28. Synthesis and Enzyme-Inhibition Studies of Phenylsemicarbazones Derived from D-Glucono-1,5-lactone and 2-Acetamido-2-deoxy-D-glucono-1,5-lactone

by **Diane R. Wolk**[a]), **Andrea Vasella**[a])*, **Fritz Schweikart**[b]), and **Martin G. Peter**[b])

[a]) Organisch-chemisches Institut der Universität Zürich, Winterthurerstrasse 190, CH–8057 Zürich
[b]) Institut für Organische Chemie und Biochemie der Universität Bonn, Gerhard-Domagk-Strasse 1, D–5300 Bonn 1

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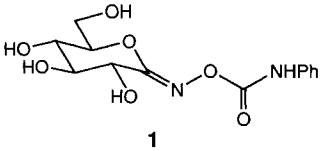
The carbohydrate-derived lactone phenylsemicarbazones **3** and **4** were prepared from **5** and **8** (*Scheme*). Treatment with 4-phenylsemicarbazide gave **6** and **7** (77:23) and **9** and **10** (76:24), respectively. Oxidation of **6** and **9** by CrO_3–pyridine to **11** and **13**, followed by deprotection, yielded **3** and **4**. The structure of **3** was established by X-ray analysis. Enzyme-inhibition studies using emulsin revealed that **3** is a competitive inhibitor with K_i = 23 μm. The activity of **4** was examined using *N*-acetylglucosaminidase from bovine kidney, *Aspergillus niger*, and *Artemia salina*. Compound **4** was found to be a competitive inhibitor of all three enzymes with K_i values of 0.13, 6.0, and 0.71 μm and K_M/K_i values of 6910, 45, and 465, respectively.

Introduction. – In 1986, *Beer* and *Vasella* reported that the *N*-phenylcarbamate **1** is a strong β-glucosidase inhibitor [1]. The 2-acetamido-2-deoxy analogue **2** [2] is a very potent *N*-acetylglucosaminidase inhibitor [3]. In both cases, the phenylcarbamates are much stronger inhibitors than the corresponding lactones or hydroximolactones, indicating an important role for the interaction of the C(1) substituent with the enzyme. To learn more about the effects of the C(1) substituents, we desired to prepare the semicarbazones **3** and **4**, and to evaluate the inhibition of emulsin by **3** and of the *N*-acetylglucosaminidases from bovine kidney, *Aspergillus niger*, and from the brine shrimp *Artemia salina* by **4**. We had demonstrated earlier that a protein preparation from *Artemia salina* contains *N*-acetylglucosaminidase activity which is susceptible to inhibition by 2-acetamido-2-deoxy-D-glucono-1,5-lactone [4].



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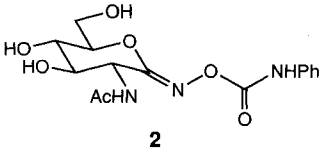

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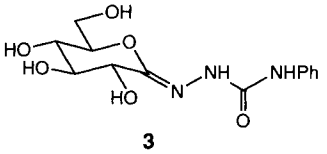

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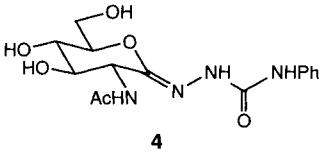

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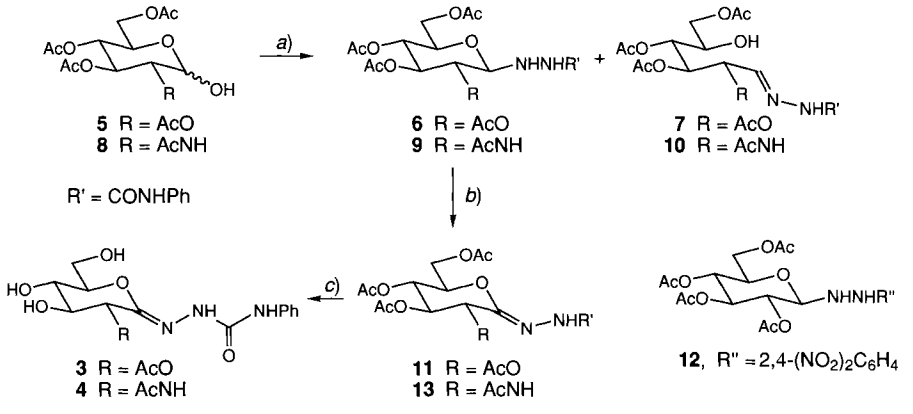
Results and Discussion. – 1. *Preparation of* **3** *and* **4**. The most direct route to the semicarbazones **3** and **4** appeared to be *via* the corresponding hydrazides (*Scheme*). A number of tautomeric, carbohydrate-derived cyclic (alkoxyalkyl)hydrazides [5] and open-chain hydrazones [6] are known. The equilibrium between the tautomers often depends on the solvent and the pH [7][8]. The standard method for the preparation of (protected) aldose hydrazones consists of heating a mixture of an aldose and a hydrazide [9]. Therefore, 1.5 equiv. of 4-phenylsemicarbazide were treated with the tetra-*O*-acetylglucose **5** [10] in 1,2-dichloroethane in the presence of a catalytic amount of oxalic acid. A precipitate was observed (polymerized 4-phenylsemicarbazide, as determined by ^{1}H-NMR spectroscopy and CI-MS (541.5 (8%)), and the reaction was sluggish unless performed at high concentrations. The crude product consisted of 4-phenylsemicarbazide and a 77:23 mixture **6**/**7**, as determined by ^{1}H-NMR spectroscopy. The mixture was freed of oxalic acid by flash chromatography. Recrystallization (EtOH) provided **6** as the major isomer in 51% yield. Further purification of the mother liquor provided 12% of the minor isomer **7**.

Scheme



a) $NH_2NHCONHPh$, cat. $(CO_2H)_2$, $ClCH_2CH_2Cl$ (**6**, 51%; **7**, 12%; **9**, 58%; **10**, 19%). *b*) CrO_3–py, CH_2Cl_2 (**11** 73%; **13**, 74%). *c*) NH_3/MeOH (**3**, 81%; **4**, 83%).

The major isomer **6** exhibits Ac and carbamate C=O stretches at 1750 and 1670 cm^{-1}, respectively, in the IR spectrum. The cyclic structure is suggested by the presence of three sharp NH absorptions at 3370, 3320, and 3240 cm^{-1} and by the ^{1}H-NMR spectrum which has three signals that are exchangeable with D_2O (δ 7.70, 6.30, and 4.42). The cyclic structure is confirmed by the ^{13}C-NMR spectrum where the C(1) signal appears as a *d* at δ 89.3. The observed chemical shift is in the range of the values observed for cyclic carbohydrate hydrazides (80 – 100 ppm), whereas the C(1) signal of the open-chain isomer is expected at *ca.* 150 ppm [8]. The ^{1}H-NMR spectrum also reveals the β-D-configuration, since H–C(1) appears as a *t* (coupled to both H–C(2) and NH) with a coupling constant of 9.4 Hz (see *Tables 1* and *2*). Carbohydrate-derived thiosemicarbazides were also reported to exist in the cyclic form [11].

The minor isomer **7** exhibits IR absorptions at 3350 and 1595 cm^{-1} which are attributable to OH and C=N groups, respectively, and suggest an acyclic structure. The ^{1}H-NMR spectrum again contains three signals which were exchanged upon addition of D_2O (δ 9.42, 8.19, and 3.48), but, in this case, the upfield signal corresponds to an OH which is coupled to a *ddd* at δ 3.81 (assigned to H–C(5)). The acyclic structure is confirmed by the chemical

Table 1. *^{1}H-NMR* (400 MHz, $CDCl_3$) *Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *for the Carbonohydrazides and Semicarbazones* (**6**, **7**, **9**, and **10**)

	H–C(1)	H–C(2)	H–C(3)	H–C(4)	H–C(5)	H–C(6)	H–C(6')
6	4.28	4.96	5.28	5.07	3.73	4.28	4.14
7	7.07	5.56	5.83	5.30	3.81	4.16	4.11
9[a])	4.29	3.81	5.12	4.82	3.85	4.19	3.99
10	7.08	5.15	5.80	5.18	3.93	4.17	4.13
	J(1,2)	*J*(2,3)	*J*(3,4)	*J*(4,5)	*J*(5,6)	*J*(5,6')	*J*(6,6')
6	9.4	9.4	9.8	10.1	4.8	2.1	12.4
7	4.4	7.8	2.1	9.1	2.9	4.9	11.9
9	9.5	9.9	9.7	9.8	4.6	2.2	12.2
10	3.5	5.1	5.1	8.5	2.0	3.3	11.7

[a]) Spectrum measured in (D_6)DMSO.

Table 2. *^{13}C-NMR* (50 MHz, $CDCl_3$) *Chemical Shifts* [ppm] *for the Carbonohydrazides and Semicarbazones* (**6**, **7**, **9**, and **10**)

	C(O)NHPh	C(1)	C(2) to C(5)	C(6)
6	155.6	89.3	73.2, 72.9, 69.0, 68.0	61.7
7	153.6	137.2	70.6, 69.9, 69.3, 67.7	64.4
9[a])	156.2	89.7	73.6, 71.9, 68.9, 51.1	62.4
10	153.6	139.6	70.3, 70.0, 68.1, 50.5	64.7

[a]) Spectrum measured in (D_6)DMSO.

shifts of H–C(1) (δ 7.07, J = 4.3 Hz) and C(1) (δ 137.2). Irradiation of H–N(2) (δ 9.42) produced an NOE at H–C(1) (δ 7.07) which reveals that the double bond in **7** is (*E*)-configurated. The majority of aldehyde-derived hydrazones are (*E*)-isomers [12].

The condensation with 4-phenylsemicarbazide was also performed using the 2-acetamido-tri-*O*-triacetyl-2-deoxyglucose **8** [13] as starting material. Again, the cyclic isomer **9** was the major product (crude ratio **9**/**10** 76:24). The two isomers were separated either by recrystallization in MeOH/MeCN (**9** is less soluble) or by flash chromatography, yielding 58% of **9** and 19% of **10**.

The structure and configuration of **9** was deduced from IR (3 sharp NH absorptions at 3365, 3310, and 3240 cm^{-1}), ^{1}H-NMR (4 exchangeable signals at δ 8.24, 8.10, 7.62, and 5.89; H–C(1) at δ 4.29, J(H–C(1), H–C(2)) = 7.4 Hz), and ^{13}C-NMR (δ 89.7 *d*, C(1)) spectral analysis (see *Tables 1* and *2*). The minor isomer was assigned the open-chain structure **10**, based on the same spectroscopic methods (IR: 3350 (OH) and 1595 cm^{-1}(C=N); ^{1}H-NMR: 4 exchangeable signals at δ 9.18, 8.10, 6.43, 3.81 with the latter coupled to H–C(5) with a coupling constant of 6.8 Hz, H–C(1) is a *d* at δ 7.08 (J = 3.5 Hz); ^{13}C-NMR: δ 139.6 (*d*, C(1))). Irradiation at H–N(2) (δ 9.18) produced an NOE at H–C(1) showing that **10** is the (*E*)-isomer.

The carbonohydrazide and semicarbazone isomers equilibrated under the reaction conditions (determined by heating **6** or **9** in 1,2-dichloroethane in the presence of a catalytic amount of oxalic acid and monitoring by TLC), and a variety of conditions were examined

to find those which would provide the largest proportion of the carbonohydrazides. In MeOH with a catalytic amount of AcOH, the ratio **6/7** was 42:58. The same ratio of isomers was reached from either pure isomer (**6** isomerized to **6/7** 41:59 and **7** to **6/7** 43:57). The 2-acetamido-2-deoxy compounds also equilibrated in MeOH/AcOH to a *ca.* 50:50 mixture of isomers (**9** isomerized to **9/10** 56:44 and **10** isomerized to **9/10** 49:51). Attempts to determine the position of the equilibrium in solvents such as MeCN or PhH failed due to the instability of the compounds under these conditions. At this point, it did not seem likely that the 3:1 (closed/open) ratio could be substantially increased.

We next oxidized **6** to the lactone semicarbazone **11**. The only known example involving a similar conversion, reported by *Gerecs* and coworkers in 1968 [14], is the oxidation of **12** with $KMnO_4$ in AcOH containing 10% H_2SO_4. Our attempts to oxidize similar hydrazides under these conditions were not successful [15], so other reagents were investigated including iodosobenzene, MnO_2, PbO_2, HgO, $FeCl_3$, and RuO_2. The best method proved to be the use of the CrO_3–pyridine complex [16] which provided **11** in 73% yield from **6**. Alternatively, the crude mixture **6/7** (77:23) could be oxidized directly to **11** (45% over two steps). Although aldose-derived hydrazones can readily equilibrate with the corresponding (alkoxyalkyl)hydrazines [17], such an equilibration was slow under these oxidation conditions, and **7** did not react. Compound **9** was also oxidized using the CrO_3–pyridine complex to provide **13** in 74% yield.

The lactone semicarbazone **11** exhibits IR absorptions at 1750 (Ac C=O), 1695 (semicarbazone C=O), and 1600 (O–C=N) cm^{-1}. The ^{1}H-NMR spectrum shows only two exchangeable signals (2 NH at δ 7.96 and 7.86) and no H–C(1) signal (see *Table 3*). In addition, the C(1) signal is at δ 139.58 in the ^{13}C-NMR spectrum, and the molecular weight is 480 ($[M + 1]^+$) by CI-MS. According to the spectral data (no extra absorptions in the ^{1}H- and ^{13}C-NMR spectra), **11** is a single diastereoisomer to which we assign the (*Z*)-configuration based on the X-ray structure of **3** (*vide infra*).

Compound **13** exhibits C=O (1750, 1675 cm^{-1}) and C=N absorptions (1595 cm^{-1}) in the IR spectrum, 3 exchangeable signals in the ^{1}H-NMR spectrum (δ 9.63, 8.65, 8.36, see *Table 3*), and a *s* at δ 142.3 for C(1) in the ^{13}C-NMR spectrum. The molecular weight, as determined by CI-MS, is 479.4 ($[M + 1]^+$).

The lactone semicarbazones **11** and **13** were deprotected in a solution of NH_3 in MeOH to provide **3** in 81% yield as stable crystals and **4** in 83% yield, respectively.

The IR, ^{1}H-NMR, and ^{13}C-NMR spectra of **3** show the absence of Ac groups. The semicarbazone and the conjugated imine bands (1645 and 1595 cm^{-1}, resp.) are still present in the IR spectrum, and a $[M + 1]^+$ peak corresponding to the expected mass (312 (20%)) is observed in the CI-MS.

Product **4** exhibits only one Ac absorption in the IR (1660 cm^{-1}), ^{1}H-NMR (δ 1.94), and ^{13}C-NMR spectra (δ 170.1). A peak corresponding to the correct molecular weight is observed by CI-MS (353, $[M + 1]^+$).

The configuration of **3** was established by X-ray analysis. It shows (*Fig. 1*) that **3** is the (*Z*)-isomer, in agreement with previous work in our group on hydroximo-lactones [18]. All ring C–C and C–O bond lengths are similar to those in D-glucono-1,5-lactone [19]. The angles around C(8) are all *ca.* 120°, and the other ring atoms are nearly tetrahedral (110–114°) except for O(2), where the angle C(12)–O(2)–C(8) is 120°. The ring exists in a distorted half-chair conformation. The phenylsemicarbazone moiety is nearly planar, similar to what is observed in phenylsemicarbazones derived from cycloalkanones [20].

The close constitutional and configurational similarity between the known glycosidase inhibitors **1** and **2**, and the new compounds **3** and **4** is reflected in the very similar ring conformations of these compounds in aqueous solution (distorted half-chairs, *Table 3*). Differences in the inhibitory activity of **1** and **3**, or **2** and **4** should thus be due to the C(1) substituents.

Table 3. *^{1}H-NMR* (400 MHz) *Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *for* D-*Glucono-1,5-lactone and* **1–4**, **11**, *and* **13**

	H–C(2)	H–C(3)	H–C(4)	H–C(5)	H–C(6)	H–C(6')
11[a])	5.51	5.21	5.13	4.61	4.36	4.32
13[b])	4.78	5.27	5.18	4.41	4.37	4.31
D-Glucono-1,5-lactone[c])	4.18	3.87	3.85	4.22	3.92	3.84
1[c])	4.35	3.85	3.78	4.20	4.03	3.87
3[c])	4.30	[d])	[d])	4.06	4.09	3.92
2[c])	4.71	[d])	[d])	4.16	4.07	3.95
4[c])	4.62	[d])	[d])	4.03	4.08	3.93
	J(2,3)	*J*(3,4)	*J*(4,5)	*J*(5,6)	*J*(5,6')	*J*(6,6')
11[a])	3.7	4.8	10.0	4.1	2.9	12.1
13[b])	9.0	9.1	9.2	3.0	2.4	12.4
D-Glucono-1,5-lactone[c])	9.0	9.0	9.0	2.5	4.0	12.5
1[c])	7.9	8.0	9.2	2.2	4.7	12.8
3[c])	9.0	[d])	9.1	2.0	4.7	12.6
2[c])	9.8	[d])	9.5	2.2	4.4	12.9
4[c])	10.0	[d])	9.5	2.0	4.8	12.6

[a]) Measured in $CDCl_3$. [b]) Measured in $(D_6)DMSO$. [c]) Measured in D_2O. [d]) Could not be determined (H–C(3) and H–C(4) = *AB* of *ABX*).

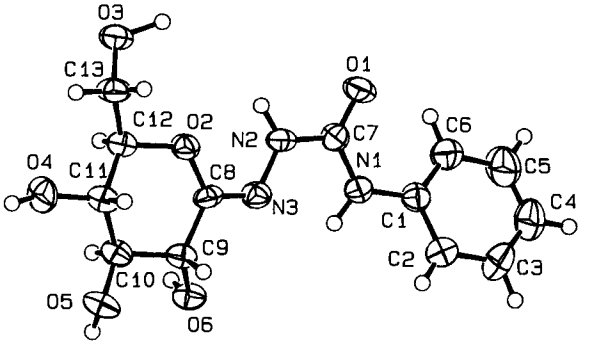


Fig. 1. *Molecular structure of* **3**. ORTEP plot, 50% probability ellipsoids with arbitrary atomic numbering.

2. *Enzymatic Measurements.* The activity of **3** was measured using β-glucosidase derived from sweet almonds (emulsin) with 4-nitrophenyl β-D-glucopyranoside as the substrate. Although **3** was not particularly stable under the conditions used for the enzyme assay ($t_{1/2}$ = 6 h, pH 4.5, 37°), it decomposed only to a very minor extent during the short periods of time required for the assay (as determined by TLC). A *Dixon* plot (*Fig. 2*) shows that **3** is a competitive inhibitor with a K_i value between those of **1** and D-glucono-1,5-lactone (*Table 4*).

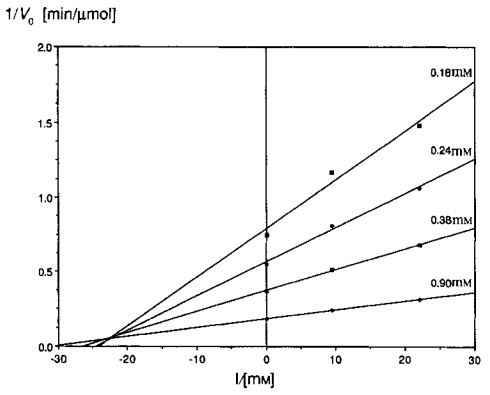


Fig. 2. Dixon *plot for inhibition of emulsin by* **3**

Table 4. *Inhibition Data for Emulsin*[a])

Inhibitor	K_i [mM]	K_M [M]/K_i [M]
D-Glucono-1,5-lactone	37	73
1[b])	2.3	1174
3	23	117

[a]) Data measured at pH 4.5. K_M was $2.7 \cdot 10^{-3}$ M. [b]) Values from [1].

Compound **4** is stable over 20 min at 37° at both pH 4.0 and 4.25, as determined by TLC. Initial kinetic analysis of *N*-acetylglucosaminidases from bovine kidney, *Aspergillus niger*, and *Artemia salina* showed a linear time course of hydrolysis of 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside over at least 15 min. *Lineweaver-Burk* transformation of *Michaelis-Menten* data yielded K_M values in the mM range (*Table 5*). The K_M value of the *Artemia* enzyme compares well with those of *N*-acetylglucosaminidases from other Arthropod species [21]. Compound **4** is a strong, competitive inhibitor of the three enzymes. K_i values, as calculated from *Dixon* transformations [22] (*Fig. 3*), are in the µM range, although they vary considerably with the enzyme source (*Table 5*). Thus, the strongest inhibition is

Table 5. *Inhibition of* N-*Acetylglucosaminidases by* **4**

	K_M [mM]	K_i [µM]	K_M [M]/K_i [M]
Bovine	0.90 ± 0.02	0.13 ± 0.02	6910 ± 670
Aspergillus	0.27 ± 0.03	6.00 ± 0.8	45 ± 4
Artemia	0.33 ± 0.03	0.71 ± 0.12	465 ± 70

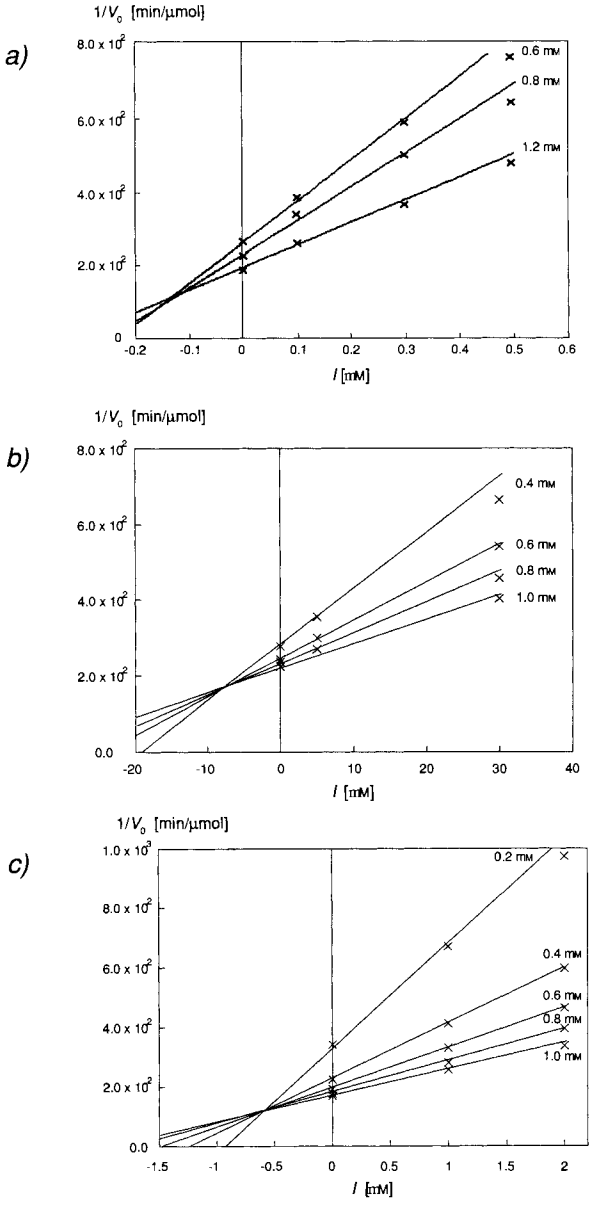


Fig. 3. Dixon *plot for inhibition of* a) *bovine*, b) Aspergillus, *and* c) Artemia N-*acetylglucosaminidase by* **4**

observed for the *N*-acetylglucosaminidase from bovine kidney, whereas the fungal enzyme, albeit showing a relatively low K_M, requires a nearly 45-fold higher inhibitor concentration in order to achieve the same levels of inhibition. In contrast, the *N*-acetylglucosaminidase from the Crustacean *Artemia salina* is inhibited more strongly than the fungal, but less than the vertebrate enzyme.

Therefore, it appears as though the replacement of an O-atom with an NH moiety is important for the inhibition of emulsin (10-fold increase in K_i, *Table 4*), whereas *N*-acetylglucosaminidase from bovine kidney does not appear to be sensitive to such a substitution (*Table 5 vs.* [3]).

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Experimental Part

General. See [18]. All reagents were purchased from *Fluka* and used without further purification. Pyridine and CH_2Cl_2 were freshly distilled from CaH_2. CrO_3 was stored over P_2O_5.

*1-(2,3,4,6-Tetra-*O*-acetyl-β-*D*-glucopyranosyl)-4-phenylsemicarbazide* (**6**) *and 2,3,4,6-Tetra-*O*-acetyl-*D*-glucose 4-Phenylsemicarbazone* (**7**). A soln. of 500 mg (1.43 mmol) of **5** [10], 324 mg (2.14 mmol) of 4-phenylsemicarbazide, and 6.4 mg (0.0715 mmol) of oxalic acid in 5 ml of 1,2-dichloroethane was boiled under reflux for 5 h. During this time, a white solid precipitated. The mixture was cooled to 25° and the precipitate filtered, rinsing with 1,2-dichloroethane. The filtrate was evaporated at 25° to provide a white foam (**6/7** 77:23) which was immediately purified by flash chromatography (FC, hexane/AcOEt 2:3). A white solid was recovered which was a mixture of **6/7** and unreacted 4-phenylsemicarbazide. Recrystallization (EtOH) provided 351 mg (51%) of the anal. pure β-D-isomer **6**. Evaporation of the mother liquor, followed by two additional FC's, afforded 98 mg (14%) of **7** as a colorless glass (*ca.* 85% pure by ^{1}H-NMR). In addition, 45 mg of mixed fractions were recovered for a combined yield of 70%. The products are stable when pure, but they slowly decompose when stored in soln.

Data for **6**: R_f (hexane/AcOEt 2:3) 0.20. M.p. 159°. $[\alpha]_D^{25} = -20.01$ ($c = 1.09$, $CHCl_3$). IR: 3370*w*, 3320*w*, 3240*w*, 1750*s* (br.), 1670*s*, 1600*m*, 1550*m*, 1530*m*, 1450*m*, 1375*m*, 1240*s* (br.), 1045*s* (br.), 910*m*, 750*m*, 690*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.70 (*s*, exchange with D_2O, NH); 7.38 (*d*, $J = 8.5$, 2 H_o); 7.30 (*t*, $J = 7.9$, 2 H_m); 7.06 (*t*, $J = 7.3$, H_p); 6.30 (*s*, exchange with D_2O, NH); 5.28 (*dd* ('*t*'), $J = 9.5$, H–C(3)); 5.07 (*dd* ('*t*'), $J = 9.8$, H–C(4)); 4.96 (*dd* ('*t*'), $J = 9.4$, H–C(2)); 4.42 (*d*, $J = 9.4$, exchange with D_2O, NH); 4.28 (*dd* ('*t*'), $J = 9.4$, H–C(1)); 4.28 (*dd*, $J = 5.1$, 12.2, H–C(6)); 4.14 (*dd*, $J = 2.1$, 12.5, H–C(6)); 3.73 (*ddd*, $J = 2.2$, 4.8, 10.1, H–C(5)); 2.12 (*s*, Me); 2.10 (*s*, Me); 2.04 (*s*, Me); 2.02 (*s*, Me). ^{13}C-NMR (50 MHz, $CDCl_3$): 170.5 (*s*); 170.0 (*s*); 169.7 (*s*); 169.4 (*s*); 155.6 (*s*); 137.8 (*s*); 129.0 (*d*); 123.3 (*d*); 118.9 (*d*); 89.3 (*d*); 73.2 (*d*); 72.9 (*d*); 69.0 (*d*); 68.0 (*d*); 61.7 (*t*); 20.6 (*q*); 20.5 (*q*). CI-MS: 482.5 (5), 460.4 (6), 423.4 (22), 422.4 (100), 303.3 (32), 213.3 (48), 94.1 (84). Anal. calc. for $C_{21}H_{27}N_3O_{10}$ (481.458): C 52.39, H 5.65, N 8.73; found: C 52.30, H 5.40, N 8.52.

Data for **7**: R_f (hexane/AcOEt 2:3) 0.16. IR: 3350*w* (br.), 2950*w*, 1740*s*, 1690*m*, 1595*w*, 1535*m*, 1445*w*, 1370*m*, 1220*s*, 1040*m*, 910*w*, 730*m*. ^{1}H-NMR (400 MHz, $CDCl_3$): 9.42 (*s*, exchange with D_2O, H–N(2)); 8.19 (*s*, exchange with D_2O, H–N(4)); 7.61 (*d*, $J = 8.6$, 2 H_o); 7.32 (*t*, $J = 8.0$, 2 H_m); 7.07 (*d*, $J = 4.3$, H–C(1)); 7.07 (*t*, $J = 7.4$, H_p); 5.83 (*dd*, $J = 2.1$, 7.8, H–C(3)); 5.56 (*dd*, $J = 4.4$, 7.8, H–C(2)); 5.30 (*dd*, $J = 2.2$, 9.1, H–C(4)); 4.16 (*dd*, $J = 2.9$, 11.9, H–C(6)); 4.11 (*dd*, $J = 4.9$, 11.9, H–C(6)); 3.81 (*dddd*, $J = 3.2$, 4.8, 5.7, 9.0, H–C(5)); 3.48 (*d*, $J = 5.7$, exchange with D_2O, OH); 2.17 (*s*, Me); 2.16 (*s*, Me); 2.12 (*s*, Me); 1.97 (*s*, Me). ^{13}C-NMR (50 MHz, $CDCl_3$): 171.2 (*s*); 171.0 (*s*); 169.9 (*s*); 169.7 (*s*); 153.6 (*s*); 137.9 (*s*); 137.2 (*d*); 128.7 (*d*); 123.3 (*d*); 119.6 (*d*); 70.6 (*d*); 69.9 (*d*); 69.3 (*d*); 67.7 (*d*); 64.4 (*t*); 20.7 (*q*); 20.6 (*q*); 20.5 (*q*).

*1-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-4-phenylsemicarbazide* (**9**) *and 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*D*-glucose 4-Phenylsemicarbazone* (**10**). A suspension of 553 mg (1.59 mmol) of **8** [13], 361 mg (2.39 mmol) of 4-phenylsemicarbazide, and 7.2 mg (0.0797 mmol) of oxalic acid in 5 ml of 1,2-dichloroethane was boiled under reflux for 6 h. During this time, a substantial amount of white solid precipitated. ^{1}H-NMR analysis of the crude mixture showed it to be **9/10** 76:24. The mixture was cooled to 25° and the solid filtered, rinsing with 1,2-dichloroethane. Recrystallization from MeOH/MeCN provided 259 mg of anal. pure **9**. FC of the mother liquor (CH_2Cl_2/MeOH 19:1) provided 101 mg of **9** and 104 mg of **10** as a colorless glass. FC of the original reaction filtrate (CH_2Cl_2/MeOH 19:1), followed by recrystallization of the high-R_f isomer (MeOH/MeCN) provided an additional 86 mg of **9** and 40 mg of **10**. Overall yield 77% (58% of **9**, 19% of **10**).

Data for **9**: R_f (CH_2Cl_2/MeOH 13:1) 0.30. M.p. 187–188°. $[\alpha]_D^{25} = -7.7977$ ($c = 0.72$, MeOH). IR: 3500*m*, 3365*m*, 3310*m*, 3240*m*, 1740*s* (br.), 1660*s*, 1600*m*, 1555*s*, 1450*m* (sh), 1375*m*, 1255*s*, 1230*s*, 1110*w*, 1050*m*, 755*m*,

695*w*. ^{1}H-NMR (400 MHz, (D_6)DMSO): 8.24 (*s*, NH); 8.10 (*d*, *J* = 8.8, exchange with D_2O, NH); 7.62 (*s*, exchange with D_2O, NH); 7.41 (*d*, *J* = 7.6, 2 H_o); 7.24 (*t*, *J* = 7.5, 2 H_m); 6.93 (*t*, *J* = 7.3, H_p); 5.89 (*d*, *J* = 7.0, exchange with D_2O, NH); 5.12 (*dd* ('*t*'), *J* = 9.9, H–C(3)); 4.82 (*dd* ('*t*'), *J* = 9.7, H–C(4)); 4.29 (*dd*, *J* = 7.4, 9.5, H–C(1)); 4.19 (*dd*, *J* = 4.6, 12.2, H–C(6)); 3.99 (*dd*, *J* = 2.2, 12.2, H–C(6)); 3.85 (*ddd*, *J* = 2.3, 4.4, 9.8, H–C(5)); 3.81 (*ddd* ('*q*'), *J* = 9.7, H–C(2)); 1.98 (*s*, Me); 1.96 (*s*, Me); 1.91 (*s*, Me); 1.79 (*s*, Me). ^{13}C-NMR (50 MHz, (D_6)DMSO): 170.3 (*s*); 169.9 (*s*); 169.5 (*s*); 156.2 (*s*); 139.5 (*s*); 128.8 (*d*); 122.0 (*d*); 118.3 (*d*); 89.7 (*d*); 73.6 (*d*); 71.9 (*d*); 68.9 (*d*); 62.4 (*t*); 51.1 (*d*); 22.9 (*q*); 20.7 (*q*); 20.6 (*q*). CI-MS: 481.4 (36), 361.3 (10), 213.3 (27), 94.2 (100). Anal. calc. for $C_{21}H_{28}N_4O_9$ (480.474): C 52.50, H 5.87, N 11.66; found: C 52.43, H, 5.73, N 11.53.

Data for **10**: R_f (CH_2Cl_2/MeOH 13:1) 0.16. IR: 3350*m* (br.), 1740*s*, 1665*s*, 1595*m*, 1535*s*, 1450*m*, 1370*m*, 1230*s* (br.), 1040*m*, 760*w*, 695*w*. ^{1}H-NMR (400 MHz, $CDCl_3$): 9.18 (*s*, exchange with D_2O, H–N(2)); 8.10 (*s*, exchange with D_2O, H–N(4)); 7.58 (*d*, *J* = 8.5, 2 H_o); 7.30 (*t*, *J* = 7.9, 2 H_m); 7.08 (*d*, *J* = 3.5, H–C(1)); 7.07 (*t*, *J* = 7.4, H_m); 6.43 (*d*, *J* = 9.1, exchange with D_2O, N*H*Ac); 5.80 (*dd* ('*t*'), *J* = 5.1, H–C(3)); 5.18 (*dd*, *J* = 5.1, 8.5, H–C(4)); 5.15 (*ddd*, *J* = 3.6, 5.2, 9.0, H–C(2)); 4.17 (*dd*, *J* = 2.0, 11.7, H–C(6)); 4.13 (*dd*, *J* = 3.3, 11.7, H–C(6)); 3.93 (*m*, H–C(5)); 3.81 (*d*, *J* = 6.8, exchange with D_2O, OH); 2.10 (*s*, 6 H, 2 Me); 2.09 (*s*, Me); 2.03 (*s*, Me). ^{13}C-NMR (50 MHz, $CDCl_3$): 170.9 (*s*); 170.9 (*s*); 170.2 (*s*); 153.6 (*s*); 139.6 (*d*); 137.8 (*s*); 128.7 (*d*); 123.3 (*d*); 119.6 (*d*); 70.3 (*d*); 70.0 (*d*); 68.1 (*d*); 64.7 (*t*); 50.5 (*d*); 22.7 (*q*); 20.6 (*q*); 20.4 (*q*).

*2,3,4, 6-Tetra-*O*-acetyl-*D*-glucono-1,5-lactone 4-phenylsemicarbazone* (**11**) was prepared similarly to the method of [16]. A soln. of 653 ml (8.08 mmol) of pyridine in 6 ml of CH_2Cl_2 was stirred at 25° as 404 mg (4.04 mmol) of dry CrO_3 were added in one portion. The reaction was slightly exothermic, and the soln. turned burgundy. After 15 min at 25°, the soln. was cooled to 0°, and a soln. of 324 mg (0.673 mmol) of **6** in 3 ml of CH_2Cl_2 was added in one portion. The resulting black soln. was stirred for 1.25 h at 0°, and then diluted with 10 ml of Et_2O. The mixture was filtered under vacuum and the filtrate concentrated. The pyridine was removed under high vacuum, and the residue was purified by FC (hexane/AcOEt 11:9): 235 mg (73%) of **11**. Anal. pure, colorless foam. R_f (hexane/AcOEt 2:3) 0.33. M.p. 50°. $[\alpha]_D^{25}$ = +45.3 (*c* = 1.02, MeOH). IR: 3400*m*, 3000*w*, 1750*s*, 1695*s*, 1600*m*, 1530*s*, 1450*m*, 1370*m*, 1215*m* (br.), 1100*s*, 1040*s*. ^{1}H-NMR (400 MHz, $CDCl_3$): 7.96 (*s*, exchange with D_2O, NH); 7.86 (*s*, NH); 7.50 (*d*, *J* = 8.5, 2 H_o); 7.32 (*t*, *J* = 7.9, 2 H_m); 7.08 (*t*, *J* = 7.4, H_p); 5.51 (*d*, *J* = 3.7, H–C(2)); 5.21 (*dd*, *J* = 3.7, 4.8, H–C(3)); 5.13 (*dd*, *J* = 4.8, 10.0, H–C(4)); 4.61 (*ddd*, *J* = 2.9, 4.1, 10.1, H–C(5)); 4.36 (*dd*, *J* = 4.1, 12.1, H–C(6)); 4.32 (*dd*, *J* = 2.9, 12.1, H–C(6)); 2.20 (*s*, Me); 2.14 (*s*, Me); 2.12 (*s*, Me); 2.10 (*s*, Me). ^{13}C-NMR (50 MHz, $CDCl_3$): 170.4 (*s*); 169.1 (*s*); 169.0 (*s*); 168.3 (*s*); 152.4 (*s*); 139.6 (*s*); 137.7 (*s*); 128.9 (*d*); 123.5 (*d*); 119.5 (*d*); 74.5 (*d*); 72.0 (*d*); 68.4 (*d*); 68.2 (*d*); 61.3 (*t*); 20.7 (*q*); 20.6 (*q*); 20.5 (*q*). CI-MS: 480 (100), 362 (21), 361 (12). Anal. calc. for $C_{21}H_{25}N_3O_{10}$ (479.443): C 52.61, H 5.26, N 8.76; found: C 52.50, H 5.50, N 8.49.

*2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*D*-glucono-1,5-lactone 4-phenylsemicarbazone* (**13**) was prepared as described for **11**, using 642 mg (6.42 mmol) of CrO_3 and 1.04 ml (12.8 mmol) of pyridine in 10 ml of CH_2Cl_2. Insoluble **9** (514 mg, 1.07 mmol) was added as a suspension in 10 ml of CH_2Cl_2. After stirring for 5 h at 0°, the soln. was treated with Et_2O and evaporated as described above. FC (CH_2Cl_2/MeOH 49:1) provided 380 mg (74%) of **13** as a white solid. Recrystallization (AcOEt/hexane) gave an anal. sample. R_f (CH_2Cl_2/MeOH 24:1) 0.30. M.p. 103°. $[\alpha]_D^{25}$ = +23.84 (*c* = 1.05, MeOH). IR: 3385*m* (br.), 1750*s*, 1675*s*, 1595*m*, 1535*s*, 1450*m* (sh), 1370*m*, 1310*w*, 1230*s*, 1130*w*, 1050*m*, 755*w*, 695*w*. ^{1}H-NMR (400 MHz, (D_6)DMSO): 9.63 (*s*, exchange with D_2O, NH); 8.65 (*s*, exchange with D_2O, NH); 8.36 (*d*, *J* = 8.4, exchange with D_2O, NH); 7.50 (*d*, *J* = 7.6, 2 H_o); 7.28 (*t*, *J* = 7.5, 2 H_m); 6.98 (*t*, *J* = 7.4, H_p); 5.27 (*dd* ('*t*'), *J* = 9.1, H–C(3)); 5.18 (*dd* ('*t*'), *J* = 9.2, H–C(4)); 4.78 (*dd* ('*t*'), *J* = 9.0, H–C(2)); 4.41 (*ddd* ('*dt*'), *J* = 2.8, 9.5, H–C(5)); 4.37 (*dd*, *J* = 3.0, 12.4, H–C(6)); 4.31 (*dd*, *J* = 2.4, 12.4, H–C(6)); 2.05 (*s*, Me); 2.00 (*s*, Me); 1.96 (*s*, Me); 1.91 (*s*, Me). ^{13}C-NMR (50 MHz, $CHCl_3$): 170.5 (*s*); 170.4 (*s*); 170.2 (*s*); 169.1 (*s*); 152.9 (*s*); 142.3 (*s*); 137.7 (*s*); 129.2 (*d*); 123.7 (*d*); 119.2 (*d*); 76.2 (*d*); 71.6 (*d*); 67.3 (*d*); 61.5 (*t*); 49.8 (*d*); 22.7 (*q*); 20.6 (*q*); 20.5 (*q*). CI-MS: 479.4 (10), 360.3 (27), 359.3 (100), 240.3 (34), 213.3 (16). Anal. calc. for $C_{21}H_{26}N_4O_9$ (478.458): C 52.72, H 5.48, N 11.71; found: C 52.45, H 5.73, N 11.72.

D-Glucono-1,5-lactone 4-Phenylsemicarbazone (**3**). A soln. of 183 mg (0.382 mmol) of **11** in 3 ml of MeOH was stirred with 2 ml of a sat. NH_3/MeOH soln. After 2 h at 25°, the soln. was evaporated and the residue recrystallized (EtOH/MeCN): 96 mg (81%) of anal. pure **3**. R_f (CH_2Cl_2/MeOH 4:1) 0.40. M.p. 183–184°. $[\alpha]_D^{25}$ = +40.0 (*c* = 1.01, MeOH). IR: 3430*s*, 3320*s*, 3200*s*, 1645*s*, 1595*s*, 1555*s*, 1455*m*, 1345*w*, 1270*m*, 1250*m*, 1200*w*, 1140*m*, 1100*m*, 1070*w*, 1045*m*, 1030*m*, 995*w*, 960*w*, 855*w*, 750*m*, 700*m*. ^{1}H-NMR (400 MHz, CD_3OD): 7.51 (*d*, *J* = 8.7, 2 H_o); 7.28 (*t*, *J* = 8.0, 2 H_m); 7.02 (*t*, *J* = 7.4, H_p); 4.12 (*d*, *J* = 8.3, H–C(2)); 4.01 (*dd*, *J* = 1.9, 12.3, H–C(6)); 3.90 (*ddd*, *J* = 1.9, 6.3, 9.6, H–C(5)); 3.77 (*dd*, *J* = 6.3, 12.3, H–C(6)); 3.58 (*dd* ('*t*'), *J* = 8.3, H–C(3)); 3.52 (*dd* ('*t*'), *J* = 9.0, H–C(4)). ^{13}C NMR (50 MHz, CD_3OD): 155.9 (*s*); 147.9 (*s*); 140.0 (*s*); 129.7 (*d*); 124.2 (*d*); 121.0 (*d*); 83.0 (*d*); 77.5 (*d*); 71.5 (*d*); 70.3 (*d*); 62.8 (*t*). CI-MS: 312 (20), 294 (66), 218 (100), 202 (38). Anal. calc. for $C_{13}H_{17}N_3O_6$ (311.294): C 50,16, H 5.50, N 13.50; found: C 50.14, H 5.73, N 13.43.

X-Ray Structure Analysis of **3**. Suitable crystals were obtained by slow evaporation of an EtOH/MeCN soln. at r.t. All non-H-atoms of the main molecule were located by direct methods. There was also one highly disordered solvent molecule in each asymmetric unit. The electron density attributed to the solvent molecule was accounted for by placing partial-occupancy C-atoms on the sites of the peaks of electron density found in several subsequent difference *Fourier* maps. Each atom of the solvent molecule was assigned one half or one quarter site occupancy, depending upon the indications given by the temperature factors. No attempt was made to differentiate between element types for the atomic positions of the solvent. No absorption corrections were applied. The positions of the OH H-atoms were tentatively assigned from appropriately positioned peaks in the difference maps. All remaining H-atoms were placed in geometrically calculated positions with C–H and N–H bond lengths of 0.95 Å. The H-atom positons were not refined and fixed isotropic temperature factors were employed, the magnitude being $1.2 \cdot B_{eq}$ of the associated C- or O-atom. The positions of the non-H-atoms of the main molecule were refined with anisotropic thermal parameters, while those of the solvent molecule were refined with individual isotropic temperature factors only. A secondary extinction coefficient was included in the refinement. The final difference *Fourier* maps were essentially featureless, with many of the highest remaining peaks being associated with the disordered solvent molecule. The highest peak of residual electron density was only 0.31 e · Å^{-3}. High-quality crystals were difficult to obtain. This, in addition to the presence of the solvent molecule, results in the relatively high *R* factor. The structure solution was performed using the direct-methods routine of SHELXS86 [23]. Data reduction and structure refinement was performed with the TEXSAN program package [24]. Experimental parameters are given in *Table 6*. Atomic coordinates, bond lengths, and bond angles are deposited with the *Cambridge Crystallographic Data Center*.

Table 6. *X-Ray Experimental Parameters for* **3**

Formula	$C_{13}H_{17}N_3O_6$ + solvent	Wavelength [Å]	1.54059
Crystal system	monoclinic	Mode	*Wyckoff* ω-scans
Space group	$P2_12_12_1$	Diffractometer	*Nicolet-R3*
a [Å]	4.8208 (9)	Scan speed [°/min]	2.5–19.3
b [Å]	12.911 (2)	2θ limits [°]	55/75
c [Å]	31.936 (6)	Octants	$+h+k+l$
V [Å^3]	1987.6 (6)	No. of independent refl.	1854
Z	4	No. of refl. in refinement	1759
$2\theta_{max}$ [°]	116	No. of refl. with $I > 2\sigma(I)$	1402
Crystal size [mm]	0.06 · 0.23 · 0.50	*R* (*F*)	0.066
Temperature [K]	293	R_w (*F*)	0.071
Radiation	CuK_α (graphite monochromated)	*GOF*	2.536
		Weighting scheme	$[\sigma^2(F)]^{-1}$

*2-Acetamido-2-deoxy-*D*-glucono-1,5-lactone 4-Phenylsemicarbazone* (**4**). A soln. of 400 mg (0.836 mmol) of **13** in 5 ml of MeOH was stirred for 4 h at 25° with 5 ml of a sat. NH_3/MeOH soln. The soln. was evaporated and the residue recrystallized (MeOH): 244 mg (83%) of anal. pure **4**. R_f (CH_2Cl_2/MeOH 4:1) 0.53. M.p. 210°. $[\alpha]_D^{25}$ = +133.2 (*c* = 0.13, MeOH). IR: 3340*s* (br.), 1660*s* (br.), 1600*s*, 1565*s*, 1530*s*, 1455*m*, 1235*m*, 1110*m*, 1055*m*, 750*m*, 690*m*. ^{1}H-NMR (400 MHz, (D_6)DMSO): 9.63 (*s*, exchange with D_2O, NH); 8.45 (*s*, exchange with D_2O, NH); 8.15 (*d*, *J* = 8.3, exchange with D_2O, NH); 7.48 (*d*, *J* = 8.0, 2 H_o); 7.28 (*t*, *J* = 8.0, 2 H_m); 6.98 (*t*, *J* = 7.4, H_p); 5.43 (*d*, *J* = 5.8, exchange with D_2O, OH); 5.38 (*d*, *J* = 5.1, exchange with D_2O, OH); 5.09 (*dd*, *J* = 4.1, 9.2, exchange with D_2O, OH); 4.35 (*dd* ('*t*'), *J* = 8.7, H–C(2)); 3.78 (*ddd*, *J* = 1.5, 9.4, 11.9, H–C(6)); 3.71 (*ddd*, *J* = 1.6, 6.6, 9.3, H–C(5)); 3.59 (*ddd*, *J* = 4.2, 6.5, 12.2, H–C(6)); 3.48 (*ddd* ('*dt*'), *J* = 5.1, 8.5, H–C(3)); 3.38 (*ddd*, *J* = 5.8, 8.5, 9.3, H–C(4)); 1.94 (*s*, Me). ^{13}C-NMR (50 MHz, (D_6)DMSO): 170.1 (*s*); 153.0 (*s*); 145.1 (*s*); 139.2 (*s*); 129.0 (*d*); 122.5 (*d*); 118.8 (*d*); 82.3 (*d*); 73.7 (*d*); 69.7 (*d*); 61.8 (*t*); 52.3 (*d*); 23.1 (*q*). CI-MS: 353.2 (28), 287.2 (32), 234.2 (88), 216.2 (32), 213.2 (61), 94.2 (100), 93.2 (41). Anal. calc. for $C_{15}H_{20}N_4O_6$ (352.346): C 51.13, H 5.72, N 15.90; found: C 51.38, H 5.72, N 15.76.

Enzyme-Inhibition Studies for **3**. Emulsin (β-glucosidase, activity 8.15 U/mg, *Fluka*) and 4-nitrophenyl β-D-glucopyranoside (Glc-Np; *Fluka*) were used without further purification. One unit of enzyme activity will hydrolyze 1 μmol of Glc-Np per min. The amount of 4-nitrophenolate liberated was determined by reading the absorption at 400 nm ($\varepsilon = 1.55 \cdot 10^4$).

For determination of the *Michaelis* and inhibition constants (K_M and K_i, resp.), aliquots of the following stock solns. were combined: 50 μl of enzyme soln. (364 mU/ml in H_2O), 250 μl of sodium citrate buffer (0.095M, pH 4.5), and 250 μl of H_2O or inhibitor **3** soln. ($3.79 \cdot 10^{-4}$ M, $8.8 \cdot 10^{-5}$ M, or $3.79 \cdot 10^{-5}$ M in H_2O). After pre-incubation for 10 min at 37°, 450-μl aliquots of substrate stock solns. ($2 \cdot 10^{-3}$ M, $8.4 \cdot 10^{-4}$ M, $5.3 \cdot 10^{-4}$ M, or $3.9 \cdot 10^{-4}$ M in H_2O) were added, and incubation was continued for 2–8 min. The reaction was stopped by the addition of sodium tetraborate ($B_4Na_2O_7 \cdot 10\ H_2O$) buffer (0.2 M, pH 9.2, 0.9 ml).

The half-life of **3** at pH 4.5 was determined by incubating 2 ml of enzyme soln. (146 mU/ml in H_2O) with a 2 ml soln. of inhibitor **3** ($3.79 \cdot 10^{-3}$ M in H_2O) for 10 min at 37°. A 2 ml soln. of sodium citrate buffer (0.095M, pH 4.5) was added, and a 0.75 ml aliquot of the resulting soln. was immediately incubated with 0.25 ml of substrate soln. ($2.1 \cdot 10^{-2}$ M). After 2 min, the reaction was stopped by the addition of sodium tetraborate buffer (0.2M, pH 9.2, 0.9 ml). The remaining enzyme, inhibitor, citrate buffer soln. were incubated further, and aliquots were taken after 3, 5, 7, and 12 h.

Enzyme-Inhibition Studies for **4**. The 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (GlcNAc-Np) was from *Sigma*, all other chemicals from *E. Merck*. The β-*N*-acetylglucosaminidase from bovine kidney (11.0 U/mg) and from *Aspergillus niger* (35 U/mg) were from *Sigma* (cat. No. A 2415 and A 7053, resp.). In the case of *Artemia salina*, *N*-acetylglucosaminidase was assayed in a $10000 \cdot g$ supernatant from a homogenate of 72-h-old nauplii [4][25]. One unit of enzyme activity will hydrolyze 1 μmol of GlcNAc-Np per min. Substrate conversion was calculated using $\varepsilon = 1.46 \cdot 10^4$ for 4-nitrophenolate at 420 nm.

The following stock solns. were prepared for the enzyme assays: 10 mM GlcNAc-Np in H_2O, 500 mM sodium citrate pH 4.0 or 4.25, 1 mM or 10 μM inhibitor in H_2O, and sat. aq. sodium tetraborate soln. The assays contained, in a total volume of 1.0 ml, 50 mM of sodium citrate pH 4.0 (bovine) or 4.25 (*Aspergillus* or *Artemia*), 5.0–6.5 mU (bovine), or 2 mU (*Aspergillus*), or 5.0–6.0 mU (*Artemia*) of enzyme, and substrate as specified in *Fig. 3* (see *General Part*). The reactions were started for determinations of K_M by the addition of enzyme. For determination of K_i, solns. containing inhibitor and enzyme were pre-incubated at 20° for 5 min, and the reaction was started by the addition of substrate. The reactions were terminated after 10 or 15 min incubation at 37° by the addition of 2 ml of sodium tetraborate soln. Each assay was performed at least in triplicate. Data are the average from double measurements.

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