

Synthesis and Properties of Mechanism-based Inhibitors and Probes for Inositol Monophosphatase derived from 6-*O*-(2'-Hydroxyethyl)-(1*R*,2*R*,4*R*,6*R*)-cyclohexane-1,2,4,6-tetraol

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The 1-phosphate, 2'-phosphate and 1,2'-cyclic phosphate of 6-*O*-(2-hydroxyethyl)-cyclohexane-1,2,4,6-tetraol are synthesised and found to be good inhibitors of inositol monophosphatase; the stereochemistry of the most potent inhibitor, the (1*R*,2*R*,4*R*,6*R*)-1-phosphate, provides useful mechanistic insight into the action of this enzyme, the putative target for lithium therapy.

In brain cells the action of inositol monophosphatase provides inositol for the biosynthesis of the secondary messenger precursor, phosphatidylinositol 4,5-bisphosphate.^{1,2} The enzyme catalyses the hydrolysis of a range of phosphate esters including both enantiomers of *myo*-inositol 1-phosphate (Ins 1-P) **1** and *myo*-inositol 4-phosphate (Ins 4-P), ethane-1,2-diol phosphate and 2'-ribonucleoside phosphates and is believed to be the target for lithium therapy.^{3,4} Recent work has established that the enzyme operates *via* a direct displacement mechanism, in which water is the attacking nucleophile,^{3,5} and that two Mg²⁺ cofactors are required for hydrolysis.^{4,6,7} While there is consensus on the structure of, and the interactions within, the active enzyme–2Mg²⁺–substrate complex (Fig. 1),^{4,6,7} the catalytic roles recently ascribed to the two Mg²⁺ ions differ slightly.^{4,7} We favour a pseudo-rotation mechanism where Mg-2 binds to and activates the nucleophilic water molecule for attack on the phosphate P-atom^{6,7} [Fig. 1(a)] while others favour an in-line displacement mechanism in which Mg-1 activates the nucleophile⁴ [Fig. 1(b)].[†] Based on our own analysis, the 6-OH group of D-Ins 1-P forms an H-bond with the nucleophilic water molecule located on Mg-2, Fig. 1(a).⁷ Here we report on the synthesis and properties of D-Ins 1-P analogues in which the nucleophile and its H-bonding interaction are replaced by a pendant 6-*O*-2'-hydroxyethyl group and, also, some related compounds.

It was well established that the 6-OH group of D-Ins 1-P **1** is essential for catalytic activity and that its replacement by a hydrogen atom or an alkoxy or alkyl group gives tight-binding inhibitors.^{8–10} It was also known that the 3- and 5-OH groups are unable to bind to the enzyme.¹⁰ Our initial target was, therefore, 6-*O*-(2'-hydroxyethyl)-cyclohexane-1,2,4,6-tetraol 1-phosphate **2** which modelling studies⁷ had indicated should bind such that the pendant 2'-OH group of the (1*R*,2*R*,4*R*,6*R*)-enantiomer could chelate to Mg-2 in place of the nucleophilic water, Fig. 2.

To test this possibility, the racemic epoxides **7** and **8** were prepared using modifications of literature procedures^{9,11–13} as outlined in Scheme 1. The separated isomer **7** was converted to the bis-benzyl ether **9** and was treated under several different sets of conditions with lithium or sodium 2-benzyloxyethoxide. In each case either no reaction ensued or elimination occurred to

give the unwanted cyclohex-5-ene-1,2,4-triol derivative. However, under mild Lewis acidic conditions in the presence of a catalytic amount of boron trifluoride–diethylether, the epoxide **9** opened smoothly to give the required alcohol **10**. Phosphorylation of **10** gave the racemic diphenylphosphate triester and transesterification yielded the required dibenzyl triester **11**.⁸ Reductive removal of the benzyl groups was achieved in 70% yield and the resulting phosphate salt was purified and

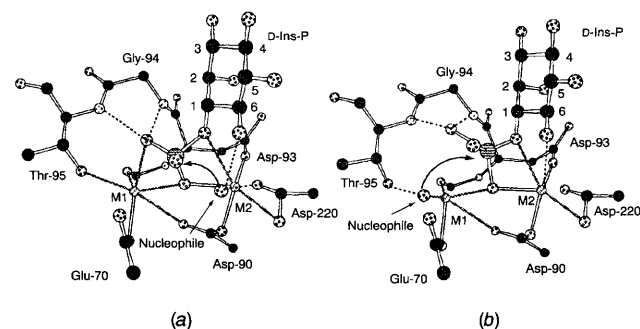
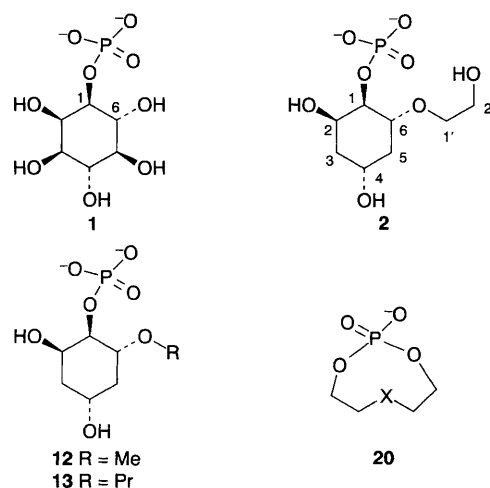


Fig. 1

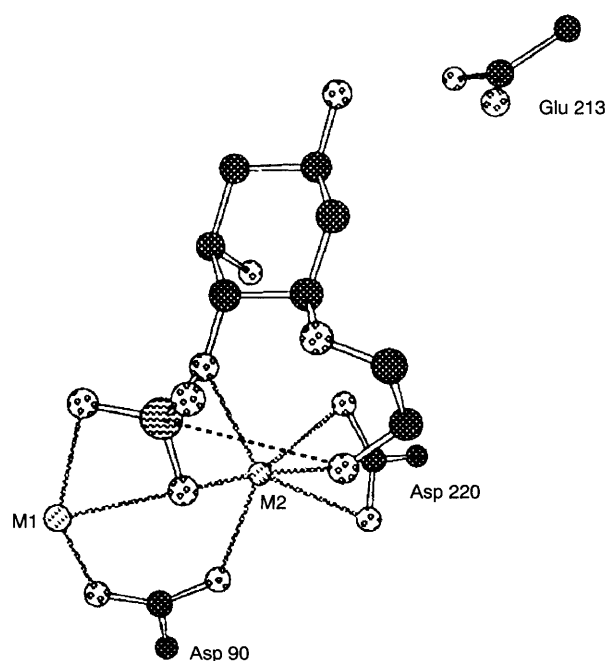


Fig. 2 Pendant arm of 2

When tested in standard enzyme assays,[§] the racemic 6-methyl ether **12** behaved as a competitive inhibitor and displayed a K_i value of $2.5 \mu\text{mol dm}^{-3}$. This value is almost identical to that reported for the 6-deoxy analogue⁸ and is in accord with the earlier finding that 6-*O*-methylinositol phosphate is an inhibitor. Also in accord with expectations⁷⁻¹⁰ the 6-propyl ether **13** served as a competitive inhibitor and gave a K_i value of $1.2 \mu\text{mol dm}^{-3}$.⁷ Most interestingly, the 6-*O*-2'-hydroxyethyl analogue **2** also served as a competitive inhibitor and displayed a K_i value of $1.8 \mu\text{mol dm}^{-3}$.

The reaction scheme illustrates the synthesis of 14a and 14b from 1,3-cyclohexanediol (3). The sequence of reactions is as follows:

- 3** (1,3-cyclohexanediol) is converted to **4** (1-(benzyloxy)-2-methylenecyclohexane) via steps i, ii.
- 4** is converted to **5** (1-(benzyloxy)-2-oxaspiro[3.5]non-2-ene) via step iii.
- 5** is converted to **6** (1-(benzyloxy)-2-methylenecyclohexanol) via step iv.
- 6** is converted to **7** (1-(benzyloxy)-2-methylenecyclohexanol) via steps v, vi.
- 7** is converted to **9** (1-(benzyloxy)-2-methylenecyclohexanol) via step vii.
- 9** is converted to **10** (1-(benzyloxy)-2-methylenecyclohexanol) via step viii.
- 10** is converted to **11** (1-(benzyloxy)-2-methylenecyclohexanol) via steps ix, x.
- 11** is converted to **2** (1-(benzyloxy)-2-methylenecyclohexanol) via step xi.
- 11** is converted to **14a and 14b** (1-(benzyloxy)-2-methylenecyclohexanol) via steps xii, xiii, and xiv.

In order to further test these ideas it was reasoned that, if the pendant arm could exist in the position predicted for the bound form of compound **2** (Fig. 2), then moving the phosphate group from the cyclohexane 1-position to the 2'-position **15** should give a potent competitive inhibitor. Note that **15** is the hypothetical intramolecular transesterification product of compound **2**, Fig. 2. To prepare isomer **15** the epoxide **9** was reacted with 2-hydroxyethyl dibenzyl phosphate to give the triester **16** (Scheme 2). Dephosphorylation of the starting material and the product caused the yield of triester **16** to be low (13%) but this strategy circumvented the extra hydroxy group protection and deprotection steps required for an alternative linear synthesis. The triester was deprotected and converted to its bis-cyclohexylammonium salt **15** in 60% yield. When tested in enzyme assays, the racemic material served as a good competitive inhibitor and displayed a K_i value of $8.5 \mu\text{mol dm}^{-3}$. Compound **15** is the most potent primary alkyl phosphate ester inhibitor known,⁶ and this finding provides strong support for the validity

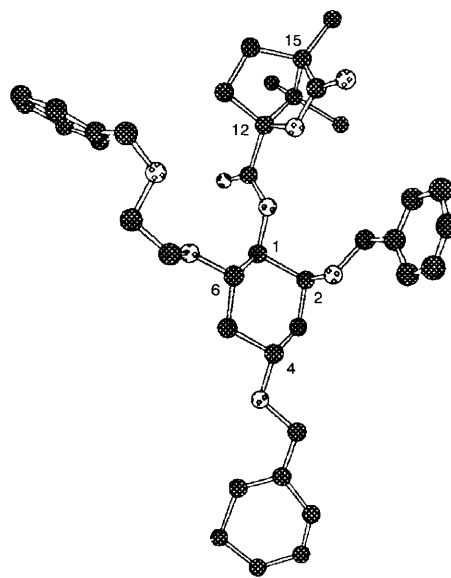
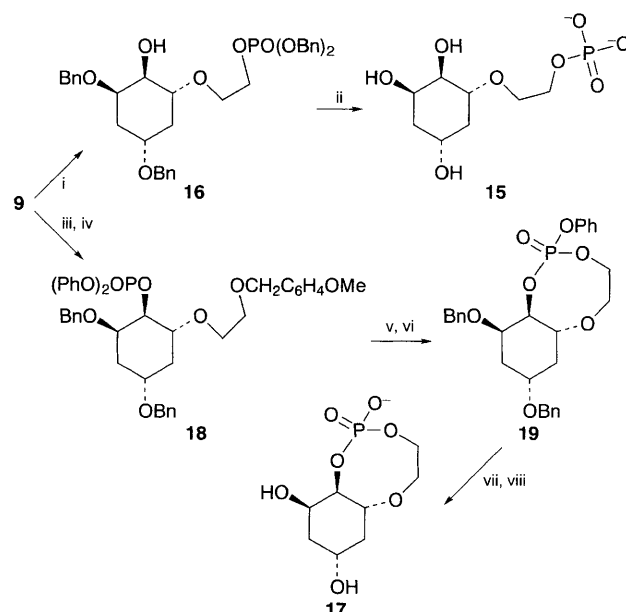


Fig. 3 X-ray structure of **14b**



Scheme 2 i, $\text{HOC}_2\text{H}_4\text{OPO}(\text{OBn})_2$, $\text{BF}_3 \cdot \text{OEt}_2$, 25 °C, 4 h, 13%; ii, Na-NH_3 , -78 °C, 0.5 h, 60%; iii, $\text{HOC}_2\text{H}_4\text{OCH}_2\text{C}_6\text{H}_4\text{OMe}$, 4 h, 50%; iv, $\text{ClPO}(\text{OPh})_2$, NEt_3 , DMAP, CH_2Cl_2 , 25 °C, 2 h, 95%; v, DDQ, CH_2Cl_2 , 25 °C, 1 h, 80%; vi, NaH , THF, -78 °C, 2 h, 50%; vii, NaH , BuOH , THF, -78 °C, 2 h, 60%; viii, Na-NH_3 , -78 °C, 0.5 h, 60%

of the modelling and for the predicted position of the pendant arm.⁷

It was also of interest to examine the properties of the cyclic phosphate diester **17** in which the 1- and 2'-O-atoms are linked. Compound **17** resembles the transition state for the hypothetical transesterification of monoester **2** to **4**, but is not an accurate mimic because the ester P-O bonds are too short and the P-atom is tetrahedral rather than pentacoordinate. Nevertheless, the diester is conformationally restrained and was expected to be able to bind to the enzyme.^{6,7}

To prepare the diester **17**, epoxide **9** was treated with 2-(*p*-methoxybenzyloxy) ethanol, as outlined in Scheme 2, and the resulting alcohol was phosphorylated to give the diphenyl phosphate ester **18**. Removal of the *p*-methoxybenzyl protection with DDQ (DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone) released the free primary alcohol which was deprotonated to facilitate 8-membered ring closure. The resulting cyclic triester **19** was converted *via* the benzyl phosphate cyclic triester to the required cyclic diester **17**[‡] which was purified and stored as its cyclohexylammonium salt. The racemic diester **17** served as a moderate competitive inhibitor and displayed a K_i value of 160 $\mu\text{mol dm}^{-3}$ suggesting that K_i for the active enantiomer is *ca.* 80 $\mu\text{mol dm}^{-3}$. This is a remarkable result given that the compound is a monoanion and strongly supports the validity of the modelling work.⁷ The K_i value is the lowest, by far, that has been obtained for any monoanion and compares favourably with the values of *ca.* 8500 $\mu\text{mol dm}^{-3}$ obtained for the cyclic diesters (**20**, X = O and CH₂)⁶ and >50 000 $\mu\text{mol dm}^{-3}$ obtained for dimethyl phosphate. Note that the dianions of Ins 1-P ($K_i = 100 \mu\text{mol dm}^{-3}$)³ and its phosphonate analogue ($K_i > 2000 \mu\text{mol dm}^{-3}$),⁷ which can form H-bonding interactions between the inositol OH groups and the enzyme, do not bind better than diester **17**.

All of the target compounds described here were also tested as substrates for the enzyme using the phosphomolybdate inorganic phosphate release assay.³ No substrate activity was detected. Since it was possible that (1*R*,2*R*,4*R*,6*R*)-tetraol 1-phosphate **2** might undergo enzyme catalysed transesterification to give **15**, (1*R*,2*R*,4*R*,6*R*)-**2** (7 μmol) was incubated with the enzyme (and in a control, without the enzyme) in a 5 mm NMR tube (total volume *ca.* 0.5 cm³) and the reaction was monitored by 500 MHz ¹H NMR spectroscopy. Except in the presence of vast excesses of enzyme, no reaction occurred. In the presence of 20 units (1 unit hydrolyses 1 μmol of substrate **1** per minute at 37 °C) of enzyme (*ca.* 1 mg) a slow phosphate ester hydrolysis reaction occurred ($t_{0.5} = 18 \text{ h}$ at 37 °C), but no transesterification was observed. The expected hydrolysis product[‡] was synthesised independently from **10** and showed identical spectral parameters. We cannot rule out the possibility that this very low level of hydrolysis activity was due to a contaminating enzyme. The entire NMR experiment was repeated for **15** and again no transesterification product was observed. All three compounds **2**, **15** and **17** were stable to hydrolysis in the presence of 1 unit of enzyme, overnight, as determined by NMR spectroscopy.

The finding that **2** and **15** are stable to enzymic interconversion is not surprising since the inclusion of the O⁶-ethylene bridge must disrupt the H-bonding network between the potential nucleophile, 6-O-atom and the side-chain of Asp-220 at the active site.^{4,7} Inhibitors **2** and **15** bind tightly to the enzyme, as they were designed to do, and form much more stable complexes (by 20–50-fold) than the equivalent complexes derived from substrates.⁹ Therefore, it seems reasonable to believe that the complexes are too stable to access the energy level of the transesterification transition state. This interpretation is completely consistent with the observation that replacement of the 6-OH group in substrates by hydrogen⁸ or small alkoxy groups gives potent competitive inhibitors which bind with 30-fold higher affinity than the parent substrates and, also, with the suggestion that H-bonding interactions involving the 6-OH group destabilise the ground-state of substrate

complexes.⁷ The dual facts that inhibitors **2** and **15** bind tightly to the enzyme and that the cyclic diester monoanion shows a marked affinity for the enzyme is in accord with earlier studies^{4,7} which indicate that a water molecule is associated with Mg-2.^{4,6}

The authors thank the BBSRC and EPSRC for grants J26649, J29893 and K35792, the Wellcome Trust for grant 040331, Merck, Sharp and Dohme, Harlow, UK for a general support award to D. G. and Roger Pybus and Emmanuelle Beushausen for performing enzyme assays.

Received, 6th July 1995; Com. 5/04403K

Footnotes

† A full consideration of the evidence supporting each mechanism is given in reference 7.

‡ All compounds and their intermediates were fully characterised and gave the expected spectral and analytic data. *Selected data* for **2** (Cha = cyclohexylamino): δ_{H} (500 MHz; ²H₂O), 1.1–1.2 (2 H, m, 4-H of Cha), 1.28–1.4 (8 H, m, 2-H and 3-H of Cha), 1.4–1.48 (1 H, m, 5-H), 1.55–1.6 (1 H, m, 3-H), 1.6–1.68 (2 H, m, 4-H of Cha), 1.75–1.85 (4 H, m, 3-H of Cha), 1.92–2.02 (4 H, m, 2-H of Cha), 2.05–2.14 (1 H, m, 3-H), 2.28–2.32 (1 H, m, 5-H), 3.1–3.2 (2 H, m, 1-H of Cha), 3.62–3.8 (5 H, m, C₂H₄, 6-H), 3.95–4.0 (1 H, m, 1-H) 4.0–4.05 (1 H, m, 4-H), 5.0–5.03 (1 H, m, 2-H); δ_{C} (125.6 MHz; ²H₂O), 24.1 (3-C of Cha), 24.6 (4-C of Cha), 30.7 (2-C of Cha), 37.3 (5-C), 37.6 (3-C), 50.7 (1-C of Cha), 61.1 (OCH₂CH₂OH), 64.8 (4-C), 68.0 (2-C), 71.3 (OCH₂CH₂OH), 76.5 (6-C, ³J_{C-P} 6.5 Hz), 77.0 (1-C, ²J_{C-P} 6.2 Hz); δ_{P} (202.5 MHz; ²H₂O) 7.05; For **15**: δ_{C} (75.4 MHz; ²H₂O), 24.1 (3-C of Cha), 24.6 (4-C of Cha), 30.7 (2-C of Cha), 37.7 (5-C), 38.5 (3-C), 50.7 (1-C of Cha), 64.3 (4-C), 64.3 (OCH₂CH₂OP, *J*_{C-P} 5.6 Hz), 69.0 (2-C), 69.5 (OCH₂CH₂OP secondary, *J*_{C-P} 5.2 Hz), 74.4 (1-C), 77.0 (6-C); δ_{P} (121.5 MHz; ²H₂O) –0.41 (br); For **17**: δ_{C} (75.4 MHz; ²H₂O), 24.1 (3-C of Cha), 24.5 (4-C of Cha), 30.6 (2-C of Cha), 38.3 (5-C), 39.2 (3-C), 50.7 (1-C of Cha), 63.9 (4-C), 66.3 (C secondary, *J*_{C-P} 7.5 Hz), 69.2 (2-C), 69.9 (C secondary), 77.2 (6-C), 81.0 (1-C, ²J_{C-P} 7 Hz); δ_{P} (121.5 MHz; ²H₂O) –0.84. Full details will appear elsewhere.

§ Inositol monophosphatase was purified and assayed as described in reference 3.

¶ *Crystal data* for **14b**: C₃₉H₄₆O₈, orthorhombic, *P*2₁2₁, *a* = 18.381(3), *b* = 29.196(6), *c* = 6.623(3) Å, *V* = 3551(1) Å³, *Z* = 4. Measurements were made on a Rigaku AFC7S diffractometer employing Mo-K α radiation. 3592 unique reflections collected of which 1615 [*I* > 3 σ (*I*)] were used for refinement. The structure was solved using SIR92 and refined using TEXSAN. Convergence at *R*(*F*) = 10.0%, *R*_w(*F*) = 7.3% for 320 variables. The crystal structure was performed in order to determine the relative stereochemistry at C-1, C-2, C-4 and C-6 compared to the known stereochemistry of the camphanic acid moiety at C-12 and C-15. Phenyl rings on C-21 and C-63 were treated as rigid groups. Atomic coordinates, bond lengths and angles and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Information for Authors, Issue No. 1.

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