Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Electronic Supplementary Information

Synthesis of Two New Enrichable and MS-Cleavable Cross-linkers to Define Protein-Protein Interactions by Mass Spectrometry

Anthony M. Burke[1], Wynne Kandur[1], Eric J. Novitsky[1], Robyn M. Kaake[2], Clinton Yu[2], Athit Kao[2], Danielle Vellucci[1], Lan Huang[2] and Scott D. Rychnovsky[1]*

[1]Department of Chemistry, University of California, Irvine, CA 92697

[2]Departments of Physiology & Biophysics, University of California, Irvine, CA 92697

I. General Experimental Details

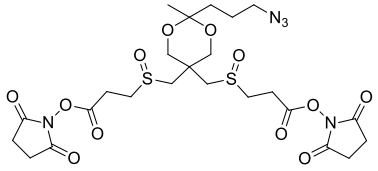
All chemicals were purchased from Sigma-Aldrich, Acros Organics, Alfa Aesar, TCI, Advanced ChemTech, or Fisher and used without further purification unless otherwise noted. 1,5-Dioxaspiro[5.5]undecane-3,3-diyldimethanol (diol **6**)[1], *N*-hydroxysuccinimidyl trifluoroacetate[2], and 5-azido pentanone (azide **11**)[3] were synthesized according to literature procedure. Ethanol was purchased from Gold Shield. Solvents were of reagent grade and used as without further purification except as follows: *N,N*-dimethylformamide (DMF), dichloromethane (DCM), and tetrahydrofuran (THF) were degassed and then passed through anhydrous neutral alumina A-2 before use, according to the procedure described by Grubbs.[4] Methanol was dried over activated 3Å molecular sieves prior to use. Triethylamine was distilled over calcium hydride and stored over activated 3Å molecular sieves prior to use. Diisopropylethylamine (DIPEA) was distilled over calcium hydride prior to use. Trifluoroacetic anhydride (TFAA) and trimethylsilyl triflate (TMSOTf) were distilled prior to use. Reported reaction temperatures refer to the temperature of the heating medium. Reactions were performed in flame- or oven-dried glassware under an atmosphere of dry argon using standard Schlenk techniques unless otherwise noted. Room temperature (rt) refers to 25 ± 3 ºC. Reactions were monitored by thin-layer chromatography (TLC) using EMD Chemicals Inc. silica gel 60 F_{256} plates. Flash chromatography was performed using Ultra Pure SiliaFlash P60, 230-400 mesh (40-63 μm) silica gel (SiO_2) following the general procedure by Still and co-workers.[5]

II. Instrumentation

Proton NMR spectra measurements were acquired at 500 MHz and 600 MHz. Carbon NMR spectra were obtained at 125 MHz. Proton NMR chemical shifts (δ) are reported in parts per million (ppm) and referenced to the residual solvent peak at 7.27 ppm for deuterated chloroform ($CDCl_3$) and 2.50 for deuterated dimethyl sulfoxide (DMSO-d_6). Carbon NMR chemical shifts (δ) are reported in ppm and referenced to the residual solvent peak at 77.23 ppm for deuterated chloroform and 39.52 for deuterated dimethylsulfoxide.[6] NMR data are reported in the following manner: chemical shift, multiplicity, (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, br = broad, app = apparent), coupling constants (J) in hertz (Hz), and integration. High Resolution Mass Spectrometry (HRMS) accurate mass experiments were performed by the University of California, Irvine mass spectrometry laboratory.

III. Experimental Procedures.

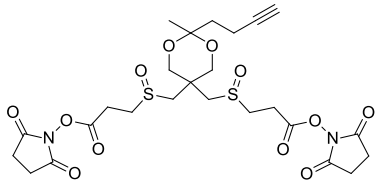
Bis(2,5-dioxopyrrolidin-1-yl)-3,3'-((2-(3-azidopropyl)-2-methyl-1,3-dioxane-5,5-diyl)bis(methylenesulfinyl))dipropanoate (azide-A-DSBSO) (3)



NHS ester **14** (1.21 g, 2.00 mmol) was dissolved in $CHCl_3$ (40 mL), and the reaction mixture was cooled to 0 °C. A solution of *m*-CPBA (0.905 g, 77% mixture with the remainder water, 4.03 mmol) in $CHCl_3$ (40 mL) was added drop-wise and the reaction mixture was stirred for 10 min. The reaction mixture was diluted with $CHCl_3$ (100 mL), and then washed with saturated

aqueous $NaHCO_3$ (3 × 125 mL). The $CHCl_3$ layer was dried over $MgSO_4$, filtered, and concentrated to afford bis-sulfoxide **3** as a white solid and mixture of diastereomers (1.13 g, 89%): ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.98–3.79 (m, 4H), 3.35 (appar. t, 2H, J = 6.8 Hz), 3.29–2.98 (m, 12H), 2.82 (s, 8H), 1.76–1.56 (m, 4H), 1.36 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.10, 170.08, 167.78, 167.76, 99.18, 99.06, 79.19 (residual $CHCl_3$), 65.82, 65.4, 65.0, 64.68, 55.03, 54.75, 54.62, 50.82, 46.11, 46.02, 45.73, 45.67, 40.02, 36.43, 36.31, 34.66, 34.60, 25.48, 25.25, 23.21, 23.18, 23.08, 23.04, 22.66, 20.12, 20.06; IR (KBr) 2931, 2850, 2098, 1782, 1739, 1624 cm^{-1}; HRMS (ESI) m / z calcd for $C_{24}H_{33}N_5O_{12}S_2Na$ $[M + Na]^+$ 670.1465, found 670.1450.

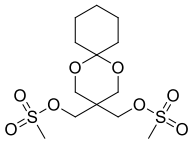
Bis(2,5-dioxopyrrolidin-1-yl) 3,3'-((2-(but-3-yn-1-yl)-2-methyl-1,3-dioxane-5,5-diyl)bis-(methylenesulfinyl))dipropanoate (alkyne-A-DSBSO) (4)



NHS ester **21** (1.82 g, 3.11 mmol) was dissolved in $CHCl_3$ (105 mL), and the solution was cooled to 0 °C. Next *m*-CPBA (1.40 g, 77% mixture with the remainder water, 6.24 mmol) was dissolved in $CHCl_3$ (56.5 mL), then was added drop-wise, and the reaction mixture was stirred for 10 min. The reaction mixture was diluted with $CHCl_3$ (175 mL), and then washed with saturated aqueous $NaHCO_3$ (5 × 40 mL). The $CHCl_3$ layer was collected, dried over $MgSO_4$, filtered, and concentrated to afford **4** as a white solid and mixture of diastereomers (1.88 g, 98%): ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.01–3.79 (m, 4H), 3.32, (s, 1H), 3.29–2.97 (m, 10H), 2.82 (s, 8H), 2.75 (s, 1H), 2.26–2.19 (m, 2H), 1.94–1.85 (m, 3H), 1.37 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.1,

167.8, 98.46, 98.36, 84.40, 79.19 (residual $CHCl_3$), 71.03, 65.8, 65.3, 65.0, 64.59, 55.1, 54.7, 54.5, 46.00, 45.69, 45.63, 40.12, 40.02, 36.7, 36.37, 36.27, 25.46, 23.20, 23.15, 23.07, 23.01, 19.81, 12.28; IR (thin film) 3294, 2989, 2934, 2877, 2117, 1813, 1782, 1736, 1427, 1365, 1207, 1134, 1088, 1068. 1034 cm^{-1}; HRMS (ESI) *m / z* calcd for $C_{25}H_{32}N_2O_{12}S_2$ $[M + Na]^+$ 639.1295, found 639.1295.

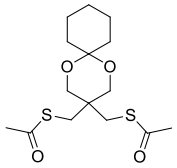
1,5-Dioxaspiro[5.5]undecane-3,3-diylbis(methylene) dimethanesulfonate (7)



Diol **6** (30.18 g, 139.5 mmol)[1] was dissolved in DMF (420 mL), and triethylamine (78 mL, 560 mmol) was added via syringe. At 0 °C, methanesulfonyl chloride (30.0 mL, 388 mmol) was added drop-wise via addition funnel. The solution was gradually warmed to rt, and stirred for 24 h. More DMF (240 mL), triethylamine (38 mL, 270 mmol) and methanesulfonyl chloride (11 mL, 140 mmol) were added at rt and the mixture was stirred another 24 h. The reaction mixture was filtered and the filter cake was rinsed with EtOAc (3 × 100 mL). Additional EtOAc (500 mL) was added, and the solution was washed with saturated aqueous $NaHCO_3$ (150 mL). The aqueous layer was back extracted with EtOAc (150 mL), and the combined organic layers were washed with saturated aqueous $NaHCO_3$ (2 × 150 mL), water (3 × 100 mL), and brine (150 mL). The EtOAc layer was dried over $MgSO_4$, filtered, and concentrated. The crude brown oil was dissolved in CH_2Cl_2 and concentrated repeatedly until a red solid formed. The red solid was scraped out of the flask and chopped into a fine powder at which point the appearance changed to a light yellow solid. The yellow solid was stirred in 900 mL boiling ether, 125 mL

CH_2Cl_2 was slowly added while maintaining a boil and then filtered hot. The clear yellow filtrate was boiled down to 600 mL and then hexanes (100 mL) were added slowly while maintaining a boil. The solution was further boiled down to 600 mL, allowed to cool to room temperature then placed in a freezer overnight. The resulting crystals were filtered, washed 3 times with cold hexanes and dried under high vacuum to afford **7** as off-white long needle shaped crystals (32.17 g, 62%). The mother liquors and hot-filtration materials were purified by column chromatography (step-gradient from 6:4 hexanes:EtOAc to 1:2 hexanes:EtOAc) to afford additional **7** as off-white crystals (15.57 g, 30%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.28 (s, 4H), 3.79 (s, 4H), 3.07 (s, 6H), 1.76–1.66 (m, 4H), 1.51–1.44 (m, 4H), 1.45–1.41 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 99.4, 68.0, 60.8, 38.4, 37.4, 32.5, 25.6, 22.6; IR (KBr pellet) 2943, 2862, 1354 cm^{-1}; HRMS (ESI) *m* / *z* calcd for $C_{13}H_{24}O_8S_2Na$ $[M + Na]^+$ 395.0810, found 395.0801.

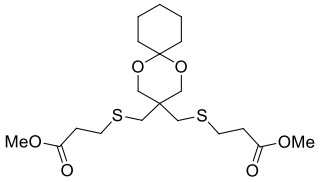
S,S'-(1,5-Dioxaspiro[5.5]undecane-3,3-diylbis(methylene)) diethanethioate (8)



Mesylate **7** (6.38 g, 17.1 mmol) was dissolved in DMF (90 mL). Potassium thioacetate (7.85 g, 68.7 mmol) was added at room temp and the solution was heated to 55 °C for 48 h. The precipitates were filtered off, washed with excess EtOAc, and the filtrate was concentrated to dryness. The red crystalline solid was recrystallized from hexanes (9.82 g in 500 mL) after hot filtration the solution was brought back to a boil (total volume 375 mL). The solution was cooled, placed in the freezer overnight, filtered, and washed with cold hexanes affording **8** as off-white small crystals (3.95 g, 69%). The mother liquors and hot-filtration materials were

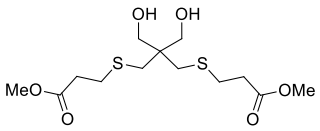
purified by column chromatography (9:1 hexanes:EtOAc) to afford additional **8** as an off-white solid (1.14 g, 20%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.65 (s, 4H), 3.09 (s, 4H), 2.37 (s, 6H), 1.75–1.67 (m, 4H), 1.58 (H_2O), 1.52–1.44 (m, 4H), 1.40 (app d, *J* = 4.2 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 195.1, 98.7, 65.3, 37.3, 32.6, 31.8, 30.9, 25.80, 22.70; IR (KBr pellet) 2927, 2866, 1693, 1446 cm^{-1}; HRMS (ESI) *m* / *z* calcd for $C_{15}H_{24}O_4S_2Na$ $[M + Na]^+$ 355.1014, found 355.1020.

Dimethyl 3,3'-((1,5-dioxaspiro[5.5]undecane-3,3-diylbis(methylene))bis(sulfanediyl))-dipropanoate (9)



Thioacetate **8** (3.95 g, 11.9 mmol) was dissolved in MeOH (300 mL), and triethylamine (8.5 mL, 61 mmol) was added. Methyl acrylate (3.20 mL, 36 mmol) was added dropwise via syringe and the solution was stirred at room temp for 6 h. The solution was concentrated, dissolved in CH_2Cl_2, and concentrated to dryness to afford **9** as a clear light brown oil (4.90 g, 98%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.73 (s, 4H), 3.71 (s, 6H), 2.82 (t, *J* = 7.4 Hz, 4H), 2.74 (s, 4H), 2.64 (t, *J* = 7.3 Hz, 4H), 1.74 (br s, 4H), 1.51 (t, *J* = 5.4 Hz, 4H), 1.41 (app d, *J* = 4.2 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.5, 98.6, 65.5, 52.0, 38.4, 36.0, 34.9, 32.8, 29.1, 25.8, 22.7; IR (neat) 2947, 2862, 1739, 1439 cm^{-1}; HRMS (ESI) *m* / *z* calcd for $C_{19}H_{32}O_6S_2Na$ $[M + Na]^+$ 443.1538, found 443.1522.

Dimethyl 3,3'-((2,2-bis(hydroxymethyl)propane-1,3-diyl)bis(sulfanediyl))dipropanoate (10)



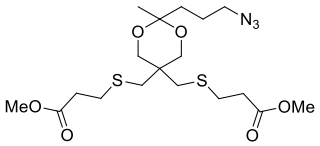
$In(OTf)_3$ Procedure: Ketal **9** (0.202 g, 0.482 mmol) was placed in a microwave tube followed by $In(OTf)_3$ (0.0079 g, 0.014 mmol), MeOH (1.9 mL), and H_2O (433 mL, 24.0 mmol). The solution was placed in a microwave reactor and heated to 70 °C at 50 psi for 30 min. The solution was concentrated and purified by column chromatography: The solution was concentrated, redissolved in a minimal amount of $CDCl_3$ and loaded onto a silica gel column of 1.8 cm O.D. packed 12 cm high with a slurry of 20 mL silica in 3:1 Hexanes:EtOAc, and eluted with 100 mL 3:1, 50 mL 2:1, 50 mL 1:1, 100 mL 1:2, 100 mL 1:3 hexanes:EtOAc. After collecting 10 mL fractions; fractions 4-8 were concentrated to afford to afford starting material **9** (0.0175 g, 8.6%) and fractions 24-38 were concentrated to afford **10** as a clear yellow oil **(**0.140 g, 86%). Characterization data were identical to that of the products using the DOWEX procedure below.

DOWEX Procedure: Ketal **9** (5.07 g, 12.05 mmol) was dissolved in MeOH (150 mL), and DOWEX 50WX8-100 resin (35 g) was added to the solution. After stirring vigorously for 18 h, the reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The crude oil was purified by column chromatography: A column of 5 cm O.D. packed 16 cm high with a slurry of 200 mL silica was loaded with the crude oil and eluted using 600 mL 3:1, 250 mL 7:3, 250 mL 6:4, 250 mL 1:1, 500 mL 1:2, 250 mL 7:3, 250 mL 8:2 hexanes:EtOAc to afford starting material **9** (0.720 g, 14%) and **10** as a clear yellow oil (2.76 g, 67%): ^{1}H NMR (500 MHz,

$CDCl_3$) δ 3.72 (s, 6H), 3.67 (d, *J* = 5.7 Hz, 4H), 2.83 (t, *J* = 7.2 Hz, 4H), 2.69 (s, 4H) 2.65 (t, *J* = 7.2 Hz, 4H), 2.39 (br s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.7, 66.1, 52.1, 44.9, 35.1, 34.8, 28.8; IR (neat) 3483, 2924, 1732, 1435 cm^{-1}; HRMS (ESI) *m* / *z* calcd for $C_{13}H_{24}O_6S_2Na$ $[M + Na]^+$ 363.0192, found 363.0904.

Alkylation Procedure from Diol 15: To a three-necked round bottom flask equipped with an overhead stirrer, a water-cooled condenser, and an argon inlet was added diol **15** (22.0 mL, 197.7 mmol), thiol **16** (17.3 g, 65.9 mmol), potassium carbonate (18.2 g, 131.8 mmol), and DMF (330 mL). The mixture was heated to 40 °C for 24 h, after which the DMF was removed directly from the vessel by vacuum distillation affording diol **10** as a clear colorless oil (22.4 g). Purification of a small sample by column chromatography produced diol **10** in a 75% yield. Characterization data were identical to that of the product using the DOWEX procedure above.

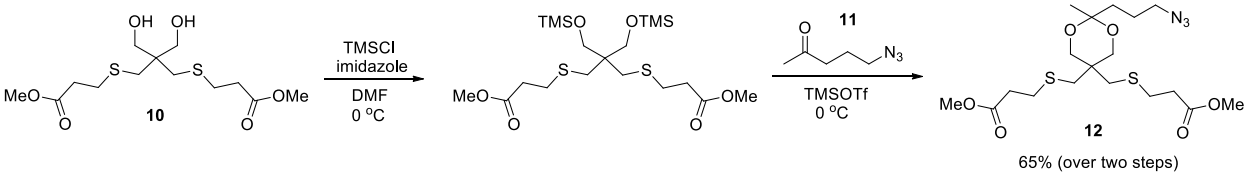
Dimethyl 3,3'-(((2-(3-azidopropyl)-2-methyl-1,3-dioxane-5,5-diyl)bis(methylene))-bis(sulfanediyl))dipropanoate (12)



Dean-Stark Procedure: Diol **10** (4.58 g, 13.5 mmol) was dissolved in benzene (120 mL). 5-Azido pentanone[3] **(11)** (1.77 g, 13.9 mmol) and CSA (0.314 g, 1.35 mmol) were added to the solution, a Dean-Stark apparatus was attached, and the reaction mixture was heated to 115 °C. After 21 h, the reaction mixture was cooled, diluted with EtOAc and partitioned between EtOAc (250

mL) and $NaHCO_3$ (125 mL). The EtOAc layer was separated, washed with brine (75 mL), dried over $MgSO_4$, filtered, and concentrated. The crude brown oil was purified by column chromatography: A 6 cm O.D. column packed 15 cm high with 325 mL silica slurry was loaded with the crude product in minimal CH_2Cl_2, eluting 750 mL 4:1, 1000 mL 3:1, 500 mL 7:3 hexanes:ethyl acetate and collecting 125 – 200 mL fractions. Fractions 8-15 were concentrated affording **12** as a clear light yellow oil (4.78 g, 79%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.78 (d, *J* = 11.9 Hz, 2H), 3.74–3.70 (m, 8H), 3.32 (app t, *J* = 3.4 Hz, 2H), 2.84 (t, *J* = 7.3 Hz, 4H), 2.80 (*J* = 7.3 Hz, 2H), 2.67–2.57 (m, 6H), 1.76–1.73 (m, 4H), 1.39 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4, 99.4, 66.02, 52.0, 51.74, 38.1, 36.0, 35.8, 35.0, 34.9, 29.1, 29.0, 23.1, 20.1; IR (neat) 2954, 2870, 2098, 1739, 1435; cm^{-1}; HRMS (ESI) *m* / *z* calcd for $C_{18}H_{31}N_3O_6S_2Na$ $[M + Na]^+$ 472.1552, found 472.1556.

Dimethyl 3,3'-(((2-(3-azidopropyl)-2-methyl-1,3-dioxane-5,5-diyl)bis(methylene))-bis(sulfanediyl))dipropanoate (12)

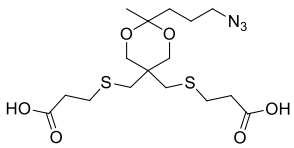


Noyori Procedure:[7] To a stirred solution of crude diol **10** from the alkylation procedure (0.756 g, 2.23 mmol) and imidazole (1.04 g, 15.3 mmol) in DMF (28 mL) was added TMSCl (1 M solution in THF, 12.6 mL) resulting in the formation of a yellow solution. After stirring for 12 h, the reaction mixture was quenched with water (150 mL) and extracted with ethyl acetate (3 × 150 mL). The combined organic portions were washed with water (3 × 150 mL), dried over

anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to afford the crude TMS ether as an orange oil which was used immediately without further purification: ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.71–3.67 (m, 10H), 2.78 (t, *J* = 7.5 Hz, 4H), 2.61 (t, *J* = 7.5 Hz, 4H), 2.57 (s, 4H), 0.08 (s, 18H).

To a cooled (–78 °C) solution of the crude TMS ether (1.00 g, 2.06 mmol) and azide **11**[3] (0.262 g, 2.06 mmol) was added TMS-OTf (50 μL, 0.1 mmol). The solution was stirred for 12 h, over which the time gradually warmed to room temperature. The reaction was quenched with two drops of pyridine (ca. 100 µL), and the mixture was diluted in ethyl acetate (100 mL). The organic layer was washed with water (2 × 100 mL) and brine (100 mL), dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to give crude **12** as a black oil. The crude product was purified by column chromatography (1:3 ethyl acetate:hexanes) to afford **12** as an orange oil (0.651 g, 65% over three steps). ^{1}H and ^{13}C NMR spectra were consistent with those previously reported above.

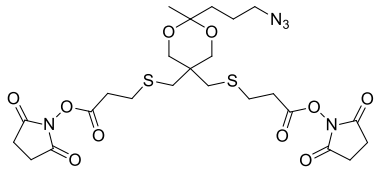
3,3'-(((2-(3-Azidopropyl)-2-methyl-1,3-dioxane-5,5-diyl)bis(methylene))bis(sulfanediyl))-dipropanoic acid (13)



Azide **12** (4.65 g, 10.3 mmol) was dissolved in 4:1 THF:H_2O (67 mL), and $LiOH{\cdot}H_2O$ (0.913 g, 21.8 mmol) was added to the reaction mixture. After 1 h, additional $LiOH{\cdot}H_2O$ (0.913 g, 21.8 mmol) was added. The reaction mixture was stirred for an additional 2 h and partitioned between H_2O

(50 mL) and hexanes (50 mL). The aqueous layer was acidified to pH 1 with 6 M HCl and extracted with EtOAc (5 × 25 mL). The combined EtOAc extracts were dried over $MgSO_4$, filtered, and concentrated to afford **13** as a clear, light yellow oil (4.58 g, quant.): ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.12 (br s, 2H), 3.78–3.69 (m, 4H), 3.29 (t, *J* = 6.0 Hz, 2H), 2.83–2.76 (m, 6H), 2.67 (dt, *J* = 12.0, 7.1 Hz, 4H), 2.60 (s, 2H), 1.73 (s, 4H), 1.33 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.0, 177.9, 99.5, 65.9, 51.6, 38.1, 35.8, 35.6, 34.9, 34.8, 28.6, 28.57, 23.0, 20.0; IR (neat) 3097, 2989, 2098, 1712, 1412 cm^{-1}; HRMS (ES/MeOH) *m* / *z* calcd for $C_{16}H_{27}N_3O_6S_2Na$ $[M + Na]^+$ 444.1239, found 444.1244.

Bis(2,5-dioxopyrrolidin-1-yl) 3,3'-(((2-(3-azidopropyl)-2-methyl-1,3-dioxane-5,5-diyl)bis(methylene))bis(sulfanediyl))dipropanoate (14)

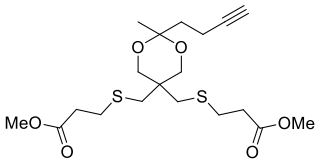


EDC Method: Diacid **13** (2.16 g, 5.12 mmol) was dissolved in DMF (52 mL), and *N*-hydroxysuccinimide was added (1.413 g, 12.3 mmol). 1-Ethyl-3(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI·HCl) (2.360 g, 12.3 mmol) was added followed by triethylamine (0.10 mL, 0.71 mmol) and the reaction mixture was stirred for 13 h. The reaction solution was concentrated by half, diluted with EtOAc (50 mL) then washed with sat. ammonium chloride (2 × 25 mL), sat. $NaHCO_3$ (2 × 25 mL), water (2 × 25 mL), and brine (25 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude oil was purified by column chromatography by loading onto a column 3.5 cm O.D packed 13 cm high

with 100 mL silica slurry in 1:1 hexanes:EtOAc, eluting with 325 mL 1:1, 600 mL 1:2, 200 mL 1:3 hexanes:EtOAc and collecting 175 mL followed by 27 mL fractions. Fractions 9-29 were concentrated affording **14** as a white solid (1.97 g, 62%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.79 (d, *J* = 11.9 Hz, 2H), 3.73 (d, *J* = 11.9 Hz, 2H) 3.32 (t, *J* = 6.0 Hz, 2H), 3.00–2.78 (m, 18H), 2.66 (s, 2H), 2.05 (acetone), 1.71 (br s, 4H), 1.40 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.23, 169.20, 167.3, 99.57, 66.0, 51.76, 38.17, 36.05, 35.89, 35.70, 32.34, 32.25, 28.31, 25.80, 25.56, 23.18, 20.01; IR (KBr) 2931, 2850, 2098, 1782, 1739, 1624 cm^{-1}; LRMS (ES/MeOH) *m* / *z* calcd for $C_{24}H_{33}N_5O_{10}S_2Na$ $[M + Na]^+$ 638.2, found 638.3.

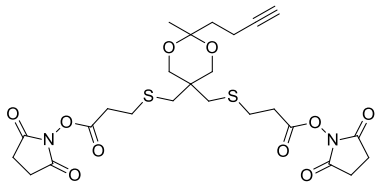
TFAA Method: To a cooled (0 °C) solution of diacid **13**, (2.45 g, 5.81 mmol), *N*-hydroxysuccinimide (2.68 g, 23.3 mmol), and DIPEA (8.10 mL, 46.4 mmol) in DMF (30 mL) was added TFAA (3.28 mL, 23.3 mmol) dropwise, slowly. The light orange solution was stirred at 0 °C for 3 h, after which the reaction was determined complete by TLC. The reaction mixture was partitioned between ethyl acetate (125 mL) and hydrochloric acid (1 M, 100 mL). The layers were separated, after which the acidic aqueous layer was extracted with ethyl acetate (2 × 125 mL), and the combined organic layers were washed with sodium bicarbonate solution (1 M, 3 × 100 mL), water (100 mL), and brine (100 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated to a dark oil which was purified by column chromatography (step-gradient from 1:1 hexanes:EtOAc to 1:3 hexanes:EtOAc) affording **14** as a white solid (2.34 g, 66%). ^{1}H and ^{13}C NMR spectra were consistent with those previously reported above.

Dimethyl 3,3'-(((2-(but-3-yn-1-yl)-2-methyl-1,3-dioxane-5,5-diyl)bis(methylene))-bis(sulfanediyl))dipropanoate (19)



Diol **10** (2.21 g, 6.48 mmol) was dissolved in benzene (45 mL). 1-Hexyne-5-one (1.33 g, 13.8 mmol) and CSA (0.152 g, 0.654 mmol) were added to the solution, a Dean-Stark apparatus was attached, and the reaction mixture was heated to 115 °C. After 27 h, the reaction mixture was cooled, diluted with EtOAc and partitioned between EtOAc (25 mL) and $NaHCO_3$ (125 mL). The EtOAc layer was separated, washed with brine (25 mL), dried over $MgSO_4$, filtered, and concentrated. The crude brown oil was purified by column chromatography using a column 6 cm O.D. packed 15 cm high with 300 mL silica slurried in 4:1 Hexanes:EtOAc. The crude was loaded after dissolution in minimal CH_2Cl_2 and the column was eluted with 250 mL 4:1, 1000 mL 3:1, 500 mL 7:3, 100 mL 65:35 hexanes:EtOAc. After collecting 2 x 200 mL fractions and 25 x 100 mL fractions, fractions 8-15 were concentrated affording **19** as a clear light yellow oil (2.08 g, 77%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.76 (d, *J* = 12.0, 2H), 3.71 (s, 6H), 3.70 (d, *J* =10.1 Hz, 2H), 2.86–2.77 (m, 6H), 2.66–2.59 (m, 6H), 2.33 (ddd, *J* = 8.2, 6.7, 2.7 Hz, 2H), 1.98–1.93 (m, 3H), 1.39 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.40, 172.37, 98.8, 84.5, 68.3, 66.0, 52.0, 38.1, 37.7, 35.9, 35.8, 35.0, 34.9, 29.10, 29.08, 20.1, 12.9; IR (thin film) 3286, 2993, 2951, 2870, 2117, 1739, 1439, 1362, 1250, 1200, 1173, 1134, 1057, 1034; HRMS (ESI) *m* / *z* calcd for $C_{19}H_{30}O_6S_2$ [M + Na]$^+$ 441.1382, found 441.1374.

Bis(2,5-dioxopyrrolidin-1-yl) 3,3'-(((2-(but-3-yn-1-yl)-2-methyl-1,3-dioxane-5,5-diyl)bis(methylene))bis(sulfanediyl))dipropanoate (20)



Dimethyl ester **19** (0.362 g, 0.864 mmol) was dissolved in 4:1 THF:H_2O (8.0 mL), and LiOH·H_2O (0.125 g, 2.98 mmol) was added to the reaction mixture. After 1 h, additional LiOH·H_2O (0.058 g, 1.38 mmol) was added. The reaction mixture was stirred for an additional 2 h and partitioned between H_2O (50 mL) and hexanes (50 mL). The aqueous layer was acidified to pH 1 with 6 M HCl and extracted with EtOAc (5 × 5 mL). The combined EtOAc extracts were dried over $MgSO_4$, filtered, and concentrated, dissolved in CH_2Cl_2 and concentrated repeatedly to afford 0.380 g of a light yellow oil, which was used immediately without any further purification: ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.20 (br s, 2H), 3.72 (q, 4H, *J* = 9.1 Hz), 2.88–2.76 (m, 6H), 2.73–2.60 (m, 6H), 2.32 (dt, 2H, *J* = 7.9, 2.8 Hz), 1.99–1.90 (m, 3H), 1.39 (s, 3H).

To a portion of the crude diacid intermediate (0.180 g, 0.461 mmol) in CH_2Cl_2 (1.6 mL) and pyridine (0.30 mL, 3.7 mmol) was added *N*-hydroxysuccinimidyl trifluoroacetate (0.620 g, 2.94 mmol) and the solution was stirred at room temperature for 3 h. The solution was diluted with CH_2Cl_2, poured into a separatory funnel, washed with sat. NH_4Cl (5 mL), sat. $NaHCO_3$ (5 mL), water (5 mL), and brine (5 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was chromatographed using a column 1.8 cm O.D. packed 12 cm high with a slurry of 20 mL silica and eluting 380 mL 1:2 hexanes:ethyl acetate. After collecting 70 mL followed by 10

mL fractions, fractions 1-13 were concentrated to afford **20** as a white solid (0.162 g, 60%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.30 (CH_2Cl_2), 3.78 (d, *J* = 12.0 Hz, 2H), 3.71 (d, *J* = 12.5 Hz, 2H), 3.01–2.79 (m, 18H), 2.68 (s, 2H), 2.31 (ddd, *J* = 9.7, 7.6, 2.6 Hz, 2H), 2.00–1.93 (m, 3H), 1.39 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.2, 167.3, 98.9, 84.6, 68.3, 66.0, 38.1, 37.4, 35.8, 35.7, 32.3, 32.2, 28.32, 28.26, 25.8, 20.2, 12.9; IR (thin film) 3282, 2947, 2870, 2252, 2114, 1813, 1786, 1739, 1431, 1369, 1250, 1207, 1134, 1068 cm^{-1}; HRMS (ESI) *m / z* calcd for $C_{25}H_{32}N_2O_{10}S_2$ $[M + Na]^+$ 607.1396, found 607.1388.

IV. Cross-Linking Experiments

In vitro cross-linking of synthetic peptide Ac-myelin

Synthetic peptide Ac-myelin was cross-linked with Azide-A-DSBSO in DMSO in a 1:1 molar ratio of peptide to cross-linker at 1 mM in the presence of 1 eq of diisopropylethylamine. Cross-linked peptide solutions were then diluted to 5 pmol/µL in a 3% CAN and 2% formic acid aqueous solution for liquid chromatography multistage tandem mass spectrometry ($LC\text{-}MS^n$) analysis.

Cytochrome C

Bovine cytochrome C was solubilized in 50 mM pH 8.0 phosphate buffer at 200 µM and reacted with 20 mM Azide-A-DSBSO dissolved in DMSO at a 1:10 molar ratio of protein to cross-linker for 1 hr at RT. The reaction was quenched with 500 mM NH_4HCO_3 and ultracentrifuged on a 10kDa NMWL Amicon Ultra centrifugal filters to remove excess cross-linker. To establish the most efficient conditions for biotin conjugation, cross-linked products were washed and

concentrated to 450 μM on filter in either 50 mM phosphate buffer or 8 M urea lysis buffer. Various amounts of BARAC were then reacted with the cross-linked cytochrome C in either phosphate or lysis buffer with agitation overnight. The reaction efficiency for each condition was evaluated by immunoblotting, with subsequent experiments carried out in optimal conditions: urea lysis buffer with 100 μM BARAC and agitation overnight. Following conjugation, excess BARAC was removed by ultracentrifugation and washed with 25 mM NH_4HCO_3. Biotin-conjugated cytochrome C was incubated with high-capacity Streptavidin beads and then digested on-bead with 1% trypsin (w/w) or 5% chymotrypsin (w/w) following reduction and alkylation of cysteine residues in 5 mM DTT at 56^{o}C and 10 mM chloroacetamide at RT, respectively. After digestion, non-cross-linked peptides were extracted and analyzed by LC-MS^{n}; cross-linked peptides bound to streptavidin beads were eluted from beads by acid cleavage in 20% FA, 10% ACN solution prior to LC-MS^{n} analysis.

Analysis of Cross-linked Peptides by LC-MS^{n}

Most of the enriched cross-linked peptides were analyzed by LC-MS^{n} using an LTQ-Orbitrap XL mass spectrometer (Thermo Scientific, San Jose, CA) coupled on-line with either an Eksigent NanoLC system (Dublin, CA), or EASY-nLC-1000 (Thermo Scientific, San Jose, CA). A few of cross-linked samples from intact cells were analyzed using an Orbitrap Elite mass spectrometer (courtesy of Thermo Scientific Demo Lab, San Jose, CA) coupled on-line with an EASY-nLC 1000 (Thermo Scientific). LC/MS^{n} data acquisition and analysis were as described.[8] Only ions with 3+ or higher in the MS1 scan were selected for MS2 analysis.

Identification of Cross-linked Peptides by Database Searching

Due to the similarity between DSBSO and DSSO, the general data analysis workflow for the identification of DSBSO inter-linked peptides by LC/MS^n is the same as the analysis of DSSO cross-linked peptides.[8,9] Using the Batch-Tag software within a developmental version of Protein Prospector (v5.10.10, University of California San Francisco), MS2 and MS3 spectra were searched against a decoy database consisting of a normal Swissprot database concatenated with its randomized version (SwissProt.2013.3.1.random.concat with a total of 454,402 protein entries). The mass tolerances for parent ions and fragment ions were set as ±20 ppm and 0.6 Da respectively. Trypsin was set as the enzyme with three maximum missed cleavages allowed. Cysteine carbamidomethylation was set as a constant modification. Protein N-terminal acetylation, asparagine deamidation, N-terminal conversion of glutamine to pyroglutamic acid, and methionine oxidation were selected as variable modifications. Similar to DSSO cross-linked peptides, DSBSO cross-linked peptides display unique and characteristic MS2 fragmentation patterns corresponding to their cross-linking types. Therefore, three additional defined modifications on uncleaved lysines and free protein N-terminus were chosen: alkene (C_3H_2O, + 54 Da), sulfenic acid ($C_3H_4O_2S$, + 254 Da), and unsaturated thiol (C_3H_2SO, + 236 Da). These are modifications resulting from CID-induced cleavage of the DSBSO cross-linked peptides. The in-house program Link-Hunter is a revised version of the previously written Link-Finder program, designed to automatically validate and summarize cross-linked peptides based MS^n data and database searching results as previously described.[8,9] In addition to checking MS2 spectra for predicted patterns, Link-Hunter automatically correlates sequence data from MS3

to MS2 and MS1 parent masses, reports identified inter-linked peptides with two associated sequences.

V. Supplemental Table 1.

Summary of Unique Inter-linked Peptides Identified from Azide-A-DSBSO Cross-linked CytC

K-K Linkage	MS^n	m/z	z	Sequence	Modification(s)
K6-K9	**MS2**	615.3107	3		
	MS3	828.41	1	MGDVEK$_A$GK	Met-loss+Acetyl@1, Alkene@6
	MS3	408.75	2	K$_A$IFVQK	Alkene@9
K6-K88	**MS2**	457.4806	4		
	MS3	478.76	2	MGDVEK$_A$GKK	Met-loss+Acetyl@1, Alkene@6
	MS3	336.20	2	K$_A$KGER	Alkene@88
K6-K89	**MS2**	555.2882	5		
	MS3	478.76	2	MGDVEK$_A$GKK	Met-loss+Acetyl@1, Alkene@6
	MS3	539.64	3	KK$_A$GEREDLIAYLK	Alkene@89
K8|9-K88*	**MS2**	482.2605	4		
	MS3	528.32	2	GK$_A$K$_A$IFVQK	Alkene@8, Alkene@9
	MS3	336.20	2	K$_A$KGER	Alkene@88
K9-K88	**MS2**	422.4780	4		
	MS3	408.75	2	K$_A$IFVQK	Alkene@9
	MS3	336.20	2	K$_A$KGER	Alkene@88
K9-K88|89**	**MS2**	527.2882	5		
	MS3	408.75	2	K$_A$IFVQK	Alkene@9
	MS3	600.31	3	K$_T$KGEREDLIAYLK or KK$_T$GEREDLIAYLK	ThiolB@88|89
K87-K89	**MS2**	530.6801	5		
	MS3	481.28	2	MIFAGIK$_A$K	Alkene@87
	MS3	496.94	3	K$_A$GEREDLIAYLK	Alkene@89

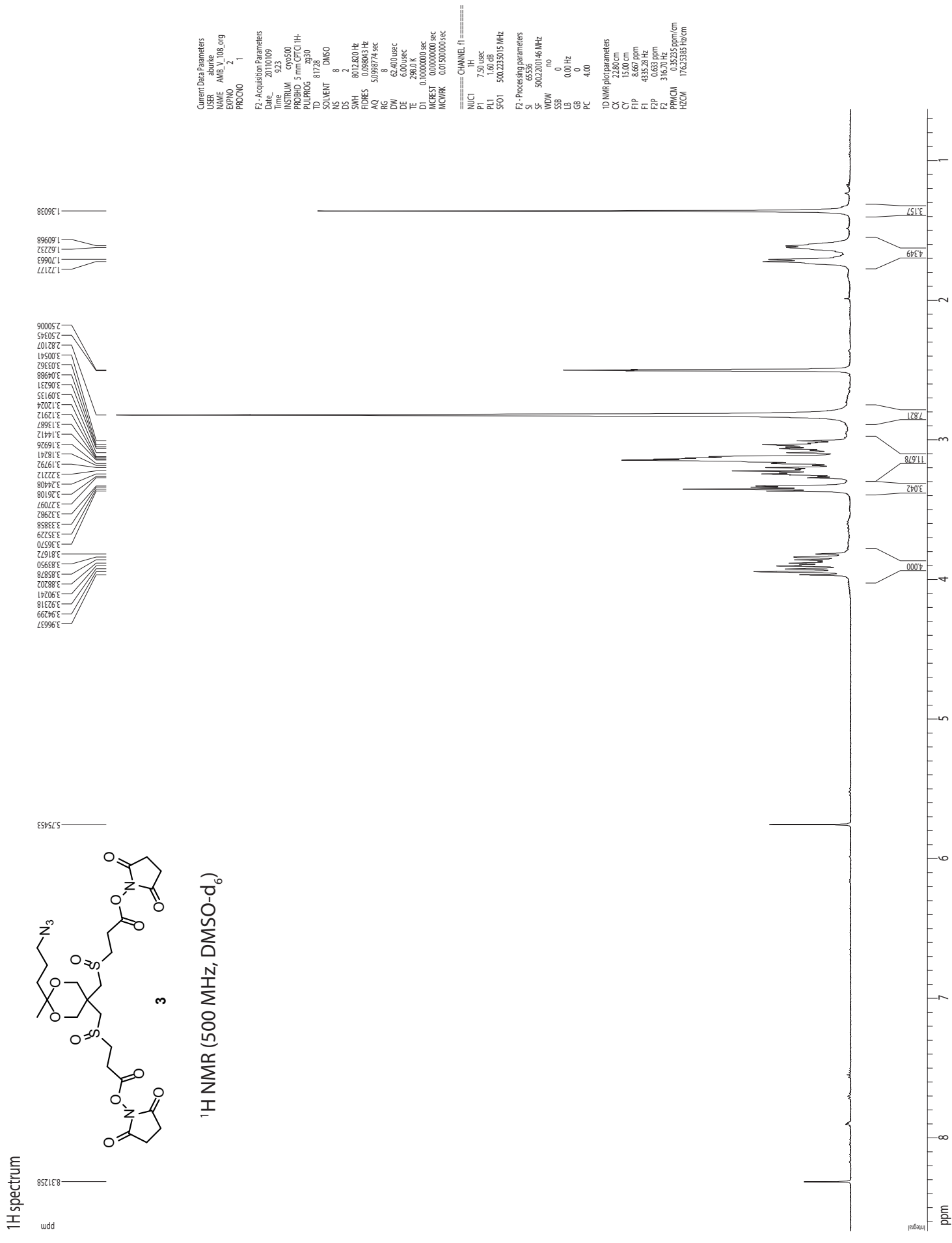
Note: K$_A$: alkene modified lysine; K$_T$:unsaturated thiol modified lysine.

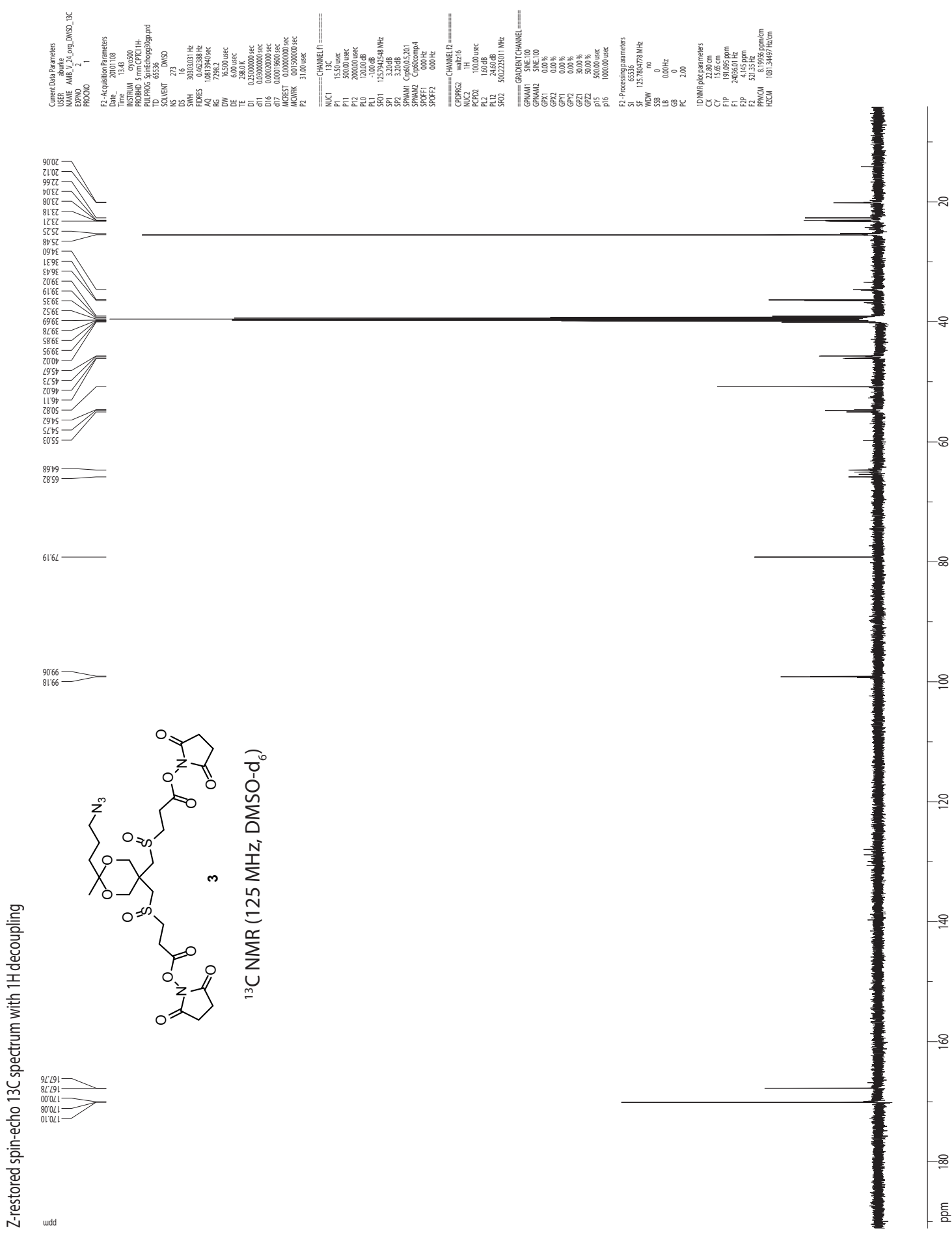
*: Either K8 or K9 was inter-linked with K88.

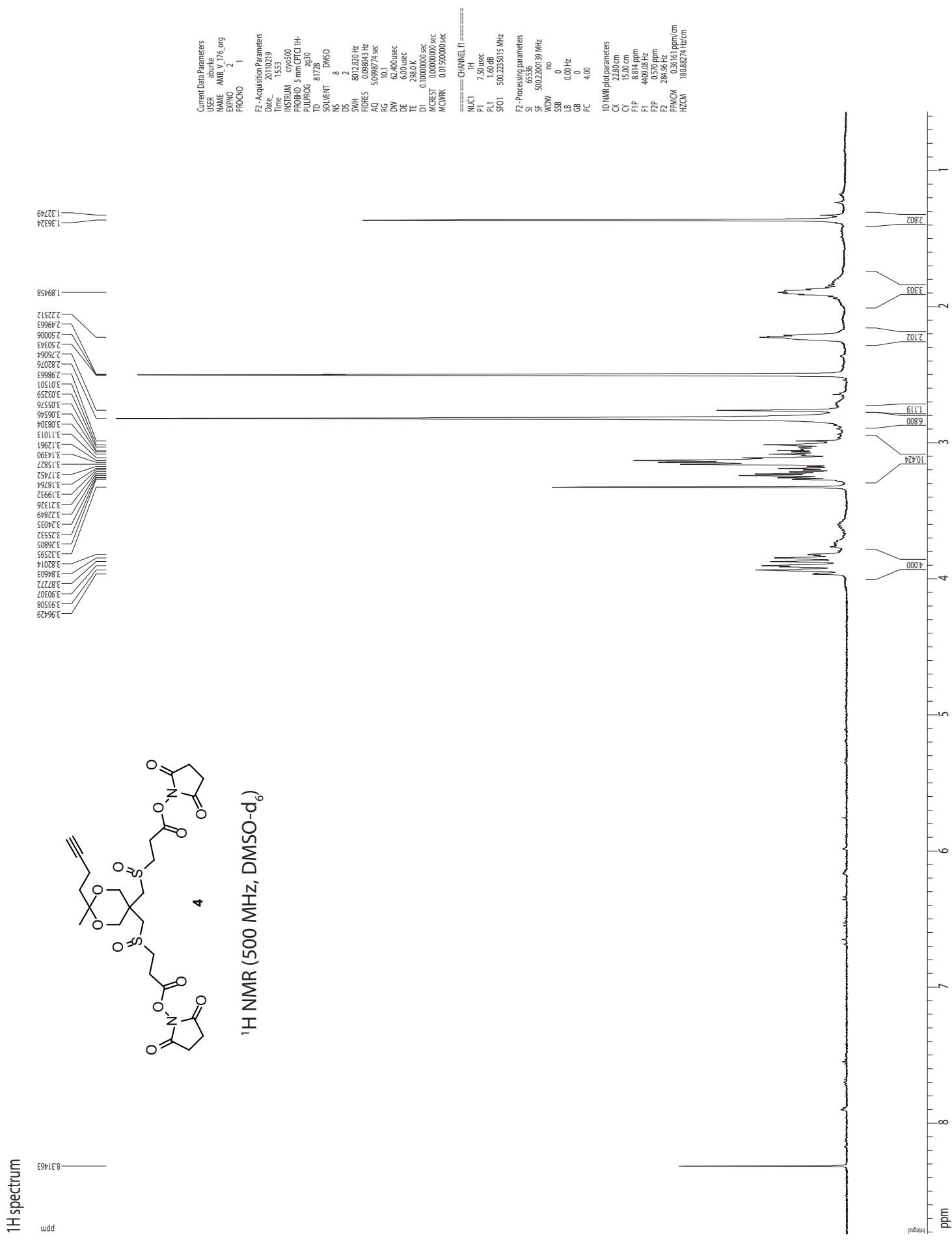
**: Either K88 or K89 was inter-linked with K9. ThiolB is the thiol fragment β_t shown in the workflow in Figure 4.

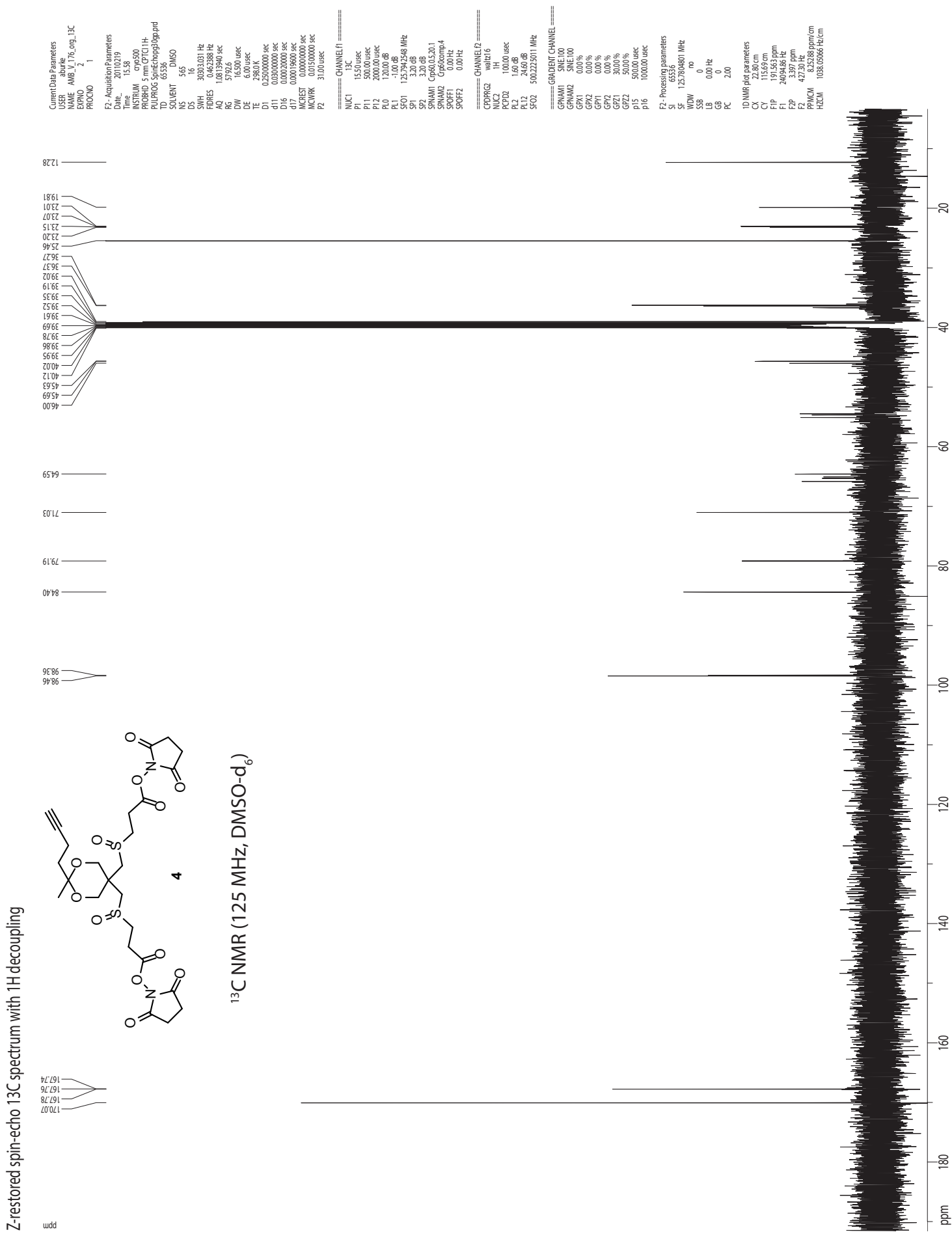
VI. References

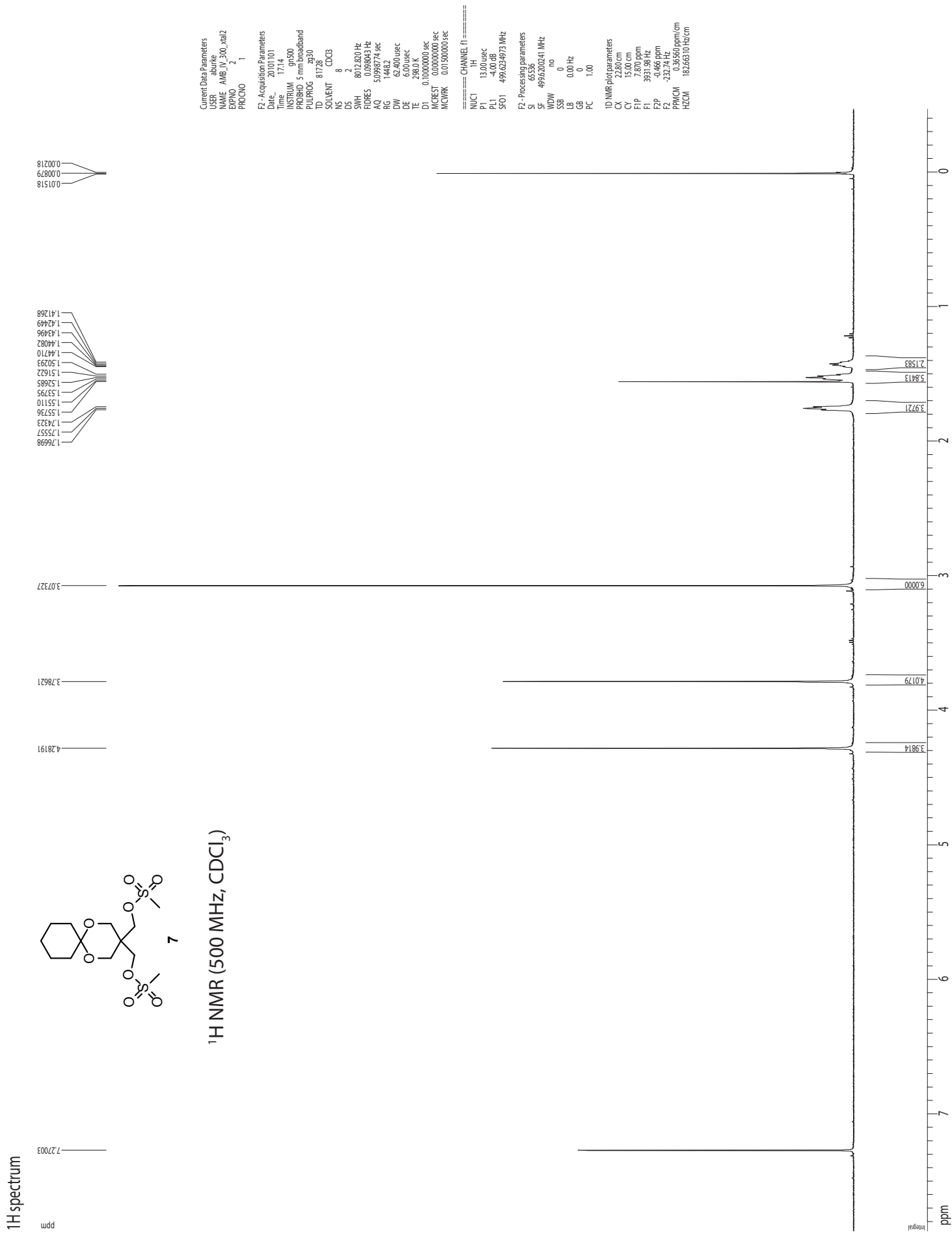
1. M. C. Murguía, S. E. Vaillard, and R. J. Grau, *SYNTHESIS-S*, 2001, **7**, 1093–1097.
2. N. M. Leonard, J. Brunckova, *J. Org. Chem.* 2011, ***76***, 9169–9174.
3. Y. Ma, *Heteroatom Chem.*, 2002, **13**, 307–309.
4. A. B. Pangborn, M. A. Giardello, R. H. Grubbs, R. K. Rosen, and F. J. Timmers, *Organometallics*, 1996, **15**, 1518–1520.
5. W. C. Still, M. Kahn, and A. Mitra, *J. Org. Chem.*, 1978, **43**, 2923–2925.
6. G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw, and K. I. Goldberg, *Organometallics*, 2010, **29**, 2176–2179.
7. T. Tsunoda, M. Suzuki, and R. Noyori, *Tetrahedron Lett.*, 1980, **21**, 1357–1358.
8. A. Kao, A. Randall, Y. Yang, V. R. Patel, W. Kandur, S. Guan, S. D. Rychnovsky, P. Baldi, and L. Huang, *Mol. Cell. Proteomics MCP*, 2012, **11**, 1566–1577.
9. A. Kao, C. Chiu, D. Vellucci, Y. Yang, V. R. Patel, S. Guan, A. Randall, P. Baldi, S. D. Rychnovsky, and L. Huang, *Mol. Cell. Proteomics MCP*, 2011, **10**, M110.002212.

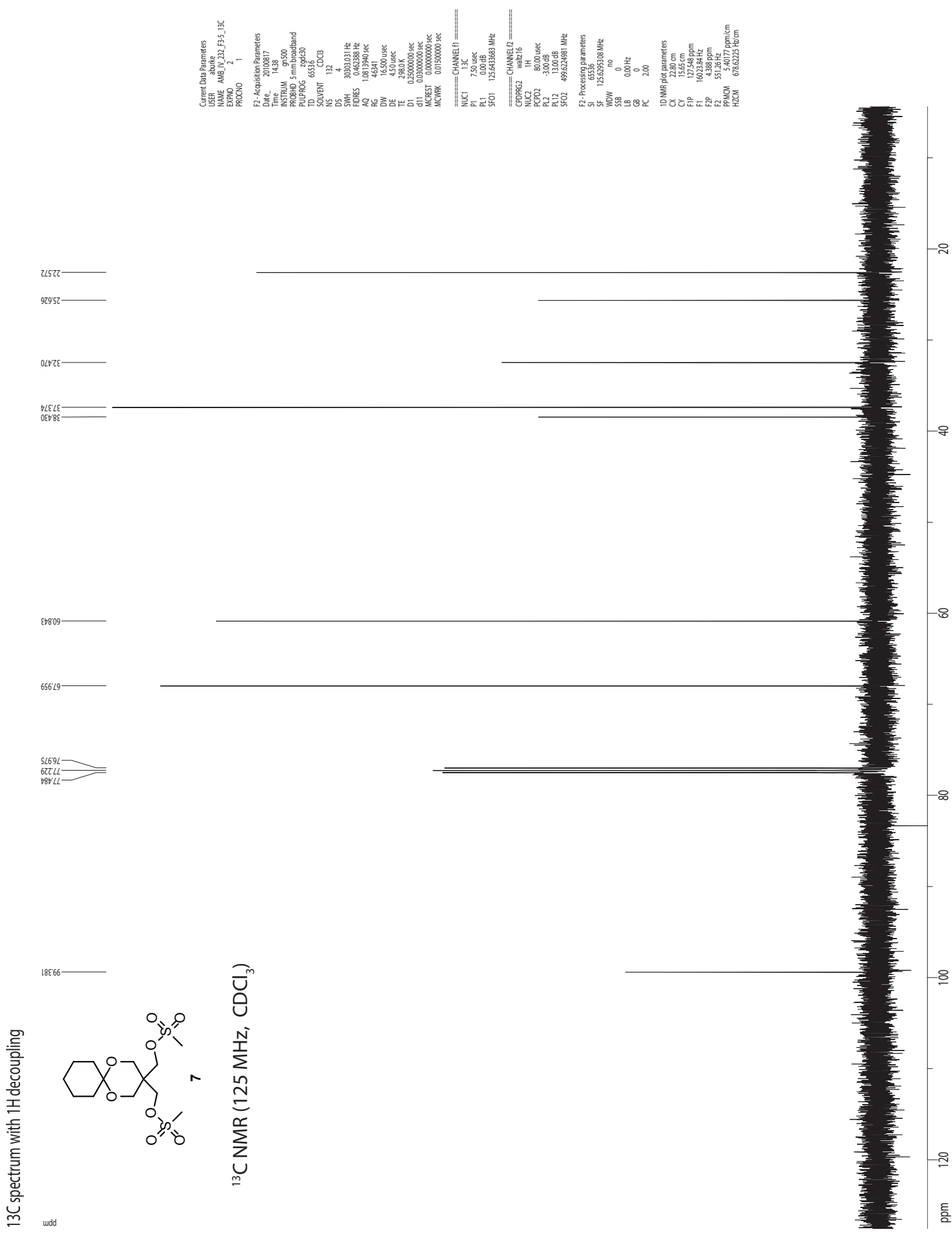

1H spectrum
Current Data Parameters
USER aburke
NAME AMB_V_108_org
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20110109
Time 9.23
INSTRUM cryo500
PROBHD 5 mm CPTCI 1H-
PULPROG zg30
TD 81728
SOLVENT DMSO
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.098043 Hz
AQ 5.0998774 sec
RG 8
DW 62.400 usec
DE 6.00 usec
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 500.2200146 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 4.00
1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 8.667 ppm
F1 4335.28 Hz
F2P 0.633 ppm
F2 316.70 Hz
PPMCM 0.35235 ppm/cm
HZCM 176.25386 Hz/cm
8.31258
5.75453
3.96637
3.94299
3.92318
3.90241
3.88202
3.85878
3.83950
3.81672
3.36570
3.33858
3.32982
3.32097
3.26108
3.24408
3.22212
3.19792
3.18241
3.16926
3.14412
3.13687
3.12912
3.12024
3.09135
3.06231
3.04988
3.03362
3.00541
2.82107
2.50345
2.50006
1.72177
1.70663
1.62232
1.60968
1.36038
3.157
4.349
7.821
11.678
3.042
4.000
integral
ppm
1
2
3
4
5
6
7
8
3
^{1}H NMR (500 MHz, DMSO-d$_6$)

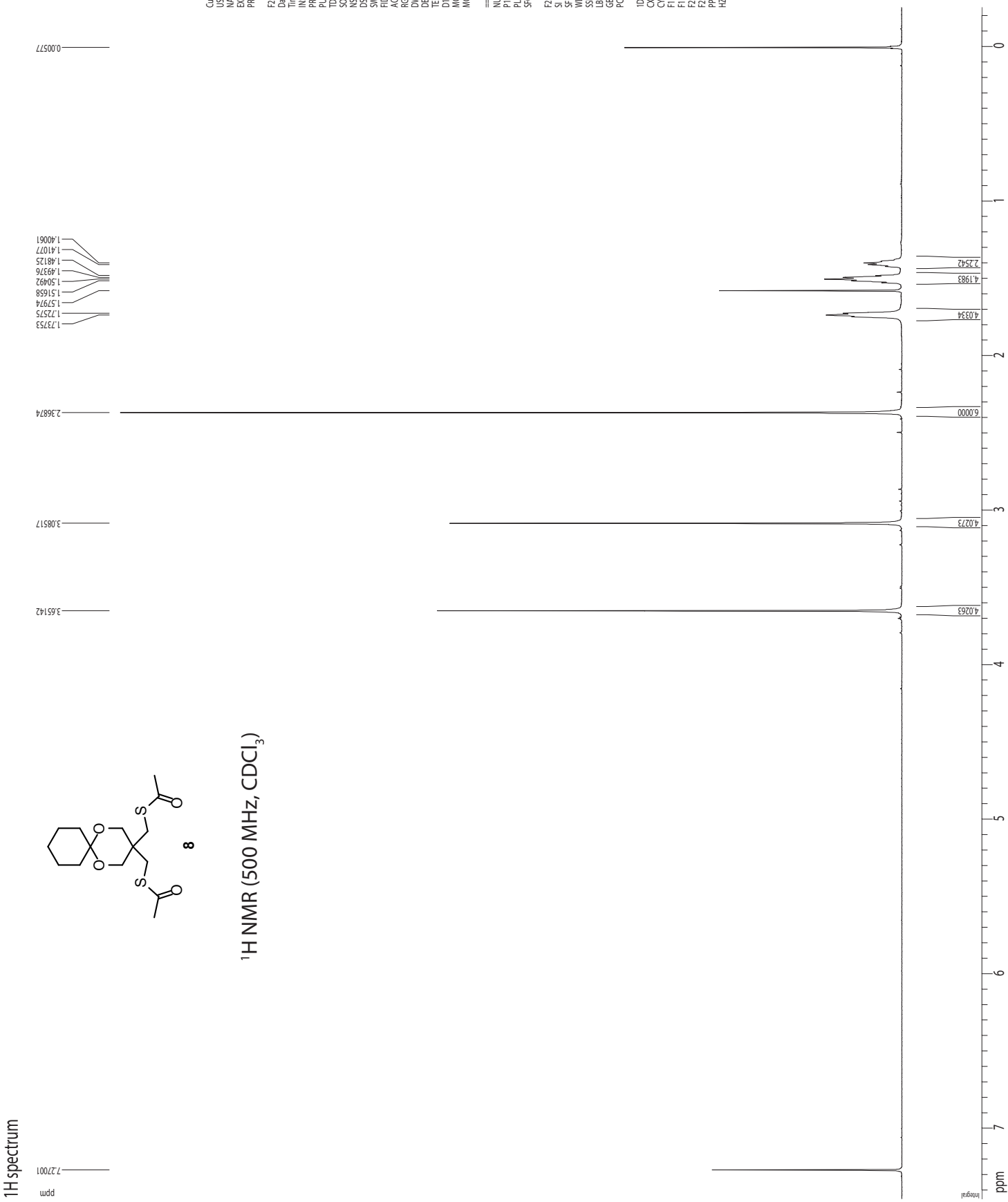

Z-restored spin-echo 13C spectrum with 1H decoupling
^{13}C NMR (125 MHz, DMSO-d$_6$)
3
ppm


1H spectrum
Current Data Parameters
USER aburke
NAME AMB_V_176_org
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20110219
Time 15:53
INSTRUM cryo500
PROBHD 5 mm CPTCI 1H-
PULPROG zg30
TD 81728
SOLVENT DMSO
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.098043 Hz
AQ 5.0998774 sec
RG 10.1
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 500.2200139 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 4.00
1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 8.814 ppm
F1 4409.08 Hz
F2P 0.570 ppm
F2 284.96 Hz
PPMCM 0.36161 ppm/cm
HZCM 180.86274 Hz/cm
8.31463
3.96429
3.93508
3.90307
3.87272
3.84603
3.82014
3.32595
3.26805
3.25532
3.24035
3.22849
3.21326
3.19932
3.18764
3.17452
3.15827
3.14390
3.12961
3.11013
3.08304
3.06546
3.05576
3.03259
3.01501
2.98663
2.82076
2.76064
2.75034
2.50006
2.49663
2.22512
1.89458
1.36324
1.32749
2.802
3.303
2.102
1.119
6.800
10.424
4.000
integral
ppm
1
2
3
4
5
6
7
8
4
^{1}H NMR (500 MHz, DMSO-d$_6$)


Z-restored spin-echo 13C spectrum with 1H decoupling
^{13}C NMR (125 MHz, DMSO-d$_6$)
4
ppm


1H spectrum
ppm
7.27003
4.28191
3.78621
3.07327
1.76698
1.75557
1.74323
1.55736
1.55110
1.53795
1.52685
1.51622
1.50293
1.44710
1.44082
1.43496
1.42449
1.41268
0.01518
0.00879
0.00218
Current Data Parameters
USER aburke
NAME AMB_IV_300_xtal2
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20101101
Time 17.14
INSTRUM gn500
PROBHD 5 mm broadband
PULPROG zg30
TD 81728
SOLVENT CDCl3
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 13.00 usec
PL1 -4.00 dB
SFO1 499.6234973 MHz
F2 - Processing parameters
SI 65536
SF 499.6200241 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.00
1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 7.870 ppm
F1 3931.98 Hz
F2P -0.466 ppm
F2 -232.74 Hz
PPMCM 0.36560 ppm/cm
HZCM 182.66310 Hz/cm
6.0000
4.0179
3.9814
2.7583
5.8413
3.9721
Integral
ppm
0
1
2
3
4
5
6
7
O
O
O
O
O
O
S
S
O
O
O
O
7
1H NMR (500 MHz, CDCl3)

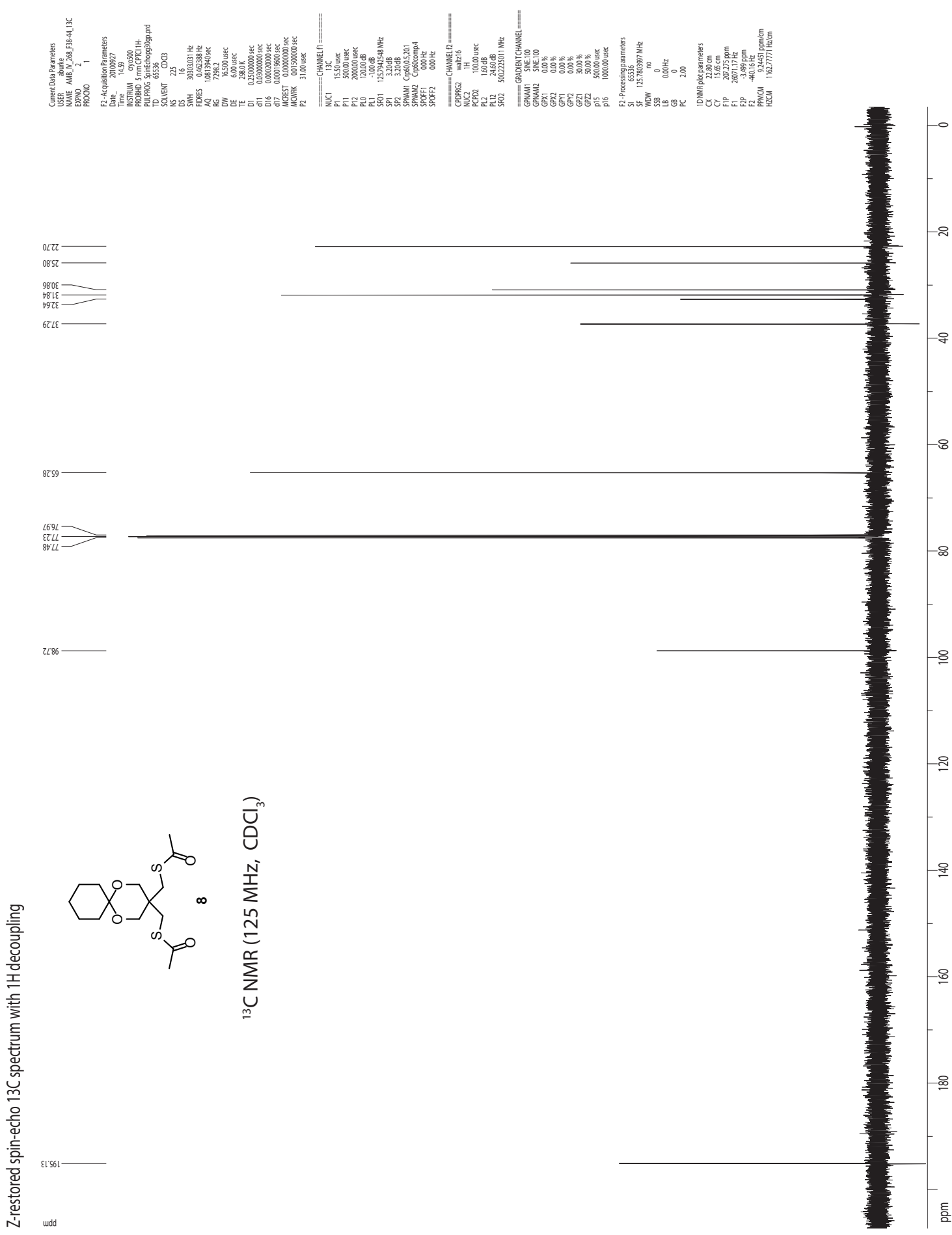

13C spectrum with 1H decoupling
^{13}C NMR (125 MHz, $CDCl_3$)
7
ppm
99.381
77.484
77.229
76.975
67.959
60.843
37.374
38.430
32.470
25.626
22.572
Current Data Parameters
USER abuirke
NAME AMB_IV_232_F3-5_13C
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20100817
Time 14.38
INSTRUM gn500
PROBHD 5 mm broadband
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 132
DS 4
SWH 30303.031 Hz
FIDRES 0.462388 Hz
AQ 1.08139440 sec
RG 46341
DW 16.500 usec
DE 4.50 usec
TE 298.0 K
D1 0.25000000 sec
D11 0.03000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
======== CHANNEL f1 ========
NUC1 13C
P1 7.50 usec
PL1 0.00 dB
SFO1 125.64435683 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 3.00 dB
PL12 17.20 dB
SFO2 499.6224981 MHz
F2 - Processing parameters
SI 65536
SF 125.6295308 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 2.00
1D NMR plot parameters
CX 22.80 cm
CY 15.65 cm
F1P 127.548 ppm
F1 16023.84 Hz
F2P 4.388 ppm
F2 551.26 Hz
PPMCM 5.40177 ppm/cm
HZCM 678.62225 Hz/cm
ppm
20
40
60
80
100
120

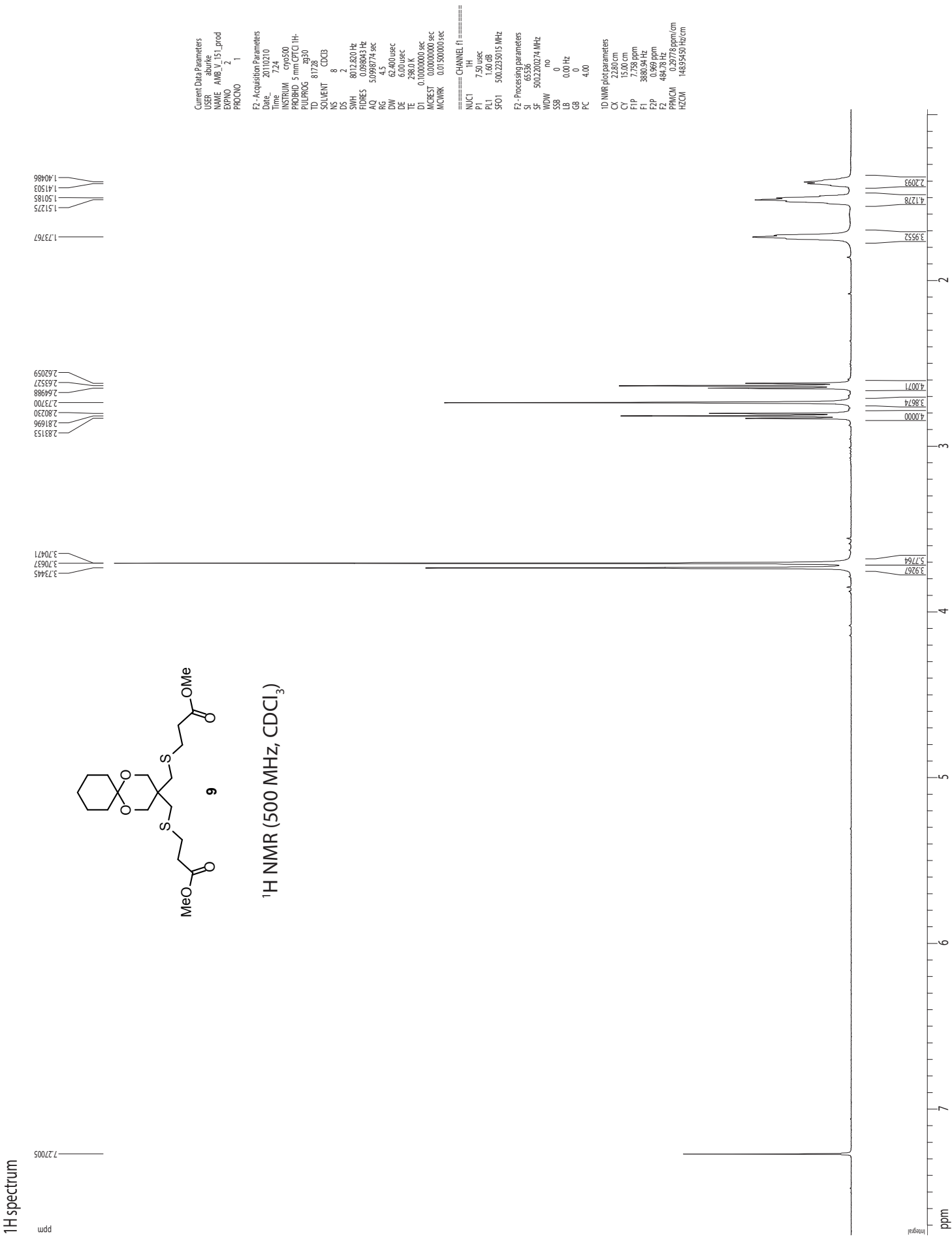


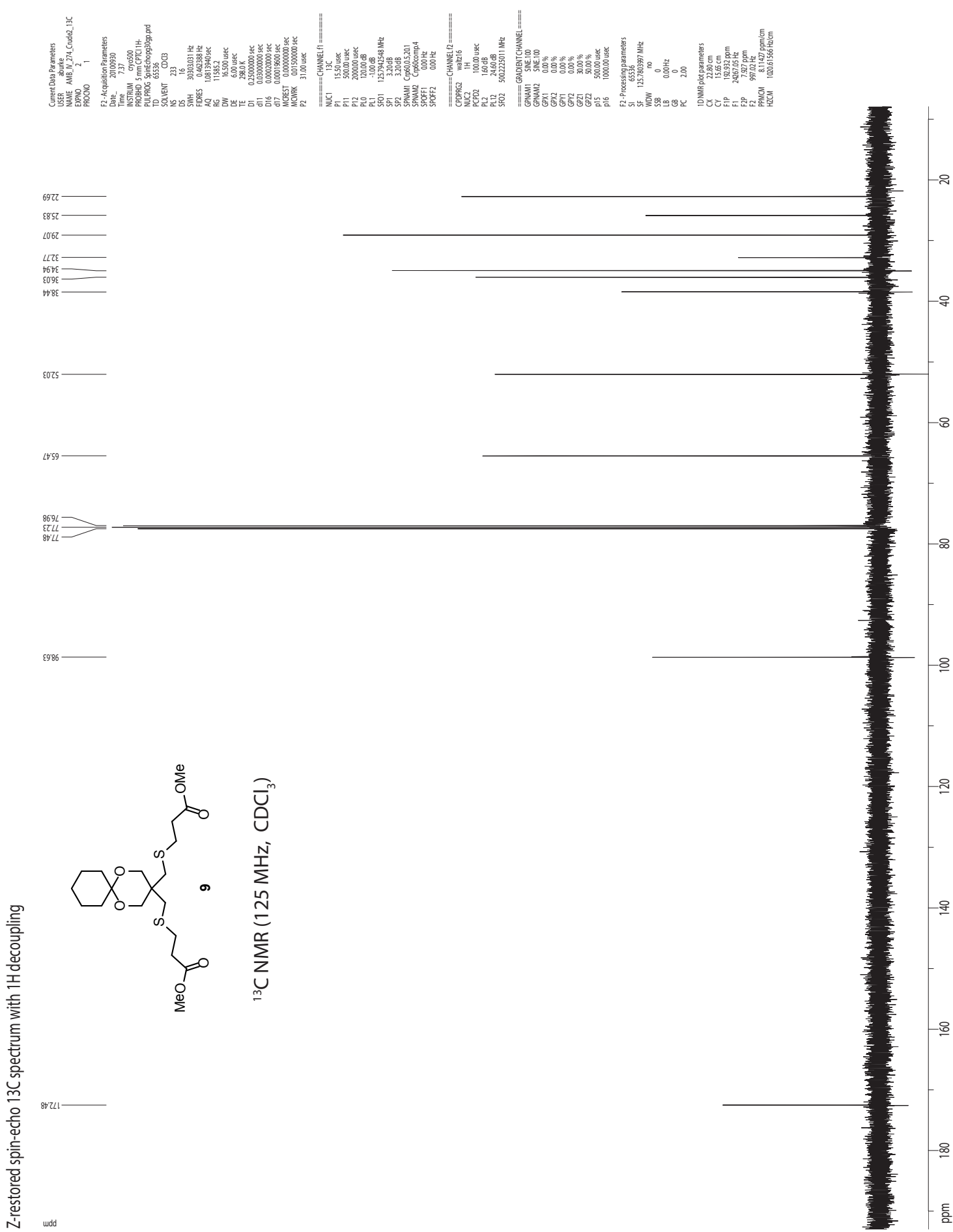
1H spectrum

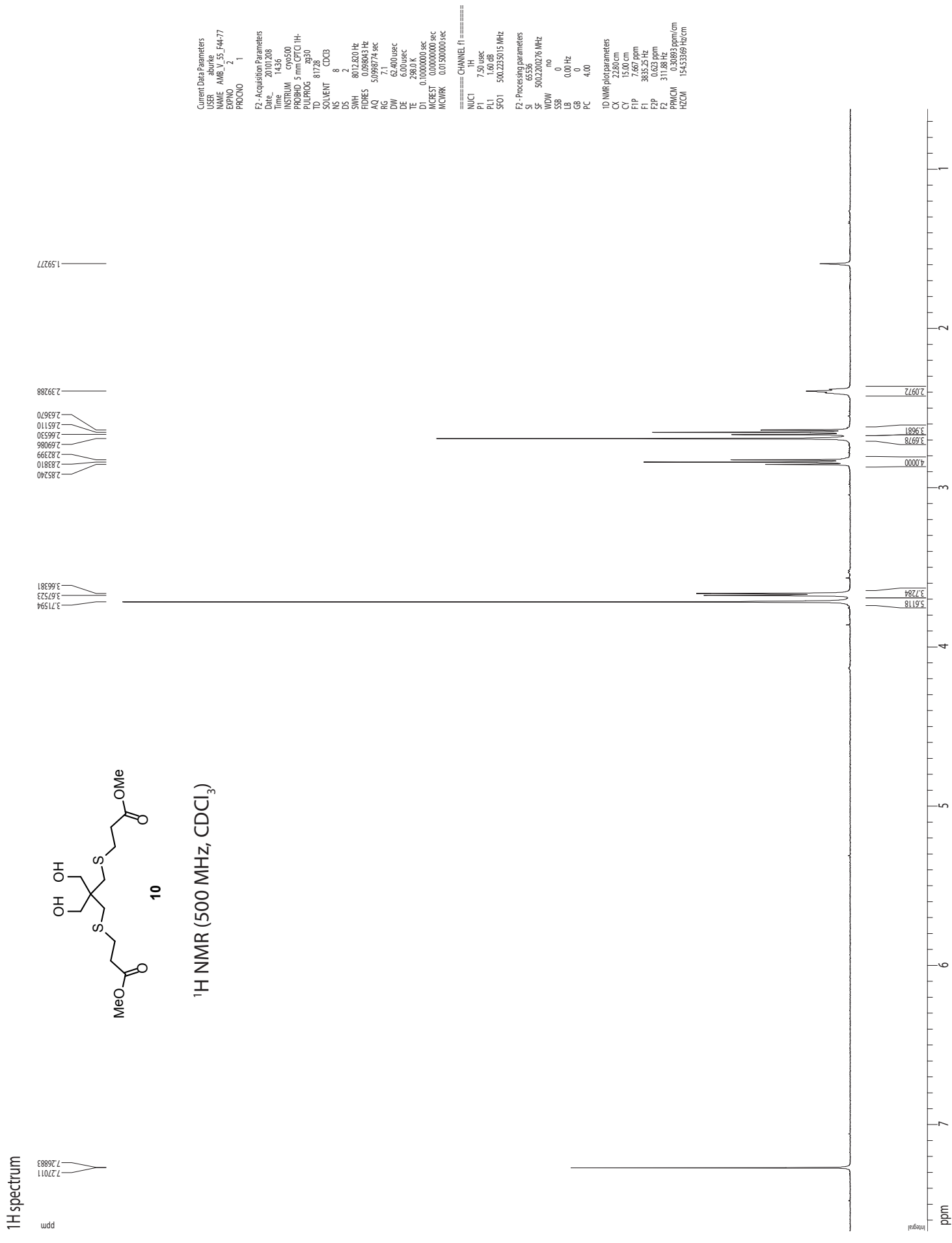
8

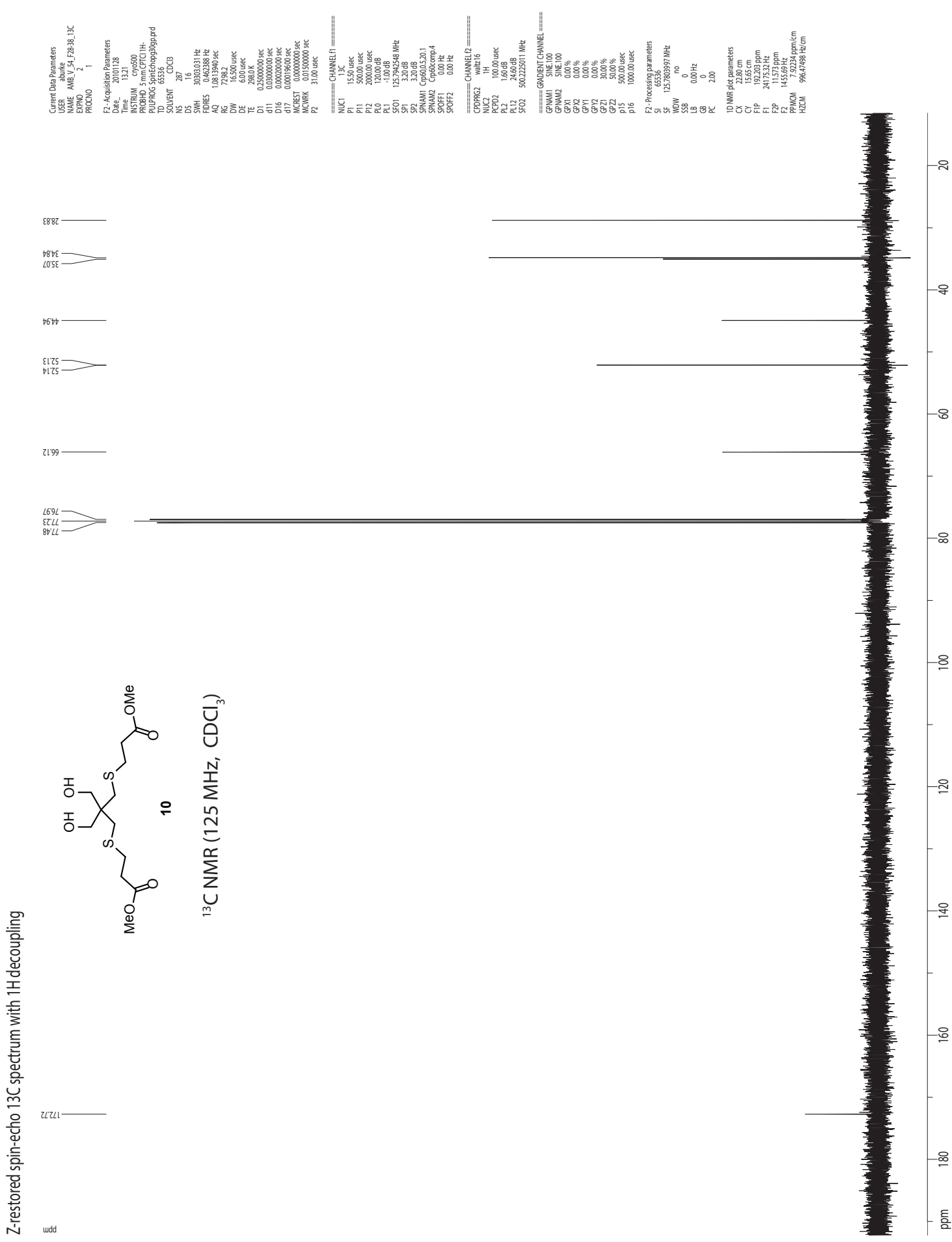
^{1}H NMR (500 MHz, $CDCl_3$)

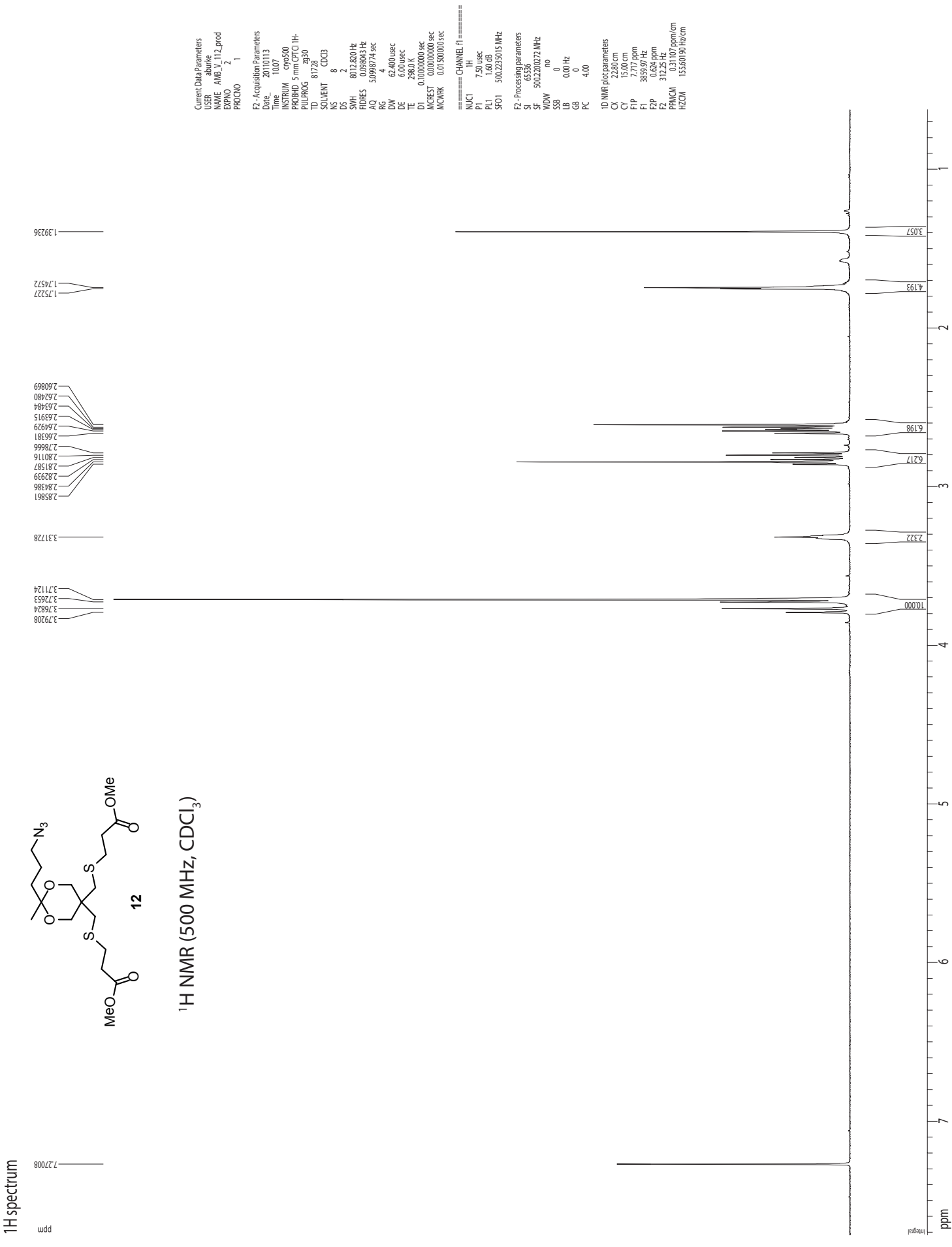

Z-restored spin-echo 13C spectrum with 1H decoupling
^{13}C NMR (125 MHz, $CDCl_3$)
8
ppm

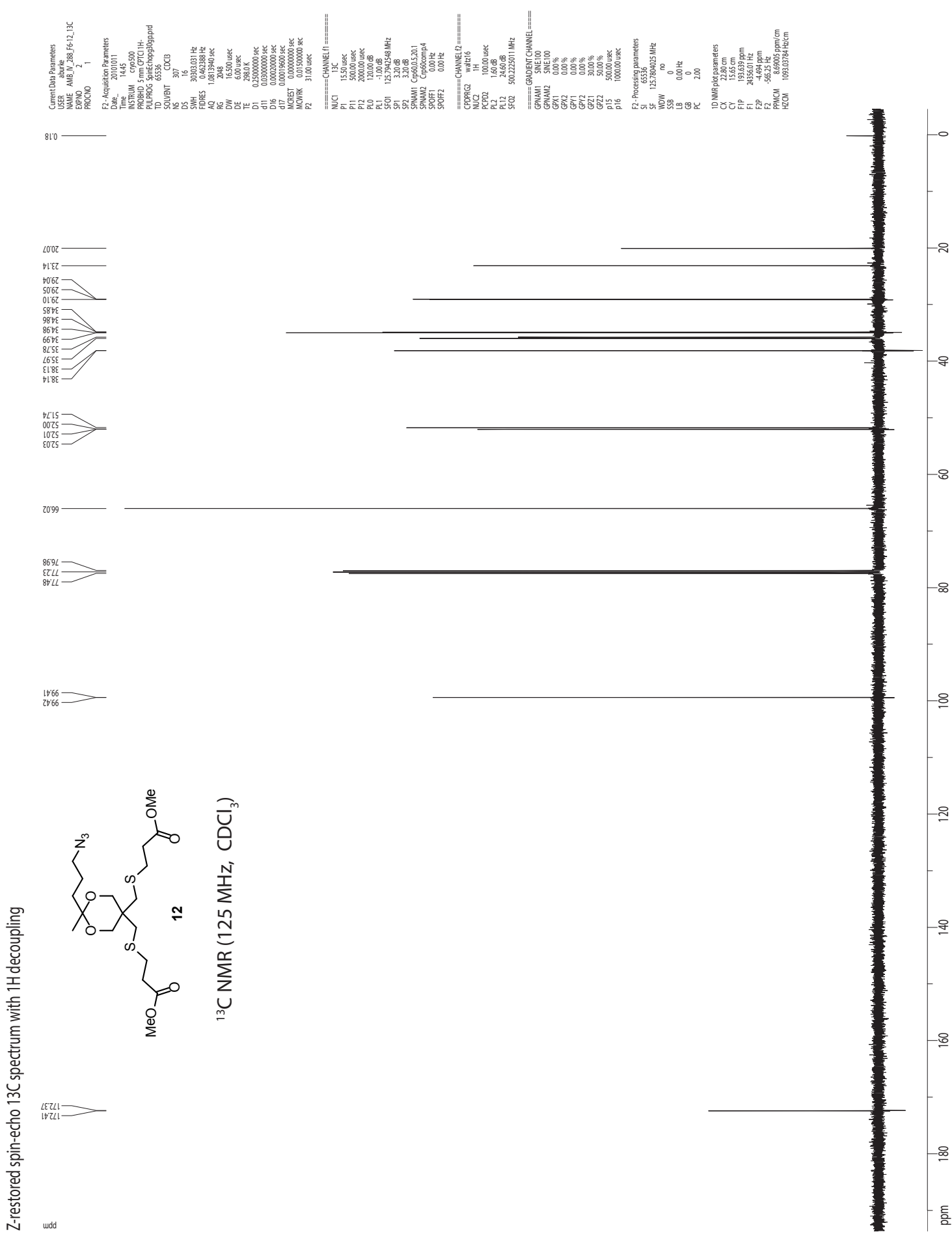

1H spectrum
Current Data Parameters
USER aburke
NAME AMB_V_151_prod
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20110210
Time 7.24
INSTRUM cryo500
PROBHD 5 mm CPTCI 1H-
PULPROG zg30
TD 81728
SOLVENT CDCl3
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.098043 Hz
AQ 5.0998774 sec
RG 4.5
DW 62.400 usec
DE 6.00 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 500.2200274 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 4.00
1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 7.758 ppm
F1 3880.94 Hz
F2P 0.969 ppm
F2 484.78 Hz
PPMCM 0.29778 ppm/cm
HZCM 148.95450 Hz/cm
7.27005
3.79445
3.76637
3.70471
2.83153
2.81696
2.80230
2.73700
2.64988
2.63527
2.62059
1.73767
1.51275
1.50185
1.41503
1.40486
3.92671
5.77641
4.00000
3.86874
4.00071
3.95521
4.12781
2.20931
MeO
OMe
S
S
O
O
O
O
9
^{1}H NMR (500 MHz, $CDCl_3$)
ppm
Integral

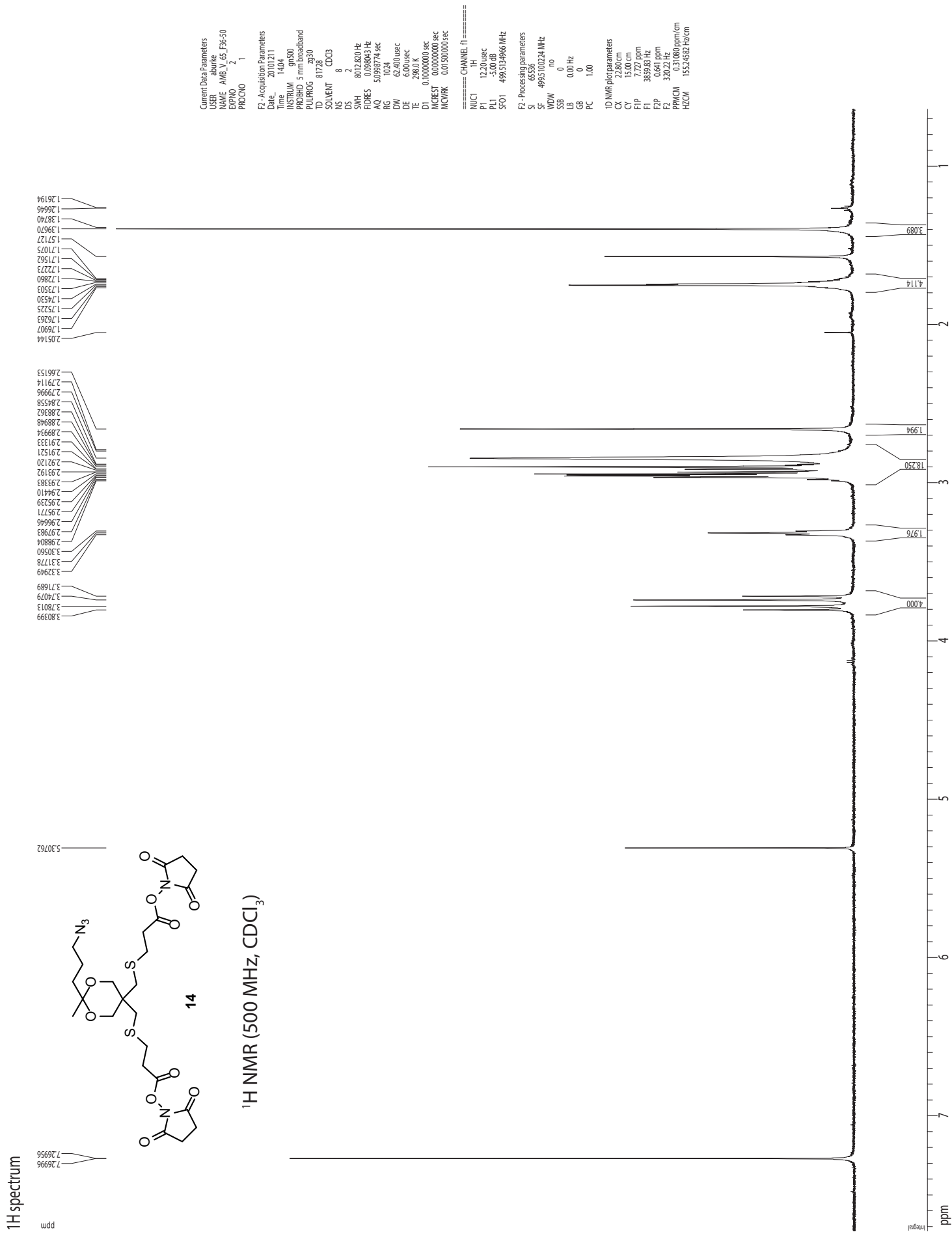

Z-restored spin-echo 13C spectrum with 1H decoupling
^{13}C NMR (125 MHz, $CDCl_3$)
9
MeO
OMe
S
S
O
O
O
O
ppm
172.48
98.63
77.48
77.23
76.98
65.47
52.03
38.44
36.03
34.94
32.77
29.07
25.83
22.69
20
40
60
80
100
120
140
160
180
ppm


1H spectrum
Current Data Parameters
USER aburke
NAME AMB_V_55_F44-77
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20101208
Time 14:36
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 7.1
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 500.2200276 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 4.00
1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 7.667 ppm
F1 3835.25 Hz
F2P 0.633 ppm
F2 311.88 Hz
PPMCM 0.30893 ppm/cm
HZCM 154.53369 Hz/cm
1.59277
2.39288
2.63670
2.65110
2.66530
2.69086
2.82399
2.88810
2.85240
3.66381
3.67523
3.71594
7.22011
7.26883
2.09972
3.69681
3.69678
4.00000
3.72844
5.61718
ppm
Integral
1
2
3
4
5
6
7
OMe
O
S
HO
OH
OH
S
O
MeO
10
1H NMR (500 MHz, CDCl3)


Z-restored spin-echo 13C spectrum with 1H decoupling
ppm
172.72
77.48
77.23
76.97
66.12
52.14
52.13
44.94
35.07
34.84
28.83
OH OH
S
S
OMe
O
MeO
O
10
^{13}C NMR (125 MHz, $CDCl_3$)
Current Data Parameters
USER abburke
NAME AMB_1'_54_F28-38_13C
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20101128
Time_ 13.21
INSTRUM cryo500
PROBHD 5 mm CPTCI 1H-
PULPROG SpinEchopg30pp.prd
TD 65536
SOLVENT CDCl3
NS 287
DS 16
SWH 30303.031 Hz
FIDRES 0.462388 Hz
AQ 1.0813940 sec
RG 7298.2
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 125.7803997 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 2.00
1D NMR plot parameters
CX 22.80 cm
CY 15.65 cm
F1P 192.203 ppm
F1 24175.32 Hz
F2P 11.573 ppm
F2 1455.69 Hz
PPMCM 7.92234 ppm/cm
HZCM 996.47498 Hz/cm
ppm
20
40
60
80
100
120
140
160
180

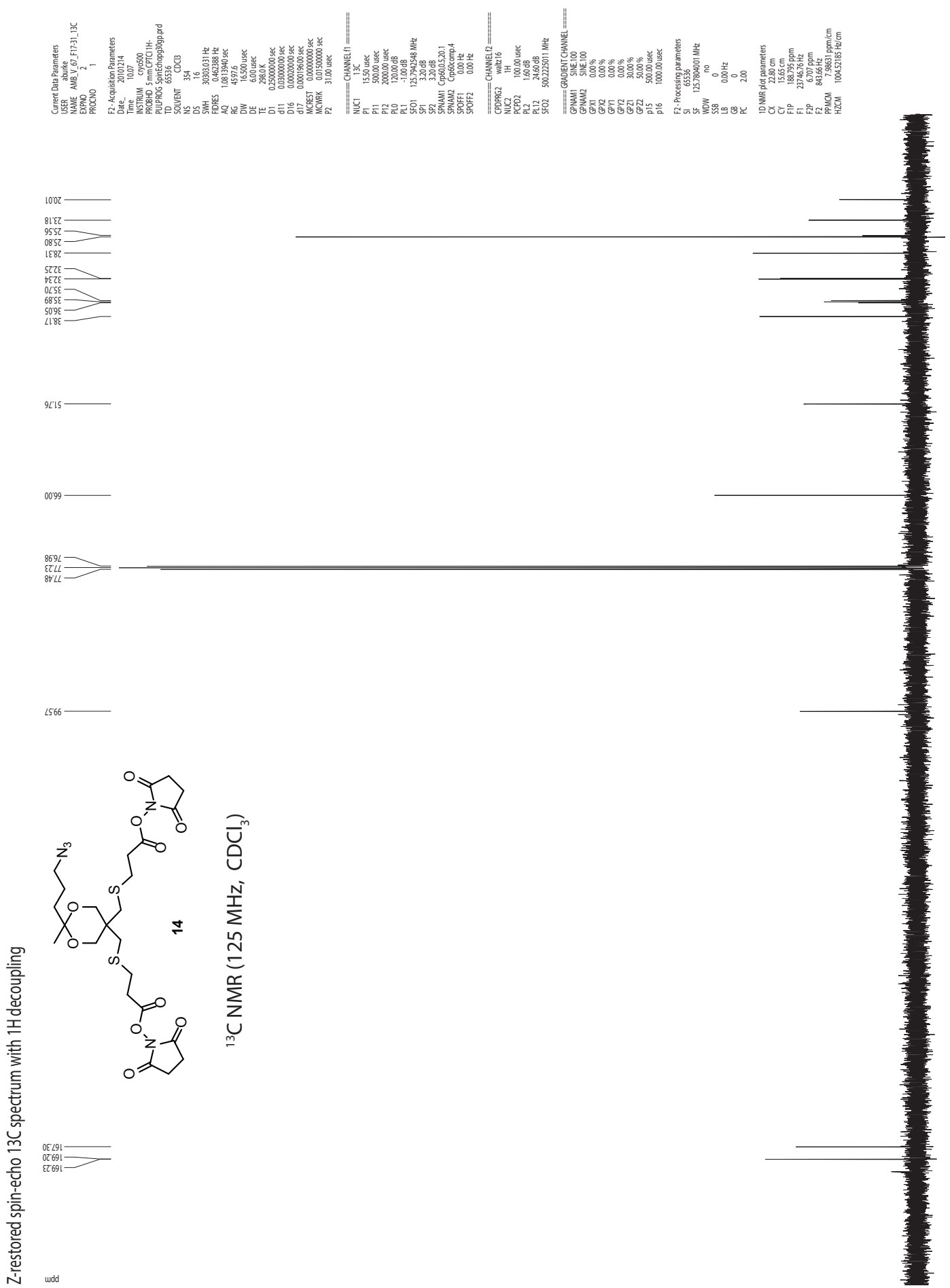

1H spectrum
Current Data Parameters
USER aburke
NAME AMB_V_112_prod
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20110113
Time 10.07
INSTRUM cryo500
PROBHD 5 mm CPTCI 1H-
PULPROG zg30
TD 81728
SOLVENT CDCl3
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.098043 Hz
AQ 5.0998774 sec
RG 4
DW 62.400 usec
DE 6.00 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 500.2200272 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 4.00
1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 7.717 ppm
F1 3859.97 Hz
F2P 0.624 ppm
F2 312.25 Hz
PPMCM 0.23107 ppm/cm
HZCM 115.56019 Hz/cm
1.39236
1.75227
1.74572
2.60869
2.62480
2.63484
2.63915
2.64929
2.66381
2.78666
2.80116
2.81587
2.82939
2.84386
2.85861
3.31728
3.71124
3.72653
3.73824
3.79208
7.27008
3.057
4.193
6.198
6.217
2.322
10.000
integral
ppm
1
2
3
4
5
6
7
ppm
MeO
O
S
S
O
O
O
O
N3
OMe
12
1H NMR (500 MHz, CDCl3)

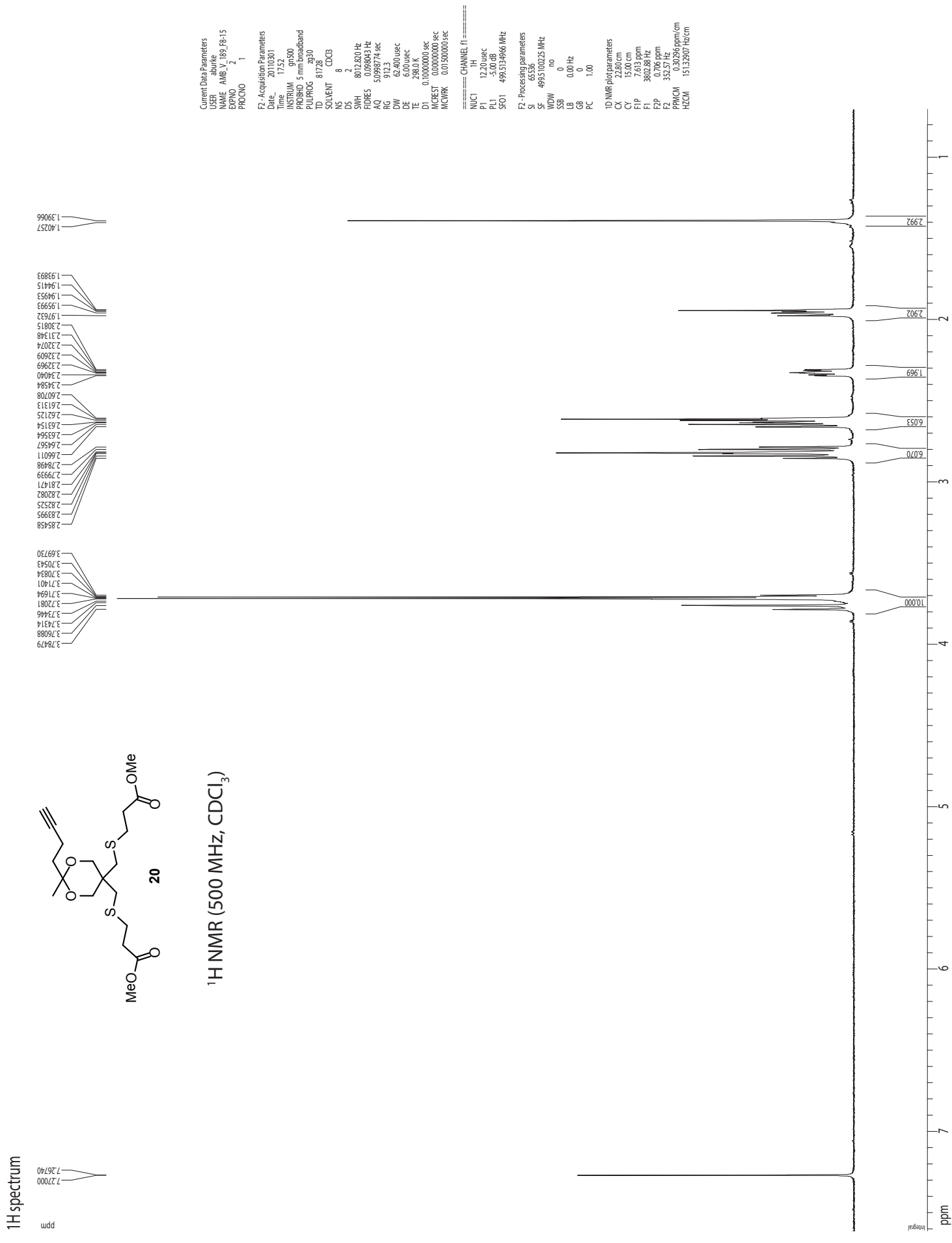

Z-restored spin-echo 13C spectrum with 1H decoupling
^{13}C NMR (125 MHz, $CDCl_3$)
12
MeO
OMe
N_3
S
O
ppm

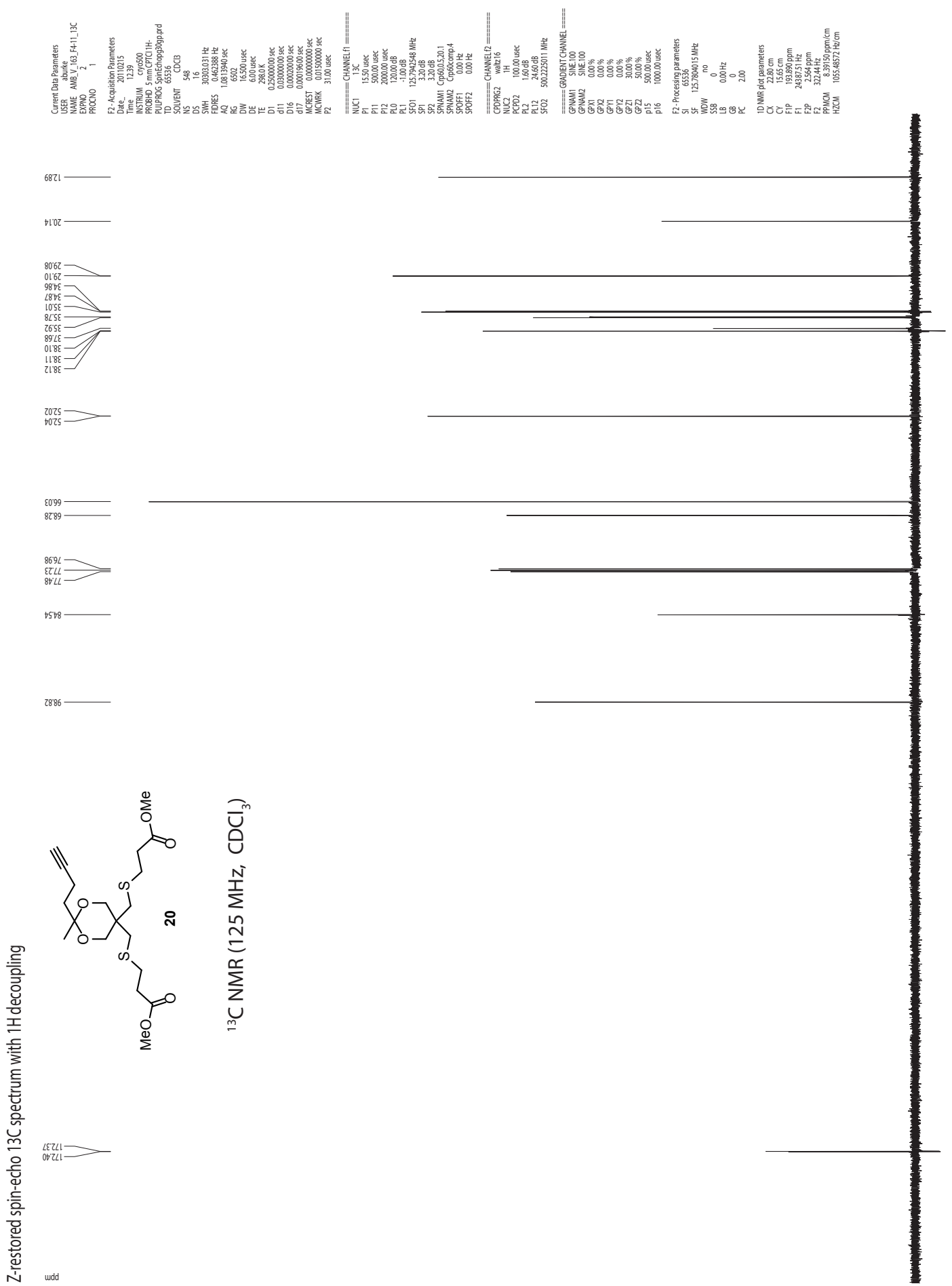


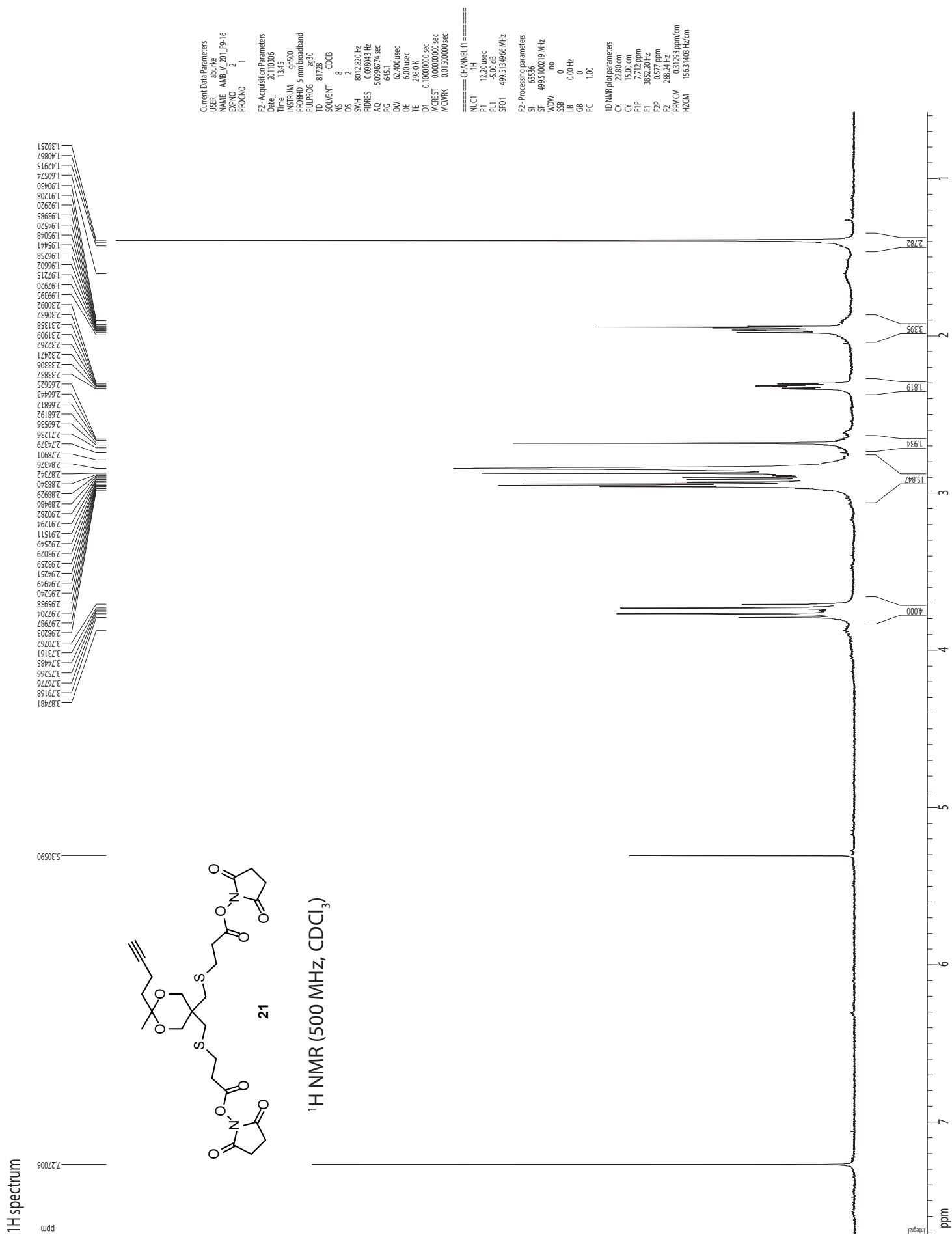
14

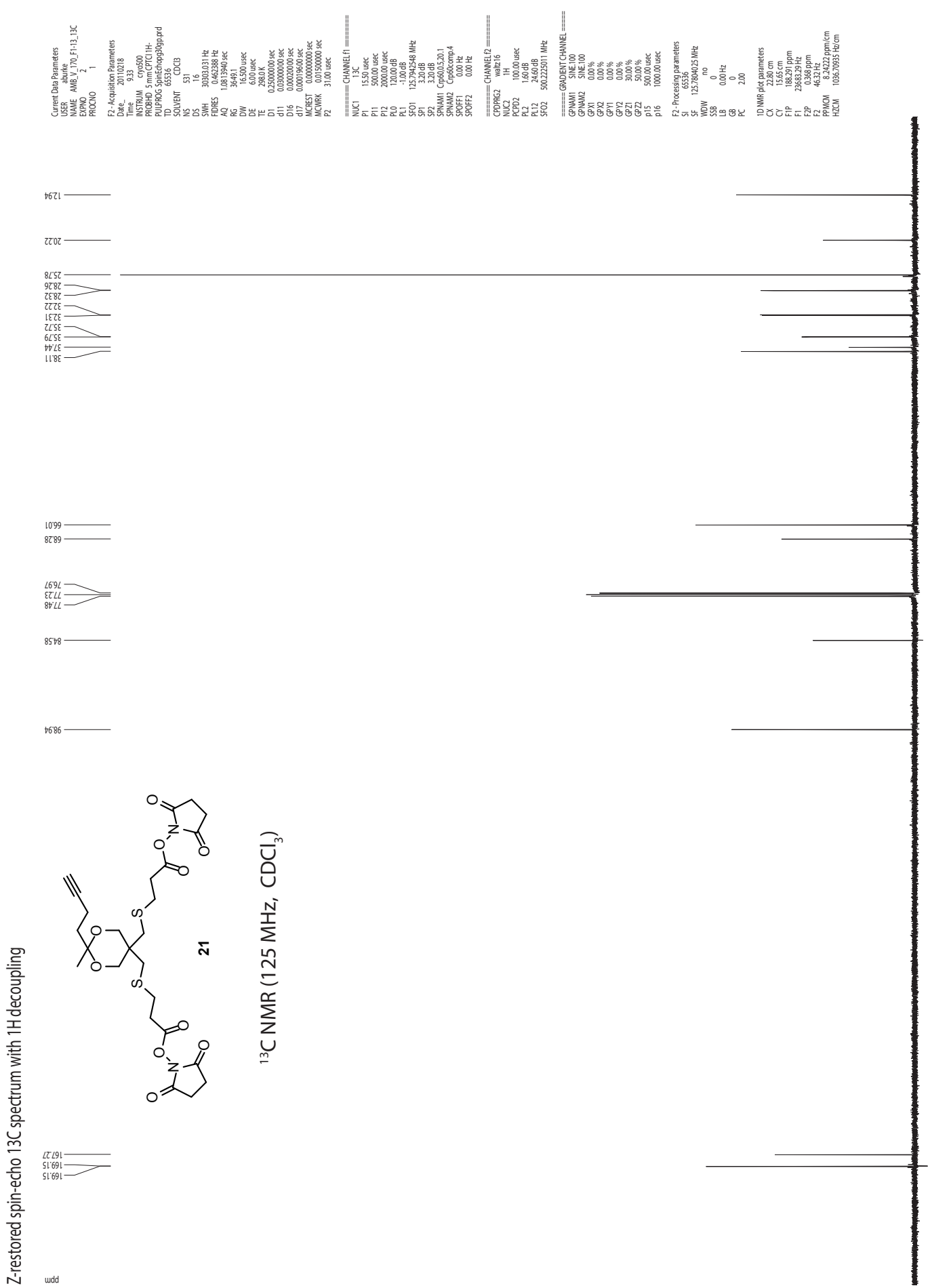
^{1}H NMR (500 MHz, $CDCl_3$)


Z-restored spin-echo 13C spectrum with 1H decoupling
^{13}C NMR (125 MHz, $CDCl_3$)
14
ppm
ppm


1H spectrum
ppm
7.27000
7.26740
1.40257
1.39066
1.93893
1.94415
1.94953
1.95993
1.97632
2.30815
2.31348
2.32074
2.32609
2.32969
2.34040
2.34584
2.60708
2.61313
2.62125
2.63154
2.63564
2.64567
2.66011
2.78498
2.79939
2.81471
2.82082
2.82525
2.83995
2.85458
3.69730
3.70543
3.70834
3.71401
3.71694
3.72081
3.73446
3.74314
3.76088
3.78479
1H NMR (500 MHz, CDCl3)
20
MeO
OMe
S
S
O
O
O
O
Current Data Parameters
USER aburke
NAME AMB_V_189_F8-15
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20110301
Time 17.52
INSTRUM gn500
PROBHD 5 mm broadband
PULPROG zg30
TD 81728
SOLVENT CDCl3
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.098043 Hz
AQ 5.0998774 sec
RG 912.3
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 499.5134966 MHz
F2 - Processing parameters
SI 65536
SF 499.5100225 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.00
1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 7.613 ppm
F1 3802.88 Hz
F2P 0.706 ppm
F2 352.57 Hz
PPMCM 0.30296 ppm/cm
HZCM 1513.2907 Hz/cm
2.992
2.902
1.969
6.053
6.070
10.000
integral
ppm
1
2
3
4
5
6
7


Z-restored spin-echo 13C spectrum with 1H decoupling
ppm
172.40
172.37
98.82
84.54
77.48
77.23
76.98
68.28
66.03
52.04
52.02
38.12
38.11
38.10
37.68
35.92
35.78
35.01
34.87
34.86
29.10
29.08
20.14
12.89
20
40
60
80
100
120
140
160
180
ppm
MeO
OMe
S
S
O
O
O
O
20
^{13}C NMR (125 MHz, $CDCl_3$)


1H spectrum
ppm
7.27006
5.30590
3.87481
3.79168
3.76776
3.75266
3.74485
3.73161
3.70762
2.98203
2.97987
2.97204
2.95938
2.95240
2.94949
2.94251
2.93259
2.93029
2.92549
2.91511
2.91294
2.90282
2.89486
2.88929
2.88340
2.87342
2.84376
2.79901
2.74379
2.71236
2.69536
2.68192
2.66812
2.66443
2.65625
2.33837
2.33306
2.32471
2.32262
2.31909
2.31358
2.30632
2.30092
1.99395
1.97920
1.97215
1.96602
1.96258
1.95441
1.95048
1.94520
1.93985
1.92920
1.91708
1.90430
1.60574
1.42915
1.40867
1.39251
21
1H NMR (500 MHz, CDCl3)
Current Data Parameters
USER aburke
NAME AMB_V_201_F9-16
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20110306
Time 13.45
INSTRUM gn500
PROBHD 5 mm broadband
PULPROG zg30
TD 81728
SOLVENT CDCl3
NS 8
DS 2
SWH 8012.820 Hz
FIDRES 0.098043 Hz
AQ 5.0998774 sec
RG 645.1
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 499.5134966 MHz
F2 - Processing parameters
SI 65536
SF 499.5100219 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.00
1D NMR plot parameters
CX 22.80 cm
CY 15.00 cm
F1P 7.772 ppm
F1 3852.20 Hz
F2P 0.577 ppm
F2 288.24 Hz
PPMCM 0.31293 ppm/cm
HZCM 156.31403 Hz/cm
2.782
3.395
1.819
1.934
15.847
4.000
Integral
ppm
1
2
3
4
5
6
7


Z-restored spin-echo 13C spectrum with 1H decoupling
ppm
169.15
169.15
167.27
98.94
84.58
77.48
77.23
76.97
68.28
66.01
38.11
37.44
35.79
35.72
32.31
32.22
28.32
28.26
25.78
20.22
12.94
21
^{13}C NMR (125 MHz, $CDCl_3$)
Current Data Parameters
USER abURKE
NAME AMB_V_170_F1-13_13C
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20110218
Time_ 9.33
INSTRUM cryo500
PROBHD 5 mm CPTCI 1H-
PULPROG SpinEchopg30gp.prd
TD 65536
SOLVENT CDCl3
NS 531
DS 16
SWH 30303.031 Hz
FIDRES 0.462388 Hz
AQ 1.0813940 sec
RG 36491
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 125.7884025 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 2.00
1D NMR plot parameters
CX 22.80 cm
CY 15.65 cm
F1P 188.291 ppm
F1 23683.29 Hz
F2P 0.368 ppm
F2 46.32 Hz
PPMCM 8.24222 ppm/cm
HZCM 1036.70935 Hz/cm
ppm
20
40
60
80
100
120
140
160