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A Concise Synthesis of the Repeating Unit of Capsular Polysaccharide *Staphylococcus aureus* Type 8

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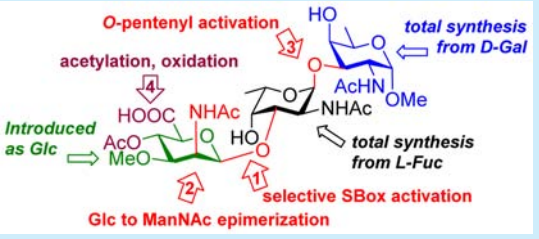
Supporting Information

ABSTRACT: The first synthesis of the repeating unit of *S. aureus* capsular polysaccharide type 8 is described. The repeating unit is an unusual trisaccharide sequence of three uncommon sugars, all connected via 1,2-*cis* linkages. The synthetic trisaccharide was equipped with capping methyl groups at the points of propagation of the polysaccharide sequence.



The Gram positive, cluster forming, bacteria *Staphylococcus aureus* normally colonizes the human nose and skin.[1] It belongs to the family of opportunistic pathogens that may cause bloodstream infections when the integrity of skin is broken and the immune system can no longer fight the infection. *S. aureus* has become one of the most frequent causes of infections in surgical patients, trauma and burn patients, patients receiving an implant, newborns, and dialysis patients with high mortality rates frequently ensuing. Public health and economic impacts of *S. aureus* is one of the highest among all bacterial pathogens. Immunocompromised individuals and patients with prosthetic devices or long-term intravascular catheters are particularly vulnerable.[2] The *S. aureus* bacteria is surrounded by a polysaccharide capsule. Therefore, preventive vaccination based on serotype-specific capsular polysaccharide (CP) or CP-protein conjugates is a suitable approach to fight against bacterial infections.[3]

Serotyping has revealed that the majority of *S. aureus* strains express either capsular polysaccharide type 5 (CP5) or type 8 (CP8). The structures of CP5 and CP8 have been established,[4] and chemical syntheses of the repeat unit of CP5 have been reported.[5,6] Herein, we report the first synthesis of the trisaccharide repeat unit of CP8. The CP8 repeating unit consists of a trisaccharide, but it is an unusual sequence of three uncommon monosaccharide residues (Figure 1). The trisaccharide bears a terminal ManNAcA (D-mannosamine uronic acid) residue, which is 4-*O*-acetylated. ManNAcA is then β-(1,2-*cis*) glycosidically linked to the L-FucNAc (L-fucosamine) unit via 3-OH. L-FucNAc is in turn linked via the 1,2-*cis*-glycosidic bond to the C-3 of D-FucNAc. In the natural polysaccharide sequence, D-FucNAc is then linked to another D-ManNAcA via α-(1−3) linkage, etc. The synthetic target **1** was designed as a tool compound to study activation and conjugation of full length CP8 derived from fermentation of *S. aureus* as previously described.[7] Hence, in order to preserve the conjugation sites the synthetic trisaccharide **1** (Figure 1) needs to be equipped with capping groups (methyl) at the points of propagation of the polysaccharide sequence.

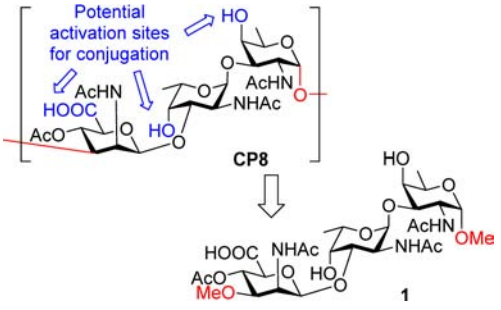


Figure 1. *S. aureus* type 8: polysaccharide CP8 and the synthetic target **1**.

From a synthetic perspective, one could anticipate a number of challenges and hurdles. For instance, the introduction of 1,2-*cis* glycosidic linkages[8] often proceeds with low selectivity, and

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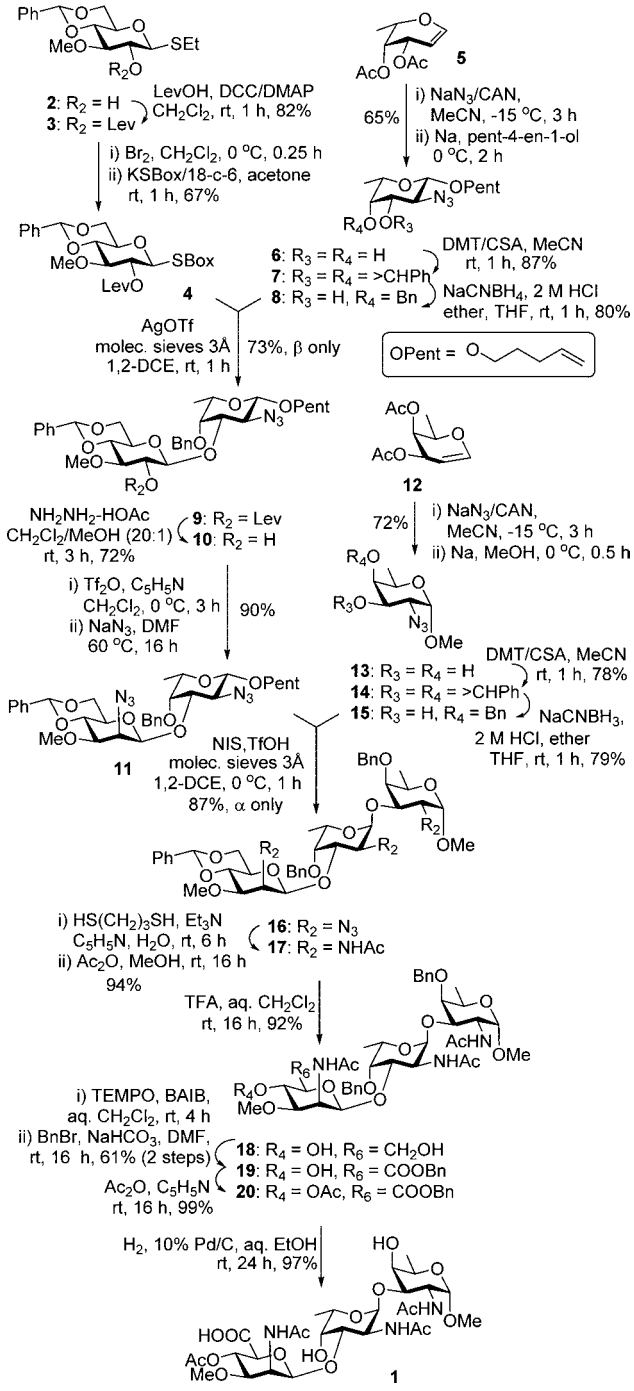
our synthetic target **1** has three such linkages. In addition, all three monosaccharide components of the repeat unit are uncommon and hence one could anticipate the necessity to perform the total synthesis of each monosaccharide. Our initial goal was to perform the direct synthesis of the β-mannosidic linkage,[9] an approach that was successfully used for other targets containing ManNAcA.[6,10] However, the direct mannosylation failed assumingly due to a very low reactivity profile for the benzylidene-protected mannosamine donors. After extended experimentation with thioglycoside and *S*-benzoxazolyl (SBox) donors,[11] we abandoned the mannosylation route and chose to proceed via a glucosylation route instead, followed by inversion of the stereocenter at C-2 postglycosylationally. According to this revised plan, the 2-OH of known thioglycoside **2**[12] was protected as a levulinoyl (Lev) ester by dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP)-mediated coupling with LevOH in dichloromethane to give **3** in 82% yield (Scheme 1). Reaction of compound **3** with bromine in the presence of molecular sieves 3 Å in dichloromethane followed by the introduction of the SBox leaving group with KSBox[13] in the presence of 18-crown-6 in acetone allowed the glucosyl donor **4** in 67% yield over two steps.

The synthesis of the L-fucosyl acceptor for the introduction of the middle unit in the trisaccharide sequence commenced from the known L-fucal **5**[14] as follows. Azidonitration of **5** with sodium azide in the presence of ceric ammonium nitrate (CAN) in MeCN at −15 °C followed by the reaction with sodium 4-penten-1-oxide gave β-pentenyl glycoside **6** in 65% yield over two steps. Diol **6** was then protected as a 3,4-*O*-benzylidene acetal using dimethoxytoluene (DMT) in the presence of camphorsulfonic acid (CSA) in MeCN to afford compound **7** in 87% yield. Reductive benzylidene ring opening in **7** with $NaCNBH_3$ in the presence of 2 M HCl in diethyl ether afforded L-fucosyl acceptor **8** in 80% yield.

With glucosyl donor **4** and fucosyl acceptor **8** in hand, we conducted selective activation of the SBox leaving group in the donor in the presence of the *O*-pentenyl anomeric group in the acceptor. This was affected in the presence of silver trifluoromethanesulfonate (AgOTf) and molecular sieves (3 Å) in 1,2-dichloroethane (1,2-DCE). As a result, disaccharide **9** was isolated in 73% yield as a pure β-anomer. In this context, thioglycoside donor **3** could also be activated over the *O*-pentenyl acceptor **8**. This activation requires MeOTf,[15] a relatively mild activator, which in this particular application gave modest results due to a highly disarmed reactivity profile for donor **3**. Selective levulinoyl group removal in **9** with hydrazine acetate in methanol and dichloromethane (1/20, v/v) gave disaccharide **10** in 72% yield. The latter was then subjected to sequential treatment with Tf_2O in the presence of pyridine in dichloromethane at 0 °C and, subsequently, sodium azide in DMF at 60 °C to obtain C-2′ epimerized[16] D-manno disaccharide **11** in 90% yield over two steps.

The synthesis of the D-fucosyl acceptor for the introduction of the reducing end unit in the trisaccharide sequence was initiated from D-galactose via the known D-fucal derivative **12**[17,18] as follows. Azidonitration of **12** with NaN_3 and CAN in MeCN at −15 °C followed by methyl glycoside formation with NaOMe at 0 °C gave α-fucoside **13** in 72% yield over two steps. Benzylidene acetal protection of diol **13** with DMT in the presence of CSA gave 3,4-*O*-benzylidene fucoside **14** in 78% yield. The reductive benzylidene ring opening in **14** with $NaCNBH_3$ in 2 M HCl in diethyl ether and THF afforded the desired D-fucosyl acceptor **15** in 79% yield. The latter was then glycosylated with disaccharide donor **11**. The *O*-pentenyl leaving group in **11** was activated with *N*-iodosuccinimide (NIS) and TfOH in the presence of molecular sieves (4 Å) in 1,2-dichloroethane at 0 °C to afford trisaccharide **16** with exclusive 1,2-*cis* stereoselectivity in 87% yield.

Scheme 1. Synthesis of CP8 Repeating Unit 1



With the backbone sequence constructed, and after thorough experimentation, we settled on the following sequence for the conversion of intermediate **16** into the target trisaccharide **1**. First, the three azide groups were reduced with propane-1,3-dithiol in the presence of triethylamine[19] in wet pyridine followed by acetylation with Ac_2O in methanol to obtain trisaccharide **17** in 94% yield over two steps. Second, the

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benzylidene acetal removal was performed with trifluoroacetic acid (TFA) in wet CH_2Cl_2 to acquire diol trisaccharide **18** in 92% yield. The oxidation of the primary alcohol in **18** was effected with (2,2,6,6-tetramethyl-piperidin-1-yl)oxyl (TEMPO) and (diacetoxyiodo)benzene (BAIB) in wet CH_2Cl_2.[20] The resulting carboxyl group was esterified with BnBr in the presence of $NaHCO_3$ in DMF to afford trisaccharide **19** in 61% yield over two steps. The 4-OH of the mannosamine subunit was then acetylated with Ac_2O in pyridine to afford trisaccharide **20** in 99% yield. Finally, the benzylic protecting groups in compound **20** were removed by hydrogenation in the presence of 10% palladium on charcoal in wet ethanol to acquire the final compound **1** in 97% yield.

In conclusion, we report the synthesis of a trisaccharide repeating unit of *S. aureus* CP8 bearing terminal methyl groups to preserve the activation sites. Characterization of the activation products derived from natural polysaccharides is very difficult owing to their large molecular size (MW = 100−2000 kDa). A study of the small model compound **1**, based on the structure of the repeat unit, offers a means to fully characterize the structure of the activated polysaccharides, allowing quantification of the level of activation. It could also allow us to begin understanding the side reactions that can occur after polysaccharide activation for conjugation. Over-activation and side reactions can lead to a loss of the epitopes required for immunogenicity of polysaccharide conjugate vaccines leading to product failure. An improved understanding of the conjugation process will, in turn, lead to a more controlled, predictable, and reproducible outcome of polysaccharide conjugations.

■ ASSOCIATED CONTENT

Supporting Information

Additional experimental details and characterization data for all new compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b00899.

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Notes

The authors declare no competing financial interest.

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