Synthesis of *N*-Acetylglucosaminyl and Diacetylchitobiosyl Amides of Heterocyclic Carboxylic Acids as Potential Chitinase Inhibitors

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2-(Dimethylamino)oxazoline-4-carboxylic acids **5** were prepared by condensation of binucleophilic amino acids **4** and *O*-ethyl-*N*,*N*-dimethylisourea **3**. New heterocyclic *N*-acetylglucosaminyl amides and chitobiosyl amides **8** were obtained by the coupling of tetraacetylglucosamine **6a** or heptaacetylchitobiosylamine **6b** with acid chlorides of heterocyclic carboxylic acids **2** or **5a** and subsequent deacetylation. Based on their substitution patterns, compounds **8** were expected to have inhibitory activity towards chitinases. Enzyme assays showed that glycosyl amides **8** indeed were moderate inhibitors of chitinases, the diacetylchitobiosyl amides **8d–f** generally having higher inhibitory activities than the *N*-acetylglucosaminyl amide derivatives **8a–c**. Inhibitory activities depended on the chitinase tested.

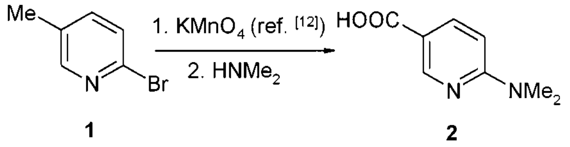
Introduction

Chitinases are enzymes occurring in many organisms, including crustaceeans, insects, bacteria, fungi, plants, and also vertebrates. They play important roles in the metabolism of chitin and higher chito-oligosaccharides (for some key references, see ref.[1]). The design of chitinase inhibitors is of interest for approaches towards an understanding of the structures and mechanisms of chitinolytic enzymes as well as for potential applications in medicine and agriculture.[2] So far, 3D structures are known for only three chitinases, namely two family 18 glycosylhydrolases,[3] hevamine from the latex of the rubber plant *Hevea brasiliensis*[4] and chitinase A from the bacterium *Serratia marcescens*,[5] and one family 19 glycosylhydrolase, a chitinase from barley, *Hordeum vulgare*.[6] The 3D structure of a complex of hevamine with the potent, naturally occurring chitinase inhibitor allosamidin has been resolved.[7] Furthermore, the 3D structure of a complex of hevamine with a less efficient, synthetic chitinase inhibitor, N^{amido}-triacetylchitotriosyl L-histidine amide[8] was determined by X-ray crystallography and compared with the structure of the hevamine-allosamidin complex.[9] The results suggest that hydrophobic interactions between Trp^{255} and the heterocyclic units of the inhibitors as well as hydrogen bonds between their oxazoline resp. imidazole group and both, Asp^{125} and Tyr^{183}, are essential features of the mechanism of inhibition. In addition, the number and spatial orientation of the sugar residues contribute to inhibitor affinities. Additional support for the structure of the active sites and the mechanism of catalysis of family 18 chitinases was obtained recently by molecular modelling of the binding interactions of hexaacetylchitohexaose with hevamine[10] and with chitinase A.[10][11]

With the goal to gain additional information on the mode of action of chitinase inhibitors, we have synthesized a series of new compounds for use in further biochemical studies. The structures consist of an 1-acylamino-2-acetamido-1,2-dideoxy-β-D-glucose unit, where the 1-*N*-acyl group is derived from a heterocyclic carboxylic acid (see **8** and Table 1). The heterocyclic units could provide both, hydrophobic interaction and a weak base, as present in the pyridine derivatives nicotinic acid and **2**, as well as mimicking of the allosamizoline unit of allosamidin, like the (dimethylamino)pyridine **2** and oxazolines **5**.

Preparation of Glycosyl Amides

N,*N*-Dimethylaminonicotinic acid **2** was prepared from commercially available 2-bromo-5-picoline (**1**) (Scheme 1).[12] The original procedure described in the literature, i.e. oxidation of **1** and heating with *N*,*N*-dimethylamine in H_2O under pressure[13] was improved by choosing DMF and MeOH as solvents. For the synthesis of oxazolines **5**, isourea **3**[14] was reacted with L-threonine (**4a**) or with L-serine (**4b**) which gave **5a** or **5b** in 59% or 28% yield, respectively (Scheme 2).



Scheme 1. Preparation of *N*,*N*-dimethylaminonicotinic acid

Usually, N^{amido}-glycosides of amino acid amides are prepared by EEDQ mediated coupling of the carboxylic acids with glycosylamines.[15] This procedure was also useful for the synthesis of glycosylamides **7c** and **7f** (Table 1). How-

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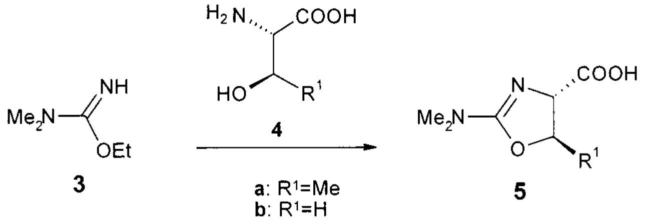
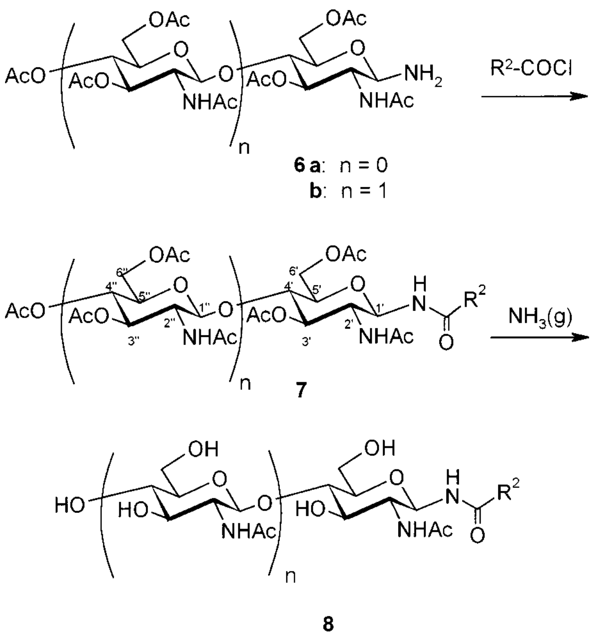


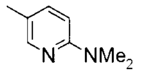
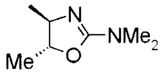
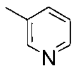
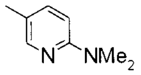
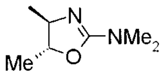
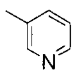
Scheme 2. Preparation of 2-dimethylaminooxazoline-4-carboxylic acids **5**

ever, it did not yield any coupling products in the case of dimethylamino-substituted carboxylic acids **2** or **5**. Attempts to achieve coupling by means of EDC/HOBT or DCC were also not successful. This was not totally unexpected since coupling of *N*-methyl amino acids to amines often requires reactive acid chlorides as acyl donors.[16] Indeed, reaction of acid chlorides, prepared from **2** or **5a** by means of oxalyl chloride, with glycosylamines **6**[17][18] yielded glycosylamides **7a**, **7b**, **7d**, and **7e** in satisfactory yields (Scheme 3 and Table 1). On the other hand, despite many attempts, decomposition was indicated always by complex TLC patterns after reaction of the acid chloride of **5b** with glycosylamines **6**. Cleavage of the *O*-acetyl groups of compounds **7** by standard procedures, employing ammonia in MeOH,[18] led to the desired glycosylamides **8**. The structures of all compounds were confirmed by NMR spectroscopy and mass spectrometry.



Scheme 3. Synthesis of the title compounds

Table 1. Formulae of heterocyclic units of **8**

7, 8	*n*	R^2
a	0	
b	0	
c	0	
d	1	
e	1	
f	1	

Enzyme Assays

A first series of experiments on the determination of the inhibitory activity (IC_{50}) of compounds **8** towards chitinase A and chitinase B from *Serratia marcescens*, with 4-methylumbelliferyl *N,N′*-diacetyl chitobioside as the substrate,[19] gave the results shown in Table 2. Moderate inhibition of chitinase A ($IC_{50} \leq 1.0$ mM) was found for the diacetylchitobiosyl amides **8d−f** and the monosaccharidic *N*-acetylglucosylaminyl derivative **8a**. Though the results are not spectacular with respect to inhibition power, some interesting features are revealed by the data. A comparison of the monosaccharidic glycosylamides **8a−c** with the disaccharide analogues **8d−f** shows a surprising behaviour of **8a**/**8d**, as both compounds give the same IC_{50} value. This is in contrast to **8b**/**8e** and **8c**/**8f** where the disaccharide derivatives are more efficient by a factor 3 or 6, respectively than the corresponding monosaccharides. Probably, hydrophobic interactions of the heterocyclic units of **8a**/**8d** dominate over the effects of the number of sugar residues, whereas the effect of the number of the latter is clearly seen with **8b**/**8e** and **8c**/**8f**.

Table 2. Inhibition of chitinase A and B from *Serratia marcescens*[19] by compounds **8**

Compound	IC_{50} (mM) Chitinase A	Chitinase B
8a	1.0	2.0
8b	3.0	4.5
8c	3.0	3.5
8d	1.0	1.5
8e	1.0	2.5
8f	0.5	1.5

Inhibitory activity towards chitinase B was generally lower, which may be because this enzyme has higher affinity for the substrate, 4-methylumbelliferyl *N,N′*-diacetyl chitobioside (K_m 20−30 μM), than chitinase A (K_m > 100 μM).[19]

The nicotinic acid amide **8f** appeared to be the most effective inhibitor synthesized in this study, with an IC$_{50}$ value for chitinase A in the range of 0.5 mM. For comparison, *N,N′*-diacetylchitobiose shows, under the same experimental conditions, IC$_{50}$ = 1.5 mM with chitinase A, and IC$_{50}$ = 3.0 mM with chitinase B. Measurements of enzyme kinetics, including pH and time dependence of inhibition, and synthesis of further potential chitinase inhibitors are in progress.

Experimental Section

General: Solvents were purified by standard procedures. − Evaporation of solvents: Rotary evaporator (ca. 1.3 kPa), bath temp. ca. 30°C. − Chromatography: Silica gel (Merck: 0.04−0.063 mm). − NMR: Bruker AMX R 300 (300 MHz and 75 MHz, for ^{1}H and ^{13}C, respectively), TMS as internal standard. − 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 analyses: LECO CHNS-932.

6-(Dimethylamino)-3-pyridinecarboxylic Acid (2): To a solution of 500 mg (2.5 mmol) of 2-bromopyridine-5-carboxylic acid[12] in 5 mL of dry DMF was added 5.5 mL of a 2 M solution of dimethylamine (11 mmol) in methanol and the mixture was stirred for 15 h at 130°C in a closed vessel. The solvent was coevaporated with toluene in vacuo at 60°C and the solid residue was recrystallized from 10 mL of toluene to yield 360 mg (87%) of **2**,[13] pale yellow solid, m.p. 195−197°C, TLC (CHCl$_3$/MeOH, 6:4): R_f = 0.46. − ^{1}H NMR (CD$_3$OD): δ = 8.57 [d, J = 1.4 Hz, 1 H, 6-H], 8.21 [dd, J = 9.4; 1.9 Hz, 1 H, 4-H], 7.04 [d, J = 9.4 Hz, 1 H, 3-H], 2.74 [s, 6 H, NMe$_2$]. − ^{13}C NMR (CD$_3$OD): δ = 167.2, 157.9, 145.5, 141.5, 116.2, 110.3, 39.6. − EI-MS: *m/z* (%) = 166.2 (72) [M]$^+$, 151.1 (64), 137.2 (100), 123.2 (20), 44.2 (46) [NMe$_2$]$^+$. − $C_8H_{10}N_2O_2$ (166.2): calcd. C 57.81, H 6.07, N 16.86; found C 57.29, H 6.36, N 16.73.

(4*S*,5*R*)-2-(Dimethylamino)-5-methyl-Δ^2-oxazoline-4-carboxylic Acid (5a): To a solution of 119 mg (1 mmol) of **4a** in 10 mL of dry methanol was added 120 mg (1.2 mmol) of **3**[14] in 2 mL of methanol and the mixture was refluxed for 5 h and then stirred at room temp. for 12 h under an argon atmosphere. The solvent was evaporated in vacuo and the solid residue was purified by flash chromatography (CHCl$_3$/MeOH, 6:4), R_f = 0.55, to yield 101 mg (59%) of **5a**, white hygroscopic crystals, m.p. 66−70°C. − $[\alpha]_D^{22}$ = −16.8 (*c* = 1, MeOH). − ^{1}H NMR (CD$_3$OD): δ = 4.22 [m, 1 H, 5-H], 3.94 [d, J = 5.0 Hz, 4-H], 3.12 [s, 6 H, NMe$_2$], 1.25 [d, J = 6.3 Hz, 3 H, Me]. − ^{13}C NMR (CD$_3$OD): δ = 174.7, 163.9, 86.9, 67.4, 38.1, 20.8. − EI-MS: *m/z* (%) = 173.2 (10) [M + H]$^+$, 172.2 (5) [M]$^+$, 127.2 (82) [M − 44]$^+$, 72 (34), 44.2 (42) [NMe$_2$]$^+$, 31.1 (100). − $C_7H_{12}N_2O_3$ (172.2): calcd. C 48.83, H 7.02, N 16.27; found C 48.68, H 6.98, N 15.94.

(4*S*)-2-Dimethylamino-Δ^2-oxazoline-4-carboxylic Acid (5b): To a solution of 500 mg (4.8 mmol) of **4b** in 50 mL of dry methanol was added 510 mg (5 mmol) of **3** in 10 mL of methanol and the mixture was refluxed for 7 h under an argon atmosphere. The solution was filtered, the solvent was evaporated in vacuo, and the residue was purified by flash chromatography (CHCl$_3$/MeOH, 6:4), R_f = 0.15, to yield 213 mg (28%) of **5b**, pale yellow hygroscopic crystals, m.p. 49−50°C. − $[\alpha]_D^{22}$ = −23.1 (*c* = 1, MeOH). − ^{1}H NMR (D$_2$O): δ = 3.86−3.99 [m, 3 H, 5-H, 4-H], 3.14 [s, 6 H, NMe$_2$]. − ^{13}C NMR (D$_2$O): δ = 177.5, 169.1, 79.1, 63.9, 41.9. − EI-MS: *m/z* (%) = 159.2 (31) [M + H]$^+$; 158.2 (12) [M]$^+$, 114.2 (23) [M − 44]$^+$, 113.2 (48), 88.3 (43), 72.2 (55), 44.2 (100) [NMe$_2$]$^+$. − $C_6H_{10}N_2O_3$ (158.2): calcd. C 45.57, H 6.37, N 17.71; found C 45.01, H 6.84, N 17.23.

***N*−(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-6-dimethylamino-3-pyridinecarboxamide (7a):** A mixture of 59.6 mg (0.36 mmol) of **2** and 6 mL (8.9 g; 69.8 mmol) of oxalyl chloride was stirred for 12 h at room temp.[16] The excess of oxalyl chloride was evaporated and the residue was dissolved in 5 mL of dry CH$_2$Cl$_2$. A solution of 125 mg (0.36 mmol) of **6a** in 3 mL CH$_2$Cl$_2$ was added at −10°C, followed by 0.05 mL (0.4 mmol) of triethylamine. The mixture was stirred at room temp. for 72 h, followed by addition of 10 mL of an aqueous saturated NH$_4$Cl solution. Extraction with CH$_2$Cl$_2$, drying of the extract with Na$_2$SO$_4$, evaporation of the solvent, and chromatography of the residue (CHCl$_3$/MeOH, 9:1), R_f = 0.81, yielded 169 mg (95%) of **7a**, colourless crystals, m.p. 140 °C (dec). − $[\alpha]_D^{22}$ = −74.1 (c = 1, CHCl$_3$). − ^{1}H NMR (CDCl$_3$): δ = 8.62 [s, 1 H, 6-H], 7.79 [d, J = 9.0 Hz, 1 H, 3-H], 7.71 [d, J = 8.0 Hz, 1 H, NH], 6.55 [d, J = 8.3 Hz, 1 H, NH], 6.42 [d, J = 9.0 Hz, 1 H, 4-H], 5.31 [dd, J 8.7; 9.1 Hz, 1 H, 3′-H], 5.19 [m, 1 H, 4′-H], 5.06 [m, 1 H, 1′-H], 4.36 [m, 2 H, 6′-H$_{A,B}$], 4.08 [d, J = 12.3 Hz, 1 H, 2′-H], 3.85 [d, J = 9.3 Hz, 1 H, 5′-H], 3.10 [s, 6 H, NMe$_2$], 2.05 [s, 6 H, 2 Me], 2.01 [s, 3 H, Me], 1.86 [s, 3 H, Me]. − ^{13}C NMR (CDCl$_3$): δ = 172.2, 171.6, 170.6, 169.4, 166.6, 160.3, 148.8, 136.1, 115.6, 104.6, 80.7, 73.2, 72.9, 68.3, 61.8, 53.2, 37.9, 23.1, 22.9, 20.7, 20.5. − MALDI-MS: *m/z* = 533.0 [M + K]$^+$, 517.0 [M + Na]$^+$, 495.0 [M + H]$^+$. − $C_{22}H_{30}N_4O_9$ (494.5): calcd. C 53.44, H 6.11, N 11.33; found C 53.03, H 6.47, N 10.82.

***N*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(4*S*,5*R*)-2-dimethylamino-5-methyl-Δ^2-oxazoline-4-carboxyamide (7b):** Following the procedure described for **7a**, 42 mg (0.25 mmol) of **5a** were treated with 4 mL (5.9 g; 4.6 mmol) of oxalyl chloride followed by 86 mg (0.25 mmol) of **6a** and 0.04 mL (0.3 mmol) of triethylamine. Chromatography (CHCl$_3$/MeOH, 8:2), R_f = 0.65, yielded 95 mg (66%) of **7b**, pale yellow crystals, m.p. 125°C (dec). − $[\alpha]_D^{22}$ = −9.0 (*c* = 1, CHCl$_3$). − ^{1}H NMR (CDCl$_3$): δ = 7.85 [d, $J \approx$ 9.4 Hz, 1 H, NH], 6.02 [d, J = 9.2 Hz, 1 H, NH], 5.01−5.10 [m, 3 H, 1′-H, 3′-H, 4′-H], 4.54 [quint, J = 5.5; 5.7, Hz, 1 H, 5-H], 4.17−4.28 [m, 2 H, 6′-H$_{A,B}$], 4.05−4.10 [dd, J = 12.3; 2.0 Hz, 1 H, 2′-H], 3.98 [d, J = 5.1 Hz, 1 H, 4-H], 3.78 [m, 1 H, 5′-H], 2.94 [s, 6 H, NMe$_2$], 2.07 [s, 3 H, Me], 2.01 [s, 6 H, 2 Me], 1.83 [s, 3 H, Me], 1.40 [d, J = 6.3 Hz, 3 H, Me]. − ^{13}C NMR (CDCl$_3$): δ = 172.2, 171.6, 170.6, 169.4, 166.6, 160.3, 148.8, 136.1, 115.6, 104.5, 80.7, 73.2, 72.9, 68.3, 61.8, 53.2, 37.9, 23.1, 22.9, 20.7, 20.5. − MALDI-MS: *m/z* = 538.9 [M + K]$^+$, 522.9 [M + Na]$^+$, 501.1 [M + H]$^+$. − $C_{21}H_{32}N_4O_{10}$ (500.5): calcd. C 50.40, H 6.44, N 11.19; found C 49.84, H 6.37, N 9.69.

***N*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-nicotinamide (7c):** To a solution of 22 mg (0.18 mmol) of nicotinic acid in 5 mL of dry CH$_2$Cl$_2$, 74 mg (0.3 mmol) of EEDQ were added.[15] After stirring for 2 h at room temp., a solution of 52 mg (0.15 mmol) of **6a** in 3 mL CH$_2$Cl$_2$ was added dropwise and stirring was continued for 120 h. Evaporation of the solvent and chromatography of the residue (CHCl$_3$/acetone, 1:3), R_f = 0.33, yielded 40 mg (64%) of **7c**, pale yellow crystals, m.p. 214°C (dec). − $[\alpha]_D^{22}$ = −72.9 (*c* = 0.32, CHCl$_3$). − ^{1}H NMR (CDCl$_3$): δ = 8.72 [s, 1 H, 2-H], 8.13 [d, J = 7.8 Hz, 1 H, 6-H], 7.88 [d, J = 8.0 Hz, 1 H, NH], 7.64−7.70 [m, 0.5 H, 5-H], 7.49−7.54 [m, 0.5 H, 5-H], 7.34−7.38 [dd, J = 4.5 Hz, 1 H, 4-H], 6.53 [d, J = 7.9 Hz, 1 H, NH], 5.27 [dd, 1 H, 3′-H], 5.11−5.19 [m, 2 H, 1′-H; 4′-H], 4.19−4.35 [m, 2 H, 6′-$H_{A,B}$], 4.09 [d, J = 10.4 Hz, 1 H, 2′-H], 3.86 [m, 1 H, 5′-H], 2.09 [s, 3 H, Me], 2.07 [s, 3 H, Me], 2.04 [s, 3 H, Me], 1.92 [s, 3 H, Me]. − ^{13}C NMR ($CDCl_3$): δ = 172.6, 171.9, 170.7, 169.3, 165.8, 152.8, 149.0, 134.9, 128.7, 123.4, 80.9, 73.5, 72.8, 67.7, 61.7, 53.5, 23.1, 22.9, 20.7, 20.6. − MALDI-MS: *m/z* = 490.1 $[M + K]^+$, 474.1 $[M + Na]^+$, 452.1 $[M + H]^+$. − $C_{20}H_{25}N_3O_9$ (451.4): calcd. C 53.21, H 5.58, N 9.31; found C 52.86, H 5.36, N 8.63.

***N*-[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]-6-dimethylamino-3-pyridinecarboxyamide (7d):** Following the procedure for **7a**, 41 mg (0.25 mmol) of **2** was treated with 4 mL (5.9 g; 46.6 mmol) of oxalyl chloride followed by 151 mg (0.24 mmol) of **6b** and 0.034 mL (0.25 mmol) triethylamine. Chromatography ($CHCl_3$/acetone, 1:3), R_f = 0.27, yielded 85 mg (44%) of **7d**, colourless crystals, m.p. 198°C (dec). − $[\alpha]_D^{22}$ = −55.5 (*c* = 1, MeOH). − ^{1}H NMR ($CDCl_3$): δ = 8.56 [s, 1 H, 6-H], 7.70−7.75 [m, 2 H, 3-H; NH], 7.31 [d, 1 H, J = 11.2 Hz, NH], 6.42 [d, J = 9.0 Hz, 1 H, 4-H], 5.70 t, J = 9.6 Hz, 1 H, 3′′-H], 5.42 [d, J = 9.0 Hz, 1 H, 3′-H], 5.36 [d, J = 9.9 Hz, 1 H, 1′′-H], 5.15 [br s, 1 H, NH], 4.92−4.99 [t, J = 9.6 Hz, 1 H, 4′′-H], 4.81−4.87 [t, J = 8−9 Hz, 1 H, 1′-H], 4.43−4.51 [m, 2 H, 6′′-$H_{A,B}$], 4.26 [d, J = 9.2 Hz, 1 H, 2′-H], 3.97 [d, J = 12.9 Hz, 2 H, 6′-$H_{A,B}$], 3.91 [d, J = 9.5 Hz, 1 H, 4′-H], 3.67−3.75 [m, 2 H, 5-H; 5′-H], 3.11 [s, 6 H, NMe_2], 2.93 [m, 1 H, 2′′-H], 2.11 [s, 3 H, Me], 2.09 [s, 3 H, Me], 2.06 [s, 3 H, Me], 2.03 [s, 3 H, Me], 2.00 [s, 3 H, Me], 1.96 [s, 3 H, Me], 1.92 [s, 3 H, Me]. − ^{13}C NMR ($CDCl_3$): δ = 172.9, 172.1, 172.0, 170.4, 170.1, 169.9, 169.8, 167.2, 160.6, 149.2, 135.9, 115.5, 104.5, 99.4, 81.4, 74.8, 73.9, 72.8, 71.8, 70.8, 68.6, 62.0, 61.8, 57.2, 52.5, 38.0, 23.3, 23.2, 21.3, 21.0, 20.7 (2), 20.6. − MALDI-MS: *m/z* = 820.1 $[M + K]^+$, 804.1 $[M + Na]^+$, 782.1 $[M + H]^+$. − $C_{34}H_{47}N_5O_{16} \cdot H_2O$ (799.8): calcd. C 51.06, H 6.18, N 8.76; found C 51.30, H 5.61, N 8.11.

***N*-[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]-(4*S*,5*R*)-2-dimethylamino-5-methyl-Δ^2-oxazoline-4-carboxyamide (7e):** Following the procedure for **7a**, 68 mg (0.4 mmol) of **5a** was treated with 6 mL (8.9 g; 69.8 mmol) of oxalyl chloride followed by 22.1 mg (0.35 mmol) of **6b** and 0.05 mL (0.25 mmol) of triethylamine. Chromatography ($CHCl_3$/acetone, 1:3), R_f = 0.22, yielded 156 mg (59%) of **7e**, pale yellow crystals, m. p. 185°C (dec). − $[\alpha]_D^{22}$ = −27.6 (*c* = 1, $CHCl_3$). − ^{1}H NMR ($CDCl_3$): δ = 7.95 [d, J = 8.6 Hz, 1 H, 1′-NH], 7.62 [s, 1 H, 2′-NH], 6.86 [d, J = 8.9 Hz, 1 H, 2′′-NH], 5.53 [dd, J = 9.8 Hz, 1 H, 3′-H], 4.99−5.24 [m, 2 H, 1′′-H, 3′′-H], 4.97 [dd, J = 9.5 Hz, 1 H, 4′-H], 4.69 [dd, J = 8.6 Hz, 1 H, 1′-H], 4.62 [dd, J = 6.1 Hz, 1 H, 5-H], 4.38−4.47 [m, 2 H, 6′-H_A; 6′′-H_A], 4.18 [dd, J = 9.8 Hz, 1 H, 2′′-H], 3.94−4.01 [m, 3 H, 6′-H_B; 6′′-H_B; 4-H], 3.84 [dd, J = 8.1 Hz, 1 H, 4′′-H], 3.72 [m, 1 H, 5′-H], 3.59 [m, 1 H, 5′′-H], 3.10 [m, 1 H, 2′-H], 2.96 [s, 6 H, NMe_2], 2.11 [s, 3 H, Me], 2.07 [s, 3 H, Me], 2.06 [s, 3 H, Me], 2.01 [s, 3 H, Me], 1.99 [s, 3 H, Me], 1.91 [s, 3 H, Me], 1.86 [s, 3 H, Me], 1.36 [d, J = 6.2 Hz, 3 H, Me]. − ^{13}C NMR ($CDCl_3$): δ = 175.8, 171.7 (3), 170.9, 170.4, 170.0, 169.8, 163.2, 99.3, 80.2, 79.7, 74.4, 73.9, 73.2, 72.7, 71.8, 70.9, 68.6, 62.0, 61.4, 56.8, 51.9, 37.4, 23.1, 22.8, 21.9, 21.1, 20.9, 20.7, 20.6, 20.5. − MALDI-MS: *m/z* = 826.3 $[M + K]^+$, 810.3 $[M + Na]^+$, 788.3 $[M + H]^+$. − $C_{33}H_{49}N_5O_{17} \cdot H_2O$ (805.8): calcd. C 49.19, H 6.38, N 8.69; found C 49.54, H 6.12, N 8.14.

***N*-[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]-nicotinamide (7f):** To a solution of 25 mg (0.20 mmol) of nicotinic acid in 5 mL of dry CH_2Cl_2 was added 74 mg (0.4 mmol) of EEDQ. After stirring for 3 h at room temp., a solution of 95 mg (0.15 mmol) of **6b** in 3 mL of CH_2Cl_2 was added dropwise and stirring was continued for 120 h. Evaporation of the solvent and chromatography ($CHCl_3$/acetone, 1:3), R_f = 0.19, yielded 62 mg (56%) of crude **7f**, pale yellow crystals, which was used for the preparation of **8f** without further purification. − MALDI-MS: *m/z* = 777.2 $[M + K]^+$, 761.2 $[M + Na]^+$, 739.7 $[M + H]^+$.

***N*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-6-dimethylamino-3-pyridinecarboxyamide (8a):** Ammonia was passed through a stirred solution of 60 mg (0.12 mmol) of **7a** dissolved in 5 mL of dry methanol at 0°C for 1 h. The reaction vessel was sealed and stirring was continued at room temp. for 24 h. The solvent was evaporated, and the residue was purified by flash chromatography ($CHCl_3$/MeOH, 6:4), R_f = 0.48, to yield 31 mg (71%) of **8a**, colourless crystals, m.p. 198 °C (dec). − $[\alpha]_D^{22}$ = −66.3 (*c* = 0.68, MeOH). − ^{1}H NMR (CD_3OD): δ = 8.56 [s, 1 H, 6-H], 7.89 [d, J = 9.1 Hz, 1 H, 3-H], 6.64 [d, J = 9.1 Hz, 1 H, 4-H), 5.11 [d, J = 9.7 Hz, 1 H, 1′-H], 3.85−3.91 [m, 2 H, 6′-H], 3.71 [m, 1 H, 2′-H], 3.55−3.61 [m, 2 H, 3′-H; 4′-H], 3.39 [d, J = 6.1 Hz, 1 H, 5′-H], 3.13 [s, 6 H, NMe_2], 1.95 [s, 3 H, Me]. − ^{13}C NMR (CD_3OD): δ = 174.8, 169.1, 161.9, 149.8, 137.4, 117.4, 106.3, 81.5, 79.7, 75.9, 71.9, 62.6, 56.1, 38.4, 22.8. − MALDI-MS: *m/z* = 407.1 $[M + K]^+$, 391.2 $[M + Na]^+$, 369.2 $[M + H]^+$. − EI-MS: *m/z* (%) = 368.2 (6) $[M]^+$, 309.3 (11), 284.2 (16), 194.2 (26), 166.2 (10), 149.1 (100), 94.1 (13). − $C_{16}H_{24}N_4O_6$ (368.3): exact mass calcd. 391.159354 $[M + Na]^+$; found 391.158512 $[M + Na]^+$ (FAB-HR-MS).

***N*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(4*S*,5*R*)-2-dimethylamino-5-methyl-Δ^2-oxazoline-4-carboxyamide (8b):** As described above for the preparation of **8a**, 45 mg (0.09 mmol) of **7b** was treated with ammonia. Chromatography ($CHCl_3$/MeOH, 6:4), R_f = 0.52, yielded 20 mg (94%) of **8b**, colourless crystals, m.p. 174 °C (dec). − $[\alpha]_D^{22}$ = +5.3 (*c* = 1.0, MeOH). − ^{1}H NMR (CD_3OD): δ = 4.48 [d, J = 9.8 Hz, 1 H, 1′-H], 4.52−4.59 [quint, 1 H, 5-H], 3.94 [d, J = 5.0 Hz, 1 H, 4-H], 3.85 [d, J = 5.2 Hz, 1 H, 6′-H_A], 3.81 [dd, J = 10.0 Hz, 1 H, 2′-H], 3.61−3.69 [m, 1 H, 6′-H_B], 3.44−3.49 [m, 1 H, 5′-H], 3.39 [m, 2 H, 3′-H; 4′-H], 2.98 [s, 6 H, NMe_2], 1.91 [s, 3 H, Me], 1.41 [d, J = 6.2 Hz, 3 H, Me]. − ^{13}C NMR (CD_3OD): δ = 176.6, 174.1, 165.3, 82.7, 80.9, 79.8, 75.6, 73.9, 71.9, 62.6, 55.6, 37.8, 22.6, 21.9. − MALDI-MS: *m/z* = 413.2 $[M + K]^+$, 397.3 $[M + Na]^+$, 375.3 $[M + H]^+$. − EI-MS: *m/z* (%) = 374.3 (22) $[M]^+$, 354.7 (8), 200.3 (8), 127.2 (100), 72.2 (10), 43.2 (17). − $C_{15}H_{26}N_4O_7$ (374.4): exact mass calcd. 397.169919; found. 397.169771 $[M + Na]^+$ (FAB-HR-MS).

***N*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)nicotinamide (8c):** As described for **8a**, 40 mg (0.09 mmol) of **7c** was treated with ammonia. Chromatography ($CHCl_3$/MeOH, 6:4), R_f = 0.28, yielded 15 mg (100%) of **8c**, pale yellow crystals, m.p. 129°C (dec). − $[\alpha]_D^{22}$ = −73.9 (*c* = 0.75, MeOH). − ^{1}H NMR (CD_3OD): δ = 8.95 [s, 1 H, 2-H], 8.68 [d, J = 4.7 Hz, 1 H, 6-H], 8.21 [d, J = 8.3 Hz, 1 H, 4-H], 7.53 [dd, J = 8.0; J = 4.9 Hz, 1 H, 5-H], 5.16 [d, J = 9.7 Hz, 1 H, 1′-H], 3.93 [d, J = 9.9 Hz, 1 H, 3′-H], 3.86 [d, J = 12.3 Hz, 1 H, 4′-H], 3.70 [dd, J = 12.0; J = 4.2 Hz, 1 H, 5′-H], 3.54 [t, J = 8.6 Hz, 1 H, 2′-H], 3.40 [m, 2 H, 6′-H], 1.92 [s, 3 H, Me]. − ^{13}C NMR (CD_3OD): δ = 174.8, 167.9, 153.1, 149.5, 137.2, 131.3, 125.2, 81.2, 79.9, 76.0, 71.8, 62.6, 56.3, 24.9. − MALDI-MS: *m/z* = 364.0 $[M + K]^+$, 348.0 $[M + Na]^+$, 326.0 $[M + H]^+$. − EI-MS: *m/z* (%) = 326.3 (9) $[M + H]^+$, 205.2 (91), 163.2 (29), 106.9 (62), 78.1 (60), 59.1 (41), 43.1 (100). − $C_{14}H_{19}N_3O_6$ (325.3): exact mass calcd. 348.11716; found 348.11720 $[M + Na]^+$ (ESI-HR-MS).

***N*-[2-Acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranosyl]-6-dimethylamino-3-pyridinecarboxamide (8d):** As described for **8a** 45 mg (0.06 mmol) of **7d** was treated with ammonia. Chromatography ($CHCl_3$/MeOH, 2:8), R_f = 0.42, yielded 28 mg (85%) of **8d**, colourless crystals, m.p. 225°C (dec). − $[\alpha]_D^{22}$ = −5.9 (*c* = 0.5, MeOH). − ^{1}H NMR (D_2O): δ = 8.38 [s, 1 H, 6-H], 7.78−7.82 [dd, *J* = 9.1 Hz, 1 H, 3-H], 6.63−6.66 [d, *J* = 9.2 Hz, 1 H, 4-H], 5.21−5.24 [d, *J* = 9.9 Hz, 1 H, 1′-H], 4.61−4.64 [d, *J* = 8.3 Hz, 1 H, 1′′-H], 3.88−4.03 [m, 3 H, 2′-H, 6′-H$_A$, 6′′-H$_A$], 3.84−3.85 [d, *J* = 4.6 Hz, 1 H, 3′-H], 3.77−3.80 [m, 2 H, 6′′-H$_B$, 2′′-H], 3.66−3.74 [m, 3 H, 6′-H$_B$, 4′-H, 5′-H], 3.57−3.63 [m, 1 H, 3′′-H], 3.48−3.51 [m, 2 H, 4′′-H, 5′′-H], 3.08 [s, 6 H, NMe_2], 2.08 [s, 3 H, Me], 1.97 [s, 3 H, Me]. − ^{13}C NMR (D_2O): δ = 179.5, 179.6, 173.9, 164.9, 152.8, 141.1, 119.5, 110.9, 105.9, 83.8, 83.4, 80.8, 80.4, 77.9, 77.2, 74.2, 65.1, 64.5, 60.1, 58.4, 42.5, 26.6, 26.4. − MALDI-MS: *m/z* = 610.1 $[M + K]^+$, 594.1 $[M + Na]^+$, 572.1 $[M + H]^+$. − $C_{24}H_{37}N_5O_{11}$ (571.6): exact mass calcd. 572.2568; found 572.2563 $[M + H]^+$ (FAB-HR-MS).

***N*-[2-Acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranosyl]-(4*S*,5*R*)-2-dimethylamino-5-methyl-Δ^2-oxazoline-4-carboxamide (8e):** As described for the preparation of **8a**, 76 mg (0.1 mmol) of **7e** was treated with ammonia. Chromatography ($CHCl_3$/MeOH, 3:7), R_f = 0.22, yielded 36 mg (71%) of **8e**, colourless crystals, m.p. 172°C (dec). − $[\alpha]_D^{22}$ = +6.8 (*c* = 0.5, MeOH). − ^{1}H NMR (D_2O): δ = 4.98 [d, *J* = 9.5 Hz, 1 H, 1′-H], 4.64 [t, *J* = 5.6 Hz, 1 H, 5-H], 4.58−4.61 [d, *J* = 8.6 Hz, 1 H, 1′′-H], 4.02−4.04 [d, *J* = 4.7 Hz, 1 H, 4-H], 3.82−3.94 [m, 3 H, 6′′-H$_A$, 2′-H, 6′-H$_B$], 3.72−3.82 [m, 3 H, 2′′-H, 3′-H, 6′′-H$_B$], 3.57−3.67 [m, 4 H, 6′-H$_A$, 5′-H, 4′-H, 3′′-H], 3.46−3.49 [m, 2 H, 4′′-H, 5′-H], 2.95 [s, 6 H, NMe_2], 2.07 [s, 3 H, Me], 1.97 [s, 3 H, Me], 1.41 [d, *J* = 6.2 Hz, 3 H, Me]. − ^{13}C NMR (D_2O): δ = 181.6, 179.3, 179.2, 169.1, 106.1, 86.1, 83.6, 83.3, 80.9, 80.5, 78.0, 76.9, 76.4, 74.3, 65.1, 64.5, 60.1, 57.9, 41.6, 26.7, 26.3, 25.4. − MALDI-MS: *m/z* = 616.7 $[M + K]^+$, 600.7 $[M + Na]^+$, 578.7 $[M + H]^+$. − $C_{23}H_{39}N_5O_{12}$ (577.6): exact mass calcd. 578.2673, found 578.2707 $[M + H]^+$ (FAB-HR-MS).

***N*-[2-Acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranosyl]nicotinamide (8f):** As described for the preparation of **8a**, 50 mg (0.07 mmol) of crude **7f** was treated with ammonia. Chromatography ($CHCl_3$/MeOH, 4:6), R_f = 0.36, yielded 34 mg (94%) of **8f**, colourless crystals, m.p. 222°C (dec). − $[\alpha]_D^{22}$ = −48.5 (*c* = 0.5, H_2O). − ^{1}H NMR (D_2O): δ = 8.90 [s, 1 H, 2-H], 8.76 [br s, 1 H, 6-H], 8.19 [d, *J* = 8.0 Hz, 1 H, 4-H], 7.62 [m, 1 H, 5-H], 5.33 [d, *J* = 9.6 Hz, 1 H, 1′-H], 4.66 [d, *J* = 8.4 Hz, 1 H, 1′′-H], 3.95 [t, *J* = 7.6 Hz, 1 H, 2′-H], 3.91−3.69 [m, 7 H, 2′′-H, 6′-H′$_{AB}$, 6′′-H$_{AB}$, 3′-H, 4′-H], 3.51−3.62 [m, 4 H, 3′′-H, 4′′-H, 5′-H, 5′′-H], 2.03 [s, 3 H, Me], 2.01 [s, 3 H, Me]. − ^{13}C NMR (D_2O): δ = 179.4, 179.2, 173.9, 156.7, 152.2, 140.9, 122.1, 124.0, 105.9, 83.9, 83.3, 80.9, 80.4, 77.9, 77.1, 74.2, 65.1, 64.4, 60.1, 58.5, 26.6, 26.4. − MALDI-MS: *m/z* = 567.08 $[M + K]^+$, 551.11 $[M + Na]^+$, 529.12 $[M + H]^+$. − $C_{22}H_{32}N_4O_{11} \cdot 3.5\ H_2O$ (591.6): calcd. C 44.66, H 6.64, N 9.47; found C 44.37, H 6.45, N 9.21.

Enzyme Assays: Chitinases A and B were purified and assayed for activity towards 4-methylumbelliferyl-β-D-*N*,*N*′-diacetylchitobioside (Sigma) as described previously.[19] For inhibition studies, 100 μL reaction mixtures containing 0.43 nM (chitinase A) or 0.87 nM (chitinase B) enzyme, 12 μM substrate, 0.1 mg/mL BSA, and varying concentrations of compounds **8** in 50 mM citrate phosphate buffer, pH 6.1, were incubated for 10 min at 37 °C. The reaction was stopped by adding 1.9 mL of an aqueous 0.2 M Na_2CO_3 solution and release of the methylumbelliferyl group was measured (TKO 100 Mini Fluorimeter, Hoefer Scientific Instruments). Estimates of IC_{50} values were obtained by extrapolating direct plots of the percentage of inhibition versus the concentration of compounds **8**. Highest inhibitor concentrations tested with chitinase A were 2.0 mM (**8a**), 4.0 mM (**8b** or **8c**), and 1.5 mM (**8d**, **8e**, or **8f**), and with chitinase B 3.0 mM (**8a**, **8b**, **8c**, **8e**, or **8f**) and 2.0 mM (**8d**). The plots contained at least four points, each derived from at least three independent measurements.

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