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Synthesis of the Repeating Unit of the O-specific Polysaccharide of *Shigella sonnei* and Quantitation of its Serologic Activity

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Abstract—The chemical synthesis of the zwitterionic disaccharide **2** is described that corresponds to the repeating unit of the O-specific polysaccharide (**1**) of the Gram-negative human pathogen *Shigella sonnei*. Passive hemolysis inhibition tests using a hyper-immune rabbit serum raised against *S. sonnei* showed that the serologic activity of the disaccharide **2** is nearly 2- to 3-fold higher than those of its component monosaccharides. NMR data of **2** are in support of the proposed structure of the O-specific polysaccharide.

Shigella sonnei is a Gram-negative bacterium that can cause diarrhea and dysentery in humans. A major cell surface component of this bacterium is its O-specific polysaccharide[1,2] (O-SP) that is the outermost, serodeterminant domain of the highly complex lipopolysaccharide. The O-SP is an essential virulence factor of Shigellae including *S. sonnei*: only strains that have their O-SP fully expressed are virulent. Additionally, the O-SP is related to host immunity in that protection against infection is a correlate of the IgG antibody level against the O-SP.[3] The O-SP of *S. sonnei* is a non-immunogenic molecule (MW ~25 kDa) that must be conjugated to an immunogenic protein carrier to induce antibodies. Clinical trials have confirmed that protein conjugates of the O-SP elicited IgG levels in adult volunteers that were similar to those in patients convalescent from shigellosis.[4] The O-SP of *S. sonnei* is composed of the zwitterionic disaccharide repeating unit **1** consisting of the rare sugars 2,4,6-trideoxy-2-acetamino-4-amino-D-galactose and 2-acetamido-2-deoxy-L-altruronic acid that are connected through 1,2-*trans* interglycosidic linkages.[1,2]

Our approach to vaccine development is based on the assumption that fragments of O-SPs mimic epitopes of the native polymers and thus may induce polysaccharide-specific antibodies when conjugated to an immunogenic carrier. Recent data confirmed this assumption and showed that albumin conjugates of chemically synthesized extended oligosaccharide fragments[5,6] of the O-SP of *S. dysenteriae* type 1 elicited higher levels of the homologous O-SP-specific IgG antibodies than did the conjugate of the native polysaccharide.[7]

As a prelude to chemical synthesis of oligosaccharides corresponding to **1** we have reported the synthesis of the methyl glycosides of its component monosaccharides in their natural glycosidic configuration.[8] We have also presented evidence that in the native polysaccharide both pyranose residues are preferentially in the $^4C_1$ chair conformation and that the polysaccharide exists in the zwitterionic form.[9] Here we describe the first synthesis of a disaccharide part (**2**) of the O-SP that to our knowledge is also the first report of a zwitterionic disaccharide containing free amino and carboxyl groups at alternating monosaccharide residues. The disaccharide **2** was constructed from an appropriately protected altruronic acid derivative (**5**) that was used as the acceptor and from the activated donor **9**. The common

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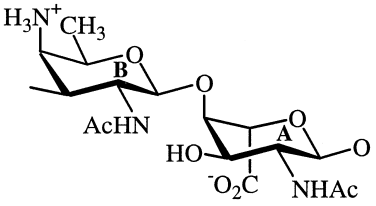

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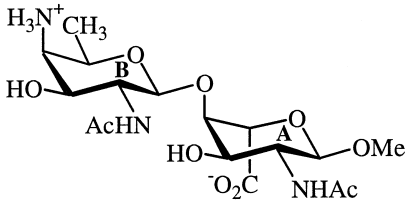

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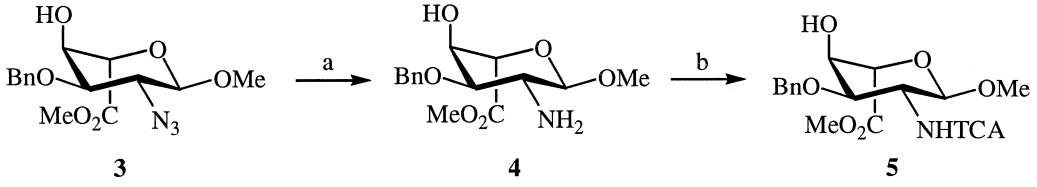
feature of both intermediates is the presence of a trichloroacetyl protecting group at their respective amino function. This protecting group has previously been shown to be suitable for *N*-protection without adverse outcomes during glycosylation reactions.[10] It was also demonstrated to have good *trans*-directing effect and to provide the substrates with acceptable solubility properties. The synthetic steps to the key donor and acceptor intermediates are described next.

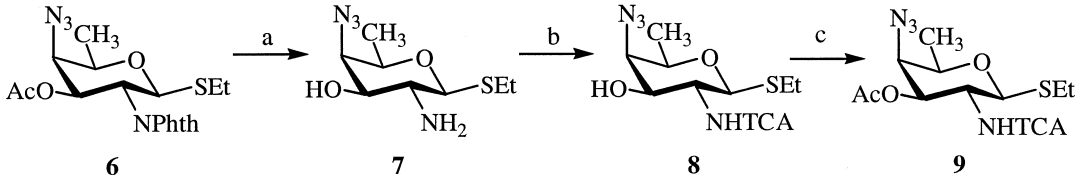
The altruronic acid residue 5 (Scheme 1). The synthetic route started from the glycoside **3** that was available from an earlier work.[8] In the first step the azido group in compound **3** was reduced to an amino group with H_2 over Pd/C to afford **4**. Hydrogenolytic cleavage of the *O*-benzyl group in this step was prevented by the use of one equivalent of pyridine. Subsequent treatment of **4** with trichloroacetyl chloride afforded **5** in 56% overall yield.

The trideoxy-galactose residue 9 (Scheme 2). Starting compound was thioglycoside[8] **6** that was converted to the amine **7** by treatment with ethylenediamine, in 94% yield. Next, a trichloroacetyl group was installed at the free amino group by exposure of **7** to trichloroacetyl chloride in the presence of triethylamine (→**8**). The synthesis of the glycosyl donor **9** was completed by acetylation of **8** with pyridine/acetic anhydride, in 94% overall yield for two steps.

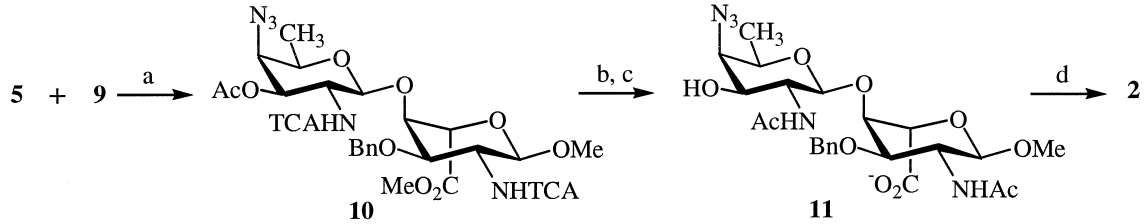
Having readied the donor (**9**) and the acceptor moieties (**5**), the fully protected disaccharide **10** was prepared by NIS/TfOH-promoted activation[11,12] of the thioglycoside **9** in the presence of 0.55 equimolar amount of the acceptor **5** in 85% yield (Scheme 3). Replacement of the *N*-trichloroacetyl groups with the acetyl groups was previously described through Bu_3SnH-mediated reductive cleavage of the chlorine atoms.[10] This approach failed to give satisfactory results when applied to **10**. Fortunately, treatment of **10** with NaOH in MeOH excellently removed the *N*-trichloroacetyl groups to afford an intermediate that was converted to the *N*-acetamido derivative **11** by conventional acetylation, in 72% overall yield for two steps. As the final phase in the synthetic sequence, compound **11** was subjected to



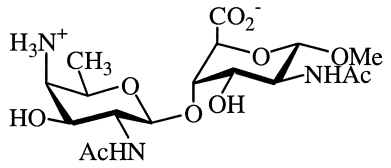
Scheme 1. Reagents and conditions: (a) H_2/Pd-C, MeOH, C_5H_5N, 25 °C, 2 h 81%; (b) 1.1. equiv of Cl_3CCOCl, Et_3N, CH_2Cl_2, 0 °C, 1 h 70%.



Scheme 2. Reagents and conditions: (a) $NH_2CH_2CH_2NH_2$ (excess), EtOH, reflux, 2 h, 94%; (b) 1.2 equiv of Cl_3CCOCl, Et_3N, CH_2Cl_2, 0 °C, 20 min; (c) C_5H_5N, Ac_2O, 25 °C, 2 h, 94% for two steps.



Scheme 3. Reagents and conditions; (a) 1.8 equiv of **9**, 2.3 equiv of NIS, TfOH (cat), CH_2Cl_2, −30 °C, 1.5 h, 85%; (b) aq NaOH, MeOH, 25 °C, 2.5 days; (c) Ac_2O, MeOH, 0 °C, 50 min, 72% for two steps; (d) H_2/$Pd(OH)_2$-C, EtOH, AcOH 25 °C, 4 days, 51%.



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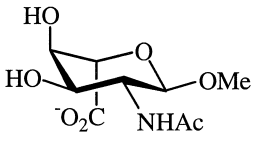

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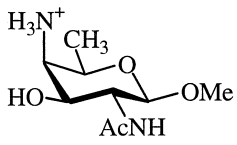

14

Table 1. Inhibition of hemolysis of *Shuigella sonnei* lipopolysaccharide-sensitized erthrocytes by the lipopolysaccharide-related antigens **2**, **13** and **14**[a]

Antigen	IC_{50} (mM)[b]
2	3.9
13	6.9
14	9.8

[a]IC_{50}: 50% inhibition concentraton.
[b]Estimated range of error: ≤10%.

hydrogenolytic cleavage of the *O*-benzyl groups that simultaneously reduced the azido group to afford the targeted disaccharide **2**.[13]

A comparison of the ^1H and ^{13}C NMR chemical shifts for compounds **1**,[9] and **2** shows an excellent agreement of the corresponding resonances that lends further support for the absolute configuration of the altruronic residue as being L and for the trideoxy-galactose residue as being D.[14] For a comparison, we have also synthesized the diastereomeric disaccharide **12** that contains the enantiomeric, D altruronic acid moiety.[15] For compound **12** the chemical shift of the C-5 carbon atom of the uronic acid residue is 70.5 ppm, distinctly different from the corresponding resonances in **1** and **2** (78.4 and 76.9, respectively). Minor differences in the chemical shifts for residues **A** in **1** and **2** are likely due to conformational differences and indicate that the disaccharide can only partially express conformational features of polysaccharide **1**.

The antigenicity of the disaccharide **2** was assayed by the passive hemolysis inhibition test as described by Kontrohr and Péterffy.[16] The method is based on complement-mediated hemolysis of lipopolysaccharide-sensitized erythrocytes after anti-LPS antibody binding. Using sheep erythrocytes, guinea pig complement, phase I lipopolysaccharide of *S. sonnei*,[1] and hyperimmune polyclonal rabbit serum raised against *S. sonnei*, the concentration of the disaccharide **2** and those of the component monosaccharides **13** and **14** were determined that were necessary for 50% inhibition of hemolysis. The results (Table 1) show that the disaccharide **2** is a better inhibitor than either one of its monosaccharide components of which the altruronic acid derivative **13** is superior. Less than 20% inhibition could be observed with the unnatural disaccharide **12** up to 15 mM concentration and no inhibition was seen with unrelated saccharides.

In summary, we have synthesized for the first time the complete repeating unit of the O-specific polysaccharide of *Shigella sonnei* and presented spectroscopic and immunochemical support for the structure of the native polysaccharide. Current work is directed at the synthesis and immunochemical study of the frame-shifted, alternative disaccharide repeating unit and higher-membered saccharides corresponding to the O-specific polysaccharide of *S. sonnei* for their eventual use as vaccine-components against enteric diseases.

Acknowledgements

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13. All new compounds had satisfactory analytical, ^1H,^{13}C NMR, and mass spectral data. For **2** ^1H NMR(D_2O): δ 4.73 (H-1_B), 4.70 (H-1_A), 4.55 (H-5_A), 4.42 (H-4_A), 3.90 (H-3_B), 3.88 (H-2_A), 3.88 (H-5_B), 3.81 (H-2_B), 3.70 (H-3_A), 1.31 (H-6_B); ^{13}C NMR: δ 102.9 (C-1_B), 100.3 (C-1_A), 77.9 (C-4_A), 76.9 (C-5_A), 69.3 (C-3_B), 69.2 (C-3_A, C-5_B), 54.6 (C-4_B), 52.8 (C-2_B), 52.1 (C-2_A), 16.3 (C-6_B).
14. We had no specific reason to question the reported structure of the O-SP of *S. sonnei*. However, in the light of numerous structural revisions of bacterial polysaccharides it is always advisable to obtain independent structural proof for these materials before undertaking a synthetic project towards their extended portions.
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