Synthesis of a Mannose Hexasaccharide Related to the Cell Wall Mannan of *Candida dubliniensis* and *Trychophyton mentagrophytes*

Guanghui Zong, Naili Yu, Yanjun Xu, Jianjun Zhang,* Daoquan Wang, Xiaomei Liang*

Key Laboratory of Pesticide Chemistry and Application Technology, Department of Applied Chemistry, China Agricultural University, Beijing 100193, P. R. of China

Fax +86(10)62732219; E-mail: zhangjianjun@cau.edu.cn; E-mail: nmrlab@cau.edu.cn

Received 14 January 2010; revised 1 March 2010

Abstract: 2,6-Branched mannose hexasaccharide **1** related to the cell wall polysaccharide of *Candida dubliniensis* and *Trychophyton mentagrophytes* was concisely synthesized from 4-methoxyphenyl α-D-mannopyranoside (**6**) in 11 steps in 26% total yield. The efficiency of the synthesis relies on the preparation of a trisaccharide acceptor **2** with three free hydroxy groups, which could be glycosylated with trichloroacetimidate **19** to provide the protected hexasaccharide **20** in one step. Compound **2** was obtained via sequential assembly from the building blocks, 2,6-di-*O*-allyloxycarbonyl-3,4-di-*O*-benzoyl-α-D-mannopyranose trichloroacetimidate (**3**), 4-methoxyphenyl 3,4-di-*O*-benzoyl-α-D-mannopyranoside (**4**), and 4-methoxyphenyl 4-*O*-acetyl-3-*O*-benzoyl-α-D-mannopyranoside (**5**). Structures of target compound and intermediates were characterized by ^{1}H and ^{13}C NMR, MS, HRMS, and elemental analysis.

Key words: synthesis, oligosaccharide, mannose, *Candida dubliniensis*, *Trychophyton mentagrophytes*

Mannans with an α-(1→6)-linked backbone and branched at O2 with varying numbers of linear oligomannosyl side chains are characteristic structures of the cell wall polysaccharides of many clinically important fungi, including *Trychophyton mentagrophytes*, *T. rubrum*, *Candida dubliniensis* and *C. albicans*.[1] *T. mentagrophytes* and *T. rubrum* are well known anthropophilic dermatophytes which cause skin infections of the feet, body, and nails.[2] While *C. dubliniensis* and *C. albicans* are important human opportunistic pathogens, they were originally identified in oral specimens from Irish HIV-infected and AIDS patients with recurrent oral candidiasis and recently have been recovered from oral samples of HIV-seropositive pediatric patients.[3] Cell wall polysaccharides are ubiquitous structures on the cell surfaces of many bacterial species, and they are involved not only in pathogenic processes, but also in mediating resistance to host defense mechanisms; the polysaccharides have been regarded as an important factor in the virulence of many animals and plant pathogens.[4] Synthetic studies on these polysaccharides are of considerable importance in view of developing novel vaccine candidates and studying structure–bioactivity relationship of carbohydrates.[5] In a collaborative project for developing novel vaccines against *C. dubliniensis* and *T. mentagrophytes* infection, we needed to prepare grams of the 2,6-branched mannose hexasaccharide related to the cell wall mannan of *C. dubliniensis* and *T. mentagrophytes* in the form of its 4-methoxyphenyl glycoside **1** (Figure 1). The choice of the 4-methoxyphenyl glycoside **1** will enable us to conjugate the synthetic oligosaccharide with suitable aglycon when necessary.

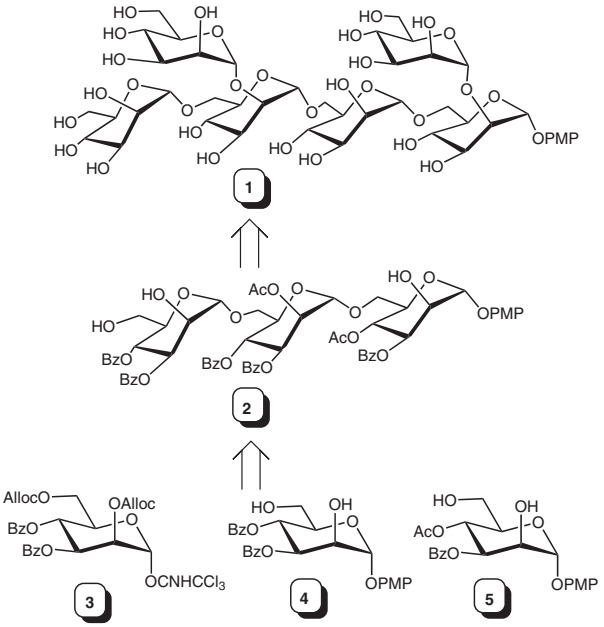
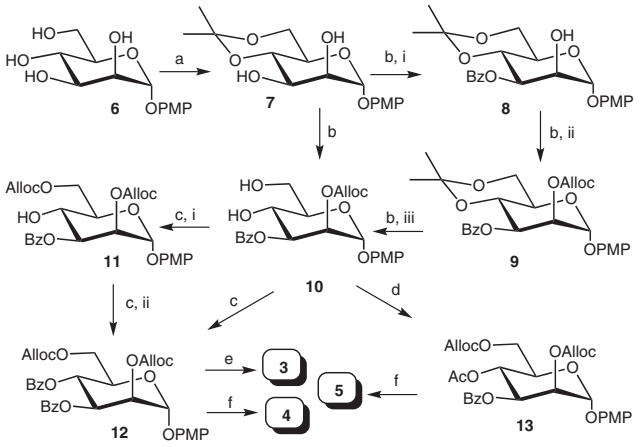


Figure 1 Structure of the target hexasaccharide **1** and the building blocks **2**–**5** used for its synthesis

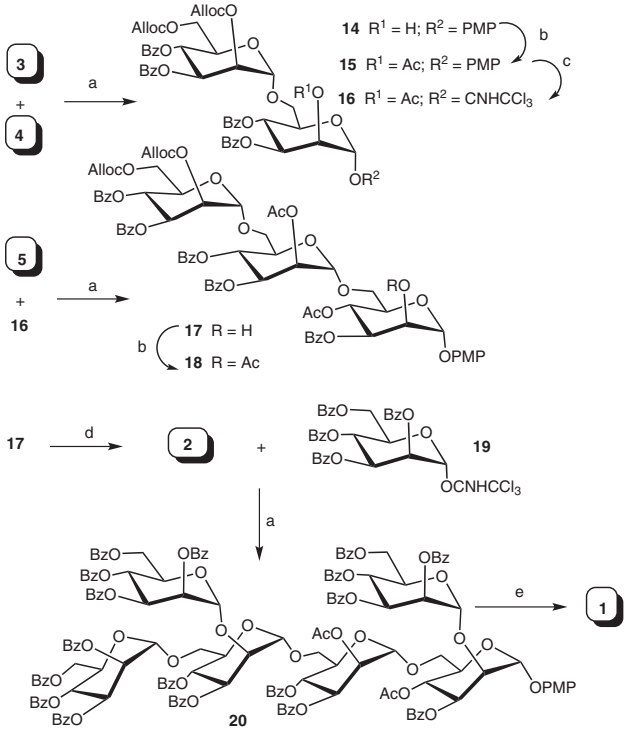
Several papers have been devoted to 2,6-branched mannose oligosaccharides synthesis,[6] however, the strategies involved in these reports were not suitable for large-scale preparation of the target hexasaccharide due to complex protection-deprotection steps and low overall yields; more efficient strategies are highly desired. In this communication, we wish to present a novel approach for the expeditious preparation of the hexasaccharide **1** (Figure 1), using trisaccharide triol acceptor **2** as the key intermediate. We envisioned that mannosylation of the three hydroxy groups in **2** will construct the hexasaccharide **1** in its protected form in only one coupling reaction and the synthetic procedure will thus be greatly simplified. The trisaccharide **2** could be produced from three suitably protected monosaccharide building blocks **3**, **4**, and **5**, using the allyloxycarbonyl function as the orthogonal protecting group.[7]

SYNTHESIS 2010, No. 10, pp 1666–1672
Advanced online publication: 26.03.2010
DOI: 10.1055/s-0029-1218719; Art ID: F01110SS



Scheme 1 Synthesis of building blocks **3**–**5**. *Reagents and conditions*: (a) 2-methoxypropene, DMF, TsOH·H_2O, 2 h, 95%; (b) (i) BzCl, py, –10 °C to r.t., 2 h, 90%; (ii) AllocCl, py, CH_2Cl_2, –10 °C to r.t., 2 h, 95%; (iii) 70% AcOH, 75 °C, 1.5 h, 91% (86% overall in one-pot from **7**); (c) (i) AllocCl, py, CH_2Cl_2, –10 °C to r.t., 2 h, 93%; (ii) BzCl, py, 0 °C to r.t., 3 h, 95% (88%, overall in one-pot from **10**); (d) (i) AllocCl, py, CH_2Cl_2, –10 °C to r.t., 2 h; (ii) Ac_2O, py, r.t., 12 h, 91%; (e) CAN, MeCN–H_2O (4:1), 30 °C, 20 min, then Cl_3CCN, DBU, CH_2Cl_2, r.t., 86% (2 steps); (f) NH_4OAc, $Pd(PPh_3)_4$, $NaBH_4$, MeOH–THF, –10 °C, 4 min, 94% (**4**); 92% (**5**).

For the synthesis of monosaccharide building blocks, 4-methoxyphenyl α-D-mannopyranoside (**6**)[8] was first transformed into 4,6-*O*-isopropylidene-α-D-mannopyranoside **7** with 2-methoxypropene in *N*,*N*-dimethylformamide in the presence of catalytic amounts of 4-toluenesulfonic acid;[9] regioselective benzoylation of **7** in pyridine gave exclusively 3-OH protected derivative **8** in 90% yield (Scheme 1). The presence of the *O*-benzoyl group at C3 was confirmed by the downfield shift of the H3 signal in the ^{1}H NMR spectrum of **8** (δ = 5.57) compared with the data for nonbenzoylated **7** (δ = 4.06); keeping the reaction temperature below –10 °C is necessary to assure the regioselectivity. Allyloxycarbonylation of **8** with allyloxycarbonyl chloride in pyridine provided **9** which was then transformed to the key intermediate **10** after removal of the isopropylidene protecting group in 70% acetic acid. It is noteworthy that these three steps could be carried out consecutively without chromatographic separation for the first two steps, making the preparation of **10** on a large scale possible in high yield (86%). Subsequently, regioselective allyloxycarbonylation of the C6-OH of compound **10** with allyloxycarbonyl chloride in pyridine provided **11** (93%), which was transformed into **12** or **13** after benzoylation or acetylation, respectively. The ^{1}H NMR spectrum of **12** or **13** showed characteristic triplets for H4 of the mannose at δ = 5.83 ($J_{3,4} = J_{4,5} = 9.8$ Hz) or 5.59 ($J_{3,4} = J_{4,5} = 9.9$ Hz), confirming the 6-selective allyloxycarbonylation of **10** (→ **11**, $\delta_{H4} = 4.60$). Importantly, **12** (or **13**) could be obtained in a one-pot reaction by adding allyloxycarbonyl chloride and benzoyl (or acetyl) chloride to the reaction mixture successively, thus greatly simplify the preparation. Finally, cleavage of the 4-methoxyphenyl group of **12** with cerium(IV) ammonium nitrate, followed by trichloroacetimidation,[10] provided the monosaccharide donor **3** in satisfactory overall yield (86%). According to our previously reported method,[7b] deallyloxycarbonylation of **12** and **13** with palladium catalyst [$Pd(PPh_3)_4$], sodium borohydride, and ammonium acetate in tetrahydrofuran–methanol gave the monosaccharide acceptors **4** and **5** in 94% and 92% yields, respectively.



Scheme 2 Synthesis of target hexasaccharide **1**. *Reagents and conditions*: (a) TMSOTf, CH_2Cl_2, – 20 °C, 0.5 h, 87% (**14**), 85% (**17**), 91% (**20**); (b) Ac_2O, py, r.t.,12 h, 95% (**15**), 92% (**18**); (c) CAN, MeCN–H_2O (4:1), 30 °C, 20 min, then Cl_3CCN, DBU, CH_2Cl_2, r.t., 77% (2 steps); (d) NH_4OAc, $Pd(PPh_3)_4$, $NaBH_4$, MeOH–THF (1:1), –10 °C, 4 min, 94%; (e) MeOH–NH_3, 120 h, then warm acetone, 83%.

With the monosaccharide synthons **3**, **4**, and **5** in hand, the hexasaccharide was prepared efficiently as outlined in Scheme 2. Thus, coupling of compounds **3** and **4** in dichloromethane at –20 °C promoted by trimethylsilyl trifluoromethanesulfonate[10] resulted in expected regio- and stereoselective products, giving exclusively α-1→6-linked mannose disaccharide **14** in 87% yield. The configuration of the glycosyl bond formed in the product was deduced from the corresponding coupling constants ($J_{1,2} = 1.5$ Hz). The regioselectivity of the coupling was confirmed by acetylation of **14** to give **15**, and the ^{1}H NMR spectrum of **15** showed a newly emerged downfield doublet of doublets at δ = 5.67 with $J_{1,2} = 1.7$ Hz and $J_{2,3} = 3.1$ Hz for H2, compared to that of **14**. Removal of the 4-methoxyphenyl group of **15** with cerium(IV) ammonium nitrate and activation with trichloroacetonitrile in the presence of DBU gave the disaccharide donor **16** in good overall yield (77%). Condensation of **16** with the acceptor **5** selectively afforded the (1→6)-linked trisaccharide **17** (85%). The regioselectivity of the reaction was

confirmed by acetylation of **17** to give **18** (92%) and the ^{1}H NMR spectrum of **18** showed a newly emerged downfield doublet of doublets at δ = 5.41 with $J_{1,2}$ = 1.8 Hz and $J_{2,3}$ = 3.1 Hz for H2, compared to that of **17**. Subsequent removal of the two Alloc groups in compound **17** with $Pd(PPh_3)_4$ provided the key triol acceptor **2** in high yield (94%). Finally, the fully protected hexasaccharide **20** was smoothly obtained by condensation of the triol **2** with 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**19**) in 91% yield. For a complete transformation of the triol **2**, excess monosaccharide donor **19** (4.5 equiv) was used in the coupling reaction. The ^{1}H NMR spectrum of **20** showed two acetyl signals (δ = 2.09, 2.01), 4-methoxyphenyl signals (7.04–6.72) and six H1 signals (δ = 5.76, 5.37, 5.35, 5.22, 5.12, and 5.01), characteristic of the structure of the hexasaccharide **20**. Deprotection of **20** in ammonia-saturated methanol gave the title 4-methoxyphenyl α-D-hexamannoside **1**, which precipitated from the solution phase as white solids when warm acetone was added to the reaction mixture at the end of deprotection.

In summary, we have successfully developed a highly efficient strategy for the preparation of 2,6-branched mannose hexasaccharide **1** related to cell-wall mannans of clinical important fungi *C. dubliniensis* and *T. mentagrophytes*. It is noteworthy that the overall yield of the entire synthesis is ~26%, and the procedure is suitable for large-scale preparation of the target hexasaccharide. Bioactivity investigations of **1** are in progress and the results will be reported in due course.

Optical rotations were determined with a Perkin-Elmer model 241-MC automatic polarimeter for soln in a 1-dm, jacketed cell. ^{1}H and ^{13}C NMR spectra were recorded with Bruker DPX300 and Bruker Avance 600 spectrometers in $CDCl_3$ or D_2O solns. Internal references: TMS (δ = 0.000 for ^{1}H), $CDCl_3$ (δ = 77.00 for ^{13}C), HOD (δ = 4.700 for ^{1}H). ^{1}H NMR signals of some compounds were assigned with the aid of COSY. Elemental analysis was performed on a Yanaco CHN Corder MF-3 automatic elemental analyzer. Matrix-assisted laser-desorption ionization mass spectra (MALDI MS) and Electrospray-ionization mass spectra (ESI-MS) were performed by the Institute of Chemistry, Chinese Academy of Sciences. TLC was performed on silica gel HF with detection by charring with 30% H_2SO_4–MeOH or by UV detection. Column chromatography used silica gel (200–300 mesh) with EtOAc–petroleum ether (PE) (bp 60–90 °C). Solns were concentrated at a temperature <60 °C under reduced pressure.

4-Methoxyphenyl 4,6-*O*-Isopropylidene-α-D-mannopyranoside (7)

To a soln of 4-methoxyphenyl α-D-mannopyranoside (**6**, 5.72 g, 20 mmol) in anhyd DMF (40 mL) was added $TsOH·H_2O$ (38 mg, 0.2 mmol) and 2-methoxypropene (2.2 mL, 22 mmol) under an N_2 atmosphere. The mixture was stirred at r.t. for 2 h; TLC (PE–EtOAc, 2:1) indicated completion. The mixture was poured onto crushed ice and the product precipitate from the mixture to give **7** (6.20 g, 95%) as white solid; R_f = 0.22 (PE–EtOAc, 2:1).

$[\alpha]_D$ +122 (*c* 1, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.99–6.94 (m, 2 H, H_{Mp}), 6.85–6.79 (m, 2 H, H_{Mp}), 5.45 (d, $J_{1,2}$ = 1.4 Hz, 1 H, H1), 4.24–3.79 (m, 5 H), 4.06 (dd, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 10.0 Hz, 1 H, H3), 3.77 (s, 3 H, OMe), 2.94, 2.89 (br s, 2 H, OH), 1.55, 1.41 (2 s, 6 H, CMe_2).

MS (ESI): *m/z* $[M + Na]^+$ calcd for $C_{16}H_{22}O_7Na$: 349.1; found: 349.5.

Anal. Calcd for $C_{16}H_{22}O_7$: C, 58.89; H, 6.79. Found: C, 58.60; H, 6.96.

4-Methoxyphenyl 3-*O*-Benzoyl-4,6-*O*-isopropylidene-α-D-mannopyranoside (8)

Compound **7** (3.26 g, 10.0 mmol) was dissolved in pyridine (20 mL), then BzCl (1.21 mL, 10.5 mmol) in pyridine (10 mL) was added dropwise to the soln at –10 °C over 30 min. The temperature was slowly allowed to rise to r.t. and the mixture was stirred for 2 h; TLC (PE–EtOAc, 2:1) indicated completion. The mixture was diluted with CH_2Cl_2 (100 mL), washed with 1 M HCl and H_2O, and dried (Na_2SO_4). The soln was concentrated and purification of the residue by column chromatography (PE–EtOAc, 4:1) gave **8** (3.86 g, 90%) as a white solid; R_f = 0.49 (PE–EtOAc, 2:1).

$[\alpha]_D$ +92 (*c* 1, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.09–7.43 (m, 5 H, H_{Bz}), 7.02–6.98 (m, 2 H, H_{Mp}), 6.86–6.82 (m, 2 H, H_{Mp}), 5.57 (dd, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 10.0 Hz, 1 H, H3), 5.46 (d, $J_{1,2}$ = 1.6 Hz, 1 H, H1), 4.45 (m, 1 H, H2), 4.36 (dd, $J_{3,4} = J_{4,5}$ = 10.0 Hz, 1 H, H4), 4.01–3.79 (m, 3 H), 3.77 (s, 3 H, OMe), 2.51 (d, *J* = 4.0 Hz, 1 H, OH), 1.53, 1.37 (2 s, 6 H, CMe_2).

MS (ESI): *m/z* $[M + Na]^+$ calcd for $C_{23}H_{26}O_8Na$: 453.2; found: 453.5.

Anal. Calcd for $C_{23}H_{26}O_8$: C, 64.18; H, 6.09. Found: C, 64.02; H, 6.33.

4-Methoxyphenyl 2-*O*-Allyloxycarbonyl-3-*O*-benzoyl-4,6-*O*-isopropylidene-α-D-mannopyranoside (9)

Compound **8** (4.30 g, 10.0 mmol) was dissolved in anhyd CH_2Cl_2 (50 mL) containing pyridine (3.2 mL, 40.0 mmol), then under an N_2 atmosphere, allyl chloroformate (1.58 mL, 15.0 mmol) in anhyd CH_2Cl_2 (10 mL) was added dropwise to the soln at –10 °C over 30 min. The temperature was slowly allowed to rise to r.t. and the mixture was stirred for 2 h; TLC (PE–EtOAc, 2:1) indicated completion. The mixture was diluted with CH_2Cl_2 (100 mL), washed with 1 M HCl and H_2O, and dried (Na_2SO_4). The soln was concentrated and purification of the residue by column chromatography (PE–EtOAc, 5:1) gave **9** (4.90 g, 95%) as a foamy solid; R_f = 0.56 (PE–EtOAc, 2:1).

$[\alpha]_D$ +46 (*c* 1, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.06–7.43 (m, 5 H, H_{Bz}), 7.03–7.00 (m, 2 H, H_{Mp}), 6.85–6.82 (m, 2 H, H_{Mp}), 5.87 (m, 1 H, CH_2=C*H*CH_2OCO), 5.70 (dd, $J_{2,3}$ = 3.6 Hz, $J_{3,4}$ = 10.2 Hz, 1 H, H3), 5.49 (d, $J_{1,2}$ = 1.6 Hz, 1 H, H1), 5.45 (dd, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 3.6 Hz, 1 H, H2), 5.36–5.21 (m, 2 H, C*H*$_2$=CHCH$_2$OCO), 4.57–4.55 (m, 2 H, CH_2=CHC*H*$_2$OCO), 4.31 (dd, $J_{3,4} = J_{4,5}$ = 10.2 Hz, 1 H, H4), 4.02 (m, 1 H, H5), 3.91–3.83 (m, 2 H, H6), 3.78 (s, 3 H, OMe), 1.57, 1.38 (2 s, 6 H, CMe_2).

MS (ESI): *m/z* $[M + Na]^+$ calcd for $C_{27}H_{30}O_{10}Na$: 537.2; found: 537.1.

Anal. Calcd for $C_{27}H_{30}O_{10}$: C, 63.03; H, 5.88. Found: C, 63.20; H, 5.91.

4-Methoxyphenyl 2-*O*-Allyloxycarbonyl-3-*O*-benzoyl-α-D-mannopyranoside (10)

Method 1: Compound **9** (5.15 g, 10.0 mmol) was dissolved in 70% AcOH (200 mL) and stirred at 75 °C for 1.5 h; TLC (PE–EtOAc, 2:1) indicated completion. The mixture was concentrated under reduced pressure and then co-evaporated with toluene (2 × 40 mL).

The residue was passed through a short silica gel column (PE–EtOAc, 3:1) to give **10** (4.31 g, 91%) as a white solid.

Method 2: Compound **7** (3.27 g, 10.0 mmol) was dissolved in pyridine (20 mL), then BzCl (1.21 mL, 10.5 mmol) was added dropwise to the soln over 30 min at –10 °C. The temperature was slowly allowed to rise to r.t. and the mixture was stirred for 2 h; TLC (PE–EtOAc, 2:1) indicated completion. The mixture was diluted with anhyd CH_2Cl_2 (50 mL) and allyl chloroformate (1.58 mL, 15.0 mmol) in anhyd CH_2Cl_2 (10 mL) was added dropwise at –10 °C over 30 min. The temperature was slowly allowed to rise to r.t. and the mixture was stirred for 2 h; TLC (PE–EtOAc, 3:1) indicated completion. The mixture was diluted with CH_2Cl_2 (100 mL) and washed with 1 M HCl and H_2O and the organic phase was concentrated to give a crude product, which was dissolved in 70% AcOH (200 mL) and stirred at 75 °C for 1.5 h. The mixture was concentrated under reduced pressure and then co-evaporated with toluene (2 × 40 mL). The residue was passed through a short silica gel column (PE–EtOAc, 3:1) to give **10** (4.10 g, 86%) as a white solid; R_f = 0.32 (PE–EtOAc, 1:1).

$[\alpha]_D$ +78 (*c* 1, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.06–7.42 (m, 5 H, H_{Bz}), 7.04–7.01 (m, 2 H, H_{Mp}), 6.85–6.82 (m, 2 H, H_{Mp}), 5.89 (m, 1 H, CH_2=C*H*CH_2OCO), 5.65 (dd, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9.8 Hz, 1 H, H3), 5.53 (d, $J_{1,2}$ = 1.8 Hz, 1 H, H1), 5.40 (dd, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.4 Hz, 1 H, H2), 5.37–5.23 (m, 2 H, C*H*$_2$=CHCH$_2$OCO), 4.65–4.52 (m, 2 H, CH_2=CHC*H*$_2$OCO), 4.30 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, 1 H, H4), 3.98–3.85 (m, 3 H, H5, H6), 3.77 (s, 3 H, OMe), 2.97, 2.21 (2 s, 2 H, 2 OH).

MS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{24}H_{26}O_{10}Na$: 497.1; found: 497.4.

Anal. Calcd for $C_{24}H_{26}O_{10}$: C, 60.76; H, 5.52. Found: C, 60.70; H, 5.41.

4-Methoxyphenyl 2,6-Di-*O*-allyloxycarbonyl-3-*O*-benzoyl-α-D-mannopyranoside (11)

Compound **10** (4.74 g, 10.0 mmol) was dissolved in anhyd CH_2Cl_2 (20 mL) containing pyridine (3.2 mL, 40.0 mmol), then under an N_2 atmosphere, allyl chloroformate (1.11 mL, 10.5 mmol) in anhyd CH_2Cl_2 (10 mL) was added dropwise to the soln at –10 °C over 30 min. The temperature was slowly allowed to rise to r.t. and the mixture was stirred for 2 h; TLC (PE–EtOAc, 3:1) indicated completion. The mixture was diluted with CH_2Cl_2 (100 mL), washed with 1 M HCl and H_2O, and dried (Na_2SO_4). The soln was concentrated and purification of the residue by column chromatography (PE–EtOAc, 4:1) gave **11** (5.20 g, 93%) as a syrup; R_f = 0.26 (PE–EtOAc, 3:1).

$[\alpha]_D$ +12 (*c* 1, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.07–7.43 (m, 5 H, H_{Bz}), 7.06–7.03 (m, 2 H, H_{Mp}), 6.85–6.82 (m, 2 H, H_{Mp}), 5.99–5.83 (m, 2 H, 2 CH_2=C*H*CH_2OCO), 5.66 (dd, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 9.6 Hz, 1 H, H3), 5.54 (d, $J_{1,2}$ = 1.8 Hz, 1 H, H1), 5.41–5.24 (m, 5 H, H2, 4 C*H*$_2$=CHCH$_2$OCO), 4.66–4.41 (m, 6 H, H4, H5, 4 CH_2=CHC*H*$_2$OCO), 4.25–4.08 (m, 2 H, H6), 3.78 (s, 3 H, OMe), 2.79 (d, *J* = 4.8 Hz, 1 H).

MS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{28}H_{30}O_{12}Na$: 581.2; found: 581.5.

Anal. Calcd for $C_{28}H_{30}O_{12}$: C, 60.21; H, 5.41. Found: C, 60.09; H, 5.25.

4-Methoxyphenyl 2,6-Di-*O*-allyloxycarbonyl-3,4-di-*O*-benzoyl-α-D-mannopyranoside (12)

Method 1: Compound **11** (5.43 g, 9.72 mmol) was dissolved in pyridine (30 mL), then BzCl (1.7 mL, 14.6 mmol) in pyridine (10 mL) was added dropwise to the soln at 0 °C over 30 min. The temperature was slowly allowed to rise to r.t. and the mixture was stirred for 3 h; TLC (PE–EtOAc, 3:1) indicated completion. The mixture was diluted with CH_2Cl_2 (100 mL), washed with 1 M HCl and H_2O, and dried (Na_2SO_4). The soln was concentrated, and purification of the residue by column chromatography (PE–EtOAc, 4:1) gave **12** (6.12 g, 95%) as a foamy solid.

Method 2: Compound **10** (4.74 g, 10.0 mmol) was dissolved in anhyd CH_2Cl_2 (20 mL) containing pyridine (3.2 mL, 40.0 mmol), then under an N_2 atmosphere, allyl chloroformate (1.11 mL, 10.5 mmol) in anhyd CH_2Cl_2 (10 mL) was added dropwise to the soln at –10 °C over 30 min. The temperature was slowly allowed to rise to r.t. and the mixture was stirred for 2 h. BzCl (1.7 mL, 14.6 mmol) in pyridine (30 mL) was then added dropwise to the soln at 0 °C over 30 min. The temperature was slowly allowed to rise to r.t. and the mixture was stirred for 12 h; TLC (PE–EtOAc, 3:1) indicated completion. The mixture was diluted with CH_2Cl_2 (100 mL), washed with 1 M HCl and H_2O, and dried (Na_2SO_4). The soln was concentrated and purification of the residue by column chromatography (PE–EtOAc, 4:1) gave **12** (5.82 g, 88%) as a foamy solid; R_f = 0.38 (PE–EtOAc, 3:1).

$[\alpha]_D$ +14 (*c* 1, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.97–7.34 (m, 10 H, H_{Bz}), 7.12–7.09 (m, 2 H, H_{Mp}), 6.87–6.84 (m, 2 H, H_{Mp}), 5.95 (dd, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9.8 Hz, 1 H, H3), 5.92–5.80 (m, 2 H, 2 CH_2=C*H*CH_2OCO), 5.83 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, 1 H, H4), 5.58 (d, $J_{1,2}$ = 1.8 Hz, 1 H, H1), 5.48 (dd, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.3 Hz, 1 H, H2), 5.37–5.20 (m, 4 H, 4 C*H*$_2$=CHCH$_2$OCO), 4.58–4.50 (m, 4 H, 4 CH_2=CHC*H*$_2$OCO), 4.45 (m, 1 H, H5), 4.38–4.29(m, 2 H, H6), 3.78 (s, 3 H, OMe).

MS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{35}H_{34}O_{13}Na$: 685.2; found: 685.5.

Anal. Calcd for $C_{35}H_{34}O_{13}$: C, 63.44; H, 5.17. Found: C, 63.31; H, 5.30.

2,6-Di-*O*-allyloxycarbonyl-3,4-di-*O*-benzoyl-α-D-mannopyranosyl Trichloroacetimidate (3)

To a soln of **12** (5.7 g, 8.6 mmol) in MeCN (120 mL) was added successively H_2O (30 mL) and CAN (19.0 g, 34.4 mmol). The mixture was stirred at 30 °C for 20 min; TLC (PE–EtOAc, 2:1) indicated completion. The solvent was evaporated under reduced pressure at 50 °C to give a residue that was dissolved in CH_2Cl_2 and then washed with H_2O. The organic phase was dried (Na_2SO_4) and concentrated to give a residue that was purified by chromatography (silica gel, PE–EtOAc, 3:1) to afford 2,6-di-*O*-allyloxycarbonyl-3,4-di-*O*-benzoyl-α-D-mannopyranoside as a slight yellow foamy solid. A mixture of this compound (2.78 g, 5.0 mmol), Cl_3CCN (2.0 mL, 20 mmol), and DBU (0.2 mL, 2 mmol) in anhyd CH_2Cl_2 (100 mL) was stirred for 0.5 h and then concentrated. The residue was purified by column chromatography (PE–EtOAc, 4:1) to give **3** (5.21 g, 86%, 2 steps); R_f = 0.64 (PE–EtOAc, 2:1).

$[\alpha]_D$ –14 (*c* 1, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.86 (s, 1 H, CN*H*CCl_3), 7.98–7.33 (m, 10 H, H_{Bz}), 6.50 (d, $J_{1,2}$ = 1.9 Hz, 1 H, H1), 5.95–5.79 (m, 4 H, H3, H4, 2 CH_2=C*H*CH_2OCO), 5.51 (d, $J_{1,2}$ = 1.9 Hz, $J_{2,3}$ = 3.2 Hz, 1 H, H2), 5.38–5.21 (m, 4 H, 4 C*H*$_2$=CHCH$_2$OCO), 4.59–4.56 (m, 4 H, 4 CH_2=CHC*H*$_2$OCO), 4.48 (m, 1 H, H5), 4.41–4.36 (m, 2 H, H6).

MS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{30}H_{28}Cl_3NO_{12}Na$: 722.1; found: 722.4.

Anal. Calcd for $C_{30}H_{28}Cl_3NO_{12}$: C, 51.41; H, 4.03; N, 2.00. Found: C, 51.26; H, 4.29; N, 2.39.

4-Methoxyphenyl 3,4-Di-*O*-benzoyl-α-D-mannopyranoside (4)

To a cooled (–10 °C) soln of **12** (3.5 g, 5.28 mmol) in MeOH–THF (1:1, 60 mL) was added NH_4OAc (4.1 g, 52.8 mmol). $NaBH_4$ (0.12 g, 3.24 mmol), $Pd(PPh_3)_4$ (0.25 g, 0.21 mmol), and $NaBH_4$ (0.48 g, 13.0 mmol) were added successively in 3 portions immediately one after another; 4 min after the addition of the second portion of $NaBH_4$, TLC (PE–EtOAc, 1:1) indicated completion. The mixture was concentrated under vacuum, the residue was dissolved in CH_2Cl_2 (30 mL) and washed with brine (15 mL) and then the organic phase was dried (Na_2SO_4). Concentration of the organic phase and purification of the residue by flash column chromatography (PE–EtOAc, 3:1) afforded **4** as a white solid (2.44 g, 94%); R_f = 0.21 (PE–EtOAc, 1:1).

$[\alpha]_D$ +36 (*c* 1, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.05–7.30 (m, 10 H, H_{Bz}), 7.12–7.09 (m, 2 H, H_{Mp}), 6.87–6.84 (m, 2 H, H_{Mp}), 5.95 (dd, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 10.0 Hz, 1 H, H3), 5.93 (dd, $J_{3,4} = J_{4,5}$ = 10.0 Hz, 1 H, H4), 5.62 (d, $J_{1,2}$ = 1.8 Hz, 1 H, H1), 4.49 (dd, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.1 Hz, 1 H, H2), 4.10 (m, 1 H, H5), 3.79 (s, 3 H, OMe), 3.78–3.67 (m, 2 H, 2 H6), 3.42 (br s, 1 H, OH), 3.18 (br s, 1 H, OH).

MS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{27}H_{26}O_9Na$: 517.1; found: 517.0.

Anal. Calcd for $C_{27}H_{26}O_9$: C, 65.58; H, 5.30. Found: C, 65.55; H, 5.56.

4-Methoxyphenyl 4-*O*-Acetyl-2,6-di-*O*-allyloxycarbonyl-3-*O*-benzoyl-α-D-mannopyranoside (13)

Compound **10** (4.74 g, 10.0 mmol) was dissolved in anhyd CH_2Cl_2 (20 mL) containing pyridine (3.2 mL, 40.0 mmol), then ,under an N_2 atmosphere, allyl chloroformate (1.11 mL, 10.5 mmol) in anhyd CH_2Cl_2 (10 mL) was added dropwise to the soln at –10 °C over 30 min. The temperature was slowly raised to r.t. and the mixture was stirred for 2 h. Ac_2O (4.7 mL, 50 mmol) in pyridine (30 mL) was added to the soln and the mixture was stirred at r.t. for 12 h; TLC (PE–EtOAc, 3:1) indicated completion. The mixture was concentrated, and then the residue was purified by flash column chromatography (PE–EtOAc, 3:1) to give **13** (5.46 g, 91%) as a foamy solid; R_f = 0.35 (PE–EtOAc, 3:1).

$[\alpha]_D$ +28 (*c* 1, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.03–7.42 (m, 5 H, H_{Bz}), 7.08–7.05 (m, 2 H, H_{Mp}), 6.85–6.82 (m, 2 H, H_{Mp}), 5.98–5.82 (m, 2 H, 2 CH_2=C*H*CH_2OCO), 5.77 (dd, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 9.9 Hz, 1 H, H3), 5.59 (dd, $J_{3,4} = J_{4,5}$ = 9.9 Hz, 1 H, H4), 5.54 (d, $J_{1,2}$ = 1.8 Hz, 1 H, H1), 5.42 (dd, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.4 Hz, 1 H, H2), 5.38–5.20 (m, 4 H, 4 C*H*$_2$=CHCH_2OCO), 4.62–4.54 (m, 4 H, 4 CH_2=CHC*H*$_2$OCO), 4.38–4.25 (m, 3 H, H5, 2 H6), 3.78 (s, 3 H, OMe), 1.99 (s, 3 H, CH_3CO).

MS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{30}H_{32}O_{13}Na$: 623.2; found: 623.5.

Anal. Calcd for $C_{30}H_{32}O_{13}$: C, 60.00; H, 5.37. Found: C, 59.75; H, 5.30.

4-Methoxyphenyl 4-*O*-Acetyl-3-*O*-benzoyl-α-D-mannopyranoside (5)

To a cooled (–10 °C) soln of **13** (1.22 g, 2.03 mmol) in MeOH–THF (1:1, 40 mL) was added NH_4OAc (1.56 g, 20.3 mmol). $NaBH_4$ (0.05 g, 1.22 mmol), $Pd(PPh_3)_4$ (0.10 g, 0.08 mmol), and $NaBH_4$ (0.18 g, 4.87 mmol) were successively added in 3 portions immediately one after another with vigorous stirring; 4 min after the addition of the second portion of $NaBH_4$, TLC (PE–EtOAc, 1:1) indicated completion. The mixture was concentrated under vacuum, the residue was dissolved in CH_2Cl_2 (20 mL) and washed with brine (10 mL), and the organic phase was dried (Na_2SO_4). Evaporation and purification by flash column chromatography (PE–EtOAc, 3:1) afforded **5** (0.80 g, 92%) as a white solid; R_f = 0.20 (PE–EtOAc, 1:1).

$[\alpha]_D$ +80 (*c* 1, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.11–7.41 (m, 5 H, H_{Bz}), 7.07–7.02 (m, 2 H, H_{Mp}), 6.87–6.83 (m, 2 H, H_{Mp}), 5.74 (dd, $J_{2,3}$ = 3.2 Hz, $J_{3,4}$ = 10.1 Hz, 1 H, H3), 5.67 (dd, $J_{3,4} = J_{4,5}$ = 10.1 Hz, 1 H, H4), 5.55 (d, $J_{1,2}$ = 1.8 Hz, 1 H, H1), 4.42 (dd, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.1 Hz, 1 H, H2), 3.95 (m, 1 H, H5), 3.78 (s, 3 H, OMe), 3.75–3.60 (m, 2 H, H6), 3.22 (d, *J* = 5.5 Hz, 1 H, OH), 2.85 (d, *J* = 6.8 Hz, 1 H, OH), 1.99 (s, 3 H, CH_3CO).

MS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{22}H_{24}O_9Na$: 455.1; found: 455.5.

Anal. Calcd for $C_{22}H_{24}O_9$: C, 61.11; H, 5.59. Found: C, 60.96; H, 5.77.

4-Methoxyphenyl 2,6-Di-*O*-allyloxycarbonyl-3,4-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-3,4-di-*O*-benzoyl-α-D-mannopyranoside (14)

Compound **4** (2.47 g, 5.00 mmol), **3** (3.85 g, 5.50 mmol) and 4 Å molecular sieves (3 g) were dried together under high vacuum (0.0067 mbar) for 2 h, then dissolved in anhyd, redistilled CH_2Cl_2 (50 mL). TMSOTf (36 μL, 0.2 mmol) was added dropwise at –20 °C under an N_2 atmosphere. The mixture was stirred for 0.5 h, during the course of which time the mixture was allowed to gradually warm to r.t.; TLC (PE–EtOAc, 1:1) indicated completion. Then the mixture was neutralized with Et_3N and filtered and the filtrate was concentrated. Purification of the residue by column chromatography (PE–EtOAc, 4:1) gave **14** (4.50 g, 87%) as a foamy solid; R_f = 0.39 (PE–EtOAc, 1:1).

$[\alpha]_D$ +25 (*c* 1, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.05–7.30 (m, 20 H, H_{Bz}), 7.19–7.16 (m, 2 H, H_{Mp}), 6.94–6.91 (m, 2 H, H_{Mp}), 6.01 (dd, $J_{3',4'} = J_{4',5'}$ = 10.0 Hz, 1 H, H4′), 5.94–5.80 (m, 3 H, H3′, 2 CH_2=C*H*CH_2OCO), 5.79–5.71(m, 2 H, H3, H4), 5.58 (d, $J_{1,2}$ = 1.6 Hz, 1 H, H1), 5.38 (dd, $J_{1',2'}$ = 1.5 Hz, $J_{2',3'}$ = 3.1 Hz, 1 H, H2′), 5.34–5.19 (m, 4 H, 4 C*H*$_2$=CHCH_2OCO), 5.07 (d, $J_{1',2'}$ = 1.5 Hz, 1 H, H1′), 4.61–4.52 (m, 5 H, H2, 4 C*H*$_2$=CHCH_2OCO), 4.43 (m, 1 H, H5′), 4.34–4.21 (m, 2 H, H5, H6), 4.15 (d, *J* = 11.5 Hz, 1 H, H6), 4.02 (d, *J* = 11.2 Hz, 1 H, H6), 3.70 (m, 1 H, H6), 3.65 (s, 3 H, OMe), 2.87 (d, *J* = 5.2 Hz, 1 H, OH).

MS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{55}H_{52}O_{20}Na$: 1055.3; found:1055.1.

Anal. Calcd for $C_{55}H_{52}O_{20}$: C, 63.95; H, 5.07. Found: C, 63.83; H, 5.11.

4-Methoxyphenyl 2,6-Di-*O*-allyloxycarbonyl-3,4-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2-*O*-acetyl-3,4-di-*O*-benzoyl-α-D-mannopyranoside (15)

To a soln of **14** (5.17 g, 5.0 mmol) in pyridine (20 mL) was added Ac_2O (4.7 mL, 50 mmol). The mixture was stirred at r.t. for 12 h; TLC (PE–EtOAc, 2:1) indicated completion. The mixture was concentrated and then the residue was purified by flash column chromatography (PE–EtOAc, 3:1) to give **15** (5.11 g, 95%) as a foamy solid; R_f = 0.35 (PE–EtOAc, 2:1).

$[\alpha]_D$ +28 (*c* 1, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.01–7.33 (m, 20 H, H_{Bz}), 7.18–7.15 (m, 2 H, H_{Mp}), 6.94–6.91 (m, 2 H, H_{Mp}), 5.99–5.97 (m, 2 H, H3, H3′), 5.93–5.72 (m, 2 H, 2 CH_2=C*H*CH_2OCO), 5.81–5.71 (m, 2 H, H4, H4′), 5.67 (dd, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.1 Hz,1 H, H2), 5.53 (d, $J_{1,2}$ = 1.7 Hz, 1 H, H1), 5.38 (dd, $J_{1',2'}$ = 1.8 Hz, $J_{2',3'}$ = 2.8 Hz, 1 H, H2′), 5.34–5.18 (m, 4 H, 4 C*H*$_2$=CHCH_2OCO), 5.06 (d, $J_{1',2'}$ = 1.8 Hz, 1 H, H1′), 4.54–4.50 (m, 4 H, 4 CH_2=CHC*H*$_2$OCO), 4.45 (m, 1

H, H5), 4.28–4.13 (m, 3 H, H5, H6), 4.01–3.70 (m, 2 H, H6), 3.66 (s, 3 H, OMe), 2.24 (s, 3 H, CH_3CO).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.2 (*C*OCH_3), 165.5, 165.4, 165.3, 165.1 (4 C, 4 *C*OPh), 97.6, 97.1 (2 C, 2 C1), 55.4 (OMe), 20.7 (*C*H_3CO).

MS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{57}H_{54}O_{21}Na$: 1097.3; found: 1097.7.

Anal. Calcd for $C_{57}H_{54}O_{21}$: C, 63.68; H, 5.06. Found: C, 63.82; H, 4.91.

2,6-Di-*O*-allyloxycarbonyl-3,4-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2-*O*-acetyl-3,4-di-*O*-benzoyl-α-D-mannopyranosyl Trichloroacetimidate (16)

To a soln of **15** (10.75 g, 10.0 mmol) in MeCN (120 mL) and H_2O (60 mL) was added CAN (21.9 g, 40.0 mmol). The mixture was stirred at 30 °C for 20 min; TLC (PE–EtOAc, 2:1) indicated completion. The solvent was evaporated under reduced pressure at 50 °C to give a residue that was dissolved in CH_2Cl_2 and washed with H_2O. The organic phase was dried (Na_2SO_4) and concentrated. Purification by column chromatography (PE–EtOAc, 3:1) afforded 2,6-di-*O*-allyloxycarbonyl-3,4-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2-*O*-acetyl-3,4-di-*O*-benzoyl-α-D-mannopyranoside as a slight yellow foamy solid. The foamy solid was dried under high vacuum (0.0067 mbar) for 2 h, then dissolved in anhyd CH_2Cl_2 (100 mL), and Cl_3CCN (4.0 mL, 40 mmol) and DBU (0.05 mL, 0.4 mmol) were added successively under an N_2 atmosphere. The mixture was stirred for 0.5 h and then concentrated. The residue was purified by chromatography (PE–EtOAc, 4:1) to give **16** (8.61 g, 77%) as a foamy solid; R_f = 0.38 (PE–EtOAc, 2:1).

$[\alpha]_D$ +6 (*c* 1, $CHCl_3$).

^1H NMR (300 MHz, $CDCl_3$): δ = 8.91 (s, 1 H, $CNHCCl_3$), 8.04–7.34 (m, 20 H, H_{Bz}), 6.41 (d, $J_{1,2}$ = 1.8 Hz, 1 H, H1), 6.02 (dd, $J_{3,4} = J_{4,5}$ = 10.2 Hz, 1 H, H4), 5.92–5.71 (m, 6 H, H2, H3, H3′, H4′, 2 CH_2=C*H*CH_2OCO), 5.35 (dd, $J_{1',2'}$ = 1.7 Hz, $J_{2',3'}$ = 3.0 Hz, 1 H, H2′), 5.33–5.19 (m, 4 H, 4 CH_2=CHCH_2OCO), 5.06 (d, $J_{1',2'}$ = 1.7 Hz, 1 H), 4.55–4.50 (m, 4 H, 4 CH_2=CHCH_2OCO), 4.45 (m, 1 H, H5′), 4.30 (m, 1 H, H5), 4.23–4.14 (m, 2 H, H6′), 4.01–3.79 (m, 1 H, H6), 2.26 (s, 3 H, CH_3CO).

MS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{52}H_{48}Cl_3NO_{20}Na$: 1136.2; found: 1136.5.

Anal. Calcd for $C_{52}H_{48}Cl_3NO_{20}$: C, 56.10; H, 4.35, N, 1.26. Found: C, 56.28; H, 4.44; N, 1.43.

4-Methoxyphenyl 2,6-Di-*O*-allyloxycarbonyl-3,4-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2-*O*-acetyl-3,4-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-4-*O*-acetyl-3-*O*-benzoyl-α-D-mannopyranoside (17)

Compound **5** (3.24 g, 7.50 mmol), **16** (8.50 g, 7.65 mmol), and 4 Å molecular sieves (3 g) were dried together under high vacuum (0.0067 mbar) for 2 h, then dissolved in anhyd, redistilled CH_2Cl_2 (80 mL). TMSOTf (36 μL, 0.2 mmol) was added dropwise at –20 °C under an N_2 atmosphere. The mixture was stirred for 0.5 h, during which time the mixture was allowed to gradually warm to r.t; TLC (PE–EtOAc, 2:1) indicated completion. The mixture was neutralized with Et_3N and filtered, and the filtrate was concentrated. Purification of the residue by column chromatography (PE–toluene–EtOAc, 4:2:1) gave **17** (8.83 g, 85%) as a foamy solid; R_f = 0.53 (PE–EtOAc, 1:1).

$[\alpha]_D$ +16 (*c* 1, $CHCl_3$).

^1H NMR (300 MHz, $CDCl_3$): δ = 8.04–7.26 (m, 25 H, H_{Bz}), 7.24–7.21 (m, 2 H, H_{Mp}), 6.99–6.86 (m, 2 H, H_{Mp}), 5.95–5.84 (m, 2 H, 2 CH_2=C*H*CH_2OCO), 5.82–5.67 (m, 6 H, 3 H3, 3 H4), 5.53 (dd, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 3.2 Hz, 1 H, H2), 5.51 (dd, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 3.1 Hz, 1 H, H2), 3.36 (d, $J_{1,2}$ = 1.6 Hz, 1 H, H1), 5.32–5.17 (m, 4 H, 4 CH_2=CHCH_2OCO), 5.05 (d, $J_{1,2}$ = 1.6 Hz, 1 H, H1), 5.00 (d, $J_{1,2}$ = 1.3 Hz, 1 H, H1), 4.58–4.50 (m, 4 H, 4 CH_2=CHCH_2OCO), 4.47–4.39 (m, 3 H, 3 H5), 4.33–4.22 (m, 3 H, H2, H6), 4.14–3.98 (m, 2 H, H6), 3.67–3.63 (m, 2 H, H6), 3.58 (s, 3 H, OMe), 3.45 (d, *J* = 8.1 Hz, 1 H, OH), 2.19, 1.97 (2 s, 6 H, 2 CH_3CO).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.1, 169.8 (2 C, 2 CH_3CO), 166.0, 165.7, 165.6, 165.4, 165.1 (5 C, 5 Ph*C*O), 99.7, 97.2, 97.1 (3 C, 3 C1), 55.2 (1 C, OMe), 20.7, 20.6 (2 C, 2 *C*H_3CO).

MS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{72}H_{70}O_{28}Na$: 1405.4; found: 1405.7.

Anal. Calcd for $C_{72}H_{70}O_{28}$: C, 62.51; H, 5.10. Found: C, 62.36; H, 5.25.

4-Methoxyphenyl 2,6-Di-*O*-allyloxycarbonyl-3,4-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2-*O*-acetyl-3,4-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,4-di-*O*-acetyl-3-*O*-benzoyl-α-D-mannopyranoside (18)

To a soln of **17** (120 mg, 0.087 mmol) in pyridine (1.0 mL) was added Ac_2O (0.5 mL, 0.85 mmol). The mixture was stirred at r.t. for 12 h; TLC (PE–EtOAc, 2:1) indicated completion. The mixture was co-evaporated with toluene and the residue was purified by flash column chromatography (PE–EtOAc, 2:1) to give **18** (110 mg, 92%) as a white foamy solid; R_f = 0.39 (PE–EtOAc, 2:1).

$[\alpha]_D$ +36 (*c* 1, $CHCl_3$).

^1H NMR (300 MHz, $CDCl_3$): δ = 8.03–7.31 (m, 25 H, H_{Bz}), 7.17–7.14 (m, 2 H, H_{Mp}), 6.92–6.88 (m, 2 H, H_{Mp}), 5.96 (dd, $J_{3,4} = J_{4,5}$ = 10.1 Hz, 1 H, H4), 5.92–5.70 (m, 7 H, 3 H3, 2 H4, 2 CH_2=C*H*CH_2OCO), 5.62 (dd, $J_{1,2}$ = 1.9 Hz, $J_{2,3}$ = 3.2 Hz, 1 H, H2), 5.56 (dd, $J_{1,2}$ = 1.7 Hz, $J_{2,3}$ = 3.3 Hz, 1 H, H2), 5.50 (d, $J_{1,2}$ = 1.7 Hz, 1 H, H1), 5.41 (dd, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.1 Hz, 1 H, H2), 5.34–5.19 (m, 4 H, 4 CH_2=CHCH_2OCO), 5.09 (d, $J_{1,2}$ = 1.9 Hz, 1 H, H1), 5.01 (d, $J_{1,2}$ = 1.8 Hz, 1 H, H1), 4.46–4.44 (m, 4 H, 4 CH_2=CHCH_2OCO), 4.35 (m, 1 H, H5), 4.30–4.25 (m, 2 H, H5), 4.22–4.11 (m, 2 H, H6), 4.05–3.97 (m, 2 H, H6), 3.74–3.68 (m, 2 H, H6), 3.61 (s, 3 H, OMe), 2.22, 2.11, 2.04 (3 s, 9 H, 3 CH_3CO).

MS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{74}H_{72}O_{29}Na$: 1447.4; found: 1447.7.

Anal. Calcd for $C_{74}H_{72}O_{29}$: C, 62.36; H, 5.09. Found: C, 62.30; H, 4.88.

4-Methoxyphenyl 3,4-Di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2-*O*-acetyl-3,4-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-4-*O*-acetyl-3-*O*-benzoyl-α-D-mannopyranoside (2)

To a cooled (–10 °C) soln of **17** (8.3 g, 6.0 mmol) in MeOH–THF (1:1, 150 mL) was added NH_4OAc (4.62 g, 60 mmol). $NaBH_4$ (0.22 g, 6.0 mmol), $Pd(PPh_3)_4$ (0.35 g, 0.30 mmol), and $NaBH_4$ (0.11 g, 3.0 mmol) were added successively in 3 portions one immediately after another with vigorous stirring; 4 min after the addition of the second portion of $NaBH_4$, TLC (PE–EtOAc, 1:3) indicated completion. The mixture was concentrated under vacuum at 30 °C, the residue was dissolved in CH_2Cl_2 (10 mL) and washed with brine (10 mL), then the organic phase was dried (Na_2SO_4). Evaporation and purification by flash column chromatography (PE–EtOAc, 1:2) afforded **2** (6.85 g, 94%) as a white foamy solid; R_f = 0.26 (PE–EtOAc, 1:3).

$[\alpha]_D$ +14 (*c* 1, $CHCl_3$).

^1H NMR (300 MHz, $CDCl_3$): δ = 8.06–7.26 (m, 25 H, H_{Bz}), 7.23–7.20 (m, 2 H, H_{Mp}), 7.00–6.97 (m, 2 H, H_{Mp}), 5.78 (dd, $J_{2,3} = J_{3,4}$ = 10.0 Hz, 1 H, H4), 5.72–5.67 (m, 4 H, 3 H3, H4), 5.62 (dd, $J_{2,3} = J_{3,4}$ = 10.0 Hz, 1 H, H4), 5.50 (d, $J_{1,2}$ = 1.3 Hz, 1 H, H1), 5.49 (m, 1 H, H2), 5.02 (d, $J_{1,2}$ = 1.5 Hz, 1 H, H1), 4.96 (d, $J_{1,2}$ = 1.4 Hz, 1 H, H1), 4.46 (m, 4 H, 2 H2, 2 H5), 4.15–3.60 (m, 7 H, H5, 6

H6), 3.50 (s, 3 H, OMe), 2.73 (t, J = 6.5 Hz, OH), 2.17, 2.00 (2 s, 6 H, 2 CH_3CO), 1.79 (s, 2 H, 2 OH).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.1, 169.8 (2 *C*OCH_3), 166.4, 166.1, 165.7, 165.6, 165.1 (5 *C*OPh), 99.5, 99.1, 96.9 (3 C1), 55.1 (OMe), 20.8, 20.7 (2 CO*C*H_3).

MS (ESI): *m/z* $[M + Na]^+$ calcd for $C_{64}H_{62}O_{24}Na$: 1237.4; found: 1237.6.

Anal. Calcd for $C_{64}H_{62}O_{24}$: C, 63.26; H, 5.14. Found: C, 63.01; H, 5.35.

4-Methoxyphenyl 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→2)]-3,4-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2-*O*-acetyl-3,4-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→2)]-4-*O*-acetyl-3-*O*-benzoyl-α-D-mannopyranoside (20)

Compound **2** (6.08 g, 5.0 mmol), 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**19**, 14.81 g, 20.00 mmol) and 4 Å molecular sieves (8 g) were dried together under high vacuum (0.0067 mbar) for 2 h, then dissolved in anhyd CH_2Cl_2 (120 mL). TMSOTf (54 μL, 0.3 mmol) was added dropwise at –20 °C under an N_2 atmosphere. The mixture was stirred for 0.5 h, during the course of which time the mixture was gradually warmed to r.t.; TLC (PE–EtOAc, 1:1) indicated completion. Then the mixture was neutralized with Et_3N and filtered, and the filtrate was concentrated. Purification of the residue by column chromatography (PE–toluene–EtOAc, 4:2:1) gave **20** (13.43 g, 91%) as a foamy solid; R_f = 0.37 (PE–EtOAc, 1:1).

$[\alpha]_D$ +56 (*c* 1, $CHCl_3$).

^1H NMR (300 MHz, $CDCl_3$): δ = 8.15–7.15 (m, 85 H, H_{Bz}), 7.04–6.72 (m, 4 H, H_{Mp}), 6.29–5.81 (m, 15 H), 5.76 (d, $J_{1,2}$ = 1.8 Hz, 1 H, H1), 5.67 (dd, $J_{1,2}$ = 1.0 Hz, $J_{2,3}$ = 3.3 Hz, 1 H, H2), 5.37 (d, $J_{1,2}$ = 1.0 Hz, 1 H, H1), 5.35 (d, $J_{1,2}$ = 0.7 Hz, 1 H, H1), 5.22 (d, $J_{1,2}$ = 0.6 Hz, 1 H, H1), 5.12 (d, $J_{1,2}$ = 0.9 Hz, 1 H, H1), 5.01 (d, $J_{1,2}$ = 1.1 Hz, 1 H, H1), 4.85–3.73 (m, 19 H), 3.66 (s, 3 H, OMe), 3.51 (m, 1 H), 2.09, 2.01 (2 s, 6 H, 2 CH_3CO).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.1, 169.5 (2 *C*OCH_3), 166.1, 166.0, 166.0, 165.8, 165.8, 165.7, 165.7, 165.5, 165.4, 165.2, 165.1, 165.1, 164.8, 164.8, 164.8, 164.7, 164.7 (17 *C*OPh), 100.1, 99.3, 98.9, 98.1, 97.9, 97.8 (6 C1), 55.5 (OMe), 20.7, 20.6 (2 CO*C*H_3).

Anal. Calcd for $C_{166}H_{140}O_{51}$: C, 67.57; H, 4.78; found: C, 67.70; H, 4.59.

4-Methoxyphenyl α-D-Mannopyranosyl-(1→6)-[α-D-mannopyranosyl-(1→2)]-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-[α-D-mannopyranosyl-(1→2)]-α-D-mannopyranoside (1)

Compound **20** (13.28 g, 4.5 mmol) was dissolved in sat. NH_3 in MeOH (1500 mL). After 120 h at r.t., the mixture was concentrated to a total volume of ~50 mL, then warm acetone (500 mL, 50 °C) was added to the mixture under vigorous stirring, and a white solid precipitate from the soln, after kept at 0 °C for 24 h, filtration gave target compound **1** (4.1 g, 83%) as a white solid.

$[\alpha]_D$ +35 (*c* 1.0, H_2O).

^1H NMR (300 MHz, D_2O): δ = 7.07–7.04 (m, 2 H, H_{Mp}), 6.94–6.92 (m, 2 H, H_{Mp}), 5.73 (d, $J_{1,2}$ = 1.3 Hz, 1 H, H1), 5.05 (d, $J_{1,2}$ = 1.2 Hz, 1 H, H1), 5.03 (d, $J_{1,2}$ = 1.3 Hz, 1 H, H1), 4.97 (d, $J_{1,2}$ = 1.4 Hz, 1 H, H1), 4.87 (d, $J_{1,2}$ = 1.5 Hz, 1 H, H1), 4.70 (d, $J_{1,2}$ = 1.1 Hz, 1 H, H1), 3.76 (s, 3 H, OMe), 4.13–3.58 (m, 39 H, H2–6).

^{13}C NMR (75 MHz, D_2O): δ = 102.3, 102.0, 99.1, 98.9, 97.8, 97.1 (6 C, 6 C1), 55.6 (1 C, OCH_3).

MS (MALDI-TOF): *m/z* $[M + Na]^+$ calcd for $C_{43}H_{68}O_{32}Na$: 1119.36; found: 1119.42.

MS (ESI): *m/z* $[M + Na]^+$ calcd for $C_{43}H_{68}O_{32}Na$: 1119.3591; found: 1119.3582.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (No. 20902108); the Chinese Universities Scientific Fund (No. 2009-1-43).

References

(1) (a) Kawarasaki, I.; Takeda, T.; Ogihara, Y.; Shimonaka, H.; Nozawa, Y. *Chem. Pharm. Bull.* **1979**, *27*, 2073. (b) Takeda, T.; Kawarasaki, I.; Ogihara, Y. *Carbohydr. Res.* **1981**, *89*, 301. (c) Liqibárová, I.; Matulová, M.; Machová, E.; Capek, P. *Carbohydr. Polym.* **2007**, *68*, 191.
(2) Hay, R. J. *J. Dermatol.* **1982**, *106*, 1.
(3) (a) Sullivan, D. J.; Westerneng, T. J.; Haynes, K. A.; Bennet, D. E.; Coleman, D. C. *Microbiology* **1995**, *141*, 1507. (b) Brown, D. M.; Jabra-Rizk, M. A.; Falkler, W. A.; Baqui, A. A. M. A.; Meiller, T. F. *Pediatr. Dent.* **2000**, *22*, 234.
(4) Roberts, I. S. *Annu. Rev. Microbiol.* **1996**, *50*, 285.
(5) (a) Jones, C. *An. Acad. Bras. Cienc.* **2005**, *77*, 293. (b) Takamatsu-Matsushita, N.; Yamaguchi, N.; Kawasaki, M.; Yamashita, Y.; Koga, T. *Oral Microbiol. Immunol.* **1996**, *11*, 220. (c) Zuurmond, H. M.; Veeneman, G. H.; Marel, G. A.; Boom, J. H. *Carbohydr. Res.* **1993**, *241*, 153. (d) Heidelberg, T.; Martin, O. R. *J. Org. Chem.* **2004**, *69*, 2290. (e) Fekete, A.; Gyergyoi, K.; Kover, K. E.; Bajza, I.; Liptak, A. *Carbohydr. Res.* **2006**, *341*, 1312.
(6) (a) Nin, J.; Kong, F. *Tetrahedron Lett.* **1999**, *40*, 1357. (b) Nin, J.; Heng, L.; Kong, F. *Carbohydr. Res.* **2002**, *337*, 1159. (c) Nin, J.; Heng, L.; Kong, F. *Tetrahedron Lett.* **2002**, *43*, 673. (d) Xing, Y.; Nin, J. *Tetrahedron: Asymmetry* **2003**, *14*, 1275.
(7) (a) Yan, S.; Wu, X.; Liang, X.; Zhang, J. *Chin. Chem. Lett.* **2009**, *20*, 582. (b) Zong, G.; Yan, S.; Liang, X.; Zhang, J.; Wang, D. *Chin. Chem. Lett.* **2009**, *20*, 127. (c) Yan, S.; Liang, X.; Diao, P.; Yang, Y.; Zhang, J.; Wang, D. *Carbohydr. Res.* **2008**, *343*, 3107.
(8) Faure, R.; Shiao, T. C.; Damerval, S.; Roy, R. *Tetrahedron Lett.* **2007**, *48*, 2385.
(9) Copeland, C.; Stick, R. V. *Aust. J. Chem.* **1978**, *31*, 1371.
(10) Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21.