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Synthetic carbohydrate-based cell wall components from *Staphylococcus aureus*

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Glycopolymers are found surrounding the outer layer of many bacterial species. The first uses as immunogenic component in vaccines are reported since the beginning of the XX century, but it is only in the last decades that glycoconjugate based vaccines have been effectively applied for controlling and preventing several infectious diseases, such as *H. influenzae* type b (Hib), *N. meningitidis*, *S. pneumoniae* or group B *Streptococcus*. Methicillin resistant *S. aureus* (MRSA) strains has been appointed by the WHO as one of those pathogens, for which new treatments are urgently needed. Herein we present an overview of the carbohydrate-based cell wall polymers associated with different *S. aureus* strains and the related affords to deliver well-defined fragments through synthetic chemistry.

Introduction

Staphylococcus aureus is a commensal ubiquitous Gram-positive bacterium associated with a range of severe infections (skin and soft tissue infections, sepsis, arthritis, pneumonia, bacteremia and others) [1]. Although *S. aureus* can be present as asymptomatic colonizer, most of the infections are hospital acquired and

affect mainly young children, elderly, immunocompromised and post-surgical patients, leading to high health-care costs and higher risk of in-hospital deaths [2]. The rise of highly antibiotic resistant strains, such as Methicillin resistance *S. aureus* (MRSA), urges the development of new treatments [3]. In the last decade much attention has been focused on the development of active or passive immunization strategies [4].

The structurally complex cell envelope of *S. aureus* is composed of peptidoglycan, cell wall glycopolymers and proteins [5]. All of these are involved in several physiological processes and they play a key role in staphylococcal virulence, making them potential antigen candidates. Fig. 1 shows a schematic representation of *S. aureus* cell wall, highlighting the major classes of glyco-based cell wall components that have been found to be promising antigen candidates: (1) biofilm, (2) capsular polysaccharides [6], the structure of which varies between strain types and which may also be absent; (3) wall teichoic acids (WTAs) and lipoteichoic acids (LTA) [7], which are anionic glycopolymers either covalently attached (as for WTA) to the peptidoglycan or anchored to the lipid bilayer (for LTA) through hydrophobic interactions, respectively; (4) the thick peptidoglycan layer. Herein we present an overview of the major carbohydrate-based antigen candidates of *S. aureus* for which organic synthesis efforts have delivered well-defined fragments to delineate clear structure-activity relationships. These fragments are attractive tools not only for vaccine applications but also for diagnostics as well as other interaction studies (such as lectin binding) and biosynthesis studies.

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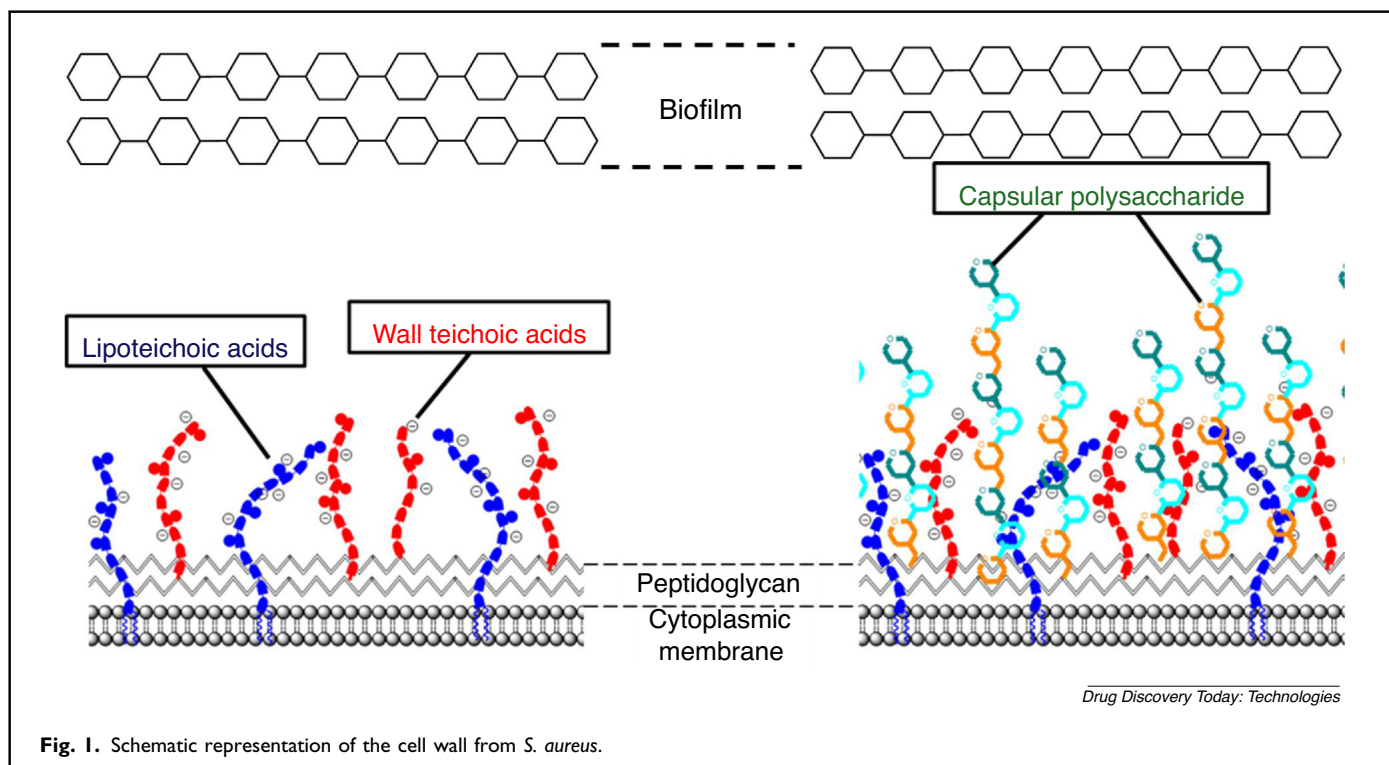


Fig. 1. Schematic representation of the cell wall from *S. aureus*.

PNAG

Biofilm formation represents one of the bacterial defence mechanisms against both host immune response and antibiotics. Poly- β -(1-6)-*N*-acetylglucosamine (PNAG) is a cell surface polysaccharide produced not only by many bacterial species, including *S. aureus*, but also fungi and protozoal parasites [8]. Thus, in the last decades much attention has been addressed to the immunological properties of PNAG and its potential application in vaccine development [9]. The structure of PNAG is shown in Fig. 2A and it has been reported that about 10-20% of the amino groups are not *N*-acetylated (dPNAG) [10].

Native PNAG and chemically deacetylated dPNAG have been conjugated to diphtheria toxoid to generate model vaccines that were evaluated in different animal models and it was observed that antibodies against dPNAG were more effective in mediating opsonophagocytic killing than the ones raised against the PNAG conjugate [11]. Native polysaccharides are generally obtained as a heterogeneous mixture of oligosaccharides, varying in length and substitution pattern with different immunological activity. Chemically synthesized, well-defined fragments, on the other hand, can be used to define the structure-immunogenicity relationships. In 2007, Nifantiev and co-workers reported the synthesis of well-defined GlcNAc and GlcN based fragments up to the undecamer level, equipped with an aminopropyl linker for further functionalization [12]. The strategy relied on the building blocks **1**, **2** and **3** (Fig. 2B) [13], in which the C-6-OH were temporarily protected with acetyl groups, while the C-3 and C-4 hydroxyl groups were protected as benzoyl esters.

The presence of a phthalimide group at the C-2-nitrogen allowed to direct the stereoselectivity of the glycosylation reactions via neighbouring group participation. In order to define structural requirements for immunogenicity, penta-saccharide **4** and nonasaccharide **5** were selected to be functionalized and conjugated to a carrier protein for immunization experiments [14]. The *N*-acetylated derivatives **6** and **7** were also generated and coupled to modified tetanus toxoid (TT) protein generating four glycoconjugates. Mice were immunized with the four synthetic glycoconjugates, after which the opsonic activity of the generated sera was evaluated. These studies confirmed that antibodies raised against the GlcN conjugates from **4** or **5** had greater opsonic activity than the ones raised against GlcNAc conjugates derived from **6** or **7**. Interestingly, the former antibodies were cross-reactive towards native PNAG and dPNAG, while dPNAG was not recognized by antibodies raised against conjugates of *N*-acetylated oligosaccharide ligands. The summary of potential cases for the use of PNAG and dPNAG related vaccine conjugates against different types of pathogens is described in the review in the same thematic issue of this journal [15].

Capsular polysaccharides (CPs)

Capsular polysaccharides (CPs) are extracellular cell-wall components comprising long polysaccharide chains covalently attached to the peptidoglycan layer. They represent a first line of defence for bacteria by shielding important cell-wall constituents and providing a mechanism to evade phagocytic uptake and killing by the host immune system [16]. They also contribute to host colonization and biofilm

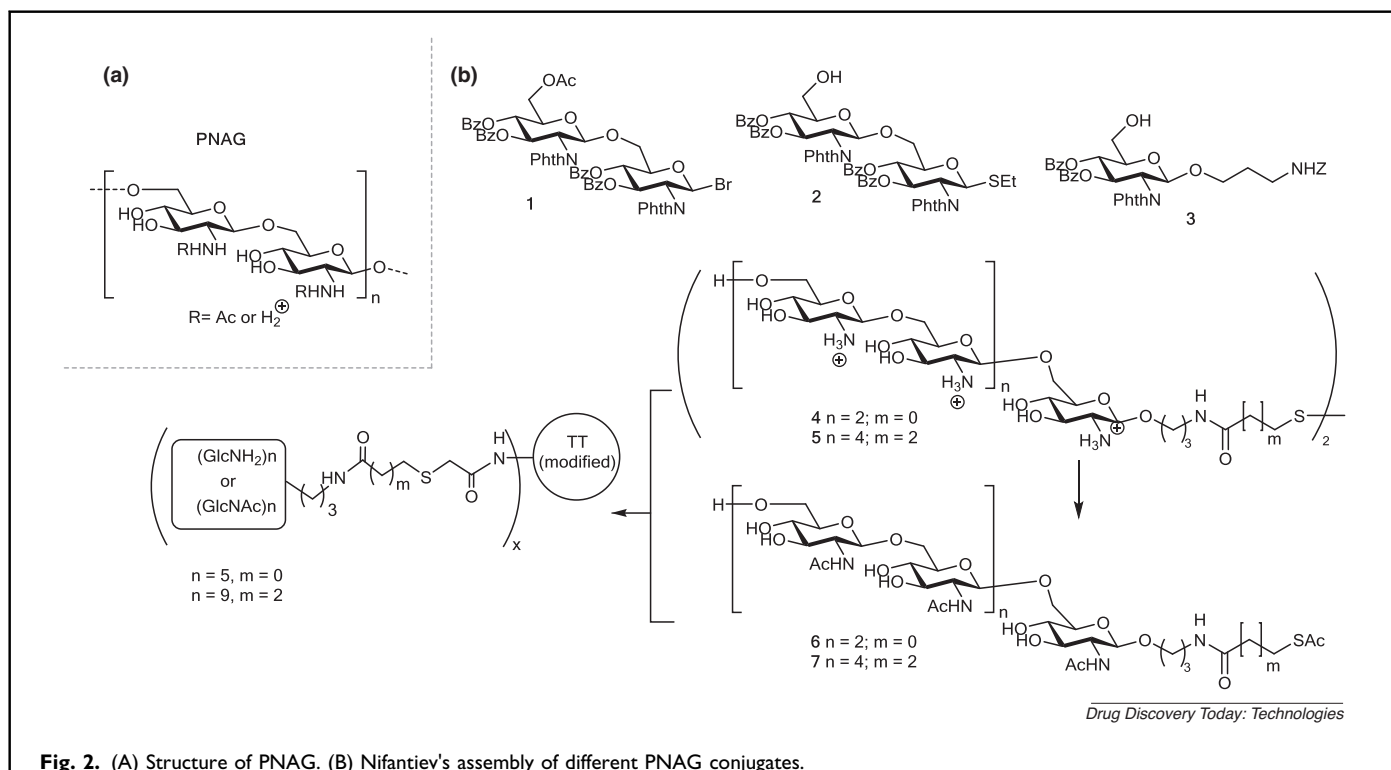


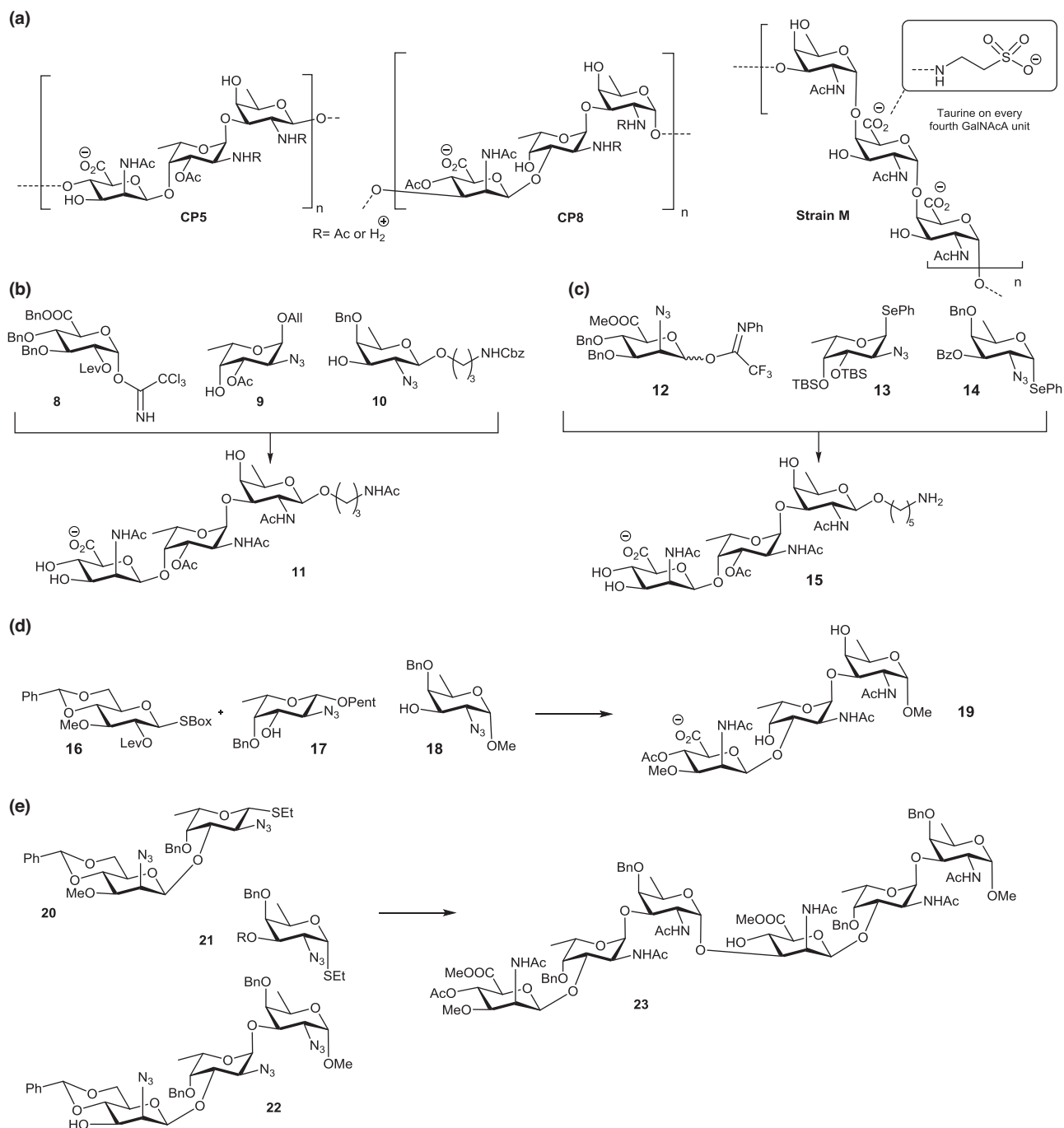
Fig. 2. (A) Structure of PNAG. (B) Nifantiev's assembly of different PNAG conjugates.

formation and thereby in the progression of invasive diseases. To date, 13 serotypes have been identified among the clinical *S. aureus* isolates, carrying different CPs [17].

S. aureus CP5 and CP8 are the most abundant among the clinical isolates, and they account for the 25%-50% of clinically encountered MRSA [18]. Most of the vaccine candidates that reached an advanced stage in clinical trials, contained either of these two CPs as antigenic component [19]. CP5 and CP8 are structurally very similar [20]. The first is constituted by a trisaccharide repeating unit composed of an *N*-acetyl mannuronic acid, β-(1,4) linked to an *N*-acetyl-*L*-fucose moiety, bearing an acetyl group at the C-3-alcohol, which is α-(1,3)-linked to an *N*-acetyl-*D*-fucose. The repeating units are linked through an β-(1,4)-linkage between the latter fucose and the mannuronic of the next repeating unit (Fig. 3A). The trisaccharide unit from CP8 is composed of the same monosaccharides, but the mannuronic acid is β-(1,3) linked to an *N*-acetyl-*L*-fucose and the acetyl substituent is placed on the C-4-hydroxy of the mannuronic acid unit and the *N*-acetyl-*D*-fucose is linked to the C-3 of the mannuronic acid through an α-linkage (Fig. 3A). These structures have been shown to possess zwitterionic character originating from the presence of a negative charge from the mannuronic acid and a positive charge, resulting from (random) deacetylation of one of the two fucose residues. Currently it is not known how many positive charges are found in these structures and where exactly they reside in the polysaccharides [21].

The synthesis of these two CP trisaccharide units represents a great challenge due to the presence of several 1,2-*cis* glycosidic linkages, the anionic charges and the *O*-acetyl

substituents. Fig. 3B and C summarizes two approaches developed to date to assemble these challenging structures. The first synthesis of the CP5 repeating unit was reported by Adamo and co-workers (Fig. 3B) [22]. In their strategy, a benzyl glucuronate imidate donor **8** was used to introduce the β-linkage to the C-4 of the *L*-fucose, after which it was transformed into the required mannosaminuronic acid. The final glycosylation with a spacer equipped fucose building block **10** proceeded with poor selectivity, but enough material was generated to allow for completion of the synthesis. From the immunological evaluation by competitive ELISA and immunodot blot experiments, it has become clear that longer fragments are needed to be sufficiently antigenic and effectively mimic the native CP structures. After this first synthesis, different approaches to generate the same trisaccharide have been reported by Boons [23], Demchenko [24], Codée [25], and Kulkarni [26]. The approach by Codée differed from the other strategies as a mannosaminuronic acid building block was employed (Fig. 3C). The use of compound **12** obviated the need for a late stage oxidation reaction and it secured the stereoselective construction of the challenging 1,2-*cis* mannosaminuronic acid linkage. The stereoselectivity in the construction of the fucosyl linkages was controlled by adjusting the reactivity of the fucosyl donors. Thus, the relatively disarmed donor **13** was used to construct the 1,2-*cis* fucosamine linkage, likely through the generation of an α-triflate intermediate, whereas a relatively armed fucoside synthon (**14**) was employed for the formation of the 1,2-*trans* fucosyl linkage with aminopentanol linker.



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Fig. 3. (A) Structures of trisaccharide repeating units of *S. aureus* CPS type 5 and type 8 and strain M. (B) Adamo's synthesis of CP5 trisaccharide repeating unit **11**. (C) Codée's synthesis of CP5 trisaccharide repeating unit **15** using a mannosaziduronic acid building block. (D) Demchenko's assembly of the CP8 trisaccharide **19**. (E) Demchenko's synthesis of a protected CP8 hexasaccharide.

Up to now only one synthetic route towards the CP8 trisaccharide unit has been described [27]. In 2015 Demchenko and co-workers reported the synthesis of the trisaccharide bearing methyl groups at the sites of propagation (**19**, Fig. 3D). In line with Adamo's strategy for the assembly of CP5 trisaccharide **11**, a glucosyl donor was used as a precursor for the mannosaminuronic acid. Later Demchenko and

co-workers also reported the assembly of a hexamer fragment (Fig. 3E) [28]. At first a [3+3] strategy was explored, but no glycosylation occurred probably due to steric hindrance. Therefore, a different synthetic approach was adopted based on a [2+1+3] strategy using building blocks **20**, **21** and **22**. Unfortunately, deprotection proved challenging and the required deprotected fragment could not be obtained. This

illustrates well the difficulties associated with the assembly of these complex glycans. To date, no extensive biological studies have been reported using the synthetic CP5 or CP8 trisaccharides. However, as described above, it can be concluded from the competitive ELISA assay and immunodot blot analysis performed by Adamo, that longer fragments will be necessary to understand the key structural immunogenic elements of these saccharides. This is a clear incentive to develop more effective synthetic chemistry to procure these challenging structures.

Fig. 3A also depicts the capsular polysaccharide from *S. aureus* strain M, the isolation of which was reported in 1969 by Smith [29]. The complete molecular structure was elucidated by Murthy *et al.* in 1974 [30] and it was shown that the repeating unit consists of an *N*-acetyl-D-fucose $\alpha(1,4)$ -linked to an *N*-acetyl-D-galacturonic acid, which is $\alpha(1,4)$ linked to a second *N*-acetyl-D-galacturonic acid, connecting to the next fucose moiety of the next trisaccharide repeating unit through an $\alpha(1,3)$ -linkage. It has been described that a taurine unit is incorporated in one out of four GalNAcA sugars through an amide bond. One synthesis of the trisaccharide repeating unit has been reported [31]. For the construction of the challenging α -galactosaminuronic acid linkages, silylidene protected galactosamine building blocks were used as these represent the most reliable type of galactosamine donors to furnish the required 1,2-*cis* linkages [32].

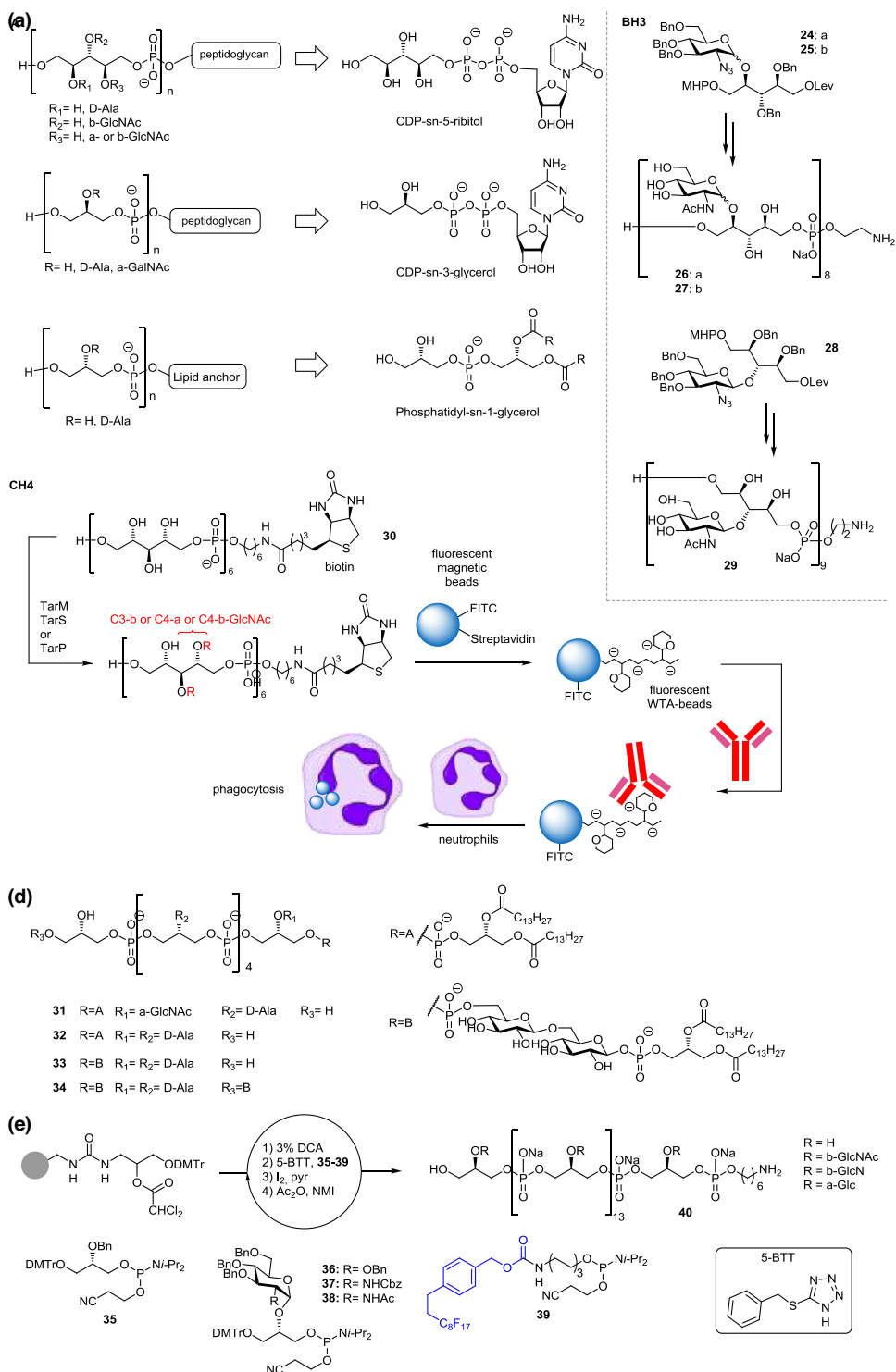
Wall and lipoteichoic acids

Teichoic acids (from the Greek word τεχοζ, fortified wall) are structurally diverse anionic carbohydrate-based polymers that can be found in the cell wall of the majority of Gram-positive bacteria. They are divided in two main classes: wall teichoic acids (WTAs), which are covalently attached to the peptidoglycan matrix, while lipoteichoic acids (LTAs) are inserted in the lipid bilayer through a diacyl glycerol lipid anchor. They are involved in several important biological processes contributing to bacterial fitness and virulence [33]. Since TAs are exposed to the extracellular milieu, they constitute possible recognition sites for host cell surface lectins, antibodies of the host immune system and phage binding [34]. TAs have been considered to be suitable antigen candidates for vaccine development [35]. The WTA from *S. aureus* is generally constituted by 1 \rightarrow 5-linked ribitol phosphate (RboP) units, which can be modified with D-alanine substituents at the C-2 and α - or β -*N*-acetylglucosamine appendages at C3 or C4 (Fig. 4A) [32]. However, some strains carry structurally different WTAs. For example, the WTA from *S. aureus* ST395 is composed of 3 \rightarrow 1 linked glycerol phosphate (GroP) chains, where the C-2 position can be decorated with either D-Ala or α -GalNAc substituents (Fig. 4A) [36]. In contrast, *S. aureus* LTAs feature 1 \rightarrow 3 linked GroP-oligomers having D-Ala or α -GlcNAc substituents [37]. Notably, the GroP-backbone of WTA and LTA are enantiomeric structures,

whose stereochemistry has been assigned based on their biosynthetic pathways. While GroP-WTA is built by oligomerization of CDP-*sn*-3-glycerol, LTAs are constructed using phosphatidyl-*sn*-1-glycerol (Fig. 4A) [38].

In 2006 Pozsgay and co-workers reported the synthesis of an RboP-octamer and dodecamer, equipped with an amino spacer for further conjugation to BSA as carrier protein [39]. Their synthetic strategy relied on the introduction of the phosphodiester linkage using the phosphoramidite approach, developed for solid-phase oligonucleotide assembly. While the solution phase chemistry proceeded uneventfully, it proved too difficult to translate the chemistry to an automated solid phase format. The generated octa- and dodecamer were conjugated to BSA to generate a prototype vaccine, but no evaluation of the WTA-BSA conjugates has been reported to date. Three RboP oligomers were recently synthesized by a team at Sanofi Pasteur, featuring either α -D-GlcNAc or β -D-GlcNAc at all C-4 hydroxyls of a RboP octamer (**26** and **27**) or a β -D-GlcNAc at all C-3 hydroxyls of a RboP nonamer (**29**, Fig. 4B) [40]. Also this group used phosphoramidite chemistry to construct the phosphodiester linkages. A protecting group scheme was developed that allowed for a convergent assembly approach. To this end the orthogonal pair of a levulinoyl ester and *para*-methoxyphenyl (MP) ether was used. All coupling events proceeded uneventfully, showing the reliability of the phosphoramidite chemistry even in the condensation of large blocks. All fragments were equipped with a linker to generate TA-protein conjugates. Notably, the spacer was attached to these WTA oligomers at the 'non-peptidoglycan' side. To access the immunological properties of the different glycosylated RboP oligomers, the synthetic fragments **26**, **27** and **29**, alongside native RboP isolated from different strains, were conjugated to rEPA or *S. aureus* alpha toxin (HladM). The conjugation was achieved via carbodiimide condensation reactions using hydrazide linker derivatives. Immunization in mice was performed using conjugated and non-conjugated fragments, with or without adjuvant, and IgG1 and IgG2 titers were determined after 0, 21, 35 and 42 days. A strong and robust immune response was elicited when the conjugates were used in combination with an adjuvant. No differences were detected between synthetic and native TAs, but it was observed that the C-4- β -GlcNAc RboP-conjugate was able to induce antibodies that were cross-reactive towards different *S. aureus* strains, carrying either C-4- β -GlcNAc or C-3- β -GlcNAc substituents, suggesting that this antigen can be used for the development of a broad-spectrum *S. aureus* vaccine.

The role of the different glycosylation patterns on *S. aureus* WTA is under active investigation. Recently it has been suggested that changing Rbo-GlcNAc glycosylation might contribute to *S. aureus* escape from host immune surveillance [41]. In a study by Peschel and coworkers, a screen on *S. aureus* genomes was performed with the intent of identifying paralogues of



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Fig. 4. (A) General structure of a RboP-WTA, GroP-WTA and GroP-LTA from *S. aureus* and their biosynthetic precursors CDP-sn-5-ribitol CDP-sn-3-glycerol and phosphatidyl-sn-1-glycerol. (B) The assembly of WTA oligomers at Sanofi-Pasteur. (C) Chemo-enzymatic synthesis of glycosylated WTA hexamers for the generation of fluorescent magnetic WTA-beads for antibody binding. (D) A selection of GroP-LTA fragments of *S. aureus* synthesized by Schmidt's group. (E) Automated solid phase assembly of LTA-pentadecamers bearing different glycan appendages.

WTA biosynthesis genes. It was found that three *S. aureus* prophages were able to encode for a glycosyl transferase, TarP, which was shown to be 27% identical to the well-known TarS enzyme, that transfers the β -GlcNAc to the RboP units. Synthetic RboP fragments were used to unravel the activity of this glycosyl transferase and it was shown that while TarS places the carbohydrate on the C4 of the ribitol backbone, TarP places the β -GlcNAc on the C3 position. In order to probe the effect on immunogenicity of these subtle differences in glycosylation activity, sera from healthy human donors were evaluated for binding with the different β -GlcNAc-WTA types. A relatively low IgG titer towards TarP glycosylated Rbo-WTA was found as compared to the TarS homologue, leading to the hypothesis that the former enzyme can be used by *S. aureus* for immune evasion. Different results however were obtained by Van Sorge's group, who used the synthetic hexamer for the generation of enzymatically glycosylated Rbo-WTA structures (Fig. 4C) [42]. In this study compound **30** was first biotinylated and subsequently glycosylated with either TarS (delivering C4- β GlcNAc), TarM (leading to C4- α GlcNAc) or TarP (yielding C3-GlcNAc). The enzymatically glycosylated fragments were immobilized on streptavidin coated beads and used to probe binding of IgG antibodies present in pooled sera of healthy human donors. It was observed that the titer of IgGs against the TarS glycosylated WTA was higher than the titer against the TarP glycosylated WTA, but the level of antibodies against the latter was still significant. The designed protocol (Fig. 4C) using well defined fragments proved to be much more sensitive than the assay used by Gerlach *et al.* [40]. The WTA-beads have also been used to probe antibody mediated phagocytosis, showing effective uptake of the TarS and TarP modified WTA-beads.

A large set of *S. aureus* type LTA-oligomers has been synthesized by Schmidt and co-workers. Fig. 4D shows a selection of these fragments, in which a lipid anchor as well as α -GlcNAc and D-Ala substituents were incorporated [43]. For the assembly of these structures, Schmidt and co-workers used phosphoramidite building blocks. To introduce the labile D-Ala moieties, the oligomers were assembled masking the alcohols that were to carry the ester with PMB-ethers. After oxidative cleave of these ethers, the D-Ala esters were formed, after which a final hydrogenation delivered the target compounds. The set of oligomers were used to establish the structural requirements needed to elicit an innate immune response. As assessed by cytokine production in a whole blood assay, it was observed that both lipid anchor and positively charged D-Ala substituents were important structural features for the innate immune stimulating activity [44].

Different solid phase approaches have been developed for the construction of GroP oligomers. Snapper and co-workers [45] used a phosphoramidite building block, having a DMTr as orthogonal protecting group at the site of elongation, and a benzoyl group at the secondary alcohol, even though it is

known that migration to the primary position readily takes place. Snapper generated a GroP decamer on a controlled pore glass solid support, and after release from the resin and concomitant removal of all protecting groups the crude product (no purification or characterisation of the material has been reported) was conjugated to tetanus toxoid (TT). Mice serum raised against this glycoconjugate showed to mediate opsonophagocytic killing of *S. aureus in vitro* and protection in an *in vivo* bacteremia model. Codée and co-workers reported the synthesis of a large set of GroP-based TA fragments using automated solid phase synthesis (Scheme 4E) [46]. A universal solid support was used in order to avoid the need of linker functionalized building blocks and at the end of the assembly a fluororous aminospacer phosphoramidite **39** was used to facilitate purification of the long fragments. Several pentadecamers were synthesized having different carbohydrate substituents, including α -GlcNAc from *S. aureus* LTA, at different position along the chain. The generated library was applied for the generation of a TA-based microarray. This technology allows the rapid, simultaneous screening of biomolecules using low amounts of ligands and it was used to explore the binding of different antibodies. First a monoclonal antibody raised against *S. epidermidis* LTA was used to access the feasibility of the assay. Subsequently, rabbit serum raised against native LTA from *E. faecalis* 12030 was used and a high titer of IgG antibodies that were directed exclusively towards glycosylated fragments was detected. In the case of serum raised against a synthetic LTA GroP-glycoconjugate, carrying a single terminal glucose appendage (WH7-BSA), a very specific interaction was observed not only depending on the type of substituent (Glc vs GlcNH₂ or GlcNAc), but also regarding the position of the monosaccharide along the chain, suggesting that a specific immune response can be elicited when a vaccine modality with a well-defined TA fragment is used.

Conclusion

Because of the rise of antibiotic resistant *S. aureus* strains much effort has been focussed on the development of vaccines to combat these bacteria. Initial immunization experiments based on the use of cell wall glycans have proved to be promising, although these vaccines have not yet progressed into further advanced stages of clinical testing. Many questions remain regarding the different and varying sugar coats of *S. aureus* in relation to host-pathogen interactions and vaccination purposes. Establishing detailed structure activity/immunogenicity relationship will be required to unravel which structures are optimal for the development of effective *S. aureus* treatments. In the last decade, much progress has been made in the generation of well-defined carbohydrate-based structures. This review has provided an overview of the synthetic strategies reported for the assembly of cell surface and capsular polysaccharides, as well different glycosyl

substituted teichoic acids oligomers. For the latter class of compounds DNA-type chemistry has been harnessed to enable the automated assembly of libraries of different oligomers, varying in length and substitution pattern. An outstanding challenge remains the incorporation of the labile D-alanine residues in the structures. This will have to be done in conjunction with the development of a strategy that enables the rapid generation of a larger library of TAs with varying glycosylation patterns. The assembly of *S. aureus* capsular polysaccharides has proven to be significantly more challenging and although single repeating units have been synthesized of the most prevalent serotypes, longer fragments, encompassing more than one repeating unit has not been achieved. From the limited interaction studies reported to date it has become clear that longer structures are required for high affinity interactions. Synthetic fragments cannot only be employed for vaccine purposes, but also to probe binding to various cell-surface receptors and phage proteins and to unravel biosynthesis pathways.

Conflict of interest

None.

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References

- [1] a) Lowy FD. Staphylococcus aureus infections. *N Engl J Med* 1998;339:520–32;Cb) Wertheim HFL, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, et al. The role of nasal carriage in Staphylococcus aureus infections. *Lancet Infect Dis* 2005;5:751–62;Cc) Miller LS, Cho JS. Immunity against Staphylococcus aureus cutaneous infections. *Nat Rev Immun* 2011;11:505–18.
- [2] a) Klein E, Smith DL, Laxminarayan R. Hospitalizations and deaths caused by methicillin-resistant Staphylococcus aureus, United States, 1999–2005. *EID J* 2007;13:1840–6;Cb) Anderson DJ, Arduino JM, Reed SD, Sexton DJ, Kaye KS, Grussemeyer CA, et al. Variation in the type and frequency of postoperative invasive Staphylococcus aureus infections according to type of surgical procedure. *Infect Control Hosp Epidemiol* 2010;31:701–9;Cc) Noskin GA, Rubin RJ, Schentag JJ, Kluytmans J, Hedblom EC, Jacobson C, et al. Infectious diseases consultation lowers mortality from Staphylococcus aureus bacteremia. *Clin Infect Dis* 2007;45:1132–40;Cd) Founou RC, Founou LL, Essack SY. Clinical and economic impact of antibiotic resistance in developing countries: a systematic review and meta-analysis. *PLoS One* 2017;12(12). [eo189621](https://doi.org/10.1371/journal.pone.0189621).
- [3] a) Jevons M. 'Celbenin'-resistant Staphylococci. *BMJ* 1961;1:124–5;Cb) Monaco M, de Araujo FP, Cruciani M, Coccia EM, Pantosti A. Worldwide epidemiology and antibiotic resistance of Staphylococcus aureus. *Curr Top Microbiol Immun* 2017;409:21–56;Cc) Lee AS, de Lencastre H, Garau J, Kluytmans J, Malhorta-Kumar S, Peschel A, et al. Methicillin-resistant Staphylococcus aureus. *Nat Rev Dis Prim* 2018;4:18033;Cd) Taconelli E, Carrara E, Savoldi A, Harbath S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis* 2018;18:318–27;Ce) Mulani MS, Kamble EE, Kumbar SN, Tawre MS, Pardesi KR. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. *Front Microbiol* 2019;10(539).
- [4] Pozzi C, Olaniyi R, Liljeroos L, Galgani I, Rappuoli R, Bagnoli F. Vaccines for Staphylococcus aureus and target populations. *Curr Top Microbiol Immun* 2016;409:491–528.
- [5] a) Vollmer W, Blanot D, de Pedro MA. Peptidoglycan structure and architecture. *FEMS Microbiol Rev* 2008;32:149–67;Cb) Silhavy TJ, Kahne D, Walker S. The bacterial cell envelope. *Cold Spring Harb Perspect Biol* 2010;2:a000414;Cc) Hanson BR, Neely MN. Coordinate regulation of Gram-positive cell surface components. *Curr Opin Microbiol* 2012;15:204–10.
- [6] Weidenmaier C, Lee JC. Structure and function of surface polysaccharides of Staphylococcus aureus. *Curr Top Microbiol Immun* 2017;409:57–93.
- [7] Xia G, Kohler T, Peschel A. The wall teichoic acid and lipoteichoic acid polymers of Staphylococcus aureus. *Int J Med Microbiol* 2010;300:148–54.
- [8] Cywes-Bentley C, Skurnik D, Zaidi T, Roux D, Deoliveira RB, Garrett WS, et al. Antibody to a conserved antigenic target is protective against diverse prokaryotic and eukaryotic pathogens. *PNAS* 2013;110(24):E2209–18.
- [9] a) Maira-Litran T, Kropec A, Abeygunawardana C, Joyce J, Mark G, Goldmann DA, et al. Immunochemical properties of the staphylococcal poly-N-acetylglucosamine surface polysaccharide. *Infect Immun* 2002;70(8):4433–40;Cb) Cerca N, Jefferson KK, Maira-Litran T, Pier DB, Kelly-Quintos C, Goldmann DA, et al. Molecular basis for preferential protective efficacy of antibodies directed to the poorly acetylated form of staphylococcal poly-N-acetyl-(1-6)-glucosamine. *Infect Immun* 2007;75(13):3406–13;Cc) McKenney D, Pouliot KL, Wang Y, Murthy V, Ulrich M, Doring G, et al. Broadly protective vaccine for Staphylococcus aureus based on an in vivo-expressed antigen. *Science* 1999;284:1523–7.
- [10] a) Sadovskaya I, Vinogradov E, Flahaut S, Kogan G, Jabbouri S. Extracellular carbohydrate-containing polymers of a model biofilm-producing strain, Staphylococcus epidermidis RP62A. *Infect Immun* 2005;73(5):3007–17;Cb) Vuong C, Kocianova S, Voyich JM, Yao Y, Fischer ER, DeLeo FR, et al. A crucial role for exopolysaccharide modification in bacterial biofilm formation, immune evasion, and virulence. *J Biol Chem* 2004;279(52):54881–86.
- [11] Maira-Litran T, Kropec A, Goldmann DA, Pier GB. Comparative opsonic and protective activities of Staphylococcus aureus conjugate vaccines containing native or deacetylated Staphylococcal poly-N-Acetyl-β-(1-6)-glucosamine. *Infect Immun* 2005;73:6752–62.
- [12] Gening ML, Tsvetkov YE, Pier GB, Nifantiev NE. Synthesis of beta-(1→6)-linked glucosamine oligosaccharides corresponding to fragments of the bacterial surface polysaccharide poly-N-acetylglucosamine. *Carbohydr Res* 2007;342:567–75.
- [13] Gening ML, Tsvetkov YE, Pier GB, Nifantiev NE. The study of the reaction of terminated oligomerization in the synthesis of oligo-(β1-6)-glucosamines. *Russ J Bioorg Chem* 2006;32:432–43.
- [14] Gening ML, Maira-Litran T, Kropec A, Skurnik D, Grout M, Tsvetkov YE, et al. Synthetic β-(1→6)-linked N-acetylated and nonacetylated oligoglucosamines used to produce conjugate vaccines for bacterial pathogens. *Infect Immun* 2010;78(2):764–72.
- [15] Gening ML, Pier GB, Nifantiev NE. Broadly protective semi-synthetic glycoconjugate vaccine against pathogens capable of producing poly-b-(1→6)-N-acetyl-D-glucosamine exopolysaccharide. *Drug Discov Today Technol* 2020. [http://dx.doi.org/10.1016/j.ddtec.2020.09.002](https://doi.org/10.1016/j.ddtec.2020.09.002).
- [16] Karakawa WW, Sutton A, Schneerson R, Karpas A, Vann WF. Capsular antibodies induce type-specific phagocytosis of capsulated Staphylococcus aureus by human polymorphonuclear leukocytes. *Infect Immun* 1988;56:1090–5.
- [17] O'Riordan K, Lee JC. Staphylococcus aureus capsular polysaccharides. *Clin Microbiol Rev* 2004;17(1):218–34.
- [18] a) Roghmann M, Taylor KL, Gupte A, Zhan M, Johnson JA, Cross A, et al. Epidemiology of capsular and surface polysaccharide in Staphylococcus aureus infections complicated by bacteraemia. *J Hosp Infect* 2005;59(1):27–32;Cb) Verdier I, Durand G, Bes M, Taylor KL, Lina G, Vandenesch F, et al. Identification of the capsular polysaccharides in Staphylococcus aureus clinical isolates by PCR and agglutination tests. *J Clin Microbiol* 2007;45(3):725–9.

- [19] Giersing BK, Dastgheyb SS, Modjarrad K, Moorthy V. Status of vaccine research and development of vaccines for *Staphylococcus aureus*. *Vaccine* 2016;34:2962–6.
- [20] a) Vann WF, Moreau M, Sutton A, Byrd RA, Karakawa WW, Vann WF, et al. Structure and immunochemistry of *Staphylococcus aureus* capsular polysaccharides. *UCLA Symp Mol Cell Biol* 1988;64:187–98;Cb) Moreau M, Richards JC, Fournier J-M, Byrd RA, Karakawa WW, Vann WF. Structure of the type 5 capsular polysaccharide of *Staphylococcus aureus*. *Carbohydr Res* 1990;201:285–97;Cc) Jones C. Revised structures for the capsular polysaccharides from *Staphylococcus aureus* Types 5 and 8, components of novel glycoconjugate vaccines. *Carbohydr Res* 2005;340:1097–106.
- [21] Tzianabos AO, Wang JY, Lee JC. Structural rationale for the modulation of abscess formation by *Staphylococcus aureus* capsular polysaccharides. *PNAS* 2001;98(16):9365–70.
- [22] Danieli E, Proietti D, Brogioni G, Romano MR, Cappelletti E, Tontini M, et al. Synthesis of *Staphylococcus aureus* type 5 capsular polysaccharide repeating unit using novel L-FucNAc and D-FucNAc synthons and immunochemical evaluation. *Bioorg Med Chem* 2012;20:6403–15.
- [23] Gagarinov IA, Fang T, Liu L, Srivastava AD, Boons GJ. Synthesis of *Staphylococcus aureus* Type 5 trisaccharide repeating unit: solving the problem of lactamization. *Org Lett* 2015;17:928–31.
- [24] Yasomane JP, Visansirikul S, Pornsuriyasak P, Thompson M, Kolodziej SA, Demchenko AV. Synthesis of the repeating unit of capsular polysaccharide *Staphylococcus aureus* Type 5 to study chemical activation and conjugation of native CPS. *J Org Lett* 2016;17:2382–4.
- [25] Hagen B, Ali S, Overkleef HS, van der Marel GA, Codée JDC. Mapping the reactivity and selectivity of 2-azidofucosyl donors for the assembly of N-acetylfucosamine-containing bacterial oligosaccharides. *J Org Chem* 2017;82:848–68.
- [26] Behera A, Rai D, Kulkarni SS. Total syntheses of conjugation-ready trisaccharide repeating units of *Pseudomonas aeruginosa* O11 and *Staphylococcus aureus* Type 5 Capsular Polysaccharide for vaccine development. *J Am Chem Soc* 2020;142(1):456–67.
- [27] Visansirikul S, Yasomane JP, Pornsuriyasak P, Kamat MN, Podvalnyy NM, Gobble CP, et al. A Concise Synthesis of the Repeating Unit of Capsular Polysaccharide *Staphylococcus aureus* Type 8. *Org Lett* 2015;17:2382–4.
- [28] Visansirikul S, Kolodziej SA, Demchenko AV. Synthesis of oligosaccharide fragments of capsular polysaccharide *Staphylococcus aureus* type 8. *J Carb Chem* 2020. <http://dx.doi.org/10.1080/07328303.2020.1821042>.
- [29] Scott AC. A capsulate *Staphylococcus aureus*. *J Med Microbiol* 1969;2:253–60.
- [30] Liau D-F, Melly MA, Hash JH. Surface polysaccharide from *Staphylococcus aureus* M that contains taurine, D-aminogalacturonic acid, and D-fucosamine. *J Bacteriol* 1974;119:913–22.
- [31] Hagen B, van Dijk MJH, Zhang Q, Overkleef HS, van der Marel GA, Codée JDC. Synthesis of the *Staphylococcus aureus* Strain M Capsular Polysaccharide Repeating Unit. *Org Lett* 2017;19:2514–7.
- [32] Akihiro I, Hiromune A, Hideharu I, Makoto K. Di-tert-butylsilylene-directed α -selective synthesis of 4-Methylumbelliferyl T-antigen. *Org Lett* 2005;7(20):4415–8.
- [33] Xia G, Kohler T, Peschel A. The wall teichoic acid and lipoteichoic acid polymers of *Staphylococcus aureus*. *Int J Med Microbiol* 2010;300:148–54.
- [34] a) Matias VR, Beveridge TJ. Native cell wall organization shown by cryo-electron microscopy confirms the existence of a periplasmic space in *Staphylococcus aureus*. *J Bacteriol* 2006;188(3):1011–21;Cb) Xia G, Corrigan RM, Winstel V, Goerke C, Grundling A, Peschel A. Wall teichoic Acid-dependent adsorption of staphylococcal siphovirus and myovirus. *J Bacteriol* 2011;193(15):4006–9;Cc) Takahashi K, Kurokawa K, Moyo P, Jung DJ, An JH, Chigweshe L, et al. Intradermal immunization with wall teichoic acid (WTA) elicits and augments an anti-WTA IgG response that protects mice from methicillin-resistant *Staphylococcus aureus* infection independent of mannose-binding lectin status. *PLoS One* 2013;8(8):e69739.
- [35] Theilacker C, Kaczynski Z, Kropec A, Fabretti F, Sange T, Holst O, et al. Opsonic antibodies to *Enterococcus faecalis* Strain 12030 are directed against Lipoteichoic Acid. *J Infect Immun* 2006;74(10):5703–12.
- [36] Endl J, Seidl PH, Fiedler F, Schleifer KH. Determination of cell wall teichoic acid structure of staphylococci by rapid chemical and serological screening methods. *Arch Microbiol* 1984;137:272–80.
- [37] Kho K, Meredith TC. Salt-induced stress stimulates a Lipoteichoic Acid-specific three-component glycosylation system in *Staphylococcus aureus*. *J Bacteriol* 2018;200:e00017–18.
- [38] Brown S, Santa Maria JP, Walker S. Wall teichoic acids of gram-positive bacteria. *Annu Rev Microbiol* 2013;67:313–36.
- [39] Fekete A, Hoogerhout P, Zomer G, Kubler-Kielb J, Schneerson R, Robbins JB, et al. Synthesis of octa- and dodecamers of D-ribitol-1-phosphate and their protein conjugates. *Carbohydr Res* 2006;341(12):2037–48.
- [40] Driguez P-A, Guillo N, Rokbi B, Mistretta N, Talaga P, WO Patent WO 2017/064190 A1. 2017, 2016/10/13
- [41] Gerlach D, Guo Y, De Castro C, Kim SH, Schlatterer K, Xu FF, et al. Methicillin-resistant *Staphylococcus aureus* alters cell wall glycosylation to evade immunity. *Nature* 2018;563(7733):705–9.
- [42] van Dalen R, Molendijk MM, Ali S, van Kessel KPM, Aerts P, van Strijp JAG, et al. Do not discard *Staphylococcus aureus* WTA as a vaccine antigen. *Nature* 2019;572.
- [43] Stadelmaier A, Morath S, Hartung T, Schmidt RR. Synthesis of the first fully active lipoteichoic acid. *Angew Chem Int Ed Engl* 2003;42(8):916–20.
- [44] Morath S, Stadelmaier A, Geyer A, Schmidt RR, Hartung TJ. Synthetic Lipoteichoic Acid from *Staphylococcus aureus* is a potent stimulus of cytokine release. *J Exp Med* 2002;195(12):1635–40.
- [45] Chen Q, Dintaman J, Lees A, Sen G, Schwartz D, Shirtliff ME, et al. Novel Synthetic (Poly)Glycerolphosphate-Based Antistaphylococcal Conjugate Vaccine. *Infect Immun* 2013;81(7):2554–61.
- [46] van der Es D, Berni F, Hogendorf WFJ, Meeuwenoord N, Laverde D, van Diepen A, et al. Streamlined synthesis and evaluation of Teichoic Acid fragments. *Chem Eur J* 2018;24(16):4014–8.