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Synthesis of the *Staphylococcus aureus* Strain M Capsular Polysaccharide Repeating Unit

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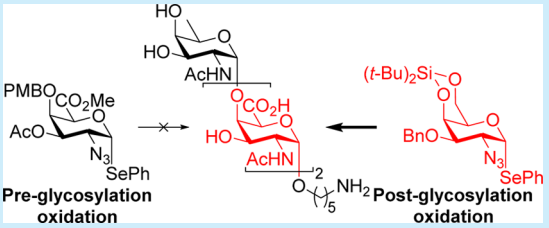
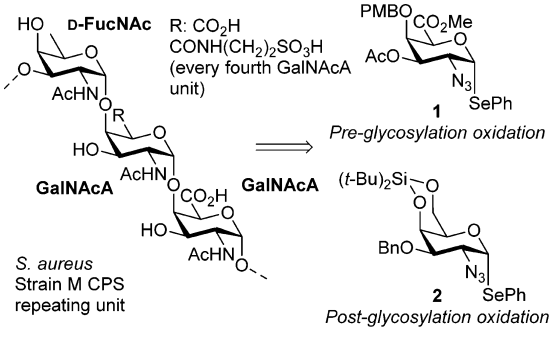
Supporting Information

ABSTRACT: The synthesis of the *Staphylococcus aureus* strain M capsular polysaccharide repeating unit is reported. A postglycosylation oxidation strategy was utilized for the construction of the α-galactosaminuronic acid linkages, relying on a stereoselective 2-azido-4,6-*O*-di-*tert*-butylsilylidene galactopyranoside donor, for which the selectivity was assessed by model glycosylations. The α-fucosamine linkage was installed stereoselectively, using a reactive 2-azidofucosyl donor. An unexpected glycosidic bond cleavage during the TEMPO/PhI(OAc)$_2$-mediated oxidation of a disaccharide intermediate was circumvented by a TEMPO/PhI(OAc)$_2$–Pinnick oxidation protocol.



The Gram-positive bacterium *Staphylococcus aureus* is a major causative agent of infections of the skin, lungs, and joints and can cause life-threatening conditions such as endocarditis or toxic shock syndrome. *S. aureus* M is a particular strain whose polysaccharide capsule is associated with increased mortality and increased resistance to phagocytosis in mouse models.[1] The capsular polysaccharide (CPS) was shown to have a repeating unit that consists of rare *N*-acetylgalactosaminuronic acid (GalNAcA) and *N*-acetylfucosamine (FucNAc) units as shown in Scheme 1.[2] A taurine residue is present on every other repeating unit, presumably by an amide bond on one of the GalNAcA moieties. Synthetic fragments of bacterial polysaccharides are valuable molecules for the generation of well-defined synthetic vaccines and as tools to unravel how the polysaccharide interacts with receptors of our immune system or to probe biosynthesis pathways.[3] These potential applications, and the synthetic challenges—the *cis*-glycosidic linkages and the rare monosaccharide constituents—associated with the structure of the polysaccharide, spurred our interest in developing an effective assembly route toward the repeating unit.

Scheme 1. Repeating Unit Structure of *S. aureus* Strain M CPS



At the onset of our synthesis campaign, we contemplated that the galactosaminuronic (GalNAcA) residues could be incorporated through either a pre- or postglycosylation strategy.[4] The advantage of the former approach lies in the fact that an (often complicated) oxidation event of multiple alcohols is prevented. An advantage of the latter approach is the higher reactivity of the non-oxidized building blocks and the use of stereodirecting protecting groups. We have recently introduced a quick "scanning method" that uses a set of partially fluorinated ethanols to map the stereoselectivity of a donor glycoside as a function of the reactivity of the acceptor.[5,6] This method rapidly provides insight into mechanistic pathways that are operational during the glycosylation event, and it provides an indication of the robustness of a glycosylation procedure. We therefore decided to probe a galactosaminuronic donor (**1**, Scheme 1) with this acceptor toolset to gauge the plausibility of using preoxidized building blocks.[4,7−10]

As a donor, we selected selenogalacturonide **1**, which bears an azide as a nonparticipating precursor for the required acetamido functions (see Scheme 1). We decided to use phenylselenoglycosides,[11,12] as the azidoselenylation of galactal enables rapid access of 2-azidogalactosyl building blocks[13] and selenophenyl donors are readily activated using the Ph$_2$SO/Tf$_2$O couple.[5,14,15] GalN$_3$A donor **1** contains a C4-*O*-*p*-methoxybenzyl (PMB) ether as an orthogonal protecting group, while the remaining C3-alcohol and C-6 uronic acid functionalities are protected as esters, cleavable by saponification (see the Supporting Information for details on the synthesis of **1**).

We first studied the activation of donor **1** by low-temperature NMR experiments to identify any covalent reactive intermediates.[16] Activation of **1** with Ph$_2$SO and Tf$_2$O in dichloromethane-d_2

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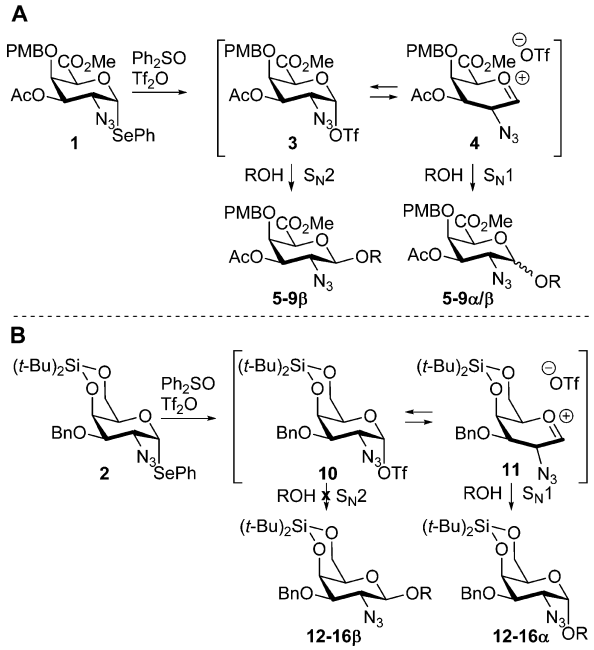
Scheme 2. Mechanistic Pathways in Glycosylations of Donors 1 and 2



at −80 °C resulted in the formation of a new species that was identified as triflate **3** (see Scheme 2) based on the chemical shift and J coupling of the anomeric signal (δ 6.16 ppm, J = 3.0 Hz; see the SI for NMR spectra).[16] This intermediate started to decompose around −30 °C. Next we coupled donor **1** with the panel of partially fluorinated alcohol acceptors (ethanol and its 2-mono-, -di-, and -trifluorinated analogues, as well as cyclohexanol, Table 1) to probe the stereoselectivity of the donor. The glycosylations of GalN_3A donor **1** show a clear dependence on acceptor nucleophilicity (Table 1, entries 1−5).[8] The highly reactive acceptors ethanol, monofluoroethanol, and cyclohexanol (entries 1, 2, and 5) gave the corresponding β-glycosides **5**, **6**, and **9**, exclusively, consistent with an S_N2-like scenario,[17]

Table 1. Glycosylations of 1 and 2 with Model Acceptors[a]

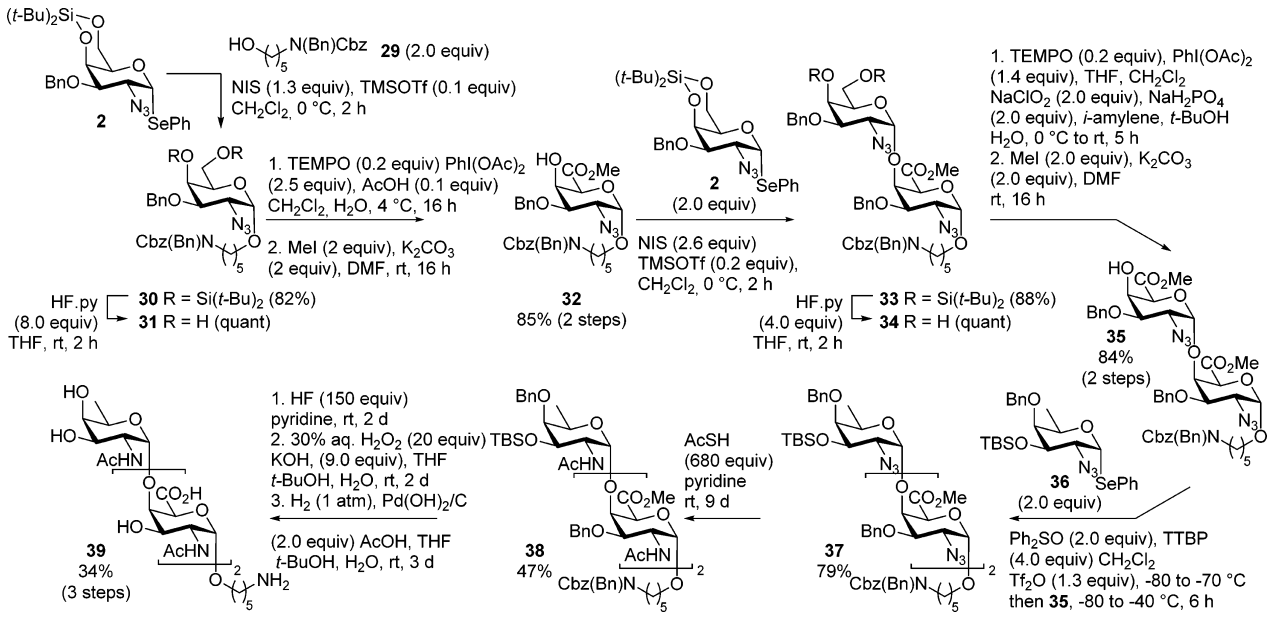
entry	donor	R	product	yield, % (α/β)[b]
1	**1**	CH_3	**5**	68 (>1:19)
2	**1**	CH_2F	**6**	79 (>1:19)
3	**1**	CHF_2	**7**	72 (1:9)
4	**1**	CF_3	**8**	55 (2:5)
5	**1**		**9**	36 (>1:19)
6	**2**	CH_3	**12**	60 (>19:1)
7	**2**	CH_2F	**13**	79 (>19:1)
8	**2**	CHF_2	**14**	90 (>19:1)
9	**2**	CF_3	**15**	81 (>19:1)
10	**2**		**16**	54 (>19:1)

[a]Glycosylation conditions: **1** or **2**, Ph_2SO (1.3 equiv), TTBP (2.5 equiv), 3 Å MS, CH_2Cl_2; Tf_2O (1.3 equiv), −80 to −70 °C, 15 min; ROH (2.0 equiv), −80 to −40 °C, 90 min. [b]α/β ratios were determined by ^{1}H NMR of the product mixture.

in which the acceptor directly displaces the anomeric triflate. The presence of more F atoms in the acceptor alcohol led to a gradual decay of selectivity, and the condensation of 2,2,2-trifluoroethanol (entry 4) proceeded with poor selectivity (α/β 2:5). The changing stereoselectivity is likely due to a change in reaction pathway, with less reactive nucleophiles preferring to react in an S_N1 manner, involving oxocarbenium ion-like intermediates.[5,6,18,19] We have previously shown that the reactivity of a secondary hydroxyl group of a carbohydrate building block falls in the range of the difluoro- and trifluoroethanols.[5,6] Based on the poor selectivity for these acceptors in the reactivity−selectivity scan, we decided to abandon the use of preoxidized building blocks and shift our attention to an alternative 2-azidogalactoside donor.

We opted to use the di-*tert*-butylsilylidene (DTBS)-functionalized galactopyranosyl donor **2** to install the *cis*-galactosamine

Scheme 3. Synthesis of *S. aureus* Strain M CPS



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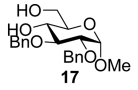
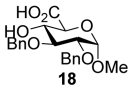
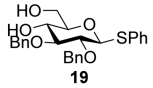
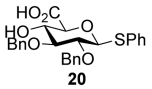
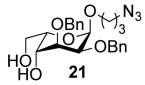
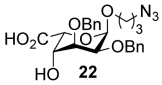
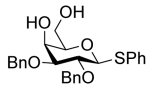
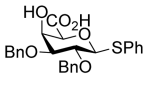
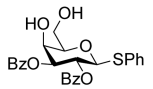
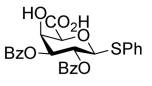
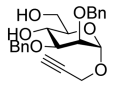
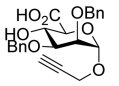
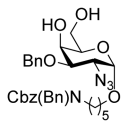
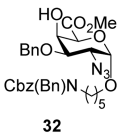
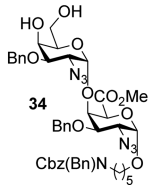
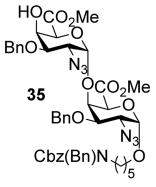
linkages. Kiso and co-workers have shown that DTBS-protected galactosyl donors exhibit exceptional α-selectivity, with the DTBS group even overriding the influence of C2-acyl functionalities, which generally give the 1,2-*trans*-products through neighboring group participation.[20,21] The influence of a C2-azido group on this system has not been investigated so far; nor have potential reactive intermediates been characterized. Thus, we preactivated the $GalN_3$ donor **2** as described above (Scheme 2). This led to the clean formation of a new species, which, on the basis of its chemical shift and J_{H1-H2}-coupling, likely is triflate **10**.[16] Intermediate **10** proved to be more stable than $GalN_3A$ triflate **3**, and decomposition was observed to start around −10 °C. Next, we subjected donor **2** to the acceptor reactivity−selectivity scan employing a set of fluorinated ethanols. As tabulated in Table 1, entries 6−10, the α-galactosides (**12**−**16**) were obtained exclusively. Even though the silylidene donor had been completely transformed into the corresponding α-triflate, and ethanol and cyclohexanol are nucleophlilic enough to engage in S_N2-type glycosylation reactions, no direct displacement of the anomeric triflate was observed. The selectivities obtained here, with the preactivation conditions used, thus attest to the extremely powerful stereodirecting effect of the DTBS group. The pathway followed by these galactosylations probably follows an attack of the nucleophiles on the α-face of an $^4H_3$-oxocarbenium ion-like intermediate.

Having established selenogalactoside **2** as a productive donor for the construction of *cis*-linkages, we next set out to synthesize the repeating unit trisaccharide of *S. aureus* strain M (Scheme 3).

The first glycosylation of **2** with aminopentanol **29**[22] proceeded uneventfully, leading to the expected α-linked product **30** exclusively. Here, we found that both the Ph_2SO/Tf_2O preactivation system and direct activation with NIS and TMSOTf were equally effective.[23] Removal of the DTBS group, using HF in pyridine, was followed by regioselective oxidation of the primary alcohol using the TEMPO/$PhI(OAc)_2$ system.[24,7] Addition of a catalytic amount of AcOH led to shorter reaction times and higher yields, likely by action of AcOH in the regeneration of the oxidant. Subsequent methylation delivered monosaccharide acceptor **32** in good yield.

Introduction of the second galactosyl unit gave disaccharide **33** as the sole anomer in 88% yield. Removal of the DTBS group with HF in pyridine gave the diol **34**, setting the stage for the oxidation to the uronic acid. Using the TEMPO/$PhI(OAc)_2$ system led, however, to monosaccharide **32** as the main product, with only small amounts of the target disaccharide **35**. The mechanism of the formation of **32** is unclear at present, but careful monitoring of the oxidation reaction by TLC indicated rapid consumption of **34** to the more apolar aldehyde. Consumption of this aldehyde was very slow, and it is likely that the glycosidic bond cleavage event occurs at this stage. The low rate of the second oxidation step in the unmodified TEMPO/$PhI(OAc)_2$ reaction may, in part, be attributable to the heterogeneity of the reaction mixture (CH_2Cl_2/H_2O) and a slow hydration step that is required to prepare the α,α-diol for the oxidation step. We therefore sought a protocol that circumvented these rate-diminishing factors. Of the many methods that exist for the oxidation of aldehydes to carboxylic acids, the Pinnick oxidation is one of the few that does not proceed by intermediate hydration of the aldehyde.[25,26] In order to form the aldehyde, however, the TEMPO/$PhI(OAc)_2$ system was deemed useful, owing to its high selectivity for primary alcohols. Thus, we devised a new two-step, one-pot TEMPO/$PhI(OAc)_2$−Pinnick oxidation protocol. To establish

Table 2. Substrate Scope of the TEMPO/$PhI(OAc)_2$−Pinnick Oxidation Sequence[a]

entry	substrate	product	time (h)	yield (%)
1	 **17**	 **18**	7	87
2	 **19**	 **20**	10	81
3	 **21**	 **22**	7	82
4	 **23**	 **24**	7	73
5	 **25**	 **26**	7	73
6	 **27**	 **28**	5	87
7	 **31**	 **32**	6	80[b]
8	 **34**	 **35**	5	84[b]

[a]Conditions: TEMPO (0.2 equiv), $PhI(OAc)_2$ (1.4 equiv), THF, H_2O, 0 °C to rt; $NaClO_2$ (2.0 equiv), NaH_2PO_4 (2.0 equiv), isoamylene (2.4 equiv), *t*-BuOH, H_2O. [b]Isolated yield after methylation of the crude carboxylic acid (MeI (2.0 equiv), K_2CO_3 (2.0 equiv), DMF, 16 h).

the feasibility of this protocol, we screened several model carbohydrate diols as summarized in Table 2. Glucose, galactose, gulose, and mannose building blocks bearing different protecting groups were probed, and in each case, the regioselectivity of the first oxidation step proved to be excellent and the ensuing Pinnick oxidation effective to deliver the desired carboxylic acids in good yield. It must be noted, however, that in the case of thioglycosides a small amount of sulfoxide was found as a byproduct.[27] Gratifyingly, when we applied the TEMPO/$PhI(OAc)_2$−Pinnick protocol to disaccharide **34**, the desired carboxylate was formed successfully, yielding the desired disaccharide **35** after methylation of the crude carboxylic acid in 84% yield (Scheme 2).

With the disaccharide **35** in hand, the final glycosylation was performed using azido-fucoside donor **36**. We have recently described that azido-fucoside donors bearing "arming" protecting groups, such as benzyl and silyl ethers, provide *cis*-selective fucosylation reactions, probably by favoring the formation of an

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intermediate azido-fucoside oxocarbenium ion that can be attacked in a stereoselective manner to give the α-products.[5] Thus, $FucN_3$ donor **36** was activated with the Ph_2SO/Tf_2O system, and ensuing addition of disaccharide **35** gave fully protected trisaccharide **37** in 79% yield with complete stereoselectivity. Deprotection of the *S. aureus* strain M repeating unit commenced with the AcSH-mediated conversion of the azides to their corresponding acetamido units to give the triacetamide **38**.[28] Next the TBS ether was removed using HF in pyridine, and the methyl esters were saponified to give the diacid in 49% yield over two steps. Finally, catalytic hydrogenolysis of the benzyl carbamate and ethers provided fully deprotected target trisaccharide **39** in 69% yield.

In conclusion, an efficient synthesis of the *S. aureus* strain M CPS repeating unit has been accomplished using a post-glycosylation oxidation strategy. Even under preactivation conditions that generate a full equivalent of α-triflate, the used 4,6-O-DTBS protected 2-azidogalactosyl donor solely provides α-linked products, independent of the reactivity of the acceptor used. A novel one-pot, two-step oxidation protocol was invented to allow for the effective regioselective oxidation of primary alcohols to give the corresponding acids. The final glycosylation, connecting the digalactosaminuronic acid and the fucosamine, was achieved in high yield and with complete stereoselectivity, building on the stereoselectivity of a reactive 2-azidofucosyl donor. The robustness of the donors, combined with their ease of preparation and their protecting group pattern, opens up avenues for the preparation of longer fragments. The trisaccharide and longer congeners will facilitate the evaluation of the immunological properties of the *S. aureus* strain M oligosaccharides.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b00747.

Synthesis of donors **1** and **2**, full experimental procedures, NMR spectra of reactive intermediates, and characterization of new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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(19) At present, it is not known what conformation (or suite of conformations) 2-azidogalacturonic acid oxocarbenium ions prefer to adopt, and it is difficult to identify a most favorable structure based on the stereoelectronic substituent preferences.

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