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Anomer-Selective Inhibition of Glycosidases Using Aminocyclopentanols

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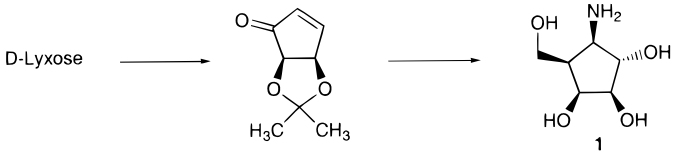
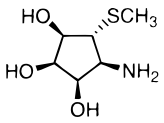
ABSTRACT

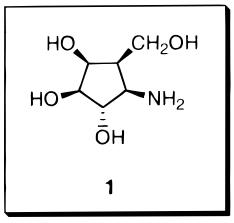


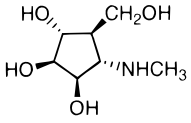
(1*S*,2*S*,3*S*,4*R*,5*R*)-4-Amino-5-(hydroxymethyl)cyclopentane-1,2,3-triol 1 is prepared stereoselectively from D-lyxose and displays anomer-selective inhibition for β-galactosidase ($K_i = 3.0 \times 10^{-6}$ M) and β-glucosidase ($K_i = 1.5 \times 10^{-7}$ M), over α-galactosidase ($K_i = 2.3 \times 10^{-5}$ M) and α-glucosidase ($IC_{50} = 1.0 \times 10^{-4}$ M). There is no observable cross-reactivity with α-mannosidase, β-mannosidase, or α-L-fucosidase.

Glycosidase inhibitors can be used for treating diabetes, cancer, and viral (HIV, influenza) and bacterial infections and as insecticides. Structurally most of these inhibitors are analogues of the carbohydrate cleaved by the glycosidase enzyme that contains a basic nitrogen containing function near the anomeric center. Canonical examples are the piperidines related to deoxy-nojirimycin and isofagomine.[1] Aminocyclopentanols such as mannostatin, a mannosidase inhibitor, are also powerful inhibitors of glycosidases.[2] However the structural relationship between carbohydrate and inhibitor is not well understood in this series. Here we show that aminocyclopentanols such as **1** are anomer-selective glycosidase inhibitors.



mannostatin





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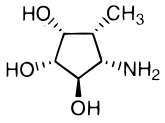

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In 1990 Farr et al. reported the synthesis of aminocyclopentanol **2** and its inhibition of α-mannosidase.[3] By contrast to most cyclopentanol glycosidase inhibitors, which lack a simple relationship to the parent carbohydrate, compound **2** is essentially a ring-contracted analogue of mannose where the oxygen atom of the pyranose ring is missing. Indeed Farr et al. proposed the idea that **2**·H^+ might be an analogue of either the mannosyl cation or of the protonated α-mannoside.

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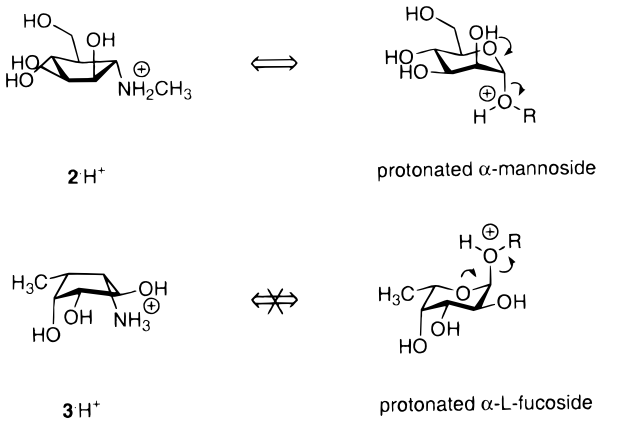
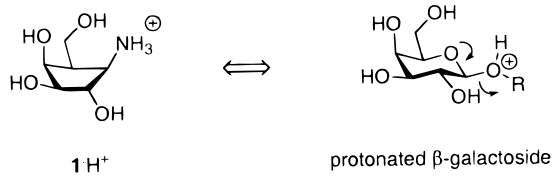
We have recently prepared the related aminocyclopentanol **3** and showed that it selectively inhibits α-L-fucosidase.[4] Both compounds match the substitution pattern of the parent carbohydrates; however, inhibitor **2** has the amino group oriented on the α-face and gives potent inhibition of α-mannosidase (IC_{50} = 62 nM for jack-beans α-mannosidase), while **3** has its amino group on the β-face and does not inhibit α-L-fucosidase as strongly (K_i = 28 μM for bovine kidney α-L-fucosidase). These data suggest that **2**·H^+ or **3**·H^+ should be viewed as analogues of the protonated glycosides rather than of the glycosyl cations. Thus potent



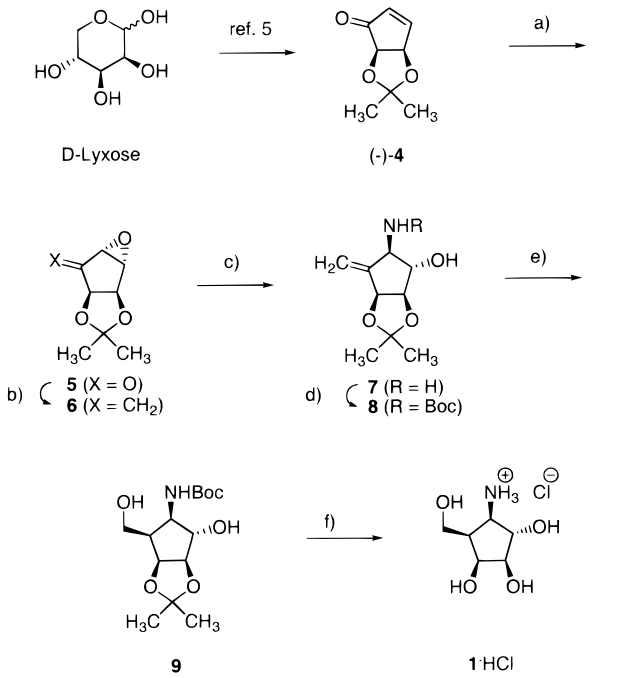
inhibition would require a match between the orientation α or β of the amino substituent of the cyclopentane and the anomeric configuration of the glycosidic bond.

This hypothesis can be tested by synthesizing aminocyclopentanol **1**, which is almost enantiomeric to **3** and can therefore be prepared by following a very similar route starting with the enantiomeric precursor. Aminocyclopentanol **1** has, like **3**, an amino group on the β-face. Therefore **1**·H^+ could be considered as an analogue of a protonated β-galactoside and is predicted to be a selective inhibitor of β-galactosidases.



Compound **1** was prepared starting from cyclopentenone (−)-**4**, which is readily prepared from D-lyxose,[5] following a route similar to that used for the preparation of **3**. Epoxidation with basic hydrogen peroxide gave stereoselectively epoxy ketone **5**. Methylenation, followed by aminolysis of the volatile epoxy olefin **6** with aqueous ammonia selectively at the allylic carbon and finally Boc protection of the resulting amine **7**, gave **8** (40% over three steps). As observed for hydrogenation in the enantiomeric series leading to **3**, hydroboration of **8** proceeded from the less hindered face of the olefin to give stereoselectively alcohol **9**.[6] Finally deprotection led to the free amino alcohol **1** as its HCl salt quantitatively[7] (Scheme 1).

Scheme 1[a]



[a] Reagents and conditions: (a) H_2O_2, NaOH, −50 °C, 1 h (65%); (b) Ph_3PCH_2, THF, 20 °C, 1 h; (c) aqueous NH_3 in EtOH; (d) Boc_2O, aqueous $NaHCO_3$, AcOEt (40% over three steps); (e) 10 equiv of BH_3·THF, then H_2O_2, NaOH (60%); (f) 3 N HCl, 60 °C, 12 h (100%).

Aminocyclopentanol **1** was assayed for its inhibition of a series of glycosidases using nitrophenyl glycosides as test substrates in aqueous buffer pH 6.8, 25 °C, under which conditions all enzymes were found to display satisfactory

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(6) All new compounds gave satisfactory spectral and MS data. Data for **9**: ^{1}H NMR (300 MHz, $CDCl_3$) 5.30 (d, J = 8.1 Hz, NH), 4.74 (dd, J = 5.9 Hz, 5.5 Hz, H−C(1)), 4.50 (d, J = 5.5 Hz, H−C(2)), 4.19 (s, H−C(3)), 4.03 (dd, J = 8.8 Hz, 5.5 Hz, H−C(4)), 3.86 (dd, J = 12.1 Hz, 8.8 Hz, H−C(6)), 3.79 (dd, J = 12.1 Hz, 5.5 Hz, H−C(6)), 2.63 (dtd, J = 8.8 Hz, 5.5 Hz, 5.5 Hz, H−C(5)), 1.48, 1.29 (2s, $(CH_3)_2$C), 1.45 (s, 9H, Boc); NOE observed H−C(5)/H−C(4), H−C(5)/H−C(1), H−C(1)/H−C(2), H−C(3)/NHBoc; ^{13}C NMR (75 MHz, $CDCl_3$) 157.2 (s), 111.4 (s), 86.7 (d), 81.3 (d), 79.1 (d), 77.2 (s), 59.6 (d), 59.0 (t), 47.9 (d), 29.0 (q), 26.5 (q), 23.7 (q); EI-MS 304 ([M + 1]$^+$), 289 ([M − 15]$^+$).
(7) Data for **1**·HCl: ^{1}H NMR (300 MHz, D_2O) 4.11 (dd, J = 7.7 Hz, 4.8 Hz, H−C(3)), 4.07 (t, J = 4.05 Hz, H−C(1)), 3.83 (m, H−C(2) + H−C(6)), 3.74 (dd, J = 11.4 Hz, 8.2 Hz, H−C(6)), 3.51 (dd, J = 8.8 Hz, 4.8 Hz, H−C(4)), 2.52 (dtd, J = 8 Hz, 8.5 Hz, 4.05 Hz, H−C(5)); NOE observed H−C(5)/H−C(1), H−C(5)/H−C(4), H−C(5)/H−C(6); ^{13}C NMR (75 MHz, D_2O + 5% DMSO-d_6) 81.3 (d, C(3)), 79.7 (d, C(2), 73.8 (d, C(1)), 58.4 (d, C(4)), 58.4 (t, C(6)), 43.0 (d, C(5)); EI-MS 162 ([M − 1]$^+$).
(8) Molecular modeling (geometry optimization by semiempirical methods using Spartan 5.0, solvent = water) does not resolve this contradiction. In the calculated most stable conformations, the C(5) hydroxylmethyl substituent of **1** is placed in pseudoequatorial position and the C(1)−OH in a pseudoaxial position, which matches very well with the shape of *galacto*- and not *gluco*-configurated glycosides.
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activity (Table 1 and Figure 1). Aminocyclopentanol **1** inhibits β-galactosidase selectively over α-galactosidase. While there is no cross-inhibition with mannosidases or α-L-fucosidase, we observe a strong cross-inhibition with glucosidases, here also with a strong preference for β-glucosidases over α-glucosidase.

Table 1. Inhibition Data on Glycosidases for Aminocyclopentanol **1**[a]

enzyme	origin	inhibition[b]
β-galactosidase	*E. coli*	$K_i = 3.0 \times 10^{-6}$ M
β-galactosidase	bovine liver	$K_i = 3.0 \times 10^{-6}$ M[c]
α-galactosidase	green coffee beans	$K_i = 2.3 \times 10^{-5}$ M[d]
β-glucosidase	almond	$K_i = 3.3 \times 10^{-7}$ M[e]
β-glucosidase	*C. saccharolyticum*	$K_i = 1.6 \times 10^{-7}$ M
α-glucosidase	yeast	$IC_{50} = 1.0 \times 10^{-4}$ M[f]
β-mannosidase	snail acetone powder	N. I.[g]
α-mannosidase	jack beans	N. I.[g]
α-L-fucosidase	bovine kidney	N. I.[g]

[a] One hundred milliliter assays contained the indicated enzyme at 0.1 U/mL, inhibitor **1**, and the corresponding nitrophenyl glycosides in 0.1 M HEPES−buffer at pH 6.8, at 25 °C. [b] Competitive inhibition constant K_i as determined by Dixon plot of inhibition data using: [c] $S = 130$, 400 μM and [**1**] = 0, 3, 5, 10, 15, 20 μM; [d] $S = 180$, 540 μM and [**1**] = 0, 30, 50, 100, 150, 200, 400 μM; [e] $S = 1$ mM and [**1**] = 0, 0.05, 0.1, 0.15, 0.20 μM. [f] This enzyme gives complex kinetics. IC_{50} value measured with 1 mM 4-nitrophenyl α-glucoside. [g] N.I. = no inhibition observed with [**1**] = 0.1 mM. Enzymes and substrates were purchased from Fluka or Sigma.

The potent and selective inhibition of β-glycosidases by **1** complements the inhibition data with **2** for α-mannosidase and confirms our hypothesis that aminocyclopentanols can be potent anomer-selective inhibitors for both α- and β-glycosidases, if the carbohydrate's anomeric configuration and the relative configuration of the cyclopentane's amino substituent are matched. The K_i values show that **1** and **2** clearly belong to the tighter binding glycosidase inhibitors known. Conversely, in the case of unmatched configurations, the weaker inhibition of α-glycosidases by **1** falls in the same range ($K_i \approx 10^{-4}$ M) as the inhibition of **3** for α-L-fucosidase.

A strong cross-inhibition is observed for **1** with β-glucosidases, which give even lower K_i values than the β-galactosidases, in contradiction to the relative configuration of the C(1)−OH group in **1**, which by design matches the C(4)−OH group of the carbohydrate with *galacto* and not *gluco* configuration.[8] This observation is explained by the fact that both β-glucosidases tested belong to family 1 of glycosidases, which displays dual galactosidase/glucosidase specificity.[1,9] Similar cross-reactivities have been observed with both *gluco*- and *galacto*-configured glycosidase inhibitors.[1,10]

In summary, a stereoselective synthesis of aminocyclopentanol **1** from D-lyxose has been devised. Aminocyclopentanol **1** is a potent and selective inhibitor for β-glucosidase and β-galactosidase. These data complement the data with compound **2**, which strongly inhibits α-mannosidase,[3] and show that potent anomer-selective glycosidase inhibitors can be designed using aminocyclopentanols as analogues of protonated glycosides. We are pursuing the synthesis of further aminocyclopentanols to test the generality of this concept.

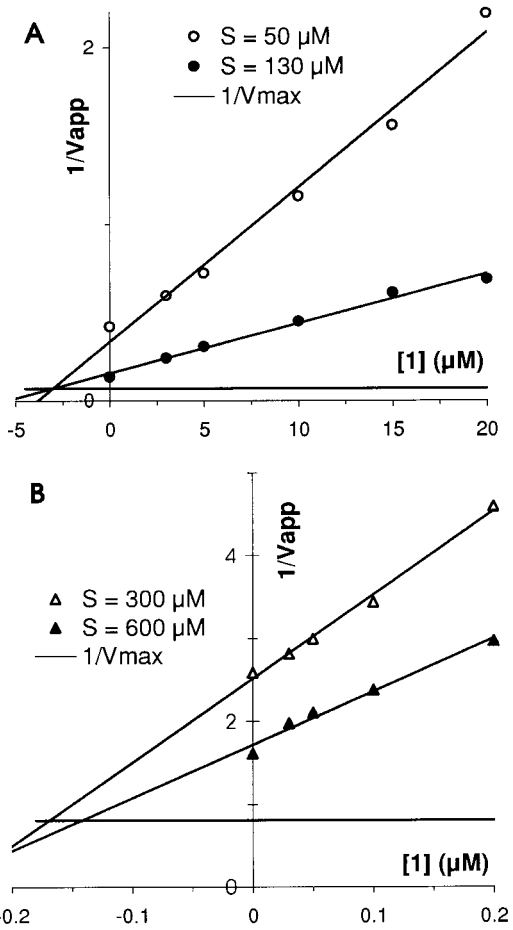

Figure 1. Dixon plot of inhibition by **1** for *Escherichia coli* β-galactosidase (A) and for *Caldocellum saccharolyticum* β-glucosidase (B). They plots were measured in 0.1 M HEPES−buffer at pH 6.8, at 25 °C, with (A) 0.1 U/mL *E. coli* β-galactosidase and 2-nitrophenyl-β-D-galactoside as substrate S (Michaelis−Menten constant $K_M = 130$ μM under these conditions), or (B) 0.1 U/mL *C. saccharolyticum* β-glucosidase and 4-nitrophenyl-β-D-glucoside as substrate S ($K_M = 630$ μM under these conditions), and inhibitor **1** at the indicated concentrations. The *x* coordinate of the intersection between the two lines and the horizontal line at $1/V_{max}$ gives $-K_i$ (**1**). One hundred milliliter assays were followed in individual wells of flat-bottom 96-well half-area polystyrene cell culture plates (*Costar*) using a UV Spectramax 250 instrument from Molecular Devices. V_{app} is given in relative units derived from the absorbance change at 405 nm as given by the instrument.

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