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Synthesis of a disaccharide repeating unit of the O-antigen from *Burkholderia ambifaria* and its oligomers

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ABSTRACT

A disaccharide repeating unit of the O-antigen from *Burkholderia ambifaria*, 6-deoxy-β-D-Alt-(1 → 4)-α-D-Rha-O$(CH_2)_3NH_2$ (**1**), and its dimer and trimer, 6-deoxy-β-D-Alt-(1 → 4)-α-D-Rha-(1 → 3)-6-deoxy-β-D-Alt-(1 → 4)-α-D-Rha-O$(CH_2)_3NH_2$ (**2**) and 6-deoxy-β-D-Alt-(1 → 4)-α-D-Rha-(1 → 3)-6-deoxy-β-D-Alt-(1 → 4)-α-D-Rha-(1 → 3)-6-deoxy-β-D-Alt-(1 → 4)-α-D-Rha-O$(CH_2)_3NH_2$ (**3**), were synthesized via a convergent strategy. The key disaccharyl thioglycoside **4** as a glycosyl donor was stereoselectively assembled by glycosylation of rhammnosyl acceptor **5** with 6-deoxy-altrosyl trichloroacetimidate donor **6b**. The glycosidation of **4** with 3-azidopropanol followed by global deprotection afforded the target disaccharide **1**. Further elongation of the carbohydrate chain of this glycosidation product with the disaccharyl donor **4** followed by global deprotection generated rapidly the dimeric tetrasaccharide **2** and the trimeric hexasaccharide **3** in a convergent [2 + 2] and [2 + 2 + 2] manner, respectively.

1. Introduction

Burkholderia cepacia complex (Bcc) is a group of phenotypically similar but genotypically different Gram-negative bacteria [1,2]. Bcc bacteria are important opportunistic human pathogens that can cause fatally infections in vulnerable patients, especially for those with cystic fibrosis (CF) or chronic granulomatous disease (CGD) [3–7]. For example, Bcc infection can result in rapid and clinically uncontrollable necrotizing pneumonia and septicemia in CF patients, which lead to high mortality [4]. Thus, the development of new strategies for the effective prevention and treatment of Bcc infection are urgently needed. The lipopolysaccharide (LPS) O-antigens of Bcc are implicated in bacterial invasion and virulence, such as promoting inflammatory cytokine IL-1β production, modulating phagocytosis of macrophages and interfering with bacterial adhesion to bronchial epithelial cells [8,9]. Therefore, these LPS O-antigens are useful targets in developing carbohydrate-based vaccines against Bcc infection [10–14].

Recently, Molinaro and coworkers have isolated and characterized two O-antigens from the *B. ambifaria* strain 1918 [15]. One of them, OPS-2 (Fig. 1), consists of a disaccharide repeating unit, →4)-α-D-Rha*p*-(1 → 3)-β-D-6dAlt*p*-(1 → . Interestingly, a unique 6-deoxyaltrose residue is present in it structure, which is considered as a special determinant of the biosynthetic pathway and the pathogenic mechanism of *B. ambifaria* [15]. As a part of our ongoing research project to develop vaccines from LPS O-antigens for the control of Bcc [13,14], we studied the chemical synthesis of a derivative of the OPS-2 repeat unit **1** and its dimer **2** and trimer **3** (Fig. 1). These synthetic targets were designed to contain a free amino group linked to their reducing end, which would allow for their regioselective conjugation with carrier molecules to generate glycoconjugates useful for biological and immunological studies.

2. Results and discussion

Retrosynthetic analysis of the synthetic targets **1**–**3**, as depicted in Scheme 1, afforded disaccharyl thioglycoside **4** as the key and common glycosyl donor, which can be utilized for introducing a 3-aminopropyl group at the reducing end via reaction with 3-azidopropynol and for elongation of the carbohydrate chain for preparing the oligomers. We chose benzyl group as the main protecting group in **4** which could allowed for one-step global deprotection in final step. In turn, **4** could be assembled from glycosylation of thiorhamnoside derivative **5** with a 6-deoxy-D-altrosyl donor **6**, *e.g.*, X = F, $OC(NH)CCl_3$ or $OP(O)(OBu)_2$. We

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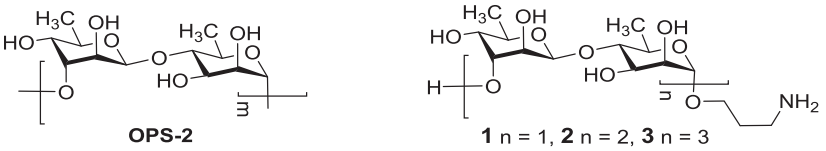
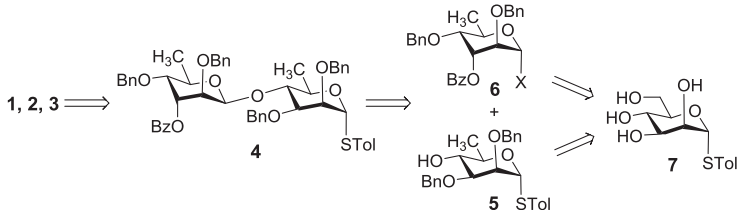


Fig. 1. Structures of the O-antigens of *B. ambifaria* strain 19182, **OPS-2**, and the designed synthetic targets **1**–**3**.



Scheme 1. Retrosynthetic analysis of the synthetic targets **1**–**3**.

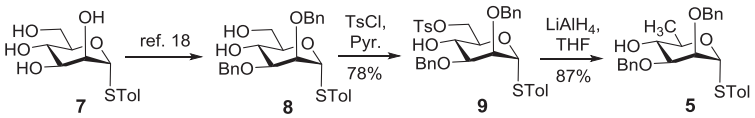
planned to use a benzoyl group to protect the 3-O-position in **6** to facilitate the formation of difficult 1,2-*cis* β-glycosidic linkage of altrose through 1,3-anchimeric assistance [16]. Moreover, the benzoyl group could be selectively removed under basic conditions to allow for additional glycosylation reaction at this position. Both **5** and **6** would be prepared from *para*-tolyl 1-thio-D-mannopyranoside **7** [17] through a series of well-documented transformations.

The synthesis of glycosyl acceptor **5** (Scheme 2) was started from *para*-tolyl 2,3-di-*O*-benzyl-1-thio-D-mannopyranoside **8**, which was derived from **7** according to a reported procedure [18]. Selective tosylation of 6-OH in **8** with tosyl chloride (TsCl) in pyridine gave tosylate **9** (78%), which was treated with lithium aluminum hydride ($LiAlH_4$) in THF [19] to afford smoothly 6-deoxy sugar **5** in an 87% yield. A double peak of the H-6 signal at δ 1.28 ppm in the ^{1}H NMR spectrum of **5** indicated the formation of a new C-6 methyl group.

The synthesis of glycosyl donors **6a-c** was delineated in Scheme 3. First, regioselective tosylation of **7** with TsCl (1.2 equiv) in pyridine followed by 2,3-*O*-isopropylidenation with 2,2-dimethoxypropane under the promotion of *p*-toluenesulfonic acid (*p*-TsOH) generated **10** [20] in an overall yield of 76%. Next, treatment of tosylate **10** with benzyl bromide and sodium hydride in DMF and then reduction of the 6-toylate with $LiAlH_4$ afforded smoothly 6-deoxy sugar **11** (82%), which was followed by removal of acetonide with 80% TFA in dichloromethane to give 2,3-diol **12** in a 90% yield. Tin complex-directed regioselective 4-methoxybenzylation of the less hindered 3-OH [21] in **12** using Bu_2SnO and 4-methoxybenzyl chloride (PMBCl) and subsequent benzylation of the remaining 2-OH using BnBr led to fully protected rhamnosyl derivative **13**. Its PMB group was removed by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) [22] to yield **14** with free 3-OH in an overall yield of 70%. Subsequently, inversion of the configuration of C-3 in **14** was achieved in two steps [21] including Dess-Martin oxidation of the equatorial 3-OH into ketone and $NaBH_4$ reduction of resultant ketone to axial 3-OH to afford **15** in a 69% yield. The coupling constants of H-3 (J = 3.6 Hz) and H-4 (J = 9.6 and 3.6 Hz) signals at δ 4.14 and 3.54 ppm, respectively, in ^{1}H NMR spectrum of **15**, compared to those of **14** (H-3: δ 3.95 ppm, J = 9.0 and 3.6 Hz; H-4: δ 3.38 ppm, J = 9.0 and 9.0 Hz), confirmed the correct relative configuration of **15**. Finally, **15** was benzoylated with BzCl in pyridine to give fully protected thioglycoside of 6-deoxy-D-altrose **16**, which was then used to prepare diversified glycosyl donors **6a-c**. Conversion of **16** into hemiacetal by *N*-iodosuccinamide (NIS) and silver triflate (AgOTf) in wet CH_2Cl_2 was followed by treatment with diethylaminosulfur trifluoride (DAST) [23] to furnish glycosyl fluoride **6a** (68%). The resultant hemiacetal intermediate was also treated with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) [24] to provide glycosyl imidate **6b** (63%). In the meantime, glycosylation of dibutyl phosphate [25] with **16** in the presence of NIS and trifluoromethanesulfonic acid (TfOH) afforded glycosyl phosphate **6c** (74%).

First, glycosylation reactions of **5** with all three glycosyl donors **6a, 6b** and **6c** (Scheme 4) were explored for the assembly of difficult β-linked disaccharide **4**. The results were shown in Table 1. The reaction of **5** with glycosyl fluoride **6a** in CH_2Cl_2-CH_3CN promoted by $SnCl_2$ and $AgClO_4$ [26] at −15 °C generated mainly α-linked disaccharide **4a** (α/β = 1.5:1) in a 47% yield (Table 1, entry a1). When employing dry Et_2O as solvent, the reaction gave β-linked disaccharide **4** as the major product (α/β = 1:3, Table 1, entry a2), suggesting that Et_2O was probably the preferred solvent for this glycosylation. The reaction of **5** with trichloroacetimidate **6b** promoted by TMSOTf in dry Et_2O afforded predominantly β-disaccharide **4** with excellent stereoselectivity (α/β = 1:11, Table 1, entry b). Finally, the reaction of **5** with glycosyl phosphate **6c** in presence of TMSOTf in dry Et_2O also furnished **4** as the major product, but the stereoselectivity was moderate (α/β = 1:2.2, Table 1, entry c). Thus, condition b (Table 1) was eventually adopted in our synthesis of the target molecules. The $^1J_{C\text{-}1',\ H\text{-}1'}$ coupling constants of **4** (158.2 Hz) and **4a** (171.6 Hz) in their ^{1}H-coupled gHSQC spectra confirmed unambiguously their correct glycosidic linkages.

The reaction of **4** with 3-azidopropanol (Scheme 4) promoted by NIS and AgOTf in dry Et_2O gave a mixture of α- and β-products (3:1) that were readily separated by column chromatography to obtain



Scheme 2. Synthesis of rhamnosyl acceptor **5**.