Synthesis of the enantiomers of *myo*-inositol 1,2,4,5-tetrakisphosphate, a regioisomer of *myo*-inositol 1,3,4,5-tetrakisphosphate

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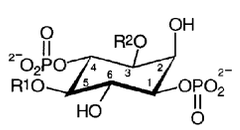
Routes for the synthesis of racemic *myo*-inositol 1,2,4,5-tetrakisphosphate DL-Ins(1,2,4,5)P_4 5ab and the chiral antipodes D- and L-*myo*-inositol 1,2,4,5-tetrakisphosphate 5a and 5b, respectively, are described. For the synthesis of racemate 5ab, 3,6-di-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol 7ab is prepared in two steps from *myo*-inositol. The ketals are hydrolysed under acidic conditions to give DL-1,4-di-*O*-benzoyl-*myo*-inositol 8ab. Phosphitylation of compounds 8ab using chloro(diethoxy)-phosphine in the presence of base, followed by oxidation and a three-step deprotection strategy, gives DL-Ins(1,2,4,5)P_4 5ab.

The chiral tetrakisphosphates 5a and 5b are synthesized using a different route. The 4,5-isopropylidene group of DL-3,6-di-*O*-benzyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol 13ab are selectively removed under mild acidic conditions to give diol 14ab. *p*-Methoxybenzylation at the 4,5-positions followed by acid hydrolysis of the *cis*-isopropylidene ketal affords *cis*-diol 16ab. Selective coupling of (*S*)-(+)-*O*-acetyl-mandelic acid with diol 16ab at the equatorial hydroxy group provides two diastereoisomers 18 and 19, which are separated by chromatography. Basic hydrolysis of the individual diastereoisomers provides the enantiomers 16a and 16b. Acidic hydrolysis gives D- and L-3,6-di-*O*-benzyl-*myo*-inositol 20a and 20b, respectively. Phosphitylation and oxidation of tetraols 20a and 20b gives the fully blocked derivatives, which are deprotected to give tetrakisphosphates 5a and 5b, respectively. The absolute configuration of compound 20a is established by a chemical method. DL-1,2:4,5-Di-*O*-isopropylidene-*myo*-inositol 12ab is coupled to (*S*)-(+)-*O*-acetylmandelic acid to give a mixture of bis-esters 26 and 27 and crystallisation of the mixture of diastereoisomers affords pure isomer 27. Basic hydrolysis gives the pure enantiomer 12a (for which the absolute configuration is known) and benzylation followed by acid hydrolysis gives tetraol 20a with the same physical properties as compound 20a prepared by a different route described previously. D-Ins(1,2,4,5)P_4 5a is a potent mobiliser of intracellular Ca^{2+} ions in permeabilised platelets, while L-Ins(1,2,4,5)P_4 5b is inactive.

Introduction

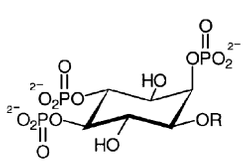
The involvement of *myo*-inositol polyphosphates in signal transduction *via* the polyphosphoinositide pathway has stimulated the need for the synthesis of molecules that will somehow interfere with, or modulate, the processes of cellular signalling,[1] whether it be at phospholipase C, the intracellular D-*myo*-inositol 1,4,5-trisphosphate receptor, or even further downstream, where the second messenger D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **1**] is deactivated and the signal is terminated. The process of signal transduction *via* Ins(1,4,5)P_3 starts when a cell-surface receptor activates the enzyme phospholipase C-β *via* a G-protein. This enzyme hydrolyses the minor membrane phospholipid, phosphatidylinositol 4,5-bisphosphate, to provide the hydrophobic diacylglycerol and hydrophilic Ins(1,4,5)P_3 as signalling molecules. Ins(1,4,5)P_3 interacts specifically at an N-terminal binding site of a tetrameric Ins(1,4,5)P_3 receptor-operated Ca^{2+} channel, in order to release Ca^{2+} from non-mitochondrial stores.[1] After the Ca^{2+}-release event, the signal must be deactivated by one or more metabolic pathways. First, an Ins(1,4,5)P_3 5-phosphatase removes a 5-phosphate moiety from Ins(1,4,5)P_3 to give D-*myo*-inositol 1,4-bisphosphate Ins(1,4)P_2 **2** which is inactive for Ca^{2+} release, but has been reported to be an allosteric activator of the enzyme 6-phosphofructo-1-kinase,[2] and also activates the enzyme DNA polymerase α.[3] Second, Ins(1,4,5)P_3 can also be phosphorylated to give D-*myo*-inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4, **3**], and the production of Ins(1,4)P_2 and Ins(1,3,4,5)P_4 is considered as an off signal. The function of Ins(1,3,4,5)P_4 has not been unambiguously resolved; however, it may gate a plasma membrane Ca^{2+} channel.[4] An Ins(1,3,4,5)P_4 binding protein has been purified from platelets[5] and is a GTPase activating protein-1 (GAP-1) family member. The GTPase activating protein-1 site has been designated GAP1^{IP4BP}. When Ins(1,3,4,5)P_4 binds at this site it may possibly have a second messenger function in its own right. The synthesis of regioisomeric inositol tetrakisphosphates is therefore of clear current interest.



$R^1 = PO_3^{2-}$, 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**



D-configuration shown

R = H, Ins(2,4,5)P_3 (racemic mixture, **4ab**)

R = PO_3^{2-}, Ins(1,2,4,5)P_4 (racemic mixture, **5ab**)

D-Ins(1,2,4,5)P_4 **5a**; L-Ins(1,2,4,5)P_4 **5b**

DL-*myo*-Inositol 2,4,5-trisphosphate [Ins(2,4,5)P_3, **4ab**] is an unnatural trisphosphate, which has a potency some 30-fold lower than Ins(1,4,5)P_3[6,7] but has been used as a metabolic

resistant analogue[6] of Ins(1,4,5)P_3, since it is 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.[6,8] Recently, Bird and Putney, Jr,[9] microinjected Ins(2,4,5)P_3 into mouse lacrimal acinar cells and it stimulated intracellular Ca^{2+} mobilisation and Ca^{2+} entry. However, microinjection of purified D-Ins(1,3,4,5)P_4 into these cells was ineffective at Ca^{2+} mobilisation or activation of Ca^{2+} entry. Thus, the introduction of high concentrations (final cellular concentration 100–200 μM) of Ins(1,3,4,5)P_4 somehow blocked the Ins(2,4,5)P_3 Ca^{2+} entry phase. These results indicated that physiological concentrations of Ins(1,3,4,5)P_4 in this cell type do not cause Ca^{2+} mobilisation[10–12] nor do they potentiate Ins(1,4,5)P_3 induced Ca^{2+} entry.

Since it was unclear whether substitution at the 2-hydroxy group or the lack of a phosphate at the 1-hydroxy moiety was responsible for the properties described above, we synthesized the unnatural tetrakisphosphate *myo*-inositol 1,2,4,5-tetrakisphosphate, DL-Ins(1,2,4,5)P_4 **5ab**, first in racemic form and then as the individual enantioners D-Ins(1,2,4,5)P_4 **5a** and L-Ins(1,2,4,5)P_4 **5b**. D-Ins(1,2,4,5)P_4 **5a** could be considered as a relative of Ins(1,4,5)P_3 but with a charged phosphate at the 2-position (several articles have focused upon substitution at the 2-position with neutral bulky groups).[1,7,13] This analogue can also be related to Ins(1,3,4,5)P_4, but with a 3-phosphate being transposed onto the adjacent 2-hydroxy moiety. These compounds were synthesized in order to evaluate structure–activity profiles with respect to the Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 binding proteins and the enzymes Ins(1,4,5)P_3 3-kinase and Ins(1,4,5)P_3 5-phosphatase, the latter of which also hydrolyses Ins(1,3,4,5)P_4.

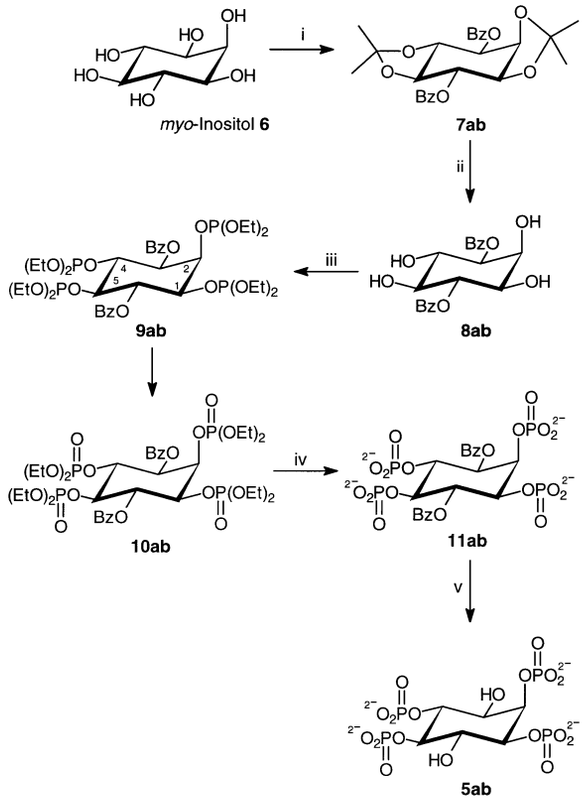
Ins(1,2,4,5)P_4 has previously been synthesized in racemic[14] and chiral[15] form and a preliminary report of the present work concerning the racemic modification has appeared.[14a] However, only D-Ins(1,2,4,5)P_4 **5a** has been reported in chiral form. We now report here a useful route to the synthesis of both enantiomers of Ins(1,2,4,5)P_4 (**5a** and **5b**) by resolution of partially blocked *myo*-inositol derivatives, using the chiral auxiliary (*S*)-(+)-*O*-acetylmandelic acid **17**.

The enantiomers of Ins(1,2,4,5)P_4 were synthesized by a different route from that for the racemic mixture. Previously,[14a] we demonstrated that DL-Ins(1,2,4,5)P_4 **5ab** competitively inhibited the dephosphorylation of [^{3}H]Ins(1,4,5)P_3 by human erythrocyte membrane Ins(1,4,5)P_3 5-phosphatase with a K_i-value of 15.9 μM. However, either isomer could potentially inhibit the enzyme. The reasons for synthesizing the title compounds were, first, to establish which isomer was responsible for the inhibition of the 5-phosphatase, and second, to discover the true EC_{50}-value for Ca^{2+} release of D-Ins(1,2,4,5)P_4 **5a** in comparison with those of Ins(1,4,5)P_3 and *scyllo*-Ins(1,2,4,5)P_4. It is also known that L-Ins(2,4,5)P_3 can release Ca^{2+} from intracellular stores, albeit with a low potency (EC_{50}-value of 110 μM) and thus it would be interesting to discover if L-Ins(1,2,4,5)P_4 **5b** made some contribution to the Ca^{2+}-releasing properties observed for DL-Ins(1,2,4,5)P_4.

Results and discussion

DL-3,6-Di-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol **7ab** (Scheme 1) was prepared using the method developed by Gigg *et al.*[16] A mixture of *myo*-inositol **6**, 2,2-dimethoxypropane and toluene-*p*-sulfonic acid (PTSA) was stirred at 100 °C in *N*,*N*-dimethylformamide (DMF). After benzoylation the highly insoluble product **7ab** was suspended in aq. acetic acid and the mixture was heated under reflux to give DL-1,4-di-*O*-benzoyl-*myo*-inositol **8ab**. This tetraol has also been synthesized by Meek *et al.*[17]

Commerically available chloro(diethoxy)phosphine (δ_P 167) was used to phosphitylate tetraol **8ab**. The ^{31}P NMR spectrum of the intermediate 1,2,4,5-tetrakisphosphite **9ab**, operating at 36.2 MHz with a sweep width of 2500 KHz (higher field

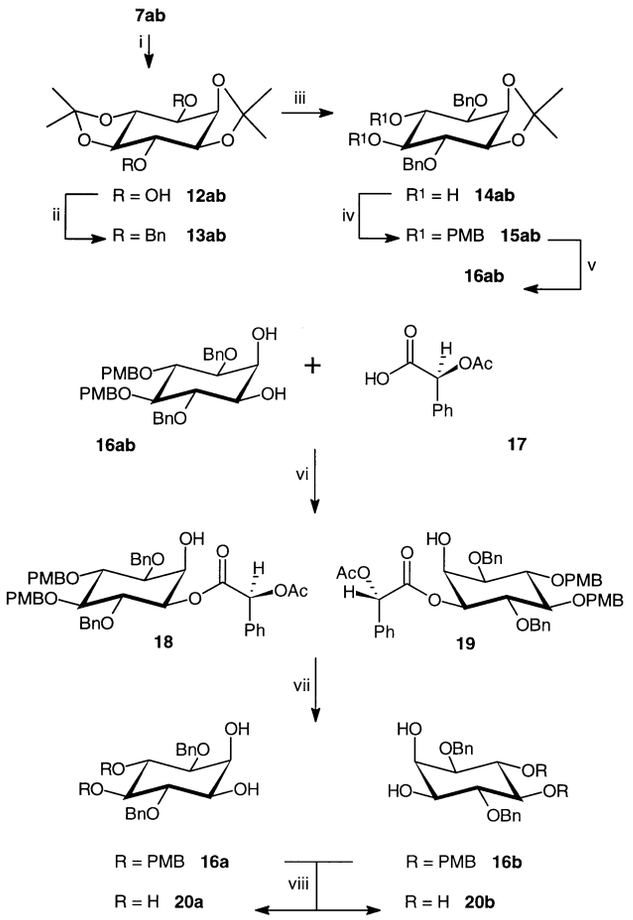


Scheme 1 *Reagents and conditions:* i, 2,2-dimethoxypropane, PTSA (cat), DMF, 100–120 °C, 2 h; then pyridine, benzoyl chloride, 2 h (30%); ii, 80% (aq.) AcOH, reflux, 30 min (93%); iii, $(EtO)_2PCl$, DIPE, DMF, 1 h; then 70% *tert*-butyl hydroperoxide (83%); iv, TMSBr, CH_2Cl_2, room temp. overnight then water, and final purification by Q-Sepharose Fast Flow ion-exchange chromatography (81%); v, 1 mol dm^{-3} NaOH, 60 °C, 1 h; then purification by Q-Sepharose Fast Flow ion exchange chromatography (80%). All compounds are racemic.

strengths may not show the following effect due to chemical-shift anisotropy), showed eight peaks resulting from two $^5J_{PP}$ AB coupling systems[18] centred around $\delta_P = 141.8$ and 141.3 (for the 4,5-positions) and $\delta_P = 140.4$ and 139.8 (for the 1,2-positions), for each doublet of the AB coupling pattern demonstrating phosphitylation of a pair of vicinal diols at the 1,2-positions ($^5J_{PP}$ 1.8 Hz) and for the 4,5-positions ($^5J_{PP}$ 3.7 Hz). Oxidation of phosphites **9ab** provided crystalline DL-3,6-di-*O*-benzoyl-1,2,4,5-tetrakis-*O*-(diethoxyphosphoryl)-*myo*-inositol **10ab**. The eight ethyl groups of compound **10ab** were replaced by transesterification quantitatively (checked by ^{31}P NMR spectroscopy) using bromotrimethylsilane in methylene dichloride. Hydrolysis with water gave DL-3,6-di-*O*-benzoyl-Ins(1,2,4,5)P_4 **11ab** quantitatively. A small sample was purified by ion-exchange chromatography using a gradient of triethylammonium hydrogen carbonate (TEAB) on Q-Sepharose Fast Flow to give pure tetraol **11ab**. DL-Ins(1,2,4,5)P_4 **5ab** was prepared by basic hydrolysis of the two benzoate esters. Pure DL-Ins(1,2,4,5)P_4 **5ab** was obtained as the triethylammonium salt after ion exchange chromatography and eluted at *ca.* 550 mmol dm^{-3} TEAB buffer and was quantified by phosphate analysis.

Racemic 1,4-di-*O*-benzyl-5,6-bis-*O*-(*p*-methoxybenzyl)-*myo*-inositol **16ab** was prepared according to Scheme 2 and has also been synthesized by another group.[19] Basic methanolysis of the two benzoyl esters of compound **7ab** gave DL-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol **12ab**. Benzylation of diol **12ab** with benzyl bromide provided fully blocked DL-3,6-di-*O*-benzyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol **13ab**. The less stable *trans*-acetal was removed selectively using a catalytic quantity of PTSA and ethane-1,2-diol to provide DL-1,4-di-*O*-benzyl-2,3-*O*-isopropylidene-*myo*-inositol **14ab** in 80% yield.

This compound has also been prepared from **13ab** by Gigg *et al.*[20] under different acidic conditions in only 55% yield. Diol **14ab** was alkylated with *p*-methoxybenzyl chloride to provide fully blocked product **15ab**. The *cis* isopropylidene group was removed by careful acid treatment to give diol **16ab**. Caution must be taken at this stage, to avoid hydrolysis of the *p*-methoxybenzyl group. It was envisaged that the introduction of a chiral auxiliary at the equatorial position of the *cis* diol **16ab** would result in the formation of two separable diastereoisomers.



Scheme 2 *Reagents and conditions:* i, NaOH, MeOH, reflux, 30 min (82%); ii, BnBr, NaH, DMF, 2 h; room temp. (91%); iii, ethane-1,2-diol, methylene dichloride, PTSA (cat), rt (80%); iv, *p*-methoxybenzyl chloride, NaH, DMF, rt, 2 h (80%); v, MeOH–1 mol dm^{-3} HCl (aq), (9:1), 50 °C, 30 min (90%); vi, DMAP, DCC, methylene dichloride, −20 °C, (36% for **18**; 37% for **19**); vii, NaOH, MeOH, reflux, 30 min (99% for **16a**, 91% for **16b**); viii, EtOH–1 mol dm^{-3} HCl (aq), (2:1), reflux, 4 h (86% for **20a**, 83% for **20b**)

(*S*)-(+)-*O*-Acetylmandelic acid **17** was chosen for resolution of the *cis*-diol because it is relatively inexpensive and is 99% pure by GLC, and unlike the enantiomers of the commonly used camphanic acid chloride, both *R* and *S* isomers are cheaply available. (*S*)-(+)-*O*-Acetylmandelic acid has not been widely used for the resolution of *myo*-inositol derivatives, so we took the opportunity to investigate its potential as a resolving reagent. Previously, it was used successfully to resolve several blocked *myo*-inositol derivatives. Two diastereoisomers were derived, by coupling (*S*)-(+)-*O*-acetylmandelic acid to DL-1,4,5,6-tetra-*O*-benzyl-*myo*-inositol[21] at the equatorial 1-hydroxy position and were easily separated by flash chromatography and used to synthesize previously inaccessible hexoses[21] and the β-glucosidase and α-mannosidase inhibitors (+)- and (−)-norjirimycin.[22] We have recently employed (*S*)-(+)-*O*-acetylmandelic acid as a chiral auxiliary to resolve partially blocked *myo*-inositol derivatives for the synthesis of D- and L-Ins(1,4,6)P_4.[23]

Coupling of DL-1,4-di-*O*-benzyl-5,6-bis-*O*-(*p*-methoxybenzyl)-*myo*-inositol **16ab** with (*S*)-(+)-*O*-acetylmandelic acid **17** at low temperature afforded the two diastereoisomers **18** and **19** (structure established retrospectively, after the determination of the chirality of derived tetraols **20a** and **20b** respectively, *vide infra*). By keeping the temperature at −20 °C, selectivity was achieved and there was no acylation at the 2-hydroxy position (by ^{1}H NMR analysis); isomers **18** and **19** were separated by flash chromatography and were obtained as crystalline solids.

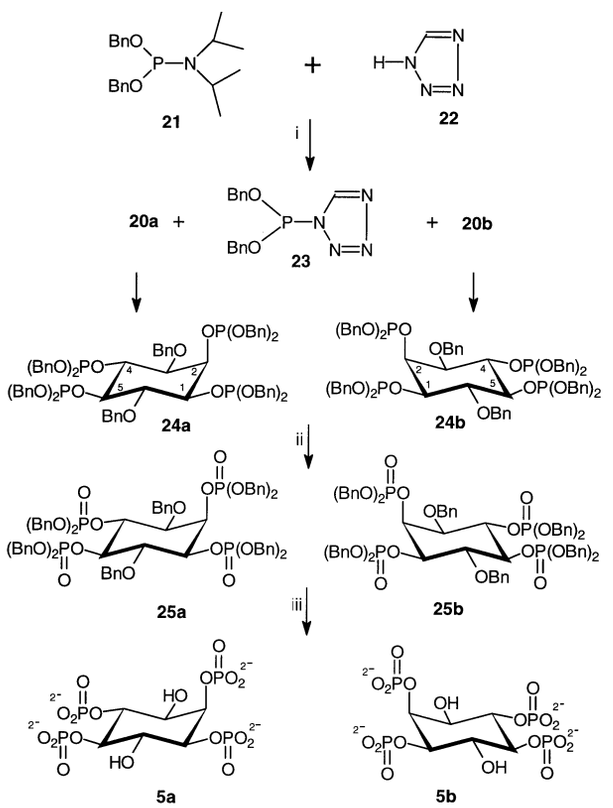
The proton 1-H (shifted downfield due to esterification of 1-OH) could not be identified in either diastereoisomer (**18** and **19**) because the methylene AB coupling pattern of the benzyl group overlapped with the expected dd for 1-H. However, 2-H was identified as a broad doublet at δ 4.15 (J 1.8 Hz) for compound **18** and as a broad doublet (J 1.8 Hz) at δ 4.40 for compound **19**. The 2-OH signal was also significant because it was seen at δ 2.16 for compound **18** and at δ 2.69 for compound **19**, which indicated that the proton and hydroxy groups attached to C-2 were more deshielded than for the less polar diastereoisomer **18**. The unique singlet at δ 5.94 for isomer **18**, and at δ 5.98 for isomer **19**, of $CH_3CO_2CH(Ph)CO_2$Ins is indicative of the high purity of each diastereoisomer and no other impurities were detected in either the ^{1}H or the ^{13}C NMR spectra.

Deacylation of isomers **18** and **19** under basic conditions gave the pure enantiomers **16a** and **16b** and the optical rotations of products **16a** and **16b** were equal and opposite. The two chiral 1,2,4,5-tetraols were prepared by acid hydrolysis of the *p*-methoxybenzyl ethers in aq. HCl at reflux temperature. The resulting solid was filtered off, and recrystallised from ethanol to give the pure enantiomers D- **20a** and L-3,6-di-*O*-benzyl-*myo*-inositol **20b** which had specific rotations of +16 and -16×10^{-1} deg cm^2 g^{-1}, respectively. The mp of the racemic mixture (lit.,[16] 205–207 °C) was considerably higher than for the chiral antipodes (172–173 °C).

The absolute configuration of antipodes **20a** and **20b** was determined by a chemical method. A simple way to establish the absolute configuration of D-3,6-di-*O*-benzyl-*myo*-inositol **20a** would be to resolve DL-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol **12ab**, followed by benzylation and acidic hydrolysis of the isopropylidene groups to give the individual enantiomers **20a** and **20b**. The resolution of DL-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol has been previously accomplished using the chiral auxiliary (*S*)-(−)-ω-camphanoyl chloride.[24] In this resolution, the 3-position was blocked by using *tert*-butyldiphenylsilyl chloride, the 6-position was acylated with the chiral auxiliary, and separation of the diastereoisomers was achieved by tedious HPLC. Therefore, a simple resolution to provide the chiral diol would be appropriate, because single-crystal X-ray analysis of the 6-*O*-camphanate has been determined, and derived from this the specific rotation, $[\alpha]_D$ +22, and mp (159–161 °C) for L-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol has been established.[24] Moreover, in a recent article, D-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol was synthesized from D-mannitol, however, the mp was found to be 176–177 °C with a specific rotation of $[\alpha]_D$ −21.7.[25] Thus, the specific rotation is in agreement for both enantiomers, but there appears to be some discrepancy over the mp

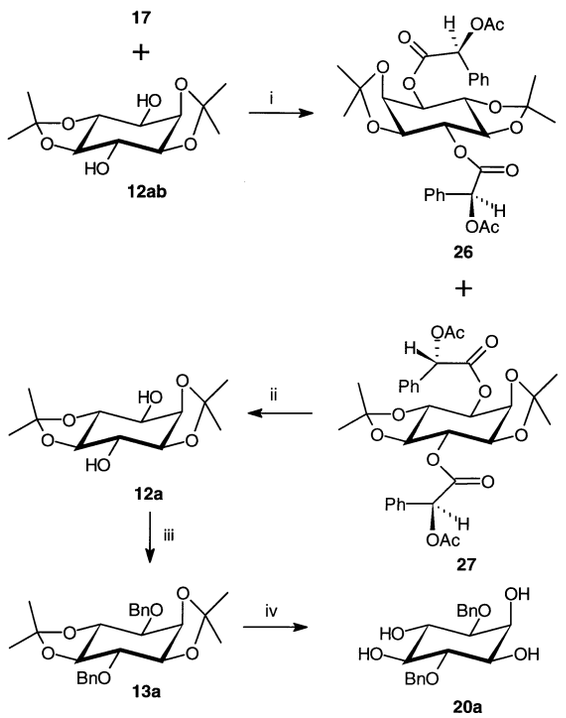
DL-1,2:4,5-Di-*O*-isopropylidene-*myo*-inositol **12ab** was acylated with (*S*)-(+)-*O*-acetylmandelic acid **17** in the presence of a coupling reagent to afford a mixture of diastereoisomers (**26** and **27**) which could not be separated by chromatography (see later, Scheme 4). The mixture of diastereoisomers was recrystallised from hot methanol and one compound, **27**, was in abundance by a factor of 2.5 by ^{1}H NMR spectroscopy; further recrystallisation from the same solvent gave the pure diastereoisomer **27** in 18% yield (unoptimised). Basic methanolysis of the two acyl groups followed by chromatography and recrystallisation of the diol from ethyl acetate gave D-1,2:4,5-di-*O*-

isopropylidene-*myo*-inositol **12a**, $[\alpha]_D$ −22, with a mp of 174–176 °C. These physical properties agreed with the data published by Chiara and Martín-Lomas.[25] Gigg and co-workers[26] have synthesized L-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol by a different route, $[\alpha]_D$ +23.3, (mp 175–177 °C). The dispute over the mp of the chiral diol has now been resolved because the value (159–161 °C) stated by Young and co-workers[24] appeared to be a little low. Compound **12a** was then benzylated to give D-3,6-di-*O*-benzyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol **13a**, ($[\alpha]_D$ −44, mp 157–159 °C). Recently, Gigg and co-workers[26] synthesized L-3,6-di-*O*-benzyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol **13b**, $[\alpha]_D$ +85, which had a mp of 159–161 °C. The acetal protecting groups at the 1,2- and 4,5-positions were removed by acid hydrolysis and the solvents were evaporated off *in vacuo*. The resulting solid was recrystallised from ethanol to give D-3,6-di-*O*-benzyl-*myo*-inositol **20a**, which was identical (^{1}H NMR, mp, specific rotation) with the previously described compound.



Scheme 3 *Reagents and conditions:* i, 1*H*-tetrazole **22**, methylene dichloride, reagent **21**, room temp., 15 min, add tetraols **20a** and **20b** in separate experiments, 10 min; ii, MCPBA, 0 °C, 30 min (94% for **25a**, 88% for **25b**); iii, Na/liquid NH_3, then purification by ion-exchange chromatography

A P^{III} approach was adopted to introduce the phosphate substituents, as shown in Scheme 3. Thus, phosphitylating agent[27] **21** (2 mole equivalents per hydroxy group) and 1*H*-tetrazole **22** (4 mole equivalents per hydroxy group) reacted to form the tetrazolide intermediate **23** (δ_P +126.73 ppm; *cf.* δ_P +147.86 ppm for compound **21**). Any moisture present in the solvent is indicated by the formation of *H*-phosphonate (δ_P +7.54 ppm). The procedure was carried out for both enantiomers in the same way. Thus, in separate experiments the enantiomers of 3,6-di-*O*-benzyl-*myo*-inositol **20a** and **20b** were allowed to react with intermediate **23**. The ^{31}P NMR spectrum again showed eight peaks and the distinctive five-bond ^{31}P–^{31}P spin–spin coupling systems[18] for isomers **24a** and **24b** ($^5J_{1,2}$ 1.8 Hz; $^5J_{4,5}$ 3.7 Hz). Oxidation of the tetrakisphosphite intermediates afforded the fully protected D **25a** and L-3,6-di-*O*-benzyl-1,2,4,5-tetrakis-*O*-(dibenzyloxyphosphoryl)-*myo*-inositol **25b** in high yields. Use of *m*-chloroperbenzoic acid (MCPBA) is preferable to *tert*-butyl hydroperoxide since the latter gives lower yields resulting from the formation of polar by-products. All the benzyl protective groups were removed from the fully blocked compound in one step by using sodium in liquid ammonia.[28] Purification of crude D-Ins(1,2,4,5)P_4 **5a** and L-Ins(1,2,4,5)P_4 **5b** was carried out by ion-exchange chromatography on Q-Sepharose Fast Flow and both compounds were eluted at ~700 mmol dm^{-3} TEAB buffer and were isolated as their triethylammonium salts and quantified by phosphate analysis.



Scheme 4 *Reagents and conditions:* i, DCC, DMAP, methylene dichloride, 0 °C (18% for **27**); ii, NaOH, MeOH, reflux, 30 min (86%); iii, NaH, BnBr, DMF, 2 h (91%); iv, 1 mol dm^{-3} HCl–MeOH (1:9), reflux, 30 min (95%)

Full experimental data for DL-Ins(1,2,4,5)P_4 **5ab** have been published for Ca^{2+} release[29] and its interaction with the enzymes Ins(1,4,5)P_3 3-kinase and 5-phosphatase.[30] The full data for antipodes **5a** and **5b** will be published elsewhere. However, notably L-Ins(1,2,4,5)P_4 **5b** was not found to release intracellular Ca^{2+} and D-Ins(1,2,4,5)P_4 **5a** was only ~2-fold less potent than was Ins(1,4,5)P_3 at Ca^{2+} release in rabbit platelets.

Experimental

TLC was performed on pre-coated plates (Merck TLC aluminium sheets silica 60 F_{254}, Art. no. 5554): the product was visualised by spraying with methanolic phosphomolybdic acid, followed by heating. Flash chromatography refers to the procedure developed by Still *et al.*[31] and was carried out on Sorbsil C60 silica gel.

NMR spectra for the nuclei ^{31}P, ^{1}H and ^{13}C were recorded on JEOL FX-90Q, GX270 and GX400 spectrometers. Chemical shifts were measured in parts per million (ppm) relative to tetramethylsilane (TMS), deuterium oxide (D_2O) or [$^2H_6$]-dimethyl sulfoxide ([$^2H_6$]DMSO). 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. M.p.s (uncorrected) were determined using a Reichert-Jung Thermo Galen Kofler block. Microanalysis was carried out by the University of Bath microanalysis service. Low-resolution mass spectra were recorded by the University of Bath Mass Spectrometry Service using +ve and −ve Fast Atom Bombardment

(FAB) with 3-nitrobenzyl alcohol (NBA) as the matrix. High-resolution accurate mass spectrometry was carried out by the EPSERC Mass Spectrometry Service in Swansea. Optical rotations were measured using an Optical Activity Ltd. AA-10 polarimeter; $[a]_D$-values are given in 10^{-1} deg cm^2 g^{-1} and all rotations were measured at ambient temperature.

Ion-exchange chromatography was performed on an LKB-Pharmacia Medium-Pressure Ion Exchange Chromatograph using Q-Sepharose and gradients of TEAB as eluent. Fractions containing phosphate were assayed by a modification of the Briggs phosphate test.[32]

Light petroleum refers to the fraction with distillation range 60–80 °C.

DL-1,4-Di-*O*-benzoyl-*myo*-inositol 8ab

DL-3,6-Di-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol **7ab** (9.36 g, 20 mmol) was suspended in 80% aq. acetic acid (200 cm^3). The mixture was heated under reflux for 30 min, cooled, and poured into an ice–water mixture (1000 cm^3). The precipitated solid was filtered off, washed thoroughly with diethyl ether and recrystallised from DMF–water to give the *title compound* **8ab** (7.25 g, 93%), mp 253–254 °C (from DMF–water) (Found: C, 61.9; H, 5.11. $C_{20}H_{20}O_8$ requires C, 61.85; H, 5.15%); δ_H(270 MHz; [$^2H_6$]DMSO) 3.48 (1 H, dt, *J* 4.65 and 9.3, 5-H), 3.73 (1 H, ddd, *J* 2.25, 6.9 and 9.5, 3-H), 3.93 (1 H, dt, *J* 5.5 and 9.5, 6-H), 4.07 (1 H, br t, *J* 2.0, 2-H), 4.83 (1 H, dd, *J* 2.0 and 10.0, 1-H), 4.97 (1 H, d, *J* 6.6, D_2O ex, OH), 5.25–5.33 (2 H, m, D_2O ex, OH), 5.35 (1 H, t, *J* 9.9, 4-H), 5.38 (1 H, d, *J* 4.2, D_2O ex, OH), 7.50–7.69 [6 H, m, OC(O)Ph] and 8.00–8.09 [4 H, m, OC(O)Ph]; δ_C(68 MHz; [$^2H_6$]DMSO) 69.83, 70.77, 70.93, 73.07, 75.70, 76.28, 129.34, 130.12, 130.25, 130.64, 131.13, 133.85, 134.14 and 166.58; *m/z* (FAB$^+$) 389 [M + H (100%)], 306 (62), 274 (28), 243 (20), 199 (40) and 105 (88).

DL-3,6-Di-*O*-benzoyl-1,2,4,5-tetrakis-*O*-(diethoxyphosphoryl)-*myo*-inositol 10ab

A mixture of DL-1,4-di-*O*-benzoyl-*myo*-inositol **8ab** (0.776 g, 2 mmol), dry DMF (10 cm^3) and dry *N*,*N*-diisopropylethylamine (2.8 cm^3, 16 mmol) was stirred under nitrogen at room temperature. The solution was cooled in an ice-bath and chloro(diethoxy)phosphine (2.32 cm^3, 16 mmol) was added dropwise over a period of 5 min and then the mixture was warmed to room temperature. After being stirred for 1 h, 70% *tert*-butyl hydroperoxide, (3 cm^3, 21.8 mmol) was added to the reaction mixture at −78 °C to give the crude product, R_f (ethyl acetate) 0.20. The solvents were evaporated off *in vacuo* and the remaining solid was partitioned between water and methylene dichloride (50 cm^3 of each). The organic layer was washed successively with 10% aq. sodium metabisulfite, brine (20 cm^3 of each) and finally water (2 × 20 cm^3). The organic layer was dried over ($MgSO_4$) and evaporated off to give a solid. The crude product was purified over silica gel (ethyl acetate) and the solvent was evaporated off to give the pure *title compound* **10ab** (1.55 g, 83%), mp 122–123 °C (from ethyl acetate–hexane) (Found: C, 46.1; H, 6.03. $C_{36}H_{56}O_{20}P_4$ requires C, 46.35; H, 6.00%); δ_H(400 MHz; $CDCl_3$) 0.82–0.88 [9 H, m, OP(O)OCH$_2$C*H*$_3$], 1.20–1.33 [15 H, m, OP(O)OCH$_2$C*H*$_3$], 3.52–3.85 [6 H, m, OP(O)OC*H*$_2$CH$_3$], 4.01–4.21 [10 H, m, OP(O)OC*H*$_2$CH$_3$], 4.79 (2 H, q, *J* 9.5, 1- and 5-H), 5.15 (1 H, q, *J* 9.2 and 9.5, 4-H), 5.25 (1 H, td, *J* 2.4 and 9.2, 2- or 3-H), 5.29 (1 H, td, *J* 2.1 and 10.1, 3- or 2-H), 5.90 (1 H, t, *J* 10.1, 6-H), 7.43–7.59 [6 H, m, OC(O)Ph] and 8.17–8.24 [4 H, m, OC(O)Ph]; δ_C(68 MHz; $CDCl_3$) 14.34, 15.24, 15.79, 15.89, 63.66, 63.79, 64.22, 70.02, 73.27, 75.05, 75.37, 76.35, 129.02, 129.70, 128.11, 128.21, 130.12, 130.38, 133.14, 133.37 and 165.32; δ_P(162 MHz; $CDCl_3$) −1.53, −2.11, −2.18 and −2.49 (^{1}H–^{31}P decoupled); *m/z* (FAB$^+$) 933 [M + H (18%)], 779 (5) and 105 (100).

DL-3,6-Di-*O*-benzoyl-*myo*-inositol 1,2,4,5-tetrakisphosphate 11ab

DL-3,6-Di-*O*-benzoyl-1,2,4,5-tetrakis-*O*-(diethoxyphosphoryl)-*myo*-inositol **10ab** (0.932 g, 0.1 mmol) in dry methylene dichloride (5 cm^3) was stirred at room temperature under nitrogen. Bromotrimethylsilane (0.264 cm^3, 2 mmol) was added to the dry solution, and the mixture was stirred overnight. The solvents were evaporated off and the residue was stirred with water (2 cm^3) for 1 h. Final purification of one-third of the compound was carried out by ion-exchange chromatography, on Q-Sepharose Fast Flow, using a buffer gradient of TEAB (200–1000 mmol dm^{-3}) and flow rate 5 cm^3 min^{-1}. The fractions which gave a positive Briggs test and eluted at ~500 mmol dm^{-3} buffer were pooled to give pure *title compound* **11ab** (27 μmol, 81%), δ_H(270 MHz; D_2O) 4.54–4.62 (2 H, m, 1- and 5-H), 4.80 (1 H, 4-H, obscured by HDO peak), 5.07 (1 H, d, *J* 10.25, 3-H), 5.23 (1 H, d, *J* 10.1, 2-H), 5.61 (1 H, t, *J* 9.9, 6-H), 7.47–7.66 [6 H, m, Ins-OC(O)Ph] and 8.09–8.16 [4 H, m, Ins-OC(O)Ph]; δ_C(68 MHz; D_2O) 71.51, 72.39, 73.88, 74.95, 128.04, 128.56, 128.79, 128.98, 129.54, 129.70, 133.22, 167.52 and 167.74; δ_P(162 MHz; D_2O) −0.22 (d, *J* 9.8, $CHOPO_3^{2-}$), −0.39 (d, *J* 10.7, $CHOPO_3^{2-}$), −0.49 (d, *J* 11.9, $CHOPO_3^{2-}$) and −0.79 (d, *J*, 8.8, $CHOPO_3^{2-}$); *m/z* (FAB$^-$) 707 [M − H (100%)], 460 (12), 387 (30), 232 (95), 177 (20), 159 (44) and 97 (30) [Found: *m/z* 706.9730. $C_{20}H_{24}O_{20}P_4$ requires (M − H)$^-$, 706.9732].

DL-*myo*-Inositol 1,2,4,5-tetrakisphosphate 5ab

Crude DL-3,6-di-*O*-benzoyl-*myo*-inositol 1,2,4,5-tetrakisphosphate **11ab** (0.1 mmol) was heated with 1 mol dm^{-3} sodium hydroxide (3 cm^3) at 60 °C for 1 h. Dowex (H$^+$-form) was added with water (30 cm^3) until the pH was ~6. The Dowex was filtered off, washed with water (2 × 20 cm^3), and the benzoic acid was removed by washing with methylene dichloride (2 × 30 cm^3). The aqueous layer was then concentrated and the residue was purified by ion-exchange chromatography, using a gradient of TEAB (0–1000 mmol dm^{-3}). The pure *title compound* **5ab** eluted at ~550 mmol dm^{-3} TEAB (80 μmol, 80%), δ_H(400 MHz; D_2O) 3.70 (1 H, d, *J* 10.1, 3-H), 3.90 (1 H, t, *J* 9.5, 6-H), 3.97–4.04 (2 H, m, 1- and 5-H), 4.29 (1 H, q, *J* 9.2, 4-H) and 4.80 (1 H, 2-H, obscured by HDO peak); δ_C(68 MHz; D_2O) 69.86, 70.86, 73.91, 74.88, 76.41 and 77.71; δ_P(162 MHz; D_2O) +1.31 (d, *J* 8.0, $CHOPO_3^{2-}$), +1.15 (d, *J* 7.9, $CHOPO_3^{2-}$), +1.04 (d, *J* 9.9, $CHOPO_3^{2-}$) and −0.04 (d, *J* 6.0, $CHOPO_3^{2-}$); *m/z* (FAB$^-$) 499 [M − H (100%)], 481 (5), 401 (5), 154 (10) and 97 (7) [Found: *m/z*, 498.9210. $C_6H_{16}O_{18}P_4$ requires (M − H)$^-$, 498.9210].

DL-1,4-Di-*O*-benzyl-2,3-*O*-isopropylidene-*myo*-inositol 14ab

DL-3,6-Di-*O*-benzyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol[16] **13ab** (5.28 g, 12 mmol) was dissolved in methylene dichloride (100 cm^3), followed by the addition of a catalytic amount of PTSA (20 mg, 0.1 mmol) and one mole equivalent of ethane-1,2-diol (0.57 cm^3, 12 mmol). The mixture was stirred at room temperature until the solvent became slightly turbid. TLC (Et_2O) showed a major product R_f = 0.30, a trace product R_f = 0.06, and a trace of starting material R_f = 0.80. Triethylamine (2 cm^3) was added to the reaction mixture and the solvent was evaporated off. Purification by flash chromatography (methylene dichloride–ethyl acetate, 1:1) gave the title compound **14ab** (3.84 g, 80%), mp 160–161 °C (from ethyl acetate) (lit.,[20] 161–163 °C), δ_H($CDCl_3$; 270 MHz) 1.33, 1.48 (6 H, 2 s, CMe_2), 2.96 (1 H, br s, D_2O ex, OH), 3.01 (1 H, br s, D_2O ex, OH), 3.35 (1 H, t, *J* 9.3, 5-H), 3.51 (1 H, d, *J* 10.25, 3-H), 3.52 (1 H, t, *J* 9.9, 6-H), 3.92 (1 H, t, *J* 9.5, 4-H), 4.06 (1 H, dd, *J* 5.3 and 6.8, 1-H), 4.27 (1 H, dd, *J* 4.2 and 5.1, 2-H), 4.68 and 4.91 (2 H, AB, *J* 11.5, OC*H*$_2$Ph), 4.77 (2 H, apparent s, OC*H*$_2$Ph) and 7.24–7.41 (10 H, m, OCH$_2$*Ph*); δ_C(68 MHz; $CDCl_3$) 25.88, 27.99, 71.51, 72.55, 72.97, 73.27, 73.98, 76.93, 79.17, 81.89, 109.85, 127.66, 127.96, 128.02, 128.31, 128.44, 137.78 and 138.07.

DL-3,6-Di-*O*-benzyl-1,2-*O*-isopropylidene-4,5-bis-*O*-(*p*-methoxybenzyl)-*myo*-inositol 15ab

A mixture of DL-1,4-di-*O*-benzyl-2,3-*O*-isopropylidene-*myo*-

inositol **14ab** (2.8 g, 7 mmol) and sodium hydride (0.72 g, 30 mmol) was dissolved in dry DMF (50 cm^3). *p*-Methoxybenzyl chloride (2.9 cm^3, 20 mmol) was added dropwise at room temperature and the mixture was stirred for 2 h. TLC (diethyl ether–light petroleum, 2:1) showed a new product, $R_f = 0.40$. The excess of sodium hydride was destroyed with methanol (10 cm^3) and the solvents were evaporated off *in vacuo*. The remaining syrup was partitioned between water (100 cm^3) and diethyl ether (100 cm^3), and washed successively with aq. 0.1 mol dm^{-3} HCl (100 cm^3), saturated aq. sodium hydrogen carbonate (100 cm^3), and water (100 cm^3). The organic layer was dried ($MgSO_4$), the remaining syrup was purified by flash chromatography (diethyl ether–light petroleum, 2:1) and the *product* **15ab** was isolated as a syrup (3.60 g, 80%) (Found: C, 73.0; H, 6.64. $C_{39}H_{44}O_8$ requires C, 73.09; H, 6.93%); δ_H(270 MHz; $CDCl_3$) 1.35 and 1.51 (6 H, 2 s, CMe_2), 3.39 (1 H, t, *J* 8.8, 5-H), 3.67 (1 H, dd, *J* 3.6 and 8.8, 3- or 1-H), 3.74–3.80 (1 H, obscured, 1- or 3-H), 3.77 (3 H, s, $OCH_2C_6H_4OMe$), 3.79 (3 H, s, $OCH_2C_6H_4OMe$), 3.92 (1 H, t, *J* 8.6, 4- or 6-H), 4.09 (1 H, t, *J* 6.6, 6- or 4-H), 4.25 (1 H, dd, *J* 4.0 and 5.3, 2-H), 4.71–4.88 (8 H, m, OCH_2Ph), 6.84 (4 H, 2 d, *J* 9.1, $OCH_2C_6H_4OMe$) and 7.21–7.41 (14 H, m, OCH_2Ph and $OCH_2C_6H_4OMe$); δ_C(68 MHz; $CDCl_3$) 25.53, 27.59, 55.04, 73.10, 73.65, 74.37, 74.73, 77.00, 78.91, 80.47, 81.70, 82.35, 109.56, 113.52, 113.58, 114.10, 127.30, 127.63, 127.72, 127.82, 128.05, 128.21, 129.44, 130.61, 131.78, 138.04, 138.40 and 158.96; *m/z* (FAB^-) 549 (M − benzyl, 8%), 519 (M − *p*-methoxybenzyl, 40)], 335 (10), 258 (30), 137 ($OCH_2C_6H_4OMe$, 100) and 107 (OCH_2Ph, 70).

DL-1,4-Di-*O*-benzyl-5,6-bis-*O*-(*p*-methoxybenzyl)-*myo*-inositol 16ab

DL-3,6-Di-*O*-benzyl-1,2-*O*-isopropylidene-4,5-bis-*O*-(*p*-methoxybenzyl)-*myo*-inositol **15ab** (2.25 g, 3.5 mmol) was dissolved in a mixture of methanol–1 mol dm^{-3} aq. HCl (9:1; 30 cm^3), which solution was kept at 50 °C for 30 min. TLC (Et_2O) showed a new product, R_f 0.40. Sodium hydrogen carbonate (2 g) was added and the solvents were evaporated off under reduced pressure. The product was extracted with methylene dichloride (3 × 100 cm^3), and the organic solvent was evaporated off to give a solid. The crude product was purified by flash chromatography (diethyl ether–chloroform, 3:1) to give the title compound **16ab** (1.9 g, 90%), mp 130–132 °C (from ethyl acetate–hexane) (lit.,[19] 130.4–130.6 °C); δ_H(270 MHz; $CDCl_3$) 2.53 (1 H, d, *J* 4.4, D_2O ex, OH), 2.62 (1 H, s, D_2O ex, OH), 3.43 (2 H, overlapping, 3- and 1-H), 3.44 (1 H, t, *J* 9.3, 5-H), 3.78 (3 H, s, $OCH_2C_6H_4OMe$), 3.79 (3 H, s, $OCH_2C_6H_4OMe$), 3.81 (1 H, t, *J* 9.3, 4- or 6-H), 3.94 (1 H, t, *J* 9.5, 6- or 4-H), 4.25 (1 H, br s, 2-H), 4.70–4.96 (8 H, m, OCH_2Ph and $OCH_2C_6H_4OMe$), 6.83 (2 H, d, *J* 8.8, $OCH_2C_6H_4OMe$), 6.84 (2 H, d, *J* 8.8, $OCH_2C_6H_4OMe$) and 7.21–7.36 (14 H, m, OCH_2Ph and $OCH_2C_6H_4OMe$); δ_C(68 MHz; $CDCl_3$) 55.04, 69.15, 71.71, 72.68, 75.34, 75.50, 80.01, 81.28, 81.37, 82.93, 113.74, 127.82, 127.89, 128.54, 129.41, 129.54, 130.68, 130.84, 138.49, 138.81 and 159.12; *m/z* (FAB^-) 753 (M + NBA, 40%), 599 (M − H, 100), 509 (M − benzyl, 10), 479 (M − *p*-methoxybenzyl, 20), 335 (15), 137 ($OCH_2C_6H_4OMe$, 30) and 107 (OCH_2Ph, 30).

D- 18 and L-1-*O*-[(*S*)-(+)-*O*-Acetylmandelyl]-3,6-di-*O*-benzyl-4,5-bis-*O*-(*p*-methoxybenzyl)-*myo*-inositol 19

A mixture of DL-1,4-di-*O*-benzyl-5,6-bis-*O*-(*p*-methoxybenzyl)-*myo*-inositol **16ab** (2.5 g, 4.17 mmol), (*S*)-(+)-*O*-acetylmandelic acid **17** (0.835 g, 4.3 mmol) and 4-(dimethylamino)pyridine (DMAP) (0.03 g, 0.25 mmol) was stirred in methylene dichloride (15 cm^3) at −20 °C (solid CO_2 alone). A solution of dicyclohexylcarbodiimide (DCC) (0.877 g, 4.33 mmol) in methylene dichloride (5 cm^3) was added dropwise over a period of 90 min with stirring of the reaction mixture, which was then stirred at room temperature overnight after which TLC (chloroform–acetone, 30:1) showed two products, R_f 0.44 and 0.34. The mixture was filtered through Celite and washed thoroughly with methylene dichloride (100 cm^3). The solvent was evaporated off to give a solid, and the individual diastereoisomers were separated by flash chromatography (chloroform–acetone, 30:1) to give isomers **18**, R_f 0.44 (36% yield); mp 120–121 °C (from EtOH); $[\alpha]_D$ +12 (*c* 1, CH_2Cl_2) and **19**, R_f 0.34 (37% yield); mp 147–148 °C (from EtOH); $[\alpha]_D$ +42 (*c* 1, CH_2Cl_2); for *isomer* **18** (Found: C, 71.1; H, 6.27. $C_{46}H_{48}O_{11}$ requires C, 71.10; H, 6.23%) and for *isomer* **19** (Found: C, 70.8; H, 6.22%); isomer **18** δ_H(400 MHz; $CDCl_3$) 2.16 (1 H, s, D_2O ex, OH), 2.19 [3 H, s, $O_2CCH(OAc)Ph$], 3.44 (1 H, dd, *J* 2.45 and 9.5, 3-H), 3.49 (1 H, t, *J* 9.5, 5-H), 3.77 (3 H, s, $OCH_2C_6H_4OMe$), 3.78 (3 H, s, $OCH_2C_6H_4OMe$), 3.91 (1 H, t, *J* 9.5, 4-H), 4.05 (1 H, t, *J* 10.1, 6-H), 4.15 (1 H, br d, *J* 1.8), 4.61–4.81 (9 H, m, OCH_2Ph, OCH_2OMe, and 1-H), 5.94 [1 H, s, $O_2CCH(OAc)Ph$], 6.82 (2 H, d, *J* 8.5, $OCH_2C_6H_4OMe$), 6.83 (2 H, d, *J* 8.85, $OCH_2C_6H_4OMe$) and 7.16–7.44 [19 H, m, OCH_2Ph, $OCH_2C_6H_4OMe$ and $O_2CCH(OAc)Ph$]; δ_C(100 MHz; $CDCl_3$) 20.70, 55.25, 67.32, 72.80, 74.78, 75.25, 75.46, 75.58, 78.42, 79.61, 80.72, 82.73, 113.77, 127.34, 127.52, 127.76, 127.91, 128.29, 128.47, 128.82, 129.26, 129.42, 129.55, 130.76, 130.81, 133.37, 137.71, 138.31, 159.14, 159.18, 168.27 and 170.75; *m/z* (FAB^-) 929 (M + NBA, 30%), 775 (M − H, 58), 599 (50), 193 (55) and 149 (100).

For isomer **19** δ_H(400 MHz; $CDCl_3$) 2.17 [3 H, s, $O_2CCH(OAc)Ph$], 2.69 (1 H, s, D_2O ex, OH), 3.43 (1 H, t, *J* 9.5, 5-H), 3.49 (1 H, dd, *J* 2.75 and 9.8, 3-H), 3.75 (3 H, s, $OCH_2C_6H_4OMe$), 3.77 (3 H, s, $OCH_2C_6H_4OMe$), 3.93 (1 H, t, *J* 9.8, 4-H), 4.00 (1 H, t, *J* 9.8, 6-H), 4.14 and 4.46 (2 H, AB, *J* 11.0, OCH_2Ph or $OCH_2C_6H_4OMe$), 4.40 (1 H, br d, *J* 1.8, 2-H), 4.61–4.84 (7 H, m, OCH_2Ph, $OCH_2C_6H_4OMe$, and 1-H), 5.98 [1 H, s, $O_2CCH(OAc)Ph$], 6.75 (2 H, d, *J* 8.5, $OCH_2C_6H_4OMe$), 6.82 (2 H, d, *J* 8.85, $OCH_2C_6H_4OMe$) and 6.83–7.46 [19 H, m, OCH_2Ph, $OCH_2C_6H_4OMe$ and $O_2CCH(OAc)Ph$]; δ_C(100 MHz; $CDCl_3$) 20.65, 55.23, 55.27, 67.43, 72.80, 74.94, 74.98, 75.40, 75.58, 78.40, 79.79, 80.74, 82.70, 113.68, 113.75, 127.19, 127.25, 127.89, 128.02, 128.49, 128.84, 129.41, 129.50, 130.65, 130.83, 132.92, 137.63, 138.20, 159.08, 159.16, 168.58 and 170.66; *m/z* (FAB^-) 929 (M + NBA, 15%), 775 (M − H, 28), 599 (25), 193 (55) and 149 (100).

D-3,6-Di-*O*-benzyl-4,5-bis-*O*-(*p*-methoxybenzyl)-*myo*-inositol 16a

A mixture of D-1-*O*-[(*S*)-(+)-*O*-acetylmandelyl]-3,6-di-*O*-benzyl-4,5-bis-*O*-(*p*-methoxybenzyl)-*myo*-inositol **18** (0.956 g, 1.23 mmol), sodium hydroxide (0.40 g, 10 mmol) and methanol (100 cm^3) was heated at reflux temperature for 30 min. The mixture was cooled, and neutralised with carbon dioxide. The resulting solid was diluted with water (50 cm^3) and evaporated to dryness *in vacuo*. The crude product was extracted with methylene dichloride (4 × 100 cm^3) which was then evaporated off to give a solid, *compound* **16a**, R_f (Et_2O) 0.40 (0.729 g, 99%); mp 133–134 °C (from ethyl acetate–hexane); $[\alpha]_D$ −25 (*c* 1, CH_2Cl_2) (Found: C, 72.1; H, 6.77. $C_{36}H_{40}O_8$ requires C, 71.98; H, 6.71%). The mass spectrum and NMR data were identical with those of racemate **16ab**.

L-3,6-Di-*O*-benzyl-4,5-bis-*O*-(*p*-methoxybenzyl)-*myo*-inositol 16b

A mixture of L-1-*O*-[(*S*)-(+)-*O*-acetylmandelyl]-3,6-di-*O*-benzyl-4,5-bis-*O*-(*p*-methoxybenzyl)-*myo*-inositol **19** (0.929 g, 1.19 mmol), sodium hydroxide (0.40 g, 10 mmol) and methanol (100 cm^3) was heated at reflux temperature for 30 min. Work-up as for the D-enantiomer gave the title compound **16b** R_f (Et_2O) 0.40 (0.655 g, 91%); mp 133–134 °C (from ethyl acetate–hexane); $[\alpha]_D$ +25 (*c* 1, CH_2Cl_2) (Found: C, 72.0; H, 6.86%). The mass spectrum and NMR data were identical with those of racemate **16ab**.

D-3,6-Di-*O*-benzyl-*myo*-inositol 20a

D-3,6-Di-*O*-benzyl-4,5-bis-*O*-(*p*-methoxybenzyl)-*myo*-inositol

16a (0.624 g, 1.04 mmol) was suspended in 1 mol dm^{-3} aq. HCl–ethanol (60 cm^3; 1:2). The mixture was heated at reflux temperature for 4 h, cooled and the solvents were evaporated *in vacuo*. The resulting solid was filtered off and washed with water (10 cm^3) and ether (2 × 10 cm^3). The solid was then recrystallised from ethanol to give the pure title compound **20a**, R_f (chloroform–methanol, 6:1) 0.60 (0.323 g, 86%); mp 172–173 °C (from ethanol); $[\alpha]_D$ +16 (*c* 1, MeOH) (Found: C, 66.6; H, 6.73. $C_{20}H_{24}O_6$ requires C, 66.65; H, 6.71%); δ_H(400 MHz; [$^2H_6$]DMSO) 3.11 (1 H, dd, *J* 2.4 and 9.8, 3-H), 3.15 (1 H, dt, *J* 4.9 and 8.85, D_2O ex, t, *J* 9.15, 5-H), 3.31 (1 H, ddd, *J* 2.4, 6.7 and 9.5, D_2O ex, dd, *J* 2.4 and 9.8, 1-H), 3.44 (1 H, t, *J* 9.5, 6-H), 3.60 (1 H, dt, *J* 2.4 and 5.8, D_2O ex, t, *J* 2.4, 2-H), 4.51 and 4.60 (2 H, AB, *J* 12.2, OC*H*$_2$Ph), 4.67 (1 H, d, *J* 6.7, D_2O ex, OH), 4.74–4.81 (4 H, m, OH and OC*H*$_2$Ph), 4.84 (1 H, d, *J* 4.9, D_2O ex, OH) and 7.11–7.44 (10 H, m, OCH$_2$*Ph*); δ_C(100 MHz; [$^2H_6$]DMSO) 69.73, 70.72, 71.43, 72.25, 73.59, 75.03, 79.79, 81.82, 126.92, 127.08, 127.48, 127.52, 127.63, 127.85, 127.99, 139.32 and 139.94; *m/z* (FAB^-) 513 (M + NBA, 100%), 359 (M − H, 75), 291 (50) and 228 (30).

L-3,6-Di-*O*-benzyl-*myo*-inositol 20b

L-3,6-Di-*O*-benzyl-4,5-bis-*O*-(*p*-methoxybenzyl)-*myo*-inositol **16b** (0.590 g, 0.98 mmol) was suspended in 1 mol dm^{-3} aq. HCl–ethanol (60 cm^3; 1:2). The mixture was heated at reflux temperature for 4 h, cooled, and evaporated *in vacuo*. The resulting solid was filtered off, and washed successively with water (10 cm^3) and diethyl ether (2 × 10 cm^3). The solid was then recrystallised from ethanol to give the pure *title compound* **20b**, R_f (chloroform–methanol, 6:1) 0.60 (0.293 g, 83%); mp 172–173 °C (from EtOH); $[\alpha]_D$ −16 (*c* 1, MeOH) (Found: C, 66.4; H, 6.73%). The mass spectrum and NMR data were identical with those of compound **20a**.

D-3,6-Di-*O*-benzyl-1,2,4,5-tetrakis-*O*-[di(benzyloxy)phosphoryl]-*myo*-inositol 25a

A mixture of bis(benzyloxy)diisopropylaminophosphine **21** (0.69 g, 2 mmol) and 1*H*-tetrazole **22** (0.28 g, 4 mmol) in dry methylene dichloride (5 cm^3) was stirred at room temperature for 15 min in order to form the tetrazolide intermediate **23**. D-3,6-Di-*O*-benzyl-*myo*-inositol **20a** (0.108 g, 0.30 mmol) was added to compound **23** and the mixture was stirred for a further 10 min before being cooled to 0 °C; MCPBA (0.8 g, 2.3 mmol) (50–60%) was added and the mixture was stirred for a further 30 min, diluted with ethyl acetate (50 cm^3), and washed successively with 10% aq. sodium metabisulfite (50 cm^3), 1 mol dm^{-3} HCl, saturated aq. sodium hydrogen carbonate, brine and water (50 cm^3 of each). The organic layer was separated, then dried ($MgSO_4$), and evaporated to give a syrup. The product was purified by flash chromatography, R_f (chloroform–acetone, 5:1) 0.20, then (ethyl acetate–pentane, 2:1), in order to obtain the *pure title compound* **25a** as a syrup (0.395 g, 94%); $[\alpha]_D$ −3.5 (*c* 2, CH_2Cl_2) (Found: C, 65.2; H, 5.54. $C_{76}H_{76}O_{18}P_4$ requires C, 65.14; H, 5.47%); δ_H(400 MHz; $CDCl_3$) 3.55 (1 H, d, *J* 9.8, 3-H), 3.98 (1 H, t, *J* 9.5, 6-H), 4.41 (1 H, t, *J* 9.5, 5-H), 4.48–5.11 [22 H, m, O(O)POC*H*$_2$Ph, OC*H*$_2$Ph, 1- and 4-H], 5.43 (1 H, d, *J* 8.9, 2-H) and 6.94–7.41 [50 H, m, O(O)POCH$_2$*Ph* and OCH$_2$*Ph*]; δ_C(100 MHz; $CDCl_3$) 69.18, 69.23, 69.29, 69.24, 69.42, 69.47, 69.53, 69.60, 69.65, 72.24, 74.37, 74.37, 74.63, 75.43, 77.38, 78.66, 127.19, 127.39, 127.57, 127.74, 127.85, 127.96, 128.09, 128,16, 128.29, 128.45, 128.51, 128.65, 135.48, 135.55, 135.60, 135.73, 135.78, 135.84, 135.91, 136.01, 136.46 and 137.94; δ_P(162 MHz; $CDCl_3$) −1.16, −1.66, −1.71 and −2.07 (^{31}P–^1H-decoupled); *m/z* (FAB^+) 1401 (M + H, 7%), 181 (5), 107 (2) and 91 (100).

L-3,6-Di-*O*-benzyl-1,2,4,5-tetrakis-*O*-[di(benzyloxy)phosphoryl]-*myo*-inositol 25b

A mixture of bis(benzyloxy)diisopropylaminophosphine **21** (0.69 g, 2 mmol) and 1*H*-tetrazole **22** (0.28 g, 4 mmol) in dry methylene dichloride (5 cm^3) was stirred at room temperature for 15 min in order to form the tetrazolide intermediate **23**. L-3,6-Di-*O*-benzyl-*myo*-inositol **20b** (0.108 g, 0.30 mmol) was added to the solution, which was stirred for a further 10 min. The reaction mixture was cooled to 0 °C, MCPBA (0.8 g, 2.3 mmol) (50–60%) was added and the mixture was stirred for a further 30 min. The *product* **25b** was extracted, and purified by chromatography in the same way as its antipode **25a** (0.37 g, 88%), $[\alpha]_D$ +3.3 (*c* 1.26, CH_2Cl_2) (Found: C, 65.0; H, 5.72%. The mass spectrum and NMR data were identical with those of isomer **25a**.

D-*myo*-Inositol 1,2,4,5-tetrakisphosphate 5a

Ammonia (80 cm^3) was distilled into a three-neck flask (cooled with solid CO_2) and small pieces of freshly cut sodium metal (0.80 g, 34.8 mmol) were added until the solution remained blue. The solid-CO_2 condenser was moved to the reaction flask and ammonia (40 cm^3) was gently transferred to the flask by heating. Small slivers of sodium (0.40 g, 17.4 mmol) were added to the ammonia until the colour remained blue once again. D-3,6-Di-*O*-benzyl-1,2,4,5-tetrakis-*o*-[di(benzyloxy)-phosphoryl]-*myo*-inositol **25a** (0.178 g, 126 μmol) was dissolved in dry 1,4-dioxane (1 cm^3), and the solution was then added to the mixture of sodium in liquid ammonia. The reaction was left for 2 min and was then quenched with methanol (20 cm^3). The ammonia was evaporated off under a stream of nitrogen and MilliQ water was then added to the residue, which was evaporated to dryness *in vacuo*. The deprotected phosphate was purified by ion-exchange chromatography on Q-Sepharose, using a gradient of TEAB buffer (0–1000 mmol dm^{-3}) and eluted at ~800 mmol dm^{-3}, to give *title compound* **5a** (50.02 μmol, 40%), $[\alpha]_D$ −27.2 (*c* 0.50, TEAB, pH 8.6); δ_H(400 MHz; D_2O) 3.59 (1 H, d, *J* 9.8, 3-H), 3.76 (1 H, t, *J* 9.5, 6-H), 3.90 (2 H, q, *J* 9.15, 1- and 5-H), 4.16 (1 H, q, *J* 9.5, 4-H) and 4.59 (1 H, d, *J* 9.8, 2-H); δ_P(162 MHz; D_2O) +0.65 (d, *J* 9.3, $CHOPO_3^{2-}$), +0.27 (d, *J* 9.0, $CHOPO_3^{2-}$), −0.01 (d, *J* 9.0, $CHOPO_3^{2-}$) and −0.33 (d, *J* 8.1, $CHOPO_3^{2-}$); *m/z* (FAB^-) 499 [M − H (100%)], 419 (5), 159 (10) and 97 (9) [Found: *m/z*, 498.9226 $(M - H)^-$ requires *m/z*, 498.9208].

L-*myo*-Inositol 1,2,4,5-tetrakisphosphate 5b

L-3,6-Di-*O*-benzyl-1,2,4,5-tetrakis-*O*-[di(benzyloxy)phosphoryl]-*myo*-inositol **25b** (0.10 g, 71 μmol) was deprotected as for its antipode **25a** to give pure L-myo-*inositol* 1,2,4,5-*tetrakisphosphate* **5b** after ion-exchange chromatography (15.56 μmol, 22%). The NMR spectra were slightly different due to the different pH of the solution sample; $[\alpha]_D$ +25.8 (*c* 0.31, TEAB, pH 8.6); δ_H(270 MHz; D_2O) 3.72 (1 H, d, *J* 9.7, 3-H), 3.90 (1 H, t, *J* 9.5, 6-H), 4.03 (2 H, q, *J* 9.3, 1- and 5-H), 4.30 (1 H, q, *J* 9.5, 4-H) and 4.71 (1 H, br d, *J* 9.7, 2-H); δ_P(109 MHz; D_2O) +1.78 (d, *J* 10.1, $CHOPO_3^{2-}$), +1.44 (d, *J* 6.7, $CHOPO_3^{2-}$), +1.20 (d, *J* 6.7, $CHOPO_3^{2-}$) and +0.67 (d, *J* 6.7, $CHOPO_3^{2-}$); *m/z* (FAB^-) 499 [M − H (100%)], 419 (10), 159 (10) and 97 (10) [Found: *m/z*, 498.9187].

D-3,6-Bis-*O*-[(*S*)-(+)-*O*-acetylmandelyl]-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol 27

A mixture of DL-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol **12ab** (2.08 g, 8 mmol), DCC (4.13 g, 20 mmol) and DMAP (0.05 g, 0.4 mmol) in dry methylene dichloride (50 cm^3) was stirred at 0 °C under nitrogen. A solution of (*S*)-(+)-*O*-acetylmandelic acid **17** (3.88 g, 20 mmol) in dry methylene dichloride (30 cm^3) was added dropwise over a period of 15 min and the mixture was stirred overnight. The precipitated dicyclohexylurea was filtered off over Celite and the filtrate was evaporated to give a solid. The mixture of diastereoisomers was purified by flash chromatography [R_f (chloroform–acetone, 16:1) 0.30] but they could not be separated. The mixture was recrystallised four times from methanol to give the *single pure diastereoisomer* **27** (0.89 g, 18%) in an unoptimised yield, mp

212–214 °C; $[\alpha]_D$ +64 (*c* 1, CH_2Cl_2) (Found: C, 62.7; H, 5.88. $C_{32}H_{36}O_{12}$ requires C, 62.74; H, 5.92%); δ_H(270 MHz; $CDCl_3$) 1.21, 1.29, 1.32 and 1.57 (12 H, 4 s, CMe_2), 2.17 and 2.18 [6 H, 2 s, $O_2CCH(OAc)Ph$], 3.23 (1 H, dd, *J* 9.3 and 11.0, 5-H), 4.04 (1 H, t, *J* 10.45, 4-H), 4.15 (1 H, dd, *J* 4.8 and 6.6, 1-H), 4.54 (1 H, t, *J* 4.6, 2-H), 5.08 (1 H, dd, *J* 4.2 and 10.6, 3-H), 5.22 (1 H, dd, *J* 6.6 and 11.0, 6-H), 6.01 [1 H, s, $O_2CCH(OAc)Ph$], 6.11 [1 H, s, $O_2CCH(OAc)Ph$] and 7.33–7.52 [10 H, m, $O_2CCH(OAc)Ph$]; δ_C($CDCl_3$; 68 MHz) 20.59, 20.72, 25.72, 26.56, 26.69, 27.73, 71.34, 74.05, 74.34, 74.48, 75.04, 75.67, 76.37, 78.65, 110.72, 112.61, 127.92, 128.17, 128.54, 128.59, 129.07, 129.20, 167.74, 168.40, 169.85 and 170.54; *m/z* (FAB^+) 613 (M + H, 50%), 555 (30), 149 (90) and 107 (100).

D-1,2:4,5-Di-*O*-isopropylidene-*myo*-inositol 12a

A mixture of D-3,6-di-*O*-[(*S*)-(+)-*O*-acetylmandelyl]-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol **27** (0.74 g, 1.21 mmol), sodium hydroxide (0.40 g, 10 mmol) and methanol (100 cm^3) was heated at reflux temperature for 30 min. The mixture was cooled, and neutralised with carbon dioxide. The solid was then diluted with water (50 cm^3) and evaporated to dryness *in vacuo*. The crude product was extracted with methylene dichloride (4 × 100 cm^3) and the solvent was evaporated off to give a solid. The title compound **12a** was purified by flash chromatography (ethyl acetate–methylene dichloride, 1:1), R_f (Et_2O) 0.20, and dried ($MgSO_4$), and the solvent was evaporated off. The remaining solid was recrystallised from ethyl acetate to give compound **12a** (0.27 g, 86%), mp 174–176 °C (from ethyl acetate) (lit.,[25] 176–177 °C); $[\alpha]_D$ −22 (*c* 1, MeCN) (lit.,[24] −21.7, *c* 0.46 MeCN) (Found: C, 55.6; H, 7.88. $C_{12}H_{20}O_6$ requires: C, 55.37; H, 7.74%). The NMR data were identical with those for racemate **12ab**.[33]

D-3,6-Di-*O*-benzyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol 13a

A mixture of D-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol **12a** (0.209 g, 0.80 mmol), DMF (10 cm^3) and sodium hydride (0.096 g, 4 mmol) was stirred at room temperature. Benzyl bromide (0.2 cm^3, 2 mmol) was added and the mixture was stirred for a further 2 h. TLC (diethyl ether–light petroleum, 1:1) then showed a new product, R_f 0.60. Methanol (2 cm^3) was added to destroy the excess of sodium hydride and the solvents were evaporated off *in vacuo*. The residue was partitioned between water and diethyl ether (30 cm^3 each) and the organic layer was evaporated off to give a solid. The title compound **13a** was purified by flash chromatography (diethyl ether–pentane, 1:2) and recrystallised from hexane (0.32 g, 91%); mp 157–159 °C (from hexane) (lit.,[26] 159–161 °C, for L-enantiomer); $[\alpha]_D$ −44 (*c* 1, CH_2Cl_2) (lit.,[26] +85, *c* 1, $CHCl_3$, for L-enantiomer) (Found: C, 71.1; H, 7.35. $C_{26}H_{32}O_6$ requires C, 70.89; H, 7.32%). The NMR data were identical with those of racemate **13ab**.[33]

D-3,6-Di-*O*-benzyl-*myo*-inositol 20a

A mixture of D-3,6-di-*O*-benzyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol **13a** (0.27 g, 0.62 mmol) and methanol–1 mol dm^{-3} HCl (50 cm^3; 9:1) was heated at reflux temperature for 30 min. The solution was cooled and the solvents were evaporated off to give a solid, which was recrystallised from ethanol [R_f (chloroform–methanol; 6:1) 0.60] (0.21 g, 95%), mp 172–173 °C (from EtOH); $[\alpha]_D$ +16 (*c* 1, MeOH). The mass spectrum and NMR data were identical with those of compound **20a** as described previously.

Acknowledgements

We thank the Biotechnology and Biological Sciences Research Council for financial support. B. V. L. P. is a Lister Institute Research Professor. We also thank Dr Christine Murphy and Professor John Westwick for the preliminary biological data.

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Paper 6/08337D
Received 11*th December* 1996
Accepted 21*st January* 1997