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AN EXTREMELY POTENT INHIBITOR FOR β-GALACTOSIDASE

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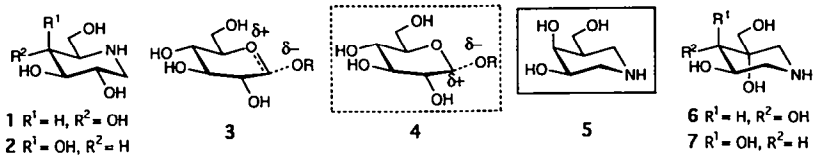
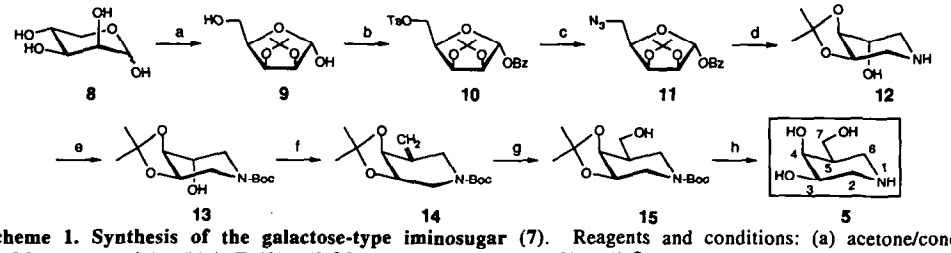
Abstract: A new galactose-type iminosugar in which a nitrogen atom is in the anomeric position was synthesized and was found to be an extremely potent inhibitor for β-galactosidase with *Ki* = 4 nM.

Iminosugars such as deoxynojirimycin (**1**) and galactostatin (**2**) are very potent inhibitors for the respective α-glucosidase from yeast (*Ki* = 12 μM) and α-galactosidase from coffee beans (*Ki* = 1.6 nM), and they mimic the currently accepted structure of glycosidic bond-cleaving reaction (**3**) where a positive charge is generated on the ring oxygen.[1,2] Interestingly, the inhibitory potency of **2** is generally weak for β-galactosidase by ca. 1000-fold compared to those for α-galactosidase.[1] In the course of our study to design a new type of inhibitor for glycosidases[3,4] based on the new model of β-glycosidic-cleaving reaction intermediate[5] (**4**) where a positive charge is generated at the anomeric position, we have found that a galactose-type iminosugar (**5**) is an extremely potent inhibitor for β-galactosidase with *Ki*= 4.1 nM.

The synthesis of **5** started with D-lyxose (**8**) (Scheme 1): Isopropylidenation[6] of **8** gave **9**, which was successively treated with TsCl and BzCl to give **10**. Azidolysis of **10** gave **11**, which was then O-debenzolylated and subsequently hydrogenated to give an iminopyranose derivative (**12**). Treatment of **12** with BocON[7] afforded **13**, which was oxidized with DMSO-$(COCl)_2$,[8] and then subjected to Wittig methylenation to give **14**. Hydroboration of **14** with a sterically bulky hydroborating agent, 9-BBN followed by oxidative work-up gave a single product (**15**) in 56% yield. Deprotection of **15** with 1N HCl afforded the galactose-type iminosugar (**5**) in 65% yield. The structure of **5** was confirmed by ^{1}H NMR experiment:[9] H-4 appeared at δ 3.93 ppm as a broad singlet, and H-3 at δ 3.77 ppm as a doublet of doublet of doublets with two small (*J* 2.69 and 7.05 Hz) and one large coupling constant (*J* 11.55 Hz), suggesting galacto-configuration.

The inhibitory potency of **5** was examined against several commercially available glycosidases (Table 1). It was found to be an extremely potent inhibitor for β-galactosidase from *Aspergillus orizae* with *Ki* = 4.1 nM at pH 6.8; however, **5** does not inhibit α-galactosidase as strongly as β-galactosidase with IC_{50} 200 μM.





Scheme 1. Synthesis of the galactose-type iminosugar (7). Reagents and conditions: (a) acetone/conc. H_2SO_4, rt. overnight; (b) i) TsCl/pyr/0 °C to rt./overnight, ii) BzCl/pyr/0 °C to rt.. overnight (41% overall); (c) NaN_3/DMF/120 °C/overnight (74%); (d) i) NaOMe/MeOH/rt./30 min, ii) H_2/Pd(OH)$_2$/MeOH/rt. overnight (62% overall); (e) BocON/Et_3N/MeOH/0 °C to rt./ overnight (85%); (f) i) DMSO/$(COCl)_2$/CH_2Cl_2/-60 °C, ii) Et_3N, iii) $Ph_3P{=}CH_2$/DME/-60 °C (62% overall); (g) i) 9-BBN/THF/0 °C to rt./3 hr, ii) NaOH/30% H_2O_2/rt./overnight (56%); (h) i) 1N HCl/rt./ 2 days, ii) Dowex 50W-X8 [H^+] chromatography (65% overall).

This is in sharp contrast with the conventional type iminosugar deoxygalactonojirimycin (**3**) that inhibits α-galactosidase stronger than β-galactosidase. A similar galactose-type iminosugar **7** with an additional 5-OH is less potent inhibitor for β-galactosidase by 1,500-fold.[3] Such a difference was also observed in glucose-type iminosugars: **6** has Ki= 4.3 μM[4] and the Ki of the one without 5-OH was reported to be 0.11 μM.[10]

Table 1. Inhibition of glycosidases by iminosugars 5 and 7.

Glycosidase	IC_{50} (nM)	
	5	7
α-Galactosidase from coffee beans (Sigma G8507)	200,000	610,000
β-Galactosidase from *Aspergillus oryzae* (Sigma G7138)	12	17,500
α-Glucosidase from yeast (Sigma G7256)	>2,000,000	>2,000,000
β-Glucosidase from almond (Sigma G4511)	190	420,000
α-Mannosidase from jack beans (Sigma M7257)	>2,000,000	

In summary, a highly potent inhibitor for β-galactosidase was stereoselectively synthesized. Iminosugar with a nitrogen at the anomeric position may indicate a new direction for the design of inhibitors of glycosidases, especially β-glycosidases that are mostly involved in the catabolic pathway.

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9. Compound **5** as a HCl salt in D_2O: ^1H NMR: δ 1.89-1.99 (1H, m, H-5), 2.73 (1H, t, J 12.6 Hz, H-2ax), 2.86 (1H, t, J 11.9 Hz, H-6ax), 3.05 (1H, dd, J 4.29, 12.21 Hz, H-6eq), 3.09 (1H, dd, J 7.07, 11.89 Hz, H-2eq), 3.40 (1H, dd, J 7.24 11.29 Hz, H-7a), 3.40 (1H, dd, J 6.70, 11.26 Hz, H-7b), 3.77 (1H, ddd, 2.69, 7.05, 11.55 Hz, H-3), 3.93 (1H, br s, H-4); ^{13}C NMR: δ 41.54, 42.31, 44.65, 62.71, 68.32, 68.70.
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