Supporting Information

General. All chemicals were purchased and used without further purification. Dichloromethane (CH_2Cl_2) was distilled over calcium hydride. Diethyl ether (Et_2O) was distilled over sodium. Molecular sieves (AW-300) used in glycosylations were crushed and activated before use. Reactions were monitored with analytical TLC on silica gel 60 F254 plates and visualized under UV (254 nm) and/or by staining with acidic cerium ammonium molybdate. Flash column chromatography was performed on silica gel (35–75 μm) or LiChroprep RP18. ^{1}H-NMR spectra were recorded on a Bruker DRX-500 (500 MHz) or DRX-600 (600 MHZ) spectrometer at 20°C. Chemical shifts (in ppm) were determined relative to either tetramethylsilane in deuterated chloroform (δ = 0 ppm) or acetone in deuterated water (δ = 2.05 ppm). Coupling constants in Hz were measured from 1D spectra. ^{13}C attached proton test (^{13}C-APT) NMR spectra were obtained by using the same Bruker NMR spectrometer (125 or 150 MHz) and calibrated with $CDCl_3$ (δ = 77 ppm). Coupling constants (*J*) are reported in Hz. Splitting patterns are described by using the following abbreviations: s, singlet; brs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. ^{1}H NMR spectra are reported in this order: chemical shift; multiplicity; number(s) of proton; coupling constant(s).

One-Pot Synthesis of Protected Globo H (13). Compound **13** was prepared from fucosyl donor **12** (118 mg, 1.2 equiv), disaccharide building block **11** (200 mg, 1 equiv), and trisaccharide **12** (263 mg, 1.0 equiv) following the general one-pot procedure withg NIS/TfOH. Yield after chromatography was 429 mg (83%). ^{1}H NMR (500 MHz, $CDCl_3$) d 7.96 (dt, 4H, *J* = 1.45, 9.50 Hz), 7.48–7.37 (m, 6H), 7.36–7.13 (m, 67H), 7.08–6.97 (m, 8H), 6.09 (d, 1H, *J* = 6.2 Hz), 5.62 (d, 1H, *J* = 2.6 Hz), 5.53 (d, 1H, *J* = 2.2 Hz), 5.24 (s, 1H), 5.13–5.06 (m, 5H), 5.00 (d, 1H, *J* = 1.5 Hz), 4.97–4.90 (m, 2H), 4.88–4.66 (m, 7H), 4.64–4.55 (m, 4H), 4.54–4.28 (m, 15H), 4.27–4.22 (m, 3H), 4.19–3.96 (m, 9H), 3.95–3.54 (m, 13H), 3.51–3.45 (m, 2H), 3.41–3.22 (m, 10H), 3.14–3.07 (m, 2H), 1.66–1.57 (m, 2H), 1.52–1.43 (m, 2H), 1.41–1.30 (m, 2H), 0.78 (d, 3H, *J* = 5.9 Hz). ^{13}C-APT NMR (125 MHz, $CDCl_3$) δ 165.9, 165.1, 156.3, 153.7, 139.3, 139.1, 139.0, 138.72, 138.7,

138.6, 138.3, 138.26, 138.2, 138.1, 138.0, 137.9, 136.6, 129.8, 129.6, 129.59, 129.5, 129.0, 128.5, 128.4, 128.3, 128.2, 128.18, 128.1, 127.9, 127.8, 127.79, 127.77, 127.6, 127.54, 127.5, 127.4, 127.39, 127.23, 127.2, 127.1, 127.0, 126.7, 126.2, 103.9, 103.4, 103.1, 10.9, 100.4, 96.6, 82.8, 81.9, 81.4, 81.0, 79.3, 78.8, 77.5, 77.1, 75.0, 74.9, 74.7, 74.66, 74.0, 73.8, 73.7, 73.6, 73.5, 73.1, 72.9, 72.8, 72.7, 72.4, 72.3, 71.8, 71.0, 70.3, 69.6, 69.0, 68.6, 68.5, 67.4, 66.9, 66.5, 63.0, 62.9, 55.7, 40.9, 29.7, 29.6, 29.3, 23.3, 16.4. Unit MS: $C_{164}H_{171}Cl_3N_2O_{35}Na$ $[M+Na]^+$ calculated: 2,856; found: 2,856.

One-Pot Synthesis of Protected Tetrasaccharide (15). Compound **15** was prepared from fucosyl donor **12** (32.8 mg, 1.2 equiv), disaccharide building block **11** (55.7 mg, 1 equiv), and galactosyl acceptor **14** (29.2 mg, 1.0 equiv) following the general one-pot procedure with NIS/TfOH. Yield after chromatography was 42.6 mg (42%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08–7.95 (m, 4H), 7.59–6.95 (m, 51H), 5.58 (d, 1H, *J* = 2.6 Hz), 5.36 (s, 1H), 5.09 (s, 2H), 4.88–4.70 (m, 6H), 4.60–4.25 (m, 16H), 4.10–3.87 (m, 9H), 3.83–3.76 (m, 2H), 3.65 (d, 1H, *J* = 11.75 Hz), 3.61–3.51 (m, 3H), 3.50–3.38 (m, 3H), 3.36–3.10 (m, 2H), 3.21–3.13 (m, 2H), 1.60–1.47 (m, 4H), 1.39–1.31 (m, 2H), 0.89 (s, 3H). ^{13}C-APT NMR (150 MHz, $CDCl_3$) δ165.9, 165.3, 156.3, 154.0, 138.9, 138.7, 138.24, 138.2, 137.93, 137.9, 136.6, 133.15, 133.1, 129.9, 129.8, 129.6, 128.5, 128.4, 128.37, 128.32, 128.3, 128.2, 128.11, 128.1, 128.0, 127.9, 127.85, 127.73. 127.7, 127.6, 127.56, 127.4, 127.37, 127.2, 127.1, 127.0717, 127.0, 126.7, 126.3, 126.2, 123.8, 101.7, 100.6, 97.9, 96.6, 95.7, 82.8, 78.9, 77.5, 77.4, 74.9, 74.7, 74.6, 74.1, 74.0, 73.6, 73.4, 73.0, 72.9, 72.86, 72.8, 72.4, 72.2, 71.6, 70.4, 69.0, 68.4, 67.9, 67.3, 66.5, 63.2, 62.6, 55.7, 40.9, 29.7, 29.6, 28.8, 23.3, 16.4. High-resolution MS (HRMS): $C_{110}H_{115}Cl_3N_2O_{25}Na$ $[M+Na]^+$ calculated: 1,991.6746, found: 1,991.6776.

One-Pot Synthesis of Protected Trisaccharide (16). Compound **16** was prepared from fucosyl donor **12** (31.5 mg, 1.2 equiv), disaccharide building block **11** (53.6 mg, 1 equiv), and *N*-Cbz-5-hydroxylpentamine linker (11.6 mg, 1 equiv) following the general one-pot procedure with NIS/TfOH. Yield after chromatography was 43.4 mg (54%). ^{1}H NMR (600 MHz, $CDCl_3$) δ8.08–7.95 (m, 4H), 7.59–7.01 (m, 41H), 5.58 (d, 1H, *J* = 3.06 Hz), 5.08 (s, 2H), 4.87–4.72 (m, 5H), 4.64–4.52 (m, 6H), 4.48–4.32 (m, 9H), 4.13–4.04 (m,

2H), 3.97–3.89 (m, 2H), 3.87–3.72 (m, 4H), 3.64–3.58 (m, 1H), 3.57–3.38 (m, 5H), 3.18–3.09 (m, 2H), 1.61–1.49 (m, 2H), 1.47–1.39 (m, 2H), 1.35–1.28 (m, 2H), 0.99 (s, 3H). ^{13}C-APT NMR (125 MHz, $CDCl_3$) δ 166.0, 165.2, 156.3, 154.1, 139.0, 138.7, 138.6, 138.3, 138.1, 137.9, 136.6, 133.1, 133.0, 129.96, 129.8, 129.7, 129.5, 128.4, 128.38, 128.2, 128.14, 128.1, 128.07, 128.0, 127.9, 127.8, 127.7, 127.68, 127.6, 127.4, 127.3, 127.2, 127.1, 126.7, 102.8, 101.0, 96.7, 83.1, 79.1, 77.6, 76.6, 74.6, 74.3, 74.1, 73.7, 73.5, 72.9, 72.86, 72.6, 72.2, 71.8, 70.1, 69.8, 68.7, 67.2, 66.5, 63.0, 55.4, 40.9, 29.6, 29.5, 29.0, 23.0, 16.4. HRMS: $C_{90}H_{95}Cl_3N_2O_{20}Na$ $[M+Na]^+$ calculated: 1651.5436; found: 1,651.5483.

The Protected Disaccharide (18). To fucosyl donor **10** (471.5 mg, 1.2 equiv) and galactosyl acceptor **17** (428.7 mg, 1 equiv) in 1,4-dioxane:CH_2Cl_2 1:2 (6 ml) was added molecular sieves at room temperature, and the reaction was stirred for 1 h. The reaction mixture was cooled to –40°C and then NIS (1.2 equiv) and TfOH (0.2 equiv) were added. The reaction mixture was warmed to –20°C for 2 h, then quenched with saturated aqueous (sat. aq.) $NaHCO_3$ and sat. aq. $Na_2S_2O_3$, diluted with CH_2Cl_2, and filtered through Celite. The organic layer was washed with sat. aq. $NaHCO_3$, sat. aq. $Na_2S_2O_3$, and brine, and then dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography on silica gel (Hex/EtOAc = 4:1 to 1:1) to give the product (α isomer 254.0 mg, 39%, β isomer 126.1 mg, 20%). α-isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51–6.95 (m, 35H), 5.68 (d, 1H, *J* = 3.65 Hz), 5.07 (s, 2H), 4.94 (d, 1H, *J* = 11.35 Hz), 4.86–4.74 (m, 4H), 4.66–4.37 (m, 9H), 4.21 (dd, 1H, *J* = 9.55 Hz, 8.05 Hz), 4.03 (dd, 1H, *J* = 9.9Hz, 3.65 Hz), 3.98–3.91 (m, 2H), 3.84 (dt, 1H, *J* = 6.6 Hz, 8.8 Hz), 3.72 (dd, 1H, *J* = 9.9 Hz, 2.2 Hz), 3.66 (s, 1H), 3.62–3.54 (m, 3H), 3.39 (dt, 1H, *J* = 6.6 Hz, 8.8 Hz), 3.12 (q, 2H, *J* = 6.6 Hz), 1.61–1.37 (m, 4H), 1.32–1.22 (m, 2H), 1.11 (d, 3H, *J* = 6.75Hz). ^{13}C-APT NMR (125 MHz, $CDCl_3$) δ 156.3, 138.9, 138.7, 138.3, 138.2, 137.8, 136.5, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.87, 127.8, 127.7, 127.5, 127.3, 127.29, 127.2, 127.17, 126.2, 102.0, 97.1, 84.3, 79.5, 77.9, 75.6, 74.6, 74.3, 73.5, 73.2, 72.9, 72.5, 72.2, 71.9, 71.2, 69.2, 68.8, 66.5, 66.1, 40.9, 29.7, 29.3, 23.2, 16.5. HRMS: $C_{67}H_{75}NO_{12}Na$ $[M+Na]^+$ calculated: 1,108.5181; found: 1,108.5171.

Procedure for Deprotection of the Disaccharide 18 (1a). Fully protected disaccharide **18** (190 mg) was dissolved in 5% formic acid in MeOH (3 ml), and Pd black (150 mg) was added. The flask was purged three times with H_2, and then stirred under an atmosphere of H_2 overnight. The reaction was neutralized with NH_4OH, filtered through Celite, and concentrated. The product was purified by column chromatography (LiChroprep R18, water to 10% MeOH) to give a white solid **1a** (42.3 mg, 59%). ^{1}H NMR (500 MHz, D_2O) δ 5.12 (d, 1H, *J* = 4.05 Hz), 4.37 (d, 1H, *J* = 8.05 Hz), 4.20 (q, 1H, *J* = 6.6 Hz), 3.87–3.77 (m, 2H), 3.76–3.68 (m, 2H), 3.67–3.52 (m, 6H), 3.46 (dd, 1H, *J* = 8.1 Hz, 9.5 Hz), 2.88 (t, 2H, *J* = 7.7 Hz), 1.62–1.51 (m, 4H), 1.37–1.28 (m, 2H), 1.09 (d, 3H, *J* = 6.6 Hz). ^{13}C-APT NMR (125 MHz, D_2O) δ 102.2, 100.1, 77.5, 74.4, 72.5, 70.7, 70.2, 69.6, 69.0, 67.5, 61.6, 40.0, 29.1, 27.2, 22.9, 16.1. HRMS: $C_{17}H_{33}NO_{10}Na$ $[M+Na]^+$ calculated: 434.1997; found: 434.1988.

General Procedure for Deprotection of Trisaccharide (2a), Tetrasaccharide (3a), and Globo H (4a). Fully protected oligosaccharide was dissolved in acetic acid. Nanosize activated Zn powder (Aldrich) was added, and the reaction was stirred vigorously for 1 h. The reaction mixture was filtered and the filtrate was concentrated to residue, which was dissolved in pyridine and acetic anhydride and a catalytic amount of 4-dimethylaminopyridine. After stirring overnight, the reaction was quenched with MeOH and the solvent was removed. The residue was dissolved in CH_2Cl_2 and washed with 2% HCl, sat. aq. $NaHCO_3$, and brine. After removal of solvent, the crude material was dissolved in MeOH (2 ml) and CH_2Cl_2 (2 ml). NaOMe solution was then added, and the reaction mixture was stirred for 2 h, then neutralized with DOWEX 50WX2-200 and filtered, and solvent was removed. The material was dissolved in 5% formic acid in MeOH, and Pd black was added. The flask was purged three times with H_2, and then stirred under an atmosphere of H_2 overnight. The mixture was neutralized with NH_4OH, filtered through Celite, and concentrated. The product was purified by column chromatography (LiChroprep R18, water to 10% MeOH) to give the product as a white solid.

Compound 2a. ^{1}H NMR (500 MHz, D_2O) δ 5.14 (d, 1H, *J* = 4.05 Hz), 4.51 (d, 1H, *J* = 7.7 Hz), 4.22 (d, 1H, *J* = 8.1 Hz), 4.13 (q, 1H, *J* = 6.6 Hz), 4.01 (d, 1H, *J* = 2.6 Hz), 3.90–3.78 (m, 4H), 3.76–3.59 (m, 8H), 3.58–3.50 (m, 3H), 3.47–3.41 (m, 1H), 2.89 (t, 2H, *J* = 7.5 Hz), 1.94 (s, 3H), 1.61–1.52 (m, 2H), 1.51–1.42 (m, 2H), 1.35–1.22 (m, 2H), 1.11 (d, 3H, *J* = 6.6 Hz). ^{13}C-APT NMR (125 MHz, D_2O) δ174.3, 103.4, 102.8, 99.8, 77.4, 76.6, 75.7, 75.5, 74.2, 72.5, 70.7, 70.2, 69.8, 69.2, 68.7, 67.5, 61.7, 61.6, 52.1, 40.0, 28.8, 27.0, 22.9, 22.8, 15.9. HRMS: $C_{25}H_{47}N_2O_{15}$ $[M+H]^+$ calculated: 615.2971; found: 615.2976.

Compound 3a. ^{1}H NMR (600 MHz, D_2O) δ 5.08 (d, 1H, *J* = 3.96 Hz), 4.73 (d, 1H, *J* = 3.96 Hz), 4.46 (d, 1H, *J* = 7.44 Hz), 4.39 (d, 1H, *J* = 7.44 Hz), 4.08 (q, 1H, *J* = 6.54 Hz), 4.03 (d, 1H, *J* = 2.7 Hz), 3.95 (s, 1H), 3.84–3.72 (m, 5H), 3.70–3.53 (m, 12H), 3.52–3.46 (m, 3H), 3.39–3.35 (m, 1H), 2.85 (t, 2H, *J* = 7.5 Hz), 1.89 (s, 3H), 1.58–1.47 (m, 4H), 1.38–1.28 (m, 2H), 1.06 (d, 3H, *J* = 6.6 Hz). ^{13}C-APT NMR (150 MHz, D_2O) δ175.02, 104.6, 102.7, 99.9, 99.1, 79.3, 77.0, 76.8, 75.7, 75.3, 74.2, 72.5, 72.2, 71.0, 70.2, 69.7, 69.1, 68.7, 68.4, 68.3, 67.4, 61.8, 61.6, 52.3, 40.0, 28.7, 27.1, 23.0, 22.9, 16.0. HRMS: $C_{31}H_{56}N_2O_{20}Na$ $[M+Na]^+$ calculated: 799.3318; found: 799.3323.

Compound 4a. ^{1}H NMR (500 MHz, D_2O) δ 5.22 (d, 1H, *J* = 4.04 Hz), 4.87 (d, 1H, *J* = 4.03 Hz), 4.59 (d, 1H, *J* = 7.71 Hz), 4.52 (d, 1H, *J* = 7.70 Hz), 4.49 (d, 1H, *J* = 7.70 Hz), 4.46 (d, 1H, *J* = 7.07 Hz), 4.37 (t, 1H, *J* = 6.4 Hz), 4.24–4.18 (m, 2H), 4.08 (d, 1H, *J* = 1.83 Hz), 4.01 (d, 1H, 3.3 Hz), 3.99–3.53 (m, 33H), 3.28 (t, 1H, *J* = 8.5 Hz), 2.98 (t, 2H, *J* = 7.52 Hz), 2.02 (s, 3H), 1.71–1.60 (m, 4H), 1.47–1.40 (m, 2H), 1.19 (d, 3H, *J* = 6.6 Hz,). ^{13}C-APT NMR (125 MHz, D_2O) δ 175.9, 105.6, 105.0, 103.7, 103.6, 102.1, 100.9, 80.4, 79.9, 78.8, 78.0, 77.4, 77.1, 76.7, 76.4, 76.3, 76.2, 75.2, 74.6, 73.7, 73.5, 72.5, 71.8, 71.7, 71.14, 71.1, 70.8, 70.7, 70.1, 69.7, 69.5, 68.4, 62.6, 62.59, 62.0, 61.7, 53.3, 41.0, 29.8, 28.1, 23.9, 23.7, 17.0 MALDI-Fourier transform (FT)-MS calculated for $C_{43}H_{76}N_2O_{30}$ $[M+Na]^+$ 1,101.4555; found 1,101.4525.

Compound 1b. ^{1}H NMR (500 MHz, D_2O) δ 5.12 (d, 1H, *J* = 3.65 Hz), 4.35 (d, 1H, *J* = 8.1 Hz), 4.21 (q, 1H, *J* = 6.6 Hz), 3.82–3.51 (m, 10H), 3.45 (dd, 1H, *J* = 9.55 Hz, 8.05 Hz), 3.20 (t, 2H, *J* = 6.75 Hz), 1.58–1.45 (m, 4H), 1.35–1.24 (m, 2H), 1.08 (d, 3H, *J* = 6.6

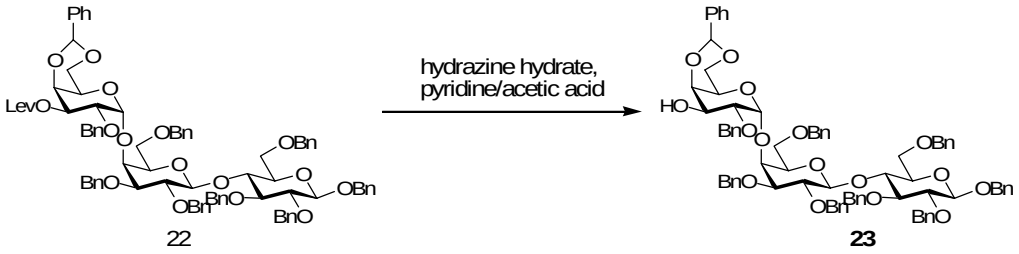
Hz). ^{13}C-APT NMR (125 MHz, D_2O) δ 102.1, 100.0, 77.2, 75.6, 74.5, 72.5, 70.9, 70.2, 69.6, 69.0, 67.4, 61.6, 51.7, 29.2, 28.5, 23.3, 16.1. HRMS: $C_{17}H_{31}N_3O_{10}Na$ $[M+Na]^+$ calculated: 460.1902; found: 460.1899.

Compound 2b. ^{1}H NMR (600 MHz, D_2O) δ 5.10 (d, 1H *J* = 3.48 Hz), 4.47 (d, 1H, *J* = 7.44 Hz), 4.18 (d, 1H, *J* = 7.86 Hz), 4.10 (q, 1H, *J* = 6.6 Hz), 3.97 (d, 1H, *J* = 1.74 Hz), 3.87–3.73 (m, 4H), 3.72–3.55 (m, 8H), 3.54–3.47 (m, 3H), 3.42–3.37 (m, 1H), 3.23–3.15 (m, 2H), 1.91 (s, 3H), 1.50–1.39 (m, 4H), 1.28–1.19 (m, 2H), 1.08 (d, 3H, *J* = 6.6 Hz). ^{13}C-APT NMR (150 MHz, D_2O) δ 174.3, 103.4, 102.8, 99.8, 77.4, 76.6, 75.7, 75.5, 74.2, 72.5, 70.7, 70.2, 69.8, 69.2, 68.7, 67.5, 61.7, 61.6, 52.1, 40.0, 28.8, 27.0, 22.9, 22.8, 15.9. HRMS: $C_{25}H_{44}N_4O_{15}Na$ $[M+Na]^+$ calculated: 663.2695; found: 663.2689.

Compound 3b. ^{1}H NMR (600MHz, D_2O) δ 5.09 (d, 1H, *J* = 3.5 Hz), 4.74 (d, 1H, *J* = 4.0 Hz), 4.47 (d, 1H, *J* = 7.4 Hz), 4.40 (d, 1H, *J* = 7.4 Hz), 4.09 (q, 1H, *J* = 6.54 Hz), 4.05 (d, 1H, *J* = 2.22 Hz), 3.96 (s, 1H), 3.87–3.73 (m, 5H), 3.71–3.54 (m, 12H), 3.53–3.47 (m, 3H), 3.41–3.36 (m, 1H), 3.20 (dt, 2H, *J* = 7.02 Hz, 6.12 Hz), 1.90 (s, 3H), 1.58–1.47 (m, 4H), 1.37–1.28 (m, 2H), 1.08 (d, 3H, *J* = 6.6 Hz). ^{13}C-APT NMR (150 MHz, D_2O) δ 175.1, 104.6, 102.7, 100.0, 99.1, 79.4, 77.1, 76.8, 75.8, 75.3, 74.2, 72.9, 72.5, 71.1, 70.20, 70.19, 69.8, 69.2, 68.6, 68.3, 67.5, 61.8, 61.7, 52.4, 51.7, 28.8, 28.5, 23.4, 22.9, 16.0. HRMS: $C_{31}H_{54}N_4O_{20}Na$ $[M+Na]^+$ calculated: 825.3223; found: 825.3232.

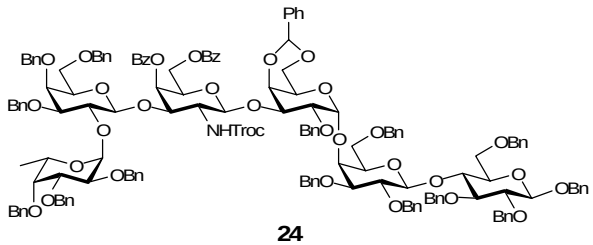
Compound 4b. ^{1}H NMR (600 MHz, D_2O) δ 5.09 (d, 1H, *J* = 3.96 Hz), 4.75 (d, 1H, *J* = 3.96 Hz), 4.48 (d, 1H, *J* = 7.86 Hz), 4.40 (d, 1H, *J* = 7.44 Hz), 4.37 (d, 1H, *J* = 7.44 Hz), 4.34 (d, 1H, *J* = 8.28 Hz), 4.26 (t, 1H, *J* = 6.6 Hz), 4.14–4.07 (m, 2H), 3.97 (br, s, 1H), 3.89 (d, 1H, *J* = 3.06 Hz), 3.87–3.74 (m, 7H), 3.73–3.43 (m, 24H), 3.20 (t, 2H, *J* = 6.96 Hz), 3.16 (t, 2H, *J* = 8.34 Hz), 1.90 (s, 3H), 1.59–1.45 (m, 4H), 1.36–1.24 (m, 2H), 1.08 (d, 3H, *J* = 6.6Hz). ^{13}C-APT NMR (150 MHz, D_2O) δ175.1, 104.8, 104.1, 102.82, 102.80, 101.2, 100.1, 79.5, 79.1, 77.9, 77.1, 76.9, 76.3, 75.9, 75.6, 75.4, 75.3, 74.4, 73.8, 72.9, 72.7, 71.6, 70.9, 70.3, 70.0, 69.9, 68.8, 68.6, 67.6, 62.2, 61.8, 61.1, 52.4, 51.9, 50.5, 29.1, 28.5, 23.4, 22.8, 16.1. HRMS: $C_{43}H_{74}N_4O_{30}Na$ $[M+Na]^+$ calculated: 1,149.4280; found: 1,149.4215.

Globo H C5 Biotin Conjugate (7). Deprotected Globo H (**4a**, 7.8 mg, 0.007 mmol) was dissolved in 25 mM $NaHCO_3$ (1 ml). Sulfo-NHS Biotin was added (9.4 mg, 3 equiv), and the reaction was shaken overnight at room temperature. Purification by Sep-Pak C18 chromatography ($MeOH/H_2O$ gradient, 0–50%) and lyophilization gave a fluffy white solid. Yield 7.7 mg (0.006 mmol, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.06 (d, *J* = 4.04 Hz, 1H), 4.72 (d, *J* = 4.03 Hz, 1H), 4.44 (m, 2H), 4.35 (m, 2H), 4.30 (d, *J* = 8.07 Hz, 1H), 4.25 (m, 1H), 4.22 (t, *J* = 6.97 Hz, 6.6), 4.07 (m, 2H), 3.93 (d, *J* = 2.56 Hz, 1H), 3.86 (d, *J* = 3.3 Hz, 1H), 3.4–3.8 (m, 33 H), 3.17 (m, 1H), 3.12 (t, *J* = 8.8, 8.44 Hz, 1H), 3.02 (m, 2H), 2.82 (dd, *J* = 4.77, 5.14 Hz, 1H), 2.6 (d, *J* = 12.84 Hz, 1H), 2.07 (m, 1H), 1.87 (s, 3H), 1.34–1.6 (m, 9H), 1.2–1.26 (m, 4H), 1.04 (d, *J* = 6.6 Hz, 3H) ^{13}C-APT NMR (125 MHz, $CDCl_3$) δ 177.03, 174.66, 104.98, 104.37, 102.42, 100.80, 99.65, 92.41, 88.65, 79.14, 78.65, 77.50, 76.72, 76.48, 75.85, 75.43, 75.16, 74.99, 74.90, 73.93, 73.33, 72.45, 72.21, 71.21, 70.79, 70.49, 69.87, 69.56, 69.48, 68.84, 68.39, 68.20, 62.44, 61.35, 60.71, 60.61, 55.75, 54.46, 52.00, 40.06, 39.49, 35.87, 28.75, 28.40, 28.18, 28.02, 25.54, 22.82, 22.61, 15.68. MALDI-FTMS calculated for $C_{53}H_{90}N_4O_{32}S$ $[M+Na]^+$ 1,349.5151, found 1,349.5141.



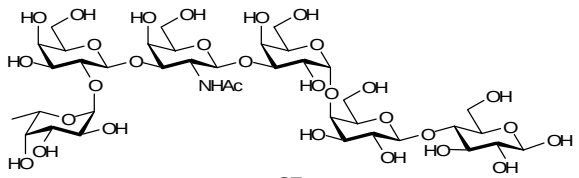
Trisaccharide Building Block Anomeric Benzyl (23). This compound was prepared from **22** (491 mg, 0.35 mmol) following the same procedures are reported for **12**. Chromatography 1:1:0.1 to 1:1:0.3 hexanes/CH_2Cl_2/EtOAc. Yield 428 mg (0.33 mmol, 94%) as white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.1–7.4 (m, 45 H), 5.36 (s, 1H), 5.17 (d, *J* = 2.94 Hz, 1H), 5.05 (d, *J* = 11.74 Hz, 1H), 4.94 (d, *J* = 12.1 Hz, 1H), 4.89 (d, *J* = 10.64 Hz), 4.84 (d, *J* = 11.37 Hz, 1H), 4.75–4.80 (m, 3H), 4.63–4.72 (m, 4H), 4.56–4.60

(m, 2H), 4.48 (d, *J* = 7.7 Hz, 1H), 4.40 (d, *J* = 12.1 Hz, 1H), 4.29 (d, *J* = 12.11 Hz, 1H), 4.24 (d, *J* = 11.74 Hz, 1H), 4.16 (m, *J* = 3.3, 1.83 Hz, 1H), 4.07–4.18 (m, 4H), 4.04–3.97 (m, 2H), 3.82–3.87 (ddd, *J* = 4.4, 3.3 Hz, 2H), 3.75 (dd, *J* = 9.17 Hz, 1H), 3.28–3.62 (m, 9H), 2.13 (d, *J* = 7.71 Hz, 1H). ^{13}C-APT NMR (125 MHz, $CDCl_3$) δ 139.27, 138.48, 138.33, 138.18, 138.14, 137.66, 137.46, 128.87, 128.34, 128.31, 128.27, 128.17, 128.15, 128.12, 128.03, 128.00, 127.96, 127.89, 127.80, 127.59, 127.56, 127.53, 127.48, 127.46, 127.45, 127.41, 127.39, 127.37, 127.03, 126.16, 102.81, 102.36, 100.76, 100.17, 82.59, 81.66, 81.17, 78.59, 77.07, 76.70, 76.33, 75.03, 74.88, 74.77, 73.99, 73.31, 73.04, 72.80, 72.03, 70.83, 69.05, 68.59, 68.30, 67.10, 62.70, 60.26.



24

One-Pot Synthesis of Protected Globo H (24). Fucosyl donor **10** (211 mg, 1.2 equiv), disaccharide building block **11** (360 mg, 0.326 mmol, 1 equiv), and molecular sieves were stirred in CH_2Cl_2 (10 ml) for 1 h at room temperature. Reaction was cooled to –50°C, and NIS (88 mg, 1.2 equiv) was added, followed by TfOH (1M solution in ether, 0.098 ml, 0.3 equiv). The mixture was stirred for 2 h at –40°C to –50°C, and the completion of the reaction was checked by TLC. Trisaccharide **23** (428 mg, 1.0 equiv) is dissolved in CH_2Cl_2 (5 ml) and added to the reaction mixture. NIS (88 mg, 1.2 equiv) is added, followed by TfOH (1 M solution in ether, 0.030 ml, 0.1 equiv). The reaction was stirred at –30°C for 2 h and then quenched with a few drops of triethylamine. The reaction mixture was then diluted with CH_2Cl_2, washed with sat. aq. $Na_2S_2O_3$ (two times) and with brine (one time), and then dried over Na_2SO_4. Purification by column chromatography (3:1:0.25 to 3:1:1 hexanes/CH_2Cl_2/EtOAc) provided **24** (643 mg, 0.238 mmol, 73%) as a white foam.



25

Deprotected Globo H Anomeric Hydroxyl (25). Protected Globo H-anomeric benzyl **24** (150 mg, 0.056 mmol) was dissolved in acetic acid (5 ml). Nanosize activated Zn powder (Aldrich, 600 mg) was added, and reaction was stirred vigorously for 1 h. The reaction was filtered and the solvent was removed. The crude residue was then dissolved in pyridine (4 ml) and acetic anhydride (2 ml) and a catalytic amount of 4-dimethylaminopyridine was added. After stirring overnight, the reaction was quenched with methanol (5 ml) and the solvent was removed. The residue was dissolved in CH_2Cl_2, washed with 2% HCl, sat. aq. $NaHCO_3$, and brine. After removal of solvent, the crude material was then dissolved in methanol (2 ml) and CH_2Cl_2 (2 ml). Freshly prepared NaOMe solution (2 ml) was then added, and the reaction was stirred for 2 h. The reaction was neutralized with DOWEX 50WX2-200 and filtered, and solvent was removed. The material was then dissolved in 5% formic acid in methanol (5 ml), and Pd black (40 mg) was added. The flask was purged three times with hydrogen, and then stirred under an atmosphere of hydrogen overnight. By TLC (3:1:0.1 MeOH/H_2O/NH_4OH) the starting material was consumed. The reaction was neutralized with NH_4OH, filtered through celite, and concentrated. The product was purified by column chromatography (LiChroprep R18, water) to give **25** as a white solid. Yield 17 mg (0.017 mmol, 30% over four steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.08 (d, *J* = 4.03 Hz, 1H), 4.75 (d, *J* = 4.03 Hz, 1H), 4.52 (d, *J* = 8.07 Hz, 1H), 4.47 (d, *J* = 7.71 Hz, 1H), 4.40 (d, *J* = 7.70 Hz, 1H), 4.37 (d, *J* = 8.07 Hz, 1H), 4.25 (t, *J* = 6.24, 6.6, 1H), 4.09 (m, 2H), 3.96 (d, *J* = 2.2 Hz, 1H), 3.89 (d, 2.94 Hz, 1H). ^{13}C-APT NMR (125 MHz, $CDCl_3$) δ 174.69, 104.39, 103.69, 102.45, 100.82, 99.68, 96.11, 79.27, 79.11, 78.76, 78.72, 77.56, 76.78, 76.52, 75.89, 75.47, 75.23, 75.02, 74.85, 74.32, 73.98, 72.51, 72.25, 71.88, 71.61, 71.27, 70.55, 69.92, 69.59, 69.51, 68.89.68.44, 68.23, 67.19, 52.03, 22.66, 15.72. MALDI-FTMS calculated for $C_{38}H_{65}NO_{30}$ $[M+Na]^+$ 1,038.3483, found 1,038.3483.

Globo H-Biotin Conjugate (8). Deprotected Globo H with anomeric hydroxyl group (17 mg, 0.017 mmol) was dissolved in sat. aq. NH_4HCO_3 (8 ml). The reaction was stirred for 3 days and monitored by TLC (4:1 MeOH/H_2O). The reaction was lyophilized repeatedly over a period of 1 week until the mass remained constant. Product was used directly in the next reaction. Yield 17 mg (100%). This Globo H amine (14 mg, 0.014 mmol) was used to prepare **8** as was described for Globo H C5 biotin conjugate **7**. Yield 9 mg (0.007 mmol, 50%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.13 (d, *J* = 4.03 Hz, 1H), 4.89 (d, *J* = 11.54 Hz, 1H), 4.79 (d, *J* = 4.03 Hz, 1H), 4.51 (m, 2H), 4.44 (m, 2H), 4.33 (m, 2H), 4.15 (m, 2H), 4.01 (d, *J* = 2.2 Hz, 1H), 3.93 (d, *J* = 2.94 Hz, 1H), 3.49–3.9 (m, 30 H), 3.34 (t, *J* = 9.17, 1H), 3.25 (m, *J* = 4.4, %.87 Hz, 1H), 2.90 (dd, *J* = 4.76, 5.14, 13.21, 12.83 Hz, 1H), 2.68 (d, *J* = 12.84 Hz, 1H), 2.25 (t, *J* = 6.97, 7.34 Hz, 2H), 1.95 (s, 3H), 1.47–1.66 (m, 6H), 1.32–1.38 (m, 2H), 1.12 (d, *J* = 6.6 Hz, 3H) ^{13}C-APT NMR (125 MHz, $CDCl_3$) δ 179.58, 175.58, 105.29, 104.55, 103.33, 101.69, 100.56, 80.31, 79.62, 79.49, 78.34, 77.71, 77.64, 77.40, 76.79, 76.48, 76.34, 75.90, 74.85, 73.34, 73.13, 72.82, 72.11, 71.39, 70.79, 70.47, 70.39, 69.76, 69.31, 69.10, 68.07, 63.26, 62.25, 61.62, 61.54, 61.13, 56.52, 52.91, 40.96, 36.71, 29.04, 28.84, 26.05, 23.52, 16.59. MALDI-FTMS calculated for $C_{48}H_{80}N_4O_{31}S$ $[M+Na]^+$ 1,263.4419, found 1,263.4410.

Globo H-Fluorescein Conjugate (9). Fluorescein dialkyne (46 mg, 10 equiv) was dissolved in tetrahydrofuran (THF) (1 ml), followed by *t*-BuOH (1 ml). Globo H azide **4b** (8.6 mg, 0.0076 mmol) was dissolved in H_2O (0.8 ml) and added to the solution of dialkyne. Sodium ascorbate (0.1 ml of 30 mg/ml aq. solution, 0.1 equiv of dialkyne) and $CuSO_4$ (0.1 ml of a 12 mg/ml aq. solution, 0.2 equiv of dialkyne) were added, and the reaction was allowed to stir for 24 h. The THF was removed by evaporation, and the excess fluorescein dialkyne was removed by filtration through Celite. Purification by size exclusion chromatography (Biogel P-2) used 10 mM NH_4HCO_3 as eluent. Yield 12 mg (0.0071 mmol, 93%). ESI-TOF calculated for $C_{76}H_{95}N_5O_{38}$ $[M+Na]^+$ 1,708.5547, found 1,708.5518.