Universiteit
Leiden
The Netherlands

Synthesis, structure and epitope mapping of well-defined Staphylococcus aureus capsular polysaccharides

Østerlid, K.E.

Citation

Østerlid, K. E. (2025, May 22). *Synthesis, structure and epitope mapping of well-defined Staphylococcus aureus capsular polysaccharides*. Retrieved from https://hdl.handle.net/1887/4246935

Version:	Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/4246935

Note: To cite this publication please use the final published version (if applicable).

Chapter 1

General introduction and outline

In the modern world, the rise of antibiotic-resistant bacteria has become a major threat to the health care system. A large percentage of the infections caused by multi-drug resistant bacteria today arises from the ESKAPE pathogens,[1] which have been marked by WHO as high priority pathogens. The ESKAPE pathogens include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp. All these bacteria have learned to adapt to the modern health care environment. In the recent years much effort has been invested in finding new treatment regiments for the ESKAPE pathaogens,[2] including therapeutic strategies using different antibiotic combinations, bacteriophages and photodynamic therapies.[3,4] *Staphylococcus aureus* (*S. aureus*), is one of the ESKAPE pathogens causing hospital-acquired infections, and with the rise of antibiotic-resistant strains,[5] such as methicillin-resistant *S. aureus* (MRSA)[6] and vancomycin-resistant *S. aureus* (VRSA)[7] the development of new therapeutic strategies is urging. To face the problems associated with antimicrobial resistance (AMR), efforts have been directed on improving clinical management, increasing the speed of diagnosis, developing (new) antimicrobial treatment,[8,9] but also on active or passive vaccination strategies.[4,10] *S. aureus* is a Gram-positive human pathogen and it is found in the environment, but is also part of the normal human microbiome[11] and it is one of the most common opportunistic pathogens. It is found in human mucous membranes and skin, and does normally not cause infections, but when the bacterium penetrates the protective skin and mucous barriers, it can cause various infections, ranging from minor skin abscesses to deadly bloodstream infections (bacteremia), heart valve infections (endocarditis), bone infections (osteomyelitis), lung infections (pneumonia), meningitis and septic shock.[11,12] It especially poses a threat to newborns and immunocompromised patients, such as, elderly, post-surgical and dialysis patients.

The cell wall of *S. aureus* (Figure 1) is complex and is composed of a peptidoglycan layer with different characteristic glycopolymers,[13–15] and cell wall proteins. The cell wall glycopolymers are involved in several physiological processes and play a key role in staphylococcal virulence. The major classes of cell wall glycopolymers include capsular polysaccharides (CPs), wall teichoic acids

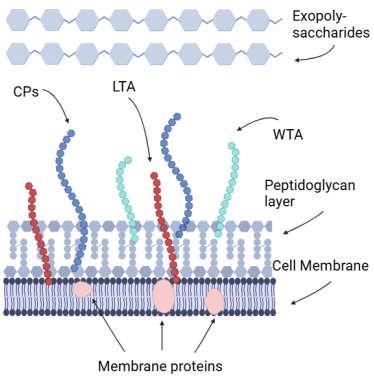


Figure 1: A schematic representation of the cell wall of *S. aureus*.

(WTAs) and lipoteichoic acids (LTAs). Exopolysaccharides can be generated by the bacterium as part of a protective biofilm. All these glycopolymer structures may be used as targets for a protective immune response.[16,17]

To date 13 different *S. aureus* CP serotypes have been identified from clinical isolates, but only four of the 13 serotypes have been structural characterized: serotype 1, 2, 5 and 8 as depicted in Table 1. Serotype 3, 4, 6, 7, 9, 10 and 11 have only been identified by immunological methods and no complete structural characterization has been reported yet.[18] The serotypes can be classified into two main groups depending on colony morphology. Serotype 1 and 2 belong to the mucoid-type species as they are heavily encapsulated forming mucoid colonies, while type 3-11 form non-mucoid colonies, generating only thin layer capsules.[19,20] Serotype 1 and 2 are rarely encountered among clinical isolates while serotype 5 and 8 are amongst the most abundant clinical isolates, comprising more than 80% of all isolates.[20–23] CP type 5 and 8 can serve to target protective antibodies and are widely investigated as vaccine candidates for protection against *S. aureus*.

Table 1: The serotypes of *S. aureus* capsular polysaccharides.

Type	Strain	Final chemical structure of the repeating unit	Established
1	D	→4)-α-D-GalNAcA-(1→4)-α-D-GalNAcA-(1→3)-α-D-FucNAc-(1→	Karakawa, 1982[24]
	M	→4)-α-D-GalNAcA-(1→4)-α-D-GalNAcA-(1→3)-α-D-FucNAc-(1→ [(a)]	Murthy, 1983[25]
2	Smith or K-93M	→4)-β-D-GlcNAcA-(1→4)-β-D-GlcNAcA-(L-alanyl)	Hanessian, 1964[26,27]
3	Mardi	Unknown	NA
4	T	D-ManNAcA-(1→3)-D-FucNAc [(b)]	Wu, 1971[28]
	7007	ManNAcA-(1→3)-FucNAc [(b)]	Karakawa, 1974 [29]
5	Reynold	→4)-β-D-ManNAcA-(1→4)-α-L-FucNAc(3-OAc)-(1→3)-α-D-FucNAc-(1→	Jones, 2005[30]
6	C	Unknown	NA
7	207	Unknown	NA
8	Becker	→3)-β-D-ManNAcA(4-OAc)-(1→4)-α-L-FucNAc-(1→3)-α-D-FucNAc-(1→	Jones, 2005[30]
9	91	Unknown	NA
10	537	Unknown	NA
11	797	Unknown	NA

[(a)] A taurine is found on every fourth α-D-GalNAcA connected through an amide bond with the carboxylic acid. [(b)] No complete structure has been published yet.

Biosynthesis of CP5 and CP8

The structures of CP5 and CP8 are very similar, and they consist of the same three monomeric sugar residues: *N*-acetyl-D-fucosamine (D-FucNAc), *N*-acetyl-L-fucosamine (L-FucNAc) and *N*-acetyl-D-mannosaminuronic acid (D-ManNAcA), but differ in glycosylic linkages and *O*-acetylation pattern as depicted in Figure 2.

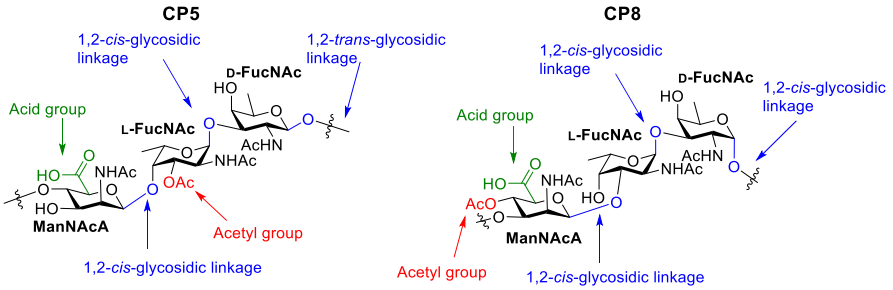


Figure 2: A schematic representation of the common repeating units of CP5 and CP8.

The biosynthetic pathways by which CP type 5 and 8 are assembled are also very similar. Several of the same genes are involved in the biosynthesis of both polysaccharides, encoding the proteins required for polymer synthesis,[31–33] *O*-acetylation,[34] transport and regulation of CP production.[35,36] The proposed pathway for the biosynthesis of the CPs is depicted in Figure 3 with CP5 on the left and CP8 on the right. The synthesis of the soluble uridine diphosphate (UDP) building blocks begins in the cytoplasm via three reaction cascades, wherein the universal precursor UDP-D-*N*-acetyl-glucosamine (UDP-D-*N*-GlcNAc) is converted into the three different nucleotide-coupled sugars, UDP-D-FucNAc, UDP-L-FucNAc and UDP-D-ManNAcA. The biosynthesis of the universal UDP-D-FucNAc (pathway I) starts with the enzymes CapD (a dehydratase) and CapN (a reductase) to transform D-GlcNAc into D-FucNAc. This is followed by transfer to the phosphosugar onto the membrane lipid carrier undecaprenol phosphate ($C_{55}P$) catalyzed by CapM, generating membrane-bound D-FucNAc (Lipid I_{cap}). From here, both CP5 and CP8 can be generated. The CP5 pathway relies on catalysis by Cap5I, whereas CP8 depends on catalysis by Cap8H.[18] UDP-L-FucNAc (pathway II for both CP5 and CP8) is formed from UDP-D-*N*-GlcNAc by the action of the trifunctional enzyme CapE (having dehydratase, 3-epimerase and 5-epimerase activity) followed by CapG (an epimerase) and CapF (a reductase).[32,37] For CP5, the acetyl transferase Cap5H catalyzes the *O*-acetylation of the UDP-L-FucNAc C-3-OH. The L-FucNAc is then transferred to the lipid carrier by the transferase CapL to give the disaccharide Lipid II_{cap}. For the final building block, UDP-D-

ManNAcA (pathway III), CapP epimerizes the C-2 of D-GlcNAc and the dehydrogenase CapO subsequently oxidizes the C-6-OH. In the CP8 biosynthesis route, Cap8J acetylates the C-4-OH of UDP-D-*N*-ManNAcA.[31,33] CapI then transfers the monosaccharide to the lipid carrier to complete the trisaccharide precursor Lipid III_{cap}.[34] The modified trisaccharides are transferred to the outer surface of the cell membrane by the flippase CapK, after which the polymerase Cap5J (for CP5) or Cap8J (for CP8) generates the longer fragments.[20,38] Finally, the attachment of the CP precursor to the *N*-acetylmuramic acid of the peptidoglycan is achieved by an yet unknown pathway.[37]

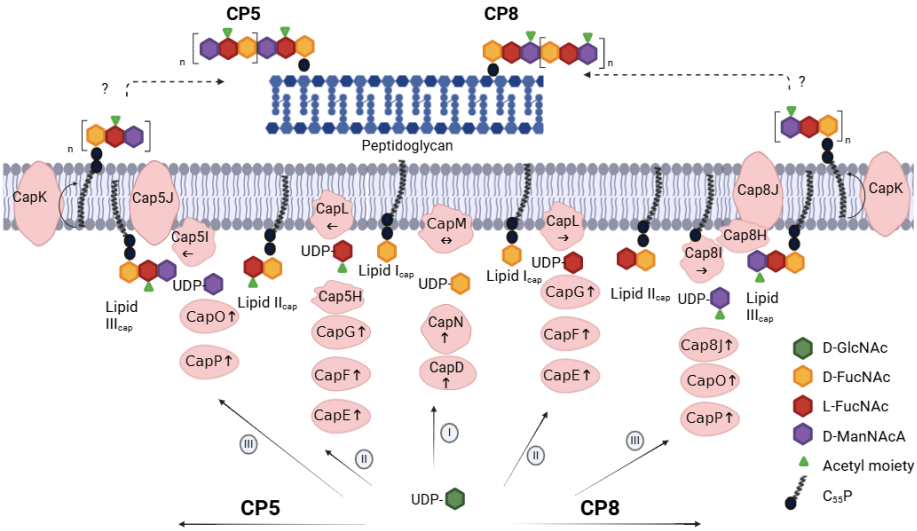


Figure 3: Model for the proposed biosynthetic pathway for CP5 (left) and CP8 (right) in *S. aureus*.

Glycoconjugate vaccines

As mentioned above, the cell wall components of bacteria can be used as targets to direct an immune response, and various bacterial CPs have been used to develop anti-bacterial vaccines.[39] Glycoconjugate vaccines have become one of the most effective and safe preventive treatments to combat bacterial infections with successful vaccines against several bacterial pathogens, including *Haemophilus influenzae* type b, *Streptococcus pneumoniae* and *Neisseria meningitidis* serogroups A, C, W and Y.[40–42] With promising results for other pathogens, and the high demand for new treatment strategies, developing a vaccine against *S.*

aureus is of high interest.[43] When designing a vaccine using polysaccharides (PS), it is necessary to conjugate these to a carrier protein as the PS itself is not sufficiently immunogenic and cannot elicit a strong and lasting immune response. This is because polysaccharides by themselves cannot engage in T-cells activation and are therefore generally referred to as T cell independent antigens.[44] When the PSs are conjugated to a carrier protein, the immunogenic properties are characterized by the generation of high affinity IgG antibodies and the development of immunological memory.[39] The generally accepted immunological mechanism of a glycoconjugate vaccine depends on the PS and the carrier protein as illustrated in Figure 4. First, the conjugated polysaccharide is taken up by the B-cell receptor (BCR), and after internalization the glycoconjugate is processed during which the protein is degraded to form smaller peptides that can bind to the major histocompatibility complex II (MHC II) and can subsequently be presented to the T-cell. Cross talk between the B and T cells then leads to T cell activation and B cell maturation. The T cell stimulates the B cell to develop into polysaccharide specific memory B cells and plasma cells, and the development of high affinity antibodies by IgM to IgG class switching.[45,46] The carrier protein is especially import for a response in infants and toddlers, as no response against a stand-alone polysaccharide vaccine can be elicited. For adults, stand-alone polysaccharides can induce a short-term antibody response, but, because of the lack of T cell help, no memory effect or booster response can be achieved.[44]

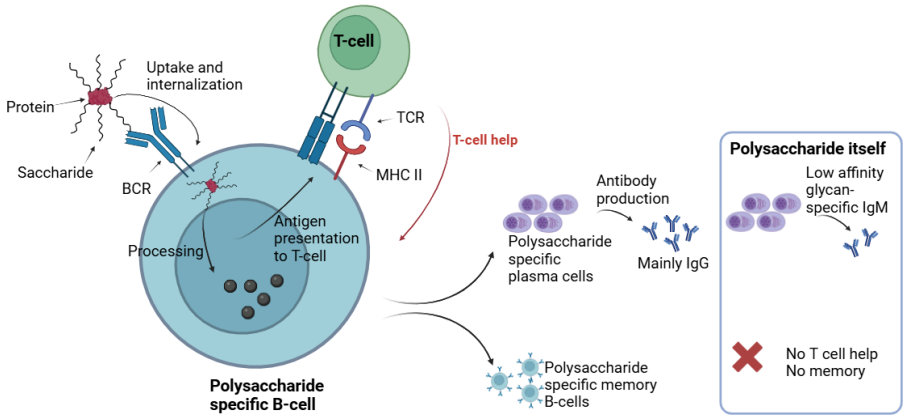


Figure 4: Schematic representation of the generally accepted immunological mechanism of a glycoconjugate vaccine.

Several glycoconjugate vaccine candidates using capsular polysaccharides and teichoic acids of *S. aureus* have been developed.[47] The polysaccharides used in these formulations are normally produced by isolation and purification from

bacterial sources. Generally, this leads to relatively heterogeneous material, varying in length and differences in substitution pattern of the polysaccharides, especially when labile substituents such as *O*-acetyl esters are present (Figure 5A).[48] When using isolated polysaccharides in combination with random conjugation chemistry, the resulting vaccine modalities are high molecular weight, crosslinked, heterogeneous and ill-defined structures.[46,49] Through the use of chemical polysaccharide sizing, often using acidic or oxidative conditions or enzymatic fragmentation and size exclusion chromatography, smaller polysaccharide fragments with a more defined degree of polymerization (DP) can be obtained.[50–52] In addition, the controlled site selective cleavage of the PS, often at the anomeric center of one of the constituting monosaccharides, can enable site selective conjugation chemistry to generate conjugates that are better defined. Even more control over glycoconjugate structure and composition can be achieved using minimal oligosaccharide (OS), generated through chemical or enzymatic synthesis (Figure 5B), which also enables standardized and reproducible vaccine production.[48] While the length can impact the effectiveness of a glycoconjugate vaccine as the oligosaccharide has to present the active epitope and allow for B cell receptor crosslinking, also the protein, the linker and type of conjugation pattern in the design of a vaccine candidate can make a difference.[48]

Various glycoconjugate vaccines comprising isolated *S. aureus* CPs and different carrier proteins have been reported. The most notable has been the StaphVAX in the 1990's, which was explored up to a phase III trial. StaphVAX, a bivalent conjugate vaccine generated using isolated *S. aureus* CP5 and CP8 polysaccharides conjugated to the nontoxic recombinant *Pseudomonas aeruginosa* exotoxin A, was developed by Nabi Pharmaceuticals.[53] StaphVAX did show prevention of infections up to 10 month after one injection, however when explored in a phase III trial, it showed limited efficacy,[54] as the vaccine was found to fail to reduce incidence of invasive infections in hemodialysis patients.[55] StaphV, developed by GSK, combines conjugates of CP5 and CP8 polysaccharides to tetanus toxid (TT) carrier protein mixed with detoxified α-toxin (Hla H35L) and clumping factor A and was evaluated with and without adjuvant AS03B. The phase I trial showed to induce adequate antibody responses and no safety concerns.[56] Another tetravalent antigen *S. aureus* vaccine SA4G was investigated by Pfizer using CP5 and CP8 polysaccharides conjugated to cross-reacting material 197 (CRM_{197}) with two additional protein antigens. It was shown to be safe and well tolerated and to induce antibody responses in healthy older adults in a phase II clinical trial.[43,57] However SA4G failed in the phase IIb trial, conducted with recipients

that underwent spinal surgery as no reduction in the incidence of *S. aureus* bloodstream infections were detected (NCT02388165).

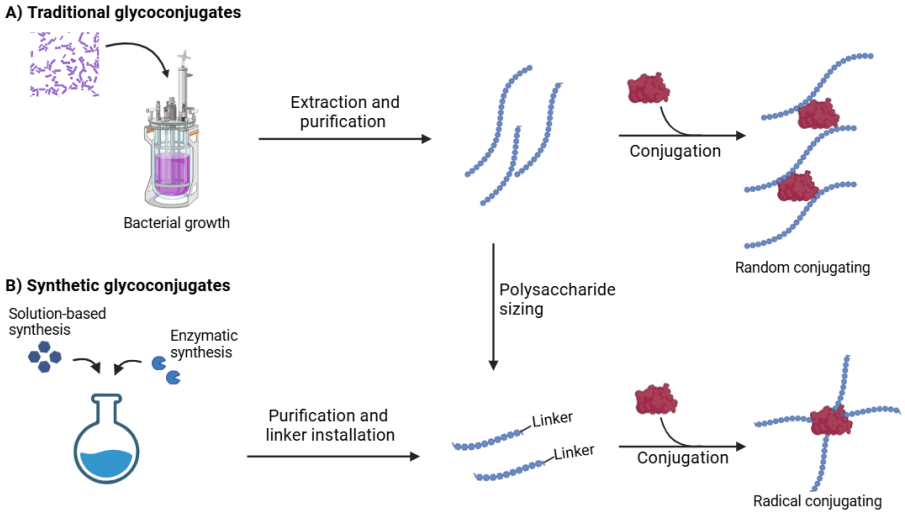


Figure 5: Different approaches to generate glycoconjugate vaccines. A) Traditional approach towards formation of glycoconjugate vaccines by bacterial fermentation, followed by extraction, purification and conjugation of the CP to a carrier protein. B) Synthetic approach towards glycoconjugates by chemical or enzymatic synthesis followed by purification, linker installation and then conjugation to a carrier protein.

The vaccine candidates tested so far have not led to any successful vaccine yet, emphasizing the challenges associated with the development, which includes lability of the linker and side-effects from impurities and non-protective epitopes that may hinder eliciting an adequate immune response against heterogeneous polysaccharides.[54,58] Another reason for the lack of a vaccine has been reasoned to be due to the lack of successful translation of vaccine protectivity, that is observed in the pre-clinical trials performed on animal subject, to human subjects.[43] The solution could include different animal models, representative *in vitro* models and *ex vivo* human tissue to study the pathogenicity. Currently different vaccine candidates that do not contain CPs are being tested in various stages of clinical trials and using either cell-wall proteins,[59,60] antigen proteins[61] or toxoids.[62] Preclinical research using CP5 and CP8 is still ongoing, implementing bioconjugation strategies and semisynthetic glycoconjugates.[63,64] Success of vaccination has been hindered by lack of established correlates of protection in humans and the complexity of the staphylococcal pathogenesis. Vaccines against different infections using synthetic material have been already developed, including the *H. influenzae* type b (Hib) vaccine using a synthetic capsular polysaccharide, which

has been licensed in Cuba[65] and a vaccine against *Shigella flexneri* 2a using a synthetic O-specific polysaccharide, which has progressed to phase II trial.[66] The synthetic oligosaccharides have thus attracted attention as antigen candidates and could therefore be considered as antigen candidates in a vaccine against *S. aureus*.

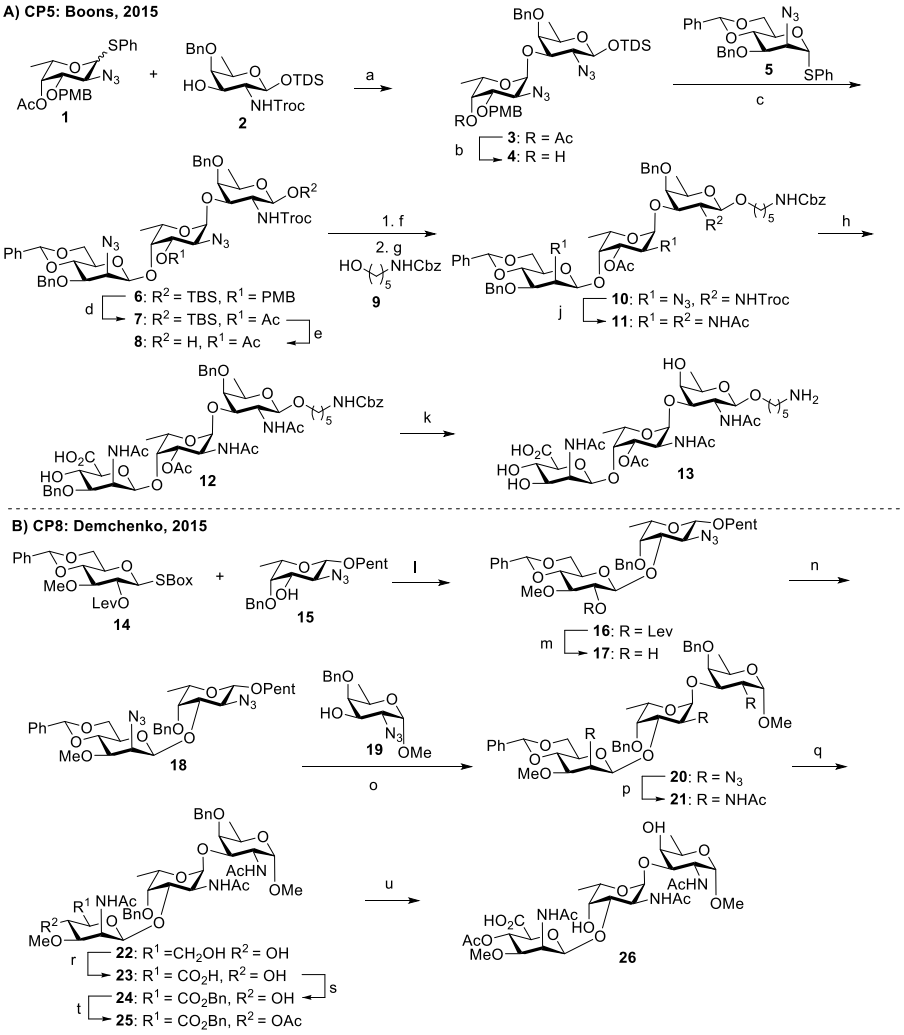
Synthetic capsular polysaccharides

Because of their biological relevance, significant efforts have been devoted to the synthesis of different fragments of the capsular polysaccharides of *S. aureus*. These CPs are complex molecules consisting of different rare monosaccharides, that are interconnected through linkages that are difficult to construct, and they carry different functional groups including a varying *N*- and *O*-acetylation pattern, which makes their synthesis a challenging task.[67] Out of the 13 different serotypes only four of them (serotype 1, 2, 5 and 8, see Table 1) have been structurally characterized and only the synthesis of serotype 1, 5 and 8 has been reported to date. Only small fragments (trisaccharide units) or protected larger fragments of CP1,[68,69] CP5[71–75] and CP8[64,70,76,77] have been published, indicating the difficulty of the synthesis.

In 2015, the group of Boons described the synthesis of a conjugation ready CP5 trisaccharide, shown in Scheme 1A.[72] Their synthesis relied on the use of the monosaccharide building blocks **1**, **2** and **5** as precursors for the L-fucosamine, D-fucosamine and D-mannosaminuronic acid residues, respectively and the trisaccharide was built from the reducing end towards the non-reducing end. Glycosylation between donor **1** and acceptor **2** in the presence of *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) gave disaccharide **3** in a yield of 73% and with an α/β ratio of 4:1. The promoter was found to influence the yield and stereoselectivity of the reaction as the promoter systems 1-benzenesulfinyl piperidine (BSP) or diphenylsulfoxide (DPS) with trifluoromethanesulfonic anhydride (Tf_2O) and 2,4,6-tri-*tert*-butylpyrimidine (TTBP) gave the α-product in only 30 % yield. Removal of the *O*-acetyl ester gave acceptor **8**. The protecting group on the C-3-OH of the L-FucN_3 building block was found to be important in the glycosylation reaction towards the trisaccharide, as an electron-withdrawing acetyl group at this position lowers the reactivity of the axial fucosyl C-4-OH.[78,79] With the *p*-methoxybenzyl (PMB)-acceptor **8**, the glycosylation to give trisaccharide **6** proceeded in 73% yield and with excellent β-selectivity, as a result of the stereoselective nucleophilic displacement of the *in situ* formed mannosazide α-triflate. Next, the PMB ether was exchanged for an *O*-acetyl ester,

which was followed by installation of a 5-(benzyloxycarbonyl)aminopentanol linker (**9**) using the generated imidate donor. Trisaccharide **10** was formed as the sole β-anomer arising from effective neighboring group participation by the trichloroethyl carbamate (Troc) on the C-2-nitrogen. Next, the azides and the Troc carbamate were transformed into the corresponding acetamides, after which the benzylidene on C-4 and C-6 of the mannose residue was hydrolyzed to set the stage for the regioselective oxidation of the C-6-OH which gave **12** in 61% yield.[80] This protecting/functional group manipulation scheme was implemented to avoid possible intramolecular lactam formation of the mannosaminuronic acid residue. Lastly, hydrogenation afforded trisaccharide **13**. While this strategy could allow both for the generation of longer oligomers, and the conjugation to a carrier protein, no investigation towards these goals has been published.

Demchenko and co-workers synthesized the CP8 trisaccharide **26**, shown in Scheme 1B, having methyl groups at both the reducing and non-reducing end, in 2015.[76] They built the trisaccharide from the non-reducing end to the reducing end relying on building blocks **14**, **15** and **19**. The chemoselective glycosylation between donor **14** and acceptor **15** in the presence of AgOTf in 1,2-dichloroethane (1,2-DCE) proceeded in 73% yield and delivered the β-glucosyl product due to neighboring group participation. They chose to use a glucosyl precursor to generate the β-mannosamine residue, as direct mannosylation failed. Next, the C-2 position of the glucosyl residue was epimerized by first removal of the levulinoyl (Lev) group in 72% yield followed by installation of the triflate and azide displacement[81] to give disaccharide **16** in 90% over two steps. The so-formed **16** was used directly as a donor in the glycosylation with acceptor **19** in the presence of NIS and triflic acid (TfOH) in 1,2-DCE to give trisaccharide **20** in 87% yield and with excellent α-selectivity. Now the intermediate trisaccharide **16** was deprotected by reduction of the azide with propane-1,3-dithiol followed by acetylation to give the acetamide in 94% over two steps. The benzylidene was hydrolyzed and the liberated C-6-OH was subsequently selectively oxidized and esterified. The free C-4-OH was acetylated and finally the benzyl protecting groups were removed by hydrogenation yielding trisaccharide **26**. Because both capping ends are equipped with methyl groups the trisaccharide cannot be elongated or conjugated.



Scheme 1: Literature synthetic approaches towards a trisaccharide repeating unit of CP5 from Boons (A) and a CP8 trisaccharide from Demchenko (B). *Reaction conditions*: A) a) NIS, TMSOTf, DCM, Et_2O 4:1, -60 ºC, 72%, α/β = 4:1, b) Na (cat), MeOH, guanidine·HCl, rt, quant, c) DPS, Tf_2O, DCM, -60 ºC to -30 ºC, 72%, d) i) DDQ, DCM/H_2O 9:1, rt, ii) Ac_2O, pyridine, DCMAP, rt, 85% over two steps, e) HF/pyridine, THF, rt, 90%, f) $CF_3(NPh)CCl$, Cs_2CO_3, DCM, g) TMSOTf, DCM/MeCN 1:1, -78 ºC, 72% over two steps, j) zinc, THF:AcOH:Ac_2O, 63%, h) i) 80% aq. AcOH, 90%, ii) TEMPO, BAIB, DCM/H_2O then $NaClO_2$, 2-methyl-2-butene, *t*-BuOH, 61% over two steps, k) $Pd(OH)_2$, MeOH, AcOH, quant. B) l) AgOTf, 1,2-DCE, rt, 73%, m) hydrazine acetate, DCM/MeOH 20:1, 0 ºC, 72%, n) i)Tf_2O, pyridine, DCM, 0 ºC, ii) NaN_3, DMF 60 ºC, 90%, o) NIS, TfOH, 1,2-DCE, 0 ºC, 87%, p) i) $HS(CH_2)_3SH$, Et_3N, pyridine, H_2, rt, ii) Ac_2O, MeOH, rt, 94%, q) TFA aq., DCM, 92%, r) TEMPO, BAIB, aq. DCM, rt, s) BnBr, $NaHCO_3$, DMF, rt, 61% over two steps, t) Ac_2O, pyridine, rt, 99%, u) H_2, Pd/C, aq. EtOH, rt, 97%.

Both the shown synthetic procedures,[72,76] and most of the published synthetic strategies to date to generate CP5 and CP8 oligosaccharides,[64,73,75,77,82] either use a glucosyl synthon or a mannosamine building block to construct the *N*-acetyl β-mannosaminuronic acid, implementing a late-stage modification on larger saccharides. While these procedures can be efficient on relatively small saccharides, implementing them on oligomers comprising several repeating units can be more challenging, as this requires multiple transformations to be executed on the same molecule. Especially the late-stage oxidation of multiple alcohol into the corresponding carboxylates, requiring two oxidation steps for each transformation, has been found difficult.[77,83,84] In 2023, Kulkarni and co-workers implemented a pre-glycosylation oxidation strategy using a $ManN_3A$ donor for the synthesis of a CP8-trisaccharide. However, because of the low reactivity of the donor, a large excess was needed for the glycosylation reaction. With this procedure late-stage modifications were minimized and the deprotection strategy only involved three steps.[70]

Neither of the described molecules above have been used for biological evaluation. Adamo and co-workers reported a synthetic protocol towards a CP5 trisaccharide, bearing a linker for conjugation purposes in 2012.[71] However, during the deprotection steps the amino group in the linker, intended for conjugation, was transformed into an acetamide, rendering the linker unsuitable for functionalization. Therefore, the trisaccharide was only evaluated in competitive ELISA and dot blot studies using murine anti-CP5 serum, generated against a CP5-polysaccharide conjugate. Recognition of the synthetic structure could be shown, but the interaction was significantly weaker than the natural polysaccharide indicating that larger structures are likely needed as potential vaccine candidates. Hu *et al*. reached the same conclusion in their report on a synthetic CP8 trisaccharide protein conjugate.[64] Mice immunization with their CP8-trisaccharide conjugate did lead to the production of anti-CP8 antibodies as revealed by glycan micro array, but longer structures were deemed necessary to generate a more potent vaccine candidate.

Outline of the thesis

In this Thesis synthetic chemistry has been developed to generate well-defined fragments of three different *S. aureus* serotype capsular polysaccharides. All of the synthetic saccharides are equipped with an aminoalkyl linker to conjugate them to a carrier protein to generate synthetic vaccine modalities. **Chapter 2** describes the synthesis of fragments of the capsular polysaccharide type 8, with the oligosaccharides ranging in length from a trisaccharide to a dodecasaccharide. The synthetic approach developed relies on a [3+3n] strategy and a pre-glycosylation oxidation and *O*-acetylation strategy has been implemented to simplify the deprotection at the end of the synthesis. The four generated saccharides are conjugated to a carrier protein, and their binding to monoclonal and polyclonal antibodies is described using Western Blot and ELISA. Conformational investigations and NMR interaction studies have revealed the epitopes recognized by the antibodies. Immunization studies are reported showing the longer synthetic fragments to serve as potent analogues of the natural polysaccharide. In **Chapter 3** the synthesis of fragments of capsular polysaccharide type 5 are described. The assembly of tri- hexa- and nonasaccharides is presented and again a pre-glycosylation oxidation and [3+3n] glycosylation strategy is implemented to obtain the saccharides. The synthetic saccharides were conjugated to a carrier protein and tested for binding with monoclonal and polyclonal antibodies using Western Blot and Surface plasmon Resonance (SPR). Conformational studies have revealed the three-dimensional structure of the oligosaccharides. In **Chapter 4** the synthesis of four frameshifted CP5 and CP8 trisaccharides is presented, complementing the trisaccharides generated in Chapter 2 and Chapter 3. Again, the saccharides are investigated for interaction with monoclonal antibodies raised against native CP5 and CP8, and quite surprisingly one of the CP5 trisaccharides was revealed to be a potent binder for anti-CP5 antibodies. **Chapter 5** describes the synthesis of oligosaccharides derived from CP type 1. Four different trisaccharides with a different taurine substitution pattern is presented together with a non-taurinated hexasaccharide. In contrast to the work of CP5 and CP8, now post-glycosylation modifications are implemented to be able to tune the taurination pattern. In **Chapter 6** the work in Chapter 2-5 is summarized and an outline for potential future directions is presented.

References

(1) Rice, L. B. Federal Funding for the Study of Antimicrobial Resistance in Nosocomial Pathogens: No ESKAPE. *J. Infect. Dis.* **2008**, *197* (8), 1079–1081. https://doi.org/10.1086/533452.

(2) Miller, W. R.; Arias, C. A. ESKAPE Pathogens: Antimicrobial Resistance, Epidemiology, Clinical Impact and Therapeutics. *Nat. Rev. Microbiol.* **2024**, *22*, 598–616. https://doi.org/10.1038/s41579-024-01054-w.

(3) Aloke, C.; Achilonu, I. Coping with the ESKAPE Pathogens: Evolving Strategies, Challenges and Future Prospects. *Microb. Pathog.* **2023**, *175* (November 2022), 105963. https://doi.org/10.1016/j.micpath.2022.105963.

(4) Mulani, M. S.; Kamble, E. E.; Kumkar, S. N.; Tawre, M. S.; Pardesi, K. R. Emerging Strategies to Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review. *Front. Microbiol.* **2019**, *10* (APR), 1–24. https://doi.org/10.3389/fmicb.2019.00539.

(5) Stryjewski, M. E.; Chambers, H. F. Skin and Soft-Tissue Infections Caused by Community-Acquired Methicillin-Resistant Staphylococcus Aureus. *Clin. Infect. Dis.* **2008**, *46* (SUPPL. 5). https://doi.org/10.1086/533593.

(6) Miller, L. G. Community-Associated Methicillin Resistant Staphylococcus Aureus. *Antimicrob. Resist.* **2010**, *6* (9725), 1–20. https://doi.org/10.1159/000298753.

(7) CDC. Staphylococcus Aureus Resistant to Vancomycin-United States, 2002. *MMWR. Morb. Mortal. Wkly. Rep.* **2002**, *51* (26), 565–567.

(8) Nandhini, P.; Kumar, P.; Mickymaray, S.; Alothaim, A. S.; Somasundaram, J.; Rajan, M. Recent Developments in Methicillin-Resistant Staphylococcus Aureus (MRSA) Treatment: A Review. *Antibiotics* **2022**, *11* (5), 1–21. https://doi.org/10.3390/antibiotics11050606.

(9) Esposito, S.; Blasi, F.; Curtis, N.; Kaplan, S.; Lazzarotto, T.; Meschiari, M.; Mussini, C.; Peghin, M.; Rodrigo, C.; Vena, A.; Principi, N.; Bassetti, M. New Antibiotics for Staphylococcus Aureus Infection: An Update from the World Association of Infectious Diseases and Immunological Disorders (WAidid) and the Italian Society of Anti-Infective Therapy (SITA). *Antibiotics* **2023**, *12* (4). https://doi.org/10.3390/antibiotics12040742.

(10) Tacconelli, E.; Carrara, E.; Savoldi, A.; Harbarth, S.; Mendelson, M.; Monnet, D. L.; Pulcini, C.; Kahlmeter, G.; Kluytmans, J.; Carmeli, Y.; Ouellette, M.; Outterson, K.; Patel, J.; Cavaleri, M.; Cox, E. M.; Houchens, C. R.; Grayson, M. L.; Hansen, P.; Singh, N.; Theuretzbacher, U.; Magrini, N.; Aboderin, A. O.; Al-Abri, S. S.; Awang Jalil, N.; Benzonana, N.; Bhattacharya, S.; Brink, A. J.; Burkert, F. R.; Cars, O.; Cornaglia, G.; Dyar, O. J.; Friedrich, A. W.; Gales, A. C.; Gandra, S.; Giske, C. G.; Goff, D. A.; Goossens, H.; Gottlieb, T.; Guzman Blanco, M.; Hryniewicz, W.; Kattula, D.; Jinks, T.; Kanj, S. S.; Kerr, L.; Kieny, M. P.; Kim, Y. S.; Kozlov, R. S.; Labarca, J.; Laxminarayan, R.; Leder, K.; Leibovici, L.; Levy-Hara, G.; Littman, J.; Malhotra-Kumar, S.; Manchanda, V.; Moja, L.; Ndoye, B.; Pan, A.; Paterson, D. L.; Paul, M.; Qiu, H.; Ramon-Pardo, P.; Rodríguez-Baño, J.; Sanguinetti, M.; Sengupta, S.; Sharland, M.; Si-Mehand, M.; Silver, L. L.; Song, W.; Steinbakk, M.; Thomsen, J.; Thwaites, G. E.; van der Meer, J. W.; van Kinh, N.; Vega, S.; Villegas, M. V.; Wechsler-Fördös, A.; Wertheim, H. F. L.; Wesangula, E.; Woodford, N.; Yilmaz, F. O.; Zorzet, A. Discovery, Research, and Development of New Antibiotics: The WHO Priority List of Antibiotic-Resistant Bacteria and Tuberculosis. *Lancet Infect. Dis.* **2018**, *18* (3), 318–327. https://doi.org/10.1016/S1473-3099(17)30753-3.

(11) Franklin, D.; Lowy, F. Staphylococcus Aureus Infections. *N. Engl. J. Med.* **1998**, *339* (8), 520–532. https://doi.org/10.1056/NEJM199808203390806.

(12) Miller, L. S.; Cho, J. S. Immunity against Staphylococcus Aureus Cutaneous

Infections. *Nat. Rev. Immunol.* **2011**, *11* (8), 505–518. https://doi.org/10.1038/nri3010.
(13) Vollmer, W.; Blanot, D.; de Pedro, M. Peptidoglycan Structure and Architecture. *FEMS Microbiol. Rev.* **2008**, *32* (2), 149–168. https://doi.org/10.1111/j.1574-6976.2007.00094.x.
(14) Silhavy, T. J.; Kahne, D.; Walker, S. The Bacterial Cell Envelope. *Cold Spring Harb. Perspect. Biol.* **2010**, *2* (5), a000414. https://doi.org/10.1101/cshperspect.a000414.
(15) Hanson, B. R.; Neely, M. N. Coordinate Regulation of Gram-Positive Cell Surface Components. *Curr. Opin. Microbiol.* **2012**, *15* (2), 204–210. https://doi.org/10.1016/j.mib.2011.12.011.
(16) Robbins, J. B.; Schneerson, R.; Horwith, G.; Naso, R.; Fattom, A. I. Staphylococcus Aureus Types 5 and 8 Capsular Polysaccharide-Protein Conjugate Vaccines. *Am. Heart J.* **2004**, *147* (4), 593–598. https://doi.org/10.1016/j.ahj.2004.01.012.
(17) Tollersrud, T.; Zernichow, L.; Rune, S.; Kenny, K. Staphylococcus Aureus Capsular Polysaccharide Type 5 Conjugate and Whole Cell Vaccines Stimulate Antibody Responses in Cattle. *Vaccine* **2001**, *19* (28–29), 3896–3903. https://doi.org/10.1016/s0264-410x(01)00124-4.
(18) Visansirikul, S.; Kolodziej, S. A.; Demchenko, A. V. Staphylococcus Aureus Capsular Polysaccharides: A Structural and Synthetic Perspective. *Org. Biomol. Chem.* **2020**, *18* (5), 783–798. https://doi.org/10.1039/c9ob02546d.
(19) Lee, C. Y. *Staphylococcus Aureus Infection and Disease*; Honeyman, A. L., Friedman, H., Bandinelli, M., Eds.; Kluwer Acedemic/Plenum Publishers: New York, 2001.
(20) O'Riordan, K.; Lee, J. C. Staphylococcus Aureus Capsular Polysaccharides. *Clin. Microbiol. Rev.* **2004**, *17* (1), 218–234. https://doi.org/10.1128/CMR.17.1.218-234.2004.
(21) Roghmann, M.; Taylor, K. L.; Gupte, A.; Zhan, M.; Johnson, J. A.; Cross, A.; Edelman, R.; Fattom, A. I. Epidemiology of Capsular and Surface Polysaccharide in Staphylococcus Aureus Infections Complicated by Bacteraemia. *J. Hosp. Infect.* **2005**, *59* (1), 27–32. https://doi.org/10.1016/j.jhin.2004.07.014.
(22) Verdier, I.; Durand, G.; Bes, M.; Taylor, K. L.; Lina, G.; Vandenesch, F.; Fattom, A. I.; Etienne, J. Identification of the Capsular Polysaccharides in Staphylococcus Aureus Clinical Isolates by PCR and Agglutnation Tests. *J. Clin. Microbiol.* **2007**, *45* (3), 725–729. https://doi.org/10.1128/JCM.01572-06.
(23) Luong, T.; Sau, S.; Gomez, M.; Lee, J. C.; Lee, C. Y. Regulation of Staphylococcus Aureus Capsular Polysaccharide Expression by Agr and SarA. *Am. Soc. Microbiol.* **2002**, *70* (2), 444–450. https://doi.org/10.1128/IAI.70.2.444.
(24) Karakawa, W. W.; Kane, J. A. *Seminars in Infectious Disease. Vol IV. Bacterial Vaccines*; Wienstein, L., Field, B. N., Eds.; Thieme-Stratton Inc.: Stuttgard, Germany, 1982.
(25) Murthy, S. V. K. N.; Ann Melly, M.; Harris, T. M.; Hellerqvist, C. G.; Hash, J. H. The Repeating Sequence of the Capsular Polysaccharide of Staphylococcus Aureus M. *Carbohydr. Res.* **1983**, *117*, 113–123. https://doi.org/10.1016/0008-6215(83)88080-X.
(26) Hanessian, S.; Haskell, T. H. Structural Studies on Staphylococcal Polysaccharide Antigen. *J. Bol. Chem.* **1964**, *239* (9), 2758–2764. https://doi.org/10.1016/S0021-9258(18)93811-1.
(27) Karakawa, W. W.; Kane, J. A. Characterization of the Surface Antigens of Staphylococcus Aureus, Strain K-93M. *J. Immunol.* **1972**, *108* (5), 1199–1208. https://doi.org/10.4049/jimmunol.108.5.1199.
(28) Wu, T. C.; Park, J. T. Chemical Characterization of a New Surface Antigenic Polysaccharide from a Mutant of Staphylococcus Aureus. *J. Bacteriol.* **1971**, *108* (2), 874–884. https://doi.org/10.1128/jb.108.2.874-884.1971.
(29) Karakawa, W. W.; Kane, A. J.; Smith, R. M. Isolation of an Acidic Surface Antigen

from a Conventional Strain of Staphylococcus Aureus. *Infect. Immun.* **1974**, *9* (3), 511–518. https://doi.org/10.1128/iai.9.3.511-518.1974.

(30) Jones, C. Revised Structures for the Capsular Polysaccharides from Staphylococcus Aureus Types 5 and 8, Components of Novel Glycoconjugate Vaccines. *Carbohydr. Res.* **2005**, *340* (6), 1097–1106. https://doi.org/10.1016/j.carres.2005.02.001.

(31) Portolés, M.; Kiser, K. B.; Bhasin, N.; Chan, K. H. N.; Lee, J. C. Staphylococcus Aureus Cap50 Has UDP-ManNAc Dehydrogenase Activity and Is Essential for Capsule Expression. *Infect. Immun.* **2001**, *69* (2), 917–923. https://doi.org/10.1128/IAI.69.2.917-923.2001.

(32) Kneidinger, B.; O'Riordan, K.; Li, J.; Brisson, J. R.; Lee, J. C.; Lam, J. S. Three Highly Conserved Proteins Catalyze the Conversion of UDP-N-Acetyl-D-Glucosamine to Precursors for the Biosynthesis of O Antigen in Pseudomonas Aeruginosa O11 and Capsule in Staphylococcus Aureus Type 5: Implications for the UDP-N-Acetyl-L-Fucosamine. *J. Biol. Chem.* **2003**, *278* (6), 3615–3627. https://doi.org/10.1074/jbc.M203867200.

(33) Kiser, K. B.; Bhasin, N.; Deng, L.; Lee, J. C. Staphylococcus Aureus Cap5P Encodes a UDP-N-Acetylglucosamine 2- Epimerase with Functional Redundancy. *J. Bacteriol.* **1999**, *181* (16), 4818–4824. https://doi.org/10.1128/jb.181.16.4818-4824.1999.

(34) Bhasin, N.; Albus, A.; Michon, F.; Livolsi, P. J.; Park, J.-S.; Lee, J. C. Identification of a Gene Essential for O-acetylation of the Staphylococcus Aureus. *Mol. Microbiol.* **1998**, *27* (1), 9–2. https://doi.org/10.1046/j.1365-2958.1998.00646.x.

(35) Soulat, D.; Jault, J. M.; Duclos, B.; Geourjon, C.; Cozzone, A. J.; Grangeasse, C. Staphylococcus Aureus Operates Protein-Tyrosine Phosphorylation through a Specific Mechanism. *J. Biol. Chem.* **2006**, *281* (20), 14048–14056. https://doi.org/10.1074/jbc.M513600200.

(36) Soulat, D.; Grangeasse, C.; Vaganay, E.; Cozzone, A. J.; Duclos, B. UDP-Acetyl-Mannosamine Dehydrogenase Is an Endogenous Protein Substrate of Staphylococcus Aureus Protein-Tyrosine Kinase Activity. *J. Mol. Microbiol. Biotechnol.* **2007**, *13* (1–3), 45–54. https://doi.org/10.1159/000103596.

(37) Rausch, M.; Deisinger, J. P.; Ulm, H.; Müller, A.; Li, W.; Hardt, P.; Wang, X.; Li, X.; Sylvester, M.; Engeser, M.; Vollmer, W.; Müller, C. E.; Sahl, H. G.; Lee, J. C.; Schneider, T. Coordination of Capsule Assembly and Cell Wall Biosynthesis in Staphylococcus Aureus. *Nat. Commun.* **2019**, *10* (1), 1410. https://doi.org/10.1038/s41467-019-09356-x.

(38) Sau, S.; Bhasin, N.; Wann, E. R.; Lee, J. C.; Foster, T. J.; Lee, C. Y. The Staphylococcus Aureus Allelic Genetic Loci for Serotype 5 and 8 Capsule Expression Contain the Type-Specific Genes Flanked by Common Genes. *Microbiology* **1997**, *143* (7), 2395–2405. https://doi.org/10.1099/00221287-143-7-2395.

(39) Sorieul, C.; Dolce, M.; Romano, M. R.; Codée, J. D. C.; Adamo, R. Glycoconjugate Vaccines against Antimicrobial Resistant Pathogens. *Expert Rev. Vaccines* **2023**, *22* (1), 1055–1078. https://doi.org/10.1080/14760584.2023.2274955.

(40) Trotter, C. L.; McVernon, J.; Ramsay, M. E.; Whitney, C. G.; Mulholland, E. K.; Goldblatt, D.; Hombach, J.; Kieny, M. P. Optimising the Use of Conjugate Vaccines to Prevent Disease Caused by Haemophilus Influenzae Type b, Neisseria Meningitidis and Streptococcus Pneumoniae. *Vaccine* **2008**, *26* (35), 4434–4445. https://doi.org/10.1016/j.vaccine.2008.05.073.

(41) De Montalembert, Mariane Abboud, M. R.; Fiquet, A.; Inati, A.; Lebensburger, J. D.; Kaddah, N.; Mokhtar, G.; Piga, A.; Halasa, N.; Inusa, B.; Rees, D. C.; Heath, P. T.; Telfer, P.; Driscoll, C.; Hajjar, S. Al; Tozzi, A.; Jiang, Q.; Emini, E. A.; Gruber, W. C.; Gurtman, A.; Scott, D. A. 13-Valent Pneumococcal Conjugate Vaccine (PCV13) Is Immunogenic and Safe in Children 6-17 Years of Age With Sickle Cell Disease Previously Vaccinated With 23-Valent Pneumococcal Polysaccharide Vaccine

(PPSV23): Results of a Phase 3 Study. *Pediatr. Blood Cancer* **2015**, *62* (8), 1427–1436. https://doi.org/10.1002/pbc.25502.

(42) Block, S. L.; Shepard, J.; Garfield, H.; Xie, F.; Han, L.; Dull, P. M.; Smolenov, I. Immunogenicity and Safety of a 3-and 4-Dose Vaccination Series of a Meningococcal ACWY Conjugate Vaccine in Infants: Results of a Phase 3b, Randomized, Open-Label Trial. *Pediatr. Infect. Dis. J.* **2016**, *35* (2), e48–e59. https://doi.org/10.1097/INF.0000000000000965.

(43) Clegg, J.; Soldaini, E.; McLoughlin, R. M.; Rittenhouse, S.; Bagnoli, F.; Phogat, S. Staphylococcus Aureus Vaccine Research and Development: The Past, Present and Future, Including Novel Therapeutic Strategies. *Front. Immunol.* **2021**, *12* (624310), 1–19. https://doi.org/10.3389/fimmu.2021.705360.

(44) Rappuoli, R. Glycoconjugate Vaccines: Principles and Mechanisms. *Sci. Transl. Med.* **2018**, *10* (456), 1–6. https://doi.org/10.1126/scitranslmed.aat4615.

(45) Pollard, A. J.; Perrett, K. P.; Beverley, P. C. Maintaining Protection against Invasive Bacteria with Protein- Polysaccharide Conjugate Vaccines. *Nat. Rev. Immunol.* **2009**, *9* (3), 213–220. https://doi.org/10.1038/nri2494.

(46) Costantino, P.; Rappuoli, R.; Berti, F. The Design of Semi-Synthetic and Synthetic Glycoconjugate Vaccines. *Expert Opin. Drug Discov.* **2011**, *6* (10), 1045–1066. https://doi.org/10.1517/17460441.2011.609554.

(47) Jahantigh, H. R.; Faezi, S.; Habibi, M.; Mahdavi, M.; Stufano, A.; Lovreglio, P.; Ahmadi, K. The Candidate Antigens to Achieving an Effective Vaccine against Staphylococcus Aureus. *Vaccines* **2022**, *10* (2), 1–18. https://doi.org/10.3390/vaccines10020199.

(48) Anish, C.; Beurret, M.; Poolman, J. Combined Effects of Glycan Chain Length and Linkage Type on the Immunogenicity of Glycoconjugate Vaccines. *npj Vaccines* **2021**, *6* (1). https://doi.org/10.1038/s41541-021-00409-1.

(49) Khatun, F.; Stephenson, R. J.; Toth, I. An Overview of Structural Features of Antibacterial Glycoconjugate Vaccines That Influence Their Immunogenicity. *Chem. - A Eur. J.* **2017**, *23* (18), 4233–4254. https://doi.org/10.1002/chem.201603599.

(50) Bröker, M.; Dull, P. M.; Rappuoli, R.; Costantino, P. Chemistry of a New Investigational Quadrivalent Meningococcal Conjugate Vaccine That Is Immunogenic at All Ages. *Vaccine* **2009**, *27* (41), 5574–5580. https://doi.org/10.1016/j.vaccine.2009.07.036.

(51) Bardotti, A.; Averani, G.; Berti, F.; Berti, S.; Carinci, V.; D'Ascenzi, S.; Fabbri, B.; Giannini, S.; Giannozzi, A.; Magagnoli, C.; Proietti, D.; Norelli, F.; Rappuoli, R.; Ricci, S.; Costantino, P. Physicochemical Characterisation of Glycoconjugate Vaccines for Prevention of Meningococcal Diseases. *Vaccine* **2008**, *26* (18), 2284–2296. https://doi.org/10.1016/j.vaccine.2008.01.022.

(52) Costantino, P.; Norelli, F.; Giannozzi, A.; D'Ascenzi, S.; Bartoloni, A.; Kaur, S.; Tang, D.; Seid, R.; Viti, S.; Paffetti, R.; Bigio, M.; Pennatini, C.; Averani, G.; Guarnieri, V.; Gallo, E.; Ravenscroft, N.; Lazzeroni, C.; Rappuoli, R.; Ceccarini, C. Size Fractionation of Bacterial Capsular Polysaccharides for Their Use in Conjugate Vaccines. *Vaccine* **1999**, *17* (9–10), 1251–1263. https://doi.org/10.1016/s0264-410x(98)00348-x.

(53) Fattom, A. I.; Schneerson, R.; Watson, D. C.; Karakawa, W. W.; Fitzgerald, D.; Pastan, I.; Li, X.; Shiloach, J.; Bryla, D. A.; Robbins, J. B. Laboratory and Clinical Evaluation of Conjugate Vaccines Composed of Staphylococcus Aureus Type 5 and Type 8 Capsular Polysaccharides Bound to Pseudomonas Aeruginosa Recombinant Exoprotein A. *Infect. Immun.* **1993**, *61* (3), 1023–1032. https://doi.org/10.1128/iai.61.3.1023-1032.1993.

(54) Fattom, A. I.; Horwith, G.; Fuller, S.; Propst, M.; Naso, R. Development of StaphVAX TM , a Polysaccharide Conjugate Vaccine against S . Aureus Infection: From the Lab

Bench to Phase III Clinical Trials. *Vaccine* **2004**, *22* (7), 880–887. https://doi.org/10.1016/j.vaccine.2003.11.034.

(55) Spellberg, B.; Daum, R. S. A New View on Development of a Staphylococcus Aureus Vaccine: Insights from Mice and Men. *Hum. Vaccin.* **2010**, *6* (10), 857–859. https://doi.org/10.4161/hv.6.10.12469.

(56) Levy, J.; Licini, L.; Haelterman, E.; Moris, P.; Lestrate, P.; Damaso, S.; Van Belle, P.; Boutriau, D. Safety and Immunogenicity of an Investigational 4-Component Staphylococcus Aureus Vaccine with or without AS03B Adjuvant: Results of a Randomized Phase I Trial. *Hum. Vaccines Immunother.* **2015**, *11* (3), 620–631. https://doi.org/10.1080/21645515.2015.1011021.

(57) Creech, C. B.; Frenck, R. W.; Sheldon, E. A.; Seiden, D. J.; Kankam, M. K.; Zito, E. T.; Girgenti, D.; Severs, J. M.; Immermann, F. W.; McNeil, L. K.; Cooper, D.; Jansen, K. U.; Gruber, W. C.; Eiden, J.; Anderson, A. S.; Baber, J. Safety, Tolerability, and Immunogenicity of a Single Dose 4-Antigen or 3-Antigen Staphylococcus Aureus Vaccine in Healthy Older Adults: Results of a Randomised Trial. *Vaccine* **2017**, *35* (2), 385–394. https://doi.org/10.1016/j.vaccine.2016.11.032.

(58) Anish, C.; Schumann, B.; Pereira, C. L.; Seeberger, P. H. Chemical Biology Approaches to Designing Defined Carbohydrate Vaccines. *Chem. Biol.* **2014**, *21* (1), 38–50. https://doi.org/10.1016/j.chembiol.2014.01.002.

(59) Yeaman, M. R.; Filler, S. G.; Chaili, S.; Barr, K.; Wang, H.; Kupferwasser, D.; Hennessey, J. P. J.; Fu, Y.; Schmidt, C. S.; Edwards, J. E. J.; Xiong, Y. Q.; Ibrahim, A. S. Mechanisms of NDV-3 Vaccine Efficacy in MRSA Skin versus Invasive Infection. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111* (51), E5555-63. https://doi.org/10.1073/pnas.1415610111.

(60) Schmidt, C. S.; White, C. J.; Ibrahim, A. S.; Filler, S. G.; Fu, Y.; Yeaman, M. R.; Edwards, J. E. J.; Hennessey, J. P. J. NDV-3, a Recombinant Alum-Adjuvanted Vaccine for Candida and Staphylococcus Aureus, Is Safe and Immunogenic in Healthy Adults. *Vaccine* **2012**, *30* (52), 7594–7600. https://doi.org/10.1016/j.vaccine.2012.10.038.

(61) Zeng, H.; Yang, F.; Feng, Q.; Zhang, J.; Gu, J.; Jing, H.; Cai, C.; Xu, L.; Yang, X.; Xia, X.; Zeng, N.; Fan, S.; Zou, Q. Rapid and Broad Immune Efficacy of a Recombinant Five-Antigen Vaccine against Staphylococcus Aureus Infection in Animal Models. *Vaccines* **2020**, *8* (1), 134. https://doi.org/10.3390/vaccines8010134.

(62) Karauzum, H.; Venkatasubramaniam, A.; Adhikari, R. P.; Kort, T.; Holtsberg, F. W.; Mukherjee, I.; Mednikov, M.; Ortines, R.; Nguyen, N. T. Q.; Doan, T. M. N.; Diep, B. A.; Lee, J. C.; Aman, M. J. IBT-V02: A Multicomponent Toxoid Vaccine Protects Against Primary and Secondary Skin Infections Caused by Staphylococcus Aureus. *Front. Immunol.* **2021**, *12*, 624310. https://doi.org/10.3389/fimmu.2021.624310.

(63) Cheng, B. L.; Nielsen, T. B.; Pantapalangkoor, P.; Zhao, F.; Lee, J. C.; Montgomery, C. P.; Luna, B.; Spellberg, B.; Daum, R. S. Evaluation of Serotypes 5 and 8 Capsular Polysaccharides in Protection against Staphylococcus Aureus in Murine Models of Infection. *Hum. Vaccines Immunother.* **2017**, *13* (7), 1609–1614. https://doi.org/10.1080/21645515.2017.1304334.

(64) Zhao, M.; Qin, C.; Li, L.; Xie, H.; Ma, B.; Zhou, Z.; Yin, J.; Hu, J. Conjugation of Synthetic Trisaccharide of Staphylococcus Aureus Type 8 Capsular Polysaccharide Elicits Antibodies Recognizing Intact Bacterium. *Front. Chem.* **2020**, *8* (April), 1–10. https://doi.org/10.3389/fchem.2020.00258.

(65) Verez-Bencomo, V.; Fernández-Santana, V.; Hardy, E.; Toledo, M. E.; Rodriguez, M. C.; Heynngnezz, L.; Rodriguez, A.; Baly, A.; Herrera, L.; Izquierdo, M.; Villar, A.; Valdés, Y.; Cosme, K.; Deler, M. L.; Montane, M.; Garcia, E.; Ramos, A.; Aguilar, A.; Medina, E.; Toraño, G.; Sosa, I.; Hernandez, I.; Martínez, R.; Muzachio, A.; Carmenates, A.; Costa, L.; Cardoso, F.; Campa, C.; Diaz, M.; Roy, R. A Synthetic

Conjugate Polysaccharide Vaccine against Haemophilus Influenzae Type B. *Science (80-.).* **2004**, *305* (5683), 522–525. https://doi.org/10.1126/science.1095209.

(66) Cohen, D.; Atsmon, J.; Artaud, C.; Meron-Sudai, S.; Gougeon, M. L.; Bialik, A.; Goren, S.; Asato, V.; Ariel-Cohen, O.; Reizis, A.; Dorman, A.; Hoitink, C. W. G.; Westdijk, J.; Ashkenazi, S.; Sansonetti, P.; Mulard, L. A.; Phalipon, A. Safety and Immunogenicity of a Synthetic Carbohydrate Conjugate Vaccine against Shigella Flexneri 2a in Healthy Adult Volunteers: A Phase 1, Dose-Escalating, Single-Blind, Randomised, Placebo-Controlled Study. *Lancet Infect. Dis.* **2021**, *21* (4), 546–558. https://doi.org/10.1016/S1473-3099(20)30488-6.

(67) Cescutti, P. *Microbial Glycobiology - Chapter 6 - Bacterial Capsular Polysaccharides and Exopolysaccharides*; Academic Press, 2010.

(68) Hagen, B.; Van Dijk, J. H. M.; Zhang, Q.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. Synthesis of the Staphylococcus Aureus Strain M Capsular Polysaccharide Repeating Unit. *Org. Lett.* **2017**, *19* (10), 2514–2517. https://doi.org/10.1021/acs.orglett.7b00747.

(69) Shirsat, A. A.; Rai, D.; Ghotekar, B. K.; Kulkarni, S. S. Total Synthesis of Trisaccharide Repeating Unit of Staphylococcus Aureus Strain M. *Org. Lett.* **2023**, *25* (16), 2913–2917. https://doi.org/10.1021/acs.orglett.3c00997.

(70) Rai, D.; Kulkarni, S. S. Total Synthesis of Trisaccharide Repeating Unit of Staphylococcus Aureus Type 8 (CP8) Capsular Polysaccharide. *Org. Lett.* **2023**, *25* (9), 1509–1513. https://doi.org/10.1021/acs.orglett.3c00290.

(71) Danieli, E.; Proietti, D.; Brogioni, G.; Romano, M. R.; Cappelletti, E.; Tontini, M.; Berti, F.; Lay, L.; Costantino, P.; Adamo, R. Synthesis of Staphylococcus Aureus Type 5 Capsular Polysaccharide Repeating Unit Using Novel L-FucNAc Synthons and Immunochemical Evaluation. *Bioorganic Med. Chem.* **2012**, *20* (21), 6403–6415. https://doi.org/10.1016/j.bmc.2012.08.048.

(72) Gagarinov, I. A.; Fang, T.; Liu, L.; Srivastava, A. D.; Boons, G.-J. Synthesis of Staphylococcus Aureus Type 5 Trisaccharide Repeating Unit: Solving the Problem of Lactamization. *Org. Lett.* **2015**, *17* (4), 928–931. https://doi.org/10.1021/acs.orglett.5b00031.

(73) Yasomanee, J. P.; Visansirikul, S.; Papapida, P.; Thompson, M.; Kolodziej, S. A.; Demchenko, A. V. Synthesis of the Repeating Unit of Capsular Polysaccharide Staphylococcus Aureus Type 5 To Study Chemical Activation and Conjugation of Native CP5. *J. Org. Chem.* **2016**, *81* (14), 5981–5987. https://doi.org/10.1021/acs.joc.6b00910.

(74) Hagen, B.; Ali, S.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. Mapping the Reactivity and Selectivity of 2-Azidofucosyl Donors for the Assembly of N-Acetylfucosamine-Containing Bacterial Oligosaccharides. *J. Org. Chem.* **2017**, *82* (2), 848–868. https://doi.org/10.1021/acs.joc.6b02593.

(75) Behera, A.; Rai, D.; Kulkarni, S. S. 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), 456–467. https://doi.org/10.1021/jacs.9b11309.

(76) Visansirikul, S.; Yasomanee, J. P.; Papapida, P.; Kamat, M. N.; Podvalnyy, N. M.; Gobble, C. P.; Thompson, M.; Kolodziej, S. A.; Demchenko, A. V. A Concise Synthesis of the Repeating Unit of Capsular Polysaccharide Staphylococcus Aureus Type 8. *Org. Lett.* **2015**, *17* (10), 2382–2384. https://doi.org/10.1021/acs.orglett.5b00899.

(77) Visansirikul, S.; Kolodziej, S. A.; Demchenko, A. V. Synthesis of Oligosaccharide Fragments of Capsular Polysaccharide Staphylococcus Aureus Type 8. *J. Carbohydr. Chem.* **2020**, *39* (7), 301–333. https://doi.org/10.1080/07328303.2020.1821042.

(78) Lichtenthaler, F. W.; Oberthür, M.; Peters, S. Directed and Efficient Syntheses of

β(1→4)-Linked Galacto-Oligosaccharides. *European J. Org. Chem.* **2001**, *2001* (20), 3849–3869. https://doi.org/10.1002/1099-0690(200110)2001:20<3849::AID-EJOC3849>3.0.CO;2-Q.

(79) van Hengst, J. M. A.; Hellemons, R. J. C.; Remmerswaal, W. A.; van de Vrande, K. N. A.; Hansen, T.; van der Vorm, S.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. Mapping the Effect of Configuration and Protecting Group Pattern on Glycosyl Acceptor Reactivity. *Chem. Sci.* **2023**, *14* (6), 1532–1542. https://doi.org/10.1039/d2sc06139b.

(80) Huang, L.; Teumelsan, N.; Huang, X. A Facile Method for Oxidation of Primary Alcohols to Carboxylic Acids and Its Application in Glycosaminoglycan Syntheses. *Chem. - A Eur. J.* **2006**, *12* (20), 5246–5252. https://doi.org/10.1002/chem.200600290.

(81) Hale, K. J.; Hough, L.; Manaviazar, S.; Calabrese, A. An Update of the Rules for Pyranoside Sulfonate Displacement. *Org. Lett.* **2014**, *16* (18), 4838–4841. https://doi.org/10.1021/ol502193j.

(82) Danieli, E.; Proietti, D.; Brogioni, G.; Romano, M. R.; Cappelletti, E.; Tontini, M.; Berti, F.; Lay, L.; Costantino, P.; Adamo, R. Synthesis of Staphylococcus Aureus Type 5 Capsular Polysaccharide Repeating Unit Using Novel L-FucNAc and D-FucNAc Synthons and Immunochemical Evaluation. *Bioorganic Med. Chem.* **2012**, *20* (21), 6403–6415. https://doi.org/10.1016/j.bmc.2012.08.048.

(83) Zhang, Q.; Gimeno, A.; Santana, D.; Wang, Z.; Valdes-Balbin, Y.; Rodríguez-Noda, L. M.; Hansen, T.; Kong, L.; Shen, M.; Overkleeft, H. S.; Verez-Bencomo, V.; van der Marel, G. A.; Jimenez-Barbero, Jesus Chiodo, F.; Codée, J. D. C. Synthetic, Zwitterionic Sp1 Oligosaccharides Adopt a Helical Structure Crucial for Antibody Interaction. *ACS Cent. Sci.* **2019**, *5* (8), 1407–1416. https://doi.org/10.1021/acscentsci.9b00454.

(84) Wang, Z.; Gimeno, A.; Lete, M. G.; Overkleeft, H. S.; van der Marel, G. A.; Chiodo, F.; Jiménez-Barbero, J.; Codée, J. D. C. Synthetic Zwitterionic Streptococcus Pneumoniae Type 1 Oligosaccharides Carrying Labile O-Acetyl Esters. *Angew. Chemie Int. Ed.* **2023**, *62* (1), e202211940. https://doi.org/10.1002/anie.202211940.