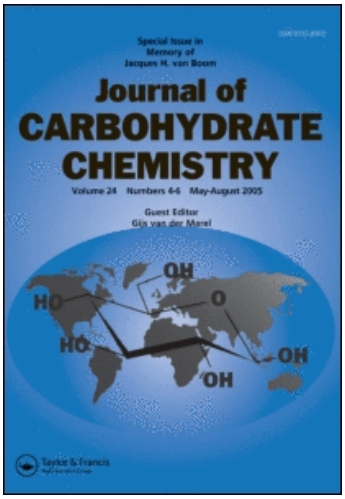

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SYNTHESIS OF L-HISTIDINE AND (-)-SPINACINE CHITOOLIGOSYL AMIDES[1]

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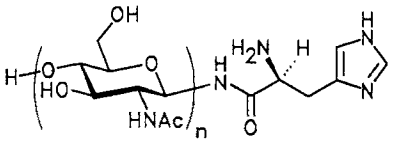
ABSTRACT

The synthesis of *N*-(2-acetamido-4-*O*-[2-acetamido-4-*O*-{2-acetamido-2-deoxy-β-D-glucopyranosyl}-2-deoxy-β-D-glucopyranosyl]-2-deoxy-β-D-glucopyranosyl)-L-histidine amide (**3**), *N*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(6*S*)-4,5,6,7-tetrahydroimidazo[4,5-c]pyridine-6-carboxylic amide (**4**) and *N*-(2-acetamido-4-*O*-[2-acetamido-2-deoxy-β-D-glucopyranosyl]-2-deoxy-β-D-glucopyranosyl)-(6*S*)-4,5,6,7-tetrahydroimidazo[4,5-c]pyridine-6-carboxamide (**5**) is achieved *via* coupling of the appropiate glycosylamines with Boc-protected L-histidine or with (6*S*)-4,5,6,7-tetrahydroimidazo[4,5-c]pyridine-6-carboxylic acid ((-)-spinacine), respectively, by means of EEDQ and subsequent deprotection.

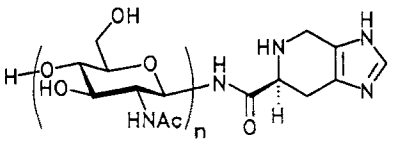
INTRODUCTION

In order to gain information about the substrate specificity of chitinases,[3] we have embarked on a program towards the synthesis of potential inhibitors. It has been shown in several cases that substrate analogs composed of a glycosidase inhibitor and a glycosidically linked oligosaccharide show endoglycosidase inhibiting specificity.[4] Furthermore, it is important for the design of endoglycosidase-inhibitors

to increase the stability of the glycosidic bond towards hydrolysis. Field *et al.* reported about some hydrophobic imidazole derivatives that are very strong inhibitors of sweet almond β-glycosidase.[5] It was proposed that the imidazole nucleus interferes with an acid base catalysis mechanism for glycoside hydrolysis. In an earlier study on chitooligosyl L-histidine amides[6] we have shown that the chitobiosyl derivative **2** is a moderate inhibitor of chitinases from *Artemia salina* (IC_{50} ca. 0.5 mM). The corresponding *N*-acetylglucosaminyl amide **1** shows no inhibition at all.



1: n=1
2: n=2
3: n=3



4: n=1
5: n=2

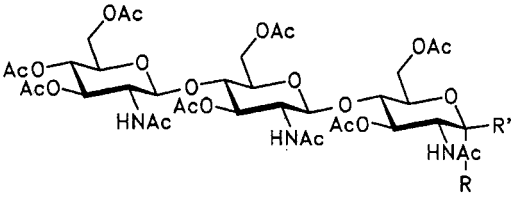
In view of these results we were encouraged to synthesize the *N,N',N"*-triacetylchitotriosyl-L-histidine amide (**3**) and to test whether it is a better inhibitor of chitinase as compared with **2**. It seemed also of interest to us to decrease the conformational mobility in the aglycon. Therefore, we have prepared the Pictet-Spengler product of L-histidine with formaldehyde, (6*S*)-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-c]pyridine-6-carboxylic acid ((-)-spinacine) and attached it to the glycosylamines of chitobiose and glucosamine to afford the glycosylamides **4** and **5**.

Many spinacine derivatives show biological activity,[7] e.g., 1-phenylpiperazinylspinacine amide protects mice against lung lesions caused by influenza APR 8 virus.[8] The synthesis of glycosidic spinacine derivatives is also of interest because of the deazapurine character of the imidazo[4,5-c]pyridine structure.

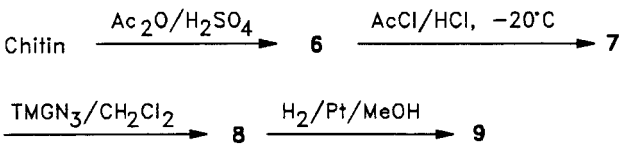
RESULTS AND DISCUSSION

For the synthesis of glycosyl amides **3**, **4** and **5** we have employed principally known reactions,[6] the key step consisting of *N*-acylation of a suitably protected glycosylamine with an appropiate activated amino acid derivative (for reviews see [9]).

For preparation of the glycosylamine **9** we used the α-glycosyl chloride methodology described before[6, 10, 11] (Scheme 1). Starting from undecaacetylchitotriose (**6**),[12] the corresponding glycosyl-α-chloride **7**[13] was prepared in yields up to 94% by the method described by Zurabyan and coworkers[14] for the chitobiosyl derivative. A suspension of **6** in acetyl chloride was saturated with HCl at -25 °C and subsequently warmed up. The conversion was not complete when the partial pressure of HCl was



	R	R'	yield
6	OAc	H	6%
7	Cl	H	94%
8	H	N_3	92%
9	H	NH_2	62%



Scheme 1

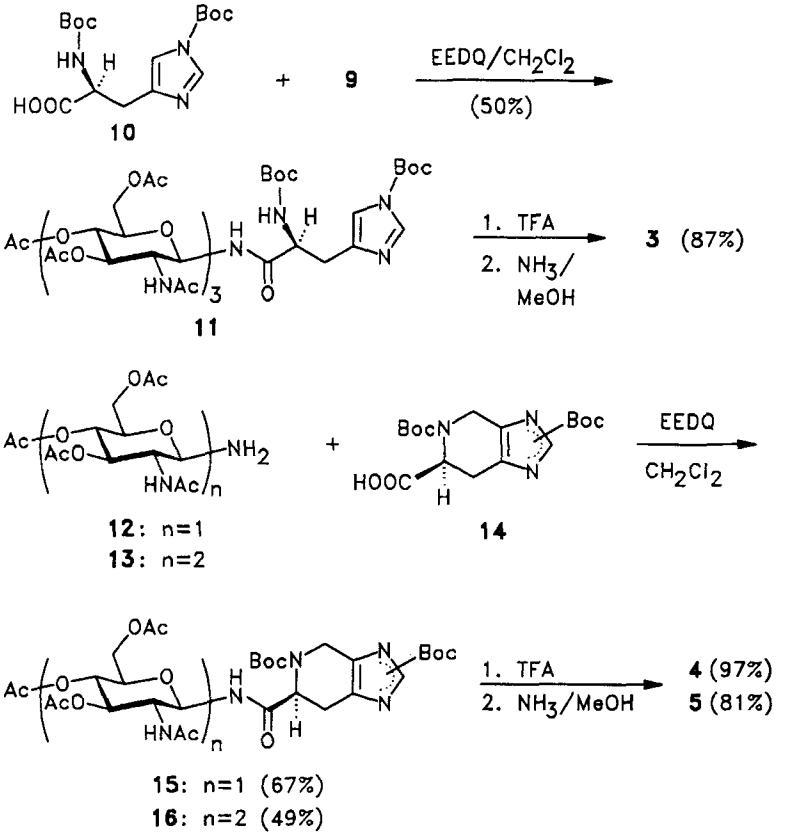
too low. Good yields of **7** were obtained after tightly sealing the reaction vessel during warming up.

Conversion of **7** into the azide **8** by the method of Waldmann *et al.*[10] afforded **8** in low yields (ca. 20%). The yield was increased to 75% by concentrating the reaction mixture containing $CHCl_3$ and water after completion of the reaction, followed by immediate chromatography of the crude product.

With *N,N,N',N'*-tetramethylguanidinium azide ($TMGN_3$)[15] the yield of **8** was increased further to 92%. Compound **8** shows the characteristic azido IR-absorption at 2118 cm^{-1}. The β-configuration was deduced from the signal of the anomeric proton at 4.54 ppm, which shows a doublet, $J = 9.3$ Hz.

Reduction of azide **8** to amine **9** was achieved by hydrogenation in methanolic solution in the presence of 10% (mass) Adams catalyst at 23 °C. When the reaction was carried out at lower temperature (0 °C) the yield decreased. The product was not further purified because it decomposed on silica gel or alumina and recrystallization was not satisfactory.

Coupling of amine **9** with N^{α},N^{τ}-di-Boc-L-histidine (**10**)[16] by means of 2-ethoxy-1-*N*-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) yielded the protected glycosylamide **11** (50%, Scheme 2). The ^1H NMR signals of the anomeric proton of the first pyranosyl ring appeared as two sets of doublets ($J = 9.6$ Hz) in a ratio of 8:2. The same ratio of signal intensities was observed in all intermediates throughout the subsequent deprotection steps (see Experimental) which indicates a partial racemisation of the histidine moiety rather than the presence of conformers. The diastereomeric mixture was partially separated by chromatography and subsequently recrystallized to give a mixture of diastereomers in a 20:1 ratio.



Scheme 2

Since the known N^{im}-benzylspinacine derivatives[17] are not suitable for glycosylamide synthesis, we have prepared the di-Boc derivative **14** from (-)-spinacine[18] by the conventional method with Boc_2O in an Et_3N/H_2O/dioxane-mixture in moderate yield (40%). The crystalline product is a 3 : 2 mixture of the 1*N*- and 3*N*-Boc regioisomers. Separation was not necessary, because the Boc-groups were removed in the final step of the synthesis.

Coupling of glycosylamines **12** and **13**,[6, 10, 11] respectively, with the protected spinacine **14** in the presence of EEDQ yielded the fully protected glycosylspinacine amides **15** (67%) and **16** (49%), respectively (Scheme 2). Both products were mixtures of the two Boc-regioisomers. This caused a line-broadening of all amino acid signals in the ^1H NMR spectra and a splitting of the acetyl signal of the 2-acetamido group at the first glucopyranosyl ring. The high field *tert*-butyl protons appeared as a

Table Inhibition data of compounds **1** - **5** ([IC_{50}] = mM).

Compound	*Chironomus* NAcGlc-ase	*Lucilia cuprina*
1	> 2	> 10
2	0.5	> 4
3	0.7	0.24
4	-	> 4
5	-	> 4
N,N',N"-Triacetyl-chitotriose	-	0.2

set of two signals, too. On the other hand the proton at C-2 of the imidazole nucleus was a sharp singlet.

The Boc-groups of glycosylamides **11**, **15** and **16**, respectively, were removed by anhydrous TFA and the crude products obtained were de-*O*-acetylated by the action of ammonia in methanolic solution[6, 10] (Scheme 2). The ratio of the two diastereomers of **3** was not affected by the deprotection procedures. Starting from the mixtures of regioisomers of **15** and **16**, respectively, we obtained uniform products **4** and **5**, respectively. The NMR spectra of both compounds indicated, within the limit of detection, that racemisation of the amino acid had not occurred. In the ^{13}C NMR spectra of **4** and **5** the signals of the quarternary atoms in the spinacine moiety of both molecules are very weak and broadened. This is possibly caused by dynamic or relaxation effects. Only the β-anomers of **3**, **4** and **5**, respectively, were found: the ^1H NMR -signals for H-1 were doublets with a coupling constant of 8 to 9 Hz.

As preliminary studies indicated, **3** showed no increase of the inhibitory effect against the chitinase of *Artemia salina* (IC_{50} ca. 0.6 mM). Substances **1**, **2**, and **3** were tested on the *N*-acetylglucosaminidase of *Chironomus tentans* and *Lucilia cuprina* (Table). The spinacine amides **4** and **5** were tested only with the chitinase of *Lucilia cuprina* (Table). Allosamidin, so far the best known chitinase inhibitor,[19] inhibits this chitinase with an IC_{50} of 0.1 μM, whereas the substrate *N,N',N"*-triacetylchitotriose shows an apparent IC_{50} of 0.2 mM.

CONCLUSIONS

All tested compounds were no or only weak inhibitors of several chitinases. Only the trisaccharide **3** showed a significant decrease in enzyme activity on the

chitinase of *Lucilia cuprina*. Comparison of the IC_{50} of **1** - **3** with the apparent IC_{50} of *N,N',N"*-triacetylchitotriose (see Table) suggests that the inhibition results from increased binding of the higher oligosaccharide rather than from the imidazole moiety. This casts some doubt on the assumption of the interference of the imidazole with acid-base catalysis.[5] The *N*-acetylglucosaminidase of *Chironomus* was partially inhibited by the di- and trisaccharidic L-histidine amides within the same range.

EXPERIMENTAL

General Methods. Melting points were determined with a Büchi SMP20 apparatus and are uncorrected. Optical rotations were measured with a Perkin Elmer 241 polarimeter. NMR spectra were recorded at 20 °C on Bruker spectrometers AM 400, AM 250 or AC 200 (400 MHz and 250 MHz for ^1H, 100.6 MHz, 62.7 MHz, and 50.3 MHz for ^{13}C) with solvent signals as internal standard unless otherwise stated. Mass spectra were obtained on Kratos Concept in FAB-technique with the matrices *m*-nitrobenzyl alcohol (mNBA), glycerol (glyc) or thioglycerol (thio). TLC analyses were performed on silica gel G 60 F_{254} with detection by iodine vapour and/or with AcOH/concd. H_2SO_4/anisaldehyde 50:1:0.5 (v/v) and heating at 150 °C for a few minutes. Flash chromatography (FC) was carried out on silica gel 60 (40 to 63 μm). Enzymatic studies were carried out with 4-methylumbelliferyl-N,N',N"-triacetylchitotrioside as substrate at 20 °C and recording the fluorescence after 15 min incubating time.

2-Acetamido-4-*O*-(2-acetamido-4-*O*-[2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl]-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranosylchloride (7). A suspension of **6** (1 g, 1.04 mmol) in acetyl chloride (20 mL) in an oven dried Schlenk flask was saturated with dry HCl gas at -25 °C for 0.5 h. The reaction vessel was tightly sealed, warmed up to 23 °C and the resulting clear solution was stirred for 24 h. The solvent was removed *in vacuo* and the resulting white solid recrystallized from acetone / $CHCl_3$ / Et_2O to afford a white powder of **7** (0.92 g, 94%): mp 195-197 °C; $[\alpha]_D^{20}$ +7.6° (*c* 0.570, $CHCl_3$);[13] (+)-FAB-MS (mNBA): *m/z* 940.3 (M^+).

2-Acetamido-4-*O*-(2-acetamido-4-*O*-[2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl]-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosylazide (8). To a solution of **7** (503 mg, 0.53 mmol) in CH_2Cl_2 (75 mL) was added $TMGN_3$ (140 mg, 0.88 mmol) and the resulting yellowish reaction mixture was stirred for 24 h at 23 °C. The solvent was removed *in vacuo* and the brownish residue purified by FC ($CHCl_3$ / MeOH

5:1) to afford **8** as a white solid (460 mg, 92%): mp 219-220 °C (decomp.); $[\alpha]_D^{23}$ -35.6° (*c* 0.383, $CHCl_3$ / MeOH 2:1 (v/v)); IR (KBr): $\tilde{\nu}$ 2118 cm^{-1} (azide); (+)-FAB-MS (mNBA): *m/z* 947.6 ($[M + H]^+$), 905.6 ($[M - N_3]^+$); IR (KBr): $\tilde{\nu}$ 3429 (ss), 2950 (w), 2118 (m, N_3), 1744 (ss, ester-C=O), 1656 (s, amide-C=O), 1542 (m), 1374 (m), 1234 (ss), 1049 cm^{-1} (s). ^1H NMR (400 MHz, $CDCl_3$ / CD_3OD) δ 5.14 (dd, 1 H, $J = 10.5, 9.3$ Hz, H-3"), 4.98 (dd, 1 H, $J = 10.2, 8.7$ Hz, H-3' or 3), 4.96 (dd, 1 H, $J = 10, 8$ Hz, H-3 or 3'), 4.94 (dd, 1 H, $J = 10, 9.3$ Hz, H-4"), 4.54 (d, 1 H, $J = 9.3$ Hz, H-1), 4.51 (d, 1 H, $J = 8.4$ Hz, H-1"), 4.39 (d, 1 H, $J = 8.4$ Hz, H-1'), 4.37 (dd, 1 H, $J = 12.5, 2$ Hz), 4.33 (dd, 1 H, $J = 12.5, 4.2$ Hz), 4.25 (dd, 1 H, $J = 12, 2$ Hz), 4.07 (dd, 1 H, $J = 12, 5.5$ Hz), 4.06 (dd, 1 H, $J = 12.5, 5.7$ Hz), 3.94 (d, 1/2 H, $J = 2$ Hz), 3.87 (dd, 1 H, $J = 10.2, 9.3$ Hz, H-2), 3.81 (dd, 1 H, $J = 10.2, 8$ Hz, H-2'), 3.71 (dd, 1 H, $J = 10.2, 8.4$ Hz, H-4), 3.66 (dd, 1 H, $J = 9.3, 9.3$ Hz, H-4'), 3.64 (dd, 1 H, $J = 9.3, 8.4$ Hz, H-2"), 3.66 - 3.62 (m, 1 H, H-5), 3.61 (ddd, 1 H, $J = 10, 4, 2.5$ Hz, H-5"), 3.53 (ddd, 1 H, $J = 9.6, 5.1, 2$ Hz, H-5'), 2.09 (s, 3 H), 2.08 (s, 3 H), 2.02 (s, 3 H), 1.97 (s, 3 H), 1.95 (s, 6 H), 1.94 (s, 3 H), 1.88 (s, 3 H), 1.86 (s, 3 H), 1.85 (s, 3 H, all acetyl-CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$, MeOH as int. standard) δ 172.7 (C), 172.6 (2×C), 172.0 (C), 171.9 (C), 171.7 (C), 171.4 (C), 171.3 (C), 171.1 (C), 170.6 (C), 101.2 (CH), 101.1 (CH), 88.6 (CH), 76.5 (2×CH), 75.3 (CH), 73.6 (CH), 73.5 (CH), 73.1 (CH), 72.8 (CH), 72.8 (CH), 72.1 (CH), 69.0 (CH), 63.3 (CH_2), 63.0 (CH_2), 62.4 (CH_2), 55.5 (CH), 55.1 (CH), 54.1 (CH), 22.8 (2×CH_3), 22.7 (CH_3), 21.0 (CH_3), 20.9 (2×CH_3), 20.8 (2×CH_3), 20.7 (CH_3), 20.6 (CH_3).

Anal. Calcd for $C_{38}H_{54}N_6O_{22}$: C, 48.20; H, 5.75; N, 8.88. Found C, 47.89; H, 5.85; N, 8.80.

2-Acetamido-4-*O*-(2-acetamido-4-*O*-[2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl]-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosylamine (9). A solution of **8** (373 mg, 0.39 mmol) in EtOH (130 mL) and $CHCl_3$ (10 mL) was hydrogenated in the presence of $PtO_2 \cdot xH_2O$ (39 mg) at 24 °C and atmospheric pressure for 5 h. The platinum was removed by filtration through celite and the resulting clear solution was concentrated at 25 °C *in vacuo* to yield **9** as a white solid (223 mg, 62%): mp 235-236 °C; $[\alpha]_D^{20}$ -24.7° (*c* 0.475, $CHCl_3$ / MeOH 2:1 (v/v)); (+)-FAB-MS (mNBA): *m/z* 921.4 ($[M + H]^+$), 904.3 ($[M - NH]^+$); IR (KBr): $\tilde{\nu}$ 3325 (m,b), 2926m(w), 1746 (ss, ester-C=O), 1663 (s, amide-C=O), 1542 (m), 1437 (w), 1374 (s), 1233 (ss), 1119 (m), 1046 cm^{-1} (s). ^1H NMR (400 MHz, d_6 – DMSO) δ 7.99 (d, 1 H, $J = 9.2$ Hz, NHAc), 7.89 (d, 1 H, $J = 9.2$ Hz, NHAc), 7.74 (d, 1 H, $J = 9.2$ Hz, NHAc), 5.11 (dd, 1 H, $J = 10, 9.6$ Hz), 4.99 (dd, 1 H, $J = 10, 9.2$ Hz), 4.86 (dd, 1 H, $J = 10, 8.8$ Hz), 4.81 (dd, 1 H, $J = 9.6, 9.6$ Hz, H-4"), 4.63 (d, 1 H, $J = 8.4$ Hz, H-1"), 4.55 (d, 1 H, $J = 8.4$ Hz, H-1'), 4.30 (dd, 1 H, $J = 11.6, \approx 2.5$ Hz), 4.26 (dd, 1 H, $J = 12.4, \approx 4$ Hz),

4.24 (dd, 1 H, J = 12.4, ≈ 2.5 Hz), 4.04 (d, 1 H, J = 9.6 Hz, H-1), 4.02 (dd, 1 H, J = 13, ≈ 6 Hz), 3.95 (dd, 1 H, J = 12, ≈ 6 Hz), 3.89 (dd, 1 H, J = 12.4, ≈ 2 Hz), 3.80 (ddd, 1 H, J = 9.6, ≈ 3.5, ≈ 2.5 Hz, H-5), 3.70 (dd , 1 H, J ≈ 9, ≈ 9 Hz, H-4), 3.62 - 3.52 (m, 3 H), 3.50 (ddd, 1 H, 3 × J ≈ 9.5 Hz, H-2"), 3.45 (ddd, 1 H, J = 8.4, ≈ 4, ≈ 2.5 Hz, H-5"), 3.33 (m , HDO and H-5'), 2.08 (s, 3 H), 2.04 (s, 3 H), 2.00 (s, 3 H), 1.94 (s, 3 H), 1.93 (s, 3 H), 1.89 (s, 6 H), 1.74 (s, 3 H), 1.73 (s, 3 H), 1.72 (s, 3 H, all acetyl-CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$ / CD_3OD) δ 171.7* (C), 171.6* (C), 171.3 (2·C), 171.2 (C), 171.1 (C), 171.0 (C), 170.9 (C), 170.7 (C), 170.6 (C), 170.5 (C), 169.4 (C), 100.9 (CH), 100.8 (CH), 84.9 (CH), 76.2 (CH), 75.6 (CH), 73.4 (CH), 72.5 (CH), 72.3 (CH), 72.2 (CH), 72.1 (CH), 71.4 (CH), 67.9 (CH), 62.7 (CH_2), 62.4 (CH_2), 61.5 (CH_2), 54.3* (CH), 54.25 (CH), 54.1* (CH), 54.0 (CH), 53.6* (CH), 53.5 (CH), 22.6* (CH_3), 22.55 (CH_3), 22.5* (CH_3), 22.45 (CH_3), 22.4 (CH_3), 20.6 (CH_3), 20.55 (CH_3), 20.4 (CH_3), 20.3 (CH_3), 20.25 (3·CH_3);*Intensity ca. 0.1 to 0.3 in relation to pairwise assigned signal of diastereomers.

***N*-(2-Acetamido-4-*O*-[2-acetamido-4-*O*-{2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl}-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl]-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-N^{α},N^{τ}-di-Boc-L-histidine Amide (11).** To a solution of **10**[16] (132 mg, 0.372 mmol) and **9** (223 mg, 0.242 mmol) in dry CH_2Cl_2 (75 mL) EEDQ (121 mg, 0.488 mmol) was added under an argon atmosphere and the solution was stirred at 23 °C for 5 d. The solvent was evaporated at 30 °C *in vacuo* and the resulting residue was purified by FC ($CHCl_3$ / MeOH 10:1) and subsequent recrystallization from MeOH/*n*-hexane to afford **11** as white crystalline powder (152 mg, 50%): mp 266-268°C; $[\alpha]_D^{20}$ -16.9° (*c* 0.305, $CHCl_3$ / MeOH 4:1 (v/v)); (+)-FAB-MS (mNBA): *m/z* 1258.3 (M^+), 1158.3 ($[M-Boc+H]^+$), 1058.3 ($[M-2\cdot Boc+H]^+$); IR (KBr): $\tilde{\nu}$ 3324 (m,b), 2980 (w), 1747 (ss, ester-C=O), 1653 (s,b, amide-C=O), 1542 (m), 1431 (m), 1374 (s), 1234 (ss,b), 1162 (s), 1115 (m), 1045 cm^{-1} (s). ^1H NMR (400 MHz, $CDCl_3$ / CD_3OD) δ 7.94 (s , 1 H, H-6), 7.16 (s , 0.84 H, H-8), 7.12 (s , 0.16 H, H-8), 5.15 (dd, 1 H, J = 10.6, 9.4 Hz, H-3"'), 5.02 (dd, 1 H, J = 10.2, 8.6 Hz, H-3' or H-3"), 5.00 (dd, 1 H, J = 10.3, 8.5 Hz, H-3" or H-3'), 4.97 (d , 1 H, J = 9.4 Hz, H-1'), 4.94 (dd, 1 H, J = 10, 9.3 Hz, H-4"'), 4.53 (d , 1 H, J = 8.4 Hz, H-1"'), 4.43 (d , 1 H, J = 8.2 Hz, H-1"), 4.32 (dd, 1 H, J = 12.4, 4.1 Hz, H-6"'i), 4.31 (dd, 1 H, J = 11.6, ≈ 2.5 Hz, H-6'ii), 4.28 - 4.23 (m, 1 H, H-2), 4.26 (dd , 1 H, J = 11.6, ≈ 2 Hz, H-6"ii), 4.06 (dd , 1 H, J = 12, 5.5 Hz, H-6"i), 4.05 - 4.00 (m , 1 H, H-6'i), 3.96 (dd , 1 H, J = 12.2, ≈ 2 Hz, H-6"'ii), 3.96 - 3.93 (m , 1 H, H-2'), 3.78 (dd , 1 H, J = 10.2, 8.3 Hz, H-2"), 3.71 (dd , 1 H, J = 10.4, 8.4 Hz, H-2"'), 3.67 (dd , 1 H, J = 9.8, ≈ 6 Hz, H-4'), 3.66 - 3.62 (m , 2 H, H-4' and H-5'), 3.62 (ddd, 1 H, J = 10, 4, 2.4 Hz, H-5"'), 3.54 (ddd, 1 H,

J = 9.6, 5.5, 2.2 Hz, H-5"), 2.94 (dd , 1 H, J = 14.4, ≈ 4 Hz, H-3i), 2.71 (dd , 1 H, J = 14.2, ≈ 9 Hz, H-3ii), 2.09 (s, 3 H), 2.05 (s, 3 H), 2.02 (s, 3 H), 1.97 (s, 3 H), 1.95 (s, 6 H), 1.94 (s, 3 H), 1.85 (s, 6 H), 1.84 (s, 3 H, all acetyl-CH_3), 1.55 (s, 9 H), 1.33 (s, 9 H, all *tert*-Bu); ^{13}C NMR (100.6 MHz, $CDCl_3$ / CD_3OD) 172.7 (C, C-1), 171.9 (C, C-1*), 171.8 (2×C), 171.7 (C), 171.2 (2×C), 171.1 (C), 171.0 (2×C), 170.0 (2×C, all acetyl-CO), 156.0 (C, urethane-CO) 146.9 (C, urethane-CO) 138.7 (C, C-4), 136.9 (CH, C-6), 115.0 (CH, C-8), 101.0 (CH, C-1" or 1"'), 100.7 (CH, C-1"' or 1"), 86.2 (C, *tert*-Bu-C), 80.1 (C, *tert*-Bu-C), 78.8 (CH, C-1'), 76.0 (CH), 75.9 (CH), 74.6 (CH), 73.5 (CH), 73.0 (CH), 72.7 (CH), 72.5 (CH), 71.7 (CH), 68.5 (CH), 62.9 (CH_2), 62.7 (CH_2), 61.9 (CH_2), 54.7 (CH), 54.5 (CH, C-2), 54.4 (CH, C-2*), 54.3 (CH), 53.0 (CH), 30.6 (CH_2, C-3), 28.2 (CH_3, *tert*-Bu-CH_3), 27.8 (CH_3, *tert*-Bu-CH_3), 22.7 (2×CH_3), 22.6 (CH_3), 20.8 (2×CH_3), 20.7 (2×CH_3), 20.6 (2×CH_3), 20.5 (CH_3, all acetyl-CH_3); *pairwise assigned signals of diastereomers.

Anal. Calcd for $C_{54}H_{79}N_7O_{27} \cdot 1.5\,H_2O$: C, 50.46; H, 6.43; N, 7.63. Found C, 50.47; H, 6.38; N, 7.54.

(6 *S*)-N^{im},5-Di-Boc- 4,5,6,7-tetrahydroimidazo[4,5-c]pyridine-6-carboxylic Acid (14). To (-)-spinacine-dihydrate[18] (1 g, 4.92 mmol) dissolved in water (30 mL) and Et_3N (2.3 mL) was added dropwise a solution of Boc_2O (3.3 g, 15.1 mmol) in dioxane (30 mL) over a period of 0.5 h. The mixture was stirred for 16 h at 24 °C. The yellowish solution was diluted with water (30 mL) and washed successively twice with Et_2O (20 mL) and twice with EtOAc (20 mL). The organic phases were discarded. EtOAc (100 mL) was added to the aqueous phase. The mixture was stirred and brought to pH 3 with diluted H_2SO_4 (c = 3 mol/L). After the organic phase was separated, the aqueous phase was extracted twice with EtOAc (40 mL). The combined organic phases were washed three times with brine (40 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The oily residue was crystallized from EtOAc / *n*-hexane to afford colourless needles (731 mg, 40%): mp 163-165°C; (-)-FAB-MS (glyc): m/z 366.1 ($[M-H]^-$); IR (KBr): $\tilde{\nu}$ 3147 (w), 2984 (s), 2929 (m), 2535 (w), 1762.6 (s, carboxylic-C=O), 1724 (s), 1695 (s, amide-C=O), 1489 (m), 1457.2 (m), 1400 (ss), 1377 (ss), 1318 (s), 1259 (s), 1229 (s), 1162 (ss), 1129 (ss), 1051 (m) cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 10.3 (br s, 1 H, COOH), 8.15 (s, 1 H, H-2), 5.35 (d, 0.61 H, J = 6.3 Hz, H-6), 5.16 (d, 0.39 H, J = 6.3 Hz, H-6), 4.98 (d, 0.37 H, J = 17.4 Hz, H-4ii), 4.86 (d, 0.63 H, J = 17 Hz, H-4ii), 4.54 (d, 0.6 H, J = 16.7 Hz, H-4i), 4.47 (d, 0.4 H, J = 16.7 Hz, H-4i), 3.28 (dm, 1 H, J = 16.2 Hz, H-7ii), 2.95 (dm, 1 H, J = 16.2 Hz, H-7i), 1.58 (s, 9 H, *tert*-Bu), 1.49, 1.489 (2 × s, 9 H, *tert*-Bu); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 173.7 (C, C-8), 173.5 (C, C-8*), 155.6 (C, urethane-CO), 155.1 (C, urethane-CO*), 146.7 (C, urethane-CO), 137.4 (CH, C-2), 137.3 (CH,

C-2*), 133.4 (C, C-7a), 132.9 (C, C-7a*), 122.6 (C, C-3a*), 122.3 (C, C-3a), 86.7 (C, *tert*-Bu-C*), 86.5 (C, *tert*-Bu-C), 81.0 (C, *tert*-Bu-C*), 80.9 (C, *tert*-Bu-C), 53.0 (CH, C-6*), 51.9 (CH, C-6), 41.0 (CH_2, C-4), 40.2 (CH_2, C-4*), 28.4 (CH_3, *tert*-Bu-CH_3), 25.8 (CH_3, *tert*-Bu-CH_3), 26.0 (CH_2, C-7*), 25.7 (CH_2, C-7);*pairwise assigned signals of diastereomers (1:2).

Anal. Calcd for $C_{17}H_{25}N_3O_6$: C, 55.58; H, 6.86; N, 11.44. Found C, 55.72; H, 6.65; N, 11.53.

***N*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(6*S*)-N^{im},5-di-Boc-4,5,6,7-tetrahydroimidazo[4,5-c]pyridine-6-carboxylic Amide (15).** To a solution of **14** (280 mg, 0.76 mmol) and **12** (241 mg, 0.69 mmol) in dry CH_2Cl_2 (15 mL) EEDQ (278 mg, 1.12 mmol) was added under an argon atmosphere and the solution was stirred at 23 °C for 24 h. The solvent was evaporated at 30 °C *in vacuo* and the residue was purified by FC. The column was eluted first with *n*-hexane / EtOAc in order to remove quinoline. The product then was eluted with EtOAc / MeOH 15:1. After concentration at 40 °C *in vacuo*, the product was isolated as a pale yellow foam. The residue was crystallized from Et_2O / *n*-hexane to yield colourless needles (319 mg, 67%): mp 180-182 °C; (+)-FAB-MS (mNBA): *m/z* 1391.6 ($[2\cdot M+H]^+$), 718.3 ($[M+Na]^+$), 696.3 ($[M+H]^+$); IR (KBr): $\tilde{\nu}$ 3386 (w), 2979 (w), 1754 (ss, ester-C=O), 1692 (s, amide-C=O), 1524 (m), 1375 (s), 1239 (ss), 1163 (m), 1128 (m), 1047 cm^{-1} (m-s); ^1H NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 1 H, H-2), 7.26 (d, 1 H, J = 8.5 Hz, amide-NH), 6.09 (br, 0.4 H, amide-NH), 6.04 (br d, 0.6 H $J \approx 8$ Hz, amide-NH), 5.26-5.10 (br, 1 H, H-6), 5.07 (dd, 1 H, J = 9.5, 9.5 Hz, H-3'), 5.00-4.90 (br, 1 H, H-4ii), 4.99 (dd, 1 H, J = 9.8, 8.5 Hz, H-1'), 4.98 (dd, 1 H, J = 10.5, 9.6 Hz, H-4'), 4.2-4.3 (br m, 1 H, H-4), 4.23 (dd, 1 H, J = 12.5, 4.3 Hz, H-6'ii), 4.11 (ddd, 1 H, J = 10, 10, 8.8 Hz, H-2'), 4.01 (br d, 1 H, $J \approx 12$ Hz, H-6'i), 3.66 (br m, 1 H, H-5'), 3.23 (br d, 1 H, $J \approx 15$ Hz, H-7ii), 2.84 (br d, 1 H, $J \approx 15$ Hz, H-7i), 2.05 (s, 3 H), 2.00 (s, 3 H), 1.99 (s, 3 H), 1.81 (s, 1.8 H), 1.72 (s, 1.2 H, all acetyl-CH_3), 1.59 (s, 9 H), 1.49 (s, 9 H, all *tert*-Bu-CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 171.7 (C), 171.6 (C), 171.4 (C), 170.8 (C), 169.3 (C, all acyl-CO), 155.4 (C, urethane-CO), 147.2 (C, urethane-CO), 136.9 (CH, C-2), 135.0 (C, C-3a or 7a), 134.6 (C, C-3a or 7a*), 121.2 (C, C-7a or 3a), 85.8 (C, *tert*-Bu-C), 81.5 (C, *tert*-Bu-C), 80.5 (CH, C-1'), 73.7 (CH) 73.0 (CH), 67.8 (CH), 61.8 (CH_2, C-6'), 54.6 (CH, C-2' or 6*), 53.2 (CH, C-2' or 6), 52.9 (CH, C-6 o. 2'), 40.9 (CH_2, C-4), 39.9 (CH_2, C-4*), 28.3 (CH_3, *tert*-Bu-CH_3), 27.9 (CH_3, *tert*-Bu-CH_3), 24.8 (CH_2, C-7), 22.8 (CH_3), 20.8 (CH_3), 20.7 (CH_3), 20.6 (CH_3, acetyl-CH_3); *pairwise assigned signals of regioisomers.

Anal. Calcd for $C_{31}H_{45}N_5O_{13} \cdot 0.5\,H_2O$: C, 52.83; H, 6.58; N, 9.94. Found C, 52.90; H, 6.65; N, 10.04.

N-(2-Acetamido-4-O-[2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl]-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(6 S)-N^{im},5-di-Boc-4,5,6,7-tetrahydroimidazo[4,5-c]pyridine-6-carboxylic Amide (16). Heptaacetylchitobiosylamine (**13**) (495 mg, 0.78 mmol), **14** (371 mg, 1.01 mmol) and EEDQ (368 mg, 1.49 mmol) were dissolved in dry CH_2Cl_2 (35 mL) in an argon atmosphere. After stirring for 24 h the solution was concentrated and the residue purified by FC (EtOAc / MeOH 15:1) to afford a white solid (376 mg, 49%): mp 195-200 °C (decomp.); (+)-FAB-MS (mNBA): m/z 983.4 ($[M+H]^+$), 883.3 ($[M\text{-}Boc+H]^+$); IR (KBr): $\tilde{\nu}$ 3389 (m), 2979 (w), 1749 (ss, ester-C=O), 1682 (s, amide-C=O), 1537 (m), 1377 (s), 1238 (ss), 1045 cm^{-1} (s); ^1H NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 1 H, H-2), 7.23 (d, 1 H, J = 8.3 Hz, amide-NH), 6.76 (b, 0.38 H, amide-NH), 6.59 (b, 0.62 H, amide-NH), 6.38 (b, 0.4 H, amide-NH), 6.34 (b, 0.6 H, amide-NH), 5.28 (br m, 2 H), 5.15-4.70 (m, 7 H), 4.40-4.09 (m, 4 H), 4.03 (ddd, 1 H, $J \approx 10, \approx 10, \approx 9$ Hz), 3.99 (dd, 1 H, J = 12.4, 2.2 Hz), 3.76-3.67 (m, 1 H), 3.63 (ddd, 1 H, J = 10.2, 4.3, 2.1 Hz), 3.56 (dm, 1 H, $J \approx 9$ Hz), 3.45 (br, 1 H), 3.23 (d, 1 H, J = 16.4 Hz, H-7ii), 2.86 (dd, 1 H, J = 16.2, 6.7 Hz, H-7i), 2.12 (s, 3 H), 2.06 (s, 3 H), 2.05 (s, 3 H), 2.00 (s, 3 H), 1.98 (s, 3 H), 1.88 (s, 3 H), 1.87 (s, 3 H, all acetyl-CH_3), 1.60 (s, 9 H), 1.50 (br s, 9 H, all *tert*-Bu-CH_3); ^{13}C NMR (50.3 MHz, $CDCl_3$) 172.4 (C), 172.3 (C), 171.9 (C), 171.4 (C), 171.3 (C), 171.2 (C), 171.1 (C), 170.2 (C, all acyl-CO), 156.0 (C, urethane-CO*), 147.8 (C, urethane-CO), 137.5 (CH, C-2), 135.5 (C, C-3a or 7a*), 121.9 (C, C-7a or 3a*), 100.9 (CH, C-1"*), 86.6 (C, *tert*-Bu-C), 82.2 (C, *tert*-Bu-C), 80.9 (CH, C-1'), 75.7 (CH*), 74.8 (CH), 73.3 (CH), 72.5 (CH), 72.3 (CH*), 62.6 (CH_2, C-6' or 6'), 62.3 (CH_2, C-6' or 6'), 56.3 (CH*), 55.3 (CH*), 53.2 (CH*), 41.3 (CH_2, C-4*), 28.9 (CH_3, *tert*-Bu-CH_3), 28.5 (CH_3, *tert*-Bu-CH_3), 25.5 (CH_2, C-7), 23.7 (CH_3), 23.5 (CH_3), 21.6 (CH_3), 21.4 (2 × CH_3), 21.3 (CH_3), 21.2 (CH_3, all acetyl-CH_3); *two broadened singlets.

Anal. Calcd for $C_{43}H_{66}N_6O_{22} \cdot 2\,H_2O$: C, 50.68; H, 6.52; N, 8.25. Found C, 50.97; H, 6.58; N, 8.19.

N-(2-Acetamido-4-O-[2-acetamido-4-O-{2-acetamido-2-deoxy-β-D-glucopyranosyl}-2-deoxy-β-D-glucopyranosyl]-2-deoxy-β-D-glucopyranosyl)-L-histidine Amide (3). The protected glycosylamide **11** (390 mg, 0.31 mmol) was stirred in anhydrous TFA (10 mL) under an argon atmosphere for 0.5 h. The solvent was removed *in vacuo* at 25 to 30 °C and the oily residue was coevaporated several times with $CHCl_3$. The resulting foam was dried and dissolved in MeOH (20 mL). A stream of ammonia was passed through the solution at 0 °C for 1 h and the mixture was stirred at 23 °C for 5 d. The precipitated product was collected by filtration and washed with MeOH to yield an off-white solid (205 mg, 87%).

Recrystallization from water afforded an analytical pure crystalline sample: mp > 255 °C; $[\alpha]_D^{20}$ -4° (*c* 0.33, H_2O); (+)-FAB-MS (thio): *m/z* 764.3 ($[M+H]^+$); ^1H NMR (400 MHz, D_2O) δ 7.69 (s, 1 H, H-6"'), 6.93 (s, 0.8 H, H-8"'), 6.90 (s, 0.2 H, H-8"'), 5.03 (d, 0.2 H, $J \approx 9.5$ Hz, H-1), 4.99 (d, 0.8 H, $J \approx 9.5$ Hz, H-1), 4.59 (d, 1 H, $J \approx 8$ Hz, H-1" or H-1'), 4.58 (d, 1 H, $J \approx 8$ Hz, H-1' or H-1"), 4.00 - 3.40 (m, 19 H), 2.93 (d, 2 H, $J \approx 6$ Hz, H-3"'), 2.06 (s, 6 H, amide-CH_3), 1.95 (s, 2.4 H, amide-CH_3), 1.91 (s, 0.6 H, amide-CH_3); ^{13}C NMR (62.5 MHz, D_2O / HCl, acetone as int. stand.) δ 175.3 (C), 174.9 (2×C), 169.1 (C, all acyl-CO), 134.7 (C-2"'), 125.7 (C-4"'), 119.1 (C-5"'), 101.8 (CH, C-1' or 1"), 101.5 (CH, C-1' or 1"), 79.6 (CH), 79.0 (CH), 78.6 (CH), 76.6 (CH), 76.3 (CH), 74.9 (CH), 73.8 (CH), 73.1 (CH), 72.5 (CH), 70.1 (CH), 60.9 (CH_2), 60.4 (2·CH_2), 56.0 (CH), 55.4 (CH), 54.0 (CH), 52.6 (CH), 26.3 (CH_2, C-3"'), 22.5 (3·CH_3, amide-CH_3).

Anal. Calcd for $C_{30}H_{49}N_7O_{16} \cdot H_2O$: C, 46.09; H, 6.58; N, 12.54. Found C, 46.02; H, 6.78; N, 12.61.

***N*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(6*S*)-4,5,6,7-tetrahydroimidazo[4,5-c]pyridine-6-carboxylic Amide (4).** The protected glycosylamide **15** (245 mg, 0.35 mmol) was treated with TFA (10 mL) as described above for **3**. The subsequent deacetylation was carried out analogously in MeOH (7.5 mL). The precipitated product was collected by filtration. The filtrate was concentrated and the solid residue was recrystallized from water. The combined solids were again recrystallized from water to afford colourless needles (130 mg, 97%): mp 244-246 °C; $[\alpha]_D^{20}$ -15.6° (*c* 0.334, H_2O); (+)-FAB-MS (thio): *m/z* 392.1 ($[M+Na]^+$), 370.2 ($[M+H]^+$); IR (KBr): $\tilde{\nu}$ 3400 (ss, b), 3287 (ss), 2900 (w), 1668 (s, amide-C=O), 1551 (s), 1378 (w-m), 1077 cm^{-1} (m); ^1H NMR (400 MHz, D_2O) δ 7.61 (s, 1 H, H-2), 5.11 (d, 1 H, J = 9.7 Hz, H-1'), 3.88 (dd, 1 H, J = 13, 1.4 Hz, H-6'ii), 3.87 (dd, 1 H, J = 9.9, 9.9 Hz, H-2'), 3.80 (dd, 2 H, J = 3.8, 1.6 Hz, H-4), 3.75 (dd, 1 H, J = 12.4, 4.8 Hz, H-6'i), 3.72 (dd, 1 H, J = 8.5, 5 Hz, H-6), 3.62 (dd, 1 H, J = 10.2, 8.4 Hz, H-3'), 3.53 (ddd, 1 H, J = 9.7, 5, 2.1 Hz, H-5'), 3.49 (dd, 1 H, J = 9.8, 8.2 Hz, H-4'), 2.89 (ddt, 1 H, J = 15.6, 5, ≈ 1 Hz, H-7ii), 2.76 (ddt, 1 H, J = 15.6, 8.5, ≈ 1.6 Hz, H-7i), 1.99 (s, 3 H, amide-CH_3); ^{13}C NMR (100.6 MHz, D_2O) δ 177.9 (C, C-8 or amide-CO), 177.4 (C, amide-CO or C-8), 137.7 (CH, C-2), 130.7 (C, br, C-3a or 7a), 128.5 (C, br, C-7a or 3a), 81.1 (CH, C-1'), 80.3 (CH), 76.8 (CH), 72.1 (CH), 63.1 (CH_2, C-6'), 58.4 (CH, C-2' o. 6), 56.9 (CH, C-6 o. 2'), 43.1 (CH_2, C-4), 27.8 (CH_2, C-7), 24.6 (CH_3, acetyl-CH_3).

Anal. Calcd for $C_{15}H_{23}N_5O_6 \cdot 0.75\,H_2O$: C, 47.05; H, 6.45; N, 18.29. Found C, 47.37; H, 6.32; N, 18.06.

***N*-(2-Acetamido-4-*O*-[2-acetamido-2-deoxy-β-D-glucopyranosyl]-2-deoxy-β-D-glucopyranosyl)-(6*S*)-4,5,6,7-tetrahydroimidazo[4,5-c]pyridine-6-**

carboxylic Amide (5). Glycosylamide **16** (297 mg, 0.3 mmol) was deprotected in the same manner as described for **4**. The product precipitated from the methanolic ammonia solution and was collected by filtration to obtain fine colourless needles (139 mg, 81%): mp 248-250 °C; $[\alpha]_D^{24}$ -16.8° (*c* 0.291, H_2O); (+)-FAB-MS (mNBA): *m/z* 595.2 ($[M+Na]^+$), 573.3 ($[M+H]^+$); IR (KBr): $\tilde{\nu}$ 3423 (ss, b), 2900 (w), 1646 (m, amide-C=O), 1558 (m), 1375 (w), 1072 cm^{-1} (m); ^1H NMR (400 MHz, D_2O) δ 7.61 (s, 1 H, H-2), 5.10 (d, 1 H, J = 9.8 Hz, H-1'), 4.59 (d, 1 H, J = 8.4 Hz, H-1"), 3.91 (dd, 1 H, J = 12.3, 2 Hz, H-6"ii), 3.90 (dd, 1 H, J = 10, 10 Hz, H-2'), 3.83 (dd, 1 H, J = 12, 1.8 Hz, H-6'ii), 3.80 (m, 2 H, H-4), 3.76 (dd, 1 H, J = 10.2, 8.4 Hz, H-3' or 2"), 3.75 (dd, 1 H, J = 10.4, 8.3 Hz, H-2" or 3'), 3.74 (dd, 1 H, J = 12.5, $\approx$ 6 Hz, H-6"i), 3.71 (dd, 1 H, J = 8.4, 5 Hz, H-6), 3.67 (dd, 1 H, J = 9.5, 8.4 Hz, H-4'), 3.64 (dd, 1 H, J = 12, 4.5 Hz, H-6'i), 3.58 (ddd, 1 H, J = 9.6, 4.6, $\approx$ 2 Hz, H-5'), 3.56 (dd, 1 H, J = 10.4, 8.6 Hz, H-3"), 3.50 (ddd, 1 H, J = 9.6, 5.6, 2.1 Hz, H-5"), 3.46 (dd, 1 H, J = 9.6, $\approx$ 8 Hz, H-4"), 2.89 (ddm, 1 H, J = 15.6, 4.9 Hz, H-7ii), 2.76 (ddt, 1 H, J = 15.6, 8.6, 1.7 Hz, H-7i), 2.06 (s, 3 H, amide-CH_3), 1.98 (s, 3 H, amide-CH_3); ^{13}C NMR (100.6 MHz, D_2O, MeOH as int. standard) δ 177.9 (C), 177.4 (C), 177.3 (C, all acyl-CO), 137.7 (CH, C-2), 130.7 (C, br, C-3a or 7a), 128.4 (C, br, C-7a or 3a), 104.1 (CH, C-1"), 81.5 (CH), 81.0 (CH), 78.9 (CH), 78.6 (CH), 76.1 (CH), 75.4 (CH), 72.3 (CH), 63.2 (CH_2, C-6' or 6"), 62.6 (CH_2, C-6" or 6'), 58.4 (CH), 58.2 (CH), 56.3 (CH), 43.0 (CH_2, C-4), 27.8 (CH_2, C-7), 24.7 (CH_3, acetyl-CH_3), 24.6 (CH_3, acetyl-CH_3).

Anal. Calcd for $C_{23}H_{36}N_6O_{11} \cdot 2.5\,H_2O$: C, 44.73; H, 6.69; N, 13.61. Found C, 44.89; H, 7.06; N, 13.42.

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