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Synthesis of Thioether-Linked Analogs of the 2,3-STF and MECA-79 Antigens: Mucin-Type Oligosaccharides Associated with Cancer and Inflammation

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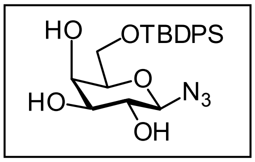
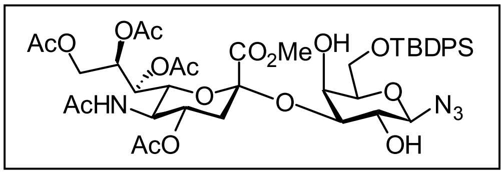
Materials and Methods. *N*$^{\alpha}$-Fmoc-amino acids, MBHA rink amide resin, HBTU, HOBt and DCC were purchased from Novabiochem. All other chemical reagents were obtained from commercial suppliers and used without further purification. The following solvents were distilled under a nitrogen atmosphere prior to use: THF was dried and deoxygenated over Na and benzophenone; CH_2Cl_2 and CH_3CN were dried over CaH_2; methanol was dried over Mg and I_2. Unless otherwise noted, all air and moisture-sensitive reactions were performed under an argon or nitrogen atmosphere. Analytical thin-layer chromatography (TLC) was conducted on Analtech Uniplate silica gel plates with detection by ceric ammonium molybdate (CAM), sulfuric acid/MeOH or UV light. For flash chromatography, 60 Å silical gel (Bodman) was employed. Reversed-phase high-pressure liquid chromatography (RP-HPLC) was performed on a Rainin Dynamax SD-200 HPLC system using Microsorb and Dynamax C_{18} reversed-phase columns (analytical: 4.6 x 250 mm, 1 mL/min; preparative: 25 x 250 mm, 20 mL/min) and UV detection was performed with a Rainin Dynamax UV-1 detector.

Infrared spectra were recorded on a Perkin-Elmer FT-IR 1600 spectrometer. ^{1}H NMR spectra were obtained at 500 MHz with a Bruker DRX 500 spectrometer. Chemical shifts are reported in parts per million (δ) relative to $CHCl_3$ (7.26 ppm) for spectra acquired in $CDCl_3$. ^{13}C NMR spectra were obtained at 125 MHz on a Bruker DRX 500 spectrometer. High-resolution FAB mass spectra were recorded at the Mass Spectrometry Facility at the University of California at Berkeley. Electrospray ionization mass spectrometry (ESI-MS) was performed on a Hewlett-Packard 1100 mass spectrometer.

6-*O*-(*Tert*-butyldiphenylsilyl)-β-D-galactosyl azide (5). To the known galactosyl azide **6** (8.40 g, 22.5 mmol) in MeOH (200 mL) was added NaOMe (4 mL, 1 M solution in MeOH). The reaction mixture was stirred at rt for 3 h, neutralized with Amberlite IRC-50® (H^+) ion-exchange resin, filtered and concentrated. After drying under high vacuum for 12 h, the crude product was dissolved in anhydrous DMF (60 mL) and cooled to 0 °C. Imidazole (3.20 g, 47.3 mmol) was added followed by *t*-Bu(Ph)$_2$SiCl (6.10 mL, 23.6 mmol). After stirring for 6 h, the reaction mixture was concentrated under high vacuum and the resulting product was purified by silica gel chromatography (EtOAc) to afford 8.9 g (89%) of **5** as a white foam: IR (film) 3387, 3071, 3050, 2930, 2887, 2857, 2358, 2342, 2118, 1651, 1589, 1471, 1428, 1248, 1113 cm^{-1}; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.68 (m, 4 H), 7.40 (m, 6 H), 4.48 (d, 1 H, *J* = 8.5 Hz), 4.08 (app d, 1 H, *J* = 2.5 Hz), 4.01 (br s, 3 H), 3.92 (m, 2 H), 3.66 (app t, 1 H, *J* =), 3.54 (m, 2 H), 1.05 (s, 9 H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 135.6, 135.5, 132.8, 132.6, 129.9, 128.3, 127.8, 90.5, 76.1, 73.8, 71.1, 69.2, 63.3, 26.7, 19.1; HRMS (FAB) calcd for $C_{22}H_{29}N_3O_5SiLi$ $[M + Li]^+$ 450.2037, found 450.2034.

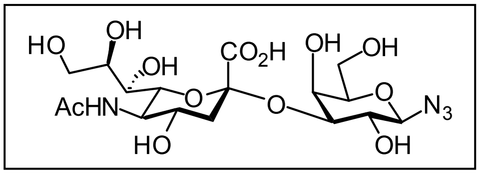


Compound 8. Phosphite donor **7** (855 mg, 1.16 mmol) and triol **5** (619 mg, 1.39 mmol) were combined, azeotroped with anhydrous



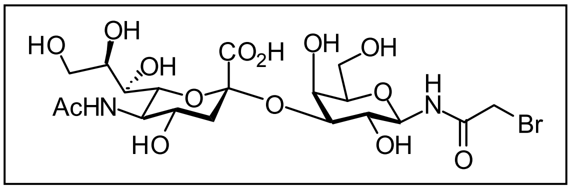
toluene, and placed under high vacuum for 2 h. The mixture was dissolved in anhydrous CH_3CN (25 mL) and freshly activated 3 Å molecular sieves were added. The mixture was cooled to –40 °C and stirred for ~15 min before the addition of TMSOTf (32 µL, 0.18 mmol). After being stirred for 1 h at -40 °C, the reaction mixture was quenched by the addition of saturated aqueous $NaHCO_3$ and warmed to rt. The reaction mixture was then added to EtOAc and the solution was washed with saturated aqueous $NaHCO_3$. The organic layer was dried (Na_2SO_4) and concentrated. The resultant material was purified by silica gel chromatography (hexanes/acetone, 2:1) to yield 468 mg (44%) of disaccharide **8** as a white foam: IR (film) 3501, 3316, 3072, 2957, 2932, 2887, 2858, 2117, 1745, 1682, 1651, 1548, 1428, 1370, 1223, 1113, 1084, 1039 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.68 (m, 4 H), 7.42 (m, 6 H), 5.45 (m, 1 H), 5.33 (d, 1 H, *J* = 9.0 Hz), 5.25 (d, 1 H, *J* = 9.0 Hz), 4.97 (m, 1 H), 4.68 (d, 1 H, *J* = 8.5 Hz), 4.28 (d, 1 H, *J* = 12.5 Hz), 4.13 (m, 3 H), 4.08 (dd, 1 H, *J* = 12.5, 5.5 Hz), 3.99 (m, 2 H), 3.87 (m, 1 H), 3.78 (s, 3 H), 3.72 (app t, 1 H, *J* = 6.5 Hz), 3.66 (m, 1 H), 2.99 (br s, 1 H), 2.90 (br s, 1 H), 2.78 (dd, 1 H, *J* = 13.0, 3.5 Hz), 2.18 (app s, 6 H), 2.05 (app s, 6 H), 1.90 (s, 3 H), 1.05 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 170.5, 170.3, 170.1, 169.9, 168.1, 135.6, 135.5, 132.8, 132.5, 129.9, 127.8, 127.7, 97.2, 90.2, 75.3, 72.6, 68.7, 68.3, 68.1, 67.9, 66.7, 62.9, 62.3, 53.2, 49.6, 38.0, 26.7, 23.2, 21.3, 20.8, 20.7, 19.1; HRMS (FAB) calcd for $C_{42}H_{56}N_4O_{17}Li$ $[M + Li]^+$ 923.3570, found 923.3594.

Compound 9. To a solution of disaccharide **8** (237 mg, 0.260 mmol) in THF (8 mL) at 0 °C were added AcOH (35 µL, 0.60 mmol) and



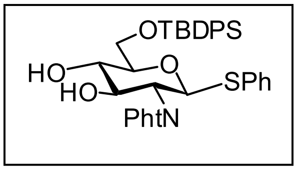
TBAF (1 M in THF, 600 μL). The reaction mixture was allowed to warm to rt overnight and then concentrated under high vacuum. The resultant product was purified by silica gel chromatography ($CHCl_3$/MeOH, 10:1) and then treated with 2% NaOMe in MeOH (10 mL). After 24 h, H_2O (2 mL) and THF (2 mL) were added. After stirring for an additional 48 h, the reaction was cooled to 0 °C, neutralized with Amberlite IRC-50® (H^+) ion-exchange resin, filtered and concentrated to afford 114 mg of (92%) of **9** as a white solid: ^1H NMR (500 MHz, D_2O) δ 4.70 (d, 1 H, *J* = 9.0 Hz), 4.09 (dd, 1 H, *J* = 9.5, 3.0 Hz), 3.94 (d, 1 H, *J* = 3.0 Hz), 3.82 (m, 3 H), 3.73 (m, 3 H), 3.63 (m, 3 H), 3.55 (m, 2 H), 3.51 (app t, 1 H, *J* = 9.5 Hz), 2.72 (dd, 1 H, *J* = 12.5, 5.0 Hz), 1.99 (s, 3 H), 1.76 (app t, 1 H, *J* = 12.0 Hz); ^{13}C NMR (125 MHz, D_2O) δ 174.9, 173.8, 99.8, 90.2, 76.9, 75.6, 72.8, 71.7, 68.7, 68.3, 68.0, 67.4, 62.5, 60.9, 51.6, 39.5, 22.0; HRMS (ESI) calcd for $C_{17}H_{27}N_4O_{13}$ $[M - H]^-$ 495.1575, found 495.1568.

Compound 2. Compound **9** (247 mg, 0.480 mmol) was dissolved in anhydrous MeOH (10 mL) and stirred in the presence of 10% Pd/C (80 mg) under an atmosphere of H_2. After 30 min the reaction mixture was filtered through Celite and then concentrated *in vacuo*. The crude product was dissolved in 1 M $NaHCO_3$ (5 mL) and bromoacetic anhydride (620 mg, 2.4 mmol) was added. After stirring for 4 h at rt, the mixture was loaded onto a size exclusion column (Bio-Gel P-2) and eluted with water. Fractions containing the desired product were combined and lyophilized to give 187 mg (66%) of **2** as a white solid: ^1H NMR (500 MHz, D_2O) δ 5.01 (d, 1 H, *J* = 9.0 Hz), 4.15 (m, 2 H), 3.97 (m, 1 H), 3.82 (m, 4 H), 3.78 (m, 2 H), 3.67 (m,

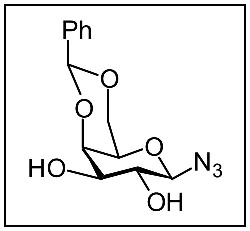


3 H), 3.60 (m, 3.0), 2.75 (dd, 1 H, J = 7.5, 4.5 Hz), 2.02 (s, 3 H), 1.81 (app t, 1 H, J = 12.0 Hz); ^{13}C NMR (125 MHz, D_2O) δ 176.2, 174.9, 173.8, 171.0, 99.9, 79.2, 76.5, 76.2, 72.8, 71.6, 68.3, 68.0, 67.6, 67.5, 62.5, 60.8, 51.6, 39.4, 21.9; LRMS (ESI) calcd for $C_{19}H_{31}BrN_2O_{14}$ $[M - H]^-$ 590.4, found 591.1.

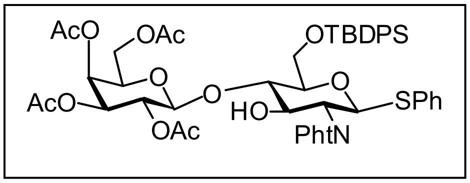
Phenyl 6-O-*t*-butyldiphenylsilyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (11). To a solution of NaOMe in MeOH (200 mL) at pH 12 was added **13** (2.25 g, 4.27 mmol). After stirring for 4 h, Amberlite IRC-50® (H^+) ion-exchange resin was added to the reaction mixture until a pH of 5 was obtained. The solution was then filtered and concentrated to afford an off-white solid. The solid was immediately dissolved in anhydrous DMF (100 mL) and imidazole (871 mg, 12.8 mmol) and *t*-butyldiphenylsilylchloride (1.22 mL, 4.69 mmol) were added. The reaction was stirred at rt for 16 h and then diluted with EtOAc (200 mL) and washed with aqueous $NaHCO_3$, H_2O, and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (20% EtOAc in hexanes) yielded 2.27 g (83%) of **11** as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (br s, 2 H), 7.73 (m, 5 H), 7.42 (m, 9 H), 7.20 (m, 3 H), 5.63 (d, 1 H J = 10.4 Hz), 4.38 (app t, 1 H, J = 8.6 Hz), 4.22 (app t, 1 H, J = 10.4 Hz), 4.07 (app t, 1 H, J = 6.7 Hz), 3.99 (dd, 1 H, J = 4.7, 1.2 Hz), 3.73 (app t, 1 H, J = 7.3 Hz), 3.64 (m, 1 H), 1.09 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.7, 135.6, 134.8, 132.2, 132.8, 132.7, 132.4, 132.1, 129.9, 128.8, 127.8, 127.8, 127.7, 127.7, 83.4, 78.9, 73.2, 72.8, 64.6, 55.3, 26.8, 19.2; FAB-HRMS calcd. for $C_{36}H_{37}NO_6SSiLi$ $[M + Li]^+$ 646.2284, found 646.2271.



4,6-*O*-Benzylidene-β-D-galactosyl azide (12). To β-galactosyl azide **6** (8.90 g, 24.0 mmol) in MeOH (200 mL) was added NaOMe (1 mL, 25% by wt in MeOH). After stirring for 12 h at rt, the mixture was neutralized with Amberlite IRC-50 (H^+) resin, filtered and concentrated. The crude product was dissolved in anhydrous CH_3CN (175 mL) and treated with camphor sulfonic acid (2.80 g, 12.0 mmol) and α,α-dimethoxytoluene (5.40 mL, 36.0 mmol) at rt overnight. The reaction was quenched with Et_3N (5 mL) and the solvents were removed *in vacuo*. The crude mixture was purified by flash chromatography (2:1 EtOAc/hexanes) to afford 4.5 g (64%) of **12** as a white solid: ^1H NMR (500 MHz, $CDCl_3$) δ 7.49 (m, 2 H), 7.38 (m, 5 H), 5.53 (s, 1 H), 4.54 (d, 1 H, *J* = 8.0 Hz), 4.35 (dd, 1 H, *J* = 12.5, 1.0 Hz), 4.18 (app d, 1 H, *J* = 3.5 Hz), 4.04 (dd, 1 H, *J* = 12.5, 1.5 Hz), 3.66 (m, 2 H), 3.53 (app s, 1 H), 2.86 (br s, 2 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.3, 129.4, 128.3, 126.3, 101.4, 90.2, 75.0, 72.8, 71.1, 68.8, 68.3; HRMS (FAB) calcd for $C_{13}H_{15}N_3O_5Li$ $[M + Li]^+$ 300.1172, found 300.1175.

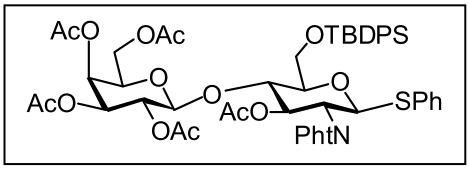


Phenyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1,4)-2-deoxy-2-phthalimido-6-*O*-(*t*ert-butyldiphenylsilyl)-1-thio-β-D-glucopyranoside (14). A mixture of donor **10** (515 mg, 1.37 mmol), acceptor **11** (730 mg, 1.14 mmol), $SnCl_2$ (389 mg, 2.05 mmol) and 4 Å MS (10 g) in 5:1 CH_2Cl_2/toluene (100 mL) was cooled to –10 °C. After 1 h, AgOTf (527 mg, 2.05 mmol) was added and the mixture was stirred



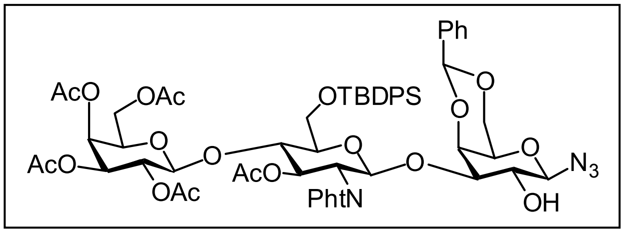
in the dark at –10 to 0 °C for 16 h. The reaction was quenched with Et_3N (1 mL), filtered through Celite and washed thoroughly with CH_2Cl_2. The filtrate was washed with $NaHCO_3$, H_2O and brine, dried ($MgSO_4$) and concentrated. The crude product was purified by silica gel chromatography (2:1 toluene/EtOAc) to give 756 mg (69%) of **14**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88 (m, 1 H), 7.83 (m, 1 H), 7.80 (m, 2 H), 7.75 (m, 4 H), 7.41 (m, 6 H), 7.35 (m, 2 H), 7.20 (m, 3 H), 5.65 (d, 1 H, *J* = 10.5 Hz), 5.34 (d, 1 H, *J* = 3.0 Hz), 5.20 (dd, 1 H, *J* = 10.5, 8.0 Hz), 4.96 (dd, 1 H, *J* = 10.0, 3.0 Hz), 4.71 (d, 1 H, *J* = 8.0 Hz), 4.47 (app t, 1 H, *J* = 9.0 Hz), 4.29 (app t, 1 H, *J* = 10.0 Hz), 4.09 (m, 2 H), 4.04 (d, 1 H, *J* = 9.0 Hz), 3.97 (m, 2 H), 3.84 (m, 2 H), 3.61 (d, 1 H, *J* = 8.5 Hz), 2.12 (s, 3 H), 1.98 (app s, 6 H), 1.69 (s, 3 H), 1.21 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 170.1, 169.9, 169.0, 168.1, 167.5, 135.9, 135.6, 134.1, 133.4, 132.6, 132.4, 132.2, 131.8, 131.7, 129.9, 129.8, 128.8, 127.9, 127.7, 127.6, 123.7, 123.2, 101.1, 83.5, 80.4, 78.5, 77.3, 71.2, 70.7, 70.5, 68.6, 66.8, 61.8, 61.1, 55.3, 26.9, 26.8, 20.5, 20.5, 20.4, 20.3, 19.4; LRMS (FAB) $C_{52}H_{57}NO_{16}SSiNa$ $[M + Na]^+$ 993.4.

Phenyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1,4)-3-*O*-acetyl-2-deoxy-2-phthalimido-6-*O*-(*t*ert-butyldiphenylsilyl)-1-thio-β-D-glucopyranoside (15).

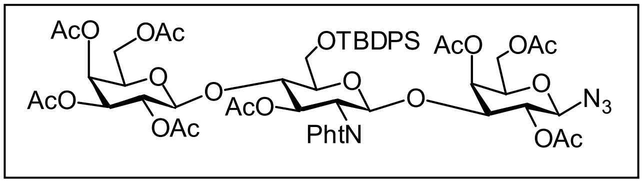


Disaccharide **14** (750 mg, 0.770 mmol) was dissolved in 1:1 Ac_2O/pyridine (20 mL) and stirred at rt overnight in the presence of a catalytic amount of DMAP (10 mg). The mixture was concentrated *in vacuo*, co-evaporated with toluene and purified by silica gel chromatography (1:1 hexanes/EtOAc) to give 674 mg (86%) of **15**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (m, 8 H), 7.40 (m, 8 H), 7.20 (m, 3 H), 5.73 (m, 2 H), 5.30 (d, 1 H, *J* = 3.0 Hz), 5.04 (dd, 1 H, *J* = 10.0, 8.0 Hz), 4.88 (dd, 1 H, *J* = 10.0, 3.5 Hz) 4.82 (d, 1 H, *J* = 8.0 Hz), 4.33 (app t, 1 H, *J* = 10.5 Hz), 4.22 (app t, 1 H, *J* = 9.5 Hz), 4.09 (m, 3 H), 3.95 (dd, 1 H, *J* = 11.5, 2.0 Hz), 3.75 (app t, 1 H, *J* = 6.5 Hz), 3.58 (d, 1 H, *J* = 10.0 Hz), 2.12 (s, 3 H), 2.09 (s, 3 H), 2.07 (s, 3 H), 1.98 (s, 3 H), 1.89 (s, 3 H), 1.79 (s, 3 H), 1.18 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 170.2, 170.1, 169.9, 168.7, 167.8, 167.2, 135.9, 135.4, 134.3, 134.1, 133.3, 132.7, 132.0, 131.8, 131.6, 131.2, 130.0, 129.9, 128.9, 128.0, 127.9, 127.8, 123.7, 123.6, 100.1, 83.1, 79.3, 74.3, 71.6, 71.1, 70.5, 69.1, 66.8, 61.2, 61.0, 53.9, 26.9, 20.6, 20.6, 20.5, 20.4, 19.4; HRMS (FAB) calcd for $C_{52}H_{58}NO_{16}SSi$ $[M + H]^+$ 1012.3246, found 1012.3258.

Compound 16. To a solution of **15** (150 mg, 0.148 mmol) and **12** (57.0 mg, 0.193 mmol) in freshly distilled CH_2Cl_2 (10 mL) was added 4 Å molecular sieves (100 mg). NIS (100 mg, 0.445 mmol) was then added at rt, and TfOH (4.00 μL, 0.044 mmol) was subsequently added dropwise over a period of 5 min. The reaction mixture was stirred for 20 min and then filtered through Celite. The filtrate was washed successively with aqueous $NaHCO_3$, aqueous $Na_2S_2O_3$, and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated to give a yellow oil. Flash chromatography (35% EtOAc in hexanes) yielded 133 mg (75%) of **16** as a clear oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (br s, 2 H), 7.73 (m, 4 H), 7.40 (m, 8 H), 7.28 (m, 5 H), 5.81 (d, 1 H, *J* = 8.5 Hz), 5.66 (dd, 1 H, *J* = 10.6, 9.1 Hz), 5.41 (s, 1 H), 5.26 (app d, 1 H, *J* = 3.3 Hz), 5.02 (dd, 1 H, *J* = 10.3, 8.0 Hz), 4.80 (dd, 1 H, *J* = 10.4, 3.6 Hz), 4.65 (d, 1 H, *J* = 8.0 Hz), 4.45 (d, 1 H, *J* = 7.8 Hz), 4.32 (dd, 1 H, *J* = 10.7, 8.5 Hz), 4.26 (d, 1 H, *J* = 11.1 Hz), 4.17

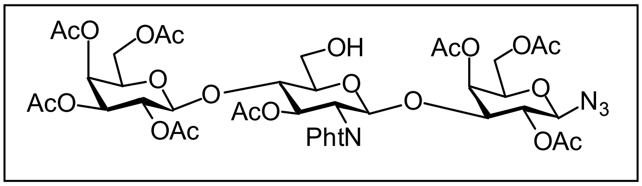


(app d, 1 H, *J* = 2.6 Hz), 4.1 (m, 4 H), 3.96 (dd, 1 H, *J* = 11.3, 3.6 Hz), 3.89 (d, 1 H, *J* = 11.1 Hz), 3.68 (m, 4 H), 2.11 (s, 3 H), 2.07 (s, 3 H), 1.96 (s, 3 H), 1.90 (s, 3 H), 1.78 (s, 3 H), 1.14 (s, 9 H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 170.3, 170.2, 170.1, 169.7, 168.5, 137.0, 136.3, 136.0, 133.9, 133.3, 130.2, 128.3, 127.6, 127.5, 126.4, 110.3, 100.4, 100.2, 98.0, 90.1, 79.9, 76.0, 75.8, 71.0, 70.5, 70.2, 69.8, 69.0, 68.9, 66.2, 60.8, 60.6, 55.2, 30.0, 25.8, 20.6, 20.5, 20.1; FAB-HRMS calcd for $C_{59}H_{66}N_4O_{21}SiLi$ $[M + Li]^+$ 1201.4149, found 1201.4124.



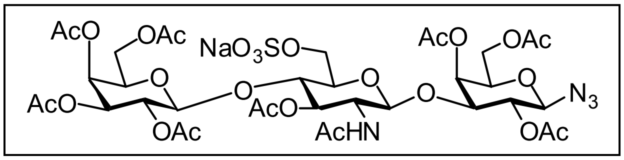
Compound 17. Trisaccharide **16** (1.20 g, 0.999 mmol) was dissolved in 80% aqueous AcOH (30 mL). The reaction mixture was heated to 80 °C and allowed to stir for 1.5 h. The reaction mixture was then concentrated to give a white solid. The solid was azeotroped with toluene (3 x) and then dissolved in pyridine (20 mL) and Ac$_2$O (10 mL). The mixture was stirred for 16 h at rt and then concentrated to give a yellow oil. Flash chromatography (30% EtOAc in hexanes) yielded 887 mg (72%) of **17** as a colorless oil: ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.75 (m, 8 H), 7.45 (m, 6 H), 5.75 (dd, 1 H, *J* = 10.8, 9.0 Hz), 5.45 (app d, 1 H, *J* = 3.0 Hz), 5.40 (d, 1 H, *J* = 8.2 Hz), 5.26 (app d, 1 H, *J* = 3.0 Hz), 5.03 (dd, 1 H, *J* = 10.3, 8.0 Hz), 4.84 (m, 2 H), 4.66 (d, 1 H, *J* = 8.0 Hz), 4.24 (d, 1 H, *J* = 8.9 Hz), 3.92 (m, 7 H), 3.94 (dd, 1 H, *J* = 11.3, 4.3 Hz), 3.91 (dd, 1 H, *J* = 9.8, 3.6 Hz), 2.11 (m, 3 H), 2.10 (s, 3 H), 2.09 (s, 3 H), 2.07 (s, 3 H), 1.96 (s, 3 H), 1.94 (s, 3 H), 1.87 (s, 3 H), 1.78 (s, 3 H), 1.15 (s, 9 H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 170.3, 170.2, 169.9, 169.8, 169.7, 169.4, 168.7, 135.9, 135.3, 133.7, 132.3, 130.1, 130.0, 129.0, 128.2,

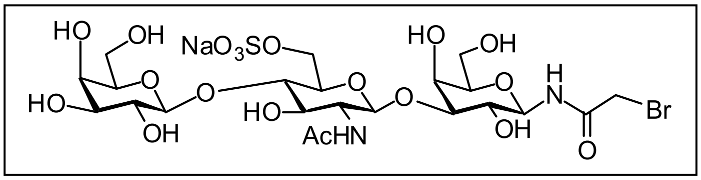
128.0, 127.9, 125.3, 123.4, 100.3, 97.4, 87.9, 75.5, 73.2, 70.9, 70.6, 70.1, 69.2, 68.7, 66.8, 61.7, 61.6, 61.0, 55.0, 29.7, 26.8, 20.9, 20.7, 20.6, 20.5, 20.4, 20.4, 19.5; FAB-HRMS calcd for $C_{58}H_{68}N_4O_{24}SiLi$ $[M + Li]^+$ 1239.4153, found 1239.4157.



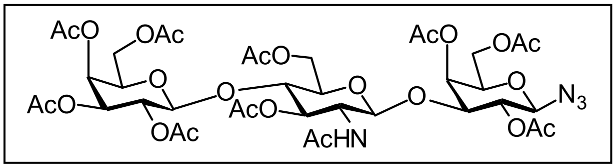
Compound 18. Trisaccharide **17** (3.34 g, 2.71 mmol) was dissolved in THF (50 mL) and cooled to 0 °C. Glacial AcOH (542 μL, 5.42 mmol) and 1.0 M TBAF in THF (13.5 mL) were added. The reaction was allowed to warm slowly to rt while stirring. After 16 h, the reaction mixture was diluted with EtOAc (100 mL) and washed, successively, with aqueous $NaHCO_3$, H_2O, and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. Flash chromatography (60% EtOAc in hexanes) yielded 2.64 g (98%) of **18** as a white foam: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (s, 2 H), 7.75 (m, 2 H), 5.68 (dd, 1 H, *J* = 10.8, 9.0 Hz), 5.62 (app d, 1 H, *J* = 3.0 Hz), 5.50 (d, 1 H, *J* = 8.3 Hz), 5.32 (app d, 1 H, *J* = 2.4 Hz), 5.13 (dd, 1 H, *J* = 10.5, 7.9 Hz), 4.98 (dd, 1 H, *J* = 10.4, 3.4 Hz), 4.90 (app t, 1 H, *J* = 9.1 Hz), 4.62 (d, 1 H, *J* = 7.9 Hz), 4.38 (d, 1 H, *J* = 8.9 Hz), 4.13 (m, 1 H), 4.05 (m, 4 H), 3.96 (app t, 1 H, *J* = 9.5 Hz), 3.88 (m, 3 H), 3.72 (m, 2 H), 3.56 (d, 1 H, *J* = 9.8 Hz), 3.02 (m, 1 H), 2.21 (s, 3 H), 2.13 (s, 3 H), 2.09 (s, 3 H), 2.08 (s, 3 H), 2.03 (s, 3 H), 1.97 (s, 3 H), 1.88 (s, 3 H), 1.37 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 170.2, 169.9, 169.8, 169.4, 168.7, 133.7, 132.3, 130.1, 130.0, 129.0, 128.2, 128.0, 127.9, 125.3, 123.4, 100.3, 97.4, 87.9, 75.5, 73.2, 70.9, 70.6, 70.1, 69.2, 68.7, 66.8, 61.7, 61.6, 61.0, 55.0, 29.7, 20.9, 20.7, 20.6, 20.5, 20.4, 20.4, 19.5; FAB-HRMS calcd for $C_{42}H_{50}N_4O_{24}Li$ $[M + Li]^+$ 1001.2975, found 1001.2978.

Compound 19. A solution of compound **18** (500 mg, 0.500 mmol) and SO_3·pyridine complex (120 mg, 0.75 mmol) in dry pyridine (10 mL) was stirred at 0 to 5 °C for 6 h. The reaction mixture was quenched with MeOH and co-evaporated with toluene. The crude product was dissolved in MeOH (25 mL) and treated with $N_2H_4·H_2O$ (5 mL) for 4 h at 70 °C. The crude product was then treated with Ac_2O/pyridine (1:1) in the presence of a catalytic amount of DMAP (10 mg) at rt overnight. The reaction mixture was co-evaporated several times with toluene and then dissolved in MeOH (25 mL) and stirred in the presence of IR-120 (Na^+) ion-exchange resin for 1.5 h at rt. The mixture was filtered, and the filtrate was concentrated and purified by silica gel chromatography (10:1 CH_2Cl_2/MeOH) to give **19** (258 mg, 51%) as a white solid: ^1H NMR (CD_3OD, 500 MHz) δ 5.53 (app d, 1 H, *J* = 3.5 Hz), 5.36 (app d, 1 H, *J* = 3.5 Hz), 5.15 (m, 1 H), 5.11 (dd, 1 H, *J* = 10.5, 7.0 Hz), 4.99 (m, 2 H), 4.82 (d, 1 H, *J* = 8.0 Hz), 4.73 (d, 1 H, *J* = 5.0 Hz), 4.65 (d, 1 H, *J* = 8.5 Hz), 4.31 (dd, 1 H, *J* = 11.0, 2.0 Hz), 4.20 (dd, 1 H, *J* = 1.0, 5.5 Hz), 4.15 (m, 7 H), 3.83 (app t, 1 H, *J* = 10.0 Hz), 3.64 (m, 2 H), 2.15 (s, 3 H), 2.12 (s, 3 H), 2.11 (s, 6 H), 2.05 (s, 3 H), 2.04 (s, 3 H), 2.03 (s, 3 H), 1.91 (s, 3 H), 1.87 (s, 3 H); ^{13}C NMR (CD_3OD, 125 MHz) δ 171.8, 171.0, 170.6, 170.6, 170.5, 170.2, 170.0, 169.9, 106.8, 100.2, 87.7, 75.5, 75.3, 73.3, 72.4, 71.2, 70.2, 69.9, 69.8, 69.1, 67.3, 65.3, 62.1, 60.9, 54.5, 21.4, 19.6, 19.5, 19.4, 19.3, 19.2, 19.1, 19.0, 18.9; HRMS (ESI) calcd for $C_{36}H_{49}N_4O_{26}S$ [M-Na]$^-$ 985.2356, found 985.2387.



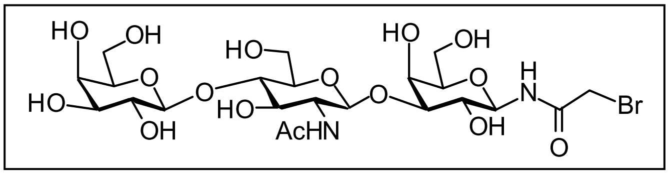


Compound 3. To a solution of **19** (244 mg, 0.24 mmol) in MeOH/H_2O (10 mL, 1:1) was added 1 M NaOMe (250 μL). The mixture was stirred at rt for 24 h, concentrated and purified by silica gel chromatography (3:1:1 *n*-PrOH/AcOH/H_2O). After removal of the solvents under reduced pressure the purified product was dissolved in water and lyophilized. The resulting white solid was dissolved in H_2O (5 mL) and stirred in the presence of 10% Pd/C (40 mg) at rt under an atmosphere of hydrogen. After 20 min, the reaction mixture was filtered through Celite, washed with H_2O (1 mL) and immediately treated with bromoacetic anhydride (312 mg, 1.20 mmol) in the presence of $NaHCO_3$ (500 mg). After 3 h the mixture was loaded onto a column of Bio-Gel P-2 and eluted with H_2O to give **3** (46 mg, 25**%**) as a white solid after lyophilization: ^{1}H NMR (D_2O, 500 MHz) δ 4.59 (app d, 1 H, *J* = 1.5 Hz), 4.43 (d, 1 H, *J* = 8.0 Hz), 4.39 (d, 1 H, *J* = 8.0 Hz), 4.26 (m, 1 H), 4.18 (dd, 1 H, *J* = 11.5, 4.5 Hz), 4.10 (app d, 1 H, *J* = 3.0 Hz), 4.04 (app d, 1 H, *J* = 4.0 Hz), 3.79 (app d, 1 H, *J* = 3.0 Hz), 3.69-3.57 (m, 12 H), 3.55 (dd, 1 H, *J* = 10.0, 3.0 Hz), 3.40 (app t, 1 H, *J* = 8.0 Hz), 1.94 (s, 3 H); HRMS (FAB) calcd for $C_{22}H_{36}N_2O_{19}SBr$ $[M-H]^-$ 743.0816, found 743.0816.



Compound 20. A solution of **18** (350 mg, 0.35 mmol) in MeOH (25 mL) and $N_2H_4{\cdot}H_2O$ (5 mL) was stirred at 70 °C for 4 h. The mixture was concentrated under reduced pressure and co-evaporated with toluene. The crude product was then treated with Ac_2O/pyridine (1:1) in the presence of

a catalytic amount of DMAP (10 mg) at rt overnight. The peracetylated product was co-evaporated with toluene several times and purified by silica gel chromatography (50% hexanes in EtOAc → 100% EtOAc) to give **20** (223 mg, 67%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.51 (d, 1 H, *J* = 8.5 Hz), 5.36 (app d, 1 H, *J* = 3.0 Hz), 5.33 (app d, 1 H, *J* = 2.5 Hz), 5.19 (dd, 1 H, *J* = 10.0, 8.5 Hz), 5.06 (m, 2 H), 4.96 (dd, 1 H, *J* = 10.5, 3.5 Hz), 4.74 (d, 1 H, *J* = 7.5 Hz), 4.72 (dd, 1 H, *J* =12.0, 2.5 Hz), 4.52 (d, 1 H, *J* = 7.5 Hz), 4.45 (d, 1 H, *J* = 8.5 Hz), 4.41 (m, 4 H), 3.97 (dd, 1 H, *J* = 12.5, 4.0 Hz), 3.91 (app t, 1 H, *J* = 6.5 Hz), 3.87 (app t, 1 H, *J* = 7.5 Hz), 3.80 (dd, 1 H, *J* = 10.0, 3.0 Hz), 3.78 (app t, 1 H, *J* = 9.0 Hz), 3.52 (m, 2 H), 2.14 (s, 3 H), 2.13 (s, 3 H), 2.12 (s, 3 H), 2.11 (s, 3 H), 2.08 (s, 3 H), 2.05 (s, 3 H), 2.04 (s, 3 H), 2.03 (s, 3 H), 2.03 (s, 3 H), 1.95 (s, 3 H), 1.89 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 170.4, 170.4, 170.3, 170.2, 170.1, 170.0, 169.8, 169.2, 169.1, 101.0, 100.4, 88.0, 75.7, 75.6, 73.3, 72.3, 71.7, 70.8, 70.7, 69.8, 69.1, 69.0, 66.6, 61.9, 60.7, 60.4, 54.9, 23.1, 20.9, 20.8, 20.8, 20.7, 20.7, 20.6, 20.5, 20.4; HRMS (FAB) calcd for $C_{38}H_{52}N_4O_{24}Li$ $[M + Li]^+$ 955.3132, found 955.3154.

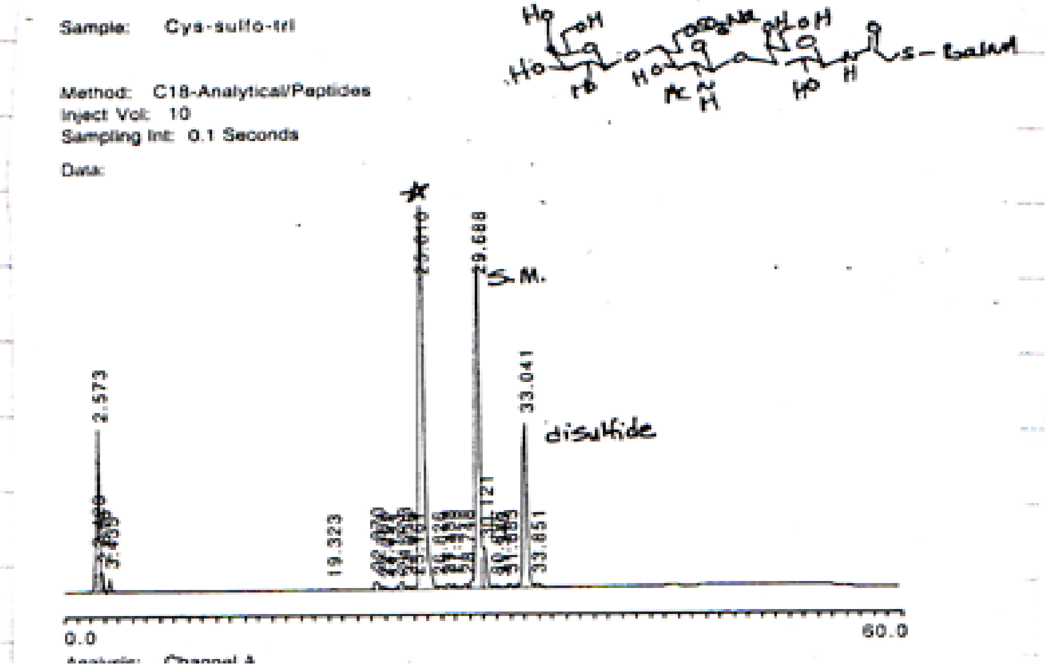


Compound 4. To a solution of **20** (196 mg, 0.200 mmol) in MeOH/H_2O (10 mL, 1:1) was added 1 M NaOMe in MeOH (250 μL). After stirring for 12 h at rt the mixture was neutralized with Amberlite IRC-50® (H^+) resin, filtered and concentrated. A mixture of the crude product, H_2O (5 mL) and 10% Pd/C (35 mg) was stirred at rt, under a hydrogen atmosphere. After 20 min the reaction mixture was filtered through Celite, washed with

H_2O (1 mL) and immediately treated with bromoacetic anhydride (260 mg, 1.0 mmol) and $NaHCO_3$ (500 mg). After stirring for 4 h at rt, the mixture was loaded onto a column of Bio-Gel P-2 and eluted with water. Fractions containing the desired product were combined and lyophilized to give 60 mg (45%) of **4** as a white solid: ^1H NMR (500 MHz, D_2O) δ 4.80 (d, 1 H, *J* = 9.0 Hz), 4.60 (d, 1 H, *J* = 8.5 Hz), 4.35 (d, 1 H, *J* = 8.0 Hz), 4.08 (d, 1 H, *J* = 2.5 Hz), 3.83 (m, 4 H), 3.73 (m, 2 H), 3.65 (m, 10 H), 3.55 (dd, 1 H, *J* = 10.0, 3.5 Hz), 3.47 (m, 1 H), 3.42 (dd, 1 H, *J* = 10.0, 7.5 Hz), 1.92 (s, 3 H); ^{13}C NMR (125 MHz, D_2O) δ 174.9, 171.0, 102.8, 102.6, 82.8, 80.2, 78.1, 76.4, 75.3, 74.5, 72.4, 72.2, 70.9, 68.5, 68.2, 68.1, 61.0, 60.8, 59.8, 55.2, 27.8, 22.2; HRMS (FAB) calcd for $C_{22}H_{38}BrN_2O_{16}$ $[M+H]^+$ 665.1405, found 665.1416.

HPLC and MS Data

5



Sample: Cys-sulfo-tri

Method: C18-Analytical/Peptides
Inject Vol: 10
Sampling Int: 0.1 Seconds

Data:

Analysis: Channel A

Date: Tue, May 1, 2001 7:37 PM
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Sample: Cys-sulfo tri

Method: C18-Analytical/Peptides
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Data:

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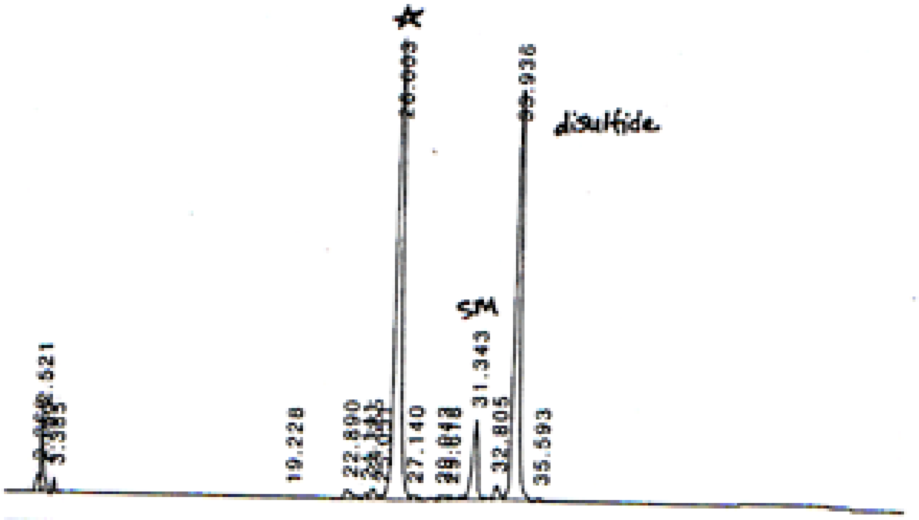

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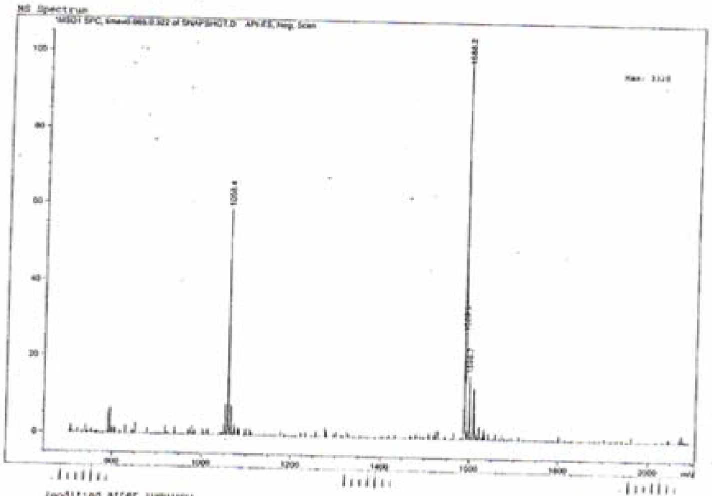
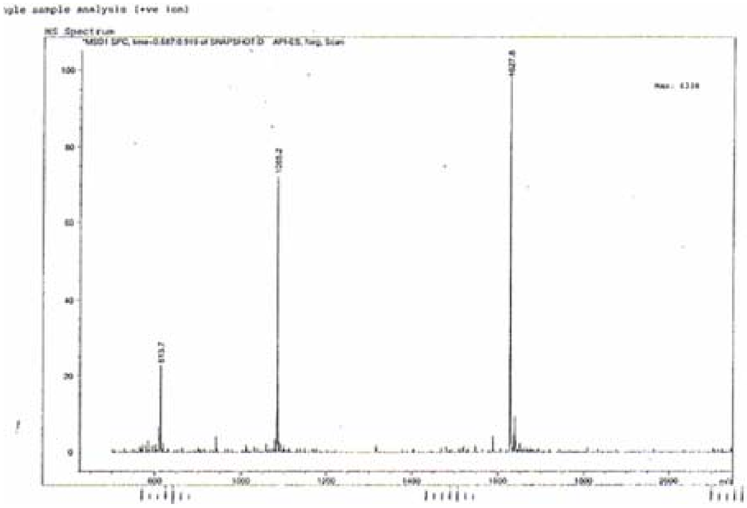


ThioGalNAc
w/ Tri

calcd 3179.
found 3178
[M-H]-

$C_{140}H_{198}N_{23}O_{57}S$



ThioGalNAc
w/
Ⓢ Tri

calcd 3258.40
found 3259

$C_{140}H_{197}N_{23}O_{60}S$