Novel Glucose-Derived Gemini Surfactants with a 1,1′-Ethylenebisurea Spacer: Preparation, Thermotropic Behavior, and Biological Properties

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ABSTRACT: In the search for environmentally safe surfactants made from inexpensive and renewable sources, the interest has mainly been focused on new saccharide derivatives. This report describes the synthesis of newly designed nonionic gemini compounds comprising two reduced sugar headgroups, two alkyl tails, and a 1,1′-ethylenebisurea entity as the spacer linking two amphiphilic glucose-derived moieties. Thus, the series of N,N′-bis[(3-alkyl-3-deoxy-D-glucitol)ureido]ethylenediamines (bis(C_nGT), with C_n = *n*-C_4H_9, *n*-C_6H_{13}, *n*-C_8H_{17}, *n*-$C_{10}H_{21}$, or *n*-$C_{12}H_{25}$), were prepared using a convenient procedure starting from easily accessible reagents such as D-glucose, *n*-alkylamines, urea, and ethylenediamine. Their structure and purity were confirmed by means of elemental analysis, electrospray ionization mass spectrometry, and ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy. Additionally, the present contribution introduces selected properties of these surfactants, including their thermotropic behavior and biological properties. The presence of two phase transition points, determined using the differential scanning calorimetry method, indicates liquid-crystalline mesophase formation upon heating. Furthermore, using the closed-bottle test (OECD Guideline 301D) as well as the biological oxygen demand test for insoluble substances for biodegradability measurements, it has been concluded that the tested glucose-derived gemini structures achieve more than 60% biodegradation after 64–75 test days. All tested surfactants were practically nontoxic to bacteria, yeast, and molds. Owing to their fitting aggregation ability as well as their nontoxicity, they constitute an interesting group of surfactants for various applications.

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KEY WORDS: Antimicrobial activity, biodegradability, DSC, 1,1′-ethylenebisurea derivatives, nonionic glucose-derived gemini surfactants, thermotropic behavior.

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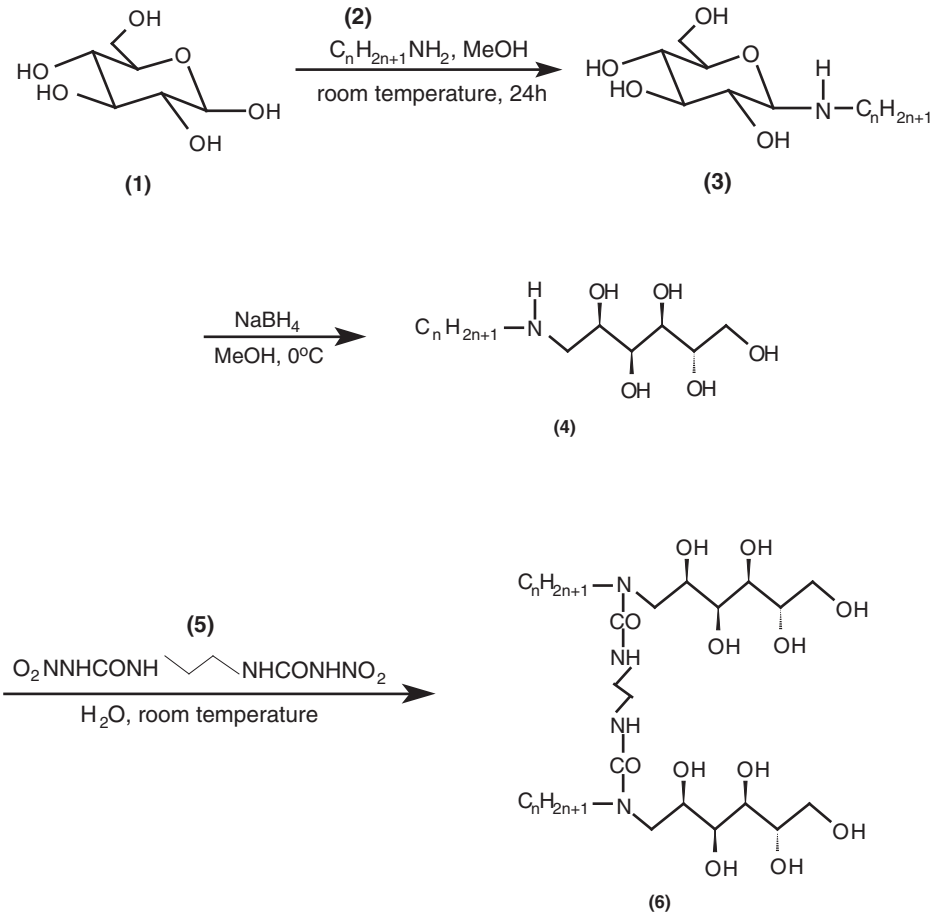
Abbreviations: bis(C_nGT), N,N′-bis[(3-alkyl-3-deoxy-D-glucitol)ureido]ethylenediamines; BOD, biological oxygen demand; BODIS, biological oxygen demand test for insoluble substances; cfu, colony forming units; DSC, differential scanning calorimetry; ESI-MS, electrospray ionization mass spectrometry; MIC, minimal inhibitory concentration; NMR, nuclear magnetic resonance; PCM, Polish Collection of Microorganisms; TOD, theoretical oxygen demand

Over the past decade, there has been an increasing interest in the creation of novel amphiphilic materials that possess structural motifs borrowed from biological systems (carbohydrates, peptides, and nucleic acid polymers). Such representatives belong to the "green chemistry" area. The incorporation of such bio-inspired groupings into the amphiphile structure results in new and interesting physicochemical and biological functionality (1–5). Sugar surfactants are the most prominent of these new groupings because of both their biocompatibility and the broad spectrum of their molecular architecture: the hydrophilic headgroup could consist of innumerable carbohydrate moieties, showing both stronger lipophobicity and stronger hydrophilicity as compared with surfactants with oligoethylene oxide headgroups; and the hydrophobic part could consist of one, two, or even more chains (e.g., hydrocarbon chains of fatty acids, long-chain fatty alcohols, and fatty amines) (5–15). Moreover, they can be synthesized by linking hydrophilic and hydrophobic moieties in different ways, which would imply, on one hand, varying surfactant stability and broadening application areas, and on the other hand, a strong demand for new preparation methodologies. Most of the methods described in the literature are examples of nonselective synthetic approaches (15–17); they often require special activation procedures (5,18), prior protections of the sugar substrates (18), or selective catalysis by enzymes (19–21), and, furthermore, result in long and costly preparation routes. The key step of amphiphilic carbohydrate syntheses is the covalent coupling of a hydrophilic sugar with a lipophilic unit through either an oxygen (ether, ester) (3–5,19–21) or a nitrogen (amine, amide) (5–15) linkage. These linkages possess various degrees of ionization or polarizability.

Following the well-known preparation procedures for "classical" structures of sugar amphiphiles, researchers have attempted to design more complex molecular architectures, including gemini (or dimeric) ones (18–31). The present contribution describes a dimeric environmentally acceptable structure, recently designed in our laboratory, that forms highly organized supramolecular aggregates at interfaces. According to literature data, short-chain saccharide-

SCHEME 1



bis(C_nGT)

C_nH_{2n+1}	Abbreviation
C_4H_9	bis(C_4GT)
C_6H_{13}	bis(C_6GT)
C_8H_{17}	bis(C_8GT)
$C_{10}H_{21}$	bis(C_{10}GT)
$C_{12}H_{25}$	bis(C_{12}GT)

SCHEME 1. Synthetic route of N,N′-bis[(3-alkyl-3-deoxy-D-glucitol)ureido]ethylenediamines, bis(C_nGT).

based dimeric surfactants have been obtained by linking suitable butyl glucopyranosides with various acyl dichlorides. However, such a preparation route demands prior protection of the carbohydrate hydroxyl groups to avoid obtaining a mixture of products (18). In our previous papers (22,23) we described a new family of bisaldobionamide gemini compounds, in which the saccharide lactone or acid residues are linked to the dimeric molecule through the amide groups. Another class of sugar gemini amphiphiles has been synthesized from D-glucose without any prior protection of carbohydrates. In this case, hydrophobic tails have been incorporated into the bolaform diamines that bear two terminal reduced sugar moieties, either by reductive alkylation with aliphatic *n*-aldehydes (24,25) or by acylation with anhydride (26) or with methyl esters of fatty acids, the latter being a subject of our patents (27,28). It has also been reported in the literature that the synthesis of sugar-based gemini structures can also employ a combination of enzymatic and chemical methods: partially protected mono- and disaccharides have been regioselectively acylated using lipases, and the products have been coupled using conventional chemistry to obtain sugar dimeric products in which the hydrocarbon tails are connected with carbohydrate entities via the ester bonds (19–21).

The main purpose of this work is to report on a relatively simple and practical synthesis of new environmentally friendly gemini surfactants (structure and abbreviations shown in Scheme 1), as well as to describe their thermotropic and ecological characteristics. Thus, N,N′-bis[(3-alkyl-3-deoxy-D-glucitol)ureido]ethylenediamines were designed based on easily accessible, inexpensive, and renewable materials, such as D-glucose and *n*-alkylamines, derived from fats and plants. Furthermore, the surfactant dimeric structure bearing two reduced saccharide headgroups and two alkyl chains of various lengths, was enhanced by incorporation of a spacer in the form of a 1,1′-ethylenebisurea entity, which can promote gel formation in a variety of solvents by means of a hydrogen-bonding network (29–32). Differential scanning calorimetry (DSC) revealed the thermotropic phase behavior of all of the studied products. The present contribution also considers how changes in the molecular structure of the compounds under study affect their antimicrobial properties and biodegradability, which are the crucial factors in determining their influence on the environment.

EXPERIMENTAL PROCEDURES

Materials. All starting materials and solvents were the highest grade available commercially, and were dried or freshly distilled as required. D(+)-Glucose, ethylenediamine, and *n*-alkylamines (*n*-butylamine, *n*-hexylamine, *n*-octylamine, *n*-decylamine, and *n*-dodecylamine) were purchased from Fluka Chemie GmbH (Buchs, Switzerland), and urea and $NaBH_4$ were purchased from Aldrich Chemical Co. (Milwaukee, WI). Pyrene was purchased from Molecular Probes, Inc. (Eugene, OR). Water used for all experiments was doubly distilled and purified by means of a Millipore (Bedford, MA) Milli-Q purification system.

*Synthesis of 3,3′-dinitro-1,1′-ethylenebisurea (**5**).* First, starting from urea and ethylenediamine, a known procedure (33) was applied to obtain 3,3′-dinitro-1,1′-ethylenebisurea (5) with a 90% yield, m.p. 197–199°C. The elemental analysis calculated for $C_4H_8N_6O_6$ was C, 20.34; H, 3.41; and N, 35.59. The elemental analysis found for the prepared samples was C, 20.47; H, 3.80; and N, 35.65.

*Synthesis of N-alkyl-1-amino-1-deoxy-D-glucitol (**4**): general procedure.* Following the method described by Rico-Lattes and Lattes (17), a solution of 0.25 mol of D-glucose (1) and 0.25 mol of the proper *n*-alkylamine (2) in 500 mL of methanol was stirred at room temperature for 24 h. The reaction mixture was then cooled to 0°C, and a small molar excess of $NaBH_4$ (10.21 g, 0.27 mol) was added stepwise. The reaction was then maintained at room temperature until the hydrogen evolution ceased. After reduction, concentrated HCl was added dropwise to a pH of 2–3; then the product was converted into its hydrochloride salt, cooled in a refrigerator, filtered, rinsed with a small amount of ice water and ice-cold ethanol, and dried *in vacuo* over KOH. The dried precipitate was stirred with methanol and a slight molar excess of sodium methylate for 24 h at room temperature. Then the stirring was continued and the reaction mixture was heated under reflux to decompose the hydrochloride salt and liberate the pure product. After cooling, the white precipitate was isolated, washed with a small amount of ice water and ice-cold methanol, and dried.

*N-Butyl-1-amino-1-deoxy-D-glucitol (**4a**).* The elemental analysis calculated for $C_{10}H_{23}NO_5$ was C, 50.62; H, 9.77; and N, 5.90. The elemental analysis found for the prepared sample was C, 50.56; H, 9.81; and N, 5.93. The structure was verified by ^{1}H nuclear magnetic resonance (NMR) ($CDCl_3$): δ = 0.89 (t, 3H, C<u>H</u>$_3$, *J* = 6.71 Hz); δ = 1.32 (m, 2H, $CH_3C\underline{H}_2$); δ = 1.41–1.57 (m, 2H, N-$CH_2C\underline{H}_2CH_2$); δ = 3.38–3.44 (m, 2H, N-C<u>H</u>$_2CH_2$); δ = 3.54–3.72 (m, 8H, C<u>H</u>, C<u>H</u>$_2$, sugar); and δ = 4.18–5.23 (m, 5H, O<u>H</u>, carbohydrate).

*N-Hexyl-1-amino-1-deoxy-D-glucitol (**4b**).* The elemental analysis calculated for $C_{12}H_{27}NO_5$ was C, 54.32; H, 10.25; and N, 5.28. The elemental analysis found for the prepared sample was C, 54.25; H, 10.31; and N, 5.21. The structure was verified by ^{1}H NMR ($CDCl_3$): δ = 0.87 (t, 3H, C<u>H</u>$_3$, *J* = 6.67 Hz); δ = 1.26 (m, 6H, $CH_3(C\underline{H}_2)_3$); δ = 1.45–1.58 (m, 2H, N-CH2CH2(CH2)3); δ = 3.42–3.54 (m, 2H, N-C<u>H</u>$_2CH_2$); δ = 3.62–3.79 (m, 8H, C<u>H</u>, C<u>H</u>$_2$, sugar); and δ = 3.98–4.53 (m, 5H, O<u>H</u>, carbohydrate).

*N-Octyl-1-amino-1-deoxy-D-glucitol (**4c**).* The elemental analysis calculated for $C_{14}H_{31}NO_5$ was C, 57.31; H, 10.65; and N, 4.78. The elemental analysis found for the prepared sample was C, 57.24; H, 10.71; and N, 4.71. The structure was verified by ^{1}H NMR ($CDCl_3$): δ = 0.90 (t, 3H, C<u>H</u>$_3$, *J* = 6.57 Hz); δ = 1.35 (brs, 10H, $CH_3(C\underline{H}_2)_5$); δ = 1.47–1.56 (m, 2H, N-$CH_2C\underline{H}_2(CH_2)_5$); δ = 3.28–3.46 (m, 2H, N-C<u>H</u>$_2CH_2$); δ = 3.52-3.67 (m, 8H, C<u>H</u>, C<u>H</u>$_2$, sugar); and δ = 3.81–5.12 (m, 5H, O<u>H</u>, carbohydrate).

*N-Decyl-1-amino-1-deoxy-D-glucitol (**4d**).* The elemental analysis calculated for $C_{16}H_{35}NO_5$ was C, 59.78; H, 10.97; and N, 4.36. The elemental analysis found for the prepared sample was C, 59.70; H, 11.06; and N, 4.30. The structure was verified by ^{1}H NMR (CDCl3): δ = 0.80 (t, 3H, C<u>H</u>$_3$, J = 6.42 Hz); δ = 1.16 (brs, 14H, $CH_3(C\underline{H}_2)_7$); δ = 1.41–1.66 (m, 2H, N-$CH_2C\underline{H}_2(CH_2)_7$); δ = 3.37–3.49 (m, 2H, N-$C\underline{H}_2CH_2$); δ = 3.59–3.70 (m, 8H, C<u>H</u>, C<u>H</u>$_2$, sugar); and δ = 4.09–5.19 (m, 5H, O<u>H</u>, carbohydrate).

*N-Dodecyl-1-amino-1-deoxy-D-glucitol (**4e**).* The elemental analysis calculated for $C_{18}H_{39}NO_5$ was C, 61.86; H, 11.25; and N, 4.01. The elemental analysis found for the prepared sample was C, 61.78; H, 11.30; and N, 3.98. The structure was verified by ^{1}H NMR ($CDCl_3$): δ = 0.89 (t, 3H, CH_3, J = 6.65 Hz); δ = 1.19 (brs, 18H, $CH_3(CH_2)_9$); δ = 1.42–1.49 (m, 2H, N-$H_2CH_2(CH_2)_9$); δ = 3.27–3.42 (m, 2H, N-CH_2CH_2); δ = 3.53–3.72 (m, 8H, CH, CH_2, sugar); and 4.10–5.21 (m, 5H, OH, carbohydrate).

*Synthesis of N,N′-bis[(3-alkyl-3-deoxy-D-glucitol)ureido]ethylenediamines (**6**): general procedure.* The obtained N-alkyl-1-amino-1-deoxy-D-glucitols (0.2 mol) were each dissolved in a small amount of water and 3,3′-dinitro-1,1′-ethylenebisurea (23.61 g, 0.1 mol) was added stepwise. The temperature increased spontaneously, and the reaction mixture was subsequently heated from 60 to 85°C during approximately 20 min. Intense evolution of N_2O was observed. External water cooling was used as needed. After the given time, the reaction mixture was heated to 90–100°C until the evolution of gas subsided. The obtained liquid was then heated with 0.2 g of activated charcoal and, after hot-filtration, the majority of the water was removed. The residue was mixed with 50 mL of ethanol and it was heated until completely dissolved. After cooling, white crystals precipitated. The crystals were isolated by filtration, rinsed with ice water, dried in the open air, and purified by recrystallization from ethanol. Spectroscopic data are summarized in Table 1.

Analytical methods. Elemental analyses were carried out using a PerkinElmer (Norwalk, CT) 2400 CHN analyzer. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker (Karlsruhe, Germany) AMX-300 spectrometer. ^{1}H chemical shifts (determined at 300.13 MHz) and ^{13}C chemical shifts (determined at 75.5 MHz) are given in ppm, and, depending on the solvent used, are referenced to residual protons of dimethyl sulfoxide (DMSO)-d_6 (2.50 ppm) or to ^{13}C DMSO-d_6 signals. Electrospray ionization (ESI) spectra were obtained with a Finnigan (San Jose, CA) TSQ-700 mass spectrometer. Surface tensions (mN/m) of 0.1 wt% aqueous solutions were measured at 25°C with a Krüss K12e processor tensiometer equipped with a du Nouy Pt-Ir ring. The results are shown in Table 1.

Thermotropic phase behavior. DSC was employed to characterize the thermotropic behavior and to define the phase transition temperatures. The DSC measurements were performed with a Perkin Elmer Differential Scanning Calorimeter DSC7 calibrated with the melting points of high-purity indium (156.60°C) and cyclohexane (6.54°C). The purge gas was nitrogen at a flow rate of 1 dm^3/h. Solid samples (10–12 mg) were introduced into 50-mL aluminum cells and then sealed and heated from 0 to 180°C with scanning rates of 20°C/min. An empty cell was used as a reference.

Antimicrobial activity. The antimicrobial activity of N,N′-bis[(3-alkyl-3-deoxy-D-glucitol)ureido]ethylenediamines was evaluated by the dilution series method in solid media (34). Nutrient agar and mycological agar were used for bacteria and fungi, respectively. A double dilution series of tested surfactants was prepared in water, and 0.5 mL of each concentration was added to 9.5 mL of the warm medium to give a final concentration range from 4 to 512 μg/mL. Then the media were poured into the sterile Petri dishes, and, after they solidified, were inoculated with 10 μL of tested cells or spores. Inocula of the bacteria and yeast were prepared by growing the microorganisms overnight in the liquid medium and diluting cultures to approximately 10^8 cfu (colony forming units) per mL.

All bacterial strains used for determination of antimicrobial activity were obtained from the Polish Collection of Microorganisms (PCM). Three gram-positive bacterial species (*Staphylococcus aureus* PCM 1944, *Sarcina lutea* PCM 1947, and *Bacillus subtilis* PCM1949), three gram-negative bacterial species (*Escherichia coli* PCM 2957, *Serratia marcescens* PCM 549, and *Pseudomonas putida* PCM 2124), three yeasts (*Saccharomyces cerevisiae*, *Candida albicans*, and *Rhodotorula glutinis*), and three mold strains (*Penicillum citrinum*, *Aspergillus niger*, and *Botrytis cinerea*) were used for the test. The incubation was carried out for 48 h at 37°C for gram-positive bacteria or 72 h at 28°C for the other species. The antimicrobial activity of the studied compounds was determined on the basis of their minimum inhibitory concentrations (MIC), defined as the lowest concentration of surfactant at which the microorganisms tested do not show visible growth.

Biodegradability tests. Biodegradability of the gemini sugar-derived surfactants studied was examined using the closed-bottle test according to OECD Test Guideline 301D (35), the two-phase closed-bottle test (ISO/DIS 10708) known as the BODIS test (i.e., biological oxygen demand test for insoluble substances) (36), conventionally inoculated with various inoculi, including sewage plant or activated sludge, and also in the prolonged closed-bottle test (37). The BODIS test conditions, such as medium and duration, were identical with those of OECD Test Guideline 301D. In contrast to the closed-bottle test in OECD Test Guideline 301D, however, the flasks were only about two-thirds filled with the test medium. In this experiment, the test medium, gas phase, and studied surfactants were continuously mixed by shaking. Biodegradability was determined by measuring the biological oxygen demand (BOD), similar to the closed-bottle test in OECD Test Guideline 301D. The value of the BOD for the test substance was corrected by subtracting the BOD value of the control without the test substance. All BOD mea-

TABLE 1
Spectroscopic Data, Krafft Temperatures, and Surface Tension Values of N,N′-Bis[(3-alkyl-3-deoxy-D-glucitol)ureido]ethylenediamines

Surfactant[a] [formula]	Elemental analysis found [calculated]	Chemical shifts δ (ppm) [J (Hz)] ^{1}H NMR	Chemical shifts δ (ppm) [J (Hz)] ^{13}C NMR	ESI-MS[a] (MH^+)	Krafft temp[a](°C)	γ^b (mN/m)
bis(C_4GT) [$C_{24}H_{50}N_4O_{12}$]	C: 49.13 [49.21] H: 8.59 [8.51] N: 9.55 [9.59]	0.86 (T, 6H, CH_3) [J = 6.64 Hz]; 1.28 (M, 4H, CH_3CH_2); 1.46–1.51 (M, 4H, N-$CH_2CH_2CH_2$); 3.42–3.49 (M, 4H, N-CH_2CH_2); 3.10–3.18 (M, 4H, CONHCH_2); 8.02 (brs, 2H, CONHCH_2); 3.50–3.77 (M,16H, CH, CH_2, sugar); 4.22–5.18 (M, 10H, OH, carbohydrate)	158.70 (C=O); 75.02, 74.20, 72.64, 69.95 ($CH(OH)_4$); 63.60 (CH_2OH); 52.25 (CH_2N); 47.93 (CH_2N); 32.56 ($NHCH_2$); 29.61–22.52 (CH_2 of alkyl group); 14.40 (CH_3)	587.0	<0	70.01
bis(C_6GT) [$C_{28}H_{58}N_4O_{12}$]	C: 52.32 [52.40] H: 9.09 [9.02] N: 8.72 [8.77]	0.79 (T, 6H, CH_3) [J = 6.74 Hz]; 1.19 (M, 12H, $CH_3(CH_2)_3$); 1.50–1.54 (M, 4H, N-$CH_2CH_2(CH_2)_3$); 3.34–3.41 (M, 4H, N-CH_2CH_2); 3.29–3.32 (M, 4H, CONHCH_2); 8.45 (brs, 2H, CONHCH_2); 3.48–3.58 (M, 16H, CH, CH_2, sugar); 3.63–4.09 (M, 10H, OH, carbohydrate)	159.01 (C=O); 74.72, 72.12, 71.65, 69.70 ($CH(OH)_4$); 63.71 (CH_2OH); 52.64 (CH_2N); 48.06 (CH_2N); 32.17 ($NHCH_2$); 29.74–22.58 (CH_2 of alkyl group); 14.32 (CH_3)	642.1	<0	55.59
bis(C_8GT) [$C_{32}H_{66}N_4O_{12}$]	C: 54.99 [55.06] H: 9.52 [9.46] N: 8.01 [8.06]	0.82 (T, 6H, CH_3) [J = 6.46 Hz]; 1.26 (brs, 20H, $CH_3(CH_2)_5$); 1.43–1.50 (M, 4H, N-$CH_2CH_2(CH_2)_5$); 3.37–3.43 (M, 4H, N-CH_2CH_2); 3.17–3.22 (M, 4H, CONHCH_2); 8.16 (brs, 2H, CONHCH_2); 3.38–3.49 (M, 16H, CH, CH_2, sugar); 3.76–4.99 (M, 10H, OH, carbohydrate)	159.10 (C=O); 75.01, 74.24, 72.51, 70.06 ($CH(OH)_4$); 63.73 (CH_2OH); 51.19 (CH_2N); 47.98 (CH_2N); 32.61 ($NHCH_2$); 29.45–22.63 (CH_2 of alkyl group); 14.43 (CH_3)	699.8	<0	51.77
bis(C_{10}GT) [$C_{36}H_{74}N_4O_{12}$]	C: 57.27 [57.33] H: 9.88 [9.82] N: 7.42 [7.46]	0.86 (T, 6H, CH_3) [J = 6.38 Hz]; 1.22 (brs, 28H, $CH_3(CH_2)_7$); 1.49–1.57 (M, 4H, N-$CH_2CH_2(CH_2)_7$); 3.32–3.38 (M, 4H, N-CH_2CH_2); 3.20–3.27 (M, 4H, CONHCH_2); 8.25 (brs, 2H, CONHCH_2); 3.52–3.64 (M, 16H, CH, CH_2, sugar); 3.93–5.28 (M, 10H, OH, carbohydrate)	158.96 (C=O); 74.13, 72.37, 70.81, 69.98 ($CH(OH)_4$); 63.70 (CH_2OH); 52.02 (CH_2N); 48.12 (CH_2N); 32.24 ($NHCH_2$); 29.57–22.51 (CH_2 of alkyl group); 14.33 (CH_3)	754.1	<0	47.95
bis(C_{12}GT) [$C_{40}H_{82}N_4O_{12}$]	C: 59.23 [59.28] H: 10.19 [10.11] N: 6.91 [6.95]	0.84 (T, 6H, CH_3) [J = 6.32 Hz]; 1.23 (brs, 36H, $CH_3(CH_2)_9$); 1.40–1.44 (M, 4H, N-$H_2CH_2(CH_2)_9$); 3.30–3.32 (M, 4H, N-CH_2CH_2); 3.06–3.14 (M, 4H, CONHCH_2); 8.41 (brs, 2H, CONHCH_2); 3.45–3.69 (M, 16H, CH, CH_2, sugar); 4.31–5.01 (M, 10H, OH, carbohydrate)	159.14 (C=O); 73.02, 72.82, 71.86, 69.60 ($CH(OH)_4$); 63.76 (CH_2OH); 50.12 (CH_2N); 47.82 (CH_2N); 31.77 ($NHCH_2$); 29.64–22.55 (CH_2 of alkyl group); 14.38 (CH_3)	812.2	<0	44.13

[a]bis(C_nGT) and ESI-MS denote, respectively N,N′-bis[(3-alkyl-3-deoxy-D-glucitol)ureido]ethylenediamines, where n=4,6,8,10,12, GT=glucitol and Electrospray Ionization Mass Spectroscopy.
[b]Measured for 0.1 wt% solution

surements were made by titration for dissolved oxygen following the azide modification of the Winkler method. Theoretical oxygen demand (TOD) was calculated according to the International Standard ISO 9408 (annex A). Biodegradability was calculated using the following formula:

$$\text{biodegradability } (\%) = \text{BOD}/\text{TOD} \times 100 \qquad [1]$$

RESULTS AND DISCUSSION

The designed N,N′-bis[(3-alkyl-3-deoxy-D-glucitol)ureido]ethylenediamines (alkyl: *n*-butyl, *n*-hexyl, *n*-octyl, *n*-decyl, or *n*-dodecyl), referred to as bis(C_nGT), comprise a promising group of surfactants. We are continuing to investigate their surface properties in detail. All bis(C_nGT) dissolve only on heating and vigorous stirring, which may indicate extensive intra- and intermolecular hydrogen bonding between the D-glucitol hydroxyl groups and the amide groups from the urea-derived entities. The Krafft points of aqueous solutions of all compounds studied lie below 0°C (see Table 1); in each case no precipitation of surfactant crystals was observed at the lowest temperature.

Additionally, this bis(C_nGT) series showed no cloud points in their 0.1 wt% aqueous solutions with increasing temperature, even to the boiling point of the solutions. This finding is in agreement with the observations reported by other authors for nonionic surfactants containing a saccharide moiety in their structures (3–5). This is evidence that phase separation at higher temperatures, which is a common feature of oxyethylene-based surfactants, does not occur in these systems. For the *n*-dodecyl derivative, however, thickening and gelation phenomena were observed at 85°C.

Synthesis of glucitol-based gemini surfactants. A series of bis(C_nGT) (n = 4, 6, 8, 10, 12) was synthesized following the straightforward three-step pathway from readily available and bio-based reagents (Scheme 1). In many cases, the selection of the starting material can be the most significant factor in determining the impact of the synthesis on the environment. Because of this, naturally formed D-glucose was selected as the starting material. In the first stage of the preparation procedure, the reductive amination of the carbohydrate substrate (**1**) was carried out with the appropriate *n*-alkylamine (**2**). The preferential addition of *n*-alkylamine at C-1 of the carbohydrate molecule in the absence of steric effects—thereby allowing the synthesis to start from unprotected sugar—has been known for some time (18). This is an evident benefit of the applied procedure with respect to the "green chemistry" principles, which assume that unnecessary derivatization (blocking group, protection/deprotection) should be avoided whenever possible (38). The reduction to open-ring sugar form was performed using sodium borohydride under laboratory conditions (methanol, 0°C, 24 h). To cleave the boron complexes formed during the reduction, the intermediate products were acidified with HCl, and N-alkyl-1-amino-deoxy-D-glucitols (**4**) were obtained in the forms of their hydrochloride salts. The yields after both treatment with sodium alkoxide and recrystallization ranged from 63 to 78%. However, the use of H_2 on the C/Pd catalyst or of Raney nickel as the reduction agent could make the reaction more efficient and applicable under industrial conditions. Moreover, the latter maximizes the incorporation of all materials used in the process into the final product. In the key coupling step, the resultant amines were reacted with 3,3′-dinitro-1,1′-ethylenebisurea (water, 60–100°C), and, after a repeated recrystallization from ethanol, gave the final gemini surfactants in 60–72% yields. Generally, the synthesis was performed at relatively mild temperature and pressure conditions. The above method provided surfactants of a high purity, confirmed by the applied analytical methods.

Structural characterization. The structures of the obtained bis(C_nGT) surfactants have been confirmed by elemental analysis, NMR spetroscopy, and ESI-MS. All of the data are in good agreement with the proposed structures. The ^{1}H and ^{13}C chemical shifts for the studied surfactants bis(C_4GT), bis(C_6GT), bis(C_8GT), bis(C_{10}GT), and bis(C_{12}GT) are summarized in Table 1.

Proton spectra for all studied compounds show many overlapping signals due to the presence of nonequivalent protons attached to carbon atoms as well as to oxygen atoms. It is, however, easy to distinguish signals related to the terminal CH_3 groups (triplet peaks at ~0.79–0.86 ppm), the mid-chain CH_2 groups (broad peaks at ~1.19–1.28 ppm), the β-CH_2 groups two carbons removed from the nitrogen atoms (multiplets in the 1.40–1.57 ppm region), and the α-CH_2 groups adjacent to the nitrogen atoms (multiplets in the 3.30–3.49 ppm region). All spectra show distinct peaks due to the NH linked to CO group (37,38), at 8.02, 8.45, 8.16, 8.25, and 8.41 ppm for structures bis(C_nGT) (for n = 4, 6, 8, 10, and 12, respectively). Signals from the spacer CH_2 groups can also be seen at ~3.06–3.32 ppm. The spectra of the cyclic sugar portion of bis(C_nGT) consist of groups of signals with chemical shifts between 3.38 and 3.77 as well as between 3.63 and 5.28 ppm. They lie in the sugar region of the spectrum and correspond to the D-glucitol CH, CH_2, and OH groups, respectively.

For all studied compounds, the ^{13}C spectra (Table 1) show the following characteristic signals (39,40): $\underline{C}$O carbon nuclei (at 173 ppm), $\underline{C}H_2OH$ (at about 63.6 ppm), and the $\underline{C}H_2$ group linked to CONH (at about 37.5 ppm). For the cyclic sugar part, an acetal carbon atom signal appears at around 105 ppm. This remains in agreement with the literature data related to the acetal grouping (39,40). Finally, all signals for the aliphatic carbon atoms are found below 35 ppm.

Thermotropic phase behavior. DSC measurements were performed in order to study the thermotropic behavior of the pure surfactants. The DSC traces prove the enantiotropic liquid-crystalline behavior and thus show both "melting" peaks and clearing peaks in heating scans (41). In the solid form, saccharide-derived amphiphiles form a close bimolecular system, with the carbohydrate-based hydrophilic groups arranged head-to-head and with alkyl chains in an interdigitated structure (26,42). Upon heating, the alkyl chains start to melt,

whereas the saccharide fragments are still stable due to the extensive intra- and intermolecular hydrogen bonds between the D-glucitol hydroxyl groups as well as the amide groups from the urea-derived entities. At this stage, the compound is in an intermediate phase (a mesophase) between the liquid and the crystalline phase (26). The melting behavior is revealed by a transition of the crystalline phase to the liquid-crystalline phase by 123.76, 67.74, 84.52, and 68.25°C for the derivatives with hydrophobic chains containing from 6 to 12 carbon atoms, respectively. If heating is continued, the hydrogen bonds break down, and an isotropic liquid is formed at the clearing point. The clearing points lie between 166.95 and 101.48°C for this series of compounds. However, different behavior was observed for *n*-butyl derivative, which underwent only one phase transition.

The melting points and the accompanying melting enthalpies of the bis(C_nGT) series are shown in Table 2. The melting point decreases with increasing hydrophobic part length of the surfactant molecule. Thus, the *n*-butyl and *n*-hexyl derivatives have a relatively high melting point compared to the other members of the bis(C_nGT) series. The short side chains may be able to fold along the spacer, thus allowing a closer packing and, consequently, higher phase transition temperature. The melting and clearing temperatures as a function of length of the alkyl chains are plotted in Figure 1. Similar observations have been described in respect to the other carbohydrate-based surfactants comprising a D-glucitol headgroup and two hydrophobic tails; N-acyl-N-dodecyl-1-amino-1-deoxy-D-glucitols and N-alkyl-N-tetradecanoyl-1-amino-1-deoxy-D-glucitols show the mesophase as well (43). Accordingly, the melting points decrease with increasing acyl chain length down to a minimum around *n*-hexanoyl and *n*-heptanoyl derivatives, and for longer chain lengths a gradual increase is observed.

Figure 2 shows a typical example of the bis(C_8GT) thermotropic behavior. Two peaks (67.74 and 148.96°C) mark a two-phase transition. At 67.74°C, the crystals transform to the liquid-crystalline state; after heating beyond the second peak at 148.96°C, only liquid exists.

Antimicrobial activity. According to the "green chemistry" philosophy, synthetic methodologies should be based on substances that possess little or no toxicity to human health and environment (38). The results of antimicrobial activity tests of various saccharide-based gemini surfactants are shown in Table 3. As shown, independent of the chemical structure, the studied glucitol-based surfactants were completely inactive toward yeast and molds studied, up to the concentration of 512 µg/mL. Also, it was found that these

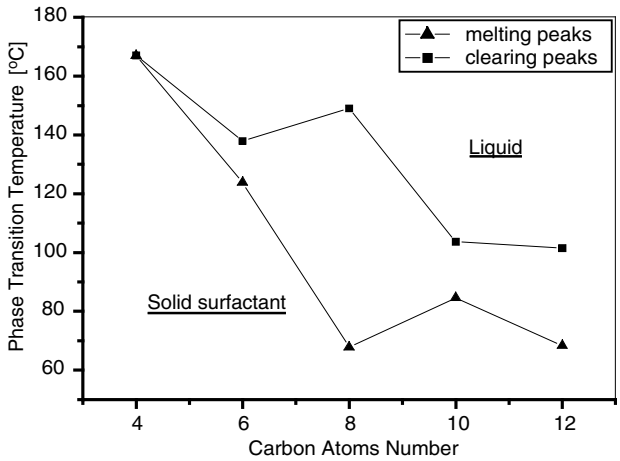


FIG. 1. Thermotropic transition temperatures for crystal to liquid crystal and for liquid crystal to isotropic liquid for the series of N,N´-bis[(3-alkyl-3-deoxy-D-glucitol)ureido]ethylenediamines tested.

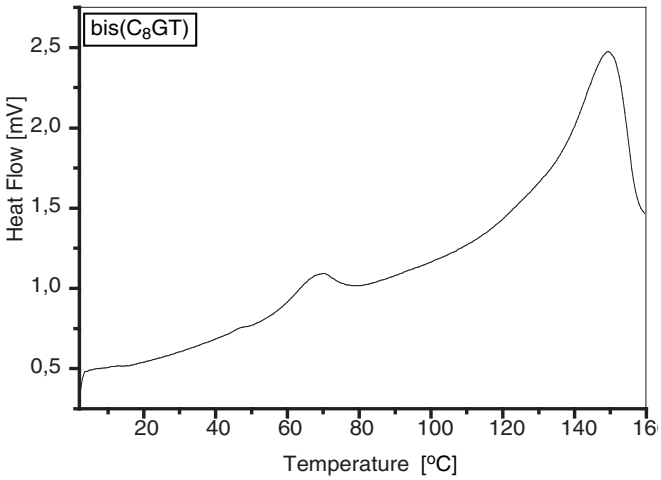


FIG. 2. Differential scaning thermogram (specific heat flow in mV vs. temperature) obtained for N,N´-bis[(3-octyl-3-deoxy-D-glucitol)ureido]ethylenediamine.

TABLE 2
The Mean Values of the Onsets and Peaks of Fusion and the Heats of Fusion for Studied Gemini Surfactants[a]

Surfactant studied	Peak (°C)	Onset (°C)	Δ*H* (kJ/mol)	Peak (°C)	Onset (°C)	Δ*H* (kJ/mol)
bis(C_4GT)	—	—	—	166.95	160.29	9.440
bis(C_6GT)	123.76	114.43	192.124	137.86	155.15	0.553
bis(C_8GT)	67.74	57.23	3.816	148.96	132.34	26.823
bis(C_{10}GT)	84.52	72.31	21.706	103.64	96.10	1.593
bis(C_{12}GT)	68.25	57.45	46.671	101.48	86.44	10.423

[a](Quantities Measured Using Differential Scanning Calorimetry)

TABLE 3
Minimal Inhibitory Concentrations (MIC)[a] (μg/mL) and Degradation of N,N′-bis[(3-alkyl-3-deoxy-D-glucitol)ureido]ethylenediamines in the Closed-Bottle Test 301D and in the Two-Phase Closed-Bottle Test

Surfactant	Microorganism												Biodegradation[b]	
	Gram-positive bacterial strains			Gram-negative bacterial strains			Yeasts			Molds			BOD/TOD (%) after 28 d	
	S. aureus	*S. lutea*	*B. subtilis*	*E. coli*	*S. marcescens*	*P. putida*	*S. cerevisiae*	*C. albicans*	*R. glutinis*	*P. citrinum*	*A. niger*	*B. cinerea*	Closed-Bottle Test 301D	BODIS test
bis(C_4GT)	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	55	59
bis(C_6GT)	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	53	56
bis(C_8GT)	512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	52	54
bis(C_{10}GT)	128	>512	512	>512	>512	>512	>512	>512	>512	>512	>512	>512	51	53
bis(C_{12}GT)	128	>512	512	>512	>512	>512	>512	>512	>512	>512	>512	>512	48	51
bis(C_8GA)[b]	64	8	512	256	>512	256	>512	—	—	—	>512	—	36.0	—
bis(C_{12}GA)[b]	512	32	>512	>512	256	>512	>512	—	—	—	>512	—	42.0	—

[a]Each MIC value (μg/mL) was defined as the lowest concentration of the tested compounds that completely inhibited bacterial growth.
[b]BOD, TOD and BODIS denote, respectively biochemical Oxygen Demand, Theoretical Oxygen Demand and Biological Oxygen Demand test for Insoluble Substances.
[c]From Wilk *et al.* (20).

dimeric representatives were practically inactive toward gram-negative bacteria, which follows from the data collected in Table 3.

Among the N,N′-bis[(3-alkyl-3-deoxy-D-glucitol)ureido]ethylenediamines studied, only bis(C_{10}GT) and bis(C_{12}GT) showed negligible inhibitory effect against gram-positive bacteria, *S. aureus* (MIC = 128 μg/mL). In comparison with other gemini compounds, such as N,N′-bisalkyl-N,N′-bis[(3-gluconylamido)propyl]ethylenediamines (referred as bis(C_nGA), where n = 8, 12), which showed a broad spectrum of antimicrobial activity particularly toward cocci (*S. aureus* and *S. lutea*, as shown in Table 3, (22)), the studied bis(C_nT)'s are characterized by particularly low toxicity toward environmental microorganisms. The improved antimicrobial properties of bis(C_nGT) in comparison with bis(C_nGA) come from more concerted amphipathic architecture of the glucose-urea–derived compounds and increased hydrophilicity of the 1,1′-ethylenebisurea spacer. All bis(C_nGT) probably make it impossible to have deeper surfactant penetration into the cell wall material, known as peptidoglycan, and furthermore, may protect cell membranes against destruction.

Biodegradability. New chemical products should be designed so they do not persist in the environment at the end of their function, breaking down into innocuous degradation products (38). The biodegradation susceptibility of surfactants in natural ecosystems is one of the most important factors determining current product requirements. In these experiments, the degradation behavior of the gemini glucose-based surfactants was investigated by the closed-bottle test (OECD 301D) and by the two-phase closed-bottle test (ISO/DIS 10708).

The data collected in Table 3 indicate that the studied gemini derivatives produced comparable results in both tests. The bis(C_4GT) surfactant showed the highest biodegradability. It reached 55% degradation on day 28 in the closed-bottle test 301D, and 59% in the BODIS test. The results obtained for other compounds tested (bis(C_6GT), bis(C_8GT), bis(C_{10}GT), and bis(C_{12}GT)) demonstrated that their biodegradability rates are a little slower, and after 28 d degraded 48–53% in the closed-bottle test 301D, and 51–56% in the BODIS test. Generally, "ready biodegradability" is a legislative concept applied to compounds reaching 60% biodegradation within 28 d. All the glucitol-based compounds revealed data below the pass level of 60%, as can be seen from Table 3. Therefore, the biodegradation results do not allow classification of the tested N,N′-bis[(3-alkyl-3-deoxy-D-glucitol)ureido]ethylenediamines as readily biodegradable. However, in the prolonged closed-bottle test, all surfactants reached more than 60% biodegradation; they required 64–75 d to degrade to 62–68% levels, thus indicating slow but complete mineralization. Additionally, compounds containing *n*-butyl, *n*-hexyl, or *n*-octyl tails are slightly more extensively degraded than those with *n*-decyl or *n*-dodecyl ones.

The BODIS degradation screening test permits the use of an inoculum at a concentration of up to 30 mg activated sludge/L, and therefore is considered less informative for real surface water conditions compared with the OECD Test 301D, which uses inoculation with ≤5 mL of sewage treatment plant effluent per liter. It has been suggested that the BODIS test has an unrealistically high degradation potential. Thus, the N,N′-bis[(3-alkyl-3-deoxy-D-glucitol)ureido]ethylenediamines were comparatively tested in the two-phase closed-bottle test at different inoculum concentration levels (50 mL effluent/L and 5 mL effluent/L). The results obtained after weekly degradation measurement over the 4-wk test period are shown in the form of the biodegradation profiles for bis(C_nGT) under the conditions described (Fig. 3). It was observed that all sugar-based compounds studied were biodegraded extensively, irrespective of the number of microorganisms present in the inoculum. Moreover, biodegradation of these surfactants started immediately, without a lag phase.

According to the biodegradation profiles for bis(C_nGT),

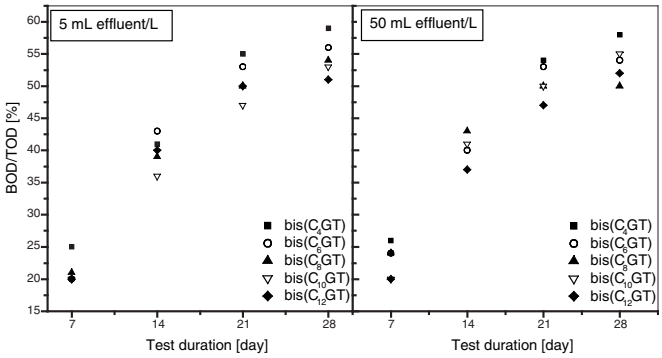


FIG. 3. Comparison of the degradation curves of N,N′-bis[(3-alkyl-3-deoxy-D-glucitol)ureido]ethylenediamines in the two-phase closed-bottle tests inoculated with 5 and 50 mL effluent/L.

n-butyl derivative was biodegraded the most extensively and after 28 d reached 58–59%. The degradation of bis(C_6GT), bis(C_8GT), bis(C_{10}GT), and bis(C_{12}GT) derivatives on day 28 reached 54–56%, 50–54%, 53–55%, and 51–52%, respectively. There were no significant differences between the intermediate and final degradation values obtained, or between the final degradation values obtained in the BODIS test when samples were inoculated with 5 mL and 50 mL sewage plant effluent/L. The values were within the expected range of variation for biological tests with chemical compounds. In all biodegradation experiments performed in this study, biodegradation of sodium acetate reached 97–98% within 5 d. Because this compound is known to be readily biodegradable, these results provide further confirmation that the tests are operating correctly. Generally, the N,N′-bis[(3-alkyl-3-deoxy-D-glucitol)ureido]ethylenediamines studied have low ecotoxicity and possess the biological properties needed for environmental acceptance.

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