Probing the Conformational Changes in the Enzymatic Hydrolysis of 2-Acetamido-2-deoxy-β-D-glucopyranosides

by **Matthias Böhm** and **Andrea Vasella***

Laboratorium für Organische Chemie, ETH-Hönggerberg, Wolfgang-Pauli Strasse 10, CH-8093 Zürich

Dedicated to Prof. *K. K. Balasubramaniam* (*K.K.B.*) on the occasion of his 65th birthday

The isoquinuclidines **7** and **8** were synthesised and tested as inhibitors of hexosaminidases from jack beans and from bovine kidney. These isoquinuclidines mimick the $^{1,4}B$-conformer of a *N*-acetyl-glucosamine-derived β-D-glucopyranoside; they are competitive inhibitors with K_i values from 0.014 to 0.30 μM. The strong inhibition of these enzymes agrees with the hypothesis that the enzymatic hydrolysis of 2-acetamido-2-deoxy-β-D-glucopyranosides proceeds *via* a boat-like conformer with a *pseudo*-axial scissile glycosidic bond and a *pseudo*-axial acetamido substituent optimally oriented to effect an intramolecular substitution of the aglycon.

Introduction. – According to the principles of stereoelectronic control, the enzymatic hydrolysis of β-D-glycopyranosides[1]) requires coplanar orientation of the scissile bond and a non-bonding, doubly occupied orbital of the endocyclic *O*-atom [3][4]. This implies that the reaction coordinate is characterised by an initial conformational change of the substrate. Several conformations of the substrate satisfy the principles of stereoelectronic control. It has been proposed, on the basis of crystallographic evidence, that all of them are induced by individual glycosidases [1]. Thus, a $^{1,4}B$- or $^{1}S_3$-conformation of the -1 unit has been deduced from the crystal structure of cellulases belonging to families[2]) 5 [6] and 7 [7] in complexes with substrate analogues and, similarly, for hexosaminidases belonging to families 18 [8][9] and 20 [10][11] in complexes with substrates.

We have synthesised the isoquinuclidines **1**–**6** (*Fig.*) and tested them as glycosidase inhibitors to evidence the conformational changes induced on their substrate by β-mannosidases and β-glucosidases [12–14]. These isoquinuclidines mimick the $^{1,4}B$-conformer, of a β-D-manno- and β-D-glucopyranoside, and possess a basic N-atom that can interact with the catalytic acid of the glycosidase. A comparison of the inhibition of snail β-mannosidase by the D-*manno*-isoquinuclidines **1** and **2**, and of the β-glucosidase from *Caldocellum saccharolyticum* (family 1) by the D-*gluco*-configured isoquinuclidines **3** and **4** evidenced that the β-mannosidase induces a conformational change of its substrate towards a $^{1,4}B$-conformer, while the β-glucosidase induces a different conformational change [14]. A further comparison of the inhibition of these

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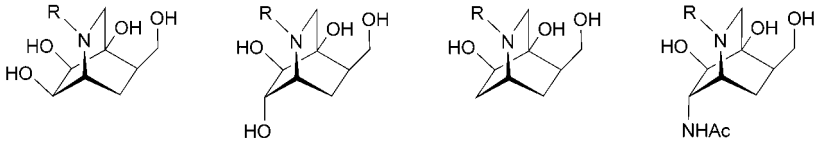
[1]) For recent review articles on the mechanism of glycoside hydrolysis, see [1][2].

[2]) Glycosidases have been classified according to their amino acid sequence [5]; enzymes belonging to the same family should have a similar tertiary structure and hydrolyse their substrates according to a similar mechanisms. A regularly updated database is available on the internet (http://afmb.cnrs-mrs.fr/CAZY/index.html).

glycosidases by D-*gluco*- and D-*manno*-configured tetrahydropyridoimidazoles, *i.e.*, inhibitors mimicking the reactive intermediates, suggests that there is no similar difference between the transition states for glycoside hydrolysis by these enzymes [15]. These observations imply that inhibitors mimicking an early part of the reaction coordinate have a particularly high chance of being selective.



1 R = Bn **2** R = H **3** R = Bn **4** R = H **5** R = Bn **6** R = H **7** R = Bn **8** R = H

Figure. *The isoquinuclidines* **1** *and* **2** *mimicking the* ^{1,4}B-*conformer of* β-D-*mannopyranosides, the isoquinuclidines* **3**–**6** *mimicking* β-D-*gluco- and 2-deoxy-*β-D-*arabinohexopyranosides, and the acetamido-isoquinuclidines* **7** *and* **8** *mimicking 2-acetamido-2-deoxy-*β-D-*glucopyranosides*

The above-mentioned crystal structures of hexosaminidases of families 18 and 20 suggest that the isoquinuclidine-derived mimicks **7** and **8** of a $^{1,4}B$-conformer of a 2-acetamido-2-deoxy-β-D-hexopyranoside should be good inhibitors of these glycosidases. We report the synthesis and inhibitory activity of **7** and **8**.

Synthesis. – Treatment of the stable trifluoromethanesulfonate **9** [12–14] with NaN_3 yielded 96% of the azide **10** (*Scheme*). Reduction of **10** with $LiAlH_4$, followed by acetylation of the crude amino alcohol **12**, gave the acetamido-isoquinuclidine **13** (96%). Acetal cleavage with Me_3SiBr yielded 76% of the tertiary alcohol **14** that was deacetylated to the *N*-benzylated isoquinuclidine **7** (85%). Hydrogenolysis of the benzyl derivative **7** followed by ion-exchange chromatography yielded 93% of the *N*-unsubstituted isoquinuclidine **8** that was, thus, obtained in six steps and 55% yield from the trifluoromethanesulfonate **9**. To exclude a contamination of **8** by the *N*-benzylated precursor **7** [12][13] or by Pd[3]), we converted the isoquinuclidine **8** to the carbamate **15**, which was chromatographed on silica gel and deprotected with HCl in $MeOH/H_2O$ to again provide **8**.

The *gluco*-configuration of the azide **10** was deduced by comparison of its NMR spectra with those of its *manno*-configured precursor **9** and with those of the D-*gluco*-configured acetate **11** [14][4]). The ^{1}H-NMR spectra of the *manno*-configured isoquinuclidine **9** and the *gluco*-configured isoquinuclidines **10** and **11** show characteristic differences (see *Table 2* in the *Exper. Part* and [12–14]); *e.g.*, the coupling constant $J(1,7) = 1.7$ Hz of **9** *vs.* 4.0 of **10** and 4.4 Hz of **11**. All the *gluco*-configured isoquinuclidines **10**–**14**, **7**, and **8** show long-range W coupling ($J(6_{exo},7) = 1.9$ Hz for **10** and **11**, and 2.0–2.3 Hz for **13**, **14**, **7**, and **8**) that is not visible in the ^{1}H-NMR spectra of *manno*-configured isoquinuclidines.

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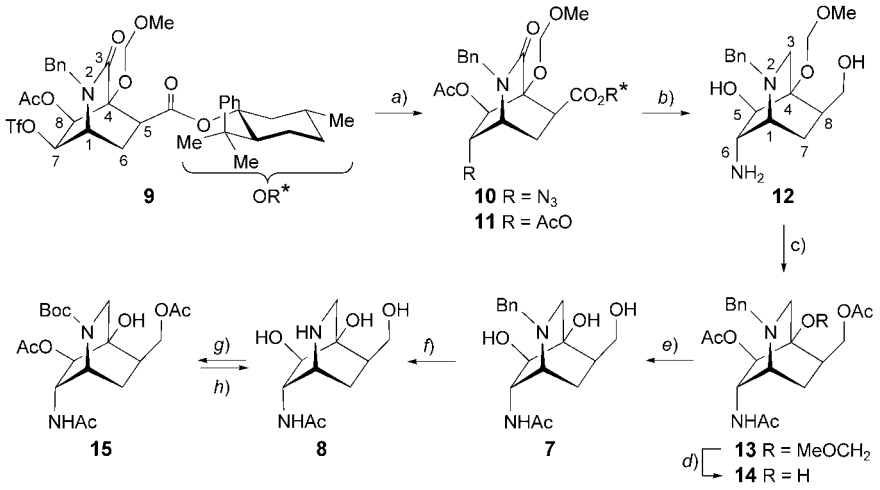
[3]) Pd and its complexes inhibit glycosidases; for examples, see [16][17].

[4]) The D-*gluco*-configured acetate **11** was obtained by treatment of **9** with CsOAc. Its structure was established by X-ray crystal-structure analysis [14].

Scheme



a) NaN_3, DMF; 96%. *b*) $LiAlH_4$, THF. *c*) 4-(Dimethylamino)pyridine (DMAP), pyridine/Ac_2O; 96% from **10**. *d*) Me_3SiBr, mol. sieves 3-Å, CH_2Cl_2; 76%. *e*) NH_3, MeOH; 85%. *f*) $Pd(OH)_2$/C, H_2, MeOH/H_2O/HCl; 93%. *g*) 1. $(t\text{-BuO})_2C(O)$, MeOH/Et_3N. 2. pyridine/Ac_2O; 75%. *h*) HCl, MeOH/H_2O; 90%.

Inhibition Studies. – The acetamido-isoquinuclidines **7** and **8**, the D-*gluco*-isoquinuclidine **4**, and the 2-deoxy-D-*arabino*-isoquinuclidine **6** are competitive inhibitors of the *N*-acetyl-β-hexosaminidases from jack beans and from bovine kidney (*Table 1*)[5]). The same inhibition constants were obtained for samples of the *N*-unsubstituted isoquinuclidine **8** prepared by hydrogenolysis of the *N*-benzylated isoquinuclidine **7** and for samples prepared by deacylation of the carbamate **15**. A comparison of the inhibition of these enzymes by the isoquinuclidines **7** and **8** that takes both their pK_{HA} values and the pH of the enzyme assays into account evidences that the glycosidases are inhibited by the free amines **7** and **8** rather than by the corresponding ammonium salts $\mathbf{1}\cdot H^+$ and $\mathbf{2}\cdot H^+$.

Table 1. *Inhibition Constants* K_i [μM] *for the Inhibition of the* N-*Acetyl-β-hexosaminidases from Jack Beans and from Bovine Kidney by the Isoquinuclidines* **4**, **6**, **7**, *and* **8**

Inhibitor	K_{HA}	*N*-Acetyl-β-hexosaminidase from jack beans (pH 5.0)	*N*-Acetyl-β-hexosaminidase from bovine kidney (pH 4.1)
4	7.8	11	210
6	–	25	310
7	5.9	0.081	0.30
8	7.7	0.014	0.067

[5]) The *N*-acetyl-β-hexosaminidase from jack beans belongs to the glycosyl hydrolase family 18 [18]. Its 3D structure was determined by X-ray crystal-structure analysis [19]. The amino acid sequence and the 3D structure of the *N*-acetyl-β-hexosaminidase from bovine kidney are not known.

The stronger inhibition of the hexosaminidases by the *N*-unsubstituted isoquinuclidine **8** than by the *N*-benzylated isoquinuclidine **7** evidences that the inhibition is due to specific interactions between these enzymes and the isoquinuclidines and not to unspecific hydrophobic interactions (with the *N*-Bn substituent)[6]). The relative strength of the inhibition of these *N*-acetyl-β-hexosaminidases by the acetamido-isoquinuclines **7** and **8**, the *gluco*-configured isoquinuclidine **4**, and the *arabino*-configured isoquinuclidine **6** confirms the strong interactions of these enzymes with the acetamido substituent[7]).

The inhibition of the *N*-acetyl-β-hexosaminidases from jack beans and from bovine kidney by the isoquinuclidines **4** and **6**–**8** evidences that the enzymatic hydrolysis of 2-acetamido-2-deoxy-β-D-glucopyranosides proceeds *via* a $^{1,4}B$- (or closely related) conformer with a *pseudo*-axial scissile glycosidic bond, and also with a *pseudo*-axial *C(2)*-acetamido subsitutent. The *pseudo*-axial orientation of the AcNH group is optimal for a nucleophilic attack at the anomeric center, displacing the aglycon, as suggested on the basis of crystal structures [8][10].

The agreement between the inhibition of the two hexosaminidases by **7** and **8** with the above-mentioned crystal-structure analyses lends additional weight to the significance of the strong difference between the inhibition of snail β-mannosidase by the *manno*-configured isoquinuclidines **1** and **2**, and the inhibition of the β-glucosidase from *C. saccharolyticum* by the *gluco*-configured isoquinuclidines **3** and **4**. While snail β-mannosidase and the hexosaminidases of families 18 and 20 appear to distort their substrates towards a $^{1,4}B$ (or related)-conformer, the β-glucosidase from *C. saccharolyticum* (and presumably all glucosidases of the same family of glycohydrolases) choose a different conformation to comply with the principle of stereoelectronic control.

We thank *D. Manser* and *M. Schneider* for the pK_{HA} determinations, Dr. *B. Bernet* for checking the *Exper. Part*, and the *Swiss National Science Foundation* and *Oxford Glycosciences Ltd.*, Abingdon (UK), for generous support.

Experimental Part

General. Solvents were distilled before use. If not specified otherwise, all reactions were carried under a N_2 atmosphere. 'Normal workup' implies pouring the reaction mixture into the indicated sat. aq. soln., extracting into the mentioned org. solvent, if necessary washing with the indicated sat. aq. soln., drying of the org. layer (Na_2SO_4), filtration, and evaporation of the volatiles. TLC: *Merck* silica gel *60F-254* plates; detection with I_2, by heating with Mostain (400 ml of 10% H_2SO_4 soln., 20 g of $(NH_4)_6Mo_7O_{24}\cdot 6\,H_2O$, 0.4 g of $Ce(SO_4)_2$) or with $KMnO_4$ soln. (3 g of $KMnO_4$, 20 g of K_2CO_3, 0.25 ml of AcOH in 300 ml of H_2O). Flash chromatography (FC): silica gel *Fluka 60* (0.04–0.063 mm). IR Spectra: 0.5% KBr suspension or 3% $CHCl_3$ soln. ^{1}H- (300 MHz) and ^{13}C-NMR (75 MHz spectra): chemical shifts δ in ppm and coupling constants J in Hz.

*(1*R*,2*S*,5*R*)-5-Methyl-2-(1-methyl-1-phenylethyl)cyclohexyl (1*R*,4*S*,5*S*,7*S*,8*R*)-8-Acetoxy-7-azido-2-benzyl-4-(methoxymethoxy)-3-oxo-2-azabicyclo[2.2.2]octane-5-carboxylate* (**10**). A soln. of **9** (700 mg, 0.946 mmol) and NaN_3 (92 mg, 1.4 mmol) in DMF (12 ml) was stirred at 80° for 3 h. Normal workup (AcOEt/H_2O) and FC (cyclohexane/AcOEt 3:1) gave **10** (576 mg, 96%). Colourless foam. R_f (cyclohexane/AcOEt 3:2) 0.56. M.p.:

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6) Most glycosidases possess a hydrophobic platform in the active site. Interactions with this hydrophobic site may lead to unspecific binding of hydrophobic molecules [20].

7) Unlike most other retaining glycosidases, the *N*-acetyl-β-hexosaminidases belonging to families 18 and 20 lack a catalytic nucleophile; the C(2)-acetamido substituent of the substrate acts instead as intramolecular catalytic nucleophile. For a review see [21].

80–82° (Et_2O/pentane). $[\alpha]_D^{25} = +40.9$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 2966*m*, 2928*s*, 2852*m*, 2113*s*, 1729*s*, 1693*s*, 1600*w*, 1496*m*, 1452*m*, 1372*m*, 1327*m*, 1158*m*, 1130*m*, 1054*m*, 1023*m*, 976*m*, 957*m*, 925*w*, 852*w*. ^{1}H-NMR ($CDCl_3$): see *Table 2*; additionally, 7.37–7.19 (*m*, 9 arom. H); 7.15 (*tt*, $J = 6.5$, 2.5, 1 arom. H); 4.95, 4.62 (2*d*, $J \approx 7.7$, OCH_2O); 4.85, 4.34 (2*d*, $J \approx 14.8$, $PhCH_2$); 4.77 (irrad. at 3.34 → *d*, $J = 0.6$, H–C(8)); 4.75 (*td*, $J = 10.6$, 4.4, H–C(1′)); 3.31 (*s*, MeO); 2.11–2.01 (*m*, irrad. at 4.75 → change, H–C(2′)); 2.06 (*s*, AcO); 2.03–1.97 (*m*, H_{ax}–C(3′)); 1.93 (irrad. at 3.34 → *t*, $J = 11.6$, H_{endo}–C(6)); 1.82 (*dq*, $J \approx 13.4$, 3.7, H_{eq}–C(4′)); 1.70 (irrad. at 3.34 → *ddd*, $J = 12.5$, 5.7, 1.8, H_{exo}–C(6)); 1.69–1.62 (*m*, irrad. at 4.75 → change, H_{eq}–C(6′)); 1.52–1.35 (*m*, H–C(5′)); 1.23, 1.17 (2*s*, Me_2PhC); 1.14 (*qd*, $J \approx 13.2$, 3.4, H_{ax}–C(3′)); 0.90 (*qd*, $J \approx 13.1$, 3.4, H_{ax}–C(4′)); 0.88 (*q*, $J \approx 11.7$, irrad. at 4.75 → *m*, H_{ax}–C(6′)); 0.87 (*d*, $J = 6.5$, Me–C(5′)). ^{13}C-NMR ($CDCl_3$): 171.03, 169.87, 168.56 (3*s*, 3 C=O); 152.25, 136.21 (2*s*); 128.65 (2*d*); 128.56 (2*d*); 127.96 (*d*); 127.77 (2*d*); 125.07 (2*d*); 124.90 (*d*); 93.03 (*t*, OCH_2O); 78.07 (*d*, C(7)); 76.58 (*s*, C(4)); 75.59 (*d*, C(1′)); 65.29 (*d*, C(8)); 55.93 (*q*, MeO); 52.10 (*d*, C(1)); 50.32 (*d*, C(2′)); 48.10 (*t*, $PhCH_2$); 41.44 (*d*, C(5)); 41.36 (*t*, C(6′)); 39.33 (*s*, Me_2PhC); 34.50 (*t*, C(4′)); 31.15 (*d*, C(5′)); 29.32, 22.87, 21.67, 20.67 (4*q*, *Me*–C(5′), Me_2PhC, *Me*C=O); 26.64, 26.13 (2*t*, C(6), C(3′)). ESI-MS (632.321): 1287 (3, $[2M + Na]^+$), 655 (100, $[M + Na]^+$), 633 (4, $[M + H]^+$), 132 (14), 116 (33), 87 (16). Anal. calc. for $C_{35}H_{45}NO_7$ (632.75): C 66.44, H 7.01, N 8.85; found: C 66.50, H 6.99, N 8.75.

Table 2. *Selected ^{1}H-NMR Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *of the Isoquinuclidines* **7** *and* **8** *in CD_3OD, and* **9**–**11**, **13**, *and* **14** *in $CDCl_3$*

	7	**8**[a])	**9** [14]	**10**[b])	**11** [14][b])	**13**	**14**
H–C(1)	2.57	2.77	3.62	3.34	3.53	2.91	2.84
H_a–C(3)	2.64	2.71	–	–	–	2.88	2.76
H_b–C(3)	2.76	3.06	–	–	–	2.76	2.70
H–C(5)	3.46	3.46	5.20	4.77	4.83	4.79	4.67
H–C(6)	4.19	3.95	4.80	3.28	4.47	4.23	4.32
H_{endo}–C(7)	1.84	1.98	1.91	1.93	1.78	1.82	1.84
H_{exo}–C(7)	1.51	1.45	1.46	1.70	1.66	1.68	1.57
H–C(8)	1.80	1.84	1.91	2.01	2.06	2.18	2.05
J(1,6)	2.2	1.6	1.7	4.4	4.4	2.2	2.2
J(1,7_{endo})	1.8	2.2	2.2	2.2	2.5	1.9	1.9
J(1,7_{exo})	3.1	3.5[c])	3.7	3.5	4.0	3.7	3.9
J(3a,3b)	10.0	10.9	–	–	–	9.0	10.9
J(3a,8)	1.6	1.6	–	–	–	2.5	1.5[c])
J(3b,5)	1.8	1.6	–	–	–	2.6	1.5[c])
J(5,6)	5.0	3.7	7.8	0.9	0.6	4.5	4.4
J(6,NH)	–	–	–	–	–	6.3	6.5
J(6,7_{exo})	2.3	2.0[c])	–	1.9	1.9	2.1	2.0
J(7_{endo},8)	10.9	11.3	10.3	10.3	10.0	10.9	11.2
J(7_{exo},8)	4.0	5.5[c])	5.9	5.7	6.7	3.7	3.7
J(7_{endo},7_{exo})	10.9	13.7	14.0	12.1	13.6	14.0	14.0
J(8,CH_a–C(8))	6.2	6.5	–	–	–	4.4	7.5
J(8,CH_b–C(8))	5.9	5.9	–	–	–	10.0	8.1

[a]) Assignment based on a DQFCOSY.GP and a HSQC.GP spectrum. [b]) Numbering according to the numbering of **7**. [c]) Broad signals; coupling constants deduced from the width of the signals at the half of their height.

*(1*R*,4*S*,5*R*,6*S*,8*R*)-6-Acetamido-8-(acetoxymethyl)-2-benzyl-4-(methoxymethoxy)-2-azabicyclo[2.2.2]oct-5-yl Acetate* (**13**). A mixture of **10** (575 mg, 0.909 mmol) and $LiAlH_4$ (533 mg, 13.6 mmol) in THF (30 ml) was heated under reflux for 2 h, cooled to 0°, and added dropwise to cold (0°) MeOH (30 ml). After evaporation, the residue was suspended in pyridine/Ac_2O 2:1 (9 ml), treated with DMAP (4 mg, 0.032 mmol), and stirred at 24° for 12 h. The mixture was filtered through *Celite*, and the residue was washed with AcOEt (3 × 10 ml). Normal workup (AcOEt/$NaHCO_3$ soln., brine) and FC (toluene/THF 1:0 → 2:1) gave (1*R*,2*S*,5*R*)-5-methyl-2-(1-

methyl-1-phenylethyl)cyclohexyl acetate [22] (240 mg, 96%) as a slightly yellow oil and **13** (375 mg, 92%) as a colourless foam. R_f (CH_2Cl_2/MeOH/Et_3N 20:1:0.1) 0.46. $[\alpha]_D^{25} = -2.8$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3437*m*, 3380*w* (br.), 2955*m*, 2826*m*, 1734*s*, 1674*s*, 1508*s*, 1453*m*, 1369*s*, 1150*s*, 1071*m*, 1046*s*, 909*m*, 829*w*. ^{1}H-NMR ($CDCl_3$): see *Table 2*; additionally, 7.36–7.25 (*m*, 4 arom. H); 7.24 (*tt*, $J \approx 6.8, 2.9$, 1 arom. H); 5.84 (br. *d*, $J = 6.2$, NH); 4.79 (irrad. at 4.23 → *d*, $J = 2.6$, H–C(5)); 4.70, 4.58 (2*d*, $J = 7.5$, OCH_2O); 4.43 (*dd*, $J = 10.6, 4.4$, irrad. at 2.18 → *d*, $J = 11.5$, CH_a–C(8)); 4.23 (irrad. at 4.79 → br. *d*, $J \approx 6.2$, irrad. at 2.91 → *t*, $J \approx 5.0$, H–C(6)); 4.19 (*t*, $J \approx 10.0$, irrad. at 2.18 → *d*, $J = 10.0$, CH_b–C(8)); 3.86, 3.76 (2*d*, $J = 13.4$, PhCH_2); 3.31 (*s*, MeO); 2.91 (irrad. at 4.23 → change, H–C(1)); 2.88 (irrad. at 2.18 → *d*, $J = 9.0$, H_a–C(3)); 2.76 (irrad. at 4.79 → *d*, $J = 9.0$, H_b–C(3)); 2.13, 2.07, 1.94 (3*s*, 3 Ac); 1.82 (irrad. at 2.91 → *dd*, $J = 13.7, 10.0$, irrad. at 2.18 → br. *d*, $J \approx 14.0$, H_{endo}–C(7)); 1.68 (irrad. at 4.23 → *dt*, $J \approx 14.0, 3.7$, irrad. at 2.91 → br. *d*, $J \approx 13.7$, irrad. at 2.18 → br. *d*, $J \approx 13.7$, H_{exo}–C(7)). ^{13}C-NMR ($CDCl_3$): 170.95, 170.83, 169.98 (3*s*, 3 C=O); 138.34 (*s*); 128.33 (2*d*); 128.09 (2*d*); 126.86 (*d*); 91.30 (*t*, OCH_2O); 76.68 (*d*, C(5)); 75.55 (*s*, C(4)); 65.58 (*t*, CH_2–C(8)); 59.28 (*t*, PhCH_2); 56.05 (*q*, MeO); 54.11, 52.83 (2*d*, C(1), C(6)); 49.16 (*t*, C(3)); 35.52 (*d*, C(8)); 25.45 (*t*, C(7)); 23.20, 21.24, 21.04 (3*q*, 3 *Me*C=O). ESI-MS: 919 (6, $[2M + Na]^+$), 471 (38, $[M + Na]^+$), 449 (100, $[M + H]^+$). Anal. calc. for $C_{23}H_{32}N_2O_7$ (448.52): C 61.59, H 7.19, N 6.25; found: C 61.58, H 7.30, N 6.23.

*(1*R,*4*R,5R,*6*S,*8*R*)-6-Acetamido-8-(acetoxymethyl)-2-benzyl-4-hydroxy-2-azabicyclo[2.2.2]oct-5-yl Acetate* (**14**). A cooled (0°) mixture of **13** (100 mg, 0.223 mmol) and mol. sieves (3 Å, 20 mg) in CH_2Cl_2 (6 ml) was treated dropwise with Me_3SiBr (60 µl, 0.45 mmol) and heated to reflux for 2 h. Normal workup (AcOEt/$NaHCO_3$ soln., brine) and FC (CH_2Cl_2/i-PrOH 9:1) gave **14** (69 mg, 76%). Colourless oil. R_f (CH_2Cl_2/MeOH 10:1) 0.71. $[\alpha]_D^{25} = -15.4$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3439*w*, 3388*w*, 2958*w*, 2837*w*, 1732*s*, 1673*s*, 1509*m*, 1453*m*, 1369*s*, 1248*s*, 1172*w*, 1126*m*, 1074*m*, 1030*m*, 977*w*, 909*m*, 852*w*. ^{1}H-NMR ($CDCl_3$): see *Table 2*; additionally, 7.36–7.24 (*m*, 4 H); 7.21 (*tt*, $J \approx 7.2$, 2.2, 1 H); 5.93 (br. *d*, $J = 6.5$, NH); 4.46 (*dd*, $J = 10.9$, 7.5, CH_a–C(8)); 4.21 (*dd*, $J = 10.9$, 8.1, CH_b–C(8)); 3.86, 3.75 (2*d*, $J = 13.4$, PhCH_2); 2.54 (*s*, OH); 2.15, 2.05, 1.95 (3*s*, 3 Ac). ^{13}C-NMR ($CDCl_3$): 171.83, 170.98, 169.84 (3*s*, 3 *C*=*O*); 138.45 (*s*); 128.39 (2*d*); 128.15 (2*d*); 126.94 (*d*); 79.18 (*d*, C(5)); 71.26 (*s*, C(4)); 65.76 (*t*, CH_2–C(8)); 59.30 (*t*, $PhCH_2$); 52.92, 52.46 (2*d*, C(1), C(6)); 50.52 (*t*, C(3)); 37.70 (*d*, C(8)); 25.45 (*t*, C(7)); 23.35, 21.24, 21.16 (3*q*, 3 *Me*C=O). ESI-MS: 831 (4, $[2M + Na]^+$), 427 (18, $[M + Na]^+$), 405 (100, $[M + H]^+$).

N-*(1*R,*4*R,5R,*6*S,*8*R*)-2-Benzyl-4,5-dihydroxy-8-(hydroxymethyl)-2-azabicyclo[2.2.2]oct-5-yl]acetamide* (**7**). A soln. of **14** (40 mg, 0.074 mmol) in MeOH (2 ml) was cooled to 0°, saturated with NH_3, stirred at 0° for 5 h, diluted with MeOH (10 ml), and evaporated. Ion-exchange chromatography (*Amberlite* CG-120, H^+ form, 0.1M aq. NH_3) gave **7** (27 mg, 85%). Colourless solid. R_f (CH_2Cl_2/MeOH 10:1) 0.26. $[\alpha]_D^{25} = -23.9$ ($c = 1.0$, EtOH). pK_{HA} 5.90. IR (KBr): 3405*s* (br.), 3073*m*, 2926*m*, 1654*s*, 1560*s*, 1496*m*, 1420*s*, 1376*m*, 1316*m*, 1259*w*, 1154*w*, 1120*m*, 1077*m*, 1042*m*, 962*w*. ^{1}H-NMR (CD_3OD): see *Table 2*; additionally, 7.35 (br. *dd*, $J \approx 7.8$, 1.2, 2 arom. H); 7.27 (*tt*, $J \approx 7.2$, 1.7, 2 arom. H); 7.20 (*tt*, $J \approx 7.2$, 2.0, 1 arom. H); 3.90 (*dd*, $J = 10.3$, 6.2, irrad. at 1.80 → *d*, $J = 10.3$, CH_a–C(8)); 3.85, 3.73 (2*d*, $J = 13.4$, PhCH_2); 3.72 (*dd*, $J = 10.3$, 5.9, irrad. at 1.80 → *d*, $J = 10.0$, CH_b–C(8)); 2.64 (irrad. at 1.80 → *d*, $J = 10.0$, H_b–C(3)); 1.95 (*s*, AcN); 1.51 (irrad. at 1.80 → *ddd*, $J \approx 10.6, 3.1, 2.2$, H_{exo}–C(7)). ^{13}C-NMR (CD_3OD): 172.85 (*s*, C=O); 139.87 (*s*); 128.75 (2*d*); 129.10 (2*d*); 127.93 (*d*); 78.69 (*d*, C(5)); 73.33 (*s*, C(4)); 64.06 (*t*, CH_2–C(8)); 60.21 (*t*, PhCH_2); 55.35, 54.27 (2*d*, C(1), C(6)); 51.20 (*t*, C(3)); 41.26 (*d*, C(8)); 26.11. (*t*, C(7)); 22.57 (*q*, *Me*C=O). HR-MALDI-MS: 343.1622 (21, $[M + Na]^+$, $C_{17}H_{24}N_2NaO_4^+$; calc.: 343.1634), 321.1811 (21, $[M + H]^+$, $C_{17}H_{25}N_2O_4^+$; calc.: 321.1814). Anal. calc. for $C_{17}H_{24}N_2O_4 \cdot H_2O$ (338.40): C 60.34, H 7.74, N 8.28; found: C 60.81, H 7.74, N 8.04.

For the enzyme tests, a sample of **7** was crystallized from MeOH. Anal. calc. for $C_{17}H_{24}N_2O_4 \cdot MeOH$ (336.60): C 61.34, H 8.01, N 7.95; found: C 61.50, H 8.01, N 7.95.

N-*(1*R,*4*R,5R,*6*S,*8*R*)-4,5-Dihydroxy-8-(hydroxymethyl)-2-azabicyclo[2.2.2]oct-6-yl]acetamide* (**8**). A suspension of **7** (30 mg, 0.094 mmol) and 20% Pd(OH)/C (6 mg) in MeOH/H_2O/conc. aq. HCl 1:1:0.1 (2.1 ml) was stirred at 24° for 12 h under H_2 (5 bar). The suspension was filtered through *Celite*, and the residue was washed with MeOH (3 × 5 ml). The combined filtrate and washings were diluted with toluene (5 ml) and evaporated. Ion-exchange chromatography (*Amberlite CG-120*, 0.1M aq. NH_3) gave **8** (20 mg, 93%). Colourless solid. R_f (MeOH/25% aq. NH_3 soln. 3:1) 0.44. $[\alpha]_D^{25} = +50.7$ ($c = 0.4$, H_2O). pK_{HA} 7.7. IR ($CHCl_3$): 3405*s* (br.), 2937*m*, 1636*s*, 1544*s*, 1378*m*, 1314*m*, 1174*m*, 1119*m*, 1084*m*, 1042*m*, 1011*m*, 925*w*. ^{1}H-NMR (CD_3OD, assignment based on a DQFCOSY.GP and a HSQC.GP spectrum): see *Table 2*; additionally, 3.92 (*dd*, $J = 10.6$, 6.5, CH_a–C(8)); 3.64 (*dd*, $J = 10.3$, 5.9, CH_b–C(8)). ^{13}C-NMR (CD_3OD, assignment based on a HSQC.GP spectrum): 173.02 (C=O); 78.69 (*d*, C(5)); 72.25 (*s*, C(4)); 63.82 (*t*, CH_2–C(8)); 60.22 (*d*, C(6)); 48.68 (*d*, C(1)); 40.93 (*t*, C(3)); 40.16 (*d*, C(8)); 26.61 (*t*, C(7)); 22.52 (*q*, *Me*C=O). HR-ESI-MS: 253.1159 (20, $[M + Na]^+$, $C_{10}H_{18}N_2NaO_4^+$; calc.: 231.1345), 231.1339 (100, $[M + H]^+$, $C_{10}H_{19}N_2O_4^+$; calc.: 231.1345).

*(1*R,*4*S,*5*R,*6*S,*8*R*)-6-Acetamido-8-(acetoxymethyl)-2-[(*tert*-*butoxy*)carbonyl]-4-hydroxy-2-azabicyclo[2.2.2]-oct-5-yl Acetate* (**15**). A soln. of **8** (20 mg, 0.087 mmol) in MeOH/Et_3N 10:1 (1.1 ml) was treated with di(*tert*-butyl) carbonate (49 mg, 0.175 mmol), stirred at 28° for 4 h, and evaporated. A soln. of the residue in pyridine/Ac_2O 2:1 (3 ml) was stirred at 5° for 24 h. Normal workup (AcOEt/$NaHCO_3$ soln., brine) and FC (toluene/AcOEt 2:1 → 0:1) gave **15** (27 mg, 75%). Colourless foam. R_f (AcOEt) 0.16. M.p.: 103–108°. $[\alpha]_D^{25} = +20.1$ ($c = 1.0$, $CHCl_3$). IR ($CHCl_3$): 3440*w*, 3332*w* (br.), 3031*w*, 3008*w*, 1735*s*, 1683*s*, 1503*w*, 1478*w*, 1458*w*, 1408*m*, 1368*s*, 1162*m*, 1102*w*, 1070*w*, 1032*w*, 978*w*, 910*w*, 870*w*. ^{1}H-NMR ($CDCl_3$, 6:4 mixture of diastereoisomers): 6.81 (*d*, $J = 7.2$, 0.6 H), 6.41 (*d*, $J = 6.5$, irrad. at 4.02 → *s*, 0.4 H) (NH); 4.79 (*d*, $J = 2.4$, 0.6 H), 4.70 (*d*, $J = 2.4$, irrad. at 4.02 → *s*, 0.4 H) (H–C(5)); 4.37 (br. *dd*, $J \approx 10.8$, 5.5, irrad. at 2.19 → *d*, $J \approx 10.8$, CH_a–C(8)); 4.22 (*dd*, $J \approx 11.5$, 2.8, irrad. at 2.19 → *d*, $J \approx 10.8$, CH_b–C(8)); 4.17–4.08 (*m*, irrad. at 4.02 → change, irrad. at 2.19 → change, irrad. at 1.57 → change, H–C(1), 0.6 H–C(6)); 4.06–3.96 (*m*, 0.4 H–C(6)); 3.82 (*s*, 0.6 H), 3.48 (*s*, 0.4 H) (OH); 3.49 (br. *d*, $J = 10.8$, 0.6 H), 3.33 (br. *d*, $J = 11.2$, 0.4 H) (H_a–C(3)); 3.44 (br. *d*, $J = 10.8$, 0.6 H), 3.29 (br. *d*, $J = 11.2$, 0.4 H) (H_b–C(3)); 2.26–2.12 (*m*, irrad. at 1.57 → change, H_{endo}–C(7), H–C(8)); 2.13 (*s*, 1.2 H), 2.12 (*s*, 1.8 H), 2.05 (*s*, 3 H), 1.98 (*s*, 1.8 H), 1.91 (*s*, 1.2 H), (3 Ac); 1.57 (br. *d*, $J \approx 13.1$, irrad. at 2.19 → br. *s*, H_{exo}–C(7)); 1.48 (*s*, t-Bu). ^{13}C-NMR ($CDCl_3$, 6:4 mixture of diastereoisomers): 171.44, 170.91 (2*s*, Me*C*=*O*); 170.11, 169.99 (2*s*, 2 Me*C*=*O*); 153.98, 153.80 (2*s*, *t*-BuO*C*=O); 80.44 (*s*, Me_3C); 78.98, 78.89 (2*d*, C(5)); 71.21, 70.11 (2*s*, C(4)); 64.59, 64.32 (2*t*, CH_2–C(8)); 56.36, 55.73 (2*d*, C(6)); 46.83, 45.74 (2*d*, C(1)); 43.35, 42.75 (2*t*, C(3)); 36.91, 36.67 (2*d*, C(8)); 28.55, 28.47 (2*q*, *Me*$_3$C); 25.94, 25.75 (2*t*, C(7)); 23.37, 23.26 (2*q*, *Me*C=O); 21.15, 21.08 (2*q*, 2 *Me*C=O). HR-MALDI-MS: 437.1891 (56, $[M + Na]^+$, $C_{19}H_{30}N_2NaO_8^+$; calc.: 437.1900), 381.1263 (100, $[M - t\text{-Bu} + H + Na]^+$, $C_{15}H_{22}N_2NaO_8^+$; calc.: 381.1274), 315.1540 (35, $[M - t\text{-BuO}_2C + 2\,H]^+$, $C_{14}H_{23}N_2O_6^+$; calc.: 315.1556). Anal. calc. for $C_{19}H_{30}N_2O_8$ (414.45): C 55.06, H 7.30, N 6.76; found: C 55.31, H 7.43, N 6.61.

Hydrolysis of **15** *to* **8**. A soln. of **15** (20 mg, 0.048 mmol) in MeOH/H_2O/conc. aq. HCl 1:1:0.1 (2.1 ml) was stirred at 25° for 12 h, diluted with toluene (3 × 5 ml) and evaporated. Ion-exchange chromatography (*Amberlite CG-120*, H^+ form, 0.1 M aq. NH_3) gave **8** (10 mg, 90%). Colourless solid.

Inhibition of N-*Acetyl-β-hexosaminidases.* IC_{50} Values were determined at a substrate concentration corresponding to the K_M of each enzyme. The IC_{50} values were calculated by plotting the inhibitor concentration *vs.* the rate of hydrolysis. Determination of the inhibition constants (K_i) was performed at five different concentrations of the inhibitor bracketing the IC_{50} value. K_i Values and α values were determined from the replot of the slopes and the replot of the $1/v$ axis intercepts of *Lineweawer–Burk* plots [23].

a) *From Jack Beans.* $K_M = 0.72$ mM ([24]: 0.62 mM). The inhibition studies were carried out at pH 5.0 (0.04M citric acid/sodium citrate buffer, containing 0.8M NaCl) and 25°. The reaction was started by the addition of 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside after pre-incubating the enzyme in the presence of the inhibitor for 30 min at 25°. After 10 min, the reaction was quenched by addition of borate buffer (pH 9.0, 0.02M). The rate of hydrolysis was determined by measuring the absorption at $\lambda = 405$ nm and subsequently substracting the absorption of a blank probe (H_2O, buffer, substrate).

b) *From Bovine Kidney.* $K_M = 1.24$ mM ([25]: 1.87 mM). The inhibition studies were carried out in an analogous way as for the inhibition of the *N*-acetyl-β-hexosaminidase from jack beans, but at pH 4.1 (0.06M citric acid/sodium citrate buffer) and 37°.

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