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Stereoselective Inhibition of α-L-Fucosidases by *N*-Benzyl Aminocyclopentitols

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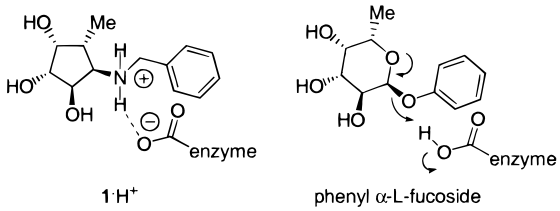
ABSTRACT



(1*R*,2*R*,3*R*,4*R*,5*R*)-4-Amino-5-methylcyclopentane-1,2,3-triol 8, its 4*S* stereoisomer 9, and their acyclic analogues (*R*)- and (*S*)-2-aminobutanol 11 and 12 are selective but moderate inhibitors of α-L-fucosidases. N-Benzylation selectively enhances inhibition potency for aminocyclopentitol 8 (→ 1, $K_i = 6.8 \times 10^{-7}$ M) but decreases inhibition for its 4*S*-stereoisomer 9 (→ 2, $K_i = 1.1 \times 10^{-4}$ M) and for the aminobutanols 11 (→ 13, no inhibition) and 12 (→ 14, no inhibition).

Glycosidase inhibitors can be used for treating diabetes, cancer, and viral (HIV, influenza) and bacterial infections and as insecticides.[1] Aminocyclopentitols such as mannostatin are powerful inhibitors of glycosidases.[2] We have shown recently that aminocyclopentitols designed as mimics of α- or β-configured protonated glycosides are potent anomer-selective inhibitors of glycosidases.[3] However, until now no comparisons of α/β stereoisomeric pairs of such inhibitors have been carried out. Herein we report the first such comparative study using L-*fuco* configured aminocyclopentitols. We show that the *N*-benzyl α-L-*fuco* configured aminocyclopentitol **1** inhibits α-L-fucosidases with high stereoselectivity over its β-configured stereoisomer **2** (Figure 1). This observation supports our originally proposed analogy between aminocyclopentitols and protonated glycosides and opens the way for further developments of these structures as glycosidase inhibitors.

Figure 1. Structure of *N*-benzyl aminocyclopentitol inhibitors of fucosidases.

The mechanism of enzymatic glycosidic bond cleavage, illustrated below for the hydrolysis of phenyl α-L-fucoside **3**, involves heterolytic scission of the C(1)−O(1) bond to form an oxocarbonium ion intermediate (e.g. **4**), which then reacts with a molecule of water to give the product (Scheme 1). Bond scission is generally facilitated by protonation of the leaving group oxygen atom O(1) by a carboxylic acid side chain on the enzyme, while a second carboxylate (not shown) stabilizes the oxocarbonium ion by either electrostatic or covalent interactions.[1]

The majority of glycosidase inhibitors have been designed as analogues of the oxocarbonium ion intermediate (e.g. **4**), where the anomeric configuration is lost. By contrast,

(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.

(2) Berecibar, A.; Grandjean, C.; Siriwardena, A. *Chem. Rev.* **1999**, *99*, 779.

(3) (a) Leroy, E.; Reymond, J.-L. *Org. Lett.* **1999**, *1*, 775. (b) Boss, O.; Leroy, E.; Blaser, A.; Reymond, J.-L. *Org. Lett.* **2000**, *2*, 151.

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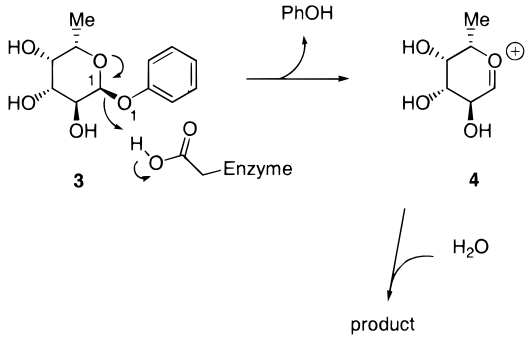
Scheme 1. Mechanism of Enzymatic Glycosidic Bond Cleavage with an α-L-Fucoside



glycosidases are generally highly stereospecific at the anomeric center. To obtain anomer-selective inhibitors of glycosidases, one should mimic an intermediate preceding C(1)−O(1) bond cleavage, where the anomeric configuration is still present. Inspired by the original work of Farr et al.,[4] who reported that compound **5** is a potent inhibitor of α-mannosidases, we have recently shown that aminocyclopentitol **6** (*β-galacto*) inhibits *β*-galactosidases and *β*-glucosidases selectively over α-galactosidase and α-glucosidase, while **7** (*β-gluco*) inhibits *β*-glucosidases selectively over α-glucosidase (Figure 2).[3] The anomer specificity of amino-

Figure 2. Aminocyclopentitol inhibitors of mannosidase, galactosidase, and glucosidase.

cyclopentitols **5**−**7** suggests that they can be considered, in their protonated form, as analogues of protonated glycosides.[5] Indeed, their cyclopentane ring, with five asymmetric centers, reproduces the full stereochemical pattern of the parent glycopyrannoside.

The analogy between aminocyclopentitols and protonated glycosides should also be tested by examining the relative potency of aminocyclopentitols bearing complementary α- and *β*-oriented amino substituents toward the same enzyme. We decided to investigate this point with the example of α-L-fucosidase inhibition, for which we have recently developed a stereoselective synthesis of both α-L-*fuco* (**8**) and *β*-L-*fuco* (**9**) configured aminocyclopentitols.[6] To probe our design further, we attached a substituent at nitrogen to mimic a glycosidic leaving group. This *N*-substitution could be beneficial for the inhibitor matching the enzyme's anomeric selectivity. Conversely, it might also decrease the potency of the corresponding unmatched stereoisomer, since it would increase the structural differences between the aminocyclopentitol and the glycoside substrate. Since α-L-fucosidases, like most glycosidases, accept a variety of leaving groups, such as simple phenols, a benzyl group on nitrogen could be used as a mimic of phenol as the leaving group. This choice was also dictated by the fact that many glycosidase inhibitor structures can be optimized by introducing an aromatic group at a position corresponding to a glycoside leaving group.[7]

N-benzyl aminocyclopentitol **1** was obtained as the HCl salt by deprotection of the cyclic carbamate **10** (1 M NaOH in EtOH/H_2O, 3.5 h, 60 °C, then 3 N HCl, 5 h, 60 °C, 91%), an intermediate along the reaction pathway leading to **8** (Figure 3).[8] Its corresponding *β*-stereoisomer **2** was prepared

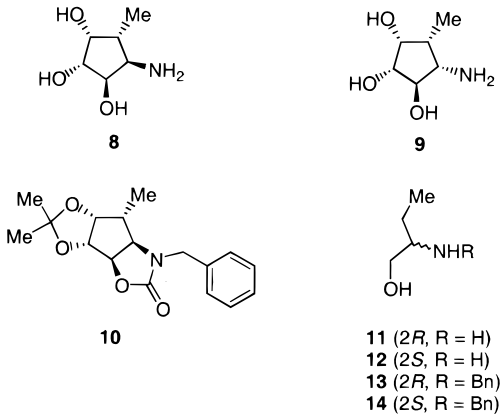


Figure 3. Structure of fucosidase inhibitors and synthetic intermediate **10**.

by reductive amination of **9** (PhCHO, $Ti(OiPr)_4$, EtOH, $NaBH_3CN$, 3 d, 27%)[9] and then purified by preparative reverse-phase HPLC as the trifluoroacetate salt.[10] In addition, (*R*)- and (*S*)-2-aminobutanols **11** and **12**, which we had identified as inhibitors of α-L-fucosidases in a screen against commercially available amino alcohols[11] and represent truncated versions of the aminocyclopentitols **8** and **9**, were

(4) Farr, R. A.; Peet, N. P.; Kand, M. S. *Tetrahedron Lett.* **1990**, *31*, 7109.

(5) In glycosidases, cleavage of the glycosidic C(1)−O(1) bond begins before protonation of the leaving group oxygen atom O(1) by the catalyzing acid; therefore the protonated glycoside only appears with a strongly elongated C(1)−O(1) bond (see ref 1c).

(6) (a) Blaser, A.; Reymond, J.-L. *Helv. Chim. Acta* **1999**, *82*, 760. (b) Blaser, A.; Reymond, J.-L. *Synlett*, in press.

(7) (a) Legler, G.; Herrchen, M. *Carbohydr. Res.* **1983**, *116*, 95. (b) Beer, D.; Vasella, A. *Helv. Chim. Acta* **1986**, *69*, 267. (c) Panday, N.; Canac, Y.; Vasella, A. *Helv. Chim. Acta* **2000**, *83*, 58.

(8) Data for **1**·HCl. $[\alpha]^{20}_D = +20.3°$ ($c = 0.34$, MeOH). ^{1}H NMR (300 MHz, CD_3OD): 7.55−7.4 (m, 5H, H_5C_5), 4.31 (d, ^2J = 13.2 Hz, 1H, PhCH_2), 4.22 (dd, $^3J_{(HC(3)-HC(2))}$ = 4.4 Hz, $^3J_{(HC(3)-HC(4))}$ = 7.0 Hz, HC(3)), 4.16 (d, ^2J = 13.2 Hz, 1H, PhCH_2), 4.05 (dd, $^3J_{(HC(1)-HC(2))}$ = 4.1 Hz, $^3J_{(HC(1)-HC(5))}$ = 5.2 Hz, HC(1)), 3.96 (dd, $^3J_{(HC(2)-HC(1))}$ = 4.1 Hz, $^3J_{(HC(2)-HC(3))}$ = 4.4 Hz, HC(2)), 3.34 (m, HC(4)), 2.20 (m, HC(5)), 1.15 (d, J = 7.0, C(5)-CH_3). ^{13}C NMR (50 MHz, CD_3OD): 132.7 (s), 131.0 (d), 130.6 (d), 130.2 (d), 80.4 (d), 73.6 (d), 73.5 (d), 64.8 (d), 51.8 (t), 39.3 (d), 13.3 (q). EI-MS: 237 (M^+ − HCl), 220, 208, 202, 190, 162, 106.

(9) Mattson, R. J.; Pham, K. M.; Leuck, D. J.; Cowen, K. A. *J. Org. Chem.* **1990**, *55*, 2552.

Table 1. Inhibition Data for α-L-Fucosidases[a]

source/K_i, μM	1	2	8	9	11	12	13	14
bovine kidney[b]	0.68	110	15	28	400	200	(13%)[i]	(26%)[i]
bovine epididymis[c]	0.57	28	12	18	350	125	(9%)[i]	(24%)[i]
human placenta[d]	0.30[f]	18	14	22	200	100	(6%)[i]	(26%)[i]
Fusarium oxysporum 377[e]	6.0	180	125	58	1100[g]	1300[h]	(15%)[i]	(22%)[i]

[a] 100 microliter assays containing the indicated α-L-fucosidase, 4-nitrophenyl α-L-fucoside, and the inhibitors in 0.1 M HEPES buffer at pH 6.8, 25 °C, were followed over 15 min in individual wells of 96-well half-area flat-bottom clear polystyrene cell-culture plates (Corning-Costar) by measuring the increase in UV absorbency at 405 nm using a UV spectramax instrument from Molecular Devices, Inc. Enzyme concentration was adjusted to give an increase of 0.2−0.5 OD during the measurement. The competitive inhibition constants K_i, given in micromolar, were determined by a Dixon plot of inhibition data using [S] = K_M and $^1/_3$ K_M, each with six different inhibitor concentrations spanning K_i. [b] K_M = 0.75 mM. [c] K_M = 0.33 mM. [d] K_M = 0.25 mM. [e] K_M = 0.75 mM. [f] Measured by Dixon replot of K_M/V_{max} vs [I], with [S] = 2, 1, 0.5, 0.25, 0.125 mM, each with [I] = 1, 0.5, 0.25, 0.125, and 0 μM. [g] 30% inhibition with [S] = 1 mM and [**11**] = 1 mM. [h] 29% inhibition with [S] = 1 mM and [**12**] = 1 mM. [i] Percent inhibition measured with 1 mM of the inhibitor and 1 mM of substrate.

converted to the *N*-benzyl derivatives **13** and **14** using the same procedure.

All compounds were assayed for inhibition of four different α-L-fucosidases, three mammalian enzymes and one fungal enzyme. All four α-L-fucosidases showed satisfactory activity at pH 6.8 at 25 °C. Competitive inhibition constants were determined by a Dixon plot of inhibition data (Figure 4) and are reported in Table 1.

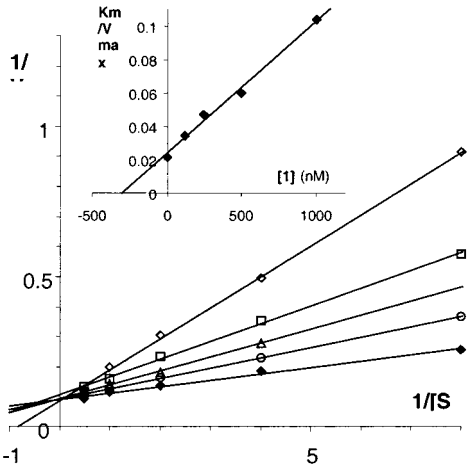


Figure 4. Lineweaver−Burk plot and Dixon replot for inhibition of human placenta α-L-fucosidase by α-L-*fuco* aminocyclopentitol **1**.

All α-L-fucosidases showed very similar inhibition patterns. For the series of primary amine inhibitors (**11**, **12**, **8**, and **9**), the increase in structural analogy with a protonated α-L-fucoside is associated with relatively modest gains in inhibition potency. Thus, going from acyclic (**11** and **12**) to cyclic (**8** and **9**) structures only results in an approximately 10- to 15-fold increase in inhibition potency. In addition, the free α-L-*fuco* aminocyclopentitol **8** is only slightly better than the β-L-*fuco* stereoisomer **9** for the three mammalian fucosidases and is even weaker than **9** with the *Fusarium oxysporum* 377 enzyme.

The data for the *N*-benzyl compounds show a much greater variation. *N*-Benzylation causes a large increase in inhibition potency selectively with α-L-*fuco* configured aminocyclopentitol **8** to give submicromolar inhibitor **1**, but there is either a minimal or a negative effect on inhibition potency with the other three aminocyclopentitols **9**, **11**, and **12**. Overall the α-L-*fuco* *N*-benzyl aminocyclopentitol **1** is between 30-fold (*Fusarium oxysporum* 377 α-L-fucosidase) and 160-fold (bovine kidney α-L-fucosidase) stronger than its β-stereoisomer **2**, while the acyclic inhibitors **13** and **14** are in all cases only marginally active. The inhibition by **1** is completely selective for α-L-fucosidases and does not occur with other common glycosidases.[12] This shows that a favorable binding interaction involving both the aminocyclopentitol and the *N*-benzyl group is selectively accessible to inhibitor **1** and not to the other *N*-benzyl inhibitors. Specifically, inhibitor **1** probably binds to the α-L-fucosidases in the same manner as a reacting α-L-fucoside substrate, with the amine forming a salt bridge with the catalyzing acid. These results, observed with four α-L-fucosidases of different origins, clearly support the analogy design relating aminocyclopentitols, in their protonated form, to protonated glycosides.

These experiments with α-L-fucosidase inhibition provide the first example of increasing the inhibition potency of an

(10) Data for **2**·TFA. $[\alpha]^{20}_D = -6.2°$ (c = 0.13, MeOH). ^{1}H NMR (300 MHz, CD_3OD): 7.55−7.4 (m, 5H, H_5C_5), 4.44 (d, ^2J = 13.2 Hz, 1H, PhCH_2), 4.26 (dd, $^3J_{(HC(3)-HC(2))}$ = 6.6 Hz, $^3J_{(HC(3)-HC(4))}$ = 5.2 Hz, HC(3)), 4.22 (d, ^2J = 13.2 Hz, 1H, PhCH_2), 3.88 (dd, $^3J_{(HC(1)-HC(2))}$ = 4.4 Hz, $^3J_{(HC(1)-HC(5))}$ = 4.1 Hz, HC(1)), 3.82 (dd, $^3J_{(HC(2)-HC(1))}$ = 4.4 Hz, $^3J_{(HC(2)-HC(3))}$ = 6.6 Hz, HC(2)), 3.35 (dd, $^3J_{(HC(4)-HC(3))}$ = 5.2 Hz, $^3J_{(HC(4)-HC(5))}$ = 8.2 Hz, HC(4)), 2.48 (m, HC(5)), 1.12 (d, J = 7.4, C(5)-CH_3). ^{13}C NMR (50 MHz, CD_3OD): 132.7 (s), 131.0 (d), 130.6 (d), 130.2 (d), 80.9 (d), 80.0 (d), 75.1 (d), 65.4 (d), 51.37 (t), 37.3 (d), 9.2 (q). HR-EI-MS: 237.1354 ($C_{13}H_{19}NO_3$ calcd 237.1365).

(11) Other acyclic amino-alcohol glycosidase inhibitors are known, such as tris(hydroxymethyl) aminomethane: (a) Larner, J.; Gillespie, R. E. *J Biol. Chem.* **1956**, *233*, 709. (b) Dale, M. P.; Ensley, H. E.; Sastry, K. A. R.; Byers, L. D. *Biochemistry* **1985**, *24*, 3530. (c) Fowler, P. A.; Haines, A. H.; Taylor, R. J. K.; Chrystal, E. J. T.; Gravestock, M. B. *J. Chem. Soc., Perkin Trans. 1* **1994**, 2229.

(12) There was no observable inhibition by **1**, **8**, or **9** for the following glycosidases (1 mM nitrophenyl glycoside, 0.1 mM inhibitor in 0.1 M HEPES pH 6.8, 25 °C): green coffee beans α-galactosidase, *Escherichia coli* β-galactosidase, yeast α-glucosidase, almond β-glucosidase, jack beans α-mannosidase, snail acetone powder β-mannosidase. **2** was not tested with these enzymes.

aminocyclopentitol analogue of a protonated glycoside by attachement of a leaving group mimic at nitrogen. That this increase in potency occurs selectively with the α-L-*fuco* configured aminocyclopentitol (**8** → **1**) over the corresponding *β*-stereoisomer or the acyclic aminobutanols fully supports the design concept and opens the way for engineering potency and selectivity of these inhibitors by manipulating the amino substituent; this applies to the inhibition of α-L-fucosidases[13] as well as for other glycosidases.

Acknowledgment. We thank Prof. Dr. Vladimír Křen of the Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague (Czech Republic), for a sample of *Fusarium oxysporum* 377 α-L-fucosidase. This work was supported by the University of Bern, the Swiss National Science Foundation, the Wander Stiftung, the European COST program D13, and the swiss Office Fédéral de l'Education et de la Science.

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(13) Recent papers on α-L-fucosidase inhibitors: (a) Nishimura, Y.; Shitara, E.; Takeuchi, T. *Tetrahedron Lett.* **1999**, *40*, 2351. (b) Peer, A.; Vasella, A. *Helv. Chim. Acta* **1999**, *82*, 1044 and references cited therein. (c) Shitara, E.; Nishimura, Y.; Kojima, F.; Takeuchi, T. *Bioorg. Med. Chem.* **2000**, *8*, 343.