General Methods. Most of the general procedures, instrumentation and biochemical methods have been previously been described.[7,15,18] All reagents were purchased from Sigma or Aldrich and were used without further purification unless otherwise stated. All solvents were dried and distilled prior to use according to standard procedures.[19] L-propargyl glycine was obtained from Advanced Chem. Tech. Deionized water was purified with a Milli-Q apparatus (Millipore, Pitscataway, NJ). Spectropor 2 dialysis tubing was obtained from Fischer Chemicals. Pure DAP isomers (LL and meso) were obtained by enzymatic resolution as previously described.[15] Protein concentrations were determined using bovine serum albumin as a standard with a Biorad Bradford protein assay kit following manufacturer's instructions.

Inhibition Studies with DAP D-Dehydrogenase. DAP D-dehydrogenase was isolated and purified from *Bacillus spaericus* IFO 3525 as previously reported.[7,14] Purity of the enzyme samples was greater than 90% as determined by SDS-Page analysis (data not shown). Spectrophotometric assays were performed on a GBC Cintra 40 UV spectrophotometer equipped with a Neslab RTE-111 variable temperature bath.

The enzyme was assayed at 25.0 °C in 0.1 M Tris-HCl, 1 mM EDTA, 1 mM dithiothreitol, 1.10 mM *meso*-DAP and 0.3 mM NADP at pH 7.8 in a total volume of 1 mL. The increase of absorbance at 340 nm, using a 1 cm light path was then followed. One unit of enzyme activity is defined as the production of 1 μmol of NADPH produced per min under the assay conditions. For examination of the inhibitors as substrates , *meso*-DAP was omitted from the assay mixture and 1.8 mU of enzyme, together with a constant inhibitor concentration of 2.5 mM was present. For inhibiton tests, the assay buffer contained varying concentrations of the DAP analogue (100 μM-1 mM), 1.06 mM *meso*-DAP, and 3.6 mU of enzyme. These results were compared against a control performed simultaneously, where the Tris-HCl buffer replaced the inhibitor.

Inhibition Studies with DAP Epimerase. DAP epimerase was isolated and purified from an *E. coli* BL21 (DE3) pLysS mutant according to a modification of the literature procedure.[6a] All operations were performed at 4 °C. Harvested *E. coli* cells (suspended in 20 mM potassium phosphate buffer containing 1 mM ETDA, 10 mM DTT, pH 7.2) were lysed using a French

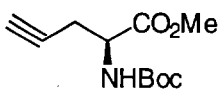
Pressure Cell (AMINCO) at 20,000 psi. The crude lysate was centrifuged at 5000 rpm and the supernatant was decanted. The solution (830 mL) was concentrated to 150 mL using an Amicon Diaflo membrane system. The concentrate was dialyzed for 15 h with 5 mM potassium phosphate buffer (1 mM EDTA, 10 mM DTT, pH 7.2). The crude, dialyzed enzyme solution was further applied to a DEAE-52 cellulose column (4 x 25 cm) at 1.0 ml/min, flushed with buffer A (200 mL) and then the epimerase activity was eluted using a continuous linear gradient (4 L) from 5 mM to 100 mM potassium phosphate (1 mM EDTA, 10 mM DTT, pH 7.2) at a flow rate of 3.0 ml/min. Protein concentration was followed by absorbance at 280 nm. All fractions containing active enzyme were combined and concentrated to a final volume of 60 mL. Thereafter, the enzyme solution was brought to 20% saturation with $(NH_4)_2SO_4$. The solution was allowed to stir for 1 h and then centrifuged at 20,000 rpm for 65 min. The pellet obtained was separated from the supernatant and dissolved in 30 mL of 100 mM phosphate buffer (1 mM EDTA, 10 mM DTT, pH 8.0). This solution was then dialyzed against the same buffer three times over 18 h (2.5 L dialyzing buffer in total). The dialyzed solution was then applied to a Reactive Blue-2 agarose column (4 ml bed volume) equilibrated in the same buffer and the activity was eluted with the same buffer containing 0.5 M KCl. All active fractions were combined, concentrated to 40 mL and dialyzed against 100 mM phosphate buffer (1 mM EDTA, 10 mM DTT, pH 7.2). The final specific acitivity was 75 U/mg.

Epimerase activity was monitored using a coupled enzyme assay which monitors the production of NADPH at 340 nm at 25 °C. The assay is performed in a 1 mL quartz cuvette filled with 1 mL of buffer solution (0.1 M Tris-HCl, 1 mM EDTA, 1 mM DTT, pH 7.8) containing 0.4 mM LL-DAP, 0.3 mM NADP, and 0.06 units of DAP D-dehydrogenase. One unit of epimerase activity corresponds to the production of one μmol of NADPH per minute. For inhibition studies with DAP analogues, the assay buffer contained varying concentrations of the DAP analogue (0.100 mM-1 mM), 0.4 mM LL-DAP, 0.3 mM NADP, 18-50 mU of DAP dehydrogenase, and 15 mU of DAP epimerase. These results were compared against a control performed simultaneously, where the Tris-HCl buffer replaced the inhibitor.

Synthesis of L-aspartate semialdehyde (For DHDP reductase studies). L–aspartate semialdehyde (L-ASA) was prepared by the ozonolysis of L-allyl-glycine according to the method of Black and Wright in 1 N HCl at 0 °C.[20] L-ASA was purified by applying the solution to a 1 x 30 cm AG-X8 cation-exchange column (H^+ form), washing with water and eluting with a 0-1 M HCl gradient. Fractions containing L-ASA were pooled and concentrated by rotary evaporation, and the concentration of L-ASA was determined enzymatically using aspartate semialdehyde dehydrogenase.

Inhibition Studies with DHDP reductase. Determination of the initial rates of dihydrodipicolinate reductase activity were based on following the decrease in absorbance at 340 nm of NADPH using 1 cm pathlength quartz cuvettes in a Gilford 260 spectrophotometer maintained at 25 °C with a circulating water bath and thermospacers. Typical assays contained 100 mM Hepes, pH 7.8, 100 μM NADPH, 1 mM pyruvic acid, 10 μg of dihydrodipicolinate synthase, 25-100 μM L-ASA and 0-200 μM concentrations of inhibitor. Reaction mixtures were incubated for five minutes to allow for the complete conversion of pyruvate and L-ASA to dihydrodipicolinate, and initiated by the addition of a small volume (2-10 μL) of dihydrodipicolinate reductase.

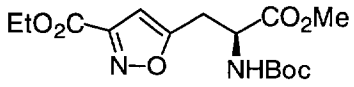
Data Analysis for DHDP Reductase. Reciprocal initial velocities were plotted against the reciprocal of the L-DHDP concentration, at various inhibitor concentrations and the data fitted to the equation describing competitive inhibition: $v = V^*A/ [K(1. + I/K_i) + A]$, where v is the initial velocity, A is the concentration of L-DHDP, I is the concentration of inhibitor, K_i is the slope inhibition constant and V is the maximal velocity.



Methyl *N*-(*tert*-butoxycarbonyl)-L-propargyl glycinate (8).

To dry MeOH (10 mL) at 0 °C was added thionyl chloride (1.0 mL, 13 mmol) dropwise over 5 min. The solution was stirred for 10 min and L-propargylglycine hydrochloride was added (0.65 g, 4.35 mmol) in one portion. The resulting solution was stirred overnight at room temperature,

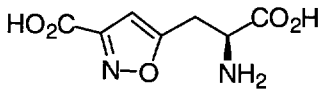
solvent and excess thionyl chloride removed *in vacuo* to give an oily residue. This residue was subsequently dissolved in dry MeCN (10 mL), then triethylamine (0.73 mL, 5.21 mmol) and di-*tert*-butyl pyrocarbonate (1.14 g, 5.21 mmoL) were added. The mixture was stirred for 2 h at ambient temperature, the solvent evaporated and the resulting residue suspended in 1 M $NaHSO_4$. The mixture was extracted with CH_2Cl_2 (3 x 15 mL), and the combined organic extracts were washed with 1 M $NaHCO_3$ (5 mL), dried (Na_2SO_4) and solvent removed *in vacuo* to afford a crude oil which was purified by flash chromatography (10% EtOAc in hexane) to give **8** as a colourless oil (0.96 g, 95%): $[\alpha]_D^{26}$ -5.0° (*c* 3.0, MeOH) (lit[10] $[\alpha]_D^{26}$ -5.2°(*c* 3.0, MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.49 (s, 9H, ($C\underline{H}_3$)$_3$), 2.05 (t, 1H, *J* = 2.6 Hz, C<u>H</u>), 2.74 (m, 2H, C<u>H</u>$_2$CHNH), 3.79 (s, 3H, OC<u>H</u>$_3$), 4.49 (ddd, 1H, *J* = 9.0, 7.9, 4.8 Hz, CH$_2$C<u>H</u>NH), 5.35 (d, 1H, *J* = 7.9 Hz, N<u>H</u>); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 22.91, 28.33, 51.98, 52.67, 71.64, 77.48, 80.27, 155.13, 171.17; HRMS (ES) Calcd for $C_{11}H_{17}NO_4$ 250.1055, found 250.1057.



5-((2S)-((*N*-*tert*-Butoxycarbonyl)amino)-2-methoxycarbonyl-ethyl)-isoxazole-3-carboxylic acid ethyl ester (10).

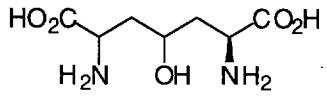
To a vigorously stirred solution of ethyl chlorooximidoacetate (1.93 g, 12.7 mmol) and methyl *N*-*tert*-butoxycarbonyl propargylglycinate **8** (0.963 g, 4.24 mmol) in Et_2O (15 mL) was added sodium carbonate (1.35 g, 12.7 mmol) in H_2O (10 mL) via syringe pump over a 5 h period. The mixture was diluted with Et_2O (20 mL), the organic layer separated, washed with H_2O (10 mL), dried (Na_2SO_4) and concentrated *in vacuo* to afford an oil which was purified by flash chromatography (10-20% EtOAc in hexane) to give **10** as a white solid (0.94 g, 70%): mp 62-65 °C; $[\alpha]_D^{26}$ +43° (*c* 1.0, CH_2Cl_2); IR ($CHCl_3$ cast) 3372, 1732, 1715, 1596 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.36 (t, 3H, *J* = 7.0 Hz, OCH$_2$C<u>H</u>$_3$), 1.40 (s, 9H, ($C\underline{H}_3$)$_3$), 3.20-3.34 (dd, 1H, *J* = 15.5, 5.5 Hz, C<u>H</u>$_2$CHNH), 3.35-3.48 (dd, 1 H, *J* = 15.5, 6.0 Hz, C<u>H</u>$_2$CHNH), 3.74 (s, 3H,

OC<u>H</u>$_3$), 4.39 (q, 2H, J = 7.0 Hz, OC<u>H</u>$_2$CH$_3$), 4.60 (m, 1H, CH$_2$C<u>H</u>NH), 5.20 (m 1H, N<u>H</u>), 6.42 (s, 1H, C<u>H</u>=C(O)CH$_2$); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 14.02, 28.13, 29.67, 51.87, 52.77, 62.05, 80.37, 103.44, 154.89, 156.42, 159.76, 170.77, 170.21; HRMS (ES) Calcd for $C_{15}H_{22}N_2O_7Na$ 365.1325, found 365.1327; Anal. Calcd for $C_{15}H_{22}N_2O_7$: C, 52.63; H, 6.48; N, 8.18. Found: C, 52.57; H, 6.43; N, 8.09.

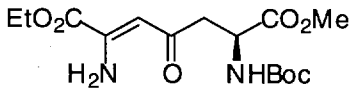


5-((2S)-Amino-2-carboxy-ethyl)-isoxazole-3-carboxylic acid (11).

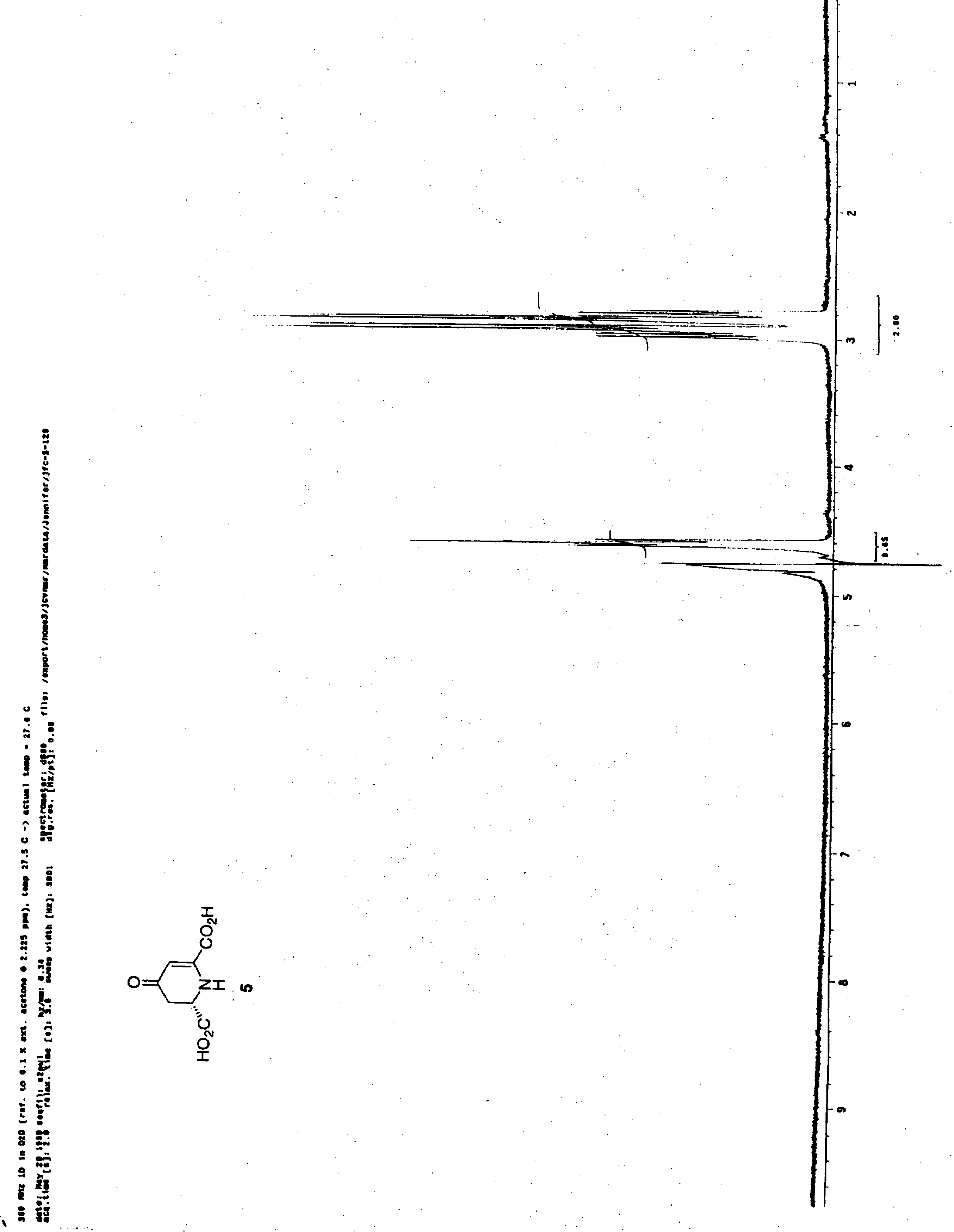
To a solution of **10** (200 mg, 0.584 mmol) in MeCN/H_2O (1:1, 6 mL) was added lithium hydroxide monohydrate (58 mg, 1.29 mmol). The solution was allowed to stir for 17 h at ambient temperature and the solvent was removed *in vacuo*. The residue was dissolved in water and extracted with EtOAc (3 x 10 mL). The aqueous layer was acidifed to pH 2 with 6M HCl and extracted with EtOAc (3 x 15 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated *in vacuo* to give a colourless residue that was dissolved in CH_2Cl_2 (2 mL) and treated with TFA (450 μL, 5.8 mmol). The solution was stirred for 45 min and the solvent removed in vacuo to afford a crude solid which was purified by flash chromatography (30% NH_3 in isopropanol) to give **11** as white crystals (120 mg, 88%): $[\alpha]_D^{26}$ -10.7° (*c* 0.15, H_2O); IR (μscope) 3229-2400, 1634, cm^{-1}; ^{1}H NMR (D_2O, 300 MHz) δ 3.41 (dd, A of ABX, 1H, J = 16.1, 7.5 Hz C<u>H</u>$_2$CHNH), 3.51 (dd, B of ABX, 1H, J = 16.1, 5.1 Hz, C<u>H</u>$_2$CHNH), 4.12 (dd, 1H, J = 5.1, 7.5 Hz, CH$_2$C<u>H</u>NH); 6.51 (s, 1H, C=C<u>H</u>); ^{13}C NMR (D_2O, 75.5 MHz) δ 28.72, 53.79, 104.72, 162.20, 167.03, 169.47, 173.55; HRMS (ES) Calcd for $C_7H_9N_2O_5$ 201.0511, found 201.0516.

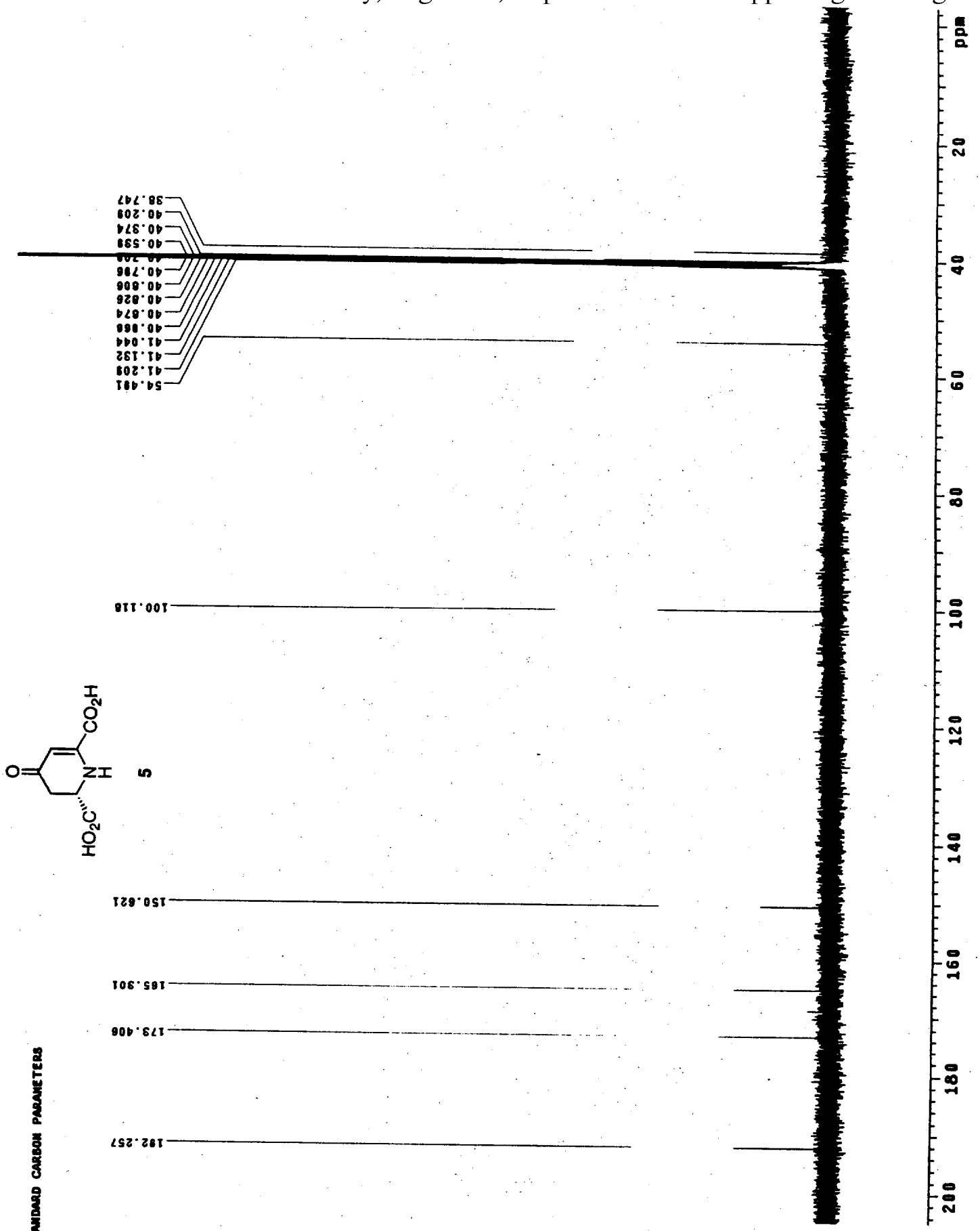


(2RS, 4RS, 6S)-2,6-diamino-4-hydroxy-heptane-1,7-dioic acid (12). To a solution of **11** (10 mg, 0.043 mmol) in water (4 mL), was added 10% Pd/C (8 mg). The suspension was stirred under 1 atm of H_2 for 10 h, then filtered through a bed of celite which was subsequently washed with H_2O. The filtrate was evaporated *in vacuo* to give an oily residue which was purified by reverse-phase C_{18} HPLC (R_t = 2.8 min, 5% MeCN/H_2O over 5 min) to give **12** as a colourless oil (7 mg, 79%): IR (μscope) 3500-3060, 1594, 1402 cm^{-1}; ^1H NMR (D_2O, 300 MHz) δ 1.82-2.20 (m, 4H, 2 x C$\underline{H}_2$), 3.68-3.83 (m, 2H, 2 x C$\underline{H}NH_2$), 3.86 (s, 1H, O$\underline{H}$), 4.06 (m, 1H, C$\underline{H}$OH); ^{13}C NMR (D_2O, 125 MHz) δ 37.86, 38.32, 50.74, 53.32 54.03, 54.18, 66.68, 67.84, 68.89, 174.18, 175.15; HRMS (ES) Calcd for $C_7H_{14}N_2O_5Na$ 229.0800, found 229.0806.



(6*S*)-2-Amino-6-(*N*-(*tert*-butoxycarbonyl)amino)-4-oxo-2-heptene-1,7-dioic acid 1-ethyl ester 7-methyl ester (13). To a solution of isoxazole **10** (0.45 g, 1.31 mmol), in acetonitrile (10 mL) under argon was added molybdenum hexacarbonyl (0.22 g, 0.85 mmol) and H_2O (24 μL, 1.31 mmol). The mixture was heated under reflux for 6 h and the solvent removed *in vacuo* to give a crude black oil which was purified by flash chromatography (5-25% EtOAc in hexane) to afford a light yellow oil (0.285 g, 65%): $[\alpha]_D^{26}$ +77.5°(*c* 1.0, CH_2Cl_2); IR (CH_2Cl_2 cast) 3431, 1717, 1639, 1593, 1215 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.33 (t, 3H, *J* = 7.0 Hz, OCH$_2$C$\underline{H}_3$), 1.40 (s, 9H, $(CH_3)_3$), 2.93 (dd, 1H, A of ABX, *J* = 17.3, 4.2 Hz, C$\underline{H}_2$CHNH), 3.16 (dd, 1H, B of ABX, *J* = 17.3, 3.9 Hz, C$\underline{H}_2$CHNH), 3.71 (s, 3H, OC$\underline{H}_3$), 4.30 (q, 2H, OC$\underline{H}_2$CH$_3$), 4.48 (m, 1H, CH$_2$C$\underline{H}$NH), 5.52 (m, 1H, N$\underline{H}$), 5.85 (s, 1H, C$\underline{H}$=C(NH$_2$)), 8.9 (br s, 2H, N$\underline{H}_2$); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 14.04, 28.33, 44.21, 50.02, 52.48, 62.72, 79.83,

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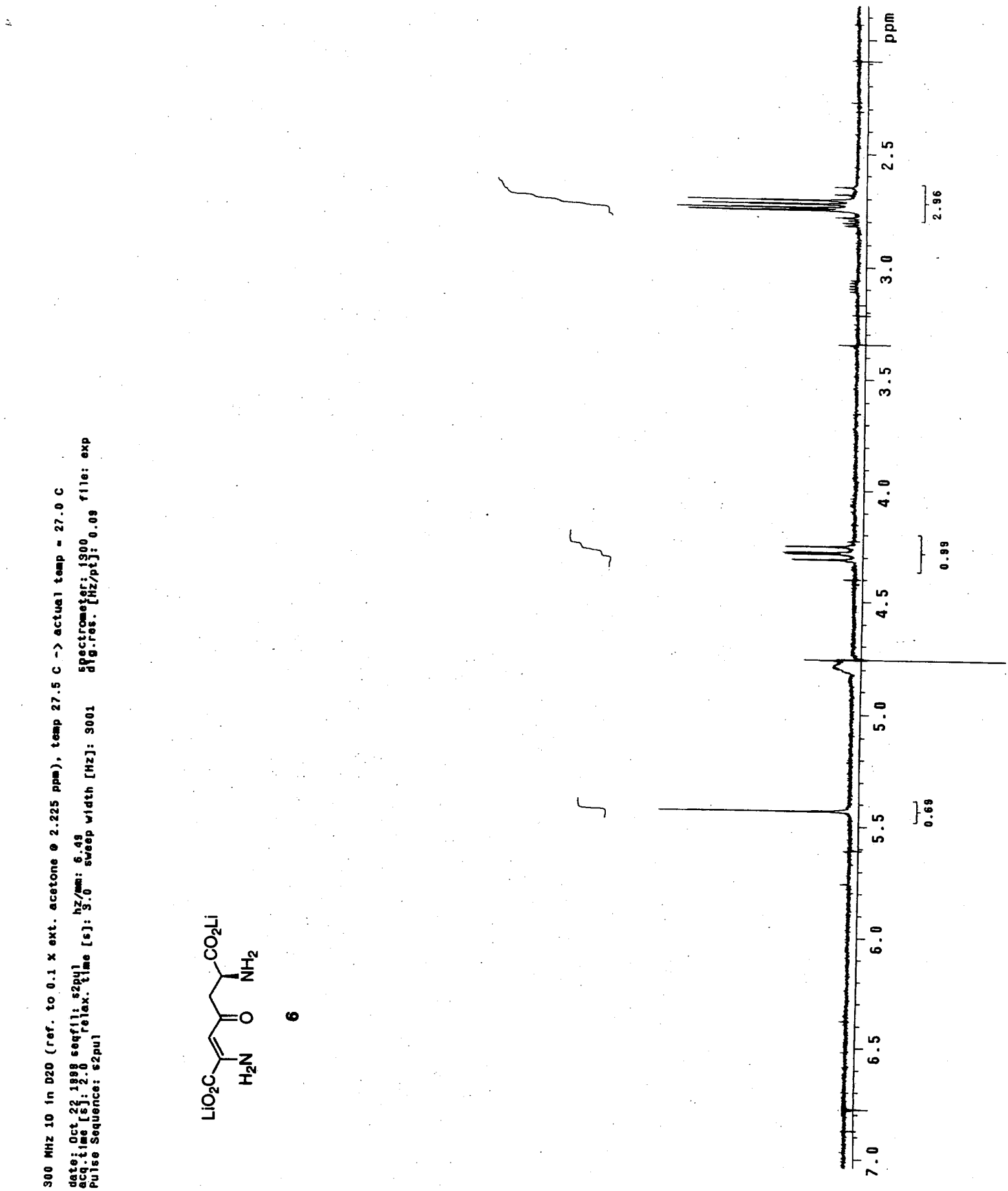

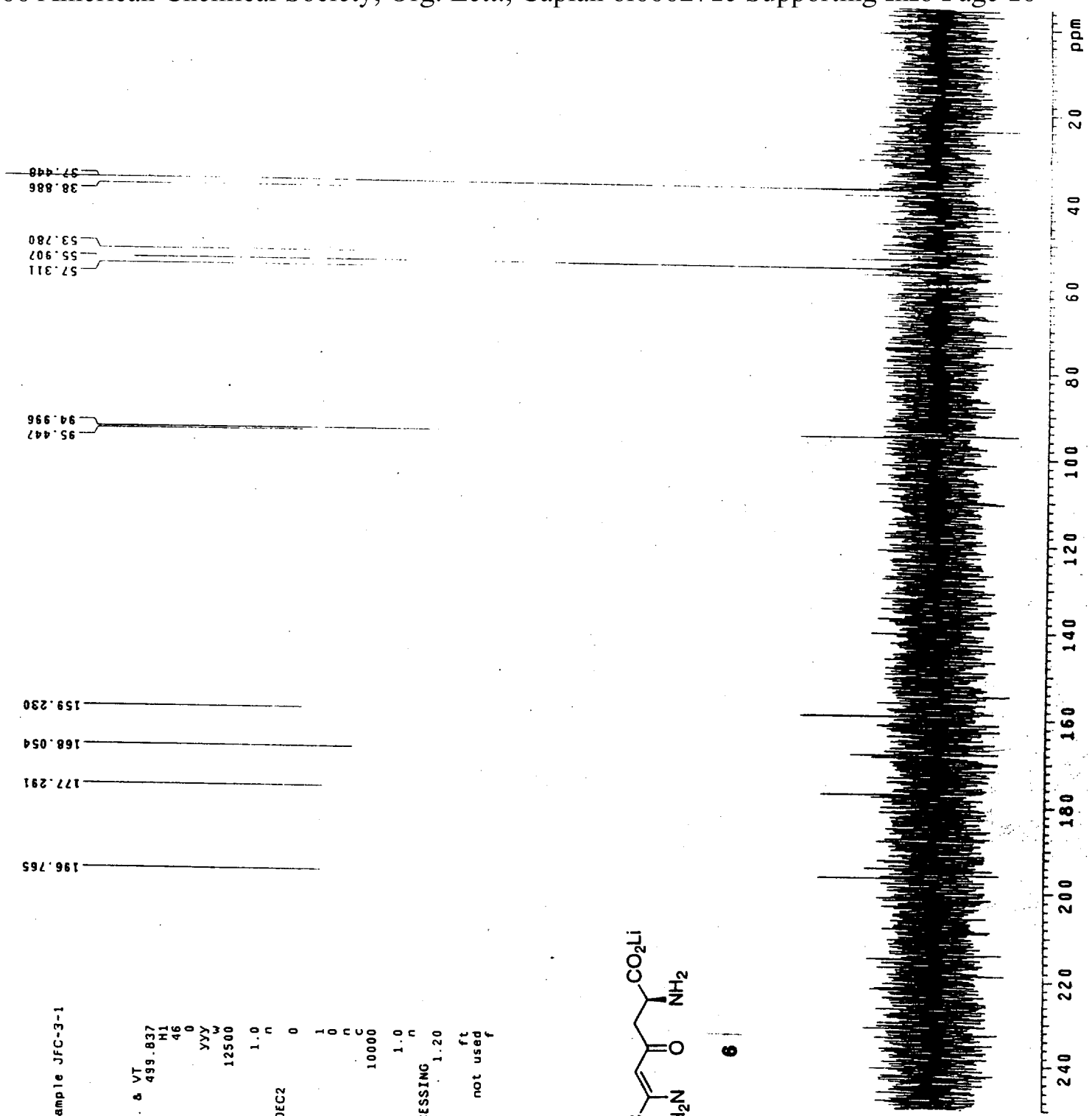

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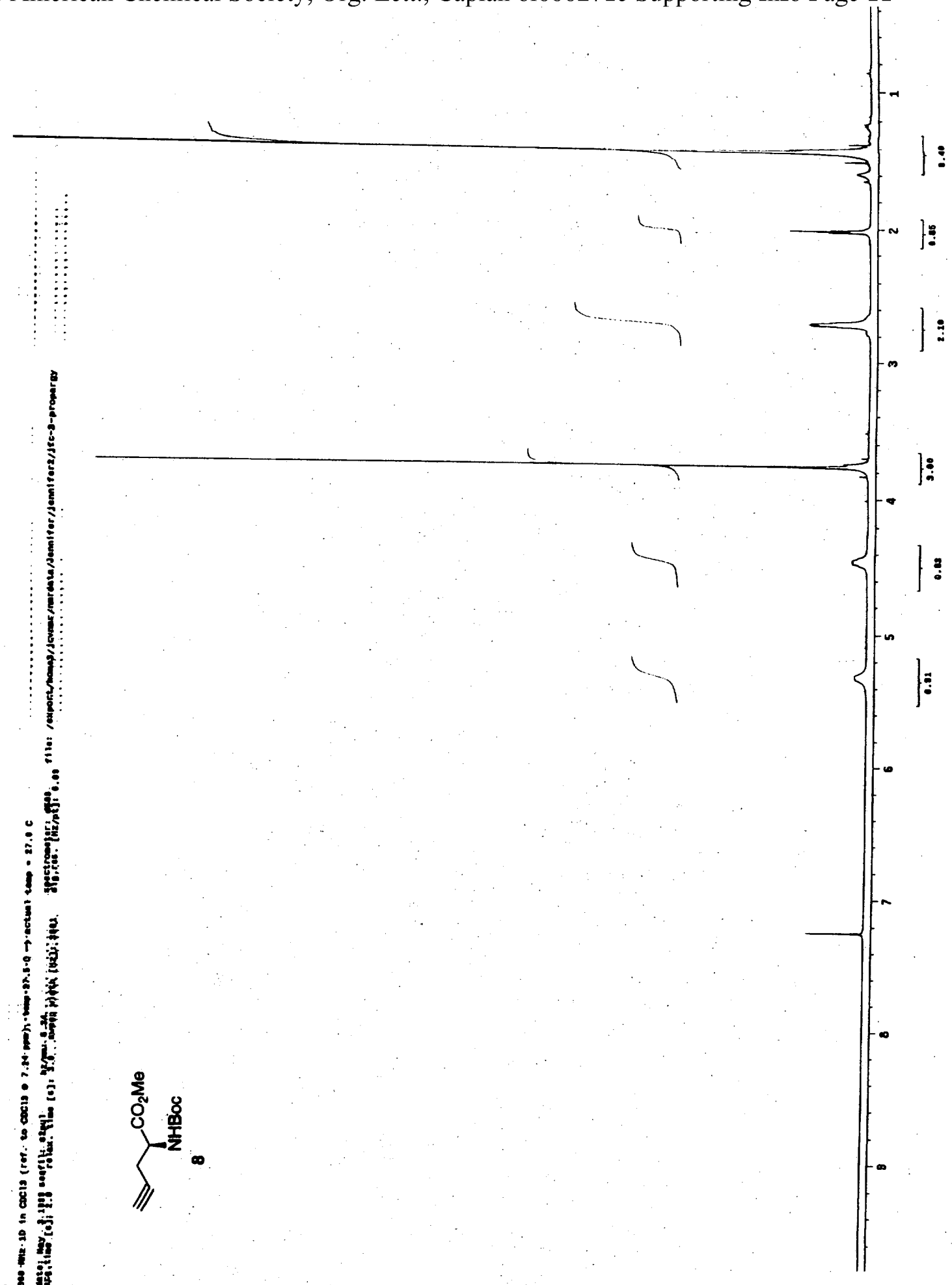

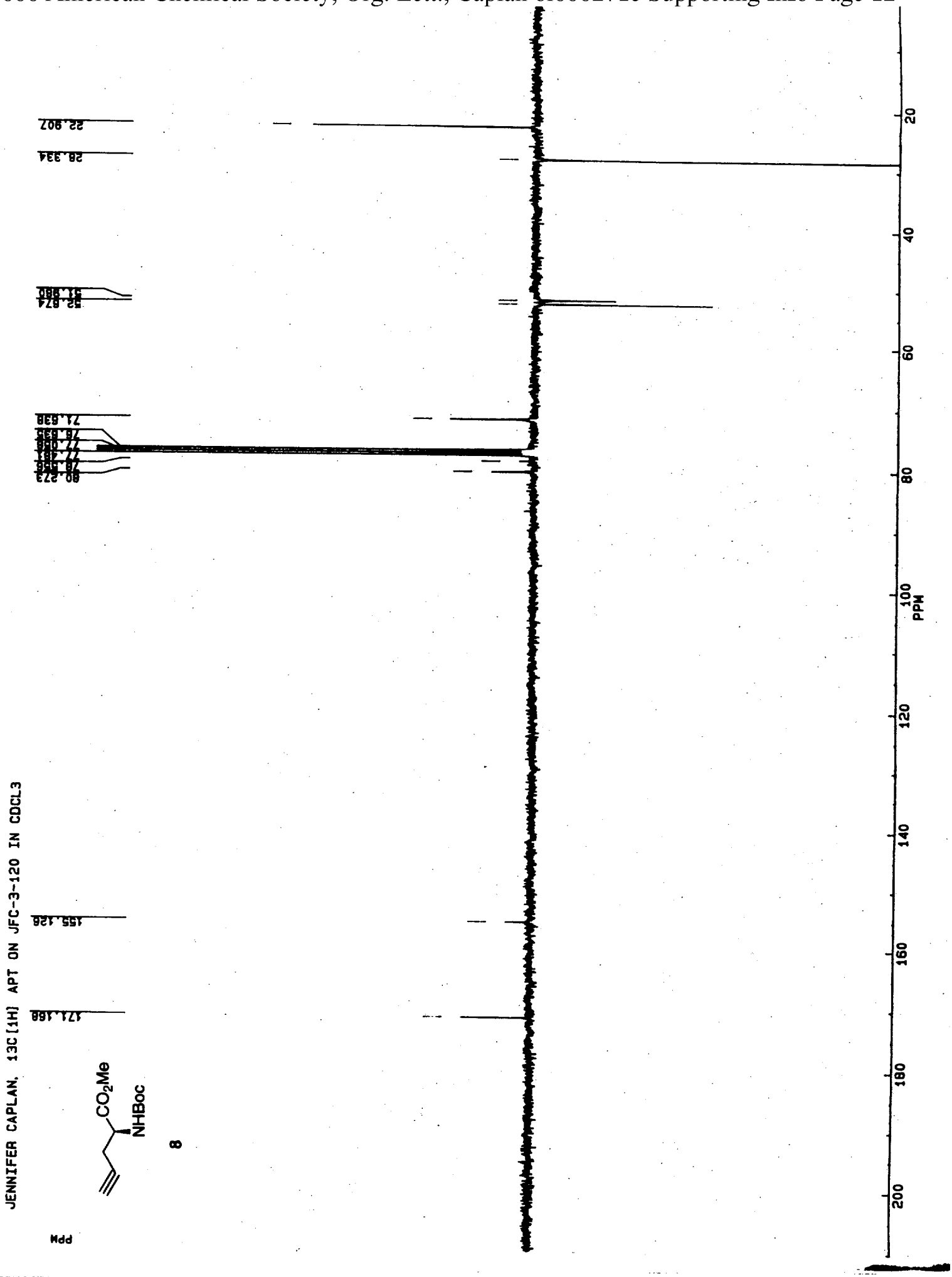
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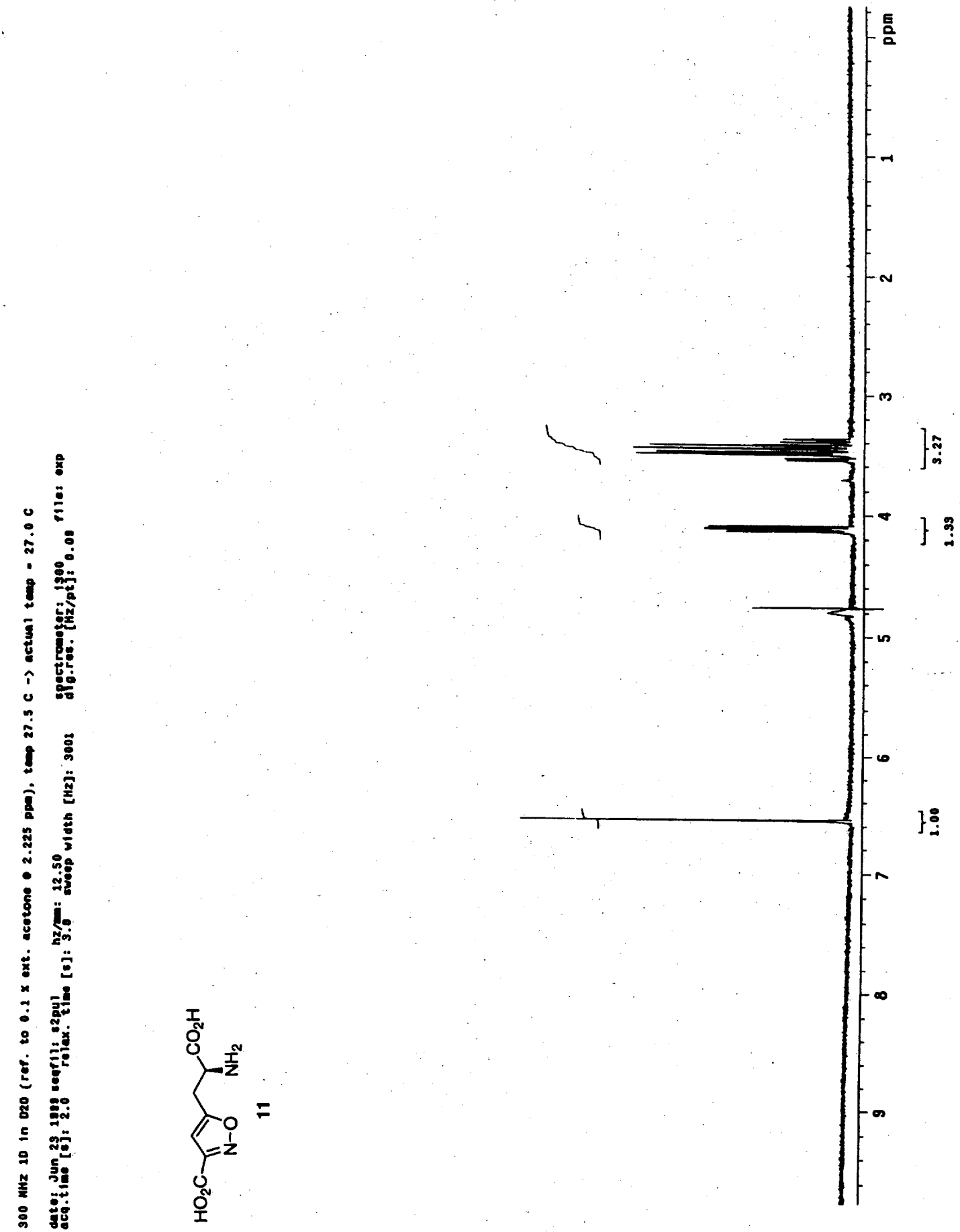

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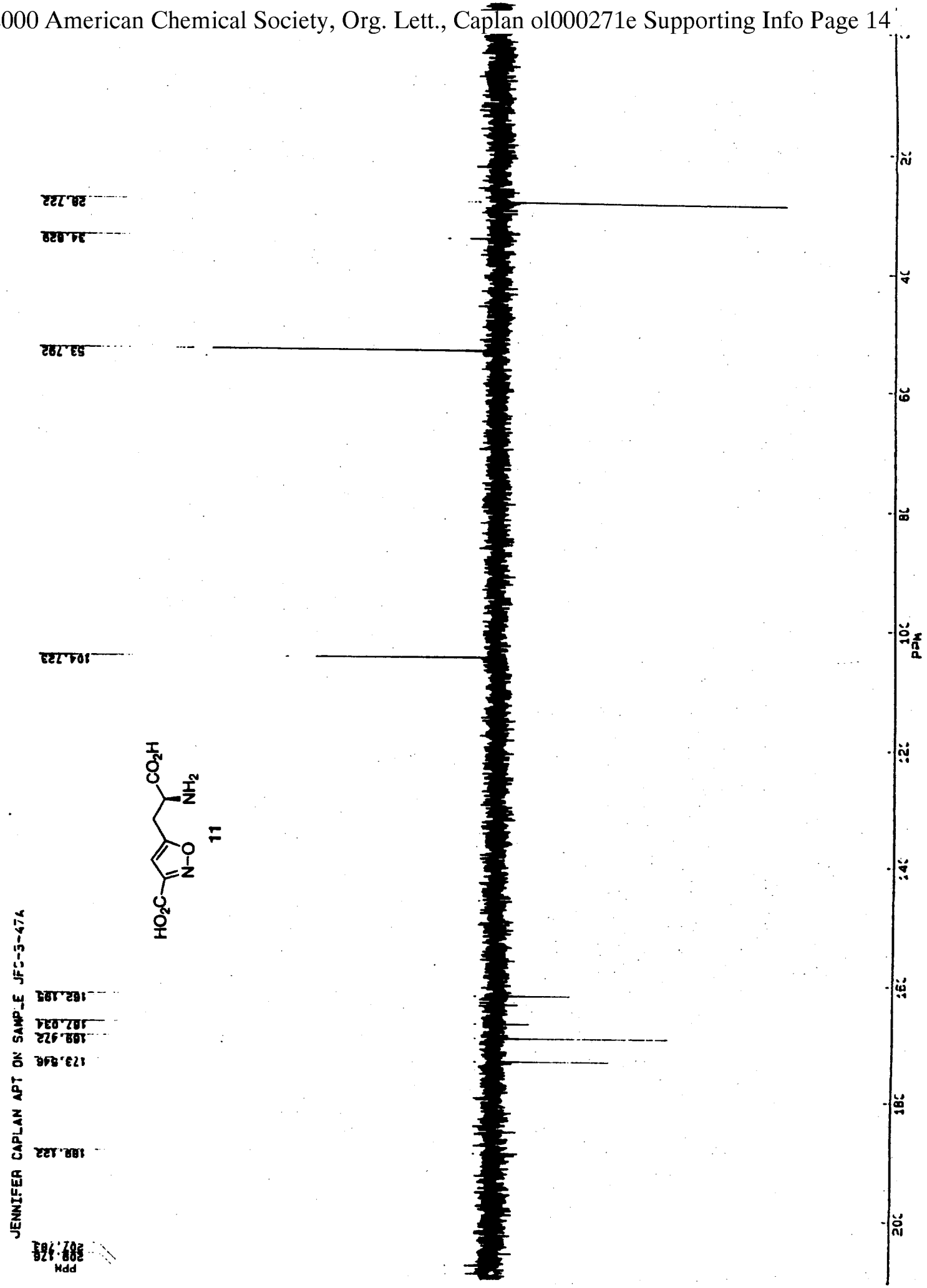

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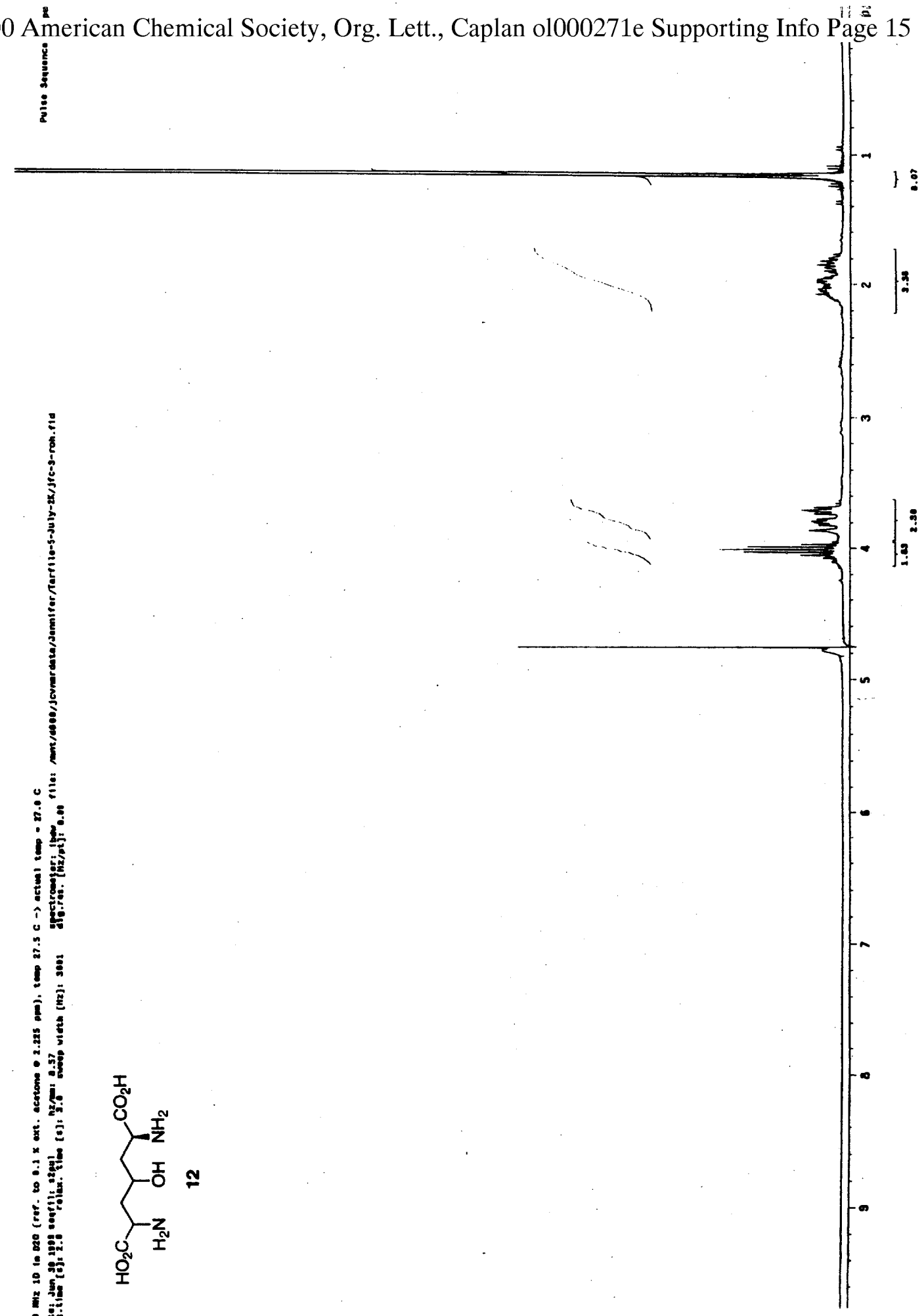

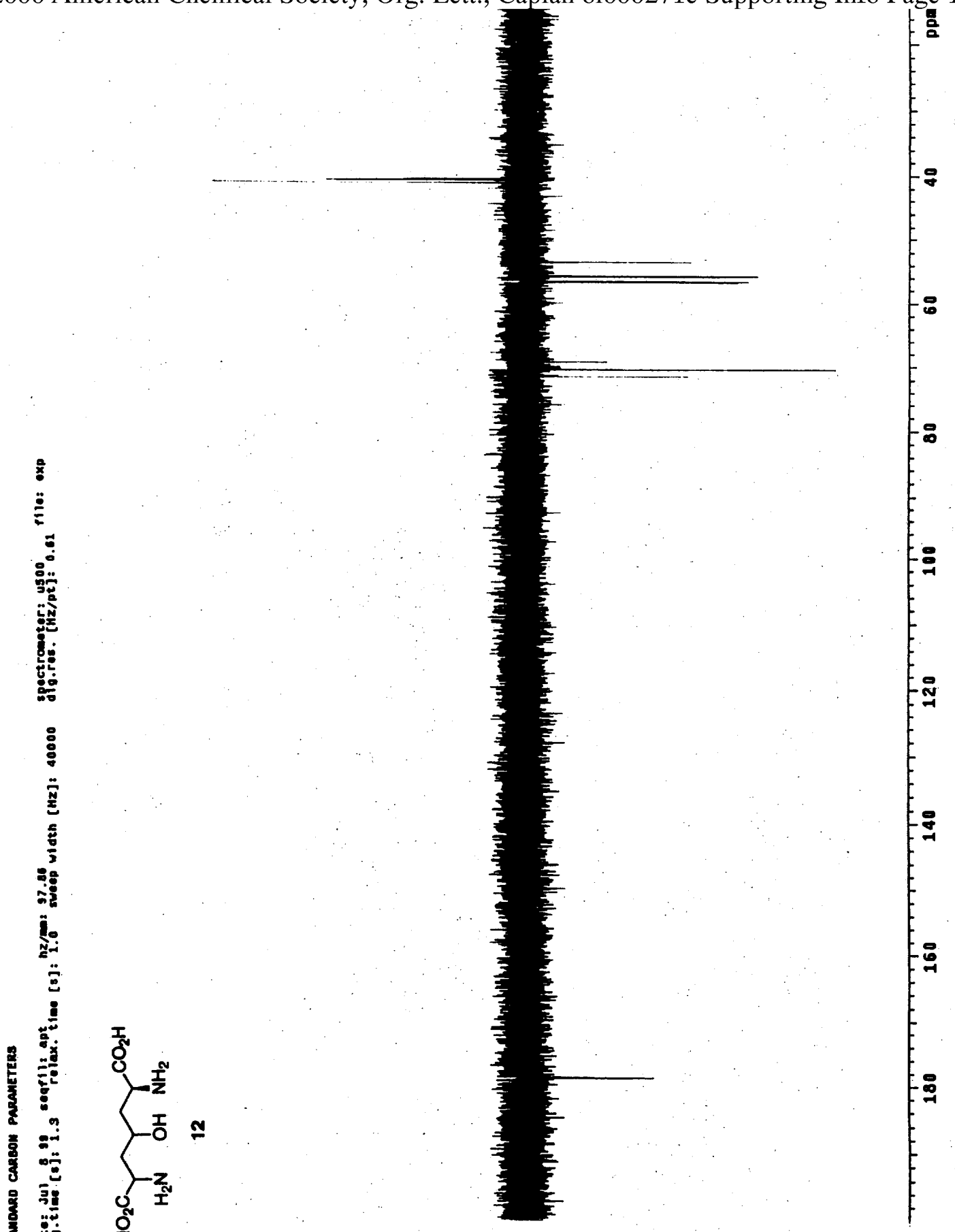

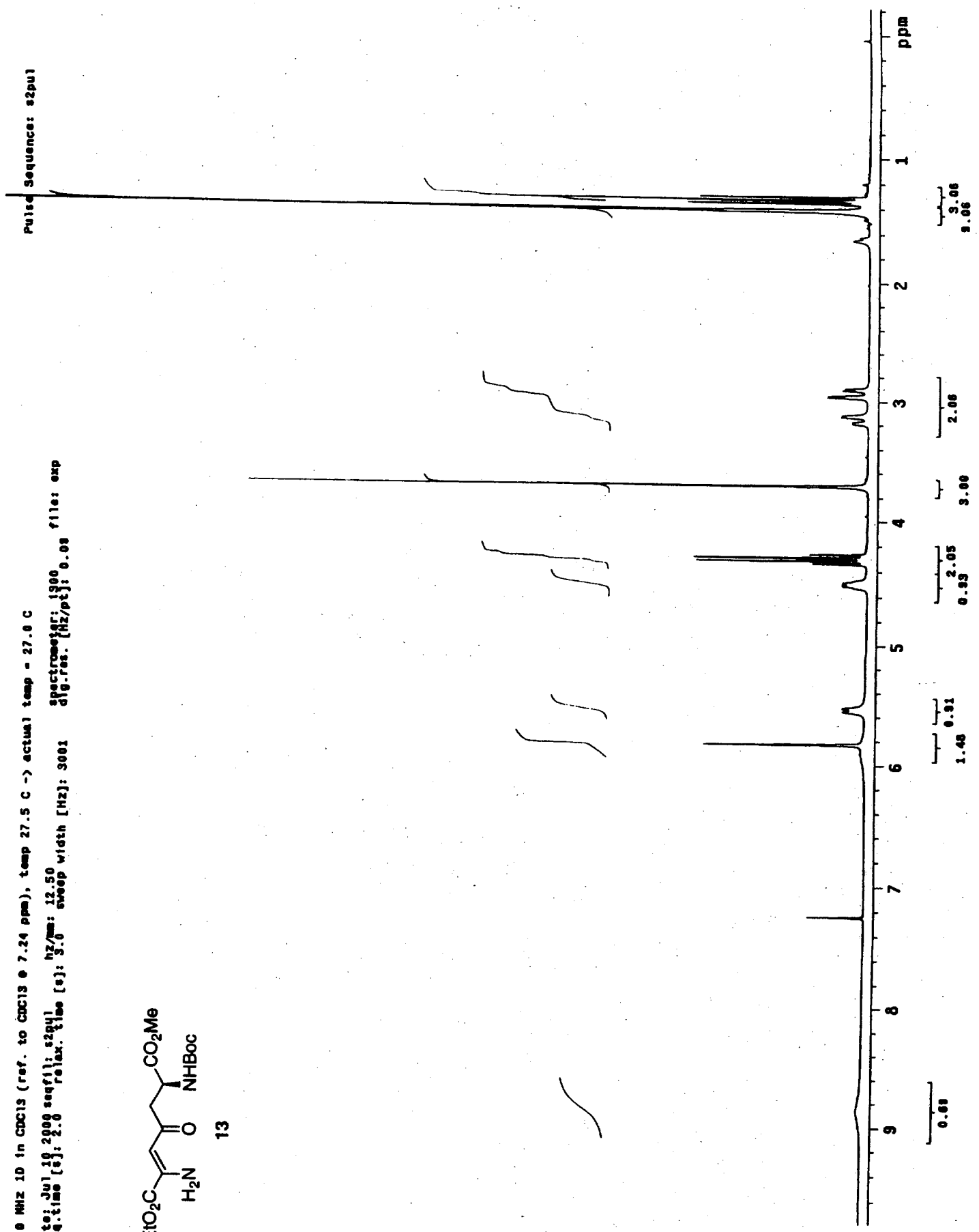

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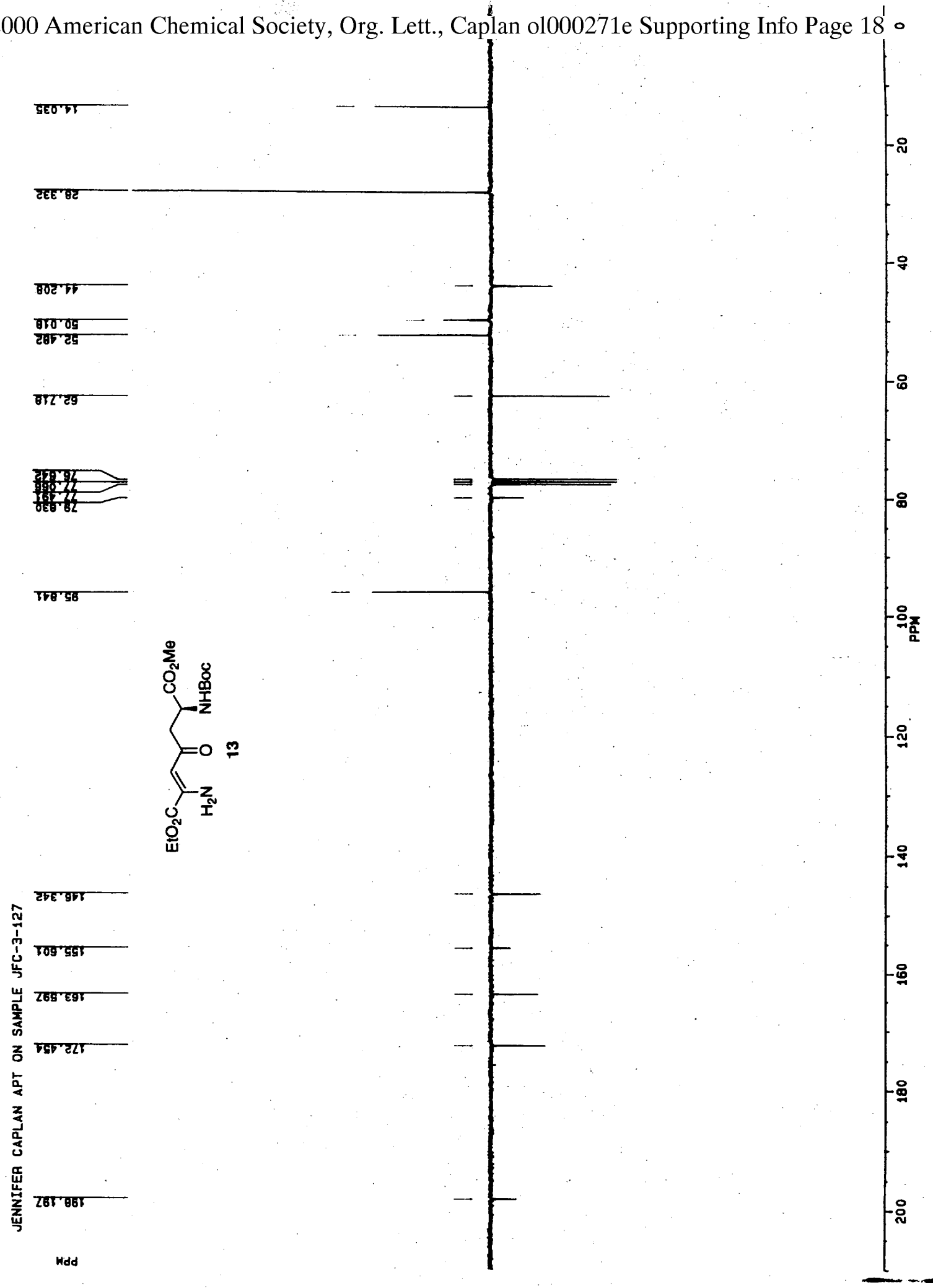
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