The Total Synthesis of *myo*-Inositol Phosphates *via* *myo*-Inositol Orthoformate

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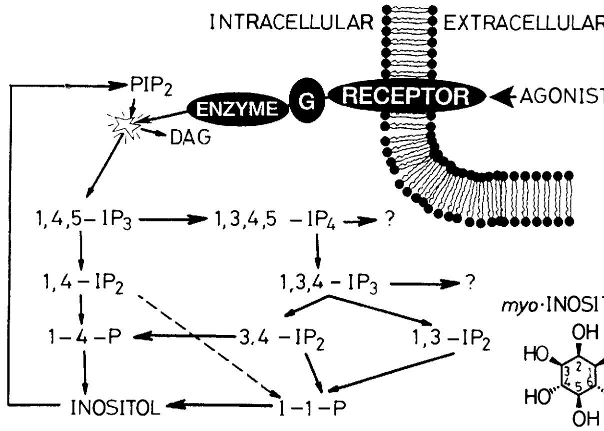
Novel selective alkylations of *myo*-inositol orthoformate (**4**) have been used to prepare a series of protected *myo*-inositol derivatives, (**5a—e**), (**7**), (**10**), (**12**), and (**16**). These intermediates have been used in efficient total syntheses of *myo*-inositol 2-phosphate, (**9**); *myo*-inositol 4-phosphate, (**6**); *myo*-inositol 1,3-bisphosphate, (**18**); and *myo*-inositol 1,3,4,5-tetrakisphosphate (**14**). This report represents the first total synthesis of the important natural metabolites (**14**) and (**18**) and significantly improved methods of preparation of (**6**) and (**9**).

The receptor controlled hydrolysis of phosphatidylinositol † 4,5-bisphosphate (PIP_2), leading to the formation of inositol 1,4,5-trisphosphate (1,4,5-IP_3) and diacylglycerol (DAG), is now firmly established as a fundamental mechanism for cellular signal transduction.[1-5] A large number of neurotransmitters and hormones use this transduction/amplification mechanism to evoke responses in target cells. Both 1,4,5-IP_3 and DAG act as second messengers in the target cell, the former binding to specific receptors on the endoplasmic reticulum and releasing calcium from intracellular stores, and the latter binding to protein kinase C. 1,4,5-IP_3 is metabolised by a series of specific dephosphorylations (see Figure) *via* inositol 1,4-bisphosphate (1,4-IP_2) and inositol 4-phosphate (I-4-P)[6] to inositol, which is used for the resynthesis of the inositol phospholipids. Evidence has recently been obtained for a second metabolic route from 1,4,5-IP_3 to inositol, *via* phosphorylation to inositol 1,3,4,5-tetrakisphosphate (1,3,4,5-IP_4) and subsequent dephosphorylation *via* inositol 1,3,4-trisphosphate (1,3,4-IP_3) and inositol 1,3- or 3,4-bisphosphates (1,3-IP_2 and 3,4-IP_2) to inositol.[7] The tetrakisphosphate 1,3,4,5-IP_4 has also been shown to have an important role in regulating the influx of calcium into stimulated cells from the extracellular fluid.[8]

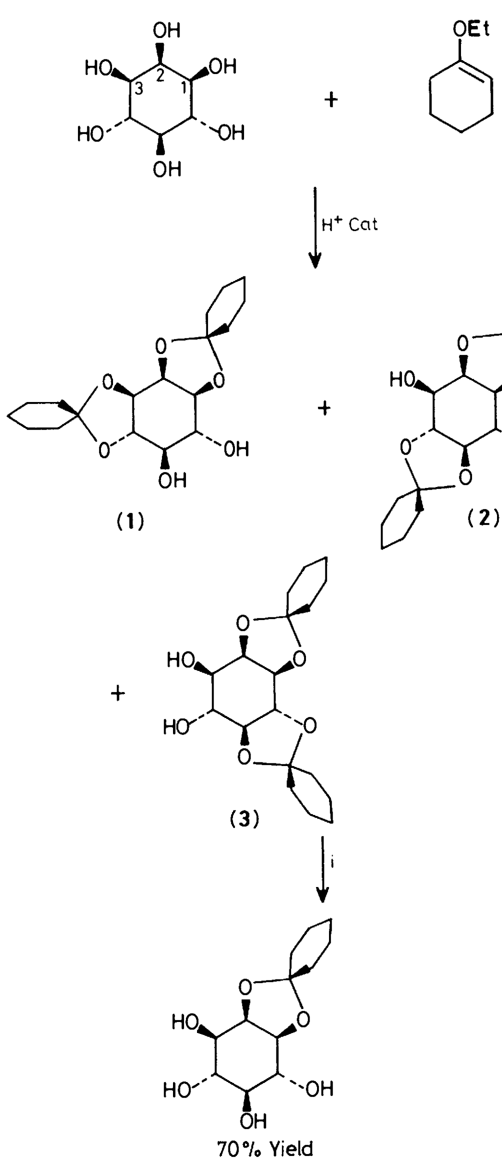


PIP_2 = Phosphatidylinositol 4,5-bisphosphate
DAG = Diacylglycerol
1,3,4,5-IP_4 = inositol 1,3,4,5-tetrakisphosphate
1,4,5-IP_3 = inositol 1,4,5-trisphosphate
1,3,4-IP_3 = inositol 1,3,4-trisphosphate
1,4-IP_2 = inositol 1,4-bisphosphate
3,4-IP_2 = inositol 3,4-bisphosphate
1,3-IP_2 = inositol 1,3-bisphosphate
I-1-P = inositol 1-phosphate
I-4-P = inositol 4-phosphate
Inositol = *myo*-inositol

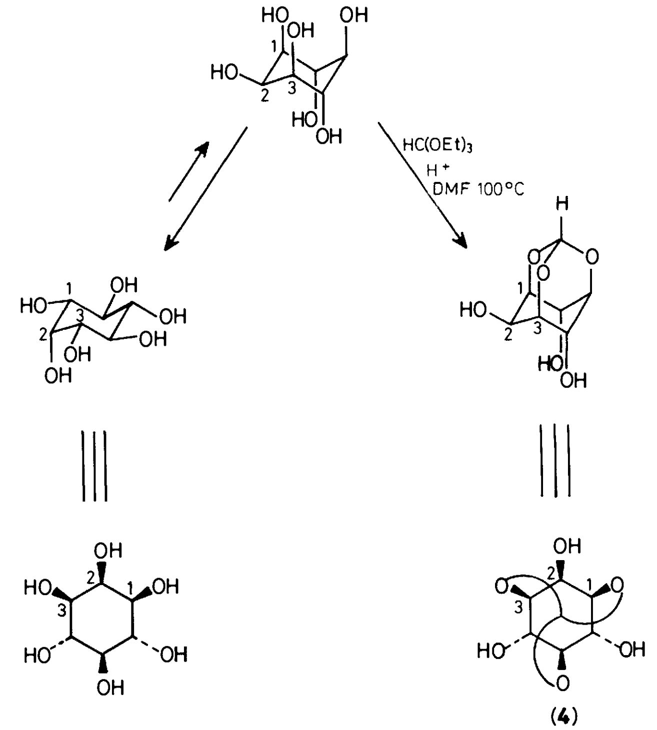
Many of the facets of this fundamental signalling system are unclear, and efficient syntheses of all of the naturally occurring inositol phosphates were required to allow detailed biochemical investigations to proceed. A classical solution to the problem of obtaining selectively protected inositols has been to make use of the three isomeric bis-acetals of inositol [Scheme 1, (**1**)—(**3**)], and the chemistry of the bis-acetals (**1**)—(**3**) has been widely explored.[9] The free hydroxy groups in (**1**)—(**3**) may be selectively protected under carefully controlled conditions,[10,11] and coupled with the selective hydrolysis of the less stable *trans* acetal linkage;[12,13] this has led to their wide spread use in synthetic approaches to inositol polyphosphates.[14] The parallel development of P^{III} based phosphorylation techniques[15,16] has allowed the successful synthesis of many inositol polyphosphates, and a comprehensive review of this area has appeared.[14]

The key problems posed in the synthesis of the inositol phosphates are (1) the synthesis of a suitable selectively protected inositol derivative, (2) efficient phosphorylation in high yield, a particularly difficult problem where vicinal diols are involved due to steric crowding and the formation of cyclic phosphate by-products, (3) deprotection of phosphate and hydroxy functions *without* migration of phosphate substituents to adjacent hydroxy groups, a major problem when *cis* groups are present. We have previously reported the successful use of benzyl phosphate esters in conjunction with benzyl ethers, allowing a single hydrogenolysis to be used for complete deprotection without migration of phosphate groups.[12,17-19] We have reported elsewhere[20] full details of our studies on the polyphosphorylation of inositol derivatives, and our development of the use of the reaction of polyalkoxide anions with tetrabenzylpyrophosphate as a general approach.[13,20] We concentrate in this paper on methodology for the synthesis of selectively protected inositols from inositol orthoformate (**4**) which together with our other studies provides all of the inositol phosphates identified in the crucial phosphatidylinositol secondary messenger pathway. A preliminary account of part of this work has appeared.[18,19]

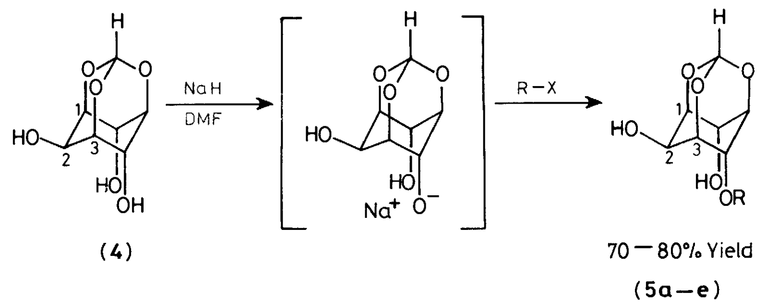
† Inositol refers to the *myo*-inositol stereochemistry throughout.



Scheme 1. *Conditions* i, H$^+$ hydrolysis



Scheme 2.



			Yields (%)
(**5a**)	R = Bn	X = Br	75
(**5b**)	R = $CH_2C_6H_4OMe$	X = Cl	67
(**5c**)	R = CH_2=$CHCH_2$	X = Br	80
(**5d**)	R = $PO(OBn)_2$	X = $OPO(OBn)_2$	72
(**5e**)	R = CH_2OBn	X = Cl	67

Scheme 3.

Results and Discussion

Since each of the bis-acetals (**1**)—(**3**) (Scheme 1) contains the more stable *cis*-1,2-acetal plus one other *trans* acetal, these intermediates are *not* suitable for the preparation of inositol phosphates having 1,3 substituents by direct means. To overcome this problem, we examined the use of inositol orthoformate (**4**) (Scheme 2) whose synthesis and structural determination was recently reported.[21] We were attracted to this intermediate since it provides simultaneous protection of the hydroxy groups at C-1, C-3 and C-5, and results in inversion of the normal axial/equatorial relationship of the remaining free hydroxy groups.

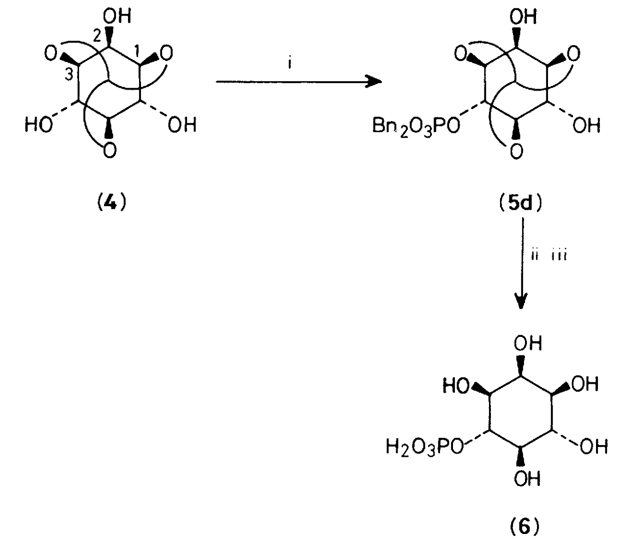
Initial attempts to introduce silyl protecting groups selectively into (**4**) met with failure, with only mixtures of isomeric mono- and bis-silyl ethers being isolated.[21] In stark contrast, formation of the alkoxide of (**4**) with sodium hydride (NaH) in dimethylformamide (DMF), followed by alkylation with benzyl bromide resulted in the formation of the 4-monobenzyl ether (**5a**) in very high yield, together with a trace of the 4,6-dibenzyl ether. The very high regioselectivity of this alkylation, together with the high degree of monoalkylation are probably due to internal co-ordination in an intermediate anion (Scheme 3). Similar selective alkylations were achieved with a series of alkylating agents, and with the phosphorylating agent tetrabenzyl pyrophosphate (TBPP). The intermediacy of a chelated anion is further indicated by the observation that changes in either counter ion or solvent lead to losses in selectivity. Subsequent treatment of (**5a**) with NaH in DMF followed by benzyl bromide (BnBr) gave a *ca.* 5:1 mixture of the 4,6- and 2,4-dibenzyl ethers, together with some tribenzyl material. Direct treatment of (**4**) with NaH (2 equiv.) in DMF, followed by BnBr (2 equiv.) gave a similar mixture of 2,4- and 4,6-dibenzyl ethers, in somewhat lower yield.

The regiochemistry of these substituted compounds was readily deduced from their 360 MHz ^1H n.m.r. spectra. The 2-benzyl and 4,6-dibenzyl compounds, having a plane of symmetry through C-2 and C-5, display symmetric spectra (1-H ≡ 3-H, 4-H ≡ 6-H) with 4 signals for the inositol ring protons. In contrast, the 4-benzyl compound displays a dissymmetric spectrum with 6 individual signals for the inositol ring protons.

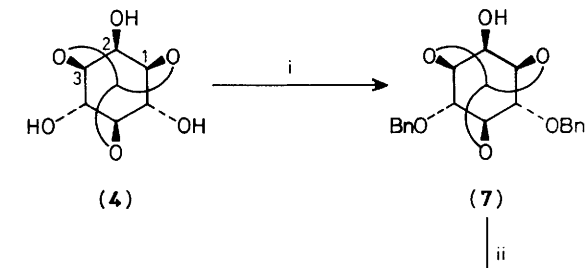
We have used these selective alkylations of inositol orthoformate to prepare a series of protected inositol derivatives, (**5a—e**), (**7**), (**10**), (**12**), and (**16**). Efficient phosphorylation and deprotection procedures have led to total synthesis of *myo*-inositol 2-phosphate, 4-phosphate, 1,3-bisphosphate, and 1,3,4,5-tetrakisphosphate (**9**), (**6**), (**18**), and (**14**). Following our original communication a synthesis of the enantiomers of inositol 1,3,4,5-tetrakisphosphate which uses the orthoformate (**4**) as an intermediate has appeared.[22]

(±)-*Inositol* 4-*Phosphate* (**6**) (Scheme 4).—Direct phosphorylation of the mono anion of inositol orthoformate (generated in DMF using NaH) with tetrabenzyl pyrophosphate (TBPP) gave the 4-dibenzylphosphate (**5d**) in 72% isolated yield. Hydrogenolysis of the benzyl esters followed by treatment with aqueous TFA to remove the orthoformate protecting group gave (±)-inositol 4-phosphate (**6**), which was isolated as its biscyclohexylammonium salt, in quantitative yield. This route provides (±)-inositol 4-phosphate in only 2 steps and 72% overall yield from the orthoformate (**4**).

Inositol 2-*Phosphate* (**9**) (Scheme 5).—Treatment of inositol orthoformate with NaH (2 equiv.) in DMF, followed by alkylation with BnBr gave a 5:1 mixture of 2,4- and 4,6-dibenzyl ethers together with some tribenzyl ether and some 4-monobenzyl ether. Chromatographic separation of the dibenzylated material, followed by recrystallisation from ethyl acetate–hexane gave the pure 4,6-dibenzyl orthoformate (**7**) in 30% yield (not optimised). Phosphorylation using NaH and TBPP gave the fully protected 2-phosphate (**8**) in 72% yield. Deprotection by hydrogenolysis, followed by treatment with aqueous TFA to remove the orthoformate protecting group gave inositol 2-phosphate (**9**), isolated as its biscyclohexylammonium salt, in 97% yield. This new synthesis of inositol 2-phosphate provides [redacted]



Scheme 4. *Reagents and conditions:* i, NaH (1 equiv.), DMF, TBPP (1 equiv.), 25 °C; ii, 10% Pd on C, EtOH–H_2O (80:20), H_2 (50 p.s.i.); iii, TFA–H_2O (80:20), 25 °C



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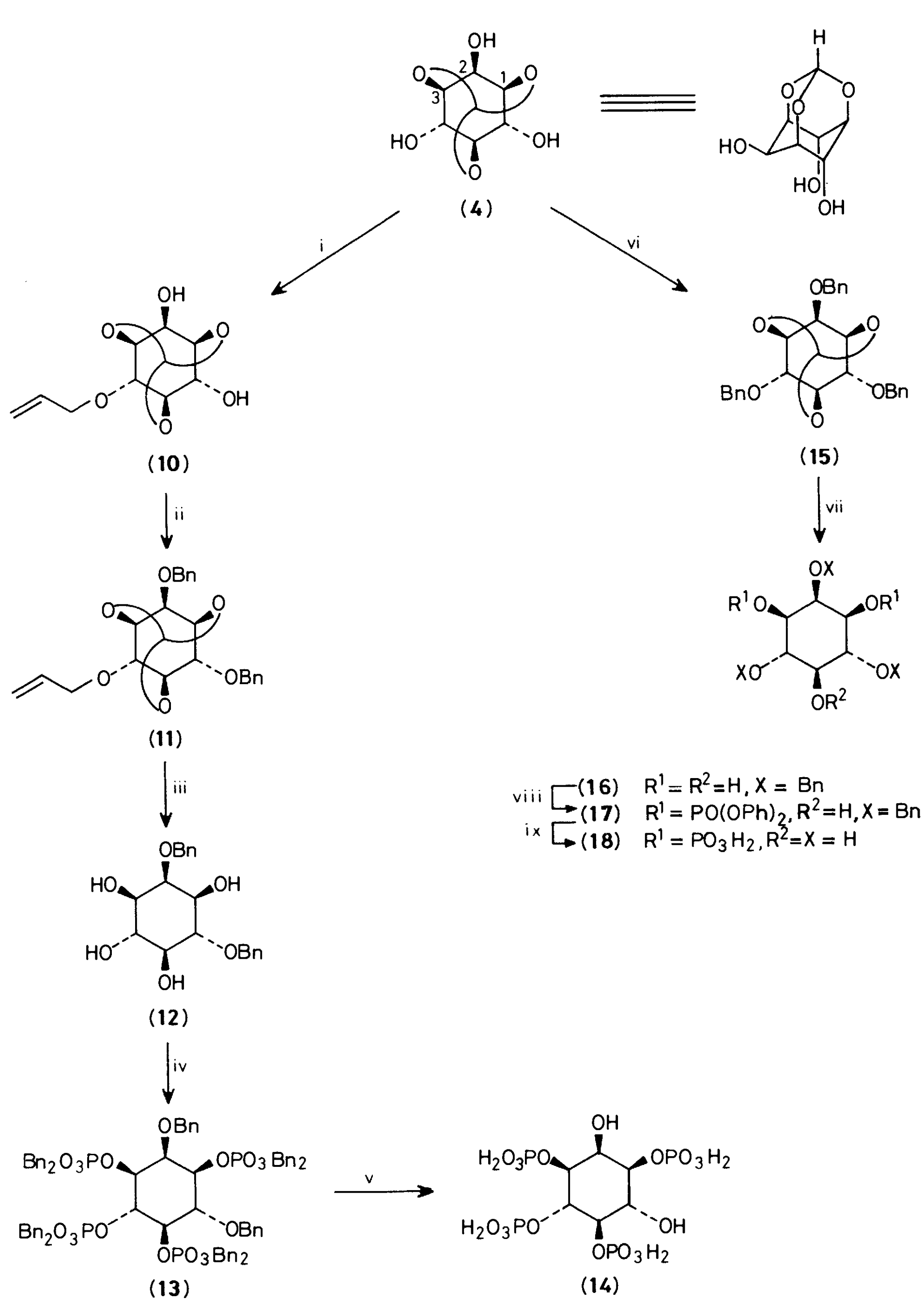

Scheme 6. *Reagents and conditions:* i, NaH (1 equiv.), DMF, allyl bromide (1 equiv.), 25 °C; ii, NaH (2.5 equiv.), DMF, BnBr (2.5 equiv.), 25 °C; iii, (*a*) 90% EtOH, $RhCl(PPh_3)_3$, diazabicyclo[2.2.0]octane, reflux; (*b*) 0.1M HCl–MeOH, reflux, 15 min; iv, NaH, THF, tetrabenzyl pyrophosphate {[(BnO)₂PO]₂O}, catalytic imidazole or 18-crown-6, 25 °C; v, 10% Pd on C, EtOH–H_2O (80:20), H_2, (50 p.s.i.), 25 °C; vi, NaH (3 equiv.), DMF, BnBr (4 equiv.), 25 °C; vii, 0.1M HCl–MeOH, reflux, 15 min; viii, $(PhO)_2POCl$, CH_2Cl_2, Et_3N, catalytic 4-dimethylaminopyridine, 25 °C; ix, Li in THF–liquid NH_3, −78 °C

under an atmosphere of nitrogen. All reactions were performed under dry nitrogen in glassware which had been dried at 180 °C and 50 mmHg for 30 min and allowed to cool under dry nitrogen. Light petroleum refers to the fraction boiling in the range 60—80 °C. Solvents were removed under reduced pressure (<10 mmHg) on a Buchi rotary evaporator at 30—50 °C. Organic solutions were dried over anhydrous magnesium sulphate.

Yields refer to isolated yields of chromatographically (t.l.c. and h.p.l.c.) homogeneous materials.

myo-*Inositol Orthoformate* (**4**).—*myo*-Inositol orthoformate (**4**) was prepared from *myo*-inositol by a modification of the method of Kishi *et al.*,[21] involving the use of DMF in place of dimethylsulphoxide. In our hands this led to a cleaner product, and gave more reproducible yields. The orthoformate was conveniently purified by column chromatography on silica gel with CH_3OH–$CHCl_3$ (1:4) as eluant, followed by recrystallization from CH_3OH, m.p. 300—302 °C.

4-(*Dibenzyloxyphosphoryloxy*)-myo-*inositol Orthoformate* (**5d**).—Sodium hydride (80% dispersion; 0.08 g, 2.6 mmol) was added in one portion to a stirred solution of *myo*-inositol orthoformate (**4**) (0.5 g, 2.6 mmol) in anhydrous DMF (250 ml) under N_2 at 25 °C. The solution was stirred for 10 min and then TBPP (1.4 g, 2.6 mmol) was added in one portion and the mixture was stirred for a further 48 h. The reaction was quenched with water (1 ml) and the solvent evaporated *in vacuo* (< 1 mmHg). The residual oil was taken up in CH_2Cl_2 (100 ml), filtered, and evaporated under reduced pressure to a thick oil. Chromatography on silica gel with EtOAc as eluant gave the title compound (**5d**) (0.84 g, 72%) as a white solid m.p. 97—99 °C (from Et_2O) (Found: C, 55.75; H, 5.15: Calc. for $C_{21}H_{23}O_9P$: C, 56.0; H, 5.1); δ_H 3.93 (1 H, br s), 4.05 (1 H, m), 4.14 (1 H, t, *J* 2 Hz), 4.29 (1 H, m), 4.50 (1 H, br s), 4.94 (1 H, complex m) 5.00—5.13 (4 H, complex m), 5.40 (1 H, d, *J* 1 Hz) and 7.37 (10 H, complex m); *m/z* f.a.b.$^+$ 451 [$(M + H)^+$, 100%], 185 (25), and 91 (90), f.a.b. 449 $(M - H)^-$ 359 (100%), 277 (30), and 183 (40).

(±) myo-*Inositol* 4-*Phosphate* (**6**).—A solution of the dibenzyl phosphate (**5d**) (0.44 g, 0.97 mmol) in EtOH–H_2O (4:1) (250 ml) was hydrogenated at 50 p.s.i. H_2 over 10% Pd/C (0.25 g) for 18 h. The catalyst was filtered off and the solvents removed under reduced pressure. The residue was taken up in TFA–H_2O (4:1) (50 ml) and allowed to stand for 4 h. The solvents were removed under reduced pressure and the residue in water (10 ml) passed through a column of Dowex 50W X-8 (100 mm × 20 mm) in the protonated form. The eluate was treated with an excess of cyclohexylamine (CHA) (1 ml) and after being stirred for 1 h under N_2 the solution was extracted with Et_2O (3 × 50 ml) and the aqueous solution freeze-dried to a white powder. Crystallization from aqueous acetone gave the title compound (**6**) as its biscyclohexylammonium salt (0.443 g 100%), m.p. 133—134 °C (Found: C, 46.85; H, 8.45; N, 6.0; Calc. for $C_{18}H_{39}N_2O_9P$: C, 47.15; H, 8.57; N, 6.11); δ_H (360 MHz; D_2O), 3.41 (1 H, t, *J* 9 Hz), 3.55 (1 H, dd, *J* 9 and 2.5 Hz), 3.63 (1 H, dd, *J* 9 and 2.5 Hz), 3.70 (1 H, t, *J* 9 Hz), 4.05 (1 H, m), and 4.11 (1 H, t, *J* 9 Hz); *m/z* (f.a.b.$^+$) 360 $(M + CHA)^+$; f.a.b.$^-$ 259 $(M - H)^-$.

4,6-*Di*-O-*benzyl*-myo-*inositol Orthoformate* (**7**).—Sodium hydride (80% dispersion in oil; 1.35 g, 45 mmol) was added to a solution of the orthoformate (**4**) (3.80 g, 20 mmol) in dry DMF (100 ml) containing imidazole (50 mg). The mixture was stirred at room temperature for 1 h, after which time benzyl bromide (7.18 g, 42 mmol) was added. After being stirred for a further 1 h the mixture was quenched with saturated aqueous ammonium chloride (5 ml) and evaporated. The residue was partitioned between water (100 ml) and dichloromethane (2 × 100 ml), and the combined organic layers were washed with brine (50 ml), dried ($MgSO_4$), and evaporated. Flash chromatography, with gradient elution using ethyl acetate in hexane (25—100%), gave, in order of elution: the tribenzyl ether, and dibenzylated material (2.91 g), consisting of an approximately 5:1 mixture of 4,6- and 2,4-dibenzyl orthoformates, respectively. Recrystallisation from ethyl acetate–hexane gave the pure 4,6-dibenzyl ether (**7**) (1.96 g, 27%), m.p. 125 °C (lit.,[21] 124—125 °C) (Found: C, 68.05; H, 6.0. Calc. for $C_{21}H_{22}O_6$: C, 68.10; H, 5.99); δ_H (360 MHz; $CDCl_3$), 3.02 (1 H, br d, *J* 10.8 Hz), 4.23 (3 H, m), 4.37 (2 H, t, *J* 3.6 Hz), 4.45 (1 H, m), 4.62 (4 H, AB, *J* 11.7 Hz), 5.47 (1 H, s), and 7.28 (10 H, m); *m/z* 371 $(M^+ + 1$, 29%), 279 (19), 173 (20), 108 (10), and 91 (100).

4,6-*Di*-O-*benzyl*-myo-*inositol* 2-*Dibenzylphosphate* (**8**).—Sodium hydride (80% dispersion in oil; 45 mg, 1.5 mmol) was added to a solution of the dibenzyl ether (**7**) (360 mg, 1 mmol) in dry THF (20 ml) containing a trace of imidazole, and the mixture heated under reflux for 0.5 h. When the mixture was cool, a solution of tetrabenzyl pyrophosphate (592 mg, 1.1 mmol) in dry THF (4 ml) was added to it; it was then heated under reflux for a further 3.5 h. After this time, the mixture was allowed to cool and the solid removed by careful filtration. The filtrate was evaporated, and the residue subjected to m.p.l.c. with 35% ethyl acetate in hexane as eluant to afford, in order of elution: recovered alcohol (**7**) (60 mg, 17% recovery), and the desired phosphate (**8**) as a solid (452 mg, 72%), m.p. 102—103 °C (from ethyl acetate–hexane). (Found: C, 66.5; H, 5.7; P, 4.75. Calc. for $C_{35}H_{35}O_9P$: C, 66.66; H, 5.59; P, 4.91%); δ_H (360 MHz; $CDCl_3$), 4.33 (2 H, m), 4.38 (2 H, m), 4.41 (1 H, m), 4.55 (4 H, AB, *J* 11.5 Hz), 4.97 (1 H, d, *J* CHN 7.0 Hz), 5.08 (4 H, d, *J* 7.9 Hz), 5.50 (1 H, d, *J* 1.2 Hz), and 7.28 (2 H, m); *m/z* (f.a.b.$^-$) 551 (16%), 275 (20), 183 (100), and 91 (80).

myo-*Inositol* 2-*Phosphate* (**9**).—A solution of the dibenzyl phosphate (**8**) (445 mg, 0.71 mmol) in ethanol (35 ml) and water (7 ml) was shaken with palladium on carbon (10%; 175 mg) under an atmosphere of hydrogen at 50 p.s.i. for 4 h. The suspension was filtered, the filtrate evaporated, and the residue taken up in TFA–H_2O (4:1) (10 ml). After being stirred for 2.5 h, the solution was evaporated, water (5 ml) added to the residue, and the resulting solution freeze-dried. The residue was once again redissolved in water, and passed through a short column of Dowex 50W X-8 resin, in the H^+ form; an excess of cyclohexylamine was added to the eluant, and the mixture freeze-dried to give the title compound (**9**) as its biscyclohexylammonium salt (318 mg, 97%), m.p. 183–185 °C (Found: C, 46.8; H, 8.35; N, 5.95; P, 6.4. Calc. for $C_{18}H_{39}N_2O_9P$: C, 47.15; H, 8.57; N, 6.11; P, 6.76); δ_H (360 MHz; D_2O), 1.19 (3 H, m), 1.34 (10 H, m), 1.65 (3 H, m), 1.80 (5 H, m), 1.98 (5 H, m), 3.23 (2 H, m), 3.25 (1 H, t, *J* 9.3 Hz), 3.47 (2 H, m), 3.73 (2 H, t, *J* 9.6 Hz), and 4.50 (1 H, dt, *J* 7.2, 2.5 Hz); *m/z* (f.a.b.$^-$) 259 [$(M - H)^-$, 100%], 113 (37), and 79 (36).

(±) 4-O-*Allyl*-myo-*inositol Orthoformate* (**10**) ≡ (**5c**).—Sodium hydride (80% dispersion; 0.8 g, 26 mmol) was added in one portion to a stirred solution of the orthoformate (**4**) (5 g, 26 mmol) in anhydrous DMF (500 ml) under N_2 at 25 °C. H_2 Evolution had ceased after 10 min and allyl bromide (3.0 ml, 2.4 g, 34 mmol) was added in one portion. The solution was stirred under N_2 for 2 h at 25 °C, and then quenched with water (5 ml). The solvent was removed under reduced pressure (1 mmHg) and the residual paste was taken up in $CHCl_3$ (500 ml) and filtered to remove NaBr. T.l.c. on silica (EtOAc–light petroleum, 3:1) showed a trace of the orthoformate (**4**) R_F 0.15, the desired product R_F 0.35 and a trace of bisallyl compounds R_F 0.53. Flash chromatography on silica gel with EtOAc–light petroleum (3:1) as eluant gave the title compound (**10**) (4.8 g, 80%) as as thick oil; δ_H (360 MHz; $CDCl_3$) 3.68 (1 H, d, *J* 10 Hz), 4.16 (2 H, m), 4.22 (1 H, m), 4.3 (2 H, m), 4.38 (1 H, m), 4.46 (1 H, m), 5.28 (2 H, m), 5.44 (1 H, s) and 5.90 (1 H, m); *m/z* 231 [$(M + H)^+$, 100%], 155 (60), 113 (60). [Found: $(M + H)^+$ 231.0085. Calc. for $C_{10}H_{15}O_6$: 231.0868].

(±) 2,6-*Di*-O-*benzyl*-4-O-*allyl*-myo-*inositol Orthoformate* (**11**).—Sodium hydride (80% dispersion; 2.3 g, 83 mmol) was added in a single portion to a stirred solution of allyl orthoformate (**10**) (4.8 g, 20.7 mmol) in anhydrous DMF (250 ml) under N_2 at 25 °C. The suspension was stirred for 20 min and then benzyl bromide (14.2 g, 83 mmol) was added in one portion. The solution was stirred at 25 °C for 18 h, and then quenched with water (5 ml). The solvent was removed *in vacuo* (<1 mmHg) and the residue partitioned between $CHCl_3$ (500 ml) and water (50 ml). The organic layer was separated, washed with water (50 ml) and brine (50 ml), dried and evaporated under reduced pressure to a thick oil. Chromatography on silica gel with EtOAc–light petroleum (1:2) as eluant gave the title compound (**11**) (7.1 g, 86%) as a thick oil (homogeneous by t.l.c. and h.p.l.c.); δ_H (360 MHz; $CDCl_3$), 4.0 (2 H, m), 4.28 (2 H, m), 4.40 (1 H, m), 4.54 (2 H, dd, *J* 11 and 41 Hz), 4.7 (2 H, s), 5.20 (2 H, m), 5.54 (1 H, s), 5.84 (1 H, m), and 7.3 (10 H, m); *m/z* 410 $(M^+$, 10%), 253 (8), 203 (15), 131 (10), and 91 (100). (Found: M^+ 410.1740. Calc. for $C_{24}H_{26}O_6$: 410.172 g).

(±) 2,6-*Di*-O-*benzyl*-myo-*inositol* (**12**).—Wilkinsons catalyst (0.5 g) and 1,4-diazabicyclo[2.2.2]octane (DABCO) (0.2 g)[23] were added to a stirred solution of the dibenzyl orthoformate (**11**) (7.1 g, 17 mmol) in EtOH–H_2O, 9:1 (55 ml). The solution was boiled under reflux under N_2 for 9 h when t.l.c. (EtOAc–light petroleum, 2:3) showed complete conversion into the enol ether. The solution was cooled, filtered, and evaporated under reduced pressure. The residue was taken up in MeOH (700 ml) and 10M HCl (70 ml) was added. The solution was boiled under reflux for 20 min, cooled, and adjusted to pH 8 by the addition of ammonia solution (*d* 0.88). The solvents were removed under reduced pressure and the semi-solid residue extracted with hot EtOAc (3 × 500 ml). The solvent was evaporated under reduced pressure, and the residue recrystallized from $CHCl_3$–light petroleum to give the title compound (**12**) (3.3 g, 53%), m.p. 119—120.5 °C (Found: C, 66.65; H, 6.75. Calc. for $C_{20}H_{24}O_6$: C, 66.65; H, 6.71); δ_H (360 MHz; $CDCl_3$), 3.45 (1 H, t, *J* 9 Hz), 3.47 (1 H, m), 3.63 (2 H, complex m), 3.71 (1 H, t, *J* 9 Hz), 4.03 (1 H, t, *J* 2.5 Hz), 4.76 (2 H, br multiplet), 4.84 (2 H, q, *J* 13 Hz), and 7.36 (10 H, m); *m/z* (f.a.b.$^-$) 359 [$(M - H)^-$, 100%], 297 (20), 269 (25), and 183 (25).

(±) 2,6-*Di*-O-*benzyl*-myo-*inositol* 1,3,4,5-*Tetrakis*(*dibenzylphosphate*) (**13**).—Sodium hydride (80% dispersion; 0.24 g, 8 mmol) was added in one portion to a stirred solution of dibenzylinositol (**12**) (0.36 g, 1 mmol) in anhydrous THF (70 ml) under N_2. The solution was heated to 60 °C and allowed to cool to 25 °C. TBPP (3.26 g, 6 mmol) was added in a single portion, followed by imidazole (0.05 g), and the solution was stirred at 25 °C for 18 h. The suspension was filtered and the filter cake washed with THF (2 × 25 ml). The filtrate was evaporated under reduced pressure and the residual oil chromatographed on silica gel, with EtOAc–light petroleum (3:2) as eluant to give the title compound (**13**) (0.92 g, 66%) as a thick oil (homogenous by t.l.c. and h.p.l.c.); δ_H (360 MHz; $CDCl_3$), 4.06 (1 H, t, *J* 9 Hz), 4.22 (1 H, m), 4.30 (1 H, m), 4.44 (1 H, q, *J* 9 Hz), 4.6—5.1 (22 H, complex m), and 7.20 (50 H, m); *m/z* (f.a.b.$^+$) 1 401 [$(M + H)^+$, 100%], 1 310 [$(M - 91 - H)^+$, 95], 1 218 (20), 1 172 (30), f.a.b.$^-$ 1 309 [$(M - 91)^-$, 100%], 1 219 (45), and 1 130 (20).

(±) myo-*Inositol* 1,3,4,5-*Tetrakisphosphate* (**14**).—The fully protected compound (**13**) (0.15 g, 0.1 mmol) was dissolved in EtOH–H_2O (4:1) (100 ml) and hydrogenated at 50 p.s.i. H_2 over 10% Pd/C (0.1 g) for 10 h. The catalyst was filtered off and the solution was evaporated under reduced pressure. The residue was taken up in water (10 ml) and passed through a column of Dowex 50W X-8 (100 mm × 20 mm) in the H^+ form. Excess CHA was added to the aqueous eluate, and the solution was stirred for 1 h under N_2. The solution was extracted with Et_2O (3 × 50 ml) and freeze dried to a fawn powder. Crystallisation from aqueous acetone gave the title compound (**14**) (0.115 g, 88%) as its pentacyclohexylammonium salt, m.p. 175—177 °C (Found: C, 43.65; H, 8.0; N, 7.35. Calc. for $C_{42}H_{94}N_6O_{18}P_4$: C, 43.45; H, 8.10; N, 7.03); δ_H (360 MHz, D_2O), 3.84 (1 H, t, *J* 10 Hz), 3.93 (1 H, td, *J* 10 and 3 Hz), 3.95 (1 H, m), 4.02 (1 H, td, *J* 10 and 3 Hz), 4.31 (1 H, q, *J* 10 Hz), and 4.32 (1 H, br s); δ_P (145.78 MHz, D_2O pH 7.0; ^1H coupled); δ_H 1.54 (d, *J* 9.28 Hz), 2.82 (d, *J* 9.62 Hz), 2.90 (d, *J* 7.27 Hz), and 3.72 (d, *J* 6.88 Hz); *m/z* (f.a.b.$^+$) 600 [$(M + CHA + H)^+$, 10%], 192 (10), and 100 (100); f.a.b.$^-$ 499 [$(M - H)^-$, 100%] 401 (10), 159 (15), and 79 (10).

2,4,6-*Tri*-O-*benzyl*-myo-*inositol Orthoformate* (**15**).—NaH (4 g of 80% dispersion; 4 g, 133 mmol) was added to a stirred solution of orthoformate (**4**) (5 g, 26 mmol) in anhydrous DMF (250 ml) under N_2 at 25 °C. The mixture was stirred for 20 min, after which benzyl bromide (21.6 g, 130 mmol) was added to it and the suspension stirred at 25 °C under N_2 for 18 h. The reaction was quenched by the addition of water (10 ml) and the solvents were removed *in vacuo* (<1 mmHg). The residue was partitioned between $CHCl_3$ (300 ml) and water (50 ml) and the $CHCl_3$ solution was washed with brine (50 ml), dried, and evaporated under reduced pressure to give a gum. Trituration under cold hexane converted this gum into a white solid, which was recrystallized from light petroleum to give the title compound (**15**) (9.5 g, 79%) as a white crystalline solid, m.p. 102—104 °C (Found: C, 73.05; H, 6.30. Calc. for $C_{28}H_{28}O_6$: C, 73.02; H, 6.13); δ_H (360 MHz; $CDCl_3$), 4.04 (1 H, d, *J* 1.4 Hz), 4.28 (2 H, t, *J* 2.5 Hz), 4.33 (2 H, t, *J* 4 Hz), 4.43 (1 H, m), 4.55 (6 H, m), 5.52 (1 H, d, *J* 1.1 Hz), and 7.28 (15 H, complex m); *m/z* (f.a.b.$^+$) 461 [$(M + H)^+$, 100%], and 181 (50); f.a.b.$^-$ 459 [$(M - H)^-$, 100%], 305 (30), 199 (50), and 153 (20).

2,4,6-*Tri*-O-*benzyl*-myo-*inositol* (**16**).—10M HCl (5 ml) was added to a solution of the benzyl orthoformate (**15**) (6 g, 13 mmol) in MeOH (500 ml) and the mixture was boiled under reflux for 20 min. The solution was cooled to 25 °C, and adjusted to pH 8 with ammonia solution (*d* 0.88). The solvents were removed under reduced pressure, and the residue extracted into EtOAc (2 × 250 ml). The combined extracts were then filtered and evaporated under reduced pressure to leave a thick oil. This was dissolved in hot Et_2O and induced to crystallize by the addition of light petroleum. Recrystallization from Et_2O–light petroleum gave the title compound (**16**) (5.1 g, 87%) as a white crystalline solid, m.p. 83—84.5 °C (Found: C, 72.2; H, 6.65. Calc. for $C_{27}H_{30}O_6$: C, 71.98; H, 6.71); δ_H (360 MHz; $CDCl_3$), 3.56 (3 H, m), 3.66 (2 H, t, *J* 9.3 Hz), 4.00 (1 H, t, *J* 2.7 Hz), 4.85 (6 H, d, *J* 7 Hz), and 7.33 (15 H, complex m); *m/z* (f.a.b.$^+$) 451 [$(M + H)^+$, 5%], 181 (20), and 91 (100); *m/z* (f.a.b.$^-$) 449 [$(M - H)^-$, 100%], 359 (50), and 183 (60).

2,4,6-*Tri*-O-*benzyl*-myo-*inositol* 1,3-*Bis*(*diphenylphosphate*) (**17**).—4-Dimethylaminopyridine (0.3 g) and triethylamine (12 ml, excess) were added to a stirred solution of tribenzylinositol (**16**) (4 g, 8.8 mmol) in anhydrous CH_2Cl_2 (300 ml) under N_2 at 25 °C. The solution was stirred for 10 min, diphenyl chlorophosphate (4.0 ml, 19.3 mmol) was added, and the solution was then stirred for a further 5 h. The solvent was removed under reduced pressure, the residue partitioned between Et_2O (800 ml) and water (100 ml); the organic phase was then washed with water (50 ml) and brine (50 ml), dried, and evaporated under reduced pressure to an oil. This was dissolved in Et_2O (400 ml) and light petroleum (400 ml) was added. The solution was evaporated to *ca.* 90% of the original volume without external heating at *ca.* 10 mmHg at which point crystallization commenced. The cold solution was scratched vigorously for 10 min and the resulting solid filtered off to give the title compound (**17**) (3.1 g, 38%) as a white crystalline solid, m.p. 109—110 °C (Found: C, 66.65; H, 5.25. Calc. for $C_{51}H_{48}O_{12}P_2$: C, 66.95; H, 5.29); δ_H (360 MHz; $CDCl_3$), 3.61 (1 H, dt, *J* 11 and 2.5 Hz), 3.96 (2 H, t, *J* 9.5 Hz), 4.49 (2 H, m), 4.62 (1 H, dt, *J* 7.5 and 2.5 Hz), 4.72 (6 H, m), and 7.23 (35 H, complex m); *m/z* (d.c.i.) 915 (M^+, 60%), 341 (25), and 251 (100).

After several days at 0 °C, the mother liquor deposited a white solid (1.6 g, 20%) which was composed of 53% of the 1,3-isomer (**17**) and 47% the 1,5-diphosphorylated material (**17a**). The isomers were separable by h.p.l.c. (μ-Porasil, Waters Associates, 3.9 mm × 300 mm EtOAc–light petroleum, 1:3), which confirmed the isomeric purity of the first crop crystalline material as >99.5%.

myo-*Inositol* 1,3-*Bisphosphate* (**18**).—A solution of diphenyl phosphate (**17**) (0.5 g, 0.54 mmol) in anhydrous THF (10 ml) was added dropwise to a stirred solution of lithium metal (*ca.* 5.0 mg) in liquid ammonia–THF (2:1) which was maintained at −78 °C under nitrogen. After *ca.* 8 ml of solution had been added the blue colour of the reaction mixture was discharged. A pellet of lithium metal (*ca.* 20 mg) was added to the reaction and, after being stirred for 5 min, more substrate was added dropwise to the blue solution until the colour was again discharged. This titration procedure[24] was repeated until all the substrate had been added (40 min) and then a pellet of lithium metal (*ca.* 20 mg) was added to the reaction which was stirred for 15 min. Water (1.5 ml) was added to the blue solution and the ammonia was evaporated under a stream of N_2 at 25 °C overnight. The residue from the reaction was taken up in water (10 ml) and passed through a 20 mm × 100 mm column of Amberlite IR 120 in the H^+ form, eluting with water. The acidic eluate was treated with an excess of CHA (1.2 ml) and after being stirred for 1 h was extracted with Et_2O (5 × 50 ml) and freeze dried to a white powder. Recrystallization from aqueous acetone gave the title compound (**18**) as its tetracyclohexylammonium salt (0.27 g, 66%), m.p. 165—166 °C (Found: C, 48.75; H, 8.9; N, 7.85. Calc. for $C_{30}H_{66}N_4O_{12}P_2$: C, 48.90; H, 9.03 N, 7.60); δ_H (360 MHz; D_2O), 3.4 (1 H, t, *J* 9 Hz), 3.78 (2 H, t, *J* 9 Hz), 3.96 (2 H, dt, *J* 9 and 3 Hz), and 4.28 (1 H, t, *J* 3 Hz); *m/z* (f.a.b.$^+$) 440 [$(M + \text{CHA} + H)^+$, 20%], and 100 (100); *m/z* (f.a.b.$^-$) 339 [$(M - H)^-$, 100%] 241 (20), and 159 (15).

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