Sugar-Derived Di- and Tetrahydropyridazinones: Synthesis of New Glycosidase Inhibitors

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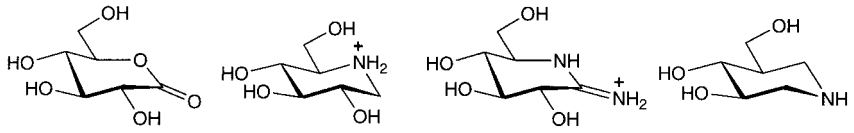
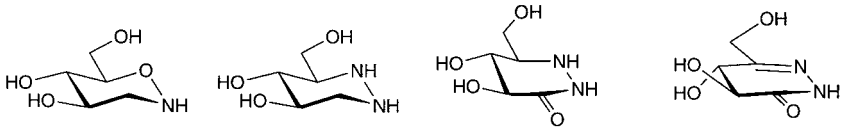
The *N*-unsubstituted D-*arabino*-tetrahydropyridazinone **7** is a micromolar inhibitor of β-glucosidases from sweet almonds (competitive), *Caldocellum saccharolyticum* (mixed), yeast α-glucosidase (competitive), jack bean α-mannosidase (competitive), and snail β-mannosidase (competitive). The *N*-substituted tetrahydropyridazinones **22**, **24**, and **26** are weak inhibitors of these glycosidases, and so are the dihydropyridazinones **8** and **17**–**19**, where the best inhibition was observed for **8** ($K_i = 56$ μM for jack bean α-mannosidase). The tetrahydropyridazinones were obtained by reduction of the corresponding dihydropyridazinones with $NaCNBH_3$, and the dihydropyridazinones were prepared by treatment with hydrazine or substituted hydrazines of the known and readily available D-*threo*-pent-2-uluronate **11**.

Introduction. – The competitive and selective inhibition of retaining β-D-pyranoside-cleaving glycosidases by 1,5-glyconolactones such as **1** has been rationalised by correlating their structure with that of related oxycarbenium cations [1][2]. The similarity between glyconolactones and oxycarbenium cations has led to the double hypothesis that oxycarbenium cations, or closely related species, are reactive intermediates of the enzymic glycoside hydrolysis, and thus similar to the transition state, and that glycono-1,5-lactones are transition-state analogues. While it has become clear that taking oxycarbenium cations as lead compounds for the design of transition-state analogues neglects the important interaction with the catalytic acid [3][4], the imitation of glycosyl cations has been fruitful in suggesting valuable modifications of the structure of the archetypical lactone and piperidine-type inhibitors. Thus, *Ganem* and co-workers have combined elements of shape (as dominant in the lactone **1**) and charge (as in deoxynojirimycin **2**) in their amidinium cations **3** [5][6]. *Bols* and co-workers [7] and later *Ichikawa et al.* [8] have changed the position of the positively charged N-centre, resulting in the synthesis of isofagomine **4** and the azaglucose **5** [9]. *Bols* and co-workers have also combined the basic sites of deoxynojirimycin and isofagomine in the hexahydropyridazine (piperidazine) **6** [10–12], and *Zhang et al.* have prepared 1,4,5,6-tetrahydropyridazine derivatives as inhibitors of influenza neuraminidase [13]. Additional modulations and combinations of shape- and charge-related structural motifs are of interest to learn more about the details of the reaction mechanism of glycosidases.

We wondered about the inhibitory power, selectivity, and pH dependence of tetrahydro- and dihydropyridazinones of type **7** and **8**. These compounds possess two structurally differentiated inhibitory motifs, *viz.* a ring N-atom corresponding to the basic site of nojirimycin, and a lactam group. The lactam C=O group may function as H-bond acceptor for the catalytic acid of β- and possibly also of α-glycosidases [14],



1 **2** **3** **4**



5 **6** **7** **8**

while the ring N-atom of **7** corresponding to that of nojirimycin might be protonated at sufficiently low pH values and lead to a charge/charge interaction with the catalytic base. Thus, the inhibition of β-glycosidases might give information about the location of the catalytic acid – since the C=O group is shifted from its position in **1** – and provide a pH-modulated selectivity of the inhibition of β- and α-glycosidases.

An established method for the construction of tetrahydropyridazinone derivatives involves the reduction by catalytic hydrogenation [15][16] or by hydride reagents [17–19] of dihydropyridazinones[1]). Dihydropyridazinones are accessible by several methods ([22] and references cited therein), and particularly from γ-oxo acids or γ-oxo esters [15–19][23], and hydrazine or substituted hydrazines. The known methyl 1,3,4-tri-*O*-acetyl-D-*threo*-pent-2-uluronate (**11**; *Scheme*), readily available from 3,4,6-tri-*O*-acetyl-D-glucal (**10**) [24], appeared to be an ideal starting compound for our needs. Condensation of the oxo ester **11** with hydrazine and mono-substituted hydrazines, followed by deacetylation should lead to dihydropyridazinones and, hence, also to the desired tetrahydropyridazinones.

Results and Discussion. – Oxidative cleavage of triacetylglucal **10** with $RuO_2/NaIO_4$ in CCl_4/H_2O [24], followed by esterification with Me_3SiCHN_2 in MeOH/PhH [25] yielded 63% of the oxo ester **11** on a 60-mmol scale. This ester was treated with a slight excess of hydrazine monohydrate in MeOH [26] to afford the crystalline dihydropyridazinone **12** in a yield of 75% after chromatography. This dihydropyridazinone was completely deacetylated with aq. NH_3 in MeOH, to afford the triol **8** (80%). In a similar fashion, **11** was treated with methyl-, (2-hydroxyethyl)-, or (2-phenethyl)hydrazine [27]. Methylhydrazine yielded predominantly the diacetyl *N*-methyldihydro-

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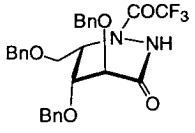
[1]) *Meng* and *Hesse* have reported the synthesis of the protected tetrahydropyridazinone **9** [20]. This derivative has not been deprotected, and the implication that deprotection may not be trivial has been confirmed by exploratory experiments with a related derivative [21].



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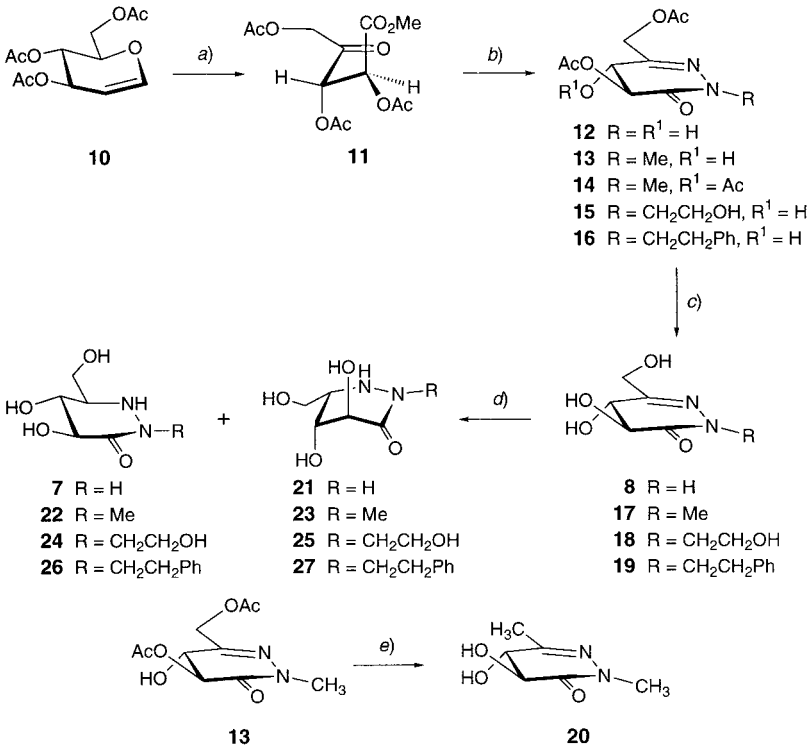
Scheme



a) $RuO_2 \cdot x\ H_2O$, $NaIO_4$, CCl_4/H_2O, then Me_3SiCHN_2, MeOH/PhH; 63%. *b*) *i*) Hydrazine monohydrate, MeOH; 75% (**12**). *ii*) Methylhydrazine, EtOH; 82% (**13**/**14**, 40:1). *iii*) (2-Hydroxyethyl)hydrazine, EtOH; 77% (**15**). *iv*) (2-Phenethyl)hydrazine, EtOH; 65% (**16**). *c*) Aq. NH_3, MeOH; 80% (**8**); 90% (**17**); 85% (**18**); 70% (**19** from **10**, with(2-phenethyl)hydrazine, EtOH and then aq. NH_3). *d*) $NaCNBH_3$, MeOH, AcOH; 58% (**7**/**21** 2:3); 75% (**22**/**23** 1:1); 87% (**24**/**25** 1:1); 89% (**26**/**27** 3:2). *e*) 5% Pd/C, 5 bar of H_2, MeOH/AcOH, then aq. NH_3, MeOH; 63%.

pyridazinone **13** (80%) besides a small amount of the triacetate **14** (2%). Deacetylation of **13** provided the *N*-methyldihydropyridazinone **17** in 90% yield. The deprotected *N*-(2-hydroxyethyl)dihydropyridazinone **18** was obtained in an overall yield of 65% from **11**. (2-Phenethyl)hydrazine reacted only slowly with **11**. Purification of the resulting diacetyl *N*-(2-phenethyl)dihydropyridazinone **16** (65%) proved cumbersome, while the deacetylated dihydropyridazinone **19** (70%) was readily purified.

Hydrogenation of the diacetyl *N*-methyldihydropyridazinone **13** in the presence of 5% Pd/C proceeded with reductive cleavage of the allylic C–O bond [28][29], and deacetylation of the reduction product yielded **20** (63%). Hydrogenation of **13** in the presence of PtO_2/Al_2O_3 [19] provided a complex mixture, while reduction of the deprotected dihydropyridazinones **8** and **17**–**19** with $NaCNBH_3$/MeOH [18] in the presence of AcOH proceeded smoothly to yield a 3:2 to 1:1 mixture of the tetrahydropyridazinones **7**, **22**, **24**, and **26** and their respective diastereoisomers **21**, **23**, **25**, and **27**.

The dihydropyridazinone **12** is characterised by a *singlet* (C=N) at 154.45 and another *singlet* at 167.12 (N–C=O) ppm in the ^{13}C-NMR spectrum. H–C(2) resonated at much higher field than to H–C(3). J(2,3) of 12.5 Hz indicates a diaxial relation. The position of the free OH group is evidenced by the chemical shift of H–C(3) and a long-range coupling between H–C(3) and both H–C(5) and H′–C(5) of 0.6 and 0.9 Hz, respectively, evidences the C(3)–OH group. The structure of **12** and of **17** was confirmed by crystal structure analysis[2]) (*Fig. 1*); structural parameters agree with those of known dihydropyridazinone derivatives [30]. Two molecules of **17** are connected by H_2O, resulting in a 1:2 ratio for **17**/H_2O in the unit cell. The NMR spectra of the dihydropyridazinone **20** are characterised by a *quadruplet* for (C(5)) at 18.54 and another *quadruplet* (NMe) at 34.91 ppm, a C(4) *singlet* at 157.16, a C(1) *singlet* at 167.11 ppm, and a br. *singlet* (1.94 ppm) for CH_3(5) with a weak long-range coupling to H–C(3), resonating as a broad *dd* (4.04 ppm).

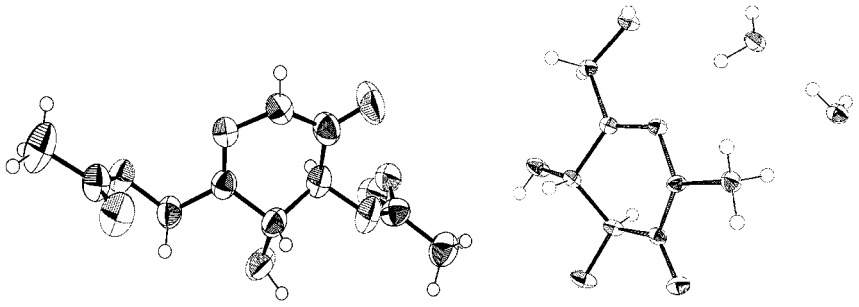


Fig. 1. *ORTEP Representation of* **12** *and* **17**·*2 H_2O*

The assignment of the D-*arabino* configuration to **7**, **22**, **24**, and **26**, and of the L-*xylo*-configuration to **21**, **23**, **25**, and **27** is based on NOE experiments. NOE's of 4.5 to 7.1% were observed for H–C(2) and H–C(4) of **22**. No analogous NOE was found for **23**, evidencing that H–C(2) and H–C(4) are *cis* in **22**, and *trans* in **23**. The D-*arabino*-configuration of **7**, **24**, and **26** is indicated by the close similarity of their NMR spectra with the one of **22**, whereas the spectra of **21**, **25**, and **27** resemble that of **23**. For **7**, **22**, **24**, and **26**, the large J(2,3) value of 9.7–9.0 Hz and the medium J(3,4) value of 6.2–5.4 Hz evidence a $^{N}S_2$ conformation of the D-*arabino*-tetrahydropyridazinones. This is corroborated by *Amber** force-field calculations (Macromodel V.6, gas phase) leading to a calculated J(2,3) value of 9.5 and a calculated J(3,4) value of 6.5 Hz for the $^{N}S_2$ conformation that is by *ca.* 2 kcal/mol more stable than the corresponding $^{3}H_4$ conformer. The epimers **20**, **22**, **24**, and **26** show medium J(2,3) and J(3,4) values of 6.2–5.3 and 5.3–3.9 Hz, respectively, in agreement with a $^{1}S_N$ conformation (calculated $J(2,3) = 6.3$ and $J(3,4) = 6.0$ Hz).

Inhibition Studies. – The dihydropyridazinones **8**, **17**, **18**, and **19**, and the D-*arabino*-tetrahydropyridazinones **7**, **22**, **24**, and **26** were tested against α-glucosidase from brewer's yeast, β-glucosidases from sweet almonds and *Caldocellum saccharolyticum*, α-mannosidase from jack beans, and β-mannosidase from snails. Among the four

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[2]) The crystallographic data have been deposited with the *Cambridge Crystallographic Data Centre* as deposition No. CCDC-142596 (**12**) and CCDC-142597 (**17**). Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44(1223)336033; e-mail: deposit@ccdc.cam.ac.uk).

dihydropyridazinones **8**, **17**, **18**, and **19**, only **8** proved to be a (weak) inhibitor of the β-glucosidases from sweet almonds (IC_{50} 0.9 mM) and *C. saccharolyticum* (IC_{50} 0.6 mM), α-mannosidase from jack beans (IC_{50} 142 μM, K_i 46 μM, competitive inhibition), and β-mannosidase from snails (IC_{50} 4.6 mM). A weak inhibition of yeast α-glucosidase was observed with **19** (IC_{50} 6.5 mM). Among the tetrahydropyridazinones, only the *N*-unsubstituted **7** is a good inhibitor of β-glucosidases from almonds (IC_{50} 21 μM, K_i 13 μM, competitive inhibition) and from *C. saccharolyticum* (IC_{50} 9 μM, K_i 9 μM, mixed inhibition), but not of yeast α-glucosidase (IC_{50} 4.0 mM). Surprisingly, **7** is also a good inhibitor of jack bean α-mannosidase (IC_{50} 47 μM, K_i 25 μM, competitive inhibition) and snail β-mannosidase (IC_{50} 10 μM, K_i 6 μM, competitive inhibition). Among the *N*-substituted tetrahydropyridazinones, only **26** was a weak inhibitor (IC_{50} 3.5 mM) of yeast α-glucosidase. Also, the *N*-substituted tetrahydropyridazinones **22** (IC_{50} 2.8 mM for jack bean α-mannosidase), **24**, and **26** are poor inhibitors of mannosidases.

The effect of pH on the inhibition by **7** of β-glucosidases from sweet almonds (pH 4.2–7.4, pH optimum 5.6 [31]) and *C. saccharolyticum* (pH 4.2–7.4, pH optimum 6.2 [32]), α-mannosidase from jack beans (pH 4.0–6.2, pH optimum 4.0–5.0 [33][34]), and β-mannosidase from snails (pH 4.0–5.6, pH optimum 4.5 [35]) was studied. The pH dependence of the inhibition is represented by the IC_{50} *vs.* pH plot in *Fig. 2*. Except for the β-glucosidases from sweet almonds, the pH dependence of the inhibition of **7** is in accordance with the pH dependence of the enzymic activity. The optimum inhibition of β-glucosidases from sweet almonds at pH 4.2 may not be informative, as it is a mixture of glycosidases. The results indicate that **7** is not fully protonated over the pH range examined. Related acylhydrazines possess pK_{HA} values between 1.5 and 3.4 [36]. Attempts to determine the pK_{HA} value of **7** by titration showed no inflection of titration curve above pH 3, where the determination of pH is rather inaccurate. The expected interaction of α-glucosidases with protonated **7** could not be tested, as the enzyme is almost inactive below pH 4.5, and the pH dependence of the inhibition shows that realisation of the concept indicated in the *Introduction* requires a more strongly basic center corresponding to O–C(5).

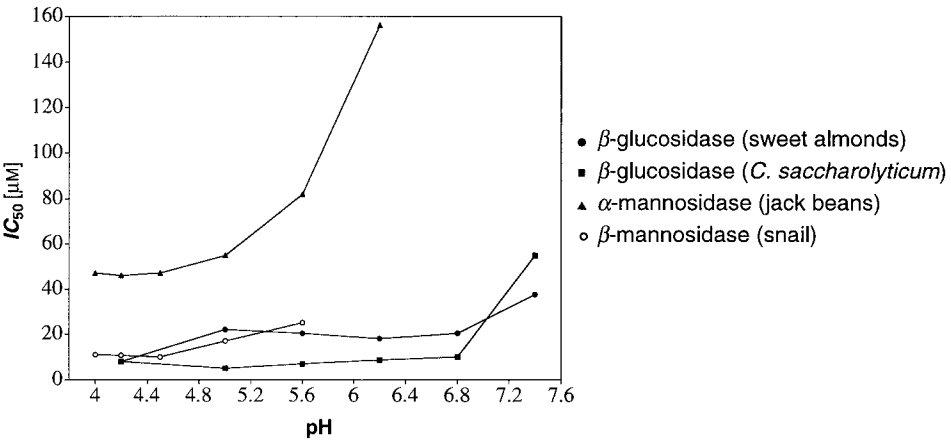

Fig. 2. *pH Dependence of the inhibition of glycosidases by the tetrahydropyridazinone* **7**

The stronger inhibition of β-glucosidases than of yeast α-glucosidase by **7** is in keeping with the hypothesis of a (bidentate?) interaction between the catalytic acid and the lactam moiety of **7**. This interaction may also explain the inhibition of β-mannosidases, and denote a flexibility of the catalytic acid, as it may be required in conjunction with the postulated conformational change of the aglycon (-1) moiety during enzyme hydrolysis [37][38]. However, the requirement for an unsubstituted lactam N–H group, suggesting a H-bond to a suitable acceptor, does not allow us to exclude a possible cooperative interaction with the catalytic nucleophile. The inhibition of α-mannosidase from jack beans by **7** and **8** is suprising, considering that only a moderate inhibition of this enzyme has been observed for deoxynojirimycin **2** [7] and deoxymannojirimycin [7], isofagomine **4** [7][8], and azafagomine **6** [10], and considering that **7** is almost inactive against yeast α-glucosidase.

We thank Dr. *B. Bernet* for valuable analytical contributions, Dr. *B. Schweizer* for the crystal-structure determinations, *K. Piens* (University of Gent, Belgium) and *M. Böhm* for the enzymic tests, *T. Mäder* for HPLC purifications, *B. Brandenberg* for NOE experiments, and the *Swiss National Science Foundation* and *F. Hoffmann La Roche AG*, Basel, for generous support.

Experimental Part

General. Solvents were distilled before use. TLC: *Merck* silica gel *60F-254* plates. HPTLC: *Macherey-Nagel Nano-Sil NH$_2$/UV254* plates; detection by heating with mostain (400 ml of 10% H_2SO_4 soln., 20 g of $(NH_4)_6Mo_7O_{24}\cdot 6H_2O$, 0.4 g of $Ce(SO_4)_2$). Flash chromatography (FC): silica gel *Fluka 60* (0.04–0.063 mm). HPLC: *Merck LiChrosorb RP-18* (7 mm) 250 × 25 nm. M.p. uncorrected. Optical rotations: 1-dm cell. UV Spectra (λ_{max} in nm (log ε)). IR spectra: KBr, neat or 2% $CHCl_3$ soln. ^{1}H- (300 MHz) and ^{13}C-NMR (75 MHz): chemical shifts δ in ppm and coupling constants J in Hz. CI- and FAB-MS: 3-Nitrobenzyl alcohol and NH_3 as matrix. α-Glucosidase (maltase, *3.2.1.20*, *Type VI, G-4634*, as lyophilised powder) from brewer's yeast, β-glucosidase (*3.2.1.21*, as lyophilised powder) from *C. saccharolyticum*, α-mannosidase (*3.2.1.24*, as suspension in 3.0M $(NH_4)_2SO_4$ and 0.1 mM $ZnCl_2$, pH 7.5) from jack beans, β-mannosidase (*3.2.1.24*, as suspension in 3.0M $(NH_4)_2SO_4$ and 10 mM AcONa, pH around 4.0) from snails and all nitrophenyl glycosides were purchased from *Sigma* and used without any further purification. β-Glucosidase (*3.2.1.21*, as lyophilised powder) from sweet almonds was purchased from *Fluka* and used without any further purification.

Methyl 1,3,4-Tri-O-*acetyl*-D-threo-*pent-2-uluronate* (**11**) [24]. At r.t., a vigorously stirred biphasic mixture of CCl_4/H_2O 1:1 (1.2 l) was treated with **10** (16.2 g, 60 mmol), sodium (meta)periodate (78.12 g, 365 mmol), and ruthenium(IV) dioxide · hydrate (240 mg, 60% Ru, 2.4 mol-%), whereupon the mixture became bright yellow. After stirring for 2 d, the dark purple CCl_4 layer was separated and discarded. The black aq. layer was diluted with H_2O (300 ml) and extracted with AcOEt (3 × 400 ml). The combined org. extracts were washed with brine, treated with charcoal and anh. Na_2SO_4, and filtered through *Celite* (washed previously with AcOEt). After evaporation, the crude oxo acid (colourless oil, 14.0 g) was dissolved in MeOH/C_6H_6 1:3 (400 ml), cooled in an ice-water bath, treated with a 2M soln. of Me_3SiCHN_2 in hexane (*ca.* 37 ml) until persistence of the yellow colour, and stirred for 15 min. Evaporation and FC (AcOEt/hexane 1:4) afforded **11** as pale yellow oil (13 g). Crystallization from Et_2O/hexane gave **11** as colourless needles (11.6 g, 63%). R_f(AcOEt/hexane 3:1) 0.83. M.p. 61–62° (Et_2O/hexane) ([24]: 57–58°). $[\alpha]_D^{25} = -17.2$ ($c = 1$, $CHCl_3$) ([24]: $[\alpha]_D^{17} = -10.2$ ($c = 6$, $CHCl_3$)). IR (KBr): 2940*m*, 1767*s*, 1750*s*, 1445*m*, 1430*m*, 1404*m*, 1379*s*, 1257*m*, 1224*s*, 1200*s*, 1162*m*, 1123*m*, 1075*s*, 1011*m*, 957*m*, 939*m*, 901*w*, 871*w*, 794*w*. ^{1}H-NMR ($CDCl_3$): 2.16, 2.18, 2.20 (3*s*, 3 AcO); 3.77 (*s*, MeO); 4.75 (*d*, $J = 17.4$, H–C(1)); 4.94 (*d*, $J = 17.4$, H′–C(1)); 5.62, 5.77 (2*d*, $J = 2.5$, H–C(3), H–C(4)). ^{13}C-NMR ($CDCl_3$): 20.36 (*q*, Me); 20.39 (*q*, 2 Me); 53.13 (*q*, MeO); 66.71 (*t*, C(1)); 70.67, 75.43 (2*d*, C(3), C(4)); 166.94 (*s*, C=O); 169.90 (*s*, 2 C=O); 170.13 (*s*, C=O); 197.50 (*s*, C(2)). CI-MS: 305 (30, $[M+H]^+$), 292 (5), 275 (21), 262 (18), 245 (59), 203 (89), 43 (100). Anal. calc. for $C_{12}H_{16}O_9$ (304.25): C 47.37, H 5.30; found: C 47.52, H 5.53.

2,5-Di-O-*acetyl-1^1,4^1-anhydro-4-deoxy-4-(hydroxyimino)*-D-threo-*pentonamide* (**12**). At 0°, a soln. of **11** (1.218 g, 4 mmol) in MeOH (80 ml) was treated with a soln. of 1M hydrazine monohydrate in EtOH (5.6 ml, 5.6 mmol), and stirred at 0° for 2 h, and then at 23° for 8 h. Evaporation and FC (AcOEt/hexane 1:1) gave **12** (710 mg, 75%). Colourless solid. R_f(AcOEt/hexane 3:1) 0.47. M.p. 160–161° (MeOH/$CHCl_3$). UV (MeOH):

253 (3.8). $[\alpha]_D^{25} = -282$ ($c = 1$, MeOH). IR (KBr). 3313*s*, 3253*s*, 2988*s*, 1747*s*, 1704*s*, 1646*m*, 1439*m*, 1398*s*, 1366*m*, 1331*s*, 1150*s*, 1255*m*, 1199*m*, 1119*m*, 1100*m*, 1066*m*, 1053*s*, 1017*w*, 926*m*, 904*w*, 831*w*. ^{1}H-NMR (CD_3OD): 2.07, 2.18 (2*s*, 2 AcO); 4.37 (br. *d*, $J = 12.5$, H–C(3)); 4.76 (*dd*, $J \approx 14$, 0.6, H–C(5)); 4.96 (*dd*, $J =$ 13.7, 0.9, H′–C(5)); 5.44 (*d*, $J = 12.5$, H–C(2)). ^{13}C-NMR (CD_3OD): 20.50 (*q*, 2 Me); 63.35 (*t*, C(5)); 68.02, 71.63 (2*d*, C(2), C(3)); 154.45 (*s*, C(4)); 167.12 (*s*, C(1)); 171.87, 172.37 (2*s*, 2 C=O). ESI-MS: 511 (45, $[2M +$ Na$]^+$), 299 (45), 267 (65, $[M +$ Na$]^+$), 262 (100, $[M + NH_4]^+$), 245 (8, $[M + H]^+$). Anal. calc. for $C_9H_{12}N_2O_6 \cdot$ 0.25 H_2O (248.71): C 43.46, H 5.07, N 11.26; found: C 43.43, H 4.95, N 11.11.

X-Ray Crystal-Structure Analysis of **12**. Monoclinic *P*21; $a = 6.0370(4)$ Å, $b = 10.059(3)$ Å, $c = 9.7125(7)$ Å; $\alpha = 90°$, $\beta = 101.783(5)°$, $\gamma = 90°$. $V = 577.4(2)$ Å^3, $Z = 2$; $D_{calc} = 1.405$ Mg/m^3. From a crystal of size $0.30 \times 0.10 \times$ 0.10 mm, 1067 reflexions were measured on an *Enraf-Nonius CAD-4* diffractometer [39], with CuK_α radiation (graphite monochromator, $\lambda = 1.54184$ Å) at 293 K. $R = 0.0462$, $R_w = 0.1344$. The structure was solved by direct methods with SHELXS-96 [40]. The non-H-atoms were refined anisotropically with SHELXL-97 [41]. H-Atoms at O(13) and N(2) were obtained from a difference *Fourier* map and refined with isotropic displacement parameters. The rest of the H-atom positions were calculated and included in the structure factor calculation. Drawings of the molecule were done with PLUTO [42] and ORTEP [43].

Treatment of **11** *with Methylhydrazine*. At 0°, a soln. of **11** (415 mg, 1.36 mmol) in EtOH (28 ml) was treated with 1M methylhydrazine in EtOH (1.9 ml, 1.9 mmol) and stirred at 0° for 2 h and at 23° for 2 h. Evaporation and FC (AcOEt/hexane 1:3 → 1:1) gave **13** (283 mg, 80%) and **14** (7 mg, 2%).

*2,5-Di-*O*-acetyl-1^1,4^1-anhydro-4-deoxy-4-(hydroxyimino)-1-*N*-methyl-*D*-threo-pentonamide* (**13**). R_f(AcOEt/hexane 3:1) 0.47. M.p. 123–124° ($CHCl_3$/hexane). UV ($CHCl_3$): 264 (3.8). $[\alpha]_D^{25} = -415.4$ ($c = 1$, $CHCl_3$). IR ($CHCl_3$): 3399*w*, 3038*w*, 2947*w*, 1749*s*, 1693*s*, 1602*w*, 1426*m*, 1378*s*, 1341*w*, 1248*s*, 1123*w*, 1064*s*, 1032*m*, 970*w*, 930*w*, 825*w*. ^{1}H-NMR ($CDCl_3$): 2.13, 2.23 (2*s*, 2 AcO); 3.34 (*s*, MeN); 3.50 (*d*, $J = 5.9$, exchanged with D_2O, HO–C(3)); 4.68 (br. *dd*, $J \approx 12.9$, 5.8, irrad. at 3.50 → br. *d*, $J \approx 12.8$, H–C(3)); 4.75 (*dd*, $J = 13.1$, 0.6, irrad. at 4.68 → *d*, $J = 13.1$, H–C(5)); 5.0 (*dd*, $J = 13.1$, 0.9, irrad. at 4.68 → *d*, $J = 13.1$, H′–C(5)); 5.45 (*d*, $J = 12.8$, H–C(2)). ^{13}C-NMR ($CHCl_3$): 20.66, 20.72 (2*q*, 2 Me); 36.59 (*q*, MeN); 62.18 (*t*, C(5)); 67.62, 70.24 (2*d*, C(2), C(3)); 151.87 (*s*, C(4)); 163.23 (*s*, C(1)); 170.71, 171.50 (2*s*, 2 C=O). CI-MS: 259 (24, $[M + H]^+$), 241 (24), 216 (26), 199 (43), 156 (100). Anal. calc. for $C_{10}H_{14}N_2O_6$ (258.23): C 46.51, H 5.46, N 10.85; found: C 46.49, H 5.44, N 10.78.

*2,3,5-Tri-O-acetyl-1^1,4^1-anhydro-4-deoxy-4-(hydroxyimino)-1-*N*-methyl-*D*-threo-pentonamide* (**14**). Colourless oil. R_f(AcOEt/hexane 1:1) 0.53. ^{1}H-NMR ($CDCl_3$): 2.06, 2.11, 2.16 (3*s*, 3 AcO); 3.36 (*s*, MeN); 4.62 (*d*, $J = 13.1$, H–C(5)); 4.92 (*dd*, $J = 13.1$, 0.9, H′–C(5)); 5.51 (br. *d*, $J \approx 12.1$, H–C(3)); 6.00 (*d*, $J = 12.4$, H–C(2)). ^{13}C-NMR ($CHCl_3$): 20.49 (*q*, Me); 20.53 (*q*, 2 Me); 36.76 (*q*, MeN); 62.39 (*t*, C(5)); 66.93, 68.15 (2*d*, C(2), C(3)); 147.42 (*s*, C(4)); 161.83 (*s*, C(1)); 169.44, 170.08, 170.36 (3*s*, 3 C=O). FAB-MS: 601 (20, $[2M +$ H$]^+$), 447 (78), 301 (100, $[M +$ H$]^+$), 199 (100).

*2,5-Di-*O*-acetyl-1^1,4^1-anhydro-4-deoxy-1-*N*-(2-hydroxyethyl)-4-(hydroxyimino)-*D*-threo-pentonamide* (**15**). A soln. of **11** (380 mg, 1.25 mmol) in EtOH (25 ml) was cooled to 0°, treated with a 1M soln. of (2-hydroxyethyl)hydrazine in EtOH (1.75 ml, 1.75 mmol), and stirred at 0° for 2 h and at 23° for 2 h. Evaporation and FC (AcOEt/hexane 7:3) gave **15** (278 mg, 77%). Colourless solid. R_f(AcOEt/hexane 3:1) 0.12. M.p. 99–99.5° ($CHCl_3$/hexane). UV ($CHCl_3$): 263 (3.75). $[\alpha]_D^{25} = -326.4$ ($c = 1$, $CHCl_3$). IR ($CHCl_3$): 3467*m*, 3038*w*, 2945*w*, 1749*s*, 1691*s*, 1427*m*, 1382*s*, 1342*w*, 1248*s*, 1144*w*, 1112*w*, 1066*s*, 979*w*, 930*w*, 860*w*. ^{1}H-NMR ($CDCl_3$): 2.13, 2.23 (2*s*, 2 AcO); 2.55 (br. *s*, exchanged with D_2O, CH_2OH); 3.70 (*d*, $J = 5.3$, exchanged with D_2O, HO–C(3)); 3.82–3.87 (*m*, 2 H, irrad. at 2.55 → change, CH_2OH); 3.90–3.94 (*m*, CH_2N); 4.71 (br. *dd*, $J \approx 12.9$, 4.2, irrad. at 3.70 → *d*, $J = 12.8$, H–C(3)); 4.81 (*dd*, $J = 13.7$, 0.9, irrad. at 4.71 → *d*, $J \approx 13.7$, H–C(5)); 5.01 (*dd*, $J = 13.7$, 0.9, irrad. at 4.71 → *d*, $J = 13.7$, H′–C(5)); 5.47 (*d*, $J = 12.8$, H–C(2)). ^{13}C-NMR ($CHCl_3$): 20.71, 20.75 (2*q*, 2 Me); 50.90 (*t*, CH_2N); 60.53 (*t*, CH_2OH); 62.25 (*t*, C(5)); 67.54, 70.27 (2*d*, C(2), C(3)); 152.68 (*s*, C(4)); 164.09 (*s*, C(1)); 170.83, 171.56 (2*s*, 2 C=O). FAB-MS: 577 (10, $[2M + H]^+$), 289 (100, $[M + H]^+$), 229 (60). Anal. calc. for $C_{11}H_{16}N_2O_7$ (288.25): C 45.83, H 5.59, N 9.72; found: C 45.66, H 5.44, N 9.63.

*2,5-Di-*O*-acetyl-1^1,4^1-anhydro-4-deoxy-4-(hydroxyimino)-1-*N*-(2-phenethyl)-*D*-threo-pentonamide* (**16**). A soln. of **11** (566 mg, 1.86 mmol) in MeOH was cooled to –10°, treated with a 1M soln. of (2-phenethyl)hydrazine (2.6 ml, 2.6 mmol), and stirred at –10° for 2 h and at 23° for 12 h. Evaporation and FC (AcOEt/hexane 1:3) gave **16** (414 mg, 64%). Colourless solid. R_f(AcOEt/hexane 3:1) 0.70. M.p. 104–105° (CH_2Cl_2/hexane). UV ($CHCl_3$): 265 (3.8). $[\alpha]_D^{25} = -335.4$ ($c = 1$, $CHCl_3$). IR ($CHCl_3$): 3399*w*, 3038*w*, 2947*w*, 1749*s*, 1693*s*, 1602*w*, 1497*w*, 1453*w*, 1433*w*, 1381*m*, 1248*m*, 1146*w*, 1102*m*, 1075*w*, 1031*w*, 971*w*, 930*w*, 826*w*. ^{1}H-NMR ($CHCl_3$): 2.14, 2.20 (2*s*, 2 AcO); 2.93 (br. *t*, $J \approx 7.6$, irrad. at 3.92 → *s*, $PhCH_2$); 3.33 (*d*, $J = 5.6$, exchanged with D_2O, HO–C(3)); 3.92 (*dt*, $J \approx 13.6$, 7.5, irrad. at 2.93 → *d*, $J = 13.1$, CHN); 4.02 (*dt*, $J \approx 13.6$, 7.7, irrad. at 2.93 → *d*, $J = 13.4$, CH′N); 4.58 (br. *dd*, $J = 12.8$, 5.6, irrad. at 5.40 → br. *d*, $J = 4.4$, irrad. at 3.33 → *d*, $J = 12.8$, H–C(3)); 4.72 (*dd*, $J \approx 13.0$,

0.6, irrad. at 4.58 → *d*, *J* = 12.8, H–C(5)); 4.98 (*dd*, *J* ≈ 12.9, 0.8 irrad. at 4.58 → *d*, *J* = 12.8, H′–C(5)); 5.40 (*d*, *J* = 12.8, H–C(2)); 7.18–7.32 (*m*, 5 arom. H). ^{13}C-NMR ($CHCl_3$): 20.74, 20.82 (2*q*, 2 Me); 34.09 (*t*, PhCH_2); 49.95 (*t*, CH_2N); 62.25 (*t*, C(5)); 67.78, 70.21 (2*d*, C(2), C(3)); 126.80 (*d*); 128.71 (*d*, 2 C); 129.16 (*d*, 2 C); 138.37 (*s*); 151.52 (*s*, C(4)); 162.76 (*s*, C(1)); 171.61, 171.66 (2*s*, 2 C=O). ESI-MS: 719 (81, $[2M + Na]^+$), 387 (40), 371 (100, $[M + Na]^+$). Anal. calc. for $C_{17}H_{20}N_2O_6$ (348.35): C 58.61, H 5.79, N 8.04; found: C 58.58, H 5.75, N 8.18.

1′,4′-Anhydro-4-deoxy-4-(hydroxyimino)-D-threo-*pentonamide* (**8**). A soln. of **12** (210 mg, 0.86 mmol) in MeOH (15 ml) was cooled to −20°, treated with aq. NH_3 (1 ml), kept at −20° for 12 h, warmed to r.t., and evaporated. The residue was dissolved in H_2O (2 ml). Lyophilisation gave **8** (120 mg, 80%). Colourless solid. M.p. 163–164° (dec., MeOH/THF). UV (H_2O): 286 (2.5). $[\alpha]_D^{25} = -349$ (*c* = 1.5, H_2O). IR (KBr): 3385*s*, 2886*w*, 1686*s*, 1653*m*, 1474*w*, 1437*w*, 1329*w*, 1281*w*, 1140*m*, 1097*m*, 1074*m*, 1038*m*, 969*w*, 850*w*. ^{1}H-NMR ((D$_6$)DMSO): 3.82 (*dd*, *J* = 9.6, 5.0, irrad. at 5.70 → *d*, *J* = 9.6, H–C(3)); 4.01 (*dd*, *J* = 14.6, 5.6, irrad. at 4.76 → *d*, *J* = 14.0, H–C(5)); 4.12 (*dd*, *J* = 9.9, 5.9, irrad. at 5.70 → change, H–C(2); 4.18 (*dd*, *J* = 14.0, 6.5, irrad. at 4.76 → *d*, *J* ≈ 14.0, H′–C(5)); 4.76 (*dd*, *J* = 6.6, 5.4, exchanged with D_2O, HO–C(5)); 5.70 (*d*, *J* ≈ 5.5, exchanged with D_2O, HO–C(2), HO–C(3)); 10.62 (br. *s*, H–N). ^{13}C-NMR ((D$_6$)DMSO): 61.11 (*t*, C(5)); 67.91, 69.26, (2*d*, C(2), C(3)); 156.83 (*s*, C(4)); 168.95 (*s*, C(1)). ESI-MS: 319 (84, $[2M - H]^+$), 249 (25), 219 (65), 159 (100, $[M - H]^+$). Anal. calc. for $C_5H_8N_2O_4$ (160.13): C 37.50, H 5.04, N 17.49; found: C 37.84, H 5.07, N 17.35.

1′,4′-Anhydro-4-deoxy-4-(hydroxyimino)-1-N-*methyl*-D-threo-*pentonamide* (**17**). A soln. of **13** (270 mg, 1.05 mmol) in MeOH (15 ml) was cooled to −20°, treated with aq. NH_3 (3 ml), kept at −20°, stirred for 12 h, warmed to r.t., and evaporated. The residue was dissolved in H_2O (4 ml). Lyophilisation gave **17** (158 mg, 90%). Colourless solid. M.p. 95–96° (MeOH). UV (H_2O): 288 (3.03). $[\alpha]_D^{25} = -341.9$ (*c* = 1, H_2O). IR (KBr): 3287*s*, 2972*m*, 2879*m*, 1684*s*, 1471*m*, 1456*m*, 1415*w*, 1384*w*, 1350*s*, 1308*m*, 1264*w*, 1152*s*, 1125*m*, 1072*s*, 1034*m*, 862*m*. ^{1}H-NMR ((D$_6$)DMSO): 3.16 (*s*, MeN); 3.85 (br. *dd*, *J* ≈ 9.2, 3.6, irrad. at 5.77 → br. *d*, *J* = 9.3, H–C(3)); 4.04 (*dd*, *J* = 14.6, 3.4, irrad. at 4.86 → *d*, *J* = 14.6, H–C(5)); 4.14 (*dd*, *J* ≈ 9.2, 3.7, irrad. at 5.77 → *d*, *J* = 9.0, H–C(2)); 4.20 (*dd*, *J* = 14.6, 3.6, irrad. at 4.90 → *d*, *J* = 14.6, H′–C(5)); 4.86 (*t*, *J* ≈ 5.7, exchanged with D_2O, HO–C(5)); 5.77 (br. *s*, 2 H, exchanged with D_2O, HO–C(2), HO–C(3)). ^{13}C-NMR ((D$_6$)DMSO): 35.20 (*q*, MeN); 60.40 (*t*, C(5)); 67.01, 68.64 (2*d*, C(2), C(3)); 156.81 (*s*, C(4)); 168.61 (*s*, C(1)). CI-MS: 175 (100, $[M + H]^+$), 157 (51), 145 (39), 141 (41). Anal. calc. for $C_6H_{10}N_2O_4 \cdot 2\,H_2O$ (208.43): C 34.29, H 6.71, N 13.33; found: C 34.76, H 6.45, N 13.22.

X-Ray Crystal-Structure Analysis of **17** · *2 H$_2$O*. Monoclinic *P*21: *a* = 4.7022(14) Å, *b* = 12.026(7) Å, *c* = 8.286(2) Å; *α* = 90.00(4)°, *β* = 103.63(2)°, *γ* = 90.00(4)°. *V* = 455.4(3) Å^3; *Z* = 2; D_{calc} = 1.533 Mg/m^3. *R* = 0.0298, R_w = 0.0777. From a crystal of size 0.2 × 0.15 × 0.05 mm 2709 reflexions were measured on an *Enraf Nonius CAD-4* diffractometer [39] with MoK_α radiation (graphite monochromator, *λ* = 0.71073 Å) at 220(2) K. The structure was solved by direct methods with SIR 97 [44]. The non-H-atoms were refined anisotropically with SHELXL-97 [40]. H-Atoms were obtained from a difference *Fourier* map and refined isotropically. Drawings of the molecule were done with PLUTO [42], ORTEP [43].

1′,4′-Anhydro-4-deoxy-1-N-*(2-hydroxyethyl)-4-(hydroxyimino)*-D-threo-*pentonamide* (**18**). A soln. of **15** (190 mg, 0.66 mmol) in MeOH (10 ml) at −20° was treated with aq. NH_3 (3 ml). The mixture was kept at −20° for 12 h, warmed to r.t., and evaporated. The residue was dissolved in H_2O (2 ml). Lyophilisation gave **18** (115 mg, 85%). Colourless solid. M.p. 132–134° (MeOH). UV (H_2O): 287 (2.96). $[\alpha]_D^{25} = -363.3$ (*c* = 1, H_2O). IR (KBr): 3442*s*, 2986*m*, 2881*m*, 1631*s* (sh); 1481*w*, 1450*s*, 1420*m*, 1372*m*, 1330*s*, 1316*m*, 1287*m*, 1257*m*, 1124*s*, 1092*s*, 1062*s*, 1038*m*, 994*w*, 960*w*, 940*w*, 862*w*, 842*m*. ^{1}H-NMR ((D$_6$)DMSO): 3.45–3.52 (*m*, irrad. at 4.6 → change, CH_2OH); 3.62–3.66 (*m*, irrad. at 3.48 → change, NCH_2); 3.87 (*dd*, *J* = 10.0, 4.7, irrad. at 5.72 → *d*, *J* = 10.0, H–C(3)); 4.06 (*dd*, *J* = 14.6, 5.3, irrad. at 4.81 → *d*, *J* = 14.3, H–C(5)); 4.17 (*dd*, *J* ≈ 9.8, 5.8, irrad. at 5.77 → *d*, *J* = 10.0, H–C(2)); 4.21 (*dd*, *J* ≈ 14.5, 6.1, irrad. at 4.81 → *d*, *J* = 14.3, H′–C(5)); 4.60 (*t*, *J* ≈ 5.8, irrad. at 3.48 → *s*, exchanged with D_2O, CH_2OH); 4.81 (*t*, *J* ≈ 5.9, exchanged with D_2O, HO–C(5)); 5.72 (*d*, *J* = 4.7, exchanged with D_2O, HO–C(3)); 5.77 (*d*, *J* = 5.9, exchanged with D_2O, HO–C(2)). ^{13}C-NMR ((D$_6$)DMSO): 49.19 (*t*, NCH_2); 57.52 (*t*, CH_2OH); 60.30 (*t*, C(5)); 67.16, 68.73 (2*d*, C(2), C(3)); 156.93 (*s*, C(4)); 166.85 (*s*, C(1)). CI-MS: 205 (100, $[M + H]^+$), 187 (66), 175 (29), 169 (25), 161 (49). Anal. calc. for $C_7H_{12}N_2O_5$ (204.18): C 41.18, H 5.92, N 13.72; found: C 41.26, H 5.82, N 13.57.

1′,4′-Anhydro-4-deoxy-4-(hydroxyimino)-1-N-*(2-phenethyl)*-D-threo-*pentonamide* (**19**). A soln. of **11** (1.064 mg, 3.5 mmol) in MeOH (80 ml) was cooled to −10°, treated with a 1M soln. of (2-phenethyl)hydrazine (5 ml, 5 mmol), stirred at −10° for 2 h and at 23° for 12 h, treated with aq. NH_3 (6 ml), and again stirred for 4 h. After evaporation and FC (AcOEt/hexane 3:7 → 3:2), the product was dissolved in MeOH, treated with charcoal, and filtered through *Celite*. Evaporation gave **19** (650 mg, 70% yield). Colourless solid. R_f(MeOH/AcOEt 1:4) 0.8. M.p. 92–94° (MeOH/$CHCl_3$/hexane). UV (H_2O): 286 (2.85). $[\alpha]_D^{25} = -248.4$ (*c* = 0.75, H_2O). IR (KBr): 3382*s*, 2942*w*, 2854*w*, 1654*s*, 1508*w*, 1456*m*, 1400*w*, 1349*m*, 1294*w*, 1277*w*, 1153*m*, 1132*w*, 1105*w*, 1076*m*,

1040*m*, 855*m*, 641*m*. ^{1}H-NMR (D_2O): 2.93 (*dt*, $J \approx 14.0$, 7.0, PhC*H*); 3.0 (*dt*, $J \approx 13.9$, 6.9, PhC*H'*); 3.88 (*dt*, $J \approx 13.5$, 6.8, CHN); 4.10 (*dt*, $J \approx 14$, 6.8, CH'N); 4.20, 4.34 (2*d*, $J = 13.1$, H–C(2), H–C(3)); 4.44 (br. *s*, H–C(5), H'–C(5)); 7.18–7.32 (*m*, 5 arom. H). ^{13}C-NMR (D_2O): 35.80 (*t*, Ph*C*H$_2$); 51.54 (*t*, CH$_2$N); 62.78 (*t*, C(5)); 71.31 (*d*, C(2), C(3)); 129.36 (*d*); 131.32 (*d*, 2 C); 131.85 (*d*, 2 C); 141.30 (*s*); 162.64 (*s*, C(4)); 171.00 (*s*, C(1)). ESI-MS: 551 (20, $[2M + Na]^+$), 287 (100, $[M + Na]^+$). Anal. calc. for $C_{13}H_{16}N_2O_4 \cdot 0.25\ H_2O$ (268.79): C 58.09, H 6.19, N 10.42; found: C 58.14, H 6.18, N 10.14.

*1',4'-Anhydro-4,5-dideoxy-4-(hydroxyimino)-1-*N*-methyl-*D-threo-*pentonamide* (**20**). A soln. of **13** (140 mg, 0.5 mmol) in MeOH/AcOH (5 ml, 95 : 5) was hydrogenated at 5 bar for 8 h in the presence of 5% Pd-C (14 mg), filtered, and concentrated. A soln. of the product in MeOH (5 ml) was treated with aq. NH_3 (0.8 ml), stirred for 4 h, and evaporated. FC (AcOEt/hexane 3 : 7 → 3 : 2) gave **20** (50 mg, 63%). Colourless waxy solid. UV (MeOH): 286 (4.05). $[\alpha]_D^{25} = -402$ ($c = 0.6$, H_2O). ^{1}H-NMR ((D_6)DMSO): 1.94 (br. *s*, irrad. at 4.04 → *s*, Me); 3.12 (*s*, MeN); 3.84 (*dd*, $J = 11.2$, 4.4, irrad. at 5.64 → *d*, $J = 11.2$, H–C(2)); 4.04 (br. *dd*, $J \approx 11.1$, 4.8, irrad. at 1.94 → *dd*, $J = 11.2$, 5.0, irrad. at 5.83 → br. *d*, $J \approx 11.4$, H–C(3)); 5.64 (*d*, $J = 4.7$, exchanged with D_2O, HO–C(2)); 5.77 (*d*, $J = 5.9$, exchanged with D_2O, HO–C(3)). ^{13}C-NMR ((D_6)DMSO): 18.52 (*q*, C(5)); 34.91 (*q*, MeN); 68.43, 69.21 (2*d*, C(2), C(3)); 157.16 (*s*, C(4)); 167.11 (*s*, C(1)). ESI-MS: 229 (55), 213 (100), 197 (44), 181 (36, $[M + Na]^+$), 176 (15), 157 (8, $[M - H]^+$). Anal. calc. for $C_6H_{10}N_2O_3$ (158.16): C 45.57, H 6.37, N 17.71; found: C 45.59, H 6.39, N 17.54.

General Procedure for the Reduction of Dihydropyridazinones **8**, *and* **17**–**19** *with* $NaCNBH_3$. A soln. or suspension of the substrate in MeOH/AcOH (99 : 1 to 95 : 5) was treated with $NaCNBH_3$ (1.5 or 2 equiv. (for **19**)). The mixture was stirred for 24 h, treated with $NaCNBH_3$ (1.5 or 2 (for **19**)), and again stirred for 2 d. Evaporation and FC (AcOEt → AcOEt/MeOH 5 : 1) gave a mixture of diastereoisomers. Their ratio was determined by ^{1}H-NMR spectroscopy. Pure samples of all diastereoisomers were obtained by prep. HPLC, with the solvent system as for anal. HPTLC.

Reduction of **8**. Treatment of **8** (260 mg, 1.62 mmol) in MeOH/AcOH (10 ml, 98 : 2) with $NaCNBH_3$ (300 mg, 4.8 mmol), and FC gave **8** (37 mg) and **7**/**21** 2 : 3 (153 mg, 58%).

*1',4-Anhydro-*D*-arabinohydrazide* (**7**). R_f(i-ProH/$CHCl_3$/H_2O, 4 : 3 : 0.3) 0.07. IR (KBr): 3433*s*, 3255*s*, 2944*m*, 2888*m*, 1677*s*, 1458*m*, 1407*w*, 1355*m*, 1307*w*, 1263*w*, 1151*m*, 1135*w*, 1074*m*, 1057*m*, 1029*w*, 996*m*, 957*w*, 841*m*. ^{1}H-NMR (D_2O): 3.08 (br. *q*, $J \approx 6.2$, H–C(4)); 3.57 (*dd*, $J = 9.3$, 6.2, irrad. at 3.08 → change, irrad. at 4.38 → *d*, $J = 6.2$, H–C(3)); 3.68 (*d*, $J = 6.2$, 2 H, irrad. at 3.08 → br. *s*, CH_2(5)); 4.38 (*d*, $J = 9.3$, H–C(2)). ^{13}C-NMR (D_2O): 63.27 (*t*, C(5)); 64.65 (*d*, C(4)); 74.63, 75.45 (2*d*, C(2), C(3)); 178.68 (*s*, C(1)). ESI-MS: 217 (100), 185 (44, $[M + Na]^+$), 163 (6, $[M + H]^+$). Anal. calc. for $C_5H_{10}N_2O_4$ (162.14): C 37.04, H 6.22, N 17.28; found: C 37.07, H 6.22, N 17.08.

*1',4-Anhydro-*L*-xylonohydrazide* (**21**). R_f(i-PrOH/$CHCl_3$/H_2O (4 : 3 : 0.3) 0.13. ^{1}H-NMR (D_2O): 3.31 (*ddd*, $J \approx 7.2$, 5.0, 4.0, irrad. at 4.07 → *dd*, $J = 7.2$, 5.4, H–C(4)); 3.74 (*dd*, $J = 12.4$, 7.2, irrad. at 3.31 → change, H–C(5)); 3.81 (*dd*, $J \approx 12.0$, 5.2, irrad. at 3.31 → change, H'–C(5)); 4.07 (br. *dd*, $J \approx 5.4$, 3.9, irrad. at. 3.31 → br. *d*, $J \approx 5.7$, H–C(3)); 4.15 (*d*, $J = 5.3$, H–C(2)). ^{13}C-NMR (D_2O): 60.53 (*d*, C(4)); 61.22 (*t*, C(5)); 73.36, 73.48 (2*d*, C(2), C(3)); 175.85 (*s*, C(1)). ESI-MS: 217 (100), 185 (44, $[M + Na]^+$). Anal. calc. for $C_5H_{10}N_2O_4$ (162.14): C 37.04, H 6.22, N 17.28; found: C 36.90, H 6.15, N 17.18.

Reduction of **17**. Reduction of **17** (330 mg, 1.89 mmol) in MeOH/AcOH (15 ml, 96 : 4) with $NaCNBH_3$ (356 mg, 5.67 mmol) and FC gave **22**/**23** 1 : 1 (239 mg, 75%).

*1',4-Anhydro-1-*N*-methyl-*D*-arabinohydrazide* (**22**). R_f(i-PrOH/$CHCl_3$/H_2O 1 : 1 : 0.02) 0.18. UV (H_2O): 286 (2.04). $[\alpha]_D^{25} = +4.2$ ($c = 1$, H_2O). IR (neat): 3375*s*, 3266*s*, 2936*m*, 2866*m*, 1650*s*, 1485*m*, 1445*m*, 1395*m*, 1341*m*, 1237*w*, 1207*w*, 1157*w*, 1102*m*, 1064*s*, 961*w*, 882*m*. ^{1}H-NMR (D_2O): 3.11 (*s*, MeN); 3.12 (*ddd*, $J \approx 8.2$, 5.6, 5.0, irrad. at 4.35 → NOE of 4.5%, H–C(4)); 3.54 (br. *dd*, $J = 9.3$, 5.9, irrad. at 3.08 → br. *dd*, $J \approx 9.0$, 1.3, irrad. at 3.08 → NOE of 2.2%, irrad. at 4.35 → *dd*, $J \approx 5.6$,1.6, irrad. at 4.35 → NOE of 3.9%, H–C(3)); 3.65 (*dd*, $J = 11.8$, 7.8, irrad. at 3.08 → change, irrad. at 3.08 → NOE of 2.9%, H–C(5)); 3.72 (*dd*, $J = 11.8$, 5.0, irrad. at 3.08 → change, irrad. at 3.08 → NOE of 2.7%, H'–C(5)); 4.38 (*d*, $J = 9.3$, irrad. at 3.08 → NOE of 7.1%, H–C(2)). ^{13}C-NMR (D_2O): 39.10 (*q*, MeN); 63.48 (*t*, C(5)); 64.58 (*d*, C(4)); 74.91, 75.63 (2*d*, C(2), C(3)); 176.07 (*s*, C(1)). ESI-MS: 375 (100, $[2M + Na]^+$), 230 (27), 199 (36, $[M + Na]^+$), 182 (16).

*1',4-Anhydro-1-*N*-methyl-*L*-xylonohydrazide* (**23**). R_f(i-PrOH/$CHCl_3$/H_2O, 1 : 1 : 0.02) 0.25. UV (H_2O): 287 (2.3). $[\alpha]_D^{25} = -137.7$ ($c = 0.7$, H_2O). IR (neat): 3361*s*, 2935*w*, 1650*s*, 1451*w*, 1398*m*, 1344*w*, 1229*w*, 1094*s*, 1054*s*, 832*w*. ^{1}H-NMR (D_2O): 3.14 (*s*, MeN); 3.38 (*ddd*, $J \approx 7.2$, 5.0, 4.0, irrad. at 4.07 → *dd*, $J = 7.2$, 5.4, irrad. at 3.38 → NOE of 5%, H–C(4)); 3.74 (*dd*, $J = 11.8$, 7.2, irrad. at 3.38 → change, irrad. at 3.38 → NOE of 1.4%, H–C(5)); 3.82 (*dd*, $J \approx 12.0$, 5.2, irrad. at 3.38 → change, irrad. at 3.38 → NOE of 2.1%, H'–C(5)); 4.07 (*dd*, $J \approx 5.6$, 4.3, irrad. at 3.38 → *d*, $J = 5.7$, H–C(3)); 4.15 (*d*, $J = 5.6$, H–C(2)). ^{13}C-NMR ((D_2O): 38.54 (*q*, MeN); 60.26 (*d*, C(4)); 61.44 (*t*, C(5)); 73.87, 73.98 (2*d*, C(2), C(3)); 173.57 (*s*, C(1)). ESI-MS: 351 (66, $[2M - H]^+$), 265 (56),

211 (47), 175 (100, $[M-H]^+$), 149 (42). Anal. calc. for $C_6H_{12}N_2O_4 \cdot 0.4\ H_2O$ (183.38): C 39.30, H 7.04, N 15.28; found: C 39.20, H 6.84, N 14.96.

Reduction of **18**. Reduction of **18** (110 mg, 0.54 mmol) in MeOH/AcOH (10 ml, 98:2) with $NaCNBH_3$ (101 mg, 1.6 mmol) and FC gave **24/25** 1:1 (98 mg, 87%).

1',4-Anhydro-1-N-*(2-hydroxyethyl)*-D-*arabinohydrazide* (**24**). R_f(i-PrOH/$CHCl_3$/H_2O 4:3:0.2) 0.14. UV (H_2O): 291 (2.2). $[\alpha]_D^{25} = 19.7$ ($c = 0.63$, H_2O). IR (neat): 3353*s*, 3277*s*, 2941*w*, 2877*m*, 1651*s*, 1453*m*, 1420*m*, 1348*m*, 1273*m*, 1150*m*, 1061*s*, 986*w*, 875*w*. ^{1}H-NMR (D_2O): 3.09 (*ddd*, $J \approx 8.3$, 5.4, 4.2, H–C(4)); 3.53–3.47 (*m*, irrad. at 3.77 → change, irrad. at 4.41 → change, H–C(3), H–CN); 3.60 (*dd*, $J = 11.8$, 8.7, H–C(5)); 3.69 (*dd*, $J = 11.8$, 4.7, H′–C(5)); 3.78–3.70 (*m*, 3 H); 4.41 (*d*, $J = 9.7$, H–C(2)). ^{13}C-NMR (D_2O): 52.96 (*t*, NCH_2); 60.94 (*t*, CH_2HO); 63.58 (*t*, C(5)); 64.61 (*d*, C(4)); 74.75, 75.60 (2*d*, C(2), C(3)); 176.67 (*s*, C(1)). ESI-MS: 435 (100, $[2M+Na]^+$), 408 (18), 360 (28), 261 (36), 229 (89, $[M+Na]^+$). Anal. calc. for $C_7H_{14}N_2O_5 \cdot 0.33\ H_2O$ (212.15): C 39.77, H 6.91, N 13.18; found: C 39.62, H 6.97, N 13.20.

1',4-Anhydro-1-N-*(2-hydroxyethyl)*-L-*xylonohydrazide* (**25**). R_f(i-PrOH/$CHCl_3$/H_2O, 4:3:0.2) 0.20. UV (H_2O): 287 (2.3). $[\alpha]_D^{25} = -86.1$ ($c = 0.8$, H_2O). IR (neat): 3361*s*, 2941*m*, 1651*s*, 1427*m*, 1353*m*, 1248*m*, 1065*s*, 865*w*, 832*w*. ^{1}H-NMR (D_2O): 3.36 (br. *ddd*, $J \approx 7.5$, 5.0, 3.4, irrad. at 4.07 → *dd*, $J = 7.2$, 4.7, H–C(4)); 3.62–3.66 (*m*, 2 H); 3.70–3.78 (*m*, 3 H); 3.82 (*dd*, $J \approx 12$, 4.6, H–C(5)); 4.07 (br. *dd*, $J \approx 6.2$, 5.0, irrad. at 3.36 → *d*, $J = 6.5$, H–C(3)); 4.22 (*d*, $J = 6.2$, H–C(2)). ^{13}C-NMR (D_2O): 52.62 (*t*, CH_2N); 60.82 (*t*, CH_2OH); 61.24 (*t*, C(5)); 61.50 (*d*, C(4)); 74.60, 75.09 (2*d*, C(2), C(3)); 175.05 (*s*, C(1)). ESI-MS: 435 (100, $[2M+Na]^+$), 413 (12), 315 (38), 261 (20), 229 (89, $[M+Na]^+$), 207 (40, $[M+H]^+$). Anal. calc. for $C_7H_{14}N_2O_5$ (206.20): C 40.77, H 6.84, N 13.59; found: C 40.53, H 6.88, N 13.34.

Reduction of **19**. Reduction of **19** (199 mg, 0.75 mmol) in MeOH/AcOH (5 ml, 95:5) with $NaCNBH_3$ (185 mg, 3 mmol) FC gave **26/27** 3:2 (180 mg, 89%).

1',4-Anhydro-1-N-*(2-phenethyl)*-D-*arabinohydrazide* (**26**). R_f(i-PrOH/$CHCl_3$/H_2O 1:1:0.02) 0.34. UV (H_2O): 290 (1.3). $[\alpha]_D^{25} = +10.1$ ($c = 0.83$, H_2O). IR (neat): 3374*s*, 3277*s*, 2938*m*, 1659*s*, 1497*w*, 1454*m*, 1360*w*, 1288*w*, 1199*w*, 1143*w*, 1082*m*, 1038*m*. ^{1}H-NMR (D_2O): 2.94 (*t*, $J = 7.2$, PhCH_2); 3.04 (*ddd*, $J \approx 9.0$, 5.9, 4.7, H–C(4)); 3.44 (*dd*, $J = 9.3$, 5.9, H–C(3)); 3.49–3.59 (*m*, H–C(5), NCH); 3.63 (*dd*, $J \approx 12.0$, 4.5, H′–C(5)); 3.90 (*dt*, $J \approx 13.7$, 7.8, CH′N); 4.29 (*d*, $J = 9.3$, H–C(2)); 7.31–7.44 (*m*, 5 arom. H). ^{13}C-NMR (D_2O): 35.38 (*t*, PhCH_2); 52.87 (*t*, CH_2N); 63.57 (*t*, C(5)); 64.63 (*d*, C(4)); 74.86, 75.67 (2*d*, C(2), C(3)); 129.68 (*d*); 131.79 (*d*, 2 C); 131.88 (*d*, 2 C); 141.82 (*s*); 176.05 (*s*, C(1)). ESI-MS: 531 (40, $[2M-H]^+$), 355 (72), 301 (65), 265 (100, $[M-H]^+$). Anal. calc. for $C_{13}H_{18}N_2O_4$ (266.29): C 58.64, H 6.81, N 10.52; found: C 58.63, H 6.94, N 10.51.

1',4-Anhydro-1-N-*(2-phenethyl)*-L-*xylonohydrazide* (**27**): R_f(i-PrOH/$CHCl_3$/H_2O 1:1:0.02) 0.43. UV (H_2O): 286 (2). $[\alpha]_D^{25} = -98.4$ ($c = 1$, H_2O). IR (neat): 3365*s*, 3237*s*, 3026*w*, 2937*w*, 1651*s*, 1497*w*, 1454*m*, 1426*m*, 1359*w*, 1292*w*, 1236*w*, 1120*m*, 1058*m*, 981*w*, 958*w*, 908*w*. ^{1}H-NMR (D_2O): 2.96 (*t*, $J = 7.2$, PhCH_2); 3.19 (*ddd*, $J \approx 7.2$, 5.0, H–C(4)); 3.69 (*dd*, $J \approx 12.0$, 7.0, irrad. at 3.19 → change, H–C(5)); 3.73 (*dt*, $J \approx 14.2$, 7.2, irrad. at 2.96 → change. CHN); 3.77 (*dd*, $J \approx 12.0$, 5.0, irrad. at 3.19 → change, H′–C(5)); 3.83 (*dt*, $J \approx 14.0$, 7.0, irrad. at 2.96 → change, CH′N); 3.97 (*dd*, $J = 6.2$, 5.3, H–C(3)); 4.13 (*d*, $J = 6.2$, H–C(2)); 7.31–7.44 (*m*, 5 arom. H). ^{13}C-NMR (D_2O): 35.17 (*t*, PhCH_2); 52.61 (*t*, CH_2N); 61.24 (*d*, C(4)); 61.43 (*t*, C(5)); 74.48, 74.86 (2*d*, C(2), C(3)); 129.68 (*d*); 131.75 (*d*, 2 C); 131.98 (*d*, 2 C); 141.87 (*s*); 174.45 (*s*, C(1)). ESI-MS: 531 (69, $[2M-H]^+$), 355 (52), 301 (36), 265 (100, $[M-H]^+$). Anal. calc. for $C_{13}H_{18}N_2O_4 \cdot 0.25\ H_2O$ (270.80): C 57.66, H 6.89, N 10.34; found: C 57.55, H 6.88, N 10.20.

Inhibition of Glycosidases. IC_{50} Values were determined at a substrate concentration corresponding to K_m of each enzyme. Determination of the inhibition constants (K_i) were performed at different concentrations of the inhibitor (usually 4–8 concentrations) bracketing the K_i or IC_{50} value.

a) *Inhibition of Brewer's Yeast α-Glucosidase.* IC_{50} Values were determined at 37° with a 0.08M KH_2PO_4/K_2HPO_4/NaCl buffer (pH 6.8), and 4-nitrophenyl α-D-glucopyranoside as substrate. Measurements were started by addition of the substrate to pre-incubated cuvets containing inhibitor or H_2O, enzyme and buffer. The increase of absorption per min at 400 nm was taken as velocity for the hydrolysis of the substrate. The increase was linear during all measurements (3 min). IC_{50} Values were calculated by plotting the inhibitor concentration *vs.* the rate of hydrolysis.

b) *Inhibition of Sweet Almond β-Glucosidase.* Inhibition studies were carried out at 37° with a 0.08M KH_2PO_4/K_2HPO_4 buffer (pH 6.8), and 4-nitrophenyl β-D-glucopyranoside as substrate. Measurements were started by addition of the substrate to a pre-incubated cuvets containing inhibitor or H_2O, enzyme and buffer. The increase of absorption per min at 400 nm was taken as velocity for the hydrolysis of the substrate. The increase was linear during all measurements (3 min). For inhibition studies below pH 6.0, reactions were started by adding substrate and quenching the reaction after 5 min with 0.2M borate buffer (pH 9.2) and taking the

absorption of the soln. at 400 nm. IC_{50} Values were calculated by plotting the inhibitor concentration *vs.* the rate of hydrolysis or absorption. K_i Values were determined from *Dixon* plots.

c) *Inhibition of* C. saccharolyticum *β-Glucosidase.* Inhibition studies were carried out at 55°, as mentioned under *b*.

d) *Inhibition of Jack Bean α-Mannosidase.* Inhibition studies were carried out at 37° in 50 mmol acetate buffer (pH 4.5) with 2 mmol of $ZnCl_2$, and 4-nitrophenyl α-D-mannopyranoside as substrate. Reactions were initiated by adding the enzyme (75 µl) to a pre-incubated cuvet containing the substrate solution in buffer (1350 µl) and H_2O and/or inhibitor solution (75 µl). The velocity of the substrate hydrolysis was measured by quenching 500 µl aliquots after 1, 2 and 3 min, with 500 µl of 0.2M borate buffer (pH 9.2) and measuring the absorption at 400 nm, and the increase of absorption was found to be linear. K_i Values were obtained from *Dixon* plots.

e) *Inhibition of Snail β-Mannosidase. K_i* and IC_{50} values were determined at 25°, with 50 mmol acetate buffer (pH 4.5) and 4-nitrophenyl β-D-mannopyranoside as substrate. The velocity of substrate hydrolysis was determined by quenching the reaction after 5 min with 0.2M borate buffer (pH 9.2) and measuring the absorption. K_i Values were obtained from *Dixon* plots.

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