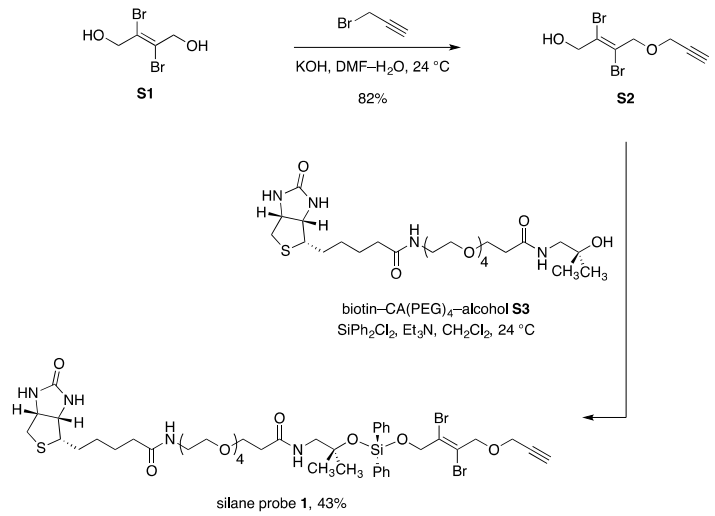
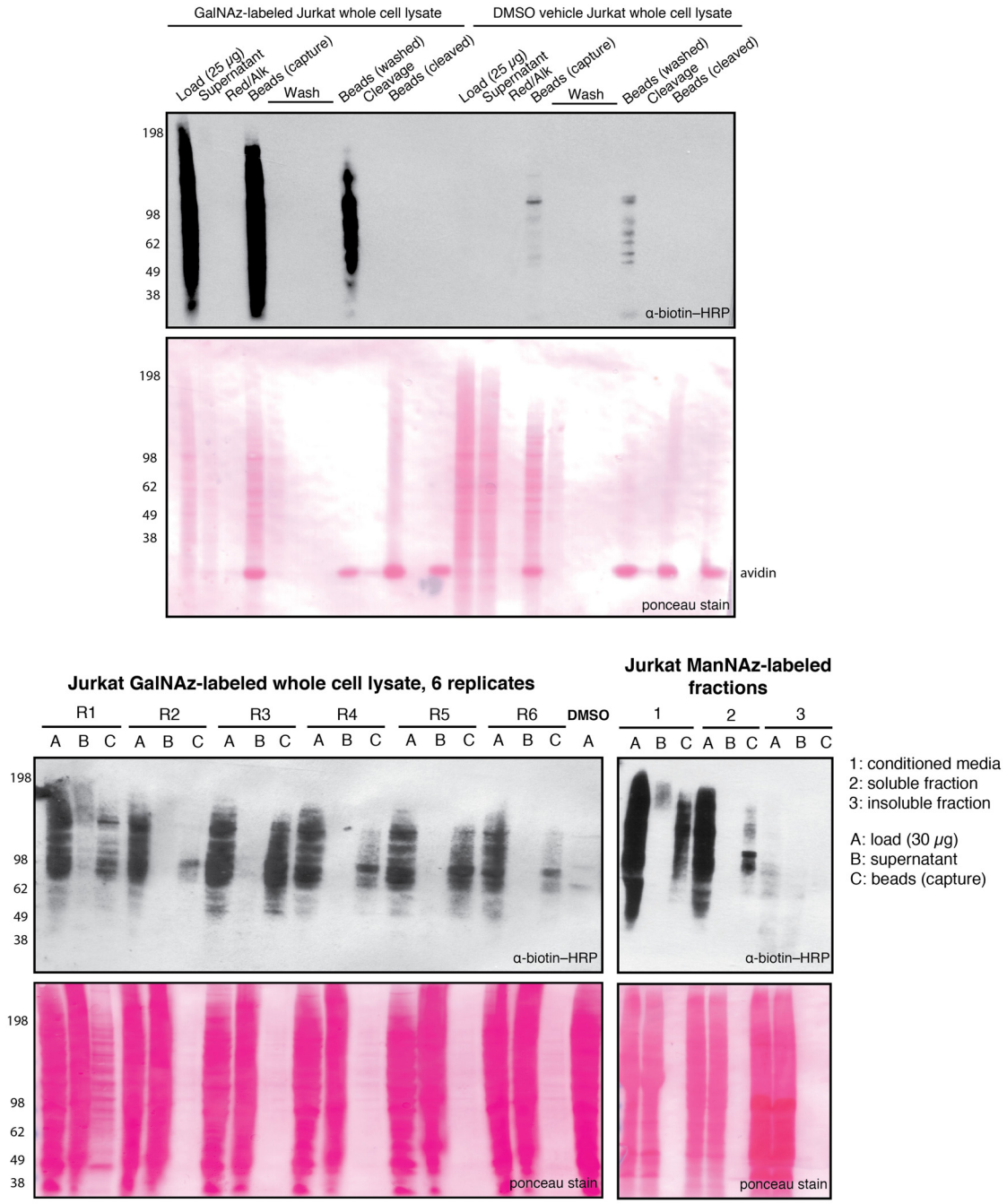


Supplementary Figure 1

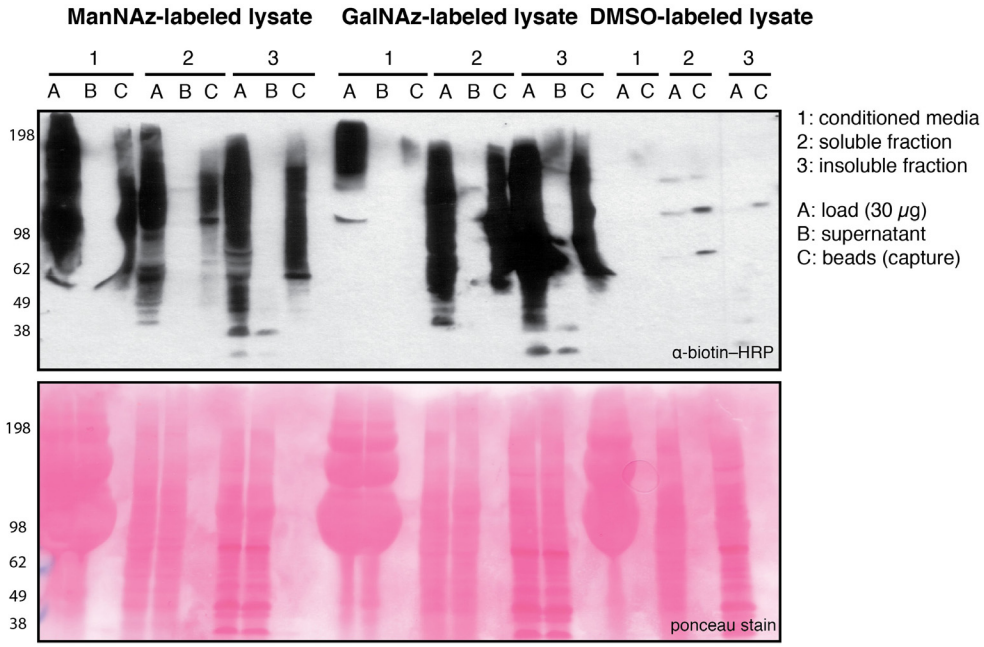
Synthesis of the dibrominated silane probe **1**.



Supplementary Figure 2

Representative western blot analysis of enrichment with probe **1** and Jurkat whole-cell lysate labeled with 100 μM Ac_4GalNAz, Ac_4ManNAz or DMSO vehicle.

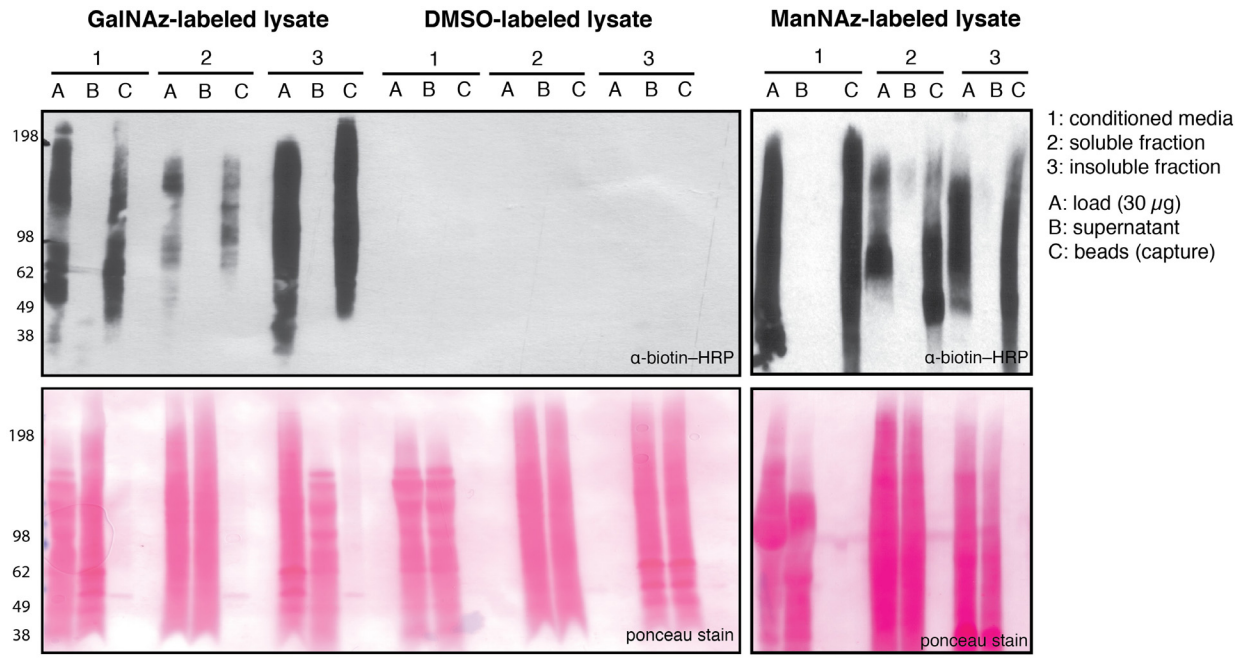
Biotinylated proteins (load) were enriched from the supernatant by affinity-capture with avidin–agarose beads. Avidin–agarose beads were reduced and alkylated, and washed with 1% RapiGest, 6 M urea, and PBS. Beads were checked for anti-biotin signal before and after washing. Treatment with 2% formic acid cleaved probe **1** and released glycoproteins from the agarose beads.



Supplementary Figure 3

Representative western blot analysis of enrichment with probe **1** and MCF7 conditioned medium and lysate labeled with 100 μM Ac_4GalNAz, Ac_4ManNAz or DMSO vehicle.

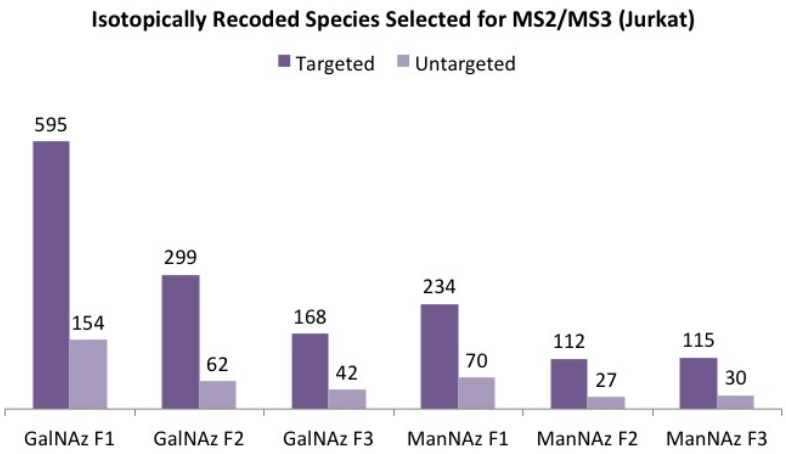
A. Protein after tagging by probe **1**. B. Protein after enrichment with streptavidin–agarose. C. A sample of the streptavidin–agarose beads after washing (1% RapiGest, 6 M urea, PBS) and reduction/alkylation.



Supplementary Figure 4

Representative western blot analysis of enrichment with probe **1** and PC-3 conditioned medium and lysate labeled with 100 μM Ac_4GalNAz, Ac_4ManNAz or DMSO vehicle.

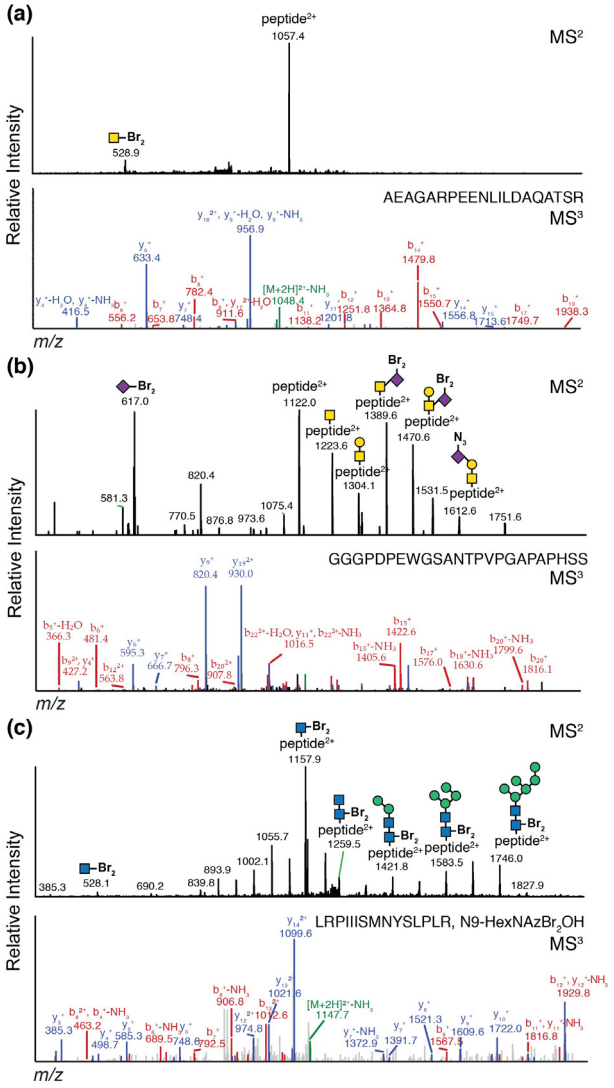
A. Protein after tagging by probe **1**. B. Protein after enrichment with streptavidin–agarose. C. A sample of the streptavidin–agarose beads after washing (1% RapiGest, 6 M urea, PBS) and reduction/alkylation.



Supplementary Figure 5

IsoStamp-directed glycoproteomics selects isotopically recoded species at a fourfold higher rate across fractions and glycan type from Jurkat cell lysates.

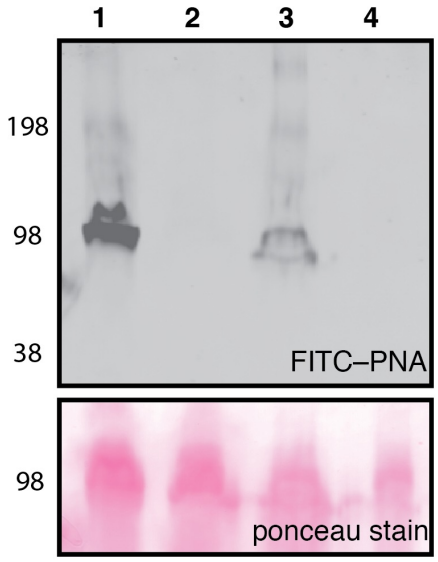
Jurkat cells were labeled with 100 μM Ac_4GalNAz or Ac_4ManNAz for 48 h. Media (F1), soluble (F2), and insoluble (F3) cellular fractions were enriched for isotopically recoded glycopeptides and analyzed by MS. Tandem MS was collected with an inclusion list (targeted) or by data-dependent analysis of the six most intense ions detected in each full-scan mass spectrum (untargeted).



Supplementary Figure 6

Representative assignments for glycopeptides from MS^2 and MS^3 spectra for O-GalNAz (a), bis-sialylated **O8** (b), and N-glycan **N5** (c) glycoforms.

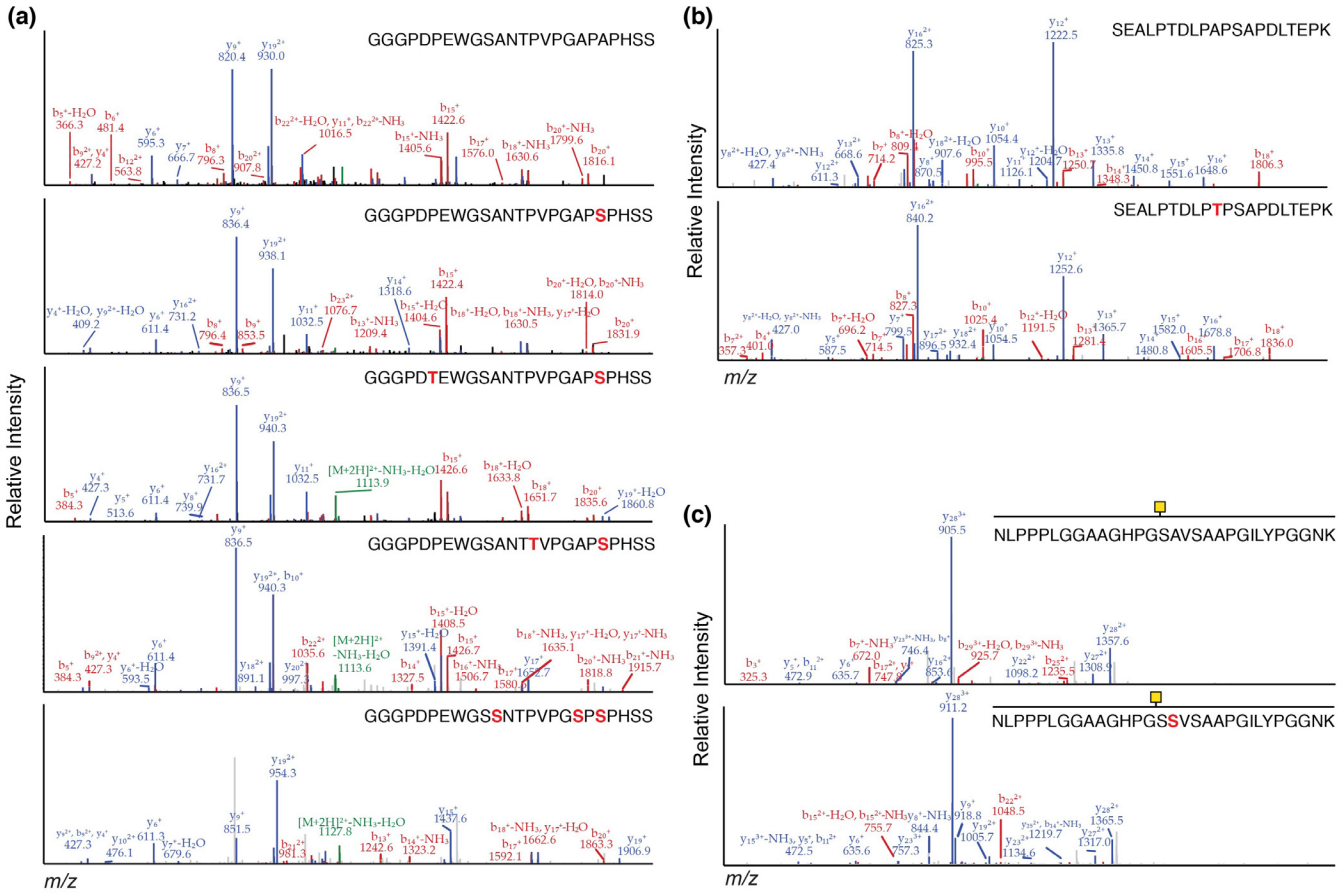
The tagged glycan is denoted with "Br_2." The metabolically labeled glycan (but not tagged) is denoted with "N_3."



Supplementary Figure 7

Medium from Jurkat cells displays core 1 O-glycans.

Medium from Jurkat cells metabolically labeled with 100 μM Ac_4GalNAz or DMSO vehicle was treated with neuraminidase and analyzed by staining with FITC–PNA that detects the core 1 O-glycan. Ponceau staining shows equal protein loading (20 μg protein per lane). Lane 1: GalNAz-labeled Jurkat media + neuraminidase. Lane 2: GalNAz-labeled Jurkat media – neuraminidase. Lane 3: DMSO treated Jurkat media + neuraminidase. Lane 4: DMSO treated Jurkat media – neuraminidase.



Supplementary Figure 8

Assigned tandem mass spectra of peptide isoforms identified from Ac_4ManNAz-labeled PC-3 cells.

Assigned tandem mass spectra of peptide isoforms identified from Ac_4ManNAz-labeled PC-3 cells from (a) prostate-specific microseminoprotein (Q1L6U9), (b) Glucosidase 2 subunit beta (P14314), and (c) Dickkopf-related protein 1 (O94907). Amino acid substitutions are in bold red font. Spectra were assigned with Byonic as a node in Proteome Discoverer.

#

Supplementary Table 1. Representative glycopeptides and associated glycoforms identified using IsoTaG. Amino acid substitutions [i.e., peptide sequence polymorphisms, (pSPs)] are in bold red font. Assigned glycosites (N-glycan) are underlined and in bold blue font.

Accession	Protein (Gene Name)	Peptide	Glycan Isoform
O00468	Agrin (AGRN)	NLEEVEFCVEDKPGTHFTPVPPTPPDACR	O2, O5, O6
O94907	Dickkopf-related protein 1 (DKK1)	GEIEETITESFGNDHSTLDGYSR	O7
		MYHTKGQEGSVCLR	O6, O8
		NLPPPLGGAAGHPGSAVSAAPGILYPGGNK	O6, O8
		NLPPPLGG**S**AGHPGSAVSAAPGILYPGGNK	O6, O8
		YQTIDNYQPYPCAEDEECGTDEYCASPTR	O6, O7
P02786	Transferrin receptor protein 1 (TFRC)	LAGTESPVREEPGEDFPAAR	O2, O5, O6, O8
P07339	Cathepsin D (CTSD)	YSQAVPAVTEGPIPEVLK	O6, O8
P07602	Prosaposin (PSAP)	LPALTVHVTQPK	O2, O6
P14314	Glucosidase 2 subunit beta (PRKCSH)	SEALPTDLPAPSAPDLTEPK	O2, O6, O8
		SEALPTDLP**T**PSAPDLTEPK	O2, O4, O6, O8, 2 × O2
P26006	Integrin alpha-3 (ITGA3)	LRPIIISM**N**YSLPLR	N4
P26572	Alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase (MGAT1)	GRVPTAAPPAQPR	O6, O8
		VPVTPAPAVIPILVIACDR	O8
P27824	Calnexin (CANX)	HDGHDDDVIDIEDDLDDVIEEVEDSKPDTTAPPSSPK	O6, O8
		VTYKAPVPTGEVYFADSFDR	O2, O4, 2 × O2
P42785	Lysosomal Pro-X carboxypeptidase (PRCP)	ALGSLHLPTNPTSLPAVAK	O2, O6
P43026	Growth/differentiation factor 5 (GDF5)	QATARTVTPK	O2, O6, O8
P46977	Dolichyl-diphosphooligosaccharide–protein glycosyltransferase subunit STT3A (STT3A)	TILVD**N**NTWNNTHISR	N4
P51610	Host cell factor 1 (HCFC1)	SGTVTVAQQAQVVTTVVGGVTK	O1, 2 × O1
		TAAAQVGTSVSSATNTSTRPIITVHK	O1, 2 × O1, 3 × O1
Q02818	Nucleobindin-1 (NUCB1)	GAPNKEETPATESPDTGLYYHR	O2, O6, O8, 2 × O2
Q08629	Testican-1 (SPOCK1)	VIKPTSSNTAQGR	O6, O7
Q12841	Follistatin-related protein 1 (FSTL1)	SVSPSASPVVCYQSNR	O2, O3, O6, O8
Q14118	Dystroglycan (DAG1)	DWENQLEASMHSVLSDLHEAVPTVVGIPDGTAVVGR	O6, O8
Q14242	P-selectin glycoprotein ligand 1 (SELPLG)	GLFIPFSVSSTHK	O2, O4, 3 × O2, O2+O4
Q14766	Latent-transforming growth factor beta-binding protein 1 (LTBP1)	EHGPGVAEPEVATAPPEKEIPSLDQEK	O8
		IKVVFTPSICK	O8
		STHPPPLPAKEEPVEALTFSR	O6
Q1L6U9	Prostate-associated microseminoprotein (MSMP)	GGGPDPEWGSANTPVPGAPAPHSS	O6, O8
		GGGPDPEWGSANTPVPGAP**S**PHSS	O6, O8
		GGGPD**T**EWGSANTPVPGAP**S**PHSS	O6, O8
		GGGPDPEWGSANT**T**VPGAP**S**PHSS	O6
		GGGPDPEWGS**S**NTPVPG**S**P**S**PHSS	O8
Q8NBS9	Thioredoxin domain-containing protein 5 (TXNDC5)	DFQTLENWMLQTLNEEPVTPEPEVEPPSAPELK	O2, O6
Q8NCH0	Carbohydrate sulfotransferase 14 (CHST14)	AGAGPSPAGDDVTFPEFLR	O2, O6
Q8TCJ2	Dolichyl-diphosphooligosaccharide–protein glycosyltransferase subunit STT3B (STT3B)	TTLVD**N**NTWNNSHIALVGK	N2, N3, N4
		TTLVDNNTW**N**NSHIALVGK	N2, N3, N4
Q92854	Semaphorin-4D (SEMA4D)	VVPKPVVAPTLSVVQTEGSR	O2, O6, 2 × O2, O2+O5
Q9NTZ6	RNA-binding protein 12 (RBM12)	VNLPTTVSNFNNPSPSVVTATTSVHESNK	O1, 2 × O1, 3 × O1
Q9Y4L1	Hypoxia up-regulated protein 1 (HYOU1)	**N**ATLAEQAK	N3, N4

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Supplementary Methods.

General Chemical 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) and/or submersion in aqueous ceric ammonium molybdate solution (CAM) followed by brief heating on a hot plate (120 °C, 10–15 s).

Instrumentation. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded at 400 or 600 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 100 or 125 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 QB3/Chemistry Mass Spectrometry Facility at the University of California, Berkeley, using a Thermo LTQ-FT mass spectrometer.

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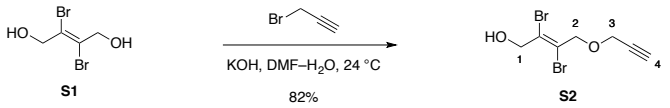
Synthetic Procedures.



*Synthesis of (E)-2,3-Dibromo-4-(prop-2-yn-1-yloxy)but-2-en-1-ol (**S2**):*

Propargyl bromide (80% solution in toluene, 1.25 mL, 11.6 mmol, 1 equiv) was added to a stirred solution of (*E*)-2,3-dibromobut-2-ene-1,4-diol (**S1**, 8.56 g, 34.8 mmol, 3.00 equiv) and potassium hydroxide (1.30 g, 23.2 mmol, 2.00 equiv) in 50% *N,N*-dimethylformamide–water (30 mL) at 24 °C. The resulting mixture was stirred for 12 h at 24 °C. The product mixture was purified by flash-column chromatography (eluting with 25% ethyl acetate–hexanes, grading to 100% ethyl acetate, one step) to afford (*E*)-2,3-dibromo-4-(prop-2-yn-1-yloxy)but-2-en-1-ol (**S2**) as a white solid (2.69 g, 82%).

R_f = 0.26 (20% ethyl acetate–hexanes; CAM). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.49 (s, 4H, H_1/H_2), 4.14 (d, 2H, J = 2.4 Hz, H_3), 3.28 (t, 1H, J = 6.8 Hz, OH), 2.49 (t, 1H, J = 2.4 Hz, H_4). ^{13}C NMR (600 MHz, $CDCl_3$): δ 125.3 (C), 118.6 (C), 78.8 (C), 75.5 (CH), 72.8 (CH_2), 66.8 (CH_2), 57.2 (CH_2). IR (NaCl), cm^{-1}: 3295 (br), 1092 (m), 642 (m). HRMS-EI (*m/z*): [M+H] calculated for $C_7H_8{}^{79/81}Br_2O_2$, 283.8871; found, 283.8878.

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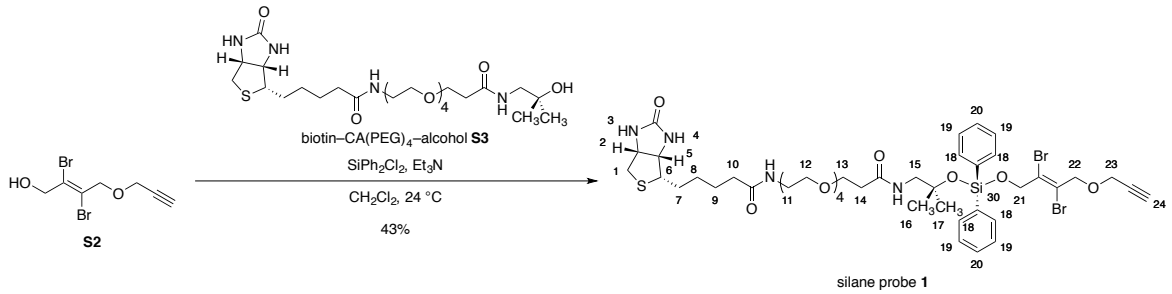

*Synthesis of the Silane Probe **1**:*

Triethylamine (43.2 μL, 312 μmol, 9.00 equiv) and dichlorodiphenylsilane (21.9 μL, 104 μmol, 3.00 equiv) were added in sequence to a stirred solution of the biotin–CA(PEG)$_4$–alcohol **S3**[2] (19.5 mg, 34.6 μmol, 1 equiv) in dichloromethane (690 μL) at 24 °C. The resulting solution was stirred for 2 h at 24 °C. (*E*)-2,3-dibromo-4-(prop-2-yn-1-yloxy)but-2-en-1-ol (**S2**, 59.0 mg, 208 μmol, 6.00 equiv) was added to the stirred solution at 24 °C. The resulting solution was stirred for an additional 1 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. The residue obtained was purified by flash-column chromatography (eluting with 1% methanol–dichloromethane, grading to 10% methanol–dichloromethane, 3 steps) to afford the silane probe **1** as a clear oil (15.4 mg, 43%).

R_f = 0.54 (5% methanol–dichloromethane; CAM). ^{1}H NMR (400 MHz, CD_3OD): δ 7.69 (d, 4H, J = 6.4 Hz, H_{18}), 7.45 (t, 2H, J = 7.2 Hz, H_{20}), 7.39 (t, 4H, J = 7.2 Hz, H_{19}), 4.72 (s, 2H, H_{21}/H_{22}), 4.50 (s, 2H, H_{21}/H_{22}), 4.48 (dd, 1H, J = 8.0, 4.8 Hz, H_2), 4.29 (dd, 1H, J = 8.0, 4.8 Hz, H_5), 4.11 (d, 2H, J = 2.4 Hz, H_{23}), 3.72 (t, 2H, J = 6.0 Hz, H_{14}), 3.64–3.53 (m, 16H, H_{11}/H_{12}/H_{15}), 3.35–3.33 (m, 4H, H_{11}/H_{12}), 3.19 (dt, 1H, J = 8.4, 5.6 Hz, H_6), 2.93–2.89 (m, 2H, H_{24}/H_1), 2.70 (d, 1H, J = 12.4 Hz, H_1), 2.48 (t, 2H, J = 6.4 Hz, H_{13}), 2.21 (t, 2H, J = 7.2 Hz, H_{10}), 1.75–1.54 (m, 4H, H_7/H_9), 1.47–1.39 (m, 2H, H_8), 1.28 (s, 6H, H_{16}/H_{17}). ^{13}C NMR (600 MHz, CD_3OD): δ 176.1 (C), 174.1 (C), 166.0 (C), 136.2 (3 × CH), 135.0 (2 × C), 131.5 (2 × CH), 128.9 (3 × CH), 125.5 (C), 120.2 (C), 80.1 (C), 77.3 (CH_2), 76.6 (CH_2), 73.7 (CH_2), 71.6 (CH_2), 71.5 (2 × CH_2), 71.4 (CH_2), 71.3 (CH_2), 71.2 (CH_2), 70.7 (CH_2), 68.4 (CH_2), 68.2 (CH_2), 63.4 (CH), 61.6 (CH), 58.0 (CH_2), 57.0 (CH_2), 51.6 (C), 50.7 (2 × CH_2, determined indirectly by HSQC), 41.1 (CH_2), 40.4 (CH_2), 37.8 (CH_2), 36.7 (CH_2), 29.8 (CH_2), 29.5 (CH_2), 28.2 (2 × CH_3), 26.8 (CH_2). IR (NaCl), cm^{-1}: 2900 (br), 1643 (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.

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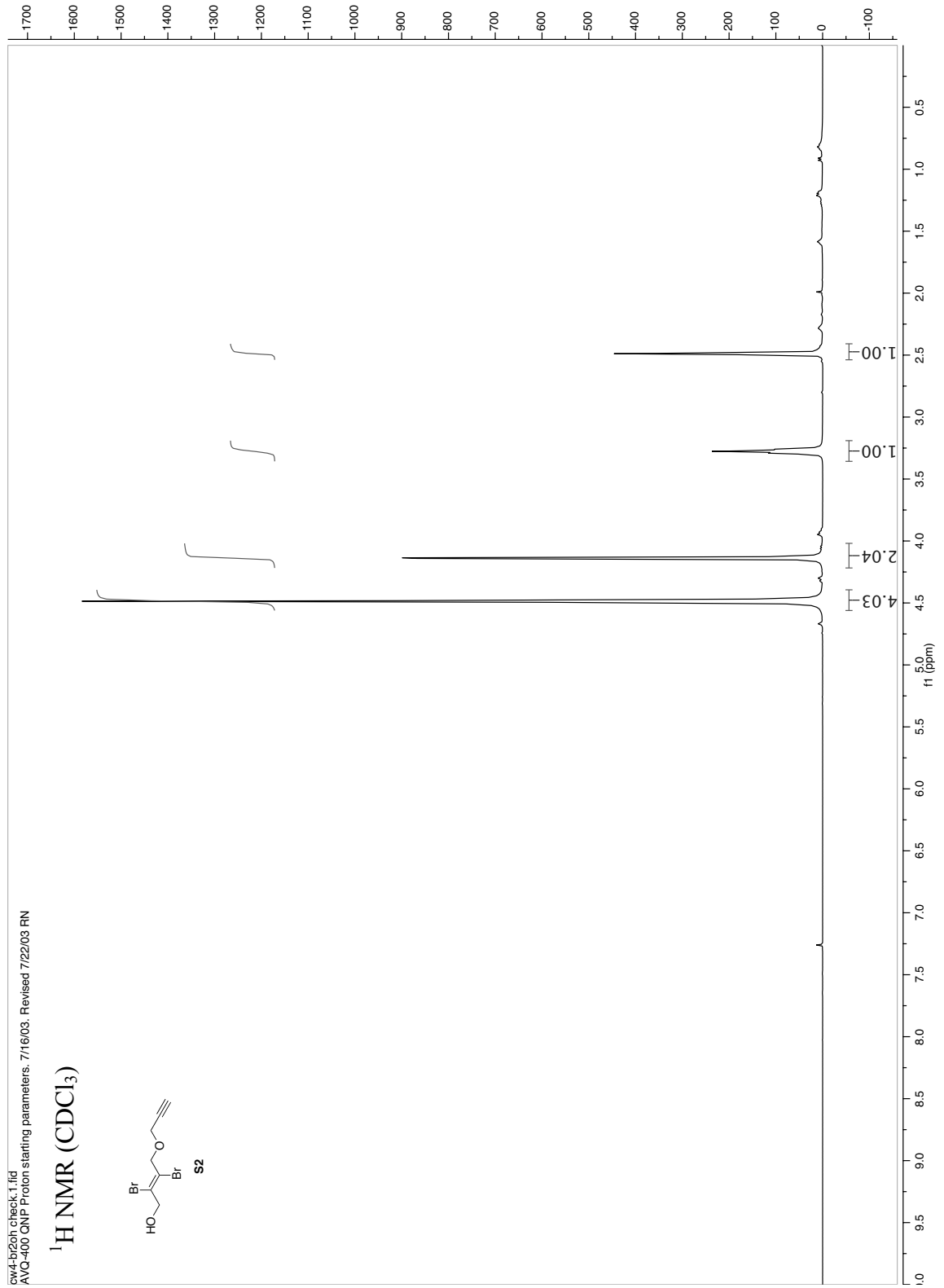
Supplementary Data.



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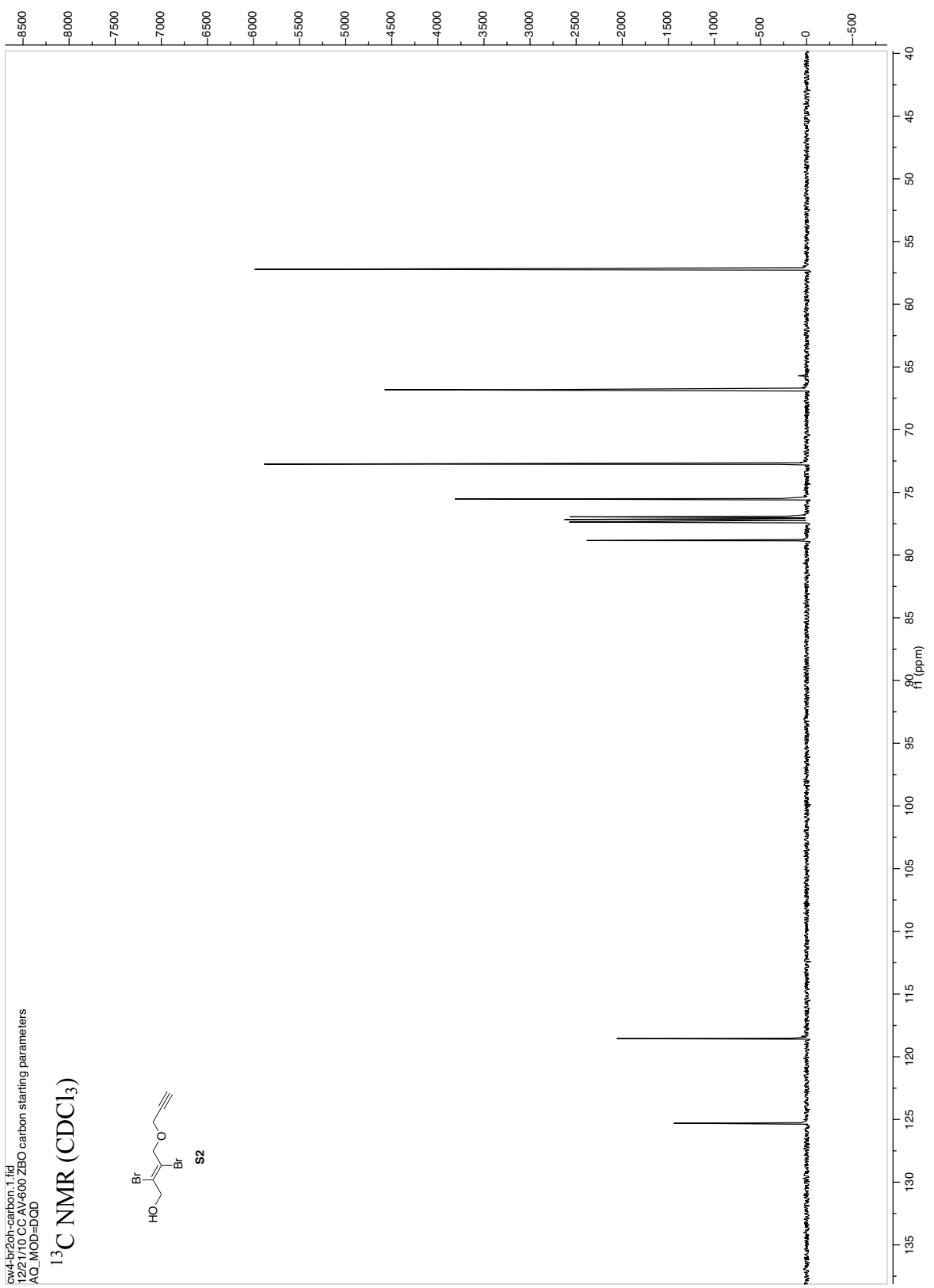

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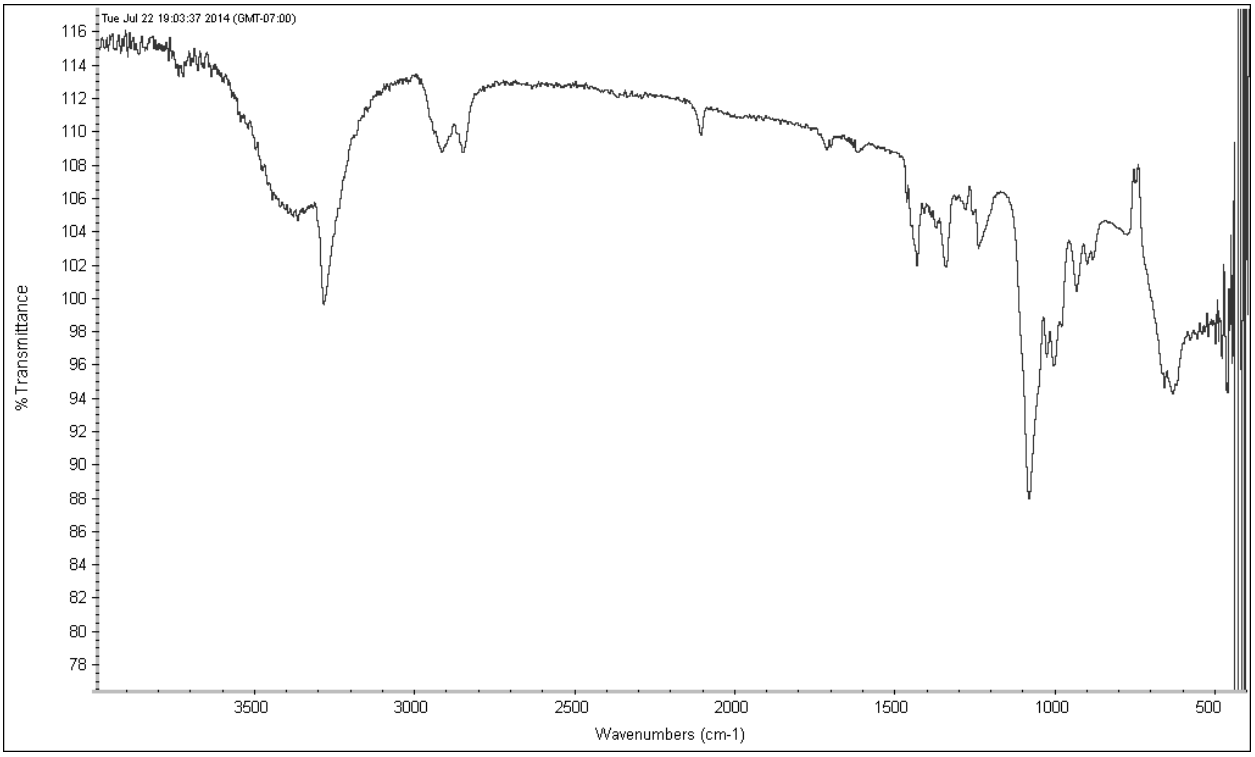

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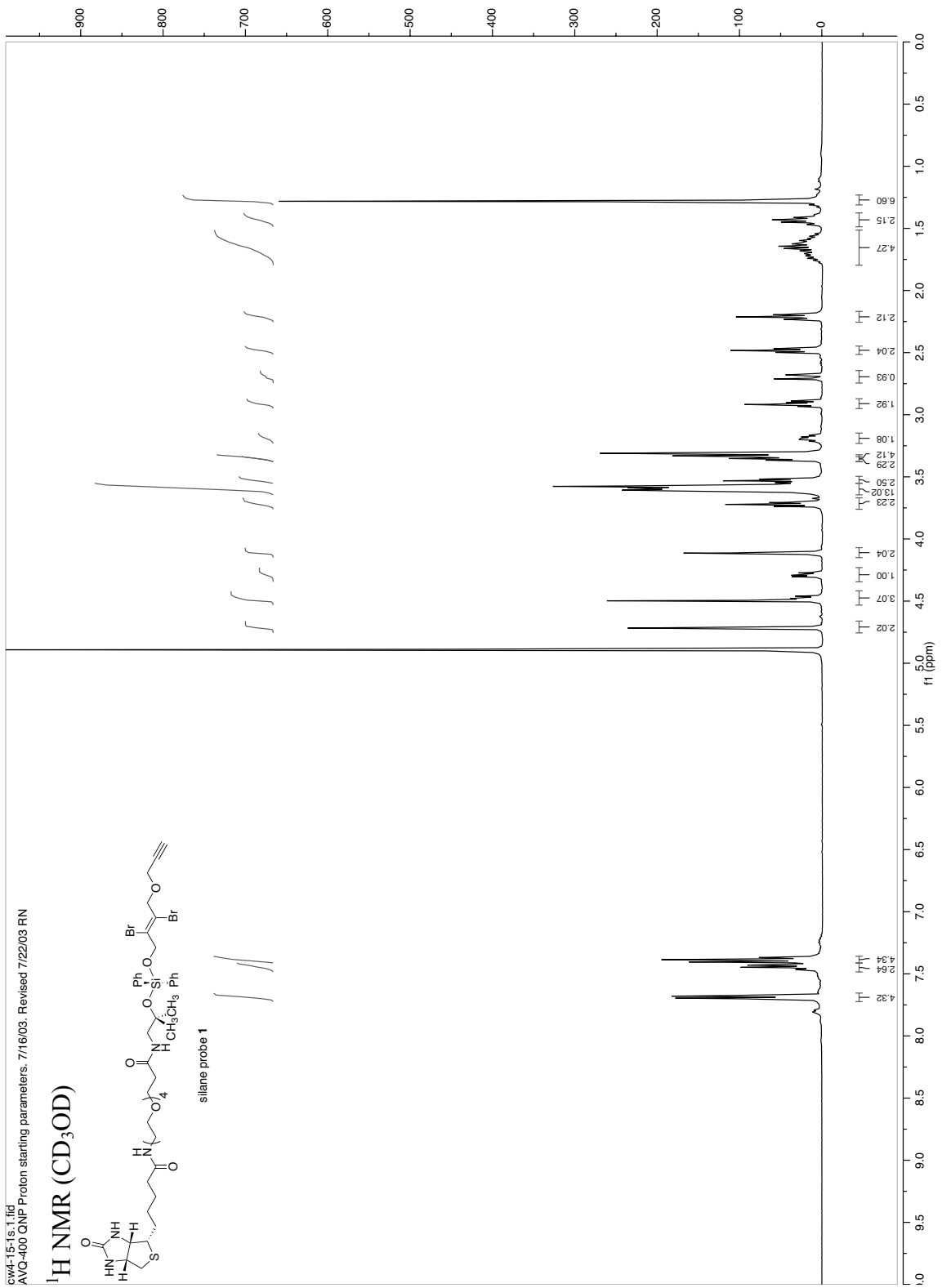

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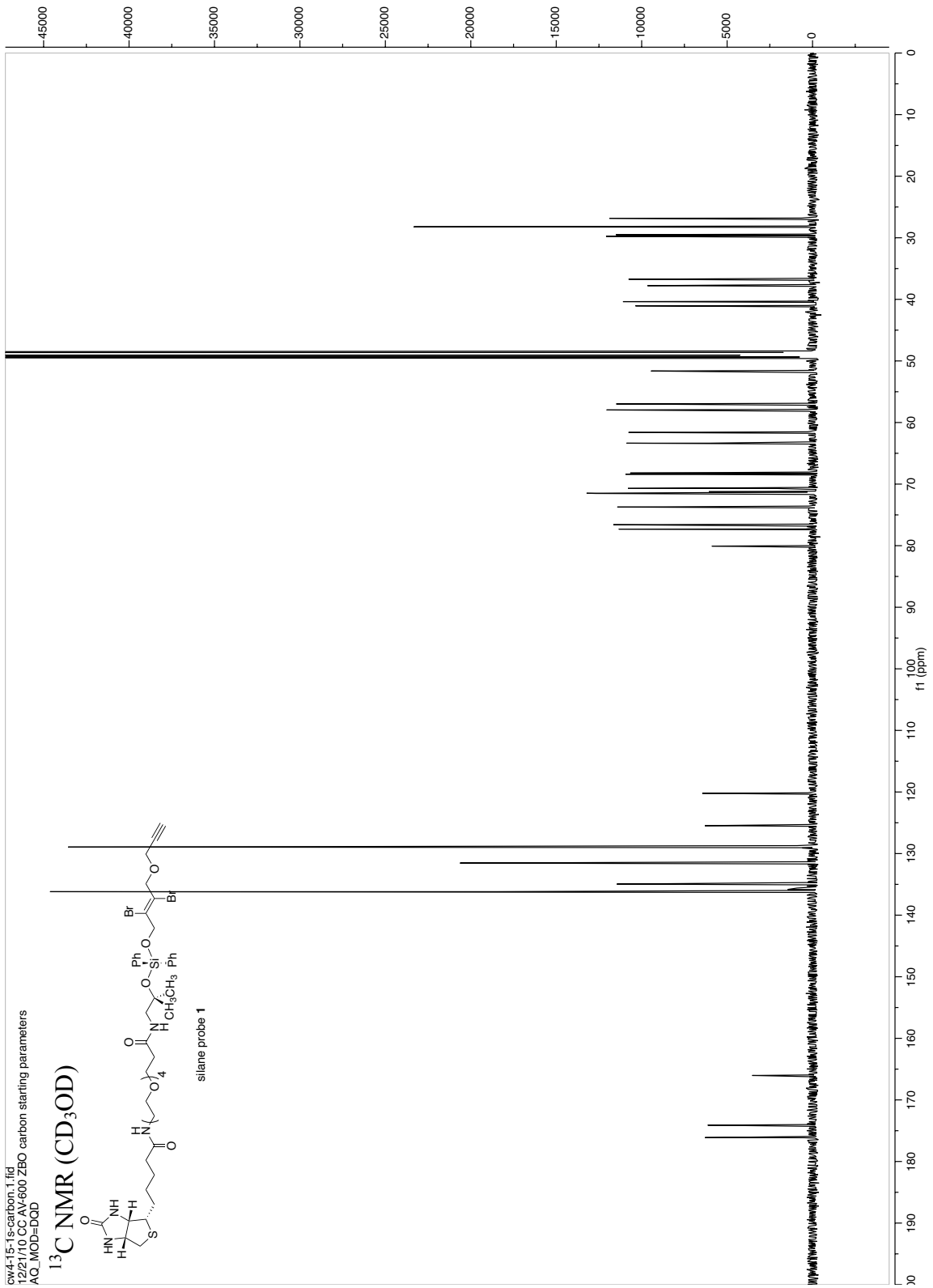

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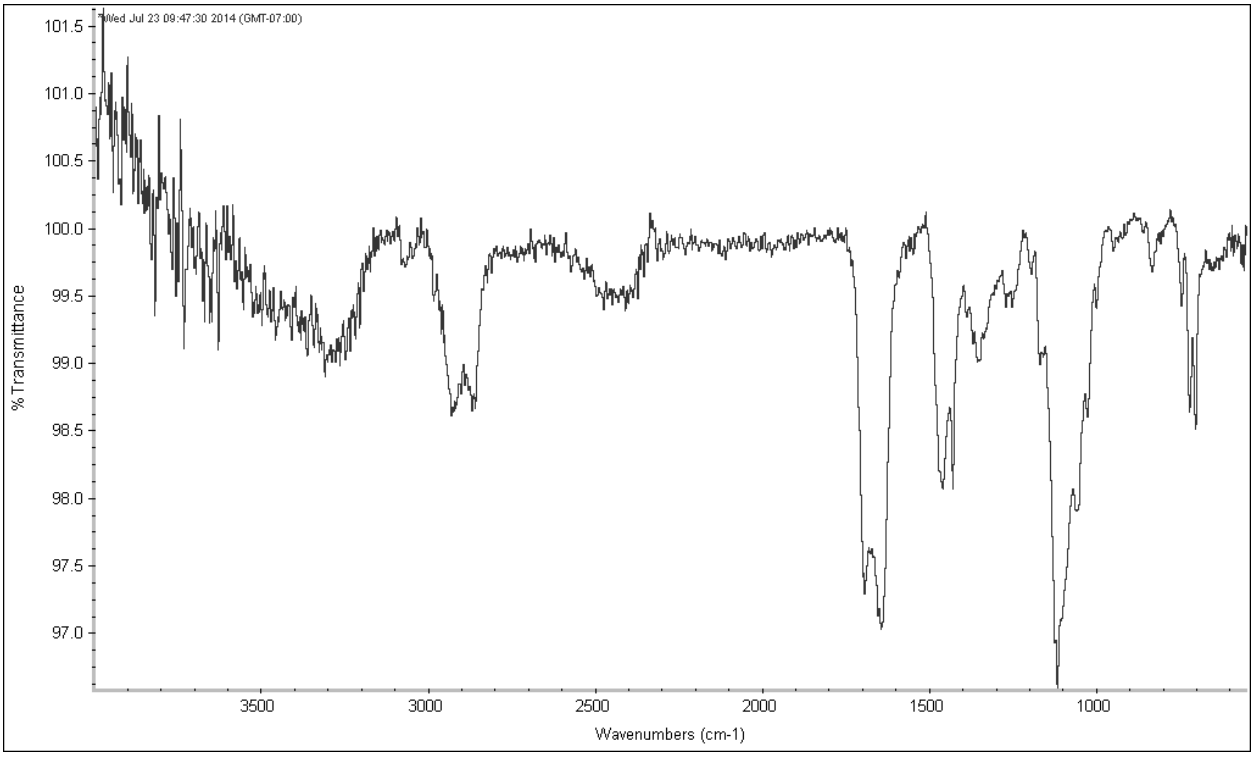

Woo, C. M., et al. "Isotope Targeted Glycoproteomics (IsoTaG): A Mass Independent Platform for Intact N- and O-Glycopeptide Discovery" *Nature Methods*, 2014.

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