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ARTICLE

Synthesis of *N*-Alkyl-β-D-glucosylamines and Their Antimicrobial Activity against *Fusarium proliferatum*, *Salmonella typhimurium*, and *Listeria innocua*

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In this study, different *N*-alkyl-β-D-glucosylamines were evaluated for both antifungal and antibacterial activity against *Fusarium proliferatum* (INRA, MUCL 1807.7), *Listeria innocua* (ISTAB, Université Bordeaux 1), and *Salmonella typhimurium* (Institut Pasteur 5858). The tested glucosylamines were β-D-glucosylamine (GPA), *N*-ethyl-β-D-glucosylamine (EtGPA), *N*-butyl-β-D-glucosylamine (BuGPA), *N*-hexyl-β-D-glucosylamine (HeGPA), *N*-octyl-β-D-glucosylamine (OcGPA), *N*-dodecyl-β-D-glucosylamine (DoGPA), *N*-(2-hydroxyethyl)-β-D-glucosylamine (HEtGPA), *N,N*-di(2-hydroxyethyl)-β-D-glucosylamine (DHEtGPA) and *N,N*-diethyl-β-D-glucosylamine (DEtGPA). The effectiveness of *N*-alkyl length, N-substitution, and *N*-hydroxyalkyl groups on both antibacterial and antifungal activity were evaluated. Results indicated that these compounds exhibited different biological activities and their effectiveness was highly increased from short to long *N*-alkyl chains. DoGPA exhibited more potent biological activity against all target strains than other *N*-alkyl glucosylamines tested. Using a radial growth method, we demonstrated that this compound completely inhibited fungal growth at 0.5×10^{-4} mol mL^{-1}, while OcGPA and HeGPA lead to 71% and 43% fungal inhibition, respectively. Using the coating method, we demonstrated that DoGPA completely inhibited bacterial growth at 0.025×10^{-4} and 0.05×10^{-4} mol mL^{-1} for *L. innocua* and *S. typhimurium*, respectively, while at the same concentrations, OcGPA exhibited weaker antibacterial activity of 12% and 27%, respectively, for *L. innocua* and *S. typhimurium*. The hole plate method enabled us to estimate the minimum inhibitory concentration (MIC) of DoGPA found to be 0.02×10^{-4} and 0.025×10^{-4} mol mL^{-1} for *L. innocua* and *S. typhimurium*, respectively. Glucosylamines with *N*-hydroxyalkyl and short *N*-alkyl chains varying from C-2 to C-4 exhibited weaker antimicrobial activity.

KEYWORDS: Antifungal activity; antibacterial activity; glucosylamines; *Fusarium proliferatum*; *Listeria innocua*; *Salmonella typhimurium*

INTRODUCTION

Human illness due to contaminated food is frequently reported and sometimes found to be the source of fatal diseases. Despite the great effort of industries for food safety, bacterial and fungal contaminations of food are still reported in different regions of the world. This is because food microorganisms are ubiquitous, quickly multiply, and resist considerably at all main conditions used to conserve foods (*1*−*6*). Thus, food contamination during processing, transport, and storage not only constitutes a substantial problem for public health but also is economically undesirable (*5*, *7*−*14*). *Fusarium*, *Listeria*, and *Salmonella* genera are widely found among those microorganisms contaminating food. Some of them are pathogenic strains and cause various disorders such as human cancer, listeriosis, and salmonellosis, diseases with fatality rates (*15*−*23*). For example, fungi such as *Fusarium* species not only are implicated annually in the worldwide harvest loss of 55 million tons of mainly cereal food (*9*) but also produce a variety of toxins such as fumonisins, zearalenone, and trichothecenes in these, thus exhibiting chronic toxicity (*15*−*19*). In addition, the mortality rate due to listeriosis can reach 20−30% of patients, and this disease frequently leaves complicated neurological after effects in about 40% of patients (*1*, *24*, *25*). The pathogen is able to infect a vast range of host tissues and spread many infections of the nervous system such as meningitis and septicemia (*26*). Furthermore, in pregnant patients, this illness can lead to spontaneous abortion, stillbirth, or fetal death (*26*). About *Salmonella* risks, the mortality of 15% of patients worldwide due to infantile diarrhea from salmonellosis was reported (*25*, *27*−*29*).

In this decade, the resistance of these microorganisms toward conventional biocides is increasing (*3*−*5*, *30*−*34*) and constitutes public health problems. For example, depending on their species, studies have shown that about 11−95% of *Listeria* species were resistant to one or more antibiotics (*30*, *31*). This higher resistance may show the dangers that some species of *Listeria* can cause if consumed in foods. Furthermore, resistance of *Fusarium* and *Salmonella* species to biocides was also reported (*3*−*5*, *32*−*34*). Consequently, it is the responsibility of scientists to find new ways

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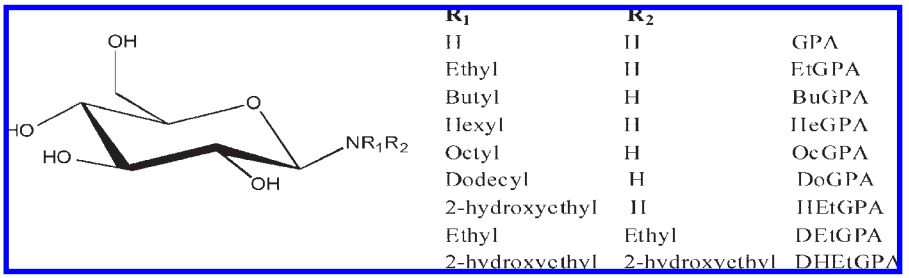

R_1	R_2	
H	H	GPA
Ethyl	H	EtGPA
Butyl	H	BuGPA
Hexyl	H	HeGPA
Octyl	H	OcGPA
Dodecyl	H	DoGPA
2-hydroxyethyl	H	HEtGPA
Ethyl	Ethyl	DEtGPA
2-hydroxyethyl	2-hydroxyethyl	DHEtGPA

Figure 1. Structure of different glucosylamines.

for controlling these microorganisms and the different illnesses they cause. The one way to do this is to discover new effective and friendly environmental biocides notably from renewable resources, which can be used to combat these pathogenic agents. In this way, biological study of amino polysaccharides was done and has shown satisfactory results (*35*−*42*). Because of the suspected close relationship between amino groups and biological activity of these polysaccharides (*39*, *43*) and considering the higher antifungal activity recently reported from glucosylamines (*43*), we evaluated in the present study the bioactivity of various synthesized glucosylamines against food microorganisms in order to determine the possible application they can have in food preservation. The impact of their amino group features in biological activity against *F. proliferatum* and both bacteria, one Gram-negative, *S. typhimurium*, and one Gram-positive, *L. innocua*, was also studied. Because of its known lower risk and the close relationship it has with *L. monocytogenes* toward inhibitors, *L. innocua* was selected as model strain of this pathogenic species to conduct the present study. Results found from this surrogate will be verified against *L. monocytogenes* before any application.

MATERIALS AND METHODS

Synthesis and Analysis Methods. The synthesis and characterization of β-D-glucosylamine and different *N*-alkyl-β-D-glucosylamines (**Figure 1**) were detailed in our previously report (*43*). *N*,*N*-Diethyl-β-D-glucosylamine (DEtGPA) was synthesized using the reported method (*44*). Briefly, D-glucose (3 g, 0.0166 mol) was suspended in 10 mL of methanol, and 4.4 mL of diethylamine was added. The mixture was refluxed for 24 h. Then, solvent was removed under reduced pressure, and 3.18 g (0.013 mol) of viscous residue was obtained. Yield: 81.5%. R_F: 0.15 (2:8, CH_3OH/CH_2Cl_2). ^{1}H NMR: δ_H in ppm (300 MHz, D_2O) 4.11 (1H, d, H_β-1), 3.90−3.86 (1 H, dd, H-6a), 3.71−3.68 (1H, dd, H-6b), 3.58−3.52 (1H, t, H-2, H-4, H-5), 3.49−3.32 (3H, m, H-3, H-4, H-5), 2.89−2.65 (4H, m, $NHCH_2$−), 1.08−1.04 (t, 6H, 2-CH_3).

Decomposition of various glucosylamines was followed by ^{1}H NMR spectroscopy. For this, 40 mg of each compound was dissolved in 1 mL of appropriate solvent chosen between D_2O and CD_3OD. Degradation of glucosylamines was estimated by integration and comparison of H_β-1 signals of their NMR spectra daily obtained during 7 days.

Microbial Strains and Preparation of Inocula. The *F. proliferatum* strain, INRA 212, used throughout the study was from the INRA collection and predominantly produces FB1 fumonisin. The strain was maintained on potato dextrose agar (PDA) (Sigma-Aldrich Co, St Louis, MO) slants at 4 °C. When inocula were required, spore suspensions were prepared by adding sterile distilled water to the slants followed by gentle shaking. This suspension was used to test the antifungal activity of glucosylamines. *L. innocua* (ISTAB, University of Bordeaux 1) and *S. typhimurium* (Institut Pasteur 5858) were used for antibacterial activity assessment. Both bacteria were maintained at −70 °C in 20% glycerol. Overnight precultures were performed as follows: *L. innocua* and *S. typhimurium* were grown in tryptose broth (Difco 262200) and nutrient broth (Difco 234000), respectively at 37 °C for 18 h.

Antifungal Activity Assessment. The antifungal activity of glucosylamines was evaluated by radial growth assays (*43*, *45*) on PDA medium with different concentrations of bioactive agents. The bioactive agents HeGPA, OcGPA, and DoGPA were incorporated into the culture medium using methanol as solvent. Prior to the fungal inoculation, the evaporation of methanol in Petri dishes was obtained after 40 min under a laminar flow. Plates were inoculated in the middle with one drop of strain suspension. In parallel, control experiments with methanol without any glucosylamines were also tested. The dishes were incubated at 75% relative humidity (RH) and 25 °C, and the fungal colony diameter was measured daily. The percentage of inhibition of different glucosylamines was calculated after 9 days of incubation when mycelium in the control experiments completely covered the dishes. It was expressed as an average diameter and calculated using the following equation:

$$\text{Percentageofinhibition} = \frac{\text{CD} - D}{\text{CD}} \times 100$$

where CD is the control diameter without any bioactive compound and *D* is the test diameter.

Each test and control experiment consisted of three replicates, and each bioactive agent was tested three times.

Antibacterial Activity Assessment. The antibacterial assessment of *N*-alkyl-β-D-glucosylamines was conducted using both coating and diffusion methods (*46*−*49*). To do this, 20 mL of culture media prepared by mixing tryptose broth (Difco 262200) or nutrient broth (Difco 234000) with 15% (w/w) agar (Difco 215530) for *L. innocua* and *S. typhimurium*, respectively, was poured to each Petri dish.

Coating method. Compounds were tested at concentrations in the range of $(0.25-2) \times 10^{-4}$ mol mL^{-1}. Two milliliters of the tested compound was poured in each test Petri dish and spread on the surface of the agar medium. Dishes were then left opened for 1 h in a sterilized chamber to enable solvent to evaporate. Then, about 100−300 cells/plate of an 18 h microbial culture were deposited on the medium prior to incubation at 37 °C for 48 h before to colony counting. The initial microbial charge of an 18 h culture was evaluated from preliminary numeration experiments on tryptose and nutrient broth, respectively, for listerial and nonlisterial strains. Moreover, the initial cell number was confirmed by the control assays conducted in parallel without biocide. The control experiments without any compounds to be tested were conducted in parallel and all experiments were in triplicate. The effectiveness of *N*-alkyl-β-D-glucosylamines was determined as percentage of inhibition calculated as follows:

$$\text{Percentageofinhibition} = \frac{\text{CFUincontrolplates} - \text{CFUintestplates}}{\text{CFUincontrolplates}} \times 100$$

where CFU is colony-forming units.

Diffusion Methods

Method 1: Disk Diffusion Method. One-hundred microliters of inoculum from the microbial culture was gently spread on the surface of agar medium. Six-millimeter-diameter cellulosic disks dipped in the test solutions were deposited on the agar medium surface. A range of concentrations from 0.1×10^{-5} to 0.5×10^{-5} mol mL^{-1} was used for each assay. Control disks dipped in the selected solvents were concurrently tested. Thereafter plates were incubated at 37 °C for 24 h prior to determination of the diameters of inhibition zones surrounding the disks. Each test was performed three times, and means of diameters of inhibition zones were calculated.

Method 2: Well Diffusion Method. The well diffusion method was performed based on previous work (*50*). Thirty milliliters of inoculated agar medium with the fresh culture (0.1%, v/v) was poured into Petri

dishes. After solidification, wells of 6-mm diameter were created. Seventy microliters of different concentrations of glucosylamines diluted in selected solvents were deposited in wells. Petri dishes were then incubated at 37 °C for 24 h after 4 h at 4 °C. Control wells were filled with solvents used to dissolve compounds. Inhibition zones around the wells were measured in millimeters and all experiments were conducted in triplicate. The minimum inhibitory concentration (MIC) was the lowest concentration of the test compounds that completely prevented growth.

Analysis of Results. Results from both antifungal and antibacterial assays were statistically analyzed using one-way analysis of variance (ANOVA). The significant difference between activities of compounds tested was obtained when the probability (p) found was higher than the significance threshold of 0.05. Student t test was also used as an additive analysis for comparing every pair of significantly different data to confirm results obtained from ANOVA.

RESULTS AND DISCUSSION

^{1}H NMR Analysis of Glucosylamines. The synthesis process and analysis of some glucosylamines used in this study was previously reported (*43*). DEtGPA was synthesized and characterized in this study. Its structure was confirmed by ^{1}H NMR spectra. Signals in the form of two symmetric multiplets appeared at chemical shifts ranging from 2.89 to 2.65 ppm and indicated four protons associated with two methylene groups neighboring the nitrogen atom. Furthermore, another signal in the form of a triplet was found at the chemical shift of 1.06 ppm and indicated six protons of two methyl groups. Signals appeared at chemical shifts ranging from 4.11 to 3.32 ppm and represented the pyranosyl group protons. In addition, an H_β-1 doublet of DEtGPA was found at a chemical shift of 4.11 ppm lower than that of β-glucose, 4.65 ppm (*51*), and this indicated the electron donor effects of both *N*-ethyl groups of this compound. Hydrolysis of all glucosylamines was followed daily by ^{1}H NMR by estimating the percentage of glucosylamines released in the solutions (**Figure 2**). Integration and comparison of H_β-1 signals of glucosylamines were taken as reference, and plots of hydrolysis kinetics are represented in **Figures 3** and **4**. The glucosylamine soluble in D_2O showed relatively high hydrolysis rates. GPA was more stable in water than others, while DHEtGPA was the least stable (**Figure 3**). In contrast, the hydrolysis rates of compounds in CD_3OD were moderate, and HeGPA was quite stable compared with OcGPA and DoGPA (**Figure 4**). These results suggested that the hydrolysis rate may be closely connected with the type of aglycon and thus to the amine basicity. The stability of GPA in water is in accordance with the results of Pigman et al. (*44*). The moderate hydrolysis of glucosylamines in CD_3OD may be due notably to the lower acidity of methanol compared with water, being less favorable to N-protonation and thus to the hydrolysis process. According to Pigman et al. (*44*), the hydrolysis of glucosylamines is strongly dependent on the solution acidity.

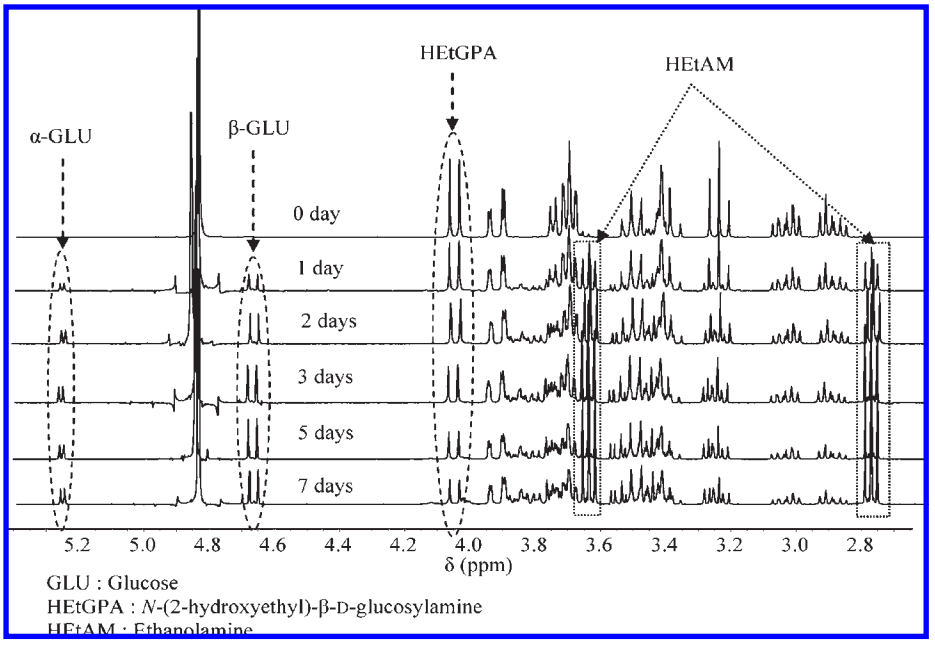

Figure 2. Example of glucosylamine decomposition analysis by ^{1}H NMR spectroscopy.

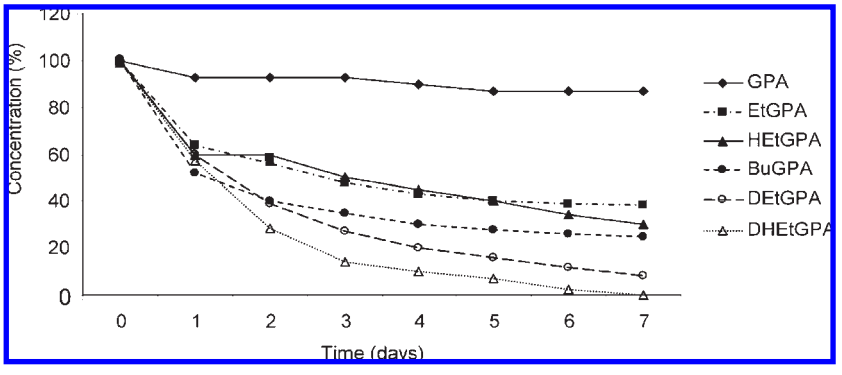

Figure 3. Estimated hydrolysis kinetics of D_2O soluble glucosylamines.

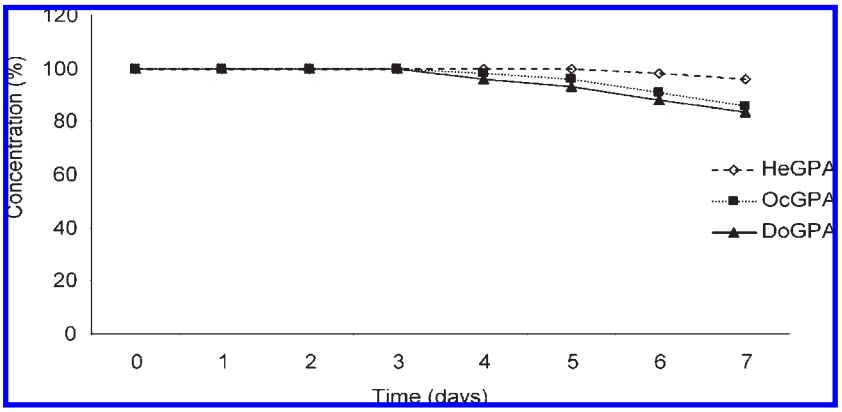

Figure 4. Estimated hydrolysis kinetics of CD_3OD soluble glucosylamines.

Table 1. Antifungal Activity of Different *N*-Alkyl Glucosylamines at Different Concentrations against *F. proliferatum* Growth

	percentage of inhibition ± SEM		
glucosylamines	0.5×10^{-4} mol mL^{-1}	1×10^{-4} mol mL^{-1}	1.5×10^{-4} mol mL^{-1}
GPA	4.3 ± 0.4	10.2 ± 1.1	22.4 ± 1.2
EtGPA	3.1 ± 0.4	9.4 ± 0.7	20.3 ± 0.9
BuGPA	17.2 ± 0.4	44.4 ± 0.3	46.7 ± 0.4
HeGPA	43.2 ± 2.0	76.5 ± 0.7	100.0 ± 0.0
OcGPA	71.0 ± 0.4	100.0 ± 0.0	100.0 ± 0.0
DoGPA	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
HEtGPA	11.8 ± 0.7	20.4 ± 0.4	29.2 ± 0.5
DHEtGPA	10.1 ± 0.5	22.5 ± 0.5	27.2 ± 0.4
DEtGPA	5.9 ± 1.2	17.2 ± 0.4	28.1 ± 3.1

Antifungal Activity Assessment. The antifungal activity of glucosylamines against *F. proliferatum* was evaluated by the radial growth method. After 9 days of incubation, the inhibition percentage was calculated. The impact of the alkyl chain length, the degree of N-substitution, and the N-hydroxyalkylation of glucosylamines was evaluated. The results are presented in **Table 1**.

Results are in accordance with a previous report in which the antifungal activity of glucosylamines directly increased with the length of alkyl chain (*43*). At the concentration of 1.5×10^{-4} mol mL^{-1}, the most active glucosylamines, HeGPA, OcGPA, and DoGPA, completely inhibited the fungal growth. At concentrations of $(1-1.5) \times 10^{-4}$ mol mL^{-1}, there is no significant difference between the activities obtained from DoGPA and OcGPA. However at lower concentration of 0.5×10^{-4} mol mL^{-1}, DoGPA showed significantly higher activity ($p < 0.05$) than OcGPA. It completely inhibited the mycelium growth of *F. proliferatum*, while OcGPA led to 71% inhibition. Furthermore, during this study, OcGPA was significantly more effective than HeGPA. Glucosylamines with short alkyl chains, until BuGPA, were significantly less active than glucosylamines such as HeGPA, OcGPA, and DoGPA (**Table 1**). The high efficiency of these more active glucosylamines could be due to high hydrophobic and surfactant characters. Indeed, the ability of surfactant compounds to inhibit fungi and their mechanism of action were previously reported (*52*). In addition, the impact of alkyl chain on the biological activity of molecules was also mentioned (*34*, *43*, *53*−*58*). The lack of activity for the water-soluble glucosylamines, those with short alkyl chains, may be partially explained by their rapid hydrolysis during the first 24 h compared with the methanol-soluble glucosylamines (**Figures 3** and **4**); however, further statistical analysis enabled us to observe significant ($p < 0.05$) differences in the biological activity due to the introduction of hydroxyl group to ethyl chain and this between EtGPA and HEtGPA. Nevertheless results from DEtGPA, HEtGPA, and DHEtGPA were not statically significantly different, and further studies may be conducted to confirm this. Due to interesting antifungal activity obtained from DoGPA, OcGPA, and HeGPA, their impact on the mycotoxin production, such as the fumonisin biosynthesis, will be studied in the future.

Antibacterial Activity Assessment. Two bacteria, one Gram-positive, *L. innocua*, and one Gram-negative, *S. typhimurium*, were selected to evaluate the antibacterial activity of synthesized glucosylamines. *L. innocua* was chosen instead of the pathogenic *L. monocytogenes* not only because of its closely similar behavior toward inhibitors with the pathogenic listerial strain but also because it can be easily manipulated (*48*, *59*). The assessment was made by three methods, and the results complemented each other. Results from the numeration in coating method were used to calculate the percentage of inhibition and are grouped in the **Tables 2** and **3**. Glucosylamines with short alkyl chains, from C-2 to C-4, did not show any significant antibacterial activity against *L. innocua* and *S. typhimurium*, while HeGPA, OcGPA, and DoGPA significantly ($p < 0.05$) decreased the number of CFUs of both bacteria. For concentrations of $(0.01-1) \times 10^{-4}$ mol mL^{-1}, DoGPA was found to be the most effective glucosylamine against the selected bacteria. This compound completely inhibited *L. innocua* and *S. typhimurium* growth at the low concentrations of 0.025×10^{-4} and 0.05×10^{-4} mol mL^{-1}, respectively, while HeGPA and OcGPA exhibited quite similar antibacterial activity at higher concentrations of 2×10^{-4} mol mL^{-1}. At all concentrations tested, the antibacterial activities from HeGPA and OcGPA are not significantly different. The mechanism of action of these compounds was not studied; however, their biological activities were reported in the past. For example, according to Srivastava et al. (*60*), glucosylamines inhibited DNA-dependent ligases and their vital biological roles like replication and repair process, which catalyze the joining of nicks between adjacent bases of double-stranded DNA. Furthermore, it was reported that these compounds can be used as chelating agents (*61*−*63*). In this way, they can bind trace of metals, which are actually needed by microorganisms from culture medium and thereby inhibit their growth.

As for antifungal properties, the increasing antibacterial activity with the length of alkyl chains may be due to the hydrophobic and surfactant characters, which increase with the length of alkyl chains (*64*, *65*). In addition, Brenner-Hénaff et al. (*66*) reported that surfactant compounds exhibit a good solubilizing power toward membrane proteins especially for spiralin, a bacterial surface antigen.

Table 2. Inhibition of *L. innocua* by Both Glucosylamines and Corresponding Free Amines at Different Concentrations

	percentage of inhibition ± SEM							
	concentrations ($\times 10^{-4}$ mol mL^{-1})							
biocides	0.01	0.025	0.05	0.1	0.25	0.5	1	2
EtGPA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.1 (0.0 ± 0.0)[a]	1.1 ± 1	4.8 ± 2
BuGPA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.5 ± 1 (0.0 ± 0.0)[a]	6.2 ± 3	19.2 ± 1.7
HeGPA	0.0 ± 0.0	8.2 ± 2	19.8 ± 1.4 (8.9 ± 3.5)[a]	46.6 ± 3.6	51.2 ± 2.9	75.4 ± 14.3 (44.4 ± 3.1)[a]	90 ± 4.5	100 ± 0
OcGPA	0.0 ± 0.0	11.6 ± 7	29.8 ± 1.7 (17.1 ± 1.2)[a]	45.7 ± 4.8	58.5 ± 4.5	79.4 ± 10.7 (48.2 ± 3.5)[a]	89.7 ± 9.2	100 ± 0
DoGPA	77.6 ± 11.2 (42.5 ± 4.7)[a]	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0 (75.3 ± 2.6)[a]	100 ± 0	100 ± 0
DEtGPA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.2 ± 1 (0.0 ± 0.0)[a]	4 ± 2	7.8 ± 3
HEtGPA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.7 ± 1 (0.0 ± 0.0)[a]	5.9 ± 3	8.4 ± 7
DHEtGPA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.7 ± 1 (0.0 ± 0.0)[a]	4.8 ± 3	9.5 ± 2

[a] Antibacterial activity of corresponding free amines.

Table 3. Inhibition of *S. typhimurium* by Both Glucosylamines and Corresponding Free Amines at Different Concentrations

	percentage of inhibition ± SEM							
	concentrations ($\times 10^{-4}$mol mL^{-1})							
biocides	0.01	0.025	0.05	0.1	0.25	0.5	1	2
EtGPA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 (0.0 ± 0.0)[a]	1.3 ± 1.0	2.1 ± 1.0
BuGPA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.3 ± 1 (0.0 ± 0.0)[a]	4.3 ± 1.0	9.5 ± 4.1
HeGPA	0.0 ± 0.0	4.7 ± 2.0	15.6 ± 4.0 (6.7 ± 5.7)[a]	25.5 ± 1.7	33.5 ± 2.0	51.2 ± 2.7 (17.5 ± 3.6)[a]	65.2 ± 7.1	79.8 ± 10.0
OcGPA	0.0 ± 0.0	8.9 ± 5.0	27.4 ± 1.4 (10 ± 2)[a]	34.3 ± 1.1	44.2 ± 2.4	58.6 ± 2.9 (20.0 ± 4.4)[a]	67.7 ± 5.9	84.2 ± 8.6
DoGPA	84.7 ± 1.0 (30.0 ± 2.0)[a]	92.8 ± 5.6	100 ± 0	100 ± 0	100 ± 0	100 ± 0 (51.8 ± 2.0)[a]	100 ± 0	100 ± 0
DEtGPA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 (0.0 ± 0.0)[a]	2.6 ± 1.0	5.9 ± 2.0
HEtGPA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 (0.0 ± 0.0)[a]	1.5 ± 1.0	4.3 ± 1.0
DHEtGPA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0 (0.0 ± 0.0)[a]	2.6 ± 1.0	5.9 ± 2.0

[a] Antibacterial activity of corresponding free amines.

Because of the noted *N*-alkyl glucosylamine hydrolysis in solution, the antibacterial activity of alkylamines used to synthesize these products was also tested in this study to determine their possible interference in the obtained results (**Tables 2** and **3**). Hexylamine, octylamine, and dodecylamine showed pronounced antibacterial activity; however, the antibacterial activity of corresponding glucosylamines such us HeGPA, OcGPA, and DoGPA, respectively, was significantly ($p < 0.05$) more pronounced than those of these alkylamines. These results conformed to those previously reported and confirmed the role of glucosyl groups in cell recognition (*67*−*69*). The antibacterial activity obtained from EtGPA, HEtGPA, DHEtGPA, and DEtGPA was not significantly pronounced. During this study, contribution of both N-hydroxyalkylation and degree of N-substitution of glucosylamines on their biological activity was suspected; however, statistical analysis done between antibacterial activities of EtGPA and DEtGPA and HEtGPA and DHEtGPA, on one hand, to assess the contribution of the degree of N-substitution and those of EtGPA and HEtGPA and HEtGPA and DHEtGPA, on the other hand, to assess the contribution of N-hydroxyalkylation, did not show any significant differences at all concentrations tested; this may be due to the higher hydrolysis rate observed from both DEtGPA and DHEtGPA (**Figure 3**). Consequently, further study of these compounds may be conducted to verify this assumption.

The antibacterial activity assessment of different glucosylamines was also evaluated using the disk diffusion method. All glucosylamines were evaluated at the concentrations ranging from 0.1×10^{-4} to 0.5×10^{-4} mol mL^{-1} (**Figure 5**). Only DoGPA inhibited the growth of both bacteria in the considered range of concentrations. Other glucosylamines did not show any clear zones of inhibition surrounding the disks. The well diffusion method was used (1) to eliminate a possible interaction between the paper-based disk and the biocide, (2) to confirm the results obtained from DoGPA, and (3) to estimate the minimal inhibitory concentration of this new antibacterial inhibitor. A concentration range similar to that previously used in the disk diffusion method was used. Diameters of clear inhibition zones surrounding the wells were measured after 48 h of incubation, and results are grouped in **Table 4**.

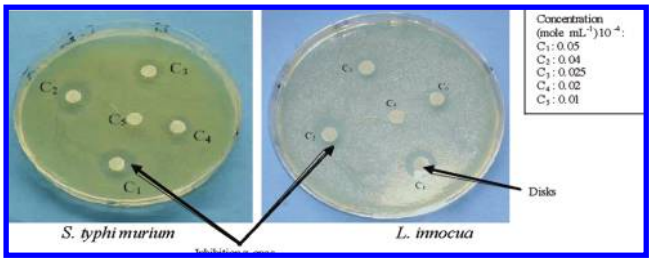

Figure 5. Inhibition clear zones of *L. innocua* and *S. typhimurium* obtained from DoGPA by the disk diffusion method.

With the disk-diffusion method, DoGPA inhibited the growth of *L. innocua* and *S. typhimurium* at the concentration of 0.05×10^{-4} mol mL^{-1}, with inhibition diameters of about 16 and 14 mm, respectively. At the same concentration, inhibition diameters of 15 and 13 mm for *L. innocua* and *S. typhimurium*, respectively, were obtained for DoGPA when the well-diffusion method was

Table 4. Antibacterial Activity of DoGPA at Different Concentrations against *L. innocua* and *S. typhimurium*, Evaluated by the Disk Diffusion Method

	diameters of inhibition ± SEM (mm)				
	concentration ($\times 10^{-4}$ mol mL^{-1})				
bacteria	0.01	0.02	0.025	0.04	0.05
L. innocua	0	6.7 ± 0.3	8.3 ± 0.9	13.3 ± 0.3	16.3 ± 0.7
S. typhimurium	0	6.5 ± 0.3	7.9 ± 0.5	12.3 ± 0.3	14.3 ± 0.9

used. According to Johnson and Case (*70*), the activity of biocides can be considered as effective against bacteria when the inhibition diameter is over 16 mm. In this study, a DoGPA concentration of 0.05×10^{-4} mol mL^{-1} led to an inhibition diameter close to 16 mm, and thus it might be used as the minimal effective concentration against both bacteria. In addition, the well-diffusion method was enabled to estimate the MIC of DoGPA at 0.02×10^{-4} ($0.8\,\mu$g mL^{-1}) and 0.025×10^{-4} ($0.7\,\mu$g mL^{-1}) mol mL^{-1} for *L. innocua* and *S. typhimurium*, respectively.

The combination of the results from coating methods with MIC values suggested that *L. innocua* is the most sensitive toward these compounds. The mechanism of action was not determined but different sensitivity between these bacteria toward inhibitors can be explained by the difference noted in the chemical compositions of their cell walls. *S. typhimurium* is a Gram-negative bacterium with phospholipids and porines in its external wall, leading to a strong barrier to external agents. In contrast, *L. innocua* as a Gram-positive bacterium possesses a cell wall essentially composed of peptidoglycans. It was reported that this wall composition is not strong enough to stop the entrance of all external substances (*71*−*73*). The higher sensitivity of Gram-positive bacteria than Gram-negative bacteria toward hydrophobic biocides was also reported in the past (*64*). In addition, it was mentioned that the presence of porines and lipopolysaccharides in the external membrane of Gram-negative bacteria strongly increases their defense ability (*71*). In this study, *L. innocua*, a Gram-positive bacterium, is more sensitive to the compounds tested than *S. typhimurium*, a Gram-negative bacterium. This is in accordance with the mentioned literature and supported our observations. However, a further biological study of these products is needed to well understand their mechanisms of action.

In conclusion, DoGPA was more effective than other glucosylamines tested and may be exploited to fight *F. proliferatum*, *L. innocua*, and *S. typhimurium*. However, the study of its toxicological profiles must be carefully done before its application to avoid any environmental contamination.

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LITERATURE CITED

(1) FAO-OMS. Evaluation des risques liés à *Listeria monocytogenes* dans les aliments prêts à consommer, Rome, 2004, 87 pp.
(2) Guillier, L.; Pardon, P.; Augustin, J. C. Influence of stress on individual lag time distributions of *Listeria monocytogenes*. *Appl. Environ. Microbiol.* **2005**, *71* (6), 2940–2948.
(3) Corrégé, I. La problématique salmonelles en filière porcine. *Tech. Porc.* **2001**, *24* (2), 25–31.
(4) White, D. G.; Zhao, S.; Sudler, R.; Ayers, S.; Friedman, S.; Chen, S.; McDermott, P. F.; McDermott, S.; Wagner, D. D.; Meng, J. The isolation of antibiotic-resistant salmonella from retail ground meats. *N. Engl. J. Med.* **2001**, *345* (16), 1147–1154.
(5) Korsak, N.; Clinquart, A.; Daube, G. Salmonella spp. dans les denrées alimentaires d'origine animale: Un réel problème de santé publique. *Ann. Méd. Vét.* **2004**, *148*, 174–193.
(6) Lin, M.; Al-Holy, M.; Al-Qadiri, H.; Kang, D.; Cavinato, A. G.; Huang, Y.; Rasco, B. A. Discrimination of intact and injured *Listeria monocytogenes* by Fourier transform infrared spectroscopy and principal component analysis. *J. Agric. Food Chem.* **2004**, *52*, 5769–5772.
(7) Roberts, D. *Listeria monocytogenes* in foods-results of PHLS (Public Health Laboratory service) In Annual general meeting and summer conference. *J. Appl. Bacteriol.* **1989**, *67* (6), xix.
(8) Roberts, T.; Pinner, R. *Economic impact of disease caused by* L. *monocytogenes*. *Topics in Industrial Microbiology: Foodborne listeriosis*; Elvisier Science pub.: New York, 1990; pp 137−149.
(9) Fandohan, P.; Hell, K.; Marasas, W. F. O.; Wingfield, M. J. Infection of maize by *Fusarium* species and contamination with fumonisin in Africa. *Afr. J. Biotechnol.* **2003**, *2* (12), 570–579.
(10) Parry, D. W.; Jenkinson, P.; McLeod, L. *Fusarium* ear blight (scab) in small grain cereals- a review. *Plant Pathol.* **1995**, *44* (2), 207–238.
(11) Mesterházy, A. Types and components of resistance to fusarium head blight of wheat. *Plant Breeding* **1995**, *114* (5), 377–386.
(12) McMullen, M.; Jones, R.; Gallenberg, D. Scab of wheat and barley: A re-emerging disease of devastating impact. *Plant Dis.* **1997**, *81* (12), 1340–1348.
(13) Spahr, U.; Walther, B.; Sieber, R.; Gafner, J. L; Guidon, D. Occurrence of mycotoxins in feeds and carry over into milk: A review. *Mitt. Lebensmitteluntersuchung Hyg.* **1999**, *90* (5), 575–609.
(14) Gärtner, B. H.; Munich, M.; Kleijer, G.; Mascher, F. Characterisation of kernel resistance against *Fusarium* infection in spring wheat by baking quality and mycotoxin assessments. *Eur. J. Plant. Pathol.* **2008**, *120*, 61–68.
(15) Yiannikouris, A.; Jouany, J.-P. Les mycotoxines dans les aliments des ruminants, leur devenir et leurs effets chez l'animal. *INRA Prod. Anim.* **2002**, *15* (1), 3–16.
(16) Cavret, S.; Lecoeur, S. Les fusariotoxicoses des animaux d'élevage. *Ann. Méd. Vét.* **2006**, *150*, 43–55.
(17) Smith, T. K.; McMillan, E. G.; Castillo, J. B. Effect of feeding blends of *Fusarium* mycotoxin-contaminated grains containing deoxynivalenol and fusaric acid on growth and feed consumption of immature swine. *J. Anim. Sci.* **1997**, *75*, 2184–2191.
(18) Gouze, M. E.; Oswald, I. P. Effets d'une mycotoxine inféodée au maïs, la fumonisine B_1, sur les lymphocytes porcins. *J Rech. Porcine France* **2001**, *33*, 277–281.
(19) Bi, Z.; Wang, Z.; Xu, L. Chemical constituents of *Dendrobium moniliforme*. *Acta Bot. Sin.* **2004**, *46* (1), 124–126.
(20) Gandhi, M.; Chilindas, M. L. Review, Listeria: A foodborne pathogen that knows how to survive. *Int. J. Food Microbiol.* **2007**, *113*, 1–15.
(21) Broome, C. V.; Gellin, B.; Schwartz, B. Epidemiology of listeriosis in the United States. In *Topics in Industrial Microbiology*; Miller, A. J., Smith, J. L., Somkuti, G. A., Eds.; Elsevier Science Pub.: New York, 1990; pp 61−65.
(22) Bille, J. Epidemiology of listeriosis in Europe, with special reference to the Swis outbreak. In *Topics in Industrial Microbiology*; Miller, A. J., Smith, J. L., Somkuti, G. A., Eds.; Elsevier Science Pub.: New York, 1990; pp 25−29.
(23) Hu, L.; Kopecko, D. Typhoid Salmonella. In *International handbook of foodborne pathogens*; Miliotis, N., Bier, J., Eds.; Marcel Dekker: New York, 2003; pp 151−165.
(24) McLauchlin, J. The pathogenicity of *Listeria monocytogenes*: A public health perspective. *Rev. Med. Microbiol.* **1997**, *8*, 1–14.
(25) Roccourt, J.; Cossart, P. *Listeria monocytogenes*. In *Food Microbiology − Fundamentals and Frontiers*; Doyle, M. P., Buechat, L. R., Montville, T. J., Eds.; American Society for Microbiology (ASM) press: Washingon DC, 1997; pp 337−352.
(26) Mead, P. S.; Slutsker, L.; Dietz, V.; McCraig, L. F.; Bresee, S.; Shapiro, C.; Griffin, P. M.; Tauxe, R. V. Food-related illness and death in United States. *Emerg. Infect. Dis.* **1999**, *5*, 607–625.

(27) Graham, S. M. Salmonellosis in children in developed countries and populations. *Curr. Opin. Infect. Dis.* **2002**, *15*, 507–512.
(28) Lalande, M.; Guyon, G.; Morin, C.; Rodière, M.; Astruc, J. Salmonella infections in children: A retrospective study over a four-year period. *Arch. Pédriatr.* **2005**, *12*, 23–27.
(29) Dagnra, A. Y.; Akolly, K.; Gbadoe, A.; Aho, K.; David, M. Emergence of multidrug resistant *Salmonella* strains in Lomé (Togo). *Méd. Mal. Infect.* **2007**, *37*, 266–269.
(30) Walsh, D.; Duffy, G.; Sheridan, J. J.; Blair, I. S.; McDowell, D. A. Antibiotic resistance among *Listeria*, including *Listeria monocytogenes*, in retail foods. *J. Appl. Microbiol.* **2001**, *90* (4), 517–522.
(31) Prazak, M. A.; Murano, E. A.; Mercado, I.; Acuff, G. R. Antimicrobial resistance of *Listeria monocytogenes* isolated from various cabbage farms and packing sheds in Texas. *J. Food. Prot.* **2002**, *65* (11), 1796–1799.
(32) Summerbell, R. C.; Richardson, S. E.; Kane, J. *Fusarium proliferatum* as an agent of disseminated infection in an imunosuppressed patient. *J. Clin. Microbiol.* **1988**, *26* (1), 82–87.
(33) Ferrer, C.; Alio, J.; Rodriguez, A.; Andreu, M.; Colom, F. Endophthalmitis caused by *Fusarium proliferatum*. *J. Clin. Microbiol.* **2005**, *43* (10), 5372–5375.
(34) Mizobuchi, S.; Sato, Y. Antifungal activities of 2,4,6-trihydroxyacylophenones and related compounds. *Agric. Biol. Chem.* **1985**, *49* (3), 719–724.
(35) Coma, V.; Martial-Gros, A.; Garreau, S.; Copinet, A.; Salin, F.; deschamps, A. Edible antimicrobial films based on chitosan matrix. *J. Food Sci.* **2002**, *67*, 1162–1169.
(36) Coma, V.; Deschamps, A.; Martial-Gros, A. Bioactive packaging materials from edible chitosan polymer-antimicrobial activity assessment on dairy-related contaminants. *J. Food Sci.* **2003**, *68*, 2788–2792.
(37) El-Tahlawy, K. F.; El-bendary, M. A.; El-hendawy, A. G.; Hundson, S. M. The antimicrobial activity of cotton fabrics with different crosslinking agents and chitosan. *Carbohydr. Polym.* **2005**, *60*, 421–430.
(38) Bordenave, N.; Grelier, S.; Pichavant, F.; Coma, V. Water and moisture susceptibility of chitosan and paper-based materials: Structure−property relationships. *J. Agric. Food Chem.* **2007**, *55*, 9479–9488.
(39) Belalia, R.; Grelier, S.; Benaissa, M.; Coma, V. New bioactive biomaterials based on quaternized chitosan. *J. Agric. Food Chem.* **2008**, *56*, 1582–1588.
(40) Coma, V. Bioactive packaging technologies for extended shelf life of meat-based products. *Meat Sci.* **2008**, *78*, 90–103.
(41) Portes, E.; Gardrat, C.; Castellan, A.; Coma, V. Environmentally friendly films based on chitosan and tetrahydrocurcuminoid derivatives exhibiting antibacterial and antioxidative properties. *Carbohydr. Polym.* **2009**, *76*, 578–584.
(42) Fernandez-Saiz, P.; Lagarón, J. M.; Ocio, M. J. Optimization of the film-forming and storage conditions of chitosan as an antimicrobial agent. *J. Agric. Food Chem.* **2009**, *57* (8), 3298–3307.
(43) Muhizi, T.; Coma, V.; Grelier, S. Synthesis and evaluation of N-alkyl-β-D-glucosylamines on the growth of to wood fungi, *Coriolus versicolor* and *Poria placenta*. *Carbohydr. Res.* **2008**, *343*, 2369–2375.
(44) 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.* **1951**, *73* (5), 1976–1979.
(45) Mota, M.; Campos, A. K.; Araújo, J. V. Sporulation, radial growth and biomass production of *A. robusta* and *M. thaumasium* submitted to different methods of preservation. *Braz. J. Microbiol.* **2003**, *34*, 157–160.
(46) Perlman, D.; Schwartz, J. L. An agar diffusion method for evaluation of antimycoplasma substances. *J. Antibiot.* **1968**, *21* (6), 399–401.
(47) Sebti, I.; Pichavant, F.; Coma, V. Edible bioactive fatty acid-cellulosic derivative composites used in food-packaging applications. *J. Agric. Food Chem.* **2002**, *50*, 4290–4294.
(48) Sebti, I.; Coma, V. Active edible polysaccharide coating and interactions between solution coating compounds. *Carbohydr. Polym.* **2002**, *49*, 139–144.
(49) Dasturi, M. R.; Fakhimzadeh, K.; Shayeg, J.; Dolgari-Sharaf, J.; Valilou, M. R.; Maheri-Sis, N. Evaluating antibacterial activity of the Iranian honey through MIC method on some dermal and intestinal pathogenic bacteria. *J. Anim. Vet. Adv.* **2008**, *7* (4), 409–412.
(50) Coma, V.; Sebti, I.; Pardon, P.; Deschamps, A.; Pichavant, H. F. Anti-microbial edible packaging based on cellulosic ethers, fatty acids and nisin incorporation to inhibit *Listeria monocytogenes* and *Staphylococcus aureus*. *J. Food Prot.* **2001**, *64*, 470–475.
(51) Blasko, A.; Bunton, A. B.; Bunel, S.; Ibarra, C.; Moraga, E. Determination of acid dissociation constants of anomers of amino sugars by ^{1}H NMR spectroscopy. *Carbohydr. Res.* **1997**, *298*, 163–172.
(52) Nihei, K.; Nihei, A.; Kubo, I. Rational design of antimicrobial agents: Antifungal activity of alk(en)yl dihydroxybenzoates and dihydroxyphenyl alkanoates. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3993–3996.
(53) Cotoras, M.; Garcia, C.; Lagos, C.; Folch, C.; Mendoza, L. Antifungal activity on *Botrytis cinerea* of flavonoids and diterpenoids isolated from the surface of *Pseudognaphalium* spp. *Bol. Soc. Chil. Quim.* **2001**, *46* (4), 1–27.
(54) Katiyar, D.; Tiwari, V. K.; Tewari, N.; Verma, S. S.; Sinha, S.; Gaikwad, A.; Srivastava, A.; Chaturvedi, V.; Srivastava, R.; Srivastava, B. S.; Tripathi, R. P. Synthesis and antimycobacterial activities of glycosylated amino alcohols and amines. *Eur. J. Med. Chem.* **2005**, *40*, 351–360.
(55) Kuča, K.; Kivala, M.; Dohnal, V. A general method for quaternisation of N,N-dimethyl benzylamines with long chain n-alkylbromides. *J. Appl. Biomed.* **2004**, *2*, 195–198.
(56) Rittich, B.; Pirochtová, M.; Hřib, J.; Jurtíková, K.; Doležal, P. The antifungal activity of some aliphatic and aromatic acids. *Collect. Czech. Chem. Commun.* **1992**, *57*, 1134–1142.
(57) Bradley, H.; Fitzpatrick, G.; Glass, W. K.; Kunz, H.; Murphy, P. V. Application of Ugi reactions in synthesis of divalent neoglycoconjugates: Evidence that the sugars are presented in restricted conformation. *Org. Lett.* **2001**, *3* (17), 2629–2632.
(58) Kubo, I.; Fujita, T.; Kubo, A.; Fujita, K. Modes of antifungal action of alkanols against *Saccharomyces cerevisiae*. *Bioorg. Med. Chem.* **2003**, *11* (6), 1117–1122.
(59) Perrin, M.; Bemer, M.; Delamare, C. Fatal case of *Listeria innocua* bacteremia. *J. Clin. Microbiol.* **2003**, *41* (11), 5308–5309.
(60) Srivastava, S. K.; Dube, D.; Tewari, N.; Dwivedi, N.; Tripathi, R. P.; Ramachandran, R. *Mycobacterium tuberculosis* NAD^+-dependent DNA ligase is selectively inhibited by glycosylamines compared with human DNA ligase I. *Nucleic Acids Res.* **2005**, *33* (22), 7090–7101.
(61) Das, T. M.; Rao, C. P.; Kolehmainen, E. Interaction of metal ions with *N*-glucosylamines: Isolation and characterisation of products of 4,6-O-benzylidene-*N*-(o-carboxyphenyl)-β-D-glucopyranosylamine with different metal ions. *Carbohydr. Res.* **2001**, *335*, 151–158.
(62) Das, T. M.; Rao, C. P.; Kolehmainen, E. Synthesis and characterisation of N-glycosyl amines from the reaction between 4,6-O-benzylidene-D-glucopyranose and substituted aromatic amines and also between 2-(O-aminophenyl) benzimidazole and pentoses or hexoses. *Carbohydr. Res.* **2001**, *334*, 261–269.
(63) Shahidi, F.; Archchi, J. K. V.; Jeon, Y. Food applications of chitin and chitosans. *Trends Food Sci. Technol.* **1999**, *10*, 37–51.
(64) Kubo, I.; Muroi, H.; Kubo, A. Structural functions of antimicrobial long chain alcohols and phenols. *Bioorg. Med. Chem.* **1995**, *3* (7), 873–880.
(65) Wozniak, E.; Mozrzymas, A.; Czarny, A.; Kocieba, M.; Rozycka-Roszak, B.; Dega-Szafran, Z.; Dulewicz, E.; Petryna, M. Antimcrobial activity of *N*-alkoxycarbonylmethyl-*N*-alkyl-piperidinium chlorides. *Z. Naturforsch.* **2004**, *59c*, 782–786.
(66) Brenner-Hénaff, C.; Valdor, J. F.; Plusquellec, D.; Wlóblewski, H. Synthesis and characterization of *N*-octanoyl-β-D-glucosylamine, a new surfactant for membrane studies. *Anal. Biochem.* **1993**, *212* (1), 117–127.
(67) Ben, R. N.; Orellana, A.; Arya, P. Stereoselective synthesis of carbon-linked analogues of α- and β-galactoserine glycoconjuguates using asymmetric enolate methodology. *J. Org. Chem.* **1998**, *63*, 4817–4820.

(68) Moniru, M.; Horoyuki, S.; Yoshihiro, S. Control of functions of chitosan by chemical modification. *Trends Glycosci. Glycotechnol.* **2002**, *14* (78), 205–222.
(69) 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* (3), 508–510.
(70) Johnson, T.; Case, C. *Laboratory Experiments in Microbiology*, 4th ed.; Benjamin Cummings Publishing Co: Redwood City, CA, 1995; 445 pp.
(71) Page, D. P.; Curtis, M. J.; Sutter, M. C.; Walker, M. J.; Hoffman, B. B. *Pharmacology intégrée*; De Boeeck Université: Bruxelles, 1999, 616 pp.
(72) Campbell, N. A.; Reece, J. B. *Biologie*, 2e éd., De Boeck Université: Bruxelles, 2004; 1482 pp.
(73) Sun, L.; Du, Y.; Fan, L.; Chen, X.; Yang, J. Preparation, characterization and antimicrobial activity of quaternized carboxymethyl chitosan and application as pulp-cap. *Polymer* **2006**, *47*, 1796–1804.

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