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Supporting Information

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Supporting Information for:

Metabolic Labeling of Sialic Acids in Living Animals with Alkynyl Sugars

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General methods and materials

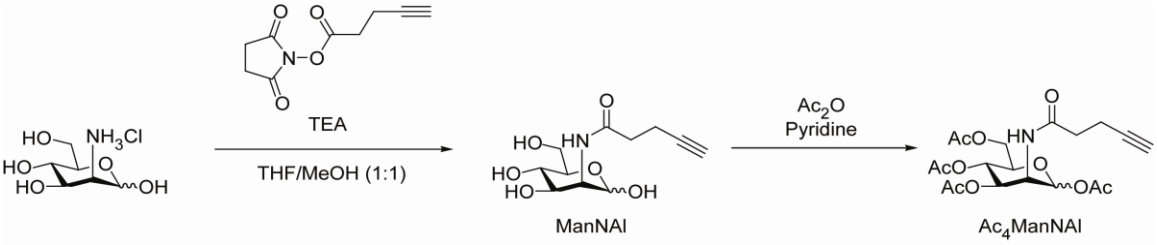
All chemical reagents were obtained from Sigma-Aldrich and GFS Chemicals and used without further purification unless otherwise noted. Flash chromatography was performed using Merck 60 Å 230-400 mesh silica gel. Analytical thin layer chromatography (TLC) was performed on glass-backed Analtech Uniplate silica gel plates, and compounds were visualized by staining with phosphomolybdic acid or 10% H_2SO_4 in ethanol. CH_2Cl_2 and THF were dried *in vacuo* over alumina. Anhydrous MeOH and pyridine were purchased from Acros Organics. Organic extracts were dried over Na_2SO_4, and the drying agent was removed by vacuum filtration. Unless otherwise specified, all solvents were removed under reduced pressure using a rotary evaporator. ^1H

NMR spectra were obtained at 500 and 600 MHz, and $^{13}C[^{1}H]$ NMR spectra were obtained at 75 and 125 MHz. Chemical shifts are reported in δ ppm relative to tetramethylsilane, and coupling constants (*J*) are reported in hertz (Hz). Mass spectra were obtained at the Albert Einstein Laboratory for Macromolecular Analysis and Proteomics. High resolution (HR) FT-ICR MS was performed on a Varian 12.0 T QFT mass spectrometer. Samples were dissolved in 50% methanol/H_2O containing 0.1% formic acid and introduced into the FT-ICR MS using electrospray ionization. Media, fetal bovine serum (FBS), and Dulbecco's Phosphate Buffered Saline (PBS) were purchased from Invitrogen (Carlsbad, CA). Cells were counted by hand using a hemocytometer. FITC-conjugated streptavidin, penicillin and streptomycin were purchased from Sigma-Aldrich (St. Louis, MO). Detergents, *DC* protein assay kits, and gels for electrophoresis were purchased from Bio-Rad Laboratories (Hercules, CA). Horseradish peroxidase-conjugated anti-biotin antibody (HRP-anti-biotin antibody) was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). SuperSignal West Pico Chemiluminescent Substrate was obtained from Pierce Biotechnology (Rockford, IL). Protease inhibitor Complete (EDTA-free) was purchased from Roche (Nutley, NJ). Wild-type B6D2F1/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME), and the animals were handled in accordance with Animal Use Protocol R234-0609B (approved by the Animal Care and Use Committee at the University of California, Berkeley).

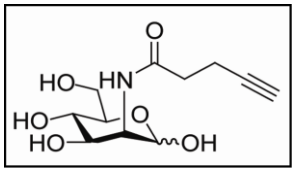
Tissue culture/cell growth conditions

Unless otherwise specified, Jurkat and LNCaP cells were grown in RPMI 1640 media supplemented with 10% FBS, 100 units/mL penicillin and 0.1 mg/mL streptomycin (P/S). HEK 293T cells were grown in Dulbecco's modified Eagle's media, supplemented with 10% FBS and P/S. CHO cells were grown in Ham's F12 media, supplemented with 10% FBS and P/S. DU145 cells were grown in Eagle's minimal essential media, supplemented with 10% FBS and P/S. PC3 cells were grown in F-12k media, supplemented with 10% FBS and P/S. In all cases, cells were incubated in a 5% carbon dioxide, water-saturated incubator at 37 °C.

Scheme S1. Synthesis of Ac_4ManNAl



Synthesis of *N*-(4-pentynoyl) mannosamine (ManNAl)[a]

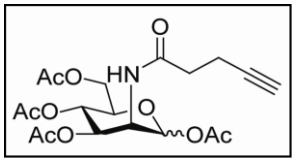


4-pentynoic acid (0.78 g, 8.0 mmol) and *N*-hydroxysuccinimide (0.92 g, 8.0 mmol) were suspended in 40 mL anhydrous THF followed by addition of DCC (1.8 g, 8.8 mmol). The reaction mixture was stirred at 0 °C for 5 min and was then warmed up to rt and stirred for another 6 h. The precipitate was removed by filtration. The eluent was concentrated to give a white solid, which was used for the next step without purification. Mannosamine hydrochloride (1.29 g, 6.00 mmol) and the succinimidyl ester (1.76 g, 9.00 mmol) obtained from the previous step were suspended in 50 mL of a 1:1 mixture of anhydrous THF and MeOH, followed by addition of anhydrous Et_3N (8.7 mL, 6.3 mmol). The

reaction mixture was stirred under N_2 at 0 °C for 5 min and was then warmed up to rt overnight. After removal of the solvent, the crude product was purified by flash chromatography on silica gel, eluting with $CHCl_3$ (200 mL) and gradually increasing the polarity to $CHCl_3$:MeOH (9:1, 500 mL), followed by $CHCl_3$:MeOH (5:1, 800 mL) to give the product as a colorless oil. Yield: 1.32 g (85 %).

[a]ManNAl was synthesized according to procedures reported by Wong *et al*. with slight modifications.[1]

Synthesis of 1,3,4,6-Tetra-*O*-acetyl-*N*-4-pentynoylmannosamine (Ac_4ManNAl)[b]



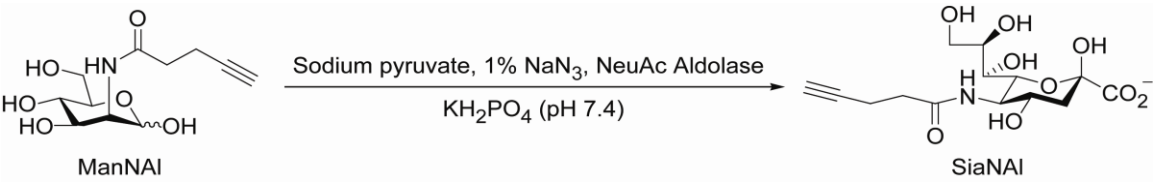
N-4-pentynoylmannosamine (ManNAl, 1.04 g, 4.00 mmol) was dissolved in 20 mL of dry pyridine followed by the addition of 4 mL of acetic anhydride. The reaction mixture was stirred at rt for 5 h and the solvent was removed *in vacuo.* The reaction mixture was redissolved in 160 mL of CH_2Cl_2 and was washed with 10% $NaHSO_4$ (100 mL), saturated $NaHCO_3$ (100 mL) and brine (100 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was purified using silica gel chromatography (hexanes:ethyl acetate, 1:1) to afford a white solid as a mixture of anomers (1.3:1 α:β). Yield: 1.62 g (95%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 2.036 (s, 3H), 2.041 (s, 3H), 2.09 (s, 6H), 2.13 (s, 3H), 2.14 (s, 3H), 2.21 (m, 8H), 2.49-2.61 (m, 8H), 3.82-3.84 (m, 1H), 4.06-4.15 (m, 3H), 4.29-4.33 (m, 2H), 4.69-4.72 (m, 1H, β-anomer), 4.82-4.85 (m, 1H, α-anomer), 5.08 (dd, *J* = 9.6, 4.2 Hz, 1H, β-anomer), 5.20 (t, *J* = 9.6 Hz, 1H, β-anomer), 5.25 (t, *J* = 10.2 Hz, 1H, α-anomer), 5.36 (dd, *J* = 10.8, 4.2 Hz, 1H, α-anomer), 5.90 (d, *J* = 1.8 Hz, 1H, β-anomer), 6.04 (dd, *J* = 9.0, 3.6 Hz, 2H), 6.08 (d, *J* = 1.8 Hz, 1H, α-anomer). ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.4, 15.5, 21.08, 21.10, 21.11, 21.17, 21.19, 21.2, 21.3, 35.8, 36.0,

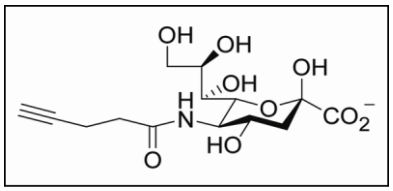
49.7, 49.9, 62.3, 62.4, 65.7, 65.9, 69.3, 70.6, 71.0, 71.8, 73.9, 82.97, 83.04, 91.0, 92.1, 168.6, 168.8, 170.1, 170.5, 170.6, 170.96, 170.98, 171.7, 172.1. HRMS (ESI): Calcd for $C_{19}H_{25}NO_{10}Na$ $[M+Na]^+$ 450.1371, found 450.1373.

[b]Ac_4ManNAl was synthesized according to procedures reported by Wong *et al*. with slight modifications.[1]

Scheme S2. Synthesis of SiaNAl



Synthesis of *N*-4-pentynoylneuraminic acid (SiaNAl)



ManNAl (290 mg, 0.0011 mol) was dissolved in 11 mL of 0.050 M potassium phosphate (pH 7.4), followed by addition of sodium pyruvate (1.2 g, 0.011 mol), NaN_3 (final concentration of 1% (w/v)) and NeuAc aldolase (20-25 U, Toyobo, lot no. 85211). The reaction mixture was placed in a shaking incubator at 37 °C for 14 h, after which ^{1}H NMR analysis indicated that the reaction was complete. The reaction mixture was then diluted with 90 mL of H_2O and purified by anion-exchange chromatography using AG1-X2 resin, formate form (Bio-Rad). The product was eluted with a gradient of 1.0 M to 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.[2] The fractions containing the desired product were combined and concentrated *in vacuo* to give a white solid as exclusively the β-anomer. Yield: 341 mg (89%).

^{1}H NMR (500 MHz, D_2O) δ 1.87 (dd, *J* = 13.0, 11.5 Hz, 1H), 2.31 (dd, *J* = 13.0, 5.0 Hz, 1H), 2.39 (br, 1H), 2.49-2.52 (m, 4H), 3.59 (dd, *J* = 12.0, 6.5 Hz, 1H), 3.66 (dd, *J* = 9.0,

1.0 Hz, 1H), 3.73-3.76 (m, 1H), 3.83 (dd, *J* = 12.0, 3.0 Hz, 1H), 3.96 (app t, *J* = 10.3 Hz, 1H), 4.05-4.10 (m, 2H). ^{13}C NMR (75 MHz, D_2O) δ 14.5, 34.7, 52.0, 63.2, 66.6, 68.3, 70.2, 70.3, 70.4, 83.5, 95.3, 173.4, 175.4. HRMS (ESI): Calcd for $C_{14}H_{22}NO_9$ $[M+H]^+$ 348.1289, found 348.1294.

Preparation of cell lysates and detection of glycoproteins by Western blot analysis

Ac_4ManNAl was maintained as a 50 mM stock solution in filter-sterilized ethanol, which was allowed to evaporate in the cell culture flasks prior to the addition of cells. Cells were seeded at a density of 1.5 x 10^6 cells in 10 mL of media with no alkynyl sugar or 50 μM of Ac_4ManNAl and incubated for three days. The cells were harvested by centrifugation at 2851 × g, and the cell pellets were homogenized in 750 μL of lysis buffer (150 mM NaCl, 1.0% NP-40, 50 mM Tris-HCl, pH 7.4) containing protease inhibitors (Complete, EDTA-free) by ten freeze-thaw cycles. The insoluble debris was removed by centrifugation at 10,000 × g for 10 min. The soluble protein concentration was determined using the *DC* protein assay kit. Biotin-azide (100 μM from a 50× stock in DMSO) was added to each sample (25 μg protein in 30 μL lysis buffer), followed by 1 mM freshly prepared sodium ascorbate (from a 50× stock in water) and 100 μM TBTA ligand (from a 40× stock in a 1:4 mixture of DMSO:*t*-butanol). Samples were gently vortexed, and 1 mM $CuSO_4{\cdot}5H_2O$ (from a 50× stock in water) was added to each sample, making the total reaction volume 32.5 μL. Samples were vortexed again and allowed to react at rt for 1 h. After adding 8.1 μL of 4× SDS-PAGE loading buffer containing β-mercaptoethanol, the samples were resolved on a Bis-Tris Criterion polyacrylamide gel (12%), transferred to nitrocellulose, and blocked with 5% bovine serum albumin in PBST (Dulbecco's Phosphate Buffered Saline with 0.1% Tween-20) for 1 h at rt. The blocked

membrane was incubated for 1 h at rt with an HRP-anti-biotin antibody (1:100,000 dilution) in blocking buffer, washed with PBST (4 x 15 min per wash), and developed using SuperSignal West Pico Chemiluminescent Substrate.

Compound administration and preparation of tissue lysates

Mice (B6D2F1/J) were injected intraperitoneally with Ac_4ManNAl, Ac_4ManNAz, or vehicle (300 mg/kg in 70% aqueous DMSO) once daily for 7 d. On day 8, the mice were euthanized 24 h post-injection, and the organs were collected, rinsed with cold PBS, and minced. The organs were then transferred into 1.5 mL of lysis buffer (150 mM NaCl, 1.0% NP-40, 50 mM Tris-HCl, pH 7.4) containing protease inhibitors (Complete, EDTA-free) and homogenized using a Dounce homogenizer. The cell debris was removed by centrifugation (13,500 × g for 10 min), and the supernatant was collected. Protein concentrations were determined using the *DC* protein assay kit. Click chemistry with biotin-azide or biotin-alkyne probe, SDS-PAGE and Western blot analysis were performed as described in the previous section.

Fluorescence microscopy

Chinese hamster ovary (CHO) cells were seeded onto glass slides mounted with tissue culture wells (Lab-Tek, Nunc) and incubated with Ac_4ManNAl (50 μM) or no sugar for 3 d. The cells were washed three times with PBS and then fixed with 3% paraformaldehyde in PBS at 4 °C for 20 min. After three washes, cells were treated with biotin-azide (50 μM) in the presence of sodium ascorbate (200 μM), TBTA (100 μM), $CuSO_4 \cdot 5H_2O$ (100 μM) in 200 μL PBS for 10 min at rt. The cells were then washed three times with PBS and blocked in PBS with 1% bovine serum albumin for 20 min, followed by staining with a FITC-streptavidin conjugate (1 μg/mL in PBS). After incubation at rt

for 30 min in the dark, the cells were washed three times and then mounted using Vectashield with 4,6-diamidino-2-phenylindole (DAPI, Vector Laboratories). A Zeiss Axiovert 200M inverted microscope equipped with a 63 × 1.4 NA Plan-Apochromat oil immersion lens was employed for imaging. A 175W xenon lamp housed in a Sutter DG4 illuminator linked to the microscope by an optical fiber assured shuttering and illumination. Images were acquired using a CoolSNAP HQ CCD camera (Roper Scientific). Images were deconvolved using the nearest neighbor algorithm in Slidebook software version 4.2 (Intelligent Imaging Innovations) and are shown as a single z-plane.

Sialic acid identification and quantification as 1,2-diamino-4,5-methylenedioxybenzene (DMB) derivatives by RP-HPLC with fluorescence detection[3]

Lysates from cells treated with 50 μM of Ac_4ManNAl, Ac_4ManNAz, or no sugar for 3 d were dissolved in a final concentration of 2 M acetic acid and heated to 80 °C for 3 h to release sialic acids. The sialic acids were collected by ultra filtration through a 3,000 NMWCO ultrafilter and derivatized with 1,2-diamino-4,5-methylenedioxybenzene (DMB). The sialic acid-DMB derivatives were analyzed by reversed phase HPLC coupled with fluorescence detection. Identification and quantification of SiaNAl and SiaNAz was determined by comparison with the synthetic standards of *N*-glycolylneuraminic acid (Neu5Gc), *N*-acetylneuraminic acid (Neu5Ac), SiaNAl, or SiaNAz.[4]

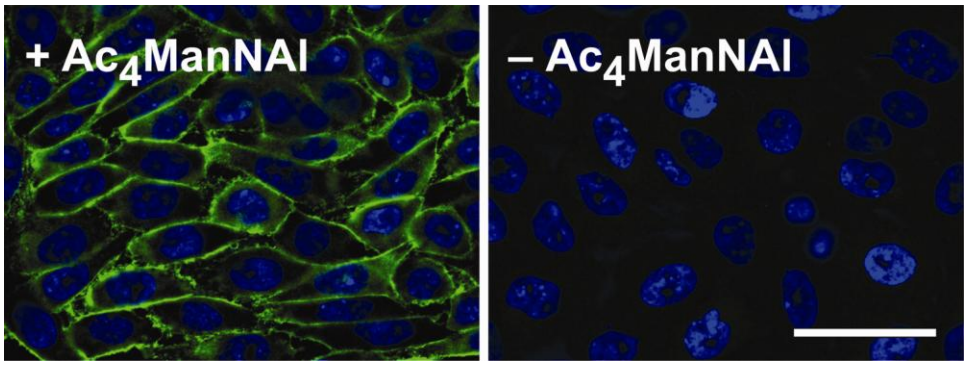


Figure S1: Fluorescence micrographs of CHO cells treated with 50 μM Ac_4ManNAl (left) or no sugar (right) for 3 d. The cells were rinsed, fixed with 3% paraformaldehyde, and labeled with biotin-azide (50 μM) in the presence of $CuSO_4$ (100 μM), sodium ascorbate (200 μM), and TBTA (100 μM) for 10 min at rt, followed by secondary labeling with a FITC-streptavidin conjugate. The cells were labeled with DAPI nuclear stain before imaging. (Blue = DAPI channel, Green = FITC channel). Scale bar = 40 μm.

3 Std mixt. (Neu5Ac+Neu5Gc) 1, 10pM

Sample Name:	**Std mixt. (Neu5Ac+Neu5Gc) 1, 10pM**		
Vial Number:	**B7**	*Injection Volume:*	**20ul**
Sample Type:	**standard**	*Sequence Name:*	**09-11-07**
Control Program:	**DMBTSK 2rev6**	*Quantif. Method:*	**SYS2 BSM**
Recording Time:	**11.09.07 22:56**		
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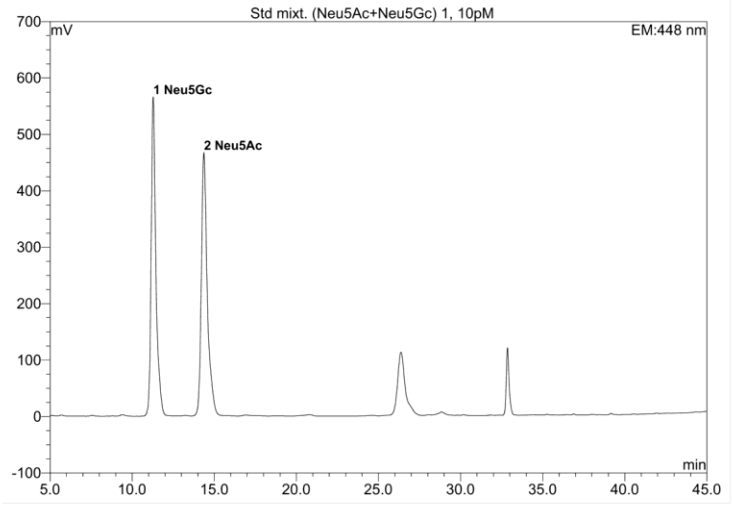

No.	Ret.Time	Peak Name	Height	Area	Rel.Area	Amount
	min		mV	mV*min	%	pMol
1	11.28	Neu5Gc	565.598	190.072	49.23	10.000
2	14.36	Neu5Ac	466.779	196.051	50.77	10.000
Total:			1032.378	386.123	100.00	20.000

Figure S2: RP-HPLC trace of authentic standards (Neu5Gc+Neu5Ac).

14 SiaNAl, 5 pM

Sample Name:	**SiaNAl, 5 pM**		
Vial Number:	**B12**	*Injection Volume:*	**20.0**
Sample Type:	**standard**	*Sequence Name:*	**09-11-07**
Control Program:	**DMBTSK 2rev6**	*Quantif. Method:*	**SYS2 BSM**
Recording Time:	**13.09.07 13:17**		
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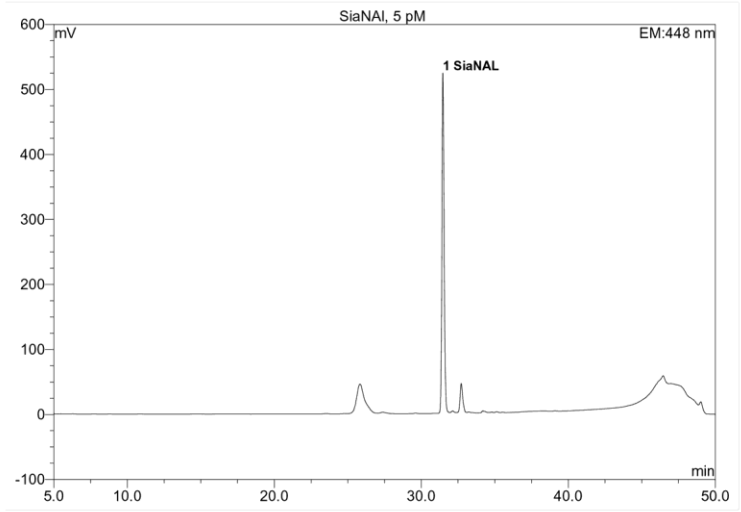

No.	Ret.Time	Peak Name	Height	Area	Rel.Area	Amount
	min		mV	mV*min	%	pMol
1	31.47	SiaNAL	522.112	89.438	100.00	5.000
Total:			522.112	89.438	100.00	5.000

Figure S3: RP-HPLC trace of authentic standard (SiaNAl).

8 Std. Mixt. + Az, 10 pM

Sample Name:	**Std. Mixt. + Az, 10 pM**		
Vial Number:	**C10**	*Injection Volume:*	**20.0**
Sample Type:	**standard**	*Sequence Name:*	**08-15-07 AZ**
Control Program:	**DMBTSK2 new 3**	*Quantif. Method:*	**SYS2 BSM**
Recording Time:	**21.08.07 17:10**		
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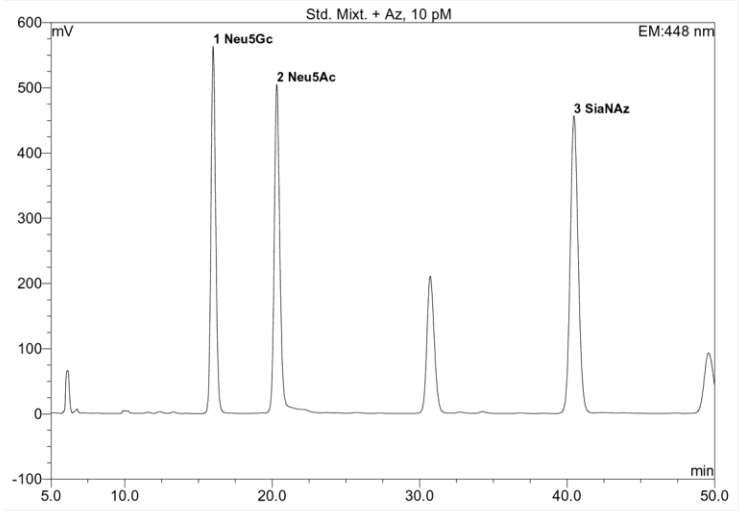
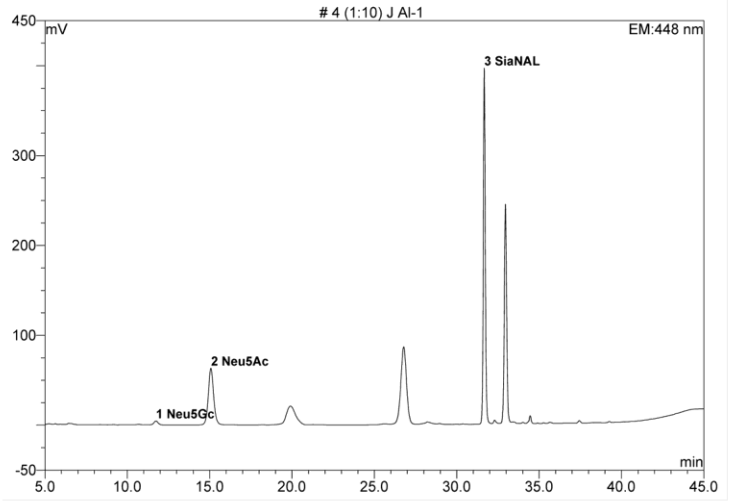


No.	Ret.Time	Peak Name	Height	Area	Rel.Area	Amount
	min		mV	mV*min	%	pMol
1	16.00	Neu5Gc	562.826	207.899	29.55	10.000
2	20.31	Neu5Ac	499.820	212.087	30.14	10.000
3	40.47	SiaNAz	455.661	283.677	40.31	20.000
Total:			1518.307	703.663	100.00	40.000

Figure S4: RP-HPLC trace of authentic standards (Neu5Gc+Neu5Ac+SiaNAz).

16 # 4 (1:10) J AI-1

Sample Name:	**# 4 (1:10) J AI-1**		
Vial Number:	**C21**	*Injection Volume:*	**20ul**
Sample Type:	**unknown**	*Sequence Name:*	**06-13-07-AI**
Control Program:	**DMBTSK 2rev6**	*Quantif. Method:*	**SYS2 BSM**
Recording Time:	**14.06.07 18:27**		
Directory:	**GLYCODX600FLOUR_2\2007-RF2000\DMB\PWu**		



No.	Ret.Time	Peak Name	Height	Area	Rel.Area	Amount
	min		mV	mV*min	%	pMol
1	11.73	Neu5Gc	4.163	1.115	1.41	0.056
2	15.07	Neu5Ac	62.763	23.096	29.27	1.124
3	31.67	SiaNAL	395.855	54.685	69.31	3.131
Total:			462.781	78.896	100.00	4.311

Figure S5: RP-HPLC trace of cell lysates from Jurkat cells cultured with Ac_4ManNAl.

5 # 1 J N3-1

Sample Name:	**# 1 J N3-1**		
Vial Number:	**C4**	*Injection Volume:*	**20ul**
Sample Type:	**unknown**	*Sequence Name:*	**06-12-07**
Control Program:	**DMBTSK2 new 3**	*Quantif. Method:*	**SYS2 BSM**
Recording Time:	**12.06.07 16:57**		
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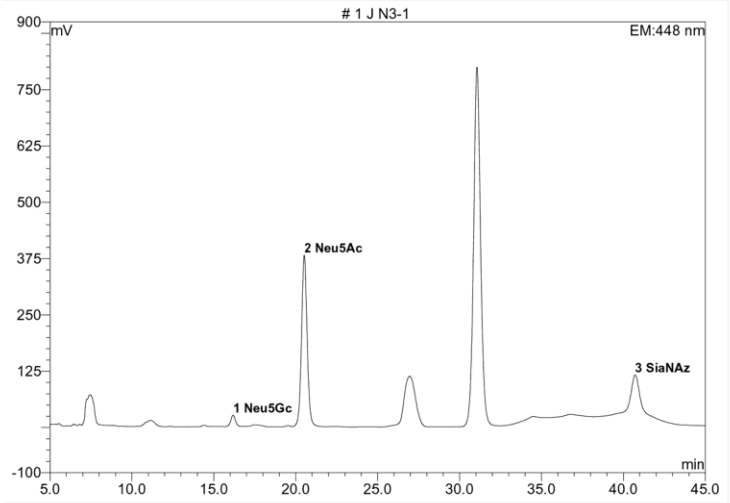
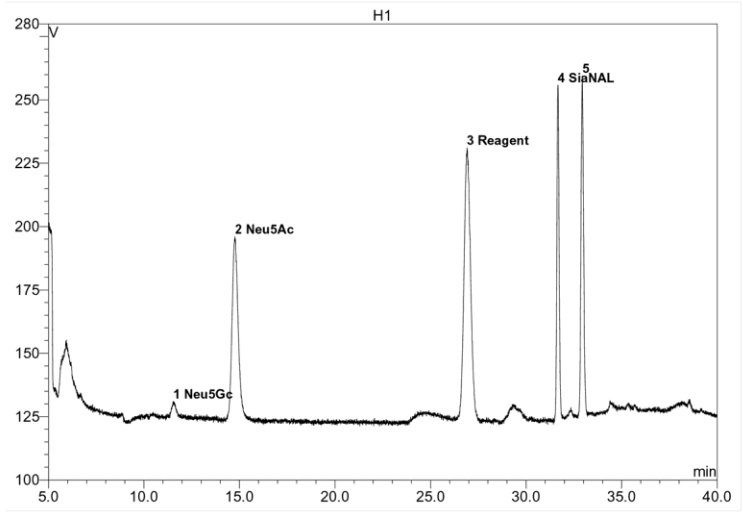


No.	Ret.Time	Peak Name	Height	Area	Rel.Area	Amount
	min		mV	mV*min	%	pMol
1	16.18	Neu5Gc	24.728	8.011	3.83	0.448
2	20.52	Neu5Ac	380.319	150.030	71.74	8.306
3	40.72	SiaNAz	86.231	51.101	24.43	3.578
Total:			491.278	209.142	100.00	12.331

Figure S6: RP-HPLC trace of cell lysates from Jurkat cells cultured with Ac_4ManNAz.

3 H1

Sample Name:	**H1**		
Vial Number:	**C5**	*Injection Volume:*	**20.0**
Sample Type:	**unknown**	*Sequence Name:*	**100607-4rpt**
Control Program:	**DMBTSK 2rev6**	*Quantif. Method:*	**SYS2 BSM**
Recording Time:	**21.12.06 00:24**		
Directory:	**GLYCODX600FLOUR_1\SA-DMB\2006\DMB Analysis\Peng Wu**		



No.	Ret.Time	Peak Name	Height	Area	Rel.Area	Amount
	min		V	V*min	%	pMol
1	11.52	Neu5Gc	4.772	0.906	0.84	0.282
2	14.76	Neu5Ac	71.374	25.478	23.75	8.033
3	26.91	Reagent	107.387	42.273	39.41	n.a.
4	31.66	SiaNAL	132.522	17.985	16.77	7.525
5	32.94	n.a.	133.315	20.634	19.23	n.a.
Total:			449.370	107.275	100.00	15.840

Figure S7: RP-HPLC trace of cell lysates from HEK 293T cells cultured with Ac_4ManNAl.

9 # 8 HEK N3-2

Sample Name:	**# 8 HEK N3-2**		
Vial Number:	**C8**	*Injection Volume:*	**20ul**
Sample Type:	**unknown**	*Sequence Name:*	**06-12-07**
Control Program:	**DMBTSK2 new 3**	*Quantif. Method:*	**SYS2 BSM**
Recording Time:	**12.06.07 22:22**		
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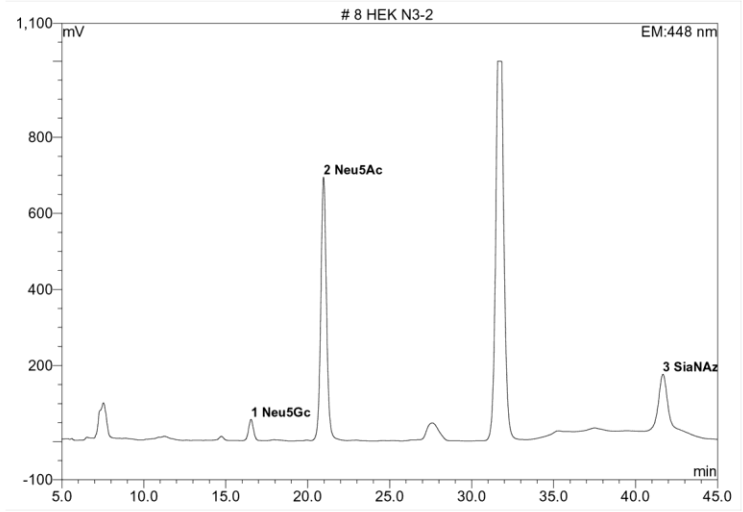
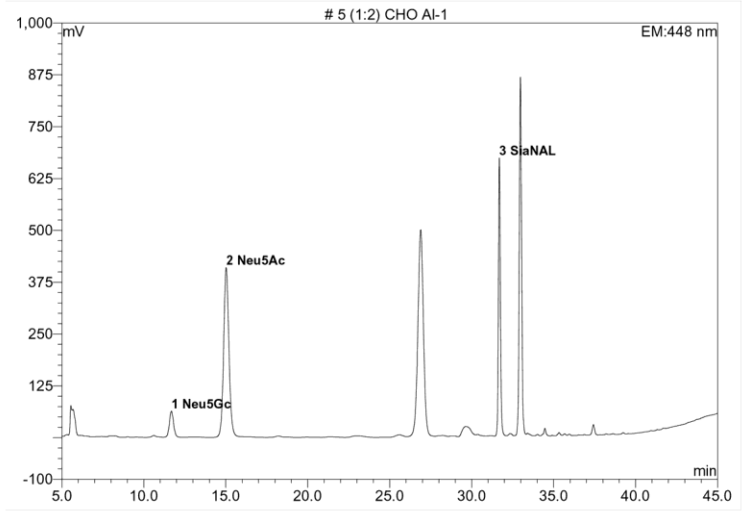


No.	Ret.Time	Peak Name	Height	Area	Rel.Area	Amount
	min		mV	mV*min	%	pMol
1	16.54	Neu5Gc	55.747	19.166	4.98	1.071
2	20.97	Neu5Ac	692.790	278.379	72.37	15.412
3	41.67	SiaNAz	144.368	87.142	22.65	6.101
Total:			892.905	384.686	100.00	22.583

Figure S8: RP-HPLC trace of cell lysates from HEK 293T cells cultured with Ac_4ManNAz.

5 # 5 (1:2) CHO AI-1

Sample Name:	**# 5 (1:2) CHO AI-1**		
Vial Number:	**C22**	*Injection Volume:*	**20ul**
Sample Type:	**unknown**	*Sequence Name:*	**06-13-07-AI**
Control Program:	**DMBTSK 2rev6**	*Quantif. Method:*	**SYS2 BSM**
Recording Time:	**14.06.07 02:47**		
Directory:	**GLYCODX600FLOUR_2\2007-RF2000\DMB\PWu**		

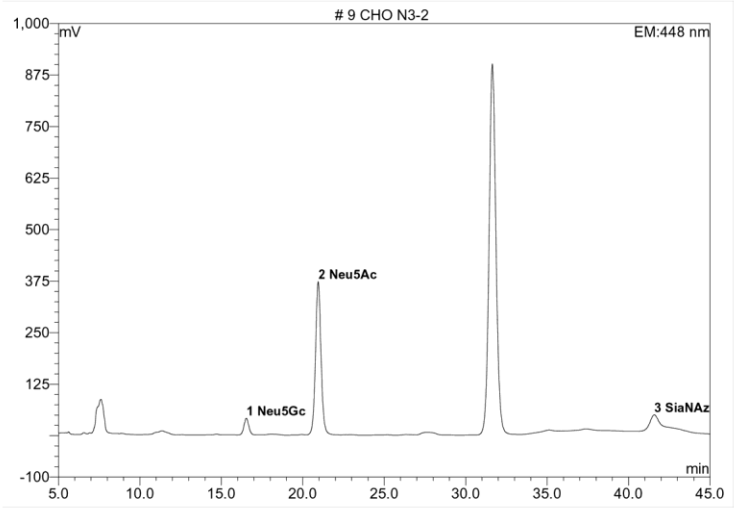


No.	Ret.Time	Peak Name	Height	Area	Rel.Area	Amount
	min		mV	mV*min	%	pMol
1	11.68	Neu5Gc	63.095	18.542	7.01	0.925
2	15.02	Neu5Ac	408.852	153.058	57.90	7.450
3	31.69	SiaNAL	670.426	92.764	35.09	5.312
Total:			1142.373	264.365	100.00	13.687

Figure S9: RP-HPLC trace of cell lysates from CHO cells cultured with Ac_4ManNAl.

10 # 9 CHO N3-2

Sample Name:	**# 9 CHO N3-2**		
Vial Number:	**C9**	*Injection Volume:*	**20ul**
Sample Type:	**unknown**	*Sequence Name:*	**06-12-07**
Control Program:	**DMBTSK2 new 3**	*Quantif. Method:*	**SYS2 BSM**
Recording Time:	**12.06.07 23:43**		
Directory:	**GLYCODX600FLOUR_2\2007-RF2000\DMB\PWu**		

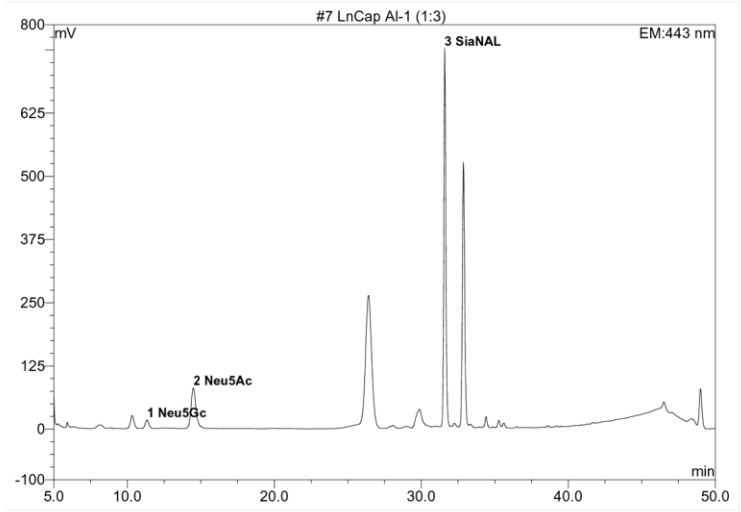


No.	Ret.Time	Peak Name	Height	Area	Rel.Area	Amount
	min		mV	mV*min	%	pMol
1	16.54	Neu5Gc	40.533	14.005	7.51	0.782
2	20.95	Neu5Ac	372.148	147.139	78.88	8.146
3	41.58	SiaNAz	36.950	25.385	13.61	1.777
Total:			449.632	186.529	100.00	10.706

Figure S10: RP-HPLC trace of cell lysates from CHO cells cultured with Ac_4ManNAz.

10 #7 LnCap Al-1 (1:3)

Sample Name:	**#7 LnCap Al-1 (1:3)**		
Vial Number:	**C17**	*Injection Volume:*	**20.0**
Sample Type:	**unknown**	*Sequence Name:*	**08-20-07 Al**
Control Program:	**DMBTSK 2rev6**	*Quantif. Method:*	**SYS2 BSM**
Recording Time:	**20.08.07 23:10**		
Directory:	**GLYCODX600FLOUR_2\2007-RF2000\DMB\PWu**		

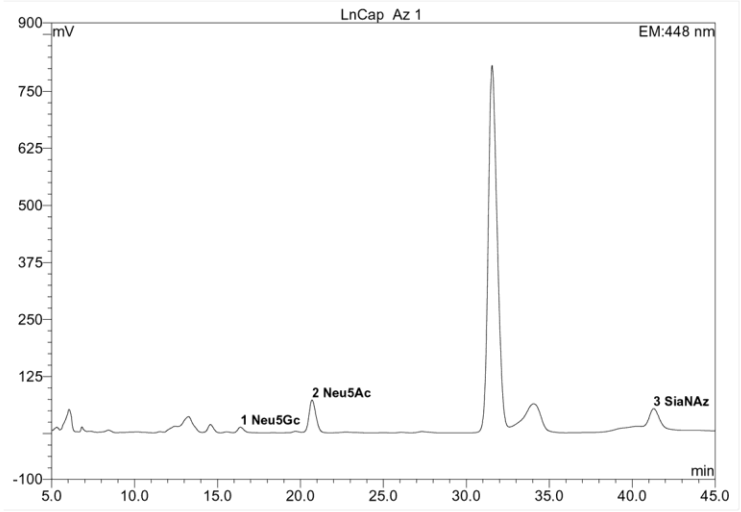


No.	Ret.Time	Peak Name	Height	Area	Rel.Area	Amount
	min		mV	mV*min	%	pMol
1	11.34	Neu5Gc	16.705	4.934	3.19	0.265
2	14.49	Neu5Ac	79.540	30.075	19.43	1.577
3	31.59	SiaNAL	747.736	119.769	77.38	6.920
Total:			843.981	154.777	100.00	8.761

Figure S11: RP-HPLC trace of cell lysates from LNCaP cells cultured with Ac_4ManNAl.

5 LnCap Az 1

Sample Name:	**LnCap Az 1**		
Vial Number:	**B9**	*Injection Volume:*	**20ul**
Sample Type:	**unknown**	*Sequence Name:*	**09-07-07**
Control Program:	**DMBTSK2 new 3**	*Quantif. Method:*	**SYS2 BSM**
Recording Time:	**08.09.07 07:49**		
Directory:	**GLYCODX600FLOUR_2\2007-RF2000\DMB\PWu**		

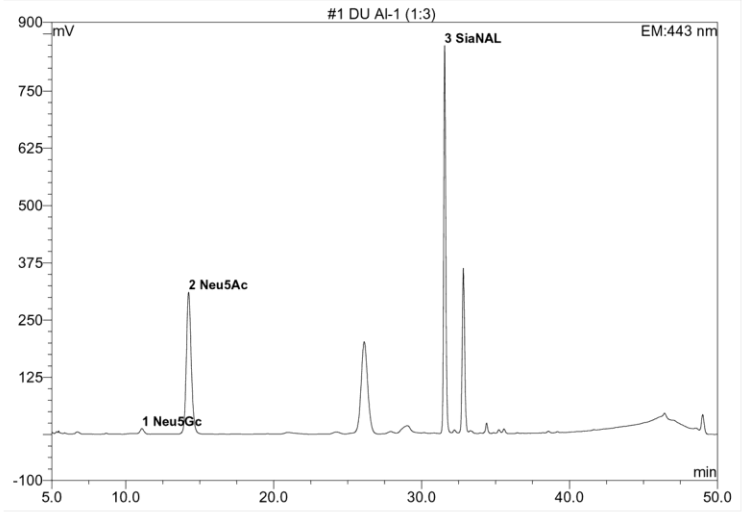


No.	Ret.Time	Peak Name	Height	Area	Rel.Area	Amount
	min		mV	mV*min	%	pMol
1	16.38	Neu5Gc	12.107	4.931	7.55	0.303
2	20.70	Neu5Ac	71.249	33.348	51.06	1.973
3	41.29	SiaNAz	41.375	27.036	41.39	2.372
Total:			124.732	65.315	100.00	4.649

Figure S12: RP-HPLC trace of cell lysates from LNCaP cells cultured with Ac_4ManNAz.

6 #1 DU AI-1 (1:3)

Sample Name:	**#1 DU AI-1 (1:3)**		
Vial Number:	**C13**	*Injection Volume:*	**20.0**
Sample Type:	**unknown**	*Sequence Name:*	**08-20-07 AI**
Control Program:	**DMBTSK 2rev6**	*Quantif. Method:*	**SYS2 BSM**
Recording Time:	**20.08.07 17:45**		
Directory:	**GLYCODX600FLOUR_2\2007-RF2000\DMB\PWu**		

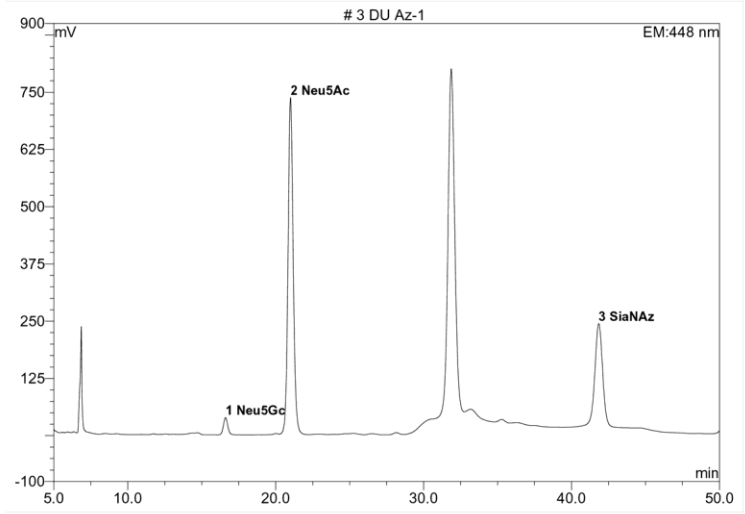


No.	Ret.Time	Peak Name	Height	Area	Rel.Area	Amount
	min		mV	mV*min	%	pMol
1	11.10	Neu5Gc	11.013	3.045	1.18	0.163
2	14.25	Neu5Ac	308.686	117.120	45.44	6.140
3	31.56	SiaNAL	845.860	137.559	53.37	7.948
Total:			1165.559	257.724	100.00	14.251

Figure S13: RP-HPLC trace of cell lysates from DU145 cells cultured with Ac_4ManNAl.

4 # 3 DU Az-1

Sample Name:	**# 3 DU Az-1**		
Vial Number:	**C4**	*Injection Volume:*	**20.0**
Sample Type:	**unknown**	*Sequence Name:*	**08-15-07 AZ**
Control Program:	**DMBTSK2 new 3**	*Quantif. Method:*	**SYS2 BSM**
Recording Time:	**15.08.07 21:45**		
Directory:	**GLYCODX600FLOUR_2\2007-RF2000\DMB\PWu**		

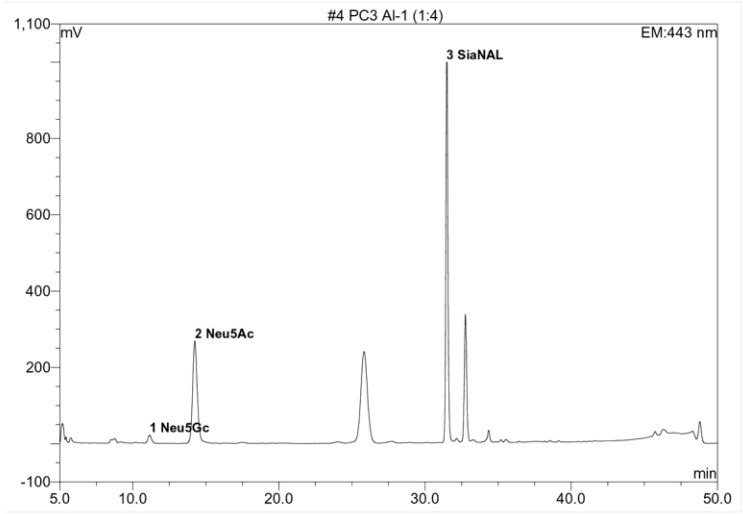


No.	Ret.Time	Peak Name	Height	Area	Rel.Area	Amount
	min		mV	mV*min	%	pMol
1	16.61	Neu5Gc	37.647	12.692	2.85	0.610
2	21.00	Neu5Ac	734.960	295.505	66.39	13.933
3	41.84	SiaNAz	224.710	136.939	30.76	9.655
Total:			997.317	445.136	100.00	24.198

Figure S14: RP-HPLC trace of cell lysates from DU145 cells cultured with Ac_4ManNAz.

14 #4 PC3 Al-1 (1:4)

Sample Name:	**#4 PC3 Al-1 (1:4)**		
Vial Number:	**C15**	*Injection Volume:*	**20.0**
Sample Type:	**unknown**	*Sequence Name:*	**08-20-07 Al**
Control Program:	**DMBTSK 2rev6**	*Quantif. Method:*	**SYS2 BSM**
Recording Time:	**21.08.07 11:45**		
Directory:	**GLYCODX600FLOUR_2\2007-RF2000\DMB\PWu**		

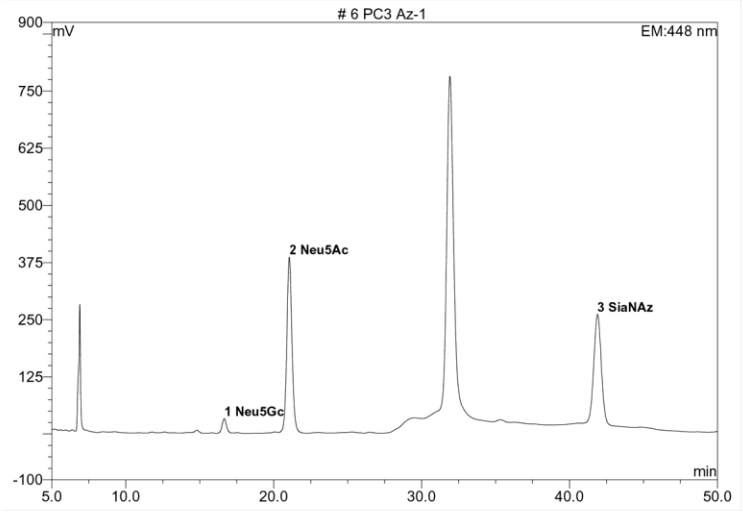


No.	Ret.Time	Peak Name	Height	Area	Rel.Area	Amount
	min		mV	mV*min	%	pMol
1	11.15	Neu5Gc	21.545	6.245	2.27	0.335
2	14.24	Neu5Ac	268.336	99.457	36.23	5.214
3	31.46	SiaNAL	995.859	168.806	61.49	9.753
Total:			1285.740	274.508	100.00	15.302

Figure S15: RP-HPLC trace of cell lysates from PC3 cells cultured with Ac_4ManNAl.

5 # 6 PC3 Az-1

Sample Name:	**# 6 PC3 Az-1**		
Vial Number:	**C5**	*Injection Volume:*	**20.0**
Sample Type:	**unknown**	*Sequence Name:*	**08-15-07 AZ**
Control Program:	**DMBTSK2 new 3**	*Quantif. Method:*	**SYS2 BSM**
Recording Time:	**15.08.07 23:06**		
Directory:	**GLYCODX600FLOUR_2\2007-RF2000\DMB\PWu**		



No.	Ret.Time	Peak Name	Height	Area	Rel.Area	Amount
	min		mV	mV*min	%	pMol
1	16.66	Neu5Gc	31.402	10.415	3.41	0.501
2	21.05	Neu5Ac	383.699	154.258	50.46	7.273
3	41.89	SiaNAz	240.028	141.002	46.13	9.941
Total:			655.129	305.676	100.00	17.715

Figure S16: RP-HPLC trace of cell lysates from PC3 cells cultured with Ac_4ManNAz.

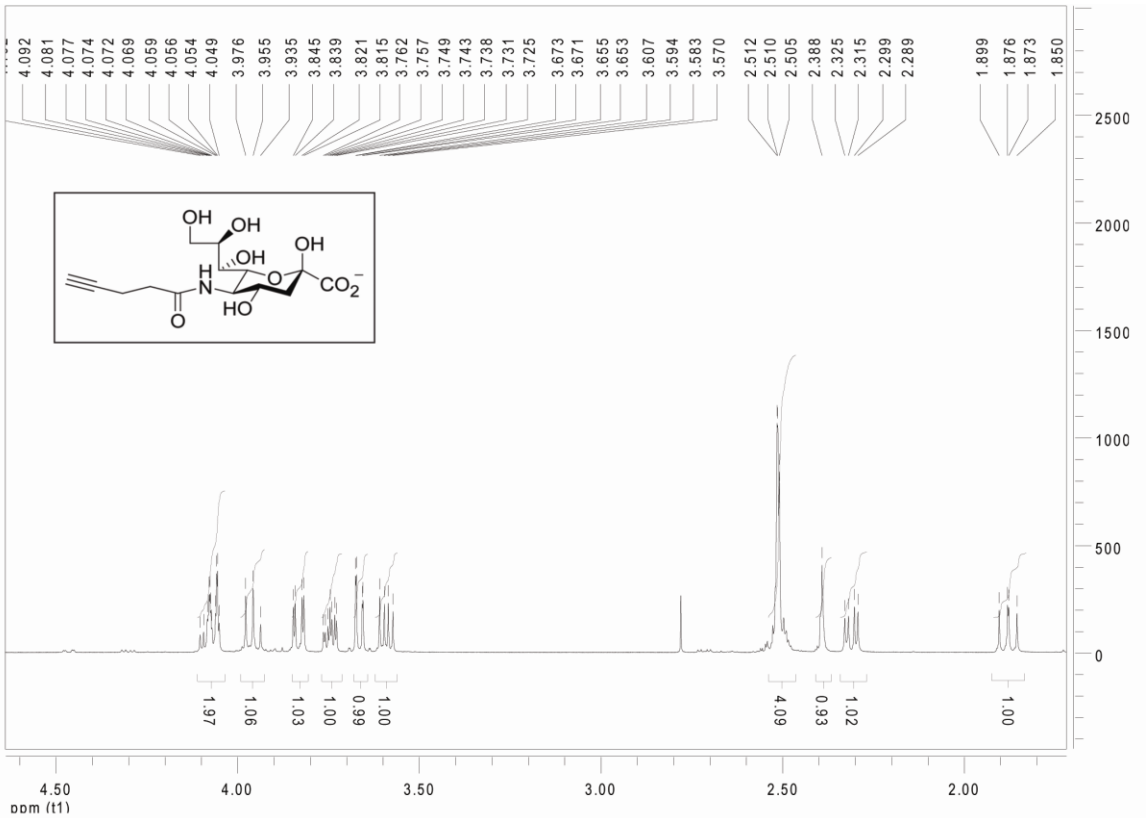


Figure S17: ^{1}H NMR of SiaNAl.

Table S1. Incorporation efficiencies of SiaNAl and SiaNAz in organ lysates[a]

Organ	Liver	Thymus	Spleen	Gut
SiaNAl/SiaNAz	1.66	1.25	1.25	1.26

[a]Based on densitometry measurements of Western blots using the Image J program provided by the National Institutes of Health (http://rsb.info.nih.gov/ij/docs/menus/analyze.html#gels)

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[1] T. L. Hsu, S. R. Hanson, K. Kishikawa, S. K. Wang, M. Sawa, C. H. Wong, *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 2614-2619.

[2] G. W. Jourdian, L. Dean, S. Roseman, *J. Biol. Chem.* **1971**, *246*, 430-435.

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[4] S. J. Luchansky, S. Goon, C. R. Bertozzi, *Chembiochem* **2004**, *5*, 371-374.