Supporting Information

for

Expanding the Diversity of Unnatural Cell Surface Sialic Acids

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Materials and General Procedures

All chemical reagents were obtained from commercial suppliers and used without further purification unless otherwise noted. All NMR spectra were measured with a Bruker AMX-300, AMX-400 or DRX-500 MHz spectrometer as noted. All chemical shifts are reported in $\delta$ ppm values downfield from tetramethylsilane and coupling constants ($J$) are reported in Hz. Mass spectral data were obtained from the UC Berkeley Mass Spectrometry Laboratory. Reversed-phase HPLC was performed using a Rainin Dynamax SD-200 HPLC system with 220 nm detection on a Microsorb C$_{18}$ analytical column (4.6 x 250 mm) at a flow rate of 1.0 mL/min or a preparative column (25 x 250 mm) at a flow rate of 20 mL/min.

RPMI-1640 media and sodium pyruvate were purchased from Invitrogen Life Technologies. FITC-avidin, penicillin, streptomycin, and biotin hydrazide were purchased from Sigma. Fetal calf serum (FCS) was from Hyclone. NeuAc Aldolase was from Toyobo. N-Acetylmannosamine hydrochloride was from Pfanstiehl. Cell densities were determined using a Coulter Z2 cell counter. Flow cytometry data were acquired using a Coulter EPICS XL-MCL flow cytometer or a BD Biosciences FACSCalibur flow cytometer equipped with 488 nm argon lasers. For flow cytometry experiments, 10,000 live cells were analyzed as determined by size and granularity and all data points were collected in triplicate.

Compounds 1d,$^{[1]}$ 3d$^{[2]}$ and 4-azidophenylacetic acid$^{[3,4]}$ were synthesized according to literature procedures.
2-Para-azidophenylacetamido-2-deoxy-\(\alpha\),\(\beta\)-d-mannopyranose (1e). To a solution of d-mannosamine hydrochloride (2.0 g, 0.0093 mol) in methanol (47 mL) was added triethylamine (2.9 mL, 0.020 mol) and 4-azidophenylacetic acid (2.0 g, 0.011 mol). The mixture was cooled on ice for 5 min prior to the addition of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (3.6 g, 0.019 mol) and hydroxybenzotriazole (1.1 g, 0.0093 mol). The reaction was stirred on ice and warmed to RT overnight. The crude reaction mixture was concentrated and purified by silica gel chromatography. Compound 1e was eluted with 7:1 CHCl\(_3\):MeOH (1.8 g, 57%). 

\(^1\)H NMR (500 MHz, D\(_2\)O): \(\delta = 3.28\) (1H, m), 3.37 (1H, t, \(J = 9.8\)), 3.42-3.56 (5H, m), 3.63-3.75 (6H, m), 3.93 (1H, dd, \(J = 5.5, 9.5\)), 4.16 (1H, app d, \(J = 4.5\)), 4.31 (1H, app d, \(J = 4.5\)), 4.89 (1H, app s), 4.95 (1H, app s), 6.92 (4H, d, \(J = 8.5\)), 7.16 (2H, d, \(J = 7.5\)), 7.20 (2H, d, \(J = 7.5\)) ppm. 

\(^{13}\)C NMR (125 MHz, D\(_2\)O): \(\delta = 41.2, 41.3, 53.3, 54.2, 60.3, 66.4, 66.6, 68.7, 71.9, 72.0, 76.2, 92.8, 93.0, 119.1, 119.1, 130.5, 130.7, 131.5, 131.5, 138.6, 138.6, 175.0, 175.8 ppm. FAB-HRMS calcd. for C\(_{14}\)H\(_{19}\)N\(_4\)O\(_6\) (M+H\(^+\)) 339.1305, found 339.1305.

**General procedure for the synthesis of unnatural sialic acid derivatives**

To the desired N-acylmannosamine (0.0011 mol) dissolved in 0.05 M potassium phosphate buffer (pH 7.2, 11 mL) were added sodium pyruvate (0.011 mol), 1% NaN\(_3\) (0.5 mL) and NeuAc Aldolase (20-25 U). The reaction was placed in a 37 °C shaking incubator for 12 h, after which the reaction was typically complete as determined by \(^1\)H NMR spectroscopy. When necessary, additional NeuAc Aldolase (10 U) was added. The reaction mixture was diluted with H\(_2\)O (90 mL) and purified by low-pressure chromatography over anion-exchange resin (Bio-Rad AG1-X2, formate form), eluting with a gradient of 1.0 M-2.5 M formic acid at 1.0 mL/min. Fractions were analyzed for the presence of the desired sialic acid derivative using the periodate-resorcinol method.\(^5,6\)

Fractions containing the desired compound were combined and concentrated in vacuo. Compounds 2b-2c were passed over cation exchange resin (AG50W-X8, sodium form). Compounds 2d and 2e were purified by reversed-phase HPLC, eluting with a gradient of CH\(_3\)CN/0.1%TFA (0-45%) and H\(_2\)O/0.1% TFA. Compounds 2a-2d were dissolved in PBS (pH 7.4, 500 mM stock solution) and compound 2e was dissolved in water:/ethanol
(50:50, 250 mM stock solution) and all solutions were filtered (0.2 µm sterile filter) prior to incubation with cells.

**5-Levulinamido-3,5-dideoxy-D-glycero-α,β-D-galacto-non-2-ulosonic acid (2a).** NeuAc Aldolase (20 U) was added to a mixture of compound 1a (0.29 g, 1.1 mmol), 1% NaN₃ (0.5 mL), and sodium pyruvate (1.16 g, 10.6 mmol) in potassium phosphate buffer (0.05 M, pH 7.2, 10.6 mL) as described above to afford compound 2a (0.16 g, 39%) after anion exchange chromatography. ¹H NMR (500 MHz, D₂O): δ = 1.75 (1H, dd, J = 11.7, 12.8), 2.17 (3H, s), 2.45-2.50 (2H, m), 2.80-2.86 (2H, m), 3.44 (1H, dd, J = 0.9, 9.1), 3.55 (1H, dd, J = 6.6, 11.9), 3.69 (1H, ddd, J = 2.6, 6.6, 9.2), 3.78 (1H, dd, J = 2.7, 11.8), 3.84 (1H, d, J = 10.2), 3.93 (1H, dd, J = 1.0, 10.5), 3.95-4.00 (1H, m) ppm. ¹³C NMR (125 MHz, D₂O): δ = 29.0, 29.4, 38.1, 39.3, 52.1, 63.3, 67.1, 68.5, 70.1, 70.3, 96.3, 175.7, 176.7, 213.8 ppm. FAB-HRMS calcd. for C₁₄H₂₄NO₁₀ (M+H⁺) 366.1400, found 366.1402.

**5-[5-Oxo-hexanamido]-3,5-dideoxy-D-glycero-α,β-D-galacto-non-2-ulosonic acid (2b).** NeuAc Aldolase (20 U) was added to a mixture of compound 1b (0.21 g, 0.73 mmol), 1% NaN₃ (0.365 mL), and sodium pyruvate (0.80 g, 7.3 mmol) in potassium phosphate buffer (0.05 M, pH 7.2, 7.3 mL) as described above to afford compound 2b (0.29 g, 98%) after anion exchange chromatography. ¹H NMR (500 MHz, D₂O): δ = 1.75-1.83 (3H, m), 2.16 (4H, m), 2.27 (2H, app t, J = 7.5), 2.57 (2H, app t, J = 7.4) 3.46 (1H, d, J = 9.1), 3.57 (1H, dd, J = 6.3, 11.8), 3.72 (1H, ddd, J = 2.6, 6.3, 9.0), 3.79 (1H, dd, J = 2.6, 11.8), 3.87 (1H, app t, J = 10.1), 3.94-4.01 (2H, m) ppm. ¹³C NMR (125 MHz, D₂O): δ = 19.5, 29.3, 34.9, 39.5, 42.1, 52.1, 63.2, 67.1, 68.5, 70.2, 70.3, 96.4, 171.0, 176.7, 176.7, 215.6 ppm. FAB-HRMS calcd. for C₁₅H₂₆NO₁₀Na (M+Na⁺) 402.1376, found 402.1386.

**5-[5-Oxo-heptanamido]-3,5-dideoxy-D-glycero-α,β-D-galacto-non-2-ulosonic acid (2c).** NeuAc Aldolase (20 U) was added to a mixture of compound 1c (0.26 g, 0.85 mmol), 1% NaN₃ (0.425 mL), and sodium pyruvate (0.94 g, 8.5 mmol) in potassium phosphate buffer (0.05 M, pH 7.2, 8.5 mL) as described above to afford compound 2c (0.27 g, 77%) after anion exchange chromatography. ¹H NMR (500 MHz, D₂O): δ = 1.49-1.56 (4H, m), 1.76 (1H, dd, J = 11.7, 12.8), 2.15 (4H, m), 2.26 (2H, app t, J = 7.0), 2.54 (1H, app t, J = 7.0), 3.44 (1H, dd, J = 6.5, 11.9), 3.70 (1H, dd, J = 2.6, 11.8), 3.84 (1H, d, J = 10.2), 3.93 (1H, dd, J = 1.0, 10.5), 3.95-4.00 (1H, m) ppm. ¹³C NMR (125 MHz, D₂O): δ = 19.3, 28.9, 34.7, 39.4, 42.1, 52.1, 63.2, 67.1, 68.5, 70.2, 70.3, 96.4, 171.0, 176.7, 176.7, 215.6 ppm. FAB-HRMS calcd. for C₁₅H₂₆NO₁₀Na (M+Na⁺) 402.1376, found 402.1386.
ddd, \( J = 2.7, 6.4, 9.9 \), 3.77 (1H, dd, \( J = 2.7, 11.9 \)), 3.86 (1H, app t, \( J = 10.2 \)), 3.93 - 4.00 (2H, m) ppm. \(^{13}\)C NMR (125 MHz, D\(_2\)O): \( \delta = 22.7, 24.7, 29.2, 35.6, 39.5, 42.7, 52.1, 63.2, 67.1, 68.6, 70.2, 70.3, 96.3, 171.0, 176.7, 177.3, 216.7 \) ppm. FAB-HRMS calcd. for C\(_{16}\)H\(_{27}\)NO\(_{10}\)Na (M+Na\(^+\)) 416.1533, found 416.1534.

5-Azidoacetamido-3,5-dideoxy-\( \alpha,\beta \)-\( \alpha,\beta \)-galacto-non-2-ulosonic acid (2d). NeuAc Aldolase (20 U) was added to a mixture of compound 1d (0.29 g, 1.1 mmol), 1% NaN\(_3\) (0.55 mL), and sodium pyruvate (1.2 g, 11 mmol) in potassium phosphate buffer (0.05 M, pH 7.2, 11 mL) as described above to afford compound 2d (0.32 g, 84%) after anion exchange chromatography. \(^1\)H NMR (300 MHz, D\(_2\)O): \( \delta = 1.77-1.85 \) (1H, app t, \( J = 12.2 \)), 2.21-2.26 (1H, dd, \( J = 4.8, 12.7 \)), 3.48 (1H, d, \( J = 9.2 \)), 3.51-3.57 (1H, dd, \( J = 6.2, 11.7 \)), 3.65-3.70 (1H, m), 3.74-3.79 (1H, app t, \( J = 10.2 \)), 4.02-4.08 (4H, m) ppm. \(^{13}\)C NMR (125 MHz, D\(_2\)O): \( \delta = 38.8, 51.9, 52.1, 63.1, 66.4, 68.1, 70.0, 70.1, 95.3, 171.0, 173.3 \) ppm. FAB-HRMS calcd. for C\(_{11}\)H\(_{19}\)N\(_4\)O\(_9\)(M+H\(^+\)) 351.1152, found 351.1152.

2-Para-azidophenylacetamido-3,5-dIDEOXY-\( \alpha,\beta \)-\( \alpha,\beta \)-galacto-non-2-ulosonic acid (2e). NeuAc Aldolase (25 U) was added to a mixture of compound 1e (0.44 g, 1.3 mmol), 1% NaN\(_3\) (0.619 mL), and sodium pyruvate (1.4 g, 13 mmol) in potassium phosphate buffer (0.05 M, pH 7.2, 13 mL) as described above to afford compound 2e (0.33 g, 60%) after anion exchange chromatography. \(^1\)H NMR (500 MHz, D\(_2\)O): \( \delta = 1.73 \) (1H, t, \( J = 12.0 \)), 2.17 (1H, dd, \( J = 5.0, 13.0 \)), 3.20 (1H, d, \( J = 9.5 \)), 3.37 (1H, dd, \( J = 6.0, 12.0 \)), 3.50 (2H, d, \( J = 5.0 \)), 3.53-3.58 (1H, m), 3.63 (1H, dd, \( J = 2.5 \), 12.0), 3.79 (1H, app t, \( J = 10.3 \)), 3.95 (2H, m), 6.97 (2H, d, \( J = 8.0 \)), 7.21 (2H, d, \( J = 8.5 \)) ppm. \(^{13}\)C NMR (125 MHz, D\(_2\)O): \( \delta = 38.9, 41.9, 52.1, 63.1, 66.4, 68.2, 70.1, 70.3, 92.5, 95.2, 119.1, 130.4, 131.6, 138.7, 173.1, 174.8, 175.2 \) ppm. FAB-HRMS calcd. for C\(_{11}\)H\(_{19}\)N\(_4\)O\(_9\) (M+H\(^+\)) 427.1465, found 427.1464.

1,3,4,6-TETRA-O-ACETYL-2-para-azidophenylacetamido-2-deoxy-\( \alpha,\beta \)-\( \alpha,\beta \)-mannopyranose (3e). Acetic anhydride (2.0 mL, 0.020 mol) was added to compound 1e (0.1 g, 0.3 mmol) in pyridine (4.0 mL) and the reaction was stirred overnight at RT. TLC analysis (1:1 ethyl acetate/hexanes) indicated the disappearance of starting material and the crude reaction was concentrated in vacuo. The crude product was purified by reversed-phase HPLC, eluting with a gradient of CH\(_3\)CN (10-60%) and H\(_2\)O.
The isolated product was lyophilized and compound 3e (0.11 g, 73%) was dissolved in ethanol (5.0 mM stock solution) and filtered (0.2 µm sterile filter) prior to incubation with cells. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 1.90 (3H, s), 1.93 (3H, s), 2.02-2.04 (15H, m), 2.15 (3H, s), 3.58 (2H, s), 3.60 (2H, s), 3.75 (1H, m), 3.96-4.04 (3H, m), 4.16-4.20 (2H, m), 4.62-4.65 (1H, m), 4.74 (1H, d, $J$ = 9.0), 5.00 (3H, m), 5.26 (1H, dd, $J$ = 4.5, 10.0), 5.82 (1H, d, $J$ = 1.5), 5.89 (2H, app t, $J$ = 9.0), 5.95 (1H, d, $J$ = 1.0), 7.02 (4H, m), 7.26-7.30 (4H, m) ppm. $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ = 20.6, 20.6, 20.6, 20.7, 20.8, 42.7, 42.9, 49.3, 49.5, 61.7, 61.8, 64.9, 65.1, 69.0, 70.0, 71.3, 73.2, 90.4, 91.5, 119.5, 119.6, 130.6, 130.6, 131.0, 131.3, 139.2, 139.3, 168.1, 168.2, 169.5, 170.0, 170.4, 170.8, 171.3 ppm. FAB-HRMS calcd. for C$_{22}$H$_{27}$N$_4$O$_{10}$ (M+H$^+$) 507.1727, found 507.1727.

**General procedure for the synthesis of protected sialic acid derivatives**

Acetyl chloride (catalytic, 3 drops) was added to a solution of the appropriate unnatural sialic acid derivative (0.2 mmol) in methanol (3.0 mL) and the reaction was stirred at RT overnight. TLC analysis (5:1 CHCl$_3$/MeOH) indicated the disappearance of starting material and the reaction was concentrated in vacuo. The crude material was either HPLC-purified directly to obtain compound 4d, or the intermediates were acetylated with acetic anhydride (0.016 mol) in pyridine (3.0 mL) at RT overnight. TLC analysis indicated the disappearance of the starting material (5:1 CHCl$_3$/MeOH) and the reactions were concentrated in vacuo. Purification over silica gel, eluting with ethyl acetate:hexanes (70:30), afforded the desired compounds. Prior to incubation with cells, derivatives 5d and 5e were purified by reversed-phase HPLC, eluting with a gradient of CH$_3$CN (10-60%) and H$_2$O. The purified compounds were dissolved in ethanol (5.0 mM stock solution) and filtered (0.2 µm sterile filter) prior to incubation with cells.

**5-Azidoacetamido-3,5-dideoxy-D-glycero-\(\alpha,\beta\)-galacto-non-2-ulosonic-1-methyl ester (4d).** Compound 2d (0.15 g, 0.43 mmol) was esterified with catalytic acetyl chloride in methanol (5.0 mL) as described above. The crude reaction mixture was purified by reversed-phase HPLC, eluting with a gradient of CH$_3$CN (0-45%) and H$_2$O and the solution was lyophilized to afford compound 4d (0.12 g, 80%). Compound 4d was dissolved in PBS (pH 7.4, 500 mM stock solution) and the solution was filtered (0.2 µm sterile filter) prior to incubation with cells. $^1$H NMR (500 MHz, D$_2$O): $\delta$ = 1.77 (1H, dd, $J$ = 11.5, 13.0), 2.17 (1H, dd, $J$ = 5.0, 13.0), 3.39 (1H, d, $J$ = 9.5), 3.46 (1H, dd,
\[ J = 6.5, 12.0 \), 3.58 (1H, m), 3.68 (3H, s), 3.85 (1H, t, \( J = 10.3 \)), 3.94 (2H, d, \( J = 1.0 \)), 3.98 (2H, m) ppm. \]  \[ ^{13}\text{C} \text{ NMR (125 MHz, D}_2\text{O):} \]  \[ \delta = 38.6, 51.8, 52.0, 53.4, 63.0, 66.3, 68.0, 69.9, 70.0, 95.2, 171.0, 171.3 \text{ ppm. FAB-HRMS calcd. for} \text{C}_{12}\text{H}_{21}\text{N}_4\text{O}_9 \] (M+H\(^+\)) 365.1309, found 365.1309.

**2,4,7,8,9-Penta-O-acetyl-5-azidoacetamido-3,5-dideoxy-\(\alpha,\beta\)D-glycero-\(\alpha,\beta\)D-galacto-non-2-ulosonic-1-methyl ester (5d).** Compound 2d (0.07 g, 0.2 mmol) was esterified with catalytic acetyl chloride in methanol (2.0 mL) and subsequently acetylated with acetic anhydride (1.5 mL, 0.016 mol) in pyridine (3.0 mL) as described above to afford compound 5d (0.07 g, 64\%) after silica gel chromatography. \[ ^{1}\text{H} \text{ NMR (500 MHz, D}_2\text{O):} \]  \[ \delta = 1.91 (10H, m), 2.04 (3H, s), 2.08 (3H, s), 2.46 (1H, dd, \( J = 4.5, 14.0 \)), 3.67 (3H, s), 3.80 (2H, s), 3.90 (1H, app t, \( J = 10.5 \)), 4.06 (1H, dd, \( J = 4.5, 12.5 \)), 4.21 (1H, d, \( J = 11.0 \)), 4.28 (1H, dd, \( J = 2.5, 13.0 \)), 5.02-5.04 (1H, m), 5.20-5.22 (1H, m), 5.30 (1H, d, \( J = 8.0 \)) ppm. \[ ^{13}\text{C} \text{ NMR (125 MHz, D}_2\text{O):} \]  \[ \delta = 19.9, 20.0, 20.1, 20.2, 35.4, 48.6, 51.8, 53.8, 61.6, 66.9, 68.4, 69.3, 70.8, 96.8, 168.2, 170.8, 171.1, 172.2, 172.4, 173.0, 173.7 \text{ ppm. FAB-HRMS calcd. for} \text{C}_{22}\text{H}_{30}\text{N}_4\text{O}_{14} \] (M+Li\(^+\)) 581.1919, found 581.1919.

**2,4,7,8,9-Penta-O-acetyl-2-para-azidophenylacetamido-3,5-dideoxy-\(\alpha,\beta\)D-glycero-\(\alpha,\beta\)D-galacto-non-2-ulosonic-1-methyl ester (5e).** Compound 2e (0.08 g, 0.02 mmol) was esterified with catalytic acetyl chloride in methanol (3.0 mL) and subsequently acetylated with acetic anhydride (1.5 mL, 0.016 mol) in pyridine (3.0 mL) as described above to afford compound 5e (0.08 g, 63\%) after silica gel chromatography. \[ ^{1}\text{H} \text{ NMR (500 MHz, CDCl}_3\):} \]  \[ \delta = 1.88 (3H, s), 2.02-2.07 (7H, m), 2.12 (3H, s), 2.14 (3H, s), 2.49 (1H, dd, \( J = 5.0, 13.5 \)), 3.42 (2H, s), 3.77 (3H, s), 4.02-4.13 (3H, m) 4.47 (1H, dd, \( J = 2.0, 12.5 \)), 4.98-5.01 (1H, m), 5.15 (1H, m), 5.25-5.30 (2H, m), 7.01 (2H, d, \( J = 8.5 \)), 7.23 (2H, d, \( J = 8.5 \)) ppm. \[ ^{13}\text{C} \text{ NMR (125 MHz, CDCl}_3\):} \]  \[ \delta = 20.7, 20.7, 20.8, 20.8, 35.9, 42.9, 49.4, 53.2, 62.0, 67.6, 67.8, 71.4, 72.7, 97.4, 119.5, 130.7, 131.0, 139.3, 166.2, 168.2, 170.1, 170.3, 170.5, 170.7, 170.8 \text{ ppm. FAB-HRMS calcd. for} \text{C}_{28}\text{H}_{34}\text{N}_4\text{O}_{14} \] (M+Li\(^+\)) 657.2232, found 657.2232.

**Cell culture conditions**

Jurkat cells were maintained in a 5.0\% CO\(_2\), water-saturated atmosphere at 37 °C and grown in RPMI-1640 media supplemented with fetal calf serum (FCS), penicillin
(100 units/mL) and streptomycin (0.1 mg/mL). Typically, cell densities were maintained between $2.0 \times 10^5$ and $1.6 \times 10^6$ cells/mL.

**Flow cytometry**

Cells were seeded at a density of $2.0 \times 10^5$ cells/mL and incubated for three days with the compounds indicated. When necessary, ethanol was pre-evaporated prior to addition of the cells. Following incubation cells were labeled with either biotin hydrazide and FITC-avidin to detect ketones,[7] or phosphine-FLAG and anti-FLAG-FITC to detect azides[1,8] and analyzed by flow cytometry as previously described.