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Carbohydrate Research 331 (2001) 431–437

CARBOHYDRATE RESEARCH

Facile synthesis of a comb-like mannohexaose: a trimer of the disaccharide repeating unit of the cell-wall mannans of *Aphanoascus mephitatus* and related species

Linsen Heng, Jun Ning, Fanzuo Kong*

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

Received 27 November 2000; accepted 16 February 2001

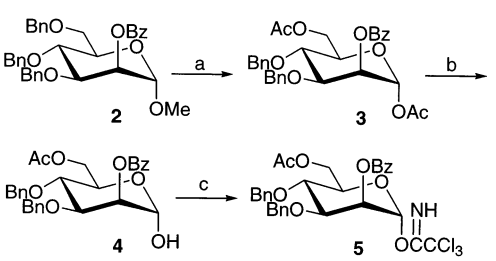
Abstract

An efficient method for the preparation of a comb-like mannohexaose having α-(1→6) and α-(1→2) linkages has been described using 6-*O*-acetyl-2-*O*-benzoyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate as the key glycosyl donor in an 'inverse Schmidt' procedure.

Keywords: Comb-like oligosaccharide; Synthesis; Mannose

1. Introduction

Mannans are constituents of many fungal cell-walls. They are considered to have important physiological functions. Besides physical protection of the cell-wall, they may serve to anchor such enzymes as invertase and acid phosphatase to the cell-wall,[1] and they may participate in cell–cell recognition as well as in the adhesion of the microorganism to host cells.



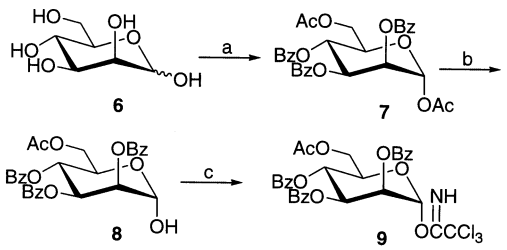
Scheme 1. Reagents and conditions: (a) 6:1:0.05 (v/v) AcOH–Ac_2O–H_2SO_4, rt, 16 h, 90%; (b) anhyd Et_2O satd with dry NH_3, rt, 24 h, 97%; (c) CCl_3CN (1.2 equiv), DBU (0.2 equiv), 0 °C, 2 h, 92%.

In 1993 Jiménez-Barbero and co-workers[2] investigated the structures of cell-wall mannans isolated from *Aphanoascus mephitatus*, *Aphanoascus fulvescens*, *Aphanoascus verrucosus*, and *Aphanoascus reticulisporus*, and found that they invariably consist of a relatively simple comb-like structure of a disaccharide repeating block {→6}-[α-Man *p*-(1→2)]-α-Man *p*-(1→). It would be helpful to synthesize its fragments for the elucidation of the biological functions of the cell-wall polysaccharides. In a previous communication,[3] we described an efficient method to construct a mannooligosaccharide having α-(1→6) and α-(1→2) linkages. In this paper, as a part of our continuing effort to develop new synthetic approaches directed toward oligosaccharide fragments of fungal cell-wall mannans, we report the synthesis of a comb-like haxasac-

* Corresponding author. Tel.: +86-10-62936613; fax: +86-10-62923563.

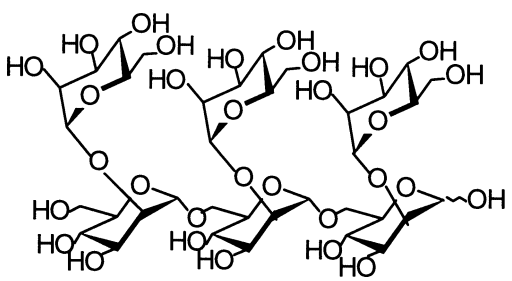
E-mail address: fzkong@mail.rcees.ac.cn (F. Kong).

0008-6215/01/$ - see front matter
PII: S0008-6215(01)00059-3



Scheme 2. Reagents and conditions: (a) (i) TrCl (1.2 equiv), pyridine, 50 °C, 32 h; (ii) BzCl (4.4 equiv), 40 °C, 24 h; (iii) 1:1:0.6:0.175 (v/v) CH_2Cl_2–HOAc–Ac_2O–H_2SO_4, rt, 20 h, 71.3%. (b) THF, $BnNH_2$ (4.2 equiv), rt, 24 h, 86.2%. (c) CCl_3CN (2.8 equiv), DBU (0.22 equiv), 0 °C, 2 h, 88.1%.

charide **1**, which is a trimer of the disaccharide repeating unit of mannans isolated from *A. mephitatus* and related species.



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2. Results and discussion

First we synthesized the synthons **5** and **9**. Thus, methyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**2**) was prepared in 65% overall yield according to the literature[4] using D-mannose as the starting material. Selective acetolysis of **2** using 6:1:0.05 HOAc–Ac_2O–H_2SO_4[5] gave the corresponding diacetate **3** (Scheme 1). Compound **4** was obtained in nearly quantitative yield by amonolysis of **3** in anhydrous ether saturated with dry NH_3 according to Lemieux and Howard.[6] Subsequent reaction of **4** with CCl_3CN–DBU in CH_2Cl_2 afforded the key glycosyl donor **5**. Tritylation of the mannose (**6**),[7] followed by benzoylation in one pot, gave the 1,2,3,4-tetra-*O*-benzoyl-6-*O*-trityl-D-mannopyranose (Scheme 2), selective acetolysis of which using 1:1:0.6:0.175 CH_2Cl_2–AcOH–Ac_2O–H_2SO_4 afforded the corresponding 1,6-diacetate **7** in 85% yield (three steps). The diacetate **7** was selectively deacetylated in high yield at the anomeric position with benzylamine in THF to the corresponding 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-D-mannopyranose (**8**). Subsequent reaction of **8** with CCl_3CN–DBU in CH_2Cl_2 afforded another glycosyl donor **9**.

With the synthons **5** and **9** in hand, construction of the target compound was readily carried out. As shown in Scheme 3, allyl 6-*O*-acetyl-2-*O*-benzoyl-3,4-di-*O*-benzyl-α-D-mannopyranoside (**10**) was prepared by the Helferich reaction[8] using **3** as the glycosyl donor and allyl alcohol as the acceptor. Selective removal of the acetyl group of **10** using a 0.5% methanolic HCl quantitatively gave the glycosyl acceptor **11**. The disaccharide **12** was prepared using the 'inverse Schmidt' strategy.[9] Thus the glycosyl acceptor **11** and the catalyst TMSOTf were mixed first in dry CH_2Cl_2, and after stirring for 15 min, the glycosyl donor **5** was added dropwise within 30 min. Selective removal of the acetyl group of **12** gave the glycosyl acceptor **13**, 'inverse Schmidt' coupling of which with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl trichloro-acetimidate[10] afforded the trisaccharide **14** in 85% yield. The ^1H NMR spectrum of **14** showed one acetyl signal (δ 2.14), one allyl methine signal (5.87) and three downfield H-2 signals (δ 5.78, 5.67, and 5.52), characteristic of the structure of the trisaccharide **14**. Removal of the acetyl and benzoyl groups of **14** with NH_3 in MeOH quantitatively gave the glycosyl acceptor **15** having free 2-OH, 2′-OH, and 2″-OH groups. The fully protected comb-like hexasaccharide **16** was smoothly obtained by coupling of **15** with 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**9**). The ^1H and ^{13}C NMR data of **16** contained structural information, i.e., three acetyl signals (δ 1.91, 1.93, and 1.98) and one allyl methine signal (δ 5.93), and six anomeric carbon signals (δ 99.6, 99.4, 99.3, 99.2. 99.1, and 98.3). The use of 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**9**) as the glycosyl donor afforded three hydroxy groups at C-6 for potential further transformation. Deprotection of **16** gave the title mannohexaose **1**.

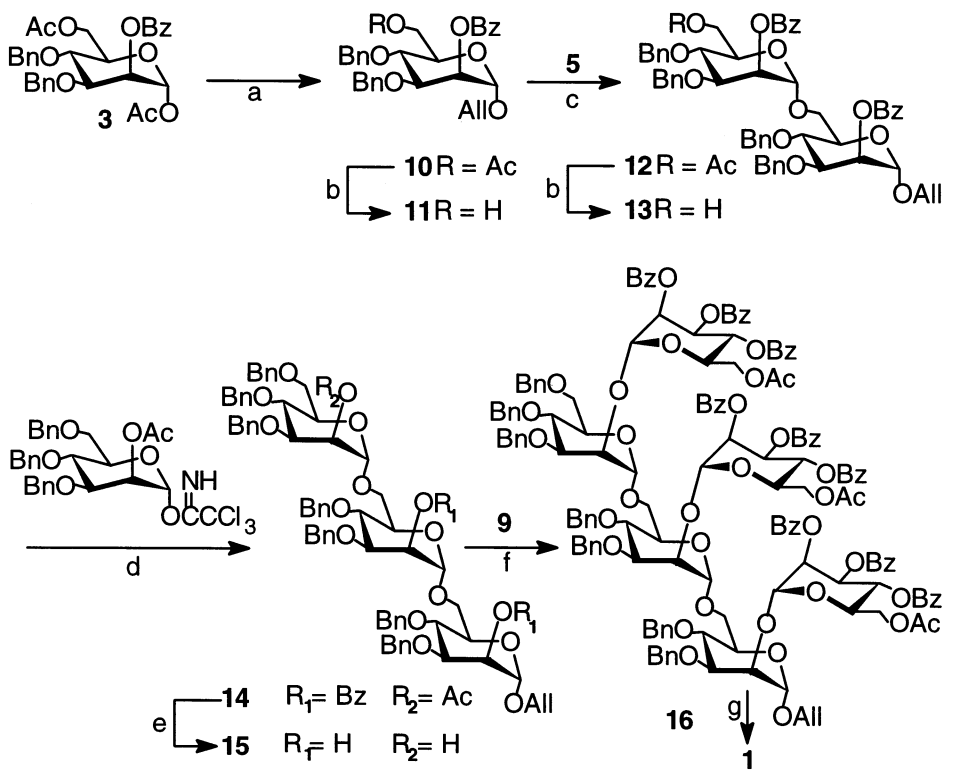
In summary, we have successfully developed a highly efficient strategy for the preparation

of a comb-like mannohexaose containing α-(1→6) and α-(1→2) linkages. It is anticipated that octa- and higher comb-like oligosaccharides can be synthesized by this strategy.

3. Experimental

General methods.—Optical rotations were determined at 25 °C with a Perkin–Elmer model 241-MC automatic polarimeter. Melting points were determined with a 'Mel-Temp' apparatus. ^{1}H NMR spectra were recorded in $CDCl_3$ with Bruker ARX 400 spectrometers. Chemical shifts are given in parts per million (ppm) downfield from internal Me_4Si. Mass spectra were recorded with a JMS-D300S mass spectrometer using a sample probe. Thin-layer chromatography (TLC) was performed on Silica Gel HF_{254} (E. Merck) with detection by charring with 30% (v/v) H_2SO_4 in MeOH or in some cases by UV detection. Column chromatography utilized silica gel with EtOAc–petroleum ether (60–90 °C) as the eluent. Solutions were concentrated at < 60 °C under diminished pressure.

*1,6-Di-*O*-acetyl-2-*O*-benzoyl-3,4-di-*O*-benzyl-α-*D*-mannopyranose* (**3**).—A solution of compound **2**[4] (6 g, 10.55 mmol) in AcOH (36 mL) and Ac_2O (6 mL) cooled to 0 °C in an ice bath, and H_2SO_4 (0.3 mL) was added dropwise over 10 min. The ice bath was removed, and the reaction was allowed to continue for 16 h at rt, then poured into ice water (100 mL). Stirring was continued for an additional 15 min, at the end of which time it was extracted with $CHCl_3$ (3 × 30 mL). The combined $CHCl_3$ extracts were washed with 10% aq $NaHCO_3$ (3 × 60 mL), dried over Na_2SO_4, and concentrated to a syrup. The crude product was then subjected to column chromatography with 3:1 petroleum ether–EtOAc as the eluent. Compound **3** was obtained as crystals (5.21 g, 90%,); mp 102–104 °C; $[\alpha]_D$ + 3.2° (*c* 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.20–7.20 (m, 15 H, 3 Ph*H*), 6.20 (d, 1 H, $J_{1,2}$ 2.1 Hz, H-1), 5.62 (dd, 1 H, $J_{1,2}$ 2.1, $J_{2,3}$ 2.9 Hz, H-2), 4.89, 4.60 (ABq, 2 H, *J* 10.7



Scheme 3. Reagents and conditions: (a) allyl alcohol (1.9 equiv), TMSOTf (0.24 equiv), CH_2Cl_2, rt, 1 h, 88%. (b) MeOH–0.5% HCl, rt, 14–18 h, 95.6–98.1%. (c) **5** (1.4 equiv), CH_2Cl_2, TMSOTf (0.1 equiv), rt, 3 h, 86.5%. (d) 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (1.5 equiv), CH_2Cl_2, TMSOTf (0.1 equiv), rt, 3 h, 85.7%. (e) MeOH satd with dry NH_3, rt, 72 h, 97%. (f) **9** (6.0 equiv), CH_2Cl_2, TMSOTf (0.2 equiv), rt, 3 h, 75.8%. (g) (i) $PdCl_2$, MeOH, rt, 8 h; (ii) MeOH satd with dry NH_3; (iii) H_2, $Pd(OH)_2$/C 20%, MeOH, rt, 24 h, 61.8%.

Hz, PhCH_2), 4.83, 4.61 (ABq, 2 H, J 11.9 Hz, PhCH_2), 4.34 (m, 2 H, H-6, 6′), 4.11 (m, 1 H, H-5), 3.96 (m, 2 H, H-3,4), 2.10, 2.06 (2 s, 6 H, 2 COCH_3). Anal. Calcd for $C_{31}H_{32}O_9$: C, 67.87; H, 5.88. Found: C, 67.95; H, 5.79.

6-O-*Acetyl*-*2*-O-*benzoyl*-*3,4*-*di*-O-*benzyl*-α-D-*mannopyranose* (**4**).—A solution of compound **3** (3 g, 5.47 mmol) in anhyd ether (100 mL) saturated with dry NH_3 was stirred for 24 h at rt. TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The solution was concentrated, and purification of the crude product by flash-column chromatography on silica gel (2:1 petroleum ether–EtOAc) gave compound **4** as crystals (2.69 g, 97%,); mp 108–110 °C; $[\alpha]_D$ +6.9° (*c* 2.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.07–7.23 (m, 15 H, 3 Ph*H*), 5.60 (dd, 1 H, $J_{1,2}$ 1.8, $J_{3,2}$ 2.8 Hz, H-2), 5.31 (d, 1 H, $J_{2,1}$ 1.8 Hz, H-1), 4.89, 4.59 (ABq, 2 H, J 10.9 Hz, PhCH_2), 4.79, 4.58 (ABq, 2 H, J 11.3 Hz, PhCH_2), 4.38 (dd, 1 H, $J_{6b,6a}$ 11.9, $J_{5,6a}$ 2.2 Hz, H-6a), 4.27 (dd, 1 H, $J_{6a,6b}$ 11.9, $J_{5,6b}$ 4.4 Hz, H-6b), 4.18 (dd, 1 H, $J_{2,3}$ 2.8, $J_{4,3}$ 9.1 Hz, H-3), 4.12 (m, 1 H, $J_{6a,5}$ 2.2, $J_{6b,5}$ 4.4, $J_{4,5}$ 9.1 Hz, H-5), 3.90 (t, 1 H, $J_{3,4} = J_{5,4} = 9.1$ Hz, H-4), 2.04 (s, 3 H, $COCH_3$). Anal. Calcd for $C_{29}H_{30}O_8$: C, 68.76; H, 5.97. Found: C, 68.61; H, 5.92.

6-O-*Acetyl*-*2*-O-*benzoyl*-*3,4*-*di*-O-*benzyl*-α-D-*mannopyranosyl trichloroacetimidate* (**5**).—A mixture of **4** (4.5 g, 8.88 mmol), CCl_3CN (1.05 mL, 10.5 mmol), and 1,8-diazabicyclo[5,4,0]undecene (DBU) (0.27 mL, 1.8 mmol) in dry CH_2Cl_2 (30 mL) was stirred under N_2 for 2 h at 0 °C and then concentrated in vacuo. The residue was purified by flash chromatography (3:1 petroleum ether–EtOAc) to give **5** (5.32 g, 92%) as crystals; mp 91–93 °C; $[\alpha]_D$ +16.9° (*c* 2.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.72 (s, 1 H, OC(N*H*)CCl_3), 8.10–7.10 (m, 15 H, 3 Ph*H*), 6.36 (d, 1 H, $J_{1,2}$ 2.1 Hz, H-1), 5.73 (t, 1 H, $J_{1,2} = J_{2,3} = 2.1$ Hz, H-2), 4.90, 4.64 (ABq, 2 H, J 10.8 Hz, PhCH_2), 4.82, 4.61 (ABq, 2 H, J 11.3 Hz, PhCH_2), 4.35 (dd, 1 H, $J_{6,6'}$ 11.9, $J_{5,6}$ 1.7 Hz, H-6), 4.30 (dd, 1 H, $J_{6,6'}$ 11.9, $J_{5,6'}$ 3.4 Hz, H-6′), 4.17 (dd, 1 H, $J_{2,3}$ 2.1, $J_{3,4}$ 8.7 Hz, H-3), 4.1 (t, 1 H, $J_{3,4} = J_{4,5} = 8.7$ Hz, H-4), 4.02 (m, 1 H, $J_{5,6}$ 1.7, $J_{5,6'}$ 3.4, $J_{4,5}$ 8.7 Hz, H-5), 2.03 (s, 3 H, $COCH_3$). Anal. Calcd for $C_{31}H_{30}Cl_3NO_8$: C, 57.20; H, 4.65. Found: C, 57.42; H, 4.60.

1,6-*Di*-O-*acetyl*-*2,3,4*-*tri*-O-*benzoyl*-α-D-*mannopyranose* (**7**).—A solution of mannose **6** (15 g, 83.3 mmol) and chlorotriphenylmethane (28 g, 100.5 mmol) in pyridine (60 mL) was stirred at 50 °C for 32 h until TLC (4:1 EtOAc–MeOH) indicated that the reaction was complete. The reaction mixture was cooled to 0 °C, and BzCl (46.5 mL, 370 mmol) was then added over 30 min keeping the reaction temperature under 40 °C. After 24 h, water (300 mL) was added to the reaction mixture and stirring was continued for 30 min. The aq solution was extracted with CH_2Cl_2 (3 × 100 mL), and the extract was washed sequentially with HCl (1 N) and satd aq $NaHCO_3$ solution, dried (Na_2SO_4), and concentrated. Without further separation, the residue was redissolved with CH_2Cl_2 (50 mL), Ac_2O (50 mL) and AcOH (30 mL), and cooled to 10 °C in an ice bath. Concd H_2SO_4 (8.8 mL) was added dropwise over 20 min, then held for 20 h at rt. The reaction solution was poured into ice water (400 mL), stirred for an additional 15 min, and extracted with $CHCl_3$ (3 × 100 mL). Combined extracts were washed with 10% aq $NaHCO_3$ (3 × 60 mL), dried, and concentrated to give a syrup. Column chromatography with 4:1 petroleum ether–EtOAc as the eluent afforded compound **7** as a syrup (34.2 g, 71.3%); $[\alpha]_D$ +9.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11–7.28 (m, 15 H, 3 Ph*H*), 6.36 (d, 1 H, $J_{1,2}$ 2.0 Hz, H–1), 6.01 (t, 1 H, $J_{3,4} = J_{4,5} = 10.2$ Hz, H-4), 5.88 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 10.2 Hz, H-3), 5.70 (dd, 1 H, $J_{1,2}$ 2.0, $J_{2,3}$ 3.2 Hz, H-2), 4.34 (m, 2 H, H-5, 6a), 4.27 (dd, 1 H, $J_{6a,6b}$ 13.4, $J_{5,6b}$ 4.2 Hz, H-6b), 2.28, 2.08 (2 s, 6 H, 2COCH_3). Anal. Calcd for $C_{31}H_{28}O_{11}$: C, 64.58; H, 4.89. Found: C, 64.67; H, 4.94.

6-O-*Acetyl*-*2,3,4*-*tri*-O-*benzoyl*-α-D-*mannopyranose* (**8**).—A solution of compound **7** (5 g, 8.67 mmol) and benzylamine (4 mL, 36.6 mmol) in anhyd THF (30 mL) was stirred at rt for 24 h until TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The solution was concentrated, and the crude product was purified by flash-column chromatography on silica gel (3:1 petroleum ether–EtOAc) to give compound **8** as a syrup

(4.0 g, 86.2%); $[\alpha]_D$ +16.8° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.11–7.27 (m, 15 H, 3 Ph*H*), 5.98 (m, 2 H, H-3, 4), 5.71 (dd, 1 H, $J_{1,2}$ 1.6, $J_{2,3}$ 2.4 Hz, H-2), 5.53 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.55 (m, 1 H, H-5), 4.33 (m, 2 H, H-6a, 6b), 2.09 (s, 3 H,COCH_3). Anal. Calcd for $C_{29}H_{26}O_{10}$: C, 65.16; H, 4.90. Found: C, 65.36; H, 4.84.

6-O-*Acetyl-2,3,4-tri*-O-*benzoyl*-α-D-*mannopyranosyl trichloroacetimidate* (**9**).—A mixture of **8** (4.0 g, 7.48 mmol), CCl_3CN (2.1 mL, 20.9 mmol), and 1,8-diazabicyclo[5,4,0]undecene (DBU) (0.25 mL, 1.67 mmol) in dry CH_2Cl_2 (25 mL) was stirred under N_2 for 2 h at 0 °C and then concentrated in vacuo. The residue was purified by flash chromatography (4:1 petroleum ether–EtOAc) to give **9** (4.47 g, 88.1%) as crystals; mp 88–91 °C; $[\alpha]_D$ +20.3° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.87 (s, 1 H, OC(N*H*)CCl_3), 8.12–7.27 (m, 15 H, 3 Ph*H*), 6.55 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1), 6.06 (t, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 5.93 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 10.0 Hz, H-3), 5.90 (dd, 1 H, $J_{1,2}$ 2.0, $J_{2,3}$ 3.2 Hz, H-2), 4.49 (m, 1 H, H-5), 4.34 (m, 2 H, H-6a, 6b), 2.07 (s, 3 H, COCH_3). Anal. Calcd for $C_{31}H_{26}Cl_3NO_{10}$: C, 54.84; H, 3.86. Found: C, 54.72; H, 3.90.

Allyl 6-O-*acetyl-2*-O-*benzoyl-3,4-di*-O-*benzyl*-α-D-*mannopyranoside* (**10**).—A solution of **3** (2.5 g, 4.56 mmol) and allyl alcohol (0.6 mL, 8.8 mmol) in dry CH_2Cl_2 (40 mL) was stirred with dried molecular sieves (4 Å, 1 g) under N_2 for 15 min, and then trimethylsilyl trifluoromethanesulfonate (0.2 mL, 1.1 mmol) was added dropwise. After 1 h, the reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with satd aq $NaHCO_3$ solution (15 mL). The organic layer was dried and concentrated in vacuo. Purification of the residue by flash chromatography (3:1 petroleum ether–EtOAc) gave **10** as a syrup (2.19 g, 88%); $[\alpha]_D$ +5.9° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.09–7.24 (m, 15 H, 3 Ph*H*), 5.88 (m, 1 H, CH_2CH=CH_2), 5.63 (dd, $J_{1,2}$ 2.0, $J_{3,2}$ 3.0 Hz, H-2), 5.28 (dd, 1 H, ^2J 1.6, $^3J_{trans}$ = 17.2 Hz, CH_2CH=CH_2), 5.21 (dd, 1 H, ^2J 1.6, $^3J_{cis}$ 10.4 Hz, CH_2CH=CH_2), 4.98 (d, $J_{1,2}$ 2.0 Hz, H-1), 4.88, 4.59 (ABq, 2 H, *J* 10.8 Hz, PhCH_2), 4.80, 4.58 (ABq, *J* 11.2 Hz, PhCH_2), 4.36 (m, 2 H, H-6a, 6b), 4.20–3.90 (m, 5 H, H-3, 4, 5, CH_2CH=CH_2), 2.07 (s, 3 H,COCH_3). Anal. Calcd for $C_{32}H_{34}O_8$: C, 70.31; H, 6.27. Found: C, 70.24; H, 6.31.

Allyl 2-O-*benzoyl-3,4-di*-O-*benzyl*-α-D-*mannopyranoside* (**11**).—A solution of **10** (1.6 g, 2.93 mmol) in MeOH (80 mL) containing 0.5% HCl was stirred at rt for 18 h, neutralized with Et_3N, and then evaporated to dryness. The residue was partitioned between water and CH_2Cl_2, and the organic layer was dried and concentrated to a syrup. Purification of the residue by flash chromatography (2:1 petroleum ether–EtOAc) gave **11** as a syrup (1.45 g, 98.1%); $[\alpha]_D$ +9.2° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.08–7.23 (m, 15 H, 3 Ph*H*), 5.88 (m, 1 H, CH_2CH=CH_2), 5.61 (dd, $J_{1,2}$ 1.8, $J_{2,3}$ 3.6 Hz, H-2), 5.28 (dd, 1 H, ^2J 1.6, $^3J_{trans}$ 17.2 Hz, CH_2CH=CH_2), 5.21 (dd, 1 H, ^2J 1.6, $^3J_{cis}$ 10.4 Hz, CH_2CH=CH_2), 4.97 (d, $J_{1,2}$ 1.8 Hz, H-1), 4.92, 4.65 (ABq, 2 H, *J* 10.8 Hz, PhCH_2), 4.78, 4.58 (ABq, 2 H, *J* 11.2 Hz, PhCH_2), 4.15–3.78 (m, 7 H, H-3, 4, 5, 6a, 6b, CH_2CH=CH_2). Anal. Calcd for $C_{30}H_{32}O_7$: C, 71.41; H, 6.39. Found: C, 71.04; H, 6.35.

Allyl 6-O-*acetyl-2*-O-*benzoyl-3,4-di*-O-*benzyl*-α-D-*mannopyranosyl-(1→6)-2*-O-*benzoyl-3,4-di*-O-*benzyl*-α-D-*mannopyranoside* (**12**).—A solution of **11** (0.37 g, 0.73 mmol) and trimethylsilyl trifluoromethanesulfonate (13 μL, 0.073 mmol) in dry CH_2Cl_2 (6 mL) was stirred with dried molecular sieves (4 Å, 0.4 g) under N_2 for 15 min, and then **5** (0.65 g, 1.0 mmol) in CH_2Cl_2 (4 mL) was added over 20 min at rt. After 3 h, the reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with satd aq $NaHCO_3$ solution (5 mL). The organic layer was dried and concentrated in vacuo. Purification of the residue by flash chromatography (2:1 petroleum ether–EtOAc) gave **12** as a syrup (0.63 g, 86.5%); $[\alpha]_D$ +19.3° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.11–7.22 (m, 30 H, 6 Ph*H*), 5.88 (m, 1 H, CH_2CH=CH_2), 5.73 (dd, 1 H, $J_{1,2}$ 1.6, $J_{2,3}$ 3.2 Hz, H-2), 5.67 (dd, 1 H, $J_{1',2'}$ 1.6, $J_{2',3'}$ 3.2 Hz, H-2′), 5.28 (dd, 1 H, ^2J 1.6, $^3J_{trans}$ 17.2 Hz, CH_2CH=CH_2), 5.21 (dd, 1 H, ^2J 1.6, $^3J_{cis}$ = 10.4 Hz, CH_2CH=CH_2), 5.08 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.97 (d, 1 H, $J_{1',2'}$ 1.6 Hz, H-1′), 4.90–4.45 (m, 8 H, 4 PhCH_2), 4.28–3.76 (m, 12 H), 1.99 (s, 3 H, $COCH_3$). Anal. Calcd for $C_{59}H_{60}O_{14}$: C, 71.36; H, 6.09. Found: C, 71.45; H, 6.11.

Allyl 2-O-benzoyl-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→6)-2-O-benzoyl-3,4-di-O-benzyl-α-D-mannopyranoside (**13**).—A solution of **12** (0.55 g, 0.55 mmol) in MeOH (25 mL) containing 0.5% HCl was stirred at rt for 14 h. The mixture was carefully neutralized with Et_3N, and then evaporated to dryness. The residue was partitioned between water and CH_2Cl_2, then the organic layer was dried and evaporated to a syrup. Purification of the residue by flash chromatography (2:1 petroleum ether–EtOAc) gave **13** as a syrup (0.50 g, 95.6%); $[\alpha]_D$ +13.0° (*c* 1.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08–7.15 (m, 30 H, 6 Ph*H*), 5.87 (m, 1 H, $CH_2CH{=}CH_2$), 5.72 (dd, 1 H, $J_{1,2}$ 1.6, $J_{2,3}$ 3.2 Hz, H-2), 5.66 (dd, 1 H, $J_{1',2'}$ 1.6, $J_{2',3'}$ 3.2 Hz, H-2′), 5.28 (dd, 1 H, ^2J 1.6, $^3J_{trans}$ 17.2 Hz, $CH_2CH{=}CH_2$), 5.20 (dd, 1 H, ^2J 1.6, $^3J_{cis}$ 10.4 Hz, $CH_2CH{=}CH_2$), 5.07 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.96 (d, 1 H, $J_{1',2'}$ 1.6 Hz, H-1′), 4.90–4.45 (m, 8 H, 4 PhC*H*$_2$), 4.14–3.71 (m, 12 H). Anal. Calcd for $C_{57}H_{58}O_{13}$: C, 71.98; H, 6.15. Found: C, 71.87; H, 6.18.

Allyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2-O-benzoyl-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→6)-2-O-benzoyl-3,4-di-O-benzyl-α-D-mannopyranoside (**14**).—A solution of **13** (0.25 g, 0.26 mmol) and trimethylsilyl trifluoromethanesulfonate (5 μL, 0.028 mmol) in dry CH_2Cl_2 (20 mL) was stirred with dried molecular sieves (4 Å, 1 g) under N_2 for 15 min, and then 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl trichloroacetimidate (**12**) (0.25 g, 0.39 mmol) in dry CH_2Cl_2 (10 mL) was added over 30 min at rt. After 3 h the reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with satd aq $NaHCO_3$ solution (8 mL). The organic layer was dried and concentrated in vacuo. Purification of the residue by column chromatography (2:1 petroleum ether–EtOAc) gave **14** as a syrup (0.32 g, 85.7%); $[\alpha]_D$ +30.7° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12–7.13 (m, 45 H, 9 PhH), 5.87 (m, 1 H, $CH_2CH{=}CH_2$), 5.78 (dd, 1 H, $J_{1,2}$ 1.6, $J_{2,3}$ 3.2 Hz, H-2), 5.67 (dd, 1 H, $J_{1',2'}$ 1.6, $J_{2',3'}$ 3.2 Hz, H-2′), 5.52 (dd, 1 H, $J_{1'',2''}$ 1.6, $J_{2'',3''}$ 3.2 Hz, H-2″), 5.28 (dd, 1 H, ^2J 1.6, $^3J_{trans}$17.2 Hz, $CH_2CH{=}CH_2$), 5.19 (dd, 1 H, ^2J 1.6, $^3J_{cis}$ 10.4 Hz, $CH_2CH{=}CH_2$), 5.07 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.98 (d, 1 H, $J_{1',2'}$ 1.6 Hz, H-1′), 4.96 (d, 1 H, $J_{1'',2''}$ 1.6 Hz, H-1″), 4.87–4.37 (m, 14 H, 7 PhCH_2), 4.14–3.61 (m, 17 H), 2.14 (s, 3 H, $COCH_3$). Anal. Calcd for $C_{86}H_{88}O_{19}$: C, 72.46; H, 6.22. Found: C, 72.53; H, 6.17.

Allyl 3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→6)-3,4-di-O-benzyl-α-D-mannopyranoside (**15**).—A solution of **14** (0.45 g, 0.32 mmol) in MeOH (50 mL) saturated with dry NH_3 was stirred at rt for 72 h, until TLC (1:1.5 petroleum ether–EtOAc) indicated that the reaction was complete. The solution was concentrated to afford compound **15** (0.36 g, 97%) as a syrup; $[\alpha]_D$ +21.4° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81–7.15 (m, 35 H, 7 Ph*H*), 5.85 (m, 1 H, $CH_2CH{=}CH_2$), 5.23 (dd, 1 H, ^2J 1.6, $^3J_{trans}$ 17.2 Hz, $CH_2CH{=}CH_2$), 5.12 (dd, 1 H, ^2J 1.6, $^3J_{cis}$ 10.4 Hz, $CH_2CH{=}CH_2$), 4.96 (1 H, $J_{1,2}$ 2.0 Hz, H-1), 4.90 (d, 1 H, $J_{1',2'}$ 2.0 Hz, H-1′), 4.85 (d, 1 H, $J_{1'',2''}$ 2.0 Hz, H-1″), 4.82–4.46 (m, 14 H, 7 PhCH_2), 4.07–3.64 (m, 20 H). Anal. Calcd for $C_{70}H_{78}O_{16}$: C, 71.53; H, 6.69. Found: C, 71.61; H, 6.73.

Allyl 6-O-acetyl-2,3,4-tri-O-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-[(6-O-acetyl-2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→2)]-(3,4-di-O-benzyl-α-D-mannopyranosyl)-(1→6)-[(6-O-acetyl-2,3,4-tri-O-benzoyl-α-D-mannopyranosyl)-(1→2)]-3,4-di-O-benzyl-α-D-mannopyranoside (**16**).—A solution of **15** (0.13 g, 0.11 mmol) and trimethylsilyl trifluoromethanesulfonate (4 μL, 0.022 mmol) in dry CH_2Cl_2 (15 mL) was stirred with dried molecular sieves (4 Å, 0.4 g) under N_2 for 15 min, and then 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**9**) (0.45 g, 0.66 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise for 30 min at rt. After 3 h the reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with satd aq $NaHCO_3$ solution (5 mL). The organic layer was dried and concentrated in vacuo. Purification of the residue by column chromatography (2:1 petroleum ether–EtOAc) gave **16** as a syrup (0.23 g, 75.8%); $[\alpha]_D$ +13.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03–7.14 (m, 80 H, 16 PhH), 6.02–5.92 (m, 10 H, 2_B, 2_D, 2_F, 3_B, 3_D, 3_F, 4_B, 4_D, 4_F,

$CH_2CH{=}CH_2$), 5.56 (d, 1 H, $J_{2,1}$ 1.1 Hz, H_B-1), 5.46 (d, 1 H, $J_{2,1}$ 1.1 Hz, H_D-1), 5.40 (d, 1 H, $J_{2,1}$ 1.1 Hz, H_F-1), 5.28 (dd, 1 H, ^2J 1.6, $^3J_{trans}$ 17.2 Hz, $CH_2CH{=}CH_2$), 5.21 (d, 1 H, $J_{2,1}$ 1.1 Hz, H_A-1), 5.18 (dd, 1 H, ^2J 1.6, $^3J_{cis}$ 10.4 Hz, $CH_2CH{=}CH_2$), 5.14 (d, 1 H, $J_{2,1}$ 1.1 Hz, H_C-1), 4.97 (d, 1 H, $J_{2,1}$ 1.1 Hz, H_E-1), 2.07, 1.99, 1.97 (3 s, 9 H, 3 $COCH_3$); ^{13}C NMR (400 MHz, $CDCI_3$): δ 170.5–170.4 (CH_3CO), 165.7–164.8 (9 PhCO), 138.8–137.7 (7 $PhCH_2$, C-1), 133.4–132.7 (7 PhCO, C-1, $CH_2{=}CH{-}CH_2$), 129.9–127.2 (aromatic C), 117.5 ($CH_2{=}CH{-}CH_2$), 99.6, 99.4, 99.3, 99.2, 99.1, 98.3 (C-1_A, 1_B, 1_C, 1_D, 1_E, 1_F), 80.2, 80.0, 79.0 (C-2_B, 2_C, 2_E), 67.9 ($CH_2{=}CH{-}CH_2$), 20.61, 20.58, 20.53 (3 CH_3CO); Anal. Calcd for $C_{157}H_{150}O_{43}$: C, 69.20; H, 5.55. Found: C, 69.14; H, 5.59.

α-D-Mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-[α-D-mannopyranosyl-(1→2)]-α-D-mannopyranosyl-(1→6)-[(α-D-mannopyranose)-(1→2)]-D-mannopyranose (**1**).—A mixture of compound **16** (0.1 g, 0.037 mmol) and $PdCl_2$ (5 mg, 0.028 mmol) in dry MeOH (10 mL) was stirred vigorously for 8 h at rt, then filtered through Celite. The filtrate was concentrated to dryness, redissolved in MeOH (20 mL) saturated with dry NH_3, and stirred at rt for 72 h. After concentrating again, the residue was treated with 20 mg of 20% $Pd(OH)_2$/C in MeOH (20 mL) (caution: extreme fire hazard!) at rt under H_2 for 24 h. The catalyst was removed by filtration through a Celite pad, and washed twice with MeOH (30 mL). The combined filtrate was concentrated to a residue which was chromatographed (MeOH) on a column of Sephadex LH-20 to afford **1** (0.022 g, 61.8%) as an amorphous mass; $[\alpha]_D$ +1.4° (*c* 0.1, MeOH); ESMS for $C_{36}H_{62}O_{31}$ (990.87): 989.5 [M − H].

Acknowledgements

This work was supported by Chinese Academy of Sciences (KJ952$J_1$510 and KIPRCEES9904) and The National Natural Science Foundation of China (59973026 and 29905004). Mr Linsen Heng is a visiting scholar from Da Xian Normal College, Department of Chemistry, Si Chuan, People's Republic of China.

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