Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Isotope-targeted glycoproteomics (IsoTaG) analysis of sialylated *N*- and *O*-glycopeptides on an Orbitrap Fusion Tribrid using azido and alkynyl sugars

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Supplementary Tables - 216_2016_9934_MOESM2_ESM.xlsx

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Additional Experimental Information.

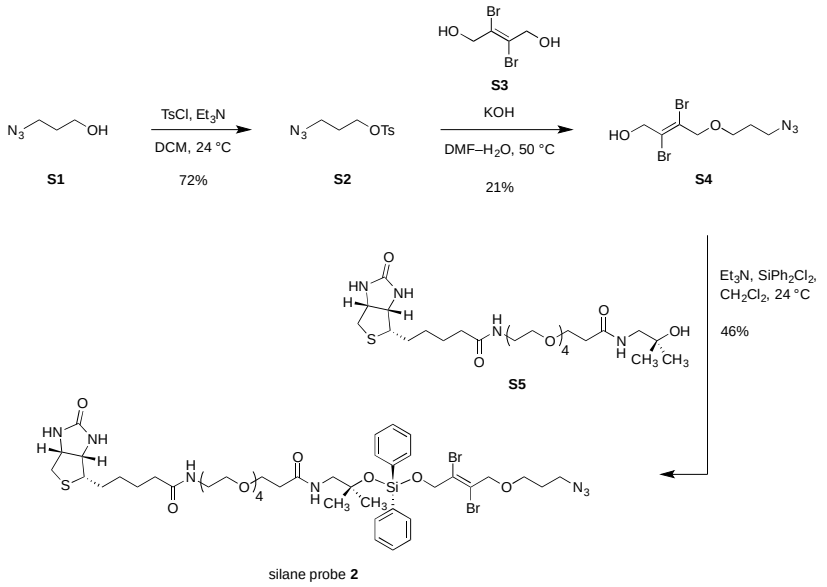


Figure S1. Synthesis of the dibrominated silane probe **2**.

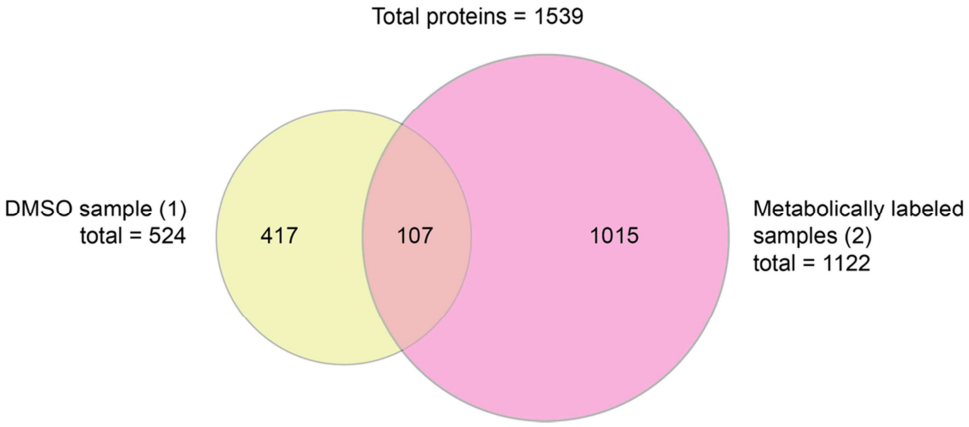


Figure S2. Venn diagram of glycoprotein overlap between metabolically labeled samples (2) and DMSO control sample (1). Overlap proteins are defined as having fewer than 5 spectral counts between DMSO control and enriched proteins from each treatment (Ac_4ManNAz, Ac_4ManAl, respectively).

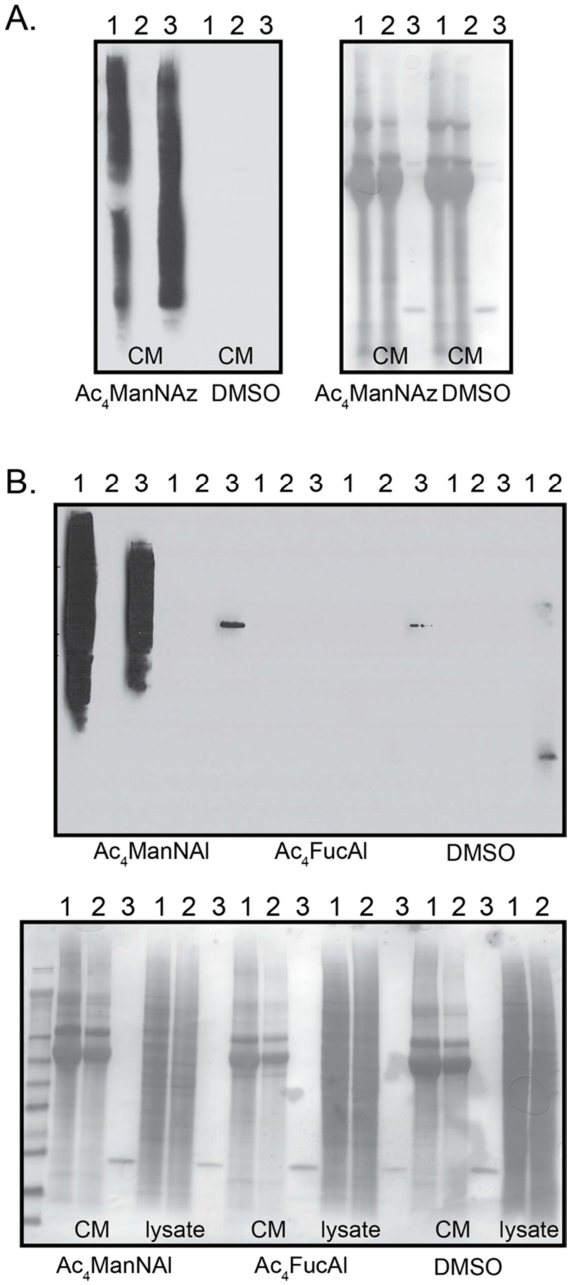


Figure S3. Anti-biotin Western blot analysis of tagging and enrichment efficiency. (A) Ac_4ManNAz and DMSO control for PC-3 conditioned media. (B) Ac_4ManNAl, Ac_4FucAl and DMSO control for PC-3 conditioned media (CM) and whole cell lysate. Total protein levels shown alongside (ponceau). Lanes: (1) protein input following CuAAC reaction with probe **1** or **2**, (2) protein supernatant following overnight incubation with streptavidin–agarose beads, (3) aliquot of streptavidin–agarose beads (10 µL) after enrichment.

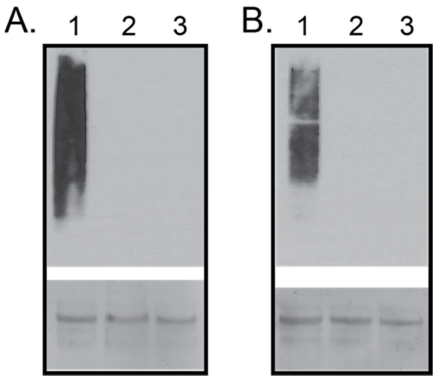
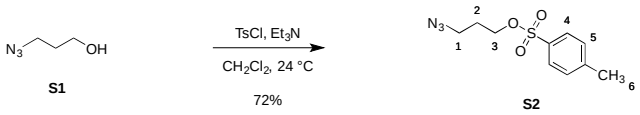


Figure S4. Anti-biotin Western blot (top) and ponceau (bottom) analysis of tagging efficiency between commercially available biotin-azide and probe **2**. A. Conditioned media from PC-3 cells tagged with biotin-azide. B. Conditioned media from PC-3 cells tagged with probe **2**. Lanes: (1) Ac_4ManNAl (2) Ac_4FucAl (3) DMSO labeled conditioned media.

General Experimental Procedures. All reactions were performed in single-neck, flame-dried, round-bottomed flasks fitted with rubber septa under a positive pressure of nitrogen, unless otherwise noted. Air- and moisture-sensitive liquids were transferred via syringe or stainless steel cannula. Organic solutions were concentrated by rotary evaporation at 30–33 °C. Normal and reverse phase flash-column chromatography was performed as described by Still and co-workers [1]. Normal phase purifications employ silica gel (60 Å, 40–63 µm particle size) purchased from Silicycle (Quebec, Canada). Analytical thin-layer chromatography (TLC) was performed using glass plates pre-coated with silica gel (0.25 mm, 60 Å pore size) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light (UV), iodine (I_2), and/or submersion in aqueous ceric ammonium molybdate solution (CAM) followed by brief heating on a hot plate (120 °C, 10–15 s).

Chemical Instrumentation. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded at 400 or 500 MHz at 24 °C, unless otherwise noted. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to residual protium in the NMR solvent ($CHCl_3$, δ 7.26; CHD_2OD, δ 3.31). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet and/or multiple resonances, br = broad, app = apparent), integration, coupling constant in Hertz, and assignment. Proton-decoupled carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded at 400 or 500 MHz at 24 °C, unless otherwise noted. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to the carbon resonances of the solvent ($CDCl_3$, δ 77.0; CD_3OD, δ 49.0). ^{13}C NMR and data are represented as follows: chemical shift, carbon type [determined from HSQC]. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane. Infrared (IR) spectra were obtained using a Thermo Electron Corporation Nicolet 8500 FTIR spectrometer referenced to a polystyrene standard. Data are represented as follows: frequency of absorption (cm^{-1}), intensity of absorption (s = strong, m = medium, w = weak, br = broad). High-resolution mass spectrometry (HRMS) measurements were obtained at the Stanford University Mass Spectrometry Facility using a Bruker micrOTOF-Q II hybrid quadrupole-time of flight, Agilent 1260 UPLC-MS.

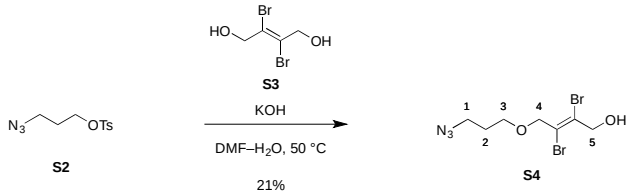
Synthetic Procedures.



*Synthesis of 3-azidopropyl 4-methylbenzenesulfonate (**S2**):*

3-Azidopropan-1-ol (**S1**, 500 mg, 4.95 mmol, 1 equiv) was dissolved in dichloromethane (10 mL) at 24 °C. Triethylamine (2.00 mL, 14.85 mmol, 3.00 equiv) and 4-methylbenzenesulfonyl chloride (1.90 g, 9.90 mmol, 2.00 equiv) were added in sequence to the stirred solution at 24 °C. The resulting mixture was stirred for 48 h at 24 °C. The product mixture was purified by flash-column chromatography (eluting with 10% ethyl acetate–hexanes, grading to 20% ethyl acetate–hexanes, one step) to afford 3-azidopropyl 4-methylbenzenesulfonate (**S2**) as clear yellow oil (890 mg, 72%).

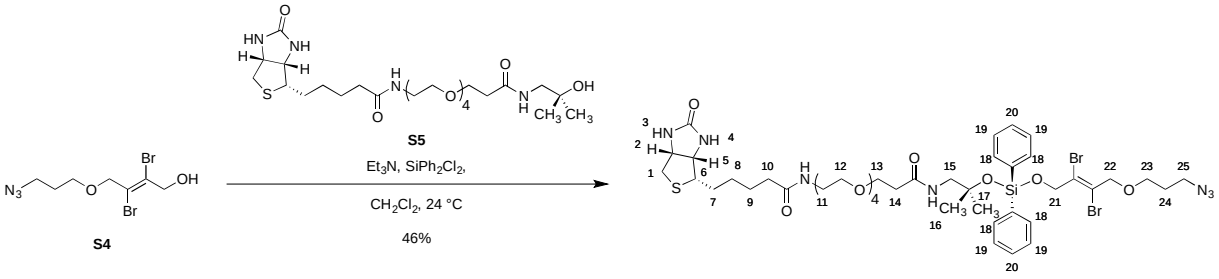
R_f = 0.39 (20% ethyl acetate–hexanes; CAM). ^{1}H NMR (400 Hz, $CDCl_3$): δ 7.80 (d, 2H, J = 8.0 Hz, H_4), 7.37 (d, 2H, J = 7.6 Hz, H_5), 4.11 (t, 2H, J = 6.0 Hz, H_3), 3.38 (t, 2H, J = 6.4 Hz, H_1), 2.46 (s, 3H, H_6), 1.89 (q, 2H, J = 6.4 Hz, H_2). ^{13}C NMR (400 Hz, $CDCl_3$): δ 145.2 (C), 132.9 (C), 130.1 (2 • CH), 128.1 (2 • CH), 67.1 (CH_2), 47.4 (CH_2), 28.6 (CH_2), 21.8 (CH_3). IR (NaCl), cm^{-1}: 2093 (s), 1355 (s), 1172 (s), 940 (s), 812 (s) ,661 (s), 551 (s). HRMS-ESI (*m/z*): [M+Na] calculated for $C_{10}H_{13}N_3O_3SNa$, 278.0575; found, 278.0570.



*Synthesis of (E)-4-(3-azidopropoxy)-2,3-dibromobut-2-en-1-ol (**S4**):*

3-Azidopropyl 4-methylbenzenesulfonate (**S2,** 400 mg, 1.56 mmol, 1 equiv) was dissolved in *N*,*N*-dimethylformamide (1.5 mL) at 24 °C. (*E*)-2,3-Dibromobut-2-ene-1,4-diol (**S3**, 1.33 g, 4.68 mmol, 3.00 equiv) and potassium hydroxide (131 mg, 2.34 mmol, 1.50 equiv) in water (3 mL) was added in sequence to the stirred solution at 24 °C. The resulting mixture was stirred for 3 h at 50 °C. The product mixture was diluted sequentially with diethyl ether (10 mL) and 6.7 mM phosphate buffered saline (pH 7.4, 10 mL). The resulting biphasic mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with diethyl ether (3 × 10 mL), and the organic layers were combined. The combined organic layers were dried over sodium sulfate. The dried solution was filtered, and the filtrate was concentrated by rotary evaporation. The product mixture was purified by flash-column chromatography (eluting with 10% ethyl acetate–hexanes, grading to 30% ethyl acetate–hexanes, two steps) to afford (*E*)-4-(3-azidopropoxy)-2,3-dibromobut-2-en-1-ol (**S4**) as a white solid (107 mg, 21%).

R_f = 0.26 (20% ethyl acetate–hexanes; CAM). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.57 (s, 2H, H_4/H_5), 4.44 (s, 2H, H_4/H_5), 3.55 (t, 2H, J = 6.0 Hz, H_3), 3.45 (t, 2H, J = 6.5 Hz, H_1), 1.87 (q, 2H, J = 6.0 Hz, H_2). ^{13}C NMR (500 MHz, $CDCl_3$): δ 124.9 (C), 120.7 (C), 74.7 (CH_2), 67.9 (CH_2), 67.2 (CH_2), 48.9 (CH_2), 29.8 (CH_2). IR (NaCl), cm^{-1}: 3405 (br), 2926 (m), 2869 (m), 2094 (s), 1115 (s). HRMS-ESI (*m/z*): [M+Na] calculated for $C_7H_{11}{}^{79/79}Br_2N_3NaO_2$, 349.9116; found, 349.9100.



*Synthesis of the IsoTaG azido silane probe **2**:*

Triethylamine (69.6 μL, 502 μmol, 20.0 equiv) and dichlorodiphenylsilane (26.4 μL, 125 μmol, 5.00 equiv) were added in sequence to a stirred solution of the biotin–CA(PEG)$_4$–alcohol **S5**[2] (14.1 mg, 25.1 μmol, 1 equiv) in dichloromethane (500 μL). The resulting solution was stirred for 3 h at 24 °C. (*E*)-4-(3-Azidopropoxy)-2,3-dibromobut-2-en-1-ol (**S4**, 165 mg, 502 μmol, 20.0 equiv) was added to the stirred solution. The resulting solution was stirred for an additional 12 h at 24 °C. The product mixture was diluted sequentially with dichloromethane (10 mL) and saturated aqueous sodium bicarbonate solution (10 mL). The resulting biphasic mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with dichloromethane (3 × 10 mL), and the organic layers were combined. The combined organic layers were dried over sodium sulfate. The dried solution was filtered, and the filtrate was concentrated by rotary evaporation. The residue obtained was purified by flash-column chromatography (eluting with 1% methanol–dichloromethane, grading to 10% methanol–dichloromethane, 3 steps) to afford the IsoTaG azido silane probe **2** as a clear oil (12.3 mg, 46%).

R_f = 0.43 (5% methanol–dichloromethane; I_2). ^{1}H NMR (500 MHz, CD_3OD): δ 7.67 (d, 4H, J = 6.5 Hz, H_{18}), 7.43 (t, 2H, J = 15.0 Hz, H_{20}), 7.36 (t, 4H, J = 7.5 Hz, H_{19}), 4.70 (s, 2H, H_{21}/H_{22}), 4.46 (dd, 1H, J = 5.5, 4.5 Hz, H_2), 4.39 (s, 2H, H_{21}/H_{22}), 4.27 (dd, 1H, J = 4.5, 3.0 Hz, H_5), 3.70 (t, 2H, J = 6.0 Hz, H_{14}), 3.36–3.28 (m, 18H, H_{11}/H_{12}/H_{15}), 3.39 (t, 2H, J = 6.5 Hz, H_{23}), 3.32 (t, 2H, J = 5.0 Hz, H_{25}), 3.16 (dt, 1H, J = 5.0, 4.5 Hz, H_6), 2.92 (d, 1H, J = 5.0 Hz, H_1), 2.90 (d, 1H, J = 5.0 Hz, H_1), 2.45 (t, 2H, J = 6.0 Hz, H_{13}), 2.19 (t, 2H, J = 7.5 Hz, H_{10}), 1.80 (q, 2H, J = 6.0 Hz, H_{24}), 1.72–1.55 (m, 4H, H_7/H_9), 1.42 (quint, 2H, J = 7.5, H_8), 1.26 (s, 6H, H_{16}/H_{17}). ^{13}C NMR (500 MHz, CD_3OD): δ 176.1 (C), 174.1 (C), 166.1 (C), 136.2 (3 • CH), 135.0 (2 • C), 131.5 (4 • CH_2), 128.9 (3 • CH), 124.8 (C), 121.2 (C), 77.3 (CH_2), 75.0 (CH_2), 71.6 (2 • CH_2), 71.5(2 • CH_2) 71.4 (CH_2), 71.3 (CH_2), 68.4 (CH_2), 68.3 (CH_2), 67.7 (CH_2), 63.4 (CH), 61.6 (CH), 57.0 (CH_2), 51.6 (CH_2), 49.5 (C) 48.8 (CH_2), 41.1 (CH_2), 40.4 (CH_2), 37.8 (CH_2), 36.7 (CH_2), 30.1 (CH_2), 29.8 (CH_2), 29.5 (CH_2), 28.2 (2 × CH_3), 26.8 (CH_2). IR (ATR-FTIR), cm^{-1}: 3302 (br), 2925 (m), 2868 (m), 2097 (s), 1696 (s), 1647 (s), 1115 (s). HRMS-ESI (*m/z*): [M+Na] calculated for $C_{44}H_{62}{}^{79/79;79/81;81/81}Br_2N_4O_{10}SSiNa$, 1047.2215/1049.2199/1051.2179; found, 1047.2216/1049.2183/1051.2182.

Byonic glycan modifications input file

For Ac_4ManNAz labeled samples:
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HexNAc(1)Hex(1)NeuAzBr2OH(1)NeuAc(1) @ OGlycan | common1
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HexNAc(1)NeuAzBr2OH(1) @ OGlycan | common1
HexNAc(4)Hex(5)NeuAzBr2OH(1) @ NGlycan | common1
HexNAc(4)Hex(5)Fuc(1)NeuAzBr2OH(1) @ NGlycan | common1
HexNAc(4)Hex(5)NeuAzBr2OH(2) @ NGlycan | common1
HexNAc(5)Hex(5)NeuAzBr2OH(2) @ NGlycan | common1
HexNAc(4)Hex(5)Fuc(1)NeuAzBr2OH(2) @ NGlycan | common1
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HexNAc(4)Hex(5)NeuAzBr2OH(1)NeuAc(1) @ NGlycan | common1
HexNAc(5)Hex(5)NeuAzBr2OH(1)NeuAc(1) @ NGlycan | common1
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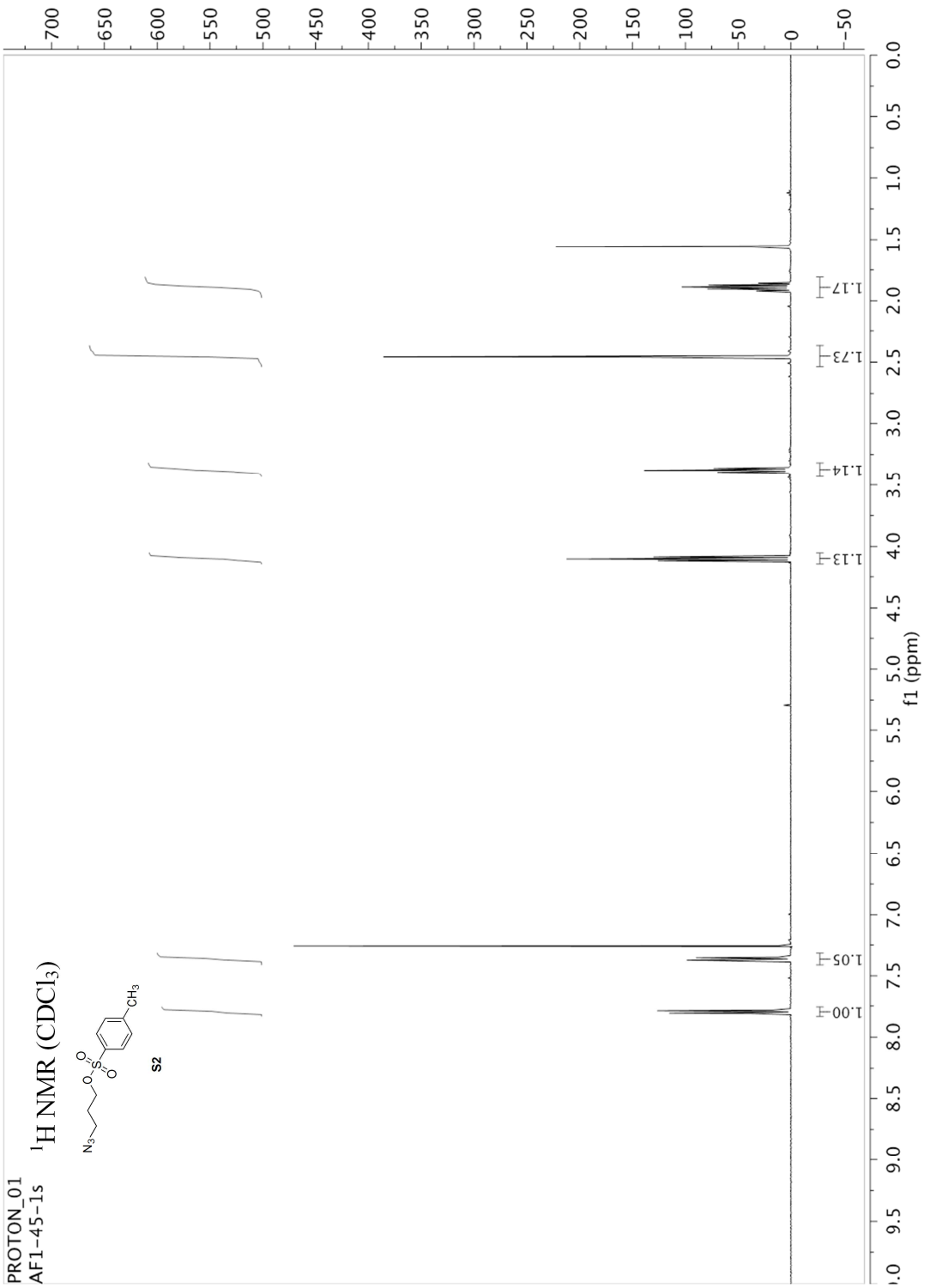
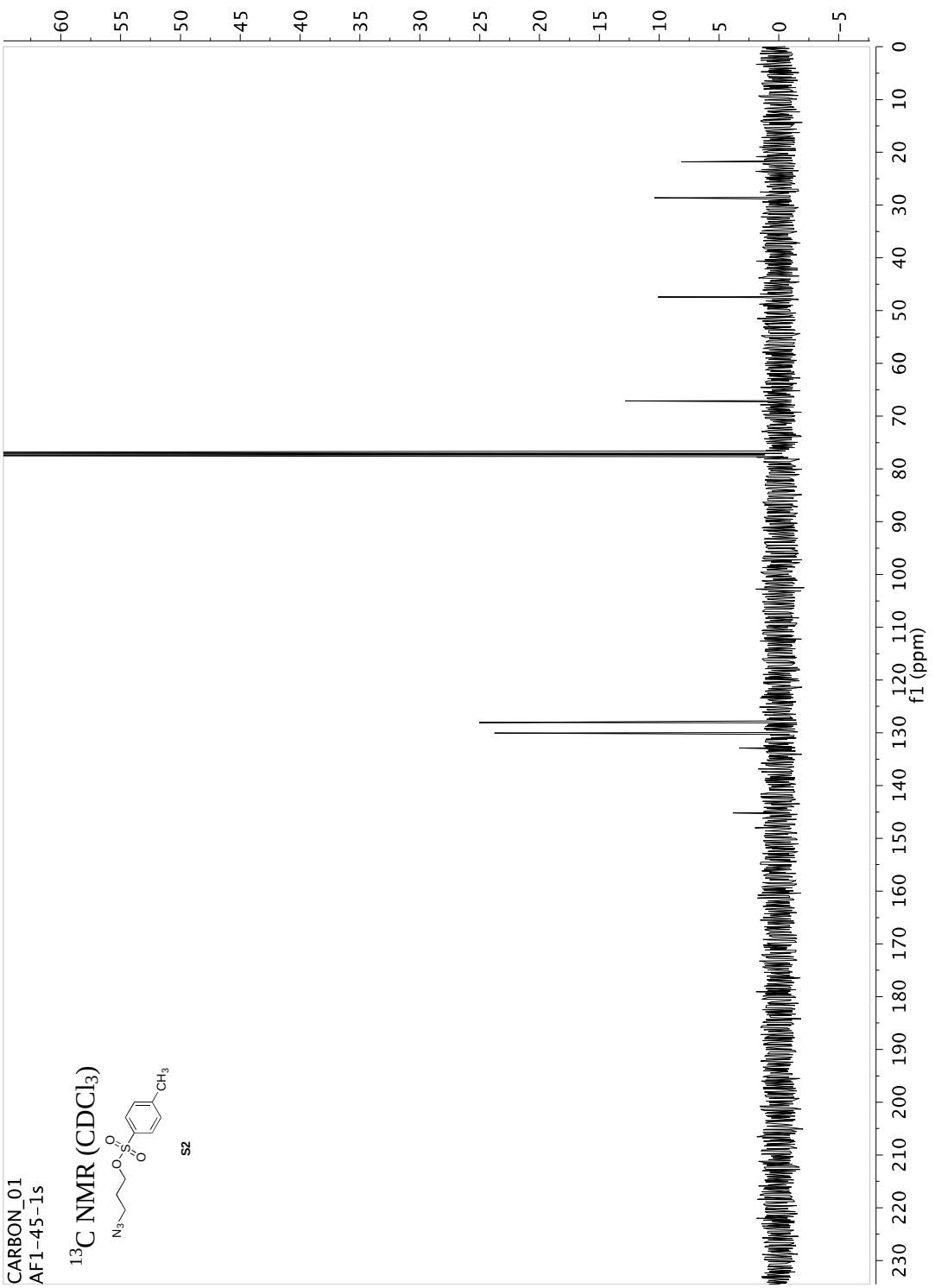
Glycan weights used: HexNAc = $C_8H_{13}NO_5$ (+203.0794), NeuAzBr2OH = $C_{18}H_{24}Br_2N_4O_{10}$ (+615.9839) NeuAz = $C_{11}H_{16}N_4O_8$ (+332.0968), NeuAcNH$_2$ = $C_{11}H_{18}N_2O_8$ (+306.1063), NeuAc = $C_{11}H_{17}NO_8$ (+291.0954), Hex = $C_6H_{10}O_5$ (+162.0528), Fuc = $C_6H_{10}O_4$ (+146.0579), FucAlBr2OH = $C_{14}H_{19}Br_2N_3O_6$ (+484.96201), NeuAlBr2OH = $C_{21}H_{30}Br_2N_4O_{10}$ (+658.03082).

Catalog of Nuclear Magnetic Resonance and Infrared Spectra.





Woo, C. M., et al. "Isotope Targeted Glycoproteomics (IsoTaG) analysis of sialylated N- and O-glycopeptides on an Orbitrap Fusion Tribrid using azido and alkynyl sugars." *Anal. Bioanal. Chem.*, 2016.

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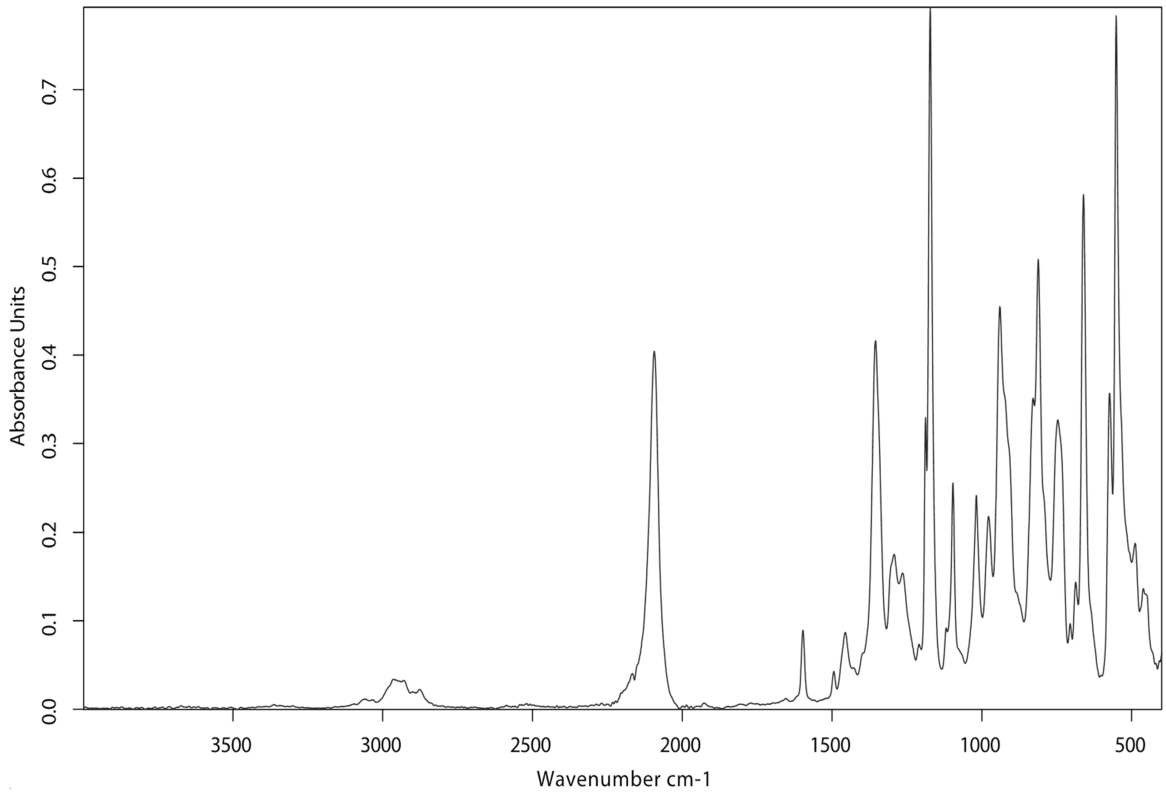
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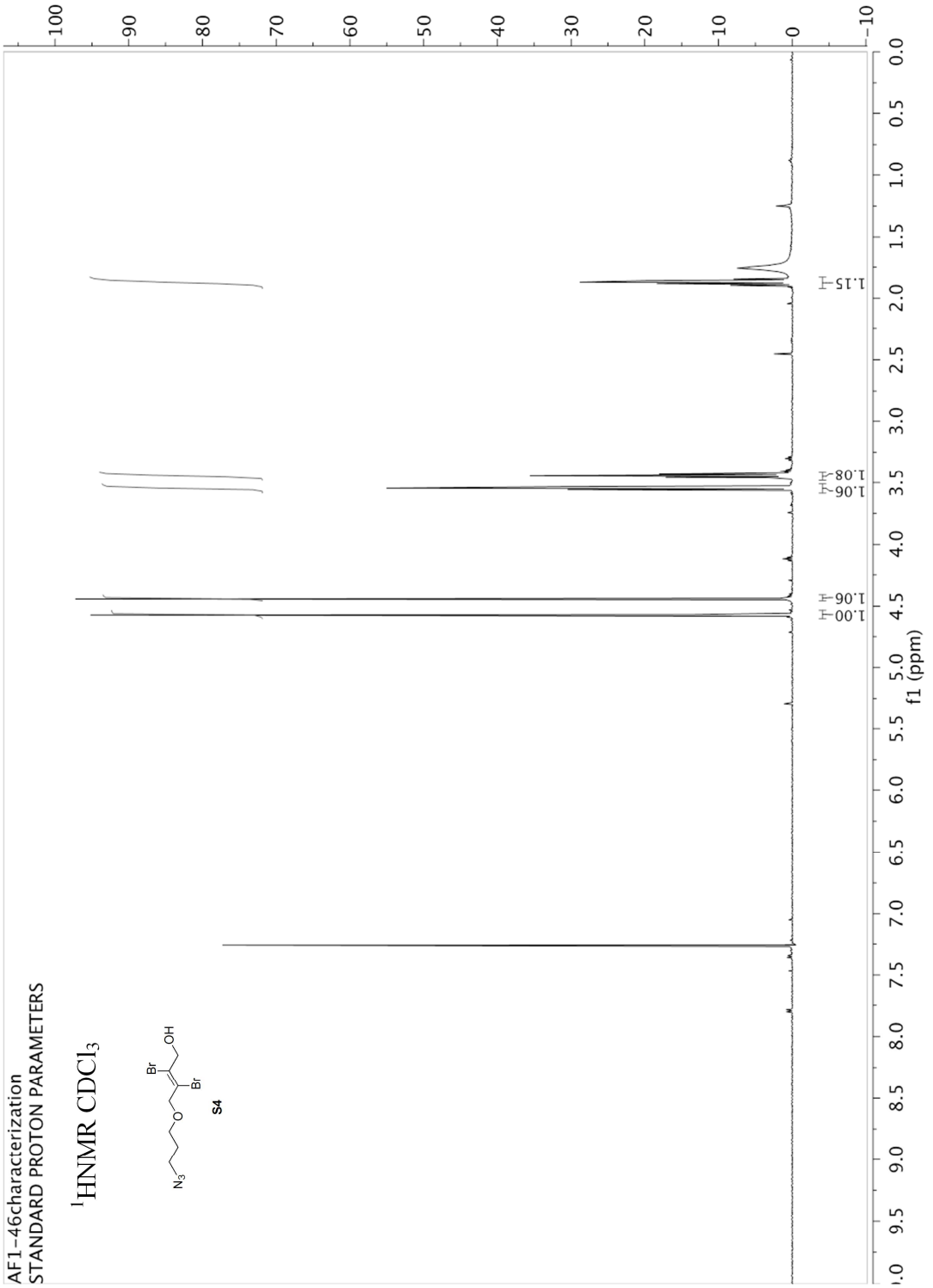
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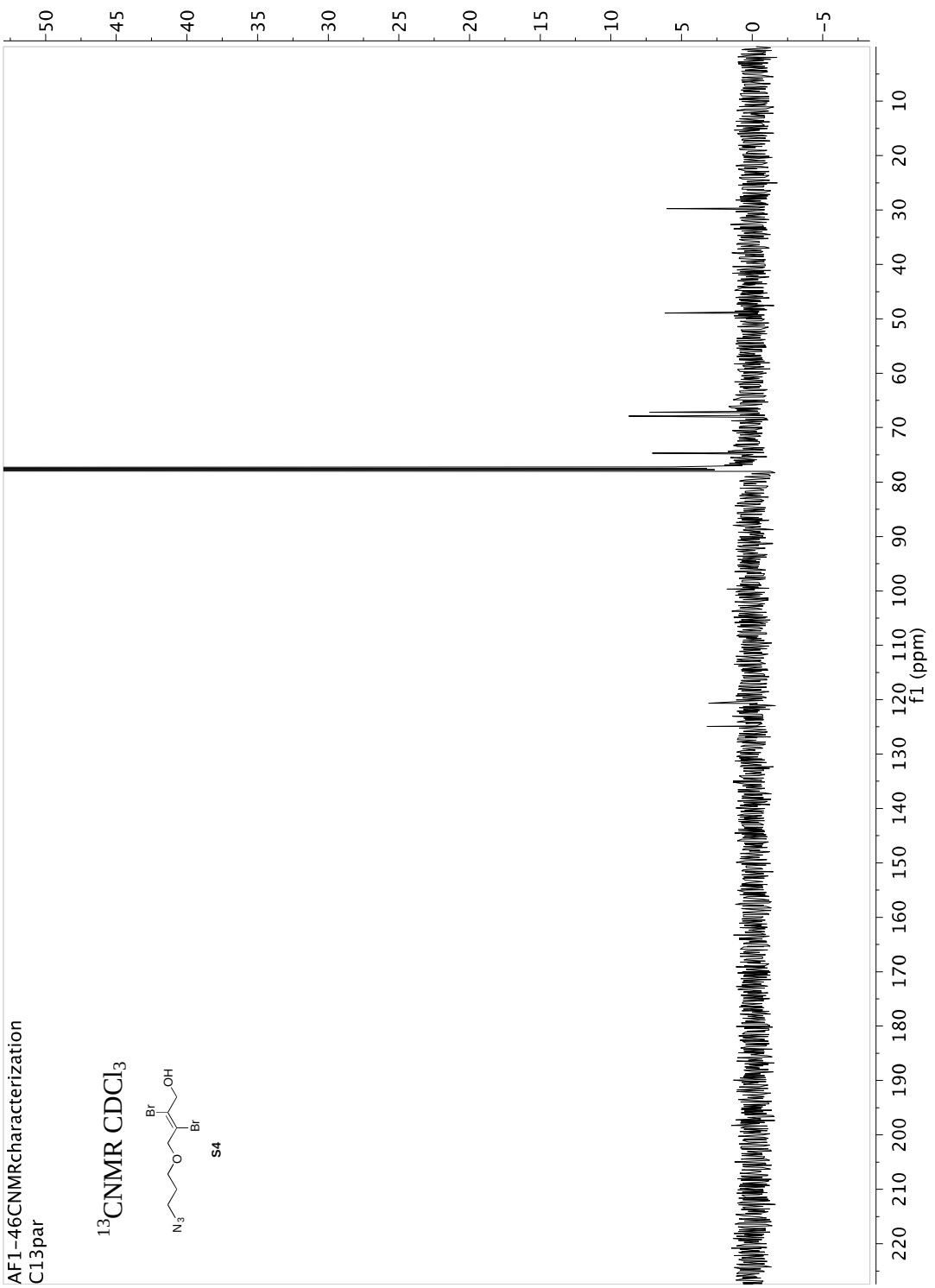
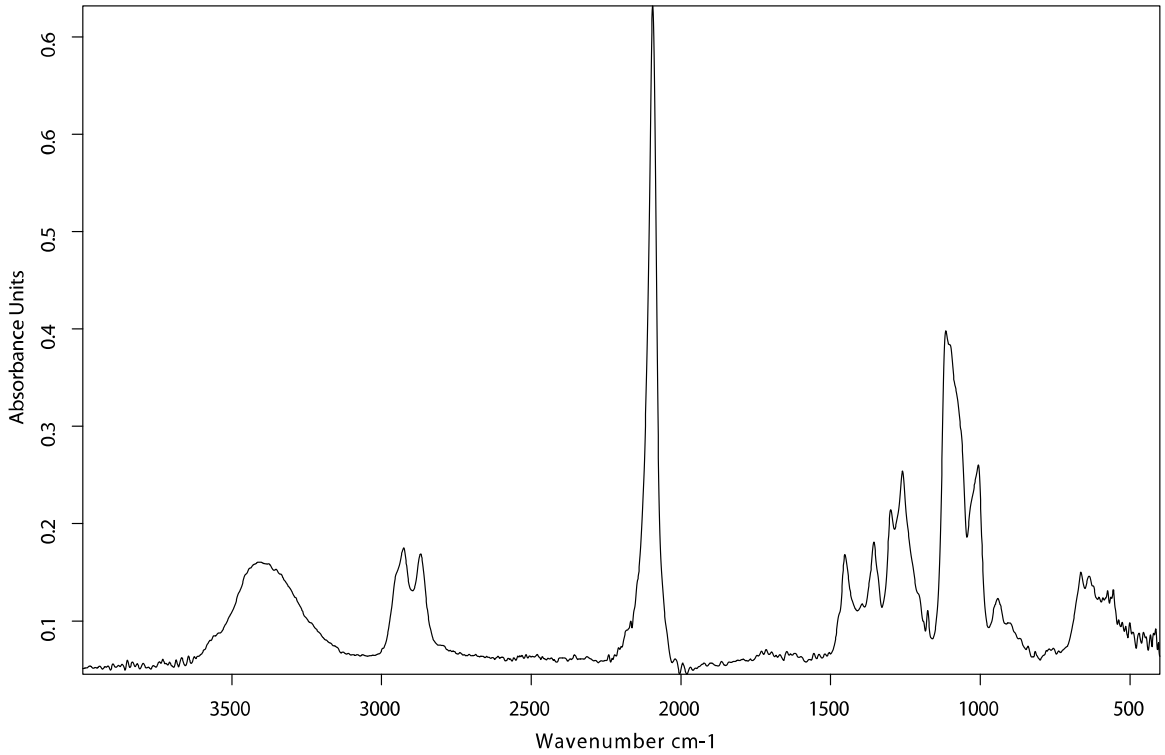


Woo, C. M., et al. "Isotope Targeted Glycoproteomics (IsoTaG) analysis of sialylated N- and O-glycopeptides on an Orbitrap Fusion Tribrid using azido and alkynyl sugars." *Anal. Bioanal. Chem.*, 2016.

IR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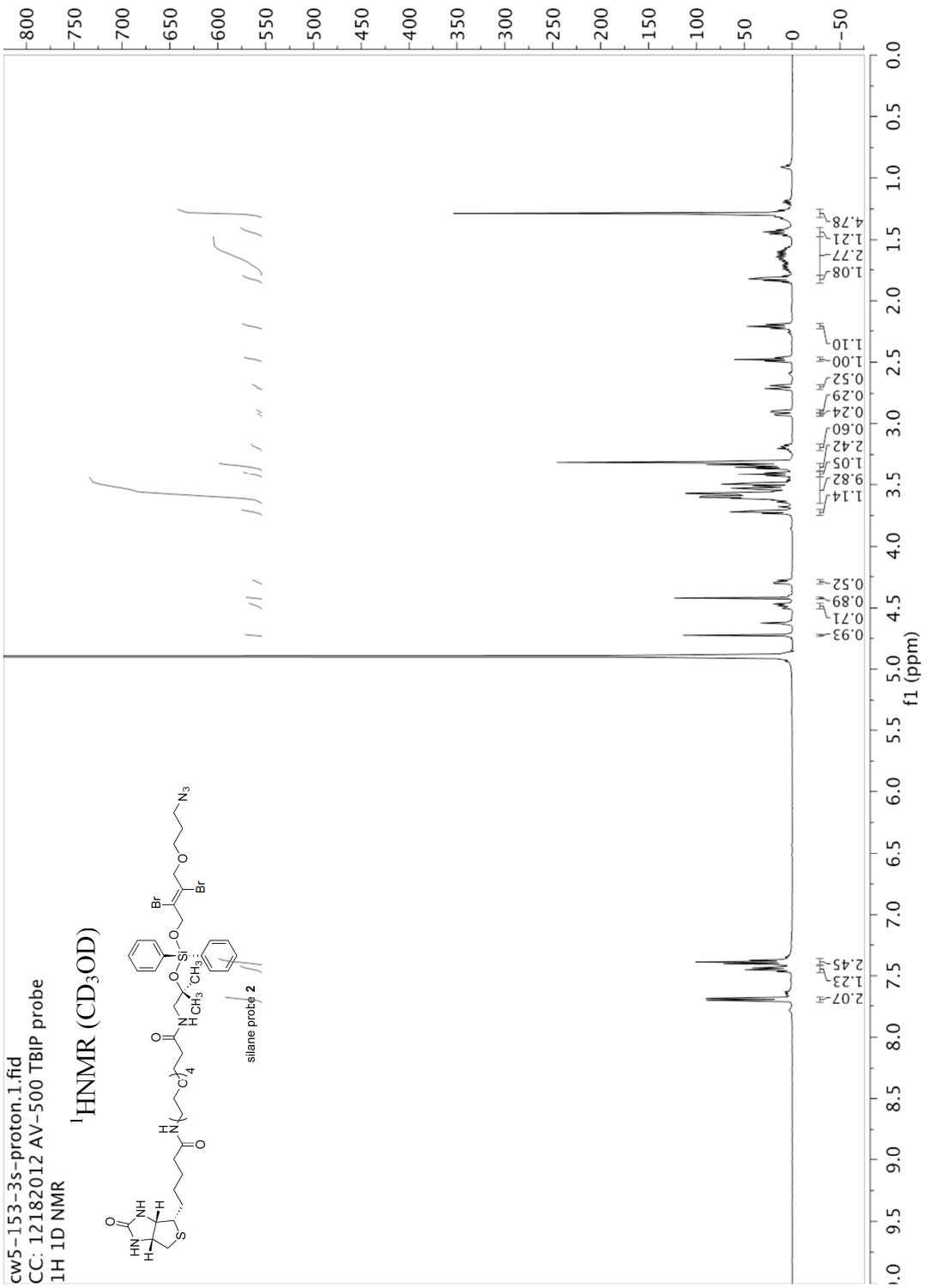
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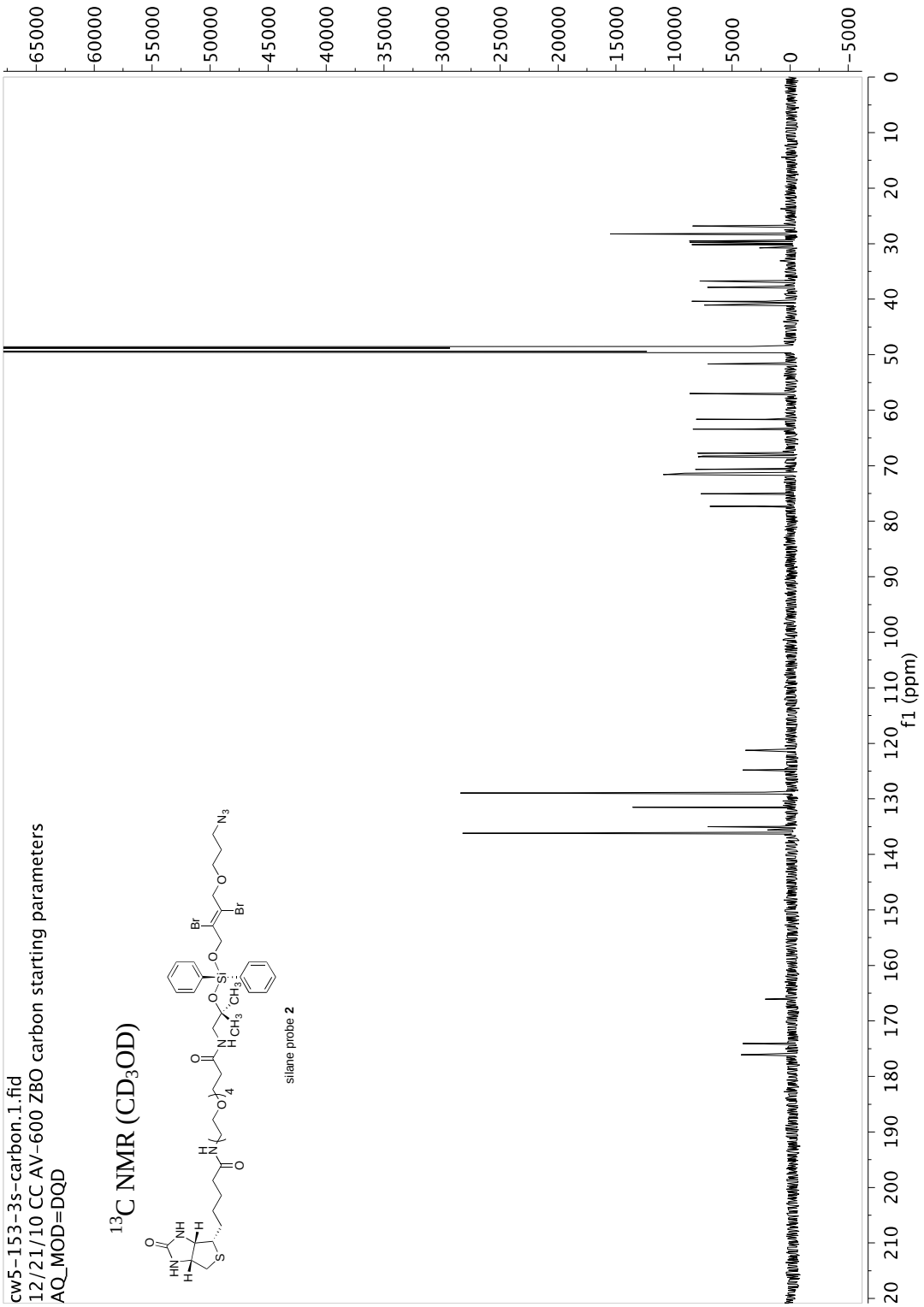
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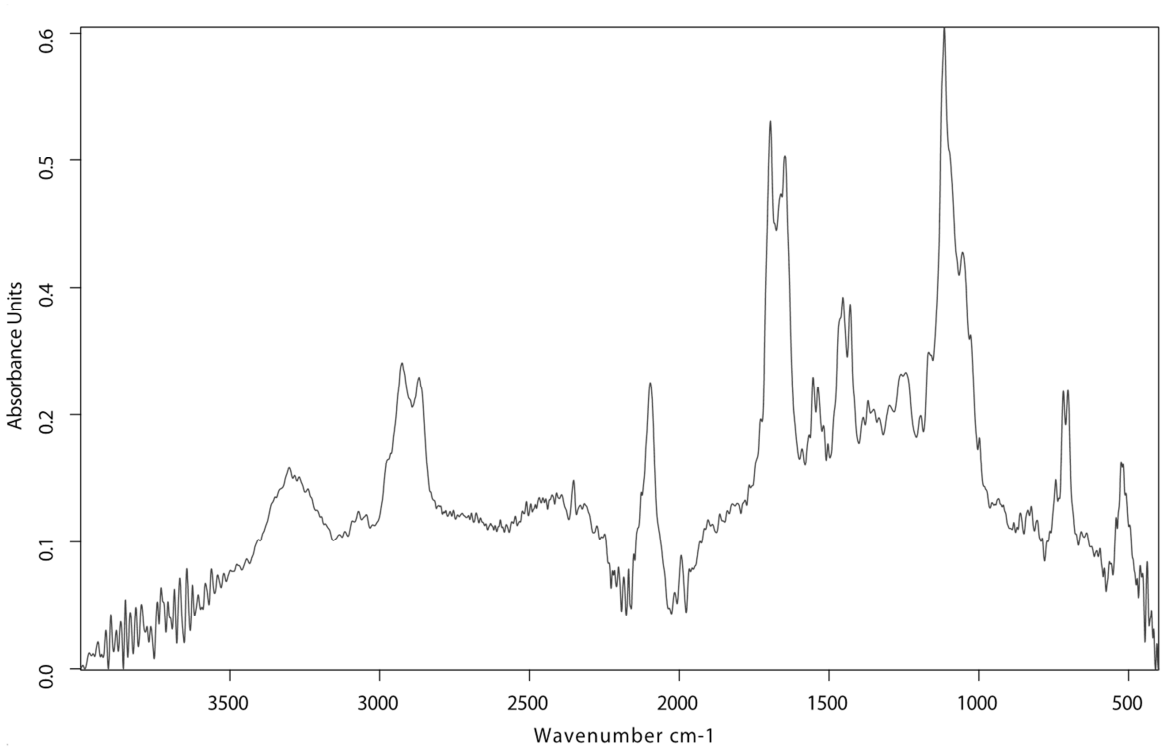
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Wavenumber cm-1

Bibliography.

1. Still WC, Kahn M, Mitra A (1978). J Org Chem 43:2923.
2. Szychowski J, Mahdavi A, Hodas JJ, Bagert JD, Ngo JT, Landgraf P, Dieterich DC, Schuman EM, Tirrell DA (2010) Cleavable biotin probes for labeling of biomolecules via azide-alkyne cycloaddition. J Am Chem Soc 132 (51):18351.