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ADENOSINE-2'-MONOPHOSPHATE DERIVATIVES: STRUCTURAL REQUIREMENTS AS SUBSTRATES FOR INOSITOL MONOPHOSPHATASE

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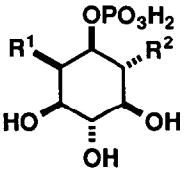
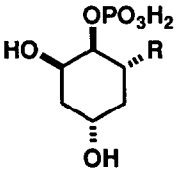
Abstract. Synthesis of derivatives and carbocyclic analogues of adenosine-2'-monophosphate, a substrate for bovine inositol monophosphatase, shows that the 1-purine heterocycle and the 4-hydroxymethyl are not required for binding to the enzyme. In contrast, the cyclic ether oxygen is necessary for efficient phosphate hydrolysis.

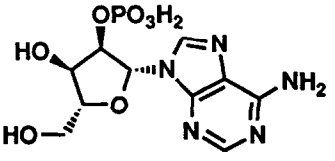
Inhibition of the enzyme inositol monophosphatase in the brain has been implicated as the mechanism responsible for the effectiveness of lithium ion in the treatment of manic depression.[1] Selective organic inhibitors of this enzyme may therefore have therapeutic utility as antidepressant agents. Using the natural substrate inositol-1-phosphate (**1**) as a lead, we employed a strategy of hydroxyl deletion and alkylation, leading to a series of phosphoryloxy cyclohexanes (**2-6**), which are competitive inhibitors of the enzyme. Both enantiomers of **1** are equally good substrates, but the inhibitory activity of the deshydroxy inhibitors **2**[2] and **5**[3] is confined to the 1-*S* enantiomers. In contrast, the 1-*R*-enantiomers of **2** and **5** were found to be weakly bound substrates. These findings led to the proposal that the 2- and 6-hydroxyls, flanking the phosphate group in the substrate **1**, have individual roles in binding to the enzyme and in the mechanism of phosphate cleavage.[2]



1 $R^1 = R^2 = OH$
2 $R^1 = H$; $R^2 = OH$
3 $R^1 = OH$; $R^2 = H$
4 $R^1 = OCH_3$; $R^2 = OH$



5 R = H
6 $R = O(CH_2)_4Ph$-2-OH



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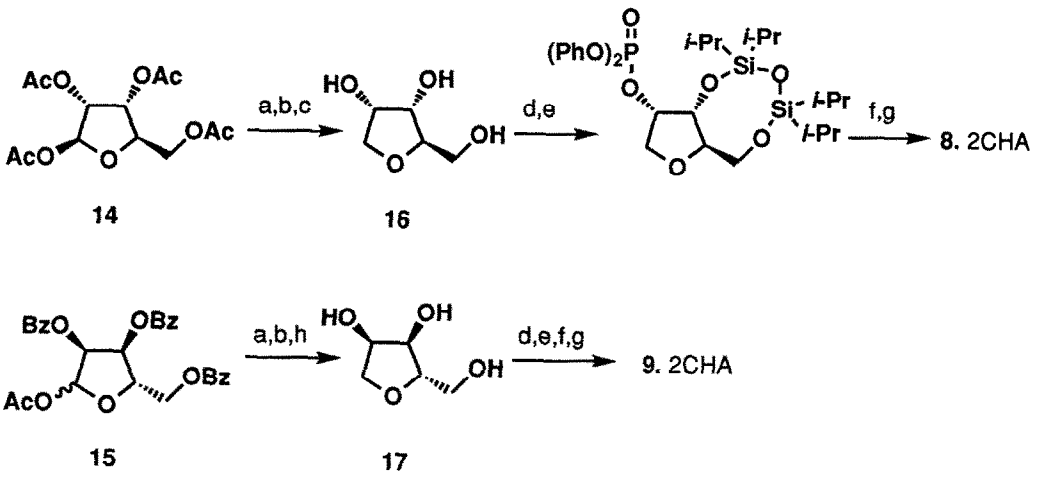
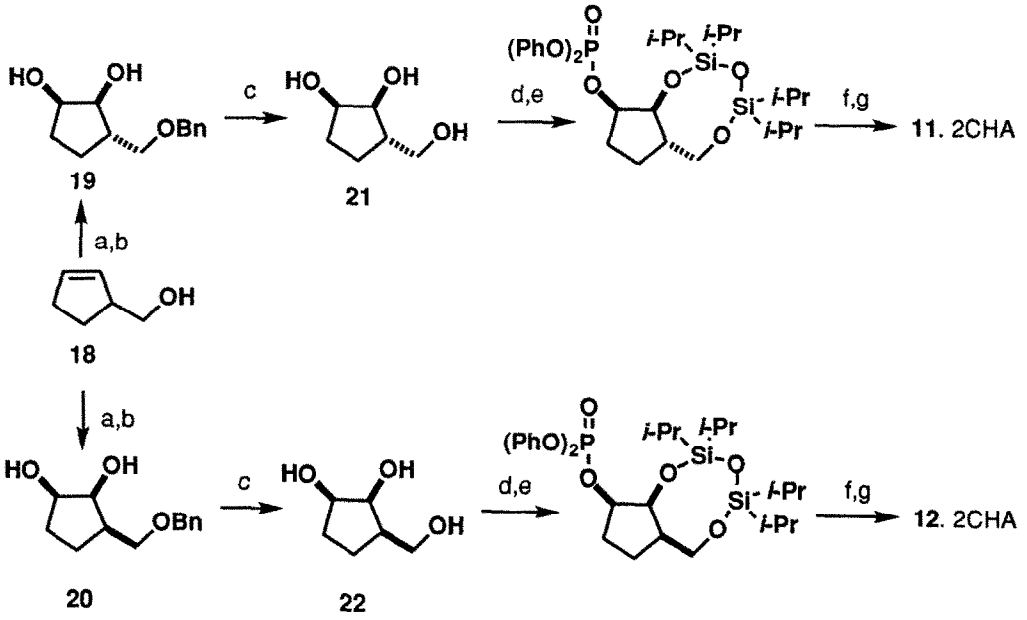


Scheme 1. a) HBr, CH_2Cl_2, RT, 15 min; b) Bu_3SnH, AIBN, PhMe, reflux, 18 hr; c) Dowex 1-x8 (OH^- form), 50°C, 18 hr; d) $(ClSi\textit{i}\text{-}Pr_2)_2O$, imidazole, DMF, RT, 18 hr; e) $(PhO)_2P(O)Cl$, DMAP, CH_2Cl_2, RT, 5hr; f) H_2, Pt, EtOH, H_2O, 50 psi, RT, 18 hr; g) i, H_2O, RT, 18 hr; ii, cyclohexylamine (CHA); h; KOH, H_2O, EtOH, RT, 2 hr. [Bz = PhCO].



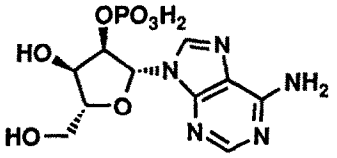
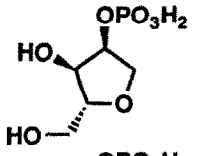
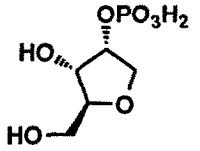
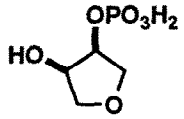
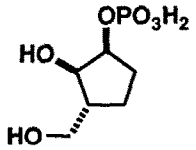
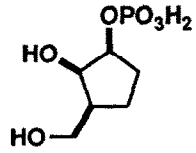
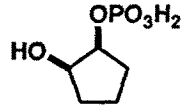
Scheme 2. a) NaH, BnBr, DMF, RT, 2 hr; b) i, OsO_4 (cat.), N-methylmorpholine N-oxide, t-BuOH, H_2O, RT, 18 hr; ii, separate by SiO_2 chromatography; c) H_2, $Pd(OH)_2$, EtOH, H_2O, 50 psi, 1 hr; d) $(ClSi\textit{i}\text{-}Pr_2)_2O$, imidazole, DMF, RT, 1 hr; e) $(PhO)_2P(O)Cl$, DMAP, CH_2Cl_2, RT, 5hr; f) H_2, Pt, EtOH, H_2O, 50 psi, RT, 18 hr; g) i, H_2O, RT, 18 hr; ii, cyclohexylamine (CHA). [Bn = CH_2Ph]

2'-Nucleotides, incuding adenosine-2'-monophosphate (**7**), are efficiently hydrolysed by inositol monophosphatase,[4] showing that there is a bulk tolerance region, close to the active site, which can accommodate the 1-heterocyclic ring. Comparisons of inhibitor **5** and substrate **7** led[5] to the design of 6-substituted derivatives of **5**, exemplified by the potent inhibitor **6**, where the 6-aryloxy group improves affinity by 100-fold. These results led us to further question the role of the purine heterocycle in **7** in both binding to the enzyme and in inducing substrate activity. In this paper we describe the synthesis of derivatives of **7** lacking the heterocycle (compounds **8-10**), together with analogous carbocyclic cyclopentane phosphates (**11-13**). These compounds serve to define the minimum requirements for both binding and phosphate hydroysis of 2'-nucleotides by inositol monophosphatase, and lead us to refine our existing model[2,3] of substrate-enzyme interactions.

Synthesis of the enantiomers **8** and **9** proceeded (Scheme 1) from D-[6] and L-1-deoxy ribofuranose (**16** and **17**) which were obtained from β-D-ribofuranose-1,2,3,5-tetraacetate (**14**) and 1-O-acetyl-2,3,5-tri-O-benzoyl-L-ribose (**15**) via tributyltin hydride reduction of the intermediate 1-bromides. Tetraisopropyldisiloxane protection[7] of **16** ($[\alpha]_D$ +57° (c 1.3, H_2O)), followed by phosphorylation and hydrogenolysis,[8] then *in situ* removal of the siloxane protecting group, led to the required phosphate **8**, which was isolated as its di(cyclohexylamine) salt (m.p. 171-173 °C, $[\alpha]_D$ +18.2° (c 0.98, H_2O)). The L-enantiomer **17** ($[\alpha]_D$ -52° (c 1.7, H_2O)) was similarly converted to **9** (m.p. 165-167 °C, $[\alpha]_D$ -16.7° (c 0.8, H_2O)). For the preparation of the cyclopentane phosphates **11** and **12** (Scheme 2), triols **21** and **22** were required. Alcohol **18**[9] was successively benzylated and *cis* hydroxylated with osmium tetroxide to give the separable diols **19** and **20**, whose structures followed from their ^1H NMR spectra and nOe experiments. Debenzylation provided **21** and **22**, which were both selectively protected as their tetraisopropyldisiloxane derivatives. Phosphorylation and deprotection then provided **11** (m.p. 140-141 °C) and **12** (m.p.185-186°C), isolated as their di(cyclohexylamine) salts. The phosphates **10** and **13** are known compounds.[10]

Compounds **7-13** (see Table) were assessed for their ability to inhibit the hydrolysis of **1** by bovine inositol monophosphatase (IC_{50} values) and to act as substrates for the enzyme (rate relative to **1**).[11] Removal of the purine ring from **7** to give **8** was found to result in no change to both affinity and relative substrate activity. A striking result is the lack of enantioselectivity in the des-purine compounds, revealed by the identical affinities and substrate activities of **8** and **9**. The *trans* 4-(hydroxymethyl) group in **7** has little effect on enzyme recognition, as shown by the comparable profiles of **8**, **9** and the racemic tetrahydrofuryloxy phosphate **10**. The α-hydroxyl in **10** is required for binding, since racemic 3-phosphoryloxy tetrahydrofuran is a very weak inhibitor (22% inhibition was observed at 5 mM). Compound **10** therefore contains the minimum structural requirements for binding and substrate activity of nucleotide derivatives by inositol monophosphatase. The carbocyclic derivatives **11** and **12** proved to be inhibitors of the enzyme, having no measurable substrate activity, suggesting that the cyclic ether oxygen atoms in **7-10** are in some way required for phosphate hydrolysis. The *cis* hydroxymethyl substituent in the inhibitor **12** enhances binding relative to the unsubstituted parent compound **13**. In agreement with the relative affinities of compounds **8-10**, the *trans* hydroxymethyl group in **11** has little effect on binding.

Table. Activities against bovine inositol monophosphatase

No.[a]	Structure	IC_{50} (mM), enzyme inhibition[b]	Relative substrate activity[c]
7		1.3	0.48
8		2.9	0.65
9		1.8	0.53
10		3.6	0.53
11		2.1	<0.05
12		0.31	<0.05
13		5	-

[a] Compounds **7,8** and **9** are single enantiomers with the absolute configurations shown. Compounds **10-13** are racemic, with the relative stereochemistries as shown.
[b] Concentration inhibiting the production of [^{14}C]-inositol from racemic inositol monophosphate (**1**) containing [^{14}C]-**1** (see ref. 11 for details).
[c] Rate of release of inorganic phosphate at 1mM substrate concentration, relative to 1 mM **1** (= 1).

These results demonstrate that i) the purine ring of **7** has no role in binding to inositol monophosphatase or in inducing substrate activity; ii) the orientation of the *trans* 4-(hydroxymethyl) in **7** is not optimal for binding to the enzyme and iii) the cyclic ether oxygen atom is necessary for efficient phosphate hydrolysis. Previous results with the enantiomers of **2** and **5** suggested a role for one of the flanking α-hydroxyls in **1** in facilitating enzymatic phosphate hydrolysis.[2,3] The inhibitory activity of the racemic 2-O-methyl derivative **4** led to the suggestion[12] that the hydrogen atom of the α-hydroxyl is required for phosphate cleavage, consistent with the proposal[13] of a proton transfer mechanism to the adjacent phosphate ester oxygen. The present results show that an ether oxygen atom interaction with the 'mechanism' binding site results in efficient phosphate hydrolysis, suggesting that partial negative charge on the 'mechanistic' oxygen α- to the phosphate is the essential feature required for substrate activity.

The lack of enantiospecificity seen with **8** and **9** contrasts with the cyclohexane derivatives **2** and **5**,[2,3] where the individual enantiomers have differing affinities and substrate activity is confined to the more weakly bound 1-*S* enantiomers. Relatively small energy differences between the allowed envelope and half chair conformers in the 5-membered tetrahydrofuran rings of **8** and **9** may result in different conformations of each enantiomer being bound to the active site. In the cyclohexanes **2** and **5**, the conformers having the phosphoryloxy substitutent equatorial are clearly favoured.[2,3] In addition, the reduced overall steric bulk of the tetrahydrofuran ring in the substrate **8** (IC_{50} 2900 μM) may permit alternative modes of binding relative to the analogous, but more considerably more potent cyclohexane inhibitor **5** (IC_{50} 7 μM). The improved affinity of **5** relative to **8** and **9** can be explained by the relative contributions to binding of the 4-hydroxyl in **5**, which is essential for high affinity,[3] and the 4-hydroxymethyl in **8** and **9**, which clearly does not influence binding. Although the 6-substituent in the inhibitor **6** enhances affinity for the enzyme,[5] the purine substitutent in the substrate **7** does not. Molecular models suggest that comparability in the spatial orientations of the substitutents in **6** and **7** is possible,[5] but the results indicate that these compounds interact differently with the enzyme.

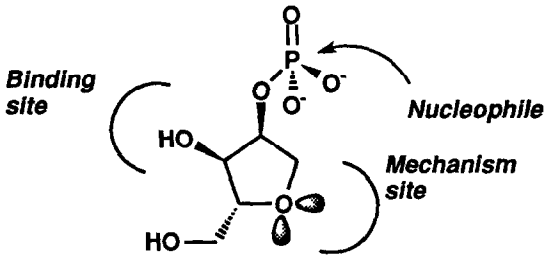


Figure. Postulated interactions of 2'-nucleotides with inositol monophosphatase. The cyclic ether oxygen assists phosphate hydrolysis by an unknown mechanism.

To explain the overall results, we suggest that the α-hydroxyl in the 2'-nucleotides and their substrate analogues interacts with an enzyme binding site, and that the ether oxygen, by an as yet undefined mechanism, functions to assist phosphate cleavage (see Figure). The enantiomers **8** and **9** will fit to

the model if they both bind to the enzyme with their α-hydroxy and ether oxygen moieties interacting respectively with the 'binding' and 'mechanism' sites adjacent to the phosphate binding pocket.

The tolerance of the 'mechanism' site to an ether oxygen would suggest that the methyl ether **4** should be a substrate. Racemic **4** is in fact a potent inhibitor,[12] showing that the binding of the methyl ether to the 'mechanism' pocket does not result in phosphate cleavage. Reasons for the inhibitory activity of **4** could include inappropriate orientation of the 2-methoxy oxygen lone pairs in the active site, or steric inhibition of the hydrolysis mechanism by the added methyl group. Testing of the individual enantiomers of **4** would provide valuable additional information.

The 3-dimensional structure of human inositol monophosphatase has recently been determined.[14] When the nature of the enzyme nucleophile is established, together with details of the interactions of substrates and inhibitors with the active site, it should be possible to establish a refined description of the chemical processes involved in the enzyme catalytic pathway. The observed structure-substrate requirements, described here and previously,[2,3,12] should assist such studies.

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