Organic Letters

Letter

pubs.acs.org/OrgLett

Synthesis of *Staphylococcus aureus* Type 5 Trisaccharide Repeating Unit: Solving the Problem of Lactamization

Ivan A. Gagarinov,[†,‡] Tao Fang,[†,‡] Lin Liu,[†] Apoorva D. Srivastava,[†,‡] and Geert-Jan Boons*[,†,‡]

[†]Complex Carbohydrate Research Center, The University of Georgia, 315 Riverbend Road, Athens, Georgia 30602, United States

[‡]Department of Chemistry, The University of Georgia, Athens, Georgia 30602, United States

Supporting Information

ABSTRACT: The chemical synthesis of an orthogonally protected trisaccharide derived from the polysaccharide of *Staphylococcus aureus* Type 5, which is an attractive candidate for the development of immunotherapies, is described. The challenging α-fucosylation and β-mannosylation are addressed through the careful choice of protecting groups. Lactamization of a β-D-Man*p*NAcA moiety during deprotection was avoided by a late stage oxidation approach. Versatility of the trisaccharide was demonstrated by its transformation into a spacer-containing repeating unit suitable for immunological investigations.

Staphylococcus aureus is a commensal of the human skin that can cause skin and soft tissue infections (SSTIs). As many as 20% of individuals receiving surgical and antibiotic therapy suffer from recurrent SSTIs, which can lead to invasive disease with bacteremia, sepsis of endocarditis.[1] Of particular concern are infections caused by antibiotic (methicillin)-resistant strains (MRSA) for which there are limited treatment options.[2] Addressing SSTIs through the development of new antibiotics alone is not sustainable due to the continued increase in antibiotic-resistance of MRSA isolates.[3] Passive or active immunization of patients may offer an attractive alternative for the treatment of infections caused by MRSAs, and particularly immunotherapies based on the capsular polysaccharide of *S. aureus* are offering exciting avenues.[1]

There are 12 known serotypes of the *S. aureus* capsular polysaccharides. However, types 5 and 8 comprise the majority of reported isolates.[4] The structures of the type 5 and 8 polysaccharides have been deduced by NMR as being →4)-β-D-Man*p*NAcA-(1→4)-α-L-Fuc*p*NAc(3-OAc)-(1→3)-β-D-Fuc*p*NAc-(1→ and →3)-β-D-Man*p*NAcA(4-OAc)-(1→3)-α-L-Fuc*p*NAc-(1→3)-β-D-Fuc*p*NAc-(1→, respectively.[5] Thus, the two polysaccharides share common monosaccharides and only differ in the location of the acetyl esters and position of an anomeric linkage. A bivalent vaccine composed of *S. aureus* capsular polysaccharide types 5 and 8 conjugated to *Pseudomonas aeruginosa* exotoxin A (rEPA) gave 60% protection. However, it failed to confer long-term protection for end stage renal disease patients, who are often affected by these infections.[6] It is thought that well-defined oligosaccharides obtained by chemical synthesis could facilitate the development of more efficacious vaccines and therapeutic antibodies. In this respect, chemical synthesis makes it possible to install a reactive linker for controlled conjugation to carrier proteins, which is often required for immunological studies. This feature is particularly relevant for the *S. aureus* polysaccharides, which are highly functionalized, and contain sensitive functional groups such as acetyl esters that complicate conjugation chemistry. Furthermore, chemical synthesis can provide substructures and structural analogs required for establishing structure−activity relationships, or be used to determine minimal epitope requirements to elicit protective immune responses.[7]

Previous efforts to prepare a repeating unit of *S. aureus* type 5 polysaccharide that can be conjugated to a carrier protein in a controlled manner failed due to difficulties of differentiating amino groups in the saccharide moiety and the amino propyl linker that was needed for conjugation purposes.[8] Specifically, reduction of azido moieties of the sugar moiety led to lactam formation of the mannosaminuronic moiety, whereas hydrogenation of the same compound resulted in the reduction of the azides and removal of the benzyloxycarbonyl-protecting group of the amino propyl linker. Birch reduction had been reported for the deprotection of a similar oligosaccharide.[9] However, this method could not be applied to the target compound due to the presence of a base-sensitive acetyl ester.

In addition to the difficulties associated with deprotection, the preparation of the repeating unit of an *S. aureas* type 5 polysaccharide (Figure 1) has a number of other synthetic challenges. Specifically, it requires efficient syntheses of rare monosaccharides, such as D- and L-fucosamines, and the installation of glycosidic linkages having a 1,2-*cis*-configuration including a *N*-acetyl-β-D-mannosaminuronic acid (β-D-Man*p*NAcA) and *N*-acetyl-α-L-fucosamine (α-L-Fuc*p*NAc) glycosides. Furthermore, the target compound contains acetyl esters, and therefore, base-sensitive protecting groups need to be avoided. Finally, orthogonal protecting groups need to be

Received: January 5, 2015
Published: February 6, 2015

ACS Publications © 2015 American Chemical Society

DOI: 10.1021/acs.orglett.5b00031
Org. Lett. 2015, 17, 928−931

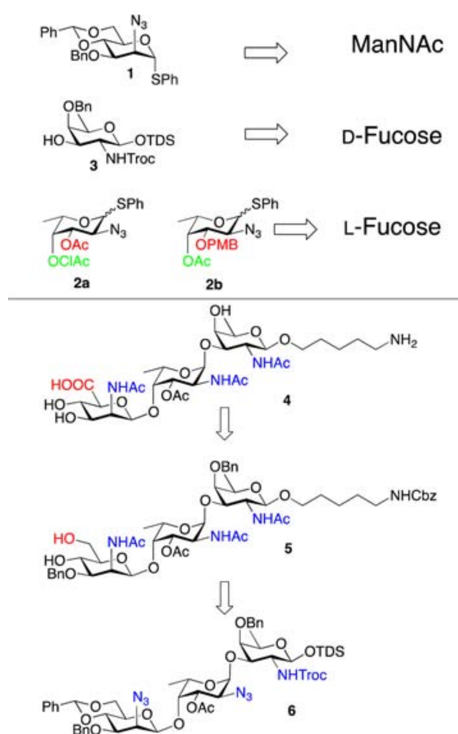


Figure 1. Retrosynthetic analysis.

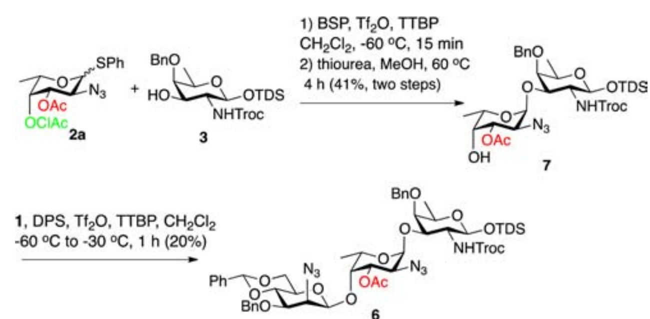
identified that allow the preparation of a glycosyl donor and acceptor for further oligosaccharide assembly.

We envisaged that monosaccharide building blocks **1**, **2a–b**, and **3** would make it possible to assemble trisaccharide **6**, which could then be converted into the spacer containing repeating unit of the *S. aureus* type 5 polysaccharide (**4**, Figure 1). Selective removal of the anomeric dimethylthexylsilyl (TDS)[10] ether of **6** would provide a route to a glycosyl donor whereas hydrolysis of the benzylidene acetal followed by regioselective protection of the C-6 hydroxyl of the resulting diol would give a potential acceptor for oligosaccharide assembly. Furthermore, it was anticipated that the β-D-Man$p$N$_3$ glycoside of **6** could be installed by employing glycosyl donor **1** by *in situ* conversion into an α-anomeric triflate and subsequent nucleophilic displacement with a sugar alcohol.[11] Lactam formation of the β-D-Man$p$NAcA moiety would be avoided by a late stage oxidation of the C-6 hydroxyl of the β-D-Man$p$NAc residue after reduction of the C-2 azido group, followed by acetylation of the resulting amine. Finally, the azido moiety at C-2 of the glycosyl donors **2a–b** would allow the installation of an α-2-amino-fucoside, whereas the 2,2,2-trichloroethoxycarbamate (Troc) moiety function at C-2 of **3** would make it possible to form a β-2-amino-fucoside.

Monosaccharide building block **1** was synthesized by a modification of a reported procedure[11d] starting from inexpensive *N*-acetyl-D-mannosamine (see Supporting Information (SI)). Building blocks **2a–b** and **3** were prepared by efficient approaches starting from commercially available L- and D-fucose, respectively (see SI).

Previous studies employing 2-azido-L-fucose as a glycosyl donor[12] indicated that methyl triflate (MeOTf) is an appropriate promoter to obtain the corresponding glycosides in good yield. However, MeOTf-promoted coupling of **2a** with **3** proceeded sluggishly resulting in the degradation of acceptor **3**, probably due to the acid sensitivity of the anomeric TDS group (Scheme 1). The use of 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) as

Scheme 1. Attempted Assembly of Trisaccharide 6



an acid scavenger did prevent acceptor decomposition.[13] However, the glycosylation still proceeded very slowly, and after a reaction time of 18 h, **7** was isolated in only a trace amount (<5%) after deprotection of C-4. An *N*-iodosuccinimide (NIS)/trimethylsilyl trifluoromethanesulfonate (TMSOTf) promoted glycosylation of **2a** with **3** gave disaccharide **7** in a low yield of 32% along with unidentified byproducts possibly resulting from acid-mediated degradation of the acceptor. The target fucoside was obtained in an improved yield of 41% when the glycosylation was performed under neutral conditions employing 1-benzenesulfinyl piperidine (BSP)/trifluoromethanesulfonic anhydride (Tf_2O) as the activator system[14] at −60 °C. Use of the more powerful diphenylsulfoxide (DPS) activator did not improve the yield of the glycosylation. However, in each case, only α-fucoside formation was observed, and although the yields of the glycosylations were modest, a sufficient quantity of **7** could be prepared to examine installation of the β-mannosamine residue.

β-Mannosides are difficult to install due to the axial C-2 substituent, which hinders the attack of an incoming nucleophile from the β-face and the Δ-anomeric effect that stabilizes the α-anomer.[15] Recent studies indicate that preactivation of phenyl 2-azido-2-deoxy-3-*O*-benzyl-4,6-benzylidene-1-thio-α-D-mannopyranoside **1** with the powerful DPS/Tf_2O promoter system[11b] offers an attractive approach for selective β-glycosylations. Thus, activation of donor **1** was achieved at low temperature (−60 °C) with DPS/Tf_2O in the presence of 2,4,6-tri-*tert*-butylpyrimidine (TTBP) in CH_2Cl_2. Subsequent dropwise addition of **7** followed by slow warming to −30 °C afforded the desired β-mannoside **6** in a disappointing yield of 20%.

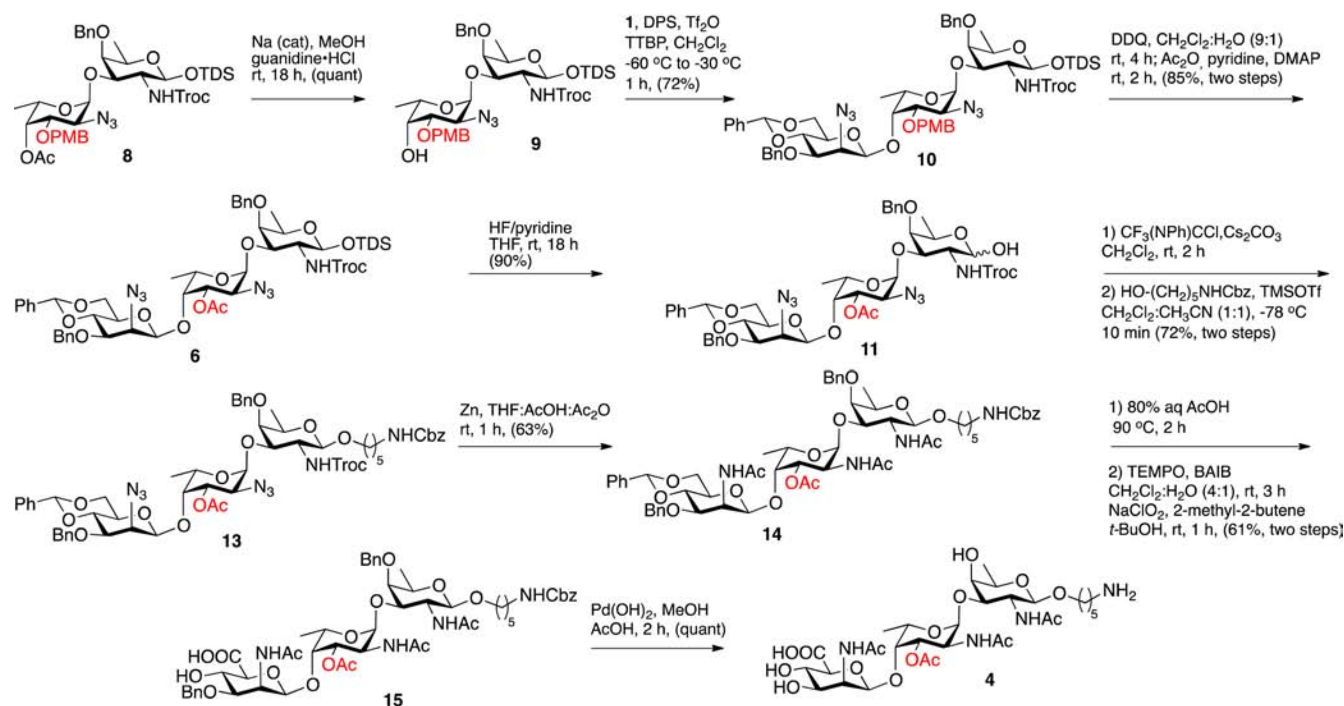
It is known that the axial C-4 hydroxyl of galactosides and fucosides are of low reactivity,[16] which in the case of glycosyl acceptor **7** is attenuated by the neighboring electron-withdrawing acetyl ester at C-3. To improve the glycosyl accepting properties of **7**, the acetyl ester at C-3 was replaced by an electron-donating *p*-methoxybenzyl (PMB) group.[17] It was

Table 1. Coupling Optimization of 2b with 3

promoter/conditions	yield	β/α
BSP, Tf_2O, TTBP, CH_2Cl_2, −60 °C	30%	α only
DPS, Tf_2O, TTBP, CH_2Cl_2, −60 °C	30%	α only
NIS, TMSOTf, CH_2Cl_2, −60 °C	73%	1/4
NIS, AgOTf, CH_2Cl_2, −60 °C	70%	1/4
NIS, TMSOTf, CH_2Cl_2/Et_2O (4:1), −60 °C	72%	1/4

DOI: 10.1021/acs.orglett.5b00031
Org. Lett. 2015, 17, 928–931

Scheme 2. Improved Assembly of Target Trisaccharide 4



expected that the newly required glycosyl donor **2b** would be more reactive than **2a**, thus providing an opportunity to improve the challenging α-fucosylation. For the purpose of the latter glycosylation, multiple reaction conditions were examined by varying the solvent, temperature, and the use of additives (Table 1). It was found that a coupling of **2b** with **3** in the presence of NIS/TMSOTf in CH_2Cl_2 at −60 °C gave disaccharide **8** in a much improved yield of 73% as mainly the α-anomer (α:β, 4:1). Surprisingly, the nature of the promoter influenced the outcome of the glycosylation and the use of BSP/Tf_2O in CH_2Cl_2 at −60 °C gave only the α-anomer ($J_{1,2}$ = 3.6 Hz), albeit in a significantly lower yield. Next, removal of the acetyl ester of **8** using sodium methoxide in methanol (Scheme 2) gave acceptor **9**, which was condensed with **1** using DPS/Tf_2O as the promoter to give the expected β-mannoside **10** in an excellent yield of 72%. The β-configuration of the newly formed glycosidic linkage was unambiguously confirmed by ^{13}C NMR spectroscopy ($^1J_{CH,\beta}$ = 159.8 Hz)[18] and the chemical shift of H-5 (~3.1 ppm). The minor α-anomer was isolated by silica gel chromatography in a yield of 5−8% (see SI).

Encouraged by these results, the flexibility of synthon **10** was demonstrated by performing a glycosylation−deprotection−oxidation−deprotection sequence to prepare spacer-containing repeating unit **4**. First, the PMB ether of **10** was removed by oxidation with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ),[19] which was followed by acetylation of the resulting hydroxyl to give **6** in an 85% yield over two steps. Subsequent cleavage of the anomeric TDS group using a hydrogen fluoride−pyridine complex gave hemiacetal **11**, which was transformed into the corresponding *N*-phenyl trifluoroacetimidate **12** by reaction with 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride in the presence of Cs_2CO_3. A TMSOTf-catalyzed glycosylation of **12** with 5-(benzyloxycarbonyl)aminopentanol in CH_2Cl_2/CH_3CN (1:1, v/v) gave **13** as only the β-anomer (72%). Trisaccharide **13** was then treated with Zn in a solution of THF/AcOH/Ac_2O[10] to simultaneously convert the azido and Troc groups into acetamido moieties, affording compound **14** in high yield. Hydrolysis of the benzylidene acetal followed by selective oxidation of the primary hydroxyl of the resulting diol **5** by a modification of Huang's one-pot TEMPO/NaOCl−$NaClO_2$ procedure[20] gave carboxylate **15** in a yield of 61% over two steps. Finally, the remaining benzyl ethers and benzyloxycarbamate were removed by hydrogenation to furnish the target trisaccharide **4**.

In conclusion, a flexible orthogonally protected trisaccharide β-D-Man*p*N_3-(1→4)-α-L-Fuc*p*N_3-(1→3)-β-D-Fuc*p*NHTroc has been prepared in high yield with excellent anomeric selectivity for the challenging α-fucosylation and β-mannosylation. Systematic modification of the protecting groups of these unusual glycosyl donors was critical for achieving high anomeric selectivities. Late-stage regioselective oxidation of a highly functionalized repeating unit containing β-D-Man*p*NAcA was key to avoid the commonly observed lactam formation. The strategic principles described in this letter will be relevant to the preparation of other biologically important polysaccharides containing β-D-Man*p*NAcA moieties.[21] Furthermore, our findings will guide the synthesis of larger *S. aureus* oligosaccharide fragments required for the development of a fully synthetic vaccine.

■ ASSOCIATED CONTENT

Supporting Information

Full experimental procedures and spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: gjboons@ccrc.uga.edu.

Notes

The authors declare no competing financial interest.

DOI: 10.1021/acs.orglett.5b00031
Org. Lett. 2015, 17, 928−931

ACKNOWLEDGMENTS

Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health (GM065248, G.-J.B.).

REFERENCES

(1) Daum, R. S.; Spellberg, B. *Clin. Infect. Dis.* **2012**, *54*, 560−567.

(2) (a) Lowy, F. D. *New Engl. J. Med.* **1998**, *339*, 520−532. (b) Fridkin, S. K.; Hageman, J. C.; Morrison, M.; Sanza, L. T.; Como-Sabetti, K.; Jernigan, J. A.; Harriman, K.; Harrison, L. H.; Lynfield, R.; Farley, M. M. *New Engl. J. Med.* **2005**, *352*, 1436−1444.

(3) Chambers, H. F.; DeLeo, F. R. *Nat. Rev. Microbiol.* **2009**, *7*, 629−641.

(4) O'Riordan, K.; Lee, J. C. *Clin. Microbiol. Rev.* **2004**, *17*, 218−234.

(5) Jones, C. *Carbohydr. Res.* **2005**, *340*, 1097−1106.

(6) Fattom, A. I.; Sarwar, J.; Ortiz, A.; Naso, R. *Infect. Immun.* **1996**, *64*, 1659−1665.

(7) (a) Astronomo, R. D.; Burton, D. R. *Nat. Rev. Drug Discovery* **2010**, *9*, 308−324. (b) Lepenies, B.; Seeberger, P. H. *Immunopharmacol. Immunotoxicol.* **2010**, *32*, 196−207.

(8) Danieli, E.; Proietti, D.; Brogioni, G.; Romano, M. R.; Cappelletti, E.; Tontini, M.; Berti, F.; Lay, L.; Costantino, P.; Adamo, R. *Bioorg. Med. Chem.* **2012**, *20*, 6403−6415.

(9) Walvoort, M. T. C.; Lodder, G.; Overkleeft, H. S.; Codée, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2010**, *75*, 7990−8002.

(10) Mo, K.-F.; Li, X.; Li, H.; Low, L. Y.; Quinn, C. P.; Boons, G.-J. *J. Am. Chem. Soc.* **2012**, *134*, 15556−15562.

(11) (a) Walvoort, M. T. C.; de Witte, W.; van Dijk, J.; Dinkelaar, J.; Lodder, G.; Overkleeft, H. S.; Codé, J. D. C.; van der Marel, G. A. *Org. Lett.* **2011**, *13*, 4360−4363. (b) Litjens, R. E. J. N.; van den Bos, L. J.; Codée, J. D. C.; van den Berg, R. J. B. H. N.; Overkleeft, H. S.; van der Marel, G. A. *Eur. J. Org. Chem.* **2005**, *2005*, 918−924. (c) van den Bos, L. J.; Duivenvoorden, B. A.; de Koning, M. C.; Filippov, D. V.; Overkleeft, H. S.; van der Marel, G. A. *Eur. J. Org. Chem.* **2007**, *2007*, 116−124. (d) Litjens, R. E. J. N.; Leeuwenburgh, M. A.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **2001**, *42*, 8693−8696.

(12) Ludewig, M.; Thiem, J. *Eur. J. Org. Chem.* **1998**, *1998*, 1189−1191.

(13) Boltje, T. J.; Li, C.; Boons, G.-J. *Org. Lett.* **2010**, *12*, 4636−4639.

(14) Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015−9020.

(15) Cai, F.; Wu, B.; Crich, D. In *Advances in Carbohydrate Chemistry and Biochemistry*; Derek, H., Ed.; Academic Press: 2009; Vol. *62*, pp 251−309.

(16) Lichtenthaler, F. W.; Oberthür, M.; Peters, S. *Eur. J. Org. Chem.* **2001**, *2001*, 3849−3869.

(17) (a) Fraser-Reid, B.; Wu, Z.; Udodong, U. E.; Ottosson, H. *J. Org. Chem.* **1990**, *55*, 6068−6070. (b) Zhu, T.; Boons, G.-J. *Org. Lett.* **2001**, *3*, 4201−4203.

(18) Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293−297.

(19) Huang, L.; Huang, X. *Chem.—Eur. J.* **2007**, *13*, 529−540.

(20) Huang, L.; Teumelsan, N.; Huang, X. *Chem.—Eur. J.* **2006**, *12*, 5246−5252.

(21) (a) Lugowski, C.; Romanowska, E.; Kenne, L.; Lindberg, B. *Carbohydr. Res.* **1983**, *118*, 173−181. (b) Tsui, F.-P.; Boykins, R. A.; Egan, W. *Carbohydr. Res.* **1982**, *102*, 263−271. (c) Branefors-Helander, P.; Kenne, L.; Lindberg, B.; Petersson, K.; Unger, P. *Carbohydr. Res.* **1981**, *88*, 77−84. (d) Van Der Kaaden, A.; Gerwig, G. J.; Kamerling, J. P.; Vliegenthart, J. F. G.; Tiesjema, R. H. *Eur. J. Biochem.* **1985**, *152*, 663−668.

DOI: 10.1021/acs.orglett.5b00031
Org. Lett. 2015, 17, 928−931