Synthesis of 2- and 6-Deoxyinositol 1-Phosphate and the Role of the Adjacent Hydroxy Groups in the Mechanism of Inositol Monophosphatase

Raymond Baker,* Janusz J. Kulagowski, David C. Billington, Paul D. Leeson, Ian C. Lennon, and Nigel Liverton

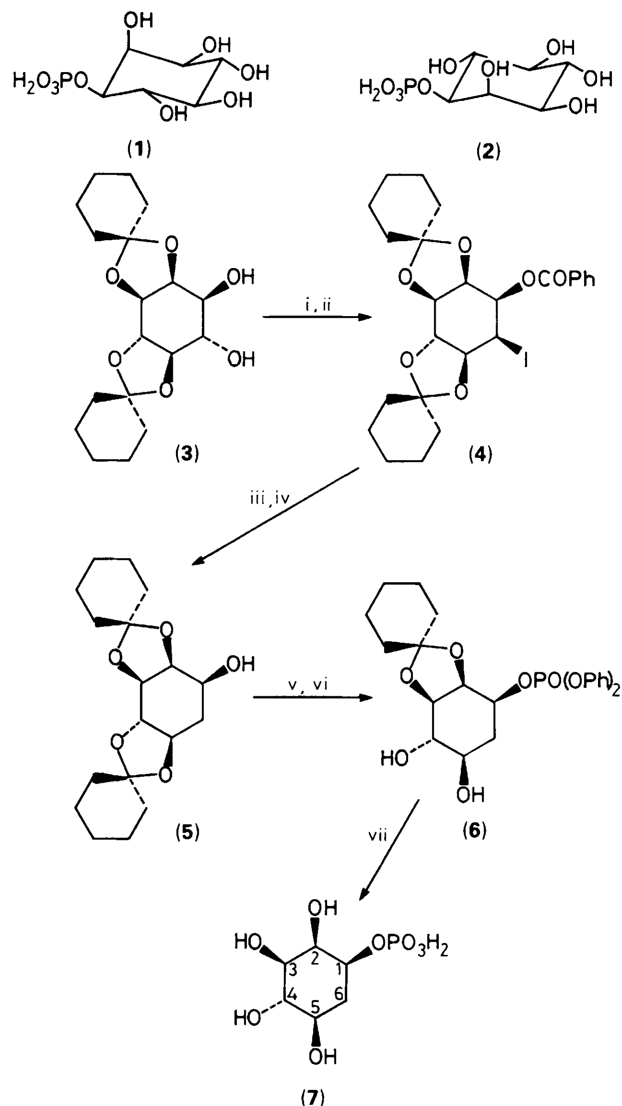
Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, U.K.

The 2- and 6-hydroxy groups of *myo*-inositol 1-phosphate have been shown to be independently associated with the mechanisms of hydrolysis and binding in the dephosphorylation of the substrate by inositol monophosphatase.

The receptor-mediated action of phospholipase C on phosphatidylinositol 4,5-bisphosphate† has been firmly established to yield the secondary messenger, *myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] which mediates the release of Ca^{2+} from intracellular stores.[1] Metabolism of Ins(1,4,5)P_3 then takes place *via* inositol 1,4-bisphosphate [Ins(1,4)P_2] and, subsequently inositol 4-phosphate [Ins(4)P] to yield inositol which is recycled in the brain to provide phosphatidylinositol 4,5-bisphosphate.[2] An additional metabolic pathway involves the phosphorylation of Ins(1,4,5)P_3 to inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4], which may play a role in controlling Ca^{2+} influx into the stimulated cell.[3] Ins(1,3,4,5)P_4 is sequentially dephosphorylated to inositol 1,3,4-trisphosphate [Ins(1,3,4)P_3] and then *via* inositol bisphosphates to the enantiomers of inositol 1-phosphate. These monophosphates are then hydrolysed to inositol by the action of a single monophosphatase enzyme.[4] Inositol monophosphatase hydrolyses both enantiomers of Ins(1)P and Ins(4)P, and has been purified to homogeneity from bovine brain.[5] The observation that both enantiomers of Ins(1)P, (**1**) and (**2**), and β-glycerophosphate derivatives,[6] are substrates for this enzyme suggests a notable lack of substrate specificity, and led us to question the roles and interactions of the 2- and 6-hydroxy groups of inositol 1-phosphate in the active site. In this Communication we report the synthesis of 2-deoxy and 6-deoxyinositol 1-phosphate and the resolution of the 2-deoxy derivative into its individual enantiomers. The relative substrate reactivities and binding affinities of these analogues for inositol monophosphatase indicate the importance of both the 2- and 6-hydroxy groups of the natural substrates in maintaining efficient enzymic hydrolysis.

Reaction of 2,3:4,5-dicyclohexylidine *myo*-inositol (**3**)[7] with benzoyl chloride in pyridine gave the 1-benzoyl derivative (70% yield) which on treatment with iodine and triphenylphosphine in the presence of imidazole gave the 6-iodo

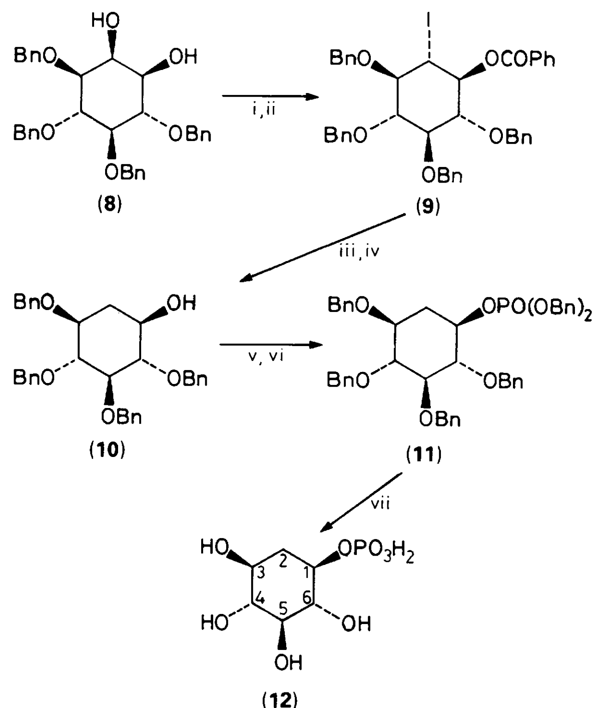
† 'Inositol' refers to the *myo*-inositol stereochemistry throughout.



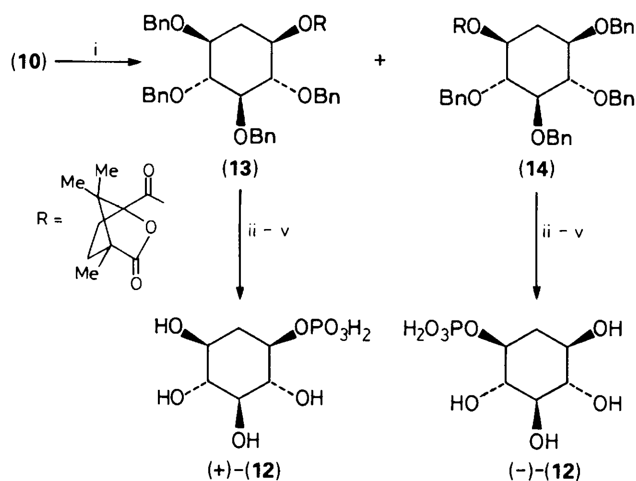
Scheme 1. *Reagents and conditions*: i, PhCOCl, pyridine, 25 °C; ii, I_2, imidazole, $(Ph)_3P$, toluene, reflux; iii, $(Bu)_3SnH$, azoisobutyronitrile (AIBN, catalytic), toluene, reflux; iv, KOH, EtOH, 25 °C; v, $(PhO)_2POCl$, CH_2Cl_2, $(Et)_3N$, 4-*N*,*N*-dimethylaminopyridine (DMAP), 25 °C; vi, SiO_2, Et_2O then Me_2CO; vii, PtO_2, EtOH, H_2 atmospheric pressure, 25 °C.

derivative (**4**) in 72% yield (Scheme 1). Reductive dehalogenation of (**4**) with tributyltinhydride in the presence of a radical initiator, followed by hydrolysis of the benzoyl group gave alcohol (**5**) in 61% overall yield from (**4**). Phosphorylation of (**5**) with diphenylchlorophosphate followed by chromatography on silica gel gave a 68% yield of the protected diol phosphate (**6**). Hydrogenation of (**6**) over a platinum catalyst caused concomitant phosphate deprotection and acetal hydrolysis and gave (±)-6-deoxyinositol 1-phosphate (**7**) isolated in 82% yield, as its crystalline biscyclohexylammonium salt.‡

Synthesis of (±)-2-deoxyinositol 1-phosphate (**12**) followed a similar strategy, starting from 3,4,5,6-tetra-*O*-benzyl inositol[8] (**8**) (Scheme 2). Formation of the cyclic stannylene from



Scheme 2. *Reagents and conditions*: i, (a), $(Bu)_2SnO$, MeOH, 25 °C; (b), PhCOCl, $(Et)_3N$, 25 °C; ii, I_2, $(Ph)_3P$, imidazole, toluene, reflux; iii, $(Bu)_3SnH$, AIBN (catalytic), toluene, reflux; iv, KOH, EtOH, 25 °C; v, $(PhO)_2POCl$, CH_2Cl_2, $(Et)_3N$, DMAP, 25 °C; vi, $PhCH_2OH$, NaH, tetrahydrofuran (THF), 25 °C; vii, 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.

(**8**) using dibutyltin oxide,[9] followed by reaction with benzoyl chloride gave the 1-benzoyl derivative (62% yield) which was halogenated as previously to give the 2-iodo derivative (**9**) in 83% yield. Dehalogenation of (**9**) and ester hydrolysis as for the 6-deoxy series gave the alcohol (**10**) in 53% overall yield from (**9**). Phosphorylation of (**9**) with diphenylchlorophosphate, followed by transesterification using the anion of benzyl alcohol[10] gave the dibenzylphosphate (**11**) in 80% yield from (**10**). Hydrogenolysis of (**11**) over palladium at 50 psi H_2 resulted in cleavage of all of the benzyl groups, and gave

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

(±)-2-deoxyinositol phosphate (**12**) isolated, in 95% yield, as its crystalline biscyclohexylammonium salt.

The abilities of (±)-2-deoxyinositol 1-phosphate (**12**) and (±)-6-deoxyinositol 1-phosphate (**7**) to act as substrates or inhibitors of the hydrolysis of (±)-inositol 1-phosphate by inositol monophosphatase was examined using standard methods.[2,5] Both (**7**) and (**12**) were competitive inhibitors of inositol monophosphatase activity, with $IC_{50} \simeq 70$ μM. Incubation of (**7**) or (**12**) with inositol monophosphatase led to no release of inorganic phosphate, indicating that both (**7**) and (**12**) were not substrates of the monophosphatase. From these observations it was concluded that both hydroxy groups α to the phosphate are required for efficient substrate hydrolysis by the monophosphatase. The role of these flanking hydroxy groups was further examined following resolution of 2-deoxyinositol 1-phosphate.

Resolution of the alcohol (**10**) was achieved by conversion into the diastereoisomeric camphanate esters[10] (**13**) and (**14**) by treatment with (−)-camphanic acid chloride (Scheme 3). Separation of these diastereoisomers by column chromatography on silica gel (mobile phase $EtOAc/CH_2Cl_2$ gradient 0—5% EtOAc) gave (−)-(**13**) ($[\alpha]_D^{25} = -10.5°$, *c* 1.5, CH_2Cl_2) and (+)-(**14**) ($[\alpha]_D^{25} = +3.5°$, *c* 1.8, CH_2Cl_2) in greater than 98% diastereoisomeric excess [as determined by h.p.l.c., μ porasil (Waters Associates) 3.9 mm × 30 cm, $EtOAc/CH_2Cl_2$, 2.5: 97.5 at 1 cm^3/min]. The absolute configuration of (+)-(**14**) was determined by single crystal *X*-ray analysis§ to be *S,R,S,R,R* as shown in Scheme 3. Hydrolysis of the ester groups in (−)-(**13**) and (+)-(**14**) gave the enantiomers of alcohol (**10**), which were converted into (+)-(**12**) and (−)-(**12**) respectively by the methods previously used in the racemic series {(+)-(**12**), $[\alpha]_D^{25} = +7.10°$, *c* 1.7, H_2O pH 9; (−)-(**12**), $[\alpha]_D^{25} = -8.2°$, *c* 1.6, H_2O pH 9}. Evaluation of the biological activity of (+)- and (−)-(**12**) suggests differing roles for the α-hydroxy groups in recognition by the enzyme. One enantiomer, (−)-(**12**), was a competitive inhibitor of monophosphatase activity, with IC_{50} of 50 μM, and was not hydrolysed by the pure enzyme. In contrast, the other enantiomer [(+)-(**12**)] showed little inhibitory potency against monophosphatase activity, but was slowly hydrolysed by the pure enzyme, indicating that it has substrate activity [V_{max} 78% of Ins(1)P; K_m 1.3 mM]. These results taken together with those previously obtained for (±)-(**7**) and (±)-(**12**) suggest that the α-hydroxy groups have discrete and different roles in inositol monophosphatase activity towards substrates.

We propose that substrate recognition by inositol monophosphatase requires a binding interaction of one α-hydroxy with the active site, whilst the other flanking hydroxy contributes little to the binding of the substrate, but is, in some way, involved in the mechanism of phosphate hydrolysis. Thus (+)-(**12**) possesses the mechanistic hydroxy, but not the binding hydroxy, resulting in weak substrate activity. In

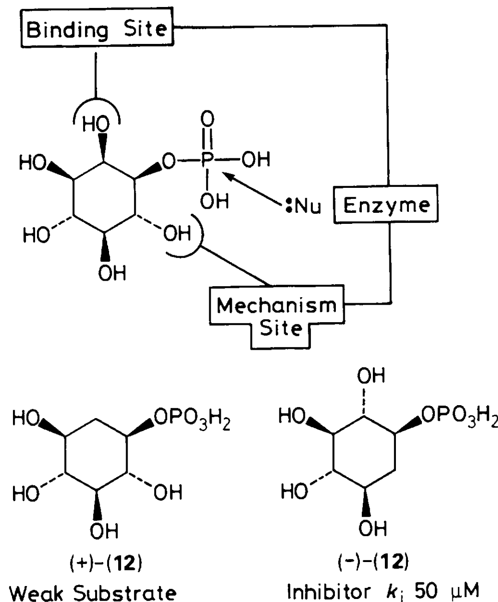


Figure 1

contrast (−)-(**12**) displays good competitive inhibitory potency but no substrate activity which can be correlated with the presence of the binding hydroxy but absence of the mechanistic hydroxy group (Figure 1). The function of the mechanistic hydroxy in the dephosphorylation reaction has not yet been established. Proton transfer from this hydroxy to the phosphate ester oxygen, or co-ordination with magnesium, a necessary cofactor, may be involved. The results obtained have, therefore, yielded important information on the differing roles played by two hydroxy groups in the hydrolysis of *myo*-inositol 1-phosphate by inositol monophosphatase.

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§ *Crystal data*: $C_{44}H_{48}O_8$, M = 704.9, monoclinic, space group $P2_1$, crystallized from ethyl acetate/hexane, a = 12.321(2), b = 9.742(1), c = 15.954(2) Å, β = 93.74(1)°, D_c = 1.225, Z = 2. An automatic four circle diffractometer equipped with Cu-K_α radiation (λ = 1.5418 Å) was used to measure 2798 potential diffraction peaks of which 1901 were observed ($I \geqslant 3\sigma I$). The following library of crystallographic programs was used to solve the structure: SHELXS-86, G. M. Sheldrick, Univ. of Gottingen, Gottingen, West Germany (1986); PLUTO, W. D. S. Motherwell and W. Clegg, University of Cambridge, Cambridge, England (1978); SDP Plus V1.1, Y. Okaya and B. A. Frenz, B. A. Frenz and associates, College Station, Texas (1984). The function $\Sigma\omega(|F_o|-|F_c|)^2$ with $\omega = 1/(\sigma F_o)^2$ was minimized with full matrix least squares to give an unweighted residual of 0.046 (R_w = 0.053). Atomic co-ordinates, thermal parameters, bond lengths, and bond angles have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

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