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Synthesis of 6-Amino-3,5-deoxyinositol 1-phosphates *via* (1R,2R,4R,6S)-1,6-Epoxy-2,4-*bis*-benzyloxycyclohexane Aminolysis in Aqueous Ytterbium Triflate Solution

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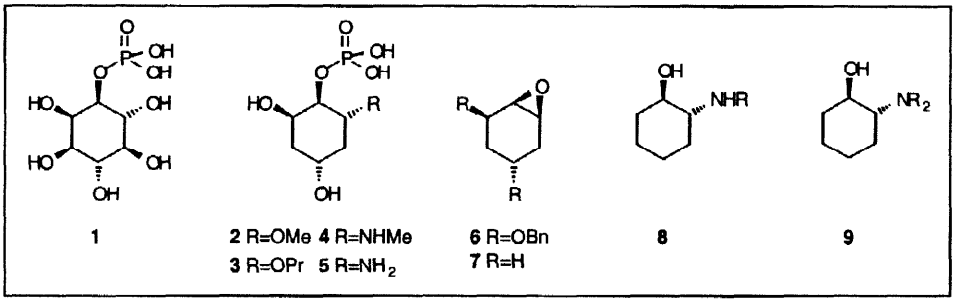
Abstract. (1R,2R,4R,6S)-1,6-Epoxy-2,4-*bis*-benzyloxycyclohexane was prepared from (-)-quinic acid and this, and cyclohexene oxide, were treated with various N-nucleophiles under a variety of conditions. In aqueous solution containing catalytic amounts of ytterbium (III) triflate, ammonia and alkylamines reacted smoothly to give the required *trans*-1,2-amino alcohols in quantitative recovery. Conversion of the 6-amino-2,4-*bis*-benzyloxycyclohexan-1-ols to 6-amino-1,2,4-trihydroxycyclohexane 1-phosphates, probes for the mechanism of inositol monophosphatase, was achieved in good overall yield.

Understanding the mechanism of action of inositol monophosphatase (IMPase, EC 3.1.3.25) and its inhibition by lithium ion is important in manic depression therapy. While it is now established that Li^+ blocks the action of the enzyme by occupying the site of the second of two essential Mg^{2+} ions in the phosphate-bound enzyme product complex,[1] the catalytic mechanism for the hydrolysis of the substrate could follow one of two mechanisms.[2,3] In order to resolve these ambiguities, we sought substrate analogues that would address the question of whether the catalytically important 6-OH group of the substrate, *myo*-inositol 1-phosphate **1**, was an H-bond donor or an H-bond acceptor. Since analogues **2** and **3** possessing 6-OMe and 6-OPr groups, respectively, were known to be inhibitors, but not substrates,[4,5] it appeared that the 6-OH group served as an H-bond donor. However, it could not be excluded that 6-O alkylated analogues were too large to bind to the enzyme in the correct conformation for catalytic hydrolysis. Thus, it was necessary to prepare an isosteric analogue of one of the inhibitors that could serve as an H-bond donor, and assess it as a substrate.



The 6-methylamino analogue **4** was considered to be a suitable probe with which to test the hypothesis, providing that it could be established that the 6-amino analogue **5** served as a substrate. Therefore, protocols for the preparation of 6-amino-1,2,4-trihydroxycyclohexane 1-phosphates **4** and **5** were sought.

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Recently, we reported on the preparation of (1R,2R,4R,6S)-1,6-epoxy-2,4-*bis*-benzyloxy-cyclohexane **6** from (-)-quinic acid.[6] The absolute stereochemistry of this intermediate facilitated the conversion of the epoxide, with inversion of configuration at C-6, to 6-alkyloxy derivatives that could be subsequently converted to 1-phosphate ester derivatives that were known to be the most active stereoisomers for inositol monophosphatase inhibition.[4-6] It, therefore seemed expedient to employ the epoxide in the synthesis of the amine derivatives. The choice of N-nucleophile was deferred, but given that primary and secondary amine targets **4** and **5** were sought, the direct aminolysis of the epoxide **6** with the naked amines (or, masked amines, for example, benzylamines or O-benzyl hydroxylamines, the derivatives of which could be deprotected at the same time as the 2- and 4-benzyloxy groups and the benzyl phosphate ester protection) seemed preferable over the use of azide.

In the synthesis of the alkoxy derivatives from the epoxide, it had been shown that alkali metal alkoxides were too basic and caused C-5 deprotonation and oxirane ring-opening to give the allylic alcohol.[5] However, under acidic conditions, in the presence of catalytic quantities of boron trifluoride, good yields of the required 6-ethers were obtained.[5] Attempts to react ammonia and primary and secondary amines directly with the epoxide failed, and likewise, in the presence of BF_3 etherate under similar conditions to those utilised for the alcoholyses, only starting materials were recovered. Presumably, in the latter case, the failure of the reaction is due to the complete sequestation of the Lewis acid catalyst by the excess amine employed.

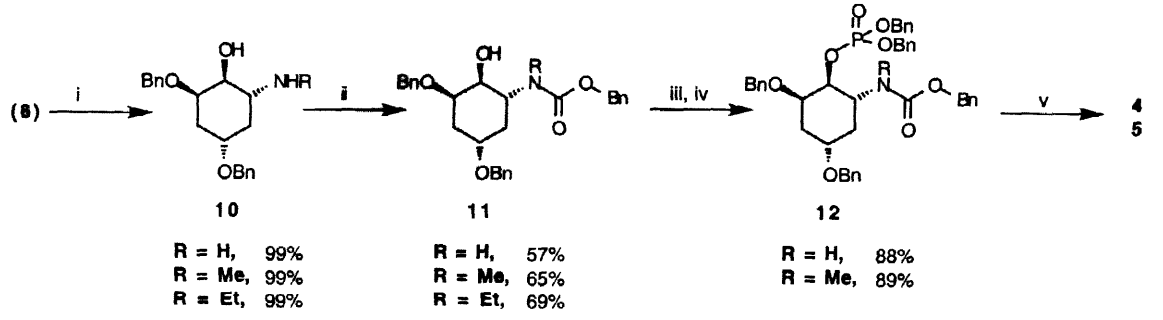
It had been reported that magnesium amides were very good reagents for the conversion of epoxides to 1,2-amino alcohols.[7] In our own hands, in model reactions using cyclohexene oxide **7**, useful yields of the required *trans* 1,2-amino alcohols **8** were obtained. For example, magnesium benzylamide freshly prepared from ethyl magnesium bromide and benzylamine, gave 2-N-benzylaminocyclohexanol **8** (R = Bn) in 76% yield. Under similar conditions the reaction with epoxide **6** gave a much poorer 19% yield of the required amino alcohol together with other products, and so other protocols were sought and tested.

Lithium tetrafluoroborate and perchlorate salts were claimed to be excellent catalysts for epoxide aminolysis.[8] In model reactions in the presence of $LiBF_4$ and benzylamine, epoxide **7** was converted to the amino alcohol **8** (R = Bn) in 43% yield using the reported conditions, but epoxide **6** failed to react.

Crotti and co-workers had shown that ytterbium (III) triflate,[9] another Lewis acid, was useful in the preparation of 2-amino alcohols from oxiranes in aprotic solvents. Treatment of cyclohexene oxide **7** with benzylamine and 0.2 equivalents of the salt in DCM solution at 20 °C quantitatively afforded the amino alcohol **8** (R = Bn). Likewise, dibenzylamine reacted with epoxide **7** to afford amino alcohol **9** (R = Bn) in 100% yield and O-benzyl hydroxylamine gave the amino alcohol **8** (R = OBn) in 90% yield after chromatographic purification. The reaction of the epoxide **7** with 15M aqueous ammonia at 20 °C gave the amino alcohol **8** (R = H) in 60% conversion (51% isolated yield), but although longer reaction times gave better conversions, under similar aqueous conditions, and also in dioxane, the epoxide **6** failed to react.

Crotti had noted that treatment of 4-benzyloxy-1,2-epoxycyclohexane with diethylamine in the presence of 0.5 equivalents of $Yb(OTf)_3$ in ethanol for 5 days at 55 °C had afforded the amino alcohol in only 50% yield.[9] It was also noted that non-protic, non-coordinating solvents worked best and that increasing amounts of water severely inhibited catalysis. However, when epoxide **6** or **7** was heated to 65 °C with either aqueous ammonia, methylamine or ethylamine in a sealed tube in the presence of 0.2 equivalents of $Yb(OTf)_3$ for 24 h, each of the amino alcohols **8** (R = H, Me and Et) and **10** (R = H, Me and Et) were obtained in quantitative conversion. The compounds displayed the expected analytical and spectroscopic properties and analysis of the ^1H NMR spectra for compounds **10** (R = H and Me) showed that the reaction had proceeded with inversion of configuration at C-6

and that no or very little reaction had occurred *via* attack at C-1. Thus, it was possible to convert the key epoxide **6** to amino alcohols of the required configuration through direct aminolysis.



Scheme 1 *Reagents and conditions* : **i.** aqueous amine, 0.2 eq. $Yb(III)(OTf)_3$, 65 °C, Sealed tube, 12-48 h; **ii.** 2M NaOH, benzylchloroformate, 0 °C, 2 h; **iii.** diphenylchlorophosphate, TEA, DMAP, DCM, 20 °C, 12 h; **iv.** BnONa, THF, -70 °C, 2 h; **v.** Pd/C (10%), $H_{2(g)}$, MeOH, 24 h.

The 6-amino cyclohexanols **10** (R= H and Me) were treated with benzyl chloroformate (Scheme 1) to give the benzyl ureathanes **11** (R = H and Me) in moderate yields of 57 and 65%, respectively, which were fully characterised. It was not essential to protect the amines prior to phosphorylation, but trial reactions in which phosphorylation with diphenyl chlorophosphate was performed directly on compounds **10** (R = H and Me), followed by hydrolysis of the resulting phosphoramides, gave poor yields of the the required phosphate triesters. Also, chromatographic purification of the triesters was hampered by the high polarity of the amino groups and their salt derivatives. Accordingly, the amines were protected and the ureathane products **11** (R = H and Me) and were treated with diphenyl chlorophosphate in the presence of TEA and DMAP to give the required ureathane triesters in excellent yield. The compounds displayed the required spectral and analytical properties and were transesterified with sodium benzyloxide to give the dibenzyl phosphate esters **12** (R = H and Me) using procedures previously optimised for the 6-alkyloxy derivatives **2** and **3**,[5] in yields of 88 and 89%, respectively. The dibenzyl phosphate esters **12** (R = H and Me)[10] were subjected to catalytic hydrogenolysis to remove all five benzyl protecting groups in quantitative recovery and the resulting amino phosphates **5** and **4** were converted to the cyclohexylammonium salts using previously described procedures.[5] These salts displayed the expected spectral data and were ready to be tested for biological activity.

Buffered deuterium oxide solutions (0.5 cm^3) at pH 8.0 of each of the amino phosphate esters (15 mM) containing magnesium chloride (2mM) and IMPase[1] (30 units) were monitored by ^1H NMR spectroscopy and spectra were acquired at 15 minute intervals. The 6-amino phosphate **5** was hydrolysed completely within 6 h to give the expected product, indicating that it is a moderate substrate. Note that the physiological substrate **1** is hydrolysed completely within 15 minutes under these conditions. The result indicates that a 6-amino group is able to fully support catalysis and, indeed, is the first example of a compound possessing any substituent other that a hydroxyl group at C-6 to have been shown to display substrate activity. It is not known whether the free base form or protonated form of the compound, or both forms can bind to the enzyme, but we would expect the pK_a value of theamino group to be *ca.* 8.5 such that a significant amount of each form is available at pH 8.0.

The 6-methylamino phosphate **4** took 10 h before an obvious time-dependent change could be observed. Nevertheless, the activity was inhibited by 200 mM lithium ion and repeat experiments performed using more enzyme gave faster rates indicating that the 6-methylamino phosphate **4** is a substrate for IMPase. Thus, it is

established that the 6-amino phosphate is a moderate substrate and that analogues of the compound with which to probe the mechanism further can be prepared in useful quantities from the key epoxide **6** through its direct aminolysis. A full kinetic evaluation of the activity of 6-methylamino phosphate **4** as a substrate, and also as an inhibitor is presently underway. Because it is at best a slow substrate, a detailed comparison of its properties to those of the 6-methylether phosphate inhibitor **2** will be required to address the catalytic role of the 6-OH group in the substrate **1** in serving as an H-bond acceptor or donor.

Acknowledgements

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Abbreviations.

Bn, benzyl; Ins 1-P, *myo*-Inositol 1-phosphate; TEA, triethylamine; DMAP, dimethylaminopyridine; P_i, inorganic phosphate; DCM, dichloromethane.

References and Notes

1. Gani, D., Downes, C. P., Batty, I. and Bramham, J. (1993) *Biochim. Biophys. Acta* **1177**, 253-269; Leech, A. P., Baker, G. R., Shute, J. K., Cohen, M. A. and Gani, D. (1993) *Eur. J. Biochem.* **212**, 693-704.
2. a) Pollack, S. J., Atack, J. R., Knowles, M. R., McAllister, G., Ragan, C. I., Baker, R., Fletcher, S. R., Iverson, L. L. and Broughton, H. B. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 5766-5770. b) Bone, R., Frank, L., Springer, J. P. and Atack, J. R. (1994) *Biochemistry* **33**, 9468-9476.
3. Cole, A. G. and Gani, D. (1994) *J. Chem. Soc., Chem. Commun.* 1139-1141; Wilkie, J., Cole, A. G. and Gani, D. (1995) *J. Chem. Soc., Perkin Trans. 1* 2709-2727.
4. Schulz, J., Wilkie, J., Lightfoot, P., Rutherford, T. and Gani, D. (1995) *J. Chem. Soc., Chem. Commun.* 2353-2356.
5. Schulz, J. and Gani, D. (1997) *J. Chem. Soc., Perkin Trans.* 1 657-670 and references cited therein.
6. Schulz, J. and Gani, D. (1997) *Tetrahedron Lett.* **38**, 111-114 and references cited therein.
7. Carre, M. C., Houmounou, J. P. and Caubere, P. (1985) *Tetrahedron Lett.* **26**, 3107-3110.
8. Chini, M., Crotti, P. and Macchia, F. (1990) *Tetrahedron Lett.* **31**, 4661-4664.
9. Chini, M., Crotti, P. and Macchia, F. (1994) *Tetrahedron Lett.* **35**, 433-436.
10. NMR data for **12** (R = H); δ_H(300 MHz; C^2HCl_3) 1.36-2.19 (4 H, m, 3 and 5-CH_2), 3.69-3.83 (2 H, m, 4-CH, 6-CH), 4.08-4.21 (1 H, m, 2-CH), 4.32 (1 H, m, 1-CH), 4.36 (2 H, s, CH_2Ph), 4.52 (2 H, d, $^2J_{HH}$ 11.5, CH_2Ph), 4.81-5.07 (6 H, m, 3 x CH_2Ph), 5.73 (1 H, b, NH) and 7.17-7.24 (25 H, m, Ar-H); δ_C(75 MHz; C^2HCl_3) 32.04 (3-C), 32.07 (5-C), 49.30 (6-C), 66.49 (CH_2Ph), 69.32 (CH_2Ph), 70.73 (CH_2Ph), 71.32 (4-C), 72.70 (2-C), 72.90 (1-C), 127.56, 127.74, 127.88, 128.02, 128.29, 128.40, 128.48 and 129.66 (Ar-CH), 135.88, 135.97, 136.57, 138.03 and 138.24 (quat. Ar-C) and 155.66 [quat. Ar-C (Cbz)]; δ_P(121.5 MHz; C^2HCl_3) -0.50; FAB$^+$ *m/z* (%): 744 ([M+Na]$^+$, 100); HRMS Calcd. $C_{42}H_{44}NO_8NaP$: 744.2702. Found 744.2689; for **12** (R = Me); δ_H(300 MHz; C^2HCl_3) 1.21-2.38 (4 H, m, 3 and 5-CH_2), 2.83 and 2.85 (3 H, 2 x s, NMe of *cis* and *trans* ureathane), 3.81-3.92 (1 H, m, 4-CH), 4.02-4.12 (1 H, m, 2-CH), 4.03-4.12 (1 H, m, 2-CH), 4.36-4.60 (5 H, m, 6-H and 2 x CH_2Ph), 4.82-5.19 (7 H, m, 1-CH, 3 x CH_2Ph) and 7.18-7.36 (25 H, m, Ar-H); δ_C(75 MHz; C^2HCl_3) 29.56 (3-C), 33.83 (5-C), 34.63 (NMe), 67.02 (CH_2Ph), 69.27 (CH_2Ph), 70.82 (6-C), 71.30 (4-C), 71.44 & 71.49 (2 x CH_2Ph), 72.41 (CH_2Ph), 75.22 (2-C), 79.90 (1-C), 126.92, 127.28, 127.49, 127.45, 127.68, 127.81, 127.94, 128.09, 128.32, 128.48, 128.55 (Ar-CH), 135.73, 136.11, 136.73, 138.43, 138.59 (quat. Ar-C) and 156.54 [quat. Ar-C (Cbz)]; δ_P(121.5 MHz; C^2HCl_3) -1.45 and -1.63; FAB$^+$ *m/z* (%): 758 ([M+Na]$^+$, 100); HRMS Calcd. $C_{43}H_{46}NO_8NaP$: 758.2859. Found 758.2880. Note: Compound **12** (R = Me) exists as two N-methyl benzylureathane rotomers.