Synthesis of *myo*-Inositol 1,3,4,5-Tetraphosphate and *myo*-Inositol 1,3-Bisphosphate

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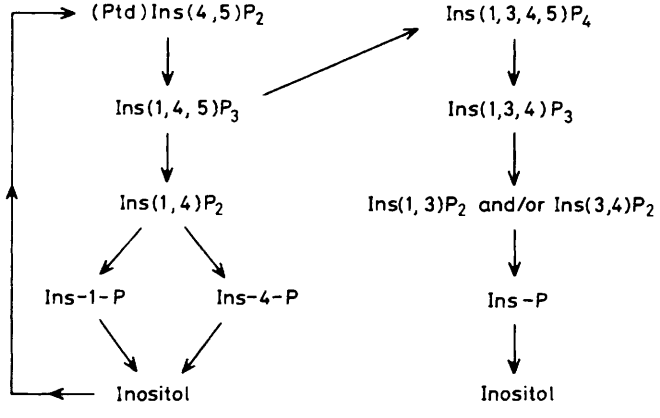
myo-Inositol 1,3,4,5-tetraphosphate, *myo*-inositol 3,4-bisphosphate, and *myo*-inositol 4-phosphate have been prepared *via* routes in which a key step is a novel, highly regioselective monoalkylation of *myo*-inositol orthoformate.

The hydrolysis of phosphatidylinositol 4,5-bisphosphate to give inositol 1,4,5-trisphosphate and diacylglycerol (Scheme 1) is now firmly established as an important secondary messenger pathway resulting from activation of membrane receptors by a substantial number of neurotransmitters and hormones. Both of these products act as second messengers in the stimulated cell, the former mediating the release of Ca^{2+} from an intracellular store and the latter activating protein kinase C.[1—3] Further metabolism of Ins(1,4,5)P_3 then takes place *via* inositol 1,4-bisphosphate and subsequently inositol 1- and 4-phosphate to yield *myo*-inositol; the quantitative importance of Ins-4-P in this pathway has still to be determined.[4] The inositol produced is then used for resynthesis of more (Ptd)Ins(4,5)P_2 in the target cell.[4,5] Recently evidence has been obtained for the existence of an important alternative pathway for the rapid metabolism of Ins(1,4,5)P_3 *via* a 3-kinase to inositol 1,3,4,5-tetraphosphate.[6] This tetraphosphate appears to be an important secondary messenger regulating the influx of Ca^{2+} into stimulated cells.[7] Further metabolism of this tetraphosphate is then proposed to occur *via* inositol 1,3,4-trisphosphate and subsequently either or both inositol 1,3-bisphosphate and inositol 3,4-bisphosphate.[8] Degradation to inositol would then involve inositol monophosphates (Scheme 1). Many of the facets of this fundamental signal transduction system are not yet clear. To allow investigations into the details of these important biochemical pathways we required effective syntheses of these naturally occurring substrates. We report the first synthesis of inositol 1,3,4,5-tetraphosphate and of inositol 1,3-bisphosphate. The methodology developed has also allowed a new and efficient synthesis of inositol 4-phosphate.



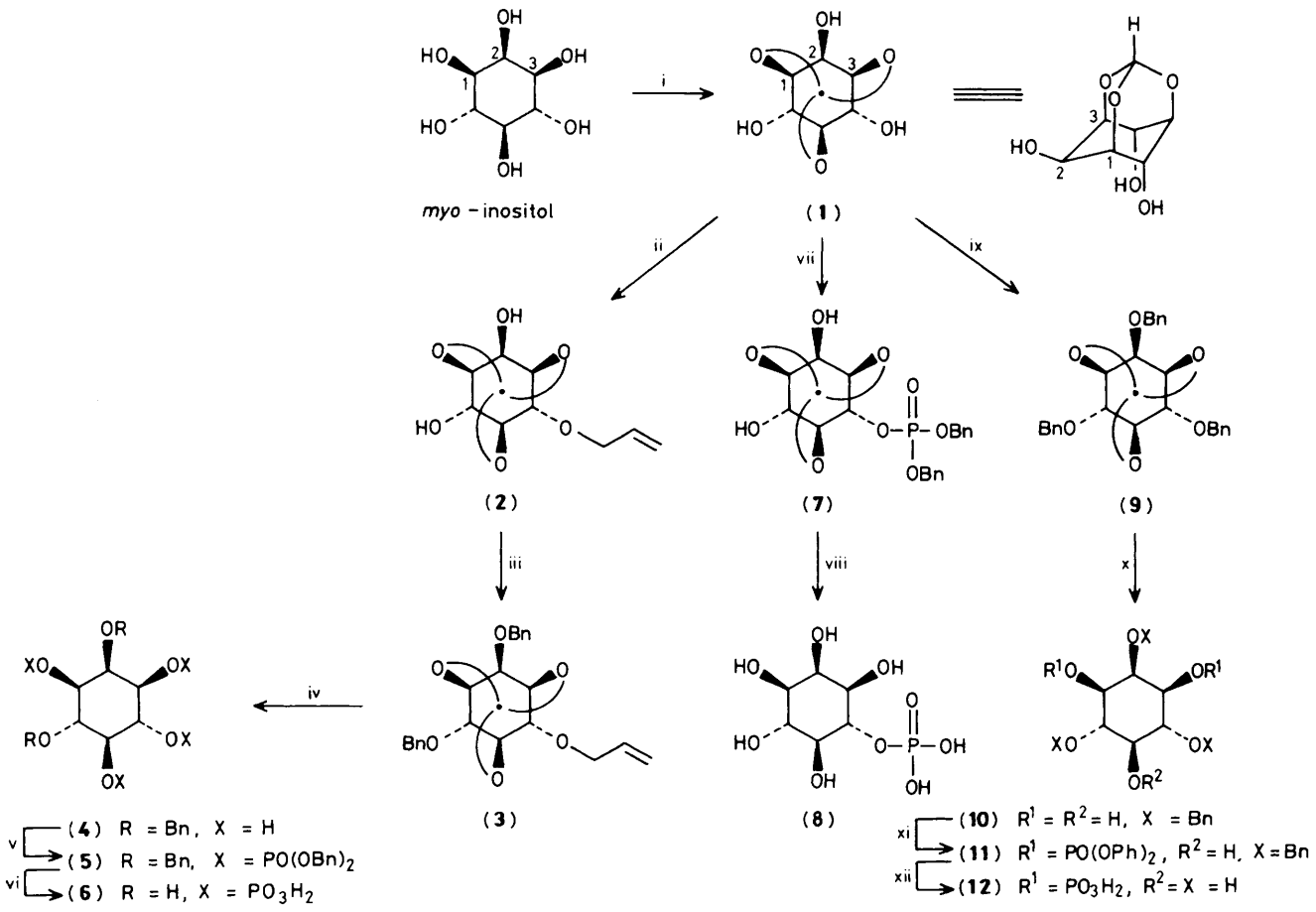
Scheme 1. *Abbreviations*: (Ptd)Ins(4,5)P_2 = Phosphatidylinositol 4,5-bisphosphate; Ins(1,3,4,5)P_4 = Inositol 1,3,4,5-tetraphosphate; Ins(1,3,4)P_3 = Inositol 1,3,4-trisphosphate; Ins(1,4,5)P_3 = Inositol 1,4,5-trisphosphate; Ins(1,3)P_2 = Inositol 1,3-bisphosphate; Ins(1,4)P_2 = Inositol 1,4-bisphosphate; Ins(3,4)P_2 = Inositol 3,4-bisphosphate; Ins-1-P = Inositol 1-phosphate; Ins-4-P = Inositol 4-phosphate; Ins-P = Inositol monophosphate(s).

The key problems posed in syntheses of the inositol polyphosphates[9] are: (i) synthesis of a suitable selectively protected inositol derivative, (ii) polyphosphorylation in high yield, a particular problem when vicinal hydroxy groups are involved owing to steric crowding and the ready formation of cyclic phosphates, and (iii) deprotection without migration of phosphate substituents to neighbouring hydroxy groups. Our synthetic approach to Ins(1,3,4,5)P_4 uses a novel, highly selective alkylation of *myo*-inositol orthoformate (**1**) (Scheme 2) to generate the desired protected inositol, and the reaction of a sodium alkoxide with tetrabenzyl pyrophosphate, catalysed by imidazole or 18-crown-6, to introduce four dibenzyl phosphate groups in high overall yield.[10,11] We have previously shown that in the synthesis of inositol derivatives,[12] dibenzyl phosphate esters and adjacent benzyl protected hydroxy groups may be simultaneously deprotected by hydrogenolysis, without phosphate migration.

The starting point for our synthetic approach utilised *myo*-inositol orthoformate (**1**), the synthesis and characterisation of which has been recently reported (Scheme 2).[13] Formation of the mono-anion of (**1**) [NaH, dimethylformamide (DMF), 25 °C] followed by alkylation in DMF with a range of electrophiles gave high yields of 4-substituted products (Scheme 2), in a highly selective manner. Thus reaction with allyl bromide and tetrabenzyl pyrophosphate {[$(BnO)_2$-PO]$_2$O} gave (**2**) as an oil, and (**7**) as a crystalline solid, m.p. 97—99 °C (Et_2O), in 80 and 72% yields respectively.† A similar reaction using benzyl bromide gave the 4-substituted monobenzyl ether as an oil in 73% yield. The regiochemistry of these 4-substituted products was clear from the dissymetric nature of their 360 MHz ^{1}H n.m.r. spectra. This high degree of selectivity for monoalkylation at position 4 of the orthoformate (**1**) would appear to be due to a chelation effect in the intermediate mono-anion. Treatment with a second mole of base and subsequent alkylation led to *ca.* 5 : 1 mixtures of the 4,6- and 2,4-dialkylated orthoformates, again in high overall yields.

Benzylation of the two free hydroxy groups in the 4-substituted allyl compound (**2**) gave the fully protected inositol (**3**) as an oil in 86% yield. Isomerisation of the allyl group to the enol ether,[14] followed by acidic hydrolysis of both the enol ether and orthformate protecting groups gave 2,6-dibenzyl-*myo*-inositol (**4**), m.p. 119—120.5 °C ($CHCl_3$–light petroleum, b.p. 60—80 °C), in 66% yield from (**3**). Phosphorylation of (**4**) with NaH and tetrabenzyl pyrophosphate in tetrahydrofuran (THF) proceeded readily in the presence of a catalytic quantity of either imidazole or 18-crown-6 to give the fully benzylated tetraphosphate (**5**) as an oil in 66—70% yield. Hydrogenolysis of the benzyl esters and ethers gave *myo*-inositol 1,3,4,5-tetraphosphate (**6**) in quantitative yield, conveniently isolated and stored as its crystalline cyclohexylammonium salt, m.p. 175—177 °C (H_2O–acetone); ^{1}H n.m.r.

† All new compounds displayed physical and spectral (360 MHz ^{1}H n.m.r. and mass spectra, h.p.l.c., elemental analysis, *etc.*) characteristics in full accord with their assigned structure.



Scheme 2. *Reagents and conditions*; Bn = CH_2Ph throughout. i, triethyl orthoformate, dimethylsulphoxide, toluene-*p*-sulphonic acid, 100 °C; ii, NaH (1 equiv.), *N*,*N*-dimethylformamide (DMF), allyl bromide (1 equiv.), 25 °C; iii, NaH (2.5 equiv.), DMF, BnBr (2.5 equiv.), 25 °C; iv, a, 90% EtOH, $RhCl(PPh_3)_3$, diazabicyclo[2.2.0]octane, reflux, b, 0.1 M HCl–MeOH, reflux, 15 min; v, NaH, THF, tetrabenzyl pyrophosphate $\{[(BnO)_2PO]_2O\}$, catalytic imidazole or 18-crown-6, 25 °C; vi, 10% Pd on C, EtOH–H_2O (80:20), H_2 (50 psi), 25 °C; vii, NaH (1 equiv.), DMF, $[(BnO)_2PO]_2O$ (1 equiv.), 25 °C; viii, a, as step vi, b, 80% trifluoroacetic acid–H_2O, 25 °C; ix, NaH (3 equiv.), DMF, BnBr (4 equiv.), 25 °C; x, 0.1 M HCl–MeOH, reflux, 15 min; xi, $(PhO)_2POCl$, CH_2Cl_2, Et_3N, catalytic 4-dimethylaminopyridine, 25 °C; xii, Li in liquid NH_3, −78 °C.

(D_2O) δ 3.84 (t, *J* 10 Hz, 1H, 6-H), 3.93 (td, *J* 10 and 3 Hz, 1H, 1-H), 3.95 (m, 1H, 5-H), 4.02 (td, *J* 10 and 3 Hz, 1H, 3-H), 4.31 (q, *J* 10 Hz, 1H, 4-H), and 4.32 (br. s, 1H, 2-H),[15] *m/z* fast-atom bombardment (FAB)$^+$ 600 (*M* + cyclohexylamine + H)$^+$, FAB$^-$ 499 (*M* − H)$^-$.

Benzylation of *myo*-inositol orthoformate (**1**) gave the fully-protected compound (**9**), m.p. 102—104 °C (light petroleum, b.p. 60—80 °C). Acidic hydrolysis of (**9**) gave 2,4,6-tribenzyl-*myo*-inositol (**10**), m.p. 83—84.5 °C (Et_2O–light petroleum, b.p. 60—80 °C) in 87% yield. Phosphorylation of (**10**) with diphenyl chlorophosphate gave an 82:18 mixture of the 1,3- and 1,5-diphosphorylated inositols, respectively, in 58% combined yield. The desired 1,3-isomer (**11**), m.p. 109—110 °C, was conveniently isolated from this mixture by selective crystallisation from diethyl ether–light petroleum (b.p. 60—80 °C), resulting in an 80% recovery of (**11**). Attempts to transesterify the diphenyl phosphate esters of (**11**) to dibenzyl phosphates resulted in decomposition. The alcohol (**11**) was deprotected directly using lithium in liquid ammonia[16] at −78 °C, which cleaved the diphenyl phosphate esters and the adjacent benzyl ethers without migration, giving *myo*-inositol 1,3-bisphosphate (**12**) in quantitative yield. The bisphosphate was isolated as its crystalline cyclohexylammonium salt, by ion exchange chromatography on Amberlite IR 120 in the H^+ form using water as eluant, m.p. 165—166 °C (H_2O–acetone), ^1H n.m.r. δ (D_2O) 3.40 (t, *J* 9 Hz, 1H, 5-H), 3.78 (t, *J* 9 Hz, 2H, 4- + 6-H), 3.96 (dt, *J* 9 and 3 Hz, 2H, 1- + 3-H), and 4.28 (t, *J* 3 Hz, 1H, 2-H); *m/z* FAB$^+$ 440 (*M* + cyclohexylamine + H)$^+$, FAB$^-$ 339 (*M* − H)$^-$.

The selective phosphorylation of (**1**) giving (**7**) allows a very short and efficient synthesis of *myo*-inositol 4-phosphate. Thus, hydrogenolysis of (**7**) followed by acidic hydrolysis of the orthoformate protecting group gave *myo*-inositol 4-phosphate (**8**) isolated as its crystalline cyclohexylammonium salt, m.p. 133—134 °C (H_2O–acetone), ^1H n.m.r. δ (D_2O) 3.41 (t, *J* 9 Hz, 1H, 5- or 6-H), 3.55 (dd, *J* 9 and 2.5 Hz, 1H, 1- or 3-H), 3.63 (dd, *J* 9 and 2.5 Hz, 1- or 3-H), 3.70 (t, *J* 9 Hz, 1H, 5- or 6-H), 4.05 (m, 1H, 2-H), and 4.11 (t, *J* 9 Hz, 1H, 4-H); *m/z* FAB$^+$ 360 (*M* + cyclohexylamine)$^+$, FAB$^-$ 259 (*M* − H)$^-$, in quantitative yield.

These new and efficient syntheses which make *myo*-inositol

1,3-bisphosphate and 1,3,4,5-tetraphosphate available for the first time will allow the use of these substrates in detailed investigations of this fundamental secondary messenger pathway.

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