Electronic Supplementary Material (ESI) for Molecular BioSystems.

SUPPORTING INFORMATION

Improved cyclopropene reporters for probing protein glycosylation

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Materials and Methods

Metabolic labeling studies with cultured cells

Jurkat cells were plated at a density of ~500,000 cells/mL in RPMI media (Corning) supplemented with 10% fetal bovine serum (FBS, Life Technologies), penicillin (100 U/mL), and streptomycin (100 μg/mL). HEK293 and 4T1 cells were plated at ~500,000 cells/well in 2 mL DMEM media (Corning) supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 μg/mL). Cells were incubated with **Ac_4ManNCyc**, **Ac_4ManCCp**, **Ac_4GalCCp**, or **Ac_4GlcCCp** (0-50 μM) for 24 h in a 5% CO_2, water-saturated incubator at 37 °C. The cells were rinsed with PBS containing 1% bovine serum albumin (FACS buffer, 3 x 200 μL), then reacted with **Tz-biotin** (10 μM, 30 min, 37 °C). The cells were subsequently pelleted (1500 rpm), washed with FACS buffer (3 x 200 μL), and stained with streptavidin-APC (eBioscence, 1:500 dilution in FACS buffer) for 20 min on ice. The cells were pelleted and washed with additional FACS buffer (3 x 200 μL), then analyzed by flow cytometry on an LSR-II flow cytometer (BD Biosciences). For each sample, data were acquired for 10,000 live cells. Cells were analyzed in triplicate, and three replicate experiments were performed for each study. Cellular fluorescence data were analyzed using FloJo software (Tree Star, Inc.).

Western blot analyses

Cells were cultured as above and incubated with azido sugars **Ac_4ManNAz**, **Ac_4GalNAz**, or **Ac_4GlcNAz** (75 μM) or cyclopropene sugars **Ac_4ManCCp**, **Ac_4GalCCp**, or **Ac_4GlcCCp** (75 μM) for 36 h in a 5% CO_2, water-saturated incubator at 37 °C. The cells were pelleted (1500 rpm), rinsed with PBS (3 x 0.5 mL), and then lysed with 100 μL lysis buffer (1% Igepal™ CA-630, 150 mM NaCl, 50 mM triethanolamine, pH 7.4) on ice for 30 min. The lysates were pelleted (13,000 rpm for 10 min at 4 °C) and protein concentrations were measured using a BCA protein assay kit (Pierce). Lysates (~1 mg/mL, 50 μL) were treated with either freshly prepared "click" chemistry cocktail containing **alkyne-biotin** (100 μM); sodium ascorbate (1 mM); tris[(1-benzyl-1-H-1,2,3-triazol-4-yl)methyl]amine (TBTA) (100 μM); $CuSO_4 \bullet 5H_2O$ (1 mM)] or **Tz-biotin** (100 μM, 1 h, 37 °C). To precipitate the labeled proteins, ice-cold methanol (1 mL) was added and the samples were stored at -80 °C overnight. Protein precipitates were pelleted via centrifugation (13,000 rpm for 10 min at 4 °C), aspirated and dried for 1 h at rt. The protein isolates were then re-suspended in 10 μL buffer (4% SDS, 150 mM NaCl, 50 mM triethanolamine, pH 7.4), then treated with SDS-PAGE loading buffer (10 μL of a 2X stock containing 20% glycerol, 0.2% bromophenol blue, 1.4% β-mercaptoethanol). The samples were heated at 90 °C for 5–10 min, separated by gel electrophoresis using 12% polyacrylamide gels, and then electroblotted to nitrocellulose membranes (0.2 μm; Bio-Rad). Transfer efficiency was analyzed with Ponceau S staining. The membranes were rinsed with water and incubated with blocking buffer (7% bovine serum albumin in PBS containing 1% Tween® 20, PBS-T) for 1 h at rt, followed by IRDye® 800CW streptavadin (LI-COR Biosciences; 1:10,000 dilution in blocking buffer) for at least 1 h. The membranes were subsequently washed with PBS-T (5 x 10 min) and imaged using an Odyssey infared imaging system (Li-Cor, Odyssey version 3.0).

Microscopy

4T1Luc2 cells were grown on glass cover slips submerged in 0.5 mL DMEM media (Corning) supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 μg/mL) (in 24-well culture dishes). The media also contained **Ac_4ManCCp** (25 μM), **Ac_4GalCCp** (25 μM), **Ac_4GlcCCp** (25 μM), or no sugar. After 36 h, the cells were washed with FACS buffer (3 x 0.25 mL). Cells were then treated with **Tz-biotin** (25 μM) in media for 15 min at 37 °C. The cells were washed with FACS buffer (3 x 0.25 mL) and fixed with 4% paraformaldehyde in PBS for 15 min at rt. After washing with PBS (3 x 0.25 mL), the cells were blocked for 1 h at rt with PBS + 5% BSA (0.5 mL). The cells were treated with streptavidin-AlexaFluor594 (Jackson Labs; 1:1000 in FACS buffer) for 30 min at rt, then washed with FACS buffer (3 x 0.25mL). The cover slips were mounted on glass slides with Vectashield® mounting media (Vector Laboratories) for imaging. Images were acquired on a Nikon Eclipse Ti inverted microscope with NIS-Elements Microscope Imaging Software and analyzed with ImageJ.

For dual labeling experiments, 4T1Luc2 cells were grown on glass cover slips submerged in 0.5 DMEM media (Corning) supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 μg/mL) (in 24-well culture dishes). The media also contained **Ac_4ManCCp** (25 μM), **Ac_4GalNAz** (25 μM), both sugars (25 μM each), or no sugar. After 36 h, the cells were washed with PBS + 1% BSA (3 x 0.25 mL). Cells were then treated with **Tz-Biotin** (25 μM), **DBCO-FLAG** (100 μM), both reagents, or no reagent in media for 1 h at 37 °C. The cells were then washed with FACS buffer (3 x 0.25 mL) and blocked for 1 h at rt with PBS + 5% BSA (0.5 mL). The cells were treated with streptavidin-AlexaFluor594 (Jackson Labs; 1:1000 in FACS buffer) and FITC-α-FLAG (Sigma-Aldrich; 10 μg/mL in FACS buffer) for 1 h at rt. The cover slips were washed with FACS buffer (3 x 0.25 mL), then fixed with 4% paraformaldehyde in PBS for 15 min at rt. The cover slips were washed with FACS buffer (3 x 0.25 mL) and mounted on glass slides with Vectashield® mounting media (Vector Laboratories) for imaging. Images were acquired on a Nikon Eclipse Ti inverted microscope with NIS-Elements Microscope Imaging Software and analyzed with ImageJ.

General Synthetic Procedures

Compounds **Ac_4ManNCyc**,[1] **Tz-biotin**,[2] **alkyne-biotin**,[3] **S1**,[4] and **S2**[5] were synthesized as previously reported. All other reagents were purchased from commercial sources and used as received without further purification. Reactions were carried out under an inert atmosphere of nitrogen in oven- or flame-dried glassware. Dichloromethane (CH_2Cl_2), tetrahydrofuran (THF), *N,N*-dimethylformamide (DMF), and triethylamine (NEt_3) were degassed with argon and passed through two 4 x 36 inch columns of anhydrous neutral A-2 (8 x 14 mesh; LaRoche Chemicals; activated under a flow of argon at 350 °C for 12 h). The remaining solvents were of analytical grade and purchased from commercial suppliers. Thin-layer chromatography was performed using Silica Gel 60 F_{254} plates. Plates were visualized with UV radiation or staining with 10% sulfuric acid in ethanol or $KMnO_4$. Flash column chromatography was performed with SiliaFlash® F60 40-63 μM (230-400 mesh) silica gel from Silicycle. ^1H and ^{13}C NMR spectra were recorded on CRYO-500 (500 MHz ^1H, 125.7 MHz ^{13}C) or DRX-400 (400 MHz ^1H) spectrometers. All spectra were collected at 298 K. Chemical shifts are reported in ppm values relative to residual non-deuterated NMR solvent and coupling constants (*J*) are reported in Hertz (Hz). High-resolution mass spectrometry was performed by the University of California, Irvine Mass Spectrometry Center. HPLC runs were conducted on a Varian ProStar equipped with 325 Dual Wavelength UV-Vis Detector. Semi-preparative runs were performed using an Agilent Prep-C18 Scalar column (9.4 x 150 mm, 5 μm) with a 5 mL/min flow rate. The elution gradients for the relevant separations are specified below.

Synthetic Procedures

3-Hydroxymethyl-2-methyl-trimethylsilylcyclopropene (1). To a stirring mixture of TMS-propyne (4.0 mL, 27 mmol) and rhodium acetate dimer (15 mg, 0.034 mmol) was slowly added ethyl diazoacetate (1.0 mL, 8.6 mmol) dissolved in 15 mL CH_2Cl_2 at a rate of 0.5-1.0 mL/min. Once the addition was complete, the reaction was stirred for an additional 30 min. The mixture was then partially concentrated under reduced pressure and eluted through a plug of silica gel (eluting with CH_2Cl_2) to remove the rhodium catalyst. The eluant was gently concentrated under reduced pressure and added dropwise (over 1 min) to a solution of DIBAL-H (14.0 mL of a 25% wt/wt solution in hexanes, 17.2 mmol) in 15 mL Et_2O at 4 °C. The reaction was stirred until the cyclopropene ester was consumed (30–60 min). Saturated Rochelle's salt was then added and the mixture was stirred until a white gel formed. The organic layer was isolated and the aqueous layer was further extracted with Et_2O (2 x 20 mL). The combined organic layers were dried with $MgSO_4$, filtered, and concentrated *in vacuo* to afford the crude product as a faint yellow oil. The product was purified by flash column chromatography (eluting with 30% Et_2O in hexanes) to yield **1** as a faint yellow oil (714 mg, 53% over two steps): ^1H NMR (500 MHz, $CDCl_3$) δ 3.48 (d, *J* = 4.6 Hz, 2H), 2.21 (s, 3H), 1.56 (t, *J* = 4.6 Hz, 1H), 0.17 (s, 9H). Data are in agreement with previously reported spectrum.[2]

(2-Methylcycloprop-2-enyl)methyl (4-nitrophenyl) carbonate (3). Cyclopropene **1** (392 mg, 2.51 mmol) was dissolved in 10 mL THF. Anhydrous 18-crown-6 (729 mg, 2.76 mmol) and anhydrous cesium fluoride (400 mg, 2.63 mmol) were added, and the solution was stirred until **1** was fully consumed (2.5 h). The reaction was then diluted with CH_2Cl_2 (25 mL). Pyridine (1.2 mL, 15 mmol) was added, followed by 4-nitrophenyl chloroformate (1.01 g, 5.01 mmol), and the

reaction was stirred overnight. The reaction mixture was then concentrated *in vacuo*, dissolved in Et_2O (30 mL), rinsed with concentrated $NaHCO_3$ (3 x 30 mL), and dried with $MgSO_4$. The crude product was filtered, then gently concentrated and purified by flash column chromatography (eluting with 5% Et_2O in petroleum ether) to afford carbonate **3** as a clear oil (459 mg, 73%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (m, 2H), 7.38 (m, 2H), 6.61 (s, 1H), 4.20 (dd, *J* = 10.9, 5.2 Hz, 1H), 4.13 (dd, *J* = 10.9, 5.5 Hz, 1Hz), 2.17 (d, *J* = 1.2 Hz, 3H), 1.78 (m, 1H); ^{13}C NMR 125 MHz, $CDCl_3$) δ 155.8, 152.7, 145.3, 125.3, 121.9, 120.2, 101.7, 77.5, 16.7, 11.7; LRMS (ESI) calcd for $C_{12}H_{11}O_5N$ $[M+Na]^+$ 272.0535, found 272.0460.

General procedure for the synthesis of carbamate sugars

The hydrochloride salt of mannosamine (**4**), galactosamine (**5**), or glucosamine (**6**) (20.1 mg, 0.0932 mmol) was added to a solution of DMF (2 mL) and *N,N*-diisopropylethylamine (65 μL, 0.37 mmol) and heated to 60 °C. The solution was cooled to ambient temperature and treated with a solution of carbonate **3** (91.0 mg, 0.365 mmol) dissolved in 0.5 mL DMF. The solution quickly turned yellow, indicating the release of 4-nitrophenolate. The reaction was stirred for 4-12 h. The solvent was removed *in vacuo* onto silica gel and was run through a plug of silica gel (flushed with 5% and eluted with 10% MeOH in CH_2Cl_2). The isolated sugar was dissolved in 1 mL pyridine and treated with acetic anhydride (0.5 mL, 4 mmol). The reactions were stirred overnight and concentrated *in vacuo*. The crude acetylated sugar was diluted with CH_2Cl_2 and rinsed with $NaHSO_4$ (3 x 10 mL) and washed with brine (10 mL). The product was purified by flash column chromagraphy (eluting with 3:2 hexanes:ethyl acetate) or HPLC (eluting with 30–70% CH_3CN in H_2O over 20 min). The desired fractions were combined and dried to yield **Ac$_4$ManCCp**, **Ac$_4$GalCCp**, or **Ac$_4$GlcCCp**.

Ac$_4$ManCCp. Mixture of anomers isolated (16.1 mg, 0.0352 mmol, 38%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$, 2:1 α:β) δ (α anomer) 6.58 (d, *J* = 4.8 Hz, 1H), 6.09 (s, 1H), 5.31 (dd, *J* = 10.2, 4.2 Hz, 1H), 5.20 (app t, *J* = 9.8 Hz, 1H), 5.02 (m, 1H), 4.34 (dd, *J* = 8.6, 2.9 Hz, 1H), 4.25 (dd, *J* = 12.2, 4.2 Hz, 1H), 4.02 (m, 1H), 3.95 (app t, *J* = 5.2 Hz, 2H), 2.17 (s, 3H), 2.14 (s, 3H), 2.10 (s 3H), 2.05 (s, 3H), 2.02 (s, 3H), 1.65 (m, 1H); (β anomer) 6.58 (d, *J* = 4.8 Hz, 1H), 5.85 (s, 1H), 5.16 (app t, *J* = 9.7 Hz, 1H), 5.09 (d, *J* = 9.2 Hz, 1H), 5.02 (m, 1H), 4.47 (m, 1H), 4.25 (dd, *J* = 12.2, 4.2 Hz, 1H), 4.13-4.04 (m, 2H), 3.78 (ddd, *J* = 9.5, 4.8, 2.6 Hz, 1H), 2.14 (s, 3H), 2.12 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.65 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$, 298 K) δ 170.7, 170.6, 170.2, 170.2, 169.7, 169.7, 168.5, 168.2, 156.9, 156.3, 120.7, 120.5, 102.3, 102.2, 102.1, 92.0, 90.8, 73.4, 73.2, 73.2, 73.0, 71.6, 70.2, 69.2, 65.4, 65.3, 62.0, 61.9, 51.3, 51.1, 21.0, 20.9, 20.8, 20.8, 20.8, 20.8, 20.7, 20.7, 17.1, 17.1, 11.7, 11.7; ^{13}C NMR (125 MHz, $CDCl_3$, 318 K) δ 170.4, 170.4, 169.9, 169.9, 169.4, 169.4, 168.3, 168.0, 156.2, 120.5, 120.5, 102.2, 102.1, 92.1, 90.9, 73.5, 73.2, 72.9, 71.5, 70.3, 69.2, 65.6, 65.6, 62.1, 62.0, 51.3, 51.3, 20.7, 20.7, 20.6, 20.6, 20.6, 20.6, 20.5, 20.5, 17.2, 17.1, 11.5, 11.5; HRMS (ESI) calcd for $C_{20}H_{27}O_{11}N$ $[M+Na]^+$ 480.1482, found 480.1460.

Ac$_4$GalCCp. Mixture of anomers isolated (14.3 mg, 0.0313 mmol, 17%) as a white solid: ^{1}H NMR (500 MHz, DMSO-*d*$_6$, 3:1 α:β) δ (α anomer) 7.53 (m, 1H), 6.86 (d, *J* = 9.0 Hz, 1H), 6.08 (dd, *J* = 5.1, 3.6 Hz, 1H), 5.41 (d, *J* = 2.3 Hz, 1H), 5.09 (dd, *J* = 11.7, 3.0 Hz, 1H), 4.34 (app t, *J* = 6.2 Hz, 1H), 4.15-3.97 (m, 3H), 3.92-3.74 (m, 2H), 2.16 (s, 3H), 2.14 (s, 3H), 2.11 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H), 1.53 (m, 1H); (β anomer) 7.23 (m, 1H), 6.86 (d, *J* = 9.0 Hz, 1H), 5.66

(d, J = 8.7 Hz, 1H), 5.29 (d, J = 3.0 Hz, 1H), 5.09 (dd, J = 11.7, 3.0 Hz, 1H), 4.17 (app t, J = 6.1 Hz, 1H), 4.15-3.97 (m, 3H), 3.92-3.74 (m, 2H), 2.14 (s, 3H), 2.11 (s, 3H), 2.07 (s, 3H), 2.01 (s, 3H), 1.94 (s, 3H), 1.53 (m, 1H);^{13}C NMR (125 MHz, DMSO-d_6, α anomer) δ 170.5, 170.4, 170.1, 169.7, 156.9, 120.5, 102.7, 90.7, 71.8, 68.7, 67.9, 67.1, 61.8, 48.7, 21.3, 21.0, 21.0, 20.9, 17.1, 11.8; HRMS (ESI) calcd for $C_{20}H_{27}O_{11}N$ $[M+Na]^+$ 480.1482, found 480.1466.

Ac_4GlcCCp. Single isomer isolated (57.9 mg, 0.127 mmol, 51%) as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6, α anomer only) δ 7.51 (d, J = 8.6 Hz, 1H), 6.86 (s, 1H), 6.00 (s, 1H), 5.17 (app t, J = 10.2 Hz, 1H), 5.01 (app t, J = 9.8, 1H) 4.19 (dd, J = 12.4, 3.8 Hz, 1H), 4.11 (ddd, J = 10.3, 3.5, 2.2 Hz, 1H), 4.01 (dd, J = 12.0, 1.5 Hz, 2H), 3.93-3.75 (m, 2H), 2.18 (s, 3H), 2.11 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H), 1.96 (s, 3H), 1.53 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.5, 170.1, 169.7, 169.7, 156.9, 120.6, 102.6, 90.3, 71.8, 70.9, 69.5, 68.5, 61.8, 52.6, 21.3, 21.0, 20.9, 20.9, 17.1, 11.8; HRMS (ESI) calcd for $C_{20}H_{27}O_{11}N$ $[M+Na]^+$ 480.1482, found 480.1464.

Dibenzocyclooctyne-FLAG peptide conjugate (DBCO-FLAG). The peptide conjugate was prepared as previously described.[5] Briefly, DYKDDDDKC (20 mg, 0.018 mmol) was dissolved in 0.25 mL H_2O and added to a solution of DBCO-maleimide (Click Chemistry Tools; 5.0 mg, 0.012 mmol) in 0.2 mL DMF. The reaction was stirred overnight. The product was isolated via HPLC (eluting with 20–80% MeCN in H_2O over 20 min). The desired fractions were combined and lyophilized to yield **DBCO-FLAG** (7.5 mg, 41%) as a white solid; LRMS (ESI) calcd for $C_{69}H_{86}N_{14}O_{25}S$ $[M+2H]^{2+}$ 772.27, found 772.26.

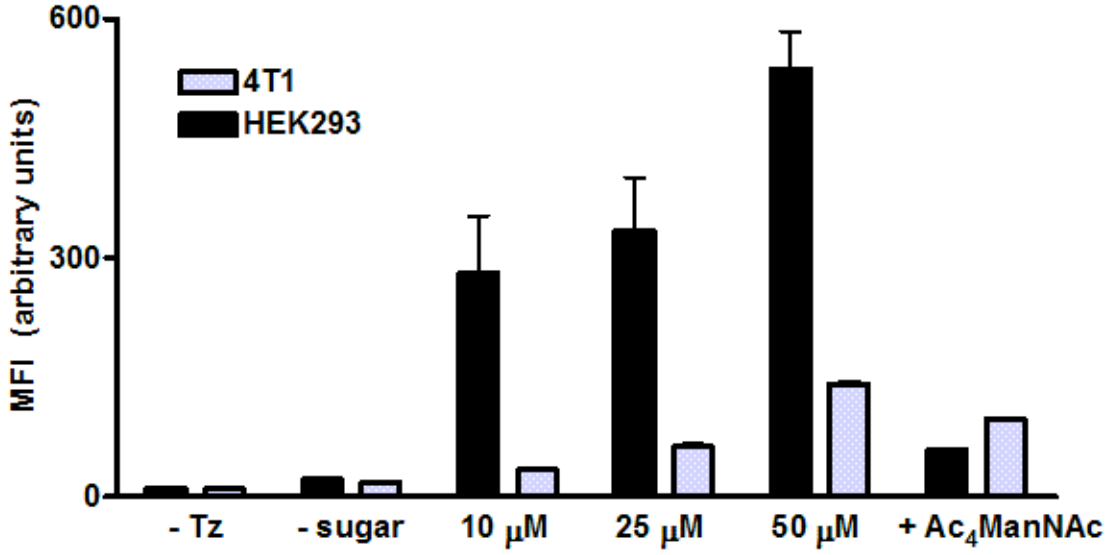


Figure S1. **Ac_4ManCCp** is metabolically incorporated into 4T1 and HEK293 cell surface glycans. Cells were incubated with **Ac_4ManCCp** (0-50 μM), **Ac_4ManCCp** plus a control sugar (+ Ac_4ManNAc, 10 μM), or no sugar (- sugar) for 24 h. After washing, the cells were treated with **Tz-biotin** (10 μM) for 30 min or no secondary reagent (-Tz) at 37 °C, stained with streptavadin-APC, and analyzed by flow cytometry. The mean fluorescence intensity (MFI) values for the cell populations are plotted. Error bars represent the standard deviation of the mean for three experiments.

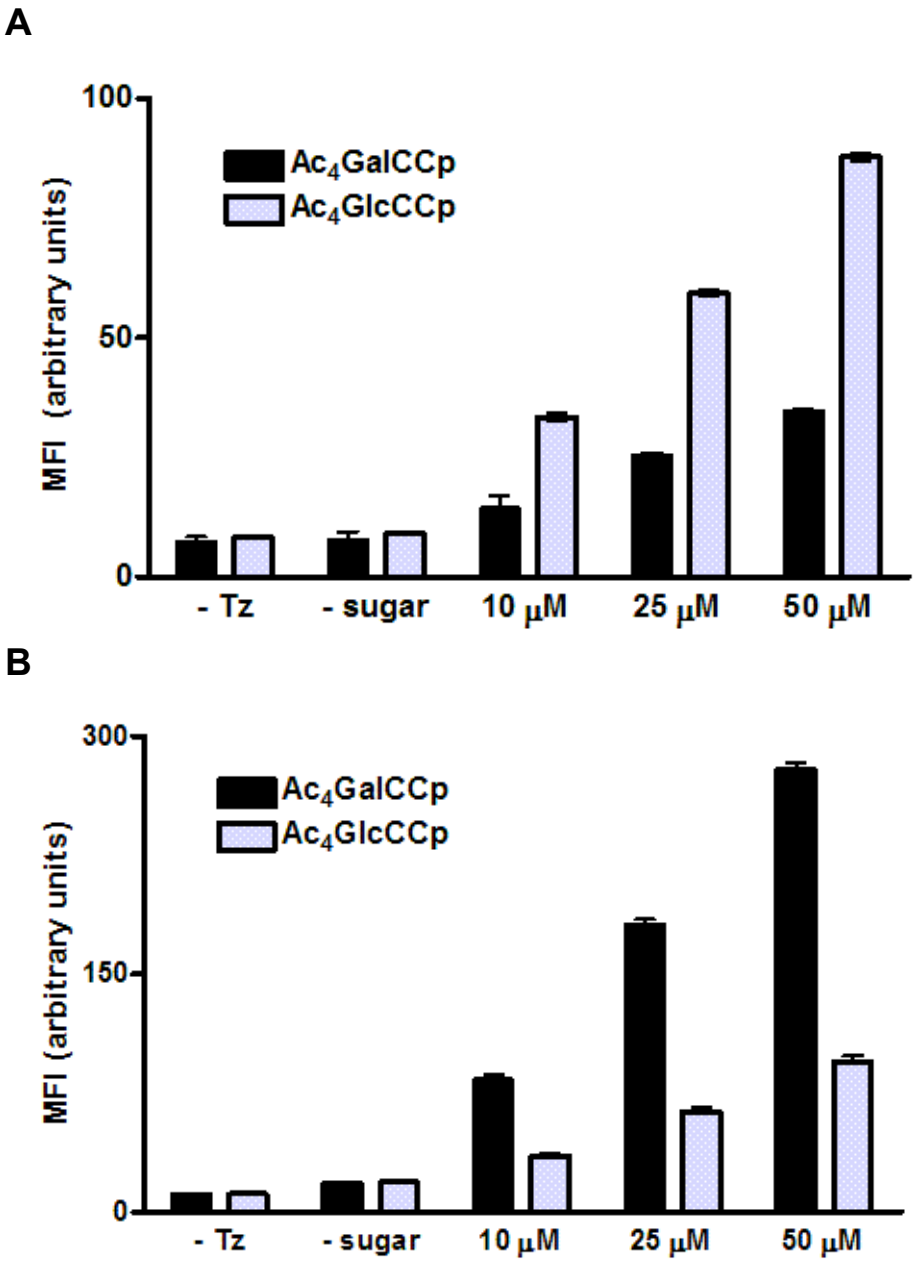


Figure S2. **Ac_4GalCCp** and **Ac_4GlcCCp** are metabolically incorporated into cell surface glycans. Jurkat (A) and HEK293 (B) cells were incubated in the presence of **Ac_4GalCCp** (0-50 μM), **Ac_4GlcCCp** (0-50 μM), or no sugar (-sugar) for 24 h. After washing, the cells were treated with **Tz-biotin** (10 μM) or no reagent (-Tz) for 30 min at 37 °C, stained with streptavadin-APC, and analyzed by flow cytometry. The mean fluorescence intensity (MFI) values for the cell populations are plotted. Error bars represent the standard deviation of the mean for three experiments.

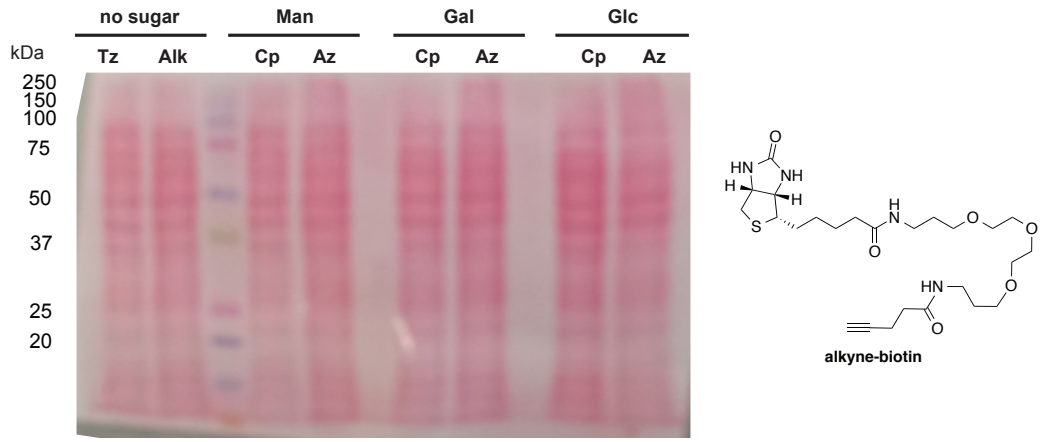


Figure S3. Equivalent protein loading was observed via Ponceau S staining. Jurkat cells were incubated with cyclopropene (Cp) or azido (Az) analogs of ManNAc (Man), GalNAc (Gal), or GlcNAc (Glc) (75 μM) for 36 h, then lysed. Protein isolates were treated with either 100 μM **Tz-biotin** to tag Cp-modified proteins or **alkyne-biotin** (100 μM) to tag Az-modified proteins via CuAAC. The labeled proteins were separated by gel electrophoresis and transferred to nitrocellulose prior to Ponceau S staining.

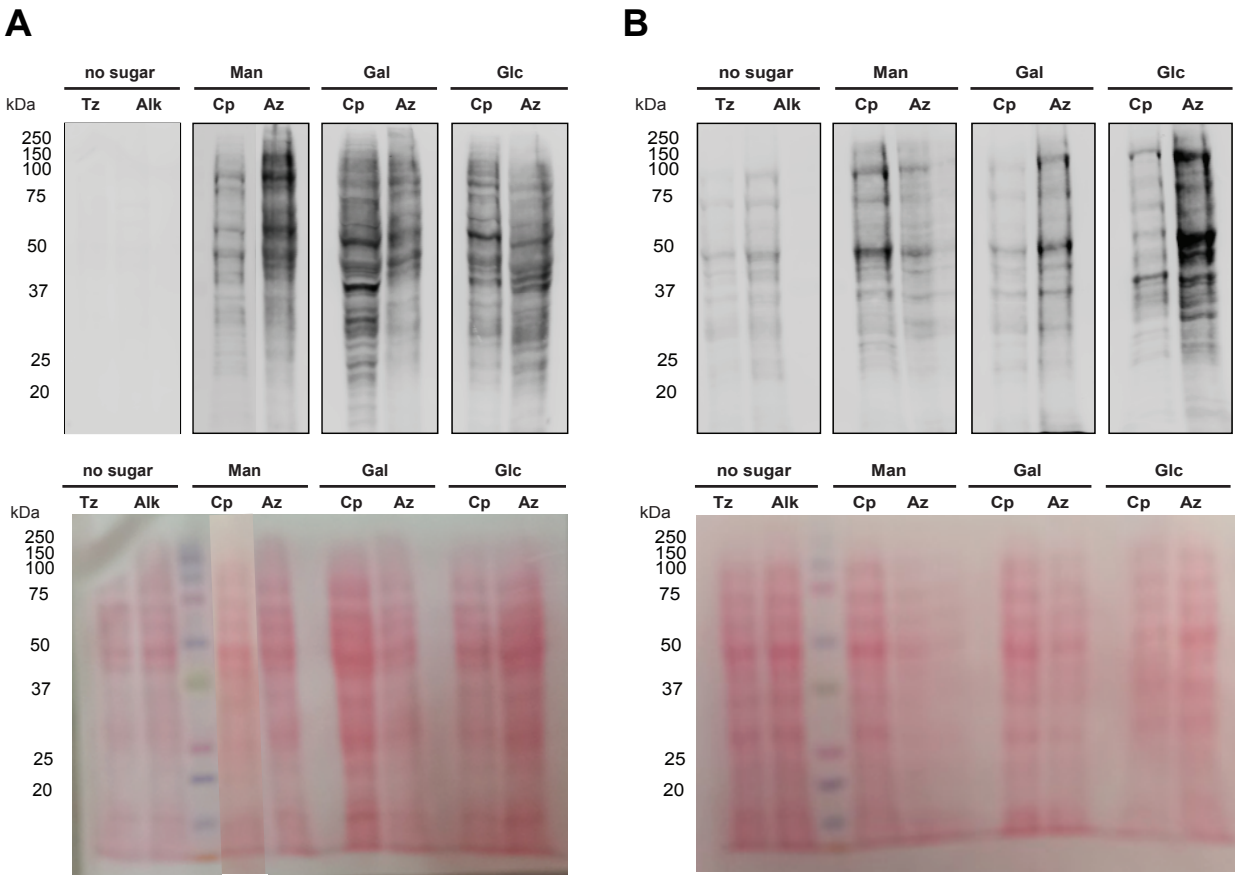


Figure S4. Carbamate-linked cyclopropene sugars label cellular glycoproteins. (A) HEK293 and (B) 4T1 cells were incubated with cyclopropene (Cp) or azido (Az) analogs of ManNAc (Man), GalNAc (Gal), or GlcNAc (Glc) (75 μM) for 36 h, then lysed. Soluble protein isolates were treated with either 100 μM **Tz-biotin** to tag Cp-modified proteins or 100 μM **alkyne-biotin** (structure shown in Figure S3) to tag Az-modified proteins via CuAAC. All samples were separated by gel electrophoresis and analyzed via Western blot. Protein loading was confirmed using Ponceau S stain.

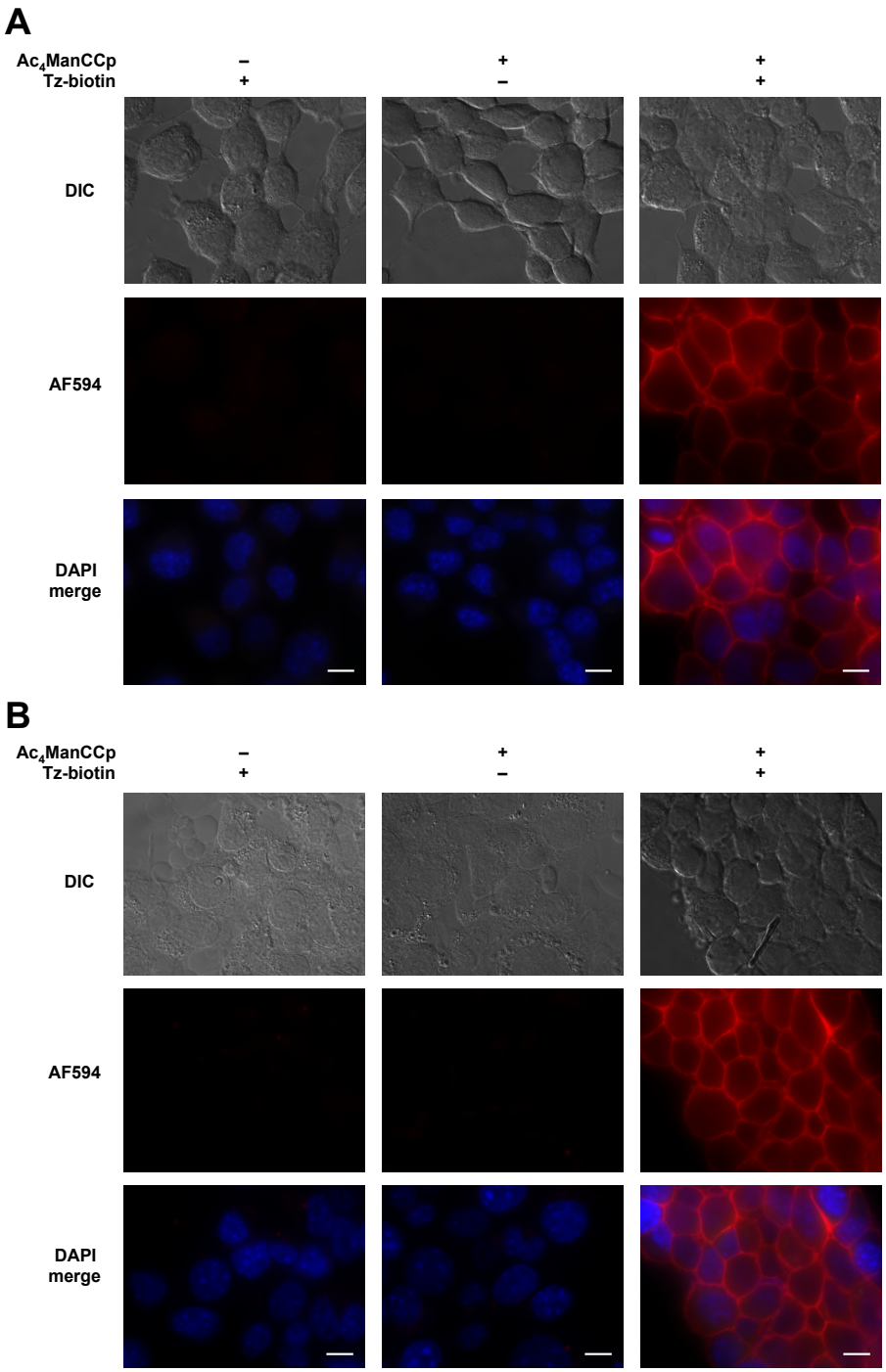


Figure S5. Carbamate cyclopropene sugars can be metabolically introduced and visualized in 4T1 mammalian cells. Cells were incubated in the presence of **Ac_4ManCCp** (25 μM, +) or no sugar (–) for 36 h. After washing, the cells were treated with **Tz-biotin** (25 μM, +) or no reagent (–) for (A) 1 h or (B) 15 min at 37 °C, stained with streptavadin-APC and DAPI, and analyzed by flourescence microscopy. Cell surface fluorescence (red) was only observed in samples treated with both **Ac_4ManCCp** and **biotin**). Representative bright-field (DIC) images AF594 images, and merged images are shown. Scale bar = 10 μm.

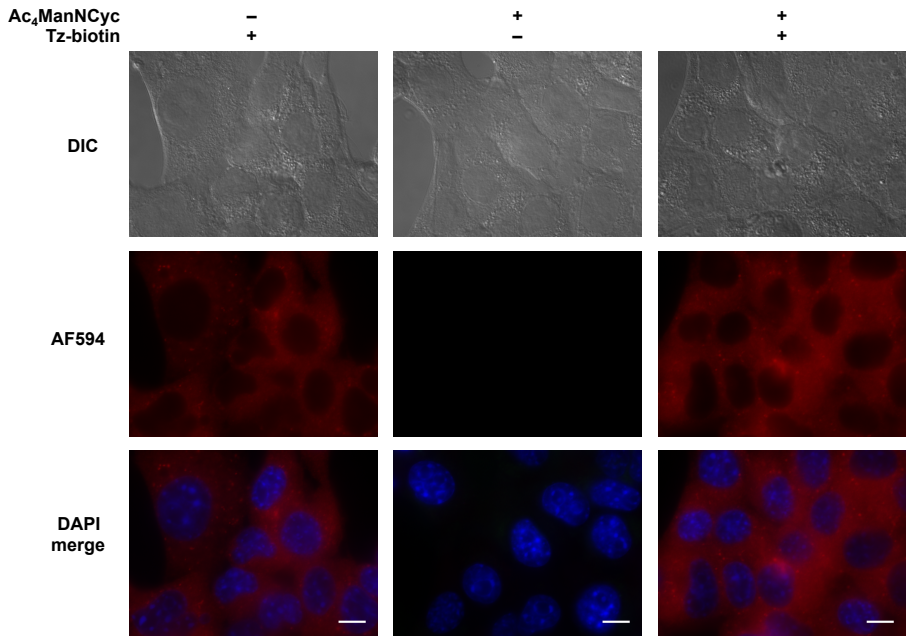


Figure S6. No cell surface labeling observed with **Ac_4ManNCyc**. Cells were incubated with **Ac_4ManNCyc** (25 μM, +) or no sugar (–) for 36 h. After washing, the cells were treated with **Tz-biotin** (25 μM, +) or no reagent (–) for 1 h at 37 °C, stained with streptavadin-APC and DAPI, and analyzed by flourescence microscopy. Representative bright-field (DIC) images, AF594 images, and merged images are shown. Scale bar = 10 μm. (Note: the exposure times used for the AF954 panels in this experiment were ~3X greater than those used to generate the images in Figure S4).

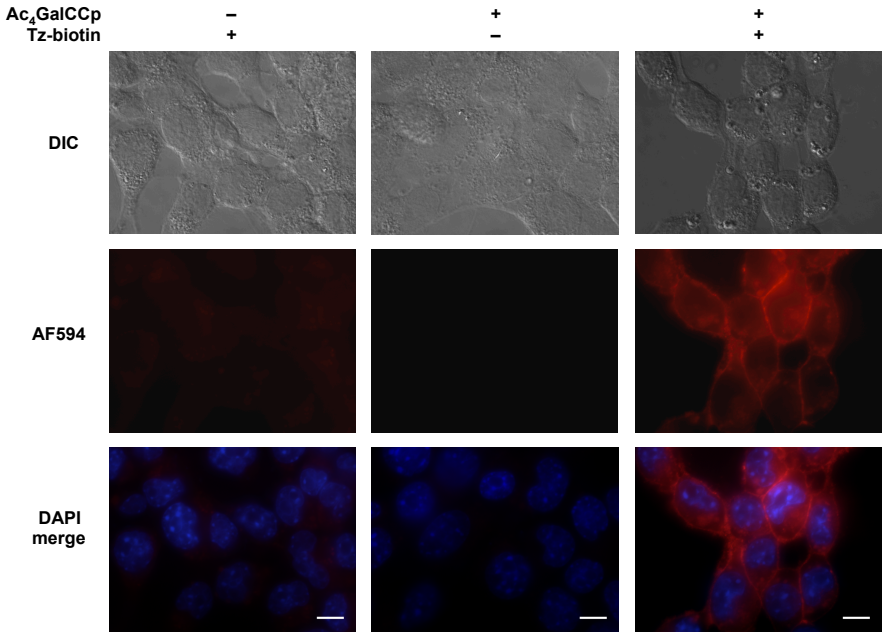


Figure S7. Cyclopropene-GalNAc reporters can be metabolically incorporated and visualized in 4T1 cells. Cells were incubated in the presence of **Ac_4GalCCp** (25 μM, +) or no sugar (–) for 36 h. After washing, the cells were treated with **Tz-biotin** (25 μM, +) or no reagent (–) for 1 h at 37 °C, stained with streptavadin-APC and DAPI, and analyzed by flourescence microscopy. Representative bright-field (DIC) images, AF594 images, and merged images are shown. Scale bar = 10 μm.

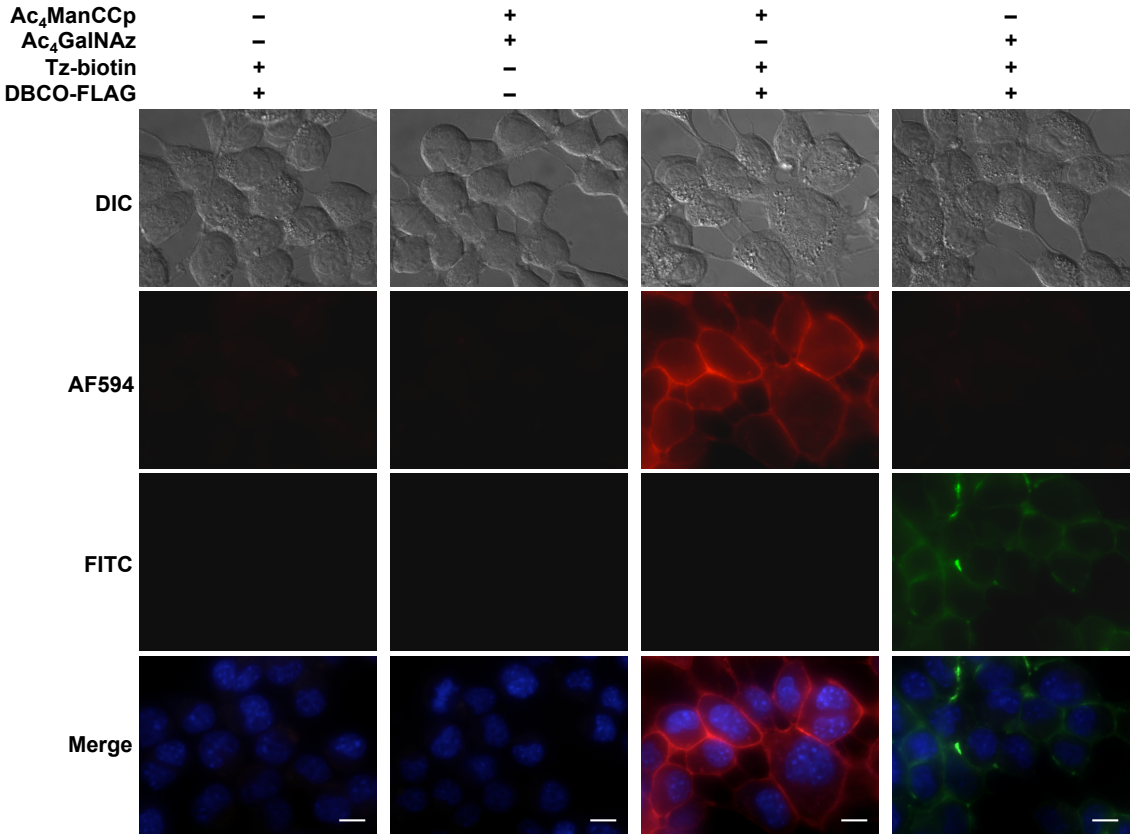
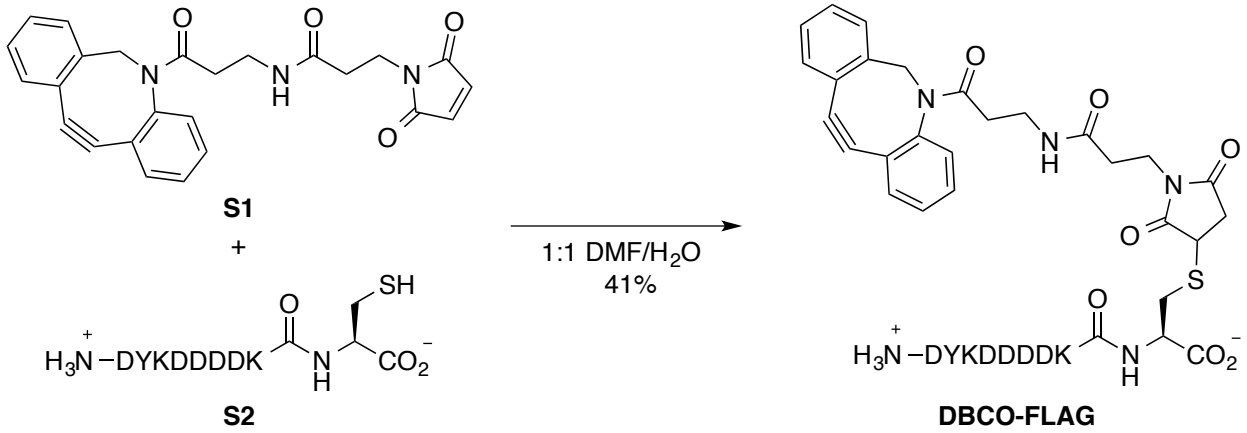


Figure S8. Control images for the dual labeling experiment in Figure 3. 4T1 cells were cultured in the presence of **Ac_4ManCCp** (25 μM, +), **Ac_4GalNAz** (25 μM, +), both sugars (25 μM each), or no sugar (–) for 36 hours. The cells were then treated with **Tz-biotin** (25 μM, +), **DBCO-FLAG** (100 μM, +), both reagents (+) or no reagent (–) for 1 h at 37 °C. Cells were then stained (streptavidin-AF594, FITC-α-FLAG, and DAPI) and imaged by fluorescence microscopy. Representative bright-field (DIC), AF594, FITC, and merged images are shown. Scale bar = 10 μm.

Scheme S1. Synthesis of **DBCO-FLAG**.



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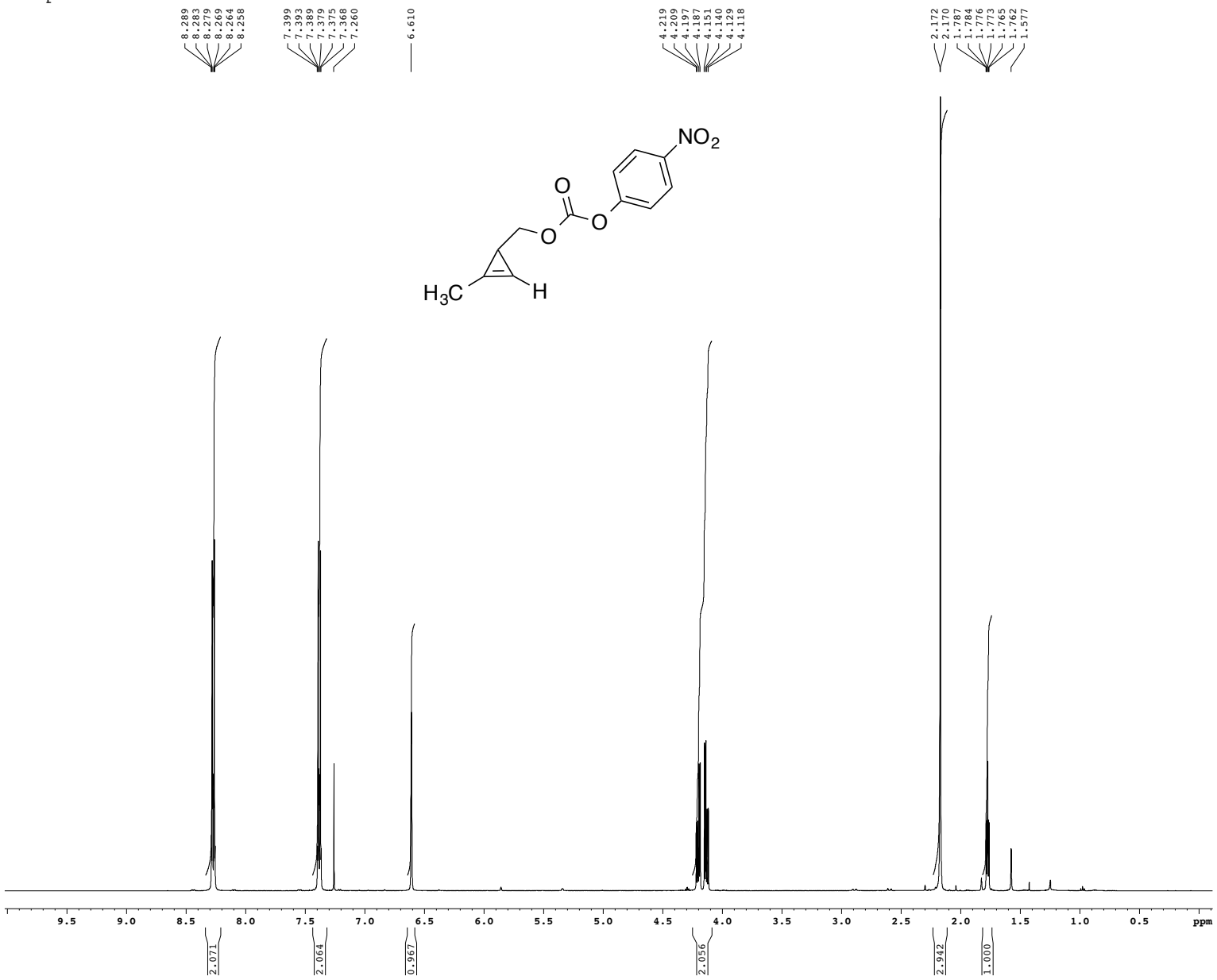
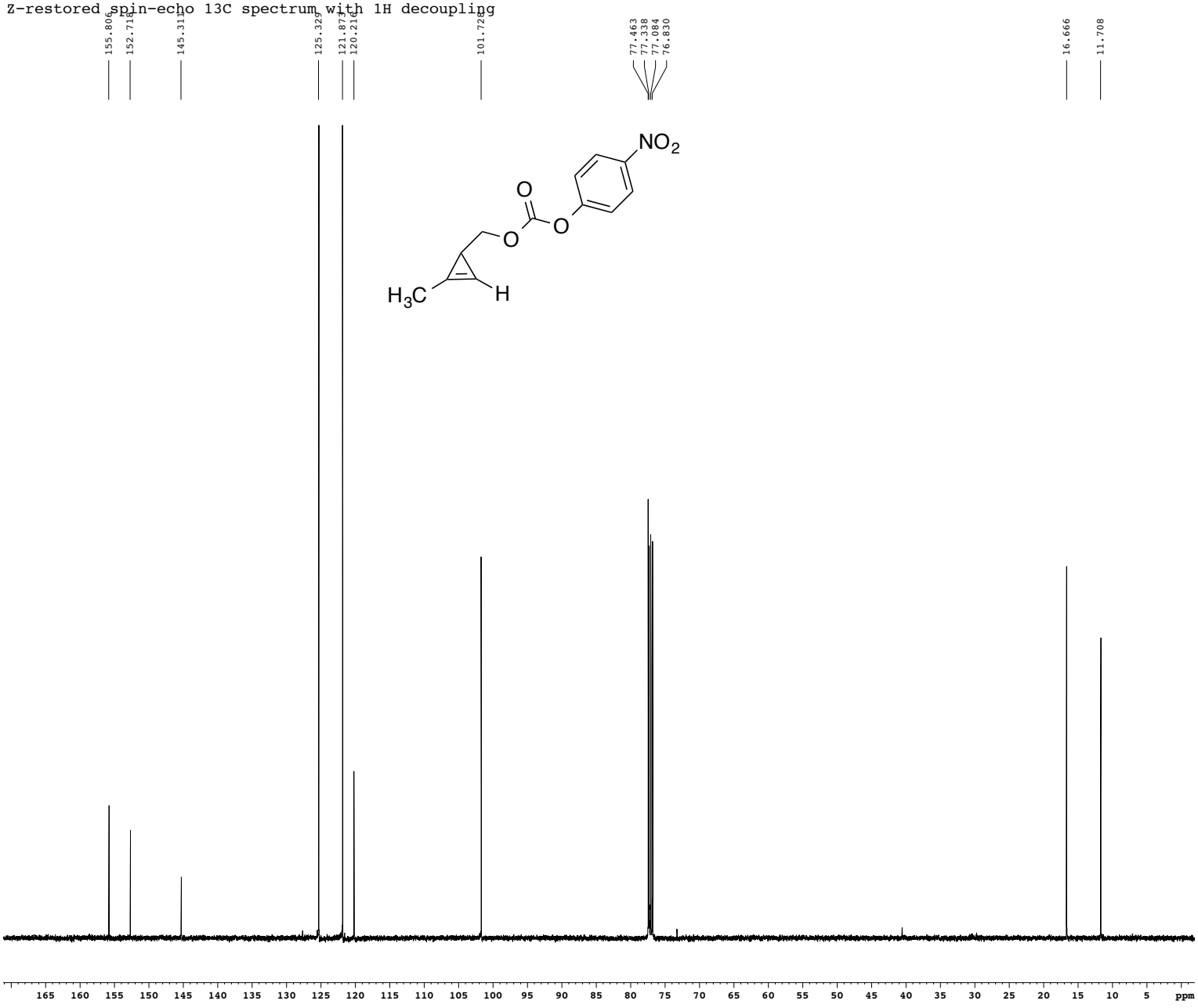
1H spectrum



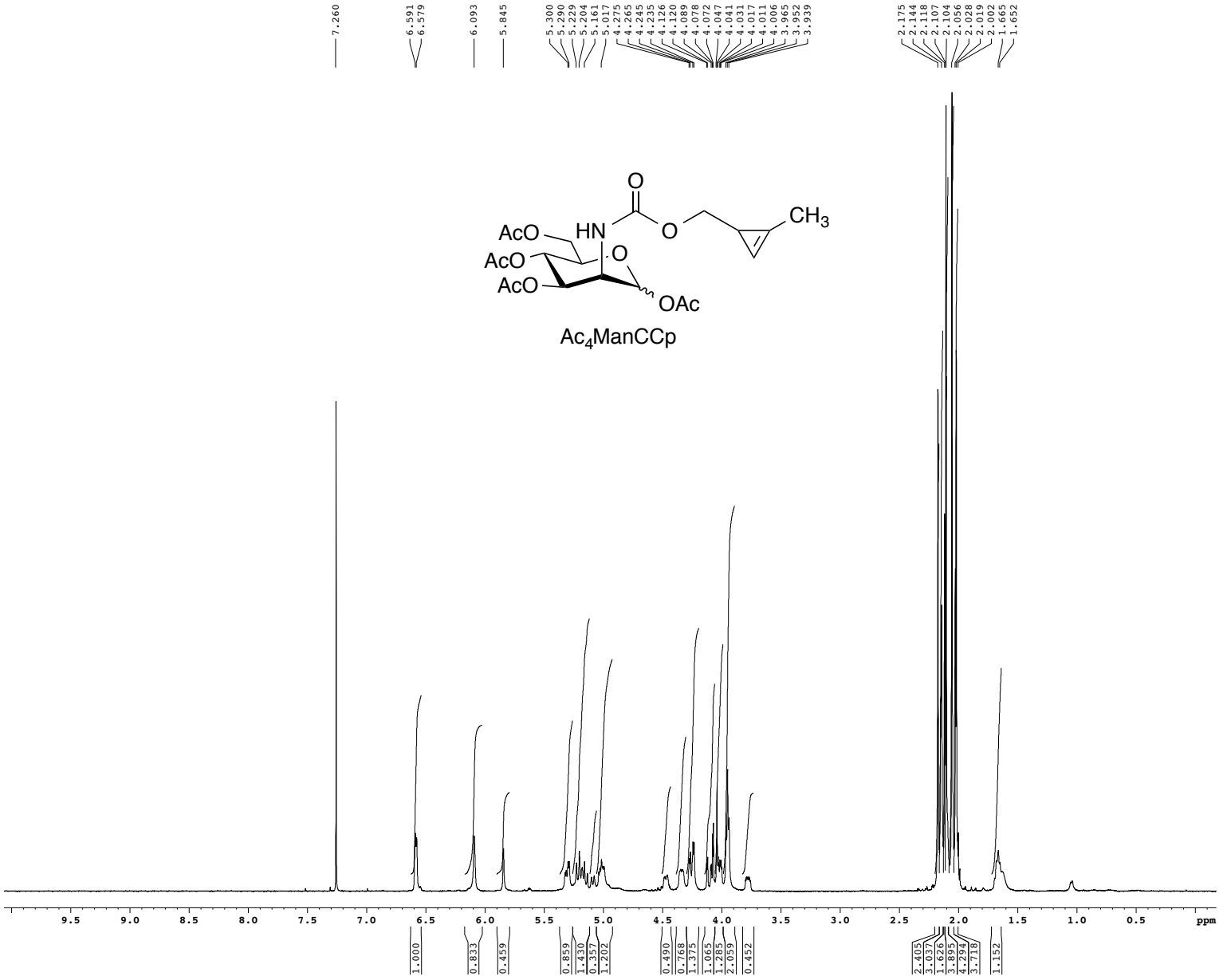

```
Current Data Parameters
USER                dpatte
NAME              DMP4_060
EXPNO                    4
PROCNO                   1

F2 - Acquisition Parameters
Date_             20130909
Time                 12.56
INSTRUM           cryo500
PROBHD   5 mm CPTCI 1H-
PULPROG               zg30
TD                   81728
SOLVENT              CDCl3
NS                       8
DS                       2
SWH               8012.820 Hz
FIDRES               0.098043 Hz
AQ                5.0998774 sec
RG                       5
DW                  62.400 usec
DE                   6.00 usec
TE                  298.0 K
D1                0.10000000 sec
MCREST            0.00000000 sec
MCWRK             0.01500000 sec

======== CHANNEL f1 ========
NUC1                     1H
P1                    7.50 usec
PL1                   1.60 dB
SFO1           500.2235015 MHz

F2 - Processing parameters
SI                       65536
SF             500.2200308 MHz
WDW                        EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    4.00
```

Z-restored spin-echo 13C spectrum with 1H decoupling



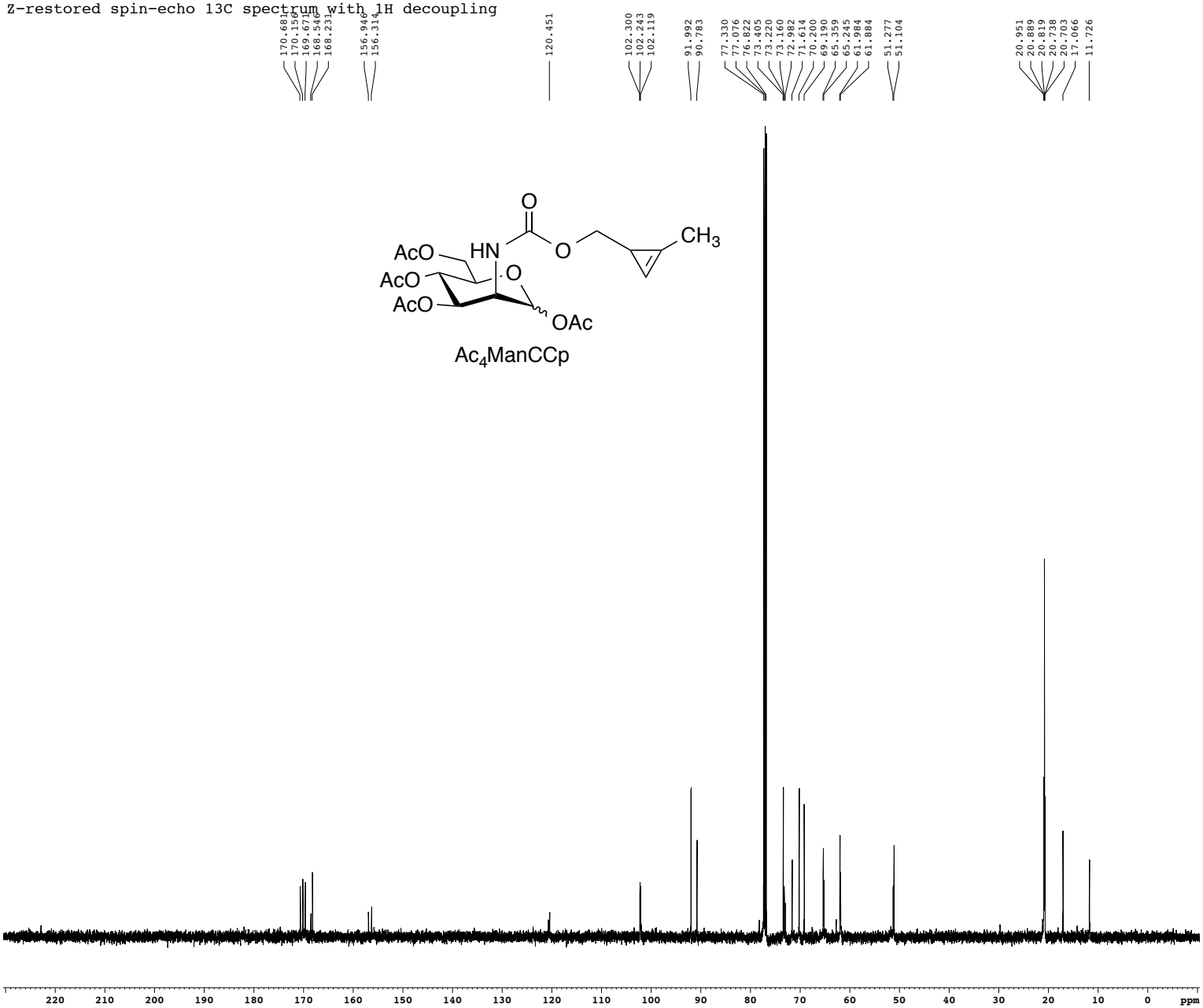

```
Current Data Parameters
USER                dpatte
NAME              DMP4_060
EXPNO                    5
PROCNO                   1

F2 - Acquisition Parameters
Date_            20130909
Time                13.21
INSTRUM           cryo500
PROBHD   5 mm CPTCI 1H-
PULPROG  SpinEchopg30gp.prd
TD                  65536
SOLVENT               CDC13
NS                      456
DS                       16
SWH              30303.031 Hz
FIDRES           0.462388 Hz
AQ               1.0813940 sec
RG                    4096
DW                  16.500 usec
DE                   6.00 usec
TE                  298.0 K
D1              0.25000000 sec
d11             0.03000000 sec
D16             0.00020000 sec
d17             0.00019600 sec
MCREST          0.00000000 sec
MCWRK           0.01500000 sec
P2                  31.00 usec

======== CHANNEL f1 ========
NUC1                   13c
P1                   15.50 usec
P11                 500.00 usec
P12                2000.00 usec
PL0                 120.00 dB
PL1                  -1.00 dB
SFO1           125.7942548 MHz
SP1                   3.20 dB
SP2                   3.20 dB
SPNAM1    Crp60,0.5,20.1
SPNAM2         Crp60comp.4
SPOFF1                0.00 Hz
SPOFF2                0.00 Hz

======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                   1H
PCPD2               100.00 usec
PL2                   1.60 dB
PL12                 24.60 dB
SFO2           500.2225011 MHz

====== GRADIENT CHANNEL =====
GPNAM1           SINE.100
GPNAM2           SINE.100
GPX1                  0.00 %
GPX2                  0.00 %
GPY1                  0.00 %
GPY2                  0.00 %
GPZ1                 30.00 %
GPZ2                 50.00 %
p15                 500.00 usec
p16                1000.00 usec

F2 - Processing parameters
SI                    65536
SF             125.7804190 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    2.00
```

1H spectrum



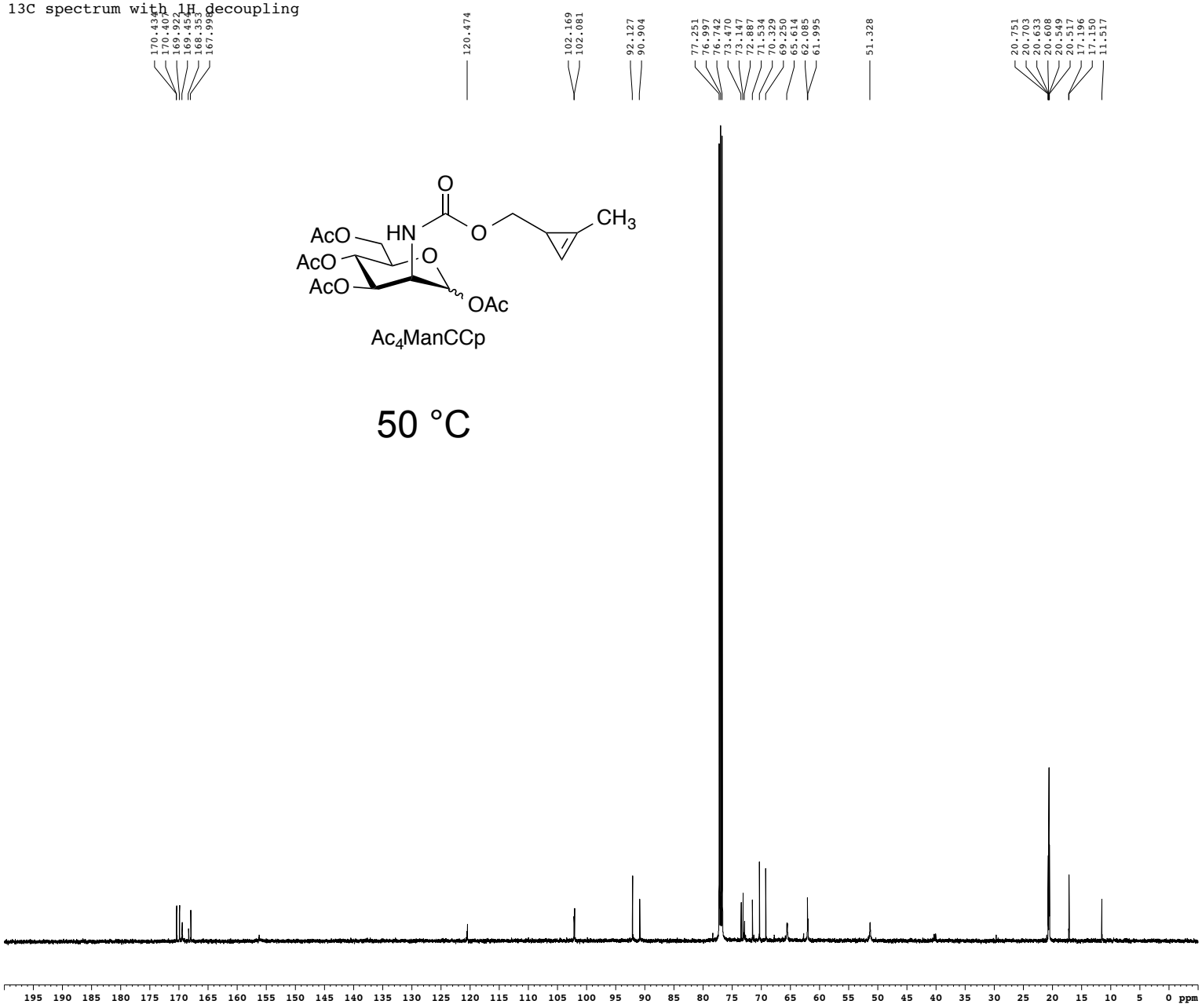

```
Current Data Parameters
USER              dpatte
NAME            DMP4_066
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20130916
Time_              10.37
INSTRUM          drx400
PROBHD   5 mm Multinucl
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                     8
DS                     2
SWH             6410.256 Hz
FIDRES          0.097813 Hz
AQ             5.1118579 sec
RG                 406.4
DW                78.000 usec
DE                 4.50 usec
TE                298.0 K
D1           0.10000000 sec
MCREST       0.00000000 sec
MCWRK        0.01500000 sec

======== CHANNEL f1 ========
NUC1                1H
P1                 12.00 usec
PL1                -0.90 dB
SFO1        400.1328009 MHz

F2 - Processing parameters
SI                 65536
SF          400.1300218 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  2.00
```

Z-restored spin-echo 13C spectrum with 1H decoupling



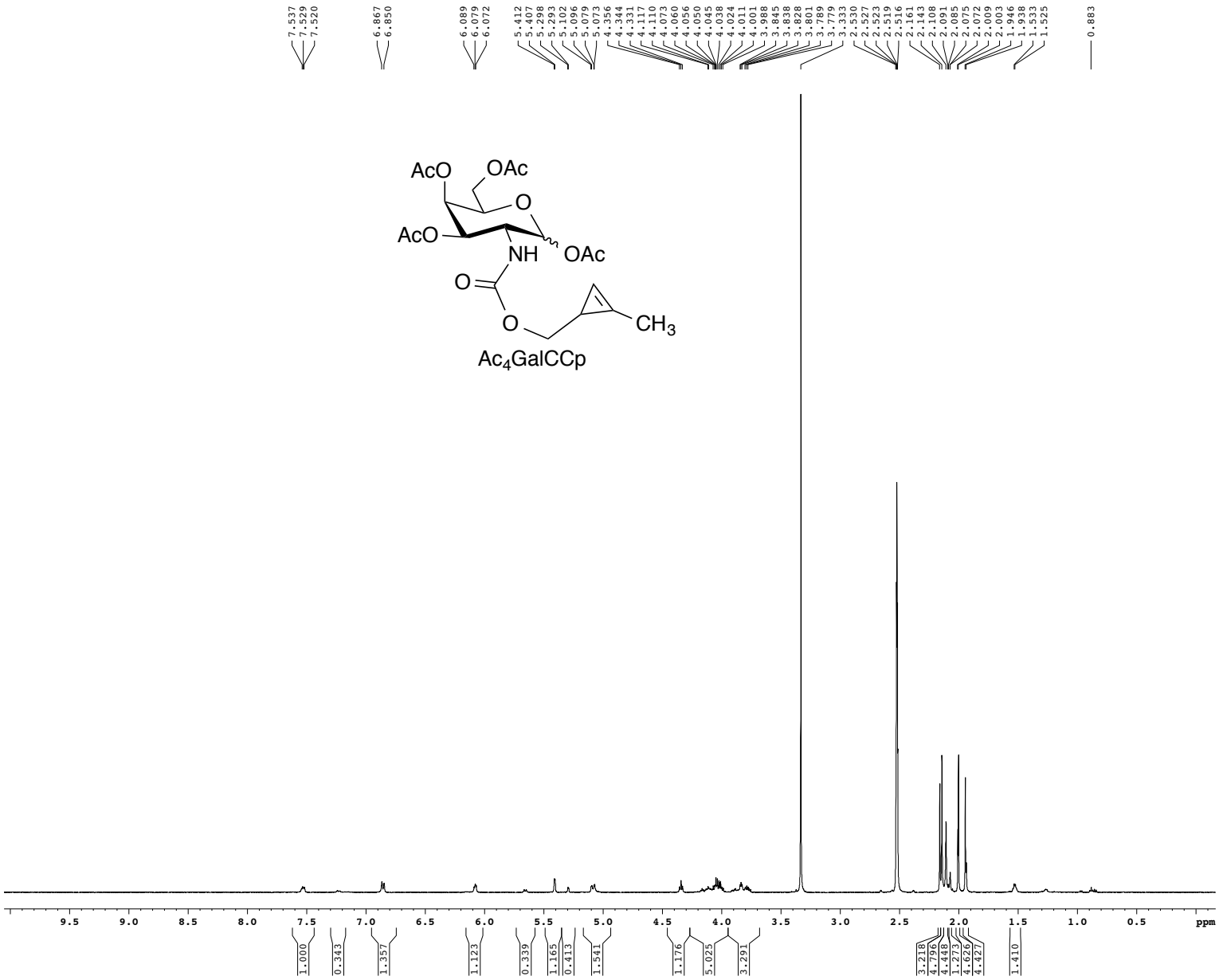

```
Current Data Parameters
USER                dpatte
NAME              DMP4_126
EXPNO                    2
PROCNO                   1

F2 - Acquisition Parameters
Date_             20131220
Time                  9.54
INSTRUM           cryo500
PROBHD    5 mm CPTCI 1H-
PULPROG   SpinEchopg30gp.prd
TD                   65536
SOLVENT              CDCl3
NS                    1024
DS                      16
SWH              30303.031 Hz
FIDRES           0.462388 Hz
AQ               1.0813940 sec
RG                    6502
DW                 16.500 usec
DE                  6.00 usec
TE                  298.0 K
D1             0.25000000 sec
d11            0.03000000 sec
D16            0.00020000 sec
d17            0.00019600 sec
MCREST         0.00000000 sec
MCWRK          0.01500000 sec
P2                 31.00 usec

======== CHANNEL f1 ========
NUC1                  13C
P1                  15.50 usec
P11                500.00 usec
P12               2000.00 usec
PL0                120.00 dB
PL1                 -1.00 dB
SFO1          125.7942548 MHz
SP1                  3.20 dB
SP2                  3.20 dB
SPNAM1    Crp60,0.5,20.1
SPNAM2       Crp60comp.4
SPOFF1               0.00 Hz
SPOFF2               0.00 Hz

======== CHANNEL f2 ========
CPDPRG2           waltz16
NUC2                  1H
PCPD2              100.00 usec
PL2                  1.60 dB
PL12                24.60 dB
SFO2          500.2225011 MHz

====== GRADIENT CHANNEL =====
GPNAM1           SINE.100
GPNAM2           SINE.100
GPX1                 0.00 %
GPX2                 0.00 %
GPY1                 0.00 %
GPY2                 0.00 %
GPZ1                30.00 %
GPZ2                50.00 %
p15                500.00 usec
p16               1000.00 usec

F2 - Processing parameters
SI                   65536
SF            125.7804190 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    2.00
```

13C spectrum with 1H decoupling



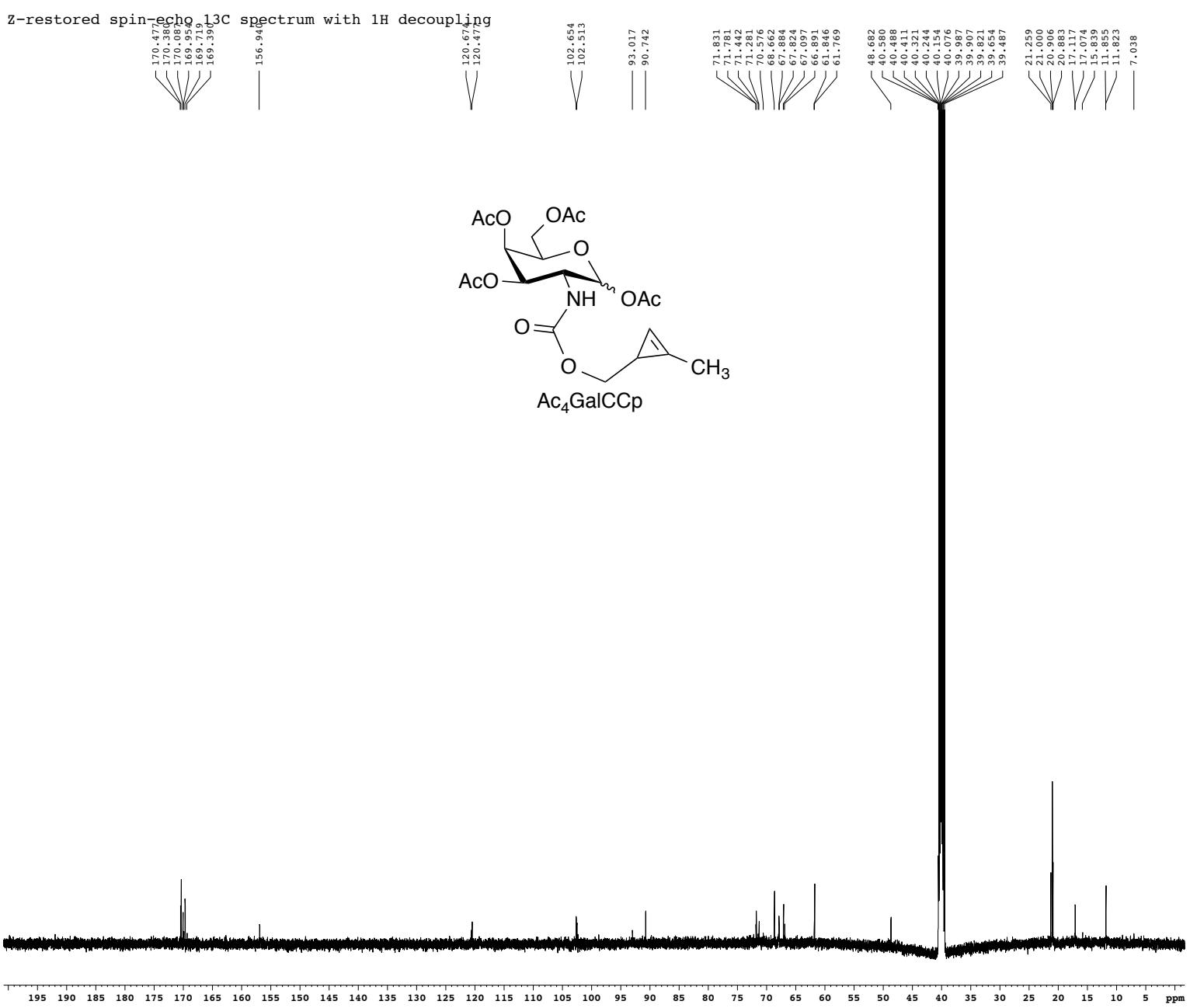

```
Current Data Parameters
USER                dpatte
NAME        DMP4_126_temp
EXPNO                   6
PROCNO                  1

F2 - Acquisition Parameters
Date_            20140207
Time                7.13
INSTRUM             gn500
PROBHD   5 mm broadband
PULPROG          zgdc30
TD                 65536
SOLVENT            CDCl3
NS                 20480
DS                     4
SWH            30303.031 Hz
FIDRES         0.462388 Hz
AQ             1.0813940 sec
RG                5792.6
DW                16.500 usec
DE                 4.50 usec
TE                318.0 K
D1             0.25000000 sec
d11            0.03000000 sec
MCREST         0.00000000 sec
MCWRK          0.01500000 sec

======== CHANNEL f1 ========
NUC1                 13C
P1                  7.70 usec
PL1                 0.00 dB
SFO1         125.5603801 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2              80.00 usec
PL2                -3.00 dB
PL12               13.20 dB
SFO2         499.2924964 MHz

F2 - Processing parameters
SI                 65536
SF           125.5465700 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 2.00
```

1H spectrum



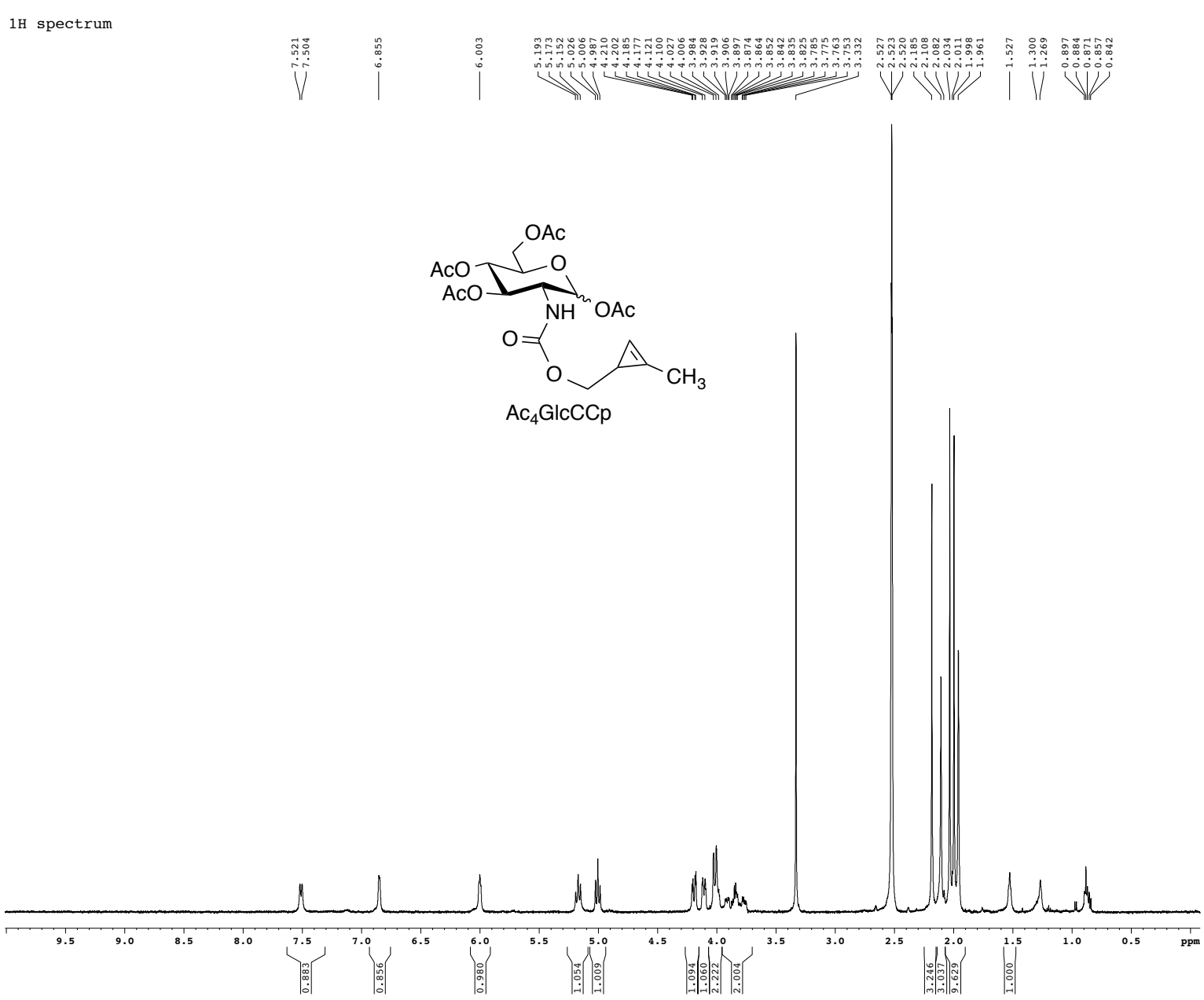

```
Current Data Parameters
USER                dpatte
NAME            DMP4_133B
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_            20140107
Time                21.32
INSTRUM             gn500
PROBHD   5 mm broadband
PULPROG              zg30
TD                  81728
SOLVENT            CDCl3T
NS                      8
DS                      2
SWH              8012.820 Hz
FIDRES           0.098043 Hz
AQ               5.0998774 sec
RG                 1824.6
DW                62.400 usec
DE                 6.00 usec
TE                298.0 K
D1             0.10000000 sec
MCREST         0.00000000 sec
MCWRK          0.01500000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                 12.20 usec
PL1                -5.00 dB
SFO1         499.2934950 MHz

F2 - Processing parameters
SI                  65536
SF           499.2900000 MHz
WDW                    EM
SSB                     0
LB                 0.50 Hz
GB                      0
PC                   1.00
```

Z-restored spin-echo 13C spectrum with 1H decoupling



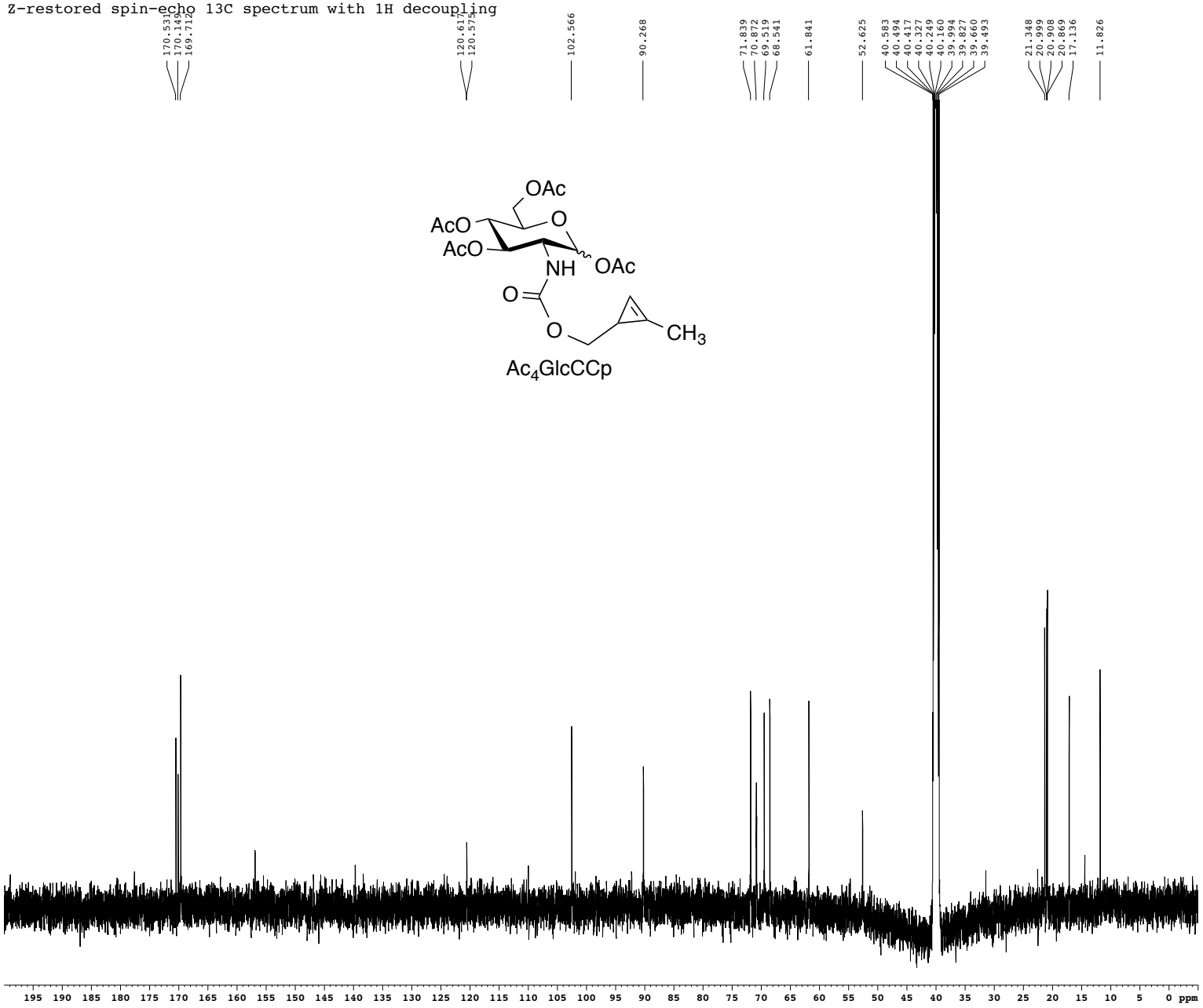

```
Current Data Parameters
USER              dpatte
NAME           DMP4_133B
EXPNO                 3
PROCNO                1

F2 - Acquisition Parameters
Date_          20140108
Time              23.02
INSTRUM         cryo500
PROBHD   5 mm CPTCI 1H-
PULPROG  SpinEchopg30gp.prd
TD                65536
SOLVENT             DMSO
NS                14336
DS                   16
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ            1.0813940 sec
RG               9195.2
DW               16.500 usec
DE                6.00 usec
TE                298.0 K
D1            0.25000000 sec
d11           0.03000000 sec
D16           0.00020000 sec
d17           0.00019600 sec
MCREST        0.00000000 sec
MCWRK         0.01500000 sec
P2               31.00 usec

======== CHANNEL f1 ========
NUC1               13c
P1                15.50 usec
P11              500.00 usec
P12             2000.00 usec
PL0              120.00 dB
PL1               -1.00 dB
SFO1        125.7942548 MHz
SP1               3.20 dB
SP2               3.20 dB
SPNAM1   Crp60,0.5,20.1
SPNAM2       Crp60comp.4
SPOFF1            0.00 Hz
SPOFF2            0.00 Hz

======== CHANNEL f2 ========
CPDPRG2         waltz16
NUC2               1H
PCPD2            100.00 usec
PL2               1.60 dB
PL12             24.60 dB
SFO2        500.2225011 MHz

====== GRADIENT CHANNEL =====
GPNAM1         SINE.100
GPNAM2         SINE.100
GPX1              0.00 %
GPX2              0.00 %
GPY1              0.00 %
GPY2              0.00 %
GPZ1             30.00 %
GPZ2             50.00 %
p15             500.00 usec
p16            1000.00 usec

F2 - Processing parameters
SI                65536
SF          125.7804190 MHz
WDW                EM
SSB                 0
LB                0.50 Hz
GB                  0
PC                1.00
```

1H spectrum




```
Current Data Parameters
USER                dpatte
NAME       DMP4_107B_DMSO
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_             20140107
Time                 21.00
INSTRUM              gn500
PROBHD   5 mm broadband
PULPROG               zg30
TD                   81728
SOLVENT             CDCl3T
NS                       8
DS                       2
SWH               8012.820 Hz
FIDRES               0.098043 Hz
AQ                5.0998774 sec
RG                   1448.2
DW                  62.400 usec
DE                   6.00 usec
TE                  298.0 K
D1             0.10000000 sec
MCREST         0.00000000 sec
MCWRK          0.01500000 sec

======== CHANNEL f1 ========
NUC1                     1H
P1                   12.20 usec
PL1                  -5.00 dB
SFO1          499.2934950 MHz

F2 - Processing parameters
SI                     65536
SF            499.2900000 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.00
```

Z-restored spin-echo 13C spectrum with 1H decoupling




```
Current Data Parameters
USER                dpatte
NAME      DMP4_107B_DMSO
EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
Date_           20140107
Time_              21.48
INSTRUM           cryo500
PROBHD   5 mm CPTCI 1H-
PULPROG  SpinEchopg30gp.prd
TD                 65536
SOLVENT             DMSO
NS                  3072
DS                    16
SWH           30303.031 Hz
FIDRES         0.462388 Hz
AQ            1.0813940 sec
RG                  8192
DW               16.500 usec
DE                6.00 usec
TE               298.0 K
D1            0.25000000 sec
d11           0.03000000 sec
D16           0.00020000 sec
d17           0.00019600 sec
MCREST        0.00000000 sec
MCWRK         0.01500000 sec
P2               31.00 usec

======== CHANNEL f1 ========
NUC1                13C
P1               15.50 usec
P11             500.00 usec
P12            2000.00 usec
PL0             120.00 dB
PL1              -1.00 dB
SFO1        125.7942548 MHz
SP1               3.20 dB
SP2               3.20 dB
SPNAM1   Crp60,0.5,20.1
SPNAM2     Crp60comp.4
SPOFF1              0.00 Hz
SPOFF2              0.00 Hz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2           100.00 usec
PL2               1.60 dB
PL12             24.60 dB
SFO2        500.2225011 MHz

====== GRADIENT CHANNEL =====
GPNAM1          SINE.100
GPNAM2          SINE.100
GPX1                0.00 %
GPX2                0.00 %
GPY1                0.00 %
GPY2                0.00 %
GPZ1               30.00 %
GPZ2               50.00 %
p15             500.00 usec
p16            1000.00 usec

F2 - Processing parameters
SI                 65536
SF          125.7804190 MHz
WDW                   EM
SSB                    0
LB                1.00 Hz
GB                     0
PC                1.00
```