# Inhibitors of *myo*-Inositol Monophosphatase containing Methylenebisphosphonic Acid as a Replacement for a Phosphate Group

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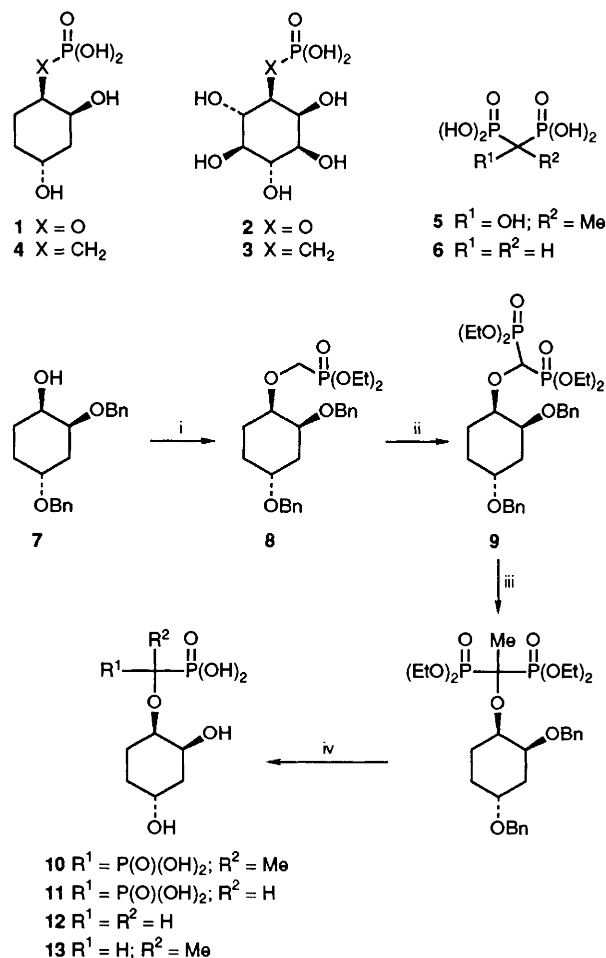
Replacement of the phosphate group in 2β,4α-dihydroxy-1β-phosphoryloxycyclohexane **1** with methylenebisphosphonic acid gives **11**, a potent inhibitor of *myo*-inositol monophosphatase.

The phosphoinositide (PI) pathway has become firmly established as a fundamental mechanism for cellular signal transduction and calcium mobilisation.[1] Our interest in regulating this process within the central nervous system has led to the design and synthesis of inhibitors of the pivotal enzyme *myo*-inositol monophosphatase. Thus, 2β,4α-dihydroxy-1β-phosphoryloxycyclohexane **1** was identified as a potent and competitive inhibitor of the bovine enzyme ($K_i$ = 4.3 μmol $dm^{-3}$),[2] and more recently, it has been shown that appropriate substitution at the 6α-position leads to a significant improvement in inhibitory potency.[3] Whilst this strategy has resulted in a series of potent, specific inhibitors of *myo*-inositol monophosphatase, the utility of these compounds in whole-cell and *in vivo* studies is compromised by their lability towards ubiquitous, non-specific phosphatases. We therefore sought to identify an alternative functionality, not subject to hydrolysis by the action of phosphatases, with which we could replace the phosphate group in our series of inhibitors.

Our initial efforts in this area led to the replacement of the ester oxygen with a methylene group in both the natural substrate for the monophosphatase enzyme, inositol-1-phosphate **2** and also the inhibitor **1**, to provide the methylene-linked phosphonates **3**[4] and **4**† respectively. These phosphonates however exhibited no enzyme inhibitory activity. Although proposed as an isosteric replacement for a phosphate group in biologically active molecules, the altered electronic properties of a methylene phosphonate often precludes its use as a direct phosphate mimic.[5] α-Fluorinated phosphonates have recently found favour as more appropriate, isoelectronic replacements of some phosphates.[6,7] We now report the finding that a methylenebisphosphonic acid group can function as a replacement for a phosphate group in inhibitors of *myo*-inositol monophosphatase such as **1**.

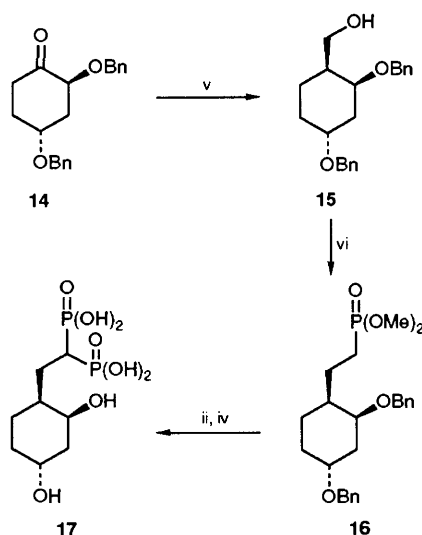
Screening of a number of diacids for inhibitory activity revealed the hydroxymethylenebisphosphonic acid **5** to be a moderately potent ($K_i$ = 0.18 mmol $dm^{-3}$) and competitive inhibitor of *myo*-inositol monophosphatase, whereas methylenebisphosphonic acid **6** was inactive. Since inorganic phosphate is also known to act as a competitive inhibitor of the phosphatase ($K_i$ = 0.52 mmol $dm^{-3}$),[8] we were intrigued by the possibility that **5** may be mimicking the action of phosphate in binding to the enzyme-active site. This idea was tested by combining the bisphosphonate **5** with the dihydroxycyclohexane moiety present in **1**. This was accomplished (Scheme 1) by alkylation of the anion of alcohol **7**[2] with the trifluoromethanesulphonate of diethyl hydroxymethylphosphonate.[9] Phosphonylation of the alkoxy phosphonate **8** proceeded smoothly using two equivalents of lithium diisopropylamide (LDA) followed by diethyl chlorophosphate to give bisphosphonate **9**. Methylation followed by hydrolysis of the phosphonate esters using an excess of bromotrimethylsilane and subsequent hydrogenolysis of benzyl ethers gave the desired diacid **10**. Compound **10** proved to be a potent, competitive inhibitor of bovine *myo*-inositol monophosphatase having similar affinity to the corresponding phosphate **1** ($K_i$ = 2.5 μmol $dm^{-3}$).

A number of simplifications were made to determine which features of this molecule were required for recognition by the enzyme. The corresponding bisphosphonate lacking a methyl group **11** was prepared by deprotection of **9** as described above, and also proved to be an inhibitor, albeit of slightly reduced affinity ($K_i$ = 7.4 μmol $dm^{-3}$). The beneficial effect on introduction of the methyl group may reflect an increase in steric compression around the quaternary carbon, leading to a closer proximity of the two phosphonate groups. Both phosphonate groups are necessary for binding since the monophosphonates **12** and **13** have greatly reduced affinity ($K_i$ 1.8 and 1.2 mmol $dm^{-3}$ respectively). The role played by the ethereal oxygen in the binding of **11** was investigated by its replacement with a methylene (Scheme 2). Wittig methenylation of the ketone **14**[2] and subsequent hydroboration with 9-borabicyclo[3.3.1]nonane (9-BBN) gave the desired *cis* alcohol **15** exclusively. Oxidation to the aldehyde, then Horner–Emmons alkenation, and reduction afforded the monophosphonate **16** which was phosphonylated and de-




**Scheme 1** *Reagents and conditions*: i, BuLi, $TfOCH_2P(O)(OEt)_2$; ii, LDA, $ClP(O)(OEt)_2$; iii, NaH, MeI; iv, $Me_3SiBr$, $H_2O$, then, Pd–C, $H_2$ (Tf = $CF_3SO_2$; Bn = $PhCH_2$)

† Prepared by Horner–Emmons reaction of the anion of tetramethyl methylenebisphosphonate with ketone **14**, catalytic hydrogenation over platinum and subsequent deprotection as described for **10**.




**Scheme 2** *Reagents and conditions*: v, $MePPh_3^+Br^-$, BuLi, then 9-BBN, $H_2O_2$, $OH^-$; vi, pyridinium chlorochromate (PCC), then $(MeO)_2(O)PCH_2P(O)(OMe)_2$, BuLi, then Pd–C, $H_2$

protected as before to provide the required methylene analogue **17**. The very considerable reduction in affinity observed with this compound ($K_i$ = 0.68 mmol $dm^{-3}$) relative to **11** confirms that the oxygen at C-1 participates in an important interaction with the enzyme. This interaction is likely to resemble the binding of the ester oxygen of substrate phosphates such as **2**, where, during hydrolysis, activation by protonation or association with a Lewis acid precedes P–O bond cleavage.

Finally, the stereospecificity of binding of bisphosphonate **11** was determined by resolution. Previously, resolution of the corresponding phosphate **1** demonstrated that the (1*S*,2*R*,4*S*)-enantiomer [(−)-**1**] was responsible for the inhibitory activity, whereas (+)-**1** was a weak substrate for the enzyme.[2] Resolution of the alcohol **7** *via* its diastereoisomeric camphanate esters[2] and conversion of each enantiomer individually to a bisphosphonate gave (−)-**11** ($[\alpha]^{20}$ −12.7°, *c* 0.24, $H_2O$) and (+)-**11** ($[\alpha]^{20}$ + 11.0°, *c* 0.25, $H_2O$). The former was confirmed to have an absolute configuration corresponding to the inhibitory enantiomer of **1**, and proved to have enhanced affinity ($K_i$ = 4.3 μmol $dm^{-3}$) relative to the racemate, whilst (+)-**11** exhibited reduced activity ($K_i$ 34 = μmol $dm^{-3}$).

In conclusion, we have shown that the methylenebisphosphonic acid group can function as a replacement for a phosphate ester in inhibitors of *myo*-inositol monophosphatase such as **1**. Moreover, the evidence presented suggests that both phosphate **1** and methylenebisphosphonates **10** and **11** have similar interactions with the enzyme. The precise mode of recognition of bisphosphonates by the enzyme is unclear. It may be that one phosphonate group directly mimics the phosphate whilst the other is used to modify its electronic properties or to participate in ancilliary hydrogen bonding interactions. Alternatively, both phosphonates may interact equally, each providing part of the electron density required to mimic the phosphate group. The removal of the phosphorus–oxygen bond in the bisphosphonates compared to phosphate-containing inhibitors has introduced a point of stability with respect to the action of phosphatases. Indeed structurally related bisphosphonates have been reported[10] to be stable to metabolism under *in vivo* conditions.

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