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Efficient and practical syntheses of three pentasaccharides core structures corresponding to *N*-glycans

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Dedicated to Professor Joachim Thiem on the occasion of his 60th birthday

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Abstract—Three branched pentasaccharide derivatives and one tetrasaccharide were synthesized efficiently. The advantages of this method include a one-pot facile synthesis of 3,6-differentially protected mannose building block and an efficient strategy for oligosaccharide assembly.

1. Introduction

Asparagine-linked glycoprotein oligosaccharides play a vital role in fundamental biological processes such as cell differentiation, malignant transformation, viral, bacterial and parasitic infections and protein transportations.[1] They are usually divided into three major subgroups, i.e. high-mannose type, complex type and hybrid type, and each of them may consist of diverse structures in living cells.[2] Pentasaccharides A, B, C (Fig. 1) are typical sub-structures corresponding to the aforementioned three different types of *N*-glycan, respectively. Obviously, they are the bases for the synthesis of advanced *N*-glycans. Intensive research into the biological role of these carbohydrates and related glycoconjugates has led to an increased need for the practical synthesis of these core structures. Although remarkable progress has been made in the field of *N*-glycan synthesis,[3] further innovations are still required since the synthesis remains a highly specialized and time consuming task. We present here, a very efficient and practical method[4] for the synthesis of 3,6-branched oligosaccharide moieties that occur in most complex *N*-glycans.

2. Results and discussion

To supply enough samples for glycobiology studies on the relationship between *N*-glycans and diseases, we have been seeking practical methods for the synthesis of *N*-glycan core structures. Scheme 1 depicts the synthesis of key synthon **2** via a one-pot manner. The transformation of allyl α-D-mannopyranoside (**1**) into allyl 2,4-di-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilyl-6-*O*-triphenylmethyl-α-D-mannopyranoside (**2**) was successfully achieved through three sequential reactions. Thus, **1** was selectively tritylated with triphenylmethyl chloride (TrCl, 1.25 equiv.) in pyridine at 80°C for

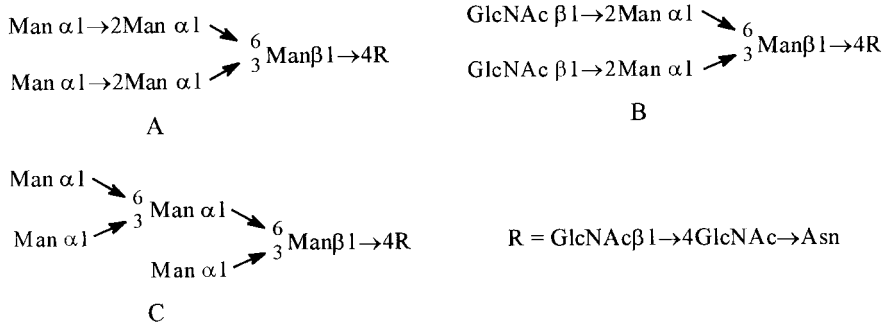

Figure 1.

Keywords: carbohydrates; glycosylations; glycopeptides.

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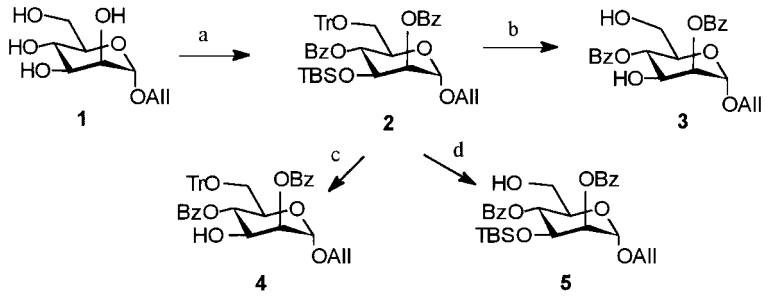
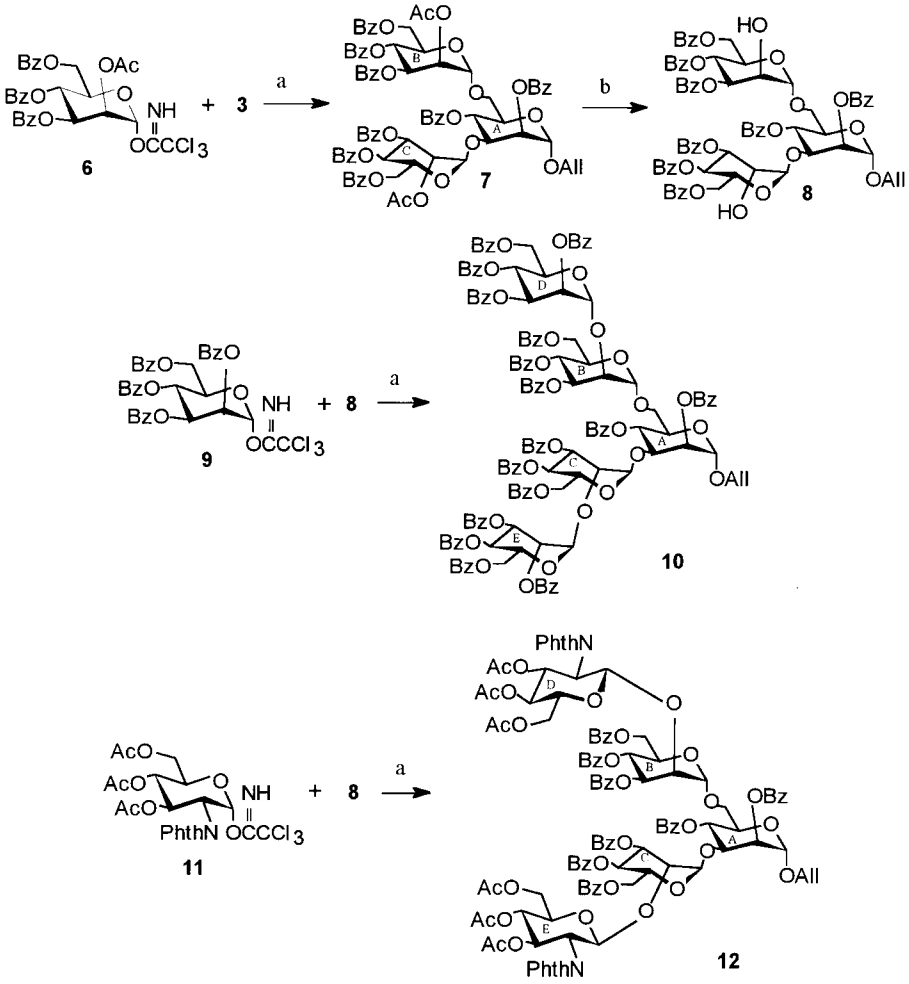


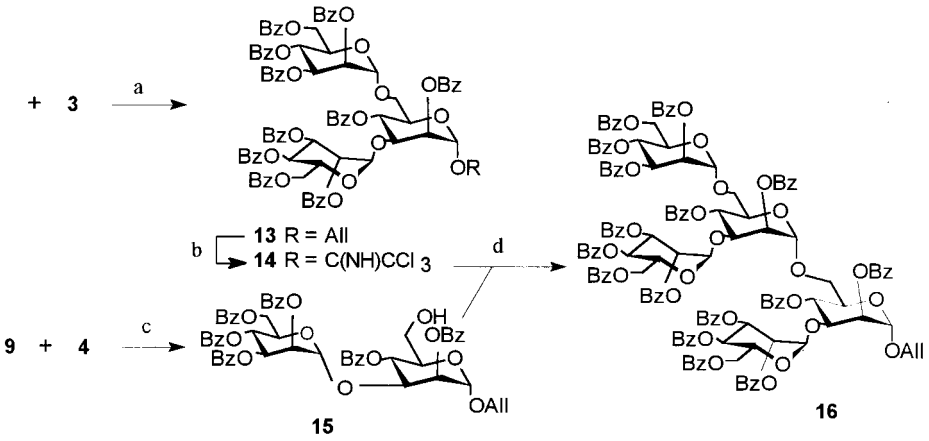
Scheme 1. Regioselective synthesis of versatile building block **2**. *Reaction conditions*: (a) TrCl, Py, DMAP; TBSCl in DMF; BzCl in Py, 79% in one-pot; (b) 90% TFA, 91%; (c) TBAF, THF, 66%; (d) $FeCl_3{\cdot}6H_2O$, CH_2Cl_2, 88%.

16 h, then cooled down to 0°C. This mixture was treated with 2 equiv. of imidazole, and finally 1.1 equiv. of *tert*-butylchlorodimethylsilane (TBDMSCl) in DMF was added into the reaction flask portion by portion during 2 h. The mixture was stirred at rt overnight, then premixed benzoyl chloride (2.5 equiv.) and pyridine was added. The reaction mixture was stirred at 50°C overnight to afford compound **2** in 79% overall yield.

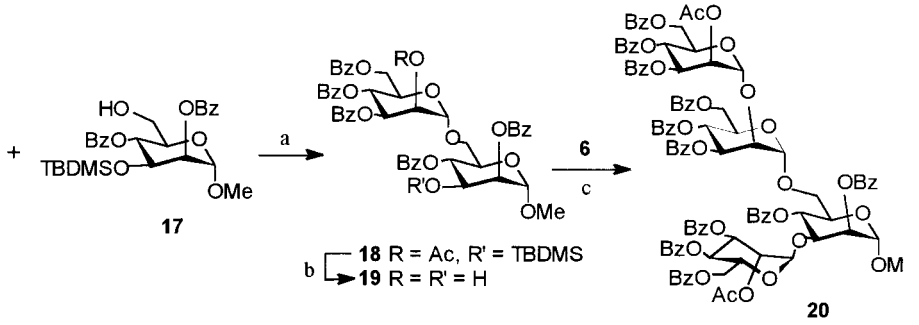
It is noteworthy that **2** could be easily transformed into a suitable acceptor for the synthesis of either symmetrical (C-3 and C-6 connected with identical sugar residues) or asymmetrical (C-3 and C-6 linked by non-identical sugar residues) trisaccharide core structure. Treatment of compound **2** with 90% trifluoroacetic acid (TFA)[5] gave diol **3** in 91% yield which could be used for the synthesis of symmetrical analogs (see Scheme 2). When **2** was treated with tetrabutylammonium fluoride in tetrahedrofuran (THF),[6] 3-OH derivative **4** was obtained in 66% yield, and **4** could be used to synthesize $1 \rightarrow 3$ linked structure (see Scheme 3). Treatment of **2** with ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$)[7] afforded **5** (88%) as a useful synthon for the synthesis of $1 \rightarrow 6$ linked structure (see Scheme 4).



Scheme 2. Synthesis of pentasaccharides **10** and **12**. *Reaction conditions*: (a) TMSOTf, CH_2Cl_2, 0°C, 80% (for **7**); 82% (for **10**); 50% (for **12**); (b) 5% HCl (gas) in MeOH–CH_2Cl_2 (1:1), 78%.



Scheme 3. Synthesis of pentasaccharide **16**. *Reaction conditions*: (a) TMSOTf, CH_2Cl_2, 0°C, 85%; (b) $PdCl_2$, NaOAc, 90% HOAc, then CCl_3CN, DBU, 83% (2 steps); (c) TMSOTf, CH_2Cl_2, −15°C, then TMSOTf (50 μL), rt, 80%; (d) TMSOTf, CH_2Cl_2, 0°C, 85%.



Scheme 4. Synthesis of asymmetrical tetrasaccharide **20**. *Reaction conditions*: (a) TMSOTf, CH_2Cl_2, 0°C, 84%; (b) 5% HCl (gas) in MeOH–CH_2Cl_2 (1:1), 70%; (c) 2.2 equiv. of **6**, TMSOTf, CH_2Cl_2, 0°C, 73%.

Pentasaccharide **10** or **12** consists of three differently linked sugar residues, which necessitates a corresponding protective group pattern for these building blocks. Previous research focused on the use of benzyl ether as hydroxyl protection group. However, readily removable acyl groups are generally more attractive. We chose acetyl as a temporary protective group and benzoyl as a permanent one. Coupling of diol **3** with 2.1 equiv. of 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl 2,2,2 trichloroacetimidate (**6**) in anhydrous CH_2Cl_2 using TMSOTf as catalyst gave symmetrical trisaccharide **7** in 80% yield. Selective deacetylation[8] of **7** in the presence of benzoyl groups was carried out smoothly using 5% HCl (gas) in methanol–CH_2Cl_2 (1:1) co-solvent to give deacetylated trisaccharide **8** in 78% yield. Glycosylation of trisaccharide diol **8** with donor **9** under the same reaction conditions as described in the preparation of **7** furnished pentasaccharide **10** in 82% yield. Similarly, coupling of **8** with 2.5 equiv. of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-α-D-glucopyranosyl 2,2,2 trichloroacetimidate (**11**) afforded pentasaccharide **12** in 50% yield, together with some unidentified tetrasaccharides. The quick consumption of the donor **11** may be responsible for the low yield in this reaction.

Pentasaccharide **16**, which contains both 1 → 3 and 1 → 6 linkages, best exhibits the advantages of our synthetic strategy. Coupling of diol **3** with trichloroacetimidate **9** (2.1 equiv.) in anhydrous CH_2Cl_2 using TMSOTf (10% equiv.) as catalyst gave trisaccharide **13** in 85% yield. Deallylation on **13** with $PdCl_2$ (2 equiv.) and NaOAc (4 equiv.) in 90% aqueous acetic acid,[9] followed by anomeric Schmidt activation[10] with trichloroactonitrile furnished trisaccharide donor **14** in 83% yield (2 steps). Convergently, coupling of donor **9** with acceptor **4** was accomplished in 40 min using TMSOTf (0.1 equiv.) as catalyst at −15°C. Trifluoacetic acid (TFA, 1 mL) or more TMSOTf was added into this reaction mixture and the mixture was stirred at room temperature for 2 h to afford disaccharide acceptor **15** in one-pot (80%). Trisaccharide donor **14** and disaccharide acceptor **15** were treated under the same glycosylation conditions affording pentasaccharide **16** in 85% yield. Coupled ^{13}C NMR spectrum of **10**, **13** and **16** showed all $^1J_{C\text{-}1,H\text{-}1}$ values in a range 170–173 Hz, indicating the α-linkage of mannose residues in them.

Mono-hydroxyl acceptor **17** was synthesized starting from its corresponding methyl α-D-mannopyranoside using the same procedure as described in the preparation of **5**. TMSOTf promoted coupling reaction of **17** with donor **6** in anhydrous CH_2Cl_2 at 0°C afforded disaccharide **18** in 84% yield. Simultaneous desilylation and deacetylation of **18** in the presence of benzoyl groups was carried out using 5% HCl (gas) in methanol–CH_2Cl_2 (1:1) co-solvent giving disaccharide diol **19** in 70% yield. Further glycosylation of diol **19** with donor **6** under the same reaction conditions furnished asymmetrical tetrasaccharide **20** in 73% yield.

3. Experimental

3.1. 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, ^{13}C NMR and ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY spectra were recorded with Bruker ARX 400 spectrometers for solutions in $CDCl_3$. Chemical shifts are given in ppm downfield from internal Me_4Si. Mass spectra were measured using MALTI–TOF–MS with CCA as matrix or recorded with a VG PLATFORM mass spectrometer using the ESI technique to introduce the sample. IR spectra were recorded with a Hitachi 270-30 spectrometer. Thin-layer chromatography (TLC) was performed on silica gel HF_{254} with detection by charring with 30% (v/v) H_2SO_4 in MeOH or in some cases by a UV detector. Column chromatography was conducted by elution of a column (16×240 mm^2, 18×300 mm^2, 35×400 mm^2) of silica gel (100–200 mesh) with EtOAc–petroleum ether (60–90°C) as the eluent. Solutions were concentrated at <60°C under reduced pressure.

3.1.1. Allyl 2,4-di-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilyl-6-*O*-triphenylmethyl-α-D-mannopyranoside (2). To a solution of allyl α-D-mannopyranoside (**1**) (2.2 g, 10 mmol) in pyridine (15 mL) was added TrCl (3.5 g, 12.5 mmol) and catalytic amount of 4-dimethylaminopyridine (DMAP). The mixture was stirred at 80°C for 16 h, then cooled down to 0°C. To the above reaction mixture was added imidazole (1.36 g, 20 mmol) in one portion. A solution of TBDMSCl (1.66 g, 11 mmol) in DMF (5 mL) was finally added portion by portion during 2 h. The mixture was stirred at rt overnight, then a premixed solution of benzoyl chloride (2.9 mL, 25 mmol) and pyridine (3 mL) was added. The reaction mixture was stirred at 50°C overnight, and then poured into ice-cold water, extracted with EtOAc. The organic phase was concentrated to dryness by repeating co-evaporation with toluene. The residue was subjected to column chromatography on silica gel with petroleum ether–EtOAc as the eluent (12:1) to give **2** as a syrup (6.19 g, 79%); $[\alpha]_D^{25}=-31$ (*c* 1, $CHCl_3$); ν_{max} (liquid film): 2932, 1734, 1602; δ_H (400 MHz, $CDCl_3$) −0.15, 0.04 (2s, 2×3H, $(CH_3)^2Si$), 0.62, (s, 9H, *t*-Bu), 3.75 (dd, 1H, $J_{5,6a}$=1.8 Hz, $J_{6a,6b}$=11.2 Hz, H-6a), 3.84 (dd, 1H, $J_{5,6b}$=5.3 Hz, $J_{6a,6b}$=11.2 Hz, H-6b), 3.98 (ddd, $J_{5,6a}$=1.8 Hz, $J_{5,6b}$=5.3 Hz, $J_{4,5}$=9.9 Hz,1H, H-5), 4.09 (m, 1H, CH_2=CH–CH_2–), 4.28 (m, 1H, CH_2=CH–CH_2–), 4.38 (dd, 1H, $J_{2,3}$=3.5 Hz, $J_{3,4}$=9.4 Hz, H-3), 5.03 (d, 1H, $J_{1,2}$=1.4 Hz, H-1), 5.24–5.36 (m, 2H, CH_2=CH–CH_2–), 5.40 (dd, $J_{1,2}$=1.4 Hz, $J_{2,3}$=3.5 Hz, 1H, H-2), 5.73 (dd, 1H, $J_{3,4}$=9.4 Hz, $J_{4,5}$=9.9 Hz, H-4), 5.91–5.96 (m, 1H, CH_2=C*H*–CH_2–), 7.20–8.30 (m, 25H, Ph). Anal. Calcd for $C_{48}H_{52}O_8Si$: C, 73.47; H, 6.63. Found: C, 73.51; H, 6.58.

3.1.2. Allyl 2,4-di-*O*-benzoyl-α-D-mannopyranoside (3). Compound **2** (2.5 g, 3.19 mmol) was dissolved into 90% aqueous TFA (15 mL) and the solution was stirred at rt for 4 h. Toluene (50 mL) was added and then the solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (petroleum ether–EtOAc, 1:1) to give **3** as a white foam (1.24 g, 91%); $[\alpha]_D^{25}=-26$ (*c* 1, $CHCl_3$); ν_{max} (liquid film): 3443, 2925, 1608; δ_H (400 MHz, $CDCl_3$) 3.74 (dd, 1H, $J_{5,6a}$=4.1 Hz, $J_{6a,6b}$=12.6 Hz, H-6a), 3.81 (dd, 1H, $J_{5,6b}$=2.3 Hz, $J_{6a,6b}$=12.6 Hz, H-6b), 3.98 (ddd, 1H, $J_{5,6b}$=2.3 Hz, $J_{5,6a}$=4.1 Hz, $J_{4,5}$=9.9 Hz, H-5), 4.09 (m, 1H, CH_2=CH–CH_2–), 4.26 (m, 1H, CH_2=CH–CH_2–), 4.46 (dd, 1H, $J_{2,3}$=3.5 Hz, $J_{3,4}$=9.9 Hz, H-3), 5.10 (d, 1H, $J_{1,2}$=1.6 Hz, H-1), 5.25–5.34 (m, 2H, CH_2=CH–CH_2–), 5.43 (dd, 1H, $J_{2,3}$=3.5 Hz, $J_{1,2}$=1.6 Hz, H-2), 5.51 (t, 1H, $J_{3,4}$=9.9 Hz, $J_{4,5}$=9.9 Hz, H-4), 5.91–5.96 (m, 1H, CH_2=C*H*–CH_2–), 7.25–8.10 (m, 10H, Ph). Anal. Calcd for $C_{23}H_{24}O_8$: C, 64.48; H, 5.61. Found: C, 64.40; H, 5.66.

3.1.3. Allyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)-[2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 6)]-2,4-di-*O*-benzoyl-α-D-mannopyranoside (7). To a cooled solution (0°C) of **3** (2.1 g, 4.91 mmol) and **6** (6.99 g, 10.3 mmol) in anhydrous CH_2Cl_2 (80 mL) was added TMSOTf (40 μL, 0.22 mmol). The mixture was stirred at this temperature for 2 h, and then quenched with Et_3N (2 drops). The solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (petroleum ether–EtOAc, 1.5:1) to give trisaccharide **7** as a syrup (5.73 g, 80%); $[\alpha]_D^{25}=-12$ (*c* 1, $CHCl_3$); ν_{max} (liquid film): 3442, 2926, 1730, 1452, 1270, 1110, 1070, 711 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.882, 2.095 (2s, 6H, 2CH_3CO), 3.726 (dd, 1H, $J_{5,6a}$=1.8 Hz, $J_{6a,6b}$=10.6 Hz, H-6a^A), 4.080–4.190 (m, 2H, H-6b^A, one proton of CH_2=CH–CH_2–), 4.285 (ddd, 1H, $J_{5,6a}$=1.8 Hz, $J_{5,6b}$=3.5 Hz, $J_{4,5}$=9.7 Hz, 1H, H-5^A), 4.300–4.550 (m, 7H, H-5^{B,C}, 2×H-6^{B,C}, one proton of CH_2=CH–CH_2–), 4.617 (dd, 1H, $J_{2,3}$=3.3 Hz, $J_{3,4}$=9.7 Hz, H-3^A), 4.979 (d, 1H, $J_{1,2}$=1.6 Hz, H-1^B), 5.110–5.130 (m, 2H, H-1^A, H-2^C), 5.189 (d, 1H, $J_{1,2}$=1.8 Hz, H-1^C), 5.290–5.507 (m, 2H, CH_2=CH–CH_2–), 5.513 (dd, 1H, $J_{1,2}$=1.6 Hz, $J_{2,3}$=2.9 Hz, H-2^B), 5.582 (dd, 1H, $J_{2,3}$=3.3 Hz, $J_{3,4}$=9.8 Hz, H-3^C), 5.690 (dd, 1H, $J_{1,2}$=1.6 Hz, $J_{2,3}$=3.6 Hz, H-2^A), 5.793 (t, 1H, $J_{3,4}$=9.8 Hz, $J_{4,5}$=9.8 Hz, H-4^C), 5.841 (t, 1H, $J_{3,4}$=9.7 Hz, $J_{4,5}$=9.7 Hz, H-4^A), 5.865–5.890 (m, 3H, H-3^B, H-4^B, CH_2=C*H*–CH_2–), 7.252–8.093 (m, 40H, Ph). Anal. Calcd for $C_{81}H_{72}O_{26}$: C, 66.58; H, 4.93. Found: C, 66.61; H, 4.94.

3.1.4. Allyl 3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)-[3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 6)]-2,4-di-*O*-benzoyl-α-D-mannopyranoside (8). To a cooled solution (0°C) of **7** (720 mg, 0.49 mmol) in CH_2Cl_2 (10 mL) and MeOH (10 mL) was added acetyl chloride (1 mL). The mixture was stirred at rt overnight, then diluted with CH_2Cl_2 (200 mL), neutralized with Et_3N and washed with water. The organic phase was dried over anhydrous Na_2SO_4 and concentrated. The residue was purified on a silica gel column using EtOAc–petroleum ether (1:2) as the elutant giving **8** as a syrup (526 mg, 78%); $[\alpha]_D^{25}=-6$ (*c* 1, $CHCl_3$); ν_{max} (liquid film): 3444, 2960, 1731, 1603; δ_H (400 MHz, $CDCl_3$) 2.300–2.500 (br d, 2H, 2O*H*), 3.797 (dd, 1H, $J_{5,6a}$=1.2 Hz, $J_{6a,6b}$=10.8 Hz, H-6a^A), 4.05–4.460 (m, 12H, H-6b^A, H-2^{B,C}, CH_2=CH–CH_2–, H-5^{A,B,C}, 2×H-6^{B,C}), 4.618 (dd, 1H, $J_{2,3}$=3.4 Hz, $J_{3,4}$=8.7 Hz, H-3^A), 5.070 (bs, 1H, H-1^B), 5.154 (d, 1H, $J_{1,2}$=2.0 Hz, H-1^A), 5.229 (bs, 1H, H-1^C), 5.260–5.440 (m, 2H, CH_2=CH–CH_2–), 5.450 (dd, 1H, $J_{2,3}$=3.1 Hz, $J_{3,4}$=9.7 Hz, H-3^C), 5.673 (dd, 1H, $J_{1,2}$=2.0 Hz, $J_{2,3}$=3.4 Hz, H-2^A), 5.714 (dd,

1H, $J_{2,3}$=3.1 Hz, $J_{3,4}$=10.0 Hz, H-3^{B}), 5.841 (t, 1H, $J_{3,4}$=9.7 Hz, $J_{4,5}$=9.7 Hz, H-4^{C}), 5.865 (t, 1H, $J_{3,4}$=10.0 Hz, $J_{4,5}$=10.0 Hz, H-4^{B}), 5.930 (t, 1H, $J_{3,4}$=8.7 Hz, $J_{4,5}$=8.7 Hz, H-4^{A}), 5.955 (m, 1H, CH_2=C*H*–CH_2–), 7.258–8.201 (m, 40H, Ph). Anal. Calcd for $C_{77}H_{68}O_{24}$: C, 67.15; H, 4.94. Found: C, 67.09; H, 4.99.

3.1.5. Allyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 6)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)]-2,4-di-*O*-benzoyl-α-D-mannopyranoside (10). To a cooled solution (0°C) of **8** (200 mg, 0.14 mmol) and **9** (290 mg, 0.39 mmol) in anhydrous CH_2Cl_2 (7 mL) was added TMSOTf (5 μL, 0.028 mmol). The mixture was stirred at this temperature for 2 h, and then quenched with Et_3N (1 drop). The solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography twice (petroleum ether–EtOAc, 2:1 and toluene–petroleum ether–EtOAc, 0.75:1.5:1.0) to give pentasaccharide **10** as white solid (290 mg, 82%); $[\alpha]_D^{25}$=−49 (*c* 1, $CHCl_3$); ν_{max} (liquid film): 3440, 2958, 1730, 1603, 1452, 1270, 1110, 1071; δ_H (400 MHz, $CDCl_3$) 3.647 (dd, 1H, $J_{5,6a}$<1 Hz, $J_{6a,6b}$=10.4 Hz, H-6a^{A}), 3.987 (dd, 1H, $J_{5,6b}$=5.0 Hz, $J_{6a,6b}$=10.4 Hz, H-6b^{A}), 4.032 (bs, 1H, H-2^{C}), 4.179–4.230 (m, 3H, H-5^{A}, *J*=5.1 Hz, 12.0 Hz, 2H-6), 4.319 (bs, 1H, H-2^{B}), 4.320–4.500 (m, 6H), 4.502 (s, 1H, H-1^{E}), 4.505–4.635 (m, 6H), 4.640 (dd, 1H, $J_{2,3}$=2.5 Hz, $J_{3,4}$=9.9 Hz, H-3^{A}), 5.090 (s, 1H, H-1^{D}), 5.184 (s, 1H, H-1^{A}), 5.205 (s, 1H, H-1^{B}), 5.312, 5.550 (2dd, 2H, *J*=1.2 Hz, 11.2, 16.8 Hz, C*H*$_2$=CH–CH_2–), 5.481 (s, 1H, H-1^{C}), 5.563 (bs, 1H, H-2^{E}), 5.688 (dd, $J_{2,3}$=2.1 Hz, $J_{3,4}$=10.6 Hz, H-3^{C}), 5.741 (bs, 1H, H-2^{A}), 5.825 (s, 1H, H-2^{D}), 5.827–6.005 (m, 8H, H-4^{A}, H-3^{E}, H-4^{E}, H-4^{C}, H-3^{B}, H-4^{B}, H-3^{D}, CH_2=C*H*–CH_2–), 6.095 (t, 1H, $J_{3,4}$=10.1 Hz, $J_{4,5}$=10.1 Hz, H-4^{D}), 7.245–8.140 (m, 80H, Ph). δ_C (100 MHz, $CDCl_3$) 166.31, 166.19 (2C), 166.00, 165.94, 165.57, 165.50, 165.35, 165.27, 165.23, 165.03, 164.94, 164.88, 164.83, 164.80, 164.51 (16PhCO), 118.89 (*C*H_2=CH–CH_2–), 100.00, 99.72, 99.39, 98.21, 96.66 (5C-1, $^{1}J_{C\text{-}1,H\text{-}1}$=169, 170, 170, 171, 173 Hz), 78.4, 77.47, 77.21, 75.21, 71.96, 70.57, 70.07, 69.89, 69.74 (3C), 69.63, 69.53, 69.47, 69.23, 68.70 (CH_2=CH–*C*H_2–), 68.75 (2C), 67.19, 67.13, 66.61, 66.37, 63.65, 63.50, 62.82, 62.62 (sugar carbons). MALDI-TOF MS: M+Na calcd: 2555, found: 2555.62; Anal. Calcd for $C_{145}H_{120}O_{42}$: C, 68.72; H, 4.94. Found: C, 68.76; H, 4.88.

3.1.6. Allyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 6)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)]-2,4-di-*O*-benzoyl-α-D-mannopyranoside (12). To a cooled solution (0°C) of **8** (200 mg, 0.14 mmol) and **11** (208 mg, 0.36 mmol) in anhydrous CH_2Cl_2 (5 mL) was added TMSOTf (3 μL, 0.016 mmol). The mixture was stirred at this temperature for 2 h, and then quenched with Et_3N (1 drop). The solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography twice (petroleum ether–EtOAc, 1.0:1 and toluene–petroleum ether–EtOAc, 0.2:1.0:1.0) to give pentasaccharide **12** as a syrup (167 mg, 50%); $[\alpha]_D^{25}$=−48 (*c* 1, $CHCl_3$); ν_{max} (liquid film): 3442, 2932, 1743, 1455, 1259; δ_H (400 MHz, $CDCl_3$) 1.780, 1.821, 1.834, 1.852, 1.964, 1.974 (6s, 6×3H, 6C*H*$_3$CO), 3.370 (dd, 1H, $J_{5,6a}$=1.6 Hz, $J_{6a,6b}$=11.4 Hz, H-6a^{A}), 3.529 (dd, 1H, $J_{5,6b}$=3.7 Hz, $J_{6a,6b}$=11.4 Hz, H-6b^{A}), 3.631 (dd, 1H, $J_{5,6}$=1.1 Hz, $J_{6a,6b}$=10.8 Hz, H-6), 3.620–3.690 (m, 2H, H-6), 3.702–3.780 (m, 2H, H-5, H-6), 3.800 (dd, 1H, $J_{5,6}$=3.8 Hz, $J_{6a,6b}$=10.8 Hz, H-6), 3.900 (dd, 1H, $J_{5,6}$=3.7, $J_{6a,6b}$=11.0 Hz, H-6), 3.995 (dd, 1H, $J_{1,2}$<1.0 Hz, $J_{2,3}$=3.2 Hz, H-2^{C}), 4.050–4.260 (m, 6H, H-2^{E}, H-5^{A,C,D,E}, 2H-6) 4.269 (m, 1H, H-5^{B}), 4.350–4.437 (m, 3H, H-3^{A}, CH_2=CH–C*H*$_2$–), 4.462 (dd, 1H, $J_{1,2}$<1.0 Hz, $J_{2,3}$=3.4 Hz, H-2^{B}), 4.627 (d, 1H, $J_{1,2}$<1.0 Hz, H-1^{B}), 4.688 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1^{E}), 4.795 (t, $J_{1,2}$=8.9 Hz, $J_{2,3}$=8.9 Hz, H-2^{D}), 4.914 (s, 1H, H-1^{C}), 5.011 (t, 1H, $J_{3,4}$=8.7 Hz, $J_{4,5}$=8.7 Hz, H-4^{E}), 5.070 (d, 1H, $J_{1,2}$<1 Hz, H-1^{A}), 5.163 (dd, 1H, $J_{2,3}$=3.2 Hz, $J_{3,4}$=10.1 Hz, H-3^{C}), 5.300–5.480 (m, 4H, $J_{1,2}$=8.8 Hz, $J_{3,4}$=9.8 Hz, H-1^{D}, H-4^{D}, C*H*$_2$=CH–CH_2–), 5.493 (t, 1H, $J_{3,4}$=10.1 Hz, $J_{4,5}$=10.1 Hz, H-4^{C}), 5.520 (dd, 1H, $J_{1,2}$<1 Hz, $J_{2,3}$=3.2 Hz, H-2^{A}), 5.555 (dd, 1H, $J_{2,3}$=3.4 Hz, $J_{3,4}$=9.2 Hz, H-3^{B}), 5.677 (t, 1H, $J_{3,4}$=10.4 Hz, $J_{4,5}$=10.4 Hz, H-4^{A}), 5.701 (t, 1H, $J_{2,3}$=9.3 Hz, $J_{3,4}$=9.3 Hz, H-3^{D}), 5.783 (t, 1H, $J_{2,3}$=10.3 Hz, $J_{3,4}$=10.3 Hz, H-3^{E}), 5.900–6.000 (m, 1H, CH_2=C*H*–CH_2–), 7.250–8.307 (m, 48H, Ph). δ_C (100 MHz, $CDCl_3$) 96.60 ($^{1}J_{C\text{-}1,H\text{-}1}$=170 Hz), 98.20 ($^{1}J_{C\text{-}1,H\text{-}1}$=173 Hz), 98.50 ($^{1}J_{C\text{-}1,H\text{-}1}$=162 Hz), 99.10 ($^{1}J_{C\text{-}1,H\text{-}1}$=165 Hz), 99.40 ($^{1}J_{C\text{-}1,H\text{-}1}$=174 Hz). MALDI-TOF MS: M+Na calcd: 2233, found: 2233.99; Anal. Calcd for $C_{117}H_{106}N_2O_{42}$: C, 63.53; H, 4.80. Found: C, 63.47; H, 4.76.

3.1.7. Allyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 6)]-2,4-di-*O*-benzoyl-α-D-mannopyranoside (13). To a cooled solution (0°C) of **3** (1.5 g, 3.5 mmol) and **9** (5.45 g, 7.36 mmol) in anhydrous CH_2Cl_2 (50 mL) was added TMSOTf (25 μL, 0.14 mmol). The mixture was stirred at this temperature for 2 h, and then quenched with Et_3N (2 drops). The solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (petroleum ether–EtOAc, 1.5:1) to give trisaccharide **13** as a syrup (4.72 g, 85%); $[\alpha]_D^{25}$=−271 (*c* 1, $CHCl_3$); ν_{max} (liquid film): 3444, 2956, 1731, 1602, 1452, 1267, 1109, 1070, 710 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 3.80 (dd, 1H, $J_{5,6}$<1 Hz, $J_{6a,6b}$=9.1 Hz, H-6), 4.15–4.25 (m, 2H, 2H-6), 4.32–4.42 (m, 4H, H-5, 3H-6), 4.50–4.56 (m, 2H, 2H-5), 4.58–4.64 (m, 2H, CH_2=CH–C*H*$_2$–), 4.70 (dd, 1H, $J_{2,3}$=3.2 Hz, $J_{3,4}$=11.0 Hz, H-3), 5.16 (d, 1H, $J_{1,2}$=1.4 Hz, H-1), 5.20 (d, 1H, $J_{1,2}$=1.2 Hz, H-1), 5.30–5.35 (m, 1H, C*H*$_2$=CH–CH_2–), 5.36–5.39 (m, 2H, H-1, H-2), 5.41–5.50 (m, 1H, C*H*$_2$=CH–CH_2–), 5.76 (dd, 1H, $J_{2,3}$=3.1 Hz, $J_{3,4}$=12 Hz, H-3), 5.77 (dd, 1H, $J_{1,2}$=1.6 Hz, $J_{2,3}$=3.3 Hz, H-2), 5.79 (dd, 1H, $J_{1,2}$=1.8 Hz, $J_{2,3}$=3.1 Hz, H-2), 5.94 (t, 1H, $J_{3,4}$=10.4 Hz, $J_{4,5}$=10.4 Hz, H-4), 6.00–6.06 (m, 3H, H-3, H-4, CH_2=C*H*–CH_2–), 6.15 (t, 1H, $J_{3,4}$=10.6 Hz, $J_{4,5}$=10.6 Hz, H-4), 7.25–8.17 (m, 50H, Ph). δ_C (100 MHz, $CDCl_3$) 166.14, 166.02, 165.99, 165.56, 165.41, 165.18, 165.11 (2C), 164.59, 164.57 (Ph*C*O), 118.66 (*C*H_2=CH–CH_2–), 99.54, 97.43, 96.48 (3C-1, $^{1}J_{C\text{-}1,H\text{-}1}$=170, 170, 173 Hz), 76.52, 71.87, 70.19, 70.13, 70.00, 69.68, 69.53, 69.27, 68.79, 68.71 (CH_2=CH–*C*H_2–), 68.45, 66.88, 66.53, 66.35, 62.57, 62.42. Anal. Calcd for $C_{91}H_{76}O_{26}$: C, 68.94; H, 4.80. Found: C, 68.97; H, 4.78.

3.1.8. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 6)]-2,4-di-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (14). A solution of **13** (850 mg, 0.54 mmol) in 90% aqueous acetic acid (10 mL) was added NaOAc (177 mg, 2.16 mmol) and $PdCl_2$ (191 mg, 1.08 mmol). The mixture was stirred at rt overnight and then neutralized with saturated aqueous $NaHCO_3$. The mixture was extracted with CH_2Cl_2 (3×50 mL) and the organic phase was concentrated. The residue was purified by a silica gel column chromatography to give hemiacetal trisaccharide intermediate as syrup. A solution of the above syrup, CCl_3CN (0.22 mL, 4 equiv.) and DBU (0.02 mL) in dry CH_2Cl_2 (5 mL) was stirred at rt for 2 h. The solvents were removed in vacuo. The residue was purified by silica gel flash column chromatography to give trichloroacetimidate **14** (752 mg, 83%) as a white foam; $[\alpha]_D^{25}=-40$ (*c* 1, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 3.84 (dd, 1H, $J_{5,6a}<1$ Hz, $J_{6a,6b}=9.2$ Hz, H-6a), 4.15 (dd, 1H, $J_{5,6b}=6.3$ Hz, $J_{6a,6b}=11.0$ Hz, H-6b), 4.30–4.37 (m, 2H), 4.48–4.58 (m, 5H), 4.73 (dd, 1H, $J_{2,3}=3.4$ Hz, $J_{3,4}=9.8$ Hz, H-3), 5.10 (d, 1H, $J_{1,2}=1.2$ Hz, H-1), 5.38–5.41 (m, 2H, H-1, H-2), 5.69–5.73 (m, 2H, H-2, H-3), 5.90–5.95 (m, 2H), 6.01 (t, 1H, $J_{3,4}=10.8$ Hz, $J_{4,5}=10.8$ Hz, H-4), 6.07 (t, 1H, $J_{3,4}=10.0$ Hz, $J_{4,5}=10.0$ Hz, H-4), 6.12 (t, 1H, $J_{3,4}=10.0$ Hz, $J_{4,5}=10.0$ Hz, H-4), 6.59 (d, 1H, $J_{1,2}=1.5$ Hz, H-1), 7.00–8.13 (m, 50H, Ph), 9.00 (s, 1H, N*H*). Anal. Calcd for $C_{90}H_{72}Cl_3NO_{26}$: C, 63.96; H, 4.26; found: C, 63.88; H, 4.24.

3.1.9. Allyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)-2,4-di-*O*-benzoyl-α-D-mannopyranoside (15). To a stirred solution of **2** (2 g, 2.55 mmol) in THF (50 mL) was added TBAF (800 mg, 2.55 mmol). The mixture was stirred at rt for 4 h, then evaporated to dryness (<40°C water both) under reduced pressure. The residue was purified by silica gel column chromatography to give **4** as a white solid (1.13 g, 66%). To a cooled solution (−15°C) of **4** (1.062 g, 1.58 mmol) and **9** (1.23 g, 1.66 mmol) in dry CH_2Cl_2 (15 mL) was added TMSOTf (20 μL, 0.11 mmol), and the mixture was stirred at this temperature for 40 min, another portion of TMSOTf (50 μL) was added. The reaction was warmed up to rt and stirred for 2 h further, then concentrated. The residue was purified by silica gel column chromatography to give **15** as a white solid (1.276 g, 80%); $[\alpha]_D^{25}=-42$ (*c* 1.4, $CHCl_3$); ν_{max} (liquid film): 3444, 2926, 1731, 1602, 1452, 1317, 1266, 1110, 1070, 1028, 711 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 2.89 (bs, H, O*H*), 3.75–3.80 (m, 2H, 2H-6), 3.92–3.95 (m, 1H, H-5), 4.07 (ddd, 1H, $J_{4',5'}=10.0$ Hz, $J_{5',6'a}=3.6$ Hz, $J_{5',6'b}=2.4$ Hz, H-5′), 4.18–4.25 (m, 1H, CH_2═CH–CH_2–), 4.35 (dd, 1H, $J_{5',6'a}=3.6$ Hz, $J_{6'a,6'b}=12.3$ Hz, H-6′a), 4.44–4.50 (m, 1H, CH_2═CH–CH_2–), 4.61 (dd, 1H, $J_{5',6'b}=2.4$ Hz, $J_{6'a,6'b}=12.3$ Hz, H-6′b), 4.70 (dd, 1H, $J_{2,3}=3.5$ Hz, $J_{3,4}=9.8$ Hz, H-3), 5.15 (d, 1H, $J_{1,2}=1.4$ Hz, H-1), 5.23–5.36 (m, 3H, H-2 and CH_2═CH–CH_2–), 5.41 (d, 1H, $J_{1',2'}=1.7$ Hz, H-1′), 5.65–5.69 (m, 2H, H-2′, H-3′), 5.72 (t, 1H, $J_{3,4}=10.0$ Hz, $J_{4,5}=10.0$ Hz, H-4), 5.89 (m, 1H, CH_2═C*H*–CH_2–), 6.02 (t, 1H, $J_{3',4'}=10.0$ Hz, $J_{4',5'}=10.0$ Hz, H-4′), 7.19–8.25 (m, 30H, Ph). Anal. Calcd for $C_{57}H_{50}O_{17}$: C, 67.99; H, 4.97. Found: C, 67.96; H, 4.99.

3.1.10. Allyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 6)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)]-2,4-di-*O*-benzoyl-α-D-mannopyranosyl-(1 → 6)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)]-2,4-di-*O*-benzoyl-α-D-mannopyranoside (16). To a cooled solution (0°C) of **14** (2 g, 1.18 mmol) and **15** (1.13 g, 1.12 mmol) in dry CH_2Cl_2 (15 mL) was added TMSOTf (20 μL). The mixture was stirred at this temperature for 2 h, then neutralized with Et_3N (2 drops), and concentrated. The residue was purified by silica gel column chromatography to give **16** as a syrup (2.42 g, 85%); $[\alpha]_D^{25}=-54$ (*c* 1.5, $CHCl_3$); ν_{max} (liquid film): 3443, 2957, 1730, 1603, 1452, 1266, 1110, 1070, 711; δ_H (400 MHz, $CDCl_3$) 3.44 (br d, 1H, $J_{6a,6b}=9.2$ Hz, H-6), 3.82 (dd, 1H, $J_{5,6b}=1.7$ Hz, $J_{6a,6b}=9.2$ Hz, H-6), 4.01 (dd, 1H, $J_{5,6b}=6.8$ Hz, $J_{6a,6b}=12.0$ Hz, H-6), 4.10–4.15 (m, 1H, H-5), 4.20–4.53 (m, 12H), 4.58 (dd, 1H, $J_{5,6b}=3.5$ Hz, $J_{6a,6b}=10.1$ Hz, H-6), 4.67 (dd, 1H, $J_{2,3}=3.2$ Hz, $J_{3,4}=10.2$ Hz, H-3), 4.73 (dd, 1H, $J_{2,3}=3.4$ Hz, $J_{3,4}=10.0$ Hz, H-3), 4.78 (d, 1H, $J_{1,2}=1.5$ Hz, H-1), 5.17–5.19 (m, 2H, H-1 and one proton of CH_2═CH–CH_2–), 5.21 (d, 1H, $J_{1,2}=1.2$ Hz, H-1), 5.29–5.32 (m, 3H, H-1, H-2, and one proton of CH_2═CH–CH_2–), 5.37 (d, 1H, $J_{1,2}=1.6$ Hz, H-1), 5.44 (dd, 1H, $J_{1,2}=2.0$ Hz, $J_{2,3}=3.0$ Hz, H-2), 5.57 (dd, 1H, $J_{1,2}=2.0$ Hz, $J_{2,3}=3.0$ Hz, H-2), 5.65 (dd, 1H, $J_{2,3}=2.8$ Hz, $J_{3,4}=10.0$ Hz, H-3), 5.69–5.74 (m, 2H, H-2, H-3), 5.82 (dd, 1H, $J_{1,2}=1.5$ Hz, $J_{2,3}=3.4$ Hz, H-2), 5.85–6.01 (m, 4H, H-3, 2H-4, CH_2═C*H*–CH_2–), 6.04 (t, 1H, $J_{2,3}=10.1$ Hz, $J_{3,4}=10.1$ Hz, H-4), 6.07 (t, 1H, $J_{2,3}=10.8$ Hz, $J_{3,4}=10.8$ Hz, H-4), 6.08 (t, 1H, $J_{2,3}=10.0$ Hz, $J_{3,4}=10.0$ Hz, H-4), 7.18–8.35 (m, 80H, Ph); δ_C (100 MHz, $CDCl_3$) 166.13, 166.10, 166.08, 166.05, 165.92, 165.75, 165.57, 165.47, 165.17, 165.14, 165.06, 165.02, 164.65 (2C), 164.62, 164.55 (16 Ph*C*O), 118.41 (*C*H_2═CH–CH_2–), 100.07, 99.61, 97.54, 97.31, 96.67 (5C-1, $^1J_{C\text{-}1,H\text{-}1}=171$, 172, 172, 173, 174 Hz), 77.71, 77.24, 72.06, 72.00, 70.20, 70.14, 69.57, 69.50, 69.45, 69.37, 69.32, 68.77, 68.63, 68.20, 68.08, 66.45, 66.27, 66.13, 62.50, 62.43. Anal. Calcd for $C_{145}H_{120}O_{42}$: C, 68.72; H, 4.74. Found: C, 68.79; H, 4.81.

3.1.11. Methyl 2,4-di-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilyl-α-D-mannopyranoside (17). 2.0 equiv. of $FeCl_3{\cdot}6H_2O$ was added to a mixture of methyl 2,4-di-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilyl-6-*O*-triphenylmethyl-α-D-mannopyranoside (3 g, 3.96 mmol) in CH_2Cl_2 (25 mL). The mixture was stirred for 2 h at rt, then diluted with more CH_2Cl_2 (50 mL), washed with ice-cold water twice. The washings were re-extracted with CH_2Cl_2 (20 mL). The organic phase was combined, dried and concentrated, then subjected to column chromatography on silica gel with petroleum ether–EtOAc (4:1) as the eluent to give syrupy **17** (1.78 g, 87%); $[\alpha]_D^{25}=-255$ (*c* 0.8, $CHCl_3$); ν_{max} (liquid film): 3443, 3066, 2927, 1319, 1452, 1267, 1110, 1070, 1030; δ_H (400 MHz, $CDCl_3$) −0.08, 0.06 (2s, 2×3H, Si(CH_3)$_2$), 0.62 (s, 9H, *t*-Bu), 2.50 (bs, 1H, O*H*), 3.46 (s, 3H, OCH_3), 3.71 (dd, 1H, $J_{5,6a}=4.4$ Hz, $J_{6a,6b}=12.6$ Hz, H-6a), 3.75 (dd, 1H, $J_{5,6b}=2.4$ Hz, $J_{6a,6b}=12.6$ Hz, H-6b), 3.88 (ddd, 1H, $J_{4,5}=9.4$ Hz, $J_{5,6a}=4.4$ Hz, $J_{5,6b}=2.4$ Hz, H-5), 4.44 (dd, 1H, $J_{2,3}=3.6$ Hz, $J_{3,4}=9.4$ Hz, H-3), 4.89 (d, 1H, $J_{1,2}=1.6$ Hz, H-1), 5.37 (dd, 1H, $J_{1,2}=1.6$ Hz, $J_{2,3}=3.6$ Hz, H-2), 5.55 (t, 1H, $J_{3,4}=9.4$ Hz, $J_{4,5}=9.4$ Hz, H-4), 7.40–8.14 (m, 10H, Ph). Anal. Calcd for $C_{27}H_{36}O_8Si$: C, 62.79; H, 6.98. Found: C, 62.82; H, 6.92.

3.1.12. Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 6)-2,4-di-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilyl-α-D-mannopyranoside (18). To a cooled solution (0°C) of **17** (850 mg, 1.65 mmol) and **6** (1.28 g, 1.89 mmol) in anhydrous CH_2Cl_2 (15 mL) was added TMSOTf (25 μL, 0.14 mmol). The mixture was stirred at this temperature for 1 h, and then quenched with Et_3N (2 drops). The solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (petroleum ether–EtOAc, 2:1) to give disaccharide **18** as a syrup (1.43 g, 84%); $[\alpha]_D^{25}=-103$ (*c* 1.1, $CHCl_3$); ν_{max} (liquid film): 3440, 2925, 1730; δ_H (400 MHz, $CDCl_3$) −0.11, 0.06 (2s, 2×3H, Si(CH_3)$_2$), 0.63 (s, 9H, *t*-Bu), 2.05 (s, 3H, CH_3CO), 3.56 (s, 3H, OCH_3), 3.67 (dd, 1H, $J_{5,6a}$=2.2 Hz, $J_{6a,6b}$=10.5 Hz, H-6a), 4.04 (dd, 1H, $J_{5,6b}$=6.4 Hz, $J_{6a,6b}$=10.5 Hz, H-6b), 4.15 (ddd, 1H, $J_{4,5}$=9.6 Hz, $J_{5,6a}$=2.2 Hz, $J_{5,6b}$=6.4 Hz, H-5), 4.32 (ddd, 1H, $J_{4',5'}$=9.6 Hz, $J_{5',6a'}$<1.0 Hz, $J_{5',6b'}$=5.2 Hz, H-5′), 4.38–4.47 (m, 3H, H-3, 2H-6′), 4.88 (d, 1H, $J_{1,2}$=1.5 Hz, H-1), 4.94 (d, 1H, $J_{1',2'}$=1.7 Hz, H-1′), 5.40 (dd, 1H, $J_{1,2}$=1.5 Hz, $J_{2,3}$=3.6 Hz, H-2), 5.44 (dd, 1H, $J_{1',2'}$=1.7 Hz, $J_{2',3'}$=3.1 Hz, H-2′), 5.67 (dd, 1H, $J_{3,4}$=10.0 Hz, $J_{4,5}$=9.6 Hz, H-4), 5.82 (dd, $J_{2',3'}$=3.1 Hz, $J_{3',4'}$=10.8 Hz, H-3′), 5.88 (dd, 1H, $J_{3',4'}$=10.8 Hz, $J_{4',5'}$=9.6 Hz, H-4′), 7.36–8.11 (m, 25H, Ph). Anal. Calcd for $C_{56}H_{60}O_{17}Si$: C, 65.12; H, 5.81. Found: C, 65.09; H, 5.88.

3.1.13. Methyl 3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 6)-2,4-di-*O*-benzoyl-α-D-mannopyranoside (19). To a cooled solution (0°C) of **18** (916 mg, 0.89 mmol) in CH_2Cl_2 (7 mL) and MeOH (7 mL) was added acetyl chloride (1 mL). After 12 h, another portion of AcCl (0.4 mL) was added. The mixture was further stirred at rt for 24 h, then diluted with CH_2Cl_2 (50 mL), neutralized with Et_3N and washed with water. The organic phase was concentrated. The residue was purified on a silica gel column using EtOAc–petroleum ether (5:4) as the elutant gave **19** as a syrup (544 mg, 70%); $[\alpha]_D^{25}=+25$ (*c* 0.5, $CHCl_3$); ν_{max} (liquid film): 3444, 3067, 2926, 1731; δ_H (400 MHz, $CDCl_3$) 2.04 (bs, 1H, O*H*), 2.31 (bs, 1H, O*H*), 3.52 (s, 3H, OCH_3), 3.75 (dd, 1H, $J_{5,6a}$=1.9 Hz, $J_{6a,6b}$=10.9 Hz, H-6a), 4.08–4.28 (m, 5H, H-5, H-5′, H-6b, H-6′a, H-6′b), 4.36 (bs, 1H, H-2′), 4.40 (dd, 1H, $J_{2,3}$=3.5 Hz, $J_{3,4}$=10.1 Hz, H-3), 4.96 (d, 1H, $J_{1,2}$=1.3 Hz, H-1), 5.04 (d, 1H, $J_{1',2'}$=1.2 Hz, H-1′), 5.44 (dd, 1H, $J_{1,2}$=1.3 Hz, $J_{2,3}$=3.5 Hz, H-2), 5.74 (dd, 1H, $J_{2',3'}$=3.2 Hz, $J_{3',4'}$=6.1 Hz, H-3′), 5.77 (t, 1H, $J_{3,4}$=10.1 Hz, $J_{4,5}$=10.1 Hz, H-4), 7.25–8.17 (m, 25H, Ph). Anal. Calcd for $C_{48}H_{44}O_{16}$: C, 65.75; H, 5.02. Found: C, 65.69; H, 5.10.

3.1.14. Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 6)-[2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)]-2,4-di-*O*-benzoyl-α-D-mannopyranoside (20). To a cooled solution (0°C) of **19** (370 mg, 0.42 mmol) and **6** (630 mg, 0.93 mmol) in anhydrous CH_2Cl_2 (15 mL) was added TMSOTf (20 μL, 0.11 mmol). The mixture was stirred at this temperature for 3 h, and then quenched with Et_3N (2 drops). The solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (petroleum ether–EtOAc, 2:3) to give tetrasaccharide **20** as a syrup (588 mg, 73%); $[\alpha]_D^{25}=-16$ (*c* 1.5, $CHCl_3$); ν_{max} (liquid film): 3441, 2928, 1730, 1452, 1269, 1108, 1068, 756, 710 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.87, 2.05 (2 s, 6H, 2CH_3CO), 3.48 (s, 3H, CH_3), 3.63 (dd, 1H, $J_{5,6a}$=2.4 Hz, $J_{6a,6b=}$11 Hz, H-6a^A), 3.98 (dd, 1H, $J_{5,6b}$=5.8 Hz, $J_{6a,6b}$=11 Hz, H-6b^A), 4.15 (ddd, 1H, $J_{4,5}$=9.5 Hz, $J_{5,6a}$=2.4 Hz, $J_{5,6b}$=5.8 Hz, H-5^A), 4.26 (dd, $J_{1,2}$=1.6 Hz, $J_{2,3}$=2.1 Hz, H-2^B), 4.35–4.56 (m, 10H, H-3^A, H-5^B,5^C,5^D, 2H-6^B, 2H-6^C, 2H-6^D), 4.95 (d, 1H, $J_{1,2}$=1.5 Hz, H-1^A), 4.96 (d, 1H, $J_{1,2}$=1.6 Hz, H-1^B), 5.12 (dd, 1H, $J_{1,2}$=1.2 Hz, $J_{2,3}$=3.2 Hz, H-2^C), 5.14 (d, 1H, $J_{1,2}$=1.7 Hz, H-1^D), 5.16 (d, 1H, $J_{1,2}$=1.2 Hz, H-1^C), 5.57 (dd, 1H, $J_{2,3}$=3.2 Hz, $J_{3,4}$=9.9 Hz, H-3^C), 5.60 (dd, 1H, $J_{1,2}$=1.7 Hz, $J_{2,3}$=3.6 Hz, H-2^D), 5.66 (dd, 1H, $J_{1,2}$= 1.5 Hz, $J_{2,3}$=3.8 Hz, H-2^A), 5.75 (t, 1H, $J_{3,4}$=9.9 Hz, $J_{4,5}$=9.9 Hz, H-4^C), 5.80 (t, 1H, $J_{3,4}$=9.5 Hz, $J_{4,5}$=9.5 Hz, H-4^A), 5.83–5.89 (m, 3H, H-3^B, H-3^D, H-4^D), 5.96 (t, 1H, $J_{3,4}$=9.8 Hz, $J_{4,5}$=9.8 Hz, H-4^B), 7.25–8.40 (m, 55H, Ph). Anal. Calcd for $C_{106}H_{92}O_{34}$: C, 66.67; H, 4.82. Found: C, 66.71; H, 4.78.

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