Synthesis of *N*-Acetylglucosamine-Derived Nagstatin Analogues and Their Evaluation as Glycosidase Inhibitors

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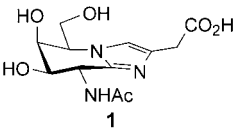
The *gluco*-configured analogue **15** of nagstatin (**1**) and the methyl ester **14** were synthesized *via* condensation of the thionolactams **17** or **18** with the β-amino ester **19**. The silyl ethers **20** and **21** resulting from **17** were desilylated to **22** and **23**; these alcohols were directly obtained by condensing **18** and **19**. The attempted substitution of the C(8)–OH group of **22** by azide under *Mitsunobu* conditions led unexpectedly to the deoxygenated α-azido esters **24**. The desired azide **25** was obtained by treating the *manno*-configured alcohol **23** with diphenyl phosphorazidate. The azide was transformed to the debenzylated acetamido ester **14** that was hydrolyzed to the nagstatin analogue **15**. The imidazole-2-acetates **14** and **15** are nanomolar inhibitors of the *N*-acetyl-β-glucosaminidases from *Jack* beans and from bovine kidney, submicromolar to micromolar inhibitors of the β-glucosidase from *Caldocellum saccharolyticum*, and rather weak inhibitors of the snail β-mannosidase. In all cases, the ester was a stronger inhibitor than the corresponding acid. As expected from their *gluco*-configuration, both imidazopyridines **14** and **15** are stronger inhibitors of the β-*N*-acetylglucosaminidase from bovine kidney than nagstatin.

Introduction. – Nagstatin (**1**), a strong inhibitor of several hexosaminidases [1–4], is a *N*-acetylgalactosamine-derived tetrahydropyridoimidazole-2-acetic acid [5]. Its inhibitory activity is essentially associated with the imidazole ring and not with the carboxymethyl substituent [2], although substituents on the imidazole ring may strongly affect the inhibition of β (and α-)-glycosidases [6–8]. In the preceding paper [9], we described the influence of the hydrophobic character of *C(2)*-methyl ester and carboxylic acid substituents on the inhibition of β-glycosidases. Imidazole-2-propionates **10**–**13** are stronger inhibitors of the β-glucosidase from *Caldocellum saccharolyticum* and of the β-mannosidase from snail than imidazole-2-acetates **6**–**9**, and these are stronger than imidazole-2-carboxylates **2**–**5**. There is a parallel sequence of inhibitory activity for the methyl esters and the corresponding acids, with the esters being stronger inhibitors.



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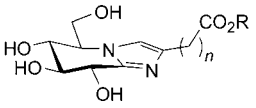
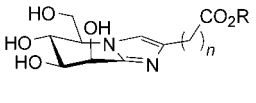
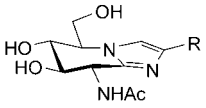


n = 0: **2** R = Me **3** R = H
n = 1: **6** R = Me **7** R = H
n = 2: **10** R = Me **11** R = H



n = 0: **4** R = Me **5** R = H
n = 1: **8** R = Me **9** R = H
n = 2: **12** R = Me **13** R = H



14 R = CH_2CO_2Me
15 R = CH_2CO_2H
16 R = H

We wanted to know if the relationship between the inhibitory activity of esters and acids is also valid for the inhibition of the *N*-acetyl-β-glucosaminidase from bovine kidney[1]) by the *N*-acetylglucosamine-derived imidazole-2-acetate **14** and the corresponding acid **15**. These compounds will also allow testing the effect of the *galacto- vs. gluco*-configuration on the inhibition.

We report the synthesis of **14** and **15**, their evaluation as inhibitors of the hexosaminidases from bovine kidney (unknown family) and from *Jack* beans (family 18 [11][12]), and a comparison of the inhibition to that of the unsubstituted GlcNAc-imidazole **16** [2][13].

Synthesis. – We planned to synthesize the *gluco*-analogue **15** of nagstatin (**1**) *via* the *gluco*- and *manno*-configured hydroxy-imidazoles **22** and **23**. These imidazoles should be obtained by condensing the selectively protected thionolactams **17** [14] or **18** [15] with the β-amino ester **19** [9], followed by selective deprotection of the products. The resulting alcohols **22** and **23** should be transformed into **15** by a *Mitsunobu* substitution, similarly as described for the *galacto*- and *talo*-analogues in the synthesis of nagstatin [3][4] (*Scheme 1*).

Condensation of the *O*-silylated thionolactam **17**[2]) with the β-amino ester **19** [9] followed by treatment of the crude with $TsOH \cdot H_2O$ provided the *gluco*- and *manno*-configured imidazoles **20** and **21** (53%; 55:45), which were readily separated by chromatography to give 29% of **20** and 23% of **21**. Standard desilylation [16][17] of **20** and **21** led to the hydroxy-imidazoles **22** (93%) and **23** (93%). The alcohols **22** and **23** were obtained in higher yields by condensing **19** with the acetoxy-thionolactam **18** [15] followed by acid treatment of the crude. This yielded 81% of a 45:55 mixture **22**/**23**; chromatography provided 32% of **22**, 33% of **23** and 16% of a 28:72 mixture **22**/**23**.

Substitution of the OH by an N_3 group under *Mitsunobu* conditions [18–20] using PBu_3 was reported to proceed well for the *galacto*-analogue of the imidazopyridine **22**, but the same conditions did not affect **22**. Replacing Bu_3P by Ph_3P, however, led to a mixture of the diastereoisomeric α-azido esters **24** (47%; (2*R**)/(2*S**) 2:1); 32% of **22** were recovered. The unexpected and non-trivial formation of **24** is rationalized (*Scheme 2*) by assuming that **22** is transformed *via* a betaine formed from Ph_3P and azodicarboxylate [21] into the phosphonium cation **A**. Elimination of Ph_3PO from **A** generates the diazafulvenium cation **B**. A 1,5-H shift transforms **B** into the (*E*)- and/or (*Z*)-diazafulvenium cations **C** and is followed by addition of azide leading to the α-azido esters **24**.

The *gluco*-azide **25** was finally obtained in a yield of 69% by treating the *manno*-configured hydroxy-imidazole **23** with diphenyl phosphorazidate (DPPA) and DBU [22]. Under otherwise identical conditions, the *gluco*-imidazole **22** decomposed to a complex mixture from which the *gluco*- and *manno*-configured azides **25** and **26** were isolated in 6 and 3% yield, respectively. In contradistinction, the substitution of

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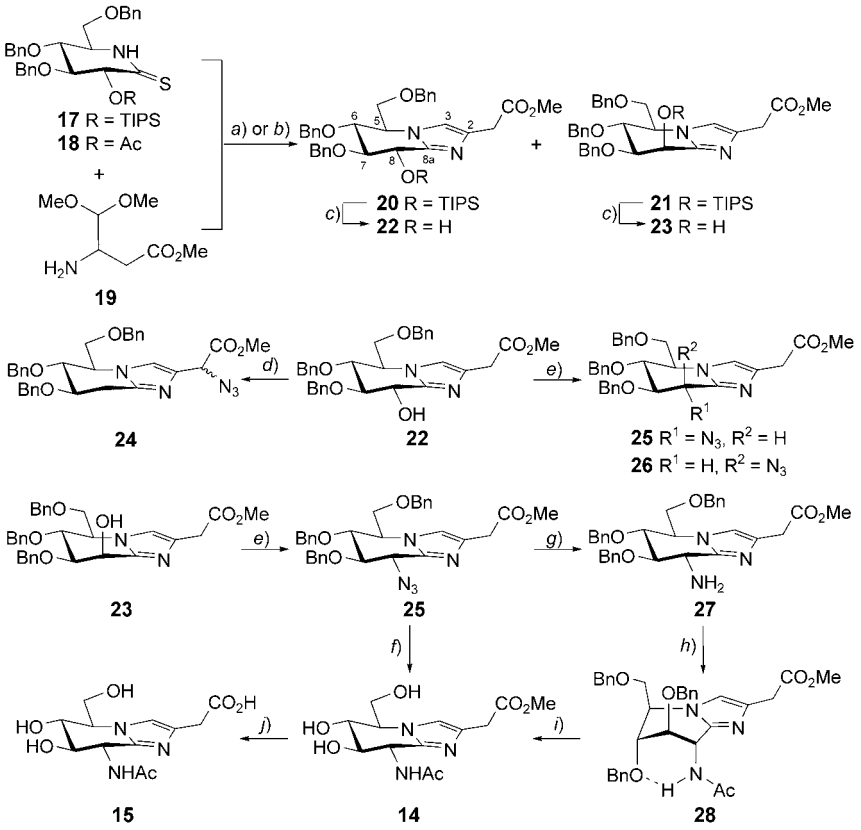
[1]) The inhibition of the *N*-acetyl-β-glucosaminidase from bovine kidney and the β-glucosidase from *C. saccharolyticum* was correlated with the basicity of GlcNAc- and glucose-derived inhibitors of the azolopyridine type [10].

[2]) The thionolactam **17** will be described in a forthcoming paper on the synthesis of the *manno*-configured 8-amino-8-deoxy-tetrahydroimidazopyridine [14].

Scheme 1

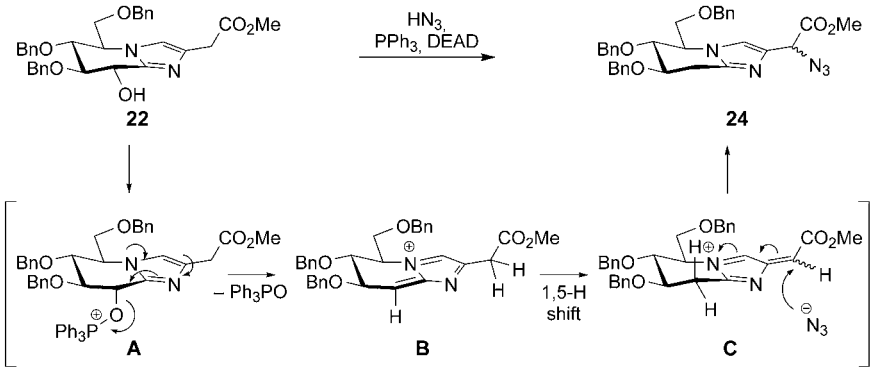


a) 1. $HgCl_2$, THF; 2. $TsOH \cdot H_2O$, toluene, 60°; 53% of **20**/**21** 55:45 (from **17**). *b*) As *a*; 81% of **22**/**23** 45:55 (from **18**). *c*) Bu_4NF, THF, 0°; 93% of **22**; 93% of **23**. *d*) HN_3, Ph_3P, diethyl azodicarboxylate (DEAD), THF, 0→23°; 47% of **24** (2*R**)/(2*S**) 2:1. *e*) Diphenyl phosporazidate (DPPA), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), toluene; 9% of **25**/**26** 2:1 (from **22**); 69% of **25** (from **23**). *f*) 1. H_2, 10% Pd/C, AcOH; 2. Ac_2O, MeOH; 3. NH_3, MeOH, 40°; 60%. *g*) $HS(CH_2)_3SH$, Et_3N, MeOH; 85%. *h*) Ac_2O, pyridine; 81%. *i*) H_2, 10% Pd/C, AcOH; 98%. *j*) KOH, $EtOH/H_2O$, 0°; 76%.

C(8)–OH of the *galacto*-analogue of **22** (HN_3, Bu_3P, DEAD) provided 77% of the *galacto*-azide [3][4]. The inversion of configuration observed for the transformation of **23** to **25** marks only a formal contrast to the retention of configuration observed by *Tatsuta et al.* for the substitution of the *galacto*-configured analogue of **23** [4] (*cf.* [15][23]), considering the preferred pseudoaxial addition of azide to an intermediate azoniafulvenium cation of type **B** (*Scheme 2*) presumably formed in both cases.

Hydrogenation of the azido-imidazole **25** (10% Pd/C, AcOH), followed by acetylation (Ac_2O, MeOH) and *O*-deacetylation (NH_3, MeOH), led to the debenzylated methyl ester **14** in 60% yield. In a longer but higher-yielding route to **14**, we reduced the azide **25** with propane-1,3-dithiol and Et_3N [24] and acetylated the resulting amino-imidazole **27** (85%) to **28** (81%). Hydrogenolysis of **28** yielded 98% of

Scheme 2



the desired methyl ester **14** (67% over three steps). Finally, hydrolysis of **14** at 0° provided 76% of the nagstatin analogue **15** that was thus prepared from **18** in five steps and an overall yield of 14%, or, *via* the protected amino- and acetamido-imidazoles **27** and **28**, in seven steps and an overall yield of 16%. The methyl ester **14** was prepared in 19 and 21% yield over four and six steps, respectively.

Similarly to the other *C(2)*-substituted imidazoles [6–8][25], the protected *gluco*- and *manno*-tetrahydroimidazopyridines **21**–**23** and **25**–**27** exist in solution as 2:1 mixtures of the $^{7}H_{6}$ and the $^{6}H_{7}$ conformers[3]), while the unprotected *gluco*-imidazopyridines **14** and **15** adopt a $^{7}H_{6}$ conformation (see *Tables 3* and *5* in *Exper. Part*). The *gluco*-configured imidazole **20** adopts in $CDCl_3$ a $^{5,8}B$ conformation with the C(8)–O bond in the pseudo-axial position, as evidenced by *J*(5,6), *J*(6,7), and *J*(7,8) values of 8.4, 3.4, and 3.4 Hz, respectively. The coupling constants indicate a $^{6}H_{7}$ conformation for the *gluco*-acetamide **28** which is stabilized by an intramolecular H-bond between the N–H and O–C(6)[4]). The ^{13}C signals of C(5)–C(8) of all imidazoles (*Table 4* in *Exper. Part*) were assigned by analogy to the *gluco*- and *manno*-analogues described in the preceding paper [9]. The formation of the methyl imidazole-2-acetates **20** and **21** is confirmed by the disappearance of the NH signal of **17**, ^{13}C *singlets* at 133.43 (**20**) and 134.32 ppm (**21**), ^{13}C *doublets* at 115.09 (**20**) and 116.70 ppm (**21**), and strong C=O bands at 1736 cm^{-1}. The configuration of the alcohols **22** and **23** (and thus also of **20** and **21**) was assigned on the basis of *J*(7,8) values (7.5 and 3.4 Hz, resp.). The structure the α-azido esters **24** was deduced on the basis of the replacement of a broad *s* at 3.67 ppm for CH_2–C(2) by two broad *s*s at 4.87 and 4.88 ppm in the ratio 2:1, and of a *d* at 4.87 ppm for H–C(8) for **22** by two *dd*s at 3.00 and 3.25 ppm with $J_{gem} = 16.8$ Hz for $CH_2(8)$. The structure of **24** is supported by a strong IR band at 2109 cm^{-1} for the N_3 group and by the $[M+H]^+$ peak at *m/z* 568.2550.

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[3]) The direction of numbering of imidazopyridines (*cf.* **20** in *Scheme 1*) is opposite to that of pyranosides. Thus, the sides above and below the plane of the imidazoles, as defined by the clockwise and counterclockwise numbering, are interchanged relative to those defined by carbohydrate nomenclature.

[4]) A similar stabilization of the $^{6}H_{7}$ conformation by an intramolecular H-bond was observed for the corresponding *gluco*-configured tetrahydrotetrazolopyridine [26] and tetrahydroindolizines [23].

Enzymatic Tests and Discussion. – The nagstatin analogue **15** and the corresponding methyl ester **14** were tested as inhibitors of the *N*-acetyl-β-glucosaminidases from *Jack* beans (citrate buffer, pH 5.0, 25°) and from bovine kidney (citrate buffer, pH 4.1, 37°), the β-glucosidase from *C. saccharolyticum* (phosphate buffer, pH 6.8, 55°), and the β-mannosidase from snail (acetate buffer, pH 4.5, 25°), using the corresponding 4-nitrophenyl glycopyranosides as substrates. The inhibition data of **14** and **15** for the hexosaminidases are summarized in *Table 1* and compared to the inhibition by the parent acetamido-imidazole **16** and by nagstatin [2]. A comparison of the inhibition of the β-glucosidase and β-mannosidase by **14** and **15** to the inhibition by the glucose- and mannose-related analogues **6**–**9** [9] is shown in *Table 2*.

Table 1. *Data* (IC_{50} and K_i Values in [nM]) *for the Inhibition of the* N-*Acetyl-β-glucosaminidase from* Jack *Beans and from Bovine Kidney by the Acetamido-imidazoles* **1** *and* **14**–**16**

Inhibitor	pK_{HA}	*N*-Acetyl-β-glucosaminidase from *Jack* beans[a])		*N*-Acetyl-β-glucosaminidase from bovine kidney[b])	
		IC_{50}	K_i	IC_{50}	K_i
1[c])	–	–	–	13.4	–
16[c])	–	–	–	7.0	–
14	4.62	2.0	1.5 ($\alpha = 1.5$)	4.5	4.0 ($\alpha = 1.1$)
15	5.32[d])	7.8	5.9 ($\alpha = 1.5$)	8.1	5.2 ($\alpha = 3.0$)

[a]) At 25° and pH 5.0. [b]) At 37° and pH 4.1. [c]) Data taken from [2]. [d]) No additional pK_{HA} was observed between pH 2.3–5.7.

Table 2. *Comparison of the Inhibition* (K_i Values in [nM]) *of the β-Glucosidase from* C. saccharolyticum *and β-Mannosidase from Snail by the Acetamido-imidazoles* **14** *and* **15**, *and by Their Glucose- and Mannose-Derived Analogues* **6**–**9**

Inhibitor	pK_{HA}	β-Glucosidase from *C. saccharolyticum*[a])	β-Mannosidase from snail[b])
14	4.62	167 ($\alpha = 1.3$)	$20 \cdot 10^3$ (competitive)
15	5.32[c])	$110 \cdot 10^3$ ($\alpha = 1.6$)	$145 \cdot 10^3$ (competitive)
6[d])	5.03	5.0 (non-competitive)	–
7[d])	6.40	95 ($\alpha = 1.6$)	–
8[d])	4.61	–	117 ($\alpha = 3.4$)
9[d])	5.08	–	810 (competitive)

[a]) At 55° and pH 6.8. [b]) At 25° and pH 4.5. [c]) No additional pK_{HA} was observed between pH 2.3–5.7. [d]) Data taken from [9].

The acetamido-imidazoles **14** and **15** are nanomolar, albeit essentially non-competitive inhibitors of the *N*-acetyl-β-glucosaminidases (α-values between 1.1 and 3.0 [27]; *Table 1*). As expected from the *gluco*-configuration of **14** and **15**, these acetates are 2–3 times stronger inhibitors of the *N*-acetyl-β-glucosaminidase from bovine kidney than the *galacto*-configured nagstatin. The ester **14** is a stronger inhibitor of this enzyme than the *C(2)*-unsubstituted acetamide **16**, which is slightly but not significantly stronger than the acid **15**. That both glucosaminidases are more strongly

inhibited by the ester **14** is in agreement with the inhibition of other β-glycosidases by the *gluco*- and *manno*-analogues of **14** and **15** [9].

The methyl ester **14** is also a (mixed-type) inhibitor ($K_i = 167$ nM) of the β-glucosidase from *C. saccharolyticum*. Although **14** is 30–35 times weaker than the corresponding C(8)–OH analogue **6**, the submicromolar inhibition constant for **14** may imply that there is still a binding interaction of the enzyme with the acetamido group. The acid **15** (essentially dissociated at the pH of the assay) is a weaker inhibitor by about one order of magnitude than the ester **14**, evidencing a disrupting influence of the negatively charged carboxylate group. As expected, the imidazoles **14** and **15**, which bear an equatorial acetamido group, are only weak, albeit competitive, inhibitors of the β-mannosidase from snail.

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Experimental Part

General. Solvents were distilled: THF and toluene from Na and benzophenone, MeOH, Et_3N, and DBU from CaH_2. Reactions were carried out under Ar, unless stated otherwise. Qual. TLC: precoated silica-gel plates (*Merck* silica gel *60 F_{254}*); detection 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 ninhydrin. Flash chromatography (FC): silica gel *Fluka 60* (0.04–0.063 mm). M.p.'s uncorrected. Optical rotations: 1-dm cell at 25°, 589 nm. UV spectra (*ca.* 0.2 mM solns.) were taken in 1-cm cell at 25° in the range of 190 to 500 nm (log ε values in parenthesis). FT-IR spectra: KBr or *ca.* 2% soln. in $CHCl_3$, absorption in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: chemical shifts δ in ppm rel. to TMS as external standard, and coupling constants J in Hz. HR-MALDI-MS: in gentisic acid (=2,5-dihydroxybenzoic acid, DHB) matrix. The pK_{HA} values were determined in H_2O by potentiometric titration with HCl at 25°. The β-*N*-acetylglucosaminidase from *Jack* beans (EC 3.2.1.52, as a suspension in 2.5M $(NH_4)_2SO_4$, pH 7.0, *Sigma A-2264*), β-*N*-acetylglucosaminidase from bovine kidney (EC 3.2.1.52, as a suspension in 3.2M $(NH_4)_2SO_4$, pH *ca.* 6.0, *Sigma A-2415*), β-glucosidase from *C. saccharolyticum* (EC 3.2.1.21, as a lyophilized powder, *Sigma G-6906*), β-mannosidase from snail acetone powder (EC 3.2.1.25, as a suspension in 3.0M $(NH_4)_2SO_4$ containing 10 mM AcONa, pH *ca.* 4.0, *Sigma M-9400*), 4-nitrophenyl *N*-acetyl-β-D-glucosaminide (*Sigma N-9376*), 4-nitrophenyl β-D-glucopyranoside (*Fluka 73676*), and 4-nitrophenyl β-D-mannopyranoside (*Sigma N-1268*) were used without further purification.

Preparation of **20** *and* **21**. A suspension of **17** [14] (90 mg, 0.145 mmol) and $HgCl_2$ (56 mg, 0.206 mmol) in THF (1.7 ml) was treated with **19** [9] (162 mg, 0.914 mmol), stirred for 12 h at 22°, and filtered over *Celite*. The combined filtrate and washing (50 ml of AcOEt) were washed with brine (30 ml), dried ($MgSO_4$), filtered, and evaporated. A soln. of the residue (180 mg) in toluene (3.5 ml) was treated with $TsOH\cdot H_2O$ (129 mg, 0.678 mmol), stirred for 40 h at 60°, cooled to 22°, diluted with AcOEt (40 ml), and washed with sat. $NaHCO_3$ soln. (3 × 20 ml). The combined aq. layers were extracted with AcOEt (2 × 20 ml). The combined org. layers were washed with H_2O (40 ml) and brine (40 ml), dried ($MgSO_4$), filtered, and evaporated. FC (hexane/AcOEt 5:1 → 3:1) gave **20** (29 mg, 29%) and **21** (24 mg, 24%). Repetition of this experiment with 350 mg of **17** (0.565 mmol) gave **20** (97 mg, 25%), **20**/**21** 55:45 (6 mg, 2%) and **21** (99 mg, 25%).

*Methyl (5*R*,6*R*,7*S*,8*S*)-6,7-Bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydro-8-(triisopropylsilyloxy)-imidazo[1,2-a]pyridine-2-acetate* (**20**). Colourless oil. R_f (hexane/AcOEt 1:1) 0.65. $[\alpha]_D^{25} = +29.3$ ($c = 1.00$, $CHCl_3$). UV ($CHCl_3$): 285 (2.38), 264 (2.77), 259 (2.82), 240 (3.33). IR ($CHCl_3$): 3090*w*, 3067*w*, 3032*w*, 3012*m*, 2946*s*, 2867*s*, 1952*w*, 1877*w*, 1808*w*, 1736*s*, 1604*w*, 1528*w*, 1497*w*, 1455*m*, 1438*m*, 1363*m*, 1263*m*, 1223*m*, 1096*s*, 1018*m*, 913*w*, 883*m*. ^{1}H-NMR ($CDCl_3$, 300 MHz): see *Table 3*; additionally, 0.97, 1.02 (2*d*, $J = 7.2$, $(Me_2CH)_3Si$); 1.08–1.22 (*m*, $(Me_2CH)_3Si$); 3.57 (*d*, $J = 16.5$, CH–C(2)); 3.65 (*d*, $J = 16.5$, CH′–C(2)); 3.68 (*s*, MeO); 3.73 (irrad. at 4.07 → *d*, $J = 8.1$, irrad. at 4.31 → *d*, $J = 3.1$); 3.79 (irrad. at 4.31 → *d*, $J = 10.9$); 3.89 (irrad. at 3.79 → change, irrad. at 4.31 → *d*, $J = 10.3$); 4.07 (irrad. at 3.73 → *d*, $J = 3.1$, irrad. at 5.05 → *d*, $J = 3.4$); 4.31 (irrad. at 3.89 → *dd*, $J = 4.4, 8.4$); 4.36 (*d*, $J = 11.5$, PhC*H*); 4.50 (br. *s*, PhCH_2); 4.55 (*d*, $J = 11.5$, PhC*H*); 4.58 (*d*, $J = 11.2$, PhC*H*); 4.71 (*d*, $J = 11.5$, PhC*H*); 5.05 (irrad. at 4.07 → *s*); 7.15–7.19 (*m*, 2 arom. H); 7.26–7.38 (*m*, 13 arom. H). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 4*; additionally, 12.43 (*d*, $(Me_2CH)_3Si$); 17.99, 18.18 (2*q*, $(Me_2CH)_3Si$);

Table 3. *Selected ^{1}H-NMR Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *of the Protected and Deprotected* gluco-*Imidazoles* **14**, **15**, **20**, **22**, **25**, **27**, *and* **28**

	14 CD_3OD	**14** D_2O	**15** D_2O	**20** $CDCl_3$	**22** $CDCl_3$	**22** $(D_6)DMSO$	**25** $CDCl_3$	**27** $CDCl_3$	**28** $CDCl_3$
H–C(3)	7.22	7.20	7.23	6.97	6.98	7.07	6.98	6.93	7.00
H–C(5)	3.84–3.91	3.94–4.06	4.02–4.12	4.31	4.12	4.15	4.11	4.18	4.40–4.47
H–C(6)	3.84–3.91	3.94–4.06	4.03	3.73	3.93	3.82–3.90	3.97	3.98	4.02[a])
H–C(7)	3.73	3.90	3.93	4.07	3.98	3.82–3.90	3.90	3.84	4.06[b])
H–C(8)	4.95	4.89	4.98	5.05	4.87	4.59[c])	4.67	4.12	5.38[d])
CH–C(5)	3.94	4.08	4.02–4.12	3.79	3.70	3.71	3.68	3.69	3.72
CH′–C(5)	4.16	4.22	4.23	3.89	3.79	3.90	3.77	3.76	3.76
J(5,6)	[e])	[e])	9.3	8.4	7.5	7.5	7.2	6.2	2.5
J(6,7)	9.0	9.7	9.3	3.4	8.4	[e])	8.4	7.5	5.0
J(7,8)	8.7	9.7	9.0	3.4	7.5	6.5	7.2	6.5	2.5
J(5,CH)	3.7	[e])	[e])	5.6	5.0	4.7	4.4	5.6	6.9
J(5,CH′)	1.9	[e])	2.2	2.8	3.4	2.8	3.1	3.7	5.6
J(CH,CH′)	12.1	12.5	12.8	10.6	10.3	10.3	10.0	10.0	10.0

[a]) $J(6,8) \approx 0.6$ Hz. [b]) $J(5,7) \approx 0.6$ Hz. [c]) $J(8,OH) = 5.6$ Hz. [d]) $J(8,NH) = 8.1$ Hz. [e]) Not assigned.

Table 4. *Selected ^{13}C-NMR Chemical Shifts* [ppm] *of the Protected and Deprotected* gluco-*Imidazoles* **14**, **15**, **20**, **22**, **25**, **27**, *and* **28** *and of the Protected* manno-*Imidazoles* **21** *and* **23**

	Solvent	C(2)	C(3)	C(5)	CH_2–C(5)	C(6)	C(7)	C(8)	C(8a)
gluco									
14	D_2O	133.93	116.26	60.18	58.65	67.87	72.01	49.87	143.68
15	D_2O	134.11	116.16	60.87	58.34	67.59	71.35	49.11	142.67
20	$CDCl_3$	133.43	115.09	56.60	67.66	78.57	82.73	66.25	144.97
22	$CDCl_3$	134.80	115.19	58.66	68.63	75.02	81.69	67.63	145.95
25	$CDCl_3$	135.55	115.87	58.73[a])	68.18	75.22	81.05	59.09[a])	140.21
27	$CDCl_3$	134.75	115.44	58.81	69.49	75.65	82.27	50.39	146.25
28	$CDCl_3$	134.67	117.12	58.81	71.33	74.19[a])	75.87[a])	46.10	140.42
manno									
21	$CDCl_3$	134.32	116.70	59.74	70.82	73.62	81.63	64.26	144.76
23	$CDCl_3$	134.75	116.28	59.03	70.36	73.25	78.77	62.92	144.00

[a]) Assignments may be interchanged.

34.73 (*t*, CH_2–C(2)); 51.88 (*q*, MeO); 72.10, 72.41, 73.16 (3*t*, 3 PhCH_2); 127.64–128.37 (several *d*); 137.26, 137.51, 137.56 (3*s*); 171.60 (*s*, C=O). HR-MALDI-MS: 721.3647 (29, $[M+Na]^+$, $C_{41}H_{54}N_2NaO_6Si^+$; calc. 721.3649), 699.3822 (24, $[M+H]^+$, $C_{41}H_{55}N_2O_6Si^+$; calc. 699.3829), 655.3203 (4, $[M-i\text{-}Pr]^+$, $C_{38}H_{47}N_2O_6Si^+$; calc. 655.3203), 525.2386 (100, $[M-(i\text{-}Pr)_3SiO]^+$, $C_{32}H_{33}N_2O_5^+$; calc. 525.2389). Anal. calc. for $C_{41}H_{54}N_2O_6Si$ (698.97): C 70.45, H 7.79, N 4.01; found: C 70.31, H 7.80, N 4.08.

*Methyl (5*R*,6*R*,7*S*,8*R*)-6,7-Bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydro-8-(triisopropylsilyloxy)-imidazo[1,2-*a*]pyridine-2-acetate* (**21**). Colourless oil. R_f (hexane/AcOEt 1:1) 0.52. $[\alpha]_D^{25} = -20.3$ ($c = 0.69$, $CHCl_3$). UV ($CHCl_3$): 264 (2.83), 259 (2.86), 240 (3.27). IR ($CHCl_3$): 3090*w*, 3067*w*, 3031*m*, 3013*m*, 2946*s*, 2893*m*, 2867*s*, 1952*w*, 1875*w*, 1810*w*, 1736*s*, 1604*w*, 1561*w*, 1497*w*, 1455*s*, 1438*m*, 1408*w*, 1363*m*, 1266*m*, 1222*m*, 1102*s*, 1017*s*, 952*w*, 915*w*, 883*m*. ^{1}H-NMR ($CDCl_3$, 300 MHz): see *Table 5*; additionally, 0.94, 1.03 (2*d*, $J \approx 7.2$, $(Me_2CH)_3Si$); 1.08–1.22 (*m*, $(Me_2CH)_3Si$); 3.55 (irrad. at 3.65 → *d*, $J \approx 5.0$, irrad. at 4.05 → change); 3.59 (br. *s*, CH_2–C(2)); 3.65 (irrad. at 4.05 → *d*, $J \approx 9.3$); 3.68 (*s*, MeO); 3.76 (irrad. at 5.17 → *d*, $J = 9.3$); 4.05 (irrad. at 3.65 → *t*, $J = 6.9$); 4.30 (irrad. at 3.76 → *d*, $J = 7.5$, irrad. at 4.05 → *d*, $J = 9.3$); 4.43 (br. *s*, PhCH_2); 4.61 (*d*, $J = 11.5$, PhCH); 4.65 (*d*, $J = 11.8$, PhCH); 4.82 (*d*, $J = 11.5$, PhCH); 4.93 (*d*, $J = 11.2$, PhCH); 5.17 (irrad. at 3.76 → *s*);

7.22 – 7.38 (*m*, 15 arom. H). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 4*; additionally, 12.50 (*d*, $(Me_2CH)_3Si$); 18.02, 18.22 (2*q*, $(Me_2CH)_3Si$); 34.56 (*t*, CH_2–C(2)); 51.92 (*q*, MeO); 71.97, 73.06, 74.68 (3*t*, 3 PhCH_2); 127.48 – 128.32 (several *d*); 137.46 (*s*); 137.88 (2*s*); 171.47 (*s*, C=O). HR-MALDI-MS: 721.3641 (28, $[M+Na]^+$, $C_{41}H_{54}N_2NaO_6Si^+$; calc. 721.3649), 699.3817 (82, $[M+H]^+$, $C_{41}H_{55}N_2O_6Si^+$; calc. 699.3829), 655.3196 (6, $[M-$i-Pr$]^+$, $C_{38}H_{47}N_2O_6Si^+$; calc. 655.3203), 525.2380 (100, $[M-$(i-Pr)$_3$SiO$]^+$, $C_{32}H_{33}N_2O_5^+$; calc. 525.2389). Anal. calc. for $C_{41}H_{54}N_2O_6Si$ (698.97): C 70.45, H 7.79, N 4.01; found: C 70.32, H 7.79, N 4.15.

Table 5. *Selected ^{1}H-NMR Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *of the Protected* manno*-Imidazoles* **21**, **23**, *and* **26**

	21 $CDCl_3$	**23** $CDCl_3$	**23** (D$_6$)DMSO	**26** $CDCl_3$
H–C(3)	6.99	7.04	7.07	7.04
H–C(5)	4.05	4.12	4.10	4.06
H–C(6)	4.30	4.19	4.17	4.12
H–C(7)	3.76	3.95	3.845	3.99
H–C(8)	5.17	5.07	4.86[a])	4.875
CH–C(5)	3.55	3.70	3.67	3.648
CH′–C(5)	3.65	3.77	3.847	3.74
J(5,6)	7.5	6.2	6.8	7.2
J(6,7)	9.3	7.8	8.7	8.1
J(7,8)	2.2	3.4	3.4	3.7
J(5,CH)	6.9	6.2	5.6	5.6
J(5,CH′)	3.1	3.7	3.4	3.4
J(CH,CH′)	10.0	10.0	10.0	10.0

[a]) *J*(8,OH) = 5.0 Hz

Preparation of **22** *and* **23**. a) *Treatment of* **18** *with the β-Amino Ester* **19** *and* $HgCl_2$. A suspension of **18** [15] (300 mg, 0.593 mmol) and $HgCl_2$ (225 mg, 0.829 mmol) in THF (6.9 ml) was treated with **19** [9] (635 mg, 3.58 mmol), stirred for 19 h at 23°, and filtered over *Celite*. The combined filtrate and washing (80 ml of AcOEt) were washed with brine (50 ml), dried ($MgSO_4$), filtered, and evaporated. A soln. of the residue (660 mg) in toluene (15 ml) was treated with TsOH · H_2O (525 mg, 2.76 mmol), stirred for 40 h at 60°, cooled to 22°, diluted with AcOEt (100 ml), and washed with sat. $NaHCO_3$ soln. (3 × 70 ml). The combined aq. layers were extracted with AcOEt (2 × 60 ml). The combined org. layers were washed with H_2O (120 ml) and brine (120 ml), dried ($MgSO_4$), filtered, and evaporated. FC (hexane/AcOEt/MeOH 1:3:0 → 0:1:0 → 0:20:1) gave **22** (102 mg, 32%), **22**/**23** 28:72 (53 mg, 16%) and **23** (107 mg, 33%). Repetition of this experiment with 980 mg of **18** (1.94 mmol) gave **22** (299 mg, 28%), **22**/**23** 1:4 (236 mg, 22%), and **23** (299 mg, 28%).

b) *Desilylation of* **20**. At 0°, a soln. of **20** (68 mg, 97.3 μmol) in THF (1.3 ml) was treated with 1M Bu_4NF in THF (0.20 ml, 0.20 mmol), stirred at 0° for 2 h, and treated with sat. NH_4Cl soln. (5 ml). The mixture was diluted with Et_2O (30 ml) and washed with sat. NH_4Cl soln. (3 × 15 ml). The combined aq. layers were extracted with Et_2O (2 × 15 ml). The combined org. layers were washed with H_2O (30 ml) and brine (30 ml), dried ($MgSO_4$), filtered, and evaporated. FC (AcOEt) gave **22** (49 mg, 93%).

c) *Desilylation of* **21**. At 0°, a soln. of **21** (80 mg, 0.115 mmol) in THF (1.5 ml) was treated with 1M Bu_4NF in THF (0.22 ml, 0.22 mmol), stirred at 0° for 2 h, and treated with sat. NH_4Cl soln. (5 ml). Workup and FC (as described in a) gave **23** (55 mg, 93%).

*Methyl (5*R,*6*R,*7*S,*8*S*)-6,7-Bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydro-8-hydroxyimidazo[1,2-*a*]pyridine-2-acetate* (**22**). Colourless oil. R_f (AcOEt) 0.15. R_f (AcOEt/MeOH 10:1) 0.44. $[\alpha]_D^{25} = -3.1$ ($c = 0.74$, $CHCl_3$). UV ($CHCl_3$): 258 (3.07), 240 (3.44). IR ($CHCl_3$): 3305*w*, 3163*w*, 3090*w*, 3067*w*, 3031*m*, 3011*m*, 2954*m*, 2918*m*, 2869*m*, 1952*w*, 1877*w*, 1810*w*, 1736*s*, 1604*w*, 1565*w*, 1497*m*, 1454*s*, 1438*m*, 1408*w*, 1361*m*, 1308*m*, 1269*m*, 1144*s*, 1111*s*, 1027*s*, 912*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): see *Table 3*; additionally, 3.67 (br. *s*, CH_2–C(2)); 3.68 (*s*, MeO); 3.70 (irrad. at 4.12 → *d*, *J* = 10.2); 3.79 (irrad. at 4.12 → *d*, *J* = 10.2); 3.93 (irrad. at 4.12 → *d*, *J* = 8.2); 3.98 (irrad. at 4.87 → *d*, *J* = 8.2); 4.42 (*d*, *J* = 12.1, PhC*H*); 4.46 (*d*, *J* = 12.1, PhC*H*); 4.53 (*d*, *J* = 11.2, PhC*H*); 4.84 (*d*, *J* = 11.5, PhC*H*); 4.87 (irrad. at 3.98 → br. *s*); 4.91 (*d*, *J* = 11.2, PhC*H*); 5.07 (*d*, *J* = 11.2, PhC*H*);

6.26–6.36 (br. *s*, OH); 7.18–7.21 (*m*, 2 arom. H); 7.24–7.40 (*m*, 13 arom. H). ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): see *Table 3*; additionally, 3.52 (br. *s*, CH$_2$–C(2)); 3.59 (*s*, MeO); 3.71 (irrad. at 4.15 → *d*, *J* = 10.3); 3.82–3.90 (irrad. at 4.15 → change); 3.90 (irrad. at 4.15 → *d*, *J* = 10.6); 4.44 (*d*, *J* = 12.1, PhC*H*); 4.45 (*d*, *J* = 10.9, PhC*H*); 4.50 (*d*, *J* = 12.1, PhC*H*); 4.59 (irrad. at 5.84 → *d*, *J* = 6.5); 4.73 (*d*, *J* = 11.5, PhC*H*); 4.77 (*d*, *J* = 11.5, PhC*H*); 4.88 (*d*, *J* = 11.8, PhC*H*); 5.84 (*d*, *J* = 5.6, exchange with CD$_3$OD, OH); 7.15–7.20 (*m*, 2 arom. H); 7.23–7.37 (*m*, 13 arom. H). ^{13}C-NMR (CDCl$_3$, 75 MHz): see *Table 4*; additionally, 34.06 (*t*, *C*H$_2$–C(2)); 51.95 (*q*, MeO); 73.20, 74.60, 74.67 (3*t*, 3 PhCH$_2$); 127.57–128.37 (several *d*); 137.20, 137.26, 138.10 (3*s*); 171.34 (*s*, C=O). HR-MALDI-MS: 581.2053 (2, $[M+K]^+$, $C_{32}H_{34}KN_2O_6^+$; calc. 581.2054), 565.2304 (32, $[M+Na]^+$, $C_{32}H_{34}N_2NaO_6^+$; calc. 565.2314), 543.2490 (100, $[M+H]^+$, $C_{32}H_{35}N_2O_6^+$; calc. 543.2495), 525.2366 (18, $[M-OH]^+$, $C_{32}H_{33}N_2O_5^+$; calc. 525.2389). Anal. calc. for $C_{32}H_{34}N_2O_6$ (542.63): C 70.83, H 6.32, N 5.16; found: C 70.56, H 6.52, N 5.24.

*Methyl (5*R*,6*R*,7*S*,8*R*)-6,7-Bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydro-8-hydroxyimidazo[1,2-*a*]pyridine-2-acetate* (**23**). White solid. M. p. 109–111° (AcOEt/hexane 1:1). R_f (AcOEt) 0.11. R_f (AcOEt/MeOH 10:1) 0.39. $[\alpha]_D^{25} = +0.7$ (*c* = 1.01, CHCl$_3$). UV (CHCl$_3$): 259 (2.91), 240 (3.33). IR (CHCl$_3$): 3304*w*, 3163*w*, 3089*w*, 3067*w*, 3031*m*, 3011*m*, 2954*m*, 2925*m*, 2869*m*, 1953*w*, 1877*w*, 1810*w*, 1736*s*, 1603*w*, 1564*w*, 1496*w*, 1454*m*, 1438*m*, 1407*w*, 1363*m*, 1306*m*, 1269*m*, 1099*s*, 1018*m*, 911*w*. ^{1}H-NMR (CDCl$_3$, 300 MHz): see *Table 5*; additionally, 3.57 (*d*, *J* = 16.5, CH–C(2)); 3.64 (*d*, *J* = 17.1, CH′–C(2)); 3.68 (*s*, MeO); 4.45 (*d*, *J* = 12.1, PhC*H*); 4.50 (*d*, *J* = 12.1, PhC*H*); 4.57 (*d*, *J* = 11.5, PhC*H*); 4.71 (*d*, *J* = 12.1, PhC*H*); 4.79 (*d*, *J* = 11.8, PhC*H*); 4.87 (*d*, *J* = 11.2, PhC*H*); 7.22–7.38 (*m*, 15 arom. H). ^{1}H-NMR ((D$_6$)DMSO, 300 MHz): see *Table 5*; additionally, 3.47 (*d*, *J* = 16.2, CH–C(2)); 3.53 (*d*, *J* = 16.8, CH′–C(2)); 3.58 (*s*, MeO); 3.67 (irrad. at 4.10 → *d*, *J* = 10.3); 3.845 (irrad. at 4.17 → *d*, *J* = 3.1, irrad. at 4.86 → *d*, *J* = 8.4); 3.847 (irrad. at 3.67 → change, irrad. at 4.10 → change); 4.10 (irrad. at 3.67 → change); 4.44 (*d*, *J* = 12.1, PhC*H*); 4.51 (br. *d*, *J* = 11.2, 2 PhC*H*); 4.59 (*d*, *J* = 12.1, PhC*H*); 4.77 (*d*, *J* = 11.8, PhC*H*); 4.79 (*d*, *J* = 11.2, PhC*H*); 4.86 (irrad. at 5.60 → *d*, *J* = 3.4); 5.60 (*d*, *J* = 5.0, irrad. at 4.86 → *s*, exchange with CD$_3$OD, OH); 7.19–7.39 (*m*, 15 arom. H). ^{13}C-NMR (CDCl$_3$, 75 MHz): see *Table 4*; additionally, 34.22 (*t*, *C*H$_2$–C(2)); 51.97 (*q*, MeO); 72.66, 73.19, 74.21 (3*t*, 3 PhCH$_2$); 127.69–128.37 (several *d*); 137.27, 137.43, 137.46 (3*s*); 171.42 (*s*, C=O). HR-MALDI-MS: 581.2054 (7, $[M+K]^+$, $C_{32}H_{34}KN_2O_6^+$; calc. 581.2054), 565.2297 (37, $[M+Na]^+$, $C_{32}H_{34}N_2NaO_6^+$; calc. 565.2314), 543.2484 (100, $[M+H]^+$, $C_{32}H_{35}N_2O_6^+$; calc. 543.2495), 525.2357 (18, $[M-OH]^+$, $C_{32}H_{33}N_2O_5^+$; calc. 525.2389). Anal. calc. for $C_{32}H_{34}N_2O_6$ (542.63): C 70.83, H 6.32, N 5.16; found: C 70.84, H 6.45, N 5.08.

*Methyl (*R**)- and (*S**)-2-Azido-2-[(5*R*,6*R*,7*S*)-6,7-bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridin-2-yl]acetate* (**24**). At 0°, a soln. of **22** (35 mg, 64.5 μmol) and Ph$_3$P (25 mg, 95.3 μmol) in THF (3 ml) was successively treated with 0.67M HN$_3$ in toluene[5]) (0.19 ml, 0.127 mmol) and *ca.* 40% DEAD in toluene (44 μl, 96 μmol), stirred for 5 min at 0° and 3 h at 23°, diluted with Et$_2$O (40 ml), and washed with sat. NH$_4$Cl soln. (3 × 15 ml). The combined aq. layers were extracted with Et$_2$O (2 × 15 ml). The combined org. layers were washed with H$_2$O (30 ml) and brine (30 ml), dried (MgSO$_4$), filtered, and evaporated. FC (hexane/AcOEt/MeOH 1:1:0 → 1:3:0 → 0:1:0 → 0:10:1) gave **24** (17.3 mg, 47%, (2*R**)/(2*S**) 2:1) and **22** (11.2 mg, 32%).

Data of **24**: Colourless oil. R_f (hexane/AcOEt 1:1) 0.16. IR (CHCl$_3$): 3090*w*, 3067*w*, 3032*w*, 3012*m*, 2956*m*, 2926*m*, 2868*m*, 2109*s*, 1951*w*, 1879*w*, 1810*w*, 1747*s*, 1673*w*, 1603*w*, 1521*w*, 1496*w*, 1454*m*, 1437*m*, 1364*m*, 1334*m*, 1266*m*, 1163*m*, 1097*s*, 1076*s*, 1027*m*, 910*w*. ^{1}H-NMR (CDCl$_3$, 300 MHz, (2*R**)/(2*S**) 2:1): 3.00 (*dd*, *J* = 5.9, 16.8, irrad. at 3.25 → *d*, *J* ≈ 7.2, H–C(8)); 3.25 (*dd*, *J* = 4.7, 16.8, irrad. at 3.00 → change, H′–C(8)); 3.69 (*dd*, *J* = 6.2, 10.0, irrad. at 4.24 → *d*, *J* = 9.7, CH–C(5)); 3.75 (*dd*, *J* = 4.7, 10.0, irrad. at 3.69 → *d*, *J* ≈ 7.5, irrad. at 4.24 → *d*, *J* = 9.7, 0.7 H), 3.76 (*dd*, *J* = 4.1, 10.0, 0.3 H) (CH′–C(5)); 3.78 (*s*, 2.1 H), 3.79 (*s*, 0.9 H) (MeO); 3.92 (*dd*, *J* = 4.7, 6.2, irrad. at 4.24 → *d*, *J* = 6.5, 0.7 H), 3.93 (*dd*, *J* ≈ 4.4, 6.5, 0.3 H) (H–C(6)); 4.00 (*dt*, *J* = 4.7, 5.9, irrad. at 3.00 → change, irrad. at 3.25 → *dd*, *J* = 4.7, 6.2, H–C(7)); 4.22 (*td*, *J* = 4.7, 6.2, 0.3 H), 4.24 (*td*, *J* = 4.7, 6.2, irrad. at 3.69 → *t*, *J* = 4.4, 0.7 H) (H–C(5)); 4.41 (*d*, *J* = 12.1, 0.3 H), 4.42 (*d*, *J* = 12.1, 0.7 H) (PhC*H*); 4.47 (*d*, *J* = 11.8, 0.7 H), 4.49 (*d*, *J* = 12.5, 0.3 H) (PhC*H*); 4.54 (*d*, *J* ≈ 11.8, 0.3 H), 4.57 (*d*, *J* = 11.5, 0.7 H) (PhC*H*); 4.61 (*d*, *J* = 11.5, PhC*H*); 4.642 (*d*, *J* = 11.8, 0.7 H), 4.644 (*d*, *J* = 12.1, 0.3 H) (PhC*H*); 4.77 (*d*, *J* = 11.5, PhC*H*); 4.87 (br. *s*, $w_{1/2}$ = 1.4, 0.7 H), 4.88 (br. *s*, $w_{1/2}$ = 1.4, 0.3 H) (CH–C(2)); 7.108 (*d*, *J* = 0.6, irrad. at 4.875 → *s*, 0.7 H), 7.112 (*d*, *J* = 0.6, irrad. at 4.875 → *s*, 0.3 H) (H–C(3)); 7.22–7.38 (*m*, 15 arom. H). ^{13}C-NMR (CDCl$_3$, 75 MHz, (2*R**)/(2*S**) 2:1): 27.29 (*t*, C(8)); 52.88 (*q*, MeO); 58.72 (*d*, 0.7 C), 58.80 (*d*, 0.3 C), 59.94 (*d*, 0.7 C), 60.08 (*d*, 0.3 C) (C(5), *C*H–C(2)); 70.36 (*t*, *C*H$_2$–C(5)); 71.55 (*t*, PhCH$_2$); 73.25, 73.30, 73.35 (3*t*, 2 PhCH$_2$); 74.52, 74.62 (2*d*, C(6), C(7)); 116.53 (*d*, 0.3 C), 116.65 (*d*, 0.7 C) (C(3)); 127.47–128.40 (several *d*); 134.16 (*s*, 0.3 C), 134.20 (*s*,

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[5]) Prepared according to [28]. The molar concentration was determined by titration with 0.92M NaOH using phenolphthalein as indicator.

0.7 C) (C(2)); 137.26 (2*s*); 137.40 (*s*); 142.82 (br. *s*, C(8a)); 169.37 (*s*, 0.7 C), 169.47 (*s*, 0.3 C) (C=O). HR-MALDI-MS: 568.2550 (59, $[M+H]^+$, $C_{32}H_{34}N_5O_5^+$; calc. 568.2560), 562.2319 (22, $[M+Na-N_2]^+$, $C_{32}H_{33}N_3NaO_5^+$; calc. 562.2318), 540.2489 (100, $[M+H-N_2]^+$, $C_{32}H_{34}N_3O_5^+$; calc. 540.2498), 527.2535 (27, $[M+2\,H-N_3]^+$, $C_{32}H_{35}N_2O_5^+$; calc. 527.2546), 525.2375 (15, $[M-N_3]^+$, $C_{32}H_{33}N_2O_5^+$; calc. 525.2389), 480.2281 (27, $[M-N_2-COOMe]^+$, $C_{30}H_{30}N_3O_3^+$; calc. 480.2287), 419.1958 (32, $[M+H-N_3-BnO]^+$, $C_{25}H_{27}N_2O_4^+$; calc. 419.1971).

*Methyl (5*R*,6*R*,7*S*,8*S*)-8-Azido-6,7-bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-2-acetate* (**25**). *a*) A soln. of **23** (51 mg, 94.0 µmol) in toluene (1 ml) was successively treated with DPPA (0.1 ml, 0.464 mmol) and DBU (70 µl, 0.469 mmol), stirred for 5 h at 23°, diluted with CH_2Cl_2 (50 ml), and washed with sat. NH_4Cl soln. (3×25 ml). The combined aq. layers were extracted with CH_2Cl_2 (2×30 ml). The combined org. layers were washed with H_2O (60 ml) and brine (60 ml), dried ($MgSO_4$), filtered, and evaporated. FC (hexane/AcOEt 1:0 → 3:1 → 1:1) gave **25** (37 mg, 69%).

b) Repetition of the experiment *a*): a mixture of 295 mg **23** (0.544 mmol), DPPA (0.59 ml, 2.74 mmol), and DBU (0.41 ml, 2.75 mmol) in toluene (5.5 ml) was stirred for 4 h at 23°. Workup and FC gave **25** (180 mg, 58%).

Data of **25**: Yellowish oil. R_f (hexane/AcOEt 1:1) 0.25. $[\alpha]_D^{25}=+28.5$ ($c=1.04$, $CHCl_3$). UV ($CHCl_3$): 241 (3.53). IR ($CHCl_3$): 3089*w*, 3067*w*, 3032*w*, 3011*w*, 2954*w*, 2921*w*, 2870*w*, 2109*s*, 1951*w*, 1877*w*, 1810*w*, 1736*m*, 1589*w*, 1564*w*, 1496*w*, 1454*m*, 1438*w*, 1407*w*, 1362*m*, 1332*w*, 1269*m*, 1145*m*, 1098*m*, 1018*m*, 917*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): see *Table 3*; additionally, 3.66 (br. *s*, CH_2–C(2)); 3.68 (irrad. at 3.77 → change, irrad. at 4.11 → *d*, $J=10.0$); 3.71 (*s*, MeO); 3.77 (irrad. at 4.11 → *d*, $J=10.0$); 3.90 (irrad. at 3.97 → change, irrad. at 4.67 → *d*, $J=8.4$); 3.97 (irrad. at 3.90 → change, irrad. at 4.11 → change); 4.11 (irrad. at 3.77 → *dd*, $J=2.8$, 7.2, irrad. at 3.97 → change); 4.39 (*d*, $J=11.8$, PhC*H*); 4.45 (*d*, $J=12.1$, PhC*H*); 4.54 (*d*, $J=11.5$, PhC*H*); 4.67 (irrad. at 3.90 → *s*); 4.82 (*d*, $J=11.2$, PhC*H*); 4.87 (*d*, $J=11.2$, PhC*H*); 4.88 (*d*, $J=11.2$, PhC*H*); 7.18–7.25 (*m*, 4 arom. H); 7.27–7.37 (*m*, 11 arom. H). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 4*; additionally, 34.39 (*t*, *C*H_2–C(2)); 51.98 (*q*, MeO); 73.19, 74.59, 74.91 (3*t*, 3 Ph*C*H_2); 127.75–128.42 (several *d*); 136.95 (*s*); 137.04 (2*s*); 171.34 (*s*, C=O). HR-MALDI-MS: 590.2363 (25, $[M+Na]^+$, $C_{32}H_{33}N_5NaO_5^+$; calc. 590.2379), 568.2549 (86, $[M+H]^+$, $C_{32}H_{34}N_5O_5^+$; calc. 568.2560), 562.2317 (82, $[M+Na-N_2]^+$, $C_{32}H_{33}N_3NaO_5^+$; calc. 562.2318), 540.2486 (57, $[M+H-N_2]^+$, $C_{32}H_{34}N_3O_5^+$; calc. 540.2498), 527.2537 (64, $[M+2\,H-N_3]^+$, $C_{32}H_{35}N_2O_5^+$; calc. 527.2546), 525.2374 (100, $[M-N_3]^+$, $C_{32}H_{33}N_2O_5^+$; calc. 525.2389), 434.2066 (18), 324.1337 (18), 310.1182 (24). Anal. calc. for $C_{32}H_{33}N_5O_5$ (567.64): C 67.71, H 5.86, N 12.34; found: C 67.85, H 5.83, N 12.33.

Treatment of **22** *with DPPA and DBU.* A soln. of **22** (50 mg, 92.1 µmol) in toluene (1 ml) was successively treated with DPPA (99 µl, 0.459 mmol) and DBU (69 µl, 0.462 mmol) and stirred for 22 h at 23°. Workup (as described for the preparation of **25**) and FC (hexane/AcOEt 1:0 → 3:1 → 1:1 → 1:3) of the mixture gave **25** (3.2 mg, 6%) and **26** (1.6 mg, 3%).

*Methyl (5*R*,6*R*,7*S*,8*R*)-8-Azido-6,7-bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-2-acetate* (**26**). Yellow oil. R_f (hexane/AcOEt 1:1) 0.13. ^{1}H-NMR ($CDCl_3$, 300 MHz): see *Table 5*; additionally, 3.59 (*d*, $J=14.6$, CH–C(2)); 3.646 (*d*, $J=14.6$, CH′–C(2)); 3.70 (*s*, MeO); 4.42 (*d*, $J=12.1$, PhC*H*); 4.47 (*d*, $J=11.8$, PhC*H*); 4.56 (*d*, $J=11.2$, PhC*H*); 4.71 (*d*, $J=11.8$, PhC*H*); 4.76 (*d*, $J=11.8$, PhC*H*); 4.875 (*d*, $J=10.9$, PhC*H*); 7.18–7.39 (*m*, 15 arom. H).

*Methyl (5*R*,6*R*,7*S*,8*S*)-8-Amino-6,7-bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-2-acetate* (**27**). A soln. of **25** (31 mg, 54.6 mmol) in MeOH (0.5 ml) was successively treated with Et_3N (0.15 ml, 1.08 mmol) and propane-1,3-dithiol (0.15 ml, 1.49 mmol) and stirred for 23 h at 23°. The mixture was diluted with CH_2Cl_2 (40 ml) and poured onto 0.1M NaOH/ice (40 ml). The layers were separated, and the aq. layer was extracted with CH_2Cl_2 (3×20 ml). The combined org. layers were dried ($MgSO_4$). Evaporation and FC (AcOEt/MeOH 1:0 → 10:1 → 5:1) gave **27** (25.3 mg, 85%). Yellowish oil. R_f (AcOEt/MeOH 10:1) 0.11. $[\alpha]_D^{25}=+12.8$ ($c=0.98$, $CHCl_3$). UV ($CHCl_3$): 258 (2.86), 240 (3.40). IR ($CHCl_3$): 3378*w*, 3308*w*, 3167*w*, 3090*w*, 3067*w*, 3032*m*, 3009*m*, 2954*m*, 2929*m*, 2869*m*, 1952*w*, 1875*w*, 1810*w*, 1736*s*, 1587*w*, 1497*m*, 1454*s*, 1438*m*, 1407*w*, 1363*m*, 1269*m*, 1137*s*, 1098*s*, 1027*m*, 1020*m*, 940*w*, 912*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): see *Table 3*; additionally, 2.00 (br. *s*, exchange with CD_3OD, NH_2); 3.62 (br. *s*, CH_2–C(2)); 3.69 (irrad. at 3.76 → *d*, $J\approx5.0$, irrad. at 4.18 → *d*, $J\approx10.0$); 3.70 (*s*, MeO); 3.76 (irrad. at 4.18 → *d*, $J=10.0$); 3.84 (irrad. at 3.98 → *d*, $J\approx5.9$, irrad. at 4.12 → *d*, $J=7.5$); 3.98 (irrad. at 3.84 → change, irrad. at 4.18 → *d*, $J=7.5$); 4.12 (irrad. at 3.84 → *s*); 4.18 (irrad. at 3.76 → br. *t*, $J\approx5.6$, irrad. at 3.98 → change); 4.41 (*d*, $J=12.1$, PhC*H*); 4.46 (*d*, $J=12.1$, PhC*H*); 4.56 (*d*, $J=11.2$, PhC*H*); 4.80 (br. *s*, PhCH_2); 4.82 (*d*, $J=11.2$, PhC*H*); 7.20–7.36 (*m*, 15 arom. H). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 4*; additionally, 34.38 (*t*, *C*H_2–C(2)); 51.92 (*q*, MeO); 73.24, 74.11, 74.30 (3*t*, 3 Ph*C*H_2); 127.80–128.53 (several *d*); 137.33, 137.42, 137.88 (3*s*); 171.69 (*s*, C=O). HR-MALDI-MS: 564.2461 (15, $[M+Na]^+$, $C_{32}H_{35}N_3NaO_5^+$; calc. 564.2474), 542.2639 (14, $[M+H]^+$, $C_{32}H_{36}N_3O_5^+$; calc. 542.2655), 525.2368 (100,

$[M-NH_2]^+$, $C_{32}H_{33}N_2O_5^+$; calc. 525.2389). Anal. calc. for $C_{32}H_{35}N_3O_5 \cdot 0.5\ H_2O$ (550.65): C 69.80, H 6.59, N 7.63; found: C 69.86, H 6.63, N 7.67.

*Methyl (5*R*,6*R*,7*S*,8*S*)-8-Acetamido-6,7-bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine-2-acetate* (**28**). A soln. of **27** (10 mg, 18.5 μmol) in pyridine/Ac_2O 3:1 (0.4 ml) was stirred for 3 h at 23°, evaporated, and co-evaporated with toluene (3 × 5 ml). FC (AcOEt/MeOH 1:0 → 20:1) gave **28** (8.8 mg, 81%). Colourless oil. R_f (AcOEt/MeOH 10:1) 0.35. $[\alpha]_D^{25} = +8.0$ ($c = 0.40$, $CHCl_3$). UV ($CHCl_3$): 258 (2.84), 240 (3.39). IR ($CHCl_3$): 3441*w*, 3308*w* (br.), 3089*w*, 3066*w*, 3019*m*, 3009*m*, 2954*m*, 2929*m*, 2869*m*, 1951*w*, 1875*w*, 1810*w*, 1736*s*, 1669*s*, 1603*w*, 1497*s*, 1455*s*, 1438*m*, 1408*w*, 1368*m*, 1268*m*, 1153*m*, 1097*s*, 1028*m*, 1018*m*, 911*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): see *Table 3*; additionally, 1.89 (*s*, AcN); 3.63 (br. *s*, $CH_2-C(2)$); 3.70 (*s*, MeO); 3.72 (irrad. at 4.44 → *d*, $J \approx 10.3$); 3.76 (irrad. at 4.44 → *d*, $J \approx 11.5$); 4.02 (irrad. at 4.44 → br. *d*, $J = 5.0$, irrad. at 5.38 → *dd*, $J = 2.5, 4.7$); 4.06 (irrad. at 4.44 → *dd*, $J = 2.8, 4.7$, irrad. at 5.38 → br. *d*, $J = 4.7$); 4.42 (*d*, $J =$ 11.8, PhC*H*); 4.48 (*d*, $J = 11.8$, PhC*H*); 4.52 (*d*, $J = 12.5$, PhC*H*); 4.56 (*d*, $J = 11.8$, PhC*H*); 4.68 (*d*, $J = 12.1$, PhC*H*); 4.84 (*d*, $J = 11.8$, PhC*H*); 5.38 (irrad. at 6.02 → br. *d*, $J = 2.5$); 6.02 (*d*, $J = 8.1$, irrad. at 5.38 → *s*, NH); 7.15–7.20 (*m*, 2 arom. H); 7.23–7.38 (*m*, 13 arom. H). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 4*; additionally, 23.38 (*q*, *Me*C=O); 34.38 (*t*, *C*$H_2-C(2)$); 52.00 (*q*, MeO); 72.50, 72.55, 73.39 (3*t*, 3 Ph*C*H_2); 127.59–128.52 (several *d*); 136.81, 137.40, 137.49 (3*s*); 168.92 (*s*, NHC=O); 171.54 (*s*, *C*O_2Me). HR-MALDI-MS: 622.2312 (2, $[M+K]^+$, $C_{34}H_{37}KN_3O_6^+$; calc. 622.2319), 606.2564 (95, $[M+Na]^+$, $C_{34}H_{37}N_3NaO_6^+$; calc. 606.2580), 584.2748 (100, $[M+H]^+$, $C_{34}H_{38}N_3O_6^+$; calc. 584.2761), 525.2361 (4, $[M-AcNH]^+$, $C_{32}H_{33}N_2O_5^+$; calc. 525.2389), 476.2168 (73, $[M-BnO]^+$, $C_{27}H_{30}N_3O_5^+$; calc. 476.2185), 434.2067 (14, $[M+H-BnO-Ac]^+$, $C_{25}H_{28}N_3O_4^+$; calc. 434.2080), 338.1499 (12), 260.1023 (15). Anal. calc. for $C_{34}H_{37}N_3O_6$ (583.68): C 69.97, H 6.39, N 7.20; found: C 69.69, H 6.60, N 7.00.

*Methyl (5*R*,6*R*,7*S*,8*S*)-8-Acetamido-5,6,7,8-tetrahydro-6,7-dihydroxy-5-(hydroxymethyl)imidazo[1,2-*a*]pyridine-2-acetate* (**14**). *a*) A mixture of **25** (142 mg, 0.250 mmol) and 10% Pd/C (140 mg) in AcOH (6 ml) was hydrogenated for 22 h at 6 bar and filtered over *Celite* (washing with 40 ml MeOH). The combined filtrates were evaporated and co-evaporated with toluene (5 × 5 ml). The residue (91 mg) was dissolved in MeOH (9 ml), treated with Ac_2O (4.5 ml), and stirred for 4 h at 23°. Evaporation of the mixture and co-evaporation with toluene (4 × 5 ml) yielded a yellow oil (122 mg), which was dissolved in 2M NH_3 in MeOH (3 ml) and heated for 1 h at 40°. Evaporation and FC (AcOEt/MeOH/H_2O 1:0:0 → 5:1:1) gave **14** (47.2 mg, 60%).

b) A soln. of **28** (47 mg, 80.5 μmol) in AcOH (2 ml) was treated with 10% Pd/C (27 mg) and hydrogenated for 22 h at 6 bar. Filtration over *Celite* (washing with 30 ml MeOH), evaporation of the combined filtrates, co-evaporation with toluene (3 × 5 ml), and FC (AcOEt/MeOH/H_2O 1:0:0 → 5:1:1) gave **14** (24.7 mg, 98%).

Data of **14**: White solid. M. p. 208–212°. R_f (AcOEt/MeOH/H_2O 5:1:1) 0.12. $[\alpha]_D^{25} = +24.0$ ($c = 0.89$, H_2O). p$K_{HA} = 4.62$. UV (H_2O): 216 (3.77), 192 (3.91). IR (0.4% in KBr): 3600–2600*s* (br.), 3585*s*, 3415*s*, 3261*s*, 3098*s*, 2956*m*, 2918*m*, 2887*m*, 1725*s*, 1650*s*, 1566*s*, 1508*m*, 1440*s*, 1408*m*, 1375*m*, 1349*m*, 1317*m*, 1288*s*, 1228*m*, 1205*m*, 1164*m*, 1128*m*, 1087*m*, 1070*m*, 1055*m*, 1018*m*, 990*m*, 951*w*, 905*w*, 867*w*, 760*w*, 700*m*, 635*m*, 594*m*. ^{1}H-NMR (D_2O, 300 MHz): see *Table 3*; additionally, 2.12 (*s*, AcN); 3.68 (br. *s*, $CH_2-C(2)$); 3.73 (*s*, MeO); 3.90 (with virtual coupling, irrad. at 4.89 → *d*, $J = 9.7$, with virtual coupling); 3.94–4.06 (irrad. at 3.90 → change, irrad. at 4.22 → change); 4.08 (irrad. at 4.22 → br. *s*); 4.89 (irrad. at 3.90 → *s*). ^{1}H-NMR (CD_3OD, 300 MHz): see *Table 3*; additionally, 2.06 (*s*, AcN); 3.59 (br. *s*, $CH_2-C(2)$); 3.68 (*s*, MeO); 3.73 (with virtual coupling, irrad. at 4.95 → br. *d*, $J = 8.7$); 3.84–3.91 (irrad. at 3.73 → change, irrad. at 4.16 → change); 3.94 (irrad. at 4.16 → *d*, $J \approx$ 4.4); 4.95 (irrad. at 3.73 → *s*). ^{13}C-NMR (D_2O, 75 MHz): see *Table 4*; additionally, 22.18 (*q*, *Me*C=O); 32.84 (*t*, *C*$H_2-C(2)$); 52.56 (*q*, MeO); 174.10, 174.18 (2*s*, NHC=O, *C*O_2Me). HR-MALDI-MS: 336.1163 (55, $[M+Na]^+$, $C_{13}H_{19}N_3NaO_6^+$; calc. 336.1172), 314.1343 (99, $[M+H]^+$, $C_{13}H_{20}N_3O_6^+$; calc. 314.1352), 296.1240 (100, $[M-OH]^+$, $C_{13}H_{18}N_3O_5^+$; calc. 296.1246), 254.1131 (42, $[M-CO_2Me]^+$, $C_{11}H_{16}N_3O_4^+$; calc. 254.1141).

*(5*R*,6*R*,7*S*,8*S*)-8-Acetamido-5,6,7,8-tetrahydro-6,7-dihydroxy-5-(hydroxymethyl)imidazo[1,2-*a*]pyridine-2-acetic Acid* (**15**). At 0°, a soln. of **14** (11.2 mg, 35.7 μmol) in H_2O (0.3 ml) was treated with 0.4M KOH in EtOH/H_2O 4:1 (0.5 ml), stirred for 45 min at 0°, treated with 0.1M HCl (5 ml), and evaporated. The residue was taken up in H_2O (2 ml) and subjected to ion-exchange chromatography (*Amberlite CG-120*, H^+ form, elution with 0.1M aq. NH_3). Evaporation and lyophilisation gave **15** (8.1 mg, 76%). Colourless hygroscopic resin containing substantial amounts of H_2O. The sample for microanalysis was dried for 8 d at 10^{-4} Torr. R_f (AcOEt/MeOH/H_2O 3:1:1) 0.09. $[\alpha]_D^{25} = +28.0$ ($c = 0.39$, H_2O). p$K_{HA} = 5.32$ (no additional pK value was observed in the pH range 2.3–5.7). UV (H_2O): 222 (3.81). IR (0.4% in KBr): 3600–2400*s* (br.), 2923*m*, 2852*m*, 1647*s*, 1580*s*, 1460*m*, 1414*m*, 1383*s*, 1319*m*, 1273*m*, 1202*w*, 1167*w*, 1101*m*, 1075*m*, 1042*m*, 909*w*, 873*w*, 704*m*, 660*m*, 586*m*. ^{1}H-NMR (D_2O, 300 MHz): see *Table 3*; additionally, 2.10 (*s*, AcN); 3.49 (br. *s*, $CH_2-C(2)$). ^{13}C-NMR (D_2O, 75 MHz): see *Table 4*; additionally, 22.09 (*q*, *Me*C=O); 34.68 (*t*, *C*$H_2-C(2)$); 174.39 (*s*, NHC=O); 177.62 (*s*, CO_2H). HR-MALDI-MS: 344.0817 (9, $[M-H+2\ Na]^+$, $C_{12}H_{16}N_3Na_2O_6^+$; calc. 344.0834), 322.1005 (83, $[M+Na]^+$,

$C_{12}H_{17}N_3NaO_6^+$; calc. 322.1015), 300.1189 (100, $[M+H]^+$, $C_{12}H_{18}N_3O_6^+$; calc. 300.1196), 282.1084 (43, $[M-OH]^+$, $C_{12}H_{16}N_3O_5^+$; calc. 282.1090), 240.0977 (9, $[M-CH_2CO_2H]^+$, $C_{10}H_{14}N_3O_4^+$; calc. 240.0984). Anal. calc. for $C_{12}H_{17}N_3O_6 \cdot H_2O$ (317.30): C 45.42, H 6.04, N 13.24; found: C 45.25, H 5.63, N 13.42.

Inhibition Studies. The inhibition constants (K_i) or the IC_{50} values were determined for a range of inhibitor concentrations (typically 4–7 concentrations) that bracket the K_i or IC_{50} value, and the substrate concentrations that bracket the K_M of each enzyme (for K_i, typically 5–7 concentrations), or correspond to it (for IC_{50}).

a) *Inhibition of* Jack *Beans* N-*Acetyl-β-glucosaminidase.* $K_M = 0.57-0.81$ mM ([29]: $K_M = 0.62$ mM; [30]: $K_M = 0.72$ mM). Inhibition constants (K_i) and IC_{50} values were determined at 25° at an enzyme concentration of 0.2 units/ml, with a 0.04M citric acid/sodium citrate buffer (pH 5.0), containing 0.08M NaCl, and 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside as the substrate. The enzymatic reaction was started, after incubation of the enzyme (15 µl) in the presence of the inhibitor (15 µl) and buffer (100 µl) for 1 h at 25°, by addition of the substrate (20 µl). The enzyme reaction was quenched by the addition of 0.2M borate buffer (pH 9.0, 150 µl) after 10 min, and the absorption at 405 nm was taken as rate of hydrolysis of the substrate after subtraction of the absorption of a blank probe (H_2O, buffer, substrate). IC_{50} Values were determined by plotting the reciprocal value of the rate of substrate hydrolysis *vs.* the inhibitor concentration. After fitting a straight line to the data by linear regression, the negative $[I]$-intercept of this plot provided the appropriate IC_{50} value. K_i Values were determined by taking the slopes from the *Lineweaver-Burk* plots [31] and plotting them *vs.* the inhibitor concentrations [27]. After fitting a straight line to the data by linear regression, the negative $[I]$-intercept of this plot provided the appropriate K_i value. α Values were determined by plotting the $1/v$ axis intercepts of the *Lineweaver-Burk* plots *vs.* the inhibitor concentrations [27]. After fitting a straight line to the data by linear regression, the negative $[I]$-intercept of this plot provided the appropriate $\alpha \cdot K_i$ value.

b) *Inhibition of Bovine Kidney* N-*Acetyl-β-glucosaminidase.* $K_M = 1.17-1.62$ mM ([32]: $K_M = 1.87$ mM; [30]: $K_M = 1.24$ mM). As described in *a*, inhibition studies were carried out at 37° at an enzyme concentration of 0.5 units/ml, using a 0.05M citric acid/sodium citrate buffer (pH 4.1). The enzymatic reaction was started after the incubation at 37° for 1 h and quenched by the addition of 0.2M borate buffer (pH 9.8, 150 µl).

c) *Inhibition of* C. saccharolyticum *β-Glucosidase.* $K_M = 0.68-0.77$ mM ([33]: $K_M = 0.51$ mM; [34]: $K_M = 0.51-0.78$ mM). Inhibition studies were carried out at 55° at an enzyme concentration of 0.005 units/ml, with a 0.08M KH_2PO_4/K_2HPO_4 buffer (pH 6.8) and 4-nitrophenyl β-D-glucopyranoside as the substrate. The enzymatic reaction was started after incubation of the enzyme (150 µl) in the presence of the inhibitor (40 µl) for 1 h at 55° by addition of the substrate (10 µl). The enzyme reaction was quenched by addition of 0.2M borate buffer (pH 9.0, 100 µl) after 30 min, and the absorption at 405 nm was taken as rate of hydrolysis of the substrate after subtraction of the absorption of a blank probe (H_2O, buffer, substrate). IC_{50}, K_i, and α values were determined by plots as described in *a*.

d) *Inhibition of Snail β-Mannosidase.* $K_M = 0.53-0.58$ mM ([8]: $K_M = 0.42-0.80$ mM). Inhibition constants (K_i) and IC_{50} values were determined at 25° at an enzyme concentration of 0.048 units/ml, using a 0.04M acetate buffer (pH 4.5) and 4-nitrophenyl β-D-mannopyranoside as the substrate. The enzymatic reaction was started, after incubation of the enzyme (100 µl) in the presence of the inhibitor (50 µl) for 1 h at 25°, by addition of the substrate (50 µl). The enzyme reaction was quenched by addition of 0.2M borate buffer (pH 9.0, 100 µl) after 5 min, and the absorption at 405 nm was taken as rate of the hydrolysis of the substrate after subtraction of the absorption of a blank probe (H_2O, buffer, substrate). IC_{50}, K_i, and α values were determined by plots as described in *a*.

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