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ORIGINAL PAPER

Antimicrobial activity of mannose-derived glycosides

Andrea Bilková[1] · Ema Paulovičová[2] · Lucia Paulovičová[2] · Monika Poláková[2]

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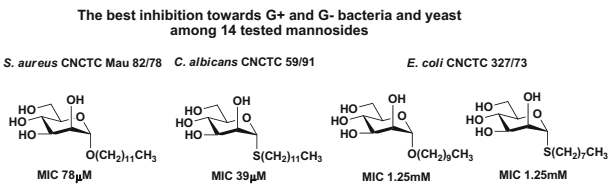
Abstract A set of 14 synthetic mannolipid-mimicking O-mannosides, S-mannosides, and mannosylsulfones varying in aglycone length were evaluated for their antimicrobial activity towards yeast *Candida albicans* as well as Gram-positive and Gram-negative bacteria, *Staphylococcus aureus* and *Escherichia coli*, respectively. *S. aureus* was the most susceptible to dodecyl α-D-mannopyranoside showing MIC value of 78 μM, while dodecyl α-D-thiomannopyranoside was superior yeast inhibitor exhibiting twofold lower MIC value. On the other hand, *E. coli* was resistant to both dodecyl glycosides. Mannosides exposure on RAW 264.7 cell line murine macrophages revealed the tight structure—immunobiological activity pattern. No significant cytotoxic effect and suppression of proliferation as a consequence of cell injury following 24 h exposure with 1–100 μg/cm^3 mannosides were observed.

Graphical abstract



✉ Monika Poláková
chemonca@savba.sk

[1] Department of Cellular and Molecular Biology of Drugs, Faculty of Pharmacy, Comenius University, Kalinčiakova 8, 832 32 Bratislava, Slovakia

[2] Institute of Chemistry, Center for Glycomics, Slovak Academy of Sciences, Dúbravska cesta 9, 845 38 Bratislava, Slovakia

Keywords Mannoside · Antimicrobial activity · *Candida albicans* · *Escherichia coli* · *Staphylococcus aureus*

Introduction

Glycosides are compounds consisting of a saccharide core linked to a simple or more complex aglycone. Such compounds occur in nature and are a matter of interest because many of them exhibit interesting biological activities [1, 2]. Although their spectrum is wide, there are only several reports on antimicrobial activity of carbohydrate fatty acid esters [3–5], and alkyl glycosides have also been scarcely studied in this respect [6–8].

The most often evaluated carbohydrate esters have been those derived from lauric and palmitic acids. For example, sucrose laurate inhibited the growth of *Bacillus cereus* a food poisoning bacterium [5]. This ester along with maltose and maltotriose laurates completely inhibited *Streptococcus sobrinus*, a dental pathogen [3]. *Escherichia coli*, *Lactobacillus plantarum*, and *Bacillus* spp. are even more susceptible because they have also been affected by maltose palmitate, in addition to the three oligosaccharide laurates [4].

Alkyl glycosides are attractive compounds because many of them can be made from naturally occurring renewable sources (such as sugar and fatty alcohols). Moreover, chemical synthesis of alkyl glycosides as glycolipid mimetics is straightforward and requires only limited number of reaction steps. Due to their biocompatibility and biodegradability, the alkyl glycosides have potential biological and pharmaceutical applications as detergents, pharmaceuticals, agrochemicals, oral-care

products, and medical supplies [9–11]. Additional benefits result from their low toxicity which is particularly relevant for expanding their applicability in fields of food and cosmetic industries as well as personal hygiene.

Alkyl glycosides target the cell membrane. Their incorporation into the lipid bilayer may lead to either a disruption or a modification of the membrane structure. It is known that many of them are able to dissolve the lipid membrane and in this way disrupts the cells. This causes a reduction of the surface tension of the membrane that allows water to flow into the cell and finally results in lysis and bactericidal action. To make this action as efficient as possible, correct balance between the hydrophilic and hydrophobic parts of the alkyl glycoside is essential [12]. The factors that determine the physical and chemical properties of the alkyl glycosides are structure of hydrophobic aglycone, the sugar core, and its stereochemistry. Searching for the active alkyl glycosides is of importance because they could be a valuable environment-friendly alternative for some common products.

Regarding the synthetic alkyl glycosides, octyl and dodecyl 2-deoxy-β-D-*arabino*-hexopyranosides have been reported to inhibit the growth of *Enterococcus faecalis*, which was also highly susceptible to dodecyl 2,6-dideoxy-α-L-*arabino*-hexopyranoside. The latter compound was particularly active towards *Bacillus* sp. [6]. To the best of our knowledge, antimicrobial potential of only three mannopyranosides has been tested [8]. In this study, a series of D-mannosides having lipid aglycone of the length varying from 6 to 20 carbon atoms that is attached by O-glycosidic, S-glycosidic, or sulfonyl linkage were evaluated. *Staphylococcus aureus*, *E. coli*, and *Candida albicans* as the representatives of Gram-positive bacteria, Gram-negative bacteria, and yeasts, respectively, were chosen with the aim to better understand the factors affecting the antimicrobial activity and specificity of the glycolipid-like compounds.

Results and discussion

A list of 14 tested compounds is depicted in Fig. 1. We have previously published the synthesis of **1**, **2**, **4**–**10**, **13**, **14** [13]. Mannosides **3**, **11**, and **12** were prepared by a similar procedure (Scheme 1, "Experimental" section). Briefly, $SnCl_4$-promoted glycosylation of the corresponding alcohol with peracetylated mannose provided after column chromatography O- and S-mannosides with α-selectivity. Subsequent removal of the acetyl protective groups afforded the target mannosides **3**, **11**, and **12** in moderate yields. Their structures were confirmed on the basis of a typical chemical shift [13] of the anomeric proton

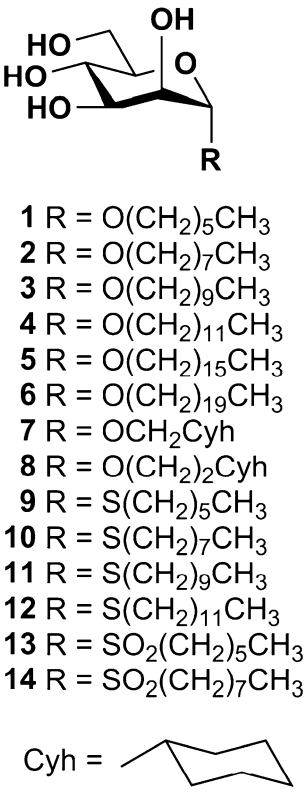


Fig. 1 The tested mannosides

which gave a doublet at δ 4.73 for O-mannoside **3**, while for S-mannosides **11** and **12** was downfielded and found at δ 5.23 and 5.24, respectively. On the other hand, anomeric carbon signals for **3** appeared at δ 101.5, and for both **11** and **12** was upfielded to δ 86.4.

The efficacy of the glycosides **1**–**14** towards the 3 above-mentioned microorganisms was expressed as MIC value. The results are summarized in Table 1. The reference compounds, mannose, methyl α-D-mannopyranoside as well as 1-dodecanethiol, were inactive, and 1-dodecanol did not inhibit *E. coli* and showed MIC value of 1.25 mM towards *S. aureus* and 10 mM towards *C. albicans*.

Escherichia coli was the least affected microorganism by the tested compounds. However, elongation of the aglycone chain of the O-glycosides from C-6 to C-10 led to 16-fold increase in inhibitory activity giving MIC value of 1.25 mM for **3**, which was the most efficient O-mannoside. In contrast, further elongation of the alkyl chain to C-12 resulted in a complete loss of activity. A similar trend was also observed for S-glycosides. The lowest MIC value of 1.25 mM was determined for **10** bearing octyl aglycone, which was fourfold and 32-fold more active than hexyl thiomannoside **9** and decyl thiomannoside **11**, respectively. Also in this case, dodecyl derivative **12** was inactive.

The efficacy of *S. aureus* inhibition was also increasing with the elongation of the aglycone alkyl chain. Dodecyl α-

Table 1 MIC values of the mannosides (mM)

Compound	*E. coli* CNCTC 327/73	*S. aureus* CNCTC Mau 82/78	*C. albicans* CNCTC 59/91
1	20	n.i.	n.i.
2	2.5	5	10
3	1.25	0.625	0.625
4	n.i.	0.078	0.078
5[a]	n.i.	n.i.	0.31
6[a]	n.i.	n.i.	0.31
7	20	n.i.	n.i.
8	10	10	20
9	5	10	10
10	1.25	1.25	2.5
11	40	0.625	0.31
12	n.i.	0.625	0.039
13	40	40	n.i.
14	20	10	n.i.
Ciprofloxacine	$<3 \times 10^{-4}$	6.8×10^{-4}	n.a.

n.a. not applicable, *n.i.* no inhibition observed at the highest concentration (40 mM) tested, or [a] due to limited solubility of the compound its highest concentration tested was 0.625 mM

D-mannopyranoside **4** showed the MIC value of 0.078 mM, which is the lowest among all tested compounds towards this bacterium. It was 64-fold more potent than octyl glycoside **2**. The most efficient thioglycosides **11** and **12** showed the same MIC value of 0.625 mM, and hence, **12** was eightfold less active than its O-linked analog **4**.

Candida albicans was the most susceptible to the studied compounds, and thiomannosides were more active than mannosides. Moreover, the elongation of the aglycone chain of both S- and O-glycosides resulted in a significant increase in their efficacy. In this case, however, dodecyl thiomannoside **12**, exhibiting MIC value of 0.039 mM, was twofold better inhibitor than dodecyl mannoside **4** having MIC value of 0.078 mM. Both decyl S- and O-mannosides were one order of magnitude poorer inhibitors than the corresponding dodecyl counterparts, and further shortening of the aglycone was accompanied with a concomitant reduction of the inhibition efficiency. This trend resulted in an inactivity of hexyl mannoside **1** at 40 mM concentration.

The potency of sulfones **13** and **14** was reduced towards all tested strains in comparison with the corresponding thiomannosides **9** and **10**. This biological activity reduction caused by the oxidation of alkylthio group to sulfone was only slightly compensated by the effect of alkylsulfonyl aglycone prolongation.

Furthermore, replacement of alkyl aglycone in compounds **1** and **2** with cyclohexylalkyl one of similar length (compounds **7** and **8**) did not result in improval of the potency. Evaluation of another alkyl mannosides **5** and **6** having the aglycone chain length of C-16 and C-20 was affected by their limited solubility that resembles the solubility of C-16 and C-20 ethers of methyl glycosides [14].

Considering the selectivity and potency of the tested compounds, Gram-negative bacterium *E. coli* appeared to be the most resistant to the inhibition. The most efficient glycosides were decyl mannoside **3** and octyl thiomannoside **10**, both exhibiting MIC value of 1.25 mM. The aglycone lengths of these glycosides are similar to that of short-chain fatty acids and their carbohydrate esters [14], which exhibited comparable low inhibitory activity towards the Gram-negative bacteria. In contrast, longer free fatty acids [15] and their carbohydrate esters [3, 4, 14] are more efficient towards the Gram-positive bacteria, which agrees well with our findings that dodecyl thiomannoside **12** and, in particular, dodecyl mannoside **4** were the most efficient inhibitors of *S. aureus*. The susceptibility of *C. albicans* resembles that of *S. aureus*, although thiomannoside **12** was the most potent inhibitor of the yeast (Fig. 2). It is also noteworthy that among the tested O-mannosides, dodecyl glycoside **4** has been previously found to be the best inhibitor of *S. aureus*, while *E. coli* has been to the greatest extent affected by decyl mannoside **3,** and inhibitory activity of the both glycosides towards *C. albicans* was the same [8]. The data reported are qualitatively consistent with ours, although in our case the influence of the aglycone length is much more pronounced. These differences may be explained by the different microbial strains used.

Based on the results of the growth inhibition of *S. aureus*, *E. coli*, and *C. albicans* and resulting MICs, the in vitro cytotoxicity and cell proliferation tests with RAW 264.7 cell line murine macrophages following the 24 h exposure with the most effective glycosides i.e., **3**, **4**, **10**, and **12** (Fig. 2) have been performed. In comparison with non-treated cells, the non-cytotoxic, rather stimulation effect of alkyl (thio)mannosides used at the concentrations ranged from 1 to 100 $\mu g/cm^3/10^5$ cells on cell proliferation could be observed with RAW 264.7 cell line murine macrophages, following 24 h exposure (Fig. 3). The only exception represented mannoside **3**, where the 95.3 % ($P < 0.001$) decrease of the proliferation with the highest concentration of 100 $\mu g/cm^3$ has been revealed. Generally, concentration-dependent proliferation activity was observed, the highest concentrations of 10 and 100 $\mu g/cm^3/10^5$ cells being stimulative to lesser degree as the dose of 1 $\mu g/cm^3/10^5$ cells. Concentration of mannosides higher than 10 $\mu g/cm^3$ exerted significant dose-dependent decrease of cell proliferation if compared to those of 1 and 10 $\mu g/cm^3$, but not in comparison with non-treated baseline. The concentration-dependent kinetics of proliferation index supports these findings (Fig. 3). The only different pattern of proliferation was observed for thiomannoside **10**

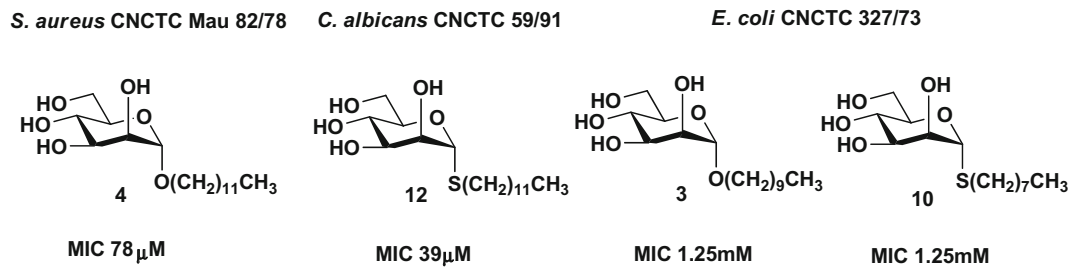


Fig. 2 The most potent mannosides against each of the tested microbial strains

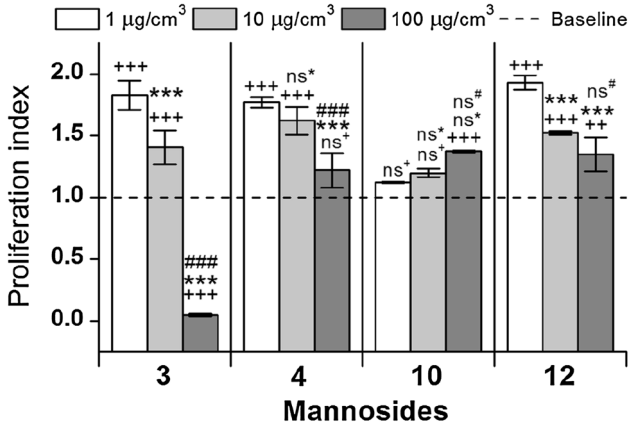


Fig. 3 The effect of mannosides' exposure on RAW 264.7 cell line murine macrophages. The experimental data are expressed as geometric means of double measurements ± SD. Comparisons of data groups were performed vs untreated baseline proliferation, and statistical significance of differences is expressed as follows: $^{+++}P < 0.001$, $^{++}0.001 < P < 0.01$, $^{+}0.01 < P < 0.05$. Experimental datasets comparisons with 1 (μg/cm^3) dose treatment: $P < 0.001$, $^{**}0.001 < P < 0.01$, $^{*}0.01 < P < 0.05$; comparison between 10 and 100 (μg/cm^3) dose treatment: $^{###}P < 0.001$, $^{##}0.001 < P < 0.01$, $^{#}0.01 < P < 0.05$. Differences were considered significant when $0.01 < P < 0.05$

with shortest aglycone length, where the highest concentration of 100 μg/cm^3 triggered the most effective enhancement of proliferation. Based on the comparison of the proliferation activity of O- and S-mannosides (**4** and **12**), both with dodecyl aglycone, the statistically more significant concentration-dependent pattern ($P < 0.001$) has been revealed with the mannoside **12** (Fig. 3). The growth inhibition experiments (Table 1; Fig. 2) resembled also the tight structure—immunobiological activity relations of these mannosides. Evidently, the length of aglycone and the type of glycosidic linkage (O- or S-) are of great importance.

In summary, a correlation between increased hydrophobicity of mannosides and their antimicrobial activity was not observed. However, the results strongly suggest that only glycoside bearing aglycone of a certain length possesses significant activity, and the type of glycosidic linkage (O- or S-) also affects the potency and selectivity. Therefore, it was reasonable to synthesize a set of model compounds varying in the aglycone length and shape (alkyl and cycloalkyl) because it helps to identify appropriate structure of aglycone that is necessary for mannolipid inhibitory activity against given bacterial strains. The microorganisms used in this study are of importance to food and health areas. *E. coli* induces water and food poisoning symptoms. *S. aureus* infection results in a range of mild to severe infection as well as food poisoning and toxic shock syndrome. Human pathogen *C. albicans* is well-known causing agent of fungal infection. The most susceptible to the tested mannolipids were found to be *S. aureus* and *C. albicans*, both being of significant medicinal importance. Against them, two mannolipids, **4** and **12**, showed potent activity at micromolar level. In addition, the latter exhibited also selectivity towards the Gram-positive bacterium [comp **12**: IC_{50} (*S. aureus*/IC_{50} (*C. albicans*) = 16, *E. coli* n.i.].

Therefore, the effort to find out as much as efficient and selective microbial strains inhibitors based on saccharides is ongoing interest due to their environmental acceptance. Moreover, they represent suitable and safe alternative to standard therapy, to which many of microbial strains have become resistant over the years.

The tested compounds provide starting templates for further development of carbohydrate-based antimicrobial agents. Obtained results encourage us to synthesize a larger set of glycosides to further explore the influence of several structural variations (such as e.g., aglycone length, carbohydrate core and its size, anomeric configuration) on their biological activity as well as to determine bacteriostatic and bactericidal activity on a wider scale of microorganisms that are of interest in various areas such as food, cosmetics, health, and others. Work is currently under way and results will be reported in due course.

Conclusions

A series of alkyl O- and S-mannosides were synthesized and evaluated as inhibitors of three microbial strains. The most efficient were those having dodecyl aglycone showing MIC values at micromolar level. While *S. aureus* was the

most susceptible to *O*-mannoside, thiomannoside was superior yeast inhibitor. Antimicrobial activity of glycolipids is still an open field. Therefore, it is desirable to investigate further potential of alkyl glycosides as antimicrobial agents.

Experimental

TLC was performed on aluminum sheets precoated with silica gel 60 F_{254} (Merck). Flash column chromatography was carried out on silica gel 60 (0.040–0.060 mm, Merck) with distilled solvents (hexane, ethyl acetate, and methanol). Dichloromethane was dried (CaH_2) and distilled before use. All reactions containing sensitive reagents were carried out under argon atmosphere. All products were fully characterized with ^{1}H NMR and ^{13}C NMR in combination with the 2D NMR techniques (COSY, HSQC), which were recorded at 25 °C with a VNMRS 400 MHz Varian spectrometer. Chemical shifts are referenced to either TMS ($\delta = 0.00$ ppm, $CDCl_3$ for ^{1}H) or HOD (4.87 ppm, CD_3OD for ^{1}H), and to internal $CDCl_3$ ($\delta = 77.23$ ppm) or CD_3OD (49.15 ppm) for ^{13}C. 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. For bacterial cultures incubation, Incubator BD 23 (Binder GmbH, Germany) was used. Turbidity of bacterial cultures was determined spectrophotometrically at 600 nm (Lambda 35 UV/VIS Spectrophotometer, PerkinElmer, USA). Ciprofloxacine was used as a reference.

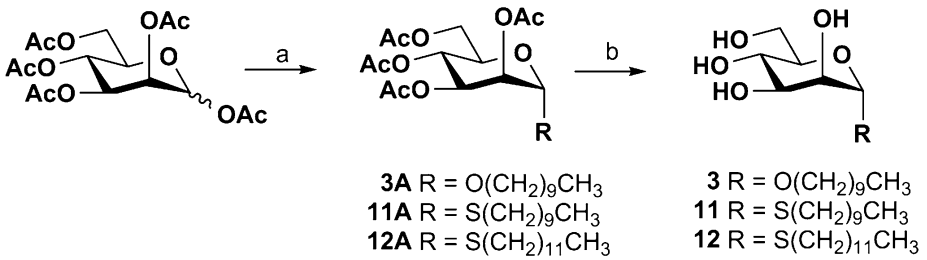
General procedure for glycosylation (Scheme 1)

A stirred solution containing 1 g 1,2,3,4,6-penta-*O*-acetyl-D-mannopyranose (2.56 mmol, 1 eq) in 20 cm^3 CH_2Cl_2 was cooled down on an ice bath, and 1 M $SnCl_4$ in CH_2Cl_2 (1 eq) was added. The reaction mixture was stirred for 10 min, and then, the corresponding alcohol (1.2 eq) was added dropwise. The resulting mixture was stirred for 15 min, brought to r.t. and stirring was continued for 16 h. The reaction mixture was diluted with 80 cm^3 CH_2Cl_2 and poured into 100 cm^3 ice-cold satd $NaHCO_3$ under stirring. The organic phase was separated, washed with 100 cm^3 water, dried with anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (hexane:EtOAc 7:1 → 3:1).

Decyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (**3A**, $C_{24}H_{40}O_{10}$)
Yield 0.44 g (36 %); colorless oil; $[\alpha]_D = +18.0$ ($c = 0.5$, $CHCl_3$) [Ref. [16] $[\alpha]_D = +45.0$ ($c = 1.56$, $CHCl_3$), Ref. [17] $[\alpha]_D^{20} = +37.8$ ($c = 1.0$, CH_2Cl_2)]; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.36$ (dd, 1H, $J_{2,3} = 3.4$ Hz, H-3), 5.27 (dd, 1H, $J_{3,4} = 9.9$ Hz, H-4), 5.23 (dd, 1H, H-3), 4.80 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1), 4.28 (dd, 1H, $J_{5,6b} = 5.4$ Hz, $J_{6a,6b} = 12.2$ Hz, H-6b), 4.10 (dd, 1H, $J_{5,6a} = 2.4$ Hz, H-6a), 3.98 (ddd, 1H, H-5), 3.67 (dt, 1H, $J = 6.8$ Hz, $J = 9.6$ Hz, $OCH_2(CH_2)_8CH_3$), 3.44 (dt, 1H, $J = 6.6$ Hz, $J = 9.6$ Hz, $OCH_2(CH_2)_8CH_3$), 2.15, 2.10, 2.04, 1.99 (each s, each 3H, 4× CH_3CO), 1.61–1.27 (m, 16H, $OCH_2(CH_2)_8CH_3$), 0.88 (t, 3H, $J = 7.0$ Hz, $O(CH_2)_9CH_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.8$, 170.3, 170.1, 170.0 (4× CH_3CO), 97.7 (C-1), 69.9 (C-2), 69.3 (C-3), 68.7, 68.6 (C-5, $OCH_2(CH_2)_8CH_3$), 66.5 (C-4), 62.7 (C-6), 32.1, 29.7 (2×), 29.6, 29.5, 29.4, 26.3, 22.9 ($OCH_2(CH_2)_8CH_3$), 21.1, 20.9 (3×) (4× CH_3CO), 14.3 ($OCH_2(CH_2)_8CH_3$) ppm; HRMS (ESI): *m/z* calcd for $C_{24}H_{40}O_{10}Na$ $[M + Na]^+$ 511.2519, found 511.2502.

Decyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside (**11A**, $C_{24}H_{40}O_9S$)
Yield 0.70 g (54 %); yellow oil; $[\alpha]_D = +68.0$ ($c = 0.5$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.34$–5.25 (m, 4H, H-1, H-2, H-3, H-4), 4.40 (m, 1H, H-5), 4.32 (dd, 1H, $J_{5,6b} = 5.3$ Hz, $J_{6a,6b} = 12.2$ Hz, H-6b), 4.09 (dd, 1H,

Scheme 1



3A R = $O(CH_2)_9CH_3$
11A R = $S(CH_2)_9CH_3$
12A R = $S(CH_2)_{11}CH_3$

3 R = $O(CH_2)_9CH_3$
11 R = $S(CH_2)_9CH_3$
12 R = $S(CH_2)_{11}CH_3$

Reagents and conditions. **(a)** $SnCl_4$, alcohol, CH_2Cl_2, 16 h, rt; **(b)** MeONa, MeOH, 16 h, rt.

$J_{5,6a}$ = 2.2 Hz, H-6a), 2.68–2.53 (m, 2H, $SCH_2(CH_2)_8CH_3$), 2.16, 2.09, 2.05, 2.00 (each s, each 3H, 4× CH_3CO), 1.63–1.26 [m, 16H, $SCH_2(CH_2)_8CH_3$], 0.88 [t, 3H, J = 6.6 Hz, $S(CH_2)_9CH_3$] ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.2, 170.1, 170.0, 169.9 (4× CH_3CO), 82.7 (C-1), 71.3 (C-2), 69.6 (C-3), 69.1 (C-5), 66.5 (C-4), 62.6 (C-6), 32.0, 31.5, 29.7, 29.6, 29.5, 29.4, 29.2, 28.9, 22.8 ($S(CH_2)_9CH_3$), 21.1, 20.9 (2×), 20.8 (4× CH_3CO), 14.2 ($SCH_2(CH_2)_8CH_3$) ppm; HRMS (ESI): *m/z* calcd for $C_{24}H_{40}O_9SNa$ $[M + Na]^+$ 527.2291, found 527.2285.

Dodecyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside (**12A**, $C_{26}H_{44}O_9S$)
Yield 0.63 g (46 %); yellow oil; $[\alpha]_D$ = +72.0 (c = 0.5, $CHCl_3$) [Ref. [18] $[\alpha]_D^{20}$ = +76.8 (c = 0.37, $CHCl_3$)]; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.35–5.26 (m, 4H, H-1, H-2, H-3, H-4), 4.39 (m, 1H, H-5), 4.32 (dd, 1H, $J_{5,6b}$ = 5.3 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6b), 4.08 (dd, 1H, $J_{5,6a}$ = 2.3 Hz, H-6a), 2.68–2.53 [m, 2H, $SCH_2(CH_2)_{10}CH_3$], 2.17, 2.10, 2.05, 2.00 (each s, each 3H, 4× CH_3CO), 1.66–1.24 [m, 20H, $SCH_2(CH_2)_{10}CH_3$], 0.88 [t, 3H, J = 6.7 Hz, $S(CH_2)_{11}CH_3$] ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.7, 170.1, 170.0, 169.9 (4× CH_3CO), 82.7 (C-1), 71.3 (C-2), 69.6 (C-3), 69.0 (C-5), 66.5 (C-4), 62.6 (C-6), 32.0, 31.5, 29.8, 29.7 (2×), 29.5 (2×), 29.4, 29.3, 28.9, 22.8 ($S(CH_2)_9CH_3$), 21.1, 21.0, 20.9, 20.8 (4× CH_3CO), 14.3 ($SCH_2(CH_2)_8CH_3$) ppm; HRMS (ESI): *m/z* calcd for $C_{26}H_{44}O_9SNa$ $[M + Na]^+$ 555.2604, found 555.2594.

General procedure for removal of acetyl groups

Acetylated glycoside (0.2 g) was dissolved in 5 cm^3 MeOH, and 0.15 cm^3 1 M MeONa was added. The reaction mixture was stirred for 16 h at r.t., filtered, and concentrated. The crude product was purified by column chromatography (EtOAc:MeOH 1:0 → 10:1).

Decyl α-D-mannopyranoside (**3**, $C_{16}H_{32}O_6$)
Yield 0.12 g (90 %); colorless oil; $[\alpha]_D$ = +50.0 (c = 0.5, methanol); ^{1}H NMR (400 MHz, CD_3OD): δ = 4.73 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 3.83 (dd, 1H, $J_{5,6a}$ = 2.4 Hz, $J_{6a,6b}$ = 11.9 Hz, H-6a), 3.78 [dd, 1H, $J_{2,3}$ = 3.3 Hz, H-2), 3.76–3.67 (m, 3H, H-3, H-6b, $OCH_2(CH_2)_8CH_3$], 3.61 (t, $J_{3,4}$ = 9.5 Hz, $J_{4,5}$ = 9.5 Hz, H-4), 3.52 (m, 1H, H-5), 3.41 [dt, 1H, J = 6.3 Hz, J = 9.6 Hz, $OCH_2(CH_2)_8CH_3$], 1.64–1.29 [m, 16H, $OCH_2(CH_2)_8CH_3$], 0.90 [t, 3H, J = 6.9 Hz, $O(CH_2)_9CH_3$] ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 101.5 (C-1), 74.6 (C-5), 72.7 (C-3), 72.3 (C-2), 68.6 (2×) (C-4, $OCH_2(CH_2)_8CH_3$), 62.9 (C-6), 33.1, 30.7 (2×), 30.6 (2×), 30.5, 27.4, 23.7 ($OCH_2(CH_2)_8CH_3$), 14.4 ($O(CH_2)_9CH_3$) ppm; HRMS (ESI): *m/z* calcd for $C_{16}H_{32}O_6Na$ $[M + Na]^+$ 343.2097, found 343.2090.

Decyl 1-thio-α-D-mannopyranoside (**11**, $C_{16}H_{32}O_5S$)
Yield 0.12 g (89 %); colorless oil; $[\alpha]_D$ = +160.0 (c = 0.5, methanol); ^{1}H NMR (400 MHz, CD_3OD): δ = 5.24 (d, 1H, $J_{1,2}$ = 1.4 Hz, H-1), 3.94–3.90 (m, 2H, H-2, H-3), 3.84 (dd, 1H, $J_{5,6a}$ = 2.4 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6a), 3.78 (dd, 1H, 1H, $J_{5,6b}$ = 5.6 Hz, H-6b), 3.70–3.64 (m, 2H, H-4, H-5), 2.73–2.67 [m, 1H, $SCH_2(CH_2)_8CH_3$], 2.64–2.58 [m, 1H, $SCH_2(CH_2)_8CH_3$], 1.70–1.32 [m, 16H, $SCH_2(CH_2)_8CH_3$], 0.92 [t, 3H, J = 6.8 Hz, $S(CH_2)_9CH_3$] ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 86.4 (C-1), 74.8, 73.8, 73.2, 68.9 (C-2, C-3, C-4, C-5), 62.7 (C-6), 33.1, 31.9, 30.7 (3×), 30.4, 30.3, 30.0, 23.7 ($S(CH_2)_9CH_3$), 14.4 ($S(CH_2)_9CH_3$) ppm; HRMS (ESI): *m/z* calcd for $C_{16}H_{32}O_5SNa$ $[M + Na]^+$ 359.1868, found 359.1861.

Dodecyl 1-thio-α-D-mannopyranoside (**12**, $C_{18}H_{36}O_5S$)
Yield 0.12 g (88 %); colorless oil; $[\alpha]_D$ = +164.0 (c = 0.5, methanol); ^{1}H NMR (400 MHz, CD_3OD): δ = 5.23 (d, 1H, $J_{1,2}$ = 1.4 Hz, H-1), 3.94–3.90 (m, 2H, H-2, H-3), 3.84 (dd, 1H, $J_{5,6a}$ = 2.4 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6a), 3.78 (dd, 1H, 1H, $J_{5,6b}$ = 5.6 Hz, H-6b), 3.70–3.66 (m, 2H, H-4, H-5), 2.73–2.66 [m, 1H, $SCH_2(CH_2)_{10}CH_3$], 2.64–2.57 [m, 1H, $SCH_2(CH_2)_{10}CH_3$], 1.69–1.31 [m, 20H, $SCH_2(CH_2)_{10}CH_3$], 0.96 [t, 3H, J = 7.4 Hz, $S(CH_2)_{11}CH_3$] ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 86.4 (C-1), 74.8, 73.8, 73.2, 68.9 (C-2, C-3, C-4, C-5), 62.7 (C-6), 33.1, 31.9, 30.8 (2×), 30.7 (3×), 30.5, 30.3, 29.9, 23.7 ($S(CH_2)_{11}CH_3$), 14.4 ($S(CH_2)_9CH_3$) ppm; HRMS (ESI): *m/z* calcd for $C_{18}H_{36}O_5SNa$ $[M + Na]^+$ 387.2181, found 387.2176.

Evaluation of the antimicrobial activity

Preparation of bacterial cultures
For testing of antimicrobial activity, bacteria *E. coli* CNCTC 327/73, *S. aureus* CNCTC Mau 82/78, and yeast *C. albicans* CNCTC 59/91 [all purchased from Czech National Collection of Type Cultures, Czech Republic and recommended for antimicrobial susceptibility [19] and preservative efficacy tests [20] (European Pharmacopoeia)] were used. Bacteria were grown aerobically in nutrient broth (NB, Imuna, Slovakia) and yeast in Sabouraud dextrose broth (SDB, *Difco,* France) for 18 h at 37 °C or 48 h at 24 °C, respectively. Cultures were then maintained at 4 °C on appropriate solid medium: Endo agar (EA, Oxoid, England) for *E. coli*, blood agar (BA, Biomark, India) for *S. aureus,* and Sabouraud dextrose agar (SDA, *Difco,* France) for *C. albicans*. Working cultures were prepared by incubation of single colony of each microorganism in NB (bacteria) or SDB (yeast) for 18 h at 37 °C or 48 h at 24 °C, respectively. A microbial suspension was prepared in saline solution (0.85 % NaCl) according to McFarland standard No 0.5 using Lambda 35 UV/VIS

Spectrophotometer (PerkinElmer, USA), to obtain a concentration of ca 1.5×10^8 cfu/cm^3. After a dilution in appropriate liquid medium (NB for bacteria and SDB for yeast), a working concentration 1.5×10^7 cfu/cm^3 was prepared.

Antimicrobial activity assay

The antimicrobial activity assay was performed according to Malík et al. [21] with some modifications. Stock solutions (80 mM) of test mannosides and standards were prepared in sterile 50 % MeOH (1:1 MeOH-distilled H_2O), immediately before use. Working test mannosides and standards were prepared by serial dilution of stock solutions in sterile doubly concentrated NB or SDB to a final volume 100 mm^3 within the 96-well microplates. Freshly prepared inoculum (5 mm^3) of the tested microorganism was added to each appropriate well (bacteria into the plates with NB, *C. albicans* into the plates with SDB). The final concentration of each organism in each well was ca. 7.5×10^5 cfu/cm^3. The concentration of tested compounds ranged from 40 mM to 0.3 μM, except of mannosides **5** and **6**, which were tested in the range from 0.625 mM to 0.3 μM due to their low solubility under experimental conditions used. Each concentration was assayed in triplicates. For each test compound and microorganism, the following controls were used: blank, uninoculated media without test compound to account for changes in the media during the experiment; negative control, uninoculated media containing only the test compound; positive control 1, inoculated media without compound; positive control 2, inoculated media without compound but including mannose to evaluate any effect of the sugar alone; and the positive control 3, inoculated media with serial dilution of 50 % MeOH without test compound, thereby assessing any activity of the alcohol. The 96-well plates were incubated aerobically for 24 h at 37 °C or 24 °C, in dependence if bacteria or yeast were grown. Then, 5 mm^3 of each well was inoculated on the appropriate agar plate (EA for *E. coli*, BA for *S. aureus*, and SDA for *C. albicans*). Bacteria and yeast were grown aerobically 24 h at 37 °C or 24 °C, respectively. The MIC was defined as the lowest concentration of compound that inhibited the growth of microorganism on agar plates for all parallel samples compared to the positive control after 24 h.

Immunobiological experiments

Proliferation

Proliferation and cytotoxicity of mammalian cell line RAW 264.7 in culture was evaluated based on the bioluminescent measurement of adenosine triphosphate (ATP) levels, marker of metabolically active cells, using the ViaLight™ plus kit (Lonza, Walkersville, Maryland, US) according to the manufacturer's instructions. The emitted light intensity was measured with a 96-microtitre plate computer-driven luminometer Immunotech LM-01T (Immunotech, Prague, Czech Republic). Light emission, expressed as relative light units (RLU), was recorded continuously for one second and evaluated on the basis of integral (peak) values. Proliferation observed with unstimulated cells was considered to be the baseline value. The proliferation index represents ratio of induced proliferation (stimulated cells) to baseline (unstimulated cells) proliferation.

Cell cultivation and activation

RAW 264.7 cell line murine macrophages (ATCC®TIB-71™) were cultured in complete DMEM for 24 h (until approx 60 percentage confluence), after then, the aliquots of 1×10^5 cells/cm^3/well (with 93.1 % viability) were seeded in 24-well cell culture plate (Sigma, US), and exposed to different concentrations of mannosides (**3**, **4**, **10**, and **12**), ranged from 1 to 100 μg/cm^3/well. In vitro exposure has been performed for next 24 h, respectively, at 37 °C in an atmosphere of 5 % CO_2 and at 90–100 % relative humidity. Cell viability has been assessed using the trypan blue exclusion method.

Computational and statistical analysis

Results from in vitro experiments were calculated as mean values with ± standard error of the mean (s.e.m.). Normality of distribution was evaluated according to Shapiro–Wilk's test at the 0.05 level of significance. Statistical comparison between individual groups was performed using one-way analysis of variance (ANOVA) and post hoc Bonferroni's test. The results were significant if the difference between the analyzed groups equaled or exceeded the 95 % confidence level ($P < 0.05$). Statistics were performed with Origin 7.5 Pro software (OriginLab Corporation, Northampton, MA, USA).

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