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A uronic acid analogue of isofagomine lactam as a nanomolar glucuronidase inhibitor

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ABSTRACT

The synthesis of (3*S*,4*R*,5*R*)-3,4-dihydroxypiperidin-2-one-5-carboxylic acid ('isofagomine lactam uronate') from D-arabinose is reported. The product is a potent inhibitor in the low nanomolar range (K_i 36 nM) for bovine liver β-glucuronidase.

The polar monosaccharide D-glucuronic acid (**1**) plays an important role in human biochemistry by forming glucuronide conjugates with xenobiotics and other undesired and lipophilic substances to allow them to be excreted (Fig. 1).[1] A large number of toxic compounds including many drug metabolites are eliminated safely from the body as glucuronides before they can cause harm. β-Glucuronidase, a glycosidase, catalyses the hydrolysis of glucuronides back to **1** and aglycone, and this enzyme is therefore potentially counterproductive to the natural catabolic protection system against xenobiotics.[2] Indeed the inhibition of β-glucuronidases has recently been shown to be useful in alleviating drug toxicity in cancer chemotherapy.[3] Therefore potent and selective β-glucuronidase inhibitors could become important supplement drugs in chemotherapy.

Iminosugars are among the most potent and selective glycosidase inhibitors.[4,5] They are monosaccharide analogues in which the endocyclic oxygen[6,7] and/or anomeric carbon[8,9] has been replaced by nitrogen. Their inhibitory activity stems from their amine functional group either through their ionic interactions in conjugate acid form, with carboxylate groups in the enzyme active site or, and hotly debated, as mimics of a transition state or intermediate. Yet the neutral lactam analogues of iminosugars are also occasionally surprisingly potent inhibitors.[10] Several iminosugars mimicking **1** have been prepared and found to be β-glucuronidase inhibitors. Uronic-1-deoxynojirimycin (**2**) is a moderate inhibitor (K_i 6.5 μM) of bovine liver β-glucuronidase (Fig. 1, Table 1).[11] A more potent inhibitor is the uronic acid derivative of isofagomine (**3**, K_i 79 nM),[12] while the uronic azafagomine **4** has intermediary inhibitory potency (K_i 1 μM).[13] Recently, an analogue of **3**, compound **5**

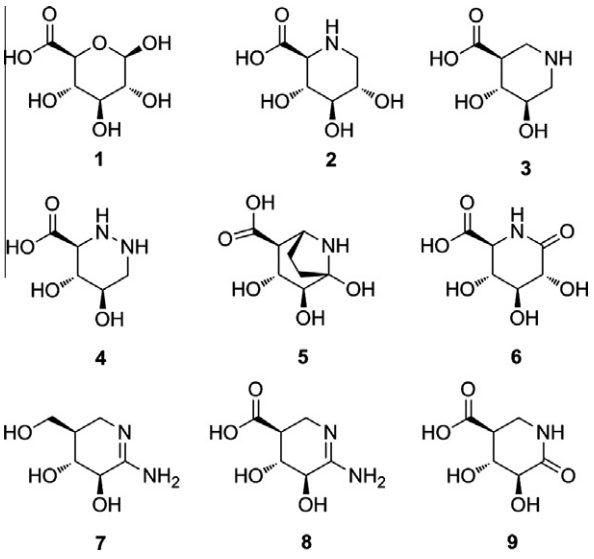

Figure 1. D-Glucuronic acid (**1**) and the known nitrogen-containing β-glucuronidase inhibitors **2**–**6**, amidine **7** and the target compounds **8** and **9**.

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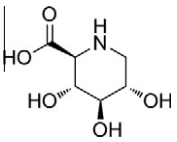
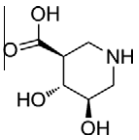
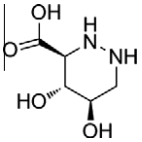
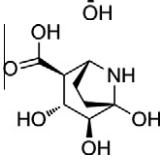
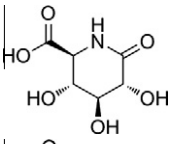
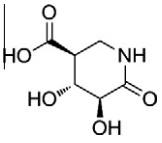
Table 1
Inhibition constants (K_i, nM) of iminosugars **2**–**6** and **9**

Compound	Structure	Bovine liver β-glucuronidase
2		6500[11]
3		79[12]
4		1000[13]
5		2300[14]
6		32[15]
9		36

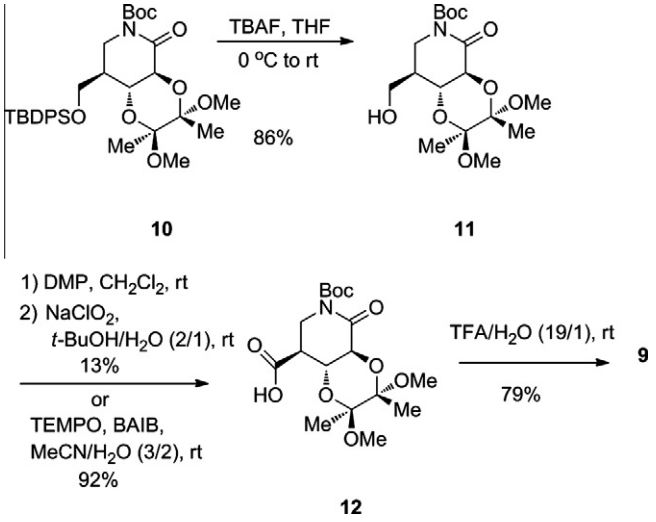
(Fig. 1), with the 2-OH intact and a calystegin-like structure was reported.[14] This compound is less potent versus the mammalian enzyme (K_i 2.3 μM), but very potent against the *Escherichia coli* enzyme (K_i 60 nM).

Surprisingly D-glucurono-1,5-lactam (**6**)[15] was reported to be a much more potent bovine liver β-glucuronidase inhibitor than **2**, which suggests that for this mammalian enzyme, lactams are actually better inhibitors than amines. We recently prepared the amidine **7**,[16] and our interest in glucuronidase inhibitors led us to target the synthesis of amidine **8** and lactam **9**. The synthesis of **8** ultimately failed as the product was too unstable, but we present here the synthesis and inhibitory testing of uronic-isofagomine lactam **9** which showed it to be a very potent β-glucuronidase inhibitor.

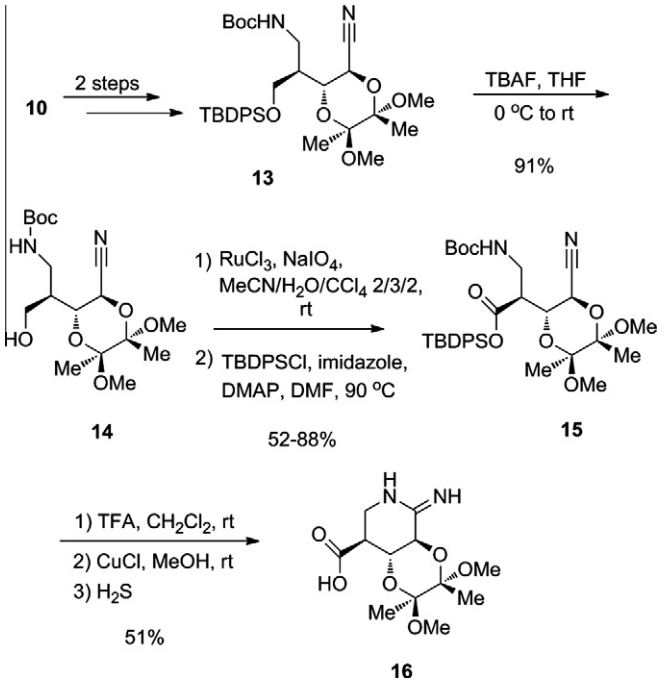
The synthesis of uronic-isofagomine lactam **9** started from building block **10**, itself prepared from D-arabinose (Scheme 1).[16]

Treatment of **10** with tetrabutylammonium fluoride (TBAF) gave the alcohol **11** in an 86% yield.[17] Alcohol **11** was then subjected to a Dess–Martin periodinane (DMP)/sodium chlorite oxidation sequence to furnish carboxylic acid **12** in low yield (13%). The yield was significantly improved when **11** was reacted with (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) and bisacetoxyiodobenzene (BAIB)[18] in aqueous acetonitrile (MeCN); this furnished **12** in a 92% yield. Finally, the *trans*-diacetal and Boc protecting groups of **12** were removed by treatment with aqueous trifluoroacetic acid (TFA) to give uronic-isofagomine lactam **9** in a 79% yield (Scheme 1).

As part of this β-glucuronidase inhibitor project, we also tried to synthesize uronic-isofagomidine **8** (Fig. 1). The synthesis started from the reported building block **13** (Scheme 2),[16] treatment of which with TBAF gave **14** in high yield (91%). Ruthenium-catalyzed



Scheme 1. Synthesis of uronic-isofagomine lactam (**9**).



Scheme 2. Toward the synthesis of uronic-isofagomidine **8**.

oxidation of **14** gave **15** in 52–88% yield, after TBDPS protection. The unpredictable yield of the silyl ester **15** was caused by its hydrolytic instability during purification by flash chromatography. Compound **15** was then subjected to TFA-mediated selective deprotection of the Boc group and subsequently CuCl-mediated ring-closure in methanol. The copper salt complexes strongly to the amidine, but treatment with H_2S gas precipitated the copper salt as the sulfide. Subsequent column chromatography gave **16** as its acetate salt in a 51% yield. Removal of the protecting group from **16** turned out to be more difficult than anticipated. Treatment of **16** with concentrated hydrochloric acid in 1,4-dioxane or using other deprotection methods did not give **8** as an analytically pure compound.

Uronic-isofagomine lactam **9** showed inhibition in the low nanomolar range (with a K_i value of 36 nM) for bovine liver β-glucuronidase (Table 1).[19] This inhibitor was 181-fold more potent than uronic-1-deoxynojirimycin (**2**, K_i 6.5 μM), and more than two-fold as potent as uronic-isofagomine (**3**, K_i 79 nM). Compound **9** and

D-glucolactam (**6**, K_i 32 nM) had almost identical K_i values for bovine liver β-glucuronidase.

In summary, we have synthesized a uronic acid derivative of isofagomine lactam **9** from D-arabinose. Compound **9** was found to be a very potent β-glucuronidase inhibitor in the nanomolar range (with a K_i value of 36 nM).

Acknowledgments

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Supplementary data

Supplementary data (copies of ^{13}C and ^{1}H NMR spectra of **9** as well as synthetic procedures for compounds **14**, **15** and **16**) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2012.02.009.

References and notes

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17. Compounds were prepared using the following procedures:
(3*S*,4*R*,5*R*,2′*S*,3′*S*)-3.4-(2′,3′-Dimethoxybutylene-2′,3′-dioxy)-5-hydroxymethyl-1-(*tert*-butyloxycarbonyl)piperidin-2-one (11). To a solution of silyl ether **10** (600 mg, 0.978 mmol) in THF (9.5 mL) at 0 °C was slowly added TBAF (1.08 mL, 1 M in THF). The mixture was stirred at this temperature for 30 min, and then the temperature was increased to room temperature and the mixture stirred for 5.5 h. The solvent was removed under reduced pressure. Purification of the concentrate by dry column chromatography (for a description of this technique see Pedersen, D. S.; Rosenbohm, C. *Synthesis* **2001**, 2431 and references therein, heptane–EtOAc, 1:0 → 1:1) gave alcohol 11 (315 mg, 86%) as a white foam. R_f: 0.27 (EtOAc–heptane, 1:1); $[\alpha]_D$ +97 (*c* 0.3, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 4.28 (d, 1H, $J_{3,4}$ = 10.7 Hz, H-3), 3.85 (dd, 1H, $J_{4,5}$ = 9.3 Hz, H-4), 3.80–3.76 (m, 1H, CHaOH), 3.72–3.66 (m, 3H, CHbOH, H-6a, H-6b), 3.29 (s, 3H, OCH_3), 3.22 (s, 3H, OCH_3), 2.25–2.20 (m, 2H, H-5, OH), 1.49 (s, 9H, $(CH_3)_3C$, Boc), 1.37 (s, 3H, CH_3), 1.29 (s, 3H, CH_3); ^{13}C NMR (125 MHz, CDCl3) δ 167.3 (C-2), 152.8 (C=O), 100.5, 99.3 (C-2′, C-3′), 83.9 (C$(CH_3)_3$, Boc), 70.2 (C-3), 67.4 (C-4), 62.1 (CH_2OH), 48.6, 48.1 (OCH_3), 43.9 (C-6), 39.0 (C-5), 28.1 ($(CH_3)_3C$, Boc), 17.7, 17.6 (CH_3); HRMS (ESP) *m*/*z* calcd for $C_{17}H_{29}NNaO_8$ ($[M+Na]^+$): 398.1791, found: 398.1781.
(3*S*,4*R*,5*R*,2′*S*,3′*S*)-5-Carboxylic acid-3.4-(2′,3′-dimethoxybutylene-2′,3′-dioxy)-1-(*tert*-butyloxycarbonyl)piperidin-2-one (12). To a solution of alcohol **11** (111 mg, 0.296 mmol) in MeCN–H_2O (3:2, 2 mL) at room temperature was added TEMPO (13.6 mg, 0.0887 mmol) and BAIB (238.4 mg, 0.74 mmol). The reaction mixture was left stirring for 4.5 h, and then the solvent was removed under reduced pressure. Purification of the concentrate by dry column chromatography ($CHCl_3$–MeCN, 1:0 → 0:1) furnished carboxylic acid **12** (106 mg, 92%) as a white solid. R_f: 0.52 (MeCN); $[\alpha]_D$ +188 (*c* 0.15, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 4.35-4.31 (m, 2H, H-3, H-4), 4.27 (dd, 1H, $J_{6a,5}$ = 5.4 Hz, $J_{6a,6b}$ = 14.1 Hz, H-6a), 3.78 (dd, 1H, $J_{6b,5}$ = 6.7 Hz, H-6b), 3.31 (s, 3H, OCH_3), 3.27 (s, 3H, OCH_3), 3.02–2.98 (m, 1H, H-5), 1.51 (s, 9H, $(CH_3)_3C$, Boc), 1.40 (s, 3H, CH_3), 1.32 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.7, 166.6 (C-2, CO_2H), 151.7 (C=O), 100.7, 99.8 (C-2′, C-3′), 84.2 (C$(CH_3)_3$, Boc), 69.4, 66.5 (C-3, C-4), 48.7, 48.3 (OCH_3), 43.0 (C-5), 42.3 (C-6), 28.1 ($(CH_3)_3C$, Boc), 17.6 (2×CH_3); HRMS (ESP) *m*/*z* calcd for $C_{17}H_{27}NNaO_9$ ($[M+Na]^+$): 412.1584, found 412.1606.
(3*S*,4*R*,5*R*)-5-Carboxylic acid-3,4-dihydroxypiperidin-2-one (9). A solution of compound **13** (58 mg, 0.149 mml) in TFA–H_2O (19:1, 4 mL) was stirred at room temperature for 16 h. Then the solvent was removed under reduced pressure and the residue was purified by column chromatography (50 mN aqueous HCl–MeCN, 1:199 → 1:19 → 1:4) to furnish the title compound (20.5 mg, 79%) as a colorless oil. R_f: 0.49 (50 mN aqueous HCl–MeCN, 7:13); ^1H NMR (500 MHz, D_2O) δ 4.00–3.95 (m, 2H, H-3, H-4), 4.43 (dd, 1H, $J_{6a,5}$ = 5.8 Hz, $J_{6a,6b}$ = 12.9 Hz, H-6a), 3.34 (dd, 1H, $J_{6b,5}$ = 9.9 Hz, H-6b), 2.96–2.91 (m, 1H, H-5); ^{13}C NMR (125 MHz, D_2O) δ 174.9, 173.2 (C-2, CO_2H), 72.1, 71.0 (C-3, C-4), 46.0 (C-5), 39.8 (C-6); HRMS (ES) *m*/*z* calcd for $C_6H_9NNaO_5$ ($[M+Na]^+$): 198.0378, found: 198.0374.
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19. The enzyme inhibition constant K_i was determined in the following manner: Measurement of inhibition of bovine liver β-glucuronidase was carried out in 50 mM acetate buffer (pH 5.0) at 37 °C. The substrate used was phenolphthalein β-D-glucuronide. Each second minute, 0.100 mL of the reaction mixture was transferred to a cuvette consisting of 0.900 mL of 0.100 M glycine–NaOH buffer (pH 12.0). The formation of phenolphthalein was monitored by measurement of the absorbance at 553 nm. Initial velocities were calculated from the slopes from each reaction and used to construct two Hanes plots ([S]/v vs [S]), one with and one without inhibitor. From the Michaelis–Menten constants K'_M and K_M the K_i value was calculated.