Inhibition of Cellobiohydrolases from *Trichoderma reesei*. Synthesis and Evaluation of Some Glucose-, Cellobiose-, and Cellotriose-Derived Hydroximolactams and Imidazoles

by **Stefan Vonhoff**[a]), **Kathleen Piens**[b])*, **Muriel Pipelier**[a]), **Christophe Braet**[b]), **Marc Claeyssens**[b]), and **Andrea Vasella**[a])*

[a]) Laboratory of Organic Chemistry, ETH-Zentrum, Universitätstrasse 16, CH-8092 Zürich
[b]) Department of Biochemistry, Microbiology and Physiology, University of Gent, K.L. Ledeganckstraat 35, B-9000 Gent

The lactam **16**, the hydroximolactams **8**, **20**, **23**, and **27**, and the imidazole **32** were prepared following known methods. They were tested together with the known tetrazole **35** and the hydroximolactams **2** and **36** as inhibitors of the cellobiohydrolases Cel7A and Cel6A from *Trichoderma reesei*. Cel7A is only weakly inhibited by these compounds. Comparing their inhibitory activity evidences the importance of occupying subsites +1 and +2. The results strongly suggest that the shape of none of the variants of the lactone-type inhibitor motif embodied by these inhibitors is complementary to the subsite −1, *i.e.*, analogous to the transition state. Cel6A is rather strongly inhibited by the cellobiose analogues **20**, **23**, and **32**, and by the cellotriose analogue **27**. Their relative inhibitory activities evidence that binding at subsite −2 depends upon the shape of the moiety occupying subsite −1. There is only a small difference between the inhibition by the hydroximolactams **20** and **23**, which may be (partially) protonated by the catalytic acid of either *anti*- or *syn*-protonating glycosidases, and the imidazole **32**, which can only be protonated by *anti*-protonating glycosidases. The results strongly suggest that shape requirements must be met by glycosidase inhibitors before they can be used to characterize the proton trajectory of glycosidases.

1. Introduction. – *Trichoderma reesei* produces two cellobiohydrolases, Cel7A and Cel6A (formerly CBH I and CBH II [1]), which degrade crystalline cellulose very efficiently, releasing cellobiose from one or the other chain end [2]. X-Ray crystallography revealed that both enzymes have extended tunnels containing the active site [3][4].

For Cel7A, the ten structurally defined subsites for glucosyl units are designated −7 to +3 [3]. Hydrolysis occurs between subsites −1 and +1 with retention of configuration at the anomeric C-atom [5][6]. The Cel6A active site tunnel contains four well-defined binding sites (−2 to +2) and two additional subsites (+3, +4) [4][7]. As with all family-6 enzymes, the β-1,4 linkages of cellulose are cleaved with inversion of configuration [5][6].

On the basis of the crystal structure of endoglucanase I of *Fusarium oxysporum* complexed to a nonhydrolysable thiooligosaccharide [8], and according to a recently proposed classification for retaining glycosidases [9], enzymes of family 7 are expected to be *syn*-protonators. Family-6 enzymes cannot be classified as yet according to the *syn*- or *anti*-protonation trajectory. To distinguish between these is of obvious importance for the design of glycosidase inhibitors.

Monosaccharide-derived lactam oximes and imidazoles are established, strong inhibitors of *anti*-protonating exoglycosidases [9]. None of these inhibitors have been

studied with inverting *or syn*-protonating glycosidases. They could be useful for distinguishing *syn*- from *anti*-protonators. As far as proton transfer from the catalytic acid is concerned, tetrahydropyridoimidazoles of type **1** are expected to be selective inhibitors of *anti*-glycosidases, as illustrated in the *Figure*, while hydroximolactams like **2** may interact in an analogous way with either *anti*- or *syn*-protonators. Obviously, this does not exclude other factors related to shape or electrostatic interactions.

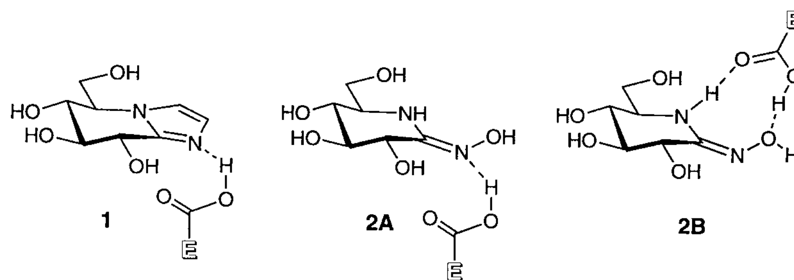
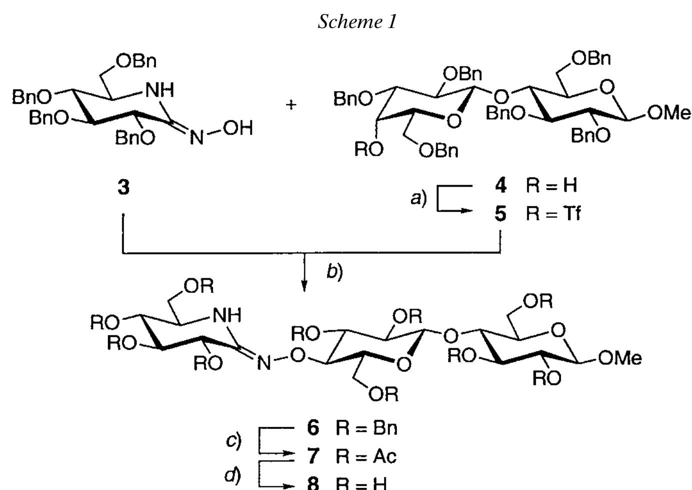


Figure. anti- *and* syn-*Protonation trajectory for imidazoles and for hydroximolactams*

To address these issues, we prepared a number of mono- to trisaccharide-related lactam oximes and a cellobiose-derived imidazole and tetrazole, and tested them as inhibitors of *T. reesei* Cel7A and Cel6A.

2. Results and Discussion. – 1. *Synthesis of the Cellotrioside Analogue* **8**. Alkylation under phase-transfer conditions [10] of the hydroximolactam **3** [11] with the triflate **5**, obtained in 91% yield from the lactoside **4** [12], yielded 70% of the benzylated cellotrioside analogue **6** (*Scheme 1*). Debenzylation with Li in $EtNH_2$ afforded the unprotected, crystalline analogue **8** in 62% yield after purification *via* the acetate **7** [10].

Scheme 1

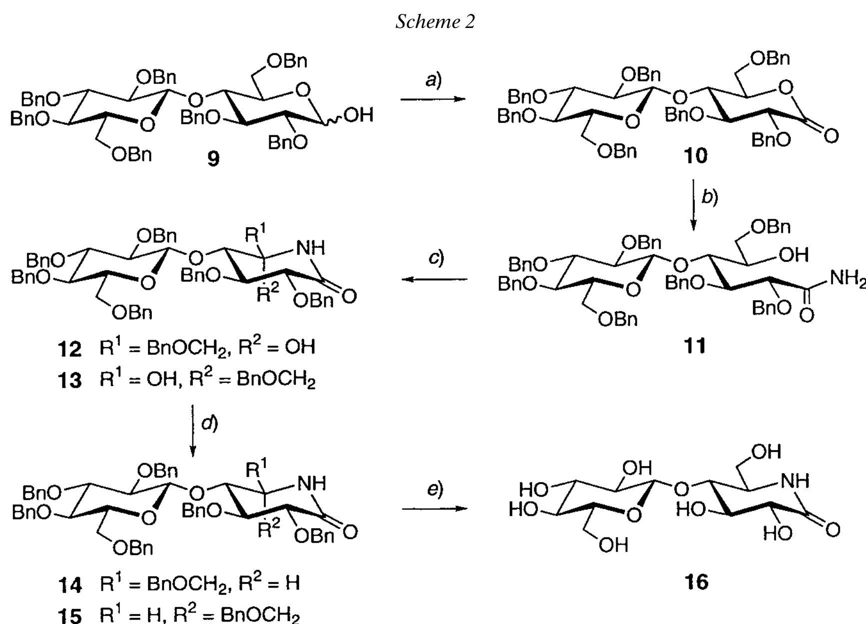


a) Tf_2O, pyridine, CH_2Cl_2; 91%. *b*) NaOH, Et_4NBr, H_2O, toluene; 70%. *c*) Li, $EtNH_2$, THF, $-60°$; Ac_2O, pyridine; 84%. *d*) NaOMe, MeOH; 75%.

The benzylated oxime ether **6** is characterized by an exchangeable NH signal at 5.49 ppm, by $J(1,2) = 7.5$, and $J(1',2') = 7.7$ Hz. The imino group gives rise to a C(1'') *s* at 149.68 ppm, characteristic for the (*Z*)-configuration [11], and a C=N band at 1653 cm^{-1}. $J(2'',3'') = 5.4$, $J(3'',4'') = 5.6$, and $J(4'',5'') = 9.3$ Hz of the acetylated cellotrioside analogue **7** indicate a $B_{2,5}$ conformation (in $CDCl_3$) of the hydroximolactam moiety, as reported for acetylated D-gluconohydroximo-1,5-lactams [10][11], while $J(2'',3'') = 9.1$, $J(3'',4'') = J(4'',5'') = 9.2$ Hz are in agreement with a $^4C_1$ conformation (in D_2O) of the hydroximolactam moiety of **8**.

2. *Synthesis of the Cellobionolactam* **16**. This lactam was prepared by analogy to D-gluconolactam [13] from the benzylated cellobiose **9** [14] without purification of the intermediates (*Scheme 2*). Oxidation of **9** with *Dess-Martin*'s periodinane [15] yielded the known cellobionolactone **10** [16][1]), which was treated with ammonia to give the hydroxy amide **11**. Oxidation of **11** with 4 equiv. of 1-hydroxy-1,2-benziodoxol-3(1*H*)-one 1-oxide [15] gave the intermediate oxo amide[2]), which cyclized under workup conditions to a mixture of the D-*gluco*- and L-*ido*-configured lactams **12** and **13**. Reductive deoxygenation of the mixture **12/13** with $NaBH_3CN$ and HCO_2H in MeCN yielded the D-gluconolactam **14** and the L-*ido*-epimer **15** in 38 and 7%, respectively, from **9**. In contrast to the reduction of the glucose-derived analogues, reduction of **12/**

Scheme 2



a) *Dess-Martin* periodinane, CH_2Cl_2. *b*) NH_3, CH_2Cl_2, $-60°$. *c*) 1-Hydroxy-1,2-benziodoxol-3(1*H*)-one 1-oxide, DMSO; H_2O, Et_2O. *d*) $NaCNBH_3$, HCO_2H, MeCN, 38% of **14** and 7% of **15** (from **9**). *e*) $Pd(OH)_2$, H_2, THF/MeOH/H_2O 3:2:1, AcOH; 69%.

---

[1]) On a 20-g scale, the *Dess-Martin* oxidation proceeded in higher yields and more reproducibly than the *Swern* oxidation.

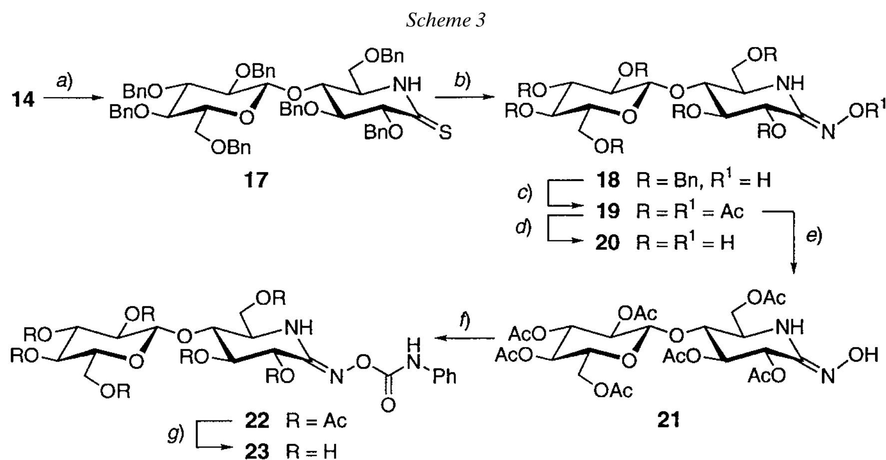
[2]) Oxidation of **11** with DMSO/Ac_2O, pyridine·SO_3 complex, or pyridinium chlorochromate (PCC) was incomplete. *Swern* oxidation gave a less polar product than **12** or **13**, which was not isolated. Oxidation with *Dess-Martin* periodinane led a product with a higher R_f value than **12** and **13**.

13 with $NaBH_4$ in HCO_2H [17] or with Et_3SiH and $BF_3 \cdot OEt_2$ [13] was lower yielding and less diastereoselective[3]). The D-glucono- and L-idonolactams **14** and **15** were separated by MPLC. Hydrogenolytic debenzylation of **14** in the presence of $Pd(OH)_2$ yielded 69% of the cellobionolactam **16**[4]).

The D-*gluco*- and L-*ido*-configured hydroxylactams **12** and **13** are characterized by $J(2,3) = 6.7$ and $J(3,4) = 6.3$, and $J(2,3) = 2.2$ and $J(3,4) = 5.9$ Hz, as well as by an exchangeable NH signal at 6.05 and 6.14 ppm, respectively. For the D-glucono- and L-idonolactams **14** and **15**, $J(2,3) = 5.6$, $J(3,4) = 5.5$, and $J(4,5) = 8.0$, and $J(2,3) = 5.2$, $J(3,4) = 2.8$, and $J(4,5) = 2.2$ Hz, respectively, were observed for the lactam moiety. The J values of the deprotected cellobionolactam **16** ($J(2,3) = 5.6$, $J(3,4) = 5.5$, $J(4,5) = 8.0$, $J(2',3') = 9.3$, $J(3',4') = 9.2$, and $J(4',5') = 9.4$ Hz) in D_2O are in agreement with a $B_{2,5}$ and $^4C_1$ conformation of the lactam and pyranosyl moiety, respectively.

3. *Synthesis of the Cellobionohydroximolactam* **20** *and the Phenylcarbamate* **23**. Conversion of the lactam **14** into the thiolactam **17**, followed by treatment with NH_2OH in boiling MeOH (*cf.* [11]) yielded 79% of the Bn-protected hydroximolactam **18** (*Scheme 3*). Debenzylation of **18** with Li in $EtNH_2$ and acetylation afforded the octaacetate **19**, which was deprotected under *Zemplen* conditions to yield 66% of the cellobionohydroximolactam **20**.

Scheme 3



a) *Lawesson*'s reagent, toluene; 87%. *b*) $NH_2OH \cdot HCl$, $NaHCO_3$, MeOH; 91%. *c*) Li, $EtNH_2$, THF, −70°; Ac_2O, pyridine; 76%. *d*) NaOMe, MeOH; 66%. *e*) $N_2H_4 \cdot AcOH$, DMF. *f*) PhNCO, Et_3N, THF; 59% from **17**. g) NH_3, MeOH; 64%.

The phenylcarbamate **23** was obtained in 38% overall yield from the octaacetate **19** by selective deacetylation of the acetoximo group with hydrazine acetate, treatment of the resulting oxime **21** with PhNCO, and deacetylation of **22** with NH_3 in MeOH [11][5]).

---

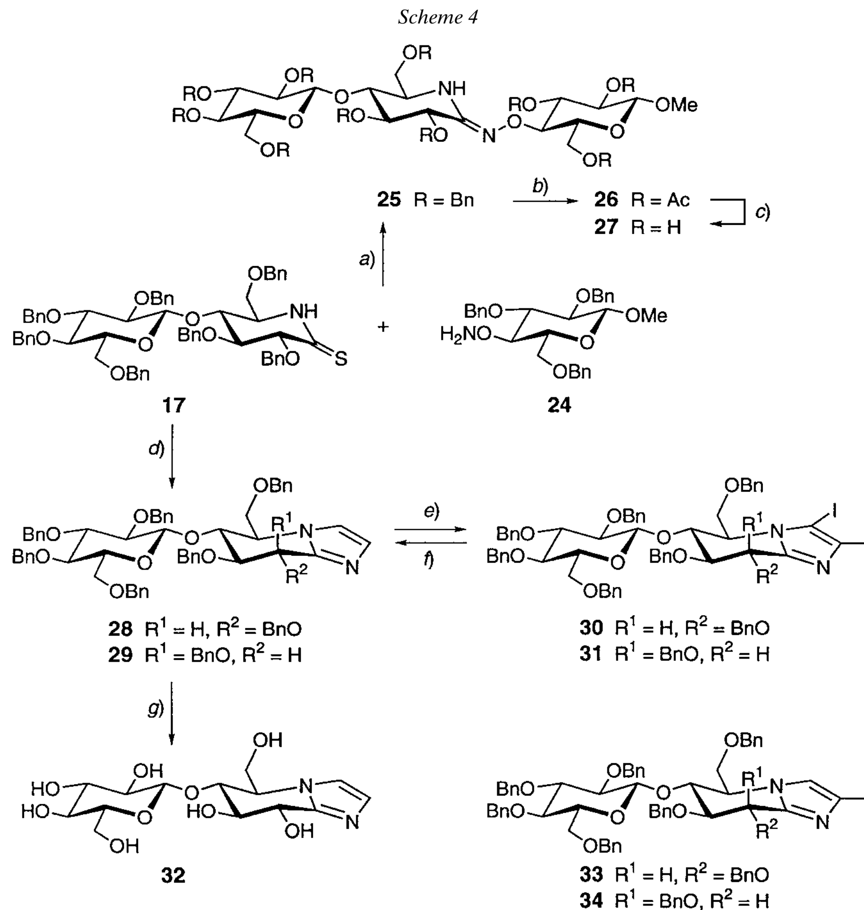
[3]) Trialkylsilanes (R_3SiH with R = Me, Pr, or Bu) did not improve the diastereoselectivity [18]. Conversion of the mixture of hydroxylactams **12** and **13** to the corresponding (ethylthio)lactams [19] followed by radical reduction with Bu_3SnH and AIBN [20] gave preferentially the L-*ido*-configured **15**.

[4]) No reduction was observed with Pd-black in EtOH as catalyst.

[5]) Deacetylation with NaOMe in MeOH at 0° resulted in methanolysis of the carbamoyl group.

4. *Synthesis of the Cellotrioside Analogue* **27**. The benzylated cellotrioside analogue **25** (*Scheme 4*) was synthesized by a $Hg(OAc)_2$-assisted condensation of the Bn-protected cellobiothionolactam **17** with the hydroxylamine **24** [10]. Debenzylation of **25** with Li in $EtNH_2$ afforded the cellotrioside analogue **27**, which was purified *via* the peracetylated derivative **26**.

Scheme 4



a) (i-Pr)$_2$EtN, $Hg(OAc)_2$, THF; 78%. *b*) Li, $EtNH_2$, THF, –60°; Ac_2O, pyridine; 42%. *c*) NaOMe, MeOH; 91%; *d*) $(MeO)_2CHCH_2NH_2$, $Hg(OAc)_2$, THF, 0°; TsOH · H_2O, toluene, 60°; 69%. *e*) NIS, DMF, 70°; 82%. *f*) EtMgBr, then H_2O (twice); 77% of **28** from **30**, 66% of **29** from **31**. *g*) $Pd(OH)_2$, 6 bar of H_2, AcOEt/MeOH/AcOH; 69%.

The imino group of **25** gives rise to a C(1′) *s* at 148.85 ppm, and a C=N band at 1653 cm^{-1}. The peracetate **26** is characterized by $J(2',3') = 4.3$, $J(3',4') = 1.2$, and $J(4',5') = 1.2$ Hz and a *W* coupling of 2.6 Hz between H–C(2′) and H–C(4′), which is in agreement with a ^3E conformation of the hydroximolactam moiety. The *J* values of the deprotected cellotrioside analogue **27** ($J(2',3') = J(3',4') = 6.8$, and $J(4',5') = 9.2$ Hz) in CD_3OD indicate a $B_{2,5}$ conformation of the hydroximolactam moiety.

5. *Synthesis of the Cellobiose Analogue* **32**. We followed the procedure for the preparation of the benzylated *gluco*-imidazole **1** [21]. Thionolactam **17** (*Scheme 4*) was

treated with aminoacetaldehyde dimethyl acetal and $Hg(OAc)_2$ in THF at 0°. The crude amidines were cyclized by treatment with $TsOH \cdot H_2O$ in toluene[6]), yielding 69% of an 8:2 mixture of the *gluco-* and *manno*-imidazoles **28/29**, the ratio of the isomers depending upon the exact reaction conditions (compare [21]). Separation of these isomers proved exceptionally difficult[7]). They were, however, separated *via* their diiodo derivatives **30/31** obtained in 82% yield by treating **28/29** with excess *N*-iodosuccinimide in DMF at 80°, following a known procedure [21]. The diiodides were separated by standard flash chromatography. The pure diiodides **30** and **31** were deiodinated by repeated treatment with a *ca.* 1.5 molar excess of EtMgBr in THF at 0°, followed by workup with aqueous sat. NH_4Cl solution, yielding 77 and 66% of the imidazoles **28** and **29**[8]), respectively. Hydrogenolytic debenzylation of **28** in AcOH under slightly elevated pressure (6 bar) in the presence of $Pd(OH)_2$ yielded the desired deprotected cellobioimidazole **32** in 69% yield.

The I substituents in **30**, **31**, **33**, and **34** are evidenced by a strong upfield shift for the C(2) *s* at 81.6–82.2 and the C(3) *s* at 96.2–96.7 ppm as compared to the unsubstituted imidazoles (C(2) and C(3) *d* of **28**, **29**, and **32**–**34** at 118.5–129.1 ppm). H–C(2) and H–C(3) of **28** and **29** in $CDCl_3$ and of **32** in D_2O resonating at 6.91–7.20 ppm show small vicinal couplings ($J \leq 1.4$ Hz). Signal overlap in the ^{1}H-NMR spectra of **28**–**31** required the measurement of ^{1}H,^{1}H-COSY spectra to allow an unambiguous assignment of H–C(5) to H–C(8) and of most of the vicinal coupling constants. The coupling constants of the *manno*-imidazoles **29** ($J(5,6) = 2.5$, $J(6,7) = 6.7$, and $J(7,8) = 3.3$ Hz) and **32** ($J(5,6) = 1.0$, $J(6,7) = 6.5$, and $J(7,8) = 3.3$ Hz) suggest a $^7S_5$ conformation. The *gluco*-imidazoles **28** and **30** probably possess a similar conformation as indicated by $J(7,8)$ of 2.8 Hz for both. The values for $J(5,6)$ and $J(6,7)$ of **28** are 4.2 Hz. The corresponding couplings of **30** could not be exactly determined due to signal overlap. Nevertheless, the signal widths suggest medium values. Thus, the vicinal couplings of **28** and **30** suggest a *ca.* 2:1 equilibrium of the $^6H_7$ and $^7H_6$ conformers. The unprotected cellobioimidazole **32**, however, adopts a $^7H_6$ conformation, as evidenced by the rather large coupling constants ($J(6,7) = 8.4$ and $J(7,8) = 8.2$ Hz).

6. *Inhibition Studies.* Cel7A is subject to strong product inhibition by cellobiose (K_i *ca.* 20 μM) [24]. As evidenced by crystal-structure analysis [25], this is probably due to H-bonding between cellobiose and the product binding sites +1 and +2 and to ring-stacking interactions with Trp 376 in the +1 site. As discussed in [9], the factors relevant for the inhibition by transition-state analogues are shape, protonation trajectory, and non-directional charge effects.

While the inhibition of Cel7A by the lactam **16**, by the hydroximolactams **2** [11], **8**, **20**. **23**, **27**, and **36** [10], and by the azoles **32** and **35** [26] is always weaker than the one by cellobiose, considerable differences in strength and inhibition type are observed (see *Table*).

The poor inhibition by the hydroximolactam **2** [11] is in keeping with the requirement for the substrate to fill subsites +1 and +2. It also indicates that **2** does not meet the requirements of a transition-state analogue binding to subsite −1. Surprisingly, the cellobiose analogue **36** does not inhibit; *i.e.*, it binds neither at subsites

---

[6]) Treatment in aqueous toluene led to cleavage of the glycosidic bond.

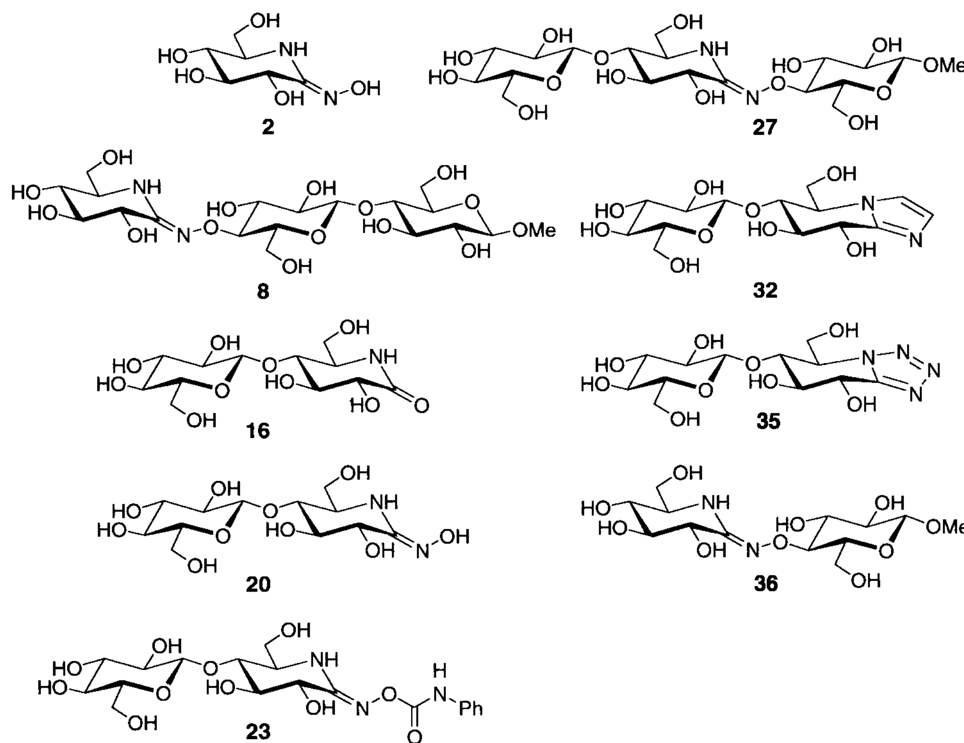
[7]) The isomers were partially separated by HPLC on a CN phase; neither the unprotected isomers (obtained by hydrogenolytic debenzylation) nor their acetylation products could be separated.

[8]) The first treatment with EtMgBr led selectively to the monoiodides **33** and **34** as evidenced by their ^{13}C-NMR spectra. The regioselectivity is assumed by analogy to the selective dehalogenation of related diiodides [22] and dibromides [23].

Table. *Inhibition Constants* K_i [µM] *of Cellobiose and Compounds* **2**, **8**, **16**, **20**, **23**, **27**, **32**, **35**, and **36**

Inhibitor	Cel7A[a])		Cel6A[b])
	K_i	Type of inhibition	K_i
Cellobiose	20	competitive	*ca.* 1800[c])
2	600	[d])	[e])
8	40	competitive	700
16	*ca.* 1300	[d])	300
20	190	mixed-type ($\alpha = 2.3$)	5
23	740	competitive	1
27	*ca.* 6000	[d])	14
32	130	non-competitive	1
35	1000	[d])	130
36	[e])	[d])	[e])

[a]) Activity measurements with 2-chloro-4-nitrophenyl β-lactoside at pH 5.7. [b]) Activity measurement on cellotriose at pH 5; type of inhibition not determined. [c]) Determined in [29]. [d]) Not determined. [e]) No inhibition at a concentration of 2 mM.



$+1/+2$, nor at $-2/-1$, nor at $-1/+1$. This may be rationalized by the greater distance between the two monosaccharide moieties, impairing binding at $+1/+2$, and at $-2/-1$; it again evidences the poor complementarity of the hydroximolactam moiety to the -1 subsite. In keeping with this, **8** is a much better inhibitor than **36**, although it is only half as strong as cellobiose. This suggests that the cellobiosyl moiety

of **8** binds to the $+1/+2$ subsites, and that this binding is (weakly) impaired by the hydroximolactam moiety. This interpretation is in keeping with the poor inhibition of **27**, formally derived from **36** by attaching a glucosyl moiety to the hydroximolactam ring. The assumption that the hydroximolactam moiety is a poor inhibition motif for Cel7A, *i.e.*, that it fits the -1 site poorly, is confirmed by the weak inhibition by the lactam **16** and the hydroximolactam **20**. Remarkably, **20** is a mixed type inhibitor, meaning that it does not bind at subsites occupied by the substrate, *i.e.*, -2, -1, and $+1$. In contradistinction to this, the phenylcarbamate **23**, derived from **20**, is a (weak) competitive inhibitor. This is in agreement with the ring-stacking interaction, at the $+1$ site, of a glucosyl unit in the cellulose substrate, and of the aryl ring in the aryl-lactoside substrate, meaning that **23** binds at the $-2/-1/+1$ subsites. Apparently, the stacking interaction compensates partially for the poor fit of the hydroximoyl moiety at the -1 site. The similarity between the hydroximolactam and the tetrahydropyridoimidazole moiety as inhibition motifs is expressed by the similar inhibition constants for **20** and **32**, and by their mode of inhibition (mixed for **20** and non-competitive for **32**). The tetrazole **35** is almost ten times weaker than the imidazole **32**.

These results confirm the importance of binding to the subsites $+1$ and $+2$, as expressed by the product inhibition. They also show that none of the variants of the 'lactone-type' inhibitor motif [9] leads to a binding interaction at the -1 site. The similarity of the inhibition by **20**, which can be protonated by a *syn*-protonating glycosidase, and by **32**, which cannot, suggests that the main factor responsible for the poor inhibition is the difference between the half-chair conformation common to the inhibitors and the conformation at the -1 site of the transition state. This is in keeping with the distortion of the glucosyl unit in the -1 subsite observed in the crystal structure of one of the members of the family-7 glycosidases, EGI of *Fusarium oxysporum*, in complex with a thioglucoside [8].

For Cel7A, the inhibition constant of 130 μM for the imidazole **32** compares to a K_i of 88 μM for the inhibition by **32** of Cel5A [27], a retaining, *anti*-protonating endoglucosidase for which a conformational change of the substrate moiety bound to the -1 subsite has been strongly evidenced [28]. This means that the use of inhibitors for the characterization of glycosidases as *syn*- or *anti*-protonators is restricted to inhibitors that possess a shape close enough to that of the transition state.

That Cel6A cleaves cellulose from the non-reducing end [2] explains the weak product inhibition by cellobiose [4][29]. The released cellobiose corresponds to the cellobiosyl moiety of the substrate bound at subsites -2 and -1, where it must be most strongly bound in the transition-state conformation. Neither **2** nor **36** inhibit this enzyme, suggesting that the inhibition motif is not optimal. The trisaccharide analogue **8** is a weak inhibitor. Introduction of the additional glucosyl moiety that should bind at the $+2$ site has a small but positive effect. A stronger effect is seen for the glucosyl moiety that should bind at the -2 site, suggesting that the hydroximoyl moiety, while not matching the requirements of the transition state, still has a positive effect on the binding of the glucosyl moiety to the -2 site. Among the disaccharide analogues that correspond the disaccharide moiety at the non-reducing end of **27**, the more strongly basic hydroximolactam **20**, carbamate **23**, and imidazole **32** are clearly stronger inhibitors than the less basic lactam **16** and tetrazole **35**. The inhibition constants for these disaccharide analogues do not differ much. Although these results are more in

keeping with the hypothesis that Cel6A is an *anti*-protonator, we are cautioned by the results obtained in the inhibition of Cel7A and Cel5A, and the lack of inhibition by **2** and **36**, suggesting that the shape requirements are not well met, and that non-directional electrostatic interactions may be responsible for the stronger inhibition by the basic disaccharide analogues. Apart from evidencing a dependence of the interaction at the -2 site on the conformation of the glucosyl moiety at the -1 site, these results show that the geometry of the transition state must differ significantly from the one of lactone type inhibitors. A twist boat conformation and a (laterally) protonable site that lies above the mean plane of the pyranosyl ring appear necessary for strong and selective inhibitors that can be used to unambiguously characterize a glycosidase as *syn*- or *anti*-protonator.

We thank the *EU Biotechnology Programme* (BBW Nr. 96.0375), the *Swiss National Science Foundation*, the *Belgium FWO,* and *F. Hoffmann-La Roche AG*, Basel, for generous support, and Dr. *B. Bernet* for the critical reading of the manuscript.

Experimental Part

General. Solvents were distilled before use. Moisture-sensitive reactions were run under Ar or N_2 in dry solvents. TLC: *Merck* silica gel *60 F_{254}* plates; detection by heating with 'mostain' (400 ml of aq. 10% H_2SO_4 soln., 20 g of $(NH_4)_6Mo_7O_{24} \cdot H_2O$, 0.4 g of $Ce(SO_4)_2$) or 20% aq. H_2SO_4 soln. Flash chromatography (FC): silica gel *Merck 60* (0.040 – 0.063 mm). HPLC: *Spherisorb* silica gel (5 μm) column (20 × 250 mm). Reversed-phase HPLC: *RP-18* silica gel. M.p.: uncorrected. IR Spectra: *Perkin Elmer 1600 FT-IR* spectrometer. ^{1}H-, ^{13}C-, and ^{19}F-NMR Spectra: at 200 – 500 MHz on *Varian* instruments, chemical shifts δ in ppm and coupling constants J in Hz. FAB-MS: *VG-SABSEQ* instrument; 3-NOBA = 3-nitrobenzyl alcohol. ESI-MS: *Finnigan MAT TSQ 7000* instrument. The inhibition studies were carried out with purified Cel7A [30] and Cel6A [31] from *Trichoderma reesei*.

*Methyl 2,3,6-Tri-*O*-benzyl-4-*O*-{2,3,6-tri-*O*-benzyl-4-*O*-[(trifluoromethyl)sulfonyl]-β-*D*-galactopyranosyl}-β-*D*-glucopyranoside* (**5**). At $-15°$, Tf_2O (2.0 ml, 12.8 mmol) was added dropwise within 20 min to a stirred suspension of **4** (5.8 g, 6.4 mmol) [12], pyridine (1.0 ml, 12.8 mmol), and 3-Å molecular sieves (0.1 g) in CH_2Cl_2 (25 ml). The suspension was warmed to 0° within 3 h, poured into cold 1M aq. HCl (25 ml). After separation, the org. layer was washed with H_2O (3 × 30 ml). Evaporation and FC (hexane/AcOEt 4 : 1) gave **5** (6.0 g, 91%). Colourless oil. R_f (hexane/AcOEt 7 : 3) 0.75. IR (CH_2Cl_2): 3031*w*, 2880*w*, 1496*w*, 1454*m*, 1410*s*, 1212*s*, 1139*s*, 1099*s*, 1082*s*, 1022*m*, 925*s*, 631*m*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 3.39 – 3.52 (*m*, H–C(2), H–C(5), H–C(2′), H–C(3′), H–C(5′), H–C(6′)); 3.62 (*t*, $J \approx 8.7$, H–C(4)); 3.63 – 3.65 (*m*, H′–C(6′)); 3.64 (*s*, MeO); 3.74 (*dd*, $J = 10.3$, < 1.0, H–C(6)); 3.90 (*dd*, $J = 10.9$, 3.1, H′–C(6)); 4.02 (*t*, $J \approx 9.3$, H–C(3)); 4.33 (*d*, $J = 11.5$, PhC*H*); 4.37 (*d*, $J = 7.1$, H–C(1)); 4.38 (*d*, $J = 11.5$, PhC*H*); 4.49 (*d*, $J = 8.1$, H–C(1′)); 4.53 (*d*, $J = 11.5$), 4.63 (*d*, $J = 12.4$), 4.65 (*d*, $J = 12.4$), 4.79 (*d*, $J = 11.5$), 4.83 (*d*, $J = 11.5$), 4.85 (*d*, $J = 10.2$), 4.87 (*d*, $J = 10.9$), 4.94 (*d*, $J = 11.2$), 4.98 (*d*, $J = 10.9$), 4.99 (*d*, $J = 10.6$, 10 PhC*H*); 5.42 (*d*, $J = 2.5$, H–C(4′)); 7.26 – 7.42 (*m*, 30 H). ^{13}C-NMR ($CDCl_3$, 75 MHz): 57.31 (*q*, MeO); 66.71, 68.15 (2*t*, C(6), C(6′)); 70.93 (*d*, C(4′)); 72.92, 73.32, 73.71 (3*t*, 3 Ph*C*H_2); 75.10 (*d*); 75.17, 75.68, 75.75 (3*t*, 3 Ph*C*H_2); 77.23 (*d*); 78.60 (*d*); 78.89 (*d*); 81.83 (*d*); 82.01 (*d*); 82.71 (*d*); 102.72 (*d*, C(1′)); 105.03 (*d*, C(1)); 119.41 (*q*, J(C,F) = 321, CF_3); 127.73 – 128.81 (several *d*); 137.52, 137.81, 138.47, 138.65, 139.00, 139.26 (6*s*). ^{19}F-NMR ($CDCl_3$, 282 MHz): -73.83. FAB-MS (3-NOBA): 1027 (15, [*M* − H]$^+$), 271 (30), 181 (80), 91 (100).

*Methyl {[(*Z*)-5-Amino-2,3,4,6-tetra-*O*-benzyl-5-deoxy-β-*D*-glucopyranosylidene]amino}-(1-*N* → 4)-2,3,6-tri-*O*-benzyl-β-*D*-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-β-*D*-glucopyranoside* (**6**). A vigorously stirred mixture of **3** [11] (3.0 g, 5.4 mmol), **5** (5.5 g, 5.4 mmol), Et_4NBr (50 mg) in toluene (30 ml) and an aq. soln. of NaOH (10.0 g in 30 ml) was heated to reflux for 16 h. The layers were separated, and the aq. layer was extracted with CH_2Cl_2 (2 × 25 ml). Drying of the combined org. layers ($MgSO_4$), evaporation, and FC (hexane/AcOEt 4 : 1) afforded **6** (5.4 g, 70%). Colourless oil. R_f (hexane/AcOEt 7 : 3) 0.80. IR (CH_2Cl_2): 3412*w*, 3068*s*, 2987*s*, 2925*m*, 2868*m*, 1653*m*, 1605*w*, 1550*w*, 1496*m*, 1453*s*, 1422*s*, 1389*m*, 1362*m*, 1288*s*, 1212*m*, 1094*s*, 1059*s*, 1028*m*, 896*s*. ^{1}H-NMR ($CDCl_3$, 200 MHz): 3.36 – 3.55 (*m*, 7 H); 3.59 (*s*, MeO); 3.63 – 3.68 (*m*, 1 H); 3.73 – 3.78 (*m*, 2 H); 3.83 – 3.86 (*m*, 1 H); 3.89 – 3.96 (*m*, 3 H); 3.99 – 4.09 (*m*, 4 H); 4.33 (*d*, $J = 7.5$, H–C(1)); 4.34 (*d*, $J = 11.2$), 4.35 (*d*, $J = 11.6$), 4.36 (*d*, $J = 12.0$), 4.38 (*d*, $J = 11.2$), 4.43 (*d*, $J = 12.0$, 5 PhC*H*); 4.49 (*AB*, $J = 12.0$, PhCH_2); 4.52 (*d*,

$J = 7.7$, H–C(1′)); 4.56 (*d*, $J = 12.0$), 4.57 (*d*, $J = 12.0$), 4.58 (*d*, $J = 12.0$, 3 PhC*H*); 4.59 (*s*, PhCH_2); 4.64 (*d*, $J = 12.0$), 4.73 (*d*, $J = 10.8$), 4.77 (*d*, $J = 12.1$), 4.78 (*d*, $J = 12.1$, 4 PhC*H*); 4.80 (*s*, PhCH_2); 4.88 (*d*, $J = 10.8$), 5.09 (*d*, $J = 11.2$, 2 PhC*H*); 5.49 (br. *s*, NH); 7.15–7.43 (m, 50 H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 52.39 (*d*, C(5″)); 57.16 (*q*, MeO); 70.88 (*t*), 71.67 (2*t*, C(6), C(6′), C(6″)); 71.96 (2*t*), 72.83(2*t*), 73.72 (3*t*, 7 PhCH_2); 73.83 (*d*); 74.42 (*d*); 74.20 (*t*), 75.18 (*t*), 76.09 (*t*, 3 PhCH_2); 77.11 (*d*); 79.67 (2*d*); 81.40 (*d*); 82.39 (2*d*); 83.05(*d*); 83.43 (*d*); 83.90(*d*); 102.98 (*d*, C(1′)); 104.31 (*d*, C(1)); 127.77–129.20 (several *d*); 135.14–139.52 (several *s*); 149.68 (*s*, C(1″)). FAB-MS (3-NOBA): 1432 (100, $[M+H]^+$), 1400(41), 1340(12), 1324(19). Anal. calc. for $C_{89}H_{94}N_2O_{15}$ (1431.73): C 74.66, H 6.62, N 1.96; found: C 74.41, H 6.52, N 1.94.

*Methyl {[(*Z*)-2,3,4,6-Tetra-*O*-acetyl-5-amino-5-deoxy-β-*D*-glucopyranosylidene]amino}-(1-*N*→4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranoside* (**7**). At −60°, a soln. of **6** (5.0 g, 3.5 mmol) in THF (12 ml) was added to a deep blue soln. of Li (0.73 g, 0.11 mol) in condensed $EtNH_2$ (*ca.* 100 ml) within 10 min. The mixture was stirred at −60° for 10 min and treated with NH_4Cl (5.0 g). After evaporation, the residue was dried, dissolved in pyridine (40 ml), and treated with Ac_2O (20 ml) at 0°, and the mixture stirred for 14 h at 20°. After evaporation, the residue was dissolved in CH_2Cl_2 (100 ml) and washed with sat. aq. $NaHCO_3$ soln. (2 × 50 ml). Drying of the org. phase ($MgSO_4$), evaporation, FC (hexane/AcOEt 1:2) and HPLC (hexane/AcOEt 2:3), afforded pure **7** (2.8 g, 84%). Colourless oil. R_f (hexane/AcOEt 2:3) 0.20. IR (CH_2Cl_2): 3448*w*, 3068*m*, 2987*m*, 1754*s*, 1666*m*, 1518*w*, 1422*m*, 1370*m*, 1234*s*, 1230*s*, 1162*m*, 1043*m*, 896*m*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 1.97, 1.98, 1.99, 2.00, 2.04, 2.05, 2.06, 2.07, 2.09, 2.10 (10*s*, 10 AcO); 3.44 (*s*, MeO); 3.56 (*ddd*, $J = 9.6$, 4.7, 1.9, H–C(5)); 3.58 (*dddd*, $J = 9.4$, 6.1, 2.8, 1.3, H–C(5″)); 3.73 (*t*, $J \approx 9.0$, H–C(4)); 3.75 (*ddd*, $J = 10.0$, 4.7, 2.2, H–C(5′)); 3.98 (*dd*, $J = 12.1$, 6.7, H–C(6″)); 3.99 (*t*, $J \approx 9.7$, H–C(4′)); 4.07 (*dd*, $J = 11.8$, 5.0, H–C(6)); 4.19 (*dd*, $J = 12.2$, 2.2, H–C(6′)); 4.22–4.25 (*m*, H′–C(6′)); 4.26 (*dd*, $J = 12.2$, 2.8, H′–C(6″)); 4.34 (*d*, $J = 7.8$, H–C(1)); 4.45 (*d*, $J = 7.8$, H–C(1′)); 4.49 (*dd*, $J = 12.1$, 1.9, H′–C(6)); 4.84 (*dd*, $J = 9.7$, 7.9), 4.85 (*dd*, $J = 9.7$, 7.8, H–C(2), H–C(2′)); 4.94 (*dd*, $J = 9.3$, 5.6, H–C(4″)); 5.13 (*t*, $J \approx 9.0$, H–C(3)); 5.17 (*t*, $J \approx 5.6$, H–C(3″)); 5.25 (*d*, $J = 5.4$, H–C(2″)); 5.26 (*t*, $J \approx 9.6$, H–C(3′)); 5.32 (br. *s*, NH). ^{13}C-NMR ($CDCl_3$, 50 MHz): 20.58–20.73 (several *q*); 52.21 (*d*, C(5″)); 57.03 (*q*, MeO); 61.98, 62.66, 63.03 (3*t*, C(6), C(6′), C(6″)); 67.66 (*d*); 70.01 (*d*); 71.66 (*d*); 71.87 (*d*); 72.16 (2*d*); 72.27 (*d*); 72.64 (*d*); 72.85 (*d*); 76.49, 77.84 (2*d*, C(5), C(5′)); 101.59, 101.86 (2*d*, C(1), C(1′)); 148.00 (*s*, C(1″)); 168.90, 169.53 (2*s*); 169.74 (2*s*); 169.97, 170.18, 170.34, 170.70, 170.86, 171.30 (6*s*). FAB-MS (3-NOBA): 952 (39, $[M+H]^+$), 951 (100, M^+), 631 (25).

*Methyl {[(*Z*)-5-Amino-5-deoxy-β-*D*-glucopyranosylidene]amino}-(1-*N*→4)-β-*D*-glucopyranosyl-(1→4)-β-*D*-glucopyranoside* (**8**). At 5°, a soln. of **7** (2.4 g, 2.5 mmol) in MeOH (30 ml) was treated dropwise with a freshly prepared 0.5N soln. of NaOMe in MeOH (*ca.* 1 ml). After 4 h, the mixture was stirred for 15 min with *Amberlite* IR-120 (H^+ form), filtered, and evaporated. Reversed-phase HPLC (*RP18* silica gel, H_2O/MeOH 100:0 → 20:80) and crystallization from MeOH afforded **8** (1.0 g, 75%). Colourless solid. R_f (AcOEt/MeOH/H_2O 4:2:1) 0.12. M.p. 159.5–160.5° (MeOH). $[\alpha]_D^{25} = +103.6$ ($c = 0.59$, H_2O). IR (KBr): 3440*s*, 3340*s*, 3200*s*, 2990*m*, 2940*m*, 1665*s*, 1640*s*, 1455*m*, 1330*s*, 1300*s*, 1150*m*, 1080*s*, 970*s*. ^{1}H-NMR (D_2O, 500 MHz): 3.18 (*ddd*, $J = 9.2$, 4.9, 2.8, H–C(5″)); 3.24 (*m*, 10 lines, $J = 9.4$, 8.0, H–C(2)); 3.32 (*dd*, $J = 9.2$, 8.0, H–C(2′)); 3.51 (*s*, MeO); 3.53 (*t*, $J \approx 9.2$, H–C(4″)); 3.54–3.60 (*m*, H–C(3), H–C(4), H–C(5)); 3.61 (*t*, $J \approx 9.2$, H–C(3″)); 3.64 (*dd*, $J = 12.2$, 5.5, H–C(6′)); 3.65 (*dd*, $J = 11.9$, 4.8, H–C(6″)); 3.68 (*ddd*, $J = 9.2$, 5.4, 1.9, H–C(5′)); 3.76 (*dd*, $J = 12.1$, 4.8, H–C(6)); 3.77 (*dd*, $J = 12.0$, 2.8, H′–C(6″)); 3.78 (*t*, $J \approx 9.5$, H–C(4′)); 3.79 (*dd*, $J = 12.1$, 2.3, H′–C(6′)); 3.84 (*t*, $J \approx 9.3$, H–C(3′)); 3.94 (*dd*, $J = 11.8$, 1.9, H′–C(6)); 4.09 (*d*, $J = 9.1$, H–C(2″)); 4.35 (*d*, $J = 8.0$, H–C(1)); 4.46 (*d*, $J = 8.0$, H–C(1′)). ^{13}C-NMR (CD_3OD, 75 MHz): 58.17 (*q*, MeO); 58.66 (*d*, C(5″)); 61.62, 62.18, 63.19 (3*t*, C(6), C(6′), C(6″)); 70.58 (*d*); 70.71 (*d*); 74.56 (*d*); 74.60 (*d*); 75.22 (*d*); 76.13 (*d*); 76.24 (*d*); 76.37 (*d*); 76.74 (*d*); 80.61, 81.05 (2*d*, C(4), C(4′)); 104.33, 104.99 (2*d*, C(1), C(1′)); 155.13 (*s*, C(1″)). FAB-MS (3-NOBA): 531 (25, $[M+H]^+$), 460(70), 307(100), 154(60). Anal. calc. for $C_{19}H_{34}N_2O_{15} \cdot H_2O$ (548.48): C 41.60, H 6.62, N 5.11; found: C 41.85, H 6.53, N 5.18.

*2,3,6-Tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-*D*-glucono-1,5-lactone* (**10**) [16]. At 23°, a soln. of **9** [14] (22.2 g, 22.8 mmol) in CH_2Cl_2 (330 ml) was treated with 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one (*Dess-Martin* periodinane) [15] (24.0 g, 57.0 mmol), and stirred for 5 h. The mixture was diluted with Et_2O (300 ml), poured into 200 ml of a 1:1 mixture of sat. aq. $NaHCO_3$ soln. and Na_2SO_3 soln. (20 g/100 ml H_2O), and stirred at 23° for 20 min. After separation, the aq. layer was extracted with CH_2Cl_2 (2 × 200 ml). Drying of the combined org. layers ($MgSO_4$) and evaporation yielded crude **10** (21.7 g) which was sufficiently pure to be used for the next step. Crystallization of a sample from EtOH gave colourless needles. M.p. 110–112° ([16]: M.p. 114–115°).

*2,3,6-Tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-*D*-gluconamide* (**11**). At −60°, a soln. of crude **10** (21.7 g, 23 mmol) in dry CH_2Cl_2 (280 ml) was added dropwise to condensed NH_3 (*ca.* 40 ml). The cooling bath was removed, and the soln. was kept under reflux for 2 h. Evaporation gave crude **11** (21.8 g) as an

oil which was used for the next step. A sample was purified by FC (hexane/AcOEt 6 : 1). R_f (hexane/AcOEt 4 : 1) 0.33. IR ($CHCl_3$): 3517*w*, 3485*w*, 3400*w*, 3066*w*, 3007*m*, 2911*w*, 2869*w*, 1687*s*, 1497*w*, 1454*m*, 1359*m*, 1261*s*, 1091*s*, 1070*s*, 1024*s*. ^{1}H-NMR ($CDCl_3$, 500 MHz): 3.23 (br. *s*, OH); 3.35 (*t*, $J \approx 8.6$, H−C(2′)); 3.34 – 3.37 (*m*, H−C(5′)); 3.50 (*t*, $J \approx 9.1$, H−C(4′)); 3.54 (*t*, $J \approx 8.8$, H−C(3′)); 3.55 (*dd*, $J = 10.0$, 3.3, H−C(6)); 3.54 – 3.58 (*m*, 2 H−C(6′)); 3.64 (*dd*, $J = 10.1$, 4.6, H′−C(6)); 3.99 (*m*, H−C(5)); 4.09 (*dd*, $J = 7.6$, 3.6, H−C(4)); 4.26 (*dd*, $J =$ 4.7, 3.6, H−C(3)); 4.33 (*d*, $J = 11.9$, PhC*H*); 4.38 (*d*, $J = 4.8$, H−C(2)); 4.41 (*d*, $J = 11.9$), 4.42 (*d*, $J = 11.9$), 4.44 (*d*, $J = 12.1$, 3 PhC*H*); 4.45 (*d*, $J = 7.8$, H−C(1′)); 4.52 (*d*, $J = 11.0$), 4.57 (*d*, $J = 11.3$), 4.62 (*d*, $J = 11.4$), 4.66 (*d*, $J = 11.2$), 4.70 (*d*, $J = 11.1$), 4.72 (*d*, $J = 11.2$), 4.78 (*d*, $J = 11.1$), 4.79 (*d*, $J = 11.0$), 4.80 (*d*, $J = 11.0$), 4.89 (*d*, $J =$ 11.0, 10 PhC*H*); 5.29 (br. *s*, NH); 6.69 (br. *s*, NH); 7.15 – 7.33 (*m*, 35 H). ^{13}C-NMR ($CDCl_3$, 63 MHz): 69.14, 70.71 (2*t*, C(6), C(6′)); 71.49 (*d*, C(5)); 73.37 (*t*), 73.44 (*t*), 73.58 (*t*), 74.77 (2*t*), 75.09 (2*t*, 7 Ph*C*H); 75.77 (*d*); 76.82 (*d*); 77.90 (*d*); 79.37 (*d*); 80.13 (*d*); 82.30 (*d*); 84.84 (*d*); 103.54 (*d*, C(1′)); 127.87 – 128.76 (several *d*); 137.70, 138.16, 138.38, 138.41, 138.53 (5*s*); 138.83 (2*s*); 174.26 (*s*, C(1)). FAB-MS (3-NOBA): 988 (40, [*M* + H]$^+$), 466 (35), 181 (95), 901 (100).

*5-Amino-2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-*D*-glucono-1,5-lactam* (**12**) *and 5-Amino-2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-*L*-idono-1,5-lactam* (**13**). At 23°, a soln. of crude **11** (21.7 g, 22 mmol) in DMSO (90 ml) was added to a 1M soln. of 1-hydroxy-1,2-benziodoxol-3(1*H*)-one 1-oxide [15] (25.0 g, 88 mmol) in DMSO (90 ml). After stirring for 5 h and the addition of H_2O (200 ml) and Et_2O (300 ml) at 5°, the mixture was stirred for 2 h at 23° and filtered through *Celite*. After separation, the aq. layer was extracted with Et_2O (3 × 150 ml). The combined org. layers were dried and evaporated to give **12**/**13** (21.3 g) as an oil which was dried at 0.01 Torr and used without further purification for the next step. FC (hexane/AcOEt 6 : 1) gave pure samples of **12** and **13**.

Data of **12**: R_f (hexane/AcOEt 4 : 1) 0.47. IR (CH_2Cl_2): 3350*w*, 3068*s*, 2986*s*, 1697*m*, 1550*w*, 1496*w*, 1422*s*, 1361*m*, 1288*s*, 1154*m*, 1070*m*, 1028*m*, 988*w*, 895*s*. ^{1}H-NMR ($CDCl_3$, 500 MHz): 3.36 – 3.40 (*m*, H−C(5′)); 3.43 (*d*, $J = 9.7$, H−C(6)); 3.46 (*dd*, $J = 9.2$, 7.8, H−C(2′)); 3.52 (*d*, $J = 9.7$, H′−C(6)); 3.54 (*dd*, $J = 11.0$, 5.0, H−C(6′)); 3.59 – 3.61 (*m*, H−C(3′), H−C(4′)); 3.65 (*dd*, $J = 11.0$, 1.8, H−C(6′)); 3.79 (br. *s*, OH); 3.92 (*d*, $J = 6.3$, H−C(4)); 4.07 (*d*, $J = 6.7$, H−C(2)); 4.17 (*t*, $J \approx 6.5$, H−C(3)); 4.32 (*d*, $J = 11.8$), 4.39 (*d*, $J = 11.1$), 4.40 (*d*, $J =$ 11.9), 4.42 (*d*, $J = 11.1$, 4 PhC*H*); 4.43 (*d*, $J = 7.9$, H−C(1′)); 4.55 (*d*, $J = 10.9$), 4.62 (*d*, $J = 11.6$), 4.66 (*d*, $J =$ 11.3), 4.70 (*d*, $J = 11.5$, 4 PhC*H*); 4.80 (*d*, $J = 11.8$, 2 PhC*H*); 4.84 (*d*, $J = 10.9$), 4.85 (*d*, $J = 11.2$), 4.91 (*d*, $J =$ 10.9), 4.97 (*d*, $J = 11.3$, 4 PhC*H*); 6.05 (br. *s*, NH); 7.12 – 7.41 (*m*, 35 H). ^{13}C-NMR ($CDCl_3$, 63 MHz): 68.89 (*t*, C(6′)); 72.47, 73.52, 73.59, 73.81, 74.09, 75.08, 75.20 (7*t*, C(6), 6 PhCH_2); 75.39 (*d*, C(5′)); 75.77 (*t*, PhCH_2); 78.03 (*d*); 78.51 (2*d*); 78.89 (*d*); 82.28 (*d*); 82.73 (*s*, C(5)); 85.07 (*d*); 103.71 (*d*, C(1′)); 127.77 – 128.70 (several *d*); 137.29, 137.70, 138.21, 138.27, 138.40 (5*s*); 138.56 (2*s*); 170.46 (*s*, C(1)). FAB-MS (Na): 1008 (35, [*M* + Na]$^+$), 984 (55, [*M* − 1]$^+$), 860 (69), 446 (100).

Data of **13**: R_f (hexane/AcOEt 4 : 1) 0.38. IR (CH_2Cl_2): 3345*w*, 3070*s*, 2987*s*, 1697*m*, 1548*w*, 1497*w*, 1419*s*, 1360*m*, 1290*s*, 1152*w*, 1082*m*, 1028*m*, 896*s*. ^{1}H-NMR ($CDCl_3$, 500 MHz): 3.39 (*t*, $J \approx 8.0$, H−C(2′)); 3.40 – 3.43 (*m*, H−C(5′)); 3.43 (*d*, $J = 9.1$, H−C(6)); 3.50 (*d*, $J = 9.1$, H′−C(6)); 3.56 – 3.62 (*m*, H−C(3′), H−C(4′), H−C(6′)); 3.61 (*dd*, $J = 10.8$, 4.6, H′−C(6′)); 3.72 (br. *s*, OH); 4.10 (*d*, $J = 2.2$, H−C(2)); 4.20 (*dd*, $J = 5.9$, 2.8, H−C(3)); 4.22 (*s*, PhCH_2); 4.30 (*d*, $J = 5.9$, H−C(4)); 4.36 (*d*, $J = 11.1$), 4.42 (*d*, $J = 12.3$, 2 PhC*H*); 4.50 (*d*, $J =$ 7.8, H−C(1′)); 4.53 (*d*, $J = 10.9$), 4.64 (*d*, $J = 11.8$), 4.67 (*d*, $J = 11.5$), 4.69 (*d*, $J = 11.4$), 4.73 (*d*, $J = 11.7$), 4.78 (*d*, $J = 11.5$), 4.80 (*d*, $J = 10.9$), 4.83 (*d*, $J = 11.0$), 4.91 (*d*, $J = 11.0$), 5.02 (*d*, $J = 11.5$, 10 PhC*H*); 6.14 (br. *s*, NH); 7.10 – 7.45 (*m*, 35 H). ^{13}C-NMR ($CDCl_3$, 75 MHz): 68.95 (*t*, C(6′)); 72.37, 73.21, 73.53, 73.57, 74.00, 75.09, 75.18 (7*t*, C(6), 6 PhCH_2); 75.28 (*d*, C(5′)); 75.79 (*t*, PhCH_2); 77.91 (*d*); 78.09 (*d*); 78.40 (*d*); 82.26 (*d*); 82.43 (*d*); 83.90 (*s*, C(5)); 84.89 (*d*); 104.27 (*d*, C(1′)); 127.77 – 128.76 (several *d*); 137.29, 138.17 (2*s*); 138.26 (2*s*); 138.39, 138.57, 138.63 (3*s*); 170.89 (*s*, C(1)). FAB-MS (Na): 1008 (22, [*M* + Na]$^+$), 860 (50), 446 (21), 181 (23), 91 (100).

*5-Amino-2,3,6-tri-*O*-benzyl-5-deoxy-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-*D*-glucono-1,5-lactam* (**14**) *and 5-Amino-2,3,6-tri-*O*-benzyl-5-deoxy-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-*L*-idono-1,5-lactam* (**15**). At 23°, sodium cyanoborohydride (6.7 g, 107 mmol) was added to a soln. of crude **12**/**13** (21.3 g, *ca.* 22 mmol) in MeCN (310 ml) and HCO_2H (80 ml). After 5 h at 35°, the mixture was cooled to 5° and treated slowly with a sat. aq. $NaHCO_3$ soln. (300 ml). After separation, the aq. layer was extracted with CH_2Cl_2 (3 × 200 ml). The combined org. layers were dried ($MgSO_4$) and evaporated. FC (CH_2Cl_2/Et_2O 95 : 5) and MPLC (1 kg of silica gel, CH_2Cl_2/THF 97 : 3, 10 ml/min) gave **14** (8.8 g, 38% from **9**) and **15** (1.5 g, 7% from **9**).

Data of **14**: Colourless solid. R_f (hexane/AcOEt 3 : 2) 0.29. M.p. 141 – 142° (EtOH). $[\alpha]_D^{25} = +52.8$ ($c = 0.5$, $CHCl_3$). IR ($CHCl_3$): 3392*w*, 3066*w*, 3007*m*, 2931*w*, 2867*m*, 1684*s*, 1497*m*, 1454*s*, 1361*m*, 1311*w*, 1070*s*, 1031*m*, 909*w*. ^{1}H-NMR ($CDCl_3$, 500 MHz): 3.35 (*ddd*, $J = 9.3$, 4.5, 2.2, H−C(5′)); 3.41 – 3.44 (*m*, H−C(2′), H−C(6)); 3.56 – 3.63 (*m*, H−C(3′), H−C(4′), H′−C(6), 2 H−C(6′)); 3.80 (*ddd*, $J = 7.6$, 3.4, 1.6, H−C(5)); 3.87 (*dd*, $J =$ 8.0, 4.9, H−C(4)); 3.95 (*d*, $J = 5.6$, H−C(2)); 4.08 (*t*, $J \approx 5.5$, H−C(3)); 4.25 (*d*, $J = 11.8$), 4.32 (*d*, $J = 11.7$, 2

Ph*CH*); 4.40 (*d*, *J* = 7.8, H–C(1')); 4.43 (*s*, PhC*H*$_2$); 4.52 (*d*, *J* = 10.9), 4.61 (*d*, *J* = 11.7), 4.65 (*d*, *J* = 11.4), 4.72 (*d*, *J* = 11.7, 4 PhC*H*); 4.77 (*s*, PhC*H*$_2$); 4.80 (*d*, *J* = 10.9), 4.81 (*d*, *J* = 11.0), 4.89 (*d*, *J* = 11.5), 4.96 (*d*, *J* = 10.9, 4 PhC*H*); 5.81 (br. *s*, NH); 7.16–7.39 (*m*, 35 H). ^{13}C-NMR ($CDCl_3$, 75 MHz): 54.03 (*d*, C(5)); 68.85, 69.68 (2*t*, C(6), C(6')); 73.20, 73.26, 73.39, 73.52 (4*t*, 4 Ph*C*H$_2$); 75.11 (2*t*, 2 Ph*C*H$_2$); 75.17 (*d*, C(5')); 75.84 (*t*, Ph*C*H$_2$); 77.87 (*d*); 78.29 (*d*); 79.07 (*d*); 81.30 (*d*); 82.41 (*d*); 84.95 (*d*); 104.12 (*d*, C(1')); 127.07–128.72 (several *d*); 137.51, 137.89 (2*s*); 138.36 (2*s*); 138.49 (2*s*); 138.65 (*s*); 170.33 (*s*, C(1)). FAB-MS (3-NOBA): 1939 (18, [2 *M* + H]$^+$), 970 (80, [*M* + H]$^+$), 754 (52), 448 (50), 91 (100). Anal. calc. for $C_{61}H_{63}NO_{10}$ (970.17): C 75.52, H 6.55, N 1.44; found: C 75.41, H 6.49, N 1.46.

Data of **15**: Colourless solid. R_f (hexane/AcOEt 3 : 2) 0.22. M.p. 114–115° (EtOH). IR ($CHCl_3$): 3399*w*, 3007*m*, 2926*m*, 2867*m*, 1689*s*, 1469*m*, 1454*s*, 1360*m*, 1309*w*, 1070*s*, 1030*m*, 909*w*. ^{1}H-NMR ($CDCl_3$, 500 MHz): 3.40 (*dd*, *J* = 9.1, 7.9, H–C(2')); 3.40–3.43 (*m*, H–C(5')); 3.46 (*t*, *J* ≈ 9.0, H–C(6)); 3.53 (*dd*, *J* = 9.0, 5.0, H–C(6)); 3.55 (*t*, *J* ≈ 9.5, H–C(4')); 3.58–3.64 (*m*, H–C(3'), 2 H–C(6')); 3.80 (*ddd*, *J* = 9.1, 4.8, 2.2, H–C(5)); 3.96 (*t*, *J* ≈ 2.1, H–C(4)); 3.98 (*d*, *J* = 5.2, H–C(2)); 4.15 (*d*, *J* = 12.0, PhC*H*); 4.16 (*dd*, *J* = 5.3, 2.8, H–C(3)); 4.19 (*d*, *J* = 11.8, PhC*H*); 4.37 (*d*, *J* = 7.8, H–C(1')); 4.41 (*d*, *J* = 12.2), 4.44 (*d*, *J* = 12.2), 4.53 (*d*, *J* = 10.9), 4.58 (*d*, *J* = 11.7), 4.63 (*d*, *J* = 11.7), 4.71 (*d*, *J* = 11.8), 4.77 (*d*, *J* = 11.8), 4.79 (*d*, *J* = 11.2), 4.80 (*d*, *J* = 11.4), 4.81 (*d*, *J* = 10.9), 4.91 (*d*, *J* = 11.0), 5.02 (*d*, *J* = 11.8, 12 PhC*H*); 5.76 (br. *s*, NH); 7.17–7.40 (*m*, 35 H). ^{13}C-NMR ($CDCl_3$, 75 MHz): 52.10 (*d*, C(5)); 68.55, 69.21 (2*t*, C(6), C(6')); 72.79, 73.42 (2*t*, 2 Ph*C*H$_2$); 73.66 (*t*, 2 Ph*C*H$_2$); 75.25, 75.32 (2*t*, 2 Ph*C*H$_2$); 75.36 (*d*); 75.81 (*d*); 75.92 (*t*, Ph*C*H$_2$); 78.06 (*d*); 78.85 (*d*); 82.36 (2*d*); 84.94 (*d*); 104.48 (*d*, C(1')); 128.04–128.81 (several *d*); 138.01, 138.38, 138.49, 138.60, 138.69, 138.81, 138.94 (7*s*); 171.78 (*s*, C(1)). FAB-MS (Na): 992 (2, [*M* + Na]$^+$), 448 (20), 147 (34), 91 (100).

*5-Amino-5-deoxy-4-*O*-(β-*D*-glucopyranosyl)-*D*-glucono-1,5-lactam* (**16**). At 24°, a soln. of **14** (0.30 g, 0.31 mmol) in THF/MeOH/H_2O 3 : 2 : 1 (12 ml) and AcOH (3 ml) was treated with 20% $Pd(OH)_2$/C (0.3 g) and hydrogenated at 6 bar for 15 h. The suspension was filtered through *Celite* and the residue washed thoroughly with MeOH and H_2O. Evaporation of the combined filtrate and crystallization from H_2O/EtOH 1 : 9 afforded **16** (58 mg, 69%). Colourless solid. R_f (AcOEt/MeOH/H_2O 7 : 3 : 2) 0.31. M.p. 125.3–125.8° (H_2O/EtOH 1 : 9). $[\alpha]^{25}_D = +16.1$ (*c* = 0.36, MeOH). IR (KBr): 3415*s*, 3340*s*, 3200*s*, 3030*m*, 2945*m*, 1662*s*, 1645*s*, 1540*w*, 1495*m*, 1385*s*, 1345*s*, 1320*s*, 1155*s*, 1075*s*, 1025*s*, 990*m*, 890*m*. ^{1}H-NMR (D_2O, 500 MHz): 3.29 (*dd*, *J* = 9.3, 8.0, H–C(2')); 3.37 (*t*, *J* ≈ 9.2, H–C(4')); 3.43 (*ddd*, *J* = 9.7, 5.7, 2.2, H–C(5')); 3.45 (*t*, *J* ≈ 9.2, H–C(3')); 3.50 (*dt*, *J* = 8.2, 3.5, H–C(5)); 3.69 (*dd*, *J* = 12.4, 5.7, H–C(6')); 3.75–3.78 (*AB*, 2 H–C(6)); 3.82 (*t*, *J* ≈ 9.8, H–C(3)); 3.86 (*dd*, *J* = 12.4, 2.1, H'–C(6')); 3.94 (*dd*, *J* = 9.4, 8.4, H–C(4)); 3.99 (*d*, *J* = 9.9, H–C(2)); 4.51 (*d*, *J* = 7.8, H–C(1')). ^{13}C-NMR (D_2O, 75 MHz): 58.95 (*d*, C(5)); 62.92, 63.45 (2*t*, C(6), C(6')); 72.32 (*d*); 73.03 (*d*); 74.98 (*d*); 76.07 (*d*); 78.38 (*d*); 78.98 (*d*); 80.02 (*d*, C(4)); 105.74 (*d*, C(1')); 176.21 (*s*, C(1)). ESI-MS: 677 (60, [2*M* – H]$^+$), 428 (8), 384 (12), 374 (18), 338 (100, [*M* – H]$^+$). Anal. calc. for $C_{12}H_{21}NO_{10} \cdot H_2O$ (411.35): C 40.34, H 6.49, N 3.91; found: C 40.55, H 6.48, N 3.93.

*5-Amino-2,3,6-tri-*O*-benzyl-5-deoxy-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-1-thio-*D*-gluconolactam* (**17**). A mixture of **14** (1.40 g, 1.44 mmol) and *Lawesson*'s reagent (435 mg, 1.08 mmol) in toluene (25 ml) was stirred at 40° for 2 h. Evaporation and FC (hexane/AcOEt 4 : 1) gave **17** (1.24 g, 87%). Pale-yellow oil. R_f (hexane/AcOEt 4 : 1) 0.43. IR (CH_2Cl_2): 3367*w*, 3068*s*, 2987*s*, 1604*w*, 1550*m*, 1512*m*, 1497*m*, 1421*s*, 1362*m*, 1288*s*, 1153*m*, 1072*m*, 1028*m*, 988*m*, 896*s*. ^{1}H-NMR ($CDCl_3$, 500 MHz): 3.30 (*ddd*, *J* = 9.7, 4.1, 1.9, H–C(5')); 3.42 (*dd*, *J* = 9.0, 7.9, H–C(2')); 3.47 (*dd*, *J* = 9.9, 7.9, H-C(6)); 3.55 (*dd*, *J* = 10.7, 2.1, H–C(6')); 3.57 (*t*, *J* ≈ 9.0, H–C(3')); 3.61 (*dd*, *J* = 10.2, 3.4, H'–C(6)); 3.63 (*dd*, *J* = 10.9, 4.1, H'–C(6')); 3.64 (*t*, *J* ≈ 9.2, H–C(4')); 3.79 (*dd*, *J* = 8.8, 2.2, H–C(4)); 4.01–4.05 (*m*, H–C(5)); 4.19 (*t*, *J* = 2.5, H–C(3)); 4.24 (*d*, *J* = 11.8), 4.31 (*d*, *J* = 11.8, 2 PhC*H*); 4.37 (*d*, *J* = 7.8, H-C(1')); 4.41 (*d*, *J* = 12.1), 4.46 (*d*, *J* = 12.1, 2 PhC*H*); 4.49–4.51 (*m*, H–C(2)); 4.50 (*d*, *J* = 11.7), 4.54 (*d*, *J* = 10.9), 4.55 (*d*, *J* = 11.8), 4.68 (*d*, *J* = 11.7, 4 PhC*H*); 4.74 (*s*, PhC*H*$_2$); 4.80 (*d*, *J* = 10.9), 4.81 (*d*, *J* = 10.9), 4.86 (*d*, *J* = 11.7), 4.89 (*d, J* = 11.0, 4 PhC*H*); 7.15–7.39 (*m*, 35 H); 7.99 (br. *s*, NH). ^{13}C-NMR ($CDCl_3$, 75 MHz): 55.95 (*d*, C(5)); 68.45, 68.73 (2*t*, C(6), C(6')); 71.82, 72.21, 73.39, 73.58, 75.00 (5*t*, 5 Ph*C*H$_2$); 75.03 (*d*, C(5')); 75.10 (*t*, 2 Ph*C*H$_2$); 77.72 (*d*); 80.59 (*d*); 81.17 (*d*); 82.25 (*d*); 82.85 (*d*); 84.89 (*d*); 104.97 (*d*); 127.82–18.88 (several *d*); 137.39, 137.84, 138.21 (3*s*); 138.39 (2*s*); 138.55, 138.74 (2*s*); 200.38 (*s*, C(1)). FAB-MS (3-NOBA): 1076 (18), 986 (100, *M*$^+$), 878 (15), 91 (83).

*(*Z*)-5-Amino-2,3,6-tri-*O*-benzyl-5-deoxy-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyl)-*D*-gluconohydroximo-1,5-lactam* (**18**). A mixture of **17** (1.0 g, 1.0 mmol), $NH_2OH \cdot HCl$ (175 mg, 2.53 mmol), and $NaHCO_3$ (212 mg, 2.53 mmol) in MeOH (13 ml) was heated under reflux for 2.5 h. Filtration, evaporation, and FC (hexane/AcOEt 4 : 1) afforded **18** (910 mg, 91%), which was further purified by HPLC (SiO_2; hexane/Et_2O 2 : 1, 10 ml/min). Colourless oil. R_f (hexane/AcOEt 4 : 1) 0.17. IR (CH_2Cl_2): 3588*w*, 3423*w*, 3068*s*, 2987*s*, 2867*m*, 1662*w*, 1550*w*, 1496*w*, 1421*s*, 1363*m*, 1288*s*, 1154*m*, 1071*m*, 1028*m*, 987*w*, 896*s*. ^{1}H-NMR ($CDCl_3$, 500 MHz): 3.31 (*ddd*, *J* = 9.7, 4.0, 1.8, H–C(5')); 3.43 (*dd*, *J* = 9.1, 7.9, H–C(2')); 3.55 (*dd*, *J* = 10.8, 1.8, H–C(6')); 3.56 (*dd*,

$J = 9.5$, 6.7, H–C(6)); 3.57 (*t*, $J \approx 9.1$, H–C(3′)); 3.64 (*dd*, $J = 10.7$, 4.1, H′–C(6′)); 3.65 (*t*, $J \approx 9.7$, H–C(4′)); 3.68 (*dd*, $J = 9.5$, 2.9, H′–C(6)); 3.79 (*dd*, $J = 9.5$, 2.2, H–C(4)); 3.85 (*m*, H–C(5)); 4.02 (br. *s*, H–C(2)); 4.17 (*t*, $J \approx 2.5$, H–C(3)); 4.26 (*d*, $J = 12.0$), 4.29 (*d*, $J = 11.9$), 4.38 (*d*, $J = 12.1$, 3 PhC*H*); 4.43 (*d*, $J = 7.8$, H–C(1′)); 4.44 (*d*, $J = 11.1$), 4.48 (*d*, $J = 11.9$, 2 PhC*H*); 4.53 (*d*, $J = 11.6$, 2 PhC*H*); 4.57 (*d*, $J = 12.3$), 4.67 (*d*, $J = 11.9$), 4.73 (*d*, $J = 11.2$), 4.79 (*d*, $J = 10.9$, 4 PhC*H*); 4.80 (*d*, $J = 10.9$, 2 PhC*H*); 4.89 (*d*, $J = 10.9$, PhC*H*); 5.44 (*s*, NH); 6.52 (br. *s*, OH); 7.11–7.35 (*m*, 35 H). ^{13}C-NMR ($CDCl_3$, 75 MHz): 51.95 (*d*, C(5)); 68.08, 69.63 (2*t*, C(6), C(6′)); 70.47, 71.96, 73.11, 73.58 (4*t*, 4 PhCH_2); 74.52, 74.99 (2*d*, C(2), C(5′)); 75.08, 75.12, 75.85 (3*t*, 3 PhCH_2); 77.84 (*d*); 81.43 (*d*); 82.28 (*d*); 82.40 (*d*); 84.97 (*d*); 104.90 (*d*, C(1′)); 127.71–128.75 (several *d*); 138.10, 138.16, 138.25, 138.51 (4*s*); 138.70 (2*s*); 138.89 (*s*); 149.71 (*s*, C(1)). FAB-MS (3-NOBA): 1970 (10, $[2M + H]^+$), 985 (100, $[M + H]^+$), 793 (15), 91 (67). Anal. calc. for $C_{61}H_{64}N_2O_{10}$ (985.12): C 74.37, H 6.55, N 2.84; found: C 74.35, H 6.68, N 2.58.

*(*Z*)-*N*^{1},2,3,6-Tetra-*O*-acetyl-5-amino-5-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-D-glucono-hydroximo-1,5-lactam* (**19**). At −70°, a soln. of **18** (670 mg, 0.68 mmol) in THF (3.5 ml) was added dropwise to a deep blue soln. of Li (0.17 g, 24.5 mmol) in condensed $EtNH_2$ (*ca.* 20 ml). The mixture was stirred at −70° for 10 min and treated with NH_4Cl (1.44 g). After evaporation, the residue was dried, dissolved in pyridine (10 ml), treated with Ac_2O (5 ml) at −15°, and stirred for 4 h at 0°. After evaporation, the residue was dissolved in CH_2Cl_2 (25 ml), washed with brine (2 × 25 ml), and dried ($MgSO_4$). Evaporation and FC (CH_2Cl_2/acetone 17:3) afforded **19** (590 mg, 76%). Colourless oil. R_f (hexane/AcOEt 17:3) 0.24. IR ($CHCl_3$): 3400*w*, 3008*w*, 1756*s*, 1656*m*, 1434*w*, 1368*m*, 1066*m*, 1042*m*, 841*w*, 818*w*. ^{1}H-NMR ($CDCl_3$, 500 MHz): 2.01, 2.02, 2.06, 2.07, 2.09, 2.10, 2.16, 2.19 (8*s*, 8 AcO); 3.64 (*dt*, $J = 8.8$, 1.5, H–C(4)); 3.75 (*dddd*, $J = 8.7$, 6.0, 2.3, 0.9, H–C(5)); 3.79 (*ddd*, $J = 10.1$, 4.5, 2.4, H–C(5′)); 4.04 (*dd*, $J = 12.0$, 6.0, H–C(6)); 4.08 (*dd*, $J = 12.4$, 2.4, H–C(6′)); 4.27 (*dd*, $J = 12.4$, 4.5, H′–C(6′)); 4.47 (*dd*, $J = 12.0$, 2.5, H′–C(6)); 4.79 (*d*, $J = 8.1$, H–C(1′)); 4.96 (*dd*, $J = 9.6$, 8.1, H–C(2′)); 5.07 (*t*, $J \approx 10.1$, H–C(4′)); 5.20 (*t*, $J \approx 9.5$, H–C(3′)); 5.38 (*dt*, $J = 3.8$, 1.3, H–C(2)); 5.65 (*dd*, $J = 3.7$, 1.8, H–C(3)); 5.79 (br. *s*, NH). ^{13}C-NMR ($CDCl_3$, 50 MHz): 19.63 (*q*, Me); 20.55 (*q*, 4 Me); 20.61, 20.77, 20.81 (3*q*, 3 Me); 53.01 (*d*, C(5)); 61.75, 62.96 (2*t*, C(6), C(6′)); 67.48 (*d*); 68.08 (*d*); 69.63 (*d*); 71.57 (*d*); 72.14 (*d*); 72.92 (*d*); 79.69 (*d*, C(4)); 101.13 (*d*, C(1′)); 151.36 (*s*, C(1)); 168.62, 169.52 (2*s*, 2 C=O); 169.61 (*s*, 2 C=O); 169.69, 170.48, 170.91, 171.78 (4*s*, 4 C=O). FAB-MS (3-NOBA): 691 (100, $[M + H]^+$), 649 (25).

*(*Z*)-4-*O*-(β-D-Glucopyranosyl)-D-gluconohydroximo-1,5-lactam* (**20**). At 0°, a soln. of **19** (150 mg, 0.22 mmol) in MeOH (3.1 ml) was treated dropwise with a 1M soln. of MeONa in MeOH. After 6 h, the mixture was stirred with *Amberlite IRC-50* (H^+ form), filtered, and evaporated. Reversed-phase HPLC (*RP18* silica gel, H_2O/MeCN 100:0 → 10:90) and lyophilization afforded **20** (51 mg, 66%). Colourless solid. R_f (AcOEt/MeOH/H_2O 7:3:2) 0.23. $[\alpha]_D^{25} = +32.8$ ($c = 0.36$, MeOH). IR (KBr): 3390*s*, 3150*s*, 2980*m*, 2890*w*, 1655*s*, 1565*m*, 1365*m*, 1115*s*, 1020*s*, 955*m*. ^{1}H-NMR (D_2O, 500 MHz): 3.28 (*dd*, $J = 9.3$, 8.0, H–C(2′)); 3.37 (*t*, $J \approx 9.2$, H–C(4′)); 3.39–3.45 (*m*, H–C(5), H–C(5′)); 3.45 (*t*, $J \approx 9.1$, H–C(3′)); 3.69 (*dd*, $J = 12.4$, 5.7, H–C(6′)); 3.74 (*dd*, $J = 12.0$, 4.6, H–C(6)); 3.79–3.85 (*m*, H–C(3), H–C(4), H′–C(6)); 3.86 (*dd*, $J = 12.4$, 2.2, H′–C(6′)); 4.15 (*d*, $J = 8.0$, H–C(2)); 4.51 (*d*, $J = 7.9$, H–C(1′)). ^{13}C-NMR (D_2O, 50 MHz): 58.66 (*d*, C(5)); 63.48 (*t*, C(6), C(6′)); 71.25 (*d*); 72.38 (*d*); 76.10 (*d*); 76.38 (*d*); 78.41 (*d*); 78.93 (*d*); 81.57 (*d*, C(4)); 105.90 (*d*, C(1′)); 156.96 (*s*, C(1)). ESI-MS: 355 (100, $[M + H]^+$), 193 (82). Anal. calc. for $C_{12}H_{22}N_2O_{10} \cdot H_2O$ (372.31): C 38.71, H 6.49, N 7.52; found: C 38.75, H 6.36, N 7.43.

*(*Z*)-2,3,6-Tri-*O*-acetyl-5-amino-5-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-D-gluconohydroximo-1,5-lactam* (**21**). At 22°, a soln. of **19** (100 mg, 0.144 mmol) and hydrazine acetate (30 mg, 0.32 mmol) in DMF (3 ml) was stirred for 30 min. After addition of AcOEt (20 ml) and washing with 0.1M aq. soln. of HCl, the org. layer was dried ($MgSO_4$) and evaporated. Drying at 0.01 mbar afforded **21** as a colourless oil, which was used without further purification for the next step. R_f (hexane/AcOEt 4:1) 0.43. IR ($CHCl_3$): 3591*w*, 3402*w*, 3007*w*, 2928*w*, 1754*s*, 1671*w*, 1430*w*, 1370*m*, 1066*m*, 1041*s*, 908*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 1.99, 2.01, 2.06, 2.07, 2.08, 2.09, 2.10, 2.13 (7*s*, 7 AcO); 3.66–3.68 (*m*, H–C(4), H–C(5)); 3.76 (*ddd*, $J = 9.7$, 4.1, 2.2, H–C(5′)); 3.98 (*dd*, $J = 11.2$, 5.6, H–C(6)); 4.07 (*dd*, $J = 12.5$, 2.2, H–C(6′)); 4.28 (*dd*, $J = 12.5$, 4.7, H′–C(6′)); 4.48 (*dd*, $J = 11.0$, 2.2, H′–C(6)); 4.73 (*d*, $J = 8.1$, H–C(1′)); 4.97 (*dd*, $J = 9.7$, 8.1, H–C(2′)); 5.08 (*t*, $J \approx 9.7$, H–C(4′)); 5.19 (*t*, $J \approx 9.7$, H–C(3′)); 5.30 (br. *d*, $J = 4.4$, H–C(2)); 5.46 (br. *s*, NH); 5.50 (*dd*, $J = 4.4$, 2.5, H–C(3)); 7.08 (br. *s*, OH). ^{13}C-NMR ($CDCl_3$, 53 MHz): 20.61 (*q*, 3 Me); 20.70 (*q*, Me); 20.76 (*q*, 3 Me); 52.57 (*d*, C(5)); 61.74, 63.30 (2*t*, C(6), C(6′)); 67.55 (*d*); 68.03 (*d*); 70.85 (*d*); 71.55 (*d*); 72.09 (*d*); 72.98 (*d*); 79.83 (*d*, C(4)); 101.33 (*d*, C(1′)); 147.99 (*s*, C(1)); 169.60 (*s*, 2 C=O); 169.66 (*s*, 2 C=O); 170.51, 170.90, 171.16 (3*s*, 3 C=O). FAB-MS (3-NOBA): 1297 (3, $[2M + H]^+$), 649 (100, $[M + H]^+$), 607 (25).

*(*Z*)-*O*-[2,3,6-Tri-*O*-acetyl-5-amino-5-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-D-glucopyranosylidene]amino *N*-Phenylcarbamate* (**22**). At 23°, a soln. of crude **21** (68 mg, 0.104 mmol) in THF (1 ml) was treated with Et_3N (43 µl, 0.312 mmol) and PhNCO (12.5 µl, 0.156 mmol) and was stirred for 30 min.

Evaporation and FC (CH_2Cl_2/acetone 9:1) afforded **22** (80.5 mg, 59%). Colourless foam. R_f (hexane/AcOEt 1:2) 0.45. IR ($CHCl_3$): 3448*w*, 3372*w*, 3007*w*, 1754*s*, 1669*m*, 1595*w*, 1521*m*, 1446*w*, 1434*w*, 1370*m*, 1067*m*, 1040*m*, 907*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 1.99, 2.02, 2.07, 2.08, 2.10, 2.13, 2.14 (7*s*, 7 Me); 3.65 (br. *d*, J = 8.7, H−C(4)); 3.75 – 3.80 (*m*, H−C(5), H−C(5′)); 3.99 (*dd*, J = 11.8, 6.5, H−C(6)); 4.09 (*dd*, J = 12.5, 2.5, H−C(6′)); 4.26 (*dd*, J = 12.5, 4.4, H′−C(6′)); 4.39 (*dd*, J = 11.8, 2.5, H′−C(6)); 4.75 (*d*, J = 8.1, H−C(1′)); 4.97 (*dd*, J = 9.3, 8.1, H−C(2′)); 5.08 (*t*, J ≈ 9.3, H−C(4′)); 5.19 (*t*, J ≈ 9.3, H−C(3′)); 5.37 (*d*, J = 3.7, H−C(2)); 5.59 (*dd*, J = 3.7, 1.9, H−C(3)); 5.84 (br. *s*, NH); 7.11 (*t*, J ≈ 7.5, 1 arom. H); 7.33 (*t*, J ≈ 7.5, 2 arom. H); 7.48 (*d*, J = 7.8, 2 arom. H); 8.41 (br. *s*, NHCO). ^{13}C-NMR ($CDCl_3$, 50 MHz): 20.47 (*q*, 3 Me); 20.59 (*q*, 2 Me); 20.69 (*q*, 2 Me); 52.24 (*d*, C(5)); 62.63, 62.87 (2*t*, C(6), C(6′)); 66.87 (*d*); 67.89 (*d*); 70.05 (*d*); 71.23 (*d*); 71.98 (*d*); 72.71 (*d*); 79.63 (*d*, C(4)); 101.31 (*d*, C(1′)); 119.66 (*d*, 2 C); 124.29 (*d*); 129.15 (*d*, 2 C); 137.06 (*s*); 149.49 (*s*, C(1)); 152.07 (*s*, NC=O); 168.73, 169.35 (2*s*, 2 C=O); 169.53 (*s*, 2 C=O); 170.29 (*s*, C=O); 170.73 (*s*, 2 C=O). FAB-MS (3-NOBA): 768 (65, $[M + H]^+$), 73 (100).

(Z)-O-[5-Amino-5-deoxy-4-O-(β-D-glucopyranosyl)-D-glucopyranosylidene]amino N-Phenylcarbamate (**23**). At 23°, a soln. of **22** (86.7 mg, 0.113 mmol) in MeOH (4 ml) was treated with a sat. soln. of NH_3 in MeOH (0.4 ml) and stirred for 2 h. Evaporation at 28°, FC (AcOEt/MeOH/H_2O 9:1:1), and lyophilization afforded **23** (34.2 mg, 64%). Colourless solid. R_f (AcOEt/MeOH/H_2O 7:3:2) 0.41. $[\alpha]^{25}_D = -11.2$ (c = 0.17, MeOH). IR (KBr): 3480*s*, 3290*s*, 1720*s*, 1650*s*, 1610*s*, 1520*m*, 1450*w*, 1225*m*, 1120*m*, 1090*m*, 1035*m*, 960*m*. ^{1}H-NMR (D_2O, 500 MHz): 3.28 (*dd*, J = 9.3, 8.0, H−C(2′)); 3.36 (*t*, J ≈ 9.2, H−C(4′)); 3.43 (*ddd*, J = 9.7, 5.7, 2.3, H−C(5′)); 3.45 (*t*, J = 9.2, H−C(3′)); 3.50 (*dt*, J ≈ 7.5, 4.1, H−C(5)); 3.68 (*dd*, J = 12.3, 5.8, H−C(6′)); 3.74 (*dd*, J = 12.0, 4.5, H−C(6)); 3.82 (*dd*, J = 12.0, 3.3, H′−C(6)); 3.84 (*t*, J ≈ 7.8, H−C(4)); 3.86 (*dd*, J = 12.3, 2.2, H′−C(6′)); 3.93 (*t*, J ≈ 8.2, H−C(3)); 4.20 (*d*, J = 8.3, H−C(2)); 4.50 (*d*, J = 7.9, H−C(1′)); 7.16 – 7.20 (*m*, 1 arom. H); 7.37 – 7.38 (*m*, 4 arom. H). ^{13}C-NMR (D_2O, 75 MHz): 56.21(*d*, C(5)); 60.56, 60.72 (2*t*, C(6), C(6′)); 68.43 (*d*); 69.61 (*d*); 73.07 (*d*); 73.29 (*d*); 75.64 (*d*); 76.18 (*d*); 78.53 (*d*, C(4)); 103.27 (*d*, C(1′)); 121.54 (*d*, 2 C); 125.31 (*d*); 129.50 (*d*, 2 C); 136.84 (*s*); 155.69, 157.02 (2*s*, C(1), NC=O). ESI-MS: 496 (80), 475 (23, $[M + H]^+$), 474 (100, M^+). Anal. calc. for $C_{19}H_{27}N_3O_{11} \cdot 1.5\,H_2O$ (500.45): C 45.60, H 5.43, N 8.39; found: C 45.29, H 5.43, N 8.05.

Methyl (2,3,4,6-Tetra-O-benzyl-β-D-glucopyranosyl)-(1 → 4)-{[(Z)-5-amino-2,3,6-tri-O-benzyl-5-deoxy-D-glucopyranosylidene]amino}-(1-N → 4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**25**). At 22°, a soln. of **17** (0.40 g, 0.41 mmol) and **24** [10] (0.29 g, 0.62 mmol) in THF (15 ml, freshly distilled) was treated with $(i\text{-}Pr)_2EtN$ (1.0 ml, 5.8 mmol) and $Hg(OAc)_2$ (0.30 g, 0.94 mmol) and stirred for 8 h at 22°. After filtration through *Celite* and evaporation, the residue was dissolved in CH_2Cl_2 (25 ml) and washed with sat. aq. $NaHCO_3$ soln. (2 × 25 ml). Drying of the org. phase ($MgSO_4$), evaporation, and FC (hexane/AcOEt 9:1) afforded **25** (0.43 g), which was sufficiently pure to be used for the next step. Colourless oil. R_f (hexane/AcOEt 4:1) 0.25. IR (CH_2Cl_2): 3400*w*, 3068*s*, 2986*s*, 2914*m*, 2868*m*, 1726*w*, 1653*m*, 1497*m*, 1454*s*, 1422*s*, 1361*m*, 1288*s*, 1212*m*, 1062*s*, 1028*s*, 896*s*. ^{1}H-NMR ($CDCl_3$, 200 MHz): 3.31 – 3.36 (*m*, 1 H); 3.41 – 3.56 (*m*, 4 H); 3.61 – 3.77 (*m*, 6 H); 3.64 (*s*, MeO); 3.88 – 3.94 (*m*, 2 H); 4.03 – 4.07 (*m*, 4 H); 4.21 (*t*, J ≈ 3.0, H−C(2′)); 4.27 (*d*, J = 12.0), 4.35 (*d*, J = 12.1, 2 PhC*H*); 4.36 (*d*, J = 8.8, H−C(1)); 4.39 – 4.44 (*m*, 4 PhC*H*); 4.50 – 4.61 (*m*, H−C(1″), 7 PhC*H*); 4.70 (*d*, J = 12.0), 4.75 (*d*, J = 11.2), 4.77 (*d*, J = 12.0), 4.85 (*d*, J = 12.0), 4.86 (*d*, J = 11.5), 4.93 (*d*, J = 11.2), 4.95 (*d*, J = 10.8, 7 PhC*H*); 5.44 (br. *s*, NH); 7.15 – 7.39 (*m*, 50 H). ^{13}C-NMR ($CDCl_3$, 50 MHz): 51.76 (*d*); 57.06 (*q*); 71.10 (*t*); 71.75 (*t*, 2 C); 72.96 (*t*, 2 C); 73.34 (*t*, 2 C); 73.50 (*t*, 3 C); 73.66 (*d*); 74.04 (*d*); 74.90 (*t*); 75.18 (*t*); 75.69 (*t*); 77.63 (*d*, 2C); 80.51 (*d*); 81.42 (*d*); 82.17 (*d*, 2 C); 82.78 (*d*); 83.36 (*d*); 84.77 (*d*); 104.67, 104.89 (2*d*, C(1), C(1″)); 127.30 – 128.64 (several *d*); 137.84 – 139.33 (several *s*); 148.85 (*s*, C(1′)). FAB-MS (3-NOBA): 1522 (8), 1432 (100, $[M + H]^+$), 1400 (23), 1340 (19), 1324 (10). Anal. calc. for $C_{89}H_{94}N_2O_{15}$ (1431.73): C 74.66, 6.62, N 1.96; found: C 74.49, H 6.72, N 2.12.

Methyl (2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-(1 → 4)-{[(Z)-2,3,6-tri-O-acetyl-5-amino-5-deoxy-D-glucopyranosylidene]amino}-(1-N → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**26**). At −60°, a soln. of **25** (189 mg, 0.13 mmol) in THF (2 ml) was added to a deep blue soln. of Li (0.10 g, 14.3 mmol) in condensed $EtNH_2$ (*ca.* 10 ml) within 2 min. The mixture was stirred at −60° for 10 min and treated with NH_4Cl (0.10 g). After evaporation, the residue was dried, dissolved in pyridine (10 ml), treated with Ac_2O (5 ml) at 0°, and stirred for 1 h at 20°. After evaporation, the residue was dissolved in CH_2Cl_2 (25 ml) and washed with sat. aq. $NaHCO_3$ soln. (2 × 25 ml). Drying of the org. phase ($MgSO_4$), evaporation, and FC (hexane/AcOEt 1:2) gave, after crystallization from Et_2O, impure **26** (105 mg, 85%). HPLC (hexane/AcOEt 2:3) afforded pure **26** (0.39 g, 69%). Colourless solid. R_f (hexane/AcOEt 1:2) 0.22. M.p. 188 – 189° (Et_2O/hexane). $[\alpha]^{25}_D = -50.1$ (c = 0.27, $CHCl_3$). IR (CH_2Cl_2): 3400*w*, 3068*s*, 2987*s*, 1755*s*, 1659*w*, 1551*w*, 1422*s*, 1369*m*, 1237*s*, 1161*m*, 1040*m*, 987*w*, 896*s*. ^{1}H-NMR ($CDCl_3$, 500 MHz): 2.00, 2.01, 2.02, 2.05, 2.06, 2.07, 2.08, 2.09, 2.10, 2.15 (10*s*, 10 AcO); 3.49 (*s*, MeO); 3.60 (*ddd*, J = 10.5, 2.5, 1.6, H−C(4′)); 3.63 – 3.67 (*m*, H−C(5′)); 3.77 (*ddd*, J = 10.1, 4.5, 2.4, H−C(5″)); 3.85 (*ddd*, J = 10.0, 5.3, 2.2, H−C(5)); 3.96 (*dd*, J = 11.7, 6.5, H−C(6′)); 4.07 (*dd*, J = 12.4, 2.3, H−C(6″)); 4.08 (*t*,

$J \approx 9.7$, H–C(4)); 4.25 (*dd*, $J = 12.3$, 5.3, H–C(6)); 4.28 (*dd*, $J = 12.2$, 4.5, H'–C(6'')); 4.35 (*dd*, $J = 12.0$, 2.1, H'–C(6)); 4.40 (*d*, $J = 8.0$, H–C(1)); 4.40 (*dd*, $J = 11.5$, 2.5, H'–C(6')); 4.73 (*d*, $J = 8.1$, H–C(1'')); 4.94 (*dd*, $J =$ 8.0, 7.4); 4.96 (*dd*, $J = 8.1$, 7.5, H–C(2), H–C(2'')); 5.07 (*t*, $J \approx 9.5$, H–C(4'')); 5.19 (*t*, $J \approx 9.6$, H–C(3'')); 5.20 (*dt*, $J \approx 4.4$, 1.2, H–C(3')); 5.31 (br. *s*, NH); 5.36 (*t*, $J \approx 9.5$, H–C(3)); 5.49 (*dd*, $J = 4.3$, 2.6, H–C(2')). ^{13}C-NMR ($CDCl_3$, 50 MHz): 21.06–21.38 (several *q*, Me); 53.39 (*d*, C(5')); 57.53 (*q*, MeO); 62.26, 63.34, 63.78 (3*t*, C(6), C(6'), C(6'')); 67.88 (*d*); 68.54 (*d*); 70.67 (*d*); 72.00 (*d*); 72.39 (*d*); 72.48 (*d*); 72.58 (*d*); 72.73 (*d*); 73.43 (*d*); 78.73, 80.35 (2*d*, C(4), C(4')); 101.75, 102.22 (2*d*, C(1), C(1'')); 148.47 (*s*, C(1')); 169.36, 170.05, 170.12, 170.21, 170.34, 170.78, 170.94, 171.36, 171.55, 171.77 (10*s*, 10 C=O). FAB-MS (3-NOBA): 952 (100, $[M+H]^+$), 169 (30), 109 (38). Anal. calc. for $C_{39}H_{54}N_2O_{25}$ (950.85): C 49.26, H 5.72, N 2.95; found: C 49.19, H 5.78, N 3.01.

Methyl β-D-Glucopyranosyl-(1→4)-{[(Z)-5-amino-5-deoxy-D-glucopyranosylidene]amino}-(1-N→4)-β-D-glucopyranoside (**27**). At 5°, a soln. of **26** (90 mg, 0.1 mmol) in MeOH (5 ml) was treated dropwise with a freshly prepared 0.5N soln. of NaOMe in MeOH (*ca.* 0.1 ml). After 1 h, the mixture was treated with *Amberlite IR-120* (H^+ form), stirred for 15 min, and filtered. After evaporation, lyophilization gave **27** (46 mg, 91%). Colourless solid. R_f (AcOEt/MeOH/H_2O 4 : 2 : 1) 0.10. IR (KBr): 3425*s*, 3340*s*, 2990*m*, 2945*m*, 1662*s*, 1643*s*, 1455*m*, 1335*s*, 1090*m*, 1078*s*, 970*s*, 935*m*. ^{1}H-NMR (CD_3OD, 500 MHz): 3.21–3.24 (*m*, H–C(5')); 3.26 (*t*, $J \approx$ 8.7), 3.30 (*t*, $J \approx 8.8$, H–C(2), H–C(2'')); 3.32–3.36 (*m*), 3.36 (*t*, $J \approx 8.8$), 3.40–3.44 (*m*, H–C(3''), H–C(4''), H–C(5'')); 3.53 (*s*, MeO); 3.55–3.58 (*m*, H–C(5)); 3.63 (*dd*, $J = 9.2$, 6.8, H–C(4')); 3.64 (*dd*, $J = 11.8$, 6.2, H–C(6')); 3.71 (*dd*, $J = 12.3$, 5.0, H–C(6)); 3.73–3.79 (*m*, H–C(3), H–C(4), H–C(6'')); 3.82 (*dd*, $J = 12.3$, 3.1, H'–C(6)); 3.83 (*t*, $J \approx 6.8$, H–C(3')); 3.88 (*dd*, $J = 11.5$, 3.1, H'–C(6'')); 3.89 (*dd*, $J = 11.8$, 2.3, H'–C(6')); 4.04 (*d*, $J = 6.8$, H–C(2')); 4.18 (*d*, $J = 7.8$, H–C(1)); 4.40 (*d*, $J = 7.8$, H–C(1'')). ^{13}C-NMR (CD_3OD, 50 MHz): 56.87 (*d*, C(5')); 57.32 (*q*, MeO); 62.52, 62.58, 62.71 (3*t*, C(6), C(6'), C(6'')); 70.84 (*d*); 71.63 (*d*); 75.06 (*d*, 2 C); 75.47 (*d*); 76.01 (*d*); 76.17 (*d*); 77.95 (*d*); 78.17 (*d*); 81.50, 81.85 (2*d*, C(4), C(4')); 105.18, 106.40 (2*d*, C(1), C(1'')); 154.60 (*s*, C(1')). FAB-MS (3-NOBA): 1084 (3), 553 (55), 531 (55, $[M+H]^+$), 383 (56), 307 (100), 154 (85).

Transformation of **17** *into* **28**/**29**. A soln. of **17** (1.0 g, 1.01 mmol) in THF (6 ml, freshly distilled) was treated with aminoacetaldehyde dimethyl acetal (570 µl, 5.23 mmol) and $Hg(OAc)_2$ (450 mg, 1.37 mmol) and kept at 0° for 2 h. Normal workup and drying gave a crude product which was dissolved in toluene (36 ml), treated with TsOH · H_2O (250 mg, 1.3 mmol), and stirred overnight at 60°. Normal workup and FC (hexane/AcOEt/Et_3N 4 : 1 : 0.03) gave **28**/**29** 4 : 1 (692 mg, 69%) which could not be separated by HPLC.

Iodination of **28**/**29**. A soln. of **28**/**29** (350 mg, 0.353 mmol) in DMF (3.5 ml), was treated with *N*-iodosuccinimide (793 mg, 3.53 mmol) and stirred at 70° for 24 h. The mixture was cooled, diluted with Et_2O, and washed with a sat. NH_4Cl soln. The aq. phase was extracted several times with Et_2O. The combined org. layers were dried ($MgSO_4$) and evaporated. FC (hexane/AcOEt 4 : 1) gave **31** (133 mg, 30%), **30**/**31** (22 mg, 5%), and **30** (208 mg, 47%).

*(5*R*,6*R*,7*S*,8*S*)-7,8-Bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydro-2,3-diiodo-6-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyloxy)imidazo[1,2-*a*]pyridine* (**30**). R_f (hexane/AcOEt 4 : 1) 0.29. IR ($CHCl_3$): 3067*w*, 3007*w*, 2868*m*, 1610*w*, 1595*w*, 1497*m*, 1453*m*, 1432*w*, 1392*w*, 1361*m*, 1309*w*, 1261*m*, 1175*s*, 1070*s*, 1028*m*, 912*w*. ^{1}H-NMR (500 MHz, $CDCl_3$, assignment based on a DQFCOSY.GRASP spectrum): 3.33 (*dd*, $J = 8.8$, 7.9, H–C(2')); 3.45 (*ddd*, $J = 9.5$, 4.0, 2.3, H–C(5')); 3.57 (*t*, $J = 9.2$, H–C(4')); 3.62 (*t*, $J = 8.9$, H–C(3')); 3.64–3.70 (*m*, 2 H–C(6'), CH_2–C(5)); 4.33 (*d*, $J \approx 11.0$, PhC*H*); 4.34–4.35 (*m*, H–C(7)); 4.41 (*d*, $J = 12.0$, PhC*H*); 4.44 (*d*, $J = 11.8$, PhC*H*); 4.47 (*d*, $J = 12.0$, PhC*H*); 4.49–4.56 (*m*, 4 PhC*H*, H–C(5), H–C(1')); 4.57 (*d*, $J = 11.7$, PhC*H*); 4.66 (*d*, $J = 2.6$, H–C(8)); 4.72–4.77 (*m*, H–C(6), 2 PhC*H*); 4.80 (*d*, $J = 10.8$, PhC*H*); 4.89 (*d*, $J = 10.8$, PhC*H*); 4.91 (*d*, $J = 11.0$, PhC*H*); 6.99–7.01 (*m*, 2 arom. *H*); 7.15–7.34 (m, 33 arom. H). ^{13}C-NMR (50.6 MHz, $CDCl_3$): 60.06 (*d*, C(5)); 68.95, 69.14 (2*t*, CH_2–C(5), C(6')); 71.78, 71.90 (2*d*); 72.03, 72.60, 73.24, 73.52, 74.69, 75.01 (6*t*, 6 PhCH_2); 75.01 (*d*, C(5')); 75.65 (*t*, PhCH_2); 77.78 (*d*, C(4')); 79.04 (*d*); 81.55 (*d*, C(2')); 81.55 (*s*, C(2)); 84.50 (*d*, C(3')); 96.16 (*s*, C(3)); 102.86 (*d*, C(1')); 127.64–128.59 (several *d*); 137.58 (2*s*); 138.12 (*s*); 138.18 (2s); 138.24, 136.68 (2*s*); 148.91 (*s*, C(8a)).

*(5*R*,6*R*,7*S*,8*R*)-7,8-Bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydro-2,3-diiodo-6-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyloxy)imidazo[1,2-*a*]pyridine* (**31**). R_f (hexane/AcOEt 4 : 1) 0.34. IR ($CHCl_3$): 3066*w*, 3007*m*, 2867*m*, 1605*w*, 1497*m*, 1454*m*, 1435*w*, 1394*w*, 1361*m*, 1309*w*, 1262*w*, 1175*s*, 1070*s*, 1028*m*, 912*w*. ^{1}H-NMR (500 MHz, $CDCl_3$, assignment based on a DQFCOSY.GRASP spectrum): 3.18 (*dt*, $J = 9.7$, 2.5, H–C(5')); 3.37 (*dd*, $J = 9.0$, 7.9, H–C(2')); 3.55 (*dd*, $J = 9.0$, 5.1, CH–C(5)); 3.59 (*t*, $J = 9.1$, H–C(3')); 3.61–3.66 (*m*, CH'–C(5), 2 H–C(6')); 3.66 (*t*, $J = 9.3$, H–C(4')); 3.75 (*dd*, $J = 6.5$, 3.3, H–C(7)); 4.35 (*ddd*, $J = 9.2$, 5.1, 1.0, H–C(5)); 4.39 (*d*, $J = 11.9$, PhC*H*); 4.43 (*d*, $J = 11.9$, PhC*H*); 4.50 (*d*, $J = 12.2$, PhC*H*); 4.545 (*d*, $J = 12.0$, PhC*H*); 4.550 (*d*, $J = 12.2$, PhC*H*); 4.58 (*d*, $J = 12.0$, PhC*H*); 4.647 (*s*, PhCH_2); 4.650 (*d*, $J = 12.1$, PhC*H*); 4.654 (*d*, $J \approx 12.2$, PhC*H*); 4.660 (*d*, $J \approx 8.0$, H–C(1')); 4.69 (*d*, $J = 11.4$, PhC*H*); 4.73 (*d*, $J = 3.3$, H–C(8)); 4.74 (*dd*,

$J = 6.5$, 1.1, H–C(6)); 4.800 (*d*, $J = 10.9$, PhC*H*); 4.805 (*d*, $J = 11.0$, PhC*H*); 4.89 (*d*, $J = 10.9$, PhC*H*); 7.16–7.33 (*m*, 35 arom. H). ^{13}C-NMR (50.6 MHz, $CDCl_3$): 60.23 (*d*, C(5)); 69.21, 69.41 (2*t*, CH_2–C(5), C(6′)); 72.03, 72.16 (2*d*); 72.25, 72.84, 73.47, 73.74, 74.91, 75.21 (6*t*, 6 PhCH_2); 75.28 (*d*, C(5′)); 75.85 (*t*, PhCH_2); 77.93 (*d*, C(4′)); 79.34 (*d*); 81.78 (*d*, C(2′)); 81.78 (*s*, C(2)); 84.76 (*d*, C(3′)); 96.66 (*s*, C(3)); 102.62 (*d*, C(1′)); 127.84–128.83 (several *d*); 138.86 (2*s*); 138.39 (*s*); 138.45 (2*s*); 138.52, 138.97 (2*s*); 149.18 (*s*, C(8a)).

Deiodination of **30**. A soln. of **30** (130 mg, 0.106 mmol) in THF (2 ml) was treated at 0° with 1M EtMgBr in Et_2O (170 µl, 0.17 mmol), stirred for 15 min, and treated with sat. aq. NH_4Cl soln. Extraction with CH_2Cl_2, drying of the combined org. layers ($MgSO_4$), and evaporation gave crude **33** (115 mg) which was completely deiodinated by the repetition of this procedure. FC (hexane/AcOEt 7:3) gave **28** (80 mg, 77%).

*(5*R*,6*R*,7*S*,8*S*)-7,8-Bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydro-2-iodo-6-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyloxy)imidazo[1,2-*a*]pyridine* (**33**). R_f (hexane/AcOEt 7:3) 0.22. ^{13}C-NMR (50.6 MHz, $CDCl_3$): 58.09 (*d*, C(5)); 69.07, 69.83 (2*t*, CH_2–C(5), C(6′)); 71.27 (*d*); 71.85, 72.83, 73.48, 73.71 (4*t*, 4 PhCH_2); 74.55 (*d*); 75.08, 75.23 (2*t*, 2 PhCH_2); 75.32 (*d*, C(5′)); 75.89 (*t*, PhCH_2); 77.95 (*d*, C(4′)); 79.16 (*d*); 82.13 (*d*, C(2′)); 82.20 (*s*, C(2)); 84.87 (*d*, C(3′)); 102.63 (*d*, C(1′)); 124.48 (*d*, C(3)); 127.82–128.91 (several *d*); 137.58, 137.93, 138.34, 138.42 (4*s*); 138.47 (2*s*); 138.88 (*s*); 145.17 (*s*, C(8a)). FAB-MS: 1119 ($[M+1]^+$).

*(5*R*,6*R*,7*S*,8*S*)-7,8-Bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydro-6-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyloxy)imidazo[1,2-*a*]pyridine* (**28**). R_f (hexane/AcOEt/Et_3N 1:1:0.03) 0.29. IR ($CHCl_3$): 3066*w*, 3007*m*, 2961*w*, 2868*m*, 1495*m*, 1454*m*, 1362*m*, 1312*w*, 1262*m*, 1173*s*, 1070*s*, 1028*m*, 909*w*. ^{1}H-NMR (500 MHz, $CDCl_3$; assignment based on a DQFCOSY.GRASP spectrum): 3.37–3.40 (*m*, H–C(2′), H–C(5′)); 3.56–3.62 (*m*, 2 H–C(6′)); 3.62–3.65 (*m*, H–C(3′), H–C(4′)); 3.75 (*d*, $J = 5.6$, CH_2–C(5)); 4.318 (*t*, $J \approx 3.5$, H–C(7)); 4.337 (*t*, $J = 4.2$, H–C(6)); 4.38 (*d*, $J = 11.8$, PhC*H*); 4.39–4.43 (*m*, H–C(5)); 4.41 (*d*, $J = 11.9$, PhC*H*); 4.465 (*d*, $J = 12.0$, PhC*H*); 4.488 (*d*, $J \approx 11.0$, PhC*H*); 4.492 (*d*, $J \approx 7.7$, H–C(1′)); 4.51 (*d*, $J \approx 12.1$, PhC*H*); 4.54 (*d*, $J = 10.9$, PhC*H*); 4.59 (*s*, PhCH_2); 4.60 (*d*, $J \approx 11.6$, PhC*H*); 4.73 (*d*, $J = 2.8$, H–C(8)); 4.73 (*d*, $J = 11.7$, PhC*H*); 4.78 (*d*, $J = 11.0$, PhC*H*); 4.81 (*d*, $J = 10.9$, PhC*H*); 4.84 (*d*, $J = 11.8$, PhC*H*); 4.89 (*d*, $J = 11.0$, PhC*H*); 6.96 (*d*, $J = 1.0$), 7.10 (*d*, $J = 1.0$, H–C(2), H–C(3)); 7.03–7.06 (*m*, 2 arom. H); 7.15–7.34 (*m*, 33 arom. H). ^{13}C-NMR (75.9 MHz, $CDCl_3$): 57.90 (*d*, C(5)); 69.02, 69.87 (2*t*, CH_2–C(5), C(6′)); 71.65 (*t*, PhCH_2); 71.70 (*d*); 72.75, 73.38, 73.63 (3*t*, 3 PhCH_2); 74.91 (*d*); 74.94, 75.13 (2*t*, 2 PhCH_2); 75.21 (*d*, C(5′)); 75.78 (*t*, PhCH_2); 77.88 (*d*, C(4′)); 79.73 (*d*); 82.06 (*d*, C(2′)); 84.82 (*d*, C(3′)); 102.86 (*d*, C(1′)); 118.54 (*d*); 127.64–128.74 (several *d*); 128.97 (*d*); 137.69 (*s*); 138.41 (2*s*); 138.49 (2*s*); 138.52, 138.99 (2*s*); 143.03 (*s*, C(8a)). FAB-MS: 993 ($[M+1]^+$).

Deiodination of **31**. A soln. of **31** (208 mg, 0.167 mmol) in THF (2.8 ml) was treated at 0° with 1M EtMgBr in Et_2O (250 µl, 0.25 mmol), stirred for 15 min, and treated with a sat. aqueous NH_4Cl soln. Extraction with CH_2Cl_2, drying of the combined org. layers ($MgSO_4$), and evaporation gave crude **34** (165 mg) which was completely deiodinated by the repetition of this procedure. FC (hexane/AcOEt 7:3) gave **29** (110 mg, 66%).

*(5*R*,6*R*,7*S*,8*R*)-7,8-Bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydro-2-iodo-6-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyloxy)-imidazo[1,2-*a*]pyridine* (**34**). ^{13}C-NMR (50.6 MHz, $CDCl_3$): 60.25 (*d*, C(5)); 68.82 (*t*, C(6′)); 70.07 (*d*); 71.90, 71.93, 72.97, 73.43, 73.73, 75.12, 75.18 (7*t*, CH_2–C(5), 6 PhCH_2); 75.26 (*d*, C(5′)); 75.66 (*d*); 75.86 (*t*, PhCH_2); 77.59 (*d*); 77.87 (*d*, C(4′)); 81.93 (*s*, C(2)); 82.30 (*d*, C(2′)); 84.97 (*d*, C(3′)); 102.07 (*d*, C(1′)); 125.37 (*d*, C(3)); 127.87–128.79 (several *d*); 137.67, 138.79, 138.73, 138.41 (4*s*); 138.45 (2*s*); 138.72 (*s*); 145.67 (*s*, C(8a)).

*(5*R*,6*R*,7*S*,8*R*)-7,8-Bis(benzyloxy)-5-[(benzyloxy)methyl]-5,6,7,8-tetrahydro-6-(2,3,4,6-tetra-*O*-benzyl-β-*D*-glucopyranosyloxy)imidazo[1,2-*a*]pyridine* (**29**). R_f (hexane/AcOEt/Et_3N 1:1:0.03) 0.29. IR ($CHCl_3$): 3066*w*, 3008*m*, 2926*w*, 2868*m*, 1605*w*, 1595*w*, 1496*m*, 1454*m*, 1360*m*, 1309*w*, 1263*m*, 1175*s*, 1070*s*, 1028*m*, 909*m*. ^{1}H-NMR (500 MHz, $CDCl_3$, assignment based on a DQFCOSY.GRASP spectrum): 3.26 (*ddd*, $J = 9.4$, 3.9, 2.1, H–C(5′)); 3.37 (*t*, $J \approx 8.4$, H–C(2′)); 3.58–3.64 (*m*, CH–C(5), 2 H–C(6′)); 3.59 (*t*, $J = 9.1$, H–C(3′)); 3.64 (*t*, $J = 9.2$, H–C(4′)); 3.70 (*dd*, $J = 9.5$, 6.1, CH′–C(5)); 3.97 (*dd*, $J = 6.7$, 3.3, H–C(7)); 4.31 (*d*, $J = 11.9$, PhC*H*); 4.32–4.37 (*m*, H–C(5)); 4.35 (*d*, $J = 11.9$, PhC*H*); 4.47 (*d*, $J = 12.1$, PhC*H*); 4.49 (*dd*, $J = 6.8$, 2.5, H–C(6)); 4.535 (*d*, $J = 11.9$, PhC*H*); 4.557 (*d*, $J \approx 11.5$, PhC*H*); 4.561 (*d*, $J \approx 7.7$, H–C(1′)); 4.595 (*d*, $J = 11.2$, PhC*H*); 4.643 (*d*, $J = 12.1$, PhC*H*); 4.652 (*d*, $J \approx 12.4$, 2 PhC*H*); 4.70 (*d*, $J = 12.3$, PhC*H*); 4.802 (*d*, $J \approx 11.0$, PhC*H*); 4.805 (*d*, $J \approx 11.3$, 2 PhC*H*); 4.811 (*d*, $J = 3.3$, H–C(8)); 4.88 (*d*, $J = 11.0$, PhC*H*); 6.91 (*d*, $J = 1.3$), 7.07 (*d*, $J = 1.4$, H–C(2), H–C(3)); 7.12–7.31 (*m*, 35 arom. H). ^{13}C-NMR (75.9 MHz, $CDCl_3$): 60.10 (*d*, C(5)); 68.81 (*t*, C(6′)); 70.17 (*d*); 71.56, 72.22, 72.75, 73.39, 73.71 (5*t*, CH_2–C(5), 4 PhCH_2); 75.08 (*t*, 2 PhCH_2); 75.23 (*d*, C(5′)); 75.83 (*t*, PhCH_2); 76.04 (*d*); 77.90 (*d*, C(4′)); 78.16 (*d*); 82.27 (*d*, C(2′)); 85.04 (*d*, C(3′)); 102.07 (*d*, C(1′)); 119.69 (*d*); 127.81–128.71 (several *d*); 129.14 (*d*); 137.88 (*s*); 138.34 (2*s*); 138.45 (2*s*); 138.60, 138.81 (2*s*); 143.50 (*s*, C(8a)).

*(5*R*,6*R*,7*S*,8*S*)-6-(β-*D*-Glucopyranosyloxy)-5,6,7,8-tetrahydro-5-[(hydroxy)methyl]imidazo[1,2-*a*]pyridine-7,8-diol* (**32**). A soln. of **28** (108 mg, 0.11 mmol) in AcOEt/MeOH/H_2O 5:17:3 (0.45 ml, 0.26M) was treated with AcOH (0.1 ml) and 20% $Pd(OH)_2$/C (110 mg) and hydrogenated at 6 bar for 2 d. The suspension was filtered

through *Celite* (eluted with MeOH and H_2O). Evaporation, FC (AcOEt/MeOH/H_2O 7:3:2), and lyophilization gave **32** (27 mg, 69%). R_f (AcOEt/MeOH/H_2O 7:3:2) 0.14. ^{1}H-NMR (500 MHz, D_2O; assignment based on a DQFCOSY.GRASP spectrum): 3.32 (*dd*, $J = 9.4$, 7.9, H–C(2')); 3.39 (*t*, $J \approx 9.4$, H–C(4')); 3.45 (*ddd*, $J = 9.7$, 5.5, 2.2, H–C(5')); 3.485 (*t*, $J = 9.2$, H–C(3')); 3.71 (*dd*, $J = 12.4$, 5.6, H–C(6')); 3.87 (*dd*, $J = 12.4$, 2.2, H'–C(6')); 3.90 (*t*, $J = 8.4$, H–C(7)); 4.09 (*dd*, $J = 12.6$, 2.3, CH–C(5)); 4.15–4.22 (*m*, H–C(5), H–C(6), CH'–C(5)); 4.59 (*d*, $J = 7.9$, H–C(1')); 4.62 (*d*, $J = 8.2$, H–C(8)); 7.06, 7.20 (2 br. *s*, H–C(2), H–C(3)). ^{13}C-NMR (75.9 MHz, D_2O): 61.32 (*t*, C(6')); 63.44 (*d*, C(5)); 63.89 (*t*, CH_2–C(5)); 70.08 (*d*); 72.28 (*d*); 74.95 (*d*); 76.01 (*d*); 78.35 (*d*); 79.08 (*d*); 79.14 (*d*); 105.89 (*d*, C(1')); 122.44, 125.46 (2*d*, C(2), C(3)); 147.99 (*s*, C(8a)). Anal. calc. for $C_{14}H_{22}N_2O_9 \cdot 3\,H_2O$ (416.39): C 40.38, H 6.77, N 6.72; found: C 40.61, H 6.93, N 6.14. ESI-MS: 363 ($[M+1]^+$).

Inhibition Studies. Determination of the inhibition constants (K_i) was performed in the presence of five inhibitor concentrations which bracket the K_i value.

a) *Inhibition of Cel7A.* The inhibition constants were determined at 30° using a 50 mM KH_2PO_4/Na_2HPO_4 buffer (pH 5.7) and 2-chloro-4-nitrophenyl β-lactoside as substrate [32]. The release of 2-chloro-4-nitrophenol was monitored continuously by measuring absorbance at 405 nm for 10 minutes. The K_m value of this substrate is 460 μM [30]. The K_i values were determined classically either by *Lineweaver-Burk* plots or *Dixon* plots [33].

b) *Inhibition of Cel6A.* The inhibition experiments were performed at 27° in 10 mM NaOAc buffer (pH 5) by following the hydrolysis of cellotriose, which has a K_m value of 22 μM [31]. Samples were taken at 8 different time points over 12 min and the amount of hydrolysed cellotriose was determined by high-performance anion-exchange chromatography (HPAEC) equipped with a pulsed amperometric detector (PAD) and using the conditions specified by the manufacture (*Dionex*, Sunnyvale, USA). The K_i values were obtained from *Dixon* plots.

REFERENCES

[1] B. Henrissat, T. T. Teeri, R. A. J. Warren, *FEBS Lett.* **1998**, *425*, 352.
[2] B. K. Barr, Y.-L. Hsieh, B. Ganem, D. B. Wilson, *Biochemistry* **1996**, *35*, 586.
[3] C. Divne, J. Ståhlberg, T. Reinikainen, L. Ruohonen, G. Pettersson, J. K. C. Knowles, T. T. Teeri, T. A. Jones, *Science* **1994**, *265*, 524.
[4] J. Rouvinen, T. Bergfors, T. Teeri, J. K. C. Knowles, T. A. Jones, *Science* **1990**, *249*, 380.
[5] J. K. C. Knowles, P. Lehtovaara, M. Murray, M. L. Sinnott, *J. Chem. Soc., Chem. Commun.* **1988**, 1401.
[6] M. Claeyssens, P. Tomme, C. F. Brewer, E. J. Hehre, *FEBS Lett.* **1990**, *263*, 89.
[7] A. Koivula, T. Kinnari, V. Harjunpää, L. Ruohonen, A. Teleman, T. Drakenberg, J. Rouvinen, T. A. Jones, T. T. Teeri, *FEBS Lett.* **1998**, *429*, 341.
[8] G. Sulzenbacher, H. Driguez, B. Henrissat, M. Schülein, G. J. Davies, *Biochemistry* **1996**, *35*, 15280.
[9] T. D. Heightman, A. Vasella, *Angew. Chem., Int. Ed.* **1999**, *38*, 751.
[10] S. Vonhoff, T. D. Heightman, A. Vasella, *Helv. Chim. Acta* **1998**, *81*, 1710.
[11] R. Hoos, A. B. Naughton, W. Thiel, A. Vasella, W. Weber, K. Rupitz, S. G. Withers, *Helv. Chim. Acta* **1993**, *76*, 2666.
[12] A. Rivera-Sagredo, J. Jimenez-Barbero, M. Martin-Lomas, D. Solis, T. Diaz-Maurino, *Carbohydr. Res.* **1992**, *232*, 207.
[13] R. Hoos, A. B. Naughton, A. Vasella, *Helv. Chim. Acta* **1993**, *76*, 1802.
[14] S. Koto, N. Morishima, T. Irisawa, Y. Hashimoto, M. Yamazaki, S. Zen, *Nippon Kagaku Kaishi (J. Chem. Soc. Jpn.)* **1982**, 1651.
[15] D. B. Dess, J. C. Martin, *J. Am. Chem. Soc.* **1991**, *113*, 7277.
[16] H. Fukase, S. Horii, *J. Org. Chem.* **1992**, *57*, 3651.
[17] H. S. Overkleeft, J. v. Wiltenburg, U. K. Pandit, *Tetrahedron* **1994**, *50*, 4215.
[18] Y. Wang, S. A. Babirad, Y. Kishi, *J. Org. Chem.* **1992**, *57*, 468.
[19] R. Claus, R. Hunter, *J. Chem. Soc., Perkin Trans. 1* **1997**, 71.
[20] K. C. Nicolaou, D. G. McGarry, P. K. Somers, C. A. Veale, G. T. Furst, *J. Am. Chem. Soc.* **1987**, *109*, 2504.
[21] T. Granier, N. Panday, A. Vasella, *Helv. Chim. Acta.* **1997**, *80*, 979.
[22] N. Panday, A. Vasella, in preparation.
[23] K. Tatsuta, S. Miura, H. Gunji, *Bull. Chem. Soc. Jpn.* **1997**, *70*, 427.
[24] M. Claeyssens, H. van Tilbeurgh, P. Tomme, T. M. Wood, S. McRae, *Biochem. J.* **1989**, *261*, 819.
[25] C. Divne, J. Ståhlberg, T. T. Teeri, T. A. Jones, *J. Mol. Biol.* **1998**, *275*, 309.

[26] S. Vonhoff, A. Vasella, *Synth. Commun.* **1999**, *29*, 551.
[27] A. Varrot, M. Schülein, M. Pipelier, A. Vasella, G. J. Davies, *J. Am. Chem. Soc.* **1999**, *121*, 2621.
[28] G. J. Davies, L. Mackenzie, A. Varrot, M. Dauter, A. M. Brzozowski, M. Schülein, S. G. Withers, *Biochemistry* **1998**, *37*, 11707.
[29] H. van Tilbeurgh, G. Pettersson, R. Bhikabhai, H. De Boeck, M. Claeyssens, *Eur. J. Biochem.* **1985**, *148*, 329.
[30] J. Ståhlberg, C. Divne, A. Koivula, K. Piens, M. Claeyssens, T. T. Teeri, T. A. Jones, *J. Mol. Biol.* **1996**, *264*, 337.
[31] A. Koivula, T. Reinikainen, L. Ruohonen, A. Valkeajärvi, M. Claeyssens, O. Teleman, G. J. Kleywegt, M. Szardenings, J. Rouvinen, T. A. Jones, T. T. Teeri, *Protein Eng.* **1996**, *9*, 691.
[32] H. van Tilbeurgh, F. G. Loontiens, C. K. de Bruyne, M. Claeyssens, *Methods Enzymol.* **1988**, *160*, 45.
[33] A. Cornish-Bowden, 'Fundamentals of Enzyme Kinetics', Portland Press, London, 1995, p. 93.

Received March 18, 1999