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Efficient and practical syntheses of mannose tri-, tetra-, penta-, hexa-, hepta-, and octasaccharides existing in *N*-glycans

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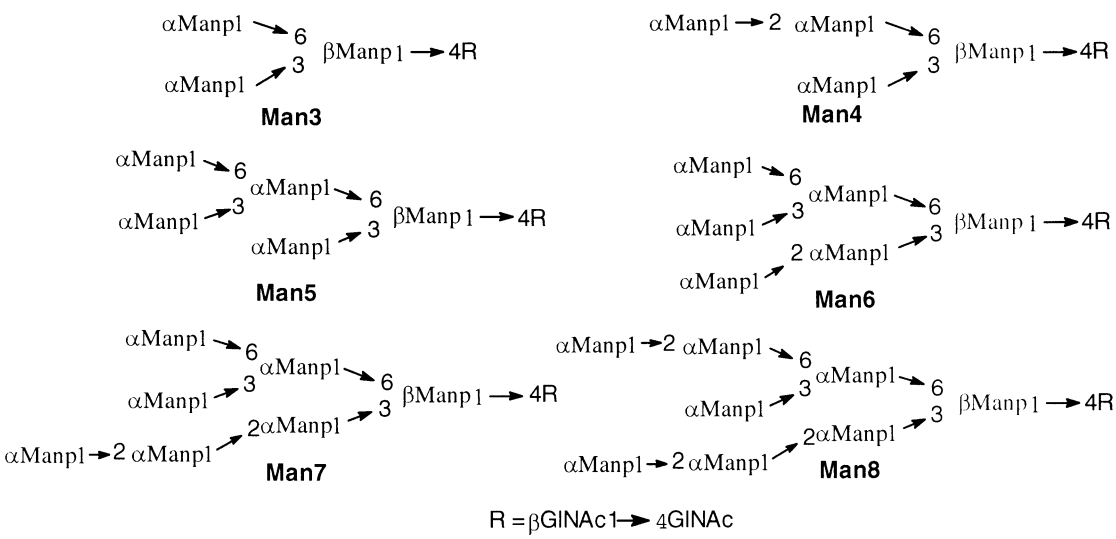
Abstract—An efficient and practical regio- and stereoselective synthesis of mannose tri-, tetra-, penta-, hexa-, hepta-, and octasaccharides of *N*-glycans was achieved using simple mannosyl derivatives as the starting materials.

1. Introduction

N-Linked glycans play a vital role in fundamental biological processes such as cell differentiation, malignant transformation, viral, bacterial and parasitic infections and protein transportations.[1] Recent studies have revealed that the mannose oligosaccharides in *N*-glycans are required for human CD2 adhesion function[2] and high mannose oligosaccharides are related to HIV infection.[3] For a study of the details of the recognition mechanism, chemically synthesized oligosaccharides are required because such homogeneous oligosaccharide samples are obtainable in only very small amounts from natural sources. Remarkable progress has been made in the field of *N*-glycan synthesis,[4] however, multiple steps and orthogonal masking groups are needed in most of the syntheses making the work complex and time consuming. We present herein a very efficient and practical method for the synthesis of mannose oligosaccharides (**Man3**, **Man4**, **Man5**, **Man6**, **Man7**, and **Man8**) for *N*-glycans,[5] as shown in Scheme 1.



Scheme 1.

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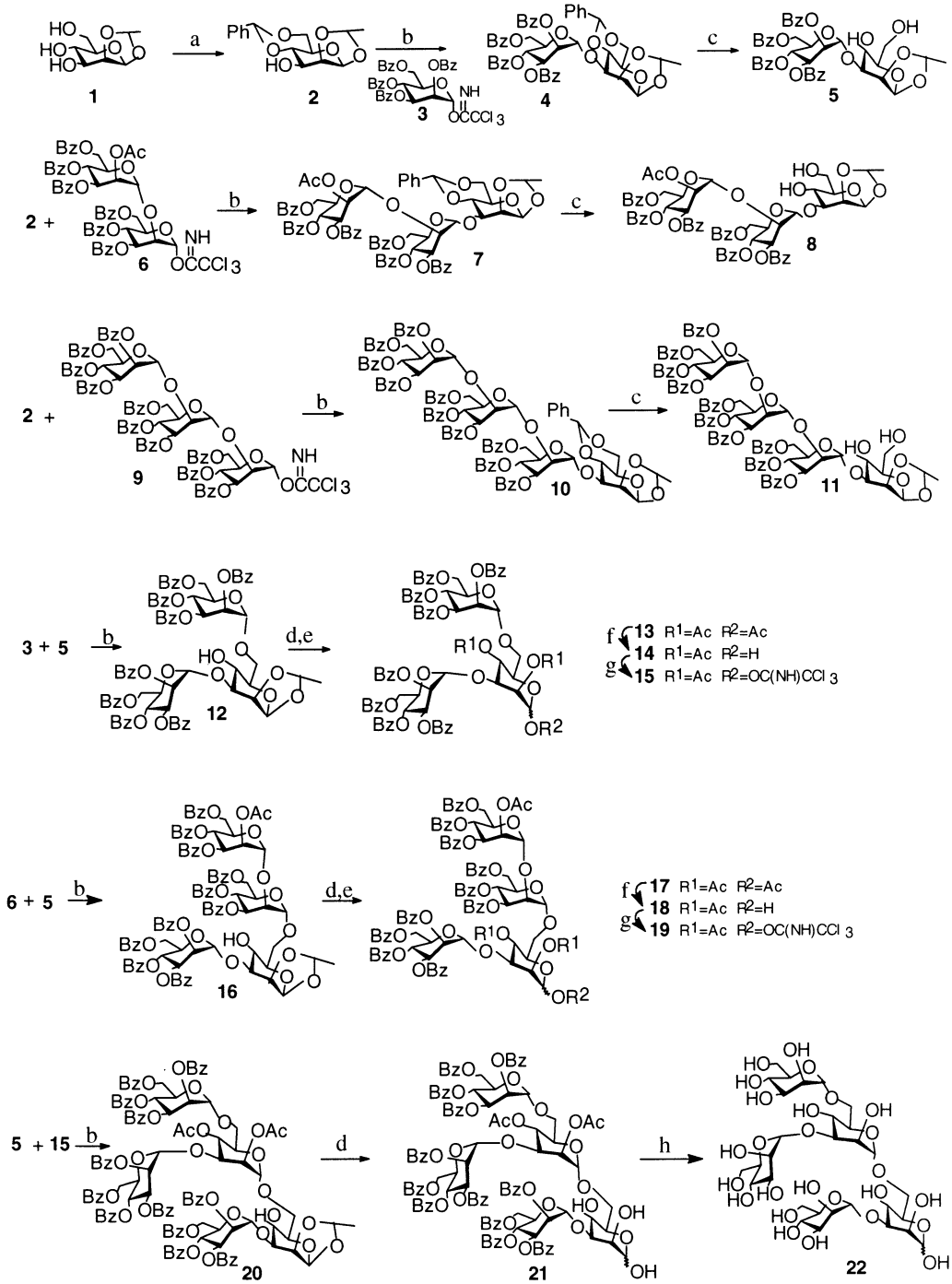
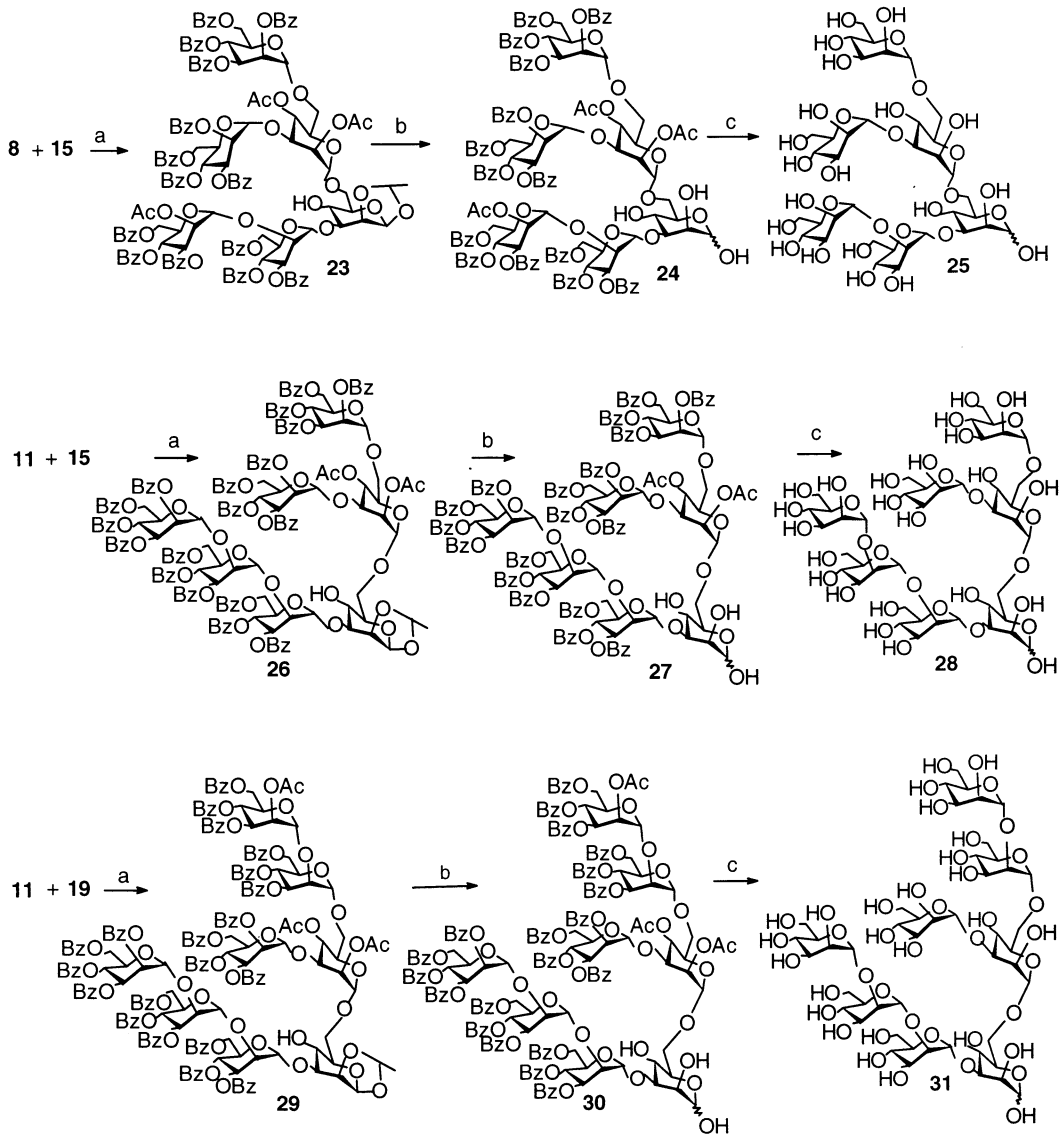
2. Results and discussion

Previous syntheses of mannose oligosaccharides involved the use of permanent and temporary protecting groups as well as complex protection and deprotection procedures.[4] Thus, it is difficult to obtain the oligosaccharide samples in sufficient quantity through these syntheses. In the work described here we designed a new method for mannose oligosaccharide syntheses using solely temporary protecting groups in combination with regioselective glycosylation and one-pot multistep transformation protocols.

Scheme 2 depicts the syntheses of **Man3**, **Man4**, and **Man 5**, and Scheme 3 shows the syntheses of **Man6**, **Man7**, and **Man8**. In our synthetic route, the target oligosaccharides were constructed from the coupling of fully acylated mannose mono- and oligosaccharide trichloroacetimidate donors with the mannose di-, tri-, and terasaccharide acceptors having free 4,6-hydroxyl



Scheme 2. *Reagents and conditions*: (a) PhCHO, $HC(OEt)_3$, toluenesulfonic acid, rt, 12 h (88%); (b) TMSOTf, CH_2Cl_2, 0°C, 2 h; (c) 0.1% HCl–MeOH, rt, 12 h (88%); (d) 90% CF_3COOH, rt, 2–5 h; (e) Ac_2O/pyridine (dry), rt, 6 h; (f) 1.0 M NH_3/MeOH, 3 h; (g) CCl_3CN, DBU, CH_2Cl_2 3 h (for **15**: 82% from **12**; for **19**: 82% from **16**); (h) satd NH_3/MeOH, rt, 7 days (76%).



Scheme 3. *Reagents and conditions*: (a) TMSOTf, CH_2Cl_2, 0°C, 2 h (79% for **23**, 82% for **26**, 80% for **29**); (b) 90% CF_3COOH, rt, 2–5 h (81% for **24**, 87% for **27**, 85% for **30**); (c) satd NH_3/MeOH, rt, 7 days (72% for **25**, 76% for **28**, 82% for **31**).

groups at the reducing end. This strategy ensures good reactivity and selectivity of the couplings because of the primary hydroxyl in the acceptors,[6] while the preparations of the trichloroacetimidate donors are relatively easy. Thus, 4,6-*O*-benzylidene-1,2-*O*-ethylidene-β-D-mannopyranose **2**, which was obtained from 1,2-*O*-ethylidene-β-D-mannopyranose,[7] was chosen as the starting material. Condensation of **2** with perbenzoylated mannopyransyl trichloroacetimidate **3** afforded the disaccharide **4** in high yield (83%). Selective removal of the benzylidene protecting group with 0.1% HCl–MeOH at room temperature from **4** was carried out smoothly, yielding the disaccharide acceptor **5** (88%). Coupling of **2** with the disaccharide donor **6**, which was readily prepared by self condensation of 1,2-*O*-allyloxyethylidene-3,4,6-tri-*O*-benzoyl-β-D-mannopyranose followed by deallylation and trichloroacetimidation,[8] gave **7** in high yield (92%), and subsequent debenzylidenation gave the trisaccharide acceptor **8** (95%). Coupling of **2** with the trisaccharide donor **9**[9] (→**10**, 86%), followed by debenzylidenation furnished the tetrasaccharide acceptor **11** (92%).

With these acceptors in hand, the required mannosyl oligosaccharides were synthesized readily. Thus, condensation of **5** with the monosaccharide donor **3** gave the 6-linked trisaccharide **12** with high selectivity (87%). Subsequent hydrolysis to remove the ethylidene protecting group, acetylation with Ac_2O in pyridine, selective 1-*O*-deacetylation with dilute NH_3–MeOH solution, followed by trichloroacetimidation produced the trisaccharide donor **15** (overall yield for the four steps 82%). These four steps were carried out consecutively without chromatographic separation for the first three steps, making large scale preparation possible with high yields. The ^1H NMR spectrum of **15** showed three triplets for C(4)H of the mannose at δ 6.22 ($J_{3,4}=J_{4,5}=$ 9.8 Hz), 6.11 ($J_{3,4}=J_{4,5}=9.9$ Hz), 5.51 ppm ($J_{3,4}=J_{4,5}=$ 9.8 Hz), confirming the 6-selective mannosylation of **5** as the third downfield triplet was obtained through

acetylation of the upfield C(4)H of **12**. The tetrasaccharide donor **19** was prepared in the same way, i.e. coupling of **5** with **6** gave **16** (78%), and subsequent hydrolysis, acetylation, 1-*O*-deacetylation, and trichloroacetimidation gave **19** (overall yield 84%). Condensation of the trisaccharide donor **15** with the disaccharide acceptor **5** gave the pentasaccharide **20** in satisfactory yield (85%). Subsequent hydrolysis to remove the ethylidene group, followed by acetylation and deacylation afforded the free pentasaccharide **22**.

The same strategy was applied for the synthesis of higher mannose oligosaccharides. Therefore, condensation of the trisaccharide donor **15** with the trisaccharide acceptor **8** gave the hexasaccharide **23**, while condensation with the tetrasaccharide acceptor **11** gave the heptasaccharide **26**. Meanwhile, coupling of the tetrasaccharide acceptor **11** with the tetrasaccharide donor **19** afforded the octasaccharide **29**. Deethylidenation of **23**, **26**, and **29**, followed by deacylation gave the free hexasaccharide **25**, heptasaccharide **28**, and octasaccharide **31**, respectively. To the best of our knowledge, these are the first reported syntheses of mannoheptaose **28** and mannooctaose **31**.

It was found that the size of either donor or acceptor did not have a significant influence on the yield of the coupling. Owing to the use of convenient materials and the simplicity of the procedure, this method is readily amenable to large-scale production of mannose oligosaccharides.

3. Conclusion

In summary, we have presented herein a very facile and practical method for the synthesis of multi branched mannose oligosaccharides of *N*-glycans. Sufficient quantities of mannose oligosaccharide samples can be obtained and this will greatly facilitate the biological studies.

4. Experimental

4.1. General

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 and ^{13}C NMR spectra were recorded with Bruker ARX 400 spectrometers (400 MHz for ^{1}H, 100 MHz for ^{13}C) for solutions in $CDCl_3$ or D_2O as indicated. 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 mode. 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, 18×300 mm, 35×400 mm) of silica gel (100–200 mesh) with EtOAc–petroleum ether (60–90°C) as the eluent. Solutions were concentrated at <60°C under reduced pressure. In the syntheses, compound **2** containing an ethylidene group was composed of (*R*)- and (*S*)-isomers in a 4:1 ratio. The two isomers were separated on a silica gel column with 2:1 petroleum ether–EtOAc as the eluent. Thus, pure (*R*)-**2** was used in further reactions in most cases, yielding products predominantly consisting of the (*R*)-isomer as the major compound and less than 10% of the (*S*)-form as the minor isomer. An exception was the synthesis of **26**, in which (*S*)-**2** was used at the beginning and thus the (*S*)-form was obtained as the predominant isomer for **26**. The (*R*)- and (*S*)-isomers had no difference in reactivity and thus no separation was conducted in the synthesis. However, for convenience of identification by NMR spectrometry, the predominant isomer in each synthesis was isolated in pure form. The assignments of (*R*)- and (*S*)-forms were based on the data reported by Kochetkov.[7]

4.2. 4,6-*O*-Benzylidene-1,2-*O*-ethylidene-β-D-mannopyranose 2

p-Toluenesulfonic acid monohydrate (190 mg, 1 mmol) was added to a solution of **1** (2.06 g, 10 mmol), triethyl orthoformate (4.2 mL, 25 mmol) and benzaldehyde (3.0 mL, 30 mmol) in anhydrous DMF (25 mL). The mixture was stirred at room temperature for 12 h, when TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. Sodium bicarbonate (2.52 g, 30 mmol) was added to the reaction mixture, and the mixture was stirred for an additional hour. After filtration, the solvents were evaporated in vacuo to give a residue, which was subjected to silica gel column chromatography (2:1 petroleum ether–EtOAc) to give **2** as a white solid (2.58 g, 88%) consisting of (*R*)- and (*S*)-isomers in a 4:1 ratio. Separation of the mixture with 2:1 petroleum ether–EtOAc as the eluent gave pure (*R*)- and (*S*)-isomers. For the (*R*)-isomer: $[\alpha]_D$ −47.6 (*c* 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.50 (m, 5H, *Ph*), 5.58 (s, 1H, PhC*H*), 5.33 (q, 1H, J=4.8, MeC*H*), 5.26 (d, 1H, $J_{1,2}$=2.0, H-1), 4.31 (dd, 1H, $J_{5,6}$=5.1, $J_{6,6'}$=10.6, H-6), 4.20 (dd, 1H, $J_{1,2}$=2.0, $J_{2,3}$=3.2, H-2), 4.07 (dd, 1H, $J_{2,3}$=3.2, $J_{3,4}$=9.4, H-3), 3.92 (dd, 1H, $J_{3,4}$=$J_{4,5}$=9.4, H-4), 3.77 (dd, 1H, $J_{5,6'}$=2.7, $J_{6,6'}$=10.6, H-6′), 3.38 (ddd, 1H, $J_{4,5}$=9.4, $J_{5,6}$=5.1, $J_{5,6'}$=2.7, H-5), 2.44–2.48 (br, 1H, OH), 1.52 (d, 3H, J=4.9, *Me*CH). Anal. calcd for $C_{15}H_{18}O_6$: C, 61.21; H, 6.16. Found: C, 61.32; H, 6.25%. For the (*S*)-isomer: $[\alpha]_D$ −42.1 (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.51 (m, 5H, *Ph*), 5.57 (q, 1H, J=4.8, MeC*H*), 5.56 (s, 1H, PhC*H*), 5.40 (d, 1H, $J_{1,2}$=1.9, H-1), 4.00 (dd, 1H, $J_{5,6}$=5.1, $J_{6,6'}$=10.6, H-6), 3.83–3.73 (m, 4H, H-2, H-3, H-4 and H-6′), 3.35 (ddd, 1H, $J_{4,5}$=9.4, $J_{5,6}$=5.1, $J_{5,6'}$=2.7, H-5), 2.44–2.48 (br, 1H, OH), 1.36 (d, 3H, J=4.9, *Me*CH). Anal. calcd for $C_{15}H_{18}O_6$: C, 61.21; H, 6.16. Found: C, 61.18; H, 6.15%.

4.3. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-4,6-*O*-benzylidene-1,2-*O*-ethylidene-β-D-mannopyranose 4

To a cooled solution (0°C) of **2** (2.94 g, 10 mmol) and **3** (7.40 g, 10 mmol) in anhydrous CH_2Cl_2 (50 mL) was added TMSOTf (50 μL, 0.28 mmol). The mixture was stirred at this temperature for 2 h, and then quenched with Et_3N (two drops). The solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give disaccharide **4** as a syrup (4.72 g, 83%). For the (*R*)-isomer: $[\alpha]_D$ −39.7 (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07–7.37 (m, 25H, 5*Ph*), 6.07 (dd, 1H, $J_{3,4}=J_{4,5}=9.8$, H-4), 6.01 (dd, 1H, $J_{2,3}=3.0$, $J_{3,4}=9.8$, H-3), 5.86 (dd, 1H, $J_{1,2}=1.0$, $J_{2,3}=3.0$, H-2), 5.66 (s, 1H, PhC*H*), 5.59 (d, 1H, $J_{1,2}=1.0$, H-1), 5.41 (q, 1H, $J=4.7$, MeC*H*), 5.07 (d, 1H, $J_{1,2}=1.6$, H-1), 4.69 (dd, 1H, $J_{2,3}=2.0$, $J_{3,4}=9.7$, H-3), 4.62 (ddd, 1H, $J_{4,5}=9.8$, $J_{5,6}=4.2$, $J_{5,6'}=2.6$, H-5), 4.70 (dd, 1H, $J_{5,6}=4.5$, $J_{6,6'}=13.5$, H-6), 4.34–4.23 (m, 4H, H-2, H-6 and 2H-6′), 3.81 (dd, 1H, $J_{3,4}=J_{4,5}=9.7$, H-4), 3.36 (ddd, 1H, $J_{4,5}=9.7$, $J_{5,6}=4.5$, $J_{5,6'}=4.3$, H-5), 1.58 (d, 3H, $J=4.7$, *Me*CH). Anal. calcd for $C_{49}H_{44}O_{15}$: C, 67.42; H, 5.08. Found: C, 67.56; H, 5.12%.

4.4. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-1,2-*O*-ethylidene-β-D-mannopyranose 5

To a solution of **4** (8.72 g, 10.0 mmol) in anhydrous MeOH (100 mL) was added acetyl chloride (0.1 mL). The solution was sealed in a flask and stirred at rt until TLC (2:1 petroleum ether–EtOAc) showed that all starting material was consumed. The solution was neutralized with Et_3N, then concentrated to dryness. The residue was passed through a short silica gel column (1:2 petroleum ether–EtOAc) to give **5** as a white solid (6.90 g, 88%). For the (*R*)-isomer: $[\alpha]_D$ −49.7 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08–7.27 (m, 20H, 4*Ph*), 6.13 (dd, 1H, $J_{3,4}=J_{4,5}=10.2$, H-4), 5.97 (dd, 1H, $J_{2,3}=3.4$, $J_{3,4}=10.2$, H-3), 5.83 (dd, 1H, $J_{1,2}=1.1$, $J_{2,3}=3.4$, H-2), 5.42 (d, 1H, $J_{1,2}=1.1$, H-1), 5.31 (q, 1H, $J=4.8$, MeC*H*), 5.23 (d, 1H, $J_{1,2}=2.1$, H-1), 4.74 (ddd, 1H, $J_{4,5}=10.2$, $J_{5,6}=4.4$, $J_{5,6'}=2.3$, H-5), 4.65 (dd, 1H, $J_{2,3}=2.4$, $J_{3,4}=9.4$, H-3), 4.51 (dd, 1H, $J_{5,6}=4.4$, $J_{6,6'}=12.1$, H-6), 4.38 (dd, 1H, $J_{1,2}=2.0$, $J_{2,3}=2.4$, H-2) 4.17 (dd, 1H, $J_{3,4}=J_{4,5}=9.4$, H-4), 3.91–3.83 (m, 3H, H-6, and 2H-6′), 3.43 (ddd, 1H, $J_{4,5}=9.4$, $J_{5,6}=5.1$, $J_{5,6'}=2.2$, H-5), 1.53 (d, 3H, $J=4.8$, *Me*CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 165.3, 165.1, 164.9 (4C, 4Ph*CO*), 133.0–127.9 (*Ph*CO), 103.9 (CH_3CH), 99.5, 96.4 (2C-1), 81.3, 78.9, 74.8, 69.9, 69.8, 69.0, 66.4, 65.8, 62.5, 61.9 (10C, C-2–6), 21.3 (CH_3CH). Anal. calcd for $C_{42}H_{40}O_{15}$: C, 64.28; H, 5.14. Found: C, 64.24; H, 5.15%.

4.5. 2-*O*-Acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-4,6-*O*-benzylidene-1,2-*O*-ethylidene-β-D-mannopyranose 7

To a cooled solution (0°C) of **2** (0.74 g, 2.5 mmol) and **6** (2.88 g, 2.5 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 (two drops). After evaporation of the solvents, the crude residue was subjected to silica gel column chromatography (2:1 petroleum ether–EtOAc) to give trisaccharide **7** as a foamy solid (2.94 g, 92%). For the (*R*)-isomer: $[\alpha]_D^{25}$ −31.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06–7.18 (m, 35H, 7Ph), 5.98 (dd, 1H, $J_{2,3}=3.1$, $J_{3,4}=9.6$, H-3), 5.95 (dd, 1H, $J_{3,4}=J_{3,4}=9.6$, H-4), 5.89 (dd, 1H, $J_{2,3}=3.2$, $J_{3,4}=9.8$, H-3), 5.78 (dd, 1H, $J_{3,4}=J_{3,4}=9.8$, H-4), 5.66 (dd, 1H, $J_{1,2}=1.6$, $J_{2,3}=3.1$, H-2), 5.58 (d, 1H, $J_{1,2}=1.6$, H-1), 5.37 (q, 1H, $J=4.9$, MeC*H*), 5.34 (s, 1H, PhC*H*), 5.06 (d, 1H, $J_{1,2}=1.4$, H-1), 4.93 (d, 1H, $J_{1,2}=0.9$, H-1), 4.68 (dd, 1H, $J_{2,3}=3.2$, $J_{3,4}=10.0$, H-3), 4.63 (dd, 1H, $J_{5,6}=4.9$, $J_{6,6'}=11.7$, H-6), 4.54 (ddd, 1H, $J_{4,5}=9.4$, $J_{5,6}=5.1$, $J_{5,6'}=3.3$, H-5), 4.48 (dd, 1H, $J_{1,2}=1.9$, $J_{2,3}=3.1$, H-2), 4.31 (ddd, 1H, $J_{4,5}=9.6$, $J_{5,6}=4.9$, $J_{5,6'}=4.3$, H-5), 4.19 (dd, 1H, $J_{5,6}=5.1$, $J_{6,6'}=10.6$, H-6), 4.13–4.00 (m, 5H), 3.65 (dd, 1H, $J_{3,4}=J_{3,4}=10.0$, H-4), 3.13 (ddd, 1H, $J_{4,5}=9.8$, $J_{5,6}=5.5$, $J_{5,6'}=2.4$, H-5), 2.03 (s, 3H, CH_3CO), 1.56 (d, 1H, $J=4.9$, *Me*CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8 (CH_3CO), 165.8, 165.3, 165.2, 165.1, 165.0, 164.6 (6C, Ph*CO*), 136.5–125.5 (*Ph*CO and *Ph*CH), 104.3 (CH_3CH), 101.1 (PhCH), 99.5, 98.6, 96.4 (3C-1), 79.2, 75.7, 73.6, 70.1, 69.2, 69.2, 68.8, 68.1, 67.7, 66.6, 65.1, 63.6, 62.5 (15C, C-2–6, some signals overlapped), 21.3, 20.1 (CH_3CH and CH_3CO). Anal. calcd for $C_{71}H_{64}O_{23}$: C, 66.35; H, 5.02. Found: C, 66.48; H, 4.93%.

4.6. 2-*O*-Acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-1,2-*O*-ethylidene-β-D-mannopyranose 8

To a solution of **7** (2.57 g, 2 mmol) in anhydrous MeOH (100 mL) was added acetyl chloride (0.1 mL). The solution was sealed in a flask and stirred at room temperature until TLC (2:1 petroleum ether–EtOAc) showed that the starting material disappeared. The solution was neutralized with Et_3N, then concentrated to dryness. The residue was passed through a short silica gel column (1:2 petroleum ether–EtOAc) to give **8** as a white solid (2.27 g, 95%). For the (*R*)-isomer: $[\alpha]_D^{25}$ −3.8 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06–7.18 (m, 30H, 6Ph), 6.05–5.86 (m, 4H, 2H-3, 2H-4), 5.63 (dd, 1H, $J_{1,2}=1.5$, $J_{2,3}=3.1$, H-2), 5.58 (d, 1H, $J_{1,2}=1.5$, H-1), 5.24 (q, 1H, $J=4.7$, MeC*H*), 5.16 (d, 1H, $J_{1,2}=1.7$, H-1), 5.04 (d, 1H, $J_{1,2}=2.2$, H-1), 4.64–4.69 (m, 7H), 4.18 (dd, 1H, $J_{1,2}=2.2$, $J_{2,3}=3.6$, H-2), 4.08 (dd, 1H, $J_{3,4}=J_{3,4}=9.5$, H-4), 3.83–3.76 (m, 3H), 3.26 (ddd, 1H, $J_{4,5}=9.5$, $J_{5,6}=5.1$, $J_{5,6'}=2.3$, H-5), 2.04 (s, 3H, CH_3CO), 1.49 (d, 1H, $J=4.7$, *Me*CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.9 (CH_3CO), 166.0, 165.8, 165.4, 165.2, 164.9, 164.7 (6C, Ph*CO*), 133.0–127.9 (*Ph*CO), 103.9 (CH_3CH), 100.3, 98.7, 96.3 (3C, 3C-1), 79.6, 78.8, 74.8, 70.4, 69.2, 68.9, 67.2, 67.0, 66.1, 63.4, 63.0, 61.8 (15C, 3C-2–6, some signals overlapped), 21.2 (CH_3CH), 20.1 (CH_3CO). Anal. calcd for $C_{42}H_{40}O_{15}$: C, 64.21; H, 5.05. Found: C, 64.04; H, 4.90%.

4.7. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-4,6-*O*-benzylidene-1,2-*O*-ethylidene-β-D-mannopyranose 10

TMSOTf (25 μL, 0.14 mmol) was added to a cooled solution (0°C) of **2** (0.59 g, 2.0 mmol) and **9** (3.38 g, 2.0 mmol) in anhydrous CH_2Cl_2 (50 mL). The mixture was stirred at this temperature for 2 h, and then quenched with Et_3N (two drops). The solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give tetrasaccharide **10** as a syrup (3.17 g, 86%). For the (*R*)-isomer: $[\alpha]_D^{25}$ −33.1 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11–7.27 (m, 55H, 11Ph), 6.06 (dd, 1H, $J_{3,4}=J_{4,5}=9.6$, H-4), 5.99–5.94 (m, 2H, 2H-3), 5.93 (dd, 1H, $J_{3,4}=J_{3,4}=9.8$, H-4), 5.88 (dd, 1H, $J_{2,3}=3.0$, $J_{3,4}=9.8$, H-3), 5.81 (dd, 1H, $J_{1,2}=1.5$, $J_{2,3}=3.1$, H-2), 5.73 (dd, 1H, $J_{3,4}=J_{3,4}=9.7$, H-4), 5.60 (d, 1H, $J_{1,2}=1.6$, H-1), 5.52 (s, 1H, PhC*H*), 5.38 (d, 1H, $J_{1,2}=1.0$, H-1), 5.31 (q, 1H, $J=4.7$, MeC*H*), 5.11 (d, 1H, $J_{1,2}=1.5$, H-1), 4.99 (d, 1H, $J_{1,2}=1.9$, H-1), 4.74 (dd, 1H, $J_{2,3}=3.2$, $J_{3,4}=9.7$, H-3), 4.65 (dd, 1H, $J_{5,6}=5.2$, $J_{6,6'}=11.8$, H-6), 4.54 (ddd, 1H, $J_{4,5}=9.4$, $J_{5,6}=5.0$, $J_{5,6'}=2.3$, H-5), 4.50–4.45 (m, 3H), 4.28 (ddd, 1H, $J_{4,5}=9.6$, $J_{5,6}=5.0$, $J_{5,6'}=2.1$, H-5), 4.20 (dd, 1H, $J_{5,6}=4.9$, $J_{6,6'}=10.5$, H-6), 4.15–4.04 (m, 7H), 3.82 (dd, 1H, $J_{3,4}=J_{3,4}=9.9$, H-4), 3.40 (ddd, 1H, $J_{4,5}=9.8$, $J_{5,6}=5.4$, $J_{5,6'}=2.6$, H-5), 1.52 (d, 1H, $J=4.9$, *Me*CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.9, 165.8, 165.4, 165.3, 165.2, 165.1, 165.0, 164.4 (10C, 10Ph*CO*, some signals overlapped), 136.5–125.5 (*Ph*CO and *Ph*CH), 103.9 (CH_3CH), 100.8 (Ph*C*H), 99.1, 99.0, 97.3, 96.9 (4C, 4C-1), 79.8, 75.5, 74.9, 73.5, 73.6, 70.1, 69.3, 69.2, 68.9, 68.1, 67.0, 66.7, 65.4, 64.0, 62.1 (20C, 4C-2–6, some signals overlapped), 21.3 (CH_3CH). Anal. calcd for $C_{103}H_{88}O_{31}$: C, 67.91; H, 4.87. Found: C, 68.12; H, 4.85.

4.8. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-1,2-*O*-ethylidene-β-D-mannopyranose 11

To a solution of **10** (3.00 g, 1.65 mmol) in anhydrous MeOH (100 mL) was added acetyl chloride (0.1 mL). The solution was sealed in a flask and stirred at rt until TLC (1:1 petroleum ether–EtOAc) showed that the starting material disappeared. The solution was neutralized with Et_3N, and then concentrated. Purification of the residue on a short silica gel column (1:2 petroleum ether–EtOAc) gave acceptor **11** as a white foam (2.60 g, 92%). For the (*R*)-isomer: $[\alpha]_D^{25}$ −33.3 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06–7.18 (m, 50H, 10Ph), 6.10 (dd, 1H, $J_{3,4}=J_{4,5}=10.1$, H-4), 5.99 (dd, 1H, $J_{3,4}=J_{4,5}=9.9$, H-4), 5.95–5.93 (m, 2H, H-2 and H-3), 5.84–5.80 (m, 3H, 2H-3 and H-4), 5.57 (d, 1H, $J_{1,2}=2.1$, H-1), 5.53 (d, 1H, $J_{1,2}=1.0$, H-1), 5.23 (q, 1H, $J=4.8$, MeC*H*), 5.05 (2d, 2H, 2H-1, overlapped), 4.63–4.50 (m, 9H), 4.39 (dd, 1H, $J_{1,2}=1.0$, $J_{2,3}=3.1$, H-2), 4.24 (dd, 1H, $J_{2,3}=3.1$, $J_{3,4}=9.6$, H-3), 4.17 (dd, 1H, $J_{1,2}=2.1$, $J_{2,3}=3.1$, H-2), 4.11 (dd, 1H, $J_{3,4}=J_{3,4}=9.7$, H-4), 3.89–3.78 (m, 3H), 3.26 (ddd, 1H, $J_{4,5}=9.6$, $J_{5,6}=5.3$, $J_{5,6'}=2.0$, H-5), 1.45 (d, 1H, $J=4.8$, *Me*CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 165.9, 165.7, 165.3, 165.2, 165.1, 165.0, 164.9, 164.6, 164.4 (10C, Ph*CO*), 132.9–127.8 (*Ph*CO), 103.8 (CH_3CH), 100.2, 99.1, 98.6, 96.3 (4C, 4C-1), 78.9, 78.8, 75.0, 70.0, 69.8, 69.6, 69.4, 69.1, 69.0, 68.9, 67.8, 67.4, 66.8, 66.2, 63.5, 63.3, 62.2, 62.0 (20C, 4C-2–6, some signals overlapped), 21.1 (CH_3CH). Anal. calcd for $C_{42}H_{40}O_{15}$: C, 66.51; H, 4.88. Found: C, 66.32; H, 4.96%.

4.9. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)]-1,2-*O*-ethylidene-β-D-mannopyranose 12

To a cooled solution (0°C) of **5** (3.92 g, 5 mmol) and **3** (3.70 g, 5 mmol) in anhydrous CH_2Cl_2 (50 mL) was added TMSOTf (50 μL, 0.28 mmol). The mixture was stirred at this temperature for 2 h, and then quenched with Et_3N (four drops). The solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give trisaccharide **12** as a syrup (5.92 g, 87%). For the (*R*)-isomer: $[\alpha]_D^{25}$ −42.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06–7.28 (m, 40H, 8Ph), 6.18 (dd, 1H, $J_{3,4}=J_{3,4}=9.8$, H-4), 6.15 (dd, 1H, $J_{3,4}=J_{3,4}=9.6$, H-4), 6.03 (dd, 1H, $J_{2,3}=2.9$, $J_{3,4}=9.8$, H-3), 5.98 (dd, 1H, $J_{2,3}=3.1$, $J_{3,4}=9.6$, H-3), 5.89 (dd, 1H, $J_{1,2}=0.9$, $J_{2,3}=3.0$, H-2), 5.81 (dd, 1H, $J_{1,2}=1.2$, $J_{2,3}=3.1$, H-2), 5.44 (d, 1H, $J_{1,2}=0.9$, H-1), 5.37 (q, 1H, $J=4.7$, MeC*H*), 5.28 (d, 1H, $J_{1,2}=1.2$, H-1), 5.21 (d, 1H, $J_{1,2}=1.5$, H-1), 4.85–4.72 (m, 2H), 4.67 (dd, 1H, $J_{2,3}=2.7$, $J_{3,4}=9.9$, H-3), 4.55–4.49 (m, 3H), 4.45 (dd, 1H, $J_{1,2}=1.5$, $J_{2,3}=2.7$, H-2), 4.24 (dd, 1H, $J_{3,4}=J_{3,4}=9.9$, H-4), 4.15 (dd, 1H, $J_{5,6}=4.9$, $J_{6,6'}=10.8$, H-6), 3.59 (ddd, 1H, $J_{4,5}=9.8$, $J_{5,6}=4.9$, $J_{5,6'}=2.2$, H-5), 1.58 (d, 1H, $J=4.7$, *Me*CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8, 165.7, 165.3, 165.2, 165.1, 165.0, 164.9, 164.8 (8C, 8Ph*CO*), 133.1–127.8 (*Ph*CO and *Ph*CH), 104.2 (CH_3CH), 99.6, 97.5, 96.4 (3C, 3C-1), 82.9, 78.8, 73.7, 70.0, 69.1, 68.3, 66.7, 66.6, 66.4, 65.5, 62.5 (15C, 3C-2–6, some signals overlapped), 21.5 (CH_3CH). Anal. calcd for $C_{76}H_{66}O_{24}$: C, 66.95; H, 4.88. Found: C, 66.76; H, 4.85%.

4.10. 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*-acetyl-α-D-mannopyranosyl trichloroacetimidate 15

Compound **12** (5.45 g, 6.69 mmol) was dissolved in 90% TFA (70 mL) and stirred for 2 h, at the end of which time the reaction mixture was poured directly into 250 mL toluene and concentrated. Drying the residue under high vacuum gave a white foamy solid. This foamy solid was dissolved in pyridine (20 mL), and then Ac_2O (10 mL) was added. The reaction mixture was stirred at rt for 12 h, and TLC (4:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was concentrated to dryness. The resultant crude product **13** was dissolved in a 1 M solution of ammonia–methanol (200 mL) and stirred at rt for 3 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The

solution was concentrated to give compound **14** as a syrup. A mixture of **14**, trichloroacetonitrile (4.2 mL, 20 mmol), and 1,8-diazabicyclo[5.4.0]undecene (DBU) (0.50 mL, 4.04 mmol) in dry dichloromethane (50 mL) was stirred under nitrogen for 3 h and then concentrated. The residue was purified by flash chromatography (4:1 petroleum ether–EtOAc) to give **15** as a white foam (5.10 g, 82% for four steps): $[\alpha]_D$ −33.9 (*c* 1.4, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.96 (s, 1H, CN*H*CCl_3), 8.06–7.27 (m, 40H, 8Ph), 6.35 (d, 1H, $J_{1,2}=1.4$, H-1), 6.22 (dd, 1H, $J_{3,4}=J_{4,5}=9.8$, H-4), 6.11 (dd, 1H, $J_{3,4}=J_{4,5}=9.9$, H-4), 5.89 (dd, 1H, $J_{2,3}=3.2$, $J_{3,4}=9.8$, H-3), 5.81 (dd, 1H, $J_{2,3}=3.2$, $J_{3,4}=9.8$, H-3), 5.69 (dd, 1H, $J_{1,2}=1.8$, $J_{2,3}=3.2$, H-2), 5.64 (dd, 1H, $J_{1,2}=1.5$, $J_{2,3}=3.1$, H-2), 5.54 (dd, 1H, $J_{1,2}=1.0$, $J_{2,3}=3.1$, H-2), 5.51 (dd, 1H, $J_{3,4}=J_{4,5}=9.8$, H-4), 5.42 (d, 1H, $J_{1,2}=1.8$, H-1), 5.11 (d, 1H, $J_{1,2}=1.5$, H-1), 4.68 (dd, 1H, $J_{2,3}=3.1$, $J_{3,4}=9.9$, H-3), 4.64–4.46 (m, 3H, H-5, H-6, H-6′), 4.51–4.46 (m, 3H, H-5, H-6, H-6′), 4.22 (ddd, 1H, $J_{4,5}=9.8$, $J_{5,6}=4.7$, $J_{5,6'}=2.2$, H-5), 3.99 (dd, 1H, $J_{5,6}=4.7$, $J_{6,6'}=11.0$, H-6), 3.75 (dd, 1H, $J_{5,6'}=2.1$, $J_{6,6'}=11.0$, H-6), 2.37 (s, 3H, *Me*CO), 2.29 (s, 3H, *Me*CO). Anal. calcd for $C_{80}H_{68}Cl_3NO_{26}$: C, 61.36; H, 4.38. Found: C, 61.52; H, 4.40%.

4.11. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[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)]-1,2-*O*-ethylidene-β-D-mannopyranose 16

TMSOTf (25 μL, 0.14 mmol) was added to a cooled solution (0°C) of **5** (1.57 g, 2 mmol) and **6** (2.30 g, 2 mmol) in anhydrous CH_2Cl_2 (50 mL), and the mixture was stirred at this temperature for 2 h and then quenched with Et_3N (two drops). The solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give tetrasaccharide **16** as a white foamy solid (2.93 g, 83%). For the (*R*)-isomer: $[\alpha]_D^{25}$ −21.6 (*c* 1.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.09–7.27 (m, 50H, 10Ph), 6.17 (dd, 1H, $J_{3,4}=J_{4,5}=10.1$, H-4), 6.02 (dd, 1H, $J_{3,4}=J_{4,5}=9.8$, H-4), 5.99 (dd, 1H, $J_{2,3}=3.2$, $J_{3,4}=9.8$, H-3), 5.91–5.88 (m, 4H, H-2, 2H-3 and H-4), 5.68 (dd, 1H, $J_{1,2}=1.0$, $J_{2,3}=2.6$, H-2), 5.41 (d, 1H, $J_{1,2}=1.3$, H-1), 5.37 (q, 1H, $J=4.9$, MeC*H*), 5.24 (2d, 2H, 2H-1, overlapped), 5.09 (d, 1H, $J_{1,2}=1.9$, H-1), 4.45–4.68 (m, 10H), 4.41 (dd, 1H, $J_{1,2}=1.3$, $J_{2,3}=3.1$, H-2), 4.11 (dd, 1H, $J_{3,4}=J_{3,4}=9.6$, H-4), 4.01 (dd, 1H, $J_{5,6}=5.5$, $J_{6,6'}=10.9$, H-6), 3.90 (dd, 1H, $J_{2,3}=3.1$, $J_{3,4}=9.6$, H-3), 3.76 (dd, 1H, $J_{5,6'}=2.1$, $J_{6,6'}=10.9$, H-6′), 3.48 (ddd, 1H, $J_{4,5}=9.6$, $J_{5,6}=5.5$, $J_{5,6'}=2.1$, H-5), 2.02 (s, 3H, *Me*CO), 1.53 (d, 1H, $J=4.8$, *Me*CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.9 (Me*CO*), 166.1, 165.7, 165.7, 165.3, 165.2, 165.2, 165.1, 165.0, 164.9, 164.6 (10C, Ph*CO*), 132.1–127.8 (*Ph*CO), 104.2 (CH_3CH), 99.7, 99.3, 98.1, 96.4 (4C, 4C-1), 81.8, 78.8, 73.4, 70.5, 69.9, 69.8, 69.3, 69.2, 69.1, 68.1, 67.1, 66.7, 66.6, 66.3, 65.6, 63.3, 62.9, 62.4 (20C, 4C-2–6, some signals overlapped), 21.5 (*Me*CO), 20.1 (CH_3CH). Anal. calcd for $C_{98}H_{86}O_{32}$: C, 66.28; H, 4.88. Found: C, 66.11; H, 5.02%.

4.12. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[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)]-2,4-di-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate 19

Compound **16** (2.50 g, 1.41 mmol) was dissolved in 30 mL 90% TFA and stirred for 2 h, and then the reaction mixture was poured directly into toluene (150 mL) and concentrated. Drying the residue under high vacuum gave a white foamy solid. This foamy solid was dissolved in pyridine (20 mL) and Ac_2O (10 mL) was added. The reaction mixture was stirred at rt for 12 h, TLC (4:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was concentrated to dryness. The resultant crude product **17** was dissolved in a 1 M solution of ammonia–methanol (100 mL) and stirred at rt for 3 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The solution was concentrated to give compound **18** as a syrup. A mixture of **18**, trichloroacetonitrile (1.1 mL, 5 mmol), and 1,8-diazabicyclo[5.4.0]undecene (DBU) (0.10 mL, 0.80 mmol) in dry dichloromethane (10 mL) was stirred under nitrogen for 3 h and then concentrated. Purification of the residue by flash chromatography (3:1 petroleum ether–EtOAc) gave **19** as a white foam (2.34 g, 84% for four steps): $[\alpha]_D$ +3.6 (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 9.04 (s, 1H, CN*H*CCl_3), 8.11–7.27 (m, 50H, 10Ph), 6.33 (d, 1H, $J_{1,2}=0.8$, H-1), 6.22 (dd, 1H, $J_{3,4}=J_{4,5}=10.0$, H-4), 5.99 (dd, 1H, $J_{3,4}=J_{4,5}=9.7$, H-4), 5.90–5.79 (m, 4H, H-2, 3H-3 and H-4), 5.68 (dd, 1H, $J_{1,2}=0.8$, $J_{2,3}=2.6$, H-2), 5.64 (dd, 1H, $J_{1,2}=1.0$, $J_{2,3}=3.1$, H-2), 5.52–5.48 (m, 2H, H-2 and H-4), 5.42 (d, 1H, $J_{1,2}=1.0$, H-1), 5.17 (d, 1H, $J_{1,2}=0.9$, H-1), 5.09 (d, 1H, $J_{1,2}=1.1$, H-1), 4.65–4.49 (m, 10H), 4.33 (dd, 1H, $J_{1,2}=1.0$, $J_{2,3}=2.9$, H-2), 4.19 (ddd, 1H, $J_{4,5}=9.8$, $J_{5,6}=4.7$, $J_{5,6'}=2.0$, H-5), 3.93 (dd, 1H, $J_{5,6}=4.3$, $J_{6,6}=11.2$, H-6), 3.61 (dd, 1H, $J_{5,6'}=2.3$, $J_{6,6'}=11.2$, H-6′), 2.35 (s, 3H, *Me*CO), 2.29 (s, 3H, *Me*CO), 2.22 (s, 3H, *Me*CO). Anal. calcd for $C_{102}H_{88}Cl_3NO_{34}$: C, 61.93; H, 4.48. Found: C, 61.71; H, 4.41%.

4.13. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-{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*-acetyl-α-D-mannopyranosyl-(1→6)}-1,2-*O*-ethylidene-β-D-mannopyranose 20

To a cooled solution (0°C) of **5** (784 mg, 1 mmol) and **15** (1.56 g, 1 mmol) in anhydrous CH_2Cl_2 (25 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 (two drops). The solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (1:1 petroleum ether–EtOAc) to give pentasaccharide **20** as a syrup (1.86 g, 85%). For (*R*)-isomer: $[\alpha]_D^{25}$ −44.3 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.05–7.04 (m, 60H, 12Ph), 6.17 (dd, 1H, $J_{3,4}=J_{4,5}=10.0$, H-4), 6.03 (dd, 1H, $J_{3,4}=J_{4,5}=10.0$, H-4), 6.00 (dd, 1H, $J_{3,4}=J_{4,5}=10.4$, H-4), 5.93 (dd, 1H, $J_{2,3}=3.5$, $J_{3,4}=10.0$, H-3), 5.87 (dd, 1H, $J_{2,3}=3.0$, $J_{3,4}=10.0$, H-3), 5.75–5.72 (m, 2H, H-2 and H-3),

5.66 (dd, 1H, $J_{1,2}=1.3$, $J_{2,3}=3.2$, H-2), 5.48 (dd, 1H, $J_{1,2}=1.0$, $J_{2,3}=3.5$, H-2), 5.46 (dd, 1H, $J_{1,2}=1.5$, $J_{2,3}=3.0$, H-2), 5.38 (d, 1H, $J_{1,2}=1.3$, H-1), 5.32 (dd, 1H, $J_{3,4}=J_{4,5}=10.5$, H-4), 5.26 (q, 1H, $J=4.5$, MeC*H*), 5.22 (d, 1H, $J_{1,2}=0.9$, H-1), 5.20 (d, 1H, $J_{1,2}=1.0$, H-1), 5.13 (d, 1H, $J_{1,2}=0.8$, H-1), 5.10 (d, 1H, $J_{1,2}=1.0$, H-1), 4.68–4.62 (m, 3H), 4.57–4.50 (m, 3H), 4.45–4.36 (m, 4H), 4.29 (dd, 1H, $J_{1,2}=2.0$, $J_{2,3}=2.9$, H-2), 4.19–4.09 (m, 3H, H-4 and 2H-6), 3.97 (ddd, 1H, $J_{4,5}=9.8$, $J_{5,6}=4.9$, $J_{5,6'}=2.4$, H-5), 3.85 (dd, 1H, $J_{5,6}=5.2$, $J_{6,6}=11.3$, H-6), 3.78 (dd, 1H, $J_{2,3}=2.9$, $J_{3,4}=9.5$, H-3), 3.76 (dd, 1H, $J_{5,6'}=1.9$, $J_{6,6'}=11.9$, H-6′), 3.48 (ddd, 1H, $J_{4,5}=9.8$, $J_{5,6}=5.2$, $J_{5,6'}=2.2$, H-5), 2.21 (s, 3H, *Me*CO), 2.20 (s, 3H, *Me*CO), 1.48 (d, 1H, $J=4.5$, *Me*CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 170.3 (2C, 2Me*CO*), 166.1, 166.0, 165.9, 165.8, 165.7, 165.4, 165.3, 165.2, 165.1, 165.0, 164.9, 164.8 (12C, 12Ph*CO*), 133.2–128.1 (*Ph*CO), 104.6 (CH_3CH), 100.1, 98.7, 97.2, 96.9, 96.6 (5C, 5C-1), 81.6, 79.3, 74.4, 73.6, 70.6, 70.5, 70.4, 70.1, 69.9, 69.8, 69.4, 69.2, 68.8, 68.6, 66.7, 66.6, 66.2, 66.0, 65.8, 62.8, 62.7, 62.3 (25C, 5C-2–6, some signals overlapped), 21.9 (*Me*CO), 20.9, 20.7 (2C, 2CH_3CH). Anal. calcd for $C_{120}H_{106}O_{40}$: C, 65.86; H, 4.88. Found C, 65.62; H, 4.80%.

4.14. α-D-Mannopyranosyl-(1→3)-{α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-α-D-mannopyranosyl-(1→6)}-α-D-mannopyranose 22

Compound **20** (1.00 g, 0.457 mmol) was dissolved in 90% TFA (15 mL) and stirred for 2 h, and then the reaction mixture was poured directly into toluene (50 mL) and concentrated. The residue was purified by flash chromatography (1:2 petroleum ether–EtOAc) to give **21** as a white foam (0.931 g, 94%). Compound **21** was dissolved in a saturated ammonia–MeOH solution (50 mL). After 1 week at rt, the reaction mixture was concentrated and the residue was purified by chromatography on Sephadex LH-20 (MeOH) to afford **22** (288 mg, 76%) as a syrup with predominant α-anomer at the reducing end; $[\alpha]_D^{25}$ +46.5 (*c* 1.0, D_2O); ^{1}H NMR (400 MHz, D_2O) δ 5.03, 5.01, 4.94, 4.71, 4.70 (d, 5H, $J_{1,2}\approx 0$, 5H-1), 4.13–3.50 (m, 30H, 5H-2–6); ^{13}C NMR (100 MHz, D_2O) δ 105.0, 104.8, 102.1, 101.9, 96.5 (5C, 5C-1); MALDI-TOF MS calcd for $C_{30}H_{52}O_{26}$: 828.72 [M]. Found: 851.55 [M+Na].

4.15. 2-*O*-Acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-{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*-acetyl-α-D-mannopyranosyl-(1→6)}-1,2-*O*-ethylidene-β-D-mannopyranose 23

To a cooled solution (0°C) of **8** (598 mg, 0.5 mmol) and **15** (782 mg, 0.5 mmol) in anhydrous CH_2Cl_2 (25 mL) was added TMSOTf (9.0 μL, 0.05 mmol). The mixture was stirred at this temperature for 2 h, and then quenched with Et_3N (two drops). The solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (1:1 petroleum ether–EtOAc) to give hexaccharide **23** as a foamy solid (1.02 g, 79%). For the (*R*)-isomer: $[\alpha]_D^{25}$ −14.3 (*c* 1.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14–7.18 (m, 70H, 14Ph), 6.24 (dd, 1H, $J_{3,4}=J_{4,5}=9.8$, H-4), 6.13 (dd, 1H, $J_{3,4}=J_{4,5}=10.2$, H-4), 5.99 (dd, 1H, $J_{3,4}=J_{4,5}=10.1$, H-4), 5.96 (dd, 1H, $J_{2,3}=3.2$, $J_{3,4}=10.1$, H-3), 5.87 (dd, 1H, $J_{2,3}=3.1$, $J_{3,4}=9.8$, H-3), 5.89 (dd, 1H, $J_{3,4}=J_{4,5}=9.7$, H-4), 5.86 (dd, 1H, $J_{1,2}=1.4$, $J_{2,3}=3.1$, H-2), 5.81 (dd, 1H, $J_{2,3}=3.0$, $J_{3,4}=10.5$, H-3), 5.76 (dd, 1H, $J_{1,2}=1.5$, $J_{2,3}=3.2$, H-2), 5.64 (dd, 1H, $J_{1,2}=1.3$, $J_{2,3}=3.1$, H-2), 5.55–5.52 (m, 3H, H-1, H-2 and H-3), 5.44 (dd, 1H, $J_{3,4}=J_{4,5}=9.6$, H-4), 5.41 (d, 1H, $J_{1,2}=1.0$, H-1), 5.28 (q, 1H, $J=4.6$, MeC*H*), 5.20 (d, 1H, $J_{1,2}=1.5$, H-1), 5.04 (d, 1H, $J_{1,2}=0.7$, H-1), 5.02 (d, 1H, $J_{1,2}=1.1$, H-1), 5.00 (d, 1H, $J_{1,2}=0.8$, H-1), 4.73–4.46 (m, 15H), 4.25–4.16 (m, 3H), 4.04 (ddd, 1H, $J_{4,5}=9.6$, $J_{5,6}=5.1$, $J_{5,6'}=2.2$, H-5), 3.92 (dd, 1H, $J_{5,6}=5.6$, $J_{6,6}=12.4$, H-6), 3.78 (dd, 1H, $J_{2,3}=3.3$, $J_{3,4}=9.8$, H-3), 3.70 (dd, 1H, $J_{5,6'}=2.3$, $J_{6,6'}=12.4$, H-6′), 3.38 (ddd, 1H, $J_{4,5}=9.7$, $J_{5,6}=5.1$, $J_{5,6'}=2.3$, H-5), 2.30 (s, 3H, *Me*CO), 2.26 (s, 3H, *Me*CO), 1.93 (s, 3H, *Me*CO), 1.52 (d, 1H, $J=4.6$, *Me*CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 170.0, 168.7 (3C, 3Me*CO*), 165.8, 165.7, 165.6, 165.5, 165.3, 165.1, 165.0, 164.9, 164.8, 164.6 (14C, 14Ph*CO*, some signals overlapped), 133.1–127.9 (*Ph*CO), 104.2 (CH_3CH), 100.6, 99.1, 98.5, 97.2, 96.9, 96.3 (6C, 6C-1), 80.5, 78.9, 74.4, 73.8, 70.6, 70.4, 70.2, 70.0, 69.2, 69.0, 68.9, 68.8, 68.5, 68.1, 67.3, 67.0, 66.4, 66.0, 65.7, 63.4, 63.0, 62.5, 61.9 (30C, 6C-2–6, some signals overlapped), 21.6 (CH_3CH), 20.5, 20.3, 20.1 (3C, 3*Me*CO). Anal. calcd for $C_{142}H_{126}O_{48}$: C, 65.58; H, 4.88. Found C, 65.78; H, 4.75%.

4.16. α-D-Mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-{α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-α-D-mannopyranosyl-(1→6)}-α-D-mannopyranose 25

Compound **23** (910 mg, 0.35 mmol) was dissolved in 90% TFA (15 mL) and stirred for 2 h, and then the reaction mixture was poured directly into toluene (50 mL) and concentrated. The residue was purified by flash chromatography (1:2 petroleum ether–EtOAc) to give **24** as a foamy solid (730 mg, 81%). Compound **24** was dissolved in a saturated ammonia–MeOH solution (50 mL). After 1 week at rt, the reaction mixture was concentrated and the residue was purified by chromatography on Sephadex LH-20 (MeOH) to afford **25** as a syrup (203 mg, 72%) with predominant α-anomer at the reducing end: $[\alpha]_D^{25}$ +80.0 (*c* 1.0, D_2O); ^{1}H NMR (400 MHz, D_2O) δ 5.24, 5.03, 4.93, 4.78, 4.73, 4.71 (d, 6H, $J_{1,2}\approx 0$, 6H-1), 4.13–3.50 (m, 36H, 6H-2–6); ^{13}C NMR (100 MHz, D_2O) δ 104.7, 104.6, 103.0, 102.0, 101.8, 101.7 (6C, 6C-1). MALDI-TOF MS calcd for $C_{36}H_{62}O_{31}$: 990.86 [M]. Found: 1013.74 [M+Na].

4.17. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-{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*-acetyl-α-D-mannopyranosyl-(1→6)}-1,2-*O*-ethylidene-β-D-mannopyranose 26

To a cooled solution (0°C) of **11** (866 mg, 0.5 mmol) and **15** (782 mg, 0.5 mmol) in anhydrous CH_2Cl_2 (25 mL) was added TMSOTf (9 μL, 0.05 mmol). The mixture was

stirred at this temperature for 2 h, and then quenched with Et_3N (two drops). Evaporation of the solvents in vacuo gave a residue, and purification of the residue by silica gel column chromatography (1:1 petroleum ether–EtOAc) gave heptasaccharide **26** as a white foam (1.29 g, 82%). For the (*S*)-isomer: $[\alpha]_D^{25}$ −17.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05–7.22 (m, 90H, 18Ph), 6.22 (dd, 1H, $J_{3,4}=J_{4,5}=10.1$, H-4), 6.14 (dd, 1H, $J_{3,4}=J_{4,5}=10.0$, H-4), 6.06–5.89 (m, 6H), 5.82–5.76 (m, 4H), 5.66 (q, 1H, $J=4.9$, MeC*H*), 5.54–5.52 (m, 2H, 2H-2), 5.02 (dd, 1H, $J_{3,4}=J_{4,5}=9.9$, H-4), 5.47–5.46 (2d, 2H, $J_{1,2}=1.4$, 2H-2), 5.36 (d, 1H, $J_{1,2}=1.3$, H-1), 5.23 (d, 1H, $J_{1,2}=1.5$, H-1), 5.16 (d, 1H, $J_{1,2}=1.0$, H-1), 5.02 (d, 1H, $J_{1,2}=1.3$, H-1), 4.98 (d, 1H, $J_{1,2}=1.0$, H-1), 4.73–4.39 (m, 18H), 4.19–3.96 (m, 6H), 3.78 (dd, 1H, $J_{2,3}=3.4$, $J_{3,4}=9.4$, H-3), 3.70 (dd, 1H, $J_{5,6'}=2.2$, $J_{6,6'}=11.3$, H-6′), 3.38 (ddd, 1H, $J_{4,5}=9.4$, $J_{5,6}=5.5$, $J_{5,6'}=2.2$, H-5), 2.35 (s, 3H, *Me*CO), 2.24 (s, 3H, *Me*CO), 1.32 (d, 1H, $J=4.9$, *Me*CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.4 (2C, 2Me*CO*), 166.6, 166.4, 166.3, 166.2, 166.1, 165.6, 165.5, 165.4, 165.1, 164.8 (18C, 18Ph*CO*, some signals overlapped), 133.5–128.4 (*Ph*CO), 103.8 (CH_3CH), 101.3, 99.7, 99.7, 99.1, 97.7, 97.6, 97.5 (7C, 7C-1), 81.5, 75.3, 74.4, 71.0, 70.8, 70.5, 70.3, 70.1, 69.9, 69.6, 69.2, 69.0, 68.3, 67.9, 66.9, 66.7, 66.4, 66.1, 64.0, 63.5, 63.0, 62.6, 62.5 (35C, 7C-2–6, some signals overlapped), 21.2 (CH_3CH), 21.0, 20.8 (2C, 2*Me*CO). Anal. calcd for $C_{174}H_{150}O_{56}$: C, 66.62; H, 4.82. Found C, 66.84; H, 4.77%.

4.18. α-D-Mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-{α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-α-D-mannopyranosyl-(1→6)}-α-D-mannopyranose 28

Compound **26** (1.00 g, 0.32 mmol) was dissolved in 90% TFA (15 mL) and stirred for 2 h, and then the reaction mixture was poured directly into toluene (50 mL) and concentrated. The residue was purified by flash chromatography (1:2 petroleum ether–EtOAc) to give **27** (860 mg, 87%) as a white foam. Compound **27** was dissolved in a saturated ammonia–MeOH solution (50 mL). After 1 week at rt, the reaction mixture was concentrated and the residue was purified by chromatography on Sephadex LH-20 (MeOH) to afford **28** (242 mg, 76%) as a foamy solid with predominant α-anomer at the reducing end: $[\alpha]_D^{25}$ +74.5 (*c* 1.0, D_2O); ^{1}H NMR (400 MHz, D_2O) δ 5.22, 5.17, 5.01, 5.00, 4.91, 4.77, 4.72 (d, 7H, $J_{1,2}\approx 0$, 7H-1), 4.02–3.50 (m, 42H, 7H-2–6); ^{13}C NMR (100 MHz, D_2O) δ 103.4, 103.1, 103.0, 102.9, 102.1, 101.9, 101.9 (7C, 7C-1). MALDI-TOF MS calcd for $C_{42}H_{72}O_{36}$: 1152.99 [M]. Found: 1175.87 [M+Na].

4.19. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-{2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[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)]-2,4-di-*O*-acetyl-α-D-mannopyranosyl-(1→6)}-1,2-*O*-ethylidene-β-D-mannopyranose 29

To a cooled solution (0°C) of **11** (433 mg, 0.25 mmol) and **19** (494 mg, 0.25 mmol) in anhydrous CH_2Cl_2 (25 mL) was added TMSOTf (9 μL, 0.05 mmol). The mixture was stirred at this temperature for 2 h, and then quenched with Et_3N (two drops). The solvents were evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (1:1 petroleum ether–EtOAc) to give octasaccharide **29** as a syrup (710 mg, 80%). For the (*R*)-isomer: $[\alpha]_D^{25}$ −9.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05–7.22 (m, 100H, 20Ph), 6.21 (dd, 1H, $J_{3,4}=J_{4,5}=10.0$, H-4), 6.04–5.90 (m, 9H), 5.81–5.78 (m, 3H), 5.65 (dd, 1H, $J_{1,2}=1.1$, $J_{2,3}=3.2$, H-2), 5.53–5.50 (m, 3H), 5.46 (d, 1H, $J_{1,2}=1.2$, H-1), 5.43 (d, 1H, $J_{1,2}=1.5$, H-1), 5.37 (d, 1H, $J_{1,2}=1.2$, H-1), 5.27 (q, 1H, $J=4.8$, MeC*H*), 5.25 (d, 1H, $J_{1,2}=1.3$, H-1), 5.14 (d, 1H, $J_{1,2}=1.5$, H-1), 5.07 (d, 1H, $J_{1,2}=1.4$, H-1), 5.01 (d, 1H, $J_{1,2}=1.4$, H-1), 4.97 (d, 1H, $J_{1,2}=1.2$, H-1), 4.70–4.26 (m, 21H), 4.18–4.08 (m, 5H), 3.98–3.92 (m, 2H), 3.88 (dd, 1H, $J_{2,3}=3.2$, $J_{3,4}=9.6$, H-3), 3.57 (dd, 1H, $J_{5,6'}=2.0$, $J_{6,6'}=10.8$, H-6′), 3.42 (ddd, 1H, $J_{4,5}=9.8$, $J_{5,6}=4.9$, $J_{5,6'}=2.1$, H-5), 2.21 (s, 3H, *Me*CO), 2.18 (s, 3H, *Me*CO), 1.95 (s, 3H, *Me*CO), 1.48 (d, 1H, $J=4.8$, *Me*CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 169.8, 167.4 (3C, 3Me*CO*), 165.6–164.6 (Ph*CO*), 133.0–128.0 (*Ph*CO), 104.1 (CH_3CH), 100.8, 99.5, 99.3, 99.2, 98.5, 97.6, 97.2, 96.2 (7C, 7C-1), 81.6–61.9 (C-2–6), 21.6 (CH_3CH), 20.5, 20.4, 20.1 (3C, 3*Me*CO). Anal. calcd for $C_{196}H_{170}O_{64}$: C, 66.32; H, 4.83. Found C, 66.55; H, 4.88%.

4.20. α-D-Mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-{α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-α-D-mannopyranosyl-(1→6)}-α-D-mannopyranose 31

Compound **29** (600 mg, 0.17 mmol) was dissolved in 90% TFA (15 mL) and stirred for 2 h, and then the reaction mixture was poured directly into toluene (50 mL) and concentrated. The residue was purified by flash chromatography (1:2 petroleum ether–EtOAc) to give **30** (510 mg, 86%) as a white foam. Compound **30** was dissolved in a saturated ammonia–MeOH solution (50 mL). After 1 week at rt, the reaction mixture was concentrated and the residue was purified by chromatography on Sephadex LH-20 (MeOH) to afford **31** (154 mg, 82%) as a syrup with predominant α-anomer at the reducing end: $[\alpha]_D^{25}$ +30.7 (*c* 1.0, D_2O); ^{1}H NMR (400 MHz, D_2O) δ 5.43, 5.39, 5.34, 5.27, 5.11, 5.08, 5.02, 5.00 (d, 8H, $J_{1,2}\approx 0$, 8H-1), 4.11–3.31 (m, 48H, 8H-2–6); ^{13}C NMR (100 MHz, D_2O) δ 105.4, 105.3, 105.0, 104.8, 103.9, 101.8, 101.4, 100.9 (8C, 8C-1). MALDI-TOF MS calcd for $C_{48}H_{82}O_{41}$: 1315.14 [M]. Found: 1338.27 [M+Na].

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References

1. *Essentials of Glycobiology*, Varki, A.; Cummings, R.; Esko, J.; Freeze, H.; Hart, G.; Marth, J., Eds.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York, 1999.
2. (a) Wyss, D. F.; Choi, J. S.; Li, J.; Knoppers, M. H.; Willis, K. J.; Arulanandam, A. R. N.; Smolyar, A.; Reinherz, E. L.; Wagner, G. *Science* **1995**, *269*, 1273; (b) Recny, M. A.; Luther, M. A.; Knoppers, M. H.; Neidhardt, E. A.; Khandekar, S. S.; Concino, M. F.; Shimke, P. A.; Francis, M. A.; Moebius, U. *J. Biol. Chem.* **1992**, *267*, 22428.
3. (a) Bewley, C. A.; Otero-Quintero, S. *J. Am. Chem. Soc.* **2001**, *123*, 3892; (b) Hansen, J.-E. S.; Nielsen, C.; Arendrup, M.; Olofsson, S.; Mathiesen, L.; Nielsen, J. O.; Clausen, H. *J. Virol.* **1991**, *65*, 6461; (c) Hansen, J.-E. S.; Clausen, H.; Sorensen, T.; White, T.; Wandall, H. H. *Alfred Benzon Symp.* **1994**, *36*, 297.
4. (a) Rademann, J.; Geyer, A.; Schmidt, R. R. *Angew. Chem., Int. Ed.* **1998**, *37*, 1241; (b) Ogawa, T.; Katano, K.; Sasajima, K.; Matsui, M. *Tetrahedron* **1981**, *37*, 2779; (c) Ogawa, T.; Sasajima, K. *Tetrahedron* **1981**, *37*, 2787; (d) Guo, Z.; Nakahara, Y.; Ogawa, T. *Tetrahedron Lett.* **1997**, *38*, 4799; (e) Depre, D.; Duffels, A.; Green, L. G.; Lenz, R.; Ley, S. V.; Wong, C.-H. *Chem. Eur. J.* **1999**, *5*, 3326; (f) Dan, A.; Lergenmuller, M.; Amano, M.; Nakahara, Y.; Ogawa, T.; Ito, Y. *Chem. Eur. J.* **1998**, *4*, 2182; (g) Unverzagt, C.; Seifert, J. *Tetrahedron Lett.* **1997**, *38*, 7857; (h) Weiler, S.; Schmidt, R. R. *Tetrahedron Lett.* **1998**, *39*, 2292; (i) Wang, W.; Kong, F. *Angew. Chem., Int. Ed.* **1999**, *38*, 1247; (j) Becker, B.; Furneaux, R. H.; Reck, F.; Zubkov, O. A. *Carbohydr. Res.* **1999**, *315*, 148; (k) Ogawa, T.; Katano, K.; Matsui, M. *Carbohydr. Res.* **1978**, *64*, c3.
5. Bachhawat, K.; Thomas, C. J.; Amutha, B.; Krishnasastry, M. V.; Khan, M. I.; Surolia, A. *J. Biol. Chem.* **2001**, *276*, 5541.
6. (a) Zhu, Y.; Kong, F. *Synlett* **2000**, 663; (b) Unverzagt, C. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1102.
7. Betaneli, V. I.; Ovchinnikov, M. V.; Kochetkov, N. K. *Carbohydr. Res.* **1982**, *107*, 285.
8. Zhu, Y.; Kong, F. *Synlett* **2000**, 1783.
9. Zhu, Y.; Kong, F. *Synlett* **2001**, 1217.