Identification of (1*S*)-Phosphoryloxy-(2*R*,4*S*)-dihydroxycyclohexane as a Potent Inhibitor of Inositol Monophosphatase

Raymond Baker, Paul D. Leeson, Nigel J. Liverton,* and Janusz J. Kulagowski

Merck, Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, U.K.

The 3,5,6-trisdeoxy derivative of *myo*-inositol 1-monophosphate, (1*S*)-phosphoryloxy-(2*R*,4*S*)-dihydroxycyclohexane [(−)-(**1**)], derived from the parent substrate by a strategy of hydroxy deletion, has been synthesised and shown to be the most potent inhibitor of inositol monophosphatase yet identified.

Inositol 1,4,5-trisphosphate† and inositol 1,3,4,5-tetrakisphosphate act as second messengers by stimulating calcium release from intracellular stores as well as external calcium entry.[1] These polyphosphates are formed from hydrolysis of phosphatidylinositol 3-phosphate and are subsequently metabolised by sequential dephosphorylations catalysed by specific phosphatase enzymes. The final dephosphorylation step is mediated by the enzyme inositol monophosphatase, which converts both enantiomers of inositol 1-phosphate and inositol 4-phosphate to free inositol.[2] Peripheral inositol does not penetrate the blood–brain barrier effectively, and in the central nervous system (CNS), the levels of free inositol available for phosphoinositide formation may be regulated by the action of inositol monophosphatase. Potent and selective inhibitors of inositol monophosphatase would provide valuable tools to evaluate the pivotal role of this enzyme in the phosphoinositide pathway. Herein we report the design and synthesis of the trisdeoxy derivatives [(**1**) and (**2**)] of inositol 1-monophosphate (**3**). Compound (**1**) was resolved into its individual enantiomers and (−)-(**1**) proved to be a highly potent and specific inhibitor of inositol monophosphatase.

Inositol monophosphatase displays a notable lack of substrate specificity, as shown by its ability to catalyse the hydrolysis of both enantiomers of inositol 1- and 4-phosphates,[2] as well as other phosphates possessing at least one hydroxy group α- to the phosphate.[3,4] Our strategy[4] for the development of inhibitors has focused on the systematic

† Inositol refers to *myo*-inositol chemistry in all cases.

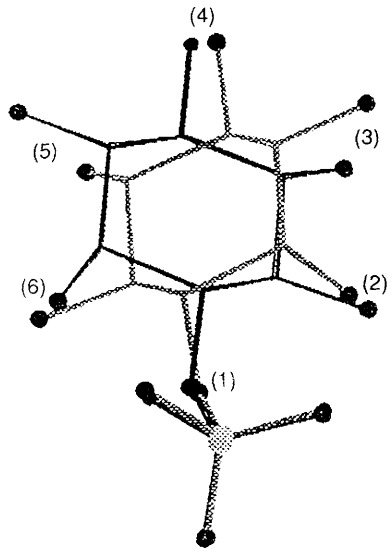
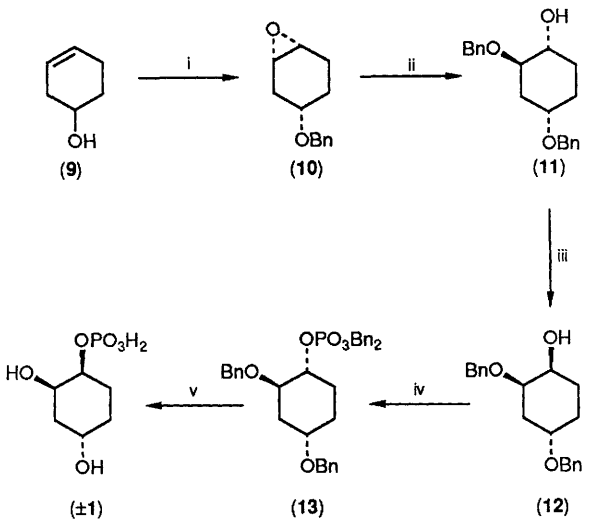
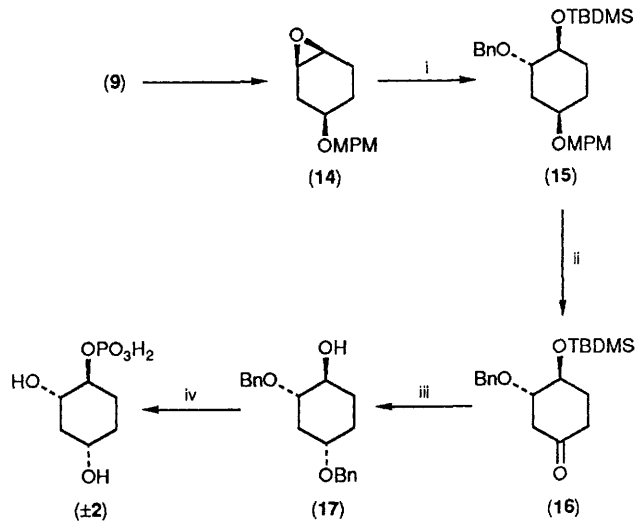


Figure 1. Superimposition of the 1,2,4,6-oxygens and phosphorus atoms of the structures of D-*myo*-inositol 1-phosphate (solid bonds) and L-*myo*-inositol 1-phosphate (D-*myo*-inositol 3-phosphate, hatched bonds). Distances in Å between the superimposed atoms: O(1), 0.14; O(2), 0.37; O(4), 0.39; O(6), 0.38; P, 0.06. Distances in Å between non-superimposed atoms: O(3), 2.45; O(5), 2.39. Structures were built and energy minimised using MOLEDIT and OPTIMOL (MSDRL molecular modelling programmes written by the Molecular Systems Group, MSRDL Rahway) and were superimposed and displayed using CHEMX (Chemical Design Limited, Oxford).



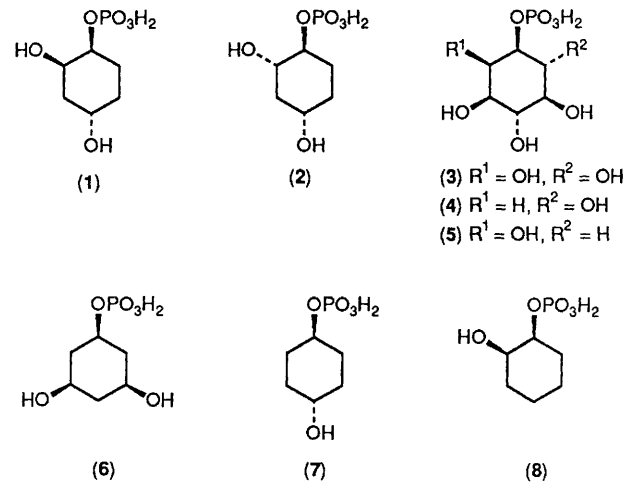
Scheme 1. *Reagents and conditions*: i, (a) $VO(acac)_2$, Bu^tO_2H, (b) NaH, BnBr (Bn = $PhCH_2$); ii, BnOH, Al_2O_3, PhMe, reflux; iii, (a) dimethyl sulphoxide (DMSO), $(COCl)_2$, (b) L-selectride; iv, $[(BnO)_2PO]_2O$; v, H_2, Pd/C.

deletion of hydroxy groups from the substrate (**3**). Initial studies with the 2-deoxy (**4**) and 6-deoxy (**5**) derivatives led us to conclude that the hydroxy groups α- to the phosphate in (**3**) and other substrates are independently associated with the mechanisms of phosphate hydrolysis and binding to the enzyme.[4] We next addressed the roles of the hydroxy groups at the 3-, 4-, and 5-positions of (**4**) and (**5**). Comparisons of the three-dimensional structures of the enantiomers of the substrate (**3**), using molecular modelling techniques, revealed



MPM =
TBDMS = Bu^tMe_2Si

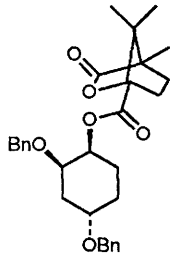
Scheme 2. *Reagents and conditions*: i, (a) BnOH, Al_2O_3, PhMe, reflux, (b) $Bu^tMe_2SiOSO_2CF_3$; ii, (a) DDQ, (b) DMSO, $(COCl)_2$; iii, (a) $NaBH_4$, EtOH, (b) KH, BnBr, tetrahydrofuran (THF), (c) Bu^n_4F, THF; iv, (a) $[(BnO)_2PO]_2O$, NaH, (b) H_2, Pd/C.



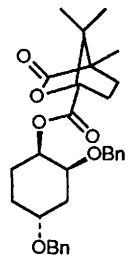
that the phosphate group, both α-hydroxys, and the 4-hydroxy of each enantiomer could be superimposed (Figure 1). In this comparison, the 3- and 5-hydroxys of the enantiomers could not be superimposed, suggesting that these groups may not be required for binding by the enzyme. This conclusion was supported by the lack of substrate or inhibitor activity of the *cis* 3,5-dihydroxy analogue (**6**). In addition the *trans*-4-hydroxy- (**7**) and *cis*-2-hydroxy- (**8**) cyclohexane phosphates were the only monohydroxy derivatives to show any activity. Consequently the 3,5-deoxy derivatives [(**1**) and (**2**)] of the inhibitors [(**5**) and (**4**) respectively] were synthesised (Schemes 1 and 2).

Cyclohex-3-en-1-ol[5] [(**9**), Scheme 1] was epoxidised[6] and benzylated to give the *cis*-regioisomer (**10**).‡ Treatment of

‡ All new compounds displayed spectral characteristics consistent with their proposed structures, and were characterised by elemental analysis and/or high resolution mass spectra.



(18) [⟶ (−)-(1)]



(19) [⟶ (+)-(1)]

(**10**) with benzyl alcohol in refluxing toluene containing activated alumina[7] resulted in regioselective epoxide ring opening, giving the alcohol (**11**) exclusively. Inversion of the stereochemistry of alcohol (**11**) was accomplished by Swern oxidation to the ketone, followed by stereoselective reduction with L-selectride. Conversion of (**12**) to the racemic phosphate (**1**), *via* the tetrabenzyl derivative (**13**), was completed using previously established methodology.[4,8] The alcohol (**17**) required for the synthesis of the regioisomer (**2**) was also prepared from (**9**), Scheme 2. Protection in this case as the *p*-methoxybenzyl (MPM) ether gave epoxide (**14**) which was ring opened with benzyl alcohol as described above and silylated to give the protected derivative (**15**). Selective removal of the *p*-methoxybenzyl group with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) followed by oxidation of the alcohol afforded the ketone (**16**). Stereoselective reduction of (**16**) with sodium borohydride provided the inverted alcohol in 77% yield. Benzylation followed by removal of the silyl protecting group gave the required precursor alcohol (**17**) which was converted as described above to the isomeric phosphate (**2**).

The racemic compounds [(**1**) and (**2**)] were found to be potent competitive inhibitors of inositol monophosphatase, lacking any substrate activity, having IC_{50}'s of 7 and 90 μM respectively. The more potent inhibitor (**1**) was resolved into its individual enantiomers by treatment of the alcohol (**12**) with (−)-camphanic acid chloride, giving a mixture of diastereoisomeric esters [(**18**) and (**19**)], which were separated by chromatography. These were hydrolysed to the enantiomers of (**12**) which were separately converted to (−)-(**1**) ($[\alpha]_D^{21}$ −7.08, *c* 1.10, H_2O, pH 9) and (+)-(**1**) ($[\alpha]_D^{21}$ +7.0, *c* 1.02, H_2O, pH 9). The absolute stereochemistries of (−)- and (+)-(**1**) were shown to be 1*S*, 2*R*, 4*S* and 1*R*, 2*S*, 4*R* respectively following single crystal *X*-ray analysis of the ester (**18**). In keeping with the activities of the corresponding enantiomers of (**4**),[4] (−)-(**1**) proved to be a potent inhibitor of inositol monophosphatase (IC_{50} 3 μM) and (+)-(**1**) was a weak substrate [K_{cat}/K_m 0.19 min^{-1} μmol^{-1}; K_{cat}/K_m for (±)-(**1**), 4.8 min^{-1} μmol^{-1}].

These results demonstrate that the hydroxy groups at the 3- and 5-positions in both inositol monophosphates and their α-deoxy derivatives are not necessary for enzyme recognition by inositol monophosphatase. Hydroxy groups at the 2- and 4-positions of the cyclohexane ring are however essential for binding to the enzyme. The 2,4-dihydroxy phosphate (−)-(**1**) contains the minimum essential structural features for enzyme recognition, in the optimal stereochemical configuration. Compound (−)-(**1**) is the most potent and selective inhibitor of inositol monophosphatase yet reported, and should have utility in unravelling the role of this enzyme in the phosphoinositide cycle.

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