Synthesis of *myo*-Inositol 1-Phosphate and 4-Phosphate, and of their Individual Enantiomers

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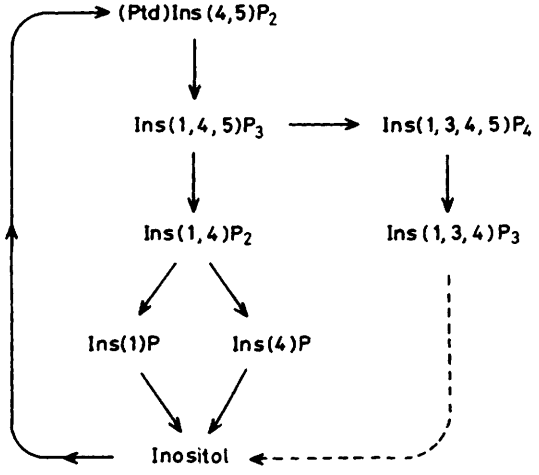
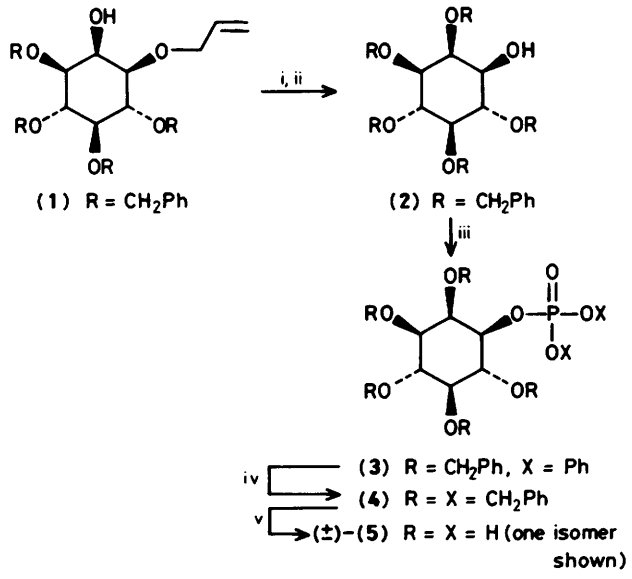
New methodology is described which allows the synthesis of *myo*-inositol 1-phosphate completely free of contamination by the 2-isomer, and novel resolution procedures have been developed for the preparation of the enantiomers of *myo*-inositol 1- and 4-phosphates.

A number of receptors of neurotransmitters, hormones, and other signals cause the hydrolysis of phosphatidyl inositol 4,5-bisphosphate [(Ptd)Ins(4,5)P_2] (Scheme 1), and effect a rise in cytosolic Ca^{2+} concentration.[1—3] The inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] liberated in this hydrolysis seems to act as a secondary messenger within the target cell, activating the release of Ca^{2+} from an intracellular store.[2] In addition the diacylglycerol released by this hydrolysis is also a second messenger, which is involved in the activation of protein kinase C. The major mechanism for terminating the action of Ins(1,4,5)P_3 is considered to be removal of the 5-phosphate group by a specific 5-phosphatase located in plasma membranes, and in the cytosol of stimulated cells.[4] Other phosphatases are then responsible for the degradation of the inositol 1,4-bisphosphate [Ins(1,4)P_2] formed in this hydrolysis, *via* inositol 1- and 4-phosphates, giving finally free inositol (Scheme 1), which is recycled in the brain to provide more (Ptd)Ins(4,5)P_2.[4,5] More recently another pathway of inositol phosphate metabolism has been demonstrated, since inositol 1,3,4-trisphosphate [Ins(1,3,4)P_3] has been detected in stimulated tissues, and evidence has been presented[6,7] that this arises *via* phosphorylation of Ins(1,4,5)P_3 to Ins(1,3,4,5)P_4 followed by hydrolysis of the 5-phosphate group giving Ins(1,3,4)P_3. As part of a programme to investigate the details of these fundamental pathways, we required effective syntheses of the individual enantiomers of a number of these naturally-occurring inositol phosphates. We report the first synthesis of inositol 1-phosphate free of contamination by the 2-phosphate isomer, a considerably improved synthesis of inositol 4-phosphate, and new and efficient procedures for the resolution of intermediates in these syntheses, giving access to the individual enantiomers of inositol 1-phosphate and inositol 4-phosphate.

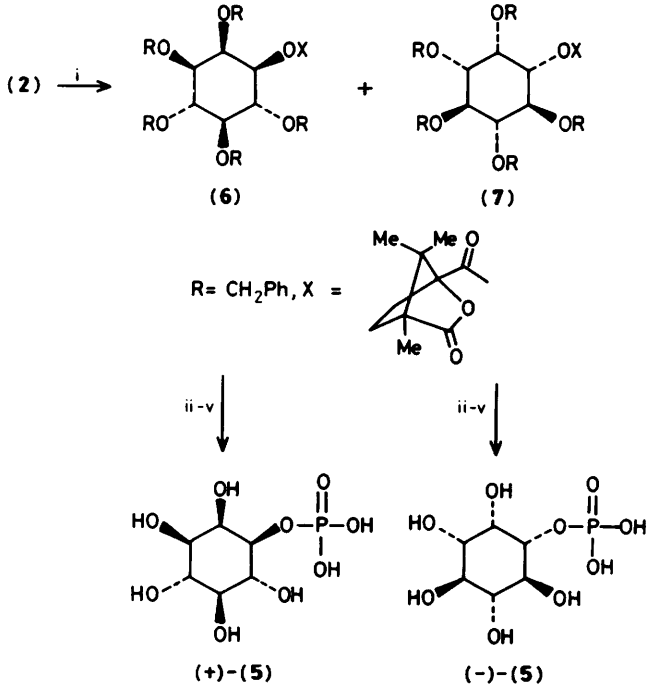
1-*O*-Allyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**1**) was prepared *via* 1,2-cyclohexylidene-*myo*-inositol by an amalgamation of the reported procedures.[8,9] Benzylation of (**1**) followed by removal of the allyl protecting group[10] gave 2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (**2**) in 70% yield (Scheme 2). Phosphorylation of (**2**) gave the diphenyl phosphate (**3**) in 90% yield. Deprotection of (**3**) using the reported conditions[8] (Pd/C, EtOH, H_2 and then PtO_2, EtOH, H_2) consistently gave mixtures of inositol 1-phosphate and inositol 2-phosphate. This migration presumably occurs *via* formation of a cyclic phenyl phosphate ester from the intermediate inositol 1-diphenyl phosphate which is formed during deprotection. We reasoned that replacement of the



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

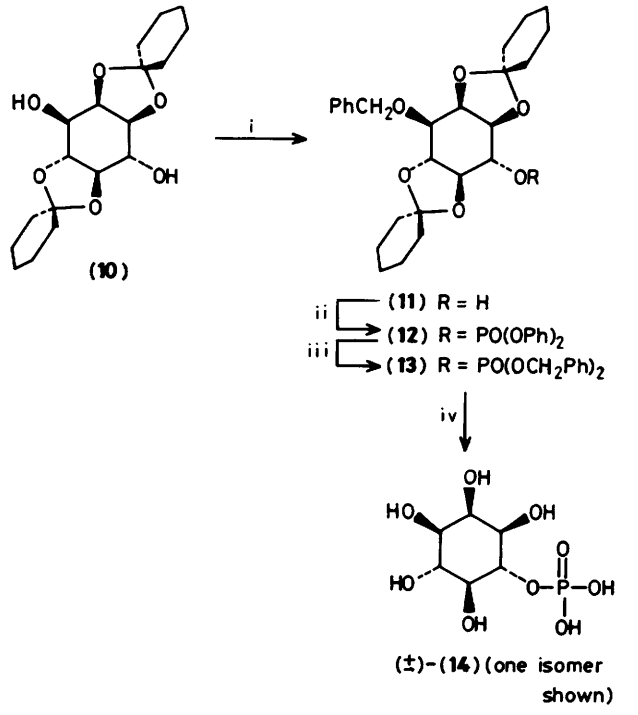


Scheme 2. *Reagents and conditions:* i, $PhCH_2Br$, NaH, dimethylformamide, 25 °C; ii, (a) 90% EtOH, $RhCl(PPh_3)_3$, diazabicyclo-[2.2.0]octane, reflux, (b) HOAc–tetrahydrofuran (THF)–H_2O (3 : 1 : 1), reflux; iii, $(PhO)_2POCl$, CH_2Cl_2, $(Et)_3N$, catalytic 4-dimethylaminopyridine (DMAP), 25 °C; iv, $PhCH_2OH$, NaH, THF, 25 °C; v, 10% Pd on C, EtOH–H_2O (80 : 20), H_2, 50 psi, 25 °C.



Scheme 3. *Reagents and conditions:* i, *R*(−)-camphanic acid chloride, CH_2Cl_2, $(Et)_3N$, DMAP, 25 °C; ii, KOH, EtOH, 25 °C; iii, $(PhO)_2POCl$, CH_2Cl_2, $(Et)_3N$, DMAP, 25 °C; iv, $PhCH_2OH$, NaH, THF, 25 °C; v, 10% Pd on C, EtOH–H_2O (80 : 20), H_2, 50 psi, 25 °C.

phenyl ester by a benzyl ester would suppress this migration and allow a clean single step deprotection, due to the very rapid cleavage of the benzyl esters. Alcohol (**2**) failed to react with either dibenzyl chlorophosphate or tetrabenzyl pyrophosphate directly; however, transesterification of (**3**) using

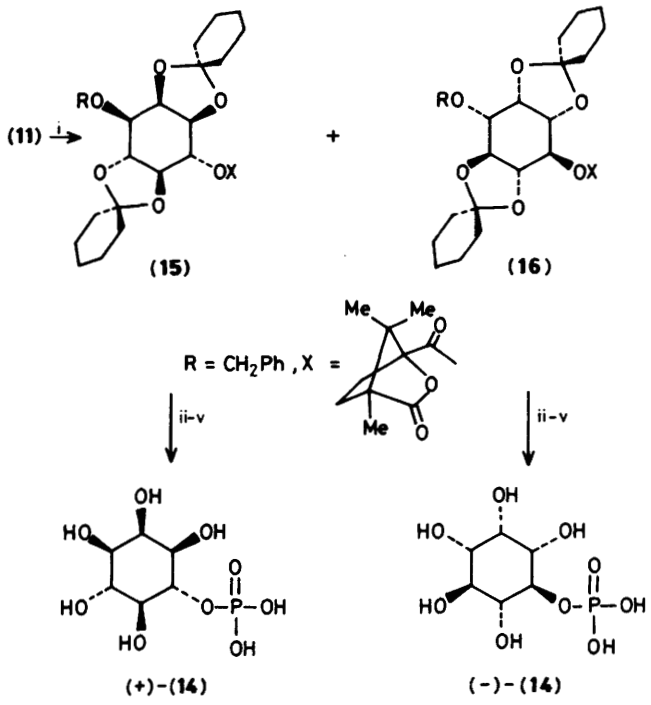


Scheme 4. *Reagents and conditions:* i, $PhCH_2Br$, NaH, PhMe, reflux; ii, $(PhO)_2POCl$, CH_2Cl_2, $(Et)_3N$, DMAP, 25 °C; iii, $PhCH_2OH$, NaH, THF, 25 °C; iv, 10% Pd on C, EtOH–H_2O (80 : 20), H_2, 50 psi, 25 °C.

the anion of benzyl alcohol in tetrahydrofuran (THF) as nucleophile gave the desired heptabenzyl inositol 1-phosphate (**4**) in 83% yield. Hydrogenolysis of (**4**) (Pd/C, EtOH–H_2O, H_2) cleanly cleaved all of the benzyl groups to give racemic inositol 1-phosphate (**5**), which was isolated as its crystalline biscyclohexylammonium salt, in 95% yield. Racemic inositol 1-phosphate prepared in this way has been shown to contain no detectable inositol 2-phosphate [by 360 MHz ^1H n.m.r. spectroscopy and h.p.l.c.: μ Bondapak NH_2, 3.9 mm × 30 cm (Waters Associates); 75mM ammonium formate buffer at pH 4, 1 cm^3/min].

The enantiomers of inositol 1-phosphate were obtained by resolution of the intermediate alcohol (**2**) (Scheme 3). Treatment of (**2**) with *R*(−)-camphanic acid chloride gave a mixture of diastereoisomeric camphanate esters (**6**) and (**7**) in quantitative yield. These esters were conveniently separated on a 10 g scale by flash chromatography [1% $(Et)_2O$–CH_2Cl_2], with a 70% overall recovery of the individual diastereoisomers [> 99.5% by h.p.l.c., μ Porasil (Waters Associates) 3.9 mm × 30 cm $(Et)_2O$–CH_2Cl_2 5 : 95 at 1 cm^3/min]. Single crystal *X*-ray analysis allowed the absolute configuration of the less polar diastereoisomer to be established as (**6**).† Hydrolysis of these

† *Crystal data* for (**6**): $C_{51}H_{54}O_9$, M = 810.98. Crystals formed from CH_2Cl_2, monoclinic, a = 6.147(1), b = 23.976(6), c = 15.030(3) Å, β = 98.68(2)°, U = 2189.65 $Å^3$, space group $P2_1$, Z = 2, D_c = 1.230 g cm^{-3}. Of the 3085 reflections measured using an automatic four-circle diffractometer with Cu radiation, 2806 were observed ($I > 3\sigma I$). The structure was solved with a multi-solution tangent formula approach and difference Fourier analysis and refined using full-matrix least squares techniques.[11] $\Sigma w(|F_o|-|F_c|)^2$ with $w = 1/\sigma F_o)^2$ was minimised to give R = 0.070. No abnormally short intermolecular contacts were noted. Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.



Scheme 5. *Reagents and conditions:* i, *R*(−)-camphanic acid chloride, CH_2Cl_2, $(Et)_3N$, DMAP, 25 °C; ii, KOH, EtOH, 25 °C; iii, $(PhO)_2POCl$, CH_2Cl_2, $(Et)_3N$, DMAP, 25 °C; iv, $PhCH_2OH$, NaH, THF, 25 °C; v, 10% Pd on C, EtOH–H_2O (80 : 20), H_2, 50 psi, 25 °C.

esters gave the corresponding enantiomeric forms of alcohol (**2**) in quantitative yield {$[\alpha]_D^{20}$ +9.10° and −9.0° (*c* 0.3, $CHCl_3$)}. Phosphorylation, transesterification, and hydrogenolysis of the benzyl protecting groups as for the racemic series gave the (+)- and (−)-inositol 1-phosphates (+)-(**5**) and (−)-(**5**), isolated as their crystalline biscyclohexylammonium salts {$[\alpha]_D^{20}$ +3.55° and −3.45° (*c* 1, H_2O at pH 9)}, in similar yields to the racemic series (Scheme 3).

It should be noted that a plane of symmetry between C-2 and C-5 is to be found in *myo*-inositol, and so C-3 is equivalent to C-1 and C-4 to C-6. Thus phosphorylation of a suitably protected inositol derivative at C-6 leads to a synthesis of *myo*-inositol 4-phosphate. 1,2 : 4,5-Dicyclohexylidene-*myo*-inositol (**10**) was prepared by the method of Garegg *et al.*[12] Selective benzylation of the more reactive 3-hydroxy group was achieved in 70% yield giving (**11**) which was phosphorylated in 75% yield to give (**12**) (Scheme 4). Transesterification of (**12**) to give the dibenzyl ester (**13**) proceeded in 70% yield. Hydrogenolysis of (**13**) with palladium on carbon in aqueous ethanol resulted in concomitant cleavage of the cyclohexylidene acetals to give (±)-inositol 4-phosphate (±)-(**14**) directly, in 60% yield. No evidence of the formation of isomeric inositol phosphates was apparent from 360 MHz ^1H n.m.r. spectroscopy or h.p.l.c. analysis (conditions as for the 1-phosphate).

Treatment of the alcohol (**11**) with *R*(−)-camphanic acid chloride gave a mixture of the diastereoisomeric camphanate esters (**15**) and (**16**) in quantitative yield (Scheme 5). The more polar diastereoisomer was obtained in >99% purity by direct crystallisation from the mixture, followed by two recrystallisations [2 : 1, light petroleum (b.p. 40—60) : ethyl acetate], and the less polar diastereoisomer was recovered from the combined mother liquors by medium pressure liquid chromatography on silica gel [Licroprep Silica 60 (Merck) 3 : 1 light petroleum (b.p. 40—60) : ethyl acetate at 5 cm^3/min]. H.p.l.c. analysis [μ Porasil (Waters Associates) 3.9 mm × 30 cm, 3 : 1, light petroleum (b.p. 40—60) : ethyl acetate at 2 cm^3/min] indicated purity of >99% for each diastereoisomer. Hydrolysis of the esters gave the corresponding enantiomeric forms of the alcohol (**11**) in quantitative yield, {$[\alpha]_D^{20}$ = −26.07 and +25.90° (*c* 1.1, $CHCl_3$)}. Phosphorylation, transesterification, and hydrogenolysis of the resulting enantiomeric benzyl esters as in the racemic case gave the enantiomers of inositol 4-phosphate (+)-(**14**) and (−)-(**14**) {$[\alpha]_D^{20}$ = + 1.1 ± 0.3° and −1.30 ± 0.2° (*c* 5, H_2O, pH 9)} in similar yields to the racemic series.

The resolved alcohol (**11**) should also be a useful intermediate for the preparation of the enantiomers of inositol 1,4-bisphosphate. These novel resolution procedures thus represent a significant advance in the synthesis of these interesting natural substrates and enable sufficiently pure materials to be provided for investigation into the detailed biochemical pathways.

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