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Antimicrobial and cytotoxic activity of (thio)alkyl hexopyranosides, nonionic glycolipid mimetics

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ABSTRACT

A series of 19 synthetic alkyl and thioalkyl glycosides derived from D-mannose, D-glucose and D-galactose and having C₁₀–C₁₆ aglycone were investigated for cytotoxic activity against 7 human cancer and 2 non-tumor cell lines as well as for antimicrobial potential on 12 bacterial and yeast strains. The most potent compounds were found to be tetradecyl and hexadecyl β-D-galactopyranosides (**18**, **19**), which showed the best cytotoxicity and therapeutic index against CCRF-CEM cancer cell line. Similar cytotoxic activity showed hexadecyl α-D-mannopyranoside (**5**) but it also inhibited non-tumor cell lines. Because these two galactosides (**18**, **19**) were inactive against all tested bacteria and yeast strains, they could be a target-specific for eukaryotic cells. On the other hand, β-D-glucopyranosides with tetradecyl (**11**) and hexadecyl (**12**) aglycone inhibited only Gram-positive bacterial strain *Enterococcus faecalis*. The studied glycosides induce changes in the lipid bilayer thickness and lateral phase separation at high concentration, as derived from SAXS experiments on POPC model membranes. In general, glucosides and galactosides exhibit more specific properties. Those with longer aglycone show high cytotoxicity and therefore, they are more promising candidates for cancer cell line targeted inhibition.

1. Introduction

Glycolipids are cell membrane amphiphilic components present in all living organisms. The hydrophilic carbohydrate portion is composed of one or more monosaccharide units linked by a glycosidic bond while the hydrophobic lipid moiety anchors them to the membrane. It is known that these compounds fulfill a large variety of functions that are important for many biological processes, such as recognition, cell adhesion and signaling that are influenced by membrane properties [1].

The glycolipid ability to be incorporated into the lipid bilayer may lead to either disruption or modification of the membrane structure. Many of glycolipids are also able to dissolve the lipid membrane yielding a disruption of the cells. Therefore, the surface tension of the membranes reduces, allowing water to flow into the cell and finally results in cell lysis. To enhance the efficiency of this action, a tuned balance between the hydrophilic and hydrophobic parts of the glycolipid is essential [2].

Among others, the sugar core, its stereochemistry and the structure of hydrophobic aglycone are the factors that determine the physical and chemical properties of the glycolipids [3].

The ability of the glycolipids to destabilize biological membrane determines their biomedical application as antifungal and antibacterial agents. Due to their antiviral and cytotoxic properties, they are a subject of interest as therapeutics and pharmaceuticals.

Their low toxicity is an additional benefit, important for applications in agro, food and cosmetic industries [4].

The large potential of these compounds was an impulse for synthesis and evaluation of a series of synthetic glycolipid mimetics [5]. Alkyl glycosides is one group of such mimetics. The synthesis of such sugar-based amphiphiles is rapid [6] and, often, it requires just a few reaction steps. Moreover, these non-ionic surfactants are environmentally friendly, biocompatible and biodegradable.

The discovery of anticancer activity of fractions containing mixtures of diverse glycolipids isolated from edible plants [7] motivated the

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preparation of a series of synthetic decyl to hexadecyl α - and β -D-galactopyranosides. Some of them were moderately cytotoxic against 4 cancer cell lines with IC_{50} values at μ M level. However, in some cases, equimolar mixtures of both anomers were even more cytotoxic towards several cancer cell lines than single anomers [8]. This was the case of the mixture of tetradecyl α/β -D-galactopyranosides, which accentuates apoptosis of multiple myeloma cells by inducing DNA damage [9]. These are the only report on cytotoxicity of sugar-based amphiphiles.

Reports on antimicrobial activity of alkyl glycosides are rare. Nevertheless, the synthesis of a series of alkyl 2-, 3-, 4-deoxy, 2,3- and 3,4-dideoxy glycosides was reported [10,11]. Among them, octyl and dodecyl 2-deoxy- β -D-*arabino*-hexopyranosides inhibit the growth of *Enterococcus faecalis*. This Gram-positive species is also highly sensitive to dodecyl 2,6-dideoxy α -L-*arabino*-hexopyranoside, which is particularly active against another Gram-positive bacterium, *Bacillus* sp. [10]. Additionally, Hu et al. have shown that triazole glycolipids can reverse the bacterial MRSA phenotype and restore sensitivity to beta-lactam antibiotics [12]. Such activity may be important for the eradication of resistant bacteria in cystic fibrosis or, more generally, in the therapy of nosocomial infections.

In our previous study, we showed that antimicrobial activity of 14 synthetic alkyl and thioalkyl α -D-mannopyranosides, with aglycone from hexyl to eicosyl, against 3 species (Gram-positive *Staphylococcus aureus*, Gram-negative *Escherichia coli* and yeast *Candida albicans*), depends on the aglycone length and on the type of glycosidic linkage (O- vs. S-mannosides). Mannosides having (thio)dodecyl aglycones are the most potent antimicrobials, with IC_{50} at the micromolar level [13].

The eco-friendly nature of alkyl glycosides offers a broad potential for their study. We evaluated a broad variety of synthetic alkyl D-glycosides (glucosides, galactosides and mannosides), which include aglycone of selected length from decyl to hexadecyl. Moreover, because (thio)dodecyl mannosides are very active [13], both anomers of the corresponding glucoside and galactoside analogs were screened in this study.

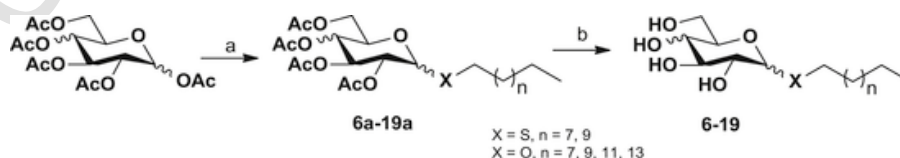
This paper reports the investigation of the inhibitory potential of these synthetic sugar based amphiphiles against several Gram-positive (including methicillin-resistant *Staphylococcus aureus*), Gram-negative bacteria and yeasts, and 7 human cancer and 2 non-tumor lines. Due to the amphiphilic character of these molecules, the lipid bilayer of the cell membrane is a target of their biological activity. We examined structural changes of the model membrane palmitoyl-oleoyl-phosphatidylcholine (POPC) induced by selected glycosides, by Small angle X-ray scattering (SAXS).

2. Results and discussion

2.1. Synthesis

Tetradecyl α -D-mannopyranoside (**4**) was prepared by a procedure previously utilized in the synthesis of various alkyl mannosides, including **1** [13], **2** [6], **3** [13] and **5** [6]. This 2 steps sequence led to the exclusive formation of **4** in 45% overall yield.

The synthesis of another neutral (thio)alkyl α - and β -D-glycopyranosides bearing hydrocarbon chains of different length (C_{10} – C_{16}) was performed by a glycosylation reaction between the corresponding alcohol or thiol as an acceptor with peracetylated sugar donor upon activation with $BF_3 \cdot OEt_2$ at room temperature (Scheme 1, Experimental section).



Scheme 1. Synthesis of target nonionic glycolipid mimetics **6–19**. *Reagents and conditions.* a) $BF_3 \cdot OEt_2$, alcohol, solvent, 5–24 h, rt; b) MeONa, MeOH, 16 h, rt. Synthesis and analytical data for compounds **1a** [13], **2a** [6], **3a** [13] and **5a** [6] were reported.

When the reaction was proceeded for 5 h in DCM, desired decyl glycosides **6a** and **13a** were obtained in moderate yields (~30%). Similar yields of all glycosides having longer, tetradecyl and hexadecyl aglycones, were achieved after 22 h in 1,2-dichloroethane.

α - and β -anomers of (thio)dodecyl glucoside and galactoside were separated from the corresponding anomeric mixtures. While dodecyl α/β glycosides were formed in approximately equimolar ratio after 24 h, in the case of thio analogs, β -anomers were observed as major products within 5 h. The purified isomers were identified based on a typical chemical shift for H-1. For dodecyl glycosides (**7a**, **8a** and **14a**, **15a**), the H-1 signal of α -anomers was shifted more downfield by 0.6–1.1 ppm than that of β -anomers. The same trend in chemical shifts of the anomeric H-1 was observed for the corresponding thio analogs **9a**, **10a** and **16a**, **17a**. In addition, for β -anomers of all (thio)dodecyl glycosides, C-1 atom resonated at higher ppm than that of the corresponding α -anomer.

Finally, deacetylation of **6a–19a** under Zemplén conditions (sodium methoxide in methanol) gave deprotected glycosides **6–19** with good yields.

2.2. Biological assay

2.2.1. Cytotoxicity

Compounds **1–19** (Fig. 1) were tested *in vitro* for their cytotoxic activity on seven cancer cell lines: A549 (human lung adenocarcinoma), CCRF-CEM (T-lymphoblastic leukemia), CEM-DNR (T-lymphoblastic leukemia, daunorubicin resistant), K562 (acute myeloid leukemia), K562-TAX (acute myeloid leukemia, overexpressing the P-glycoprotein), HCT116 (human colorectal cancer with wild-type p53), HCT116p53–/– (human colorectal cancer with deleted p53), and on the two non-malignant cell lines BJ (human fibroblast) and MRC-5 (human lung fibroblasts). Cytotoxic activities are presented in Table 1 and are expressed as IC_{50} values.

The tested compounds showed IC_{50} values between 9.4 μ M and more than 100 μ M.

The acute lymphoblastic leukemia CCRF-CEM cell line was the most sensitive to tested glycosides, particularly to **5**, **11**, **18** and **19** (IC_{50} in the range of 9.4–20.3 μ M) bearing tetradecyl or hexadecyl aglycone, what implies correlation between aglycone length and cytotoxic activity. All the compounds were less active against its daunorubicin resistant CEM-DNR counterpart.

However, in the case of K562 and the corresponding drug-resistant K562-TAX lines, the cytotoxic activities of glucosides and galactosides are similar. A more significant difference was observed for decyl α -D-mannoside (**1**) having 2.6 times higher cytotoxicity against K562 than K562-TAX (IC_{50} 27.7 μ M vs. 69.4 μ M). On the other hand, hexadecyl α -D-mannoside (**5**) was 2.9 times more potent against resistant K562-TAX than K562 cell line (IC_{50} 19.7 μ M vs. 57.3 μ M). These results indicate that for the resistance are responsible other mechanisms than P-glycoprotein, which is common for both cell lines.

Except for decyl β -D-glucopyranoside (**6**) and decyl β -D-galactopyranoside (**13**), all other compounds are active against human lung adenocarcinoma A549. Only a slight increase in cytotoxic activity is observed with an elongation of alkyl lipid chain. Moreover, the impact of anomeric configuration is not significant. α -Linked dodecyl O- and S-glucosides **7** and **9**, as well as galactosides **14** and **16**, were slightly

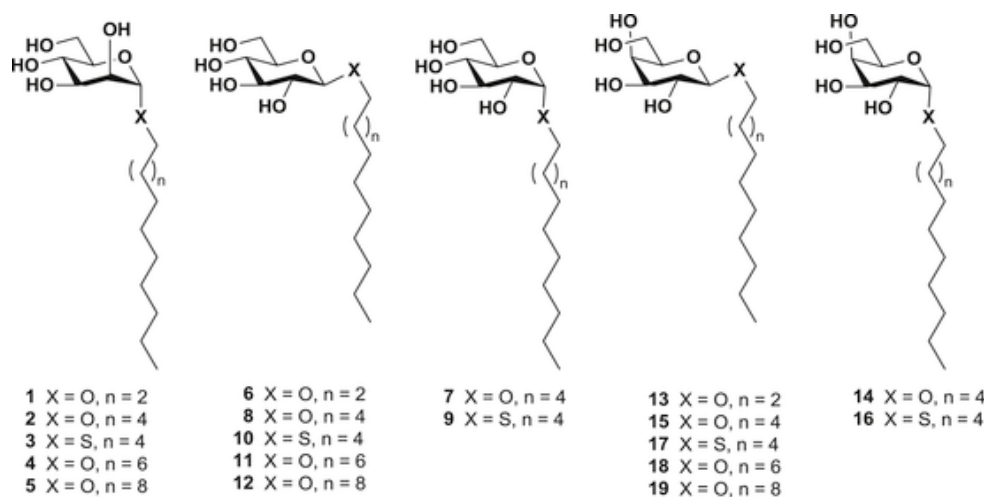


Fig. 1. Structure of the nonionic glycosides used in this study.

Table 1
Cytotoxic activity of derivatives 1–19 on human malignant cell lines different tissue origin and drug resistance profile.

Comp	IC ₅₀ (μM)								
	A549	CCRF-CEM	CEM-DNR	HCT116	HCT116p53--	K562	K562-TAX	BJ	MRC-5
1	74.1	75.7	91.7	73.7	86.5	27.2	69.4	85.8	>100
2	57.1	44.5	55.5	37.0	58.4	18.8	31.9	49.2	60.5
3	62.3	56.6	61.4	52.6	60.3	56.5	57.5	88.1	96.5
4	45.9	29.2	47.3	45.1	53.1	44.5	37.0	55.3	45.0
5	47.9	15.6	26.2	47.6	52.8	57.3	19.7	35.1	22.7
6	>100	66.0	87.6	>100	>100	94.9	>100	>100	>100
7	58.7	37.2	45.4	66.5	72.3	36.2	43.2	86.1	71.3
8	74.2	54.8	>100	56.7	77.5	61.8	68.1	84.1	>100
9	53.9	54.4	80.2	52.1	64.7	62.2	64.2	61.7	66.3
10	70.0	47.7	96.3	56.1	65.5	62.1	68.8	91.8	>100
11	58.8	20.3	47.9	55.9	60.7	47.8	51.9	>100	>100
12	55.5	51.6	74.9	57.8	61.1	73.0	56.2	76.0	83.3
13	>100	81.9	94.7	>100	>100	98.2	>100	>100	>100
14	61.2	54.5	98.2	52.3	77.4	58.0	69.4	68.8	>100
15	79.8	53.4	>100	64.3	89.4	62.8	72.2	95.4	>100
16	63.1	53.2	60.7	48.3	58.6	53.2	60.7	89.2	96.4
17	77.3	55.6	>100	65.6	79.6	55.2	69.6	>100	>100
18	54.2	9.4	50.4	68.8	55.4	48.5	52.3	>100	62.0
19	50.1	16.4	46.2	67.9	64.6	62.2	43.6	62.7	74.9

IC₅₀ is the lowest concentration that kills 50% of cells. The standard deviation in cytotoxicity assays is typically up to 20% of the average value. Compounds with IC₅₀ > 100 μM are considered inactive.

more active than their β-analogs **8**, **10**, **15** and **17**. The most potent glycolipid is tetradecyl α-D-mannoside (**4**) (IC₅₀ 45.9 μM).

Cytotoxicity of all glycosides tested against HCT116 and HCT116p53-- were similar. The most efficient were mannosides **2**, **4** and **5**, with IC₅₀ below 50 μM in the case of HCT116. Conversely, any effect of the aglycone length on the cytotoxicity of the glycosides against non-tumor lines BJ and MRC-5 was not observed. The most toxic are mannosides, in particular hexadecyl α-D-mannoside (**5**) (IC₅₀ < 35.1 μM). Some of the glucosides and galactosides inhibit these lines only weakly. Therefore, these glycosides are more suitable as anticancer drugs with favorable therapeutic index (TI).

TI value is the ratio between the average IC₅₀ value of noncancer cell lines (MRC-5 and BJ) and the IC₅₀ value of a given cancer cell line.

In general, glycolipids have the best selectivity (TI in a range from 9.1 to 2.27) for hematological CCRF-CEM (T-lymphoblastic leukemia), K562 (acute myeloid leukemia) and CEM-DNR (T-lymphoblastic

leukemia, daunorubicin resistant) cell lines. The highest TI value among all tested compounds is observed for tetradecyl β-D-galactoside (**18**) (TI_{CCRF-CEM} 9.10). Tetradecyl β-D-glucoside (**11**) also showed high TI (TI_{CCRF-CEM} 5.36) against the same line and a similar TI (TI_{CCRF-CEM} 4.20) was calculated for another galactoside **19** having two more carbon atoms in the aglycone chain. The β-glucoside **11** is the only compound with TI > 2 against some of the multidrug-resistant cell lines (TI_{CEM-DNR} 2.27). It exhibits the same TI value for the K562 cell line (TI_{K562} 2.27). Higher TI values for the K562 cell line were calculated for α-D-mannosides **1** and **2** (TI_{K562} 3.76 and 2.88, respectively) having shorter aglycones. Some selectivity on the K562 line (TI_{K562} 2.17) also showed dodecyl α-D-glucoside (**7**).

In a recent report [8], the cytotoxic activity of decyl to hexadecyl galactosides was assayed against 4 cancer cell lines, from which A549 and HCT116 cell lines were also used in this study. Cytotoxic activity of dodecyl galactosides **14** and **15** against the A549 cell line determined

in the present study is very similar to those reported, while IC₅₀ values for galactoside **19** were rather different. In case of HTC116 cell line, the IC₅₀ values for these 3 galactosides differed, but there was a similar trend, elongation of the aglycone chain resulted in a decrease in cytotoxicity.

2.2.2. Cell cycle analysis

The effect of the most active tetradecyl β-D-galactoside (**18**) on the cell cycle was analyzed on CCRF-CEM cell line at 1 ×/5 × IC₅₀ concentrations (9.39/46.95 μM) with 24-h treatment (Table 2). Accumulation of cells in the G0/G1 and reduction in the S phase of the cell cycle was observed, which was supported by the decrease of the DNA and RNA synthetic activity and increase of apoptotic cells percentage following the 5 × IC₅₀ treatment.

2.2.3. Antimicrobial activity

Antimicrobial activity of the glycolipid mimetic **1–19** were tested against Gram-positive (*Enterococcus faecalis* CCM 4224, *Staphylococcus aureus* CCM 3953, methicillin-resistant *S. aureus* (MRSA) 4591 and fluoroquinolone-resistant *S. haemolyticus* A/16568) and Gram-negative (*Escherichia coli* CCM 3954, *E. coli* C/16702, *Pseudomonas aeruginosa* CCM 3955, *P. aeruginosa* A/16575) bacterial strains and yeasts (*Candida albicans*, *C. krusei*, *C. tropicalis* and *C. parapsilosis*).

Inhibitory activity of the glycolipids (Table 3) was determined and is expressed as minimal bactericidal concentration (MBC) and minimal inhibitory concentration (MIC).

All tested glycolipids were inactive against Gram-negative bacterial strains. These species (*Escherichia coli* and *Pseudomonas aeruginosa*) represent two of the most frequently Gram-negative pathogens isolated in humans. Moreover, none of the decyl glycosides inhibited any of the other 10 microorganisms tested.

All tested *Candida* strains were sensitive only to (thio)dodecyl β-glycosides, α-galactosides and α-mannosides.

The best inhibitors of *C. albicans* are α-thioglycosides, mannoside **3** and galactoside **16** (both having MBC 50 μM), which were more bactericidal than their O-counterparts **2** and **14**. β-Glucosides **8** and **10** exhibited weaker activity.

The same inhibition pattern is observed for *C. krusei*. Dodecyl glycosides **8** and **14** are less potent than their thio-counterparts **10** and **16**. The latter compound is the most efficient *C. krusei* inhibitor, along with mannosides **2** and **3**, all showing the same MBC 50 μM.

C. parapsilosis showed an inhibitory pattern identical to that of *C. krusei*, but the potency of the glycosides is slightly weaker. *C. parapsilosis* is the most sensitive to thiomannoside **3**, which showed bactericidal activity equal to that against the two *Candida* sp. strains (*C. albicans*, *C. krusei*) mentioned above.

C. tropicalis is sensitive to the same glycosides as *C. albicans*, but it is the weakest inhibited strain among the tested *Candida* sp. strains. The most potent glycosides (**2**, **3** and **16**) out of 6 active ones have the same bactericidal activity (MBC 100 μM).

In general, S-glycosides were more efficient *Candida* sp. strains inhibitors than their O-counterparts.

The Gram-positive strain *E. faecalis* is the most susceptible strain, since it was inhibited by ten glycosides. Comparing two active α-galactosides, S-glycoside **16** (MBC 25 μM) is more efficient than its O-analogue **14**, thus resembling the inhibitory pattern of all *Candida* sp. strains. Five out of seven tested glucosides possess bactericidal activity which increases with the elongation of the aglycone hydrocarbon chain. Hexadecyl β-D-glucoside **12** (MBC 25 μM) is the most bactericidal. It was inactive against all other microbes. Similarly, tetradecyl β-D-glucoside **11**, as well as hexadecyl α-D-mannoside **5** (both MBC 50 μM), are selective inhibitors of *E. faecalis*. However, (thio)dodecyl mannosides **2** and **3** are more bactericidal glycosides (MBC 25 μM), reaching the efficiency of **16**.

Another Gram-positive strain, represented by the more virulent *S. aureus*, is sensitive to the same (thio)dodecyl glycosides as *Candida* sp. strains, and S-glycosides showed again better inhibitory potential. Among 19 tested glycosides, tetradecyl α-D-mannoside (**4**) selectively inhibits this strain. Together with another mannoside **3**, they were the most bactericidal (MBC 25 μM). On the other hand, reduced potential of glucosides was observed. *S. aureus* was affected only by two of them.

4 (Thio)dodecyl glycosides (**2**, **3**, **10** and **14**) exhibit a bactericidal activity against MRSA at a level similar to that observed against *S. aureus*.

S. haemolyticus 16568 was the least sensitive to the tested glycolipids. Only two of them show some activity, but both bactericidal activity of dodecyl α-D-mannoside (**2**) (MBC 200 μM) and bacteriostatic activity of dodecyl α-D-galactoside (**14**) (MBC 200 μM) are quite weak.

The study reveals that alkyl glycosides as nonionic glycolipid mimetics can induce a bactericidal action. In general, glycosides were the most efficient inhibitors of *E. faecalis*, which is sensitive to ten of them. On the other hand, the mannosides as the broadest spectrum antimicrobials are able to inhibit 8 out of 12 tested microbial strains. Next, the length of the aglycone affects inhibitory activity. Decyl glycosides were completely inactive, while those with (thio)dodecyl aglycone were efficient on the majority of the tested strains. In addition, further elongation of glycolipid aglycone chain leads to selective inhibition of some strains (namely *E. faecalis* (compounds **5**, **11** and **12**) and *S. aureus* (comp. **4**)). These conclusions are in agreement with our previous paper reporting on a brief study of antimicrobial properties of a series of α-D-mannosides. Nonionic alkyl glycosides presented here affect *S. aureus*, *E. faecalis* and *Candida* sp. strains, but also show inactivity against *E. coli* and *P. aeruginosa*. The bactericidal activity of the most active glycolipids mimetics (MBC 25 μM) demonstrate their promising antimicrobial potential against some Gram-positive bacterial strains and yeast.

2.3. Structural study of the model membrane

As reported above, some of the studied glycosides are biologically active. These nonionic amphiphiles interact readily with the phospholipid bilayer of the biological membrane.

Table 2

Summary of the cell cycle, apoptosis (sub G1), mitosis (pH3), and DNA (BrDU+) and RNA (BrU+) synthesis analyses for tetradecyl β-D-galactopyranoside (**18**). Data are expressed as a percentage of the total cellular population.

Comp.	Concentration (μM)	% of total cell populations						
		Sub-G1 (apoptosis)	G0/G1	S	G2/M	pH3 ^{Ser10} + (mitosis)	BrDU + (DNA synthesis)	BrU + (RNA synthesis)
Control	0	2.18	38.40	42.36	19.25	2.10	37.48	42.05
18	9.39	2.24	39.19	39.61	21.20	2.07	42.98	45.87
18	46.95	12.73	48.24	31.25	20.51	1.62	26.90	15.16

Table 3
Antimicrobial activity of derivatives 1–19 on bacterial strains.

Comp	C. albicans		IC ₅₀ (µM)																					
			C. krusei		C. parapsilosis		C. tropicalis		E. faecalis CCM 4224		S. aureus CCM 3953		S. aureus (MRSA) 4591		S. haemolyticus 16568		E. coli 16702		E. coli CCM 3954		P. aeruginosa 16575		P. aeruginosa CCM 3955	
	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC
1	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
2	100	50	50	50	200	100	100	100	25	25	50	50	50	50	200	200	>200	>200	>200	>200	>200	>200	>200	>200
3	50	50	50	50	50	50	100	100	25	25	25	25	50	50	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
4	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	25	25	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
5	>200	>200	>200	>200	>200	>200	>200	>200	50	25	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
6	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
7	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
8	200	200	>200	>200	>200	200	200	200	100	100	200	200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
9	>200	>200	>200	>200	>200	>200	>200	>200	50	50	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
10	200	100	100	100	200	200	200	200	50	50	100	100	100	100	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
11	>200	>200	>200	>200	>200	>200	>200	>200	50	50	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
12	>200	>200	>200	>200	>200	>200	>200	>200	25	25	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
13	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
14	200	200	200	200	200	200	200	200	100	100	200	200	200	200	>200	200	>200	>200	>200	>200	>200	>200	>200	>200
15	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
16	50	50	50	50	100	100	100	100	25	25	50	50	200	200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
17	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
18	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
19	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200

The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of an antimicrobial ingredient or agent that is bacteriostatic; the Minimum Bactericidal Concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a bacterium. The MBC is complementary to the MIC; whereas the MIC test demonstrates the lowest level of antimicrobial agent that significantly inhibits growth, the MBC demonstrates the lowest level of antimicrobial agent resulting in microbial death.

We studied the effect of selected glycosides (**4**, **11**, **12**, **18** and **19**) on the lipid bilayer formed by zwitterionic phosphatidylcholine (POPC), intending to better understand the mechanism of their biological activity.

Hydrated POPC forms a liquid-crystalline lamellar phase (L_α) in our studied temperature range (25–60 °C) [14]. Small angle X-ray scattering (SAXS) experiments were performed to follow structural changes of bilayers induced by glycoside (GL) at 2 M ratios (GL:POPC = 0.2 and 0.5 mol/mol). Fig. 2 depicts typical SAXS patterns. Fully hydrated POPC shows two peaks (L1 and L2) characterizing a lamellar phase (Fig. 2, A bottom) with repeat distance $d = 65.30 \pm 0.01 \text{ \AA}$ (at 25 °C), a value that agrees with the value $d = 64.3 \text{ \AA}$ reported for the same POPC in 200 mmol/l of NaCl [15]. All studied mixtures show lamellar phases. Table S1 (see Supplementary material) summarizes the repeat distances (d) of GL/POPC mixtures.

Glycosides inserted into the POPC bilayer decrease d : $\Delta d = d_{\text{POPC}} - d_{\text{GL/POPC}} \sim 0.4\text{--}3.3 \text{ \AA}$ (Fig. 2, C). Fig. 2A shows SAXS patterns of **11**/POPC, where the change of d is the highest one: $\Delta d = d_{\text{POPC}} - d_{\text{11/POPC}} = 65.3 - 62.0 = 3.3 \text{ \AA}$ at **11**/POPC = 0.2 mol/mol. However, the highest content of **11** in POPC bilayer (**11**/POPC = 0.5 mol/mol) induces a smaller change of d , $\Delta d = 2.5 \text{ \AA}$. Generally, d values of zwitterionic phosphatidylcholines in the L_α state do not exceed $\sim 65\text{--}67 \text{ \AA}$ [16]. d is the sum $d = d_L + d_w$, where d_L is the thickness of the lipid bilayer and d_w is the thickness of the water layer between two neighboring lipid bilayers. For zwitterionic phosphatidylcholines, the thickness $d_w \sim 1.8\text{--}2 \text{ nm}$ is the result of the balance between repulsive interbilayer interactions (steric, hydration and fluctuations) and attractive van der Waals forces [16]. Thus, changes of both thicknesses, the lipid bilayer (d_L) as well as the water layer (d_w), can contribute to the detected Δd . Detailed inspection of data in Table S1 reveals that Δd induced by the glycoside alkyl aglycone ($n = \text{number of carbon atoms in aglycone alkyl chain, } n = 14 \text{ or } 16$) is larger for $n = 14$. Actually, in-

duced changes can be quantified in the order glycosides > galacto \approx manno (as it is summarised in Fig. 2, C). POPC molecule is formed by two acyl chains, one saturated palmitic acid ($n = 16$), the other monounsaturated oleic acid ($n = 18$, 1 double bond). The length of the glycoside alkyl aglycone with $n = 16$ fits well inside the hydrophobic POPC region, creating smaller disturbance than the glycoside with $n = 14$. The studied glycosides are nonionic compounds and their molecules differ in the stereochemical arrangement of hydroxyl groups attached to a tetrahydropyran ring. In the bilayer, the hydrophilic part of the glycoside, localized in the polar region of POPC, causes a lateral expansion of the bilayer that, in synergy with the hydrophobic mismatch between POPC and tetradecyl aglycone, explains the decrease of the lipid bilayer thickness (d_L). Thus, we attribute Δd by changes of the lipid bilayer thickness (d_L). Our results indicate small differences in localization of glycoside in the POPC bilayer due to the stereochemical arrangement of hydroxyl groups. The asymmetry of the peak (second order reflection, L2) in the pattern of **18**/POPC = 0.5 mol/mol (Fig. 2, A, marked by an arrow) indicates a phase separation in the mixture. Another lamellar phase was detected with repeat distance $d = 64.0 \pm 0.22 \text{ \AA}$. Similar structural changes were observed in mixtures with the highest amount of **4** in the bilayer (0.5 mol/mol) (Fig. 2 B). Higher temperature promotes a phase separation as it is clearly seen for **4**/POPC = 0.5 mol/mol at 60 °C. The pattern was fitted assuming the superposition of two coexisting lamellar phases. Dashed lines show individual peaks (L1_a, L1_b, etc.). Two phases (L_a and L_b) with periodicities $d_a = 65.3 \pm 0.13 \text{ \AA}$ and $d_b = 61.5 \pm 0.23 \text{ \AA}$ indicate absence of ideal mixing of the glycoside with the lipid. It was identified that L_a is a phase rich of POPC, likely with a low content of **4** ($d_a \sim d_{\text{POPC}}$), while a phase L_b , has a higher amount of **4** ($d_b < d_{\text{POPC}}$). d of fully hydrated POPC bilayers increases $\sim 2 \text{ \AA}$ in the temperature range 25–60 °C [14]. Glycosidic surfactants with alkyl aglycone (C14–C16) and their interactions with lipid mem-

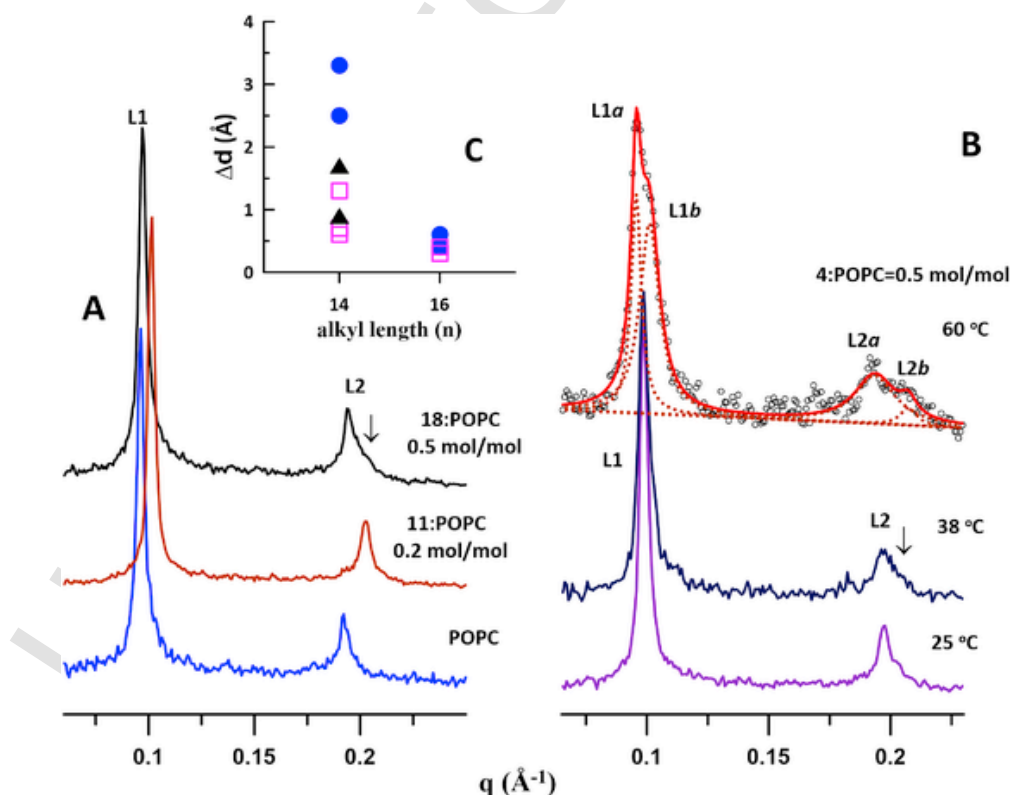


Fig. 2. SAXS patterns of POPC and GL/phospholipid bilayer mixtures: A) POPC and selected glycosides (**18**, **11**) + POPC at 25 °C. The asymmetry of L2 peak is marked by an arrow; B) **4**:POPC = 0.5 mol/mol at different temperatures. Dashed lines represent the fit of individual peaks of two lamellar phases (L_a , L_b). Full line represents the fitted curve of two phases. C) $\Delta d = d_{\text{POPC}} - d_{\text{GL/POPC}}$ as a function of the length n of alkyl aglycone of GL: gluco- (full circles); galacto- (empty squares) and manno- (full triangles).

branes are rarely discussed. In accord with our findings, Carion-Taravella et al. [17] reported the reduction of the lipid bilayer thickness of saturated dipalmitoylphosphatidylcholine (DPPC) due to its interaction with dodecyl- β -D-glucopyranoside, and coexistence of lamellar phases.

In summary, we did not observe changes of the long-range organization of POPC bilayer in presence of glycosides, meaning that the lamellar phase was preserved in all studied mixtures. Instead, SAXS experiments revealed that glycosides affect the structure of the POPC membrane, changing the lipid bilayer thickness. High glycoside content induces lateral phase separation resulting in two phases (L_a , L_b) with slightly different periodicities, although without macroscopic phase separation. The small difference between the two repeat distances supports the model of coexistence of two phases within one supramolecular structure, which can be due to non-ideal mixing of compounds. Lipid components of biological membranes are not uniformly distributed in the membrane, they create domains. Experiments have shown that glycosides can alter this distribution by their clustering with neutral phospholipids, main component of the membrane. This can be toxic for the cell either by preventing the interaction of lipids with other membrane components, or by disrupting existing natural domains. Finally, we found that even minor amounts of glycosides affect the lipid bilayer thickness which plays a crucial role in the activity of transmembrane proteins [18].

3. Conclusions

It is known that lipophilicity of the compounds plays an important role in their penetration into cells and in cell permeability. The length of aglycone is a factor determining lipophilicity of the investigated non-ionic alkyl glycosides. The most lipophilic ones show the highest cytotoxicity, but this dependence was not clear for all tested cancer cell lines. In addition, the most lipophilic mannosides also do affect negatively non-tumor lines, what is a serious limitation for their use as anticancer drugs. The most lipophilic glycosides are not the strongest antibacterials, but, in some cases, their selectivity against different bacterial strains depends on the length of the aglycone chain.

Among the 19 tested sugar based amphiphiles, we identified those specific for eukaryotes.

Tetradecyl β -D-galactopyranoside (**18**) shows the most promising cytotoxicity and the highest TI (9.1) against CCRF-CEM cancer cell line. At higher concentrations ($5 \times IC_{50}$), we observe the accumulation of the cells in G0/G1 cell phase and inhibition of DNA/RNA synthesis, which can be a non-specific phenotype of the pre-/apoptotic cells. Along with hexadecyl β -D-galactopyranoside (**19**) having TI (4.2, CCRF-CEM), these two glycolipids have no antimicrobial activity. Therefore, they could be a target specific for eukaryotic cells.

Regarding prokaryotes, tetradecyl and hexadecyl β -D-glucopyranosides (**11**) and (**12**) are selective inhibitors of *E. faecalis*. The *Candida* sp. strains were sensitive only to glycosides having (thio)dodecyl aglycone, which are the broadest spectrum antimicrobials inhibiting enterococci as well as staphylococci.

The structural study reveals small but not negligible changes in the lipid bilayer from zwitterionic POPC induced by glycosides selected for these pilot experiments. However, the detected "mild effect" can generate lateral disorder in membrane components resulting in malfunction of the cell. On the other hand, long-range order in lamellar phase is preserved up to rather high content of the additive in the lipid bilayer. Therefore, the antimicrobial activity is not due to membrane fragmentation and formation of non-lamellar phases, as often observed with antimicrobial peptides [19]. On another hand, one can take advantage of this "mild effect" when designing a lipid-based carrier for glycosides at targeted drug delivery.

The results presented in this study reveal an interesting potential of nonionic glycosides. This is in line with recent demands for bioactive compounds, which are strongly preferred because based on natural ori-

gin, contrary to synthetic compounds. The tested glycosides fulfill these trends for bioprotection, since the combination of saccharides with lipids, two biodegradable components, fully meet the requirements for nature-like composition. Therefore, the active glycolipid mimetic should become an environmentally friendly alternative to common products.

4. Experimental

4.1. General

TLC was performed on aluminum sheets precoated with silica gel 60 F₂₅₄ (Merck). Flash column chromatography was carried out on silica gel 60 (0.040-0.060 mm, Merck) with distilled solvents (hexanes, ethyl acetate, methanol). Dry dichloromethane and dry 1,2-dichloroethane were purchased from Aldrich. POPC (1-palmitoyl-2-oleoyl-glycero-3-phosphocholine) was purchased from Avanti Polar Lipids, Inc., USA. All reactions containing sensitive reagents were carried out under an argon atmosphere. ¹H NMR and ¹³C NMR spectra were recorded at 25 °C with Bruker AVANCE III HD 400 spectrometer. Chemical shifts are referenced to either TMS (δ 0.00, CDCl₃ for ¹H) or HOD (δ 4.87, CD₃OD for ¹H), and to internal CDCl₃ (δ 77.00) or CD₃OD (δ 49.00) for ¹³C. Optical rotations were measured on a Jasco P2000 polarimeter at 20 °C. High-resolution mass determination was performed by ESI-MS on a Thermo Scientific Orbitrap Exactive instrument operating in positive mode.

All the tested compounds used in biological tests were lyophilized before the use. Peracetylated donors were synthesized according to published procedure [20].

4.2. General procedure for glycosylation

A stirred solution containing peracetylated donor (1 g, 2.56 mmol, 1 eq) in dry solvent (10 mL) was cooled down on an ice bath and BF₃·OEt₂ was added. After being stirred for 10 min, the corresponding alcohol (1.5 eq) was added dropwise. Then the resulting mixture was stirred at rt. The reaction mixture was then diluted with solvent (50 mL) and poured into ice cold satd NaHCO₃ (100 mL) under stirring. The organic phase was separated, washed with water (100 mL), dried with anhydrous Na₂SO₄, filtered and concentrated. The crude product was purified by flash chromatography (Hexane:EtOAc 7:1 → 3:1).

The conditions (catalyst, solvent, reaction time) used in the synthesis of acetylated glycosides: β -C10 glycosides **6a** and **13a**: BF₃·OEt₂ (5eq), DCM, 5 h; α/β C12 glycosides **7a**, **8a**, **14a**, **15a**: BF₃·OEt₂ (5eq), DCM, 24 h; α/β SC12 glycosides **9a**, **10a**, **16a**, **17a**: BF₃·OEt₂ (1.5 eq), DCE, 5 h; β -C14 glycosides **11a** and **18a**: BF₃·OEt₂ (3eq), DCE, 22 h; β -C16 glycosides **12a** and **19a**: BF₃·OEt₂ (3eq), DCE, 22 h.

4.3. General procedure for deprotection

To a solution of peracetylated glycoside (1eq) in MeOH (9.5 mL) MeONa (1 M, 0.50 mL) was added. The reaction mixture was stirred for 16 h, neutralized with Dowex 50 H⁺-form and filtered. After removal of the solvent, the residue was purified by column chromatography (hexane:EtOAc 1:9 → EtOAc → EtOAc:MeOH 10:1).

4.3.1. Tetradecyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranoside (**4a**)

Synthesis was carried out as reported previously [6]. Yield 0.82 g, 59%, clear oil. [α]_D + 23.3 (c 0.5, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 5.37 (dd, 1H, $J_{2,3} = 3.4$ Hz, H-3), 5.29 (dd, 1H, $J_{3,4} = 9.9$ Hz, $J_{4,5} = 9.8$ Hz, H-4), 5.23 (dd, 1H, H-2), 4.81 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1), 4.29 (dd, 1H, $J_{5,6b} = 5.3$ Hz, $J_{6a,6b} = 12.1$ Hz, H-6b), 4.11 (dd, 1H, $J_{5,6a} = 2.4$ Hz, H-6a), 3.99 (ddd, 1H, H-5), 3.68 (dt, 1H, $J = 6.7$ Hz, $J = 9.6$ Hz, OCH₂(CH₂)₁₂CH₃), 3.45 (dt, 1H, $J = 6.5$ Hz, $J = 9.5$ Hz, OCH₂(CH₂)₁₂CH₃), 2.15, 2.10, 2.04, 1.99 (each s, each 3H,

4 × CH₃CO), 1.64–1.24 (m, 24H, OCH₂(CH₂)₁₂CH₃), 0.88 (t, 3H, *J* = 6.5 Hz, O(CH₂)₁₃CH₃). ¹³C NMR (100 MHz, CDCl₃): δ 170.6, 170.1, 170.0, 169.8 (4 × CH₃CO), 97.6 (C-1), 69.9 (C-2), 69.2 (C-3), 68.6, 68.5 (C-5, OCH₂(CH₂)₁₂CH₃), 66.4 (C-4), 62.6 (C-6), 32.0, 29.8(3 ×), 29.7(2 ×), 29.6(2 ×), 29.4, 29.3, 26.1, 22.7 (OCH₂(CH₂)₁₂CH₃), 20.9, 20.7(3 ×) (4 × CH₃CO), 14.2 (O(CH₂)₁₃CH₃). HRMS (ESI) *m/z*: calcd for C₂₈H₄₈O₁₀Na [M + Na]⁺: 567.3140; found: 567.3135.

4.3.2. Decyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (6a)

Yield 0.39 g, 31%, clear oil. [α]_D - 16.5 (c 1.1, CHCl₃). Analytical data, [α]_D - 15.0 (c 1.19, CHCl₃) [21], ¹H NMR [22] and ¹³C NMR [21] data were reported previously. HRMS (ESI) *m/z*: calcd for C₂₄H₄₀O₁₀Na [M + Na]⁺: 511.2514; found: 511.2518.

4.3.3. Dodecyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (7a)

Yield 0.45 g, 34%, clear oil. [α]_D + 44.5 (c 0.2, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 5.48 (t, 1H, *J*_{3,4} = 9.8 Hz, H-3), 5.08–5.03 (m, 2H, H-1, H-4), 4.85 (dd, 1H, *J*_{1,2} = 3.7 Hz, *J*_{2,3} = 10.2 Hz, H-2), 4.26 (dd, 1H, *J*_{5,6a} = 4.5 Hz, *J*_{6a,6b} = 12.4 Hz, H-6b), 4.09 (dd, 1H, *J*_{5,6a} = 2.3 Hz, H-6a), 4.02 (m, 1H, H-5), 3.67 (dt, 1H, *J* = 6.6 Hz, *J* = 9.9 Hz, OCH₂(CH₂)₁₀CH₃), 3.43 (dt, 1H, *J* = 6.5 Hz, *J* = 9.9 Hz, OCH₂(CH₂)₁₀CH₃), 2.09, 2.06, 2.03, 2.01 (each s, each 3H, 4 × CH₃CO), 1.59–1.26 (m, 20H, OCH₂(CH₂)₁₀CH₃), 0.88 (t, 3H, *J* = 6.5 Hz, O(CH₂)₁₁CH₃). ¹³C NMR (100 MHz, CDCl₃): δ 170.7, 170.2(2 ×), 169.7 (4 × CH₃CO), 95.6 (C-1), 71.0 (C-2), 70.3 (C-3), 68.8, 68.7 (C-4, OCH₂(CH₂)₁₀CH₃), 67.1 (C-5), 62.0 (C-6), 31.9, 29.7(2 ×), 29.6(3 ×), 29.4, 29.3, 26.1, 22.7 (OCH₂(CH₂)₁₀CH₃), 20.8(2 ×), 20.7(2 ×) (4 × CH₃CO), 14.1 (O(CH₂)₁₁CH₃). HRMS (ESI) *m/z*: calcd for C₂₆H₄₄O₁₀Na [M + Na]⁺: 539.2827; found: 539.2829.

4.3.4. Dodecyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (8a)

Yield 0.42 g, 32%, clear oil. [α]_D - 16.0 (c 0.5, CHCl₃). ¹H NMR data were reported [23]. ¹³C NMR (100 MHz, CDCl₃): δ 170.7, 170.4, 169.5, 169.3 (4 × CH₃CO), 100.9 (C-1), 73.0 (C-3), 71.8 (C-5), 71.5 (C-2), 70.4 (OCH₂(CH₂)₁₀CH₃), 68.6 (C-4), 62.1 (C-6), 32.0, 29.8, 29.7(3 ×), 29.5, 29.4(2 ×), 25.9, 22.8 (OCH₂(CH₂)₁₀CH₃), 20.8, 20.7(3 ×) (4 × CH₃CO), 14.2 (O(CH₂)₁₁CH₃). HRMS (ESI) *m/z*: calcd for C₂₆H₄₄O₁₀Na [M + Na]⁺: 539.2827; found: 539.2823.

4.3.5. Dodecyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-glucopyranoside (9a)

Yield 0.16 g, 12%, yellowish oil. [α]_D + 120.0 (c 0.25, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 5.61 (d, 1H, *J*_{1,2} = 5.8 Hz, H-1), 5.32 (dd, 1H, *J*_{4,5} = 9.7 Hz, H-4), 5.01 (dd, 1H, *J*_{3,4} = 9.7 Hz, H-3), 4.98 (dd, 1H, *J*_{2,3} = 10.2 Hz, H-2), 4.39 (ddd, 1H, *J*_{5,6a} = 2.3 Hz, *J*_{5,6b} = 4.7 Hz, H-5), 4.26 (dd, 1H, *J*_{6a,6b} = 12.3 Hz, H-6b), 4.02 (dd, 1H, H-6a), 2.55–2.41 (m, 2H, SCH₂(CH₂)₁₀CH₃), 2.04, 2.02, 2.00, 1.97 (each s, each 3H, 4 × CH₃CO), 1.58–1.19 (m, 20H, SCH₂(CH₂)₁₀CH₃), 0.83 (t, 3H, *J* = 6.6 Hz, S(CH₂)₁₁CH₃). ¹³C NMR (100 MHz, CDCl₃): δ 170.7, 170.4, 170.3, 169.9 (4 × CH₃CO), 81.8 (C-1), 70.6 (C-2), 70.2 (C-4), 68.5 (C-3), 67.3 (C-5), 62.6 (C-6), 30.3 (SCH₂(CH₂)₁₀CH₃), 32.0, 30.0, 29.7(2 ×), 29.6(2 ×), 29.5, 29.4, 28.9, 22.7 (SCH₂(CH₂)₁₀CH₃), 20.6(2 ×), 20.5(2 ×) (4 × CH₃CO), 14.2 (S(CH₂)₁₁CH₃). HRMS (ESI) *m/z*: calcd for C₂₆H₄₄O₉SNa [M + Na]⁺: 555.2598; found: 555.2596.

4.3.6. Dodecyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (10a)

Yield 0.70 g, 38%, yellowish oil. [α]_D - 28.0 (c 0.15, CHCl₃). Analytical data, [α]_D²⁰ - 29.2 (c 0.12, CHCl₃) [24], ¹H and ¹³C NMR [25] data were reported. HRMS (ESI) *m/z*: calcd for C₂₆H₄₄O₉SNa [M + Na]⁺: 555.2598; found: 555.2593.

4.3.7. Tetradecyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (11a)

Yield 0.49 g, 35%, white solid. [α]_D - 29.4 (c 0.5, CHCl₃). Analytical data, [α]_D²⁰ - 63.8 (c 0.2, CHCl₃) [26], ¹H and ¹³C NMR data were re-

ported [27]. HRMS (ESI) *m/z*: calcd for C₂₈H₄₈O₁₀Na [M + Na]⁺: 567.3140; found: 567.3143.

4.3.8. Hexadecyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (12a)

Yield 0.56 g, 38%, white solid. [α]_D - 18.0 (c 0.5, CHCl₃). ¹H and ¹³C NMR data were reported [27]. HRMS (ESI) *m/z*: calcd for C₃₀H₅₂O₁₀Na [M + Na]⁺: 595.3453; found: 595.3445.

4.3.9. Decyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (13a)

Yield 0.40 g, 32%, clear oil. [α]_D - 15.6 (c 1.1, CHCl₃). Analytical data, [α]_D²⁰ - 11.6 (c 1.5, CHCl₃) [22], ¹H NMR data were reported [8]. ¹³C NMR (100 MHz, CDCl₃): δ 170.5, 170.4, 170.3, 169.5 (4 × CH₃CO), 101.5 (C-1), 71.1 (C-3), 70.7 (C-5), 70.5 (OCH₂(CH₂)₈CH₃), 69.1 (C-2), 67.2 (C-4), 61.4 (C-6), 32.0, 29.7(2 ×), 29.6, 29.5(2 ×), 26.0, 22.8 (OCH₂(CH₂)₈CH₃), 20.9, 20.8(2 ×), 20.7 (4 × CH₃CO), 14.3 (O(CH₂)₉CH₃). HRMS (ESI) *m/z*: calcd for C₂₄H₄₀O₁₀Na [M + Na]⁺: 511.2514; found: 511.2513.

4.3.10. Dodecyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside (14a)

Yield 0.29 g, 22%, white semisolid. [α]_D + 10.0 (c 0.5, CHCl₃). ¹H NMR data were reported [8]. ¹³C NMR (100 MHz, CDCl₃): δ 170.4, 170.3(2 ×), 170.0 (4 × CH₃CO), 96.2 (C-1), 68.7 (OCH₂(CH₂)₁₀CH₃), 68.3, 68.2 (C-3, C-4), 67.7 (C-2), 66.2 (C-5), 61.9 (C-6), 32.0, 29.7(3 ×), 29.6(2 ×), 29.4(2 ×), 26.2, 22.7 (OCH₂(CH₂)₁₀CH₃), 20.8, 20.7(3 ×) (4 × CH₃CO), 14.2 (O(CH₂)₁₁CH₃). HRMS (ESI) *m/z*: calcd for C₂₆H₄₄O₁₀Na [M + Na]⁺: 539.2827; found: 539.2833.

4.3.11. Dodecyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (15a)

Yield 0.40 g, 30%, white solid. [α]_D - 10.0 (c 0.5, CHCl₃). Analytical data, [α]_D²⁵ - 14.0 (c 0.8, CHCl₃) [28] and ¹H NMR data were reported [23]. ¹³C NMR (100 MHz, CDCl₃): δ 170.5, 170.4, 170.2, 169.4 (4 × CH₃CO), 101.4 (C-1), 71.1 (C-3), 70.76 (C-5), 70.4 (OCH₂(CH₂)₁₀CH₃), 69.1 (C-2), 67.2 (C-4), 61.4 (C-6), 32.0, 29.7(3 ×), 29.5(2 ×), 25.9, 22.8 (OCH₂(CH₂)₁₀CH₃), 20.8, 20.7(3 ×) (4 × CH₃CO), 14.3 (O(CH₂)₁₁CH₃). HRMS (ESI) *m/z*: calcd for C₂₆H₄₄O₁₀Na [M + Na]⁺: 539.2827; found: 539.2831.

4.3.12. Dodecyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-galactopyranoside (16a)

Yield 0.17 g, 12%, yellowish oil. [α]_D + 144.0 (c 0.5, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 5.71 (d, 1H, *J*_{1,2} = 5.2 Hz, H-1), 5.45 (dd, 1H, *J*_{4,5} = 1.3 Hz, H-4), 5.26 (dd, 1H, *J*_{2,3} = 10.8 Hz, H-2), 5.22 (dd, 1H, *J*_{3,4} = 10.8 Hz, H-3), 4.60–4.57 (m, 1H, H-5), 4.14–4.06 (m, 2H, H-6a, H-6b), 2.60–2.45 (m, 2H, SCH₂(CH₂)₁₀CH₃), 2.14, 2.07, 2.04, 1.99 (each s, each 3H, 4 × CH₃CO), 1.53–1.25 (m, 20H, SCH₂(CH₂)₁₀CH₃), 0.87 (t, 3H, *J* = 6.5 Hz, S(CH₂)₁₁CH₃). ¹³C NMR (100 MHz, CDCl₃): δ 170.5, 170.3(2 ×), 170.0 (4 × CH₃CO), 82.3 (C-1), 68.3, 68.2(2 ×) (C-2, C-3, C-4), 66.6 (C-5), 62.0 (C-6), 32.1 (SCH₂(CH₂)₁₀CH₃), 30.0, 29.8(2 ×), 29.7(2 ×), 29.5, 29.3, 29.1, 26.1, 22.8 (SCH₂(CH₂)₁₀CH₃), 21.0, 20.8(2 ×), 20.7 (4 × CH₃CO), 14.3 (S(CH₂)₁₁CH₃). HRMS (ESI) *m/z*: calcd for C₂₆H₄₄O₉SNa [M + Na]⁺: 555.2598; found: 555.2601.

4.3.13. Dodecyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranoside (17a)

Yield 0.50 g, 37%, white solid. [α]_D - 8.0 (c 0.5, CHCl₃). Analytical data, [α]_D²⁰ - 16.0 (c 0.3, CHCl₃) [24], ¹H and ¹³C NMR data were reported [29]. HRMS (ESI) *m/z*: calcd for C₂₆H₄₄O₉SNa [M + Na]⁺: 555.2598; found: 555.2600.

4.3.14. Tetradecyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (18a)

Yield 0.39 g, 28%, white solid. [α]_D - 35.7 (c 0.5, CHCl₃). ¹H NMR data were reported [23]. ¹³C NMR (100 MHz, CDCl₃): δ 170.5, 170.4, 170.3, 169.5 (4 × CH₃CO), 101.5 (C-1), 71.1 (C-3), 70.7 (C-5), 70.5 (OCH₂(CH₂)₁₂CH₃), 69.1 (C-2), 67.2 (C-4), 61.4 (C-6), 32.1, 29.8(4 ×), 29.6(2 ×), 29.5(3 ×), 26.0, 22.8 (OCH₂(CH₂)₁₂CH₃), 20.9, 20.8(2 ×),

20.7 (4 × CH₃CO), 14.3 (O(CH₂)₁₃CH₃). HRMS (ESI) *m/z*: calcd for C₂₈H₄₈O₁₀Na [M + Na]⁺: 567.3140; found: 567.3138.

4.3.15. Hexadecyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (19a)

Yield 0.29 g, 20%, white solid. [α]_D - 22.8 (c 0.5, CHCl₃). ¹H NMR data were reported [8]. ¹³C NMR (100 MHz, CDCl₃): δ 170.5, 170.4, 170.3, 169.5 (4 × CH₃CO), 101.5 (C-1), 71.1 (C-3), 70.7 (C-5), 70.5 (OCH₂(CH₂)₁₄CH₃), 69.1 (C-2), 67.2 (C-4), 61.4 (C-6), 32.1, 29.9, 29.8(4 ×), 29.7 (2 ×), 29.6(2 ×), 29.5(2 ×), 26.0, 22.8 (OCH₂(CH₂)₁₄CH₃), 20.9, 20.8(2 ×), 20.7 (4 × CH₃CO), 14.3 (O(CH₂)₁₅CH₃). HRMS (ESI) *m/z*: calcd for C₃₀H₅₂O₁₀Na [M + Na]⁺: 595.3453; found: 595.3456.

Synthesis and analytical data for compounds 1 [13], 2 [6], 3 [13] and 5 [6] were reported.

4.3.16. Tetradecyl α-D-mannopyranoside (4)

Yield 0.42 g, 77%, white solid. [α]_D + 35.6 (c 0.5, MeOH), ref [30] [α]_D + 50.0 (c 1.0, MeOH). ¹H NMR (400 MHz, CD₃OD): δ 4.76 (d, 1H, J_{1,2} = 1.7 Hz, H-1), 3.86 (dd, 1H, J_{5,6a} = 2.4 Hz, J_{6a,6b} = 11.9 Hz, H-6a), 3.80 (dd, 1H, J_{2,3} = 3.3 Hz, H-2), 3.77–3.68 (m, 3H, H-3, H-6b, OCH₂(CH₂)₁₂CH₃), 3.63 (t, J_{3,4} = 9.5 Hz, J_{4,5} = 9.5 Hz, H-4), 3.56 (m, 1H, H-5), 3.44 (dt, 1H, J = 6.3 Hz, J = 9.5 Hz, OCH₂(CH₂)₁₂CH₃), 1.65–1.29 (m, 24H, OCH₂(CH₂)₁₂CH₃), 0.93 (t, 3H, J = 6.6 Hz, O(CH₂)₁₃CH₃). ¹³C NMR (100 MHz, CD₃OD): δ 101.5 (C-1), 74.6 (C-5), 72.7 (C-3), 72.3 (C-2), 68.6(2 ×) (C-4, OCH₂(CH₂)₁₂CH₃), 62.9 (C-6), 33.1, 30.8(3 ×), 30.7(3 ×), 30.6(2 ×), 30.5, 27.4, 23.7 (OCH₂(CH₂)₁₂CH₃), 14.4 (O(CH₂)₁₃CH₃). HRMS (ESI) *m/z*: calcd for C₂₀H₄₀O₆Na [M + Na]⁺: 399.2917; found: 399.2920.

4.3.17. Decyl β-D-glucopyranoside (6)

Yield 0.20 g, 79%, white powder. [α]_D - 35.0 (c 1.1, MeOH). Analytical data, [α]_D²⁰ - 25.4 (c 1.15, MeOH) [31], ¹H and ¹³C NMR data were reported [32]. HRMS (ESI) *m/z*: calcd for C₁₆H₃₂O₆Na [M + Na]⁺: 343.2091; found: 343.2090.

4.3.18. Dodecyl α-D-glucopyranoside (7)

Yield 0.27 g, 90%, white powder. [α]_D + 54.5 (c 0.9, MeOH), ref [31] [α]_D²⁰ + 101 (c 1.72, MeOH). ¹H NMR (400 MHz, CD₃OD): δ 4.79 (d, 1H, J_{1,2} = 3.7 Hz, H-1), 3.81 (dd, 1H, J_{5,6a} = 2.4 Hz, J_{6a,6b} = 11.9 Hz, H-6a), 3.75 (dt, 1H, J = 6.9 Hz, J = 9.5 Hz, OCH₂(CH₂)₁₀CH₃), 3.69 (dd, 1H, J_{5,6b} = 5.6 Hz, H-6b), 3.66 (t, 1H, J_{3,4} = 9.4 Hz, H-3), 3.59 (ddd, 1H, J_{4,5} = 9.9 Hz, H-5), 3.46 (dt, 1H, J = 6.5 Hz, J = 9.6 Hz, OCH₂(CH₂)₁₀CH₃), 3.40 (dd, 1H, J_{2,3} = 9.7 Hz, H-2), 3.30 (dd, 1H, H-4), 1.69–1.29 (m, 20H, OCH₂(CH₂)₁₀CH₃), 0.91 (t, 3H, J = 6.8 Hz, O(CH₂)₁₁CH₃). ¹³C NMR (100 MHz, CD₃OD): δ 100.1 (C-1), 75.1 (C-3), 73.6 (2 ×) (C-2, C-5), 71.8 (C-4), 69.1 (OCH₂(CH₂)₁₀CH₃), 62.7 (C-6), 33.1, 30.8(2 ×), 30.7(3 ×), 30.6, 30.5, 27.3, 23.7 (OCH₂(CH₂)₁₀CH₃), 14.4 (O(CH₂)₁₁CH₃). HRMS (ESI) *m/z*: calcd for C₁₈H₃₆O₆Na [M + Na]⁺: 371.2404; found: 371.2409.

4.3.19. Dodecyl β-D-glucopyranoside (8)

Yield 0.24 g, 87%, white powder. [α]_D - 26.3 (c 0.9, MeOH). Analytical data, [α]_D²⁰ - 24.1 (c 1.06, MeOH) [31], ¹H and ¹³C NMR data were reported [32]. HRMS (ESI) *m/z*: calcd for C₁₈H₃₆O₆Na [M + Na]⁺: 371.2404; found: 371.2405.

4.3.20. Dodecyl 1-thio-α-D-glucopyranoside (9)

Yield 0.09 g, 81%, yellowish powder. [α]_D²⁰ + 171.8 (c 0.9, MeOH), ref [33] [α]_D²⁰ + 179 (c 1, MeOH). ¹H NMR (400 MHz, CD₃OD): δ 5.33 (d, 1H, J_{1,2} = 5.4 Hz, H-1), 3.97 (ddd, 1H, J_{4,5} = 9.9 Hz, J_{5,6b} = 5.3 Hz, H-5), 3.83 (dd, 1H, J_{5,6a} = 2.4 Hz, J_{6a,6b} = 12.0 Hz, H-6a), 3.75–3.68 (m, 2H, H-2, H-6b), 3.55 (t, 1H,

J_{2,3} = 9.3 Hz, J_{3,4} = 9.3 Hz, H-3), 3.33 (dd, 1H, H-4), 2.68–2.55 (m, 2H, SCH₂(CH₂)₁₀CH₃), 1.68–1.32 (m, 20H, SCH₂(CH₂)₁₀CH₃), 0.92 (t, 3H, J = 6.8 Hz, S(CH₂)₁₁CH₃). ¹³C NMR (100 MHz, CD₃OD): δ 87.2 (C-1), 75.7 (C-3), 73.9 (C-5), 73.2 (C-2), 71.8 (C-4), 62.6 (C-6), 33.1 (SCH₂(CH₂)₁₀CH₃), 31.1, 30.8(3 ×), 30.7(2 ×), 30.5, 30.4, 30.0, 23.7 (SCH₂(CH₂)₁₀CH₃), 14.4 (S(CH₂)₁₁CH₃). HRMS (ESI) *m/z*: calcd for C₁₈H₃₆O₅SNa [M + Na]⁺: 387.2176; found: 387.2179.

4.3.21. Dodecyl 1-thio-β-D-glucopyranoside (10)

Yield 0.38 g, 79%, yellowish powder. [α]_D²⁰ - 100.4 (c 0.8, MeOH). ¹H and ¹³C NMR data were reported [25]. HRMS (ESI) *m/z*: calcd for C₁₈H₃₆O₅SNa [M + Na]⁺: 387.2176; found: 387.2173.

4.3.22. Tetradecyl β-D-glucopyranoside (11)

Yield 0.28 g, 82%, white powder. [α]_D²⁰ - 10.1 (c 0.25, MeOH). Analytical data, [α]_D²⁵ - 10.8 (c 0.2, MeOH) [26], ¹H and ¹³C NMR data were reported [27]. HRMS (ESI) *m/z*: calcd for C₂₀H₄₀O₆Na [M + Na]⁺: 399.2717; found: 399.2710.

4.3.23. Hexadecyl β-D-glucopyranoside (12)

Yield 0.31 g, 73%, white powder. [α]_D²⁰ - 64.5 (c 0.33, MeOH). ¹H and ¹³C NMR data were reported [27]. HRMS (ESI) *m/z*: calcd for C₂₂H₄₄O₆Na [M + Na]⁺: 427.3030; found: 427.3035.

4.3.24. Decyl β-D-galactopyranoside (13)

Yield 0.18 g, 70%, white powder. [α]_D - 23.2 (c 0.45, MeOH). ¹H NMR data were reported [8]. ¹³C NMR (100 MHz, CD₃OD): δ 104.9 (C-1), 76.5 (C-5), 75.0 (C-3), 72.5 (C-2), 70.8 (OCH₂(CH₂)₈CH₃), 70.3 (C-4), 62.4 (C-6), 33.1, 30.8, 30.7(2 ×), 30.6, 30.5, 27.1, 23.7 (OCH₂(CH₂)₈CH₃), 14.4 (O(CH₂)₉CH₃). HRMS (ESI) *m/z*: calcd for C₁₆H₃₂O₆Na [M + Na]⁺: 343.2091; found: 343.2089.

4.3.25. Dodecyl α-D-galactopyranoside (14)

Yield 0.17 g, 85%, white powder. [α]_D + 104.2 (c 0.35, MeOH). ¹H NMR (400 MHz, CD₃OD): δ 4.78 (d, 1H, J_{1,2} = 3.3 Hz, H-1), 3.88 (dd, 1H, J_{3,4} = 2.8 Hz, J_{4,5} = 1.2 Hz, H-4), 3.80–3.66 (m, 6H, H-2, H-3, H-5, H-6a, H-6b, OCH₂(CH₂)₁₀CH₃), 3.41 (dt, 1H, J = 6.5 Hz, J = 9.6 Hz, OCH₂(CH₂)₁₀CH₃), 1.67–1.27 (m, 20H, OCH₂(CH₂)₁₀CH₃), 0.88 (t, 3H, J = 6.8 Hz, O(CH₂)₁₁CH₃). ¹³C NMR (100 MHz, CD₃OD): δ 100.2 (C-1), 72.2 (C-5), 71.5 (C-3), 71.0 (C-4), 70.2 (C-2), 69.2 (OCH₂(CH₂)₁₀CH₃), 62.6 (C-6), 33.0, 30.8(2 ×), 30.7(2 ×), 30.6(2 ×), 30.5, 27.3, 23.7 (OCH₂(CH₂)₁₀CH₃), 14.5 (O(CH₂)₁₁CH₃). HRMS (ESI) *m/z*: calcd for C₁₈H₃₇O₆ [M + H]⁺: 349.2585; found: 349.2584; calcd for C₁₈H₃₆O₆Na [M + Na]⁺: 371.2404; found: 371.2404.

4.3.26. Dodecyl β-D-galactopyranoside (15)

Yield 0.23 g, 87%, white powder. [α]_D - 13.2 (c 0.26, MeOH). Analytical data, [α]_D²⁵ - 15.8 (c 0.54, MeOH) [34], ¹H and ¹³C NMR data were reported [35]. HRMS (ESI) *m/z*: calcd for C₁₈H₃₆O₆Na [M + Na]⁺: 371.2404; found: 371.2407.

4.3.27. Dodecyl 1-thio-α-D-galactopyranoside (16)

Yield 98 mg, 82%, yellowish powder. [α]_D + 217.5 (c 0.23, MeOH). ¹H NMR (400 MHz, CD₃OD): δ 5.37 (d, 1H, J_{1,2} = 5.6 Hz, H-1), 4.18 (m, 1H, H-5), 4.07 (dd, 1H, J_{2,3} = 10.1 Hz, H-2), 3.90 (dd, 1H, J_{4,5} = 1.3 Hz, H-4), 3.72–3.70 (m, 2H, H-6a, H-6b), 3.61 (dd, 1H, J_{3,4} = 3.4 Hz, H-3), 2.65–2.51 (m, 2H, SCH₂(CH₂)₁₀CH₃), 1.67–1.26 (m, 20H, SCH₂(CH₂)₁₀CH₃), 0.89 (t, 3H, J = 6.7 Hz, S(CH₂)₁₁CH₃). ¹³C NMR (100 MHz, CD₃OD): δ 87.4 (C-1), 72.6 (C-5), 72.2 (C-3), 70.9 (C-4), 69.8 (C-2), 62.6 (C-6), 33.1 (SCH₂(CH₂)₁₀CH₃), 30.8(3 ×), 30.7(3 ×), 30.5, 30.4, 30.1, 23.7 (SCH₂(CH₂)₁₀CH₃), 14.4 (S(CH₂)₁₁CH₃). HRMS (ESI) *m/z*: calcd for C₁₈H₃₆O₅SNa [M + Na]⁺: 387.2176; found: 387.2179.

4.3.28. Dodecyl 1-thio- β -D-galactopyranoside (17)

Yield 0.30 g, 85%, yellowish powder. $[\alpha]_D - 18.9$ (c 0.21, MeOH). ^1H NMR (400 MHz, CD_3OD): δ 4.31 (d, 1H, $J_{1,2} = 9.5$ Hz, H-1), 3.88 (dd, 1H, $J_{4,5} = 1.2$ Hz, H-4), 3.73 (dd, 1H, $J_{5,6a} = 6.7$ Hz, $J_{6a,6a} = 11.4$ Hz, H-6a), 3.68 (dd, 1H, $J_{5,6b} = 5.4$ Hz, H-6b), 3.56–3.48 (m, 2H, H-2, H-5), 3.44 (dd, 1H, $J_{3,4} = 3.3$ Hz, H-3), 2.79–2.65 (m, 2H, $\text{SCH}_2(\text{CH}_2)_{10}\text{CH}_3$), 1.67–1.27 (m, 20H, $\text{SCH}_2(\text{CH}_2)_{10}\text{CH}_3$), 0.89 (t, 3H, $J = 6.7$ Hz, $\text{S}(\text{CH}_2)_{11}\text{CH}_3$). ^{13}C NMR (100 MHz, CD_3OD): δ 87.7 (C-1), 80.6 (C-5), 76.3 (C-3), 71.5 (C-2), 70.5 (C-4), 62.6 (C-6), 33.1 ($\text{SCH}_2(\text{CH}_2)_{10}\text{CH}_3$), 31.1, 30.9, 30.8(2 \times), 30.7(2 \times), 30.5, 30.4, 30.0, 23.7 ($\text{SCH}_2(\text{CH}_2)_{10}\text{CH}_3$), 14.4 ($\text{S}(\text{CH}_2)_{11}\text{CH}_3$). HRMS (ESI) m/z : calcd for $\text{C}_{18}\text{H}_{36}\text{O}_5\text{SNa}$ $[\text{M} + \text{Na}]^+$: 387.2176; found: 387.2176.

4.3.29. Tetradecyl β -D-galactopyranoside (18)

Yield 0.20 g, 75%, white powder. $[\alpha]_D - 10.6$ (c 0.7, MeOH). Analytical data, $[\alpha]_D^{20} - 8.1$ (c 0.8, MeOH) [23], ^1H [23] and ^{13}C NMR [36] data were reported. HRMS (ESI) m/z : calcd for $\text{C}_{20}\text{H}_{40}\text{O}_6\text{Na}$ $[\text{M} + \text{Na}]^+$: 399.2717; found: 399.2720.

4.3.30. Hexadecyl β -D-galactopyranoside (19)

Yield 0.17 g, 82%, white powder. $[\alpha]_D - 8.1$ (c 0.35, MeOH), ref [34] $[\alpha]_D^{25} - 5.9$ (c 0.51, MeOH). ^1H NMR (400 MHz, CD_3OD): δ 4.23 (d, 1H, $J_{1,2} = 7.3$ Hz, H-1), 3.92 (dt, 1H, $J = 6.8$ Hz, $J = 9.5$ Hz, $\text{OCH}_2(\text{CH}_2)_{14}\text{CH}_3$), 3.86 (dd, 1H, $J_{3,4} = 3.2$ Hz, $J_{4,5} = 1.1$ Hz, H-4), 3.76 (dd, 1H, $J_{5,6a} = 6.7$ Hz, $J_{6a,6a} = 11.4$ Hz, H-6a), 3.60–3.46 (m, 4H, H-2, H-3, H-5, $\text{OCH}_2(\text{CH}_2)_{14}\text{CH}_3$), 1.67–1.32 (m, 28H, $\text{OCH}_2(\text{CH}_2)_{14}\text{CH}_3$), 0.93 (t, 3H, $J = 6.9$ Hz, $\text{O}(\text{CH}_2)_{15}\text{CH}_3$). ^{13}C NMR (100 MHz, CD_3OD): δ 105.0 (C-1), 76.6 (C-5), 75.1 (C-3), 72.6 (C-2), 70.8 ($\text{OCH}_2(\text{CH}_2)_{14}\text{CH}_3$), 70.3 (C-4), 62.5 (C-6), 33.1, 30.9(5 \times), 30.8(3 \times), 30.6(2 \times), 30.5, 27.1, 23.7 ($\text{OCH}_2(\text{CH}_2)_{14}\text{CH}_3$), 14.4 ($\text{O}(\text{CH}_2)_{15}\text{CH}_3$). HRMS (ESI) m/z : calcd for $\text{C}_{22}\text{H}_{44}\text{O}_6\text{Na}$ $[\text{M} + \text{Na}]^+$: 427.3030; found: 427.3033.

4.4. Biology

4.4.1. In vitro MTS assays

Cell lines. All cell lines (if not indicated otherwise) were purchased from the American Tissue Culture Collection (ATCC). The CCRF-CEM line is derived from T lymphoblastic leukemia, evincing high chemosensitivity, K562 represent cells from an acute myeloid leukemia patient sample with bcr-abl translocation, U2OS line is derived from osteosarcoma, HCT116 is colorectal tumor cell line and its p53 gene knock-down counterpart (HCT116p53 $^{-/-}$, Horizon Discovery Ltd, UK) is a model of human cancers with p53 mutation frequently associated with poor prognosis, A549 line is lung adenocarcinoma. The daunorubicin resistant subline of CCRF-CEM cells (CEM-DNR bulk) and paclitaxel-resistant subline K562-TAX were selected in our laboratory by the cultivation of maternal cell lines in increasing concentrations of daunorubicin or paclitaxel, respectively. The CEM-DNR bulk cells overexpress MRP-1 and P-glycoprotein protein, while K562-TAX cells overexpress P-glycoprotein only. Both proteins belong to the family of ABC transporters and are involved in the primary and/or acquired multidrug resistance phenomenon [37,38]. MRC-5 and BJ cell lines were used as a non-tumor control and represent human fibroblasts. The cells were maintained in nunc/corning 80 cm² plastic tissue culture flasks and cultured in cell culture medium according to ATCC or Horizon recommendations (DMEM/RPMI 1640 with 5 g/L glucose, 2 mM glutamine, 100 U/mL penicillin, 100 mg/mL streptomycin, 10% fetal calf serum, and NaHCO_3).

Cytotoxic MTS assay. MTS assay was performed at the Institute of Molecular and Translational Medicine by robotic platform (High-ResBiosolutions). Cell suspensions were prepared and diluted according to the particular cell type and the expected target cell density

(25000–35000 cells/mL based on cell growth characteristics). Cells were added by automatic pipettor (30 μL) into 384 well microtiter plates. All tested compounds were dissolved in 100% DMSO and four-fold dilutions of the intended test concentration were added in 0.15 μL aliquots at time zero to the microtiter plate wells by the echo acoustic non-contact liquid handler Echo550 (Labcyte). The experiments were performed in technical duplicates and three biological replicates at least. The cells were incubated with the tested compounds for 72 h at 37 $^\circ\text{C}$, in a 5% CO_2 atmosphere at 100% humidity. At the end of the incubation period, the cells were assayed by using the MTS test. Aliquots (5 μL) of the MTS stock solution were pipetted into each well and incubated for an additional 1–4 h. After this incubation period, the optical density (OD) was measured at 490 nm with an Envision reader (PerkinElmer). Tumor cell survival (TCS) was calculated by using the following equation: $\text{TCS} = (\text{OD}_{\text{drug-exposed well}} / \text{mean OD}_{\text{control wells}}) \times 100\%$. The IC_{50} value, the drug concentration that is lethal to 50% of the tumor cells, was calculated from the appropriate dose-response curves in Dotmatics software [37,38].

Cell cycle and apoptosis analysis. CCRF-CEM cells were seeded at a density of 1×10^6 cells mL^{-1} in 6-well plates and were cultivated with compounds at concentrations corresponding to 1 \times or 5 \times IC_{50} . After 24 h, cells were washed with cold $1 \times$ PBS and fixed in 70% ethanol added dropwise and stored overnight at -20 $^\circ\text{C}$. Afterward, cells were washed in hypotonic citrate buffer, treated with RNase (50 $\mu\text{g mL}^{-1}$) and stained with propidium iodide. Flow cytometry using a 488 nm single beam laser (Becton Dickinson) was used for measurement. Cell cycle was analyzed by the software ModFitLT (Verity), and apoptosis was measured in logarithmic model expressing the percentage of the particles with propidium content lower than cells in G0/G1 phase ($< \text{G1}$) of the cell cycle. Half of the sample was used for pH3^{Ser10} antibody (Sigma) labeling and subsequent flow cytometry analysis of the cells in mitosis.

BrDU incorporation analysis. Cells were cultivated as in the previous method. Before harvesting, 10 μM 5-bromo-2-deoxyuridine (BrDU) was added to the cells for pulse labeling for 30 min. The cells were washed by PBS and fixed with ice-cold 70% ethanol and stored overnight. Before analysis, they were incubated on ice for 30 min, washed once with PBS and re-suspended in 2 M HCl for 30 min at room temperature to denature their DNA. Following neutralization with 0.1 M $\text{Na}_2\text{B}_4\text{O}_7$ (borax), the cells were washed with PBS containing 0.5% Tween-20 and 1% BSA. Staining with primary anti-BrDU antibody (Exbio) for 30 min at room temperature in the dark followed. Cells were then washed with PBS and stained with secondary anti-mouse-FITC antibody (Sigma). After another wash with PBS and incubation with propidium iodide (0.1 mg mL^{-1}) and RNase A (0.5 mg mL^{-1}) for 1 h at room temperature in the dark, cells were analyzed by flow cytometry using a 488 nm single beam laser (FACSCalibur, Becton Dickinson).

BrU incorporation analysis. Cells were cultured and treated as above. Before harvesting, pulse-labeling with 1 mM 5-bromouridine (BrU) for 30 min followed. The cells were then fixed in 1% buffered paraformaldehyde with 0.05% NP-40 at room temperature for 15 min, and then stored at 4 $^\circ\text{C}$ overnight. Before measurement, they were washed with 1% glycine in PBS, washed with PBS again and stained with primary anti-BrDU antibody cross-reacting to BrU (Exbio) for 30 min at room temperature in the dark. From this point, the experiment was performed exactly as in the previous method [39].

4.4.2. Antimicrobial assay

To assess antimicrobial potential of tested compounds, the determination of MIC (minimal inhibitory concentration) and MBC (minimal bactericidal concentration) was performed. The antimicrobial effects of compounds were tested on the reference bacterial strains: Gram-positive *Staphylococcus aureus* CCM 3953 and *Enterococcus faecalis* CCM

4224, Gram-negative *Escherichia coli* CCM 3954 and *Pseudomonas aeruginosa* CCM 3955. Additionally, multiresistant bacterial strains: methicillin-resistant *Staphylococcus aureus* 4591 (MRSA), fluoroquinolone-resistant *Staphylococcus aureus* 16568 (FQR), fluoroquinolone-resistant *Escherichia coli* 16702 (FQR) and fluoroquinolone-resistant *Pseudomonas aeruginosa* 16575 (FQR) were used. Also, four strains of yeasts were tested: *Candida albicans*, *Candida krusei*, *Candida parapsilosis* and *Candida tropicalis*. These strains are part of the culture collection of Department of Microbiology (Faculty of Medicine and Dentistry, Palacký University Olomouc). ITTEST Kryobanka B (ITTEST plus s. r. o) was used for bacterial strains storage at $-80\text{ }^{\circ}\text{C}$. For non-reference bacterial strains and yeasts an automatic system Phoenix (Becton Dickinson) and system MALDI-TOF Biotyper (Bruker Daltonics) were used for identification. The *in vitro* testing of antimicrobial activity was performed according to standard testing protocols and minimum inhibitory concentration (MIC) was determined as the concentration of the tested substance that visibly inhibits the growth of the bacterial/yeast strain (EUCAST). Bacteria and yeasts were grown for 24 h at $35\text{ }^{\circ}\text{C}$ on blood agar (bacteria) and Sabouraud agar (yeasts) (TRIOS, Czech Republic), and microbial suspensions (10^{5-6} CFU/mL) were prepared in 2 mL of buffer saline (TRIOS, Czech Republic) and used for inoculation. Tested samples were diluted exponentially in microtiter plates in Brain Heart Infusion broth (BHI, Hi-Media) and inoculated with prepared suspension followed by incubation for 24/48 h for bacteria/yeasts respectively at $35\text{ }^{\circ}\text{C}$. MICs were then determined as the lowest concentration of tested compounds that visibly inhibited microbial growth. To determine bactericidal concentration the microtiter well's content with visibly inhibited growth was inoculated on cultivation agar and incubated for the subsequent 24/48 h at $35\text{ }^{\circ}\text{C}$. Negative growth of microbial colonies then determined the minimal bactericidal concentration (MBC) of tested compounds. The MIC (minimum inhibitory concentration) of selected antibiotics in the reference bacterial strains is defined by standard methods of EUCAST (European Committee on Antimicrobial Susceptibility Testing) [40].

4.5. Structural study

Sample preparation. POPC and selected glycosides (GL) (4, 11, 12, 18, 19) were dissolved in chloroform/methanol and mixed at 2 M ratios GL:PL = 0.2 and 0.5. Lipid mixtures were dried under a stream of gaseous nitrogen and the residue of organic solvents was removed under vacuum. Dried lipid films (8 mg/sample) were hydrated with 300 μL of 150 mmol/L NaCl. Samples were thoroughly homogenized (by vortexing and freezing-thawing, in several cycles). For SAXS measurement, the lipid mixture was transferred into a capillary made of special glass with a diameter of 1.5 mm. Capillaries were sealed and prepared for X-ray scattering experiments.

Small-angle X-ray scattering (SAXS). SAXS experiments were performed at LLB CEA Saclay, France using the SAXS laboratory beamline XEUSS 2.0 (Xenocs, Sassenage, France) equipped with a Pilatus detector (1 M). The measurement wavelength was $\lambda = 0.154\text{ nm}$ ($\text{CuK}\alpha$) and sample-detector distance 120 cm covering a q -range $0.03\text{--}0.5\text{ \AA}^{-1}$. The sample in a capillary was equilibrated at selected temperature using Linkam heating stage (Linkam Scientific, Tadworth, UK). The detector was calibrated using silver behenate. The scattered intensity is plotted as a function of the scattering vector $q = 4\pi \sin \delta/\lambda$, where 2δ is the scattering angle and λ the wavelength of the incident beam. The patterns were corrected for the used solvent. Each peak was fitted with a Lorentzian curve above a linear background. The Lorentzian is defined by $I = I_n/(1 + ((q-q_n)/\Delta q_n)^2)$, where q_n ($n = 1, 2 \dots$) are positions of maxima, I_n is the intensity of the peak, and Δq_n is its half width at half maximum. The repeat distance d was determined from $d = 2\pi/k$, where k is the slope of $q(\text{\AA}^{-1}) = f(n)$ including the origin (0,0). The uncertainty in d is expressed from the standard deviation of the slope.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.carres.2019.107905>.

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