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A NEW FAMILY OF FIVE-CARBON IMINOALDITOLS WHICH ARE POTENT GLYCOSIDASE INHIBITORS

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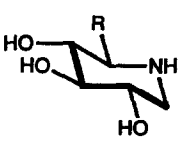
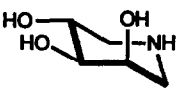
Abstract -- The synthesis of 1,5-dideoxy-1,5-imino-D-xylitol **2** (and the enantiomeric iminoalditols **3** and **4**) is described. Alditol **2** compares favorably with 1-deoxynojirimycin **1** as a potent inhibitor of β-glucosidase.

Deoxynojirimycin **1** effectively inhibits the enzymatic hydrolysis of β-D-glucosides.[2] The potential chemotherapeutic importance of such inhibitors of glucose metabolism[3-7] has prompted considerable synthetic interest in these alkaloids.[8-10] We reasoned that improved inhibitors might be rationally designed by examining how each hydroxyl substituent in the glycone (e.g. D-glucose) contributed to the free energy of binding with the enzyme. Dale *et al.* have systematically studied the inhibition of sweet almond β-glucosidase by a wide variety of normal and deoxysugars.[11] While the stereochemical configurations of individual ring hydroxyls were important, removing the C6-hydroxymethyl substituent altogether had remarkably little effect on enzyme-substrate interactions. This surprising finding suggested that stereochemically simpler *nor*-analogs of **1** might also inhibit glycoside hydrolysis. We now wish to report an extremely rapid and efficient synthesis of des(hydroxymethyl)-nojirimycin **2**. As expected, this achiral triol is in its own right a potent and highly selective almond β-glucosidase inhibitor. We have also prepared the mannose and galactose analogs **3** and **4**. These enantiomeric structures likewise inhibit glycosidases and shed new light on H-bonding and other enzyme-substrate interactions in the catalyzed hydrolysis of glycosides.



1 R= CH_2OH
2 R= H



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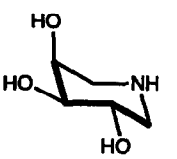

4

We planned a general synthesis of five-carbon iminoalditols like **2** from D-hexoses as outlined in the Scheme. The reductive ring opening of bromopyranose sugars, first developed by Vasella[12] and modified by us[8] to incorporate an *in situ* reductive amination of the incipient ω-alkenylaldehyde, was successful in transforming methyl 6-bromo-6-deoxy-α-D-glucopyranoside[13] **5** into aminoalkenetriol **6** in a one-pot reaction (70% yield).[14] To our knowledge this represents the first example of direct, reductive opening of an unprotected glycoside. Moreover the reaction proceeded without complications arising from aldehyde epimerization or acetal/hemiacetal formation. To prevent oxidation of the benzylic amine in the next step,[15] ozonolysis of **6** was performed on the corresponding trifluoroacetate salt. When the first-formed ozonide was reductively cleaved using dimethylsulfide in the presence of sodium cyanoborohydride (2.5 equiv in CH_3OH), trihydroxypiperidine **8** was obtained directly in 57% yield.[16] Hydrogenolysis of **8** quantitatively afforded the desired 1,5-dideoxy-1,5-imino-D-xylitol **2**.[17] In similar fashion D-mannose and D-galactose were converted via intermediates **9** and **10** to the enantiomerically related **3**[18] and **4**,[19] respectively.

At pH 5.0, alditol **2** inhibited sweet almond β-glucosidase (35% of control activity at 1 mM) but had no effect whatsoever on yeast α-glucosidase, jackbean α-mannosidase, coffee α-galactosidase, β-galactosidase, β-glucuronidase, or β-hexosaminidase (all bovine). Kinetic measurements with **2** on almond β–glucosidase using *p*-nitrophenyl β-D-glucopyranoside as substrate (K_M= 2.5 mM) resulted in Lineweaver-Burk plots indicating competitive inhibition.[20] Additionally, Dixon plots of 1/V versus [I] gave a value of K_I for **2** (0.43 ±0.1 mM) similar to that for **1** (0.37 mM at pH 5).[11] These data indicate that the hydroxymethylene sidechain of **1** is relatively unimportant for inhibitor binding.

Likewise, in exploratory screens against the same group of enzymes, mannose analog **3** competitively inhibited only jackbean α-mannosidase. Its effect was comparable to that of 1-deoxymannojirimycin (40% of control activity at 1 mM). However galactose analog **4** had only marginal effects on α- or β-galactosidase, suggesting that the -CH_2OH group of galactose may serve as a much more important recognition unit for catalyzed glycoside hydrolysis.

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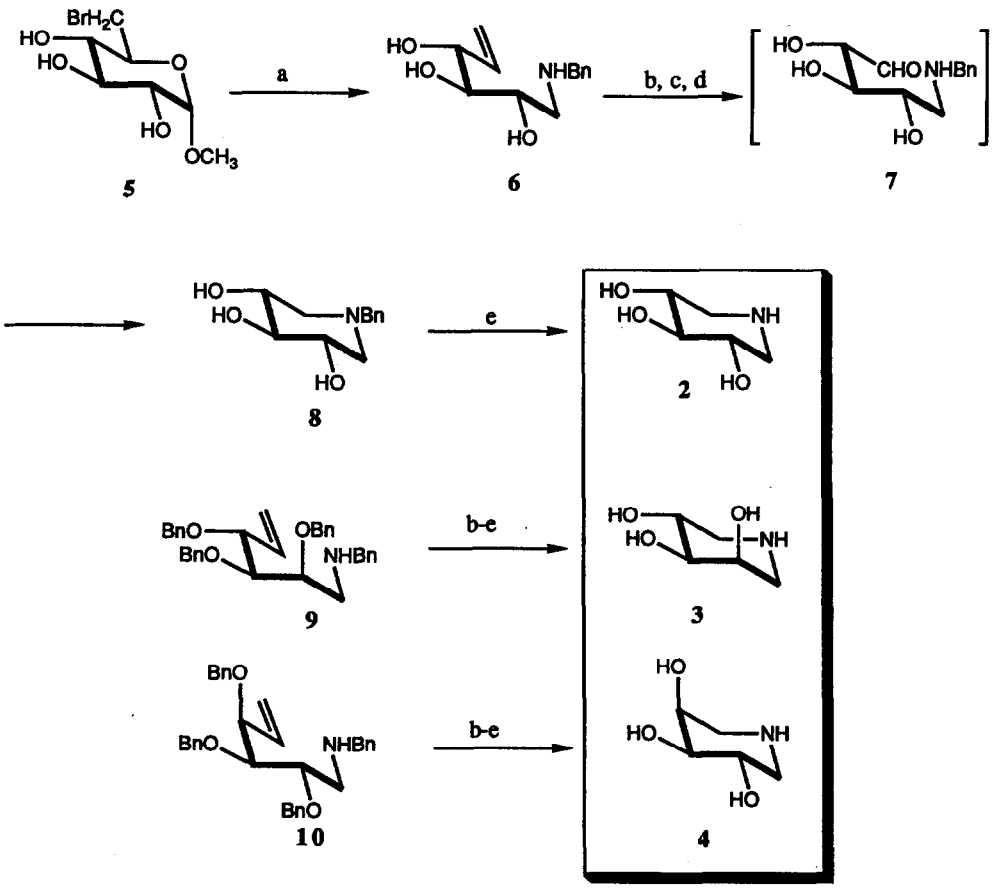
SCHEME



(a) excess Zn, 19:1 PrOH:H_2O, $NaBH_3CN$ (2 equiv), $PhCH_2NH_2$ (15 equiv), reflux, 2h; (b) CH_2Cl_2, excess CF_3CO_2H, evaporation; (c) CH_2Cl_2, O_3 (1.1 equiv), -78°C; (d) $(CH_3)_2S$ (1.5 equiv), $NaBH_3CN$ (2.5 equiv), CH_3OH, rt, 3h; (e) Pd-C, H_2, CH_3OH.

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14. For **6**: $[\alpha]_D$= -26° (c=0.9, $CHCl_3$); ^{1}H-NMR δ ($CDCl_3$) 7.26-7.14 (m, 5 H), 5.82 (ddd, 1 H, J=17.2, 10.5, 5.7 Hz), 5.26 (dd, 1 H, J=17.2, 2.8 Hz), 5.12 (dd, 1 H, J= 10.5, 2.8 Hz), 4.16 (m, 1 H), 3.74 (m, 1 H), 3.67 (d, 2 H, J= 4.8 Hz), 3.40 (dd, 1 H, J= 4.0, 2.9 Hz), 2.79 (dd, 1 H, J= 12.2, 4.1 Hz), 2.69 (dd, 1 H, J= 12.2, 5.3 Hz); CMR ($CDCl_3$) 139.2, 137.5, 128.5, 128.1, 127.2, 116.5, 75.4, 73.8, 70.4, 53.7, 51.9; IR (film) 3400, 2960, 1520, 1480, 1140, 1080, 1020 cm^{-1}; CIMS (isobutane) *m/e* 238 (M+1, 100%)
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16. For **8**: $[\alpha]_D$= 0.0°; ^{1}H-NMR δ (CD_3OD) 7.22-7.15 (m, 5 H), 3.46 (s, 2 H), 3.33 (m, 2 H), 2.98 (t, 1 H, J=8.9 Hz), 2.83 (m, 2 H), 1.83 (m, 2 H); CMR (CD_3OD) 138.7, 130.4, 129.3, 128.4, 80.6, 71.6, 63.1, 59.2; IR (KBr) 3330, 3270, 3050, 2935, 1450, 1365, 1145, 1075, 1020; CIMS *m/e* 224 (M+1, 39%).
17. For **2**: $[\alpha]_D$= 0.0°; ^{1}H-NMR (D_2O) 3.56 (m, H-3, H-5), 3.33 (t, H-4, J=8.9 Hz), 3.18 (dd, H-2_{eq}, H-6_{eq}, J= 12.5, 4.8 Hz), 2.53 (dd, H-2_{ax}, H-6_{ax}, J=12.7, 12.2 Hz); CMR (D_2O) 77.2, 69.8, 48.4; IR (KBr) 3400, 2920, 1645, 1455, 1075, 1045, 1025, 1010; CIMS *m/e* 116 (M+1-H_2O, 100%).
18. For **3**-HCl salt (73% overall yield from the corresponding aminoalkene): mp 191-192°C; $[\alpha]_D$= -16° (c= 0.9, CH_3OH); ^{1}H-NMR (D_2O) 4.26 (m, 1 H), 4.13 (ddd, 1 H, J= 8.3, 7.7, 4.0 Hz), 3.81 (dd, 1 H, J= 7.7, 2.7 Hz), 3.44 (dd, 1 H, J= 12.8, 4.0 Hz), 3.33 (dd, 1 H, J= 13.0, 5.9 Hz), 3.24 (dd, 1 H, J= 13.0, 2.7 Hz), 2.99 (dd, 1 H, J= 12.8, 8.3 Hz); IR (KBr) 3300 3080, 2930, 2910, 2860, 1615, 1480, 1450, 1410, 1370 cm^{-1}; CIMS *m/e* 134 (M+1, 100%).
19. For **4**-HCl (82% overall yield from the corresponding aminoalkene): mp 191-192°C; $[\alpha]_D$= +16° (c= 0.5, CH_3OH); all other spectral data identical with **3**-HCl.
20. At very low concentrations (≤30 μM), **2** behaved as a mild *activator* of β-glucosidase, judging from plots of absorbance versus [I] at several different substrate concentrations.

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