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Synthesis of a tetrasaccharide fragment of cobra venom factor oligosaccharide

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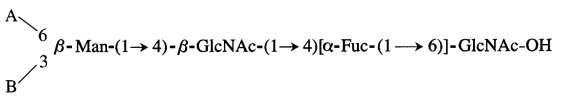
Abstract

Synthesis of a tetrasaccharide fragment, α-L-Fuc-(1→3)-β-D-GlcNAc-(1→2)-α-D-Man-(1→6)-α-D-Man-OMe of the cobra venom factor (CVF) oligosaccharide is described.

Keywords: Cobra venom factor; Tetrasaccharide; Synthesis

1. Introduction

Cobra venom factor (CVF) is a non-toxic glycoprotein present in cobra venom with strong complement-activating activity through the alternative pathway [1,2]. When conjugated to a tumor monoclonal antibody or its fragment F(ab)′2, CVF selectively generates cytotoxicity, killing cells of melanonoma, leukemia, and neuroblastoma [3–5]. According to Gowda et al. [6], the CVF molecule contains three chains α, β, and γ, among which the α and β chains have two or three oligosaccharides with the same biantennal structure.



A = α-Gal-(1→3)-β-Gal-(1→4)[α-Fuc-(1→3)]-β-GlcNAc-(1→2)-α-Man1

B = α-Gal-(1→3)-β-Gal-(1→4)[α-Fuc-(1→3)]-β-GlcNAc-(1→2)-α-Man1

This carbohydrate fragment, which contains the Le^x antigen, a well-known tumor antigen, is related to the immunogencity of the CVF [7]. Accordingly, we chose the outer biantennal structure as a synthesis target and used block-building strategy to accomplish the synthesis of a tetrasaccharide fragment, namely α-L-Fuc-(1→3)-β-D-GlcNAc-(1→2)-α-D-Man-(1→6)-α-D-Man-OMe. The terminal methyl group was introduced as a signal to confirm the structure in NMR.

2. Results and discussion

The tetrasaccharide fragment was synthesized via a 2 + 2 model (see Scheme 1). Building block A was prepared according to Lönn's method [8], in which ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-fucopyranoside was converted into the corresponding glycosyl bromide and then immediately coupled to the glycosyl acceptor. The ethylthio group at C-1 of the disaccharide was introduced as both a protective and a leaving group.

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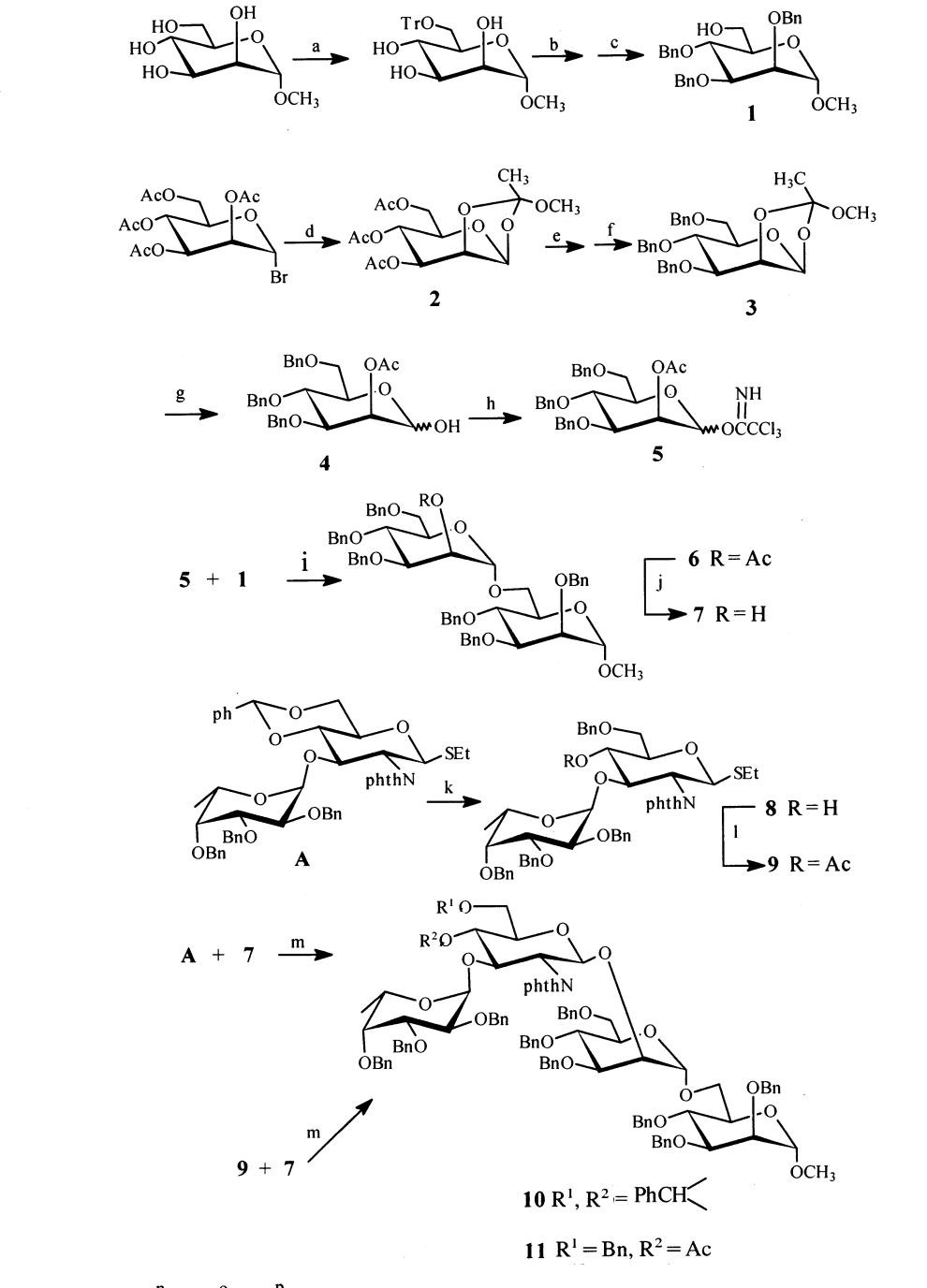
The other disaccharide building block was obtained from the coupling of glycosyl donor **5** and acceptor **1**. Compound **1** was prepared in three steps from the starting material methyl α-D-mannopyranoside; the trityl group was introduced selectively at O-6 of the start-



Scheme 1. (a) $Ph_3CCl–C_5H_5N$; (b) BnBr–NaH–DMF; (c) HBr–HOAc; (d) MeOH–C_5H_5N; (e) NH_3–MeOH; (f) BnBr–NaH–DMF; (g) 80% HOAc–H_2O; (h) $CNCCl_3$, DBU–CH_2Cl_2; (i) Me_3SiOTf, 4 Å molecular sieves–CH_2Cl_2, −12 °C; (j) NaOMe–MeOH; (k) $NaBH_3CN$–HCl–Et_2O; (l) $Ac_2O–C_5H_5N$; (m) MeOTf, 4 Å molecular sieves–ether; (n) $NH_2–NH_2·H_2O$–EtOH, reflux; (o) $Ac_2O–C_5H_5N$; (p) H_2, Pd–C.

ing material with chlorotriphenylmethane in pyridine. Conventional benzylation at O-2,3,4 followed by detritylation with HBr–HOAc solution gave **1** in total yield over three steps of 38.9%. The mannosyl trichloroacetimidate **5** was prepared in five steps. The starting material 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide [9] was stirred in dry MeOH–pyridine to give the methyl 1,2-*O*-orthoacetate **2**. The conventional deacetylation with NaOMe–MeOH unexpectedly gave no compound **3**, but **3** was obtained in good yield (64% in two steps) by using NH_3–MeOH instead of NaOMe–MeOH for deacetylation (and then benzylation). Compound **3** was stirred in 80% acetic acid solution at room temperature (rt) to give compound **4** in satisfactory yield (92.5%). The imidate **5** was then prepared from compound **4** with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The yield was also satisfactory (90.2%).

The blocked disaccharide **6** was prepared in excellent yield (93.2%) by coupling compounds **1** and **5** according to the Schmidt method [10], in which trimethylsilyl triflate (Me_3SiOTf) was used as promoter in the presence of 4 Å molecular sieves and dry CH_2Cl_2. Deacetylation of compound **6** gave the disaccharide glycosyl acceptor **7** in quantitative yield.

The coupling of disaccharide **A** and disaccharide **7** in the presence of methyl triflate as a promoter gave the blocked tetrasaccharide **10** (53%), which was not very stable, even when stored below −10 °C. We therefore converted compound **A** into compound **8** by selective reduction of the benzylidene group in the presence of $NaBH_3CN$ and HCl–ether. Compound **9** resulting from the acetylation of compound **8** was coupled to the glycosyl donor to give another blocked tetrasaccharide **11** (38%), which was more stable and could be stored for a long time. Another advantage of compound **11** is that the acetyl group could then be readily removed, allowing further glycosylation.

The deblocking of compound **10** involved three steps: dephthaloylation with hydrazine hydrate, acetylation of the amino group, and hydrogenolysis. The resultant tetrasaccharide **12** was affirmed by 1D and 2D NMR spectra.

3. Experimental

General.—Melting points (uncorrected) were determined with an X_4 model micromelting apparatus. Thin-layer chromatography was performed on glass plates coated with Silica Gel GF254 (Qingdao) with detection by quenching of fluorescence and/or charring with 5% H_2SO_4–EtOH. Column chromatography was performed on Silica Gel H (Qingdao). Optical rotations were recorded with a Perkin–Elmer model 243B polarimeter. IR spectra were recorded with a Perkin–Elmer model 240C spectrophotometer, using KBr pellets for crystalline samples and films for syrupy samples. ^1H NMR and ^{13}C NMR were recorded on a Bruker ARX-400 spectrometer. High-resolution mass spectra were recorded with a VG-ZAB-2F mass spectrometer.

Methyl 2,3,4-tri-O-*benzyl*-α-D-*mannopyranoside* (**1**).—Powdered methyl *O*-α-D-mannopyranoside (2.0 g) was dissolved in dry pyridine (30 mL). To the solution was added Ph_3CCl (3.5 g) and the mixture was stirred overnight at rt. The mixture was then poured into ice–water (150 mL) and stirred vigorously. The waxy solid obtained through filtration and washing successively with water and light petroleum was dissolved in dry DMF (40 mL). Sodium hydride (80%, Serva, 2.5 g) was added carefully to the solution and the mixture was stirred vigorously for 30 min. Benzyl bromide (7.0 mL) was then added dropwise. After stirring overnight at rt, MeOH (5 mL) was added to the mixture to decompose unreacted NaH and the mixture was poured into ice–water (100 mL). Toluene (3 × 60 mL) was used to extract the mixture and the dried (Na_2SO_4) extract was evaporated in vacuo. The crude syrup was dissolved in a mixture of 80% AcOH (40 mL) and 20% HBr–HOAc solution (20 mL). The mixture was stirred in a 60 °C water bath for 1 h. After cooling to rt, the mixture was poured into ice–water (200 mL) and the solution was extracted with $CHCl_3$ (50 mL). The organic layer was washed successively with water, saturated $NaHCO_3$ solution, and water to pH 7, and then dried (Na_2SO_4). The solvent was evaporated off in vacuo and the residue purified by column chromatography [1:5 to 1:4 EtOAc–light

petroleum (60–90 °C)] to give compound **1**, 1.86 g (total yield 38.9%), as a colorless syrup, $[\alpha]_D$ +34.8° (*c* 0.28, $CHCl_3$); IR (cm^{-1}): 3456 (OH). Anal. Calcd for $C_{28}H_{33}O_6$: C, 72.41; H, 6.90. Found: C, 72.10; H, 6.97.

*3,4,6-Tri-*O*-acetyl-1,2-*O*-(methoxyethylidene)-β-*D*-mannopyranoside* (**2**).—Absolute MeOH (10 mL) was added to a solution of 2,3,4,6-*O*-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (10.2 g) in dry pyridine (20 mL). The mixture was stirred at rt for 24 h, when TLC showed that most of the material had been converted, and then poured into ice–water (200 mL). Dichloromethane (3 × 50 mL) was used for extraction. The separated organic layer was washed with water, dried (Na_2SO_4), and concentrated. Toluene (50 mL) was added to the residue to remove traces of pyridine and water by azeotropic distillation in vacuo. The resultant residue was purified by column chromatography [3:1 to 2:1 light petroleum (60–90 °C)–EtOAc] to give compound **2**, yield 5.8 g (70%) as a white powder, mp 110–112 °C (lit. [11] 109–110 °C, 111–113 °C [12]).

*3,4,6-Tri-*O*-benzyl-1,2-*O*-(methoxyethylidene)-β-*D*-mannopyranoside* (**3**).—To a solution of compound **2** (2.0 g) in abs. MeOH (20 mL), saturated NH_3–MeOH (10 mL) was added and the mixture was stirred overnight at rt. After removing the solvent in vacuo, the residue was benzylated according to the general method used in the preparation of compound **1**. Column chromatography [10:1 to 8:1 light petroleum (60–90 °C)–EtOAc] gave compound **3**, 1.80 g (yield over two steps, 64%), as colorless needles, mp 74–76 °C (lit. [11] 76–78 °C); $[\alpha]_D$ +32.8° (*c* 0.37, $CHCl_3$). ^1H NMR (400 MHz): δ_H (ppm) 1.57 (s, 3 H, C*H*$_3$), 3.28 (s, 3 H, OC*H*$_3$), 3.44–5.35 (m, 13 H, H-1, 2, 3, 4, 5, 6, 3 × PhC*H*$_2$), 7.24–7.89 (m, 15 H, aromatic H); ^{13}C NMR (100 MHz): δ_C (ppm) 24.42 (*C*H$_3$), 49.77 (O*C*H$_3$), 68.99–79.02 (8C, C-2, 3, 4, 5, 6, 3 × Ph*C*H$_2$), 97.55 (C-1), 124.0–138.2 (aromatic C). Anal. Calcd for $C_{30}H_{34}O_7$: C, 71.18; H, 6.71. Found: C, 71.25; H, 6.56.

*2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α,β-*D*-mannopyranose* (**4**).—Compound **3** (1.50 g) was suspended in 80% HOAc (30 mL) and stirred at rt for 24 h. The mixture was poured into a mixture of toluene (100 mL) and water (100 mL) and the aq layer was removed. The organic layer was washed successively with water, saturated $NaHCO_3$, and water, to pH 7, and then dried (Na_2SO_4). After removal of the solvent in vacuo, the residue was purified by column chromatography [3:1 to 2:1 petroleum ether (60–90 °C)–EtOAc] to give compound **4**, 1.35 g (92.5%), as a colorless syrup, $[\alpha]_D$ +6.2° (*c* 1.0, $CHCl_3$); IR (cm^{-1}): 3400 (OH), 1738 (C=O). Anal. Calcd for $C_{29}H_{32}O_7$: C, 70.73; H, 6.50. Found: C, 70.59; H, 6.68.

*2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α,β-*D*-mannopyranosyl trichloroacetimidate* (**5**).—To a solution of compound **4** (1.02 g) in dry CH_2Cl_2 (15 mL), trichloroacetonitrile (1 mL) was added. The mixture was cooled and stirred in an ice–water bath, and then five drops of DBU was added. After stirring for 1 h, TLC showed complete conversion and the mixture was concentrated in vacuo. Column chromatography of the resulting residue (5:1 light petroleum (60–90 °C)–EtOAc) gave compound **5**, 1.19 g (90.2%) as a colorless syrup.

*Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside* (**6**).—To a stirred solution of compound **5** (1.04 g) and compound **1** (0.75 g) in dry CH_2Cl_2 (10 mL), powdered 4 Å molecular sieves (1.0 g) were added. The mixture was kept at rt for 30 min and then in an ice–salt bath (−12 °C) for 20 min, and ten drops of Me_3SiOTf were added to the mixture. The mixture was stirred for 3 h, when TLC showed that the material had been converted into a new compound. The mixture was filtered through a layer of Celite and washed with CH_2Cl_2. The combined filtrate was washed with water, saturated $NaHCO_3$ and water, and dried (Na_2SO_4). The solvent was concentrated in vacuo to a syrup which was then chromatographed [6:1 light petroleum (60–90 °C)–EtOAc] to give compound **6**, 1.10 g (93.2%) as a colorless syrup; $[\alpha]_D$ +39.2° (*c* 0.14, $CHCl_3$); IR (cm^{-1}): 1739 (C=O); ^1H NMR (400 MHz): δ_H (ppm) 2.16 (s, 3 H, C*H*$_3$CO), 3.25 (s, 3 H, OC*H*$_3$), 3.67–5.50 (m, 36 H, H-1, 2, 3, 4, 5, 6, H-1′,2′,3′,4′,5′,6′,6 × PhC*H*$_2$), 7.30 (m, 30 H, aromatic H); ^{13}C NMR (100 MHz): δ_C (ppm) 21.17 (*C*H$_3$CO), 54.70 (O*C*H$_3$), 68.57–80.25 (16C, C-2, 3, 4, 5,

6, C-2′, 3′, 4′, 5′, 6′, 6 × PhCH_2), 97.97, 98.71 (C-1, C-1′), 127.40–138.60 (36 C, aromatic C), 170.37 (CH_3CO). Anal. Calcd for $C_{52}H_{62}O_{12}$: C, 72.92; H, 6.61. Found: C, 72.65; H, 6.50.

Methyl 2,3,4-tri-O-*benzyl-6*-O-*(3,4,6-tri*-O-*benzyl*-α-D-*mannopyranosyl)*-α-D-*mannopyranoside* (**7**).—Sodium (20 mg) was added to a solution of compound **6** (950 mg) in abs MeOH (20 mL). The mixture was stirred for 12 h at rt and then neutralized with AcOH. After removal of the solvent in vacuo, the residue was purified by column chromatography [3:1 light petroleum (60–90 °C)–acetone] to give compound **7** in quantitative yield, as a colorless syrup; $[\alpha]_D$ +60.0° (*c* 0.24, $CHCl_3$); IR (cm^{-1}): 3457 (OH); ^{1}H NMR (400 MHz) δ_H (ppm) 2.46 (s, 1 H, OH), 3.26 (s, 3 H, CH_3), 3.60–5.08 (m, 26 H, H-1, 2, 3, 4, 5, 6, H-1′, 2′, 3′, 4′, 5′, 6′, 6 × PhCH_2), 7.31 (m, 30 H, aromatic H); ^{13}C NMR (100 MHz): δ_C (ppm) 54.59 (OCH_3), 66.17, 67.92, 68.67, 70.93, 71.19, 71.30, 71.91, 73.24, 74.08 74.43, 74.64, 74.88, 79.32, 80.08 (16C, C-2, 3, 4, 5, 6, C-2′, 3′, 4′, 5′, 6′, 6 × PhCH_2), 98.64, 99.51 (C-1, C-1′), 127.40–138.39 (36C, aromatic C). Anal. Calcd for $C_{55}H_{60}O_{11}$: C, 73.66; H, 6.70. Found: C, 73.59; H, 6.70.

Ethyl 6-O-*benzyl-2-deoxy-2-phthalimido-3*-O-*(2,3,4-tri*-O-*benzyl*-α-L-*fucopyranosyl)-1-thio*-β-D-*glucopyranoside* (**8**).—Ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-3-*O*-(2,3,4-*O*-tribenzyl-α-L-fucopyransyl)-1-thio-β-D-glucopyranoside (0.42 g) [8] was dissolved in dry THF (15 mL), and then powdered 4 Å molecular sieves (1.0 g) and $NaBH_3CN$ (0.2 g) were added slowly. After stirring at rt for 20 min, the mixture was added to saturated HCl–dry ether solution until the bubbles disappeared and pH 3 was reached. The mixture was stirred for another 2 h, when TLC showed that the reactant had been converted. The mixture was then filtered through a layer of Celite and washed with ether. The combined filtrate was washed with water, saturated $NaHCO_3$ solution, and the solution was concentrated to a syrup, which was then purified by column chromatography [6:1 light petroleum (60–90 °C)–acetone] to give compound **8**, 0.38 g (90%) as a colorless syrup; $[\alpha]_D$ +27.6° (*c* 0.6, $CHCl_3$); IR (cm^{-1}): 3416 (OH), 1770, 1707 (phth C=O); FABMS: (*m*/*z*) 860 $[M+1]^+$.

Ethyl 4-O-*acetyl-6*-O-*benzyl-2-deoxy-2-phthalimido-3*-O-*(2,3,4-tri*-O-*benzyl*-α-L-*fucopyranosyl)-1-thio*-β-*glucopyranoside* (**9**).—Compound **8** (0.31 g) was dissolved in 1:1 Ac_2O–pyridine (8 mL) and stirred overnight. The mixture was poured into ice–water (50 mL), and $CHCl_3$ (3 × 10 mL) was used to extracted the solution. The extract was washed successively with water, saturated $NaHCO_3$ solution, and water, and then dried (Na_2SO_4). The resultant residue was purified by PTLC [5:1 light petroleum (60–90 °C)–EtOAc] to give compound **9**, 0.27 g, as a colorless syrup; IR (cm^{-1}): 1766, 1740, 1707 (phth and acetyl C=O); ^{1}H NMR (400 MHz): δ_H (ppm) 0.95 (d, 3 H, $J_{5,6}$ = 6.5 Hz, H-6′), 1.23 (t, 3 H, SCH_2CH_3), 1.88 (s, 3 H, CH_3CO), 2.71 (m, 2 H, SCH_2CH_3), 4.55 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1′), 5.53 (d, 1 H, $J_{1,2}$ = 10.0 Hz, H-1), 3.41–4.80 (18H, H-2, 3, 4, 5, 6, H-2′, 3′, 4′, 5′, 4 × PhCH_2), 7.02–7.80 (m, 24 H, aromatic H); ^{13}C NMR (100 MHz): δ_C (ppm) 14.85 (s, SCH_2CH_3), 15.71 (C-6′), 21.05 (CH_3CO), 23.81 (SCH_2CH_3), 54.02 (C-2), 60.12, 67.90, 69.64, 72.72, 73.23, 73.28, 74.56, 74.88, 77.33, 78.25, 79.16, 80.05, 80.67 (13C, C-1, 3, 4, 5, 6, C-2′, 3′, 4′, 5′, 4 × PhCH_2), 101.55 (C-1′), 122.83–138.65 (30C, aromatic C), 167.68, 168.41 (phth, 2 × C=O), 169.95 (CH_3CO); FABMS: (*m*/*z*) 908 $[M+Li]^+$, 924 $[M+Na]^+$.

Methyl 2,3,4-tri-O-*benzyl-6*-O-*{3,4,6-tri*-O-*benzyl-2*-O-*[4,6*-O-*benzylidene-2-deoxy-2-phthalimido-3*-O-*(2,3,4-tri*-O-*benzyl*-α-L-*fucopyranosyl)*-β-D-*glucopyranosyl]*-α-D-*mannopyranosyl}*-α-D-*mannopyranoside* (**10**).—Compound **A** (0.55 g) and compound **7** (0.62 g) were dissolved in dry ether (20 mL), the solution was stirred for 1 h at rt with powdered 4 Å molecular sieves (1.0 g), and then methyl triflate (MeOTf, 100 μL) was added to the mixture. After 48 h, Et_3N (0.5 mL) was added to the mixture to quench the reaction. The mixture was filtered through a layer of Celite and washed with CH_2Cl_2. The combined filtrate was washed with water, saturated $NaHCO_3$ solution and water, and then dried (Na_2SO_4). The product was purified by column chromatography [5:5:2 benzene–light petroleum (60–90 °C)–EtOAc) to give compound **10**, 0.58 g (53.0%) as a colorless syrup;

$[\alpha]_D$ +9.4° (*c* 0.3, $CHCl_3$); IR (cm^{-1}): 1772, 1708 (phth, C=O). ^1H NMR (400 MHz): δ_H (ppm) 0.88 (d, 3 H, $J_{3,6} = 6.5$ Hz), H-6‴), 3.30 (s, 3 H, OCH_3), 5.55 (s, 1 H, phC*H*), 5.47 (d, 1 H, $J_{1,2} = 8.5$ Hz, H-1″), 3.21–4.83 (43 H, H-1, 2, 3, 4, 5, 6, H-1′, 2′, 3′, 4′, 5′, 6′, H-2″, 3″, 4″, 5″, 6″, H-1‴, 2‴, 3‴, 4‴, 5‴, 9 × PhCH_2), 7.17–7.61 (m, 54 H, aromatic H); ^{13}C NMR (100 MHz): δ_C (ppm) 16.41 (C-6‴), 54.72 (OCH_3), 55.60 (C-2″), 66.20–82.20 (27C, C-2, 3, 4, 5, 6, C-2′, 3′, 4′, 5′, 6′, C-3″, 4″, 5″, 6″, C-2‴, 3‴, 4‴, 5‴, 9 × Ph*C*H_2), 97.61 (C-1′), 98.93 (C-1), 99.45 (C-1‴), 101.15 (ph*C*H), 102.25 (C-1″), 167.90, 168.83 (phth, 2 × C=O); FABMS: (*m*/*z*) 1714 $[M + Na]^+$. Anal. Calcd for $C_{103}H_{105}NO_{21}$: C, 73.09; H, 6.21; N, 0.83. Found: C, 73.24; H, 6.31; N, 0.76.

Methyl 2,3,4-tri-O-*benzyl-6*-O-*{3,4,6-tri*-O-*benzyl-2*-O-*[4*-O-*acetyl-6*-O-*benzyl-2-deoxy-2-phthalimido-3*-O-*(2,3,4-tri*-O-*benzyl-α*-L-*fucopyranosyl)-β*-D-*glucopyranosyl]-α*-D-*mannopyranosyl}-α*-D-*mannopyranoside* (**11**).—Compound **9** (0.21 g) and compound **7** (0.26 g) were coupled as in the procedure for the preparation of the tetrasaccharide **10**. The result of chromatography (10:1 benzene–EtOAc) gave compound **11**, 0.15 g (38%), as a colorless syrup; IR (cm^{-1}): 1769, 1744, 1706 (phth and acetyl, C=O); ^1H NMR (400 MHz): δ_H (ppm) 0.94 (d, 3 H, $J_{5,6} = 6.4$ Hz, H-6‴), 1.87 (s, 3 H, CH_3C=O), 3.27 (s, 3 H, OCH_3), 3.42–5.59 (m, 46 H, H-1, 2, 3, 4, 5, 6, H-1′, 2′, 3′, 4′, 5′, 6′, H-1″, 2″, 3″, 4″, 5″, 6″, H-1‴, 2‴, 3‴, 4‴, 5‴, 10 × PhCH_2), 7.17–7.30 (m, 54 H, aromatic H); ^{13}C NMR (100 MHz): δ_C (ppm) 15.85 (C-6‴), 21.19 (CH_3C=O), 54.57 (OCH_3), 54.89 (C-2″), 67.96–80.37 (28C, C-2, 3, 4, 5, 6, C-2′, 3′, 4′, 5′, 6′, C-3″, 4″, 5″, 6″, C-2‴, 3‴, 4‴, 5‴, 10 × Ph*C*H_2), 96.79, 97.46 (C-1, C-1′), 98.87 (C-1‴), 101.73 (C-1″), 167.70, 168.99, 170.14 (phth 2 × C=O, CH_3C=O).

Methyl 6-O-*{2*-O-*[3*-O-*(α*-L-*fucopyranosyl)-2-deoxy-2-acetamido-β*-D-*glucopyranosyl]-α*-D-*mannopyranosyl}-α*-D-*mannopyranoside* (**12**).—Compound **10** (112 mg) was dissolved in 95% EtOH (20 mL), hydrazine hydrate (50%, 4 mL) was added to the solution and the mixture was kept for 13 h under reflux and then concentrated in vacuo. Anhydrous toluene (30 mL) was added to the residue to remove the hydrazine hydrate and water. The residue was dissolved in 1:2 Ac_2O–pyridine (12 mL) and stirred for 24 h at rt. Conventional processing gave a white waxy solid, 76 mg (71.6%); FABMS: (*m*/*z*) 1611 $[M + Li + 1]^+$, 1627 $[M + Na + 1]^+$. The foregoing waxy solid (63 mg) was dissolved in MeOH (40 mL) and hydrogenolyzed with 10% Pd–C (100 mg) and 330 kPa of hydrogen pressure for 24 h. The mixture was filtered and washed with MeOH and distilled water. The combined filtrate was concentrated and purified by PTLC (8:1 to 5:1 $CHCl_3$–MeOH), and the resulting syrup was lyophilized to give compound **12**, 17 mg (61.4%), as a yellowish foam. $[\alpha]_D$ −19.5° (*c* 0.4, H_2O); ^1H NMR (400 MHz, D_2O): δ_H (ppm) 1.19 (d, 3 H, $J_{5,6} = 6.6$ Hz, H-6‴), 2.11 (s, 3 H, CH_3C=O), 3.44 (s, 3 H, OCH_3), 3.43–4.39 (22H, H-2, 3, 4, 5, 6, H-2′, 3′, 4′, 5′, 6′, H-2″, 3″, 4″, 5″, 6″, H-2‴, 3‴, 4″, 5″), 4.62 (d, 1 H $J_{1,2} = 8.56$ Hz, H-1″), 4.78 (d, 1 H, $J_{1,2} = 2.2$ Hz, H-1′), 4.96 (s, 1 H, H-1), 5.02 (d, 1 H, $J_{1,2} = 4.08$ Hz, H-1‴); ^{13}C NMR (100 MHz), D_2O): δ_C (ppm) 17.77 (C-6‴), 22.23 (CH_3C=O), 57.35 (OCH_3), 57.74 (C-2″), 63.24–82.43 (18C, C-2, 3, 4, 5, 6, C-2′, 3′, 4′, 5′, 6′, C-3″, 4″, 5″, 6″, C-2‴, 3‴, 4‴, 5‴), 99.37 (C-1‴), 101.84 (C-1″), 102.37, 103.64 (C-1, C-1′), 170.20 (CH_3C=O).

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