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Note

Synthesis of α- and β-L-Fuc *p*-(1 → 2)-α-L-Fuc *p*-(1 → 3)-β-D-Gal *p*NAc glycosides identified as termini of *Schistosoma mansoni* oligosaccharides

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Caulfield and colleagues have reported [1–5] that cercariae, the freshwater stage of *Schistosoma mansoni* that are infectious to humans, are covered by a 1–2 μm-thick carbohydrate-rich glycocalyx layer that has immunomodulating properties. Structural studies carried out to identify the detailed carbohydrate structures have revealed that this glycocalyx is a complex structure composed of oligosaccharides with a high fucose content that are probably linked to a peptide. The proposed structures contain repeating units of a trifucosyl side chain attached to *N*-acetylglucosamine and terminated by an unusual linear difucosylated *N*-acetylgalactosamine sequence [6,7].

Mass-spectrometric structural studies utilizing fast atom bombardment ionization identified the terminal sequence as Fuc-(1 → 2)-Fuc-(1 → 3)-GalNAc [6,7]. Establishing the unambiguous anomeric configuration of these terminal glycosidic linkages will be greatly facilitated by having available reference standards to be used as substrates for fucosidases (in conjunction with mass spectral studies) or for ^{1}H NMR chemical shift correlations. For these purposes, we have prepared two trisaccharides, namely α-L-Fuc *p*-(1 → 2)-α-L-Fuc *p*-(1 → 3)-β-D-Gal *p*NAc-O-R (**1**) and β-L-Fuc *p*-(1 → 2)-α-L-Fuc *p*-(1 → 3)-β-D-Gal *p*NAc-O-R (**2**). We chose the aglycon, R = $(CH_2)_8COOMe$, to permit the preparation of neoglycoproteins for eventual immunological studies.

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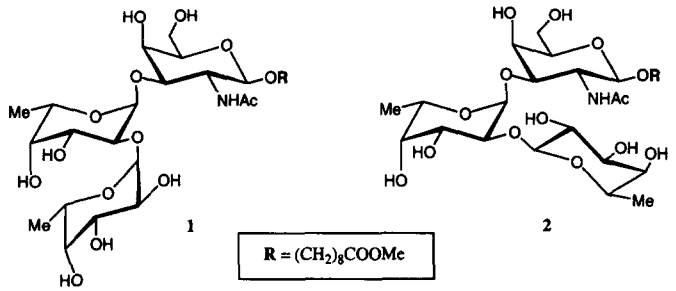
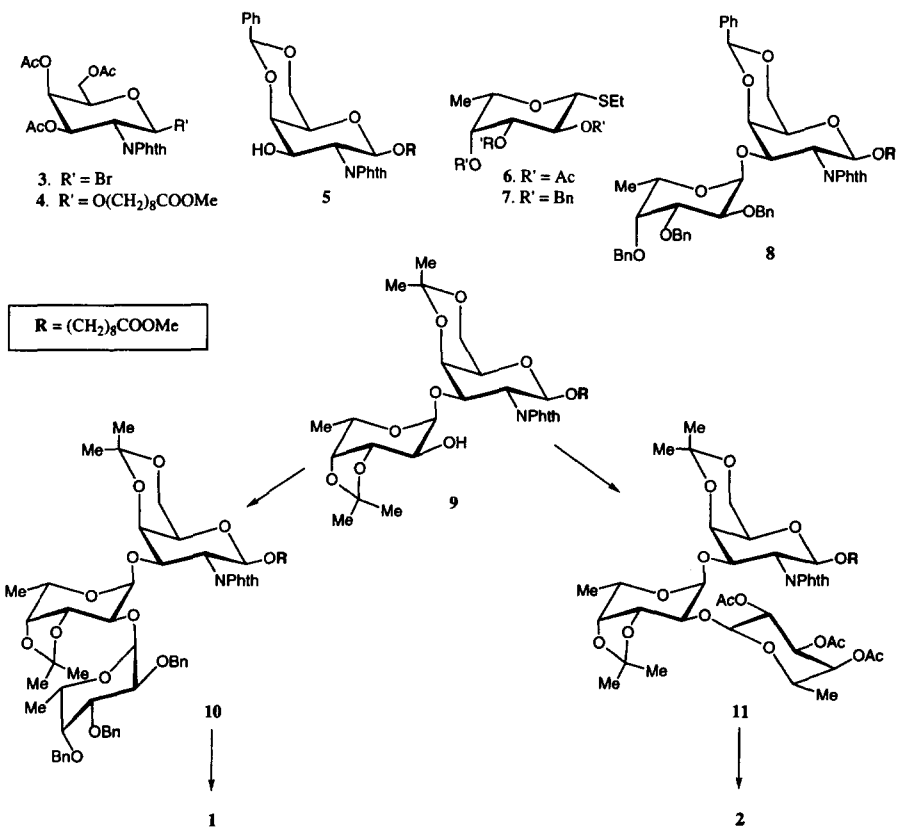


Treatment of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-galactopyranosyl bromide (**3**) with 8-methoxycarbonyl-1-octanol furnished the desired β-glycoside **4** in 87% yield. Removal of the *O*-acetyl protecting groups (NaOMe–MeOH) gave a crude syrup that was selectively benzylidenated [9] to give the 4,6-*O*-benzylidene derivative **5** in 80% yield. Fucosylation of **5** with ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-fucopyranoside (**7**), [10] using dimethyl(methylthio)sulfonium triflate (DMTST) [11] as promoter and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) as base, furnished the disaccharide **8** in 80% yield.



Removal of the benzyl and the benzylidene protecting groups (H_2, 5% Pd–C) furnished a syrup that was used directly in the next step. Compound **9** was obtained by reaction of the crude syrup with 2,2-dimethoxypropane and a catalytic amount of *p*-toluenesulfonic acid. Glycosylation of **9** with fucosyl donor **7** in the presence of silver triflate, followed by slow addition of bromine [12], gave the desired trisaccharide **10** in 61% yield. The isopropylidene groups were then removed using 60% aq acetic acid. Removal of the phthalimido group using hydrazine acetate [9] in refluxing methanol, followed by acetylation, then gave the *N*-acetylated derivative that was directly debenzylated (H_2, 5% Pd–C) to give the desired trisaccharide **1** in 42% yield. Similarly, glycosylation of **9** with ethyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-fucopyranoside (**6**) [10] using methylsulfenyl bromide (MSB) [13] as promoter, along with DTBMP as acid acceptor, furnished the desired β-fucosylated trisaccharide **11** in 90% yield. Removal of the isopropylidene groups was achieved under controlled conditions using 60% aq acetic acid at 50 °C for 30 min. Prolonging the reaction time led to decomposition of the product. Removal of the phthalimido group using hydrazine acetate, followed by acetylation of the free amine using 10% acetic anhydride in methanol, furnished the desired trisaccharide **2** in 75% yield. The structure and the purity of trisaccharides **1** and **2** were established by NMR spectroscopy and high-resolution FAB mass spectrometry.

1. Experimental

General methods.—Optical rotations were measured with a Perkin–Elmer 241 polarimeter at 22 ± 2 °C. Analytical TLC was performed on Silica Gel 60-F_{254} (E. Merck, Darmstadt) with detection by quenching of fluorescence and/or by charring with H_2SO_4. All commercial reagents were used as supplied, and chromatography solvents were distilled prior to use. Column chromatography was performed on Silica Gel 60 (E. Merck 40–60 μm, Darmstadt). Millex-GV (0.22 μm) filter units were from Millipore (Missisauga, ON), and C_{18} Sep-Pak sample preparation cartridges were from Waters Associates (Missisauga, ON). Iatrobeads refers to a silica gel (product 6RS-8060) produced by Iatron Laboratories (Tokyo, Japan). ^{1}H NMR spectra were recorded at 360 MHz (Bruker WM 360) or 300 MHz (Bruker AM 300) with either internal $(CH_3)_4Si$ (δ 7.26, $CDCl_3$) or DOH (δ 4.80, D_2O). ^{13}C NMR spectra were recorded at 75.5 MHz (Bruker AM 300) with internal standards. ^{1}H NMR data are reported as though they were first order. All ^{13}C chemical-shift assignments are tentatively assigned. Unless otherwise noted, all reactions were carried out at room temperature. Organic solutions were dried (Na_2SO_4) prior to concentration under vacuum at < 40 °C (bath). Microanalyses were carried out by the analytical services of this department, and all the samples submitted for elemental analyses were dried overnight under vacuum over P_2O_5 at 56 °C (refluxing Me_2CO). Mass spectra were recorded on samples suspended in a matrix of glycerol and HCl using a Kratos AEIMS9 instrument with Xe as the bombarding gas.

8-Methoxycarbonyloctyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-galacto-pyranoside (**4**).—A mixture of 8-methoxycarbonyl-1-octanol (5.66 g, 30 mmol), silver trifluoromethanesulfonate (3.35 g, 13 mmol) and 4-Å molecular sieves (1.6 g) in dry

dichloromethane (50 mL) was stirred for 1 h at room temperature. The reaction mixture was cooled to −78 °C, followed by slow addition of compound **3** (5.0 g, 10 mmol) [8] dissolved in dichloromethane (25 mL). The reaction was stirred at this temperature for 2 h. The reaction mixture was then diluted with dichloromethane (30 mL), and the solids were removed by filtration over Celite. The filtrate was washed sequentially with satd $NaHCO_3$ and water. After solvent evaporation, the residue was purified by column chromatography using 2:1 EtOAc–hexane as eluent to furnish **4** as a white foam (5.25 g, 87%): $[\alpha]_D$ −10.1° (*c* 0.8, $CHCl_3$); R_f 0.69 (1:9 methanol–chloroform); ^{1}H NMR ($CDCl_3$): δ 7.70–7.90 (m, 4 H, Ar), 5.80 (dd, 1 H, $J_{2,3}$ 11.5 Hz, $J_{3,4}$ 3.4 Hz, H-3), 5.44 (dd, 1 H, $J_{3,4}$ 3.4 Hz, H-4), 5.25 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.55 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 11.5 Hz, H-2), 4.22 (m, 2 H, H-6), 4.08 (dd, 1 H, H-5), 3.85 (m, 1 H, OC*H* H), 3.66 (s, 3 H, CO*OMe*), 3.43 (m, 1 H, OCH*H*), 2.30 (t, 2 H, *J* 7.5 Hz, CH_2COOMe), 2.20, 2.08, 1.86 (s each, 3 × 3 H, Ac), 0.9–1.50 (m, 12 H, -(CH_2)$_6$-); ^{13}C NMR ($CDCl_3$): δ 174.26 (*CO*OMe), 170.47, 170.39, 169.89 (O*CO*Me), 134.33, 131.55, 123.59 (Ph), 96.65 (C-1), 70.82 (C-5), 70.17 (O*C*H$_2$), 68.20 (C-3), 66.85 (C-4), 61.48 (C-6), 51.57 (C-2), 51.49 (COO*C*H$_3$), 34.07, 29.23, 29.07, 28.97, 25.75, 24.91 (-OCH$_2$(*CH*$_2$)$_7$-), 20.78, 20.58 (2 × Ac). Anal. Calcd for $C_{30}H_{39}NO_{12}$: C, 59.50; H, 6.49; N, 2.31. Found: C, 59.35; H, 6.65; N, 2.28.

*8-Methoxycarbonyloctyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-*D*-galacto-pyranoside* (**5**).—Compound **4** (4.0 g, 6.6 mmol) was dissolved in dry methanol (20 mL) containing sodium methoxide (0.2 g). After 1 h, the solution was neutralized by addition of Amberlite IR-120 (H^+) cation-exchange resin, which was removed by filtration, and the solvent was evaporated to furnish the intermediate 8-methoxycarbonyloctyl 2-deoxy-2-phthalimido-β-D-galactopyranoside, as a clear syrup (3.25 g, quant). This material was not further characterized but was used directly for the preparation of **5**.

The crude syrup was dissolved in 1:1 acetonitrile–*N*,*N*-dimethylformamide, *p*-toluenesulfonic acid (0.4 g, 2.6 mmol) was added, and the mixture was stirred for 1 h. α,α-Dimethoxytoluene (5.15 mL, 34 mmol) was added dropwise, and stirring was continued for 4 h. After neutralization with Et_3N (4 mL) and solvent evaporation, the residue was dissolved in dichloromethane (25 mL) and washed with water. Solvent evaporation left a residue that was purified by chromatography using 2:1 EtOAc–hexane as eluent to furnish **5** as a white solid (3.07 g, 80%): $[\alpha]_D$ +16.9° (*c* 0.7, $CHCl_3$); R_f 0.51 (1:9 methanol–chloroform); ^{1}H NMR ($CDCl_3$): δ 7.20–7.90 (m, 9 H, Ar), 5.62 (s, 1 H, C*H* Ph), 5.25 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.51 (dd, 1 H, $J_{2,3}$ 11.5, $J_{3,4}$ 4.0 Hz, H-3), 4.43 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 11.5 Hz, H-2), 4.42 (dd, 1 H, H-6a), 4.30 (dd, 1 H, $J_{3,4}$ 4.0, $J_{4,5}$ 1.0 Hz, H-4), 4.15 (dd, 1 H, H-6b), 3.88 (m, 1 H, OC*H* H), 3.67 (s, 3 H, CO*OMe*), 3.56 (d, 1 H, *J* 2.5 Hz, H-5), 3.43 (m, 1 H, OCH*H*), 2.45 (d, 1 H, D_2O exchange, *J* 11.5 Hz, O*H*), 2.23 (t, 2 H, *J* 8.0 Hz, CH_2COOMe); ^{13}C NMR ($CDCl_3$): δ 174.28 (*C*OOCH$_3$), 137.42, 134.04, 131.92, 129.38, 128.34, 126.58, 123.57, 123.06, (Ph), 101.6 (*C*HPh), 98.43 (C-1), 76.62 (C-5), 69.54 (O*C* H$_2$), 69.31 (C-6), 68.05 (C-3), 66.80 (C-4), 54.92 (C-2), 51.45 (COO*C*H$_3$). Anal. Calcd for $C_{31}H_{37}NO_9$: C, 65.60; H, 6.57; N, 2.47. Found: C, 65.59; H, 6.72; N, 2.49.

*8-Methoxycarbonyloctyl 2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl-(1 → 3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-*D*-galactopyranoside* (**8**).—A solution of DMTST

(1.45 g, 5.60 mmol) in dry dichloromethane (10 mL) was added to a stirred mixture of **5** (0.70 g, 1.41 mmol), **7** (0.70 g, 1.48 mmol), DTBMP (1.30 g, 6.30 mmol) and molecular sieves 4 Å (400 mg) in dry dichloromethane under an inert atmosphere of Ar. The mixture was stirred for 3 h at room temperature, followed by addition of Et_3N (5 mL). The mixture was filtered through Celite and the filtrate was evaporated. The residue was dissolved in dichloromethane (20 mL) and washed with satd $NaHCO_3$, dried, and the solvent was evaporated to a syrup. Column chromatography of the residue using 1:4 EtOAc–hexane as a eluent yielded **8** as a syrup (1.11 g, 80%): $[\alpha]_D$ +53.5° (*c* 1.3, $CHCl_3$); R_f 0.48 (2:3 EtOAc–hexane); ^1H NMR ($CDCl_3$): δ 6.90–7.80 (m, 24 H, Ar), 5.54 (s, 1 H, C*H* Ph), 5.32 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.76 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 11.5 Hz, H-2), 4.39 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1'), 4.36 (dd, 1 H, H-2'), 4.12 (dd, 1 H, $J < 1.5$ Hz, H-4'), 4.08 (dd, 1 H, H-3'), 3.77 (dd, 1 H, H-5), 3.67 (dd, $J_{2,3}$ 10.0, $J_{3,4}$ 3.5 Hz, H-3), 3.65 (s, 3 H, CO*OMe*), 3.60 (bs, 1 H, H-5'); ^{13}C NMR ($CDCl_3$): δ 174.28 (*C*OOCH$_3$), 101.35 (*C*HPh), 100.81 (C-1), 99.65 (C-1'), 69.48 (O*C*H$_2$), 69.29 (C-6), 51.84 (C-2), 16.57 (C-6'). Anal. Calcd for $C_{58}H_{65}NO_{13}$: C, 70.79; H, 6.66; N, 1.42. Found: C, 70.53; H, 6.80; N, 1.40.

*8-Methoxycarbonyloctyl 3,4-*O*-isopropylidene-α-*L*-fucopyranosyl-(1 → 3)-2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-β-*D*-galactopyranoside* (**9**).—Compound **8** (0.47 g, 0.47 mmol) was dissolved in absolute ethanol (10 mL) containing 5% Pd–C (0.30 g) and stirred under an H_2 atmosphere (0.1 MPa) for 36 h. The catalyst was removed by filtration, washed with 95% ethanol, and the solvent was evaporated. The residue was not further characterized but was used directly for the preparation of **9**.

The residue (0.27 g) was dissolved in 1:1 acetonitrile–*N,N*-dimethylformamide (10 mL). To this solution was added *p*-toluenesulfonic acid (80 mg, 0.41 mmol), followed by slow addition of 2,2-dimethoxypropane (0.31 mL, 2.52 mmol). The reaction mixture was stirred at 50 °C for 2 h. The reaction was neutralized with 0.8 mL of Et_3N and concentrated. The residue was dissolved in dichloromethane (10 mL) and washed with satd $NaHCO_3$, dried and evaporated. The crude product was purified by column chromatography using 2:3 EtOAc–hexane as eluent to furnish **9** (0.33 g, 100%) as a foam: $[\alpha]_D$ −18.9° (*c* 0.9, $CHCl_3$); R_f 0.33 (1:19 methanol–chloroform); ^1H NMR ($CDCl_3$): δ 7.65–7.80 (m, 4 H Ar), 5.14 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.73 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1'), 4.64 (dd, 1 H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 11.0 Hz, H-2), 4.52 (dd, 1 H, $J_{2,3}$ 11.5 Hz, $J_{3,4}$ 3.5 Hz, H-3), 4.35 (dd, 1 H, $J_{4,5} < 1.0$ Hz, H-4), 1.54, 1.49, 1.40, 1.29 (4 s, 3 H each, 4 × CH_3); ^{13}C NMR ($CDCl_3$): δ 174.29 (*C*OOCH$_3$), 100.72 (C-1), 98.43 (C-1'), 69.48 (O*C* H$_2$), 62.91 (C-6), 52.00 (C-2), 51.47 (COO*C*H$_3$), 16.43 (C-6'). Anal. Calcd for $C_{36}H_{51}NO_{13}$: C, 61.26; H, 7.28; N, 1.98. Found: C, 61.23; H, 7.15; N, 2.12.

*8-Methoxycarbonyloctyl 2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl-(1 → 2)-3,4-*O*-isopropylidene-α-*L*-fucopyranosyl-(1 → 3)-2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-β-*D*-galactopyranoside* (**10**).—Compounds **9** (0.40 g, 0.57 mmol) and **7** (0.32 g, 0.68 mmol) were dissolved in dry dichloromethane (15 mL) and stirred at room temperature for 1 h in presence of 4-Å molecular sieves under an inert atmosphere of Ar. To this mixture was added silver trifluoromethanesulfonate (0.44 g, 1.72 mmol), and DTBMP (0.23 g, 1.14 mmol), and the reaction mixture was further stirred for 20 min. This was followed by dropwise addition of bromine (29.4 μL, 0.57 mmol), and stirring was continued for 2 h. The reaction mixture was neutralized with addition of Et_3N (3 mL).

The solids were removed by filtration over Celite, and the filtrate was extracted with dichloromethane, washed with satd $NaHCO_3$, dried and concentrated to a syrup. The residue was purified by column chromatography using 1:3 EtOAc–hexane as eluent to furnish **10** as a foam (0.39 g, 61%): $[\alpha]_D$ −42.7° (*c* 0.3, $CHCl_3$); R_f 0.71 (1:1 EtOAc–hexane); ^1H NMR ($CDCl_3$): δ 7.10–7.80 (m, 19 H, Ar), 5.15 (d, 1 H, *J* 8.5 Hz, H-1), 4.95 (d, 1 H, *J* 3.5 Hz, H-1′), 4.88 (d, 1 H, *J* 5.5 Hz, H-1″), 4.62 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 11.0 Hz, H-2), 4.35 (dd, 1 H, $J_{2,3}$ 11.0, $J_{3,4}$ 4.0 Hz, H-3), 4.22 (dd, 1 H, $J_{4',5'} < 1.0$ Hz, H-4), 1.26 (d, 3 H, H-6′), 0.78 (d, 3 H, H-6″); ^{13}C NMR ($CDCl_3$): δ 98.25, 98.00 (C-1, C-1′, C-1″), 79.26 (C-2″), 77.49 (C-2′), 69.29 (O*C* H_2), 62.94 (C-6), 51.80 (C-2), 16.51 (C-6′, C-6″). Anal. Calcd for $C_{63}H_{79}NO_{17}$: C, 67.42; H, 7.10; N, 1.25. Found: C, 67.50; H, 7.13; N, 1.33.

*8-Methoxycarbonyloctyl α-*L*-fucopyranosyl-(1 → 2)-α-*L*-fucopyranosyl-(1 → 3)-2-acetamido-2-deoxy-β-*D*-galactopyranoside* (**1**).—Compound **10** (0.38 g, 0.33 mmol) was dissolved in 60% aq acetic acid, and the reaction mixture was heated at 50 °C for 1 h. The solvent was evaporated, and the residue was coevaporated several times with dry toluene to afford a syrup (0.35 g): (R_f 0.45 1:9 methanol–chloroform). The crude product was not further characterized but was used directly in the next reaction.

Hydrazine acetate (0.31 g, 3.44 mmol) was added to a stirred solution of the crude material (0.35 g, 0.34 mmol) in dry methanol (8 mL). The reaction was refluxed for 20 h. Evaporation of the solvent furnished a syrupy residue that was then dissolved in 10 mL of moist methanol and 0.5 mL of acetic anhydride. The reaction was stirred for 3 h during which time the reaction went to completion. Evaporation of the solvent furnished a foamy residue that was then debenzylated using 5% Pd–C in absolute methanol (8 mL) for 20 h under a H_2 atmosphere (0.1 MPa). The catalyst was removed by filtration, washed with 95% ethanol, and the solvent was evaporated. The residue was purified by chromatography on Iatrobeads using 10:4:1 chloroform–methanol–water as eluent to furnish the desired compound. The compound was dissolved in 8 mL of deionized water and loaded onto a Sep-Pak C_{18} cartridge that was then washed with water (20 mL). The product was eluted with methanol (10 mL), and concentrated. The residue was redissolved in deionised water (8 mL) and passed through a Millipore (0.22 μm) filter. Lyophilization of the filtrate furnished **1** as a white fluffy solid (98.7 mg, 42%): $[\alpha]_D$ −202° (*c* 0.2, H_2O); R_f 0.28 (10:4:1 chloroform–methanol–water); ^1H NMR (D_2O): δ 5.28 (d, 1 H, *J* 3.5 Hz, H-1″), 4.95 (d, 1 H, *J* 3.5 Hz, H-1′), 4.50 (d, 1 H, *J* 8.0 Hz, H-1), 4.21 (q, 1 H, H-5′), 4.13 (q, 1 H, H-5″), 4.01 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 3.5 Hz, H-3), 4.00 (t, 1 H, *J* 2.0 Hz, H-3″), 3.94 (dd, 1 H, $J_{2',3'}$ 10.0, $J_{3',4'}$ 3.5 Hz, H-3′), 3.90 (m, 1 H, OC*H* H), 3.88 (d, 1 H, *J* 1.5 Hz, H-4), 3.86 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, H-2), 3.85 (d, 1 H, *J* 1.5 Hz, H-4″), 3.82 (dd, 1 H, *J* 8.5 Hz, H-2″), 3.80 (dd, 1 H, *J* 8.5 Hz, H-2′), 3.81 (d, 1 H, *J* 2.0 Hz, H-4′), 3.69 (s, 3 H, CO*OMe*), 3.67 (dd, 1 H, H-5), 3.57 (m, 1 H, OCH*H*), 2.38 (t, 2 H, *J* 8.0 Hz, CH_2 COOMe), 2.05 (s, 3 H, NH*Ac*), 1.50–1.64 and 1.26–1.34 (m, 12 H, -(CH_2)$_6$-), 1.22 (d, 3 H, H-6′), 1.21 (d, 3 H, H-6″); ^{13}C NMR (D_2O): δ 178.71 (*C*OOCH$_3$), 175.49 (NH*C*O CH$_3$), 102.43 (C-1, *J* 163 Hz), 96.85 (C-1″, *J* 169.2 Hz), 95.89 (C-1′, *J* 168.9 Hz), 76.66 (C-2″), 75.72 (C-5), 72.62 (C-4″), 72.54 (C-2′), 72.00 (C-4), 71.33 (O*C* H_2), 70.21 (C-3″), 68.71 (C-3′), 68.55 (C-4′), 68.26 (C-3), 68.17 (C-5″), 67.80 (C-5′), 61.70 (C-6), 52.91 (C-2), 52.80 (COO*C*H$_3$), 34.55, 29.42, 29.15, 29.03, 29.01, 25.86, 25.13 (7 C, *C*H_2), 23.52

($NHCOCH_3$), 16.14 (C-6′, C-6″). MS: Calcd for $C_{30}H_{54}NO_{16}$ (M + 1): *m/z* 684.3443; found: *m/z* 684.3432.

*8-Methoxycarbonyloctyl 2,3,4-tri-*O*-acetyl-β-*L*-fucopyranosyl-(1 → 2)-3,4-*O*-isopropylidene-α-*L*-fucopyranosyl-(1 → 3)-2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-β-*D*-galactopyranoside* (**11**).—Compounds **9** (0.36 g, 0.51 mmol) and **6** (0.24 g, 0.61 mmol) were dissolved in a 1:1 mixture of dry dichloromethane and acetonitrile (15 mL). To this mixture was added 3-Å molecular sieves (200 mg), DTBMP (0.12 g, 0.61 mmol) and silver trifluoromethanesulfonate (0.21 g, 0.51 mmol). The slurry was stirred for 1 h under an inert atmosphere of Ar. The temperature was then lowered to −40 °C, and a solution of MSB (84 mg, 0.66 mmol) in dichloromethane was injected slowly through the septum into the stirred mixture. The reaction temperature was then raised slowly to 0 °C over 45 min. Upon completion of the reaction as indicated by TLC, the reaction mixture was neutralized by addition of Et_3N (0.7 mL) and filtered through Celite. The filtrate was washed with satd $NaHCO_3$, dried and evaporated to a syrup. The crude product was purified by column chromatography using 1:2 EtOAc–toluene to furnish compound **11** as a foam (0.44 g, 90%): $[\alpha]_D$ −19.6° (*c* 1.1, $CHCl_3$); R_f 0.76 (1:1 EtOAc–toluene); ^1H NMR ($CDCl_3$): δ 7.70–7.90 (m, 4 H, Ar), 5.23 (d, 1 H, *J* 8.5 Hz, H-1), 4.97 (d, 1 H, *J* 3.5 Hz, H-1′), 4.94 (dd, 1 H, $J_{2,3} = J_{3,4}$ 3.5 Hz, H-3′), 4.36 (d, 1 H, *J* 8.5 Hz, H-1″), 4.21 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 4.10 (dd, 1 H, $J < 1.0$ Hz, H-4″), 3.67 (s, 3 H, $COOCH_3$), 1.26 (d, 3 H, $J_{5',6'}$ 6.5 Hz, H-6′), 1.18 (d, 3 H, $J_{5'',6''}$ 6.5 Hz, H-6″), 2.31, 1.98 and 1.93 (each s, 3 H, OAc); ^{13}C NMR ($CDCl_3$): δ 101.91 (C-1), 98.94 (C-1′), 98.43 (C-1″), 69.20 (OCH_2), 63.50 (C-6), 51.59 (C-2), 51.45 ($COOCH_3$). Anal. Calcd for $C_{48}H_{67}NO_{20}$: C, 58.95; H, 6.90; N, 1.43. Found: C, 58.64; H, 7.28; N, 2.00.

*8-Methoxycarbonyloctyl β-*L*-fucopyranosyl-(1 → 2)-α-*L*-fucopyranosyl-(1 → 3)-2-acetamido-2-deoxy-β-*D*-galactopyranoside* (**2**).—Compound **11** (0.47 g, 0.47 mmol) was dissolved in 60% aq acetic acid (15 mL), and the reaction was heated at 50 °C for 30 min. The solvent was evaporated, and the residue was coevaporated several times with dry toluene to afford a syrup (0.42 g, 98%); R_f 0.44 (1:9 methanol–dichloromethane). The crude product was not further characterized but was used directly for the next reaction.

Hydrazine acetate (0.17 g, 1.94 mmol) was added to a stirred solution of the crude material (88.4 mg, 97.1 μmol) in dry methanol (4 mL). The reaction was refluxed for 20 h. Evaporation of the solvent furnished a foamy residue that was then dissolved in 10% acetic anhydride in methanol (10 mL) and stirred for 1 h. Evaporation of the solvent, followed by purification of the crude product by column chromatography on Iatrobeads using 3:7 methanol–chloroform, furnished the desired product. The compound was dissolved in 4 mL of deionized water and loaded onto a Sep-Pak C_{18} cartridge that was then washed with water (20 mL). The product was eluted with methanol (8 mL) and concentrated. The residue was redissolved in deionized water (6 mL) and passed through a Millipore (0.22 μm) filter. Lyophilization of the filtrate furnished **2** as a white fluffy solid (50 mg, 75%): $[\alpha]_D$ −102.6° (*c* 0.3, water): R_f 0.25 (35:14:1 chloroform–methanol–water); ^1H NMR (D_2O): δ 5.16 (d, 1 H, *J* 3.5 Hz, H-1′), 4.52 (d, 1 H, *J* 8.0 Hz, H-1″), 4.44 (d, 1 H, *J* 8.5 Hz, H-1), 4.24 (q, 1 H, H-5′), 4.05 (d, 1 H, *J* 2.5 Hz, H-4), 4.02 (dd, 1 H, $J_{2',3'}$ 10, $J_{3',4'}$ 3.5 Hz, H-3′), 3.99 (dd, 1 H,

$J_{1,2}$ 8.0, $J_{2,3}$ 10.0 Hz, H-2), 3.89 (m, 1 H, OC*H* H), 3.83 (d, 1 H, *J* 2.0 Hz, H-4′), 3.82 (dd, 1 H, H-2′), 3.79 (d, 1 H, H-5′), 3.76 (m, 2 H, H-6a, H-6b), 3.75 (dd, 1 H, H-3), 3.73 (dd, 1 H, *J* 1.5 Hz, H-4″), 3.68 (s, 3 H, CO*OMe*), 3.66 (dd, 1 H, $J_{2'',3''}$ 10.5, $J_{3'',4''}$ 1.5 Hz, H-3″), 3.65 (d, 1 H, H-5), 3.59 (m, 1 H, OCH*H*), 3.53 (dd, 1 H, $J_{1'',2''}$ 8.5, $J_{2'',3''}$ 10.5 Hz, H-2″), 2.38 (t, 2 H, CH_2 COOMe), 2.08 (s, 3 H, NH*Ac*), 1.50–1.62 and 1.29–1.32 (m, 12 H, -(CH_2)$_6$-), 1.28 (d, 3 H, H-6″), 1.18 (d, 3 H, H-6′); ^{13}C NMR (D_2O): δ 178.74 (*C*OOMe), 175.46 (NH*C*O CH_3), 105.27 (C-1″, *J* 160.8 Hz), 102.57 (C-1, *J* 160.4 Hz), 101.06 (C-1′, *J* 170.6 Hz), 80.45 (C-4″), 78.24 (C-2′), 75.88 (C-5), 73.69 (C-3″), 72.65 (C-4′), 71.98 (C-2″), 71.78 (C-5″), 71.71 (C-3), 71.25 (O*C* H_2), 69.34 (C-3′), 68.24 (C-4), 68.19 (C-5′), 61.79 (C-6), 52.91 (COO*C*H_3), 51.69 (C-2), 34.55, 29.37, 29.16, 29.00, 25.82, 25.13 (7C, *C* H_2), 23.44 (NHCO*C*H_3), 16.58 (C-6″), 16.13 (C-6′). MS: Calcd for $C_{30}H_{54}NO_{16}$ (M + 1): *m/z* 684.3443; found: *m/z* 684.3417.

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