European Journal of Medicinal Chemistry 62 (2013) 177–186

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis, surface tension properties and antibacterial activities of amphiphilic D-galactopyranose derivatives

Ludovic Chaveriat [a,*], Isabelle Gosselin [b,**], Cécile Machut [c], Patrick Martin [a]

[a] Centre Technologique des Agroressources (CTA), Université d'Artois, IUT de Béthune, 1230 rue de l'Université, BP 819, 62408 Béthune Cedex, France
[b] Unité de Génie Enzymatique et Cellulaire (GEC), UMR CNRS 6022, Université de Picardie Jules Verne, UFR des Sciences, 33 rue Saint Leu, 80039 Amiens cedex, France
[c] Unité de Catalyse et de Chimie du Solide (UCCS), UMR CNRS 8181, Université d'Artois, IUT de Béthune, 1230 rue de l'Université, BP 819, 62408 Béthune Cedex, France

ARTICLE INFO

Article history:
Received 12 July 2012
Received in revised form
11 December 2012
Accepted 16 December 2012
Available online 25 December 2012

Keywords:
D-galactopyranoside
Alkyl chain
Spacer arm
Surfactant
Antimicrobial

ABSTRACT

Several amphiphilic D-galactopyranose derivatives were synthesized in which the glycosidic moiety was separated from the hydrophobic alkyl chain (along 8 or 12 carbon atoms) by a spacer arm (butyl, butynyl or benzyl) in order to increase their surfactant properties and to obtain new antibacterial compounds. The surface tensions of the products were analyzed by Critical Micelle Concentration (CMC) and γ_{CMC} measurements and the antimicrobial activities were assayed against 10 bacterial species by Minimum Inhibitory Concentration (MIC) determination in liquid broth. The introduction of an aliphatic spacer arm increased the amphiphilic properties of the compounds and the CMC values were 40–500 times lower than their analogs without spacer arm. In the same manner, the spacer arms significantly increased the antibacterial power of the compounds. The products **4d** and **4e** exhibiting a C12 alkyl chain and an aliphatic spacer arm (butyl and butynyl) were the best surfactants (CMC = 0.023 and 0.032 mmol/L, respectively) and presented also the best antibacterial activities (MIC = 15.62 and 3.91 μg/mL for *Micrococcus luteus*, respectively). But the antibacterial activity of the newly synthesized products seemed to depend more on the cell wall composition of the bacteria than only on the amphiphilic character of the compounds.

1. Introduction

Carbohydrate-derived surfactants are non-ionic amphiphilic compounds in which the carbohydrate moiety is linked to a long alkyl chain. In recent years, this class of detergents has been extensively studied [1–7] due to several interesting properties in addition to their emulsifying properties such as non-toxicity or biodegradability, and because carbohydrates are attractive starting materials for chemical elaboration due to their status as renewable resources [8,9]. An overall review on the synthesis and the properties of carbohydrate-based amphiphilic molecules has been published recently [10]. Carbohydrate-derived surfactants are also known for their antimicrobial activity due to their capacity to interact with biological membranes [11,12]. Indeed surfactants can solubilize the lipids constituting the membrane bilayer, impair the membrane integrity and cause the cellular lysis [13,14]. But generally, the carbohydrate-derived surfactants synthesized for antimicrobial purposes were derivatives of amino ether [6,15–19], thio ether [20,21], aminoglycosides [22–25], or fatty acid ester [26–29]. Only few works deal with the antimicrobial activity of carbohydrate ether derivatives [30–34].

The aim of this study was to deepen the knowledge on the antimicrobial properties of carbohydrate ether derivatives in the actual context of bacterial resistance to antibiotics and the increasing need to discover new antibacterial molecules [35,36]. In that purpose, we synthesized carbohydrate ethers deriving from caprylic and lauric acids, C8 and C12 fatty acid respectively, well known for their strong antibacterial properties [33,37–43]. Concerning the glycosidic part, previous studies on carbohydrate esters showed that the configuration of hydroxyl groups in the carbohydrate moiety influenced the antibacterial activities, and the galactose derivatives were more bactericide than the glucose, fructose or sucrose ones [44]. Then, we synthesized 6-*O*-alkyl-galactose derivatives with a C8 or a C12 alkyl chain and tested the tension surface characteristics as well as the antimicrobial activities of these compounds. In previous works, the amphiphilic and liquid crystal properties of these molecules have been shown to strongly decrease

Abbreviations: CMC, critical micelle concentration; MIC, minimum inhibitory concentration.

* Corresponding author. Tel.: +33 3 21 63 23 25; fax: +33 3 21 63 02 94.
** Corresponding author. Tel.: +33 3 22 82 74 73; fax: +33 3 22 82 75 95.
E-mail addresses: Ludovic.Chaveriat@univ-artois.fr (L. Chaveriat), Isabelle.Gosselin@u-picardie.fr (I. Gosselin).

0223-5234/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2012.12.032

when the alkyl chain contained more than eight carbon atoms [45,46]. This phenomenon, which decreased the hydrophilic character, resulted from the intramolecular hydrogen bonding between the cis-hydroxyl groups located on C-3 and C-4 [47]. It was proposed that the C-6 hydrophobic alkyl chain with a certain length, sterically crowded the C-3 and C-4 hydroxyl groups, thus preventing them to be engaged in intermolecular hydrogen bonding with water molecules [48]. In order to circumvent this problem, we prepared glucidoamphiphile derivatives from D-galactose in which the glucidic moiety and the hydrophobic alkyl chain were separated by a spacer arm with various length and rigidity (butyl, butynyl, benzyl). Afterward these molecules and their derivatives without spacer arm were all tested for their surface-active properties and their antimicrobial activities on a large range of bacterial strains.

2. Results and discussion

Firstly, the synthesis of the hydrophobic alkyl chain including spacer arm began with the monoalkylation of commercial diol with *n*-dodecyl bromide or *n*-octyl bromide in DMF (Scheme 1). For this step, sodium hydride was used when the starting material was butan-1,4-diol or butyn-1,4-diol and sodium hydroxide to prevent degradation of hydroquinone. The corresponding alkylated alcohol **1a**–**f** was obtained with a yield of 60–90%. Then, the activation of hydroxyl group was performed using *p*-toluenesulfonyl chloride and dimethylaminopyridine in dichloromethane. Tosylated derivatives **2a**–**f** were isolated with a 73–92% yield.

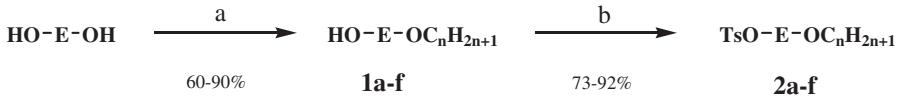
The 6-*O*-alkyl-D-galactopyranoses were prepared by alkylation of 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose using appropriate tosylated alkyl chain and potassium hydroxide for derivatives with spacer arm (Scheme 2), or suitable bromoalkanes and sodium hydride for derivatives without spacer arm (Scheme 3).

The final condensation of the tosylated derivatives with 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose in toluene–Me_2SO in the presence of potassium hydroxide produced the **3a**–**f** intermediate 6-*O*-alkyl-1,2:3,4-di-*O*-isopropylidene-D-galactopyranoses. These compounds were subsequently deprotected with CF_3COOH to obtain **4a**–**f** with a 75–90% yield. The experimental conditions for the deacetalization reaction allowed the maintenance of the anomeric integrity to provide deprotected compounds in the α-configuration [45,46,49] (see representative NMR spectra in Supplementary Data).

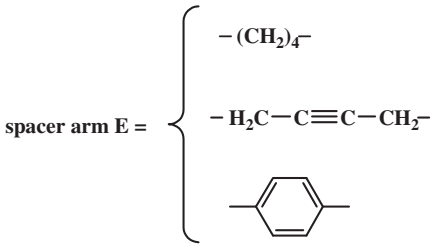
After synthesis, the deprotected compounds **4a**–**f** were evaluated for their surfactant properties. The Critical Micelle Concentrations (CMC) was determined by surface tension measurement (γ) using the Wilhelmy method and the measured values were plotted against the molar concentrations. The surface tension characteristics of the galactose derivatives were reported in Table 1 and the corresponding graphs in Figs. 1 and 2. The results were compared to the corresponding analogs without spacer arm **6a** and **6b** (6-*O*-alkyl-D-galactopyranoses), which were already described in the literature [48] but were synthesized again here (Scheme 3) to compare the influence of the spacer arm on the tension surface properties and hereafter on the antimicrobial efficiency.

The compounds **4a**, **4b**, **4d** and **4e** (derivatives with non aromatic spacer arms) exhibited a large decrease in surface tension from 72 to 30–42 mN/m approximatively when the concentration raised (Figs. 1 and 2) which is a classical surfactant behavior. In addition, all of them presented a characteristic phenomenon of micellization. For the compounds **4c** and **4f** with an aromatic spacer arm, the profiles were different. The surface tension for the compound **4c** decreased from 72 to 54 mN/m (Fig. 1), but no CMC or γ_{cmc} could be determined because the solution became turbid over 0.6 mmol/L. This compound seemed to have a hydrotropic behavior which prevented the molecule organization in micelle and induced no break in the γ slope (Fig. 1). For the compound **4f**, the same decrease in surface tension as **4c** was observed; however, a CMC could be measured but with a 100 times worse value than the compounds with aliphatic spacer arm, *i.e.* 3.1 mmol/L instead of 0.023 and 0.032 mmol/L for **4d** and **4e**, respectively.

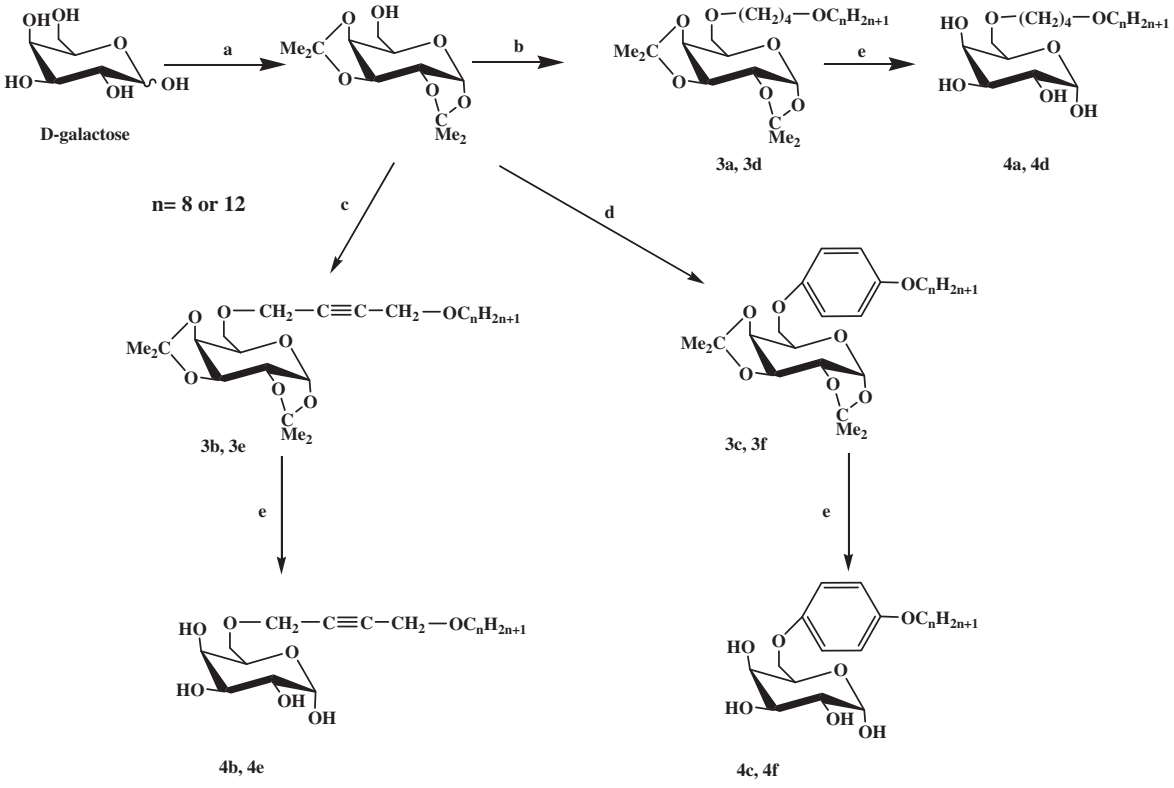
The comparison between the CMC values obtained for the galactose derivatives with and without non aromatic spacer arm (Table 1) showed that the distance between the glucidic moiety and the hydrophobic alkyl chain greatly improved the surfactant properties of the molecules. For the C8 derivatives, the CMC decreased from 12 mmol/L for **6a** to 0.3 and 0.06 mmol/L for butyl **4a** and butynyl **4b** spacer arm, respectively. And for the C12 derivatives, no CMC could be measured for the product **6b** without spacer arm, in accordance to the literature [48], whereas the values were 0.023 and 0.032 mmol/L for butyl **4d** and butynyl **4e** spacer arm, respectively.



n	E	Product
8	butyl	**1a, 2a**
8	butynyl	**1b, 2b**
8	benzyl	**1c, 2c**
12	butyl	**1d, 2d**
12	butynyl	**1e, 2e**
12	benzyl	**1f, 2f**



Scheme 1. Synthesis of alkyl chains including spacer arm. Reagents and conditions: (a) BrC_nH_{2n+1}, NaH or NaOH, DMF, RT; (b) TsCl, DMAP, CH_2Cl_2, 0 °C.



Scheme 2. Synthesis of 6-*O*-alkyl-D-galactopyranose derivatives with spacer arm: Reagents and conditions: (a) acetone, H_2SO_4, 54%; (b) $TsO(CH_2)_4OC_nH_{2n+1}$, KOH, DMF, RT, 85–91%; (c) $TsOCH_2C{\equiv}CCH_2OC_nH_{2n+1}$, KOH, DMF, 74–81%; (d) $TsOPhOC_nH_{2n+1}$, KOH, DMF, 62–66%; (e) TFA–H_20, 0 °C, 75–90%.

The nature of the spacer arm affected the surface tension properties in relation to the length of the alkyl chain of the galactose derivatives (Table 1). Therefore, for the galactose derivatives with butyl spacer arm, the C8 derivative showed a CMC value 10 times higher than the C12 derivatives (CMC = 0.3 and 0.023 mmol/L for **4a** and **4d**, respectively) and 2 times higher with the butynyl spacer arm (CMC = 0.06 and 0.032 mmol/L for **4b** and **4e**, respectively). However when the alkyl chain was longer (**4d**, **4e**), the nature of the spacer arm (alkyne or alkane) had less influence and resulted in equivalent CMC values (0.023 and 0.032 mmol/L, respectively). The lengthening of the hydrocarbon chain adjusted the hydrophilic–lipophilic balance in favor of a more important emulsifying power.

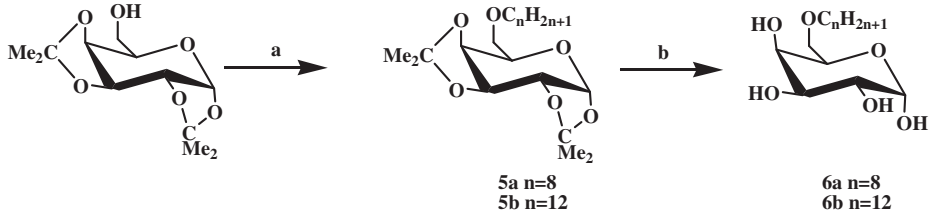
For molecules with a C8 alkyl chain, the introduction of a rigid spacer arm was effective to prevent the folding of the alkyl chain only when the spacer was aliphatic. The compounds **4a** and **4b** showed very interesting surface tension properties with a CMC = 0.3 and 0.06 mmol/L, respectively, instead of 12 mmol/L for C8 derivative without spacer arm **6a**.

When the alkyl chain was relatively short (8 carbons), the insertion of a spacer arm a little more rigid supported the deployment of the alkyl chain and the supramolecular assembly of the compounds in aqueous solution. However the nature of the spacer arm became less important when the length of the alkyl chain increased and no significant difference was observed between the CMC of the compounds **4d** and **4e**.

The aromatic spacer arm didn't seem appropriate since the micellization didn't appear clearly in the surface tension graphs (**4c** in Fig. 1 and **4f** in Fig. 2). The benzene ring made the final sugar completely hydrophobic and was probably too rigid to form a stable supramolecular structure.

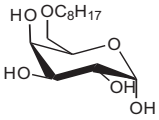
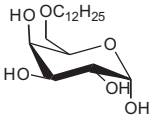
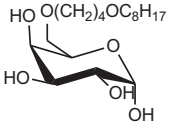
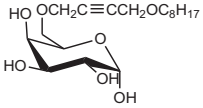
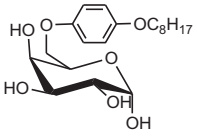
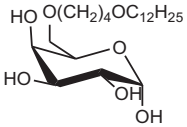
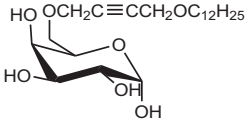
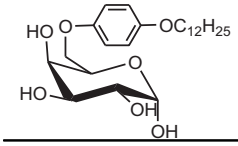
The surface tension properties of all the synthesized derivatives, except those with an aromatic spacer arm, were comparable to the most effective neutral surfactant, Tween 20, exhibiting a CMC = 0.05 mmol/L [50].

To complete this study, the antimicrobial activity of all the 6-*O*-alkyl-galactose derivatives (C8 or C12 alkyl chain, with or without spacer arm, protected or deprotected) was determined by liquid broth serial dilutions. The Minimum Inhibitory Concentration (MIC) was assayed on 10 bacterial species potentially pathogens for humans, animals or plants: 6 Gram negative bacteria (*Citrobacter freundii* ATCC 6750, *Enterobacter cloacae* ATCC 13047, *Erwinia chrysanthemi* EC3937, *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* CIP A22 and *Sinorhizobium meliloti* Rm1021) and 4 Gram positive bacteria (*Bacillus stearothermophilus* ATTC 12980, *Micrococcus luteus* CIP 53.45, *Mycobacterium smegmatis* CIP 73.26 and *Staphylococcus aureus* subsp *aureus* CIP 53.154). Chloramphenicol



Scheme 3. Synthesis of 6-*O*-alkyl-D-galactopyranose derivatives without spacer arm: Reagents and conditions: (a) BrC_nH_{2n+1}, NaH, DMF, 69–81%, (b) TFA–H_20, 0 °C, 95%.

Table 1
Surface tension characteristics of the 6-*O*-alkyl-D-galactopyranose derivatives.

Compound		CMC (mmol/L)	γ_{CMC} (mN/m)
	**6a**	12	28
	**6b**	–[a]	–[a]
	**4a**	0.3	30
	**4b**	0.06	30
	**4c**	–[a]	–[a]
	**4d**	0.023	48
	**4e**	0.032	35
	**4f**	3.1	54

[a] No CMC or γ_{CMC} were recorded during surface tension experiment.

was used as a standard to compare the synthesized products to a large spectrum antibiotic. The results are presented in Table 2.

The alkyl-galactose derivatives exhibiting the best antimicrobial activities were the C12 derivatives with non aromatic spacer arms, whether they were protected or not, *i.e.* the products **3d**, **3e**, **4d** and **4e**. These compounds gave MIC values as low as 3.91 μg/mL depending on the strain, which was similar to the chloramphenicol action. The aromatic spacer arm seemed to cancel the antimicrobial activity of the C12-derived products and no significant MIC could be detected for compounds **3f** or **4f**, with only one exception of the **3f** tested on *M. luteus* (MIC = 31.25 μg/mL).

The MIC results were independant of the nature of the aliphatic spacer arm and were equivalent with a butyl or a butynyl group. Moreover, the protected galactose derivatives had similar antimicrobial activities as the deprotected ones (**3d** and **4d**, or **3e** and **4e**) exhibiting analogous MIC values.

The antibacterial effects were clearly strain-dependant. The compounds **3d**, **3e**, **4d** and **4e** had a better activity against Gram positive strains, whereas the Gram negative bacteria seemed to be more resistant to these products, with the only exception of *S. meliloti* with **4e** (MIC = 31.25 μg/mL). Among Gram positive strains, the lowest MIC values were obtained on *M. luteus* and *B. stearothermophilus* (MIC = 3.91–31.25 μg/mL).

On another hand, the C8 derivatives (compounds **3a**–**c**, **4a**–**c**) showed poor antibacterial activities and no MIC values under 62.5 μg/mL could be measured whatever was the strain tested.

The products without spacer arm **5a**–**6b** gave no MIC, showing the importance to move the alkyl chain away from the glycosidic part to present antibacterial properties.

The newly synthesized 6-*O*-alkyl-galactose derivatives exhibited tension surface properties related to the antibacterial activities and the best surfactants **4d** and **4e** were also the best antimicrobials. Nevertheless, the amphiphilic character is not enough to explain the bactericidal power and the compounds exhibited a strain-dependant action in which the Gram negative species were more resistant than the Gram positive bacteria. This was already observed with fatty acid derivatives [29,37,51] and this resistance was attributed to the presence of the cell wall lipopolysaccharide (LPS) and of the outer membrane, which are two structures both absent in Gram positive bacteria and could prevent the active compounds to reach the cytoplasmic membrane of the Gram negative species [37,52].

The only exception in the resistance to the products by the Gram negative species is the case of *S. meliloti* with **4e**, which was the best antibacterial compound. This special feature could be explained by the unusual LPS structure of *S. meliloti* which is composed of an atypical very long-chain fatty acid with up to 30 carbon atoms [53] and could interact differently with the alkyl chain of the compounds.

Among the Gram positive bacteria, some species were more sensitive to the 6-*O*-alkyl-galactose derivatives like *M. luteus* or *B. stearothermophilus*, whereas others were more resistant like *M. smegmatis* or *S. aureus*. This implies that the specific strain composition of the cell wall or the cytoplasmic membrane is important to explain the bactericidal effects of the products [54]. For example, *M. luteus* is known to possess mannosyl residus in its membrane phospholipids [55] and *M. smegmatis* a unique arabinogalactan feature [53]. Each strain presents a specific cell wall composition and a deeper study on the molecular interactions between the products and the bacterial components is necessary to understand the bactericidal mechanisms.

3. Conclusion

The introduction of a spacer arm between the galactose moiety and the alkyl chain prevented the invasion of the hydrophobic part in the region of the OH groups. Hence the intermolecular hydrogen bonds with water molecules were allowed and the newly synthesized compounds showed good amphiphilic properties. In order to check the correlation between the glucidoamphiphile structure and the surface tension characteristics, as well as the antimicrobial activities of the surfactant molecules, we currently extend the synthesis pathway to different molecules containing other types of rigid spacer arm and one or more alkyl groups.

4. Experimental section

All chemicals were purchased from Acros and Aldrich Chemicals in their highest purity degree. All solvents were used as-supplied without further purification. Distilled water was used in all experiments. Analytical thin-layer chromatography (TLC) was performed on E. Merck aluminum-backed silica gel (Silica Gel F254).

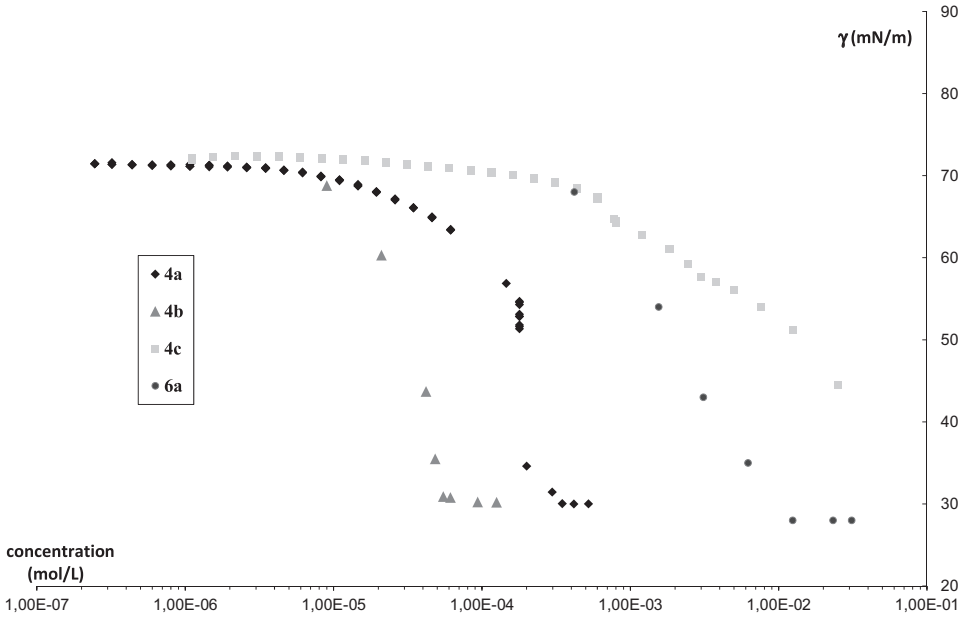


Fig. 1. Surface tension of 6-*O*-octyl-D-galactopyranose derivatives **4a**, **4b** and **4c** with butyl-, butynyl- and benzyl-spacer arm respectively, and **6a** without spacer arm, as a function of concentration.

Compounds were identified using UV fluorescence and/or staining with a solution of phosphomolybdic acid in aqueous sulfuric acid and ethanol.

Melting points were determined on an electrothermal apparatus and are uncorrected. Optical rotations, for solutions in $CHCl_3$ or methanol, were measured at room temperature with a digital polarimeter ATAGO model Polax-2L.

NMR spectra were recorded on a Bruker DRX300 spectrometer operating at 300 MHz for ^1H nuclei and 75 MHz for ^{13}C nuclei. $CDCl_3$ (99.50% isotopic purity) and DMSO d_6 (99.80% isotopic purity) were purchased from Euriso-Top. ^1H NMR data are reported as chemical shift, multiplicity (s, singlet; d, doublet; m, multiplet), relative integral, coupling constant (*J* in hertz).

The processor tensiometer Sigma 70 (KSV) and the Wilhelmy plate method for air–water interface have been used for the surface tension measurements at 293 K. A concentrated solution was installed in a syringe and the addition of small volumes to ultrapure water enhanced the solution concentration. After each addition, the solution was gently stirred for 30 s. Equilibrium surface tension was measured for each concentration. All surface tension values were mean quantities of at least three measurements. The standard deviation of the mean never deviated more than ±1.5% of the mean. The precision of the force transducer of the surface tension apparatus was 0.1 mN/m and before each experiment, the platinum plate was cleaned in red/orange color flame.

4.1. General procedure of monoalkylation using sodium hydroxide

Hydroquinone (9.08 mmol, 1 equiv) and potassium hydroxide (9.08 mmol, 1 equiv) were stirred at room temperature for 1 h in

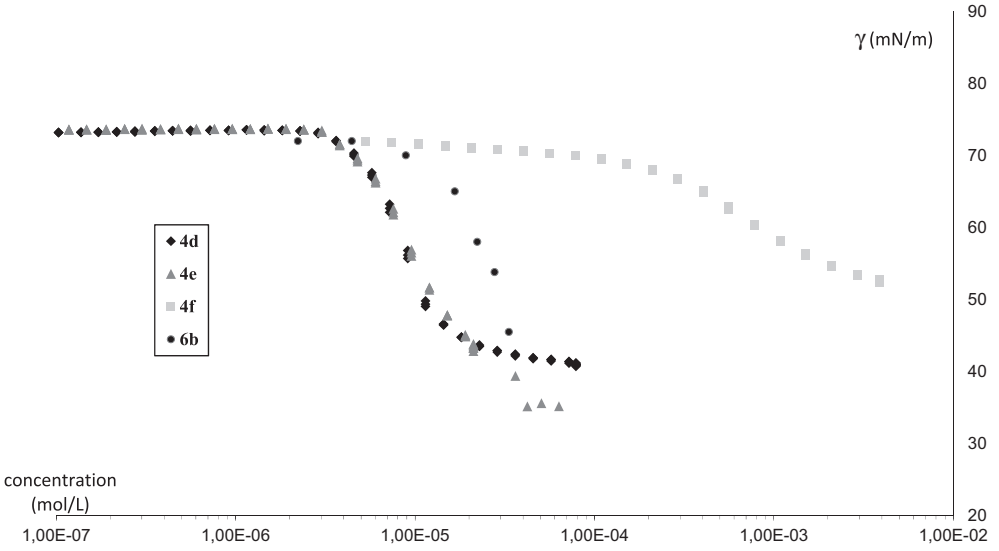


Fig. 2. Surface tension of 6-*O*-dodecyl-D-galactopyranose derivatives **4d**, **4e** and **4f** with butyl-, butynyl- and benzyl-spacer arm respectively, and **6b** without spacer arm, as a function of concentration.

Table 2
Antimicrobial activity of the compounds with spacer arm **3a–4f** and without spacer arm **5a–6b** against Gram negative and Gram positive bacterial species.

		MIC (μg/mL)															
		Without spacer arm				With spacer arm											Chloramphenicol
		5a	**5b**	**6a**	**6b**	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**	
Negative	*Citrobacter freundii*	≥250	≥250	≥250	≥250	62.5	62.5	125	62.5	62.5	62.5	125	125	125	62.5	62.5	125	7.8
	Enterobacter cloacae	≥250	≥250	≥250	≥250	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	< 0.98
	Erwinia chrysanthemi	≥250	≥250	≥250	≥250	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	0.98
	Escherichia coli	≥250	≥250	≥250	≥250	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	< 0.98
	Pseudomonas aeruginosa	≥250	≥250	≥250	≥250	62.5	62.5	125	62.5	62.5	125	62.5	125	125	62.5	62.5	62.5	1.95
	Sinorhizobium meliloti	≥250	≥250	≥250	≥250	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	31.25	62.5	< 0.98
Positive	*Bacillus stearothermophilus*	≥250	≥250	≥250	≥250	62.5	62.5	62.5	31.25	15.62	62.5	62.5	62.5	62.5	31.25	7.81	62.5	3.9
	Micrococcus luteus	≥250	≥250	≥250	≥250	62.5	62.5	62.5	15.62	7.81	31.25	62.5	62.5	62.5	15.62	3.91	62.5	< 0.98
	Mycobacterium smegmatis	≥250	≥250	≥250	≥250	62.5	62.5	62.5	31.25	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	1.95
	Staphylococcus aureus	≥250	≥250	≥250	≥250	125	125	125	125	125	125	125	125	125	125	62.5	125	< 0.98

DMF (40 mL), then *n*-alkyl bromide (10.9 mmol, 1.2 equiv) was added slowly. After 24 h, the reaction was quenched with acid resin DOWEX 50WX4 and the organic layer was extracted with ethyl acetate, dried with Na_2SO_4 and concentrated under reduced pressure. The monoalkylated derivative was isolated as a white solid after purification by column chromatography with 7:3 hexane–ethyl acetate.

4.2. General procedure of monoalkylation using sodium hydride

Sodium hydride (3.3 mmol, 1.1 equiv) was added at room temperature to a solution of alcohol (3 mmol, 1 equiv) in DMF (20 mL). The medium was stirred at room temperature during 1 h and *n*-alkyl bromide (3.6 mmol, 1.2 equiv) was added slowly. After 1 h at room temperature, water was added to quench the reaction and the organic layer was extracted with ethyl acetate, dried with Na_2SO_4 and concentrated under reduced pressure. The monoalkylated derivative was isolated as a colorless oil after purification by column chromatography with 7:3 hexane–ethyl acetate.

4.3. General procedure of tosylation

p-Toluenesulfonyl chloride (5.91 mmol, 1.5 equiv) in dichloromethane (10 mL) was added dropwise to a solution of monoalkylated diol (3.94 mmol, 1 equiv) and dimethylaminopyridine (5.91 mmol, 1.5 equiv) in dichloromethane (20 mL) at −10 °C. After 7 h at room temperature, the solution was filtered and concentrated under reduced pressure. The tosylated derivative was isolated as a viscous liquid after purification by column chromatography with 9:1 hexane–ethyl acetate.

4.4. General procedure of condensation between tosylated derivatives and protected sugar

Finely powdered potassium hydroxide (10.6 mmol, 2 equiv) was added to a solution of 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose (5.3 mmol, 1 equiv) in toluene–Me_2SO 3:2 (10 mL) at room temperature. The reaction was stirred during 1 h at room temperature and a solution of the tosylated derivative (5.3 mmol, 1 equiv) in toluene–Me_2SO 3:2 (5 mL) was added to the medium. After 15 h, the mixture was filtered and the filtrate neutralized with saturated aq. NH_4Cl. The organic phase was separated, washed with water (twice), dried (Na_2SO_4) and concentrated under reduced pressure. The 6-*O*-alkyl-1,2:3,4-di-*O*-isopropylidene-D-galactopyranose was isolated as a white solid after purification by column chromatography with 9:1 hexane–ethyl acetate.

4.5. General procedure for deprotection of diacetal derivatives

Diacetal derivative (3.2 mmol, 1 equiv) was added to a stirred solution of 9:1 CF_3COOH–H_2O (5 mL) at room temperature. Cold diethyl ether was added after 1 h and the solution was cooled at −20 °C. The desired products were filtered off, sucked dry, washed with diethyl ether (twice) and recrystallized from THF to give 6-*O*-*n*-alkyl-α-D-galactopyranose.

4.6. 4-O-n-Octyl-butan-1-ol (***1a***)

Colorless oil (8.70 g, 43.06 mmol, 78%); ^{1}H NMR ($CDCl_3$): 3.51 (2H, t, *J* 6.8 Hz, C**H**$_2$OH), 3.39 (4H, m, C**H**$_2$OR), 1.53 (m, 6H, C**H**$_2$CH$_2$O), 1.29 (m, H alkyl chain), 0.82 (t, 3H, *J* 6.4 Hz, C**H**$_3$); ^{13}C NMR ($CDCl_3$): 71.4, 71.1 (**C**H$_2$OR), 62.5 (**C**H$_2$OH), 32.4–22.9 (**C**H$_2$ alkyl chain), 14.4 (**C**H$_3$).

*4.7. 4-O-n-Dodecyl-butan-1-ol (**1d**)*

Colorless oil (3.46 g, 13.43 mmol, 90%); ^{1}H NMR ($CDCl_3$): 3.53 (2H, t, *J* 6.8 Hz, C**H**$_2$OH), 3.35 (4H, m, C**H**$_2$OR), 1.54 (m, 6H, C**H**$_2$CH$_2$O), 1.19 (m, H alkyl chain), 0.81 (t, 3H, *J* 6.4 Hz, C**H**$_3$); ^{13}C NMR ($CDCl_3$): 71.4, 71.1 (**C**H$_2$OR), 62.6 (**C**H$_2$OH), 32.3–23.0 (**C**H$_2$ alkyl chain), 14.4 (**C**H$_3$).

*4.8. 4-O-n-Octyl-but-2-yn-1-ol (**1b**)*

Colorless oil (8.99 g, 45.40 mmol, 78%); ^{1}H NMR ($CDCl_3$): 4.21 (2H, m, ≡CC**H**$_2$OH), 4.12 (2H, m, ROC**H**$_2$C≡), 3.43 (t, 2H, C**H**$_2$OCH$_2$C≡, *J* 6.8 Hz), 1.48 (m, 2H, C**H**$_2$CH$_2$OCH$_2$C≡), 1.22 (m, H alkyl chain), 0.82 (t, 3H, *J* 7.0 Hz, C**H**$_3$); ^{13}C NMR ($CDCl_3$): 85.9 (**C**≡), 69.7, 58.2 (**C**H$_2$OR), 51.3 (**C**H$_2$OH), 32.2–23.0 (**C**H$_2$ alkyl chain), 14.2 (**C**H$_3$).

*4.9. 4-O-n-Dodecyl-but-2-yn-1-ol (**1e**)*

Colorless oil (5.87 g, 23.03 mmol, 79%); ^{1}H NMR ($CDCl_3$): 4.29 (2H, m, ≡CC**H**$_2$OH), 4.16 (2H, m, ROC**H**$_2$C≡), 3.49 (t, 2H, C**H**$_2$OCH$_2$C≡, *J* 6.7 Hz), 1.84 (m, 2H, CH$_3$C**H**$_2$(CH$_2$)$_{10}$), 1.58 (m, 2H, C**H**$_2$CH$_2$OCH$_2$C≡), 1.26 (m, H alkyl chain), 0.87 (t, 3H, *J* 7.0 Hz, C**H**$_3$); ^{13}C NMR ($CDCl_3$): 85.9 (**C**≡), 69.7, 58.2 (**C**H$_2$OR), 51.3 (**C**H$_2$OH), 32.2–23.0 (**C**H$_2$ alkyl chain), 14.2 (**C**H$_3$).

*4.10. p-O-n-Octyl-phenol (**1c**)*

White solid (6.05 g, 27.24 mmol, 60%); ^{1}H NMR ($CDCl_3$): 6.79 (4H, m, H$_{aro}$), 3.88 (2H, t, C**H**$_2$OR, *J* 8.9 Hz), 1.69 (m, 2H, CH$_2$C**H**$_2$OR), 1.24 (m, H alkyl chain), 0.94 (t, 3H, *J* 6.5 Hz, C**H**$_3$); ^{13}C NMR ($CDCl_3$): 153.4, 150.1, 116.2, 115.9 (4 × C$_{aro}$), 69.3 (**C**H$_2$OR), 32.3–23.1 (**C**H$_2$ alkyl chain), 14.6 (**C**H$_3$).

*4.11. p-O-n-Dodecyl-phenol (**1f**)*

White solid (8.08 g, 29.06 mmol, 64%); ^{1}H NMR ($CDCl_3$): 6.79 (4H, m, H$_{aro}$), 3.91 (2H, t, C**H**$_2$OR, *J* 9.0 Hz), 1.77 (m, 2H, CH$_2$C**H**$_2$OR, *J* 6.8 Hz), 1.29 (m, H alkyl chain), 0.91 (t, 3H, *J* 6.3 Hz, C**H**$_3$); ^{13}C NMR ($CDCl_3$): 153.6, 149.8, 116.4, 116.1 (4 × C$_{aro}$), 69.3 (**C**H$_2$OR), 32.3–23.1 (**C**H$_2$ alkyl chain), 14.6 (**C**H$_3$).

*4.12. 4-O-n-Octyl-but-1-yl tosylate (**2a**)*

White solid (2.61 g, 7.33 mmol, 74%); ^{1}H NMR ($CDCl_3$): 7.79, 7.32 (4H, d, *J* 8.4, H$_{aro}$), 4.05 (2H, t, *J* 6.3 Hz, C**H**$_2$OTs), 3.34 (4H, m, C**H**$_2$OR), 2.44 (s, 3H, C**H**$_3$ tosyl), 1.72–1.26 (m, H alkyl chain), 0.87 (t, 3H, *J* 6.4 Hz, C**H**$_3$); ^{13}C NMR ($CDCl_3$): 145.0, 133.6, 130.2, 128.3 (C$_{aro}$), 71.4, 70.9, 70.1(**C**H$_2$OR), 32.2–23.0 (**C**H$_2$ alkyl chain), 22.0 (**C**H$_3$ tosyl), 14.4 (**C**H$_3$).

*4.13. 4-O-n-Dodecyl-but-1-yl tosylate (**2d**)*

White solid (2.64 g, 6.40 mmol, 82%); ^{1}H NMR ($CDCl_3$): 7.80, 7.34 (4H, d, *J* 8.1, H$_{aro}$), 4.07 (2H, t, *J* 6.1 Hz, C**H**$_2$OTs), 3.32 (4H, m, C**H**$_2$OR), 2.46 (s, 3H, C**H**$_3$ tosyl), 1.80–1.29 (m, H alkyl chain), 0.85 (t, 3H, *J* 6.6 Hz, C**H**$_3$); ^{13}C NMR ($CDCl_3$): 144.9, 133.4, 130.2, 128.4 (C$_{aro}$), 71.2, 70.9, 69.9 (**C**H$_2$OR), 32.1–23.2 (**C**H$_2$ alkyl chain), 21.9 (**C**H$_3$ tosyl), 14.3 (**C**H$_3$).

*4.14. 4-O-n-Octyl-but-2-yn-1-yl tosylate (**2b**)*

White solid (2.59 g, 7.37 mmol, 73%); ^{1}H NMR ($CDCl_3$): 7.80, 7.34 (4H, d, *J* 8.1 Hz, H$_{aro}$), 4.75 (2H, m, ≡CC**H**$_2$OTs), 4.03 (2H, m, ROC**H**$_2$C≡), 3.39 (t, 2H, C**H**$_2$OCH$_2$C≡, *J* 6.6 Hz), 2.46 (s, 3H, C**H**$_3$ tosyl), 1.54 (m, 2H, C**H**$_2$CH$_2$OCH$_2$C≡), 1.30 (m, H alkyl chain), 0.87 (t, 3H, *J* 6.3 Hz, C**H**$_3$); ^{13}C NMR ($CDCl_3$): 145.4, 133.6, 130.2, 128.3 (C$_{aro}$), 86.3 (**C**≡), 70.8, 67.4 (**C**H$_2$OR), 58.3 (**C**H$_2$OTs), 32.2–23.0 (**C**H$_2$ alkyl chain), 22.1 (**C**H$_3$ tosyl),14.5 (**C**H$_3$).

*4.15. 4-O-n-Dodecyl-but-2-yn-1-yl tosylate (**2e**)*

White solid (2.41 g, 5.91 mmol, 75%); ^{1}H NMR ($CDCl_3$): 7.80, 7.34 (4H, d, *J* 8.1 Hz, H$_{aro}$), 4.74 (2H, m, ≡CC**H**$_2$OTs), 4.02 (2H, m, ROC**H**$_2$C≡), 3.38 (t, 2H, C**H**$_2$OCH$_2$C≡, *J* 6.7 Hz), 2.45 (s, 3H, C**H**$_3$ tosyl), 1.52 (m, 2H, C**H**$_2$CH$_2$OCH$_2$C≡), 1.29 (m, H alkyl chain), 0.88 (t, 3H, *J* 7.1 Hz, C**H**$_3$); ^{13}C NMR ($CDCl_3$): 145.4, 133.5, 130.2, 128.5 (C$_{aro}$), 86.3 (**C**≡), 70.8, 67.2 (**C**H$_2$OR), 58.3 (**C**H$_2$OTs), 32.2–23.0 (**C**H$_2$ alkyl chain), 22.0 (**C**H$_3$ tosyl),14.2 (**C**H$_3$).

*4.16. p O-n-Octyl-phenyl tosylate (**2c**)*

White solid (2.81 g, 7.47 mmol, 90%); ^{1}H NMR ($CDCl_3$): 7.66, 7.30 (4H, d, *J* 8.1 Hz, H$_{aro}$), 6.85, 6.74 (4H, d, *J* 8.0 Hz, H$_{aro}$), 3.87 (2H, t, C**H**$_2$OR, *J* 6.3 Hz), 2.45 (s, 3H, C**H**$_3$ tosyl), 1.73 (m, 2H, CH$_2$C**H**$_2$OR, *J* 6.8 Hz), 1.27 (m, H alkyl chain), 0.88 (t, 3H, *J* 7.2 Hz, C**H**$_3$); ^{13}C NMR ($CDCl_3$): 158.2, 145.7, 115.3 (4 × C$_{aro}$), 145.4, 133.5, 130.2, 128.5 (C$_{aro}$ tosyl) 68.7 (**C**H$_2$OR), 32.3–22.1 (**C**H$_2$ alkyl chain), 22.0 (**C**H$_3$ tosyl),14.5 (**C**H$_3$).

*4.17. p O-n-Dodecyl-phenyl tosylate (**2f**)*

White solid (3.12 g, 7.22 mmol, 92%); ^{1}H NMR ($CDCl_3$): 7.66, 7.30 (4H, d, *J* 8.1 Hz, H$_{aro}$), 6.85, 6.74 (4H, d, *J* 8.0 Hz, H$_{aro}$), 3.85 (2H, t, C**H**$_2$OR, *J* 6.5 Hz), 2.47 (s, 3H, C**H**$_3$ tosyl), 1.71 (m, 2H, CH$_2$C**H**$_2$OR, *J* 6.9 Hz), 1.29 (m, H alkyl chain), 0.85 (t, 3H, *J* 7.2 Hz, C**H**$_3$); ^{13}C NMR ($CDCl_3$): 158.2, 145.7, 115.3 (4 × C$_{aro}$), 145.4, 133.5, 130.2, 128.5 (C$_{aro}$ tosyl) 68.5 (**C**H$_2$OR), 32.1–22.2 (**C**H$_2$ alkyl chain), 21.8 (**C**H$_3$ tosyl), 14.4 (**C**H$_3$).

*4.18. 6-O-(4-O-n-Octyl-but-1-yl)-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (**3a**)*

White solid (1.21 g, 2.72 mmol, 85%); (Found: C, 64.7; H, 10.0. $C_{24}H_{44}O_7$ requires C, 64.8; H, 10.0%); m.p. 83 °C; $[\alpha]_D^{26}$ −18.2° (*c* 0.7; $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.51 (1H, d, J_{1-2} 5.0 Hz, H-1), 4.59 (1H,dd, $J_{3,4}$ 7.9 Hz, H-3), 4.31 (1H, dd, $J_{2,3}$ 2.3 Hz, H-2), 4.26 (1H, dd, $J_{4,5}$ 1.8 Hz, H-4), 3.97 (1H, m, H-5), 3.48 (6H, m, H-6, C**H**$_2$O), 1.65–1.40 (12H, s, CH$_3$ iso), 1.27 (m, H alkyl chain), 0.88 (t, 3H, *J* 6.4 Hz, C**H**$_3$); ^{13}C NMR ($CDCl_3$): δ 109.6, 108.9 (C$_{iso}$), 96.8 (C-1), 71.7 (C-4), 71.6 (CH$_2$O), 71.4 (C-3), 71.0 (2 × **C**H$_2$O, C-2), 69.7 (C-5), 67.1 (C-6), 32.3–23.0 (**C**H$_2$ alkyl chain), 26.7, 25.1, 23.2, 22.9 (4 × **C**H$_3$ iso), 14.5 (**C**H$_3$).

*4.19. 6-O-(4-O-n-Dodecyl-but-1-yl)-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (**3d**)*

White solid (1.83 g, 3.66 mmol, 91%); (Found: C, 67.1; H, 10.6. $C_{28}H_{52}O_7$ requires C, 67.2; H, 10.5%); m.p. 95 °C; $[\alpha]_D^{26} = -21.3°$ (*c* 0.8; $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.53 (1H, d, J_{1-2} 5.1 Hz, H-1), 4.59 (1H,dd, $J_{3,4}$ 7.8 Hz, H-3), 4.31 (1H, dd, $J_{2,3}$ 2.4 Hz, H-2), 4.23 (1H, dd, $J_{4,5}$ 1.8 Hz, H-4), 3.97 (1H, m, H-5), 3.48 (6H, m, H-6, C**H**$_2$O), 1.65–1.34 (12H, s, CH$_3$ iso), 1.25 (m, H alkyl chain), 0.89 (t, 3H, *J* 6.5 Hz, C**H**$_3$); ^{13}C NMR ($CDCl_3$): δ 109.6, 108.9 (C$_{iso}$), 96.8 (C-1), 71.7 (C-4), 71.6 (CH$_2$O), 71.4 (C-3), 71.0 (2 × **C**H$_2$O, C-2), 69.7 (C-5), 67.1 (C-6), 32.4–22.7 (**C**H$_2$ alkyl chain), 26.7, 25.1, 23.4, 23.2 (4 × **C**H$_3$ iso), 14.4 (**C**H$_3$).

*4.20. 6-O-(4-O-n-Octyl-but-2-yn-1-yl)-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (**3b**)*

White solid (1.07 g, 2.43 mmol, 74%); (Found: C, 65.4; H, 9.1. $C_{24}H_{40}O_7$ requires C, 65.4; H, 9.15%); m.p. 78 °C; $[\alpha]_D^{26}$ −20.1° (*c* 0.6;

$CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.54 (1H, d, J_{1-2} 4.8 Hz, H-1), 4.60 (1H, dd, $J_{3,4}$ 8.0 Hz, H-3), 4.31 (1H, dd, $J_{2,3}$ 2.5 Hz, H-2), 4.26 (3H, m, H-4, ≡CC**H**$_2$), 4.16 (2H, m, ROC**H**$_2$C≡), 3.90 (1H, m, H-5), 3.75 (1H, dd, J_{6a-6b} 11.2 Hz, J_{6a-5} 5.1 Hz, H-6a), 3.65 (1H, dd, J_{6b-5} 6.9 Hz, H-6b), 3.48 (2H, m, C**H**$_2$O), 1.54–1.33 (12H, s, C**H**$_3$ iso), 1.27 (m, H alkyl chain), 0.88 (t, 3H, *J* 6.4 Hz, C**H**$_3$); ^{13}C NMR ($CDCl_3$): δ 109.7, 109.0 (C_{iso}), 96.7 (C-1), 83.2, 82.3 (**C**≡), 71.6 (C-4), 71.1 (C-3), 70.9 (C-2) 70.7 (**C**H$_2$O), 69.0 (C-5), 61.8 (C-6), 59.2, 58.6 (O**C**H$_2$C≡), 32.3–23.0 (**C**H$_2$ alkyl chain), 26.5, 25.3, 23.0 (4 × **C**H$_3$ iso), 14.5 (**C**H$_3$).

4.21. 6-O-(4-O-n-Dodecyl-but-2-yn-1-yl)-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (**3e**)

White solid (1.26 g, 3.03 mmol, 81%); (Found: C, 67.7; H, 9.7. $C_{28}H_{48}O_7$ requires C, 67.7; H, 9.7%); m.p. 91 °C; $[\alpha]_D^{26}$ −23.4° (*c* 0.7; $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.56 (1H, d, J_{1-2} 5.0 Hz, H-1), 4.57 (1H, dd, $J_{3,4}$ 7.9 Hz, H-3), 4.31 (1H, dd, $J_{2,3}$ 2.6 Hz, H-2), 4.27 (3H, m, H-4, ≡CC**H**$_2$), 4.16 (2H, m, ROC**H**$_2$C≡), 3.92 (1H, m, H-5), 3.77 (1H, dd, J_{6a-6b} 10.9 Hz, J_{6a-5} 5.0 Hz, H-6a), 3.66 (1H, dd, J_{6b-5} 7.0 Hz, H-6b), 3.48 (2H, m, C**H**$_2$O), 1.52–1.28 (12H, s, C**H**$_3$ iso), 1.25 (m, H alkyl chain), 0.85 (t, 3H, *J* 6.4 Hz, C**H**$_3$); ^{13}C NMR ($CDCl_3$): δ 109.7, 109.0 (C_{iso}), 96.7 (C-1), 83.2, 82.3 (**C**≡), 71.6 (C-4), 71.1 (C-3), 70.9 (C-2) 70.7 (**C**H$_2$O), 69.0 (C-5), 61.8 (C-6), 59.2, 58.6 (O**C**H$_2$C≡), 32.6–22.8 (**C**H$_2$ alkyl chain), 26.5, 25.3, 23.0 (4 × **C**H$_3$ iso), 14.5 (**C**H$_3$).

4.22. 6-O-(p O-n-Octyl-phen-1-yl)-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (**3c**)

White solid (1.46 g, 3.15 mmol, 62%); (Found: C, 67.1; H, 8.7. $C_{26}H_{40}O_7$ requires C, 67.2; H, 8.7%); m.p. 95 °C; $[\alpha]_D^{26}$ −23.7° (*c* 0.9; $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.77, 7.29 (4H, d, *J* 8.1 Hz, H_{aro}), 5.40 (1H, d, J_{1-2} 5.1 Hz, H-1), 4.53 (1H, dd, $J_{3,4}$ 7.8 Hz, H-3), 4.25 (1H, dd, $J_{2,3}$ 5.4 Hz, H-2), 4.16 (2H, m, H-4 H-6a), 4.05 (2H, m, H-5 H-6b), 1.27 (m, H alkyl chain), 1.17 (t, 3H, *J* 6.4 Hz, C**H**$_3$); ^{13}C NMR ($CDCl_3$): δ 145.2, 133.1, 130.1, 128.4 (C_{aro}), 109.9, 109.2 (C_{iso}), 96.5 (C-1), 70.8 (C-4), 70.7 (C-3), 70.6 (C-2) 68.6 (**C**H$_2$O), 66.2 (C-5), 60.7 (C-6), 32.3–23.0 (CH_2 alkyl chain), 14.5 (**C**H$_3$).

4.23. 6-O-(p O-n-Dodecyl-phen-1-yl)-1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (**3f**)

White solid (1.02 g, 1.96 mmol, 66%); (Found: C, 69.1; H, 9.2. $C_{30}H_{48}O_7$ requires C, 69.2; H, 9.3%); m.p. 98 °C; $[\alpha]_D^{26}$ −25.2° (*c* 0.7; $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.79, 7.30 (4H, d, *J* 8.0 Hz, H_{aro}), 5.45 (1H, d, J_{1-2} 5.3 Hz, H-1), 4.53 (1H, dd, $J_{3,4}$ 7.7 Hz, H-3), 4.27 (1H, dd, $J_{2,3}$ 5.3 Hz, H-2), 4.16 (2H, m, H-4 H-6a), 4.07 (2H, m, H-5 H-6b), 1.26 (m, H alkyl chain), 1.15 (t, 3H, *J* 6.4 Hz, C**H**$_3$); ^{13}C NMR ($CDCl_3$): δ 145.2, 133.1, 130.1, 128.4 (C_{aro}), 109.8, 109.4 (C_{iso}), 96.4 (C-1), 71.1 (C-4), 70.8 (C-3), 70.7 (C-2) 68.4 (**C**H$_2$O), 66.3 (C-5), 60.7 (C-6), 32.7–22.8 (CH_2 alkyl chain), 14.3 (**C**H$_3$).

4.24. 6-O-(4-O-n-Octyl-but-1-yl)-α-D-galactopyranose (**4a**)

White solid (0.84 g, 2.31 mmol, 85%); (Found: C, 59.2; H, 10.0. $C_{18}H_{36}O_7$ requires C, 59.3; H, 10.0%); m.p. 119 °C; $[\alpha]_D^{26}$ 22.4° (*c* 0.8; MeOH); ^1H NMR (DMSO d_6): δ 4.93 (1H, d, J_{1-2} 3.1 Hz, H-1), 3.91 (1H, m, H-5), 3.62 (1H, dd, $J_{4,3}$ 2.9 Hz, H-4), 3.53 (1H, dd, $J_{3,2}$ 7.5 Hz, H-3), 3.50 (1H, dd, H-2), 3.45 (6H, m, C**H**$_2$O), 3.40 (1H, dd, J_{6a-6b} 9.4, H-6a), 3.36 (1H, dd, J_{6b-5} 5.8 Hz, H-6b), 1.49–1.27 (m, H alkyl chain), 0.85 (t, 3H, *J* 6.4 Hz, C**H**$_3$); ^{13}C NMR (DMSO d_6): δ 92.5 (C-1), 70.1 (C-6), 69.3 (C-4), 69.1 (C-3), 68.6 (CH_2O), 68.5 (C-2), 68.3 (C-5), 31.2–22.0 (**C**H$_2$ alkyl chain), 13.6 (**C**H$_3$).

4.25. 6-O-(4-O-n-Dodecyl-but-1-yl)-α-D-galactopyranose (**4d**)

White solid (1.38 g, 3.29 mmol, 90%); (Found: C, 62.8; H, 10.6. $C_{22}H_{44}O_7$ requires C, 62.8; H, 10.5%); m.p. 121 °C; $[\alpha]_D^{26}$ 24.1° (*c* 0.5; MeOH); ^1H NMR (DMSO d_6): δ 4.92 (1H, d, J_{1-2} 3.0 Hz, H-1), 3.93 (1H, m, H-5), 3.65 (1H, dd, $J_{4,3}$ 3.1 Hz, H-4), 3.50 (1H, dd, $J_{3,2}$ 7.2 Hz, H-3), 3.48 (1H, dd, H-2), 3.43 (6H, m, C**H**$_2$O), 3.38 (1H, dd, J_{6a-6b} 9.1, H-6a), 3.35 (1H, dd, J_{6b-5} 5.5 Hz, H-6b), 1.53–1.21 (m, H alkyl chain), 0.83 (t, 3H, *J* 6.9 Hz, C**H**$_3$); ^{13}C NMR (DMSO d_6): δ 92.5 (C-1), 70.1 (C-6), 69.3 (C-4), 69.1 (C-3), 68.4 (CH_2O), 68.3 (C-2), 67.9 (C-5), 31.5–21.6 (**C**H$_2$ alkyl chain), 13.3 (**C**H$_3$).

4.26. 6-O-(4-O-n-Octyl-but-2-yn-1-yl)-α-D-galactopyranose (**4b**)

White solid (0.77 g, 2.14 mmol, 88%); (Found: C, 59.9; H, 8.9. $C_{18}H_{32}O_7$ requires C, 60.0; H, 8.95%); m.p. 108 °C; $[\alpha]_D^{26}$ 20.3° (*c* 0.5; MeOH); ^1H NMR (DMSO d_6): δ 4.91 (1H, d, J_{1-2} 3.3 Hz, H-1), 4.21 (2H, m, H-4, ≡CC**H**$_2$), 4.12 (2H, m, ROC**H**$_2$C≡), 4.01 (1H, m, H-5), 3.68 (1H, dd, $J_{4,3}$ 3.1 Hz, H-4), 3.53 (1H, dd, $J_{3,2}$ 7.8 Hz, H-3), 3.48 (1H, dd, H-2), 3.45 (2H, m, C**H**$_2$O), 3.39 (1H, dd, J_{6a-6b} 9.1, H-6a), 3.32 (1H, dd, J_{6b-5} 5.7 Hz, H-6b), 1.50–1.25 (m, H alkyl chain), 0.88 (t, 3H, *J* 6.5 Hz, C**H**$_3$); ^{13}C NMR (DMSO d_6): δ 92.5 (C-1), 70.1 (C-6), 69.3 (C-4), 69.1 (C-3), 68.6 (CH_2O), 68.5 (C-2), 68.3 (C-5), 31.2–22.0 (**C**H$_2$ alkyl chain), 13.6 (**C**H$_3$).

4.27. 6-O-(4-O-n-Dodecyl-but-2-yn-1-yl)-α-D-galactopyranose (**4e**)

White solid (0.99 g, 2.39 mmol, 79%); (Found: C, 63.3; H, 9.7. $C_{22}H_{40}O_7$ requires C, 63.4; H, 9.7%); m.p. 113 °C; $[\alpha]_D^{26}$ 21.4° (*c* 0.5; MeOH); ^1H NMR (DMSO d_6): δ 4.95 (1H, d, J_{1-2} 3.7 Hz, H-1), 4.19 (2H, m, H-4, ≡CC**H**$_2$), 4.11 (2H, m, ROC**H**$_2$C≡), 3.98 (1H, m, H-5), 3.70 (1H, dd, $J_{4,3}$ 3.4 Hz, H-4), 3.53 (1H, dd, $J_{3,2}$ 7.9 Hz, H-3), 3.45 (1H, dd, H-2), 3.42 (2H, m, C**H**$_2$O), 3.39 (1H, dd, J_{6a-6b} 9.5, H-6a), 3.35 (1H, dd, J_{6b-5} 5.9 Hz, H-6b), 1.54–1.19 (m, H alkyl chain), 0.91 (t, 3H, *J* 6.5 Hz, C**H**$_3$); ^{13}C NMR (DMSO d_6): δ 92.5 (C-1), 70.3 (C-6), 69.1 (C-4), 68.9 (C-3), 68.6 (CH_2O), 68.5 (C-2), 68.1 (C-5), 31.6–21.5 (**C**H$_2$ alkyl chain), 13.6 (**C**H$_3$).

4.28. 6-O-(p O-n-Octyl-phen-1-yl)-α-D-galactopyranose (**4c**)

White solid (0.91 g, 2.36 mmol, 75%); (Found: C, 62.5; H, 8.4. $C_{20}H_{32}O_7$ requires C, 62.5; H, 8.4%); m.p. 139 °C; $[\alpha]_D^{26}$ 28.2 (*c* 0.5; MeOH); ^1H NMR (DMSO d_6): δ 7.80, 7.32 (4H, d, *J* 8.3 Hz, H_{aro}), 4.91 (1H, d, J_{1-2} 3.3 Hz, H-1), 3.96 (1H, m, H-5), 3.69 (1H, dd, $J_{4,3}$ 2.7 Hz, H-4), 3.59 (1H, dd, $J_{3,2}$ 7.7 Hz, H-3), 3.55 (1H, dd, H-2), 3.45 (2H, m, C**H**$_2$O), 3.38 (1H, dd, J_{6a-6b} 9.7 Hz, H-6a), 3.32 (1H, dd, J_{6b-5} 6.1 Hz, H-6b), 1.49–1.27 (m, H alkyl chain), 0.85 (t, 3H, *J* 6.4 Hz, C**H**$_3$); ^{13}C NMR (DMSO d_6): δ 151.2, 143.1, 138.1, 131.4 (C_{aro}), 92.8 (C-1), 71.1 (C-6), 69.1 (C-4), 68.9 (C-3), 68.4 (CH_2O), 68.1 (C-2), 59.9 (C-5), 31.2–22.0 (CH_2 alkyl chain), 13.4 (**C**H$_3$).

4.29. 6-O-(p O-n-Dodecyl-phen-1-yl)-α-D-galactopyranose (**4f**)

White solid (0.65 g, 1.49 mmol, 76%); (Found: C, 65.4; H, 9.2. $C_{24}H_{40}O_7$ requires C, 65.4; H, 9.15%); m.p. 128 °C; $[\alpha]_D^{26}$ 26.7° (*c* 0.5; MeOH); ^1H NMR (DMSO d_6): δ 7.79, 7.35 (4H, d, *J* 8.5 Hz, H_{aro}), 4.93 (1H, d, J_{1-2} 3.6 Hz, H-1), 3.95 (1H, m, H-5), 3.71 (1H, dd, $J_{4,3}$ 3.0 Hz, H-4), 3.57 (1H, dd, $J_{3,2}$ 7.9 Hz, H-3), 3.56 (1H, dd, H-2), 3.42 (2H, m, C**H**$_2$O), 3.34 (1H, dd, J_{6a-6b} 9.9 Hz, H-6a), 3.29 (1H, dd, J_{6b-5} 6.3 Hz, H-6b), 1.53–1.23 (m, H alkyl chain), 0.84 (t, 3H, *J* 6.4 Hz, C**H**$_3$); ^{13}C NMR (DMSO d_6): δ 151.2, 143.1, 138.1, 131.4 (C_{aro}), 92.7 (C-1), 71.1 (C-6), 69.3 (C-4), 68.7 (C-3), 68.5 (CH_2O), 68.1 (C-2), 59.7 (C-5), 31.6–21.5 (CH_2 alkyl chain), 13.5 (**C**H$_3$).

4.30. Analysis of antimicrobial properties

4.30.1. Organisms and culture conditions

The antimicrobial activity of the 6-*O*-alkyl-galactose derivatives was tested on ten bacterial strains: *B. stearothermophilus* ATTC 12980, *C. freundii* ATCC 6750, *E. cloacae* ATCC 13047, *E. chrysanthemi* EC3937, *E. coli* ATCC 10536, *M. luteus* CIP 53.45, *M. smegmatis* CIP 73.26, *P. aeruginosa* CIP A22, *S. meliloti* Rm1021 and *S. aureus* subsp *aureus* CIP 53.154.

Stock cultures were maintained in Luria Bertani (LB) broth supplemented with 20% glycerol at −80 °C. Cultures were realized from 1% (v/v) stock cultures in a nutritive broth identical for all the strains (Yeast extract 1 g/L, Meat extract 2 g/L, Peptone 5 g/L and NaCl 5 g/L) at 30 °C, except for *M. smegmatis* which was incubated in a LB/Tween 80 medium (Tryptone 10 g/L, Yeast extract 5 g/L, NaCl 5 g/L and Tween 80 at 0.05%) at 37 °C. Working suspensions were obtained from an overnight culture, eventually diluted in nutritive broth (or LB/Tween 80 medium for *M. smegmatis*), to reach a bacterial density equivalent to a 0.5 McFarland standard, corresponding approximatively to 1.5×10^8 cfu/mL.

4.30.2. Assay of antimicrobial activity in liquid medium

Stock solutions of the 6-*O*-alkyl-galactose derivatives were prepared at 1 g/L in DMSO and were then serially diluted in sterile nutritive broth (or LB/Tween 80 medium for *M. smegmatis*) to a final volume of 2 mL. Two milliliters of bacterial working suspension were added to each tube and the final concentration of 6-*O*-alkyl-galactose derivatives ranged from 1:2 to 1:512. Several controls were realized for each microorganism and each synthesized compound, as previously described [29]. A supplementary control consisted in inoculated nutritive broth without compounds but with chloramphenicol, which is a broad-spectrum antibiotic, in order to compare its antimicrobial activity to those of the 6-*O*-alkyl-galactose derivatives.

Then each tube was incubated for 24 h in a rotary shaker (150 rpm) at 30 °C, except *M. smegmatis* at 37 °C. Bacterial density was determined spectrophotometrically at 600 nm. The MIC was defined as the lowest concentration of compound that showed no increase in Optical Density (OD) values for all the replicates compared to the negative control after incubation. Each experiment was replicated three times.

Acknowledgments

This work was supported by the Ministère de l'Enseignement Supérieur et de la Recherche. The authors thank Adrien Valingot and Cécile Poret for their excellent microbiological support.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.12.032.

References

[1] T. Plat, R.J. Linhardt, Syntheses and applications of sucrose-based esters, J. Surfactants Deterg. 4 (2001) 415–421.
[2] M. Abert, N. Mora, J.-M. Lacombe, Synthesis and surface-active properties of a new class of surfactants derived from D-gluconic acid, Carbohydr. Res. 337 (2002) 997–1006.
[3] D. Pilakowska-Pietras, K. Lunkenheimer, A. Piasecki, Synthesis of novel *N,N*-di-*n*-alkylaldonamides and properties of their surface chemically pure adsorption layers at the air/water interface, J. Colloid Interface Sci. 271 (2004) 192–200.
[4] C. Satgé, R. Granet, B. Verneuil, Y. Champavier, P. Krausz, Synthesis and properties of new bolaform and macrocyclic galactose-based surfactants obtained by olefin metathesis, Carbohydr. Res. 339 (2004) 1243–1254.
[5] L. Chaveriat, I. Stasik, G. Demailly, D. Beaupère, Direct syntheses of *S*-alkylthio-D-galactono-, D-mannono-1,4-lactones, *S*-alkylthio-L-galactitols and D-mannitols displaying amphiphilic and mesophasic properties, Carbohydr. Res. 339 (2004) 1817–1821.
[6] M.V. de Almeida, M. Le Hyaric, G.W. Amarante, M.C.S. Lourenço, M.L.L. Brandão, Synthesis of amphiphilic galactopyranosyl diamines and amino alcohols as antitubercular agents, Eur. J. Med. Chem. 42 (2007) 1076–1083.
[7] L. Chaveriat, C. Meyer, D. Beaupère, G. Demailly, I. Stasik, 6-*S*-alkyl-6-thiohexonolactones and 6-*S*-alkyl-6-thiohexitols: efficient synthesis and new smectic liquid crystal phases, J. Mol. Liq. 142 (2008) 17–21.
[8] K. Holmberg, Novel surfactants. Preparation, applications and biodegradability, second ed., in: M. Dekker (Ed.), Surfactant Science Series, vol. 114, New York, 2003, pp. 1–192.
[9] S. Warwel, F. Brüse, C. Demes, M. Kunz, M. Rüsch gen Klaas, Polymers and surfactants on the basis of renewable resources, Chemosphere 43 (2001) 39–48.
[10] Y. Queneau, S. Chambert, C. Besset, R. Cheaib, Recent progress in the synthesis of carbohydrate-based amphiphilic materials: the examples of sucrose and isomaltulose, Carbohydr. Res. 343 (2008) 1999–2009.
[11] M. le Maire, P. Champeil, J.V. Møller, Interaction of membrane proteins and lipids with solubilizing detergents, Biochim. Biophys. Acta 1508 (2000) 86–111.
[12] J. Piao, S. Kishi, S. Adachi, Surface tensions of aqueous solutions of 1-*O*-monoacyl sugar alcohols, Colloids Surf. A Physicochem. Eng. Aspects 277 (2006) 15–19.
[13] M.N. Jones, Surfactants in membrane solubilisation, Int. J. Pharm. 177 (1999) 137–159.
[14] H. Ahyayauch, M. Bennouna, A. Alonso, F.M. Goñi, Detergent effects on membranes at subsolubilizing concentrations: transmembrane lipid motion, bilayer permeabilization, and vesicle lysis/reassembly are independent phenomena, Langmuir 26 (2010) 7307–7313.
[15] J.D. Rose, J.A. Maddry, R.N. Comber, W.J. Suling, L.N. Wilson, R.C. Reynolds, Synthesis and biological evaluation of trehalose analogs as potential inhibitors of mycobacterial cell wall biosynthesis, Carbohydr. Res. 337 (2002) 105–120.
[16] N. Tewari, V.K. Tiwari, R.P. Tripathi, V. Chaturvedi, A. Srivastava, R. Srivastava, P.K. Shukla, A.K. Chaturvedi, A. Gaikwad, S. Sinha, B.S. Srivastava, Synthesis of galactopyranosyl amino alcohols as a new class of antitubercular and antifungal agents, Bioorg. Med. Chem. Lett. 14 (2004) 329–332.
[17] D. Katiyar, V.K. Tiwari, N. Tewari, S.S. Verma, S. Sinha, A. Gaikwad, A. Srivastava, V. Chaturvedi, R. Srivastava, B.S. Srivastava, R.P. Tripathi, Synthesis and antimycobacterial activities of glycosylated amino alcohols and amines, Eur. J. Med. Chem. 40 (2005) 351–360.
[18] R.P. Tripathi, V.K. Tiwari, N. Tewari, D. Katiyar, N. Saxena, S. Sinha, A. Gaikwad, A. Srivastava, V. Chaturvedi, Y.K. Manju, R. Srivastava, B.S. Srivastava, Synthesis and antitubercular activities of bis-glycosylated diamino alcohols, Bioorg. Med. Chem. 13 (2005) 5668–5679.
[19] A.F. Taveira, M. Le Hyaric, E.F.C. Reis, D.P. Araújo, A.P. Ferreira, M. Aparecida de Souza, L.L. Alves, M.C.S. Lourenço, F.R.C. Vicente, M.V. de Almeida, Preparation and antitubercular activities of alkylated amino alcohols and their glycosylated derivatives, Bioorg. Med. Chem. 15 (2007) 7789–7794.
[20] C.B. Davis, R.D. Hartnell, P.D. Madge, D.J. Owen, R.J. Thomson, A.K. Chong, R.L. Coppel, M. von Itzstein, Synthesis and biological evaluation of galactofuranosyl alkyl thioglycosides as inhibitors of mycobacteria, Carbohydr. Res. 342 (2007) 1773–1780.
[21] A.K. Sanki, J. Boucau, P. Srivastava, S.S. Adams, D.R. Ronning, S.J. Sucheck, Synthesis of methyl 5-*S*-alkyl-5-thio-D-arabinofuranosides and evaluation of their antimycobacterial activity, Bioorg. Med. Chem. 16 (2008) 5672–5682.
[22] S. Quader, S.E. Boyd, I.D. Jenkins, T.A. Houston, Multisite modification of neomycin B: combined Mitsunobu and click chemistry approach, J. Org. Chem. 72 (2007) 1962–1979.
[23] J. Zhang, F.-I. Chiang, L. Wu, P.G. Czyryca, D. Li, C.-W. Tom Chang, Surprising alteration of antibacterial activity of 5″-modified neomycin against resistant bacteria, J. Med. Chem. 51 (2008) 7563–7573.
[24] S. Pal, K. Mitra, S. Azmi, J.K. Ghosh, T.K. Chakraborty, Towards the synthesis of sugar amino acid containing antimicrobial noncytotoxic CAP conjugates with gold nanoparticles and a mechanistic study of cell disruption, Org. Biomol. Chem. 9 (2011) 4806–4810.
[25] N. Gernigon, V. Bordeau, F. Berrée, B. Felden, B. Carboni, Synthesis and antibacterial activity of novel neamine derivatives: preponderant role of the substituent position on the neamine core, Org. Biomol. Chem. 10 (2012) 4720–4730.
[26] R.P. Tripathi, R. Tripathi, V.K. Tiwari, L. Bala, S. Sinha, A. Srivastava, R. Srivastava, B.S. Srivastava, Synthesis of glycosylated beta-amino acids as new class of antitubercular agents, Eur. J. Med. Chem. 37 (2002) 773–781.
[27] M. Ferrer, J. Soliveri, F.J. Plou, N. López-Cortés, D. Reyes-Duarte, M. Christensen, J.L. Copa-Patiño, A. Ballesteros, Synthesis of sugar esters in solvent mixtures by lipases from *Thermomyces lanuginosus* and *Candida antarctica* B, and their antimicrobial properties, Enzyme Microb. Technol. 36 (2005) 391–398.
[28] A. Smith, P. Nobmann, G. Henehan, P. Bourke, J. Dunne, Synthesis and antimicrobial evaluation of carbohydrate and polyhydroxylated non-carbohydrate fatty acid ester and ether derivatives, Carbohydr. Res. 343 (2008) 2557–2566.
[29] P. Nobmann, A. Smith, J. Dunne, G. Henehan, P. Bourke, The antimicrobial efficacy and structure activity relationship of novel carbohydrate fatty acid derivatives against *Listeria* spp. and food spoilage microorganisms, Int. J. Food Microbiol. 128 (2009) 440–445.
[30] A.K. Pathak, V. Pathak, L. Seitz, J.A. Maddry, S.S. Gurcha, G.S. Besra, W.J. Suling, R.C. Reynolds, Studies on (β,1→5) and (β,1→6) linked octyl Gal*f* disaccharides

as substrates for mycobacterial galactosyltransferase activity, Bioorg. Med. Chem. 9 (2001) 3129–3143.
[31] A.K. Pathak, V. Pathak, W.J. Suling, S.S. Gurcha, C.B. Morehouse, G.S. Besra, J.A. Maddry, R.C. Reynolds, Studies on *n*-octyl-5-(α-D-arabinofuranosyl)-β-D-galactofuranosides for mycobacterial glycosyltransferase activity, Bioorg. Med. Chem. 10 (2002) 923–928.
[32] F.V.M. Silva, M. Goulart, J. Justino, A. Neves, F. Santos, J. Caio, S. Lucas, A. Newton, D. Sacoto, E. Barbosa, M.-S. Santos, A.P. Rauter, Alkyl deoxy-*arabino*-hexopyranosides: synthesis, surface properties, and biological activities, Bioorg. Med. Chem. 16 (2008) 4083–4092.
[33] P. Nobmann, P. Bourke, J. Dunne, G. Henehan, *In vitro* antimicrobial activity and mechanism of action of novel carbohydrate fatty acid derivatives against *Staphylococcus aureus* and MRSA, J. Appl. Microbiol. 108 (2010) 2152–2161.
[34] L. Legentil, J.L. Audic, R. Daniellou, C. Nugier-Chauvin, V. Ferrières, Studies of a furanoside as antimycobacterial agent loaded into a biodegradable PBAT/sodium caseinate support, Carbohydr. Res. 346 (2011) 1541–1545.
[35] H.C. Neu, The crisis in antibiotic resistance, Science 257 (1992) 1064–1073.
[36] J.L. Martinez, A. Fajardo, L. Garmendia, A. Hernandez, J.F. Linares, L. Martínez-Solano, M.B. Sánchez, A global view of antibiotic resistance, FEMS Microbiol. Rev. 33 (2009) 44–65.
[37] B. Ouattara, R.E. Simard, R.A. Holley, G.J.-P. Piette, A. Bégin, Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms, Int. J. Food Microbiol. 37 (1997) 155–162.
[38] G. Bergsson, J. Arnfinnsson, O. Steingriacute;msson, H. Thormar, Killing of gram-positive cocci by fatty acids and monoglycerides, APMIS 109 (2001) 670–678.
[39] M.K. Nair, H. Abouelezz, T. Hoagland, K. Venkitanarayanan, Antibacterial effect of monocaprylin on *Escherichia coli* O157:H7 in apple juice, J. Food Prot. 68 (2005) 1895–1899.
[40] T. Kitahara, Y. Aoyama, Y. Hirakata, S. Kamihira, S. Kohno, N. Ichikawa, M. Nakashima, H. Sasaki, S. Higuchi, *In vitro* activity of lauric acid or myristylamine in combination with six antimicrobial agents against methicillin-resistant *Staphylococcus aureus* (MRSA), Int. J. Antimicrob. Agents 27 (2006) 51–57.
[41] A. Kollanoor, P. Vasudevan, M.K.M. Nair, T. Hoagland, K. Venkitanarayanan, Inactivation of bacterial fish pathogens by medium-chain lipid molecules (caprylic acid, monocaprylin and sodium caprylate), Aquacult. Res. 38 (2007) 1293–1300.
[42] H.I. Jang, M.S. Rhee, Inhibitory effect of caprylic acid and mild heat on *Cronobacter* spp. (*Enterobacter sakazakii*) in reconstituted infant formula and determination of injury by flow cytometry, Int. J. Food Microbiol. 133 (2009) 113–120.
[43] T. Nakatsuji, M.C. Kao, J.-Y. Fang, C.C. Zouboulis, L. Zhang, R.L. Gallo, C.-M. Huang, Antimicrobial property of lauric acid against *Propionibacterium acnes*: its therapeutic potential for inflammatory acne vulgaris, J. Invest. Dermatol. 129 (2009) 2480–2488.
[44] T. Watanabe, S. Katayama, M. Matsubara, Y. Honda, M. Kuwahara, Antibacterial carbohydrate monoesters suppressing cell growth of *Streptococcus mutans*in the presence of sucrose, Curr. Microbiol. 41 (2000) 210–213.
[45] P. Bault, P. Godé, G. Goethals, J.W. Goodby, J.A. Haley, S.M. Kelly, G.H. Mehl, G. Ronco, P. Villa, Liquid crystalline derivatives of galactose and galactitol: dependence of thermotropic mesomorphism on carbohydrate form, Liq. Cryst. 25 (1998) 31–45.
[46] P. Bault, P. Godé, G. Goethals, J.W. Goodby, J.A. Haley, S.M. Kelly, G.H. Mehl, G. Ronco, P. Villa, An homologous series of 6-*O*-*n*-alkyl-alpha-D-galactopyranoses: synthesis and thermotropic mesomorphic properties, Liq. Cryst. 24 (1998) 283–293.
[47] M.P. Savelli, P. Van Roeckeghem, O. Douillet, G. Cavé, P. Godé, G. Ronco, P. Villa, Effect of tail alkyl chain length (*n*), head group structure and junction (Z) on amphiphilic properties of 1-Z-*R*-D, L-xylitol compounds, Int. J. Pharm. 182 (1999) 221–236.
[48] G. Goethals, A. Fernàndez, P. Martin, M. Minana-Pérez, C. Scorza, P. Villa, P. Godé, Spacer arm influence on glucidoamphiphile compound properties, Carbohydr. Polym. 45 (2001) 147–154.
[49] P. Bault, P. Godé, G. Goethals, J.W. Goodby, J.A. Haley, S.M. Kelly, G.H. Mehl, P. Villa, Synthesis and mesomorphism of 6-*Z*-*n*-alkyl-α-D-galactopyranoses, Liq. Cryst. 26 (1999) 985–997.
[50] L. Di Marzio, C. Marianecci, M. Petrone, F. Rinaldi, M. Carafa, Novel pH-sensitive non-ionic surfactant vesicles: comparison between Tween 21 and Tween 20, Colloids Surf. B Biointerfaces 82 (2011) 18–24.
[51] A.D. Russell, Mechanisms of bacterial resistance to non-antibiotics: food additives and food and pharmaceutical preservatives, J. Appl. Bacteriol. 71 (1991) 191–201.
[52] T.J. Silhavy, D. Kahne, S. Walker, The bacterial cell envelope, Cold Spring Harb. Perspect. Biol. 2 (2010) a000414.
[53] A.F. Haag, S. Wehmeier, A. Muszynski, B. Kerscher, V. Fletcher, S.H. Berry, G.L. Hold, R.W. Carlson, G.P. Ferguson, Biochemical characterization of *Sinorhizobium meliloti* mutants reveals gene products involved in the biosynthesis of the unusual lipid A very long-chain fatty acid, J. Biol. Chem. 286 (2011) 17455–17466.
[54] R.M. Epand, R.F. Epand, Domains in bacterial membranes and the action of antimicrobial agents, Mol. Biosyst. 5 (2009) 580–587.
[55] J. de Bony, A. Lopez, M. Gilleron, M. Welby, G. Lanéelle, B. Rousseau, J.P. Beaucourt, J.F. Tocanne, Transverse and lateral distribution of phospholipids and glycolipids in the membrane of the bacterium *Micrococcus luteus*, Biochemistry 28 (1989) 3728–3737.