DOI:10.1021/jf901609y

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Synthesis and Antibacterial Activity of Aminodeoxyglucose Derivatives against *Listeria innocua* and *Salmonella typhimurium*

Théoneste Muhizi, Stéphane Grelier, and Véronique Coma*

UMR US2B, Unité des Sciences du Bois et des Biopolymères, Université Bordeaux 1, INRA, CNRS, 351 Cours de la Libération, F-33405 Talence, France

In this study aminodeoxyglucose derivatives were synthesized and evaluated for their antibacterial activity against two food bacteria, *Listeria innocua* and *Salmonella typhimurium*. 6-Amino-6-deoxy-α-D-methylglucopyranose (GSA-6), 3-amino-3-deoxy-D-glucopyranoside (GSA-3), and β-D-glucopyranosylamine (GSA-1) were synthesized and concurrently tested with commercially available D-glucosamine (GSA-2) for antibacterial activity. Results obtained from this study showed a pronounced antagonist effect due to the position of amino groups of aminoglucose derivatives on the antibacterial activity. GSA-3 was the most active compound. At a concentration of 2×10^{-4} mol mL^{-1}, it delayed the growth of both bacteria with percentages of inhibition of 29 and 15% for *L. innocua* and *S. typhimurium*, respectively. At the same concentration the percentages of inhibition for other aminodeoxyglucoses varied between 5 and 18% and between 2 and 11% for *L. innocua* and *S. typhimurium*, respectively. All compounds were characterized by FTIR, ^{1}H NMR, and ^{13}C NMR spectroscopy.

KEYWORDS: Antibacterial activity; aminodeoxyglucoses; *Salmonella typhimurium*; *Listeria innocua*

INTRODUCTION

Research for biocide discovery has long been known. However, because of their high multiplicity and resistance, total control of microorganisms is far from being accomplished. Some microorganisms are implicated in the contamination of food, water, and various instruments used by people. Physical changes occur during the processing, transport, and storage of food, and when contaminated elements are consumed, these microorganisms can multiply and cause dangers to humans. *Listeria* and *Salmonella* genera are widely found among those microorganisms, which necessitate our effort to control them. These two bacteria are respectively responsible for listeriosis and salmonellosis, two severe diseases with large numbers of patients and high mortality rates. The danger of these bacteria is actually due to their capacity to survive over a wide range of preservation conditions, which are, for example, low temperatures, low pH, and high concentrations of salt. Therefore, research aimed at the use of new modes of food conservation is under intense scrutiny. According to some authors, the use of active packaging can constitute a new method of achieving the control of bacteria contamination in foods (*1*−*5*). For this, chitosan and its derivatives have shown their efficiency by inhibiting the growth of *Listeria innocua*, *Listeria monocytogenes*, and *Salmonella typhimurium* in both solid and liquid media (*6*−*9*). However, the mechanism of action of these compounds is still unknown. It is suspected that there is a close relationship between amino groups and biological activity of chitosan (*9*). In this order, the biological activity from this polymer could be expanded to its monomer, D-glucosamine, and either to its isomers or other small aminoglucose derivatives. These small sugars are suspected to better enter into cell membranes than their respective polymers. The present work is principally aimed at the study of these aminodeoxyglucose derivatives by verifying the impact of amino group features in biological activity. The impact of amino group position on glucose in the study of antibacterial activity was the main objective of this study. For this, we synthesized different aminoglucose derivatives by changing the position of the amino group and studied their impact on the development of *S. typhimurium* and *L. innocua*.

*Corresponding author [telephone (33) 5 40 00 2913; fax (33) 5 40 00 64 39; e-mail v.coma@us2b.u-bordeaux1.fr].

MATERIALS AND METHODS

Chemicals. Glucose (Sigma-Aldrich), α-D-methylglucoside 99% (Janssen Chimica), zinc chloride 98% (Acros Organics), phosphorus acid 85% (Sigma-Aldrich), pyridinium dichromate 98% (Sigma-Aldrich), acetic anhydride 99% (Sigma-Aldrich), phosphorus dioxide 98% (Acros Organics), sodium borohydride 98% (Acros Organics), *p*-toluenesulfonyl chloride 98% (Sigma-Aldrich), palladium/carbon 5% (Sigma-Aldrich), sodium hydrogenocarbonate, and sodium sulfate 99% (Acros Organics) were used in this study. Solvents such as ethanol, methanol, chloroform, petroleum ether, ethyl acetate, diethyl ether, dichloromethane, toluene, pyridine 99%, *N*,*N*-dimethylformamide 99%, and acetone were purchased from BDH Prolabo and were used without any further purification.

General Methods. Aminodeoxyglucoses GSA-1, GSA-3, and GSA-6 were synthesized and characterized by FTIR, ^{1}H NMR, and ^{13}C NMR spectroscopy. The synthesis was monitored by thin layer chromatography realized on aluminum plates (silica gel 60 F254) and developed with a solution of 2% potassium permanganate in a 0.2 M aqueous solution of NaOH. Compounds obtained were purified by open column chromatography

 Published on Web 09/02/2009

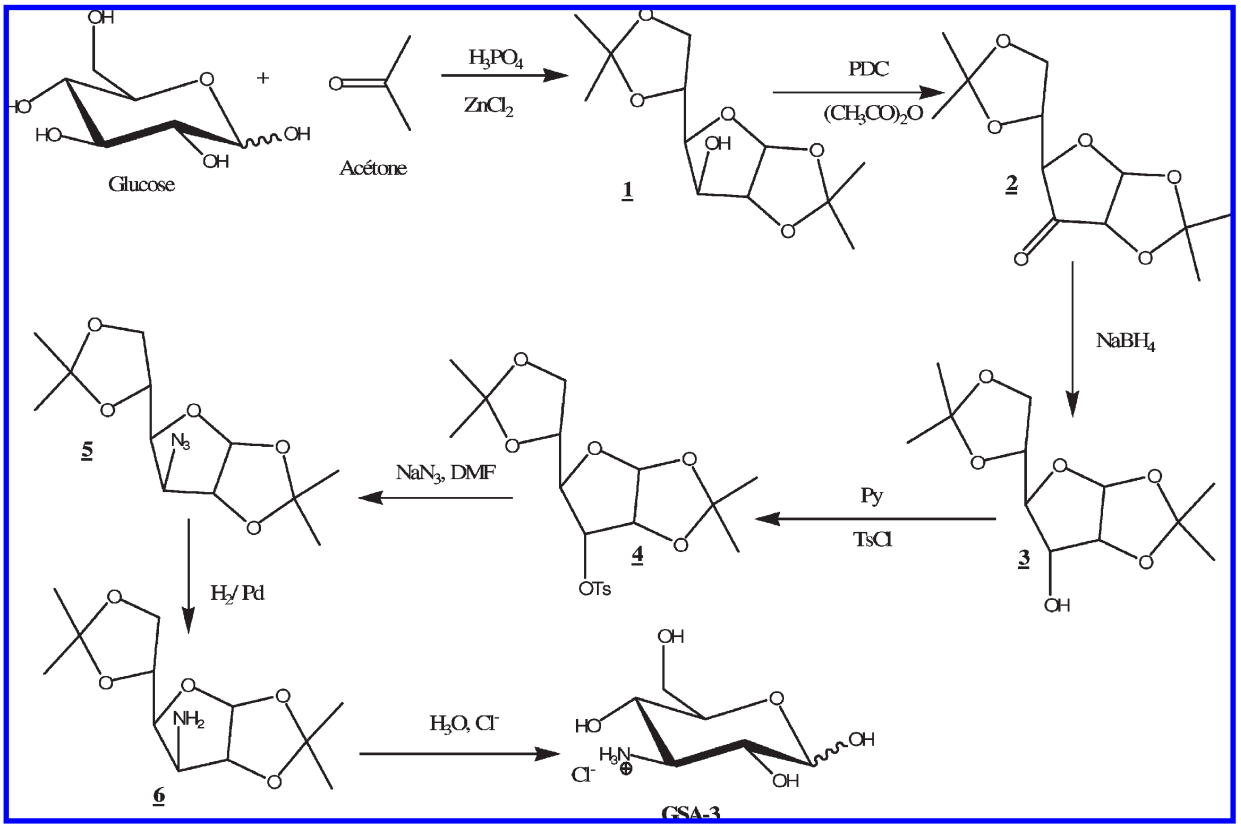

Figure 1. Synthesis of GSA-3.

(silica gel and with different eluents). FTIR spectra were recorded on a Perkin-Elmer Paragon 1000 PC spectrophotometer from 300 mg of KBr pellet disks containing 3 mg of compound. The KBr pellet disks were formed from Edwards High Vacuum Pump Es 50. Spectra were recorded between 400 and 4000 cm^{-1} using 50 scans at a resolution of 4.0 cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded at 300 MHz from a Bruker Avance 300 spectrometer. Chemical shifts are given in parts per million, and the assignments of ^1H NMR and ^{13}C NMR signals were monitored by COSY, HMBC, or HMQC NMR spectroscopy.

Chemical Synthesis. As previously reported (*10*, *11*), *β-D-glucopyranosylamine* (GSA-1) was synthesized from glucose and ammonium carbamate in methanol to give a desired compound with yield varying from 98.3 to 100%.

3-Amino-3-deoxy-D-glucose (GSA-3) was synthesized from D-glucose in seven steps (**Figure 1**) using the methods previously reported (*12*−*18*). Hydroxyl groups were protected using the method of Blakley et al. (*12*) to give 1,2,5,6-di-*O*-isopropylidene-D-glucofuranose (**1**): yield, 43.4%; mp, 107−111 °C; ^1H NMR (300 MHz, $CDCl_3$), δ_H 5.96 (1H, d, H-1), 4.52 (1 H, dd, H-2), 4.31 (2H, complex, H-4, H-5), 4.13 (1H, dd, H-3), 4.03 (1H, dd, H-6a), 3.92 (1H, dd, H-6b), 2.63 (1H, s, −OH), 1.49−1.31 (12H, s, $-CH_3$). The oxidation of OH-3 was done using a method described by Saito et al. (*17*) and gave 1,2,5,6-di-*O*-isopropylidene-3-oxo-D-glucofuranose (**2**): yield, 96.7%; mp, 130 °C; FTIR (KBr), ν_{max} (cm^{-1}) 3390, 3000−2856, 1729, 1456−1352, 1278−1215, 1067−1014; ^{13}C NMR (300 MHz, $CDCl_3$), δ_C 208.7 (C-3, C=O), 129.0 (C-2), 128.2 (C-1), 114.2 (C-8), 110.3 (C-7), 103.0 (C-4), 78.9 (C-5), 64.2 (C-6), 27.4−25.2 ($-4CH_3$). A carbonyl function of compound **2** was reduced by $NaHB_4$ in ethanol to give an epimer of **1**, which is compound **3** (*16*): yield, 50.4%; mp, 106 °C; FTIR, ν_{max} (cm^{-1}) 3390, 3000−2856, 1456−1352, 1278−1215, 1067−1014. Then, this compound easily reacted with *p*-toluenesulfonyl chloride (Ts) in dry pyridine (*13*) to give 1,2,5,6-di-*O*-isopropylidene-3-*O*-(*p*-toluenesulfonyl)-α-D-glucofuranose (**4**): yield, 91.6%; mp, 116−118 °C; ^1H NMR (300 MHz, $CDCl_3$), δ_H 7.8 (2H, d, H-arom), 7.3 (2H, d, H-arom), 5.92 (1H, dd, H-3), 4.83 (1 H, d, H-1), 4.79 (1H, dd, H-2), 4.1−3.8 (4H, m, H-4, H-5, H-6a, H-6b), 2.4 (3H, s, $-CH_3$ arom), 1.47−1.14 (12H, s, $-CH_3$). A substitution of the tosyl group by an azido group was done in DMF at 120 °C for 2 days using a modified method of Brimacombe et al. (*14*) to give 1,2,5,6-di-*O*-isopropylidene-3-azido-3-deoxy-α-D-glucofuranose (**5**): yield, 34.2%; mp, 104−109 °C; FTIR (KBr), ν_{max} (cm^{-1}) 3428, 3019−2839, 2140, 1454−1158, 1081, 702−500; ^1H NMR (300 MHz, $CDCl_3$), δ_H 5.79 (1H, d, H-1), 4.59 (1H, t, H-2), 4.29 (1H, dd, H-4), 4.06−3.90 (3H, m, H-3, H-5, H-6a), 3.85−3.8 (1H, dd, H-6b), 1.56−1.35 (12H, s, $-CH_3$). Compound **5** was dissolved in methanol and submitted to hydrogen flow in the presence of Pd/C (5%) to reduce the azido group. After filtration of catalyst, the solution was concentrated to half, and hydrochloric acid in aqueous solution (0.2 N) was added. The mixture was heated at 50 °C for 15 min and washed three times with chloroform, and the solvent was evaporated to give 3-amino-3-deoxy-α-D-glucopyranose hydrochloride (*15*) (GSA-3): yield, 33%; mp, decomposition at 125 °C; FTIR (KBr), ν_{max} (cm^{-1}) 3428, 3019−2839, 1621, 1454−1195, 1164, 1081, 1029, 882, 702−500; ^1H NMR (300 MHz, D_2O), δ_H 5.21 (1H, d, H_α-1), 3.85−3.82 (1H, dd, H-6a), 3.8 (1H, complex, H-5), 3.76 (1H, t, H-2), 3.76−3.74 (1H, dd, H-6b), 3.65 (1H, dd, H-4), 3.6−3.5 (1H, dd, H-3); ^{13}C NMR (300 MHz, D_2O), δ_C 97.2 (C-1, isomer α), 92.9 (C-1, isomer *β*), 76.2 (C-5), 75.0 (C-2), 73.2 (C-4), 70.1 (C-6), 61.1 and 60.9 (C-3).

6-Amino-6-deoxy-α-D-methylglucoside (*GSA-6*). This compound was synthesized in three steps as follows (**Figure 2**). The modified method of Kondo et al. (*19*) was used to tosylate the C-6 of glucose. Then, a substitution of tosyl by an azide group was realized using the modified methods of Brimakombe et al. (*14*) and Li et al. (*20*), and azide reduction was followed using the modified method of Jary et al. (*15*). To synthesize 6-(*p*-toluenesulfonyl)-α-D-methylglucoside, α-D-methylglucoside (10 g, 51.5 mmol) was dissolved in 20 mL of pyridine previously dried on 4A molecular sieves. At 0 °C, under shaking, a mass of 19.6 g (103 mmol) of *p*-toluenesulfonyl chloride was added. The mixture was then kept at 0 °C for 24 h before being shaken at 5 °C for 48 h and at ambient temperature for 48 h. The reaction mixture was diluted with 20 mL of distilled water and extracted three times with portions of 20 mL of chloroform. The organic layer was washed successively with a portion of 20 mL of 0.2 N hydrochloric acid, saturated sodium hydrogenocarbonate, and distilled water before being dried with sodium sulfate. Solvent was evaporated, and residue was taken in a small volume of chloroform and submitted to the open column chromatography (silica gel, ethyl acetate/cyclohexane 7:3). The product **9** was obtained: yield, 30.5%; R_f, 0.52 (ethyl acetate/

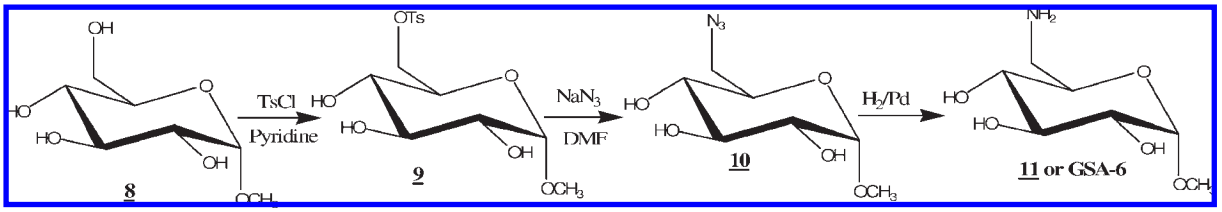

Figure 2. Synthesis of GSA-6.

cyclohexane 7:3); ^{1}H NMR (300 MHz, $CDCl_3$), δ_H 7.81−7.75 (2H, dd, H-arom), 7.35−7.30 (2H, dd, H-arom), 4.57 (1H, d, H-1), 4.25−4.06 (5H, m, H-3, H-4, H-5, H-6a, and H-6b), 3.19 (3H, s, $-OCH_3$), 2.42 (3H, s, $-CH_3$). Then, product **9** (4 g, 11.5 mmol) was dissolved in 20 mL of dried DMF, and 2.9 g of sodium azide (44 mmol) was added. The mixture was refluxed at 120 °C for 48 h. Solvent was removed. A viscous yellowish residue was purified on open column chromatography (silica gel, ethyl acetate/cyclohexane 8:2) and compound **10**, 6-azido-6-deoxy-α-D-methylglucoside, was obtained: yield, 68%; R_f, 0.9 (ethyl acetate/cyclohexane 8:2); FTIR (KBr), ν_{max} (cm^{-1}) 3569−3125, 2971−2821, 2105, 1482−1278, 1090, 713−524. Thus, compound **10** (1.2 g; 5.5 mmol) was dissolved in 20 mL of dried methanol, and 0.4 g of Pd/C (5%) was added. The mixture was submitted under a flow of hydrogen to reduce the azide group for 5 h. After the catalyst had been filtered and the solution concentrated, the yellowish residue obtained was purified by open column chromatography (silica gel, ethyl acetate/cyclohexane 8:2) and 1 g of compound 6-amino-6-deoxy-α-D-methylglucoside (**11** or GSA-3) was obtained: yield, 94.5%; FTIR (KBr), ν_{max} (cm^{-1}) 3569−3125, 3371, 3309, 2971−2821, 1607, 1482−1278, 1090, 893, 713−524; ^{1}H NMR (300 MHz, $CDCl_3$), δ_H 4.77 (1H, d, H-1), 3.73−3.67 (2H, t, H-3, H-4), 3.56−3.40 (4H, m, H-5, H-6a, H-6b, H-2,), 3.47 (3H, $-OCH_3$); ^{13}C NMR (300 MHz, $CDCl_3$), δ_C 99.4 (C-1), 74.1 (C-3), 71.9 (C-5), 70.8 (d, C-2, C-4), 55.4 ($-OCH_3$), 51.3 (C-6).

Microorganisms and Preparation of Inocula. *L. innocua* (ISTAB, Université Bordeaux1) and *Salmonella typhimurium* (Institut Pasteur 5858) were maintained at −70 °C in 20% of glycerol. Overnight precultures were performed as follows: *L. innocua* and *S. typhimurium* were grown in tryptose broth (Difco 262200) and nutrient broth (Difco 234000) at 37 °C for 18 h, respectively.

Antibacterial Activity Assessment. The antibacterial assessment of aminodeoxyglucose derivatives was conducted using an agar plate method. Compounds were tested at concentrations varying in the range from 0.25×10^{-4} to 2×10^{-4} mol mL^{-1}. To do this, 20 mL of culture medium 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 into each Petri dish. After solidification, 2 mL of the tested compound was poured into 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 be evaporated. Thus, about 100−300 cell charges per plate of an 18 h microbial culture were deposited on the medium prior to incubation at 37 °C for 48 h prior 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 aminodeoxyglucoses was determined as percentage of inhibition calculated as follows:

$$\text{percentage of inhibition} = \frac{\text{CFU in control plates} - \text{CFU in test plates}}{\text{CFU in control plates}} \times 100$$

Analysis of Results. Results from the antibacterial assays were statistically analyzed using Student's t test. The probability (p) was determined from the critical t value obtained by comparing results from control and different experiments, on the one hand, and those from test experiments on the other hand. The critical t value is expressed as

$$t = \frac{M_1 - M_2}{\text{SD}}$$

where M_1 is the control mean (or the first test experiment mean), M_2 is the test experiment mean (or the second test experiment mean), and SD is the standard deviation between means. The degree of freedom used to determine this probability was equal to 4 in our experiment. The standard error means (SEM) were calculated as SEM $= \frac{\sigma}{\sqrt{n}}$ where n is the size of sample ($n = 3$) and σ is the standard deviation.

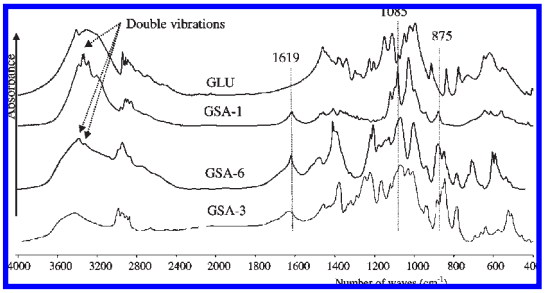

Figure 3. FTIR spectra of Glu, GSA-1, GSA-3, and GSA-6.

Table 1. FTIR Bands Attribution

wavenumber (cm^{-1})	band attributions
3389	symmetric and asymmetric stretching vibration of O−H
3344−3333 (double vibration)	symmetric and asymmetric stretching vibration of N−H
2963−2857	symmetric and asymmetric stretching vibration of CH_2
1619	bending vibration in plane scissoring of N−H
1457−1253	bending vibration in plane scissoring, out of plan wagging, and out of plan twisting of CH_2
1122	bending vibration of C−O for secondary alcohol
1085	bending vibration out of plan twisting of C−N
1029	stretching vibration of C−O for primary alcohol
875	bending vibration out of plan twisting of N−H
640−559	bending vibration in plan rocking of CH_2

RESULTS AND DISCUSSION

Physicochemical Characteristics of GSA-1, GSA-3, and GSA-6. GSA-1, GSA-3, and GSA-6 were preliminarily characterized by FTIR spectra (**Figure 3**) showing the vibration bands around 1619 cm^{-1}, which were not observed from glucose spectra and thus indicate the presence of N−H bonding. Furthermore, GSA-1 and GSA-6 showed other particular double-vibration bands around 3330 cm^{-1}, suggesting the presence of free amino groups in both molecules (*21*). These vibration bands were absent in the GSA-3 spectrum because of its amino group in the form of ammonium. Vibration bands observed around 1085 and 875 cm^{-1} were attributed to the bending vibrations of C−N and out of plan vibration bands of N−H, respectively. Other vibration bands observed from **Figure 3** were from different pyranosic ring bonds such as C−H, CH_2, C−O, and O−H as indicated in **Table 1**.

To complete the FTIR data and characterize GSA-1, GSA-3, and GSA-6, ^{1}H NMR and ^{13}C NMR spectroscopy were used (**Figures 4**−**6**). As reported in our recent paper (*11*) the chemical shifts of anomeric proton and C-1 of GSA-1 were taken as references in NMR spectroscopies to confirm the fixation of NH_2 group, in comparison to glucose. For this, a signal in the form of doublet of H_β-1 and another of C-1 were obtained at chemical shifts of 4.15 and 85.08 ppm despite 4.65 and 92.3 ppm found for glucopyranose (*22*, *23*). In the synthesis of GSA-3 different melting points obtained from intermediary products conformed to these reported (*12*−*15*, *17*, *24*) and thus suggest purity; however, their further characterization using either FTIR or NMR spectroscopy was needed to complete these literature data. The structure of product **2** was confirmed by a signal at a chemical shift of 208.7 ppm in ^{13}C NMR and by a vibration band at 1729 cm^{-1}, both indicating the presence of a carbonyl group in FTIR (*21*, *25*); this band was disappeared by the complete reduction done with $NaBH_4$ to give the product **3**. Substitution of the hydrogen of OH-3 by a tosyl group was indicated by signals at chemical shifts of 7.8 and 7.3 ppm in the form of doublets, which was attributed to aromatic protons. These signals disappeared when compound **4** was reacted with sodium azide to give compound **5**, which was confirmed by FTIR spectra showing a strong vibration band at 2140 cm^{-1}. This group was characterized in other papers by the vibration bands observed around 2100 cm^{-1} (*4*,*26*). Reduction of the azido group of **5** followed by a deprotection of the hydroxyl group gave GSA-3, which was characterized by both FTIR ad NMR spectroscopies. The FTIR spectrum of this compound showed a vibration band at 1624 cm^{-1}, characterizing a bending vibration in plane scissoring of N−H. This was confirmed by both ^{1}H and ^{13}C NMR spectra. An anomeric proton signal was observed at 5.21 ppm in ^{1}H NMR, which confirmed a total deprotection of OH-1, whereas other protons of GSA-3 were represented by signals in the zone of chemical shifts of 3.85−3.50 ppm, which was confirmed by the finding of Milner et al. (*27*). Furthermore, in ^{13}C NMR, signals of C-1 and C-3 were found at chemical shifts of 97.2 and 60.9 ppm, respectively, and this corroborated those described for this compound (*27*, *28*). For the GSA-6 synthesis, the characterization of intermediary products, compounds **9** and **10**, was conducted as done in the case of GSA-3. For compound **9**, two signals of aromatic protons were found at 7.81 and 7.35 ppm in ^{1}H NMR, whereas the formation of compound **10** was justified by FTIR spectra that showed a vibration band at 2105 cm^{-1} characteristic of an azide group. Reduction of compound **10** was confirmed by the FTIR spectrum of GSA-6, which showed, on the one hand, the disappearance of the vibration at 2105 cm^{-1} and, on the other hand, the appearance of a new absorption at 1607 cm^{-1}, indicating the bending vibration in plane scissoring of N−H. In ^{1}H NMR, the signal of H-1 was found at a chemical shift of 4.8 ppm. Its shifted displacement at low parts per million in comparison of that of α-D-glucopyranose, which was reported to be at 5.23 ppm (*23*), was due to the replacement of OH by OMe in this compound. In addition, protons H-6a and H-6b resonated at lower chemical shifts compared to those of the same protons of GSA-3 or GSA-1, which confirmed the substitution of OH-6 by an NH_2 group in GSA-6 (**Table 2**). Furthermore, in ^{13}C NMR C-6 resonated at a chemical shift of 51.28 ppm, and this corroborated the finding of Murphy et al. (*29*), who characterized a C-6 of 1-methyl-6-deoxy-6-azido-β-D-glucopyranoside, a compound quite similar to GSA-6.

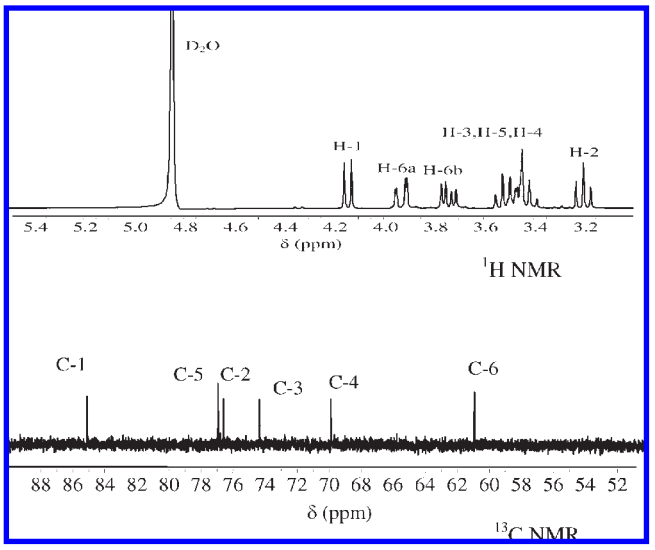

Figure 4. NMR spectra of GSA-1.

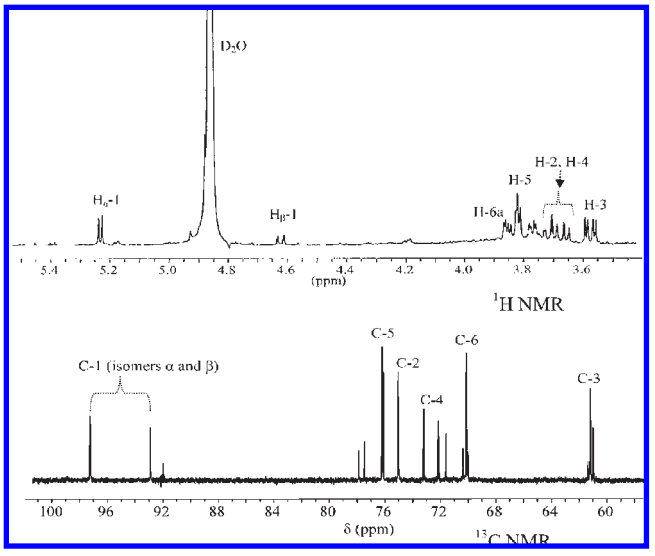

Figure 5. NMR spectra of GSA-3.

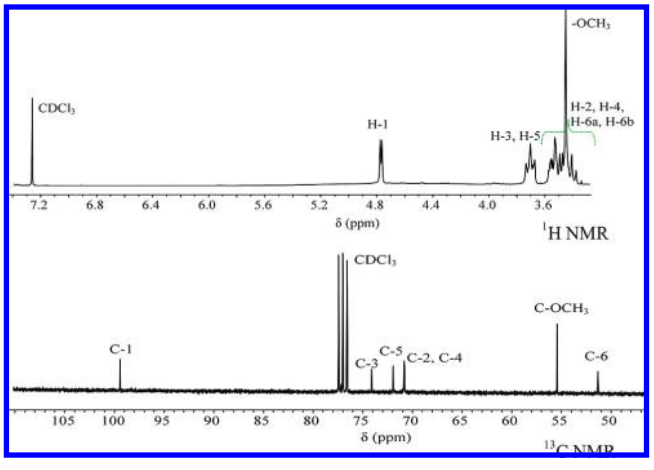

Figure 6. NMR spectra of GSA-6.

Antibacterial Activities of GSA-1, GSA-2, GSA-3, and GSA-6. To determine the effect of amino group position on the antibacterial activity of aminoglucose derivatives, four compounds, GSA-1, GSA-2, GSA-3, and GSA-6, were comparatively tested on two food bacteria, *L. innocua* and *S. typhimurium*. Concentrations ranging from 0.25×10^{-4} to 2×10^{-4} mol mL^{-1} were used in this study.

Table 2. Different Chemical Shifts of C-6, H-6a, and H-6b for GSA-1, GSA-2, and GSA-6

	chemical shifts (ppm)		
type of signal	GSA-1	GSA-3	GSA-6
C-6	60.8	60.9	51.3
H-6a	3.95	3.86−3.82	≤3.56
H-6b	3.76	3.76−3.74	≤3.56

Results showed that GSA-3 was significantly ($p < 0.05$) more anti-*L. innocua* than the other compounds tested (**Table 3**). However, there is no significant difference between the anti-*S. typhimurium* activities of GSA-3 and GSA-6, and these two compounds are significantly ($p < 0.05$) more anti-*S. typhimurium* than GSA-1 and GSA-2 (**Table 4**).

For example, at the concentration of 2×10^{-4} mol mL^{-1} GSA-3 affected the growth of *L. innocua* with a percentage of inhibition of 29%, whereas GSA-1, GSA-2, and GSA-6 exhibited inhibitions close to 5, 11, and 18%, respectively. The same observation was made for *S. typhimurium*, for which GSA-3 altered the microbial growth with 15% of inhibition, whereas GSA-1, GSA-2, and GSA-6 exhibited activities of only 2, 7, and 11% of inhibition, respectively. Thus, the biocide efficiency found in this study decreased in the order GSA-3 ≥ GSA-6 > GSA-2 > GSA-1.

The mechanism of action of these compounds was not determined in this study. However, according to different reports done on aminosugars and similar compounds (*30*, *31*), these types of biocide may exhibit their activity by inhibiting the cell wall and membrane synthesis of bacteria and also the synthesis of proteins or DNA . According to Llewellyn et al. (*31*), aminoglucosides bond at the 30S subunit, which includes 16S NRA, and blocked all of its biological mechanisms and altered the growth of bacteria. Furthermore, other studies done on D-glucosamine showed that its *N*-alkyl derivatives had high affinity with 16S NRA, which played a key role in the translation mechanism (*32*). Considering the high similarity of these compounds with the aminoglucose derivatives used in our study, it is possible that the same mechanism may be considered for the antibacterial activity found in this paper. Comparing the biological activities obtained from GSA-1, GSA-2, GSA-6, and GSA-3 and having in mind that aminoglucosides mainly act by altering the function of ribosomes (*31*), this study suggests that GSA-3 may have more affinity with ribosome of bacteria than other aminoglucoses tested. According to these results, an amino group fixed at position 3 of glucose may increase the biological effects of aminosugar on the growth of bacteria. These findings on the activity of GSA-3 are supported by the literature, where the biological activity of this compound against bacteria was already demonstrated (*27*, *28*, *33*). According to Milner et al. (*27*), GSA-3 delayed the growth of both Gram-positive and Gram-negative bacteria, as, for example, *Lactobacillus acidophilus*, *Staphylococcus aureus*, *Erwinia herbicola*, and *Cytophaga johnsonae*. Some authors (*34*, *35*) have suggested that the compound may act by inhibiting the cell wall synthesis through an inhibition of D-frucose-6-phosphate incorporation in the cell wall and, thus, by avoiding the synthesis of 6-phosphate glucosamine, known as a key product in cell wall reconstruction. The reason for the effect of the amino group position on antibacterial activity is still unknown. However, it is reported that the number and the position of amino groups on sugar rings of antibiotics play important roles in their antibacterial activity (*30*). In fact, a higher activity of kanamycin A, an antibiotic with a 6-aminohexose group, relative to that of kanamycin C, with a 2-aminohexose group, was reported (*30*, *36*). In addition, the presence of kanosamine (GSA-3) and the amino group at C-6 in antibiotic structures is reported to be essential in cell recognition and in the diversification of binding sites, respectively (*36*). These previous reports are in agreement with our results, where GSA-3 and GSA-6 exhibited better antibacterial activity against *L. innocua* and *S. typhimurium* compared to GSA-1 and GSA-2. Furthermore, in this study GSA-3 was synthesized and isolated as an ammonium compound and biologically tested as well. This chemical characteristic of GSA-3 may increase its biological effects on bacteria growth because the impact of ammonium compounds on the growth of bacteria has already been reported (*9*, *37*). The impact of quaternary ammonium on the growth of bacteria is due to its high facility to bind to the cell membrane of bacteria and thus to modify their permeability (*37*). However, even if GSA-3 and GSA-6 are more active than GSA-2 and GSA-1, their biological activity is still to be ameliorated. One way to achieve this is to N-alkylate these molecules or to manage other modifications by multiplying the number of glucosyl and amino groups introduced. The nature and position of pharmacophore groups to be introduced may be of interest because both the position of C-3 and the ammonium form of the amino group have shown high efficiency in the antibacterial activity assessment. GSA-2 is actually the essential element in the biosynthesis of peptidoglycan found in the cytoplasm walls of bacteria (*34*, *35*). These essential physiological roles of GSA-2 may explain its insignificant antibacterial activity against both *L. innocua* and *S. typhimurium* growth. In comparison to chitosan, a known bacterial inhibitor, these monomers exhibited lower antibacterial activity against selected microorganisms. In fact, according to some authors, chitosan exhibited higher antibacterial activity against both strains at the concentration close to 10^{-4} mol mL^{-1}, and at 5×10^{-4} mol mL^{-1} this compound quite completely inhibited the growth of both bacterial strains (*9*). The higher antibacterial activity of chitosan compared to small aminosugars was not studied here. However, its high viscosity and high number of ammonium groups may explain its antibacterial efficacy.

Table 3. Antibacterial Activities of GSA-1, GSA-2, GSA-3, and GSA-6 against the Growth of *L. innocua* at Different Concentrations

	percentage of inhibition ($\times 10^{-4} \pm$ SD) at a concentration of			
aminoglucose	0.25 mol mL^{-1}	0.5 mol mL^{-1}	1 mol mL^{-1}	2 mol mL^{-1}
GSA-1	0.0 ± 0.0	1.1 ± 0.7	3.3 ± 1.0	5.1 ± 3.0
GSA-2	2.2 ± 1.0	7.8 ± 3.0	8.9 ± 4.0	10.6 ± 1.1
GSA-3	10.0 ± 0.4	19.3 ± 1.5	22.8 ± 1.3	29.1 ± 2.5
GSA-6	3.6 ± 1.0	10.0 ± 7.0	12.8 ± 3.0	17.7 ± 1.4

Table 4. Antibacterial Activities of GSA-1, GSA-2, GSA-3, and GSA-6 against the Growth of *S. typhimurium* at Different Concentrations

	percentage of inhibition ($\times 10^{-4} \pm$ SD) at a concentration of			
aminoglucose	0.25 mol mL^{-1}	0.5 mol mL^{-1}	1 mol mL^{-1}	2 mol mL^{-1}
GSA-1	0.0 ± 0.0	0.3 ± 1.0	1.0 ± 1.0	2.3 ± 1.0
GSA-2	0.0 ± 0.0	0.0 ± 0.0	3.7 ± 1.0	7.2 ± 1.0
GSA-3	0.0 ± 0.0	5.8 ± 2.0	10.0 ± 3.0	14.6 ± 1.1
GSA-6	0.0 ± 0.0	4.9 ± 1.0	8.4 ± 2.0	10.5 ± 3.0

In conclusion, GSA-1, GSA-2, GSA-6, and GSA-3 have shown different antibacterial activities. According to these results, further modifications of these molecules may be envisaged to improve their antibacterial activity as well.

ACKNOWLEDGMENT

We thank Fabrice Azemar (student in training in US2B, University of Bordeaux 1) for technical assistance.

LITERATURE CITED

(1) Altieri, C.; Siniganglia, M.; Corbo, M. R.; Buonocor, G. G.; Falcone, P.; Del Nobile, M. A. Use of entrapped microorganisms as biological oxygen scavengers in food packaging applications. *Lebensm.-Wiss. -Technol.* **2004**, *37*, 9–15.

(2) Vartiainen, J.; Skytta, E.; Enqvist, J.; Ahvenainen, R. Properties of antimicrobial plastics containing traditional food preservatives. *Packag. Technol. Sci.* **2003**, *16*, 223–229.

(3) Soares, F. F.; Rutishauser, D. M.; Melo, N.; Cruz, R. S.; Andrade, N. J. Inhibition of microbial growth in bread through active packaging. *Packag. Technol. Sci.* **2002**, *15*, 129–132.

(4) Lee, C. H.; An, D. S.; Park, H. J.; Lee, D. S. Wide-spectrum antimicrobial packaging materials incorporating nisin and citosan in the coating. *Packag. Technol. Sci.* **2003**, *16*, 99–106.

(5) Vermeiren, L.; Devlieghere, F.; van Beest, M.; de Kruijf, N.; Debevere, J. Developments in the active packaging of foods. *Tends Food Sci. Technol.* **1999**, *10*, 77–86.

(6) 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.

(7) 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.

(8) 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.

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

(10) Likhosherstov, L. M.; Novikova, O. S.; Shibaev, V. N. New efficient synthesis of β-glucosylamines of mono- and disaccharides with the use of ammonium carbamate. *Dokl. Chem.* **2002**, *383* (4), 500–503.

(11) 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.

(12) Blakley, E. R. 6-Deoxy-6-fluoro-D-glucose. *Biochem. Prep.* **1960**, *7* (39), 40–44.

(13) Williams, D. T.; Jones, J. K. N. A new synthesis of 3-acetamido-3-deoxy-D-glucose. *Can. J. Chem.* **1967**, *45* (7), 7–9.

(14) Brimacombe, J. S.; Bryan, J. G. H.; Husain, A.; Stacey, M.; Tolley, M. S. The oxidation of some carbohydrate derivatives, using acid anhydride methyl sulphoxide mixtures and the Pfitzner−Moffatt reagent. Facile synthesis of 3-acetamido-3-deoxy-D-glucose and 3-amino-3-deoxy-D-xylose. *Carbohydr. Res.* **1967**, *3*, 318–324.

(15) Jary, J.; Kefurtova, Z.; Kovar, J. Aminosugars XX. Synthesis of 3-amino- and 6-amino-deoxyhexoses of the gluco and allo configuration. *Collect. Czech. Chem. Commun.* **1969**, *34*, 1452–1458.

(16) Yu, Y.; Russell, R. N.; Thorson, J. S.; Liu, L.; Liu, H. Mechanistic studies of the biosynthesis of 3,6-dideoxyhexoses in *Yersinia pseudotuberculosis*. *J. Biol. Chem.* **1992**, *267* (9), 5868–5875.

(17) Saito, Y.; Zevaco, T. A.; Agrofoglio, L. A. Chemical synthesis of ^{13}C labeled anti-HIV nucleosides as mass-internal standards. *Tetrahedron* **2002**, *58*, 9593–9603.

(18) Guo, J.; Frost, J. W. Kanosamine biosynthesis: a likely source of the aminoshikimate pathway's nitrogen atom. *J. Am. Chem. Soc.* **2002**, *124*, 10642–10643.

(19) Kondo, Y. Partial tosylation of methyl α- and β-D-galactospyranosides. *Agric. Biol. Chem.* **1988**, *52* (5), 1313–1315.

(20) Li, S. C.; Bao, X.; Cai, M. S.; Li, Z. J. Optimazed procedure for synthesis of 6-azido-6-deoxy-galactopyranosides from 6-octosyl-galactopyranosides. *Synth. Commun.* **2006**, *36* (5), 637–643.

(21) Silverstein, R. M.; Basler, G. C.; Morill, T. C. *Identification Spectrométrique de Composés Organiques*; De Boeck Université: Bruxelles, Belgium, 1998; 420 pp.

(22) Lubineau, A.; Augé, J.; Drouillat, B. Improved synthesis of glycosylamines and a straight forward preparation of *N*-acylglycosylamines as carbohydrate-based detergents. *Carbohydr. Res.* **1995**, *266*, 211–219.

(23) Blasko, A.; Bunton, A. B.; Bunel, S.; Ibarra, C.; Moraga, E. Determination of acid dissociation constants of anomers of amino sugars by ^1H NMR spectroscopy. *Carbohydr. Res.* **1997**, *298*, 163–172.

(24) Trnika, T.; Cerny, M.; Budesinsky, M.; Pacak, J. The synthesis of 3-amino-3-deoxy-D-glucose (kanosamine) and its 1,6-anhydro derivative conformation of amino derivatives of 1,6-anhydro-β-D-hexopyranoses. *Collect. Czech. Chem. Commun.* **1975**, *40*, 3038–3045.

(25) Pretsch, E.; Clerc, T.; Seibl, J.; Simon, W. *Tablas para la Elucidacion Estructural de Compuestos Organicos por Metodos Espectroscopicos*; Springer Verlag: Berlin, Germany, 1989; pp C95−U155.

(26) Borjihan, G.; Zhong, G.; Baigude, H.; Nakashima, H.; Uryu, T. Synthesis and anti-HIV activity of 6-amino-6-deoxy-(1→3)-β-D-curdlan sulfate. *Polym. Adv. Technol.* **2003**, *14*, 326–329.

(27) Milner, J. J.; Silo-Suh, L.; Lee, J. C.; He, H.; Clardy, J.; Handelsman, J. Production of kanosamine by *Bacillus cereus* UW85. *Appl. Environ. Microbiol.* **1996**, *62* (8), 3061–3065.

(28) Dolak, L. A.; Castle, T. M.; Dietz, A.; Laborde, A. L. 3-Amino-3-deoxyglucose produced by a *Streptomyces* sp. *J. Antibiot.* **1980**, *33* (8), 900–901.

(29) Murphy, P. V.; O'Brien, J. L.; Gorey-Feret, L. J.; Smith, A. B. Synthesis of novel HIV-1 protease inhibitors based on carbohydrate scaffolds. *Tetrahedron* **2003**, *58*, 2259–2271.

(30) Benveniste, R.; Davies, J. Structure−activity relationships among the aminoglycoside antibiotics: role of hydroxyl and amino groups. *Antimicrob. Agents Chemother.* **1973**, *4* (4), 402–409.

(31) Llewellyn, N. M.; Spencer, J. B. Biosynthesis of 2-deoxystreptamine containing aminoglycoside antibiotics. *Nat. Prod. Rep.* **2006**, *23*, 864–874.

(32) Wu, B.; Yang, J.; Robinson, D.; Hofstadler, S.; Griffey, R.; Swayze, E. E.; He, Y. Synthesis of linked carbohydrates and evaluation of their binding for 16S RNA by mass spectrometry. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3915–3918.

(33) Janiak, A. M.; Milewski, S. Mechanism of antifungal action of kanosamine. *Med. Mycol.* **2001**, *39* (5), 401–408.

(34) Iwai, Y.; Tanaka, H.; Oiwa, R.; Shimizu, S.; Omura, S. Studies on bacterial cell wall inhibitors. III. 3-Amino-3-deoxy-D-glucose, an inhibitor of bacterial cell wall synthesis. *Biochim. Biophys. Acta* **1977**, *498*, 223–228.

(35) Tanaka, H.; Shimizu, S.; Oiwa, R.; Iwai, Y.; Omura, S. The site of inhibition of cell wall synthesis by 3-amino-3-deoxy-D-glucose in *Staphylococcus aureus*. *J. Biochem.* **1979**, *86* (1), 155–159.

(36) Le Goffic, F.; Capmau, M.; Tangy, F.; Baillarge, M. Mechanism of action of aminoglycoside antibiotics. *Eur. J. Biochem.* **1979**, *102*, 73–81.

(37) Kim, C. H.; Choi, J. W.; Chun, H. J.; Choi, K. S. Synthesis of chitosan derivatives with quaternary ammonium salt and their antibacterial activity. *Polym. Bull.* **1997**, *38*, 387–393.

Received December 19, 2008. Accepted July 17, 2009. We are very grateful to the Ministry of Foreign Affairs of the French government for funding.