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# Total Synthesis of the *Bacteroides fragilis* Zwitterionic Polysaccharide A1 Repeating Unit

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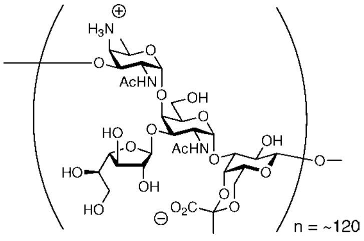
***Abstract:*** Nearly all bacteria capsular polysaccharides are T-cell-independent antigens that do not promote immunoglobulin class switching from IgM to IgG nor memory responses. In contrast, zwitterionic polysaccharides activate T-cell-dependent immune responses by major histocompatability complex class II presentation, a mechanism previously believed to be reserved for peptidic antigens. The best studied zwitterionic polysaccharide, polysaccharide A1 (PS A1) is found on the capsule of the commensal bacteria *Bacteroides fragilis*. Its potent immunomodulatory properties have been linked to postoperative intra-abdominal abscess formation. Here, we report the synthesis of the PS A1 tetrasaccharide repeating unit (**2**) as a tool to investigate the biological role of this polysaccharide. A modular synthetic strategy originating from the reducing end of the PS A1 repeating unit was unsuccessful and illustrated the limitations of glycosylation reactions between highly armed glycosylating agents and poor nucleophiles. Thus, a [3 + 1] glycosylation relying on trisaccharide **5** and pyruvalated galactose **6** was used to complete the first total synthesis of the PS A1 repeating unit (**2**).

## Introduction

Zwitterionic polysaccharides (ZPSs) are a unique class of immunomodulatory agents that can activate a major histocompatability complex class II (MHCII)-mediated T-cell-dependent immune response in the absence of protein.[1,2] The promise of ZPSs as immunotherapeutic agents is slowly being realized. Several semisynthetic polysaccharide-derived ZPSs have been constructed that exhibit potent immunostimulatory activity,[3] and a cancer vaccine candidate composed entirely of carbohydrate moieties has been developed using a conjugate of ZPS and the carbohydrate hapten, Tn.[4] Finally, it has been demonstrated that structurally different ZPSs appear to stimulate distinct immunological responses, including a host memory immune response.[1−5]



Native PS A1 (**1**)

***Figure 1.*** Structure of native PS A1 (**1**).

The best characterized natural ZPS is polysaccharide A1 (PS A1),[6] which is found on the capsule of the commensal bacteria *Bacteroides fragilis* (Figure 1). Although *B. fragilis* accounts for less than 0.5% of the normal colonic microflora in humans, it is the predominant obligate anaerobe isolated from postoperative intra-abdominal abscesses.[7] Studies have determined that PS A1's ability to strongly stimulate $CD4^+$ T-cells to produce cytokines (e.g., IL-2, IL-10, IL-12, IL-17, interferon-$\gamma$, and TNF-$\alpha$) and chemokines strengthens the localized immune response to form abscesses around foreign infectious agents. In addition to T-cell stimulation via MHCII presentation, PS A1 can also initiate an innate immune response through Toll-like receptor 2 (TLR2) signaling.[8] Curiously, the anti-inflammatory properties of PS A1, mediated by IL-10 production, can also prevent

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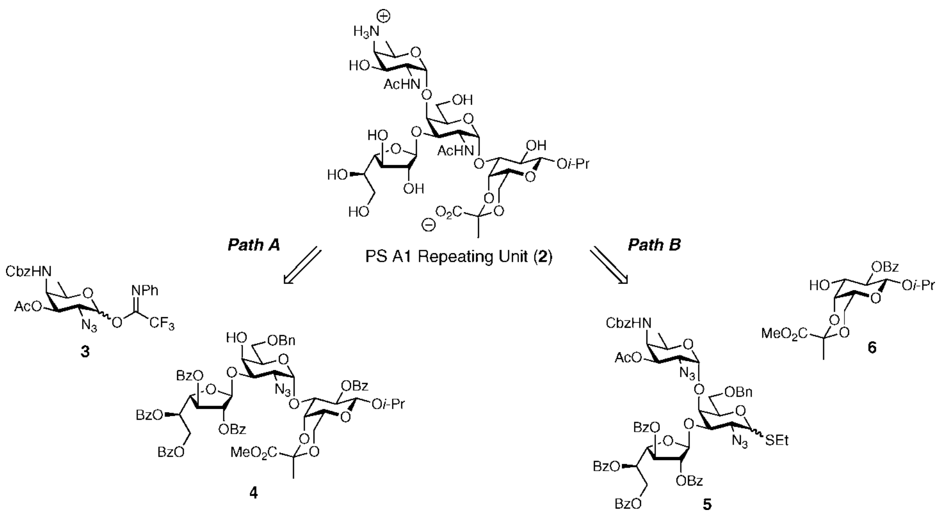
10.1021/ja1087375

***Scheme 1.*** Retrosynthetic Analysis of PS A1 Repeating Unit **2**



abscess formation against a *B. fragilis* challenge in germ-free mice.[9] Furthermore, by stimulation of IL-10 secretion, PS A1 can modulate surgical fibrosis,[9] inhibit intestinal inflammatory disease caused by *Helicobacter hepaticus*,[10] and protect against central nervous system (CNS) demyelinating disease.[11] Beyond its anti-inflammatory activities, PS A1 plays a role in the development and the maintenance of a balanced mammalian immune system.[8,12] In germ-free mice, administration of PS A1-bearing *B. fragilis* corrected systemic T-cell deficiencies, Th1/Th2 imbalances, and directed lymphoid organogenesis.

The interesting biological profile of native PS A1 (**1**) is complemented by a unique structural architecture (Figure 1). Indeed, the pyruvalated galactose, galactofuranose, and 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (AAT) are novel residues often found in immunodominant epitopes.[1,13,14] The zwitterionic charge motif of PS A1 is crucial for the activation of a T-cell-dependent immune response, since chemical removal of either the positive or negative charge eliminates MHCII binding.[1,2] The polysaccharides are around 120 repeating units long. Sp1 and PS A2, ZPSs closely related to PS A1, adopt an extended right-handed helix[15] with two repeating units per turn and a pitch of 20 Å. This α-helical architecture is necessary for MHCII binding. Circular dichroism studies[16] have shown that PS A1 fragments exhibit a similar helical architecture; however, fragments with fewer than three repeating units do not adopt this conformation and are unable to bind to MHCII and stimulate T-cell expansion.[17]

The challenging structural features of PS A1 (**1**) and the lack of chemically defined PS A1 fragments for use as mechanistic probes to study *B. fragilis* provided the impetus to synthesize the PS A1 repeating unit (**2**). Currently, only the fully protected PS A1 repeating unit has been synthesized.[18]

## Results and Discussion

**Initial Retrosynthetic Considerations.** Previous studies[18] highlighted the challenges associated with synthesizing the repeating unit (**2**) via trisaccharide nucleophile **4** (Scheme 1, path A), when an AAT thioglycoside or lactol served as the glycosylating agent. The low nucleophilicity of **4** and highly electron-rich nature of the glycosylating agents were most likely culpable for the low-yielding coupling. However, we had demonstrated in earlier studies[19] that AAT building block **3** can partake in high-yielding glycosylation reactions with a C4−OH galactosamine nucleophile. Thus, we were optimistic that glycosyl *N*-phenyl trifluoroacetimidate **3** would be able to overcome the deficiencies of previously used AAT glycosylating agents. The modular nature of path A was particularly attractive in light of a future automated synthesis.[20]

A second retrosynthetic disconnection was considered, in case path A was not viable. Path B involved the [3 + 1] glycosylation between trisaccharide glycosylating agent **5** and pyruvalated galactose nucleophile **6** (Scheme 1). This disconnection appeared attractive due to the involvement of a more electron-deficient glycosylating agent (**5**) and a more nucleophilic C3−OH galactose residue (**6**).

**Building Block Synthesis.** Our general strategy relied on *N*-phenyl trifluoroacetimidate glycosylating agents,[21] since we[19] and others[22] had found them to perform better than glycosyl trichloroacetimidates in glycosylations involving electron-rich deoxysugars. Most of the monosaccharide building blocks

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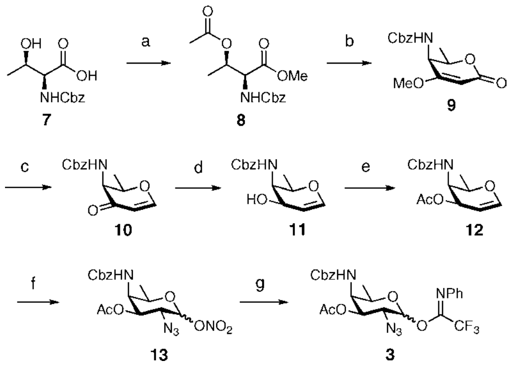
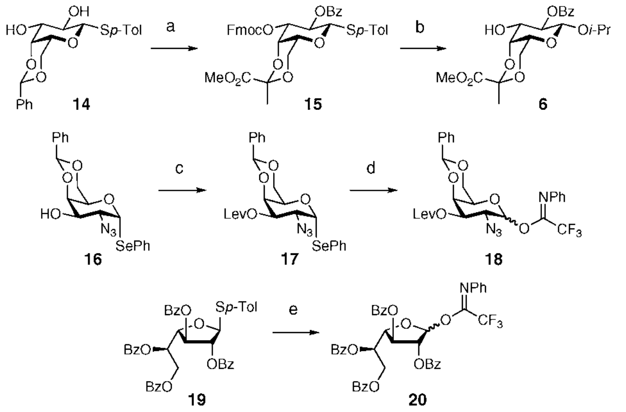
*Scheme 2.* De Novo Synthesis of AAT Building Block **3**[a]



[a] Reagents and conditions (a) i., AcCl, MeOH, 23 °C; ii., $Ac_2O$, $NEt_3$, DMAP, $CH_2Cl_2$, 0 to 23 °C, 95%, two steps; (b) i., LHMDS, THF, −78 to 23 °C; ii., $NaHCO_3$, $Me_2SO_4$, acetone, 23 °C, 73%, two steps; (c) DIBAL, THF, −78 °C, then $H^+$; (d) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, −78 °C, 77%, two steps; (e) $Ac_2O$, $NEt_3$, DMAP, $CH_2Cl_2$, 23 °C, 90%; (f) CAN, $NaN_3$, $CH_3CN$, −20 °C, 67% (3.5:1 dr); (g) i., *p*-TolSH, DIPEA, $CH_3CN$, 23 °C; ii., $F_3CC(NPh)Cl$, $Cs_2CO_3$, $CH_2Cl_2$, 23 °C, 69%, two steps.

*Scheme 3.* Synthesis of Building Blocks **6**, **18**, and **20**[a]



[a] Reagents and conditions (a) i., FmocCl, py., 0 °C, 79%; ii., BzCl, py., 0 °C, 82%; iii., TsOH, MeOH, 40 °C; iv., $CH_3C(O)CO_2Me$, $BF_3 \cdot OEt_2$, $CH_3CN$, 23 °C, 39%, two steps; (b) i., *i*-PrOH, NIS, AgOTf, $CH_2Cl_2$, 0 °C; ii., $NEt_3$, $CH_2Cl_2$, 23 °C, 49%, two steps; (c) LevOH, DIPC, DMAP, $CH_2Cl_2$, 0 to 23 °C, 90%; (d) i., NIS, THF, $H_2O$, 23 °C; ii., $F_3CC(NPh)Cl$, $Cs_2CO_3$, $CH_2Cl_2$, 0 °C, 83%, two steps; (e) i., NBS, EtOAc, $H_2O$, 23 °C, 91%; ii., $F_3CC(NPh)Cl$, $Cs_2CO_3$, $CH_2Cl_2$, 23 °C, 87%.

needed to prepare the PS A1 repeating unit (**2**) are readily accessed by using procedures modified from previously published methods. The synthesis of the AAT building block would benefit from a more efficient route when compared to known procedures.[18,23]

The C3−C6 portion of the AAT building block (**3**) was mapped back to L-threonine (Scheme 2). As a Cbz group was deemed appropriate for C4 amine protection, *N*-Cbz-L-threonine **7** was the starting point of the de novo AAT building block synthesis.[19] Conversion of **7** to a methyl ester followed by acetylation provided acetate ester **8** in 95% yield over two steps. Lithium bis(trimethylsilylamide) (LHMDS)-mediated Dieckmann cyclization[24,25] and $K_2CO_3/Me_2SO_4$ methylation then afforded enoate **9** in 73% yield over two steps. The 1,2-diisobutylaluminium hydride (DIBAL) reduction[26] of the enoate followed by acidic workup gave intermediate **10**, which was reduced under Luche conditions[27] to afford allylic alcohol **11** in 77% yield over two steps. Acetylation of the alcohol set the stage for the introduction of the C2 azide. Azidonitration[23b,28] of glycal **12** furnished nitrate **13**. Nitrate **13** was transformed into AAT building block **3** by cleavage of the anomeric nitrate with *p*-TolSH/DIPEA[29] followed by *N*-phenyl trifluoroacetimidate formation in 69% yield over two steps.

Having established a route for the AAT monosaccharide (**3**), the remaining three monosaccharide building blocks were pursued (Scheme 3). Pyruvalated galactose **6** was synthesized from known benzylidene galactoside **14**.[30] First, the C3 and C2 hydroxyl groups of **14** were selectively protected with Fmoc (79% yield)[31] and Bz (82% yield) groups, respectively. Cleavage of the benzylidene acetal and $BF_3 \cdot OEt_2$ mediated pyruvate ketal formation[32] afforded ketal **15** as a single diastereomer in 39% yield over two steps. The high diastereoselectivity of the thermodynamically driven ketalization is ascribed to the interaction of the low-lying unoccupied $\sigma^*$ orbital with the axial lone pairs of the ketal moiety in the six-membered ring of **15**. This favorable anomeric interaction[33] would not be possible for the other diastereomer. Finally, NIS/AgOTf promoted glycosylation of **15** with isopropanol followed by Fmoc cleavage furnished galactose **6**. At this stage, the stereochemistry at the pyruvate quaternary carbon in galactose **6** was verified through a nuclear Overhauser effect (NOE) interaction between the equatorial C4 hydrogen and the pyruvate methyl ester.

Galactosamine building block **18** was synthesized in a straightforward fashion from selenide **16**.[34] Levulinoylation of the C3−OH provided **17** in 90% yield. Selenide **17** was further treated with NIS to furnish an intermediate lactol that was converted into *N*-phenyl trifluoroacetimidate **18** in 83% yield over two steps. The final building block, galactofuranose **20**, was synthesized from known thiol **19**[35] by conversion to the lactol with *N*-bromosuccinimide (NBS) followed by *N*-phenyl trifluoroacetimidate formation. In storage, building block **20** was more stable than the trichloroacetimidate analog.[36]

**Forays Toward the PS A1 Repeating Unit.** With the building blocks in hand, retrosynthetic path A (Scheme 1), involving a modular approach that begins from the reducing end, was reduced to practice (Scheme 4). Pyruvalated galactose **6** was

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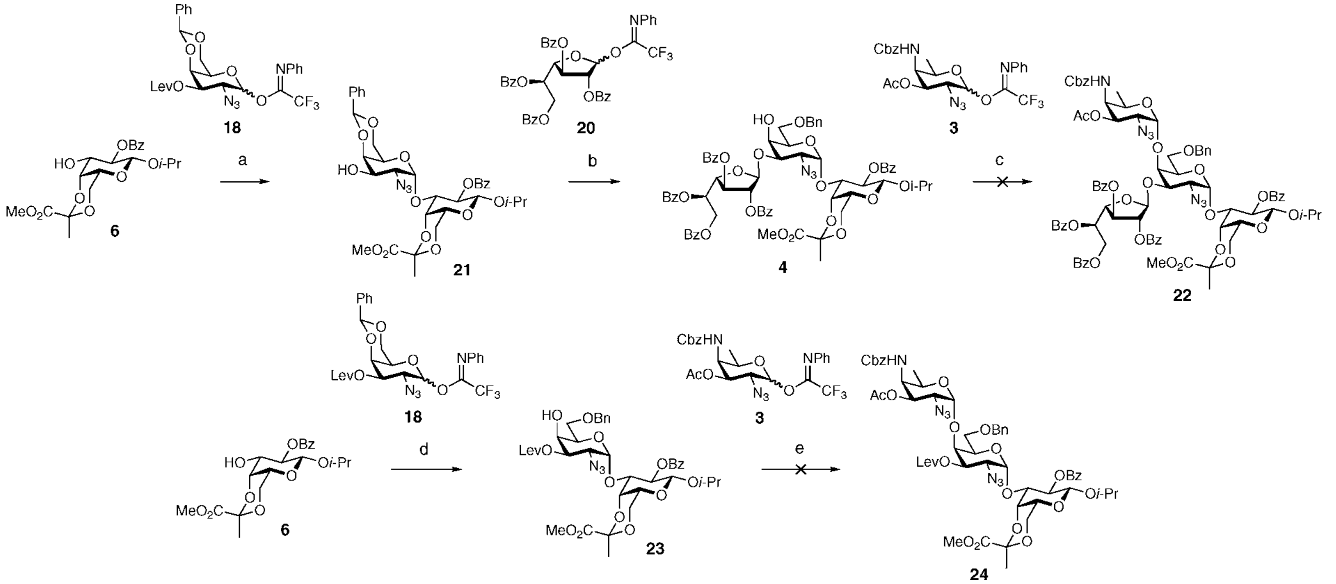
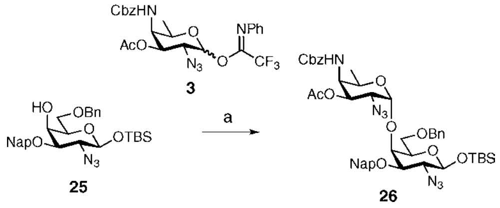
***Scheme 4.*** Attempted Glycosylations of AAT Building Block **3**[a]



[a] Reagents and conditions (a) i., TMSOTf, $CH_2Cl_2$, 0 °C, 72%; ii., $N_2H_4 \cdot H_2O$, AcOH, py., $CH_2Cl_2$, 23 °C, 89%; (b) i., TMSOTf, $CH_2Cl_2$, −60 °C, 57%; ii., TES, TfOH, 4 Å MS, $CH_2Cl_2$, −50 °C, 54%; (c) TMSOTf, $CH_2Cl_2$, 0 °C, 0%; (d) i., TMSOTf, $CH_2Cl_2$, 0 °C, 72%; ii., TES, $BF_3 \cdot OEt_2$, $CH_2Cl_2$, 0 °C, 54%; (e) TMSOTf, $CH_2Cl_2$, 0 °C, 0%.

***Scheme 5.*** Successful Glycosylation of AAT Building Block **3**[a]



[a] Reagents and conditions (a) TMSOTf, $CH_2Cl_2$, 0 °C, 74%.

successfully coupled with building block **18** in 72% yield. Lev deprotection using $N_2H_4 \cdot H_2O$ furnished disaccharide **21** in 89% yield. Galactofuranose **20** was then appended to disaccharide **21**, and the resulting trisaccharide was reductively ring-opened with TES/TfOH at −50 °C to provide alcohol **4**. Attempted glycosylation of alcohol **4** with AAT building block **3** provided no trace of product **22**. Since other building blocks, including an AAT thioglycoside activated with $Ph_2SO/Tf_2O$ or NIS/TMSOTf and an AAT lactol activated with $Ph_2SO/Tf_2O$, poorly couple with alcohol **4**,[18] glycosylation with a less complex nucleophile was attempted.

It was thought that by swapping out the bulky C3 perbenzoylated galactofuranose of **4** for a smaller protecting group, such as a levulinoyl ester, the glycosylation might proceed. Disaccharide **23** was prepared by glycosylation of building block **18** and nucleophile **6** followed by reductive $TES/BF_3 \cdot OEt_2$ opening of the benzylidene acetal ring. Unfortunately, the glycosylation between nucleophile **23** and AAT **3** was not successful.

The failure of nucleophiles **4** and **23** to couple with AAT building block **3** was surprising in light of the successful glycosylation between galactosamine **25**[19,37] and **3** (Scheme 5). The primary difference of **4** and **23** with **25** was the nature of the anomeric substituent. With nucleophiles **4** and **23**, molecular mechanics (MM2) energy minimized models showed the α-pyruvulated galactose preferentially occupying the space below the galactosamine residue near the C6 benzyl ether. This steric crowding likely forces the C6 benzyl ether to the top face of the galactosamine residue, thereby shielding the already poorly nucleophilic C4−OH. However, in nucleophile **25**, the equatorial *β*-OTBS group is projected away from the ring, permitting the C6 benzyl ether to rotate below the ring. Thus, nucleophile **25** constitutes an attractive means for glycosidic bond formation.

**Total Synthesis of the PS A1 Repeating Subunit.** Due to difficulties establishing a modular route to the tetrasaccharide repeat, AAT-containing cassette **26** was used to finish the synthesis via retrosynthetic path B (Scheme 1). Thus, the use of AAT building block **3** in a late-stage, sterically demanding glycosylation would be avoided.

The C3 naphthalene ether of disaccharide **26** was removed using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in 86% yield (Scheme 6). Glycosylation of resulting alcohol **27** with galactofuranose *N*-phenyl trifluoroacetimidate **20** proceeded best at −30 °C to afford trisaccharide adduct **28** in 90% yield as the *β*-isomer. The observed $^{13}C$ chemical shift of 106.5 ppm for the anomeric carbon of the galactofuranose is highly supportive[35,38] of a *β*-linkage. The anomeric *tert*-butyl-dimethylsilyl (TBS) protecting group was removed using tetrabutylammonium fluoride (TBAF) buffered with AcOH. The resulting lactol was finally converted into *N*-phenyl trifluoroacetimidate **29** in 82% yield over two steps. Thioethyl glycoside **5** was also prepared from glycosyl imidate **29** in 96% yield for glycosylation trials.

This approach also posed some interesting challenges: the union of *N*-phenyl trifluoroacetimidate **29** and pyruvalated galactose **6** yielded only trace amounts of adduct **22** (Table 1, entry 1). Steric interactions between the AAT residue, galactosamine C6 benzyl ether, and pyruvalated galactose that prevented the glycosylation of nucleophiles **4** and **23** with AAT **3** (Scheme 4) were likely also involved here. A breakthrough

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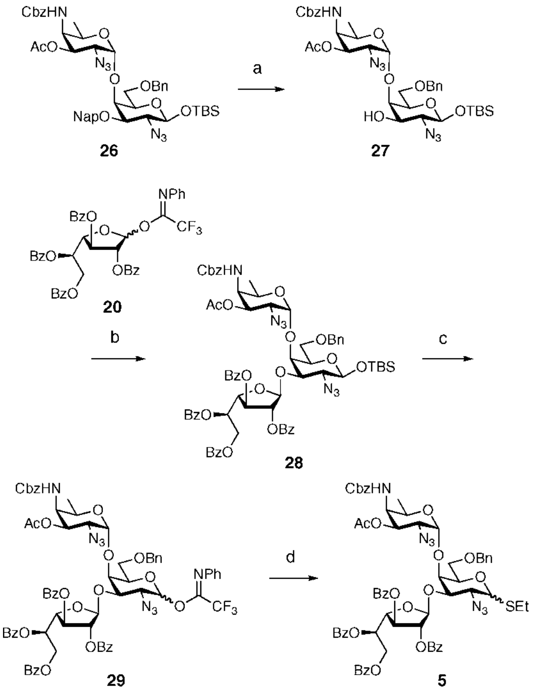
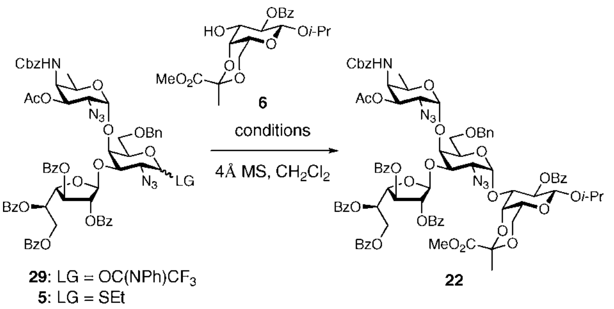
***Scheme 6.*** Synthesis of AAT-containing Trisaccharide **5**[a]



[a] Reagents and conditions (a) DDQ, MeOH, $CH_2Cl_2$, 23 °C, 86%; (b) TMSOTf, $CH_2Cl_2$, $-30$ °C, 90%; (c) i., TBAF, AcOH, THF, 0 °C; ii., $F_3CC(NPh)Cl$, $Cs_2CO_3$, $CH_2Cl_2$, 23 °C, 82%, two steps; (d) EtSH, TMSOTf, 4 Å MS, $CH_2Cl_2$, 0 °C, 96%.

***Table 1.*** Optimization of Tetrasaccharide **22** Formation



| entry | building block | conditions | yield, % |
|---|---|---|---|
| 1 | **29** | TMSOTf, 0 °C | <10 |
| 2 | **5** | NIS, AgOTf, 0 °C | 26 |
| 3 | **5** | MeOTf, 0 °C | 0 |
| 4 | **5** | DMTST, TTBP, 0 °C | 58 |
| 5 | **5** | $Ph_2SO$, $Tf_2O$, TTBP, $-60$ to 0 °C | 0 |

was finally achieved when thioglycoside **5** served as the coupling partner for **6**. Using NIS/AgOTf as the promoter system, tetrasaccharide **22** was isolated in 26% yield as the α-anomer (Table 1, entry 2). Our inspiration to use thioglycoside **5** came from Schmidt,[39] who had used a thioglycoside in place of a trichloroacetimidate for a glycosylation involving a sterically hindered C2−OH glucose nucleophile. However, succinimide addition[40,41] into the activated trisaccharide was a major side reaction of the NIS-promoted glycosylation. Thus, different promoters[42] for thioglycosides with less nucleophilic counterions were tested. Although MeOTf could not activate thioglycoside **5** (Table 1, entry 3), methylsulfenylating agent dimethyl(methylthio)sulfonium triflate (DMTST)[40,43] promoted the glycosylation to furnish tetrasaccharide **22** in 58% yield as the α-anomer (Table 1, entry 4). The stereochemistry of the newly formed anomeric linkage was verified by coupling constant analysis ($^3J_{H1,H2} = 3.0$ Hz for the anomeric position of the galactosamine residue). Low-temperature activation of thioglycoside **5** with $Ph_2SO/Tf_2O$[44] followed by addition of **6** was also unsuccessful in forming **22** (Table 1, entry 5).

The final stages of the synthesis called for the conversion of the azides in **22** to be converted to acetamides (Scheme 7). Initial studies using Staudinger conditions[45] ($PMe_3$ in $THF/H_2O$) followed by acetylation gave inconsistent yields of diacetamide **30** with poorer results associated with the absence of water during the Staudinger reduction. Employing AcSH and pyridine to effect a reduction−acetylation sequence[46,47] allowed for a one-pot reaction, ensured constant buffering of the moderately acid-labile deoxy sugars, and provided consistent and clean reactions. Diacetamide **30** was isolated in 67% yield from diazide **22**.

Global deprotection of acetamide **30** was not entirely straightforward (Scheme 7). Employing KOH in $THF/H_2O$ first to cleave the ester groups showed gradual formation of a cyclic carbonate before all benzoates had been cleaved. Hydrogenation of the intermediate with Pearlman's catalyst[48] afforded solely cyclic carbonate **31**. Since the cleavage of a cyclic carbonate in the presence of two acetamides was considered risky, the final steps were rearranged. By performing first the hydrogenation, Pearlman's catalyst cleanly removed the benzyl and Cbz groups. However, methanolysis of the esters using NaOMe in MeOH (Zemplén conditions) followed by the addition of water[49] to cleave the pyruvate ester resulted in significant quantities of triacetate **32** (along with desired **2**). This unexpected product originated from the migration of the AAT C3 acetate to the C4 amine. It has been suggested that a decrease in solvent polarity can prevent the migration of a C8 acetate to the C9 position in a neuraminic acid derivative.[50] Thus, by first dissolving the intermediate that results from hydrogenolysis in THF and then treating this mixture, in dropwise fashion, with NaOMe in $MeOH/H_2O$, only a very minor amount of acetyl migration was observed. Under these conditions, the PS A1 tetrasaccharide repeating unit (**2**) was produced in 46% yield over the two steps.

The $^1H$ NMR of PS A1 repeating unit **2** (Figure 2) compares remarkably well with native PS A1 (**1**).[51] This observation came as somewhat of a surprise since native PS A1 (**1**) is thought to exist in a helical secondary structure, whereas PS A1 fragments of less than three repeating units are too small to form helices.[16] Such a difference in secondary structure is observed in the $^1H$

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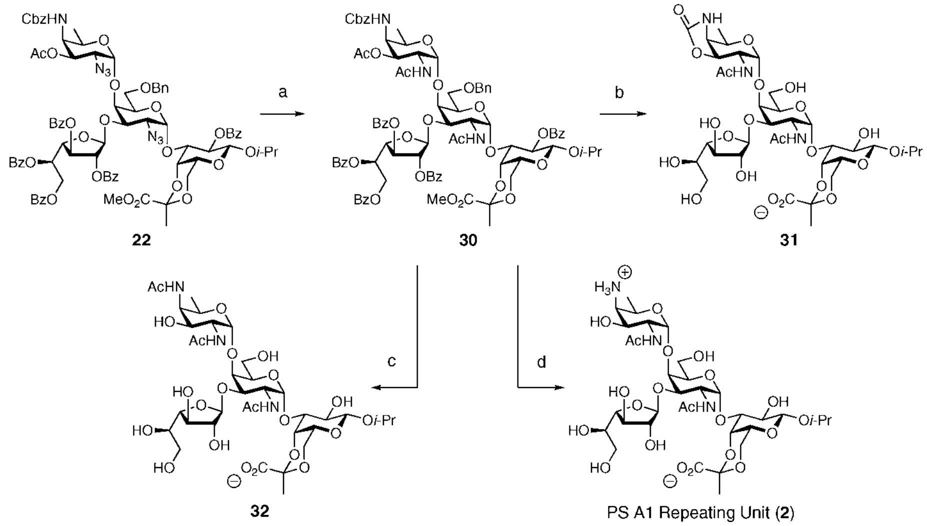
***Scheme 7.*** Completion of PS A1 Tetrasaccharide Repeating Unit **2**[a]



[a] Reagents and conditions (a) AcSH, py., 23 °C, 67%; (b) i., KOH, THF, $H_2O$, 23 °C; ii., $H_2$, $Pd(OH)_2/C$, MeOH, 23 °C, quant. conv.; (c) i., $H_2$, $Pd(OH)_2/C$, MeOH, 23 °C; ii., KOH, THF, $H_2O$, 23 °C or NaOMe, MeOH, 23 °C, 12 h, then, $H_2O$, ~50% conv. (plus, ~50% of **2**); (d) i., $H_2$, $Pd(OH)_2/C$, MeOH, 23 °C; ii., THF, then 0.5 M NaOMe in 1:1 $MeOH/H_2O$, 23 °C, 46%, two steps.

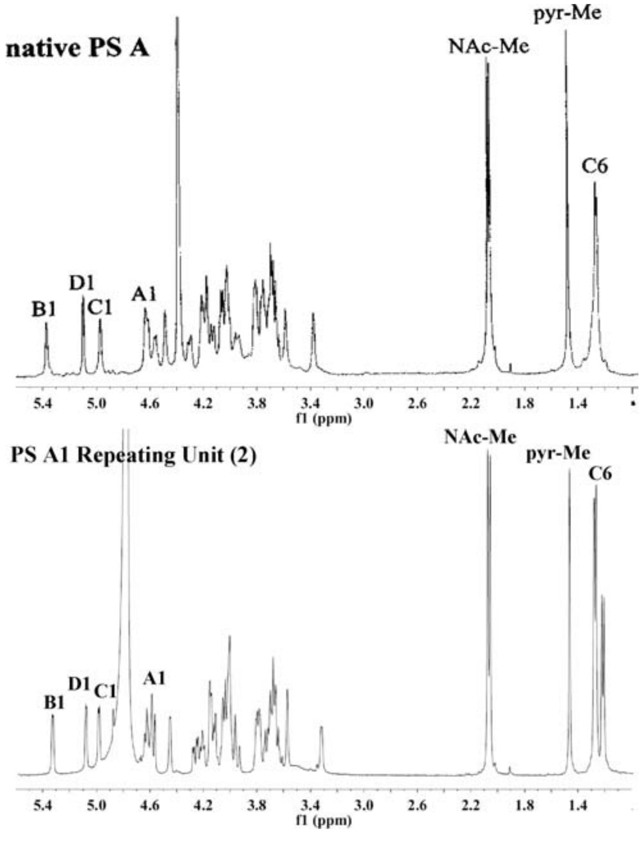


***Figure 2.*** $^1H$ NMR comparison of native PS A1 (**1**) and PS A1 repeating unit **2**.

NMR spectra of native ZPS Sp1 (which also exists as a right-handed helix) and the chemically synthesized monomeric and dimeric Sp1 repeating units.[52] However, due to the sterically congested nature of the PS A1 tetrasaccharide repeating unit (**2**), formation of the helical secondary structure of native PS A1 (**1**) may have little effect on the conformation of the rigid subunits.

## Conclusion

Reported is the first total synthesis of a deprotected PS A1 repeating unit (**2**) (see Supporting Information) from *N*-Cbz-L-threonine in 20 linear steps with a 1.8% overall yield. This synthesis highlights the de novo preparation of monosaccharide building blocks, such as AAT **3**, as an important means to fuel oligosaccharide synthesis. Furthermore, the need to further develop and understand glycosylation techniques involving unusual and complex carbohydrate targets was demonstrated. A cursory examination of target **2** would not suggest that any immediate steric complications would be encountered during its construction. Not surprisingly, it was found that the same steric and electronic issues that drive our understanding of modern synthetic organic chemistry are also applicable to carbohydrate synthesis. The methodology developed for the synthesis of PS A1 repeating unit **2** is currently being used to develop immunological probes for *B. fragilis*. We hope that these probes will help unravel the mechanism and action of zwitterionic PS A1.

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**Supporting Information Available:** Experimental procedures for the synthesis of PS A1 repeating unit **2**, NMR spectral characterization, and other characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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