatase. D-$Ins(1,2,4,6)P_4$ (Table 1 and Fig. 5) was found to be a good inhibitor of 5-phosphatase with an $IC_{50}$ value of 3.8 μM [*cf* $Ins(1,4,6)P_3$ has a $K_i$ value of 9.2μM from another study], although it was 20-fold weaker than $Ins(1,3,4,5)P_4$ and not recognised by 3-kinase. L-$Ins(1,2,4,6)P_4$ (Table 1 and Fig. 5) was a reasonable inhibitor of 5-phosphatase with an $IC_{50}$ value of 14 μM and some 74-fold

weaker than Ins(1,3,4,5)$P_4$, but not recognised by 3-kinase. To complete this study we also evaluated two other chiral tetrakisphosphates, namely, D-Ins(1,2,4,5)$P_4$ **5a** and L-Ins(1,2,4,5)$P_4$ **5b** (Table 1 and Fig. 5).[10] Previously, we[31] and others[32] have shown that racemic Ins(1,2,4,5)$P_4$ is a full agonist at the Ins(1,4,5)$P_3$ receptor and a potent 5-phosphatase inhibitor, but is not recognised by 3-kinase. The biological interaction of these enantiomers with 3-kinase and 5-phosphatase has, however, not been examined. We found that D-Ins(1,2,4,5)$P_4$ **5a** is a potent inhibitor of 5-phosphatase having an $IC_{50}$ value of 0.47 μM but is not recognised by 3-kinase. L-Ins(1,2,4,5)$P_4$ is also a good inhibitor of 5-phosphatase, with an $IC_{50}$ value of 3 μM and does not inhibit 3-kinase.

These results illustrate some recognition elements of 3-kinase. We have found that only Ins(1,3,4,5)$P_4$ inhibits 3-kinase significantly, having an equatorial 3-phosphate and an axial 2-hydroxyl moiety and a 6-hydroxyl together with a D-1,4,5 arrangement of phosphates. Deviation from these requirements results in a dramatic loss of recognition by the 3-kinase, although inverting the stereochemistry at the 3-position of Ins(1,4,5)$P_3$ **1a** to give L-*chiro*-Ins(2,3,5)$P_3$ **26** delivers a potent 3-kinase inhibitor.

For 5-phosphatase inhibition, the D-series of inositol phosphates possessing a D-1,4,5-type of phosphate arrangement plus an extra phosphate at the 2- or 3-position is more potent than the L-series of inositol tetrakisphosphates. Similarly, phosphate substitution at position-2 of Ins(1,4,6)$P_3$ to give Ins(1,2,4,6)$P_4$, delivers a more potent 5-phosphatase inhibitor than the 1,4,6-trisphosphate analogue. This corresponds with the general trend that tetrakisphosphates are more potent 5-phosphatase inhibitors than trisphosphate derivatives. However, trisphosphates having a D-1,4,5-configuration and a 6-hydroxyl [derived from L-*chiro*-Ins(2,3,5)$P_3$ and Ins(1,4,6)$P_3$] are superior inhibitors of 3-kinase than most of the tetrakisphosphates.

We have synthesised D- and L-Ins(1,2,4,6)$P_4$ and evaluated these compounds against, 5-phosphatase and 3-kinase. Broadly, our results confirm early conclusions that 5-phosphatase has loose specificity of recognition, where that for 3-kinase is very stringent.[5] In agreement with the 5-phosphatase activity of other tetrakisphosphates, D-Ins(1,2,4,6)$P_4$ is more potent than the corresponding D-Ins(1,4,6)$P_3$ **4a** and the D-series of inositol trisphosphates derived from a 1,4,5-trisphosphate motif and an additional 6-hydroxyl group is more potent than the corresponding L-derivatives. Substitution of a phosphate group at position-2 of Ins(1,4,6)$P_3$ to give Ins(1,2,4,6)$P_4$ **3a**, removes any interaction of this compound with 3-kinase.

Thus, we have synthesised the enantiomers of Ins(1,2,4,6)$P_4$ and provide the first evaluation of the activity against Ins(1,4,5)$P_3$ 5-phosphatase and 3-kinase. We have also rationalised why D-Ins(1,2,4,6)$P_4$ is apparently inactive in $Ca^{2+}$ mobilisation at the ins(1,4,5)$P_3$ receptor, relative to the active trisphosphate Ins(1,4,6)$P_3$. These results contribute to the developing structure–activity relationship amongst the soluble inositol polyphosphates.

## Experimental

### Materials and methods

Chemicals were purchased from Aldrich, Fluka and Lancaster. Sodium hydride was 60% pure in a dispersion 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, and heated at high temperature. Flash chromatography refers to the procedure developed by Still *et al.*[33] and was carried out on Sorbsil C60 silica gel. D-Ins(1,3,4,5)$P_4$ was purchased from Eurobiochem (Belgium) and D- and L-Ins(1,2,4,5)$P_4$ were synthesised according to the published procedure.[10] All final compounds were homogeneous as judged by standard spectroscopic methods and purified by ion exchange chromatography and used as their triethylammonium salts. 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.[11] (Diisopropylamino)dichlorophosphine was prepared by the method of Tanaka *et al.*[34] 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 ($\delta_P$ = +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 methylene dichloride, to afford bis(benzyloxy)(diisopropylamino)phosphine[9] ($\delta_P$ = +147.9 ppm) which was pure by $^{31}P$ NMR, ($R_f$ = 0.78, hexane–triethylamine 10 : 1). NMR spectra (proton frequency 270, or 400 MHz) were referenced to $SiMe_4$, (HDO) or [$^2H_6$]-dimethyl sulfoxide ([$^2H_6$]DMSO). The $^{31}P$ NMR shifts were measured in ppm relative to external 85% phosphoric acid. Melting points (uncorrected) were determined using a Reichert-Jung Thermo Galen Kofler block. Microanalysis was carried out by the Microanalysis Service, at the University of Bath. Mass spectra were recorded by the University of Bath Mass Spectrometry Service using positive and negative 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 $[a]_D$-values are given in $10^{-1}$ deg cm$^2$ g$^{-1}$ and were measured at ambient temperature.

### Ins(1,4,5)$P_3$ 5-phosphatase and 3-kinase activities

The activities of Ins(1,4,5)$P_3$ 5-phosphatase and 3-kinase were determined at 1 μM of Ins(1,4,5)$P_3$ substrate concentration using published procedures.[35,36] We used purified recombinant enzymes from rat brain for the Ins(1,4,5)$P_3$ 3-kinase (clone C5). Cloning and expression was carried out in *Escherichia coli* of the rat brain cDNA encoding a $Ca^{2+}$/calmodulin-sensitive *myo*-inositol 1,4,5-trisphosphate 3-kinase,[37] and the source of Ins(1,4,5)$P_3$ 5-phosphatase was derived from human brain (clone ECH11).[38] The Ins(1,4,5)$P_3$ 3-kinase activity was determined in the presence of 0.9 mM EGTA. The incubation period was 10 min in the presence of [$^3$H]Ins(1,4,5)$P_3$ which was provided by NEN; 1 μM of cold Ins(1,4,5)$P_3$ was sourced from EuroBiochem.

### Molecular modelling

Molecular docking of Ins(1,4,6)$P_3$ and Ins(1,4,5)$P_3$ to the ligand binding domain of the Ins(1,4,5)$P_3$ receptor was carried out similarly to that described by Rosenberg *et al.*[39]

### 2,4,6-Tri-*O*-allyl-*myo*-inositol orthoformate 7

A mixture of *myo*-inositol orthoformate[7] **6** (3.83 g, 20 mmol) and 60% sodium hydride (4.0 g, 100 mmol) was stirred in DMF (50 cm$^3$) at 0 °C. Allyl bromide (6.05 cm$^3$, 70 mmol) was added dropwise over 10 min, and the reaction mixture was stirred at room temperature for 3 h. TLC ($Et_2O$–hexane 1 : 1), showed a single product ($R_f$ 0.44). The reaction was cooled with ice–water and the excess sodium hydride was destroyed with methanol (10 cm$^3$). The solvents were evaporated *in vacuo* and the residue was partitioned between ether (200 cm$^3$) and water (200 cm$^3$). The organic layer was separated and dried ($MgSO_4$), then evaporated to give an oil, which was purified by flash chromatography ($Et_2O$–pentane 1 : 1) to give the title compound **7** as a syrup. Yield (5.54 g, 89%); (Found: C, 61.7; H, 7.23. $C_{16}H_{22}O_6$

requires C, 61.92; H, 7.15%); $\delta_H$ (270 MHz; $CDCl_3$) 3.91 (1 H, d, *J* 1.65, CH), 4.01–4.33 (10 H, m, 3 × $OCH_2CHCH_2$ and 4 inositol ring protons), 4.41 (1 H, m, CH), 5.18–5.37 (6 H, m, 3 × $OCH_2CHCH_2$), 5.53 (1 H, d, *J* 1.3, CH), 5.81–6.07 (3 H, m, 3 × $OCH_2CHCH_2$); $\delta_C$ (68 MHz; $CDCl_3$) 67.48, 67.58, 68.05, 73.76 (*myo*-inositol ring carbons), 70.59 ($OCH_2CHCH_2$), 103.11 (*C*H, orthoformate), 117.42, 117.67 ($OCH_2CHCH_2$), 134.12, 134.57 ($OCH_2CHCH_2$); *m*/*z* ($FAB^+$) 621.3 (46), 446.1 (39), 311.1 (100), 253.1 (20), 153.1 (32), 81.0 (34).

### 2,4,6-Tri-*O*-allyl-*myo*-inositol 8

2,4,6-Tri-*O*-allyl-*myo*-inositol orthoformate **7** (5.0 g, 16.1 mmol) was heated at reflux temperature in a mixture of ethanol and 1.0 mol $dm^{-3}$ aq. HCl (60 $cm^3$ 2 : 1) for 3 h, after which TLC ($CH_2Cl_2$–EtOAc 1 : 1) showed a single product ($R_f$ 0.30). The solvents were evaporated *in vacuo* to give a syrup, then co-evaporated with water (2 × 50 $cm^3$) to give the title compound **8** (4.5 g, 93%) as a solid; mp 77–78 °C (from EtOAc–hexane); (Found: C, 60.0; H, 8.15. $C_{15}H_{24}O_6$ requires C, 59.98; H, 8.05%); $\delta_H$ (400 MHz; $CDCl_3$) 2.55 (2 H, d, *J* 5.5, $D_2O$ ex, OH), 2.74 (1 H, d, *J* 2.1, $D_2O$ ex, OH), 3.41–3.50 (5 H, m, 1-H, 3-H, 4-H, 5-H and 6-H), 3.90 (1 H, s, 2-H), 4.31–4.40 (6 H, m, 3 × $OCH_2CHCH_2$), 5.18–5.33 (6 H, m, 3 × $OCH_2CHCH_2$), 5.90–6.02 (3 H, m, 3 × $OCH_2CHCH_2$); $\delta_C$ (100 MHz; $CDCl_3$) 73.94, 74.28 ($OCH_2CHCH_2$), 72.37, 74.76, 79.17, 81.69 (*myo*-inositol ring carbons), 116.93, 117.24 ($OCH_2CHCH_2$), 134.96, 135.07 ($OCH_2CHCH_2$); *m*/*z* ($FAB^+$) 601.2 (10), 301.1 (88), 243.1 (100), 153.1 (44), 109.0 (88), 81.0 (70).

### DL-1,2,4,6-Tetra-*O*-allyl-*myo*-inositol 9ab

A mixture of 2,4,6-tri-*O*-allyl-*myo*-inositol **8** (4.3 g, 14.3 mmol), allyl bromide (1.47 $cm^3$ 17 mmol), tetrabutylammonium sulfate[8] (5.08 g, 15 mmol) in methylene dichloride (150 $cm^3$) and 5% aq. sodium hydroxide (150 $cm^3$), was heated at reflux temperature for 24 h. The organic layer was separated and washed with water (2 × 100 $cm^3$) then dried ($MgSO_4$) and evaporated to give a syrup. Flash chromatography ($CH_2Cl_2$–EtOAc 3 : 1) gave the title compound **9ab** as a syrup (3.6 g, 74%, $R_f$ 0.62, $CH_2Cl_2$–EtOAc 1 : 1), together with other minor products $R_f$ 0.88, $R_f$ 0.44 and a tiny quantity of starting material $R_f$ 0.24, which were not isolated; (Found: C, 63.4; H, 8.36. $C_{18}H_{28}O_6$ requires C, 63.49; H, 8.30%); $\delta_H$ (400 MHz; $CDCl_3$) 2.45 (2 H, br m, $D_2O$ ex, OH), 3.23 (1 H, dd, *J* 2.45, 9.8, 1-H), 3.39 (1 H, t, *J* 8.8, 5-H), 3.42 (1 H, dd, *J* 2.9, 10.7, 3-H), 3.51 (1 H, t, *J* 9.3, 4-H), 3.64 (1 H, t, *J* 9.8, 6-H), 3.93 (1 H, t, *J* 2.9, 2-H), 4.07–4.45 (8 H, m, 4 × $OCH_2CHCH_2$), 5.18–5.33 (8 H, m, 4 × $OCH_2CHCH_2$), 5.90–6.02 (4 H, m, 4 × $OCH_2CHCH_2$); $\delta_C$ (100 MHz; $CDCl_3$) 71.52, 71.94, 73.86, 74.23 ($OCH_2CHCH_2$), 71.94, 74.65, 76.72, 80.56, 80.76 (*myo*-inositol ring carbons), 116.80, 116.90, 117.11 ($OCH_2CHCH_2$), 135.20, 135.26 ($OCH_2CHCH_2$); *m*/*z* ($FAB^+$) 681.9 (68), 341.2 (100), 283.2 (62), 225.2 (6), 111.0 (16), 97 (36).

### DL-1,2,4,6-Tetra-*O*-allyl-3,5-di-*O*-benzyl-*myo*-inositol 10ab

A mixture of DL-1,2,4,6-tetra-*O*-allyl-*myo*-inositol **9ab** (1.60 g, 4.70 mmol) and sodium hydride (1.25 g, 31.25 mmol) was stirred in dry DMF (40 $cm^3$). Benzyl bromide (1.68 $cm^3$, 14.1 mmol) was added dropwise and the solution was stirred for 2 h at room temperature. TLC ($Et_2O$–pentane 1 : 3) showed a new product $R_f$ 0.50. The excess sodium hydride was destroyed with methanol (10 $cm^3$) and the solvents were evaporated *in vacuo* to give an oil. The crude product was partitioned between water and ether (50 $cm^3$ of each) and washed with 0.10 mol $dm^{-3}$ aq. hydrochloric acid, then water again (50 $cm^3$ of each). The organic layer was dried ($MgSO_4$) and the solvent was evaporated to give an oil. The crude product was purified by flash chromatography (pentane–$Et_2O$ 3 : 1) to give the title compound **10ab** (2.15 g, 88%) as an oil which solidified, but could not be crystallised; (Found: C, 73.5; H, 7.68. $C_{32}H_{40}O_6$ requires C, 73.81; H, 7.75%); $\delta_H$ (400 MHz; $CDCl_3$) 3.14 (1 H, dd, *J* 2.4, 9.8, 1-H or 3-H), 3.25 (1 H, t, *J* 2.1, 9.8, 1-H or 3-H), 3.32 (1 H, t, *J* 9.2, 5-H), 3.77 (1 H, t, *J* 9.5, 4-H or 6-H), 3.80 (1 H, t, *J* 9.8, 4-H or 6-H), 3.90 (1 H, t, *J* 2.1, 2-H), 4.10–4.40 (8 H, m, 4 × $OCH_2CHCH_2$), 4.66, 4.70 (2 H, AB, *J* 11.9, $OCH_2Ph$), 4.82 (2 H, s, $OCH_2Ph$), 5.12–5.31 (8 H, m, 4 × $OCH_2CHCH_2$), 5.85–6.03 (4 H, m, 4 × $OCH_2CHCH_2$), 7.22–7.38 (10 H, m, 2 × $OCH_2Ph$); $\delta_C$ (100 MHz; $CDCl_3$) 71.65, 72.82, 73.24, 74.54, 75.99 ($CH_2Ph$ and $OCH_2CHCH_2$), 74.12, 80.32, 80.58, 81.33, 83.61 (*myo*-inositol ring carbons), 116.44, 116.48, 116.55, 116.64 ($OCH_2CHCH_2$), 127.54, 128.09, 128.31, 128.34 ($CH_2Ph$), 134.96, 135.50, 135.64, 135.77 ($OCH_2CHCH_2$), 138.49, 138.88 ($C_q$, $CH_2Ph$); *m*/*z* ($FAB^+$) 521.1 (88), 249.0 (12), 181.0 (50), 91.0 (100).

### DL-1,5-Di-*O*-benzyl-*myo*-inositol 11ab

A mixture of DL-1,2,4,6-tetra-*O*-allyl-3,5-di-*O*-benzyl-*myo*-inositol **10ab** (1.20 g, 2.50 mmol), palladium on activated charcoal (10% Fluka, 1.0 g) and toluene *p*-sulfonic acid was dissolved in methanol–water (120 $cm^3$, 5 : 1) then heated under reflux for 1.5 h, after which TLC (EtOAc) showed a new product $R_f$ 0.20. The solution was filtered through Celite and evaporated to give a solid. The crude product was purified by flash chromatography ($CHCl_3$–MeOH 10 : 1) to give the title compound **11ab**, (441 mg, 49%) as a solid; mp 139–141 °C (from ethanol); (Found: C, 66.4; H, 6.72. $C_{20}H_{24}O_6$ requires C, 66.64; H, 6.72%); $\delta_H$ (270 MHz; [$^2H_6$]DMSO) 3.04 (1 H, t, *J* 9.0, 5-H), 3.14 (2 H, d, *J* 9.3, 1-H and 3-H, $D_2O$ ex, 2 dd, overlapping), 3.58 (1 H, dt, *J* 9.3, 5.0, 4-H or 6-H), 3.77 (1 H, dt, *J* 9.3, 5.5, 4-H or 6-H), 3.98 (1 H, s, 2-H), 4.56 (1 H, d, *J* 5.5, $D_2O$ ex, OH), 4.58 (1 H, d, *J* 7.0, $D_2O$ ex, OH), 4.64 (2 H, m, $OCH_2Ph$), 4.73 (1 H, d, *J* 4.9, $D_2O$ ex, OH), 4.79 (2 H, app s, $OCH_2Ph$), 4.92 (1 H, d, *J* 5.3, $D_2O$ ex, OH), 7.21–7.45 (10 H, m, 2 × $OCH_2Ph$); $\delta_C$ (68 MHz; [$^2H_6$]DMSO) 69.12, 71.86, 71.92, 72.42, 80.16, 84.33 (*myo*-inositol ring carbons), 70.79, 73.80 ($CH_2Ph$), 127.01, 127.19, 127.60, 127.94, 128.08 ($CH_2Ph$), 139.34, 139.95 ($C_q$, $CH_2Ph$); *m*/*z* ($FAB^+$) 513.1 (76), 359.1 (36), 308.0 (26), 91.0 (100).

### DL-3,5-Di-*O*-benzyl-1,2,4,6-tetrakis-*O*-[di(benzyloxy)-phosphoryl)]-*myo*-inositol 13ab

A mixture of bis(benzyloxy)diisopropylaminophosphine (0.69 g, 2 mmol) and 1*H*-tetrazole (0.28 g, 4 mmol) in dry methylene dichloride (5 $cm^3$) was stirred at room temperature for 15 min. DL-1,5-Di-*O*-benzyl-*myo*-inositol **11ab** (0.108 g, 0.30 mmol) was added to the solution which was stirred for a further 10 min. The reaction mixture was cooled with ice–water and (50–60%) MCPBA (0.80 g, 2.30 mmol) was added slowly and the mixture was stirred for a further 30 min. The solution was diluted with ethyl acetate (50 $cm^3$) and washed with 10% aq. sodium metabisulfite (50 $cm^3$), saturated aq. sodium hydrogen carbonate, brine and water (50 $cm^3$ of each). The organic layer was isolated then dried ($MgSO_4$) and evaporated to give an oil. The crude product was purified by flash chromatography, ($R_f$ 0.20, $CHCl_3$–acetone 5 : 1) then EtOAc–pentane 2 : 1, to give the title compound **13ab** (0.382 g, 91%) as a solid; mp 76–77 °C (from EtOAc–pentane); $\delta_H$ (400 MHz; $CDCl_3$) 3.63 (1 H, d, *J* 10.3, 3-H), 3.63 (1 H, t, *J* 9.8, 5-H), 4.47–5.20 (23 H, m, 1-H, 4-H, 6-H and 10 × $OCH_2Ph$), 5.58 (1 H, d, *J* 8.85, 2-H), 6.92–7.46 (50 H, m, 8 × $O(O)POCH_2Ph$, 2 × $OCH_2Ph$); $\delta_C$ (100 MHz; $CDCl_3$) 69.24, 69.29, 69.37, 69.40, 69.55, 69.60, 69.68, 70.00, 70.15, 72.16, 74.56 ($CH_2Ph$), 73.95, 74.40, 75.88, 76.97, 78.50, 79.30 (*myo*-inositol ring carbons), 127.16, 127.65, 127.80, 127.85, 127.94, 128.05, 128.11, 128.16, 128.27, 128.40, 128.53, 128.64, 129.48, 129.90 ($CH_2Ph$), 133.37, 134.22, 135.39, 135.53, 135.62, 136.56, 137.87 ($C_q$, $CH_2Ph$); $\delta_P$ (162 MHz; $D_2O$) −1.04, −1.26, −1.43, −2.75; *m*/*z* ($FAB^+$) 1401.0 (64), 1311.0

(15), 1033.0 (10), 459 (16), 361 (40), 301 (10), 181.1 (22), 91 (100); [Found: *m*/*z*, 1401.4066 $(M + H)^+$ requires *m*/*z* 1401.4060].

### DL-*myo*-Inositol 1,2,4,6-tetrakisphosphate 3ab

DL-3,5-Di-*O*-benzyl-1,2,4,6-tetrakis-*O*-[di(benzyloxyphosphoryl)]-*myo*-inositol **13ab** (0.165 g, 0.118 mmol) was hydrogenolysed in a mixture of methanol (40 $cm^3$) and water (10 $cm^3$), in the presence of palladium on carbon (10%, 0.20 g) for 20 h. The reaction mixture was filtered over a bed of Celite to remove the insoluble components, and washed with water and methanol, then evaporated *in vacuo* to give a syrup. The residue was then dissolved in MilliQ water (150 $cm^3$) and purified by ion exchange chromatography on Q-Sepharose Fast Flow with a gradient of TEAB buffer (0–1000 mmol $dm^{-3}$) at pH 8.6. The triethylammonium salt of **3ab** eluted at *ca*. 800 mmol $dm^{-3}$. Yield (39 μmol, 33%); $\delta_H$ (400 MHz; $D_2O$) 3.60 (1 H, t, *J* 9.2, 5-H), 3.69 (1 H, d, *J* 9.3, 3-H), 4.10 (1 H, *J* 9.3, 1-H), 4.23 (1 H, q, *J* 9.2, 4-H or 6-H), 4.32 (1 H, q, *J* 9.2, 4-H or 6-H), 4.69 (1 H, d, *J* 9.2, 2-H); $\delta_P$ (162 MHz; $D_2O$) 2.12 (1 P, d, *J* 10.1), 1.75 (1 P, br d, *J* 13.5), 1.62 (1 P, d, *J* 10.0), 1.32 (1 P, d, *J* 10.0); *m*/*z* ($FAB^-$) 999.1 (5), 499.0 (100), 291.2 (5); [Found: *m*/*z*, 498.9193 $(M–H)^-$ requires *m*/*z* 498.9208].

### DL-2,3-*O*-Isopropylidene-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol 16ab, DL-2,3-*O*-isopropylidene-1,4,5-tris-*O*-*p*-methoxybenzyl-*myo*-inositol 15ab and DL-2,3-*O*-isopropylidene-1,4,5,6-tetrakis-*O*-*p*-methoxybenzyl-*myo*-inositol 17ab

A mixture of DL-2,3-*O*-isopropylidene-1,4-di-*O*-*p*-methoxybenzyl-*myo*-inositol[12] **14ab** (9.21 g, 20 mmol), acetonitrile (400 $cm^3$), dibutyltin oxide (5.48 g, 22 mmol), tetrabutylammonium bromide (6.45 g, 20 mmol) and *p*-methoxybenzyl chloride (4.35 $cm^3$, 30 mmol) was heated under reflux in a Soxhlet apparatus containing 4 Å molecular sieves for 16 h. After this time a further quantity of *p*-methoxybenzyl chloride (4.35 $cm^3$, 30 mmol) was added, together with some tetrabutylammonium iodide (7.38 g, 20 mmol) and heated under reflux for a further 24 h, after which the reaction was complete according to TLC. The reaction mixture was cooled, the solvent was evaporated and the orange residue was partitioned between water and ether (250 $cm^3$ of each). The organic layer was separated and stirred with a saturated aqueous solution of sodium hydrogen carbonate (250 $cm^3$) for 1 h. The solid components were removed by filtering the solution over Celite then washed with ether and the organic layer was dried over magnesium sulfate. TLC ($CH_2Cl_2$–EtOAc 1 : 1) showed four products, *p*-methoxybenzyl halide, $R_f$ 0.78; DL-2,3-*O*-isopropylidene-1,4,5,6-tetrakis-*O*-*p*-methoxybenzyl-*myo*-inositol **17ab**, $R_f$ 0.44; DL-2,3-*O*-isopropylidene-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol **16ab**, $R_f$ 0.34 and DL-2,3-*O*-isopropylidene-1,4,5-tris-*O*-*p*-methoxybenzyl-*myo*-inositol **15ab**, $R_f$ 0.22, which were separated by flash chromatography to give the products as syrups. DL-2,3-*O*-Isopropylidene-1,4,5-tris-*O*-*p*-methoxybenzyl-*myo*-inositol **15ab** was recrystallised from $Et_2O$–pentane. **16ab** (5.34 g, 46%), **15ab** (3.01 g, 26%), **17ab** (0.87 g, 6%).

**16ab** (Found: C, 68.1; H, 6.99. $C_{33}H_{40}O_9$ requires C, 68.26; H, 6.94%); $\delta_H$ (400 MHz; [$^2H_6$]DMSO) 1.28, 1.38 (6 H, 2 s, $CMe_2$), 3.32 (1 H, br t, $D_2O$ ex, t, *J* 9.2, 5-H), 3.50 (2 H, m, 1-H or 3-H and 6-H), 3.66 (1 H, dd, *J* 3.7, 7.6, 4-H), 3.74 (9 H, s, OMe), 4.05 (1 H, t, *J* 6.7, 1-H or 3-H), 4.36 (1 H, t, *J* 4.0, 2-H), 4.52–4.69 (6 H, m, 3 × $OCH_2C_6H_4OMe$), 5.15 (1 H, br s, $D_2O$ ex, OH), 6.86–6.89 (6 H, m, $OCH_2C_6H_4OMe$), 7.23–7.32 (6 H, m, $OCH_2C_6H_4OMe$); $\delta_C$ (100 MHz; [$^2H_6$]DMSO) 25.67, 27.56 ($CMe_2$), 55.03 ($CH_2PhOMe$), 72.20, 73.06, 73.20 ($CH_2$PhOMe), 71.16, 73.66, 76.46, 78.23, 80.79, 81.84 (*myo*-inositol ring carbons), 108.50 ($C_q$, $CMe_2$), 113.38, 113.51, 127.90, 129.04, 129.20 ($CH_2PhOMe$), 130.57, 130.99, 131.09 ($C_q$, $CH_2PhOMe$), 158.55, 158.63 ($C_q$, $CH_2PhOMe$); *m*/*z* ($FAB^-$) 733.3 (100), 579.2 (60), 467.1 (50), 258.1 (44), 92 (30).

**15ab** (Found: C, 68.0; H, 6.90. $C_{33}H_{40}O_9$ requires C, 68.26; H, 6.94%); mp 84–86 °C (from $Et_2O$–pentane); $\delta_H$ (270 MHz; [$^2H_6$]DMSO) 1.27, 1.38 (6 H, 2 s, $CMe_2$), 3.32 (1 H, t, *J* 9.0, 5-H), 3.56–3.60 (2 H, m, 1-H or 3-H and 6-H), 3.73 (3 H, s, OMe), 3.74 (3 H, s, OMe), 3.75 (3 H, s, OMe), 4.10 (1 H, t, *J* 6.0, 1-H or 3-H), 4.37 (1 H, t, *J* 4.5, 2-H), 4.56–4.72 (6 H, m, 3 × $OCH_2C_6H_4OMe$), 5.23 (1 H, br s, $D_2O$ ex, OH), 6.84–6.91 (6 H, m, $OCH_2C_6H_4OMe$), 7.20–7.32 (6 H, m, $OCH_2C_6H_4OMe$); $\delta_C$ (68 MHz; [$^2H_6$]DMSO) 25.64, 27.53 ($CMe_2$), 55.05 ($CH_2PhOMe$), 71.35, 72.33, 73.15 ($CH_2$PhOMe), 71.66, 73.72, 77.25, 78.34, 81.28, 82.35 (*myo*-inositol ring carbons), 108.56 ($C_q$, $CMe_2$), 113.49, 129.14, 129.23, 129.30 ($CH_2PhOMe$), 130.82, 130.89, 131.15 ($C_q$, $CH_2PhOMe$), 158.67 ($C_q$, $CH_2PhOMe$); *m*/*z* ($FAB^-$) 733.5 (100), 626.3 (50), 579.3 (16), 355.1 (20), 299.2 (18), 181.1 (16), 106.0 (16).

**17ab** (Found: C, 70.0; H, 6.96. $C_{41}H_{48}O_{10}$ requires C, 70.27; H, 6.90%); $\delta_H$ (400 MHz; $CDCl_3$) 1.35, 1.51 (6 H, 2 s, $CMe_2$), 3.36 (1 H, t, *J* 8.85, 5-H), 3.64 (1 H, dd, *J* 3.7, 8.85, 1-H or 3-H), 3.74–3.79 (1 H, obscured, 1-H or 3-H), 3.77 (3 H, s, OMe), 3.74 (6 H, s, OMe), 3.79 (3 H, s OMe), 3.89 (1 H, t, *J* 8.85, 4-H or 6-H), 4.06 (1 H, t, *J* 6.7, 4-H or 6-H), 4.21 (1 H, t, *J* 4.0, 5.5, 2-H), 4.64–4.80 (8 H, m, 4 × $OCH_2C_6H_4OMe$), 6.83–6.87 (8 H, m, $OCH_2C_6H_4OMe$), 7.20–7.32 (8 H, m, $OCH_2C_6H_4OMe$); $\delta_C$ (100 MHz; $CDCl_3$) 25.80, 27.76 ($CMe_2$), 55.21 ($CH_2$PhO*Me*), 72.86, 73.55 ($CH_2$PhOMe), 74.60, 74.94, 79.17, 80.61, 81.87, 82.33 (*myo*-inositol ring carbons), 109.67 ($C_q$, $CMe_2$), 113.62, 113.69, 113.73, 129.52, 129.61 ($CH_2PhOMe$), 130.28, 130.74, 130.83, 130.87 ($C_q$, $CH_2PhOMe$), 159.12, 159.16, 159.31 ($C_q$, $CH_2PhOMe$); *m*/*z* ($FAB^+$) 723.3 (22), 699.3 (38), 579.3 (82), 241.1 (90), 121.0 (100).

### DL-5-*O*-Benzyl-2,3-*O*-isopropylidene-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol 18ab

A mixture of DL-2,3-*O*-isopropylidene-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol **16ab** (5.34 g, 9.18 mmol) and sodium hydride (0.96 g, 24 mmol) in dry DMF (60 $cm^3$) was stirred at room temperature. Benzyl bromide (2.38 $cm^3$, 20 mmol) was added to the solution, which was then stirred for a further 2 h. The excess sodium hydride was destroyed with methanol (10 $cm^3$) and the solvents were evaporated *in vacuo*. The crude product was partitioned between ether and water (100 $cm^3$ of each) and the organic layer was separated and dried ($MgSO_4$). The fully protected product **18ab** was purified by flash chromatography ($R_f$ 0.22, $Et_2O$–pentane 1 : 1). Yield (5.29 g, 86%); (Found: C, 71.7; H, 7.03. $C_{40}H_{46}O_9$ requires C, 71.62; H, 6.91%); $\delta_H$ (270 MHz; $CDCl_3$) 1.35, 1.52 (6 H, 2 s, $CMe_2$), 3.37 (1 H, t, *J* 9.2, 5-H), 3.64 (1 H, dd, *J* 3.7, 9.0, 1-H or 3-H), 3.75–3.79 (1 H, obscured, 1-H or 3-H), 3.77 (3 H, s, OMe), 3.78 (3 H, s, OMe), 3.79 (3 H, s, OMe), 3.90 (1 H, t, *J* 8.8, H-4 or H-6), 4.06 (1 H, t, *J* 6.6, H-4 or H-6), 4.21 (1 H, dd, *J* 4.0, 5.3, H-2), 4.63–4.80 (8 H, m, $OCH_2Ph$ and 3 × $OCH_2C_6H_4OMe$), 6.83–7.34 (17 H, m, $CH_2Ph$ and 3 × $OCH_2C_6H_4OMe$); $\delta_C$ (68 MHz; $CDCl_3$) 25.70, 27.67 ($CMe_2$), 55.09, 55.19 ($CH_2PhOMe$), 72.80, 73.46, 74.87, 75.13 ($CH_2$Ph and $CH_2$PhOMe), 74.53, 76.66, 79.09, 80.50, 82.11 (*myo*-inositol ring carbons), 109.63 ($CMe_2$), 113.67, 126.82, 127.44, 127.83, 128.20 ($CH_2Ph$ and $CH_2PhOMe$), 130.23, 130.63, 130.74 ($C_q$, $CH_2PhOMe$), 138.63 ($C_q$, $CH_2Ph$), 159.04, 159.11, 159.24 ($C_q$, $CH_2PhOMe$); *m*/*z* ($FAB^+$) 639.3 (20), 669.3 (22), 549.2 (76), 241.1 (22), 211.1 (52), 121.0 (100).

### DL-5-*O*-Benzyl-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol 19ab

DL-5-*O*-Benzyl-2,3-*O*-isopropylidene-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol **18ab** (9.46 g, 14.08 mmol) was dissolved in methanol–1.0 mol $dm^{-3}$ aq. HCl (100 $cm^3$, 9 : 1) and kept at 50 °C for 30 min. TLC ($CH_2Cl_2$–EtOAc 2 : 1) showed a new product $R_f$ 0.40, which precipitated from the reaction mixture as a white solid. The methanolic solution was cooled and the solid was filtered off. The remaining solution was kept at 50 °C

for a further 30 min, after which, most of the starting material had disappeared. The reaction mixture was cooled and the insoluble solid was filtered off. The acidic solution was neutralised with triethylamine (10 $cm^3$) and the solvents were evaporated. The remaining solid was partitioned between methylene dichloride and water (50 $cm^3$ of each) and the organic layer was dried ($MgSO_4$) and evaporated. The crude product was purified by flash chromatography ($CH_2Cl_2$–EtOAc 2 : 1) to give the pure title compound **19ab** (7.0 g, 79%); mp 148–150 °C (from EtOAc–hexane); (Found: C, 70.2; H, 6.77. $C_{37}H_{42}O_9$ requires C, 70.46; H, 6.71%); $\delta_H$ (400 MHz; [$^2H_6$]DMSO) 3.32 (1 H, t, *J* 9.5, 5-H), 3.36–3.59 (2 H, br, $D_2O$ ex, OH), 3.37 (2 H, app dt, *J* 3.35, 9.5, $D_2O$ ex, 2 dd, *J* 2.45, 9.8, 1-H, *J* 2.75, 9.8, 3-H), 3.59 (1 H, t, *J* 9.5, 4-H or 6-H), 3.66 (3 H, s, OMe), 3.67 (3 H, s, OMe), 3.69 (3 H, OMe), 3.67–3.71 (1 H, obscured, 4-H or 6-H), 3.99 (1 H, t, *J* 2.4, 2-H), 4.44–4.78 (8 H, m, $OCH_2Ph$ and 3 × $OCH_2C_6H_4OMe$), 6.79–7.50 (17 H, m, $CH_2Ph$ and 3 × $OCH_2C_6H_4OMe$); $\delta_C$ (100 MHz; [$^2H_6$]DMSO) 55.27 ($CH_2PhOMe$), 72.40, 75.25, 75.62 ($CH_2PhOMe$ and $CH_2Ph$), 69.05, 71.58, 79.74, 80.94, 81.49, 83.92 (*myo*-inositol ring carbons), 113.77, 113.92, 113.99, 127.59, 127.70, 128.42, 129.53, 129.64, 129.94 ($CH_2Ph$ and $CH_2PhOMe$), 130.65, 130.85 ($C_q$, $CH_2PhOMe$), 138.66 ($C_q$, $CH_2Ph$), 159.18, 159.34, 159.40 ($C_q$, $CH_2PhOMe$); *m*/*z* ($FAB^+$) 653.3 (54), 629.3 (14), 509.2 (88), 419.3 (44), 329.1 (68), 242.2 (64), 167.1 (56), 121.0 (100).

### L-(21) and D-1-*O*-[*R*-(−)-*O*-Acetylmandelyl]-5-*O*-benzyl-3,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol 22

A mixture of DL-5-*O*-benzyl-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol **19ab** (6.60 g, 10.45 mmol), (*R*)-(−)-*O*-acetylmandelic acid **20** (2.06 g, 10.6 mmol) and 4-(dimethylamino)pyridine (0.03 g, 0.25 mmol) was stirred in methylene dichloride (100 $cm^3$) at −20 °C. A solution of dicyclohexylcarbodiimide (DCCI) (2.15 g, 10.6 mmol) in methylene dichloride (20 $cm^3$) was added dropwise over a period of 2 h with stirring, then stirred overnight at room temperature. TLC ($CHCl_3$–acetone 15 : 1) showed two main products, $R_f$ 0.34 and 0.24, together with minor products at $R_f$ 0.44 and 0.14. The minor products were not investigated further. The solution was filtered over a bed of Celite and washed with methylene dichloride (2 × 100 $cm^3$). The solvent was evaporated off to give a solid and the individual diastereoisomers were isolated by flash chromatography ($CHCl_3$–acetone 15 : 1) to give **21** $R_f$ 0.34 (2.74 g, 32%); mp 105–107 °C (from EtOAc–hexane); $[a]_D$ −15 (*c* 1 in $CHCl_3$) and **22** $R_f$ 0.24 (2.19 g, 26%); mp 127–129 °C from EtOAc–hexane; $[a]_D$ −37 (*c* 1 in $CHCl_3$).

**21** (Found: C, 69.5; H, 6.18. $C_{47}H_{50}O_{12}$ requires C, 69.96; H, 6.25%); $\delta_H$ (400 MHz; $CDCl_3$) 2.13 (1 H, s, $D_2O$ ex, OH), 2.22 [3 H, s, $O_2CCH(OAc)Ph$], 3.41 (1 H, dd, *J* 2.4, 9.8, 3-H), 3.45 (1 H, t, *J* 9.5, 5-H), 3.77 (3 H, s, OMe), 3.78 (3 H, s, OMe), 3.79 (3 H, s, OMe), 3.88 (1 H, t, *J* 9.5, 4-H), 4.04 (1 H, t, *J* 9.5, 6-H), 4.11 (1 H, t, *J* 2.4, 2-H), 4.54–4.86 (9 H, m, 1-H, $OCH_2Ph$ and 3 × $OCH_2C_6H_4OMe$), 5.97 [1 H, s, $O_2CCH(OAc)Ph$], 6.76–7.47 (22 H, m, $CH_2Ph$, 3 × $OCH_2C_6H_4OMe$ and $O_2CCH(OAc)Ph$); $\delta_C$ (100 MHz; $CDCl_3$) 20.72 [OC(O)*Me*], 55.23 ($CH_2PhOMe$), 74.72, 75.33, 75.57, 75.78 ($CH_2PhOMe$, $CH_2Ph$), 67.34, 72.51, 75.20, 78.11, 79.28, 80.65, 82.95 ($O_2CCH(OAc)Ph$ and *myo*-inositol ring carbons), 113.68, 113.73, 113.88, 127.30, 127.49, 127.61, 128.31, 128.84, 129.28, 129.44, 129.61, 129.70, 129.79, 129.86 ($CH_2PhOMe$, $CH_2Ph$ and $O_2CCH(OAc)Ph$), 130.43, 130.76, 133.39, 138.68, 159.14, 159.36 ($C_q$, $CH_2PhOMe$, $CH_2Ph$ and $O_2CCH(OAc)Ph$), 168.25, 170.74 (O*C*(O)Me and $O_2CCH(OAc)Ph$); *m*/*z* ($FAB^-$) 959.5 (100), 805.4 (95), 629.3 (30), 419.3 (30), 331.1 (45), 258.1 (33), 181.1 (40), 149.1 (35).

**22** (Found: C, 69.6; H, 6.18. $C_{47}H_{50}O_{12}$ requires C, 69.96; H, 6.25%); $\delta_H$ (400 MHz; $CDCl_3$) 2.19 [3 H, s, $O_2CCH(OAc)Ph$], 2.72 (1 H, s, $D_2O$ ex, OH), 3.41 (1 H, t, *J* 9.15, 5-H), 3.47 (1 H, dd, *J* 2.4, 9.5, 3-H), 3.75 (3 H, s, OMe), 3.76 (3 H, s, OMe), 3.79 (3 H, s, OMe), 3.92 (1 H, t, *J* 9.2, 4-H), 4.04 (1 H, t, *J* 9.8, 6-H), 4.05 and 4.36 (2 H, AB, *J* 10.3, $OCH_2C_6H_4OMe$), 4.37 (1 H, 2-H, br, obscured), 4.57–4.82 (9 H, m, $OCH_2Ph$ and 3 × $OCH_2C_6H_4OMe$ and 1-H), 5.99 [1 H, s, $O_2CCH(OAc)Ph$], 6.63–7.51 (22 H, m, $CH_2Ph$, $OCH_2C_6H_4OMe$ and $O_2CCH(OAc)Ph$); $\delta_C$ (100 MHz; $CDCl_3$) 20.66 [OC(O)*Me*], 55.17, 55.23 ($CH_2PhOMe$), 72.49, 74.82, 75.58, 75.80 ($CH_2PhOMe$ and $CH_2Ph$), 67.43, 74.94, 75.55, 78.20, 79.48, 80.67, 82.92 ($O_2CCH(OAc)Ph$, and *myo*-inositol ring carbons), 113.42, 113.72, 113.90, 127.43, 127.59, 128.03, 128.25, 128.93, 129.09, 129.55, 129.73 ($CH_2PhOMe$, $CH_2Ph$ and $O_2CCH(OAc)Ph$), 130.30, 130.78, 132.99, 138.59, 158.87, 159.12, 159.38 ($C_q$, $CH_2PhOMe$, $CH_2Ph$ and $O_2CCH(OAc)Ph$), 168.56, 170.66 ($O_2CCH(OAc)Ph$, O*C*(O)Me); *m*/*z* ($FAB^-$) 959.5 (100), 805.4 (95), 629.3 (25), 419.3 (15), 335.1 (35), 106.0 (35).

### D-5-*O*-Benzyl-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol 19a

A mixture of L-1-*O*-[*R*-(−)-*O*-acetylmandelyl]-5-*O*-benzyl-3,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol **22** (2.59 g, 4.10 mmol) and sodium hydroxide (0.8 g, 20 mmol) in methanol (100 $cm^3$) was heated under reflux for 30 min. The mixture was cooled and neutralised with carbon dioxide. The remaining solution was diluted with water (100 $cm^3$) and evaporated to dryness *in vacuo*. The title compound was extracted with methylene dichloride (4 × 100 $cm^3$) and the solvent was evaporated to give the product **19a** (1.86 g, 96%), ($R_f$ 0.40, $CH_2Cl_2$–EtOAc 2 : 1); mp 123–125 °C (from $Et_2O$–pentane); $[a]_D$ +24 (*c* 1 in $CHCl_3$); (Found: C, 70.4; H, 6.60. $C_{37}H_{42}O_9$ requires C, 70.46; H, 6.71%); Mass spectrum and NMR data were identical with those of the racemic mixture **19ab**.

### L-5-*O*-Benzyl-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol 19b

A mixture of D-1-*O*-[*R*-(−)-*O*-acetylmandelyl]-5-*O*-benzyl-3,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol **22** (2.00 g, 4.10 mmol) and sodium hydroxide (0.8 g, 20 mmol) in methanol (100 $cm^3$) was heated under reflux for 30 min. Work up as for the D-enantiomer gave the title compound **19b** (1.48 g, 99%), $R_f$ 0.40 ($CH_2Cl_2$–EtOAc 2 : 1); mp 123–125 °C (from $Et_2O$–pentane); $[a]_D$ −24 (*c* 1 in $CHCl_3$); (Found: C, 70.4; H, 6.65. $C_{37}H_{42}O_9$ requires C, 70.46; H, 6.71%); The mass spectrum and NMR data were identical with those of the racemic mixture **19ab**.

### D-3,5-Di-*O*-benzyl-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol 23a

A mixture of D-5-*O*-benzyl-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol **19a** (1.64 g, 2.6 mmol) and dibutyltin oxide (0.875 g, 3.5 mmol) was heated under reflux in toluene (250 $cm^3$) using a Dean-and-Stark apparatus for 3 h. The reaction mixture was cooled and the solvent was evaporated to give a syrup which was dried under vacuum for a further 2 h. Caesium fluoride (1.52 g, 10 mmol) and dry DMF (50 $cm^3$) were added to the dried residue under an atmosphere of nitrogen. Benzyl bromide (0.71 $cm^3$, 6 mmol) was added to the mixture and the reaction was stirred overnight at room temperature. TLC ($CH_2Cl_2$–EtOAc 10 : 1), showed a product with $R_f$ 0.40. The solvent was evaporated under reduced pressure and the residue was taken up in methylene dichloride (100 $cm^3$), washed with water (100 $cm^3$) and stirred with a saturated aqueous solution of sodium hydrogen carbonate (100 $cm^3$, 10% w/v) for 30 min. The organic layer was separated, washed with water, dried over $MgSO_4$ and the solvent was evaporated to give the crude product. The title compound **23a** was obtained by flash chromatography ($CH_2Cl_2$–EtOAc 10 : 1), to give the pure enantiomer as a solid. Yield (1.55 g, 83%); mp 100–102 °C (from EtOAc–hexane); $[a]_D$ −3 (*c* 1 in $CHCl_3$); (Found: C, 73.3; H, 6.70. $C_{44}H_{48}O_9$ requires C, 73.31; H, 6.71%); $\delta_H$ (400 MHz; $CDCl_3$) 2.50 (1 H, br s, $D_2O$ ex, OH), 3.34 (1 H, dd, *J* 2.75, 9.5, 1-H or 3-H), 3.35 (1 H, dd, *J* 2.75, 9.5, 1-H or 3-H), 3.41 (1 H, t, *J* 9.5, 5-H), 3.76 (3 H, s,

OMe), 3.77 (3 H, s, OMe), 3.79 (3 H, s, OMe), 3.95 (1 H, t, *J* 9.2, 4-H or 6-H), 3.98 (1 H, t, *J* 9.5, 4-H or 6-H), 4.18 (1 H, t, *J* 2.7, 2-H), 4.63–4.87 (10 H, m, 2 × $OCH_2Ph$ and 3 × $OCH_2C_6H_4OMe$), 6.79–7.36 (22 H, m, 2 × $CH_2Ph$ and 3 × $OCH_2C_6H_4OMe$); $\delta_C$ (100 MHz; $CDCl_3$) 55.23 ($CH_2PhOMe$), 67.56, 72.37, 72.69, 75.62, 75.82 ($CH_2Ph$ and $CH_2PhOMe$), 67.56, 79.52, 79.83, 80.94, 83.19 (*myo*-inositol ring carbons), 113.73, 113.82, 127.47, 127.67, 127.80, 128.34, 128.44, 129.48, 129.68 ($CH_2Ph$ and $CH_2PhOMe$), 130.06, 130.91, 130.94, 138.02, 138.82, 159.12, 159.31 ($C_q$, $CH_2Ph$ and $CH_2PhOMe$); *m*/*z* ($FAB^-$) 873.1 (100), 719.1 (44), 599.4 (22), 329.0 (20), 106.0 (16).

### L-3,5-Di-*O*-benzyl-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol 23b

A mixture of L-5-*O*-benzyl-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol **19b** (0.90 g, 1.43 mmol) and dibutyltin oxide (0.425 g, 1.7 mmol) was heated under reflux in toluene (100 $cm^3$) using a Dean-and-Stark apparatus for 3 h. The reaction mixture was cooled and the solvent was evaporated to give a syrup which was dried under vacuum for a further 2 h. Caesium fluoride (0.65 g, 4.29 mmol) and dry DMF (30 $cm^3$) were added to the dried residue under an atmosphere of nitrogen. Benzyl bromide (0.35 $cm^3$, 3 mmol) was added to the mixture and the reaction was stirred overnight at room temperature. TLC ($CH_2Cl_2$–EtOAc 10 : 1) showed a product with $R_f$ 0.40. Work up and purification as for the D-enantiomer provided the title compound **23b** (0.98 g, 95%); mp 100–102 °C (from EtOAc–hexane); $[\alpha]_D$ +2 (*c* 1 in $CHCl_3$); (Found: C, 73.0; H, 6.74. $C_{44}H_{48}O_9$ requires C, 73.31; H, 6.71%); The mass spectrum and NMR data were identical to those of the D-derivative.

### D-3,5-Di-*O*-benzyl-*myo*-inositol 11a

D-3,5-Di-*O*-benzyl-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol **23a** (1.31 g, 1.82 mmol) was added to 1 mol $dm^{-3}$ aq. hydrochloric acid–ethanol (60 $cm^3$, 1 : 2). The mixture was heated under reflux for 4 h, cooled and the solvents were evaporated *in vacuo* to give a solid, which was co-evaporated with methanol (50 $cm^3$). TLC (EtOAc) showed a product $R_f$ 0.20 and deprotected *p*-methoxybenzyl derivative $R_f$ 1.00. The remaining solid was purified by flash chromatography ($R_f$ 0.40 $CHCl_3$–MeOH 10 : 1), to give the title compound **11a** as a solid (654 mg, 92%); mp 162–163 °C (from ethanol); $[\alpha]_D$ +5 (*c* 1 in MeOH); (Found: C, 66.6; H, 6.70. $C_{20}H_{24}O_6$ requires C, 66.64; H, 6.72%); The mass spectrum and NMR data were identical with those of racemate **11ab**.

### L-3,5-Di-*O*-benzyl-*myo*-inositol 11b

L-3,5-Di-*O*-benzyl-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol **23b** (0.675 g, 0.936 mmol) was added to 1 mol $dm^{-3}$ aq. hydrochloric acid–ethanol (90 $cm^3$, 1 : 2). The mixture was heated under reflux for 4 h, cooled, and the solvents were evaporated *in vacuo* to give a solid, which was co-evaporated with methanol (50 $cm^3$). TLC (ethyl acetate) showed a product $R_f$ 0.20 and a deprotected *p*-methoxybenzyl derivative $R_f$ 1.00. The remaining solid was purified by flash chromatography ($CHCl_3$–MeOH 10 : 1) $R_f$ 0.40, to give the title compound **11b** as a solid (0.315 g, 93%); mp 162–163 °C (from ethanol); $[\alpha]_D$ −5 (*c* 1 in MeOH); (Found: C, 66.6; H, 6.68. $C_{20}H_{24}O_6$ requires C, 66.64; H, 6.72%); The mass spectrum and NMR data were identical with those of racemate **11ab**.

### D-3,5-Di-*O*-benzyl-1,2,4,6-tetrakis-*O*-[di(benzyloxy)-phosphoryl]-*myo*-inositol 13a

A mixture of bis(benzyloxy)diisopropylaminophosphine (1.38 g, 4 mmol) and 1*H*-tetrazole (0.56 g, 8 mmol) in dry methylene dichloride (10 $cm^3$) was stirred at room temperature for 10 min. D-3,5-Di-*O*-benzyl-*myo*-inositol **11a** (0.216 g, 0.60 mmol) was added to the reaction mixture and stirred for a further 10 min. The solution was cooled to 0 °C then (50–60%) MCPBA (1.60 g, 4.6 mmol) was added to the solution and the reaction mixture was stirred for a further 30 min. Work up and purification as for the racemate **13ab** gave the title compound **13a** as a syrup (661 mg, 79%); $[\alpha]_D$ −2.20 (*c* 7.68 in $CHCl_3$); The mass spectrum and NMR data were identical with those of racemate **13ab**.

### L-3,5-Di-*O*-benzyl-1,2,4,6-tetrakis-*O*-[di(benzyloxy)-phosphoryl]-*myo*-inositol 13b

A mixture of bis(benzyloxy)diisopropylaminophosphine (0.69 g, 2 mmol) and 1*H*-tetrazole (0.28 g, 4 mmol) in dry methylene dichloride (10 $cm^3$) was stirred at room temperature for 10 min. L-3,5-Di-*O*-benzyl-*myo*-inositol **11b** (0.108 g, 0.30 mmol) was added to the reaction mixture, which was stirred for a further 10 min. The solution was cooled to 0 °C then (50–60%) MCPBA (0.80 g, 2.3 mmol) was added to the solution and the reaction mixture was stirred for a further 30 min. Work up and purification as for the racemate **13ab** gave the title compound **13b** as a syrup (0.360 g, 86%); $[\alpha]_D$ +2.37 (*c* 5.05 in $CHCl_3$); The mass spectrum and NMR data were identical with those of racemate **13ab**.

### D-*myo*-Inositol 1,2,4,6-tetrakisphosphate 3a

D-3,5-Di-*O*-benzyl-1,2,4,6-tetrakis-*O*-[di(benzyloxy)phosphoryl]-*myo*-inositol **13a** (0.160 g, 0.114 mmol) was hydrogenolysed in a mixture of methanol (40 $cm^3$) and water (10 $cm^3$), in the presence of palladium on carbon (10%, 0.20 g) for 20 h. The reaction mixture was filtered over a bed of Celite to remove the insoluble components and washed with water and methanol then evaporated *in vacuo* to give a syrup. The residue was then dissolved in MilliQ water (150 $cm^3$) and purified by ion exchange chromatography on Q-Sepharose Fast Flow with a gradient of TEAB buffer (0–1000 mmol $dm^{-3}$) at pH 8.6. The triethylammonium salt of **4a** eluted at *ca*. 800 mmol $dm^{-3}$. Yield (74 μmol, 65%); $[\alpha]_D$ −15.4 (*c* 3.12 in MeOH), lit.[18] $[\alpha]_D$ −15.2 (*c* 2.10, $H_2O$, pH 9.5); $\delta_H$ (270 MHz; $D_2O$) 3.58 (1 H, t, *J* 9.2, 5-H), 3.67 (1 H, d, *J* 9.3, 3-H), 4.08 (1 H, t, *J* 9.3, 1-H), 4.19 (1 H, q, *J* 9.0, 4-H or 6-H), 4.29 (1 H, q, *J* 9.2, 4-H or 6-H), 4.67 (1 H, d, *J* 9.2, 2-H); $\delta_P$ (109 MHz; $D_2O$) 1.78 (1 P, d, *J* 6.7), 1.32 (2 P, d, *J* 6.7), 1.07 (1 P, d, *J* 6.7); *m*/*z* ($FAB^-$) 999.1 (5), 499.0 (100), 291.2 (5); [Found: *m*/*z*, 498.9189 $(M - H)^-$ requires *m*/*z* 498.9208].

### L-*myo*-Inositol 1,2,4,6-tetrakisphosphate 4b

L-3,5-Di-*O*-benzyl-1,2,4,6-tetrakis-*O*-[di(benzyloxy)phosphoryl]-*myo*-inositol **13b** (0.150 g, 0.107 mmol) was hydrogenolysed in a mixture of methanol (40 $cm^3$) and water (10 $cm^3$) over palladium on carbon (10%, 0.20 g) for 20 h. The reaction mixture was filtered over a bed of Celite to remove the insoluble components and washed with water and methanol then evaporated *in vacuo* to give a syrup. The residue was then dissolved in MilliQ water (150 $cm^3$) and purified by ion exchange chromatography on Q-Sepharose Fast Flow with a gradient of TEAB buffer (0–1000 mmol $dm^{-3}$) at pH 8.6. The triethylammonium salt of **4b** eluted at *ca*. 800 mmol $dm^{-3}$. Yield (38 μmol, 36%); $[\alpha]_D$ +15 (*c* 1.6 in MeOH), lit.[18] $[\alpha]_D$ + 14.7 (*c* 1.77, $H_2O$, pH 9.5); $\delta_H$ (270 MHz; $D_2O$) 3.57 (1 H, t, *J* 9.2, 5-H), 3.67 (1 H, d, *J* 10.1, 3-H), 4.08 (1 H, t, *J* 9.2, 1-H), 4.21 (1 H, q, *J* 9.15, 4-H or 6-H), 4.30 (1 H, q, *J* 9.3, 4-H or 6-H), 4.67 (1 H, d, *J* 9.2, 2-H); $\delta_P$ (109 MHz; $D_2O$) 2.05 (1 P, d, *J* 10.0), 1.72 (1 P, d, *J* 10.1), 1.56 (1 P, d, *J* 10.1), 1.29 (1 P, d, *J* 6.8); *m*/*z* ($FAB^-$) 999.1 (5), 499.0 (100), 291.2 (5); [Found: *m*/*z*, 498.9212 $(M - H)^-$ requires *m*/*z* 498.9208].

### L-2,3,5-Tri-*O*-benzyl-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol 24b

A mixture of L-5-*O*-benzyl-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol **23b** (0.50 g, 0.79 mmol) and sodium hydride (0.33 g,

8.25 mmol) was stirred at room temperature in DMF (25 $cm^3$). Benzyl bromide (0.24 $cm^3$, 2.0 mmol) was added dropwise and stirred for a further 2 h. TLC ($CHCl_3$–pentane–EtOAc 3 : 3 : 1) showed a new product $R_f$ 0.50. The excess sodium hydride was destroyed with methanol (10 $cm^3$) and the solvents were evaporated off *in vacuo*. The residue was partitioned between methylene dichloride and water (50 $cm^3$ of each) and the organic layer was separated, dried ($MgSO_4$) and the solvent was evaporated off to give the crude product. Flash chromatography using the TLC solvent, provided the pure title compound **24b** (0.523 g, 82%); mp 84–86 °C (from $Et_2O$–pentane); $[\alpha]_D$ −5 (*c* 1 in $CHCl_3$); (Found: C, 75.6; H, 6.71. $C_{51}H_{54}O_9$ requires C, 75.53; H, 6.71%); $\delta_H$ (400 MHz; $CDCl_3$) 3.31 (1 H, dd, *J* 2.4, 9.8, 1-H or 3-H), 3.32 (1 H, dd, *J* 2.1, 9.8, 1-H or 3-H), 3.44 (1 H, t, *J* 9.15, 5-H), 3.76 (3 H, s, OMe), 3.77 (3 H, s, OMe), 3.81 (3 H, s, OMe), 4.00 (1 H, t, *J* 2.1, 2-H), 4.05 (1 H, t, *J* 9.5, 4-H or 6-H), 4.06 (1 H, t, *J* 9.5, 4-H or 6-H), 4.52–4.88 (12 H, m, 3 × $OCH_2$Ph and 3 × $OCH_2C_6H_4$OMe), 6.77–7.42 (27 H, m, 3 × $CH_2Ph$ and 3 × $OCH_2C_6H_4$OMe); $\delta_C$ (100 MHz; $CDCl_3$) 55.23 ($CH_2PhOMe$), 72.44, 72.75, 74.08, 75.49, 75.82 ($CH_2$Ph, $CH_2$PhOMe), 74.47, 80.72, 80.97, 81.42, 83.76 (*myo*-inositol ring carbons), 113.70, 113.75, 127.30, 127.41, 127.50, 127.56, 127.65, 127.76, 128.13, 128.33, 129.15, 129.73 ($CH_2Ph$ and $CH_2Ph$OMe), 130.54, 131.03, 131.09, 138.48, 139.01, 159.09, 159.14 ($C_q$, $CH_2Ph$OMe and $CH_2Ph$); *m*/*z* ($FAB^-$) 833.0 (30), 689.0 (90), 391.1 (14), 241.0 (24), 211.0 (78), 121.0 (100).

#### L-2,3,5-Tri-*O*-benzyl-*myo*-inositol 25b

L-2,3,5-Tri-*O*-benzyl-1,4,6-tris-*O*-*p*-methoxybenzyl-*myo*-inositol **24b** (0.390 g, 0.48 mmol) was added to 1 mol $dm^{-3}$ aq. hydrochloric acid–ethanol (30 $cm^3$, 1 : 2). The mixture was heated under reflux for 4 h, cooled, and the solvents were evaporated *in vacuo* then the remaining solid was co-evaporated with methanol (50 $cm^3$). TLC (ether) showed a new product $R_f$ 0.30 and a deprotected *p*-methoxybenzyl derivative $R_f$ 0.80. The remaining solid was partitioned between methylene dichloride and water (50 $cm^3$ of each) and the organic layer was separated and dried ($MgSO_4$) then evaporated to give the crude product. The title compound **25b** was obtained by flash chromatography (EtOAc–$CH_2Cl_2$ 1 : 1) and isolated as a solid (0.190 g, 88%); mp 175–176.5 °C (from EtOAc–hexane); lit.,[13] 176–177 °C; $[\alpha]_D$ −29 (*c* 1 in $CHCl_3$), lit.,[13] $[\alpha]_D$ −34 (*c* 1 in $CH_2Cl_2$); [same sample as lit.,[13] $[\alpha]_D$ −28 (*c* 1 in $CHCl_3$) for the L-enantiomer]; $\delta_H$ (270 MHz; $CDCl_3$) 2.30–2.70 (3 H, br s, $D_2O$ ex, OH), 3.24 (1 H, t, *J* 9.2, 5-H), 3.30 (1 H, dd, *J* 2.4, 9.7, 1-H or 3-H), 3.40 (1 H, dd, *J* 2.75, 9.7, 1-H or 3-H), 3.82 (1 H, t, *J* 9.5, 4-H or 6-H), 4.06 (1 H, t, *J* 2.6, 2-H), 4.12 (1 H, t, *J* 9.5, 4-H or 6-H), 4.59–4.99 (6 H, m, 3 × $OCH_2$Ph), 7.25–7.40 (15 H, m, 3 × $OCH_2Ph$).

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