Synthesis and Some Properties of D-*myo*-Inositol 1,4,5-Tris(dihydrogen phosphate)

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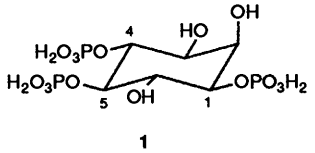
Optically active *myo*-inositol 1,4,5-tris(dihydrogen phosphate) **1**, which has now been recognized as a second messenger in a new intracellular signal transduction system, has been prepared starting from *myo*-inositol. The key step, phosphorylation of an adequately protected polyhydroxy derivative, was accomplished by three methods, among which a phosphoramidite method using a new phosphitylating agent, *o*-xylylene *N,N*-diethylphosphoramidite, gave the triphosphoric ester in quantitative yield. Optical resolution was effectively realized by derivatization into diastereoisomeric *l*-menthoxyacetic esters. NMR spectra and optical rotation are shown to depend on the pH of an aqueous solution of compound **1**.

Since the report on the biological role of D-*myo*-inositol 1,4,5-tris(dihydrogen phosphate) ($InsP_3$, **1**) as a second messenger in intracellular signalling in 1983,[1] the new intracellular signal transduction system which involves hydrolysis of phosphatidyl inositol 4,5-bis(dihydrogen phosphate) (PIP_2, **2**) to **1** and diacylglycerol has been investigated in considerable detail not only in the biological field and but also chemically. $InsP_3$ **1** has now been recognized to mediate the release of calcium ions from intracellular stores.[2] To supply compound **1** and its analogues in large quantities for investigation of their biological processes, their chemical synthesis is required. On the other hand, these inositol poly(phosphate) derivatives are structually unique in that, although their molecular weights are relatively small, they have several phosphoryl functions in the same molecule and some of these are situated vicinally. Accordingly, their chemical synthesis is quite challenging. Therefore, both the biological and the chemical interest in the PIP_2 cycle encouraged us to explore the chemical synthesis of $InsP_3$ **1**.



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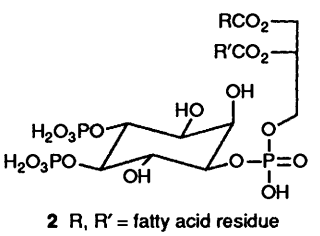


2 R, R' = fatty acid residue

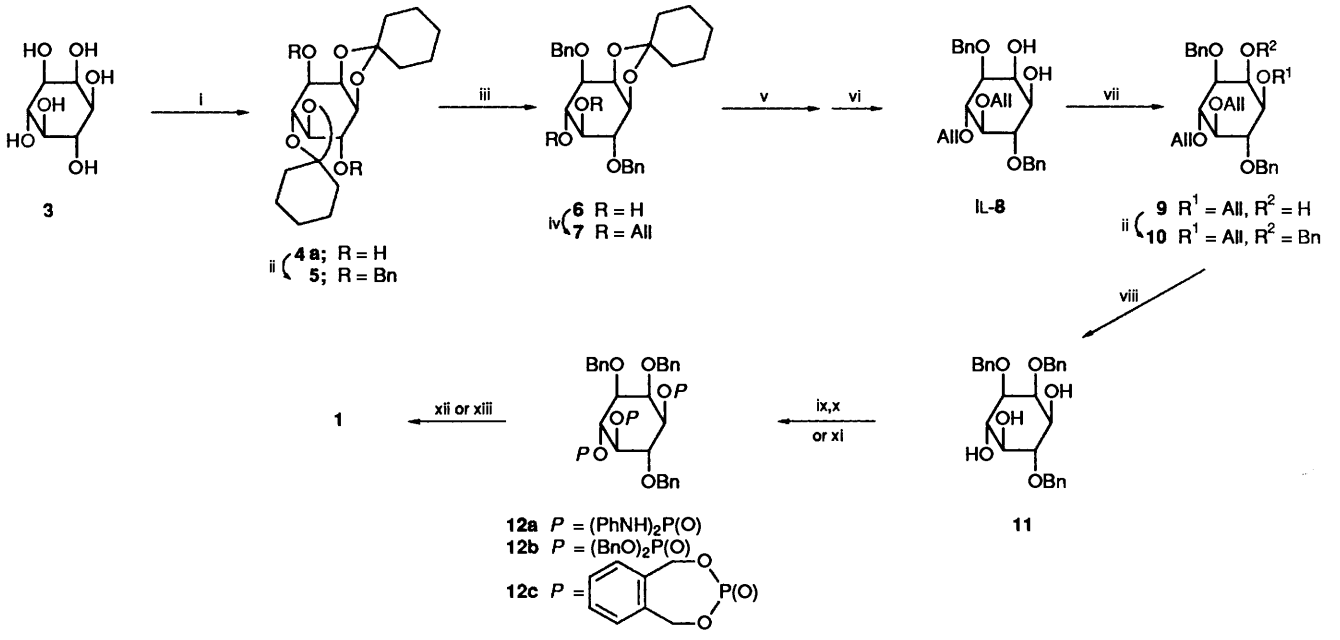
In order to reach our synthetic goal, it was particularly necessary to develop a suitable optical resolution procedure for *myo*-inositol derivatives as well as an efficient exhaustive phosphorylation of polyalcohols, especially vicinally situated polyols. The solutions to these crucial problems have led us to a synthesis of D-*myo*-inositol 1,4,5-tris (dihydrogen phosphate) **1**. These results were partly reported as the first synthesis of compound **1** in 1986.[3] Several research groups have subsequently reported its synthesis.[4] In this paper, we would like to describe our synthesis of **1** in full detail.

Results and Discussion

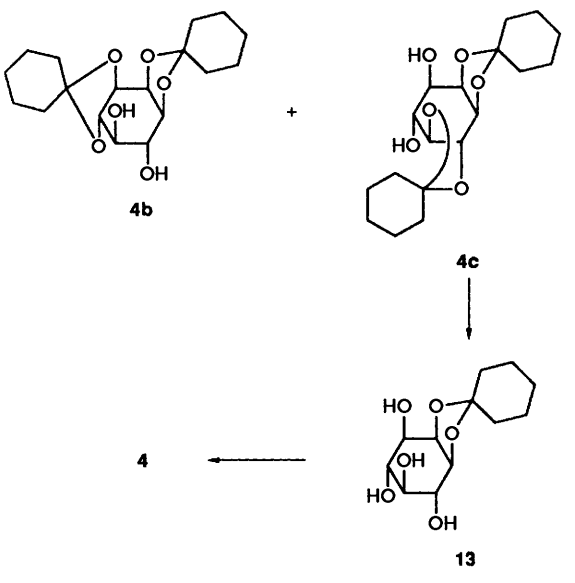
Optical Resolution of (±)-5,6-*Di*-O-*allyl*-1,4-*di*-O-*benzyl*-myo-*inositol* (±)-**8** *and Synthesis of* 1L-1,2,4-*Tri*-O-*benzyl*-myo-*inositol* **11**.—Readily available *myo*-inositol **3** was chosen as the starting material and transformed into a key synthetic intermediate, L-1,2,4-tri-*O*-benzyl-*myo*-inositol **11**, which is the substrate for phosphorylation (Scheme 1). According to the literature procedure,[5] compound **3** was treated with 1-ethoxycyclohexene in the presence of toluene-*p*-sulfonic acid (PTSA) to give 1,2:4,5-di-*O*-cyclohexylidene-*myo*-inositol **4a** accompanied with its regioisomers **4b** and **4c**. The desired isomer **4a** was readily isolated from other products by crystallization of the reaction mixture, although yields of isomer **4a** were usually lower than 20%. Treatment of the residual mixture, obtained after crystallization of isomer **4a**, with PTSA in an ethanolic solvent system[6] resulted in the precipitation of 1,2-cyclohexylidene-*myo*-inositol **13**, which was then subjected to cyclohexylidenation as described above to give the mixture of isomers **4** (Scheme 2). Thus, waste products involving the unrequired isomers **4b** and **4c** could be recycled for preparation of the required isomer **4a**. Benzylation of compound **4a** (90% yield) followed by selective removal of the 4,5-cyclohexylidene group (80% yield) afforded 4,5-diol **6** which was then allylated to give fully substituted inositol **7** in quantitative yield. After hydrolysis of compound **7** by aqueous acetic acid, racemic 1,2-diol **8** was obtained in 88% yield.

Optical resolution was examined at this stage. Some methods are known for resolution of *myo*-inositol derivatives and various chiral auxiliaries have been employed for derivatization of a racemate to the corresponding diastereoisomeric mixture.[7] Among them, only the method using the mannoside orthoester derivative reported by Shvets *et al.*[8a] was shown to be of wide use. However, yields for the formation of diastereoisomeric orthoesters are not always good and orthoesters are inherently too sensitive towards acidic media such as silica gel. These facts prompted us to devise an efficient resolution method and menthoxyacetic ester was found to offer a quite effective diastereoisomer. Thus, treatment of racemic 1,2-diol **8** with 1-*l*-menthoxyacetyl chloride yielded regioselective acylation products, which were readily separated by flash chromatography to afford esters **14a** and **14b** in 39 and 43% yield, respectively (Scheme 3). Fortunately, recrystallization of the reaction mixture from hexane with seed crystals also yielded directly the desired diastereoisomer **14a**. Each diastereoisomer thus separated was transformed into the corresponding 1,2-diol quantitatively by alkaline hydrolysis.

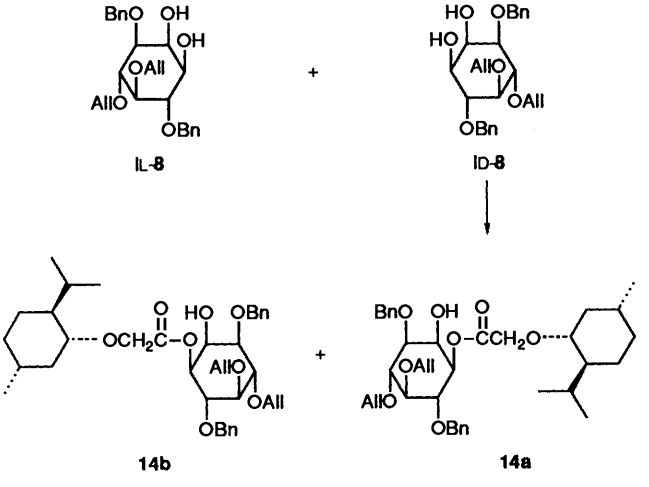
Other 1,2-diol derivatives of *myo*-inositol such as 1,4,5,6-tetra-*O*-benzyl-*myo*-inositol **17**,[9a] 1,5,6-tri-*O*-benzoyl-4-*O*-benzyl-*myo*-inositol,[9c] and 1,4-di-*O*-benzyl-5,6-bis-*O*-(dibenzylphosphoryl)-*myo*-inositol[4h] could be also resolved similarly by way of their 1-*l*-menthoxyacetates.[9] It is noteworthy that menthoxyacetic acid is economical, readily recoverable after hydrolysis of each diastereoisomer, and essentially racemization-free during its introduction and removal. After our report was



Scheme 1 Synthesis of D-*myo*-inositol 1,4,5-tris(dihydrogen phosphate) [All = allyl, Bn = benzyl]. *Reagents and conditions:* i, 1-ethoxycyclohexene, PTSA; ii, BnCl, NaH, DMF; iii, ethylene glycol, PTSA; iv, AllBr, NaH, DMF; v, aq. AcOH; (a) *l*-menthoxyacetyl chloride, pyridine, (b) separation of diastereoisomers by SiO_2 chromatography or crystallization, (c) Aq. NaOH, MeOH; viii, (a) RhCl $(PPh_3)_3$, DABCO, (b) HCl, MeOH; ix (for **12a**), **22**, DMAP, pyridine; x (for **12b**), BuLi, **28**; xi (for **12c**), (a) **30**, tetrazole, (b) MCPBA; xii (**12a** to **1**), (a) isopentyl nitrite, (b) H_2, 5% Pd–C; xiii (**12b** or **12c** to **1**), H_2, 5% Pd–C



Scheme 2 Recycle of by-products for preparation of **4a**



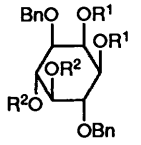
Scheme 3 Derivatization to diastereoisomeric menthoxyacetic esters

published, *l*-menthoxyacetic esters were utilized for optical resolution of some inositol derivatives by other laboratories.[4f,10] Consequently, derivatization to diastereoisomeric menthoxyacetates is a useful tool for optical resolution of racemic inositols, especially of 1,2-diol derivatives. Camphanic ester was introduced successfully for resolution of inositols by Gigg *et al.*,[11] Billington *et al.*[12] and Vacca *et al.*,[4c] and many inositol derivatives so far have been resolved by this procedure. According to our experiments, however, 1-*O*-(*l*-menthoxyacetyl)-*myo*-inositols derived from 1,2-diols gave better results than did the corresponding camphanate esters. van Boom and co-workers also reported similar results in the synthesis of optically active $InsP_3$ and *myo*-inositol 1,3,4,5-tetrakis (dihydrogen phosphate).[4f]

The absolute configuration of 1L-**8** was confirmed by transforming it into 1L-1,4,5,6-tetra-*O*-benzyl-*myo*-inositol **17**. Thus 1L-**8** was deallylated by isomerization of the double bond [RhCl $(PPh_3)_3$, diazabicycloundecane (DABCO)] and subsequent acidic methanolysis[13] to afford dibenzyl ether **15**. The ether **15** was then converted into the dicyclohexylidene derivative 1L-**5** as described above for the synthesis of compound **4a**, which was then converted selectively into 4,5-diol 1D-**6** $\{[\alpha]_D^{20}$ +33.0$\}$.* Benzylation of 4,5-diol 1D-**6** followed by acidic hydrolysis gave 1L-1,4,5,6-tetra-*O*-benzyl-*myo*-inositol **17**. Comparison of its optical rotation $\{[\alpha]_D^{20}$ −22.3$\}$ with those[8] reported for authentic **17** {for example,[8a] $[\alpha]_D^{20}$ −24.3} elucidated the stereochemistry of our product **17**.

1L-5,6-Di-*O*-allyl-1,4-di-*O*-benzyl-*myo*-inositol 1L-**8** thus resolved was then treated with allyl bromide in the presence of sodium hydroxide in boiling benzene[14] to give the 3-allyl

* The opposite enantiomer was reported to have $[\alpha]_D^{20}$ +37.0 (*c* 0.5, $CHCl_3$). Its value should read −37.0: V. N. Krylova, V. M. Kornitskayer, V. I. Shvets and R. P. Evstigneeva, *Zh. Org. Khim.*, 1975, **11**, 2034.



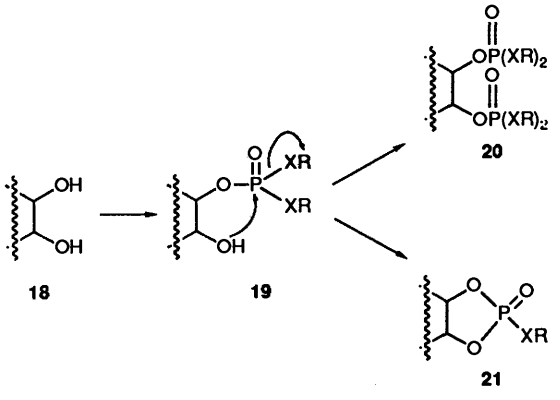
15 $R^1 = R^2 = H$

16 $R^1 = R^1 =$  , $R^2 = Bn$

17 $R^1 = H$, $R^2 = Bn$

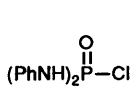
derivative **9** selectively in 74% yield. After benzylation of compound **9** under standard conditions [NaH, BnCl, dimethylformamide (DMF); 98% yield], removal of the three allyl groups was achieved as described above by isomerization of the double bond and acidic methanolysis[13] to furnish 1L-1,2,4-tri-*O*-benzyl-*myo*-inositol **11** in 58% yield. Other known deprotection procedures [Bu^{t}OK, dimethyl sulphoxide (DMSO) then HCl, MeOH:[14] 49% yield and 10% Pd–C, PTSA, aq. MeOH:[15] 24% yield] were less effective. The structure of triol **11** was further supported by transformation into the triacetate and its NMR analysis. After we had completed the synthesis of optically active triol **11**, Gigg *et al.*[16] reported a synthesis of racemic triol **11** which was synthesized by a similar route. They have subsequently described improved routes and the preparation of the opposite enantiomer of compound **11**.[11] Very recently, Sato *et al.* also prepared optically active triol **11** by using a chiral starting material, D-glucose.[17]

Strategies for the Phosphorylation of myo-*Inositols. Synthesis of Fully Protected Ins*(1,4,5)P_3.—As reported by several research groups, phosphorylation of inositol derivatives is quite difficult.[18] The reasons are as follows: (i) The reactivity of hydroxy functions in inositols is generally low compared with that of common alcohols and nucleotides because of their steric crowding and hydrogen bonding; (ii) intramolecular transesterification of a monophosphorylated intermediate **19** in the phosphorylation of 1,2-diol **18** occurs preferentially, forming the five-membered cyclic phosphate **21** instead of further phosphorylation of the remaining vicinal hydroxy function leading to bis(phosphate) **20** (Scheme 4).[18]



Scheme 4 Diphosphate formation *vs* internal ester exchange in the phosphorylation of 1,2-diol

After some unsuccessful experiments, we obtained the 1,4,5-tris(phosphate) **12a** in 60%* yield by the reaction of triol **11** with dianilinophosphoric chloride **22**[19] which has previously been utilized for phosphorylation of some inositol derivatives.[18c,20] However, the regioisomeric 2,5,6-tris(phosphate) could not be obtained at all by a similar phosphorylation of 1,3,4-tri-*O*-benzyl-*myo*-inositol **23** and small amounts of a complex mixture of bis- and mono-phosphates were detected in the organic layer. The majority of the products were observed in the alkaline aqueous solution obtained in the work-up procedure. This result suggests that the monophosphate once formed at C-4 or C-5 was converted predominantly into the five-membered cyclic phosphate which was then easily hydrolysed[21] to the water-soluble acyclic monophosphate. Therefore, reactivity of the phosphoryl chloride **22** toward inositol derivatives is too low, and prolonged reaction time sometimes causes serious side reactions. Consequently, we turned our attention to an alternative phosphorylating agent.



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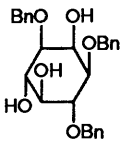

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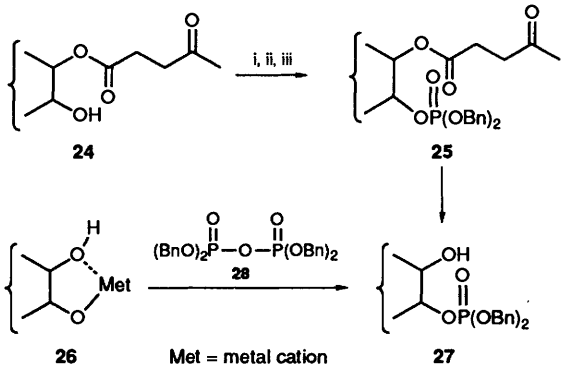
For the construction of 1,2-bis(phosphate) functionality, we reported the stepwise phosphorylation procedure[9b] which involved temporary protection of one of two vicinal hydroxy groups by the laevulinyl function as substrate **24** and employment of phosphorus trichloride (Scheme 5). In order to avoid the use of such a protecting group, we envisaged that a monoalkoxide **26** might be functionally equivalent to the monolaevulinate **24** and react with one mol equivalent of a phosphorylating agent to give the corresponding monophosphate, such as **27**. We chose tetrabenzyl pyrophosphate **28**[22] since

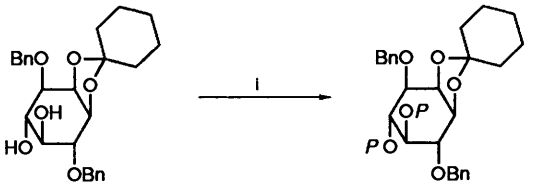


Scheme 5 Laevulinyl route and an alternative for phosphorylation of 1,2-diol. *Reagents:* i, PCl_3; ii, BnOH; iii, Bu^{t}OOH

this reagent is an easily handled and fairly stable crystalline compound. Thus, compound **6** was treated with equimolar amounts of butyllithium and the pyrophosphate **28** in tetrahydrofuran (THF) at 0 °C and the bis(phosphate) **29** (26% yield) was unexpectedly isolated, accompanied by half of the starting diol **6** (46% recovery). This result directed us to use two molar equivalents of both reagents. In fact, bis(phosphate) **29** was obtained in 81% yield when 2.1 molar equivalents of butyllithium and 2.5 molar equivalents of pyrophosphate **28** were used. Similar treatment of triol **11** with butyllithium (3.6 mol equiv.) and pyrophosphate **28** (3.7 mol equiv.) gave 1,4,5-tris(phosphate) **12b** in 72% yield (Scheme 1).

Phosphorylation by the activation of an alcohol has been well documented[23] and the method used here has already been employed by Bartlett[24] in the synthesis of shikimic acid monophosphate where lithium diisopropylamide (LDA) was used as a base instead of butyllithium. After we had submitted our paper concerning the present phosphorylation method,[25] similar procedures, except for the use of sodium hydride and potassium hydride as bases, were employed for the synthesis of

* Racemic triol **11** was first phosphorylated in 41% yield in a similar manner.

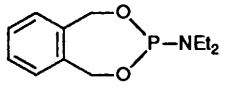


6 [P = $(BnO)_2P(O)$] 29

Scheme 6 Phosphorylation of vicinal diol by activation of the hydroxy group. *Reagents:* i, **28**, BuLi

inositol 1,3,4,5-tetrakis(phosphate)[26] and several inositol tris(phosphates.)[4c,27]

Utilization of a highly active phosphorus reagent instead of activation of the alcoholic component in the synthesis of inositol phosphates is useful, as has been demonstrated by a number of groups.[28] Among these methods, a phosphoramidite method is especially effective.[4a,29] We have also recently developed an efficient phosphorylation method which uses a new phosphitylating agent, *o*-xylylene *N,N*-diethylphosphoramidite (**30**, XEPA).[30] According to this method, a variety of inositol derivatives as well as other alcohols were smoothly phosphorylated to afford phosphoric esters in high yields. Thus, treatment of triol **11** with XEPA in the presence of tetrazole followed by addition of *m*-chloroperbenzoic acid (MCPBA) afforded tris(phosphate) **12c** in 98% yield (Scheme 1). 2,4,5-Triol **23** was similarly phosphorylated in 87% yield while dianilinophosphoric chloride **22** and pyrophosphate **28** gave the corresponding 2,4,5-triesters in 0 and 45% yield, respectively. Judging from these results, the efficacy of XEPA is now apparent.



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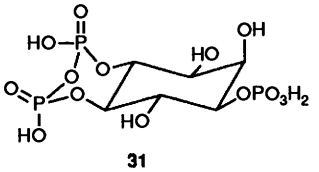

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Final Deprotection Step in the Synthesis of myo-*Inositol* 1,4,5-*Tris(dihydrogen phosphate)*.—The final stage of the synthesis of $InsP_3$ **1** is the removal of protecting groups from the fully protected tris(phosphates) **12**. The anilinophosphate derivative **12a** was deblocked by a two-step procedure. Firstly, the anilino groups in compound **12a** were removed by treatment with isoamyl (isopentyl) nitrite in a 1:1:1 mixture of acetic anhydride–acetic acid–pyridine,[31] then subsequent removal of two benzyl groups by hydrogenolysis on 5% Pd–C in aqueous methanol afforded the desired final product **1** in low yield (17% yield from **12a**) accompanied by the 4,5-cyclic pyrophosphate derivative **31** in a similar yield. Cellulose column chromatography effected separation of tris(phosphate) **1** from pyrophosphate **31**. The structure of compound **1** thus isolated as the ammonium salt was confirmed by ^1H, ^{13}C and ^{31}P NMR spectroscopy and mass spectrum analyses as well as by elemental analysis. In particular, 2D *J*-resolved NMR analysis gave useful information about coupling patterns which suggested the existence of $InsP_3$ in a typical chair form. The structure of pyrophosphate **31** was supported clearly by ^1H, ^{13}C and ^{31}P NMR analysis. Observation of resonance peaks at δ_P −9.02 and −8.65 with coupling constant $J_{P,P}$ 17.5 Hz in the ^{31}P NMR spectrum suggested the cyclic pyrophosphate structure.[32]

In the cases of the two other fully protected 1,4,5-tris(phosphates) **12b** and **12c**, removal of all protecting groups was quite easily accomplished by hydrogenolysis by using 5% Pd–C as catalyst. Simple filtration to remove the catalyst after completion of the reaction, and evaporation of the aqueous filtrate involving ammonia or pyridine, afforded essentially pure crystalline product as an ammonium or pyridinium salt quantitatively since by-products such as toluene and *o*-xylene were volatile. Thus, hydrogenolytic deprotection made isolation of highly polar $InsP_3$ easy. Further purification of the ammonium salt was accomplished by recrystallization from aqueous MeOH to afford analytically pure $InsP_3$ **1** as a crystalline solid in quantitative yield. The pyridinium salt was transformed into the corresponding sodium and potassium salts by passage through a cation-exchange column (Na^+- and K^+-forms). It should be noted that ammonium, sodium, and potassium salts of $InsP_3$ **1** crystallized from aq. MeOH possessed methanol of crystallization as observed in NMR analyses, and which would not be removed even by heating at ~90 °C *in vacuo*. In order to remove the methanol, evaporation of a solution of the crystals in water under reduced pressure was repeated (see Experimental section). Hydrogenolysis in the presence of ammonium acetate or formate (the latter is better because it can be removed during evaporation of the reaction solvents) might be useful for preventing the migration of phosphate functions by the formation of ammonium salts, although such experiments using compound **12b** as substrate on a small scale all gave the same results as did experiments in the absence of the salts. We can now prepare analytically and optically pure D-*myo*-inositol 1,4,5-tris(phosphate) ammonium, sodium and potassium salts as crystalline solids in quantity.

Physical, Spectral and Biological Properties of Compound **1**.—Elemental analysis of the ammonium salt of $InsP_3$ **1** showed that the number of ammonium ions was 3.5. That of the sodium and potassium cations in the corresponding salts was shown to be 3. All of these salts showed a pH of ~6.5 in aqueous solutions and optical rotations $[\alpha]_D$ ~ −10 in water. When the pH of the potassium salt of $InsP_3$ **1** in water was adjusted to 10.6 by addition of cyclohexylamine, the value of the rotation increased to −25.5, which was similar to those ($[\alpha]_D$ ~ −30) obtained at pH ~10 by other groups.[4j-l] Therefore we suggest that the optical rotation of $InsP_3$ **1** at higher pH shows a generally higher absolute numerical number than that at neutrality, while Ley *et al.* have recently reported $[\alpha]_D$ −24 at pH 6.9.[4p]

The three kinds of salts (NH_4, Na, K) of $InsP_3$ **1** showed essentially the same NMR spectra. Mayr and Dietrich[33] have reported a similar ^1H NMR spectrum for the ammonium salt of compound **1** which showed pD 6.03 in D_2O. Most research groups have prepared the hexaammonium and hexasodium salts of $InsP_3$ **1**, whose aqueous solutions showed pH ~9. Their fully dissociated samples showed partly different ^1H NMR spectra[4b,j,k] as was observed for the optical rotations. When the pD of an D_2O solution of our neutral sample was brought to ~9 with NaOD, the resulting solution gave identical spectra with those of an alkaline sample.[34]

Biological evaluation of synthetic $InsP_3$ **1** was carried out by using $InsP_3$ 5-phosphatase (erythrocyte ghosts prepared from human blood), $InsP_3$ 3-kinase (supernatant obtained by homogenizing rat whole brain), binding (rat cerebellum), and Ca^{2+} release (peritoneal macrophage from guinea pig) assays and its biological properties were found to be indistinguishable from those of the natural compound **1**.*

Experimental

General.—NMR spectra (^1H, ^{13}C and ^{31}P) were recorded on a JEOL JNM-GSX270, JEOL JNM-FX100S or JEOL JNM

* These biological experiments were accomplished by Dr. Masato Hirata of Kyushu University.

GX400 spectrometer. When $CDCl_3$ was used as the solvent, $SiMe_4$ (δ_H 0.0) for proton spectra and the signal of the solvent (δ_C 77.0) for carbon spectra were used as references. When D_2O was used as solvent, the signal of HOD (δ 4.64) was utilized for proton spectra as a standard and dioxane (δ_C 67.4) in a D_2O solution for carbon spectra as an external standard. In the case of ^{31}P NMR spectra, 85% H_3PO_4 (δ_P 0.0) was used as an external standard and positive chemical shifts are downfield from it. ^{13}C and ^{31}P NMR spectra were all taken under ^1H-decoupled conditions. *J*-values are given in Hz. IR spectra were recorded on a Hitachi EPI-G3 spectrophotometer. Optical rotations were measured on a Union PM-101 spectrometer, and the units of $[\alpha]_D$ are to be read as 10^{-1} deg cm^2 g^{-1}. Elemental analyses were performed on a Perkin-Elmer 240C instrument. pH was measured by a Horiba Compact pH Meter C-1. TLC analyses were performed on Merck pre-coated plates, Silica Gel 60 F254. Solvents used for chromatography are abbreviated as follows: EA = AcOEt, H = hexane. Flash chromatography was utilized for column chromatography by using Wako Pure Chemical Industries, silica gel, Wakogel C-300. Cellulose pre-coated TLC plates (SF-2020) were purchased from Funakoshi, and Whatman BioSystems microgranular cellulose powder, CC-31, was used for column chromatography. An anhydrous reaction atmosphere was achieved by nitrogen gas. Anhydrous solvents used here were prepared in the usual manner. Extracts obtained after work-up were dried over $MgSO_4$ or Na_2SO_4. Light petroleum refers to the fraction boiling in the range 30–70 °C.

1,2:4,5-*Di*-O-*cyclohexylidene*-myo-*inositol* **4a** (*Recycling Other Waste Cyclohexylidenated Products*).—The mother liquid which was obtained after crystallization of 1,2:4,5-di-*O*-cyclohexylidene-*myo*-inositol (19.4 g) produced by the reaction[5] of *myo*-inositol (71.5 g) with 1-ethoxycyclohexene (133 g) was subjected to evaporation and the residual mixture was further heated at ~65 °C under reduced pressure to remove volatile materials completely. The residue was dissolved in the solvent system of EtOH, benzene, and light petroleum (1:5:5) (400 cm^3) and the solution was stirred with PTSA·H_2O (1.2 g) at room temperature for 1 h. After neutralization of the solution with Bu^{t}OK (1.1 g), precipitated crystalline material was collected, and recrystallized from EtOH to give 1,2-*O*-cyclohexylidene-*myo*-inositol (56.3 g, m.p. 174–175 °C; lit.,[35] 179–180 °C). A DMF (220 cm^3) solution of this compound was treated with 1-ethoxycyclohexene (54.6 g) and PTSA·H_2O (0.65 g) at 95–100 °C for 1.5 h and the resulting mixture was worked up similarly to the procedure described in the literature[5] to afford title compound **4a** (12.4 g), m.p. 171–172 °C; (lit.,[5] 172–174 °C).

(±)-1,4-*Di*-O-*benzyl*-2,3-O-*cyclohexylidene*-myo-*inositol* **6**.—To a solution of 1,4-di-*O*-benzyl-2,3:5,6-di-*O*-cyclohexylidene-*myo*-inositol **5**[36] (1.0 g, 1.92 mmol) in $CHCl_3$ (35 cm^3) were added a $CHCl_3$ (16 cm^3) solution of ethylene glycol (122 mg, 1.96 mmol) and PTSA (18 mg) and the mixture was stirred at room temperature for 4 h. After addition of aq. K_2CO_3 for neutralization of the solution, the resultant organic layer was washed with water, dried, and concentrated under reduced pressure. The residue was recrystallized from benzene to afford compound **6** (678 mg, 80% yield), R_f [EA–H (1:1)] 0.4; m.p. 140–141 °C (lit.,[36] 147.6–148 °C).

(±)-4,5-*Di*-O-*allyl*-3,6-*di*-O-*benzyl*-1,2-O-*cyclohexylidene*-myo-*inositol* **7**.—To a solution of compound **6** (2.73 g, 6.20 mmol) in DMF (25 cm^3) was added a 50% mineral oil dispersion of NaH (684 mg, 14.25 mmol) and the mixture was cooled in an ice-bath. After addition of allyl bromide (1.65 g, 13.64 mmol), the resultant mixture was stirred at room temperature for 30 min and diluted with water. The solution was extracted with ethyl acetate (×3) and the extracts were washed with water, dried and concentrated under reduced pressure. The residue was chromatographed on silica gel and eluted with EA–H (1:6) to give compound **7** as an oil (3.23 g, quant), R_f[EA–H (1:3)] 0.6; ν_{max}(Nujol)/cm^{-1} 1060, 1030 and 920; δ_H(270 MHz; $CDCl_3$) 3.22 (1 H, dd, $J_{5,6}$ 10.0, $J_{5,4}$ 8.5), 3.59 (1 H, dd, $J_{3,4}$ 8.5, $J_{3,2}$ 3.8), 3.71 (1 H, dd, $J_{6,5}$ 10.0, $J_{6,1}$ 7.0), 3.75 (1 H, dd, $J_{4,3} = J_{4,5}$ = 8.5), 4.04 (1 H, dd, $J_{1,6}$ 7.0, $J_{1,2}$ 5.5), 4.23 (1 H, dd, $J_{2,1}$ 5.5, $J_{2,3}$ 3.8), 4.73 and 4.81 (2 H, ABq, *J* 12.5) and 4.76 and 4.87 (2 H, ABq, *J* 12.5); δ_C(67.8 MHz; $CDCl_3$) 72.92, 73.59, 73.84 (two unresolvable singlets, 2 C), 76.83, 78.46, 80.32, 81.58, 82.55, 110.14, 116.40, 116.42, 127.26, 127.52, 127.67, 127.69, 128.02, 128.15, 135.07, 135.11, 138.21 and 138.55.

(±)-5,6-*Di*-O-*allyl*-1,4-*di*-O-*benzyl*-myo-*inositol* **8**.—A solution of compound **7** (3.23 g, 6.20 mmol) in 80% aq. acetic acid (30 cm^3) was stirred and heated at 100 °C for 5 h. After evaporation of volatile materials under reduced pressure, the residue was chromatographed on silica gel and eluted with EA–H (1:1) to give compound **8** (2.41 g, 88%), R_f[EA–H (1:2)] 0.2; m.p. 104.5–106 °C (from benzene–hexane) (lit.,[16] 106–108 °C) (Found: C, 70.8; H, 7.35. Calc. for $C_{26}H_{32}O_6$: C, 70.89; H, 7.32%); ν_{max}(Nujol)/cm^{-1} 3395, 3300, 1135, 1090, 1060, 1000 and 915; δ_H(100 MHz; $CDCl_3$) 2.44 (2 H, br s), 3.30 (dd, $J_{1,2}$ 2.9, $J_{1,6}$ 9.1), 3.23 (1 H, dd, $J_{5,6} = J_{5,4}$ = 9.1), 3.38 (1 H, dd, $J_{3,2}$ 2.9, $J_{3,4}$ 9.1), 3.71 (1 H, dd, $J_{6,5} = J_{6,1}$ = 9.1), 3.74 (1 H, dd, $J_{4,3} = J_{4,5}$ = 9.1), 4.20 (1 H, dd, $J_{2,1} = J_{2,3}$ = 2.9), 4.20–4.30 (4 H, m), 4.67 (2 H, s), 4.69 and 4.94 (2 H, ABq, *J* 11.4), 5.02–5.42 (4 H, m), 5.70–6.18 (2 H, m) and 7.12–7.40 (10 H, m).

1D-4,5-*Di*-O-*allyl*-3,6-*di*-O-*benzyl*-1-l-*menthoxyacetyl*-myo-*inositol* **14a** *and* 1L-4,5-*Di*-O-*allyl*-3,6-*di*-O-*benzyl*-1-l-*menthoxyacetyl*-myo-*inositol* **14b**.—To a solution of racemic 1,2-diol **8** (4.66 g, 10.6 mmol) in pyridine (50 cm^3) at 0 °C was added dropwise *l*-menthoxyacetyl chloride (2.59 g, 11.1 mmol) and the reaction mixture was stirred at the temperature for 5 h. After addition of water (100 cm^3) the mixture was extracted with ethyl acetate (~50 cm^3 × 3) and the extracts were washed sequentially with water, saturated aq. $KHSO_4$, saturated aq. $NaHCO_3$, and water, and dried. After evaporation of the solvent, the residue was subjected to column chromatography on silica gel [diethyl ether–hexane (1:2)] to give diastereoisomers **14a** (2.64 g, 39%) and **14b** (2.92 g, 43%). When the residue was dissolved in hexane and cooled in an ice-bath, compound **14a** crystallized upon addition of a small amount of pure crystalline **14a** as seeds (m.p. 63–66 °C, ~2.0 g from 4.5 g of **8**). However, crystallization from the reaction mixture was not always successful. *Compound* **14a**: R_f[EA–H (3:2)] 0.52; m.p. 67.5–68.5 °C (from hexane) (Found: C, 71.9; H, 8.0. $C_{38}H_{52}O_8$ requires C, 71.67; H, 8.23%); $[\alpha]_D^{30}$ −54.1 (*c* 2.2, $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 3450 and 1750; δ_H(270 MHz; $CDCl_3$) 0.79 (3 H, d, *J* 6.7), 0.90 (6 H, d, *J* 6.7), 1.26 (2 H, m), 1.62 (2 H, m), 2.05 (1 H, m), 2.30 (1 H, m), 2.48 (1 H, br s), 3.13 (1 H, ddd, *J* 10.4, 10.4 and 3.9), 3.32 (1 H, dd, $J_{5,4} = J_{5,6}$ = 9.5), 3.42 (1 H, dd, $J_{3,4}$ 9.5, $J_{3,2}$ 2.7), 3.73 (1 H, dd, $J_{4,3} = J_{4,5}$ = 9.5), 3.94 and 4.18 (2 H, ABq, *J* 16.7), 3.95 (1 H, dd, $J_{6,1} = J_{6,5}$ = 9.5), 4.23 (1 H, dd, $J_{2,1} = J_{2,3}$ = 2.7), 4.32 (4 H, m), 4.63 and 4.69 (2 H, ABq, *J* 11.3), 4.65 and 4.83 (2 H, ABq, *J* 11.3), 4.84 (1 H, dd, $J_{1,6}$ 9.5, $J_{1,2}$ 2.7), ~5.21 (4 H, complex) and 5.98 (2 H, m); δ_C(67.8 MHz; $CDCl_3$) 16.23, 20.92, 22.22, 23.18, 25.38, 31.31, 34.28, 39.69, 48.04, 65.19, 67.89, 72.91, 73.16, 74.52, 75.47, 78.79, 79.43, 79.76, 80.59, 82.52, 116.62, 116.74, 127.41, 127.49, 127.77, 127.95, 128.25, 128.45, 134.99, 135.10, 137.48, 138.52 and 170.22.

Diastereoisomer **14b**: R_f[EA–H (3:2)] 0.61; $[\alpha]_D^{30}$ −14.7 (*c* 1.9, $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 3450 and 1750; δ_H(100 MHz; $CDCl_3$) 3.20 (1 H, dd, $J_{5,4} = J_{5,6}$ = 8.3), 3.32 (1 H, dd, $J_{3,2}$ 2.5, $J_{3,4}$ 8.3), 3.62 (1 H, dd, $J_{4,3} = J_{4,5}$ = 8.3), 3.83 (1 H, dd, $J_{6,1} = J_{6,5}$ = 8.3), 3.83 and 4.01 (2 H, ABq, *J* 15.8), 4.02–4.24 (H_2 and

4 H, m), 4.35–4.79 (4 H, m), 4.69 (1 H, dd, $J_{1,2}$ 2.5, $J_{1,6}$ 8.3), 4.88–5.24 (4 H, m) and 5.54–5.98 (2 H, m).

1L-5,6-*Di*-O-*allyl*-1,4-*di*-O-*benzyl*-myo-*inositol* 1L-**8**.—To a solution of compound **14a** (5.92 g, 9.3 mmol) in methanol (50 cm^3) was added 2.5 mol dm^{-3} NaOH (4.46 cm^3) and the mixture was stirred at room temperature overnight. After addition of water (100 cm^3), the solution was extracted with ethyl acetate (70 cm^3 × 3) and the extracts were washed successively with aq. $NaHCO_3$ (30 cm^3), water (50 cm^3), and brine (50 cm^3), and dried. After evaporation of the solvent, the residue was recrystallized from benzene–hexane to give the title compound 1L-**8** (4.01 g, 98%), R_f[EA–H (1:6)] 0.2; m.p. 105–106 °C (from benzene–hexane); $[\alpha]_D^{16}$ −14.3 (*c* 1.3, $CHCl_3$). The same IR and ^1H NMR spectra were obtained as from racemic **8**.

1D-5,6-*Di*-O-*allyl*-1,4-*di*-O-*benzyl*-myo-*inositol* 1D-**8**.—The title compound (1.85 g, 99%) was similarly obtained by treatment of compound **14b** (2.72 g, 4.27 mmol) in methanol (30 cm^3) with 2.5 mol dm^{-3} NaOH (2.05 cm^3) at room temperature for 5 h, m.p. 105–106 °C (from benzene–hexane); $[\alpha]_D^{16}$ +14.2 (*c* 1.3, $CHCl_3$). The same IR and ^1H NMR spectra were obtained as for racemic **8**.

Elucidation of the Absolute Configuration of 1L-**8**.—1,2-Diol 1L-**8** was first transformed into 1L-1,4-di-*O*-benzyl-2,3-*O*-cyclohexylidene-*myo*-inositol 1L-**6** *via* optically active dibenzyl ether **15** and the subsequent 1,2:4,5-di-*O*-cyclohexylidene derivative 1D-**5** which were briefly characterized by ^1H NMR spectroscopy. A mixture of compound 1D-**5** (182 mg, 0.35 mmol), ethylene glycol (22 mg) and PTSA·H_2O (3.3 mg) in anhydrous $CHCl_3$ (obtained by passage through basic Al_2O_3: Merck No. 1076) was stirred at room temperature for 4 h and K_2CO_3 was added for neutralization. The organic phase was washed with water, and silica gel column chromatography and then recrystallization from anhydrous ethanol gave 1L-**6** (117 mg, 76%), m.p. 119 °C; $[\alpha]_D^{20}$ +33.0 (*c* 0.5, $CHCl_3$) {lit. (for the opposite enantiomer),* m.p. 142–143 °C; $[\alpha]_D^{20}$ −37.0, for sign of the rotation see footnote*} (Found: C, 70.7; H, 7.4. Calc. for $C_{26}H_{32}O_6$: C, 70.89; H, 7.32%). The ^1H NMR spectrum (100 MHz) was superposable on that of racemic **6**. To a solution of 1L-**6** (115 mg, 0.26 mmol) in DMF (5 cm^3) was added a 50% mineral oil dispersion of NaH (28 mg, 0.57 mmol) and benzyl chloride (69 mg, 0.55 mmol), and the mixture was stirred at room temperature for 2 h. After careful addition of water, extraction with diethyl ether was carried out and the ethereal solution was washed successively with water and brine, dried, and evaporated under reduced pressure.

The residual product **16** was dissolved in 80% aq. AcOH and the solution was heated at 90–100 °C for 2 h. Volatile materials were distilled off under reduced pressure and silica gel column chromatography [EA–H (1:1)] and subsequent recrystallization from methanol gave 1L-4,5,6-tetra-*O*-benzyl-*myo*-inositol **17** (87 mg, 60%), m.p. 139–140 °C; $[\alpha]_D^{20}$ −22.3 (*c* 1.3, $CHCl_3$) {lit.,[8a] m.p. 141–143 °C; $[\alpha]_D^{20}$ −24.3 (*c*. 1.3, $CHCl_3$); for other reported m.p. and optical rotation data for **17** see reference 8*b*} (Found: C, 75.3; H, 6.7. Calc. for $C_{34}H_{36}O_6$: C, 75.53; H, 6.71%).

1D-1,4,5-*tri*-O-*allyl*-3,6-*di*-O-*benzyl*-myo-*inositol* **9**.—A mixture of 1L-**8** (721 mg, 1.64 mmol), allyl bromide (297 mg, 2.46 mmol), powdered NaOH (883 mg, 13.5 mol equiv.), and anhydrous benzene (16 cm^3) was heated under reflux for 80 min. After being cooled, the reaction mixture was washed with water (× 2), dried, and evaporated. Column chromatography of the residue [SiO_2; EA–H (1:4)] yielded triallyl derivative **9** as an oil (612 mg, 78%), R_f [EA–H (1:3)] 0.3; $[\alpha]_D^{30}$ +3.1 (*c* 1.6, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3450, 1060, 990 and 920; δ_H(100 MHz; $CDCl_3$) 2.42 (1 H, br s), 3.19 (1 H, dd, $J_{3,2}$ 2.3, $J_{3,4}$ 9.1), 3.20 (1 H, dd, $J_{5,4} = J_{5,6}$ = 9.1), 3.26 (1 H, dd, $J_{1,2}$ 2.3, $J_{1,6}$ 9.1), 3.76 (1 H, dd, $J_{4,5} = J_{4,3}$ = 9.1) and 3.82 (1 H, dd, $J_{6,5} = J_{6,1}$ = 9.1).

1D-1,4,5-*Tri*-O-*allyl*-2,3,6-*tri*-O-*benzyl*-myo-*inositol* **10**.—A mixture of compound **9** (1.57 g, 3.26 mmol), a 50% mineral oil dispersion of NaH (188 mg, 3.92 mmol), and anhydrous DMF (32 cm^3) was stirred for 10 min and benzyl chloride (454 mg, 3.58 mmol) was added. After the mixture had been stirred at room temperature for 1 h, water was added and the mixture was extracted with ethyl acetate (× 2). The extracts were washed with water, dried, and evaporated. The residue was chromatographed on silica gel [EA–H (1:7)] to afford compound **10** as an oil (1.85 g, 99%), R_f [EA–H (1:7)] 0.5; $[\alpha]_D^{30}$–2.5 (*c* 1.6, $CHCl_3$); ν_{max}(neat)/cm^{-1} 1065, 985 and 920; δ_H(270 MHz; $CDCl_3$) 3.19 (1 H, dd, $J_{3,4}$ 9.5, $J_{3,2}$ 2.4), 3.25 (1 H, dd, $J_{5,4} = J_{5,6}$ = 9.5), 3.26 (1 H, dd, $J_{1,6}$ 9.5, $J_{1,2}$ 2.4), 3.87 (1 H, dd, $J_{6,1} = J_{6,5}$ = 9.5), 3.93 (1 H, dd, $J_{4,3} = J_{4,5}$ = 9.5), 3.97 (1 H, dd, $J_{2,1} = J_{2,3}$ = 2.4), 4.07 (2 H, m), 4.34 (4 H, m), 4.57 and 4.67 (2 H, ABq, *J* 11.9), 4.77 and 4.85 (2 H, ABq, *J* 10.3) and 4.85 (2 H, s); δ_C(67.8 MHz; $CDCl_3$) 71.56, 72.78, 73.89, 74.33, 74.47, 74.53, 75.79, 80.41, 80.55, 81.29, 81.47, 83.18, 116.41, 116.47, 116.52, 127.21, 127.39, 127.47, 127.50, 127.54, 127.57, 127.61, 127.73, 128.01, 128.05, 128.10, 128.13, 128.21, 128.25, 128.27, 128.39, 128.90, 129.66, 134.86, 135.36, 135.41, 138.47, 138.84 and 138.89.

1L-1,2,4-*Tri*-O-*benzyl*-myo-*inositol* **11**.—1D-1,4,5-Tri-*O*-allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol **10** (102 mg, 0.179 mmol), tris-(triphenylphosphine)rhodium(I) chloride (34 mg, 0.037 mmol), 1,4-diazabicyclo[2.2.2]octane (DABCO) (12.4 mg, 0.111 mmol), and 90% aq. ethanol (2 cm^3) were heated together under reflux for 8 h and the mixture was diluted with water. The solution was extracted twice with ethyl acetate and the combined extracts were washed with water, dried, and then concentrated to dryness under reduced pressure. The residue was heated under reflux for 20 min with 0.1 mol dm^{-3} methanolic hydrogen chloride (2 cm^3) which was prepared from acetyl chloride and anhydrous methanol. After saturated aq. $NaHCO_3$ had been added to neutralize the reaction mixture, the resultant solution was extracted twice with ethyl acetate and the extracts were washed with water, dried, and then condensed under reduced pressure. The residue was chromatographed on silca gel [EA–H (1:1)] to give the title compound **11** (48 mg, 59%, R_f [EA–H (1:1)] 0.2; m.p. 117–119 °C (from EtOH); $[\alpha]_D^{16}$ +15.5 (*c* 1, $CHCl_3$) {lit.,[17] m.p. 117–119 °C; $[\alpha]_D$ +12.4; lit. (for the opposite enantiomer),[11] m.p. 118–120 °C; $[\alpha]_D^{25}$ −9.0} (Found: C, 72.15; H, 6.80. Calc. for $C_{27}H_{30}O_6$: C, 71.98; H, 6.71%); ν_{max} (Nujol)/cm^{-1} 3460 and 3350; δ_H(270 MHz; $CDCl_3$) 2.37 (1 H, br d, *J* 6.1), 2.75 (2 H, br s), 3.27 (1 H, dd, $J_{1,6}$ 9.8, $J_{1,2}$ 2.4), 3.45 (1 H, dd, $J_5 = J_{5,6}$ = 9.2), 3.50 (1 H, m), 3.67 (1 H, dd, $J_{4,3} = J_{4,5}$ = 9.2), 4.00 (1 H, dd, $J_{6,1} = J_{6,5}$ = 9.2), 4.06 (1 H, dd, $J_{2,1} = J_{2,3}$ = 2.4), 4.56 and 4.67 (2 H, ABq, *J* 11.8), 4.70 and 4.88 (2 H, ABq, *J* 11.9) and 4.82 and 4.92 (2 H, ABq, *J* 11.5); δ_C (67.8 MHz; $CDCl_3$–D_2O) 72.32, 72.39, 72.45, 74.64, 74.70, 75.01, 76.28, 80.14, 81.60, 127.72, 127.80 (2 C), 127.85, 128.00, 128.06, 128.39, 128.53, 128.56, 137.62, 138.44 and 138.51.

(±)-1,4,5-*Tri*-O-*acetyl*-2,3,6-*tri*-O-*benzyl*-myo-*inositol*.—The usual acetylation procedure using racemic triol **11** (23.6 mg, 0.052 mmol) and an excess of acetic anhydride in pyridine gave the title triacetate (27.5 mg, 91%; R_f[EA–H (1:3)] 0.7; m.p. 116.5–117 °C (from EtOH) (lit.,[37] 123–125 °C) (Found: C, 68.35; H, 6.3. Calc. for $C_{33}H_{36}O_9$: C, 68.72; H, 6.29%); ν_{max}(Nujol)/cm^{-1} 1735, 1100, 1040 and 1020; δ_H(100 MHz; $CDCl_3$) 1.91 (6 H, s), 1.98 (3 H, s), 3.54 (1 H, dd, $H_{3,2}$ 2.8, $J_{3,4}$ 10.5), 4.11 (1 H, dd, $J_{2,1} = J_{2,3}$ = 2.8), 4.11 (1 H, dd, $J_{6,1} = J_{6,5}$

* See footnote on page 730.

10.5), 4.46 and 4.85 (2 H, ABq, J 12), 4.60 and 4.64 (2 H, ABq, J 4), 4.74 (1 H, dd, $J_{1,2}$ 2.8, $J_{1,6}$ 10.5), 5.04 (1 H, dd, $J_{5,4} = J_{5,6} =$ 10.5) and 5.53 (1 H, dd, $J_{4,3} = J_{4,5} =$ 10.5).

1L-1,2,4-*Tri*-O-*benzyl*-3,5,6-*tris*-O-*dianilinophosphoryl*-myo-*inositol* **12a**.—To a solution of triol 1L-**11** (855 mg, 1.90 mmol) in pyridine (6.0 cm^3) at −10 °C was added a solution of dianilinophosphoric chloride (7.08 g, 26.6 mmol) in pyridine (26 cm^3) and a catalytic amount of 4-(dimethylamino)pyridine and the resulting solution was stirred at room temperature for 2 days. After addition of 5% aq. potassium acetate (48 cm^3), the resultant solution was stirred at room temperature for 30 min and extracted with chloroform (× 2). The extracts were washed successively with 20% aq. $KHSO_4$ and water, dried, and concentrated to dryness. The residue was subjected to silica gel column chromatography with a 10:1 mixture of methylene dichloride and acetone to give fractions containing the desired product {R_f[$CHCl_3$–MeOH (30:1)] 0.25} and an impurity (R_f 0.20). The combined eluates were again chromatographed on silica gel [$CHCl_3$–MeOH (30:1)] to give almost pure compound **12a** (1.30 g, ~60%), which was recrystallized from ethanol–hexane to afford crystals with m.p. 225–227 °C. This product was again recrystallized from benzene to give the product of m.p. 227–229 °C which still involved a trace of impurity, ν_{max}(Nujol)/cm^{-1} 3400, 3125, 1210 and 1030; δ_P(40.5 MHz; C_5D_5N; Instrument: JEOL PS-100) 1.816 (1 P) and 2.930 (2 P); δ_H(100 MHz; $CDCl_3$) complex; m/z (FD. Instrument: JEOL JMS-D300) 1141 ($[M + H]^+$).

1L-1,2,4-*Tri*-O-*benzyl*-3,5,6-*tris*-O-*dibenzyloxyphosphoryl*-myo-*inositol* **12b**.—Butyllithium (1.57 Mol dm^{-3} in hexane; 5.8 cm^3, 9.11 mmol) and tetrabenzyl pyrophosphate **28** (5.11 g, 9.49 mmol) were successively added to a solution of 1L-1,2,4-tri-*O*-benzyl-*myo*-inositol (1.14 g, 2.53 mmol) in THF (30 cm^3) at −78 °C and the resulting solution was cooled to 0 °C by an ice–salt–water-bath and stirred for 1 h. Precipitated lithium dibenzyl phosphate was filtered off, and washed with diethyl ether, and the combined filtrate and washings were washed with water, dried and evaporated to dryness. The residue was subjected to flash column chromatography to afford the *title compound* (2.30 g, 72%), recrystallization of which (H–EA) gave analytically pure crystals, m.p. 109–110 °C (Found: C, 67.05; H, 5.8. $C_{69}H_{69}O_{15}P_3$ requires C, 67.31; H, 5.65%); $[\alpha]_D^{28}$ −5.3 (*c* 1.7, $CHCl_3$); ν_{max}(Nujol)/cm^{-1} 1260 and 1000; δ_H(270 MHz; $CDCl_3$) 3.45 (1 H, dd, $J_{1,6}$ 10.1, $J_{1,2}$ 2.1), 4.10 (1 H, dd, $J_{4,3} = J_{4,5}$ = 9.5), 4.25 (1 H, ddd, $J_{3,4}$ 9.5, $J_{3,P3}$ 7.5, $J_{3,2}$ 2.1), 4.33 (1 H, dd, $J_{2,1} = J_{2,3}$ = 2.1), 4.53 (1 H, ddd, $J_{5,4} = J_{5,6} = J_{5,P5}$ = 9.5), 4.40–5.10 (19 H, complex, benzylic protons and 4 H) and 6.80–7.35 (45 H, m); δ_C(67.8 MHz; $CDCl_3$) 69.10 (d. J 4.9), 69.16 (d, J 4.9), 69.31 (d, J 5.5), 69.40 (d, J 6.1), 69.44 (d. J 4.9), 69.49 (d, J 6.1), 72.25, 74.56, 75.09, 75.22, 77.21, 77.75, 77.88, 77.90 (m), 77.97, 79.00 (m), 127.14, 127.57, 127.66, 127.74, 127.79, 127.82, 127.84, 128.03, 128.05, 128.07, 128.24, 128.27, 128.33, 128.51, 128.54, 135.47 (d, J 7.3), 135.50 (d, J 6.7), 135.76 (d, J 6.7), 136.06 (d, J 8.0), 136.07 (d, J 7.3), 136.09 (d, J 7.3), 137.30, 138.18 and 138.20; δ_P(109 MHz; $CDCl_3$) −1.32, −1.08 and −0.93.

1L-1,2,4-*Tri*-O-*benzyl*-3,5,6-*tris*-O-(o-*xylylenedioxyphosphoryl*)-myo-*inositol* **12c**.—To a suspension of 1L-1,2,4-tri-*O*-benzyl-*myo*-inositol **11** (0.85 g, 1.89 mmol) and 1*H*-tetrazole (0.94 g, 13.45 mmol) in methylene dichloride (19 cm^3) was added *o*-xylylene *N,N*-diethylphosphoramidite **30** (2.16 g, 9.01 mmol) at room temperature and the resulting clear solution was stirred for 10 min. Water (0.65 cm^3) was added and the mixture was stirred for an additional 10 min to destroy an excess of the amidite, the mixture was then cooled to −40 °C. MCPBA (2.83 g, 16.37 mmol) was added to the solution and the cooling bath was removed. After being stirred for 10 min, the solution was diluted with ethyl acetate and the organic layer was washed successively with 10% aq. Na_2SO_3, saturated aq. $NaHCO_3$, water, and brine, and then dried. The reaction mixture was chromatographed on silica gel [Et_2O–$CHCl_3$ (1:5), R_f 0.31] to give *tris*(*phosphate*) **12c** (1.83 g, 97%), m.p. 101–103 °C (from EA–H) (Found: C, 61.1; H, 5.4; P, 9.55. $C_{51}H_{51}O_{15}P_3$ requires C, 61.45; H, 5.16; P, 9.32%); $[\alpha]_D^{29}$ −7.6 (*c* 1.05, $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 1200 and 1010; δ_H(270 MHz; $CDCl_3$) 3.56 (1 H, dd, $J_{1,6}$ 9.8), 4.21 (1 H, dd, $J_{4,5} = J_{4,3}$ = 9.8), 4.40 (1 H, ddd, $J_{3,4}$ 9.8, $J_{3,P3}$ 7.6, $J_{3,2}$ 2.1), 4.60 (1 H, dd, $J_{2,1} = J_{2,3}$ = 2.1), 4.63–5.23 (18 H, complex, benzylic protons and 5 H), 5.25 (1 H, ddd, $J_{6,1} = J_{6,5} = J_{6,P6}$ = 9.8), 5.41–5.57 (2 H, m) and 7.06–7.44 (27 H, complex); δ_C(67.8 MHz; $CDCl_3$) 68.00 (d, J 6.7), 68.10 (d, J 6.7), 68.47 (d, J 6.7), 68.75 (2 C, d, J 7.9), 68.86 (d, J 6.7), 72.30, 74.75, 75.33, 75.47, 77.32 (m), 78.00 (2 C), 78.05 (m), 79.50 (m), 127.31, 127.44, 127.57, 127.69, 128.04, 128.10, 128.18, 128.22, 128.29, 128.44, 128.56, 128.59, 128.71, 128.81, 128.84, 128.98, 129.11, 134.46, 134.84, 135.12, 135.14, 135.18, 137.30, 137.64 and 138.04; δ_P(109 MHz; $CHCl_3$) −1.28, −0.30 and 0.48.

1D-myo-*Inositol* 1,4,5-*tris*(*dihydrogen phosphate*) **1**.—(a) *Ammonium salt of* **1** *from phosphoranilide* **12a**. To a solution of phosphoranilide **12a** (526 mg, 0.461 mmol) in a 1:1:1 mixture of acetic anhydride–acetic acid–pyridine (10 cm^3) was added isoamyl nitrite (2.8 cm^3, 20.74 mmol) and the mixture was stirred at room temperature for two days. After water had been added, volatile materials were removed under reduced pressure at below 25 °C. A triethylammonium carbonate buffer solution of the residue was passed through a plug of DEAE-Sephadex A-25 (Pharmacia) and the column was washed with the same buffer solution. The combined eluates were concentrated to dryness under reduced pressure. The residue (318 mg) was shown to be impure on TLC (cellulose) but without further purification a solution of the mixture in methanol–water (4:1; 15 cm^3) were stirred with 5% Pd–C (1.0 g) under hydrogen at room temperature overnight. After filtration to remove the catalyst, the filtrate was concentrated to dryness and the residue was subjected to column chromatography with application of pressure (cellulose). First, a solvent system of 7:2:1 propan-1-ol–28% NH_4OH–water was used as eluent and the 4,5-cyclic pyrophosphate **31**, with the higher-value R_f[0.43; cellulose; 28% NH_4OH propan-1-ol–water (5.5:5:1)], was eluted (43.8 mg, ~20%). After that, the proportions of the solvents were changed to 5:4:1 and D-*myo*-inositol 1,4,5-tris (dihydrogen phosphate) ammonium salt was obtained (36.5 mg, 17% yield from **12a**), R_f[cellulose; 28% NH_4OH–propan-1-ol–water 5.5:5:1)] 0.21.

1 (*ammonium salt*): (Found: C, 13.7; H, 5.7; N, 9.3. $C_6H_{15}O_{15}P_3$ $(NH_3)_{3.5}(H_2O)_{2.5}$ requires C, 13.73; H, 5.86; N, 9.34%); $[\alpha]_D^{23}$ −10.3 (*c* 1.80, water, pH 6.73); δ_H[400 MHz; 12 mg in D_2O (0.4 cm^3); pD ~6] 3.55 (1 H, dd, $J_{3,4}$ 8.7, $J_{3,2}$ 3.1), 3.76 (1 H, dd, $J_{6,1} = J_{6,5}$ = 8.7), 3.83–3.89 (2 H, complex), 4.08–4.15 (2 H, complex). The latter complex regions which contained 1-, 2-, 4- and 5-H were disclosed mainly by J-resolved 2D NMR experiments as follows: δ 3.85 (1 H, ddd, $J_{5,4} = J_{5,6} = J_{5,P5}$ = 8.7), 3.85 (1 H, ddd, $J_{1,6} = J_{1,P1}$ = 8.7, $J_{1,2}$ 3.1), 4.10 (1 H, ddd, $J_{4,3} = J_{4,5} = J_{4,P4}$ = 8.7) and 4.12 (1 H, dd, $J_{2,1} = J_{2,3}$ = 3.1); δ_C([100.4 MHz; 12 mg in D_2O 10.4 cm^3); pD ~6] 71.22 (C-3), 71.29 (C-2), 71.86 (m, C-6), 75.64 (d, J 5.3, C-1), 77.11 (m, C-4) and 78.75 (m, C-5); δ_P[109 MHz; 12 mg in D_2O (2.5 cm^3); pD 6.73] 1.28, 2.30 and 3.23; δ_P[109 MHz, 12 mg in D_2O (2.5 cm^3); pD 6.73] 1.28, 2.30 and 3.23; δ_P[109 MHz, 12 mg in D_2O (2.5 cm^3); pD 11.82, NH_4OH added] 3.64. 5.41 and 5.53.

Compound **31** δ_H[400 MHz, 18 mg in D_2O (0.4 cm^3)] 3.80 (1 H, dd, $J_{3,4}$ 9.9, $J_{3,2}$ 2.9), 3.95 (1 H, dd, $J_{6,1} = J_{6,5}$ = 9.9), 4.05 (1 H, ddd, $J_{1,6} = J_{1,P1}$ = 9.9, $J_{1,2}$ 2.9), 4.14 (1 H, ddd, $J_{5,4} = J_{5,6}$ = 9.9, $J_{5,P5}$ 6.2), 4.32 (1 H, ddd, $J_{2,1} = J_{2,3}$ = 2.9, $J_{2,P1}$ 1.1) and 4.42 (1 H, ddd, $J_{4,3} = J_{4,5}$ = 9.7, $J_{4,P4}$ 5.9); δ_C[100.4

MHz; 18 mg in D_2O (0.4 cm^3)] 69.57 (d, *J* 7.6), 70.61 (t, *J* 6.5), 71.48, 75.38 (m), 78.74 (d, *J* 6.9) and 79.87 (d, *J* 6.9); δ_P([161.7 MHz, 18 mg in D_2O (~1.5 cm^3); pD 6.73] −9.02 (d, $J_{P4,P5}$ 17.5), −8.65 (d, $J_{P4,P5}$ 17.5) and 2.52.

(b) *Sodium salt of* **1** *from dibenzyl phosphate derivative* **12b**. A mixture of 1L-1,2,4-tri-*O*-benzyl-3,5,6-tris-*O*-(dibenzyloxyphosphoryl)-*myo*-inositol **12b** (1.96 g, 1.48 mmol), ammonium acetate (1.0 g), and 5% Pd–C (2.0 g) in MeOH–water (4:1, 50 cm^3) was stirred under hydrogen for 16 h and the catalyst was filtered off and washed with water. The combined aq. solution was evaporated below 40 °C and evaporation of aq. solutions of the residue were repeated in order to remove some of the ammonium acetate and then subjected to column chromatography on cellulose (80 g) by using, firstly, a solvent system of propan-1-ol–28% NH_4OH–water (7:2:1) (When ammonium formate was used this procedure was not necessary, because it was removed during evaporation of the filtrate) and, after elution of remaining ammonium acetate, propan-1-ol–28% NH_4OH–water (5:4:1). The eluted fractions containing $InsP_3$ **1** were concentrated to dryness under reduced pressure and the residue was passed successively through cation-exchange (Dowex-50) columns of H^+-, pyridinium-, and then Na^+- form. The sodium salt of compound **1** thus obtained was finally dried by application of heat (below 40 °C) under reduced pressure (0.1 mmHg) to afford *crystalline* **1** *sodium salt* quantitatively, m.p. >270 °C (Found: C, 13.9; H, 3.3. $C_6H_{12}O_{15}P_3Na_3(H_2O)_{1.5}$ requires C, 14.05; H, 2.95%); $[\alpha]_D^{23}$ −11.1 (*c* 0.9, water, pH 6.9); δ_H [270 MHz, 12.6 mg in D_2O (0.56 cm^3); pD 6.8] 3.66 (1 H, dd, $J_{3,4}$ 9.3, $J_{3,2}$ 2.7), 3.85 (1 H, dd, $J_{6,1} = J_{6,5}$ = 9.3), 3.97 (1 H, ddd, $J_{1,6} = J_{1,P1}$ = 9.3, $J_{1,2}$ unreadable), 3.99 (1 H, ddd, $J_{5,4} = J_{5,6} = J_{5,P5}$ 9.3), 4.22 (1 H, dd, $J_{2,1} = J_{2,3}$ = 2.7) and 4.25 (1 H, ddd, $J_{4,3} = J_{4,5} = J_{4,P4}$ 9.3); δ_C[67.8 MHz, 12.6 mg in D_2O (0.56 cm^3); pD 6.8] 70.81 (C-3), 71.15 (C-2), 71.44 (m, C-6), 75.63 (d. *J* 6.1, C-1), 77.35 (m, C-4) and 78.73 (m, C-5).

(c) *Potassium salt of* **1** *from* o-*xylylene phosphate derivative* **12c**. 1,4,5-Tris(*o*-xylylenedioxyphosphoryl)-*myo*-inositol **12c** (1.91 g, 1.918 mmol), 5% Pd–C (2.1 g), and 80% aq. methanol (100 cm^3) were stirred together for 24 h at room temperature under hydrogen. After filtration of the catalyst and addition of pyridine to the filtrate, the aq. solution was concentrated to a small volume which was then subjected to cation-exchange chromatography of Dowex-50 (K^+-form) and elution with water. The resulting eluate and washings were concentrated to dryness by using, firstly, a rotary evaporator under reduced pressure (~15 mmHg) below 40 °C and then at vacuum-pump pressure (~0.1 mmHg) at the same temperature to give **1** *tri* (*potassium*) *salt* hemihydrate (1.02 g, quantitative yield) as a crystalline solid [Found: C, 13.4; H, 2.85. $C_6H_{12}O_{15}P_3K_3(H_2O)_{0.5}$ requires C, 13.26; H, 2.41%]; $[\alpha]_D^{23}$ −9.4 (*c* 1.27, water; pH 6.9); $[\alpha]_D^{23}$ −25.2 (*c* 1.03, water; pH 10.6 adjusted by addition of cyclohexylamine); δ_H([270 MHz, 10 mg in (0.7 cm^3); pD 6.1] 3.60 (1 H, dd, $J_{3,4}$ 9.4, $J_{3,2}$ 2.7), 3.78 (1 H, dd, $J_{6,1} = J_{6,5}$ = 9.4), 3.89 (1 H, complex), 3.91 (1 H, ddd, $J_{5,4} = J_{5,6} = J_{5,P5}$ = 9.4), 4.14 (1 H, dd, $J_{2,1} = J_{2,3}$ = 2.7) and 4.16 (1 H, ddd, $J_{4,3} = J_{4,5} = J_{4,P4}$ = 9.4).

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