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First synthesis of the immunodominant β-galactofuranose-containing tetrasaccharide present in the cell wall of *Aspergillus fumigatus*

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Abstract—β-Gal*f*-(1→5)-β-Gal*f*-(1→6)-α-Man*p*-(1→6)-α-Man*p*, the immunodominant epitope in the cell-wall galactomannan of *Aspergillus fumigatus*, was synthesized for the first time as its allyl glycoside. The key disaccharide glycosyl donor, 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2-*O*-acetyl-3,6-di-*O*-benzoyl-β-D-galactofuranosyl trichloroacetimidate (**10**), was constructed by 5-*O*-glycosylation of 1,2-*O*-isopropylidene-3,6-di-*O*-benzoyl-α-D-galactofuranose (**4**) with 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl trichloroacetimidate (**5**), followed by 1,2-*O*-deacetonation, acetylation, selective 1-*O*-deacetylation, and trichloroacetimidation. The target tetrasaccharide **16** was obtained by the condensation of allyl 2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (**14**) as glycosyl acceptor with the disaccharide glycosyl donor **10**, followed by deprotection.

Keywords: Oligosaccharide; Galactofuranose; Synthesis

1. Introduction

Aspergillus fumigatus, a fungus, is the major causative agent of several respiratory tract-related diseases such as invasive aspergillosis, airway cavity colonization, and allergic manifestations.[1] Invasive aspergillosis is a leading cause of death among those treated for hematological malignancy and those receiving a solid organ transplant, especially of the lung,[2] due to the infection in allogeneic hematopoietic stem-cell-transplant recipients.[3] The high mortality of invasive aspergillosis is due partly to difficulties in timely diagnosis because signals and symptoms are often nonspecific. For instance, only at a late stage of invasive aspergillosis did cultures of respiratory specimens become positive.[4] Hence, in the search for a potential structure that could be helpful in the diagnosis of the different forms of aspergillosis, much attention has been paid to the study of the *Aspergillus* cell wall, considering its high immunogenicity.[5] Notermans and Soentoro[6a] and Latgé[6c] have identified the diagnostic potential of the *Aspergillus* cell-wall polysaccharides and glycoproteins. Barreto-Bergter and co-workers,[6d,e] Latgé et al.,[6b] Bennnet et al.,[7] and Notermans et al.[8] have studied the structure of galactomannans from the *Aspergillus* cell wall. Later on, through extensive exploration,[9] it was found that the galactomannans from *Aspergillus* could be regarded as a marker to indicate invasive aspergillosis to improve timely diagnosis. That is to say, when galactomannans[10] released by *Aspergillus* are detected in the serum or plasma of patients, a prompt diagnosis can be used to reduce the high ratio of mortality of invasive aspergillosis. For instance, in around two-thirds of the patients, galactomannan could be detected at a mean of 8 days before diagnosis by other means.[11] Recent studies[12] shown that tetra- and hexasaccharide, that is, β-Gal*f*-(1→5)-β-Gal*f*-(1→6)-α-Man*p*-(1→6)-Man and β-Gal*f*-(1→5)-β-Gal*f*-(1→5)$_3$-β-Gal*f*-(1→6)-Man, fragments of the galactomannan, are the immunodominant epitopes of the galactomannan. The tetrasaccharide seems to be the

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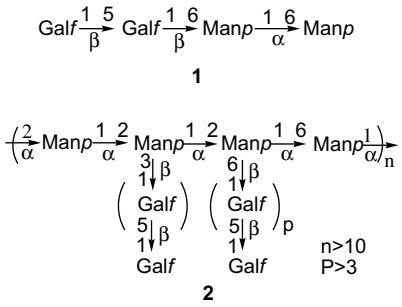

Figure 1. Structures of the immunodominant tetrasaccharide **1** of the cell wall galactomannan **2** of *Aspergillus fumigatus*.

minimum structure required for the immunodominant epitope in the mycelial cell wall of *A. fumigatus* (Fig. 1).

Providing enough sample is a basic condition for detailed studies on a compound's fundamental biochemical properties and possible biological functions. However, in the carbohydrate field, these efforts are often frustrated by the difficulty of synthesizing saccharides. Synthesis of complex oligosaccharide sequences containing two to six sugar units, both in solution and on solid phase, presents a major challenge in organic chemistry.[13] Unlike peptides and nucleic acids, oligosaccharides are typically branched rather than linear. The monosaccharide units can be connected by α or β linkages. Furthermore oligosaccharide synthesis requires multiple selective protection and deprotection steps. Although over the past few decades considerable progress[14] has been made in this field, there still is no general route for oligosaccharide synthesis.

Study on the synthesis of the immunodominant oligosaccharide present in the mycelial cell wall of *A. fumigatus* is important. On the one hand, sufficient quantities of the sample can be provided by this means for its immunoassay studies in detail; on the other hand, it could be used to further elucidate the molecular structure responsible for monitoring invasive aspergillosis. These, together with the fact that synthesis of the tetrasaccharide has never been done so far, prompted us to synthesize β-Gal*f*-(1→5)-β-Gal*f*-(1→6)-α-Man*p*-(1→6)-α-Man*p* as its allyl glycoside **16**.

2. Results and discussion

3,6-Di-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-galactofuranose (**4**) is an important synthetic intermediate in our synthesis, which was easily obtained through selective benzoylation of 3-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-galactofuranose (**3**)[15a] with BzCl in pyridine at 0 °C in 82% yield. Although a similar disaccharide was published in 1990,[15b] our method for preparing the novel disaccharide, 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-3,6-di-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-galactofuranose (**6**), is simple and is more efficient. Compound **6** was prepared by coupling compound **4** and 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl trichloroacetimidate (**5**)[16] in the presence of a catalytic amount of TMSOTf in excellent yield (93%) (Scheme 1). Both ^{1}H NMR and TLC were employed to detect the (1→5)-linked disaccharide **6**. The structure of **6** was confirmed by the ^{1}H NMR data. The characteristic resonances due to the anomeric protons H-1 and H-1′ were located as a doublet at 5.93 ppm with $J_{1,2}$ = 4.1 Hz and as a singlet at 5.69 ppm, respectively. The deisopropylidenation of **6** in 10:1 $CHCl_3$–CF_3COOH (v/v) at room temperature gave 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-3,6-di-*O*-benzoyl-β-D-galactofuranose (**7**), which after purification was treated with Ac_2O and pyridine at room temperature to afford 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2-*O*-acetyl-3,6-di-*O*-benzoyl-β-D-galactofuranose **(8)**. The diacetate **8** was selectively deacetylated at the anomeric position with benzylamine in THF in high yield to give the corresponding 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2-*O*-acetyl-3,6-di-*O*-benzoyl-β-D-galactofuranose (**9**). Subsequent reaction of **9** with CCl_3CN–K_2CO_3 in CH_2Cl_2 afforded the 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-2-*O*-acetyl-3,6-di-*O*-benzoyl-β-D-galactofuranosyl trichloroacetimidate (**10**). The structure of **10** was confirmed by ^{1}H NMR spectral analysis as follows: δ 8.63 (s, 1H, CN*H*CCl_3), δ 6.45 (s, 1H, H-1′), δ 5.74 (s, 1H, H-1).

6-*O*-Acetyl-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**11**) was prepared from D-mannose according to the reported procedure.[17] Glycosylation of **11** with allyl alcohol in the presence of TMSOTf, followed by removal of the 6-*O*-acetyl group in MeOH containing 1% HCl, gave allyl 2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside **12** in 81% yield (Scheme 2). Condensation of **11** and **12** with TMSOTf as catalyst and 4 Å molecular sieves in CH_2Cl_2 at −20 °C, followed by removal of acetyl group of allyl 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (**13**), afforded allyl 2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (**14**). The ^{1}H NMR spectra of **14** showed two characteristic signals for H-1 at 5.16 ppm with $J_{1,2}$ = 1.4 Hz and H-1′ at 5.14 ppm with $J_{1,2}$ = 1.3 Hz, respectively.

With the glycosyl donor and acceptor **10** and **14** in hand, construction of the target compound was readily carried out. As shown in Scheme 3, the fully protected tetrasaccharide, allyl 1,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-3,6-di-*O*-benzoyl-2-*O*-acetyl-β-D-galactofuranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside **15**, was obtained by coupling **10** with **14** in 85% yield. The ^{1}H NMR data of **15** contained structurally characteristic information: for instance, one acetyl signal (δ 1.86), one allyl signal (δ 5.52–5.32), and four H-1 signals (δ 5.74, 5.22, 5.20, 5.18). The ^{13}C NMR spec-