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Environmentally friendly approaches to the synthesis of new antibiotics from sugars*

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Abstract: In light of the biological importance of carbohydrates and their role when present in antibiotic agents, the design and synthesis of carbohydrate-based antibiotics has occupied a prominent place in drug discovery. This review focuses on synthetic carbohydrate antimicrobial agents, giving special emphasis to novel structures easily accessible from readily available carbohydrate precursors.

Keywords: antibiotics; antimicrobial; carbohydrates; chemical synthesis; enzyme catalysis.

INTRODUCTION

Although the term "antibiotic" earlier referred to a compound or a substance produced by a microorganism (i.e., a bacterial or a fungal metabolite), which inhibits the growth of other microorganisms [1], today this designation relates to any compound, natural, semisynthetic, or synthetic, which exhibits such inhibitory effect.

The emergence of antibiotic-resistant bacterial strains and the development of antibiotic resistance during therapy has become an important public health problem, and hence the search for new antibiotic structures with novel mechanisms of action has regained a significant interest in drug research.

Considering their chemical structure, antibiotics comprise many classes of organic compounds. In this mini-review we will only consider those which are carbohydrate-based entities. It is not intended to cover all types of carbohydrate-containing antibiotics (natural derivatives are just briefly mentioned) since some reviews have appeared in the literature [2] and recently an updated account of these antimicrobial agents was published [3]. Instead, we will highlight new bioactive structures for which synthesis involves easily available carbohydrate precursors, few steps, and environmentally safe methodologies.

NATURAL CARBOHYDRATE-CONTAINING ANTIBIOTICS

Most carbohydrate-based antibiotics are natural or semisynthetic compounds. These include the aminoglycosides, which consist of a central six-membered aminocyclitol that is glycosylated with various amino sugars, and are mostly produced by *Streptomyces* and a number of *Actinomycetes* strains. Among the most representative families of compounds of this class are the kanamycins, the gentamycins, the neomycins, or the apramycins (Fig. 1).

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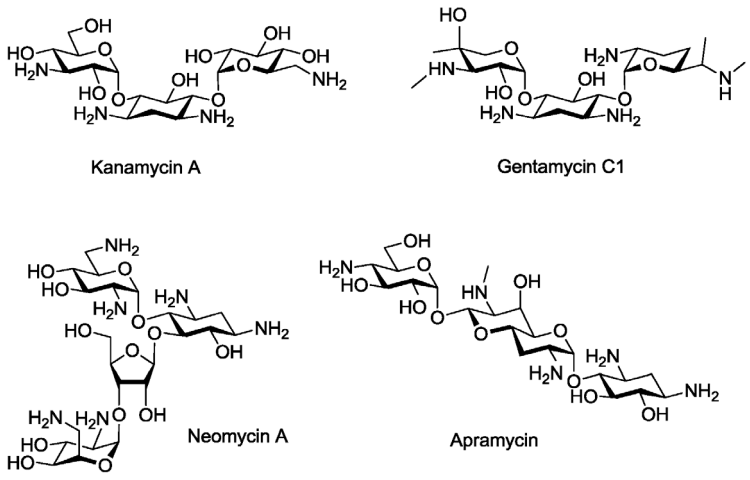


Fig. 1 Structure of aminoglycoside antibiotics.

Their mechanism of action relies mainly on the interruption of protein synthesis by binding to the small ribosomal subunit, which inhibits the translation. Aminoglycosides are frequently used for the treatment of serious infections, such as respiratory infections, urinary tract infections, septicemia, intra-abdominal infections, or bacteremia [4].

Another class of carbohydrate-containing antibiotics is that of glycopeptides, the structure of which comprises a cyclic peptide framework O-glycosylated with mono- or disaccharide moieties at up to four positions. The most significant compounds of this class are vancomycin, which was isolated from *Amycolatopsis orientalis*, and teichoplanin, which was extracted from *Actinoplanes teichomyceticus* (Fig. 2).

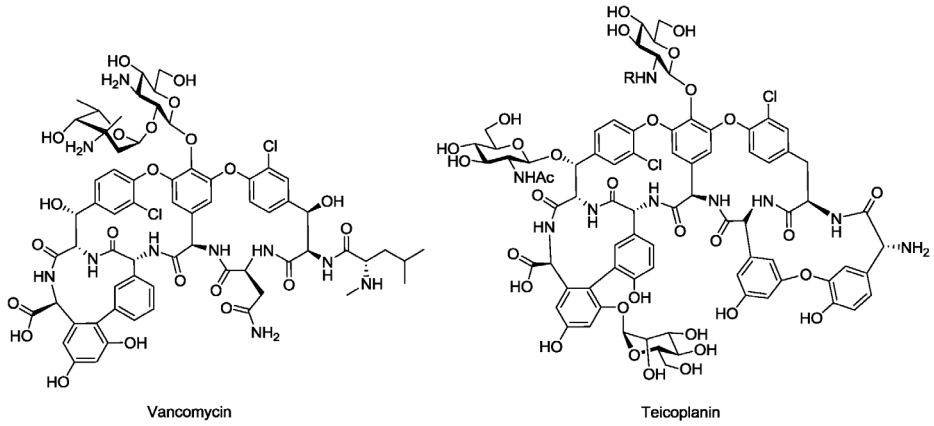


Fig. 2 Structure of vancomycin and the core structure of teichoplanin.

Vancomycin and teicoplanin are the main therapeutic agents against infections caused by Gram-positive bacteria that have acquired multiple drug resistance. Their mechanism of action is based on the interruption of cell wall peptidoglycan assembly, by inhibiting cross-linking of the peptide side chains by the transpeptidase enzyme [5]. Both antibiotics display potent activity toward a broad range of Gram-positive bacteria, especially *Staphylococcus aureus* (minimum inhibitory concentration or MIC

value for vancomycin is 0.25–10.0 μg/mL [6]) and *Enterococcus* strains. The appearance of vancomycin-resistant *Enterococci* and vancomycin-resistant *S. aureus* has prompted the development of synthetic analogs with improved antibacterial activity [7].

Macrolide antibiotics are a group of antibiotics which comprise a macrocyclic lactone that is O-glycosylated with one or more deoxy sugars, usually cladinose or desosamine and sometimes amino deoxy sugars. They exhibit a broad-spectrum antimicrobial activity and are used against respiratory and soft-tissue infections [8]. Amongst the compounds that belong to this class, erythromycin should be highlighted. It is produced by a strain of *Saccharopolyspora erythraea*, and is commonly used as an alternative to penicillin by patients who are allergic to this antibiotic. Novel erythromycin-related compounds such as clarithromycin and azithromycin (Fig. 3) have been developed [9]. Most of the macrolides exhibit potent activity against Gram-positive bacteria, and their mechanism of action is based on the inhibition of protein synthesis by binding to the 23S rRNA and, thus, by blocking elongation of the growing peptide chain.

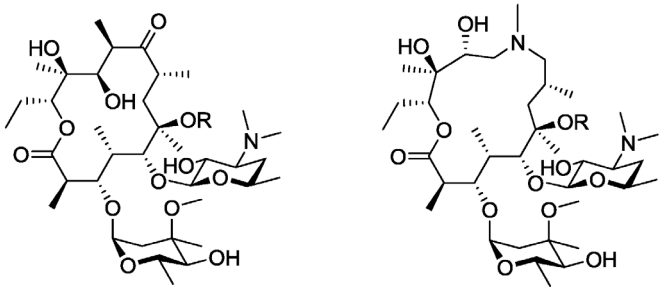


Fig. 3 Structure of erythromycin, clarithromycin, and azithromycin.

Nucleoside antibiotics are another major class of sugar-based antibiotics. They encompass a structural variety of natural products in which the nucleoside is combined with other moieties, namely, peptides, lipids, disaccharides, or higher sugars [10].

Several classes of nucleoside antibiotics act by inhibiting the bacterial cell wall assembly by targeting translocase MraY, which catalyzes the first membrane step of peptidoglycan biosynthesis [10d]. These include the tunicamycins, which are produced by several *Streptomyces sp.*, the mureidomycins, produced by *Streptomyces flavidovirens* or the liposidomycins, which were first isolated from the fermentation broth of *Streptomyces griseosporeus* (Fig. 4).

The spectrum of antimicrobial activity of nucleoside antibiotics is broad, targeting Gram-positive and Gram-negative bacteria and fungi.

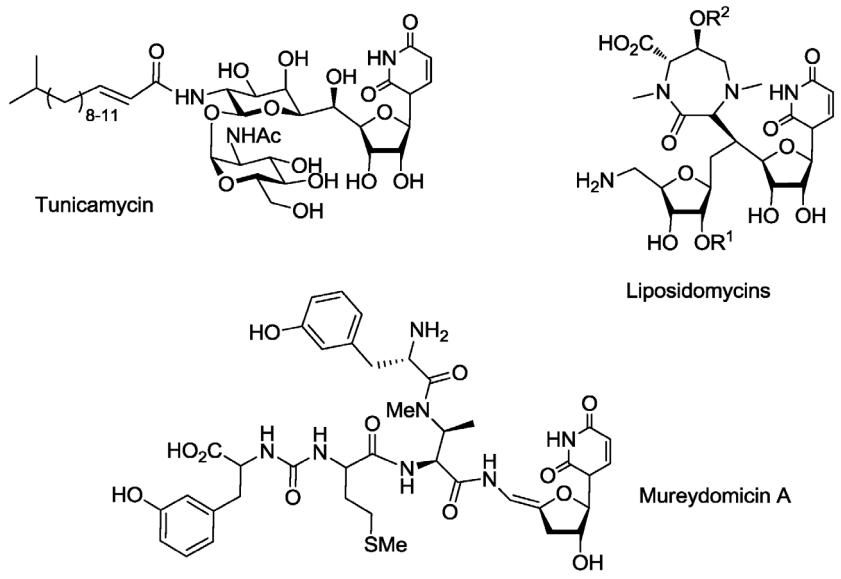


Fig. 4 Structure of tunicamycin, mureidomycin A, and the core structure of liposidomycins.

NOVEL CARBOHYDRATE-BASED ANTIBIOTICS

Targeting the cell wall biosynthesis

The increasing occurrence of bacterial resistance to existing antibiotics has motivated the research on the structural modification of the natural carbohydrate-based antibiotics as well as the development of new structures and the identification of new targets.

Carbohydrates are involved in various intercellular recognition processes and are a common feature of bacterial and fungal cell walls. Many antibiotics contain carbohydrate moieties which bind to enzymes that are associated with carbohydrate recognition, synthesis, and their assembly within the cell wall. Inhibition of the biosynthesis of key carbohydrates that are present in the microbial cell wall may therefore be an approach for the development of new antibiotics.

The peptidoglycan biosynthetic pathway is an attractive target for antibiotics [11] especially those focusing Gram-positive bacteria. In Gram-negative bacteria, the cell wall is enveloped by the outer membrane that contains lipopolysaccharides (LPS) as major components. LPS, also known as endotoxins, are essential for bacterial growth and viability [12]. When released in animals or humans, LPS induce a strong response from the immune system, which may lead to septic shock. The structure of LPS comprises three regions: the *O*-antigen, which consists of glycans; the core domain, which contains 3-deoxy-D-*manno*-octulosonic acid (KDO) [13] as well as heptoses [14], usually L-*glycero*-α-D-*manno*-heptose; and the lipid A region [15], with a bisphosphorylated and acylated β-(1 → 6)-interlinked glucosamine disaccharide backbone. The latter is the major factor for the bacterial virulence stimulating an intense host immune response [16]. Therefore, the search for inhibitors of LPS biosynthesis has been a valuable strategy in antibacterial drug design. Syntheses of inhibitors of the biosynthetic pathways leading to KDO [17], to lipid A [18], and more recently, to L-*glycero*-D-*manno*-heptose [19] have been explored.

Carbohydrate-based surfactants

Sugar-based surfactants comprise a carbohydrate hydrophilic moiety, usually a mono- or an oligosaccharide and a hydrophobic tail, generally derived from a fatty alcohol or from a fatty acid. These

compounds have the potential to interact with the lipid bilayer of cell membranes and, hence, to exhibit biological activity. The carbohydrate moiety has the propensity to bind to the hydrophilic portion of the membrane while the lipophilic portion is then able to penetrate the lipid bilayer structure. This aspect has prompted the synthesis of amphiphilic sugar derivatives for further antimicrobial activity evaluation.

Carbohydrate fatty acid esters are amongst these derivatives, whose antibacterial properties have been studied. These compounds are chemically synthesized by esterification of sugars with fatty acids or transesterification with fatty acyl methyl esters, which normally require toxic organic solvents and high temperatures and often lead to low selectivity, giving mixtures of sugar esters [20]. Another methodology involves the use of acyl chlorides which readily react with sugar hydroxyl groups owing to their higher reactivity than that of the corresponding carboxylic acids. However, this method produces hydrochloric acid as byproduct [21]. To overcome the drawbacks of these protocols, chemoenzymatic methodologies have been employed for the synthesis of new sugar fatty esters (Fig. 5). Plou and co-workers [22] succeeded in synthesizing 6-*O*-lauroylsucrose and 6-*O*-palmitoylsucrose in 70 and 80 % yield, respectively, by transesterification of sucrose with the corresponding vinyl esters in 2-methylbutan-2-ol containing 20 % of dimethyl sulfoxide in the presence of a lipase. In a further study [23], 6-*O*-lauroylsucrose, 6-*O*-lauroylmaltose, and 6-*O″*-lauroylmaltotriose were regioselectively synthesized in the presence of a lipase from *Thermomyces lanuginosus.* These compounds inhibited completely the growth of *Streptococcus sobrinus*, an oral bacteria with a key role in the initiation of dental caries, at 100 µL/mL in agar plates, which turns them into promising caries-prevention agents.

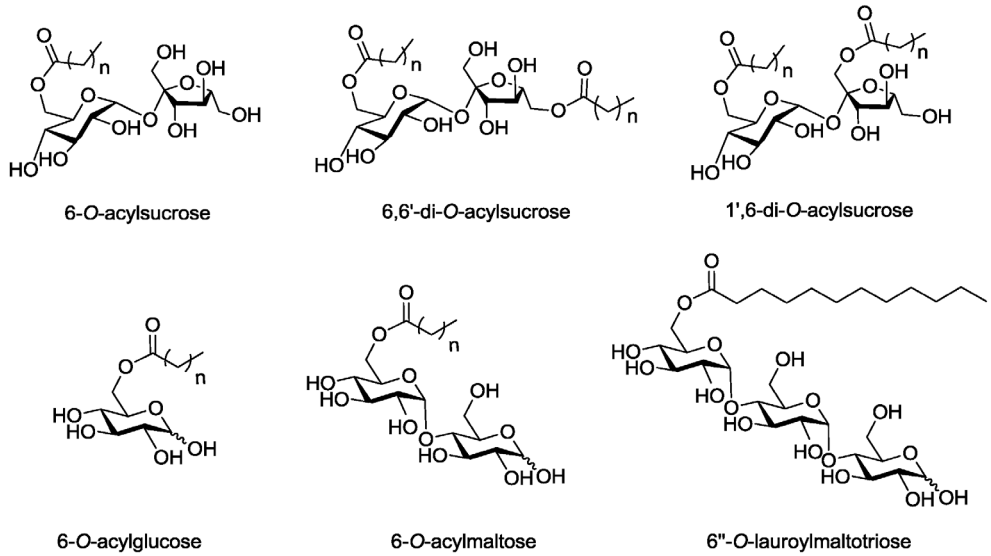


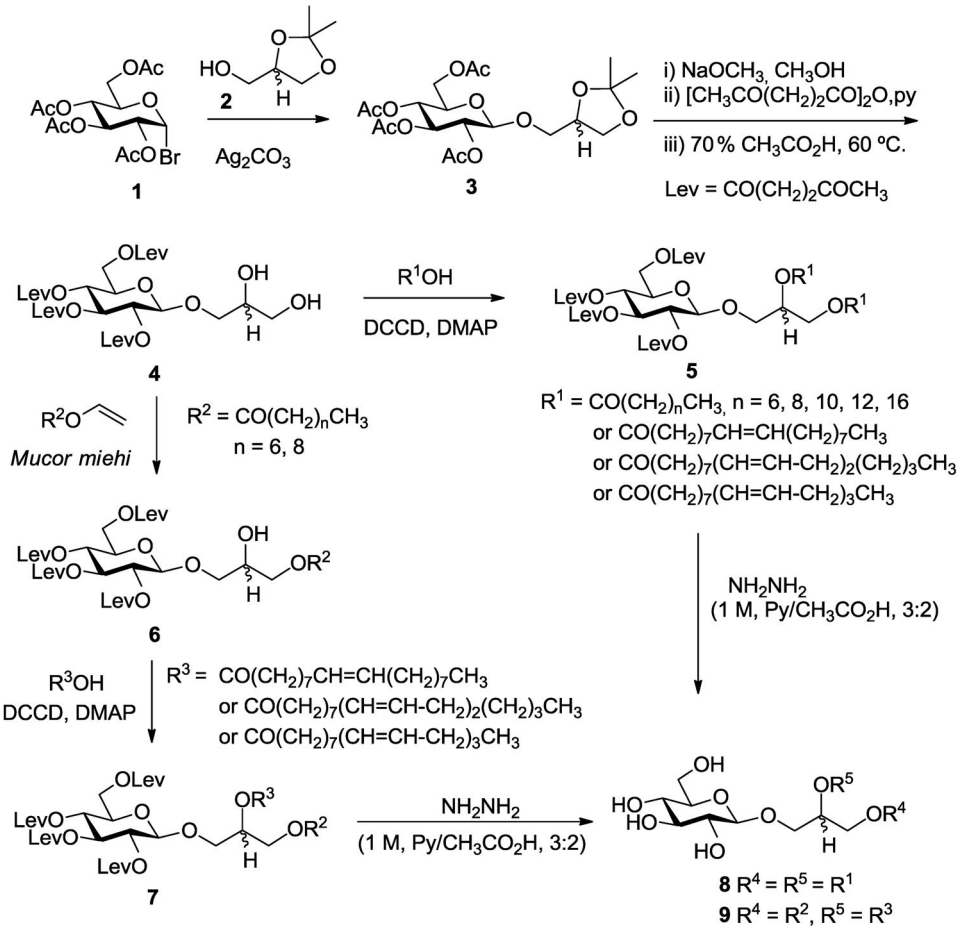
Fig. 5 Enzymatically synthesized surfactant sugar esters for the evaluation of their antibacterial effect; n = 10 (lauroyl), n =14 (palmitoyl).

The lipase from *Candida antarctica B* was also further employed and provided mainly 6,6-di-*O*-acylsucrose derivatives, although its efficiency toward the synthesis of 6-*O*-palmitoylmaltose and 6-*O*-lauroylglucose proved to be similar to that of *T. lanuginosus* [24]. The latter lipase also gave a nearly equimolar mixture of the diesters 1',6- and 6,6'-di-*O*-acyl sucrose as minor products.

Their antimicrobial activity was evaluated by examining their effect on the growth of several Gram-positive and -negative bacteria and yeasts involved in food spoilage and poisoning (*Pseudomonas fluorescens*, *Bacillus sp.*, *Lactobacillus plantarum*), and on a diversity of diseases (*S. aureus*,

Escherichia coli, *Pichia jadinii*). *Bacillus sp.* was the most susceptible bacteria, particularly to 6-*O*-lauroylsucrose and 6-*O*-lauroylmaltose, whose growth inhibition percentages were of 95 and 93 % at concentration of 4 and 0.8 mg/mL, respectively. These compounds were also effective against *E. coli* and *L. plantarum*. The 6-*O'*-palmitoylmaltose also showed a significant activity toward *Bacillus sp.*, leading to 94 % bacteria growth inhibition at the concentration of 2 mg/mL.

Cateni et al. [25] reported the synthesis and antibacterial activity of glucosyl diglycerides comprising medium-to-long-length fatty acyl chains. The synthetic pathway was straightforward, starting with glycosylation of 1,2-*O*-isopropylideneglycerol (**2**) with tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**1**, Scheme 1). Then acetyl groups of **3** were replaced by levulinyl groups and the aglycon isopropylidene acetal was cleaved leading to the diol **4**. The latter compound was diacylated with a saturated or an unsaturated fatty acid in the presence of dicyclohexylcarbodiimide (DCCD) and 4-dimethylaminopyridine (DMAP) to give **5**.



Scheme 1 Synthesis of glucosyl diglycerides comprising medium-to-long-length acyl chains.

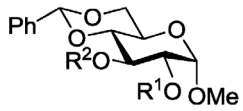
Regioselective acylation at the primary hydroxyl group of **4** was achieved by transesterification with octanoate or decanoate vinyl ester, in the presence of *Mucor miehei* lipase, in 95–98 % yields. The secondary hydroxyl group of **6** was further acylated by DCCD coupling with oleic, linoleic, and linolenic fatty acids leading to mixed diesters **7**.

Final removal of the levulinyl groups of **5** and **7** led to the desired amphiphilic glucosyl diglycerides **8** and **9** which were submitted to antimicrobial activity evaluation toward Gram-positive, Gram-negative bacteria and fungi.

Most of the derivatives bearing the same saturated acyl chains showed no activity, with the exception of that containing the octanoyl group which was active against *B. subtilis*, *S. aureus*, *E. faecalis*, and *Mycobacterium tuberculosis* with MICs of 16, 16, 32, and 64 μg/mL, respectively, and that bearing a decanoyl moiety which displayed effects toward *S. aureus* and *M. tuberculosis* with MIC values of 64 μg/mL.

The compounds with different fatty acyl moieties proved to be more active than the previous series of derivatives. All compounds showed effects against *S. aureus* with MICs ranging from 8 to 64 μg/mL, and three of them displayed moderate inhibitory activity against *M. tuberculosis* reference strain and clinical strains exhibiting MIC values from 64 to 128 μg/mL.

2-*O*-Decanoyl derivatives of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**10**) possessing different substituents at C-3 (compounds type **11**, Fig. 6) were screened for their antibacterial and antifungal activity by the paper disk diffusion method [26]. Amongst these compounds, only two of them, one with a free hydroxyl group at C-3 and the other with a benzoyl group, were active against all the bacteria tested. Both were particularly active against *B. cereus*, *Bacillus megaterium*, and *Pseudomonus sp.*, showing diameters of inhibition zones very close or even higher than those of the standard antibiotic (ampicillin). Concerning the antifungal activity, six plant pathogenic fungi were essayed and methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-*O*-decanoyl-α-D-glucopyranoside was again amongst the most active compounds for most of the fungi tested with inhibition values greater than those of nystatin, the reference compound.

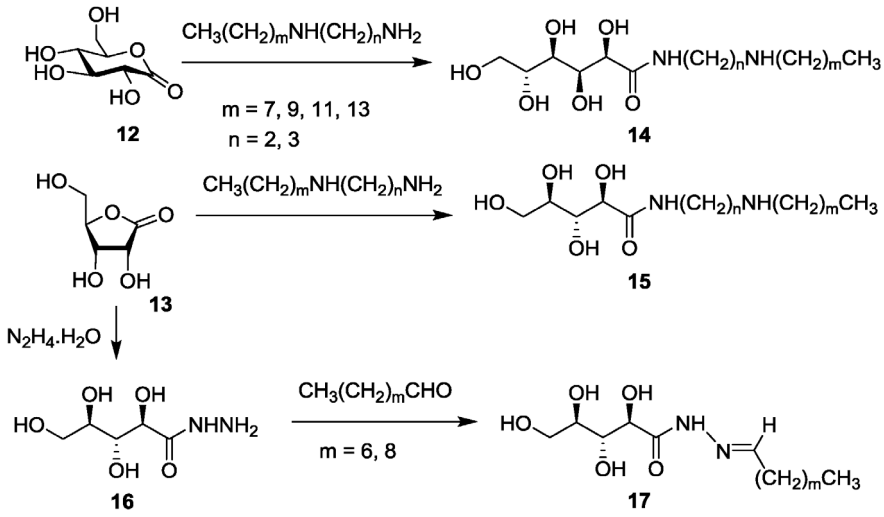


10 $R^1 = R^2 = H$

11 R^1 = Decanoyl,
R^2 = Ac, Ms, Bz, Octanoyl,
Lauroyl, 2-Cl.Bz, 2.CO_2H.Bz

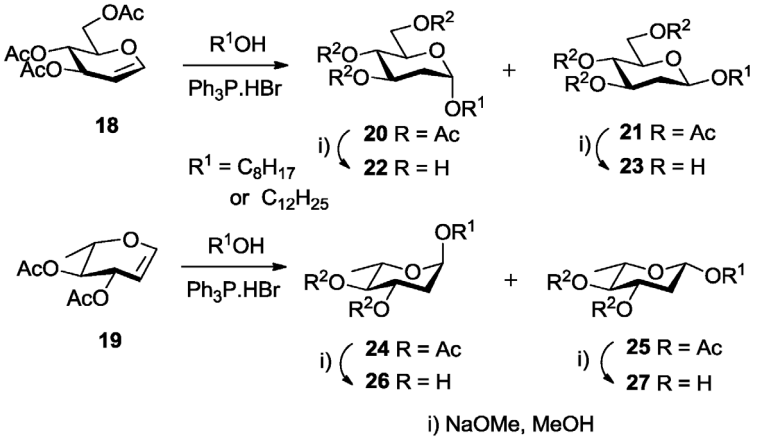
Fig. 6 2-*O*-Decanoyl derivatives of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside submitted to antimicrobial activity evaluation.

Besides sugar fatty esters, other types of amphiphilic derivatives and their antimicrobial effects have been reported, namely, the aldonoamides **14** and **15**, which were synthesized in moderate to good yields by addition of long-chain N-monoalkylated diamines to readily available D-glucono-1,5-lactone **12** or D-ribono-1,4-lactone **13** (Scheme 2) [27]. In addition, hydrazones **17** were obtained by treatment of the intermediate ribonohydrazide **16** with octanal or decanal. All compounds derived from ribonolactone showed a moderate activity against *M. tuberculosis*, and *s*ome ribonoamides were also active against *S. aureus*. The activities increased somewhat with the elongation of their hydrocarbon chain.



Scheme 2 Amphiphilic antimicrobial compounds obtained from aldonolactones.

In our group the design and synthesis of long-chain alkyl glycosides has been undertaken. In particular, the synthesis of alkyl 2-deoxy or 2,6-dideoxy glycosides has been accomplished starting from readily available glycals, namely, tri-*O*-acetyl-D-glucal (**18**, 3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol) and 3,4-di-*O*-acetyl-6-deoxy-L-glucal (**19**, 1,5-anhydro-2,6-dideoxy-L-*arabino*-hex-1-enitol), respectively [28]. Their reaction with fatty alcohols such as octanol or dodecanol catalyzed by triphenylphosphane hydrobromide afforded the corresponding 2-deoxy (**20**, **21**) or 2,6-dideoxy glycosides (**24**, **25**), and the α-anomers were the major compounds (Scheme 3). Recently, acetonitrile was used as solvent leading to higher product yield and avoiding chlorine-containing species in the reaction mixture (unpublished results). Further deacetylation gave the amphiphilic glycosides (**22–23**, **26–27**), which were studied for their surface activity properties and screened for their antimicrobial activity.



Scheme 3 Synthesis of alkyl 2-deoxy and 2,6-dideoxy glycosides.

Amongst the alkyl 2,6-dideoxy-L-*arabino*-hexopyranosides, the dodecyl α-glycoside was the most active compound, showing strong and very strong activities toward *B. subtilis* and *B. cereus* in the

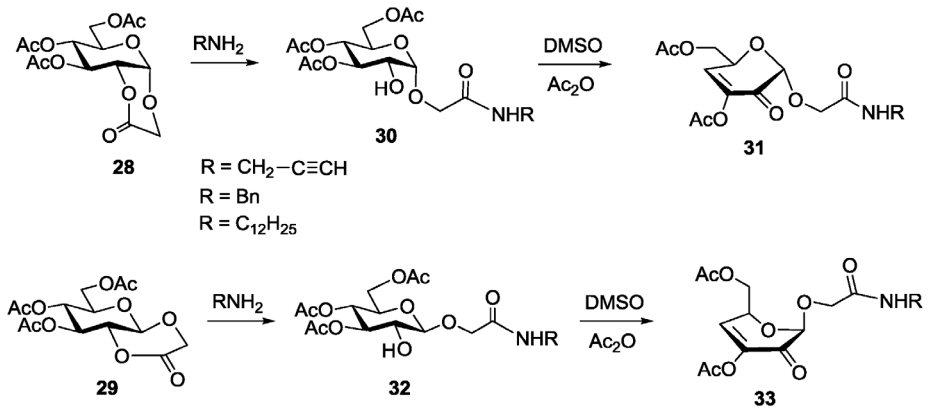
preliminary assays with the paper disk diffusion method. The latter is a clinically significant pathogen, recognized as a causative agent in both gastrointestinal and in nongastrointestinal infections [29]. Moreover, it can be a useful model for the biological weapon *Bacillus anthracis*, responsible for the acute fatal disease anthrax, owing to the genotypical and phenotypical resemblance of these microorganisms.

Using the dilution method, the dodecyl α-glycoside showed MIC values of the same order of magnitude (7.8 and 6.3 μg/mL, respectively) than those of the control chloramphenicol toward *B. cereus* and *B. subtillis*. The MIC values observed for this compound toward *Enterococcus faecalis* and *Listeria monocytogenes* were 15.6 and 31.3 μg/mL, respectively.

Considering the alkyl 2-deoxy-D-*arabino*-hexopyranosides, the most active compounds were the α- and β-dodecyl glycosides, which exhibited significant activity toward *E. faecalis*, giving an MIC value of 31.3 μg/mL (MIC value for chloramphenicol was 6.3 μg/mL). This microbe has been recognized as one of the most common bacteria involved in serious infections amongst hospitalized patients [30] and frequently acquires antibiotic resistance [30b]. Therefore, more antibiotic options toward this pathogen are required.

Another class of antibiotic glycosides with a long hydrocarbon chain in the aglycon moiety are those based on sugar enones of type 3-enopyranosid-2-ulose [31]. We were motivated to undertake the synthesis of such structures, given that the conjugated carbonyl functionality is frequently related to the bioactivity of many compounds owing to its ability to react with nucleophilic enzymes.

A straightforward methodology was implemented starting from lactones derived from carboxymethyl glycosides (CMGs). These bicyclic lactones can be accessed from readily available sugars, such as the inexpensive disaccharide isomaltulose, simply by oxidation followed by acetylation, or the unprotected monosaccharides and their 2,3,4,6-tetra-*O*-acetyl counterparts, through anomeric alkylation with *tert*-butyl bromoacetate and successive ester cleavage and cyclization [32]. These compounds offer the possibility to obtain adducts containing a free and isolated hydroxyl group at position 2 of the pyranose system, through lactone ring opening with a nucleophile, such as an amine. This was the key aspect of our synthetic approach in which oxidation of the adducts at C-2 occurs with concomitant 3,4-elimination to give the target 3-enopyranosid-2-uloses [31]. Hence, opening of α- and β-CMG-lactones (**28**, **29**) with propargyl-, benzyl-, or dodecylamine afforded the corresponding glycosides (**30**, **32**) which upon oxidation with the system DMSO/Ac_2O anhydride furnished the expected α- or β-enulosides (**31**, **33**, Scheme 4). Other mild oxidation methods, which were performed in dichloromethane, using Dess–Martin periodinane or pyridinium dichromate, less convenient because of its toxicity, proved to be less effective, giving lower yields and mixtures of the target enones with their 2-uloside counterparts.

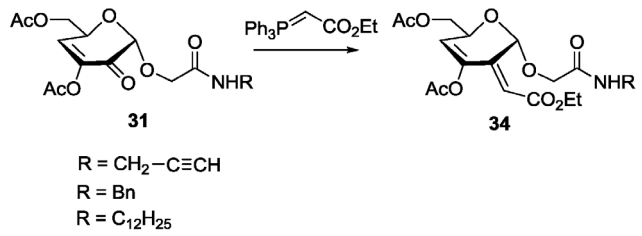


Scheme 4 Synthesis of (*N*-alkylcarbamoyl)methyl enulosides.

This strategy for 3-enopyranos-2-ulose is advantageous to the previous reported approaches starting from 2-acyloxyglycal esters, which make use of less environmentally convenient methodologies based on chlorine-containing reagents. One of them involves chlorination of the glycal to give 1,2-dichlorohexoses which, upon hydrolysis and subsequent hydrochloric acid formation, afford pyran-2-uloses, easily converted into the corresponding enones by elimination [33,34]. Another method consists of the peroxidation of 2-acetyloxyglycals with 3-chloroperbenzoic acid, providing the monohydrates of the corresponding pyranos-2-uloses, which acetylation occurs with double elimination of acetic acid to give 3-enopyranos-2-uloses [33,34].

The antimicrobial evaluation of enones **31–33** showed that only those carrying a dodecyl chain have significant antibacterial/antifungal activities.The α-enuloside showed very strong effects toward two *Bacillus* species, namely, *Bacillus subtillis* and *Bacillus cereus*, with inhibition diameters equal or higher than those of chloramphenicol. An MIC value of 2.3 μg/mL toward the pathogenic *B. cereus*, very close to that of chloramphenicol (1.2 μg/mL), was obtained. The preliminary paper disk diffusion experiments also revealed a very strong inhibitory effect of this enone against the fungal pathogen *Penicillium aurantiogriseum*, which surpassed that of the control actidione. Moreover, it showed low acute toxicity in eukaryotic hepatoma cells [31]. The β-anomer inhibited the growth of *B. cereus* at 9.4 μg/mL and displayed very strong activity against the fungi *P. aurantiogriseum* and *Aspergillus niger*, judging by the disk diffusion method, with inhibition diameters similar to or higher than those obtained for the standard antibiotic. However, this enuloside exibited moderate acute toxicity effect.

Wittig olefination of the α-enones (**31**) provided pyranoid conjugated diene esters (**34**), which were shown to display a strong activity against *E. faecalis* (Scheme 5). In particular, a low MIC value of 9.4 μg/mL was found for the N-docecyl-containing derivative, being in the same order of magnitude as that of chloramphenicol (1.2 μg/mL). However, it proved to be moderately toxic to eukaryotic cells [31].



Scheme 5 Synthesis of pyranoid-conjugated diene esters from (*N*-alkylcarbamoyl)methyl enulosides.

Antimicrobial cationic sugar-based surfactants have also appeared in the literature. D-Lysine (**35**, **36**) and D-homoarginine (**37**, **38**, Fig. 7) D-*gluco*-templated derived surfactants were prepared in a multistep synthesis starting from commercially available tetra-*O*-benzyl-D-gluconolactone [35]. These glycolipids exhibited significant antibacterial activities, particularly toward Gram-positive strains such as *S. aureus*, methicillin-resistant *S. aureus*, *Staphylococcus epidermidis* and methicillin-resistant *S. epidermidis*, with MIC values ranging from 8 to 64 μg/mL. However, it was shown that the presence of the carbohydrate moiety has little effect on the antibacterial activity, since these new derivatives showed similar effects as lysine or guanidinylated lysine-based lipid counterparts.

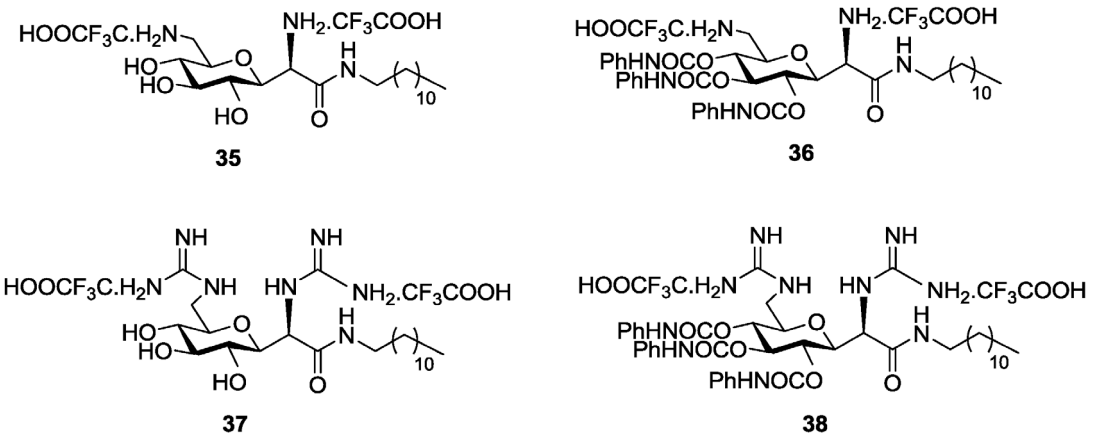
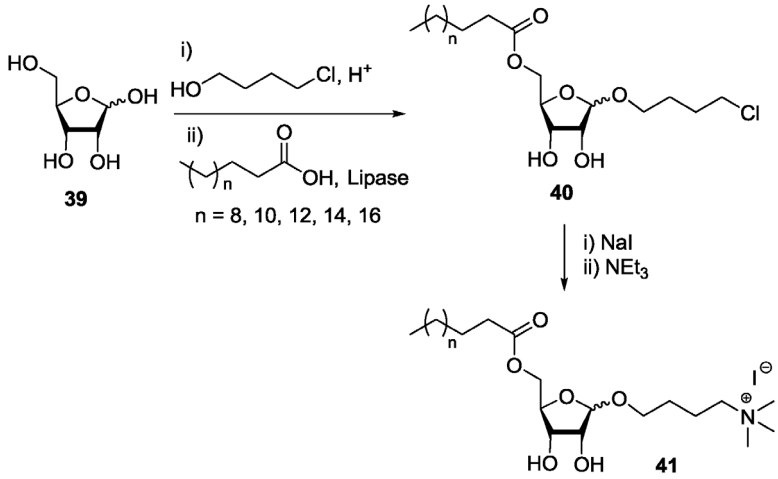


Fig. 7 D-Lysine D-*gluco*-templated surfactants **35**, **36** and D-*gluco*-templated homo-D-arginine-based surfactants **37**, **38**.

Ribose-derived surfactants were synthesized by Fischer-glycosylation of chlorohydrin with ribose (**39**, Scheme 6) and further lipase-catalyzed (from *C. antarctica*) esterification of the primary hydroxyl group with a molten fatty acid as solvent, such as dodecanoic, tetradecanoic, hexadecanoic, and octadecanoic acids [36]. Treatment of the resulting glycolipids **40** with sodium iodide, followed by addition of trimethylamine, afforded the cationic surfactants **41** (quaternary ammonium salts), which were studied for their surface tension properties and antimicrobial activity.



Scheme 6 Synthesis of ribose-derived cationic surfactants.

According to the disk diffusion method results, all compounds inhibited significantly the growth of both Gram-positive bacteria, namely, *B. subtilis* and *S. aureus*, and Gram-negative strains, such as *E. coli* and *Proteus vulgaris* and fungi as well, notably *A. niger*, *Candida albicans*, *Trichosporon beigelli*, and *Aspergillus flavus*. The most active compounds were the decanoyl, dodecanoyl, and octadecanoyl esters, which displayed diameter of inhibition zones similar or higher to those of ciprofloxacin and griseofulvin, the reference antibiotics used for bacteria and fungi, respectively.

Chitosan derivatives

Chitosan, the linear aminopolysaccharide of glucosamine and *N*-acetylglucosamine, is a biodegradable and nontoxic polymer reported to exhibit a variety of biological properties. Particularly, it has been shown to be an antimicrobial agent toward both Gram-positive and Gram-negative strains and fungi. Moreover, it is biocompatible with many organs, tissues, and mammalian cells [37].

Besides its significant activities toward human pathogenic bacteria (Table 1, [38]), chitosan has been recognized as an antimicrobial agent for plant protection and a viable alternative to the commercially available pesticides [39].

Table 1 MIC and MBC* values of chitosan solution against various bacterial strains.

Bacteria	Chitosan	
	MIC (μg/mL)	MBC (μg/mL)
E. coli K88	8	64
E. coli ATCC 25922	8	64
S. choleraesuis ATCC 50020	16	32
S. typhimurium ATCC 50013	16	64
S. aureus ATCC 25923	8	32

*MBC: minimum bactericidal concentration.

One of the most plausible mechanisms for chitosan antibacterial activity is an electrostatic interaction owing to the positive charge density of chitosan molecules (arising from the protonated NH_3^+ groups) and the negative surface charge of microbial cells owing to anionic components such as Gram-negative LPS and cell surface proteins. Such interaction may lead to disruption of the cell.

However, chitosan's biological activity is limited owing to its poor solubility in water at physiological pH. To address this drawback, chemical modifications of chitosan, particularly through quaternization of the nitrogen atoms of the amino groups, have been reported, leading to novel chitosan derivatives with improved antimicrobial properties [40].

CONCLUSIONS

Carbohydrates are major components of bacterial and fungal cell walls. Considering today's knowledge of the biosynthetic pathways that lead to their inclusion and assembly into the cell walls, one of the strategies for finding potential antimicrobial agents is to inhibit those mechanisms. The design of carbohydrate-based antibiotics addressing the enzymes involved in microbe's cell wall assembly is therefore a valuable approach.

Also, the synthesis of new amphiphilic carbohydrate derivatives, in view of their propensity to interact with the lipid bilayer of microbial cell membranes, has led to active compounds that are promising alternatives to the current antibiotics.

Design and synthesis of chlorine-free new molecules, which are interesting candidates for antibiotic research, have been developed starting from easily available carbohydrates as raw materials. The advances in existing chlorine-free methodologies for such compounds, in which chlorine-containing reagents or solvents are avoided, competing well with the known procedures, if reported, are emphasized. In this context, also environmentally benign chemoenzymatic methodologies for the production of carbohydrate fatty acid ester surfactants are shown as an alternative to their chemical synthesis with acyl chlorides.

The target compounds and synthetic approaches covered in this paper clearly show that chlorine-free chemistry concerns are rising amongst chemists and illustrate its contribution to the development of new molecular entities with promise against infectious diseases.

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