-Supporting Information-

Photoactivatable Glycopolymers for the Proteome-Wide Identification of Fucose-α(1-2)-Galactose Binding Proteins

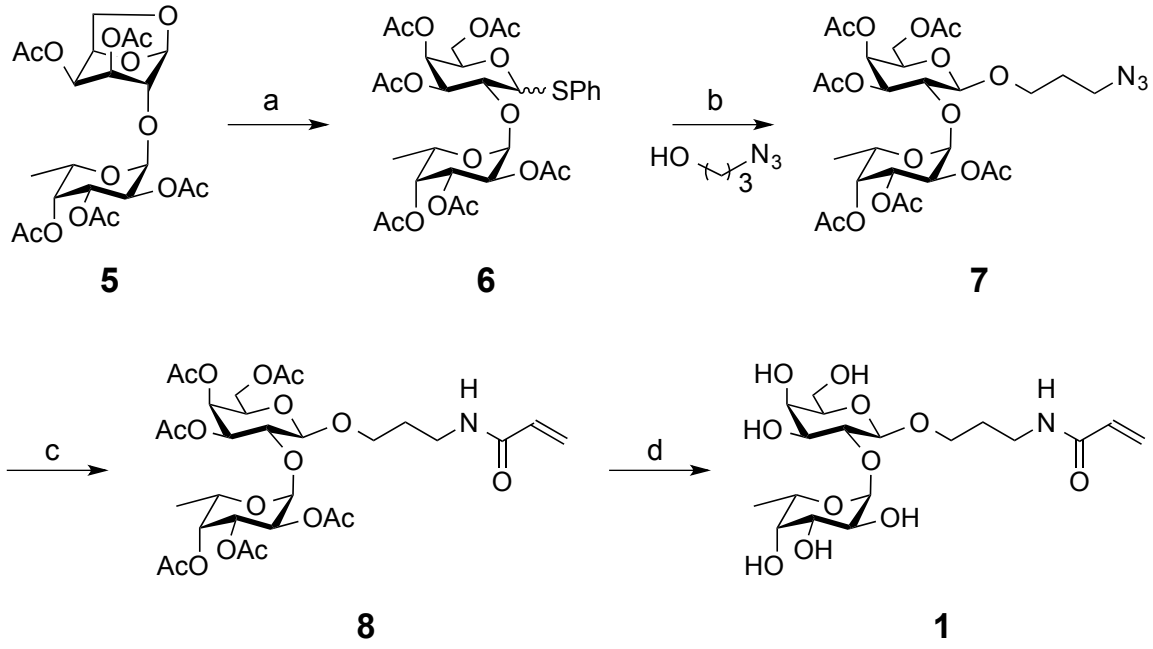
Arif Wibowo,[†] Eric C. Peters,[§] and Linda C. Hsieh-Wilson[†]

[†]Division of Chemistry and Chemical Engineering, California Institute of Technology and Howard Hughes Medical Institute, 1200 East California Boulevard, Pasadena, California 91125, United States

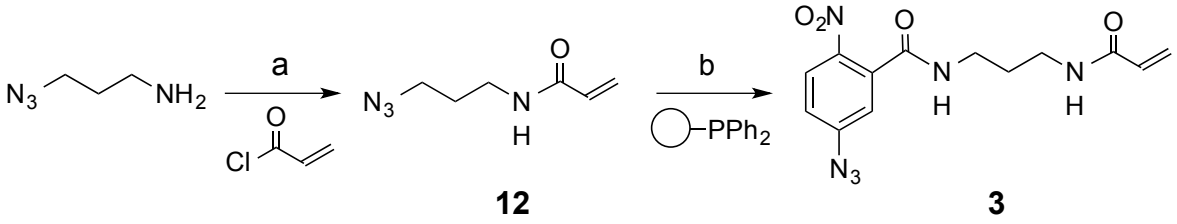
[§]Genomics Institute of the Novartis Research Foundation, San Diego, California 92121, United States

CONTENTS:

1. Supporting Schemes



Scheme S1. a) (i) TMSSPh, ZnI_2, DCM; (ii) TBAF, THF; (iii) Ac_2O, DMAP, pyr (55%, three steps). b) NIS-AgOTf, 3-azido-1-propanol, MeCN, -20 ^oC to 25 ^oC, 64%. c) (i) Pd/C, H_2, EtOAc; (ii) aq. $NaHCO_3$, acryloyl chloride, dioxane, 0 ^oC to 25 ^oC (57%, two steps). d) NaOMe, MeOH, 90%.



Scheme S2. (a) aq. $NaHCO_3$, acryloyl chloride, dioxane, 0 ^oC to 25 ^oC, 83%. (b) (i) PPh_3 polystyrene beads, THF, 37 ^oC, 24 h; (ii) 10:1 THF: H_2O, 37 ^oC, 48 h; (iii) ANB-NOS, Et_3N, DMF (33%, three steps).

2. Supporting Figure

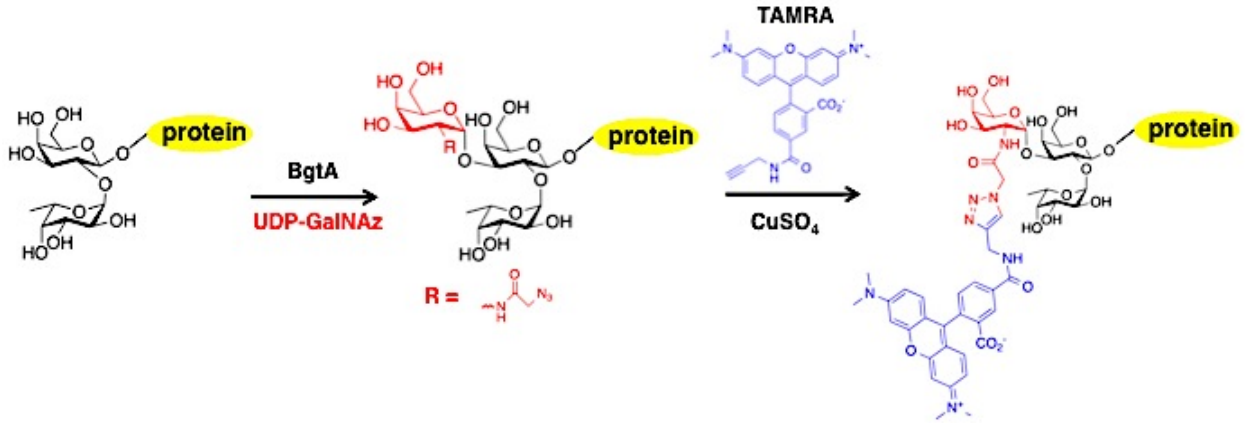


Figure S1. Chemoenzymatic strategy for the detection of Fucα(1-2)Gal glycans. Exogenous addition of the glycosyltransferase BgtA and UDP-GalNAz will covalently tag Fucα(1-2)Gal sugars with an azido functionality, which can be labeled with reporter groups such as fluorescent dyes using azide-alkyne [3+2] cycloaddition chemistry.

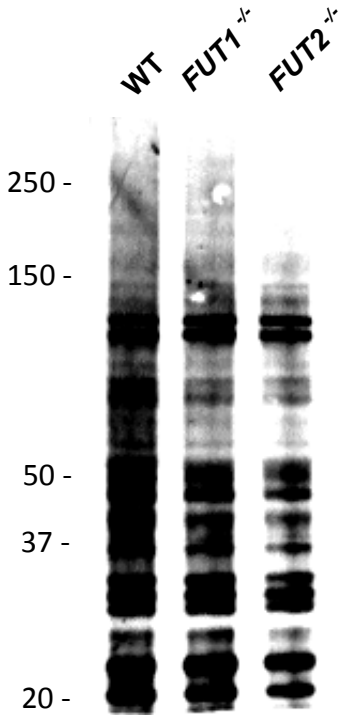


Figure S2. Expression of Fucα(1-2)Gal-containing glycoproteins in the rat brain cortex. Glycoproteins were detected using fluorescein-conjugated UEAI.

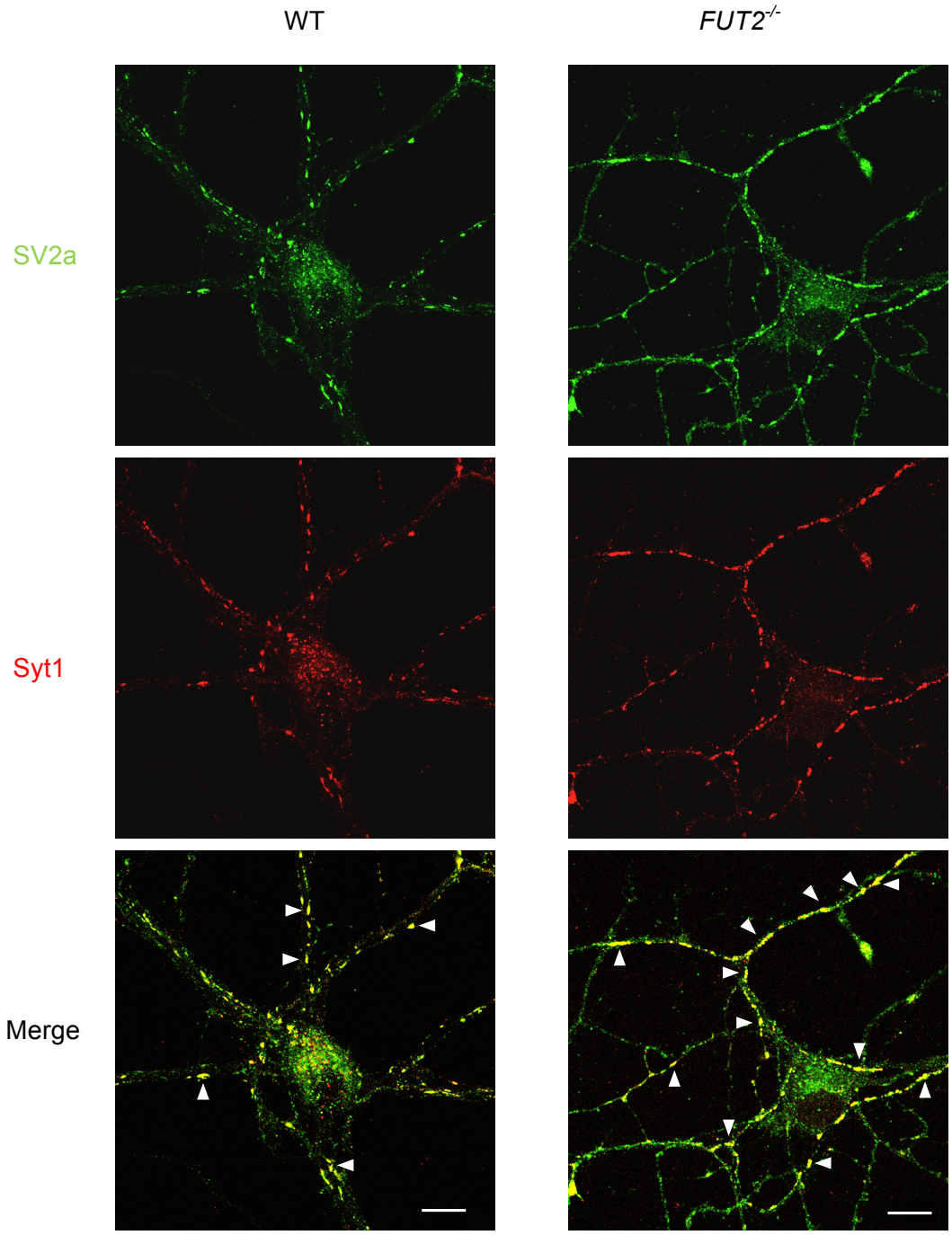


Figure S3. Coimmunostaining of WT and *FUT2*$^{-/-}$ cortical neuronal cultures for SV2a (green) and syt1 (red). Arrowheads indicate colocalized puncta (yellow) of SV2a and syt along the neuronal processes. Scale bar = 10 μm.

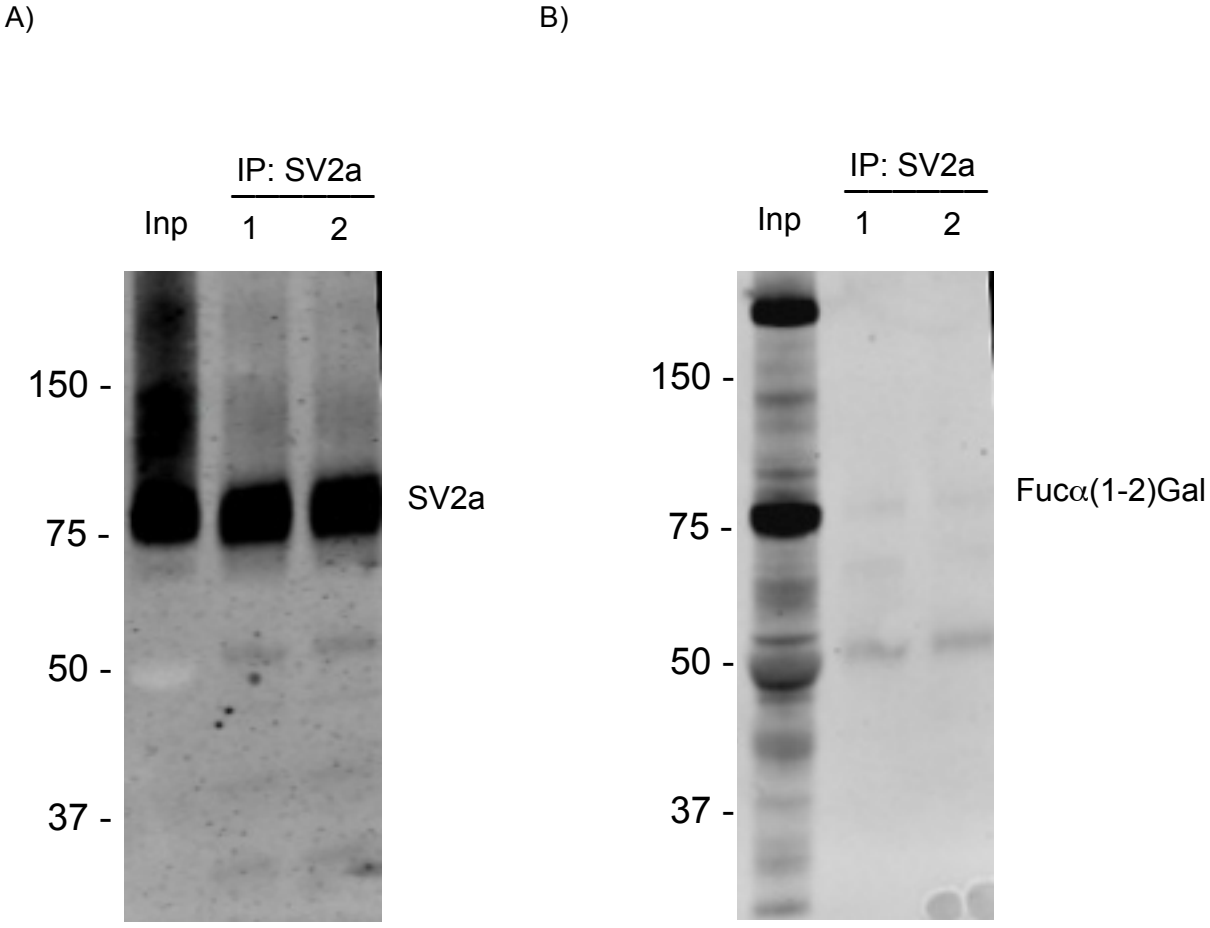


Figure S4. SV2a is not modified by Fucα(1-2)Gal glycans . SV2a was immunoprecipitated from the rat brain cortex, and the immunoprecipitated protein was analyzed by A) Western blotting using an anti-Sv2a antibody or B) lectin blotting using biotinylated-UEAI followed by streptavidin conjugated to horseradish peroxidase to detect Fucα(1-2)Gal glycans. Inp, lysate input into IP; 1 and 2 indicate duplicate samples.

3. Supporting Table

Table S1. Fucα(1-2)Gal Glycan-Binding Proteins Identified from Rat Synaptosomal and Membrane Fractions[a]

Protein	Accession Number	Unique Peptides[b]	% Coverage[b]	MW (Da)	Function
Cellular Adhesion					
Ctnna2	IPI00364916.2	8	13.6	100000	Cellular differentation
Ctnnd1	IPI00359491.3	6	10	105000	Cellular recognition
Cellular Signalling					
Dclk1 (Ania 4)	IPI00778626.1	13	23.8	84000	Neuronal migration
Centg3	IPI00358128.3	6	9	102000	Signal transduction
Crmp1	IPI00561065.2	9	16	74000	Axonal guidance
Crimp4	IPI00203250.1	7	13.6	74000	Neurite outgrowth
Gαo	IPI00231505.5	7	22.3	40000	Signal transduction
Gnaq	IPI00230868.4	6	20.1	42000	Signal transduction
Pkcε	IPI00551781.4	10	17.8	83000	Phosphorylation
Cytoskeletal-Associated Proteins					
Ckap5	IPI00764313.1	9	5.9	197000	Microtubule elongation
Cyln2	IPI00195929.1	10	12.3	111000	Microtubule associated transport
Dync1h1	IPI00327630.1	54	13.5	532000	Dynein motor protein
Kif5b	IPI00364904.2	10	15.1	110000	Kinesin motor protein
Kif5c	IPI00193402.4	16	21.2	109000	Kinesin motor protein
LOC362587 similar to Macf1	IPI00359003.4	6	1	831000	Actin regulation
LOC367171 similar to Map4	IPI00393975.2	8	10.4	110000	Microtubule assembly
LOC363309 similar to Tbcd	IPI00765967.1	6	5.6	134000	Microtubule regulation
Map2	IPI00206171.1	10	7.1	202000	Cytoskelatal organization
Cellular Metabolism					
Aco2	IPI00421539.3	8	13.1	85000	Mitochondrial metabolism
Cad	IPI00365582.3	6	3.1	243000	Enzymatic biosynthesis
Fasn	IPI00200661.1	21	11.1	273000	Lipid biosynthesis
Hk1	IPI00202543.1	10	13	102000	Mitochondrial metabolism
Immt	IPI00364895.4	7	12.4	82000	Mitochondrial homeostasis
LOC360975 similar to Odo1	IPI00215093.1	13	17.9	116000	Mitochondrial metabolism
Phgdh	IPI00475835.3	7	16.9	56000	Enzymatic biosynthesis
Prss15	IPI00205076.1	10	12.9	106000	Mitochondrial proteases
Uspx9	IPI00204923.4	11	5.4	291000	Ubiquitin Hydrolases
Protein Synthesis					
Aars	IPI00363563.3	8	11.5	107000	Translation
Cand1	IPI00205466.1	8	7.1	136000	Transcription
Ddx1	IPI00555314.1	8	12.8	82000	Transcription
Lrpprc Leucine rich protein 157	IPI00360075.2	12	10.7	157000	Transcription
Nars	IPI00565217.3	7	15.2	64000	Translation
Tufm	IPI00371236.3	7	19.7	50000	Translation
Protein Trafficking					
Dctn1	IPI00196703.1	8	7.2	142000	Retrograde vesicle transport
Hspcb	IPI00471584.7	13	18.9	83000	Chaperone for protein folding
Hspd1	IPI00339148.2	12	33	61000	Chaperone for mitochondrial protein
LOC315676 similar to Dmx-like 2	IPI00369671.3	7	3.1	344000	Synaptic vesicle scaffolding
Sv2a	IPI00208115.4	6	10.8	83000	Neurotransmitter release
Syn2	IPI00210036.1	6	17.1	63000	Neurotransmitter release
Vac14	IPI00230981.1	7	11.6	88000	Endosome regulation
Vps35	IPI00363493.2	6	9.6	92000	Retrograde protein transport
Transporters					
Atp5a1	IPI00396910.1	10	24.6	60000	ADP/ATP biosynthesis
Gria2	IPI00780113.1	6	7.5	103000	Glutamate Receptor
Slc25a4	IPI00231927.11	8	29.9	33000	ADP/ATP Translocase

[a] Proteins from Sprague Dawley rats were captured using glycopolymer **10**

[b] The number of full tryptic unique peptides and their coverage were identified from a reversed database searching strategy. See Materials and Methods for procedures and analysis criteria.

4. Experimental Methods

4-1. Material and Methods

Unless otherwise stated, reactions were performed in flame-dried glassware under an argon atmosphere, using anhydrous solvents. Solvents were dried by passage through an activated alumina column under argon. All other commercially obtained reagents were used as received unless otherwise noted. Thin layer chromatography (TLC) was performed using E. Merck silica gel 60 F254 precoated plates (0.25 mm). Visualization of the developed chromatogram was performed by UV, *p*-anisaldehyde or ninhydrin stain as necessary. ICN silica gel (particle size 0.032 - 0.063 mm) was used for flash chromatography. Gel filtration chromatography (Sephadex LH-20, and G-25 ultrafine) was used in order to achieve purification of the final products. ^{1}H NMR spectra were recorded on Varian Inova 500 (500 MHz) and 600 (600 MHz) spectrometers and are reported in parts per million (δ) relative to $CDCl_3$ (7.26 ppm), CD_3OD (4.87 ppm) or D_2O (4.80 ppm). Data for ^{1}H are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant in Hz, and integration. ^{13}C NMR spectra were recorded on a Varian Inova 500 (125 MHz) spectrometer and are reported in terms of chemical shift. High-resolution mass spectra were obtained by the Caltech Mass Spectral Facility using a Waters LCT Premier XE high-resolution mass spectrometer.

Gel Permeation Chromatography (GPC) was carried out in 100 mM $NaNO_3$ and 200 ppm NaN_3 in water using an OHpak SB – 804 HQ column (Shodex), which was connected in series with a miniDAWN TREOS MALLS detector and Optilab rEX differential refractometer (both from Wyatt Technology). The dn/dc values were obtained for each injection assuming 100% mass elution from the column using dextran (40K) as a calibration standard to confirm complete mass recovery.

4-2. Synthetic Procedures

Phenyl 3,4,6-tri-*O*-acetyl-2-O-(methyl 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-1-thio-D-galactopyranoside (6). A mixture of **5**[1] (510 mg, 0.98 mmol), (phenylthio)trimethylsilane (0.77 mL, 3.9 mmol) and zinc iodide (1.3 g, 3.9 mmol) in CH_2Cl_2 (1.0 mL) was stirred overnight at rt.[2] Following complete consumption of the starting material, the mixture was diluted with EtOAc and washed successfully with aq. $NaHCO_3$, H_2O and brine. The organic extract was dried over Na_2SO_4 and concentrated *in vacuo*. The resulting residue was dissolved in dry THF (2.8 mL), and tetrabutylammonium fluoride in THF (2.0 mL of a 1M solution) was added to the solution. After 1 h, the solvent was removed *in vacuo*, and the residue was redissolved in EtOAc, washed with H_2O, aq. $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and concentrated to afford the crude thioglycoside. To a solution of this thioglycoside in dry pyridine (9.6 mL, 120 mmol) was added a catalytic amount of DMAP (150 mg, 1.2 mmol) and acetic anhydride (6.1 mL, 64 mmol). The reaction mixture was stirred at rt overnight, then diluted with EtOAc and washed successively with aq. $CuSO_4$, aq. $NaHCO_3$, H_2O and brine. The organic extract was dried over Na_2SO_4 and concentrated *in vacuo*. Purification by silica gel flash chromatography (3:2 Hex:EtOAc) afforded **7** (360 mg, 55%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.48 (d, *J* = 7.3 Hz, 2H), 7.36 – 7.27 (m, 3H), 5.69 (d, *J* = 5.7 Hz, 1H), 5.45 (d, *J* = 3.1 Hz, 1H), 5.34 (d, *J* = 3.2 Hz, 1H), 5.30 (d, *J* = 3.7 Hz, 2H), 5.21 (dd, *J* = 10.6, 3.4 Hz, 1H), 5.03 (dd, *J* = 10.7, 3.9 Hz, 1H), 4.73 (t, *J* = 6.5 Hz, 1H), 4.36 (q, *J* = 6.5 Hz, 1H), 4.29 (dd, *J* = 10.6, 5.7 Hz, 1H), 4.07 (t, *J* = 6.4 Hz, 2H), 2.15 (s, 3H), 2.14 (s, 3H), 2.04 (s, 3H), 1.99 (s, 6H), 1.96 (s, 3H), 1.00 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.69, 170.65, 170.56, 170.27, 170.03, 169.97, 133.22, 131.70 (2C), 129.32 (2C), 127.78, 98.21, 87.81, 73.15, 70.99, 70.09, 68.16 (2C), 67.89, 67.35, 65.87, 61.83, 20.92, 20.88, 20.84 (2C), 20.81, 20.80, 16.03. HRMS *m/z* Calculated for $C_{30}H_{38}O_{15}S$ $[M]^+$: 670.1931. Found: 670.1941.

3-Azidopropyl-3,4,6-tri-*O*-acetyl-2-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-D-galactopyranoside (7). The following modified procedure was adapted from Kanie *et al*.[3] Compound **6** (360 mg, 0.54 mmol) and 3-azido-1-propanol (75 µL, 0.81 mmol) was azeotroped with toluene, dissolved in acetonitrile with 4Å molecular sieves, and cooled to -20 °C. *N*-Iodosuccinimide (190 mg, 0.81 mmol) was added, and the mixture was allowed to stir for 15 min. Silver triflate (210 mg, 0.81 mmol) was then added, and the mixture was allowed to warm to rt and stirred for 1 h. After the reaction was complete, the reaction mixture was diluted with EtOAc and washed successively with saturated aq. Na_2SO_3, aq. $NaHCO_3$, and brine. The organic extract was dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silica gel flash chromatography (3:2 Hex:EtOAc) to afford **7** (230 mg, 64%) as a pale-yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.39 (d, *J* = 3.9 Hz, 1H), 5.35 – 5.25 (m, 3H), 5.05 – 4.98 (m, 2H), 4.54 – 4.44 (m, 2H), 4.18 (dd, *J* = 11.2, 6.5 Hz, 1H), 4.10 (dd, *J* = 11.3, 6.9 Hz, 1H), 4.01 – 3.85 (m, 3H), 3.68 (dt, *J* = 9.9, 6.3 Hz, 1H), 3.43 (t, *J* = 6.6 Hz, 2H), 2.16 (s, 3H), 2.13 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H), 1.99 (d, *J* = 3.4 Hz, 6H), 1.93 – 1.86 (m, 2H), 1.13 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.78, 170.73, 170.54, 170.41 (2C), 170.20, 101.75, 95.85, 73.83, 71.91, 71.25, 70.63, 68.33, 67.73, 67.31, 67.02, 64.77, 61.38, 48.28, 29.44, 20.88, 20.84 (3C), 20.80, 20.77, 15.90. HRMS *m/z* Calculated for $C_{27}H_{39}O_{16}Na$ $[M+Na]^+$: 684.2228. Found: 684.2228.

3-*N*-Acryloyl-aminopropyl-3,4,6-tri-*O*-acetyl-2-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-D-galactopyranoside (8). A suspension of **7** (200 mg, 0.31 mmol) and Pd/C (20% by wt) in dry EtOAc (3.1 mL) was charged with H_2 gas for 3.5 h in the presence of a catalytic amount of triethylamine. The reaction mixture was diluted with EtOAc and filtered through celite. The filtrate was concentrated *in vacuo*, and the crude material was dissolved in dioxane (3.0 mL) before the addition of aq. $NaHCO_3$ (33 mg in 2.9 mL H_2O, 0.39 mmol). The reaction mixture was cooled to 0 °C, stirred for 15 min, and acryolyl chloride (37 µL, 0.45 mmol) was added

dropwise. The reaction was allowed to warm to rt overnight with minimal exposure to light, then diluted with EtOAc and washed with H_2O. The organic layer was concentrated *in vacuo*, and the crude product was purified by silica gel flash chromatography (1:9 MeOH:CH_2Cl_2) to afford **8** as colorless oil (120 mg, 57%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.30 (dd, *J* = 17.0, 1.4 Hz, 1H), 6.14 (dd, *J* = 17.0, 10.3 Hz, 1H), 5.64 (dd, *J* = 10.3, 1.4 Hz, 1H), 5.40 (d, *J* = 3.8 Hz, 1H), 5.32 – 5.27 (m, 3H), 5.04 – 4.97 (m, 2H), 4.54 (q, *J* = 6.6 Hz, 1H), 4.47 (d, *J* = 7.8 Hz, 1H), 4.18 (dd, *J* = 11.3, 6.7 Hz, 1H), 4.11 (dd, *J* = 12.4, 6.9 Hz, 1H), 3.97 – 3.86 (m, 3H), 3.76 – 3.69 (m, 1H), 3.44 (q, *J* = 6.6 Hz, 2H), 2.16 (s, 3H), 2.14 (s, 3H), 2.05 (d, *J* = 2.1 Hz, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.89 (td, *J* = 13.1, 6.6 Hz, 2H), 1.13 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.71, 170.70, 170.53, 170.47, 170.32, 170.30, 165.86, 131.02, 126.35, 101.74, 95.67, 73.72, 71.62, 71.15, 70.77, 68.24, 67.98, 67.83, 67.40, 64.79, 61.44, 37.03, 29.69, 20.88, 20.81, 20.78 (2C), 20.73 (2C), 15.83. HRMS *m/z* Calculated for $C_{30}H_{44}NO_{17}$ $[M+H]^+$: 690.2609. Found: 690.2629.

3-*N*-Acryloyl-aminopropyl-2-*O*-(α-L-fucopyranosyl)-D-galactopyranoside (1).

To a solution of **8** (120 mg, 0.17 mmol) in MeOH (1.1 mL) was added 0.5 M NaOMe (100 µL, 0.052 mmol). The reaction mixture was stirred at rt for 1 h, then neutralized to pH ~7 by the addition of Dowex 50W-X4-200 (H^+), and the resin was filtered off. The filtrate was concentrated *in vacuo* and purified with gel filtration chromatography (Sephadex LH-20) to obtain disaccharide monomer **1** as a white foam (68 mg, 90%). ^{1}H NMR (500 MHz, D_2O): δ 6.25 (dd, *J* = 17.1, 10.2 Hz, 1H), 6.17 (dd, *J* = 17.1, 1.3 Hz, 1H), 5.74 (dd, *J* = 10.2, 1.3 Hz, 1H), 5.23 (d, *J* = 3.9 Hz, 1H), 4.48 (d, *J* = 7.9 Hz, 1H), 4.29 (q, *J* = 6.5 Hz, 1H), 3.95 (dt, *J* = 10.2, 6.8 Hz, 1H), 3.89 (d, *J* = 3.3 Hz, 1H), 3.84 (dt, *J* = 9.5, 2.7 Hz, 2H), 3.81 – 3.71 (m, 5H), 3.67 (dd, *J* = 7.7, 4.5 Hz, 1H), 3.58 (dd, *J* = 9.5, 7.9 Hz, 1H), 3.35 (dd, *J* = 11.3, 6.7 Hz, 2H), 1.91 – 1.84 (m, 2H), 1.19 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (125 MHz, D_2O): δ 168.68, 130.08, 127.30, 101.68, 99.67, 77.09,

75.14, 73.83, 72.01, 69.64, 69.09, 68.48, 67.87, 66.98, 61.12, 36.57, 28.70, 15.51. HRMS *m/z* Calculated for $C_{18}H_{31}NO_{11}Na$ $[M+Na]^+$: 460.1795. Found: 460.1781.

3-*N*-Acryloyl-aminopropyl azide (12). To a solution of 3-azidopropylamine (870 mg, 8.7 mmol) in dioxane (20 mL) was added aq. $NHCO_3$ (950 mg in 12 mL H_2O, 11 mmol). The reaction mixture was cooled to 0 ^oC and stirred for 15 min. Acrylolyl chloride (1.1 mL, 13 mmol) was then added dropwise, and the reaction mixture was warmed to rt while minimizing exposure to light. After 2 h, the mixture was diluted with EtOAc, washed with H_2O, and the organic layer was concentrated *in vacuo*. The crude product was purified by silica gel flash chromatography (1:1 Hex:EtOAc) to afford **12** as a colorless oil (1.1 g, 83%). ^1H NMR (500 MHz, $CDCl_3$): δ 6.29 (dd, *J* = 17.0, 1.3 Hz, 1H), 6.08 (dd, *J* = 17.0, 10.3 Hz, 1H), 5.66 (dd, *J* = 10.3, 1.3 Hz, 1H), 3.44 (dd, *J* = 13.0, 6.5 Hz, 2H), 3.40 (t, *J* = 6.6 Hz, 2H), 1.88 – 1.80 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.84, 130.77, 126.80, 49.52, 37.36, 28.87. HRMS *m/z* Calculated for $C_6H_{10}N_4O$ $[M]^+$: 154.0855. Found: 154.0842.

3-*N*-Acryloyl-aminopropyl-2-nitrophenylazide (3). The following procedure was modified from Lindsley *et al.* and Hemming *et al.*[4] Triphenylphosphine polystyrene resin (Novabiochem) was first equilibrated by washing successively with THF, CH_2Cl_2 and MeOH. After drying *in vacuo*, a suspension of the resin (3.4 g, 3.4 mmol) and **12** (530 mg, 3.4 mmol) in THF (21 mL) was gently shaken at 37 ^oC for 24 h. The mixture was filtered, and the residual beads were washed successively with THF, CH_2Cl_2 and MeOH. After drying *in vacuo*, the resin was resuspended in 10:1 THF/H_2O (15 mL) and gently shaken at 37 ^oC for 48 h. The filtrate was collected, and the resin was washed with CH_2Cl_2 and MeOH. The filtrate and the washes were combined and concentrated *in vacuo* to afford the amine intermediate (190 mg, 43%). To a solution of the amine in dry DMF (7.5 mL) was added Et_3N (0.11 mL, 0.79 mmol) and *N*-(5-azido-2-nitrobenzoyloxy)-succinimide (ANB-NOS, Pierce; 200 mg, 0.55 mmol). The reaction was

allowed to stir overnight in the dark, concentrated *in vacuo*, and the crude product was purified by silica gel flash chromatography (10:1 EtOAc:MeOH) to afford nitrophenylazide monomer **3** as a pale yellow solid (69 mg, 33 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.13 (d, *J* = 8.7 Hz, 1H), 7.15-7.11 (m, 2H), 6.23 (dd, *J* = 17.0, 1.3 Hz, 1H), 6.10 (dd, *J* = 16.9, 10.2 Hz, 1H), 5.66 (dd, *J* = 10.2, 1.3 Hz, 1H), 3.54 – 3.45 (m, 4H), 1.89 – 1.81 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.93, 166.33, 146.52, 142.34, 135.78, 130.58, 127.16, 127.07, 120.13, 119.13, 36.51, 36.25, 29.41. HRMS *m/z* Calculated for $C_{13}H_{15}N_6O_4$ $[M+H]^+$: 319.1155. Found: 319.1154.

Polymerization reactions. The polymerization procedure was adapted from Hou *et al*.[5] In a typical experiment, 4-aminobenzyl-biotinylamide **4**[6] (17 mg, 46 μmol) was dissolved in degassed 1:1 H_2O/THF (0.8 mL) and reacted with HBF_4 (8.7 μL of a 50 wt% aq. solution, 69 μmol) at 0 °C under an Ar atmosphere. The diazonium salt was generated by the addition of $NaNO_2$ (3.9 mg, 55 μmol) into the reaction mixture. After 30 min, a degassed mixture of disaccharide monomer **1** (75 mg, 170 μmol), nitrophenylazide monomer **3** (18 mg, 57 μmol), acrylamide (49 mg, 690 μmol), and NaOCN (15 mg, 220 μmol) in 1:1 H_2O/THF (1.2 mL) was introduced to the diazonium salt, and the reaction mixture was heated to 60 °C for 16 h. The resulting mixture was dialyzed (3000 MWCO) at 4 °C against H_2O for 48 h, purified by gel filtration chromatography (G-25), and lyophilized to afford the desired polymer as fluffy powder. No further purification was necessary following dialysis, as indicated by the absence of signal for vinyl protons in the purified polymer solutions.

The number of glycosyl units incorporated into the polymer (Table1, x) was calculated by comparing the integrals of the methyl peaks of fucose (1.24 ppm, 3H) and those of the phenyl group of the biotin handle (7.18 ppm, 4H). The number of nitrophenylazide crosslinker units (Table1, y) was calculated by comparing the integrals of the phenyl proton peaks (8.27 ppm, 3H) and those of the phenyl group of the biotin handle. The number of the 3-hydroxylpropyl

units (Table1, z) was calculated by comparing the integrals of the methylene proton (3.63 ppm, 2H) and those of the phenyl group of the biotin handle.

FucGal polymer (9). Polymer **9** was prepared using the above procedure without nitrophenylazide monomer **3** to yield an orange fluffy powder (42%). M_n (SEC) = 26300. PDI (SEC) = 1.17. ^{1}H NMR (500 MHz, D_2O): δ 7.18 (d, *J* = 15.5 Hz, 5H), 5.24 (s, 11H), 5.11 – 5.01 (m, 5H), 4.55 – 4.26 (m, 32H), 4.13 – 3.51 (m, 186H), 3.48 – 3.12 (m, 38H), 2.43 – 2.04 (m, 229H), 1.91 – 1.44 (m, 500H), 1.30 – 1.15 (m, 58H).

FucGal crosslinker polymer (10). Polymer **10** was prepared using the above procedure to yield a light yellow fluffy powder (20% yield). M_n (SEC) = 23900. PDI (SEC) = 1.22. ^{1}H NMR (500 MHz, D_2O): δ 8.27 – 7.95 (m, 28H), 7.18 (d, *J* = 16.3 Hz, 6H), 5.24 (s, 25H), 5.12 – 4.99 (m, 18H), 4.55 – 4.25 (m, 58H), 4.12 – 3.49 (m, 374H), 3.42 – 3.12 (m, 80H), 2.44 – 2.00 (m, 159H), 1.89 – 1.46 (m, 338H), 1.24 – 1.14 (m, 91H).

Control crosslinker polymer (11). Polymer **11** was prepared using the above procedure, but substituting **1** with **2**, to yield a white fluffy powder (21% yield). M_n (SEC) = 23000. PDI (SEC) = 1.17. ^{1}H NMR (500 MHz, D_2O): δ 8.29 – 7.90 (m, 20H), 7.18 (d, *J* = 16.1 Hz, 5H), 3.63 (s, 85H), 3.24 (s, 98H), 2.46 – 1.94 (m, 213H), 1.70 (d, *J* = 54.2 Hz, 477H).

4-3. Biological Procedures

Animals, Tissue Isolation and Synaptosome Preparation. Sprague Dawley rats and C57BL/6 wild type, *FUT1-/-* and *FUT2-/-* mice were maintained in accordance with proper Institute of Animal Care and Use Committee (IACUC) procedures. Adult mice (3-4 months of age) and postnatal day 3 (P3) pups were anesthetized with CO_2 and dissected to remove the cortex and hippocampus. Synaptosomes (P2') were prepared from P3 rat pups by using a sucrose density gradient as previously described[7] and dissolved in lectin binding buffer (100 mM Tris pH 7.5, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.5% NP-40, and 0.2% sodium deoxycholate supplemented with EDTA-free Complete protease inhibitors (Roche)). To prepare cortical membrane fractions, cortices from P3 rat pups were extracted and fractionated using the Qproteome Cell Compartment Kit (Qiagen) to give a final protein concentration of 5 mg/mL.

UEAI Lectin Enrichment and Photocrosslinking Capture. Fluorescein-conjugated UEAI (Vector Laboratories; 100 µg) was incubated with polymer **10** or **11** (50 μM) in lectin binding buffer with gentle end-over-end mixing at 37 ^{o}C for 3 h in the dark. The samples were irradiated with UV light at 365 nm (Blak-Ray B100 AP, Upland) for 15 min at 4 ^{o}C if indicated and mixed further at rt for 45 min in the dark. The samples were diluted with binding buffer to a final concentration of 1 mg/mL and incubated with pre-treated (blocked with 0.1% fish gelatin) streptavidin agarose resin (Pierce) at 4 ^{o}C for 2 h. The resin was washed twice with 10 column volumes each of low salt buffer (0.1 M Na_2HPO_4 pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS), twice with 10 column volumes each of high salt buffer (0.1 M Na_2HPO_4 pH 7.5, 0.5 M NaCl, 0.2% Triton X-100), and once with 10 column volumes of 50 mM Tris•HCl pH 7.4. Captured protein was eluted in boiling sample buffer (50 mM Tris pH 6.8, 2.5% SDS, 2 mM DTT, 10% glycerol, 2 mM biotin) for 5 min. The sample was resolved on a NuPAGE

4-12% Bis-Tris gel (Invitrogen), transferred to polyvinylidene difluoride (PVDF) membrane (Millipore) and visualized using a Typhoon Scanner (GE Healthcare).

Fucα(1-2)Gal Glycan-Binding Protein Enrichment and Photocrosslinking Capture. Brain lysates from the synaptosomal and membrane fractions (1.2 mg) were pre-incubated with polymer **11** (35 μM) in lectin binding buffer and pre-cleared against high capacity streptavidin agarose resin (Pierce) with gentle end-over-end mixing for 2 h in the dark. After centrifugation at 1000 x *g* for 3 min, the supernatants were incubated with polymer **10** or **11** (75 μM) in lectin binding buffer with gentle end-over-end mixing at 37 ^{o}C for 3 h in the dark. The samples were irradiated with UV light at 365 nm for 15 min at 4 ^{o}C and mixed further at rt for 45 min in the dark. The samples were diluted with binding buffer to a final concentration of 1 mg/mL and incubated with pre-treated (blocked with 0.1% fish gelatin) high capacity streptavidin agarose resin at 4 ^{o}C for 4 h. The resin was then washed twice with 10 column volumes each of low salt buffer, twice with 10 column volumes each of high salt buffer, once with 20 column volumes of 4 M urea + 1% SDS and once with 10 column volumes of 50 mM Tris•HCl pH 7.4. Captured protein was eluted in boiling sample buffer for 5 min and subsequently resolved by 10% SDS-PAGE for LC-MS analysis.

Silver Staining, In-Gel Digestion, and LC-MS Analysis. All silver staining reagents were prepared fresh before they were used. The staining and destaining, in-gel tryptic digests, and peptide extractions were performed as described previously.[8] NanoLC-MS of in-gel tryptic digests was performed on a Thermo Fisher LTQ XL linear ion trap mass spectrometer using a modified vented column setup and data-dependent scanning.[9] Samples were loaded onto a 360 μm x 100 μm precolumn (2 cm, 5 μm Monitor C18) and desalted before the precolumn was placed in-line with the analytical column. Peptides were then eluted with a linear gradient from 0 to 40% B over 30 min (A, 0.1 M aq. HOAc; B, 0.1 M HOAc in CH_3CN), with a flow rate of

approximately 250 nL/min, and using a 360 μm x 75 μm self-packed column with an integrated electrospray emitter (10 cm, 5 μm Monitor C18). For data-dependent experiments, the mass spectrometer was programmed to record a full-scan ESI mass spectrum (*m/z* 400-2000), followed by five data-dependent MS/MS scans in the ion trap (relative collision energy of 35%, 3.5 Da isolation window). Dynamic exclusion parameters were set as follows: repeat count=1, repeat duration=15 s, and exclusion duration=30 s.

MS/MS spectra were searched against a rat subset of the European Bioinformatics Institute-International Protein Index (EBI-IPI) database (downloaded August 1, 2007), with an appended reversed database using Sequest 3.0. A fixed modification of Cys (+57), a variable modification of Met (+16), and trypsin cleavage were specified. Mass tolerances of 3.0 Da for the parent and 0.8 Da for the fragment ions were employed. Search results were compiled and filtered in Scaffold 2.0 (Proteome Software, Inc., Portland, OR). Peptide identifications were accepted if they could be established at ≥95.0% probability, as specified by the Peptide Prophet algorithm.[10] Protein identifications were accepted if they could be established at ≥99.0% probability (as specified by the Protein Prophet algorithm)[11] and were based on at least three unique peptide identifications. To be listed in Table 1, proteins had to be identified as specified both in at least two of the three synaptosome and/or membrane fractions from the polymer **10** samples, as well as on the basis of six unique peptides identified from among the various fractions. By contrast, proteins were eliminated as non-specific if even a single peptide was also identified in any of the polymer **11** samples. Using these highly stringent filtering criteria, 44 candidate GBPs were identified from six independent runs (three synaptosome and membrane fractions).

Protein Expression and Purification. DCLK1 (DCAMKL1) was expressed and purified from *E. coli* BL21(DE3) cells harboring the recombinant plasmid vector pET28a-DCLK1-His as previously described.[12] SV2a was expressed and purified from HEK293 cells transfected with

plasmid vector SV2a-FLAG-pIRES2 as previously described.[13] GαO protein was kindly provided by Dr. Tohru Kozasa (University of Illinois, Chicago). PKCε protein was purchased from ProspecBio.

Binding Validation of Lectin Candidates to Fucα(1-2)Gal by ELLA. Nunc Maxisorp 384-well plates were coated overnight at 4 °C with lectin (50 ng/well) in carbonate buffer (50 μL, 50 mM, pH 9.0). The wells were then washed with washing buffer (3 x 100 μL, 50 mM Tris-HCl pH 7.4, 150 mM NaCl containing 0.03% Tween 20). The washing procedure was repeated after each incubation throughout the assay. After washing, the wells were filled with serial dilutions of biotinylated polymers (Fucα(1-2)Gal, Galα(1-2)Gal and control (-OH) polymer, 25 μL/well, GlycoTech) in binding buffer (PBS, 1 mM $CaCl_2$, 4 mM $MgCl_2$) and incubated at rt for 2.5 h. The wells were washed and blocked with 10% Fetal Bovine Serum (FBS; 80 μL) at 37 °C for 1 h. The plates were then incubated with horseradish peroxidase-conjugated streptavidin (Pierce; 1:40000) at rt for 1 h, developed with 3,3',5,5-tetramethylbenzidine substrate (TMB; Pierce) and quenched with 2 M H_2SO_4. Absorbances were measured at 450 nm using a PerkinElmer Victor plate reader and were fitted to a linear regression analysis using KaleidaGraph (version 4.1.2). Experiments were performed in triplicate, and data represent the mean ± SEM.

Chemoenzymatic Labeling of Cell Lysates. This procedure was adapted from Chaubard *et al.*[14] The cortices of adult C57BL/6, *FUT1-/-* and *FUT2-/-* mice were dissected on ice and lysed in boiling 1% SDS (5 volumes/weight) with sonication until the mixture was homogeneous. Protein was precipitated with methanol/chloroform/water. Briefly, protein was diluted to 200 μL and precipitated by sequential mixing with 600 μL of MeOH, 200 μL of $CHCl_3$ and 450 μL H_2O, after which the mixture was centrifuged at 23,000 x *g* for 15 min. Precipitated protein was washed with 450 μL of MeOH and centrifuged at 23,000 x *g* for 10 min. After the protein pellet was allowed to dry briefly, the pellet was re-dissolved at 5 mg/mL in 20 mM HEPES pH 7.9

containing 1% SDS, and diluted 5-fold into a buffer with the following final concentrations: 20 mM HEPES pH 7.9, 50 mM NaCl, 2% NP-40, 5 mM $MnCl_2$. UDP-GalNAz (Invitrogen; 25 μM) and BgtA (0.16 mg/mL) were added, and the samples were incubated at 4 °C for 16-20 h. The labeled proteins were precipitated as above and resuspended in 50 mM Tris pH 7.4 containing 1% SDS at 4 mg/mL. The resuspended proteins were subsequently reacted with 5-carboxytetramethylrhodamine (TAMRA) alkyne (Invitrogen) per the Click-It™ TAMRA Protein Analysis Detection kit instructions (Invitrogen), except that EDTA-free Complete™ protease inhibitors were added during the reaction. Negative controls were performed under identical conditions except that BgtA was omitted from the labeling reaction. After the labeling reactions, protein was precipitated using methanol/chloroform/water as described above and re-dissolved in boiling 2% SDS. TAMRA-labeled proteins were resolved on a NuPAGE 4-12% Bis-Tris gel and transferred to PVDF membrane. The membrane was blocked with 5% milk (Biorad) in TBST (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.05% Tween 20) for 1 h at rt and immunoblotted with a rabbit anti-TAMRA antibody (Invitrogen; 1:1000) and a mouse anti-tubulin antibody (Sigma Aldrich 1:5000). Membranes were washed with TBST, incubated with the appropriate Alexa Fluor 680-conjugated (Invitrogen) or IR800-conjugated (Rockland) secondary antibody, and visualized using a LiCOR Odyssey Imaging System.

Detection of Fucα(1-2)Gal Glycoproteins by UEAI Lectin Blotting. Cortical proteins precipitated from C57BL/6, *FUT1-/-* and *FUT2-/-* mice were resolved on a NuPAGE 4-12% Bis-Tris gel and transferred to PVDF membrane. The membrane was blocked with 5% milk (Biorad) in PBST (PBS+ 0.05% Tween 20) at rt for 1 h and immunoblotted with fluorescein-conjugated UEAI (1:100) at rt for 1 h. The membrane was washed with TBST and visualized using a Typhoon Scanner.

Neuronal Cultures for Functional Assays. For biochemical assays, cortical neuronal cultures were prepared as previously described,[15] except that neurons from E15-16 timed-pregnant C57BL/6, *FUT1-/-* or *FUT2-/-* mice were plated onto poly-DL-lysine-coated 6-well plates (BD Bioscience). Neurons were maintained for up to 10 days in vitro (DIV) in Neurobasal medium (NBM; Life Technologies) supplemented with 2 mM Glutamax-I and 2% B-27 (Invitrogen). For staining and imaging, hippocampal neurons were prepared as previously described,[16] except that neurons from E15-16 timed-pregnant mice were plated on poly-DL-lysine-coated glass coverslips (Carolina Biological) at a density of 75 cells/mm^2. Neurons were maintained for up to 10 DIV in NBM supplemented with 2 mM Glutamax-I and 2% B-27.

Assaying SV2a Distribution in Cortical Neurons. Cortical neuron cultures (C57BL/6 *FUT1-/-* and *FUT2-/-*, 10 DIV) were washed once with ice-cold PBS, gently scraped with trypsin and suspended in Dulbecco's Modified Eagle Medium (DMEM; Life Technologies) supplemented with 10% FBS (Invitrogen) and then centrifuged for 10 min at 500 x *g* at 4 ^{o}C. Pellets were washed with cold PBS and fractionated using the Qproteome Cell Compartment Kit. The protein concentrations of the resulting membrane extracts were measured using the BCA assay (Pierce). Equal amounts of protein were resolved on a NuPAGE 4-12% Bis-Tris gel and transferred to PVDF membrane. The membrane was blocked with 5% milk, immunoblotted with a goat anti-SV2a (E-15, Santa Cruz; 1:200) or mouse anti-synaptophysin (Sigma Aldrich; 1:800) antibody and visualized using a LiCOR Odyssey Imaging System. SV2a distribution in the membranes of *FUT1-/-* and *FUT2-/-* neurons was expressed relative to the level in C57BL/6 mice (n = 3-5) and was reported as the mean ± SEM. Statistical analysis was performed using the Student's unpaired t-test to compare the SV2a level between *FUT2-/-* and C57BL/6 neurons.

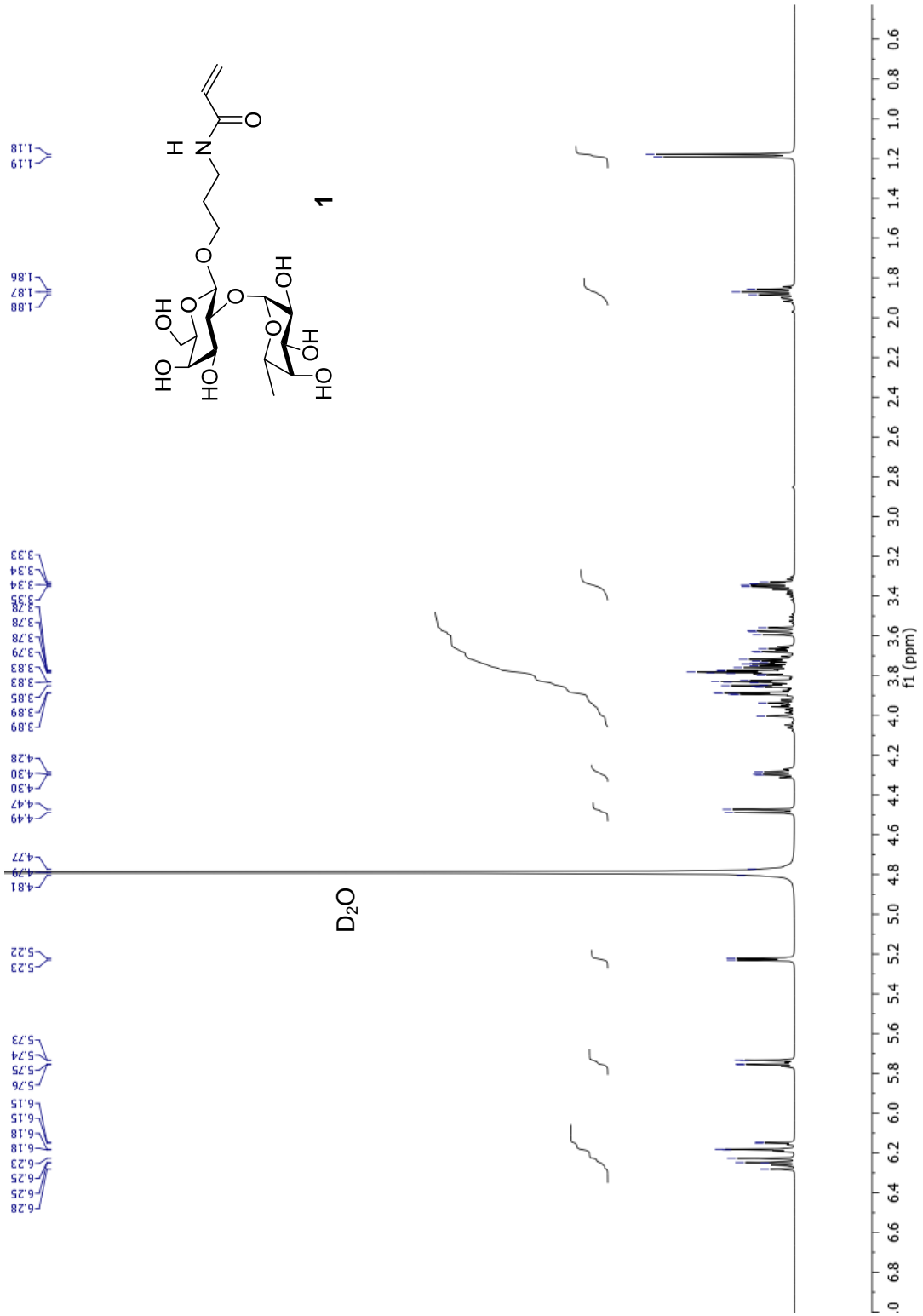
Immunostaining and Quantification. Hippocampal neurons on coverslips were rinsed once with PBS, fixed with 4% paraformaldehyde (PFA)/ 4% sucrose at rt for 20 min, washed twice

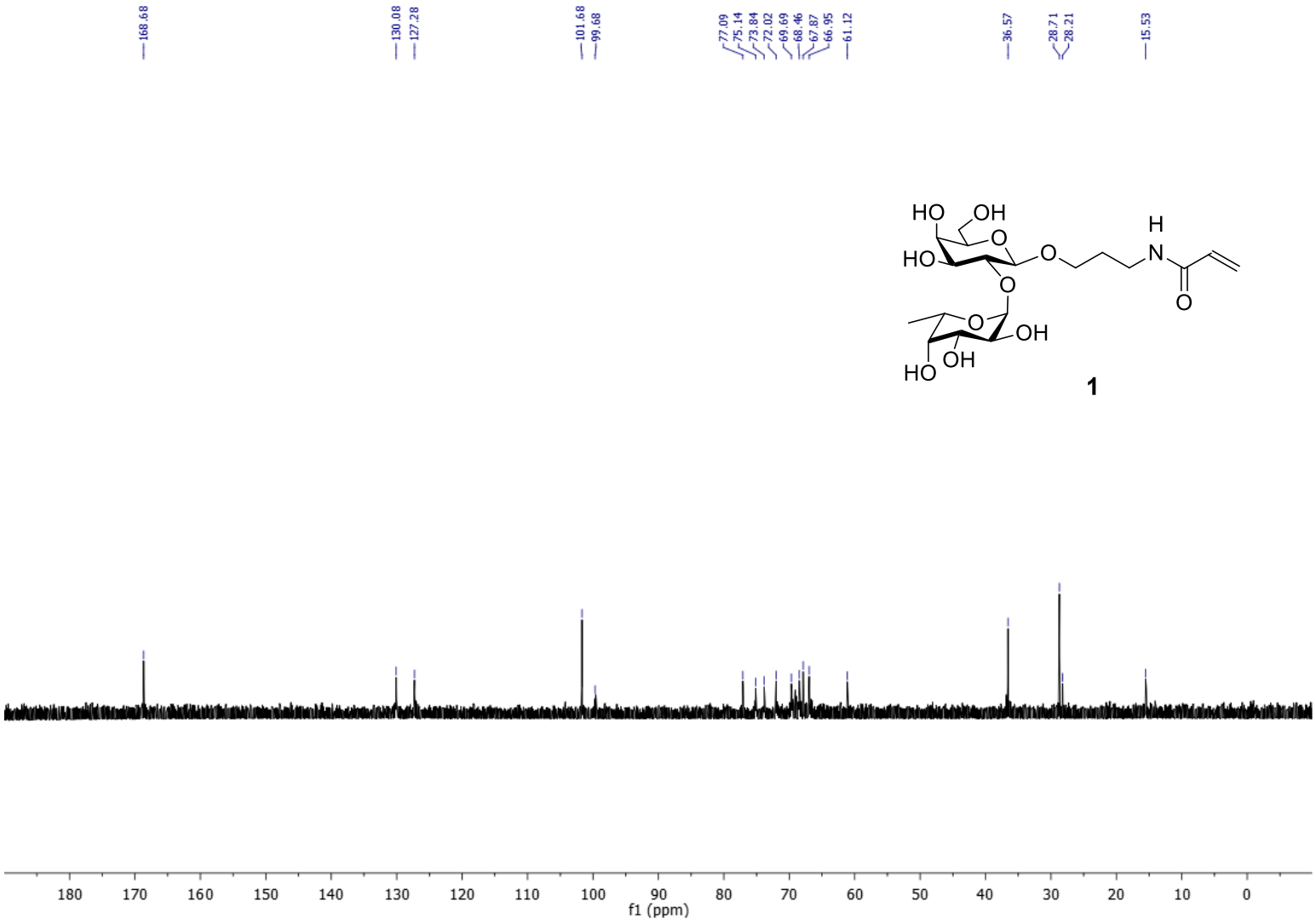
with PBS, permeabilized with 0.3% TritonX-100 at rt for 5 min, and washed twice with PBST. Non-specific binding was blocked by incubating with 5% goat serum (GS) in PBST at 4 ^{o}C for 1 h. After rinsing twice with PBST, the cells were incubated with a rabbit anti-SV2a antibody (Synaptic Systems; 1:300) in 5% GS at rt for 2 h. Excess antibody was removed by washing five times with PBST, and the cells were incubated overnight with a mouse anti-synaptophysin antibody (Millipore; 1:300) in 5% GS at 4 ^{o}C. After washing five times with PBST, the cells were treated with anti-rabbit IgG AlexaFluor 488 (Invitrogen; 1:1000) and anti-mouse IgG AlexaFluor568 (Invitrogen; 1:1000) antibodies in 5% GS at rt for 2 h. The coverslips were mounted onto glass slides and imaged using a LSM510 Meta confocal laser scanning microscope with a 100X 1.40 N.A oil immersion plan apochromat objective lens (Carl Zeiss MicroImaging, Inc.). All images were acquired blind to the experimental conditions. To minimize possible sources of bias, 7-10 neurons were randomly selected from 3 representative fields in each cover slip. Each genotype was analyzed from at least three independent experiment using three different mice (n=70-80 images).

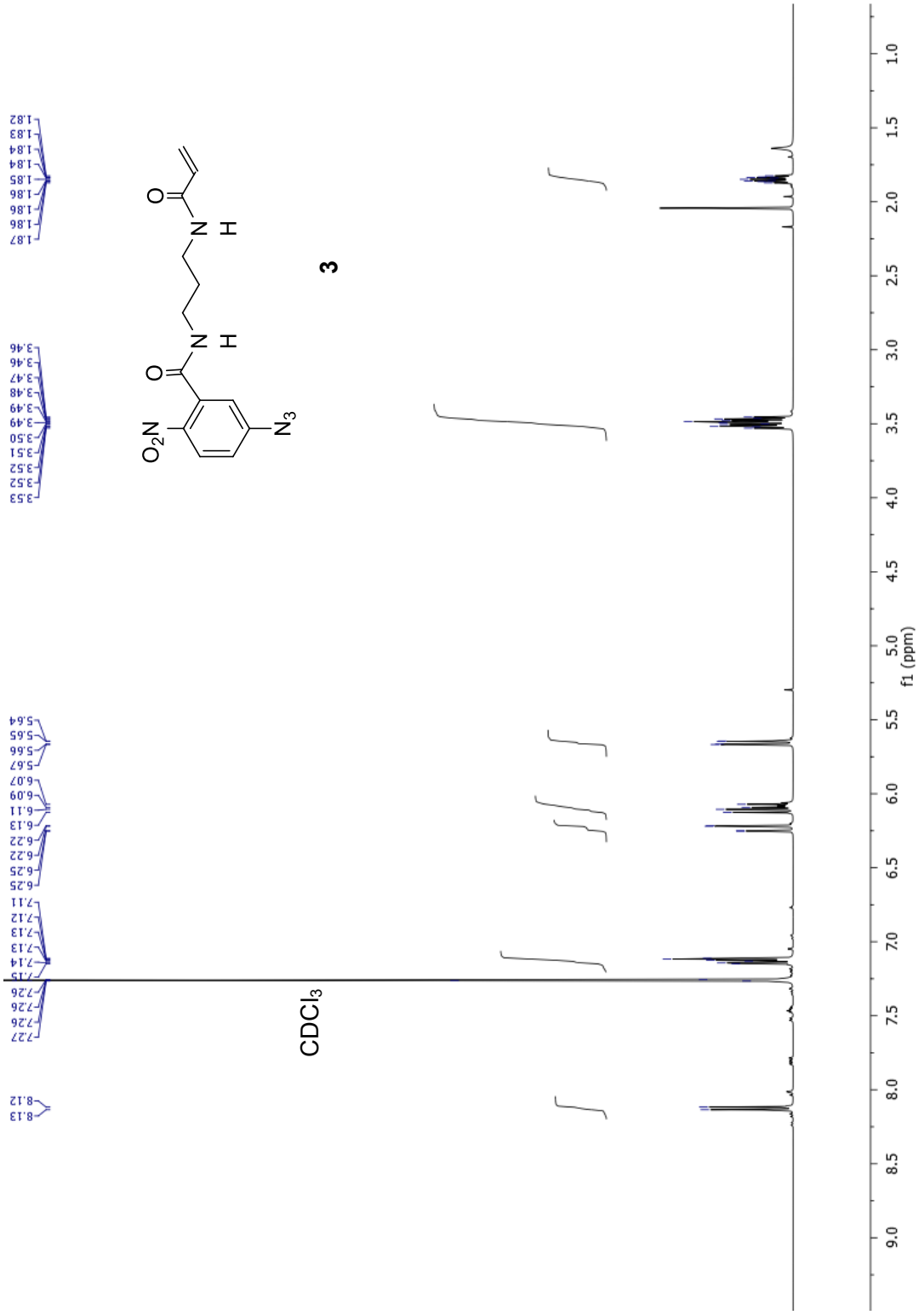
Images were analyzed blind to the experimental condition using NIH ImageJ software (http://rsbweb.nih.gov/ij/) using the JACoP Colocalization plugin, and colocalized puncta analysis was performed as previously described[17] using the Colocalization Highlighter plugin. To determine the degree of colocalization between two proteins, each channel was set with the same threshold value, and the distinct puncta in each channel were overlaid to generate a colocalized region. Colocalized puncta (i.e. yellow pixel clusters) were defined as continuous pixel clusters between 0.5 – 11 μm in size and having an intensity at least 2-fold greater than each individual (red or green) channel in the adjacent dendrite. The number of colocalized puncta per unit area (puncta density) were manually counted and then normalized with respect to the WT sample. Statistical analyses were performed using the Student's unpaired t-test, and the data were reported as the mean ± SEM.

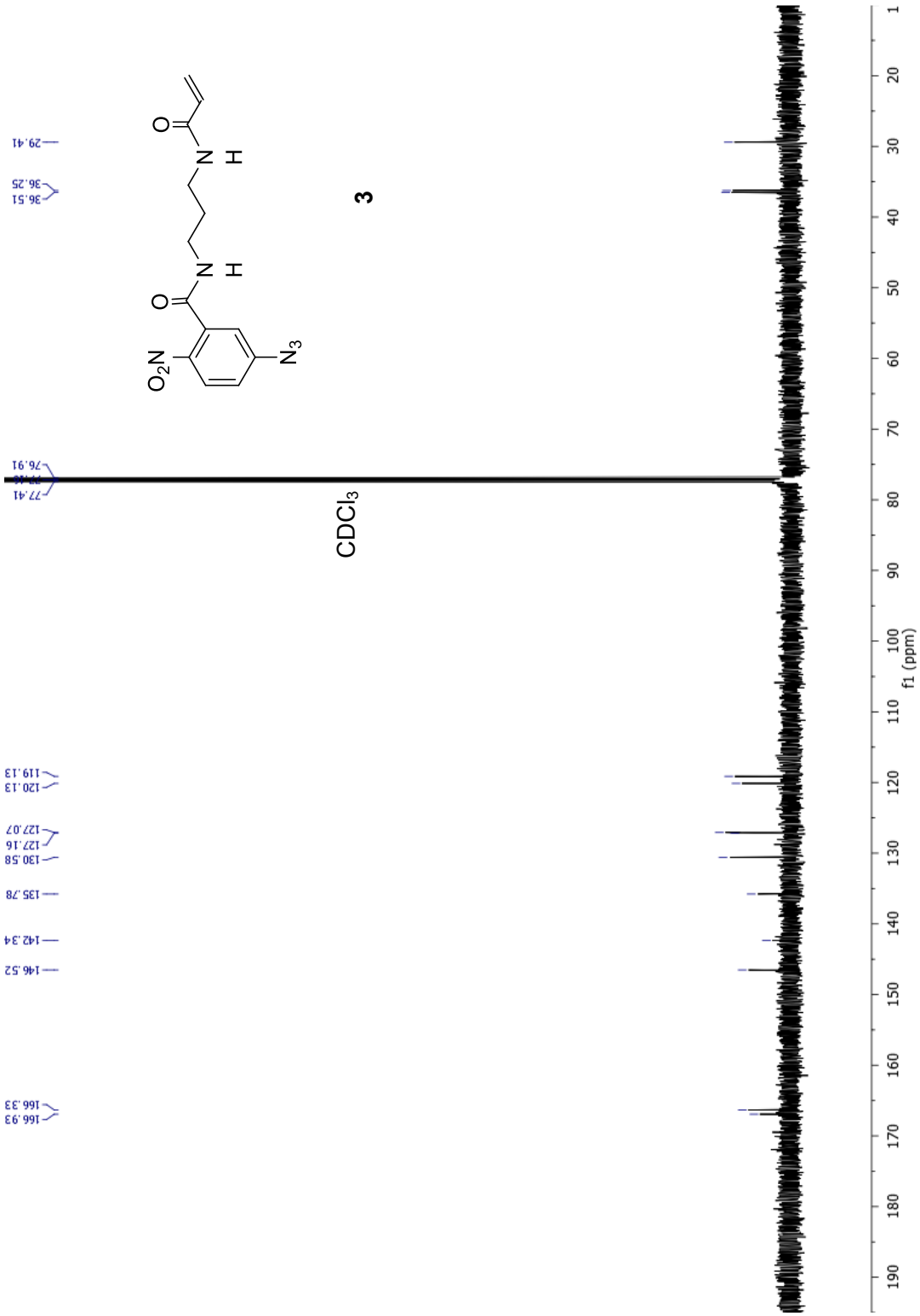
Surface Biotinylation and SV2a Immunoprecipitation. Cortical neuronal cultures (C56BL/7, *FUT1*$^{-/-}$ and *FUT2*$^{-/-}$, 10 DIV) were washed once with ice-cold PBS, pH 8.0, and biotinylated by incubating with 0.5 mg/ml EZ-Link Sulfo-NHS-LC-LC-Biotin (Pierce) for 25 min at 4 ^{o}C with gentle shaking.[18] Cultures were washed with PBS, pH 7.4, and incubated with 100 mM glycine for 15 min at 4 ^{o}C to quench the reaction. Neurons were rinsed with ice-cold PBS, pH 7.4, and extracted in solubilization buffer (150 mM NaCl, 50 mM Tris-HCl, 1 mM EDTA, 1% NP-40 and protease inhibitor) for 1 h at 4 ^{o}C. Insoluble material was removed by ultracentrifugation (19,000 x g for 20 min), and the supernatants were subjected to immunoprecipitation by incubating with an anti-SV2a antibody (E-15, Santa Cruz) overnight at 4 ^{o}C. The samples were diluted with solubilization buffer to a final concentration of 1 mg/mL and then were incubated with pre-treated (blocked with 0.1% fish gelatin) protein A/G sepharose beads (ThermoFisher) at 4 ^{o}C for 2 h. The resin was washed three times with 10 column volumes of solubilization buffer, and the captured protein was eluted with loading dye without boiling. Equal amount of protein were resolved on a NuPAGE 4-12% Bis-Tris gel and transferred to PVDF membrane. The membrane was blocked in 5% milk and immunoblotted with a rabbit anti-SV2a antibody (1:300, Synaptic Systems) for total immunoprecipitated SV2a and streptavidin-HRP (1:1000, Pierce) for biotinylated SV2a. Labeled bands were visualized and quantified using a LiCOR Odyssey Imaging System. The proportion of SV2a on the plasma membrane of *FUT2-/-* neurons was expressed as the normalized intensity of biotinylated-SV2a signal to the total SV2a, and was reported relative to the control C57BL/6 level (n=4) as the mean ± SEM. Statistical analysis was performed using the Student's unpaired t-test to compare the SV2a level between *FUT2*$^{-/-}$ and C57BL/6 neurons.

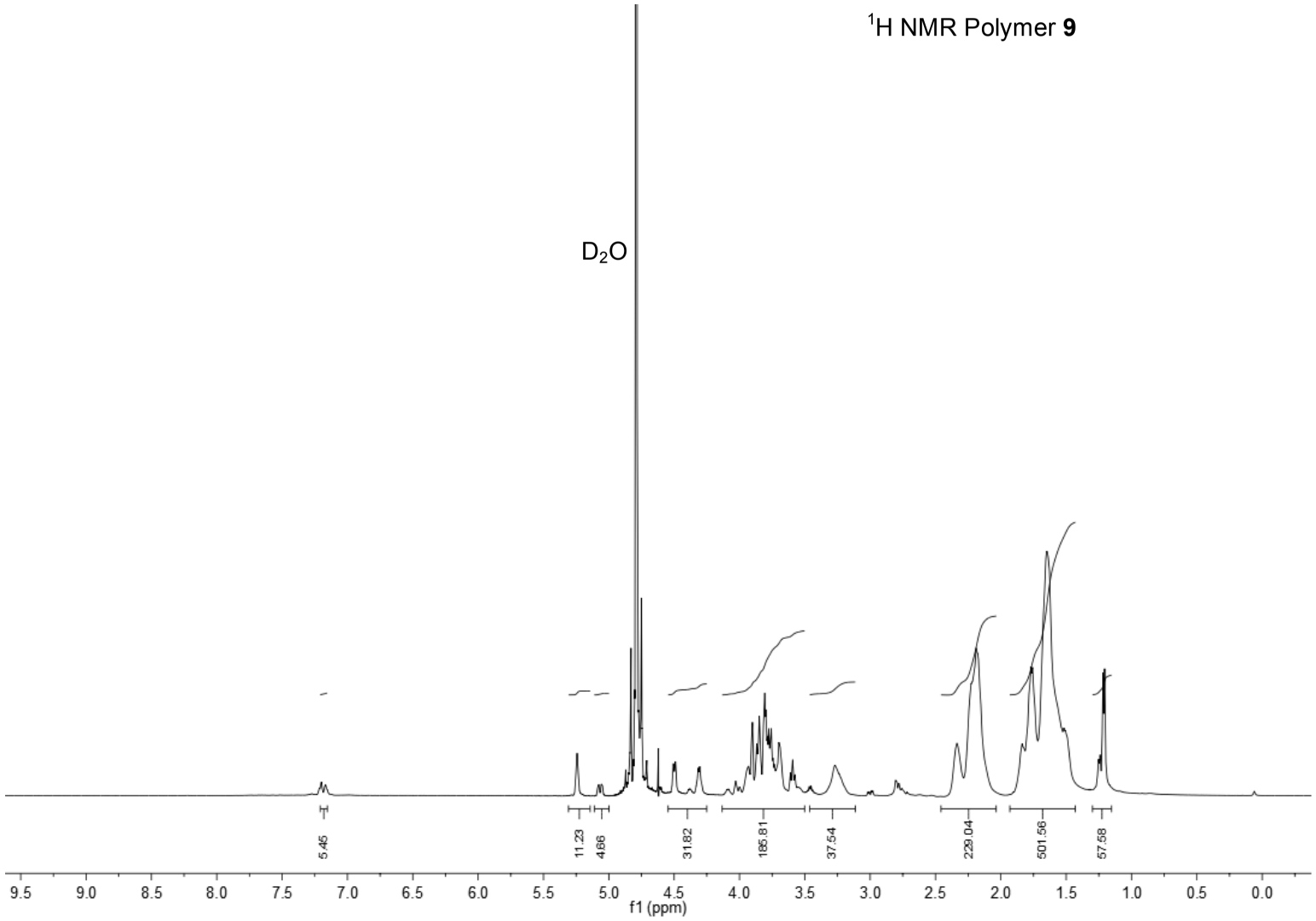
4-4. Representative ^{1}H and ^{13}C NMR Spectra



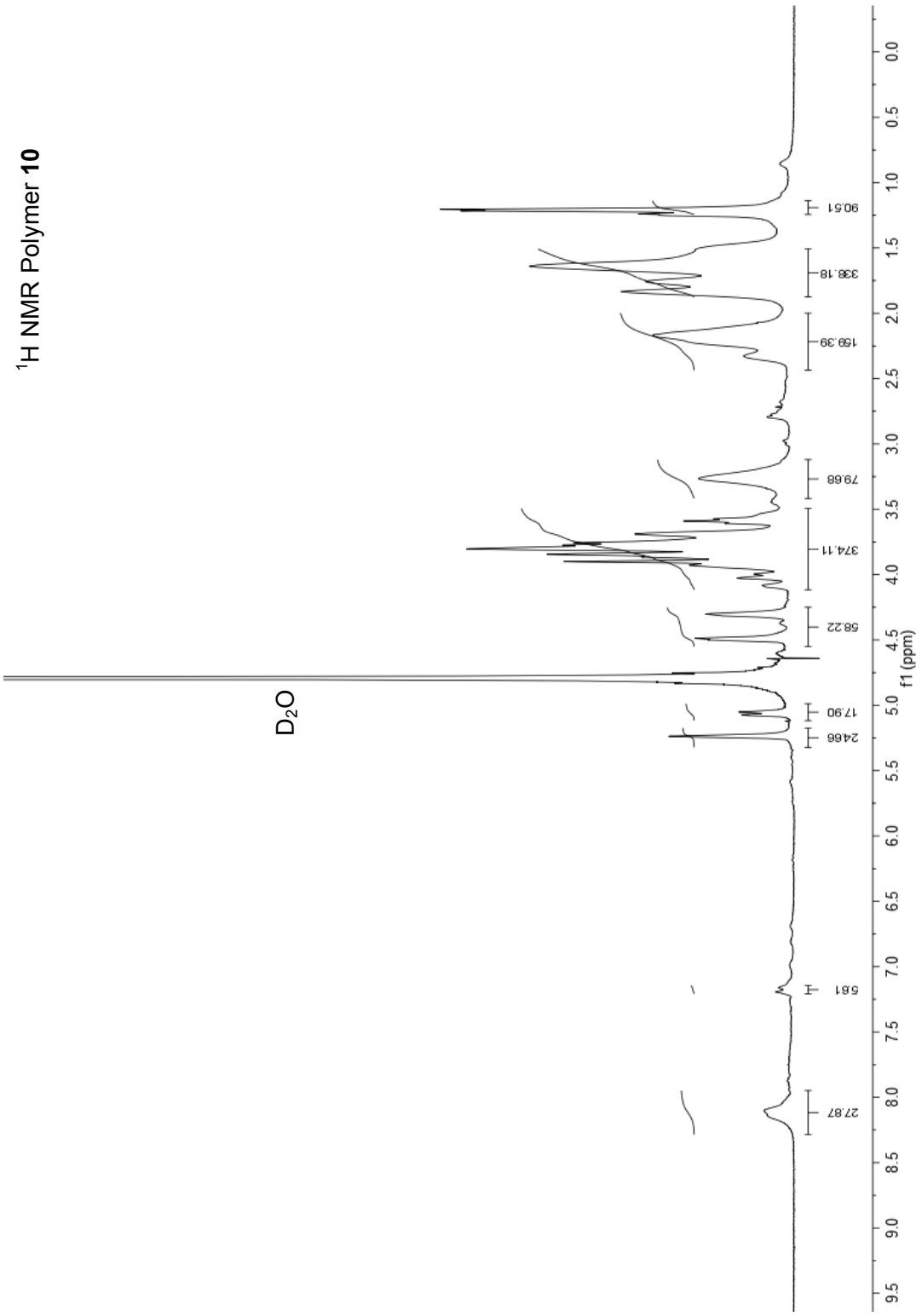

168.68
130.08
127.28
101.68
99.68
77.09
75.14
73.84
72.02
69.69
68.46
67.87
66.95
61.12
36.57
28.71
28.21
15.53
HO
OH
O
HO
O
O
H
N
O
O
OH
HO
OH
1
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

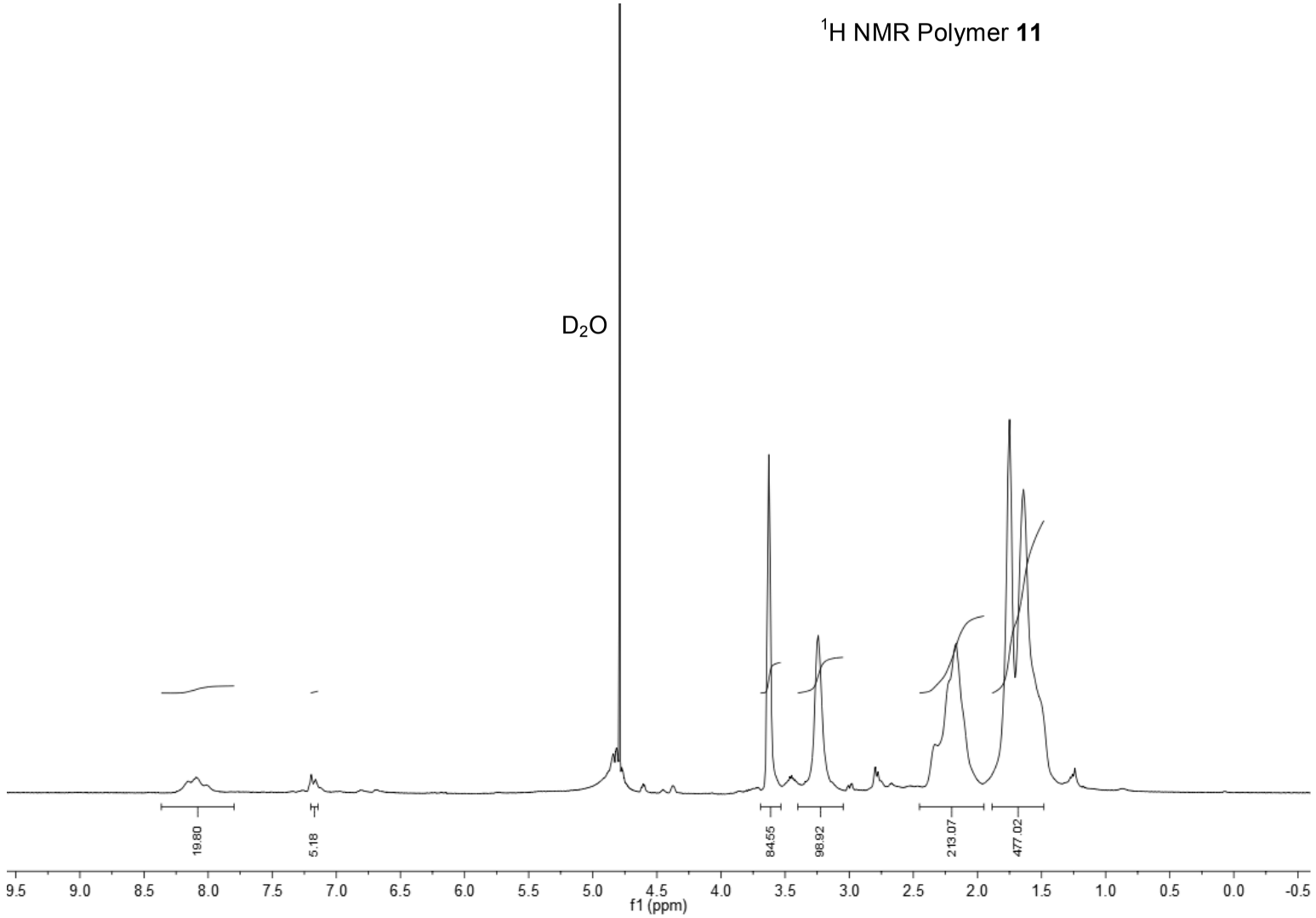

3
CDCl3
f1 (ppm)


3
CDCl3
O2N
N3
O
N H
O
N H
29.41
36.51
36.25
76.91
77.41
119.13
120.13
127.07
127.16
130.58
135.78
142.34
146.52
166.93
166.33
f1 (ppm)


^{1}H NMR Polymer **9**
D_2O
5.45
11.23
4.66
31.82
185.81
37.54
229.04
501.56
57.58
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

^{1}H NMR Polymer **10**




^{1}H NMR Polymer **11**
D_2O
19.80
5.18
84.55
98.92
213.07
477.02
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

5. References

1. Wegman, B.; Schmidt, R. R. *Carbohydr. Res.* **1988**, 184, 254.
2. Damager, I.; Olsen, C.E.; Moller, B.L.; Motawia, M.S. *Synthesis.* **2002**, 3, 418.
3. Kanie, O.; Ito, Y.; Ogawa, T. *Tet. Lett*. **1996**, 37, 4551.
4. (a) Lindsley, C.W.; Zhao, Z.; Newton, R.C.; Leister, W.H.; Strauss, K.A. *Tet. Lett.* **2002**, 43, 4467. (b) Hemming, K.; Bevan, M.J.; Loukou, C.; Patel, S.D.; Renaudeau, D. *Synlett.* **2000**, 11, 1565.
5. Hou, S.; Sun, X-L.; Dong, C-M.; Chaikof, E.L. *Bioconjugate. Chem.* **2004**, 15, 954.
6. Tseng, P-Y.; Rele, S.M.; Sun, X-L. Chaikof, E.L. *Biomaterials.* **2006**, 27, 2627.
7. Huttner, W. B.; Schiebler, W.; Greengard, P.; De Camilli, P. *J. Cell Biol.* **1983**, 96, 1374.
8. Clark, P.M.; Dweck, J.F.; Mason, D.E.; Hart, C.R.; Buck, S.B.; Peters, E.C.; Agnew, B.J.; Hsieh-Wilson, L.C. *J. Am. Chem. Soc.* **2008**, 130, 11576.
9. Murrey, H.E.; Ficarro, S.B.; Krishnamurthy, C.; Domino, S.E.; Peters, E.C.; Hsieh-Wilson, L.C. *Biochemistry*. **2009**. 48, 7261.
10. Keller, A.; Nesvizhskii, A.I.; Kolker, E.; Aebersold, R. *Anal. Chem.* **2002**, 75, 5383.
11. Nesvizhskii, A.I.; Keller, A.; Kolker, E.; Aebersold, R. *Anal. Chem.* **2003**, 75, 4646.
12. Lin, P.T.; Gleeson, J.G.; Corbo, J.C.; Flanagan, L.; Walsh, C.A. *J. Neurosci*. **2000**, 20, 9152.
13. Yao, J.; Bajjalieh, S.M. *J. Biol. Chem.* **2008**, 283, 20628.
14. Chaubard, J.-L.; Khrisnamurthy, C.; Yi, W.; Smith, D.; Hsieh-Wilson, L.C. *J. Am. Chem. Soc.* **2012**, 134, 4489
15. Rexach, J.E.; Rogers, C.J.; Yu, S-H.; Tao, J.; Sun, Y.E.; Hsieh-Wilson, L.C. *Nat. Chem. Biol.* **2010**, 6, 645.
16. Rawat, M.; Gama, C.I.; Matson, J.B.; Hsieh-Wilson, L.C. *J. Am. Chem. Soc.* **2008**, 130, 2959.

17. (a) Kayser, M.S.; McClelland, A.C.; Hughes, E.G.; Dalva, M.B. *J. Neurosci*. **2006**, 26, 12152. (b) Jones, K.A.; Srivastava, D.P.; Allen, J.A.; Strachan, R.T.; Roth, B.L.; Penzes, P. *Proc. Natl. Acad. Sci.* **2009**, 106, 19575.

18. Yao, J.; Nowack, A.; Gardner, R. G.; Kensel-Hammes, P.; Bajjalieh, S. M. *J. Neurosci.* **2010**, 30, 5569.