

# Total Synthesis of Zwitterionic Tetrasaccharide Repeating Unit from Bacteroides fragilis ATCC 25285/NCTC 9343 Capsular Polysaccharide PS A1 with Alternating Charges on Adjacent Monosaccharides

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#### Supporting Information

ABSTRACT: The tetrasaccharide repeating unit of zwitterionic polysaccharide A1 (PS A1) from Bacteroides fragilis ATCC 25285/NCTC 9343 has been synthesized using a linear glycosylation approach. One key step includes an  $\alpha(1,4)$ -stereoselective [2 + 1] glycosylation of a 2,4,6trideoxy-2-acetamido-4-amino-D-Galp (AAT) donor with a poorly reactive axial C4-OH disaccharide acceptor. Mild acidmediated deacetylation and a challenging [3 + 1]glycosylation are also highlighted. The strategy is inclusive of a single-pot, three-step deprotection affording PS A1 with alternating charges on adjacent monosaccharide units.

apsular zwitterionic polysaccharides (ZPSs), known to exist on the commensal bacteria Bacteroides fragilis ATCC 25285/NCTC 9343 (PS A1 (1) and PS B (2)),<sup>1</sup> Streptococcus pneumoniae ATCC 10015 (Sp1 (3)),<sup>2</sup> and Staphylococcus aureus (Reynolds strain - CP5 (4) and Becker strain - CP8 (5)),<sup>3</sup> all possess unique immunomodulatory properties, activating T-cell mediated immune responses through major histocompatibility complex II, so giving CD4<sup>+</sup> T-cells (Figure 1).<sup>4</sup> This novel discovery runs counter to the usual T-cell immunity trend, which has long been thought to exclusively involve peptide antigens. Thus, the emergence of ZPSs not only are considered paradigm shifting but also represent an opportunity for developing novel carbohydrate-based immunotherapeutics.<sup>4,5</sup> One of the key features to these ZPSs is a conserved alternating charge character located on adjacent monosaccharide units. The work of Kasper et al. illustrated that chemical modification, abrogating zwitterionic character on 1, rendered a T-cell independent immune response common to all carbohydrates.<sup>6</sup> The unique bioproperties of ZPSs, especially 1 and 2, have been utilized by our group to develop "antigen to carrier" approaches for entirely carbohydrate-based immunotherapeutics that conjugate to the tumor associated carbohydrate antigens Tn,7 TF,8 and STn.9 Our immunogen design allows for a hydroxyl amine to oxime strategy that takes advantage of synthetic aminooxy sugar antigens and readily oxidized vicinal hydroxyls from the D-Galf of PS A1 mediated by hypervalent iodine; no immunogenic linkers are incorporated.<sup>10</sup> Although a lengthy, hot-water phenol extraction protocol, followed by multistep chromatography, is required for the isolation and purification of 1, this has become common place in our group. Nonetheless, having pure synthetic entities for definitive mechanistic and other



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Figure 1. Zwitterionic polysaccharides PS A1 (1), PS B (2) from B. fragilis, Sp1 (3) from S. pneumoniae, and CP 5 (4) and CP 8 (5) from S. aureus.

immunological studies is of critical importance. Literature reports, documenting syntheses of the same PS A1

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tetrasaccharide backbone repeating unit,<sup>11</sup> include the work of Overkleeft et al.<sup>12a</sup> and Seeberger et al.<sup>12b</sup> In the work of Overkleeft, the tetrasaccharide was never subjected to deprotection, and therefore only a fully protected unit was reported, while, in the latter, a fully deprotected moiety was revealed.<sup>12,13</sup> Critically, in both synthetic attempts of **1**, the assembled tetrasaccharides were furnished without electrostatic charges on adjacent monosaccharide units, a property important for biorelevance.<sup>1a</sup>

Recently, an elegant synthetic biology strategy was employed that capitalized on an enzymatic-based in vitro assembly of lipidated PS A1 tetrasaccharide for the development of fluorescent polyisoprenoid chemical probes, and although the unit was furnished with alternating charge character on adjacent monosaccharides, the biosynthetic quantity was limited and compound characterization was only achieved using mass spectrometry.<sup>14</sup> Notable in the syntheses of ZPSs is the work from Bundle et al. in which tri- and hexasaccharide moieties of Sp1 (3) were accomplished.<sup>15</sup> Interestingly, synthetic units from the Seeberger and Bundle groups did not show any bioactivity when subjected to in vitro assays conducted in the Kasper group.<sup>16</sup> Furthermore, and to the best of our knowledge, none of the synthetic repeating units were ever examined in vivo and, therefore, important biological data relevant to the PS A1 T-cell dependent story remain missing. Herein, we report, for the first time, the total synthesis and characterization of the PS A1 tetrasaccharide repeating unit designed to contain an electrostatic charge on adjacent monosaccharide units. In order to synthesize the tetrasaccharide repeating unit of PS A1 and develop a strategy that would allow for future tetrasaccharide and tetrasaccharide glycosylations, a retrosynthetic disconnection of target tetrasaccharide 6 (Scheme 1) highlighted an orthogonally protected tetrasaccharide 7 inclusive of Fmoc and p-methoxyphenol (MP) protecting groups. Further retroanalysis of 7 revealed that a trisaccharide acceptor 8 and an (R)-pyruvate acetal D-Galp donor (9) could potentially be coupled via a thio-donor.

Scheme 1. Retrosynthetic Analysis of MP Protected Zwitterionic Repeating Unit of PS A1 (6)



Trisaccharide 8 could arise from coupling of disaccharide acceptor 10 and a 2-azido-4-amino-2,4,6-trideoxy-D-galactose (AAT) trichloroacetimidate donor (11) building block. Such a linear strategy would then commence from building blocks 9, 11, 12,<sup>12b</sup> and 13 $\beta$ .<sup>17</sup> (*R*)-Pyruvate acetal D-Gal*p* donor (9) (Scheme 2) was synthesized from compound 14.<sup>18</sup> Although

# Scheme 2. Synthesis of Building Blocks 9, 12,<sup>12b</sup> and $13\beta^{17}$



14 could serve as a donor in the synthesis of compound 7, we chose to introduce orthogonal functionality at C-3 for future repeating unit elongation. Therefore, debenzoylation under Zemplén NaOMe conditions gave rise to 2,3-diol 15 in 92% yield. Selective 3-OH Fmoc protection using  $Ag_2O^{19}$  with cat. KI afforded compound 16 (3-O-Fmoc/2-O-Fmoc = 3:1) in 62% isolated yield. Acetylation of the 2-OH group, using a Dakin–West-type pyridine/Ac<sub>2</sub>O, afforded (*R*)-pyruvate acetal D-Galp donor (9) in 94% yield (Scheme 2).

The preparation of AAT building block 11 (Scheme 3) from D-rhamnal (17) was accomplished by adapting the known literature methods.<sup>15,20</sup> A highly regioselective acetylation





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reaction of the 3-OH group at -78 °C, using acetyl chloride, led to compound 18 in 74% yield. Inversion at C-4, using the triflate/S<sub>N</sub>2 method, was unsuccessful due to triflate instability; however, azide-mediated inversion of C4-OH went smoothly when a DPPA<sup>21</sup>-based Bose-Mitsunobu<sup>22</sup> reaction was conducted giving 19. Compound 19 was then subjected to Zemplén conditions to obtain 20 in 61% yield, determined from compound 18. LAH reduction of the C4-azido group of 20, followed by Troc protection, using a single-pot protocol, gave rise to compound 21 in 63% over two steps. Acetylation of the 3-OH group, using acetyl chloride, led to 22 in 91% yield. Important to note is that we attempted LAH reduction to directly obtain 21 from 19. However, this led to poor yields due to acetyl group migration giving the unwanted acetamide product. With 22 in hand, conditions for azidonitration,<sup>12b,23</sup> with CAN and NaN<sub>3</sub> in acetonitrile at -25 °C, led to compound 23 in 73% yield. <sup>1</sup>H NMR was used to calculate the diastereomeric ratio of 3.5:1. The anomeric nitrate of 23 was

then deprotected using thiophenol,<sup>24</sup> which was followed by treatment of the hemiacetal with trichloroacetonitrile and cat. DBU in dichloromethane at 0 °C to afford the AAT trichloroacetimidate<sup>17b</sup> donor (11) in 67% yield over two steps.

Monosaccharide donor D-Galf trichloroacetimidate (12)<sup>12b</sup> and 2-azido-D-Galp acceptor  $(13\beta)^{17}$  were synthesized following the literature precedent (Scheme 2). Compound  $13\alpha$  was arbitrarily used as an acceptor for the synthesis of disaccharide 24. To our surprise, attempted glycosylation of compound  $13\alpha$  (Scheme 4) with the D-Galf donor (12) failed to yield the desired product due to aglycone transfer of the *p*methoxyphenolic group (Scheme 4; 24a, 82% yield from  $13\alpha$ ). We suspect that the observed aglycone transfer might be due to the anomeric effect leading to the increased electron density of the  $\alpha$ -glycosidic bond and possibly to the relative stability of the oxocarbenium ion of the acceptor versus donor causing undesired 24a.<sup>25</sup> We were elated to obtain disaccharide 24 ( $\beta$ only) in 89% yield using a [1 + 1] Schmidt glycosylation<sup>2</sup> strategy with D-Galf donor (12) and compound  $13\beta$  (Scheme 4), as  $13\alpha$  proved to be incompatible in the [1 + 1]glycosylation. Debenzoylation, followed by benzylation of disaccharide 24, gave disaccharide 25 in 87% yield over two steps. Using Et<sub>3</sub>SiH and TfOH at -78 °C, a regioselective benzylidene ring opening<sup>27</sup> of disaccharide 25, led to the desired free 4-OH acceptor 10 (Scheme 4) in 83% yield. Benzyl protecting groups were used to increase the "armed" nature of disaccharide 10 as the axial C4-OH of the 2-azido-D-Galp is known to be poorly reactive.<sup>12b,15</sup> A challenging stereoselective  $\alpha(1,4)$  glycosylation was then attempted that was inclusive of 10 and 11 (Scheme 4). After probing the scope of reaction parameters, such as equivalents of donor, activator/promoter combinations, and variation of the solvent and temperature, the desired trisaccharide 26 was obtained in 63% yield with complete  $\alpha$ -stereoselectivity (Table 1, entry 4). The observed  $\alpha$ -stereoselectivity is thought to arise due to the remote participation<sup>28</sup> of the carbonyl functionality of the NHTroc blocking the  $\beta$ -face leading to favorable attack of the nucleophile at the  $\alpha$ -face in the preferred  ${}^{4}H_{3}$  half-chair conformation (Figure 2). Important to note is that initial attempts for the acetyl deprotection of trisaccharide 26, using a catalytic amount of NaOMe, Et<sub>3</sub>N, and guanidine nitrate,<sup>2</sup> were unsuccessful. All of the noted conditions led to the oxazolidinone byproduct (Scheme 4, 26a) due to the ease of cyclization even under mild conditions. This can be attributed to the cis/syn configuration of the vicinal 3-OH and 4-NHTroc groups.<sup>12b</sup> However, deprotection of the acetyl group was achieved using mild acidic conditions of p-toluenesulfonic  $acid^{30}$  or CSA in methanol/CH<sub>2</sub>Cl<sub>2</sub> (2:1) under reflux. These conditions gave rise to trisaccharide acceptor 8 in 72% yield (Scheme 4). Orthogonally protected tetrasaccharide 7 was then assembled in the presence of acceptor 8 and donor 9 at 0

Tab	le 1.	Optimization	of $\alpha$	1,4	)-Gl	ycosy	lation
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entry <sup>a</sup>	equivalents of acceptor 10	equivalents of donor 11	reaction conditions <sup>b</sup>	(%) yield <sup>c</sup> / $\alpha$ : $\beta$ ratio
1	1	1.2	TMSOTf (20 mol %)/CH $_2$ Cl $_2$ /0 °C/30 min	$38/\alpha$ -only
2	1	1.5	TMSOTf (20 mol %)/CH $_2$ Cl $_2$ /0 °C/30 min	$47/\alpha$ -only
3	1	2	TfOH (15 mol %)/CH <sub>2</sub> Cl <sub>2</sub> /rt/20 min	$52/\alpha$ -only
4	1	2	TMSOTf (30 mol %)/CH <sub>2</sub> Cl <sub>2</sub> /ether (2:1)/rt/20 min	$63/\alpha$ -only

<sup>*a*</sup>All reactions were conducted under inert conditions and in the presence of activated 4 Å MS. <sup>*b*</sup>Anhydrous solvents were purchased from Sigma. <sup>*c*</sup>All yields correspond to chromatographically purified products.

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**Figure 2.** Mechanistic insight for an  $\alpha$ -selective [2 + 1] glycosylation driven by remote NHTroc participation.

 $^{\circ}$ C with NIS/TMSOTf<sup>17b</sup> activation in 72% yield (Scheme 5). Compound 7a was also synthesized using the same set of

# Scheme 5. Synthesis of Orthogonally Protected Tetrasaccharide 7



reaction conditions from acceptor 8 and donor 14 in 69% yield. Diacetamido tetrasaccharide 27 was obtained from compound 7a after a one-pot azide to acetamide reduction<sup>31</sup> with AcSH/pyridine (1:1) at 25 °C in 61% yield (Scheme 5). With respect to the choice of donors, for the first two glycosylations in the linear sequence, trichloroacetimidate donors gave the best yields and, for the final [3 + 1] glycosylation, the thiophenyl donor worked well as compared to other donors.

Initial attempts at the global deprotection of diacetamido tetrasaccharide 27 using a single-pot, two-step protocol employing Pd/C, followed by saponification with LiOH, were unsuccessful. We fully characterized the undesired compound **6a** (Scheme 6). We observed that the NHTroc group was not stable to hydrogenolysis,<sup>32</sup> leading to the formation of ethyl carbamate, most likely arising from dehalogenation of three chlorine atoms on the Troc functionality. Furthermore, the ethyl carbamate was observed to be stable under conditions of LiOH saponification at room temperature even for 24 h. Other deprotection methods for the removal of ethyl carbamate were deemed risky, and saponification at higher temperatures was not preferred due



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to the presence of acetamide groups. We opted for a single-pot, three-step global deprotection strategy to obtain the MP anomeric protected zwitterionic tetrasaccharide **6** (Scheme 6). Starting with deprotection of NHTroc, using activated Zn<sup>(0)</sup> in THF/AcOH,<sup>17b</sup> followed by Pd/C mediated hydrogenolysis and saponification with 1 M LiOH in THF/H<sub>2</sub>O, the target zwitterionic tetrasaccharide **6** was revealed in 56% yield over three steps. Target molecule **6** was fully characterized using NMR and HRMS techniques.

In summary, we have synthesized zwitterionic tetrasaccharide repeating unit 6 of polysaccharide PS A1 (1) as well as an orthogonally protected repeating unit 7 for future unit elongation, providing access to constructs of well-defined oligomeric length. Future work regarding the synthesis of antigenic fragment(s) of polysaccharide PS A1 (1) composed of two to four repeating units will be attempted, and *in vitro* and *in vivo* studies using zwitterionic repeating unit 6 will be reported in due course.

# ASSOCIATED CONTENT

#### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.or-glett.8b01829.

Detailed experimental procedures and the respective NMR spectra for the synthesis of all new compounds (PDF)

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#### Notes

The authors declare no competing financial interest.

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## NOTE ADDED AFTER ASAP PUBLICATION

Scheme 2 and the Table 1 column heads were corrected on August 3, 2018.