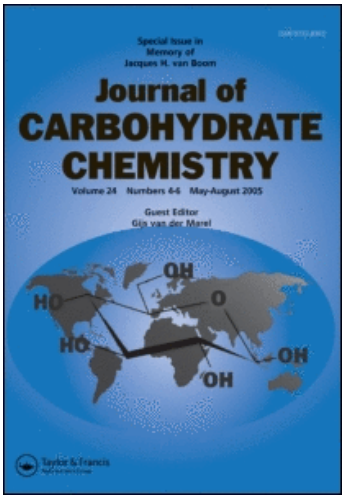

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SYNTHESIS AND CONFORMATIONAL ANALYSIS OF PSEUDOSUGAR ANALOGUES OF CHITOTRIOSE*

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ABSTRACT

EDC mediated coupling of the 4-*O*-succinoyl glycosyl azide **2** with glycosylamine **3** gave the protected glycosylazide **4**. Hydrogenation of **4** afforded the glycosylamine **5**. Chemoselective hydrolysis of the reducing end glycosylamine, followed by hydrogenation afforded *N*,*N*″-diacetylpseudochitotriose **7**. Coupling of **5** with heterocyclic or with Cbz protected aliphatic amino acids yielded glycosyl amides **8**–**12**. Deprotection of **11** and **12** afforded the *N*,*N*″-diacetylpseudochitotriosyl amides of β-alanine, **13**, and L-arginine, **14**. Molecular modelling calculations revealed that the pseudotrisaccharides exist in low energy extended conformations which show similar space filling as *N*,*N*′,*N*″-triacetylchitotriose.

Key Words: Chitotriose; Glycosyl amides; Molecular modelling; Pseudooligosaccharides; Sugar mimics; Spacers; Succinoyl amides

*Dedicated to Professor Dr. Joachim Thiem on the occasion of his 60th birthday.

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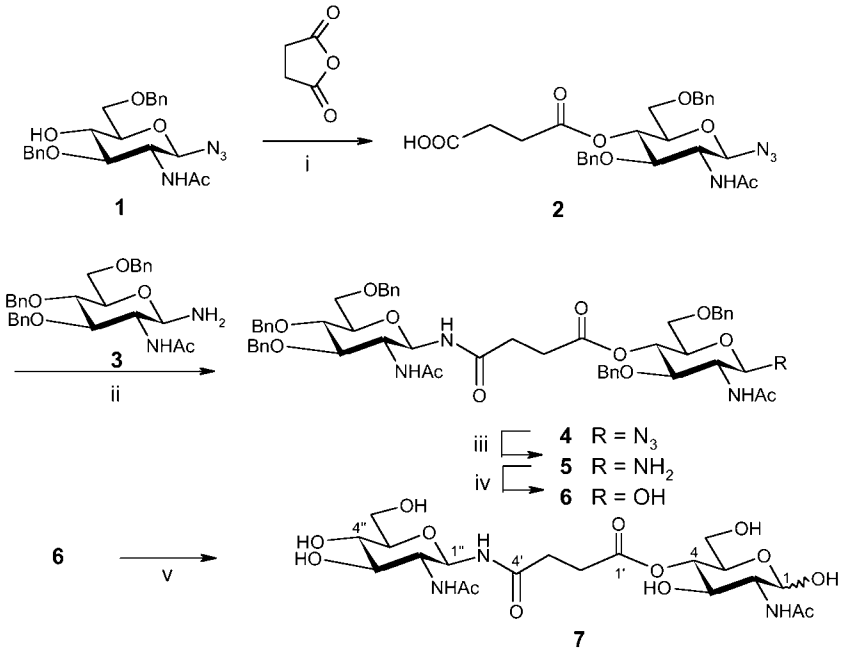
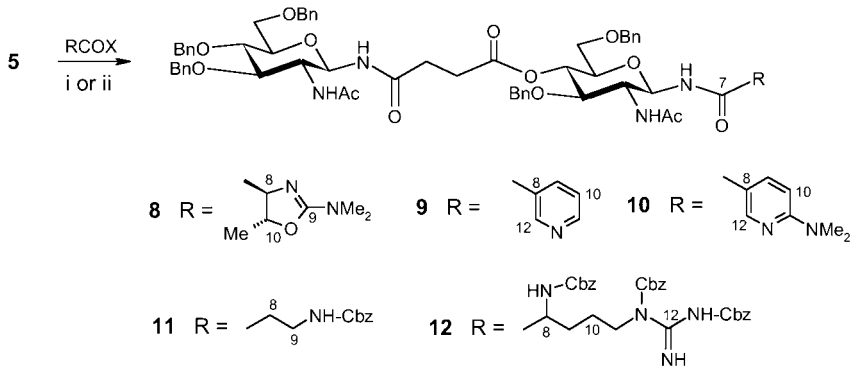
INTRODUCTION

Chitooligosaccharides are formed in nature by enzymatic degradation of chitin or chitosan as well as by de novo biosynthesis in a variety of organisms, including humans. Due to their intriguing biological activities, chitooligosaccharides [$(GlcNAc/GlcN)_n$, $n>2$] attract increasing interest (for a review, see Ref. [1]). Current concepts in carbohydrate chemistry and biology include sugar mimics as tools for investigations of carbohydrate-receptor interactions (for recent reviews, see Refs. [2-4]). A variety of spacer modified oligosaccharides are described as sugar mimics suitable for studies of certain polysaccharide binding proteins, including enzymes.[5,6] Within our work on the synthesis of derivatives of chitooligosaccharides[7–9] it appeared to be of interest to prepare spacer containing pseudochitooligosaccharides that should resist hydrolysis by chitinases. In this paper, we report on the synthesis of analogues of *N*,*N*′,*N*″-triacetylchitotriose in which the central sugar residue was replaced by a succinic acid unit. The non-reducing end GlcNAc residue was linked to one of the carboxy groups of succinic acid *via* an *N*-glycosyl amide whereas the reducing end GlcNAc residue was attached to the other as an ester *via* the 4-OH group, giving the sequence GlcNAc-1-*N*-Succ-4-*O*-GlcNAc (Scheme 1, formula **7**). This was based on the consideration that **7** could possibly bind to the -2, -1, and $+1$ or to the -3 to -1 sites of chitinases (for nomenclature of binding sites of glycosyl hydrolases, see Ref. [10]). In either case,



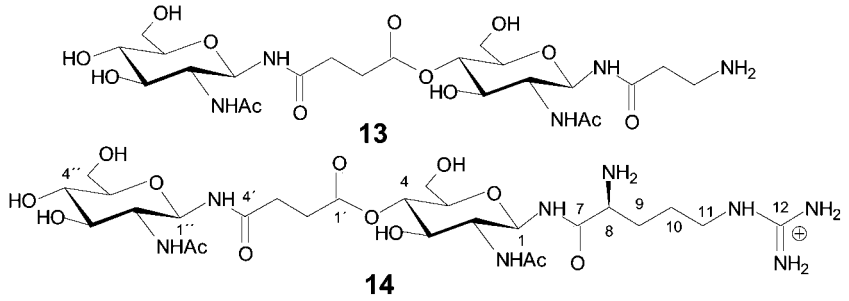
Scheme 1. Synthesis of pseudotrisaccharide 7. i:0.7 EDC, 0.5 HOBt, 0.6 NEt_3, CH_2Cl_2, rt, 10-14 d; ii:EDC, 0.2 HOBt, 0.4 NEt_3, CH_2Cl_2, rt, 30 h; iii: H_2, Pd/C, MeOH, 60 °C to rt, 35 min; iv: $MeOH/CH_2Cl_2$, HCl/H_2O, rt, 20 h; v: H_2, $Pd(OH)_2/C$, MeOH, 50°C to rt, 5 h.



Scheme 2. Synthesis of glycosylamides **8**–**12**. i: CH_2Cl_2, Pyr, -35 °C to rt; ii: EEDQ, CH_2Cl_2, rt.

enzyme inhibition could be observed when the affinities of the pseudotrisaccharide and the substrate are comparable.

Furthermore, in an extension of our earlier work on glycosyl amides as potential chitinase inhibitors,[7–9] we report on the synthesis of novel protected carboxylic acid glycosylamides of **7** (Scheme 2, formulas **8**, **9**, and **10**) as well as the glycosylamides of β-alanine, **13**, and of L-arginine, **14** (Scheme 3). Compound **13** would introduce a basic primary aliphatic amino group at the catalytic site, when the pseudotrisaccharide binds at sites -4 to -2. If compound **14** binds analogously, the positively charged arginine residue would give an ion pair with the catalytic glutamate residue, leading again to enzyme inhibition. However, very little is known on the mode of binding of chitooligosaccharides to chitinases in terms of the sequences of binding sites and contribution of individual sugar residues to binding affinity (cf. Refs. [11–13]). Likewise, the roles of the amino acid side chains involved in substrate recognition of these enzymes are largely unknown, though a few high resolution crystal structures of enzymes belonging to these highly diverse families 18 and 19 of glycosyl hydrolases have been published[14–17] (see also Ref. [1] for a review of the earlier literature).



Scheme 3. Structures of glycosylamides **13** and **14**.

None of the compounds **7**, **13**, or **14** showed appreciable competition with the fluorogenic 4-methylumbelliferone glycosides of $(GlcNAc)_2$ or $(GlcNAc)_3$ in enzyme assays, using chitinases from a bacterium (ChiA or ChiB from *Serratia marcescens*), a plant (*Hevea brasiliensis*), or an insect (*Chironomus tentans*) cell line, respectively (data not shown). Therefore, conformational analysis was of interest. Especially the flexibility and the size of the succinoyl spacer group of the substrate analogue should be compared with those of *N*,*N*′,*N*″-triacetylchitotriose. Because neither scalar (J) nor dipolar coupling (NOE) information concerning the conformation of the oligosaccharide analogues could be obtained from NMR routines, we report on molecular modelling calculations of **7** and **14**, introducing new sets of parameters for the spacer unit and for the glycosyl amide linkage.

RESULTS AND DISCUSSION

Synthesis

Glycosyl azide **1** was prepared from 2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl azide[18] by reductive opening of the benzylidene ring with $NaCNBH_3$ in THF (Scheme 1). The isolated yield of the crystalline compound **1** never exceeded 71%, as partial decomposition occurred during flash chromatography of the crude product. Reaction of **1** with succinic anhydride in the presence of EDC, HOBt and NEt_3 afforded hemisuccinate **2** in 90% yield. The exact ratio of the reagents was crucial in this reaction (see Experimental).

O-Benzylation of 2-acetamido-2-deoxy-β-D-glucopyranosyl azide[19] gave 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl azide in 52% yield (cf. Refs. [20–22]). Catalytic hydrogenation of this compound over Adam's catalyst yielded the novel tri-*O*-benzyl-*N*-acetamidoglucosylamine **3**. EDC mediated coupling of **2** with glycosylamine **3** gave, after trituration of the crude solid sequentially with toluene, MeCN and MeOH, the protected pseudotrisaccharide **4** in 65% isolated yield. Also in this reaction, the ratio of EDC, HOBt and NEt_3 had to be adjusted carefully. Hydrogenation of **4** over Pd/C (10%) afforded the protected glycosylamine **5**. In contrast to azide **4**, the amine **5** is rather unstable and, therefore, was always freshly prepared immediately before use in subsequent reactions.

Attempts to achieve complete debenzylation of **5** by catalytic hydrogenation always resulted in a mixture of the corresponding amine with pseudotrisaccharide **7** as the minor product. It is reasonable to assume that **7** was generated from traces of water which could not be removed by the method of preparation. Alternatively, **7** was prepared in 55% overall yield by chemoselective hydrolysis of glycosylamine **5**, followed by catalytic hydrogenation (Scheme 1).

The glycosyl carboxamides **9**, **11**, and **12** were prepared by EEDQ mediated coupling of the corresponding carboxylic acid with glycosylamine **5** (Scheme 2).[7–9] Optimum yields were obtained after reaction of the carboxylic acid with EEDQ prior to addition of freshly prepared **5**. Synthesis of the glycosyl carboxamides **8** and **10** was achieved by coupling of **5** with the corresponding dimethylaminooxazoline- and dimethylaminopyridinecarboxylic acid chlorides, respectively.[9]

Catalytic hydrogenation of glycosyl amides **8**, **9**, and **10** over Pd or Ni catalysts always resulted in partial reduction of the heterocyclic rings, as evidenced by MALDI-TOF mass spectrometry, thus lowering the yields of fully deprotected products. De-*O*-benzylation of **11** by hydrogenation over $Pd(OH)_2/C$ (20%) in both, MeOH or EtOH, gave always N^{β}-alkyl-β-alanine glycosyl amides as side products.[23]

Due to the insolubility of **12** in most solvents, deprotection failed under standard conditions, and complete decomposition ensued in many cases. Also, reductive *N*-alkylation of the guanidino group moiety was observed after hydrogenation of **12** in the presence of MeOH or EtOH. Attempts to suppress the *N*-alkylation by addition of small amounts of H_2O to the reaction mixture resulted in partial hydrolysis of the *O*-succinate. Finally, reproducible debenzylation of **11** and of **12** proceeded smoothly when the hydrogenation was carried out at 40°C over 15 or 18 h, respectively, in a rather large volume of MeOH, containing a few drops of H_2O, while maintaining a pressure of 35 bar. The pseudochitotriosyl amides **13** and **14** were isolated in 95 and 64% yields, respectively.

Conformational Analysis

NMR spectroscopy (1D and 2D ^1H and ^{13}C NMR routines as well as 1D and 2D NOE/NOESY/ROESY experiments) did not yield conclusive information about the conformations. In addition, the H,H coupling constants which could be extracted from the ^1H NMR spectra proved to be weighted averages of an unknown number of conformations participating in conformational equilibria of the compounds under study.

As force field calculations are useful alternatives to NMR for estimating global minima conformations, compounds **7** and **14** were studied by molecular mechanical calculations using the AMBER force field. In this approach, the molecule under study is constructed from monomers. The relevant data for amino acid residues and various linkages are available from appropriate data bases.[24–27] The 3D geometry of the molecule is defined by a z-matrix and each atom is defined by its type and net atomic charge. Torsional angles, bond lengths and bond angles are specific for the atom type selected. However, the available data sets do not contain the parameters for calculating the succinoyl units in **7** and **14**, and the arginine residue in **14**. Using the AMBER force field and based on the procedure described by Woods et al.,[27] we have derived fully compatible parameters for the carbohydrate residues as well as for the additional units in **7** and **14** (see Experimental).

The principal approach towards analyzing the conformation of the pseudotrisaccharides was dynamic simulation and systematic variation of dihedral angles, followed by energy minimization. Dihedral angles are defined as: ϕ (H1–C1–N1–C7); ϕ′ (C2′–C1′–O1′–C4); ϕ″ (H1″–C1″–N1″–C4′); ψ′ (C1′–O4–C4–H4); χ^1 (N1″–C4′–C3′–C2′); χ^2 (C4′–C3′–C2′–C1′); χ^3 (C3′–C2′–C1′–O1′); χ^4 (N1–C7–C8–C9). Some preselections were necessary, as follows: the bond angles C1″–N1″–C4′ and C1′–O4–C4 in **7** and **14** were set to 117°; the pyranose rings were treated as rigid $^4C_1$ conformations; the dihedral angles ω (O5–C5–C6–O6) and ω″ (O5″–C5″–C6″–O6″) were fixed at 60°, corresponding to the *gt* energy minimum conformations;[28,29] the

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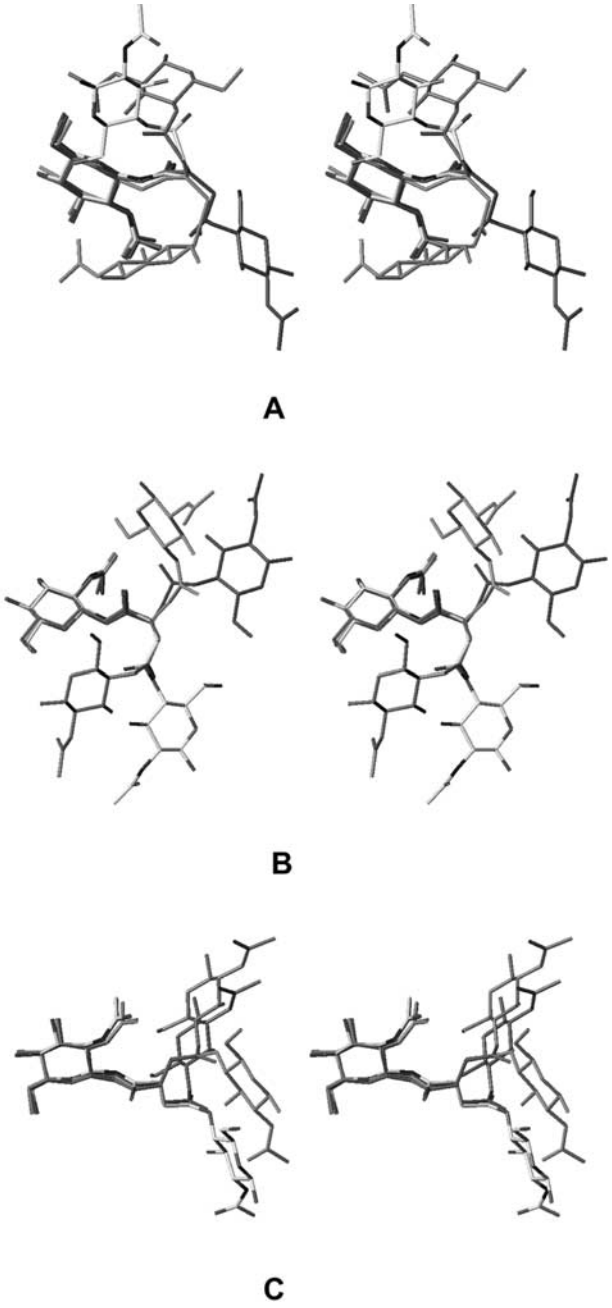

Figure 1. Stereo-view of low energy conformation of **7**, four each minimized for χ^1 and χ^3 in two *gauche* (**A**: $\chi^2 = 60°$; **B**: $-60°$) and in the *anti* (**C**: $\chi^2 = 180°$) conformations.

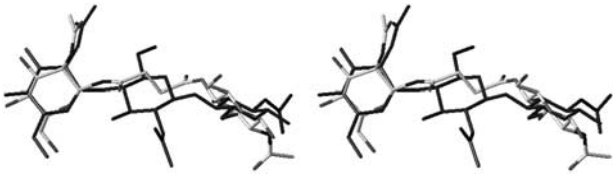

Figure 2. Stereo-view of the global minimum conformations of *N*,*N*′,*N*″-triacetyl-chitotriose superimposed with the χ^2 *anti* conformation of **7**.

N-acetyl groups of the pyranose rings were set to the energetically favoured *anti* orientation; and the amide group N1″–C4′–O4′ was treated as essentially planar. Compound **7** was chosen as the β-anomer.

The results obtained with **7** are depicted in Figure 1. Conformations with the lowest steric hindrance between the non-reducing end glycosyl amide and the succinoyl spacer appeared with $\phi'' = 10°$ and 170°. The dihedral angles χ^2, describing conformations of the succinoyl spacer, were 60°, −60°, and 180°. Consequently, two folded *gauche* (Figure 1, A and B) and one extended *anti* (Figure 1C) conformers occupy the global minima in this part of the molecule, with *anti* being the most stable one. In addition, each of the three conformers has four global minima, described by χ^1 and χ^3 (Figure 1).

When the extended conformer **C** is superimposed with triacetylchitotriose, it appears that both compounds occupy similar space, leading to the conclusion that both may a priori bind to chitinases in a similar fashion (Figure 2). However, with respect to the dihedral angles ϕ' and ψ', many conformations near the global minimum have been found, suggesting a non-restricted torsional flexibilty in the ester moiety which is also in accordance with the absence of any observable NOEs between the two GlcNAc residues of **7**.

The pseudotrisaccharide unit of **14** shows essentially the same conformational behavior as **7**. The C7 amide group in **14** favours the *E*-configuration. The four low energy conformers of **14** which are found by energy minimization of ϕ and χ^4 are shown in Figure 3. The conformation of the aliphatic arginine chain is not considered.

The fact that none of the compounds inhibits any of the chitinases tested in this study leads to the conclusion that the high conformational flexibility of the pseudotrisaccharides described in this work prevents high affinity binding, though the spacer unit leaves the reducing end at nearly the same distance in the lowest energy

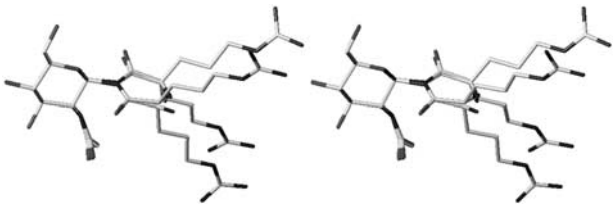

Figure 3. Stereo-view of the low energy conformers of **14** representing the energy minima of ϕ and χ^4 (the non-reducing end GlcNAc and the succinamide are not shown in this figure).

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conformation. Obviously, the decrease in entropy which must occur when **7** should bind to the enzyme, cannot be compensated by the binding enthalpy. In addition, the dipoles associated with the glycosyl amide and the ester group may cause unfavourable interactions with amino acid residues flanking the binding site. We hope that future synthesis of compounds which are designed on the basis of the results described in this work, as well as on complementary knowledge will lead to more efficient pseudooligosaccharides as chitinase inhibitors.

EXPERIMENTAL

General Methods. Solvents were purified by standard procedures. Solutions in organic solvents were dried over anhydrous $MgSO_4$. Gel filtration was performed on Biogel P2® (BioRad) with water containing 2.5% BuOH. Evaporation of solvents: Rotary evaporator (ca. 1.3 kPa), bath temp. at $\leq 40°C$. Chromatography: Silica gel (Merck: 0.04–0.063 mm). NMR: Bruker AMX R 300 (300 MHz or 75 MHz, for ^1H or ^{13}C NMR, respectively), internal standards: TMS or solvent signals for ^1H or ^{13}C NMR spectra, respectively. Assignments of chemical shifts (δ) and coupling constants (J) of compounds **7** and **14** are based on HMBC, HMQC, and NOE experiments. Optical rotations: Jasco DIP-1000. EI-MS: Finnigan SSQ 710 (70 eV). MALDI-MS: Bruker Franzen Reflex II MALDI-TOF, positive ion mode, matrix: THAP. FAB-HR-MS: Fisons VG four sector tandem MS, ZAB T, positive ion mode, matrix: thioglycerol. ESI-HR-MS: Finnigan MAT95, solvent: MeOH. Elemental analysis: LECO CHNS-932.

2-Acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl Azide (1). To an ice-cold solution of 2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl azide[18] (500 mg, 1.2 mmol) and $NaCNBH_3$ (700 mg, 11 mmol) in dry THF (100 mL), 12 mL of a 1M HCl solution in Et_2O was added dropwise during 45 min. The mixture was stirred under reflux for 1.5 h. After cooling to rt, THF was evaporated, followed by addition of $CHCl_3$ (150 mL). The suspension was filtered through Celite, and the remaining residue was repeatly washed with $CHCl_3$. The combined solutions were concentrated to a small volume. Flash chromatography ($CHCl_3$/EtOH 20:1) and recrystallization from Et_2O afforded **1** (355 mg, 71%) as colourless needles, mp 127°C (dec). $[\alpha]_D^{25} - 21.9$ (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 1.90 (s, 3 H, NHCO*Me*), 2.95 (d, 1 H, OH, $J_{OH,4}$ 2.1 Hz), 3.42 (dd, 1 H, H-2), 3.58 (m, 1 H, H-5), 3.66–3.76 (m, 3 H, H-4, H-6ab), 3.82 (dd, 1 H, H-3), 4.58 (dd, 2 H, C*H*$_2$Ph, $J_{Ha,Hb}$ 12.0 Hz), 4.76 (dd, 2 H, C*H*$_2$Ph), 4.91 (d, 1 H, H-1, $J_{1,2}$ 9.1 Hz), 5.63 (d, 1 H, NH, $J_{NH,2}$ 8.0 Hz), 7.26–7.34 (m, 10 H, 2 Ph). ^{13}C NMR ($CDCl_3$): δ 23.4 (NHCO*Me*), 55.8 (C-2), 70.0 (C-6), 72.6 (C-4), 73.7, 74.2 (2 *C*H$_2$Ph), 76.0 (C-5), 80.1 (C-3), 87.8 (C-1), 127.8–138.2 (Ph), 170.7 (CO). MALDI-MS: *m/z* 465.1 $[M+K]^+$, 449.1 $[M+Na]^+$, 427.1 $[M+H]^+$. FAB-HR-MS: *m/z* calcd for $[M+H]^+$: 427.1952, found 427.1981.

2-Acetamido-3,6-di-*O*-benzyl-4-*O*-succinoyl-2-deoxy-β-D-glucopyranosyl Azide (2). To a solution of **1** (500 mg, 1.17 mmol) and succinic anhydride (250 mg, 2.5 mmol) in CH_2Cl_2 (15 mL), EDC (157 mg, 0.82 mmol), HOBt (79 mg, 0.58 mmol), and NEt_3 (71 mg, 0.7 mmol) were added. The mixture was stirred at rt for 10–14 days,

until TLC ($CHCl_3$/EtOH 19:1) indicated complete reaction. H_2O (15 mL) was added and the mixture was stirred for 5 min. The organic layer was washed with 5% HCl and with H_2O, followed by drying and solvent concentration. The remaining residue was suspended in toluene (25 mL) under vigorous stirring overnight. Filtration gave **2** (559 mg, 90%) as a colourless solid. $[\alpha]_D^{18} - 17.5$ (*c* 1.3, DMSO). ^{1}H NMR (DMSO-d_6): δ 1.80 (s, 3 H, NHCO*Me*), 2.46 (m, 4 H, H-2′, H-3′, J 12.3 Hz, J 1.5 Hz), 3.35 (b, 1 H, COOH), 3.42–3.56 (2 dd, 2 H, H-6a, H-6b, J 11.5 Hz, J 12.1 Hz, J 5.2 Hz), 3.72–3.82 (m, 3 H, H-2, H-3, H-5), 4.44 (dd, 2 H, C*H*$_2$Ph, J 12.0 Hz, J 2.8 Hz), 4.53 (s, 2 H, C*H*$_2$Ph), 4.69 (d, 1 H, H-1, $J_{1,2}$ 8.5 Hz), 4.88 (dd, 1 H, H-4, J 9.3 Hz), 7.19–7.39 (m, 10 H, 2 Ph), 8.17 (d, 1 H, NH, $J_{NH,2}$ 7.9 Hz). ^{13}C NMR (DMSO-d_6): δ 22.8 (NHCO*Me*), 28.6, 28.9 (C-2′, C-3′), 53.8 (C-2), 68.4 (C-6), 70.1 (C-4), 72.4, 73.4 (2 C*H*$_2$Ph), 74.6 (C-5), 79.4 (C-3), 87.9 (C-1), 127.5–138.3 (Ph), 169.5 (*C*OO-1′), 171.2 (NH*C*OMe), 173.4 (COOH). MALDI-MS: *m*/*z* 565.8 $[M+K]^+$, 549.8 $[M+Na]^+$, 527.7 $[M+H]^+$.

2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosylamine (3). To an ice-cold suspension of NaH (120 mg, 5 mmol) in dry DMF (30 mL), 2-acetamido-2-deoxy-β-D-glucopyranosyl azide[20] (600 mg, 2.4 mmol) was added under an atmosphere of nitrogen, and the mixture was stirred for 15 min at 0°C. Benzyl bromide (1.5 mL, 12.6 mmol) was added, and the mixture was stirred for 30 min at rt. BaO (3.0 g, 19.6 mmol) and $Ba(OH)_2 \cdot 8\ H_2O$ (1.1 g, 3.5 mmol) were added, and the mixture was stirred overnight at rt. The suspension was diluted with dry CH_2Cl_2 (150 mL) and stirred under reflux for 4–5 h until TLC (toluene/EtOAc 1:1) indicated completeness of the reaction. CH_2Cl_2 (200 mL) was added, the mixture was filtered through Celite, and the residue was repeatly washed with CH_2Cl_2. The combined solutions were concentrated, and the remaining DMF and benzyl bromide were removed by distillation at ca. 10^{-4} kPa. Column chromatography (toluene/EtOAc 1:1) of the residue was followed by recrystallization from EtOH to afford 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl azide[20] (660 mg, 52%) as colourless crystals, mp 176°C. $[\alpha]_D^{21} + 3.8$ (*c* 1.0, $CHCl_3$), $[\alpha]_D^{24} - 39.8$ (*c* 0.4, MeOH) [lit.[20] mp 171°C, $[\alpha]_D^{21} - 34$ (*c* 0.4, MeOH)].

A solution of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl azide (500 mg, 0.97 mmol) in boiling EtOH (150 mL) was cooled to rt, $PtO_2 \cdot nH_2O$ (50 mg) was added, and the mixture was kept under hydrogen atmosphere at rt for 2.5 h. The solution was filtered through Celite, and the filtrate was concentrated to a small volume of EtOH. The solid was filtered off, affording **3** (474 mg, 100%) as colourless crystals, mp 156–162°C (dec). $[\alpha]_D^{22.5} + 21.8$ (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.83 (s, H, NHCO*Me*), 2.01 (b, 2 H, NH_2), 3.48 (ddd, 1 H, H-5), 3.57–3.69 (m, 4 H, H-3, H-4, H-6a, H-6b), 3.71 (dd, 1-H, H-2), 4.02 (d, 1 H, H-1, $J_{1,2}$ 9.0 Hz), 4.51–4.86 (3 dd, 6 H, 3 C*H*$_2$Ph, $J_{Ha,Hb}$ 12.2 Hz, $J_{Ha,Hb}$ 11.6 Hz, $J_{Ha,Hb}$ 10.8 Hz), 5.19 (d, 1 H, NH, $J_{NH,2}$ 8.6 Hz), 7.16–7.35 (m, 15 H, 3 Ph). ^{13}C NMR ($CDCl_3$): δ 23.5 (NHCO*Me*), 56.0 (C-2), 68.9 (C-6), 73.3, 73.5, 74.3 (3 *C*H$_2$Ph), 74.8 (C-5), 78.8 (C-4), 82.0 (C-3), 85.8 (C-1), 127.7–138.2 (Ph), 170.6 (CO). MALDI-MS: *m*/*z* 529.1 $[M+K]^+$, 513.1 $[M+Na]^+$, 491.1 $[M+H]^+$. FAB-HR-MS: *m*/*z* calcd for $[M+Na]^+$: 513.23654; found 513.23610.

Anal. Calcd for $C_{29}H_{34}N_2O_5$: C 70.99, H 6.98, N 5.71%; Found: C 71.01, H 7.05, N 5.55%.

2-Acetamido-4-*O*-[3-*N*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)carboxamidopropanoyl]-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl Azide (4). To a solution of **2** (500 mg, 0.95 mmol) and **3** (466 mg, 0.95 mmol) in dry CH_2Cl_2 (10 mL), freshly activated, ground molecular sieves (4 Å) were added, followed by addition of EDC (183 mg, 0.95 mmol), HOBt (26 mg, 0.19 mmol), and NEt_3 (38 mg, 0.38 mmol). After stirring at rt for 30 h, MeOH/$CHCl_3$ (1:1) was added until a clear solution was obtained (ca. 10 mL). After filtration through Celite, CH_2Cl_2 (30 mL) was added to the filtrate, and the solution was washed with 5% HCl and H_2O, dried, and the solvent was removed. The residue was triturated with toluene (25 mL) for 1 h under vigorous stirring and then filtered off. The solid was then briefly boiled with MeCN (25 mL), cooled to rt and filtered off. Finally, the solid was stirred with MeOH (15 mL) for 30 min. The suspension was filtered to afford **4** (615 mg, 65%) as a colourless solid, mp 190–221°C (dec). $[\alpha]_D^{23} - 6.9$ (*c* 0.5, DMSO). ^{1}H NMR (DMSO-d_6): δ 1.79, 1.86 (2 s, 6 H, 2 NHCO*Me*), 2.30 (m, 4 H, H-2′, H-3′), 3.41–3.66 (m, 7 H, H-5″, H-4″, H-6ab, H-6″ab, H-3″), 3.75–3.81 (m, 4 H, H-2, H-5, H-3, H-2″), 4.41–4.56 (m, 8 H, 4 C*H*$_2$Ph), 4.67–4.73 (m, 3 H, C*H*$_2$Ph, H-1), 4.88 (dd, 1 H, H-4, J 9.4 Hz), 4.97 (dd, 1 H, H-1″, $J_{1'',2''}$ 9.4 Hz), 7.16–7.37 (m, 25 H, 5 Ph), 8.01 (d, 1 H, NH-2″, $J_{NH,2''}$ 9.1 Hz), 8.18 (d, 1 H, NH-2, $J_{NH,2}$ 8.0 Hz), 8.41 (d, 1 H, NH-1″, $J_{NH,1''}$ 9.2 Hz). ^{13}C NMR (DMSO-d_6): δ 22.9 (2 NHCO*Me*), 28.7, 29.8 (C-2′, C-3′), 53.9 (C-2, C-2″), 68.3, 68.6 (C-6, C-6″), 69.9 (C-4), 72.3, 72.4, 73.5, 74.0, 74.2 (5 *C*H$_2$Ph), 74.7 (C-5), 75.9 (C-5″), 77.7 (C-4″), 78.7 (C-1″), 79.4 (C-3), 83.0 (C-3″), 87.9 (C-1), 127.2–141.6 (Ph), 169.5, 169.6, 171.1, 171.3 (4 CO). MALDI-MS: *m/z* 1037.3 $[M+K]^+$, 1021.4 $[M+Na]^+$, 999.4 $[M+H]^+$.

Anal. Calcd for $C_{55}H_{62}N_6O_{12}$: C 66.12, H 6.32, N 8.41%; Found: C 65.83, H 6.30, N 8.17%.

2-Acetamido-4-*O*-[3-*N*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)carboxamidopropanoyl]-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosylamine (5). A suspension of **4** (100 mg, 0.1 mmol) in dry MeOH was hydrogenated for 35 min over Pd/C (10%). The temp. was initially 60°C, and the mixture was allowed to cool to rt during the reaction. Filtering through Celite was followed by evaporation of the solvent at 25°C to dryness, yielding **5** (90 mg, 93%) as a colourless solid. The unstable compound was immediately used for subsequent reactions. ^{1}H NMR (DMSO-d_6): δ 1.79, 1.83 (2 s, 6 H, 2 NHCO*Me*), 2.38 (m, 4 H, H-2′, H-3′), 3.38–3.65 (m, 9 H, H-6ab, H-6″ab, H-5, H-5″, H-4″, H-3, H-3″), 3.66 (dd, 1 H, H-2, J 8.7 Hz, J 9.5 Hz), 3.78 (dd, 1 H, H-2″, J 9.3 Hz), 4.02 (d, 1 H, H-1, $J_{1,2}$ 8.7 Hz), 4.42–4.71 (m, 10 H, 5 C*H*$_2$Ph), 4.79 (dd, 1 H, H-4, J 9.3 Hz), 4.95 (dd, 1 H, H-1″, J 9.1 Hz), 6.55 (d, 1 H, N*Ha*H, $J_{NHa,1}$ 8.7 Hz), 7.17–7.32 (m, 25 H, 5 Ph), 7.95 (d, 1 H, N*Hb*H, $J_{NHb,1}$ 8.7 Hz), 8.04 (d, 2 H, 2 N*H*, $J_{NH,2}$ 8.7 Hz), 8.41 (d, 1 H, 1″-NH, $J_{NH,1''}$ 9.0 Hz). ^{13}C NMR (DMSO-d_6): δ 22.9, 23.1 (2 NHCO*Me*), 28.8, 29.8 (C-2′, C-3′), 53.9, 55.6 (C-2, C-2″), 68.5, 69.0 (C-6, C-6″), 70.9 (C-4), 72.2, 72.4, 73.1, 74.1 (4 *C*H$_2$Ph), 73.2 (C-4″), 74.5 (*C*H$_2$Ph), 75.8, 77.7 (C-5, C-5″), 78.6 (C-1″), 80.4, 82.9 (C-3, C-3″), 85.5 (C-1), 127.3–139.3 (Ph), 168.9, 169.1, 170.7, 171.0 (4 CO). MALDI-MS: *m/z* 1011.5 $[M+K]^+$, 995.5 $[M+Na]^+$, 973.5 $[M+H]^+$. FAB-HR-MS: *m/z* calcd for $[M+H]^+$: 973.461355; found 973.459899.

2-Acetamido-4-*O*-[3-*N*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)carboxamidopropanoyl]-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranose (6). To a suspension of freshly prepared **5** (100 mg, 0.1 mmol) in MeOH/$CHCl_3$ (1:1,

20 mL), H_2O (1 mL) and a 0.1 M HCl solution (1.3 mL) were added. The mixture was stirred for 15–20 h, until TLC ($CHCl_3$/EtOH 19:1) indicated completeness of the reaction. The solution was diluted with CH_2Cl_2, washed with H_2O, dried and evaporated. Flash chromatography ($CHCl_3$/EtOH 30:1) yielded **6** (68 mg, 70%) as a colourless solid, mp 227°C. $[\alpha]_D^{22}$ + 22.4 (*c* 1.0, DMSO). ^{1}H NMR (DMSO-d_6): δ 1.79, 1.86 (2 s, 6 H, NHCO*M*′), 2.36 (m, 4 H, H-2′, H-3′), 3.41–4.03 (m, 11 H, H-6ab, H-6″ab, H-5, H-5″, H-4″, H-3, H-3″, H-2, H-2″), 4.41–4.98 (m, 13 H, 5 C*H*$_2$Ph, H-4, H-1, H-1″), 7.02 (b, 1 H, OH), 7.16–7.31 (m, 25 H, 5 Ph), 8.03, 8.11 (2 d, 2 H, NH-2, NH-2″, $J_{NH,2}$, $J_{NH,2''}$ 8.9 Hz), 8.42 (d, 1 H, NH-1″, $J_{NH,1''}$ 9.2 Hz). ^{13}C NMR (DMSO-d_6): δ 22.6, 22.9 (2 NHCO*Me*), 28.8, 29.9 (C-2′, C-3′), 52.9, 53.9 (C-2, C-2″), 68.5, 68.8 (C-6, C-6″), 68.1, 70.9 (C-4, C-4″), 72.2, 72.3, 73.3, 73.9, 74.0 (5 *C*H$_2$Ph), 75.8, 76.9, 77.7, 82.9 (C-5, C-5″, C-3, C-3″), 78.6 (C-1″), 90.8 (C-1), 127.3–138.4 (Ph), 168.9, 169.1, 170.7, 171.0 (4 CO). MALDI-MS: *m/z* 1012.4 $[M+K]^+$, 996.4 $[M+Na]^+$, 974.4 $[M+H]^+$.

Anal. Calcd for $C_{55}H_{63}N_3O_{13} \times 3\ H_2O$: C 64.25, H 6.76, N 4.09%; Found: C 64.28, H 6.54, N 3.98%.

2-Acetamido-4-*O*-[3-*N*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)carbox-amidopropanoyl]-2-deoxy-D-glucopyranose (7). A suspension of **6** (68 mg, 0.07 mmol) in MeOH (30 mL), containing H_2O (1 mL), was hydrogenated over $Pd(OH)_2$/C (20%) for 5 h. The temp. was initially 50°C, and the mixture was allowed to cool to rt during the reaction. The mixture was filtered through Celite, the residue was thoroughly washed with MeOH/H_2O (1:1), and concentrated in vacuum at 25°C. Gel filtration and lyophylization afforded **7** (28 mg, 78%) as a colourless solid, mp 109–110°C. $[\alpha]_D^{21}$ + 23.8 (*c* 0.5, H_2O). ^{1}H NMR (D_2O): δ 2.02, 2.03 (2 s, 6 H, NHCO*Me*), 2.53, 2.61 (2 m, 4 H, H-2′, H-3′), 3.49 (m, 2 H, H-4″, H-5″), 3.54–4.15 (m, 9 H: 3.59 H-3″, 3.61 H-5, H-6ab, H-6″ab, 3.65 H-4β, 3.77 H-2β, 3.81 H-2″, 3.96 H-2α, 4.00 H-4α), 4.77 (d, 0.5 H, H-1β), 4.89 (m, 1 H, H-3α, H-3β), 5.06 (d, 1 H, H-1″, $J_{1'',2''}$ 9.7 Hz), 5.22 (d, 0.5 H, H-1α, $J_{1\alpha,2\alpha}$ 3.5 Hz). ^{13}C NMR (D_2O): δ 26.4, 26.6 (2 NHCO*Me*), 33.4, 34.6 (C-2′, C-3′), 58.4 (C-2α), 58.8 (C-2″), 60.9 (C-2β), 64.7, 65.0 (C-6, C-6″), 73.1 (C-4α), 74.0 (C-4β), 74.1 (C-4″), 76.2 (C-3α), 77.2 (C-3β), 78.8 (C-3″), 82.1 (C-5, C-5″), 82.9 (C-1″), 95.3 (C-1α), 99.5 (C-1β), 178.2, 179.1, 179.4, 180.0 (4 CO). MALDI-MS: *m/z* 562.3 $[M+K]^+$, 546.3 $[M+Na]^+$, 524.3 $[M+H]^+$. ESI-HR-MS *m/z* calcd for $[M+H]^+$: 524.2092; found 524.2110.

***N*-{2-Acetamido-4-*O*-[3-*N*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)carboxamidopropanoyl]-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl}-(4*S*,5*R*)-2-dimethylamino-5-methyl-Δ^2-oxazoline-4-carboxylic Acid Amide (8).** 2-Dimethylamino-4-carboxy-5-methyl-Δ^2-oxazoline[9] (40 mg, 0.23 mmol) was suspended in oxalyl chloride (3 mL) under nitrogen and stirred for 12 h at rt. The excess of oxalyl chloride was removed by evaporation. The residue was dissolved in dry CH_2Cl_2 (5 mL) under nitrogen and cooled to −30°C. This solution was then added to a cooled (−30°C) suspension of freshly prepared **5** (80 mg, 0.08 mmol) in CH_2Cl_2 (10 mL), containing ground activated molecular sieves (4 Å). The mixture was allowed to warm up to −10°C, and pyridine (1 mL) was added over a period of 45 min. Stirring was continued at rt overnight. The mixture was diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed with sat. $NaHSO_4$ (2 ×) and H_2O, dried and concentrated. Flash chro-

matography ($CHCl_3$/EtOH 12:1) afforded **8** (30 mg, 40%) as a colourless solid, mp 212–214°C. $[\alpha]_D^{18}$ +12.4 (*c* 0.5, DMSO). ^{1}H NMR (DMSO-d_6): δ 1.33 (d, 3 H, Me, $J_{Me,10}$ 6.0 Hz), 1.80 (s, 6 H, 2 NHCO*Me*), 2.43 (m, 4 H, H-2′, H-3′), 2.85, 2.87 (2 s, 6 H, NMe_2), 3.42–3.66 (m, 9 H, H-3, H-3″, H-8, H-6ab, H-6″ab, H-5, H-5″), 3.75–3.88 (3 dd, 3 H, H-2, H-2″, H-4″), 4.42–4.72 (m, 11 H, 5 C*H*$_2$Ph, H-10), 4.92, 4.96 (m, dd, 3 H, H-4, H-1, H-1″, J 9.7 Hz), 7.17–7.32 (m, 25 H, 5 Ph), 8.01 (d, 2 H, NH-2, NH-2″), 8.42 (d, 2 H, NH-1, NH-1″). ^{13}C NMR (DMSO-d_6): δ 21.5 (Me), 22.6, 22.8 (2 NHCO*Me*), 28.7, 29.8 (C-2′, C-3′), 37.0. 37.1 (NMe_2), 53.9 (C-2, C-2″), 68.6 (C-6, C-6″), 70.2 (C-4), 72.3, 72.4 (*C*H_2Ph), 74.3 (C-8), 74.0, 74.1 (*C*H_2Ph), 73.1 (C-4″), 75.9, 77.8 (C-5, C-5″), 78.7 (C-1, C-1″), 79.9, 80.0, 83.0 (C-10, C-3, C-3″), 127.5–138.6 (Ph), 166.5 (C-9), 169.4, 170.0, 171.1, 171.3, 173.6 (5 CO). MALDI-MS: *m/z* 1165.8 $[M+K]^+$, 1149.8 $[M+Na]^+$, 1127.8 $[M+H]^+$.

***N*-{2-Acetamido-4-*O*-[3-*N*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)carboxamidopropanoyl]-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl}-3-pyridinecarboxylic Acid Amide (9).** A solution of nicotinic acid (15 mg, 0.12 mmol) and EEDQ (27 mg, 0.11 mmol) in dry CH_2Cl_2 (10 mL) was stirred overnight and subsequently added to freshly prepared **5** (50 mg, 0.05 mmol). The suspension was vigorously stirred for 48 h. The solvent was evaporated to dryness, and the residue was suspended in EtOH (20 mL), stirred for 30 min, and the suspension was filtered. Flash chromatography ($CHCl_3$, then $CHCl_3$/EtOH 20:1) of the residue afforded **9** (22 mg, 41%), mp > 240°C (dec). $[\alpha]_D^{23}$ +3.9 (*c* 0.5, DMSO). ^{1}H NMR (DMSO-d_6): δ 1.83, 1.86 (2 s, 6 H, 2 NHCO*Me*), 2.53 (m, 4 H, H-2′, H-3′), 3.40–4.05 (m, 10 H, H-5, H-5″, H-6ab, H-6″ab, H-4″, H-3″, H-2″, H-3), 4.15 (dd, 1 H, H-2), 4.46–4.78 (m, 10 H, 5 C*H*$_2$Ph), 5.01 (dd, 2 H, H-4, H-1″, J 7.6 Hz), 5.33 (dd, 1 H, H-1), 7.22–7.37 (m, 25 H, 5 Ph), 7.54 (dd, 1 H, H-10, $J_{10,11}$ 7.9 Hz, $J_{9,10}$ 4.8 Hz), 8.08 (d, 1 H, NH-2″, $J_{2'',NH}$ 9.0 Hz), 8.24 (d, 1 H, H-9), 8.18 (d, H, 1 H, NH-2, $J_{2,NH}$ 8.9 Hz), 8.45 (d, 1 H, NH-1″, $J_{1'',NH}$ 9.2 Hz), 8.75 (dd, 1 H, H-11, J 1.4 Hz), 9.0 (d, 1 H, H-12, J 1.5 Hz), 9.25 (d, 1 H, NH-1, $J_{1,NH}$ 9.1 Hz). ^{13}C NMR (DMSO-d_6): δ 22.7, 22.8 (2 NHCO*Me*), 28.7, 29.8 (C-2′, C-3′), 53.0 (C-2, C-2″), 68.3, 68.6 (C-6, C-6″), 70.2 (C-4), 72.3, 72.5, 73.5, 73.9, 74.1 (5 *C*H_2Ph), 74.5 (C-4″), 75.9 (C-5), 77.8 (C-5″), 78.7, 79.3 (C-1, C-1″), 80.1, 83.0 (C-3, C-3″), 127.5–138.6 (Ph), 123.5 (C-8), 129.4 (C-10), 135.3 (C-9), 148.7 (C-11), 152.5 (C-12), 165.3, 169.4, 169.7, 171.1, 171.3, 175.2 (5 CO) . MALDI-MS: *m/z* 1116.3 $[M+K]^+$, 1100.3 $[M+Na]^+$, 1078.3 $[M+H]^+$.

***N*-{2-Acetamido-4-*O*-[3-*N*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)carboxamidopropanoyl]-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl}-2-dimethylamino-5-pyridinecarboxylic Acid Amide (10).** 2-Dimethylamino-5-pyridinecarboxylic acid[9] (20 mg, 0.12 mmol) was suspended in oxalyl chloride (3 mL) under nitrogen and stirred for 12 h at rt. The excess of oxalyl chloride was removed by evaporation. The residue was dissolved in dry CH_2Cl_2 (5 mL) under nitrogen, cooled to −35°C and subsequently added to a cooled (−35°C) suspension of freshly prepared **5** (58 mg, 0.06 mmol) in CH_2Cl_2 (8 mL), containing pyridine (3 mL) and ground activated molecular sieves (4 Å). The cooling bath was removed and the mixture was stirred for another 3 h. The mixture was then diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed with $NaHSO_4$ (2×) and with H_2O, dried and concentrated. Flash chromatography ($CHCl_3$/EtOH 10:1) afforded **10** (25 mg, 50%) as a colourless solid. ^{1}H NMR ($CDCl_3$): δ 1.74, 1.76 (2 s, 6 H, 2 NHCO*Me*), 2.35–2.75 (m,

4 H, H-2′, H-3′), 3.12, 3.16 (2 s, 6 H, NMe_2), 3.45–4.40 (4 m, 11 H, H-5, H-5″, H-6ab, H-6″ab, H-3, H-3″, H-4″, H-2, H-2″), 4.42–4.79 (m, 10 H, 5 CH_2Ph), 4.91–5.12 (3 dd, 3 H, H-4, H-1, H-1″), 6.45–8.88 (m, 28 H, Ph, H-9, H-10, H-12). MALDI-MS: *m/z* 1159.3 $[M+K]^+$, 1143.4 $[M+Na]^+$, 1121.3 $[M+H]^+$.

***N*-{2-Acetamido-4-*O*-[3-*N*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)carboxamidopropanoyl]-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl}-(3-benzyloxycarbonylamino)propanoic Acid Amide (11).** A solution of *N*-benzyloxycarbonyl-β-alanine (44 mg, 0.2 mmol) and EEDQ (47 mg, 0.19 mmol) in dry CH_2Cl_2 (10 mL) was stirred for 6 h and subsequently added to freshly prepared **5** (85 mg, 0.09 mmol). The suspension was vigorously stirred overnight. After concentration, the remaining colourless solid was suspended in EtOH (20 mL) and stirred for 30 min at rt. Filtration gave 60 mg (58%) of **11** as a colourless solid, mp 287°C. $[\alpha]_D^{22}+9.4$ (*c* 0.5, DMSO). ^{1}H NMR (DMSO-d_6): δ 1.79 (s, 6 H, 2 NHCO*Me*), 2.28 (m, 2 H, H-8), 2.41 (m, 4 H, H-2′, H-3′), 3.18 (m, 2 H, H-9), 3.35–3.81 (m, 11 H, H-5, H-5″, H-6ab, H-6″ab, H-4″, H-3, H-3″, H-2, H-2″), 4.40–4.71 (m, 10 H, 5 C*H*$_2$Ph), 4.87 (dd, 1 H, H-4, J 9.2 Hz), 4.99 (m, 4 H, COOC*H*$_2$Ph, 1-H, 1″-H), 7.18–7.24 (m, 31 H, 6 Ph, NH-9), 8.01, 8.06 (2 d, 2 H, NH-2, NH-2″, J 9.0 Hz, J 8.5 Hz), 8.42, 8.48 (2 d, 2 H, NH-1, NH-1″, J 9.1 Hz, J 9.3 Hz). ^{13}C NMR (DMSO-d_6): δ 22.7, 22.8 (2 NHCO*Me*), 28.7, 29.8 (C-2′, C-3′), 35.6 (C-8), 36.8 (C-9), 53.7 (C-2, C-2″), 65.2 (COO*C*H$_2$Ph), 68.4, 68.6 (C-6, C-6″), 70.2 (C-4), 72.3, 72.4, 73.5, 73.9, 74.1 (5 *C*H$_2$Ph), 74.3 (C-4″), 75.9, 77.8 (C-5, C-5″), 78.4, 78.7 (C-1, C-1″), 80.3, 83.0 (C-3, C-3″), 127.4–138.6 (Ph), 155.9 (CO-3), 169.4, 170.7, 171.1, 171.3 (5 CO). MALDI-MS: *m/z* 1216.7 $[M+K]^+$, 1200.8 $[M+Na]^+$, 1178.7 $[M+H]^+$.

Anal. Calcd for $C_{66}H_{75}N_5O_{15}\times 1\ H_2O$: C 66.27, H 6.49, N 5.85%; Found: C 66.09, H 6.32, N 5.57%.

***N*-{2-Acetamido-4-*O*-[3-*N*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)carboxamidopropanoyl]-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl}-(N^{α},N^{δ},N^{ω}-tris-benzyloxycarbonyl)-L-arginine Amide (12).** A solution of N^{α},N^{δ},N^{ω}-tri-benzyloxycarbonyl-L-arginine (119 mg, 0.21 mmol) and EEDQ (47 mg, 0.19 mmol) in dry CH_2Cl_2 (5 mL) was stirred for 6 h. This solution was added to 85 mg (0.09 mmol) of freshly prepared **5**. The suspension was vigorously stirred for 18 h, followed by concentration. The residue was suspended in EtOH (15 mL) and stirred for 30 min. Filtration gave **12** (100 mg, 75%) as a colourless solid, mp 249–250°C. $[\alpha]_D^{22}+10.5$ (*c* 0.5, DMSO). ^{1}H NMR (DMSO-d_6): δ 1.45–1.60 (m, 6 H, H-9, H-10, H-11), 1.79 (s, 6 H, 2 NHCO*Me*), 2.35–2.49 (m, 4 H, H-2′, H-3′), 3.35–3.84 (m, 12 H, H-5, H-5″, H-6ab, H-6″ab, H-4″, H-3, H-3″, H-2, H-2″, H-8), 4.37–4.71 (m, 10 H, 5 C*H*$_2$Ph), 4.75–5.19 (m, 9 H, H-4, H-1″, H-1, 3 C*H*$_2$COOPh), 7.16–7.44 (m, 42 H, NH-2, NH-12, 8 Ph), 8.02 (dd, 2 H, NH-2, NH-2″, J 9.6 Hz, J 11.2 Hz), 8.38 (dd, 2 H, NH-1, NH-1″, J 9.2 Hz, J 8.8 Hz), 9.15 (b, 1 H, CN*H*). ^{13}C NMR (DMSO-d_6): δ 22.7, 22.8 (2 NHCO*Me*), 25.1 (C-10), 28.7, 29.8 (C-2′, C-3′, C-9), 44.3 (C-11), 53.3, 53.9, 54.7 (C-2, C-2″, C-8), 65.4, 66.1, 68.1 (3 *C*H$_2$COOPh), 68.3, 68.6 (C-6, C-6″), 70.2 (C-4), 72.3, 72.4, 73.4, 73.9, 74.1 (5 *C*H$_2$Ph), 74.4 (C-4″), 75.9, 77.8 (C-5, C-5″), 78.7 (C-1, C-1″), 80.3, 83.0 (C-3, C-3″), 127.5–138.6 (Ph), 154.9, 155.9, 159.7 (3 *C*H$_2$COOPh), 162.9 (CN), 169.4, 169.5, 171.1, 171.2, 172.5 (5 CO). MALDI-MS: *m/z* 1569.5 $[M+K]^+$, 1553.5 $[M+Na]^+$, 1531.5 $[M+H]^+$.

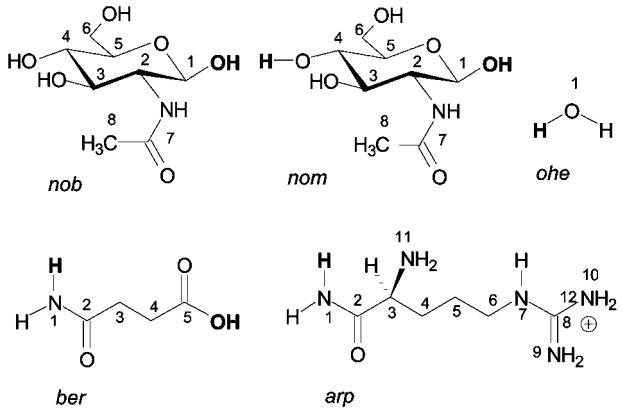
Anal. Calcd for $C_{85}H_{94}N_8O_{19} \times 1\ H_2O$: C 65.88, H 6.24, N 7.23%; Found: C 65.62, H 6.29, N 6.95%.

***N*-{2-Acetamido-4-*O*-[3-*N*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-carboxamidopropanoyl]-2-deoxy-β-D-glucopyranosyl}-3-aminopropanoic Acid Amide (13).** A suspension of **11** (63 mg, 0.05 mmol) in MeOH (100 mL), containing of H_2O (0.1 mL), was hydrogenated over Pd/C (10%) at 35 bar and 40°C overnight. The mixture was filtered through Celite, washed with MeOH/H_2O (1:1), and the combined solutions were concentrated at 25°C, followed by lyophylization to afford **13** (30 mg, 95%) as a colourless solid, mp 128–129°C. $[\alpha]_D^{23}$ +11.3 (*c* 0.5, H_2O). ^{1}H NMR (D_2O): δ 1.98, 1.99 (2 s, 6 H, NHCO*Me*), 2.58 (m, 2 H, H-2′), 2.60 (m, 4 H, H-3′, H-8), 3.23 (m, 2 H, H-9), 3.44–3.89 (m, 11 H, H-3″, H-4″, H-5, H-6ab, H-6″ab, H-5″, H-2″, H-3, H-2), 4.81 (m, 1 H, H-4), 5.03 (d, 1 H, H-1″, $J_{1'',2''}$ 9.6 Hz), 5.11 (d, 1 H, H-1, $J_{1,2}$ 8.8 Hz). ^{13}C NMR (D_2O): δ 26.5, 26.6 (2 NHCO*Me*), 33.4, 34.5 (C-2′, C-3′), 36.5 (C-8), 39.8 (C-9), 58.6, 58.8 (C-2, C-2″), 64.5, 65.0 (C-6, C-6), 74.0 (C-4″), 75.5 (C-4), 76.3 (C-3), 78.7 (C-3″), 79.9 (C-5), 82.1 (C-5″), 82.8, 82.9 (C-1, C-1″), 177.5, 178.1, 179.4, 179.9 (5 CO). MALDI-MS: *m/z* 632.2 $[M+K]^+$, 616.2 $[M+Na]^+$, 594.2 $[M+H]^+$. FAB-HR-MS: *m/z* calcd for $[M+H]^+$: 594.2591; found 594.2623.

***N*-{2-Acetamido-4-*O*-[3-*N*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-carboxamidopropanoyl]-2-deoxy-β-D-glucopyranosyl}-L-arginine Amide (14).** A suspension of **12** (100 mg, 0.065 mmol) in MeOH (80 mL), containing H_2O (0.1 mL), was hydrogenated over Pd/C (10%) at 35 bar and 40°C for 18 h. The mixture was filtered through Celite, washed with MeOH/H_2O (1:1). The combined solutions were concentrated at 25°C, followed by lyophylization to afford **14** (28 mg, 64%) as a colourless solid, mp > 212°C (dec). $[\alpha]_D^{22}$ +12.7 (*c* 0.5, H_2O). ^{1}H NMR (D_2O): δ 1.67 (m, 2 H, H-10), 1.93 (m, 2 H, H-9), 2.58, 2.73 (2 m, 4 H, H-2′, H-3′), 3.22 (dd, 2 H, H-11, $J_{10,11}$ 6.7 Hz), 3.49 (m, 2 H, H-5″, H-4″), 3.59 (dd, 1 H, H-3″, $J_{3'',4''}$ 8.1 Hz, $J_{3'',2''}$ 10.6 Hz), 3.68–3.95 (m, 8 H: 3.71 H-5, 3.57, 3.87 H-6a, H-6b, 3.73, 3.87 H-6″a, H-6″b, 3.81 H-2″, 3.86 H-3, 3.95 H-2), 3.99 (dd, 1 H, H-8, $J_{8,9}$ 6.0 Hz, $J_{8,NH}$ 6.4 Hz), 4.87 (dd, 1 H, H-4, J 4.8 Hz), 5.05 (d, 1 H, H-1″, $J_{1'',2''}$ 9.7 Hz), 5.16 (d, 1 H, H-1, $J_{1,2}$ 9.3 Hz). ^{13}C NMR (D_2O): δ 26.7 (2 NHCO*Me*), 27.9 (C-10), 32.4 (C-9), 33.5, 34.6 (C-2′, C-3′), 44.9 (C-11), 57.4 (C-8), 58.6 (C-2), 58.9 (C-2″), 64.6 (C-6), 65.1 (C-6″), 74.1 (C-4″), 75.6 (C-4), 76.6 (C-3), 78.8 (C-3″), 80.1 (C-5), 82.2 (C-5″), 83.0 (C-1), 83.1 (C-1″), 161.4 (CN), 175.0 (C-7), 178.3, 179.4, 179.6, 180.0 (4 CO). MALDI-MS: *m/z* 717.3 $[M+K]^+$, 701.3 $[M+Na]^+$, 679.3 $[M+H]^+$. FAB-HR-MS: *m/z* calcd for $[M+H]^+$: 679.3269; found 679.3263.

Enzyme assays. Hevamine A was isolated from the latex of *Hevea brasiliensis*.[30] ChiA and ChiB were isolated from *Serratia marcescens*.[31] The enzymes from *Chironomus tentans* were obtained from the cell cultures of an epithelial cell line.[32]

Chitinase activities were determined by microfluorimetric assays,[33] adapted for a microtitre plate fluorimeter. The substrates $(GlcNAc)_2$-MU or $(GlcNAc)_3$-MU, respectively, were used as described by McCreath and Gooday.[34] The final amounts of the enzymes in each assay mixture were 40 ng hevamine or ChiA, or 80 ng ChiB, respectively. Solutions of the enzymes (20 μL) were incubated with a substrate solution (50 μL, final concentration 125 μM) in 0.2 M Na-citrate-phosphate buffer (pH 5.5), and



Scheme 4. Naming of the groups *nob*, *nom*, *ohe*, *ber*, and *arp*.

a solution of the test compound (30 μL), containing at least three different concentrations, in a 96-well microtitre plate for 30 min at 37°C. The reaction was terminated by addition of a 1 M glycine-NaOH buffer, pH 10.6 (100 μL). Fluorescence (excitation: 360 nm; emission: 460 nm) was measured by means of a microtitre fluorimeter (Cytofluor 4000, Perseptive Biosystems). All measurements were performed in triplicate. Standard deviations were $< 10\%$.

Conformational analysis. The carbohydrate parameter set of Woods et al.[27] is based on the AMBER force field of Cornell et al.;[24] this force field is implemented into the SYBYL[35] program and was used for the molecular modelling throughout this work.

For force field calculations, the parameters and charges for the GlcNAc moieties (*nom*, *nob*; see Scheme 4) were taken from Ref. [27]. The parameter sets for the units *ber*, *arn*, and *ohe* were derived from ab initio quantum molecular mechanical calculations (HF/6-31G*). The glycosidic linkages *nob-ber*, *ber-nom*, *nom-ohe* and *nom-arn* of **7** and **14** were calculated from the monomers, taking the loss of water into account. The atom types for the residues *ber* and *arn* were taken from the AMBER-Cornell-P94 parameter sets.[24] The bond lengths, bond angles and torsional angles of the carbohydrate residues were again taken from Ref. [27] and supplemented by data for the *ber-nom* linkage, as shown in Table 1. The parametrization of the atomic

Table 1. Parameters for Compounds **7** and **14**

Atom Types	K	r_{eq}	Θ_{eq}	$V_n/2$	γ	n
C—OG Stretching	450.0	1.364				
CT—C—OG Bending	70		117			
C—OG—CT Bending	70		117			
O—C—OG Bending	80		126			
X—C—OG—X Torsion				0.00	0.00	3

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Table 2. Atom Types and HF/6-31G* RESP Charges of Residues *nob*, *nom*, *ber*, *arp* and *ohe*

	Atom Type, Charges (au)				
Atom	*nob*	*nom*	*ber*	*arp*	*ohe*
C1/N1/O1	EC, 0.476	EC, 0.476	N, −0.833	N, −0.797	OH, −0.5349
C2	CT, −0.098	CT, −0.098	C, 0.785	C, 0.764	
C3	CT, 0.450	CT, 0.450	CT, −0.437	CT, −0.082	
C4	CT, 0.090	CT, 0.090	CT, −0.411	CT, −0.334	
C5	CT, 0.407	CT, 0.407	C, 0.813	CT, −0.364	
C6	CT, 0.224	CT, 0.224		CT, −0.122	
C7/N7	C, 0.962	C, 0.962		N2, −0.825	
C8	CT, −0.676	CT, −0.676		CA, 1.068	
H1/H1a			H, 0.000	H, 0.000	HO, 0.0000
	H2, 0.028	H2, 0.028			
H1b			H, 0.385	H, 0.387	HO, 0.3549
H2	H1, 0.193	H1, 0.193			
H3/H3a			HC, 0.228		
	H1, −0.017	H1, −0.017		H1, 0.193	
H3b			HC, 0.231		
H4/H4a			HC, 0.242	HC, 0.220	
	H1, 0.032	H1, 0.032			
H4b			HC, 0.244	HC, 0.199	
H5/H5a				HC, 0.249	
	H1, −0.005	H1, −0.005			
H5b				HC, 0.199	
H6a	H1, −0.017	H1, −0.017		HC, 0.229	
H6b	H1, 0.006	H1, 0.006		HC, 0.225	
H8a	HC, 0.189	HC, 0.189			
H8b	HC, 0.175	HC, 0.175			
H8c	HC, 0.172	HC, 0.172			
O2/N2	N, −0.707	N, −0.707	O, −0.618	O, −0.621	
O3	OH, −0.728	OH, −0.728			
O4	OH, −0.715	OH, −0.452			
O5	OS, −0.535	OS, −0.535	O, −0.589		
O6	OH, −0.701	OH, −0.701			
O7	O, −0.672	O, −0.672			
N9				N2, −0.925	
N10				N2, −0.925	
N11				N, −0.847	
(HO)3	HO, 0.440	HO, 0.440			
(HO)4	HO, 0.443	HO, 0.00			
(HO)6	HO, 0.437	HO, 0.437			
(HN)2	H, 0.327	H, 0.327			
(HN)7				H, 0.428	
(HaN)9				H, 0.453	
(HbN)9				H, 0.440	
(HaN)10				H, 0.440	
(HbN)10				H, 0.439	
(HaN)11				H, 0.373	
(HbN)11				H, 0.356	
Total	0.18	0	0	0.82	−0.18

charges of the oligosaccharides **7** and **14** proved to depend on the conformation of the molecule and were defined as average values for each atom, as obtained from the ab initio HF/6-31G* RESP charges. In order to maintain neutrality of the molecules **7** and the positive charge of **14**, small adjustments for each atom were necessary, as shown in Table 2. Hydrogen bonds were simulated adequately without selecting a special hydrogen bonding term. The van der Waals terms employed in the present calculations were taken from Refs. [24,27].

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