Design and Synthesis of 6α-Substituted 2β,4α-Dihydroxy-1β-phosphoryloxycyclohexanes, Potent Inhibitors of Inositol Monophosphatase

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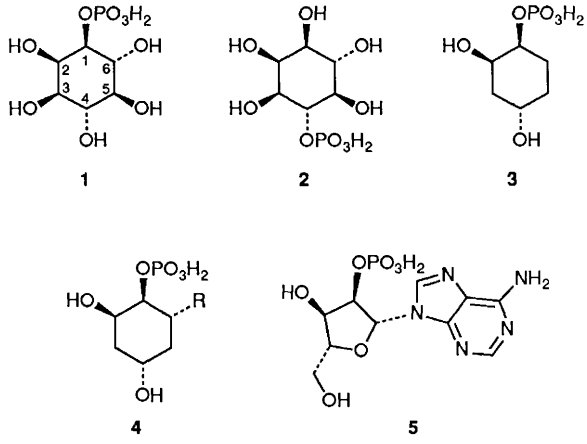
Molecular superimposition studies have led to the design and synthesis of 2β,4α-dihydroxy-6α-[5-(2-hydroxyphenyl)pentyloxy]-1β-phosphoryloxycyclohexane, a potent inhibitor of inositol monophosphatase.

The enzyme inositol monophosphatase acts at a pivotal point in the phosphatidylinositol (PI) cycle, since it can catalyse the hydrolysis of both enantiomers of inositol 1-phosphate **1** and inositol 4-phosphate **2** to free inositol.[1,2] Inositol does not readily penetrate the blood–brain barrier, and consequently this enzyme may be important in regulating the supply of inositol for PI synthesis in the central nervous system. In order to study the effects of inositol monophosphatase action in the brain, we have designed and synthesised competitive inhibitors, based on a strategy of hydroxy deletion from the natural substrates (**1** and **2**).[3,4] Initial work showed that the 2- and 6-hydroxy groups in **1** and **2** are independently associated with enzyme binding and the mechanism of hydrolysis, and further studies revealed that the 3- and 5-hydroxy groups are not necessary for inhibitor binding. These results led to the synthesis of 1β-phosphoryloxy-2α,4β-dihydroxycyclohexane, a moderately potent competitive inhibitor (IC_{50} 7 μmol dm^{-3}).[4] In this communication, we describe the design and synthesis of derivatives of **3** possessing significantly enhanced inhibitory activity. The primary clue in the search to identify a site in **3** suitable for substitution was provided by a molecular modelling study using the ligands recognized by inositol monophosphatase. This analysis led to the design and synthesis of 6α-substituted inhibitors **4** having up to 100-fold increased affinity for the enzyme.

Inositol monophosphatase is able to hydrolyse several structurally diverse phosphates.[2,5] In addition to **1** (K_m 0.16 mmol dm^{-3}; rel. V_{max} 100)[2]† and **2** (K_m 0.10 mmol dm^{-3}; rel. V_{max} 300)[2] this enzyme is also able to hydrolyse 2′-nucleotides including adenosine-2′-monophosphate (2′-AMP, **5**, K_m 0.58 mmol dm^{-3}; rel. V_{max} 157).[5] The adenine moiety in **5** does not significantly reduce binding to the enzyme, implying the tolerance of considerable bulk near the enzyme active site. Although **3** and **5** are hydrolysed at different rates by the enzyme, **3** being essentially an inhibitor possessing little or no substrate activity, comparisons of their 3-dimensional structures proved a powerful tool for inhibitor design. The hydroxy and phosphate groups in both **3** and **5** were considered to be participating in similar binding interactions with the enzyme and were therefore used in molecular superimposition studies. Fitting of **3** and **5** results in a very close correspondence of the key functional groups (Fig. 1) and clearly indicated that a 6α-substituent on the cyclohexane ring of **3** would occupy the



† All enzyme data were obtained using inositol monophosphatase purified from bovine brain. V_{max} values are given relative to V_{max} = 100 for (±)-inositol 1-phosphate; actual V_{max} for (±)-inositol 1-phosphate 13.3 μmol min^{-1} (mg of protein)$^{-1}$. For further details see ref. 2.

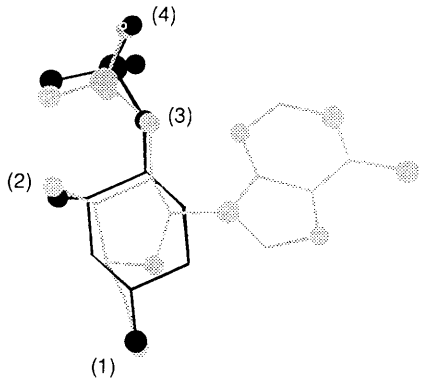
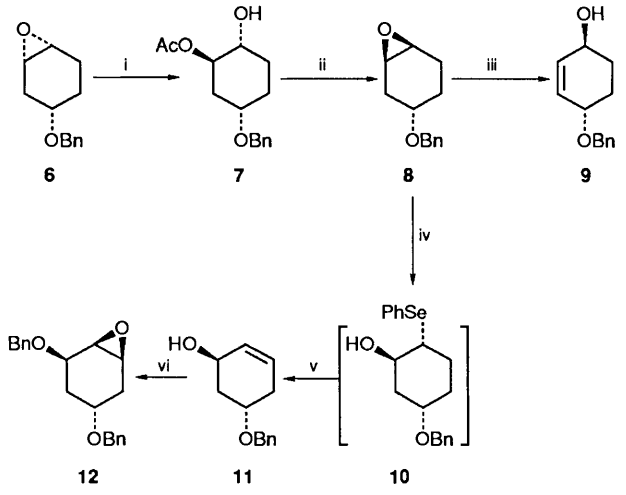


Fig. 1 Molecular superimposition of inhibitor **3** and 2′-AMP **5**. The indicated oxygen atoms were used in the fitting procedure, resulting in the following O–O interatomic-distances (Å): 1, 0.206; 2, 0.337; 3, 0.289; 4, 0.255. [The molecules were built and optimized using MOLEDIT/OPTIMOL, MSDRL's in-house molecular modelling package, and were displayed using CHEMX (Chemical Design Ltd., Oxford)].

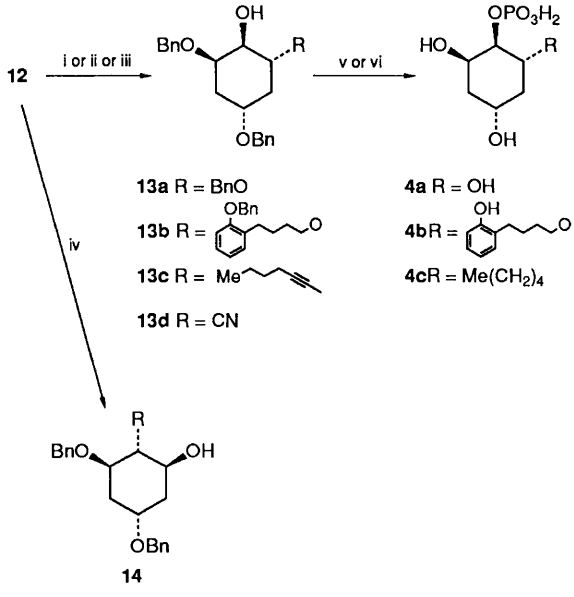


Scheme 1 *Reagents and conditions*: i, AcOH, Al_2O_3, toluene, reflux; ii, a, $MeSO_2Cl$, NEt_3, CH_2Cl_2; b, K_2CO_3, MeOH; iii, LDA, tetrahydrofuran (THF), −78 °C to room temp.; iv, PhSeSePh, $NaBH_4$, EtOH, room temp.; v, H_2O_2, EtOH, THF, reflux; vi, a, *m*-CPBA, CH_2Cl_2, room temp.; b, KH, BnBr, THF

same region in space as the adenine group in **5**. As a result of this analysis, trisubstituted cyclohexyl phosphates **4** were selected for synthesis. Tetrasubstituted cyclitols having the designed substitution pattern have not been prepared previously, and a regioselective synthesis was therefore established.

Treatment of the epoxide **6**[4] with acetic acid under alumina catalysis selectively gave the hydroxy acetate **7** (Scheme 1). Conversion of **7** to the corresponding methanesulphonate (mesylate), followed by treatment with potassium carbonate in methanol, resulted in acetate hydrolysis and subsequent ring closure to form the inverted epoxide **8**. Attempts to effect eliminative ring opening of the *trans*-epoxide **8** by treatment with lithium diisopropylamide (LDA)[6,7] did not afford the desired alcohol **11** but instead gave a poor yield of the regioisomeric allylic alcohol **9**. However, treatment of **8** with sodium phenylselenide[8] resulted in exclusive formation of selenide **10**, presumably as a consequence of specific *trans*-diaxial epoxide ring opening of the conformer of **8** in which the benzyloxy substituent is pseudoequatorial. The intermediate selenide **10** was treated *in situ* with hydrogen peroxide and heated to effect selenoxide elimination, affording allylic alcohol **11**. The route to **11** can be considerably shortened by carrying out the phenylselenide addition on a mixture of *cis* **6** and *trans* **8** epoxides derived from epoxidation of 4-benzyloxycyclohex-1-ene, the desired alcohol **11** being easily separated from the product mixture. Alcohol **11** was subjected to Henbest epoxidation with *m*-chloroperbenzoic acid (*m*-CPBA), yielding the *cis*-epoxide, which after benzylation of the free hydroxy group provided the key intermediate **12** required for the introduction of 6-substituents.

Ring opening reactions of the epoxide **12** were studied with oxygen and carbon nucleophiles (Scheme 2). Treatment of **12** with alcohols in the presence of catalytic alumina[9] led exclusively to the desired 6α-substituted products **13a,b** in good yields. Using carbon nucleophiles, however, regioselectivity was found to be highly dependent on the nature of the reagent. Reaction of the epoxide **12** with dibutyl cyanocuprate[10] afforded solely the product of undesired ring opening **14**, whereas diethylaluminium acetylides and diethylaluminium cyanide led only to the required products **13c,d**. The alcohols **13a,b,c** were phosphorylated, either with tetrabenzyl pyrophosphate–sodium hydride,[11] or more conveniently with *N,N*-diethyl dibenzylphosphoramidite,[12] followed by oxidation of the intermediate phosphite. Deprotection by hydrogenolysis as described previously[3] yielded the required phosphates **4a,b,c**.



Scheme 2 *Reagents and conditions*: i, ROH, Al_2O_3, toluene, reflux; ii, $Et_2AlC{\equiv}CR$, toluene, 0 °C; iii, Et_2AlCN, toluene, 0 °C; iv, $R_2CuCNLi$, Et_2O; v, a, NaH, tetrabenzyl pyrophosphate, THF; b, H_2, Pd/C, EtOH–H_2O; vi, a, $(BnO)_2PNEt_2$, 1*H*-tetrazole, CH_2Cl_2, room temp.; b, *m*-CPBA, CH_2Cl_2, −78 °C; c, H_2, Pd/C, EtOH–H_2O

It was reassuring to find that the n-pentyl substituent of **4c** was well tolerated within the enzyme active site, giving an inhibitor with an IC_{50} 3 μmol dm^{-3}, confirming the presence of a bulk tolerance site at this position. Results for **4b** (IC_{50} 70 nmol dm^{-3}) were far more dramatic, demonstrating a significant positive binding contribution from the C-6 substituent.

The phosphate **4a** having two α-hydroxy groups proved to be a good substrate for inositol monophosphatase, having an affinity (K_m 0.025 mmol dm^{-3}, rel. V_{max} 61) greater than any of the natural substrates. This result demonstrates that the detrimental effects of the 3,5-hydroxy groups previously found with inhibitor binding[4] also occur with substrate binding. It is interesting to speculate on the substrate activity of 2′-AMP **5** which possesses only one α-hydroxy group. In

this case presumably either the purine heterocycle or the sugar ether oxygen in some way facilitate substrate activity, but the nature of this interaction remains unclear.

These results confirm the prediction, based on the molecular modelling comparisons of **3** and **5** (Fig. 1), that there is substantial bulk tolerance close to the enzyme active site. Compound **4b** is 100 times more potent than **3** and represents the most potent inositol monophosphatase inhibitor reported to date; it may play a role in more fully understanding the biochemical effects of blockade of this crucial part of the PI cycle.

Received, 6th September 1990; Com. 0/04069J

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