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A synthetic study towards the PSA1 tetrasaccharide repeating unit

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Abstract—A synthetic study to the protected tetrasaccharide repeating unit of zwitterionic polysaccharide PSA1 using 1-thio, 1-seleno and 1-hydroxyl functionalized donor glycosides is presented. The ABC trisaccharide part was successfully assembled using an iterative dehydrative glycosylation protocol.

Zwitterionic polysaccharide A1 (PSA1), isolated from capsules of the anaerobic bacterium *Bacteriodes fragilis*, is thought to be the key pathogenic substance responsible for the development of intra-abdominal sepsis.[1,2] In contrast to that found for the majority of pathogenic polysaccharides[3] the immune response elicited by PSA1 is mediated by T-lymphocytes.[4] The unusual immunologic property of PSA1 is likely to be caused by its zwitterionic character. Neutralization of either the positively charged amino groups or the negatively charged carboxyl groups resulted in a strongly reduced biological activity as compared to the unmodified polysaccharide.[5,6] The repeating unit **1** of PSA1 is depicted in Figure 1. The positive charge is situated at the free amino group of the rare 2-acetamido-4-aminofucose moiety and the negative charge resides on the carboxylate of the pyruvate ketal spanning C4 and C6 in the galactopyranosyl residue. As part of a program aimed at elucidation of the interaction of bacterial polysaccharides with the immune system, we here present our studies on the assembly of the repeating unit of PSA1.

Retrosynthetic analysis indicates that the protected tetrasaccharide repeating unit **2** can be assembled from protected monosaccharides **3**–**8** following the sequence of glycosylation events depicted in Figure 1. The lengthy synthetic route to suitably protected 2,4-diamino-2,4,6-trideoxygalactose building blocks **7** or **8** dictates that

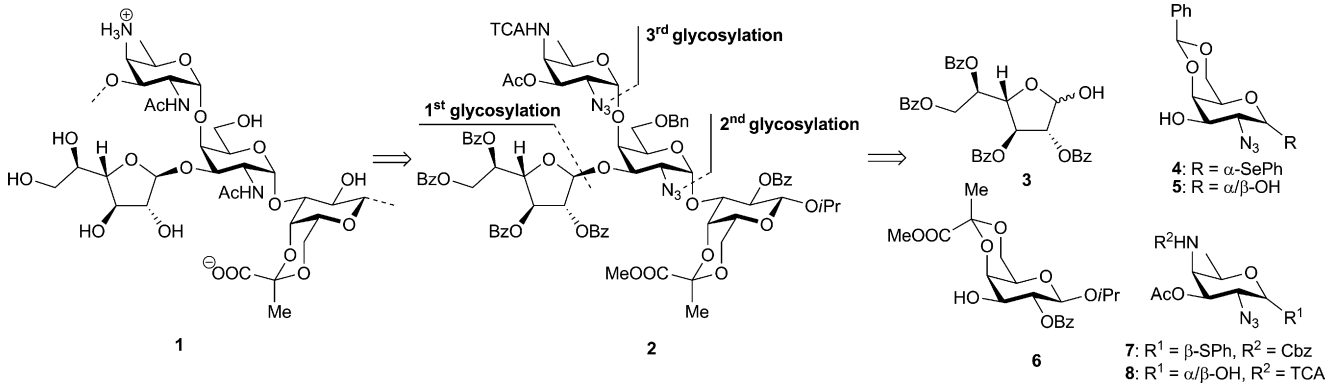

Figure 1. Retrosynthetic analysis.

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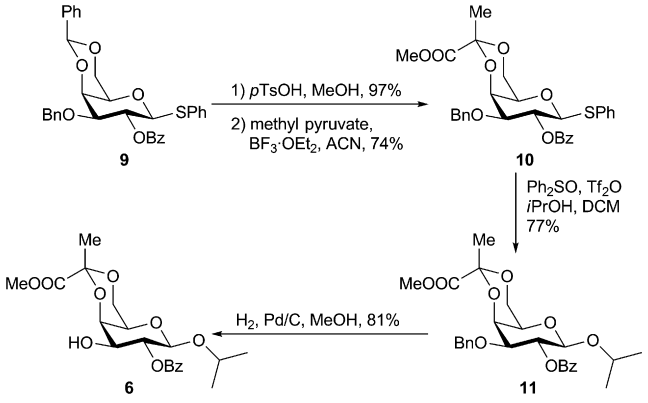
this residue is best introduced at a late stage (i.e., the third glycosylation in Fig. 1). The construction of the protected trisaccharide that follows from this reasoning was investigated using orthogonal and chemoselective glycosylation strategies in combination with Ph_2SO/Tf_2O as an activator system.[7] This orthogonal strategy[8] required the availability of furanose **3**,[9] 1-seleno galactosazide **4**[10] and terminal pyruvate residue **6**, while for the chemoselective strategy[11] the availability of furanose **3**, diol acceptor **5**[12] and pyruvate building block **6** was necessary.

Pyruvate derivative **6** was synthesized as depicted in Scheme 1. Acidolysis of the 4,6-*O*-benzylidene in known thiogalactopyranoside **9**[13] was followed by introduction of the pyruvate ketal according to the procedure developed by Ziegler and co-workers.[14] Treatment of the intermediate diol with equimolar amounts of methyl pyruvate and boron trifluoride diethyletherate ($BF_3{\cdot}OEt_2$) in acetonitrile (ACN) gave pyruvated galactopyranoside **10**. Conclusive evidence for the predicted (*R*)-stereochemistry at the pyruvate ketal came from the X-ray structure of **10**.[15] The isopropyl group was installed at the anomeric centre via a Ph_2SO/Tf_2O-mediated glycosylation to give **11**. Although the use of tri-*tert*-butylpyrimidine (TTBP)[16] in this condensation was omitted to prevent orthoester formation, no deterioration of the putative acid labile pyruvate acetal was observed. In the last step, hydrogenolysis of the C3 benzyl protecting group yielded galactopyranoside **6**.

The synthesis of trisaccharide **14** was investigated employing first an orthogonal glycosylation approach, in which a hemiacetal donor is condensed with a 1-thio acceptor (Scheme 2A).[8] Pre-activation of galactofuranose **3**[9] using the Ph_2SO/Tf_2O[17,18] reagent combination at −40 °C and subsequent addition of 1.5 equiv of partially protected 1-selenogalactoside **4** afforded β-linked digalactoside **12** in a yield of 56%.[8] Although the anomeric seleno-function was stable according to TLC analysis, the yield of this condensation could not be further improved. In the next glycosylation event, 1-selenodisaccharide **12** was successfully activated with the Ph_2SO/Tf_2O reagent combination. However, addition of acceptor **6** did not afford the expected trisaccharide **14**. The same outcome was observed employing the BSP/Tf_2O activator system.[19] Based on our experience that NIS/TMSOTf is a useful alternative when sulfonium ion mediated glycosidation of thioglycosides is unproductive,[20] a mixture of disaccharide **12** and acceptor **6** was treated with NIS/TMSOTf[21] in DCM at 0 °C to afford trisaccharide **14** in 65% yield as a single stereoisomer.[22] Most likely, the use of only a catalytic amount of TMSOTf is at the basis of this favourable result.[20]



Scheme 1. Synthesis of pyruvate residue **6**.

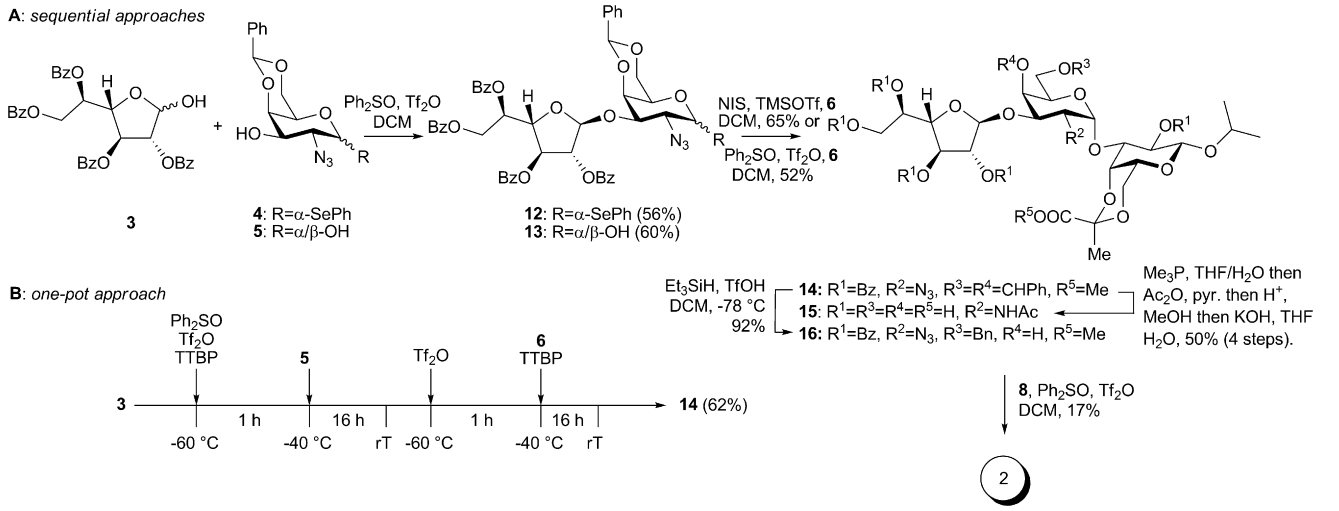
With the objective of assembling trimer **14** via a one-pot glycosylation procedure, attention was focused on a chemoselective dehydrative glycosylation strategy (Scheme 2A), as reported by Gin and co-workers.[11] Pre-activation of 1-hydroxyl donor **3** with Ph_2SO/Tf_2O for 1 h at −40 °C was followed by the addition of a solution of diol **5** in dichloromethane/dioxane[23] (10/1) to give, upon warming of the reaction mixture, disaccharide **13** in 60% yield. Analogously, Ph_2SO/Tf_2O mediated dehydrative coupling of dimer **13** with acceptor **6** afforded target trisaccharide **14** in 52% yield.

Next, attention was focused on adapting these results in a one-pot iterative dehydrative glycosylation strategy (Scheme 2B). Pre-activation of donor **3** and addition of acceptor **5** afforded hemiacetal donor **13** with concomitant regeneration of Ph_2SO. The reaction mixture was warmed to room temperature, kept overnight and then cooled to −60 °C. Activation of the newly formed hemiacetal disaccharide **13** was accomplished by the addition of a fresh amount of Tf_2O and stirring was continued for 1 h at −40 °C. Addition of 1.5 equivalents of acceptor **6** and tri-*tert*-butylpyrimidine (TTBP) followed by overnight warming to room temperature (rt) produced trisaccharide **14** in 62% yield.

Removal of all the protecting groups in **14** by, (1) conversion of the azide functionality into the acetamido group, (2) acidic cleavage of the 4,6-*O*-benzylidene acetal, and (3) alkaline hydrolysis of all the ester functions, gave unprotected trisaccharide **15** in 50% yield (Scheme 2).

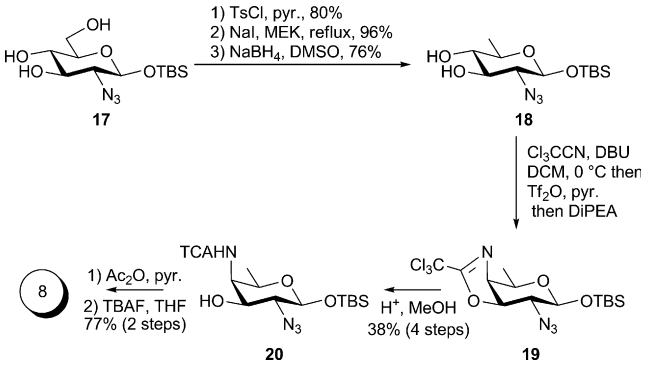
Now the stage was set to study the construction of the fully protected tetrameric repeating unit **2**. In a first attempt, we investigated the glycosylation of trisaccharide **16** with phenyl 3-*O*-acetyl-4-(*N*-benzyloxycarbonyl)-amino-2-azido-2,4-dideoxy-1-thio-β-D-fucopyranoside (**7**). Donor **7** was prepared by adaptation of the literature procedure.[15] Compound **16** was obtained after reductive opening of the benzylidene functionality in compound **14** using the triethylsilane/triflic acid reagent combination (Scheme 2).[24] Although activation of thio donor **7** with Ph_2SO/Tf_2O proceeded smoothly at −60 °C, addition of trisaccharide acceptor **16** did not lead to a productive coupling. Changing the activator to the NIS/TMSOTf system was also unsuccessful because donor **7** could not be activated under these conditions.

We then turned our attention to Gin's dehydrative glycosylation procedure[25] using 3-*O*-acetyl-2-azido-4-trichloroacetamido-2,4,6-trideoxy-β-D-galactopyranoside (**8**) (Scheme 3). The synthesis of **8** started from known 2-



Scheme 2. Construction of unprotected trisaccharide **22** and fully protected tetrasaccharide **2**.

azido glucose **17**, which was easily obtained from glucosamine hydrochloride.[26] Regioselective tosylation of compound **17** followed by the reaction with NaI in refluxing butanone and final $NaBH_4$ reduction of the intermediate C6 iodo function gave quinovosamine derivative **18** in a good overall yield. Attempts to reduce the C6 tosyl function directly using $NaBH_4$ resulted in prolonged reaction times and decreased reaction efficiencies compared to reduction of the iodinated intermediate. In the next step, the regioselective introduction of the axially oriented C4 amino function was addressed by means of a one-pot tethered nucleophilic inversion approach.[27] Treatment of compound **18** with an equimolar amount of trichloroacetonitrile (Cl_3CCN) and a catalytic amount of DBU resulted in the predominant formation of the 3-*O*-trichloroacetimidate intermediate. After triflation of the C4-OH group, excess DiPEA was added to afford oxazoline derivative **19**. Mild acid treatment of the crude reaction mixture selectively cleaved the oxazoline functionality of compound **19** to the 4-*N*-trichloroacetamide target compound **20**. Two-dimensional ^1H NMR spectroscopy confirmed the inverted chirality at the C4-position. The final steps towards donor building block **8** comprised acetylation of C3-OH and subsequent fluoride assisted removal of the anomeric silyl group. Activation of hemiacetal donor **8** using the Ph_2SO/Tf_2O reagent combination and addition of trisaccharide **16** afforded tetrasaccharide **2** in 17% yield. Trisaccharide **16** was recovered in 53%, whereas excess donor **8** was completely degraded to unidentified baseline products.



Scheme 3. Synthesis of the 2,4-diamino fucose **8**.

The poor yield in the final step forces us to consider an alternative synthetic route to tetrasaccharide **2**, and we are currently working on this. Possibly, the difficulty in introducing the 2,4-diaminofucose at this stage is caused by a combination of the steric bias in the trimeric acceptor **16** and the apparent low reactivity of the protected 2,4-diaminofucose donors **7** and **8**. On the positive side, comparative studies towards the construction of the protected trisaccharide **14** demonstrate the effectiveness of the dehydrative glycosylation procedure, and have led to the development of a one-pot iterative protocol that is characterized by the single use of the Ph_2SO/Tf_2O reagent combination.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.02.067.

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