Highly Efficient Synthesis of the Mannose Nonasaccharide of the N-Glycan Expressed on the HIV Glycoprotein gp 120

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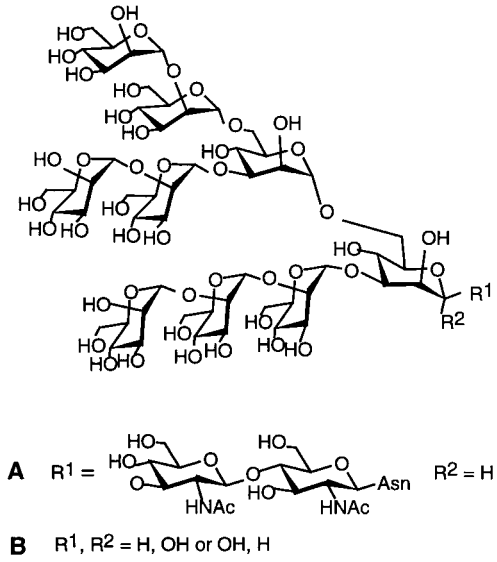
Abstract: A highly concise and effective synthesis of the mannose nonasaccharide of the glycan expressed on the HIV protein gp 120 was achieved via TMSOTf promoted selective 6-glycosylation of a tetrasaccharide 4,6-diol acceptor with a pentasaccharide donor followed by deprotection. The pentasaccharide was constructed by selective 3,6-diglycosylation of 1,2-*O*-ethylidene-β-D-mannopyranose with 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroimidate while the tetrasaccharide was obtained by selective 3-*O*-glycosylation of allyl 4,6-*O*-benzilidene-α-D-mannopyranoside with 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 trichloroimidate.

Key words: regio- and stereoselective synthesis, mannopyranosyl oligosaccharides

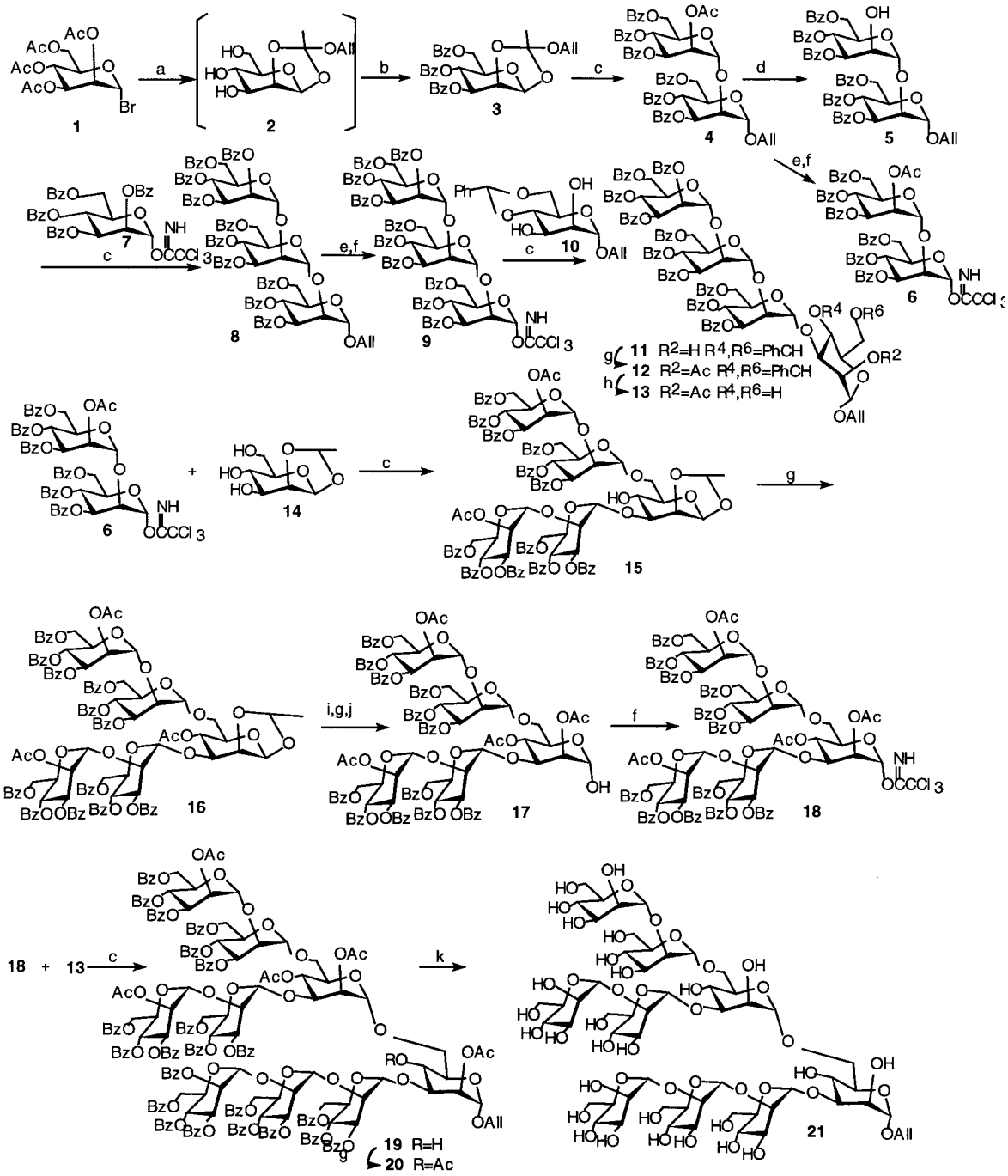
AIDS (Acquired Immunodeficiency Syndrome) is a serious disease imperiling millions people lives in the world. Despite great research efforts, the viral infection caused by the human immunodeficiency virus (HIV) remains incurable. The glycosylated surface of glycoprotein gp 120 has a critical role for HIV infection of cells. Gp 120 is essential not only for the attachment and penetration of target cells, but also for the antiviral immune response. The glycans of the viral envelope may be useful for immunotherapy or vaccine development.[1] A variety of N-linked oligosaccharides have been identified, the main fraction being a ubiquitous undecasaccharide **A**.[2] The synthesis of major segments of high mannose glycoproteins has been reported[3] and a new method for the preparation of the model nonasaccharide with cyclohexane-1,2-diacetal intermediates has been described.[4] In continuation of our effort towards regio- and stereoselective synthesis of bioactive oligosaccharides, we report herewith a highly efficient and concise synthesis of the nonasaccharide **B** of the N-glycan expressed on the glycoprotein gp 120.

Scheme 2 outlines the synthetic route. The key intermediate mannose 1,2-allyl orthoester **3** was readily prepared in large scales by reaction of acetylated mannosyl bromide **1** with allyl alcohol in the presence of 2,4-lutidine, then Zemplén deacetyaltion with MeONa/MeOH, and benzoylation with benzoyl chloride in pyridine. Self condensation of **3** produced the disaccharide **4** in a satisfactory yield (66%). Selective removal of 2-O-acetyl from **4** afforded the disaccharide acceptor **5**, while deallylation of **4** with $PdCl_2$ followed by activation with CCl_3CN in the presence of K_2CO_3 gave the disaccharide donor **6**.[5] Coupling of the benzoylated mannosyl trichloroimidate **7** with **5** yielded the trisaccharide **8**, its deallylation and subsequent trichloroimidation afforded the trisaccharide donor **9**. Condensation of **9** with allyl 4,6-O-benzylidene α-D-mannopyranoside (**10**) selectively gave 3-linked tetrasaccharide **11**. Acetylation of **11** confirmed the 3-glycosylation as the ^{1}H NMR spectrum of acetylated tetrasaccharide **12** showed a newly emerged doublet of doublets at δ 5.52 ppm for H-2. Debenzylidenation was carried out readily with CH_3COCl-CH_3OH (0.1% v/v) giving the terasaccharide acceptor **13** with 4,6-free OH.



Scheme 1

The pentasaccharide donor was obtained readily by regioselective glycosylation through orthoester intermediates.[6] Thus coupling of 2.2 equiv of the disaccharide trichloroimidate **6** with 1 equiv of 1,2-O-ethylidene-β-D-mannopyranose (**14**) promoted by TMSOTf gave 3,6-branched pentasaccharide **15** in good yield (86%). The 3,6-selective glycosylation was confirmed by acetylation of **15**, and the ^{1}H NMR spectrum of the acetylated product **16** showed a characteristic triplet for H-4 at δ 5.20 ppm. It was noted that the temperature at addition of TMSOTf was maitained below −20 °C to ensure orthoester to be the intermediate.[7] Otherwise, for example at room temperature, the regioselectivity will be lost producing 3,4,6-triglycosylated heptasaccharide as the major one. Deethylidenation of **16** with 90% CF_3COOH, acetylation



Scheme 2 Conditions and reagents: a: lutidine, CH_2Cl_2, 4Å M.S., 4 h, then MeONa, MeOH; b: BzCl/pyridine (dry); c: TMSOTf, CH_2Cl_2, 4Å M.S., N_2, -20 °C to rt, 4 h; d: CH_3OH, CH_3COCl (5%, V/V); e: $PdCl_2$, CH_2Cl_2, 2 h; f: CCl_3CN, DBU, CH_2Cl_2 8 h; g: Ac_2O/Pyridine (dry), rt, 6 h; h: CH_3OH, CH_3COCl (0.1%, V/V); i: 90% CF_3COOH, rt, 2-5 h; j: DMF, $(NH_4)_2CO_3$, rt, 4-8 h; k: NH_3/MeOH.

with acetic anhydride in pyridine, trichloroimidation with CCl_3CN in the presence of DBU or K_2CO_3 gave the pentasaccharide donor **18** (82% from **16**). With the pentasaccharide donor and tetrasaccharide acceptor at hand, selective 6-glycosylation[7] was readily carried out giving the nonasaccharide **19** in acceptable yield (84%). Acetylation with acetic anhydride in pyridine, purification by column chromatography afforded fully protected nonasaccharide **20**. The MS spectrum of **20** showed M^+ at 4057.16 (cald. 4057.36) and the ^{13}C NMR spectrum of **20** gave 9 signals for C-1 from δ 95.7-100.9 ppm with $^2J_{C1\text{-}H1}$ from 170-174 Hz indicating complete α-linkages. Finally, deacylation of **20** in ammonia-saturated methanol gave the target nonasaccharide **21**.[8]

In summary, a highly efficient and concise synthesis of the mannose nonasaccharide of the N-glycan expressed on protein gp 120 was achieved by regio- and stereoselec-

tive glycosylation using glycosyl trichloroimidates as the donors and partially protected sugars as the acceptors. In terms of the simplicity and efficiency, this method will be useful for the synthesis of high mannose oligosaccharides.

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(8) All of the new compounds were identified by ^{1}H, ^{13}C NMR, optical rotations, and elemental analysis. Selected physical data and preparations for some important compounds are presented below. For **11**: The trisaccharide donor **9** (1677 mg, 1 mmol) and the monosaccharide acceptor **10** (308 mg, 1 mmol) were dried together under high vacuum for 2 h, then dissolved in anhydrous dichloromethane (50 mL). TMSOTf (15 μL, 0.08 equiv) was added dropwise at −20 °C with N_2 protection. The reaction mixture was stirred for 2 h, during which time the reaction temperature gradually raised to ambient temperature. Then the mixture was neutralized with triethylamine, concentrated under reduced pressure to an oily residue. Purification by column chromatography (1:1.5 petroleum ether-ethyl acetate) gave **11** (1651 mg, 90%) as colorless crystals; mp 131-133 °C; $[\alpha]_D$ −11.5° (*c* 1.1, $CHCl_3$); ^{1}H NMR δ 8.07-7.24 (m, 50 H, Bz-H), 6.03 (t, 1 H, J = 9.9 Hz), 5.95-5.90 (m, 3 H), 5.81-5.76 (m, 3 H), 5.73 (d, 1 H, J = 1.6 Hz), 5.58 (s, 1 H, Ph-C-H), 5.55 (dd, 1 H, J = 3.1 Hz, J = 1.5 Hz), 5.45 (s, 1 H), 5.28 (dd, 1 H, C*H*$_2$ = CH-CH$_2$), 5.20 (dd, 1 H, C*H*$_2$ = CH-CH$_2$), 5.02 (s, 1 H), 4.93 (s, 1 H), 4.62 (dd, 1 H), 4.55-4.45 (m, 2 H), 4.43-4.33 (m, 4 H), 4.30-4.17 (m, 8 H), 4.05-3.97 (m, 2 H), 3.90-3.85 (m, 1 H), 3.81 (t, 1 H, J = 10.0 Hz). For **12**: To a solution of **11** (1834 mg, 1 mmol) in pyridine (30 mL) acetic anhydrate (1 mL, 10 mmol) was added dropwise, and the mixture was stirred overnight at room temperature. TLC (1.5:1 petroleum ether-ethyl acetate) indicated that the reaction was complete. The mixture was diluted with dichloromethane, washed with 1 N hydrochloric acid, water, and saturated aqueous solution of sodium bicarbonate subsequently. The organic layers were combined, dried, and concentrated. Purification by column chromatography (2:1 petroleum ether-ethyl acetate) gave **12** quantitatively as colorless crystals; mp 138-140 °C; $[\alpha]_D$ −5.6° (*c* 1.1, $CHCl_3$); ^{13}C NMR δ 170.31, (1 CH_3CO), 166.34, 165,92, 165.73, 165.60, 165.43, 165.25, 165.20, 165.15, 164.91, 164.64, (10C_6H_5*C*O), 99.98, 99.67, 99.05, 97.69, (C-1^{I-IV}), 79.17 (C-3), 71.18, 71.09, 70.33, 70.25, 70.00, 69.72, 69.63, 69.52, 68.60, 68.32, 66.79, 66.42, 63.68, 63.38, 62.95, 62.59, (C-2,3,4,5,6^{I-IV}, some signals were overlapped); ^{1}H NMR δ 8.07-7.24 (m, 50 H, Bz-H), 6.00-5.88 (m, 5 H), 5.88-5.77 (m, 1 H, CH_2=C*H*-CH_2-), 5.75 (dd, 1 H, J = 3.2 Hz, J = 1.6 Hz), 5.65 (dd, 1 H, J = 3.1 Hz, J = 10.0 Hz), 5.61 (s, 1 H), 5.52 (dd, 1 H, J = 3.2 Hz, J = 1.5 Hz), 5.36 (s, 2 H), 5.30 (dd, 1 H, C*H*$_2$=CH-CH$_2$), 5.24 (dd, 1 H, C*H*$_2$=CH-CH$_2$), 4.93 (s, 1 H), 4.86 (s, 1 H), 4.64-4.58 (m, 3 H), 4.53 (dd, 1 H, J = 3.2 Hz, J = 10.0 Hz), 4.52-4.48 (m, 1 H), 4.43 (dd, 1 H, J = 3.1 Hz, J = 10.0 Hz), 4.30-4.20 (m, 3 H), 4.16-4.09 (m, 3 H), 4.07-3.95 (m, 5 H), 3.77 (t, 1 H, J = 10.0 Hz), 2.35 (s, 3 H, CH_3CO). For **13**: To a solution of **12** (1876 mg, 1 mmol) in methyl alcohol (50 mL) acetyl chloride (0.05 mL, 0.7 mmol) was added dropwise, and the mixture was stirred overnight at room temperature. TLC (1.5:1 petroleum ether-ethyl acetate) indicated that the reaction was complete. The mixture was concentrated. Purification by column chromatography (1.5:1 petroleum ether-ethyl acetate) gave **13** (1628 mmg, 91%) as a colorless solid; $[\alpha]_D$ −4.4° (*c* 1.2, $CHCl_3$); ^{13}C NMR δ 170.22, (CH_3CO), 167.60, 167.23, 166.40, 166.15, 165.43, 165.43, 165.40, 165.32, 165.20, 165.15, (10C_6H_5*C*O), 99.56, 99.36, 99.06, 98.98, (C-1^{I-IV}), 76.45, (C-3), 72.39, 70.16, 70.06, 69.90, 69.64, 69.32, 69.01, 68.63, 67.97, 67.56, 66.49, 63.88, 63.76, 63.51, 62.31, 55.04, (C-2,3,4,5,6^{I-IV}, some signals were overlapped); ^{1}H NMR δ 8.11-7.26 (m, 50 H, Bz-H), 6.11 (t, 1 H, J = 9.9 Hz), 6.07 (t, 1 H, J = 10.0 Hz), 5.95-5.85 (m, 3 H), 5.85-5.80 (m, 1H, CH_2=C*H*-CH_2), 5.76 (dd, 1 H, J = 3.0 Hz, J = 1.5 Hz), 5.71 (d, 1 H, J = 1.6 Hz), 5.69 (dd, 1 H, J = 3.0 Hz, J = 10.0 Hz), 5.51 (s, 1 H), 5.38 (dd, 1 H, J = 3.0 Hz, J = 1.6 Hz), 5.31 (dd, 1 H, C*H*$_2$=CH-CH$_2$), 5.20 (dd, 1 H, C*H*$_2$=CH-CH$_2$), 5.02 (s, 1 H), 4.84 (d, 1 H, J 1.4 Hz), 4.63-4.51 (m, 9 H), 4.39 (dd, 1 H, J = 3.0 Hz, J = 1.5 Hz), 4.25 (dd, 1 H, J = 3.0 Hz, J = 10.0 Hz), 4.23-4.14 (m, 3 H), 4.00 (dd, 1 H), 3.89 (m, 2 H), 3.67-3.64 (m, 1 H), 2.18 (s, 3 H, CH_3CO). For **15**: The disaccharide donor **6** (1268 mg, 1.1 mmol) and the monosaccharide acceptor **14** (103 mg, 0.5 mmol) were dried together under high vacuum for 2 h, then dissolved in anhydrous dichloromethane (30 mL). TMSOTf (15 μL, 0.08 equiv) was added dropwise at −20 °C with N_2 protection. The reaction mixture was stirred for 3 h, during which time the reaction temperature gradually raised to ambient temperature. Then the mixture was neutralized with triethylamine, concentrated under reduced pressure to an oily residue. Purification by column chromatography (1:1.5 petroleum ether-ethyl acetate) gave **15** (940 mg, 86%) as a colorless solid; mp 141-144 °C; $[\alpha]_D$ −11.5° (*c* 1.1, $CHCl_3$); ^{1}H NMR δ 7.98-7.25 (m, 60 H, Bz-H), 6.03-5.85 (m, 8 H), 5.67 (dd, 1 H, J = 3.0 Hz, J = 1.7 Hz), 5.64 (dd, 1 H, J = 3.1 Hz, J = 1.7 Hz), 5.53 (s, 1 H), 5.26 (q, 1 H, CH_3-C*H*), 5.20 (s, 1 H), 5.12 (d, 1 H, J = 1.6 Hz), 5.08 (d, 1 H, J = 1.7 Hz), 5.05 (d, 1 H, J = 1.7 Hz), 4.60-4.46 (m, 13 H), 4.42 (dd, 1 H, J = 3.0 Hz, J = 1.7 Hz), 4.20 (dd, 1 H, J = 3.0 Hz, J = 1.7 Hz), 4.04 (t, 1 H, J = 10.0 Hz), 3.98-3.94 (m, 1 H), 3.83 (dd, 1 H, J = 3.2 Hz, J = 10.0 Hz), 3.75-3.68 (m, 1 H), 3.41-3.37 (m, 1 H), 2.01 (s, 3 H, CH_3CO), 2.00 (s, 3 H, CH_3CO), 1.50 (d, 3 H, C*H*$_3$-CH). For **17**: **16** (2228 mg, 1 mmol) was treated with 90% F_3CCOOH (10 mL) at room temperature for 1 h, the solution

was concentrated and co-concentrated with toluene. The residue was dissolved in pyridine (10 mL) and treated with Ac_2O (3 mL) for 2 h. After conventional work-up, the residue was subjected to column chromatography (1.5/1 petroleum ether/EtOAc) to yield 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-O-acetyl-3,4,6-tri-O-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzoyl-α-D-mannopyranosyl-(1→6)]-1,2,4-tri-O-acetyl-α,β-D-mannopyranose as a solid (1941 mg, 85%). A solution of the solid (1142 mg, 0.5 mmol), ammonium carbonate (790 mg, 10 mmol) in DMF (20 mL) was stirred for 12 h at r.t. At the end of which time TLC (2/1 petroleum ether/EtOAc) showed that the reaction was complete. Water was added, and the mixture was diluted with dichloromethane, washed with 1 N hydrochloric acid, water, and sat. aq. solution of sodium bicarbonate subsequently. The organic layer was combined, dried, and concentrated. The obtained residue was passed through a short silica gel column with 2/1 petroleum ether/ethyl acetate as the eluent to give **17** as a solid consisting of α anomer predominantly (1021 mg, 91%),: $[\alpha]_D$ −13.2° (*c* 1.1, $CHCl_3$); ^{13}C NMR δ 170.52, 170.04, 169.31, 169.26, ($4CH_3CO$), 166.66, 166.53, 166.45, 166.15, 165.70, 165.67, 165.65, 165.55, 165.42, 165.28, 165.11, 164.98, ($12C_6H_5CO$), 100.47, 99.76, 99.36, 98.97, 92.36 (C-1^{I-V}), 79.08 (C-3), 77.28, 77.28 (C-2), 72.86, 71.32, 70.59, 70.06, 69.94, 69.73, 69.70, 69.67, 69.64, 69.61,69.58, 69.38, 69.11, 68.47, 68.20, 67.49, 67.12, 66.88, 63.97, 63.86, 63.33, 63.04, (C-2,3,4,5,6^{I-V}); ^1H NMR δ 8.01-7.26 (m, 60 H, Bz-H), 5.95-5.83 (m, 7 H), 5.70 (dd, 1 H, J = 3.0 Hz, J = 10.0 Hz), 5.64 (dd, 1 H, J = 3.1 Hz, J = 1.5 Hz), 5.60 (dd, 1 H, J = 3.0 Hz, J = 1.5 Hz), 5.49 (dd, 1 H, J = 3.0 Hz, J = 1.5 Hz), 5.40 (s, 1 H), 5.24 (s, 1 H), 5.22 (t, 1 H, J = 10.0 Hz), 5.20 (s, 1 H), 5.08 (s, 1 H), 5.02 (s, 1 H), 4.66-4.37 (m, 14 H), 4.22 (dd, 1 H, J = 3.0 Hz, J = 1.5 Hz), 4.00-3.95 (m, 1H), 3.77-3.73 (dd, 1 H), 3.58-3.55 (m, 1 H), 2.30 (s, 3 H, CH_3CO), 2.19 (s, 3 H, CH_3CO), 2.01 (s, 3 H, CH_3CO), 2.00 (s, 3 H, CH_3CO). For **18**: The compound **17** (2244 mg, 1 mmol) was dissolved in dichloromethane (20 mL), then CCl_3CN (0.1 ml, 1 mmol) and DBU (14 μL, 0.1 mmol) were added. The reaction mixture was stirred for 2 h, at the end of which time TLC (2/1 petroleum ether/ethyl acetate) indicated that the reaction was complete. Concentration of the reaction mixture followed by purification on a silica gel column with 2/1 petroleum ether/EtOAc as the eluent furnished the pentasaccharide donor **18** as crystals in a good yield (2150 mg, 90%): mp 135-137 °C; $[\alpha]_D$ −9.5° (*c* 1.3, $CHCl_3$); ^1H NMR δ 9.03 (s, 1 H, C = NH), 8.10-7.26 (m, 60 H, Bz-H), 6.35 (d, 1 H, $J_{1,2}$ = 1.5 Hz, H-1), 6.05 (t, 1 H, J = 10.0 Hz), 5.96 (t, 2 H, J = 10.1 Hz), 5.91 (t, 1 H, J = 10.1 Hz), 5.88 (dd, 1 H, J = 3.0 Hz, J = 10.0 Hz), 5.85 (dd, 1 H, J = 3.0 Hz, J = 10.0 Hz), 5.80 (dd, 1 H, J = 3.0 Hz, J = 10.1 Hz), 5.74 (dd, 1 H, J = 3.0 Hz, J = 10.1 Hz), 5.68-5.66 (m, 2 H), 5.64 (dd, 1 H, J = 3.0 Hz, J = 1.5 Hz), 5.45 (t, 1 H, J = 10.0 Hz), 5.43 (s, 1 H), 5.17 (s, 1 H), 5.09 (s, 2 H), 4.61-4.43 (m, 13 H), 4.33 (dd, 1 H, J = 3.0 Hz, J = 1.5 Hz), 4.30 (dd, 1 H, J = 3.0 Hz, J = 1.5 Hz), 4.22-4.18 (m, 1 H), 3.89-3.84 (m, 1 H), 3.59-3.56 (m, 1 H), 2.26 (s, 3 H, CH_3CO), 2.10 (s, 3 H, CH_3CO), 2.02 (s, 3 H, CH_3CO), 2.00(s, 3 H, CH_3CO). For **19**: The pentasaccharide donor **18** (1193 mg, 0.5 mmol) and the tetrasaccharide acceptor **13** (894 mg, 0.5 mmol) were dried together under high vacuum for 2 h, then dissolved in anhydrous dichloromethane (30 mL). TMSOTf (7.5 μL, 0.08 equiv) was added dropwise at −20 °C with N_2 protection. The reaction mixture was stirred for 3 h, during which time the reaction temperature gradually raised to ambient temperature. Then the mixture was neutralized with triethylamine, concentrated under reduced pressure to an oily residue. Purification by column chromatography (1:1 petroleum ether-ethyl acetate) gave **19** (1606 mg, 80%) as a colorless solid; $[\alpha]_D$ −4.8° (*c* 1.0, $CHCl_3$); ^1H NMR δ 8.10-7.26 (m, 110 H, Bz-H), 6.17 (t, 1 H, J = 9.9 Hz), 6.14-5.70 (m, 13 H), 5.65-5.50 (m, 7 H), 5.42 (s, 1 H), 5.40 (s, 1 H), 5.38 (s, 1 H), 5.35 (dd, 1 H, C*H*$_2$=CH-CH$_2$), 5.25 (dd, 1 H, C*H*$_2$=CH-CH$_2$), 5.23 (s, 1 H), 5.18 (s, 2 H), 5.04 (s, 1 H), 4.95 (s, 1 H), 4.84 (s, 1 H), 4.70-4.25 (m, 26 H), 4.20-4.10 (m, 4 H), 4.03-3.98 (m, 2 H), 3.85-3.78 (m, 2 H), 3.65-3.61 (m, 1 H), 3.55-3.51 (m, 1 H), 2.27 (s, 3 H, CH_3CO), 2.18 (s, 3 H, CH_3CO), 2.12 (s, 3 H, CH_3CO), 2.00 (s, 3 H, CH_3CO), 1.98 (s, 3 H, CH_3CO); MALDI-TOF MS Calcd for $C_{221}H_{194}O_{73}$: 4015.15 [M]. Found: 4015.39 [M]. For **20**: To a solution of **19** (1204 mg, 0.3 mmol) in pyridine (20 mL) acetic anhydrate (1 mL, 10 mmol) was added dropwise, and the mixture was stirred overnight at room temperature. TLC (1.5:1 petroleum ether-ethyl acetate) indicated that the reaction was complete. The mixture was diluted with dichloromethane, washed with 1 N hydrochloric acid, water, and saturated aqueous solution of sodium bicarbonate subsequently. The organic layers were combined, dried, and concentrated. Purification by column chromatography (1.5:1 petroleum ether-ethyl acetate) gave **20** quantitatively as a colorless solid; $[\alpha]_D$ −2.5° (*c* 1.0, $CHCl_3$); ^{13}C NMR δ 170.10, 170.06, 169.56, 169.31, 168.79, 168.70, ($6CH_3CO$), 165.99, 165.93, 165.84, 165.75, 165.55, 165.51, 165.41, 165.28, 165.20, 165.14, 165.10, 165.05, 165.01, 164.95, 164.89, 164.78, 164.70, 164.67, 164.56, 164.42, 164.38, 164.27, ($22C_6H_5CO$), 99.94, 99.80, 99.72, 99.50, 99.41, 98.90, 98.18, 97.16, 96.05, (C-1^{I-IX}), 76.85, 76.78, (C-3), 73.43, 73.25, 70.53, 70.46, 70.25, 70.08, 69.86, 69.57, 69.32, 69.17, 68.47, 68.24, 68.11, 66.80, 66.76, 66.70, 66.60, 66.54, 66.48, 66.40, 66.30, 66.17, 66.06, 65.81, 63.26, 63.05, 62.64, 62.27, 61.90, 59.93, (C-2,3,4,5,6^{I-IX}, some signals overlapped); ^1H NMR δ 8.09-7.26 (m, 110 H, Bz-H), 6.09 (t, 1 H, J = 9.9 Hz), 6.06-5.77 (m, 17 H), 5.68-5.60 (m, 3 H), 5.40 (dd, 1 H, J = 3.0 Hz, J = 1.5 Hz), 5.38 (s, 1 H), 5.34 (s, 1 H), 5.32 (dd, 1 H, C*H*$_2$ = CH-CH$_2$), 5.27 (s, 1 H), 5.26 (dd, 1 H, C*H*$_2$ = CH-CH$_2$), 5.24 (s, 1 H), 5.21 (s, 1 H), 5.11 (s, 1 H), 5.08 (s, 1 H), 5.02 (s, 1 H), 4.88 (s, 1 H), 4.63-4.42 (m, 20 H), 4.39-4.19 (m, 6 H), 4.16-4.00 (m, 5 H), 3.89-3.80 (m, 2 H), 3.60-3.56 (m, 1 H), 3.45-3.40 (m, 1 H), 2.29 (s, 3 H, CH_3CO), 2.15 (s, 3 H, CH_3CO), 2.11 (s, 3 H, CH_3CO), 2.09 (s, 3 H, CH_3CO), 2.00 (s, 6 H, $2CH_3CO$); MALDI-TOF MS Calcd for $C_{223}H_{196}O_{74}$: 4057.16 [M]. Found: 4057.36 [M]. For **21**: A saturated solution of ammonia in MeOH (5 mL) was added to a solution of **20** (811 mg, 0.2 mol) in MeOH (4 mL). After a week at room temperature, the reaction mixture was concentrated and the residue was purified by chromatography on Sephadex LH-20 (MeOH) to afford **21** (243 mg, 80%) as a syrup; ^{13}C NMR δ 136.22, 118.17 (*C*H$_2$=*C*H-CH$_2$), 104.32, 104.32, 104.32, 102.83, 102.83, 102.72, 102.72, 101.24, 100.10, (C-1^{I-IX}), 80.73, 80.54, (C-3), 72.85, 72.46, 70.70, 70.12, 69.85, 69.56, 69.50, 69.44, 69.32, 69.10, 66.45, 66.32, 66.28, 66.24, 66.09, 65.24, 65.03, 64.76, 64.60, 60.90, 60.68, 60.48, 60.22, 59.96, 58.25, (C-2,3,4,5,6^{I-IX}, some signals overlapped); ^1H NMR δ 5.90 (m, 1 H, CH$_2$=C*H*-CH$_2$), 5.30 (s, 1 H), 5.27 (dd, 1 H, C*H*$_2$=CH-CH$_2$), 5.24 (s, 1 H), 5.22 (dd, 1 H, C*H*$_2$=CH-CH$_2$), 5.18 (s, 1 H), 5.05 (s, 1 H), 4.95 (s, 3 H), 4.72 (s, 1 H), 4.68 (s, 1H), 4.05-3.53 (m, 56 H); MALDI-TOF MS Calcd for $C_{57}H_{96}O_{46}$: 1516.52 [M]. Found: 1539.27 [M+Na].

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