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De Novo Synthesis of a 2-Acetamido-4-amino-2,4,6-trideoxy-D-galactose (AAT) Building Block for the Preparation of a *Bacteroides fragilis* A1 Polysaccharide Fragment

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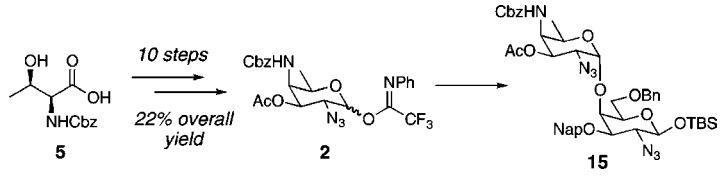
ABSTRACT



Zwitterionic polysaccharides (ZPSs) are potent T-cell activators that naturally occur on the cell surface of bacteria and show potential as immunostimulatory agents. An unusual, yet important component of many ZPSs is 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (AAT). AAT building block 2 was prepared via a de novo synthesis from *N*-Cbz-L-threonine 5. Furthermore, building block 2 was used to synthesize disaccharide 15 that constitutes a fragment of zwitterionic polysaccharide A1 (PS A1) found in *Bacteroides fragilis*.

Access to synthetic carbohydrates drives our understanding of molecular glycobiology.[1] Although many monosaccharide building blocks are readily accessed by protection of commercially available sugars, monosaccharides specific to many bacteria are only available via lenghty synthetic routes. As a result, de novo synthesis[2,3] of unusual, biologically important monsaccharide building blocks from linear fragments in turn derived from the chiral pool has emerged as an alternative to access carbohydrates.

One particular monosaccharide, 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (AAT), is an important component of zwitterionic polysaccharides (ZPSs) that are found on the cell surface of bacteria. For example, AAT is part of the lipopolysaccharide of *Shigella sonnei*,[4] on the capsules of *Streptococcus pneumoniae*[5] and *Bacteroides fragilis*,[6] and on the cell-wall polysaccharide of *Streptococcus mitis*[7] and

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S. pneumoniae.[8] Zwitterionic polysaccharide A1 (PS A1 (**1**); Figure 1) found in *B. fragilis* is the putative cause of postoperative intra-abdominal abscesses in humans and has been shown to activate a MHCII-mediated T-cell response in the absence of proteins.[9] As a potential immunostimulant, PS A1 when conjugated to tumor-associated antigen Tn (D-GalNAc) has been shown to promote a T-cell-dependent immune response against Tn in mice.[10] Due to the immunological properties of PS A1 and related ZPSs,[9−11] the development of efficient syntheses for AAT building blocks in order to access homogeneous ZPS fragments for immunological study remains important.

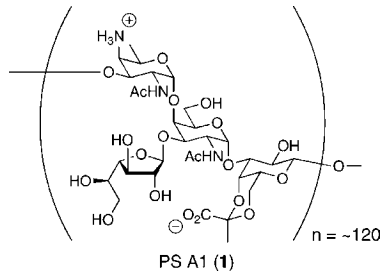
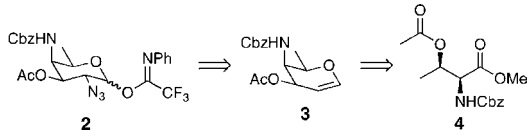


Figure 1. *B. fragilis* zwitterionic polysaccharide A1 (**1**).

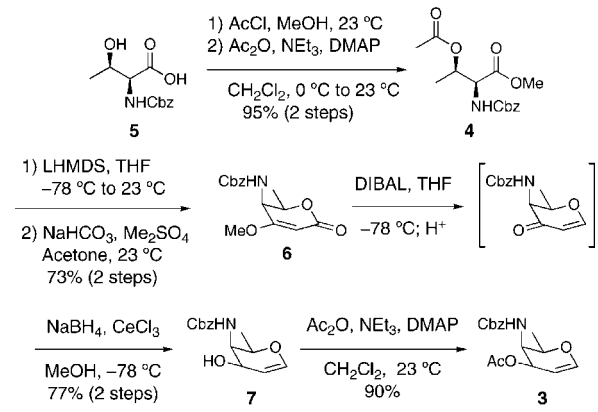
Previous syntheses[12] of AAT building blocks originate either from glucosamine or mannose and are quite lengthy. Shorter syntheses[13] provide building blocks with a participatory C2-nitrogen protecting group and thus cannot be used to form α-linked AAT disaccharides such as the one found in PS A1. We felt that a de novo approach[14] would reduce the number of steps required to generate a properly functionalized AAT building block for PS A1. Here we report a de novo synthesis of an AAT glycosylating agent, and its use in the assembly of a PS A1 disaccharide fragment.

Scheme 1. Retrosynthetic Analysis of AAT Building Block **2**



In the retrosynthetic analysis of AAT building block **2** (Scheme 1), we envisioned the installation of the C2-equatorial azide via an azido nitration reaction of glycal **3**. The carbon skeleton of this glycal could be generated by a Dieckmann cyclization of acetate **4** that is derived in two steps from *N*-Cbz-L-threonine.

Scheme 2. Synthesis of Glycal **3**



The synthesis of glycal **3** commenced with acid mediated methyl ester formation of *N*-Cbz-L-threonine **5** (Scheme 2). The resulting alcohol was acetylated to furnish acetate **4** in 95% yield over two steps. Two equivalents[15] of LHMDS were used to deprotonate the acetate and NHCbz groups to induce a Dieckmann cyclization.[16] The crude β-ketoester was methylated with K_2CO_3/Me_2SO_4 to provide enone **6** in 73% yield over two steps. Methoxy enone **6** was then reduced with DIBAL in a 1,2 manner and upon acidic workup, rearranged to an intermediate enone.[17] Luche reduction[18] of the crude enone at −78 °C gave glycal **7** in 77% yield over two steps. The free hydroxyl group was finally protected as an acetate ester to afford glycal **3** in 90% yield.

At this stage, azido functionalization of glycal **3** was attempted. Azido nitration[12b,19] generated azido nitrate **8** in 67% yield as an inseparable 3.5:1 C2-diastereomeric mixture that favored the desired equatorial azide (Table 1, entry 1).

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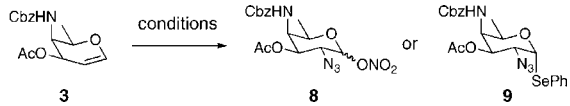
Table 1. Azido Functionalization of Glycal **3**



3 **8** **9**

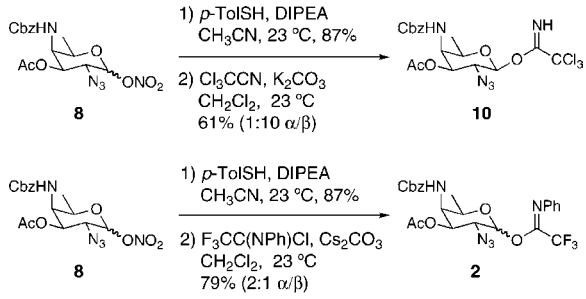
entry	conditions	product	yield, %	C2 dr[a]
1	CAN, NaN_3, CH_3CN, −20 °C	**8**	67	3.5:1[b]
2	$PhI(OAc)_2$, $TMSN_3$, Ph_2Se_2, CH_2Cl_2, −25 °C	9	68	1:1[b]

[a] Ratios determined by ^1H NMR. [b] Diastereomers could not be separated by silica gel chromatography; major isomer shown.

Surprisingly, the azido selenation[20] of glycal **3** furnished selenide **9** with poor stereoselectivity (1:1 dr), regarding the C2-position (Table 1, entry 2). This result stands in stark contrast to the high equatorial azide selectivity for the azido selenation of the structurally related di-*O*-acetyl fucal.[21]

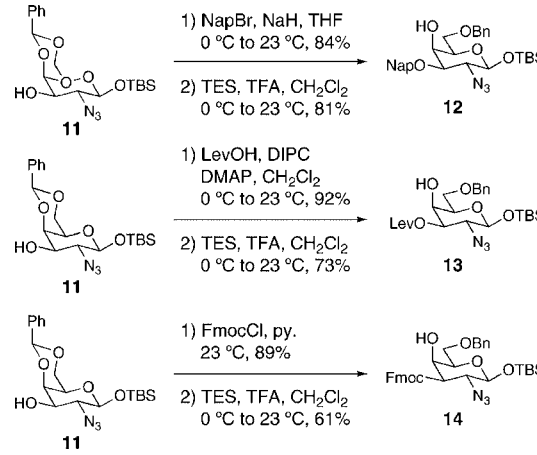
Two AAT building blocks were synthesized from nitrate **8** (Scheme 3). Glycosyl trichloroacetimidate **10** was prepared by selective hydrolysis of the anomeric nitrate[22] followed by K_2CO_3 mediated imidate formation. Glycosyl *N*-phenyl trifluoroacetimidate **2** was also prepared since recent studies[23] have shown that the *N*-phenyl trifluoroimidate leaving group performs well for armed, deoxyhexose glycosylating agents. Similar to the preparation of trichloroacetimidate **10**, the anomeric nitrate in **8** was removed followed by Cs_2CO_3 mediated imidate formation to afford **2**.

Scheme 3. Synthesis of AAT Building Blocks **2** and **10**



With building blocks **2** and **10** in hand, we wanted to test their ability to effect the glycosylation of C4-OH galactosamine nucleophiles in order to prepare a disaccharide motif found in the ZPSs of *S. mitis*, *S. pneumoniae*, and *B. fragilis*. This glycosylation was considered to be potentially problematic due to the low nucleophilicity of the axial C4-OH of galactosamine and previously described difficulties[12c] creating this glycosidic linkage. Specifically, a complex C4-OH galactosamine nucleophile performed poorly in glycosylations with an AAT thioglycoside (activated with Ph_2SO/Tf_2O or NIS/TMSOTf) and lactol (activated with Ph_2SO/Tf_2O). Thus, a variety of galactosamine nucleophiles (**12**−**14**) were prepared from known alcohol **11**[24] (which was prepared in eight steps from D-galactose; see Supporting Information). In particular, alcohol **11** was first protected with either a Lev, Fmoc, or Nap protecting group (Scheme 4). Then, the benzylidene acetal of each intermediate was reduced with TFA/TES to afford alcohols **12**−**14** in good yields.

Scheme 4. Synthesis of Galactosamine Nucleophiles **12**−**14**



Glycosylations between AAT building blocks (**2** and **10**) and nucleophiles (**12**−**14**) were then studied by employing standard activation conditions. Unfortunately, alcohol **12** did not react with trichloroacetimidate **10** to form desired disaccharide **15** (Table 2, entries 1 and 2). Although **10** has been used in a successful α-selective glycosylation with a C4-OH galactosamine nucleophile,[12b] the galactosamine was derivatized to place the hydroxyl group in an equatorial position, which rendered it more nucleophilic.

It was hoped that the attenuated reactivity of *N*-phenyl trifluoroacetimidate **2** compared to trichloroacetimidate **10** would slow the rate of building block decomposition and allow for a successful glycosylation reaction. Initial attempts to activate *N*-phenyl trifluoroacetimidate **2** with catalytic TMSOTf or $BF_3{\cdot}OEt_2$ in the presence of alcohol **12** at −78 °C (and by slowly warming to 0 °C) were indeed able to furnish desired disaccharide **15** (Table 2, entries 3 and 4). Unfortunately, both reactions did not proceed to completion, even though *N*-phenyl trifluoroacetimidate **2** was completely consumed in both cases, and the selectivity was poor with use of TMSOTf. Conducting the reaction at 0 °C improved the α-selectivity of the TMSOTf-catalyzed glycosylation (Table 2, entry 5). Further improvement of the α-selectivity was attempted by using a 4:1 Et_2O/CH_2Cl_2 solvent mixture (Table 2, entry 6). Unfortunately, under these conditions,

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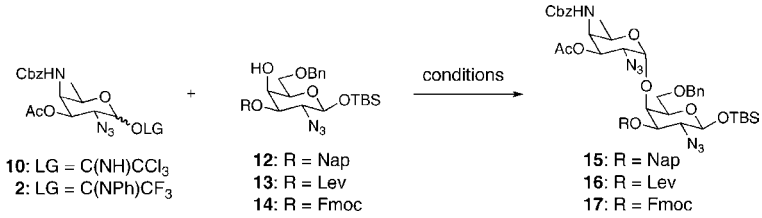
Table 2. Glycosylations between AAT Building Blocks **2** and **10** and Galactosamine Nucleophiles **12**−**14**



entry	building block[a]	nucleophile[a]	catalyst[a]	solvent	temp, °C	4 Å MS	product	conversion,[b] %	α:β selectivity[c]
1	**10**	**12**	TMSOTf	CH_2Cl_2	−78 to 0	yes	**15**	0	
2	**10**	**12**	$BF_3{\cdot}OEt_2$	CH_2Cl_2	−78 to 0	yes	**15**	0	
3	**2**	**12**	TMSOTf	CH_2Cl_2	−78 to 0	yes	**15**	55	1:2.5
4	**2**	**12**	$BF_3{\cdot}OEt_2$	CH_2Cl_2	−78 to 0	yes	**15**	30	6.5:1
5	**2**	**12**	TMSOTf	CH_2Cl_2	0	yes	**15**	57	7:1
6	**2**	**12**	TMSOTf	Et_2O/CH_2Cl_2 (4:1)	0	yes	**15**	0	
7	**2**	**12**	TMSOTf	CH_2Cl_2	0	no	**15**	>95 (74)	5:1
8	**2**	**13**	TMSOTf	CH_2Cl_2	0	no	**16**	>95 (81)	5.5:1
9	**2**	**14**	TMSOTf	CH_2Cl_2	0	no	**17**	>95 (59)	5:1

[a] Reactions were run with 1.5 equiv of building block, 1.0 equiv of nucleophile, 0.1 equiv of catalyst, and at 0.02 M concentration. [b] Conversion determined by ratio of product to nucleophile in the crude ^{1}H NMR; isolated yield of α-anomer in parentheses. [c] α/β ratios determined by ^{1}H NMR of the crude product.

N-phenyl trifluoroacetimidate **2** could not be activated and was recovered. Finally, the glycosylation between *N*-phenyl trifluoroacetimidate **2** and nucleophile **12** was attempted without preactivated 4 Å molecular sieves (Table 2, entry 7). Although the α-selectivity dropped slightly to 5:1, nucleophile **12** was completely consumed in the reaction. Desired α-linked disaccharide **15** was separated from the *β*-isomer by silica gel chromatography and isolated in 74% yield. Disaccharides **16** and **17** were prepared in analogous fashion with similar yields and selectivites (Table 2, entries 8 and 9). For the newly formed glycosidic bond, the anomeric proton of disaccharides **15**−**17** was determined by HSQC spectroscopy, and all disaccharides displayed an anomeric coupling constant ($^3J_{H1,H2}$ = 3.6−4.0 Hz) confirming the presence of an α-linkage.

In summary, we have completed the synthesis of orthogonally protected disaccharides **15**−**17** that are an essential component of the ZPS repeating subunits of *S. mitis*, *S. pneumoniae*, and *B. fragilis*. Key to this synthesis is the de novo synthesis of AAT building blocks **2** and **10** from *N*-Cbz-L-threonine **5** and the successful use of *N*-phenyl trifluoroacetimidate **2** in glycosylation reactions with galactosamine nucleophiles **12**−**14**. Further progress toward the synthesis of the repeating subunit of PS A1 (**1**) and related ZPSs, and the study of their immunological properties will be reported in due course.

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Supporting Information Available: Experimental procedures and tabulated spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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