Synthesis of a hexasaccharide, the repeating unit of O-deacetylated GXM of C. neoformans serotype A

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Received 25 March 2003; accepted 3 June 2003

Abstract

β-D-Glc-(1→2)-β-D-Manp-(1→3)-[β-D-Xylp-(1→2)]-β-D-Manp-(1→3)[-β-D-Xylp-(1→2)]-β-D-Manp, the repeating unit of the exopolysaccharide from Cryptococcus neoformans serovar A, was synthesized as its allyl glycoside. Thus, 3-O-selective acetylation of allyl 4,6-O-benzylidene-α-d-mannopyranoside afforded 2, and subsequent glycosylation of 2 with 2,3,4-tri-O-benzoyl-D-xylopyranosyl trichloroacetimidate furnished the β-(1→2)-linked disaccharide 4. Debenzylation followed by benzoylation gave allyl 2,3,4-tri-O-benzoyl-β-D-xylopyranosyl-(1→2)-3-O-acetyl-4,6-di-O-benzoyl-α-d-mannopyranoside (5), and selective 3-O-deacetylation gave the disaccharide acceptor 6. Coupling of 6 with 2-O-acetyl-3,4,6-tri-O-benzoyl-α-d-mannopyranosyl trichloroacetimidate yielded the trisaccharide 8, and subsequent deallylation and trichloroacetimidation gave 3,4,6-tri-O-benzoyl-β-D-xylopyranosyl-(1→2)-[2-O-acetyl-3,4,6-tri-O-benzoyl-α-d-mannopyranosyl-(1→3)]-4,6-di-O-benzoyl-α-d-mannopyranosyl trichloroacetimidate (9). Condensation of the trisaccharide donor 9 with the disaccharide acceptor 6 gave the pentasaccharide 10 whose 2-O-deacetylation gave the acceptor 11. Glycosylation of 11 with methyl 2,3,4-tri-O-acetyl-α-d-glucopyranosyluronate trichloroacetimidate and subsequent deprotection gave the target hexasaccharide.

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Keywords: Mannose; Xylose; Glucouronic acid

1. Introduction

Cryptococcus neoformans, a primary cause of opportunistic infections associated with AIDS, produces glucuronoxylomannan (GXM) as the major capsule component.1,2 There are four major serotypes for GXM designated A–D (Fig. 1). All four serotypes are composed of a linear α-(1→3)-linked mannosyl backbone with β-glucopyranosyluronic acid, β-xylopyranosyl, and 6-O-acetyl substituents.4

GXM is antipathogenic and poorly immunogenic, and acapsular strains have significantly reduced virulence.5 In vitro, GXM inhibits leukocyte migration.6

enhances HIV infection in human lymphocytes7 and promotes L-selectin shedding from neutrophils.8 Because of its prominent virulence, synthesis of the GXM repeating unit will be very helpful for the research on the structure–activity relationships of oligosaccharides.

The synthesis of tri- and tetrasaccharide fragments corresponding to structures in the capsular polysaccharides of C. neoformans has been reported,9 and the synthesis of a pentasaccharide, the repeating unit of the polysaccharide in C. neoformans serovar D, has appeared.10 We have reported in a preliminary communication11 the successful synthesis of the hexasaccharide repeating unit of O-deacetylated GXM of C. neoformans serotype A with 4,6-O-isopropylidened mannose derivatives as the acceptors based on our previous studies on the syntheses of cell-wall components. We now report an alternative highly efficient and conver-
gent synthesis of the hexasaccharide repeating unit of O-deacetylated GXM of *C. neoformans* serotype A.

2. Results and discussion

As outlined in Scheme 1, selective acetylation of allyl 4,6-O-benzylidene-α-D-mannopyranoside (1)\(^1\) with acetyl chloride in pyridine went smoothly giving allyl 3-O-acetyl-4,6-O-isopropylidene-α-D-mannopyranoside (2) in high yield (91%), and no acetyl migration was found. The \(^1\)H NMR spectrum of 2 showed a characteristic downfield doublet of doublets at \(\delta\) 5.36 ppm with \(J_{2,3} = 3.2\) and \(J_{3,4} = 10.0\) Hz for H-3, confirming the 3-OH regioselectivity. This selective 3-O-acetylation was the key step in the synthesis since, on the one hand, 2 contained a free hydroxyl group at the position where the xylosyl residue should be attached, and on the other hand, the coupling of 2 with perbenzoylated xylosyl trichloroacetimidate (3)\(^3\) gave a product that was readily transformed to either an acceptor or a donor for further reactions. Thus, condensation of 2 with 3

![Scheme 1](image-url)
afforded (1→2)-linked disaccharide 4 in good yield (85%). Because of the presence of a benzylidene group, selective deacetylation of 4 with 2% CH₃COCl–MeOH was not successful since a benzylidene group is more sensitive to acidic conditions than either acetyl or benzoyl groups. Thus, debenzyldenation of 4 was carried out with 90% trifluoroacetic acid (TFA), and subsequent benzylation gave disaccharide 5 (87%). Selective deacetylation of 5 with 2% CH₃COCl–MeOH gave the disaccharide acceptor 6 (73%).

Due to the unstability of methyl ester linkage of a glucuronate residue under either basic or acidic conditions, assembly of the glucuronate unit was arranged at the end of the reaction series. So, 2-O-acetyl-3,4,6-tri-O-benzoyl-α-D-mannopyranosyl trichloroacetimidate (7) was used as the donor to couple 6, and trisaccharide 8 was obtained in satisfactory yield (88%). Subsequent 1-O-deallylation with PdCl₂ and activation with trichloroacetonitrite in the presence of potassium carbonate gave the trisaccharide donor 9 (84%). Again, glycosylation of 6 with 9 readily afforded the pentasaccharide 10 (83%). Selective deacetylation of 10 with 2% CH₃COCl–MeOH was accompanied by some decomposition, perhaps caused by breaking of the xylosyl linkage, giving the pentasaccharide acceptor 11 in 65% yield. Coupling of 11 with methyl 2,3,4-tri-O-acetyl-α-D-glucopyranosyluronate trichloroacetimidate (12) went smoothly affording the protected hexasaccharide in good yield (86%). The 1H and 13C NMR spectra of 13 showed a methyl signal (δ 3.69 ppm), 13 benzoyl C=O signals (δ 165.9, 165.9, 165.8, 165.4, 165.3, 165.3, 165.2, 165.1, 165.0, 164.8, 164.6, 164.6 ppm), four C=O signals (δ 169.0, 168.5, 168.5, 168.3 ppm), and six anomeric C signals (100.9, J₁,₁,₁ = 176 Hz, Manp; 100.1, J₁C₁,₁₁ = 162 Hz, GluAp; 99.9, J₁C₁,₁₁ = 164 Hz, Xylp; 99.5 J₁C₁,₁₁ = 164 Hz, Xylp; 96.7, J₁C₁,₁₁ = 173 Hz, Manp; 96.2, J₁C₁,₁₁ = 175 Hz, Manp). It is noted that although it was difficult to assign the anomeric configuration of the two Xylp just from the relatively small J₁₁,₁₂ values (6.1 and 5.2 Hz, respectively), the chemical shifts of H-1 (δ 4.93 and 4.74, respectively) and the J₁C₁,₁₁ values clearly indicate the β linkages. Deprotection of 13 was carried out in a saturated solution of ammonia in methanol for 36 h, then water (2 equiv) was added to cleave the methyl ester. After standing at room temperature for 5 h, the reaction mixture was concentrated and purified on a Bio-Gel P2 column (elucent-water), affording the target hexasaccharide 14 as a foamy solid.

In summary, an alternative highly efficient and convergent synthesis of the hexasaccharide repeat unit of O-deacetylated GXM of C. neoformans serotype A was achieved. The strategy presented here also provides a route to the synthesis of more complex repeating units of GXM of C. neoformans serotypes B and C.

3. Experimental

3.1. General methods

Melting points were determined with a ‘Mel-Temp’ apparatus. Optical rotations were determined with a Perkin–Elmer model 241-MC automatic polarimeter for solutions in a 1-dm jacketed cell. 1H and 13C NMR spectra were recorded with Varian XL-400 and Varian XL-200 spectrometers, for solutions in CDCl₃ or in D₂O as indicated. Chemical shifts are expressed in ppm downfield from the Me₄Si absorption. Mass spectra were recorded with a VG PLATFORM mass spectrometer using the electrospray-ionization (ESI) mode. Thin-layer chromatography (TLC) was performed on silica gel HF with detection by charring with 30% (v/v) H₂SO₄ in CH₃OH or by UV detection. Column chromatography was conducted by elution of a column (8 × 100, 16 × 240, 18 × 300, 35 × 400 mm) of silica gel (100–200 mesh) with EtOAc–petroleum ether (bp 60–90 °C) as the eluent. Analytical LC was performed with a Gilson HPLC consisting of a pump (model 306), stainless steel column packed with silica gel (Spherisorb SiO₂, 10 × 300 or 4.6 × 250 mm), a differential refractometer (132-RI Detector), and a UV–Vis detector (model 118). EtOAc–petroleum ether (bp 60–90 °C) was used as the eluent at a flow rate of 1–4 mL/min. Solutions were concentrated at a temperature <60 °C under diminished pressure.

3.2. Allyl 3-O-acetyl-4,6-O-benzylidene-α-D-mannopyranoside (2)

Compound 1 (3.08 g, 10 mmol) was dissolved in dry CH₂Cl₂ (40 mL) containing pyridine (8.1 mL, 100 mmol), then under N₂ protection and stirring, a solution of acetyl chloride (0.8 mL, 11 mmol) in anhyd CH₂Cl₂ (10 mL) was added dropwise within 30 min at 0 °C. The reaction mixture was slowly raised to room temperature (rt) and stirred for 2 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was slowly diluted with CH₂Cl₂ (100 mL), washed with water, 1 N HCl, and dried over Na₂SO₄. The solution was concentrated, and purification of the residue by column chromatography on a silica gel column (3:1 petroleum ether–EtOAc) gave compound 2 (3.17 g, 90.6%) as a syrup: [α]D + 42.0° (c 1.0, CHCl₃); 1H NMR (400 MHz, CDCl₃): δ 7.46–7.34 (m, 5 H, PhH₅), 5.88 (m, 1 H, CH₂=CHCH₂O), 5.55 (s, 1 H, PhCHO₂), 5.36 (dd, 1 H, J₂,₃ 3.2, J₃,₄ 10.0 Hz, H-3), 5.20 (m, 1 H, CH₂=CHCHO₂), 5.23 (m, 1 H, CH₂=CHCH₂O), 4.89 (d, 1 H, J₁,₂ 1.5 Hz, H-1), 4.28 (dd, 1 H, J 4.8, 10.6 Hz, H-6a), 4.19 (m, 1 H, CH₂=CHCHO₂), 4.15 (dd, 1 H, J₃,₂ 1.5, J₂,₃ 3.2 Hz, H-2), 4.09 (dd, 1 H, J 10.0, 10.6 Hz, H-6b), 4.02 (m, 1 H, CH₂=CHCH₂O), 3.99 (ddd, 1 H, J 4.8, 10.0, 10.6 Hz,
3.3. Allyl 2,3,4-tri-O-benzoyl-β-D-xylopyranosyl-(1 → 2)-3-O-acetyl-4,6-O-benzylidene-α-D-mannopyranoside (4)

To a cooled solution (–10 °C) of 2 (3.50 g, 10.0 mmol) and 3 (6.70 g, 11.0 mmol) in anhyd CH₂Cl₂ (50 mL) was added TMSOTf (18 μL, 0.1 mmol). The mixture was stirred for 2 h and then quenched with Et₃N (four drops). The solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (3:1 petroleum ether–EtOAc) to give disaccharide 4 (6.76 g, 85.1%) as a foamy solid: [α]D = –38.9° (c 1.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 8.13–7.33 (m, 20 H, 4 PhH), 5.85 (m, 1 H, CH₂=CH₂CHO), 5.71 (dd, 1 H, J₂,₃ = J₃,₄ = 5.8 Hz, H-3’), 5.37 (dd, 1 H, J₁,₂ = 4.2, J₂,₃ = 5.8 Hz, H-2’), 5.36 (dd, 1 H, J₂,₃ = 3.5, J₃,₄ = 10.5 Hz, H-3), 5.28 (m, 1 H, H-4’), 5.25 (m, 1 H, CH₂=CH₂CHO), 5.24 (s, 1 H, PhCHO), 5.20 (m, 1 H, CH₂=CH₂CHO), 4.91 (dd, 1 H, J₁,₂ = 4.2 Hz, H-1’), 4.80 (dd, 1 H, J₁,₂ = 1.5 Hz, H-1), 4.59 (dd, 1 H, J₃,₅ = 12.4 Hz, H-6a), 4.30 (dd, 1 H, J₁,₂ = 1.5, J₂,₃ = 3.5 Hz, H-2), 4.10 (m, 1 H, CH₂=CH₂CHO), 4.06 (dd, 1 H, J₄,₅a = 4.8, J₅a,b = 10.3 Hz, H-5’a), 3.98 (dd, 1 H, J₄,₅b = J₅a,b = 10.3 Hz, H-5’), 3.92 (m, 1 H, CH₂=CH₂CHO), 3.88–3.76 (m, 2 H, H-5, H-6b), 3.49 (dd, 1 H, J₃,₄b = J₅a,b = 10.5 Hz, H-4’), 2.14 (s, 3 H, C₂H₅CO). Anal. Calcd for C₄₉H₄₄O₁₅: C, 67.42; H, 5.08. Found: C, 66.95; H, 5.07. Found: C, 67.09; H, 5.31.

3.5. Allyl 2,3,4-tri-O-benzoyl-β-D-xylopyranosyl-(1 → 2)-4,6-di-O-benzylidene-α-D-mannopyranoside (6)

To a solution of 5 (4.57 g, 5 mmol) in anhyd CH₂Cl₂ (10 mL) was added anhyd MeOH (40 mL). Acetylated chloride (1.0 mL) was then added to the reaction mixture at 0 °C. The solution was stoppered in a flask and stirred at rt until TLC (3:1 petroleum ether–EtOAc) showed that the starting material had disappeared. The solution was neutralized with Et₃N, then concentrated to dryness. The residue was passed through a short silica gel column to give 6 (3.20 g, 73.4%) as a foamy solid: [α]D = –7.5° (c 1.0, CHCl₃); ¹H NMR (400 MHz, CDCI₃): δ 8.05–7.30 (m, 25 H, 5 PhH), 5.88 (m, 1 H, CH₂=CH₂CHO), 5.77 (dd, 1 H, J₂,₃ = J₃,₄ = 6.9 Hz, H-3’), 5.46 (dd, 1 H, J₃,₄ = J₄,₅ = 9.8 Hz, H-4’), 5.37 (dd, 1 H, J₁,₂ = 5.2, J₂,₃ = 6.9 Hz, H-2’), 5.29 (m, 1 H, H-4’), 5.19–5.13 (m, 2 H, CH₂=CH₂CHO), 4.97 (d, 1 H, J₁,₂ = 5.2 Hz, H-1’), 4.94 (dd, 1 H, J₂,₃ = 0.8 Hz, H-1’), 4.61 (dd, 1 H, J₁,₂ = 4.0, 12.3 Hz, H-6a), 4.34 (dd, 1 H, J₄,₅b = J₅a,b = 11.9 Hz, H-5’a), 4.24 (dd, 1 H, J₄,₅b = 5.8, J₅a,b = 11.9 Hz, H-5’b), 4.18–4.12 (m, 4 H), 3.95–3.84 (m, 2 H), 1.60 (bs, 1 H, OH). Anal. Calcd for C₄₉H₄₄O₁₅: C, 67.42; H, 5.08. Found: C, 67.19; H, 5.01.

3.6. Allyl 2-O-acetyl-3,4,6-tri-O-benzoyl-β-D-xylopyranosyl-(1 → 3)[2,3,4-tri-O-benzoyl-β-D-xylopyranosyl-(1 → 2)]-4,6-di-O-benzylidene-α-D-mannopyranoside (8)

Compound 6 (2.62 g, 3.0 mmol) and 2-O-acetyl-3,4,6-tri-O-benzoyl-β-D-xylopyranosyl trichloroacetimide (7) (2.23 g, 3.3 mmol) were dried together under high vacuum for 2 h, then dissolved in anhyd CH₂Cl₂ (50 mL). TMSOTf (18 μL, 0.1 mmol) was added dropwise at –10 °C with nitrogen protection. The reaction mixture was stirred for 3 h, during which time the mixture was allowed to gradually warm to ambient temperature. The reaction mixture was neutralized with Et₃N and concentrated to dryness. Purification of the residue by column chromatography (1:1 petroleum ether–EtOAc) gave 8 (3.66 g, 87.9%) as a syrup: [α]D = –45.6° (c 1.0, CHCl₃); ¹H NMR (400 MHz, CDCI₃): δ 8.12–7.13 (m, 40 H, 8 PhH), 5.89 (dd, 1 H, J₂,₃ = 3.3, J₃,₄ = 10.0 Hz, H-3 of Manp), 5.77 (dd, 1 H, J₁,₄ = J₄,₅ = 9.9 Hz, H-4 of Manp), 5.76 (dd, 1 H, J₃,₄ = J₄,₅ = 10.0 Hz, H-4 of Manp), 5.71 (m, 1 H, CH₂=CH₂CHO), 5.65 (dd, 1 H, J₂,₃ = J₃,₄ = 4.6 Hz, H-3 of Xylp), 5.47 (m, 1 H, H-4 of Xylp), 5.38 (dd, 1 H, J₁,₂ = J₂,₃ = 4.6 Hz, H-2 of CH₂=CH₂O), 4.97 (d, 1 H, J₁,₂ = 3.7 Hz, H-1’), 4.94 (dd, 1 H, J₁,₂ = 1.4 Hz, H-1), 4.61 (dd, 1 H, J₃,₄ = 12.5 Hz, H-6a), 4.34 (dd, 1 H, J₄,₅b = J₅a,b = 11.8 Hz, H-5’a), 4.26 (dd, 1 H, J₁,₂ = 1.4, J₂,₃ = 3.3 Hz, H-2), 4.22–4.16 (m, 2 H), 4.08–3.97 (m, 2 H), 3.78 (dd, 1 H, J = 4.8, 12.5 Hz, H-6b), 1.97 (s, 3 H, CH₃CO). Anal. Calcd for C₅₁H₄₀O₁₆: C, 66.95; H, 5.07. Found: C, 67.09; H, 5.31.
3.7. 2-O-Acetyl-3,4,6-tri-O-benzoyl-α-D-mannopyranosyl-(1 → 2)-[2,3,4-tri-O-benzoyl-β-D-xlylopyranosyl-(1 → 2)]-4,6-di-O-benzoyl-α-D-mannopyranosyl trichloroacetimidate (9)

To a solution of 8 (2.08 g, 1.5 mmol) in 90% AcOH (15 mL) containing AcONa (0.44 g, 4.5 mmol) was added PdCl₂ (81 mg, 0.75 mmol), and the mixture was stirred for 12 h, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was diluted with CH₂Cl₂ (150 mL), washed with water and satd aq sodium bicarbonate. The organic layer was concentrated, and the residue was passed through a short silica gel column with 1:1 petroleum ether–EtOAc as the eluent to give crude 2-O-acetyl-3,4,6-tri-O-benzoyl-α-D-mannopyranosyl-(1 → 3)-[2,3,4-tri-O-benzoyl-β-D-xlylopyranosyl-(1 → 2)]-4,6-di-O-benzoyl-α-D-mannopyranosyl as a syrup. After drying under high vacuum for 2 h, the solid was dissolved in CH₂Cl₂ (10 mL), and CCl₄CN (0.5 mL, 5 mmol) and DBU (40 μL, 0.3 mmol) were added. The reaction mixture was stirred for 2 h, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. Concentration of the reaction mixture, followed by purification of the crude product on a silica gel column with 2:1 petroleum ether–EtOAc as the eluent, furnished the trisaccharide donor 9 (1.88 g, 83.9%) as a foamy solid: [α]D₂₅¹ − 42.¹ (c 0.8, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ 8.68 (s, 1 H, NHCCl), 8.06–7.21 (m, 40 H, 8 Ph), 6.43 (s, 1 H, H-1 of Manp), 5.89 (dd, 1 H, J₂₃ = 3.1, J₃₄ = 9.8 Hz, H-3 of Manp), 5.86 (dd, 1 H, J₃₄ = J₄₅ = 9.8 Hz, H-4 of Manp), 5.76 (dd, 1 H, J₃₄ = J₄₅ = 9.7 Hz, H-4 of Manp), 5.74 (dd, 1 H, J₁₂ = J₂₃ = 4.4 Hz, H-3 of Xylp), 5.48 (dd, 1 H, J₁₂ = 0.8, J₂₃ = 3.0 Hz, H-2 of Manp), 5.45 (dd, 1 H, J₁₂ = 4.9, J₂₃ = 4.4 Hz, H-2 of Xylp), 5.27 (m, 1 H, H-4 of Xylp), 5.26 (d, 1 H, J₁₂ = 4.9 Hz, H-1 of Xylp), 5.20 (d, 1 H, J₁₂ = 1.1 Hz, H-1 of Manp), 1.94 (s, 3 H, CH₃CO). Anal. Caled for C₇₇H₄₆Cl₃NO₂₄: C 61.91; H 4.32. Found: C 61.65; H 4.58.

3.8. Allyl 2-O-acetyl-3,4,6-tri-O-benzoyl-α-D-mannopyranosyl-(1 → 3)-[2,3,4-tri-O-benzoyl-β-D-xlylopyranosyl-(1 → 2)]-4,6-di-O-benzoyl-α-D-mannopyranosyl (10)

Compound 9 (1.64 g, 1.1 mmol) and 6 (873 mg, 1.0 mmol) were coupled under the same conditions as those used for the preparation of 8 from 7 and 6, giving 10 (1.82 g, 82.7%) as a foamy solid: [α]D₂₅¹ − 20.³ (c 1.0, CHCl₃); ¹H NMR (CDCl₃, 400 MHz): δ 8.10–7.22 (m, 65 H, 13 PhH), 5.95 (dd, 1 H, J₃₄ = J₄₅ = 10.0 Hz, H-4 of Manp), 5.79–5.70 (m, 3 H), 5.62 (dd, 1 H, J₃₄ = J₄₅ = 10.0 Hz, H-4 of Manp), 5.57 (dd, 1 H, J₃₄ = J₄₅ = 9.9 Hz, H-4 of Manp), 5.49–5.43 (m, 3 H), 5.33–5.28 (m, 2 H), 5.20 (m, 1 H, OCH₂CH₂CH₂), 5.17 (d, 1 H, J₁₂ = 5.3 Hz, H-1 of Xylp), 5.17 (m, 1 H, OCH₂CH₂CH₂), 5.13 (s, 1 H, H-1 of Manp), 5.00 (s, 1 H, H-1 of Manp), 4.78 (d, 1 H, J₁₂ = 4.9 Hz, H-1 of Xylp), 4.71 (s, 1 H, H-1 of Manp), 1.90 (s, 3 H, COCH₃); ¹³C NMR (100 MHz, CDCl₃): 168.9 (COCH₃), 166.0, 165.9, 165.8, 165.4, 165.3, 165.2, 165.1, 165.0, 164.8, 164.7, 164.6, 164.5 (13 C, 13 COPh), 118.2 (OCH₂CH₂CH₂), 100.0, 99.7, 99.7, 98.9, 96.7 (5 C, 5 C-1), 20.3 (COCH₃). Anal. Caled for C₁₂₃H₁₁₀O₃₈: C 67.56; H 4.85. Found: C, 67.75; H, 5.03.

3.9. Allyl 3,4,6-tri-O-benzoyl-α-D-mannopyranosyl-(1 → 3)-[2,3,4-tri-O-benzoyl-β-D-xlylopyranosyl-(1 → 2)]-4,6-di-O-benzoyl-α-D-mannopyranoside (11)

To a solution of 10 (1.10 g, 0.05 mmol) in anhyd CH₂Cl₂ (10 mL) was added anhyd MeOH (40 mL). Acetyl chloride (1.0 mL) was added to the reaction mixture at 0 °C. The solution was stoppered in a flask and stirred at rt until TLC (3:1 petroleum ether–EtOAc) showed that the starting material had disappeared. The solution was neutralized with Et₃N, then concentrated to dryness. The residue was passed through a short silica gel column to give 11 (700 mg, 64.7%) as a foamy solid: [α]D₂₀⁻¹ − 19.⁴ (c 1.0, CHCl₃); ¹H NMR (CDCl₃, 400 MHz): δ 8.12–7.22 (m, 65 H, 13 PhH), 6.02 (dd, 1 H, J₃₄ = J₄₅ = 9.9 Hz, H-4 of Manp), 5.77 (m, 1 H, OCH₂CH₂CH₂), 5.76 (dd, 1 H, J₁₂ = J₂₃ = 6.9 Hz, H-3 of Xylp), 5.72 (dd, 1 H, J₁₂ = J₂₃ = 6.1 Hz, H-3 of Xylp), 5.66 (dd, 1 H, J₂₃ = 3.2, J₃₄ = 9.9 Hz, H-3 of Manp), 5.61 (dd, 1 H, J₃₄ = J₄₅ = 9.8 Hz, H-4 of Manp), 5.57 (dd, 1 H, J₃₄ = J₄₅ = 9.9 Hz, H-4 of Manp), 5.47 (dd, 1 H, J₁₂ = J₂₃ = 6.1 Hz, H-2 of Xylp), 5.43–5.37 (m, 2 H, 2 H-4 of Xylp), 5.35–5.29 (m, 2 H, H-3 of Xylp; OCH₂CH₂CH₂), 5.23 (s, 1 H, H-1 of}
Manp), 5.16 (s, 1 H, OCH₂CH=CH₂), 5.12 (s, 1 H, H-1 of Manp), 4.82 (d, 1 H, J₁₂,1 5.1 Hz, H-1 of Xylp), 4.70 (d, 1 H, J₁₂,2 1.3 Hz, H-1 of Manp), 4.61 (d, 1 H, J₁₁,2 5.4 Hz, H-1 of Xylp); Anal. Calc'd for C₁₁₂H₁₉₀O₃₅: C, 67.77; H, 4.85. Found: C, 67.93; H, 4.64.

3.10. Allyl (methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronic acid)-1→2)-4,6-di-O-benzoyl-α-D-mannopyranosyl(1→3)-2,3,4-tri-O-benzoyl-β-D-xlylopyranosyl(1→2)-4,6-di-O-benzoyl-α-D-mannopyranoside (13)

To a cooled solution (0 °C) of 11 (648 mg, 0.3 mmol) and methyl 2,3,4-tri-O-acetyl-α-D-glucopyranosylurionate trichloroacetimide (12) (240 mg, 0.5 mmol) in anhyd CH₂Cl₂ (10 mL) was added TMSOTf (8 μL, 0.05 mmol). The mixture was stirred at this temperature for 2 h, and then quenched with Et₃N (one drop). The solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (1:1 petroleum ether–EtOAc) to give hexasaccharide 13 (638 mg, 85.9%); [α]D = -26.9° (c 0.5, CHCl₃); ¹H NMR (CDCl₃, 400 MHz): δ 8.08–7.23 (m, 65 H, 13 PhH), 6.00 (dd, 1 H, J₃₄,4 = 10.0 Hz, H-4 of Manp), 5.79 (m, 1 H, OCH₂CH=CH₂), 5.71 (dd, 1 H, J₂₃, ⊧ = 6.9 Hz, H-3 of Xylp), 5.15 (s, 1 H, H-1 of Manp), 4.97 (d, 1 H, J₁₁,2 0.7 Hz, H-1 of Manp), 4.93 (d, 1 H, J₁₂,1 6.1 Hz, H-1 of Xylp), 4.74 (d, 1 H, J₁₂,2 5.2 Hz, H-1 of Xylp), 4.78 (s, 1 H, H-1 of Manp), 4.07 (d, 1 H, J₁₂,3 6.8 Hz, H-1 of GluAp), 3.69 (s, 3 H, COOCH₃), 1.96, 1.93, 1.27 (3 s, 9 H, 3 COCH₃); ¹³C NMR (100 MHz, CDCl₃): 169.0, 168.5, 168.5, 168.3 (C, 3 COCH₃), COOME), 165.9, 165.9, 165.8, 165.4, 165.3, 165.3, 165.2, 165.1, 165.0, 164.8, 164.6, 164.6 (13 C, 13 COP), 118.3 (OCH₂CH=CH₂), 100.9 (C-1, J₁₁₁,11 = 176 Hz, Manp), 100.1 (C-1, J₁₁₁,11 = 162 Hz, GluAp), 99.9 (C-1, J₁₁₁,11 = 164 Hz, Xylp), 99.5 (C-1, J₁₁₁,11 = 164 Hz, Xylp), 96.7 (C-1, J₁₁₁,11 = 173 Hz, Manp), 96.2 (C-1, J₁₁₁,11 = 175 Hz, Manp), 51.9 (COOCH₃), 20.5, 20.4 (4 C, 4 COCH₃). Anal. Calc'd for C₁₃₅H₂₁₂O₄₆: C, 65.42; H, 4.88. Found: C, 65.61; H, 4.79.

3.11. Allyl (β-D-glucopyranosyluronic acid)-(1→2)-α-D-mannopyranosyl(1→3)-β-D-xlylopyranosyl(1→2)-α-D-mannopyranoside, ammonium salt (14)

Hexasaccharide 13 (490 mg, 0.2 mmol) was dissolved in a satd methanolic ammonia (50 mL). After 36 h at rt, water (1.0 mL) was added to the mixture to cleave the methyl ester. After stirring at rt for 5 h, the reaction mixture was concentrated and purified on a Bio-Gel P2 column (eluent:water), affording the target hexasaccharide 14 (126 mg, 63.0%) as a foamy solid; [α]D = +72.8° (c 0.5, H₂O); ¹H NMR (D₂O, 400 MHz): δ 5.87 (m, 1 H, OCH₂CH=CH₂), 5.31–5.17 (m, 2 H, OCH₂CH=CH₂), 5.11 (s, 1 H, H-1 of Manp), 4.92 (s, 1 H, H-1 of Manp), 4.70 (d, 1 H, J₁₁,2 7.8 Hz, H-1 of GluAp), 4.65 (d, 1 H, J₁₁,2 2.0 Hz, H-1 of Manp), 4.42 (d, 1 H, J₁₂,1 8.0 Hz, H-1 of Xylp), 4.40 (d, 1 H, J₁₂,2 8.2 Hz, H-1 of Xylp); ¹³C NMR (100 MHz, D₂O): δ 173.6 (CH₂=CHCH₂O), 118.5 (CH₂=CHCH₂O), 103.6, 103.5, 102.7, 100.5, 100.5, 97.5 (6 C-1), 79.0, 78.5, 78.4, 78.3, 78.3, 76.9, 76.2, 75.8, 75.8, 73.9, 73.4, 73.2, 72.9, 72.9, 70.7, 70.3, 69.5, 69.5, 68.3, 67.2, 67.0, 66.7, 66.5, 65.4, 65.3, 65.1, 60.7, 60.6. MALDI-TOF MS Calc'd for the ammonium salt of 14, C₇₇H₆₃N₅O₄₉: M+ 1001.9 [M]. Found: 1001.6 (M-NH₄⁺ + Na⁺).

Acknowledgements

This work was supported by The Chinese Academy of Sciences (KZCX3-J-08) and by The National Natural Science Foundation of China (Projects 30070185 and 39907864).

References