ORGANIC LETTERS

2000
Vol. 2, No. 2
151−154

# Synthesis and Evaluation of Aminocyclopentitol Inhibitors of β-Glucosidases

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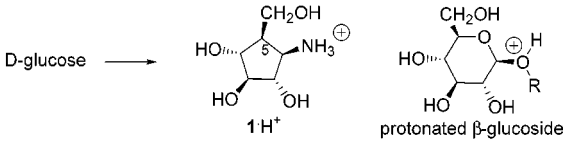
**Received November 17, 1999**

## ABSTRACT

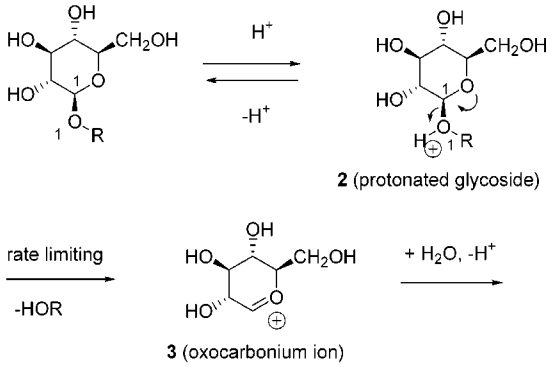



**(1*R*,2*S*,3*S*,4*R*,5*R*)-4-Amino-5-(hydroxymethyl)cyclopentane-1,2,3-triol 1, prepared from D-glucose, inhibits β-glucosidases from *Caldocellum saccharolyticum* ($K_i = 1.8 \times 10^{-7}$ M) and from almonds ($K_i = 3.4 \times 10^{-6}$ M). Inhibition is not influenced by *N*-ethylation (→ 15) but is strongly reduced upon *N*-acetylation (→ 12). Inversion of stereochemistry at C(5) (→ 14) has little effect on inhibition of β-glucosidases. These experiments suggest that 1 acts as an analogue of a protonated β-glucoside.**

Glycosidase inhibitors can be used for treating diabetes, cancer, and viral (HIV, influenza) and bacterial infections and as insecticides. Most of these inhibitors are monosaccharide analogues displaying a basic nitrogen-containing function near the anomeric center, such as deoxynojirimycin and isofagomine.[1] Aminocyclopentitols such as mannostatin (**5**) are also powerful inhibitors of glycosidases.[2] However, the relationship between inhibitor structure and inhibition remains poorly understood in this series. We have shown recently that aminocyclopentitols designed as mimics of α- or β-configured protonated glycosides are potent anomer-selective inhibitors of glycosidases.[3] Herein we report an efficient synthesis of aminocyclopentitol **1** as a mimic of a protonated β-glucoside and show that this compound is a potent inhibitor of β-glucosidases. Comparison of its inhibition pattern with close analogues provides the first evidence that these inhibitors indeed act as cationic mimics of the protonated glycosides.

The reaction mechanism of glycosidic bond cleavage is acid catalyzed and involves protonation of the exocyclic oxygen atom at the anomeric center to give a protonated glycoside **2** (Scheme 1).[4] Subsequent rate-limiting cleavage

**Scheme 1.** Mechanism of Acid-Catalyzed Glycosidic Bond Cleavage for the Example of a β-Glucoside




of the C(1)−O(1) bond leads to oxocarbonium ion intermediate **3**, which then reacts with a molecule of water to form

(1) Recent reviews: (a) Bols, M. *Acc. Chem. Res.* **1998**, *31*, 1. (b) Heightman, T. D.; Vasella, A. T. *Angew. Chem.* **1999**, *111*, 795. (c) Davies, G.; Sinnott, M. L.; Withers, S. G. In *Comprehensive Biological Catalysis*; Sinnott, M., Ed.; Academic Press: London, 1998; pp 119−208.

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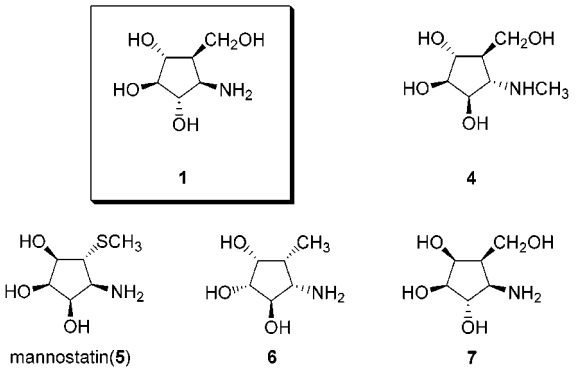
10.1021/ol991252b CCC: $19.00 
**Published on Web 12/23/1999**

the product. The true transition state of the reaction lies in between **2** and **3**, which are high-energy intermediates. The enzymatic mechanism is similar, although **2** and **3** may not occur as intermediates due to a transition from specific acid catalysis (pre-equilibrium protonation) to general acid catalysis (rate-limiting protonation) and the formation of a covalent intermediate with some enzymes.[5] Despite the differences between solution and enzymatic reaction mechanisms, analogues of the high-energy intermediates along the chemical reaction pathway are generally potent glycosidase inhibitors.

Glycosidases are generally highly specific in cleaving only one type of glycoside in a given anomeric configuration. Most glycosidase inhibitors have been designed as analogues of the oxocarbonium ion intermediate **3**, where the stereochemistry of the substrate's anomeric configuration is lost. Recently we proposed that anomer-selective glycosidase inhibitors could be rationally designed by mimicking instead the protonated glycoside **2** using aminocyclopentitols. In particular, the amino substituent, in a well-defined α or $\beta$ configuration, can be used as a mimic of the protonated oxygen atom O(1) and thus encodes for the anomeric specificity of the inhibitor. Thus, aminocyclopentitol **4** (Scheme 2), an analogue of a protonated α-mannoside related

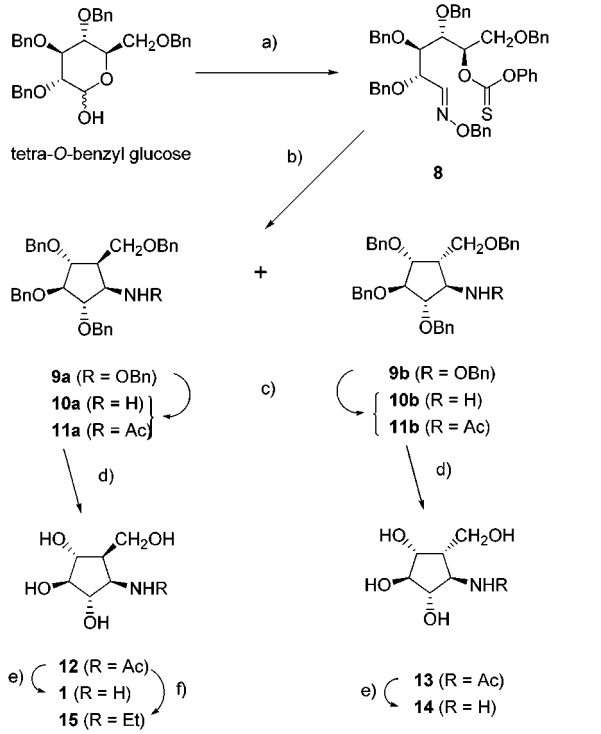
**Scheme 2.** Structure of Aminocyclopentitol Glycosidase Inhibitors




to mannostatin **5** and reported by Farr et al.,[6] is a potent inhibitor of α-mannosidase ($IC_{50}$ = 62 nM for jack beans α-mannosidase). While compound **6**, an analogue of a protonated $\beta$-L-fucoside, is only a weak inhibitor for α-L-fucosidase ($K_i$ = 28 $\mu$M for bovine kidney α-L-fucosidase),[7] the related aminocyclopentitol **7**, which mimics a protonated $\beta$-galactoside, inhibits $\beta$-galactosidases ($K_i$ = 3 $\mu$M) and $\beta$-glucosidases ($K_i$ = 0.25 $\mu$M) selectively over α-galactosidase ($K_i$ = 25 $\mu$M) and α-glucosidase ($IC_{50}$ = 100 $\mu$M).[3]

The potent inhibition of the *galacto*-configured inhibitor **7** with $\beta$-glucosidases, which can be explained by the known polyspecificity of type I $\beta$-glucosidases, prompted us to investigate the synthesis and evaluation of the parent *$\beta$-gluco*-configured aminocyclopentitol **1**. Functionalized aminocyclopentitols, including natural products such as mannostatin, can be prepared by a variety of synthetic routes.[2,8] One of the first and most straightforward approaches, reported by Bartlett et al. in 1988,[9] involves the radical cyclization of oxime **8** to form benzylated hydroxylamines **9a** and **9b**. Oxime **8** is itself easily obtained in two steps from tetra-*O*-benzylglucose (Scheme 3). The strategy of closing the

**Scheme 3.** Synthesis of Aminocyclopentitol **1** and Related Structures[a]




[a] Reagents and conditions: (a) (i) $NH_2OBn$, MeOH, pyridine, 90 °C, 3.5 h (84%), (ii) ClCSOPh, $CH_2Cl_2$, pyridine, 0°C, 3.5 h (73%); (b) $Bu_3SnH$, AIBN, benzene, reflux, 4 h (39% **9a**, 27 % **9b**); (c) Zn, AcOH, 135 °C, 4 h (32% **10a** + 43 % **11a**, 19% **10b** + 28 % **11b**); (d) 10 Atm $H_2$, Pd/C, EtOH, 25 °C, 10 days (100%); (e) HCl 1.2 N, reflux, 15 h (100%); (f) HCl 1.2 N, AcOH, $H_2$, Pd/C (100%).

cyclopentane ring by radical cyclization between C(1) and C(5) in an hexose oxime, which has been extended subsequently by a number of groups,[10] allows one to incorporate the stereochemical pattern of the carbohydrate into the target

(5) The C(1)−O(1) bond cleavage precedes the protonation step in the enzymatic hydrolysis with phenolic leaving groups. Protonation does not occur with acidic leaving groups such as pyridine or fluoride. Review on mechanisms: Sinnott, M. L. *Chem. Rev.* **1990**, *90*, 1171−1202.

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(9) Bartlett, P. A.; McLaren, K. L.; Ting, P. C. *J. Am. Chem. Soc.* **1988**, *110*, 1633.

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

| enzyme | origin | $K_i$(**1**) $\times 10^{-6}$ M | $K_i$(**12**) $\times 10^{-6}$ M | $K_i$(**13**) $\times 10^{-6}$ M | $K_i$(**14**) $\times 10^{-6}$ M | $K_i$(**15**) $\times 10^{-6}$ M |
|---|---|---|---|---|---|---|
| β-glucosidase | *C.s.*[b] | **0.18** | **170** | **5.4** | **0.60** | **0.16** |
| β-glucosidase | almond | **3.4** | | (28%) | **6.4** | **2.6** |
| α-glucosidase | yeast | **13** | | (23%) | **19** | **12** |
| β-galactosidase | *E. coli* | | | | (9%) | (9%) |
| β-galactosidase | bovine liver | **41** | (23%) | (7%) | **59** | **37** |
| α-galactosidase | green coffee beans | (11%) | (16%) | (16%) | (11%) | (20%) |
| β-mannosidase | snail acetone powder | | (19%) | (19%) | (6%) | (9%) |
| α-mannosidase | jack beans | **180** | | | **9.1** | **115** |

[a] 100 μL assays contained the indicated enzyme at 0.1 U/mL, and the corresponding nitrophenyl glycosides in 0.1 M HEPES buffer at pH 6.8, at 25 °C. The reactions 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. The competitive inhibition constants $K_i$ are given in micromoles and were determined by a Dixon plot of inhibition. No data are given when less than 5% inhibition was observed. For weak inhibition, the percentage of inhibition observed with [S] = 1 mM, [I] = 0.1 mM is given in parentheses. [b] *C.s.* = *Caldocellum saccharolyticum*. The following substrate and inhibitor concentrations were used. β-Glucosidase *C.s.*: [S] = 1.0 and 0.3 mM; [**1**] = 0, 62.5 nM, 125, 250, 500, 1000 nM; [**12**] = 0, 12.5, 25, 50, 100, 200, 400 μM; [**13**] = 0, 1, 2, 5, 10, 20, 50 μM; [**14**] = 0, 0.3125, 0.625, 1.25, 2.5, 5 μM; [**15**] = 0, 0.0625, 0.125, 0.25, 0.5, 1, 2 μM. Almond β-glucosidase: [S] = 1.0 and 0.3 mM; [**1**] = 0, 0.2, 0.5, 1, 2, 5,10 μM; [**14**] = 0, 2, 5, 10, 20, 50, 100 μM; [**15**] = 0, 0.3125, 0.625, 1.25, 2.5, 5, 10 μM. Yeast α-glucosidase: [S] = 0.3 and 0.1 mM; [**1**] = [**14**] = 0, 3.125, 6.25, 12.5, 25, 50, 100 μM; [**15**] = 0, 12.5, 25, 50, 100, 200 μM. Bovine liver β-galactosidase: [S] = 0.9 and 0.3 mM; [**1**] = [**14**] = [**15**] = 0, 6.25, 12.5, 25, 50, 100, 200 μM. Jack beans α-mannosidase: [S] = 0.78 and 0.26 mM; [**1**] = [**15**] = 0, 6.25, 12.5, 25, 50, 100, 200 μM; [**14**] = 0, 6.25, 12.5, 25, 50, 100 μM. Enzymes and substrates were purchased from Fluka or Sigma.

aminocyclopentitol and highlights the structural analogies between the two compounds.

Protected hydroxylamines **9a/b** were obtained by following the literature procedure[9] and were easily separated by column chromatography. The complete debenzylation of **9a** necessary to prepare **1**, which had not been investigated by Bartlett et al., proved unexpectedly challenging. After experimenting with a variety of schemes, deprotection succeeded by first cleaving the N−O bond in **9a** with zinc in acetic acid to give **10a** and the corresponding amide **11a**. While direct hydrogenation of amine **10a** only gave mixtures of products, extensive hydrogenation of amide **11a** proceeded cleanly and quantitatively to give **12**. Finally, acidic hydrolysis of **12** with 1.2 N HCl gave **1** as the only product as the hydrochloride salt. A similar sequence starting with isomer **9b** gave sequentially **10b** and **11b**, the amide **13**, and finally aminocyclopentitol **14**. Reduction of amide **12** with hydrogen and palladium in acidic medium gave the *N*-ethyl derivative **15**. The structure and stereochemistry of the compounds was confirmed by analytical data. In particular, NOE in the amides **12** and **13** allowed for the establishment of the relative configuration of the amino and hydroxymethyl substituents and confirmed the original structural assignment for **9a** and **9b**.[9]

Aminocyclopentitol **1** was assayed for inhibition of glycosidases together with its stereoisomer **14**, the corresponding acetamides **12** and **13**, and its *N*-ethyl derivative **15** (Table 1). All measurements were carried out with the corresponding nitrophenyl glycoside substrates in aqueous buffer at pH 6.8, 25 °C, under which conditions all enzymes displayed satisfactory activity. All enzyme−inhibitor pairs displaying more than 30% inhibition in the initial screening (100 μM inhibitor, 1 mM substrate) were characterized in more detail by measurement of the competitive inhibition constant.

The results show that **1** is a potent inhibitor of β-glucosidases. It is comparable in potency with the β-*galacto*-configured stereoisomer **7** and clearly belongs to the more potent small molecular weight glycosidase inhibitors. The isomeric aminocyclopentitol **14**, which derives from deprotection of the minor isomer formed during the radical cyclization and corresponds to an α-L-*ido* configuration, also turns out to be a potent inhibitor of β-glucosidases. As noted for the β-*galacto*-configured aminocyclopentitol **7**, this cross-reactivity is explained by the known polyspecificity of type I β-glucosidases.[1,11] This should not hide the fact that the relative configuration of aminocyclopentitols influences inhibition of the different glycosidases in a manner consistent with their design as analogues of protonated glycosides. Thus, the β-*gluco*-configured inhibitor **1** displays the most pronounced selectivity for β-glucosidases, while its β-*galacto*-configured isomer **7** is the only one in the series to strongly inhibit β-galactosidases. By contrast the α-L-*ido*-configured inhibitor **14** displays an unexpected cross-reactivity with α-mannosidase. Finally all of these inhibitors show a good β-anomer selectivity in agreement with their amino substituent being on the β-face.

*N*-Acetylation to form the neutral amides **12** and **13** essentially abolishes inhibition in both **1** and **14**. By contrast, the *N*-ethyl derivative **15** shows an inhibition pattern almost identical with that of **1**. The loss of inhibition potency in **12** or **13** is therefore probably not due to a steric effect of the added acetyl group but truly reflects the importance of the basic amino group, which is protonated and bears a positive charge at neutral pH, for inhibition by aminocyclopentitols **1** and **15**. On this basis one can reasonably propose that these inhibitors are binding to the enzymes as analogues of the

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protonated $\beta$-glucoside **2** (Scheme 1). This interpretation is further supported by the inhibition selectivities discussed above. The key interaction explaining the potent inhibition observed is probably a salt bridge between the enzyme's carboxyl group that protonates the substrate's leaving group and the inhibitor's ammonium group. A similar interaction explains that glycosylamines are also glycosidase inhibitors.[12]

In conclusion, we have shown that the $\beta$-*gluco*-configured aminocyclopentitol **1** is a potent and selective inhibitor of $\beta$-glucosidases. The presence of a basic amino group and the relative configuration of the substituents were shown to be essential for both inhibition potency and selectivity. In particular, the essential role of the positively charged amino group was demonstrated by abolishing inhibition via acetylation. The deprotection sequence reported here from the benzyl-protected hydroxylamine intermediates to the free aminocyclopentitols should be applicable for the rapid synthesis of related aminocyclopentitols.

**Acknowledgment.** This work was supported by the University of Bern, the Swiss National Science Foundation, and the Wander Stiftung.

**Supporting Information Available:** Data for compounds **9a**, **9b**, **12**, **1**·HCl, **13**, **14**·HCl, and **15**·HCl. Dixon plot of inhibition with **1** and $\beta$-glucosidases from *C. saccharolyticum* and from almonds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) $K_i$($\beta$-D-*galacto*-pyrannosylamine) = 7 $\mu$M for *Escherichia coli* $\beta$-galactosidase (in this case an enzyme-bound $Mg^{2+}$ activates the leaving group): Legler, G.; Herrchen, M. *Carbohydr. Res.* **1983**, *116*, 95. $K_i$(α-D-*manno*-pyrannosylamine) = 300 $\mu$M for jack beans α-mannosidase: Maity, S. K.; Dutta, S. K.; Banerjee, A. K.; Achari, B.; Singh, M. *Tetrahedron* **1994**, *50*, 6965. $K_i$(L-*fuco*-pyrannosylamine) = 1.2 $\mu$M for bovine kidney α-L-fucosidase: Legler, G.; Stütz, A. E.; Immich, H. *Carbohydr. Res.* **1995**, *272*, 17. $K_i$($\beta$-D-*gluco*-pyrannosylamine) = 24 $\mu$M for $\beta$-glucosidase from *Trichoderma reesei*: Kolarova, N.; Trgina, R.; Linek, K.; Farkas, V. *Carbohydr. Res.* **1995**, *273*, 109.