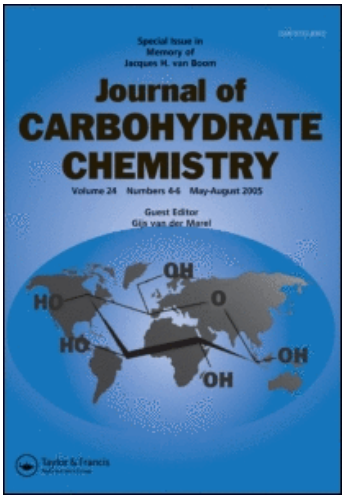

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Synthesis and Properties of Surfactants Derived from *N*-Acetyl-D-Glucosamine

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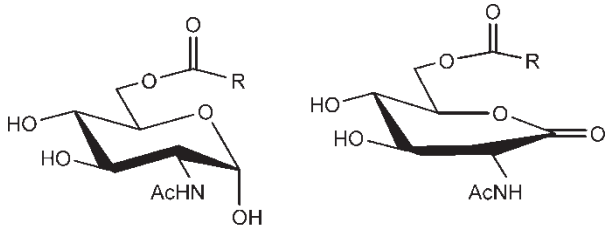
The synthesis of 2-acetamido-2-deoxy-6-*O*-octanoyl-D-glucono-1,5-lactone **9** and 2-acetamido-2-deoxy-6-*O*-octanoyl-α-D-glucopyranose **7** from 2-acetamido-2-deoxy-α-D-glucopyranose is reported. For both targets, the key intermediate was allyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-octanoyl-α-D-glucopyranoside **5**. Surface tension measurements (critical micellar concentration of 22.3 mM and 5 mM for **9** and **7**, respectively) showed up the surface activity of both compounds, while enzyme inhibition assays indicated that **9** could inhibit bovine β-*N*-acetylglucosaminidase ($K_i = 6.5$ μM) but not *Serratia marcescens* chitobiase nor hen egg-white lysozyme. Moreover, **7** was shown to induce chitinase production of *S. marcescens* and to be readily metabolized by these bacteria.



7 **9**

Keywords 2-Acetamido-2-deoxy-6-*O*-octanoyl-D-glucono-1,5-lactone, Sugar monoester*N*-Acetyl-D-glucosamine derivatives, Glycosidase inhibition, β-*N*-Acetylglucosaminidase, Chitobiase, Surface activity, Affinity surfactant, Chitinase induction, *Serratia marcescens*

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INTRODUCTION

Sugar-based surfactants are known for their use in food, cosmetics, drugs, detergents, and in the biochemical field due to some advantages over other amphiphiles, such as their dermic compatibility and biodegradability.[1–5] Depending on the properties provided either by the hydrophobic tail or by the sugar moiety, some are biologically active.[6,7] They may act as specific inhibitors of the different glycosyl hydrolases belonging to families 18 (chitinases) or 20 (β-hexosaminidases, β-*N*-acetylglucosaminidases, and chitobiases),[8] which do receive great attention.[9–15] Indeed, the inhibitory activity of 2-acetamido-2-deoxy-D-gluconolactones and some of their derivatives, on β-*N*-acetylglucosaminidases, has already been described.[16–18] To a larger extent, lactones derived from chito-oligosaccharides are potential inhibitors of family 18, 20, and 22 glycosyl hydrolases due to the sp^2 hybridization of the anomeric center and the coplanarity of the C-1, C-2, O-5, and C-5 atoms, thus mimicking the transition state.[15,18–24] Design of new analogs combining surfactant properties with the biological activity of the lactone may enlarge their field of applications, for example, by changing their distribution and persistence features in cells or organisms. Besides, the synthesis of amphiphilic ligands may allow the development of affinity liquid-liquid separation processes of several glycosyl hydrolases, as in the case of concanavalin-A, for which it has been shown that incorporation of an affinity cosurfactant (octyl β-D-glucopyranoside) in a reversed micellar system led to a great increase in the yield and the specificity of protein separation.[25] For these reasons, the synthesis of a new surfactant, 2-acetamido-2-deoxy-6-*O*-octanoyl-D-glucono-1,5-lactone **(9)**, has been carried out. Amphiphiles derived from gluconolactone,[18,26,27] glucosamine, or *N*-acetyl-D-glucosamine (GlcNAc) units[3,4,7,28] are commonly available in a few synthetic steps. However, the preparation of **9** from 2-acetamido-2-deoxy-α-D-glucose was never described and is proposed here in nine steps. Since the mechanism of β-*N*-acetylglucosaminidase inhibition by 2-acetamido-2-deoxy-D-gluconolactones involves a flattened conformation at C-1, 2-acetamido-2-deoxy-6-*O*-octanoyl-α-D-glucopyranose **(7)** was synthesized and assayed as a negative control. The surfactant properties of **7** and **9** have been characterized and preliminary inhibition tests have been performed. Besides the interest for finding new glycosyl hydrolase inhibitors, another field of application concerns the utilization of chitinases for plant protection.[29] Microbial chitinases are known to be inducible enzymes, secreted by some microorganism species when they are grown on chitin (poly-(1 → 4)-β-*N*-acetyl-D-glucosamine).[30,31] Since chitin is insoluble in water, the actual inducers must be soluble oligomers derived after partial degradation of chitin. However, few results have been published on the mechanisms of induction and on the efficiency of other potential

inducers.[30,32] In this context, compounds **7** and **1** (allyl 2-acetamido-2-deoxy-α-D-glucopyranoside) have been tested for chitinase induction in *Serratia marcescens* cultures and compared with chitin and *N*-acetyl-D-glucosamine.

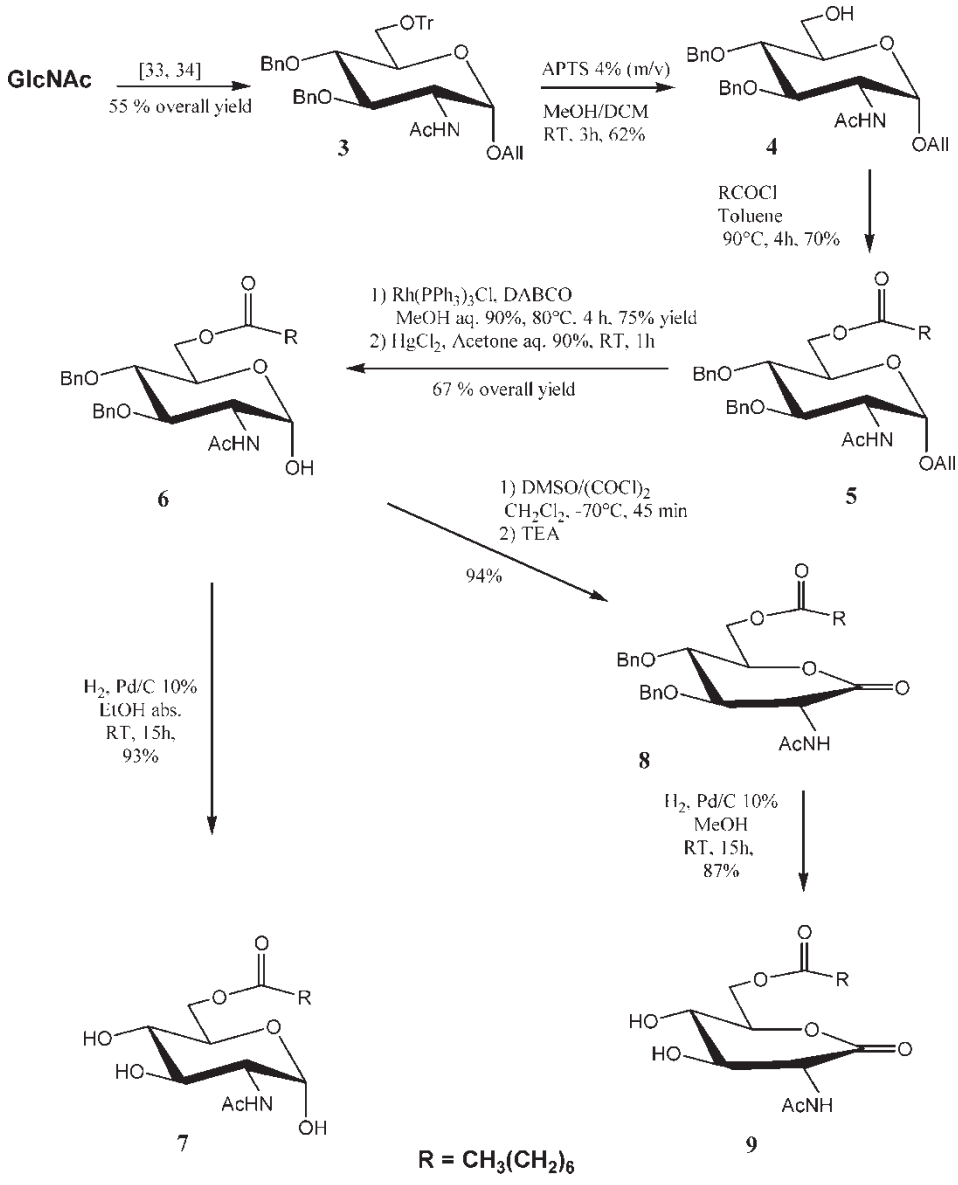
RESULTS AND DISCUSSION

Synthesis

Starting from GlcNAc, the key intermediate **3** was synthesized according to known procedures[33,34] (Sch. 1). Detritylation of **3** using *p*-toluene sulfonic acid in dichloromethane/methanol afforded allyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside **(4)** in 62% yield. The 6-*O* acylation of **4** in hot toluene was easily achieved to give **5** in 70% yield. The anomeric allyl group of **5** was isomerized in mild conditions with tris(triphenylphosphine)rhodium chloride in the presence of diazabicyclo[2.2.2]octane[35] and then hydrolyzed with mercuric chloride to give hemiacetal **6**. Purification by chromatography of the isomerized (1-propenyl) compound and subsequent hydrolysis gave the best yield of **6** (67%) containing a minimum of impurities. Oxidation of **6** by dimethyl sulfoxide-oxalyl chloride was performed at -70°C in dichloromethane[36] to give the glucono-1,5-lactone **8** in 94% yield. Classic hydrogenolysis of the benzyl groups yielded the lactone **9** in 87% from **8**, and the hemiacetal **7** in 93% from **6**. The overall yield of **9**, resp. **7** from GlcNAc was about 13%, resp. 15%. The conservation of the α-configuration from 2-acetamido-2-deoxy-α-D-glucose to **7** was proved by the NMR characteristics of the successive intermediates, for instance, the value of the ^{1}H NMR coupling constant $J_{1,2} \leq 4.0$ Hz.

Surface Tension Properties

As shown in Fig. 1, both compounds **7** and **9** show surfactant properties since the surface tension of water is lowered from 71 mN/m to 39 ± 0.2 mN/m for **7**, and to 29.7 ± 0.2 mN/m for **9**. Their critical micellar concentrations (CMC) can be estimated to 5 ± 0.2 mM for **7** and 22.3 ± 0.3 mM for **9**. These CMC values are in the same order of magnitude as those of 3-*O*-octanoyl-D-glucopyranose (1.6 mM)[37] and octyl-β-D-glucopyranoside (25 mM).[38] The higher CMC value of the lactone **9** as compared with **7** seems to indicate that **9** is more hydrophilic than **7**. It is generally assumed that the dissolution in water of gluconolactone derivatives results in a mixture of 1,5-lactone, 1,4-lactone, and open-chain forms.[16,26] After dissolution of **9** in D_2O and long-time equilibration (pH of the solution between 4 and 5), NMR spectra show one major component corresponding



Scheme 1: Synthesis of the target compounds **9** and **7**.

to the 1,5-lactone form, confirmed by HMBC and NOESY experiments. However, the possible presence of little amounts of other forms not identified by NMR, in particular the more hydrophilic 2-acetamido-2-deoxy-6-*O*-octanoyl gluconic acid, could explain the higher CMC value observed for the solution of **9**.

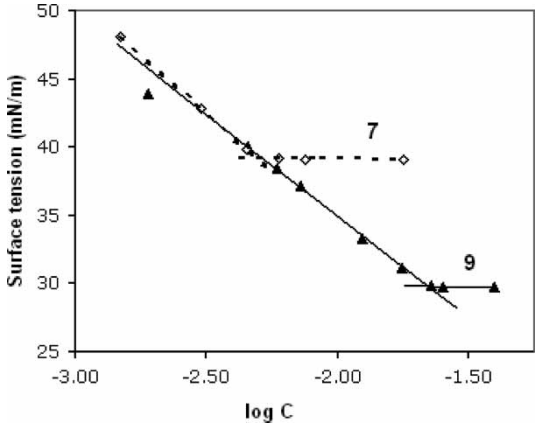


Figure 1: Surface tension of aqueous solutions of compounds **9** and **7** versus log C (mol/L) at 25°C.

Enzyme Inhibition Assays

Initial kinetic analysis of β-*N*-acetylglucosaminidase (NAGase) from bovine kidney was performed using the synthetic substrate 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (pNPGlcNAc), an analog of chitobiose $(GlcNAc)_2$. Lineweaver-Burk plots (Fig. 2) yield K_m value (2.05 ± 0.1 mM) and show that **9** behaves as a competitive inhibitor of NAGase. Its IC_{50} was 16 ± 3 μM for a 2.5 mM pNPGlcNAc concentration. The inhibition constant K_i was found to be about 6.5 ± 1 μM. By comparison, **7,** which does not have the flattened conformation of C-1, is a very moderate inhibitor with an $IC_{50} = 1 \pm 0.1$ mM.

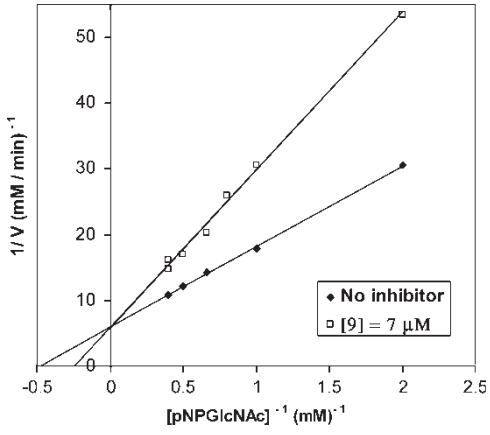


Figure 2: Lineweaver-Burk plots of β-*N*-acetylglucosaminidase activity in the presence (7 μM) or the absence of compound **9**.

The lactone **9** has also been assayed against a batch of partially purified chitinolytic enzymes from a *Serratia marcescens* culture.[39] Since the substrate used was again pNPGlcNAc, the assay concerned the chitobiase activity of the preparation.[40] The IC_{50} value (1.2 mM) indicates weak inhibition. This preliminary result has to be verified by inhibition assays on each purified enzyme separetely. However, it is in agreement with the exo-chito-oligosaccharide *β-N*-acetylglucosaminidase character of the chitobiase,[41] which presents a great decrease of affinity when decreasing chito-oligosaccharide chain length. Tests of **9** against hen egg-white lysozyme showed no inhibition up to 1.8 mM, which is in agreement with the endoglycosidase activity of this enzyme.[22]

Due to the possible interconversion of the gluconolactone into the corresponding free acid and into its other ring form, it is virtually impossible to obtain the inhibition constants of each form separately. However, as mentioned above, at the pH of our assays (pH 4.2) one major form exists (1,5-lactone), which appears to be mostly responsible of inhibition, in agreement with previous results.[16]

In conclusion, though exhibiting inhibition features lower than the nonsurfactant parent 2-acetamido-2-deoxy-D-glucono-1,5-lactone ($K_i = 0.55 \pm 0.1$ μM for bull epididymis NAGase[16]), **9** keeps a strong and selective affinity for NAGase, among retaining *β*-glycosidases likely sharing a common catalysis mechanism.[24] Its surfactant properties make it a potential affinity ligand for separation processes and could help its vectorization.

Induction of Chitinase Production in Bacterial Culture

Several *Serratia marcescens* cultures were grown in baffled flasks using different media comprising a basal medium, in which the only carbon source is yeast extract 0.5 g/L, and different additives as chitin, GlcNAc, and compounds **7** and **1** (allyl 2-acetamido-2-deoxy-*α*-D-glucopyranoside). Maximum cell growth and chitinase production usually occur after 4 to 6 days. After sampling, bacterial cell concentration expressed as colony forming unit (CFU) per mL and chitinase activity were assayed (Table 1). The results show that **7** is a substrate for the bacteria and that **7** induces their chitinase production. The consumption of **7** by *S. marcescens* is evidenced by the increase of cell concentration when it is added to the basal medium and also by the visual observation of its disappearance as growth is going on, since **7** formed insoluble mixtures at initial concentrations. This also proves that **7** would be a readily biodegradable surfactant. Concerning chitinase induction, **7** appears to be less efficient than chitin but more than GlcNAc. Whatever the concentration tested, between 1 and 16 g/L, **7** leads to approximately the same chitinolytic activity (8 ± 2 U/mL), which corresponds to about $18\% \pm 3\%$ of chitin reference assay activity. GlcNAc is known to cause catabolite repression

Table 1: Comparison of cell growth and chitinase production with and without additives.

	Additive conc. (g/L); (mM)	Cell concentration ratio C/C^{a}_{Ref}	CNase activity (U/mL)	CNase activity ratio act./ $act._{Ref}$
Basal medium	0	<0.1	0	0
Chitin (Reference)	10; 45 mM)[b]	1	40–70	1
GlcNAc	2; (9 mM)	0.4	0	0
GlcNAc	10; (45 mM)	—	<2	<0.03
7	1; (3 mM)	0.2	6	0.15
7	4; (11 mM)	0.7–1.5	6–10	0.15–0.22
7	16; (46 mM)	2	11	0.15
1	4; (15 mM)	<0.1	0	0

[a]C_{Ref} = Cell concentration in Reference (chitin 10 g/L) reachs a maximum between 10^{10} and 2 10^{10} CFU/mL, after 4–5 days of growth.
[b]Chitin molarity expressed as GlcNaC equivalent.
At initial time (inoculation) cell concentration ranges around 2 10^{8} CFU/mL.

of chitinase synthesis at high concentration (10 g/L) but induction at low concentration.[30,32] In this way, chitinase production in batch culture would be maximum when a maximal concentration of microbial cells is maintained as long as possible at an inducing level of GlcNAc, estimated to 6 mg/L.[32] The results presented in Table 1 suggest that transmembrane transport mechanisms allow **7** to enter bacterial cell, where it induces chitinase synthesis before—or after—being metabolized, while its low aqueous solubility prevents it from exerting catabolite repression. Thus, the solid reserve formed by the initial amounts of **7** added to the culture seems to play the same role as chitin, although it results finally in lower chitinase production. This difference could come from the presence of more effective inducing oligomers when chitin is used. On the other hand, it can be noted that **1** behaves neither as a substrate nor as an inducer for *Serratia*.

EXPERIMENTAL

General Methods

Physicochemical and biological characteristics were measured on crude materials without recrystallisation. ^{1}H and ^{13}C NMR spectra were recorded either with a Bruker AC 200 or a Bruker Avance 400 spectrometers. Chemical shifts are given in parts per million (ppm) and referenced to the residual solvent signals. Assignments were confirmed by COSY, HMQC, HMBC, or NOESY experiments. Mass spectra were recorded on a quadrupole Ribermag R 1010 spectrometer by DCI/NH_3. High-resolution mass spectra were conducted

at Ecole Normale Supérieure (Paris, France) on a JEOL MS 700 spectrometer. Thin layer chromatography was carried out using aluminium precoated plates of silica gel 60 F_{254} (Merck) with the solvent system chloroform-ethanol (19:1). The spots were detected by UV (254 nm) or by iodine. Column chromatography was conducted on silica gel (Merck) of 0.040–0.063 mm particle size. Concentrations were conducted *in vacuo*. Microanalyses were performed by the laboratory of Microanalysis CNRS (Gif-sur-Yvette, France).

Allyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**4**). Compound **3** (5 g, 7.3 mmol) was dissolved in a methanol-dichloromethane (30:70) mixture (125 mL) containing *p*-toluenesulfonic acid (5 g, 26.3 mmol). The mixture was stirred at rt for 3 h or until TLC showed complete conversion of **3** into **4** (R_F 0.4). The reaction mixture was neutralized with Na_2CO_3, washed with water, and dried (Na_2SO_4). Solvents were evaporated and the crude product was chromatographed (19:1 $CHCl_3$-EtOH) to give **4** (2 g, 62%) as a white amorphous mass. ^{1}H NMR (400 MHz) in $CDCl_3$: δ 1.84 (s, 3H, COCH_3), 3.65–3.83 (m, 5H, H-3, H-4, H-5, H-6), 3.93 (ddt, 1H, $^2J = 13.0$ Hz, $^3J = 6.2$ Hz, $^4J = 1.3$ Hz, OCH_2 (Allyl)), 4.12 (ddt, 1H, $^2J = 13.0$ Hz, $^3J = 5.3$ Hz, $^4J = 1.4$ Hz, OCH_2 (Allyl)), 4.21 (td, 1H, $J_{NH,2} = J_{2,3} = 9.6$ Hz, $J_{1,2} = 3.6$ Hz, H-2), 4.66 (d, 1H, $^2J = 11.7$ Hz, CH_2Ph), 4.68 (d, 1H, $^2J = 10.9$ Hz, CH_2Ph), 4.81 (d, 1H, $J_{1,2} = 3.6$ Hz, H-1), 4.87 (d, 1H, $^2J = 11.7$ Hz, CH_2Ph), 4.88 (d, 1H, $^2J = 10.9$ Hz, CH_2Ph), 5.18–5.27 (m, 2H, CH_2=CH), 5.34 (d, 1H, $J_{NH,2} = 9.6$ Hz, NH), 5.80–5.90 (m, 1H, CH_2=CH), 7.30–7.36 (m, 10H, Ar). ^{13}C NMR (100 MHz) in $CDCl_3$: δ 23.5 (CO*C*H_3), 52.6 (C-2), 61.7 (C-6), 68.2 (O*C*H_2 (Allyl)), 71.6 (C-5), 74.9, 75.2 (*C*H_2Ph), 78.2 (C-4), 80.0 (C-3), 96.9 (C-1), 117.7 (*C*H_2=CH), 127.9, 128.0, 128.2, 128.2, 128.6, 128.6, (CH Ar), 133.6 (CH_2=*C*H), 138.0, 138.4 (C_q Ar), 169.9 (C=O). MS for $C_{25}H_{31}NO_6$ (M, 441): m/z 442 $(M + H)^+$, 459 $(M + NH_4)^+$. Anal. Calcd for $C_{25}H_{31}NO_6$: C, 68.01; H, 7.07; N, 3.17. Found: C, 67.94; H, 7.07; N, 3.07.

Allyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-octanoyl-α-D-glucopyranoside (**5**). A mixture of **4** (9.5 g, 21.5 mmol) and toluene (150 mL) was heated at 90 to 95°C and capryloyl chloride (5 g, 30.7 mmol) was slowly added. Boiling under reflux was then continued for 4 h. The cooled reaction was washed with water and the product was extracted with more toluene (3 × 100 mL). The combined extracts were washed with an aq solution of $NaHCO_3$ (5% w/v) then with water, dried (Na_2SO_4), and concentrated. Column chromatography of the residue (19:1 $CHCl_3$-EtOH) afforded **5** (8.6 g, 70%) as a white solid. ^{1}H NMR (400 MHz) in $(CD_3)_2SO$: δ 0.82 (t, 3H, $^3J = 6.8$ Hz, CH_2CH_3), 1.21–1.25 (m, 8H, $(CH_2)_4CH_3$), 1.51 (quint., 2H, $^3J = 7.1$ Hz, $COCH_2CH_2$), 1.86 (s, 3H, COCH_3), 2.31 (t, 2H, $^3J = 7.2$ Hz, $COCH_2CH_2$), 3.48 (t, 1H, $J_{3,4} = J_{4,5} = 9.4$ Hz, H-4), 3.72–3.78 (m, 2H, H-3, H-5), 3.95–4.02 (m, 2H, H-2, OCH_2 (Allyl)), 4.12 (dd, 1H, $^2J = 13.3$ Hz, $^3J = 5.1$ Hz, OCH_2 (Allyl)), 4.18 (dd, 1H, $J_{6,6'} = 11.7$ Hz, $J_{5,6} = 5.1$ Hz, H-6), 4.26 (dd, 1H, $J_{6,6'} = 11.7$ Hz, $J_{5,6'} = 2.0$ Hz, H-6′), 4.54 (d, 1H, $^2J = 11.0$ Hz,

C*H*$_2$Ph), 4.67 (d, 1H, $J_{1,2} = 3.5$ Hz, H-1), 4.68 (d, 1H, $^2J = 11.0$ Hz, C*H*$_2$Ph), 4.75 (d, 1H, $^2J = 11.0$ Hz, C*H*$_2$Ph), 4.76 (d, 1H, $^2J = 11.0$ Hz, C*H*$_2$Ph), 5.19 (dd, 1H, $^3J_{cis} = 10.5$ Hz, $^2J = 1.5$ Hz, C*H*$_2$=CH cis), 5.35 (dd, 1H, $^3J_{trans} = 17.2$ Hz, $^2J = 1.5$ Hz, C*H*$_2$=CH trans), 5.87–5.97 (m, 1H, CH$_2$=C*H*), 7.24–7.36 (m, 10H, Ar), 8.12 (d, 1H, $J_{NH,2} = 9.2$ Hz, NH). ^{13}C NMR (100 MHz) in (CD$_3$)$_2$SO: δ 14.1 (CH$_2$*C*H$_3$), 22.2, 28.5, 28.6, 31.3 ((*C*H$_2$)$_4$CH$_3$), 22.7 (CO*C*H$_3$), 24.6 (COCH$_2$*C*H$_2$), 33.6 (CO*C*H$_2$), 52.8 (C-2), 62.7 (C-6), 67.6 (O*C*H$_2$ (Allyl)), 69.0 (C-5), 74.2 (*C*H$_2$Ph), 78.2 (C-4), 80.0 (C-3), 96.6 (C-1), 117.3 (*C*H$_2$=CH), 127.6, 127.7, 127.8, 127.9, 128.4, 128.5 (CH Ar), 134.5 (CH$_2$=*C*H), 138.2, 138.8 (C$_q$ Ar), 169.6 (*C*OCH$_3$), 172.9 (COO). Anal. Calcd for $C_{33}H_{45}NO_7$: C, 69.81; H, 7.98; N, 2.46. Found: C, 69.43; H, 7.89; N, 2.34.

2-Acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-octanoyl-α-D-glucopyranose (**6**). A solution of **5** (8 g, 14.1 mmol) and diazabicyclo [2.2.2] octane (1.2 g, 10.7 mmol) in 9:1 methanol-water (400 mL) was stirred at 80°C and treated with (PPh$_3$)$_3$RhCl (3 g, 3.2 mmol). After 4 h of reaction and cooling, the catalyst was filtered off and the filtrate was concentrated to give up a residue that was taken up in chloroform (250 mL). The resulting solution was successively washed with aqueous citric acid (5% w/v) and water, and dried (Na$_2$SO$_4$). Evaporation gave a syrup, which was chromatographed (19:1 CHCl$_3$-EtOH) to give a solid (6 g, 75%), identified as 1-propenyl 2-acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-octanoyl-α-D-glucopyranoside. The latter product (2 g) was dissolved in 9:1 acetone-water (50 mL) and the solution was treated with HgCl$_2$ (1 g, 3.7 mmol) and stirred at rt under Ar for 1 h or until TLC showed complete hydrolysis of the starting material to give **6** (R_F 0.3). After evaporation of most of the acetone, the residue was extracted with 50 mL of chloroform. The organic layer was washed with a saturated solution of KI then twice with water (2 × 100 mL), dried, and concentrated. The crude product was finally purified by column chromatography (19:1 CHCl$_3$-EtOH) to give **6** (1.7 g, yield from **5**: 67%). ^{1}H NMR (400 MHz) in CDCl$_3$: δ 0.87 (t, 3H, $^3J = 6.8$ Hz, CH$_2$C*H*$_3$), 1.25–1.30 (m, 8H, (C*H*$_2$)$_4$CH$_3$), 1.62 (quint, 2H, $^3J = 7.4$ Hz, COCH$_2$C*H*$_2$), 1.84 (s, 3H, COC*H*$_3$), 2.32 (t, 2H, $^3J = 7.4$ Hz, COC*H*$_2$CH$_2$), 3.64 (t, 1H, $J_{3,4} = J_{4,5} = 9.4$ Hz, H-4), 3.80 (dd, 1H, $J_{2,3} = 10.5$ Hz, $J_{3,4} = 9.4$ Hz, H-3), 4.04–4.09 (m, 1H, H-5), 4.12–4.18 (m, 1H, H-2), 4.24 (dd, 1H, $J_{6,6'} = 12.1$ Hz, $J_{5,6} = 3.9$ Hz, H-6), 4.38 (dd, 1H, $J_{6,6'} = 12.1$ Hz, $J_{5,6'} = 2.3$ Hz, H-6′), 4.61 (dd, 1H, $^2J = 11.0$ Hz, C*H*$_2$Ph), 4.67 (dd, 1H, $^2J = 11.0$ Hz, C*H*$_2$Ph), 4.87 (dd, 2H, $^2J = 11.0$ Hz, C*H*$_2$Ph), 5.18 (t, 1H, $J_{1,2} = J_{1,OH} = 3.5$ Hz, H-1), 5.44 (d, 1H, $J_{NH,2} = 9.1$ Hz, NH), 7.26–7.38 (m, 10H, Ar). ^{13}C NMR (50 MHz) in CDCl$_3$: δ 14.0 (CH$_2$*C*H$_3$), 22.5, 28.8, 29.0, 31.6, (*C*H$_2$)$_4$CH$_3$), 23.2 (CO*C*H$_3$), 24.8 (COCH$_2$*C*H$_2$), 34.1 (CO*C*H$_2$), 53.0 (C-2), 62.5 (C-6), 69.0 (C-5), 74.9 (*C*H$_2$Ph), 78.2 (C-4), 79.5 (C-3), 91.7 (C-1), 127.9, 128.0, 128.2, 128.4 (CH Ar), 137.6, 138.1 (C$_q$ Ar), 170.5 (*C*OCH$_3$), 173.7 (COO). Anal. Calcd for $C_{30}H_{41}NO_7$: C, 68.30; H, 7.78; N, 2.65. Found: C, 68.20; H, 7.51; N, 2.80.

2-Acetamido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-octanoyl-D-glucono-1,5-lactone (**8**). A mixture of dichloromethane (5 mL) and oxalyl chloride (0.16 mL, 1.9 mmol) was stirred at −70°C under argon. Dimethyl sulfoxide (0.26 mL, 3.7 mmol) in dichloromethane (4 mL) was added dropwise to the solution and stirring was continued for 10 min. A solution of **6** (200 mg, 0.38 mmol) in dichloromethane (5 mL) was then added and the reaction mixture was stirred for 45 min. Triethylamine (1 mL, 7.2 mmol) was added and stirring was continued at −70°C for an additional 30 min. The mixture was allowed to warm to rt and water (25 mL) was added. The aqueous layer was extracted twice with dichloromethane and the combined organic layers were washed successively with HCl (1M), $NaHCO_3$ (5%, w/v), and water. Drying on Na_2SO_4 and concentration gave **8** as a clear syrup (185 mg, 94%). ^{1}H NMR (200 MHz) in $CDCl_3$: δ 0.88 (t, 3H, $^3J = 6.8$ Hz, CH_2CH_3), 1.29 (bs, 8H, $(CH_2)_4CH_3$), 1.61 (quint, 2H, $COCH_2CH_2$), 1.87 (s, 3H, $COCH_3$), 2.30 (t, 2H, $^3J = 7.6$ Hz, $COCH_2CH_2$), 3.79–4.92 (m, 10H, H-2, H-3, H-4, H-5, H-6, CH_2Ph), 7.15 (d, 1H, $J_{NH,2} = 6.6$ Hz, NH), 7.27–7.40 (m, 10H, Ar).^{13}C NMR (50 MHz) in $CDCl_3$: δ 13.9 (CH_2CH_3), 22.3, 22.5, 24.7, 28.8, 29.0, 31.5, 33.9 ($CH_2)_6CH_3$, $COCH_3$), 55.5 (C-2), 61.7 (C-6), 74.5, 74.7 (CH_2Ph), 75.4 (C-5), 76.4 (C-4), 79.9 (C-3), 128.0–128.5 (CH Ar), 137.0, 137.6 (C_q Ar), 168.5 (C-1), 170.8 ($COCH_3$), 173.1 (COO). MS for $C_{30}H_{39}NO_7$ (M, 525): m/z 526 $(M + H)^+$ and 543 $(M + NH_4)^+$. Anal. Calcd for ($C_{30}H_{39}NO_7$, 0.5 H_2O): C, 67.40; H, 7.54; N, 2.62. Found: C, 67.43; H, 7.43; N, 2.53.

2-Acetamido-2-deoxy-6-*O*-octanoyl-D-glucono-1,5-lactone (**9**). To a solution of **8** (160 mg, 0.30 mmol) in methanol (10 mL) was added 10% palladium-on-carbon catalyst (180 mg) and the suspension was shaken with hydrogen overnight. The catalyst was removed by filtration and the filtrate was passed through a cotton plug. Evaporation of the solvent afforded **9** as a thick gel (92 mg, 87%). ^{1}H NMR (400 MHz) in D_2O: δ 0.74 (t, 3H, $^3J = 7.0$ Hz, CH_2CH_3), 1.18 (bs, 8H, $(CH_2)_4CH_3$), 1.47–1.54 (m, 2H, $COCH_2CH_2$), 1.96 (s, 3H, $COCH_3$), 2.32 (t, 2H, $^3J = 7.4$ Hz, $COCH_2CH_2$), 3.56 (dd, 1H, $J_{4,5} = 8.2$ Hz, $J_{3,4} = 3.1$ Hz, H-4), 3.77–3.82 (m, 1H, H-5), 4.09 (dd, 1H, $J_{6,6'} = 11.7$ Hz, $J_{5,6} = 5.8$ Hz, H-6), 4.13 (dd, 1H, $J_{2,3} = 5.0$ Hz, $J_{3,4} = 3.1$ Hz, H-3), 4.24 (dd, 1H, $J_{6,6'} = 11.7$ Hz, $J_{5,6'} = 2.7$ Hz, H-6′), 4.36 (d, 1H, $J_{2,3} = 4.7$ Hz, H-2). ^{13}C NMR (100 MHz) in D_2O: δ 13.3 (CH_2CH_3), 21.8 ($COCH_3$), 21.9, 28.0, 28.2, 30.9 (($CH_2)_4CH_3$), 24.3 ($COCH_2CH_2$), 33.8 ($COCH_2$), 56.6 (C-2), 65.5 (C-6), 68.6 (C-5), 69.3 (C-3), 71.4 (C-4), 174.1 ($COCH_3$), 175.1 (C-1), 177.4 (COO). Anal. Calcd for ($C_{16}H_{27}NO_7$, 1.5 H_2O): C, 51.60; H, 8.12; N, 3.76. Found: C, 51.77; H, 7.72; N, 4.08. HRMS (FAB, m/z) calcd for ($C_{16}H_{27}O_7$ N + H^+): 346.1866, found: 346.1872.

2-Acetamido-2-deoxy-6-*O*-octanoyl-α-D-glucopyranose (**7**). To a solution of **6** (230 mg, 0.44 mmol) in absolute ethanol (7 mL) was added 10% palladium-on-carbon catalyst (90 mg) and the suspension was shaken with hydrogen overnight. The catalyst was removed by filtration and the filtrate was

passed through a cotton plug. Evaporation of the solvent afforded **7** as a white solid (141 mg, 93%). ^{1}H NMR (400 MHz) in $(CD_3)_2SO$: δ 0.85 (t, 3H, $^3J = 6.8$ Hz, CH_2CH_3), 1.25 (bs, 8H, $(CH_2)_4CH_3$), 1.50–1.53 (m, 2H, $COCH_2CH_2$), 1.82 (s, 3H, $COCH_3$), 2.29 (t, 2H, $^3J = 7.2$ Hz, $COCH_2CH_2$), 3.07–3.13 (m, 1H, H-4), 3.47–3.52 (m, 1H, H-3), 3.56–3.62 (m, 1H, H-2), 3.76–3.81 (m, 1H, H-5), 4.01 (dd, 1H, $J_{6,6'} = 11.7$ Hz, $J_{5,6} = 6.2$ Hz, H-6), 4.28 (dd, 1H, $J_{6,6'} = 11.7$ Hz, $J_{5,6'} = 1.6$ Hz, H-6′), 4.89 (t, 1H, $J_{1,2} = J_{1,OH} = 4.0$ Hz, H-1), 7.66 (d, 1H, $J_{NH,2} = 8.2$ Hz, NH). ^{13}C NMR (50 MHz) in $(CD_3)_2SO$: δ 14.2 (CH_2CH_3), 22.3, 28.6, 28.6, 31.4, (($CH_2)_4CH_3$), 22.9 ($COCH_3$), 24.7 ($COCH_2CH_2$), 33.7 ($COCH_2$), 54.4 (C-2), 64.0 (C-6), 69.5 (C-5), 70.5 (C-3), 71.4 (C-4), 90.9 (C-1), 169.7 ($COCH_3$), 173.0 (COO). Anal. Calcd for ($C_{16}H_{29}NO_7$, 0.2 H_2O) C, 54.75; H, 8.44; N, 3.99. Found: C, 54.73; H, 8.05; N, 3.96. HRMS (FAB, m/z) calcd for ($C_{16}H_{29}O_7\,N + H^+$): 348.2022, found: 348.2033.

Determination of Critical Micellar Concentration

The surface tension (γ) of **9** and **7** solutions were measured at 25°C with a Wilhelmy tensiometer (Tensimat n°3 - Prolabo). The measurements were repeated three times and the respective mean value was considered. The critical micellar concentration was determined from the break point of each surface tension vs. concentration (on log-scale) curve.

Bacterial Culture and Chitinase Induction Experiments

The bacterial strain *Serratia marcescens* BCCM 18541 was grown at 32°C, in baffled Erlenmeyer flasks containing basal medium (g/L): yeast extract 0.5, $(NH_4)_2SO_4$ 1, $MgSO_4 \cdot 7H_2O$ 0.3, KH_2PO_4 1.36. The pH was adjusted to 8.0 with 2 M NaOH before sterilization (121°C—30 min). Each compound to be tested was added to this medium before inoculation. Purified chitin[39] was used as reference. Cell concentration (CFU) was determined by spread plate technique on LB/agar.[39]

Chitinase Activity Assay

The activity of *S. marcescens* chitinases was determined by following the amount of reducing ends released by the cleavage of colloidal chitin (100–170 mesh).[39] The reaction mixture, consisting of trial solution (0.5 mL) and colloidal chitin (20 mg) in 1.5 mL of 100 mM phosphate buffer (pH 6.6), was shaken for 1 h at 50°C. The reaction was stopped by adding 0.1 mL 10% (m/v) trichloroacetic acid to 0.6 mL of the mixture. After centrifugation, 0.5 mL supernatant was heated for 3 min at 100°C with 1.5 mL dinitrosalicylic acid (DNS) reagent (0.69 g DNS, 1.18 g NaOH, 20 g sodium potassium tartrate

per 100 mL water). After dilution, the absorbance was measured at 530 nm. The amount of reducing moieties released, expressed as GlcNAc equivalent, was determined from a calibration curve. One unit (U) of chitinase activity was defined as the amount of enzyme required to produce 1 μmol GlcNAc equivalent in 1 h at 50°C and pH 6.6.

Enzyme Inhibition Experiments

4-Nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (pNPGlcNAc) from Acros (22941-1000) and β-*N*-acetyl-β-glucosaminidase (EC 3.2.1.52) from bovine kidney from Sigma (A-2415) were used. The commercial preparation of the enzyme, a suspension in 3.2 M $(NH_4)_2SO_4$, was centrifuged (13,000 rpm, 5 min) and the pellet was dissolved in water (1 mL). β-*N*-acetyl glucosaminidase solution diluted 10 times (100 μL), citrate buffer 0.5 M, pH 4.2 (100 μL), and inhibitor solution (or water for controls) (300 μL) were incubated at 37°C for 5 min in a 1 mL spectrophotometer cuvette.[42] After addition of pNPGlcNAc (5 mM or 2.5 mM in water, 500 μL), incubation was continued for 8 min at 37°C and the amount of 4-nitrophenol released was followed vs. time by measurement of UV/vis absorption at 400 nm. Absorbance calibration curve (linear) was set from 4-nitrophenol standard solutions maintained in the same buffer and temperature conditions as the enzyme reaction pool. IC_{50} was defined as the concentration of inhibitor needed to cause 50% decrease of activity. The K_i value was obtained from comparison of K'_M and K_M affinity constants calculated in the presence or absence of a known concentration of inhibitor and by plotting 1/v vs. I for two concentrations of substrate (2.5 and 1.25 mM), where v is the rate of 4-nitrophenol release and I the concentration of inhibitor (Dixon graphical method). One unit of enzyme activity will hydrolyze 1 μmol of pNPGlcNAc per minute.

Chitinolytic enzymes were obtained from *S. marcescens* cultures and were partially purified on a FPLC anion-exchange column Mono P 5/20 (Pharmacia). Stock enzyme preparation contained 35 U/mL or 50 U/mg of albumine equivalent. The substrate used was pNPGlcNAc at 2.5 mM in order to point up chitobiase activity. Enzyme solution was diluted 4 times and the inhibition study was performed as for β-*N*-acetylglucosaminidase. A solution of hen egg-white lysozyme from Sigma (L-6876) at 0.5 g/L and a suspension (170 g/L) of lyophilized cells of *Micrococcus lysodeikticus* (Sigma, M-3770) were prepared in a phosphate buffer (100 mM, pH 6.5). Lysozyme solution (100 μL) and inhibitor solution (400 μL) were incubated at rt for 5 and 10 min. Forty microliters of the mixture were added to 3 mL of the substrate supension in a spectrophotometer cuvette. Lytic activity was determined by monitoring at 450 nm the decrease in absorbance, which occured during the lysis of the suspension.

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