Supplementary Material (ESI) for Molecular BioSystems

Direct Evaluation of Cellular Internalization Rates Using Chromogenic Disulfides

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Supplementary Information

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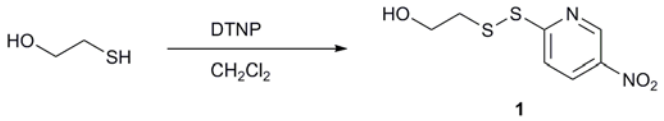
General information. Amino acids, 1-ethyl 3-(dimethylaminopropyl)carbodiimide hydrochloride (EDC), and methylamine hydrochloride were purchased and used as received from Chem-Impex, Inc. 1-Hydroxybenzotriazole hydrate (HOBt·H_2O) was purchased and used as received from Advanced ChemTech. *N,N'*-Carbonyldiimidazole (CDI) was and used as received from Oakwood Products, Inc. Triethylamine was purchased and used as received from EMD Chemicals, Inc. Sodium phosphate monobasic and sodium phosphate dibasic heptahydrate were purchased from FisherBiotech and used directly. Sodium chloride, anhydrous (anhyd.) magnesium sulfate, 29% v/v aqueous ammonium hydroxide, 30% aqueous hydrogen peroxide, and ethylenediaminetetraacetic acid disodium salt (EDTA) were obtained from Mallinckrodt Baker, Inc., and used directly. 5-Nitropyridin-2-thiolate was obtained following chromatographic purification of disulfide exchange reactions of thiols with 2,2'-dithiobis(5-nitropyridine) (DTNP). Luria-Bertani (LB) agar (casein peptone 10 g L^{-1}, yeast extract 5 g L^{-1}, sodium chloride 5 g L^{-1}, agar 15 g L^{-1}), LB broth (casein peptone 10 g L^{-1}, yeast extract 5 g L^{-1}, sodium chloride 5 g L^{-1}), sodium dodecyl sulfate (SDS), and Tris-HCl were purchased from USB Corporation. DTNP, Ellman's reagent, and all other chemicals were purchased from Aldrich Chemical Company and Acros Organics. Deuterated solvents were obtained from Cambridge Isotope Laboratories, Inc. All other solvents were purchased from either Mallinckrodt Baker, Inc., or Aldrich Chemical Company and used directly. Column chromatography was performed on silica gel (230–400 mesh) from Whatman. Thin layer chromatography (TLC) was carried out on F-254 glass plates from Dynamic Adsorbent Inc. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova-300 MHz spectrometer. Chemical shifts are expressed in parts per million (ppm) from dimethylsulfoxide-d_6 (DMSO-d_6) (δ_H = 2.50, δ_C = 39.51), methanol-d_4 (CD_3OD) (δ_H = 3.31, δ_C = 49.00), chloroform-*d* ($CDCl_3$) (δ_H = 7.26, δ_C = 77.16), or tetramethylsilane (Me_4Si) (δ_H = 0.00, δ_C = 0.00). Coupling constant (*J*) values are expressed in hertz (Hz). Infrared (IR) data were recorded on a Perkin Elmer FT-IR. Optical rotation ($[\alpha]_D$) values were recorded on a Rudolph Research Autopol III automatic polarimeter using a Rudolph Research Analytical polarimeter cell (32-2.5-100-0.5), and are reported in 10^{-1} deg cm^2 g^{-1}. Concentrations (*c*) used for specific rotation are given in g $(100\ mL)^{-1}$. Water used for making buffers and performing cellular experiments was purified using a Nanopure Infinity ultrapure water system and autoclaved to sterilize before use. Microcentrifuge tubes, disposable polystyrene petri dishes, automatic pipets, and disposable pipet tips were purchased from VWR International. Falcon polypropylene conical

centrifuge tubes were purchased from Becton Dickinson and Company. Cell cultures were grown and agitated in a Lab-Line Max Q 4000 incubated and refrigerated shaker. Absorbance measurements of solutions and cell suspensions were obtained on a Shimadzu BioSpec-mini DNA/RNA/Protein analyzer. Absorbance data for kinetic experiments, concentration measurements, and spectra were collected using a Bio-Tek Synergy HT multi-detection microplate reader, Bio-Tek KC4 V.3.4 software, and transparent, flat-bottomed 96-well microtiter plates (Nunc). Graphical analyses of the resulting data were performed using KaleidaGraph V.4 (Synergy Software).

Strains. Cell studies were performed with BL21Star(DE3) (Invitrogen) and Origami(DE3)(Δ*trxB*; Δ*gor*) (Novagen) *Escherichia coli* (*E. coli*) strains.

Synthesis and characterization of chromogenic disulfide probes 1–14 and disulfide 15

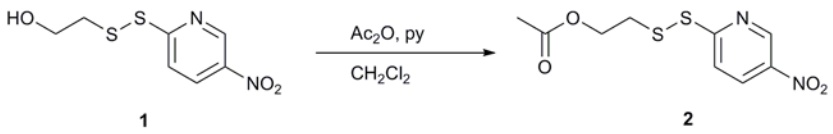
General procedure for disulfide exchange. DTNP (1.5 mol eq.) was dissolved in CH_2Cl_2 to give a 0.2 M solution. A thiol (1 mol eq.) was dissolved in the same volume of CH_2Cl_2, and added dropwise to the stirred solution at room temperature under argon atmosphere. The reaction mixture was stirred until Ellman's reagent[1] indicated full consumption of the thiol. The reaction mixture was concentrated *in vacuo* and subjected to further purification.



1

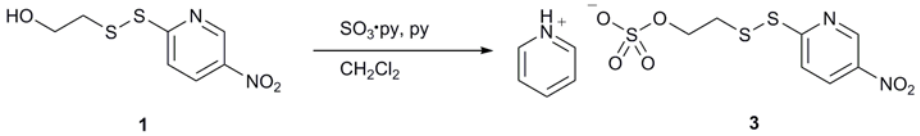
2-((5-Nitropyridin-2-yl)disulfanyl)ethanol (1). Following the general disulfide exchange procedure with 2-mercaptoethanol (83 μL, 0.97 mmol) and silica gel flash chromatography (40% EtOAc in hexanes), the disulfide product was recrystallized from 1:1 v/v ethyl acetate (EtOAc)/*n*-heptane to provide **1** (110 mg, 49%) as a light yellow solid; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3369, 3086, 2926, 2872, 1589, 1566, 1515, 1438, 1344, 1098, 856, 750 cm^{-1}; δ_H(300 MHz; $CDCl_3$; Me_4Si) 3.01 (1 H, t, *J* 5.4, CH_2), 3.79 (br t, *J* 5.4, CH_2), 4.09 (1 H, br s, OH), 7.66 (1 H, d, *J* 8.8, ArH), 8.37 (1 H, dd, *J* 8.8 and 2.6, ArH), and 9.34 (1 H, d, *J* 2.6, ArH); δ_C(75 MHz; $CDCl_3$;

$CDCl_3$) 42.34, 58.64, 120.71, 131.42, 142.40, 145.24, and 167.59; *m/z* (EI) 233.0052 (M^+ + H, $C_7H_9N_2O_3S_2$ requires 233.0055).



1 2

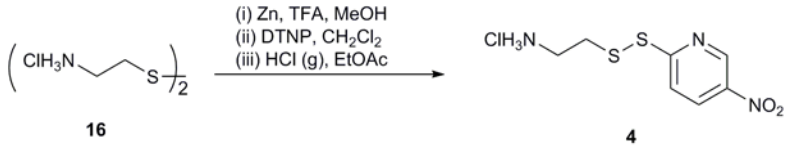
2-((5-Nitropyridin-2-yl)disulfanyl)ethyl acetate (2). Alcohol **1** (65 mg, 0.28 mmol) was combined with pyridine (113 μL, 1.40 mmol) in CH_2Cl_2 (1 mL), the solution was placed in an ice/water bath. Acetic anhydride (79 μL, 1.8 mmol) was added dropwise to the chilled reaction mixture, which was subsequently allowed to warm to room temperature over 1 h with stirring. The reaction mixture was diluted with EtOAc and washed with 1 M HCl, saturated (sat.) $NaHCO_3$, and brine. The organic layer was dried over anhyd. $MgSO_4$ and concentrated *in vacuo*. Silica gel flash chromatography (20% EtOAc in hexanes) provided **2** (62 mg, 81%) as a light yellow, viscous oil; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3087, 2949, 2850, 1741, 1589, 1566, 1518, 1438, 1345, 1226, 1098, 856, 750; δ_H(300 MHz; $CDCl_3$; Me_4Si) 2.07 (3 H, s, CH_3), 3.09 (2 H, t, *J* 6.5, CH_2), 4.34 (2 H, t, *J* 6.5, CH_2), 7.88 (1 H, d, *J* 8.8, ArH), 8.42 (1 H, dd, *J* 8.8 and 2.3, ArH), and 9.29 (1 H, d, *J* 2.3, ArH); δ_C(75 MHz; $CDCl_3$; $CDCl_3$) 20.71, 37.24, 61.81, 119.24, 131.57, 142.02, 145.08, 168.00, and 170.54; *m/z* (ESI) 275.0162 (M^+ + H. $C_9H_{11}N_2O_4S_2$ requires 275.0160).



1 3

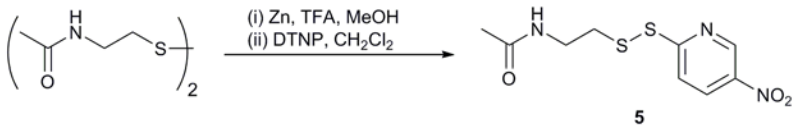
Pyridinium 2-((5-nitropyridin-2-yl)disulfanyl)ethyl sulfate (3). Alcohol **1** (20 mg, 0.086 mmol) was combined with sulfur trioxide pyridine complex (41 mg, 0.26 mmol) and pyridine (7.0 μL, 0.086 mmol) in CH_2Cl_2 (2.2 mL) at room temperature under N_2. The reaction was allowed to stir 12 h until consumption of **1** was verified by TLC. Insoluble pyridine sulfur trioxide complex removed by filtration, and volatile components were removed *in vacuo*, giving **3** (0.032 g, 95%) as a light yellow solid; ν_{max}(methanol (MeOH))/cm^{-1} 3074, 2951, 2889, 1589, 1566, 1517, 1347, 1195, 1048, 996, 856, 750; δ_H(300 MHz; CD_3OD; CD_3OD) 3.19 (2 H, t, *J* 6.0,

CH_2), 4.21 (2 H, t, *J* 6.0, CH_2), 8.12–8.17 (3 H, m, ArH), 8.54 (1 H, dd, *J* 8.8 and 2.8, ArH), 8.69 (1 H, tt, *J* 7.9 and 1.4, ArH), 8.90 (2 H, d, *J* 5.1, ArH), and 9.20 (1 H, d, *J* 2.8, ArH); δ_C(75 MHz; CD_3OD; CD_3OD) 39.71, 66.32, 120.90, 128.79, 133.47, 143.02, 143.78, 145.74, 148.30, and 169.46; *m/z* (ESI) 310.9472 (M^-. $C_7H_7N_2O_6S_3$ requires 310.9466).



16 4

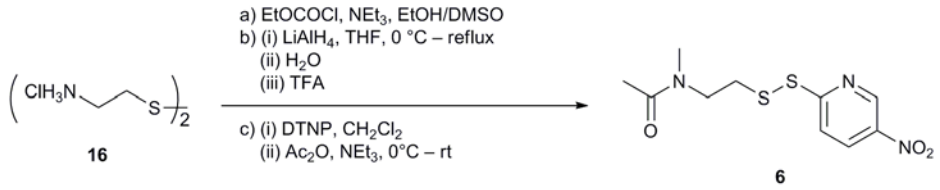
2-((5-Nitropyridin-2-yl)disulfanyl)ethylamine hydrochloride (4). Cystamine dihydrochloride (0.010 g, 0.044 mmol) was combined with zinc dust (< 10 micron, 144 mg, 2.20 mmol) in MeOH (1.5 mL). TFA (339 μL, 4.60 mmol) was added, and the suspension was sonicated at room temperature for 1 h in a vented reaction vessel. After the solids were removed via filtration through Celite and volatile components were removed *in vacuo*, the crude thiol was subjected to the general disulfide exchange procedure. The residue isolated after silica gel flash chromatography (20% MeOH in EtOAc) was redissolved in EtOAc and converted into a hydrochloride salt by bubbling HCl gas through the solution. Filtration, washing with cold EtOAc and drying in air of the precipitate provided **4** (125 mg, 53%) as a white powder; ν_{max}(KBr)/cm^{-1} 3183, 3080, 1587, 1561, 1509, 1342, 1098, 1020, 1000, 856, 748; δ_H(300 MHz; DMSO-d_6; DMSO-d_6) 3.13 (4 H, m, 2 × CH_2), 8.05 (1 H, d, *J* 8.8, ArH), 8.11 (3 H, br s, NH_3), 8.58 (1 H, dd, *J* 8.8, ArH), and 9.30 (1 H, d, *J* 2.2, ArH); δ_C(75 MHz; DMSO-d_6; DMSO-d_6) 34.95, 37.81, 120.26, 132.76, 142.49, 145.12, and 166.30; *m/z* (ESI) 232.0215 (M^+. $C_7H_{10}N_3O_3S_2$ requires 232.0214).



5

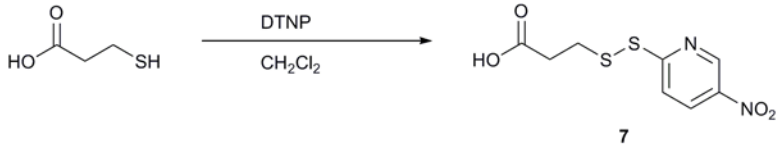
***N*-(2-((5-Nitropyridin-2-yl)disulfanyl)ethyl)acetamide (5).** Prepared as described for **4** from *N*,*N*'-diacetylcystamine[2] (100 mg, 0.44 mmol). Silica gel flash chromatography (5% MeOH in EtOAc) and recrystallization (EtOAc/*n*-heptane) provided **5** (43 mg, 18%) as a white crystalline

solid; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3281, 3084, 2930, 1652, 1588, 1566, 1516, 1437, 1344, 1097, 856, 750; δ_H(300 MHz; $CDCl_3$; Me_4Si) 2.04 (3 H, s, CH_3), 2.98 (2 H, t, *J* 6.0, CH_2), 3.56 (2 H, q, *J* 6.0, CH_2), 6.40 (1 H, br s, NH), 8.05 (1 H, d, *J* 8.6, ArH), 8.41 (1 H, dd, *J* 8.6 and 2.8, ArH), and 9.30 (1 H, d, *J* 2.5, ArH); δ_C(75 MHz; $CDCl_3$; $CDCl_3$), 23.29, 37.75, 38.49, 119.95, 131.59, 142.25, 145.15, 167.66, and 170.24; *m/z* (ESI) 296.0142 (M^+ + Na. $C_9H_{11}N_3O_3S_2Na$ requires 296.0140).



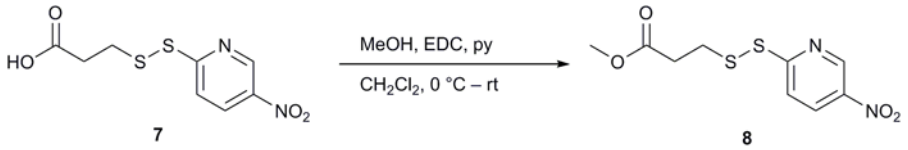
***N*-Methyl-*N*-(2-((5-nitropyridin-2-yl)disulfanyl)ethyl)acetamide (6).** Cystamine dihydrochloride (0.22 g, 0.98 mmol) was suspended in ethanol (EtOH) (4 mL). DMSO (1 mL) and triethylamine (820 μL, 5.88 mmol) were added to provide a monophasic solution, which was placed under argon atmosphere and chilled to 0˚C. Ethyl chloroformate (281 μL, 2.94 mmol) was added via a syringe, and the reaction mixture was allowed to warm up to room temperature over 1 h. Crude diethyl carbamate derivative of cystamine, which was isolated as a clear oil through the standard extraction procedure (1 M HCl, sat. $NaHCO_3$, and brine), was dissolved in anhyd. tetrahydrofuran (THF) (5 mL) and combined with lithium aluminum hydride (372 mg, 9.80 mmol) at 0˚C. The suspension was refluxed for 2 h until the disulfide was completely consumed as detected by TLC. Unreacted aluminum hydrides were quenched with water (1 mL) by a dropwise addition to the chilled and stirred suspension. Crude 2-(methylamino)ethanethiol (stench!) was isolated as trifluoroacetate salt through filtration, addition of TFA (1 mL) and distillation of volatile components from the resulting solution. The oily material was then subjected to the general disulfide exchange procedure. Upon thiol consumption, the reaction mixture was chilled to 0˚C, prior to the addition of acetic anhydride (278 μL, 2.94 mmol) and triethylamine (683 μL, 4.90 mmol). The reaction mixture was allowed to warm up to room temperature over 1 h, and then subjected to the standard extraction procedure (1 M HCl, sat. $NaHCO_3$, and brine) and silica gel flash chromatography (5% MeOH in EtOAc) to give **6** (39 mg, 7%) as a viscous oil and a 3:1 mixture of rotamers; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3078, 2928, 1640,

1588, 1566, 1516, 2437, 1404, 1344, 1097, 855, 750; δ_H(300 MHz; $CDCl_3$; Me_4Si) 2.09 and 2.12 (3 H, s, CH_3), 2.98 and 3.06 (2 H, t, *J* 7.0, CH_3), 2.93 and 3.08 (3 H, s, CH_3), 3.64 and 3.67 (2 H, t, *J* 7.0, CH_2), 7.84 and 7.90 (1 H, d, *J* 8.8, ArH), 8.42 and 8.44 (1 H, dd, *J* 8.8 and 2.8, ArH), and 9.29 and 9.32 (1 H, d, *J* 2.8, ArH); δ_C(75 MHz; $CDCl_3$; $CDCl_3$) 21.34 and 21.86, 33.29 and 35.81, 36.25 and 37.34, 47.24 and 49.74, 119.29 and 119.55, 131.67 and 131.77, 142.01 and 142.26, 145.12 and 145.31, 167.24 and 168.23, and 170.33 and 170.95; *m/z* (ESI) 310.0292 (M^+ + Na. $C_{10}H_{13}N_3O_3S_2Na$ requires 310.0296).



7

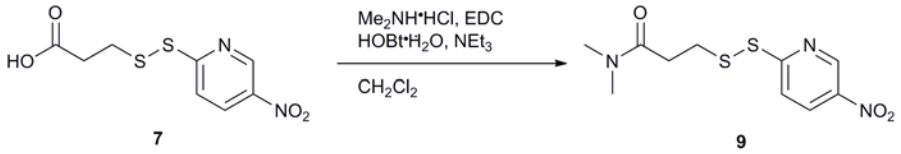
3-((5-Nitropyridin-2-yl)disulfanyl)propanoic acid (7). Following the general disulfide exchange procedure with 3-mercaptopropionic acid (56 μL, 0.64 mmol) and silica gel flash chromatography (EtOAc), the disulfide product was recrystallized from 1:1 v/v EtOAc/*n*-heptane to provide **7** (0.12 g, 72%) as off-white needles; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3091, 2890, 1702, 1592, 1566, 1508, 1343, 1096, 856, 748; δ_H(300 MHz; $CDCl_3$; Me_4Si) 2.84 (2 H, t, *J* 7.0, CH_2), 3.11 (2 H, t, *J* 7.0, CH_2), 7.88 (1 H, d, *J* 8.8, ArH), 8.42 (1 H, dd, *J* 8.8 and 2.3, ArH), 9.29 (1 H, d, *J* 2.3, ArH), and 10.56 (1 H, br s, CO_2H); δ_C(75 MHz; $CDCl_3$; $CDCl_3$) 33.15, 33.50, 119.49, 131.74, 142.16, 145.15, 167.88, and 177.14; *m/z* (ESI) 261.0006 (M^+ + H. $C_8H_9N_2O_4S_2$ requires 261.0004).



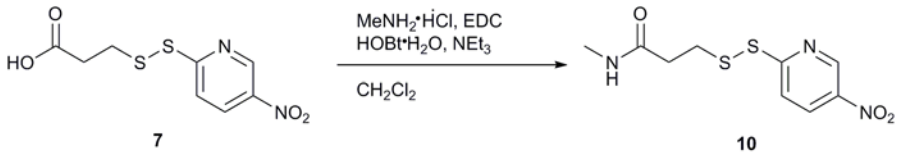
7 8

Methyl 3-((5-nitropyridin-2-yl)disulfanyl)propanoate (8). Carboxylic acid **7** (10 mg, 0.038 mmol) was combined with MeOH (5.0 μL, 0.12 mmol), EDC (15 mg, 0.077 mmol), and pyridine (9.3 μL, 0.12 mmol) in CH_2Cl_2 (0.3 mL) at 0 °C with stirring. The reaction mixture was allowed to warm to room temperature and stir for 8 h until TLC indicated complete consumption of **7**.

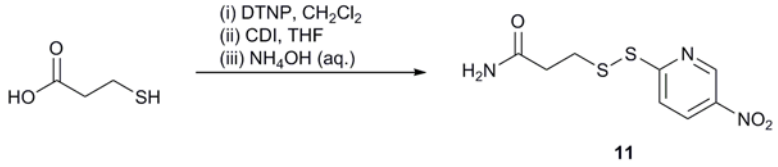
The reaction mixture was diluted with EtOAc and washed with 1 M HCl, sat. $NaHCO_3$, and brine. The organic layer was dried over anhyd. $MgSO_4$ and concentrated *in vacuo* to give **8** (10 mg, 97%) as an off-white solid; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3454, 3088, 2952, 2849, 1736, 1589, 1566, 1517, 1437, 1344, 1097, 855, 750; δ_H(300 MHz; $CDCl_3$; Me_4Si) 2.77 (2 H, t, *J* 7.0, CH_2), 3.10 (2 H, t, *J* 7.0, CH_2), 3.71 (3 H, s, CH_3), 7.89 (1 H, d, *J* 8.8, ArH), 8.42 (1 H, dd, *J* 8.8 and 2.6 Hz, ArH), and 9.29 (1 H, d, *J* 2.6, ArH); δ_C(75 MHz; $CDCl_3$; $CDCl_3$) 33.40, 51.95, 119.25, 131.60, 142.01, 145.07, 168.13, and 171.53; *m/z* (ESI) 275.0158 (M^+ + H. $C_9H_{11}N_2O_4S_2$ requires 275.0160).



***N,N'*-Dimethyl-3-((5-nitropyridin-2-yl)disulfanyl)propanamide (9).** Carboxylic acid **7** (80 mg, 0.31 mmol) was combined with dimethylamine hydrochloride (50 mg, 0.62 mmol), $HOBt{\cdot}H_2O$ (141 mg, 0.920 mmol), EDC (118 mg, 0.620 mmol), and triethylamine (129 μL, 0.920 mmol) in CH_2Cl_2 (3 mL). The reaction mixture was stirred at room temperature for 2 h until TLC indicated complete consumption of **7**. The reaction mixture was diluted with EtOAc and washed with 1 M HCl, sat. $NaHCO_3$, and brine. The organic layer was dried over anhyd. $MgSO_4$ and concentrated *in vacuo*. Recrystallization from 1:1 v/v EtOAc/*n*-heptane provided **9** (42 mg, 48%) as off-white needles; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3099, 2931, 1637, 1588, 1567, 1508, 1340, 1097, 856, 840, 750; δ_H(300 MHz; $CDCl_3$; Me_4Si) 2.77 (2 H, t, *J* 7.0, CH_2), 2.96 (3 H, s, CH_3), 2.98 (3 H, s, CH_3), 3.14 (2 H, t, *J* 7.0, CH_2), 7.97 (1 H, d, *J* 8.8, ArH), 8.42 (1 H, dd, *J* 8.8 and 2.8, ArH), and 9.27 (1 H, d, *J* 2.8, ArH); δ_C(75 MHz; $CDCl_3$; $CDCl_3$) 32.57, 34.18, 35.40, 36.91, 119.22, 131.60, 141.89, 144.99, 168.78, and 170.03; *m/z* (ESI) 310.0300 (M^+ + Na. $C_{10}H_{13}N_3O_3S_2Na$ requires 310.0296).

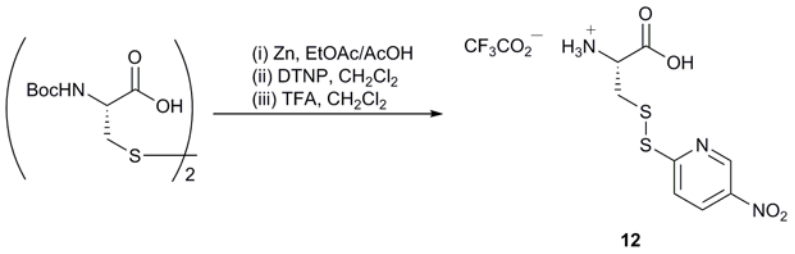


***N*-Methyl-3-((5-nitropyridin-2-yl)disulfanyl)propanamide (10).** Prepared as described for **9** with carboxylic acid **7** (55 mg, 0.21 mmol) and methylamine hydrochloride (28 mg, 0.42 mmol). Silica gel flash chromatography (EtOAc) and recrystallization from 1:1 v/v EtOAc/*n*-heptane provided **10** (30 mg, 53%) as a white crystalline solid; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3315, 3097, 2941, 1642, 1591, 1567, 1515, 1345, 1096, 857, 748; δ_H(300 MHz; $CDCl_3$; Me_4Si) 2.61 (2 H, t, *J* 6.8, CH_2), 2.85 (3 H, d, *J* 5.1, CH_3), 3.14 (2 H, t, *J* 6.8, CH_2), 5.77 (1 H, br s, NH), 7.90 (1 H, d, *J* 8.8, ArH), 8.42 (1 H, dd, *J* 8.8 and 2.3, ArH), and 9.28 (1 H, d, *J* 2.3, ArH); δ_C(75 MHz; $CDCl_3$; $CDCl_3$) 26.39, 34.20, 35.29, 119.44, 131.67, 142.04, 145.05, 168.32, and 170.61; *m/z* (ESI) 296.0141 (M^+ + Na. $C_9H_{11}N_3O_3S_2Na$ requires 296.0140).



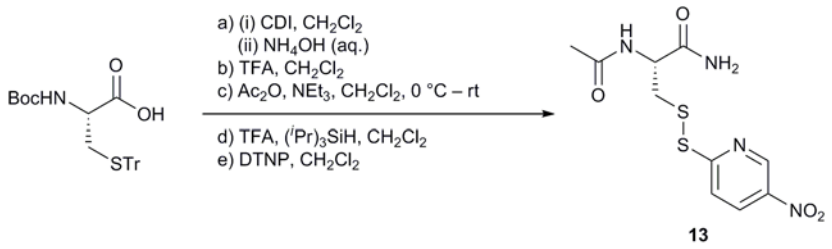
3-((5-Nitropyridin-2-yl)disulfanyl)propanamide (11). Carboxylic acid **7** was prepared using the general disulfide exchange procedure using 3-mercaptopropionic acid (21 μL, 0.24 mmol). Crude **7** was dissolved in THF (0.94 mL), and CDI (0.10 g, 0.62 mmol) was added portion-wise to the stirring reaction. Upon cessation of CO_2 evolution, 29% v/v aqueous ammonium hydroxide (52 μL, 0.94 mmol) was added, and the reaction was allowed to stir under N_2 for 14 h, at which point TLC confirmed consumption of **7**. The reaction was concentrated *in vacuo*, dissolved in EtOAc, and washed with 1 M HCl, sat. $NaHCO_3$, and brine. The EtOAc layer was dried over anhyd. $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting crude product was purified by silica gel flash chromatography (25% EtOAc in hexanes to 100% EtOAc) to give **11** (27 mg, 45%) as a yellow solid in a 9:1 mixture with an inseparable material by ^{1}H NMR, presumably a conformational isomer; ν_{max}(KBr)/cm^{-1} 3390, 3175, 3087, 2930, 1682, 1651, 1593, 1567, 1514, 1418, 1347, 1096, 857, 750; δ_H(300 MHz; DMSO-d_6; DMSO-d_6) 2.51 (2 H, t, *J* 6.8,

CH_2) 3.05 and 3.38 (2 H, t, *J* 6.8, CH_2), 6.93 (1 H, br s, NH_2), 7.37 (1 H, br s, NH_2), 7.55 and 8.01 (1 H, d, *J* 8.8, ArH), 8.35 and 8.53 (1 H, dd, *J* 8.8, ArH), and 9.22 (1 H, d, *J* 2.9, ArH); δ_C(75 MHz; DMSO-d_6; DMSO-d_6) 25.63, 34.25, 121.70, 131.20, 141.17, 144.84, 166.98, and 172.11; *m/z* (ESI) 281.9989 (M^+ + Na. $C_8H_9N_3O_3S_2Na$ requires 281.9983).

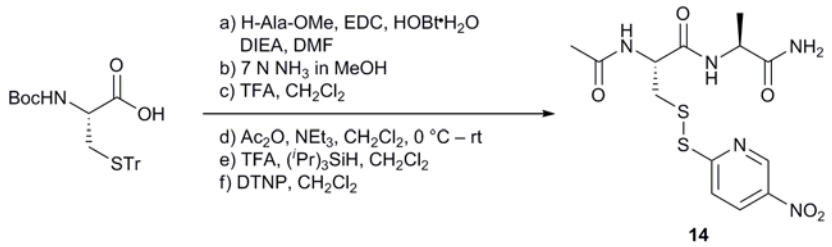


12

H-L-Cys(SNpy)-OH (12). Zinc dust (< 10 micron, 178 mg, 2.72 mmol) was added to a solution of Boc_2-L-cystine[3] (120 mg, 0.272 mmol) in 3:2 v/v EtOAc/acetic acid (AcOH), and the resulting suspension was sonicated until consumption of the cystine disulfide and emergence of Boc-L-cysteine, as detected with Ellman's reagent. The suspension was diluted, filtered through Celite, and the filtrate concentrated *in vacuo*. The residue was treated according to the general disulfide exchange procedure. Following silica gel chromatography (25% EtOAc, 0.1% AcOH in hexanes to 0.1% AcOH in EtOAc), Boc-L-Cys(SNpy)-OH was isolated as a yellow glassy semi-solid (105 mg, 51%). Boc-L-Cys(SNpy)-OH (100 mg, 0.266 mmol) was treated with 1:1 v/v TFA/CH_2Cl_2 (1.6 mL each) for 1 h. The reaction was concentrated *in vacuo* to give **12** (103 mg, 50% overall) as a sticky yellow solid; $[\alpha]_D^{20}$= +48.4 (*c* = 3.49 in MeOH); ν_{max}(MeOH)/cm^{-1} 2927, 1674, 1592, 1567, 1519, 1346, 1199, 1137, 1099, 857, 750, 722; δ_H(300 MHz; CD_3OD; CD_3OD) 3.36 (1 H, dd, 14.8 and 8.8, CH_2), 3.58 (1 H, dd, *J* 14.8 and 4.2 Hz, CH_2), 4.29 (1 H, dd, *J* 8.8 and 4.2, CH), 7.89 (1 H, d, *J* 8.8, ArH), 8.53 (1 H, dd, *J* 8.8 and 2.3, ArH), and 9.35 (1 H, d, *J* 1.8, ArH); δ_C(75 MHz; CD_3OD; CD_3OD) 39.77, 53.01, 122.26, 133.43, 144.52, 146.45, 166.78, and 169.95; *m/z* (ESI) 276.0116 (M^+. $C_8H_{10}N_3O_4S_2$ requires 276.0113).

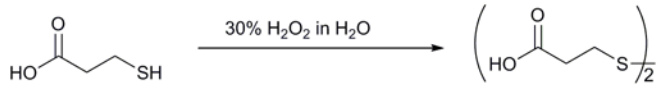


Ac-L-Cys(SNpy)-NH_2 (13). CDI (378 mg, 2.33 mmol) was added to a solution of Boc-L-Cys(Tr)-OH (900 mg, 1.94 mmol) in CH_2Cl_2 (10 mL) at room temperature. Upon the cessation of CO_2 evolution (15 min), 29% v/v aqueous ammonium hydroxide (1.0 mL, 19 mmol) was added, and the biphasic mixture was stirred for 1 h. After a conventional extraction sequence (1 M HCl, sat. $NaHCO_3$, and brine), crude Boc-L-Cys(Tr)-NH_2 (white solid) was treated with 1:1 v/v TFA/CH_2Cl_2 mixture (2 mL each) for 30 min to provide an intensely yellow solution. Evaporation of volatile components, followed by removal of residual TFA by azeotrope with toluene provided trifluoroacetate salt of H-L-Cys(Tr)-NH_2 as a clear oil. The latter was dissolved in CH_2Cl_2 (5 mL), chilled to 0°C in an ice/water bath, and combined with triethylamine (1.35 mL, 9.70 mmol) and acetic anhydride (0.55 mL, 5.8 mmol). The reaction mixture was allowed to warm up to room temperature over 1 h, after which the CH_2Cl_2 solution was diluted with EtOAc, extracted with 1 M HCl, sat. $NaHCO_3$, and brine, dried over anhyd. $MgSO_4$, and concentrated *in vacuo*. Recrystallization from EtOAc/*n*-heptane provided Ac-L-Cys(Tr)-NH_2 as a white solid (575 mg, 73% from Boc-L-Cys(Tr)-OH). Treatment of the trityl-protected cysteine derivative (100 mg 0.25 mmol) with 1:1 v/v TFA/CH_2Cl_2 mixture (2 mL each) in the presence of triisopropylsilane (154 μL, 0.752 mmol) provided *N*-acetyl-L-cysteinamide[4] upon evaporation of the volatiles *in vacuo*. This crude residue was subjected to the general disulfide exchange procedure to give **13** (35 mg, 35%) as a white solid following silica gel flash chromatography (EtOAc) and flash precipitation from EtOAc into *n*-heptane; $[\alpha]_D^{20}$= –30.5 (*c* = 1.29 in MeOH); ν_{max}(MeOH)/cm^{-1} 3313, 3090, 2925, 2853, 1682, 1591, 1567, 1520, 1439, 1346, 1208, 1137, 1399, 856, 842, 802, 750, 724; δ_H(300 MHz; CD_3OD; CD_3OD) 3.00 (1 H, dd, *J* 13.9 and 9.1, CH_2), 3.35 (1 H, dd, *J* 13.9 and 4.6, CH_2), 4.65 (1 H, dd, *J* 9.1 and 4.6, CH), 8.01 (1 H, d, *J* 8.8, ArH), 8.53 (1 H, dd, 8.8 and 2.2, ArH), and 9.24 (1 H, d, *J* 2.3, ArH); δ_C(75 MHz; CD_3OD; CD_3OD) 22.59, 41.71, 53.64, 120.94, 133.29, 143.94, 146.02, 168.89, 173.49, and 174.65; *m/z* (ESI) 339.0196 (M^+ + Na. $C_{10}H_{12}N_4O_4S_2Na$ requires 339.0198).



14

Ac-L-Cys(SNpy)-L-Ala-NH$_2$ (14). Boc-L-Cys(Tr)-OH (0.26 g, 0.56 mmol) was combined with the hydrochloride salt of H-L-Ala-OMe[5] (117 mg, 0.838 mmol), EDC (215 mg, 1.12 mmol), HOBt·H$_2$O (214 mg, 1.40 mmol) and *N*,*N*-diisopropylethylamine (DIEA) (278 μL, 1.68 mmol) in anhyd. DMF (2.5 mL) under argon atmosphere at room temperature. The reaction mixture was allowed to stir 3 h until complete consumption of Boc-L-Cys(Tr)-OH, after which the DMF solution was diluted with EtOAc, extracted with 1 M HCl, sat. NaHCO$_3$, and brine, dried over anhyd. MgSO$_4$, and concentrated *in vacuo*. Crude dipeptide was sonicated with 7 N ammonia in MeOH (800 μL, 5.60 mmol) for 5 h until completion of aminolysis, as judged by TLC. Boc-L-Cys(Tr)-L-Ala-NH$_2$, obtained as a white powder after the evaporation of volatile components, was treated as described for amino acid derivative **13** to provide, after silica gel flash chromatography (10% MeOH in EtOAc) and recrystallization (Et$_2$O/MeOH), disulfide **14** (80 mg, 37% from Boc-L-Cys(Tr)-OH); $[\alpha]_D^{20}$= –60.0 (*c* = 2.55 in DMSO); δ_H(300 MHz; DMSO-d_6; DMSO-d_6) 1.20 (3 H, d, *J* 6.8, CH$_3$), 1.88 (3 H, s, CH$_3$), 3.05 (1 H, dd, *J* 13.2 and 9.3, CH$_2$), 3.26 (1 H, dd, *J* 13.2 and 4.7, CH$_2$), 4.12–4.20 (1 H, m, CH), 4.51–4.55 (1 H, m, CH), 7.04 (1 H, br s, NH$_2$), 7.32 (1 H, br s, NH$_2$), 8.05 (1 H, d, *J* 8.8, ArH), 8.19 (1 H, d, *J* 7.3, NH), 8.43 (1 H, d, *J* 8.3, NH), 8.57 (1 H, dd, *J* 8.8 and 2.2, ArH), and 9.25 (1 H, d, *J* 2.2, ArH); δ_C(75 MHz; DMSO-d_6; DMSO-d_6) 18.20, 22.64, 40.82, 48.33, 51.92, 119.56, 132.73, 142.28, 144.98, 167.41, 169.18, 169.76, and 173.98; *m/z* (ESI) 410.0567 (M^+ + Na. $C_{13}H_{17}N_5O_5S_2Na$ requires 410.0569).



15

3,3'-Dithiodipropionic acid (15). Distilled water (1.0 mL), 30% aqueous hydrogen peroxide (0.107 mL, 0.942 mmol), and 3-mercaptopropanoic acid (0.100 g, 0.082 mL, 0.942 mmol) were

combined with stirring under ambient conditions. After 1 h, white solid began to precipitate from the reaction. The reaction was allowed to stir at room temperature under ambient conditions for 13 h, after which time TLC showed consumption of the thiol. The reaction was diluted with 1 M HCl and extracted with EtOAc (3×). The combined organic layers were washed with 1 M HCl saturated with NaCl, dried over anhyd. $MgSO_4$, and concentrated *in vacuo* to give a flakey, white solid (0.10 g, >98%).[6] δ_H(300 MHz; CD_3OD; CD_3OD) 2.72 (2 H, t, *J* 6.9, CH_2), and 2.93 (1 H, t, *J* 6.9, CH_2); δ_C(75 MHz; CD_3OD; CD_3OD) 34.26, 34.71, and 175.36; CAS Registry No. 1119-62-6.

General procedure for preparation of cell suspensions. BL21(DE3) *E. coli* strains were grown on a Luria-Bertani (LB) agar plate at 37 °C for 16 h. LB broth (4 mL) in a 15-mL centrifuge tube was inoculated with a single bacterial colony, and incubated for 16 h at 37 °C with agitation. The resulting cell suspensions were stored on ice until immediately prior to the assay. The cells were gently pelleted by centrifugation (3000 rpm, 10 min, 4 ºC), the supernatant was decanted, and the cells were resuspended in 4 mL of the assay buffer (10% DMSO in 5 mM sodium phosphate, 100 mM NaCl, pH 6.0). The centrifugation and resuspension sequence was repeated, and the cell density of the final suspension was assessed from both optical dispersion measurements at 600 nm (OD_{600} = 1.00 equivalent to approximately 7.7×10^8 cells per mL) and viable cell count of serial dilutions on LB agar.

General procedure for cellular internalization assay. After preheating the plate reader to 37 °C and completing a pre-blank scan of the 96-well plate, appropriate aliquots of chromogen samples were delivered by a multichannel pipet to wells of a 96-well plate containing cells suspended in the assay buffer to give the desired chromogen concentrations and cell density in a total volume of 200 μL. Wells containing the corresponding concentrations of Ellman's reagent with cells and chromogenic disulfides without cells in the assay buffer were used to control for permeation-independent changes in chromophore absorbance. The plate was inserted into the plate reader immediately following chromogen delivery, and absorbance changes were monitored at 392 nm and 412 nm for 2–3 hours using the minimum time interval between readings, with gentle agitation for 10 s between each reading. All chromogen samples were assayed in triplicate or greater.

General procedure for analysis of kinetic data. Absorbance values for each chromogen were corrected for light scattering caused by the cells. Initial (steady-state) rates were measured via linear regressions of contiguous absorbance points displaying extended linearity in the regions following non-linear lag periods and averaged. These values were corrected for internalization-independent production of the chromophore, using the rates obtained with the Ellman's reagent and cell-free controls. The corrected rates were plotted against the corresponding initial concentrations of chromogens, and weighted linear regressions yielded k_{obs} values.

General procedure for cell viability assessment. Following a cellular assay, cells were withdrawn from a single well of each triplicate sample, as well as a control well containing cells and assay buffer, and serially diluted with sterile water. The resulting cell suspensions were inoculated onto LB agar plates as microdroplets (3–5 μM), and colonies were counted following overnight incubation at 37 °C (see Fig. S2).

Disulfide processing by *E. coli* lysates and live cells. The BL21(DE3) and Origami(DE3) strains of *E. coli* were grown overnight in LB media, washed with sterile water and resuspended in a phosphate buffer (10% DMSO in 5 mM sodium phosphate, 100 mM NaCl, pH 7.0). The lysates were prepared by vortexing the 1-mL aliquots of the cell suspensions (6.0×10^8 cells per mL) with 10 μL chloroform and 5 μL 0.1% (w/v) aqueous SDS. Three sets of 1-mL samples were made in triplicate for each strain in microcentrifuge tubes: lysates with 100 μM DTNP, live cells (6.0×10^8 cells per mL) with 100 μM Ellman's reagent, and live cells (6.0×10^8 cells per mL) with 100 μM DTNP. The samples were incubated at 37 °C with agitation for 1 h. Cells and cell debris were pelleted by centrifugation (3000 rpm, 10 min), and the supernatants were decanted. The cleared solutions were diluted 10-fold with 0.1 M Tris-HCl (pH 8.0), and the absorbance was measured at the appropriate wavelengths and corrected for any spontaneous chromophore release in the buffer (Fig. S1).

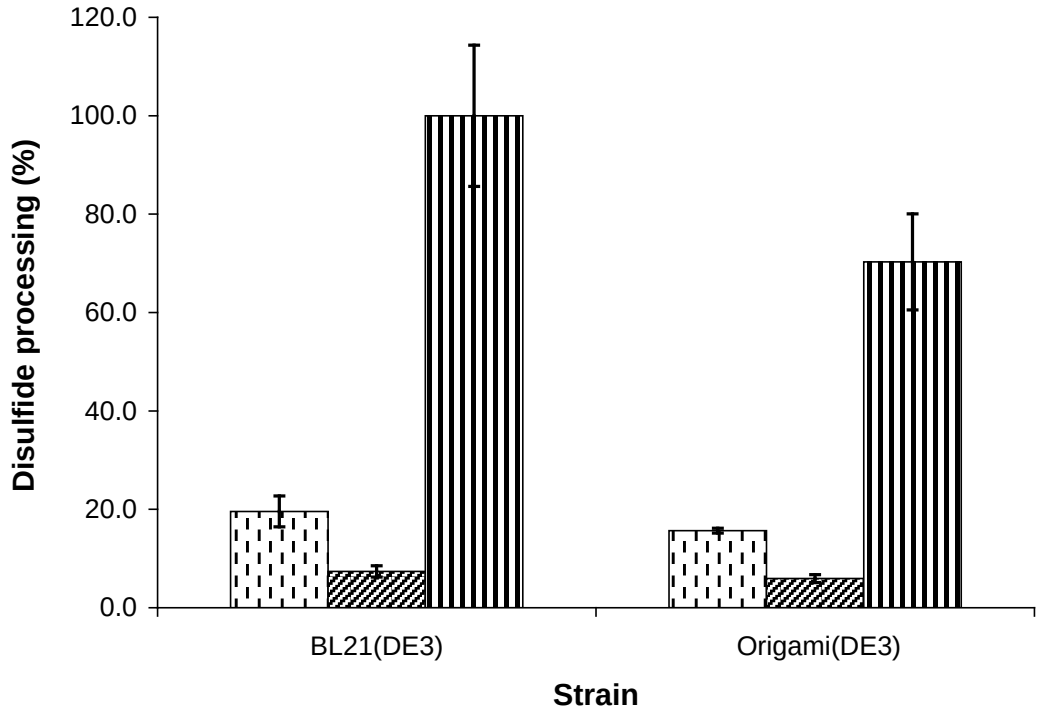


Fig. S1 Percentage of chromogenic disulfide processed by BL21(DE3) and Origami(DE3) strains after 1 h. Lysates from 6.0×10^8 cells were incubated with 100 μM DTNP (vertical dashes) in a volume of 1 mL. Live cells (6.0×10^8 cells per mL) were incubated with 100 μM Ellman's reagent (diagonal stripes) and 100 μM DTNP (vertical stripes). Live cells from both strains released approximately 5-fold more thiolate from DTNP than the corresponding lysates, indicating intracellular turnover. Conversely, the use of Ellman's reagent led to a greatly reduced disulfide processing by both strains (ca. 12- and 10-fold for BL21(DE3) and Origami(DE3) cells, respectively).

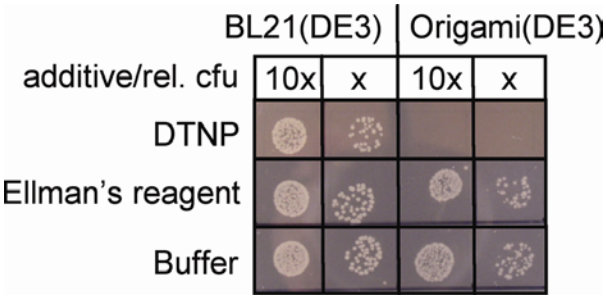


Fig. S2 Cell viability analysis of BL21(DE3) and Origami(DE3) following incubation with DTNP (20 μM), Ellman's reagent (20 μM), or the assay buffer. Following the cellular internalization assay conducted according to the general procedure, cellular viability was evaluated by inoculating LB-agar medium with 5-μL drops of serially diluted cell suspensions, to give approximately 3.0×10^2 (10×) and 3.0×10 (×) colony forming units (cfu) per drop, respectively. Origami(DE3) viability was maintained after incubation with cell-impermeable Ellman's reagent, but cell-permeable DTNP led to death of the redox-compromised cells. BL21(DE3) maintained the level of viability comparable with the buffer control upon treatment with either disulfide.

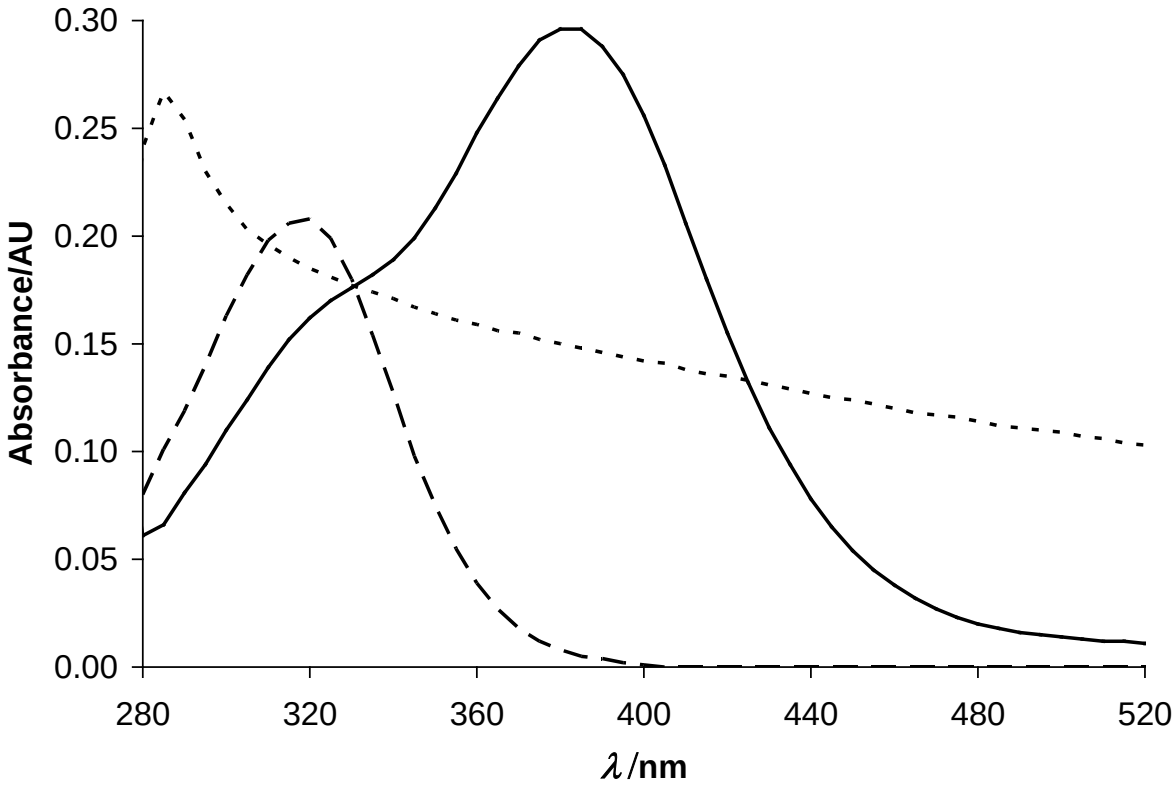


Fig. S3 Overlay of UV-Vis spectra of DTNP (20 μM) (– –), 5-nitropyridin-2-thiolate ($NpyS^-$) (40 μM) (—), and suspension of BL21(DE3) cells (1.5×10^8 cells per mL) (▪▪▪▪) in the assay buffer.

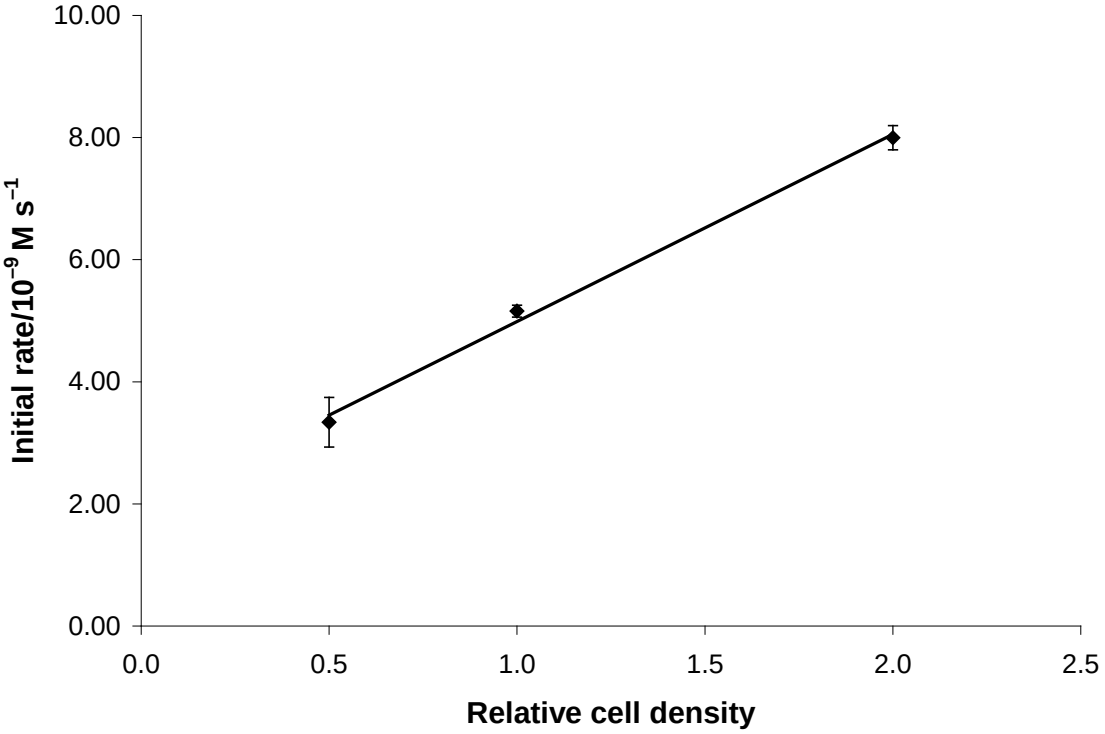


Fig. S4 Linear regression of initial rates of chromophore release for representative disulfide **5** at variable cell densities. BL21(DE3) cells were incubated with **5** (6 μM) or Ellman's reagent (6 μM) at densities of 0.75×10^8, 1.5×10^8, and 3.0×10^8 cells per mL in the assay buffer. Initial rates of $NpyS^-$ release were measured, corrected for internalization-independent chromophore release, and plotted as a function of cell density. Each point represents the mean ± standard deviation (SD) for three determinations.

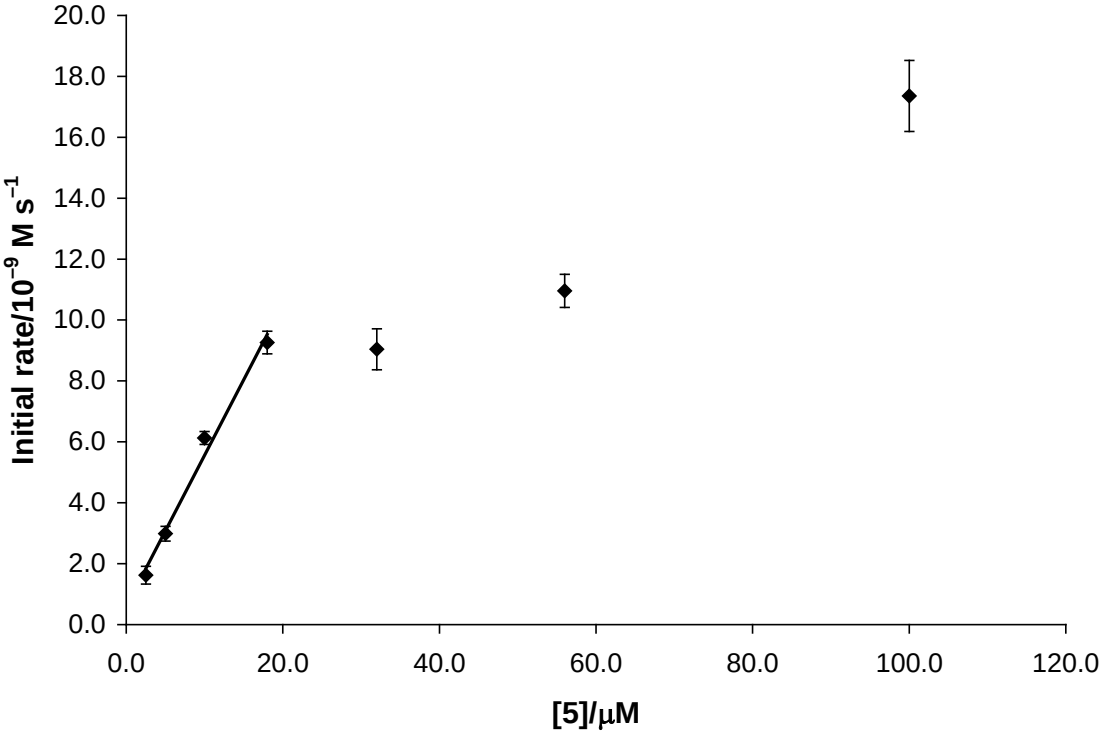


Fig. S5 Initial rates of BL21(DE3)-mediated chromophore release from various concentrations of disulfide **5**. BL21(DE3) cells (1.5×10^8 cells per mL) were incubated with **5** or Ellman's reagent in the assay buffer at the following concentrations: 2.5, 5.0, 10.0, 18.0, 32.0, 56.0, and 100.0 μM. Initial rates of $NpyS^-$ release were measured and processed according to the general procedure, and plotted as a function of concentration. Each point represents mean initial rate ± SD for three determinations. Initial rates maintained apparent first-order dependence for the concentration range of 2.5 μM–18 μM, but lost linear relationship at the concentrations greater than 18 μM.

Derivation of the rate equation. In the following derivation, subscript *C* indicates an intracellular concentration. Subscript obs signifies an observed value. A factor proportional to cell density, which is kept constant for all experiments, is denoted as [cell].

From eqn (1), the rate of chromophore (NpySH) release can be expressed as

$$v_{\text{obs}} = k_3[\text{NpySH}]_C - k_{-3}[\text{NpySH}][\text{cell}].$$

When the intracellular chromophore concentration is kept at steady state,

$$\frac{d[\text{NpySH}]_C}{dt} = 0 \rightarrow k_3[\text{NpySH}]_C = k_2[\text{RSSNpy}]_C + k_{-3}[\text{NpySH}][\text{cell}]$$

$$[\text{NpySH}]_C = \frac{k_2[\text{RSSNpy}]_C + k_{-3}[\text{NpySH}][\text{cell}]}{k_3}$$

$$\therefore v_{\text{obs}} = k_3\left(\frac{k_2[\text{RSSNpy}]_C + k_{-3}[\text{NpySH}][\text{cell}]}{k_3}\right) - k_{-3}[\text{NpySH}][\text{cell}] = k_2[\text{RSSNpy}]_C.$$

A second steady-state assumption, that the intracellular concentration of chromogenic disulfide remains constant, gives

$$\frac{d[\text{RSSNpy}]_C}{dt} = 0 \rightarrow k_2[\text{RSSNpy}]_C + k_{-1}[\text{RSSNpy}]_C = k_1[\text{RSSNpy}][\text{cell}]$$

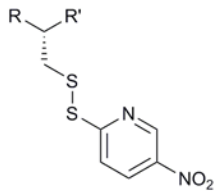
$$[\text{RSSNpy}]_C = \frac{k_1[\text{RSSNpy}][\text{cell}]}{k_2 + k_{-1}}$$

$$\therefore v_{\text{obs}} = \frac{k_2 k_1[\text{RSSNpy}][\text{cell}]}{k_2 + k_{-1}},$$

where the observed rate expression is the product of the extracellular chromogenic disulfide concentration, a value proportional to the cell density ([cell]), and a composite rate constant (see also eqn (2). Assuming that $k_2 >> k_{-1}$, the rate expression simplifies and the rate directly reports on internalization rate constant k_1:

$$k_2 >> k_{-1} \rightarrow v_{\text{obs}} \approx k_1[\text{RSSNpy}][\text{cell}].$$

Table S1 Corrected initial rates of chromophore release by BL21(DE3) cells from DTNP and disulfide probes **1**–**14**



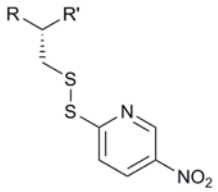
Compound	R	R'	Concentration/μM	Corrected initial rate ± SD/10^{-9} M s^{-1}[a]
DTNP	—	—	2.0	1.68 ± 0.16
			4.0	2.64 ± 0.15
			6.0	3.73 ± 0.19
			8.0	5.02 ± 0.11
1	H	OH	4.0	2.78 ± 0.21
			6.0	3.85 ± 0.37
			9.0	5.20 ± 0.56
			12.0	5.94 ± 0.47
2	H	OAc	4.0	1.88 ± 0.29
			6.0	2.69 ± 0.32
			9.0	4.59 ± 0.44
			12.0	6.12 ± 0.30
3	H	OSO_3H·py	80.2	3.18 ± 0.13
			159.9	5.71 ± 0.21
			200.0	6.82 ± 0.40
			240.1	8.21 ± 0.53
4	H	NH_3Cl	2.0	2.57 ± 0.01
			4.0	3.77 ± 0.58
			6.0	5.50 ± 0.17
			8.0	8.01 ± 0.40
5	H	NHAc	4.0	2.25 ± 0.40
			6.0	3.52 ± 0.10
			9.0	6.09 ± 0.18
			12.0	7.87 ± 0.10
6	H	N(Me)Ac	2.0	1.10 ± 0.03
			4.0	2.25 ± 0.06
			6.0	3.03 ± 0.36
			8.0	4.14 ± 0.19

Table S1 (continued)

Compound	R	R'	Concentration/μM	Corrected initial rate ± SD/10^{-9} M s^{-1}[a]
7	H	CO_2H	6.0	0.99 ± 0.30
			9.0	1.63 ± 0.26
			12.0	2.24 ± 0.04
			15.0	2.60 ± 0.09
8	H	CO_2Me	4.0	2.38 ± 0.29
			6.0	2.64 ± 0.32
			9.0	3.49 ± 0.15
			12.0	4.38 ± 0.11
9	H	$C(O)NMe_2$	6.0	2.04 ± 0.08
			9.0	2.86 ± 0.32
			12.0	4.12 ± 0.19
			15.0	4.63 ± 0.08
10	H	C(O)NHMe	4.0	3.11 ± 0.47
			6.0	4.10 ± 0.20
			9.0	5.35 ± 0.31
			12.0	6.49 ± 0.24
11	H	$C(O)NH_2$	4.0	2.14 ± 0.33
			6.0	3.08 ± 0.24
			9.0	3.75 ± 0.03
			12.0	4.21 ± 0.01
12	NH_3^+	CO_2^-	4.0	2.81 ± 0.53
			6.0	3.18 ± 0.02
			9.0	4.30 ± 0.26
			12.0	4.94 ± 0.22
13	NHAc	$C(O)NH_2$	10.0	2.60 ± 0.15
			20.1	4.98 ± 0.33
			40.0	9.19 ± 0.45
14	NHAc	C(O)–Ala–NH_2	80.1	0.312 ± 0.081
			99.9	0.537 ± 0.037
			129.9	0.648 ± 0.031
			180.0	0.942 ± 0.059

[a] Values represent mean initial rate ± SD (standard deviation) per 1.5×10^8 BL21(DE3) cells per mL for three determinations corrected for permeation-independent chromophore release.

Table S2 Observed rate constants and linear regression parameters for DTNP and disulfide probes **1**–**14**



Compound	R	R'	k_{obs} SEM/ 10^{-4} s^{-1} [a]	Relative k_{obs} [b]	Intercept SEM/ 10^{-9} M s^{-1}	R^2
4	H	NH_3Cl	7.79 ± 0.35	2.8	1.01 ± 0.07	0.989
5	H	NHAc	7.23 ± 0.23	2.6	–0.76 ± 0.22	0.996
2	H	OAc	5.41 ± 0.50	1.9	–0.38 ± 0.41	0.996
6	H	N(Me)Ac	5.37 ± 0.24	1.9	0.04 ± 0.07	0.995
1	H	OH	4.13 ± 0.60	1.5	1.19 ± 0.38	0.984
10	H	C(O)NHMe	4.07 ± 0.47	1.4	1.63 ± 0.40	0.998
12	NH_3^+	CO_2^-	3.04 ± 0.34	1.1	1.35 ± 0.21	0.989
9	H	$C(O)NMe_2$	2.90 ± 0.12	1.03	0.31 ± 0.14	0.995
DTNP	—	—	2.82 ± 0.15	1	0.46 ± 0.18	0.997
8	H	CO_2Me	2.66 ± 0.32	0.9	1.15 ± 0.33	0.991
13	NHAc	$C(O)NH_2$	2.22 ± 0.15	0.8	0.39 ± 0.25	0.999
11	H	$C(O)NH_2$	1.60 ± 0.09	0.57	2.30 ± 0.10	0.980
7	H	CO_2H	1.54 ± 0.26	0.5	0.37 ± 0.33	0.940
3	H	OSO_3H·py	0.312 ± 0.021	0.11	0.68 ± 0.26	0.999
14	NHAc	C(O)–Ala–NH_2	0.0536 ± 0.008	0.019	–0.03 ± 0.10	0.955

[a] Pseudo-first-order rate constants k_{obs} ± SEM (standard error of the mean) are per 1.5×10^8 BL21(DE3) cells per mL and derived from weighted linear regressions (see Table S1 for corrected mean initial rates); [b] Related to the DTNP rate constant.

Rate constant assessment for cell-free disulfide exchange. To transparent 96-well plate wells, pre-blanked at 392 nm and containing L-cysteine and 1 mM EDTA in the assay buffer (10% DMSO in 5 mM sodium phosphate, 100 mM NaCl, pH 6.0), **4** was added via a multichannel pipet to give the final concentration of 5 μM thiol and 50 μM disulfide in the total volume of 200 μL. The disulfide (50 μM) was also added to a control well containing only the buffer. The wells were monitored at 392 nm at 37 °C for 10 min using the minimum time increment (6 s). The absorbance data was corrected for the pre-blank scan and buffer control, and initial rates were measured for the first three data points. The average rate and a pseudo-first-order rate constant were calculated to be $7.1 \pm 1.2 \times 10^{-8}$ M s^{-1} and $1.4 \pm 0.2 \times 10^{-2}$ s^{-1}, respectively.

Competitive inhibition of disulfide 12 uptake. Aliquots (200 μL) of BL21(DE3) suspensions (1.5×10^8 cells per mL) in the assay buffer, were incubated with **12** (10 μM) or Ellman's reagent (10 μM) in the presence of L-cystine, **15**, or **16** (Fig. S6A) at variable concentrations. One set of triplicate wells contained no inhibitor. The kinetic data was collected and corrected following the general procedure. Cell survival was evaluated according to the general procedure for cell viability assessment. Loss of cell viability was observed for **15** at concentrations of 560 μM and higher, and for **16** at 5.6 mM and higher; data corresponding to these concentrations were not included in the analysis.

The initial rates of $NpyS^-$ accumulation from **12** appeared to remain largely unchanged as L-cystine concentrations were increased. The control experiment employing Ellman's reagent, however, demonstrated a direct dependence of chromophore accumulation rates on L-cystine concentrations, which we assigned to a rapid export of thiols into the extracellular media during the incubation with L-cystine.[7] Since thiolate release from **12**, therefore, resulted from both cellular reduction and extracellular disulfide exchange with exported thiols, initial rates of $NpyS^-$ evolution for **12** were corrected for the rates of chromophore release in the presence of Ellman's reagent (Fig. S6, Table S3). The same corrections were applied to experiments containing **15** and **16**, but only small absorbance increases were observed in control wells containing Ellman's reagent for these potential competitors.

The corrected initial rates, normalized to the inhibition range obtained for L-cystine, were plotted as percentage values from the inhibitor-free controls versus the inhibitor concentrations (Fig. S6B). Weighted nonlinear regression with the equation % initial rate = top – (top – bottom)[I]/([I] + [IC_{50}]) provided IC_{50} values for each inhibitor (Table S3).[8] In the cases of **15** and **16** as inhibitors of uptake, the titration curves were extrapolated to the limits of the L-cystine inhibition curve because toxic effects ensued before complete inhibition of the transport of **12**.

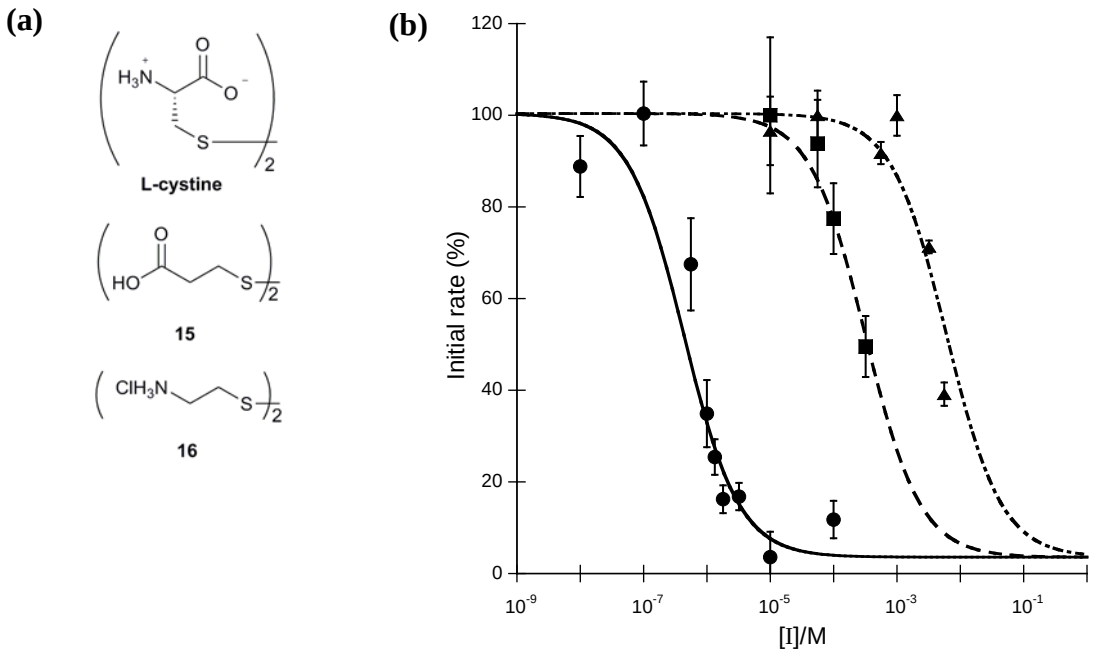


Fig. S6 Competitive inhibition of cellular processing of **12** by symmetrical disulfides. (a) Structures of symmetrical disulfide inhibitors. (b) Inhibition curves of chromophore release from **12** (10 μM) by variable concentrations of L-cystine (●), **15** (■), and **16** (▲).

Table S3 IC_{50} values for inhibition of chromophore release from **12**

Inhibitor	IC_{50} ± SEM/mM[a]
L-cystine	0.00043 ± 0.00005
15	0.32 ± 0.07
16	6.1 ± 0.3

[a] Values represent mean ± SEM (standard error of the mean) of weighted nonlinear regression.

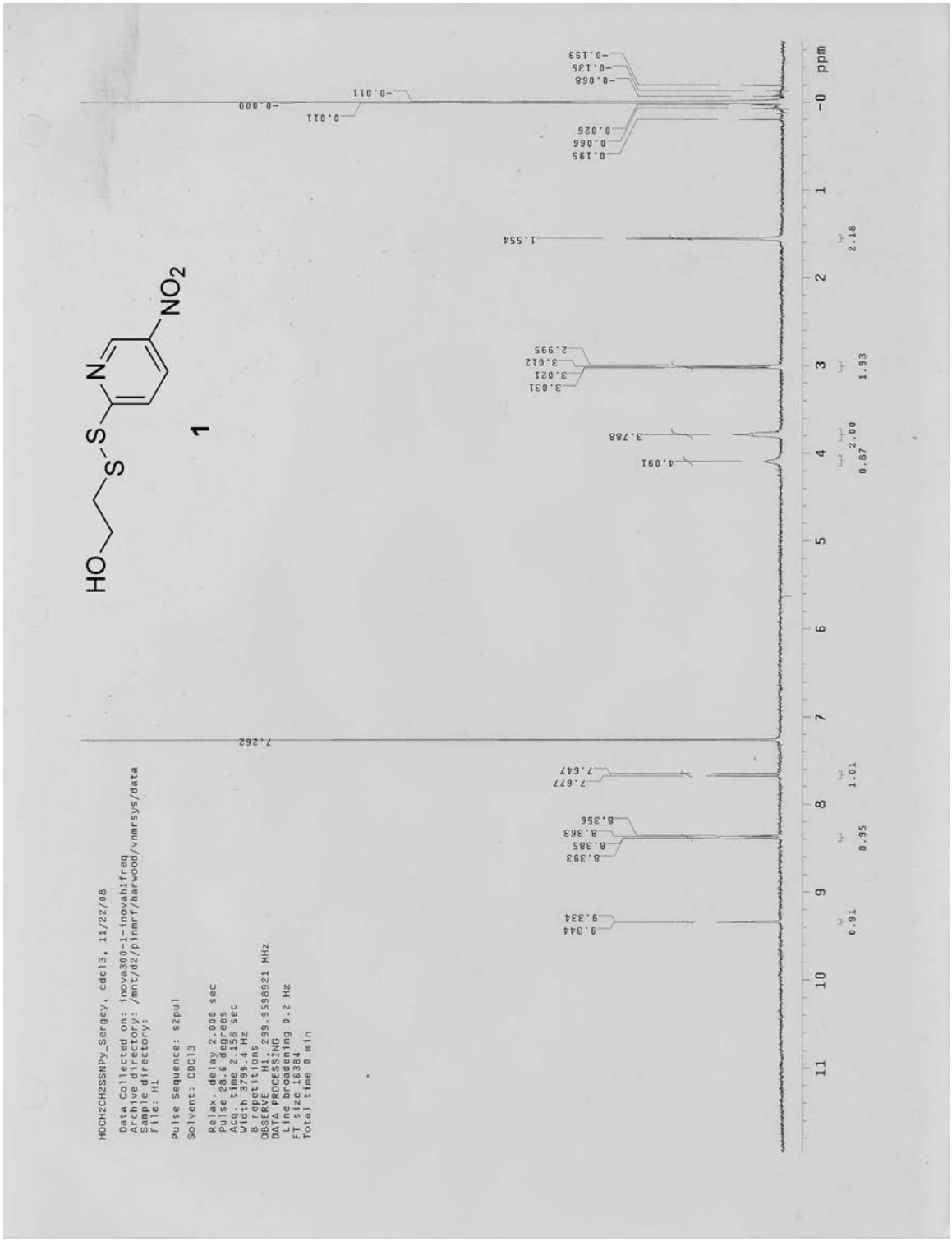


Fig. S7 ^{1}H NMR spectrum (300 MHz) of **1** in $CDCl_3$.

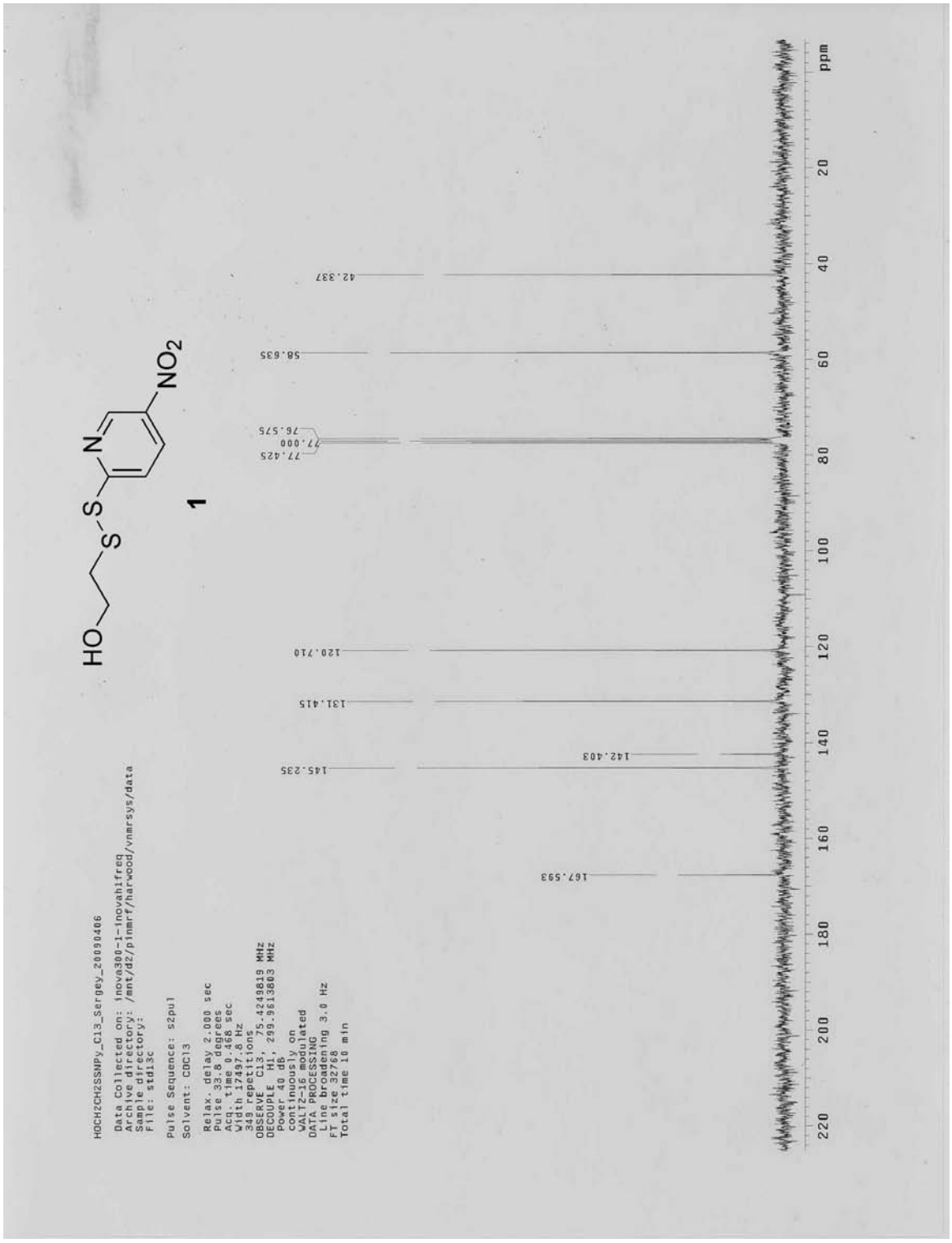


Fig. S8 ^{13}C NMR spectrum (75 MHz) of **1** in $CDCl_3$.

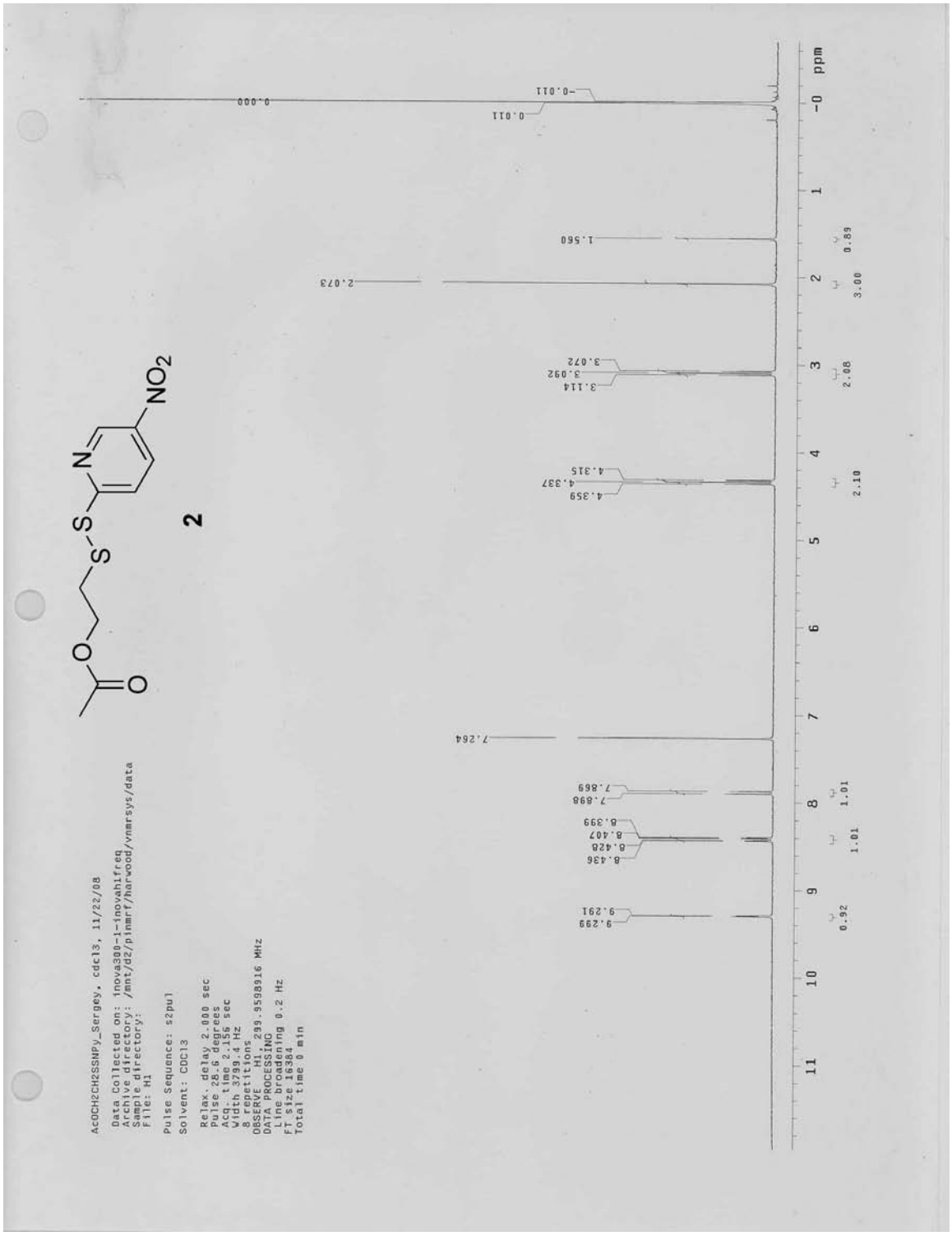


Fig. S9 ^{1}H NMR spectrum (300 MHz) of **2** in $CDCl_3$.

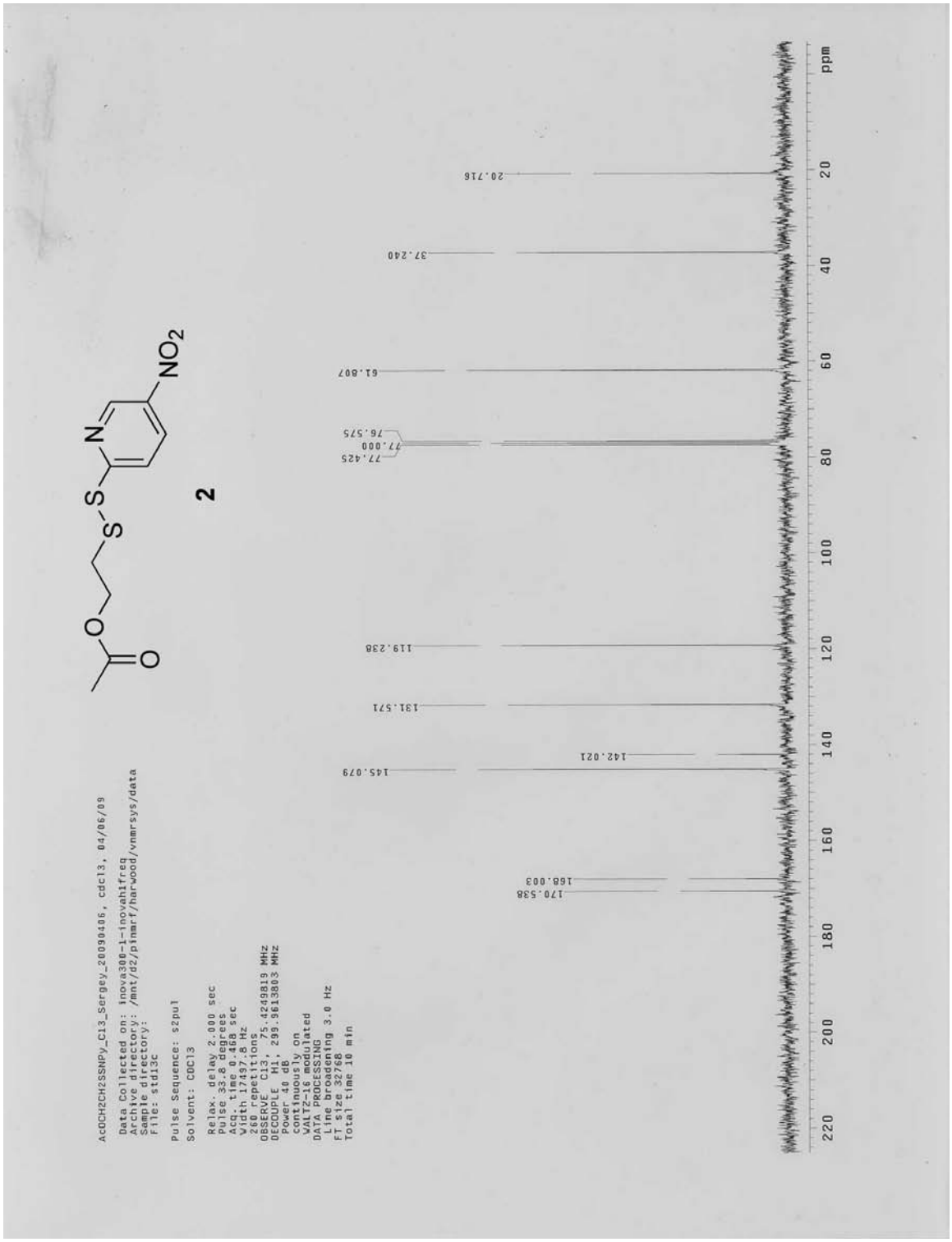


Fig. S10 ^{13}C NMR spectrum (75 MHz) of **2** in $CDCl_3$.

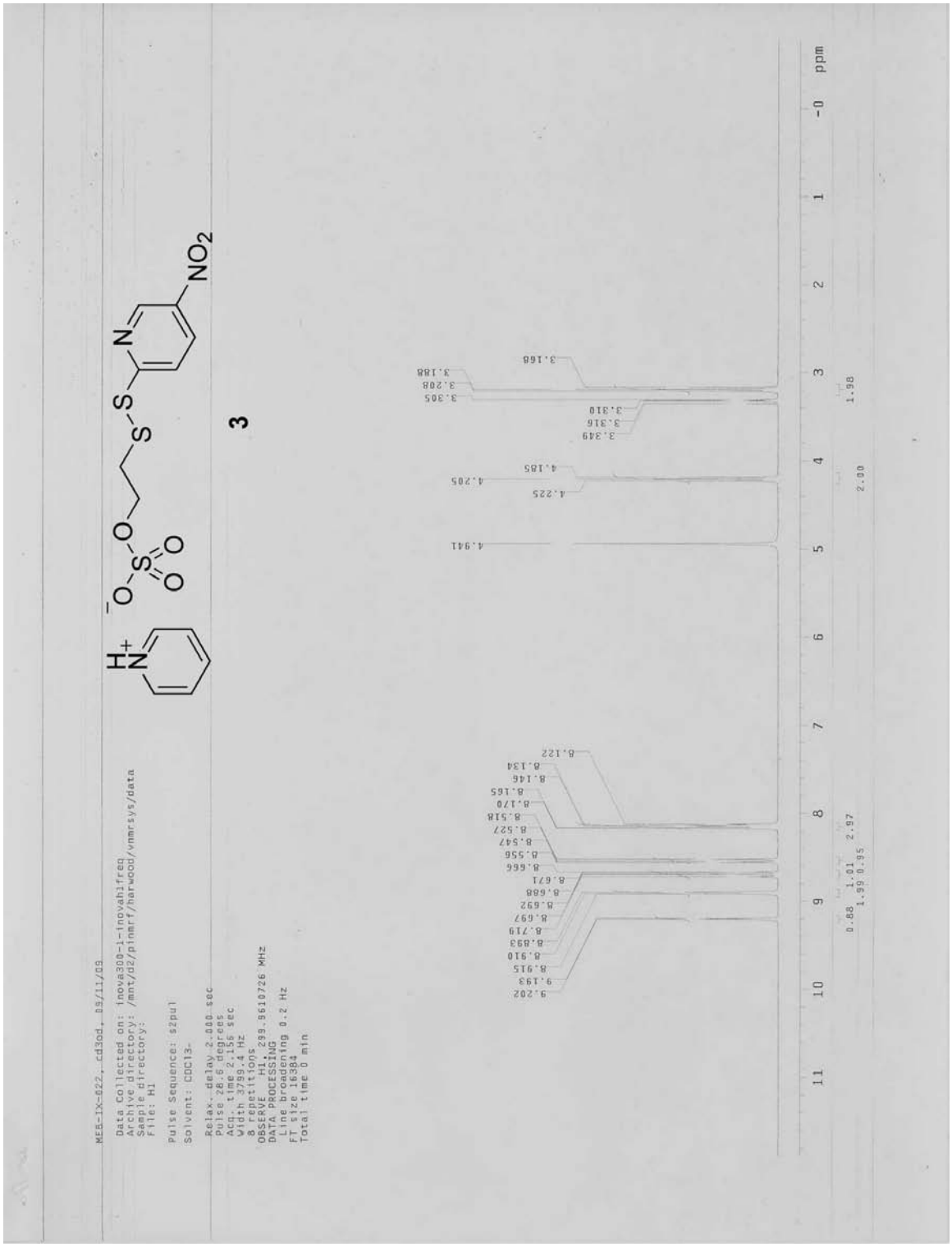


Fig. S11 ^{1}H NMR spectrum (300 MHz) of **3** in CD_3OD.

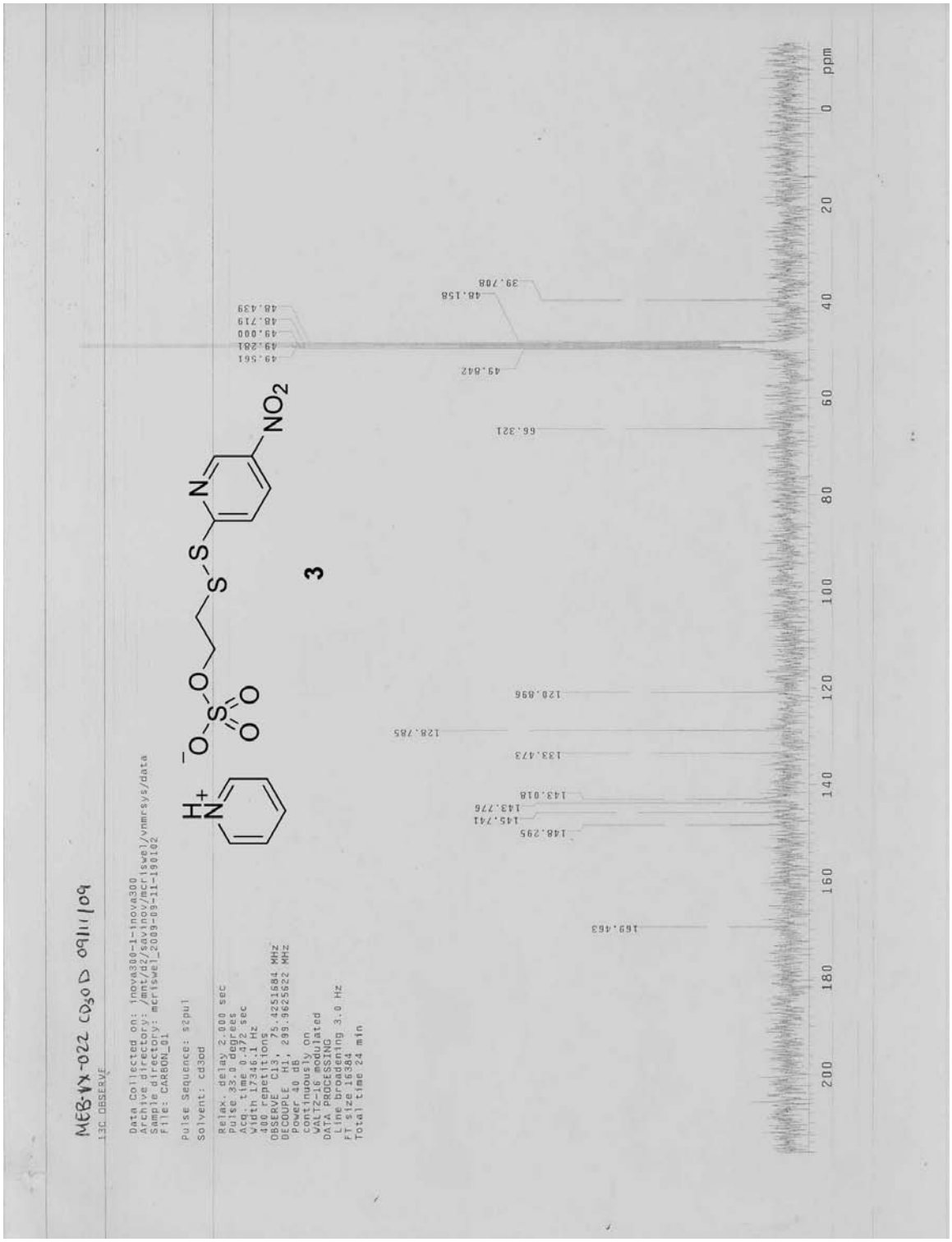


Fig. S12 ^{13}C NMR spectrum (75 MHz) of **3** in CD_3OD.

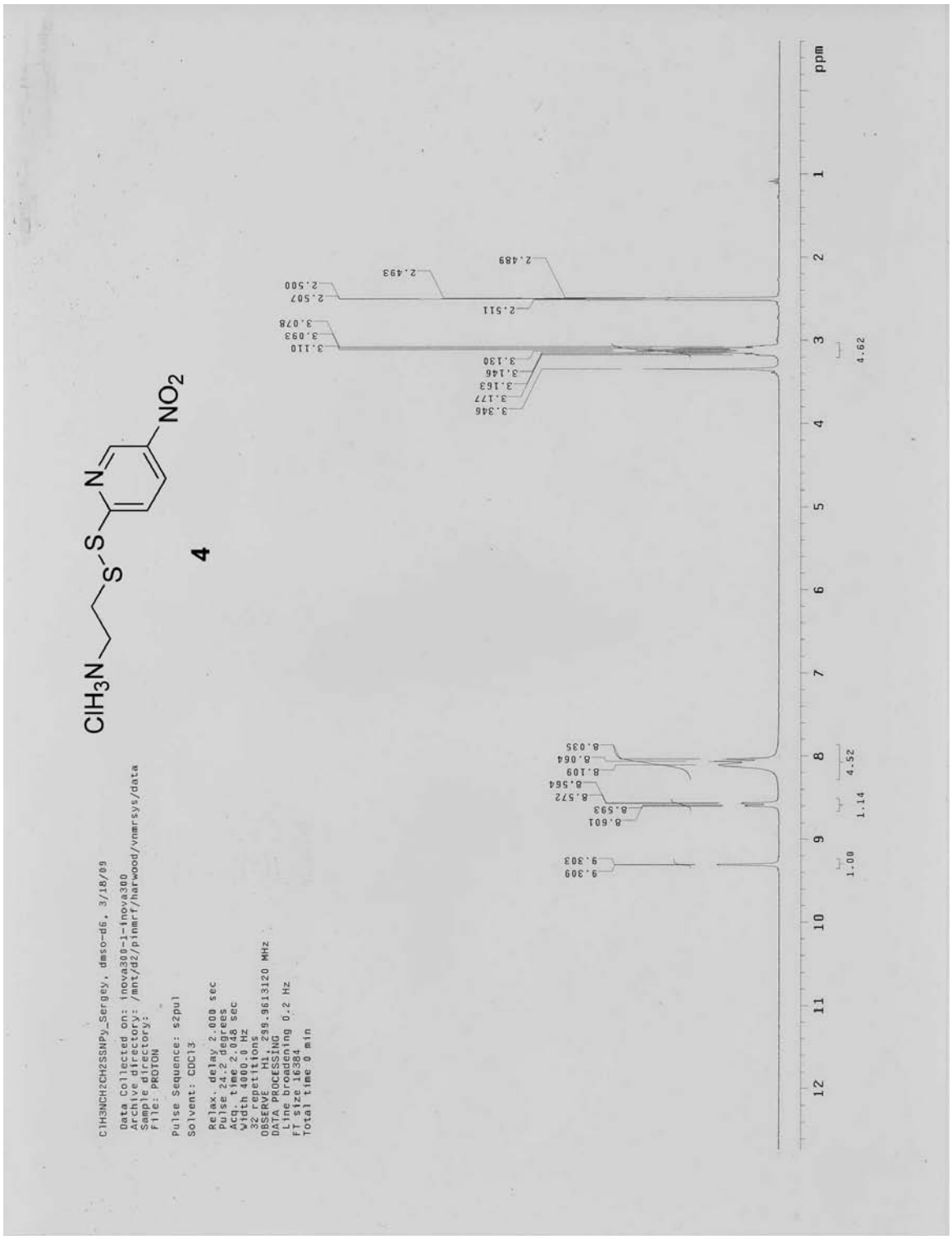


Fig. S13 ^{1}H NMR spectrum (300 MHz) of **4** in DMSO-d_6.

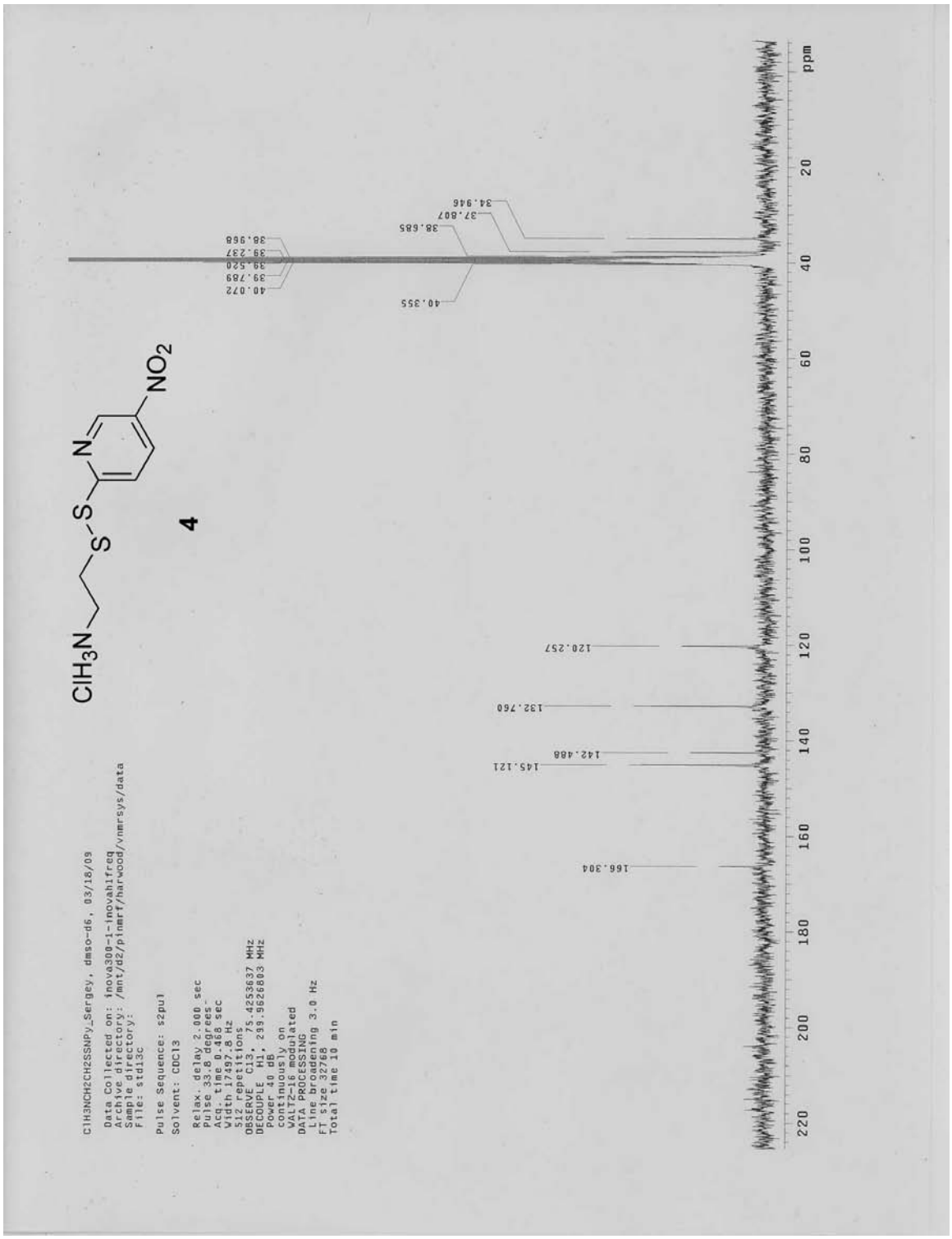


Fig. S14 ^{13}C NMR spectrum (75 MHz) of **4** in DMSO-d_6.

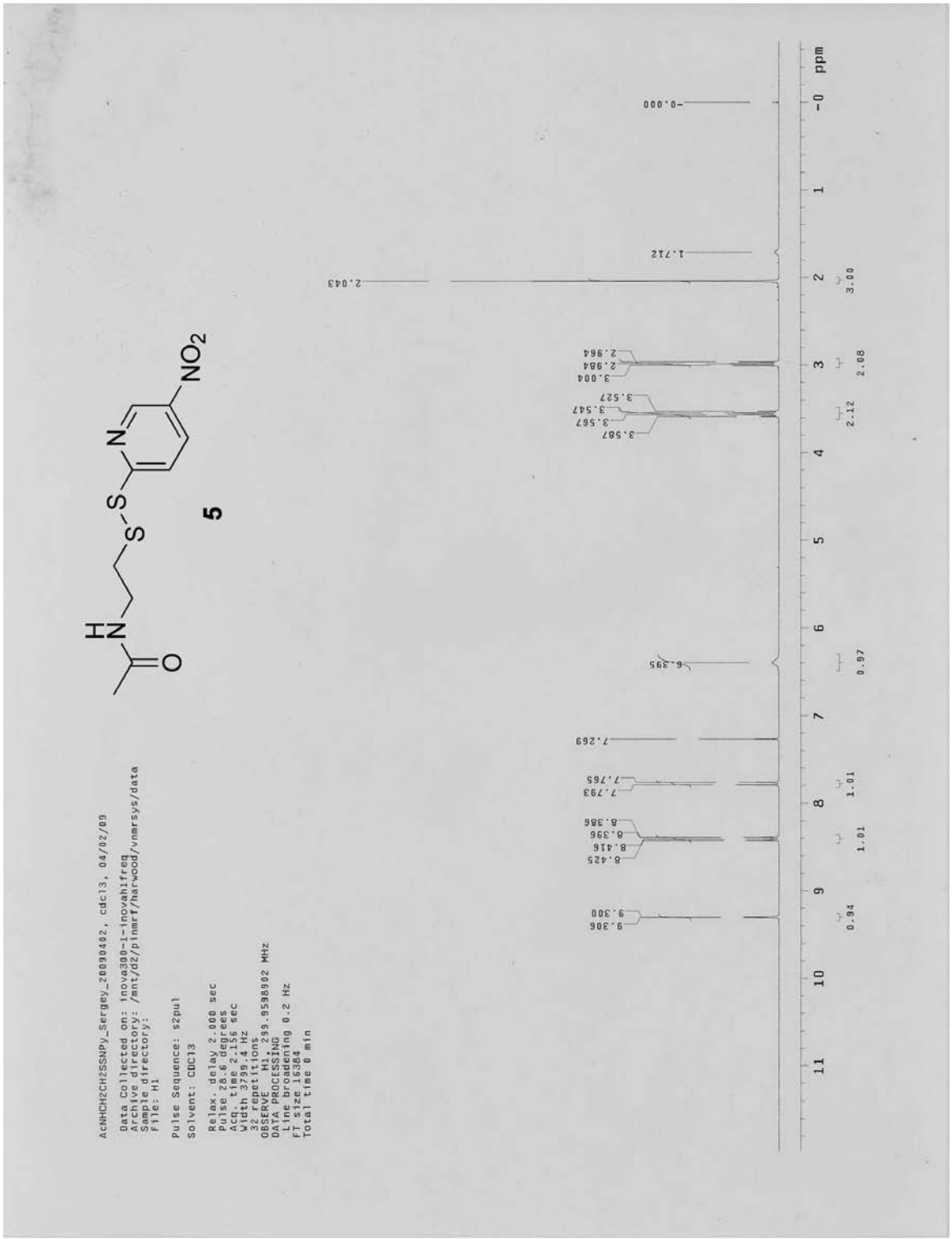


Fig. S15 ^{1}H NMR spectrum (300 MHz) of **5** in $CDCl_3$.

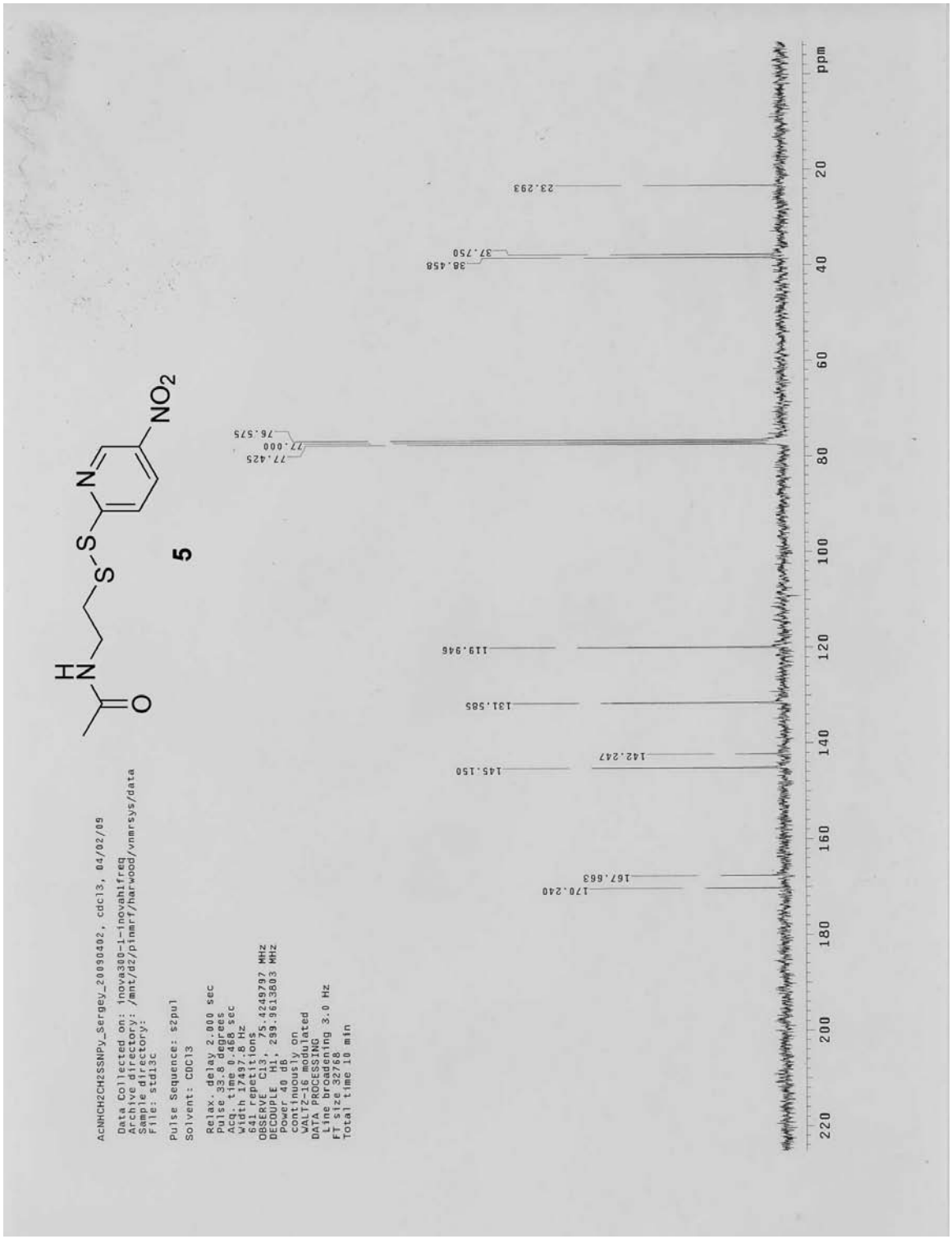


Fig. S16 ^{13}C NMR spectrum (75 MHz) of **5** in $CDCl_3$.

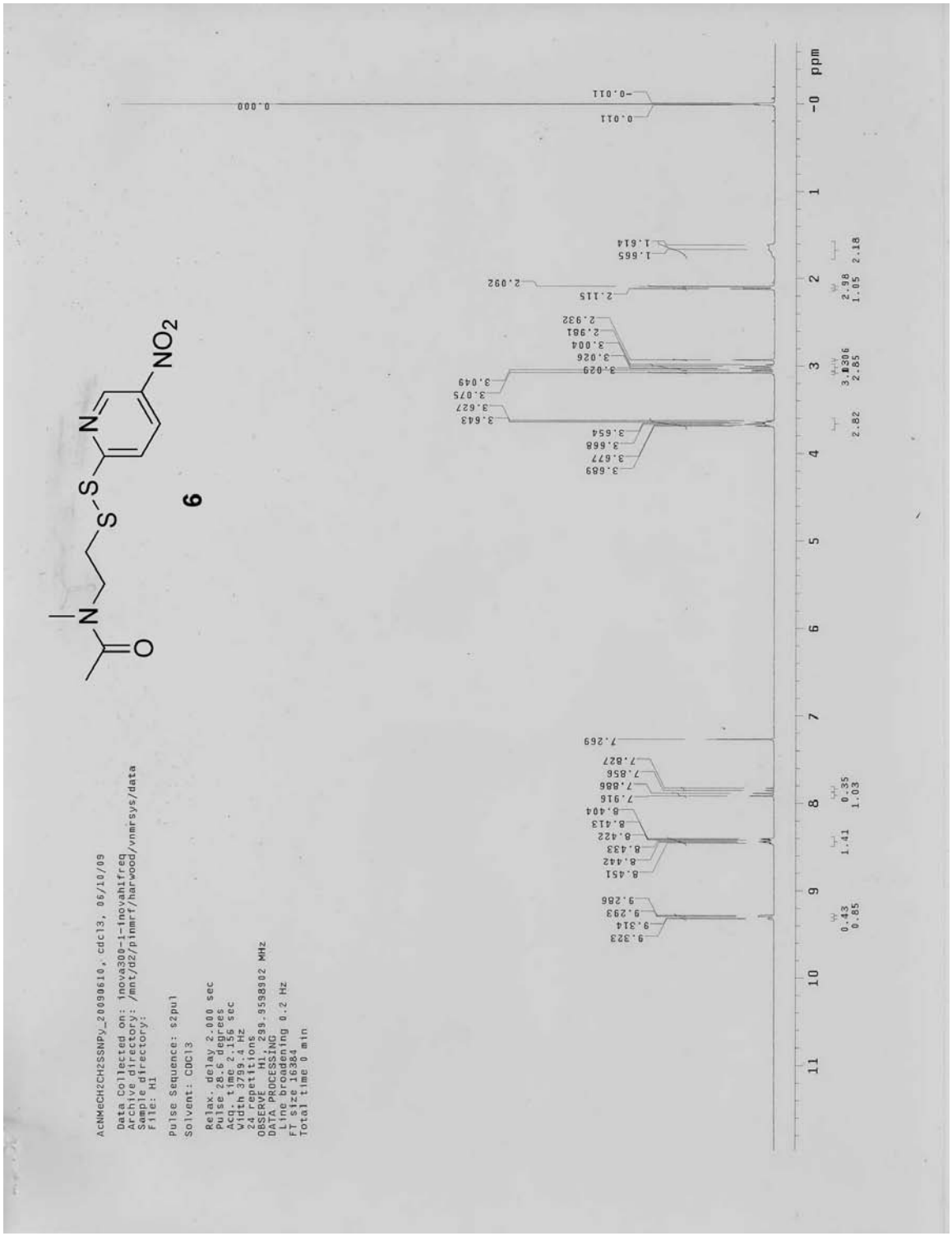


Fig. S17 ^{1}H NMR spectrum (300 MHz) of **6** in $CDCl_3$.

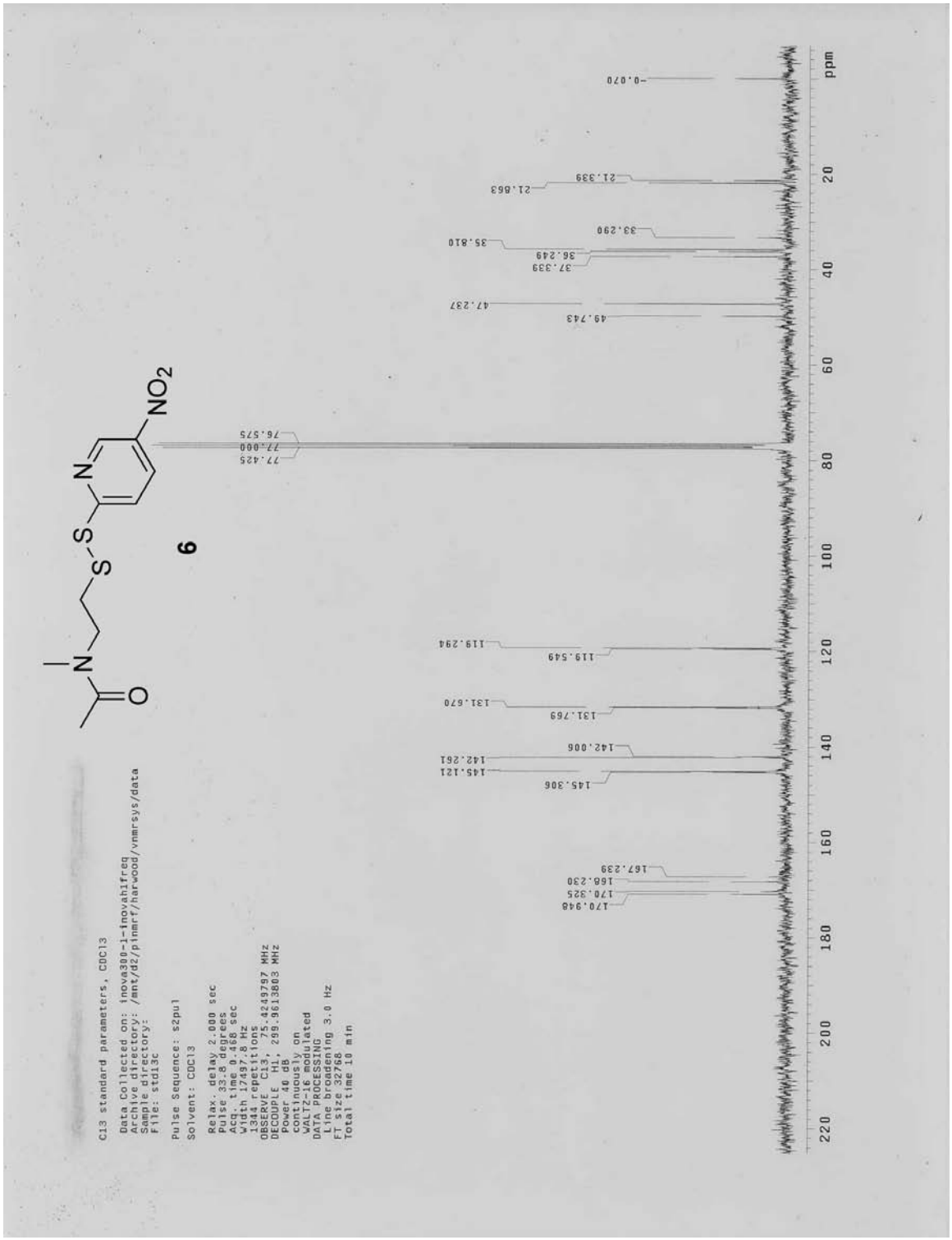


Fig. S18 ^{13}C NMR spectrum (75 MHz) of **6** in $CDCl_3$.

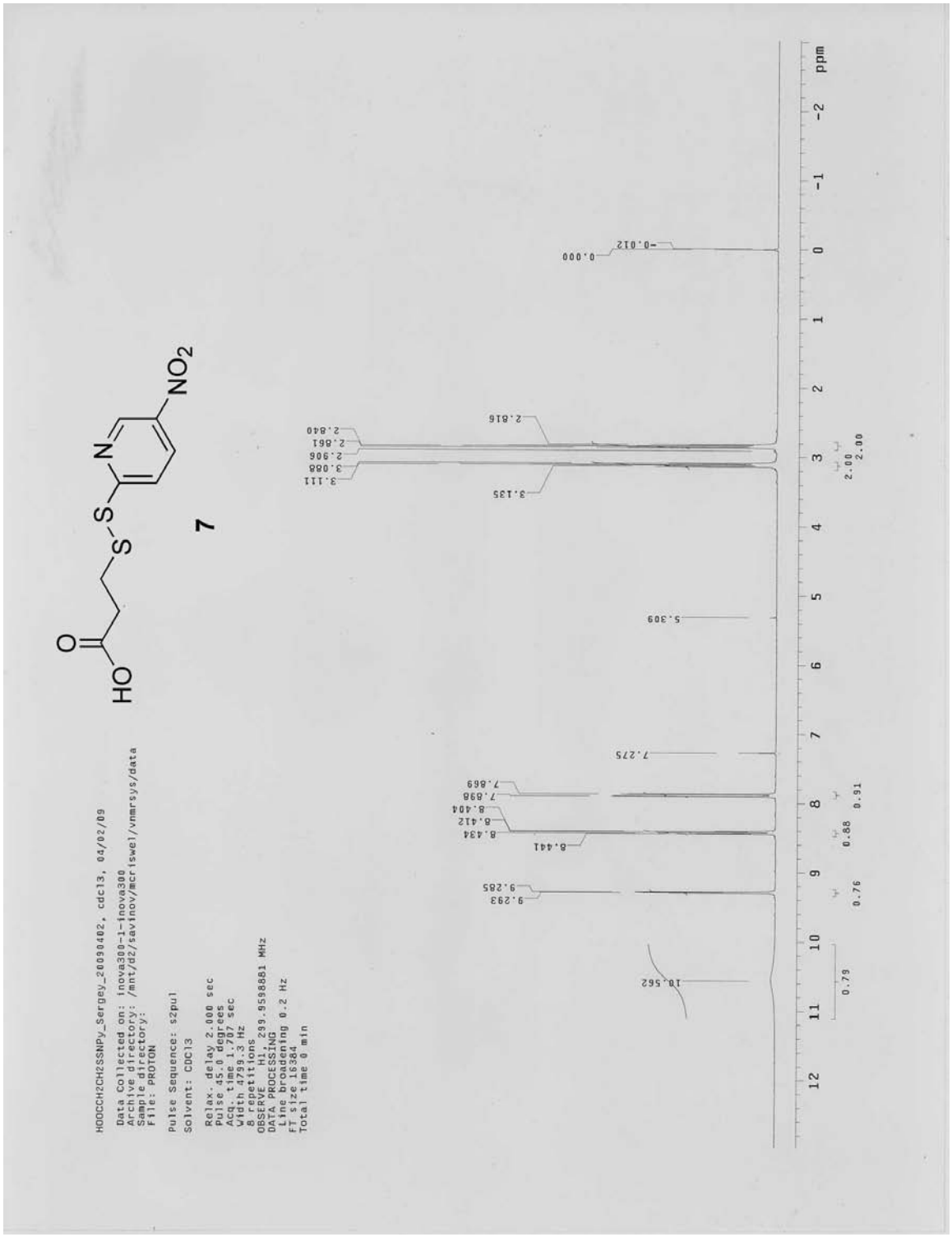


Fig. S19 ^{1}H NMR spectrum (300 MHz) of **7** in $CDCl_3$.

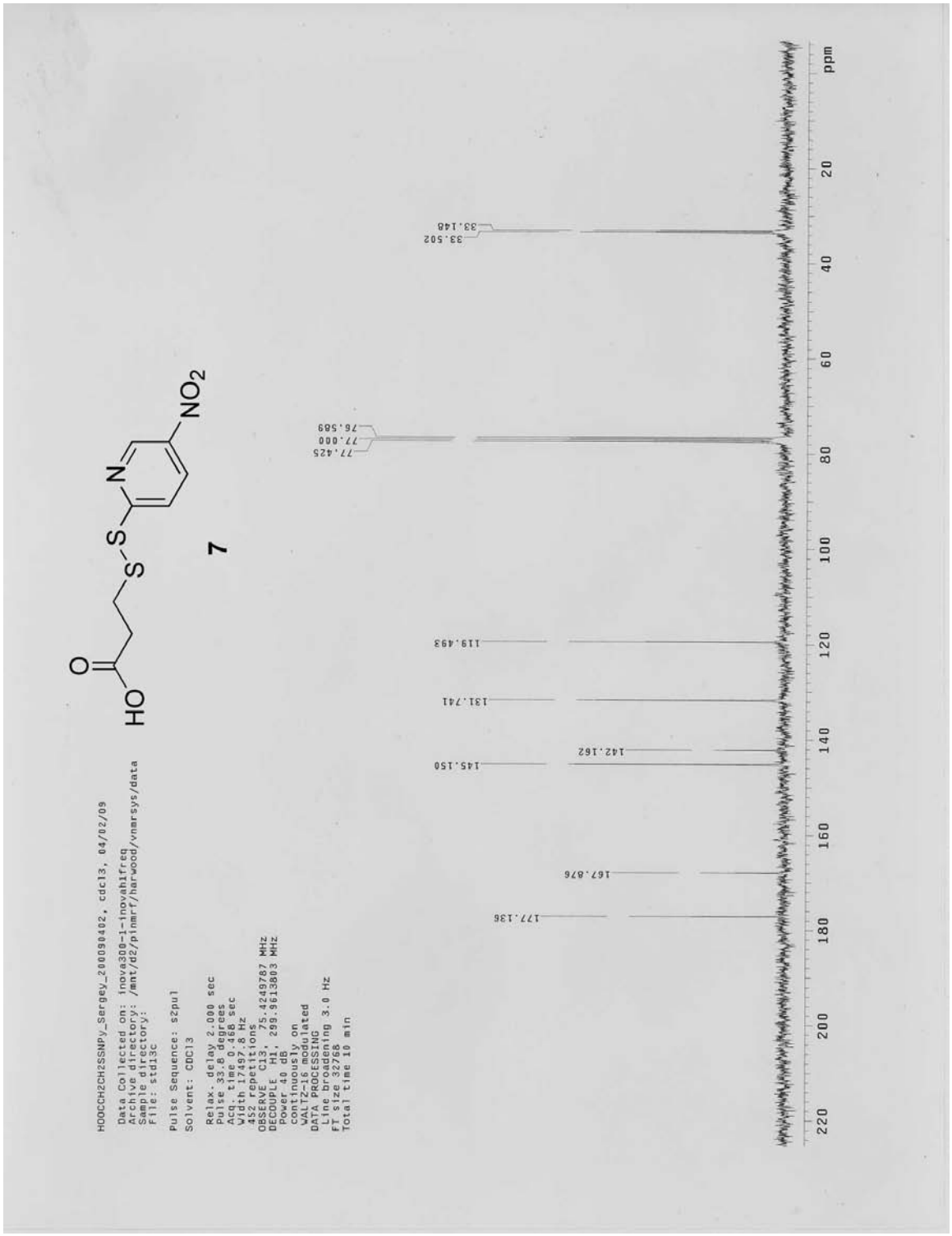


Fig. S20 ^{13}C NMR spectrum (75 MHz) of **7** in $CDCl_3$.

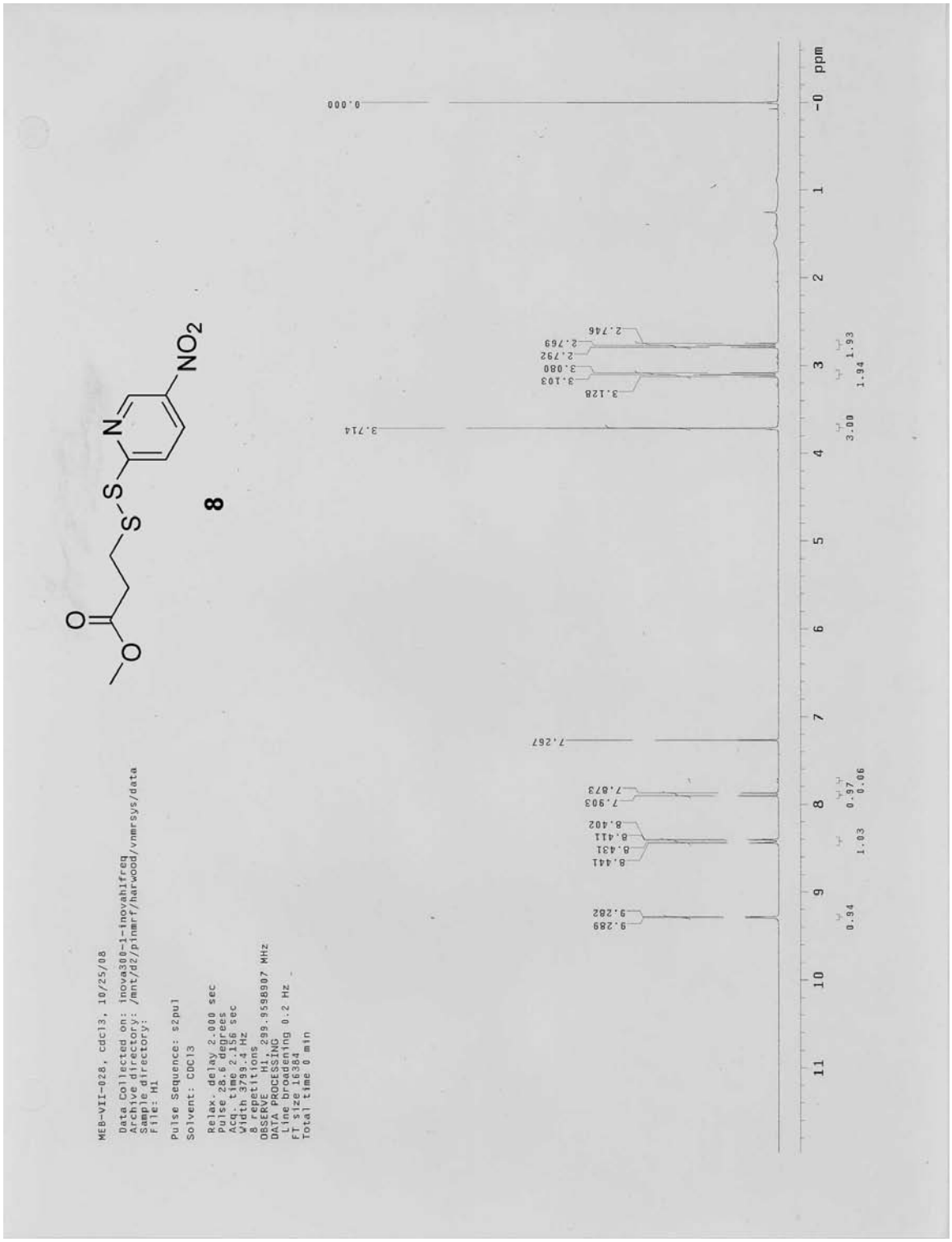


Fig. S21 ^{1}H NMR spectrum (300 MHz) of **8** in $CDCl_3$.

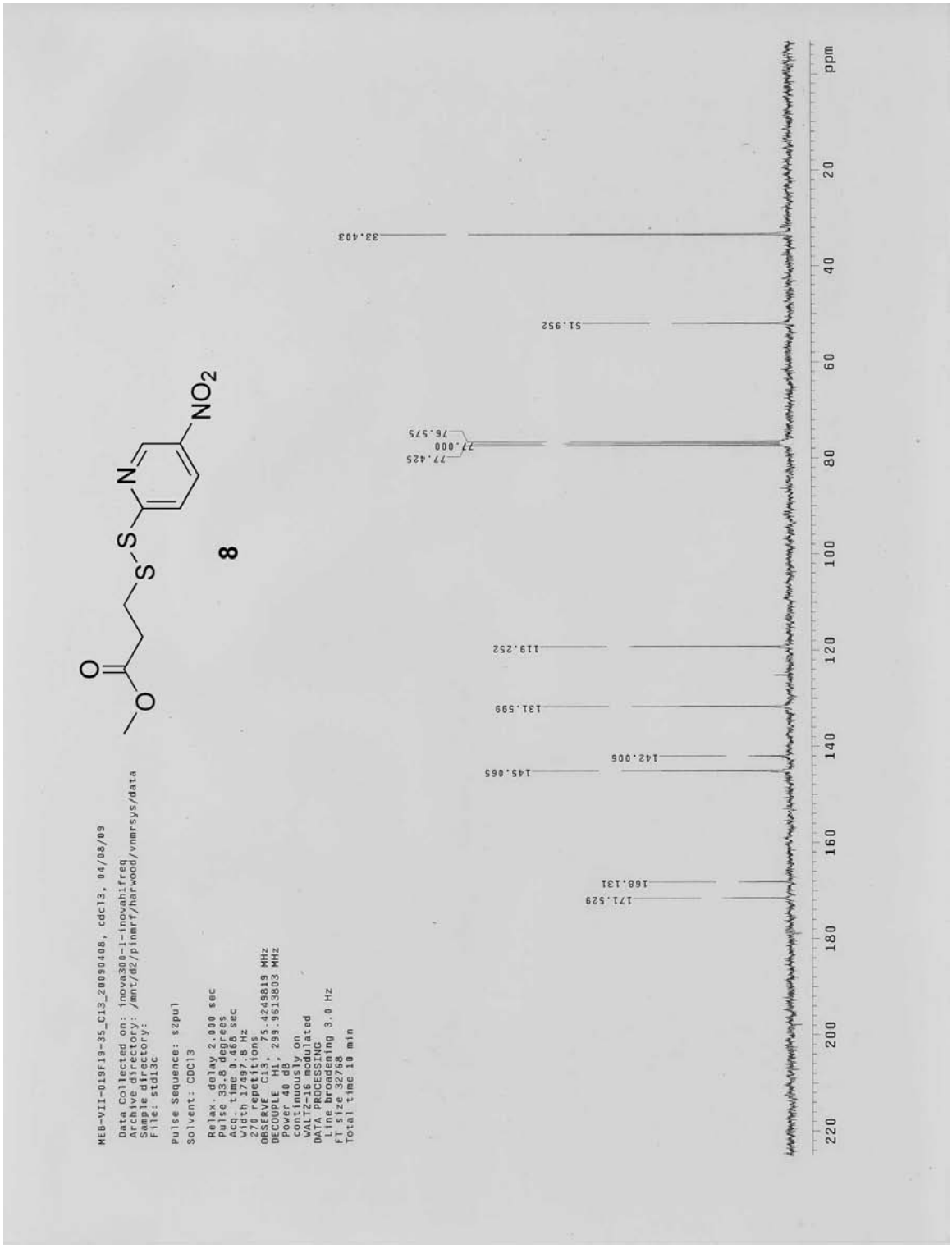


Fig. S22 ^{13}C NMR spectrum (75 MHz) of **8** in $CDCl_3$.

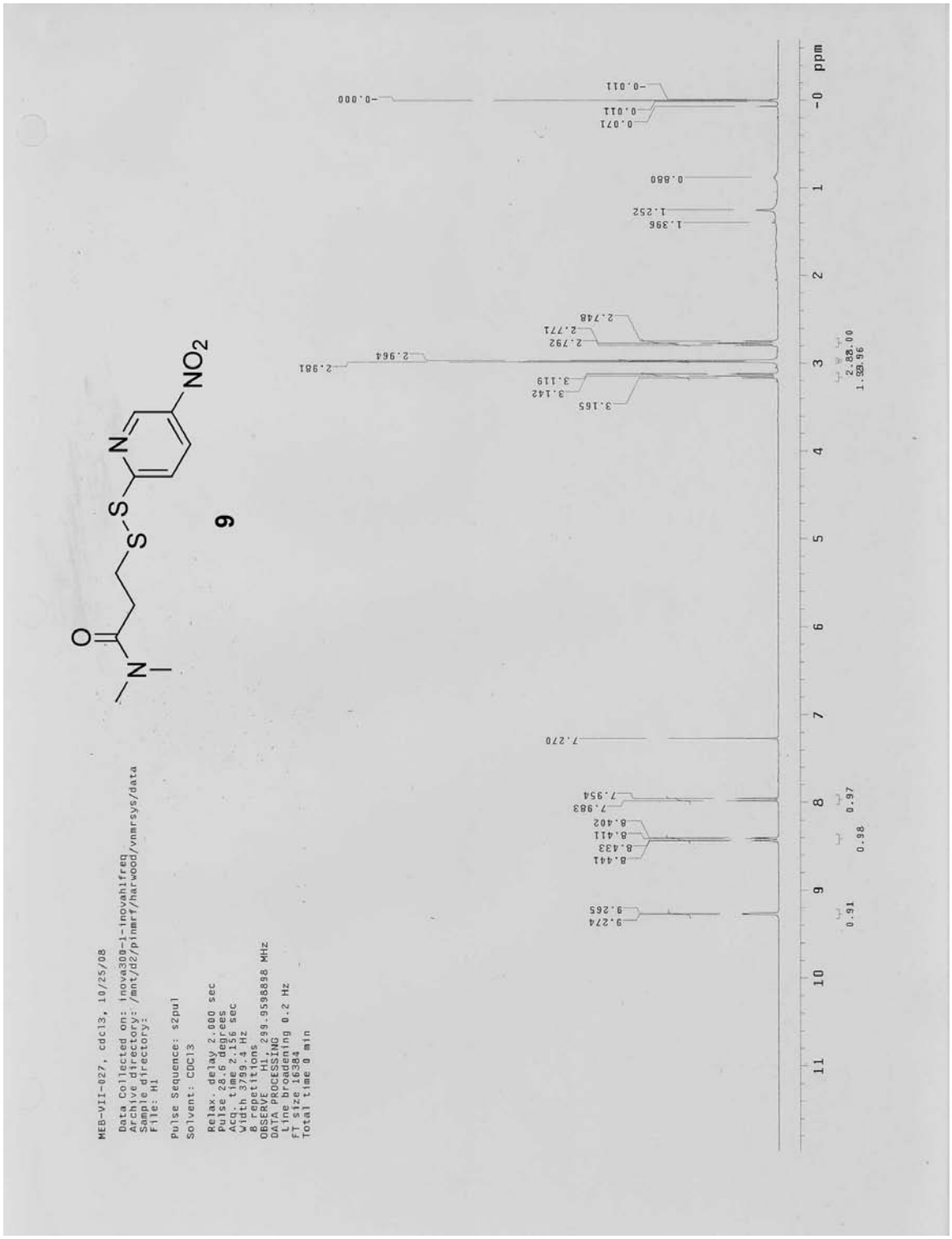


Fig. S23 ^{1}H NMR spectrum (300 MHz) of **9** in $CDCl_3$.

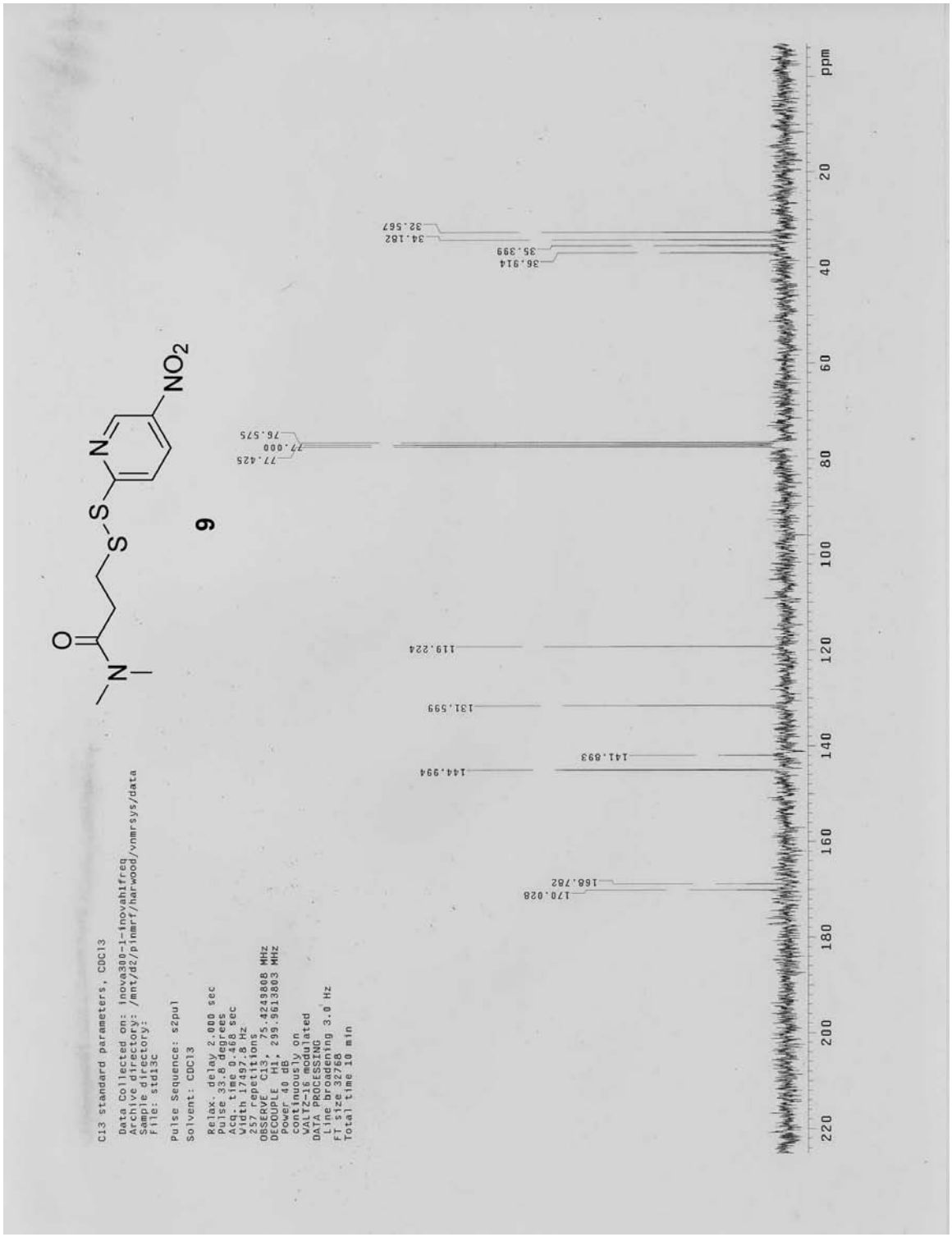


Fig. S24 ^{13}C NMR spectrum (75 MHz) of **9** in $CDCl_3$.

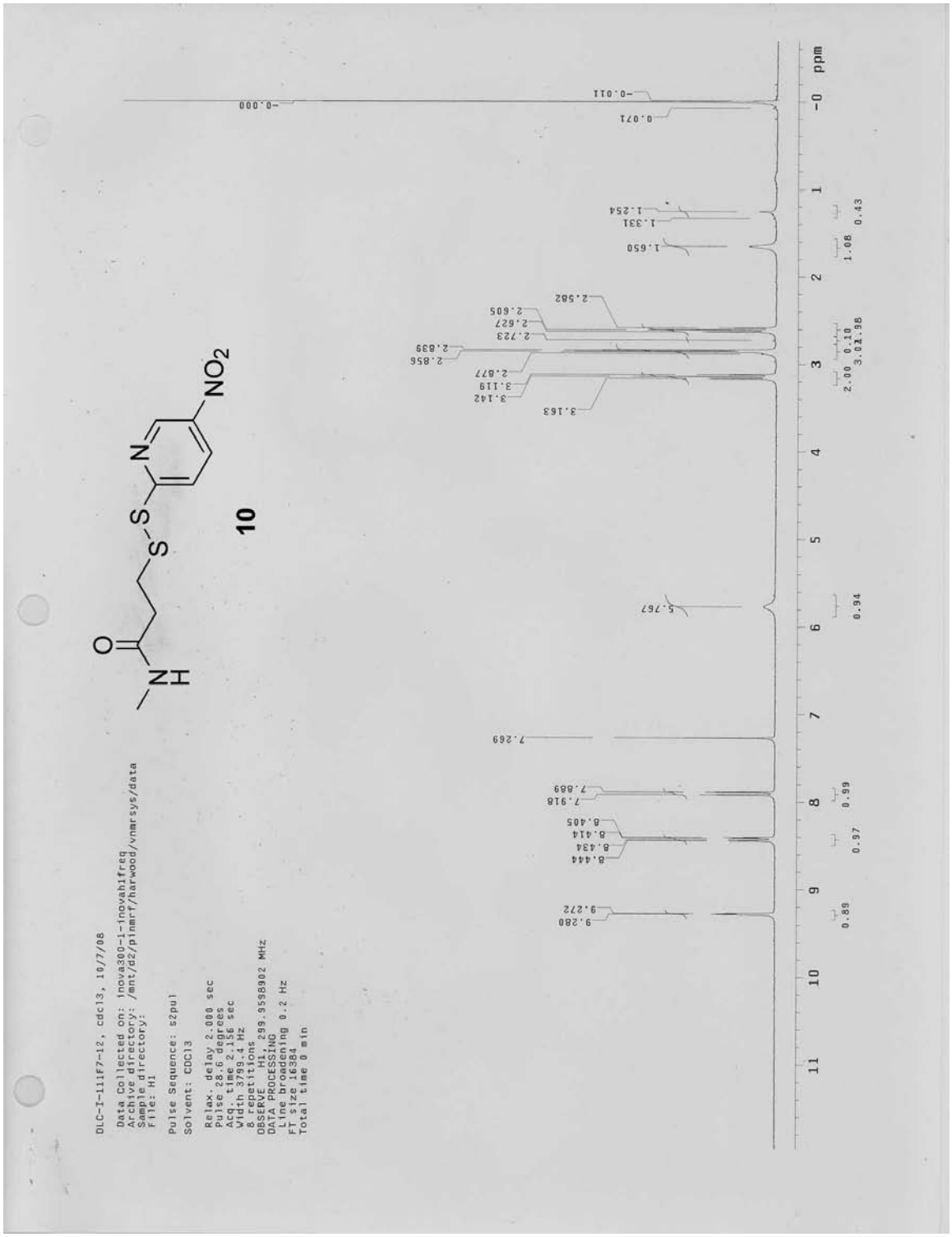


Fig. S25 ^{1}H NMR spectrum (300 MHz) of **10** in $CDCl_3$.

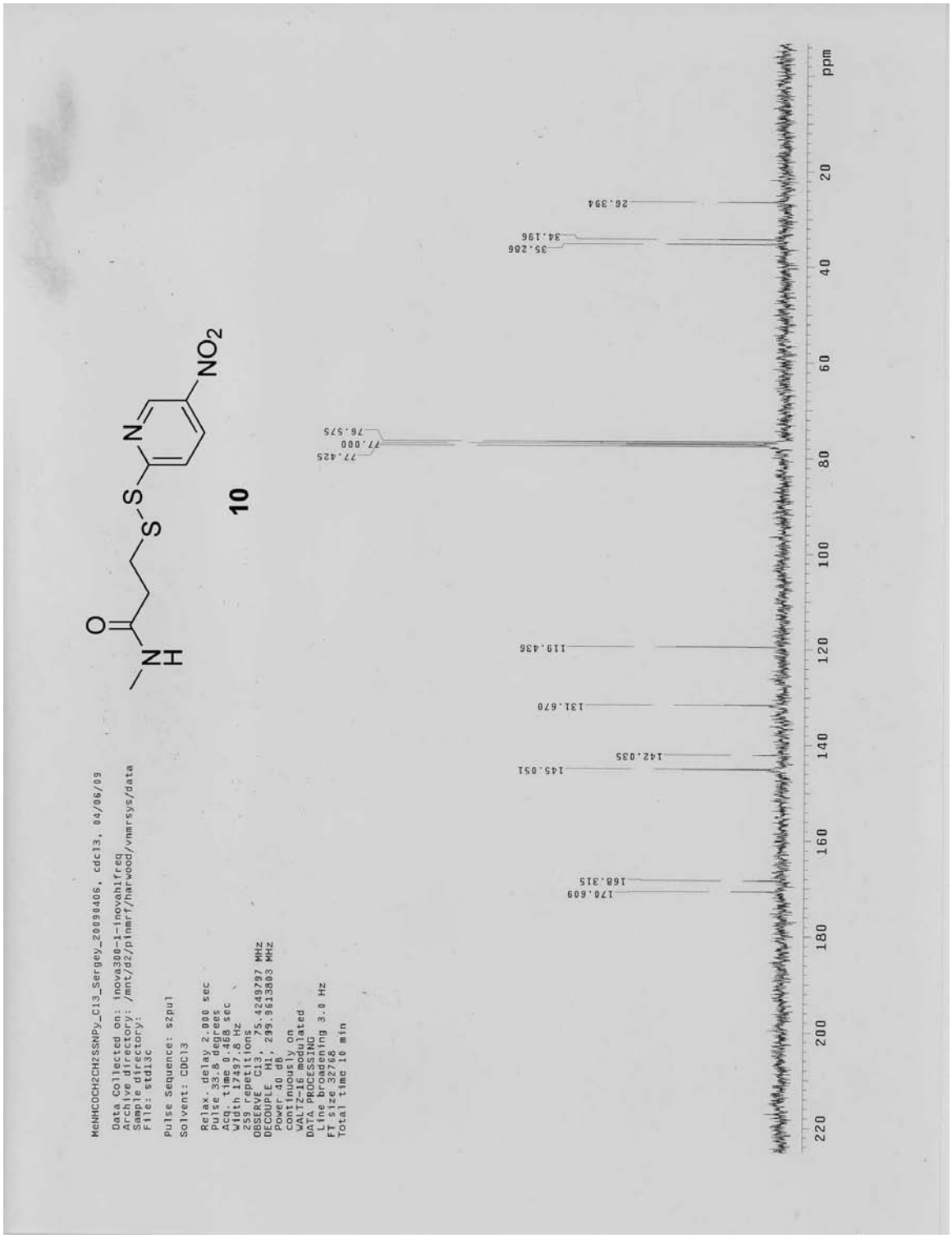


Fig. S26 ^{13}C NMR spectrum (75 MHz) of **10** in $CDCl_3$.

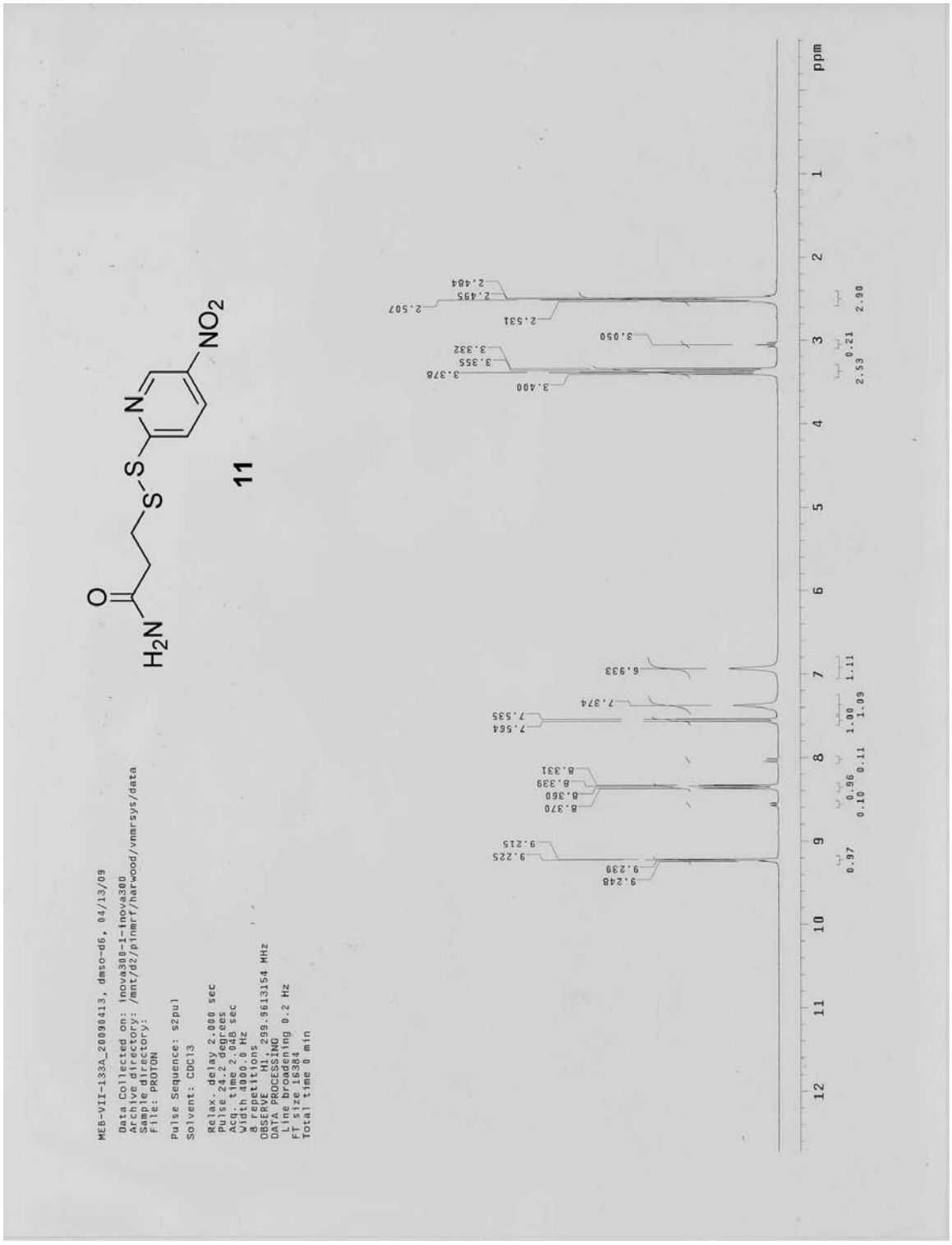


Fig. S27 ^{1}H NMR spectrum (300 MHz) of **11** in DMSO-d_6.

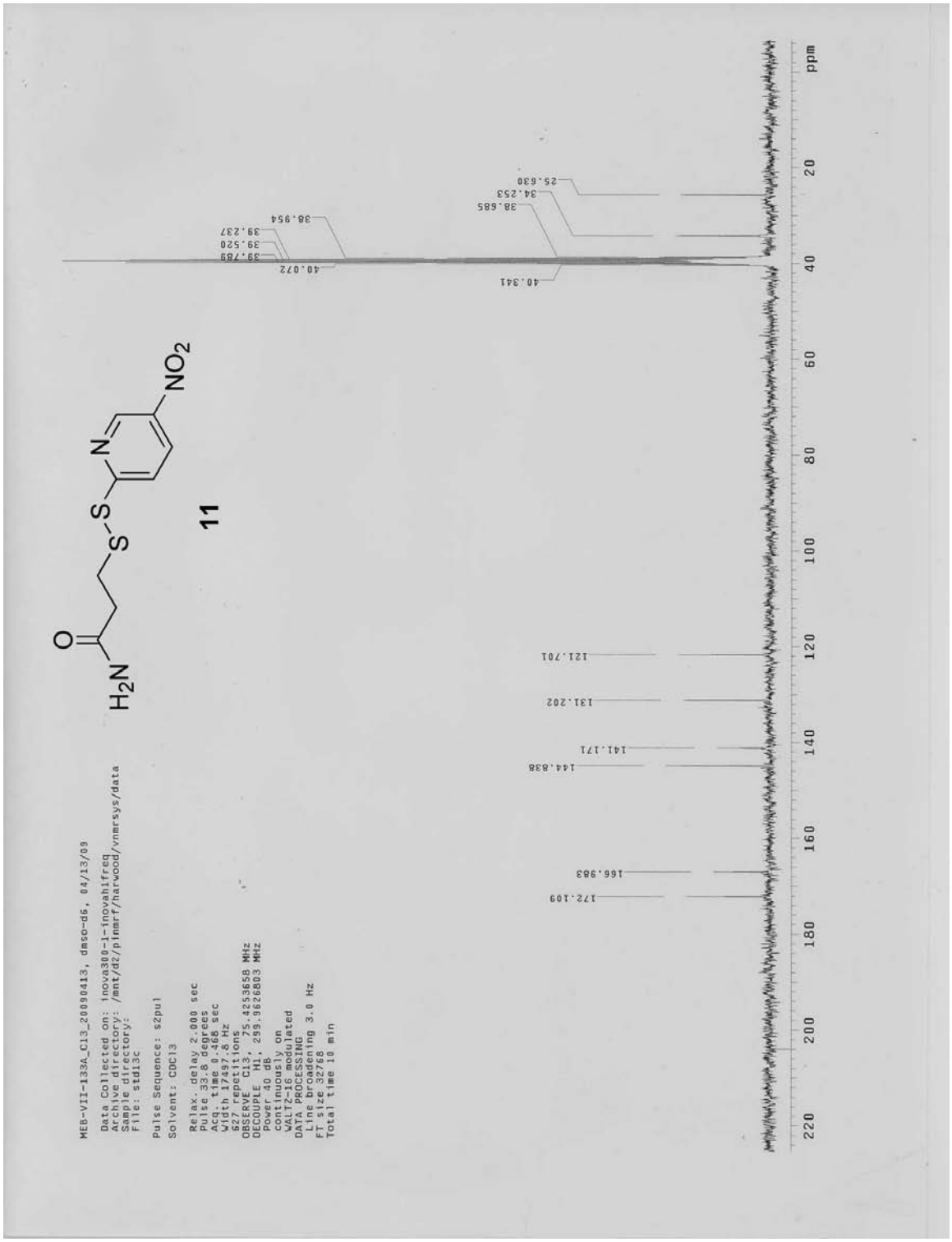


Fig. S28 ^{13}C NMR spectrum (75 MHz) of **11** in DMSO-d_6.

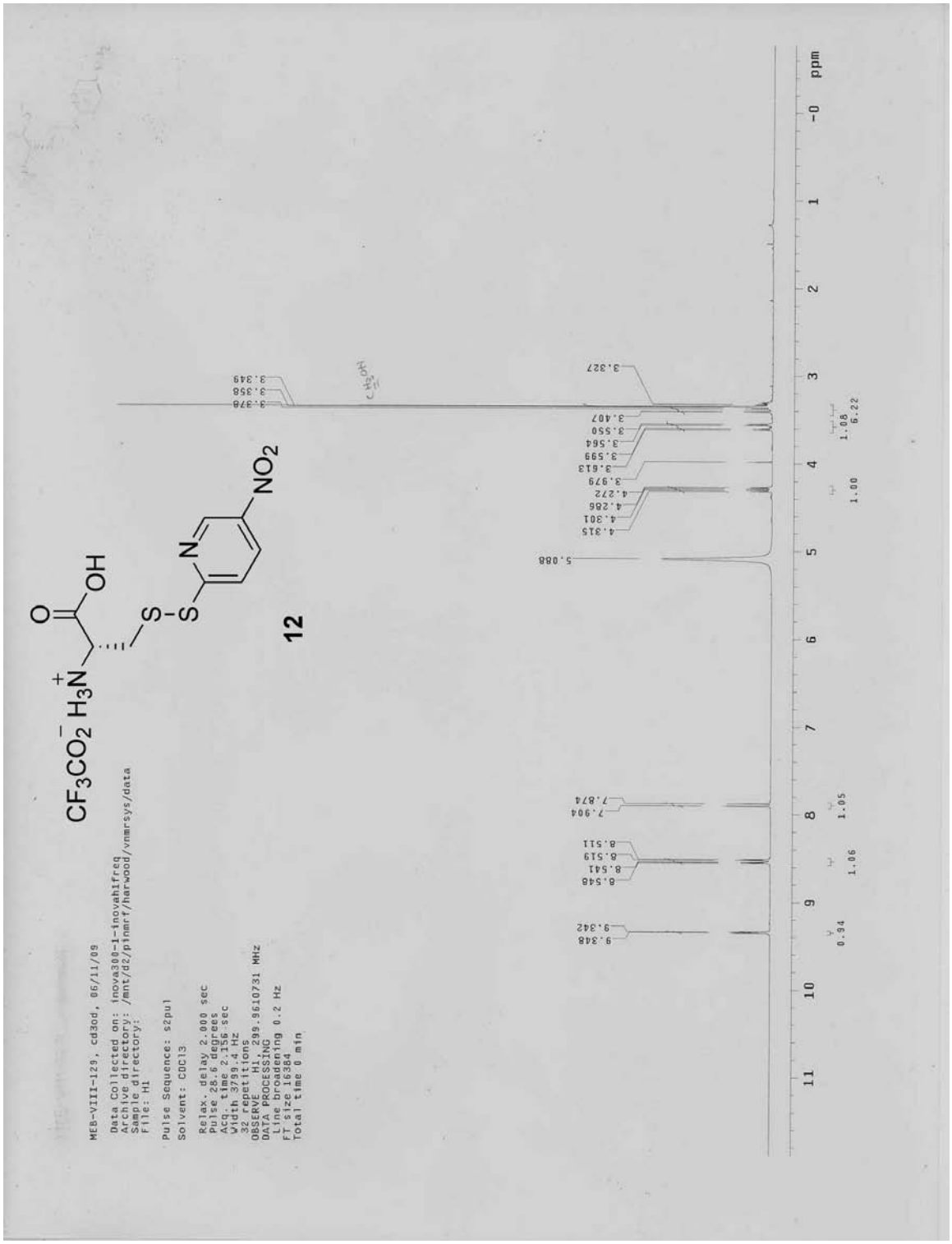


Fig. S29 ^{1}H NMR spectrum (300 MHz) of **12** in CD_3OD.

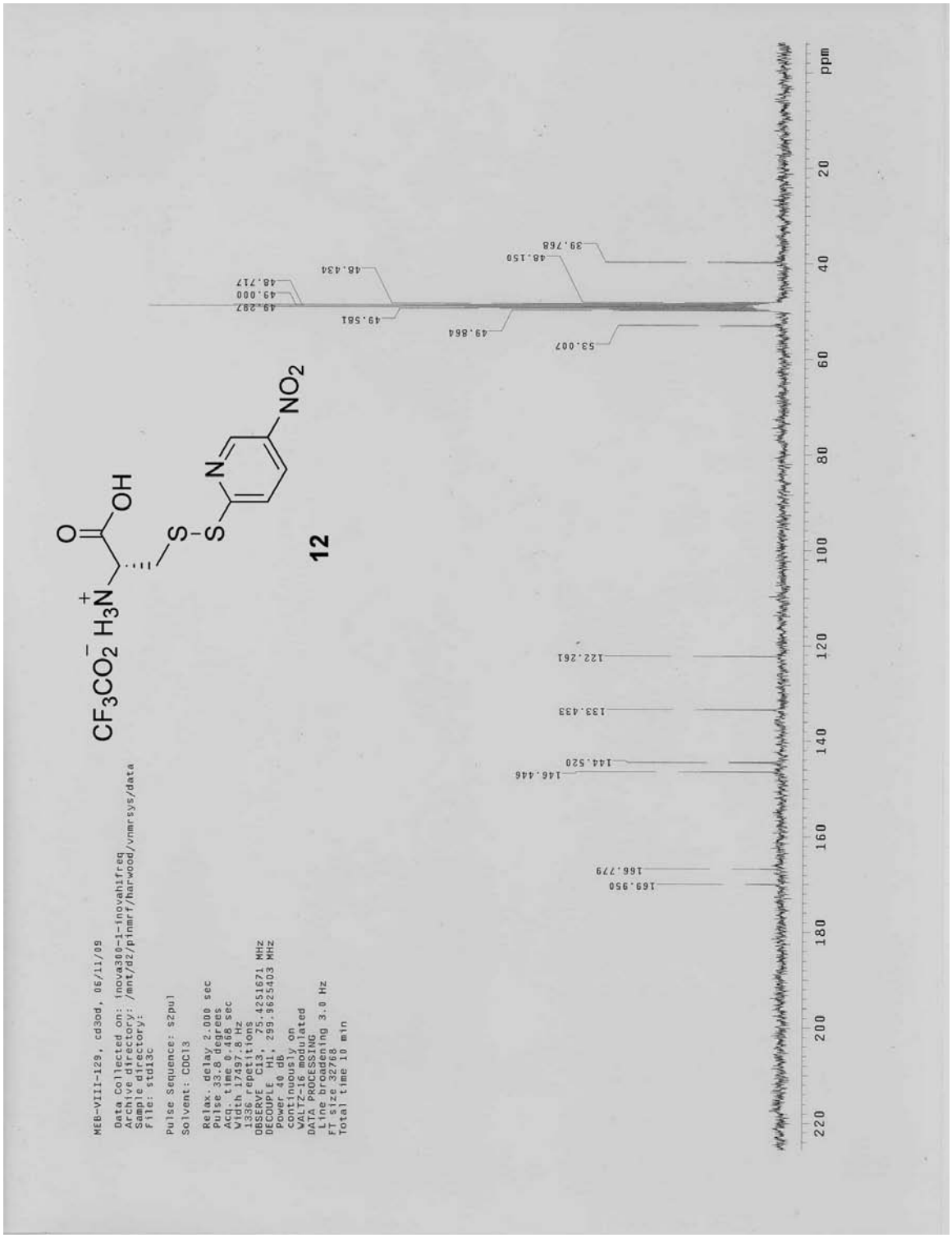


Fig. S30 ^{13}C NMR spectrum (75 MHz) of **12** in CD_3OD.

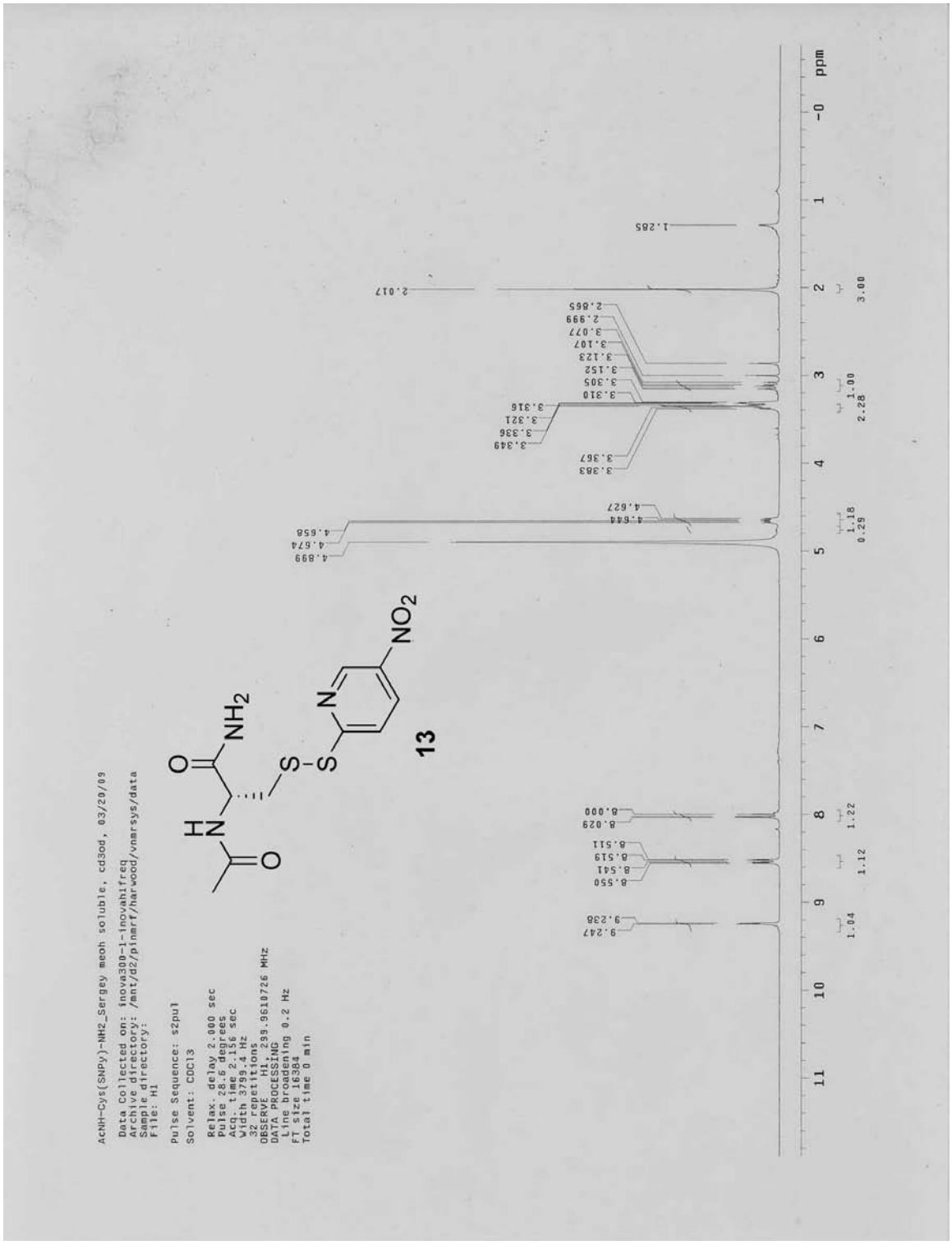


Fig. S31 ^{1}H NMR spectrum (300 MHz) of **13** in CD_3OD.

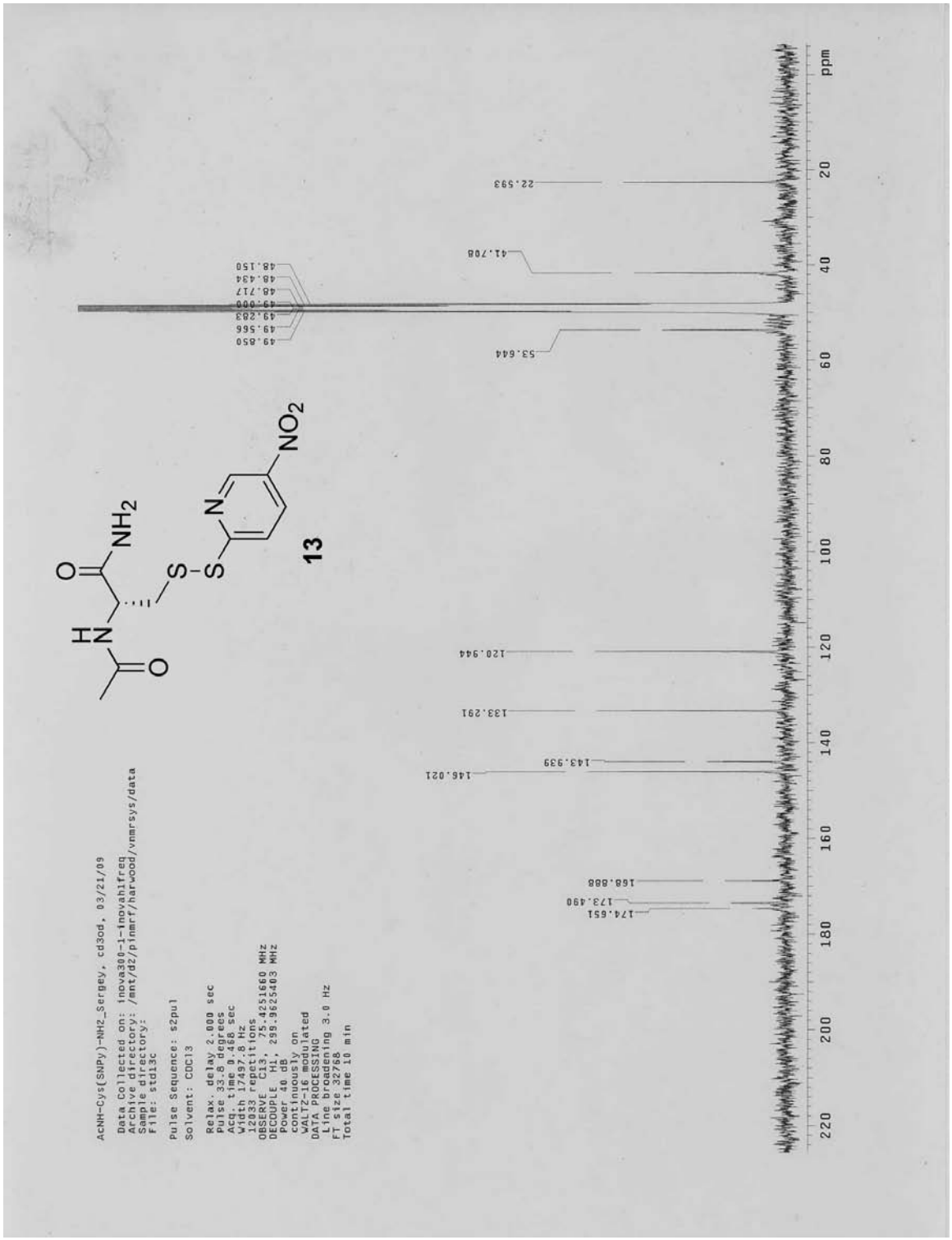


Fig. S32 ^{13}C NMR spectrum (75 MHz) of **13** in CD_3OD.

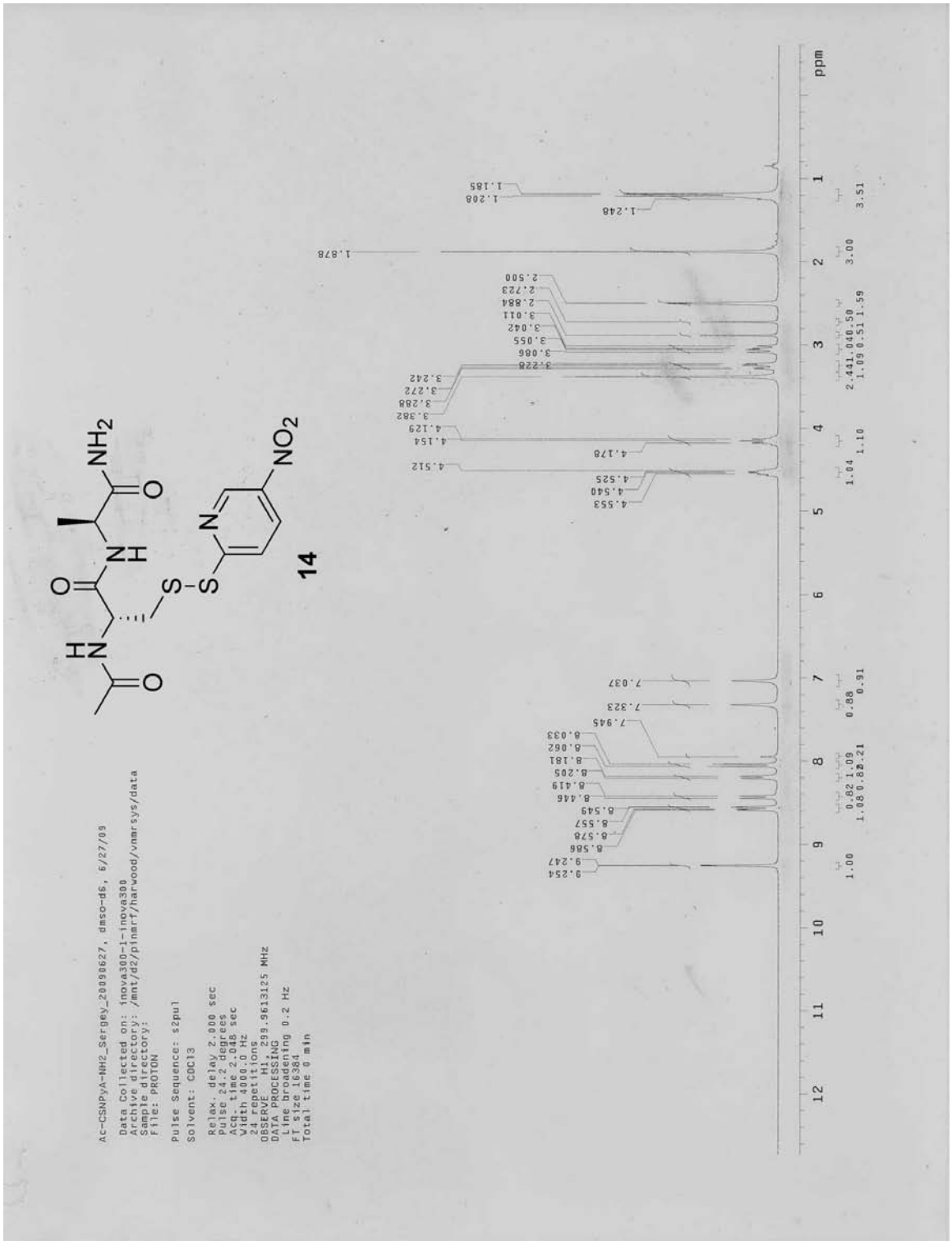


Fig. S33 ^{1}H NMR spectrum (300 MHz) of **14** in DMSO-d_6.

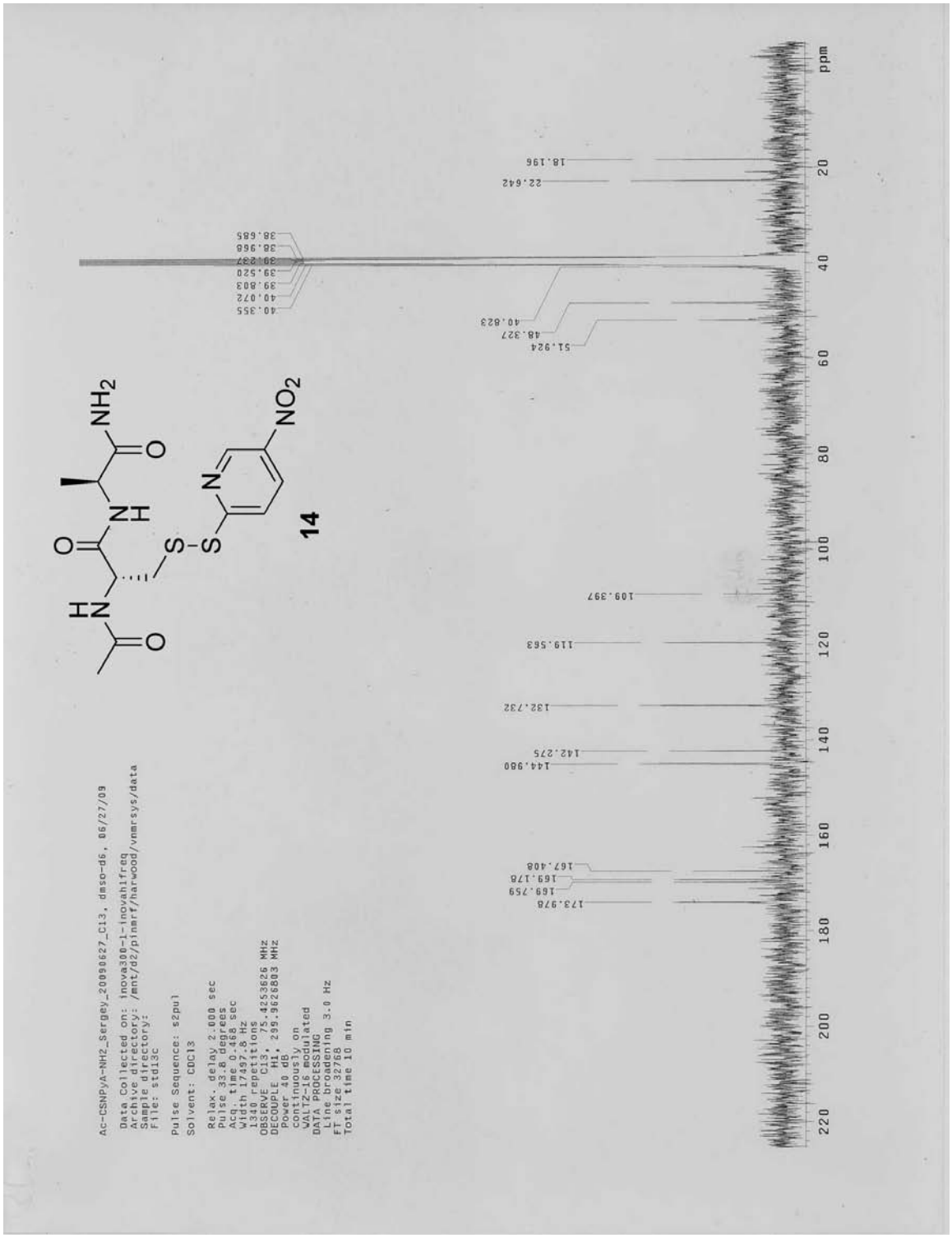


Fig. S34 ^{13}C NMR spectrum (75 MHz) of **14** in DMSO-d_6.

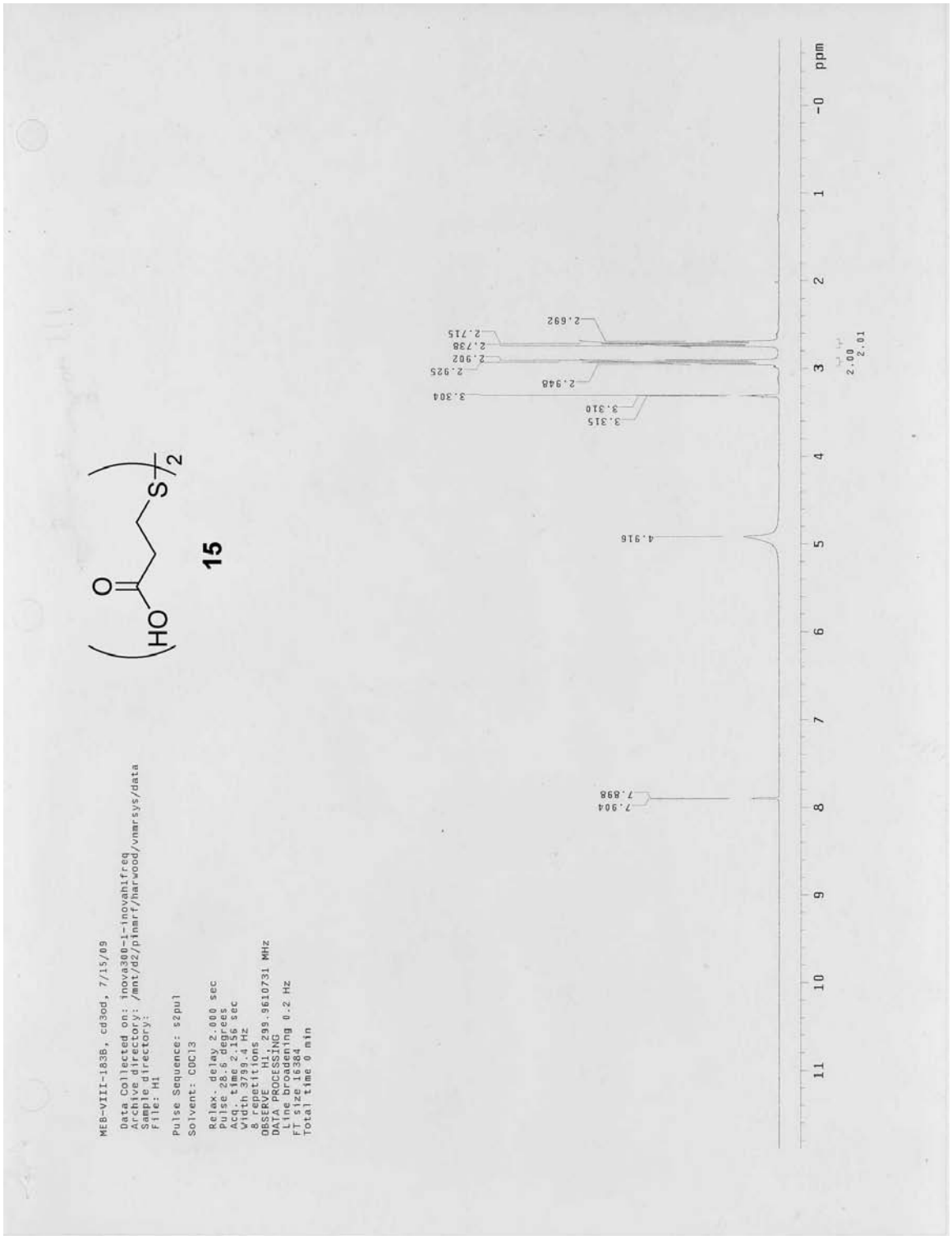


Fig. S35 ^{1}H NMR spectrum (300 MHz) of **15** in CD_3OD.

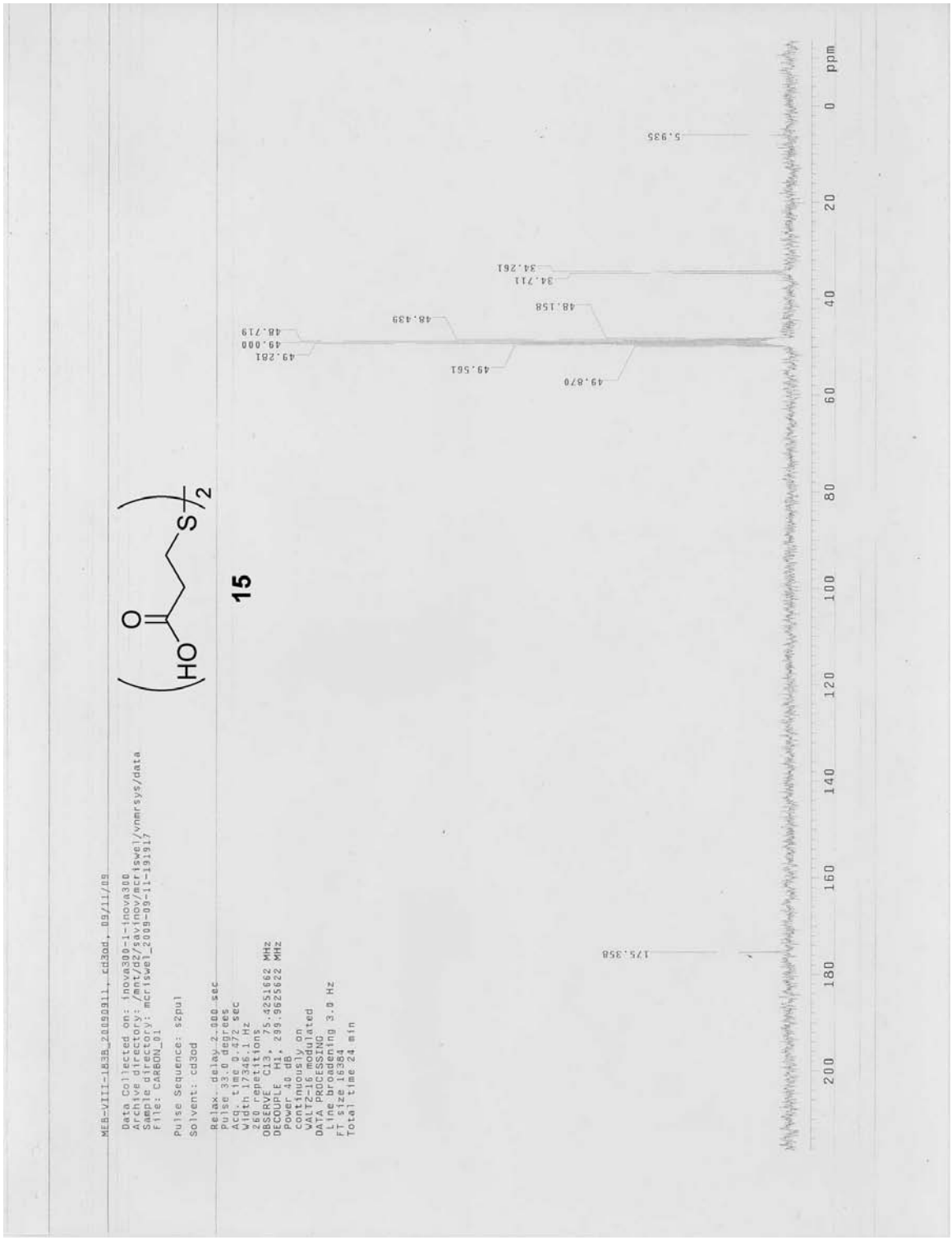


Fig. S36 ^{13}C NMR spectrum (75 MHz) of **15** in CD_3OD.

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