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SYNTHESIS OF A POTENT INHIBITOR OF β-GLUCURONIDASE

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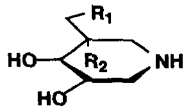
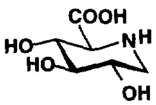
Abstract: A new glucuronic acid-type iminosugar in which a nitrogen atom is placed in the anomeric positon was synthesized and was proven to potently inhibit β-glucuronidase, with *K*i = 79 nM. Copyright © 1996 Elsevier Science Ltd

Heparanase, one of the β-glucuronidases, degrades heparan sulfate which is a constituent of extracellular matrix and of endotherial basement membranes. Because of its involvement in connective tissue degradation, heparanase is thought to play a role in tumor metastasis.[1] In fact, it has been demonstrated that heparanase activity is correlated with metastatic potentials in some types of malignant tumor cells,[2] and several studies have shown that metastasis is significantly suppressed by the inhibitors of heparanase such as heparin derivatives[2] and by β-glucuronidase inhibitors such as D-glucaro-δ-lactam (2).[3] Development of a new inhibitor of β-glucuronidase should provide useful information for the design of antitumor agents. In the course of our study to develop more potent glycosidase inhibitors, we have demonstrated that the new iminosugars (shown in 1) in which a nitrogen atom is placed in the anomeric position are potent inhibitors for β-glycosidases.[4-7] Since uronic acid derivative of deoxynojirimycin (3) has been reported to be a moderate inhibitor of β-glucuronidase, we assumed that a new iminosugar **4** would be more potent inhibitor for β-glucuronidase. We herein report a synthesis of **4** and its analysis of inhibitory potency.



1 R_1, R_2=H or OH

2



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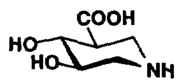

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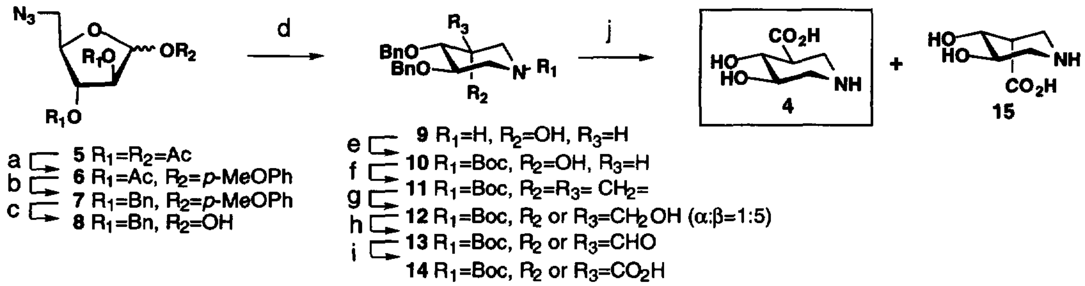
The azide **5** was prepared from D-arabinose according to the reported procedure.[8] Treatment of **5** with *p*-methoxyphenol and TMSOTf gave **6** in 84% yield. The acetyl groups of **6** were removed by NaOMe, and the following treatment with BnBr and NaH gave **7** in 99% yield. The oxidative removal of the *p*-methoxyphenyl group of **7** by $(NH_4)_2Ce(NO_3)_4$ gave **8** in 89% yield. The intramolecular reductive amination of **8** with H_2-Lindlar catalyst afforded a piperidine derivative **9**, in 67% yield, which was treated with $(Boc)_2O$ to give **10** in 75% yield. For the introduction of a hydroxymethyl group, **10** was subjected to Swern oxidation and Wittig methylenation to give an *exo*-methylene derivative (**11**) in 63% yield. Hydroboration of **11** with 9-BBN preferentially occurred from the α-face to give a 5:1 mixture of D-gluco (β-) and L-ido (α-) isomers of **12** in quantitative yield. The hydroxymethyl group of **12** was oxidized in 2 steps: 1) Swern oxidation of **12** gave an aldehyde **13** (84% yield); 2) further oxidation of **13** with $NaClO_2$-H_2O_2[9] afforded a carboxylic acid derivative **14** in 82% yield. Removal of the protective groups of **14** by catalytic hydrogenolysis and subsequent aqueous



Scheme 1. Synthesis of a glucuronic acid-type iminosugar (4). Reagents and conditions: (a) *p*-$MeOC_6H_4OH/TMSOTf/CH_2Cl_2$/0 °C to rt./5 h (84%); (b) i) NaOMe/MeOH/rt/10 min, ii) BnBr/NaH/DMF/0 °C to rt./12 h (99%); (c) $(NH_4)_2Ce(NO_3)_3/CH_3CN-H_2O$ (5:1)/0 to 5 °C/5 min (89%); (d) $H_2/Pd-CaCO_3$/MeOH/rt/18 h (67%); (e) $(Boc)_2O/Et_3N$/MeOH/0 °C to rt./8 h (75%); (f) i) $(COCl)_2/DMSO/CH_2Cl_2$/-70 °C/1 h then Et_3N/-70 to 0 °C/30 min (78%), ii) $CH_3{}^+Ph_3PBr^-/(TMS)_2NLi$/DME/0 °C to rt/18 h (81%); (g) 9-BBN/THF/0 °C to rt/12 h then 10%NaOH/35%H_2O_2/0 °C to rt/12 h (quant); (h) $(COCl)_2/DMSO/CH_2Cl_2$/-70 °C/1 h then Et_3N/-70 to 0 °C/30 min; (i) $NaClO_2$/35%$H_2O_2/NaH_2PO_4/CH_3CN-H_2O$ (1:1)/0 °C to rt/1 h (69% in 2 steps); (j) i) $H_2/Pd(OH)_2$/EtOH-EtOAc/rt/18 h, ii) 1*N* HCl iii) SiO_2 chromatography (*i*-PrOH:H_2O:30%NH_4OH = 7:2:1) and gel filtration (Sephadex G-25) (**4**: 32%; **15**: 8%).

HCl treatment gave a mixture of **4** (D-glucuronic acid-type) and **15** (L-iduronic acid-type), which were separated by silica gel chromatography to afford a pure **4** in 32% yield and **15** in 8% yield.[10]

As expected, the glucuronic acid-type iminosugar **4** strongly inhibited the hydrolysis of phenolphthalein β-D-glucuronide by β-glucuronidase from bovine liver (Sigma G0501) with a *Ki* of 79 nM at pH 5. This inhibition was 1,000-fold more potent than that of the deoxynojirimycin-type analogue **3** (*Ki*=80 μM at pH 4 against β-glucuronidase from human liver)[11] and was almost equivalent to that of D-glucaro-δ-lactam **2** (*Ki*=39 nM at pH 5.2 against β-glucuronidase from bovine liver).[3] The iduronic acid-type iminosugar **15** was a moderate inhibitor with an IC_{50} of 1.3 μM.

In summary, we have synthesized a new glucuronic acid-type 1-*N*-iminosugar (**4**) from D-arabinose and have shown **4** to be a potent inhibitor of β-glucuronidase with a *Ki* of 79 nM.

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10. **Compound 4** (D-glucuronic acid type): colorless amorphous (HCl salt); ^1H NMR (300 MHz, D_2O) δ 2.90 (ddd, 1H, *J* 4.3, 7.4, 7.8 Hz, H-5), 3.07 (dd, 1H, *J* 7.7, 12.9 Hz, H-2ax), 3.38 (dd, 1H, *J* 7.8, 13.2 Hz, H-6ax), 3.50 (dd, 1H, *J* 3.6, 12.9 Hz, H-2eq), 3.51 (dd, 1H, *J* 4.3, 13.2 Hz, H-6eq), 3.90 (ddd, 1H, *J* 3.6, 7.2, 7.7 Hz, H-3), 4.08 (t, 1H, *J* 7.0 Hz, H-4).
    Compound 15 (L-iduronic acid type): colorless amorphous (HCl salt); ^1H NMR (300 MHz, D_2O) δ 3.20-3.43 (m, 5H), 4.08 (m, 1H), 4.31 (m, 1H).
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