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Alkyl deoxy-*arabino*-hexopyranosides: Synthesis, surface properties, and biological activities

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Abstract—Octyl and dodecyl glycosides possessing 2-deoxy-*arabino*-hexopyranoside moieties belonging to the D- and L-series in their α- and β-forms were synthesized by reaction of an acetyl protected glycal with octanol or dodecanol, catalyzed by triphenylphosphine hydrobromide, followed by deprotection. Their surface properties were studied and discussed in terms of the adsorption and aggregation parameters, pC_{20}, CMC, and γ_{CMC}. The antimicrobial activities were assessed using the paper disk diffusion and broth dilution methods. Both the octyl and dodecyl 2-deoxy β-D-glycosides inhibited significantly *Enterococcus faecalis*, a microbe also highly susceptible to dodecyl 2,6-dideoxy-α-L-*arabino*-hexopyranoside. This compound was particularly active against *Bacillus cereus* and *Bacillus subtilis*, presenting for both *Bacillus* species a minimal inhibitory concentration of the same order of magnitude and a minimal lethal concentration even smaller than that obtained for chloramphenicol, a bioactivity which remained unaltered after 1 year solution storage at 4 °C. In addition, activity over *Listeria monocytogenes* was also observed. Direct cytotoxicity and genotoxicity of the glycosides were determined by proliferative index (mitotic index) evaluation in peripheral human lymphocytes of healthy donors. All compounds induced acute toxicity effects, and the response was dose dependent for the α-anomer of both the alkyl 2-deoxy-*arabino*-hexopyranosides and for the corresponding dodecyl β-anomer, what suggests that non-toxic but still bioactive concentrations may be found for these compounds.

1. Introduction

Sugar-based surfactants are a very interesting class of compounds on account of their low toxicity and their synthesis from renewable resources.[1] They are biocompatible and have various applications in personal care and food industry, as detergents, pharmaceuticals, agrochemicals, and explosives.[1–4] An overall survey on the preparation, applications, and biodegradability of sugar surfactants has been recently published.[2] The type of sugar in the headgroup, including its stereochemistry, and the nature of the hydrophobic tail are determinant for the physical and chemical properties exhibited by these compounds. Some non-ionic sugar surfactants, including the octyl β-D-glucoside, have been used for the extraction of biological proteins of the cellular membrane since 1980.[5] However, little is known about deoxy glycoside surfactants and their properties.[6] Glycon deoxygenation disturbs the hydrophilic–lipophilic balance toward lipophilicity, reinforcing the surface activity of this class of surfactants. Furthermore, drug bioavailability has been successfully related to the surface activity of amphiphilic drugs.[7,8] Hence, alkyl deoxy glycosides seem to be promising compounds regarding their bioactivity and surface properties.[6] For their synthesis, a variety of ionic and radical deoxygenation procedures are known.[9] The system glycal/triphenylphosphine hydrobromide has been successfully used to prepare 2-deoxy glycosides in good yield.[10,11] In a previous work, we have synthesized the octyl and dodecyl 2,6-dideoxy-L-*arabino*-hexopyranosides **5–8**

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(Fig. 1), studied their surface activity properties, and screened for their antimicrobial activity using the paper disk diffusion method.[6] The results obtained, in terms of surface properties and antimicrobial activity, prompted us to further study this class of compounds, being the main objectives of this work as follows:

(i) to synthesize new related alkyl 2-deoxy-*arabino*-hexopyranosides belonging to the D-series;
(ii) to evaluate their surface activity parameters providing insight on those which may be related to the observed antimicrobial activity;
(iii) to determine the minimal inhibitory concentration (MIC) and the minimal lethal concentration (MLC) for the glycosides belonging to the D- or L-series, which were recognized as active in preliminary susceptibility tests, using the paper disk diffusion method;
(iv) to rationalize the bioactivity exhibited by the studied compounds in terms of their structural features and surface properties;
(v) to evaluate the potential direct cytotoxicity for human cells of the compounds described.

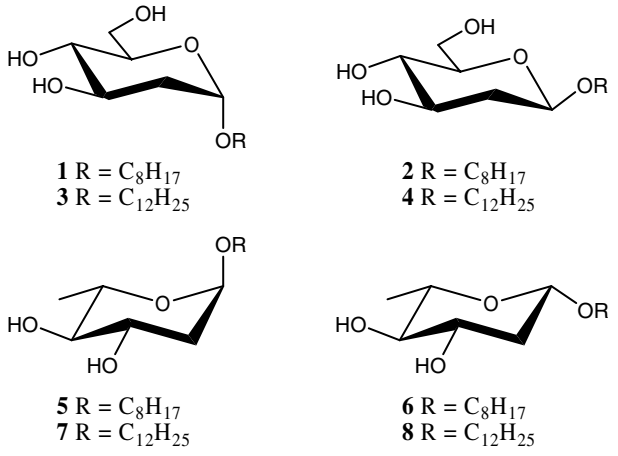
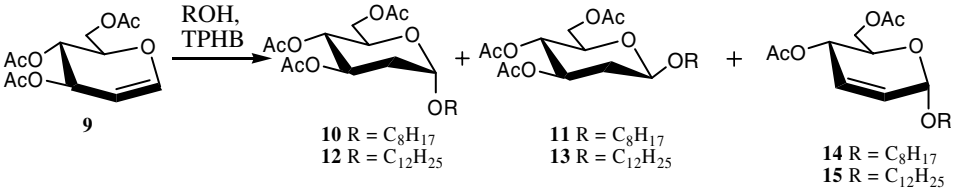


Figure 1. Structure of the studied 2-deoxy-D-*arabino*-glycosides.

2. Results and discussion

2.1. Chemistry

Synthesis of the new 2-deoxy D-glycosides **1**–**4** (Fig. 1) was accomplished in high yield (94.5–98.3%) by Zémplen deacetylation of the glycosides **10**–**13**, obtained by reaction of 3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol (**9**) with octanol or dodecanol, catalyzed by triphenylphosphine hydrobromide (TPHB) in dichloromethane at room temperature for 12 h (Scheme 1 and Table 1). The major formation of the 2-deoxy α-anomers, isolated in 69% yield (**10**) and 83% yield (**12**), results from the anomeric effect, and the corresponding 2-deoxy β-glycosides were isolated in 15% (for **11**) and 12% yield (for **13**). This glycosylation reaction afforded also the 2,3-unsaturated glycosides **14** and **15** in very low yield (1.8% and 1.7%, respectively). These products were not expected to be formed since TPHB has been described as the catalyst of choice for the synthesis of 2-deoxy glycosides from glycals without Ferrier rearrangement, when running the reaction at room temperature.[10] However, compounds of this type have been described as minor secondary products when an L-glycal reacted with dodecanol or octanol, in the presence of TPHB, in dichloromethane under reflux, the procedure followed to prepare compounds **5**–**8**.[6] In order to investigate the influence of temperature and solvent polarity on the reaction stereoselectivity and on the formation of 2,3-unsaturated products, the solvents dichloromethane and dichloroethane (both under reflux) and acetonitrile (at room temperature) were tested (Table 1). This nitrile has been reported to promote a marked effect on the stereochemical outcome of some glycosylation reactions, namely using trichloroacetimidates as glycosyl donors, leading to the stereoselective formation of the β-anomer. This occurs via nucleophilic attack of the alcohol to an intermediary α-nitrilium ion, formed by co-ordination of the glycosyl cation with a solvent molecule.[12] Recently, the system dichloromethane/acetonitrile (95:5) has also been used to increase the β-selectivity of glycosylation reactions conducted with



Scheme 1. Synthesis of the acetyl protected 2-deoxy glycosides.

Table 1. Yield for compounds **10**–**15** and α:β ratio obtained for the 2-deoxy glycosides, when the reaction was run in different solvents, time (*t*), and temperature (*T*)

Entry	Solvent	*t* (h)	*T* (°C)	Yield (%) of compounds **10**–**15**						α:β ratio	
				10	**11**	**12**	**13**	**14**	**15**	**10**:**11**	**12**:**13**
1	CH_2Cl_2	12	rt	69.0	15.0	83.0	12.0	1.8	1.7	5:1	7:1
2	CH_2Cl_2	5	40	66.0	15.0	86.0	12.0	6.2	3.7	4:1	7:1
3	$ClCH_2CH_2Cl$	5	70	46.5	10.0	59.8	11.3	9.8	6.9	5:1	5:1
4	CH_3CN	12	rt	46.3	4.4	67.8	6.6	0.8	2.3	11:1	10:1
5	CH_2Cl_2/CH_3CN (95:5)	12	rt	52.0	5.8	61.3	8.6	2.2	4.4	9:1	7:1

thioglycosides.[13] However, neither acetonitrile nor this system were effective to increase the yield of the β-anomer when using the glycal **9** as donor. In fact, the α:β ratio enhanced in the presence of acetonitrile in the reaction mixture (Table 1). Considering the donor number (DN = 14.0 kcal mol^{-1})[14] and the polarity of acetonitrile (E_T = 45.6 kcal mol^{-1}),[14] this result is rationalized in terms of the stabilization of the intermediate oxonium ion in acetonitrile, favoring the formation of the α-anomer due to the anomeric effect. The use of dichloroethane under reflux led to a lower global yield of the 2-deoxy glycosides and did not affect significantly the α:β ratio, while that of dichloromethane under reflux led to comparable results to those obtained with this solvent at room temperature, being the reaction time reduced to 5 h. However, when the reaction was run under reflux in dichoroethane or dichloromethane, the 2,3-unsaturated glycosides were obtained in higher yield than at room temperature (Table 1). The structural assignment of the 2-deoxy-D-*arabino*-α- and -β-anomers was easily accomplished considering the chemical shift of the anomeric carbon (δ 96.8 for **10**, δ 96.9 for **12**, and δ 99.6 for both **11** and **13**) and proton (δ 4.94 for **10** and **12**, and δ 4.56 for **11** and **13**), as well as the $J_{1,2a}$ coupling constant, which was of the order of magnitude characteristic for *trans*-diaxial coupling in the β-anomers (9.71 Hz for **11** and 9.73 Hz for **13**), while that of the α-glycosides was considerably lower, as expected (3.14 Hz for **10** and 3.11 Hz for **12**). The structure of the allylically rearranged unsaturated α-glycosides **14** and **15** was confirmed by the signals of the olefinic protons, which appeared as a multiplet at δ 5.92–5.81 (for **14**) and δ 5.90–5.79 (for **15**) and carbons (129.1, 128.1 for **14** and 128.9, 127.9 for **15**). The α-configuration was assigned considering the anomeric proton signals, which appeared as singlets at δ 5.03 (for **14**) and δ 4.95 (for **15**) due to their quasi-axial orientation, in agreement with the data previously reported for 2,3-unsaturated α-pyranoid compounds.[6,15]

2.2. Differential scanning calorimetry

Differential scanning calorimetry was used to ascertain compounds purity, stability, and melting temperature. DSC scans were performed from room temperature to 140 °C for compounds **1**–**4**, in sealed aluminum crucibles, at 1 °C/min, and are presented in Figure 2. These scans did not display any pretransitions or 'double melting points' indicating no solvent loss or formation of liquid crystalline phases prior to melting, features which were identified on previous studies for alkyl glucosides,[16–19] performed at higher scanning rates. All scans showed only one endothermic peak, centered at 107.0, 85.5, 114.7, and 103.4 °C for compounds **1**–**4**, respectively. These values are in agreement with the melting points determined independently in a melting point apparatus. A slow scanning rate was used to ensure maximum resolution and accuracy of the scans, and allowed the identification of peak broadening for the octyl derivatives **1** and **2**, showing a shallow curvature on the low temperature side. This behavior suggests the presence of a minor contamination, which is marked in compound **1**, where the scan allowed the identification of a small shoulder. Despite a well-defined thermal hysteresis, presented by successive heating and cooling cycles, in agreement with data previously reported for octyl glucoside,[18] the endothermic peaks were reproducible on successive scans following sample cooling to room temperature.

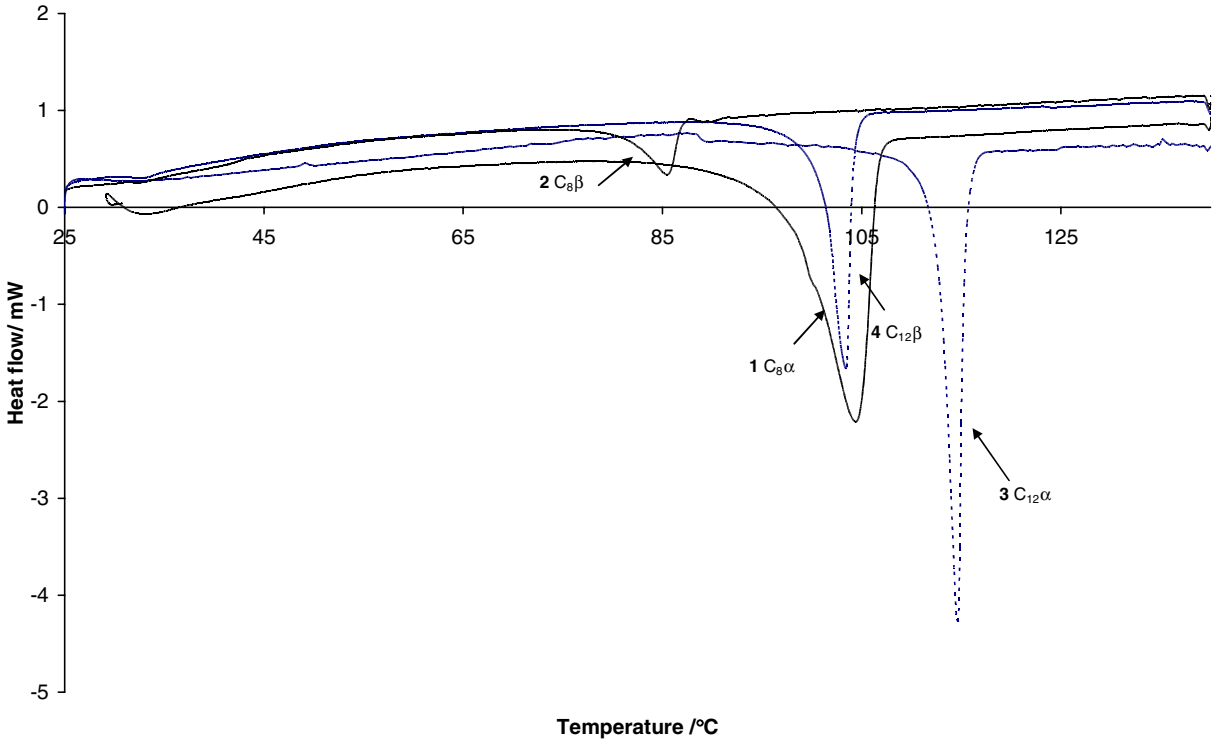


Figure 2. DSC scans obtained at 1 °C/min for compounds **1**–**4**.

2.3. Surface activity

The dependence of the surface tension, γ, with molality, m, for homogeneous solutions of the alkyl 2-deoxy-D-*arabino*-hexopyranosides **1**–**4** was studied. The general trends observed are similar to those previously reported for the 2,6-dideoxy-*arabino*-hexopyranosides.[6] Compound **3** has a very low solubility ($<3\times10^{-5}$ *m*) and solution saturation occurred prior to the onset of any constant surface tension at 35 °C. Solutions of octyl 2-deoxy-D-*arabino*-hexopyranosides **1** and **2** display higher, and similar surface tensions than solutions of dodecyl 2-deoxy-β-D-*arabino*-hexopyranoside **4**, a behavior expected in view of the longer hydrophobic carbon chain of the C_{12} sugar surfactant. The similarities found between compounds **1** and **2** are in agreement with the results previously reported for the adsorption at the air–water interface of α-D- and β-D-anomers of alkyl glucosides[16] and of octyl 2,6-dideoxy α-L- and β-L-*arabino*-hexopyranosides,[6] indicating that for soluble alkyl glycosides, the anomeric configuration has very little effect on the air–water interfacial adsorption properties of those compounds.

CMC values were determined from the break in the γ versus m plots and are presented in Table 2, as well as adsorption efficiency, quantified in terms of pC_{20}, and adsorption effectiveness, γ_{CMC}. Aggregation tendency, evaluated by CMC, and adsorption effectiveness display a decrease on going from the octyl to the dodecyl sugar surfactant, being identical for both octyl glycosides, while adsorption efficiency (pC_{20}) increases with the increase of the alkyl chain length. The same overall behavior is observed on the literature data reported for structurally related surfactants, namely alkyl glucosides[16,20] and alkyl 2,6-dideoxy-L-*arabino*-hexopyranosides,[6] also given in Table 2 for comparative purposes. These data clearly show that, for the same chain length, deoxygenation of the glycoside headgroups promotes aggregation and adsorption, in accordance with the decreasing surfactant hydrophilicity.

The ratio CMC/C_{20} is a convenient way to ascertain the role of structural microenvironmental factors on micellization and on adsorption, therefore this quantity is also included in Table 2. This ratio presents a clear-cut difference in terms of the dependence with chain length on going from glucopyranosides and 2-deoxyhexopyranosides to 2,6-dideoxyhexopyranosides. While for the former two families CMC/C_{20} decreases with chain length, for the latter the opposite is observed, indicating that the alkyl chain increase promotes adsorption more efficiently within the 2,6-dideoxyhexopyranosides. Hence, headgroup hydration is determinant for the aggregation/adsorption balance in these alkyl glycosides families. Furthermore, a closer look at the parameters of glucopyranosides and 2-deoxyhexopyranosides reinforces these conclusions, since chain length increase is more effective in promoting aggregation in glucoderivatives than in deoxygenated compounds.

2.4. Antimicrobial activity

The antimicrobial activity of compounds **1**–**4** was evaluated using the paper disk diffusion method. The results are expressed by the average diameter of the inhibition zone, detected in three replicates, being presented in Table 3 and compared to those previously reported for compounds **5**–**8**.[6] The minimal inhibitory and lethal concentrations (MIC and MLC, respectively) data were determined for those compounds, which demonstrated to be active in the preliminary paper disk tests. *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Aspergillus niger*, and *Candida albicans* were not affected by all the compounds tested and therefore no further dilution tests were conducted with these microbes. With the exception of **5**, that slightly affected *Pyricularia oryzae* (12 mm), no other compound was active against this fungus. Some inhibition of the octyl L-glycosides **5** and **6** over *Bacillus* species and *Staphylococcus aureus* was detected, while compounds **2** and **4** did not affect these microbes (MIC > 500 μg/mL) within the concentrations tested. The dodecyl α-D-glycoside **3** presented trace activity over *L. monocytogenes*, *B. subtilis*, and *C. albicans*, while the octyl α-D-glycoside **1** and the dodecyl β-L-glycoside **8** did not inhibit the growth of any of the microbial species tested.

Using the dilution method, compound **7** and chloramphenicol gave MIC values of the same order of magnitude (7.8 and 6.3 μg/mL, respectively) for *B. cereus* and *B. subtilis*. This dodecyl α-L-glycoside also inhib-

Table 2. Adsorption properties of alkyl glycosides at the air–aqueous solution interface

Compound	CMC (mol kg^{-1})	γCMC (mNm^{-1})	CMC/C_{20}	pC_{20}
1 (2-Deoxy-C_8α,D)	2.16×10^{-3}	37.1	4.6	**3.3**
2 (2-Deoxy-C_8β,D)	2.20×10^{-3}	37.1	4.9	3.3
3 (2-Deoxy-C_{12}α,D)	—	—	—	—
4 (2-Deoxy-C_{12}β,D)	2.45×10^{-5}	32.5	3.0	5.1
5 (2,6-Dideoxy-C_8α,L)[6]	3.00×10^{-4}	43.6	1.7[a]	3.8[a]
6 (2,6-Dideoxy-C_8β,L)[6]	2.86×10^{-4}	45.2	1.4[a]	3.7[a]
7 (2,6-Dideoxy-C_{12}α,L)[6]	1.1×10^{-5}	28.4	5.8[a]	5.7[a]
8 (2,6-Dideoxy-C_{12}β,L)[6]	—	—	—	—
C_8β,D[18]	1.8×10^{-2}	31.3	—	—
C_8β,D[17]	2.5×10^{-2}	30.1	8.3[a]	2.5[a]
C_{10}β,D[16]	1.96×10^{-3}	27.8	—	—
C_{12}β,D[17]	1.9×10^{-4}	39.1	3.0[a]	4.2[a]

[a] Calculated from previously published data.

Table 3. Antimicrobial activity expressed by the diameter of the inhibition zones ± standard deviation (mm) for compounds **1**–**8**, compared to that of the control, using the paper disk diffusion method[a,b]

Compound (configurational symbols)	**1** (α, D)	**2** (β, D)	**3** (α, D)	**4** (β, D)	**5** (α, L)	**6** (β, L)	**7** (α, L)	**8** (β, L)	Control[c]	
									I	II
Bacillus cereus	<6.4	11 ± 1	<6.4	10 ± 0	12 ± 2	12 ± 2	27 ± 5	<6.4	24 ± 3	38 ± 3
Bacillus subtilis	<6.4	12 ± 1	9 ± 0	10 ± 0	12 ± 2	10 ± 0	25 ± 3	<6.4	30 ± 1	46 ± 3
Enterococcus faecalis	<6.4	14 ± 0	<6.4	17 ± 2	10 ± 0	9 ± 2	13 ± 2	<6.4	29 ± 4	38 ± 4
Escherichia coli	<6.4	<6.4	<6.4	<6.4	8 ± 1	9 ± 0	<6.4	<6.4	28 ± 3	42 ± 4
Listeria monocytogenes	<6.4	<6.4	10 ± 1	<6.4	10 ± 1	10 ± 1	12 ± 2	<6.4	31 ± 4	45 ± 3
Pseudomonas aeruginosa	<6.4	<6.4	<6.4	<6.4	8 ± 0	9 ± 0	<6.4	<6.4	<6.4	22 ± 3
Salmonella enteritidis	<6.4	<6.4	<6.4	<6.4	<6.4	<6.4	<6.4	<6.4	30 ± 2	41 ± 2
Staphylococcus aureus	<6.4	<6.4	<6.4	<6.4	11 ± 1	12 ± 3	9 ± 1	<6.4	27 ± 5	40 ± 4
Aspergillus niger	<6.4	<6.4	<6.4	<6.4	<6.4	<6.4	<6.4	<6.4	13 ± 2	23 ± 1
Candida albicans	<6.4	<6.4	9 ± 0	10 ± 1	10 ± 0	11 ± 1	<6.4	<6.4	<6.4	15 ± 1
Pyricularia oryzae	<6.4	<6.4	<6.4	<6.4	13 ± 3	<6.4	<6.4	<6.4	40 ± 5	63 ± 4

[a] With the exception of *Pyricularia oryzae*, data referring to compounds **1**–**4** were published by Rauter et al.[6]

[b] A solution of the compounds (300 μg) in DMSO (5 mL) was applied on the disk.

[c] Chloramphenicol was used for all bacteria and for *C. albicans*, whereas actidione was used for the filamentous fungi *A. niger* and *P. oryzae*; A solution of the control (I, 30 μg; II, −300 μg) in DMSO (15 mL) was used.

Table 4. Antimicrobial activity expressed in MIC and MLC (μg/mL)[a] of compounds **2** and **4**–**7** compared to chloramphenicol, using the dilution method.

Compound	**2**		**4**		**5**		**6**		**7**		Chloramphenicol	
Microorganism	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC
B. cereus	>500	nd	>500	nd	250	500	>500	nd	7.8	7.8	6.3	12.5
B. subtilis	>500	nd	>500	nd	250	>500	>500	nd	7.8	15.6	3.1	>50
E. faecalis	31.3	>500	31.3	>500	nd	nd	nd	nd	15.6	>250	6.3	>50
L. monocytogenes	nd	nd	250	>500	nd	nd	>500	nd	31.3	62.5	6.3	>50
S. aureus	nd	nd	>500	nd	500	500	>500	nd	nd	nd	6.3	>50

[a] MLC was only determined when MIC ⩽ 500 μg/mL; nd, not determined.

ited *Enterococcus faecalis* and *L. monocytogenes* at the concentration of 15.6 and 31.3 μg/mL, respectively (Table 4). These MIC values were also obtained after compound's solution storage for 132 days. Furthermore, compound **7** showed a bactericide effect for *B. cereus*, *B. subtilis*, and *L. monocytogenes* since the MIC and MLC values for those bacteria were low and close to each other, giving the *Bacillus* species MLC values even lower than chloramphenicol, the reference antibiotic. The potent antibacterial activity of **7** against *B. cereus* was also observed with a compound's solution kept at 4 °C for 1 year. According to preliminary experiments,[21] the activity of the D-2,6-dideoxy analogue is comparable to that of the L-series compound **7** described in this paper, showing MIC values of the same order of magnitude. This behavior suggests that the 2,6-dideoxy pattern may be a determinant structural feature for the potent and selective bioactivity observed.

The results obtained are particularly relevant as *Bacillus* species may lead to human diseases, namely *B. cereus*, which causes toxin-mediated food poisoning. Growth inhibition of *E. faecalis* was also observed with the β-D-glycosides **2** and **4,** which presented a MIC of 31.3 μg/mL, reinforcing the results obtained with the disk diffusion methodology (14 and 17 mm, Table 3). This microorganism is a leading cause of bacterial infection among hospital patients, being vancomycin, a cell wall antibiotic, one of the last-resort antibiotics for the treatment of this infection. This bacterium lives peacefully in the human gut, but it also thrives on wounds and burns. Its transformation from harmless in the gut to a menacing invader may be associated to a group of genes already identified.[22]

The results obtained also indicate that diameters ⩽10 mm observed in the disk diffusion methodology lead to MIC values >500 μg/mL with the dilution method for the compounds tested.

2.5. Cytotoxicity and genotoxicity

All tensioactives tested induce acute toxicity effects in human lymphocytes in the concentrations used (Table 5). The difference between the ability to proliferate is strongly significant in all experiments, except in two cases: Compounds **3** and **4,** at the lower concentration tested, showed a decrease in cell proliferation that is borderline significant ($p = 0.054$). The results in the range of concentrations studied demonstrate that response is proportional to dose for compounds **1**, **3**, and **4,** suggesting that non-toxic but still bioactive concentrations may be found for these compounds.

3. Conclusions

The non-ionic glycosides studied showed antibacterial activity against some Gram-positive bacteria, being

Table 5. In vitro cytotoxicity of surfactant compounds (10 and 100 μg/mL) in peripheral blood human lymphocytes

	Mitotic index	
	10 μg/mL	100 μg/mL
Blank	23.25 ± 7.85	23.25 ± 7.85
H_20_2 5%	3.00 ± 1.41	3.00 ± 1.41
1	10.75 ± 2.22	3.00 ± 1.51
2	0.00 ± 0.00	0.00 ± 0.00
3	13.50 ± 2.38	7.75 ± 4.79
4	13.50 ± 2.38	6.75 ± 2.50
7	0.00 ± 0.00	0.00 ± 0.00

inactive towards the Gram-negative bacteria. The latter contain a strong negative charge of lipopolysaccharide at the exterior outer membrane[23] which may inhibit the adsorption of the slightly acidic and therefore weakly anionic alkyl glycosides[24] on the membrane surface of the bacteria, thus explaining this general trend. Data for *L. monocytogenes* for compounds **4**, **6**, and **7** (Table 4) suggest that the antimicrobial activity may be related to CMC and pC_{20}, increasing with the decrease on CMC and the increase of pC_{20}. However, data for *B. cereus* and *B. subtilis* show that the bioactivity of the 2,6-dideoxy-α-L-glycosides tested depends on their chain length, while that of the octyl and dodecyl 2-deoxy-β-D-derivatives **2** and **4** is quite similar, suggesting an additional and specific contribution of the headgroup, probably associated with deoxygenation at position 6.

Enterococcus faecalis was significantly inhibited by compound **7**, and also by compounds **2** and **4.** The antimicrobial activity observed was independent from compounds' CMC and pC_{20}, a feature which is probably associated with the specific differentiated membrane of this group D *Streptococcus* microbes.[25]

The cytotoxicity exhibited by the compounds tested suggests that further work needs to be carried out in order to optimize selective bioactivity and dose responses. The exhibited antimicrobial activity is linked to membrane adsorption and binding, the latter being influenced by the anomeric configuration. A preliminary correlation of the antibacterial results with the sugar configuration and hydroxy groups pattern evidences that the 2,6-dideoxy α,L- and the 2-deoxy-β,D-glycosides are more potent and selective antibacterial agents than the corresponding β-L- or α-D-glycosides. The dodecyl α-L-glycoside **7** was the most surface active and potent antimicrobial agent, presenting significant activity against *E. faecalis* and *L. monocytogenes*, giving MIC values over *B. cereus* and *B. subtilis* comparable to those of chloramphenicol, and MLC values even lower than those obtained for this antibiotic, an activity maintained by the compound after 1 year storage in DMSO solution at 4 °C.

4. Experimental

Starting materials and reagents were purchased from Sigma, Fluka, or Acros. The solvents were dried prior to use with molecular sieves 4 or 3 Å (methanol). Melting points were first obtained with a Melting Point Apparatus, SMP3, Stuart Scientific, Bibby. A Setaram TG-DSC111 was used to perform differential scanning calorimetry scans at a rate of 1 °C/min to ascertain compounds melting point and purity. Elemental analyses were performed at the Service of Microanalyses of Instituto Superior Técnico, Universidade Técnica de Lisboa. IR spectra were carried out using a Hitachi 270-50. Optical rotations were measured on a Perkin-Elmer 343 polarimeter. ^1H and ^{13}C NMR spectra, DEPT, COSY, and HMQC experiments were recorded using a BRUKER Avance 400 spectrometer at 298 K, operating at 100.62 MHz for ^{13}C and at 400.13 MHz for ^1H. The solvents used were $CDCl_3$ 0.03% v/v TMS and CD_3OD, Merck. Chemical shifts are reported as δ (ppm) and the coupling constants (J) are given in Hertz. TLC was carried out on aluminum sheets (20 × 20 cm) coated with silica gel 60 F-254, 0.2 mm thick (Merck). Detection was accomplished by spraying the plates with a solution of H_2SO_4 in ethanol (10%), followed by heating at 120 °C. Solutions were concentrated on a rotary evaporator under diminished pressure below 40 °C. The purification of the compounds was carried out by column chromatography (CC) using silica gel 60 G (0.040–0.063 mm, Merck), or silica gel 60 G (0.015–0.040 mm, Merck) and elution under low pressure.

4.1. General procedure for compounds deacetylation

A solution of NaOMe in MeOH (1%, 11.2 mL) was added to a solution of the sugar (2.1 mmol) in MeOH (106.7 mL) and the mixture was stirred at room temperature for 1 h 30 min. Neutralization with Amberlite (IR-120) was followed by filtration and evaporation of the solvent to give a residue, which was submitted to CC eluted with EtOAc affording the corresponding 2-deoxy glycosides.

4.1.1. Octyl 2-deoxy-α-D-*arabino*-hexopyranoside (1). The above-mentioned procedure gave **1** as a white solid (0.56 g, 96.8%); mp 108.7 °C; $[\alpha]_D^{20}$ +6.8 (*c* 1; MeOH); R_f 0.56 (EtOAc); IR (neat): 3371 cm^{-1} (C-OH); ^1H NMR (CD_3OD) δ 4.90 (br d, 1H, $J_{1,2a}$ = 2.71 Hz, H-1), 3.90–3.81 (m, 2H, H-6a, H-3), 3.74–3.67 (m, 2H, H-6b, H-1′a), 3.54 (ddd, 1H, H-5), 3.38 (dt, 1H, $J_{1'a,1'b}$ = 9.82 Hz, $J_{1'a,2'a} = J_{1'a,2'b}$ = 6.34 Hz, H-1′b), 3.26 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.21 Hz, H-4), 2.07 (dd, 1H, $J_{2e,3}$ = 4.5 Hz, $J_{2e,2a}$ = 12.75 Hz, H-2e), 1.67–1.56 (m, 3H, H-2a, H-2′a, H-2′b), 1.46–1.26 (m, 10H, H-3′a,b-H-7′a,b), 0.93 (t, 3H, $J_{7',8'}$ = 6.35 Hz, H-8′); ^{13}C NMR (CD_3OD) δ 99.4 (C-1), 74.8 (C-5), 74.2 (C-4), 70.8 (C-3), 69.1 (C-1′), 63.7 (C-6), 39.8 (C-2), 33.9, 31.5, 31.4, 31.3, 28.3, 24.6 (C-2′-C-7′), 15.3 (C-8′). Anal. Calcd for $C_{14}H_{28}O_5$: C, 60.84; H, 10.21. Found: C, 60.50; H, 10.50.

4.1.2. Octyl 2-deoxy-β-D-*arabino*-hexopyranoside (2). The above-mentioned procedure gave **2** as a white solid (0.57 g, 98.3%); mp 84.3 °C; $[\alpha]_D^{20}$ +6.8 (*c* 1; MeOH); R_f 0.54 (EtOAc); IR (neat): 3465 cm^{-1} (C-OH); ^1H NMR (CD_3OD) δ 4.48 (d, 1H, $J_{1,2a}$ = 9.76 Hz, H-1), 3.89–3.77 (m, 2H, H-6a, H-1′a), 3.63 (dd, 1H,

$J_{6b,5}$ = 5.03 Hz, $J_{6b,6a}$ = 11.62 Hz, H-6b), 3.49 (ddd, 1H, $J_{3,2e}$ = 4.45 Hz, $J_{3,2a}$ = 11.67 Hz, $J_{3,4}$ = 12.02 Hz, H-3), 3.42 (dt, 1H, $J_{1'b,2'a} = J_{1'b,2'b}$ = 6.8 Hz, $J_{1'b,1'a}$ = 13.6 Hz, H-1′b), 3.18–3.07 (m, 2H, H-4, H-5), 2.04 (dd, 1H, $J_{2e,2a}$ = 12.33 Hz, $J_{2e,3}$ = 4.45 Hz, H-2e), 1.58–1.47 (m, 2H, H-2′a,b), 1.42 (q, 1H, $J_{2a,2e} = J_{2a,3} = J_{2a,1}$ = 11.67 Hz, H-2a), 1.35–1.16 (m, 10H, H-3′a,b-H-7′a,b), 0.85 (t, 3H, $J_{8',7'}$ = 7.16 Hz, H-8′); ^{13}C NMR (CD_3OD) δ 101.9 (C-1), 78.8 (C-4), 73.9 (C-5), 73.3 (C-3), 71.1 (C-1′), 63.7 (C-6), 41.2 (C-2), 33.8, 31.5, 31.3, 31.2, 27.9, 24.5 (C-2′-C-7′), 15.2 (C-8′). Anal. Calcd for $C_{14}H_{28}O_5$: C, 60.84; H, 10.21. Found: C, 60.60; H, 10.40.

4.1.3. Dodecyl 2-deoxy-α-D-*arabino*-hexopyranoside (3). The above-mentioned procedure gave **3** as a white solid (0.66 g, 94.5%); mp 114.8 °C; $[\alpha]_D^{20}$ +6.4 (*c* 1; MeOH); R_f 0.48 (EtOAc); IR (neat): 3354 cm^{-1} (C-OH); ^{1}H NMR (CD_3OD) δ 4.90 (d, 1H, $J_{1,2a}$ = 2.84 Hz, H-1), 3.90–3.81 (m, 2H, H-3, H-6a), 3.75–3.67 (m, 2H, H-6b, H-1′a), 3.55 (ddd, 1H, $J_{5,4}$ = 9.22 Hz, $J_{5,6a}$ = 1.95 Hz, $J_{5,6b}$ = 5.31 Hz, H-5), 3.38 (dt, 1H, $J_{1'b,2'a} = J_{1'b,2'b}$ = 6.32 Hz, $J_{1'b,1'a}$ = 9.82 Hz, H-1′b), 3.26 (t, 1H, $J_{4,3} = J_{4,5}$ = 9.22 Hz, H-4), 2.07 (dd, 1H, $J_{2e,2a}$ = 12.84 Hz, $J_{2e,3}$ = 5.17 Hz, H-2e), 1.67–1.54 (m, 3H, H-2a, H-2′a, H-2′b), 1.45–1.26 (m, 18H, H-3′a,b-H-11′a,b), 0.93 (t, 3H, $J_{11',12'}$ = 6.48 Hz, H-12′); ^{13}C NMR (CD_3OD) δ 99.4 (C-1), 74.8 (C-4), 74.2 (C-5), 70.9 (C-3), 69.1 (C-1′), 63.7 (C-6), 39.8 (C-2), 34.0, 31.7, 31.6, 31.6, 31.5, 31.4, 28.3, 24.6 (C-2′-C-11′), 15.3 (C-12′). Anal. Calcd for $C_{14}H_{36}O_5$: C, 65.03; H, 10.91. Found: C, 65.10; H, 11.20.

4.1.4. Dodecyl 2-deoxy-β-D-*arabino*-hexopyranoside (4). The above-mentioned procedure gave **4** as a white solid (0.68 g, 97.4%); mp 103.4 °C; $[\alpha]_D^{20}$ −1.6 (*c* 1; MeOH); R_f 0.45 (EtOAc); IR (neat): 3466 cm^{-1} (C-OH); ^{1}H NMR (CD_3OD) δ 4.56 (dd, 1H, $J_{1,2e}$ = 1.67 Hz, $J_{1,2a}$ = 9.79 Hz, H-1), 3.97–3.87 (m, 2H, H-1′a, H-6a), 3.72 (dd, 1H, $J_{6b,5}$ = 5.22 Hz, $J_{6b,6a}$ = 11.75 Hz, H-6b), 3.58 (ddd, 1H, $J_{3,2e}$ = 5.05 Hz, $J_{3,2a}$ = 12.26 Hz, $J_{3,4}$ = 12.37 Hz, H-3), 3.50 (dt, 1H, $J_{1'b,2'a} = J_{1'b,2'b}$ = 6.74 Hz, $J_{1'b,1'a}$ = 9.58 Hz, H-1′b), 3.25–3.16 (m, 2H, H-5, H-4), 2.13 (ddd, 1H, $J_{2e,2a}$ = 12.26 Hz, H-2e), 1.65–1.58 (m, 2H, H-2′a, H-2′b), 1.51 (q, 1H, $J_{2a,3} = J_{2a,2e} = J_{2a,1}$ = 12.26 Hz, H-2a) 1.44–1.26 (m, 18H, H-3′a,b-H11′a,b), 0.93 (t, 3H, $J_{11'-12'}$ = 7.44 Hz, H-12′); ^{13}C NMR (CD_3OD) δ 102.0 (C-1), 78.9 (C-4), 74.0 (C-5), 73.4 (C-3), 71.2 (C-1′), 63.8 (C-6), 41.3 (C-2), 33.9, 31.7, 31.6, 31.6, 31.4, 31.3, 28.1, 24.6 (C-2′-C-11′), 15.3 (C-12′). Anal. Calcd for $C_{14}H_{36}O_5$: C, 65.03; H, 10.91. Found: C, 65.30; H, 11.10.

4.2. General procedure for the glycosylation reaction

The nucleophile (36.5 mmol) and a solution of $Ph_3P{\cdot}HBr$ (686 mg, 2.0 mmol) in the chosen dried solvent (16.0 mL) were added to a solution of **9** (5.04 g, 18.5 mmol) in the same solvent (16.0 mL). The mixture was stirred at room temperature overnight (under reflux in $ClCH_2CH_2Cl$ or in CH_2Cl_2 for 5 h). CH_2Cl_2 (30.0 mL) was added to the reaction mixture and the solution washed with a satd $NaHCO_3$ solution Evaporation and CC eluted with EtOAc/cyclohexane (1:10) afforded the two anomers of the corresponding 2-deoxyglycosides as well as the α-anomer of the Ferrier compound as a secondary product.

4.2.1. Octyl3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*arabino*-hexopyranoside (10). Reaction of **9** with octanol (3.9 mL, 36.5 mmol) gave **10** as a syrup (5.11 g, 69%, in CH_2Cl_2; 3.46 g, 46.5% in $ClCH_2CH_2Cl$; 3.44 g, 46.3%, in CH_3CN; 3.85 g, 52.0%, in CH_2Cl_2/CH_3CN 95/5), $[\alpha]_D^{20}$ +7.7 (*c* 1; CH_2Cl_2); R_f 0.53 (EtOAc/petrol ether 1:3); IR (neat): 1748 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$) δ 5.33 (ddd, 1H, $J_{2a,3}$ = 12.20 Hz, $J_{2e,3}$ = 5.62 Hz, $J_{3,4}$ = 9.98 Hz, H-3), 4.99 (t, 1H, $J_{4,5}$ = 9.98 Hz, H-4), 4.94 (d, 1H, $J_{1,2a}$ = 3.14 Hz, H-1), 4.31 (dd, 1H, $J_{5,6a}$ = 4.67 Hz, H-6a), 4.05 (dd, 1H, $J_{6b,6a}$ = 12.26 Hz, H-6b), 3.96 (ddd, 1H, $J_{5,6b}$ = 1.60 Hz, H-5), 3.62 (dt, 1H, $J_{1'a,1'b}$ = 13.5 Hz, $J_{1'a,2'a,b}$ = 6.75 Hz, H-1′a), 3.38 (dt, 1H, H-1′b), 2.23 (dd, 1H, $J_{2e,2a}$ = 12.20 Hz, $J_{2e,3}$ = 5.62 Hz, H-2e), 2.09 (s, 3H, C*H*$_3$-Ac), 2.04 (s, 3H, C*H*$_3$-Ac), 2.01 (s, 3H, C*H*$_3$-Ac), 1.82 (td, 1H, $J_{2a,3}$ = 12.20 Hz, H-2a), 1.63–1.53 (m, 2H, H-2′a,b), 1.39–1.21 (m, 10H, H-3′a,b-H-7′a,b), 0.89 (t, 3H, $J_{7',8'}$ = 7.2 Hz, H-8′); ^{13}C NMR ($CDCl_3$) δ 170.6 (C=O), 170.1 (C=O), 169.9 (C=O), 96.8 (C-1), 69.4 (C-4), 69.1 (C-3), 67.8 (C-1′), 67.7 (C-5), 62.4 (C-6), 35.0 (C-2), 31.8, 29.3, 29.2, 29.1, 26.1, 22.6 (C-2′-C-7′), 20.9, 20.7 (*C*H$_3$-Ac), 14.0 (C-8′). Anal. Calcd for $C_{20}H_{34}O_8$: C, 59.68; H, 8.51. Found: C, 59.70; H, 8.80.

4.2.2. Octyl3,4,6-tri-*O*-acetyl-2-deoxy-β-D-*arabino*-hexopyranoside (11). Reaction of **9** with octanol (3.9 mL, 36.5 mmol) gave **11** as a syrup (1.11 g, 15%, in CH_2Cl_2; 0.74 g, 10.0% in $ClCH_2CH_2Cl$; 0.33 g, 4.4%, in CH_3CN; 0.43 g, 5.8%, in CH_2Cl_2/CH_3CN 95/5), $[\alpha]_D^{20}$ −1.8 (*c* 1; CH_2Cl_2); R_f 0.47 (EtOAc/petrol ether 1:3); IR (neat): 1749 cm^{-1} (C=O) ^{1}H NMR ($CDCl_3$) δ 5.07–4.95 (m, 2H, H-3, H-4), 4.56 (dd, 1H, $J_{1,2a}$ = 9.71 Hz, $J_{1,2e}$ = 1.94 Hz, H-1), 4.30 (dd, 1H, $J_{6a,5}$ = 4.88 Hz, $J_{6a,6b}$ = 12.18 Hz, H-6a), 4.11 (dd, 1H, $J_{6b,5}$ = 2.43 Hz, H-6b), 3.87 (dt, 1H, $J_{1'a,1'b}$ = 9.40 Hz, $J_{1'a,2'a,b}$ = 6.57 Hz, H-1′a), 3.60 (ddd, 1H, $J_{5,4}$ = 9.33, H-5), 3.46 (dt, 1H, H-1′b), 2.32 (ddd, 1H, $J_{2e,3}$ = 4.77 Hz, $J_{2e,2a}$ = 12.60 Hz, H-2e), 2.09 (s, 3H, C*H*$_3$-Ac), 2.04 (s, 3H, C*H*$_3$-Ac), 2.03 (s, 3H, C*H*$_3$-Ac), 1.75 (ddd, 1H, $J_{2a,3}$ = 12.30 Hz, H-2a), 1.66–1.52 (m, 2H, H-2′a,b), 1.38–1.18 (m, 10H, H-3′a,b-H-7′a,b), 0.88 (t, 3H, $J_{7',8'}$ = 7.02 Hz, H-8′); ^{13}C NMR ($CDCl_3$) δ170.9 (C=O), 170.4 (C=O), 169.8 (C=O), 99.6 (C-1), 71.9 (C-5), 70.8 (C-4), 70.0 (C-1′), 69.1 (C-3), 62.5 (C-6), 36.2 (C-2), 31.8, 29.7, 29.5, 29.3, 29.2, 26.0, 22.7 (C-2′-C-7′), 20.9, 20.8, 20.7 (*C*H$_3$-Ac), 14.0 (C-8′). Anal. Calcd for $C_{20}H_{34}O_8$: C, 59.68; H, 8.51. Found: C, 59.60; H, 8.70.

4.2.3. Dodecyl 3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*arabino*-hexopyranoside (12). Reaction of **9** with dodecanol (5.6 mL, 36.5 mmol) gave **12** as a syrup (7.05 g, 83%, in CH_2Cl_2; 5.08 g, 59.8% in $ClCH_2CH_2Cl$; 5.76 g, 67.8%, in CH_3CN; 5.20 g, 61.3%, in CH_2Cl_2/CH_3CN 95/5); $[\alpha]_D^{20}$ +6.6 (*c* 1; CH_2Cl_2); R_f 0.41 (EtOAc/petrol ether 1:3); IR (neat): 1748 (C=O); ^{1}H NMR ($CDCl_3$): δ 5.33 (ddd, 1H, $J_{3,2a}$ = 11.93 Hz, $J_{3,2e}$ = 5.46 Hz,

$J_{3,4}$ = 9.82 Hz, H-3), 5.00 (t, 1H, $J_{4,5}$ = 10.02 Hz, H-4), 4.94 (d, 1H, $J_{1,2a}$ = 3.11 Hz, H-1), 4.32 (dd, 1H, $J_{6a,5}$ = 4.69 Hz, $J_{6a,6b}$ = 12.27 Hz, H-6a), 4.06 (dd, 1H, $J_{6b,5}$ = 2.18 Hz, H-6b), 3.97 (ddd, 1H, $J_{5,4}$ = 10.02 Hz, H-5), 3.62 (dt, 1H, $J_{1'a,1'b}$ = 9.36 Hz, $J_{1'a,2'}$ = 6.24 Hz, H-1′a), 3.38 (dt, 1H, $J_{1'b,2'a,b}$ = 6.24 Hz, H-1′b), 2.24 (dd, 1H, $J_{2e,2a}$ = 12.90 Hz, $J_{2e,3}$ = 5.65 Hz, H-2e), 2.10 (s, 3H, CH_3-Ac), 2.05 (s, 3H, CH_3-Ac), 2.02 (s, 3H, CH_3-Ac), 1.83 (td, 1H, H-2a), 1.63–1.53 (m, 2H, H-2′a,b), 1.37–1.22 (m, 18H, H-3′- H-11′), 0.89 (t, 3H, $J_{12'-11'}$ = 7.06 Hz, H-12′); ^{13}C NMR ($CDCl_3$) δ 170.8 (C═O), 170.2 (C═O), 170.0 (C═O), 96.9 (C-1), 69.5 (C-4), 69.2 (C-3), 67.9 (C-1′), 67.7 (C-5), 62.4 (C-6), 35.1 (C-2), 31.9, 29.7, 29.6, 29.5, 29.4, 29.3 26.2, 22.7 (C-3′-C-11′), 21.0, 20.8, 20.7 (*C*H_3-Ac), 14.1 (C-12′). Anal. Calcd for $C_{24}H_{42}O_8$: C, 62.86; H, 9.23. Found: C, 63.20; H, 9.50.

4.2.4. Dodecyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-*arabino*-hexopyranoside (13). Reaction of **9** with dodecanol (5.6 mL, 36.5 mmol) gave **13** as a syrup (1.01 g, 12%, in CH_2Cl_2; 0.96 g, 11.3% in $ClCH_2CH_2Cl$; 0.56 g, 6.6%, in CH_3CN; 0.73 g, 8.6%, in CH_2Cl_2/CH_3CN 95/5); $[\alpha]_D^{20}$ −1.9 (*c* 1; CH_2Cl_2); R_f 0.40 (EtOAc/petrol ether 1:3); IR (neat): 1748 cm^{-1} (C═O); ^{1}H NMR ($CDCl_3$) δ 5.03-4.99 (m, 2H, H-3, H-4), 4.56 (dd, 1H, H-1, $J_{1,2a}$ = 9.73, $J_{1,2e}$ = 1.96), 4.30 (dd, 1H, H-6a, $J_{6a,6b}$ = 11.98, $J_{5,6a}$ = 4.99), 4.11 (dd, 1H, H-6b, $J_{6b,5}$ = 2.5), 3.87 (td, 1H, H-1′a, $J_{1'a,1'b}$ = 9.6 Hz, $J_{1'a,2'a,b}$ = 6.33 Hz), 3.60 (ddd, 1H, H-5, $J_{4,5}$ = 9.38), 3.46 (ddd, 1H, H-1′b), 2.32 (ddd, 1H, H-2e, $J_{2e,3}$ = 4.41 $J_{2a,2e}$ = 12.43), 2.10(s, 3H, *CH*$_3$-Ac), 2.05 (s, 3H, *CH*$_3$-Ac), 2.02(s, 3H, *CH*$_3$-Ac), 1.75 (ddd, 1H, H-2a, $J_{2a,3}$ = 10.0), 1.63–1.53 (m, 2H, H-2′a,b), 1.33–1.22 (m, 18H, H-3′a,b-H-11′a,b), 0.84 (t, 3H, H-12′, $J_{11'-12'}$ = 6.4); ^{13}C NMR ($CDCl_3$) δ 99.6 (C-1), 71.9 (C-5), 71.0 (C-3), 70.8 (C-1′), 70.0 (C-4), 62.5 (C-6), 36.3 (C-2), 31.9, 29.7, 29.6, 29.5, 29.4, 26.0, 22.7(C-3′-C-11′), 20.9, 20.8(*C*H_3-Ac), 14.1 (C-12′). Anal. Calcd for $C_{24}H_{42}O_8$: C, 62.86; H, 9.23. Found: C, 63.20; H, 9.40.

4.2.5. Octyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-erythro-hex-2-enopyranoside (14). Reaction of **9** with octanol (3.9 mL, 36.5 mmol) gave **14** as a syrup (0.11 g, 1.8%, in CH_2Cl_2; 0.62 g, 9.8% in $ClCH_2CH_2Cl$; 0.05 g, 0.8%, in CH_3CN; 0.14 g, 2.2%, in CH_2Cl_2/CH_3CN 95/5), $[\alpha]_D^{20}$ +38 (*c* 1; CH_2Cl_2); R_f 0.38 (EtOAc/petrol ether 1:3); IR (neat): 1757 cm^{-1} (C═O); ^{1}H NMR ($CDCl_3$) δ 5.92–5.81 (m, 2H, H-2, H-3), 5.32 (brd, 1H, $J_{4,5}$= 9.68 Hz, H-4), 5.03 (br s, 1H, H-1), 4.28, 4.27, 4.25, 4.24 (Part AX of ABX system, 1H, $J_{6a,6b}$ = 11.89 Hz, $J_{6a,5}$ = 5.4 Hz, H-6a), 4.19, 4.16 (Part B of ABX system, 1H, H-6b), 4.11 (ddd, 1H, $J_{5,6b}$ = 1.72 Hz, H-5), 3.77 (dq, 1H, $J_{1'a,1'b}$ = 9.39 Hz, $J_{1'a,2'a,b}$ = 6.87 Hz, H-1′a), 3.50 (dq, 1H, $J_{1'b,2'a,b}$ = 6.68 Hz, H-1′b), 2.11 (s, 3H, CH_3-Ac), 2.09 (s, 3H, CH_3-Ac), 1.66–1.53 (m, 2H, H-2′a,b), 1.41–1.20 (m, 10H, H-3′a,b-H-7′a,b), 0.88 (t, 3H, $J_{7',8'}$ = 7.02Hz, H-8′); ^{13}C NMR ($CDCl_3$) δ 171.1 (C═O), 170.6 (C═O), 129.1, 128.1 (C-2, C-3), 94.5 (C-1), 69.1 (C-1′), 67.0 (C-5), 65.4 (C-4), 63.1 (C-6), 29.8 (C-2′), 31.9, 29.5, 29.4, 26.4, 22.8 (C-3′-C-7′), 21.1, 20.9 (*C*H_3-Ac), 14.2 (C-8′). Anal. Calcd for $C_{18}H_{30}O_6$: C, 63.14; H, 8.83. Found: C, 62.90; H, 9.10.

4.2.6. Dodecyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-erythro-hex-2-enopyranoside (15). Reaction of **9** with dodecanol (5.6 mL, 36.5 mmol) gave **15** as a syrup (0.13 g, 1.7%, in CH_2Cl_2; 0.51 g, 6.9% in $ClCH_2CH_2Cl$; 0.17 g, 2.3%, in CH_3CN; 0.32 g, 4.4%, in CH_2Cl_2/CH_3CN 95/5), $[\alpha]_D^{20}$ +38 (*c* 1; CH_2Cl_2); R_f 0.38 (EtOAc/petrol ether 1:3); IR (neat): 1757 cm^{-1} (C═O); ^{1}H NMR ($CDCl_3$) δ 5.90–5.79 (m, 2H, H-2, H-3), 5.30 (br d, 1H, $J_{4,5}$ = 9.68 Hz, H-4), 4.95 (br s, 1H, H-1), 4.28, 4.27, 4.25, 4.24 (Part AX of ABX system, 1H, $J_{6a,6b}$ = 12.13 Hz, $J_{6a,5}$ = 5.31 Hz, H-6a), 4.19, 4.16 (Part B of ABX system, 1H, $J_{5,6b}$ = 1.77 Hz, H-6b), 4.11 (ddd, 1H, H-5), 3.77 (dq, 1H, $J_{1'a,1'b}$ = 9.35 Hz,$J_{1'a,2'a,b}$ = 6.82 Hz, H-1′a), 3.50 (dq, 1H, $J_{1'b,2'a,b}$ = 6.57 Hz, H-1′b), 2.10 (s, 3H, CH_3-Ac), 2.09 (s, 3H, CH_3-Ac), 1.66–1.54 (m, 2H, H-2′a,b), 1.35–1.24 (m, 18H, H-3′a,b-H-11′a,b), 0.88 (t, 3H, $J_{11',12'}$ = 7.07 Hz, H-12′); ^{13}C NMR ($CDCl_3$) δ 170.7 (C═O), 170.2 (C═O), 128.9, 127.9 (C-2, C-3), 94.3 (C-1), 68.9 (C-1′), 66.8 (C-5), 65.2 (C-4), 63.0 (C-6), 29.7 (C-2′), 31.8, 29.6, 29.6, 29.5, 29.4, 29.3, 26.2, 22.6 (C-3′-C-11′), 20.9, 20.7 (*C*H_3-Ac), 14.1 (C-12′). Anal. Calcd for $C_{22}H_{38}O_6$: C, 66.30; H, 9.61. Found: C, 66.00; H, 9.90.

4.3. Differential scanning calorimetry

A Setaram TG-DSC111, temperature calibrated with LGC standard reference materials (Hg, In, Sn, Pb), and energy calibrated by both Joule effect and standard reference material (Sapphire NIST SRM 720).[26] Samples of compounds **1**–**4** were weighed into aluminum crucibles which were sealed under air, without protective atmosphere, and DSC scans were performed at a rate of 1 °C/min, from room temperature to 140 °C. The stability of the samples and accuracy of the determinations were ensured performing successive heating and cooling scans which showed pronounced thermal hysteresis, giving reproducible results on successive heating and cooling cycles. No pretransitions or 'double melting points' were observed demonstrating the absence of solvent loss or formation of liquid crystalline phases. In Figure 1 only heating scans are presented, for the sake of intelligibility and comparability with data from melting point, showing one endothermic peak for each compound.

4.4. Surface activity

Surface tension measurements were performed with an automatic Kruss K100MK2 Interfacial Tensiometer using the du Nouy Ring Method at 35.0 ± 0.1 °C, temperature control being ensured through continuous circulation of the thermostatic fluid in a stainless steel sleeve around the sample vessel. Glass material was carefully washed with chromic acid and then thoroughly rinsed with distilled water. The platinum–iridium ring was rinsed with distilled water and then heated in a Bunsen burner until dark-red glowing before each measurement. The tensiometer calibration was checked daily with Milli-Q water. The du Noüy ring non-detachment technique was used and the surface tension was determined as a function of surfactant concentration, being Harkins and Jordan correction automatically introduced. For the preparation of the most concentrated

solutions, the dissolution was accelerated resorting to the immersion in an ultrasound bath at 35 °C, when necessary. Further solutions were prepared by successive dilution, being also used samples from independent syntheses to check the reproducibility of the results obtained. The most dilute solutions exhibited a time dependant surface tension, with a decreasing tendency on standing consequently, the temperature of all solutions was pre-equilibrated for at least 30 min and then several measurements were performed, the values presented being the average of five successive evaluations, with a deviation smaller than 0.2 mNm^{-1}. Surface tension was determined exclusively on clear isotropic solutions thus limiting the studies to aqueous solutions of compounds **1**, **2**, and **4** as attempts to prepare 3×10^{-5} *m* solutions of compound **6** were unfruitful, and led to liquid mixtures containing dispersed crystals.

4.5. Antimicrobial activity

The microbial susceptibility of the sugar derived compounds belonging to D-and L-series, in their α- and β-forms, was initially investigated by the agar diffusion method using paper disks. The disk method was early described by Bauer et al.[27] but is presently used as a standard procedure by the National Committee for Clinical Laboratory Standards.[28] When inhibition diameter $\geqslant$11 mm, the substance was considered active and the minimum inhibitory and lethal concentrations (MIC, MLC) were assessed by the dilution method.[29] However, when the MIC > 500 μg/mL, the maximum concentration tested, no MLC determination was carried out.

The following fungi and bacteria were used in the tests: *C. albicans* (ATCC 10231), *A. niger* (ATCC 16404), *P. oryzae* (DSMZ 62938), *B. cereus* (ATCC 11778), *B. subtilis* (ATCC 6633), *E. faecalis* (ATCC 29212), *E. coli* (ATCC 8739), *L. monocytogenes* (ATCC 7644), *P. aeruginosa* (ATCC 27853), *S. enteritidis* (ATCC 13076), and *S. aureus* (ATCC 25923).

Regarding the disk diffusion method, the overnight cultures of microorganisms were spread over the appropriate media: nutrient agar was used for all bacteria except for *Listeria*, *Enterococcus*, and fungi, where triptona soy agar, azide dextrose agar, and potato dextrose agar were used, respectively. Paper disks of 6.4 mm were placed on the agar and the solution of each substance (300 μg) in DMSO (15 μL) was applied on each disk. Chloramphenicol was used as control for all microorganisms tested except for *A. niger*, for which actidione was used. Bacteria were incubated at 37 °C for 24 h and fungi at 25 °C for 48 h. After incubation, the diameter of the inhibition zone was measured. At least three replicates were made.

For the dilution method, overnight cultures were used. Serial dilutions starting at 500 μg/mL until 1.95 μg/mL were made for all the compounds tested. With the exception of *C. albicans*, chloramphenicol dilutions ranged between 50 and 0.195 μg/mL. Bacteria and the yeast were incubated at 35 °C for 16–20 h. At least three replicates were made.

4.6. Cytotoxicity and genotoxicity

Direct cytotoxicity and genotoxicity of the glycoside surfactant compounds were determined by proliferative index (mithotic index) evaluation in peripheral human lymphocytes of healthy donors. Venous peripheral blood was collected aseptically from healthy donors using heparine as anti-coaggulant. Five-hundred microliters of whole blood was added to 4.5 mL Ham's F-10 medium supplemented with 15% fetal calf serum, 4 μg/mL 2% (v/v) phytohemagglutinin, 1% L-glutamine, 100 μg/mL streptomycin, and 100 IU/mL penicillin. After 24 h of culture the test compounds (10 and 100 μg/mL) were added to the culture medium, and incubation continued until 48 h of culture. Two percent of hydrogen peroxide was used as a positive control for acute cytotoxicity. In the last 3 h of incubation, colcemid was added to the medium to a final concentration of 0.5 μg/mL. Metaphase spreads were then obtained as described in Rueff et al.,[30] scored in a Leitz microscope at 500× magnification for mitotic index.

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