A Selective α-L-Fucosidase Inhibitor Based on an Aminocyclopentane Framework

by **Adrian Blaser** and **Jean-Louis Reymond***

Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, CH-3012 Bern

(1*R*,2*R*,3*R*,4*S*,5*R*)-4-amino-5-methylcyclopentane-1,2,3-triol (**1**) was prepared stereoselectively from D-ribose (*Scheme*). Aminocyclopentanetriol **1**, which by its design may be considered an analog of the fucosyl cation, inhibits α-L-fucosidase selectively ($K_i = 28$ μM) over α- and β-glucosidase, α- and β-mannosidase, and α- and β-galactosidase (*Table*).

Introduction. – In conjunction with our efforts towards glycosidase catalytic antibodies [1], we have become interested in raising α-L-fucosidase catalytic antibodies [2]. α-L-Fucosidase catalytic antibodies might become useful therapeutic agents for modifying fucosylated cell-surface glycoconjugates [3] such as Sialyl *Lewis* X *in vivo*, and might serve as a novel therapeutic agent for treating inflammation and cancer.

A number of aza-sugars [4], such as deoxynojirimycin and castanospermine, which occur as natural products in plants, are potent inhibitors of a variety of glycosidases (*Fig. 1*). Inhibitors based on an aminocyclopentane framework have also been reported [5], such as mannostatine [6] and the closely related chitinase inhibitor trehazoline and related compounds [7]. Such glycosidase inhibitors have been investigated for treating diabetes, cancer, viral (HIV) and bacterial infections, and as insecticides [8]. As to their mechanism of action, these compounds most often act as competitive inhibitors of the enzymes. Due to their close structural and, in their protonated form, electrostatic resemblance to the oxycarbonium cation intermediate intervening in the acid-catalyzed glycosidic cleavage, these compounds are generally believed to act as transition-state-analog inhibitors of glycosidases.

A β-galactosidase catalytic antibody has recently been obtained by immunization against a derivative of a pyrrolidine-based glycosidase inhibitor [9], and a β-glucosidase catalytic antibody was obtained starting with a derivative of deoxynojirimycine [10]. These reports suggest that glycosidase inhibitors might be good starting points for preparing immunogenic haptens aimed at inducing glycosidase catalytic antibodies. In view of preparing α-L-fucosidase catalytic antibodies, we report the design, stereoselective synthesis, and inhibitory activity of aminocyclopentane **1**, whose structure is related to the known mannosidase inhibitor **2** [11] (*Fig. 2*). Compound **1** is shown to inhibit selectively α-L-fucosidase over a series of other glycosidases.

Results. – Aminocyclopentane **2** has been shown to strongly inhibit α-mannosidase [10]. We reasoned that the related aminocyclopentane **1** might similarly inhibit α-L-fucosidase [12]. In essence, compound $\mathbf{1} \cdot H^+$ mimicks the high-energy fucosyl cation intervening in the hydrolytic cleavage of fucosides by its substitution pattern as well as

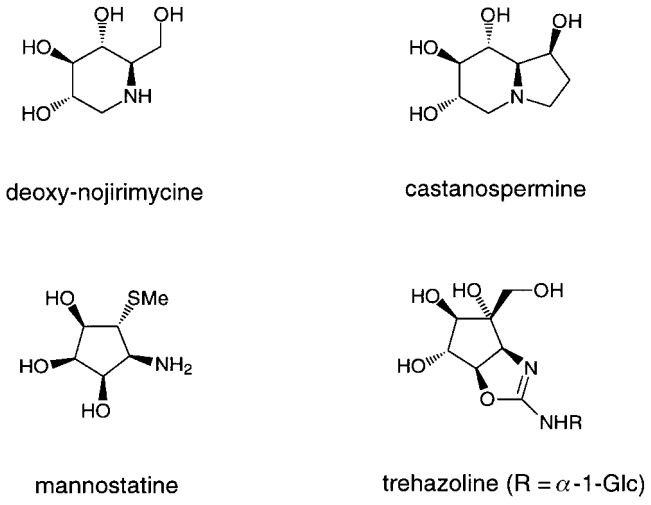


Fig. 1. *Structures of glycosidase inhibitors*

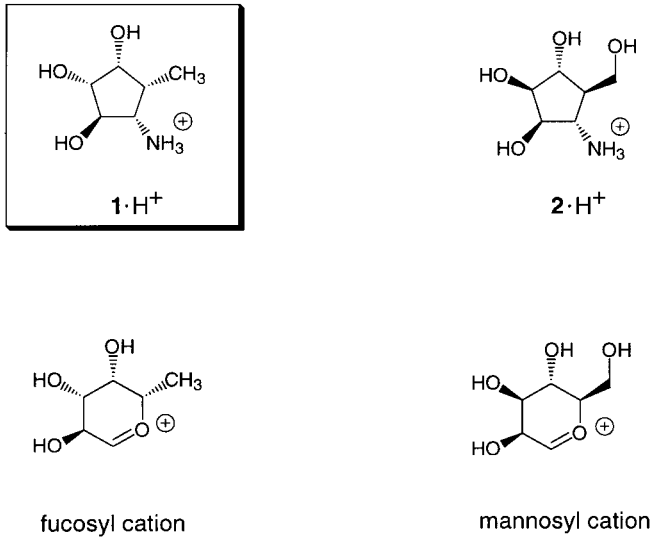


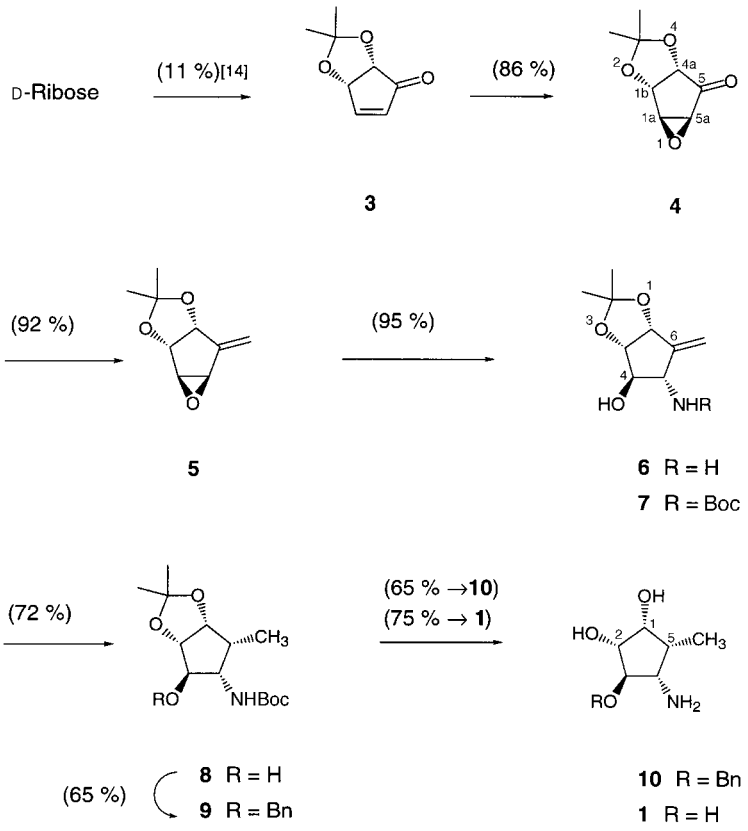
Fig. 2. *Aminocyclopentanes* **1** *and* **2** *and the related glycosyl cations*

by its positive charge, and may be considered, by design, a transition-state analog for the reaction.

Retrosynthetic analysis suggested a stereoselective access to aminocyclopentanetriol **1** from enone **3** (*Scheme*). This enone is accessible by a variety of routes [13], from which we selected the three-step preparation from D-ribose as the most convenient despite its moderate yield [14]. Epoxidation of **3** with basic hydrogen peroxide gave epoxy ketone **4** as a single product (MeOH, $-50°$, 1 h). Methylenation ($Ph_3P{=}CH_2$, THF, room temp., 1 h) gave epoxy-olefin **5** [15]. This sensitive and volatile product was

immediately aminolyzed (NH_3, EtOH, room temp., 3 h) to give amino alcohol **6** by selective epoxide opening at the allylic position. The amino alcohol **6** was then converted to the (*tert*-butoxycarbonyl)-protected (Boc) amino alcohol **7** (Boc_2O, aq. $NaHCO_3$ soln./AcOEt, room temp. 18 h). Hydrogenation (1 atm H_2, Pd/C, EtOH, room temperature 15 h) proceeded stereoselectively to give **8**, which was purified as its benzyl ether **9** (NaH, BnBr, DMF, room temp.). Deprotection (3N aq. HCl, 40°, 12 h) gave the benzylated amino-alcohol **10**. Alternatively, hydrogenation of **9** followed by acidic deprotection gave the free amino alcohol **1**.

Scheme. *Synthesis of Aminocyclopentanetriol* **1** *and of* **10** *from* D-*Ribose*



The structure and configuration of compounds **4**–**10** are readily established from the analytical data. In particular, the '*anti*'-relationship between the epoxy and isopropylidenedioxy moieties relative to the cyclopentane ring in epoxy ketone **4**, as well as the position and relative configuration of the ring subsituents in amino-alcohols **1** and **10**, are established by the ^{1}H-NMR coupling constants and NOE effects between ring protons (see *Exper. Part*).

Enzymes were assayed according to standard procedures by following the hydrolysis of nitrophenyl glycosides spectrophotometrically. The results show that **1** inhibits α-L-fucosidase selectively ($K_i = 28$ μM), with only a weak cross inhibition of

Table. *Inhibition of Glycosidases by Inhibitors* **1** *and* **10**[a])

Inhibitor	Enzyme	$U \cdot ml^{-1}$	K_i
1	bovine-kidney α-L-fucosidase	0.1	$2.8 \cdot 10^{-5}$ M[b])
10	bovine-kidney α-L-fucosidase	0.1	$> 10^{-3}$ M[c])
1	green-coffee-beans α-galactosidase	0.03	n.i.[d])
1	*E. Coli* β-galactosidase	0.3	n.i.[d])
1	yeast α-glucosidase	0.01	n.i.[d])
1	almond β-glucosidase	0.1	*ca.* 10^{-3} M[e])
1	jack-beans α-mannosidase	0.2	n.i.[d])
1	snail acetone-powder β-mannosidase	0.14	n.i.[d])

[a]) 100-µl assays contained the indicated enzyme concentration, 1 mM of inhibitor **1** or **10**, and the corresponding nitrophenyl glycoside (1 mM) in 0.1M HEPES buffer at pH 6.8, at 25°. [b]) Competitive inhibition constant as determined by *Dixon* plot of inhibition data (see *Fig. 3*). [c]) 10% inhibition with 1 mM **10**. [d]) n.i. = no inhibition observed with 1 mM **1**.[e]) 50% inhibition with 1 mM **1**.

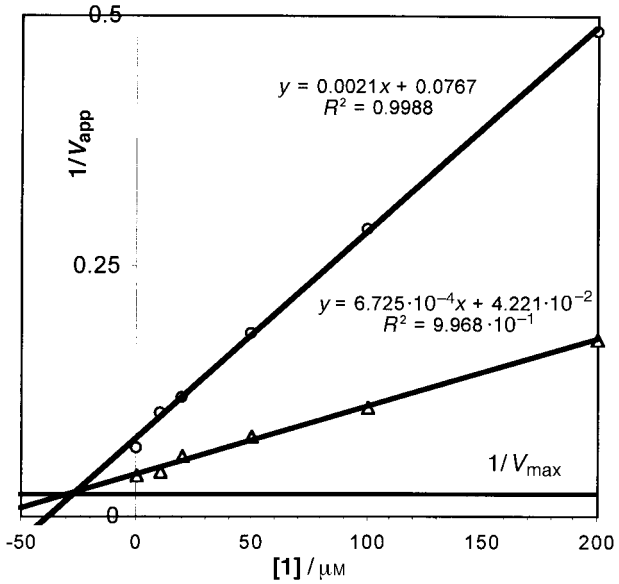


Fig. 3. Dixon *plot of inhibition data for aminocyclopentanetriol* **1** *with bovine-kidney* α-L-*fucosidase.* Measured in aq. 100 mM HEPES buffer pH 6.8 at 25° using (750 µM (△) and 250 µM (○) 4-nitrophenyl α-L-fucoside as substrate. V_{app} is given in milli$OD \cdot min^{-1}$ at 405 nm (see *Exper. Part*). The two lines intersect on the horizontal line crossing the vertical axis at $1/V_{max}$. The *x*-coordinate of the intersection point gives $-K_i$ (**1**). The measured *Michaelis-Menten* constant K_M for 4-nitrophenyl α-D-fucopyranoside was 750 µM.

almond β-glucosidase, and no measurable inhibition of five other glycosidases (*Table* and *Fig. 3*). Inhibition of α-L-fucosidase is essentially abolished when the 2-OH group is blocked as in benzyl ether derivative **10**.

Discussion. – Inhibition of α-L-fucosidase by compound **1** is competitive in nature, which implies that this inhibitor competes with the substrate for occupancy of the enzyme active site. As planned, the three OH substituents and the Me substituent of the cyclopentane match the relative configuration and substitution pattern of the

fucopyranose substrate. Selective inhibition of α-L-fucosidase over six other glycosidases is probably mediated by selective recognition of these substituents by the enzyme. This hypothesis is further supported by the fact that blocking the 2-OH group to give benzyl ether **10** abolishes inhibition.

Compound **1** ($K_i = 28$ μM) is very similar in its inhibition of α-L-fucosidase to piperidinediol **11** ($IC_{50} = 26$ μM), which is also very selective, and, similarily, only cross-reacts with β-glucosidase ($IC_{50} = 0.5$ mM) [12f] (*Fig. 4*). However both compounds are much less potent than fuco-deoxynojirimycin **12** ($K_i = 2.7$ nM) [12d] or its hydroxymethyl analog **15** ($K_i = 5.6$ nM) [12e]. The latter amines are closer analogs of the fucoside substrate than either **1** or **11**. The reaction product L-fucose **13** ($K_i = 30$ μM) has been reported to inhibit α-L-fucosidase almost as well as **1**, and its amino analog **14** is a micromolar inhibitor of this enzyme ($K_i = 0.75$ μM) [12d]. Other analogs of α-L-fucose, such as compounds **16** ($K_i = 6$ μM) [12a] and **17** ($K_i = 5.9$ μM) [12b], also inhibit α-L-fucosidase, which confirms that matching the substitution and configuration of the α-L-fucoside substrate leads to efficient inhibition of the enzyme. This might not be an exclusive criterion for design, as suggested by the fact that compounds only distantly related to α-L-fucose such as **18** also inhibit α-L-fucosidase ($K_i = 5.5$ μM), albeit with strongly reduced selectivity compared to other glycosidases ($K_i = 16.3$ μM for β-glucosidase) [12e].

Is compound $\mathbf{1} \cdot H^+$ a transition-state-analog inhibitor of bovine-kidney α-L-fucosidase? As discussed in detail by *Ermert et al.* for the case of two micromolar inhibitors of glucosidases and mannosidases [16], selective inhibition of one over other glycosidases, as observed here for **1**, is an important but insufficient condition for an inhibitor to act as a transition-state analog. The formal proof requires quantitative correlation of inhibition with catalytic potency for several diastereoisomeric inhibitor/substrate pairs and their corresponding enzyme, which is not readily possible in the fucose series. In the present case, binding of $\mathbf{1} \cdot H^+$ as transition-state analog would be expected to involve electrostatic and/or H-bonding interactions between the positively charged ammonio group in the inhibitor and the negatively charged carboxylate residues responsible for catalysis in α-L-fucosidase [17], as is generally postulated for glycosidase inhibitors that are carbohydrate mimics displaying a basic functionality near the anomeric center [18]. Although it was implicitly planned in our design by positioning of the primary ammonio group, the relatively weak inhibitory power of compound **1** towards bovine-kidney α-L-fucosidase suggests that such an interaction does not take place, or at least not in productive manner, and that **1** might not be a transition-state-analog inhibitor of bovine-kidney α-L-fucosidase. This is, however, in our view insufficient evidence to reject the possibility that a similar interaction leading to productive antibody catalysis might occur in an antibody-antigen interaction involving immunogenic derivatives of **1** and their corresponding antibodies.

Conclusion. – In summary, we reported the design, synthesis, and evaluation of a new selective α-L-fucosidase inhibitor. An efficient and stereoselective synthesis from D-ribose was devised that might be expanded to produce related glycosidase inhibitors. We are currently exploring the synthesis of further derivatives of **1**, in particular *N*-alkylated (4*R*)-stereoisomers of **1**, to serve as immunogenic haptens for inducing α-L-fucosidase catalytic antibodies.

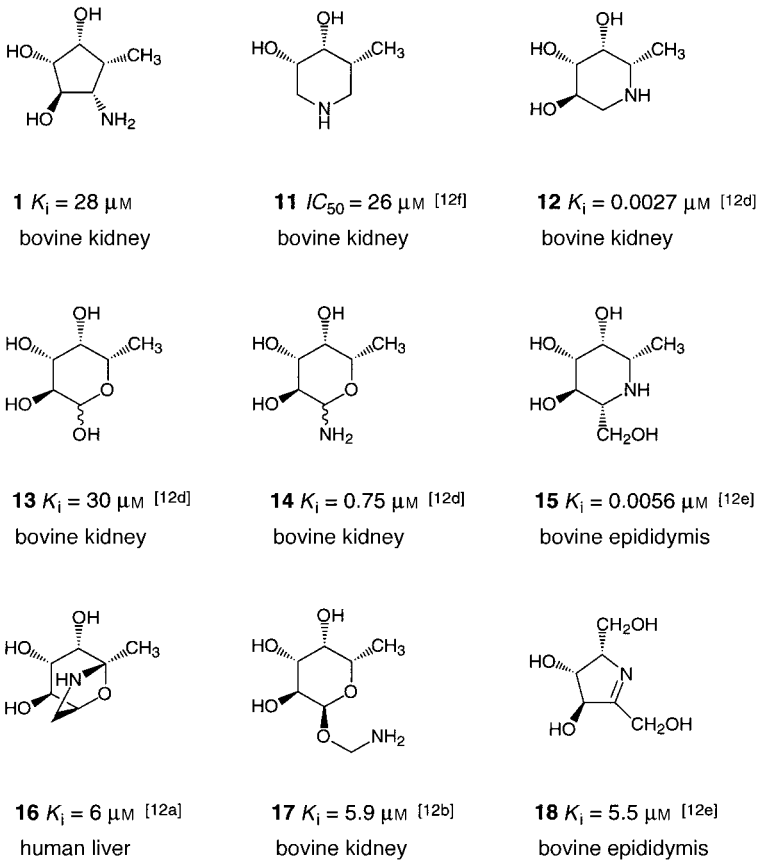


Fig. 4. *Structure and inhibition data for selected α-L-fucosidase inhibitors*

This work was supported by the *Swiss National Science Foundation*, the *Koordinationsgruppe für Forschungsfragen der Basler chemischen Industrie (KGF)*, and the *Wander Stiftung.*

Experimental Part

*(1a*S*,1b*S*,4a*S*,5a*S*)-Tetrahydro-3,3-dimethyl-5*H*-oxireno[3,4]cyclopenta[1,2-*d*][1,3]dioxol-5-one* (**4**). To a soln. of enone **3** (500 mg, 3.22 mmol) in MeOH (20 ml), 30% H_2O_2 soln. (0.7 ml, 6.44 mmol, 2 equiv.) was added and the mixture was cooled to $-50°$. Within 15 min, 2M NaOH (675 μl) was added. After 50 min at $-40°$, the mixture was poured into sat. NH_4Cl soln. (30 ml) and extracted (CH_2Cl_2). The org. layer was washed (brine), dried (Na_2SO_4), and evaporated. FC (hexane/AcOEt 5 : 1) yielded **4** (441 mg, 80%). Colourless oil. R_f (hexane/AcOEt 2 : 1) 0.63. $[\alpha]_D^{20} = +32.8$ ($c = 0.61$, $CHCl_3$). IR (Film): 3479(br.), 3028*w*, 2992*m*, 2938*m*, 1765*s*, 1456*w*, 1377*s*, 1231*m*, 1208*m*, 1156*m*, 1079*s*, 1005*m*, 969*m*, 865*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 4.91 (br. *d*, *J*(1b,4a) = 5.52, H–C(4a)); 4.44 (br. *d*, *J*(1b,4a) = 5.52, H–C(1b)); 4.03 (br. *d*, *J*(1a,5a) = 2.21, H–C(5a)); 3.59 (br. *d*, *J*(1a,5a) = 2.21, *J*(1b,1a) < 1, H–C(1a)); 1.42, 1.38 (2 *s*, Me_2C). NOE: H–C(1a)/H–C(5a), H–C(1b)/H–C(4a); no NOE between H–C(1a) and H–C(1b), and between H–C(4a) and H–C(5a). ^{13}C-NMR (75 MHz, $CDCl_3$): 203.40 (*s*); 114.16 (*s*); 77.37 (*d*); 75.36 (*d*); 57.88 (*d*); 54.29 (*d*); 26.95 (*q*); 24.93 (*q*). EI-MS: 170 (M^+), 155 ([$M-15$]$^+$), 113, 85. HR-EI-MS: 170.0575 ($C_8H_{10}O_4^+$; calc. 170.0579). Anal. calc. for $C_8H_{10}O_4$: C 56.47, H 5.92; found: C 56.30, H 6.04.

*(1a*S*,1b*R*,4a*R*,5a*R*)-Tetrahydro-3,3-dimethyl-5-methylidene-5*H*-oxireno[3,4]cyclopenta[1,2-*d*][1,3]dioxol* (**5**). A soln. of methyltriphenylphosphonium bromide/NaH ('Instant Ylid' 1.48 g, 2.4 mmol) in abs. THF (3 ml)

in an oven-dried flask was stirred for 15 min at r.t. Epoxy ketone **4** (400 mg, 2.35 mmol) in abs. THF (2 ml) was added. After stirring for 1 h at r.t., the mixture was poured into Et_2O (60 ml), filtered over *Celite*, and evaporated. FC (hexane/AcOEt 5:1) yielded **5** (359 mg, 91%). Colourless oil. R_f (hexane/AcOEt 5:1) 0.42. $[\alpha]_D^{20} = +87.3$ ($c = 0.395$, $CHCl_3$). IR (Film): 3055*w*, 2928*m*, 2856*w*, 1728*w*, 1585*w*, 1479*w*, 1435*m*, 1374*w*, 1270*w*, 1234*w*, 1158*m*, 1120*m*, 1071*m*, 1028*w*, 855*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 5.62 (*d*, $J = 1.83$, 1 H), 5.52 (*d*, $J = 1.11$, 1 H, $=CH_2$); 4.72, 4.67 (2*d*, $J = 5.52$, H–C(1b), H–C(4a′)); 3.83, 3.79 (2*d*, $J = 2.22$, H–C(1a), H–C(5a)); 1.42, 1.37 (2*s*, Me_2C). ^{13}C-NMR (75 MHz, $CDCl_3$): 146.75 (*s*); 117.17 (*t*); 112.79 (*s*); 80.32 (*d*); 78.99 (*d*); 59.19 (*d*); 58.97 (*d*); 27.41 (*q*); 25.44 (*q*). EI-MS: 153 ($[M-15]^+$), 111, 81. HR-EI-MS: 153.0552 ($C_8H_9O_3^+$, $[M-15]^+$; calc. 153.0552).

*(3a*S*,4*R*,5*S*,6a*R*)-5-Amino-tetrahydro-2,2-dimethyl-6-methylidene-4*H*-cyclopenta-1,3-dioxol-4-ol* (**6**). Olefin **5** (330 mg, 1.96 mmol) was dissolved in EtOH (20 ml), and 25% NH_3 soln. (50 ml) was added. The mixture was stirred for 2–3 h at r.t. and evaporated. FC (CH_2Cl_2/MeOH 9:1) yielded **6** (280 mg, 77%). Yellow oil. R_f (CH_2Cl_2/MeOH 9:1) 0.26. $[\alpha]_D^{20} = +18.4$ ($c = 0.75$, $CHCl_3$). IR (Film): 3361(br.), 2990*s*, 2935*s*, 1673*w*, 1590*m*, 1456*m*, 1374*s*, 1270*m*, 1222*m*, 1208*s*, 1157*s*, 1072*s*, 995*m*, 917*m*, 863*s*. ^{1}H-NMR (300 MHz, $CDCl_3$): 5.35 (*dd*, $J = 2.19$, 1.47, 1 H), 5.26 (*t*, $J = 2.19$, 1 H, $=CH_2$); 4.90 (*dt*, $J = 7.0$, 1.5, H–C(6a)); 4.40 (*dd*, $J = 7.0$, 4.0, H–C(3a)); 3.73 (*dd*, $J = 7.35$, 3.66, H–C(4)); 3.44 (*dt*, $J = 7.74$, 2.22, H–C(5)); 2.31 (br., NH_2, OH); 1.46, 1.34 (2*s*, Me_2C). ^{13}C-NMR (75 MHz, $CDCl_3$): 150.61 (*s*); 112.32 (*s*); 111.64 (*t*); 83.83 (*d*); 82.03 (*d*); 78.40 (*d*); 61.81 (*d*); 27.15 (*q*); 24.92 (*q*). EI-MS: 185 (M^+), 170 ($[M-15]^+$), 127, 110, 98. HR-EI-MS: 185.1052 ($C_9H_{15}NO_3^+$; calc. 185.1052).

*(3a*S*,4*R*,5*S*,6a*R*)-5-[[(*tert*-Butoxy)carbonyl]amino]-tetrahydro-2,2-dimethyl-6-methylidene-4*H*-cyclopenta-1,3-dioxol-4-ol* (*= [(3a*S*,4*R*,5*S*,6a*R*)-Tetrahydro-4-hydroxy-2,2-dimethyl-6-methylidene-4*H*-cyclopenta-1,3-dioxol-5-yl]carbamic Acid* tert-*Butyl Ester*; **7**). To a mixture of **6** (320 mg, 1.73 mmol), $(Boc)_2O$ (640 mg, 2.9 mmol) and $NaHCO_3$ (440 mg, 5.2 mmol), AcOEt (5 ml) and H_2O (5 ml) were added, and the mixture was stirred overnight at r.t. The aq. phase was extracted with AcOEt (80 ml) and the combined org. layer washed (brine, 2 × 15 ml) and evaporated. FC (hexane/AcOEt 2:1) yielded **7** (493 mg, quant.). White solid. M.p. 102–104°. R_f (hexane/AcOEt 2:1) 0.17. $[\alpha]_D^{20} = +5.3$ ($c = 0.48$, $CHCl_3$). IR (Film): 3443(br.), 2983*s*, 2935*m*, 1694*s*, 1504*s*, 1456*m*, 1368*s*, 1253*m*, 1207*m*, 1161*s*, 1055*s*, 1021*m*, 861*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 5.42, 5.34 (2*s*, $=CH_2$); 5.07 (br. *d*, $J = 5.88$, NH); 4.90 (*d*, $J = 5.88$, H–C(6a)); 4.45 (*dd*, $J = 6.27$, 1.47, H–C(3a)); 4.26 (*m*, H–C(5)); 3.99 (*dd*, $J = 4.05$, 1.83, H–C(4)); 3.81 (br., OH); 1.45, 1.32 (2*s*, Me_2C); 1.42 (*s*, *t*-Bu). ^{13}C-NMR (75 MHz, $CDCl_3$): 155.88 (*s*); 147.07 (*s*); 115.43 (*t*); 111.76 (*s*); 84.44 (*d*); 80.21 (*d*); 80.03 (*s*); 79.79 (*d*); 61.29 (*d*); 28.28 (*q*); 26.76 (*q*); 24.54 (*q*). EI-MS: 285 (M^+), 270 ($[M-15]^+$), 229, 214, 185, 171, 154, 127, 110, 98. HR-EI-MS: 285.1576 ($C_{14}H_{23}NO_5^+$; calc. 285.1576). Anal. calc. for $C_{14}H_{23}NO_5$: C 58.93, H 8.12, N 4.91; found: C 58.80, H 8.15, N 4.94.

*(3a*S*,4*R*,5*S*,6*R*,6a*R*)-5-[[(*tert*-Butoxy)carbonyl]amino}tetrahydro-2,2,6-trimethyl-4*H*-cyclopenta-1,3-dioxol-4-ol* (*= [(3a*S*,4*R*,5*S*,6*R*,6a*R*)-Tetrahydro-4-hydroxy-2,2,6-trimethyl-4*H*-cyclopenta-1,3-dioxol-5-yl]carbamic Acid* tert-*Butyl Ester*; **8**). To a soln. of **7** (195 mg, 0.68 mmol) in EtOH (5 ml), 10% Pd/C (5 mg) was added. The flask was evacuated and filled with H_2 (3×). After stirring overnight, the mixture was filtered over *Celite* and evaporated. FC (hexane/AcOEt 2:1) yielded **8** (151 mg, 77%). Colourless oil. R_f (hexane/AcOEt 2:1) 0.26. $[\alpha]_D^{20} = -40.3$ ($c = 0.585$, $CHCl_3$). IR (Film): 3443(br.), 2983*m*, 2936*m*, 1697*s*, 1507*s*, 1456*m*, 1386*m*, 1376*m*, 1368*m*, 1251*m*, 1207*m*, 1162*s*, 1047*s*, 994*w*, 871*w*. ^{1}H-NMR (300 MHz, $CDCl_3$): 5.22 (*d*, $J = 9.18$, NH); 4.58 (*t*, $J = 5.16$, H–C(6a)); 4.42 (*dd*, $J = 5.52$, 1.47, H–C(3a)); 4.05 (br. *s*, H–C(4)); 3.76 (*dd*, $J = 9.18$, 5.88, H–C(5)); 3.28 (br. *s*, OH); 2.52 (*m*, H–C(6)); 1.47, 1.30 (2*s*, Me_2C); 1.42 (*s*, *t*-Bu); 1.11 (*d*, $J = 7.35$, Me–C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): 155.84 (*s*); 110.21 (*s*); 86.15 (*d*); 83.15 (*d*); 79.47 (*d*); 79.34 (*s*); 61.50 (*d*); 37.46 (*d*); 28.33 (*q*); 25.85 (*q*); 23.14 (*q*); 8.69 (*q*). EI-MS: 287 (M^+), 272 ($[M-15]^+$), 231, 216, 173, 156, 155, 138, 129, 112, 103. HR-EI-MS: 287.1732 ($C_{14}H_{25}NO_5^+$; calc. 287.1733).

*[(3a*S*,4*R*,5*S*,6*R*,6a*R*)-Tetrahydro-4-hydroxy-2,2,6-trimethyl-4*H*-cyclopenta-1,3-dioxol-5-yl]carbamic Acid* tert-*Butyl Ester* (**9**). A soln. of **8** (30 mg, 0.1 mmol) in abs. DMF (1 ml) was cooled to −14° (ice/acetone bath). A 55% NaH dispersion (10 mg, 0.23 mmol) was added, and the mixture was stirred for 2 h at −14°. Benzyl bromide (14 µl, 19.7 mg, 0.115 mmol) was added and the mixture stirred for 3 h at r.t. Then, the mixture was poured into H_2O (15 ml) and extracted with AcOEt (3 × 20 ml). The org. layer was washed with brine (3 × 20 ml) and evaporated. FC (hexane/AcOEt 5:1) yielded **9** (28 mg, 70%). Colourless oil. R_f (hexane/AcOEt 5:1) 0.47. $[\alpha]_D^{20} = -20.7$ ($c = 1.015$, $CHCl_3$). IR (Film): 3446*m*, 2981*m*, 2932*m*, 1712*s*, 1498*s*, 1456*m*, 1385*m*, 1367*m*, 1270*w*, 1248*m*, 1207*m*, 1163*s*, 1062*s*, 981*w*, 871*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.35–7.28 (*m*, Ph); 5.20 (*d*, $J = 9.18$, NH); 4.70 (*d*, $J = 11.40$, 1 H, PhCH_2); 4.60–4.50 (*m*, 3 H, PhCH_2, H–C(3a), H–C(6a)); 4.04 (*dd*, $J = 9.18$, 5.88, H–C(5)); 3.82 (*s*, H–C(4)); 2.45 (*m*, H–C(6)); 1.49, 1.31 (2*s*, Me_2C); 1.45 (*s*, *t*-Bu); 1.15 (*d*, $J = 7.35$, Me–C(6)). ^{13}C-NMR (75 MHz, $CDCl_3$): 155.52 (*s*); 137.96 (*s*); 128.34 (*d*); 127.71 (*d*); 127.61 (*d*);

110.37 (*s*); 86.98 (*d*); 84.89 (*d*); 83.18 (*d*); 79.03 (*s*); 71.47 (*t*); 58.05 (*d*); 38.01 (*d*); 28.40 (*q*); 25.92 (*q*); 23.22 (*q*); 8.77 (*q*). EI-MS: 377 (M^+), 362 ($[M15]^+$), 321, 276, 215, 170, 157, 139, 128, 112, 96. HR-EI-MS: 377.2201 ($C_{21}H_{31}NO_5^+$; calc. 377.2202). Anal. calc. for $C_{21}H_{31}NO_5$: C 66.82, H 8.28, N 3.71; found: C 66.87, H 8.24, N 3.79.

*(1*R*,2*R*,3*R*,4*S*,5*R*)-4-amino-5-methylcyclopentane-1,2,3-triol* (**1**). To a soln. of **9** (40 mg, 0.1 mmol) in EtOH (3 ml), 10% Pd/C (*ca.* 5 mg) was added. The flask was evacuated and filled with H_2 (3×). After stirring overnight, the mixture was filtered over *Celite* and evaporated. FC (hexane/AcOEt 2 : 1) yielded **8** (27 mg, 88%) as a colourless oil. The residue was dissolved in H_2O (3 ml), and 37% HCl soln. (1 ml) was added. The soln. was stirred overnight at 45° and evaporated. The product was redissolved in H_2O and passed over a *DOWEX* column (50 × 8 cm, sulfonic acid): **1**·HCl (10.2 mg, 60%) eluted directly. After washing with H_2O and MeOH, elution with aq. NH_3 soln. yielded a second fraction of free **1** (3.7 mg, 27%). Both compounds were white solids after solvent evaporation and drying.

Data of **1**·HCl: $[\alpha]_D^{20} = -16.3$ ($c = 0.51$, MeOH).

Data of **1**: IR (KBr): 3387*s*(br.), 3240*s*, 3156*s*, 2975*s*, 2921*s*, 2361*w*, 2343*w*, 1617*m*, 1578*m*, 1491*s*, 1450*w*, 1378*w*, 1330*m*, 1311*m*, 1292*m*, 1195*w*, 1114*m*, 1063*s*, 1043*m*, 992*w*, 968*m*. ^{1}H-NMR (300 MHz, D_2O): 4.00 (*dd*, $J = 6.99$, 4.05, H–C(2)); 3.74–3.87 (*m*, H–C(1), H–C(3)); 3.30 (*dd*, $J = 8.10$, 3.33, H–C(4)); 2.37 (*quint. d*, $J = 7.74$, 3.33, H–C(5)); 0.96 (*d*, $J = 7.35$, 3 H, Me–C(5)). ^{13}C-NMR (50 MHz, CD_3OD): 86.15 (*d*); 80.95 (*d*); 76.75 (*d*); 61.29 (*d*); 38.43 (*d*); 9.22 (*q*). EI-MS: 147 (M^+), 130, 88, 86, 84, 73, 72. HR-EI-MS: 147.0889 ($C_6H_{13}NO_3^+$; calc. 147.0895).

*(1*R*,2*R*,3*R*,4*S*,5*R*)-4-Amino-3-(benzyloxy)-5-methylcyclopentane-1,2-diol* (**10**). To a soln. of **9** (15 mg, 40 mmol) in CH_2Cl_2 (5 ml), CF_3COOH (5 ml) was added and stirred for 1.5 h at r.t. The solvent was evaporated the residue dissolved in H_2O (14 ml), 1M HCl (1 ml) added, and the mixture stirred for 4 h at r.t. and evaporated. The product was purified over a *DOWEX* column (50 × 8 cm): free **10** (4.9 mg, 52%). White solid. $[\alpha] = +6.0$ ($c = 0.15$, MeOH). IR (KBr): 3418*s*(br.), 2925*s*, 2361*w*, 2344*w*, 1616*m*, 1456*w*, 1404*m*, 1097*m*. ^{1}H-NMR (300 MHz, D_2O): 7.36 (*m*, Ph); 4.66, 4.59 (2*d*, $J = 10.65$, PhCH_2); 4.04 (*m*, H–C(2), H–C(3)); 3.93 (*m*, H–C(1)); 3.53 (*d*, $J(4,5) = 7.35$, H–C(4)); 2.38 (*quint. d*, $J(4,5) = J(5,Me) = 7.35$, $J(1,5) = 3.30$, H–C(5)); 1.03 (*d*, $J(5,Me) = 7.35$, Me–C(5)). NOE: only H–C(1)/H–C(5), H–C(4)/H–C(5). ^{13}C-NMR (75 MHz, CD_3OD): 129.38 (*d*); 128.90 (*d*); 128.80 (*d*); 90.40 (*d*); 80.51 (*d*); 77.36 (*d*); 73.06 (*t*); 59.42 (*d*); 37.61 (*d*); 9.05 (*q*). EI-MS: 237 (M^+), 222 ($[M-15]^+$), 161, 147, 132, 129, 107, 91. HR-EI-MS: 237.1356 ($C_{13}H_{19}NO_3^+$; calc. 237.1365).

Enzyme Measurements. Enzymes were purchased from *Fluka* and *Sigma*. The following glycosidases were assayed: α-glucosidase (yeast, EC 3.2.1.20), β-glucosidase (almonds, EC 3.2.1.21), α-galactosidase (green coffee beans, EC 3.2.1.22), β-galactosidase (*E. coli*, EC 3.2.1.23), α-mannosidase (jack beans, EC 3.2.1.24), β-mannosidase (snail acetone powder, EC 3.2.1.25), and α-L-fucosidase (bovine kidney, EC 3.2.1.51). All buffers and solns. were prepared using MilliQ-deionized H_2O. Enzymes were diluted with 0.1M HEPES (4-(2-hydroxyethyl)piperazine-1-sulfonic acid) buffer pH 6.8. Substrates and inhibitors were used as 10 mM stock solns. in buffer.

Reactions were initiated by addition of substrate to a soln. containing enzyme and inhibitor in 0.1M HEPES buffer: 100-μl 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*. The release of nitrophenol was followed at 405 nm over 1 h. The concentration of each enzyme in the assays was adjusted such as to give *ca.* 0.5 to 2.0 *OD* increase over 1 h as given by the instrument. The initial rate of reaction of the first 10 min was linear and was used to calculate the rate. Rates were expressed in relative units as milli$OD \cdot min^{-1}$.

REFERENCES

[1] J.-L. Reymond, K. D. Janda, R. A. Lerner, *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1711; D. Shabat, S. C. Sinha, J.-L. Reymond, E. Keinan, *ibid.* **1996**, *35*, 2628.

[2] P. G. Schultz, R. A. Lerner, *Science* **1995**, *269*, 1835; E. Keinan, R. A. Lerner, *Isr. J. Chem.* **1996**, *36*, 113; R. A. Lerner, S. J. Benkovic, P. G. Schultz, *Science* **1991**, *252*, 659; N. R. Thomas, *Natl. Prod. Rep.* **1996**, 479; J.-L. Reymond, *Top. Curr. Chem.* **1999**, *200*, 59.

[3] 'Carbohydrate Antigens', Ed. P. J. Garegg and A. A. Lindberg, ACS Symposium Series 519, Washington DC, 1993; b) E. E. Simanek, G. J. McGarvey, J. A. Jablonowski, C.-H. Wong, *Chem. Rev.* **1998**, *98*, 833.

[4] M. Bols, *Acc. Chem. Res.* **1998**, *31*, 1.

[5] R. J. Ferrier, S. Middleton, *Chem. Rev.* **1993**, *93*, 2779.

[6] T. Aoyagi, T. Yamamoto, K. Kojiri, H. Morishima, M. Nagai, M. Hamada, T. Takeuchi, H. Umezawa, *J. Antibiot.* **1989**, *42*, 883; S. B. King, B. Ganem, *J. Org. Chem.* **1994**, *116*, 562.

[7] J.-L. Maloisel, A. Vasella, B. M. Trost, D. L. van Vranken, *Helv. Chim. Acta* **1992**, *75*, 1515; R. Blattner, R. H. Furneaux, T. Kemmitt, P. C. Tyler, R. J. Ferrier, A.-K. Tidén, *J. Chem. Soc., Perkin Trans. 1* **1994**, 3411; R. Blattner, R. H. Furneaux, G. P. Lynch, *Carbohydr. Res.* **1996**, *294*, 29; J. Li, F. Lang, B. Ganem, *J. Org. Chem*. **1998**, *63*, 3403; A. Boiron, P. Zillig, D. Faber, B. Giese, *ibid*. **1998**, *63*, 5877; I. Storch de Gracia, H. Dietrich, S. Bobo, J. L. Chiara, *ibid*. **1998**, *63*, 5883; C. Uchida, H. Kimura, S. Ogawa, *Bioorg. Med. Chem*. **1997**, *5*, 921.

[8] G. S. Jacob, *Curr. Opin. Struct. Biol*. **1995**, *5*, 605; A. J. Scheen, P. J. Lefebvre, *Drugs* **1998**, *55*, 225; W. McDowell, R. T. Schwarz, *Biochimie* **1988**, *70*, 1535; L. Ratner, *AIDS Res. Hum. Retroviruses* **1992**, *8*, 65; N. Asano, M. Takeuchi, Y. Kameda, K. Matsui, Y. Kono, *J. Antibiot*. **1990**, *43*, 722.

[9] K. D. Janda, L.-C. Lo, C.-H. L. Lo, M.-M. Sim, R. Wang, C.-H. Wong, R. A. Lerner, *Science* **1997**, *275*, 945.

[10] J. Yu, S. Y. Choi, K. D. Moon, H. H. Chung, H. J. Youn, S. Jeong, H. Park, P. G. Schultz, *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2880.

[11] R. A. Farr, N. P. Peet, M. S. Kand, *Tetrahedron Lett*. **1990**, *31*, 7109.

[12] Recent α-L-fucosidase inhibitors: a) A. R. Beacham, K. H. Smelt, K. Biggadike, C. J. Britten, L. Hackett, B. G. Winchester, R. J. Nash, R. C. Griffiths, G. W. J. Fleet, *Tetrahedron Lett*. **1998**, *39*, 151; b) Z. J. Witczak, D. Boryczewski, *Bioorg. Med. Chem. Lett*. **1998**, *8*, 3265; c) R. Wischnat, R. Martin, S. Takayama, C.-H. Wong, *ibid*. **1998**, *8*, 3353; d) G. Legler, A. E. Stütz, H. Immich, *Carbohydr. Res.* **1995**, *272*, 17; e) S. Takayama, R. Martin, J. Wu, K. Laslo, G. Siuzdak, C.-H. Wong, *J. Am. Chem. Soc*. **1997**, *119*, 8146; f) Y. Ichikawa, Y. Igarashi, M. Ichikawa, Y. Suhara, *ibid*. **1998**, *120*, 3007.

[13] S. M. Siddiqi, S. W. Schneller, *Nucleosides Nucleotides* **1993**, *12*, 185; H. Ohrui, M. Konno, H. Meguro, *Agric. Biol. Chem*. **1987**, *51*, 625; C. R. Johnson, J. L. Esker, M. C. Van Zandt, *J. Org. Chem*. **1994**, *59*, 5854; D. R. Deardorff, S. Shambayati, D. C. Myles, D. Heerding, *ibid*. **1988**, *53*, 3614; T. Hudlicky, M. G. Natchus, T. C. Nugent, *Synth. Commun*. **1992**, *22*, 151; P. Bélanger, P. Prasit, *Tetrahedron Lett*. **1988**, *29*, 5521; T. Biadatti, J. L. Esker, C. R. Johnson, *Tetrahedron: Asymmetry* **1996**, *7*, 2313; C. R. Johnson, T. D. Penning, *J. Am. Chem. Soc.* **1988**, *110*, 4726; H. Sano, S. Sugai, *Tetrahedron: Asymmetry* **1995**, *6*, 1143; C. Wesdemiotis, R. Feng, F. W. McLafferty, *J. Am. Chem. Soc*. **1986**, *108*, 5656; C.-C. Lin, Y.-C. Wang, J.-L. Hsu, C.-C. Chiang, D.-W. Su, T.-H. Yan, *J. Org. Chem*. **1997**, *62*, 3806; R. J. Parry, M. R. Burns, P. N. Skae, J. C. Hoyt, B. Pal, *Bioorg. Med. Chem.* **1996**, *4*, 1077; J. M. Hill, E. J. Hutchinson, D. M. Le Grand, S. M. Roberts, A. J. Thorpe, N. J. Turner, *J. Chem. Soc., Perkin Trans. 1* **1994**, 1483; T. Hudlicky, H. Luna, G. Barbieri, L. D. Kwart, *J. Am. Chem. Soc*. **1988**, *110*, 4735.

[14] S. M. Ali, K. Ramesh, R. T. Borchardt, *Tetrahedron Lett*. **1990**, *31*, 1509.

[15] M. Schlosser, B. Schaub, *Chimia* **1982**, *10*, 396.

[16] P. Ermert, A. Vasella, M. Weber, K. Rupitz, S. G. Withers, *Carbohydr. Res.* **1993**, *250*, 113.

[17] W. J. White, K. J. Schray, G. Legler, J. A. Alhadeff, *Biochim. Biophys. Acta* **1987**, *812*, 132.

[18] R. Hoos, A. Vasella, K. Rupitz, S. G. Withers, *Carbohydr. Res.* **1997**, *298*, 291.

Received March 5, 1999