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Synthesis, surface active and antimicrobial properties of new alkyl 2,6-dideoxy-L-*arabino*-hexopyranosides

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Abstract—Synthesis of alkyl 2,6-dideoxy-L-*arabino*-hexopyranosides was accomplished by the reaction of 1,5-anhydro-2,6-dideoxy-L-*arabino*-hex-1-enitol with fatty alcohols in dichloromethane, catalyzed by triphenylphosphine hydrobromide. Reaction with octanol and dodecanol gave the corresponding α-glycosides in 50% and 42% yield, the β-glycosides in 20% and 21% yield and the α-anomer of the Ferrier product in 10% and 9% yield, respectively. Deacetylation of the α-/β-glycosides with sodium methoxide in methanol afforded the amphiphilic L-*arabino*-hexopyranosides in 94–99% yield. The surface tension at the air–water interface of the octyl L-glycosides and of the dodecyl α-L-glycoside aqueous solutions at 35 °C was measured with a du Noüy ring tensiometer and surface properties such as critical micelle concentration (CMC), relative surface excess, molecular area at the interface and Gibbs micellization free energy were evaluated. The stereochemistry of the hexopyranoside ring in unimers and aggregates is correlated to the hydrophobicity and packing efficiency on the air–water interface. The antibacterial and antifungal activities of the surface-active glycosides were evaluated using the paper disk diffusion method. The dodecyl α-L-*arabino*-hexopyranoside was quite active over *Bacillus cereus* and *Bacillus subtilis*, while low activity was found for this glycoside over *Enterococcus faecalis* and *Listeria monocytogenes*. The octyl glycosides tested showed low activity over almost all the above-mentioned bacteria, and also over the fungus *Candida albicans*. No inhibition of *Salmonella enteritidis* and of the filamentous fungus *Aspergillus niger* was detected for any of the compounds tested.

Keywords: Alkyl 2,6-dideoxy-L-*arabino*-hexopyranosides; Synthesis; Interfacial properties; Non-ionic sugar surfactants; Antimicrobial activity

1. Introduction

Over the last decades, sugar-based surfactants have been widely studied mainly due to their environmental properties and to the fact that they are synthesized from renewable resources. Being biocompatible and biodegradable, these compounds have a wide range of applications spanning from detergents, personal care products, food, pharmaceuticals, agrochemicals to explosives.[1–4] Their low toxicity is particularly relevant in food or cosmetic industries.[2,3] Moreover some non-ionic sugar surfactants are employed in the extraction of biological membrane proteins.[5] An overall survey on the preparation, applications, and biodegradability of sugar surfactants has been recently published.[4] The type of sugar in the headgroup, its stereochemistry, and the nature of the hydrophobic tail determine the physical and chemical properties of these compounds. The most frequent types of linkage between the hydrophilic and the hydrophobic moieties are of ester, amine,

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amide, and glycosidic nature.[6] Among the wide variety of sugar surfactants known, acyl polyols such as fructose mono- and dicaprate, dipalmitate, and distearate were recently described and their properties compared with commercial sucrose esters surfactants.[7] Trehalose esters[8] and glucose esters[9] have been reported as surfactants, being for some of them commercially available.[7,10] These ester surfactants are sensitive towards hydrolysis, especially in alkaline conditions, while glucamides and glycosides are usually considered as rather stable in water solution.[8,10] The influence of the aglycone chain length, and of the anomeric configuration of alkyl glycosides in the physicochemical properties of surfactants has also been studied.[11]

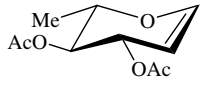
Considering the molecular diversity of sugars, these compounds open the way to the design of new surfactants with controlled performance properties, fulfilling the principles of green chemistry. In this report, we describe the preparation of new glycosides and investigate their surface activity analyzing the surface tension dependence on the molality. Critical micelle concentrations were determined at 35 °C, surface excess and molecular areas at the air–water interface were calculated and correlated to packing efficiency in the interfacial region. The aggregation tendency was also evaluated resorting to the Gibbs micellization energies. The influence of the structure of the hydrophilic moiety, namely the number of hydroxyl groups and the stereochemistry of the sugar moiety in the surface-active properties will be discussed, and these properties compared with other known sugar-based surfactants.

Surface-active agents, including non-ionic surfactants, are known to disrupt cell membranes because they dissolve in both extracellular fluid and the lipid membrane. This lowers the surface tension of the membrane, allowing water to flow into the cell and ultimately resulting in lysis and bactericidal action. The balance between the hydrophilic and lipophilic sections of the molecules is essential for these processes.[12] The irritating properties of cationic surfactants have been one of the major limitations to a widespread use of this type of surfactants with known bactericidal activity, in personal care products.[13] In personal hygiene as well as in the cosmetic industry, the association of a low antibacterial activity with emulsifying potential is desirable in order to clean and simultaneously cause the least disturbance in the normal skin flora and moisture balance.[2] Therefore, the antimicrobial activity of the new surface-active glycosides was also evaluated, using the paper disk diffusion method.[14]

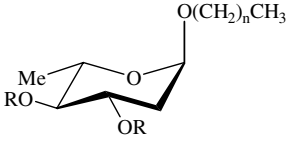
2. Results and discussion

2.1. Synthesis

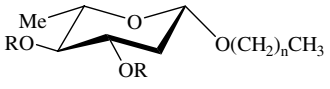
Synthesis of the new 2,6-dideoxy glycosides **2**, **3**, **5**, and **6** was accomplished by the addition of octanol and dodecanol to the double bond of the 6-deoxy glycal **1** (Chart 1). Various catalysts are known to promote this type of addition reaction resulting mainly in α-2-deoxy adducts, for example, $CeCl_3{\cdot}7H_2O$–NaI,[15] BBr_3, or



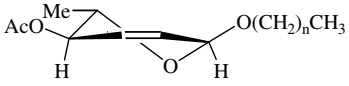
1



2 n = 7, R =Ac
5 n = 11, R =Ac
8 n = 7, R =H
10 n = 11, R = H



3 n = 7, R =Ac
6 n = 11, R =Ac
9 n = 7, R =H
11 n = 11, R = H



4 n = 7
7 n = 11

Chart 1. Structure of compounds **1**–**11**.

BCl_3,[16] sulfonic acid resins in the presence of LiBr,[17] $Ph_3P{\cdot}HBr$,[18] and a polymer-bound diphenylphosphane hydrobromide.[19] Alternatively, Montmorillonite K-10,[20] BF_3,[21] and many other Lewis acids promote the Ferrier transposition into 2,3-unsaturated glycosides.[22] In this work, the required adducts were obtained by the use of $Ph_3P{\cdot}HBr$ in 50% (for **2**) and 42% (for **5**) yield, while the β-glycosides **3** and **6** were isolated in 20% and 21% yield, respectively. The assignment of the anomeric configuration resulted from the anomeric coupling constants as $J_{1,2a}$ 9.3 Hz (for **3**) and $J_{1,2a}$ 9.6 Hz (for **6**) are consistent with an axial disposition of H-1 in these compounds, while $J_{1,2a}$ 3.3 Hz was measured for compounds **2** and **5**. These coupling constants are a consequence of the $^1C_4$ conformation adopted by these compounds, in which CH_3-6, as well as C-3 and C-4 substituents are in equatorial orientation. In addition, the resonance of the H-1 signal of **3** and **6** at lower field (δ 4.51 and 4.55, respectively), as compared to **2** and **5** (δ 4.84 and 4.77, respectively) is in agreement with the axial orientation of H-1 in **3** and **6** and its equatorial orientation in **2** and **5**. The higher chemical shift for C-1 in ^{13}C NMR spectroscopy for α-L-glycosides **3** and **6** (δ 99.2) as compared to β-L-glycosides **2** and **5** (δ 96.6 and 96.5, respectively) is also in accordance with the proposed anomeric configuration.

The stereochemistry of the major compounds **2** and **5** is in agreement with results previously reported by Kaila et al.[23] They used deuterated alcohols with catalytic amounts of $Ph_3P{\cdot}HBr$ to show that the major axial stereochemistry at the anomeric center of the glycosides, obtained by acid-catalyzed addition of alcohols to glycals, probably arises from the kinetic anomeric effect. Their results are consistent with an Ad_E2 (addition-electrophilic-bimolecular) mechanism for the first step of protonation of the glycal, followed by a glycosyl transfer usually directed by the kinetic anomeric effect.[23,24]

In addition to the synthesis of the expected dideoxy glycosides, the allylically rearranged unsaturated α-glycosides **4** and **7** were isolated in 9% and 10% yield, respectively, in contradiction with previous results which reported that the Ferrier rearrangement does not take place with this catalyst.[18] Their structure was assigned by means of the ^1H NMR, ^{13}C NMR, and NOESY spectra. The signals of the olefinic protons appeared as a multiplet at δ 5.86–5.80 for **4** and at δ 5.78–5.76 for **7**, the resonance of H-1 was detected as a singlet at δ 4.95 and δ 4.90 for **4** and **7**, respectively, and C-1 appeared at δ 94.3 for both compounds. The anomeric configuration was proposed by analysis of the NOESY spectrum of compound **7**, which presented a correlation of H-1 with H-4, and as expected, with the multiplet due to H-2 and H-3 signals, and with the signals of H-1′a and H-1′b. The singlet due to H-1 is also in agreement with its quasi-axial orientation in compounds **4** and **7** and with data previously reported for other 2,3-unsaturated pyranoid compounds in which H-1 is axially disposed, thus confirming the proposed anomeric configuration.[25]

The formation of these 2,3-unsaturated α-glycosides occurred by acid catalysed removal of the allylic acetoxy substituent of the glycal to generate a highly resonance-stabilised oxycarbenium ion intermediate, which reacted then with the alcohol, as described in the literature for other reaction conditions.[22]

Deacetylation of compounds **2**, **3**, **5**, and **6** with sodium methoxide in methanol[26] gave the corresponding deprotected compounds **8**–**11** in 98%, 94%, 99%, and 98% yield, respectively. Their physical and spectroscopic data were in full agreement with those expected, with H-3 and H-4 at higher field than the corresponding protons of the acetylated precursor molecules. The correlation between H-1 and H-5 observed in the NOESY spectrum of **11** confirmed its anomeric configuration, as well as that of its precursor **6**.

2.2. Surface activity

The dependence of the surface tension γ, with molality m, for homogeneous solutions of the alkyl L-*arabino*-hexopyranosides **8**, **9**, and **10** is presented in Figure 1. Measurements were not performed for the compound **11** due to its very low solubility ($<4\times10^{-5}m$). A preliminary analysis of these plots shows that the data obtained from independent dilutions of the compounds tested are in good agreement and also no minimum is observed near the break point in any of the plots, thus indicating the absence of hydrophobic impurities in the 2,6-dideoxy-L-glycosides previously synthesized and purified. Furthermore, a lower surface tension is displayed by compound **10** ($C_{12}\alpha$) solutions, in comparison with those of compounds **8** ($C_8\alpha$) and **9** ($C_8\beta$), which are placed well above, exhibiting very similar values (Fig. 1). This overall behavior may be understood due to the longer hydrophobic carbon chain of the C_{12} sugar surfactant. The similarities found between compounds **8** and **9** are in agreement with the results previously reported for the adsorption at the air–water interface for the α-D- and β-D-anomers of alkyl glucosides, which indicated that the anomeric configuration has very little effect on the interfacial adsorption properties of these compounds.[11] Moreover, within the concentration range studied, no solubility gaps were observed and all the plots clearly evidence three sections, two of them below a final plateau, with an almost flat pattern, assigned to the presence of micellar aggregates. Below the critical micelle concentration, CMC, the presence of two different curvatures indicates the formation of pre-micellar aggregates for the three compounds, a feature previously reported with commercial alkyl polyglucosides.[27]

Table 1. Evaluation of the CMC for the 2,6-dideoxy-L-*arabino*-glycosides **8**, **9**, and **10**

	Compound no.		
	8 ($C_8\alpha$)	**9** ($C_8\beta$)	**10** ($C_{12}\alpha$)
$(\partial\gamma/\partial m)_{T,P,m\cong 0} \times 10^5$/mN m^{-1} kg mol^{-1}	−1.90 ± 0.2	−1.51 ± 0.04	−103 ± 7
$(\partial\gamma/\partial m)_{T,P,m\,=\,\mathrm{CMC}} \times 10^5$/mN m^{-1} kg mol^{-1}	−0.52 ± 0.4	−0.525 ± 0.003	−18.1 ± 5
$(\partial\gamma/\partial m)_{T,P,m\,>\,\mathrm{CMC}} \times 10^4$/mN m^{-1} kg mol^{-1}	−1.698 ± 0.09	−1.44 ± 0.1	−0.001993 ± 0.0007
(CMC ± σ) × 10^4/mol kg^{-1} (γ vs m)	3.00 ± 0.1	2.86 ± 0.1	0.110 ± 0.004
(CMC ± σ) × 10^4/mol kg^{-1} (γ vs ln m)	2.86 ± 0.3	2.83 ± 0.1	0.113 ± 0.007

A closer look at the experimental results reveals that in the first section, that is, for the unimeric species, the modulus of the slope follows the order **10** ≫ **8** > **9** (Table 1), **10** showing the highest value as expected, in agreement with its longer hydrophobic carbon chain. The nearby but distinguishable values obtained for the two octyl anomers may be tentatively ascribed to the increased stability in solution of the β-L-glycoside. This effect is probably due to the conformation adopted by the hydrocarbon chain in this anomer, where the hydrophobic tail is less exposed to water molecules, simultaneously promoting a less efficient shielding of the headgroup oxygen atoms. In the second section, Figure 1 shows identical slopes for the octyl anomers, which are milder as compared to the corresponding one for the dodecyl glycoside **10**, a feature indicative of an aggregation process dominated by the length of the hydrophobic alkyl chain. Finally, in the third section, that is, the post-micellar region, the curve is much flatter for the dodecyl glycoside **10** than for the octyl anomers **8** and **9**, indicating the formation of a tighter and more regularly packed aggregate for the former compound.

A deeper understanding of the surface properties for these systems involves a quantitative analysis of the experimental data, which is summarized in Tables 1 and 2. The CMC was evaluated from the break in the γ versus m experimental curves (Fig. 1) and from Gibbs adsorption isotherms (Fig. 2). In both approaches, curves were fitted below and above the CMC, and the interception of both lines was calculated from the tangents to the curves around the CMC, as illustrated in Figures 1 and 2. The quantities obtained from these two methodologies agree well within the estimated uncertainties for all the compounds studied. The four carbon atoms increase in the hydrophobic alkyl chain of the sugar surfactant promotes a decrease by a factor of 30 on the determined CMC values. The slopes just below the CMC for both C_8 anomers are identical (within the estimated uncertainties), meaning that these pre-micellar aggregates have indistinguishable hydrophobicities. Nevertheless post-micellar slopes for the octyl glycosides are different, being slightly more negative for **8**, suggesting a less efficient packing on the air–water interface of this compound, and also indicating the formation of micellar aggregates with an increased degree of polydispersity, when compared to those of its anomer **9**. On the other hand, the post-micellar slope modulus for the dodecyl glycoside **10** is almost three orders of magnitude smaller than the corresponding ones for compounds **8** and **9**, thus reinforcing the hypothesis of a more compact packing at the air–water interface, as well as an increased monodisperse character of the $C_{12}\alpha$ aggregates with respect to those of the C_8 aggregates.

The estimated surface tensions at the CMC are presented in Table 2. Once again the experimental data display a decrease of γ_{CMC} with the alkyl chain length on going from the octyl to the dodecyl sugar surfactant and show a slightly higher γ_{CMC} for the $C_8\beta$ anomer **9** as a consequence of the previously mentioned, relatively more hydrophilic nature of this compound, when compared to the α-anomer **8**.

The surface excess as well as the molecular areas at the interface are included in Table 2. The application of the Gibbs adsorption isotherm to a two-component system (1+2) allows the calculation of the solute relative surface excess, $\Gamma_2^{(1)}$, considering the Gibbs dividing surface, GDS, located such that $\Gamma_1^{\sigma} = 0$ and therefore

$$\frac{\partial\gamma}{\partial \ln a_2} = \Gamma_2^{(1)} RT \qquad (1)$$

where γ is the solution surface tension, R is the gas constant, T the absolute temperature and a_2 the solute activity. In dilute solutions, the solute activity coefficient may be regarded as unity and consequently the activity is identical to the molality. The molecular areas, a_m, corre-

Table 2. Energetics and surface packing efficiency data of 2,6-dideoxy-L-*arabino*-glycosides **8**, **9**, and **10**

	Compound no.		
	8 ($C_8\alpha$)	**9** ($C_8\beta$)	**10** ($C_{12}\alpha$)
$\gamma_{CMC} \pm \sigma$/mNm^{-1}	43.6 ± 0.2	45.2 ± 0.4	28.4 ± 0.3
$(\Gamma_2^1 \pm \sigma) \times 10^6$/mol m^{-2}	4.99 ± 0.3	5.23 ± 0.1	6.1355 ± 0.0002
$(a_m \pm \sigma)$/Å^2	33 ± 1	31.7 ± 0.1	27.1065 ± 0.0003
ΔG_{mic}/kJ mol^{-1}	−46 ± 15	−46.0 ± 5	−54.3 ± 9

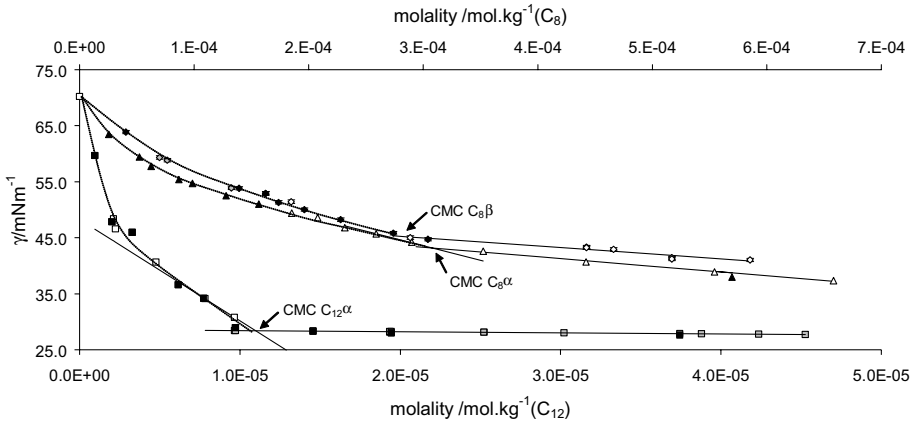


Figure 1. Surface tension versus molality for the aqueous solutions of compounds **8** ($C_8\alpha$), **9** ($C_8\beta$), and **10** ($C_{12}\alpha$) at 35 °C; compounds obtained from different synthetic batches are identified by open and filled symbols; shaded symbols indicate dilutions of independent concentrated solutions.

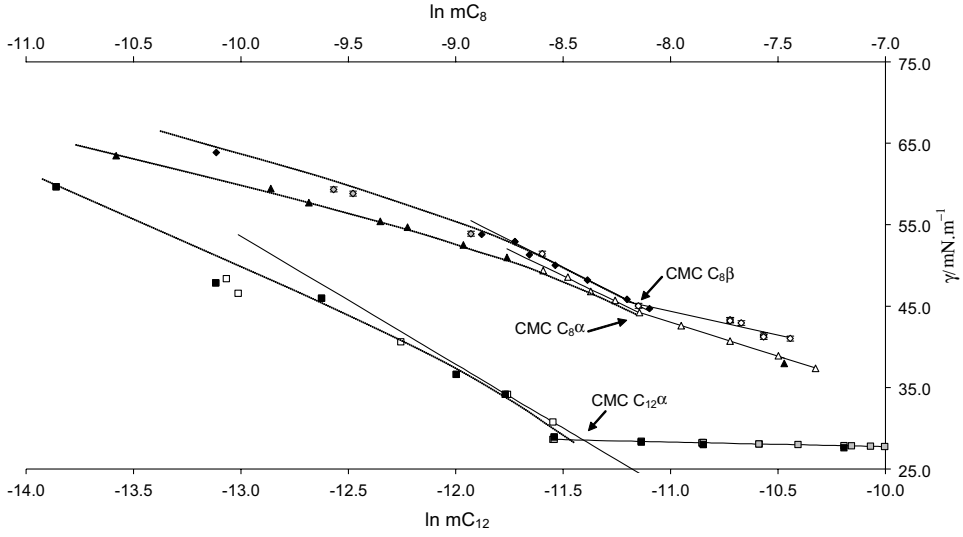


Figure 2. Gibbs adsorption isotherms for the aqueous solutions of compounds **8** ($C_8\alpha$), **9** ($C_8\beta$), and **10** ($C_{12}\alpha$) at 35 °C; compounds obtained from different synthetic batches are identified by open and filled symbols; shaded symbols indicate dilutions of independent concentrated solutions.

sponding to saturated monolayers at the air–water interface, were calculated resorting to Eq. 2:

$$a_m = \frac{1}{N_A \Gamma_2^{(1)}} \quad (2)$$

where N_A is Avogadro's constant.

The calculated surface excess and molecular areas reinforce, once again, the role of the alkyl chain hydrophobic interactions on packing efficiency at the air–water interface. However, it is interesting to note that despite the slightly different values of γ_{CMC} determined for the octyl glycoside anomers, no distinction is observed in terms of surface excess or molecular areas, thus pointing out to the delicate balance between hydrophobicity and packing efficiency sensed by excess properties.

The alkyl 2,6-dideoxy-L-*arabino*-hexopyranosides studied are a new type of surface active glycosides and therefore there is no data in the literature available for a straightforward comparison of the results obtained in this work. However, it is worth calling upon literature values for structurally related surfactants, namely those which possess the hydrophobic chain bonded to the anomeric oxygen atom (usually known as ether-linked alkyl glycosides) and glucose esters. Overall, the molecular areas for the glycoside surfactants reported in the literature are larger than those found in this work, ranging on average between 36 and 45 Å^2 on going from dodecyl to octyl glycosides.[11,28–31] Glucose esters also present molecular areas identical to those of the alkyl glucosides, namely 37 Å^2 for 6-*O*-dodecanoylglucose[31] and 38 and 42 Å^2 for methyl 6-*O*-octanoyl-α-D- and β-D-glucopyranosides, respectively.[9] These values, despite some small discrepancies ($\leqslant$3 Å^2) between data from different authors, display a general decreasing trend with

the increase of the number of carbon atoms of the alkyl chain of the hydrophobic moiety, well in accordance with the data presented here, thus supporting the role of hydrophobic interactions in the aggregation processes for the glycoside surfactants studied. In terms of magnitude, there is an overall decrease of about 9 Å^2 on going from the glucopyranosides[6,11,28] towards the corresponding 2,6-dideoxy-L-*arabino*-hexopyranosides, which may be attributed to the decrease in the number of hydroxyl substituents in the headgroup of the *arabino*-hexopyranosides, leading to a lower hydrophilicity and thereby allowing the ring to acquire a more tilted position at the air–water interface.

Micellization free energies, ΔG_{mic} (Table 2) were also calculated resorting to the pseudo-phase model according to Eq. 3:

$$\Delta G_{mic} = RT \ln\left(\frac{CMC}{(1000/18)}\right) \quad (3)$$

The calculated values clearly mean that all solutes spontaneously form aggregates in solution, and the aggregation tendency follows the expected trend line on going from dodecyl to octyl glycosides. The ΔG_{mic} values found for these alkyl 2,6-dideoxy-L-*arabino*-hexopyranosides are much more negative than those reported for the alkyl D-glucopyranosides (−20 to −28 kJ mol^{-1}),[11,28,30] probably reflecting the decreasing hydrophilicity and headgroup bulkiness on going from the D-glucopyranosides to the 2,6-dideoxy-L-*arabino*-glycosides reported in this work. Attention should also be drawn to another interesting feature in terms of methylene group contribution in the alkyl chain of homologous series of glycosides for the Gibbs free energy, which is around −3[30,32] kJ mol^{-1} for alkyl glucosides, −2.5 kJ mol^{-1} for glucose esters,[29] and −2 kJ mol^{-1} for the alkyl 2,6-dideoxy-α-L-*arabino*-glycosides studied. These values are close to the methylene group contribution in ethoxylated surfactants (−2.85 kJ mol^{-1}),[29] and suggest some headgroup hydration.

The physicochemical parameters such as $(\partial\gamma/\partial m)_{T,P}$, CMC, $\Gamma_2^{(1)}$, and ΔG_{mic} (Tables 1 and 2) demonstrate that these alkyl 2,6-dideoxy-L-*arabino*-hexopyranosides exhibit pre-micellar aggregates, are surface active and more hydrophobic than the octyl and dodecyl D-glycoside surfactants reported in the literature.[9,11,28–30,32] These data open a range of perspectives having in mind the potential applications of this class of compounds in the personal care, food, or pharmaceutical industries among others.[2]

2.3. Biological activity

The antimicrobial activity of compounds **8**–**11** was evaluated using the paper disk diffusion method[14] and the results obtained are presented in Table 3. Given the variability of the method, the results are expressed by the average diameter of the inhibition zone detected in three replicates, as well as by the symbols −, +, ++, +++, ++++, +++++, corresponding to a range of diameters, for increasing sensitivity of the microorganism to the substance tested. Miyazawa et al.[33] reported the disk diffusivity results in a similar way. The control used was chloramphenicol for all microorganisms tested, with the exception of *Aspergillus niger*, for which actidione was used. The dodecyl β-L-glycoside **11** did not exhibit any antimicrobial activity over all the microorganisms tested, as expected, due to its extremely low solubility in water. Regarding the octyl α-L-glycoside **8**, some inhibition on the growth of *Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Staphylococcus aureus*, and the yeast *Candida albicans* was observed, while trace activity was detected over *Escherichia coli* and *Pseudomonas aeruginosa*. The β-anomer **9** had a similar behavior, presenting trace activity over *E. coli*, *P. aeruginosa*, and *E. faecalis*. The dodecyl α-glycoside **10** had potent activity over *B. cereus* and *B. subtilis*, low activity over *E. faecalis* and *L. monocytogenes* and trace activity over *S. aureus*. The filamentous fungus *A. niger* was not affected by any substance tested. Reports were already made in the literature on interesting environmental properties of non-ionic surfactants with regard to their antimicrobial properties, namely those of *n*-octyl, *n*-decyl-, and *n*-dodecyl β-D-glucosides. The most hydrophobic member within this series, the C_{12} derivative, was also found to be the most active compound, exhibiting high activity against *B. subtilis*, *S. aureus*, and *S. lutea*, which are gram-positive bacteria, while being inactive towards *E. coli*, *P. aeruginosa*, *C. albicans* and *A. niger*.[4,34]

The selectivity exhibited by compound **10** over the *Bacillus* species studied is a promising result, as these bacteria are increasingly recognized as human pathogens causing food poisoning. *B. cereus* is considered of significant medical importance, together with its close relative *Bacillus anthracis*, whose genome sequences were recently compared.[35]

3. General methods

TLC was carried out on aluminum plates (20 × 20 cm) coated with Silica Gel 6F-254, 0.2 mm thick (E. Merck). Detection was accomplished by spraying the plates with a soln of vanillin in H_2SO_4 2.5%, followed by heating at 120 °C. Solutions were concentrated on a rotary evaporator under diminished pressure below 40 °C. The starting material 1,5-anhydro-2,6-dideoxy-L-*arabino*-hex-1-enitol (**1**) was purchased from Aldrich. The purification of the compounds was carried out by column chromatography (CC) using Silica Gel 60 G (0.040–0.063 mm, E. Merck) and elution under low pressure. ^{1}H and ^{13}C NMR spectra, DEPT, COSY, and HMQC

Table 3. Antimicrobial activity expressed in diameter of the inhibition zone for **8**–**11** compared with that of the control[a] using the paper disk diffusion method

Microorganism	Compound no.											
	8 (300 μg)		**9** (300 μg)		**10** (300 μg)		**11** (300 μg)		Control[b] (30 μg)		Control[b] (300 μg)	
	∅ (mm)	inhibition	∅ (mm)	inhibition	∅ (mm)	inhibition	∅ (mm)	inhibition	∅ (mm)	inhibition	∅ (mm)	inhibition
B. cereus ATCC 11778	12	+	12	+	27	+++++	<6.4	–	26	+++++	38	+++++
B. subtilis ATCC 6633	12	+	10	+	25	++++	<6.4	–	29	+++++	44	+++++
E. faecalis ATCC 29212	10	+	9	–	13	+	<6.4	–	24	++++	35	+++++
E. coli ATCC 25922	8	–	9	–	<6.4	–	<6.4	–	27	+++++	41	+++++
L. monocytogenes ATCC 7644	10	+	10	+	12	+	<6.4	–	29	+++++	42	+++++
P. aeruginosa ATCC 27853	8	–	9	–	<6.4	–	<6.4	–	<6.4	–	23	++++
S. enteritidis ATCC 13076	<6.4	–	<6.4	–	<6.4	–	<6.4	–	29	+++++	40	+++++
S. aureus ATCC 25923	11	+	12	+	8	–	<6.4	–	24	++++	38	+++++
A. niger ATCC 16404	<6.4	–	<6.4	–	<6.4	–	<6.4	–	11	+	23	++++
C. albicans ATCC 10231	10	+	11	+	<6.4	–	<6.4	–	<6.4	–	16	++

[a] Diameter of inhibition zones (∅): +++++, ∅ ⩾ 26 mm; ++++, 22 mm ⩽ ∅<26 mm; +++, 18 mm ⩽ ∅<22 mm; ++, 14 mm ⩽ ∅<18 mm; +, 10 mm ⩽ ∅<14 mm; –, ∅<10 mm.

[b] Chloramphenicol for all microorganisms tested with the exception of *A. niger* for which actidione was used.

experiments were recorded using a VARIAN Unity 300 MHz spectrometer operating at 75.4 MHz for ^{13}C, while NOESY spectra were recorded with a BRUKER Avance 400, both spectrometers operating at a constant temperature of 298 K. The solvent used was $CDCl_3$ 1% v/v Me_4Si or 0.03% v/v Me_4Si, Aldrich. IR spectra were carried out using a Hitachi 270-50. Optical rotations were measured on a Perkin–Elmer 343 polarimeter. Melting points were determined on a Reichert–Thermovar Microscope and are uncorrected. Elemental analyses were performed at the Service of Microanalyses of Instituto Superior Técnico, Universidade Técnica de Lisboa. High-resolution mass spectra were obtained on a Finingan FT/MS 2001 DT, FT-ICR/MS mass spectrometer equipped with a 3 T superconducting magnet and interfaced with a Nd:YAg laser operating at the fundamental wavelength (1064 nm).

3.1. Synthesis

General procedure for the glycosylation reaction: The nucleophile (9.3 mmol) and a soln of $Ph_3P{\cdot}HBr$ (122 mg, 0.36 mmol) in dry CH_2Cl_2 (4.0 mL) were added to a soln of **1** (1 g, 4.7 mmol) in dry CH_2Cl_2 (4.0 mL). The mixture was stirred at 40 °C for 50 min. After cooling at rt, CH_2Cl_2 (30 mL) was added to the reaction mixture and the solution washed with a satd solution of $NaHCO_3$. Evaporation and column chromatography with EtOAc/*n*-hexane afforded the two anomers of the corresponding 2,6-dideoxy glycosides as well as the α-anomer of the Ferrier compound as a secondary product.

3.1.1. Octyl 3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*arabino*-hexopyranoside (2). Reaction of **1** with octanol (1.21 g, 9.3 mmol) gave **2** as a syrup (809 mg, 50%); $[\alpha]_D^{20}$ −79 (*c* 1, CH_2Cl_2); R_f 0.72 (1:3 EtOAc–*n*-hexane); IR (neat): 1752 cm^{-1} (C=O); ^1H NMR: δ 5.28 (ddd, 1H, H-3), 4.84 (d, 1H, H-1, $J_{1,2a}$ 3.3 Hz), 4.73 (t, 1H, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4), 3.85 (dq, 1H, $J_{5,6}$ 6.3 Hz, H-5), 3.60 (dt, 1H, $J_{H\text{-}1'a,H\text{-}1'b}$ 13.5, $J_{1',2'}$ 6.6 Hz, H-1′a), 3.36 (dt, 1H, H-1′b) 2.23 (dd, 1H, $J_{2a,2e}$ 12.9, $J_{2e,3}$ 5.7 Hz, H-2e), 2.05 (s, 3H, CH_3–Ac), 2.00 (s, 3H, CH_3–Ac), 1.77 (ddd, 1H, H-2a), 1.57 (m, 2H, H_2-2′a, H-2′b), 1.29 (m, 10H, CH_2-3′–CH_2-7′), 1.17 (d, 3H, CH_3-6), 0.89 (t, 3H, $J_{7',8'}$ 6.9 Hz, CH_3-8′); ^{13}C NMR: δ 170.3 (C=O), 96.6 (C-1), 74.9 (C-4), 69.1 (C-3), 67.5 (C-1′), 65.3 (C-5), 35.2 (C-2), 31.6, 29.5, 29.3, 29.2, 26.0, 23.5 (C-2′–C-7′), 20.8 (CH_3–Ac), 20.6 (CH_3–Ac), 17.3 (C-6); 13.8 (C-8′); HRMS: calcd for $C_{18}H_{32}O_6$: 344.220014; found: *m*/*z* 344.2221989.

3.1.2. Octyl 3,4-di-*O*-acetyl-2,6-dideoxy-β-L-*arabino*-hexopyranoside (3). The above-mentioned general procedure gave **3** as a syrup (323.8 mg, 20%); $[\alpha]_D^{20}$ +3 (*c* 1, CH_2Cl_2); R_f 0.69 (1:3 EtOAc–*n*-hexane); IR (neat): 1738 cm^{-1} (C=O); ^1H NMR: δ 4.97 (ddd, 1H, $J_{3,4}$ 9.6 Hz, H-3), 4.74 (t, 1H, $J_{4,5}$ 9.6 Hz, H-4), 4.51 (dd, 1H, $J_{1,2a}$ 9.3 Hz, H-1), 3.87–3.85 (m, 1H, H-1′a), 3.47–3.44 (m, 2H, $J_{5,6}$ 6.6 Hz, H-5, H-1′b) 2.30 (ddd, 1H, $J_{1,2e}$ 1.8 Hz, $J_{2e,2a}$ 13.5 Hz, $J_{2e,3}$ 5.8 Hz, H-2e), 2.05 (s, 3H, CH_3–Ac), 2.02 (s, 3H, CH_3–Ac); 1.73 (m, 3H, H-2a, H-2a′, H-2′b), 1.26–1.23 (m, 10H, CH_2-3′–CH_2-7′), 0.98 (d, 3H, CH_3-6), 0.93 (t, 3H, $J_{7',8'}$ 7.2 Hz, CH_3-8′); ^{13}C NMR: δ 170.5 (C=O), 170.1 (C=O), 99.2 (C-1), 74.3 (C-4), 70.8 (C-3), 70.0 (C-5), 69.8 (C-1′), 36.5 (C-2), 31.8, 29.6, 29.4, 29.2, 26.0, 22.7 (C-2′–C-7′), 21.0 (CH_3–Ac), 20.9 (CH_3–Ac), 17.6 (C-6); 14.1 (C-8′); HRMS: calcd for $C_{18}H_{32}O_6$: 344.220014; found: *m*/*z* 344.219890.

3.1.3. Octyl 4-*O*-acetyl-2,3,6-trideoxy-α-L-*erythro*-hex-2-enopyranoside (4). The above indicated reaction gave **4** as a syrup (120.2 mg, 9%); $[\alpha]_D^{20}$ −50 (*c* 1, CH_2Cl_2); R_f 0.40 (EtOAc–*n*-hexane 1:10); IR (neat): 1606 (C=C), 1752 (C=C); ^1H NMR: δ 5.86–5.80 (m, 2H, H-2, H-3), 5.05 (dd, 1H, $J_{4,5}$ 7.8 Hz, $J_{3,4}$ 1.2 Hz, H-4), 4.95 (s, 1H, H-1), 3.97 (dq, 1H, $J_{5,6}$ 6.6 Hz, H-5), 3.77–3.72 (m, 1H, H-1′a), 3.52–3.45 (m, 1H, H-1′b) 2.08 (s, 3H, CH_3–Ac), 1.62–1.52 (m, 2H, H-2′a, H-2′b), 1.27–1.21 (m, 10H, CH_2-3′–CH_2-7′), 0.97 (d, 3H, CH_3-6), 0.92 (t, 3H, $J_{7',8'}$ = 7.2 Hz, CH_3-8′); ^{13}C NMR: δ 170.5 (C=O), 128.8, 127.9 (C-2, C-3), 94.3 (C-1), 70.9 (C-4), 68.1 (C-1′), 64.7 (C-5), 31.8, 30.3, 29.7, 29.3, 26.1, 23.7 (C-2′–C-7′), 20.9 (CH_3–Ac), 16.9 (C-6), 14.0 (CH_3-8′). Anal. Calcd for $C_{16}H_{28}O_4$: C, 67.57; H, 9.92. Found: C, 67.53; H, 9.99.

3.1.4. Dodecyl 3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*arabino*-hexopyranoside (5). Reaction of **1** with dodecanol (1.73 g, 9.3 mmol) gave **5** as a syrup (791 mg, 42%); $[\alpha]_D^{20}$ −80 (*c* 1, CH_2Cl_2); R_f 0.72 (1:3 EtOAc–*n*-hexane); IR (neat): 1754 (C=O); ^1H NMR: δ 5.21 (ddd, 1H, $J_{3,4}$ 9.6 Hz, H-3), 4.77 (d, 1H, $J_{1,2a}$ 3.3 Hz, H-1), 4.65 (t, 1H, $J_{4,5}$ 9.6 Hz, H-4), 3.77 (dq, 1H, $J_{5,6}$ 6.3 Hz, H-5), 3.52 (dt, 1H, $J_{1'a,1'b}$ 9.3 Hz, $J_{1'a,2'}$ 6.9 Hz, H-1′a), 3.28 (dt, 1H, $J_{1'b,2'}$ 6.6 Hz, H-1′b), 2.14 (dd, 1H, $J_{2a,2e}$ 12.9 Hz, $J_{2e,3}$ 5.7 Hz, H-2e), 1.93 (s, 3H, CH_3–Ac), 1.70 (s, 3H, CH_3–Ac), 1.52–1.48 (m, 2H, H-2′a, H-2′b), 1.20 (br, s, 18H, CH_2-3′–CH_2-11′), 1.10 (d, 3H, CH_3–Ac), 0.81 (t, 3H, $J_{11',12'}$ 6.3 Hz, CH_3-12′); ^{13}C NMR: δ 170.1 (C=O), 96.5 (C-1), 74.9 (C-4), 69.1 (C-3), 67.5 (C-1′), 65.4 (C-5), 35.3 (C-2), 31.8, 29.5, 26.1, 22.6 (C-2′–C-11′), 20.9 (CH_3–Ac), 17.5 (C-6), 14.0 (C-12′); HRMS: calcd for $C_{22}H_{40}O_6$: 400.282914; found: *m*/*z* 400.282490.

3.1.5. Dodecyl 3,4-di-*O*-acetyl-2,6-dideoxy-β-L-*arabino*-hexopyranoside (6). The above-mentioned reaction gave **6** as a syrup (395.3 mg, 21%); $[\alpha]_D^{20}$ +5 (*c* 1, CH_2Cl_2); R_f 0.68 (1:3 EtOAc–*n*-hexane); IR (neat): 1752 cm^{-1} (C=O); ^1H NMR: δ 5.00 (ddd, 1H, $J_{3,4}$

9.3 Hz, H-3), 4.76 (t, 1H, $J_{4,5}$ 9.3 Hz, H-4), 4.55 (dd, 1H, $J_{1,2a}$ 9.6 Hz, H-1), 3.89 (dt, 1H, $J_{1'a,1'b}$ 9.6 Hz, $J_{1',2'}$ 6.6 Hz, H-1′a), 3.52–3.64 (m, 2H, H-5, H-1′b) 2.35 (ddd, 1H, $J_{1,2e}$ 1.8 Hz, $J_{2e,2a}$ 12.6 Hz, $J_{2e,3}$ 5.4 Hz, H-2e), 2.08 (s, 3H, CH_3–Ac), 2.05 (s, 3H, CH_3–Ac); 1.75 (ddd, 1H, $J_{2a,3}$ 12.6 Hz, H-2a), 1.34–1.21 (m, 21H, CH_2-3′–CH_2-11′, CH_3-6), 0.94 (t, 3H, $J_{11',12'}$ 6.6 Hz, CH_3-12′); ^{13}C NMR: δ 170.5 (C═O), 170.1 (C═O), 99.2 (C-1), 76.7 (C-4), 74.3 (C-3), 70.0 (C-5), 69.8 (C-1′), 36.5 (C-2), 31.9, 29.7, 29.4, 29.3, 26.0, 22.7 (C-2′–C-11′), 21.0 (CH_3–Ac), 20.9 (CH_3–Ac), 17.6 (C-6); 14.1 (C-8′); HRMS: calcd for $C_{22}H_{40}O_6$: 400.282914; found: *m*/*z* 400.282490.

3.1.6. Dodecyl 4-*O*-acetyl-2,3,6-trideoxy-α-L-*erythro*-hex-2-enopyranoside (7). The above reaction gave **7** as a syrup (160 mg, 10%); $[\alpha]_D^{20}$ −56 (*c* 1, CH_2Cl_2); R_f 0.58 (1:7 EtOAc–*n*-hexane); IR (neat): 1606 (C═C), 1752 (C═O); ^{1}H NMR: δ 5.78–5.76 (m, 2H, H-2, H-3), 4.99 (dd, 1H, $J_{4,5}$ 9 Hz, $J_{3,4}$ 1.2 Hz, H-4), 4.90 (s, 1H, H-1), 3.93 (dq, 1H, $J_{5,6}$ 6.3 Hz, H-5), 3.71 (dt, 1H, $J_{H\text{-}1'a,H1'b}$ 6.3 Hz, H-1′a), 3.44 (dt, 1H, H-1′b, $J_{H\text{-}1'b,H\text{-}2'}$ 6.3 Hz), 2.03 (s, 3H, CH_3–Ac), 1.59–1.50 (m, 2H, CH_2-2′), 1.27–1.08 (m, 18H, CH_2-3′–CH_2-11′), 1.17 (d, 3H, CH_3-6), 0.83 (t, 3H, $J_{11',12'}$ 6.6 Hz, CH_3-12′); ^{13}C NMR: δ 170.4 (C═O), 129.4, 128.5 (C-2, C-3), 94.3 (C-1), 70.9 (C-4), 68.6 (C-1′), 64.6 (C-5), 31.8, 29.5, 28.5, 26.1, 25.8, 22.6 (C-2′–C-11′), 20.9 (CH_3–Ac), 17.1 (C-6), 14.0 (C-12′). Anal. Calcd for $C_{20}H_{36}O_4$: C, 70.55; H, 10.66. Found: C, 70.68; H, 10.32.

General procedure for the deacetylation of compounds ***2***, ***3***, ***5***, ***6***: A soln of NaOMe in MeOH (1%, 1.6 mL) was added to a soln of the sugar (0.3 mmol) in MeOH (16 mL) and the mixture was stirred at rt 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 1:1 EtOAc–*n*-hexane affording the corresponding 2,6-dideoxy glycosides.

3.1.7. Octyl 2,6-dideoxy-α-L-*arabino*-hexopyranoside (8). The above-mentioned procedure gave **8** as a syrup (76.5 mg, 98%); $[\alpha]_D^{20}$ −8.7 (*c* 1, CH_2Cl_2); R_f 0.35 (1:1 EtOAc–*n*-hexane); ^{1}H NMR: δ 4.82 (d, 1H, $J_{1,2a}$ 3.0 Hz, H-1), 4.70 (br s, 1H, OH), 3.88 (br ddd, 1H, $J_{2a,3} = J_{3,4}$ 9.3 Hz, H-3), 3.65–3.57 (m, 2H, H-5, H-1′a), 3.35 (td, 1H, $J_{1'a,1'b}$ 9.6 Hz, $J_{1'b,2'}$ 6.6 Hz, H-1′a), 3.06 (br t, 1H, $J_{4,5}$ 9.0 Hz, H-4) 2.12 (dd, 1H, $J_{2a,2e}$ 12.6 Hz, $J_{2e,3}$ 4.8 Hz, H-2e), 1.72–1.55 (m, 2H, H-2a, H-2′), 1.29–1.27 (m, 13H, CH_3-6, CH_2-3′–CH_2-7′), 0.90 (t, 3H, H-8′, $J_{7',8'}$ 6.9 Hz); ^{13}C NMR: δ 97.0 (C-1), 77.6 (C-4), 68.9 (C-3), 67.5 (C-5), 67.3 (C-1′), 37.8 (C-2), 29.4 (C-2′), 31.7, 29.3, 29.1, 26.0, 22.5, (C-3′–C-7′) 17.6 (C-6); 13.9 (C-8′). Anal. Calcd for $C_{14}H_{28}O_4$: C, 64.58; H, 10.84. Found: C, 64.19; H, 11.03.

3.1.8. Octyl 2,6-dideoxy-β-L-*arabino*-hexopyranoside (9). The general procedure gave **9** as a colorless crystalline solid (73.4 mg, 94%); mp 68 °C; $[\alpha]_D^{20}$ +5 (*c* 1, CH_2Cl_2); R_f 0.27 (1:1 EtOAc–*n*-hexane); ^{1}H NMR: δ 4.54 (dd, 1H, $J_{1,2a}$ 7.8 Hz, $J_{1,2e}$ 1.8 Hz, H-1), 3.92 (td, 1H, $J_{1'a,1'b}$ 9.3 Hz, $J_{1'a,2'}$ 6.9 Hz, H-1′a), 3.65 (ddd, 1H, $J_{2a,3}$ 10.8 Hz, $J_{3,4}$ 9.3 Hz, H-3), 3.50 (ddd, 1H, H-1′b), 3.34 (qd, 1H, $J_{5,6}$ 6.0 Hz, H-5) 3.16 (t, 1H, $J_{4,5}$ 8.7 Hz, H-4) 2.82, 2.75 (2H, OH), 2.27 (br dd, 1H, $J_{2a,2e}$ 12.3 Hz, $J_{2e,3}$ 5.1 Hz, H-2e), 1.74–1.62 (m, 2H, H-2a, H-2′), 1.40 (d, 3H, CH_3-6), 1.33 (br s, 10H, CH_2-3′–CH_2-7′), 0.94 (t, 3H, $J_{7',8'}$ 6.9 Hz CH_3-8′); ^{13}C NMR: δ 99.6 (C-1), 77.5 (C-4), 71.6 (C-3′, C-5′), 69.6 (C-1′), 39.1 (C-2), 29.6 (C-2′), 31.8, 29.4, 29.2, 26.0, 22.6, (C-3′-C-7′), 17.7 (C-6); 14.0 (C-8′). Anal. Calcd for $C_{14}H_{28}O_4$: C, 64.58; H, 10.84. Found: C, 64.89; H, 10.64.

3.1.9. Dodecyl 2,6-dideoxy-α-L-*arabino*-hexopyranoside (10). The deacetylation reaction gave **10** as a colorless crystalline solid (94 mg, 99%); mp 34 °C; $[\alpha]_D^{20}$ −8.4 (*c* 1, CH_2Cl_2); R_f 0.41 (1:1 EtOAc–*n*-hexane); ^{1}H NMR: δ 4.83 (d, 1H, $J_{1,2a}$ 3.0 Hz, H-1), 3.92 (ddd, 1H, H-3), 3.69–3.57 (m, 2H, H-5, H-1′a), 3.35 (td, 1H, $J_{1'a,1'b}$ 9.6 Hz, $J_{1'b,2'}$ 6.6 Hz, H-1′b), 3.09 (br t, 1H, $J_{4,5}$ 9.0 Hz, H-4) 2.13 (ddd, 1H, $J_{2a,2e}$ 12.6 Hz, $J_{2e,3}$ 4.8 Hz, H-2e), 1.68 (ddd, 1H, H-2a, $J_{2a,3} = J_{3,4}$ 9.6 Hz, H-2a), 1.59–1.52 (m, 1H, H-2′), 1.30–1.27 (m, 21H, CH_3-6, CH_2-3′–CH_2-11′), 0.89 (t, 3H, CH_3-12′, $J_{11',12'}$ 6.9 Hz); ^{13}C NMR: δ 97.0 (C-1), 77.6 (C-4), 68.7 (C-3), 67.5 (C-5), 67.2 (C-1′), 37.7 (C-2), 29.5 (C-2′), 31.8, 29.3, 29.2, 26.0, 22.5, (C-3′-C-11′), 17.6 (C-6); 13.9 (C-12′). Anal. Calcd for $C_{18}H_{36}O_4$: C, 68.31; H, 11.47. Found: C, 68.15; H, 11.76.

3.1.10. Dodecyl 2,6-dideoxy-β-L-*arabino*-hexopyranoside (11). The above general procedure gave **11** as a colorless crystalline solid (93 mg, 98%); mp 82 °C; $[\alpha]_D^{20}$ +3.5 (*c* 1, CH_2Cl_2); R_f 0.29 (1:1 EtOAc–*n*-hexane); ^{1}H NMR: δ 4.48 (dd, 1H, $J_{1,2a}$ 9.6 Hz, $J_{1,2e}$ 1.8 Hz, H-1), 3.86 (td, 1H, $J_{1'a,1'b}$ 9.6 Hz, $J_{1'a,2'}$ 6.6 Hz, H-1′a), 3.63 (ddd, 1H, $J_{2a,3}$ 11.1 Hz, $J_{3,4}$ 9.3 Hz, H-3), 3.43 (td, 1H, H-1′b), 3.27 (qd, 1H, $J_{5,6}$ 6.0 Hz, H-5) 3.10 (t, 1H, $J_{4,5}$ 9.0 Hz, H-4) 2.20 (ddd, 1H, $J_{2a,2e}$ 12.3 Hz, $J_{2e,3}$ 4.8 Hz, H-2e), 1.68–1.56 (m, 3H, H-2a, CH_2-2′), 1.34 (d, 3H, CH_3-6), 1.26 (br s, 18H, CH_2-3′–CH_2-11′), 0.88 (t, 3H, CH_3-12′, $J_{11',12'}$ 6.9 Hz); ^{13}C NMR: δ 99.6 (C-1), 77.6 (C-4), 71.7 (C-3), 71.6 (C-5), 69.6 (C-1′), 39.1 (C-2), 29.6 (C-2′), 31.8, 29.4, 29.3, 26.0, 22.7, (C-3′–C-11′), 17.7 (C-6); 14.1 (C-12′). Anal. Calcd for $C_{18}H_{36}O_4$: C, 68.31; H, 11.47. Found: C, 68.38; H, 11.09.

3.2. Surface activity

Surface tension measurements were performed with a Kruss K8 Interfacial Tensiometer using the du Noüy

Ring Method at 35.0 ± 0.1 °C. Adequate temperature control was ensured through continuous circulation of the thermostatic fluid in a stainless steel sleeve around the sample vessel. Cleanliness of all the materials was ensured washing all glass material with chromic acid and then thoroughly rinsing with distilled water and by glowing, until dark-red color in a Bunsen flame, the platinum ring before each measurement. The tensiometer calibration was checked with Milli-Q water before each run.

The du Noüy ring non-detachment technique was used and the surface tensions were determined as a function of surfactant concentration. The most dilute solns, $m \leqslant 1 \times 10^{-5}$ mol · kg^{-1} for **10** and $m \leqslant 2 \times 10^{-4}$ mol · kg^{-1} for **8** and **9** exhibited a time-dependant surface tension, with a decreasing tendency on standing consequently, the temperature of all solns was pre-equilibrated for at least 30 min and the measurements initiated about 5 min after the creation of a fresh air–water interface. Solns were prepared by weight in an A&D Instruments analytical balance to $\pm 2 \times 10^{-5}$ g. The dissolution of the compounds in water was slow and this process was accelerated, for the concentrated solns, resorting to the immersion of the solns in an ultrasound bath at 40 °C, during 30 min. Surface tensions were determined exclusively on clear isotropic solns thus limiting the studies to aq solns of compounds **8**, **9**, and **10** as attempts to prepare $4 \times 10^{-5}m$ solns of compound **11** were unsuccessful, and lead to liquid mixtures containing dispersed crystals. These macroscopic observations were corroborated examining under crossed polarization with a 100× magnification, mixtures of compounds **10** and **11** in water ($3.75 \times 10^{-5}m$ and $3.55 \times 10^{-5}m$, respectively), cooled down to −5 °C, prepared according to the procedure mentioned above. These microscopic observations unequivocally established the isotropic nature of the C12α (**10**) aqueous mixture at room temperature (23 °C) and evidenced crystals dispersed in an isotropic media for the $C_{12}\beta$ (**11**) aqueous mixture, even at 50 °C, thus confirming that the measurements reported for compound **10** were performed above the Krafft temperature.

The surface tension values presented are the average of at least three independent evaluations. To crosscheck the reliability of the experimental data, the dilute solutions ($m \leqslant 1 \times 10^{-4}$ mol · kg^{-1} for **10** and $m \leqslant 5 \times 10^{-4}$ mol · kg^{-1} for **8** and **9**) were prepared by successive dilution of independently prepared concentrated solns, and compounds obtained from independent synthetic batches were also used. Open and filled symbols included in Figures 1 and 2 in all experimental data sets pertain to independent synthesis of the same sugar surfactant, and in all cases the dilute solns were obtained by several independent, successive but crisscrossing dilutions of the most concentrated soln. The experimental data for **9** and **10** also include shaded symbols that are connected with dilutions of independent concentrated solns.

3.3. Biological activity

The antibacterial and antifungal activity of compounds **8**–**11** was evaluated using the paper disk diffusion method.[14] The following bacteria and fungi were used in the tests: *B. cereus* (ATCC 11778), *B. subtilis* (ATCC 6633), *E. faecalis* (ATCC 29212), *E. coli* (ATCC 25922), *L. monocytogenes* (ATCC 7644), *P. aeruginosa* (ATCC 27853), *Salmonella enteritidis* (ATCC 13076), *S. aureus* (ATCC 25923), *A. niger* (ATCC 16404), and *C. albicans* (ATCC 10231). The overnight cultures of the microorganisms were spread over the appropriate media, nutrient agar for all bacteria except *Listeria* and *Enterococcus*, where triptone soya agar and azide dextrose agar were used, respectively. Potato dextrose agar was used for fungi. Paper disks of 6.4 mm were placed on the agar and a soln of each substance (300 μg) in DMSO (15 μL) was applied on each disk. Chloramphenicol (*B. cereus*, *B. subtilis*, *E. faecalis*, *E. coli*, *L. monocytogenes*, *P. aeruginosa*, *S. enteritidis*, *S. aureus*, *C. albicans*) and actidione (*A. niger*) were used as positive controls and DMSO was used as negative control. Bacteria were incubated at 37 °C for 24 h and fungi at 25 °C for 48 h. After incubation, the plates presented a biomass lawn, and when applicable, the nearest diameter of the inhibition zones formed was measured. Results were the average of three replicates.

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