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

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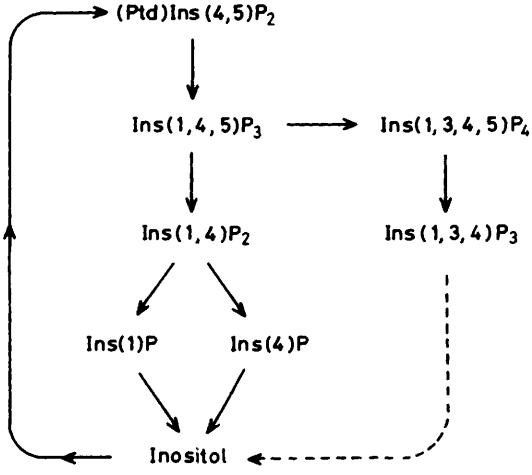
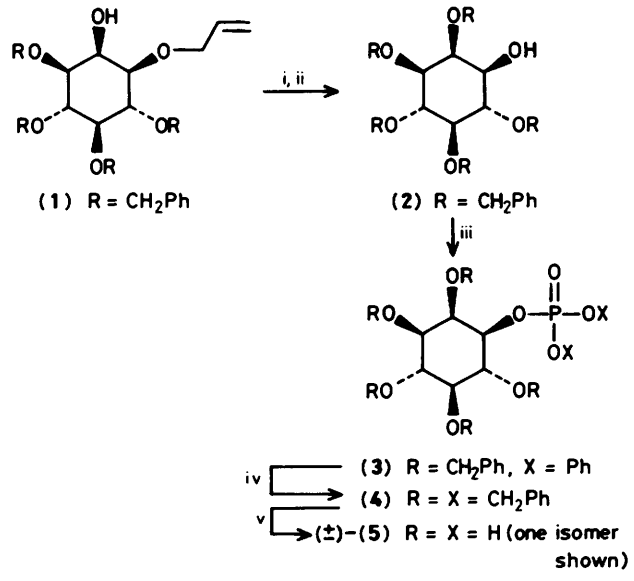
New methodology is described which allows the synthesis of *myo*-inositol 1-phosphate completely free of contamination by the 2-isomer, and novel resolution procedures have been developed for the preparation of the enantiomers of *myo*-inositol 1- and 4-phosphates.

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.

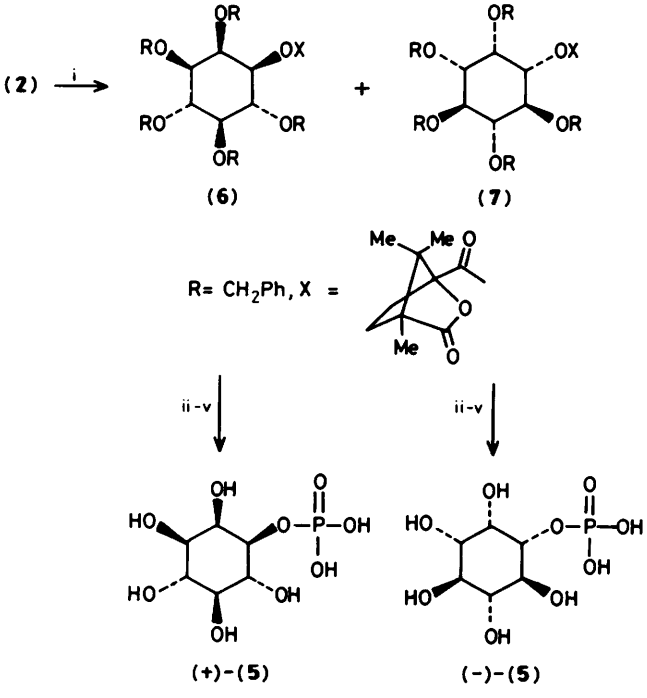
1-*O*-Allyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (**1**) was prepared *via* 1,2-cyclohexylidene-*myo*-inositol by an amalgamation of the reported procedures.[8,9] Benzylation of (**1**) followed by removal of the allyl protecting group[10] gave 2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (**2**) in 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.

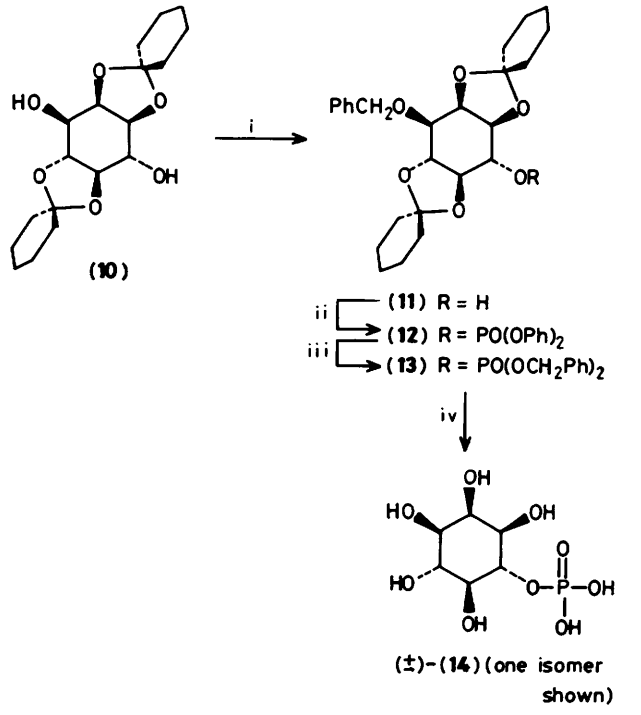


Scheme 2. *Reagents and conditions:* i, $PhCH_2Br$, NaH, dimethylformamide, 25 °C; ii, (a) 90% EtOH, $RhCl(PPh_3)_3$, diazabicyclo-[2.2.0]octane, reflux, (b) HOAc–tetrahydrofuran (THF)–H_2O (3:1:1), reflux; iii, $(PhO)_2POCl$, CH_2Cl_2, $(Et)_3N$, catalytic 4-dimethylaminopyridine (DMAP), 25 °C; iv,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

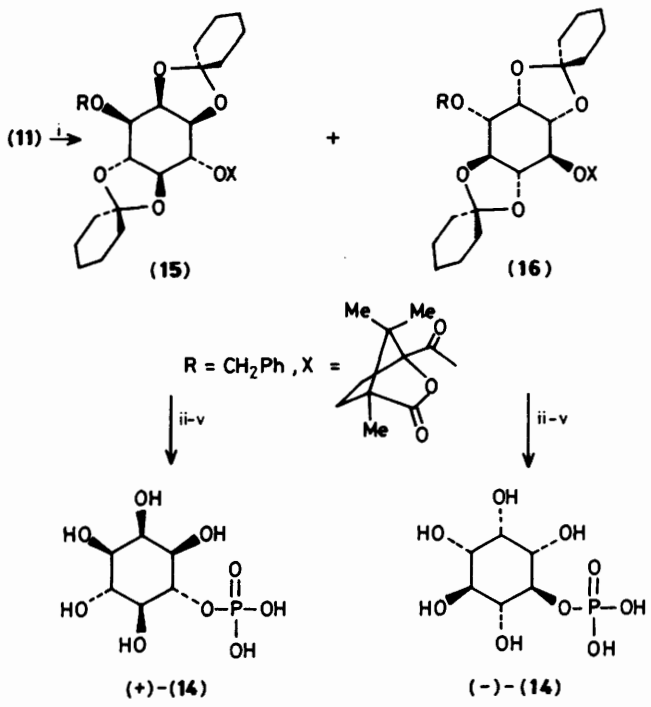


Scheme 4. *Reagents and conditions:* i, $PhCH_2Br$, NaH, PhMe, reflux; ii, $(PhO)_2POCl$, CH_2Cl_2, $(Et)_3N$, DMAP, 25 °C; iii, $PhCH_2OH$, NaH, THF, 25 °C; iv, 10% Pd on C, EtOH–H_2O (80:20), H_2, 50 psi, 25 °C.

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.

We thank Drs. J. Springer and J. Hirshfield, MSD Rahway, for the *X*-ray structure determination.

Received, 4th September 1986; Com. 1274

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Typeset and printed by Black Bear Press Limited, Cambridge, England