Supporting information for:

Metabolism of diazirine-modified *N*-acetylmannosamine analogs to photocrosslinking sialosides

Michelle R. Bond,[1,2] Haochi Zhang,[2] Jaekuk Kim,[2] Seok-Ho Yu,[2] Fan Yang,[2] Steven M. Patrie,[2] and Jennifer J. Kohler[2,*]

[1]Department of Chemistry, Stanford University, Stanford, CA 94305
[2]Division of Translational Research, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75390
* To whom correspondence should be directed. phone: 214-648-1214; fax: 214-648-4156; email: jennifer.kohler@utsouthwestern.edu

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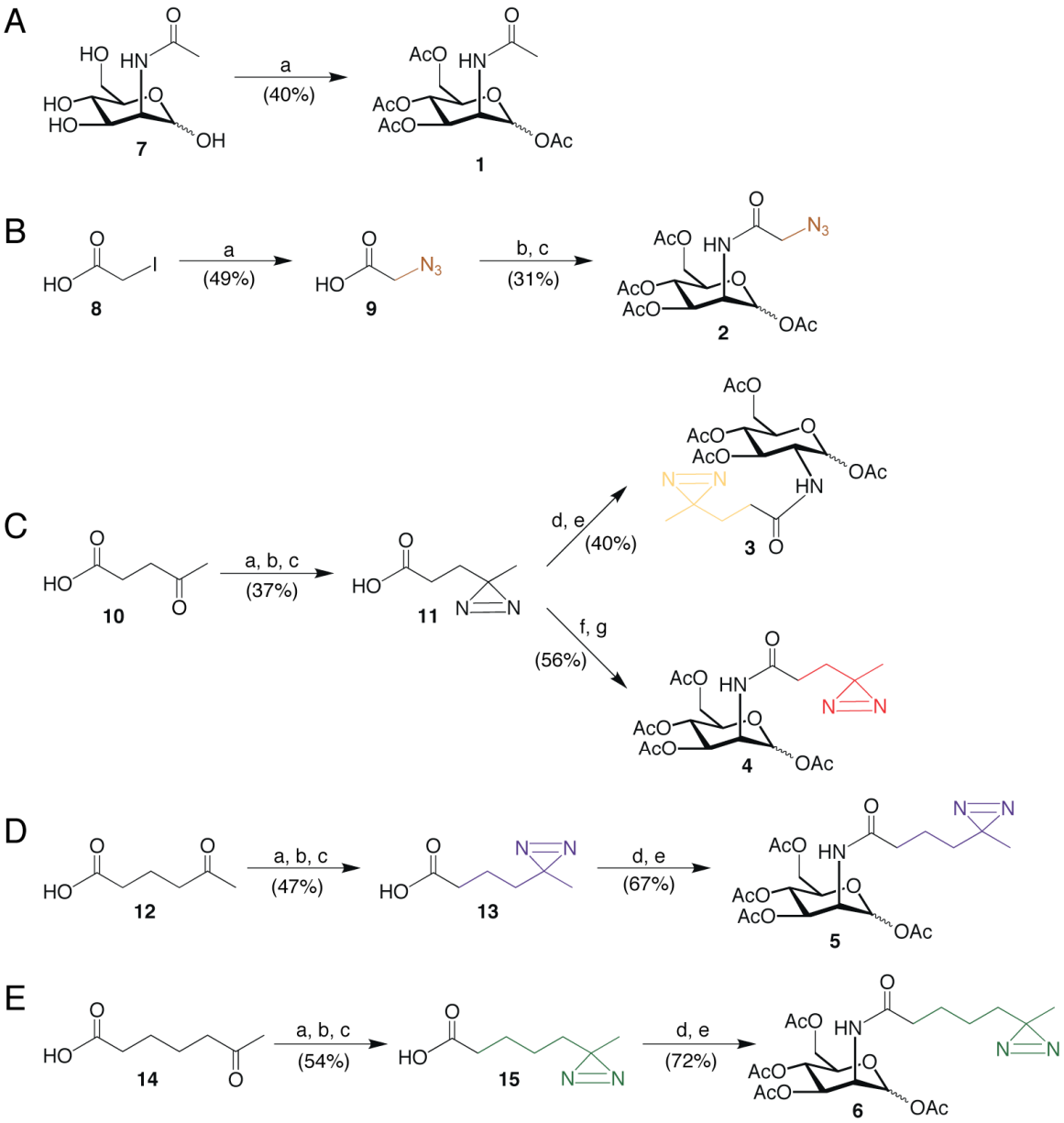
Syntheses of photocrosslinking sugars

Unless otherwise noted, chemicals were purchased from commercial suppliers and used as received without further purification. *N*-hydroxybenzyltriazole (HOBt) was purchased from AnaSpec (Fremont, CA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Waltham, MA) unless otherwise noted. To monitor reactions, analytical thin layer chromatography (TLC) on silica gel 60 F254 glass plates (EM Science; Gibbstown, NJ) was used, followed by staining with ceric ammonium molybdate (CAM). CAM stain was composed of 0.4% wt/v $Ce(SO_4)_2$, 10% wt/v ammonium molybdate tetrahydrate, and 10% sulfuric acid. Products were purified by flash column chromatography on silica gel 60 (particle size 40-63 or 60-200 mm, EM Science). ^{1}H-NMR, ^{13}C-NMR, and ^{31}P-NMR were recorded with Varian 300, 400, or 500 MHz spectrometers and reported in δ ppm scale. ^{1}H-NMR were referenced to $CDCl_3$ (7.260 ppm). ^{13}C-NMR spectra were referenced to the center of the $CDCl_3$ triplet (77.0 ppm). ^{31}P-NMR spectra were referenced to 85% phosphoric acid (0 ppm). Electrospray ionization mass spectrometry (ESI-MS) data were collected at the Protein Chemistry and Technology Center at UT Southwestern Medical Center by Dr. Yan Li. Mass spectrometry methods: samples were dissolved or diluted into either a solution that contained 50% acetonitrile and 5% NH_4OH or an acetonitrile:H_2O 1:1 solution that contained 0.1% formic acid. Proxeon nano-tips were used to infuse the samples into a QStar XL mass spectrometer (Applied Biosystems, Framingham, MA). Spectra were acquired with mass range m/z 50-1000 or 300-1000. Molecular weights were calculated using the Baysesian Protein Reconstruct tool of the Analyst QS1.1 software. To confirm the purity of the unnatural analogs, samples were assessed using reverse phase HPLC.



Scheme S1. **Synthesis of per-*O*-acetylated ManNAc and GlcNAc analogs.** (A) a) Ac_2O, pyridine. (B) a) NaN_3, ddH_2O, b) *D*-(+)-mannosamine hydrochloride, HOBt, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), triethylamine (TEA), MeOH, c) Ac_2O, pyridine. (C) a) 7 M NH_3/MeOH, b) NH_2OSO_3H, MeOH, c) MeOH, TEA, I_2, d) *D*-(+)-glucosamine hydrochloride, HOBt, EDC, TEA, MeOH, e) Ac_2O, pyridine, f) *D*-(+)-mannosamine hydrochloride, HOBt, EDC, TEA, MeOH, g) Ac_2O, pyridine. (D) a) 7 M NH_3/MeOH, b) NH_2OSO_3H, MeOH, c) MeOH, TEA, I_2, d) *D*-(+)-mannosamine hydrochloride, HOBt, EDC, TEA, MeOH, e) Ac_2O, pyridine. (E) a) 7 M NH_3/MeOH, b) NH_2OSO_3H, MeOH, c) MeOH, TEA, I_2, d) *D* -(+)-mannosamine hydrochloride, HOBt, EDC, TEA, MeOH, e) Ac_2O, pyridine.

*Synthesis of Ac_4ManNAc (**1**)*

Ac_4ManNAc was synthesized as previously reported (Scheme S1A).[1] To a solution of *N*-acetylmannosamine (ManNAc, **7**) (124 mg, 0.56 mmol) in 5.5 mL of pyridine, 1.57 mL of acetic anhydride (Ac_2O) was added at 4 ºC. The reaction was allowed to warm to room temperature (RT) as it stirred for 20 h. The reaction mixture was diluted with CH_2Cl_2 and washed

successively by 1.0 M HCl, saturated sodium bicarbonate, and brine. The organic layer was dried over sodium sulfate and the solvent evaporated *in vacuo*. The resulting residue was purified by column chromatography (hexanes / ethyl acetate (EtOAc) gradient = 5/1, 3/1, 1/1) to afford **1** in 90 mg yield as a colorless gum in 40% yield as a mixture of anomers. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.65 (3H, s), 2.02 (3H, s), 2.07 (3H, s), 2.11 (3H, s), 2.18 (3H, s), 4.10 (1H, dd, *J* = 2.3, 12.5 Hz), 4.28 (1H, t, *J* = 3.7 Hz), 4.78 (1H, ddd, *J* = 1.6, 3.9, 9.1 Hz), 5.06 (1H, d, 4.0 Hz), 5.13 (1H, t, *J* = 9.8 Hz), 5.33 (1H, d, *J* = 4.5 Hz), 5.79 (1H, d, *J* = 9.0 Hz), 5.86 (1H, d, *J* = 1.6 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 20.88, 20.90, 20.92, 20.96, 20.97, 21.01, 21.08, 23.56, 23.65, 49.52, 49.74, 62.20, 65.41, 65.62, 68.99, 70.29, 71.56, 73.68, 90.86, 91.90, 168.34, 168.55, 169.92, 169.93, 170.23, 170.32, 170.73, 170.74, 170.82. ESI-MS for $C_{16}H_{23}NO_{10}$ [M], calcd. for 389.13, found 389.12. Representative ^{1}H-NMR spectrum, ^{13}C-NMR spectrum, and HPLC trace are shown in Supplemental Figures S13, S17, and S21, respectively.

*Synthesis of 2-azido-acetic acid (**9**)*

Synthesis of 2-azido-acetic acid was based on literature procedure.[2] To 50 mL of water, sodium azide (5.6 g, 86 mmol) and iodoacetic acid (8.0 g, 43 mmol) were added. The reaction was stirred overnight at RT. The solution was diluted with 1 M HCl, followed by extraction of the product into EtOAc. The organic layer was then washed with saturated sodium bicarbonate and brine. The organic layer was the dried with sodium sulfate and solvent was evaporated *in vacuo*, yielding the 1.5 g of the **9** as a yellow oil in 49% yield.

*Synthesis of 4,4-azo-pentanoic acid (**11**)*

As described previously,[1, 3] to levulinic acid (**10**, 8.19 g, 70.5 mol) at 4 ºC, 70 mL of 7 M NH_3/MeOH was added via syringe. Solution was stirred on ice for 3 h. NH_2OSO_3H (9.16 g, 81.1 mol) in 60 mL of MeOH was then added dropwise at 4 ºC. Solution stirred and reached RT overnight, yielding a white slurry. Precipitate was removed by vacuum filtration and filtrate was reduced to oil of the diaziridine *in vacuo*. Residue was dissolved in MeOH (50 mL) and solution was stirred on ice. 15 mL of TEA was then added. Under foil, iodine was added portionwise until the solution sustained a red-brown color for several min. Solvent was removed *in vacuo*. Residue was dissolved in EtOAc and washed successively with 1M HCl, 10% sodium thiosulfate, and brine. The organic layer was dried with anhydrous sodium sulfate and concentrated *in vacuo* to afford **11** in 37% yield as an orange liquid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.41 (3H, s), 1.72 (2H, t, *J* = 7.2 Hz), 2.24 (2H, t, *J* = 7.5 Hz). ESI-MS for $C_5O_2N_2H_8$ $[M-H]^-$, calcd. for 127.05, found 127.05. Representative ^{1}H-NMR spectrum is shown in Supplemental Figure S10.

*Synthesis of 5,5-azo-hexanoic acid (**13**)*

To 5-oxohexanoic acid (**12**, 2.56 g, 19.67 mol) and 20 mL of 7 M NH_3/MeOH, NH_2OSO_3H (2.58 g, 22.81 mol) in 40 mL of MeOH was added dropwise and purified as described above. To residue of the diaziridine, 30 mL MeOH, 5 mL of TEA, and iodine were added and processed as above, yielding 1.31g **13** in 47% yield as a light orange liquid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.02 (3H, s), 1.43 (2H, m), 1.51 (2H, m), 2.35 (2H, t, *J* = 7.2 Hz). ESI-MS for $C_6O_2N_2H_{10}$ $[M-H]^-$, calcd. for 141.07, found 141.07. Representative ^{1}H-NMR spectrum is shown in Supplemental Figure S11.

*Synthesis of 6,6-azo-septanoic acid (**15**)*

To 6-oxoheptanoic acid (**14**, 0.5 g , 3.47 mmol) and 30 mL of 7 M NH_3/MeOH, NH_2OSO_3H (0.451 g, 4.0 mmol) in 20 mL of MeOH was added dropwise and purified as described above. To residue of the diaziridine, 25 mL MeOH, 10 mL of TEA, and iodine were added and processed as above, yielding 0.51g, **15** in 54% yield as a light orange liquid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.00 (3H, s), 1.23 (2H, m), 1.38 (2H, m), 1.61 (2H, q, *J* = 7.5 Hz), 2.33 (2H, t, *J* = 7.5 Hz). ESI-MS for $C_7O_2N_2H_{12}$ $[M-H]^-$, calcd. for 155.08, found 155.08. Representative ^{1}H-NMR spectrum is shown in Supplemental Figure S12.

*Synthesis of Ac_4ManNAz (**2**)*

Synthesis of Ac_4ManNAz was based on a literature procedure (Scheme S1B).[4] Briefly, to a solution of azido acetic acid (**9**, 120 mg, 1.0 mmol) in 5.0 mL MeOH, D-(+)-mannosamine hydrochloride (216 mg, 1.00 mmol), TEA (280 μL), EDC (383 mg, 2.00 mmol), and HOBt (136 mg, 1.01 mmol) were added as described above. Purification was also described above and yielded a crude product as a pale yellow gum. The crude product was immediately acetylated by the same procedure described in the synthesis of Ac_4ManNAc to afford Ac_4ManNAz, **2**. The reaction was treated as above and purification afforded 134 mg as a colorless gum in 31% yield in two steps as a mixture of anomers. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.96 (3H, s), 2.03 (3H, s), 2.08 (3H, s), 2.15 (3H, s), 4.02 (2H, s), 4.09 (1H, ddd, *J* = 2.0, 8.0, 14.4 Hz), 4.20 (1H, d, *J* = 1.8 Hz), 4.58 (1H, ddd, *J* = 4.2, 9.3, 13.5 Hz), 5.04 (1h, d, *J* = 3.9 Hz), 5.19 (1H, t, *J* = 10.1 Hz), 5.20 (1H, d, *J* = 4.3 Hz), 6.01 (1H, s), 6.65 (1H, d, *J* = 18.1 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 20.80, 20.84, 20.87, 20.91, 20.95, 20.98, 21.06, 49.44, 49.90, 52.55, 52.74, 61.84, 61.93, 65.08, 65.27, 69.02, 70.41, 71.62, 73.55, 90.42, 91.47, 166.94, 167.52, 168.30, 168.54, 169.76, 170.30, 170.35, 170.73. ESI-MS for $C_{16}H_{22}N_4O_{10}$ $[M-H]^-$, calculated for 429.13, found 429.14.

*Synthesis of 1,3,4,6-tetra-O-acetyl-2-acetyl-N-4,4-azo-pentamido-2-deoxy-α,β-D-mannopyranose (Ac_4ManNDAz(2me), **4**)*

As described previously (and see Scheme S1C), to a solution of 4,4-azo-pentanoic acid (**11**, 270 mg, 2.1 mmol) in 6 mL of methanol, D-(+)-mannosamine hydrochloride (160 mg, 0.72 mol) and TEA (350 mL) were added. The mixture was cooled to 4 ºC for the addition of EDC (475 mg, 2.5 mol) and HOBt (167 mg, 1.2 mmol). The reaction mixture was stirred for 20 h at RT. The solvent was evaporated *in vacuo*, leaving a yellow oil. The residue was purified with flash column chromatography (CH_2Cl_2 / MeOH = 1/0, 10/1, 4/1) yielding the unacetylated product as an oil. For 69 mg of the unacetylated product, acetylation was performed by the same procedure described in the synthesis of Ac_4ManNAc to afford Ac_4ManNDAz(2me), **4**. The reaction mixture was diluted with EtOAc and washed successively with 1 M HCl, saturated sodium bicarbonate, water, and brine. The organic layer was dried over anhydrous sodium sulfate and evaporated *in vacuo*. The residue was purified by flash column chromatography (hexanes/EtOAc = 1/1, 1/2). Rotary evaporation afforded 68 mg of **4** as a colorless gum in 56% yield as a mixture of anomers over two steps. ^{1}H-NMR (400 MHz, $CDCl_3$, major anomer): δ 1.06 (3H, s), 1.73- 1.85 (2H, m), 2.00-2.16 (2H, m), 2.02 (3H, s), 2.07 (3H, s), 2.11 (3H, s), 2.19 (3H, s), 4.03-4.08 (2H, m), 4.29 (1H, dd, *J* = 5.6, 12.8 Hz), 4.65 (1H, ddd, *J* = 1.2, 4.4, 9.2 Hz), 5.20 (1H, t, *J* = 10.0 Hz), 5.34 (1H, dd, *J* = 4.4, 10.4 Hz), 5.95 (1H, d, *J* = 9.2 Hz), 6.03 (1H, d, *J* = 2.0 Hz). ^{13}C-NMR (400 MHz, $CDCl_3$, major anomer): δ 19.9, 20.6, 20.7, 20.7, 20.8, 25.3, 29.7, 30.4, 49.2, 62.0, 65.3, 68.8, 70.1, 91.5, 168.1, 169.6, 170.0, 170.6, 171.5. ESI-MS for $C_{19}O_{10}N_3H_{27}$ $[M-H]^-$, calcd. for 456.16, found 456.17. Representative ^{1}H-NMR spectrum, ^{13}C-

NMR spectrum, and HPLC trace are shown in Supplemental Figures S14, S18, and S22, respectively.

*Synthesis of 1,3,4,6-tetra-O-acetyl-2-acetyl-N-5,5-azo-hexamido-2-deoxy-α,β-D-mannopyranose (Ac$_4$ManNDAz(3me), **5**)*

To a solution of 5,5-azo-hexanoic acid (**13**, 147 g, 1.03 mmol) in 4 mL MeOH, D-(+)-mannosamine hydrochloride (218 mg, 1.01 mol), TEA (278 μL), EDC (383 mg, 2.0 mol), and HOBt (132 mg, 0.97 mol) were added as described above (and see Scheme S1D). Purification was also as described above and yielded crude product as a pale yellow gum. The crude product was immediately acetylated by the same procedure described in the synthesis of Ac$_4$ManNAc to afford Ac$_4$ManNDAz(3me), **5**. The reaction was treated as above and purification afforded 319 mg as a colorless gum in 67% yield over two steps as a mixture of anomers. ^{1}H-NMR (300 MHz, $CDCl_3$, major anomer): δ 1.03 (3H, s), 1.35-1.55 (2H, m), 1.54-1.64 (2H, m), 1.99 (3H, s), 2.06 (3H, s), 2.11 (3H, s), 2.18 (3H, s), 2.27 (2H, m), 4.01-4.12 (2H, m), 4.29 (1H, dd, *J* = 5.4, 13.5 Hz), 4.63 (1H, ddd, *J* = 1.8, 4.5, 9.6 Hz), 5.17 (1H, t, *J* = 9.6 Hz), 5.32 (1H, dd, *J* = 4.5, 10.5 Hz), 5.80 (1H, d, *J* = 9.3 Hz), 6.02 (1H, d, *J* = 1.8 Hz). ^{13}C-NMR (500 MHz, $CDCl_3$, major anomer): δ 19.54, 20.15, 20.62, 20.73, 20.86, 25.48, 33.30, 35.41, 49.15, 61.94, 65.29, 68.89, 70.11, 71.37, 73.44, 91.64, 168.13, 169.59, 169.93, 170.55, 172.17. ESI-MS for $C_{20}O_{10}N_3H_{29}$ $[M-H]^-$, calcd. for 470.18, found 470.19. Representative ^{1}H-NMR spectrum, ^{13}C-NMR spectrum, and HPLC trace are shown in Supplemental Figures S15, S19, and S23, respectively.

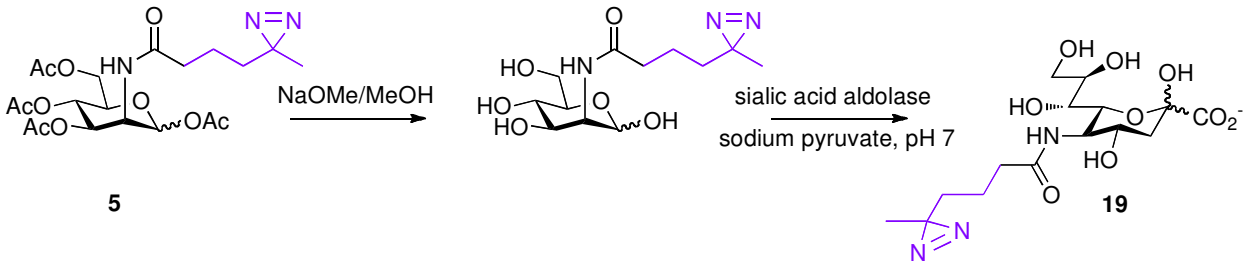
*Synthesis of 1,3,4,6-tetra-O-acetyl-2-acetyl-N-6,6-azo-septamido-2-deoxy-α,β-D-mannopyranose (Ac$_4$ManNDAz(4me), **6**)*

To a solution of 6,6-azo-heptanoic acid (**15**, 530 mg, 3.39 mmol) in 30 mL MeOH, D-(+)-mannosamine hydrochloride (25 mg, 1.00 mol), TEA (279 μL), EDC (383 mg, 2.0 mol), and HOBt (135 mg, 0.99 mol) were added as described above (and see Scheme S1E). Purification was also as described above and yielded crude product as a pale yellow gum. The crude product was immediately acetylated by the same procedure described in the synthesis of Ac$_4$ManNAc to afford Ac$_4$ManNDAz(4me), **6**. The reaction was treated as above and purification afforded 320 mg as a colorless gum in 72% yield over two steps as a mixture of anomers. ^{1}H-NMR (300 MHz, $CDCl_3$, major anomer): δ 1.00 (3H, s), 1.1641.27 (2H, m), 1.35-1.42 (2H, m), 1.57-1.66 (2H, m), 2.00 (3H, s), 2.06 (3H, s), 2.10 (3H, s), 2.18 (3H, s), 2.23 (2H, t, *J* = 7.5 Hz), 4.01-4.12 (2H, m), 4.29 (1H, ddd, *J* = 2.4, 8.1, 15.6 Hz), 4.65 (1H, ddd, *J* = 1.8, 6.0, 11.1), 5.16 (1H, t, *J* = 9.6 Hz), 5.79 (1H, t, *J* = 9.3 Hz), 6.02 (1H, d, *J* = 1.8 Hz). ^{13}C-NMR (500 MHz, $CDCl_3$, major anomer): δ 19.79, 20.64, 20.74, 20.78, 20.86, 23.57, 25.0, 25.66, 33.97, 36.18, 49.11, 61.97, 65.33, 68.85, 70.08, 91.68, 168.15, 169.65, 169.99, 170.52, 172.58. ESI-MS for $C_{21}O_{10}N_3H_{31}$ $[M-H]^-$, calcd. for 484.19, found 485.20. Representative ^{1}H-NMR spectrum, ^{13}C-NMR spectrum, and HPLC trace are shown in Supplemental Figures S16, S20, and S24, respectively.

*Synthesis of standards SiaDAz(2me) and SiaDAz(3me) (**19**)*

SiaDAz(2me) was prepared as described.[3] Synthesis of SiaDAz(3me) (**19**) followed a similar protocol (Scheme S2) and is described briefly. A NaOMe solution (20 μL, 1 M in anhydrous MeOH) was added to a solution of Ac$_4$ManNDAz(3me) (**5**) (1 μmol) in anhydrous MeOH (100 μL) at 0 °C. The solution was stirred at room temperature for 3 h. MeOH was removed by SpeedVac before the solid was redissolved in water and moved to a 1.5 mL centrifuge tube containing sodium pyruvate (100 mM), Tris-HCl buffer (100 mM, pH 7.5) and $MgCl_2$ (20 mM).

Finally *N*-acetylneuraminic acid aldolase (22.5 μL of 11.05U/mL, Cat# NAL-301, Toyobo CO., LTD) was added to bring the final volume of the reaction mixture to 50 μL. The reaction mixture was incubated with agitation at 125 rpm in a 37 °C incubator overnight. The reaction was terminated by addition of 50 μL of ice-cold EtOH followed by incubation at 4 °C for 30 min. The resulting suspension was then filtered in 10 kDa MWCO filter by centrifugation and the resulting flow-through was evaporated to yield a white powder. ESI-MS for $C_{15}H_{25}N_3O_9$ $[M-H]^-$, calcd. 390.15, found 390.14.



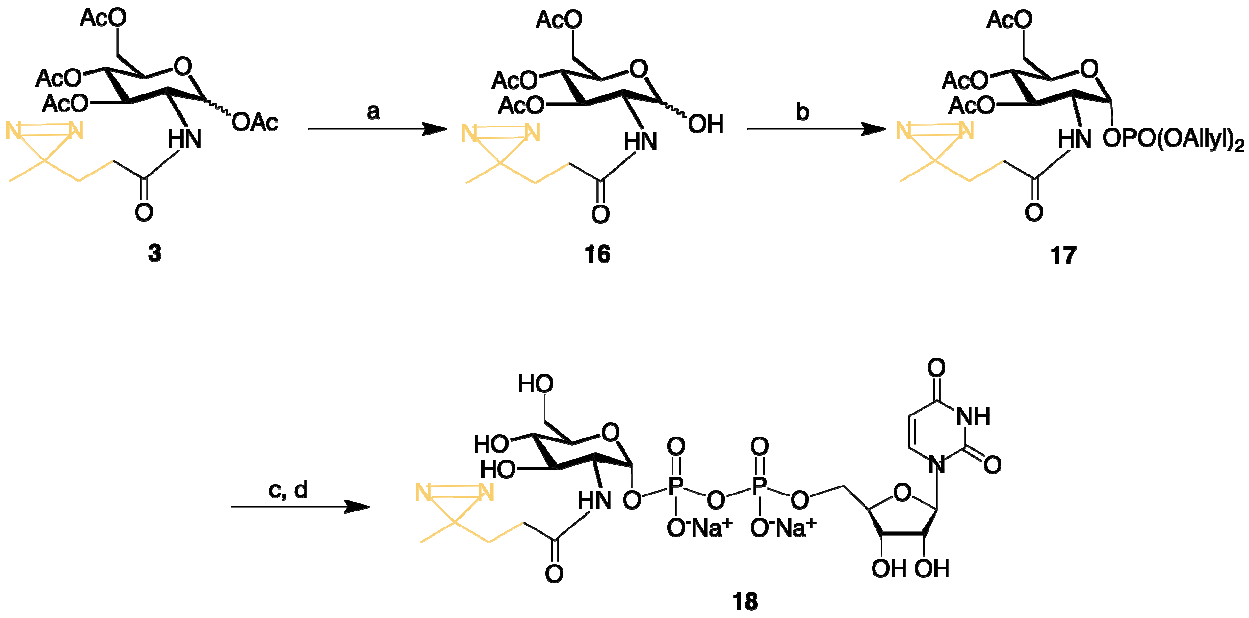
Scheme S2. **Synthesis of SiaDAz(3me) standard.** SiaDAz(3me) (**19**) was prepared from Ac_4ManNDAz(3me) for use as a standard in DMB derivatization experiments.

*Synthesis of 1,3,4,6-tetra-O-acetyl-2-acetyl-N-4,4-azo-pentamido-2-deoxy-α,β-D-glucopyranose (Ac_4GlcNDAz(2me), **3**)*

The synthesis of Ac_4GlcNDAz follows published procedures (Scheme S1C).[1, 3] Briefly, to a solution of 4,4-azo-pentanoic acid (**11**, 420 mg, 3.27 mmol) in 20 mL of methanol, D-(+)-glucosamine hydrocholride (640 mg, 2.89 mol) and TEA (1.25 mL) were added. The mixture was cooled to 4 ºC for the addition of EDC (1.09 g, 5.7 mol) and HOBt (440 mg, 3.3 mmol). Solvent was removed *in vacuo* and the sample triturated with 100% ice-cold ethanol and methylene chloride and the resulting solid filtered. The solid was dried to obtain a white, crystalline solid GlcNDAz, which was acetylated. For 107 mg (0.35 mmol) of GlcNDAz, the acetylation was performed by the same procedure described in the synthesis of Ac_4ManNAc to afford Ac_4GlcNDAz(2me), **3**. The reaction was treated as above and purification afforded 150 mg of a pale yellow oil in 40.0% yield as a mixture of anomers over two steps. ^{1}H NMR (400 MHz, $CDCl_3$, major anomer): δ 1.01 (3H, s), 1.74-1.79 (2H, m), 1.83-1.93 (2H, m), 2.05 (3H, s), 2.07 (3H, s), 2.10 (3H, s), 2.20 (3H, s), 3.98-4.02 (1H, m), 4.07 (1H, dd, J = 2.4, 12.4 Hz), 4.25-4.30 (2H, m), 5.19-5.28 (2H, m), 5.59 (1H, d, J = 8.8 Hz), 6.19 (1H, d, J = 3.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, major anomer): δ 20.0, 20.5, 20.7, 20.8, 20.9, 25.3, 29.2, 30.3, 51.1, 61.5, 67.4, 69.6, 70.5, 90.4, 168.6, 169.1, 171.2, 171.2, 171.8. HRMS (ES-TOF) for $C_{19}H_{27}N_3O_{10}Na$ $[M+Na]^+$, calcd. for 480.1594, found 480.1597.

*Synthesis of UDP-GlcNDAz(2me) (**18**)*

UDP-GlcNDAz(2me) (**18**) was prepared from Ac_4GlcNDAz (**3**), as described below (Scheme S3).



Scheme S3. **Synthesis of UDP-GlcNDAz(2me) (18).** a) $BnNH_2$, tetrahydrofuran, 72%. b) i) diallyl *N*,*N*-diisopropylphosphoramidite, tetrazole, AcCN, ii) mCPBA, 59%. c) Sodium *p*-toluenesulfinate, $Pd(PPh_3)_4$, tetrahydrofuran-MeOH (1:1). d) i) UMP salt, tetrazole, pyridine, ii) Dowex 50WX8-100 (Na^+ form), 60 %.

*Synthesis of Ac_3GlcNDAz(2me)-1-OH (**16**)*

To the solution of Ac_4GlcNDAz(2me) (**3**, 457 mg, 1.0 mmol) in THF (4.0 ml), benzylamine (164 µl, 1.5 mmol) was added and the resulting solution was stirred overnight. After removing the solvent by evaporation, the crude mixture was purified by flash silica gel chromatography (EtOAc) to give Ac_3GlcNDAz(2me)-1-OH (**16**) a colorless foam (300 mg, 72.2%). ^{1}H NMR (300 MHz, $CDCl_3$, α anomer, α: β = 6 : 1): δ 1.01 (3H, s), 1.74 (2H, m), 1.93-1.99 (2H, m), 2.03 (3H, s), 2.04 (3H, s), 2.10 (3H, s), 3.23(1H, bs , *J* = 2.7 Hz, -OH), 4.10-4.23 (2H, m, H5, H6a, H6b), 4.29(1H, td, *J* = 3.0, 9.6 Hz, H2), 5.13 (1H, t, *J* = 9.9 Hz), 5.25-5.32 (2H, m), 5.78 (1H, d, *J* = 9.6 Hz), 6.19 (1H, d, *J* = 3.6 Hz). ^{13}C NMR (125 MHz, $CDCl_3$, major anomer): δ 19.8, 20.6, 20.8, 25.4, 30.5, 52.2, 62.0, 67.5, 68.2, 70.7, 91.5, 169.4, 171.0, 171.5, 171.6, ESI MS for $C_{17}H_{26}N3O_9$ $[M+H]^+$, calcd. for 416.17, found 416.17.

*Synthesis of Ac_3GlcNDAz(2me)-1-O-P(=O)(OAllyl)$_2$ (**17**)*

To the solution of **16** (300 mg, 0.72 mmol) in acetonitrile (AcCN) (2 mL) at 0 °C, were added diallyl *N*,*N*-diisopropylphosphoramidite (228 µl, 0.864 mmol) and 1*H*-tetrazole (2.88 mL, 0.45M in AcCN, 1.3 mmol). After stirring for 1 h at RT, meta-chloroperoxybenzoic acid (290 mg, 77% mCPBA, 1.3 mmol) was added at 0 °C. After 30 min, the reaction mixture was partitioned between EtOAc (30 mL) and 10% aqueous Na_2SO_3 and the organic layer was washed with saturated aq. $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4), concentrated and purified by silica gel chromatography (CH_2Cl_2:MeOH=40:1) and then re-crystallized in EtOAc-hexane to provide **17** as white solid (245mg, 0.425 mmol, 59%). ^{1}H NMR (500 MHz, $CDCl_3$, α anomer): δ 1.00 (3H, s), 1.64-1.79 (2H, m), 1.86-2.00 (2H, m), 2.02 (6H, s), 2.07 (3H, s), 4.07(1H, bd , *J* = 12.0 Hz), 4.19 (1H, m), 4.24(1H, dd, *J* = 4.0, 12.5 Hz), 4.41(1H, m), 4.55-

4.61(m, 4H), 5.17 (1H, t, J = 10.0 Hz), 5.22(1H, t, J = 10.0), 5.28-5.42 (4H, m), 5.66 (1H, dd, J = 3.0, 5.5 Hz), 5.88-6.00 (2H, m), 6.03(1H, bd, J = 9.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.9, 20.6, 20.7 (2s), 25.3, 29.4, 30.27, 51.8(2s), 51.84, 61.3, 67.3, 68.7(2s), 68.8(2s), 69.7, 69.9, 96.0, 119.1(2s), 131.8, 131.9, 132.0, 132,1, 169.1, 170.5, 171.3, 171.4; ^{31}P NMR (200MHz, $CDCl_3$): δ -2.376 (s); ESI-MS for $C_{23}H_{35}N_3O_{12}P$ $[M+H]^+$, calcd. for 576.20, found 576.20, $C_{23}H_{34}N_3NaO_{12}P$ $[M+Na]^+$, calcd. for 598.18, found 598.18.

Synthesis of UDP-GlcNDAz(2me) ***(18)***

To the solution of Ac_3GlcNDAz(2me)-1-O-diallylphosphate (**17**, 200 mg, 0.347 mmol) in THF-MeOH (1:1, 4ml) at RT, were added sodium *p*-toluenesulfinate (124 mg, 0.695 mmol) and $Pd(PPh_3)_4$ (20 mg, 17.3 μmol). After stirring for 1 h at RT, the resulting mixture was concentrated and then co-evaporated with dry pyridine (2 mL) three times and then used for the next step without purification. To the crude mixture (Ac_3GlcNDAz(2me)-1-OPO_3^{2-} $2Na^+$) was added UMP salt (uridine 5'-monophosphomorpholidate 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidine salt, 537 mg, 0.832 mmol) and the mixture was co-evaporated in pyridine (2 mL, three times). To the residue, 1H-tetrazole (2.31 mL, 0.45M in AcCN, 1.04 mmol) was added and the resulting mixture was evaporated to remove AcCN. The resulting mixture was co-evaporated with pyridine (2mL) and 4 mL of pyridine was added and then concentrated until ~2 mL of pyridine was left, and then the resulting mixture was stirred for at RT. After 5 days, the reaction mixture was concentrated thoroughly to remove pyridine and the residue was re-dissolved in MeOH:water:TEA (8 mL, 5:2:1) and then stirred for 24 h. The resulting mixture was concentrated by evaporation and then partitioned between CH_2Cl_2 (12 mL × 3) and water (12 mL × 3). The aqueous layer was lyophilized and the residue was purified by Bio-gel P2 (2.6 cm × 60 cm) column by elution with 0.1% aq. NH_4HCO_3. The pure compound containing fractions were collected and lyophilized. The dried residue was re-dissolved in 3 mL of water and then passed through Dowex 5WX8-100 (Na^+ form) and then lyophilized to give UDP-GlcNDAz(2me) disodium salt (**18**, 149 mg, 0.207 mmol, 60% from **17**). ^{1}H NMR (500 MHz, D_2O, α anomer): δ 1.00 (3H, s), 1.60-1.70 (2H, m), 2.21-2.32 (2H, m), 3.53(1H, t, J = 9.5 Hz), 3.76-3.86(3H, m), 3.91(1H, m), 3.98(1H, m), 4.15-4.26(3H, m), 4.34 (2H, m), 5.50(1H, dd, J = 3.5, 7.0 Hz), 5.95(2H, m), 7.93(1H, d, J = 8 Hz). ^{13}C NMR (125 MHz, D_2O): δ 18.6, 26.4, 29.9, 30.25, 53.5, 53.7, 53.8, 60.4, 65.1, 69.6, 69.7, 71.0, 73.1, 73.9, 83.2(2s), 88.7, 94.7, 102.8, 141.6, 153.0, 167.9, 176.1. ^{31}P-NMR (200 MHz, D_2O): δ -11.28 (d, J = 20.6Hz), -13.04 (d, J = 20.6 Hz). ESI-MS for $C_{20}H_{30}N_5Na_2O_{17}P_2$ $[M+H]^+$, calcd. 720.09, found 720.10, $C_{20}H_{29}N_5Na_3O_{17}P_2$ $[M+Na]^+$, calcd. 742.07, found 742.08.

Reverse-phase HPLC analysis of compound purity

Acetylated sugars **1**-**6** were evaporated to near dryness and resuspended in a 2:1 ddH_2O:acetonitrile solution. Generally, a GRACE analytical Vydac 218TP c18 5u column was used with 250 mm length and an internal diameter of 4.6 mm. Sugars were separated by reverse phase HPLC with a gradient of 5-40% acetonitrile (ddH_2O) with 0.1% trifluoroacetic acid (TFA) and detected by absorbance at 210 nm. All compounds were to be at least 95% pure prior to use in experiments. In the case of sugar purification by preparatory column, a similar method was used, but no TFA was present in the solvents.

Effects of compounds on cell growth and viability

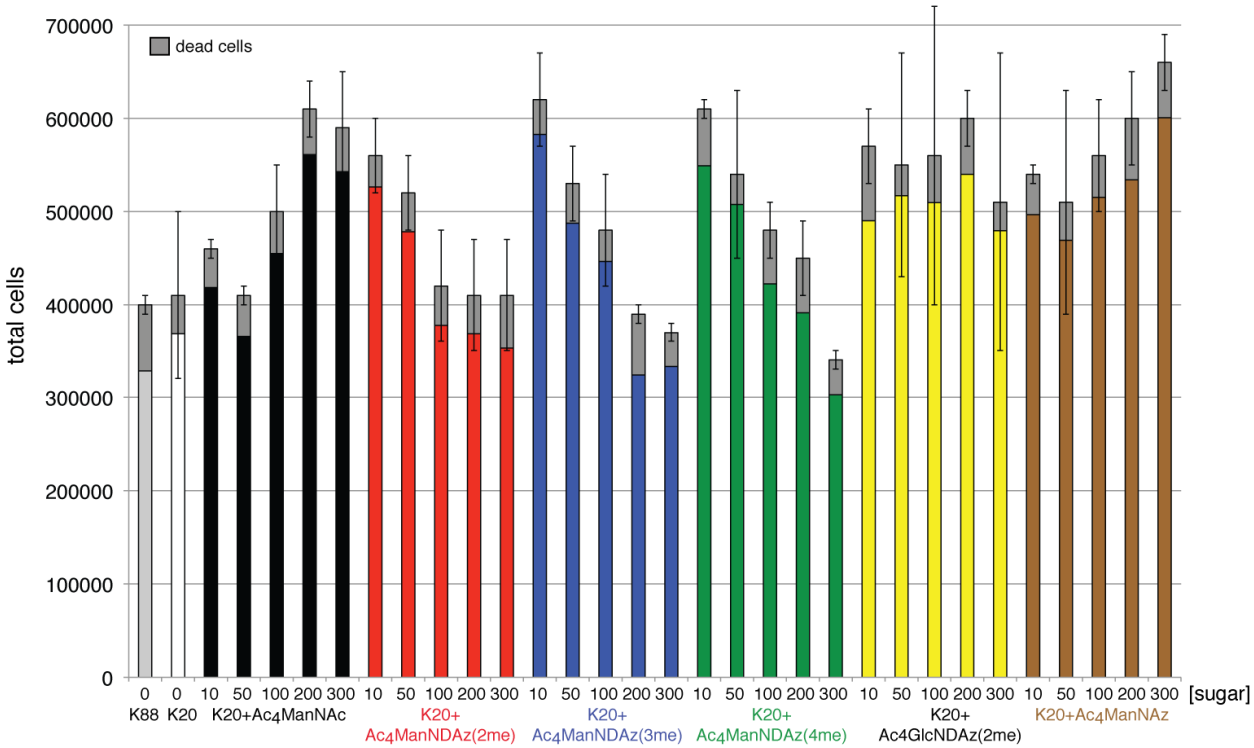


Figure S1. **Cell growth and viability of BJA-B cells grown in serum free media with photocrosslinking sugar analogs.** Cells (5 x 10^6 per sample) were treated with increasing concentrations (10, 50, 100, 200, and 300 μM) of Ac_4ManNAc, Ac_4ManNAc analogs, or Ac_4GlcNDAz(2me). With photocrosslinking sugar analogs, higher concentrations yielded less cell growth. The total number of live cells was determined by Trypan blue staining. No significant variation in the percentage of live cells was observed.

Cell culture, flow cytometry, and immunoblotting

Materials

RPMI 1640 with 2 mM L-glutamine, fetal calf serum, heat-inactivated fetal bovine serum (FBS), penicillin/streptomycin, DPBS, PBS (pH 7.4), FITC-streptavidin, DTAF-streptavidin, *N*-(aminooxyacetyl)-*N*'-(D-biotinoyl) hydrazine trifluoroacetic acid salt (ARP), and propidium iodide were purchased from Invitrogen (Carlsbad, CA). Nutridoma SP and bovine serum albumin (BSA) Fraction V were purchased from Roche Applied Science (Indianapolis, IN). Aniline was purchased from Sigma-Aldrich (St. Louis, MO). Glycerol was purchased from Fisher Scientific (Waltham, MA). Complete™ Protease Inhibitor Cocktail Tablet, EDTA-free were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The Invitrogen Countess Automated Cell Counter and Trypan Blue exclusion were used for cell counting. BD Biosciences FACSCalibur Flow Cytometer was used for flow cytometry experiments. Antibodies and proteins were obtained from the following vendors: goat anti-rabbit-HRP (Invitrogen), anti β-actin (Abcam, Cambridge, MA), rabbit anti-CD22 (Abcam), streptavidin-DTAF (Jackson Immunoresearch; West Grove, PA), goat anti-LaminB (Santa Cruz Biotechnology, Santa Cruz, CA) and rabbit anti-calnexin (Santa Cruz Biotechnology). Cholera toxin B (CTxB) subunit from *Vibrio cholerae* was purchased from Sigma Aldrich (St. Louis, MO). The antibody against the CTxB subunit was purchased from Abcam, rabbit anti-CTxB. CTxB-HRP and CTxB-488 were purchased from Invitrogen. Lectins were purchased from the following vendors: SNA-FITC (EY Laboratories, San Mateo, CA), ECA-FITC (EY Laboratories), MAL-II-biotin (Vector Labs; Burlingame, CA; B-1265), and HPA-FITC (EY Laboratories). PVDF membranes were purchased from Millipore, Immobiolon (Billerica, CA). For chemiluminescent visualization, SuperSignal WestPico Chemiluminescent Substrate (ECL reagent) was purchased from Pierce (Rockford, IL). The bicinchoninic acid (BCA) Protein Assay Kit from Pierce was used to assess protein concentration. All other reagents, chemicals, and general supplies were purchased and used as received from Fisher Scientific or Sigma-Aldrich unless otherwise noted.

The mild stripping buffer used was composed of 200 mmol glycine, 3.5 mmol SDS, 1% v/v Tween20 at pH 2.2. Tris-buffered saline Tween-20 was used in blocking solutions (TBST, 10 mM Tris base, 150 mM NaCl, 0.1% v/v Tween 20, pH 8.0).

Detection of total cell surface sialic acid by flow cytometry

We used the PAL method, as previously described.[5] Cells were resuspended in PBS (pH = 7.4) and aliquoted in a v-bottom 96 well plate to approximately 4.0 x 10^5 cells per well. Cells were pelleted by centrifugation (650*g* for 4 min at 4 °C) and the supernatant removed by inverting the plate over the sink. Cells were washed a total of three times in the same manner with PBS (pH = 7.4). Next, cells were resuspended in 200 µl 1.0 mM $NaIO_4$ in PBS (pH = 7.4) and incubated for 30 min on ice. To the sodium periodate solution, 50 µl of 5.0 mM glycerol in PBS (pH = 7.4) was added and incubated for 10 min on ice to quench the oxidation reaction. Cells were pelleted by centrifugation (650*g* for 4 min at 4 °C) and washed twice with 5.0% FBS in DPBS (pH = 6.7). Cells were resuspended in 200 µl of 0.1 mM aminooxy-biotin, 10.0 mM aniline in 5.0 % FBS in DPBS (pH = 6.7) and incubated for 90 min on ice. Following three washes with 5.0% FBS in DPBS (pH = 6.7), cells were resuspended in 200 µl of 3.2 µg/ml DTAF-streptavidin in 5.0% FBS in DPBS for 30 min on ice. Finally, cells were washed three more times with 5.0% FBS in DPBS (pH = 6.7) and resuspended in 400 µl of 5.0% FBS in PBS (pH = 6.7). Cells were

analyzed with a FACSCalibur Flow Cytometer. Live cells (10,000 cells/sample) were identified by their forward scatter versus side scatter and DTAF fluororescence was measured on the FL-1 channel of the instrument. Data were analyzed with FlowJo (Tree Star, Inc.; Ashland, OR). Representative data are shown.

Detection of α2-3-linked and α2-6-linked cell surface sialic acid by flow cytometry
Cells were resuspended in 0.1% BSA in DPBS and aliquoted in a v-bottom 96 well plate to approximately 4.0 x 10^5 cells per well. Cells were pelleted by centrifugation (650*g* for 4 min at 4 °C) and the supernatant removed by inverting the plate over the sink. Cells were washed a total of three times in the same manner with DPBS.

For the SNA binding experiment, cells were incubated with 150 µL of 10 µg/ml SNA-FITC (specific for Siaα2-6Gal) in 0.1% BSA in DPBS for 30 min on ice. After being washed twice with 0.1% BSA in DPBS, the cells were resuspended in 400 µl of 0.1% BSA in DPBS. To distinguish the difference between live and dead cells, propidium iodide (PI) was added into each tube before analysis. Using a FACSCalibur Flow Cytometer, the cells were analyzed and live cells (10,000 cells/sample) were identified by their forward scatter versus side scatter. In addition, all PI positive cells (dead cells) were excluded from analysis. FITC fluorescence was measured on the FL-1 channel of the instrument and PI was measured on the FL-2 channel of the instrument.

For the MAL-II binding experiment, after the three wash steps, cells were incubated with 150 µL of 10 µg/ml MAL-II-biotin (specific for Siaα2-3Gal)[6] in 0.1% BSA/PBS for 30 min on ice. Cells were then washed twice with 0.1% BSA in DPBS and incubated with 10 µg/ml of streptavidin-FITC for 30 min on ice. Cells were washed two final times with 0.1% BSA in DPBS and resuspended in 400 µl of 0.1% BSA in DPBS for flow cytometry analysis. Cells were treated with PI and analyzed as described above for SNA-FITC binding. Data were analyzed with FlowJo (Tree Star, Inc.). Representative data are shown.

Detection of core glycan structures by flow cytometry
Cells were resuspended in 0.1% BSA in DPBS and aliquoted in a v-bottom 96 well plate to approximately 4.0 x 10^5 cells per well. Cells were pelleted by centrifugation (650*g* for 4 min at 4 °C) and the supernatant removed by inverting the plate over the sink. Cells were washed a total of three times in the same manner with DPBS.

For ECA and HPA binding experiments, cells were incubated with 150 µL of 5 µg/ml ECA-FITC (specific for LacNAc)[7] or 10 µg/ml HPA-FITC (specific for GalNAc)[8] in 0.1% BSA in DPBS for 30 min on ice. After being washed twice with 0.1% BSA in DPBS, the cells were resuspended in 400 µl of 0.1% BSA/DPBS. To distinguish the difference between live and dead cells, PI was added to each tube before analysis. Using a FACSCalibur Flow Cytometer, the cells were analyzed and live cells (10,000 cells/sample) were first identified by their forward scatter versus side scatter. In addition, all PI positive cells (dead cells) were excluded from analysis. FITC fluorescence was measured on the FL-1 channel of the instrument and PI was measured on the FL-2 channel of the instrument. Data were analyzed with FlowJo (Tree Star, Inc.). Representative data are shown.

CD22 photocrosslinking in BJA-B cells

For crosslinking study, BJA-B K88 and K20 cells (3.0 x 10^5 cells/mL) or Daudi cells (0.25 x 10^5 cells/mL) were cultivated with or without per-*O*-acetylated sugars in serum free media (or normal media for Daudi cells) as described above. Cells were harvested and washed twice with DPBS. Cells were resuspended in an appropriate volume of DPBS to achieve a final density of 1.0 x 10^6 cells/mL. 1.0 mL of each cell suspension was added to two identical 12-well plates which where were placed on ice. One plate was photoirradiated by 365 nm UV light (Black Ray Lamp, XX-20BLB, UVP) on ice for 10 min while the other remained covered with aluminum foil. Cells were then transferred to microcentrifuge tubes and the wells of the plate washed and added to the respective tube. Cell suspensions were centrifuged at 650*g* for 5 min at RT followed by aspiration of the supernatant.

Cells were washed twice with DPBS before lysis buffer (200 μL) was added. After incubation in lysis buffer (300 mM NaCl, 50 mM Tris (pH = 8.0), 1% Triton X-100 and 1 mM EDTA and protease inhibitors) at 4 ºC for 1 h in the dark, lysates were centrifuged at 17,000*g* for 4 min at RT. Supernatants were transferred to new microcentrifuge tubes and stored at 4 ºC until used.

Cell lysates were resolved on BioRad 7.5% Tris HCl gels and transferred to PVDF membranes overnight at 4 ºC. The PVDF membrane was pre-hydrated in methanol for 2 min. The membranes were blocked with 5% milk in TBST for 5 h and then probed with anti-CD22 antibody (1:1000 dilution) in 5% milk in TBST at 4 ºC overnight followed by three washes in TBST. After being probed with HRP conjugated goat anti-rabbit antibody (1:5000 dilution) for 1 h, the membranes were then washed three times with TBST followed by incubation with ECL reagent and exposure to film. Finally, the membrane was probed by a β-actin antibody to confirm similar loading in every lane. The membrane was first stripped with a mild stripping buffer for 1 h at 37 °C, then washed twice with TBST. The PVDF was blocked with 5% milk in TBST for 1 h followed by incubation with rabbit anti-β-actin antibody (1:10,000 dilution) overnight at 4 °C in 5% milk in TBST. The membrane was then washed three times with TBST followed by incubation with goat anti-rabbit-HRP conjugate (1:5000 dilution) for 1 h at RT. The membrane was then washed three times with TBST followed by incubation with ECL reagent and exposure to film.

CTxB-GM1a photocrosslinking in Jurkat cells

Prior to the addition of cells to a 12 well plate, EtOH, Ac_4ManNAc, Ac_4ManNDAz(2me), Ac_4ManNDAz(3me), Ac_4ManNDAz(4me), Ac_4GlcNDAz(2me), or Ac_4ManNAz in EtOH were added to achieve a final concentration of 100 μM and the EtOH pre-evaporated at ambient temperature and pressure. Jurkat cells were then seeded at a density of 2.5 x 10^5 cells/mL and incubated in the presence of the monosaccharides. After culturing cells with compounds for 70 h, cells were pelleted at 650*g* followed by resuspension in RPMI 1640 media containing 2 mM L-glutamine and supplemented with 10% heat-inactivated FBS to 5.0 x 10^6 cells/mL. Cells (1.0 x 10^6 cells in 200 μL) were then aliquoted to the wells of a 24 well tissue culture plate. To each well, 0.5 mL of 1 mg/mL cholera toxin beta subunit (CTxB) was added and the plate swirled. Plates were then incubated at 4 °C for 45 min. After pre-incubation with CTxB, plates were placed on ice and those designated as +UV were exposed to 365 nm light for 45 min on ice – approximately 2 cm away from bulb. Those plates designated as –UV were stored under aluminum foil, on ice during this 45 min time period.

After photoirradiation, cells were transferred to a 1.5 mL microcentrifuge tube and centrifuged for 5 min at 650*g*. The supernatant was removed by aspiration. Cell pellets were washed twice with 200 μL of DPBS and then lysed in 100 μL of 300 mM NaCl, 1 mM EDTA, 1% Triton X-100, and protease inhibitors at 4 °C for 60 min. Insoluble material was removed by centrifugation at 17,000*g* for 10 min and the supernatant was analyzed by immunoblot analysis. Cell lysates were mixed in a 1:1 ratio with 2X SDS loading dye containing DTT (100 mM final concentration). Samples were heated at 90 °C for 5 min prior to loading. Cell lysates were resolved on BioRad 12% Tris HCl gels and transferred to PVDF membranes (pre-hydrated in MeOH) overnight at 4 ºC. Membranes were probed twice, first for the presence of CTxB or GM1a and second for β-actin.

One membrane was probed for the presence of CTxB. The PVDF was blocked with 5% milk in TBST for 1 h followed by incubation with a rabbit anti-CTxB antibody (1:10000 dilution) for 1 h at RT in 5% milk in TBST. The membrane was washed three times with TBST followed by incubation with goat-anti-rabbit-HRP conjugate (1:5000 dilution) for 1 h at RT. The membrane was then washed three times with TBST followed by incubation with ECL reagent and exposure to film.

A second membrane was probed for the presence of GM1a using HRP-conjugated CTxB. The membrane was blocked with 5% milk in TBST followed by incubation with CTxB-HRP (1:10,000 dilution) in 5% milk in TBST for 5 h at RT. The membranes were then washed three times with TBST followed by incubation with an ECL reagent and exposure to film.

All membranes were subsequently probed with a β-actin antibody to confirm that equal amounts of samples were loaded in each lane. The membrane was first stripped with a mild stripping buffer for 1 h at 37 °C, then washed twice with TBST. The PVDF was blocked with 5% milk in TBST for 1 h followed by incubation with a rabbit anti-β-actin antibody (1:10,000 dilution) overnight at 4 °C in 5% milk in TBST. The membrane was washed three times with TBST followed by incubation with goat anti-rabbit-HRP conjugate (1:5000 dilution) for 1 h at RT. The membrane was then washed three times with TBST followed by incubation with ECL reagent and exposure to film.

Diazirine-containing sugars are stable to periodate oxidation conditions

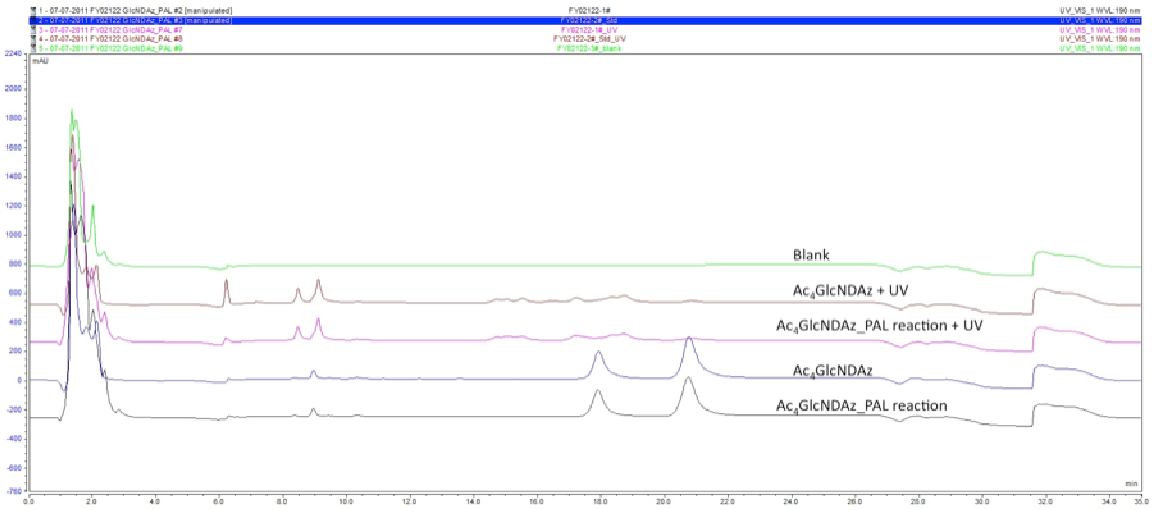


Figure S2. **PAL oxidation reaction does not affect diazirine functionality *in vitro*.** A diazirine-containing sugar subjected to periodate oxidation conditions and its integrity was analyzed by HPLC. Ac_4GlcNDAz (10 μM, 50 μL) was incubated with or without sodium periodate (10 mM in PBS buffer pH 7.4, 100 μL) for 30 min on ice. The reactions were quenched with glycerol (5 mM in PBS buffer pH 7.4, 250 μL) and incubated for 10 min on ice. Half of each sample (+ $NaIO_4$, - $NaIO_4$) was irradiated with UV (365nm, XX-20BLB, UVP) for 30 min on ice. The reaction mixtures were injected on Symmetry®C18 column (Waters, 5μm, 4.6 x 150 mm). The analysis was performed using Dionex Ultimate 3000 pump equipped with an Ultimate 3000RS variable wavelength detector. Sugars were separated using a gradient of 5-40% acetonitrile in water and monitored at 190 nm.

Sialic acid concentration determination using resorcinol/periodate assay

The following procedure is based on literature protocols.[9, 10] BJA-B cells (3.0 x 10^5 cells/mL) were cultivated with and without sugar supplementation to achieve a final concentration of 100 μM. The cells were incubated at 37 ºC for 72 h and then harvested and aliquoted with 10 million cells/sample. Cells were washed three times with 0.5 mL of Dulbecco's phosphate buffered saline (DPBS), centrifuged at 650*g* for 5 min at RT, and the supernatant aspirated. Cells were then resuspended in 250 μL of DPBS and lysed in 4 freeze/thaw cycles. All samples were oxidized with periodic acid (8 mM final concentration) on ice (for total cell sialic acid concentration) or at 37 ºC (for glycoconjugate-bound sialic acid) for 1.5 h. After adding 500 μL resorcinol solution (6.0% (w/v) resorcinol, 2.5 mM $CuSO_4$, 44% (v/v) HCl), samples were boiled at 100 ºC for 15 min. Samples were cooled on ice and 500 μL of t-butanol was added to quench the reaction. Samples were centrifuged at 13300*g* for 5 min to pellet insoluble cell debris. Immediately after centrifugation, 1 mL of samples aliquoted into cuvettes. Absorbance readings of samples were then taken at 630 nM and absorbance intensities were compared to a standard curve of known concentrations – 250, 125, 62.5, 31.3, 15.63, 7.81, 3.91, 1.95, 0 μM sialic acid – (which were treated in the same fashion as described above). Absorbance was measured on Ultrospec 2100 Pro UV/Visible spectrophotometer from Amersham Biosciences. The results are shown in Figure S3.

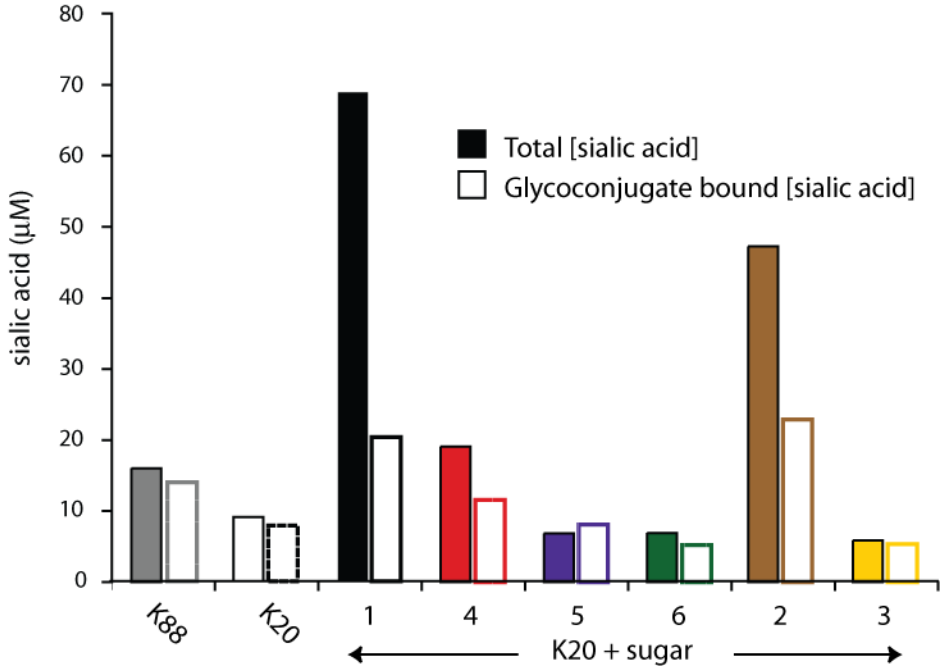


Figure S3. **Resorcinol assay to determine total and glycoconjugate bound sialic acid concentrations.** Cells treated with and without unnatural sugar analogs yield differing amounts of total cell and glycoconjugate bound sialic acids. The largest increases in absorbance were seen for Ac_4ManNAc-, Ac_4ManNAz-, and Ac_4ManNDAz(2me)-treated BJA-B K20 cells cultured in serum-starved conditions. The large differences in total and glycoconjugate bound sialic acids suggest that cells cultured with Ac_4ManNAc, Ac_4ManNAz, and Ac_4ManNDAz(2me) have a significant pool of free sialic acid present. These data represent one biological replicate and are representative of two separate experiments.

HPLC analysis of DMB derivatization

For HPLC, standards for DMB-Neu5Ac, DMB-SiaDAz(2me), and DMB-SiaDAz(3me) were used. Derivatized sugars (10 µL each) were diluted with 90 µL ddH_2O and separated on a Waters Symmetry c18, 5 µm, 4.6 x 150 mm column using a gradient of ddH_2O/acetonitrile gradient, as specified in the supplementary material. A Shimadzu SCL-10A VP HPLC with the RF-10AXL detector and Class-VP 7.4 software was used. The fluorophore was excited at 373 nm and the emission detected at 448 nm. Peaks were manually defined and the area under the curve integrated.

HPLC traces (Figures S4-S6) show raw data used to derive the percentages shown in Table 1. The identities of DMB-SiaDAz(2me) and DMB-SiaDAz(3me) were confirmed by LC-MS analysis. LC-MS for DMB-SiaDAz(2me), $C_{21}H_{27}N_5O_9$ [M], calcd. 493.18, found 493.91. LC-MS for DMB-SiaDAz(3me), $C_{22}H_{30}N_5O_9$ $[M+H]^+$, calcd. 508.20, found 508.21.

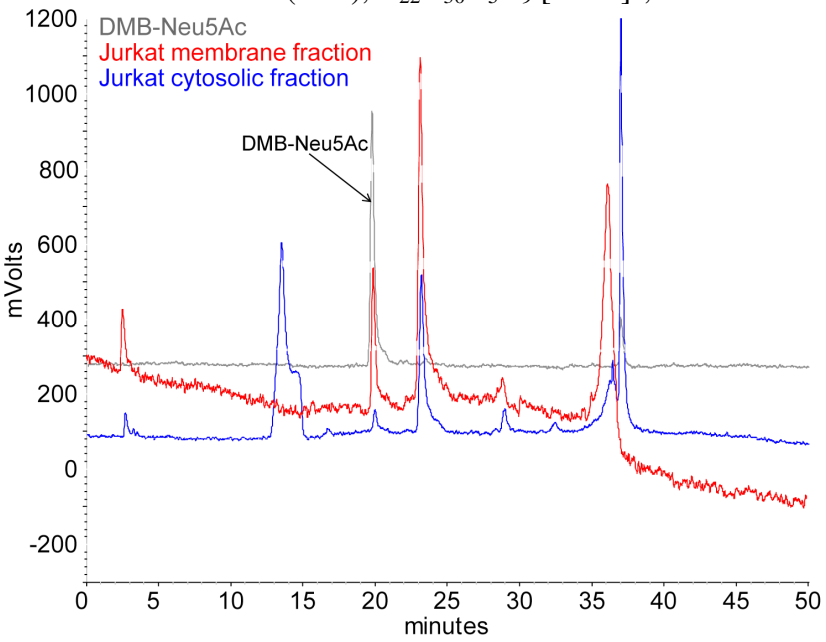


Figure S4. **HPLC traces of DMB-derivatized sialic acids from Jurkat cells.** Fluorescent molecules were excited with 373 nm light and emission detected at 448 nm. The elution gradient used the following percentages of CH_3CN in H_2O: T(0 min) 2%; T(2 min) 2%; T(30 min) 15%; T(35 min) 90%; T(40 min) 90%; T(45 min) 2%; T(50 min) 2%.

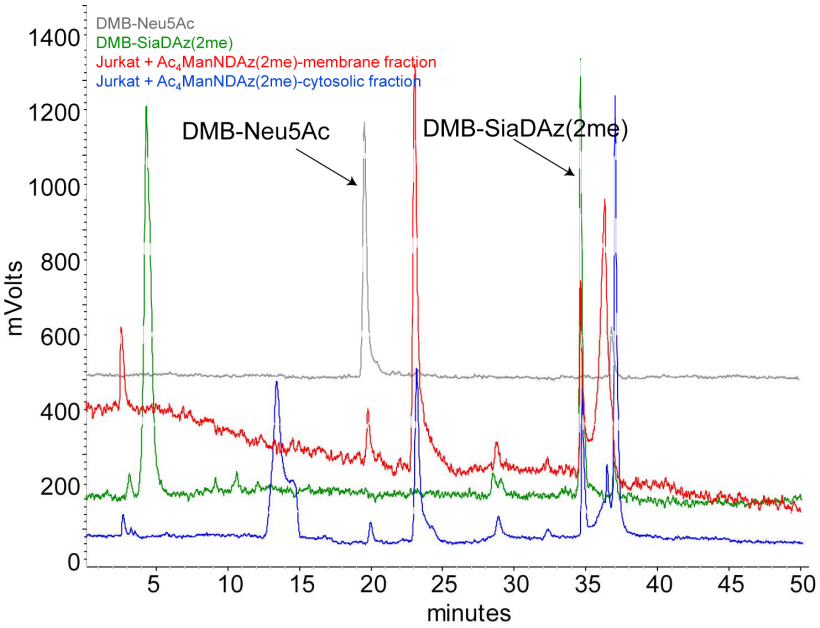


Figure S5. **HPLC traces of DMB-derivatized sialic acids from Jurkat cells cultured with Ac_4ManNDAz(2me).** Fluorescent molecules were excited with 373 nm light and emission detected at 448 nm. The peak labeled as DMB-SiaDAz(2me) displays the same retention time as the standard molecule and is observed only in samples from cells cultured with Ac_4ManNDAz(2me). The elution gradient used the following percentages of CH_3CN in H_2O: T(0 min) 2%; T(2 min) 2%; T(30 min) 15%; T(35 min) 90%; T(40 min) 90%; T(45 min) 2%; T(50 min) 2%.

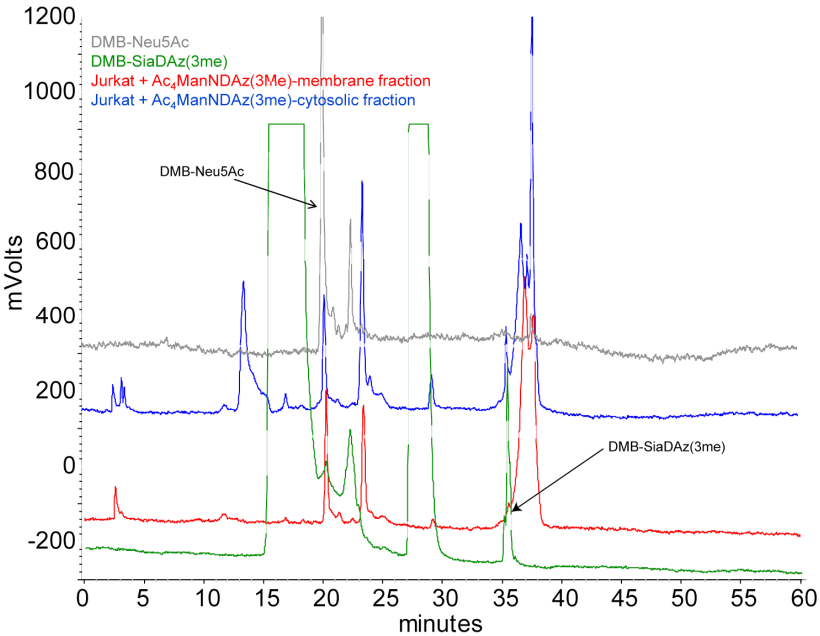


Figure S6. **HPLC traces of DMB-derivatized sialic acids from Jurkat cells cultured with Ac_4ManNDAz(3me).** Fluorescent molecules were excited with 373 nm light and emission detected at 448 nm. The peak labeled as DMB-SiaDAz(3me) displays the same retention time as the standard molecule and is observed only in samples from cells cultured with Ac_4ManNDAz(3me). A modified elution gradient was used with the following percentages of CH_3CN in H_2O: T(0 min) 2%; T(2 min) 2%; T(30 min) 15%; T(35 min) 70%; T(40 min) 70%; T(45 min) 90%; T(50 min) 90%; T(55 min) 2%; T(60 min) 2%.

Analysis of CMP-SiaDAz(2me) and UDP-GlcNDAz(2me) in Jurkat cells by HPAEC

Jurkat cells were seeded at a density of 2.5 x 10^5 cells/mL in 10 cm dishes and DMSO, 25 mM Ac_4ManNDAz(2me), or 25 mM Ac_4GlcNDAz(2me) in DMSO were added to a final concentration of 100 μM and then incubated at 37 ºC and 5% CO_2. After 72 h, cells were harvested, counted, and centrifuged at 200*g* for 4 min in 15 mL conical tubes and the cell pellet was washed two times with DPBS. The resulting pellet was lysed in 75% ethanol (700 μL) by sonication (VirSonic 100, VirTis, Gardiner, NY) and then was centrifuged at 20,000*g* for 10 min at 4 ºC. The resulting supernatant was collected and concentrated in a Savant SpeedVac in the dark. The concentrated residue was resuspended in 40 mM sodium phosphate buffer (pH 7.0, 10 μL/million cells) and then directly filtered through an Amicon Ultra centrifugal filter unit (Millipore, 10,000 MWCO). HPAEC analysis was performed by injecting 20 μL of the resulting filtrate.

HPAEC analysis was performed by injecting 20 μL of the each sample in CarboPac PA1 column (Dionex, 4 x 250 mm) through a 20 μL sample loading loop and a guard column (Dionex, CarboPac PA1, 4 x 50 mm). Elution was performed using buffers E1 (1 mM NaOH) and E2 (1 mM NaOH, 1.0 M NaOAc). For the analysis of CMP, CMP-NeuAc and CMP-SiaDAz(2me), HPAEC analysis was performed by using Dynamax SD-200, with electrochemical detector (Dionex), flow rate = 1 ml/min, gradient of T_{0min} = 2% E2, T_{2min} = 2% E2, T_{30min} = 30% E2, T_{40min} = 30% E2, T_{41min} = 55% E2, T_{60min} = 55% E2, T_{63min} = 100% E2, T_{72min} = 100% E2, T_{75min} = 2% E2, T_{81min} = 2% E2. For the analysis of UDP-GalNAc, UDP-GlcNAc and UDP-GlcNDAz(2me), HPAEC analysis was performed by using Dionex ICS-3000, PAD (UV-C, Dynamax), flow rate = 1 ml/min, gradient of T_{0min} = 2% E2, T_{2min} = 2% E2, T_{30min} = 30% E2, T_{40min} = 30% E2, T_{41min} = 55% E2, T_{60min} = 55% E2, T_{63min} = 100% E2, T_{72min} = 100% E2, T_{75min} = 2% E2, T_{81min} = 2% E2. The results are shown in Figures S7 and S8.

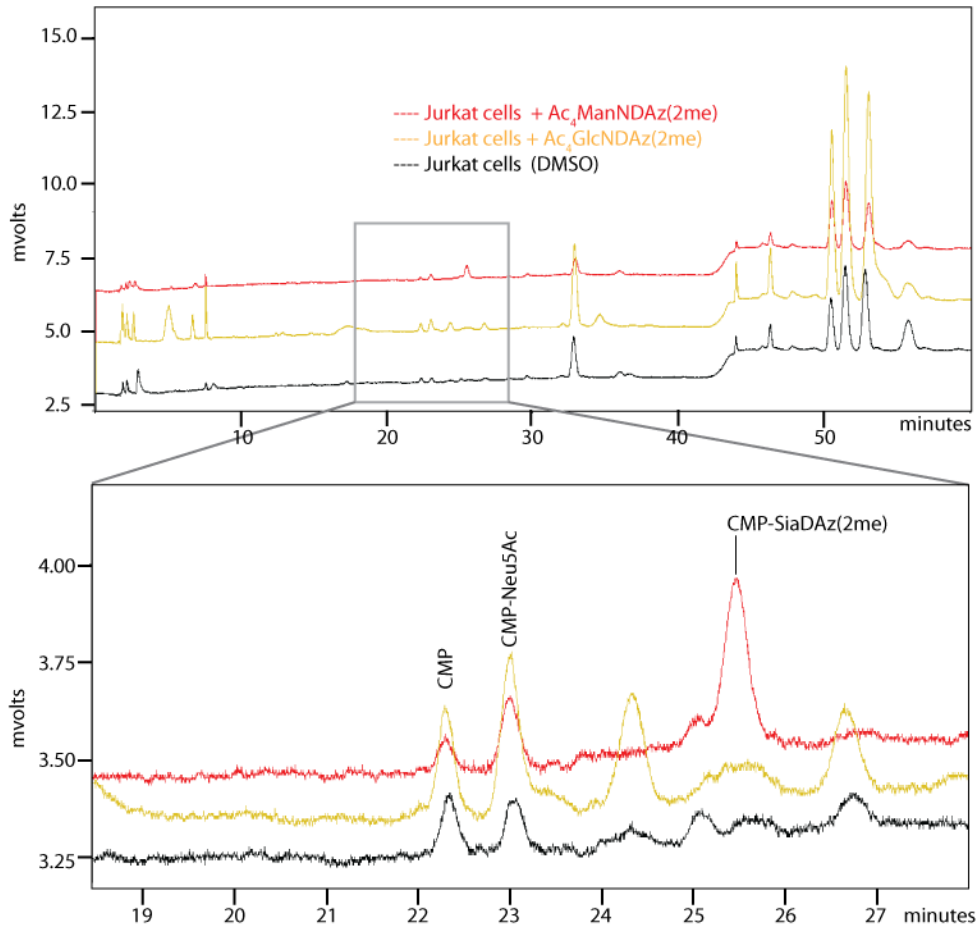


Figure S7. HPAEC analysis of Jurkat cell lysate after culturing 72 h with DMSO, Ac_4ManNDAz(2me), or Ac_4GlcNDAz(2me). UV absorbance was monitored at 260 nm with UV-C detector and CarboPac PA1 analytical column (Dionex) with Dynamax SD-2000 HPLC system. CMP and CMP-NeuAc were identified based on comparison to standards.

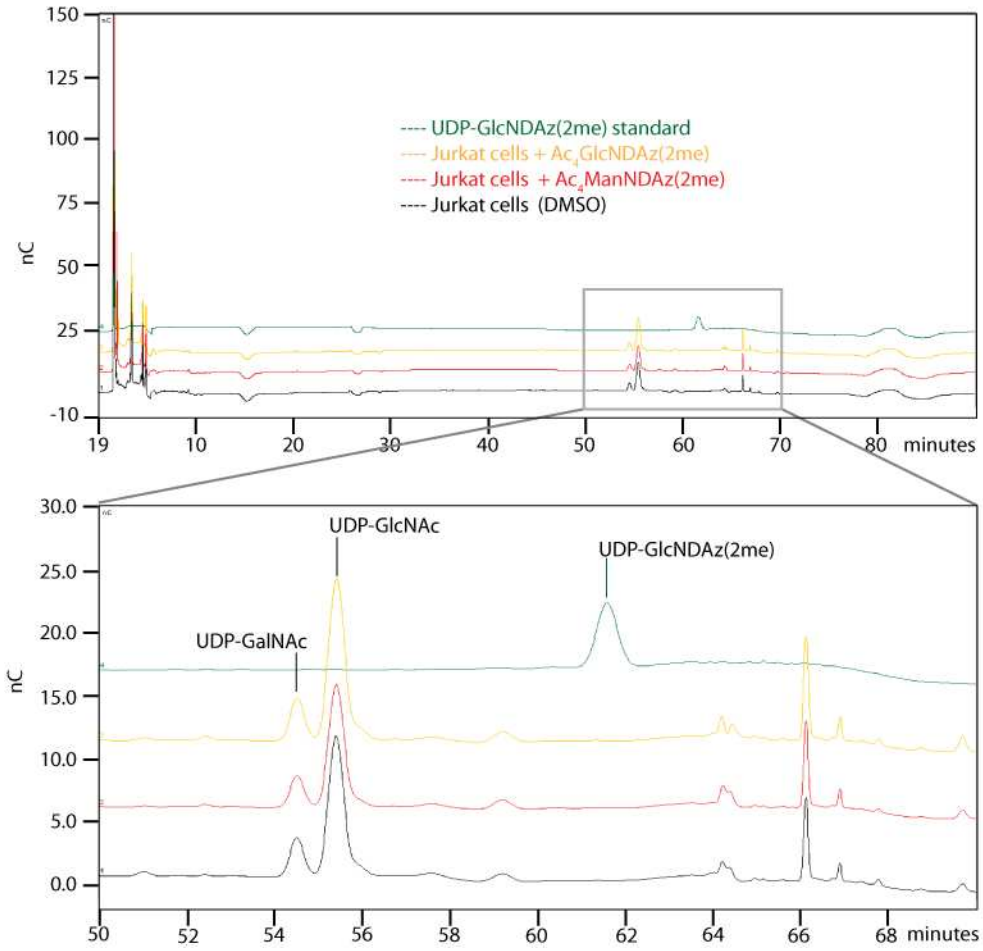


Figure S8. HPAEC analysis of Jurkat cell lysate after culturing 72 h with DMSO, Ac_4ManNDAz(2me), or Ac_4GlcNDAz(2me). UDP sugars were monitored by pulse amperometry detection (PAD) and identified based on comparison of retention times with those of known standards.

HPTLC staining with CTxB-488 is more sensitive than resorcinol staining

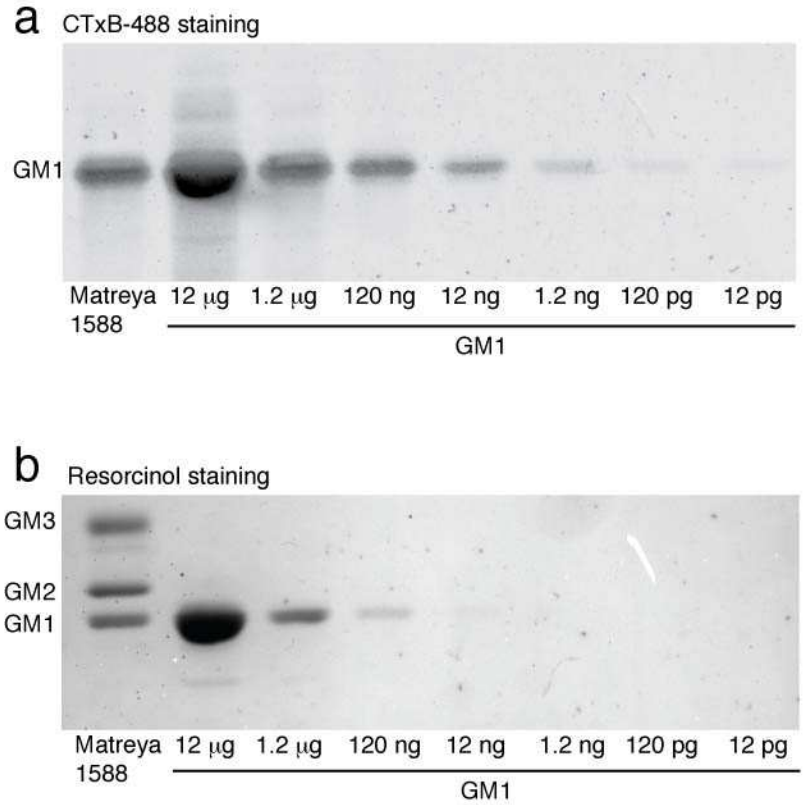


Figure S9. **Alexa Fluor 488 conjugated cholera toxin subunit B (CTxB-488) staining of HPTLC-resolved ganglioside GM1a is 1000 times more sensitive than resorcinol staining.** Pure ganglioside GM1a was spotted at decreasing concentrations and resolved on an HPTLC plate next to a ganglioside standard from Matreya. Bands corresponding to 120 pg GM1a are visible with CTxB-488 detection, whereas 120 ng GM1a is required for resorcinol detection.

Mass spectrometry analysis of CTxB-GM1a-SiaDAz(2me)

All reagents, chemicals, and general supplies were purchased and used as received from Fisher Scientific (Waltham, MA) or Sigma-Aldrich (St. Louis, MO) unless otherwise noted. The MALDI matrix, CHCA, was purchased from Fluka (Sigma-Aldrich). All solvents used for mass spectrometry were of HPLC grade. EtOH to store and wash SAM plates was absolute anhydrous 200 proof. Water was purified using a Millipore purification system to resistance of 18 MΩ. His_6-tagged protein G was purchased from Biovision Inc. (Mountain View, CA). Microscope cover glasses (24 x 50 mm size with 0.16 mm thick) were purchased from Fisher Scientific (Pittsburgh, PA). Myoglobin from equine skeletal muscle and ubiquitin from bovine erythrocytes were purchased from Sigma Aldrich.

Prior to the addition of cells to a 12 well plate, Ac_4ManNAc or Ac_4ManNDAz(2me) in EtOH were added to a final concentration of 100 μM and the EtOH pre-evaporated at ambient temperature and pressure. Jurkat cells were then seeded at a density of 2.5 x 10^5 cells/mL and incubated in the presence of the monosaccharides for 72 h at 37 °C and 5% CO_2 as described above. After growth with the appropriate compounds for 70 h, cells were pelleted at 650*g* followed by resuspension in original media to 5.0 x 10^6 cells/mL. Then 200 μL of cells (1.0 x 10^6 cells) were aliquoted to the wells of a 24 well tissue culture plate. To each well, 0.5 μL of 1 mg/mL CTxB was added and the plate swirled. Plates were then incubated at 4 °C for 45 min. After pre-incubation with CTxB, plates were placed on ice and exposed to 365 nm light approximately 2 cm away from bulb for 45 min. After photoirradiation, cells were transferred to a 1.5 mL microcentrifuge tube and centrifuged for 5 min at 650*g*. The supernatant was then removed by aspiration. Cell pellets were washed twice with 200 μL of DPBS and then lysed in 50 μL of 300 mM NaCl, 1 mM EDTA, 1% Triton X-100, and protease inhibitor cocktail at 4 °C for 1 h. Cell debris was removed by centrifugation at 17,000*g* for 10 min.

Briefly, 30 Å of chromium followed by 290 Å of gold were evaporated by electron beam onto a microscope cover glass. Self-assembled monolayers were generated by immersion of the metallized slides in an ethanolic solution containing tri(ethylene glycol)-terminated (EG3) and nitrilotriacetic acid (NTA)-terminated alkanethiolates. An 85:15 solution ratio of EG3 to NTA alkanethiolates was used and monolayers were generated for 3 days at 4 °C. The SAM slides were washed with EtOH and stored in EtOH at -20 °C prior to use.

The NTA-based monolayers on the gold-coated glass slides were treated with an aqueous solution of $NiSO_4$ for 30 min to generate the NTA/Ni^{2+} complex (10 mM, PBS, pH 7.2). They were then were cut into square pieces measuring 4 mm^2 and treated for 1 h with His_6-tagged protein G (3.84 μM in PBS pH 7.2). The resulting monolayers were treated with rabbit anti-CTxB (100 μM in PBS, pH 7.2) for 1 h. Lastly, cell lysates were diluted in equal volume of the same lysis buffer and incubated on the chips for 3 h. Chips were washed under a stream of water. Chips were dried with a stream of nitrogen after each step. α-Cyano-4-hydroxycinnamic acid (CHCA) matrix solution (0.3 μL, 10 mg/mL in acetone) was applied to each chip and dried before the MALDI-TOF-MS analysis.

The SAM chips were placed onto a MALDI sample plate (AB Sciex, V700668) that accommodates very thin SAM gold-coated glass slides (0.16 ~ 0.19 mm thickness). SAM chips

are applied directly onto the MALDI plate with vacuum grease. Mass analysis was carried out using a Voyager DE-PRO MALDI-TOF mass spectrometer (Applied Biosystems; Framingham, MA) equipped with a dual microchannel plate (MCP) detector for linear and reflectron mode (1.3 m effective flight path length) of detection with a delayed ion extraction. The instrument parameters were set such as accelerating voltage (AV) at 20 or 25 kV, grid voltage at 93% of AV, guide wire at 0.1%, and extraction delay time at 510 or 575 ns. A 337 nm nitrogen UV laser (pulse width: 3 ns, pulse energy: 120 μJ at 10 Hz) was used at a repetition rate of 3 Hz for sample ionization. The mass spectra obtained were an accumulation of 100 laser shots.

Obtained mass spectra were externally calibrated with myoglobin from equine skeletal muscle and ubiquitin from bovine erythrocytes. Raw data were baseline subtracted and Gaussian smoothed (11 points) in the DataExplorer software (Applied Biosystems; Framingham, MA). The accuracy of the external calibration for the instrument is approximately 1000 ppm. With broad peaks for both the monomeric CTxB and the CTxB-GM1a-SiaDAz(2me) complex, error propagation was handled as follows: the full width of both peaks were determined at half the maximal height. The resulting widths were divided by the median peak value corresponding to the peak's centroid. The resulting values were squared and summed. Finally, the square root was taken and multiplied by 1555 (the difference between the peaks' centroids) yielding the calculated error of ± 38 Da. Mass spectra were processed for publication with OriginPro 7.5G Software (OriginLab; Northampton, MA).

NMR spectra of purified compounds

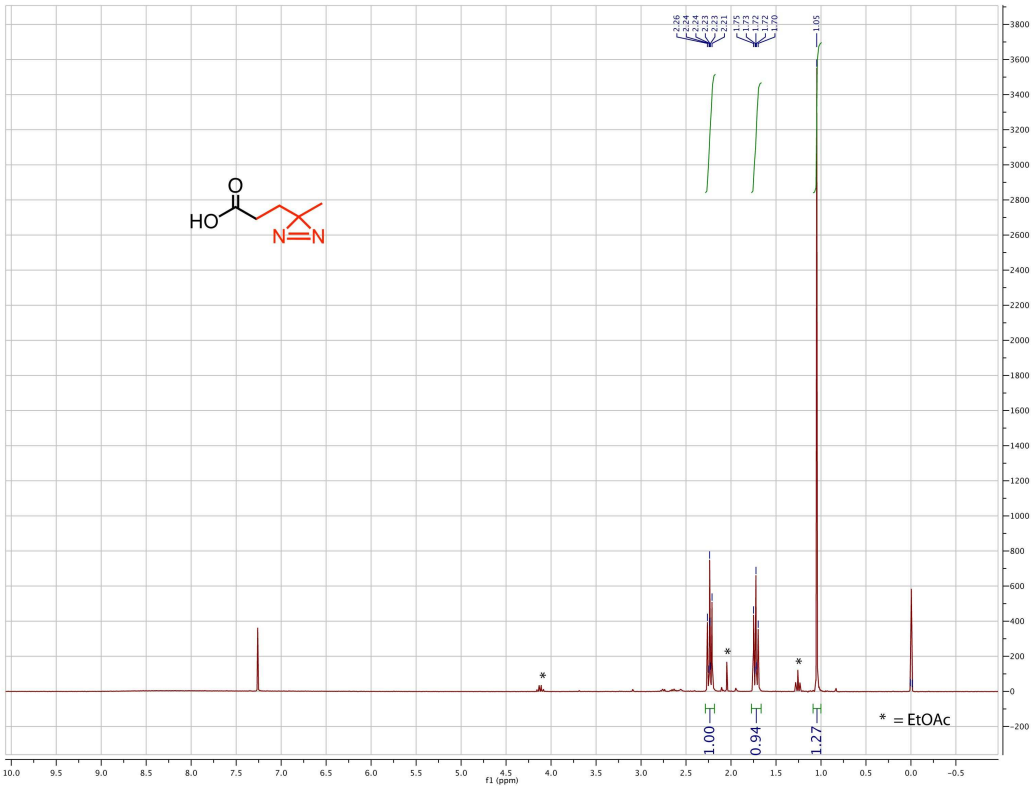


Figure S10. **^{1}H-NMR spectrum of 4,4-azo-pentanoic acid (11).**

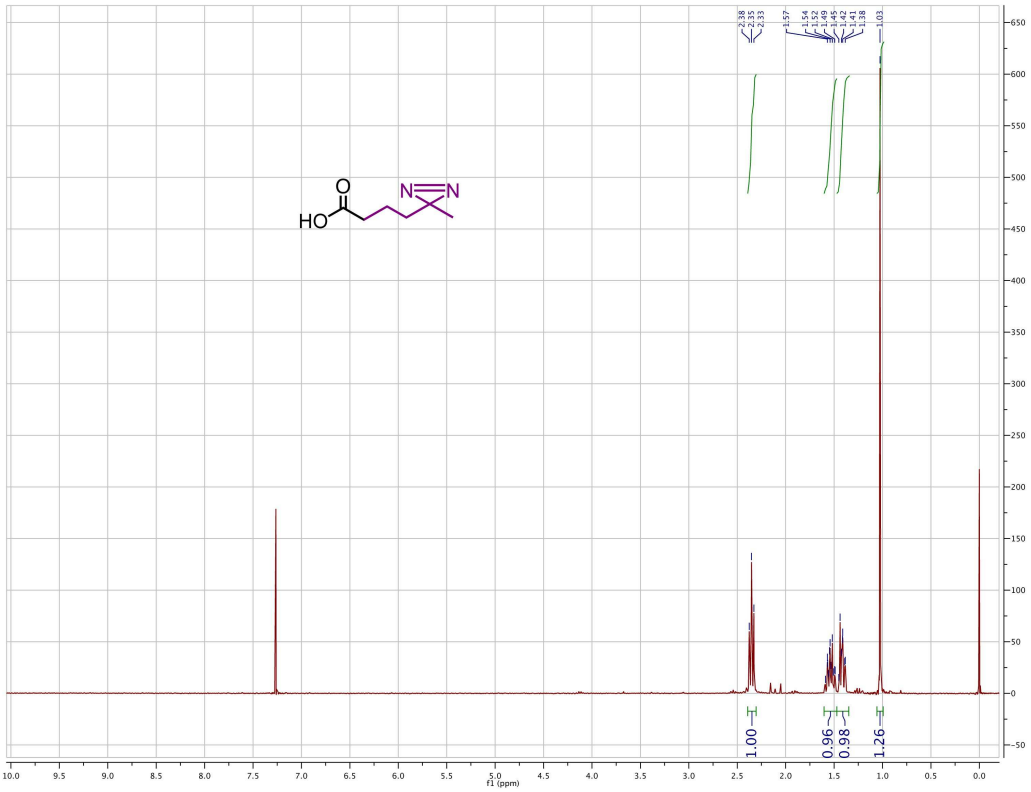


Figure S11. **^{1}H-NMR spectrum of 5,5-azo-hexanoic acid (13).**

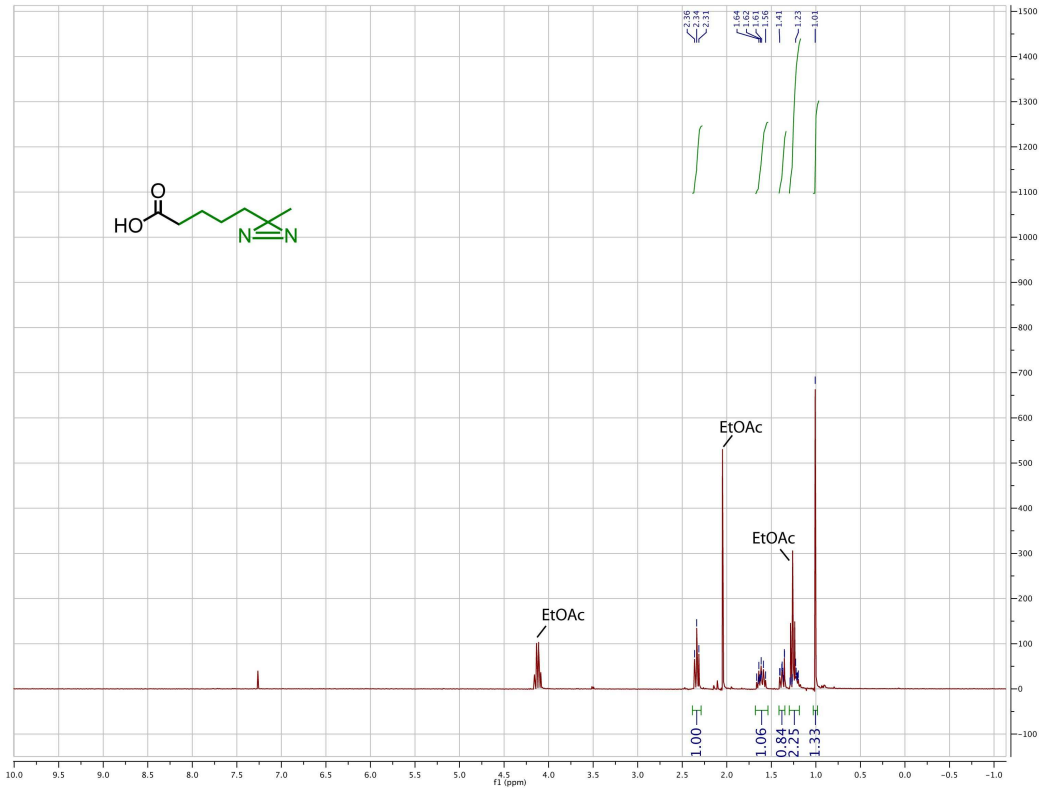


Figure S12. **^{1}H-NMR spectrum of 6,6-azo-septanoic acid (15).**

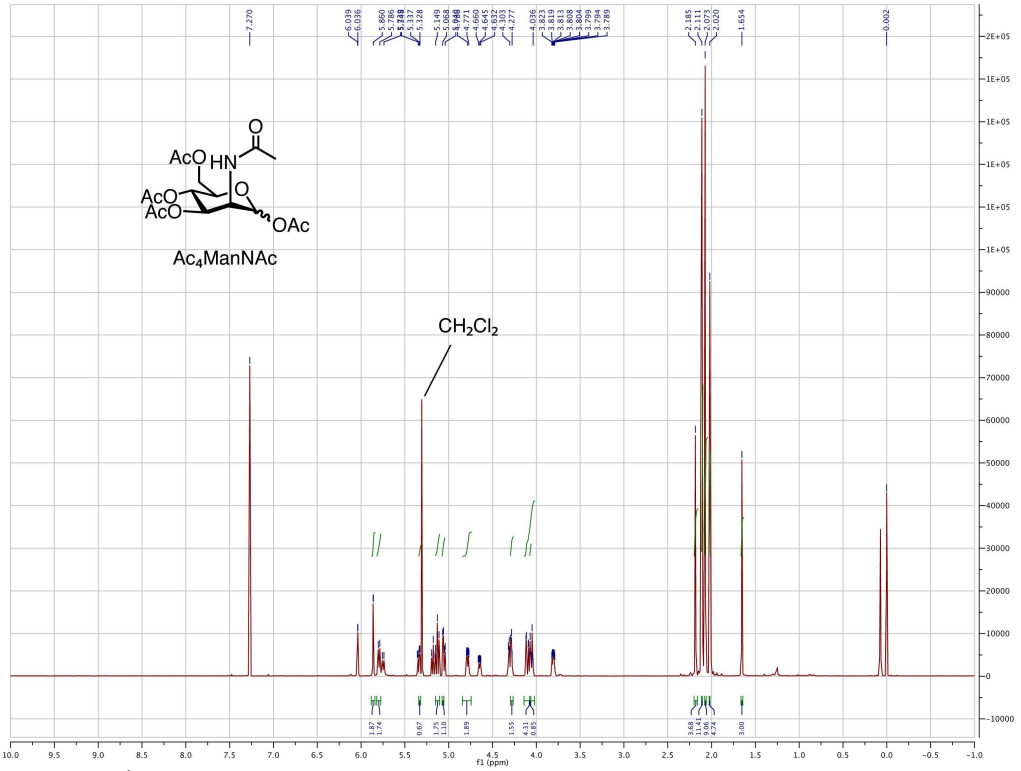


Figure S13. **^{1}H-NMR spectrum of Ac_4ManNAc (1).**

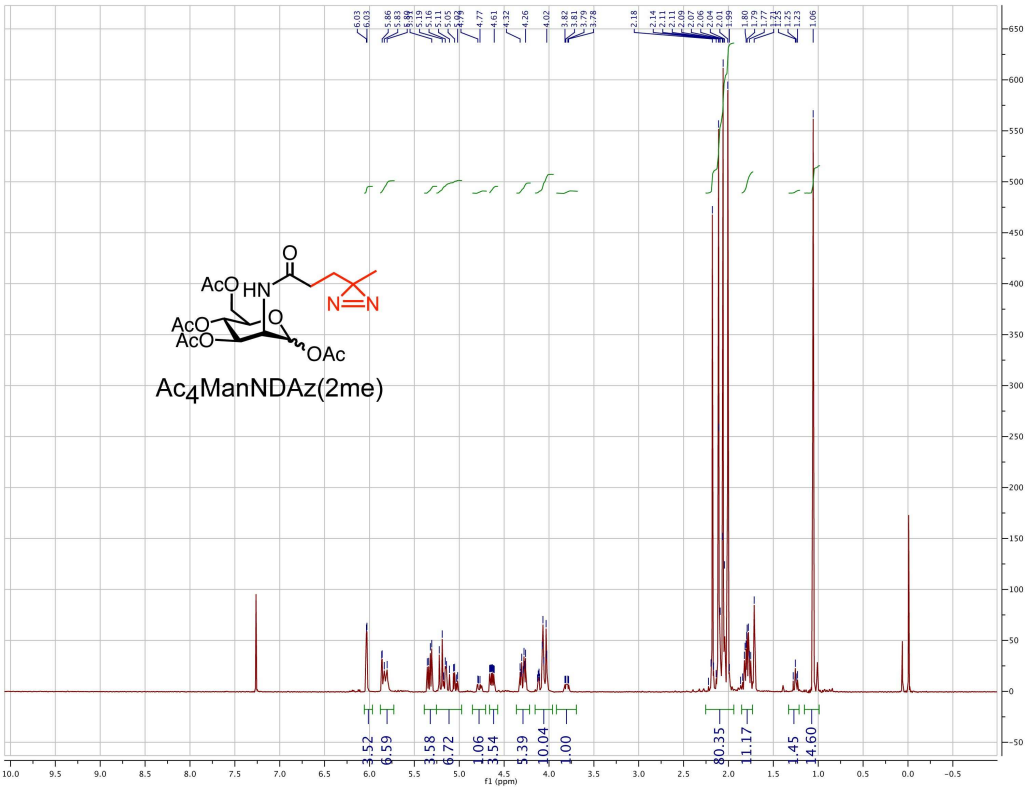


Figure S14. **^{1}H-NMR spectrum of Ac_4ManNDAz(2me) (4).**

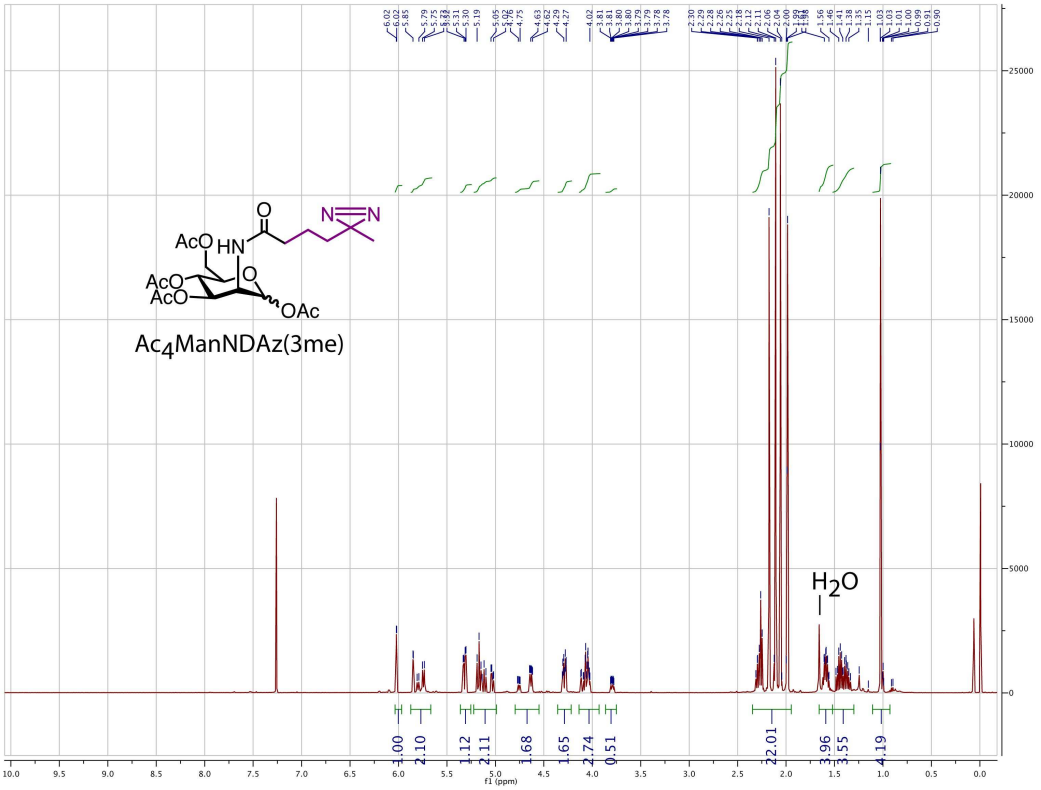


Figure S15. **^{1}H-NMR spectrum of Ac_4ManNDAz(3me) (5).**

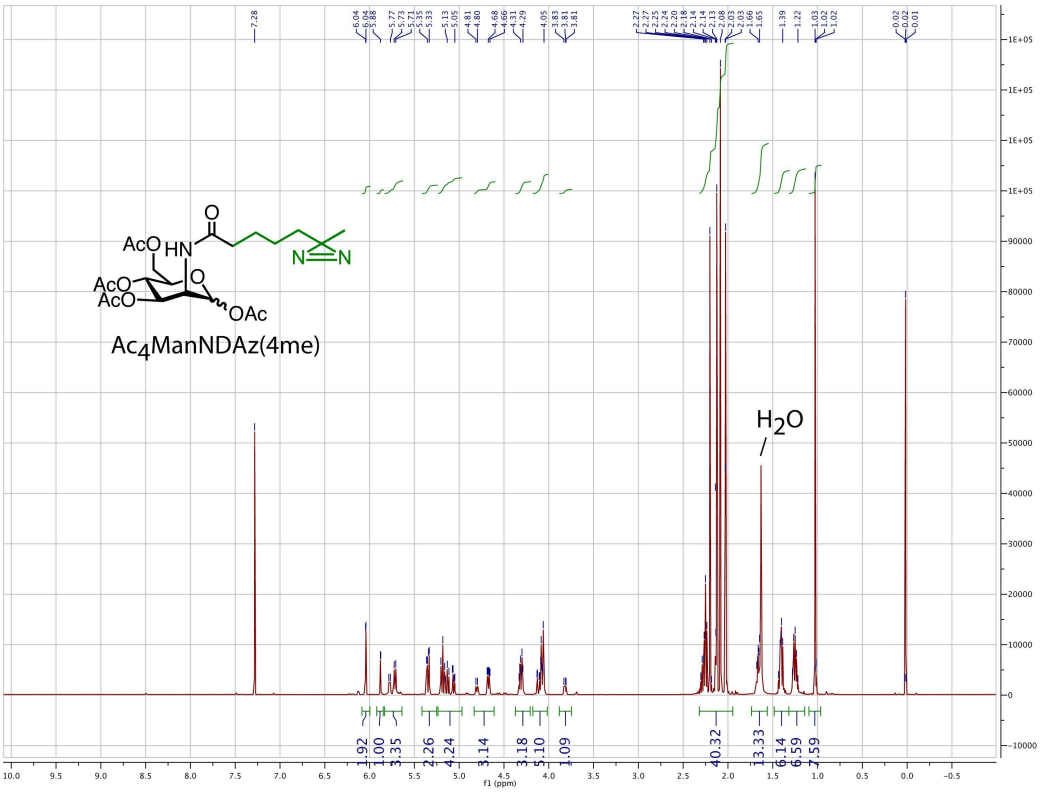


Figure S16. **^{1}H-NMR spectrum of Ac_4ManNDAz(4me) (6).**

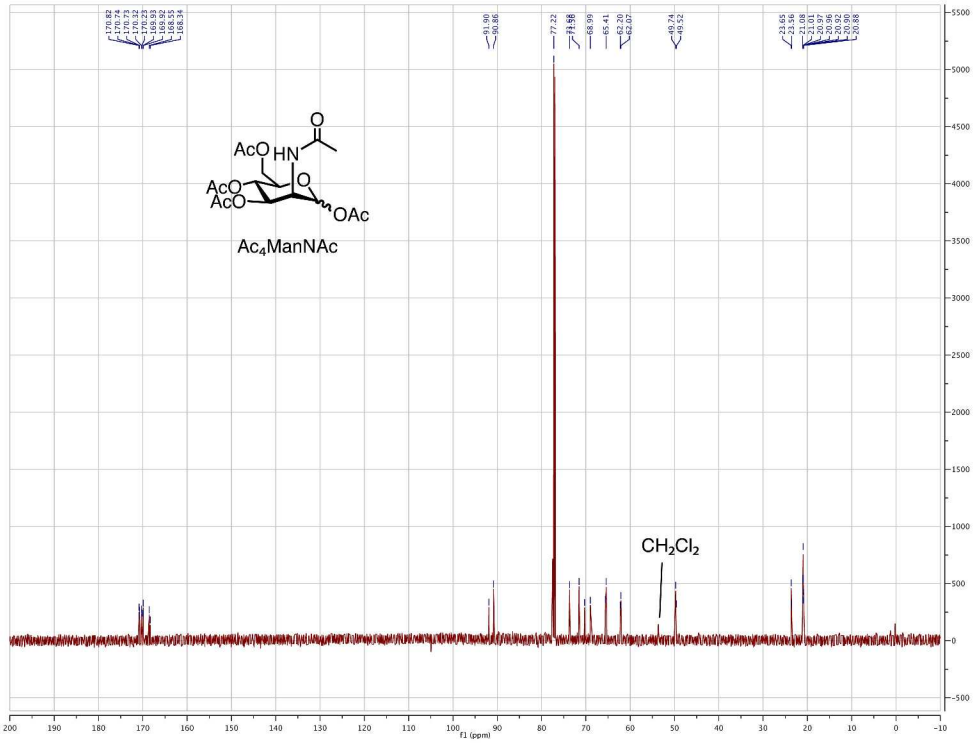


Figure S17. **^{13}C-NMR spectrum of Ac_4ManNAc (1).**

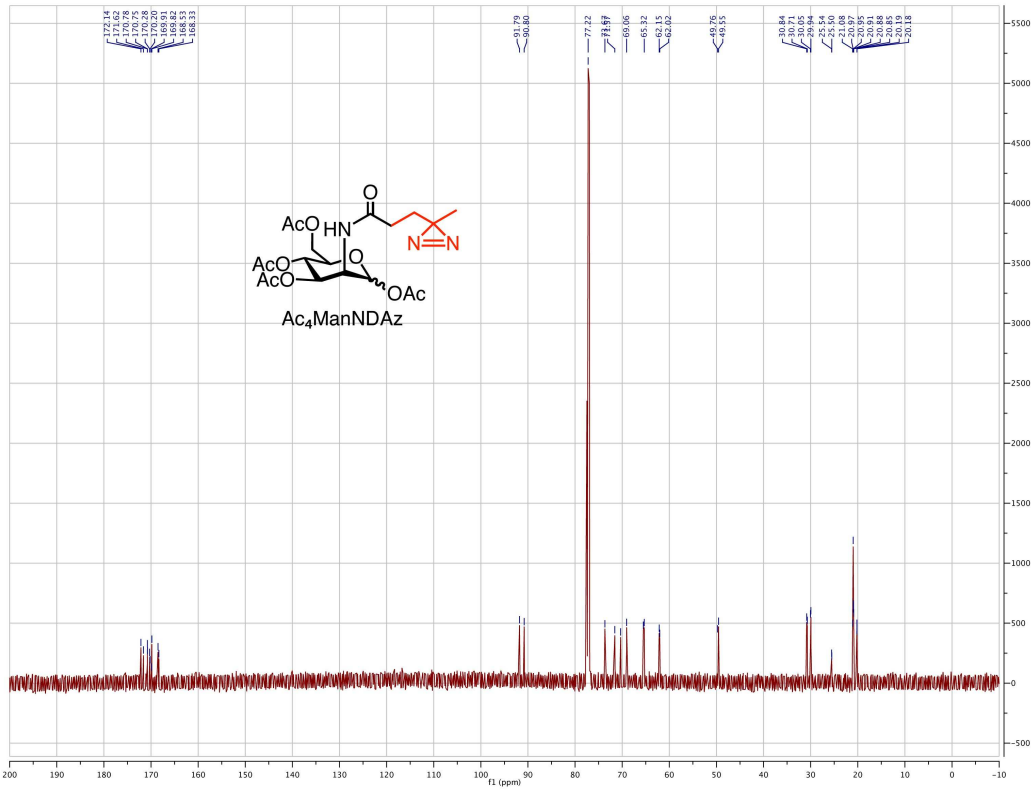


Figure S18. **^{13}C-NMR spectrum of Ac_4ManNDAz(2me) (4).**

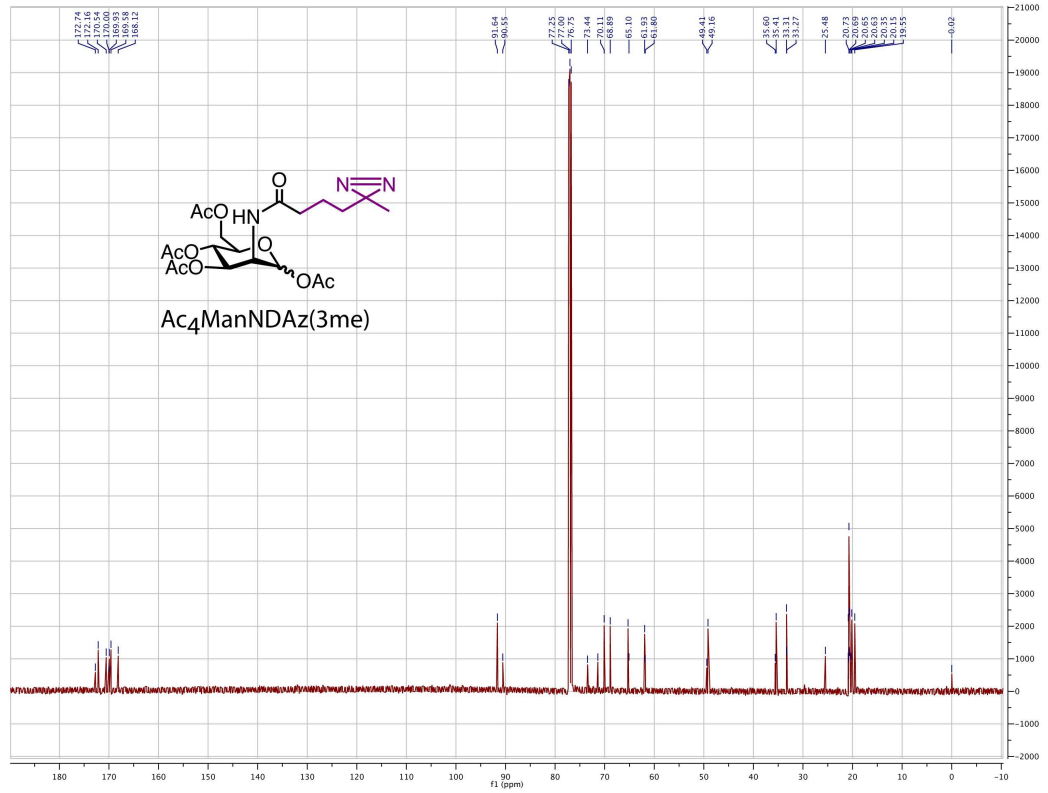


Figure S19. **^{13}C-NMR spectrum of Ac_4ManNDAz(3me) (5).**

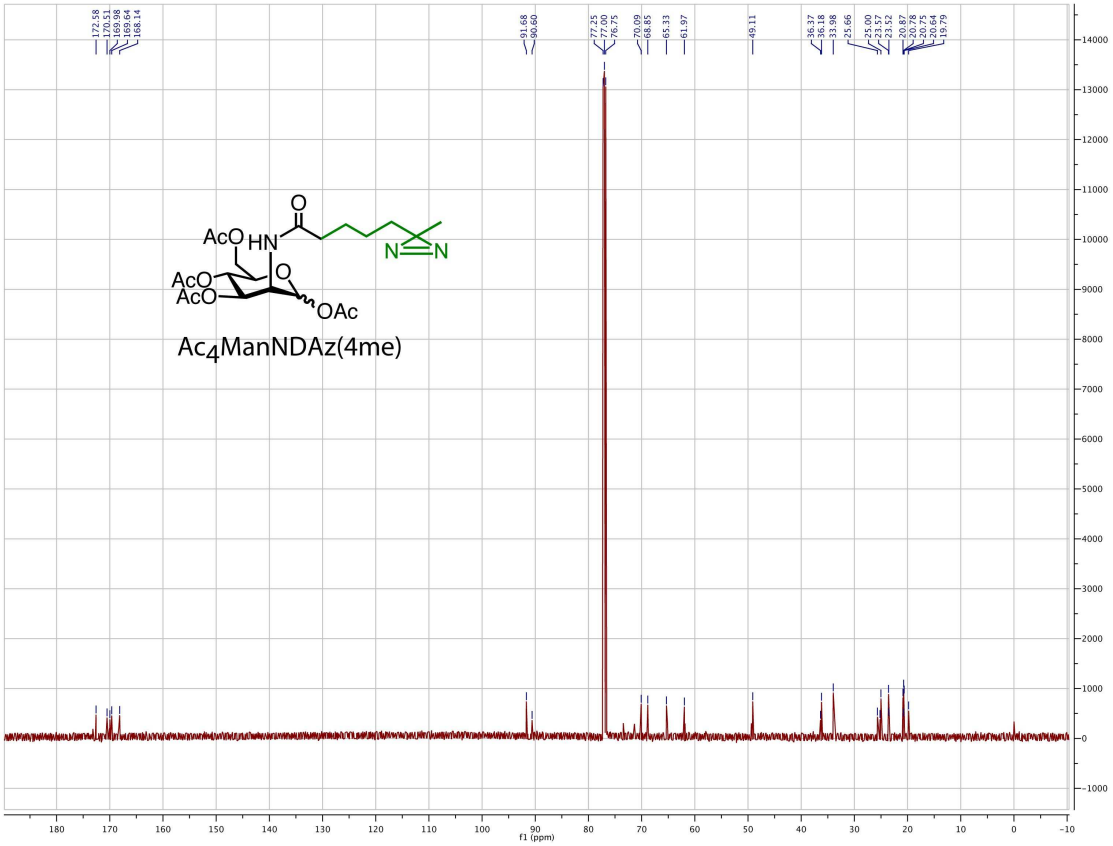


Figure S20. **^{13}C-NMR spectrum of Ac_4ManNDAz(4me) (6).**

HPLC evaluation of compound purity

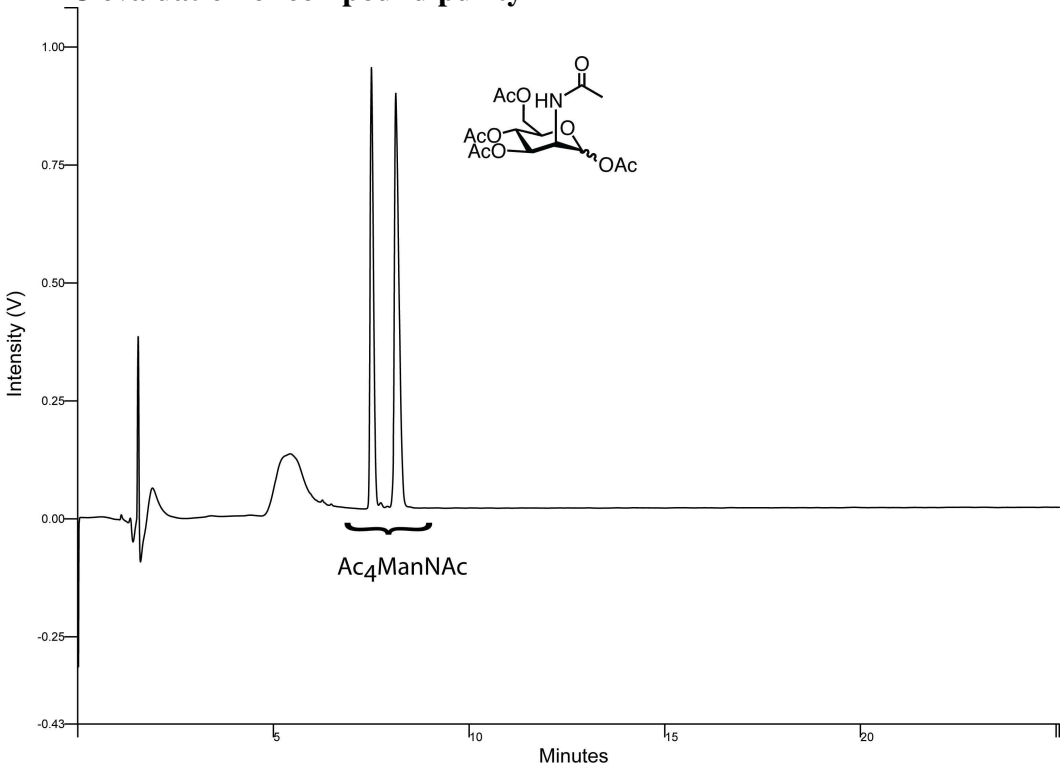


Figure S21. **HPLC trace of Ac_4ManNAc (1).** HPLC elution of acetylated sugars was monitored at 210 nm.

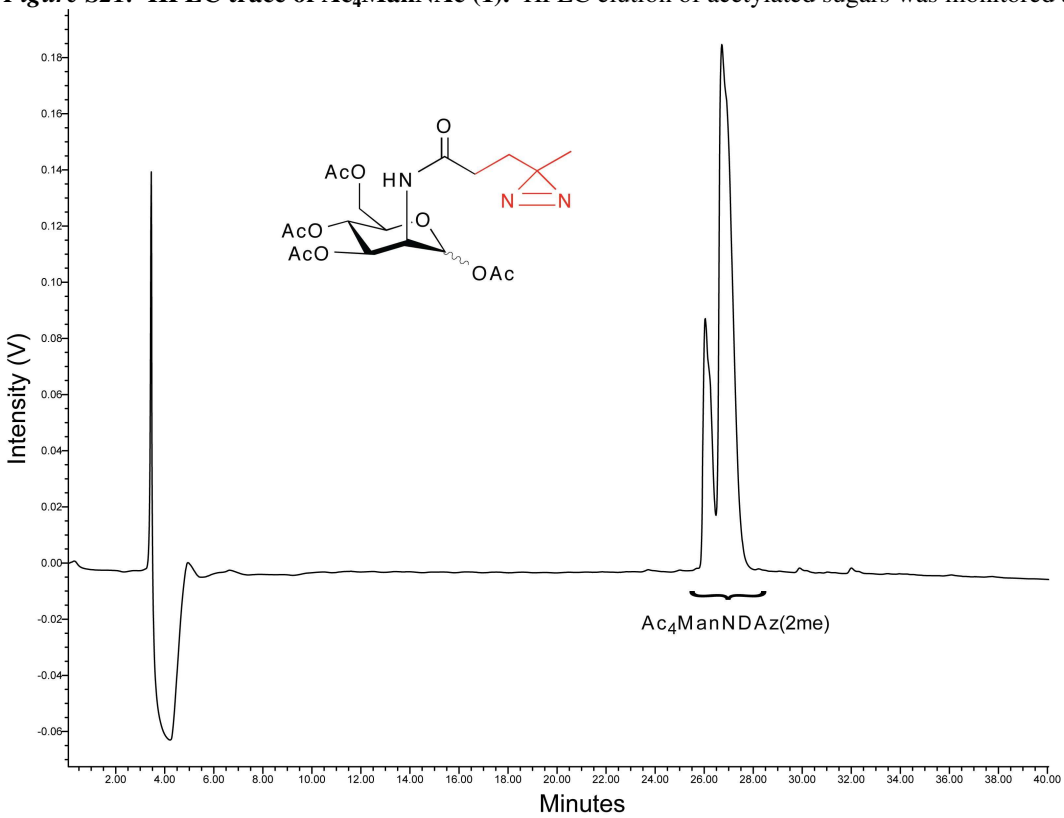


Figure S22. **HPLC trace of Ac_4ManNDAz(2me) (4).** HPLC elution of acetylated sugars was monitored at 210 nm.

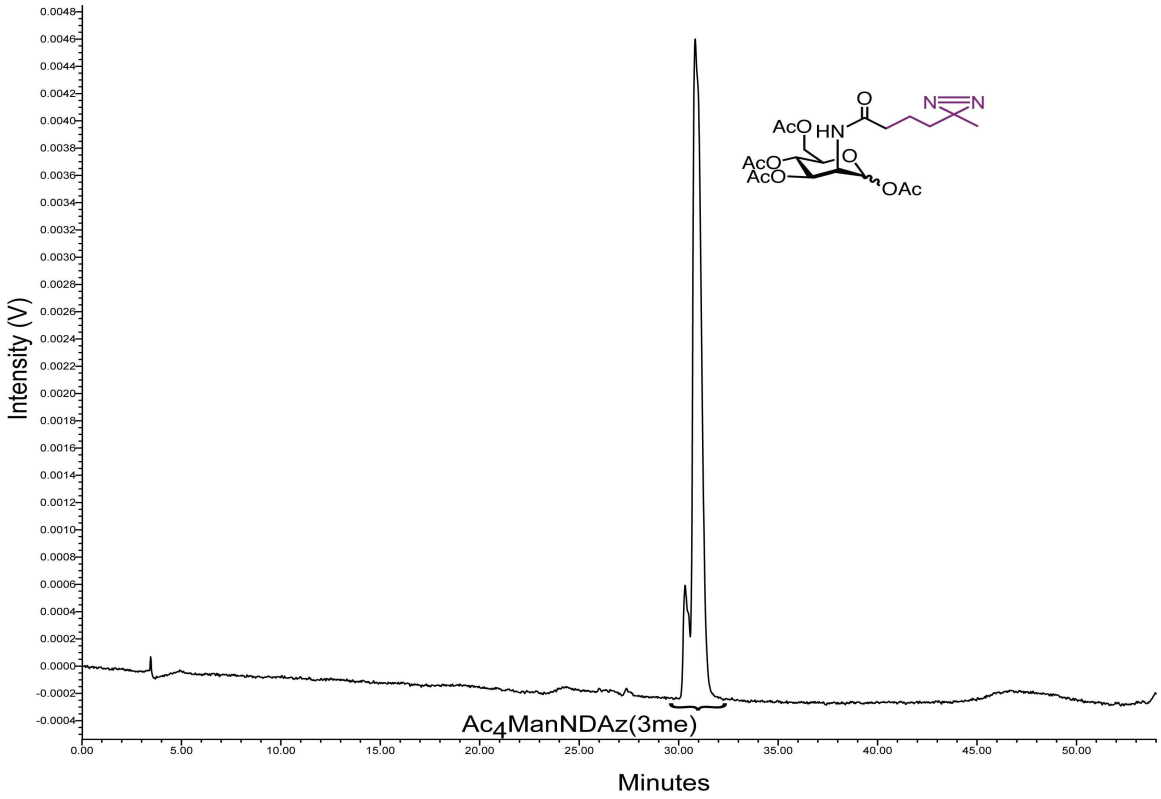


Figure S23. **HPLC trace of Ac_4ManNDAz(3me) (5).** HPLC elution of acetylated sugars was monitored at 210 nm.

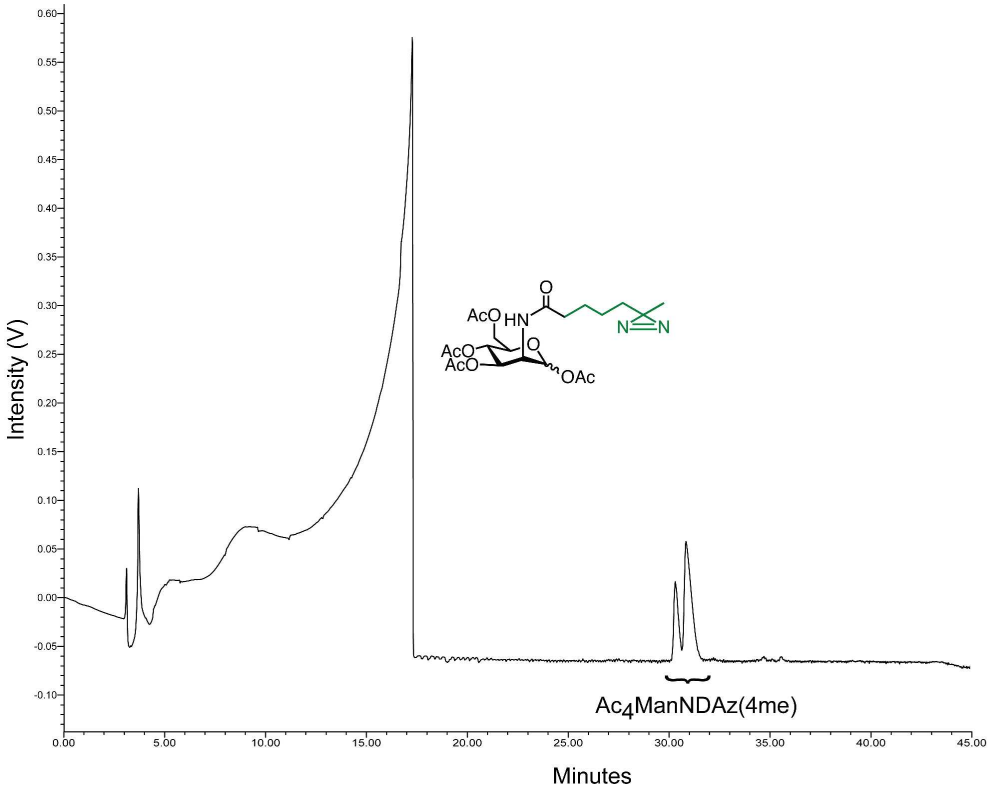


Figure S24. **HPLC trace of Ac_4ManNDAz(4me) (6).** HPLC elution of acetylated sugars was monitored at 210 nm.

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