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Synthesis of the Zwitterionic Repeating Unit of the O-Antigen from *Shigella sonnei* and Chain Elongation at Both Ends

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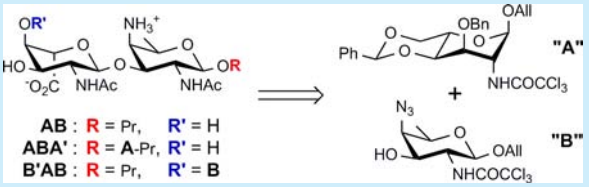
Supporting Information

ABSTRACT: *Shigella sonnei* O-antigen features a zwitterionic disaccharide repeat encompassing two rare monosaccharides. The synthesis of the **AB** repeat and of trisaccharides **ABA′** and **B′AB**, which validates chain elongation at either end, is reported. All targets were synthesized using a postglycosylation oxidation strategy in combination with imidate chemistry. Precursors to residue **A** were obtained from L-glucose. The AAT (**B**) donor and acceptor were obtained from D-glucosamine. A one-step $Pd(OH)_2/C$-mediated deprotection provided the propyl glycoside targets.



Shigellae are Gram-negative enteroinvasive bacteria causing shigellosis in humans.[1] Shigellosis remains one of the five major diarrheal diseases in children under five.[2] Epidemiological data still call for the development of a *Shigella* vaccine.[2] Out of the 4 species of *Shigella*, *S. sonnei* is of special concern.[3] It is present in developing countries where disease is endemic and is the prevalent species in transitional and industrialized countries.[2] Naturally acquired immunity against *Shigella* is in part directed against the O-specific polysaccharide (O-SP) of its lipopolysaccharide (LPS).[4] On that basis, polysaccharide conjugates have received particular attention in the search for a *Shigella* vaccine. Protective efficacy was demonstrated for the most advanced detoxified LPS-based candidates.[5] Conversely, synthetic carbohydrate haptens are being explored as promising alternatives to material of biological origin. In this context, we have proposed the first synthetic carbohydrate-based vaccine candidate against the prevalent *Shigella* serotype.[6] The designed conjugate encompasses a three-repeat segment of the O-SP from *S. flexneri* 2a.[7] To answer the need for a broadly protective vaccine against endemic shigellosis, the strategy was expanded to other *S. flexneri* serotypes.[8] Herein, the target is the O-SP from *S. sonnei*. This zwitterionic polysaccharide has a disaccharide repeating unit made of two uncommon aminosugars, a 2-acetamido-2-deoxy-L-altruronic acid (residue **A**) and a 2-acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose (AAT, residue **B**) 1,2-*trans* linked to one another (Figure 1).[9] Besides its zwitterionic nature, a peculiar feature of the *S. sonnei* O-SP is the occurrence of three amino groups, two of which present as acetamides, within a disaccharide repeat. We report an original synthesis of disaccharide **AB**, the biological repeat from *S. sonnei* O-SP, and of the frame-shifted trisaccharides **ABA′** and **B′AB**. In doing so, we validate the feasibility of chain elongation of an orthogonally protected **AB** intermediate by one residue at either end, paving the way to larger synthetic *S. sonnei* O-SP fragments.

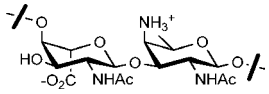
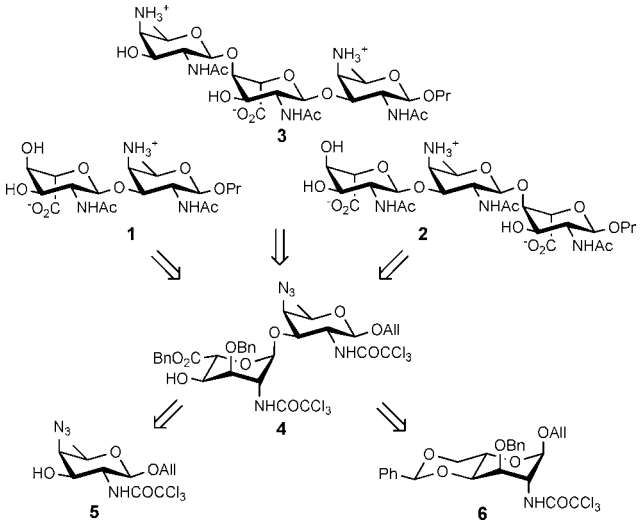


Figure 1. Biological repeat of the O-SP from *S. sonnei* (**AB**).[9]

All three targets were prepared in the form of propyl glycosides **1** (**AB**-Pr), **2** (**ABA′**-Pr), and **3** (**B′AB**-Pr) to mimic the 1,2-*trans* linkage occurring in the native polymer (Scheme 1). They were obtained from a common **AB** allyl glycoside

Scheme 1. Di- and Trisaccharide Targets 1−3 and Key Common Intermediates 4−6



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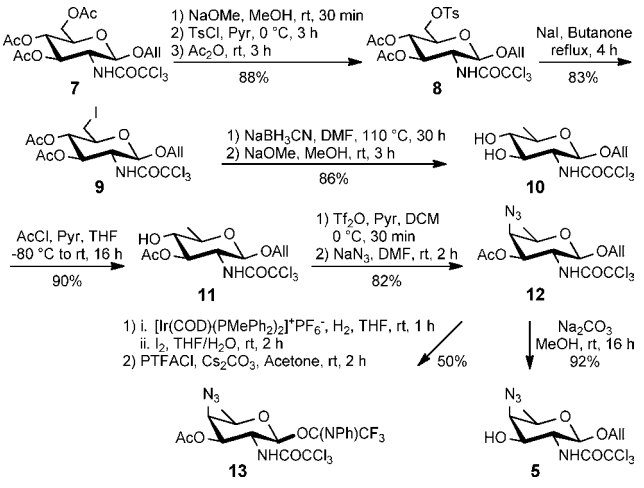
intermediate **4**, whose amino group at position 4_B is masked as an azido moiety. The latter was selected for its small size, its orthogonal properties to most protecting groups, and its easy conversion to an amine upon hydrogenation. In addition, disaccharide **4** features trichloroacetamide moieties at positions 2_A and 2_B to ensure appropriate anchimeric assistance during glycosylation,[10] and direct recovery of the acetamide moieties upon final hydrodechlorination.[8a,11] Disaccharide **4** was built from the unknown AAT acceptor **5** and allyl altroside **6**. The latter featured a 4,6-*O*-benzylidene acetal in agreement with the adopted postglycosidation oxidation strategy.[12]

Synthesis of the AAT Acceptor **5** *and Donor* **13**. Most often present as an α-linked residue and occasionally occurring as a β-linked component, AAT is a constituent of a variety of zwitterionic bacterial polysaccharides.[13] It was synthesized either as its methyl glycoside or in diverse forms compatible with donor formation (reviewed in ref 13a) and use thereof. In the latter case, most strategies employ 2-azido derivatives.[13a,14] Only on rare occasions, when AAT was involved in 1,2-*trans* glycosylation, were other *N*-protecting groups used at position 2.[15] Noticeably, in their original synthesis of the methyl glycoside of the *S. sonnei* **BA** disaccharide, Liptàk et al. used a 2-*N*-trichloroacetyl thioethyl glycoside donor, itself obtained from a 2-phtalimido AAT precursor.[15b] Instead, the strategy depicted herein is based on the early stage introduction of a 2-*N*-trichloroacetyl moiety[10,16] and on the powerful *N*-phenyltrifluoroacetimidate glycosylation chemistry.[17] Thus, starting from allyl glycoside **7**,[16] obtained from glucosamine hydrochloride via the corresponding β-tetraacetate,[18] transesterification, regioselective 6-O-tosylation of the resulting triol, and subsequent O-acetylation gave tosylate **8** (Scheme 2).

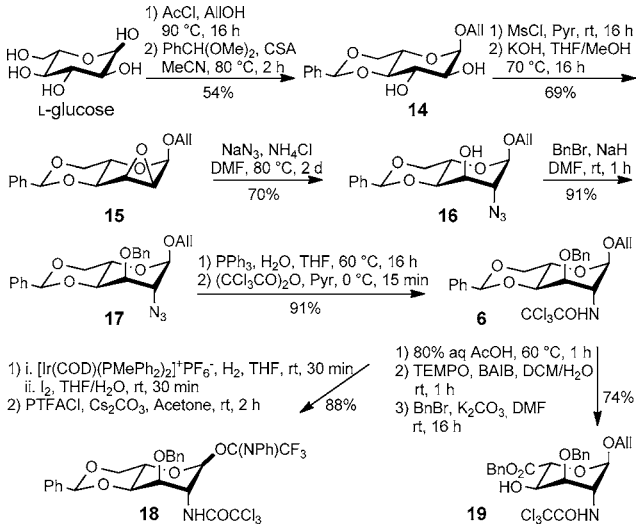
Scheme 2. Synthesis of the Protected AAT Residue 12 and Conversion into Acceptor 5 and Donor 13



Conversion of the latter into the 6-iodo analogue **9**, followed by $NaBH_3CN$-mediated reduction and acetate removal, gave the deoxy intermediate **10**. Fine-tuning of the reaction conditions enabled the critical regioselective acetylation at O-3 to give alcohol **11**. A decisive two-step process, involving formation of the intermediate triflate, and its nucleophilic displacement with sodium azide provided the fully protected 4-azido precursor **12**. On the one hand, selective acetate removal from the latter gave acceptor **5**. On the other hand, intermediate **12** was readily converted into donor **13**, following anomeric deallylation and reaction of the resulting hemiacetal with PTFACl.

Synthesis of the L*-Altrose Donor* **18** *and* L*-Altruronate Acceptor* **19** *via Orthogonally Protected* **6**. L-Altrosamine residues are not commonly encountered in bacterial polysaccharides, nor are they easily available from natural sources. Besides concern about handling *S. sonnei*,[18,19] routes to 2-amino-2-deoxy-altrose derivatives have been explored mostly owing to interest in mannosamines and their derivatives, among which are uronates.[20] Therefore, the synthesis of donor **18** was adapted from previous work.[20,18] Starting from commercially available L-glucose, Fischer-type glycosylation followed by regioselective benzylidenation gave a 3:1 α/β mixture of allyl glycosides that could be separated by flash chromatography (Scheme 3). The

Scheme 3. Synthesis of the Orthogonally Protected L**-Altrose Donor 18 and** L**-Altruronate Acceptor 19**



α-anomer **14** was converted to the *allo*-epoxide **15** by means of the 2,3-di-*O*-mesyl intermediate. Selective *trans*-diaxial opening of epoxide **15** by sodium azide in the presence of a minimal amount of ammonium chloride enabled the regioselective installation of an amino precursor at C-2 providing altropyranoside **16** as ascertained from NMR data. Yet, partial *trans*-diequatorial opening of the epoxide ring into allyl 3-azido-4,6-*O*-benzylidene-3-deoxy-α-L-glucopyranoside could not be avoided (8%, Scheme S1), adding to the scarce examples[21] that do not fully conform to the Fürst–Plattner rules.

Benzylation of alcohol **16** provided the fully protected **17**, which was converted into its trichloroacetamide analogue **6** upon selective reduction of the azido moiety under Staudinger conditions and trichloroacetylation of the resulting crude amine. In the first place,[22] trichloroacetamide **6** was isolated in poor yield together with the carbodiimide dimer (Scheme S2). The latter coeluted with 4-*N*,*N*-diethylamino-1,1,1-trichloro-3-buten-2-one,[23] arising from the oxidation of triethylamine by trichloroacetyl chloride. Isocyanate formation from a trichloroacetamide via a haloform reaction has precedent.[24] On that basis, we assumed that the carbodiimide side product was likely formed by reaction of the iminophosphorane intermediate with the corresponding isocyanate.[25] In an attempt to drive the hydrolysis of the iminophosphorane to completion, the amount of triphenylphosphine and water was increased and the reaction temper-

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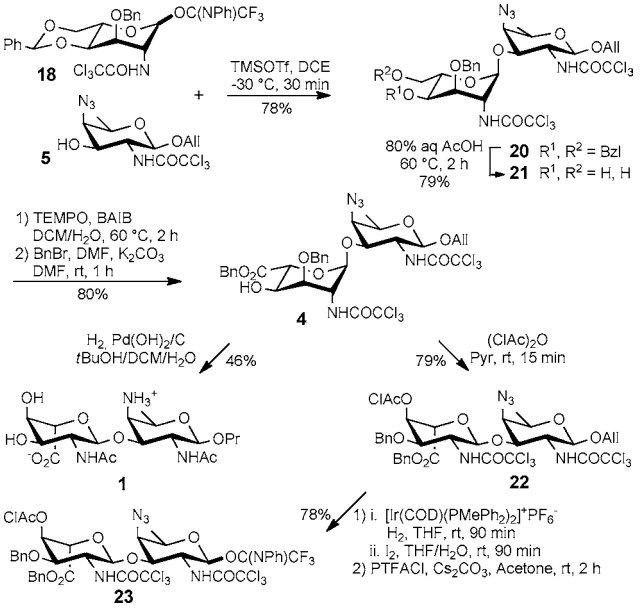
ature was raised. Unfortunately, these conditions led to an imine issued from the reaction of the excess phosphine with the newly formed trichloroacetamide **6** (Scheme S2).[26] Finally, using only a stoichiometric amount of phosphine enabled a high yielding two-step transformation (Scheme 3). Deallylation of the orthogonally protected **6**, and reaction of the formed hemiacetal with PTFACl, gave donor **18**, equipped for anchimeric assistance. Alternatively, the fully protected **6** was subjected to acetal acidolysis, selective 2,2,6,6-tetramethylpiperidine-1-oxy (TEMPO) radical/[bis(acetoxy)iodo] benzene (BAIB) oxidation[27] of the primary alcohol under conditions that left the allyl aglycon untouched,[11] and subsequent benzyl esterification to evolve into the uronate acceptor **19**.

Synthesis of Disaccharide ***AB****-Pr (****1****), Trisaccharide* ***ABA′****-Pr (****2****), and* ***BAB′****-Pr (****3****) via the* ***AB*** *Intermediate* ***4****.* TMSOTf-mediated coupling of the AAT acceptor **5** and the altrosaminyl donor **18** proceeded smoothly at −30 °C to furnish building block **20** (Scheme 4). Benzylidene cleavage provided diol **21**, which was subjected to chemo- and regioselective oxidation at C-6 using the TEMPO/BAIB system as described above. Subsequent conversion of the newly generated carboxyl group into a benzyl ester gave the **AB** disaccharide acceptor **4**. $Pd(OH)_2$/C-mediated allyl reduction, benzyl hydrogenolysis, and concomitant hydrodechlorination at two sites gave the desired **AB**-Pr disaccharide **1** following RP-HPLC purification. Alternatively, disaccharide **4** was readily converted into an **AB** donor. Thus, chloroacetylation of the 4_A-OH from intermediate **4** furnished the fully protected **22**. Anomeric deallylation of the latter and subsequent reaction of the hemiacetal intermediate with PTFACl gave donor **23**.

Scheme 4. Synthesis of AB-Pr (1) and of the AB Donor (23)



In contrast to the standard $^1C_4$ conformation adopted by allyl glucoside **14**, altrose derivatives have been shown to occupy all $^1C_4$, $^OS_2$, and $^4C_1$ conformations in solution.[28] When present in the form of a benzylidene acetal as in monosaccharide **16** and disaccharide **20**, the α-linked altrose residue is locked in a distorted conformation (Table 1, entry 1). The 1,3-transdiaxial substitution pattern and the axial substitutions at C-1, C-2, and C-3 probably account for important energy constraints destabilizing a standard $^1C_4$ chair. Instead, removal of the 4,6-acetal and subsequent oxidation drive the altropyranose ring into a significant conformational change as seen in the benzyl altruronate **4** (Table 1, entry 2).

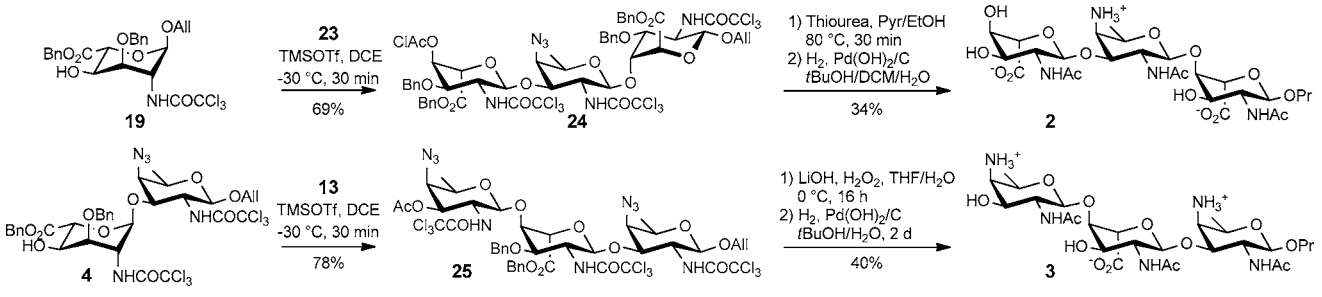
Table 1. Selected $^3J_{H,H}$ Coupling Constants (Hz) for the Altrose Residue in Compounds 1−4, 16, 20, and 25

entry	compd	$^3J_{1,2}/^3J_{2,3}$	compd	$^3J_{1,2}/^3J_{2,3}$
1	**16**	0.9/3.0	**20**	<0.5/2.7
2	**4**	3.3/5.4	**25**	7.5/10.4
3	**2**	**A**: 8.4/10.7	**1**	8.3/10.6
		A′: 8.1/10.3	**3**	8.5/10.8

With disaccharide **AB** both in the form of acceptor **4** and donor **23** in hand, the next step consisted of assessing the feasibility of chain elongation at either end. Toward this aim, the uronate acceptor **19** was reacted with donor **23** under conventional glycosylation conditions (Scheme 5). Quenching the reaction with a minimal amount of Et_3N permitted the isolation of the desired coupling product **24** in a good 69% yield. However, whereas epimerization at C-5_A was not observed, formation of the 4_A:5_A-unsaturated trisaccharide uronate, likely occurring during basic treatment,[29] could not be avoided (Scheme S3). Alternatively, trisaccharide **25** was obtained from the TMSOTf-mediated coupling of acceptor **4** with the AAT donor **13**. Interestingly, as a result of the glycosylation at O-4, the altruronate residue **A** switched from a distorted conformation in acceptor **4** to a more standard $^4C_1$ conformation in trisaccharide **25** (Table 1, entry 2). Altogether, data strongly suggest that the conformation adopted by the altruronate residues in *S. sonnei* protected oligosaccharides is highly dependent on their substitution pattern.

Trisaccharides **ABA′**-Pr (**2**) and **B′AB**-Pr (**3**) were isolated following RP-HPLC purification of the material obtained by

Scheme 5. Synthesis of ABA′-Pr (2) and B′AB-Pr (3)



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appropriate ester cleavage in the fully protected **24** and **25**, respectively, and subsequent $Pd(OH)_2$/C-mediated removal and/or reduction of all remaining protecting groups. $^3J_{H,H}$ NMR data of the propyl glycosides **1**−**3** indicated that the altropyranose residue exists preferentially in the $^4C_1$ conformation as in the *S. sonnei* O-SP[30] (Table 1, entry 3). This trend seemed less pronounced for residue **A**′ than for residue **A** in **ABA**′-Pr (**2**). As a whole, the available NMR data call for further chain elongation to reach conformational mimics of the natural O-SP.[30]

The first synthesis of the biological repeat of the zwitterionic O-SP of *S. sonnei* and of two frame-shifted trisaccharides was reported. Use of the postglycosylation oxidation strategy gave the **AB** building block **4**, designed and validated for chain elongation at both ends.

■ ASSOCIATED CONTENT

Supporting Information

Schemes S1−S4, abbreviations, experimental protocols, ^{1}H and ^{13}C NMR spectra for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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