Unambiguous Total Synthesis of the Enantiomers of *myo*-Inositol 1,3,4-Trisphosphate: 1L-*myo*-Inositol 1,3,4-Trisphosphate Mobilizes Intracellular Ca^{2+} in *Limulus* Photoreceptors

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Syntheses of the enantiomers of *myo*-inositol 1,3,4-trisphosphate are described. 1,4-Di-*O*-allyl-*myo*-inositol was regioselectively *p*-methoxybenzylated at the 3-position to give 1,4-di-*O*-allyl-3-*O*-(*p*-methoxybenzyl)-*myo*-inositol followed by benzylation of the remaining free hydroxyl groups to give the key intermediate 1,4-di-*O*-allyl-2,5,6-tri-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-*myo*-inositol. Removal of the *p*-methoxybenzyl and allyl groups gave 2,4,5-tri-*O*-benzyl-*myo*-inositol which was phosphitylated with bis(benzyloxy)(diisopropylamino)phosphine to give the fully protected trisphosphite triester. Oxidation using *tert*-butyl hydroperoxide gave 2,5,6-tri-*O*-benzyl-1,3,4-tris(dibenzylphospho)-*myo*-inositol, and deprotection using sodium in liquid ammonia gave racemic *myo*-inositol 1,3,4-trisphosphate. Deprotection of the key intermediate 1,4-di-*O*-allyl-2,5,6-tri-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-*myo*-inositol by isomerization of allyl groups followed by mild acid hydrolysis gave 2,4,5-tri-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol, which was converted to the diastereoisomeric bis-(−)-camphanates. The diastereoisomers were separated by column chromatography and the camphanates and the *p*-methoxybenzyl group removed by saponification and acid hydrolysis, respectively, for each diastereoisomer to give the enantiomers of 2,4,5-tri-*O*-benzyl-*myo*-inositol. The absolute configurations of the latter were established by conversion of 1L-2,5,6-tri-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-*myo*-inositol to the known 1L-1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol. Phosphorylation and deblocking gave the D- and L-enantiomers of *myo*-inositol 1,3,4-trisphosphate. Biological evaluation in *Limulus* photoreceptors showed that 1L-*myo*-inositol 1,3,4-trisphosphate was much more active than the D-enantiomer, producing repetitive bursts of depolarization due to mobilization of intracellular calcium.

Introduction

Receptor-mediated phospholipase C-catalyzed cleavage of phosphatidylinositol 4,5-bisphosphate releases D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **1**] (Figure 1), which has emerged within the last decade as a second messenger linking the spatially separated events of receptor stimulation and release of intracellular calcium from internal stores.[1,2] Ins(1,4,5)P_3 acts through an intracellular endoplasmic reticular receptor which has been isolated,[3] cloned, sequenced,[4,5] and reconstituted.[6] Ins(1,4,5)P_3 is metabolized *via* two pathways:[7] deactivation by a 5-phosphatase to Ins(1,4)P_2 or phosphorylation by a 3-kinase to the tetrakisphosphate Ins(1,3,4,5)P_4 (**2**). The function of the latter still remains controversial, and Ins(1,3,4,5)P_4 may gate a plasma membrane Ca^{2+} channel.[8]

The action of 5-phosphatase upon Ins(1,3,4,5)P_4 gives rise to another trisphosphate, D-*myo*-inositol 1,3,4-trisphosphate [D-Ins(1,3,4)P_3, **3a**], which occupies a central position in inositol phosphate metabolism.[9] Its hydrolysis leads to Ins(1,3)P_2 and D-Ins(3,4)P_2, whose further dephosphorylation replenishes the limited cellular inositol pool, while phosphorylation by a recently characterized 5/6-kinase[10] gives rise to Ins(1,3,4,5)P_4 and Ins(1,3,4,6)P_4, which can then be further phosphorylated to higher polyphosphates.

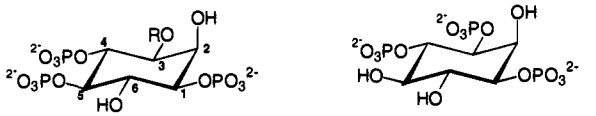

Figure 1. Structures of Ins(1,4,5)P_3, Ins(1,3,4,5)P_4, and D-Ins(1,3,4)P_3.

However, there is some uncertainty concerning the biological activity of D-Ins(1,3,4)P_3 itself. A study carried out in 1986[11] found that, in permeabilized Swiss 3T3 cells, D-Ins(1,3,4)P_3 obtained by incubation of D-Ins(1,3,4,5)P_4 with human erythrocyte membranes was active in releasing Ca^{2+} (EC_{50} = 9 μM), suggesting that D-Ins(1,3,4)P_3 may have a physiological role in keeping Ca^{2+} stores empty. Later, Polokoff *et al.*[12] found that synthetic racemic Ins(1,3,4)P_3[13,14] was active in releasing Ca^{2+} in permeabilized aortic smooth muscle cells. They naturally assumed that D-Ins(1,3,4)P_3 was the enantiomer responsible and calculated an EC_{50} of 60 μM for D-Ins(1,3,4)P_3 on the basis of this assumption. A third study[15] found that D-Ins(1,3,4)P_3 was essentially *inactive* in both GH_3 and Swiss 3T3 cells, and the authors concluded that D-Ins(1,3,4)P_3 was not involved in cellular Ca^{2+} mobilization.

The availability of synthetic optically active Ins(1,3,4)P_3 has so far not helped to resolve this confusion, as there is some uncertainty in the literature concerning the absolute configurations of the enantiomers of Ins-

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(1,3,4)P_3. Ozaki *et al.*[16] reported a synthesis of D-Ins(1,3,4)P_3 in which they gave the specific rotation of the hexa-ammonium salt as −6°. The basis for their assignment of the D-configuration was the conversion of a precursor to the known 1L-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol. No biological results were reported at that time.

Boehm and Prestwich[17] synthesized the tritiated enantiomers of Ins(1,3,4)P_3, basing their assignment of absolute configuration on the optical rotation of Ozaki *et al.*, and found that neither showed binding displaceable by D-Ins(1,3,4)P_3 to rat brain receptor proteins. A study of the metabolism of the enantiomers by saponin-permeabilized platelets[18] seemed to confirm the configurational assignments.

Gou and Chen[19] and Gou *et al.*[20] subsequently reported a synthesis, based on a chemoenzymatic method, for optical resolution of Ins(1,3,4)P_3 precursors. Their D-Ins(1,3,4)P_3 (as the hexapotassium salt) had a rotation of 14° (c = 2, H_2O, pH 8.2). Their basis for claiming the D-configuration was the conversion of an intermediate into the known D-Ins(1,3,4,5)P_4 and their use of the triol 1D-2,5,6-tri-*O*-benzyl-*myo*-inositol, whose configuration had recently been assigned by Desai *et al.*,[21] as a precursor. The authors noted the apparent discrepancies in reported optical rotations, remarking that "...unfortunately, no additional information concerning chiral Ins(1,3,4)P_3 is available in the literature". No biological results were given.

Hirata *et al.*[22] have recently reported biological data for synthetic L-Ins(1,3,4)P_3 (synthesized according to Ozaki *et al.*[16]). They found only slight ability to release Ca^{2+} from permeabilized rat basophilic leukemic cells [more than 3000-fold lower than Ins(1,4,5)P_3], although no EC_{50} could be determined and it was not possible to establish the efficacy of this agonist. In binding experiments, however, their L-Ins(1,3,4)P_3 was only 90-fold weaker than Ins(1,4,5)P_3.

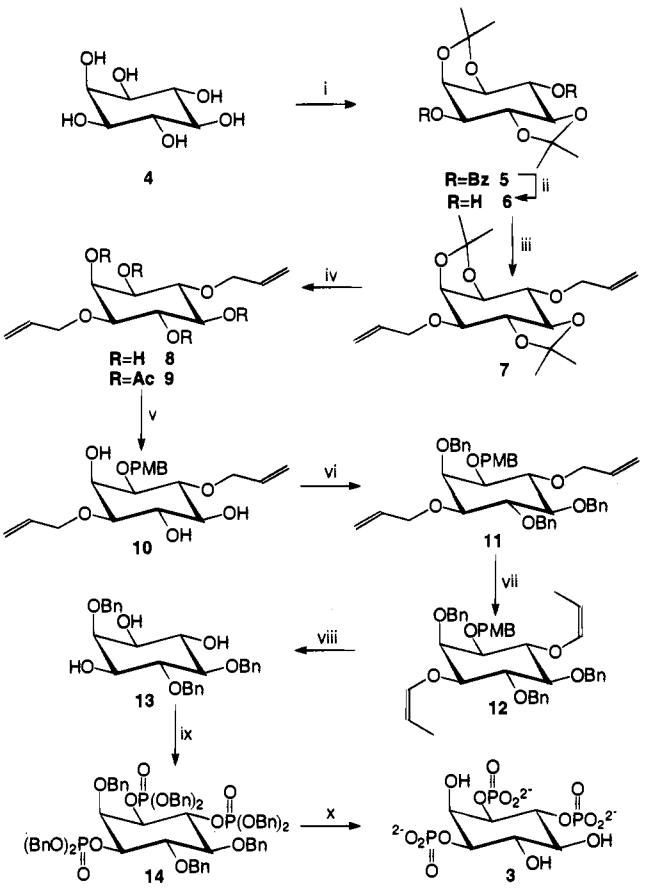
Our early investigations into the effects of D-Ins(1,3,4)P_3 in *Limulus* ventral photoreceptors (unpublished) yielded contradictory results. Synthetic D-Ins(1,3,4)P_3 prepared by Gou and Chen[19] was inactive, whereas samples from some other sources induced a Ca^{2+}-activated conductance when injected into the light-sensitive lobe of the photoreceptor.

Clearly, if synthetic D-Ins(1,3,4)P_3 is to be used biologically, there must be no doubt as to both its absolute configuration and its optical purity. Ideally, we should be able to compare the effects of both enantiomers. We therefore set out to synthesize the enantiomers of Ins(1,3,4)P_3 and to establish their absolute configurations unequivocally. We report here their total synthesis and an examination of their effects in *Limulus* ventral photoreceptors.

Results and Discussion

Racemic Ins(1,3,4)P_3 was synthesized using a novel route starting from *myo*-inositol **4** (Scheme 1). The key fully protected intermediate DL-1,4-di-*O*-allyl-2,5,6-tri-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-*myo*-inositol (**11**) was chosen for the following reasons: Firstly, partial deprotection of this compound leads to the known triol 2,4,5-tri-*O*-benzyl-*myo*-inositol (**13**) which is then used as the precursor for phosphorylation. The absolute configurations of the enantiomers of this material have been

Scheme 1[a]



[a] (i) (a) 2,2-Dimethoxypropane, PTSA, DMF, reflux, (b) BzCl, pyridine; (ii) NaOH, MeOH, reflux; (iii) allyl bromide, NaH, DMF; (iv) AcOH/H_2O, 4:1, reflux; (v) (a) Bu_2SnO, toluene, reflux, (b) PMBCl, CsF, KI, DMF, (vi) BnBr, NaH, DMF; (vii) KOBu^t, DMSO, 50 °C; (viii) MHCl/EtOH, 1:2, reflux; (ix) (a) $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, CH_2Cl_2, (b) Bu^{t}OOH; (x) Na/liquid NH_3. Bn, benzyl; Bz, benzoyl; PMB, *p*-methoxybenzyl. All compounds are racemic.

assigned recently.[21] Secondly, the use of allyl protection at positions 1 and 4, together with a *p*-methoxybenzyl at position 3, allowed for two possible strategies of resolution using diastereoisomeric camphanate esters. The diastereoisomers must be capable of separation, by either recrystallization or chromatography, and in many cases, neither is possible. Finally, the *p*-methoxybenzyl protection at position 3 allows for versatility and possible subsequent selective modification. Moreover, this intermediate could be converted, after resolution, into one of the enantiomers of the pentabenzyl ether **17** (Scheme 2), which are well known.[23-25] The absolute configurations of the enantiomers of **17** were originally determined by Shvets *et al.*[23] who assigned the structure **L-17b** to the (−)-enantiomer. In a study published very recently, Aneja *et al.* [26] discuss certain disagreements in the literature and go on to demonstrate unambiguously that this assigment is correct, thus enabling a reliable assignment of the configurations of our optically pure Ins(1,3,4)P_3.

DL-1,4-Di-*O*-allyl-2,5,6-tri-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-*myo*-inositol (**11**) was synthesized as shown in Scheme 1 *via* DL-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol (**6**).[27] Subsequent allylation using sodium hydride in DMF followed by allyl bromide gave the known 3,6-di-*O*-allyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol (**7**).[28] Finally, the ketals were removed from **7** by heating in acetic acid−water (4:1) to give the tetrol **8**.[29] NMR data

for compounds **5**–**8** have not been previously reported, and these are given in the Experimental Section.

The ^{1}H NMR spectrum of **8** initially posed some difficulties of interpretation due to overlapping signals from some of the inositol ring protons. **8** was therefore converted to the tetra-acetate **9**. In the ^{1}H NMR spectrum of this compound, the signals from protons at positions 2, 3, 5, and 6 were, as expected, shifted downfield, well away from those corresponding to H-1 and H-4. A 400 M Hz ^{1}H-^{1}H COSY NMR spectrum of **9** allowed an unambiguous assignment of the ring protons and an interpretation of the original overlapping signals.

The next step was a selective *p*-methoxybenzylation of the tetrol at position 3. This involved the protection of one equatorial hydroxyl group in the presence of three other OH groups: one axial and two equatorial. The tetrol **8** was reacted with dibutyltin oxide by refluxing in toluene with azeotropic removal of water to give a dibutylstannylene derivative, which was not isolated but reacted with *p*-methoxybenzyl chloride in DMF in the presence of cesium fluoride[30] to give the 3-*O*-*p*-methoxybenzyl ether **10**. Finally, **10** was benzylated using sodium hydride and benzyl bromide in DMF to give the fully protected key intermediate DL-1,4-di-*O*-allyl-2,5,6-tri-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-*myo*-inositol (**11**).

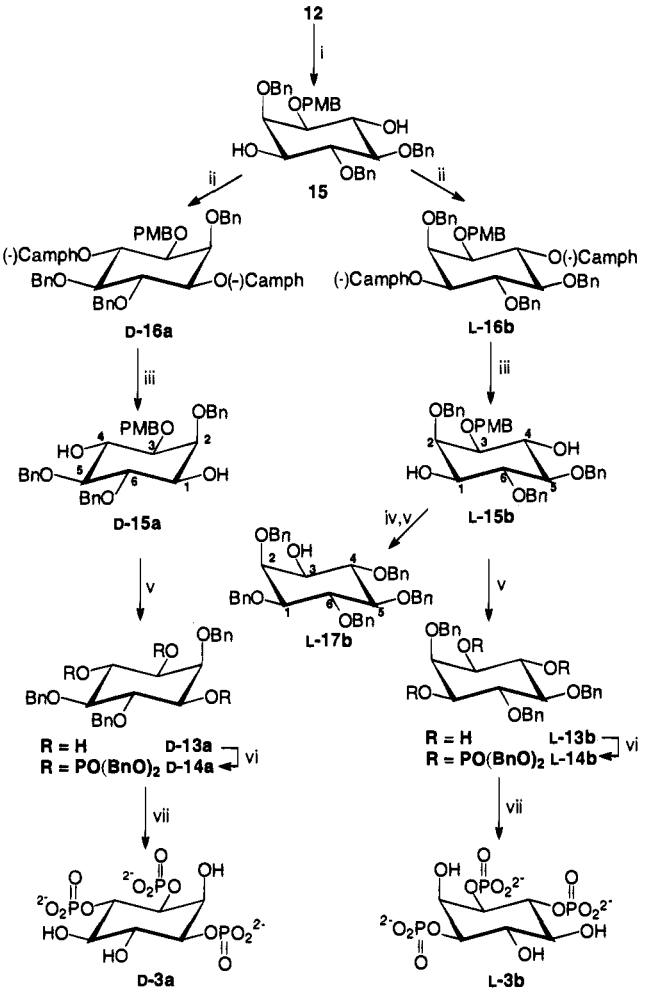
Triol **13** was generated by removing both the allyl and *p*-methoxybenzyl protecting groups from **11** (Scheme 1). The isomerization of the allyl group was carried out using potassium *tert*-butoxide in DMSO at 50 °C,[28] and the *cis*-propenyl ether **12** was obtained in high yield. **12** was deprotected to **13** in refluxing MHCl–ethanol (2:1) for 2 h, which resulted in complete conversion to the triol with no loss of benzyl protecting groups as judged by TLC.

Phosphitylation was carried out at room temperature using the P(III) reagent bis(benzyloxy)(diisopropylamino)phosphine[31] with 2 equiv of phosphitylating agent per hydroxyl group and 3 equiv of 1*H*-tetrazole, in a small volume of freshly distilled dry dichloromethane. After the solution of phosphitylating agent and tetrazole was stirred for 10 min, the ^{31}P NMR spectrum showed a signal at 127 ppm, confirming the presence of the phosphitylating agent–tetrazolide. After the triol **13** (previously dried *in vacuo*) was added, ^{31}P NMR spectroscopy showed the appearance of signals at 140.4 (2P) and 142.2 (1P) ppm, corresponding to the trisphosphite triester. Signals at 127 (excess phosphitylating agent–tetrazolide) and 7.5 (an H-phosphonate byproduct) ppm were also present. A ^{31}P NMR spectrum of the signals close to 140 ppm showed three signals, and two of these were doublets with a coupling constant of 3.7 Hz. This arises from an unusual $^5J_{PP}$ coupling[32] between the P atoms at C-3 and C-4 and was clear evidence that the compound contained a vicinal bisphosphite.

After addition of water to destroy excess phosphitylating agent, oxidation of phosphites was then carried out by adding excess *tert*-butyl hydroperoxide.[33] ^{31}P NMR now showed that oxidation to the protected trisphosphate **14** was complete, with the appearance of three clear singlets at −1.41, −1.56, and −2.01 ppm.

Final deblocking of the nine protecting benzyl groups of **14** to the racemic trisphosphate **3** used sodium in liquid ammonia.[33] Purification of the product was

Scheme 2[a]



[a] (i) MHCl/acetone, 1:10, 50 °C; (ii) (*S*)-ω-camphanic acid chloride, DMAP, pyridine; (iii) NaOH, MeOH, reflux; (iv) BnBr, NaH, DMF; (v) MHCl/EtOH, 1:2, reflux; (vi) (a) $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, (b) Bu^tOOH; (vii) Na/liquid NH_3. Bn, benzyl; PMB, *p*-methoxybenzyl; (−)Camph, (*S*)-(−)-ω-camphanate.

carried out using ion-exchange chromatography, and fractions containing phosphate were detected and then quantified after combining, using a modification of the Briggs phosphate assay.[34,35]

The proton-coupled ^{31}P NMR spectrum of **3** confirmed the presence of the three phosphate groups with heteronuclear $^3J_{HCOP}$ coupling. The identity of **3** was confirmed by one- and two-dimensional ^{1}H NMR spectroscopy. It was possible to assign all the signals, including the separate assignments of H-1 and H-3, leaving no doubt remaining as to the identity of **3**. Preliminary biological testing of DL-**3** in *Limulus* photoreceptors showed that injection of a 100 μM solution produced repetitive bursts of depolarization of the cellular membrane potential. We therefore proceeded to synthesize the enantiomers of **3** and to determine which was responsible for this effect.

The optical resolution of **15** was achieved *via* the formation of 1,4-bis-camphanates as shown in Scheme 2. Racemic 1,4-di-*O*-allyl-2,5,6-tri-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-*myo*-inositol (**11**) was converted to the 1,4-di-*O*-*cis*-propenyl isomer **12**, which was then treated with MHCl–acetone (1:10) at 50 °C. TLC showed complete conversion to the 1,4-diol within 5 min, after which the acid was rapidly neutralized. If the reaction

was continued beyond 30 min, then significant amounts of triol began to appear, arising from loss of the *p*-methoxybenzyl group at position 3. Racemic **15** was obtained in 84% yield after purification and was then converted into its bis-(−)-ω-camphanate esters by reaction with (*S*)-(−)-ω-camphanic acid chloride in the presence of 4-(dimethylamino)pyridine (DMAP) in pyridine. Reaction was complete within 30 min and TLC showed the presence of the two diastereoisomers as expected. Attempts to resolve the diastereoisomers by recrystallization from various solvent systems (methanol, ethanol, ether, ethyl acetate/hexane) were not successful. Although the two diastereoisomers showed a ΔR_f of only 0.07, excellent separation was achieved using column chromatography. The individual diastereoisomers proved to be very different: the (+)-diastereoisomer **16b** was highly crystalline, with a very low solubility in cold methanol allowing easy crystallization from hot solvent; the more polar (−)-diastereoisomer **16a** was much more soluble in methanol and ethanol and could not be induced to crystallize from any of a range of solvents, tending instead to precipitate as a gel. The NMR spectra of the two individual diastereoisomers showed no trace of the other diastereoisomer in either case.

Saponification of the individual diastereoisomers by refluxing with sodium hydroxide in methanol then gave the enantiomeric diols **15a,b**. The (−)-diol **15b** was converted to the known pentabenzyl ether **17b** by benzylation followed by removal of the *p*-methoxybenzyl group. The product was found to have an ^{1}H NMR spectrum identical to that of racemic 1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol,[25] but an optical rotation of −11.5°, identifying it as (−)-1L-1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol (**L-17b**). Thus the (−)-diol could be assigned as 1L-2,5,6-tri-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-*myo*-inositol (**L-15b**), allowing the absolute configurations of the diastereoisomeric bis-camphanates and the resolved enantiomers generated from them to be deduced.

The enantiomeric diols **15a,b** were converted to the known triols **13a,b** by removal of their *p*-methoxybenzyl groups as described for **13**. The optical rotations of these enantiomers were in agreement with, although larger than, those previously reported,[20,21] thus confirming the assignment of the absolute configuration made by Desai *et al.*[21] and the D-Ins(1,3,4)P_3 synthesized by Gou and Chen.[19] Examination of a ^{1}H-^{1}H COSY NMR spectrum of **13a** enabled us to assign unequivocally the signals corresponding to each of the six inositol ring protons and the three hydroxyl groups.

The triols were phosphorylated as described for the racemic material followed by deprotection and purification as before to give D- and L-*myo*-inositol 1,3,4-trisphosphates (**3a,b**), respectively, as the triethylammonium salts. ^{1}H NMR spectroscopy showed the number of triethylammonium cations to be between 3 and 3.5, and ethanol of crystallization was present. Solutions of the salts in water were acidic (pH 4.2), and their specific rotations, calculated for the free acid, were highly dependent on pH, being small (<10°) at acidic pH and large (>40°) at pH 10.6. A similar pH-dependency has been found for the optical rotations of D-Ins(1,4,5)P_3.[36] The rotations for D-Ins(1,3,4)P_3 were positive over this range, and those for L-Ins(1,3,4)P_3 were negative. At pH 7.8, the values were 37° and −40°, respectively (c = 0.42, TEAB buffer). The 162 M Hz proton-coupled ^{31}P NMR spectrum of **3a** and the 400 M Hz ^{1}H NMR spectrum of **3b** are shown in Figure 3. Previously published NMR spectra of Ins(1,3,4)P_3 obtained from biological sources[37] showed the material to be contaminated, probably with Ins(1,4,5)P_3. In this respect, it is also worth noting that L-Ins(1,3,4)P_3 [alternative name D-Ins(1,3,6)P_3] has been identified as a minor inositol trisphosphate in avian erythrocytes and as a product of Ins(1,3,4,6)P_4 dephosphorylation by brain cytosol.[38] It follows that any Ins(1,3,4)P_3 obtained from biological sources may contain L-Ins(1,3,4)P_3, which would not be detected by the usual chromatographical analyses.

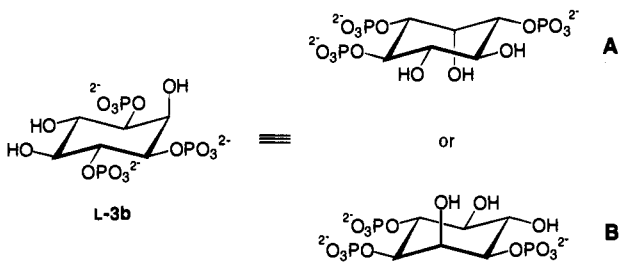


Figure 2. L-Ins(1,3,4)P_3. **3b** can be considered to contain a vicinal 4,5-bisphosphate feature similar to that found in Ins(1,4,5)P_3 and has two potential binding orientations, A and B.

Structural considerations[39] based on our earlier work predict that D-Ins(1,3,4)P_3 should show little ability to release Ca^{2+}. Our working hypothesis is that the essential requirement for Ca^{2+} release in inositol polyphosphates is the presence of a vicinal D-4,5-bisphosphate as found in D-Ins(1,4,5)P_3 and D-Ins(1,3,4,5)P_3 (Figure 1) or a pseudovicinal D-4,5-bisphosphate (e.g., in Ins(1,3,4,6)P_4). It is interesting to note that the recently discovered adenophostins A and B,[40-42] the most potent agonists yet identified at Ins(1,4,5)P_3 receptors, have been shown to contain a similar feature, although in a carbohydrate. L-Ins(1,3,4)P_3 (Figure 2) possesses this motif, and we therefore predicted that it should release Ca^{2+}. D-Ins(1,3,4)P_3 does not, and for this reason, despite the reports to the contrary cited above, we would expect it to show little, if any, activity as an agonist at the Ins(1,4,5)P_3 receptor.

The two enantiomers of Ins(1,3,4)P_3, **3a,b**, were injected into *Limulus* ventral photoreceptors in order to compare their effectiveness in a living cell. Previous work has shown that intracellular injection of Ins(1,4,5)P_3 into ventral photoreceptors results in a transient depolarization of the cellular membrane potential.[43,44] This depolarization accompanies, and is probably caused by, a burst of calcium release from intracellular stores.[45] Poorly metabolizable active analogues of Ins(1,4,5)P_3, such as D-*myo*-inositol-1,4,5 trisphosphorothioate, induce a train of bursts of depolarization due to bursts of calcium release that persist for many minutes after injection.[46]

Ventral photoreceptors were impaled in their light-sensitive region with a double-barreled micropipette, one barrel containing 100 μM D-Ins(1,3,4)P_3 **D-3a** and the other barrel either 100 μM D-Ins(1,4,5)P_3 or 100 μM L-Ins(1,3,4)P_3 **L-3b**. These solutions were injected into the photoreceptor by brief pressure pulses delivering approximately equal volumes (see the Experimental Section). Photoreceptors remained in darkness throughout the experiment. Injection of D-Ins(1,3,4)P_3 was found to be much less effective in inducing a rapid burst

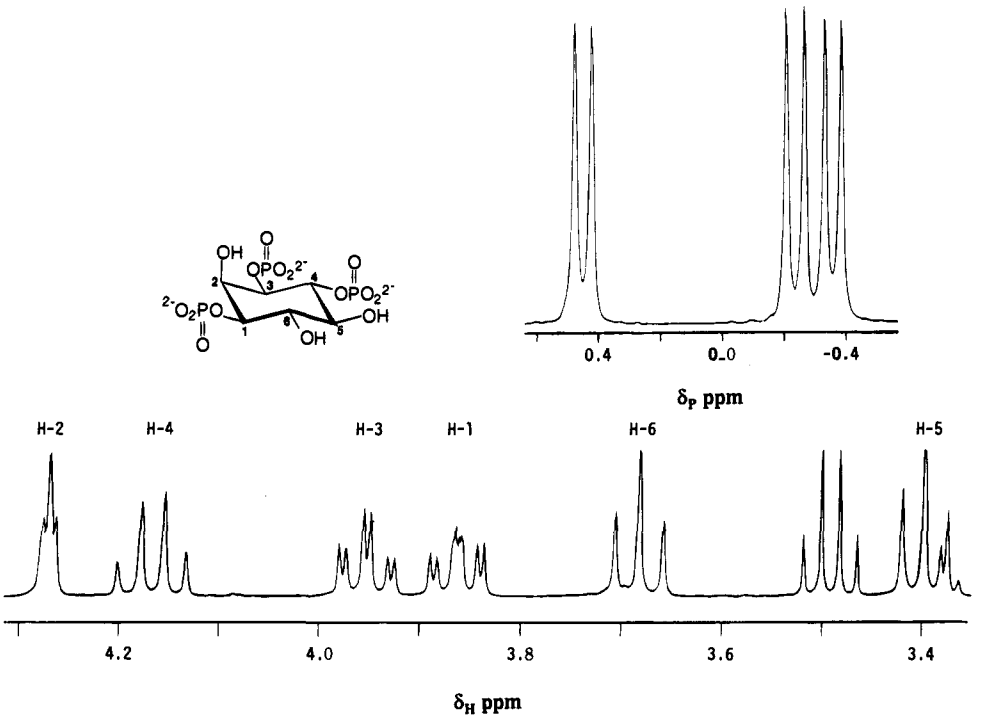


Figure 3. 400 M Hz ^{1}H NMR spectrum of **3b** in D_2O, pH ~4.2. The quartet at *ca.* δ 3.5 arises from CH_2 of ethanol of crystallization. The inset shows the 162 M Hz ^{1}H-coupled ^{31}P NMR spectrum of **3a** in D_2O, pH ~4.2.

of depolarization than injection of either D-Ins(1,4,5)P_3 or L-Ins(1,3,4)P_3 (Figure 4 and Table 1). In addition, unlike the single burst of depolarization seen following injection of D-Ins(1,4,5)P_3 (Figure 4B), repetitive bursts of depolarization were induced by L-Ins(1,3,4)P_3 (Figure 4D). These bursts continued for up to 5 min after injection of L-Ins(1,3,4)P_3, suggesting that L-Ins(1,3,4)-P_3 is metabolized at a slower rate than D-Ins(1,4,5)P_3.

Conclusion

D-Ins(1,3,4)P_3 occupies a pivotal position in the metabolism of inositol phosphates. It is essential that we be clear about its activity, or lack of it, at Ins(1,4,5)P_3 receptors and other sites. When D-Ins(1,3,4)P_3 is obtained from biological sources, there exists the possibility of contamination with D-Ins(1,4,5)P_3 or other Ca^{2+}-mobilizing inositol phosphates such as L-Ins(1,3,4)P_3.

Our results, obtained using the individual enantiomers of synthetic Ins(1,3,4)P_3, whose absolute configuration we have determined unequivocally, demonstrate that in *Limulus* photoreceptors D-Ins(1,3,4)P_3 shows much less ability to release Ca^{2+} than D-Ins(1,4,5)P_3 or L-Ins(1,3,4)P_3. These findings confirm our original proposals regarding the structural requirements for agonists at Ins(1,4,5)P_3 receptors and imply that biological data published to date on the activity of D-Ins-(1,3,4)P_3 should be re-evaluated.

Experimental Section

Chemicals were purchased from Aldrich and Fluka. Dichloromethane was dried over phosphorus pentoxide, distilled, and kept over 4 Å molecular sieves. Pyridine was dried by refluxing with sodium hydroxide pellets followed by distillation and stored over 5 Å molecular sieves. Dimethylformamide was stored over 4 Å molecular sieves.

TLC was performed on precoated plates (Merck TLC aluminum sheets silica F_{254}, Art. No. 5554) with detection by UV light or methanolic phosphomolybdic acid followed by heating. Flash column chromatography was performed on silica gel (Sorbsil C60).

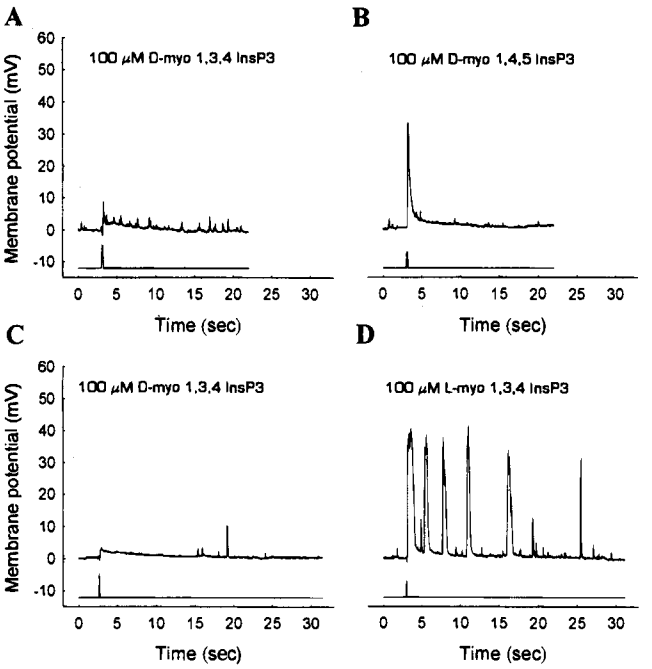


Figure 4. Injection of $InsP_3$ isomers into the rhabdomeral lobe of *Limulus* photoreceptors. Panels A and B show recordings of membrane potential made from a cell impaled with a double-barreled micropipette containing 100 μM D-Ins(1,3,4)-P_3 (**3a**) in one barrel and 100 μM D-Ins(1,4,5)P_3 in the other barrel. The pressure applied to either barrel was 60 psi for 100 ms. Panels C and D show recordings of membrane potential made from another cell impaled with a double-barreled electrode containing 100 μM D-Ins(1,3,4)P_3 (**3a**) in one barrel and 100 μM L-Ins(1,3,4)P_3 (**3b**) in the other barrel. Pressure applied to the first barrel was 20 psi for 200 ms and to the second barrel was 38 psi for 200 ms.

Table 1. Peak Depolarization Produced by Injections of 100 μM $InsP_3$ Isomers

D-Ins(1,4,5)P_3	D-Ins(1,3,4)P_3
24 ± 5 mV[a]	5.7 ± 3.3 mV[a]
L-Ins(1,3,4)P_3	D-Ins(1,3,4)P_3
32.5 ± 6.8 mV[b]	3.4 ± 2.2 mV[b]

[a] Mean ± SD; nine cells. [b] Mean ± SD; six cells.

^1H and ^{13}C NMR spectra (internal Me_4Si as reference) were recorded with a Jeol JMN-GX270 or JMN-GX-400 NMR spectrometer, and ^{31}P NMR spectra (external aqueous 85% phosphoric acid as reference) were recorded with a Jeol JMN-GX400 or FX-90Q spectrometer. Mass spectra were recorded at the SERC Mass Spectrometry Service Centre, Swansea, and the University of Bath. Microanalysis was carried out by the Microanalysis Service, University of Bath. FAB-mass spectra were carried out using *m*-nitrobenzyl alcohol as the matrix. Melting points (uncorrected) were determined using a Reichert-Jung Thermo Galen Kofler block. Optical rotations were measured using an Optical Activity Ltd. AA-10 polarimeter. Ion-exchange chromatography was performed on an LKB-Pharmacia medium pressure ion-exchange chromatograph using DEAE Sepharose or Sepharose Q Fast Flow by elution with a gradient of triethylammonium hydrogen carbonate (TEAB) buffer as eluent. Quantitative analysis of phosphate was performed using a modification of the Briggs phosphate assay.[34,35]

(Diisopropylamino)dichlorophosphine was prepared by the method of Tanaka *et al.*[47] by adding 2 equiv of diisopropylamine to a solution of PCl_3 in dry ether at −78 °C. The crude product (δ_P 166.4) was purified by distillation under reduced pressure, and reaction with 2 equiv of benzyl alcohol in the presence of 2 equiv of triethylamine afforded bis(benzyloxy)-(diisopropylamino)phosphine[48] (δ_P 145.24) which could be purified by flash chromatography.

Photoreceptors from the ventral eye of the horseshoe crab *Limulus polyphemus*, double-barreled micropipettes, and recording apparatus were prepared as previously described.[49] $InsP_3$ isomers were dissolved in a carrier solution containing 100 mM potassium aspartate, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer, pH 7.0. D-Ins(1,4,5)P_3 was obtained from LC Laboratories, Woburn, MA. The photoreceptors were bathed in artificial sea water, pH 7.2, and impaled in their light-sensitive, rhabdomeral lobe. Placement of the micropipette in the rhabdomeral lobe was confirmed by scanning the photoreceptor with a small spot of light and by the rapidity of depolarization (within 150 ms) following injection of active $InsP_3$ isomers. Before the experiment, the volume of solution ejected from each barrel of the micropipette was estimated by ejecting droplets of solution into oil.[50] The pressure applied to each barrel was adjusted so that equal size droplets, approximately 1 pL in volume, were produced from either barrel.

DL-3,6-Di-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol (5). Mp: 328−330 °C (from DMF) (lit.[27] mp 328−330 °C). ^1H NMR ($CDCl_3$, 270 M Hz): δ 1.30, 1.44, 1.51, 1.64 (12 H, 4 s, 4 CH_3), 3.72 (1 H, dd, J = 11.0 Hz, 9.3 Hz, C-5-H), 4.40 (1 H, dd, J = 10.6 Hz, 9.3 Hz, C-4-H), 4.40 (1 H, dd, J = 6.6 Hz, 4.6 Hz, C-1-H), 4.79 (1 H, dd, J = 4.6 Hz, 4.6 Hz, C-2-H), 5.43 (1 H, dd, J = 10.6 Hz, 4.4 Hz, C-3-H); 5.61 (1 H, dd, J = 11.0 Hz, 6.6 Hz, C-6-H), 7.41−7.51 (4 H, m, C_6H_5COO), 7.53−7.63 (2 H, m, C_6H_5COO), 8.08−8.18 (4 H, m, C_6H_5COO).

DL-1,2:4,5-Di-*O*-isopropylidene-*myo*-inositol (6). Mp: 166−170 °C (from ethyl acetate) (lit.[27] mp 171−173 °C). ^1H NMR ($CDCl_3$, 270 M Hz): δ 1.38, 1.46, 1.49, 1.54 (12 H, 4 s, 4 CH_3), 2.48 (1 H, d, J = 8.6 Hz, D_2O ex, C-3-OH), 2.67 (1 H, d, J = 3.1 Hz, D_2O ex, C-6-OH), 3.33 (1 H, dd, J = 10.6 Hz, 9.4 Hz, C-5-H), 3.84 (1 H, dd, J = 10.1 Hz, 10.1 Hz, C-4-H), 3.90 (1 H, ddd, J = 10.6 Hz, 6.0 Hz, 3.0 Hz, C-6-H), 4.03 (1 H, ddd, J = 10.0 Hz, 8.6 Hz, 4.4 Hz, C-3-H), 4.08 (1 H, dd, J = 6.5 Hz, 5.0 Hz, C-1-H), 4.49 (1 H, dd, J = 5.0 Hz, 5.0 Hz, C-2-H). ^{13}C NMR ($CDCl_3$, 68 M Hz): δ 25.85, 26.89, 28.06 (3 q, 4 CH_3), 69.74, 74.76, 77.56, 77.97, 81.28, 81.83 (6 d, inositol ring *C*), 110.28, 112.71 (2 s, 2 $C(CH_3)_2$).

DL-3,6-Di-*O*-allyl-1,2:4,5-di-*O*-isopropylidene-*myo*-inositol (7). Mp: 83−85 °C (from hexane) (lit.[28] mp 85−87 °C). ^1H NMR ($CDCl_3$, 270 M Hz): δ 1.38, 1.43, 1.46, 1.54 (12 H, 4 s, 4 CH_3), 3.34 (1H, dd, J = 10.5 Hz, 9.8 Hz, C-5-H), 3.66 (1 H, dd, J = 10.6 Hz, 6.4 Hz, C-6-H), 3.80 (1 H, dd, J = 10.3 Hz, 4.2 Hz, C-3-H), 3.98 (1 H, dd, J = 10.1 Hz, 10.1 Hz, C-4-H), 4.10 (1 H, dd, J = 6.1 Hz, 4.6 Hz, C-1-H), 4.21−4.37 (4 H, m, OCH_2), 4.46 (1 H, dd, J = 4.4 Hz, 4.4 Hz, C-2-H), 5.17−5.35 (4 H, m, $=CH_2$), 5.90−6.05 (2 H, m, CH=). ^{13}C NMR ($CDCl_3$, 68 M Hz): δ 25.91, 26.95, 28.06 (3 q, 4 CH_3), 71.16, 71.26 (2 t, OCH_2), 74.76, 76.42, 76.84, 78.56, 80.11, 81.38 (6 d, inositol ring), 109.99, 112.10 (2 s, $C(CH_3)_2$), 117.38, 118.16 (2 t, $=CH_2$), 134.67, 134.74 (2d, CH=).

DL-1,4-Di-*O*-allyl-*myo*-inositol (8). Mp 133−136 °C (from ethanol) (lit.[29] mp 137−139 °C). ^1H NMR (DMSO-d_6, 270 M Hz): δ 2.96 (1 H, dd, J = 9.7 Hz, 2.6 Hz, C-1-H), 3.03 (1 H, ddd, J = 9.0 Hz, 9.0 Hz, 4.8 Hz, D_2O ex gives dd, J = 9.0 Hz, 9.0 Hz, C-5-H), 3.16−3.28 (2 H, m, C-3-H and C-4-H), 3.46 (1 H, ddd, J = 9.6 Hz, 9.3 Hz, 4.6 Hz, D_2O ex gives dd, J = 9.6 Hz, 9.3 Hz, C-6-H), 3.86 (1 H, ddd, J = 3.2 Hz, 2.6 Hz, 2.6 Hz, D_2O ex gives dd, J = 2.6 Hz, 2.6 Hz, C-2-H), 3.97−4.12 (2 H, m, $CH_2CH=CH_2$), 4.16−4.26 (2 H, m, $CH_2CH=CH_2$), 4.54 (1 H, d, J = 6.0 Hz, D_2O ex, OH), 4.61 (1 H, d, J = 3.7 Hz, D_2O ex, C-2-OH), 4.64 (1 H, d, J = 4.8 Hz, D_2O ex, OH), 4.69 (1 H, d, J = 4.8 Hz, D_2O ex, OH), 5.01−5.29 (4 H, m, $CH_2CH=CH_2$), 5.80−5.98 (2 H, m, $CH_2CH=CH_2$). ^{13}C NMR (DMSO-d_6, 68 M Hz): δ 69.83, 71.42, 72.20, 75.02, 79.53, 81.41 (6 d, inositol ring *C*), 70.09, 72.94 (2 t, $CH_2CH=CH_2$), 115.30, 115.99 (2 t, $CH_2CH=CH_2$), 136.23, 137.06 (2 d, $CH_2CH=CH_2$).

DL-2,3,5,6-Tetra-*O*-acetyl-1,4-di-*O*-allyl-*myo*-inositol (9). To a solution of **8** (1.3 g, 5 mmol) in pyridine (10 mL) were added acetic anhydride (2.8 mL, 7.5 mmol) and DMAP (100 mg). The mixture was stirred at room temperature for 1 h, after which TLC (ether) showed conversion to a major product at R_f 0.60. The liquid was evaporated under reduced pressure and the product dissolved in dichloromethane (100 mL), washed with saturated sodium hydrogen carbonate solution (100 mL) and then water (100 mL), dried ($MgSO_4$), and evaporated to give an off-white solid which was recrystallized from hexane/ethyl acetate yielding **9** (1.70 g, 3.97 mmol, 79%). Mp: 118.5−119.5 °C (from hexane/ethyl acetate). ^1H NMR ($CDCl_3$, 400 M Hz): δ 2.04, 2.05, 2.06, 2.16 (12 H, 4 s, 4 CH_3), 3.55 (1 H, dd, J = 10.3 Hz, 2.9 Hz, C-1-H), 3.84 (1 H, dd, J = 10.3 Hz, 9.8 Hz, C-4-H), 3.89−4.17 (4 H, m, $CH_2CH=CH_2$), 4.87 (1 H, dd, J = 10.3 Hz, 2.9 Hz, C-3-H), 5.07 (1 H, dd, J = 9.8 Hz, 9.8 Hz, C-5-H), 5.11−5.26 (4 H, m, $CH_2CH=CH_2$), 5.31 (1 H, dd, J = 10.3 Hz, 10.3 Hz, C-6-H), 5.64 (1 H, dd, J = 2.9 Hz, 2.9 Hz, C-2-H), 5.71−5.84 (2 H, m, $CH_2CH=CH_2$). ^{13}C NMR ($CDCl_3$, 68 M Hz): δ 20.69 (q, 4 CH_3), 67.37, 71.06, 71.26, 72.52, 74.41, 76.61 (6 d, inositol ring *C*), 70.97, 73.92 (2 t, $CH_2CH=CH_2$), 116.70, 117.67 (2 t, $CH_2CH=CH_2$), 133.63, 134.28 (2 d, $CH_2CH=CH_2$), 169.73, 169.90, 170.06 (3 s, 4 C=O). MS: m/z (+ ion FAB, rel intensity) 429 [$(M + H)^+$, 80], 371 [$(M - CH_2CHCH_2O^-)^+$, 80], 369 [$(M - CH_3COO^-)^+$, 100]. Anal. ($C_{20}H_{28}O_{10}$) C, H.

DL-1,4-Di-*O*-allyl-3-*O*-(*p*-methoxybenzyl)-*myo*-inositol (10). A mixture of 1,4-di-*O*-allyl-*myo*-inositol (**8**) (13.0 g, 50 mmol), dibutyltin oxide (14.9 g, 60 mmol), and toluene (200 mL) were heated under reflux for 3 h in a Dean and Stark apparatus. The mixture was allowed to cool and the toluene removed by evaporation *in vacuo* giving an off-white solid. Cesium fluoride (19.0 g, 125 mmol), potassium iodide (12.5 g, 75 mmol), and DMF (200 mL) were added followed by *p*-methoxybenzyl chloride (10.2 mL, 75 mmol). After stirring for 3 h at room temperature, TLC (ethyl acetate) showed a major product at R_f 0.30. The mixture was left overnight at room temperature and then evaporated *in vacuo*. The residue was taken up in dichloromethane, and the suspension was washed with water followed by 1 M HCl. The insoluble tin deivatives were removed by filtration through Celite, and the solution was dried ($MgSO_4$) and evaporated to give an oil which was chromatographed on silica gel (ethyl acetate) to give **10** (12.4 g, 32.6 mmol, 65%). Mp 94−96 °C (from ethyl acetate/hexane). ^1H NMR ($CDCl_3$, 270 M Hz): δ 2.62 (1 H, br s, D_2O ex, OH), 3.11−3.16 (3 H, m, D_2O ex gives 1H, dd, J = 9.5 Hz, 2.6 Hz, 2 OH and C-1-H or C-3-H), 3.32 (1 H, dd, J = 9.5 Hz,

2.8 Hz, C-1-H or C-3-H), 3.36 (1 H, ddd, J = 9.5 Hz, 9.5 Hz, 1.5 Hz, D_2O ex gives dd, J = 9.5 Hz, 9.5 Hz, C-5-H), 3.66 (1 H, dd, J = 9.5 Hz, 9.5 Hz, C-4-H), 3.81 (3 H, s, OCH_3), 3.88 (1 H, br dd, J = 9.5 Hz, 9.5 Hz, D_2O ex gives dd, C-6-H), 4.07–4.45 (5 H, m, 2 $CH_2CH{=}CH_2$ and C-2-H), 4.63, (2 H, br s, CH_2Ph), 5.15–5.32 (4 H, m, 2 $CH_2CH{=}CH_2$), 5.86–6.05 (2 H, m, 2 $CH_2CH{=}CH_2$), 6.86–6.90 (2 H, m, *Ph*OMe), 7.27–7.31 (2 H, m, *Ph*OMe). ^{13}C NMR ($CDCl_3$, 68 M Hz): δ 55.27 (q, OCH_3), 67.01, 71.55, 74.05, 78.85, 79.34, 80.08 (6 d, inositol ring *C*), 71.29, 72.27 (2 t, $CH_2CH{=}CH_2$), 74.24 (t, CH_2Ph), 113.89, 129.51 (2 d, *Ph*OMe), 116.83, 117.87 (2 t, $CH_2CH{=}CH_2$), 129.94 (s, *Ph*OMe), 134.51, 135.26 (2 d, $CH_2CH{=}CH_2$), 159.35 (s, *Ph*OMe). MS: m/z (+ ion FAB, rel intensity) 379 [$(M - H)^+$, 5], 259 [$(M - CH_2C_6H_4OCH_3)^+$, 2], 137 (12), 121 [$(CH_2C_6H_4OCH_3)^+$, 100]. MS: m/z (− ion FAB, rel intensity) 759 [$(2M - H)^-$, 20], 533 [$(M + NBA)^-$, 92], 379 [$(M - H)^-$, 100], 339 (25), 322 (20), 249 (21). Anal. ($C_{20}O_7H_{28}$) C, H.

DL-1,4-Di-*O*-allyl-2,5,6-tri-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-*myo*-inositol (11). To a solution of **10** (6.85 g, 18 mmol) in dry *N*,*N*-dimethylformamide (100 mL) was added sodium hydride (2.0 g, 83 mmol). The mixture was cooled to 0 °C, and benzyl bromide (7.0 mL, 59 mmol) was added dropwise with stirring. The mixture was stirred at room temperature for 2 h, after which TLC (hexane/ether, 1:1) showed the reaction to be complete, with a major product at R_f 0.43. The excess sodium hydride was carefully destroyed with water and the mixture concentrated *in vacuo*. The residue was dissolved in dichloromethane (100 mL), and the solution was washed successively with water, 0.1 M HCl, saturated $NaHCO_3$ solution, and water (100 mL of each), dried ($MgSO_4$), and evaporated *in vacuo* to give 11.8 g of a solid which was recrystallized from hexane giving 11 (10.2 g, 15.7 mmol, 87%). Mp 72–74 °C (from hexane). ^1H NMR ($CDCl_3$, 270 M Hz): δ 3.23 (1 H, dd, J = 9.9 Hz, 2.2 Hz, C-1-H or C-3-H), 3.28 (1 H, dd, J = 9.9 Hz, 2.2 Hz, C-1-H or C-3-H), 3.39 (1 H, dd, J = 9.3 Hz, 9.3 Hz, C-5-H), 3.81 (3 H, s, OCH_3), 3.91 (1 H, dd, J = 9.3 Hz, 9.3 Hz, C-4-H or C-6-H), 3.97 (1 H, dd, J = 2.2 Hz, 2.2 Hz, C-2-H), 3.98 (1 H, dd, J = 9.7 Hz, 9.7 Hz, C-4-H or C-6-H), 4.07–4.14 (2 H, m, $OCH_2CH{=}CH_2$), 4.27–4.44 (2 H, m, $OCH_2CH{=}CH_2$), 4.54, 4.59 (2 H, AB, J_{AB} = 11.4 Hz, $CH_2C_6H_4OMe$), 4.76–4.90 (6 H, m, 3 $OCH_2C_6H_5$), 5.13–5.35 (4 H, m, $=CH_2$), 5.83–6.06 (2 H, m, $CH{=}$), 6.86–6.90 (2 H, m, $CH_2C_6H_4OMe$), 7.24–7.42 (17 H, m, 3 $CH_2C_6H_5$ and $CH_2C_6H_4OMe$). ^{13}C NMR ($CDCl_3$, 68 M Hz): δ 55.27 (q, OCH_3), 71.62, 72.54, 73.95, 74.54, 75.80 (6 t, OCH_2), 74.44, 80.50, 80.63, 81.41, 81.57, 83.65 (6 d, inositol ring *C*), 113.72 (d, $CH_2C_6H_4OMe$), 116.54 and 116.60 (2 t, $=CH_2$), 127.28, 127.50, 127.79, 127.92, 128.09, 128.31 (6 d, $CH_2C_6H_5$ and $CH_2C_6H_4OMe$), 130.62 (s, $CH_2C_6H_4OMe$), 134.93 and 135.48 (2 d, 2 $CH{=}$), 138.92 and 138.98 (2 s, $CH_2C_6H_5$), 159.13 (s, $CH_2C_6H_4OMe$). MS: m/z (+ ion FAB, rel intensity) 649 [$(M - H)^+$, 1.5], 121 [$(CH_2C_6H_4OCH_3)^+$, 100], 91 [$(M - C_7H_7)^+$, 70]. MS: m/z (− ion FAB, rel intensity) 803 [$(M + NBA)^-$, 100], 529 [$(M - CH_2C_6H_4OCH_3)^-$, 10]. Anal. ($C_{41}H_{46}O_7$) C, H.

DL-2,4,5-tri-*O*-Benzyl-3,6-di-*O*-(*cis*-prop-1-enyl)-1-*O*-*p*-(methoxybenzyl)-*myo*-inositol (12). A solution of 11 (1.0 g, 1.54 mmol) and freshly sublimed potassium *tert*-butoxide (2.0 g, 17.8 mmol) in dry DMSO (50 mL) was stirred for 3 h at 50 °C, after which TLC (hexane/ether, 1:1) showed complete conversion from starting material (R_f 0.44) to a product at R_f 0.56. Water (50 mL) was added to the cooled brown solution, which was then extracted with ether (3 × 100 mL). The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure to give an off-white solid which was recrystallized from ethanol to give **12** (0.83 g, 1.28 mmol, 83%). Mp: 110–112 °C (from ethanol). ^1H NMR ($CDCl_3$, 400 M Hz): δ 1.64 (3 H, dd, J = 6.8 Hz, 1.5 Hz, $CH{=}CHCH_3$), 1.66 (3 H, dd, J = 6.8 Hz, 1.5 Hz, $CH{=}CHCH_3$), 3.33 (1 H, dd, J = 9.8 Hz, 2.4 Hz, C-1-H or C-3-H), 3.44 (1 H, dd, J = 9.3 Hz, 9.3 Hz, C-5-H), 3.53 (1 H, dd, J = 9.8 Hz, 2.4 Hz, C-1-H or C-3-H), 3.81 (3 H, s, OCH_3), 3.99 (1 H, dd, J = 2.4 Hz, 2.4 Hz, C-2-H), 4.03 (1H, dd, J = 9.8 Hz, 9.8 Hz, C-4-H or C-6-H), 4.15 (1 H, dd, J = 9.3 Hz, 9.3 Hz, C-4-H or C-6-H), 4.35 (1 H, dq, J = 6.8 Hz, 6.8 Hz, $CH{=}CHCH_3$), 4.44 (1 H, dq, J = 6.8 Hz, 6.8 Hz, $CH{=}CHCH_3$), 4.51, 4.56 (2 H, AB, J_{AB} = 11.7 Hz, CH_2Ph), 4.72, 4.80 (2 H, AB, J_{AB} = 10.3 Hz, CH_2Ph), 4.73, 4.80 (2 H, AB, J_{AB} = 10.7 Hz, CH_2Ph), 4.82 (2 H, br s, CH_2Ph), 6.08 (1 H, dq, J = 6.8 Hz, 1.5 Hz, $CH{=}CHCH_3$), 6.27 (1 H, dq, J = 6.8 Hz, 1.5 Hz, $CH{=}CHCH_3$), 6.85–6.88 (2 H, m, $CH_2C_6H_4OMe$), 7.24–7.35 (15 H, m, $CH_2C_6H_5$), 7.37–7.42 (2 H, m, $CH_2C_6H_4OMe$). ^{13}C NMR ($CDCl_3$, 100 M Hz): δ 9.36, 9.42 (2 q, $CH{=}CHCH_3$), 55.29 (q, $CH_2C_6H_4OCH_3$), 72.29, 74.47, 75.69, 75.78 (4 t, CH_2Ph), 75.88, 78.35, 80.71, 82.55, 84.40, 82.94 (6 d, inositol ring), 98.19, 100.62, (2 d, $CH{=}CHCH_3$), 113.72 (d, $CH_2C_6H_4OMe$), 127.38, 127.61, 127.83, 128.13, 129.26 (6 d, $CH_2C_6H_5$ and $CH_2C_6H_4OMe$), 130.34 (s, $CH_2C_6H_4OMe$), 138.50, 138.61, 138.71 (s, $CH_2C_6H_5$), 145.66, 147.77 (2 d, $OCH{=}CH$), 159.18 (s, $CH_2C_6H_4OMe$). MS: m/z (+ ion FAB, rel intensity) 649 [$(M - H)^+$, 0.4], 559 [$(M - C_7H_7)^+$, 0.5], 121 [$(CH_2C_6H_4OCH_3)^+$, 100], 91 [$(C_7H_7)^+$, 80]. MS: m/z (− ion FAB, rel intensity) 803 [$(M + NBA)^-$, 100], 696 (32), 559 [$(M - C_7H_7)^-$, 10], 529 [$(M - CH_2C_6H_4OCH_3)^-$, 10]. Anal. ($C_{41}H_{46}O_7$) C, H.

DL-2,4,5-Tri-*O*-benzyl-*myo*-inositol (13). The *cis*-propenyl ether **12** (500 mg, 0.77 mmol) was refluxed in MHCl–ethanol (1:2) for 2 h, after which TLC (ethyl acetate/hexane, 4:1) showed conversion to a major product at R_f 0.34. After cooling, the mixture was evaporated to dryness under reduced pressure. The residue was taken up in dichloromethane (50 mL), washed with saturated $NaHCO_3$ and water (50 mL of each), dried (MgSO4), and evaporated to give a white solid, which was purified by column chromatography (ethyl acetate/hexane, 4:1) giving **13** (310 mg, 0.69 mmol, 90%). Mp: 135–136.5 °C with a phase change at 127–128 °C (from ethyl acetate/hexane) (lit.[28] mp 135–137 °C; lit.[14] mp 126–128 °C). ^1H NMR ($CDCl_3$, 270 M Hz): δ 2.38 (1 H, d, J = 5.4 Hz, D_2O ex, C-3-OH), 2.48 (1H, dd, J = 5.9 Hz, D_2O ex, C-1-OH), 2.63 (1 H, br s, D_2O ex, C-6-OH), 3.32 (1 H, dd, J = 9.3 Hz, 9.3 Hz, C-5-H), 3.44 (1 H, ddd, J = 9.7 Hz, 5.9 Hz, 2.8 Hz, D_2O ex gives dd, J = 9.7 Hz, 2.8 Hz, C-1-H), 3.57 (1 H, ddd, J = 9.7 Hz, 5.4 Hz, 2.6, D_2O ex gives dd, J = 9.7 Hz, 2.6 Hz, C-3-H), 3.78 (1 H, dd, J = 9.3 Hz, 9.3 Hz, C-4-H), 3.83 (1 H, br dd, D_2O ex gives dd, J = 9.5 Hz, 9.5 Hz, C-6-H), 3.98 (1 H, dd, J = 2.7 Hz, 2.7Hz, C-2-H), 4.75–4.94 (6 H, m, $OCH_2C_6H_5$), 7.26–7.38 (15 H, m, $OCH_2C_6H_5$). ^{13}C NMR ($CDCl_3$, 68 M Hz): δ 72.33, 72.70, 74.02, 78.89, 81.78, 82.95 (6 d, inositol ring C), 75.17, 75.23, 75.41 (3 t, $OCH_2C_6H_5$), 127.76, 127.79, 127.89, 128.04, 128.46, 128.54 (6 d, 3 $CH_2C_6H_5$), 138.35, 138.48 (2 s, 3 $CH_2C_6H_5$). MS: m/z (+ ion FAB, rel intensity) 451 [$(M + H)^+$, 3], 181 (20), 91 [$(C_7H_7)^+$, 100]. MS: m/z (− ion FAB, rel intensity) 616 (70), 603 [$(M + NBA)^-$, 92], 496 (30), 449 [$(M - H)^-$, 100], 359 [$(M - C_7H_7)^-$, 23].

DL-2,5,6-Tri-*O*-benzyl-1,3,4-tris(dibenzylphospho)-*myo*-inositol (14). To a solution of bis(benzyloxy)(diisopropylamino)phosphine[48] (920 mg, 2.66 mmol) in dry dichloromethane (3 mL) was added 1*H*-tetrazole (280 mg, 4.00 mmol). The mixture was stirred at room temperature for 10 min. **13** (200 mg, 0.44 mol) was added, and stirring continued for a further 1 h. TLC (ethyl acetate) showed complete conversion of the triol (R_f 0.44) to a product (R_f 0.70), and ^{31}P NMR spectroscopy showed phosphate triester peaks at 140.39 (2P) and 142.2 (1P) . Water (5 mL) was added and, after stirring for a further 5 min, *tert*-butyl hydroperoxide (1 mL of a 70% solution in water). Stirring was continued overnight, after which TLC showed conversion of the trisphosphite to a new product (R_f 0.64). The solvents were removed by evaporation *in vacuo*, and then ethanol (20 mL), water (20 mL), and sodium metabisulfite (1.5 g) were added. After stirring at room temperature for 15 min, the mixture was evaporated to give a paste which was taken up in ether (100 mL), washed with water (2 × 50 mL), dried ($MgSO_4$), and evaporated to give an oil (680 mg). This was purified by column chromatography (ethyl acetate) giving **14** (398 mg, 0.323 mmol, 73%) as a colorless oil.[14] ^1H NMR ($CDCl_3$, 270 M Hz): δ 3.45 (1 H, dd, J = 9.4 Hz, 9.4 Hz C-5-H), 4.06 (1 H, dd, J = 9.4 Hz, 9.4 Hz, C-6-H), 4.22–4.36 (2 H, m, C-1-H and C-3-H), 4.62 (1 H, dd, J = 2.3 Hz, 2.3 Hz, C-2-H), 4.74–5.05 (19 H, m, 9 $CH_2C_6H_5$ and C-4-H), 7.12–7.38 (45 H, m, 9 $CH_2C_6H_5$). ^{13}C NMR ($CDCl_3$, 68 M Hz): δ 69.20, 69.28, 69.36, 69.49, 69.74, 69.82 (6 t, 6 $POCH_2C_6H_5$), 75.27, 75.51, 75.74 (3 t, 3 $OCH_2C_6H_5$), 77.63, 77.92, 78.04, 79.37, 79.47, 80.53, (6 d, inositol ring C), 127.21, 127.29, 127.36, 127.42, 127.53, 127.65, 127.73, 127.79, 127.96, 128.07, 128.12, 128.25, 128.46, 128.72, 130.80 (15 d, 9 $CH_2C_6H_5$),

135.47, 137.93, 138.19 (3 s, 9 $CH_2C_6H_5$). ^{31}P NMR ($CDCl_3$, 162 M Hz): δ −1.41, −1.56, −2.01. MS: *m/z* (+ ion FAB, rel intensity) 1231 [$(M + H)^+$, 5], 1230 (6), 181 (10), 91 [$(C_7H_7)^+$, 100]. MS: *m/z* (− ion FAB, rel intensity) 1383 [$(M + NBA)^-$, 5], 1139 [$(M - C_7H_7)^-$, 30], 1138 (35), 1138 (35), 277 [$(C_6H_5CH_2O)_2PO_2)^-$, 100].

DL-*myo*-Inositol 1,3,4-Trisphosphate (3). Ammonia was condensed into a three-necked flask at −78 °C. An excess of sodium was added to dry the liquid ammonia, which was then distilled into a second three-necked flask and kept at −78 °C. Sodium was added until the solution remained blue. Compound **14** (100 mg, 81.2 μmol) was dissolved in anhydrous dioxane (2 mL) and added to the sodium−liquid ammonia mixture. After the mixture had stirred for 3 min, the reaction was quenched with ethanol. Ammonia and ethanol were evaporated. The residue was dissolved in deionized water (500 mL) and purified by ion-exchange chromatography on Q Sepharose fast flow, eluting with a gradient of triethylammonium bicarbonate buffer (0−1 M), pH 8.0. The triethylammonium salt of **3** eluted between 400 and 470 mM. Yield: 26 μmol, 32%.[13,14] ^1H NMR (D_2O, 400 M Hz, pH ~4.2): δ 3.40 (1 H, dd, J = 9.3 Hz, 9.3 Hz, C-5-H), 3.68 (1 H, dd, J = 9.3 Hz, 9.3 Hz, C-6-H), 3.85 (1 H, ddd, J = 9.3 Hz, 8.8 Hz, 2.4 Hz, C-1-H), 3.95 (1 H, ddd, J = 9.8 Hz, 9.8 Hz, 2.5 Hz, C-3-H), 4.16 (1 H, ddd, J = 9.3 Hz, 9.3 Hz, 9.3 Hz, C-4-H), 4.27 (1 H, dd, J = 2.4 Hz, 2.4 Hz, C-2-H). ^{31}P NMR (D_2O, 162 M Hz, pH ~7): δ 0.13 (1 P, d, J = 8.6 Hz), 0.24 (1 P, d, J = 9.5 Hz), 0.97 (1 P, d, J = 8.5 Hz). MS: *m/z* (+ ion FAB, rel intensity) 102 [$(C_2H_5)_3NH^+$, 100]. MS: *m/z* (− ion FAB, rel intensity) 838 ($2M^-$, 12), 419 (M^-, 100), 97 ($H_2PO_4^-$, 10). MS: *m/z* 418.954 ($M - H)^-$ (calcd for $C_6H_{14}O_{15}P_3$ 418.955).

DL-2,4,5-Tri-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol (15). A solution of **12** (0.83 g, 1.28 mmol) in acetone (45 mL) was warmed to 50 °C with stirring. MHCl (5 mL) was added, and stirring continued for 10 min, after which TLC (ether) showed complete conversion of starting material (R_f 0.76) to a major product at R_f 0.56. Sodium hydrogen carbonate (1 g) was added, and stirring continued for a further 10 min, as the solution was allowed to cool to room temperature. The solvents were removed under reduced pressure, and the residue was taken up in dichloromethane (100 mL). The suspension was washed with water (2 × 100 mL), dried ($MgSO_4$), and evaporated to give an oil which was purified by column chromatography (ether) to give **15** (0.69 g, 1.21 mmol, 94%). Mp: 100−102 °C (from hexane/ethyl acetate). ^1H NMR ($CDCl_3$, 270 M Hz): δ 2.27 (1 H, d, J = 6.4 Hz, D_2O ex, C-3-OH), 2.50 (1 H, d, J = 1.5 Hz, D_2O ex, C-6-OH), 3.26 (1 H, dd, J = 9.5 Hz, 2.2 Hz, C-1-H), 3.38 (1 H, dd, J = 9.3 Hz, 9.3 Hz, C-5-H), 3.51 (1 H, ddd, J = 9.3 Hz, 6.4 Hz, 2.2 Hz, D_2O ex gives dd, J = 9.3 Hz, 2.2 Hz, C-3-H), 3.78 (1 H, dd, J = 9.3 Hz, 9.3 Hz, C-4-H), 3.81 (3 H, s, $C_6H_4OCH_3$), 4.04 (1 H, dd, J = 2.2 Hz, 2.2 Hz, C-2-H), 4.13 (1 H, ddd, J = 9.3 Hz, 9.3 Hz, 1.5 Hz, D_2O ex gives dd, J = 9.3 Hz, 9.3 Hz, C-6-H), 4.53, 4.61 (2H, AB, J_{AB} = 11.4 Hz, OCH_2Ph), 4.67−4.93 (6 H, m, OCH_2-Ph), 6.88 (2 H, br d, J = 8.2 Hz, $CH_2C_6H_4OMe$), 7.24−7.74 (17 H, m, $CH_2C_6H_4OMe$ and 3 $CH_2C_6H_5$). ^{13}C NMR ($CDCl_3$, 68 M Hz): δ 55.27 (q, OCH_3), 72.15, 74.62, 75.20, 75.46 (4 t, OCH_2-Ph), 72.51, 73.04, 76.14, 80.03, 81.87, 83.23 (6 d, inositol ring C), 113.98 (d, $OCH_2C_6H_4OCH_3$), 127.65, 127.78, 127.91, 128.04, 128.35, 128.41, 128.48, 129.45 (8 d, 3 $CH_2C_6H_5$ and $CH_2C_6H_4$-OCH_3), 129.32 (s, $CH_2C_6H_4OMe$), 138.04, 138.17 (2 s, 3 $CH_2C_6H_5$), 158.90 (s, $CH_2C_6H_4OMe$). MS: *m/z* (+ ion FAB, rel intensity) 569 [$(M - H)^+$, 7], 479 [$(M - C_7H_7)^+$, 2], 449 [$(M - CH_2C_6H_4OCH_3)^+$, 10], 181 (60), 121 [$(CH_2C_6H_4OCH_3)^+$, 90], 91 [$(C_7H_7)^+$, 100]. MS: *m/z* (− ion FAB, rel intensity) 723 [$(M + NBA)^-$, 100], 569 [$(M - H)^-$, 50], 322 (42), 140 (52). Anal. ($C_{35}H_{38}O_7$) C, H.

Resolution of DL-2,4,5-Tri-*O*-benzyl-1-*O*-(*p*-methoxybenzyl)-*myo*-inositol. A mixture of **15** (1.35 g, 2.37 mmol) and (*S*)-(−)-ω-camphanic chloride (1.54 g, 7.11 mmol) and DMAP (50 mg, 0.41 mmol) in dry pyridine (10 mL) was stirred for 1 h at room temperature, after which TLC (chloroform/acetone, 30:1) showed complete conversion from starting material (R_f 0.13) to two products (R_f 0.17 and 0.24). The mixture was cooled in an ice bath, and water (1 mL) was added. Stirring was continued for a further 1 h. Ether (100 mL) and dichloromethane (50 mL) were added, and the organic phase was washed successively with saturated KCl, ice-cold MHCl, saturated KCl, and saturated $NaHCO_3$ (100 mL of each), dried ($MgSO_4$), and evaporated to give the mixture of diastereoisomers (2.16 g, 2.32 mmol, 98%).

The mixture of diastereoisomers was separated by column chromatography (chloroform/acetone, 30:1) into two fractions, which were recrystallized from methanol to give the two diastereoisomers: **16a**, R_f 0.17 (683 mg, 0.734 mmol, 63.3% of this diastereoisomer), and **16b**, R_f 0.24 (796 mg, 0.855 mmol, 73.7% of this diastereoisomer).

1D-2,5,6-Tri-*O*-benzyl-1,4-di-*O*-[(−)-ω-camphanoyl]-3-*O*-(*p*-methoxybenzyl)-*myo*-inositol (16a). Mp: 186−188 °C (from methanol). ^1H NMR ($CDCl_3$, 270 M Hz): δ 0.74, 0.84 (6 H, 2 s, camph-CH_3), 0.96 (6 H, br s, camph-CH_3), 1.04, 1.07 (6 H, 2 s, camph-CH_3), 1.60−1.74 (2 H, m, camph-CH_2), 1.78−1.96 (4 H, m, camph-CH_2), 2.21−2.37 (2 H, m, camph-CH_2), 3.63 (1 H, dd, J = 10.8 Hz, 1.5 Hz, C-3-H), 3.70 (1 H, dd, J = 9.5 Hz, 9.5 Hz, C-5-H), 3.79 (3 H, s, $CH_2C_6H_4OCH_3$), 4.23 (1 H, br s, C-2-H), 4.28 (1 H, dd, J = 9.7 Hz, 9.7 Hz, C-6-H), 4.47−5.05 (9 H, m, 4 CH_2Ph and C-1-H), 5.85 (1 H, dd, J = 9.9 Hz, 9.9 Hz C-4-H), 6.86 (2 H, br d, J = 8.4 Hz, C_6H_4OMe), 7.20−7.44 (17 H, m, C_6H_4OMe and 3 C_6H_5). ^{13}C NMR ($CDCl_3$, 68 M Hz): δ 15.72, 15.97, 16.06 (3 q, camph-CH_3), 28.35, 30.33, 30.47 (3 t, camph-CH_2), 53.42, 53.57, 54.18 (3 s, camph), 54.70 (q, OCH_3), 71.62, 74.03, 74.18, 74.63 (4 t, CH_2Ph), 73.24, 73.47, 74.41, 77.85, 78.22, 80.10 (6 d, inositol ring C), 90.17, 90.43 (2 s, camph), 113.31 (d, C_6H_4OMe), 126.37, 126.61, 126.82, 126.92, 127.10, 127.16, 127.70, 127.76, 128.23 (9 d, 3 $CH_2C_6H_5$ and C_6H_4OMe), 137.30, 137.40 (2 s, 3 $CH_2C_6H_5$), 158.78 (s, C_6H_4-OMe), 166.17, 166.83, 177.21, 177.70 (4 s, camph C=O). MS: *m/z* (+ ion FAB, rel intensity) 929 [$(M - H)^+$, 0.7], 839 [$(M - C_7H_7)^+$, 0.7], 809 [$(M - CH_2C_6H_4OCH_3)^+$, 1.2], 121 [$(CH_2C_6H_4$-$OCH_3)^+$, 100], 91 [$(C_7H_7)^+$, 68]. MS: *m/z* (− ion FAB, rel intensity) 1083 [$(M + NBA)^-$, 7], 197 [(camph-O)$^-$, 100]. $[\alpha]^{27}_D$ = −11° (*c* = 1, $CHCl_3$). Anal. ($C_{55}H_{62}O_{13}$) C, H.

1L-2,5,6-Tri-*O*-benzyl-1,4-di-*O*-[(−)-ω-camphanoyl]-3-*O*-(*p*-methoxybenzyl)-*myo*-inositol (16b). Mp: 194−195 °C (from methanol). ^1H NMR ($CDCl_3$, 400 M Hz): δ 0.82, 0.88, 0.96, 1.00, 1.05, 1.08 (18 H, 6 s, camph-CH_3), 1.54−1.71 (2 H, m, camph-CH_2), 1.72−1.88 (4 H, m, camph-CH_2), 2.34−2.31 (2 H, m, camph-CH_2), 3.62 (1 H, dd, J = 10.4 Hz, 2.1 Hz C-3-H), 3.68 (1 H, dd, J = 9.5 Hz, 9.5 Hz, C-5-H), 3.79 (3 H, s, $C_6H_4OCH_3$), 4.12 (1 H, dd, J = 2.1 Hz, 2.1 Hz, C-2-H), 4.24 (1 H, dd, J = 9.5 Hz, 9.5 Hz, C-6-H), 4.47, 4.58 (2 H, AB, J_{AB} = 11.3 Hz, $CH_2C_6H_4OCH_3$), 4.59−4.88 (6 H, m, 3 $CH_2C_6H_5$), 5.00 (1 H, dd, J = 10.4 Hz, 2.4 Hz, C-1-H), 5.82 (1 H, dd, J = 9.8 Hz, 9.8 Hz, C-4-H), 6.86 (2 H, br d, J = 8.9 Hz, C_6H_4OMe), 7.19−7.38 (17 H, m, C_6H_4OMe and 3 C_6H_5). ^{13}C NMR ($CDCl_3$, 68 M Hz): δ 16.45, 16.59, 16.72 (3 q, camph-CH_3), 28.85, 30.73, 30.85 (3 t, camph-CH_2), 54.18, 54.77, 54.83 (3 s, camph), 55.27 (q, OCH_3), 71.88, 75.01, 75.20, 75.27 (4 t, CH_2Ph), 74.07, 74.77, 74.81, 77.88, 78.77, 81.23 (6 d, inositol ring C), 90.75, 91.13 (2 s, camph), 113.88 (d, C_6H_4OMe), 127.21, 127.26, 127.49, 127.55, 127.67, 128.25, 128.36, 128.82, 129.38 (9d, 3 $CH_2C_6H_5$ and C_6H_4OMe), 137.96, 138.14 (2 s, 3 $CH_2C_6H_5$), 159.32 (s, C_6H_4-OMe), 166.73, 167.29, 177.84, 178.46 (4 s, camph C=O). MS: *m/z* (+ ion FAB, rel intensity) 929 [$(M - H)^+$, 0.8], 839 [$(M - C_7H_7)^+$, 1.1], 809 [$(M - CH_2C_6H_4OCH_3)^+$, 1.2], 121 [$(C_7H_6$-$OCH_3)^+$, 100], 91 [$(C_7H_7)^+$, 58]. MS: *m/z* (− ion FAB, rel intensity) 1083 [$(M + NBA)^-$, 11], 197 [(camph-O)$^-$, 100]. $[\alpha]^{27}_D$ = 5° (*c* = 1, $CHCl_3$). Anal. ($C_{55}H_{62}O_{13}$) C, H.

(+)-1D-2,5,6-Tri-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-*myo*-inositol (15a). The bis-(−)-camphanate ester **16a** (570 mg, 0.612 mmol) was dissolved in methanol (100 mL) containing sodium hydroxide pellets (4 g) and refluxed for 30 min. The mixture was cooled and then neutralized with solid carbon dioxide. After the solvents were removed by evaporation under reduced pressure, the residue was taken up in water (100 mL), extracted with chloroform (2 × 100 mL), dried ($MgSO_4$), and evaporated to give a white solid. This was recrystallized from ethyl acetate/hexane giving **15a** (334 mg, 0.585 mmol, 96%). Mp: 95− 96.5 °C (from ethyl acetate/hexane). $[\alpha]^{24}_D$ = 4° (*c* = 1, $CHCl_3$). Anal. ($C_{35}H_{38}O_7$) C, H. NMR and mass spectrometry data were identical to those for the racemic diol **15**.

(−)-1L-**2,5,6-Tri-*O*-benzyl-3-*O*-(*p*-methoxybenzyl)-*myo*-inositol (15b)**. The bis-(+)-camphanate ester **16b** (770 mg, 0.827 mmol) was converted to the diol **15b** as described for compound **16a**. Yield: 461 mg, 0.808 mmol, 98%. Mp: 95–97 °C (from ethyl acetate/hexane). $[\alpha]^{22}_D = -4°$ ($c = 1$, $CHCl_3$). Anal. ($C_{35}H_{38}O_7$) C, H. NMR and mass spectrometry data were identical to those for the racemic diol **15**.

(−)-1L-**1,2,4,5,6-Penta-*O*-benzyl-*myo*-inositol (17b)**. The absolute configuration of **15b** was determined by converting it to the known pentabenzyl ether **17b**. A sample of the (−)-diol **15b** (60 mg, 0.105 mmol) was dissolved in dry DMF (5 mL), and sodium hydride (20 mg of a 60% dispersion in oil, 0.50 mmol) was added. After stirring at room temperature for 10 min, benzyl bromide (0.05 mL, 0.42 mmol) was added and stirring continued for 2 h. The excess sodium hydride was destroyed by addition of water (5 mL) and the mixture concentrated under reduced pressure. The residue was taken up in dichloromethane (20 mL), washed with 0.1 M HCl (20 mL), and evaporated. The residue was then refluxed in methanol/MHCl (2:1) for 5 h, after which time TLC showed complete conversion to the penta-*O*-benzyl ether. The solvents were removed by evaporation *in vacuo*, and the residue was taken up in dichloromethane (30 mL), washed with saturated $NaHCO_3$ and water (20 mL of each), dried ($MgSO_4$), and evaporated. Column chromatography (pentane/ether 1:1) gave the pure penta-*O*-benzyl ether, which had an ^1H NMR spectrum identical to a racemic 1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol[25] but a specific rotation of −11.5°, allowing its absolute configuration to be assigned as L-**17b** (52 mg, 0.0824 mmol, 78% from **15b**). $[\alpha]^{22}_D = -11.5°$ ($c = 2$, $CHCl_3$) [lit.[23] $[\alpha]_D = -13.5°$ ($c = 0.5$, $CHCl_3$); lit.[24] $[\alpha]_D = 10.0°$ ($c = 1$, $CHCl_3$) for the enantiomer; lit.[26] $[\alpha]_D = 9.7°$ ($c = 1.5$, $CHCl_3$) for the enantiomer]. ^1H NMR ($CDCl_3$, 270 M Hz): δ 2.22 (1 H, d, $J = 6.0$ Hz, D_2O ex, C-3-OH), 3.43–3.53 (3 H, m, C-1-H, C-3-H, C-5-H), 3.81 (1 H, dd, $J = 9.5$ Hz, 9.5 Hz, C-6-H or C-4-H), 4.03 (1 H, dd, $J = 2.2$ Hz, 2.2 Hz, C-2-H), 4.06 (1 H, dd, $J = 9.5$ Hz, 9.5 Hz, C-6-H or C-4-H), 4.70–5.02 (10 H, m, $CH_2C_6H_5$), 7.25–7.38 (25 H, m, C_6H_5).

(−)-1D-**2,5,6-Tri-*O*-benzyl-*myo*-inositol (13a)**. The (+)-diol **15a** (300 mg, 0.526 mmol) was dissolved in ethanol (80 mL), and MHCl (40 mL) was added. The mixture was refluxed for 4 h, and the solvents were evaporated under reduced pressure. The residue was taken up in dichloromethane (50 mL), washed with water, saturated $NaHCO_3$, and brine (30 mL of each), and dried ($MgSO_4$). The solvents were removed and the residue purified by flash chromatography (ethyl acetate/hexane, 4:1) to give a white solid which was recrystallized from ethyl acetate/hexane giving the triol **13a** (213 mg, 0.473 mmol, 90%). Mp: 104–106 °C with a phase change at 92–93 °C (from ethyl acetate/hexane) (lit.[21] mp 103–105 °C). $[\alpha]^{23}_D = -32°$ ($c = 1$, $CHCl_3$) [lit.[21] $[\alpha]^{25}_D$ −27° ($c = 1$, $CHCl_3$); lit.[20] $[\alpha]_D$ −25° ($c = 0.5$, $CHCl_3$)]. NMR and mass spectrometry data were identical to those for the racemic triol **13**.

(+)-1L-**2,5,6-Tri-*O*-benzyl-*myo*-inositol (13b)**. The (−)-diol **15b** (350 mg, 0.613 mmol) was treated as described for the enantiomer above to give the (+)-triol **13b** (250 mg, 0.555 mmol, 91%). Mp: 104–106 °C with a phase change at 93–94 °C (from ethyl acetate/hexane) (lit.[21] mp 104–106 °C). $[\alpha]^{21}_D = 32°$ ($c = 1$, $CHCl_3$) [lit.[21] $[\alpha]^{25}_D = 25°$ ($c = 1$, $CHCl_3$)]. NMR and mass spectrometry data were identical to those for the racemic triol **13**.

(+)-1D-**2,5,6-Tri-*O*-benzyl-1,3,4-tris(dibenzylphospho)-*myo*-inositol (14a)**. The (−)-triol **13a** (60 mg, 0.133 mmol) was phosphitylated as described for the racemic material. Oxidation and purification as before gave **14a** as a colorless oil (126 mg, 0.102 mmol, 77%). $[\alpha]^{20}_D = 6°$ ($c = 1$, in $CHCl_3$). Anal. ($C_{69}H_{69}O_{15}P_3$) C, H. NMR and mass spectrometry data were identical to those for racemic **14**.

(−)-1L-**2,5,6-Tri-*O*-benzyl-1,3,4-tris(dibenzylphospho)-*myo*-inositol (14b)**. The (+)-triol **13b** (60 mg, 0.133 mmol) was phosphitylated as described for the racemate giving **14b** as a colorless oil. Yield: 123 mg, 0.100 mmol, 75%. $[\alpha]^{20}_D = -6°$ ($c = 1$, $CHCl_3$) [lit.[21] $[\alpha]^{25}_D = -5.8°$ ($c = 1$, in $CHCl_3$)]. NMR and mass spectrometry data were identical to those for racemic **14**.

(+)-1D-***myo*-Inositol 1,3,4-Trisphosphate (3a)**. The fully protected trisphosphate triester **14a** (80 mg, 65 μmol) was deprotected and purified as described for the racemic material to give **3a** as the glassy triethylammonium salt. Yield: 34 μmol, 52%. $[\alpha]^{26}_D = 37°$ ($c = 0.42$, TEAB buffer, pH 7.8) calculated for the free acid [lit.[16] $[\alpha]_D = -6°$ ($c = 0.5$, H_2O); lit.[20] $[\alpha]_D = 13.6°$ (c = 2, H_2O, pH 8.2)]. MS: m/z 418.956 ($M - H)^-$ (calcd for $C_6H_{14}O_{15}P_3$ 418.955). NMR data were identical to those for the racemate **3**.

(−)-1L-***myo*-Inositol 1,3,4-Trisphosphate (3b)**. Compound **14b** (100 mg, 81.2 μmol) was deprotected and purified as described for the racemic material giving the triethylammonium salt **3b**. Yield: 31 μmol, 38%. $[\alpha]^{26}_D = -40°$ ($c = 0.42$, TEAB buffer, pH 7.8) calculated for the free acid. MS: m/z 418.957 ($M - H)^-$ (calcd for $C_6H_{14}O_{15}P_3$, 418.955). NMR data were identical to those for **3**.

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