Pergamon

Tetrahedron: *Asymmetry* 14 (2003) 2595–2603

TETRAHEDRON:
ASYMMETRY

Concise syntheses of β-GlcNAc*p*-(1→6)-α-Man*p*-(1→6)-Man*p* and its dimer, and β-GlcNAc*p*-(1→2)-α-Man*p*-(1→6)-Man*p*

Zuchao Ma, Jianjun Zhang and Fanzuo Kong*

Research Center for Eco-Environmental Sciences, Academia Sinica, PO Box 2871, Beijing 100085, PR China

Received 22 May 2003; accepted 9 July 2003

Abstract—β-D-GlcNAc*p*-(1→6)-α-D-Man*p*-(1→6)-D-Man*p* and β-D-GlcNAc*p*-(1→2)-α-D-Man*p*-(1→6)-D-Man*p* were synthesized as their methyl glycosides in a regio- and stereoselective way, whereas an ethylene glycol-coupled dimer of the former was prepared in a convergent manner.

1. Introduction

The trisaccharide β-D-GlcNAc*p*-(1→6)-α-D-Man*p*-(1→6)-D-Man*p* **I** is of interest since it has been observed as increased branching of asparagine-linked oligosaccharides in murine and human tumor cells[1] and has been associated with malignancy.[2] It has also been reported that the regio isomer of the trisaccharide β-D-GlcNAc*p*-(1→2)-α-D-Man*p*-(1→6)-D-Man*p* **II** can be recognized[3] by *N*-acetylglucosaminyl transferase V and used as a transferase inhibitor.[4] To the best of our knowledge, there have been no reports so far dealing with the synthesis of **I** even though it is important in glycobiology research. Herein we report the regio- and stereoselective syntheses of the trisaccharide **I** and its ethylene glycol-linked dimer, and a concise synthesis of the trisaccharide **II** as well.

2. Results and discussion

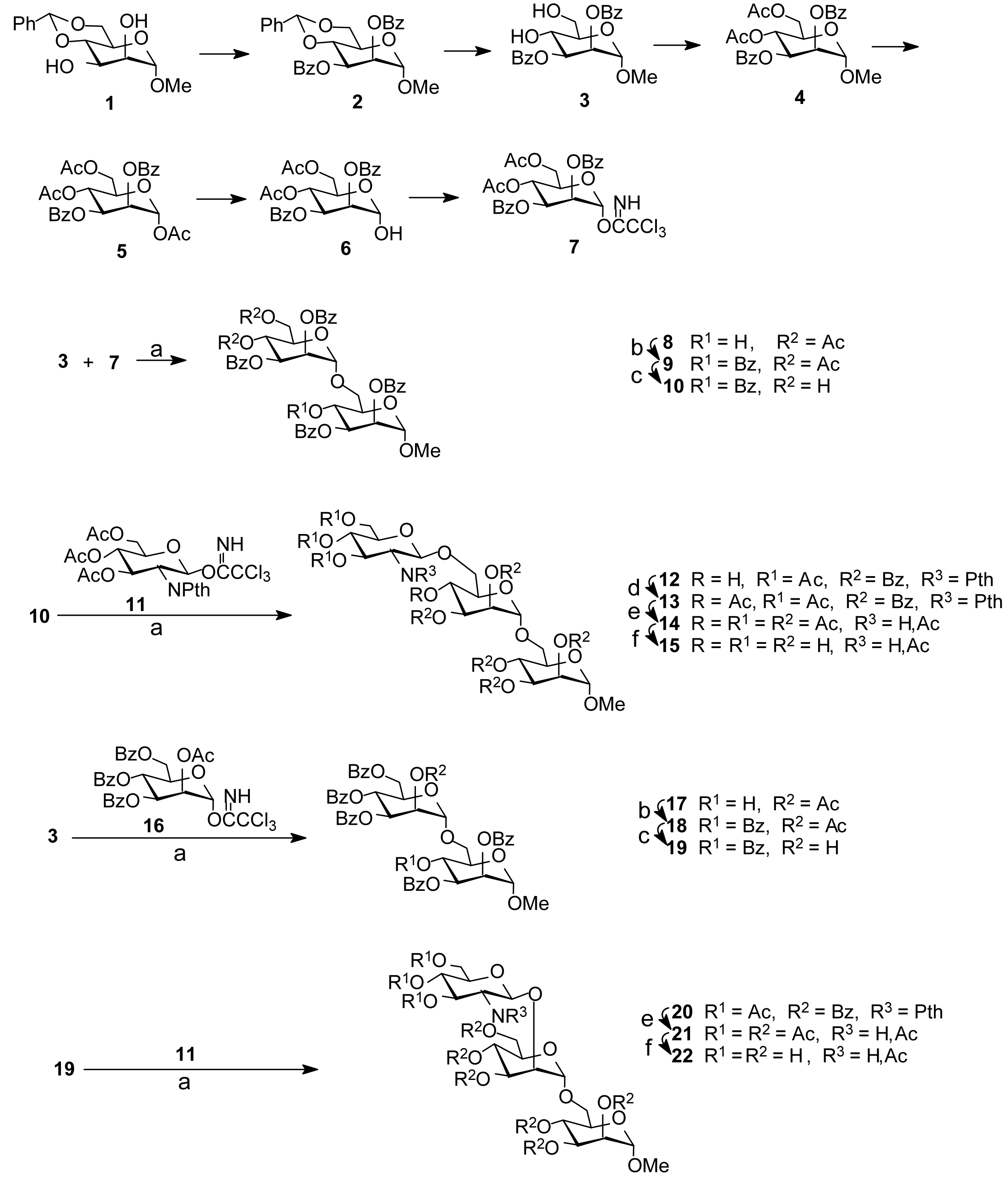
We have developed a method[5] for oligosaccharide synthesis using unprotected or lightly protected sugars as the glycosyl acceptors, with the result being a variety of complex oligosaccharides that can be synthesized efficiently.[6] In the present research, concise syntheses of the title oligosaccharides were achieved using this strategy. Scheme 1 shows the syntheses of trisaccharides **I** and **II**. Methyl 4,6-*O*-benzylidene-α-D-mannopyranoside **1** was chosen as the starting material. Benzoylation of **1** followed by debenzylidenation afforded the glycosyl acceptor **3**. Subsequent acetylation with acetic anhydride in pyridine gave **4**, and acetolysis with HOAc–Ac_2O–H_2SO_4 furnished **5**. Selective 1-*O*-deacetylation with NH_3–MeOH followed by trichloroacetimidation[7] afforded the glycosyl donor **7**. Condensation of the donor **7** with the acceptor **3** afforded the (1→6)-linked disaccharide **8** (81.3%). The regioselectivity of glycosylation was confirmed by benzoylation to give **9** which showed in its ^{1}H NMR spectrum a new signal at δ 5.96 ppm with $J_{3,4}=J_{4,5}=$ 10.6 Hz for H-4 when compared to **8**. Consecutively selective 4′6′-de-*O*-acetylation of **9** with CH_3COCl–MeOH[8] offered the disaccharide acceptor **10** in a satisfactory yield (77.6%). Selective 6-*O*-glycosylation of **10** with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate **11** proceeded smoothly giving the trisaccharide **12** (78.5%). Again, the 6-*O*-glycosylation was verified by acetylation to furnish **13** and the ^{1}H NMR spectrum of **13** showed a characteristic signal for H-4′ at δ 5.09 ppm with $J_{3,4}=J_{4,5}=9.7$ Hz. Treatment of **13** with hydrazine hydrate under reflux removed all of the acyl groups and subsequent acetylation with acetic anhydride in pyridine gave the acetylated trisaccharide **14**. Finally, deacetylation of **14** in a saturated solution of NH_3 in methanol yielded the target (1→6)-linked trisaccharide **15**.

The trisaccharide **22** was also prepared in a concise way as outlined in Scheme 1. Thus, coupling of the acceptor **3** with 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate **16**[6b] selectively afforded the (1→6)-linked disaccharide **17** (82.2%). Benzoylation of **17** followed by 2′-*O*-deacetylation furnished the disac-

---

* Corresponding author. E-mail: fzkong@mail.rcees.ac.cn

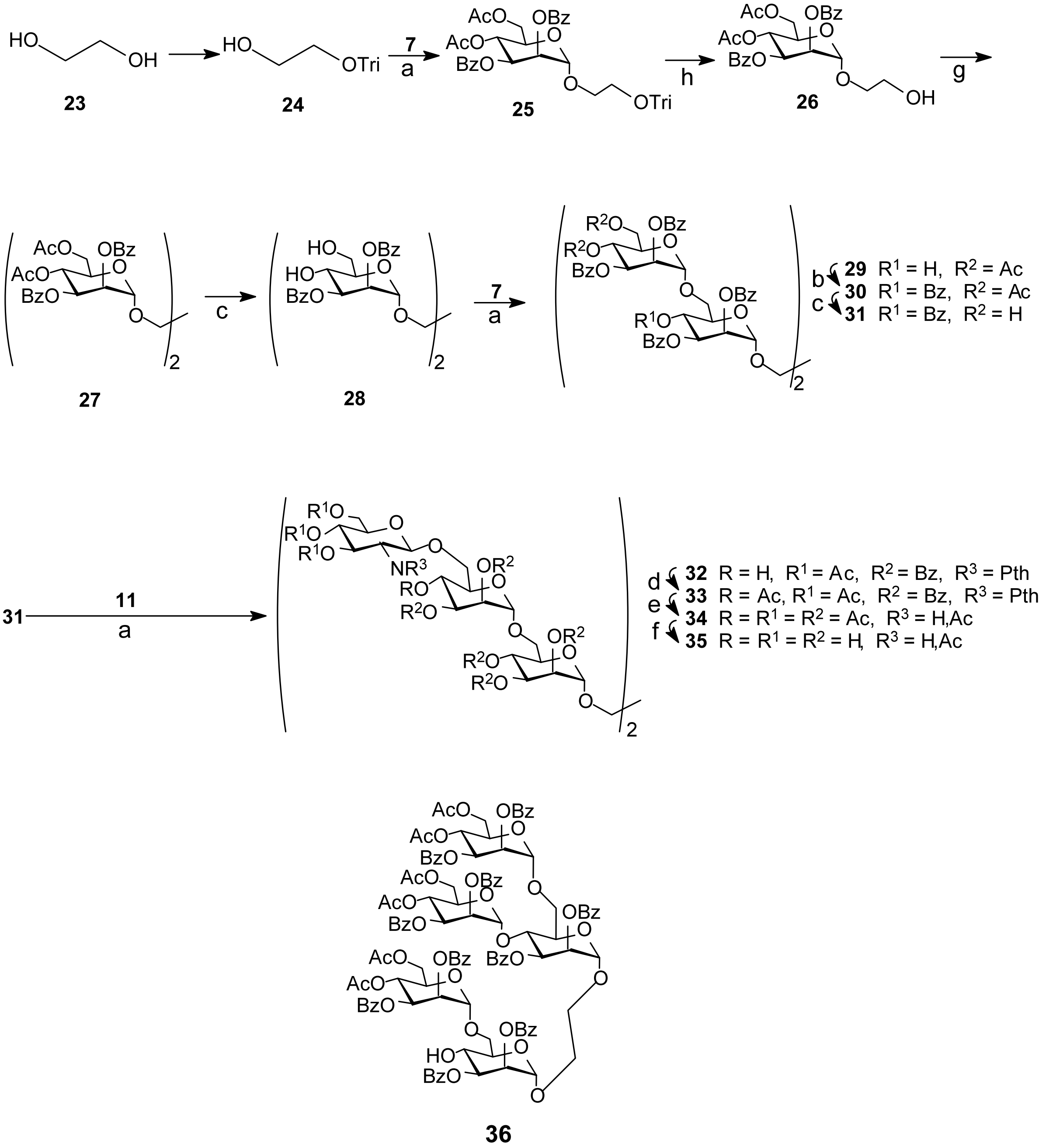
0957-4166/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0957-4166(03)00570-6



Scheme 1. *Reagents and conditions*: (a) TMSOTf (0.01 equiv.), CH_2Cl_2, −20∼0°C, 2–4 h; 81.3% for **8**, 78.5% for **12**, 82.2% for **17**, 66.6% for **20**, 82.3% for **25**, 61.5% for **29**, and 80.4% for **32**, respectively. (b) BzCl–pyridine; 83.3%, 81.5%, 88.7% for **9**, **18**, and **30**, respectively. (c) 2–4% CH_3COCl in CH_2Cl_2–CH_3OH, rt, 12 h; 77.6% for **10**, 74.7% for **19**, 75.8% for **28**, and 72.3% for **31**, respectively. (d) Ac_2O–pyridine; 84.7% for **13**, 86.9% for **33**. (e) i: EtOH–10% hydrazine hydrate, reflux, 48 h; ii: Ac_2O–pyridine (dry), rt, 12 h; 82.3%, 79.5%, 75.5% for **14**, **21**, and **34**, respectively. (f) satd NH_3–MeOH, rt, 72 h; 85.9%, 84.6%, 82.2% for **15**, **22**, and **35**, respectively.

charide acceptor **19** (74.7%). Condensation of **19** with **11** offered the protected trisaccharide **20** in good yield (66.6%). Reiteration of the dephthalylation, acetylation and deacetylation gave the target trisaccharide **22**.

Glycosyltransferases are specific with respect to both the donor and the acceptor, and irreversible inhibitors, such as those used to block glycoside hydrolases through covalent modification of the active site, are not yet well understood. Groups for the covalent modification of active sites must be attached to the ligand in such a way that they do not prevent specific binding. The first reported spacer-modified 'reducing' disaccharide consisted of two α-D-glucose residues (1→4)-linked by an acyclic six-membered spacer and competitively inhibited porcine pancreatic alpha-amylase.[9] Also a 'non-reducing' spacer-modified disaccharide, which mimicked part of the biantennary core structure of *N*-linked glycoconjugates, was also reported.[10] Dimaltoside derivatives of ethane-1,2-diol having the α,α, α,β, and β,β anomeric configurations at the linkage sites were synthesized by Tsuchiya et al.[11] In the present research, an ethylene glycol-coupled dimer of the trisaccharide **I** was also readily synthesized as indicated in Scheme 2. First, the glycosyl donor **7** was coupled with trityloxyethyl alcohol to afford **25** (82.3%). Detritylation followed by condensation with **7** offered a glycol-coupled disaccharide **27** (88.7%). Compound **27** was also obtainable by coupling of **23** with 2 equiv. of **7**, but a tedious separation of the product was necessary. Selective removal of the acetyl groups in 2% CH_3COCl–MeOH furnished **28** in satisfactory yield (75.8%). Subsequent coupling of **28** with **7** yielded tetrasaccharide **29** in a fair yield (61.5%). It was noted



Scheme 2. *Reagents and conditions*: (a) TMSOTf (0.01 equiv.), CH_2Cl_2, −20∼0°C, 2–4 h; 81.3% for **8**, 78.5% for **12**, 82.2% for **17**, 66.6% for **20**, 82.3% for **25**, 61.5% for **29**, and 80.4% for **32**, respectively. (b) BzCl–pyridine; 83.3%, 81.5%, 88.7% for **9**, **18**, and **30**, respectively. (c) 2–4% CH_3COCl in CH_2Cl_2–CH_3OH, rt, 12 h; 77.6% for **10**, 74.7% for **19**, 75.8% for **28**, and 72.3% for **31**, respectively. (d) Ac_2O–pyridine; 84.7% for **13**, 86.9% for **33**. (e) i: EtOH–10% hydrazine hydrate, reflux, 48 h; ii: Ac_2O–pyridine (dry), rt, 12 h; 82.3%, 79.5%, 75.5% for **14**, **21**, and **34**, respectively. (f) satd NH_3–MeOH, rt, 72 h; 85.9%, 84.6%, 82.2% for **15**, **22**, and **35**, respectively.

that in the preparation of **29**, the molar ratio of **7**:**28** was 1:1, otherwise, for example with 2:1, pentasaccharide **36** was the major product. Benzoylation of **29** followed by deacetylation gave the tetrasaccharide acceptor **31** (72.3%). Condensation of the acceptor **31** with the donor **11** selectively gave the (1→6)-linked oligosaccharide **32** (80.4%). Acetylation of **32** afforded **33**, with the ^{1}H NMR data of **33** showing a characteristic signal for H′-4 at δ 5.32 ppm with $J_{3,4}=J_{4,5}=9.9$ Hz. Deprotection of **33** was completed by dephthalylation, acetylation, and then by deacylation.

Bioassay of the samples **15**, **22**, and **35** is in progress, and the results will be reported in due course.

3. Conclusion

In summary, the efficient syntheses of β-D-GlcNAc*p*-(1→6)-α-D-Man*p*-(1→6)-D-Man*p* **I** and its glycol-coupled dimer, and β-D-GlcNAc*p*-(1→2)-α-D-Man*p*-(1→6)-D-Man*p* **II** were achieved with lightly protected mannose derivatives as the acceptors.

4. Experimental

4.1. General methods

Optical rotations were determined at 25°C with a Perkin–Elmer Model 241-Mc automatic polarimeter. ^{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 a MALTI-TOF-MS with CCA as the 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.

4.2. Methyl 4,6-di-*O*-acetyl-2,3-di-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate 7

Compound **3**[12] was obtained from **1**[13] by benzoylation followed by debenzylidenation. To a solution of **3** (8.04 g, 0.02 mol) in pyridine (40 mL) was added Ac_2O (25 mL, 0.25 mol). The reaction mixture was stirred at rt for 12 h and then concentrated to give the crude product **4** as a syrup, to which an acetolysis reagent ($HAc:Ac_2O:H_2SO_4=10:10:2$, 44 mL) was directly added. The reaction mixture was stirred at rt overnight, then poured onto ice (400 g). To the mixture was added CH_2Cl_2 (500 mL) and then washed with saturated aq $NaHCO_3$ and water. Concentration of the organic layer gave the crude product **5** as a syrup, which was dissolved in satd NH_3–MeOH–toluene (MeOH:toluene = 2:1, 150 mL). After stirring at rt for 1 h, TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The solvents were evaporated to give the crude product **6** as a syrup. Compound **6** was then dissolved in CH_2Cl_2 (60 mL), after which CCl_3CN (4 mL, 0.04 mol) and DBU (0.5 mL, 3.3 mmol) were added. The reaction mixture was stirred for 2 h, at the end of which TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. Concentration of the reaction mixture, followed by purification of the crude product on a silica gel column with 2.5:1 petroleum ether–EtOAc as the eluent, furnished the donor **7** (9.57 g, 77.6% for four steps) as a foamy solid. $[\alpha]_D=-68.7$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09∼7.26 (m, 10H, 2*Ph*), 6.51 (d, 1H, $J_{1,2}=2.0$, H-1), 5.85 (dd, 1H, $J_{1,2}=2.0$, $J_{2,3}=3.1$, H-2), 5.81 (dd, 1H, $J_{3,4}=J_{4,5}=9.8$, H-4), 5.75 (dd, 1H, $J_{2,3}=3.1$, $J_{3,4}=10.1$, H-3), 4.39∼4.25 (m, 3H, H-5, H-6), 2.14 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$). Anal. calcd for $C_{21}H_{26}Cl_3NO_9$: C, 52.74; H, 4.26. Found: C, 52.99; H, 4.34.

4.3. Methyl 4,6-di-*O*-acetyl-2,3-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3-di-*O*-benzoyl-α-D-mannopyranoside 8

To a cooled solution (−20°C) of **3** (0.60 g, 1.50 mmol) and **7** (1.02 g, 1.65 mmol) in anhydrous CH_2Cl_2 (25 mL) was added TMSOTf (30 μL, 0.17 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 (3:1 petroleum ether–EtOAc) to give disaccharide **8** (1.05 g, 81.3%) as a foamy solid. $[\alpha]_D=-28.5$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11∼7.34 (m, 20H, 4*Ph*), 5.78∼5.66 (m, 4H, H-2,2′,3,4′), 5.57 (dd, 1H, $J_{2',3'}=3.5$, $J_{3',4'}=9.8$, H-3′), 5.22 (d, 1H, $J_{1',2'}=1.3$, H-1′), 4.93 (d, 1H, $J_{1,2}=1.6$, H-1), 4.37∼4.18 (m, 5H), 4.03∼3.95 (m, 2H), 3.51 (s, 3H, CH_3O), 2.17 (bs, 1H, O*H*), 2.12 (s, 3H, $COCH_3$), 1.96 (s, 3H, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 169.7 (2C, $2CH_3CO$), 167.0, 165.5, 165.2, 165.1 (4C, 4PhCO), 133.4∼128.2 (*Ph*CO), 98.7, 97.5 (2C, 2C-1), 73.4, 71.5, 70.4, 70.2, 70.0, 68.6, 66.4, 66.2, 66.1, 62.4 (C-2∼6, some signals overlapped), 55.2 (CH_3O), 20.6 (CH_3CO). Anal. calcd for $C_{45}H_{44}O_{17}$: C, 63.08; H, 5.18. Found: C, 63.29; H, 5.13.

4.4. Methyl 4,6-di-*O*-acetyl-2,3-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside 9

To a solution of **8** (857 mg, 1.0 mmol) in pyridine (10 mL) was added benzoyl chloride (0.14 mL, 1.2 mmol). After stirring the mixture overnight at rt, TLC (2:1 petroleum ether–EtOAc) indicated that the reaction had gone to completion. Methanol (five drops) was added to the reaction mixture and allowed to stir for 10 min. Water (20 mL) was then added, and the mixture extracted with CH_2Cl_2 (3×20 mL). The extract was washed with 1 M HCl and saturated aq $NaHCO_3$, dried over Na_2SO_4 and concentrated. Purification by flash chromatography (3:1 petroleum ether–EtOAc) gave **9** as a foamy solid (801 mg, 83.3%). $[\alpha]_D=-98.7$ (c 1.0, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.17∼7.26 (m, 25H, 5*Ph*), 5.96 (dd, 1H, $J_{3,4}=J_{4,5}=10.6$, H-4), 5.91 (dd, 1H, $J_{2,3}=3.3$, $J_{3,4}=10.6$, H-3), 5.77∼5.66 (m, 4H), 5.09 (d, 1H, $J_{1,2}=1.1$, H-1′), 5.02 (d, 1H, $J_{1,2}=1.1$, H-1), 4.36 (m, 1H, H-5), 4.22∼4.16 (m, 2H), 4.07∼4.02 (m, 2H), 3.75 (m, 1H, H-6b), 3.60 (s, 3H, $\mathrm{C}H_3\mathrm{O}$), 2.00 (s, 3H, $\mathrm{C}H_3\mathrm{CO}$), 1.99 (s, 3H, $\mathrm{C}H_3\mathrm{CO}$). Anal. calcd for $C_{52}H_{48}O_{18}$: C, 65.00; H, 5.03. Found: C, 65.18; H, 4.97.

4.5. Methyl 2,3-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside 10

To a solution of **9** (777 mg, 0.81 mmol) in anhydrous CH$_2$Cl$_2$ (10 mL) was added anhydrous MeOH (50 mL), after which acetyl chloride (1.5 mL) was added to the reaction mixture at 0°C. The solution was stoppered in a flask and stirred at room temperature until TLC (3:1 petroleum ether–EtOAc) showed that the starting material had disappeared. The solution was neutralized with Et$_3$N and then concentrated to dryness. The residue was passed through a short silica gel column to give **10** (550 mg, 77.6%) as a foamy solid. $[\alpha]_D=-80.7$ (c 1.0, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.06∼7.27 (m, 25H, 5*Ph*), 6.06 (dd, 1H, $J_{3,4}=J_{4,5}=10.1$, H-4), 5.90 (dd, 1H, $J_{2,3}=3.3$, $J_{3,4}=10.1$, H-3′), 5.73∼5.65 (m, 3H), 5.07 (d, 1H, $J_{1,2}=1.0$, H-1′), 5.07 (d, 1H, $J_{1,2}=1.1$, H-1), 4.34∼4.27 (m, 2H), 4.05 (dd, 1H, $J_{5,6b}=4.5$, $J_{6a,6b}=10.7$, H-6b), 3.82∼3.74 (m, 4H), 3.58 (s, 3H, $\mathrm{C}H_3\mathrm{O}$), 2.17 (bs, 2H, 2O*H*); ^{13}C NMR (100 MHz, CDCl$_3$) δ 166.5, 165.8, 165.6, 165.4, 165.2 (5C, 5 Ph*CO*), 133.5∼128.2 (*Ph*CO), 98.7, 97.5 (2C, 2C-1), 72.8, 72.6, 70.3, 70.3, 70.1, 69.2, 67.1, 66.7, 66.3, 61.9 (10C, C-2∼6), 55.5 ($C\mathrm{H}_3\mathrm{O}$). Anal. calcd for $C_{48}H_{44}O_{16}$: C, 65.75; H, 5.06. Found: C, 65.84; H, 5.00.

4.6. Methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-2,3-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside 12

To a cooled solution (−20°C) of **10** (519 mg, 0.59 mmol) and **11** (361 mg, 0.62 mmol) in anhydrous CH$_2$Cl$_2$ (25 mL) was added TMSOTf (11 μL, 0.06 mmol). The mixture was stirred at this temperature for 2 h, and then quenched with Et$_3$N (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 trisaccharide **12** (602 mg, 78.5%) as a foamy solid. $[\alpha]_D=-51.5$ (c 1.0, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.13∼7.24 (m, 29H, 5*Ph*, *Pth*), 5.99 (dd, 1H, $J_{3,4}=J_{4,5}=10.0$, H-4), 5.85 (dd, 1H, $J_{2,3}=3.3$, $J_{3,4}=10.0$, H-3), 5.79 (dd, 1H, $J_{3'',4''}=9.4$, $J_{4'',5''}=10.5$, H-4″), 5.68 (dd, 1H, $J_{1',2'}=1.5$, $J_{2',3'}=3.1$, H-2′), 5.44∼5.36 (m, 3H, H-2, H-3′, H-1″), 5.12 (dd, 1H, $J_{2'',3''}=J_{3'',4''}=9.6$, H-3″), 4.95 (d, 1H, $J_{1',2'}=1.5$ Hz, H-1′), 4.66 (d, 1H, $J_{1,2}=1.0$ Hz, H-1), 4.31∼4.08 (m, 5H), 3.85∼3.79 (m, 4H), 3.64 (m, 1H), 3.55 (s, 3H, $\mathrm{C}H_3\mathrm{O}$), 3.42 (m, 1H), 2.50 (bs, 1H, O*H*), 2.07 (s, 3H, $\mathrm{C}H_3\mathrm{CO}$), 2.04 (s, 3H, $\mathrm{C}H_3\mathrm{CO}$), 1.89 (s, 3H, $\mathrm{C}H_3\mathrm{CO}$); ^{13}C NMR (100 MHz, CDCl$_3$) δ 170.6, 170.0, 169.4 (3C, $3\mathrm{CH}_3C\mathrm{O}$), 166.5, 165.7, 165.5, 165.4, 165.2 (5C, 5Ph*CO*), 133.8∼123.1 (*Ph*CO, *Pth*), 99.0, 98.6, 97.3 (3C, 3C-1), 72.9, 71.8, 71.3, 70.6, 70.3, 70.1, 70.0, 69.2, 69.1, 68.9, 66.9, 66.3, 65.9, 61.9, 54.3 (15C, C-2∼6), 55.5 ($C\mathrm{H}_3\mathrm{O}$), 20.7, 20.5, 20.4 ($C\mathrm{H}_3\mathrm{CO}$). Anal. calcd for $C_{68}H_{63}NO_{25}$: C, 63.11; H, 4.91. Found: C, 62.89; H, 4.82.

4.7. Methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-4-*O*-acetyl-2,3-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside 13

To a solution of compound **12** (688 mg, 0.53 mmol) in pyridine (10 mL) was added Ac$_2$O (5 mL, 5 mmol). The reaction mixture was stirred at rt for 12 h and then concentrated to give the crude product, which was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give trisaccharide **13** (602 mg, 84.7%) as a foamy solid. $[\alpha]_D=-62.7$ (c 1.0, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.27∼7.18 (m, 29H, 5*Ph*, *Pth*), 6.02 (dd, 1H, $J_{3,4}=J_{4,5}=10.0$, H-4), 5.88 (dd, 1H, $J_{2,3}=3.3$, $J_{3,4}=10.0$, H-3), 5.79 (dd, 1H, $J_{3'',4''}=9.3$, $J_{4'',5''}=10.4$, H-4″), 5.72 (dd, 1H, $J_{1',2'}=1.7$, $J_{2',3'}=3.3$, H-2′), 5.58 (dd, 1H, $J_{2',3'}=3.3$, $J_{3',4'}=9.7$, H-3′), 5.43 (dd, 1H, $J_{1,2}=1.7$, $J_{2,3}=3.3$, H-2), 5.35 (d, 1H, $J_{1'',2''}=8.5$, H-1″), 5.28 (dd, 1H, $J_{2,3}=J_{3,4}=10.2$, H-3″), 5.09 (dd, 1H, $J_{3',4'}=J_{4',5'}=9.7$, H-4′), 4.98 (d, 1H, $J_{1',2'}=1.7$, H-1′), 4.34∼4.1 (m, 4H), 4.93 (dd, 1H, $J_{5,6a}=1.8$, $J_{6a,6b}=12.0$, H-6a), 4.01 (m, 1H, H-5′), 3.89 (dd, 1H, $J_{5',6'a}=1.6$, $J_{6'a,6'b}=10.4$, H-6′a), 3.83 (ddd, 1H, $J_{4'',5''}=10.4$, $J_{5'',6''a}=2.1$, $J_{5'',6''b}=6.1$, H-5″), 3.72 (dd, 1H, $J_{5',6'b}=3.7$, $J_{6a',6'b}=10.4$, H-6′b), 3.58 (s, 3H, $\mathrm{C}H_3\mathrm{O}$), 3.44 (dd, 1H, $J_{5'',6''b}=6.1$, $J_{6''a,6''b}=10.3$, H-6″b), 3.25 (dd, 1H, $J_{5'',6''a}=2.1$, $J_{6''a,6''b}=10.3$, H-6″a), 2.06 (s, 3H, $\mathrm{C}H_3\mathrm{CO}$), 2.04 (s, 3H, $\mathrm{C}H_3\mathrm{CO}$), 1.91 (s, 3H, $\mathrm{C}H_3\mathrm{CO}$), 1.88 (s, 3H, $\mathrm{C}H_3\mathrm{CO}$). Anal. calcd for $C_{70}H_{65}NO_{26}$: C, 62.92; H, 4.90. Found: C, 62.73; H, 5.01.

4.8. Methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranoside 14

Trisaccharide **13** (563 mg, 0.42 mmol) was dissolved in EtOH (50 mL) to which was added 100% hydrazine hydrate (8 mL), and the solution allowed to reflux for 48 h. The solution was then concentrated, and the residue was co-evaporated several times with toluene. The residue was taken up in pyridine (10 mL) to which was added Ac$_2$O (5 mL). The solution was stirred for 12 h at rt and then evaporated to dryness. Purification of the residue by column chromatography (EtOAc) gave **14** (325 mg, 82.3% for two steps) as a foamy solid. $[\alpha]_D=+43.6$ (c 1.0, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$) δ 6.23 (d, 1H, $J_{\mathrm{H\text{-}C\text{-}N\text{-}H}}=8.9$, N*H*Ac), 5.40∼5.38 (m, 2H), 5.34∼5.32 (dd, 1H, $J_{2,3}=3.3$, $J_{3,4}=10.0$, H-3), 5.27∼5.22 (m, 2H), 5.12∼5.09 (m, 2H), 4.90 (d, 1H, $J_{1',2'}=1.7$, H-1′), 4.35 (d, 1H, $J_{1,2}=1.6$, H-1), 4.35 (d, 1H, $J_{1'',2''}=8.5$, H-1″), 4.23∼4.13 (m, 4H), 3.93∼3.91 (m, 2H), 3.75 (dd, 1H, $J_{5',6'b}=3.8$, $J_{6'a,6'b}=6.3$, H-6′b), 3.63∼3.68 (m, 1H), 3.55 (dd, 1H, $J_{5,6a}=2.5$, $J_{6a,6b}=11.1$, H-6a), 3.54 (s, 3H, $\mathrm{C}H_3\mathrm{O}$), 3.34 (m, 1H, H-6″a), 2.17 (s, 3H, $\mathrm{C}H_3\mathrm{CO}$), 2.10 (s, 3H, $\mathrm{C}H_3\mathrm{CO}$), 2.08 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO), 1.98 (s, 3H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 170.8, 170.8, 170.5, 170.3, 170.3, 170.1, 170.0, 169.6, 169.4 (10C, $10CH_3CO$), 102.2, 98.4, 97.4 (3C, 3C-1), 73.4, 72.1, 69.6, 69.3, 69.3, 69.2, 69.2, 68.8, 68.6, 68.1, 66.8, 66.7, 66.3, 62.1, 53.9 (15C, C-2 ~ 6), 55.3 (1C, CH_3O), 23.0 ~ 20.7 (CH_3CO, some signals overlapped). Anal. calcd for $C_{39}H_{55}NO_{25}$: C, 49.95; H, 5.91. Found: C, 50.11; H, 6.00.

4.9. Methyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside 15

Trisaccharide **14** (290 mg, 0.31 mmol) was dissolved in saturated NH_3–MeOH (20 mL). After 96 h at rt, the reaction mixture was concentrated, and the residue purified by chromatography on Sephadex LH-20 (MeOH) to afford **15** (149 mg, 85.9%) as a foamy solid. $[\alpha]_D = +36.2$ (*c* 1.3, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 4.75 (s, 1H, H-1′), 4.62 (s, 1H, H-1), 4.44 (d, 1H, $J_{1'',2''} = 8.4$ Hz, H-1″), 4.06 ~ 3.32 (m, 18H), 3.27 (s, 3H, CH_3O), 1.93 (s, 3H, CH_3CONH); ^{13}C NMR (100 MHz, D_2O) δ 174.6 (CH_3CONH), 101.6, 101.2, 99.7 (3C, 3C-1), 76.0, 74.0, 71.5, 70.9, 70.8, 70.1, 69.0, 66.9, 66.7, 65.7, 60.9, 54.9 (15C, C2 ~ 6, some signals overlapped), 55.7 (CH_3O), 22.4 (CH_3CONH). Anal. calcd for $C_{21}H_{37}NO_{16}$: C, 45.08; H, 6.67. Found: C, 45.22; H, 6.73.

4.10. Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3-di-*O*-benzoyl-α-D-mannopyranoside 17

Compound **3** (185 mg, 0.46 mmol) and **16** (344 mg, 0.51 mmol) were coupled under the same conditions as those used for the preparation of **8** from **3** and **7**, giving **17** (348 mg, 82.2%) as a foamy solid. $[\alpha]_D = +7.0$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 ~ 7.26 (m, 25H, 5*Ph*), 5.94 (dd, 1H, $J_{3',4'} = J_{4',5'} = 9.9$ Hz, H-4′), 5.87 (dd, 1H, $J_{2',3'} = 3.4$, $J_{3',4'} = 9.9$ Hz, H-3′), 5.66 (dd, 1H, $J_{1',2'} = 1.7$, $J_{2',3'} = 3.4$ Hz, H-2′), 5.62 (dd, 1H, $J_{1,2} = 1.6$, $J_{2,3} = 3.3$ Hz, H-2), 5.57 (dd, 1H, $J_{2,3} = 3.3$, $J_{3,4} = 9.8$, H-3), 5.14 (d, 1H, $J_{1',2'} = 1.7$, H-1′), 4.92 (d, 1H, $J_{1,2} = 1.6$, H-1), 4.64 ~ 4.39 (m, 4H), 4.25 (dd, 1H, $J_{5,6b} = 4.4$, $J_{6a,6b} = 11.3$, H-6b), 4.01 ~ 3.92 (m, 2H), 2.90 (bs, 1H, O*H*), 2.12 (s, 3H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6 (CH_3CO), 166.9, 166.1, 165.5, 165.4, 165.3 (5C, 5Ph*CO*), 98.8, 97.5 (2C, 2C-1), 133.3 ~ 128.2 (*Ph*CO), 73.4, 71.6, 70.3, 69.8, 69.7, 68.6, 67.2, 66.3, 66.0, 63.3 (10C, C-2 ~ 6), 55.2 (CH_3O), 20.6 (CH_3CO). Anal. calcd for $C_{50}H_{46}O_{17}$: C, 65.35; H, 5.05. Found: C, 65.66; H, 5.13.

4.11. Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside 18

Compound **17** (336 mg, 0.37 mmol) was benzoylated under the same conditions as those used for the preparation of **9** from **8**, giving **18** (305 mg, 81.5%) as a foamy solid. $[\alpha]_D = -27.7$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 ~ 7.26 (m, 30H, 6*Ph*), 6.05 (dd, 1H, $J_{3,4} = J_{4,5} = 10.1$, H-4), 5.94 ~ 5.90 (m3H), 5.74 (dd, 1H, $J_{1,2} = 1.7$, $J_{2,3} = 3.2$, H-2), 5.57 (s, 1H, H-2′), 5.02 (d, 1H, $J_{1,2} = 1.5$, H-1), 5.00 (d, 1H, $J_{1,2} = 1.4$, H-1′), 4.45 ~ 4.30 (m, 4H), 4.11 (dd, 1H, $J_{5,6b} = 5.3$, $J_{6a,6b} = 10.8$, H-6b), 3.75 (dd, 1H, $J_{5,6a} = 1.9$, $J_{6a,6b} = 10.8$, H-6a), 3.60 (s, 3H, CH_3O), 2.10 (s, 3H, CH_3CO). Anal. calcd for $C_{57}H_{50}O_{18}$: C, 66.92; H, 4.93. Found: C, 67.08; H, 4.99.

4.12. Methyl 3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside 19

Deacetylation of compound **18** (282 mg, 0.28 mmol) was carried out under the same conditions as those used for the preparation of **10** from **9**, giving **19** (202 mg, 74.7%) as a foamy solid. $[\alpha]_D = -37.6$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 ~ 7.25 (m, 30H, 6*Ph*), 6.10 (dd, 1H, $J_{3,4} = J_{4,5} = 10.1$, H-4), 5.95 ~ 5.90 (m, 2H), 5.77 (dd, 1H, $J_{2,3} = 3.3$, $J_{3,4} = 10.1$, H-3), 5.72 (dd, 1H, $J_{1,2} = 1.7$, $J_{2,3} = 3.3$, H-2), 5.07 (d, 1H, $J_{1,2} = 1.7$, H-1), 5.00 (d, 1H, $J_{1',2'} = 1.6$, H-1′), 4.39 ~ 4.29 (m, 5H), 4.12 (dd, 1H, $J_{5,6b} = 4.8$, $J_{6a,6b} = 11.0$, H-6b), 3.78 (dd, 1H, $J_{5,6a} = 2.2$, $J_{6a,6b} = 11.0$, H-6a), 3.59 (s, 3H, CH_3O), 2.31 (bs, 1H, O*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 165.5, 165.5, 165.4, 165.4, 165.3 (6C, 6Ph*CO*), 133.3 ~ 128.2 (*Ph*CO), 99.6, 98.8 (2C, 2C-1), 72.5, 70.5, 70.1, 69.5, 69.1, 68.7, 66.9, 66.7, 66.2, 63.2 (10C, C-2 ~ 6, C-2′ ~ 6′), 55.5 (CH_3O). Anal. calcd for $C_{55}H_{48}O_{17}$: C, 67.34; H, 4.93. Found: C, 67.08; H, 4.78.

4.13. Methyl 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)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside 20

To a cooled solution (0°C) of **19** (190 mg, 0.19 mmol) and **11** (123 mg, 0.21 mmol) in anhydrous CH_2Cl_2 (15 mL) was added TMSOTf (7 μL, 0.04 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 the trisaccharide **20** (180 mg, 66.6%) as a foamy solid. $[\alpha]_D = -42.2$ (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 ~ 7.15 (m, 34H, 6*Ph*, *Pth*), 6.07 (dd, 1H, $J_{3,4} = J_{4,5} = 10.2$, H-4), 5.87 (dd, 1H, $J_{2,3} = 3.4$, $J_{3,4} = 10.2$, H-3), 5.74 (dd, 1H, $J_{1,2} = 1.7$, $J_{2,3} = 3.3$, H-2), 5.73 ~ 5.69 (m, 2H), 5.57 (dd, 1H, $J_{1',2'} = 1.3$, H-1′), 5.38 (d, 1H, $J_{1'',2''} = 8.5$, H-1″), 4.58 (dd, 1H, $J_{1',2'} = 1.7$, $J'_{2',3'} = 3.2$, H-2′), 4.41 (dd, 1H, $J_{1'',2''} = 8.5$, $J_{2'',3''} = 10.9$, H-2″), 4.31 (m, 1H, H-5), 4.02 (m, 1H, H-5′), 3.97 ~ 3.90 (m, 2H), 3.76 ~ 3.61 (m, 5H), 3.60 (s, 3H, CH_3O), 1.96 (s, 3H, CH_3CO), 1.84 (s, 3H, CH_3CO), 1.79 (s, 3H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 170.0, 169.2 (3C, $3CH_3CO$), 165.6, 165.6, 165.5, 165.5, 165.4, 164.9 (6C, 6Ph*CO*), 134.0 ~ 125.2 (*Ph*CO, *Pth*), 98.9, 97.7, 96.9 (3C, 3C-1), 74.5, 71.7, 70.7, 70.5, 70.4, 70.2, 69.2, 68.8, 68.2, 66.5, 66.3, 65.9, 63.2, 61.8, 54.3 (15C, C-2$\sim$6), 55.5 (CH_3O), 29.6, 20.4, 20.4 (CH_3CO). Anal. calcd for $C_{75}H_{67}NO_{26}$: C, 64.42; H, 4.83. Found: C, 64.66; H, 4.91.

4.14. Methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1$\rightarrow$2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1$\rightarrow$6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranoside 21

Trisaccharide **20** (167 mg, 0.12 mmol) was dissolved in EtOH (20 mL) to which was added 100% hydrazine hydrate (4 mL), and the solution heated to reflux for 48 h. The solution was then concentrated, and the residue was co-evaporated several times with toluene. The residue was taken up in pyridine (10 mL) to which was added Ac_2O (5 mL). The solution was stirred for 12 h at rt and then evaporated to dryness. Purification of the residue by column chromatography (EtOAc) gave **21** (89 mg, 79.5% for two steps) as a foamy solid. $[\alpha]_D$= +25.7 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.72 (d, $J_{H\text{-}C\text{-}N\text{-}H}=7.8$, N*H*Ac), 5.53 (dd, 1H, $J_{3'',4''}=9.2$, $J_{4'',5''}=10.5$), 5.35$\sim$5.19 (m, 4H), 5.03 (d, 1H, $J_{1,2}=8.0$, H-1″), 5.05$\sim$4.96 (m, 2H), 4.73 (d, 1H, $J_{1,2}=1.4$, H-1′), 4.71 (d, 1H, $J_{1,2}=1.6$, H-1), 4.27$\sim$3.92 (m, 7H), 3.81$\sim$3.68 (m, 2H), 3.53$\sim$3.46 (m, 2H), 3.42 (s, 3H, CH_3O), 2.16 (s, 3H, CH_3CO), 2.09 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 1.99 (s, 6H, 2CH_3CO), 1.94 (s, 3H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 170.7, 170.3, 170.2, 170.0, 169.7, 169.6 (CH_3CO, some signals overlapped), 98.8, 98.5, 97.7 (3C, 3C-1), 74.2, 71.9, 71.6, 70.2, 69.6, 69.3, 69.1, 66.8, 66.1, 62.9, 62.3, 55.9 (15C, C-2$\sim$6), 55.3 (1C, CH_3O), 29.8$\sim$20.8 (CH_3CO). Anal. calcd for $C_{39}H_{55}NO_{25}$: C, 49.95; H, 5.91. Found: C, 49.77; H, 5.86.

4.15. Methyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1$\rightarrow$2)-α-D-mannopyranosyl-(1$\rightarrow$6)-α-D-mannopyranoside 22

Trisaccharide **21** (89 mg, 0.10 mmol) was dissolved in saturated NH_3–MeOH (20 mL). After 96 h at rt, the reaction mixture was concentrated, and the residue purified by chromatography on Sephadex LH-20 (MeOH) to afford **22** (45 mg, 84.6%) as a foamy solid. $[\alpha]_D$=+42.5 (*c* 1.3, H_2O); ^1H NMR (400 MHz, D_2O) δ 4.79 (s, 1H, H-1′), 4.62 (d, 1H, $J_{1,2}=1.2$, H-1), 4.44 (dd, 1H, $J_{1'',2''}=8.4$, H-1″), 3.99 (m, 1H, H-2), 3.90$\sim$3.31 (m, 17H), 3.27 (s, 3H, CH_3O), 1.90 (s, 3H, CH_3CONH); ^{13}C NMR (100 MHz, D_2O) δ 174.9 (CH_3CONH), 101.2, 99.7, 96.9 (3C, 3C-1), 76.5, 75.9, 73.4, 73.0, 71.0, 70.9, 70.0, 70.0, 69.7, 67.4, 66.5, 65.9, 61.7, 60.7, 54.9 (15C, C-2$\sim$6, some signals overlapped), 55.5 (CH_3O), 22.4 (CH_3CONH). Anal. calcd for $C_{21}H_{37}NO_{16}$: C, 45.08; H, 6.67. Found: C, 45.21; H, 6.59.

4.16. Trityloxyethyl 4,6-di-*O*-acetyl-2,3-di-*O*-benzoyl-α-D-mannopyranoside 25

Compound **7** (1.48 g, 2.40 mmol) and **24** (608 mg, 2 mmol) were dried together under high vacuum for 2 h and then dissolved in anhydrous CH_2Cl_2 (30 mL). TMSOTf was added dropwise at −20°C with N_2 protection. The reaction mixture was stirred for 2 h and then neutralized with Et_3N. Concentration of the reaction mixture, followed by purification on a silica gel column, gave **25** (1.07 g, 82.3%) as a foamy solid. $[\alpha]_D$=−45.3 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.08$\sim$7.24 (m, 25H, 5*Ph*), 5.86$\sim$5.69 (m, 3H), 5.13 (d, 1H, $J_{1,2}$= 1.7, H-1), 4.39$\sim$4.19 (m, 3H, H-5, H-6a, H-6b), 3.98$\sim$3.28 (m, 4H,2), 2.14 (s, 3H, CH_3CO), 1.94 (s, 3H, CH_3CO). Anal. calcd for $C_{45}H_{42}O_{11}$: C, 71.23; H, 5.58. Found: C, 71.46; H, 5.68.

4.17. Hydroxyethyl 4,6-di-*O*-acetyl-2,3-di-*O*-benzoyl-α-D-mannopyranoside 26

Compound **25** (1.06 g, 1.40 mmol) was dissolved in CH_2Cl_2 (10 mL), TsOH (110 mg) was added to the solution, and the mixture allowed to be stirred at 30°C overnight, after which it was neutralized with Et_3N. Concentration of the reaction mixture, followed by purification on a silica gel column, gave **26** (618 mg, 85.5%) as a foamy solid. $[\alpha]_D$=−47.5 (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.08$\sim$7.26 (m, 10H, 2*Ph*), 5.73$\sim$5.65 (m, 3H), 5.11 (d, 1H, $J_{1,2}=1.7$, H-1), 4.35 (dd, 1H, $J_{5,6b}=5.6$, $J_{6a,6b}=12.5$, H-6b), 4.25$\sim$4.22 (m, 2H, H-5, H-6a), 3.92$\sim$3.74 (m, 4H, 2CH_2), 2.15 (s, 3H, CH_3CO), 1.98 (s, 3H, CH_3CO). Anal. calcd for $C_{26}H_{28}O_{11}$: C, 60.46; H, 5.46. Found: C, 60.63; H, 5.55.

4.18. 1,2-Di-[4,6-di-*O*-acetyl-2,3-di-*O*-benzoyl-α-D-mannopyranosyloxy]ethane 27

Donor **7** (856 mg, 1.39 mmol) and acceptor **26** (597 mg, 1.16 mmol) were coupled under the same conditions as those used for the preparation of **8** from **3** and **7**, giving **27** (995 mg, 88.7%) as a foamy solid. $[\alpha]_D$=−78 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.09$\sim$7.26 (m, 20H, 4*Ph*), 5.80$\sim$5.73 (m, 4H), 5.68 (dd, 2H, $J_{1,2}=1.8$, $J_{2,3}=2.6$, 2H-2), 5.16 (d, 2H, $J_{1,2}=1.8$, 2H-1), 4.55 (dd, 2H, $J_{5,6b}=4.2$, $J_{6a,6b}=12.1$, 2H-6b), 4.37$\sim$4.30 (m, 4H, 2H-5, 2H-6a), 4.13$\sim$3.78 (m, 4H, 2CH_2), 2.18 (s, 6H, 2CH_3CO), 1.85 (s, 6H, 2CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 169.8 (4C, 4CH_3CO), 165.4, 165.3 (4C, 4PhCO), 113.6, 113.4, 130.0, 129.8, 129.5, 129.2, 128.6, 128.5 (*Ph*CO, some signals overlapped), 97.4 (C-1), 70.3, 70.2, 69.0, 66.3, 66.0 (C-2$\sim$6), 62.5 (CH_2), 20.9 (CH_3CO), 20.6 (CH_3CO). MALDI-TOF MS: Calcd for $C_{50}H_{50}NaO_{20}$ ([M+Na]): 993.92, found 993.28. Anal. calcd for $C_{50}H_{50}O_{20}$: C, 61.85; H, 5.19. Found: C, 62.02; H, 5.26.

4.19. 1,2-Di-[2,3-di-*O*-benzoyl-α-D-mannopyranosyloxy]-ethane 28

Compound **27** (914 mg, 0.94 mmol) was deacetylated under the same conditions as those used for the preparation of **10** from **9**, giving **28** (573 mg, 75.8%) as a foamy solid. $[\alpha]_D$=−79.7 (*c* 1.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.09$\sim$7.26 (m, 20H, 4*Ph*), 5.80 (dd, 2H, $J_{2,3}=3.2$, $J_{3,4}=9.8$, 2H-3), 5.55 (dd, 2H, $J_{1,2}$= 1.8, $J_{2,3}=3.3$, 2H-2), 5.10 (d, 2H, $J_{1,2}=1.8$, 2H-1), 4.31 (dd, 2H, $J_{3,4}=J_{4,5}=9.8$, 2H-4), 4.15$\sim$4.20 (m, 8H), 3.78$\sim$3.68 (m, 4H), 3.09 (bs, 2H, 2OH), 1.85 (s, 6H, 2CH_3CO); ^{13}C NMR (100 MHz, CDCl$_3$) δ 170.8, 169.8 (CH$_3$*C*O), 165.4, 165.3 (Ph*C*O), 113.6, 113.4, 130.0, 129.8, 129.5, 129.2, 128.6, 128.5 (*Ph*CO, some signals overlapped), 97.4 (C-1), 70.3, 70.2, 69.0, 66.3, 66.0 (C-2∼6), 62.5 (*C*H$_2$), 20.9 (*C*H$_3$CO), 20.6 (*C*H$_3$CO). Anal. calcd for $C_{42}H_{42}O_{16}$: C, 62.84; H, 5.27. Found: C, 62.98; H, 5.36.

4.20. 1,2-Di-[4,6-di-*O*-acetyl-2,3-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3-di-*O*-benzoyl-α-D-mannopyranosyloxy]ethane 29

Donor **7** (430 mg, 0.70 mmol) and acceptor **28** (560 mg, 0.70 mmol) were dried together under high vacuum for 2 h and then dissolved in anhydrous CH$_2$Cl$_2$ (25 mL). TMSOTf (10 μL, 0.06 mmol) was added dropwise at −20°C with N$_2$ protection. The reaction mixture was stirred for 2 h, during which time the temperature was gradually warmed to ambient temperature. The mixture was then neutralized with triethylamine and concentrated to dryness. Purification of the residue by column chromatography on a silica gel column (1:1 petroleum ether–EtOAc) gave recovered **28** (250 mg), pentasaccharide **36** (153 mg, 33.8%) and the target product **29** (366 mg, 61.5%, calcd based on the donor) as foamy solids. For **29**: $[\alpha]_D = -54.6$ (*c* 1.0, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 400 MHz): δ 8.04∼7.26 (m, 40H, 8*Ph*), 5.83∼5.66 (m, 10H), 5.30 (s, 2H, 2H-1′), 5.11 (d, 2H, $J_{1,2} = 1.1$, 2H-1), 4.42∼4.13 (m, 16H), 3.75 (m, 2H), 2.09 (s, 6H, 2CH_3CO), 1.98 (s, 6H, 2CH_3CO). MALDI-TOF MS: calcd for $C_{90}H_{86}NaO_{34}$ ([M+Na]): 1734.64, found 1733.92. Anal. calcd for $C_{90}H_{86}O_{34}$: C, 63.15; H, 5.06. Found: C, 62.98; H, 5.00. For **36**: $[\alpha]_D$ −71.2 (*c* 1.0, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.15∼6.99 (m, 50H, 10*Ph*), 5.95∼5.90 (m, 3H), 5.84∼5.69 (m, 7H), 5.60 (s, 1H, H-1), 5.56 (m, 1H), 5.52 (d, 1H, $J_{1,2} = 1.4$, H-1), 5.47 (dd, 1H, $J_{1,2} = 1.6$, $J_{2,3} = 3.3$, H-2), 5.46 (d, 1H, $J_{1,2} = 1.5$, H-1), 5.37 (dd, 1H, $J_{2,3} = 3.3$, $J_{3,4} = 10.3$, H-3), 5.12 (d, 1H, $J_{1,2} = 1.5$, H-1), 5.11 (d, 1H, $J_{1,2} = 1.5$, H-1), 4.69∼4.20 (m, 19H), 3.78 (m, 1H), 2.16 (s, 3H, CH_3CO), 2.17 (s, 6H, 2CH_3CO), 1.98 (s, 3H, CH_3CO), 1.74 (s, 3H, CH_3CO), 1.68 (s, 3H, CH_3CO); ^{13}C NMR (100 MHz, CDCl$_3$) δ 170.8, 170.6, 170.0, 169.8 (CH$_3$*C*O, some signals overlapped), 166.3, 165.7, 165.6, 165.5, 165.4, 165.3, 165.2, 165.0, 164.9, 164.1 (10C, 10Ph*C*O), 133.3∼127.9 (*Ph*CO), 99.5, 98.2, 98.1, 97.2, 96.9 (5C, 5C-1), 73.4, 73.3, 72.2, 71.9, 70.7, 70.5, 70.4, 70.2, 70.0, 69.9, 69.1, 68.8, 66.6, 66.3, 66.2, 66.0, 65.6, 65.4, 62.5, 62.4 (C-2∼6, *C*H$_2$, some signals overlapped), 55.5 (*C*H$_3$O), 20.8, 20.5, 20.4 (*C*H$_3$CO, some signals overlapped). Anal. calcd for $C_{114}H_{108}O_{43}$: C, 63.21; H, 5.03. Found: C, 63.01; H, 4.95.

4.21. 1,2-Di-[4,6-di-*O*-acetyl-2,3-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyloxy-]ethane 30

Compound **29** (353 mg, 0.21 mmol) was benzoylated under the same condition as those used for the preparation of **10** from **9**, giving **30** (351 mg, 88.7%) as a foamy solid. $[\alpha]_D = -84.6$ (*c* 1.0, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 400 MHz): δ 8.08∼6.72 (m, 50H, 10*Ph*), 6.16 (dd, 2H, $J_{3,4} = J_{4,5} = 10.2$, 2H-4), 6.02 (dd, 2H, $J_{2,3} = 3.3$, $J_{3,4} = 10.2$, 2H-3), 5.87∼5.82 (m, 3H), 5.69 (dd, 2H, $J_{3',4'} = J_{4',5'} = 9.7$, 2H-4′), 5.27 (d, 4H, $J_{1,2} = 1.3$, 4H-1), 4.76 (m, 2H, H-5), 4.39∼4.26 (m, 4H), 4.14 (d, 4H, $J = 9.5$, 2CH_2), 4.04∼3.92 (m, 6H), 2.04 (s, 6H, 2CH_3CO), 1.99 (s, 6H, 2CH_3CO); ^{13}C NMR (100 MHz, CDCl$_3$) δ 170.7, 169.9 (CH$_3$*C*O), 165.5, 165.4, 165.3, 165.2 (Ph*C*O), 113.4, 130.1, 133.0, 130.1, 129.9, 129.8, 129.7, 129.6, 129.4, 129.1, 128.8, 128.5, 128.4, 128.2, 128.1 (*Ph*CO, some signals overlapped), 98.0, 97.6 (C-1), 70.7, 70.6, 70.3, 70.0, 68.7, 66.9, 66.7, 66.2, 66.0 (C-2∼6, some signals overlapped), 62.2 (*C*H$_2$), 20.9 (*C*H$_3$CO), 20.7 (*C*H$_3$CO). Anal. calcd for $C_{104}H_{94}O_{36}$: C, 65.06; H, 4.94. Found: C, 64.88; H, 5.03.

4.22. 1,2-Di-[2,3-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyloxy]ethane 31

Compound **30** (324 mg, 0.17 mmol) was deacetylated under the same condition as those used for the preparation of **10** from **9**, giving **31** (214 mg, 72.3%) as a foamy solid. $[\alpha]_D = -108.5$ (*c* 1.0, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 400 MHz): δ 8.06∼6.67 (m, 50H, 10*Ph*), 6.04 (dd, 2H, $J_{3,4} = J_{4,5} = 10.2$, 2H-4), 5.94 (dd, 2H, $J_{2,3} = 3.3$, $J_{3,4} = 10.2$, 2H-3), 5.78∼5.70 (m, 6H), 5.31 (d, 2H, $J_{1,2} = 1.5$, 2H-1′), 4.93 (d, 2H, $J_{1,2} = 1.0$, 2H-1), 4.73 (m, 2H), 4.34∼4.07 (m, 8H), 3.80∼3.69 (m, 8H), 2.28 (bs, 4H, 4O*H*). Anal. calcd for $C_{96}H_{86}O_{32}$: C, 65.82; H, 4.95. Found: C, 65.55; H, 4.83.

4.23. 1,2-Di-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-2,3-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyloxy]ethane 32

Donor **11** (146 mg, 0.25 mmol) was coupled with acceptor **31** (201 mg, 0.12 mmol) to give trisaccharide dimer **32** (238 mg, 80.4%) as a foamy solid. $[\alpha]_D = -45.6$ (*c* 1.0, CHCl$_3$); ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.03∼6.86 (m, 58H, 10*Ph*, 2*Pth*), 6.05∼6.00 (m, 2H), 5.93 (dd, 2H, $J_{2,3} = 3.2$, $J_{3,4} = 10.2$, 2H-3), 5.81∼5.72 (m, 4H), 5.51 (dd, 2H, $J_{1,2} = 1.6$, $J_{2,3} = 3.4$, 2H-2), 5.44 (m, 2H), 5.35 (d, 2H, $J_{1'',2''} = 8.5$, 2H-1″), 5.19 (d, 2H, $J_{1,2} = 1.2$, 2H-1′), 5.13 (dd, 2H, $J_{2'',3''} = J_{3'',4''} = 9.6$, 2H-3″), 4.80 (d, 2H, $J_{1,2} = 1.6$, 2H-1), 4.56∼3.60 (m, 26H), 2.05 (s, 6H, 2CH_3CO), 2.03 (s, 6H, 2CH_3CO), 1.87 (s, 6H, 2CH_3CO); ^{13}C NMR (100 MHz, CDCl$_3$) δ 170.8, 170.2, 169.6 (CH$_3$*C*O), 166.6, 165.9, 165.5, 165.3, 165.3 (Ph*CO*), 133.8∼123.1 (*Ph*CO, *Pth*), 99.0, 98.6, 97.3 (C-1), 72.9, 71.8, 71.3, 70.6, 70.3, 70.1, 70.0, 69.2, 69.1, 68.9, 66.9, 66.3, 65.9 (C-2∼6), 61.9 (*C*H$_2$), 55.5 (*C*H$_3$O), 20.7, 20.5, 20.4 (*C*H$_3$CO). MALDI-TOF MS: calcd for $C_{136}H_{124}N_2NaO_{50}$ ([M+Na]): 2609.45, found 2610.36. Anal. calcd for $C_{136}H_{124}N_2O_{50}$: C, 63.15; H, 4.83. Found: C, 63.37; H, 4.90.

4.24. 1,2-Di-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-4-*O*-acetyl-2,3-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyloxy]ethane 33

Compound **32** (225 mg, 0.09 mmol) was acetylated under the same condition as those used for the preparation of **13** from **12**, giving **33** (202 mg, 86.9%) as a foamy solid. $[\alpha]_D=-58.5$ (*c* 1.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.17∼6.99 (m, 58H, 10*Ph*, 2*Pth*), 6.08 (dd, 2H, $J_{3,4}=J_{4,5}=10.0$, 2H-4), 5.99 (dd, 2H, $J_{2,3}=3.4$, $J_{3,4}=10.0$, 2H-3), 5.85 (dd, 2H, $J_{1',2'}=1.5$, $J_{2',3'}=3.4$, 2H-2′), 5.81 (dd, 2H, $J_{3'',4''}=9.6$, $J_{4'',5''}=10.7$, 2H-4″), 5.68 (dd, 2H, $J_{2',3'}=3.4$, $J_{3',4'}=9.9$, 2H-3′), 5.57 (dd, 2H, $J_{1,2}=1.7$, $J_{2,3}=3.3$, 2H-2), 5.38 (d, 2H, $J_{1'',2''}=8.5$, 2H-1″), 5.32 (dd, 2H, $J_{3',4'}=J_{4',5'}=9.9$, 2H-4′), 5.27 (d, 2H, $J_{1',2'}=1.5$, 2H-1′), 5.11 (dd, 2H, $J_{2'',3''}=J_{3'',4''}=9.6$,, 2H-3″), 4.58 (m, 2H, 2H-5), 4.52 (d, 2H, $J_{1,2}=1.7$, 2H-1), 4.35∼4.29 (m, 6H), 4.12∼4.06 (m, 4H), 3.97∼3.95 (m, 4H), 3.92∼3.82 (m, 8H), 3.57 (m, 2H), 3.46 (m, 2H), 2.05 (s, 6H, $2CH_3CO$), 2.03 (s, 6H, $2CH_3CO$), 1.89 (s, 6H, $2CH_3CO$), 1.85 (s, 6H, $2CH_3CO$). Anal. calcd for $C_{140}H_{128}N_2O_{52}$: C, 62.97; H, 4.83. Found: C, 63.11; H, 4.92.

4.25. 1,2-Di-[2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranosyloxy-]ethane 34

Trisaccharide dimer **33** (185 mg, 0.07 mmol) was dissolved in EtOH (20 mL) after which was added 100% hydrazine hydrate (4 mL) and the solution heated at reflux for 48 h. The solution was then concentrated and the residue co-evaporated several times with toluene. The residue was taken up in pyridine (10 mL) to which was added Ac_2O (5 mL). The solution was stirred for 12 h at rt and then evaporated to dryness. Purification of the residue by column chromatography (EtOAc) gave **34** (98 mg, 75.5% for two steps) as a foamy solid. $[\alpha]_D=+96.7$ (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 6.23 (d, 2H, $J_{H\text{-}C\text{-}N\text{-}H}=8.9$, 2N*H*Ac), 5.39∼5.15 (m, 16H), 5.09 (dd, 2H, $J_{3,4}=J_{4,5}=9.2$, 2H-4), 4.96 (d, 2H, $J_{1',2'}=1.5$, 2H-1′), 4.83 (d, 2H, $J_{1,2}=1.4$, 2H-1), 4.45 (d, 2H, $J_{1'',2''}=8.4$, 2H-1″), 4.25∼4.10 (m, 10H), 4.03∼3.93 (m, 2H), 3.81 (m, 2H), 3.68∼3.61 (m, 6H), 3.38 (dd, 2H, $J_{5'',6''a}=3.2$, $J_{6''a,6''b}=11.2$, 2H-6″a), 2.14 (s, 12H, $4CH_3CO$), 2.09 (s, 12H, $4CH_3CO$), 2.03 (s, 6H, $2CH_3CO$), 2.02 (s, 6H, $2CH_3CO$), 2.00 (s, 6H, $2CH_3CO$), 1.99 (s, 6H, $2CH_3CO$), 1.96 (s, 12H, $4CH_3CO$). Anal. calcd for $C_{78}H_{108}N_2O_{50}$: C, 50.00; H, 5.81. Found: C, 50.21; H, 5.73.

4.26. 1,2-Di-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyloxy-]ethane 35

Trisaccharide dimer **34** (98 mg, 0.05 mmol) was dissolved in saturated NH_3–MeOH (20 mL). After 96 h at rt, the reaction mixture was concentrated, and the residue purified by chromatography on Sephadex LH-20 (MeOH) to afford **35** (48 mg, 82.2%) as a foamy solid. $[\alpha]_D=+91.5$ (*c* 1.0, H_2O); ^1H NMR (400 MHz, D_2O) δ 4.86 (s, 4H, 4H-1), 4.54 (dd, 2H, $J_{1'',2''}=8.4$, 2H-1″), 4.15∼3.45 (m, 40H), 2.04 (s, 6H, $2CH_3CONH$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4 (CH_3CONH), 101.7, 100.1, 99.6 (C-1), 76.1, 74.0, 71.5, 71.1, 71.0, 70.9, 70.1, 69.1, 66.9, 66.4, 65.9, 60.9, 55.7 (C-2∼6, some signals overlapped), 22.5 (CH_3CONH). Anal. calcd for $C_{42}H_{72}N_2O_{32}$: C, 45.16; H, 6.50. Found: C, 45.01; H, 6.42.

Acknowledgements

This work was supported by The Chinese Academy of Sciences (KZCX3-J-08) and by The National Natural Science Foundation of China (Projects 30070185 and 39970864).

References

1. Dennis, T. W.; Laferte, S. *Cancer Res.* **1989**, *49*, 945.
2. Mitchell, B. S.; Brooks, S. A.; Leathem, A. J. C.; Schumacher, V. *Cancer Lett.* **1998**, *123*, 113.
3. Kanie, O.; Crawley, S. C.; Palcic, M. M. *Bioorg. Med. Chem. Lett.* **1994**, *2*, 1231.
4. Brockhausen, I.; Reck, F.; Kuhns, W.; Khans, W.; Khan, S.; Matta, K. L.; Meinjohanns, E.; Paulsen, H.; Shah, R. N. *Glycoconjugate J.* **1995**, *12*, 371.
5. Kong, F. *Curr. Org. Chem.* **2003**, *7*, 841–865.
6. (a) Zhang, J.; Kong, F. *Tetrahedron: Asymmetry* **2002**, *13*, 243; (b) Zhu, Y.; Kong, F. *Synlett* **2001**, 1217; (c) Zhu, Y.; Kong, F. *Synlett* **2000**, 1783; (d) Zhu, Y.; Chen, L.; Kong, F. *Chin. J. Chem.* **2001**, *19*, 1289.
7. Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21–125.
8. Byramova, N. E.; Ovchinnikov, M. V.; Backinowsky, L. V.; Kochetkov *Carbohydr. Res.* **1983**, *124*, c8–c10.
9. Jegge, S.; Lehmann, J. *Carbohydr. Res.* **1984**, *133*, 247–254.
10. Lehmann, J.; Petry, S. *Carbohydr. Res.* **1990**, *204*, 141–144.
11. Tsuzuki, M.; Tsuchiya, T. *Carbohydr. Res.* **1998**, *311*, 11–24.
12. Ziegler, T.; Vollmer, M.; Oberhaffner, S.; Eckhardt, E. *Liebigs Ann. Chem.* **1993**, 255–260.
13. Buchunan, J. G.; Schwarz, J. C. P. *J. Chem. Soc.* **1962**, 4770–4772.