CHEMBIOCHEM

Supporting Information

CHEMBIOCHEM

Supporting Information

for

Biotinylated Bi- and Tetra-antennary Glycoconjugates for *Escherichia coli* Detection

Duane M. Hatch, Alison A. Weiss,* Ramesh R. Kale, and Suri S. Iyer*

Synthesis:

General: All chemical reagents were of analytical grade, used as supplied without further purification unless indicated. Acetic anhydride and acetyl chloride were distilled and stored under an inert atmosphere. 4Å Molecular sieves were stored in an oven (>130 ^{0}C) and cooled in vacuo. The acidic ion-exchange resin used was Dowex-50 and Amberlite (H^+ form). Analytical thin layer chromatography (TLC) was conducted on silica gel 60-F254 (Merck). Plates were visualized under UV light, and/or by treatment with acidic cerium ammonium molybdate followed by heating. Column chromatography was conducted using silica gel (230-400 mesh) from Qualigens. ^{1}H and ^{13}C NMR spectra were recorded on Brüker AMX 400MHz spectrometer. Chemical shifts are reported in d (ppm) units using ^{13}C and residual ^{1}H signals from deuterated solvents as references. Spectra were analyzed with Mest-Re-C Lite (Mestrelab Research) and/or XWinPlot (Bruker Biospin). Electrospray ionization mass spectra (ESI-MS) was recorded on a Micromass Q ToF 2 (Waters) and data was analyzed with MassLynx 4.0 (Waters) software.

Compound 2: 2-(2-(2-(2azidoethoxy)ethyl)ethyl)-*O*-(2,3,4,6-tetra-*O*-acetyl-a-D-mannopyranoside): Mannose trichloroacetimidate **1**[1] (1.5 g, 3.0 mmol) and 2-(2-(2-(azidoethoxy)ethyl)ethyl)ethanol (0.80 g, 3.6 mmol) were dissolved in CH_2Cl_2 (15 mL).

The flask was cooled to 0 ^{0}C and TMSOTf (2.8 mL, 0.22 M solution in CH_2Cl_2, 0.61 mmol) was added drop wise. The reaction was monitored by TLC and after 1.5 h, ice-cold $NaHCO_3$ (25 mL) was used to quench the reaction and the product was extracted with CH_2Cl_2 (3 x 25 mL), dried over Na_2SO_4, filtered and solvent was removed in vacuo. Flash column chromatography with 60:40 EtOAc:hexane gave the desired product as a colorless oil. Yield = 1.1 g (65 %). ^{1}H NMR (400 MHz, $CDCl_3$): *d* 5.38-5.26 (m, 3H), 4.88 (d, 1H, $J_{1,2}$ = 4.0 Hz), 4.30 (dd, 1H, *J* = 12.0 Hz, *J* = 4.0 Hz), 4.09 (dd, 1H, *J* = 16.0 Hz, *J* = 4.0 Hz), 4.06, (m, 1H), 3.82 (m, 1H), 3.70-3.66 (m, 13H), 3.39 (t, 2H, *J* = 8.0 Hz), 2.16 (s, 3H), 2.11 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H).^{13}C NMR (400 MHz, $CDCl_3$, d): 170.9, 170.2, 170.1, 169.9, 97.9, 70.9, 70.8, 70.2, 70.1, 69.8, 69.3, 68.6, 67.6, 62.6, 50.9, 21.1, 21.0, 20.9. HRMS calcd for $[C_{22}H_{35}N_3O_{13}+ Na]^+$: 572.2068; found: 572.2067.

Compound 4: To **2** (0.12g, 0.21 mmol) in a 25 mL flask was added (3,5-Bis-prop-2-ynylcarbamoyl-phenyl)-carbamic acid *tert*-butyl ester[2] (0.034 g, 0.089 mmol). Next, THF:H_2O (2:2 mL), $CuSO_4.5H_2O$ (0.047 g, 0.1898 mmol) and sodium ascorbate (0.047 g, 0.24 mmol) were added in sequential order. The reaction mixture stirred at RT for 48 h. The reaction was filtered through celite and solvent removed in vacuo. Flash column chromatography with 5:95 MeOH:CH_2Cl_2 afforded the desired product as a sticky white solid. Yield = 0.12 g (77 %). ^{1}H NMR (400 MHz, $CDCl_3$): *d* 8.04 (s, 2H), 7.90 (s, br, 1H), 7.82 (s, br, 1H), 7.73 (br, 1H), 7.44 (s, br, 2H), 7.19 (s, 1H), 5.35-5.25 (m, 8H), 4.87 (d, 2H, *J* = 1 Hz), 4.76-4.71 (m, br, 4H), 4.54 (t, 4H, *J* = 4.0 Hz), 4.29 (dd, 2H, *J* = 12.0 Hz, *J* = 4.0 Hz), 4.11-4.04 (m, 5H), 3.99 (s, 1H), 3.93 (s, 1H), 3.87 (t, 4H, *J* = 4.0 Hz), 3.84-3.78 (m, 3H), 3.66-3.60 (m, 16H), 2.16 (s, 6H), 2.10 (s, 6H), 2.03 (s, 6H), 1.99 (s, 6H), 1.52 (s, 9H). ^{13}C NMR (400 MHz, $CDCl_3$, d): 170.9, 170.3, 170.2, 169.9, 166.5, 144.6, 139.7, 135.5, 120.2, 97.9, 70.9, 70.7, 70.6, 70.1, 69.8, 69.6, 69.3, 68.6, 67.6, 66.3, 62.6, 50.6, 35.7, 28.5, 21.1, 21.0, 20.9. ESI-MS calcd for $[C_{63}H_{91}N_9O_{30} + H]^+$: 1454.5945; found: 1454.5905.

Abbreviations: 2-chloro-4,6-dimethoxy-1,3,5-triazine, CDMT; *N*–methyl morpholine, NMM; N,N Dimethyl Formamide, DMF; Ethyl acetate, EtOAc; Triisopropylsilane, TIPS; Trifluroacetic acid, TFA; Acetonitrile, CH_3CN; Trifluoromethanesulfonic anhydride, Tf_2O; Benzaldehyde Dimethyl Acetal, BDA; Deuterated chloroform, $CDCl_3$; 4-N, N Dimethyl amino pyridine, DMAP; Sodium bicarbonate, $NaHCO_3$; Methanol, CH_3OH; *p*-Toluene sulfonic acid, *p*-TSA; Acetic anhydride,Ac_2O; Dichloroethane, DCE; Trimethylsilyl trifluoromethanesulfonate, TMSOTf; chloroform, $CDCl_3$; dichloromethane, CH_2Cl_2; Tetrahydrofuran, THF; high resolution mass spectroscopy, HRMS.

Compound 6: To **4** (0.11 g, 0.070 mmol) in a 25 mL flask was added CH_2Cl_2 (10 mL) via cannula. The flask was cooled to 0 ^{0}C and TIPS (.018 mL, 0.089 mmol) was added followed by the drop wise addition of TFA (.11 mL, 0.15 mmol). The reaction was stirred for 24 h while slowly warming to RT. The reaction mixture was concentrated, washed with $NaHCO_3$ (2 x 25 mL), brine (25 mL), the product extracted with EtOAc (3 x 25 mL), dried over $MgSO_4$, filtered and solvent removed in vacuo. Flash column chromatography with 10:90 MeOH:CH_2Cl_2 afforded the product as a sticky white solid. Yield = 0.069 g (69 %). This compound was directly used in the next step. D-Biotin (0.020 g, 0.081 mmol) was added to a flask, followed by CDMT (0.014 g, 0.080 mmol) and THF (2.0 mL). The flask was cooled to 0 ^{0}C and NMM (9.0 µL, 0.081 mmol) in THF (100 µL) was added dropwise and stirred overnight. In a separate flask, **5** (0.084 g, 0.062 mmol) and NMM (8.0 µL, 0.068 mmol) in DMF:THF (1:1, 2 mL) were added. This solution was added via cannula to the flask containing the activated biotin at 0 ^{0}C. The reaction was stirred overnight while warming to RT. TLC analysis indicated complete consumption of starting material **6** after 12 h. The reaction was quenched with dilute HCl (5 drops), dried over $MgSO_4$, filtered and solvent removed in vacuo. Flash column chromatography with 10:90 MeOH:CH_2Cl_2 gave the desired product as a white solid. Yield = 0.071 g (72 %). ^{1}H NMR (400 MHz, $CDCl_3$): *d* 9.24 (s, 1H), 8.36 (s, 2H), 8.14 (s, 2H), 7.92 (s, 1H), 7.80 (s, 3H), 7.00 (s, 1H), 6.55 (s, br, 2H), 5.35-5.25 (m, 6H), 4.86 (s, 2H), 4.69-4.65 (m, 4H), 4.52 (t, 5H, *J* = 8.0 Hz), 4.29 (dd, 3H, *J* = 12.0 Hz, *J* = 4.0 Hz), 4.11-4.04 (m, 4H), 3.88-3.77 (m, 6H), 3.65-3.60 (m, 19H), 3.07 (s, br, 4H), 2.83 (dd, 1H, *J* = 12.0 Hz, *J* = 4.0 Hz), 2.67 (d, 1H, *J* = 12.0 Hz), 2.15 (s, 6H), 2.10 (s, 6H), 2.03 (s, 6H), 1.98 (s, 6H), 1.58 (s, br, 3H), 1.39-1.25 (m, 3H). ^{13}C NMR (400 MHz, $CDCl_3$, d): 172.3, 170.7, 170.0, 169.9, 169.7, 166.8, 164.5, 144.7, 144.6, 139.3, 134.7, 132.6, 121.6, 120.8, 97.7, 70.7, 70.5, 70.4, 69.9, 69.5, 69.3, 69.1, 68.4, 67.4, 66.1, 62.4, 62.0, 60.3, 60.2, 40.3, 36.2, 35.3, 29.7, 28.1, 25.2, 20.9, 20.8, 20.73, 20.72. ESI-MS calcd for $[C_{68}H_{97}N_{11}O_{30}S + H]^+$: 1580.6124; found: 1580.5022.

Biotinylated biantennary a-D mannoside (MD): 6 (0.050 g, 0.030 mmol) was dissolved in MeOH (3 mL) and a solution of NaOMe in MeOH (13 µL, 0.50 M solution) was added and the reaction mixture was stirred at RT for 12h. The reaction was quenched by careful addition of Dowex H^+ resin (pH 6) and the resin was filtered. The solvent was removed in vacuo and the residue was purified by Biogel P-2 gel column chromatography, using water as eluent. The product was lyophilized to give **MD** as a

white solid. Yield = 0.30 g (77%). ^{1}H NMR (400 MHz, D_2O) *d* 8.01-7.81 (m, 10H), 4.63-4.54 (m, 10H), 4.34 (dd, 1H, *J* = 12.0 Hz, *J* = 4.0 Hz), 3.92-3.22 (m, 42H), 2.91 (dd, 1H, *J* = 12.0 Hz, *J* = 4.0 Hz), 2.70 (d, 1H, *J* = 12.0 Hz), 2.38-2.21 (m, 2H), 1.67-1.35 (m, 6H). ^{13}C NMR (400 MHz, D_2O, d) 175.2, 168.2, 165.2, 144.5, 138.4, 134.6, 124.5, 122.6, 121.9, 99.9, 69.7, 69.5, 69.4, 68.7, 66.7, 66.2, 62.0, 60.9, 60.2, 55.3, 50.0, 48.8, 39.7, 36.2, 34.9, 27.9, 27.7, 24.9. HRMS calcd for $[C_{52}H_{81}N_{11}O_{22}S + H]^+$: 1245.5424; found: 1245.5456.

Compound 10: **3** (0.090 g, 0.25 mmol) was dissolved in CH_2Cl_2 (5.0 mL) and the temperature was lowered to 0 °C. To this solution was added TIPS (0.21 mL, 1.0 mmol) and TFA (0.37 mL, 5.0 mmol) dropwise. The reaction mixture stirred at 0 °C while warming to RT over 12h. TLC analysis (MeOH/CH_2Cl_2 1:9) indicated complete deprotection. The temperature was lowered to 0 ^{0}C and the reaction mixture was neutralized with a saturated solution of $NaHCO_3$ (1 mL), washed with brine (25 mL) and extracted with EtOAc (3 x 25 mL). The combined organic phases were dried using Na_2SO_4, filtered and concentrated in vacuo. The crude product was flashed with 10:90 MeOH:CH_2Cl_2. Product-containing fractions were pooled and concentrated to a white solid. Yield = 0.042 g (66%). Spectra of this compound matched reported values.[2]

Compound 11: CDMT (0.038 g, 0.19 mmol), THF (5.0 mL) and DMF (2.0 mL) was added to a 25 mL flask. The temperature was lowered to 0 ^{0}C and NMM (28 µL, 0.22 mmol) was injected. After 30 min, D-biotin (0.044 g, 0.18 mmol) was added and stirred at 0 ^{0}C for 5 h. **10** (0.042 g, 0.16 mmol) in DMF (1.0 mL) and NMM (28 µL, 0.22 mmol) was added via cannula to the activated ester solution. The reaction stirred at 0 °C while warming to RT over 12h. The solution was neutralized with Dowex H^+ resin (pH 6), filtered through Celite and concentrated in vacuo. The crude product was preadsorbed onto SiO_2 gel and flashed with 5:95 MeOH:CH_2Cl_2. Product-containing fractions were pooled and concentrated to a white solid. Yield = 0.055 g (70%). ^{1}H NMR (400 MHz, DMSO, d): 10.1 (s, 1H), 8.96 (t, 2H, *J* = 5.6 Hz), 8.18 (d, 2H, *J* = 1.2 Hz), 7.92 (s, 1H), 6.44 (s, 1H), 6.36 (s, 1H), 4.32-4.29 (m, 1H), 4.15-4.12 (m, 1H), 4.06-4.04 (m, 4H), 3.35 (s, 13H), 3.15 (s, 3H), 2.82 (dd, 1H, *J* = 13.2 Hz, *J* = 5.2 Hz), 2.58 (d, 1H, *J* = 12 Hz), 2.33 (t, 2H, *J* = 7.6 Hz), 1.64-1.35 (m, 6H). ^{13}C NMR (400 MHz, DMSO, d) 172.0, 166.1, 163.1, 139.9, 135.2, 121.3, 81.6, 73.4, 61.5, 59.6, 55.8, 36.6, 29.1, 28.6, 28.5, 25.3. HRMS calcd for $[C_{24}H_{27}N_5O_4S + H]^+$: 482.1862; found: 482.1960.

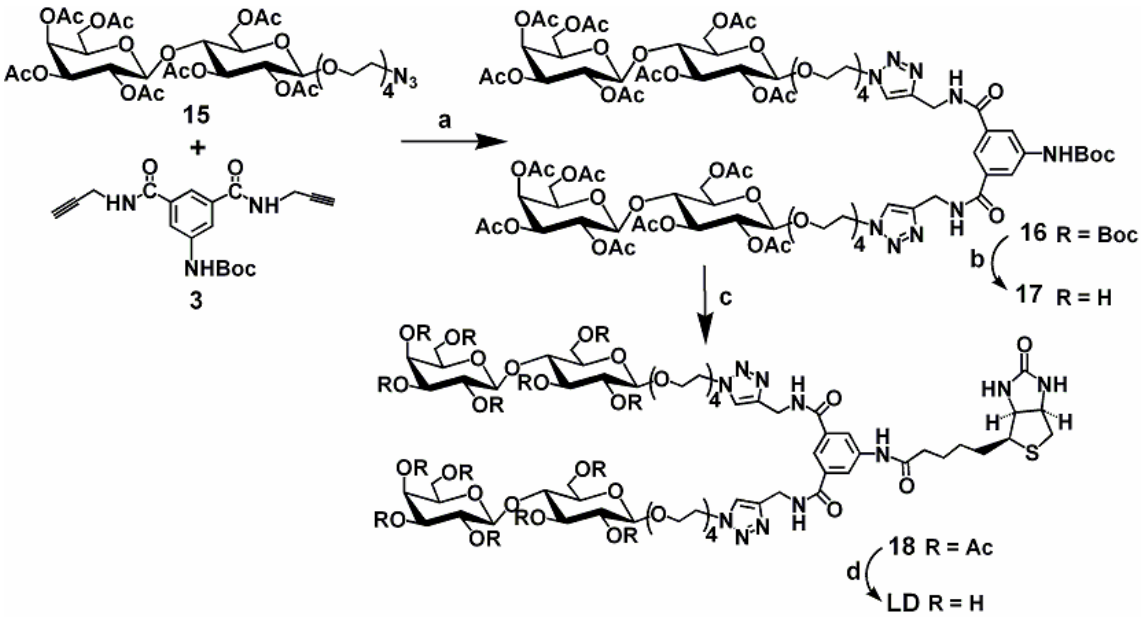
Compound 12: **5** (0.13 g, 0.090 mmol) was dissolved in CH_3CN (5.0 mL) and flame-dried Na_2CO_3 (0.015 g, 0.13 mmol) was added under argon. The temperature was lowered to 0 °C and bromoacetyl bromide (11 µL, 0.18 mmol) was injected dropwise. The reaction stirred at 0 ^{0}C while slowly warming to RT over 12 h. TLC analysis (MeOH:CH_2Cl_2 10:90) indicated complete conversion to the brominated product. The solution was filtered through a small plug of Celite and concentrated in vacuo. This compound was used in the next step without further purification.

Compound 13: **12** (0.14 g, 0.09 mmol) was dissolved in DMF (10 mL) and NaN_3 (0.015 g, 0.23 mmol) was added. The reaction mixture stirred at RT for 12 h and DMF was removed in vacuo. The crude product was pre adsorbed on SiO_2 gel and flashed with 5:95 MeOH:DCM. The product was isolated as a sticky white solid. Yield over two steps = 0.043 g (32%). ^{1}H NMR (400 MHz, $CDCl_3$): *d* 8.98 (s, 1H), 8.05 (s, 2H), 7.86-7.79 (m, 4H), 5.32-5.24 (m, 5H), 4.86 (d, 2H, *J* = 1.6 Hz), 4.67 (d, 3H, *J* = 5.2 Hz), 4.54 (t, 3H, *J* = 4.8 Hz), 4.28 (dd, 2H, *J* = 12.4 Hz, *J* = 5.2 Hz), 4.11-4.02 (m, 5 H), 3.88 (t, 3H, 5.2 Hz), 3.81-3.78 (m, 2H), 3.66-3.59 (m, 20H), 2.96 (s, 1H), 2.15 (s, 6H), 2.09 (s, 6H), 2.03 (s, 6H), 1.98 (s, 6H). ^{13}C NMR (400 MHz, D_2O, d) 170.8, 170.2, 170.1, 169.8, 166.4, 166.1, 144.7, 138.1, 135.3, 132.7, 121.7, 121.6, 97.8, 70.6, 70.1, 69.7, 69.5, 69.3, 68.6, 67.5, 66.3, 62.6, 52.7, 50.4, 35.7, 29.8, 21.0, 20.9, 20.8. HRMS calcd for $[C_{60}H_{84}N_{12}O_{29} + H]^+$: 1437.5540; found: 1437.5879.

Compound 14: **13** (43 mg, 0.032 mmol), **11** (70 mg, 0.015 mmol), THF (2.0 mL) and distilled H_2O (2.0 mL) was added to a 10 mL flask followed by the addition of $CuSO_4$. 5 H_2O (40 mg, 0.017 mmol) and sodium ascorbate (70 mg, 0.035 mmol). The reaction stirred at RT for 12h. TLC analysis (MeOH:CH_2Cl_2 10:90) showed complete consumption of starting material. The solvent was removed in vacuo and flash column chromatography (MeOH:CH_2Cl_2 10:90) afforded the purified product as a white solid. Yield = 28 mg (58%). ^{1}H NMR (400 MHz, $CDCl_3$:MeOD): *d* 7.98-7.86 (m, 14H), 5.33-5.26 (m, 16H), 4.87 (s, 4H), 4.62-4.53 (br, d, 20H), 4.28 (dd, *J* = 12 Hz, *J* = 4.4 Hz), 4.11-4.08 (m, 8H), 3.88-3.61 (m, 95H), 3.37 (s, 2H), 3.14 (s, 1H), 2.87 (br, s, 1H), 2.72 (br, s, 1H), 2.35 (br, s, 2H), 2.16 (s, 12H), 2.10 (s, 12H), 2.05 (s, 12H), 1.99 (s, 12H), 1.67-1.43 (m, 8H). ^{13}C NMR (400 MHz, $CDCl_3$:MeOD, d) 171.1, 170.3, 170.2, 170.0, 166.9, 164.5, 134.9, 121.5, 97.6, 70.4, 69.9, 69.5, 69.2, 68.3, 67.3, 66.0, 62.4, 50.3, 20.7, 20.6, 20.6. HRMS calcd for $[C_{144}H_{195}N_{29}O_{62}S + 2H]^{2+}$: 1678.6447; found: 1678.6990.

Biotinylated tetra-antennary a-D mannoside (MT): **14** (0.026 g, 0.008 mmol) was dissolved in MeOH (2.0 mL) and purged with argon. To this solution, a 0.5 M solution of NaOMe in MeOH (6.0 µL, 3.0 µmol) was added. The reaction mixture stirred under argon for 14h. TLC analysis (100% MeOH) showed complete dissappearnce of the acetlyated starting material. The solution was neutralized with Dowex H^+ resin (pH 6), filtered through a small plug of Celite and concentrated in vacuo. Biogel P-2 gel column chromatography, using water as eluent afforded the purified product as a white solid after lyophilization. Yield = 8.0 mg (37%). ^{1}H NMR (400 MHz, DMSO): *d* 10.75 (s, 2H), 10.19 (s, 1H), 9.09-9.02 (m, 6H), 8.2 (d, 6H, *J* = 8 Hz), 8.03 (d, 5H, *J* = 12 Hz), 7.95 (s, 4H), 6.45 (s, 1H), 6.37 (s, 1H), 5.34 (s, 4H), 4.73 (t, 7H, *J* = 4 Hz), 4.64-4.43 (m, 30H), 4.32-4.29 (m, 1H), 4.15-4.12 (m, 1H), 3.79 (t, 7H, *J* = 8 Hz), 3.67-3.59 (m, 11H), 3.52-3.42 (m, 48H), 3.14-3.09 (m, 2H), 2.82 (dd, 2H, *J* = 12 Hz, *J* = 4 Hz), 2.57 (br, d, 1H, *J* = 12 Hz), 2.34 (t, 3H, *J* = 4 Hz), 1.7-1.32 (m, 9H). ^{13}C NMR (400 MHz, DMSO, d) 156.3, 144.4, 123.2, 99.8, 73.7, 70.8, 70.1, 69.6, 69.58, 69.53, 69.4, 69.3, 68.6, 66.8, 65.5, 61.2, 61.1, 46.1, 34.7. HRMS calcd for $[C_{112}H_{163}N_{29}O_{46}S + 2H]^{2+}$: 342.5602; found: 1342.5651.

Synthesis of the biotinylated biantennary ß-lactoside (LD): The synthesis of the lactose-based ligand **LD**, from **8** and **3**[2] was performed in a similar manner as described for **MD** (Scheme 1). After Biogel P-2 gel filtration, **12** was lyophilized to yield a white powder. Data for selected compounds are given below.



Scheme 1 Synthesis of the lactose based ligand. *Reagents and Conditions*. a) $CuSO_4$, sodium ascorbate, THF:H_2O, 48h, 55%; b) TIPS, TFA, DCM, 0 ^{0}C? RT, 48h, 92%; c) CDMT, NMM, D-biotin, THF:DMF, 0^{0}C? RT, 48h, 53%; d) NaOMe, MeOH, RT, 12h, 92%.

Compound 16: ^{1}H NMR (400 MHz, $CDCl_3$): *d* 8.07 (br, s, 2H), 8.01-7.75 (br, 6H), 7.33 (br, 1H), 5.36 (s, 2H, *J* =11.2 Hz), 5.20 (t, 2H, *J* = 9.2 Hz), 5.12 (dd, 2H, *J* = 10.4 Hz, *J* = 8.0 Hz), 4.97 (dd, 2H, *J* = 10.4 Hz, *J* = 3.2 Hz), 4.88 (t, 2H, J = 8.0 Hz), 4.56 (br, s, 3H), 4.20-4.10 (br, m, 10H), 4.09 (m, 7H), 3.93-3.79 (m, 10H), 3.60 (br, m, 2H), 3.34 (br, m, 23H), 2.16 (s, 6H), 2.11 (s, 6H), 2.06 (s, 6H), 2.05 (s, 12H), 2.04 (s, 6H), 2.03 (s, 6H), 1.97 (s, 6H), 1.95 (s, 6H), 1.52 (s, 9H). ^{13}C NMR (400 MHz, $CDCl_3$, d): 170.4, 170.2, 169.8, 169.1, 135.0, 101.1, 100.6, 72.8, 71.7, 71.0, 70.5, 69.1, 66.6, 62.0, 60.8, 29.7, 28.3, 20.6. ESI-MS calcd for $[C_{87}H_{123}N_9O_{46}$ -2H + Na$]^+$: 2050.7303; found: 2050.0491.

Compound 18: ^{1}H NMR (400 MHz, $CDCl_3$): *d* 9.15 (br, s, 1H), 8.35 (br, s, 2H), 8.18 (s, 2H), 7.99 (s, 1H), 7.81 (s, 2H), 7.65 (s, 1H), 7.60 (s, 1H), 6.93 (s, 1H), 6.55 (br s, 1H), 5.35 (d, 2H, *J* = 14.8 Hz), 5.20 (t, 2H, *J* = 9.6 Hz), 5.10 (dd, 2H, *J* = 10.4 Hz, *J* = 8.0 Hz) 4.99 (dd, 2H, *J* = 10.4 Hz, *J* = 3.6 Hz), 4.88 (d, *J* = 8.0 Hz), 4.67 (br, m, 4H), 4.55-4.47 (m, 11H), 4.30 (br, s, 1H), 4.15-4.08 (m, 7H), 3.92-3.70 (m, 12H), 3.70-3.59 (br, s, 23H), 3.10 (br,m, 2H), 2.85 (m, 1H), 2.70 (m, 1H), 2.18 (s, 6H), 2.16 (s, 6H), 2.12 (s, 6H), 2.11 (s, 6H), 2.06 (s, 6H), 2.05 (s, 12H), 2.03 (s, 6H), 1.97 (s, 6H), 1.61 (s, br, 4H), 1.40 (m, 2H), 1.26 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$, d): 170.5, 170.4, 170.2, 170.1, 169.8, 169.7, 169.1, 166.7, 164.4, 144.7, 144.6, 139.3, 134.8, 123.6, 121.7, 101.1, 100.6, 76.7, 76.3, 72.8, 72.6, 71.6, 71.0, 70.7, 70.5, 70.6, 70.4, 70.2,

69.3, 69.1, 66.6, 62.0, 60.8, 60.3, 55.8, 50.2, 36.1, 35.2, 29.7, 28.0, 25.2, 20.9, 20.8, 20.7, 20.6, 20.5. HRMS calcd for $[C_{92}H_{129}N_{11}O_{46}S + H]^+$: 2157.8000; found: 2157.7958.

Biotinylated biantennary ß-lactoside (LD): ^{1}H NMR (400 MHz, D_2O): *d* 7.94 (s, 4H), 7.91 (s, 4H), 7.87 (2H), 4.56 (s, 4H), 4.49 (br t, 6H, *J* = 4.4 Hz), 4.31 (d, 2H, *J* = 8.0 Hz), 4.29 (br m, 2H), 3.84 (d, 2H, *J* = 8.0 Hz), 3.82 (m, 4H), 3.70-3.24 (m, 38 H), 3.23-3.17 (m, 4H), 2.85 (dd, 2H, *J* = 13.2 Hz, *J* = 4.8 Hz), 2.62 (d, 1H, *J* = 13.2 Hz), 2.33 (t, 2H, *J* = 10.2 Hz), 1.61-1.59 (m, br, 3H), 1.33 (m, 3H). ^{13}C NMR (400 MHz, D_2O): *d* 168.4, 144.5, 138.3, 134.7, 124.5, 122.8, 102.9, 102.1, 78.4, 75.3, 74.7, 74.3, 72.8, 72.5, 70.9, 69.6, 69.5, 69.4, 68.6, 68.5, 62.1, 61.0, 60.2, 55.3, 50.1, 39.7, 36.2, 35.0, 27.9, 27.7, 24.9. HRMS calcd for $[C_{64}H_{101}N_{11}O_{32}S + H]^+$: 1568.6407; found: 1568.6462.

The biantennary sialic acid derivatives, **SD** and **TD** were synthesized as described previously.[3]

Details of the *E.coli* strains used in this study.

E. coli [a]	Relevant characteristics	Ref.
ORN178	fim+, piliated	[4]
ORN208	fim-, non-piliated mutant	[4]
J96	UPEC pyelonephritis strain (serotype O4:K6), fim+, pap+, prs+	[5]
J96 pilE	J96 fim-, non-piliated mutant	[6]
B41	Calf isolate, produces K99 pili (ATCC 39303)	[7]
CFT073	UPEC pyelonephritis strain (Serotype 06:K2:H1) fim+, pap1+(P1), pap2+ (P2), foc+ (F1C)	[8]
PT22Δtox	O157:H7 clinical isolate, PT22, Stx2 deleted	[9]

[a] Fim, locus encoding pili that bind mannose; pap, locus encoding pyelonephritis-associate pili that binds Gal(αl-4)Gal; prs locus encoding pili that bind globo-A; foc locus encoding pili that bind GalNAcβ1-4Galβ; K99, pili that bind ganglioside NeuGc-GM3

References:

[1] F. Barresi, O. Hindsgaul, *J Carb Chem* **1995**, *14*, 1043.

[2] R. R. Kale, C. M. Clancy, R. M. Vermillion, E. A. Johnson, S. S. Iyer, *Bioorg Med Chem Lett* **2007**, *17*, 2459.

[3] R. R. Kale, H. Mukundan, D. N. Price, J. F. Harris, D. M. Lewallen, B. I. Swanson, J. G. Schmidt, S. S. Iyer, *J Am Chem Soc* **2008**, *130*, 8169.

[4] S. L. Harris, P. A. Spears, E. A. Havell, T. S. Hamrick, J. R. Horton, P. E. Orndorff, *J Bacteriol* **2001**, *183*, 4099.

[5] B. H. Minshew, J. Jorgensen, G. W. Counts, S. Falkow, *Infect Immun* **1978**, *20*, 50.

[6] B. R. Keith, L. Maurer, P. A. Spears, P. E. Orndorff, *Infect Immun* **1986**, *53*, 693.

[7] P. A. Guinee, W. H. Jansen, C. M. Agterberg, *Infect Immun* **1976**, *13*, 1369.

[8] H. L. Mobley, D. M. Green, A. L. Trifillis, D. E. Johnson, G. R. Chippendale, C. V. Lockatell, B. D. Jones, J. W. Warren, *Infect Immun* **1990**, *58*, 1281.

[9] S. D. Gamage, A. K. Patton, J. F. Hanson, A. A. Weiss, *Infect Immun* **2004**, *72*, 7131.