Organic LETTERS

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Synthesis of Defined and Functionalized Glycans of Lipoteichoic Acid: A Cell Surface Polysaccharide from *Clostridium difficile*

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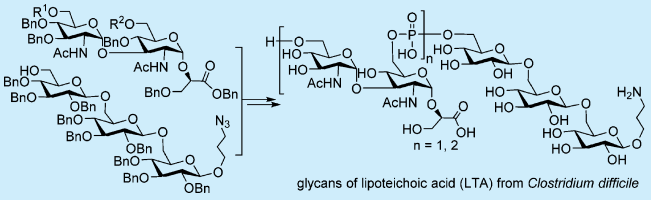
Supporting Information

ABSTRACT: Two structurally defined, functionalized glycans of lipoteichoic acid (LTA, also known as PS-III) from *C. difficile*, which have one or two repeating units of LTA linked to the core trisaccharide, were efficiently synthesized via a convergent [2 + 3] or [2 + 2 + 3] strategy. The α-linkage of both *N*-acetylglucosamine residues in the repeating unit were constructed with glycosyl imidates of azidosugars as donors, while the phosphodiester bridges between the oligosaccharides were fashioned using *H*-phosphonate chemistry. Both synthetic targets contained a 3-aminopropyl group at the core trisaccharide reducing end, facilitating their conjugation to other biomolecules to afford conjugates useful for various biological studies and applications.



Clostridium difficile is a Gram-positive, spore-forming anaerobic bacterium, which has been identified as the main cause of nosocomial diarrhea and colitis in humans that results in a high rate of mortality.[1−3] In the past two decades, the number of *C. difficile* infections (CDIs) has been growing steadily to cause an enormous medical and social problem. For example, it has been revealed that the incidence of CDIs in the US has almost doubled between 1993 and 2003 and the CDI-caused mortality has quadrupled between 1999 and 2004.[4,5] It is estimated that approximately US$3.2 billion is spent on treating CDIs every year in the US alone.[6] Currently, antibiotic therapy using vancomycin and fidaxomicin is the most common strategy to treat CDIs in the clinic.[7] Its efficacy, however, has been challenged by the emergence of hypervirulent *C. difficile* strains, such as BI/NAP1/027.[8] Therefore, novel strategies for the prevention and treatment of CDIs are highly desirable.

Vaccination is one strategy for the effective control of CDIs.[9−11] In the development of vaccines, most present efforts have been focused on *C. difficile* toxins, such as toxin A and toxin B, as target antigens.[12,13] Recently, the cell surface polysaccharides of *C. difficile*, including PS-I, PS-II, and lipoteichoic acid (LTA, also called PS-III),[14,15] have been characterized and identified as promising antigenic epitopes for prophylactic vaccine development. Accordingly, several oligosaccharides related to these polysaccharides have been synthesized[16−22] and utilized to explore conjugate vaccines that have exhibited encouraging results.[17,19,20,23,24] The structure of the LTA (Figure 1) from *C. difficile* and *Peptostreptococcus anaerobius* as well[25] is comprised of a lipophilic diacylglycerol β-1,6-triglucoside core and many repeating D-glyceric acid α-1,4-diglucosaminoside units, which are stitched together via phosphodiester bridges.[15] Seeberger and co-workers[21] described the first synthesis of the repeating unit of this LTA and its oligomers and found that they were recognized by antibodies from *C. difficile* patients. Further studies showed that the CRM_{197} protein conjugates of these oligosaccharides induced LTA-specific antibodies that could opsonize *C. difficile* and inhibit its intestinal colonization.[24] Pedersen and co-workers synthesized some LTA analogs that contained the lipidated core trisaccharide and repeating unit and used them to explore the noninnate immunity pathway.[22] Furthermore, it was shown that the protein conjugates of deacylated LTA could elicit antibodies to recognize both *C. difficile* and other *Clostridium* strains.[26] These results have highlighted the LTA glycan and related oligosaccharides as promising antigens for the development of *C. difficile* vaccines.

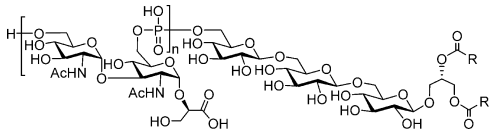


Figure 1. Structure of *C. difficile* LTA. R = lipid chains.

To facilitate the study of LTA and the development of LTA-based *C. difficile* vaccines, we established a new and efficient strategy for the synthesis of structurally defined LTA glycans. The synthetic targets **1** and **2** (Scheme 1) were designed to

Received: April 24, 2017

DOI: 10.1021/acs.orglett.7b01242
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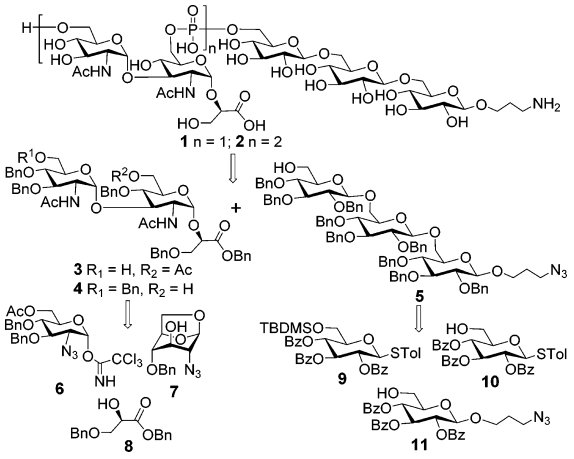
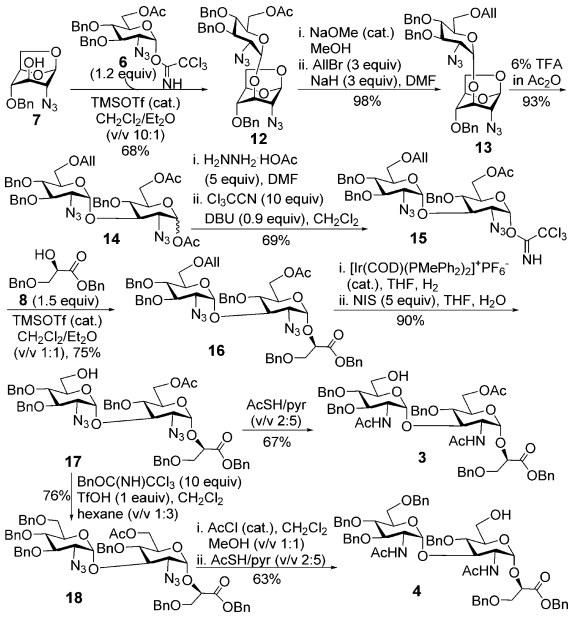
Scheme 1. Synthetic Targets 1 and 2 and the Retrosynthesis



contain both the core trisaccharide and the repeating unit of LTA, as well as a 3-aminopropyl group at the trisaccharide reducing end, to enable their expedient and efficient coupling with other molecules. Retrosynthetic disconnection of **1** and **2** (Scheme 1) led to specially protected disaccharides **3** and **4** and trisaccharide **5** as key intermediates. According to this design, **1** and **2** could be assembled by a convergent [2 + 3] or [2 + 2 + 3] strategy upon bridging 6,6′-*O*-phosphorylation. In turn, **3** and **4** could be prepared from glycosyl donor **6**,[27,28] acceptor **7**,[27,29] and D-glyceric acid **8**.[21] In **6** and **7**, the azido group was used as a latent amino group to prevent neighboring group participation and thereby promote glucosamine 1,2-*cis* α-glycosylation.[30,31] On the other hand, **5** could be prepared from **9**−**11** via preactivation-based iterative one-pot glycosylation,[32] and the 2-*O*-benzoyl group in **9**−**11** would favor 1,2-*trans* β-glycosylation as a result of the neighboring group participation effect.

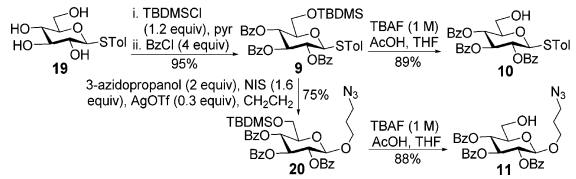
Our syntheses commenced with preparing **6**,[27,28] **7**,[27,29] and **8**[21] according to reported procedures, which were utilized to build disaccharides **3** and **4** as outlined in Scheme 2. Glycosylation of **7** with **6** using trimethylsilyl triflate (TMSOTf) as a promoter smoothly afforded the α-1,3-linked disaccharide **12** in a 68% yield with excellent stereoselectivity (α:$\beta \approx 10$:1). The newly formed α-glycosidic bond in **12** was confirmed by the small coupling constant ($J_{1',2'} = 3.6$ Hz) of its H-1′ NMR signal at δ 4.80 ppm in $CDCl_3$. Deacetylation of **12** with NaOMe in MeOH and allylation of the resultant free 6′-OH group with allyl bromide and NaH in DMF gave **13**. Regioselective opening of the 1,6-anhydro ring in **13** with trifluoroacetic acid (TFA) in acetic anhydride[33] gave **14** in an 91% overall yield. Compound **14** was converted into glycosyl donor **15** in two steps, including selective anomeric deacetylation using hydrazine acetate[34] and trichloroacetimidation of the resultant hemiacetal with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).[35] Thereafter, **8** was glycosylated with **15** under the promotion of TMSOTf to afford **16** as the only anomer. The small coupling constant ($J_{1,2} = 3.8$ Hz) of its H-1 signal at δ 5.18 ppm in $CDCl_3$ verified the α-glycosidic linkage. The 6′-*O*-allyl group in **16** was removed on iridium-mediated olefin rearrangement and *N*-iodosuccinimide (NIS)-promoted hydrolysis,[36,37] to produce **17** in an excellent yield (90%). Subsequently, its 2,2′-azido groups were converted into acetamido groups by treatment with thioacetic acid in pyridine[21,22,38] to result in **3** used to construct **2**. On the other hand, 6′-*O*-benzylation of **17** under acidic conditions with benzyl trichloroacetimidate and triflic acid (TfOH),[39] which had no influence on the base-labile *O*-acetyl and glyceric acid groups, was followed by selective 6-*O*-deacetylation using 2% acetyl chloride[40] in CH_2Cl_2 and MeOH (v/v 1:1) and reductive acetylation of the azido group with thioacetic acid in pyridine to provide **4** (63% overall yield), which was used for the assembly of both **1** and **2**.

Scheme 2. Synthesis of Intermediates 3 and 4



Monosaccharides **9**−**11** were prepared from *p*-tolyl 1-thio-β-D-glucopyranoside **19**[41] via a series of established transformations (Scheme 3). First, regioselective silylation of the 6-OH group in **19** with *tert*-butyldimethylchlorosilane (TBDMSCl) and benzoylation of the remaining hydroxyl groups with benzoyl chloride afforded **9** (95% overall yield). Treating **9** with tetrabutylammonium fluoride (TBAF) to remove the TBDMS group gave **10** (89%). Alternatively, the reaction of **9** with 3-azidopropanol in the presence of silver triflate (AgOTf) and NIS, followed by desilylation with TBAF, provided **11**.

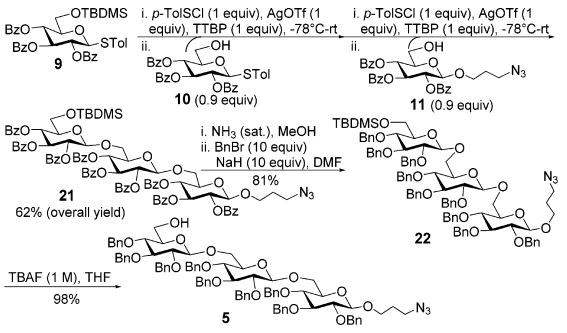
Scheme 3. Synthesis of Monosaccharides 9−11



Scheme 4 outlines the assembly of **5** from **9**, **10**, and **11** by preactivation-based iterative one-pot glycosylation.[32] For each glycosylation, the glycosyl donor was preactivated at −78 °C for 15 min with *p*-TolSOTf, generated *in situ* from *p*-toluenesulfenyl chloride (*p*-TolSCl) and AgOTf, using 2,4,6-tri*tert*-butylpyrimidine (TTBP) as a scavenger of TfOH formed from the reaction. Then, the glycosyl acceptor was added at −78 °C, and the reaction was kept at rt for 15 min and monitored with TLC to ensure completion. Moreover, 1 equiv of *p*-TolSCl and 0.9 equiv of **10** or **11** (relative to **9**) were utilized to guarantee complete consumption of glycosyl acceptors, so as to minimize the impact of unreacted substrate on subsequent reaction. Eventually, **21** was isolated in a 62% yield after two rounds of glycosylation, and all glycosidic bonds in **21** were β, verified by the coupling

DOI: 10.1021/acs.orglett.7b01242
Org. Lett. XXXX, XXX, XXX−XXX

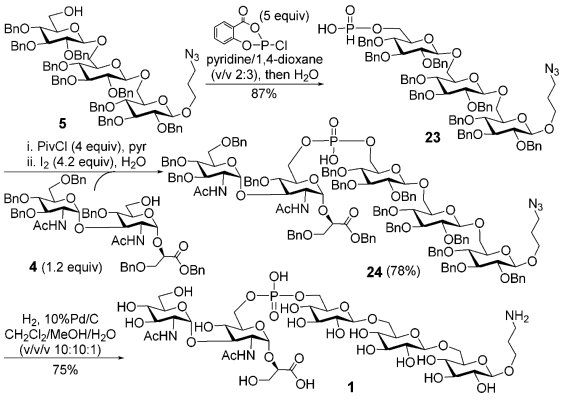
Scheme 4. Preactivation-Based Iterative One-Pot Synthesis of 5



constants (>7.2 Hz) of its H-1 NMR signals. The results indicated the excellent stereoselectivity of the glycosylations due to the neighboring group participation effect. At this stage, the benzoyl groups in **21** were swapped for benzyl groups to exploit the stability of benzyl ethers to base and acid and, in the meantime, to allow for one-step global deprotection later. Finally, the 6″-*O*-TBDMS group in **22** was removed with TBAF to complete **5** in a 98% yield.

For the synthesis of **1** depicted in Scheme 5, the key step was the coupling of **4** with **5** via a phosphoryl bridge, which was

Scheme 5. Synthesis of 1

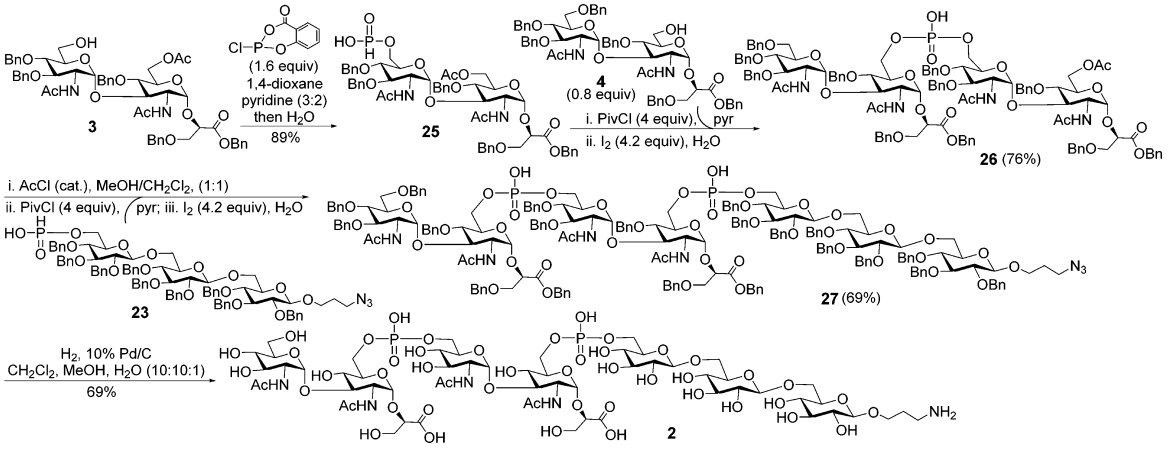


fashioned by *H*-phosphonate chemistry. First, the reaction of **5** with 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one[42] in the presence of pyridine was attempted followed by hydrolysis of the resultant phosphite, which gave *H*-phosphonate monoester **23** (87%) purified by silica gel column chromatography. Next, **23** was coupled with **4** under the influence of pivaloyl chloride (PivCl),[42] which was followed by oxidation of the resultant *H*-phosphonate diester with I_2 to afford fully protected phosphate **24** (78%) as the single isomer. Debenzylation of **24** by hydrogenolysis with concomitant azide reduction afforded synthetic target **1** in a 75% yield, after purification by size-exclusion chromatography on an LH-20 column.

Synthetic target **2** was assembled by a procedure of coupling two disaccharide repeating units first and then coupling with trisaccharide *H*-phosphonic monoester **23** (Scheme 6). Compound **3** was converted into *H*-phosphonate monoester **25** (89%) by the protocol used to synthesize **23**, which was then coupled with **4** under the above-mentioned conditions to produce tetrasaccharide phosphate **26** in a 76% yield. Removal of the 6-*O*-acetyl group in **26** under acidic conditions with 0.5% AcCl in CH_2Cl_2 and MeOH (v/v 1:1) did not affect other functional groups, but this reaction was incomplete even after an elongated time, and the deacetylated product was inseparable from **26** (5% remained). Nonetheless, **26** would not interfere with the next synthetic step, and so the mixture was directly used for conjugating with **4** by the above-mentioned two-step one-pot protocol to afford fully protected **27**, which was readily separated from **26** by size-exclusion column chromatography in a 69% overall yield. Finally, **27** was deprotected and purified as described above to produce **2**. Both synthetic targets **1** and **2**, and the intermediates involved in their syntheses, were fully characterized with MS and NMR data.

In conclusion, a highly convergent and efficient strategy was developed for synthesizing the glycans of LTA from *C. difficile*. This synthesis was highlighted by separate assembly of the α-linked disaccharide repeating unit and β-linked core trisaccharide first and then joining them together by phosphate bridges. The repeating unit was prepared in excellent overall yields and α-selectivity with glucosamine derivatives **6** and **15** as glycosyl donors. Using a reactive bicyclic derivative of glucosamine **7**[27] as a glycosyl acceptor was also helpful for the outcome. Trisaccharide **5** was prepared by iterative one-pot glycosylation, which gave a good yield and stereoselectivity as a result of the neighboring group participation. The 6,6′-*O*-phosphodiester bridge connecting oligosaccharide moieties was fashioned using *H*-phosphonate chemistry that turned out to be very efficient. Ultimately, LTA glycans **1** and **2** were synthesized in a

Scheme 6. Synthesis of 2



DOI: 10.1021/acs.orglett.7b01242
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convergent [2 + 3] or [2 + 2 + 3] manner. In the literature, two synthetic studies on this LTA have been reported. Seeberger and co-workers[21] described the synthesis of an LTA repeating unit and its oligomers and used them to study an LTA-based vaccine.[24] Pedersen and co-workers[22] reported the synthesis of some LTA analogs that contained all key structural components of LTA. Our synthesis diverged from these reports in that different building blocks and glycosylation methods were used to construct the repeating unit and the core trisaccharide, which gave improved overall synthetic efficiency. Moreover, *H*-phosphonate chemistry was employed to build the phosphate bridges between the oligosaccharides. In our hands, this chemistry, which involved stable intermediates, was more convenient and robust than the phosphoramidite method. This synthetic strategy is anticipated to be also applicable to other LTA glycans. In addition, **1** and **2** have a 3-aminopropyl group at their downstream end, which would facilitate their conjugation with other molecules. Synthetic LTA glycans and their conjugates should be useful for various studies of LTA, including the development of LTA-based *C. difficile* vaccines, which is currently pursued in our laboratory.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b01242.

Synthetic procedures; NMR and MS spectra and other analytical data for all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by grants from the National Natural Science Foundation of China (Projects 21672129 and 21472114).

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DOI: 10.1021/acs.orglett.7b01242
Org. Lett. XXXX, XXX, XXX−XXX