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D-Glyconhydroximolactams strongly inhibit α-glycosidases

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Abstract

It has been postulated that proton transfer to β-glycosides by some retaining β-glycosidases takes place in the plane of the pyranoside ring. It is now hypothesised that a similarly oriented catalytically active acidic group in α-glycosidases could interact with glyconolactone derivatives, provided that these are sufficiently basic to overcome the effect of a less favourable geometry by an energetically more favourable interaction. In keeping with this hypothesis, D-gluconolactone, D-gluconolactam, the tetrazole **3**, and the hydroximolactone **5** are weak inhibitors of yeast α-glucosidase, while the hydroximolactam **6** ($pK_a = 4.8$) is a mixed-type ($\alpha = 2$) strong inhibitor ($K_i = 2.9$ μM). A similar inhibition is observed for the arylcarbamoyl derivative **9**, while the (methylthio)methyl derivative **10** inhibits more weakly and in a purely competitive fashion. The mannonhydroximolactam **11** strongly inhibits jack bean α-mannosidase ($K_i = 0.15$ μM), while the *gluco* analogue **6** inhibits about 80 times more weakly, illustrating the dependence upon configuration.

Keywords: Yeast α-glucosidase; Jack bean α-mannosidase; D-Gluconhydroximolactam; Glucosidase inhibitors

1. Introduction

D-Gluconolactone (**1**), D-gluconolactam (**2**), and nojiritetrazole (**3**) are moderately strong, neutral inhibitors of β-glucosidases (Table 1) [1–3]. They are thought to mimic the transition-state of glycoside hydrolysis [4–6], as they possess a trigonal anomeric centre and adopt a more (**1**) or less (**2** and **3**) distorted $^4H_3$ conformation both in solution (D_2O, CD_3OD, $CDCl_3$) [3,7,8] and in the solid phase [3,8,9]. The triazole **4**, however, while possessing the same shape as **3**, is a very weak inhibitor of β-glucosidases [10]. The tetrazole **3** and the triazole **4** differ significantly only by the presence, or absence, of an anomeric

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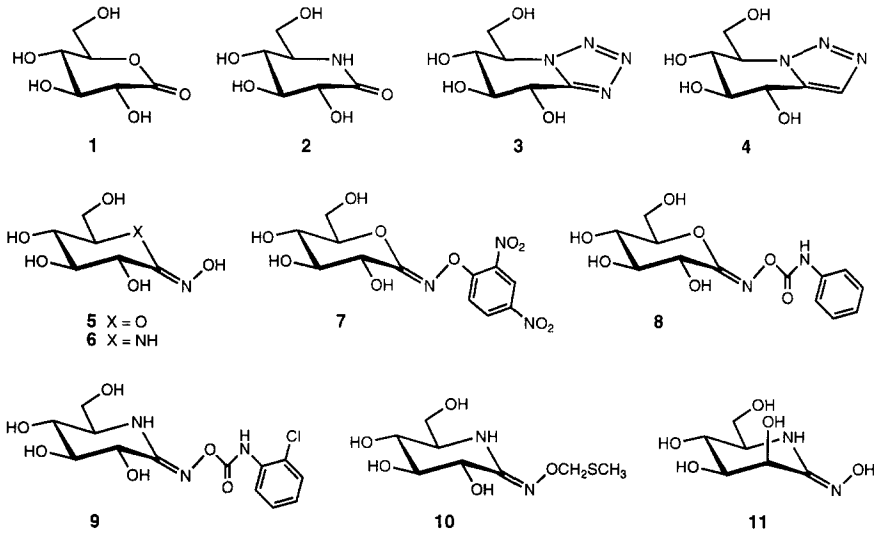
heteroatom. It has therefore been postulated that formation of a hydrogen bond to this heteroatom is required for a strong inhibition of β-glucosidases [10] and that the catalytically active acidic residue, commonly found in the active site of glycoside hydrolases [6], acts as the hydrogen bond donor.



The energies and orientation of the doubly occupied non-bonding orbitals of the anomeric heteroatom of **1–3** are only compatible with proton transfer in the ring plane. The hypothesis of a protonation in the ring plane by at least some retaining β-glycosidases is in agreement with X-ray data of three retaining exo-β-glycosidases [10–13] and with the crystal structure of the complex between the *Acidothermus cellulolyticus* endocellulase E1 and cellotetraose [14].

A priori, the lone pair of the anomeric heteroatom of **1–3** could also interact with the catalytic acid of an α-glycosidase, if this α-glycosidase protonates its substrate from the same direction as the above mentioned β-glycosidases; **1–3** might then also be strong inhibitors of at least some α-glycosidases. X-ray data of porcine pancreatic α-amylase complexed with acarbose indeed suggests that the orientation of the catalytically active acidic residue allows a protonation parallel to the cyclohexene plane [15]. However, rabbit intestine sucrase is only weakly inhibited by **1–2** [16], and yeast α-glucosidase is only weakly inhibited by **3** (Table 1) [17]. As suggested by Sinnott some years ago [18], the catalytically active acidic residue of β- but not α-glucosidases can probably form a hydrogen bond to the anomeric heteroatom of **1–3**.

We wondered if, for more strongly basic analogues of **1–3**, the effect of an unfavourable geometry for a hydrogen bond from the catalytically active acidic residue of an α-glycosidase to a neutral inhibitor may be overcome by the effect of a more energetically favourable ionic interaction. That the selectivity of the inhibition of α- versus β-glycosidases by hydroximolactone derivatives may depend on the substituent of the hydroximo group has already been shown. Thus, the 2,4-dinitrophenyl ether **7** of D-gluconhydroximolactone (**5**) is a strong competitive inhibitor of yeast α-glucosidase [1] and a moderate inhibitor of almond β-glucosidase [26], while the phenylcarbamoyl derivative **8** of **5** inhibits almond β-glucosidase better than yeast α-glucosidase (Table 1) [7,26].

To evaluate the effect of the basicity of glyconolactone analogues, we tested the lactone **1**, the lactam **2**, the tetrazole **3**, the hydroximolactone **5**, and the hydroximolactam **6** as inhibitors of yeast α-glucosidase. We were particularly interested in the inhibition by **6** [27,28], for which a pK_a of 4.8 has been reported and which is a good inhibitor of several β-glucosidases. We also wondered if the two factors basicity and substitution reinforce each other and thus tested the 2-chlorophenyl carbamate **9** [27], and the (methylthio)methyl ether **10**. Finally, to evaluate the influence of the configuration at C-2 [17,28], we

[1] For other strong α-glucosidase inhibitors, see Refs. [19–25].

Table 1
Inhibition constants K_i (μM) of compounds **1**–**11**, and D-glucose

Compound	β-Glucosidase (Almonds, pH 6.8)	β-Glucosidase (*A. faecalis*, pH 7.0)	β-Glucosidase (*C. saccharolyticum*, pH 6.8)	α-Glucosidase (Brewer's yeast, pH 6.8)	α-Mannosidase (Jack beans, pH 4.5)	Refs.
1	$0.4 \cdot 10^3$	1.4		$2 \cdot 10^3$, $\alpha = 1.3$ [b]		[31,40]
2	125	5.2		$1.1 \cdot 10^3$		[27]
3	150	1.4		$1.3\text{–}5.6 \cdot 10^3$	$\approx 8.5 \cdot 10^3$	[17,41]
4	$> 8 \cdot 10^3$		$2 \cdot 10^3$ [d]			[10]
5	$4.3 \cdot 10^3$	30		$6.8 \cdot 10^3$, $\alpha = 1.3$ [b]		[31,40]
6	16	0.6	3.3	2.9, $\alpha = 2.0$ [b]	12	[27]
7	80 [a]			5 [a]		[26]
8	43	1.4		75 [a]		[26,27,40]
9	8	0.15		3.4, $\alpha = 2.0$ [b]		[27]
10	24		2.4	50		[27]
11					0.15	
D-Glc α/β 1:2 [c]				$6.9 \cdot 10^3$		

[a] pH not given.
[b] Linear mixed-type inhibition.
[c] Ratio determined by polarography.
[d] IC_{50}.

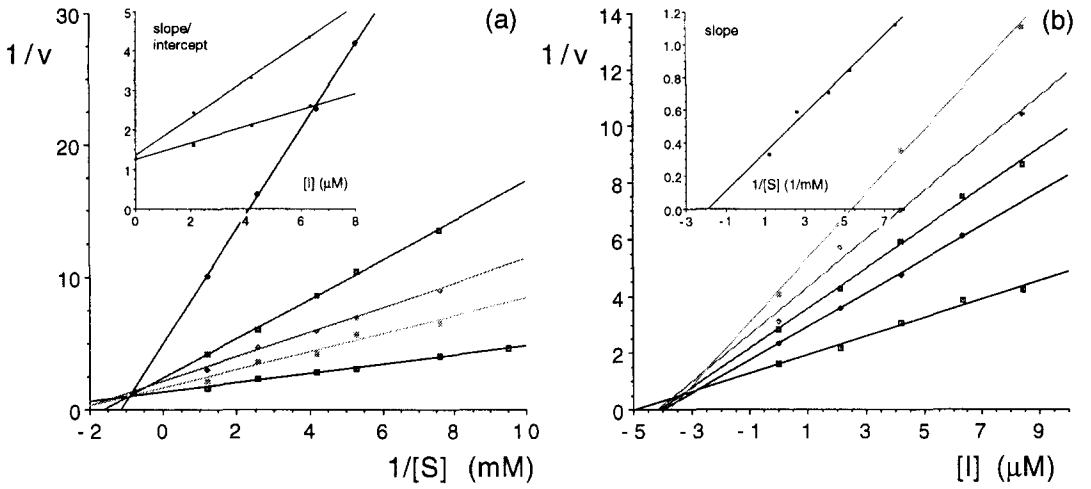


Fig. 1. Inhibition of yeast α-glucosidase by **6**. (a) Lineweaver–Burk plot, $slope_{1/[S]}$ and $1/v$-axis replots; (b) Dixon plot and Dixon-slope replot.

Table 2
^{1}H NMR spectroscopic data of **6** in the absence and presence of CF_3CO_2H (300 MHz, CD_3OD)

Compound	Chemical shift (ppm)						Coupling constants (Hz)						
	H-2	H-3	H-4	H-5	H-6	H′-6	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$	$J_{2,5}$
6	4.04	3.40	3.57	3.18	3.49	3.96	8.4	8.5	8.5	7.4	2.9	11.0	
6 · H^+	4.33	3.67	3.80	3.45	3.79	3.96	9.4	9.3	7.9	3.8	3.8	11.6	0.7 [a]

[a] Assignment was confirmed by homonuclear irradiation.

tested the hydroximolactam **6** and its 2-epimer **11** [29] as inhibitors of jack bean α-mannosidase.

2. Results and discussion

The lactone **1** and the lactam **2** bind only weakly to yeast α-glucosidase, similarly to the tetrazole **3** (Table 1) [17]. While the lactam binds competitively ($K_i = 1.1$ mM), the lactone binds mostly non-competitively ($K_i = 2$ mM, $\alpha = 1.3$) [2]. Possibly, the NH function of **2** leads to an additional interaction with the enzyme.

Similarly to the lactone **1**, the hydroximolactone **5**, a weak inhibitor of almond β-glucosidase ($K_i = 4.3$ mM at pH 6.8 [31], $K_i = 0.1$ mM at pH 4.5 [7]), inhibits yeast α-glucosidase non-competitively ($K_i = 6.8$ mM, $\alpha = 1.3$). However, D-gluconhydroximolactam (**6**) [27,28] strongly inhibits yeast α-glucosidase ($K_i = 2.9$ μM, Fig. 1). The Lineweaver–Burk plots [32], the $slope_{1/[S]}$ and $1/v$-axis intercept replots, and the Dixon-slope replot [30] show a linear mixed-type inhibition ($\alpha = 2$) for both compounds. At high inhibitor concentrations ($[I] \approx 10\ K_i$), the steady state velocity was approached after 20–30 s. At lower concentrations there was no indication of hysteresis [16,33,34].

The interaction of the catalytically active residues of yeast α-glucosidase with **6** ($pK_a = 4.8$ [27,28]) may lead to a proton transfer and an ionic interaction relaxing the geometrical constraints of a hydrogen bond, provided, evidently, that the pK_a of (protonated) **6** and of the protonating acid in the active site are sufficiently close to each other. As estimated from the high-pH branch of the pH-activity curve, the pK_a of the catalytically active acidic residue of yeast α-glucosidase is either 6.6 or 7.1 [35]. This pK_a is too high to allow a full proton transfer, unless the pK_a of the hydroximolactam bound to the enzyme is significantly higher than in water. However, a basic inhibitor will at least be a better hydrogen bond acceptor than a neutral inhibitor, and part of the energy associated with the stronger hydrogen bond might serve to induce a conformational change of the inhibitor in the active site. Protonation of **6** leads to a change of the conformation from $^4C_1$ to $^4H_3$, as

[2] For a competitive inhibition, $\alpha \to \infty$, and for a non-competitive inhibition, $\alpha = 1$ [30].

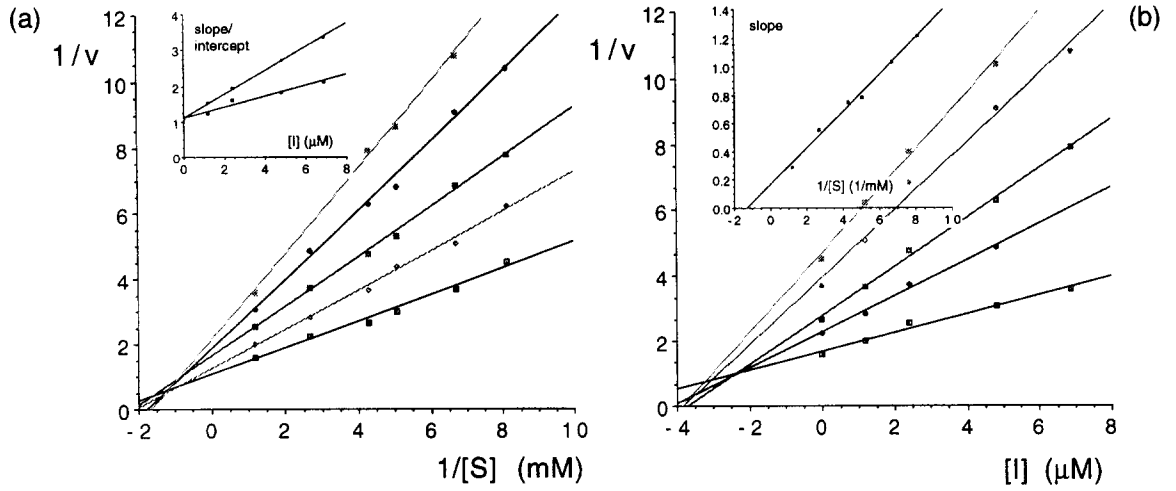
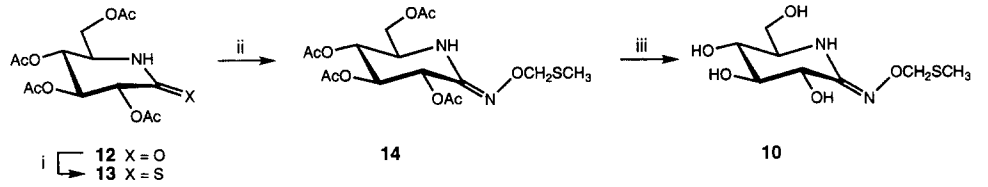


Fig. 2. Inhibition of yeast α-glucosidase by **9**. (a) Lineweaver–Burk plot, $slope_{1/[S]}$ and $1/v$-axis replots; (b) Dixon plot and Dixon-slope replot.

evidenced by the ^{15}N NMR spectra of **6** in water [27] and by the ^{1}H NMR spectra of **6** in CD_3OD in the absence and presence of acid, particularly by a homo-allylic coupling $J_{2,5} = 0.7$ that appears upon addition of CF_3CO_2H to a solution of **5** in CD_3OD (Table 2).

A very similar inhibition to that seen for **6** was observed for the 2-chlorophenyl carbamate **9** [27] (K_i = 3.4 μM, α = 2, Fig. 2). The (methylthio)methyl ether **10** however, obtained by standard procedures from the known tetra-*O*-acetylated D-gluconolactam **12** [36] (Scheme 1), binds to yeast α-glucosidase in a purely competitive mode (Fig. 3). Its inhibition constant is relatively high (K_i = 50 μM) and this is perhaps correlated in a non-trivial way to its mode of inhibition; i.e. to the



Scheme 1. (i) Lawesson's reagent, C_6H_6, reflux, 30 min, 80%; (ii) $H_2NOCH_2SCH_3L$, MeOH, 50 °C, 24 h; Ac_2O, pyridine, room temperature, 3 h, 80%; (iii) NaOMe, MeOH, room temperature, 3 h, 95%.

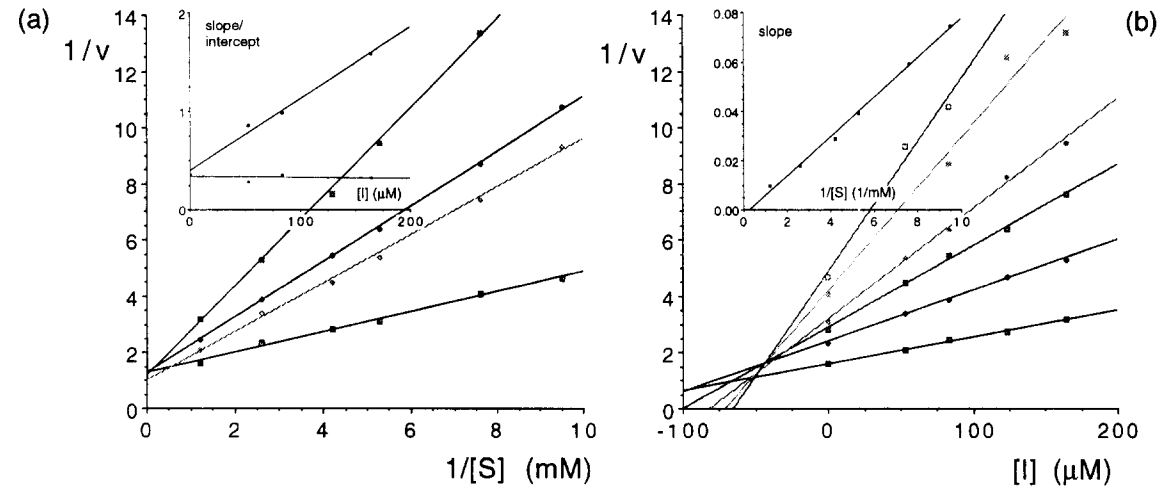


Fig. 3. Inhibition of yeast α-glucosidase by **10**. (a) Lineweaver–Burk plot, $slope_{1/[S]}$ and $1/v$-axis replots; (b) Dixon plot and Dixon-slope replot.

fact that it binds at only one site of the enzyme. The (methylthio)methyl ether **10** also inhibits the almond and the *C. saccharolyticum* β-glucosidases (K_i = 24 μM and 2.4 μM). About the same inhibition of these enzymes is observed for D-gluconhydroximolactam (**6**).

As expected, D-mannonhydroximolactam (**11**), obtained by treatment of its pentaacetate [27] with NH_3 in MeOH, proved a strong inhibitor of jack bean α-mannosidase (K_i = 0.15 μM; compare [29,37,38]). As the *gluco* hydroximolactam **6** inhibits this enzyme about eighty times more weakly (K_i = 12 μM), glyconhydroximolactams are an example of basic, configurationally selective, transition-state analogue inhibitors of glycosidases.

3. Experimental

General methods.—Moisture sensitive reactions were run under Ar or N_2 in dry, distilled solvents. TLC: Silica Gel 60F-254 plates (E. Merck); detection by heating with I_2 soln/20% H_2SO_4 1:1 (I_2 soln: 10 g of I_2, 100 g of KI, 1000 mL of H_2O), with vanillin soln (5%) in H_2SO_4, or with 'mostain' (400 mL 10% aq H_2SO_4, 20 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.6 g $Ce(SO_4)_2$). Flash chromatography (FC): Silica Gel 60 (Fluka or E. Merck; 0.040–0.063 mm). Mp's are uncorrected. NMR spectra were recorded with Me_4Si (^1H and ^{13}C) as external reference, and with HDO (^1H in D_2O and CD_3OD) as internal reference. Chemical shifts δ in ppm and coupling constants *J* in Hz.

*2,3,4,6-Tetra-*O*-acetyl-5-amino-5-deoxy-*D*-glucon-1,5-thionolactam* (**13**).—A soln of **12** [36] (2.19 g, 6.34 mmol) in C_6H_6 (40 mL) was treated with Lawesson's reagent (1.60 g, 3.96 mmol), heated to reflux for 20 min, and evaporated. FC of the residue and crystallization from Et_2O gave **13** (1.83 g, 80%); mp 132.0–133.5 °C. R_f 0.51 (1:3 hexane–EtOAc); $[\alpha]_D^{25}$ 96.8 (*c* 0.934, $CHCl_3$); ν_{max}: 3362 (NH), 1758 (CO), 1511 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.06 (s, 3 H, Ac), 2.10 (s, 3 H, Ac), 2.15 (s, 3 H, Ac), 2.17 (s, 3 H, Ac), 3.80–3.85 (m, 1 H, H-5), 4.05 (dd, 1 H, $J_{6,6'}$ 12.1, $J_{5,6}$ 6.5, H-6), 4.34 (dd, 1 H, $J_{6,6'}$ 12.1, $J_{5,6'}$ 3.1 H'-6), 5.28 (t, 1 H, $J_{3,4} = J_{4,5}$ 8.6, H-4), 5.45 (t, 1 H, $J_{2,3} = J_{3,4}$ 8.6, H-3), 5.50 (d, 1 H, $J_{2,3}$ 8.4, H-2), 8.09 (broad s, 1 H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.59 (q), 20.63 (q), 20.76 (q), 20.92 (q), 56.88 (d), 62.38 (t), 67.77 (d), 70.44 (d), 74.32 (d), 169.80 (2 s), 170.11 (s), 170.84 (s), 197.84 (s); FABMS (3-NOBA): *m/z* 363 (25), 362 (100, $[M + 1]^+$), 361 (20), 199 (34), 140 (61). Anal. Calcd for $C_{14}H_{19}NO_8S$: C, 46.53; H, 5.30; N, 3.88; S, 8.87. Found: C, 46.39; H, 5.13; N, 3.89; S, 8.88.

*2,3,4,6-Tetra-*O*-acetyl-5-amino-5-deoxy-1-*N*-[(methylthio)methyloxy]-*D*-gluconimido-1,5-lactam* (**14**).—A soln of **13** (72 mg, 0.199 mmol) in MeOH (4 mL) was treated with *O*-[(methylthio)methyl]-hydroxylamine (186 mg, 2.0 mmol) [39], heated to 50 °C for 12 h, and evaporated. The residue was dissolved in a 2:1 mixture of pyridine and Ac_2O (5 mL), stirred for 3 h, and evaporated. FC of the residue gave **14** (67 mg, 80%) as a yellow oil; R_f 0.32 (1:1 hexane–EtOAc); $[\alpha]_D^{25}$ 76.0 (*c* 1.966, $CHCl_3$); ν_{max}: 3399 (NH), 1750 (CO), 1041 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 2.06 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.11 (s, 3 H, Ac), 2.12 (s, 3 H, Ac), 2.26 (s, 3 H, SCH_3), 3.70 (dddd, 1 H, $J_{4,5}$ 9.6, $J_{5,6}$ 6.2, $J_{5,6'}$ 2.9, $J_{5,NH}$ 1.7, H-5), 4.04 (dd, 1 H, $J_{6,6'} \approx 12.0$, $J_{5,6}$ 6.2, H-6), 4.30 (dd, 1 H, $J_{6,6'}$ 11.6, $J_{5,6'}$ 2.9, H'-6), 5.02 (dd, 1 H, $J_{4,5}$ 9.6, $J_{3,4}$ 6.2, H-4), 5.08 (s, 2 H, SCH_2), 5.25 (t, 1 H, $J_{2,3} = J_{3,4} \approx 6.0$, H-3), 5.41 (d, 1 H, $J_{2,3}$ 5.8, H-2), 5.45 (broad s, 1 H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.85 (q), 20.68 (2 q), 20.79 (2 q), 52.17 (d), 62.95 (t), 67.95 (d), 70.08 (d), 72.22 (d), 78.59 (t), 147.49 (s), 169.24 (s), 169.64 (s), 169.80 (s), 171.15 (s); FABMS (3-NOBA): *m/z* 841 (15, $[2M + 1]^+$), 422 (30), 421 (100, $[M + 1]^+$), 420 (31). Anal. Calcd for $C_{16}H_{24}N_2O_9S$: C, 45.71; H, 5.75; N, 6.66; S, 7.63. Found: C, 45.64; H, 5.77; N, 6.52; S, 7.61.

*5-Amino-5-deoxy-1-*N*-[(methylthio)methyloxy]-*D*-gluconimido-1,5-lactam* (**10**).—A soln of **14** (61 mg, 0.145 mmol) in MeOH (2 mL) was treated with a 0.2 M soln of NaOMe in MeOH (0.1 mL), stirred for 1 h, filtered through SiO_2, and evaporated. The residue (38 mg) was purified by FC (LiChroprep® RP-18, 40–63 μm) to yield **10** (34 mg, 94%); mp 115–116 °C (MeOH/EtOAc). R_f 0.36 (7:2:1 EtOAc–MeOH–H_2O); ν_{max}: 2915, 1634 (N=C), 1035 cm^{-1}; ^1H NMR (200 MHz, CD_3OD): *d* 2.20 (s, 3 H, SCH_3), 3.14 (ddd, 1 H, $J_{4,5}$ 9.1, $J_{5,6}$ 7.5, $J_{5,6'}$ 2.9, H-5), 3.37 (t, 1 H, $J_{4,5} = J_{3,4}$ 8.7, H-4), 3.49 (dd, 1 H, $J_{6,6'}$ 11.2, $J_{5,6}$ 7.5, H-6), 3.60 (t, 1 H, $J_{3,4} = J_{2,3}$ 8.3, H-3), 3.90 (dd, 1 H, $J_{6,6'} = 11.2$, $J_{5,6'}$ 2.9, H'-6), 4.02 (d, $J_{2,3}$ 8.3, H-2), 5.04 (s, 2 H, SCH_2); ^{13}C NMR (75 MHz, CD_3OD): δ 13.55 (q), 57.32 (d), 62.59 (t), 69.54 (d), 70.11 (d), 75.89 (d), 77.38 (t), 154.04 (s); FABMS (3-NOBA): *m/z* 254 (20), 253 (100, $[M + 1]^+$), 252 (38). Anal. Calcd for $C_8H_{16}N_2O_5S \cdot 0.25\ H_2O$: C, 37.41; H, 6.47; N, 10.91. Found: C, 37.32; H, 6.39; N, 10.72.

*5-Amino-5-deoxy-*D*-mannonhydroximo-1,5-lactam* (**11**).—*N*,2,3,4,6-Penta-*O*-acetyl-5-amino-5-deoxy-

D-mannonhydroximo-1,5-lactam [27] (93 mg, 0.231 mmol) was dissolved in a sat soln of NH_3 in MeOH (10 mL), stirred for 2 h, and evaporated. Lyophilisation of the residue and reversed-phase HPLC ($MeCN/H_2O$ 1:20) gave **11** (40 mg, 90%); R_f 0.35 (4:2:1 EtOAc–MeOH–H_2O). ^1H NMR (300 MHz, D_2O): δ 3.14 (ddd, 1 H, $J_{4,5}$ 8.7, $J_{5,6}$ 5.5, $J_{5,6'}$ 3.0, H-5), 3.66 (dd, 1 H, $J_{6,6'}$ 11.8, $J_{5,6}$ 5.5, H-6), 3.74 (dd, 1 H, $J_{3,4}$ 9.8, $J_{2,3}$ 3.5, H-3), 3.82 (dd, 1 H, $J_{6,6'}$ 11.8, $J_{5,6'}$ 3.0, H′-6), 3.88 (t, 1 H, $J_{3,4} = J_{4,5} \approx 8.8$, H-4), 4.21 (d, 1 H, $J_{2,3}$ 3.5, H-2); ^{13}C NMR (125 MHz, D_2O): δ 57.86 (d), 61.75 (t), 66.01 (d), 67.07 (d), 71.77 (d), 154.30 (s); FABMS (3-NOBA): m/z 193 (25, $[M + H]^+$), 155 (48), 154 (100).

Enzyme kinetics.—Determinations of the inhibition constants (K_i) were performed in the presence of a range of inhibitor concentrations (typically 4–6 concentrations) which bracket the K_i value.

(a) Inhibition of almond β-glucosidase. Inhibition constants (K_i) were determined at 37 °C using a 0.08 M KH_2PO_4/K_2HPO_4 buffer (pH 6.8), and 4-nitrophenyl β-D-glucopyranoside (Fluka) as substrate. Measurements were started by addition of almond β-glucosidase (Emulsin, Sigma). Enzyme activity was ca. 0.04 U/mL. The increase of absorption per minute at 400 nm was taken as velocity for the hydrolysis of the substrate. This increase was linear during all measurements (1–3 min). The K_m value of the substrate, 4-nitrophenyl β-D-glucopyranoside, was determined by means of the Hanes–Woolf plot [30] to be 3.8 mM. The following substrate concentrations were applied: 25.56, 9.96, 5.53, 3.32, 2.49, and 1.54 mM. Inhibition constants (K_i) were determined by taking the slopes from the Lineweaver–Burk plots [32] and plotting them against five inhibitor concentrations [30]. After fitting the data to a straight line, the negative [I]-intercept of this plot gave the appropriate K_i.

(b) Inhibition of *C. saccharolyticum* β-glucosidase. See (a). The inhibition constants were determined at 55 °C. K_m (4-nitrophenyl β-D-glucopyranoside) = 1.2 mM. Substrate concentrations: 5.07, 1.46, 0.79, 0.49, 0.30, 0.25 mM.

(c) Inhibition of yeast α-glucosidase (Brewer's yeast, Sigma). See (a). Inhibition constants (K_i) were determined using a 0.025 M KH_2PO_4/K_2HPO_4/NaCl buffer (pH 6.8), and 4-nitrophenyl α-D-glucopyranoside (Fluka) as substrate. Measurements were started by addition of the substrate. K_m (4-nitrophenyl α-D-glucopyranoside) = 0.33–1.3 mM. Substrate concentrations: 0.83, 0.39, 0.24, 0.19, 0.13, and 0.11 mM.

(d) Inhibition of jack bean α-mannosidase. See (a). The assay was performed at 25 °C as a stopped assay by incubation of the enzyme–substrate mixture (45 mM sodium citrate, 0.1 mM Zn^{2+}, pH 4.5, 0.1 mL) for a 5 min period (less than 10% substrate depletion), then stopping the reaction by addition of 200 mM sodium borate buffer (pH 9.8, 0.8 mL) and measuring the absorbance at 400 nm. K_m (4-nitrophenyl α-D-mannopyranoside) = 1.4 mM. Substrate concentrations: 5.80, 3.87, 1.93, 0.97, 0.64, 0.48, 0.32 mM. Data were analyzed by non-linear regression using the programme GraFit (Leatherbarrow, 1990).

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