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Influence of the Variation of the Alkyl Chain Length of *N*-Alkyl-β-D-glycosylamine Derivatives on Antifungal Properties

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ABSTRACT: Twelve new glucosidic and galactosidic derivatives of *N*-alkylaminosugars with different alkylamines from 6 to 18 carbons were synthesized and characterized by ^{1}H and ^{13}C NMR. Their antifungal activity against the food fungal pathogen *Aspergillus niger* was evaluated using the radial growth assay. The influence of the variation of the alkyl chain length of *N*-alkylaminosugars on the mycelium growth was then discussed. Inhibition by the different alkylamines is shown as a biostatic effect rather than a biocidal effect. It was observed that alkylamines keep their antifungal properties after a thermal treatment compatible with food packaging and processing.

KEYWORDS: *aminosugars, N-alkylglycosylamine, antifungal activity, Aspergillus niger*

INTRODUCTION

Despite great efforts for food preservation and safety, bacterial and fungal contaminations are still a current issue for the food industry. Thus, there is a growing interest in developing new bioactive agents. One way to achieve this is to elaborate new effective and environmentally friendly biocides, designed to limit the development of these pathogenic agents, using renewable materials in the synthetic process. Slight modifications of carbohydrates made by introducing some targeting groups can help to obtain these desirable antimicrobial agents.

Recent studies have shown that amino-containing carbohydrate compounds exhibit antifungal activities and have indicated promising results.[1,2] In addition, aminopolysaccharides such as chitosan and its glucosamine constitutive monomer are well-known to present antifungal and antibacterial activities.[3−6] Carbohydrates and their derivatives are important synthesis targets because of their wide range of functions in living organisms.[7] Derivatives bearing an amino substituent in different ring positions on the sugar skeleton are called aminosugars[8] and are known constituents of several bioactive compounds such as antibiotics[9,10] and biopolymers.[11] On the cell surface, aminosugars play a key role as receptors for proteins and enzymes,[12] and they have been shown to interact with either RNA or the backbone phosphate of DNA.[13,14] These properties explain that some specific series of aminosugars have been considered as major target molecules for many years, owing to their biological and medicinal importance.[15]

Interest in the study of glycosylamines has grown over the years due to their widespread use. According to Stan et al.,[16] *N*-alkylglycosylamines can be used as gelators for various organic liquids. Furthermore, Fabbro and Grabowski,[17] Greenberg et al.,[18] and Legler et al.[19,20] reported that these compounds constitute effective inhibitors of glycosidase activities. Regarding their biological activity, these compounds constitute starting materials for the synthesis of different bioactive amino compounds.[21,22] Aminosugars are also constituents of many antimicrobial active compounds.[23−25] The synthesis and the evaluation of the biological activities of different *N*-alkylglycosylamines are of interest because the association of the aminoalkyl chain with a carbohydrate not only facilitates the interaction with microorganisms but also increases the compound's solubility in water and greatly enhances biological activity.[1,26−28]

Starting from studies on aminopolysaccharides and amino-antibiotics, and their good results on fungi and bacteria, we propose in this paper to investigate the impact of amino features on the growth of a fungal strain, *Aspergillus niger*, which is widely found among microorganisms contaminating food products. In addition, the influence of the nature of the carbohydrate part of aminosugars on their antifungal properties was also studied because previous work conducted with another filamentous fungus—*Aspergillus fumigatus*—has shown membrane lectins bearing the galactose moiety.[29] It is recognized that these lectins play a fundamental role in the recognition and binding of infectious agents to carbohydrate receptors on target cells.[30] For this reason, a series of glucoside- or galactoside-based amino compounds were synthesized and then tested for their antifungal activity.

MATERIALS AND METHODS

Chemicals. D-Glucose (≥99%), D-galactose (≥99%), hexylamine (99%), octylamine (99%), decylamine (99%), dodecylamine (≥99%), hexadecylamine (98%), and octadecylamine (≥99%) were provided by Sigma-Aldrich (France). Synthesis-grade solvents such as absolute ethanol, methanol, diethyl ether, hexane, and dimethyl sulfoxide (DMSO) (J.T. Baker) were provided by Atlantic Labo-ICS (France) and used without further purification.

Characterization Methods. Syntheses were monitored by thin-layer chromatography on silica gel 60 F_{254} precoated aluminum sheets (Merck) with detection by H_2SO_4 spray followed by heating. *N*-Alkylglycosylamines were characterized by ^{1}H and ^{13}C NMR

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spectroscopy. ^{1}H and ^{13}C NMR spectra were recorded respectively at 300 and 75 MHz on a Brüker Avance 300 spectrometer or at 400 and 100 MHz on a Brüker Avance 400 spectrometer. Chemical shifts are given in parts per million, and the assignments of ^{1}H and ^{13}C were made by correlation spectroscopy (COSY) and heteronuclear multiple quantum correlation (HMQC) NMR spectrometry.

Chemical Synthesis. According to previous works,[31] synthesis of *N*-dodecyl-β-D-glucopyranosylamine (**4**) was carried out using D-glucose and dodecylamine (1.2 equiv) in EtOH at 50 °C for 6 h. After precipitation in diethyl ether and filtration, the amino compound was obtained as a white powder in 72% yield. In the same way, the galactosidic homologue C_{12}Gal (**10**) was obtained in 77% yield. Hexyl-, octyl-, decyl-, hexadecyl-, and octadecylglucosylamines (C_6Glu (**1**), C_8Glu (**2**), C_{10}Glu (**3**), C_{16}Glu (**5**), C_{18}Glu (**6**)) were synthetized according to a previous method developed in the laboratory,[4] which was inspired by Lockhoff et al.[32] using 2.5 equiv of alkylamine in MeOH at 50 °C overnight. The raw products were then recrystallized in EtOH, filtered, and washed with hexane. Starting from this methodology, the corresponding galactosylamines C_6Gal (**7**), C_8Gal (**8**), C_{10}Gal (**9**), C_{16}Gal (**11**), and C_{18}Gal (**12**) were successfully obtained. All the results are summarized in Table 1.

Table 1. Yields (%) of *N*-Alkylglycosylamines with Different Alkylamines Synthesized by Method A or B

	N-alkylglucosamines		*N*-alkylgalactosamines	
alkylamine	R_1 = OH, R_2 = H		R_1 = H, R_2 = OH	
hexyl	C_6Glu (**1**)	86 (method B)	C_6Gal (**7**)	76 (method B)
octyl	C_8Glu (**2**)	60 (method B)	C_8Gal (**8**)	48 (method B)
decyl	C_{10}Glu (**3**)	83 (method B)	C_{10}Gal (**9**)	83 (method B)
dodecyl	C_{12}Glu (**4**)	86 (method A)	C_{12}Gal (**10**)	77 (method A)
hexadecyl	C_{16}Glu (**5**)	72 (method B)	C_{16}Gal (**11**)	76 (method B)
octadecyl	C_{18}Glu (**6**)	96 (method B)	C_{18}Gal (**12**)	92 (method B)

Each structure was confirmed by both ^{1}H and ^{13}C NMR spectroscopy. The synthesis and characterization of some alkylglycosylamines have previously been reported: for **1** see Pigman et al.,[33] for **2**, **3**, **4**, and **6** see Retailleau et al.,[31] and for **4** and **12** see Lockhoff et al.[32]

For compounds **5** and **10**, the following NMR results were obtained: (**5**) ^{1}H NMR (300 MHz, DMSO d_6) δ 4.81 (d, 1H, OH-4), 4.78 (d, 1H, OH-3), 4.42 (d, 1H, OH-2), 4.32 (t, 1H, OH-6), 3.63 (m, 2H, H′*glu*-6b and H′*glu*-1), 3.42 (m, 1H, H′*glu*-6a), 3.11 (m, 1H, H′*glu*-3), 3.01 (m, 2H, H′*glu*-4 and H′*glu*-5), 2.86 (m, 1H, H′*glu*-2), 2.78 (br dd, 1H, H-1a), 2.48 (m, 1H, H-1b), 2.13 (s, 1H, NH), 1.37 (quint, 2H, *J* = 6.5 Hz, H-2), 1.24 (m, 26H, H-3 to H-15), 0.85 (t, 3H, *J* = 6.8 Hz, H-16); (**10**) ^{1}H NMR (400 MHz, DMSO d_6) δ 4.60 (d, 1H, *J* = 5.4 Hz, OH-4), 4.46 (t, 1H, *J* = 5.6 Hz, OH-6), 4.29 (d, 1H, *J* = 3.9 Hz, OH-2), 4.19 (d, 1H, *J* = 4.6 Hz, OH-3), 3.63 (t, 1H, *J* = 3.8 Hz, H′*gal*-3), 3.58 (br t, 1H, *J* = 5.6 Hz, H′*gal*-1), 3.49 (dt, 1H, *J* = 6.3;10.7 Hz, H′*gal*-6a), 3.41 (dd, 1H, *J* = 5.4;10.7 Hz, H′*gal*-6b), 3.24 (m, 2H, H′*gal*-4 and H′*gal*-5), 3.17 (dd, 1H, *J* = 3.9;8.4 Hz, H′*gal*-2), 2.74 (br dd, 1H, *J* = 7.2;17.2 Hz, H-1a), 2.46 (m, 1H, H-1b), 2.11 (s, 1H, NH), 1.37 (quint, 2H, *J* = 6.5 Hz, H-2), 1.24 (m, 18H, H-3 to H-11), 0.85 (t, 3H, *J* = 6.8 Hz, H-12); ^{13}C NMR (100 MHz, DMSO d_6) δ 91.4 (C′*gal*-1β), 75.8–74.1 (C′*gal*-4 and C′*gal*-5), 70.8 (C′*gal*-2), 68.4 (C′*gal*-3), 60.5 (C′*gal*-6), 45.6 (C-1), 31.3–30.1–29.11–20.09–29.05–28.7–26.9 (C-3 to C-11), 22.12 (C-2), 14.0 (C-12).

Microbial Strains and Preparation of Spore Suspensions. The isolate of *A. niger* (ENSCBP collection, Université de Bordeaux 1, Pessac, France) was cultured for 5 days in the dark at 25 ± 1 °C and 75 ± 5% relative humidity on potato dextrose agar (PDA; DIFCO 213400) after being retrieved from the storage Petri dish. Spore suspensions were then prepared by adding sterile distilled water to the slants followed by gentle shaking. The water volume was selected to adjust the spore suspension to 10^4 spores·mL^{-1} using a Thoma cell.

Antifungal Activity Assessment. The antifungal assessment of *N*-alkyl-β-D-glycosylamines was conducted using the agar dilution method.[34] PDA medium was autoclaved at 121 °C for 15 min. Then the required concentrations of *N*-alkylglycosylamines solubilized in DMSO were incorporated into the agar medium. After homogenization, this mixture was then poured into Petri dishes. The compounds were tested at concentrations varying from 1.5×10^{-5} to 2.5×10^{-5} mol·mL^{-1}. After solidification, plates were inoculated by one drop of spore suspension deposited in the middle of the medium agar and incubated in the dark at 25 °C and 75% relative humidity. A growth control (without any growth inhibitor) and a DMSO solvent control (without the synthesized bioactive compound) were conducted in parallel. Radial growth measurements were recorded daily from the edge of the initial inoculum until the extreme area of fungal mycelium development.

The effectiveness of aminosugars was determined as the percentage of inhibition calculated after 12 days of incubation, expressed as an average diameter and calculated using the following equation:

$$\text{percentage of inhibition} = \frac{\text{control diameter} - \text{test diameter}}{\text{control diameter}} \times 100$$

In addition, the daily fungal growth rate, called the apparent growth rate (μ'_{max}) was calculated in the acceleration phase and expressed as centimeters per day. Each of the test and control experiments was made in triplicate, and each bioactive agent was tested three times.

Analysis of Results. Three independent replicates (n = 3) were obtained from each treatment. The results are presented in Figures 4 and 5 and reported as means ± standard deviations (SDs) in Tables 2–4. The results were statistically evaluated by means of one-way analysis of variance (ANOVA). The significant difference between the activities of tested *N*-alkylglycosylamines was obtained when the probability (p) was below the significance threshold of 0.05.

RESULTS AND DISCUSSION

Synthesis. In this paper, the impact of different aminoalkyl chain lengths on the antifungal activity of *N*-alkylglycosylamines against *A. niger* was studied. Convenient methods for the systematic syntheses of *N*-alkylglycosylamines, with primary alkylamines from 6 to 18 carbons, directly synthesized starting from free reducing sugars, were used. We selected two different carbohydrates as the hydrophilic head, D-glucose or D-galactose, to study the influence of the headgroup on the antifungal activity (Figure 1).

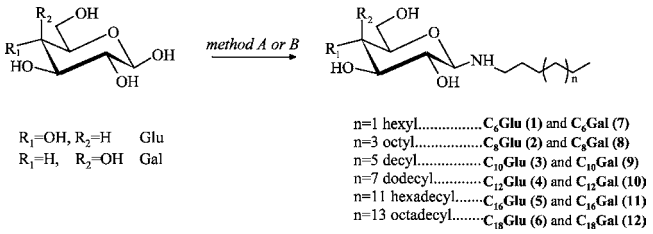

Figure 1. *N*-Alkylation of β-D-glucose or β-D-galactose with different alkylamines. Reagents and conditions: method A with 1.2 equiv of alkylamine, EtOH, 6 h, 50 °C; method B with 2.5 equiv of alkylamine, MeOH, overnight, 50 °C.

The different compounds were successfully synthesized and characterized, as shown above using NMR spectroscopy. These compounds have been designated by different numbers, which are listed in Figure 1.

The ^{1}H spectra show the presence of the H-1 proton observed at lower shifts compared to those of starting glucose and galactose (H-1α and H-1β of glucose are found respectively at 6.20 and 6.57 ppm in DMSO-d_6). The signals attributed to H-1 anomeric protons of glycosylamines were observed around

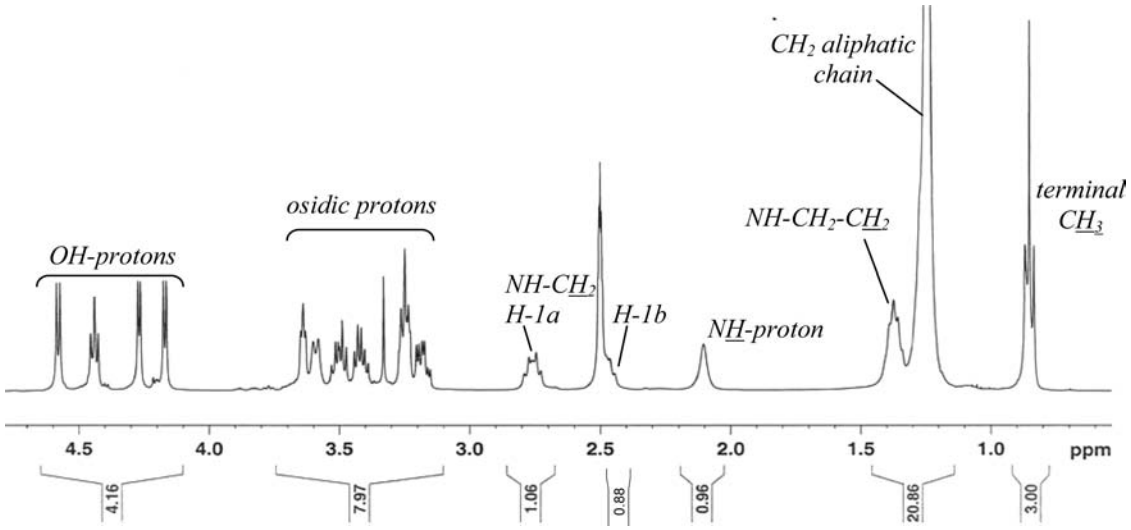

Figure 2. ^{1}H NMR spectrum of C_{12}Gal **10** obtained in DMSO-d_6 (400 MHz).

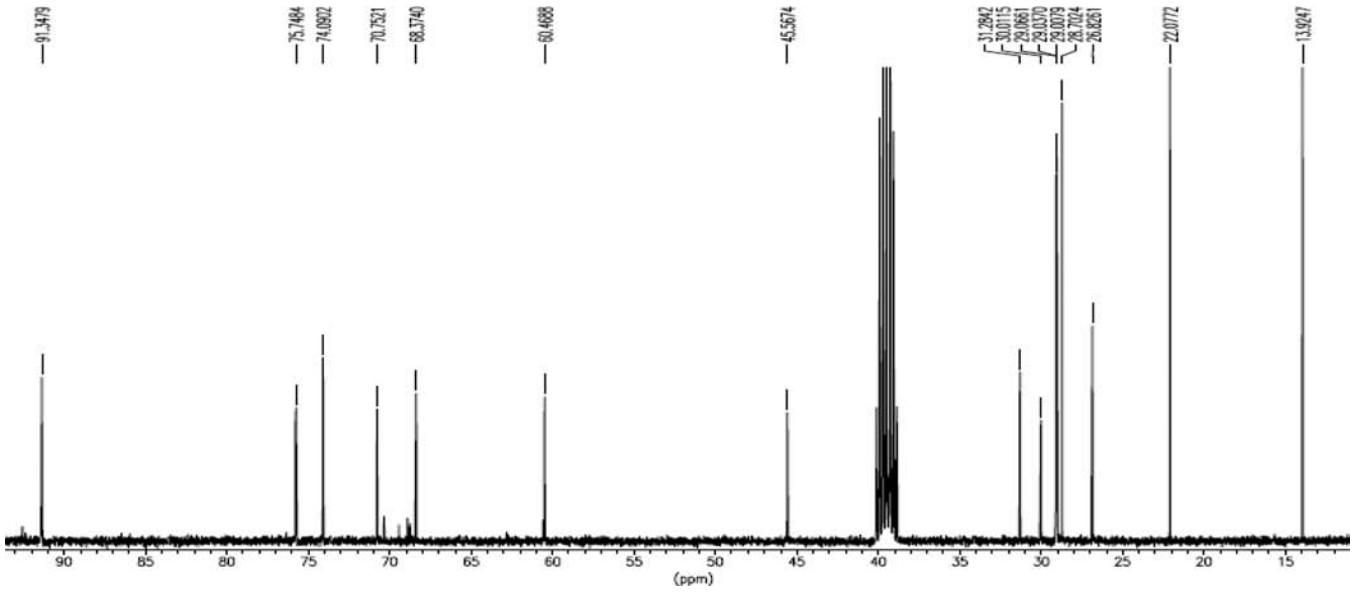

Figure 3. ^{13}C NMR spectrum of C_{12}Gal **10** obtained in DMSO-d_6 (100 MHz).

3.58 ppm, which confirms the substitution of the anomeric OH group by the amino group (an electron-donor group which has a shielding effect). The high values of the coupling constant $^{3}J_{1,2}$ (around 8 Hz) indicate that we have synthesized the β anomer of glycosylamines. Other glycosidic protons of the carbohydrate part appear from 3.63 to 3.17 ppm. The signal at 0.85 ppm integrating for 3H (triplet), attributed to the terminal CH_3 of the alkyl chain, and the signals at 2.74 and 2.46 ppm corresponding to CH_2NH also confirmed the *N*-alkylation (see, for example, the C_{12}Gal spectrum in Figure 2, obtained in DMSO-d_6).

The lengthening of the alkyl chain by one methylene unit (from a hexyl to an octadecyl compound) has no influence on the chemical shifts of the protons of the sugar part. According to the electron-donor effect (+I), the distance between alkyl protons and osidic protons is too long to generate any interaction between them. Thus, in comparison with the C_{12}Gal NMR spectrum, we can only observe a variation of integration of the multiplet at 1.24 ppm for compounds **7**, **8**, **9**, and **11**, corresponding to the internal alkyl protons. (**7**) δ 1.24 (m, 6H, H-3 to H-5); (**8**) δ 1.24 (m, 10H, H-3 to H-7); (**9**) δ 1.24 (m, 14H, H-3 to H-9); (**11**) δ 1.24 (m, 26H, H-3 to H-15).

The ^{13}C NMR characterization shows a peak at about 91 ppm corresponding to the characteristic anomeric proton of the sugar unit (Figure 3). Other carbons of the sugar skeleton appear from 76 to 60 ppm. Single peaks beyond 50 ppm are attributed to the aliphatic carbon chain. We note that the farther the carbons are from the carbohydrate part and the amine function, the weaker their chemical shifts (the CH_2NH peak is observed at 45.6 ppm, whereas the terminal CH_3 has a shielded shift at 14.0 ppm).

Standard Antifungal Activity. Radial Growth. The antifungal activity of the synthesized *N*-alkylglycosylamines bearing glucose and galactose moieties was systematically studied on a food pathogen fungal strain, *A. niger*. Three concentrations, such as 1.5, 2, and 2.5 × 10^{-5} mol·mL^{-1}, were tested for the glucosidic and galactosidic series, and for each one the percentage of fungal inhibition was determined (Tables 2 and 3). Data (n = 3) were analyzed by the one-way ANOVA method, and the differences between activities of various *N*-

Table 2. Percentage of Inhibition of the Radial Growth of *A. niger* after 12 Days of Incubation with Different Concentrations of *N*-Alkylglucosylamines[a]

	1.5×10^{-5} mol·mL^{-1}	2×10^{-5} mol·mL^{-1}	2.5×10^{-5} mol·mL^{-1}
C_6Glu (**1**)		2 ± 3 a	4 ± 5 a
C_8Glu (**2**)	0 ± 5 a	0 ± 1 a	2 ± 5 a
C_{10}Glu (**3**)	15 ± 7 b	24 ± 5 b	50 ± 6 b
C_{12}Glu (**4**)	34 ± 4 c	73 ± 13 c	100 ± 0 c
C_{16}Glu (**5**)	100 ± 0 d	100 ± 0 d	100 ± 0 c
C_{18}Glu (**6**)	42 ± 2 e	42 ± 2 e	49 ± 2 b
ANOVA *p* value	<0.0001	<0.0001	<0.0001

[a]Values are the means of three repetitions followed by the standard deviation. Means followed by different on-line Roman letters within the same column are significantly different ($p < 0.05$).

Table 3. Percentage of Inhibition of the Radial Growth of *A. niger* after 12 Days of Incubation with Different Concentrations of *N*-Alkylgalactosylamines[a]

	1.5×10^{-5} mol·mL^{-1}	2×10^{-5} mol·mL^{-1}	2.5×10^{-5} mol·mL^{-1}
C_6Gal (**7**)		0 ± 5 a	6 ± 3 a
C_8Gal (**8**)	0 ± 4 a	4 ± 3 a	0 ± 0 a
C_{10}Gal (**9**)	14 ± 3 b	21 ± 3 b	90 ± 17 b
C_{12}Gal (**10**)	84 ± 28 c	100 ± 0 c	100 ± 0 b
C_{16}Gal (**11**)	63 ± 7 d	100 ± 0 c	100 ± 0 b
C_{18}Gal (**12**)	21 ± 1 e	27 ± 1 d	28 ± 1 c
ANOVA *p* value	<0.0001	<0.0001	<0.0001

[a]Values are the means of three repetitions followed by the standard deviation. Means followed by different on-line Roman letters within the same column are significantly different ($p < 0.05$).

alkylglycosylamines were significant because of the *p* value, which was about 0.0001 (<0.05).

Results are detailed in Figure 4 for *N*-alkylglucosylamines and in Figure 5 for galactose-based derivatives. The antifungal activity of the glucosylamines increased with the alkyl chain length, from 6 to 16 carbons, which is in accordance with the literature.[3,4] At the tested concentrations ($(1.5–2.5) \times 10^{-5}$ mol·mL^{-1}), there was no significant difference between the antifungal activities of hexyl- and octylglucosylamines and the DMSO control. For the hexadecyl compound **5**, at each concentration, a complete inhibition of the mycelium growth of *A. niger* was observed, while dodecyl and octadecyl compounds **4** and **6** were less active. At 1.5×10^{-5} mol·mL^{-1}, **4** and **6** only showed a weak activity, close to 40% inhibition. With an increase in concentration in the agar medium, **4** and **6** showed a different impact on the fungal growth. About 81% inhibition was observed at 2×10^{-5} mol·mL^{-1}, and complete inhibition of mycelium growth was obtained at 2.5×10^{-5} mol·mL^{-1} for **4**. **6** did not follow the same pattern. Inhibition appeared to be constant (from 42% to 49%) at the different tested concentrations, which is less than expected. Thus, we observed an increase of bioactivity with the alkyl chain length, except with **6**. The result with the highest carbon chain could be due to the hydrophobic character of the octadecylamine chain, involving difficulties of compound solubilization in aqueous media and a lack of homogeneity in agar Petri dishes. For the galactosylamine series, the different synthesized compounds

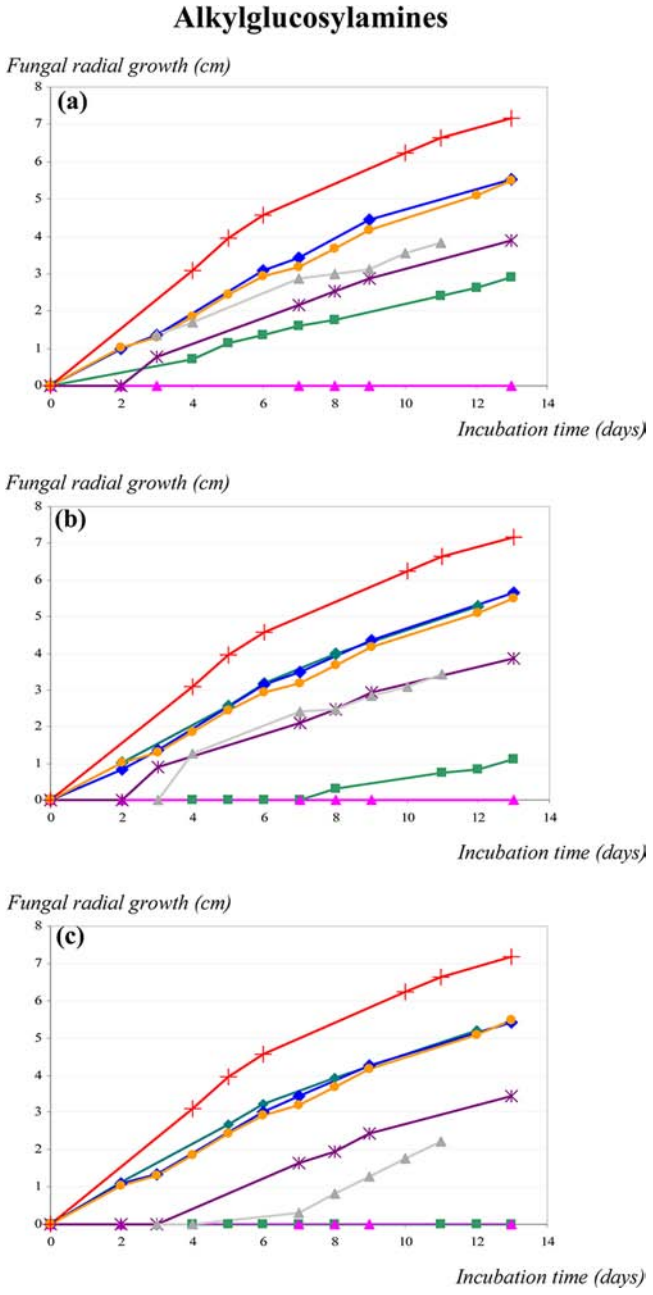

Figure 4. Influence of different *N*-alkylglucosylamines on the growth of *A. niger* at concentrations of (a) 1.5×10^{-5} mol·mL^{-1}, (b) 2×10^{-5} mol·mL^{-1}, and (c) 2.5×10^{-5} mol·mL^{-1}. Radial growth versus length (days) of incubation at 25 °C and 75% relative humidity: red plus signs, growth control; orange circles, DMSO control; green tilted squares, **1**; blue tilted squares, **2**; gray triangles, **3**; green squares, **4**; pink triangles, **5**; purple asterisks, **6**.

seemed to exhibit nearly the same behavior. Hexyl- and octylgalactosamines **7** and **8** had no influence on fungal growth. **9** exhibited 90% inhibition only for both higher concentrations. We point out that the dodecylamino compound **10** led to 100% inhibition at a lower concentration than for the glucosidic series, at 2×10^{-5} mol·mL^{-1} instead of 2.5×10^{-5} mol·mL^{-1}. As a result, galactose-bearing amino compounds are more bioactive at lower concentration than the glucosidic compounds, which is in accordance with the recognition properties of cell receptors for galactose moieties,[29,30] thus allowing better targeting. Hexadecylaminosugar **11** also presented complete

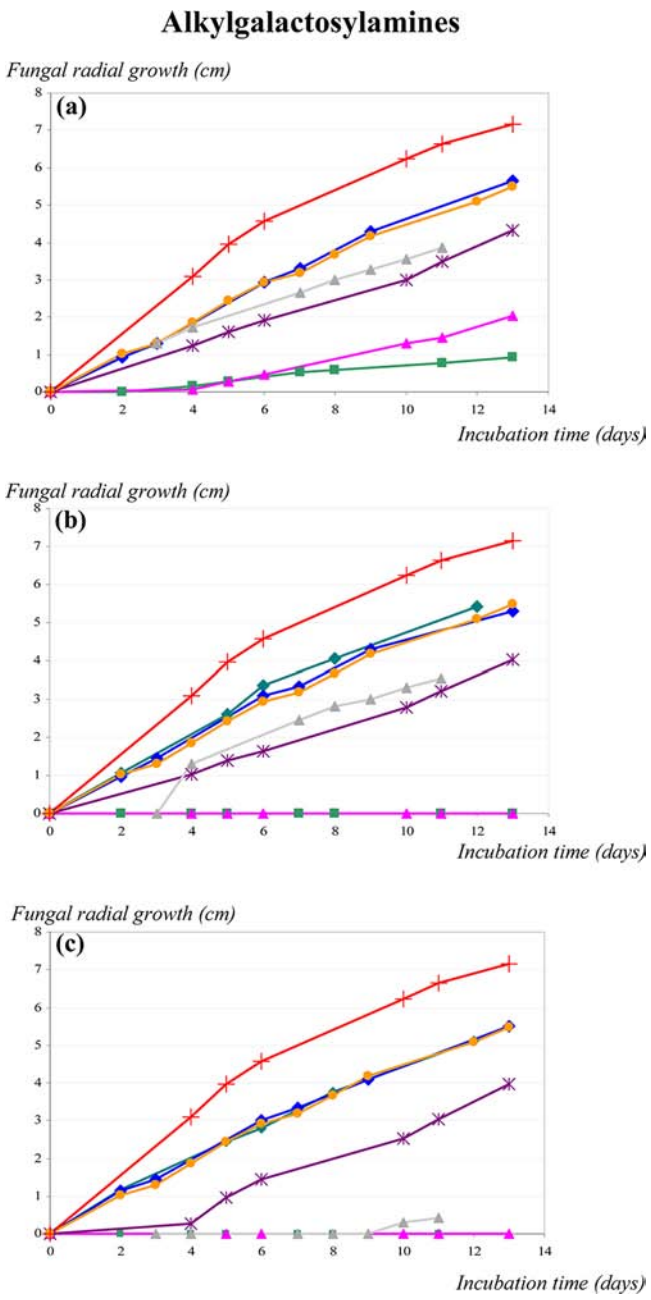

Figure 5. Influence of different *N*-alkylgalactosylamines on the growth of *A. niger* at concentrations of (a) 1.5×10^{-5} mol·mL^{-1}, (b) 2×10^{-5} mol·mL^{-1}, and (c) 2.5×10^{-5} mol·mL^{-1}. Radial growth versus length (days) of incubation at 25 °C and 75% relative humidity; red plus signs, growth control; orange circles, DMSO control; green tilted squares, **7**; blue tilted squares, **8**; gray triangles, **9**; green squares, **10**; pink triangles, **11**; purple asterisks, **12**.

growth inhibition, whereas **12** showed only weak inhibition of 20–30% depending on the concentration. To conclude, the best antifungal candidates are *N*-dodecylglycosylamines **4** and **10** at 2.5×10^{-5} and 2×10^{-5} mol·mL^{-1}, respectively, and also hexadecylamino compounds **5** and **11** at each tested concentration.

In addition to the final inhibition percentage, two fungal growth parameters have been considered to study the potential bioactivity of the synthesized compounds: the presence and duration of a lag phase—which indicates a delay of growth—and the apparent growth rate, called μ'_{max} (Table 4). Unlike for bacteria and yeasts, the growth rate of filamentous fungi cannot be established by cell counting means such as turbidimetric measurement. However, by measuring mass changes with time under excess nutrient conditions, the specific growth rate (μ) for the culture can be calculated. In our case, the apparent growth rate was calculated in the exponential phase by evaluating radial diameter changes with time. Those parameters were compared to the growth control and the DMSO control, which had no lag phase and exhibited μ'_{max} of 0.91 ± 0.02 and 0.53 ± 0.06, respectively.

First, as expected, the DMSO control had a weaker value of μ'_{max} than the growth control, which indicates that the solvent has a slowing effect on *A. niger* growth but without a significant effect on its visual development and colony morphology. Because all compounds were solubilized in this solvent, we selected the DMSO value as the positive control.

Hexylamino and octylamino compounds were in the same range as the DMSO control with μ'_{max} values of about 0.51–0.58. As a result, there was no influence of the alkylglycosylamines **1**, **2**, **7**, and **8** on the apparent μ'_{max} fungal growth. For **3** and **9**, a lag phase was observed, from 3–4 to 9 days for the highest concentration. This lag phase was associated with a reduction of the fungal growth with a μ'_{max} value around 0.4 for the less concentrated experiments to μ'_{max} = 0.22 with the highest concentration of *N*-decylgalactosylamine. *N*-Octadecylglycosylamines **6** and **12** had nearly the same behavior as the decylamino compounds. The glucoside **6** also afforded a lag phase (about 2 or 3 days), but did not really inhibit fungal growth, while **12** had the same μ'_{max} without any lag phase. Finally, the C_{12} (**4**, **10**) and C_{16} (**5**, **11**) compounds seemed to be the most active compounds because of their drastic effect on fungal growth. The more the concentration increased, the more μ'_{max} was impacted, until full inhibition of mycelium growth ($\mu'_{max} = 0$) at 2.5×10^{-5} mol·mL^{-1} for **4**, at 2×10^{-5} mol·mL^{-1} for **10** and **11**, and since the first concentration, 1.5×10^{-5} mol·mL^{-1}, for compound **5**.

Biocidal Effect. For those molecules that exhibit an antifungal activity on *A. niger* mycelium development, the question is wheter it is a *biostatic effect* or a *biocidal effect*. To answer this question, new experiments were conducted with **4**, **10**, and **5** at concentrations that completely inhibit fungal growth (2.5×10^{-5}, 2×10^{-5}, and 2.5×10^{-5} mol·mL^{-1}, respectively). First, PDA media associated with alkylamino compounds were inoculated (in triplicate) by one drop of spore suspension. After 5 days at 25 °C and 75% relative humidity, a piece of contaminated agar (0.5×0.5 cm^2) was cut out and brought onto a new pure PDA medium. Petri dishes were incubated at 25 °C and 75% relative humidity, and the potential fungal growth recovery was studied (Figure 6). After 3 days of incubation (Figure 6a), we observed a new development of the mycelium for the growth control and the DMSO control, but no growth recovery for the glycosylamine experiments. However, after 10 days (Figure 6b), all the experiments were positive (growth recovery), which indicates there is only a biostatic effect of C_{12}Glu, C_{12}Gal, and C_{16}Glu against *A. niger* development. These observations show that mycelium proliferation is stopped since the strain is in contact with the bioactive molecule. Nevertheless, as soon as there is no more *N*-alkylglycosylamine, growth recovery is observed after a few days. These experiments show the relevance of a homogeneous repartition of biocide on the surface to avoid any mycelium development.

Table 4. Growth Rate μ'_{max} and Lag Phases (in Exposant) Determined for Each *N*-Alkylglycosylamine C_6Glu (1), C_8Glu (2), C_{10}Glu (3), C_{12}Glu (4), C_{16}Glu (5), C_{18}Glu (6), C_6Glu (7), C_8Glu (8), C_{10}Glu (9), C_{12}Glu (10), C_{16}Glu (11), and C_{18}Glu (12)[a]

	N-alkylglucosylamine				*N*-alkylgalactosylamine		
	1.5×10^{-5} mol·mL^{-1}	2×10^{-5} mol·mL^{-1}	2.5×10^{-5} mol·mL^{-1}		1.5×10^{-5} mol·mL^{-1}	2×10^{-5} mol·mL^{-1}	2.5×10^{-5} mol·mL^{-1}
1		0.51 ± 0.02	0.51 ± 0.02	**7**		0.53 ± 0.06	0.58 ± 0.03
2	0.53 ± 0.06	0.53 ± 0.11	0.56 ± 0.05	**8**	0.54 ± 0.04	0.54 ± 0.10	0.52 ± 0.02
3	0.39 ± 0.10	0.38 ± 0.04^{3d}	0.43 ± 0.15^{4d}	**9**	0.43 ± 0.06	0.38 ± 0.02 ^{3d}	0.22 ± 0.37 ^{9d}
4	0.23 ± 0.03	0.14 ± 0.17^{7d}	0 ± 0.00	**10**	0.12 ± 0.21	0 ± 0.00	0 ± 0.00
5	0 ± 0.00	0 ± 0.00	0 ± 0.00	**11**	0.20 ± 0.05^{4d}	0 ± 0.00	0 ± 0.00
6	0.34 ± 0.02^{2d}	0.33 ± 0.03^{2d}	0.41 ± 0.03^{3d}	**12**	0.31 ± 0.03	0.37 ± 0.06	0.31 ± 0.01

[a]Growth control: μ'_{max} = 0.91 ± 0.02 (no lag phase). DMSO control: μ'_{max} = 0.53 ± 0.06 (no lag phase).

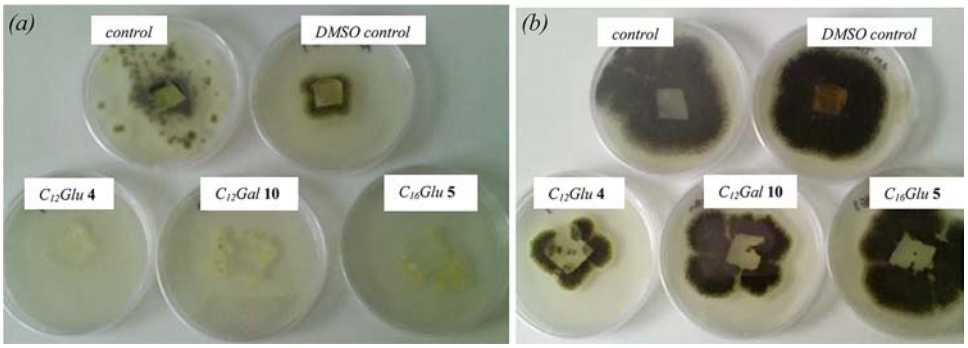

Figure 6. Restart growth assays on *A. niger* mycelium after (a) 3 days and (b) 10 days of incubation at 25 °C and 75% relative humidity.

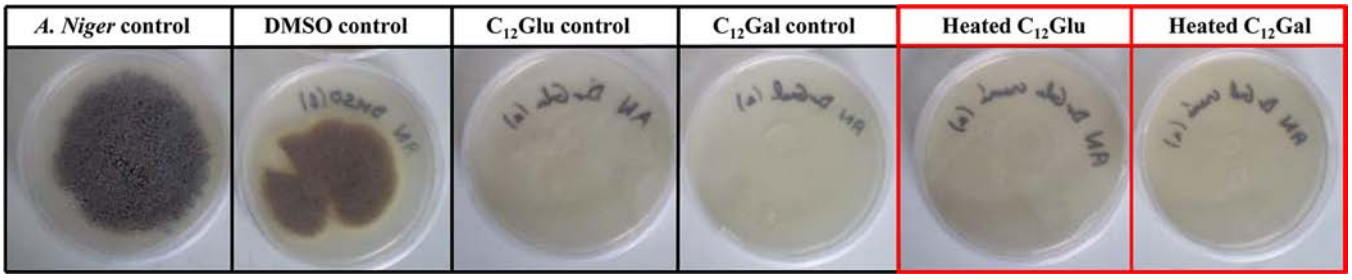

Figure 7. Growth assays on *A. niger* mycelium after 12 days of incubation with the heated aminosugars **4** and **10** at 25 °C and 75% relative humidity.

Temperature Resistance. Their being made of D-glucose and D-galactose, which are sensitive to high temperatures, we then investigated the resistance of the aminosugars **4** and **10** to temperature, to know whether they would remain bioactive after a heat treatment. For this series of experiments, the same method as for the radial growth measurements was used, except the fact that the alkylamino compounds **4** and **10** were added to the PDA medium before sterilization at a concentration of 2.5×10^{-5} mol·mL^{-1}. After homogenization, the aminosugar-containing media were autoclaved at 125 °C for 30 min. The resulting media were then poured into Petri dishes. After solidification, plates were inoculated with one drop of spore suspension deposited in the middle of the medium agar and incubated at 25 °C and 75% relative humidity, and fungal growth was examined (Figure 7). After 12 days of incubation, no difference was observed between the C_{12}Glu and C_{12}Gal controls and their heated homologues. Even though the PDA medium was slightly darker in the case of the heated alkylglycosylamines, the high-temperature treatment showed no detrimental effect on their antifungal properties. This result indicates that, in solution, both antimicrobial agents are not affected by this heat treatment and remain bioactive after a short exposure.

In conclusion, among these two series of potentially bioactive compounds made of the abundant sugars D-glucose and D-galactose and alkylamines, four of them, namely, the ones prepared by using dodecylamine and hexadecylamine as *N*-alkylating agents, fully inhibited mycelium growth of *A. niger*. The influence of the sugar head on the bioactive properties failed to be clearly identified. The galactosidic derivative C_{12}Gal had a lower inhibiting concentration than the glucosidic derivative C_{12}Glu, but both of these C_{12} compounds showed only a biostatic effect. In addition, antifungal properties remained unchanged after a high heat treatment on both dodecylglycosylamines. These aminosugar-based biocides are thus promising candidates as additives for food packaging, their thermal resistance potentially allowing their blending with polymers at high temperature and subsequent extrusion.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Gazaliev, A. M.; Nurkenov, O. A.; Kulakov, I. V.; Ainabaev, A. A.; Bessonov, D. V. Synthesis and fungicidal activity of alkaloid-containing carbohydrates. *Russ. J. Appl. Chem.* **2006**, *79*, 508−510.

(2) Liberek, B.; Melcer, A.; Osuch, A.; Wakiec, R.; Milewski, S.; Wisniewski, A. *N*-Alkyl derivatives of 2-amino-2-deoxy-D-glucose. *Carbohydr. Res.* **2005**, *340*, 1876−1884.

(3) Muhizi, T.; Coma, V.; Grelier, S. Synthesis and evaluation of *N*-alkyl-*β*-D-glucosylamines on the growth of two fungi, *Coriolus versicolor* and *Poria placenta*. *Carbohydr. Res.* **2008**, *343*, 2369−2375.

(4) Muhizi, T.; Grelier, S.; Coma, V. Synthesis of *N*-alkyl-*β*-D-glucosylamines and their antimicrobial activity against *Fusarium proliferatum*, *Salmonella typhimurium* and *Listeria innocua*. *J. Agric. Food Chem.* **2009**, *57*, 11092−11099.

(5) Sebti, I.; Martial-Gros, A.; Carnet-Pantiez, A.; Grelier, S.; Coma, V. Chitosan polymer as bioactive coating and film against *Aspergillus niger* contamination. *J. Food Sci.* **2005**, *70*, M100−M104.

(6) Belalia, R.; Grelier, S.; Benaissa, M.; Coma, V. New bioactive biomaterials based on quaternized chitosan. *J. Agric. Food Chem.* **2008**, *56*, 1582−1588.

(7) Reist, E. J.; Spencer, R. R.; Baker, B. R. Potential anticancer agents. Synthesis of alkylating agents derived from 6-amino-6-deoxy-D-glucose. *J. Am. Chem. Soc.* **1960**, *82*, 2025−2029.

(8) (a) Csuk, R. A convenient synthesis of 6-amino-6-deoxy- and 6-deoxy-D-glucopyranose. *Carbohydr. Res.* **1985**, *140*, 167−168. (b) Ermolenko, L.; Sasaki, N. A.; Potier, P. Asymmetric synthesis of amino sugars. Part 1: Stereoselective synthesis of (2*S*,3*S*,4*R*,5*S*)-2-amino-1,3,4,5,6-hexanepentol derivatives and their conversion to L-mannosamine derivatives. *Tetrahedron Lett.* **1999**, *40*, 5187−5190. (c) Xie, J. Synthesis of new sugar amino acid derivatives of D-glucosamine. *Carbohydr. Res.* **2003**, *338*, 399−406. (d) Ji, X. M.; Mo, J.; Liu, H. M.; Sun, H. P. Synthesis of new amino sugar derivatives from keto-sugars of D-xylose. *Carbohydr. Res.* **2006**, *341*, 2312−2320.

(9) Weringa, W. D.; Williams, D. H.; Feeney, J.; Brown, J. P.; King, R. W. The structure of an amino-sugar from the antibiotic vancomycin. *J. Chem. Soc., Perkin Trans. 1* **1972**, 443−446.

(10) Wang, J.; Li, J.; Tuttle, D.; Takemoto, J. Y.; Chang, C. W. T. The synthesis of L-aminosugar and the studies of L-pyranoses on the ring III of pyranmycins. *Org. Lett.* **2002**, *4*, 3997−4000.

(11) Han, M. J.; Yoo, K. S.; Kim, Y. H.; Chang, J. Y. The catalytic activity of ribose-containing polymers for the hydrolysis of phosphodiester and the cleavage of nucleic acid. *Tetrahedron Lett.* **2002**, *43*, 5597−5600.

(12) Timmons, S. C.; Thorson, J. S. Increasing carbohydrate diversity via amine oxidation: aminosugar, hydroxyaminosugar, nitrososugar, and nitrosugar biosynthesis in bacteria. *Curr. Opin. Chem. Biol.* **2008**, *12*, 297−305.

(13) Semmelhack, M. F.; Jiang, Y.; Ho, D. Synthesis of the amino sugar from C-1027. *Org. Lett.* **2001**, *3*, 2403−2406.

(14) Wu, B.; He, J. Y.; Swayze, E. E. Reexamination of neomycin B degradation: Efficient preparation of its CD and D rings as protected glycosyl donors. *Org. Lett.* **2002**, *4*, 3455−3458.

(15) San Martin, R.; Tavassoli, B.; Walsh, K. E.; Walter, D. S.; Gallagher, T. Radical-mediated synthesis of *α*-*C*-glycosides based on *N*-acylgalactosamine. *Org. Lett.* **2000**, *2*, 4051−4054.

(16) Stan, R.; Ciuculescu, E. D.; Franceschi-Messant, S.; Perez, E.; Rico-Lattes, I. New sugar based gelators for organic liquids. *Rev. Roum. Chim.* **2005**, *50*, 695−698.

(17) Fabbro, D.; Grabowski, G. A. Human acid *β*-glucosidase. Use of inhibitory and activating monoclonal antibodies to investigate the enzyme's catalytic mechanism and saposin A and C binding sites. *J. Biol. Chem.* **1991**, *266*, 15021−15027.

(18) Greenberg, P.; Merrill, A. H.; Liotta, D. C.; Grabowski, G. A. Human acid *β*-glucosidase: Use of sphingosyl and *N*-alkyl-glucosylamine inhibitors to investigate the properties of the active site. *Biochim. Biophys. Acta, Protein Struct. Mol. Enzymol.* **1990**, *1039*, 12−20.

(19) Legler, G.; Finken, M. T. *N1*-alkyl-D-gluconamidines: Are they 'perfect' mimics of the first transition state of glucosidase action? *Carbohydr. Res.* **1996**, *292*, 103−115.

(20) Legler, G. Basic monosaccharide derivatives: tools for exploring the active site of glycohydrolases and for studies in glycoprotein biosynthesis. *Pure Appl. Chem.* **1987**, *59*, 1457−1464.

(21) Kublashvili, R. *N*-glucosides of aminobenzoic acids and aminophenols. *Chem. Nat. Compd.* **2003**, *39*, 586−588.

(22) Campa, C.; Donati, I.; Vetere, A.; Gamini, A.; Paoletti, S. Synthesis of glycosylamines: Identification and quantification of side products. *J. Carbohydr. Chem.* **2001**, *20*, 263−273.

(23) Sibi, M. P.; Lu, J.; Edwards, J. A new route to 3-amino sugars. A concise synthesis of L-daunosamine and D-ristosamine derivatives. *J. Org. Chem.* **1997**, *62*, 5864−5872 and references therein.

(24) Pelyvás, I. V.; Monneret, C.; Herczegh, P. *Synthetic Aspects of Aminodeoxy Sugars of Antibiotics*; Springer: Berlin, 1988; pp 123−162.

(25) Lindberg, B. Components of bacterial polysaccharides. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 279−318.

(26) Morimoto, M.; Saimoto, H.; Shigemasa, Y. Control of functions of chitin and chitosan by chemical modification. *Trends Glycosci. Glycotechnol.* **2002**, *14*, 205−222.

(27) Ben, R. N.; Orellana, A.; Arya, P. Stereoselective synthesis of carbon-linked analogues of *α*- and *β*-galactoserine glycoconjugates using asymmetric enolate methodology. *J. Org. Chem.* **1998**, *63*, 4817−4820.

(28) Esteves, A. P.; Rodrigues, L. M.; Silva, M. E.; Gupta, S.; Oliveira-Campos, A. M. F.; Machalicky, O.; Mendonça, A. J. Synthesis and characterization of novel fluorescent *N*-glycoconjugates. *Tetrahedron* **2005**, *61*, 8625−8632.

(29) Reiss, E.; Hearn, U. M.; Poulain, D.; Shephred, M. G. Structure and function of the fungal cell wall. *J. Med. Vet. Mycol.* **1992**, *30*, 143−156.

(30) Fantini, J.; Yahi, N. Galactosyl ceramide: A new receptor for the human immunodeficiency virus (HIV). *Medecine/Sciences* **1993**, *9*, 891−900.

(31) Retailleau, L.; Laplace, A.; Fensterbank, H.; Larpent, C. Synthesis, structural analysis and properties of *N*-alkylglucosyl(meth)-acrylamides: New reactive sugar surfactants. *J. Org. Chem.* **1998**, *63*, 608−617.

(32) Lockhoff, O.; Stadler, P. Syntheses of glycosylamides as glycolipid analogs. *Carbohydr. Res.* **1998**, *314*, 13−24.

(33) Pigman, W.; Cleveland, E. A.; Couch, D. H.; Cleveland, J. H. Reactions of carbohydrates with nitrogenous substances. I. Mutarotations of some glycosylamines. *J. Am. Chem. Soc.* **1952**, *73*, 1976−1979.

(34) Bristline, R. G.; Maurer, E. W.; Smith, F. D.; Linfield, W. M. Fatty acid amides and anilides, syntheses and antimicrobial properties. *J. Am. Oil Chem. Soc.* **1980**, *57*, 98−103.