Design Synthesis and Preliminary Evaluation of a Potent α-Mannosidase Inhibitor: 1,4-Dideoxy-1,4-imino-D-mannitol

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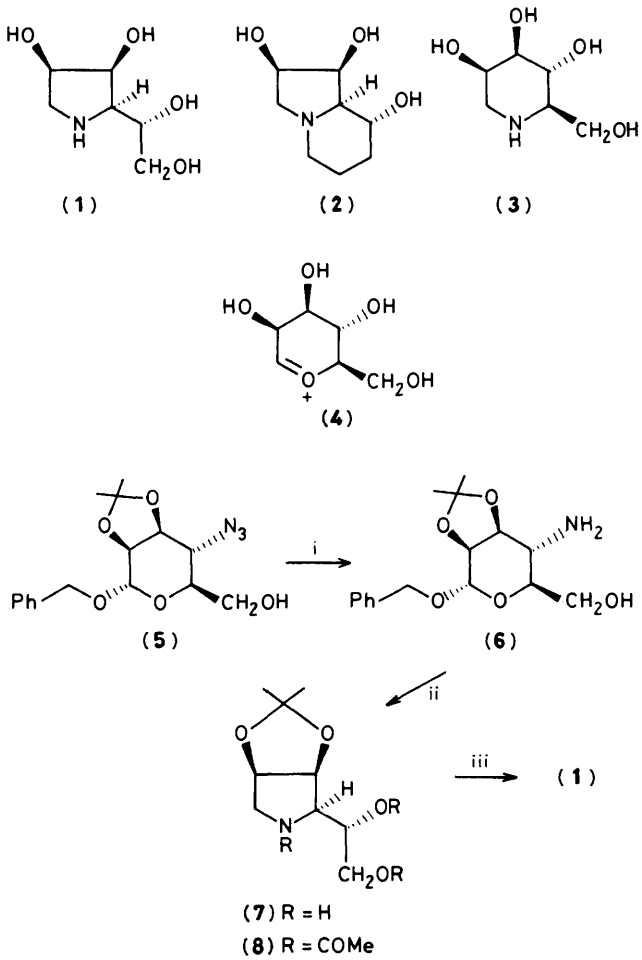
1,4-Dideoxy-1,4-imino-D-mannitol (**1**), synthesised from benzyl α-D-mannopyranoside, is a potent competitive inhibitor of the hydrolysis of *p*-nitrophenyl α-D-mannopyranoside catalysed by Jack Bean α-mannosidase (*Canavalia ensiformis*); this is the first report of the specific inhibition of a glycosidase by a pyrrolidine analogue of a furanose sugar.

There are several examples of the specific inhibition of glycosidases by hydroxylated derivatives of piperidine.[1] Various glucosidases, including a processing glucosidase of glycoprotein synthesis, are inhibited by nojirimycin,[2] deoxynojirimycin,[3] and castanospermine.[4] In contrast, swainsonine[5,6] (**2**) and 1,5-dideoxy-1,5-imino-5-mannitol (**3**)[7] are mannosidase inhibitors; each inhibits a different mannosidase of glycoprotein processing.[8] It has been proposed that the mechanisms of glucosidase and mannosidase reactions are similar, proceeding *via* intermediate glucopyranosyl or mannopyranosyl (**4**) cations, respectively.[8,9] It is apparent that (**2**) has a greater structural similarity to the azafuranose (**1**) than to the six membered ring isomer (**3**). This communication describes the synthesis of 1,4-dideoxy-1,4-imino-D-mannitol (**1**) and a preliminary evaluation of its ability to inhibit glycosidase activity.

Crystalline benzyl 4-azido-4-deoxy-2,3-*O*-isopropylidene-α-D-mannopyranoside (**5**) is readily available in an overall yield of 42% from benzyl α-D-mannopyranoside in gram quantities.[6] Hydrogenolysis of (**5**) in the presence of palladium black in methanol gives the primary amine (**6**)† in quantitative yield. Hydrogenolysis of the benzyl group in (**6**) gives a lactol which is in equilibrium with an open chain aminoaldehyde. Intramolecular reductive amination leads to the acetonide (**7**), m.p. 86—88 °C, $[\alpha]_D^{20}$ −53.3° (*c* 0.43 in $CHCl_3$) (90% yield) (Scheme 1). Acetylation of (**7**) with acetic anhydride in pyridine gave the crystalline diacetate (**8**), m.p. 80—82 °C, $[\alpha]_D^{20}$ −22.4° (*c* 0.50 in $CHCl_3$). The removal of the isopropylidene protecting group was accomplished by dissolving the acetonide in trifluoroacetic acid–deuterium oxide and observing the disappearance of the two methyl group singlets of the isopropylidene group by n.m.r. spectroscopy. The crude product (**1**) [85% yield from (**7**); 77% overall yield from (**5**)], m.p. 137 °C, $[\alpha]_D^{20}$ −10.4° (*c* 0.12 in H_2O), was purified by ion exchange chromatography. This strategy for the construction of the pyrrolidine ring is similar to that for

† Satisfactory spectral and/or analytical data were obtained for all new compounds.



Scheme 1. *Reagents and conditions:* i, H_2, Pd black, MeOH, 4 h; ii, H_2, Pd black, $MeCO_2H$, 2 days; iii, CF_3CO_2H–D_2O (9 : 1).

(**2**) from a modified 4-azidomannose;[6] ring formation is assisted by the presence of the isopropylidene protecting group.[10]

The pyrrolidine (**1**) is a weak inhibitor of the hydrolysis of *p*-nitrophenyl α-D- and β-D-glucopyranosides by yeast α-glucosidase (50% inhibition of enzymic activity at 5.0×10^{-4} M) and almond emulsin β-glucosidase (50% inhibition at 4.5×10^{-4} M), respectively.‡ However, (**1**) is a potent inhibitor of the hydrolysis of *p*-nitrophenyl α-D-mannopyranoside by Jack Bean α-mannosidase (*Canavalia ensiformis*), causing 50% inhibition of enzymic activity at a concentration of 5.0×10^{-7} M; in comparison, 50% inhibition of mannosidase activity by (**2**) requires a concentration of 8.0×10^{-6} M. A double reciprocal Lineweaver–Burk plot for Jack Bean α-mannosidase-catalysed hydrolysis of *p*-nitrophenyl α-D-mannopyranoside at different concentrations of (**1**) shows that (**1**) is a competitive inhibitor (K_i 7.6×10^{-7} M) of Jack Bean α-mannosidase (K_m 2.0×10^{-3} M);§ (**2**) also shows competitive inhibition of α-mannosidase activity (K_i 9.5×10^{-6} M).¶ Although the inhibition of mannosidase activity is pH dependent,[11] under the conditions reported here (**1**) is clearly a more potent mannosidase inhibitor than (**2**).

These results demonstrate that nitrogen analogues of furanose sugars, such as (**1**), may allow the design of specific glycosidase inhibitors. More importantly, the synthesis of polyhydroxylated piperidines and pyrrolidines may provide a general and predictive method for controlling glycosidase, glycosyl transferase, and other enzyme-mediated reactions involving carbohydrate substrates.

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‡ The *p*-nitrophenyl glycopyranosides and the enzymes used in the inhibition studies were purchased from Sigma. The assay of α-mannosidase activity contained the following components in a final volume of 1.2 ml: 0.4 ml 50 mM citric acid–sodium citrate buffer pH 4.5, 0.4 ml 2 mM *p*-nitrophenyl α-D-mannopyranoside, 0.4 ml Jack Bean α-mannosidase (*C. ensiformis*) (2.35×10^{-1} mg/ml). The mixture was incubated at 25 °C for 10 min; aqueous sodium hydroxide (0.6 ml, 0.25 M) was added and the enzymic activity was estimated as the absorbance at 410 nm relative to an uninhibited sample. The inhibitors were incorporated into the assay buffers to give inhibitor concentrations 10^{-3} and 10^{-8} M. Further details of the assay techniques are given in S. V. Evans, L. E. Fellows, and E. A. Bell, *Phytochemistry*, 1983, **22**, 768.

§ This is in good agreement with the published value for this enzyme with *p*-nitrophenyl α-D-mannopyranoside of K_m 2.5×10^{-3} M: Y.-T. Li, *J. Biol. Chem.*, 1967, **242**, 5474.

¶ This is in agreement with the value obtained [ref. 5(b)] for the inhibition of the hydrolysis of 4-methylumbelliferyl α-D-mannopyranoside by Jack Bean α-mannosidase by (**2**).