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

Mingkun Fu, Guohua Zhang and Jun Ning*

Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, PO Box 2871, Beijing 100085, China

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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

* Corresponding author. Fax: +86 10 62923563; e-mail: jning@mail.rcees.ac.cn

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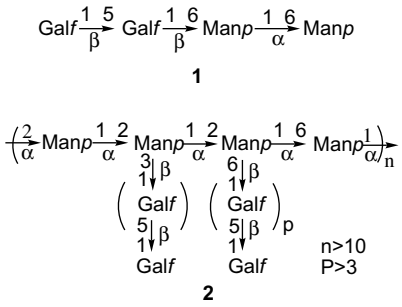


Figure 1. Structures of the immunodominant tetrasaccharide **1** of the cell wall galactomannan **2** of *Aspergillus fumigatus*.

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.

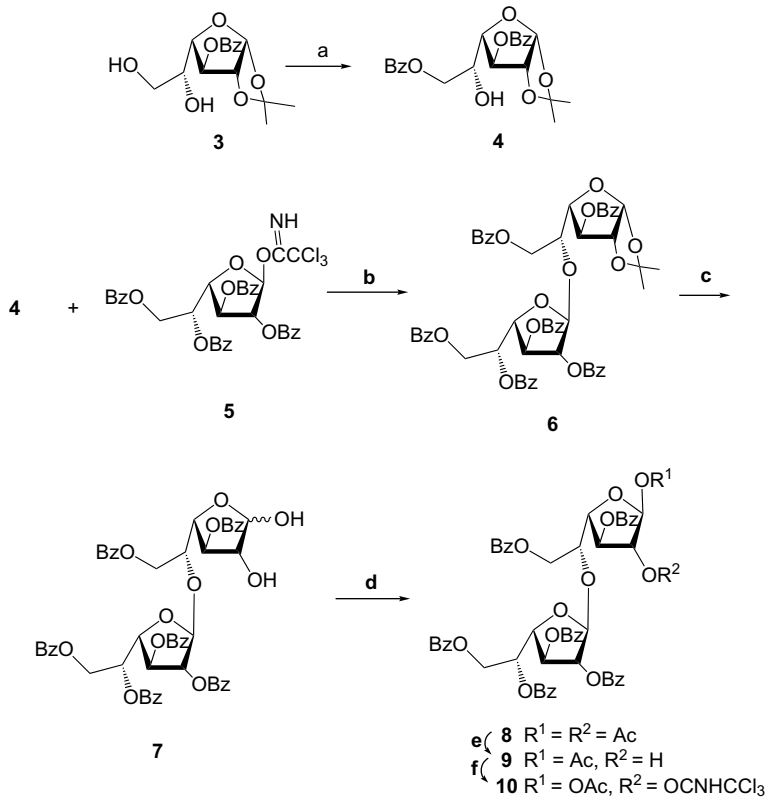
Study on the synthesis of the immunodominant oligosaccharide present in the mycelial cell wall of *A. fumigatus* is important. On the one hand, sufficient quantities of the sample can be provided by this means for its immunoassay studies in detail; on the other hand, it could be used to further elucidate 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

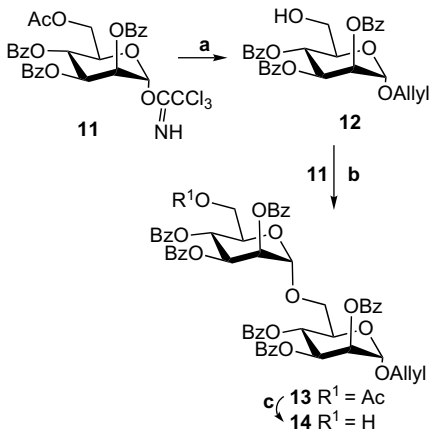
3,6-Di-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-galactofuranose (**4**) is an important synthetic intermediate in our synthesis, which was easily obtained through selective benzoylation of 3-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-galactofuranose (**3**)[15a] with BzCl in pyridine at 0 °C in 82% yield. 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-



Scheme 1. Reagents and conditions: (a) PhCOCl (1.2 equiv), pyridine, 0 °C, 6 h; (b) TMSOTf (cat), CH_2Cl_2, 4 Å MS powder, −20 °C to rt, 1.5 h, 93%; (c) 10:1 $HCCl_3$–CFCOOH, rt, 2 h; (d) Ac_2O, pyridine, rt, 12 h; (e) $PhCH_2NH_2$ (4.0 equiv), THF, rt, 24 h; (f) CCl_3CN (2 equiv), CH_2Cl_2, K_2CO_3, rt, 5 h.



Scheme 2. Reagents and conditions: (a) (i) allyl alcohol (2 equiv), TMSOTf, CH_2Cl_2, rt; (ii) 1% CH_3COCl in CH_2Cl_2–CH_3OH, rt, 10–12 h; (b) TMSOTf (cat), CH_2Cl_2, 4 Å MS powder, −20 °C to rt, 1.5 h; (c) 1% CH_3COCl in CH_2Cl_2–CH_3OH, rt, 10–12 h.

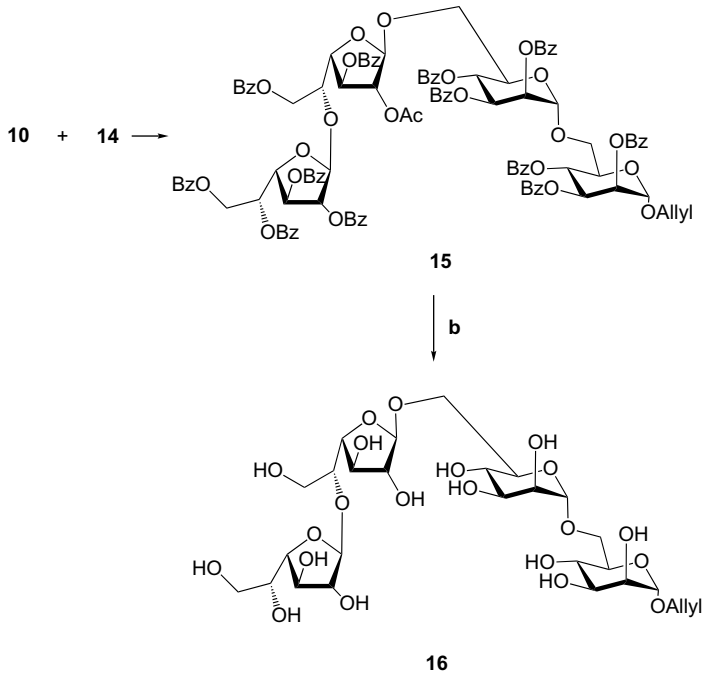
trum of **15** gave four signals for C-1 (δ 106.45, 105.94, 97.69, 96.85). Deprotection of **15** in ammonia-saturated methanol yielded the target allyl β-D-galactofuranosyl-(1→5)-β-D-galactofuranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (**16**). The signals of the ^{1}H NMR spectrum of **16** contained structurally characteristic information: one allyl signal (δ 5.92–5.84, m), a second allyl signal (δ 5.30–5.25, dd), a third allyl signal (δ 5.21–5.18, dd), and four H-1 signals (δ 5.14, 4.95, 4.81, 4.80). In addition, the chemical shifts of the anomeric carbons of **16** revealed by its ^{13}C NMR spectrum were at 107.71, 107.08, 99.57, 99.27 ppm, confirming the structure of **16**.

In summary, an efficient synthesis of the immunodominant tetrasaccharide **16** present in the cell-wall galactomannan of *A. fumigatus* was achieved for the first time. This would promote the studies on fundamental biochemical properties and biological functions about this oligosaccharide, and consequently promote the treatment of diseases caused by this pathogen.

3. Experimental

3.1. General methods

Melting points were determined with a 'Mel-Temp' apparatus. Optical rotations were measured at 25 °C in the stated solvent. ^{1}H NMR (400 Hz) and ^{13}C NMR (100 Hz) spectra were recorded in $CDCl_3$ solutions at room temperature unless otherwise specified. Chemical shift (δ) values are given in ppm; coupling constants (J) are in Hertz. Mass spectra were recorded on an Autospec mass spectrometer using the electrospray-ionization



Scheme 3. Reagents and conditions: (a) TMSOTf (cat), CH_2Cl_2, 4 Å MS powder, −20 °C to rt, 1.5 h; (b) satd aq NH_3–MeOH, rt, 24 h.

technique. Thin-layer chromatography (TLC) was performed on silica gel HF_{254} with detection by charring with 30% (v/v) H_2SO_4 in MeOH or in some cases by a UV detector. Column chromatography was conducted by elution of a column (10 × 240 mm, 18 × 300 mm, 35 × 400 mm) of silica gel (100–200 mesh) with EtOAc–petroleum ether (60–90 °C) as the eluent. Solutions were concentrated at <60 °C under diminished pressure. Dry solvents were distilled over CaH_2 and stored over molecular sieves.

3.2. Preparation of 1,2-*O*-isopropylidene-3,6-di-*O*-benzoyl-α-D-galactofuranose (4)

To a solution of **3** (5.0 g, 15.4 mmol) in dry pyridine (30 mL) was added dropwise a solution of benzoyl chloride (2.0 mL, 17 mmol) in dry CH_2Cl_2 (10 mL) at −5 °C over 30 min. Then the mixture was warmed to 0 °C and stirred until TLC (4:1 petroleum ether–EtOAc) indicated that the reaction was complete. Then the mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with 1 N HCl and satd aq $NaHCO_3$. The solution was concentrated under vacuum. The residue was purified by flash chromatography (2:1 petroleum ether–EtOAc) to give **4** as a syrup (4.8 g, 90 %): $[\alpha]_D$ −10.2 (*c* 0.8, $CHCl_3$) (lit.[18] −15.2 (*c* 1.00)).[18]

3.3. 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)

A solution of **4** (4.2 g, 9.9 mmol) and **5** (8.0 g, 10.8 mmol) in dry CH_2Cl_2 (60 mL) was stirred with activated 4 Å molecular sieves (4 g) at room temperature under an atmosphere of nitrogen for 20 min. Then the reaction mixture was cooled to −20 °C, and TMSOTf (20 μL, 0.1 mmol) was added. After 30 min, the mixture was allowed to rise to room temperature. The reaction mixture was stirred for further 1 h, at the end of which time TLC (2.5:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was neutralized with Et_3N and filtered, and the filtrate was concentrated. The resultant residue was subjected to column chromatography with 2.5:1 petroleum ether–EtOAc as eluent to afford the disaccharide **6** (11.2 g, 89%): $[\alpha]_D$ +12.7 (*c* 0.5, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.10–7.22 (m, 30H, 6Ph*H*), 6.21 (m, 1H, H-5), 5.93 (d, 1H, J = 4.1 Hz, H-1), 5.78 (s, 1H, H-3), 5.69 (s, 1H, H-1′), 5.63 (d, 1H, J = 4.1 Hz, H-3′), 5.56 (s, 1H, H-2′), 5.11 (m, 1H, H-4), 4.88 (dd, 1H, J = 4.4, 11.6 Hz, H-6′), 4.76–4.69 (m, 3H), 4.68 (m, 1H), 4.59 (dd, 1H, J = 5.2, 9.2 Hz, H-6), 4.37 (m, 1H), 1.68 (s, 3H, C*H*$_3$), 1.28 (s, 3H, C*H*$_3$). Anal. Calcd for $C_{57}H_{50}O_{17}\cdot 0.5H_2O$: C, 67.38; H, 5.06. Found: C, 67.40; H, 5.05.

3.4. 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)

To a solution of **8** (5.9 g, 5.64 mmol) and benzylamine (22.6 mmol) in a solution of THF (80 mL) was kept in the dark at room temperature for 24 h. At the end of this time, TLC (3:1 petroleum ether–EtOAc) showed that the reaction was complete, and the solution was concentrated. The resultant residue without purification was

dissolved in dry CH_2Cl_2 (50 mL), and then trichloroacetonitrile (2.6 mL, 12.4 mmol) and anhydrous K_2CO_3 (3 g, 21.8 mmol) were added. The reaction mixture was stirred at room temperature for 12 h, and the solid material was filtered off. Concentration of the filtrate, followed by purification on a silica gel column with 3:1 petroleum ether–EtOAc as eluent, gave the disaccharide donor **10** (4.6 g, 90%): $[\alpha]_D$ +15.2 (*c* 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.63 (s, 1H, N*H*), 8.06–7.24 (m, 30H, 6Ph*H*), 6.45 (s, 1H, H-1′), 6.10 (m, 1H, H-5′), 5.75 (d, J = 4.2 Hz, H-3′), 5.72 (s, 1H, H-1), 5.62 (d, 2H, J = 4.2 Hz, H-3), 5.60 (s, 1H, H-2′), 5.48 (s, 1H, H-2), 5.00 (m, 1H, H-4′), 4.81 (dd, 1H, J = 3.6, 12 Hz, H-6′), 4.73 (dd, 1H, J = 3.6, 12 Hz, H-6), 4.71–4.64 (m, 3H), 4.61–4.59 (m, 1H), 1.99 (s, 3H, C*H*$_3$CO). Anal. Calcd for $C_{58}H_{48}Cl_3NO_{18}$: C, 60.40; H, 4.19. Found: C, 60.12; H, 4.22.

3.5. Allyl 2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (12)

A solution of **11** (4.3 g, 6.4 mmol) in dry CH_2Cl_2 (40 mL) was stirred with activated 4 Å molecular sieves (1.5 g) at room temperature under an atmosphere of nitrogen for 20 min. After the reaction mixture was cooled to −20 °C, allyl alcohol (0.77 mL, 12.8 mmol) and TMSOTf (20 μL, 0.1 mmol) was added. After 30 min, the mixture was allowed to rise to room temperature, and the reaction mixture was stirred until TLC (2.5:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was neutralized with Et_3N and filtered, and the filtrate was concentrated under vacuum. Then the resultant residue was directly treated with 1% CH_3COCl in CH_2Cl_2–CH_3OH at room temperature. The solution was stirred at room temperature until TLC (2:1 petroleum ether–EtOAc) showed that the starting material disappeared. The solution was neutralized with Et_3N and concentrated under vacuum. The residue was purified by flash chromatography with 2:1 petroleum ether–EtOAc as eluent to afford **12** (2.9 g, 92%): $[\alpha]_D$ −108.2 (*c* 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12–7.24 (m, 15H, 3Ph*H*), 6.02 (dd, 1H, J = 3.6, 10.1 Hz, H-3), 5.96 (m, 1H, CH_2=C*H*–CH_2O), 5.86 (dd, 1H, J = 10.1 Hz, H-4), 5.70 (dd, 1H, J = 1.2, 3.0 Hz, H-2), 5.35 (dd, 2H, J = 10.4, 36.3 Hz, C*H*$_2$=CH–CH_2O), 5.17 (d, 1H, J = 1.2 Hz, H-1), 4.32 (dd, 1H, J = 5.2, 12.9 Hz, H-6a), 4.15 (dd, 1H, J = 5.2, 12.9 Hz, H-6), 4.10 (m, 1H, H-5), 3.85 (m, 1H, CH_2=CH–C*H*$_2O$), 3.78 (dd, 1H, J = 3.7, 12.8 Hz, CH_2=CH–C*H*$_2O$). Anal. Calcd for $C_{30}H_{28}O_9$: C, 67.66; H, 5.30. Found: C, 67.35; H, 5.23.

3.6. Allyl 2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (14)

A solution of **11** (3.6 g, 5.25 mmol) and **12** (2.8 g, 5.25 mmol) in dry CH_2Cl_2 (50 mL) was stirred with activated 4 Å molecular sieves (2 g) at room temperature under an atmosphere of nitrogen for 20 min. Then the reaction mixture was cooled to −20 °C, and TMSOTf (12 μL, 0.06 mmol) was added. After 30 min, the mixture was allowed to rise to room temperature. The reaction mixture was stirred for further 1 h, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was neutralized with Et_3N and filtered, and the filtrate was concentrated under vacuum. Then the resultant residue was directly treated with 1% CH_3COCl in CH_2Cl_2–CH_3OH at room temperature. The solution was stirred at room temperature until TLC (1.5:1 petroleum ether–EtOAc) showed that the starting material disappeared. The solution was neutralized with Et_3N and concentrated under vacuum. The resultant residue was subjected to column chromatography with 1.5:1 petroleum ether–EtOAc as eluent to afford the disaccharide acceptor **14** (4.6 g, 90%): $[\alpha]_D$ −72.3 (*c* 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17–7.25 (m, 30H, 6Ph*H*), 6.06 (m, 1H, CH_2=C*H*–CH_2O), 6.04 (dd, 1H, J = 3.2, 10 Hz, H-3′), 6.02 (dd, 1H, J = 10.1 Hz, H-4′), 5.94 (dd, 1H, J = 3.2, 10 Hz, H-3), 5.80 (dd, 1H, J = 10.1 Hz, H-4), 5.74 (m, 2H, H-2, H-2′), 5.48 (dd, 1H, J = 1.2, 17.2 Hz, C*H*$_2$=CH–CH_2O), 5.33 (dd, 1H, J = 1.2, 17.2 Hz, C*H*$_2$=CH–CH_2O), 5.16 (d, 1H, J = 1.6 Hz, H-1′), 5.14 (d, 1H, J = 1.6 Hz, H-1), 4.44–4.21 (m, 2H, CH_2=CH–C*H*$_2O$), 4.40–4.39 (m, 1H, H-5′), 4.08–4.00 (m, 2H, H-6′, H-5), 3.76 (dd, 1H, J = 1.9, 10.9 Hz, H-6′), 3.61 (dd, 1H, J = 1.9, 10.9 Hz, H-6), 3.53 (dd, 1H, J = 4.3, 12.9 Hz, H-6). Anal. Calcd for $C_{57}H_{50}O_{17}$: C, 67.99; H, 5.00. Found: C, 67.76; H, 4.83.

3.7. Allyl 2,3,5,6-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)

A solution of **10** (2.9 g, 2.52 mmol) and **14** (2.5 g, 2.52 mmol) in dry CH_2Cl_2 (50 mL) was stirred with activated 4 Å molecular sieves (2 g) at room temperature under an atmosphere of nitrogen for 20 min. Then the reaction mixture was cooled to −20 °C, and TMSOTf (15 μL, 0.07 mmol) was added. After 30 min, the mixture was allowed to rise to room temperature. The reaction mixture was stirred for further 1 h, at the end of which time TLC (2.5:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was neutralized with Et_3N and filtered, and the filtrate was concentrated. The resultant residue was subjected to the column chromatography with 2.5:1 petroleum ether–EtOAc as eluent to afford the tetrasaccharide **15** (4.9 g, 92%): $[\alpha]_D$ −64.5 (*c* 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00–7.23 (m, 60H, 12Ph*H*), 6.13 (dd, 1H, J = 10.2 Hz, H-4′), 6.12–6.04 (m, 2H, H-5‴,

CH_2=C*H*CH_2O), 6.07 (dd, 1H, J = 4, 10.2 Hz, H-3′), 6.01 (dd, 1H, J = 10.1 Hz, H-4), 5.98 (dd, 1H, J = 3.4, 10.1 Hz, H-3), 5.78 (m, 2H, H-2, H-2′), 5.74 (s, 1H, H-1‴), 5.66 (d, J = 5.2 Hz, H-3‴), 5.65 (d, J = 4.2 Hz, H-3′), 5.63 (s, 1H, H-2‴), 5.50 (dd, J = 1.3, 11.0 Hz, C*H*$_2$=CHCH$_2$O), 5.34 (dd, J = 1.3, 11.0 Hz, C*H*$_2$=CHCH$_2$O), 5.33 (s, 1H, H-2′), 5.22 (s, 1H, H-1′), 5.20 (d, 1H, J = 1.3 Hz, H-1′), 5.18 (d, 1H, J = 1.4 Hz, H-1), 5.00 (dd, J = 4.2 Hz, H-4), 4.80 (dd, 1H, J = 3.7, 12.0 Hz, H-6‴), 4.67 (dd, 1H, J = 7.8, 12.2 Hz, H-6′), 4.63–4.59 (m, 2H), 4.89–4.42 (m, 3H), 4.41–4.26 (m, 2H, CH_2=CHC*H*$_2$O), 4.24–4.21 (m, 1H, H-5), 4.13 (dd, 1H, J = 5.0, 11.1 Hz, H-6), 3.79–3.76 (m, 2H, 2 H-6′), 3.64 (dd, 1H, J = 5.0, 11.1 Hz, H-6), 1.86 (s, 3H, C*H*$_3$CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.53, 166.04, 166.01, 165.72, 165.66, 165.61, 165.47, 165.39, 165.21, 165.16, 118.46, 106.45, 105.94, 97.69, 96.85, 82.30, 82.13, 81.55, 81.39, 77.88, 77.38, 77.06, 76.99, 76.74, 73.09, 70.65, 70.59, 70.39, 70.29, 70.05, 69.76, 68.86, 66.97, 66.63, 65.93, 65.09, 63.91, 20.48. Anal. Calcd for $C_{113}H_{96}O_{34}$: C, 67.93; H, 4.85. Found: C, 67.72; H, 4.80.

3.8. Allyl β-D-galactofuranosyl-(1→5)-β-D-galactofuranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (16)

Compound **15** (210 mg, 0.105 mmol) was dissolved in satd aq NH_3–MeOH (50 mL). After 24 h at room temperature, the reaction solution was concentrated, and the residue was purified on a BioGel P2 column with MeOH in water as eluent to afford **16** (78 mg, 87%): $[\alpha]_D$ −7.9 (*c* 0.5, H_2O); ^{1}H NMR (400 MHz, D_2O): δ 5.92–5.84 (m, 1H), 5.30–5.25 (dd, 1H, J = 17.1 Hz), 5.21–5.18 (dd, 1H, J = 10.4 Hz), 5.14 (d, 1H, J = 2.0 Hz, 1H-1), 4.95 (s, 1H, 1H-1), 4.81 (d, 1H, J = 1.6 Hz, H-1), 4.80 (s, 1H, H-1); ^{13}C NMR (100 MHz, D_2O): δ 133.35, 118.49, 107.71, 107.08, 99.57, 99.27, 82.62, 81.80, 81.26, 81.01, 76.68, 76.53, 75.87, 71.66, 70.91, 70.85, 70.55, 70.06, 69.94, 68.34, 66.62, 65.74, 62.83, 61.08. Anal. Calcd for $C_{27}H_{46}O_{21}$: C, 45.89; H, 6.56. Found: C, 45.66; H, 6.40.

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