Published in final edited form as:

Org Lett. 2018 January 19; 20(2): 353–356. doi:10.1021/acs.orglett.7b03590.

Synthesis of Oligosaccharide Components of the Outer Core Domain of *P. aeruginosa* Lipopolysaccharide Using a Multifunctional Hydroquinone-Derived Reducing-End Capping Group

Abhishek Vartak, Fatma M. Hefny, and Steven J. Sucheck*

Department of Chemistry and Biochemistry, University of Toledo, 2801 West Bancroft Street, Toledo, Ohio 43606, United States

Abstract

The synthesis of a trisaccharide (common to glycoform I and II) and a tetrasaccharide (common to glycoform I) from the outer core domain of *Pseudomonas aeruginosa* lipopolysaccharide (LPS) using a novel hydroquinone-based reducingend capping group is reported. This multifunctional capping group was utilized as purification handle and was stable toward many common transformations in oligosaccharide synthesis. The access to outer-core LPS antigens with a TBDPS-protected hydroquinone (TPH) at the reducing end will be useful for glycan array and therapeutic glycoconjugate synthesis

Pseudomonas aeruginosa is a Gram-negative bacterium with intrinsic antibiotic resistance. This opportunistic human pathogen is a leading cause of death among cystic fibrosis (CF) patients. *P. aeruginosa* is also associated with hospital-acquired pneumonia and blood infections. Synthetic outer core domains from the *P. aeruginosa* lipopolysaccharide (LPS) will be valuable components of protective vaccines since the full isolated LPS is toxic.¹

According to the International Antigenic Typing System (IATS), the strains of *P. aeruginosa* are classified into 20 different serotypes on the basis of the serological reactivity of the O-polysaccharides.^{2,3} These O-polysaccharides are attached to Lipid A via an intervening core oligosaccharide. Depending on the presence or absence of O-polysaccharide, these lipopolysaccharides are termed as S (smooth)-type lipopolysaccharides or R (rough)-type

*Corresponding Author: steve.suchek@utoledo.edu.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b03590. Experimental procedures and NMR and HRMS data for all new compounds (PDF)

The authors declare no competing financial interest.

lipopolysaccharides, respectively.^{4,5} The unique feature of all lipopolysaccharides is the presence of structurally similar outer-core glycoform I (Figure 1) and closely related glycoform II. The two glycoforms share monosaccharides, consisting of one D-galactosamine residue, three D-glucose residues, and one L-rhamnose residue.^{6–8} Antibodies against these oligosaccharides have been shown to be protective against some serotypes of *P. aeruginosa*.^{9,10}

The synthesis of oligosaccharides with a protecting group at the reducing end is useful for the purification of single anomers. The reducing-end group is typically removed after synthesis for conjugation with other materials to form arrays or vaccine conjugates. While groups like *p*-methoxyphenyl can serve this purpose, they are often not amenable to postglycosylation functionalization. Groups like *p*-nitrophenyl (PNP) can be reduced to *p*-aminophenyl and coupled with linkers that enable array formatting¹¹. Nonetheless, presence of a PNP group at the reducing end limits the scope of the oligosaccharide chemistry that can be carried out and is generally more compatible with chemoenzymatic methodologies.¹² We envisioned that a latent hydroquinone would be valuable for late-stage conjugation chemistry and would confer better stability during many chemical transformations. Examples of conjugation with hydroquinones include Mitsunobu, α -haloketo, and Mannich-type chemistry.^{13,14} In addition, a hydroquinone was expected to be compatible with common oligosaccharide deprotection chemistries, including catalytic hydrogenation and Zn/AcOH reductions, under which PNP is reduced. This innovation provides additional flexibility to leave the reducing-end protecting group in place. We report herein the use of *tert*-butyldiphenylsilyl (TBDPS)-protected hydroquinone (TPH) **2** as a multifunctional reducing-end capping group and show how it can be used to assemble the outer core domain of *P. aeruginosa* LPS. Compound **2** was prepared as reported elsewhere (Figure 2).¹⁵

Syntheses of some fragments of outer core domains of *P. aeruginosa* bearing a methoxy group at the reducing end have been reported.^{16,17} In order to access conjugable outer core domains of *P. aeruginosa* LPS, we synthesized thioglycoside donor building blocks **A–D** (Figure 3). We employed fluorenylmethyloxycarbonyl (Fmoc) and levulinate (Lev) protecting groups in the donor building blocks in order to achieve selective deprotection during tri- and tetrasaccharide synthesis. The groups are compatible with emerging automated oligosaccharide synthesis technologies.

Thioglycoside intermediates **3** and **4** were obtained from D-glucose over six steps according to reported procedures.^{18–21} *S*-4-Methylphenyl-2,3,4-tri-*O*-benzoyl 1-thio- β -D-glucopyranoside (**3**) was treated with Fmoc chloride in the presence of pyridine at room temperature to afford building block **A** in 91% yield (Scheme 1). Similarly, Fmoc protection was achieved with 4-methylphenyl 2,3,4-tri-*O*-benzyl-1-thio- β -D-glucopyranoside (**4**) to obtain **B** in 89% yield. The coupling reaction of **4** with levulinic acid using dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) afforded thioglycoside donor **C** in 85% yield. The L-rhamnose-based glycosyl donor **D** was synthesized over eight steps starting from L-rhamnose according to reported literature.^{22–24}

With the donor building blocks in hand, our synthesis of the common trisaccharide of the two glycoforms was started from 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl- α -D-galactopyranosyl

bromide (**5**) (Scheme 2). The donor bromide **5** was obtained from D-galactose over five steps according to a reported procedure.²⁵ Donor **5** and glycosyl acceptor TPH **2** were coupled using silver carbonate and silver perchlorate to obtain 4-*tert*-butyldiphenylsilyloxyphenyl 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl- α -D-galactopyranoside (**6**) in 64% yield with a 4:1 α : β ratio. Both isomers were carried forward for the next reaction. Deacetylation under Zemplén conditions afforded the intermediate triol. The triol was subjected to benzylidene acetal protection in the presence of *p*-toulenesulfonic acid (*p*TsOH) to afford 4-*tert*-butyldiphenylsilyloxyphenyl 2-azido-2-deoxy-4,6-*O*-benzylidene- α -D-galactopyranoside (**7**) in 53% yield over two steps. The two isomers were separated during purification, and only the α isomer was carried forward.

Acceptor **7** and donor building block **A** were condensed using NIS:TMSOTf at -40°C to obtain disaccharide **8** in 63% yield (β -only).^{26,27} Disaccharide **8** was subjected to $\text{BF}_3\cdot\text{OEt}_2$ and Et_3SiH at 0°C to selectively open the benzylidene ring to afford acceptor **9** containing a free C-4 hydroxyl in 77% yield. The traditional method of benzylidene acetal ring opening with NaCNBH_3 and 1 N HCl in ether was initially attempted. However, it was unsuccessful in this case.

Acceptor disaccharide **9** was then coupled with thioglycoside building block **B** using NIS:TfOH in a 2:5 DCM: Et_2O mixture at -40 to -20°C to produce trisaccharide **10** in 63% yield (α -only) (Scheme 3). The Boc-alanine residue was installed by reduction of azide **10** with Zn/AcOH followed by coupling with Boc-Ala-OH in the presence of propylphosphonic anhydride (T3P) and DIPEA to obtain trisaccharide derivative **11** in 56% yield. The Fmoc group was deprotected with a Et_2NH :DCM mixture, which was followed by Zemplén deesterification to remove the benzoyl esters; this gave trisaccharide **12** in 68% yield over the two steps. The benzyl groups were removed by hydrogenation using 10% Pd/C to obtain trisaccharide **13** in 86% yield. The deprotected compound was purified using a C18 silica gel column to afford trisaccharide **13**. The TPH group was utilized as a purification handle since it was helpful for retention of the compound on the C18 column. In addition, the group locked the anomeric center for easy characterization. The TPH was removed via CAN oxidation in CH_3CN , which was followed by size-exclusion chromatography (Sephadex LH-20) to obtain the final target trisaccharide **14** with a free reducing end in 80% yield after 6 h.^{28,29} It is notable that the reducing-end deprotection was achieved in a single step without removal of the TBDPS group.

The disaccharide intermediate **9** was used to prepare the tetrasaccharide fragment of glycoform I. The coupling between acceptor **9** and glycosyl donor **C** was achieved using the NIS:TfOH promoter system to procure trisaccharide **15** in 45% yield^{26,27} (Scheme 4). The levulinate ester was deprotected using hydrazine acetate in a DCM:methanol (4:1) mixture to access trisaccharide acceptor **16**. Acceptor **16** and rhamnoside donor **D** were coupled using the NIS:TMSOTf promoter system to afford tetrasaccharide **17** in 57% yield.²⁶ Azide **17** was reduced using Zn/AcOH and coupled with Boc-Ala-OH in the presence of T3P to afford tetrasaccharide **18** in 68% yield.

In summary, we have developed a flexible strategy to assemble oligosaccharides from the outer core domain of *P. aeruginosa* LPS using Lev- and Fmoc-protected thioglycosides. The

hydroquinone-derived reducing-end capping group TPH was used for the first time in a complex oligosaccharide synthesis and was effective as a purification handle during reversed-phase and size-exclusion chromatography. The TPH group was remarkably stable toward many protection–deprotection chemistries and common glycosylation conditions. No electrophilic substitution on the hydroquinone in the presence of NIS was observed. The TPH could be efficiently removed in a single step via CAN oxidation without TBDPS deprotection to obtain free-reducing-end oligosaccharides such as **14**. The presence of a latent hydroquinone moiety at the reducing end of a polysaccharide such as **13** should be useful for direct and mild conjugation chemistry with peptides or lipids to assemble glycoconjugates such as glycopeptides or glycolipid vaccines^{30–32} using the latent phenolic hydroxyl group or the electron-rich ortho carbon as a chemoselective reaction center.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health (Grant GM094734).

REFERENCES

- (1). Erridge C; Stewart J; Bennett-Guerrero E; McIntosh TJ; Poxton IR J. Endotoxin Res 2002, 8, 39. [PubMed: 11981444]
- (2). Liu PV; Matsumoto H; Kusama H; Bergan T Int. J. Syst. Bacteriol 1983, 33, 256.
- (3). Liu PV; Wang S. J. Clin. Microbiol 1990, 28, 922. [PubMed: 2112563]
- (4). Raetz CR; Whitfield C Annu. Rev. Biochem 2002, 71, 635. [PubMed: 12045108]
- (5). Rocchetta HL; Burrows LL; Lam JS Microbiol. Mol. Biol. Rev 1999, 63, 523. [PubMed: 10477307]
- (6). Bystrova O; Lindner B; Moll H; Kocharova N; Knirel YA; Zahringer U; Pier G Biochemistry (Moscow) 2004, 69, 170. [PubMed: 15000683]
- (7). Bystrova OV; Knirel YA; Lindner B; Kocharova NA; Kondakova AN; Zaähringer U; Pier GB FEMS Immunol. Med. Microbiol 2006, 46, 85. [PubMed: 16420601]
- (8). Sadovskaya I; Brisson JR; Thibault P; Richards JC; Lam JS; Altman E Eur. J. Biochem 2000, 267, 1640. [PubMed: 10712594]
- (9). Cryz JS; Lang A; Ruådeberg A; Wedgwood J; Que J; Fuärer E; Schaad U Behring Inst. Mitt 1997, 345. [PubMed: 9382759]
- (10). Cryz SJ, Jr.; Sadoff JC; Fuärer E Infect. Immun 1988, 56, 1829. [PubMed: 2454893]
- (11). Zhang X; Xu Y; Hsieh P-H; Liu J; Lin L; Schmidt EP; Linhardt RJ Org. Biomol. Chem 2017, 15, 1222. [PubMed: 28091666]
- (12). Yang J; Hsieh P-H; Liu X; Zhou W; Zhang X; Zhao J; Xu Y; Zhang F; Linhardt RJ; Liu J Chem. Commun 2017, 53, 1743.
- (13). Banphavichit V; Bhanthumnavin W; Vilaivan T Tetrahedron 2007, 63, 8727.
- (14). Fillion H; Porte M; Bartoli M-H; Bouaziz Z; Berlion M; Villard J Chem. Pharm. Bull 1991, 39, 493. [PubMed: 2054874]
- (15). Mir R; Dudding TJ Org. Chem 2017, 82, 709.
- (16). Komarova BS; Tsvetkov YE; Knirel YA; Zaähringer U; Pier GB; Nifantiev NE Tetrahedron Lett 2006, 47, 3583.
- (17). Komarova BS; Tsvetkov YE; Pier GB; Nifantiev NE Carbohydr. Res 2012, 360, 56. [PubMed: 22940177]

- (18). Veleti SK; Lindenberger JJ; Thanna S; Ronning DR; Sucheck SJ J. Org. Chem 2014, 79, 9444. [PubMed: 25137149]
- (19). Cai C; Dickinson DM; Li L; Masuko S; Suflita M; Schultz V; Nelson SD; Bhaskar U; Liu J; Linhardt RJ Org. Lett 2014, 16, 2240. [PubMed: 24697306]
- (20). Huang X; Huang L; Wang H; Ye XS Angew. Chem 2004, 116, 5333.
- (21). Yu K; Bi N; Xiong C; Cai S; Long Z; Guo Z; Gu G Org. Lett 2017, 19, 3123. [PubMed: 28548838]
- (22). Geert Volbeda A; Reintjens NR; Overkleef HS; van der Marel GA; Codée JD Eur. J. Org. Chem 2016, 2016, 5282.
- (23). Pozsgay V Carbohydr. Res 1992, 235, 295. [PubMed: 1473109]
- (24). Pozsgay VJ Org. Chem 1998, 63, 5983.
- (25). Bay S; Huteau V; Zarantonelli M-L; Pires R; Ughetto-Monfrin J; Taha M-K; England P; Lafaye PJ Med. Chem 2004, 47, 3916.
- (26). Nicolaou K; Seitz S; Papahatjis DJ Am. Chem. Soc 1983, 105, 2430.
- (27). Toshima K; Tatsuta K Chem. Rev 1993, 93, 1503.
- (28). Gordon KH; Balasubramanian S Org. Lett 2001, 3, 53. [PubMed: 11429870]
- (29). Jarrahpour A; Zarei M Molecules 2007, 12, 2364. [PubMed: 17978763]
- (30). Ban H; Gavriluk J; Barbas CF, III J. Am. Chem. Soc 2010, 132, 1523. [PubMed: 20067259]
- (31). Bohorov O; Andersson-Sand H; Hoffmann J; Blixt O Glycobiology 2006, 16, 21C.
- (32). Peri F; Dumy P; Mutter M Tetrahedron 1998, 54, 12269.

Figure 1.
Outer core domain of *P. aeruginosa* R-type lipopolysaccharide (glycoform I).

Figure 2.
TBDPS-protected hydroquinone (TPH) **2** as a reducing- end capping group.

Figure 3.
Donor building blocks **A–D**.

Scheme 1.
Synthesis of Donor Building Blocks

Scheme 2.
 Synthesis of Intermediate Disaccharide 10

Scheme 3.

Synthesis of the Common Outer-Core Trisaccharide Component of *P. aeruginosa* LPS

Scheme 4. Synthesis of the Common Outer-Core Tetrasaccharide Component of *P. aeruginosa* LPS