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SYNTHESIS OF 1,4,6-TRIDEOXY-1,4-IMINO-D-MANNITOL: A POTENT α-MANNOSIDASE INHIBITOR

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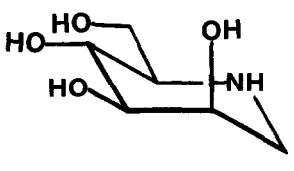
Summary: The title trihydroxypyrrolidine 4, readily synthesized in one step from the naturally-occurring sugar D-perosamine, is ten times more active than swainsonine as a competitive inhibitor of jackbean α-mannosidase.

Specific inhibitors of microsomal glycosidases have been instrumental in elucidating the step-by-step processing of complex, N-linked oligosaccharides during glycoprotein biosynthesis. Well-known glucosidase inhibitors include deoxynojirimycin[1] and castanospermine[2] whereas deoxymannonojirimycin 1[3] and swainsonine 2[4] represent specific mannosidase inhibitors. Stereochemical and configurational studies have established that these azasugars and indolizidine alkaloids are homochiral with D-gluco and D-mannopyranose structures, respectively, making them effective competitive inhibitors of enzyme-mediated glycosyl transfer. Certain hydroxylated pyrrolidines can also inhibit glycosidases,[5] and Fleet et al. recently reported a multistep synthesis of 1,4-dideoxy-1,4-imino-D-mannitol 3, a potent inhibitor of jackbean α-mannosidase.[6] Our own studies of structure-activity relationships in this area[2,7] led us to design an even simpler structure, 4 (chirality as drawn), predicted to exhibit the same biological profile but chemically accessible in one step from D-perosamine 5.



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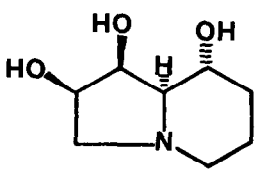

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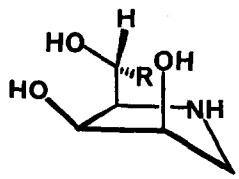
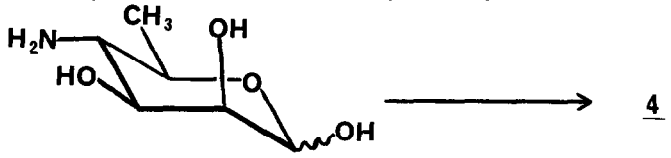


3 R = CH_2OH

4 R = CH_3

The hydrochloride salt of D-perosamine 5 (.188g, .98 mmol)[8] was treated with $NaBH_3CN$ (.19g, 3 mmol) and HOAc (40 μL) in CH_3OH (18 mL, 25°, 36h) to effect intramolecular reductive amination. After quenching (10% aqueous HCl) and lyophilization, ion exchange chromatography (Dowex 50, 100-200 mesh, 1M NH_4OH eluant) afforded 4 as the free base (60%), mp 183°C; $[\alpha]_D$ -20° (c 0.4, CH_3OH): lit mp 184-5°C; lit $[\alpha]_D$= -21.5°.[9] Pyrrolidine 4 competitively inhibited the hydrolysis of p-nitrophenyl-α-D-mannopyranoside [K_M= 1.5 x 10^{-3}M] by jack bean α-mannosidase [K_i= 5 x 10^{-7}M; 50% inhibition of enzymic activity at 6 x 10^{-7}M].[10] By comparison, Fleet reported 50% inhibition by 3 at 5 x 10^{-7}M [K_i= 7.6 x 10^{-7}M] and swainsonine required a concentration of 8 x 10^{-6}M for the same effect. Almond β-glucosidase was weakly inhibited by 4 at 10^{-3}M, but all other glycosidases tested (green coffee α-galactosidase, bovine β-galactosidase, bovine liver β-glucuronidase and bovine β-N-acetylhexosaminidase) were unaffected at 10^{-3}M.[11]



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10. All p-nitrophenylglycosides and enzymes used in these studies were purchased from Sigma. Assays were conducted at pH 5.00 (50mM HOAc-NaOAc buffer) at 0.5 mM [substrate]. Mixtures (200 μL) were incubated at 37°C for 15 min, quenched with pH 10.4 glycine buffer and absorbances read at 400nm.

11. We thank the NIH for a predoctoral traineeship to MJE (GM 97273).

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