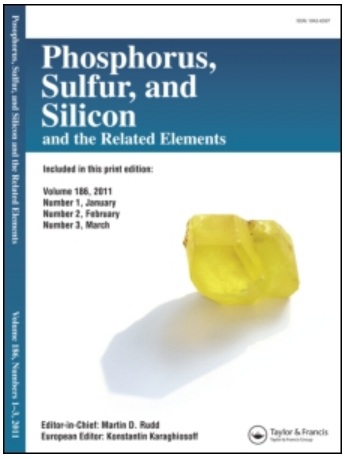

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MECHANISM AND INHIBITION OF INOSITOL MONOPHOSPHATASE

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Abstract The design and synthesis of inhibitors of inositol monophosphatase (IMPase, E.C. 3.1.3.25) based on natural substrates and lead compounds discovered by screening is discussed. The physiologically relevant form of the enzyme and a likely mechanism have been deduced from structure-activity relationships, site-directed mutagenesis experiments, X-ray crystallography and molecular modelling.

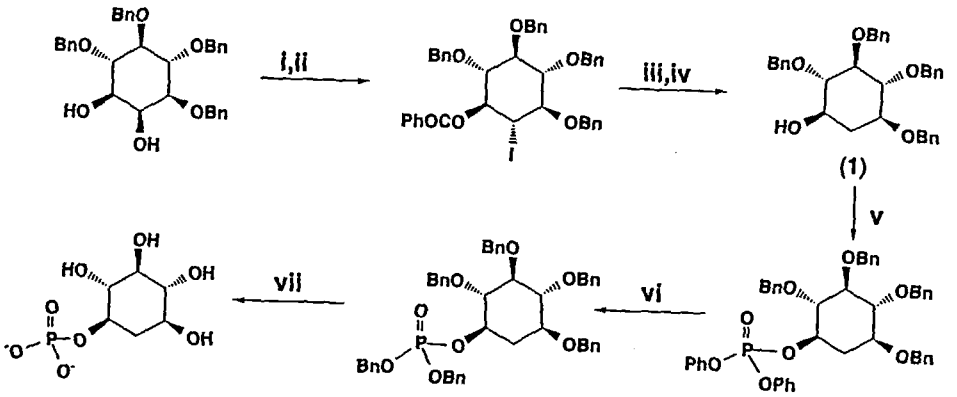
INTRODUCTION

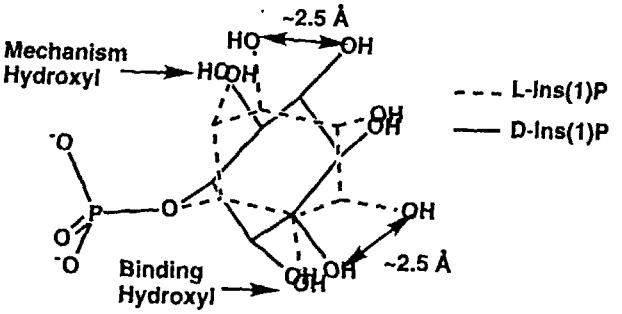
Lithium is the major drug treatment for bipolar disorder, despite a poor side effect profile and narrow therapeutic window. The hypothesis that the phosphatidylinositol (PI) cell signalling system and in particular IMPase are the site of action for the beneficial effects of lithium has found significant support and has been reviewed elsewhere[1]. On this basis, a selective organic inhibitor of IMPase might be expected to treat bipolar disorder while showing less side effects than the unselective agent lithium.

INHIBITORS

Both D- and L-inositol-1-phosphate (D- and L-Ins(1)P) are hydrolysed at equal rates by IMPase, yet the two compounds differ only in the stereochemistry of the two hydroxyl groups flanking the phosphate group. If the phosphate and the inositol ring bind in similar orientations in both enantiomers, it appears likely that neither the 2- nor the 6-hydroxyl should bind to the enzyme since they do not superimpose in such an overlay. (±) 2- Deoxyinositol-1-phosphate was therefore prepared as shown in scheme 1, while (±) 6-deoxyinositol-1-phosphate was prepared analogously using cyclohexylidene groups to provide differential protection. In the latter case, it was not necessary to transesterify the phosphate group with benzyl alcohol before removal of all the protecting groups by treatment with silica followed by hydrogenation over platinum[2].

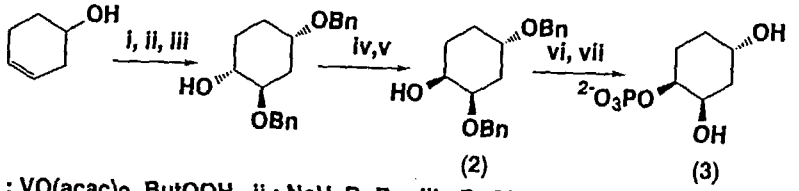
Surprisingly, *both* were found to be competitive inhibitors ($IC_{50} \approx 70\mu M$), demonstrating that both flanking hydroxyl groups were needed for substrate activity. Resolution of the enantiomers of 2-deoxyinositol-1-phosphate was achieved *via* the camphanic acid ester of racemic (**1**). Homochiral (**1**) was then treated as before to give enantiomerically pure 2-deoxyinositol-1-phosphate. The (+) enantiomer, corresponding to D-Ins(1)P, was a weak substrate, while its antipode was a competitive inhibitor (K_i 50μM). This suggested that the relative binding modes of the two substrates might be as suggested in the lower part of scheme 1, leading to the idea that the 3 and 5 hydroxyl groups might play no part in binding. In this picture, one flanking hydroxyl group (the 2-OH of L-Ins(1)P and the 6-OH of D-Ins(1)P) was necessary for catalysis to take place, while the other conferred good binding properties.





SCHEME 1 Synthesis of 2-deoxyinositol-1-phosphate, and deduced binding modes.

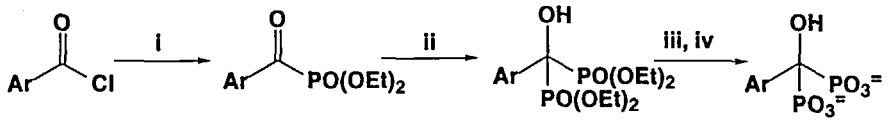
In keeping with this, the 3,5 dihydroxy and 4-hydroxy cyclohexane-1-phosphates were inactive, while (3), synthesized[3] as in scheme 2 and resolved by separation of the camphanate esters of compound (2), was a good inhibitor (IC_{50} 3μM). The 4-hydroxy group could not be removed without a drastic loss in binding. From these results, similar overlays with other substrates, notably 2'-AMP, could be generated, and suggested that bulky hydrophobic substituents at the 6-position might enhance binding and/or improve physical properties. A number of such compounds were prepared[4] following a similar route to that used previously, with optimum IC_{50}'s of 40nM.



SCHEME 2 Synthesis of 3,5,6-trideoxy-D-Ins(1)P.

In these cases, the phosphorylation was more conveniently achieved with N,N-diethyl-dibenzylphosphoramidite followed by oxidation with *m*-CPBA and hydrogenolytic removal of the protecting groups.

Hydroxymethylene-bisphosphonic acid was identified as a weak (IC_{50} 280 μM) inhibitor of the enzyme. Affinity was improved by substitution with an aryl group, synthesized according to Scheme 3, which led to sub-micromolar compounds[5].

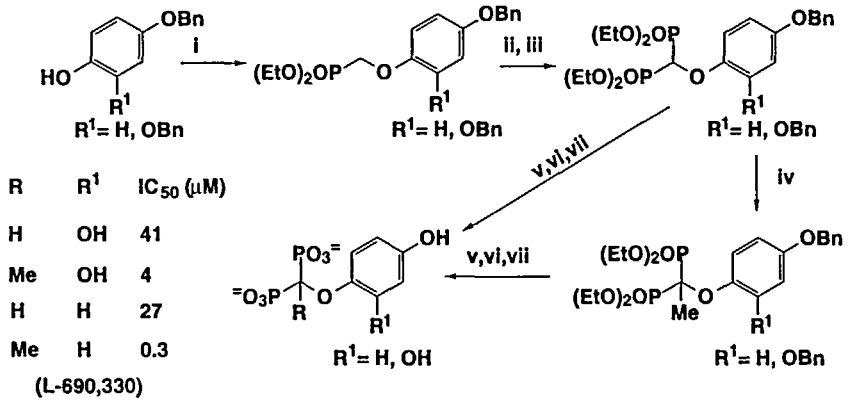


i : $(EtO)_3P$ ii : $HPO(OEt)_2$, nBu_2NH, Et_2O iii : Me_3SiBr iv : H_2O

SCHEME 3 Synthesis of arylhydroxymethylenebisphosphonates

The introduction of the second phosphonate unit required the use of di-^{n}butylamine, triethylamine giving the opposite regiochemistry of addition across the carbonyl group.

The methylenebisphosphonate unit could also be linked through oxygen to the trideoxyinositol moiety and served as a good replacement for phosphate providing new inhibitors which were stable to hydrolysis[6]. Replacement of the phosphate permitted replacement of the inositol ring with a simple phenol[7]. The synthesis of some of these compounds is illustrated in Scheme 4. Further optimisation could be achieved by simple modification of the synthetic scheme to introduce larger groups at **R** providing compounds with $IC_{50} \approx 80$ nM. However, L-690,330 and a prodrug derived from it proved sufficient to establish that an organic inhibitor of IMPase would produce similar effects on the PI cycle in cells and in whole animals to those seen with lithium[8].



R	R^1	IC_{50} (μM)
H	OH	41
Me	OH	4
H	H	27
Me	H	0.3

i : NaH, $TfOCH_2PO(OEt)_2$ ii : LDA iii : $ClPO(OEt)_2$ iv : NaH, MeI v : Me_3SiBr vi : H_2O vii : H_2 / Pd/C

SCHEME 4 Synthesis and properties of phenolic bisphosphonate inhibitors.

MECHANISM

Several crystal structures have been obtained of the enzyme in inhibited and active

forms[9]. We have blended the structures in the presence of substrate and the inhibitory metal Gd^{3+} and in the presence of the catalytically viable metal Mn^{2+}.[10] Initial studies enabled the identification of appropriate sites for site-directed mutagenesis which later assisted in confirming the validity of the model. Molecular dynamics was used on models containing a variety of different metal ion combinations to remove strain and provide a sampling of conformations available to the enzyme. A model containing Gd^{3+} together with two Li^{+} ions reproduced many of the observations for the metal environment seen in the crystal, while a model containing two Mn^{2+} ions was consistent with a large body of mutagenetic and kinetic evidence[10,11]. Analysis of potential nucleophiles revealed three possibilities. Phospho-enzyme intermediates have already been demonstrated not to form a part of the IMPase mechanism[12], leaving two water molecules which could play the role of the nucleophile. We prefer that located on the first metal site identified, which also forms interactions with Glu70, and which would be able to apically attack the phosphorus atom in a manner similar to that deduced for the structurally homologous enzyme fructose bis-phosphatase[13,14].

REFERENCES

1. See :J.R.ATACK, H.B. BROUGHTON and S.J.POLLACK, *TINS*, **18**, 343 (1995)
2. R.BAKER, J.J. KULAGOWSKI, D.C. BILLINGTON, P.D. LEESON, I.C. LENNON and N. LIVERTON, *J. Chem. Soc. Chem. Commun.*, 1383 (1989)
3. R.BAKER, P.D. LEESON, N.J. LIVERTON and J.J. KULAGOWSKI, *J. Chem. Soc. Chem. Commun.*, 462 (1990).
4. R.BAKER, C. CARRICK, P.D. LEESON, I.C. LENNON and N.J. LIVERTON, *J. Chem. Soc. Chem. Commun.*, 298 (1991).
5. S.R. FLETCHER, R. BAKER, P.D. LEESON, M. TEALL, E.A. HARLEY and C.I. RAGAN, *Bioorg. Med. Chem. Lett.*, **2**, 627 (1992)
6. J.J. KULAGOWSKI, R. BAKER and S.R. FLETCHER, *J. Chem. Soc. Chem. Commun.*, 1650 (1991).
7. S.R. FLETCHER, R. BAKER, T. LADDUWAHETTY, A. SHARPE, M. TEALL and J.R. ATACK, *Bioorg. Med. Chem. Lett.*, **3**, 141 (1993).
8. J.R. ATACK, S.M. COOK, A.P.WATT, S.R. FLETCHER and C.I. RAGAN, *J. Neurochem.*, **60**, 652 (1993)
   J.R. ATACK, A.M. PRIOR, S.R. FLETCHER, K. QUIRK, R. MCKERNAN and C.I. RAGAN, *J. Pharmacol. Exp. Ther.*, **270**, 70 (1994).
9. R. BONE, J.P. SPRINGER and J.R. ATACK, *Proc. Natl. Acad. Sci. USA*, **89**, 10031 (1992). ; R. BONE, L. FRANK J.P. SPRINGER, S.J. POLLACK, S. OSBORNE, J.R. ATACK, M.R. KNOWLES, G. MCALLISTER, C.I. RAGAN, H.B. BROUGHTON, R. BAKER and S.R. FLETCHER , *Biochemistry* , **33**, 9460 (1994). ; R. BONE, L. FRANK, J.P. SPRINGER and J.R. ATACK, *Biochemistry*, **33**, 9468 (1994).
10. S.J. POLLACK, J.R. ATACK, M.R. KNOWLES, G. MCALLISTER, C.I. RAGAN, R. BAKER, S.R. FLETCHER, L.L. IVERSEN and H.B. BROUGHTON, *Proc. Natl. Acad. Sci. USA*, **91**, 5766 (1994).
11. S.J. POLLACK, M.R. KNOWLES, J.R. ATACK, H.B. BROUGHTON, C.I. RAGAN, S. OSBORNE AND G. MCALLISTER, *Eur. J. Biochem*, **217**, 281 (1993).
12. A.P. LEECH, G.R. BAKER, J.K. SHUTE, M.A. COHEN and D. GANI, *Eur. J. Biochem.*, **212**, 693 (1993).
13. Y.ZHANG, J.-Y. LIANG and W.N. LIPSCOME, *Biochem. Biophys. Res. Commun.* , **190**, 1080 (1993) ; Y. ZHANG, J.-Y. LIANG, S. HUANG, H. KE and W.N. LIPSCOMB, *Biochemistry*, **32**, 1844 (1993).
14. See for review of mechanism : J.R. ATACK, H.B. BROUGHTON and S.J. POLLACK, *FEBS Letters*, **361** , 1 (1995).