Procaspase-3 Activation as an Anti-Cancer Strategy: Structure-Activity Relationship of PAC-1, and its Cellular Co-Localization with Procaspase-3

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Supporting Information

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Materials and Methods

Materials—All reagents were obtained from Fisher unless otherwise indicated. All buffers were made with MilliQ purified water. **PAC-1** and derivatives were synthesized as previously described and as described herein. Ac-DEVD-pNA was synthesized as described. Luria broth (LB) was obtained from EMD. Etoposide was obtained from Sigma. Caspase Activity Buffer contains 50 mM Hepes (pH 7.4), 300 mM NaCl and is Chelex® treated. Ni NTA binding buffer contains 50 mM Tris (pH 8.0), 300 mM NaCl, and 10 mM imidazole. Ni NTA Wash Buffer contains 50 mM Tris (pH 8.0), 300 mM NaCl, and 20 mM imidazole. Ni NTA Elution Buffer contains 50 mM Tris (pH 8.0), 300 mM NaCl, and 500 mM imidazole. Annexin V Binding Buffere contains 10 mM HEPES pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$, 0.1% BSA. The C-terminal 6xHis-tagged procaspase-3 proteins were expressed as described below.

Cell Culture—U937 human lymphoma cells and SK-Mel-5 human melanoma cells were obtained from ATCC. Cells were cultured in RPMI 1640 growth media supplemented with 10% FBS and 1% pen-strep. Cells were incubated at 37 °C and 5 % CO_2. Subculture of SK-Mel-5 cells was achieved through trypsinization of the cell monolayer using 0.05% Trysin-EDTA (Gibco) dilution and further culture in RPMI 1640 growth media supplemented with FBS and pen-strep as above.

Cell Death Assay—U937 human lymphoma cells were plated into the wells of 96 well plate at a density of 5000 cells per well in 200 μL of RPMI 1640 growth media with 10% FBS and 1% pen-strep. To each well was added 2 μL of 100X compound stock solutions in DMSO at varying concentrations so that the cells were treated with concentrations between 0 μM and 100 μM compound. Each concentration was tested in quintuplicate. In each plate 5 wells received 10 μM etoposide as a positive control and 5 wells received 2 μL of DMSO as a negative control. The plates were then incubated at 37 °C and 5% CO_2 for 72 hours. After the 72 hour incubation period, the plates were analyzed using a Sulforhodamine B assay as previously described.[1] Specifically, to each well of the plate was added 50 μL of an 80% (w/v) solution of TCA in H_2O and the plates were allowed to rest at 4 °C overnight. The plates were then washed gently with a steam of H_2O five times to remove the excess TCA and precipitated serum proteins. The plates were then allowed to air dry after which 100 μL of a 0.057% (w/v) Sulforhodamine B in a 1% (v/v) acetic acid solution was added to each well and allowed to stain for 30 minutes at room temperature. After staining, the wells were gently washed 5 times with 100 μL of 1% (v/v) acetic acid to remove the unbound dye. The plates were then allowed to air dry. 200 μL of 10 mM Tris base (pH 10.5) was then added to each well and the plates were

placed on an orbital shaker for five minutes. The OD was then read at 510 nm in a Molecular Dynamics plate reader and the percent cell death calculated and normalized to the positive control (100% cell death) and the negative control (0% cell death). The percent cell death was averaged for each compound concentration and plotted as a function of compound concentration. The data were fit to a logistical dose response curve using Table curve 2D and the IC_{50} value was calculated. The experiment was repeated three times and the average of the calculated IC_{50} values was reported. The standard error of the mean (SEM) was determined and reported for the triplicate experiments. The individual dose-response curves are shown in figure S1.

Recombinant expression and purification of procaspase-3/caspase-3—Procaspase-3 and caspase-3 were recombinantly expressed and purified exactly as previously described.[2] Briefly, procaspase-3 and caspase-3 were expressed from the pHC332 expression plasmid in the electrocompentent BL21(DE3) strain of *Escherichia coli* (Novagen). The C-terminally 6xHis-tagged protein was purified using the Ni NTA resin (Qiagen). Protein containing fractions were collected and pooled. The purified protein was then further purified to remove any contaminating zinc by applying the protein to a PD-10 column (GE Healthcare) charged with Caspase Activity Buffer that had been treated with Chelex ® resin. The resulting zinc free, protein containing fractions in Caspase Activity Buffer were pooled, the concentration determined, and the protein solution was flash-frozen in liquid nitrogen and stored at -80 °C.

Caspase-3 Activity Assays—Zinc-free stocks (2X) of caspase-3 (1 μM) were prepared in Caspase Activity Buffer. Compound stock solutions were made (2X) at various concentrations from 200 μM to 200nM in Caspase Activity Buffer. A stock (10X) of $ZnSO_4$ (25 μM) in Caspase Activity Buffer was prepared. To each well of 384 well plate was added 20 μL of caspase-3 (500 nM final), 20 μL compound stock, and 5 μL of $ZnSO_4$ (2.5 μM final) or buffer. Each plate contained positive control wells (0 μM zinc and no compound) and negative control wells (2.5 μM zinc and no compound). The plates were incubated at room temperature for 30 minutes. Then, to each well of the plate was added 5 μL of a 2 mM stock of Ac-DEVD-pNA substrate and the absorbance at 405 nm was immediately monitored every 1 minute for 30 minutes on a spectramax plate reader (Molecular Devices, Sunny Vale, CA). The slope of each well was used to determine the activity and was normalized to the positive and negative control wells to give a percent activity. The data for each compound concentration (4 wells) was averaged and the percent activity was plotted as a function of compound concentration. The data was analyzed using Table Curve 2D and fitted to a logistical dose-response curve. The majority of compounds achieved a maximal activity at 10 μM. The

percent activity at 10 μM was determined for each compound and reported as an average of the three replicate experiments with the corresponding SEM. The individual dose-response curves are shown in Figure S2.

EGTA Fluorescence Titration Assay—This titration assay is based on a published protocol.[ref] Before titration, cuvette was filled with EDTA (10 mM) for 10 min, followed by sterile deionized water and acetone washing for removing any residue metal ions. **PAC-1** or derivative (60 μM) was added to a cuvette containing buffer (Hepes: 50 mM, KNO_3: 100 mM, pH 7.2) with EGTA (7.3 mM) to achieve a 10-fold dilution (final **PAC-1** concentration: 6 μM). $Zn(OTf)_2$ (0 - 10 mM) was added incrementally. The formation of Zn-**PAC-1** (or derivative) complex was monitored by the increase in fluorescence intensity (ex/em: 410 nm/475nm). Fluorescence intensity at 475 nm was plotted against free Zn concentration ($[Zn]_f$/M) calculated using MaxChelator program [ref]. The data was analyzed using KaleidaGraph and fitted to a formation curve based on Eq S1 derived by published protocol.[ref]

$$I = (I_{min}K_D + I_{max}[Zn]_f)/(K_D + [Zn]_f) \qquad \text{Eq S1}$$

where Imin and Imax were defined as the fluorescence intensity of the free probe (**PAC-1** or derivative in this case) and that of the Zn-probe complex respectively.

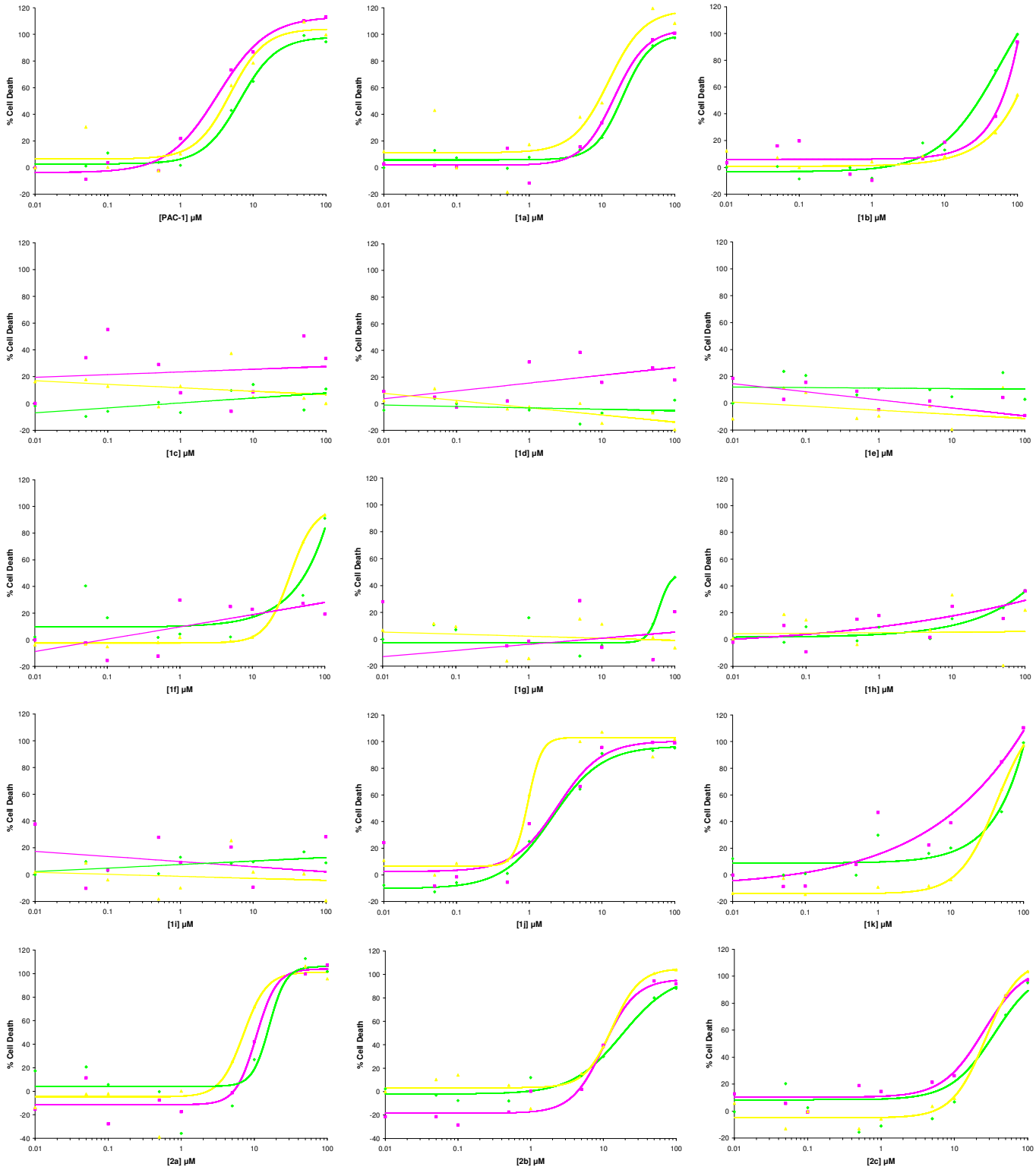
Immunofluorescent Staining—Round 18 mm no. 1 borosillicate coverglass (VWR) were coated with poly-lysine by shaking the coverglass in a poly-lysine solution (Sigma) for 1 hour and then washing with MilliQ. SK-Mel-5 human melanoma cells were grown on the poly-lysine coated coverglass in the bottom of a 12 well plate. When Cells achieved ~80% confluency, the cells were treated with either DMSO or 100 μM **PAC-1** in DMSO (total DMSO <1%) for 1 hour. The cells were then washed two times with 1 mL of PBS and fixed in 3.7% formaldehyde in PBS for 10-15 minutes at room temperature. The cells were then washed again two times with PBS and then permeabilized with 0.1% Triton X-100 in PBS for 5 minutes. The cells were then blocked in 3% BSA in PBS for 10 minutes and incubated with a 1:500 rabbit anti-procaspase-3 antibody for 1 hour. The coverglass was then washed 5 times with PBS and incubated with a 1:1000 dilution of the secondary anti-rabbit Alexafluor 647 conjugated antibody for 20 minutes protected from light. The coverglass was again washed 5 times with PBS and once with MilliQ H_2O. The coverglass were then mounted on glass slides using Fluorosave (Calbiochem) and stored in the dark until imaging. Cells were imaged on a Leica SP2 Multiphoton Confocal microscope.

Live Cell Imaging—SK-Mel-5 cells were grown on the bottom of no. 1 borosillicate growth chambers (Nunc) to a confluency of ~80%. Cells were then treated with DMSO, 25 μM FAM-DEVD-fmk in DMSO, 25 μM **AF350-PAC-1** in DMSO, or both 25 μM FAM-DEVD-fmk and 25 μM **AF350-PAC-1** concurrently for 1 hour at 37 °C and 5 % CO_2. Cells were then washed 5 times with RPMI 1640 growth media lacking phenol red. The cells were then allowed to further incubate at 37 °C for 2 hours before imaging. For cells stained with SYBR green for nuclear staining, a 1:100,000 dilution of SYBR green in media was added immediately prior to imaging. Cells were imaged on a Leica SP2 Multiphoton Confocal microscope.

Image Analysis—Images were adjusted in brightness and contrast to allow for clarity. For all images in Figure 4, the red channel was adjusted using Image J to reflect a linear intensity gradient between 10 and 28. For the green channel, the images were adjusted to a linear intensity gradient between 0 and 44. To account for the offset of the microscope optics, the red channel was manually offset by 5 pixels in the x direction and the green channel was manually offset by 6 pixels in the y direction. The images were filtered with a gausian blur with a radius of 1 pixel to improve the signal to noise ratio. Using the Image J plugin, JACoP, the % overlap was determined using the Manders overlap coefficient.

*Induction of Apoptosis by **PAC-1** derivatives*—U937 Cells (1 mL of 1 x 10^6 cells/mL) were treated with 5 μL ethanol stocks of the various compounds to achieve a final concentration of 50 μM. The cells were incubated at 37 ºC for 12 hours. The cells were centrifuged (200*g* for 5 min), washed with PBS (2 mL), resuspended in 500 μL Annexin V Binding Buffer. To each sample was added 10 μL of FITC conjugated Annexin V stain (Southern Biotech) and 10 μL of propidium iodide (Sigma) to a final concentration of of 50 μg/mL. Cell populations were analyzed on a Benton Dickinson LSR II cell flow cytometer.

Supporting Figures:



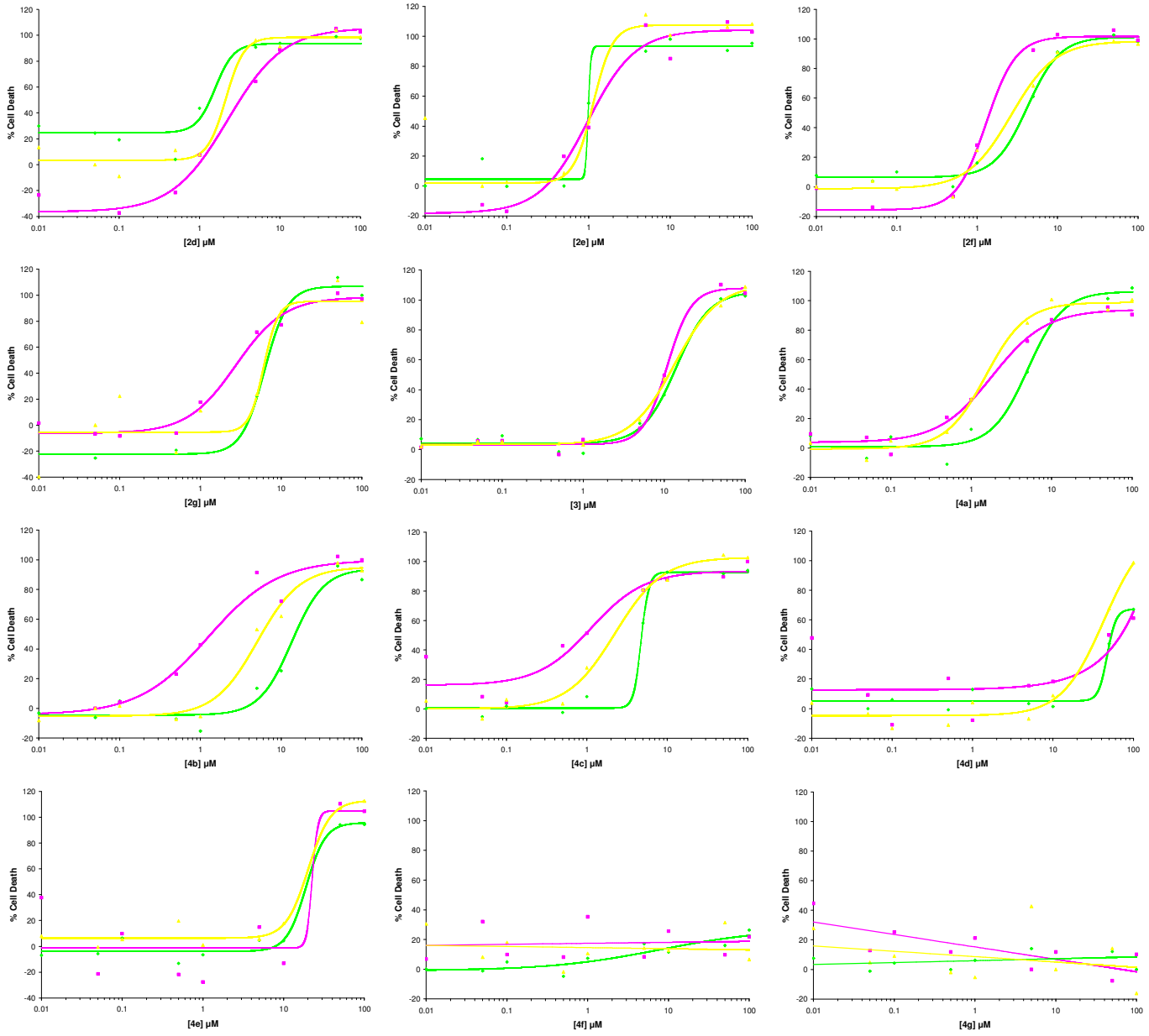
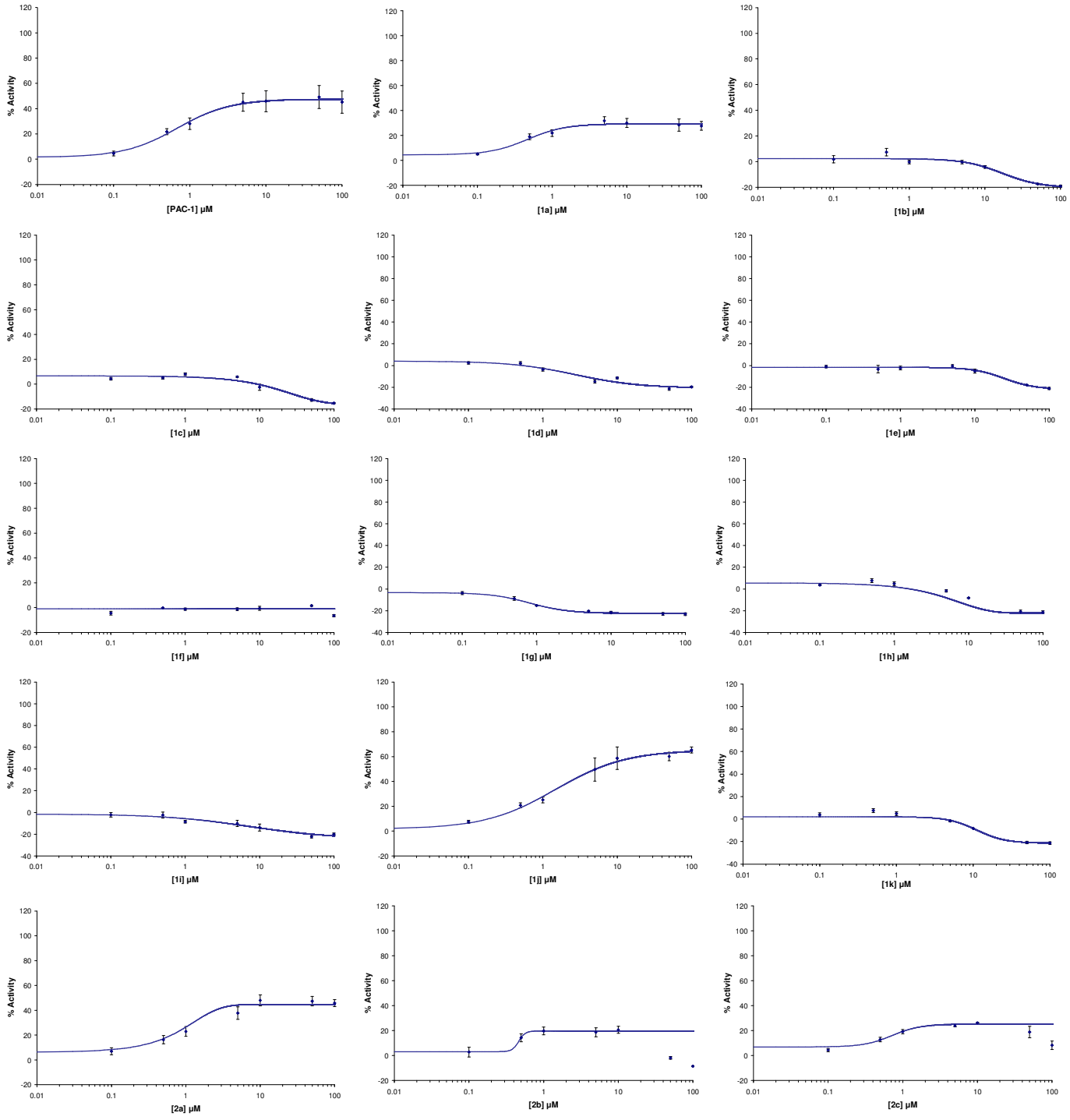


Figure S1. Cytotoxicity of **PAC-1** and derivatives. Each graph shows the dose response curves used to generate the IC_{50} value for each derivative. The results of these experiments are summarized in Table 1 of the manuscript.


% Activity
[PAC-1] µM
[1a] µM
[1b] µM
[1c] µM
[1d] µM
[1e] µM
[1f] µM
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[2c] µM

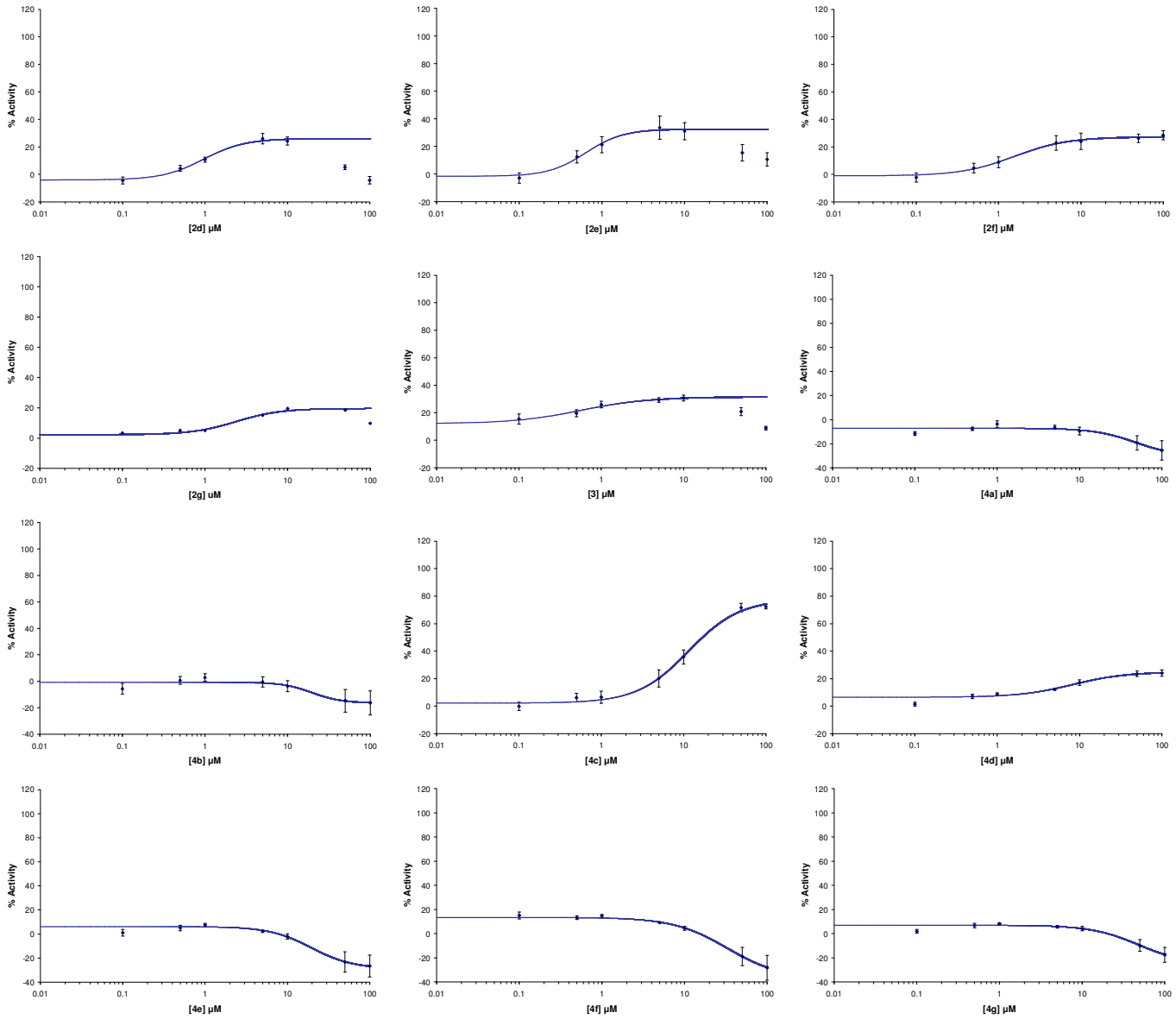
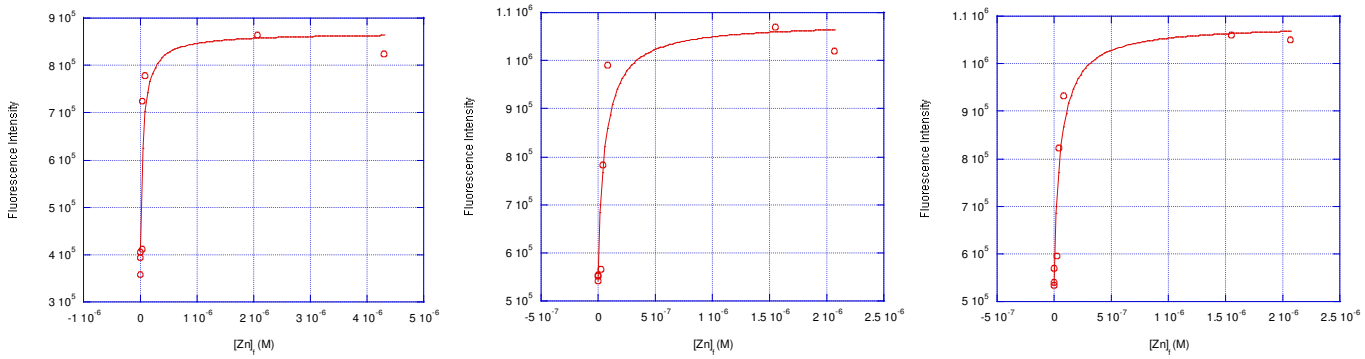
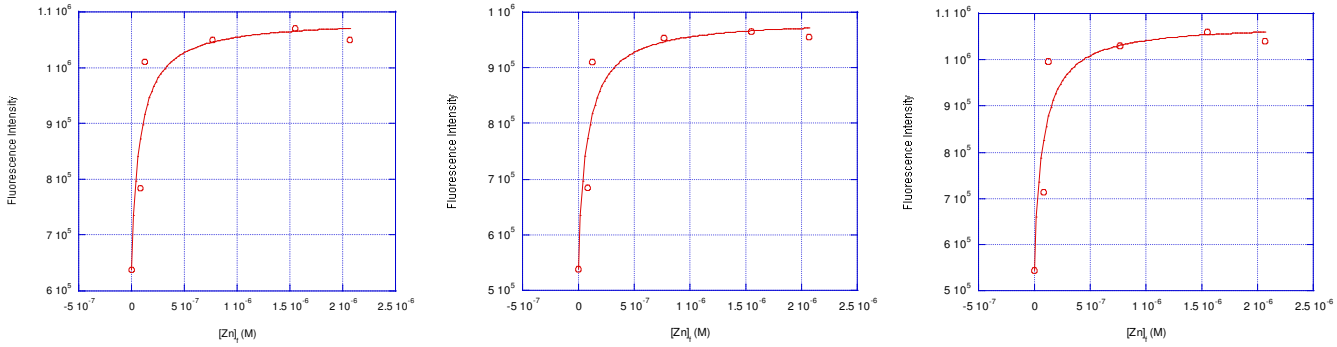


Figure S2. Relief of zinc mediated caspase-3 inhibition. Each graph represents three separate dose response experiments. The error bars represent the standard error of the mean for each data point. The majority of compounds show a maximal activity at 10 µM. The % activity at 10 µM for each compound was included in Table 1.

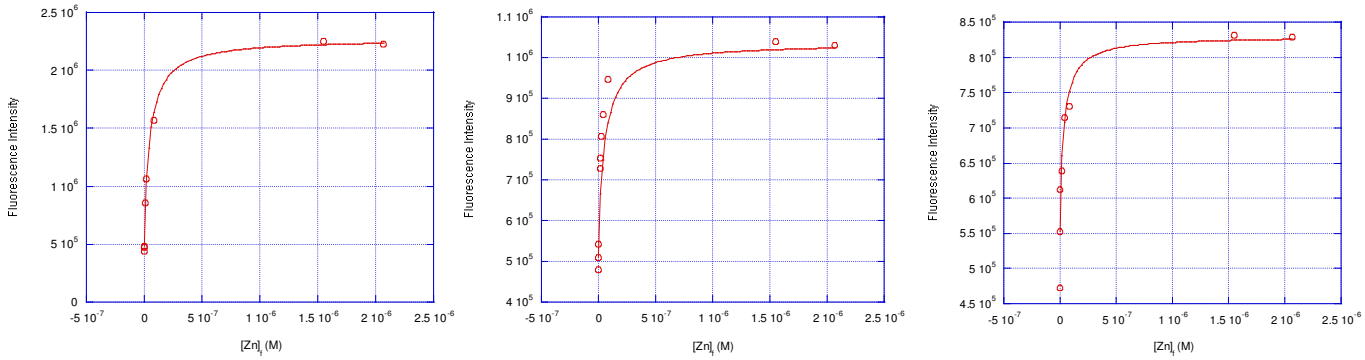
Zn-**PAC-1(1)**: K_d = 52 ± 2 nM



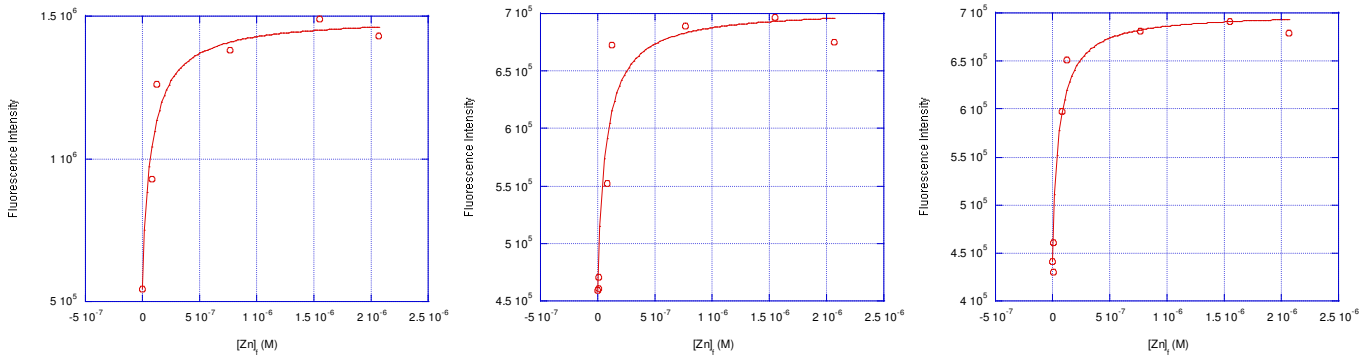
Zn-**1a**: K_d = 77 ± 2 nM



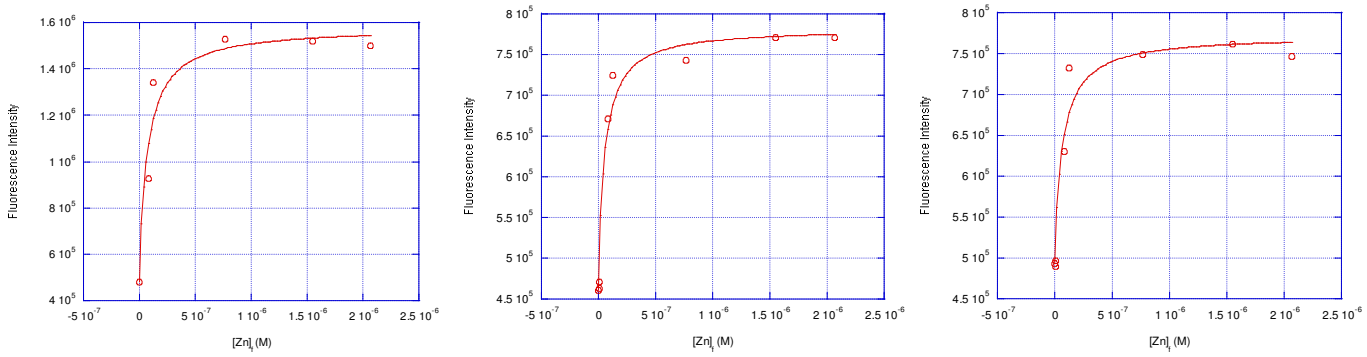
Zn-**1j**: K_d = 43 ± 9 nM



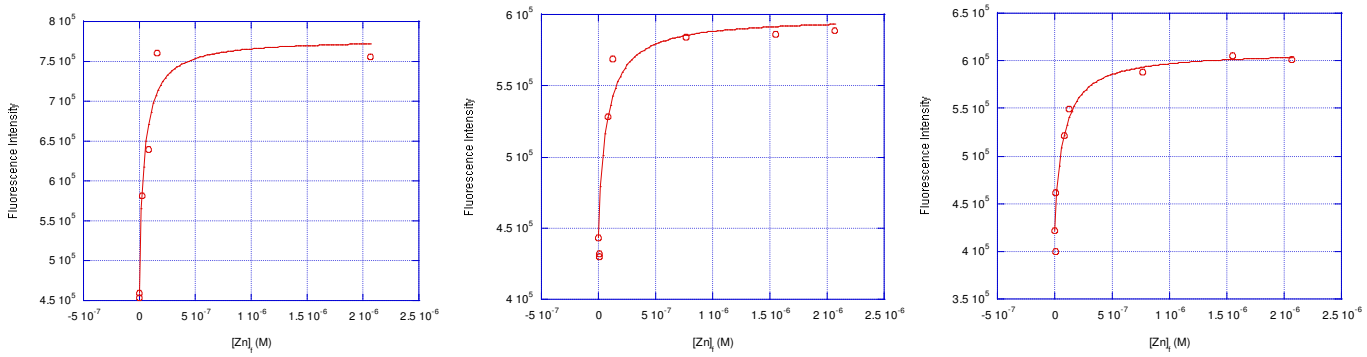
Zn-**2a**: K_d = 68 ± 10 nM



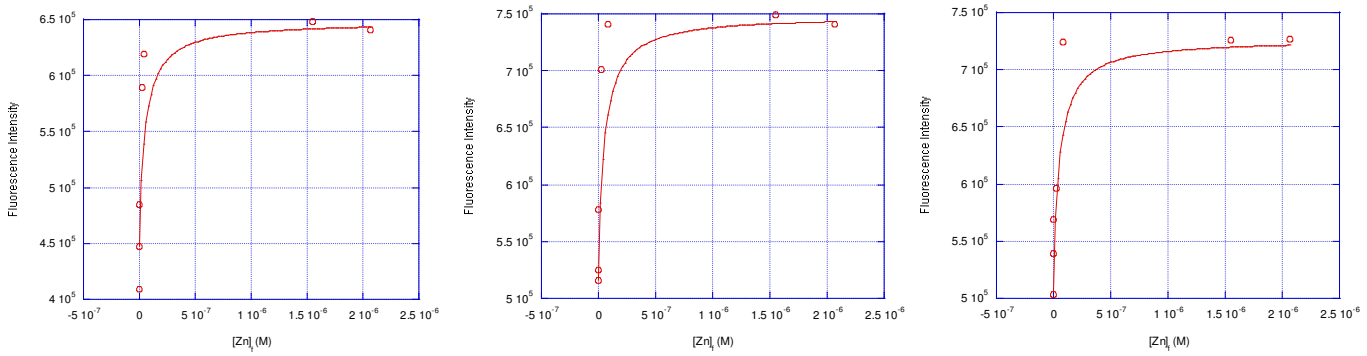
Zn-**2b**: K_d = 62 ± 9 nM



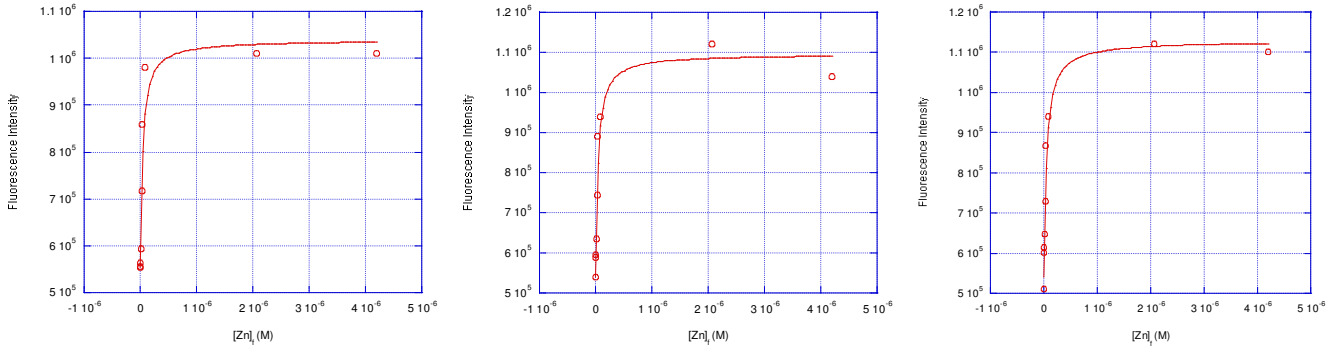
Zn-**2c**: K_d = 80 ± 14 nM



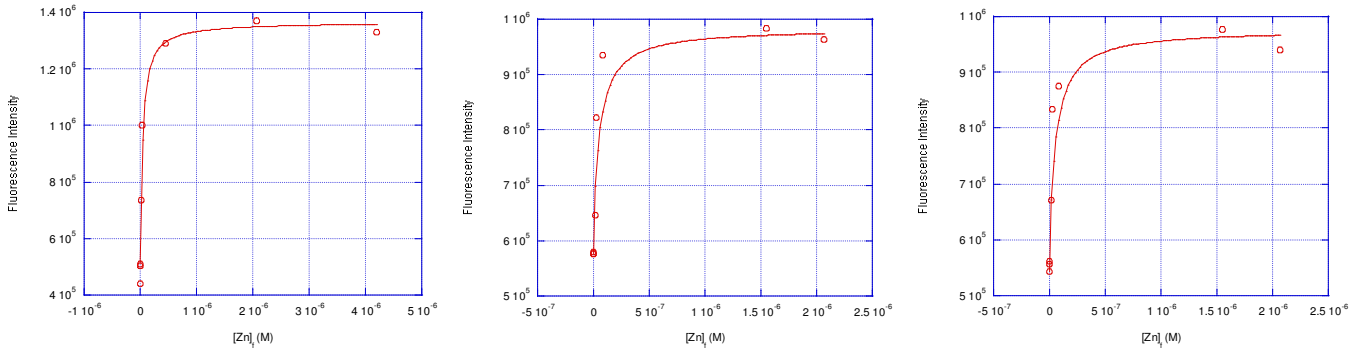
Zn-**2d**: K_d = 48 ± 3 nM



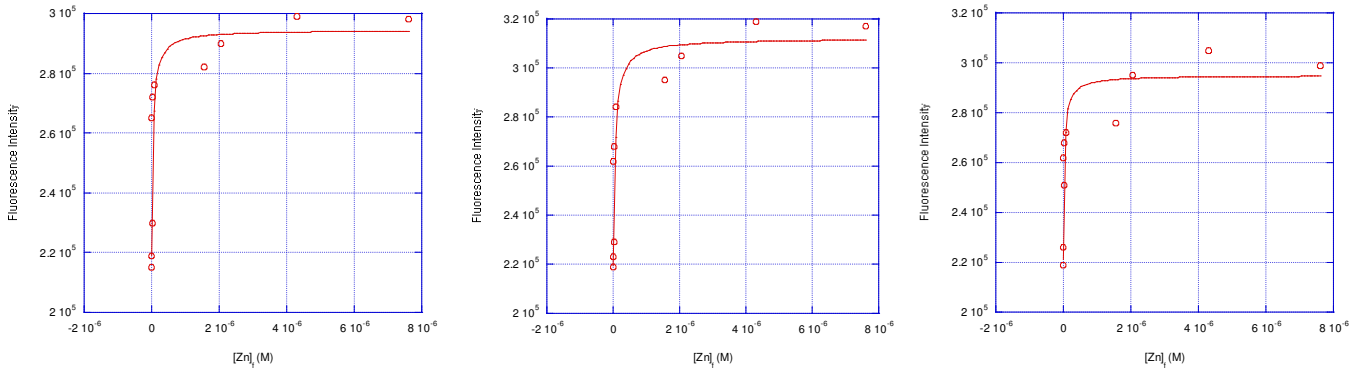
Zn-**2e**: K_d = 39 ± 3 nM



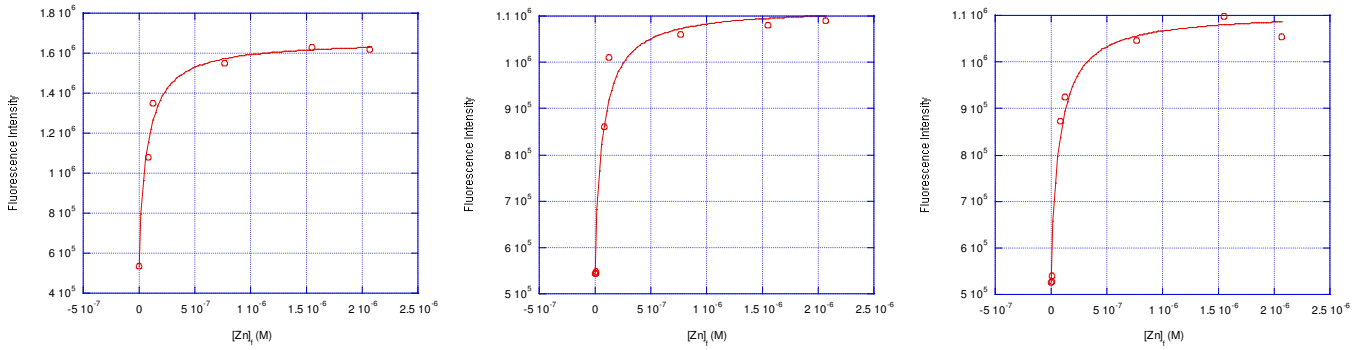
Zn-**2f**: K_d = 47 ± 5 nM



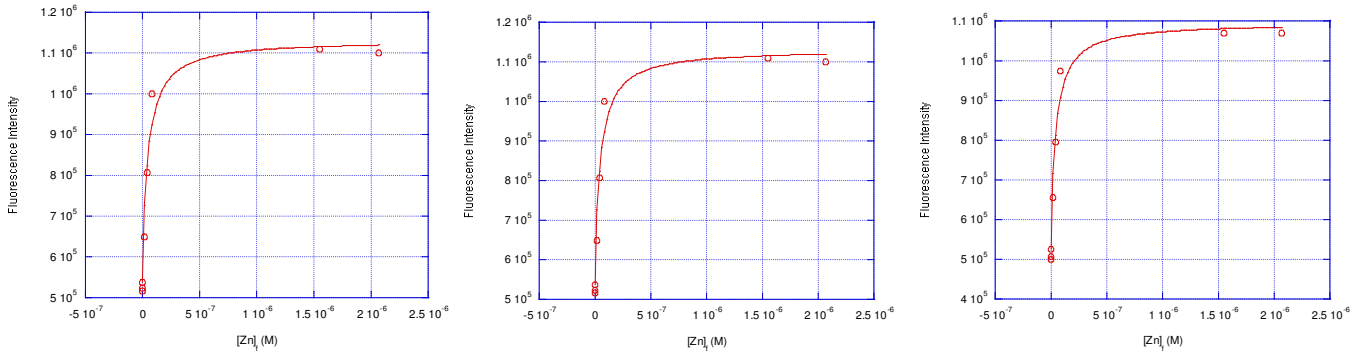
Zn-**2g**: K_d = 45 ± 13 nM



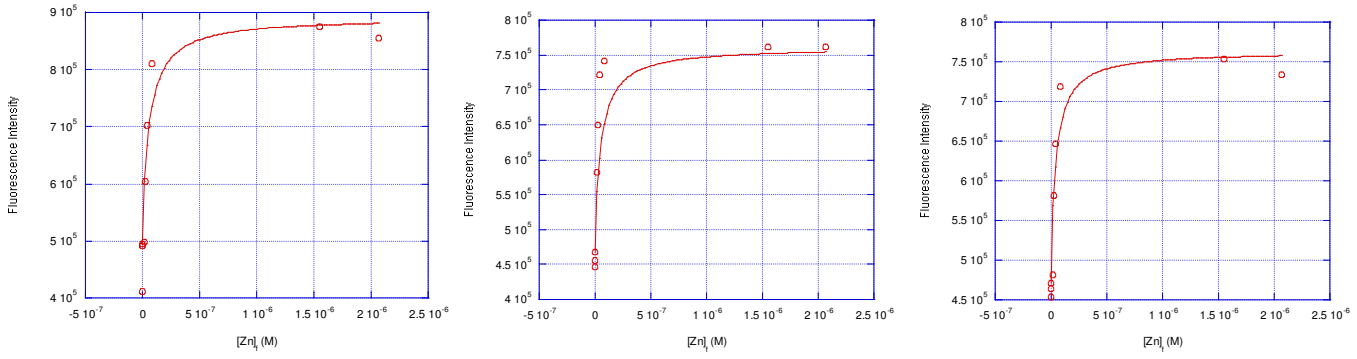
Zn-**3**: K_d = 65 ± 4 nM



Zn-**4a**: K_d = 44 ± 2 nM



Zn-**4b**: K_d = 48 ± 7 nM



Zn-**4c**: K_d = 49 ± 2 nM

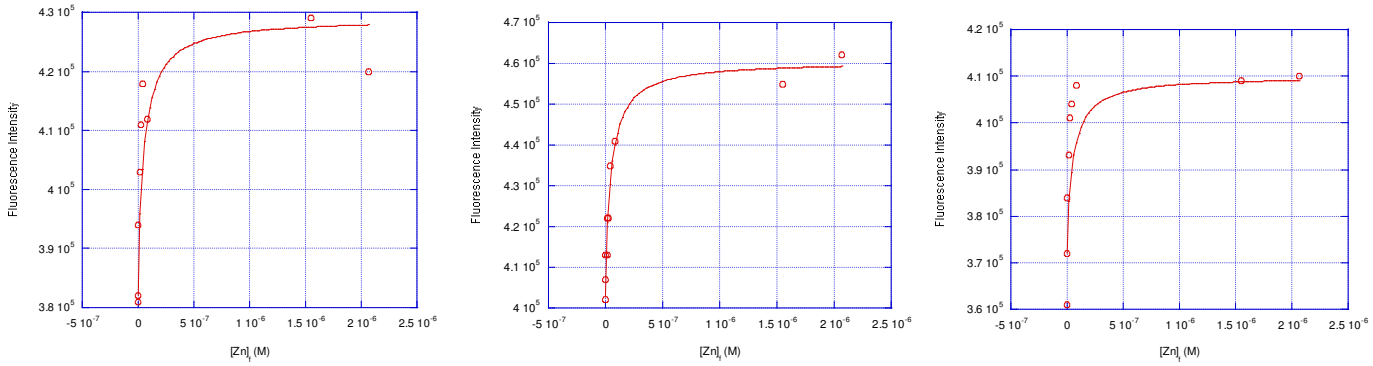


Figure S3. Formation curves for zinc binding determination. K_d values are summarized in Table 1.

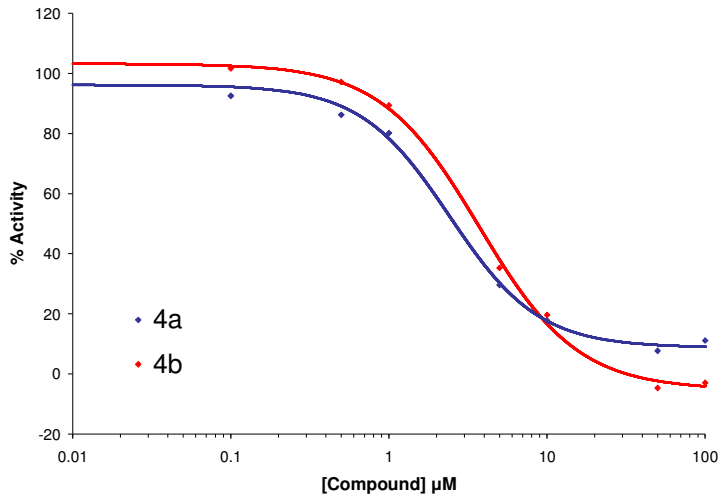


Figure S4. Inhibition by class IV compounds. **4a** and **4b** inhibit caspase-3 in the absence of zinc. This potent inhibition masks any activation at 10 µM.

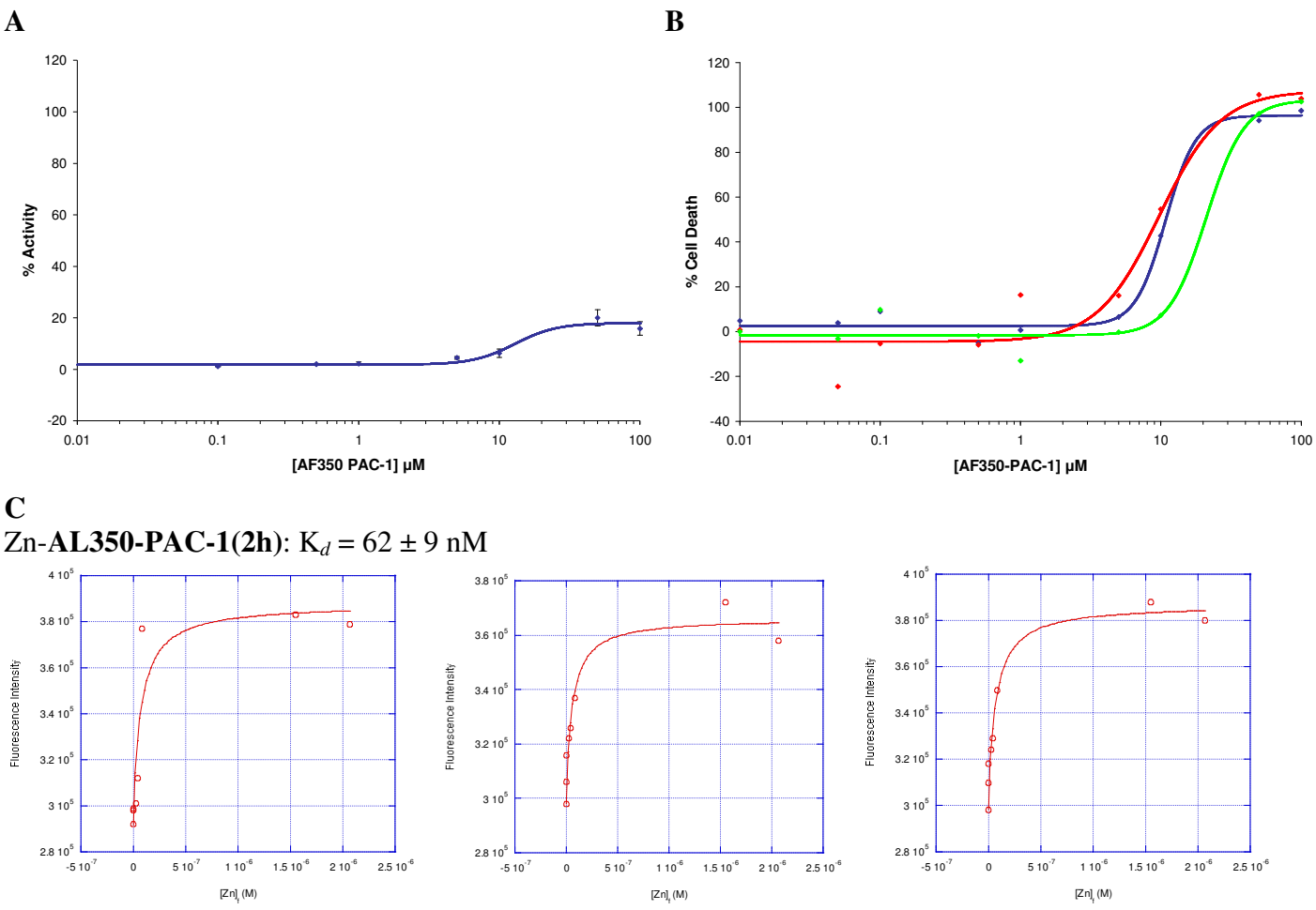


Figure S5. Biochemical assessment of **AF350-PAC-1(2h)**. A) **AF350-PAC-1(2h)** relieves zinc mediated inhibition of procaspase-3. B) **AF350-PAC-1(2h)** induces cell death in U937 human lymphoma cells with an IC_{50} of 14.8 ± 3.2 µM . C) **AF350-PAC-1(2h)** binds zinc with a K_d of 61 ± 9 nM.

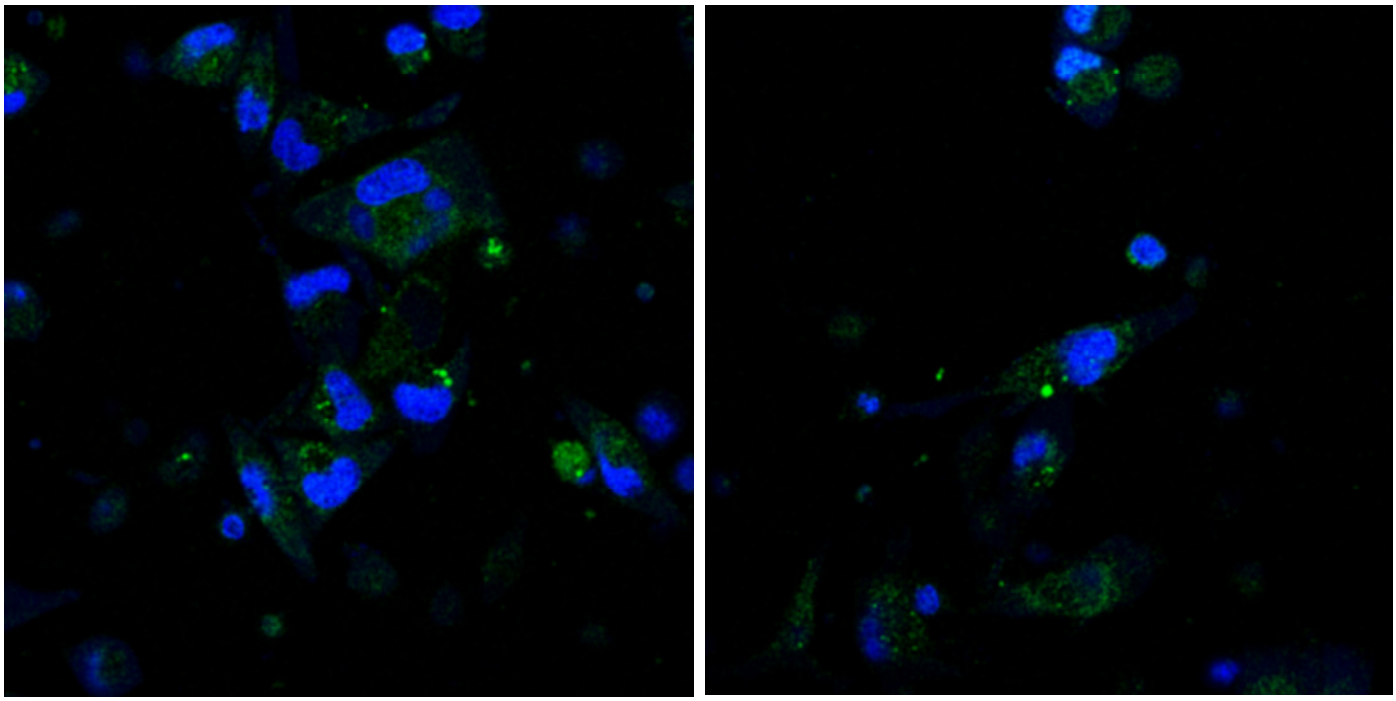


Figure S6. Cellular localization of **AF350-PAC-1**. Wide field images showing the localization of **AF350-PAC-1** in SK-MEL-5 cells.

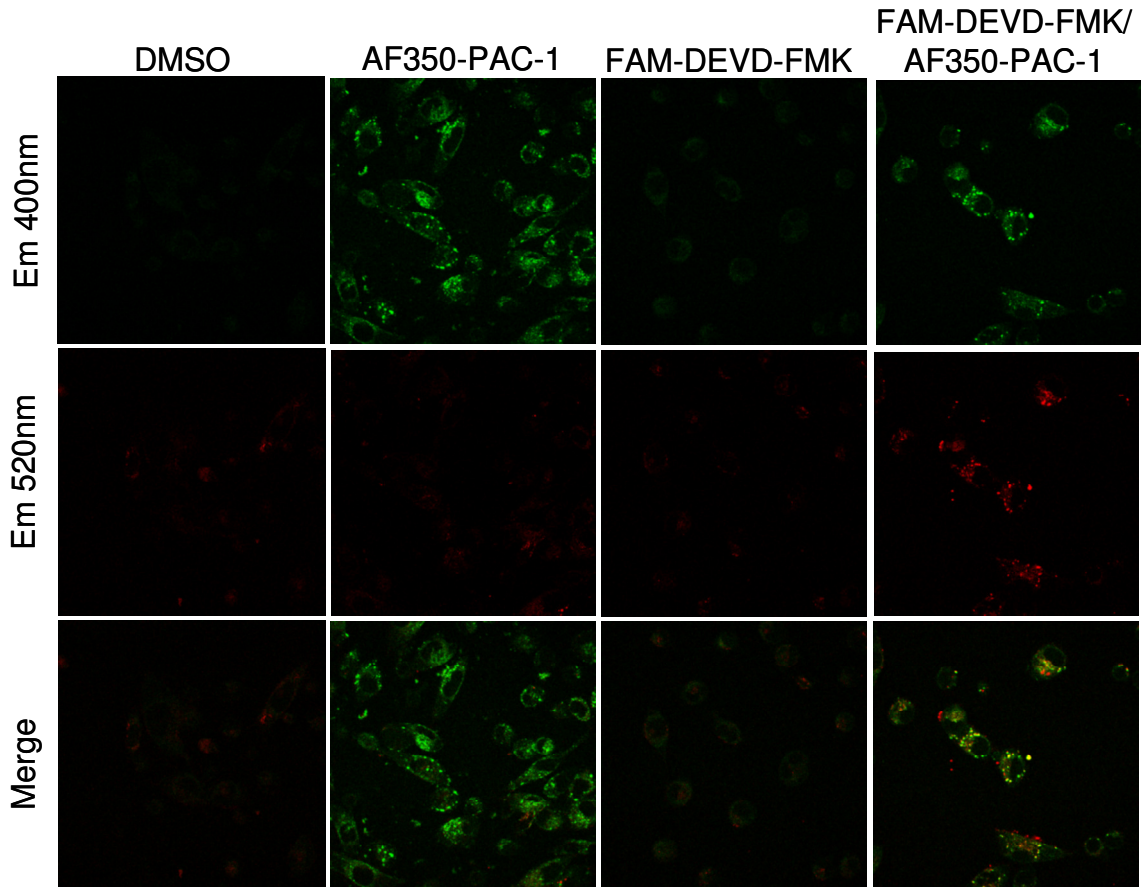


Figure S7. Colocalization of **AF350-PAC-1** and caspase-3/-7. Wide field images showing the colocalization of **AF350-PAC-1** and spots of intense caspase-3/-7 activity as visualized by FAM-DEVD-fmk.

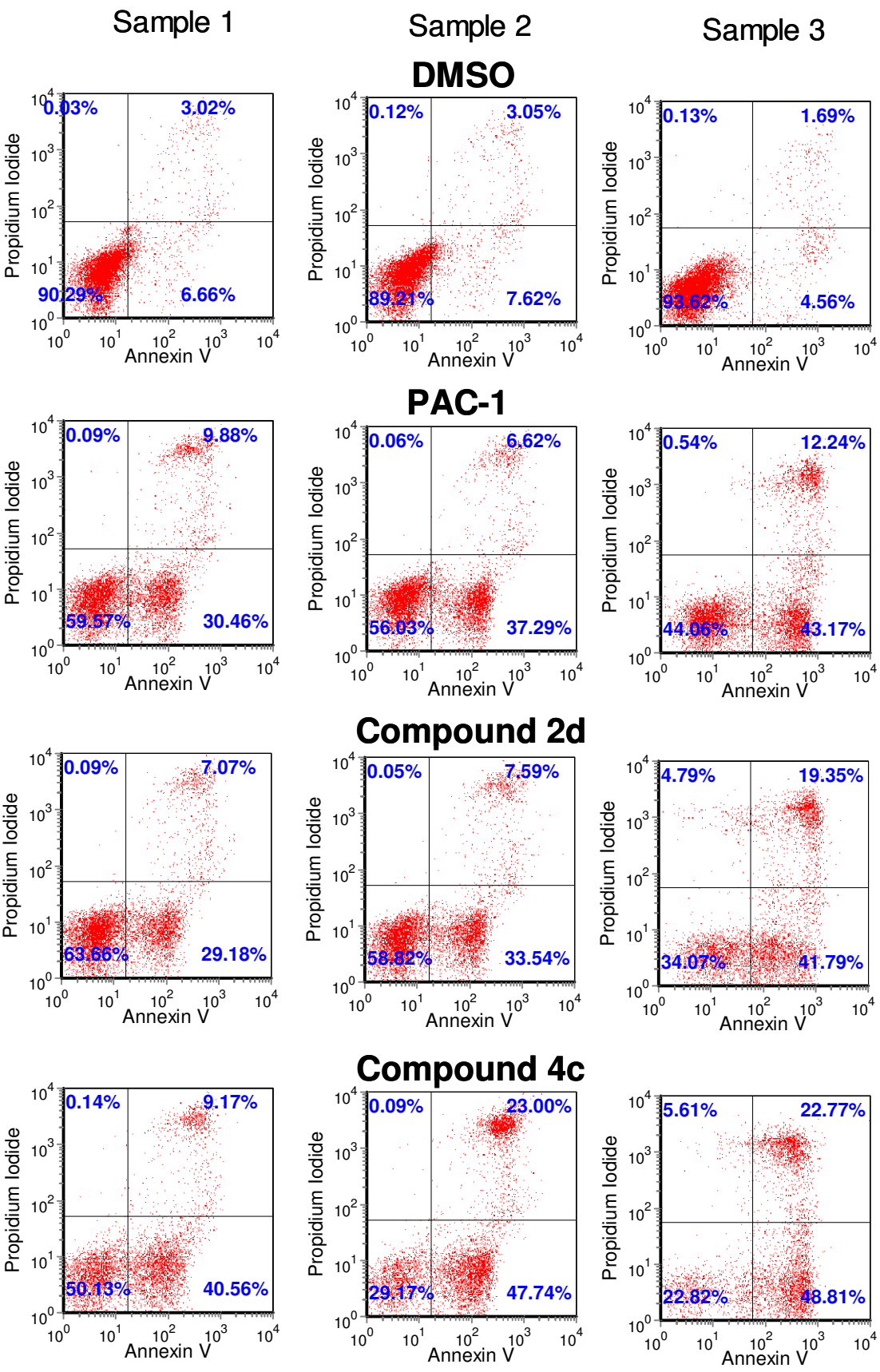

Sample 1
Sample 2
Sample 3
DMSO
0.03%
3.02%
90.29%
6.66%
0.12%
3.05%
89.21%
7.62%
0.13%
1.69%
93.62%
4.56%
PAC-1
0.09%
9.88%
59.57%
30.46%
0.06%
6.62%
56.03%
37.29%
0.54%
12.24%
44.06%
43.17%
Compound 2d
0.09%
7.07%
63.66%
29.18%
0.05%
7.59%
58.82%
33.54%
4.79%
19.35%
34.07%
41.79%
Compound 4c
0.14%
9.17%
50.13%
40.56%
0.09%
23.00%
29.17%
47.74%
5.61%
22.77%
22.82%
48.81%
Propidium Iodide
Annexin V

Figure S8. Assessment of apoptotic induction by **PAC-1** derivatives. U-937 cells were treated with **PAC-1**, **2d**, **4c** (50 μM each) or vehicle control for 12 hours, then stained with Annexin V and PI. All three compounds induce apoptosis as assessed by the Annexin V positive/PI negative populations. Results shown are of 3 separate experiments.

Chemical Information

Materials and Methods

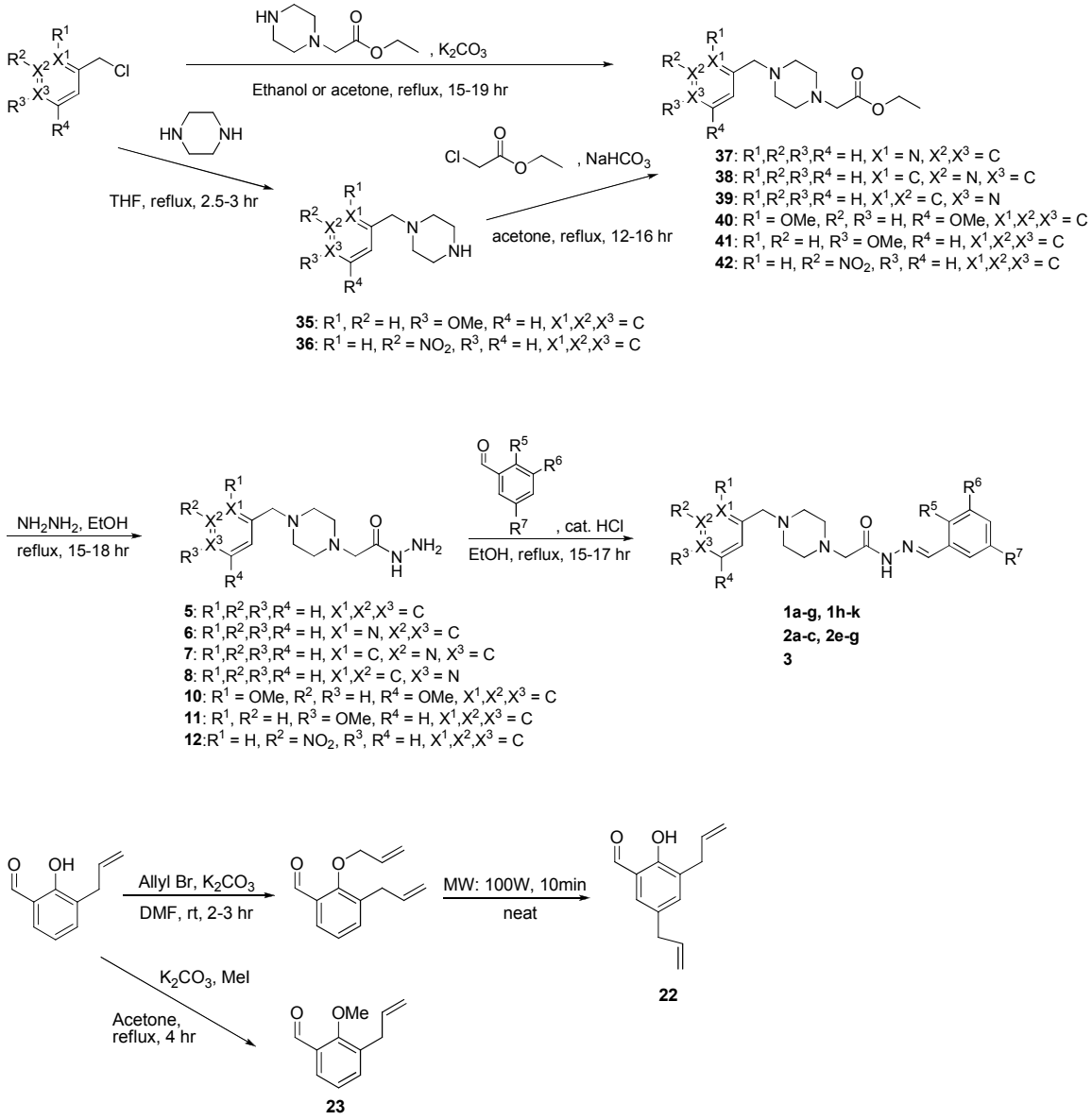
General

All reactions requiring anhydrous conditions were conducted under a positive atmosphere of nitrogen or argon in oven-dried glassware. Standard syringe techniques were used for anhydrous addition of liquids. Dry tetrahydrofuran was obtained by passing over activated alumina columns or molecular sieves in a commercial solvent purification system (Innovative Technologies). Unless otherwise noted, all starting materials, solvents, and reagents were acquired from commercial suppliers and used without further purification. Flash chromatography was performed using 230-400 mesh silica gel. Hydrazide **5**,[3] **PAC-1 (1)**,[3] **1a**,[3] **1h**,[3] 3-allylsalicylaldehyde,[4] 2-mercaptobenzaldehyde,[5] **23**,[3] **30,**[6] **44**[3] were prepared according to the literature method with modifications.

Compound Analysis.

All NMR experiments were recorded either in $CDCl_3$ (Sigma), CD_3OD (sigma) or Acetone-*d6* (Sigma) on a Varian Unity 400 MHz or 500 MHz spectrometer with residual undeuterated solvent as the internal reference. Chemical shift, δ (ppm); coupling constants, *J* (Hz); multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet); and integration are reported. High-resolution mass spectral data was recorded on a Micromass Q-Tof Ultima hybrid quadrupole/time-of-flight ESI mass spectrometer at the University of Illinois Mass Spectrometry Laboratory. All melting points are uncorrected. Analytical HPLC performed on a Altima C18 column, 2.1x20 mm, mobile phase A is 0.1% TFA in H_2O, B is acetonitrile using a gradient system from 0-45% B over 5 min, then 45-55% B from 5-10 min, then 55-100% B over 10-15 min, constant 100% over 15-20 min, and from100-0% over 20-25. LC-MS performed on a C18 column, 2.1x5 mm, mobile phase A is 0.1% TFA in H_2O, B is acetonitrile using a gradient system with constant 0% B over 0-2 min, then 0-50% B from 2-5 min, then 50-100% B over 5-7 min, constant 100% over 7-8 min, and from100-0% over 8-10.

Scheme S1. Syntheses of **PAC-1** derivatives

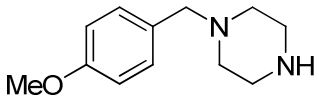


*General Procedure for 1-Benzylpiperazines **35-36***

Anhydrous piperazine (6 equiv.) was added to THF (6 mL), and the mixture was heated to reflux until the piperazine was fully dissolved. To the solution substituted benzyl chloride (1 equiv.) was added dropwise. White precipitate was formed immediately. The reaction mixture was refluxed for 2.5-3 hr monitoring by TLC. The stirring mixture was cooled and then filtered. The solids were washed with THF (3 mL) and then EtOAc (3 mL). The combined organic layer was concentrated *in vacuo*, which was then washed with basic water with 5% brine and KOH (pH >12). The aqueous layer was extracted with CH_2Cl_2 (3 x 10mL) and

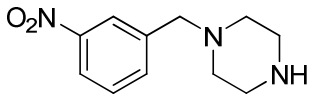
EtOAc (10 mL) at pH > 12. The organic layers were combined, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel (1:4 MeOH/EtOAc).

1-(4-Methoxybenzyl)piperazine (**35**)



The general procedure was followed: 4-Methoxybenzyl chloride (0.5 mL, 3.7 mmol, 1 equiv.), piperazine (1.9 g, 22.2 mmol, 6 equiv.), THF (8 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **35** (0.67 g, 88%) as light yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.17 (d, J = 8.6 Hz, 2H), 6.79 (d, J = 8.7 Hz, 2H), 3.73 (s, 3H), 3.37 (s, 2H), 2.81 (t, J = 4.9 Hz, 4H), 2.33 (broad s, 4H), 1.50 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.8, 130.6, 130.2, 113.7, 63.3, 55.4, 54.6, 46.3.
HRMS (ESI): found: 207.1501 (M+1); calcd for $C_{12}H_{19}N_2O$: 207.1497. IR (neat): 3288 cm^{-1}.

1-(3-Nitrobenzyl)piperazine (**36**)

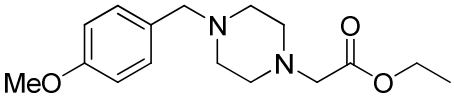


The general procedure was followed: 3-Nitrobenzyl chloride (5 g, 29.1 mmol, 1 equiv.), piperazine (15.1g, 174.6 mmol, 6 equiv.), THF (64 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **36** (6.07 g, 94%) as yellow liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.61 (d, J = 7.6 Hz, 1H), 8.13 (s, 1H), 8.02 (d, J = 8.2 Hz, 1H), 7.41 (t, J = 7.9 Hz, 1H), 3.50 (s, 2H), 2.82 (t, J = 4.8 Hz, 4H), 2.36 (broad s, 4H), 1.54 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 148.5, 141.0, 135.3, 129.3, 123.9, 122.3, 62.8, 54.7, 46.2. HRMS (ESI): found: 222.1238 (M+1); calcd for $C_{11}H_{16}N_3O_2$: 222.1243. IR (neat): 3389, 1530, 1345 cm^{-1}.

General Procedure for Ethyl 2-(4-Benzylpiperazin-1-yl)acetate ***41-42***

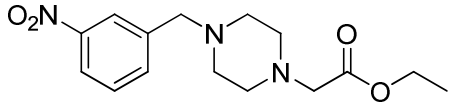
To a stirred mixture of substituted 1-benzylpiperazine (1 equiv.) and $NaHCO_3$ (1.25 equiv.) in acetone (7 mL) was added ethyl chloroacetate (1.1 equiv.). The reaction was refluxed for 20-22 hr monitoring by TLC. The solution was filtered and the solid was washed with acetone (10 mL). The filtrate was concentrated *in vacuo*, and then purified by flash column chromatography on silica gel (1:1 EtOAc/Hexanes).

Ethyl 2-(4-(4-methoxybenzyl)piperazin-1yl)acetate (**41**)



The general procedure was followed: **35** (0.67 g, 3.2 mmol, 1 equiv.), ethyl chloroacetate (0.39 mL, 3.6 mmol, 1.1 equiv.), $NaHCO_3$ (0.34 g, 4.06 mmol, 1.25 equiv.), acetone (7 mL). Purification with flash column chromatography on silica gel (1:1 EtOAc/Hexanes) afforded **41** (0.73 g, 77%) as orange oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.18 (d, *J* = 8.6 Hz, 2H), 6.80 (d, *J* = 8.6 Hz, 2H), 4.13 (q, *J* = 7.1 Hz, 2H), 3.74 (s, 3H), 3.41 (s, 2H), 3.15 (s, 2H), 2.51 (broad d, 8H), 1.22 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.5, 158.9, 130.5, 130.3, 113.7, 62.5, 60.8, 59.8, 55.4, 53.3, 52.9, 14.5. HRMS (ESI): found: 293.1869 (M+1); calcd for $C_{16}H_{25}N_2O_3$: 293.1865. IR (neat): 1742, 1660 cm^{-1}.

Ethyl 2-(4-(3-nitrobenzyl)piperazin-1-yl)acetate (**42**)

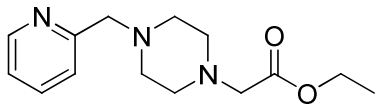


The general procedure was followed: **36** (6.01 g, 27.2 mmol, 1 equiv.), ethyl chloroacetate (3.2 mL, 29.9 mmol, 1.1 equiv.), $NaHCO_3$ (2.85 g, 34.0 mmol, 1.25 equiv.), acetone (54 mL). Purification with flash column chromatography on silica gel (1:1 EtOAc/Hexanes) afforded **42** (7.18 g, 86%) as light orange liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.13 (s, 1H), 8.02 (d, *J* = 8.2 Hz, 1H), 7.60 (d, *J* = 7.6 Hz, 1H), 7.42 (t, *J* = 7.9 Hz, 1H), 4.11 (q, *J* = 7.1 Hz, 2H), 3.54 (s, 2H), 3.15 (s, 2H), 2.52 (d, *J* = 7.1 Hz, 8H), 1.20 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.4, 148.5, 140.9, 135.2, 129.3, 123.8, 122.3, 62.1, 60.8, 59.6, 53.1, 53.0, 14.4. HRMS (ESI): found: 294.1559 (M+1); calcd for $C_{13}H_{20}N_5O_3$: 294.1566.

*General Procedure for Ethyl 2-(4-Benzylpiperazin-1-yl)acetate **37-40***

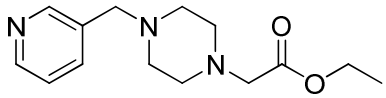
To a stirred mixture of picolyl halide hydrohalide or substituted benzyl chloride (1.2 equiv.) and K_2CO_3 (3 equiv.) in ethanol or acetone (7 mL) was added ethyl 2-(piperazin-1-yl)acetate (1 equiv.). The reaction was refluxed for 15-19 hr monitoring by TLC. The solution was filtered and the solid was washed with ethanol (10 mL). The filtrate was concentrated *in vacuo*, and then purified by flash column chromatography on silica gel (1:1 EtOAc/Hexanes).

Ethyl 2-(4-(pyridin-2-ylmethyl)piperazin-1-yl)acetate (**37**)



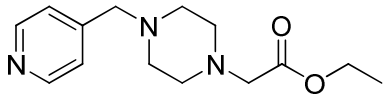
The general procedure was followed: 2-picolyl bromide hydrobromide (0.49 g, 1.9 mmol, 1.2 equiv.), ethyl 2-(piperazin-1-yl)acetate (0.28 g, 1.6 mmol, 1 equiv.), K_2CO_3 (0.67 g, 4.8 mmol, 3 equiv.), ethanol (7 mL). Purification with flash column chromatography on silica gel (1:1 EtOAc/Hexanes) afforded **37** (0.28 g, 65%) as deep yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): 8.49 (d, *J* = 4.9 Hz, 1H), 7.82 (dt, *J* = 1.7 Hz, *J* = 7.7 Hz, 1H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.32 (dd, *J* = 5.5 Hz, *J* = 7.0 Hz, 1H), 4.17 (q, *J* = 7.1 Hz, 2H), 3.67 (s, 2H), 3.24 (s, 2H), 2.61 (broad d, 8H), 1.26 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): 170.3, 157.9, 148.5, 137.5, 124.0, 122.8, 63.5, 60.5, 58.6, 52.6, 52.5, 13.4. HRMS (ESI): found: 264.1719 (M+1); calcd for $C_{14}H_{22}N_3O_2$: 264.1712

Ethyl 2-(4-(pyridin-3-ylmethyl)piperazin-1-yl)acetate (**38**)



The general procedure was followed: 3-picolyl chloride hydrochloride (0.32 g, 1.9 mmol, 1.2 equiv.), ethyl 2-(piperazin-1-yl)acetate (0.28 g, 1.6 mmol, 1 equiv.), K_2CO_3 (0.67 g, 4.8 mmol, 3 equiv.), ethanol (7 mL). Purification with flash column chromatography on silica gel (1:1 EtOAc/Hexanes) afforded **38** (0.35 g, 82%) as deep yellow oil. ^{1}H NMR (500 MHz, CD_3OD): δ 8.50 (d, *J* = 1.5 Hz, 1H), 8.44 (dd, *J* = 1.5 Hz, *J* = 4.8 Hz, 1H), 7.83 (d, *J* = 7.8 Hz, 1H), 7.41 (dd, *J* = 4.5 Hz, *J* = 7.7 Hz, 1H), 4.16 (q, *J* = 7.1 Hz, 2H), 3.58 (s, 2H), 3.23 (s, 2H), 2.57 (broad d, 8H), 1.25 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.3, 149.8, 147.8, 138.1, 134.2, 124.0, 60.5, 59.5, 58.5, 52.5, 52.3, 13.4. HRMS (ESI): found: 264.1716 (M+1); calcd for $C_{14}H_{22}N_3O_2$: 264.1712

Ethyl 2-(4-(pyridin-4-ylmethyl)piperazin-1-yl)acetate (**39**)

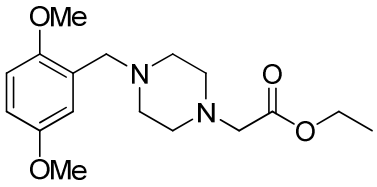


The general procedure was followed: 4-picolyl chloride hydrochloride (0.32 g, 1.9 mmol, 1.2 equiv.), ethyl 2-(piperazin-1-yl)acetate (0.28 g, 1.6 mmol, 1 equiv.), K_2CO_3 (0.67 g, 4.8 mmol, 3 equiv.), ethanol (7 mL). Purification with flash column chromatography on silica gel (1:1 EtOAc/Hexanes) afforded **39** (0.31 g, 72%) as deep yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.51 (d, *J* = 5.8 Hz, 2H), 7.25 (d, *J* = 5.7 Hz, 2H), 4.16 (q,

J = 7.1 Hz, 2H), 3.50 (s, 2H), 3.19 (s, 2H), 2.56 (broad d, 8H), 1.24 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.4, 150.0, 147.7, 124.0, 61.8, 60.8, 59.6, 53.1, 53.1, 14.4. HRMS (ESI): found: 264.1714 (M+1); calcd for $C_{14}H_{22}N_3O_2$: 264.1712

IR (neat): 1738, 1660 cm^{-1}.

Ethyl 2-(4-(2,5-dimethoxybenzyl)piperazin-1-yl)acetate (**40**)

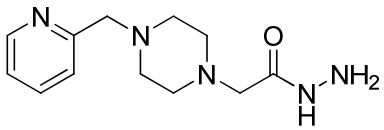


The general procedure was followed: 2,5-dimethoxybenzylchloride (5.0 g, 26.8 mmol, 1.2 equiv.), ethyl 2-(piperazin-1-yl)acetate (3.8 g, 22.3 mmol, 1 equiv.), K_2CO_3 (9.2 g, 66.9 mmol, 3 equiv.), acetone (40 mL). Purification with flash column chromatography on silica gel (1:1 EtOAc/Hexanes) afforded **40** (5.3 g, 73%) as deep yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.94 (d, *J* = 3.0 Hz, 1H), 6.76 (apparent d, *J* = 8.8 Hz, 1H), 6.71 (dd, *J* = 3.0 Hz, *J* = 8.9 Hz, 1H), 4.16 (q, *J* = 7.1 Hz, 2H), 3.75 (s, 6H), 3.54 (s, 2H), 3.18 (s, 2H), 2.58 (broad s, 8H), 1.25 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.6, 153.7, 152.3, 127.6, 116.6, 112.3, 111.7, 60.8, 59.8, 56.3, 56.1, 55.9, 53.4, 52.9, 14.5. HRMS (ESI): found: 323.1982 (M+1); calcd for $C_{17}H_{27}N_2O_4$: 323.1971. IR (neat): 1742, 1672 cm^{-1}.

*General Procedure for 2-(4-benzylpiperazin-1-yl)acetohydrazide **6-8** and **10-12***

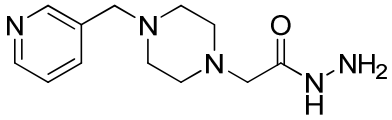
To a stirred solution of substituted ethyl 2-(4-Benzylpiperazin-1-yl)acetate (1 equiv.) in ethanol (1.9 mL, 0.5 M) was added anhydrous hydrazine (3 equiv.). The reaction was refluxed for 15-18 hr monitoring by TLC. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 and washed with water (10 mL) and brine (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL) and EtOAc (10 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel (1:4 MeOH/EtOAc).

2-(4-(Pyridin-2-ylmethyl)piperazin-1-yl)acetohydrazide (**6**)



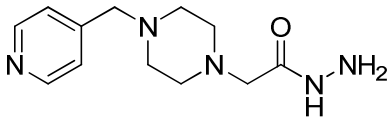
The general procedure was followed: ethyl 2-(4-(pyridin-2-ylmethyl)piperazin-1-yl)acetate (0.25 g, 0.95 mmol, 1 equiv.), anhydrous hydrazine (90 μL, 2.9 mmol, 3 equiv.), ethanol (1.9 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **6** (0.17 g, 71%) as yellow oil. ^{1}H NMR (500 MHz, CD_3OD): δ 8.49 (d, *J* = 4.9 Hz, 1H), 7.83 (dt, *J* = 1.8 Hz, *J* = 7.7 Hz, 1H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.33 (dd, *J* = 5.0 Hz, *J* = 7.4 Hz, 1H), 3.68 (s, 2H), 3.06 (s, 2H), 2.58 (broad s, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.2, 157.8, 148.4, 137.5, 124.1, 122.8, 63.5, 59.9, 53.0, 52.8. HRMS (ESI): found: 250.1670 (M+1); calcd for $C_{12}H_{20}N_5O_2$: 250.1668
IR (neat):

2-(4-(Pyridin-3-ylmethyl)piperazin-1-yl)acetohydrazide (**7**)



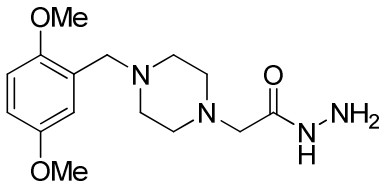
The general procedure was followed: ethyl 2-(4-(pyridin-3-ylmethyl)piperazin-1-yl)acetate (0.25 g, 0.95 mmol, 1 equiv.), anhydrous hydrazine (90 μL, 2.9 mmol, 3 equiv.), ethanol (1.9 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **7** (0.17 g, 72%) as yellow oil. ^{1}H NMR (500 MHz, CD_3OD): δ 8.51 (d, *J* = 1.5 Hz, 1H), 8.45 (dd, *J* = 1.6 Hz, *J* = 4.9 Hz, 1H), 7.84 (d, *J* = 7.9 Hz, 1H), 7.43 (dd, *J* = 4.6 Hz, *J* = 7.4 Hz, 1H), 3.60 (s, 2H), 3.05 (s, 2H), 2.55 (broad s, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.2, 149.8, 147.8, 138.2, 134.2, 124.0, 59.9, 59.5, 53.0, 52.6. HRMS (ESI): found: 250.1670 (M+1); calcd for $C_{12}H_{20}N_5O_2$: 250.1668 IR (neat):

2-(4-(Pyridin-4-ylmethyl)piperazin-1-yl)acetohydrazide (**8**)



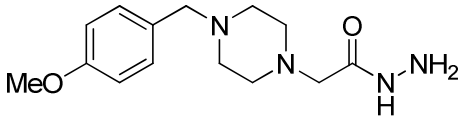
The general procedure was followed: ethyl 2-(4-(pyridin-4-ylmethyl)piperazin-1-yl)acetate (0.25 g, 0.95 mmol, 1 equiv.), anhydrous hydrazine (90 μL, 2.9 mmol, 3 equiv.), ethanol (1.9 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **8** (0.18 g, 78%) as yellow oil. ^{1}H NMR (500 MHz, CD_3OD): δ 8.47 (d, *J* = 6.0 Hz, 2H), 7.42 (d, *J* = 5.9 Hz, 2H), 3.58 (s, 2H), 3.05 (s, 2H), 2.54 (broad d, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.1, 148.9, 148.9, 124.6, 61.1, 59.9, 53.1, 52.7. HRMS (ESI): 250.1669 (M+1); calcd for $C_{12}H_{20}N_5O_2$: 250.1668. IR (neat): 3347, 1665 cm^{-1}.

2-(4-(2,5-Dimethoxybenzyl)piperazin-1-yl)acetohydrazide (**10**)



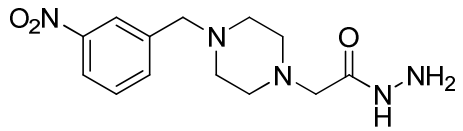
The general procedure was followed: ethyl 2-(4-(2,5-dimethoxybenzyl)piperazin-1-yl)acetate (0.19 g, 0.60 mmol, 1 equiv.), anhydrous hydrazine (58 μL, 1.81 mmol, 3 equiv.), ethanol (1.2 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **10** (0.16 g, 88%) as off-white semi-solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.16 (s, 1H), 6.93 (d, *J* = 2.9 Hz, 1H), 6.76 (m, 2H), 3.84 (s, 2H), 3.76 (s, 3H), 3.75 (s, 3H), 3.54 (s, 2H), 3.05 (s, 2H), 2.53 (s, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.7, 153.7, 152.3, 127.2, 116.7, 112.5, 111.9, 60.8, 56.4, 56.0, 55.9, 53.9, 53.2. HRMS (ESI): 309.1928 (M+1); calcd for $C_{15}H_{25}N_4O_3$: 309.1927. IR (neat): 3584, 1670 cm^{-1}.

2-(4-(4-Methoxybenzyl)piperazin-1-yl)acetohydrazide (**11**)



The general procedure was followed: ethyl 2-(4-(4-methoxybenzyl)piperazin-1-yl)acetate (0.70 g, 2.4 mmol, 1 equiv.), anhydrous hydrazine (220 μL, 7.1 mmol, 3 equiv.), ethanol (4.8 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **11** (0.54 g, 82%) as off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.13 (s, 1H), 7.20 (d, *J* = 8.6 Hz, 2H), 6.84 (d, *J* = 8.6 Hz, 2H), 3.84 (d, *J* = 3.9 Hz, 2H), 3.79 (s, 3H), 3.44 (s, 2H), 3.06 (s, 2H), 2.46 (s, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.8, 159.0, 130.5, 130.1, 113.8, 62.5, 60.8, 55.5, 53.9, 53.2. HRMS (ESI): 279.1811 (M+1); calcd for $C_{14}H_{23}N_4O_2$: 279.1821. IR (neat): 3584, 17, 1676 cm^{-1}. m.p.: 111.5-112.5 °C.

2-(4-(3-Nitrobenzyl)piperazin-1-yl)acetohydrazide (**12**)



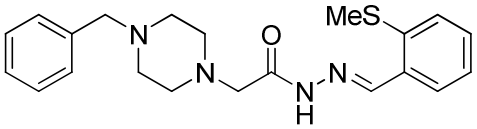
The general procedure was followed: ethyl 2-(4-(3-nitrobenzyl)piperazin-1-yl)acetate (7.0 g, 22.7 mmol, 1 equiv.), anhydrous hydrazine (2.1 mL, 68.2 mmol, 3 equiv.), ethanol (45 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **12** (5.4 g, 82%) as light yellow oil. ^{1}H NMR (500

MHz, $CDCl_3$): δ 8.13 (s, 1H), 8.02 (d, *J* = 8.2 Hz, 1H), 8.11 (s, 1H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.42 (t, *J* = 7.9 Hz, 1H), 3.82 (s, 2H), 3.52 (s, 2H), 3.01 (s, 2H), 2.45 (d, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.5, 148.5, 140.7, 135.2, 129.4, 123.8, 122.4, 62.0, 60.7, 53.7, 53.2. HRMS (ESI): 294.1559 (M+1); calcd for $C_{13}H_{20}N_5O_3$: 294.1566.

General Procedure for N'-(3-allyl-2-hydroxybenzylidene)-2-(4-benzylpiperazin-1-yl)acetohydrazide ***1b–g, 1j, 1k, 2a-g, 3***

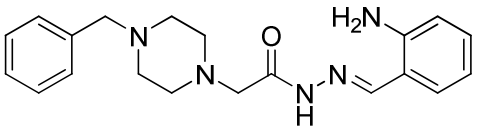
To a stirred solution of substituted benzaldehyde (1 equiv.) in ethanol (6 mL), 2-(4-benzylpiperazin-1-yl)acetohydrazide (1.8 equiv.) and HCl (10 mol%) were added. The reaction was refluxed for 12-16 hr monitoring by TLC. The solution was concentrated *in vacuo*, and then purified by flash column chromatography on silica gel (1:4 MeOH/EtOAc).

2-(4-Benzylpiperazin-1-yl)-*N'*-(2-(methylthio)benzylidene)acetohydrazide (**1b**)



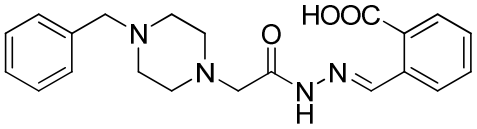
The general procedure was followed: hydrazide **5** (0.45 g, 1.8 mmol, 1.8 equiv.), 2-(methylthio)benzaldehyde (0.14 mL, 1.0 mmol, 1 equiv.), HCl (8 μL, 12M), ethanol (6 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **1b** (0.34 g, 88%) as light yellow semi-solid. ^1H NMR (500 MHz, $CDCl_3$) indicated a 87:13 ratio of two geometrical isomers: δ 10.11 (s, 1H x 0.87 isomer A), 8.67 (s, 1H x 0.13 isomer B), 8.65 (s, 1H x 0.87 isomer A), 8.12 (s, 1H x 0.13 isomer B), 8.05 (d, *J* = 8.0 Hz, 1H x 0.87 isomer A), 7.74 (d, 1H, *J* = 7.9 Hz x 0.13 isomer B), 7.36 (m, 2H), 7.33 (m, 1H), 7.28 (m, 1H), 7.21 (m, 1H), 3.70 (s, 2H x 0.13 isomer B), 3.55 (s, 2H x 0.87 isomer A), 3.53 (s, 2H x 0.13 isomer B), 3.20 (s, 2H x 0.87 isomer A), 2.58 (broad d, 8H x 0.87 isomer A), 2.50 (s, 3H), 2.46 (d, 8H x 0.13 isomer B), 1.65 (s, 3H x 0.13 isomer B). ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.3, 166.8, 146.0, 142.7, 138.7, 138.6, 138.1, 132.4, 132.2, 130.9, 130.2, 129.5, 129.3, 128.5, 128.4, 128.1, 128.0, 127.7, 127.4, 127.3, 126.1, 125.5, 63.2, 63.0, 63.0, 61.3, 58.5, 53.8, 53.7, 53.1, 17.4, 17.1. HRMS (ESI): 383.1900 (M+1); calcd for $C_{21}H_{27}N_4OS$: 383.1906. IR (neat): 1677, 1588 cm^{-1}. Purity: 99% (HPLC).

***N'*-(2-aminobenzylidene)-2-(4-benzylpiperazin-1-yl)acetohydrazide (1c)**



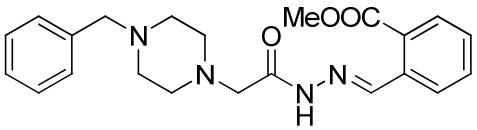
The general procedure was followed: hydrazide **5** (0.54 g, 2.2 mmol, 1.8 equiv.), 2-aminobenzaldehyde (0.15 g, 1.2 mmol, 1 equiv.), HCl (10 μL, 12M), ethanol (6 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **1c** (0.33 g, 78%) as light yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.96 (s, 1H), 8.13 (s, 1H), 7.33 (m, 2H), 7.28 (m, 1H), 7.14 (dd, *J* = 8.0 Hz, *J* = 10.2 Hz, 2H), 6.67 (m, 2H), 6.12 (s, 2H), 3.55 (s, 2H), 3.18 (s, 2H), 2.58 (d, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 165.8, 151.1, 147.7, 138.0, 132.7, 131.2, 129.4, 128.6, 127.5, 116.3, 116.0, 115.1, 63.1, 61.2, 53.9, 53.3. HRMS (ESI): 352.2137 (M+1); calcd for $C_{20}H_{26}N_5O$: 352.2137. IR (neat): 3423, 3310, 3208, 1685, 1617 cm^{-1}. m.p.: 168-169 °C. Purity: 99% (LC-MS).

2-((2-(2-(4-benzylpiperazin-1-yl)acetyl)hydrazono)methyl)benzoic acid (1d)



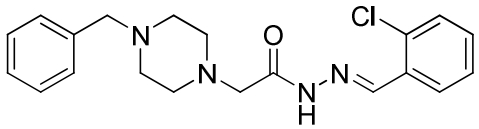
The general procedure was followed: hydrazide **5** (0.54 g, 2.2 mmol, 1.8 equiv.), 2-carboxybenzaldehyde (0.18 g, 1.2 mmol, 1 equiv.), HCl (10 μL, 12M), ethanol (6 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **1d** (0.36 g, 79%) as white solid. ^{1}H NMR (500 MHz, CD_3OD) indicated a 75:25 ratio of two geometrical isomers: δ 8.87 (s, 1H x 0.75 isomer A), 8.61 (s, 1H x 0.25 isomer B), 8.08 (m, 1H x 0.75 isomer A), 7.92 (m, 1H x 0.25 isomer B), 7.69 (m, 1H), 7.34 (m, 7H), 3.75 (s, 2H x isomer B), 3.63 (s, 2H x isomer B), 3.59 (s, 2H x isomer A), 3.14 (s, 2H x isomer A), 2.69 (broad d, 8H). ^{13}C NMR (126 MHz, CD_3OD): δ 179.1, 171.9, 153.4, 145.5, 141.0, 135.4, 135.1, 133.7, 133.5, 133.2, 132.4, 132.3, 132.1 (2 partially resolved peaks), 131.2, 130.0 (2 partially resolved peaks), 129.1, 66.6, 64.0, 56.9, 56.8, 56.4, 56.3. HRMS (ESI): 381.1938 (M+1); calcd for $C_{21}H_{25}N_4O_3$: 381.1927. IR (neat): 3364 (br), 1675 cm^{-1}. m.p.: 150-151.5 °C. Purity: 99% (HPLC).

Methyl 2-((2-(2-(4-benzylpiperazin-1-yl)acetyl)hydrazono)methyl)benzoate (1e)



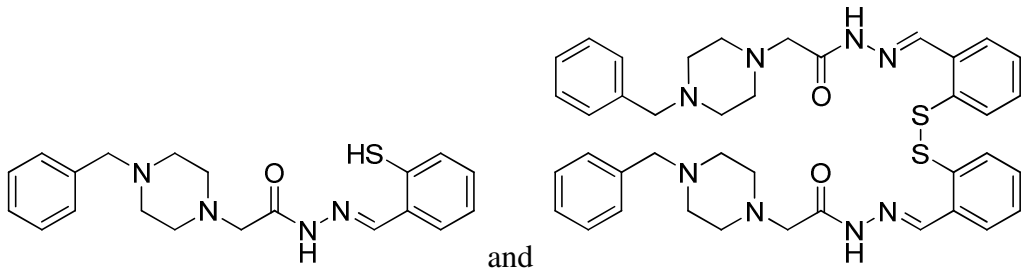
The general procedure was followed: hydrazide **5** (0.54 g, 2.2 mmol, 1.8 equiv.), methyl 2-formylbenzoate (0.20 g, 1.2 mmol, 1 equiv.), HCl (10 μL, 12M), ethanol (6 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **1e** (0.37 g, 72%) as white solid. ^{1}H NMR (500 MHz, $CDCl_3$) indicated a 87:13 ratio of two geometrical isomers: δ 10.17 (s, 1H x 0.87 isomer A), 9.11 (s, 1H x 0.13 isomer B), 9.02 (s, 1H x 0.87 isomer A), 8.63 (s, 1H x isomer B), 8.24 (d, *J* = 7.9 Hz, 1H x 0.87 isomer A), 8.01 (d, *J* = 7.5 Hz, 1H x 0.13 isomer B), 7.97 (d, *J* = 7.9 Hz, 1H x 0.87 isomer A; accidentally overlap with 1H x 0.13 isomer B), 7.54 (t, *J* = 7.2 Hz, 1H), 7.44 (t, *J* = 7.8 Hz, 1H), 7.31 (apparent d, 4H), 7.26 (m, 1H), 3.94 (s, 3H x 0.87 isomer A), 3.89 (s, 3H x 0.13 isomer B), 3.68 (s, 2H x 0.13 isomer B), 3.54 (s, 2H), 3.19 (s, 2H x isomer A), 2.58 (d, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 167.5, 166.8, 146.9, 138.1, 135.0, 132.8, 131.0, 130.8, 130.0, 129.5, 129.4, 129.0, 128.6, 128.6, 128.5, 128.4, 128.4, 127.4, 63.1, 61.4, 53.9, 53.2, 52.6. HRMS (ESI): 395.2037 (M+1); calcd for $C_{22}H_{27}N_4O_3$: 395.2083. IR (neat): 1716, 1664 cm^{-1}. m.p.: 109.5-111 °C. Purity: 98% (LC-MS).

2-(4-Benzylpiperazin-1-yl)-*N′*-(2-chlorobenzylidene)acetohydrazide (1f)



The general procedure was followed: hydrazide **5** (0.40 g, 1.6 mmol, 1.8 equiv.), 2-chlorobenzaldehyde (0.10 mL, 0.9 mmol, 1 equiv.), HCl (10 μL, 12M), ethanol (5 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **1f** (0.31 g, 94%) as yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) indicated a 78:12 ratio of two geometrical isomers: δ 10.61 (s, 1H x 0.12 isomer B), 10.40 (s, 1H x 0.78 isomer A), 8.53 (s, 1H x 0.78 isomer A), 8.23 (s, 1H x 0.12 isomer B), 8.09 (m, 1H x 0.78 isomer A), 7.88 (m, 1H x 0.12 conformer B), 7.27 (m, 5H), 7.21 (m, 3H), 3.65 (s, 2H x 0.12 conformer B), 3.49 (s, 2H), 3.17 (s, 2H x 0.78 conformer A), 2.58 (broad m, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.1, 166.9, 144.7, 140.6, 138.1, 134.4, 134.3, 131.7, 131.6, 131.3, 131.0, 130.1, 129.9, 129.5, 129.3, 128.5, 128.4, 128.1, 127.4, 127.3, 127.3, 127.2, 127.1, 63.2, 63.1, 61.3, 58.4, 53.9, 53.8, 53.1, 53.1. HRMS (ESI): 371.1624 (M+1); calcd for $C_{20}H_{24}N_4OCl$: 371.1639. IR (neat): 1682, 1595 cm^{-1}. Purity: 98% (LC-MS).

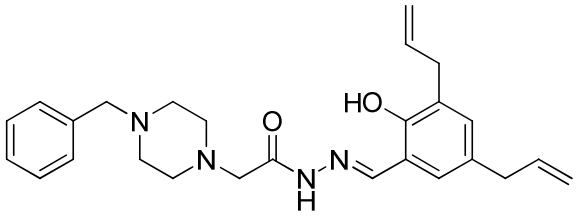
2-(4-Benzylpiperazin-1-yl)-*N′*-(2-mercaptobenzylidene)acetohydrazide (**1g**) and ***N′,N′′*-(2,2'-disulfanediylbis(2,1-phenylene)bis(methan-1-yl-1-ylidene))bis(2-(4-benzylpiperazin-1-yl)acetohydrazide)** (**1i**)



and

The general procedure was followed: hydrazide **5** (0.37 g, 1.5 mmol, 1.8 equiv.), 2-mercaptobenzaldehyde (0.11 g, 0.82 mmol, 1 equiv.), HCl (10 μL, 12M), ethanol (14 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **1g** (0.054 g, 18%) as yellow oil and **1i** (0.19 g, 63%) as yellow oil. In order to minimize the oxidation to form **1i** via disulfide bond, the product was dissolved in $CHCl_3$ as a 100 μM stock solution. **1g**: ^{1}H NMR (400 MHz, $CDCl_3$) indicated a 84:16 ratio of two geometrical isomers: δ 10.07 (s, 1H), 8.42 (s, 1H), 7.68 (dd, *J* = 1.7 Hz, *J* = 7.4 Hz, 1H), 7.26 (m, 7H), 7.14 (m, 1H), 5.23 (s, 1H x 0.84 isomer A), 4.85 (s, 1H x 0.16 isomer B), 3.67 (s, 2H x 0.16 isomer B), 3.51 (s, 2H x 0.84 isomer A), 3.17 (s, 2H x 0.16 isomer B), 3.15 (s, 2H x isomer A), 2.54 (broad d, 8H). **1i**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.98 (s, 2H), 8.43 (s, 2H), 7.95 (m, 2H), 7.47 (m, 2H), 7.24 (m, 14H), 3.50 (s, 4H), 3.14 (s, 4H), 2.53 (broad d, 16H). HRMS (ESI): 735.3259 (M+1); calcd for $C_{40}H_{47}N_8O_2S_2$: 735.3263. IR (neat): 1672 cm^{-1}. Purity: 98% (HPLC).

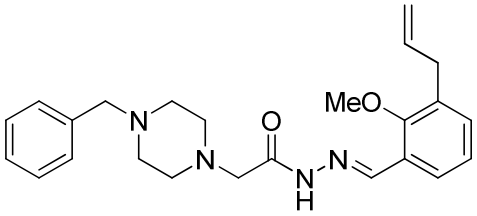
2-(4-Benzylpiperazin-1-yl)-*N′*-(3,5-diallyl-2-hydroxybenzylidene)acetohydrazide (**1j**)



The general procedure was followed: hydrazide **5** (0.54 g, 2.2 mmol, 1.8 equiv.), **22** (0.24 g, 1.2 mmol, 1 equiv.), HCl (10 μL, 12M), ethanol (6 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) and then recrystallization with EtOAc afforded **1j** (0.48 g, 93%) as beige solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.08 (s, 1H), 10.00 (s, 1H), 8.40 (s, 1H), 7.33 (m, 4H), 7.28 (m, 1H), 7.01 (d, *J* = 1.9 Hz,

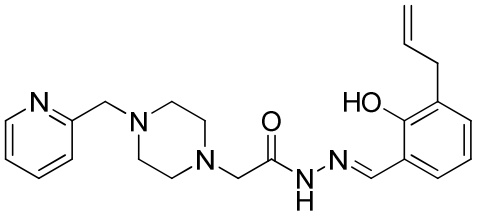
1H), 6.90 (d, *J* = 1.9 Hz, 1H), 6.03 (tdd, *J* = 6.6 Hz, *J* = 10.0 Hz, *J* = 16.7 Hz, 1H), 5.93 (tdd, *J* = 6.7 Hz, *J* = 9.5 Hz, *J* = 16.2 Hz, 1H), 5.07 (m, 4H), 3.55 (s, 2H), 3.43 (d, *J* = 6.6 Hz, 2H), 3.30 (d, *J* = 6.7 Hz, 2H), 3.18 (s, 2H), 2.58 (d, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.0, 155.0, 151.6, 145.3, 138.0, 137.8, 136.8, 133.0, 130.6, 129.4, 129.0, 128.5, 127.5, 116.9, 116.0, 115.8, 63.1, 61.2, 53.9, 53.2, 39.4, 34.1. HRMS (ESI): 433.2611 (M+1); calcd for $C_{26}H_{33}N_4O_2$: 433.2604. IR (neat): 3212 (br), 1737, 1680, 1616 cm^{-1}. m.p.: 93-93.5 °C. Purity: 95% (LC-MS).

***N'*-(3-allyl-2-methoxybenzylidene)-2-(4-benzylpiperazin-1-yl)acetohydrazide (1k)**



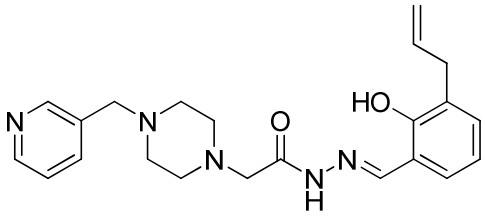
The general procedure was followed: hydrazide **5** (0.54 g, 2.2 mmol, 1.8 equiv.), **23** (0.21 g, 1.2 mmol, 1 equiv.), HCl (10 μL, 12M), ethanol (6 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) and then recrystallization with EtOAc afforded **1k** (0.41 g, 85%) as white solid. ^{1}H NMR (500 MHz, $CDCl_3$) indicated a 85:15 ratio of two geometrical isomers: δ 10.06 (s, 1H x 0.85 isomer A), 9.15 (s, 1H x 0.15 isomer B), 8.38 (s, 1H x 0.85 isomer A), 7.97 (s, 1H x 0.15 isomer B), 7.90 (dd, *J* = 1.6 Hz, *J* = 7.8 Hz, 1H x 0.85 isomer A), 7.68 (dd, *J* = 1.6 Hz, *J* = 7.8 Hz, 1H x 0.15 isomer B), 7.27 (s, 2H), 7.26 (s, 2H), 7.20 (m, 2H), 7.04 (t, *J* = 7.6 Hz, 1H), 5.92 (tdd, *J* = 6.4 Hz, *J* = 10.1 Hz, *J* = 16.6 Hz, 1H), 5.03 (m, 2H), 3.73 (s, 3H x 0.85 isomer A), 3.67 (s, 3H x 0.15 isomer B), 3.63 (s, 2H x 0.15 isomer B), 3.49 (s, 2H), 3.38 (d, *J* = 6.4 Hz, 2H), 3.14 (s, 2H x 0.85 isomer B), 2.53 (d, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.6, 157.9, 144.6, 137.0, 133.4, 133.1, 132.8, 129.4, 128.5, 127.4, 127.0, 125.9, 125.0, 124.9, 124.8, 116.4, 63.3, 63.1, 61.3, 53.9, 53.2, 33.8. HRMS (ESI): 407.2443 (M+1); calcd for $C_{24}H_{31}N_4O_2$: 403.2447. IR (neat): 1662 cm^{-1}. m.p.: 111.5-113 °C. Purity: 98% (LC-MS).

***N'*-(3-allyl-2-hydroxybenzylidene)-2-(4-(pyridin-2-ylmethyl)piperazin-1-yl)acetohydrazide (2a)**



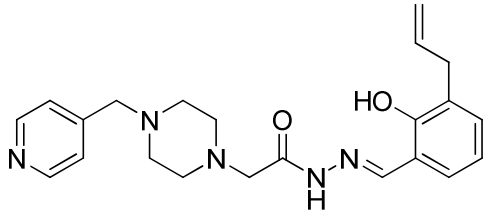
The general procedure was followed: **6** (0.54 g, 2.2 mmol, 1.8 equiv.), 3-allylsalicylaldehyde (0.19 g, 1.2 mmol, 1 equiv.), HCl (10 μL, 12M), ethanol (6 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **2a** (0.39 g, 83%) as light brown semi-solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.19 (s, 1H) 10.04 (s, 1H), 8.50 (d, *J* = 4.5 Hz, 1H), 8.35 (s, 1H), 7.61 (dt, *J* = 1.7 Hz, *J* = 7.7 Hz, 1H), 7.36 (d, *J* = 7.8 Hz, 1H), 7.12 (t, *J* = 7.2 Hz, 2H), 7.02 (d, *J* = 6.5 Hz, 1H), 6.78 (t, *J* = 7.6 Hz, 1H), 5.96 (tdd, *J* = 6.6 Hz, *J* = 10.0 Hz, *J* = 16.8 Hz, 1H), 5.00 (m, 2H), 3.67 (s, 2H), 3.38 (d, *J* = 6.6 Hz, 2H), 3.15 (s, 1H), 2.60 (d, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 165.8, 163.8, 156.6, 151.5, 149.6, 136.8, 136.7, 132.5, 129.4, 128.4, 123.6, 122.6, 119.3, 117.1, 115.9, 64.5, 61.1, 53.7, 53.4, 34.1. HRMS (ESI): 394.2235 (M+1); calcd for $C_{22}H_{28}N_5O_2$: 394.2243. IR (neat): 3209 (br), 1690, 1605 cm^{-1}. Purity: 96% (HPLC).

***N'*-(3-allyl-2-hydroxybenzylidene)-2-(4-(pyridin-3-ylmethyl)piperazin-1-yl) acetohydrazide (2b)**



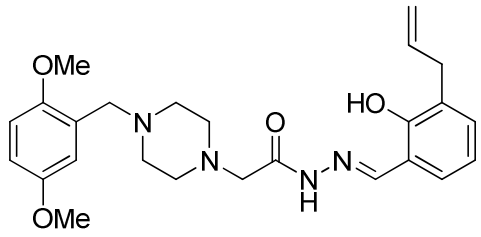
The general procedure was followed: **7** (0.10 g, 0.42 mmol, 1.8 equiv.), 3-allylsalicylaldehyde (0.038 g, 0.23 mmol, 1 equiv.), HCl (10 μL, 12M), ethanol (1.4 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **2b** (0.080 g, 88%) as light brown semi-solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.18 (s, 1H). 9.98 (s, 1H), 8.46 (m, 2H), 8.36 (s, 1H), 7.61 (d, *J* = 7.8 Hz, 1H), 7.21 (m, 1H), 7.12 (d, *J* = 7.2 Hz, 1H), 7.02 (d, *J* = 7.7 Hz, 1H), 6.78 (t, *J* = 7.6 Hz, 1H), 5.96 (tdd, *J* = 6.6 Hz, *J* = 10.1 Hz, *J* = 16.8 Hz, 1H), 5.00 (m, 2H), 3.49 (s, 2H), 3.38 (d, *J* = 6.6 Hz, 2H), 3.13 (s, 2H), 2.52 (d, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 165.9, 156.6, 151.6, 150.6, 149.1, 149.0, 136.9, 136.7, 132.5, 129.4, 128.4, 123.7, 119.3, 117.1, 115.9, 61.1, 60.2, 53.8, 53.2, 34.1. HRMS (ESI): 394.2237 (M+1); calcd for $C_{22}H_{28}N_5O_2$: 394.2243. IR (neat): 3199 (br), 1684, 1607 cm^{-1}. Purity: 95% (HPLC).

***N′*-(3-allyl-2-hydroxybenzylidene)-2-(4-(pyridin-4-ylmethyl)piperazin-1-yl) acetohydrazide (2c)**



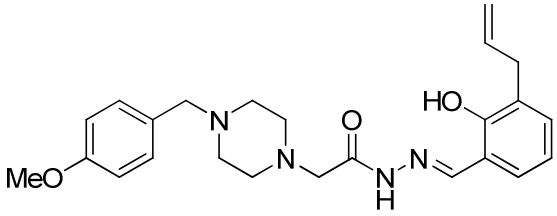
The general procedure was followed: **8** (0.075 g, 0.30 mmol, 1.8 equiv.), 3-allylsalicylaldehyde (0.027 g, 0.17 mmol, 1 equiv.), HCl (10 μL, 12M), ethanol (1 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **2c** (0.060 g, 90%) as light brown semi-solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.27 (s, 1H), 10.08 (s, 1H), 8.55 (d, *J* = 5.8 Hz, 2H), 8.39 (s, 1H), 7.27 (s, 2H), 7.18 (dd, *J* = 1.5 Hz, *J* = 7.4 Hz, 1H), 7.06 (dd, *J* = 1.5 Hz, *J* = 7.7 Hz, 1H), 6.84 (t, *J* = 7.6 Hz, 1H), 6.02 (tdd, *J* = 6.6 Hz, *J* = 10.0 Hz, *J* = 16.7 Hz, 1H), 5.07 (m, 2H), 3.54 (s, 2H), 3.44 (d, *J* = 6.6 Hz, 2H), 3.20 (s, 2H), 2.59 (d, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.0, 156.6, 151.5, 150.0, 147.5, 136.7, 132.5, 129.4, 128.4, 124.0, 119.3, 117.1, 115.9, 61.7, 61.2, 53.8, 53.3, 34.1. HRMS (ESI): 394.2238 (M+1); calcd for $C_{22}H_{28}N_5O_2$: 394.2243. IR (neat): 3189 (br), 1685, 1606 cm^{-1}. Purity: 97% (HPLC).

***N′*-(3-allyl-2-hydroxybenzylidene)-2-(4-(2,5-dimethoxybenzyl)piperazin-1-yl) acetohydrazide (2e)**



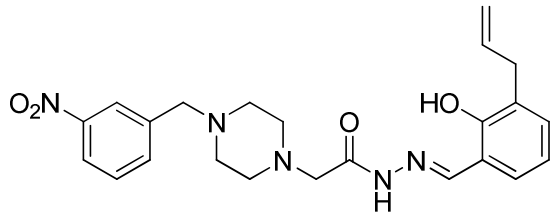
The general procedure was followed: **10** (0.67 g, 2.2 mmol, 1.8 equiv.), 3-allylsalicylaldehyde (0.19 g, 1.2 mmol, 1 equiv.), HCl (10 μL, 12M), ethanol (6 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **2e** (0.44 g, 80%) as beige solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.20 (s, 1H), 9.98 (s, 1H), 8.33 (s, 1H), 7.11 (dd, *J* = 1.5 Hz, *J* = 7.4 Hz, 1H), 7.01 (dd, *J* = 1.5 Hz, *J* = 7.7 Hz, 1H), 6.89 (d, *J* = 3.0 Hz, 1H), 6.77 (t, *J* = 7.6 Hz, 1H), 6.71 (m, 2H), 5.96 (tdd, *J* = 6.6 Hz, *J* = 10.1 Hz, *J* = 16.7 Hz, 1H), 5.00 (m, 2H), 3.71 (s, 3H), 3.70 (s, 3H), 3.52 (s, 2H), 3.38 (d, *J* = 6.6 Hz, 2H), 3.11 (s, 2H), 2.54 (d, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 164.9, 155.4, 152.5, 151.1, 150.3, 135.5, 131.3, 128.2, 127.2, 126.0, 118.0, 115.9, 115.5, 114.6, 111.3, 110.7, 60.0, 55.2, 54.8, 54.7, 52.7, 51.9, 32.8. HRMS (ESI): 453.2507 (M+1); calcd for $C_{25}H_{33}N_4O_4$: 453.2502. IR (neat): 3215 (br), 1694, 1608 cm^{-1}. m.p.: 101.2-103 °C.
Purity: 95% (LC-MS).

***N′*-(3-allyl-2-hydroxybenzylidene)-2-(4-(4-methoxybenzyl)piperazin-1-yl)acetohydrazide (2f)**



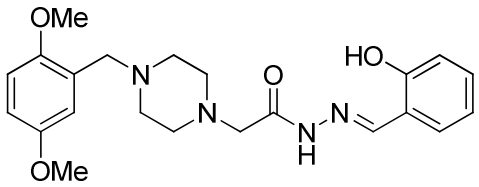
The general procedure was followed: **11** (0.60 g, 2.2 mmol, 1.8 equiv.), 3-allylsalicylaldehyde (0.19 g, 1.2 mmol, 1 equiv.), HCl (10 μL, 12M), ethanol (6 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **2f** (0.41 g, 81%) as light yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.19 (s, 1H), 9.97 (s, 1H), 8.35 (s, 1H), 7.16 (d, *J* = 8.3 Hz, 2H), 7.12 (d, *J* = 7.4 Hz, 1H), 7.02 (d, *J* = 7.6 Hz, 1H), 6.78 (m, 3H), 5.96 (m, 1H), 4.99 (m, 2H), 3.73 (s, 3H), 3.43 (s, 2H), 3.38 (d, *J* = 6.6 Hz, 2H), 3.11 (s, 2H), 2.50 (d, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 164.8, 157.9, 155.4, 150.4, 135.5, 131.3, 129.4, 128.5, 128.2, 127.2, 118.1, 115.9, 114.7, 112.7, 61.2, 59.9, 54.3, 52.6, 51.8, 32.8. HRMS (ESI): 423.2383 (M+1); calcd for $C_{22}H_{28}N_5O_2$: 423.2396. IR (neat): 3211 (br), 1686, 1609 cm^{-1}. Purity: 99% (LC-MS).

***N′*-(3-allyl-2-hydroxybenzylidene)-2-(4-(3-nitrobenzyl)piperazin-1-yl) acetohydrazide (2g)**



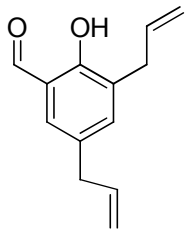
The general procedure was followed: **12** (0.63 g, 2.2 mmol, 1.8 equiv.), 3-allylsalicylaldehyde (0.19 g, 1.2 mmol, 1 equiv.), HCl (10 μL, 12M), ethanol (6 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **2g** (0.39 g, 74%) as beige solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.18 (s, 1H), 9.95 (s, 1H), 8.34 (s, 1H), 8.12 (s, 1H), 8.05 (d, *J* = 6.6 Hz, 1H), 7.61 (d, *J* = 7.6 Hz, 1H), 7.43 (t, *J* = 7.9 Hz, 1H), 7.11 (d, *J* = 7.3 Hz, 1H), 7.01 (dd, *J* = 1.4 Hz, *J* = 7.7 Hz, 1H), 6.77 (t, *J* = 7.6 Hz, 1H), 5.95 (tdd, *J* = 6.6 Hz, *J* = 10.1 Hz, *J* = 16.8 Hz, 1H), 4.99 (m, 2H), 3.56 (s, 2H), 3.37 (d, *J* = 6.6 Hz, 2H), 3.13 (s, 2H), 2.53 (d, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 165.9, 156.7, 151.8, 148.6, 140.6, 136.7, 135.2, 132.6, 129.5, 129.4, 128.5, 123.9, 122.6, 119.3, 117.1, 115.9, 62.1, 61.2, 53.8, 53.3, 34.1. HRMS (ESI): 438.2142 (M+1); calcd for $C_{23}H_{28}N_5O_4$: 438.2141. IR (neat): 3218 (br), 1682, 1526, 1349 cm^{-1}. m.p.: 84-86 °C. Purity: 96% (HPLC).

2-(4-(2,5-Dimethoxybenzyl)piperazin-1-yl)-*N'*-(2-hydroxybenzylidene) acetohydrazide (**3**)



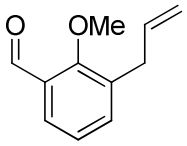
The general procedure was followed: **10** (0.67 g, 2.2 mmol, 1.8 equiv.), salicylaldehyde (0.13 g, 1.2 mmol, 1 equiv.), HCl (10 μL, 12M), ethanol (6 mL). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **3** (0.41 g, 82%) as light yellow semi-solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.98 (s, 1H), 10.05 (s, 1H), 8.46 (s, 1H), 7.30 (m, 1H), 7.21 (dd, *J* = 1.6 Hz, *J* = 7.7 Hz, 1H), 7.00 (m, 1H), 6.96 (d, *J* = 3.0 Hz, 1H), 6.90 (dt, *J* = 1.0 Hz, *J* = 7.5 Hz, 1H), 6.78 (m, 2H), 3.78 (s, 3H), 3.78 (s, 3H), 3.59 (s, 2H), 3.18 (s, 2H), 2.62 (d, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.2, 158.8, 153.7, 152.3, 151.5, 132.1, 131.2, 127.2, 119.5, 117.6, 117.5, 116.8, 112.5, 111.8, 61.2, 56.4, 56.0, 55.9, 54.0, 53.2. HRMS (ESI): 413.2182 (M+1); calcd for $C_{22}H_{29}N_4O_4$: 413.2189. IR (neat): 3214 (br), 1684, 1621 cm^{-1}. Purity: 98% (LC-MS).

3,5-Diallylsalicylaldehyde (**22**)



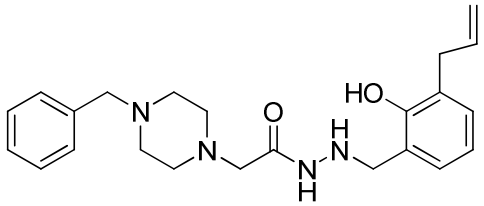
To a stirred mixture of 3-allylsalicylaldehyde (0.097 g, 0.60 mmol, 1 equiv.) and K_2CO_3 (0.10 g, 0.74 mmol, 1.25 equiv.) in DMF (5 mL) was added allyl bromide (0.077 mL, 0.89 mmol, 1.5 equiv.). The reaction mixture was stirred for 3 hr at room temperature. The solution was filtered, washed with DMF (3 mL) and then concentrated under high vaccum. The residue (neat) was microwaved at 100 W for 5 min. The crude product was purified by normal phase Prep TLC (1:4 CH_2Cl_2/Hexanes) to provide **22** (0.048 g, 40%) as yellow liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.17 (s, 1H), 9.85 (s, 1H), 7.23 (apparent dd, *J* = 2.1 Hz, *J* = 4.1 Hz, 2H), 5.97 (m, 2H), 5.10 (m, 2H), 5.07 (m, 2H), 3.41 (d, *J* = 6.6 Hz, 2H), 3.35 (d, *J* = 6.6 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 196.9, 158.2, 138.0, 137.2, 136.1, 131.5, 131.3, 129.0, 120.3, 116.6, 116.4, 39.2, 33.4. HRMS (ESI): 203.1066 (M+1); calcd for $C_{13}H_{15}O_2$: 203.1072. IR (neat): 2847, 1701 cm^{-1}.

3-Allyl-2-methoxybenzaldehyde (**23**)



To a stirred mixture of 3-allylsalicylaldehyde (0.11 g, 0.68 mmol, 1 equiv.) and K_2CO_3 (0.28 g, 2.0 mmol, 3 equiv.) in acetone was added iodomethane (0.050 mL, 0.80 mmol, 1.2 equiv.). The reaction mixture was stirred for 15 hr. The solution was filtered, washed with acetone and then concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel (1:9 EtOAc/Hexanes) to afford **23** (0.073 g, 75 %) as light yellow liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.37 (s, 1H), 7.72 (dd, *J* = 1.6 Hz, *J* = 7.7 Hz, 1H), 7.47 (dd, *J* = 1.5 Hz, *J* = 7.5 Hz, 1H), 7.19 (t, *J* = 7.6 Hz, 1H), 5.98 (tdd, *J* = 6.4 Hz, *J* = 10.1 Hz, *J* = 16.6 Hz, 1H), 5.11 (m, 2H), 3.89 (s, 3H), 3.47 (d, *J* = 6.4 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 190.3, 161.6, 137.1, 136.6, 134.5, 129.6, 127.5, 124.8, 116.8, 64.5, 33.4. HRMS (ESI): 177.0919 (M+1); calcd for $C_{11}H_{13}O_2$: 177.0916

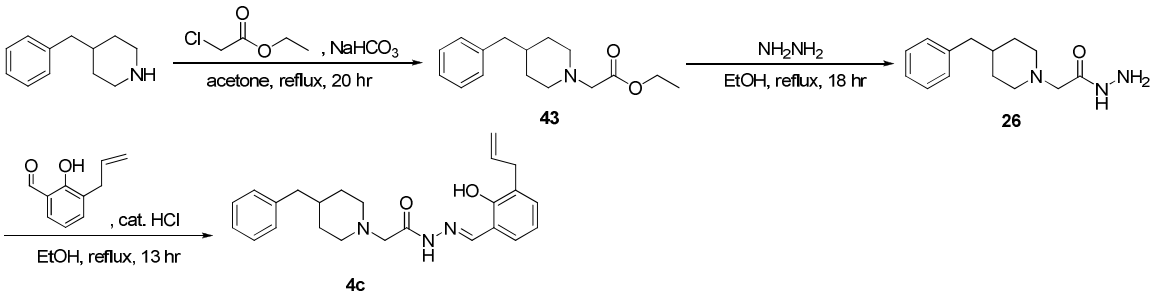
***N'*-(3-allyl-2-hydroxybenzyl)-2-(4-benzylpiperazin-1-yl)acetohydrazide** (**4d**)



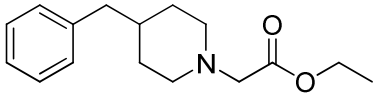
To a solution of **PAC-1** (**1**)(0.10 g, 0.25 mmol, 1 equiv.) in 3:1 THF/AcOH (8 mL in total) was added sodium cyanoborohydride (0.048 g, 0.76 mmol, 3 equiv.) at 0 °C. The reaction mixture was allowed to warm slowly to room temperature and stirred for 20 hr. The reaction mixture was then poured into a slurry of ice and concentrated hydrochloric acid (1 mL). The resulting mixture was extracted with CH_2Cl_2 (2 x 10 mL) and EtOAc (2 x 10 mL). The combined organic layer was washed with saturated sodium bicarbonate, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel (1:4 MeOH/EtOAc) to afford **4d** (0.069 g, 67%) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.26 (s, 1H), 8.42 (d, *J* = 4.8 Hz, 1H), 7.30 (m, 5H), 7.09 (dd, *J* = 1.2 Hz, *J* = 7.4 Hz, 1H), 6.87 (dd, *J* = 1.3 Hz, *J* = 7.4 Hz, 1H), 6.74 (t, *J* = 7.5 Hz, 1H), 6.05 (tdd, *J* = 6.6 Hz, *J* = 10.1 Hz, *J* = 16.7 Hz, 1H), 5.09 (m, 2H), 4.95 (s, 1H), 4.18 (s, 2H), 3.45 (s, 2H), 3.44 (s, 2H), 3.04 (s, 2H), 2.36 (d, 8H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.1, 154.9, 137.3, 130.2, 129.4, 129.3, 128.5, 128.2, 127.8, 127.4, 120.5, 119.8, 115.6, 63.0, 60.7, 54.6, 53.7, 53.1,

34.3. HRMS (ESI): 395.2428 (M+1); calcd for $C_{23}H_{31}N_4O_2$: 395.2447. IR (neat): 3268 (br), 1676 cm^{-1}. m.p.: 83-84 °C. Purity: 98% (HPLC).

Scheme S2. Synthesis of **4c**

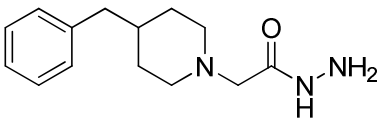


Ethyl 2-(4-benzylpiperidin-1-yl)acetate (**43**)



To a stirred mixture of 1-benzylpiperidine (0.30 mL, 1.7 mmol, 1 equiv.) and $NaHCO_3$ (0.18 g, 2.1 mmol, 1.25 equiv.) in acetone (3.5 mL) was added ethyl chloroacetate (0.20 mL, 1.9 mmol, 1.1 equiv.). The reaction was refluxed for 20 hr monitoring by TLC. The solution was filtered and the solid was washed with acetone (10 mL). The filtrate was concentrated *in vacuo*, and then purified by flash column chromatography on silica gel (1:1 EtOAc/Hexanes) to give **43** (0.36 g, 82%) as yellow liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.28 (m, 2H), 7.18 (m, 1H), 7.15 (m, 2H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.18 (s, 2H), 2.92 (d, *J* = 11.4 Hz, 2H), 2.54 (d, *J* = 7.0 Hz, 2H), 2.10 (dt, *J* = 2.1 Hz, *J* = 11.7 Hz, 2H), 1.63 (dd, *J* = 1.4 Hz, *J* = 13.4 Hz, 2H), 1.53 (m, 1H), 1.40 (ddd, *J* = 3.7 Hz, *J* = 12.2 Hz, *J* = 24.1 Hz, 2H), 1.27 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.5, 140.7, 129.3, 128.4, 126.0, 60.8, 59.9, 53.8, 43.3, 37.6, 32.0, 14.5. HRMS (ESI): 262.1819 (M+1); calcd for $C_{16}H_{24}NO_2$: 262.1807. IR (neat): 1741, 1655 cm^{-1}.

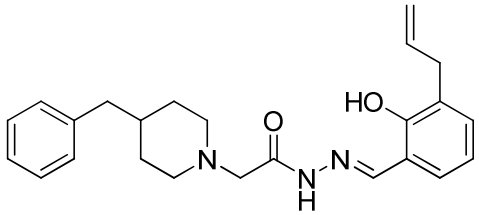
2-(4-Benzylpiperidin-1-yl)acetohydrazide (**26**)



To a stirred solution of **43** (0.29 g, 1.1 mmol, 1 equiv.) in ethanol (2.3 mL, 0.5 M) was added anhydrous hydrazine (0.11 mL, 3.4 mmol, 3 equiv.). The reaction was refluxed for 18 hr monitoring by TLC. The

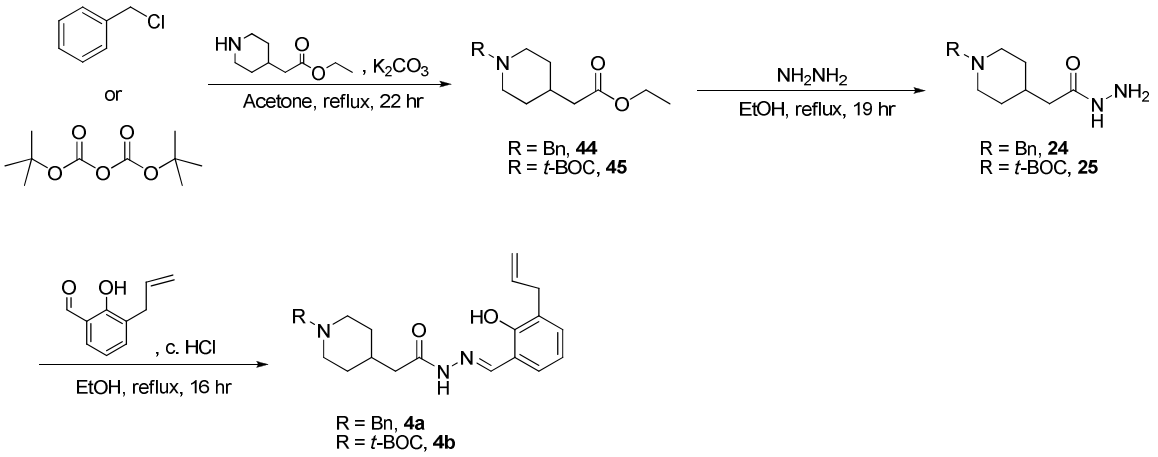
reaction mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 and washed with water (10 mL) and brine (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL) and EtOAc (10 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel (1:4 MeOH/EtOAc) to afford **26** (0.18 g, 66%) as milky oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.20 (s, 1H), 7.27 (m, 2H), 7.18 (m, 1H), 7.12 (m, 2H), 3.82 (broad s, 2H), 3.01 (s, 2H), 2.77 (d, *J* = 11.6 Hz, 2H), 2.52 (d, *J* = 7.1 Hz, 2H), 2.07 (dt, *J* = 2.4 Hz, *J* = 11.9 Hz, 2H), 1.62 (d, *J* = 13.1 Hz, 2H), 1.50 (m, 1H), 1.26 (ddd, *J* = 3.8 Hz, *J* = 12.4 Hz, *J* = 15.6 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.2, 140.6, 129.3, 128.4, 126.1, 61.2, 54.7, 43.3, 37.4, 32.6. HRMS (ESI): 248.1755 (M+1); calcd for $C_{14}H_{22}N_3O$: 248.1763. IR (neat): 3322, 1667 cm^{-1}.

***N′*-(3-allyl-2-hydroxybenzylidene)-2-(4-benzylpiperidin-1-yl)acetohydrazide (4c)**

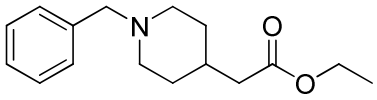


To a stirred solution of 3-allylsalicylaldehyde (0.063 g, 0.39 mmol, 1 equiv.) in ethanol (2.2 mL), **26** (0.17 g, 0.70 mmol, 1.8 equiv.) and HCl (10 mol%) were added. The reaction was refluxed for 13 hr monitoring by TLC. The solution was concentrated *in vacuo*, and then purified by flash column chromatography on silica gel (1:4 MeOH/EtOAc) to provide **4c** (0.12 g, 80%) as white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.28 (s, 1H), 10.26 (s, 1H), 8.43 (s, 1H), 7.30 (t, *J* = 7.5 Hz, 2H), 7.21 (dd, *J* = 7.1 Hz, *J* = 14.2 Hz, 2H), 7.15 (d, *J* = 7.3 Hz, 2H), 7.11 (d, *J* = 7.7 Hz, 1H), 6.86 (t, *J* = 7.6 Hz, 1H), 6.04 (tdd, *J* = 6.6 Hz, *J* = 10.1 Hz, *J* = 16.8 Hz, 1H), 5.09 (m, 2H), 3.46 (d, *J* = 6.6 Hz, 2H), 3.21 (s, 2H), 2.93 (d, *J* = 10.4 Hz, 2H), 2.58 (d, *J* = 7.1 Hz, 2H), 2.26 (t, *J* = 10.1 Hz, 2H), 1.72 (d, *J* = 13.0 Hz, 2H), 1.60 (m, 1H), 1.38 (dd, *J* = 11.1 Hz, *J* = 22.1 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.2, 156.7, 151.5, 140.4, 136.8, 132.5, 129.4, 129.3, 128.5, 128.5, 126.3, 119.3, 117.2, 115.9, 61.5, 54.7, 43.2, 37.3, 34.1, 32.4. HRMS (ESI): 392.2321 (M+1); calcd for $C_{24}H_{30}N_3O_2$: 392.2338. IR (neat): 3208 (br), 1678, 1608 cm^{-1}. m.p.: 140.8-141.3 °C. Purity: 96% (HPLC).

Scheme S3. Syntheses of **4a-b**

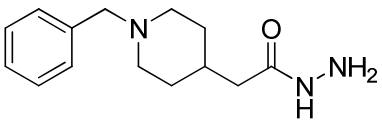


Ethyl 2-(1-benzylpiperidin-4-yl)acetate (**44**)



To a stirred mixture of benzyl chloride (0.3 mL, 2.6 mmol, 1 equiv.) and K_2CO_3 (1.1 g, 7.8 mmol, 3 equiv.) in acetone (11 mL) was added 2-(piperidin-4-yl)acetic acid ethyl ester (0.54 g, 3.1 mmol, 1.2 equiv.). The reaction was refluxed for 22 hr monitoring by TLC. The solution was filtered and the solid was washed with ethanol (10 mL). The filtrate was concentrated *in vacuo*, and then purified by flash column chromatography on silica gel (1:1 EtOAc/Hexanes) to give **44** (0.60 g, 88%) as clear liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.28 (apparent d, *J* = 4.4 Hz, 4H), 7.22 (m, 1H), 4.09 (q, *J* = 7.1 Hz, 2H), 3.46 (s, 2H), 2.83 (d, *J* = 11.7 Hz, 2H), 2.19 (d, *J* = 7.1 Hz, 2H), 1.95 (dt, *J* = 2.2 Hz, *J* = 11.8 Hz, 2H), 1.75 (m, 1H), 1.65 (d, *J* = 12.9 Hz, 2H), 1.30 (dt, *J* = 3.6 Hz, *J* = 12.3 Hz, 2H), 1.21 (t, *J* = 5.8 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 173.0, 138.7, 129.4, 128.3, 127.1, 63.6, 60.4, 53.7, 41.5, 33.2, 32.3, 14.5. HRMS (ESI): 262.1812 (M+1); calcd for $C_{16}H_{24}NO_2$: 262.1807. IR (neat): 1736 cm^{-1}.

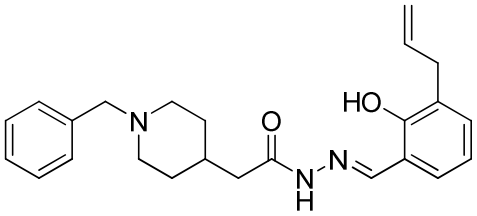
2-(1-Benzylpiperidin-4-yl)acetohydrazide (**24**)



To a stirred solution of **44** (0.58 g, 2.2 mmol, 1 equiv.) in ethanol (4.4 mL, 0.5 M) was added anhydrous hydrazine (0.21 mL, 6.6 mmol, 3 equiv.). The reaction was refluxed for 19 hr monitoring by TLC. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 and washed with water (10

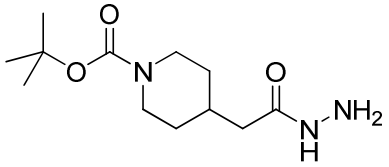
mL) and brine (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL) and EtOAc (10 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel (1:4 MeOH/EtOAc) to afford **24** (0.39 g, 72%) as milky oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29 (m, 3H), 7.24 (m, 2H), 7.15 (broad s, 1H), 3.82 (broad s, 2H), 3.47 (s, 2H), 2.86 (d, *J* = 11.7 Hz, 2H), 2.04 (d, *J* = 7.1 Hz, 2H), 1.96 (m, 2H), 1.83 (m, 1H), 1.65 (d, *J* = 12.8 Hz, 2H), 1.28 (ddd, *J* = 3.8 Hz, *J* = 12.1 Hz, *J* = 24.8 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.7, 136.9, 129.7, 128.1, 127.4, 63.0, 53.3, 40.6, 33.2, 31.3. HRMS (ESI): 248.1767 (M+1); calcd for $C_{22}H_{28}N_5O_2$: 248.1763. IR (neat): 1738 cm^{-1}.

***N'*-(3-allyl-2-hydroxybenzylidene)-2-(1-benzylpiperidin-4-yl)acetohydrazide (4a)**



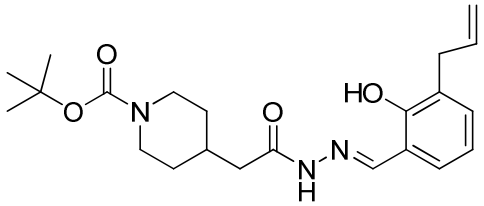
To a stirred solution of 3-allylsalicylaldehyde (0.093 g, 0.57 mmol, 1 equiv.) in ethanol (3.3 mL), **24** (0.25 g, 1.0 mmol, 1.8 equiv.) and HCl (10 mol%) were added. The reaction was refluxed for 16 hr monitoring by TLC. The solution was concentrated *in vacuo*, and then purified by flash column chromatography on silica gel (1:4 MeOH/EtOAc) to provide **4a** (0.16 g, 71%) as white solid. ^{1}H NMR (500 MHz, $CDCl_3$) indicated a 81:19 ratio of two conformers: δ 11.25 (s, 1H x 0.19 conformer B), 10.47 (s, 1H x 0.81 conformer A), 10.43 (s, 1H x 0.81 conformer A), 9.07 (s, 1H x 0.19 conformer B), 8.29 (s, 1H x 0.19 conformer B), 7.97 (s, 1H x conformer A), 7.31 (apparent d, *J* = 4.6 Hz, 4H), 7.24 (m, 1H), 7.20 (d, *J* = 7.5 Hz, 1H x 0.81 conformer A), 7.16 (d, *J* = 7.4 Hz, 1H x 0.19 conformer B), 7.12 (dd, *J* = 1.4 Hz, *J* = 7.7 Hz, 1H x 0.81 conformer A), 7.04 (dd, *J* = 1.2 Hz, *J* = 7.7 Hz, 1H x 0.19 conformer B), 6.89 (t, *J* = 7.6 Hz, 1H x 0.81 conformer A), 6.82 (t, *J* = 7.6 Hz, 1H x 0.19 conformer B), 6.02 (m, 1H), 5.11 (m, 2H x conformer A), 5.04 (m, 2H x conformer B), 3.49 (s, 2H), 3.46 (d, *J* = 6.5 Hz, 2H x 0.81 conformer A), 3.42 (d, *J* = 6.6 Hz, 2H x 0.19 conformer B), 2.89 (d, *J* = 11.6 Hz, 2H), 2.60 (d, *J* = 6.9 Hz, 2H x 0.81 conformer A), 2.19 (d, *J* = 7.1 Hz, 2H x 0.19 conformer B), 2.01 (t, *J* = 11.6 Hz, 2H), 1.94 (m, 1H), 1.78 (d, *J* = 12.5 Hz, 2H x 0.81 conformer A), 1.74 (d, *J* = 12.7 Hz, 2H x 0.19 conformer B), 1.43 (m, 2H x 0.81 conformer A), 1.31 (m, 2H x 0.19 conformer B). ^{13}C NMR (126 MHz, $CDCl_3$): δ 193.1, 174.7, 155.7, 147.9, 138.6, 136.6, 132.5, 129.6, 129.6, 129.5, 128.5, 128.4, 128.1, 127.2, 119.8, 117.1, 116.1, 63.6, 53.8, 39.6, 33.8, 32.9, 32.5. HRMS (ESI): 392.2338 (M+1); calcd for $C_{24}H_{30}N_3O_2$: 392.2338. IR (neat): 3204 (br), 1660, 1605 cm^{-1}. m.p.: 109.5-110.3. Purity: 97% (HPLC).

***Tert*-butyl 4-(2-hydrazinyl-2-oxoethyl)piperidine-1-carboxylate (25)**



To a stirred solution of **45** (0.31 g, 1.1 mmol, 1 equiv.) in ethanol (2.3 mL, 0.5 M) was added anhydrous hydrazine (0.11 mL, 3.4 mmol, 3 equiv.). The reaction was refluxed for 19 hr monitoring by TLC. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 and washed with water (10 mL) and brine (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL) and EtOAc (10 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel (1:4 MeOH/EtOAc) to afford **25** (0.18 g, 62%) as clear oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.05 (d, *J* = 12.2 Hz, 2H), 2.76 (s, 2H), 2.09 (d, *J* = 7.2 Hz, 1H), 1.95 (m, 2H), 1.68 (d, *J* = 13.3 Hz, 2H), 1.45 (s, 9H), 1.13 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 155.3, 79.8, 40.9, 40.5, 33.6, 31.7, 27.5, 17.2. HRMS (ESI): 258.1698 (M+1); calcd for $C_{11}H_{22}N_4O_3$: 258.1690. IR (neat): 3301, 1670 cm^{-1}.

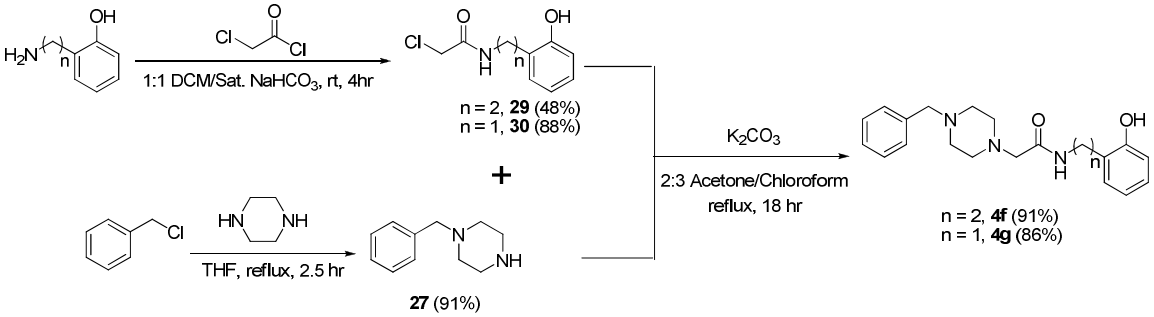
***Tert*-butyl 4-(2-(2-(3-allyl-2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperidine-1-carboxylate (4b)**



To a stirred solution of 3-allylsalicylaldehyde (0.056 g, 0.35 mmol, 1 equiv.) in ethanol (2.0 mL), **25** (0.16 g, 0.62 mmol, 1.8 equiv.) and HCl (10 mol%) were added. The reaction was refluxed for 16 hr monitoring by TLC. The solution was concentrated *in vacuo*, and then purified by flash column chromatography on silica gel (1:4 MeOH/EtOAc) to provide **4b** (0.14 g, 71%) as beige solid. ^{1}H NMR (500 MHz, $CDCl_3$) indicated a 76:24 ratio of two conformers: δ 11.43 (s, 1H x 0.24 conformer B), 10.80 (s, 1H x 0.76 conformer A), 10.49 (s, 1H x 0.76 conformer A), 10.10 (s, 1H x 0.24 conformer B), 8.29 (s, 1H x 0.24 conformer B), 8.04 (s, 1H x 0.76 conformer A), 7.19 (d, *J* = 7.4 Hz, 1H x 0.76 conformer A), 7.13 (m, 1H), 7.02 (d, *J* = 7.7 Hz, 1H x 0.24 conformer B), 6.88 (t, *J* = 7.6 Hz, 1H x 0.76 conformer A), 6.80 (t, *J* = 7.6 Hz, 1H x 0.24 conformer B), 6.01 (m, 1H), 5.08 (m, 2H x 0.76 conformer A), 5.02 (m, 2H x 0.24 conformer B), 4.10 (s, 2H), 3.45 (d, *J* = 6.5 Hz, 2H x 0.76 conformer A), 3.40 (d, *J* = 6.6 Hz, 2H x 0.24 conformer B), 2.74 (s, 2H), 2.60 (d, *J* = 6.9 Hz, 2H x 0.76 conformer A), 2.21 (d, *J* = 7.1 Hz, 2H x 0.24 conformer B), 2.09 (m, 1H), 1.79 (d, *J* = 12.2 Hz, 2H x 0.76

conformer A), 1.74 (d, J = 13.0 Hz, 2H x 0.24 conformer B), 1.45 (s, 9H x 0.76 conformer A), 1.44 (s, 9H x 0.24 conformer B), 1.27 (dq, J = 4.3 Hz, J = 12.6 Hz, 2H x 0.76 conformer A), 1.13 (dq, J = 4.0 Hz, J = 12.4 Hz, 2H x 0.24 conformer B). ^{13}C NMR (126 MHz, $CDCl_3$): δ 174.3, 168.1, 156.6, 155.7, 155.1, 150.6, 148.3, 136.7, 136.5, 132.6, 132.4, 129.7, 129.3, 128.4, 128.1, 119.8, 119.2, 117.2, 116.1, 115.9, 79.9, 79.7, 39.5, 33.8, 32.9, 32.3, 28.7. HRMS (ESI): 402.2409 (M+1); calcd for $C_{22}H_{32}N_3O_4$: 402.2393. IR (neat): 3216 (br), 1688, 1663, 1608 cm^{-1}. m.p.: 76-78 °C. Purity: 95% (HPLC).

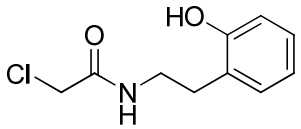
Scheme S4. Syntheses of **4f – g**



*General Procedure for haloacetamides **29** and **30***

To a stirred solution of 2-(2-aminoalkyl)phenol (1 equiv.) in 1:1 CH_2Cl_2/sat. Na_2CO_3 (40 mL in total) was added chloroacetyl chloride (1.1 – 2.0 equiv.) at room temperature. The reaction mixture was stirred for 4 hours. The organic layer was separated and the aqueous layer was extracted with DCM (3 x 10 mL). The combined organic solution was dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was purified with flash column chromatography on silica gel (1:4 EtOAc/Hexanes).

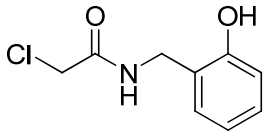
2-Chloro-*N*-(2-hydroxyphenethyl)acetamide (**29**)



The general procedure was followed: 2-(2-aminoethyl)phenol (0.05 g, 0.36 mmol, 1 equiv.), chloroacetyl chloride (0.03 mL, 0.38 mmol, 1.04 equiv.), 1:1 CH_2Cl_2/sat. Na_2CO_3 (6 mL in total). Purification with flash column chromatography on silica gel (1:4 EtOAc/Hexanes) afforded **29** (0.036 g, 46%) as light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.13 (dt, J = 1.4 Hz, J = 7.9 Hz, 2H), 7.09 (d, J = 7.7 Hz, 1H), 6.85 (m, 3H), 4.05 (s, 2H), 3.53 (dd, J = 6.9 Hz, J = 12.7 Hz, 2H), 2.89 (t, J = 7.1 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 167.3,

154.7, 130.9, 128.5, 124.8, 120.8, 116.0, 42.8, 41.0, 30.2. HRMS (ESI): 214.0629 (M+1); calcd for $C_{10}H_{13}NO_2Cl$: 214.0635. IR (neat): 3291 (br), 1650, 1608 cm^{-1}.

2-Chloro-*N*-(2-hydroxybenzyl)acetamide (30)

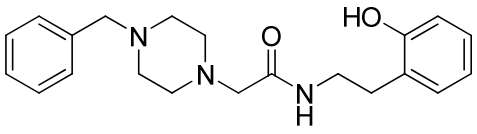


The general procedure was followed: 2-(2-aminomethyl)phenol (0.30 g, 2.4 mmol, 1 equiv.), chloroacetyl chloride (0.39 mL, 4.9 mmol, 2 equiv.), 1:1 CH_2Cl_2/sat. Na_2CO_3 (40 mL in total). Purification with flash column chromatography on silica gel (1:4 EtOAc/Hexanes) afforded **30** (0.43 g, 88%) as light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) indicated a 55:45 ratio of two conformers: δ 7.64 (broad s, 1H x 0.55 conformer A), 7.34 (m, 1H), 7.24 (m, 1H x 0.55 conformer A), 7.19 (m, 1H x 0.55 conformer A), 7.12 (dt, *J* = 1.5 Hz, *J* = 8.3 Hz, 1H), 7.08 (broad s, 1H x 0.45 conformer B), 6.91 (dd, *J* = 1.0 Hz, *J* = 8.1 Hz, 1H x 0.45 conformer B), 6.83 (dt, *J* = 1.1 Hz, *J* = 7.4 Hz, 1H), 4.43 (d, *J* = 6.0 Hz, 1H x 0.45 conformer B), 4.41 (d, *J* = 6.4 Hz, 1H x 0.55 conformer), 4.34 (s, 1H), 4.01 (overlap of two singlets of conformers A and B, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 168.3, 166.9, 166.5, 155.6, 148.9, 131.0, 130.7, 130.2, 129.8, 127.3, 123.7, 122.6, 120.4, 117.4, 42.6, 41.1, 40.7, 39.3. HRMS (ESI): 200.0473 (M+1); calcd for $C_9H_{11}NO_2Cl$: 200.0478

*General Procedure for **4f – g***

To a stirred mixture of **27**[1] (1.3 equiv.) and K_2CO_3 (2 equiv.) in 2:3 acetone/chloroform (25 mL in total) was added haloacetamide (1 equiv.). The reaction was refluxed for 18 hr monitoring by TLC. The solution was filtered and the solid was washed with chloroform (10 mL). The filtrate was concentrated *in vacuo*, and then purified by flash column chromatography on silica gel (1:4 MeOH/EtOAc)

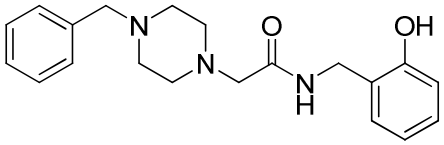
2-(4-Benzylpiperazin-1-yl)-*N*-(2-hydroxyphenethyl)acetamide (4f)



The general procedure was followed: **27** (0.15 g, 0.84 mmol, 1.3 equiv.), **29** (0.14 g, 0.64 mmol, 1 equiv.), K_2CO_3 (0.18 g, 1.3 mmol, 2 equiv.) in 2:3 acetone/chloroform (5 mL in total). Purification with flash column chromatography on silica gel (1:4 EtOAc/Hexanes) afforded **4f** (0.21 g, 91%) as white semi-solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.69 (s, 1H), 7.50 (t, *J* = 5.6 Hz, 1H), 7.30 (m, 5H), 7.08 (m, 2H), 6.83 (d, *J* = 7.9 Hz,

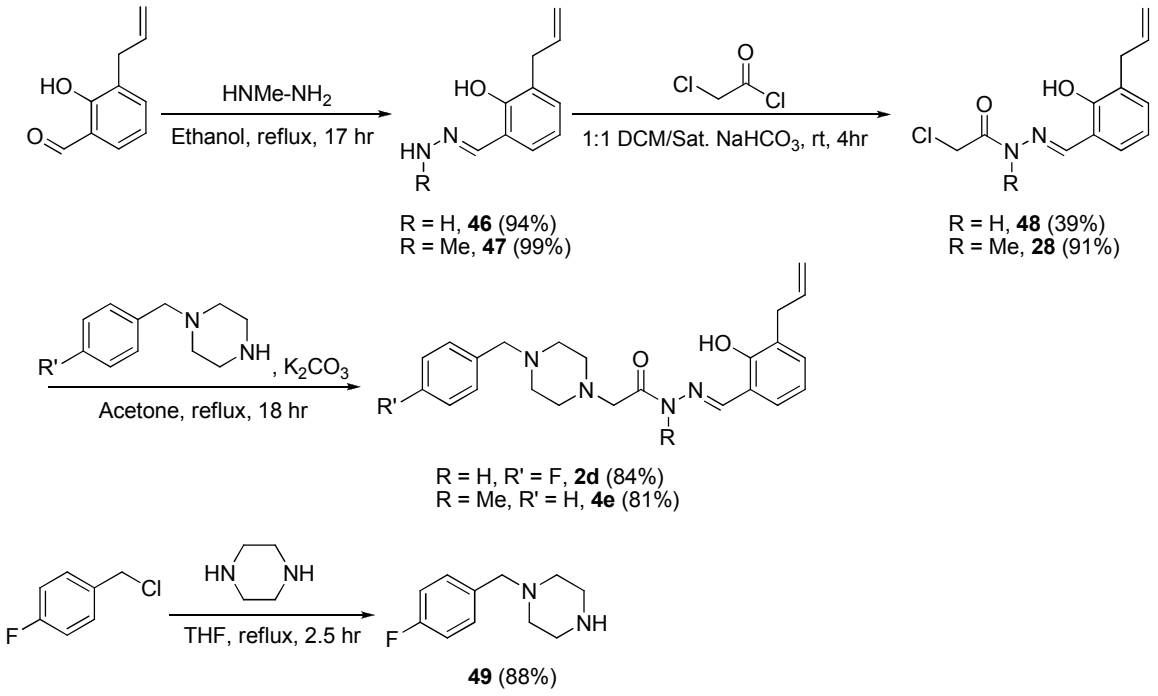
1H), 6.78 (t, *J* = 7.4 Hz, 1H), 3.57 (td, *J* = 6.7 Hz, *J* = 13.0 Hz, 2H), 3.52 (s, 2H), 3.00 (s, 2H), 2.88 (t, *J* = 6.9 Hz, 2H), 2.46 (d, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.6, 155.5, 137.8, 130.8, 129.5, 128.5, 128.2, 127.5, 125.2, 119.9, 116.1, 63.1, 61.4, 53.5, 53.1, 39.4, 30.7. HRMS (ESI): 354.2173 (M+1); calcd for $C_{21}H_{28}N_3O_2$: 354.2182. IR (neat): 3585, 3319 (br), 1650, 1607 cm^{-1}. Purity: 95% (LC-MS).

2-(4-Benzylpiperazin-1-yl)-*N*-(2-hydroxybenzyl)acetamide (**4g**)

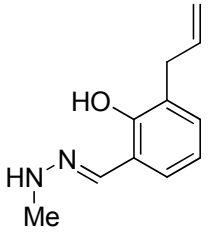


The general procedure was followed: **27** (0.48 g, 2.7 mmol, 1.3 equiv.), **30** (0.43 g, 2.1 mmol, 1 equiv.), K_2CO_3 (0.58 g, 4.2 mmol, 2 equiv.) in 2:3 acetone/chloroform (25 mL in total). Purification with flash column chromatography on silica gel (1:4 EtOAc/Hexanes) afforded **4g** (0.62 g, 86%) as white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.41 (s, 1H), 7.91 (s, 1H), 7.25 (m, 5H), 7.17 (t, *J* = 7.6 Hz, 1H), 7.09 (d, *J* = 7.3 Hz, 1H), 6.89 (d, *J* = 8.0 Hz, 1H), 6.79 (t, *J* = 7.3 Hz, 1H), 4.33 (d, *J* = 6.7 Hz, 2H), 3.47 (s, 2H), 3.00 (s, 2H), 2.45 (broad d, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 173.2, 156.2, 138.0, 130.9, 130.2, 129.4, 128.5, 127.4, 124.5, 120.0, 118.2, 63.1, 61.0, 53.8, 53.2, 40.2. HRMS (ESI): 340.2020 (M+1); calcd for $C_{20}H_{26}N_3O_2$: 340.2025. IR (neat): 3584, 3368 (br), 1653, 1600 cm^{-1}. m.p.: 119-119.8 °C. Purity: 97% (HPLC).

Scheme S5. Syntheses of **2d** and **4e**



2-Allyl-6-((2-methylhydrazono)methyl)phenol (**47**)

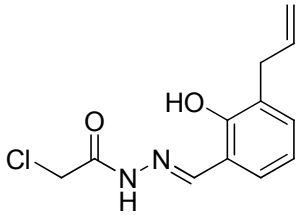


To a stirred solution of 3-allylsalicylaldehyde (0.50g, 3.1 mmol, 1 equiv.) in EtOH (47 mL) was added methylhydrazine (0.32 mL, 6.1 mmol, 2 equiv.) at room temperature. The reaction mixture was refluxed for 17 hr. The solvent was evaporated. The residue was purified by flash column chromatography on silica gel (1:9 EtOAc/Hexanes) to give **47** (0.58g, 99%) as yellow liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.65 (broad s, 1H), 7.66 (s, 1H), 7.10 (d, *J* = 7.4 Hz, 1H), 7.03 (d, *J* = 7.6Hz, 1H), 6.85 (t, *J* = 7.5 Hz, 1H), 6.09 (tdd, *J* = 6.5 Hz, *J* = 10.0 Hz, *J* = 16.8 Hz, 1H), 5.30 (broad s, 1H), 5.12 (m, 2H), 3.49 (d, *J* = 6.5 Hz, 2H), 2.95 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 155.3, 140.2, 137.2, 129.9, 127.6, 127.5, 119.1, 119.1, 115.6, 35.0, 34.2. HRMS (ESI): 191.1177 (M+1); calcd for $C_{11}H_{15}N_2O$: 191.1184. IR (neat): 3401, 1639, 1617 cm^{-1}.

*General Procedure for **48** and **28***

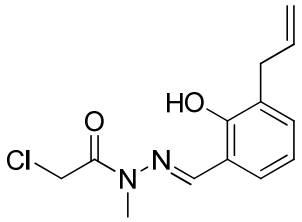
To a stirred solution of **48** or **28** (1 equiv.) in 1:1 CH_2Cl_2/sat. Na_2CO_3 (40 mL in total) was added chloroacetyl chloride (1.1 – 2.0 equiv.) at room temperature. The reaction mixture was stirred for 4 hours. The organic layer was separated and the aqueous layer was extracted with DCM (3 x 10 mL). The combined organic solution was dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was purified with flash column chromatography on silica gel (1:4 EtOAc/Hexanes).

***N'*-(3-Allyl-2-hydroxybenzylidene)-2-chloroacetohydrazide (48)**



The general procedure was followed: **46** (0.34 g, 1.9 mmol, 1 equiv.), chloroacetyl chloride (0.17 mL, 2.1 mmol, 1.1 equiv.), 1:1 CH_2Cl_2/sat. Na_2CO_3 (31 mL in total). Purification with flash column chromatography on silica gel (1:4 EtOAc/Hexanes) afforded **48** (0.19 g, 39%) as light yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) indicated a 55:45 ratio of two conformers: δ 11.05 (s, 1H x 0.45 conformer B), 10.04 (s, 1H x 0.55 conformer A), 9.42 (s, 1H x 0.55 conformer A), 8.37 (s, 2H x 0.45 conformer B), 8.05 (s, 1H x 0.45 conformer B), 7.13 (m, 2H x 0.55 conformer A), 6.89 (m, 1H), 6.04 (m, 1H), 5.09 (m, 2H x 0.55 conformer A), 4.47 (s, 2H x 0.45 conformer B), 4.23 (s, 2H x 0.55 conformer A), 3.47 (d, J = 6.4 Hz, 2H), 2.67 (m, 2H x 0.45 conformer B). HRMS (ESI): 253.0748 (M+1); calcd for $C_{11}H_{15}N_2O$: 253.0744.

***N'*-(3-Allyl-2-hydroxybenzylidene)-2-chloro-*N*-methylacetohydrazide (28)**



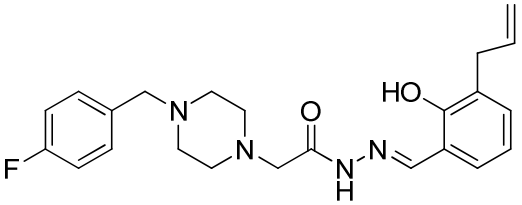
The general procedure was followed: **47** (0.30 g, 2.4 mmol, 1 equiv.), chloroacetyl chloride (0.39 mL, 4.9 mmol, 2 equiv.), 1:1 CH_2Cl_2/sat. Na_2CO_3 (40 mL in total). Purification with flash column chromatography on silica gel (1:4 EtOAc/Hexanes) afforded **28** (0.43 g, 88%) as light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) indicated a 82:18 ratio of two conformers: δ 10.20 (s, 1H x 0.82 conformer A), 7.91 (s, 1H x 0.82 conformer B), 7.84 (dd, J = 1.9 Hz, J = 7.4 Hz, 1H x 0.18 conformer B), 7.74 (s, 1H x 0.18 conformer B), 7.31 (m,

overlap of 1H x 0.18 conformer B and 1H of 0.18 conformer B), 7.23 (d, *J* = 7.4 Hz, 1H x 0.82 conformer A), 7.18 (dd, *J* = 1.3 Hz, *J* = 7.7 Hz, 1H x 0.82 conformer A), 6.93 (t, *J* = 7.6 Hz, 1H x 0.82 conformer A), 6.04 (m, 1H x 0.82 conformer A), 5.89 (m, 1H x 0.18 conformer B), 5.12 (m, 2H), 4.71 (s, 1H x 0.18 conformer B), 4.54 (s, 2H x 0.82 conformer A), 4.31 (s, 2H x 0.18 conformer B), 3.47 (overlap of two peaks of conformer A, a doublet of 2H and a singlet of 3H), 3.37 (s, 3H x 0.18 conformer A), 3.32 (d, *J* = 6.5 Hz, 2H x 0.18 conformer B). ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.4, 155.3, 145.1, 136.4, 135.4, 134.5, 133.0, 132.7, 132.5, 130.1, 128.3, 127.4, 125.1, 120.0, 117.3, 117.2, 116.2, 43.1, 42.5, 40.5, 34.9, 33.8, 28.6. HRMS (ESI): 267.0896 (M+1); calcd for $C_{13}H_{16}N_2O_2Cl$: 267.0900. IR (neat): 3330, 2107, 1645 cm^{-1}.

*General Procedure for **2d** and **4e***

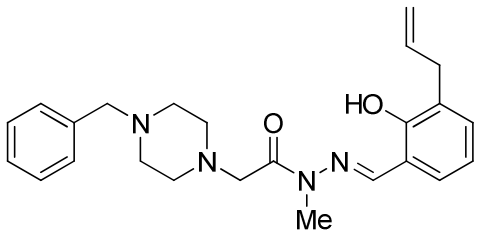
To a stirred mixture of **27** or **49** (1.3 equiv.) and K_2CO_3 (2 equiv.) in acetone (25 mL in total) was added **28** or **48** (1 equiv.). The reaction was refluxed for 18 hr monitoring by TLC. The solution was filtered and the solid was washed with chloroform (10 mL). The filtrate was concentrated *in vacuo*, and then purified by flash column chromatography on silica gel (1:4 MeOH/EtOAc).

_N'_-(3-allyl-2-hydroxybenzylidene)-2-(4-(4-fluorobenzyl)piperazin-1-yl)acetohydrazide (**2d**)



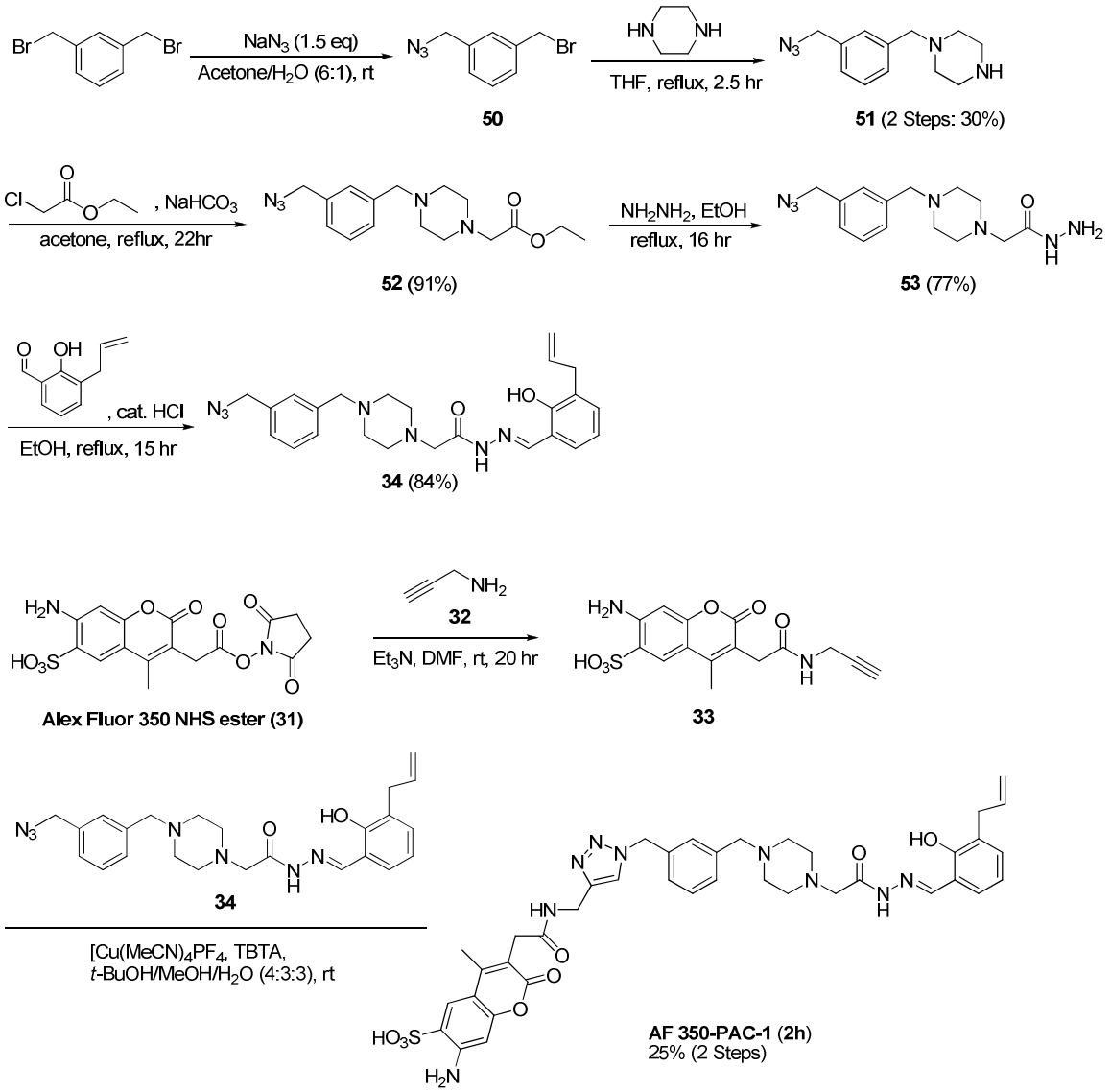
The general procedure was followed: **49** (0.61 g, 3.2 mmol, 1.5 equiv.), **48** (0.43 g, 2.1 mmol, 1 equiv.), K_2CO_3 (0.87 g, 6.3 mmol, 3 equiv.) in acetone (25 mL in total). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **2d** (0.62 g, 86%) as white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.26 (s, 1H), 10.10 (s, 1H), 8.46 (s, 1H), 7.31 (m, 2H), 7.20 (d, *J* = 7.3 Hz, 1H), 7.10 (d, *J* = 7.6 Hz, 1H), 7.03 (t, *J* = 8.6 Hz, 2H), 6.86 (t, *J* = 7.6 Hz, 1H), 6.04 (tdd, *J* = 6.6 Hz, *J* = 10.1 Hz, *J* = 16.7 Hz, 1H), 5.08 (m, 2H), 3.56 (s, 2H), 3.46 (d, *J* = 6.5 Hz, 2H), 3.22 (s, 2H), 2.62 (d, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 216.9, 165.8, 156.7, 151.8, 136.7, 132.6, 130.9, 129.4, 128.5, 119.3, 117.1, 115.9, 115.5, 115.3, 62.1, 61.1, 53.7, 53.0, 34.1. ^{19}F NMR (400 MHz, $CDCl_3$): δ 115.8. HRMS (ESI): 411.2196 (M+1); calcd for $C_{23}H_{28}N_4O_2F$: 411.2196. IR (neat): 3210, 1686, 1607 cm^{-1}. m.p.: 113.8-114.5 °C. Purity: 96% (HPLC).

***N′*-(3-allyl-2-hydroxybenzylidene)-2-(4-benzylpiperazin-1-yl)-*N*-methylacetohydrazide (4e)**

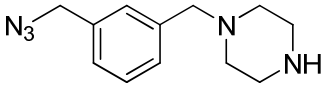


The general procedure was followed: **27** (0.15 g, 0.76 mmol, 1.5 equiv.), **28** (0.14 g, 0.51 mmol, 1 equiv.), K_2CO_3 (0.21 g, 1.5 mmol, 3 equiv.) in acetone (2.4 mL in total). Purification with flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **4e** (0.17 g, 81%) as white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.74 (s, 1H), 7.84 (s, 1H), 7.32 (m, 4H), 7.25 (m, 1H), 7.20 (dd, *J* = 0.9 Hz, *J* = 7.4 Hz, 1H), 7.15 (dd, *J* = 1.5 Hz, *J* = 7.7 Hz, 1H), 6.89 (t, *J* = 7.6 Hz, 1H), 6.06 (tdd, *J* = 6.7 Hz, *J* = 10.0 Hz, *J* = 16.8 Hz, 1H), 5.12 (m, 1H), 3.59 (s, 2H), 3.53 (s, 2H), 3.47 (d, *J* = 6.7 Hz, 2H), 3.41 (s, 2H), 2.63 (broad d, 8H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.8, 155.3, 142.9, 138.4, 136.8, 131.9, 129.6, 129.4, 128.4, 128.3, 127.2, 119.5, 117.8, 115.9, 63.2, 61.0, 53.6, 52.9, 34.2, 27.9. HRMS (ESI): 407.2441 (M+1); calcd for $C_{24}H_{31}N_4O_2$: 407.2447. IR (neat): 3062 (br), 1681, 1612 cm^{-1}. m.p.: 120-121 °C. Purity: 96% (HPLC).

Scheme S6. Synthesis of **AF350-PAC-1** (**2h**).



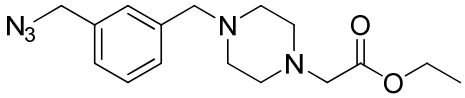
1-(3-(azidomethyl)benzyl)piperazine (**51**)



To a stirred solution of α,α'-dibromo-m-xylene (5.0 g, 18.9 mmol, 1 equiv.) in 6:1 acetone/H_2O (35 mL in total) was added sodium azide (1.8 g, 28.4 mmol, 1.5 equiv.). The reaction mixture was stirred for 10 hr at room temperature. The mixture was diluted with DCM (30 mL), washed with H_2O (20 mL) and brine (20 mL), dried over Na_2SO_4, filtered, concentrated *in vacuo*. After column chromatography on silica gel (9:1 Hexanes/ EtOAc), a mixture of **50** and minor diazido-m-xylene was obtained as light yellow liquid.

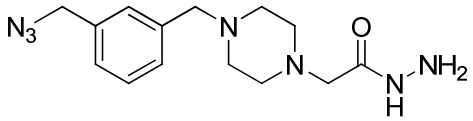
Anhydrous piperazine (4.0 g, 46.5 mmol, 6 equiv.) was added to THF (17 mL), and the mixture was heated to reflux until the piperazine was fully dissolved. To the solution the mixture of **50** and minor diazido-m-xylene (1.75g, 7.7 mmol, 1 equiv.) was added dropwise. White precipitate was formed immediately. The reaction mixture was refluxed for 3 hr. The stirring mixture was cooled and then filtered. The solids were washed with THF (15 mL) and then EtOAc (15 mL). The combined organic layer was concentrated *in vacuo*, which was then washed with basic water with 5% brine and KOH (pH >12). The aqueous layer was extracted with CH_2Cl_2 (3 x 15 mL) and EtOAc (15 mL) at pH > 12. The organic layers were combined, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel (1:4 MeOH/EtOAc) to afford **51** (0.66 g, 30% over 2 steps) as light yellow liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.29 (m, 3H), 7.18 (d, *J* = 7.2 Hz, 1H), 4.30 (s, 2H), 3.47 (s, 2H), 2.85 (t, *J* = 4.9 Hz, 4H), 2.38 (s, 4H), 1.75 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 139.2, 135.5, 129.4, 129.2, 128.9, 127.2, 63.7, 54.9, 54.8, 46.3. HRMS (ESI): 232.1552 (M+1); calcd for $C_{12}H_{18}N_5$: 232.1562

Ethyl 2-(4-(3-(azidomethyl)benzyl)piperazin-1-yl)acetate (**52**)



To a stirred mixture of **51** (0.6 g, 2.6 mmol, 1 equiv.) and $NaHCO_3$ (0.27 g, 3.3 mmol, 1.25 equiv.) in acetone (5 mL) was added ethyl chloroacetate (0.31 mL, 2.9 mmol, 1 equiv.). The reaction was refluxed for 22 hr monitoring by TLC. The solution was filtered and the solid was washed with acetone (5 mL). The filtrate was concentrated *in vacuo*, and then purified by flash column chromatography on silica gel (1:1 EtOAc/Hexanes) to give **52** (0.75 g, 91%) as yellow liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.28 (m, 3H), 7.17 (d, *J* = 7.3 Hz, 1H), 4.29 (s, 2H), 4.15 (q, *J* = 7.1 Hz, 2H), 3.50 (s, 2H), 3.17 (s, 2H), 2.54 (broad d, 8H), 1.23 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.5, 139.1, 135.5, 129.3, 129.1, 128.9, 127.2, 62.9, 60.8, 59.7, 54.9, 53.2, 53.0, 14.5. HRMS (ESI): 318.1923 (M+1); calcd for $C_{16}H_{24}N_5O_2$: 318.1930. IR (neat): 2098, 1739 cm^{-1}.

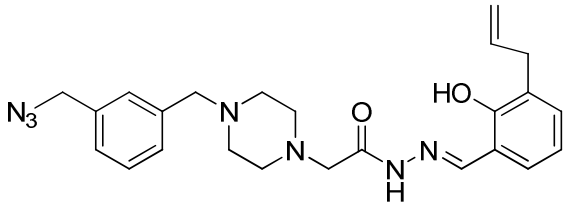
***N'*-(3-allyl-2-hydroxybenzylidene)-2-(4-(3-(azidomethyl)benzyl)piperazin-1-yl)acetohydrazide** (**53**)



To a stirred solution of **52** (0.7 g, 2.2 mmol, 1 equiv.) in ethanol (4.4 mL, 0.5 M) was added anhydrous hydrazine (0.21 mL, 6.6 mmol, 3 equiv.). The reaction was refluxed for 16 hr monitoring by TLC. The

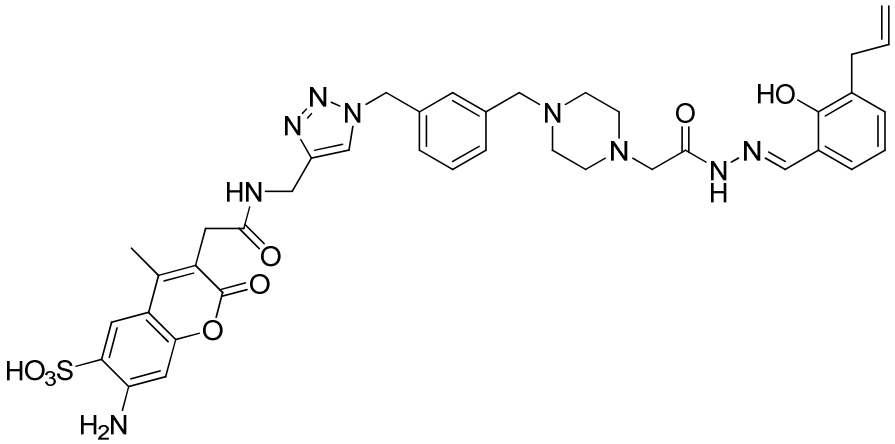
reaction mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (10 mL) and washed with water (10 mL) and brine (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL) and EtOAc (10 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel (1:4 MeOH/EtOAc) to yield **53** (0.52 g, 77%) as yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.15 (s, 1H), 7.31 (t, *J* = 7.8 Hz, 1H), 7.26 (d, *J* = 6.1 Hz, 2H), 7.19 (d, *J* = 7.4 Hz, 1H), 4.31 (s, 2H), 3.85 (s, 2H), 3.50 (s, 2H), 3.05 (s, 2H), 2.48 (d, *J* = 7.4 Hz, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.7, 138.9, 135.6, 129.3, 129.1, 129.0, 127.3, 62.8, 60.8, 54.9, 53.8, 53.3. HRMS (ESI): 304.1877 (M+1); calcd for $C_{14}H_{22}N_7O$: 304.1886

***N'*-(3-allyl-2-hydroxybenzylidene)-2-(4-(3-(azidomethyl)benzyl)piperazin-1-yl)acetohydrazide (34)**



To a stirred solution of 3-allylsalicylaldehyde (0.16 g, 0.99 mmol, 1 equiv.) in ethanol (6 mL), **53** (0.51 g1.7 mmol, 1.7 equiv.) and HCl (10 mol%) were added. The reaction was refluxed for 15 hr. The solution was concentrated *in vacuo*, and then purified by flash column chromatography on silica gel (1:4 MeOH/EtOAc) to afford **34** (0.37 g, 84%) as yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 11.28 (s, 1H), 10.04 (s, 1H), 8.38 (s, 1H), 7.35 (t, *J* = 7.7 Hz, 1H), 7.29 (d, *J* = 7.5 Hz, 2H), 7.22 (d, *J* = 7.4 Hz, 1H), 7.18 (dd, *J* = 1.4 Hz, *J* = 7.4 Hz, 1H), 7.08 (dd, *J* = 1.5 Hz, *J* = 7.7 Hz, 1H), 6.84 (t, *J* = 7.6 Hz, 1H), 6.03 (tdd, *J* = 6.6 Hz, *J* = 10.1 Hz, *J* = 16.7 Hz, 1H), 5.07 (m, 2H), 4.34 (s, 2H), 3.56 (s, 2H), 3.45 (d, *J* = 6.6 Hz, 2H), 3.19 (s, 2H), 2.58 (broad d, 8H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 166.1, 156.6, 151.5, 138.8, 136.8, 135.6, 132.5, 129.4, 129.3, 129.1, 128.4, 127.4, 119.7, 119.3, 117.1, 115.9, 62.8, 61.2, 54.9, 53.9, 53.2, 34.1. HRMS (ESI): 448.2460 (M+1); calcd for $C_{24}H_{30}N_7O_2$: 448.2461. IR (neat): 3221 (br), 2099, 1686, 1608 cm^{-1}.

3-(2-((1-(3-((4-(2-(2-(3-allyl-2-hydroxybenzylidene)hydrazinyl)-2-oxoethyl)piperazin-1-yl)methyl)benzyl)-1H-1,2,3-triazol-4-yl)methylamino)-2-oxoethyl)-7-amino-4-methyl-2-oxo-2H-chromene-6-sulfonic acid (**AF350-PAC-1, 2h**)



To a solution of propargyl amine·HCl (2.0 mg, 0.022 mmol, 1.8 equiv.) and triethylamine (5.1 μL, 0.037 mmol, 3 equiv.) in DMF (150 μL) was added Alexa Fluor 350 NHS-ester **31** (5.0 mg, 0.012 mmol, 1 equiv.) in DMF (150 μL) at minimal amount of light. The reaction was stirred for 20 hr at room temperature. The crude product was purified by flash column chromatography on silica gel (10% MeOH in EtOAc to 100% MeOH) to give **33** with a small amount of silica gel as light yellow-green solid.

To a solution of $[Cu(CH_3CN)_4]PF_6$ (5.0 mg, 0.013 mmol, 1.1 equiv.), *tris*-(benzyltriazolylmethyl)amine (TBTA, 7.1 mg, 0.013 mmol, 1.1 equiv.) and **33** (4.3 mg, 0.012 mmol, 1 equiv.) with a small amount of silica gel in 4:3:3 t-BuOH/MeOH/H2O (1 mL in total) was added **34** (37 mg, 0.082 mmol, 6.7 equiv.) at minimal amount of light. The reaction mixture was stirred for 24 hr at room temperature. Twice purification by flash column chromatography on silica gel (1:4 MeOH/EtOAc) afforded **AF350-PAC-1** (**2h**) (2.4 mg, 25% over 2 steps) as light green solid. ^{1}H NMR (500 MHz, CD_3OD): δ 8.33 (s, 1H), 8.03 (s, 1H), 7.78 (s, 1H), 7.35 (m, 3H), 7.27 (m, 2H), 7.16 (m, 2H), 6.83 (m, 1H), 6.57 (s, 1H), 6.01 (m, 1H), 5.56 (s, 2H), 5.01 (m, 2H), 4.41 (s, 1H), 4.36 (s, 1H), 3.57 (m, 4H), 3.41 (m, 2H), 3.20 (m, 2H), 2.58 (broad d, 8H), 2.26 (s, 2H). HRMS (ESI): 798.3032 (M+1); calcd for $C_{39}H_{44}N_9O_8S$: 789.3034

Purity of **PAC-1 (1)**: 100%

Purity of **1a**: 95%

Purity of **1h**: 99%

References

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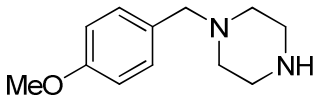
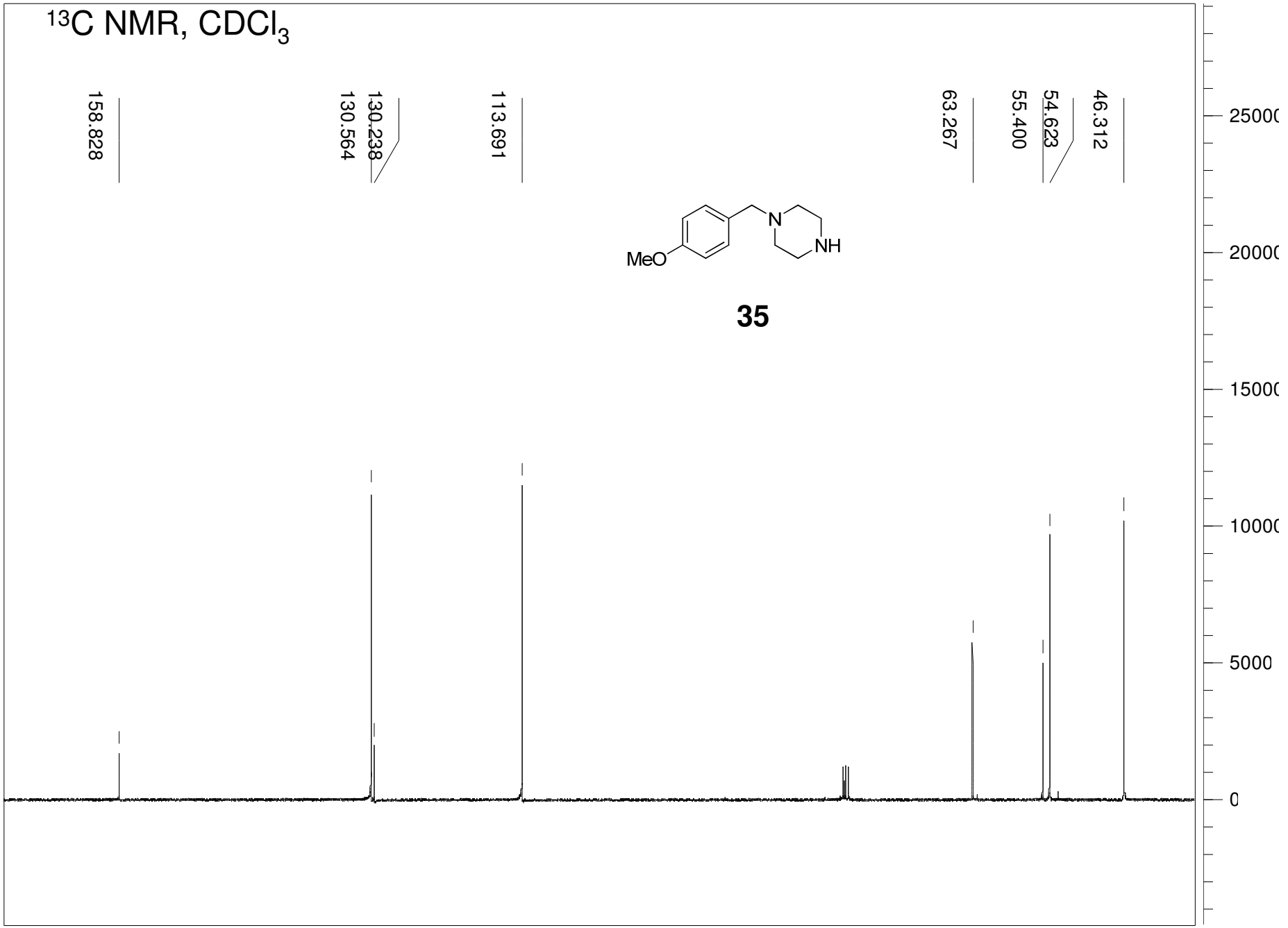
1H NMR, $CDCl_3$

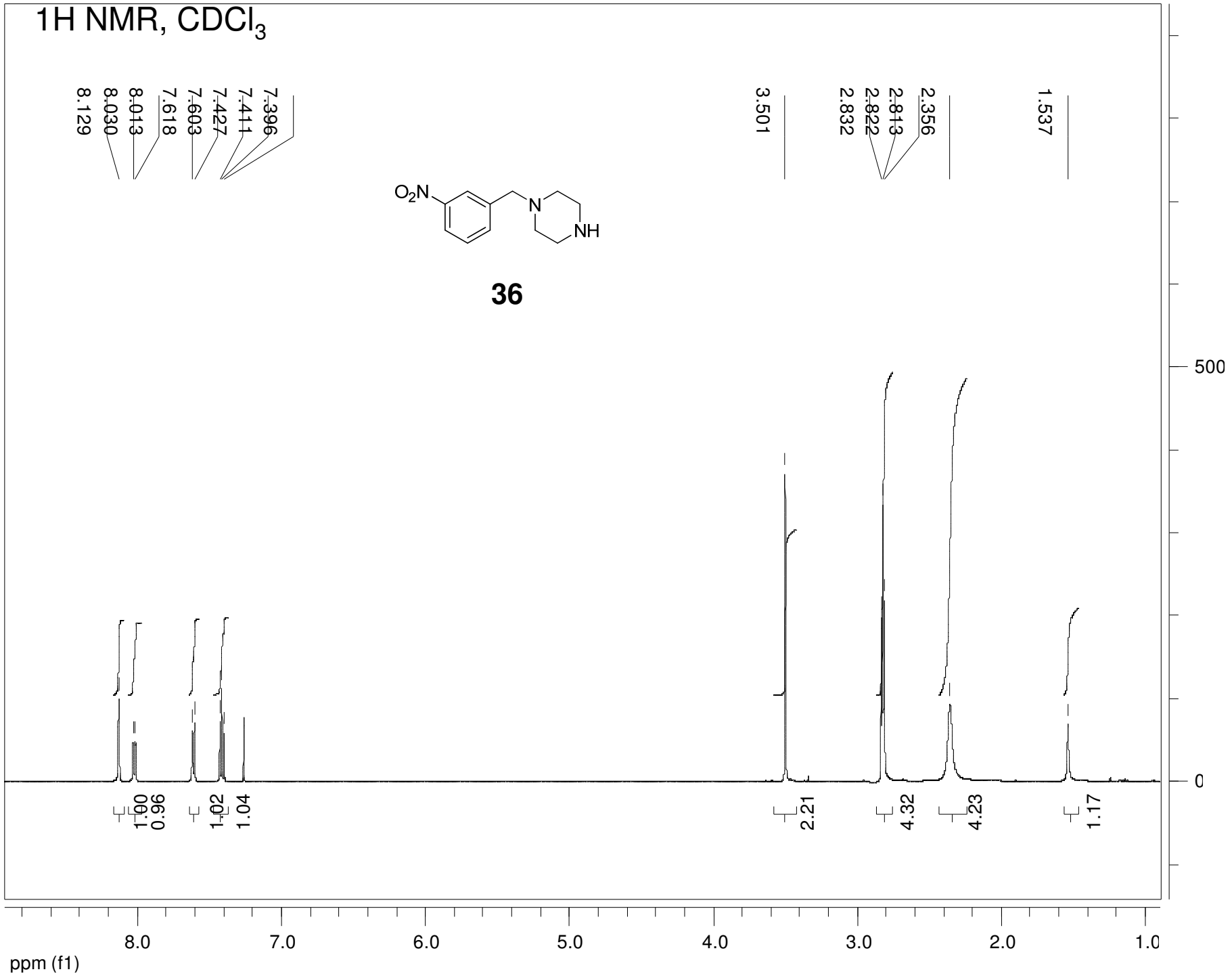


35

^{13}C NMR, $CDCl_3$



35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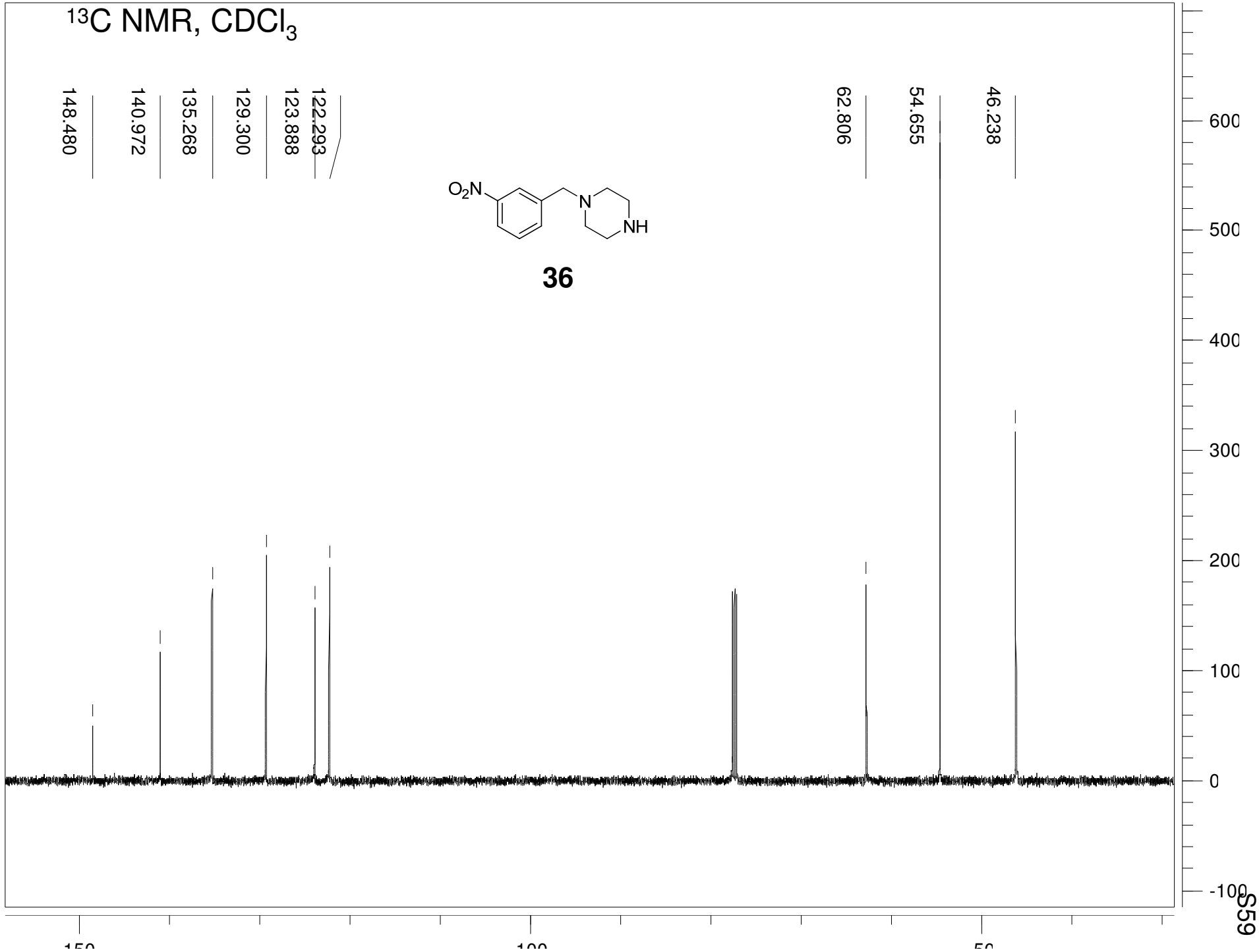
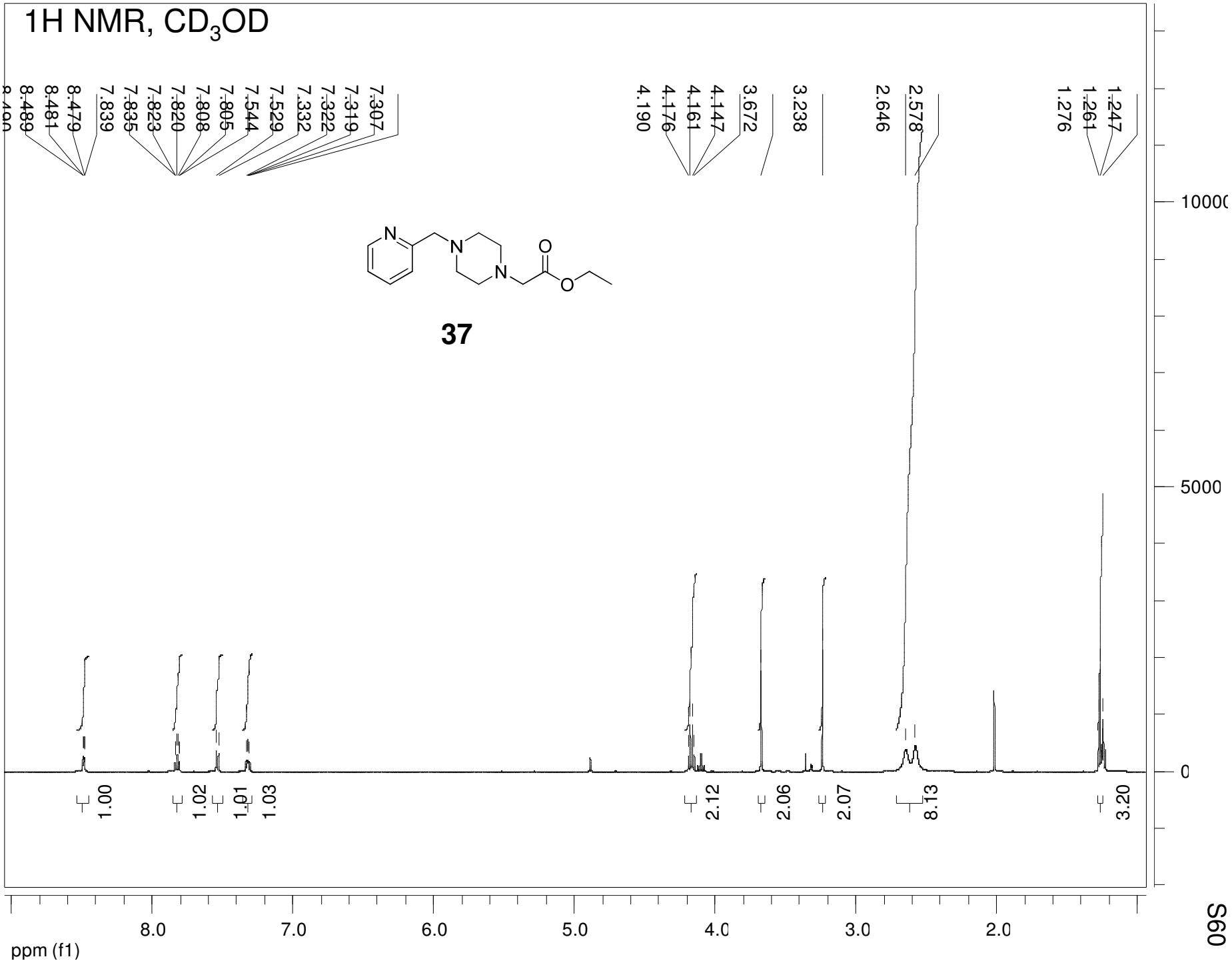
8.013
7.618
7.603
7.427
7.411
7.396
3.501
2.832
2.822
2.813
2.356
1.537
O2N
N
NH
36
500
0
1.00
0.96
1.02
1.04
2.21
4.32
4.23
1.17
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)

^{13}C NMR, $CDCl_3$



36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7.808
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7.332
7.322
7.319
7.307
4.190
4.176
4.161
4.147
3.672
3.238
2.646
2.578
1.276
1.261
1.247
N
N
N
O
O
37
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5000
0
1.00
1.02
1.01
1.03
2.12
2.06
2.07
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3.20
8.0
7.0
6.0
5.0
4.0
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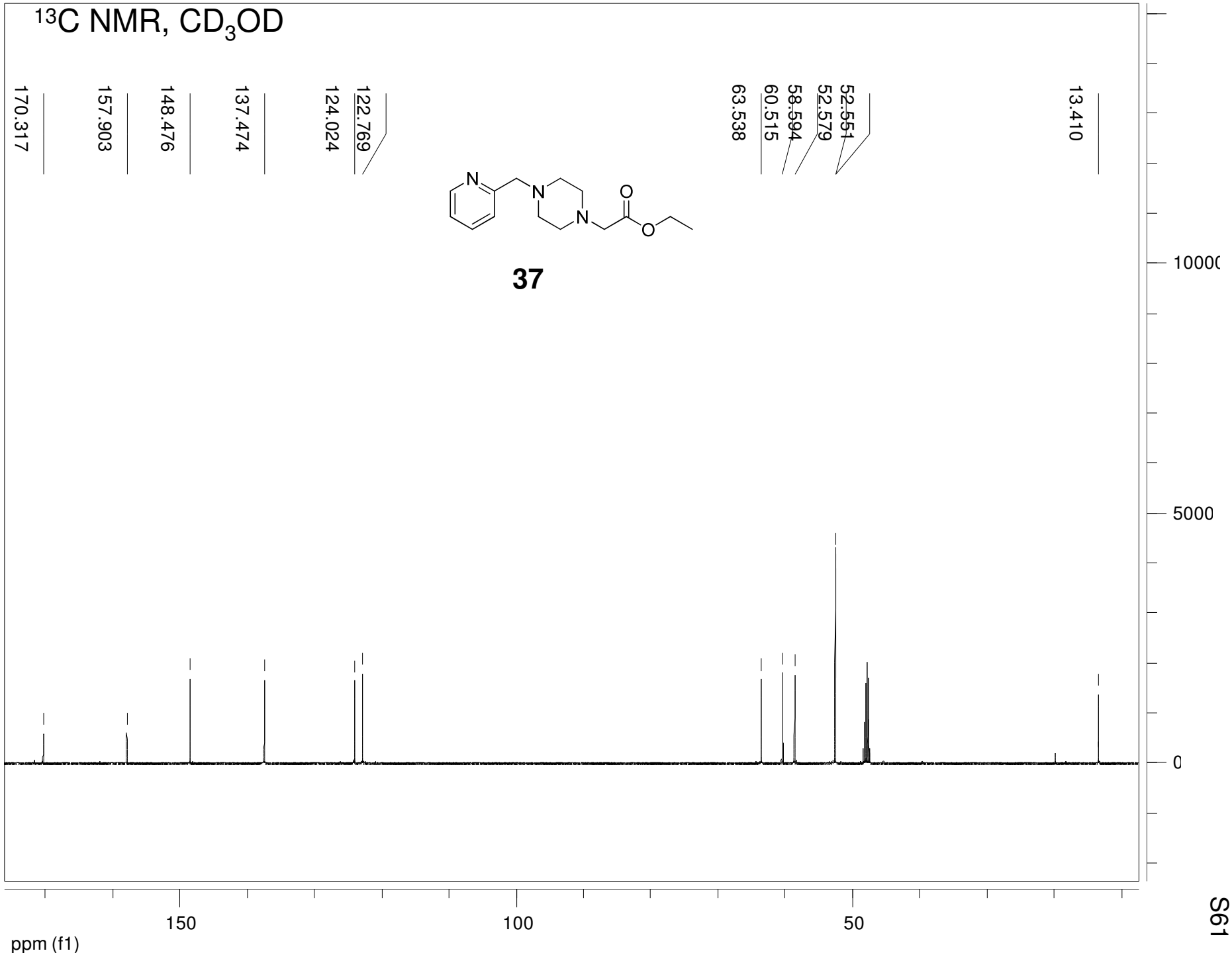
2.0
ppm (f1)


^{13}C NMR, CD_3OD
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157.903
148.476
137.474
124.024
122.769
63.538
60.515
58.594
52.579
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13.410
N
N
N
O
O
37
10000
5000
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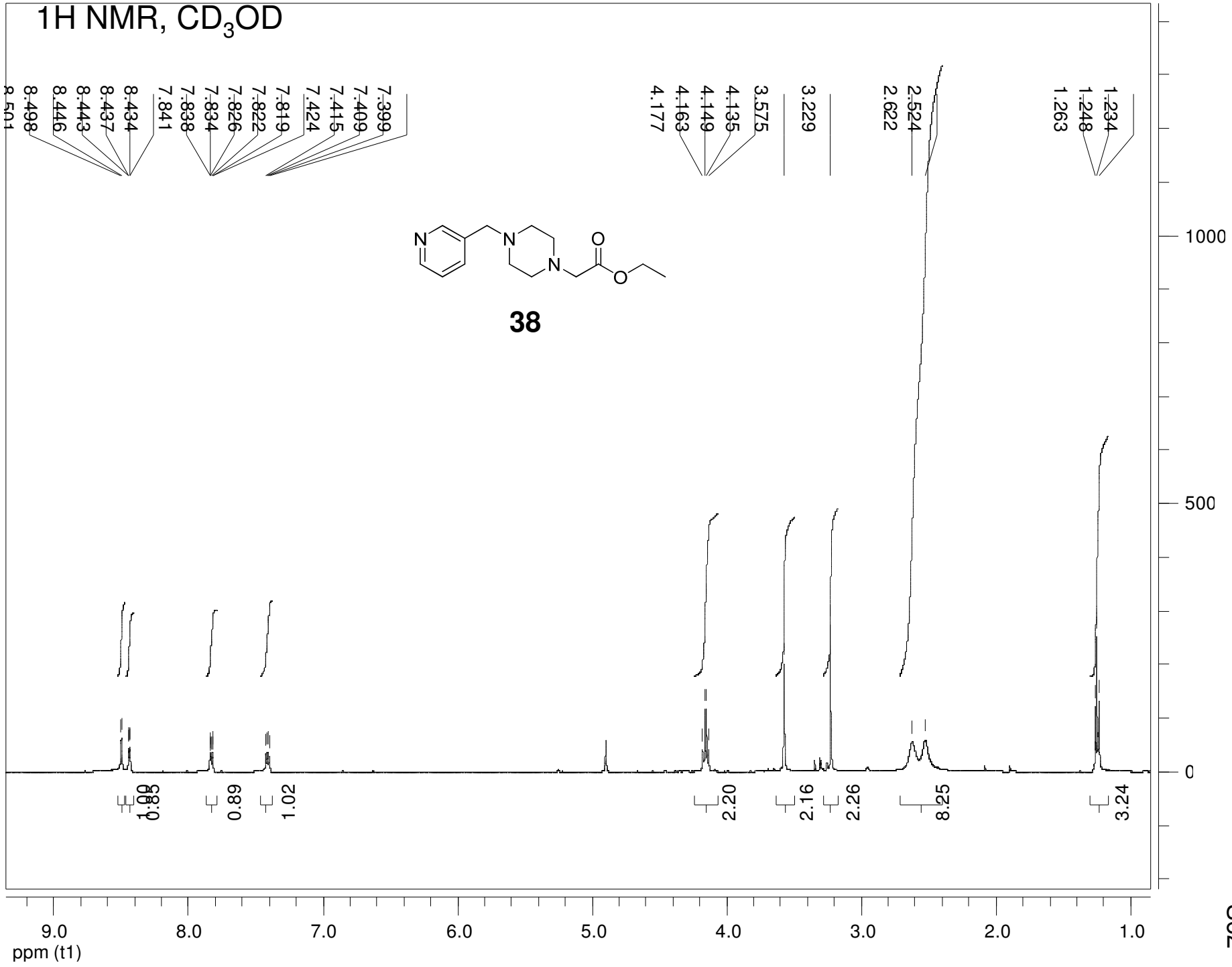
150
100
50
ppm (f1)


1H NMR, CD3OD
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8.443
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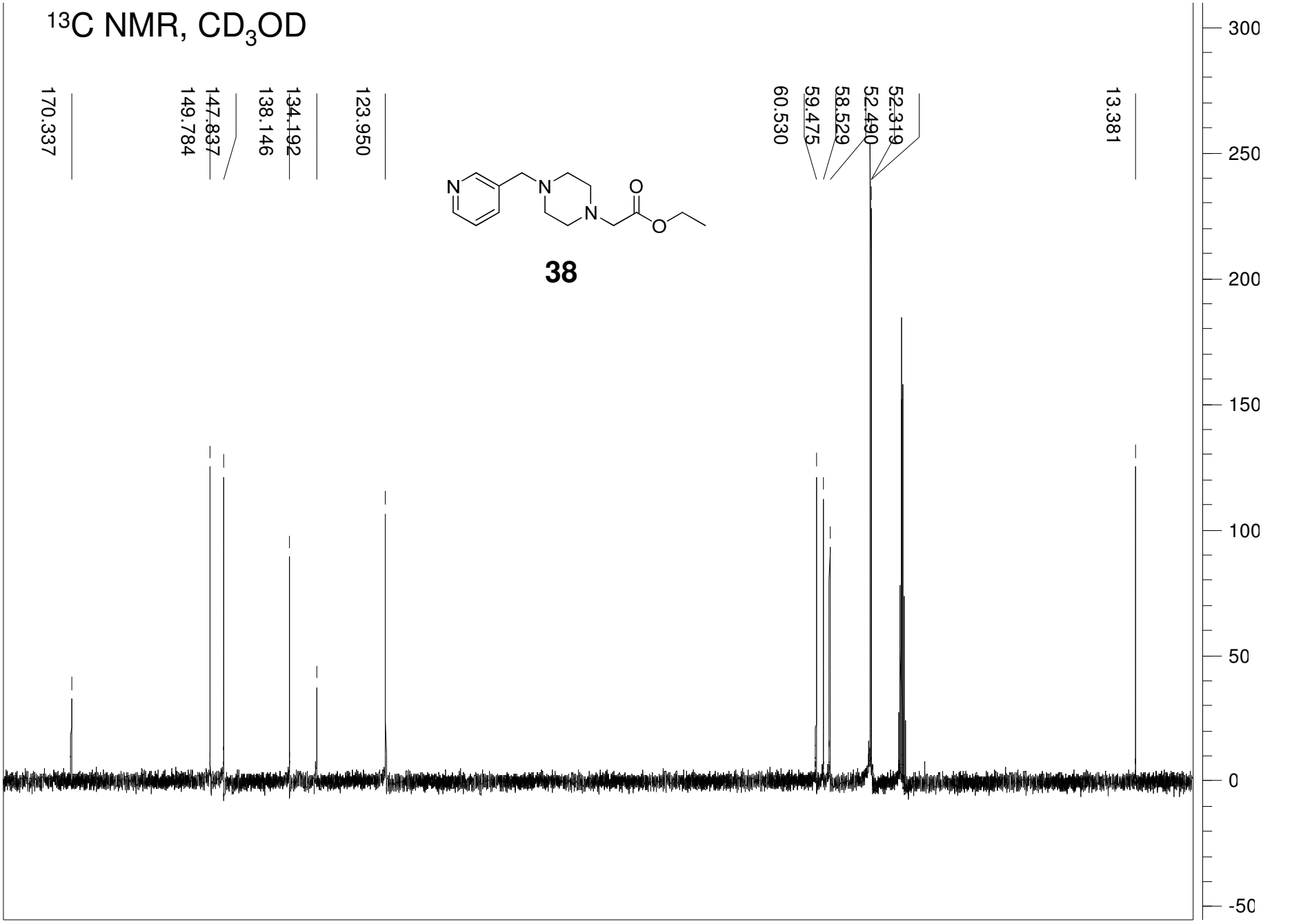
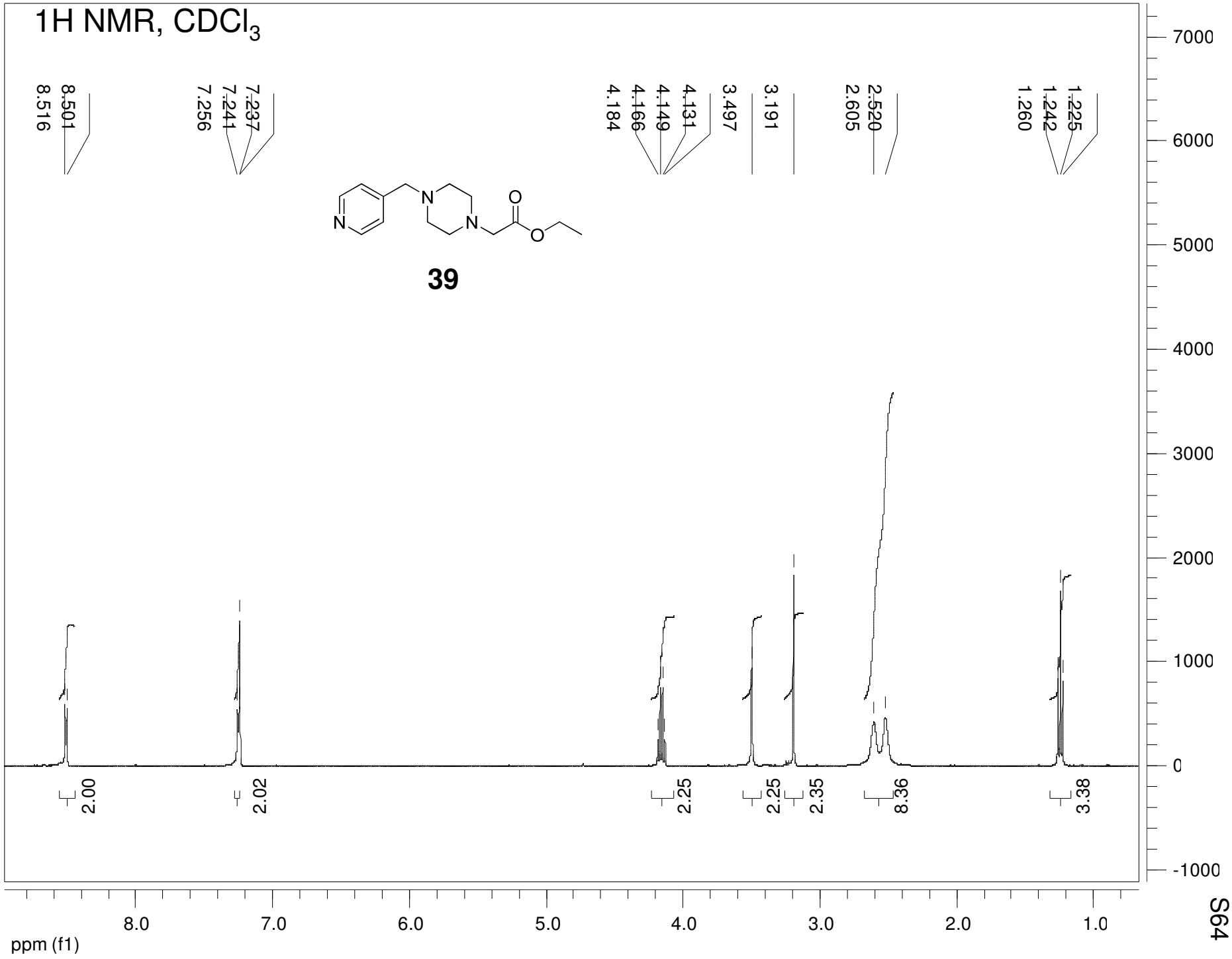
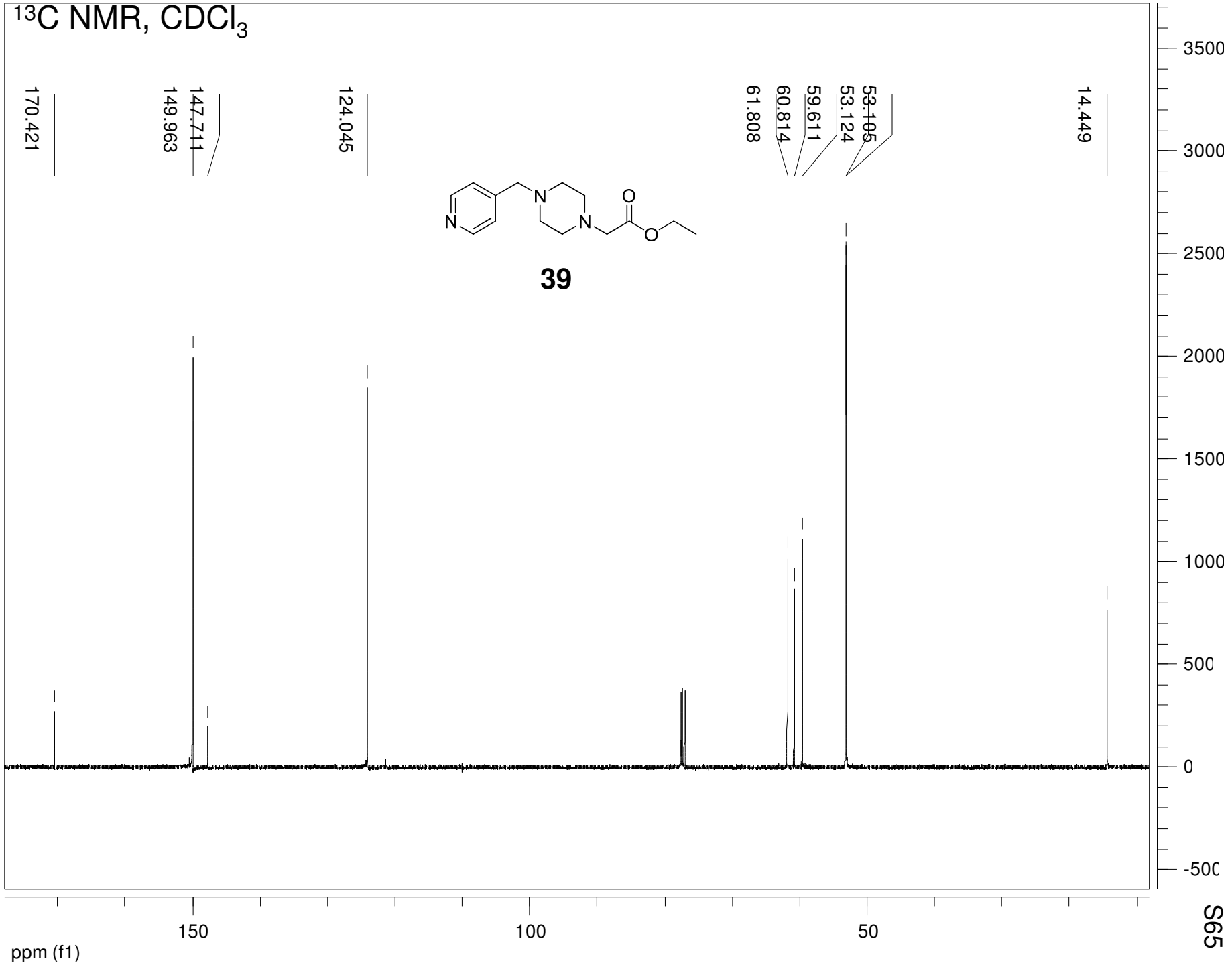
7.819
7.424
7.415
7.409
7.399
4.177
4.163
4.149
4.135
3.575
3.229
2.622
2.524
1.263
1.248
1.234
38
1000
500
0
1.00
0.85
0.89
1.02
2.20
2.16
2.26
8.25
3.24
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

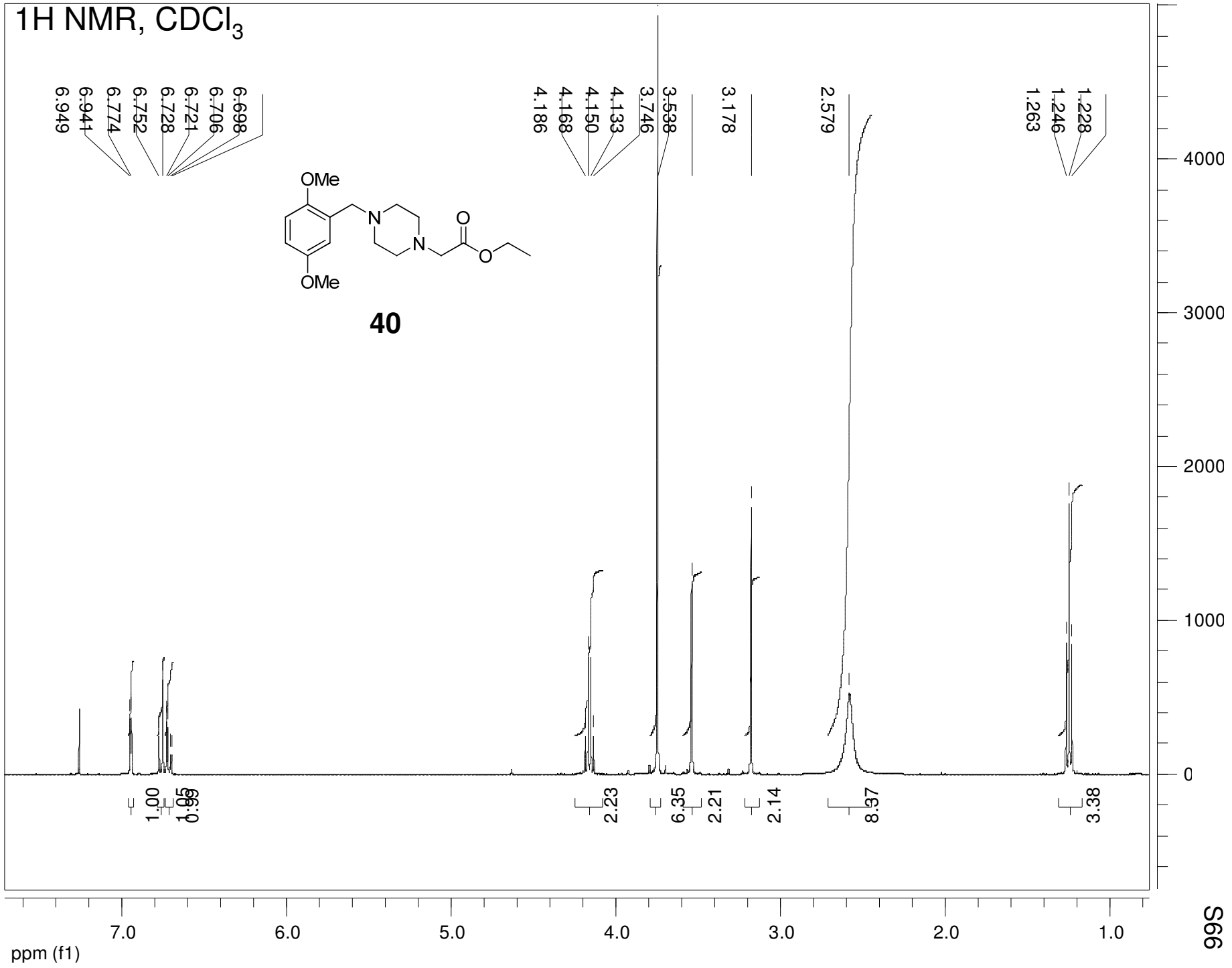
^{13}C NMR, CD_3OD



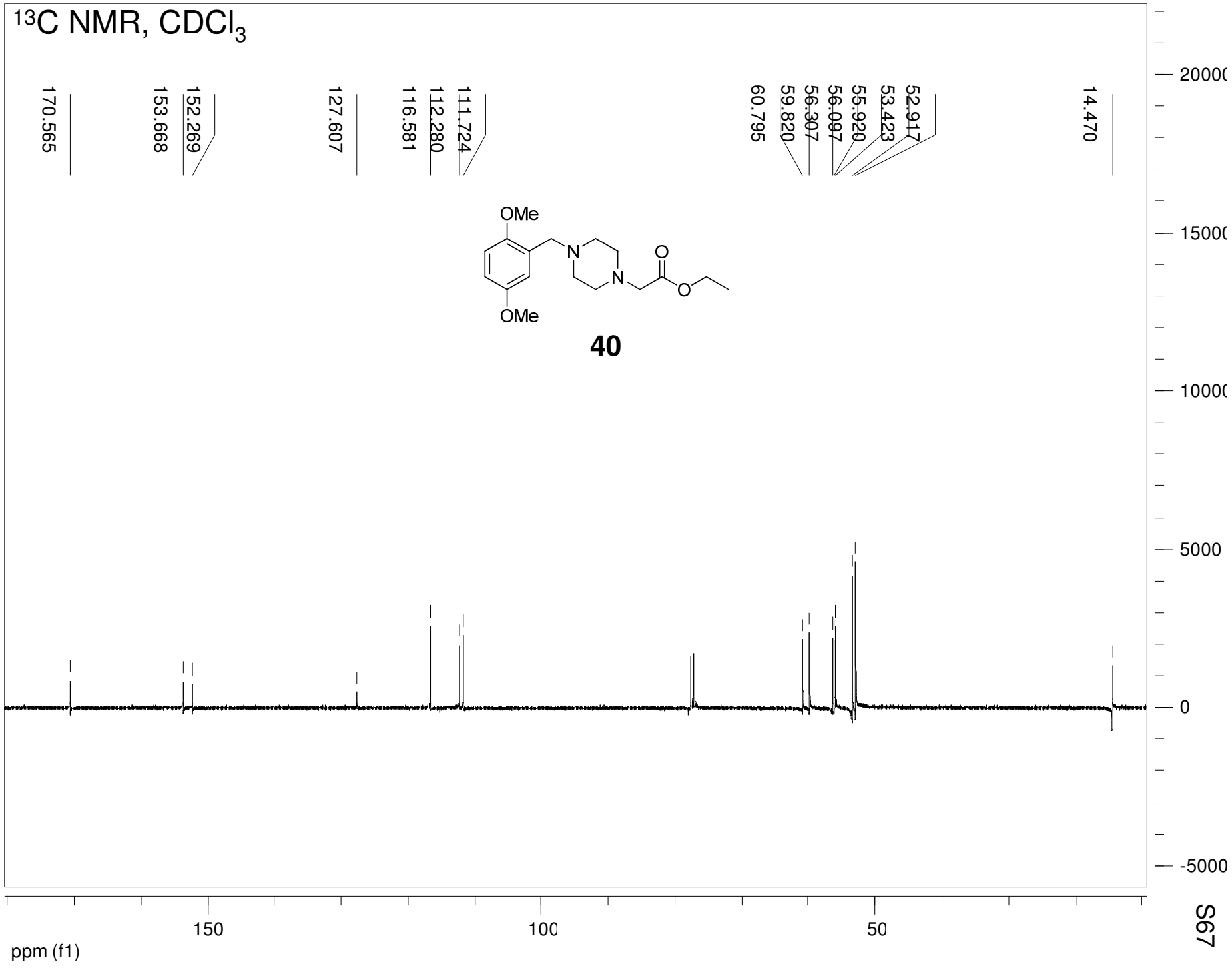
38


1H NMR, CDCl3
8.516
8.501
7.256
7.241
7.237
4.184
4.166
4.149
4.131
3.497
3.191
2.605
2.520
1.260
1.242
1.225
39
2.00
2.02
2.25
2.25
2.35
8.96
3.38
7000
6000
5000
4000
3000
2000
1000
0
-1000
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)


^{13}C NMR, $CDCl_3$
170.421
149.963
147.711
124.045
61.808
60.814
59.611
53.124
53.105
14.449
N
N
N
O
O
39
3500
3000
2500
2000
1500
1000
500
0
-500
150
100
50
ppm (f1)

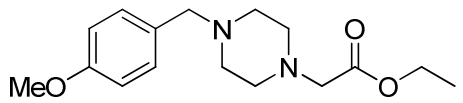

1H NMR, CDCl3
6.949
6.941
6.774
6.752
6.728
6.721
6.706
6.698
4.186
4.168
4.150
4.133
3.746
3.538
3.178
2.579
1.263
1.246
1.228
OMe
OMe
N
N
O
O
40
4000
3000
2000
1000
0
1.00
1.05
0.99
2.23
6.35
2.21
2.14
8.37
3.38
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)

^{13}C NMR, $CDCl_3$

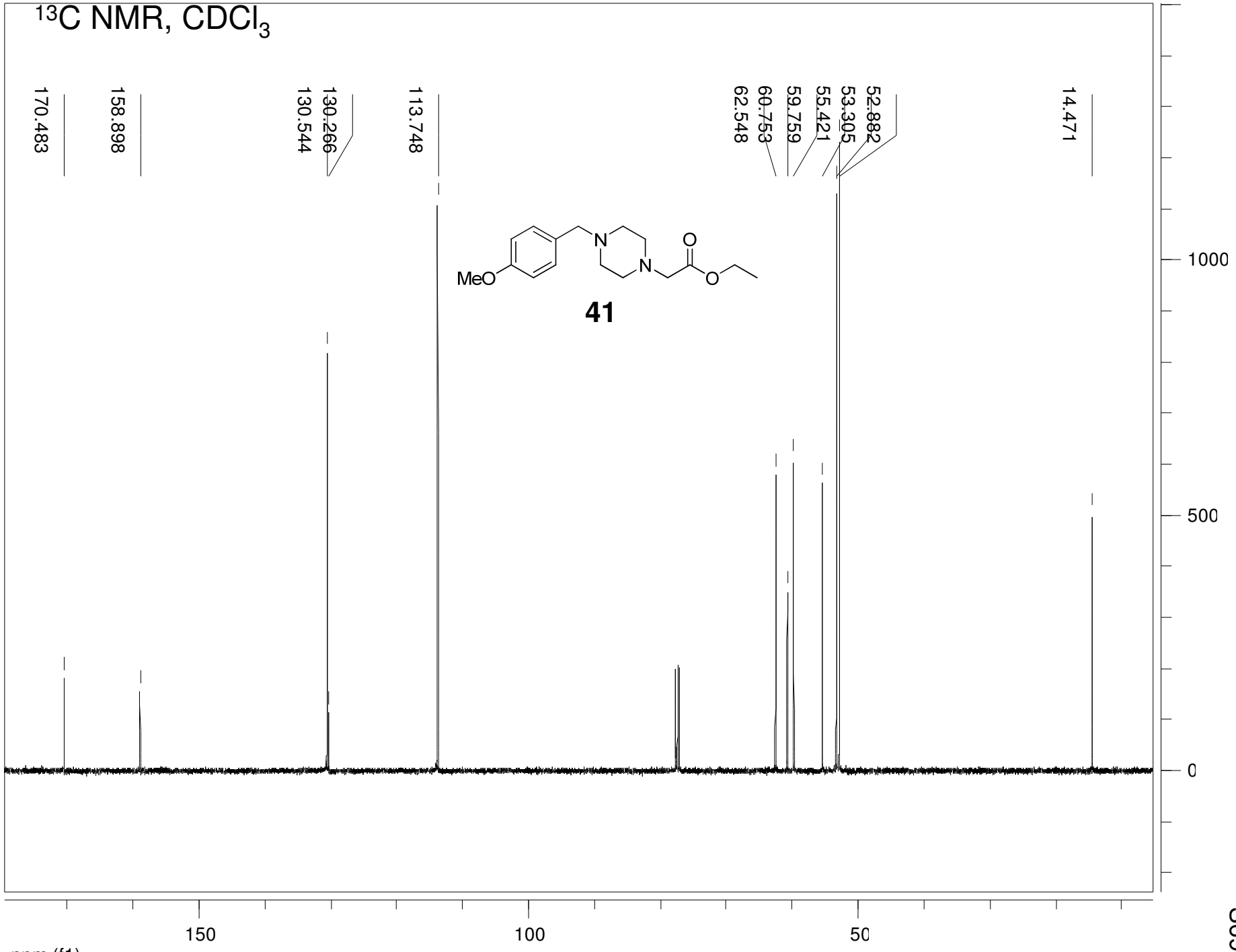


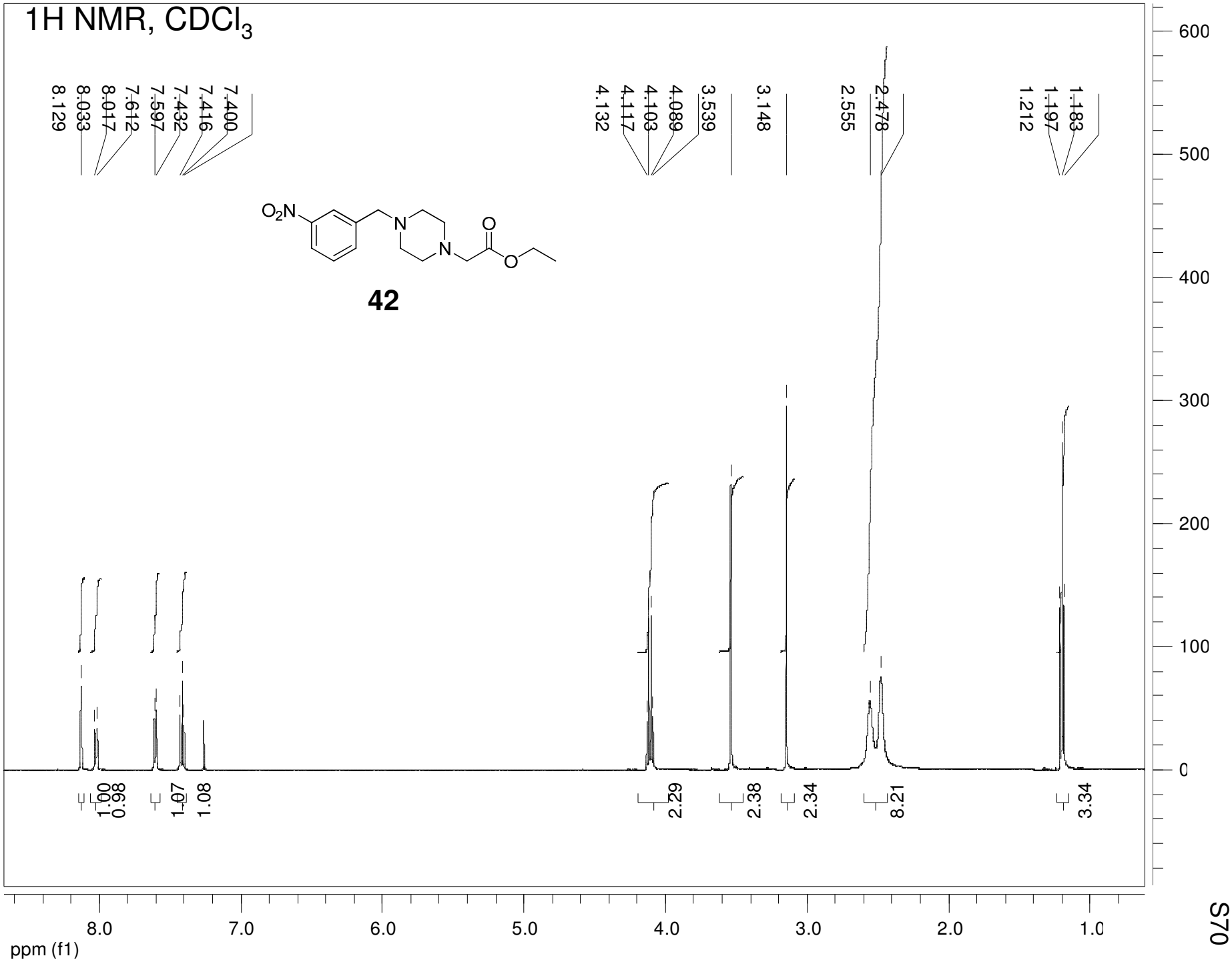
40

1H NMR, $CDCl_3$

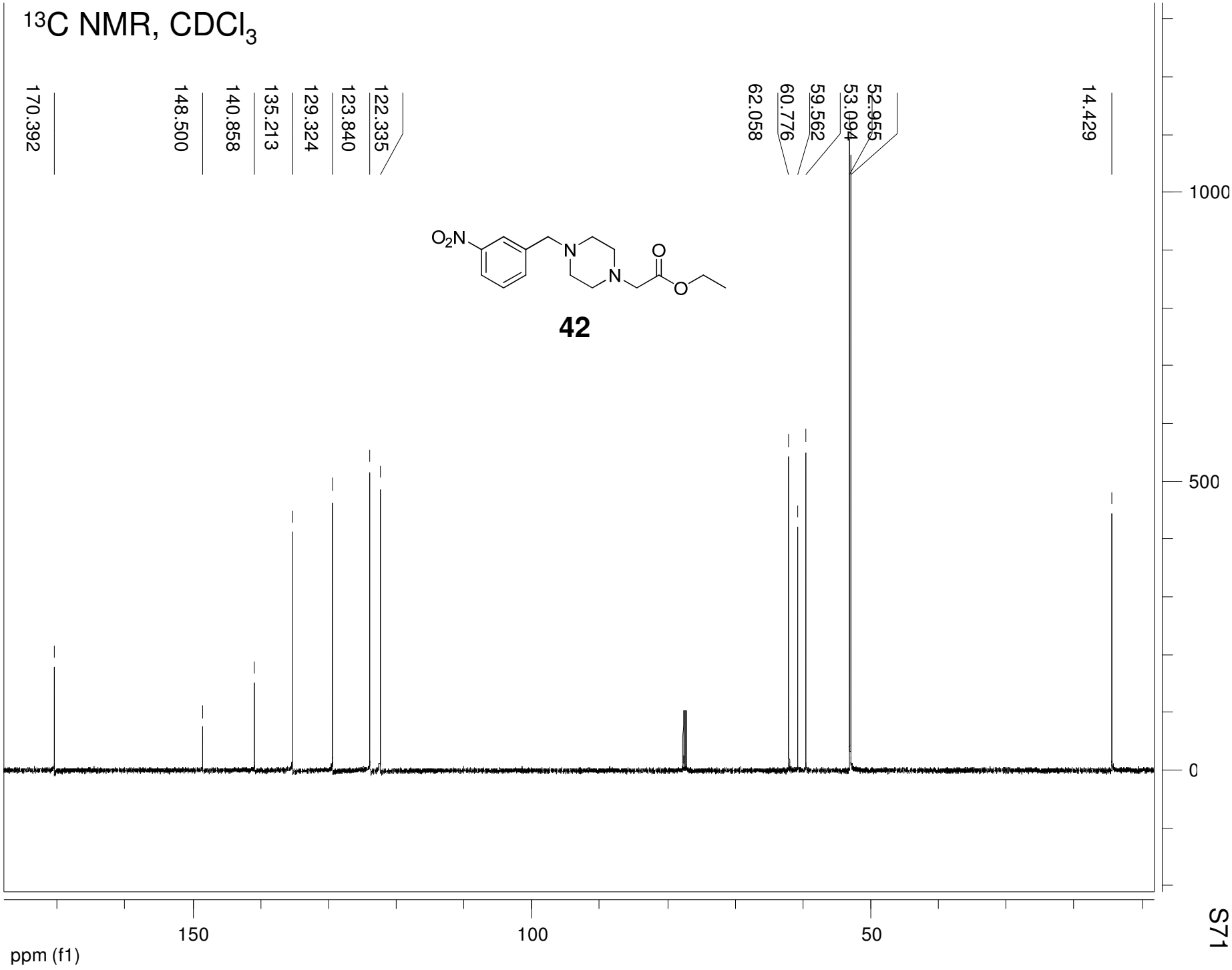


41

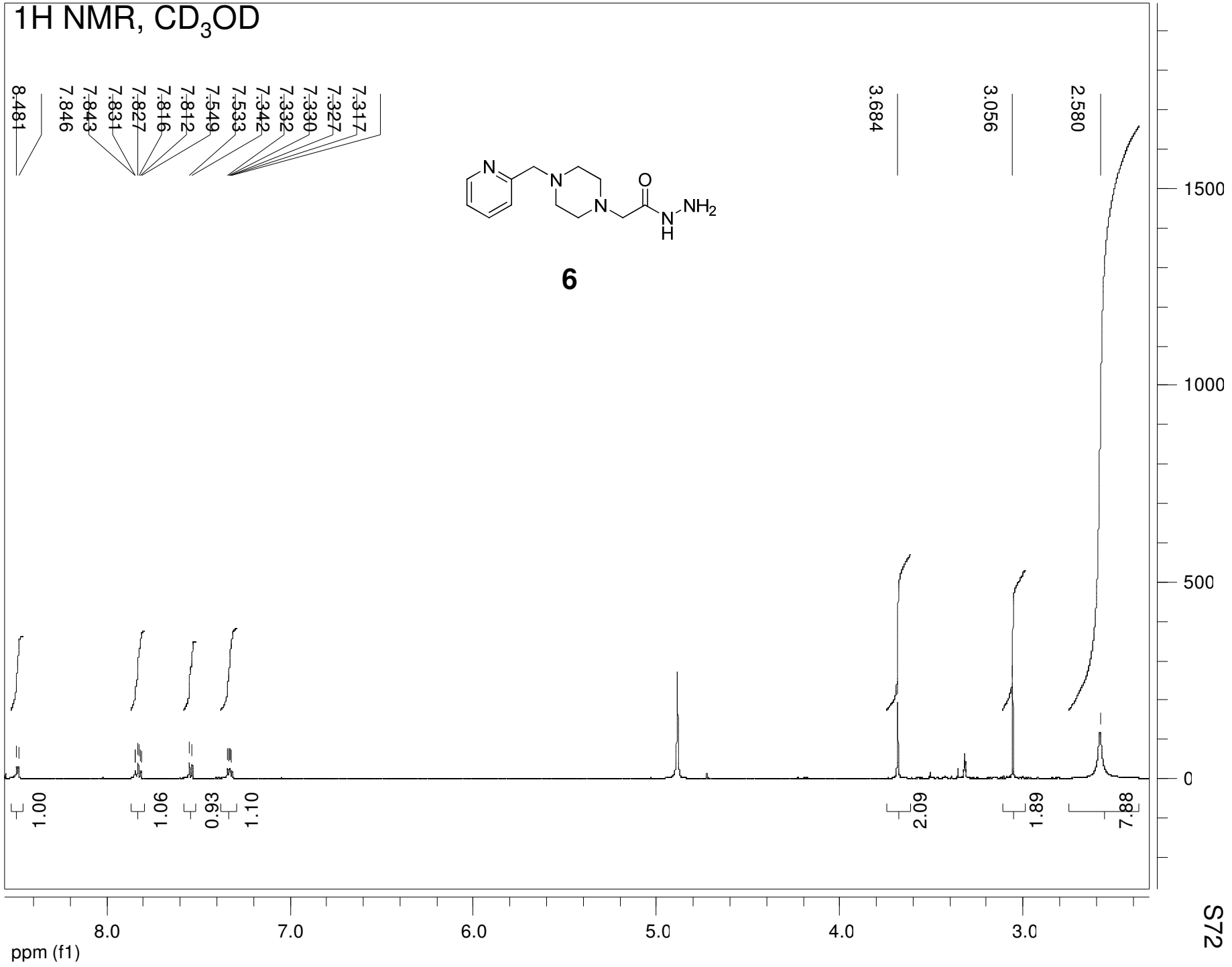

^{13}C NMR, $CDCl_3$
170.483
158.898
130.544
130.266
113.748
62.548
60.753
59.759
55.421
53.305
52.882
14.471
MeO
N
N
O
O
41
1000
500
0
150
100
50
ppm (f1)


1H NMR, CDCl3
8.129
8.033
8.017
7.612
7.597
7.432
7.416
7.400
4.132
4.117
4.103
4.089
3.539
3.148
2.555
2.478
1.212
1.197
1.183
O2N
N
N
O
O
42
1.00
0.88
1.07
1.08
2.29
2.38
2.34
8.21
3.34
600
500
400
300
200
100
0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)

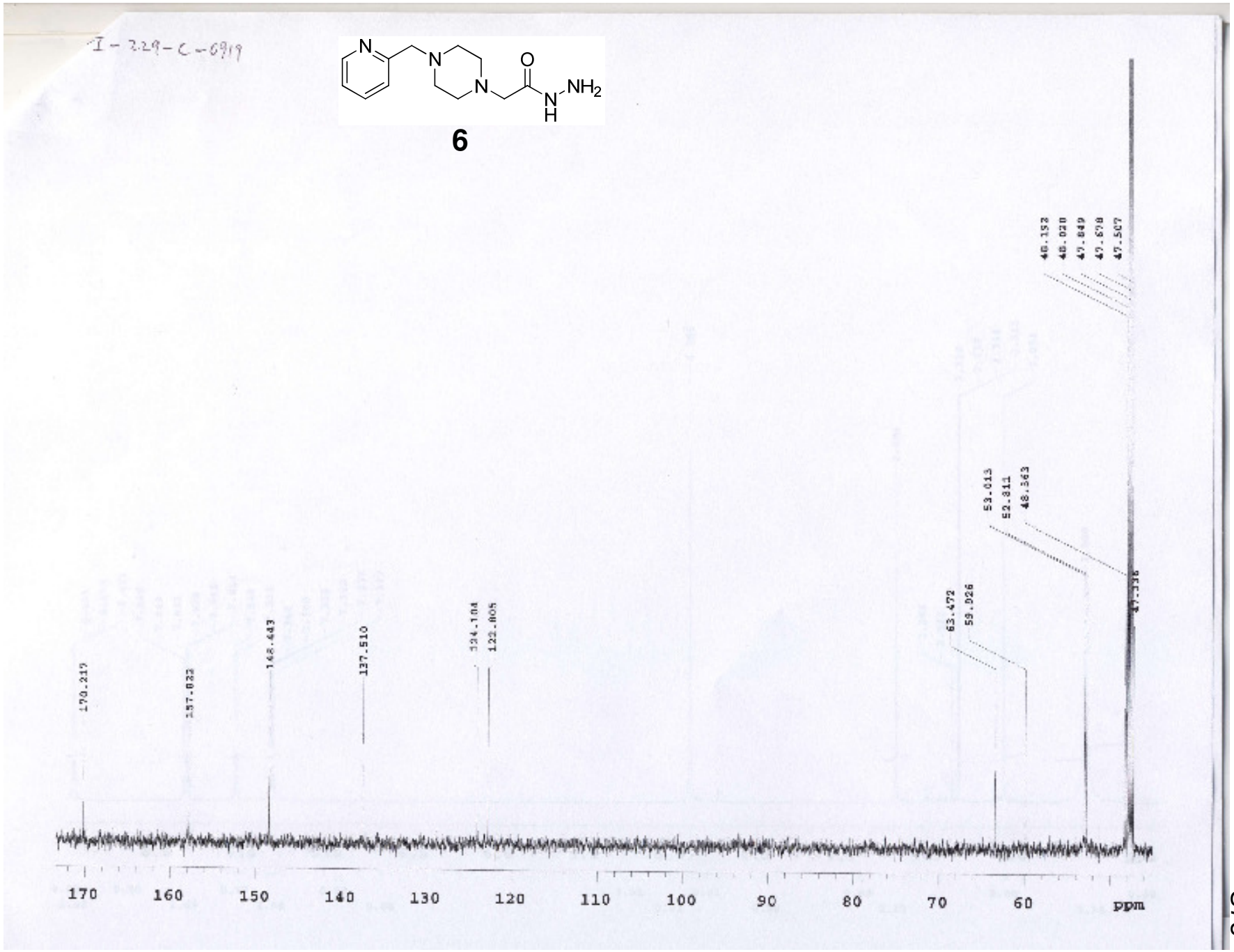
^{13}C NMR, $CDCl_3$

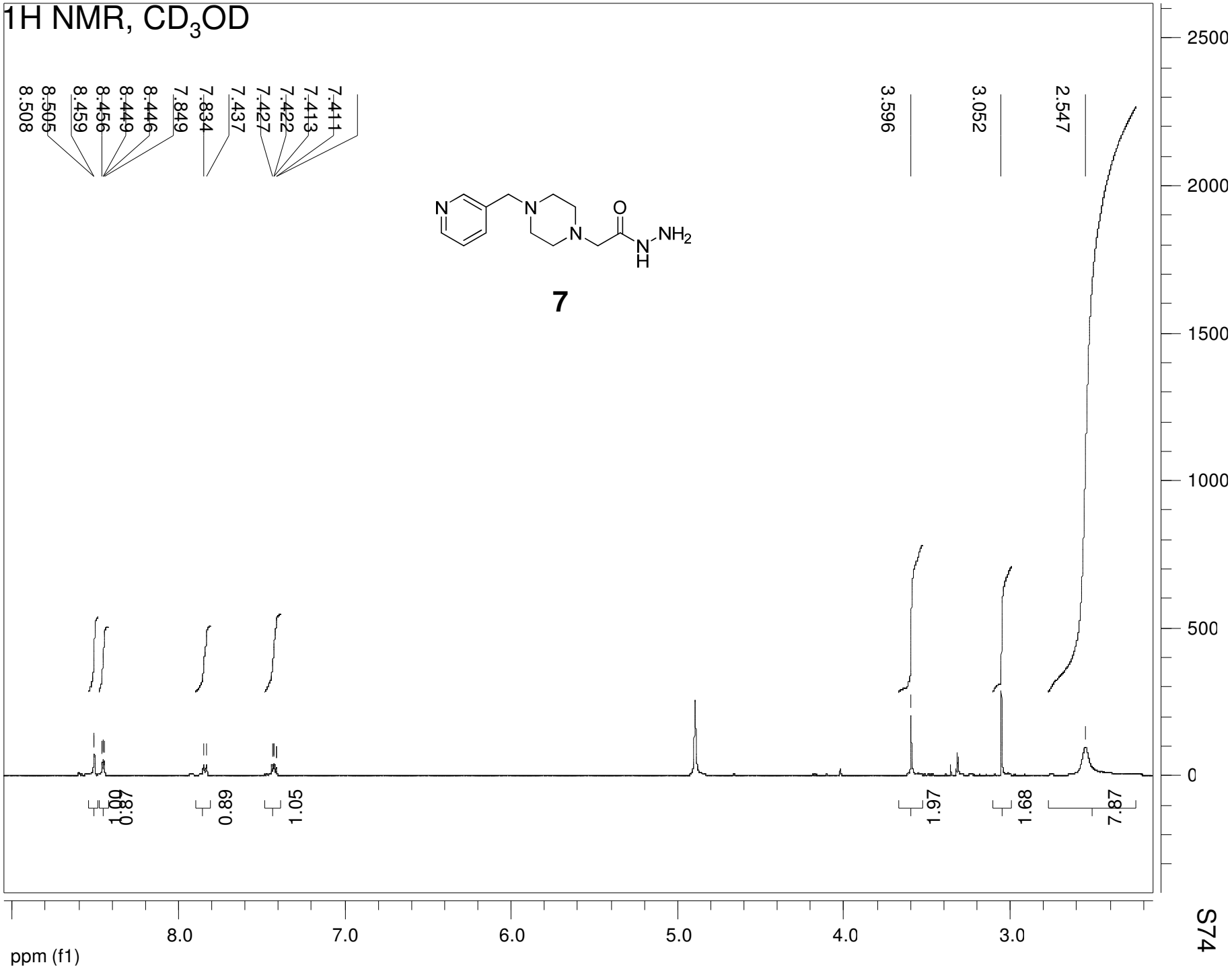


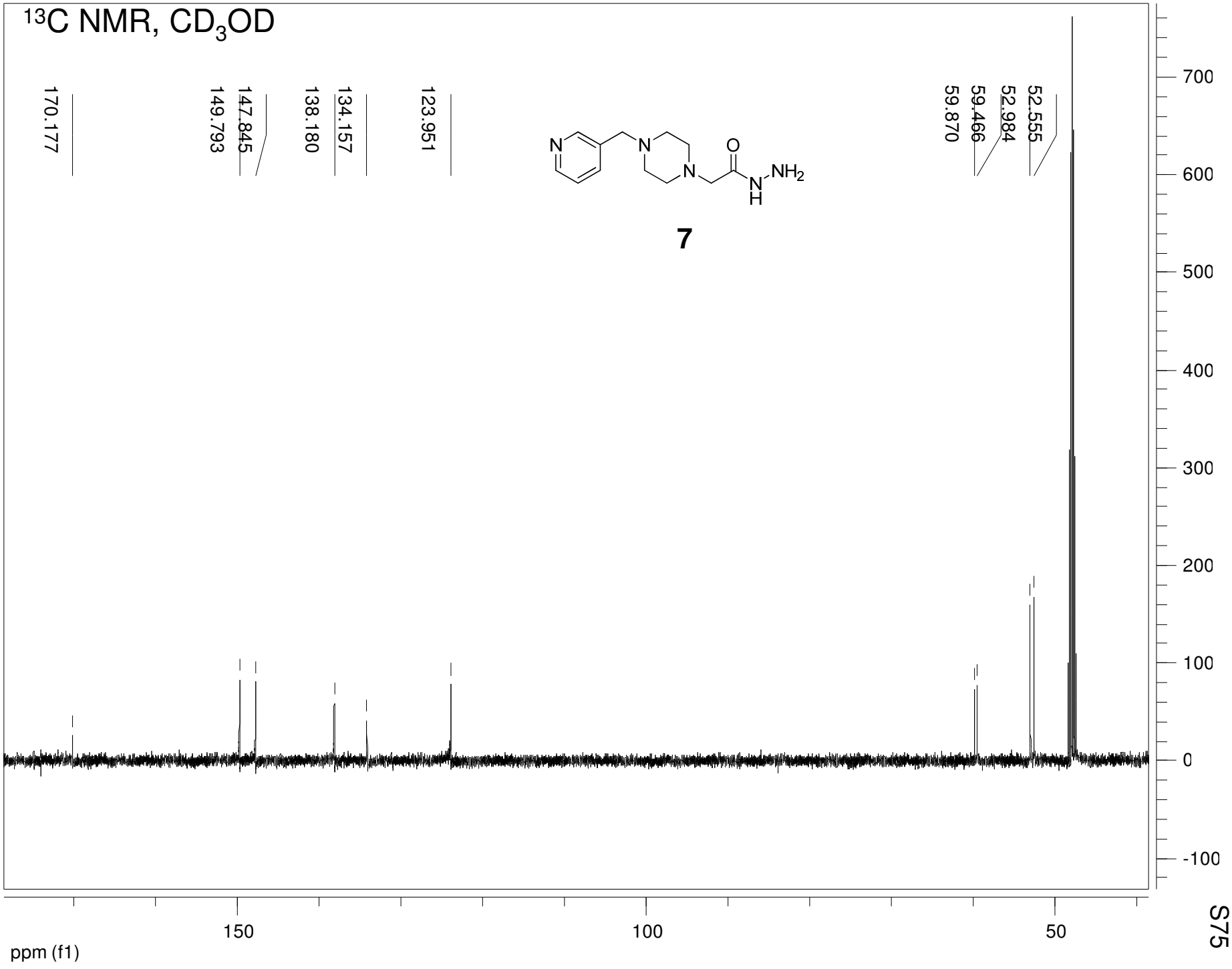
42

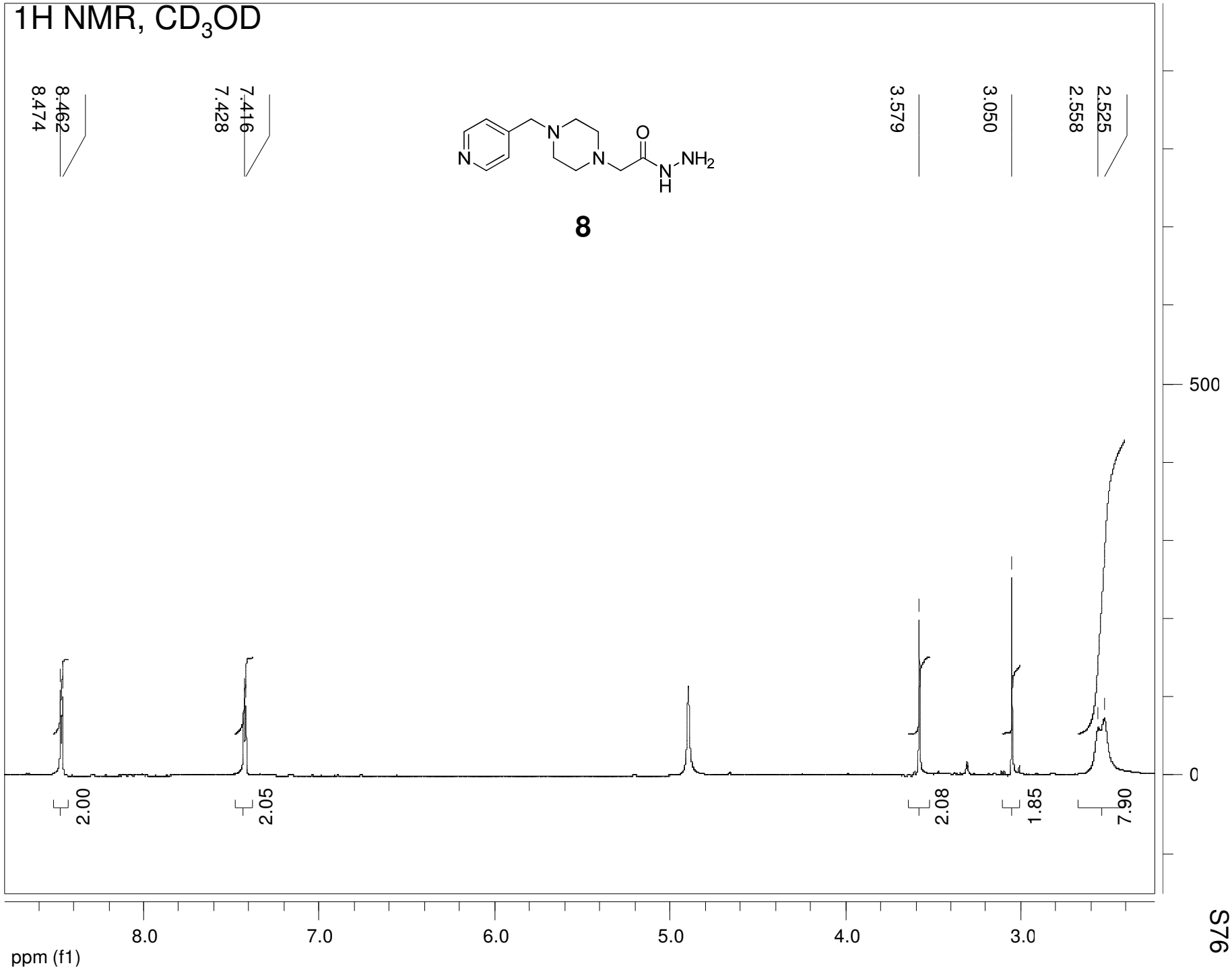

1H NMR, CD3OD
8.481
7.846
7.843
7.831
7.827
7.816
7.812
7.549
7.533
7.342
7.332
7.330
7.327
7.317
3.684
3.056
2.580
N
N
N
O
N
H
NH2
6
1500
1000
500
0
1.00
1.06
0.93
1.10
2.09
1.89
7.88
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)

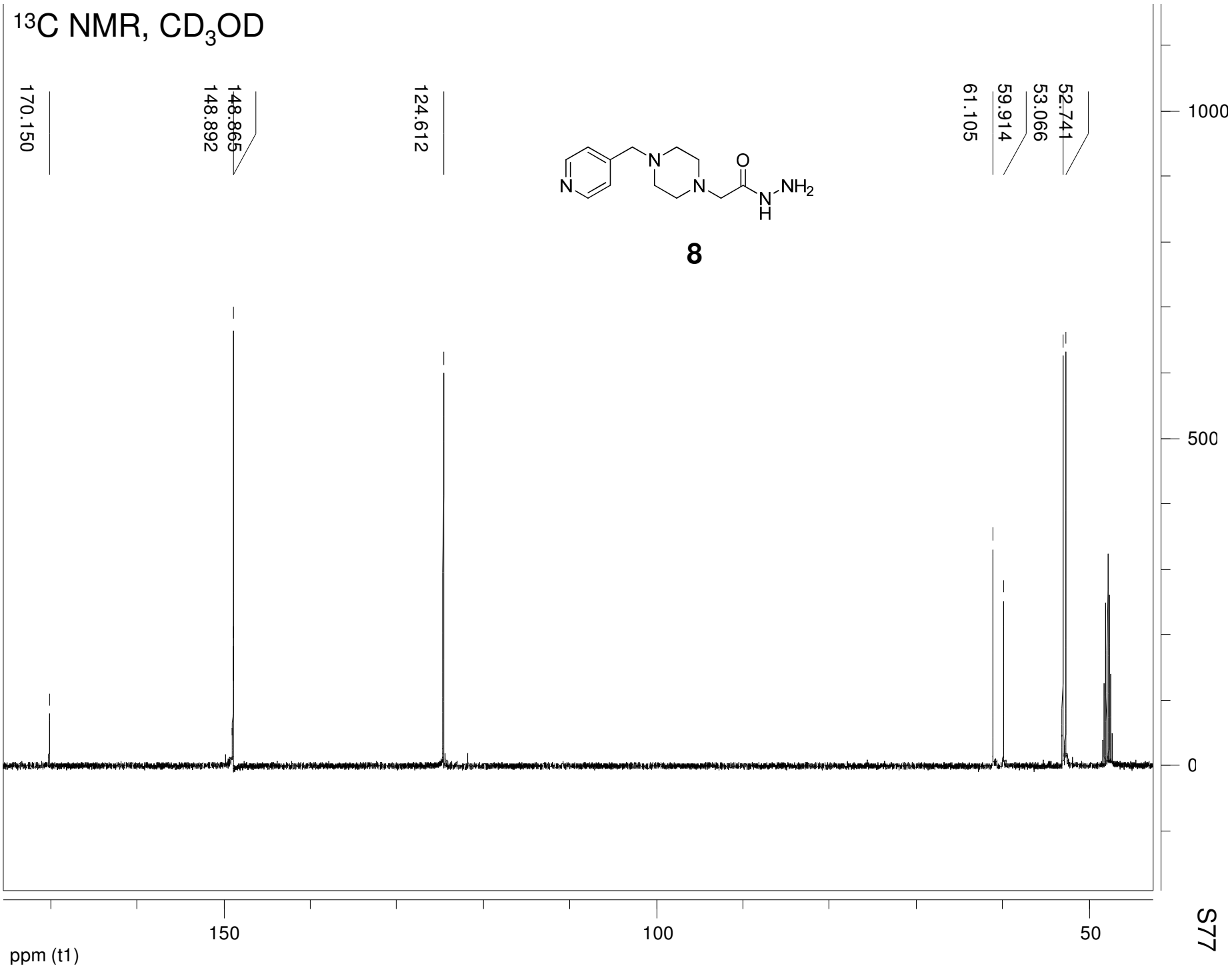
^{13}C NMR, CD_3OD



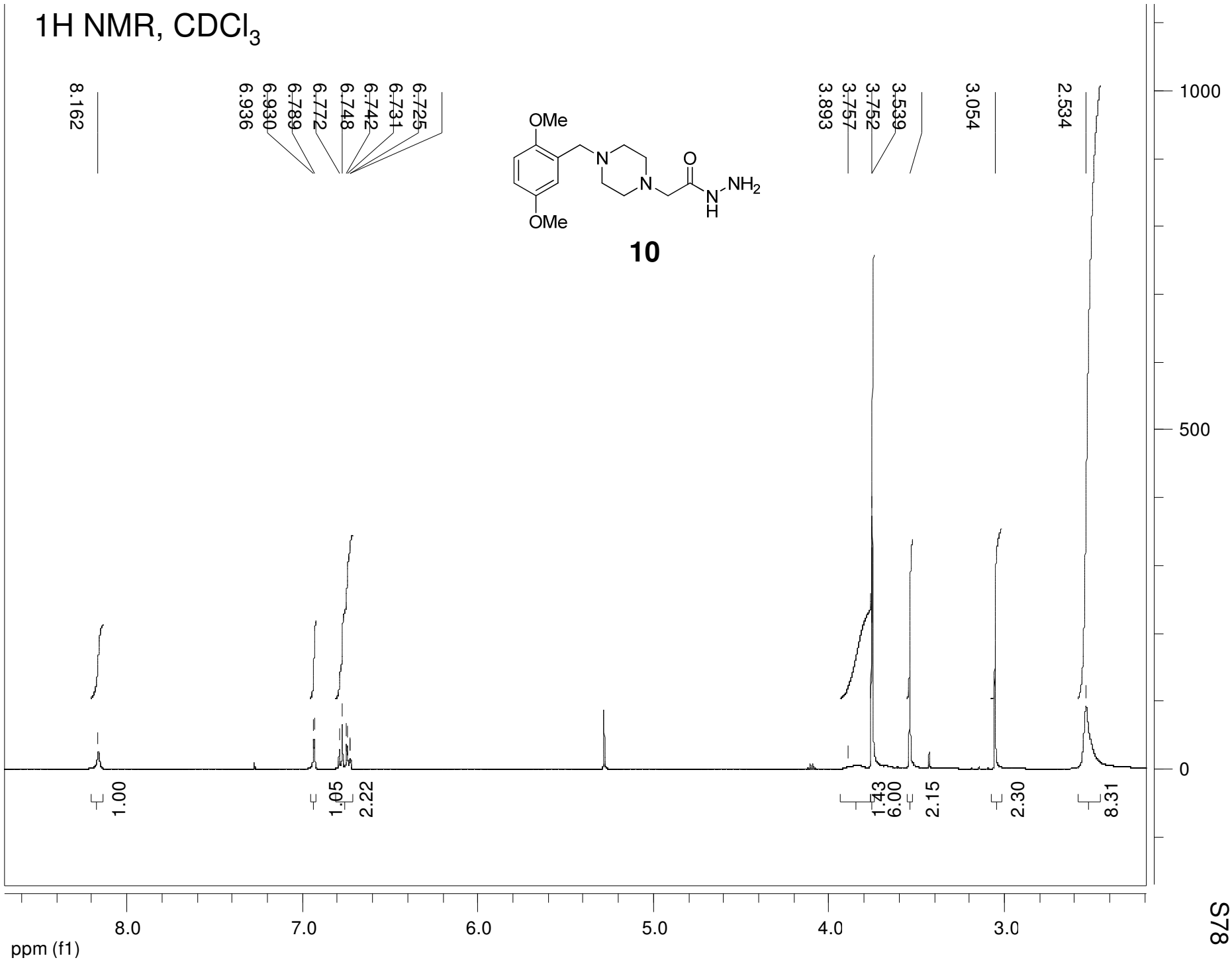

1H NMR, CD3OD
8.508
8.505
8.459
8.456
8.449
8.446
7.849
7.834
7.437
7.427
7.422
7.413
7.411
3.596
3.052
2.547
7
2500
2000
1500
1000
500
0
1.00
0.87
0.68
1.05
1.67
1.89
7.87
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)

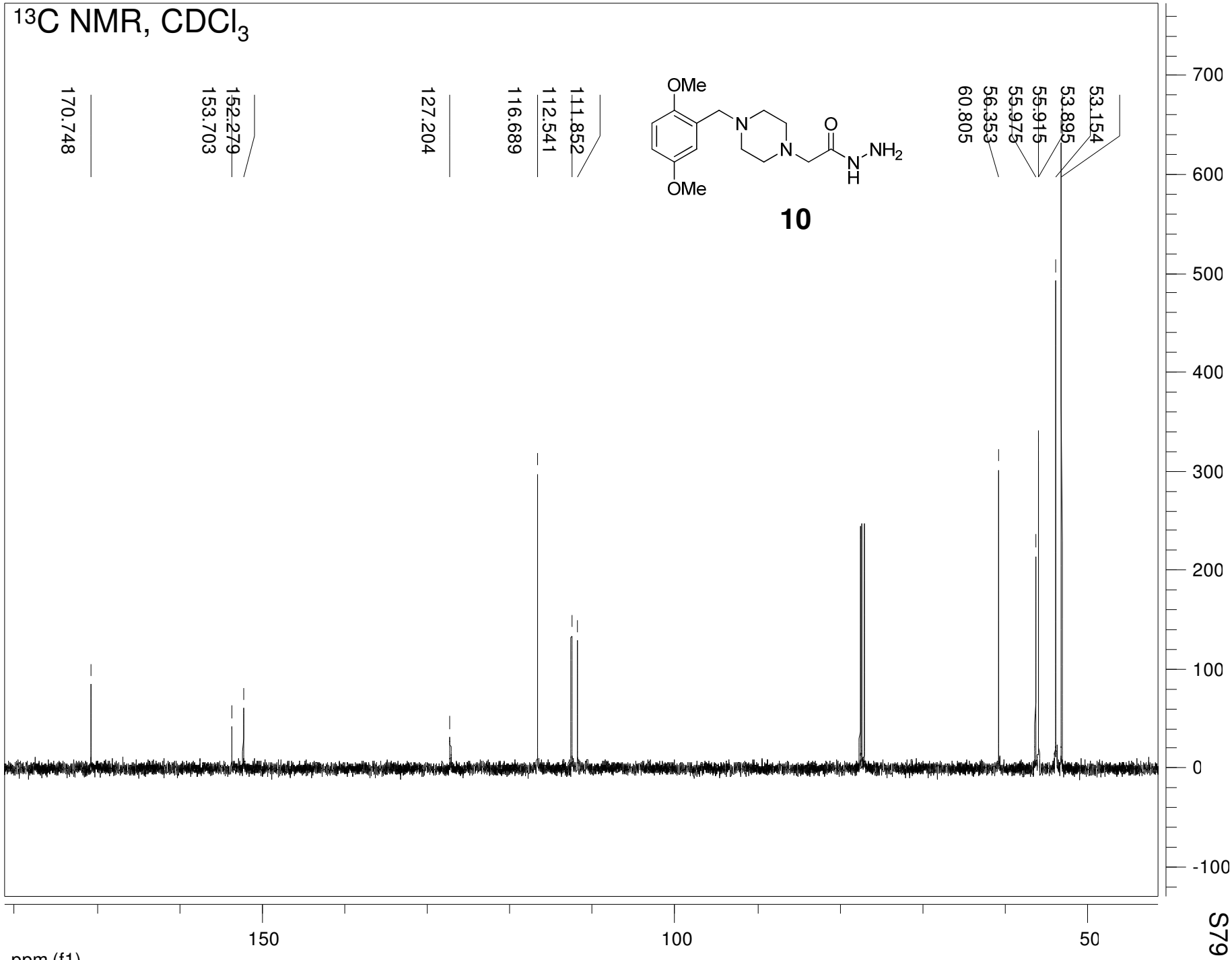

13C NMR, CD3OD
170.177
149.793
147.845
138.180
134.157
123.951
59.870
59.466
52.984
52.555
N
N
N
O
NH2
NH
7
700
600
500
400
300
200
100
0
-100
150
100
50
ppm (f1)

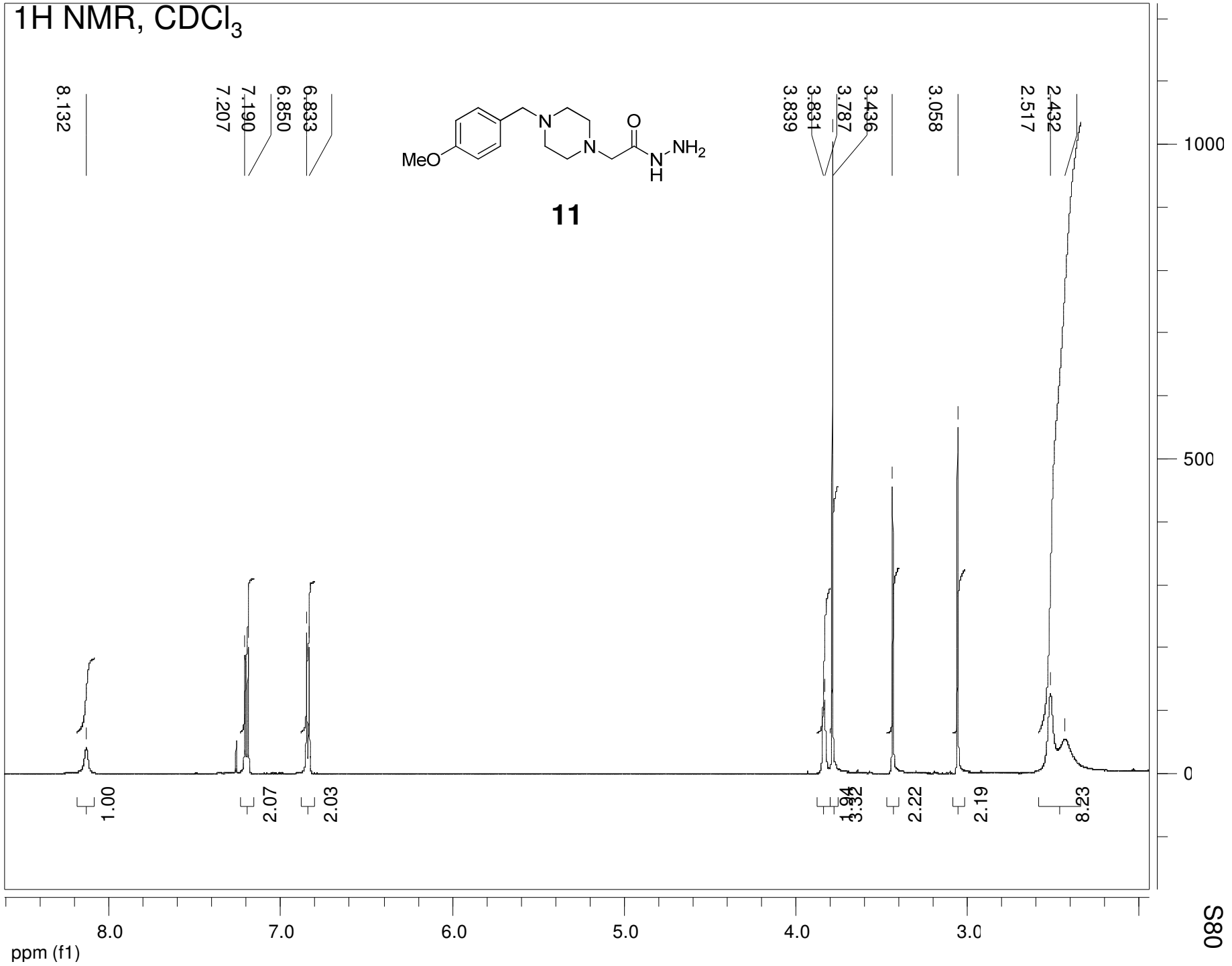

1H NMR, CD3OD
8.474
8.462
7.428
7.416
3.579
3.050
2.558
2.525
8
2.00
2.05
2.08
1.85
7.90
500
0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)

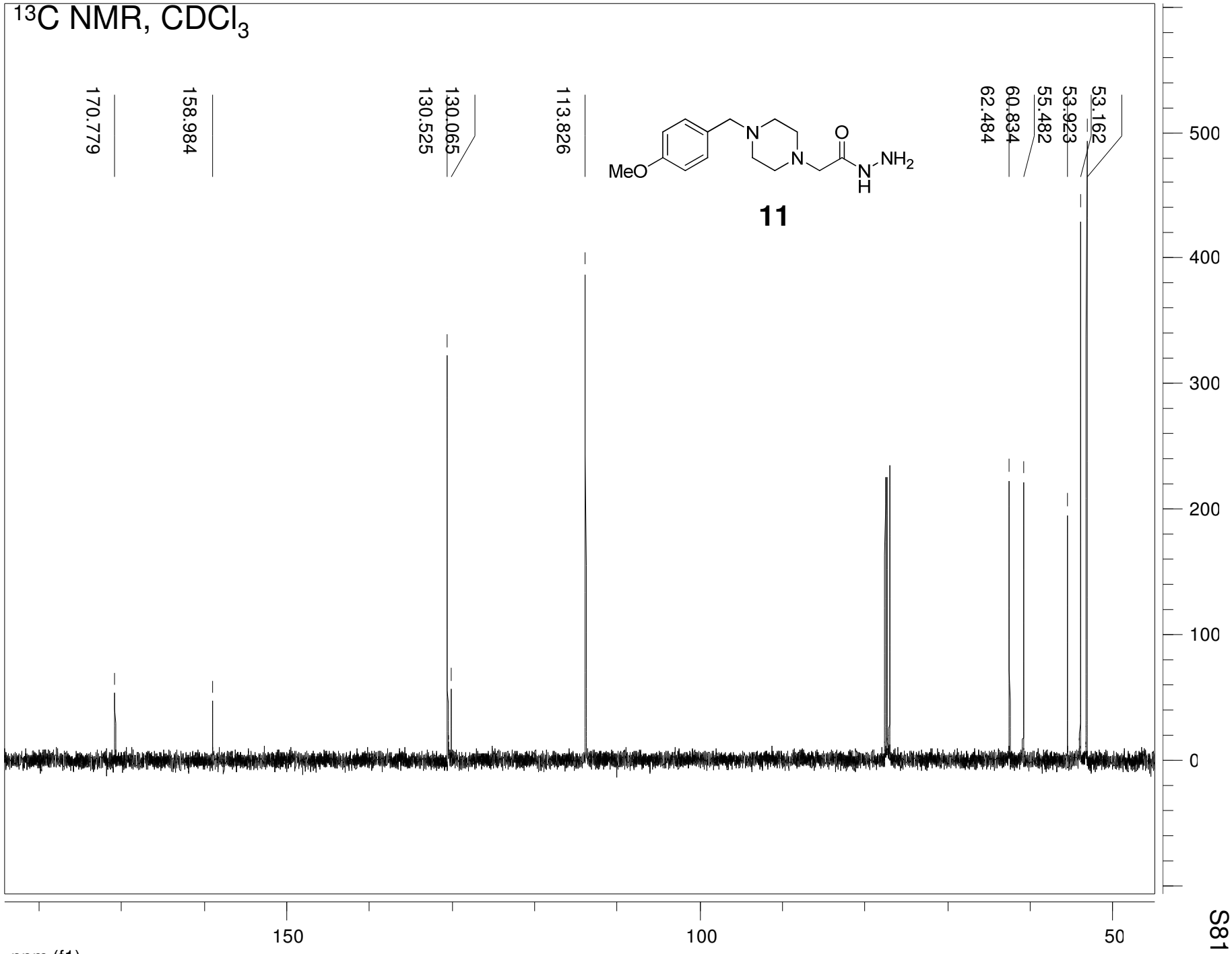

^{13}C NMR, CD_3OD
170.150
148.892
148.865
124.612
61.105
59.914
53.066
52.741
1000
500
0
150
100
50
ppm (t1)

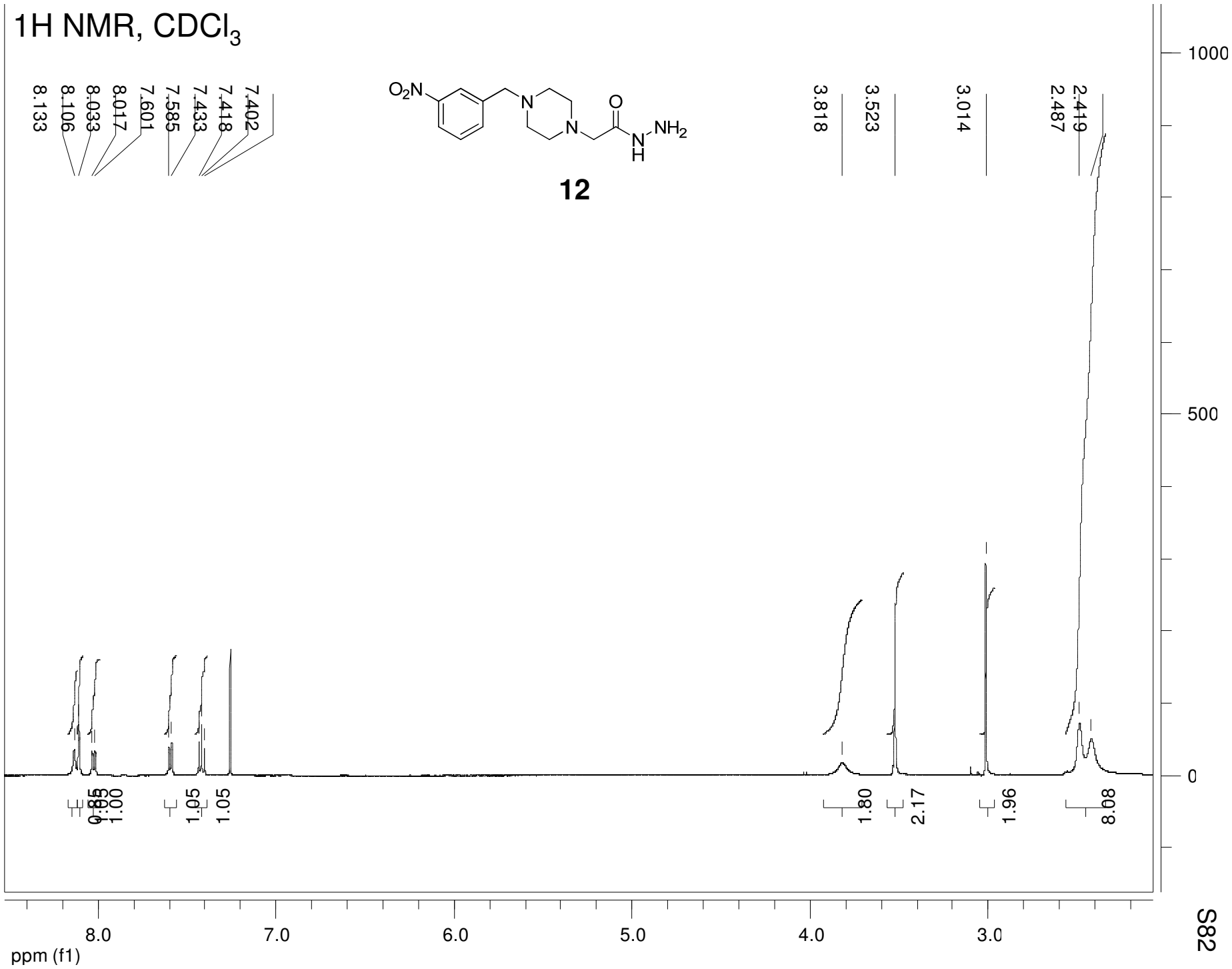
8


1H NMR, CDCl3
8.162
6.936
6.930
6.789
6.772
6.748
6.742
6.731
6.725
3.893
3.757
3.752
3.539
3.054
2.534
OMe
OMe
N
N
O
N
NH2
H
10
1000
500
0
1.00
1.05
2.22
1.43
6.00
2.15
2.30
8.31
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)

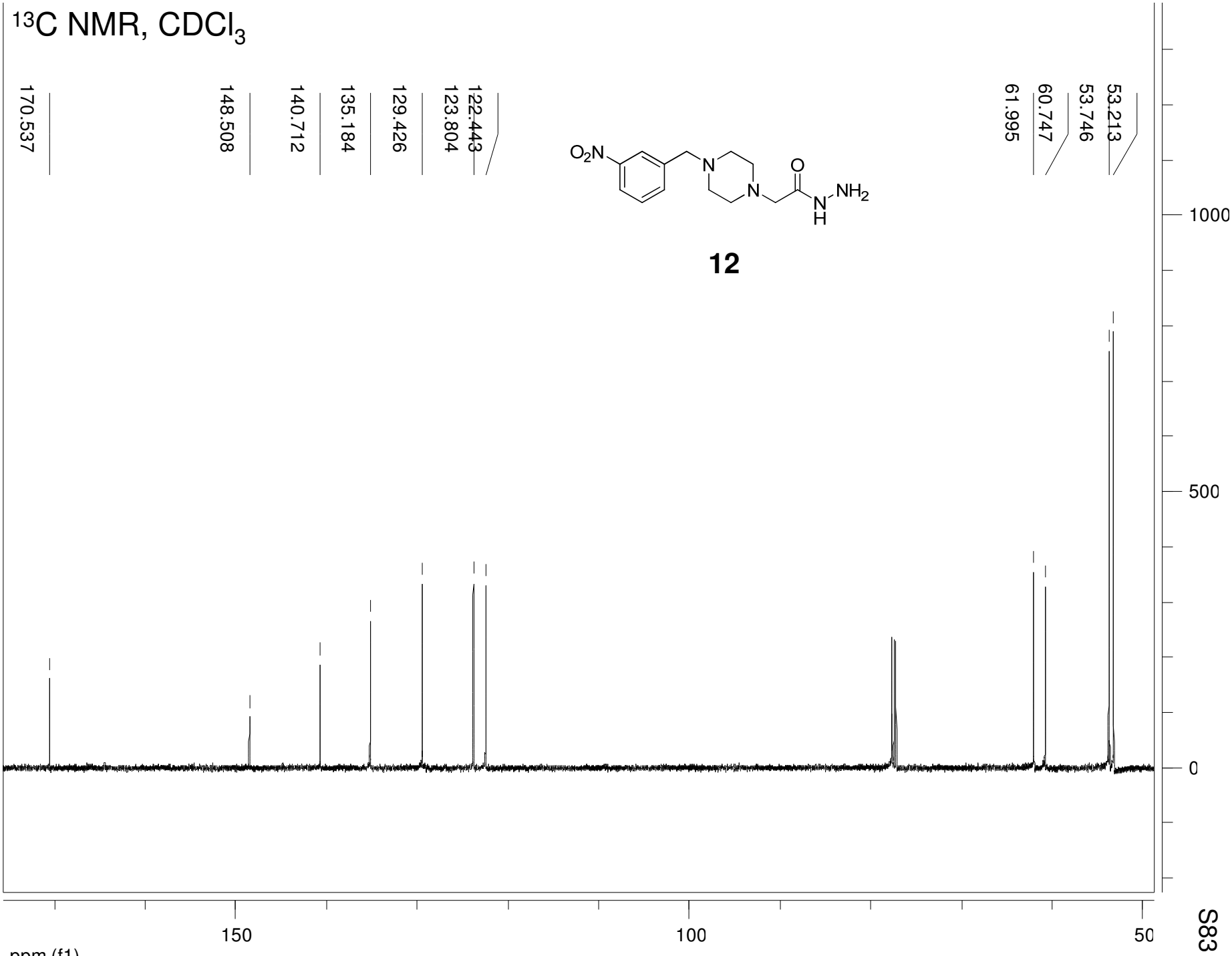

13C NMR, CDCl3
170.748
153.703
152.279
127.204
116.689
112.541
111.852
60.805
56.353
55.975
55.915
53.895
53.154
OMe
OMe
N
N
O
NH2
NH
10
700
600
500
400
300
200
100
0
-100
150
100
50
ppm (f1)


1H NMR, CDCl3
8.132
7.207
7.190
6.850
6.833
3.839
3.831
3.787
3.436
3.058
2.517
2.432
MeO
N
N
O
N
H
NH2
11
1000
500
0
1.00
2.07
2.03
2.22
2.19
8.23
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)

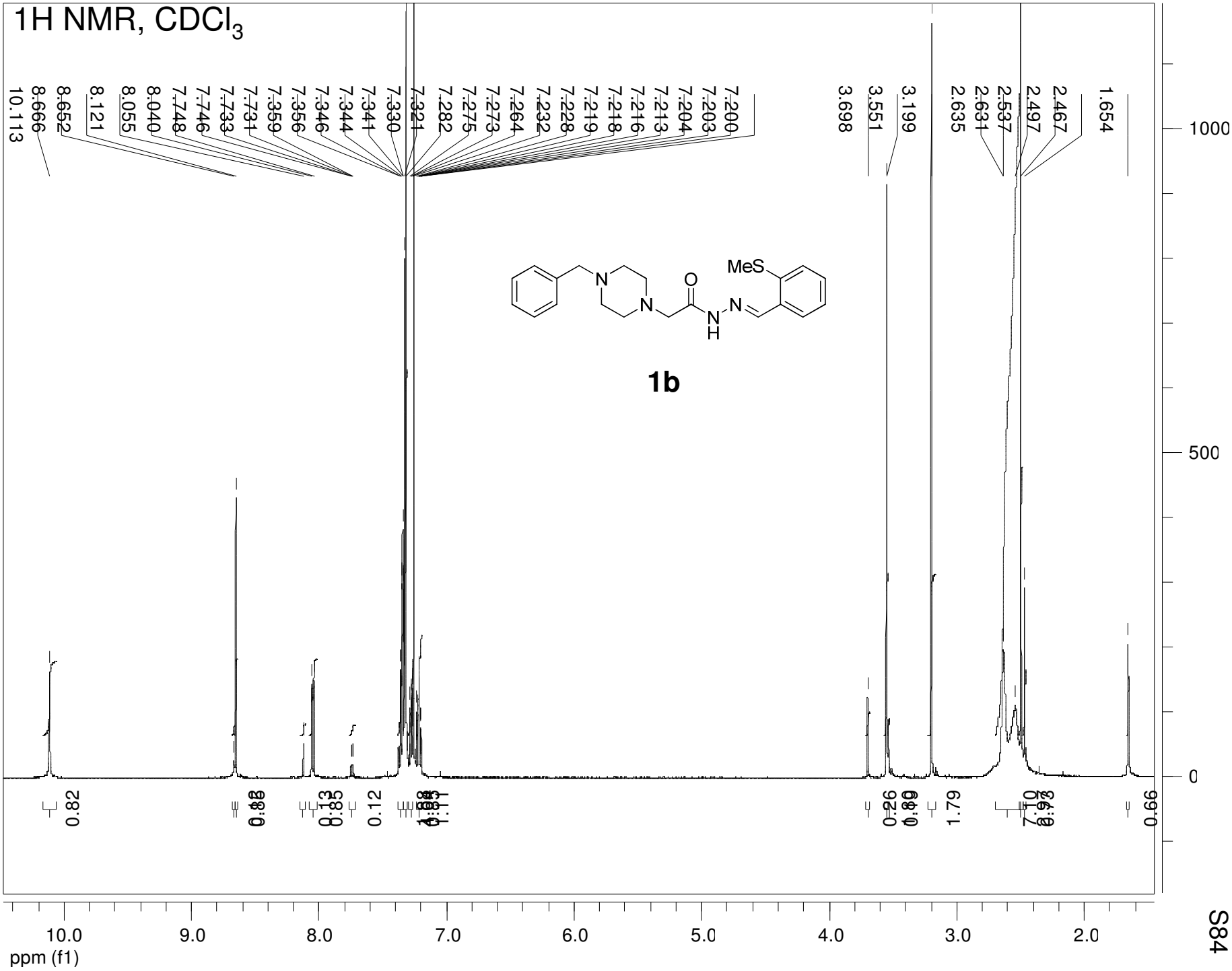

^{13}C NMR, $CDCl_3$
170.779
158.984
130.525
130.065
113.826
62.484
60.834
55.482
53.923
53.162
MeO
N
N
O
NH
NH2
11
500
400
300
200
100
0
150
100
50
ppm (f1)


1H NMR, CDCl3
8.133
8.106
8.033
8.017
7.601
7.585
7.433
7.418
7.402
O2N
N
N
O
NH2
H
12
3.818
3.523
3.014
2.487
2.419
1000
500
0
1.00
0.85
0.88
1.05
1.05
1.80
2.17
1.96
8.08
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)

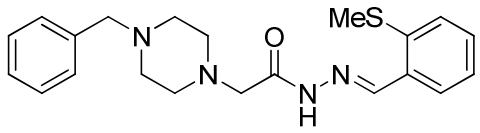
^{13}C NMR, $CDCl_3$



12

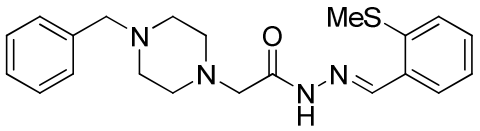

1H NMR, CDCl3
10.113
8.666
8.652
8.121
8.055
8.040
7.748
7.746
7.733
7.731
7.359
7.356
7.346
7.344
7.341
7.330
7.321
7.282
7.275
7.273
7.264
7.232
7.228
7.219
7.218
7.216
7.213
7.204
7.203
7.200
3.698
3.551
3.199
2.635
2.631
2.537
2.497
2.467
1.654
MeS
N
O
N
N
H
1b
1000
500
0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (f1)

^{13}C NMR, $CDCl_3$

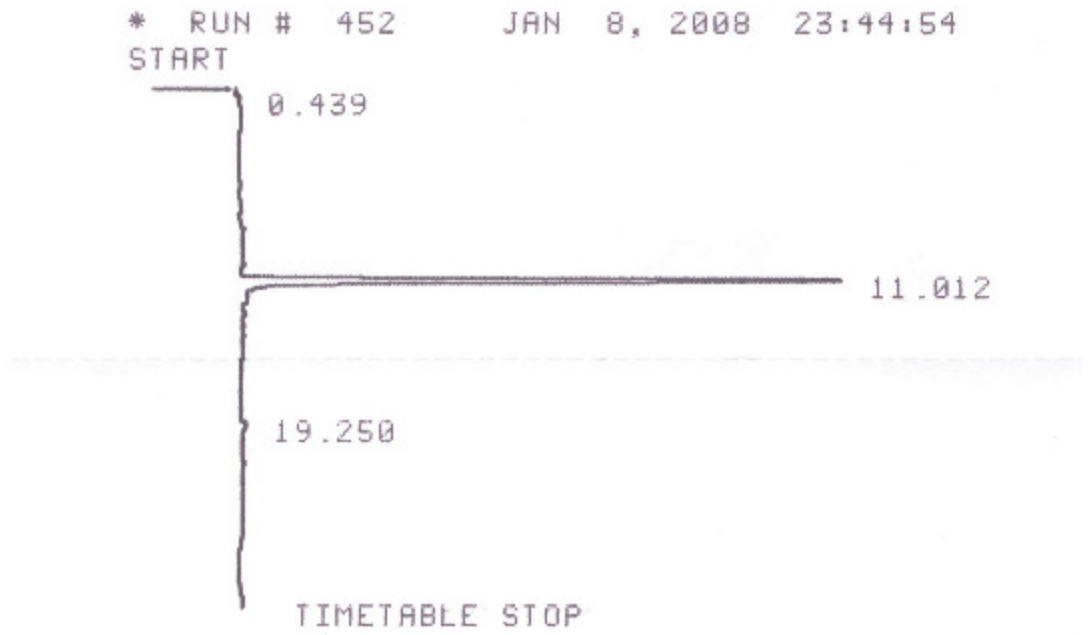


1b

HPLC



1b



RUN# 452 JAN 8, 2008 23:44:54

AREA%

RT	AREA	TYPE	WIDTH	AREA%
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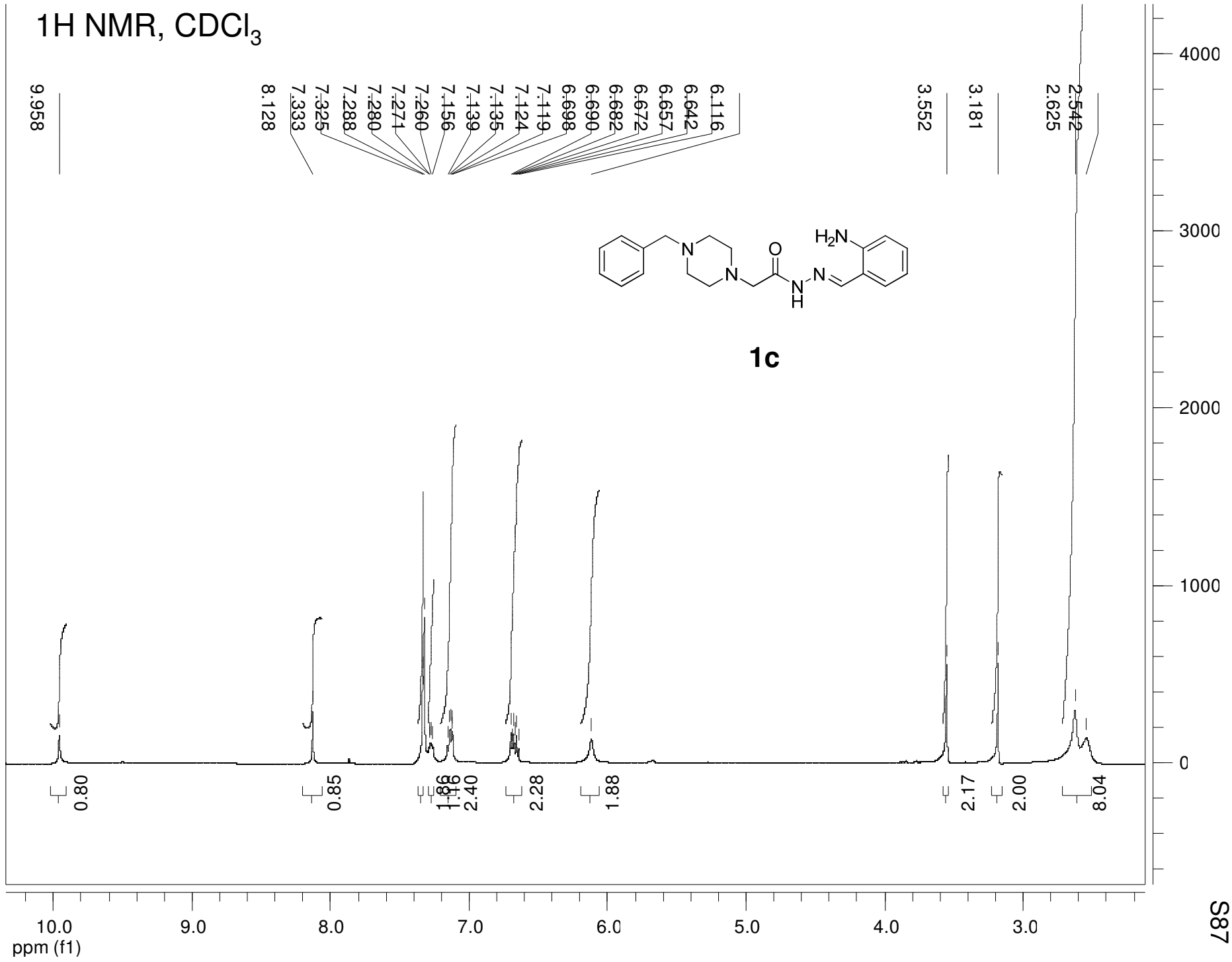
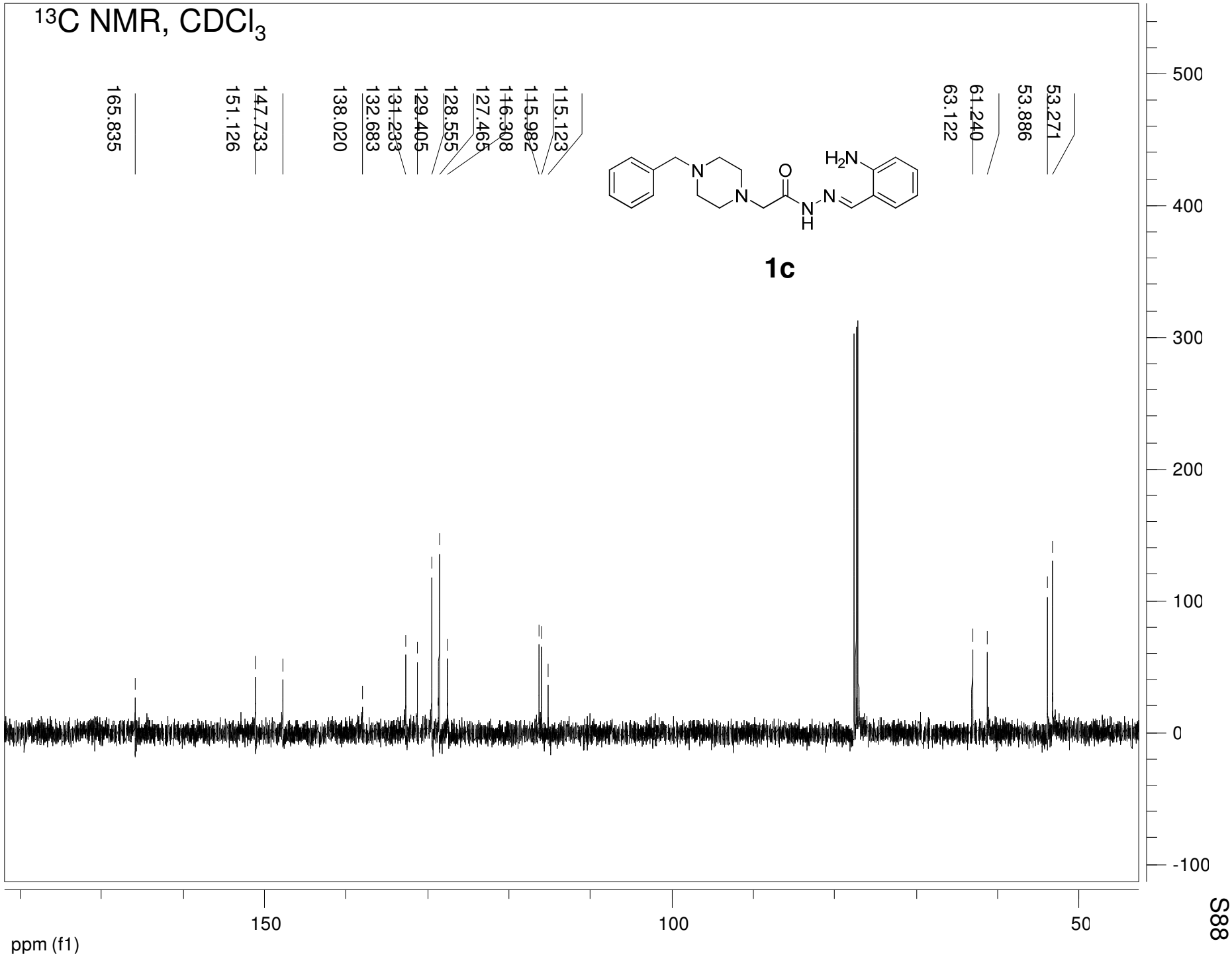
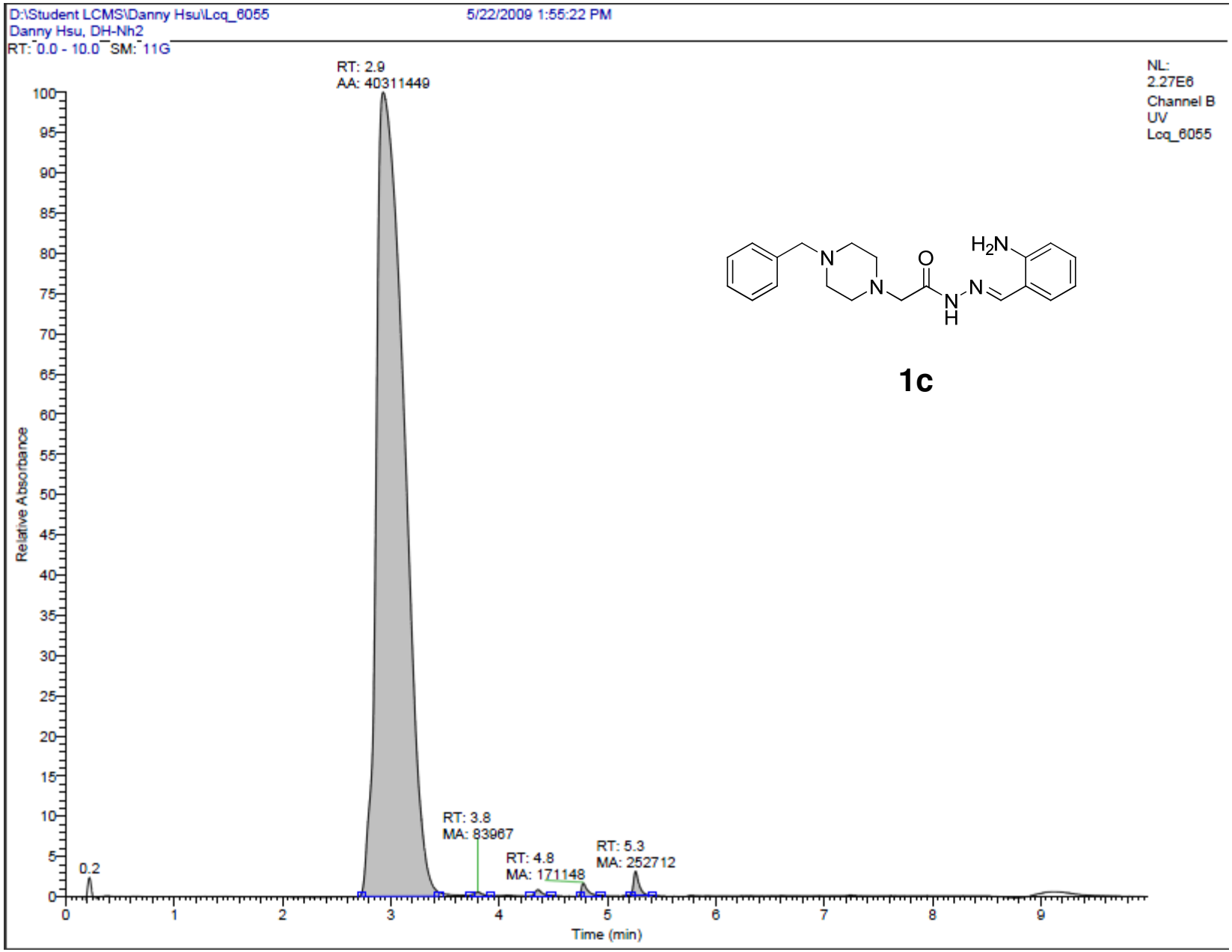
19.250	92588	BB	.194	.57620

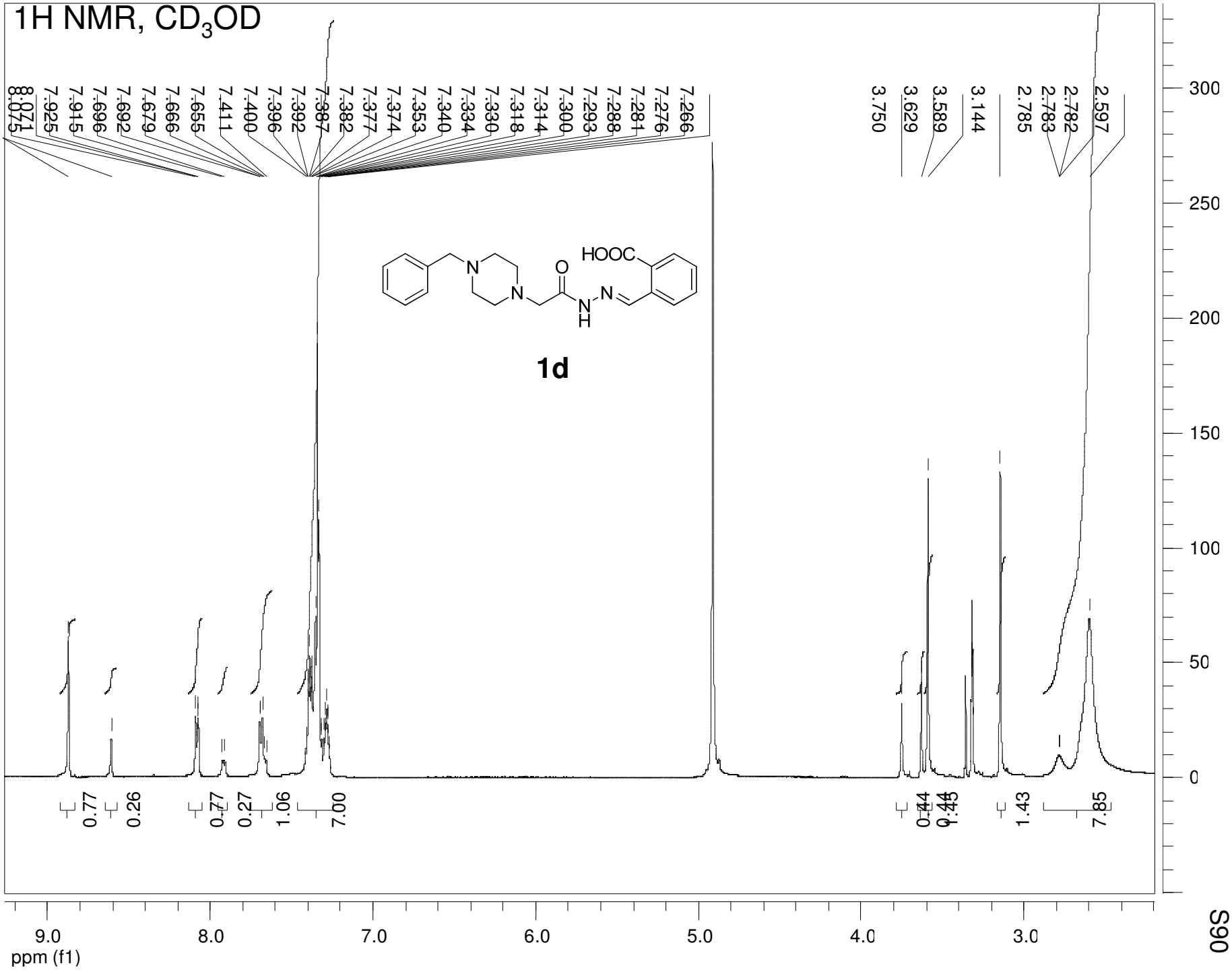
TOTAL AREA=1.6069E+07
MUL FACTOR=1.0000E+00

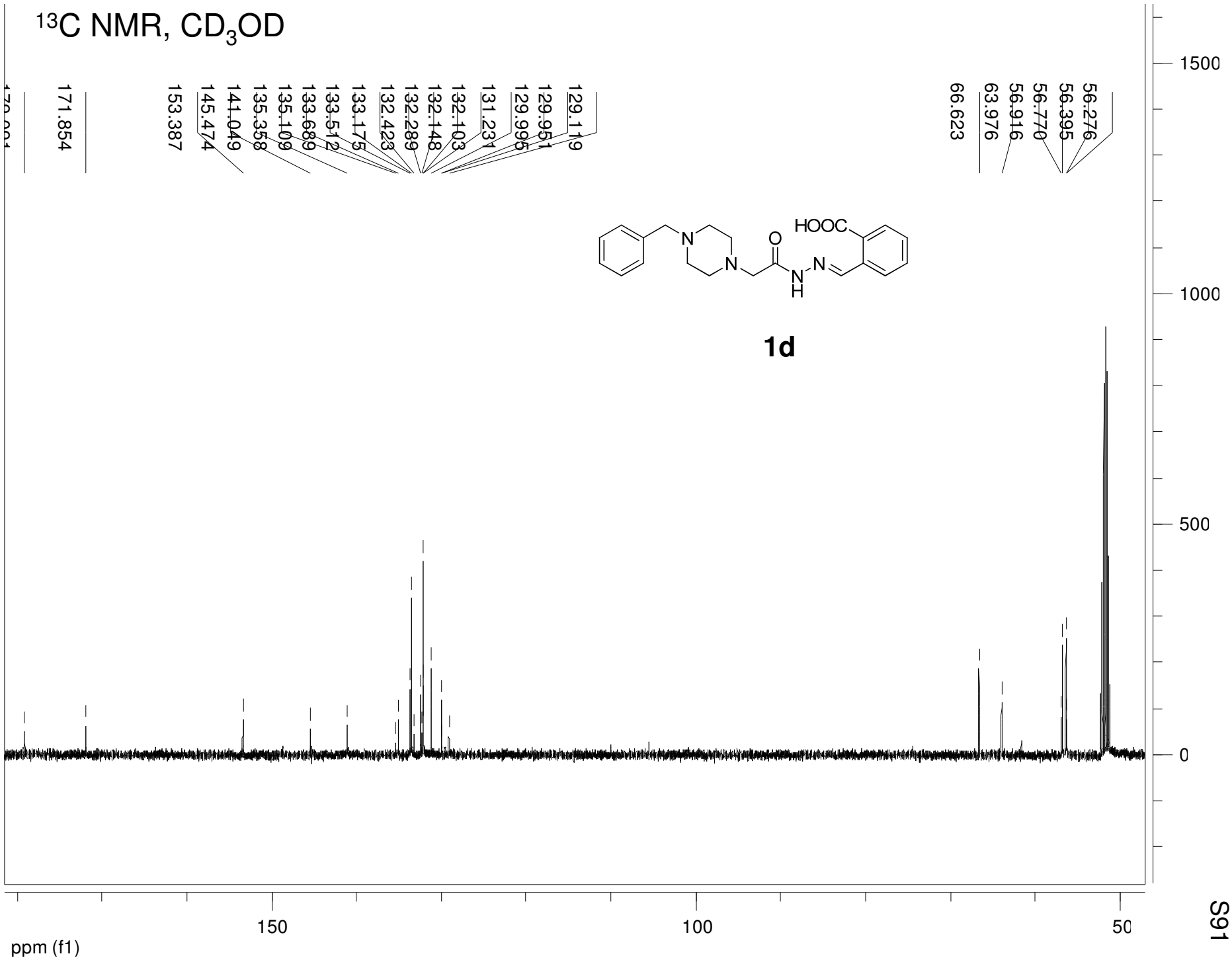

1H NMR, CDCl3
9.958
8.128
7.333
7.325
7.288
7.280
7.271
7.260
7.156
7.139
7.135
7.124
7.119
6.698
6.690
6.682
6.672
6.657
6.642
6.116
3.552
3.181
2.625
2.542
H2N
O
N
N
N
H
1c
4000
3000
2000
1000
0
0.80
0.85
1.86
1.16
2.40
2.28
1.88
2.17
2.00
8.04
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)


13C NMR, CDCl3
165.835
151.126
147.733
138.020
132.683
131.233
129.405
128.555
127.465
116.308
115.982
115.123
63.122
61.240
53.886
53.271
H2N
O
N
N
N
H
1c
500
400
300
200
100
0
-100
150
100
50
ppm (f1)

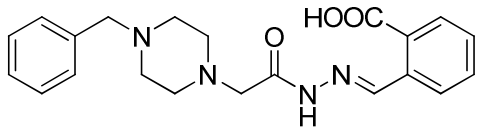
LC-MS
Purity: 99%



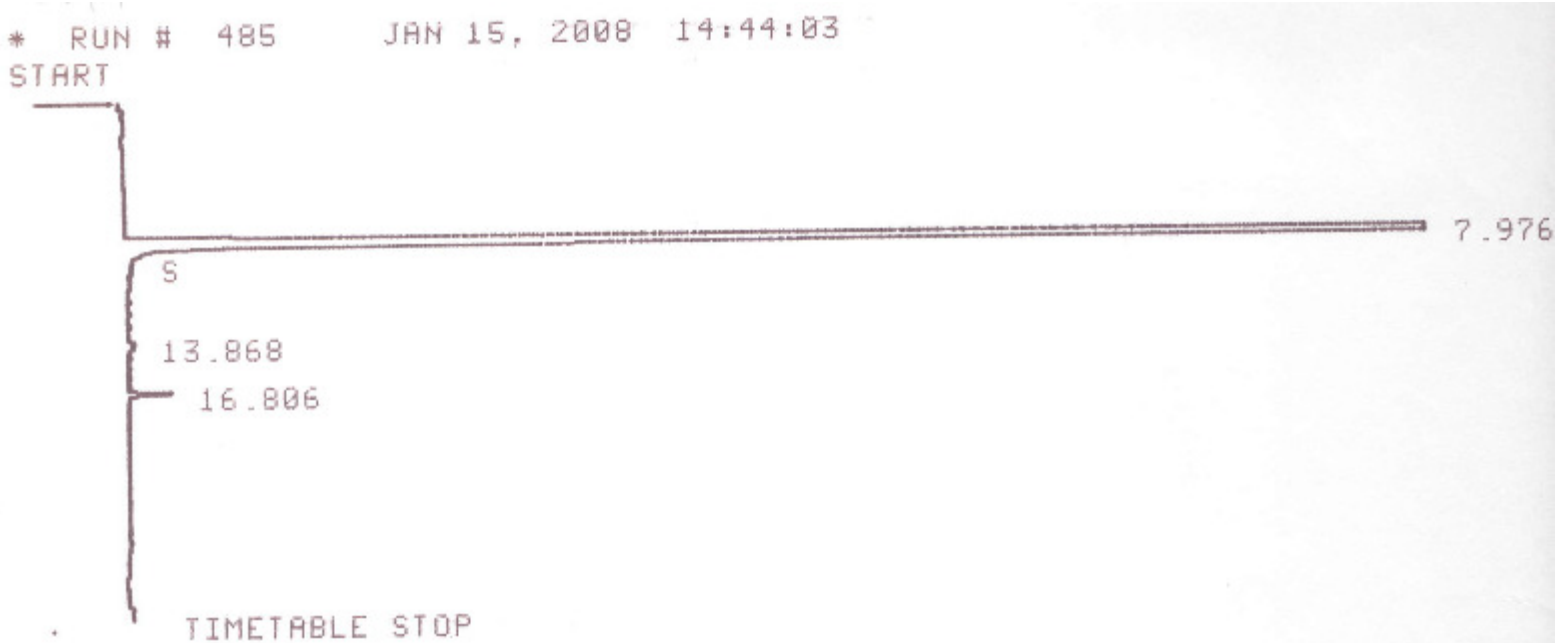

1H NMR, CD3OD
8.071
7.925
7.915
7.696
7.692
7.679
7.666
7.655
7.411
7.400
7.396
7.392
7.387
7.382
7.377
7.374
7.353
7.340
7.334
7.330
7.318
7.314
7.300
7.293
7.288
7.281
7.276
7.266
3.750
3.629
3.589
3.144
2.785
2.783
2.782
2.597
HOOC
O
N
N
N
H
1d
0.77
0.26
0.77
0.27
1.06
7.00
1.43
7.85
300
250
200
150
100
50
0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)


^{13}C NMR, CD_3OD
171.854
153.387
145.474
141.049
135.358
135.109
133.689
133.512
133.175
132.423
132.289
132.148
132.103
131.231
129.995
129.951
129.119
66.623
63.976
56.916
56.770
56.395
56.276
HOOC
O
N
N
N
H
1d
1500
1000
500
0
150
100
50
ppm (f1)

HPLC



1d

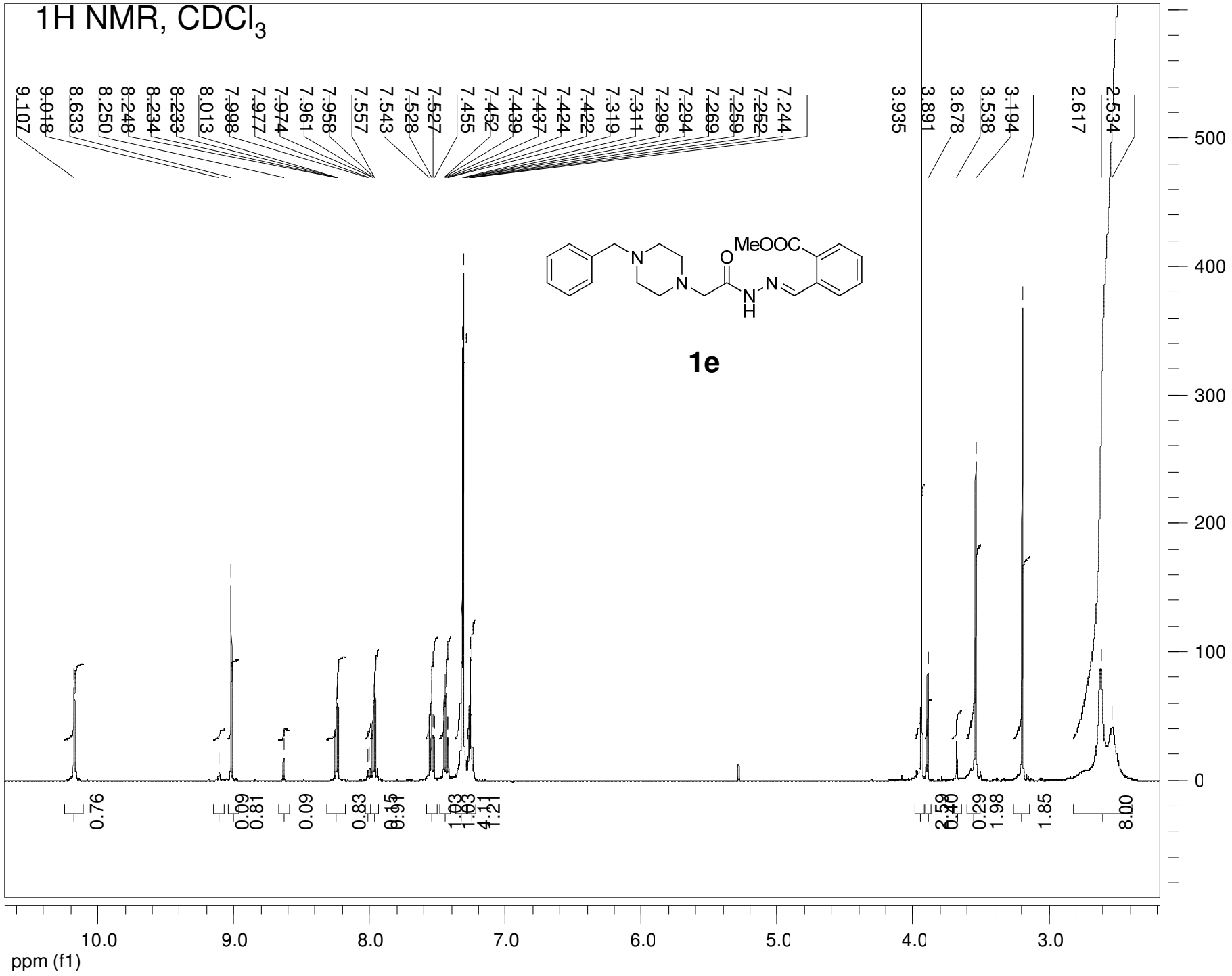


RUN# 485 JAN 15, 2008 14:44:03

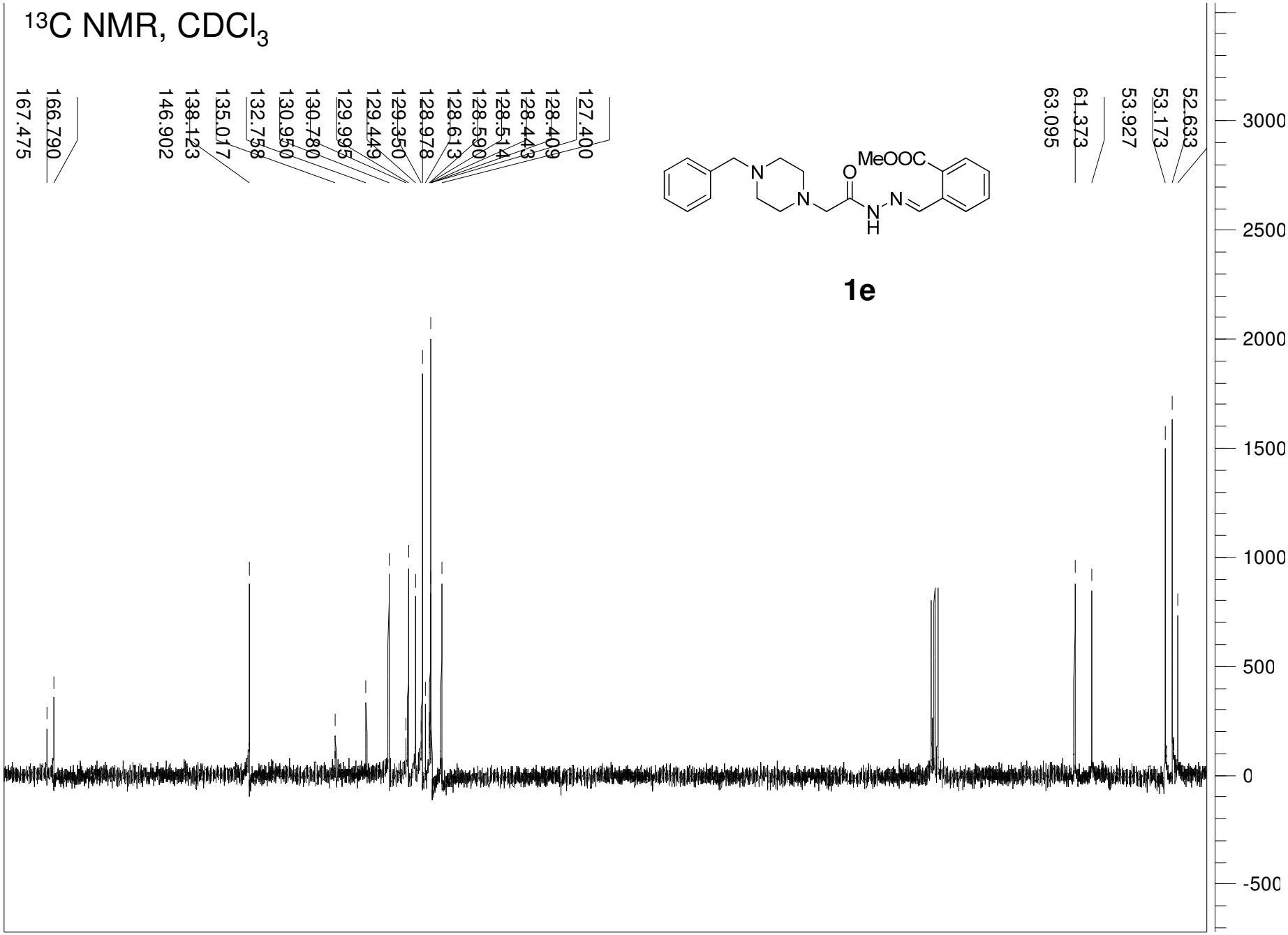
AREA%

RT	AREA	TYPE	WIDTH	AREA%
7.976	95377600	SBB	.343	99.04042
13.868	182115	BV	.259	.18911
16.806	741989	BV	.172	.77048

TOTAL AREA=9.6302E+07
MUL FACTOR=1.0000E+00

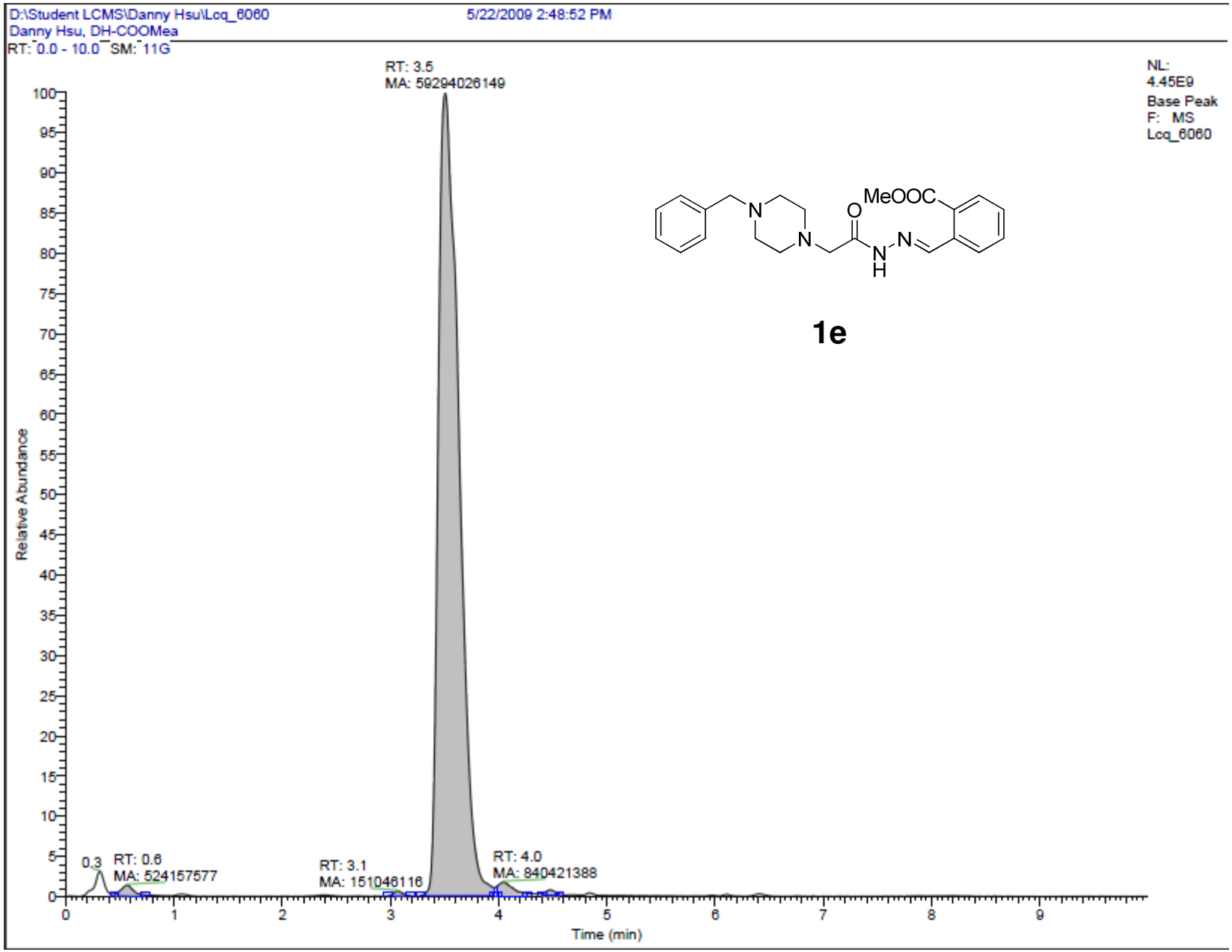

1H NMR, CDCl3
9.107
9.018
8.633
8.250
8.248
8.234
8.233
8.013
7.998
7.977
7.974
7.961
7.958
7.557
7.543
7.528
7.527
7.455
7.452
7.439
7.437
7.424
7.422
7.319
7.311
7.296
7.294
7.269
7.259
7.252
7.244
3.935
3.891
3.678
3.538
3.194
2.617
2.534
MeOOC
O
N
N
N
H
1e
500
400
300
200
100
0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)

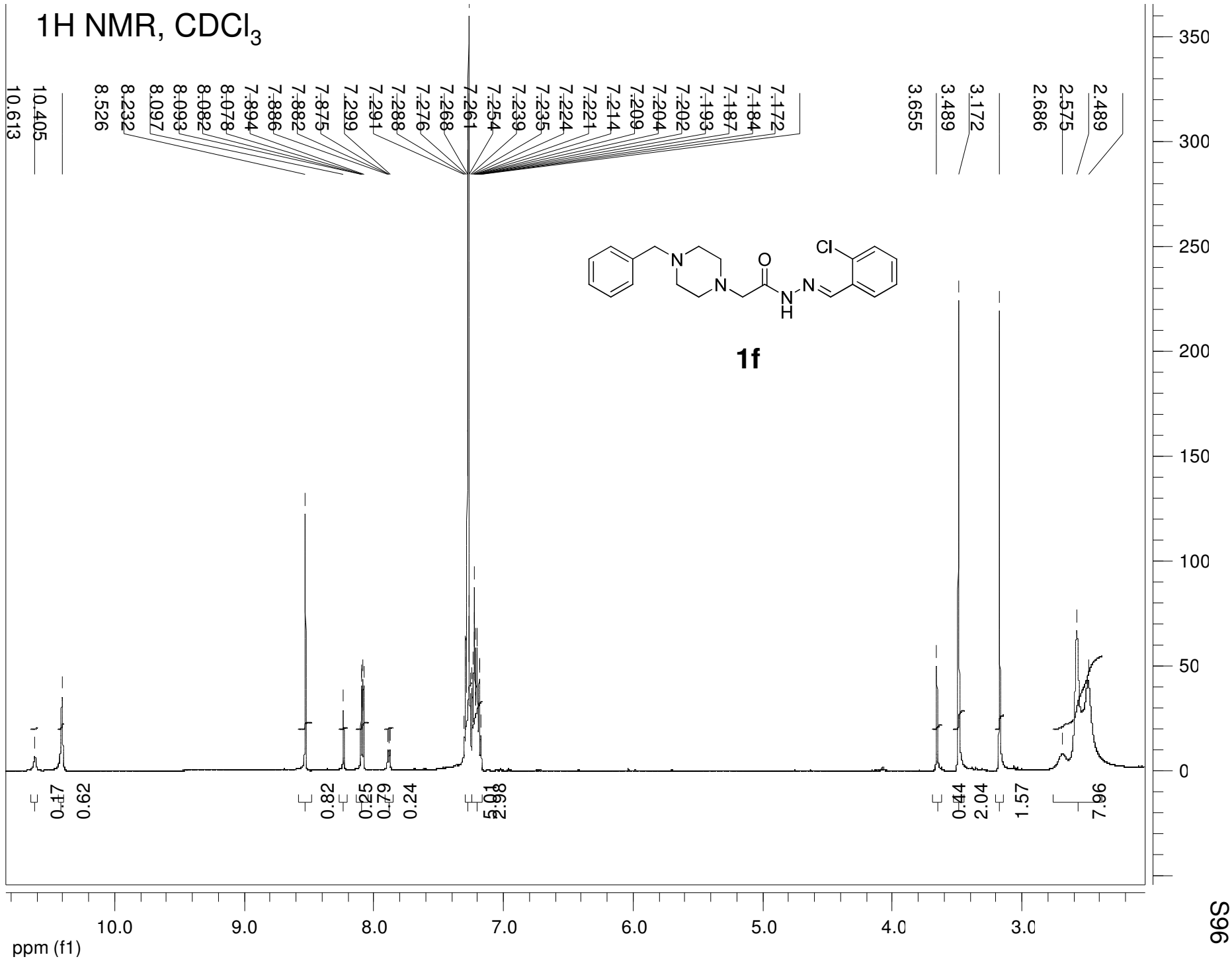
^{13}C NMR, $CDCl_3$

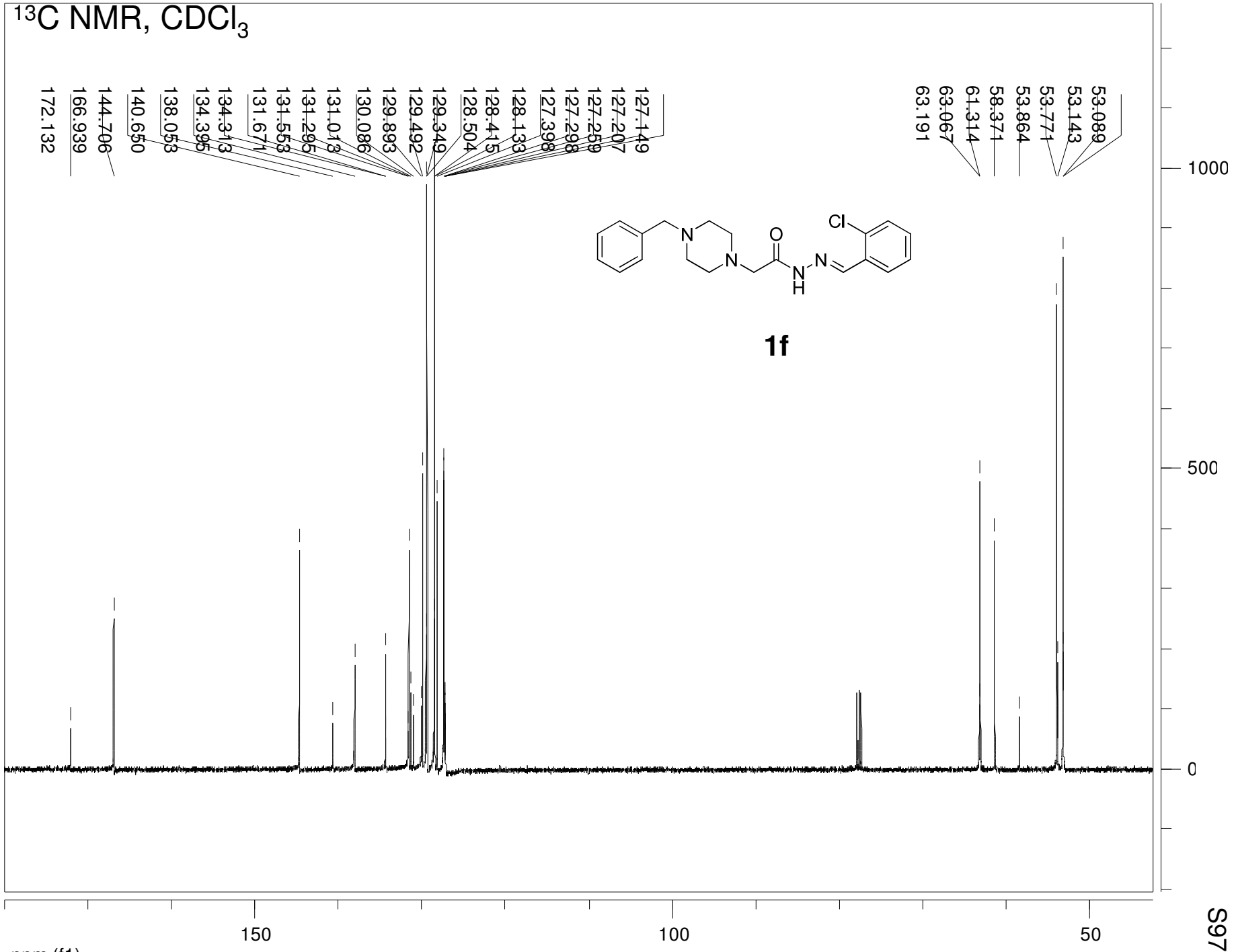


1e

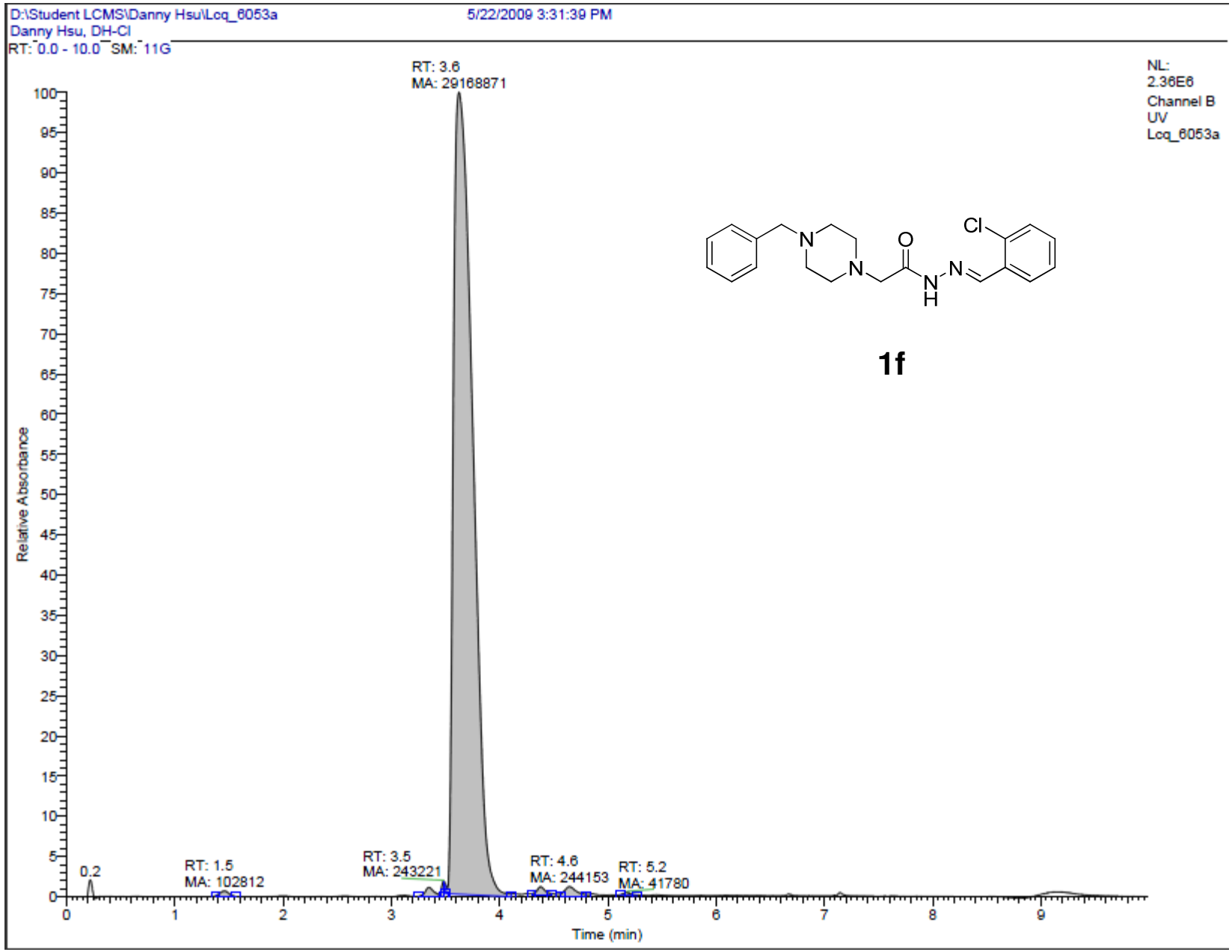
LC-MS
Purity: 98%

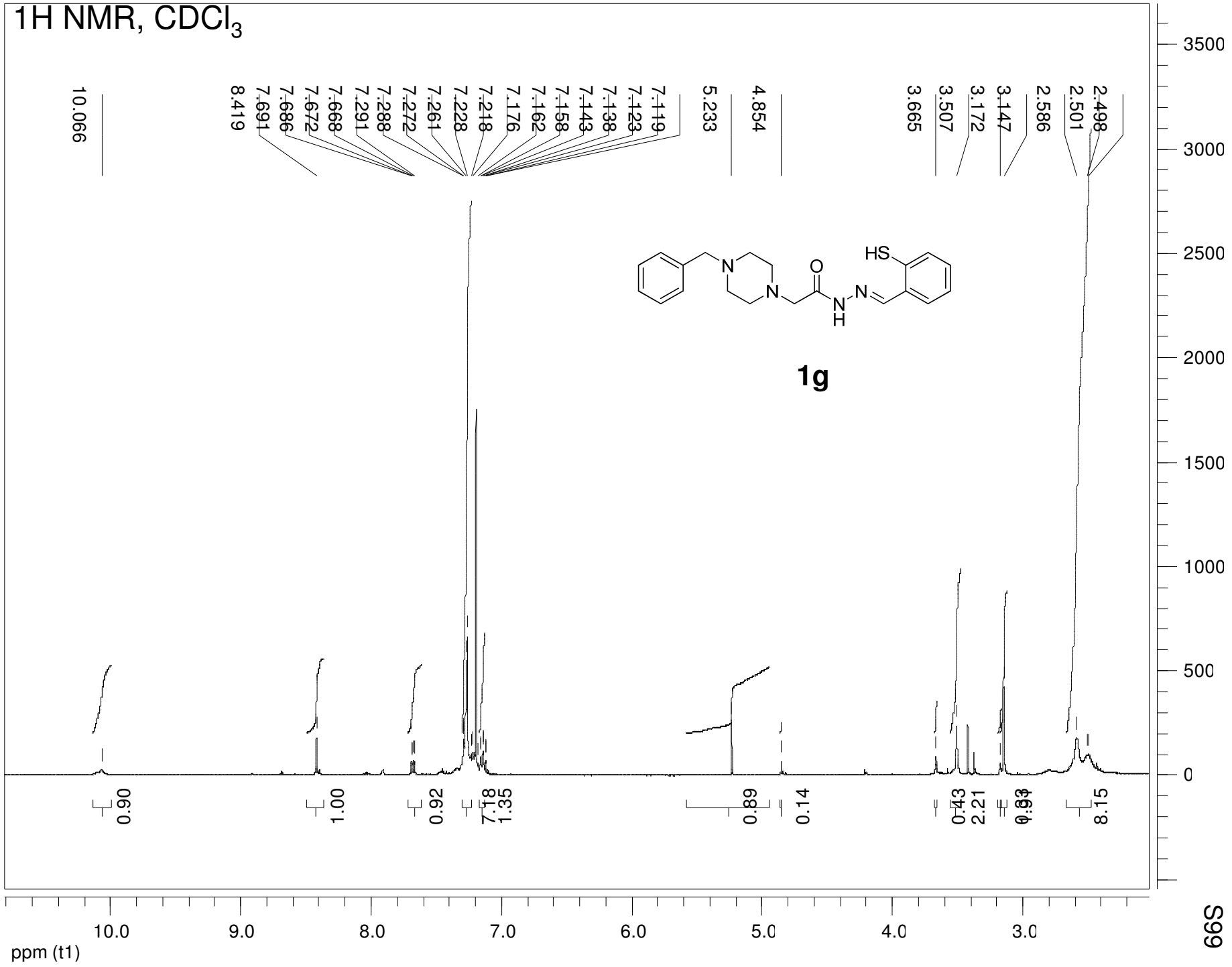


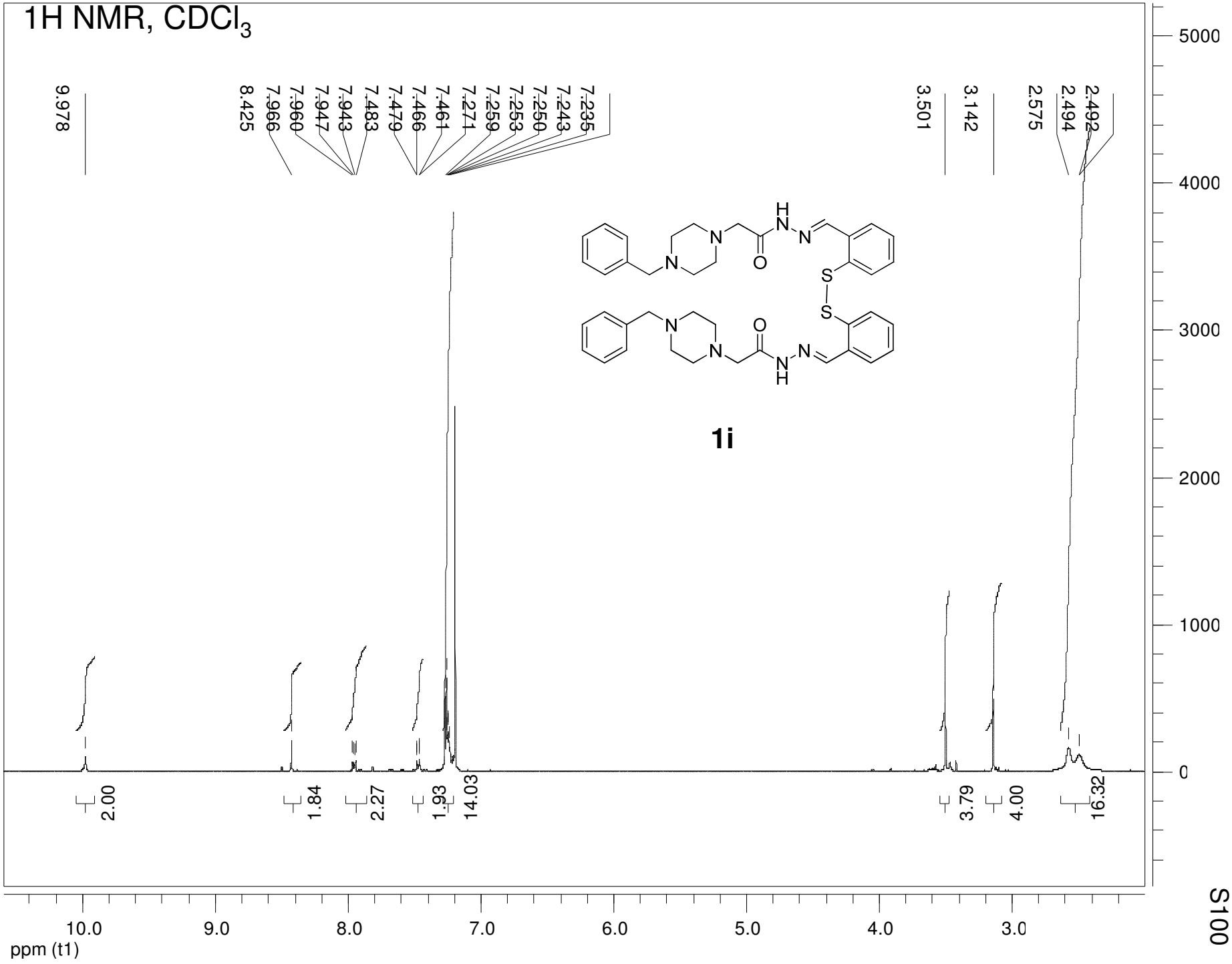

1H NMR, CDCl3
10.613
10.405
8.526
8.232
8.097
8.093
8.082
8.078
7.894
7.886
7.882
7.875
7.299
7.291
7.288
7.276
7.268
7.261
7.254
7.239
7.235
7.224
7.221
7.214
7.209
7.204
7.202
7.193
7.187
7.184
7.172
3.655
3.489
3.172
2.686
2.575
2.489
Cl
O
N
N
N
H
1f
0.17
0.92
0.82
0.55
0.79
0.24
5.01
2.98
0.44
2.04
1.57
7.96
ppm (f1)


^{13}C NMR, $CDCl_3$
172.132
166.939
144.706
140.650
138.053
134.395
134.313
131.671
131.553
131.295
131.013
130.086
129.893
129.492
129.349
128.504
128.415
128.133
127.398
127.298
127.259
127.207
127.149
63.191
63.067
61.314
58.371
53.864
53.771
53.143
53.089
Cl
O
N
N
N
NH
1f
1000
500
0
150
100
50
ppm (f1)

LC-MS
Purity: 98%




1H NMR, CDCl3
10.066
8.419
7.691
7.686
7.672
7.668
7.291
7.288
7.272
7.261
7.228
7.218
7.176
7.162
7.158
7.143
7.138
7.123
7.119
5.233
4.854
3.665
3.507
3.172
3.147
2.586
2.501
2.498
HS
N
O
N
N
H
1g
0.90
1.00
0.92
7.18
1.35
0.89
0.14
3.13
2.21
8.15
3500
3000
2500
2000
1500
1000
500
0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t1)

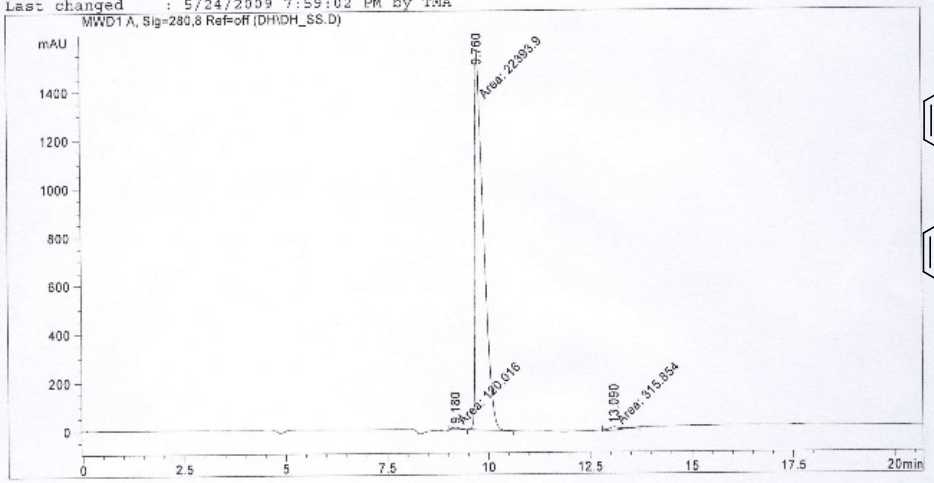

1H NMR, CDCl3
9.978
8.425
7.966
7.960
7.947
7.943
7.483
7.479
7.466
7.461
7.271
7.259
7.253
7.250
7.243
7.235
3.501
3.142
2.575
2.494
2.492
1i
2.00
1.84
2.27
1.93
14.03
3.79
4.00
16.32
5000
4000
3000
2000
1000
0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t1)

HPLC

Data File D:\HPCHEM\DATA\2009\DH\DH_SS.D
Sample Name: DH-SS

Acq. Operator : DH
Acq. Instrument : El Chupacabra Location : P1-F-06
Injection Date : 5/25/2009 12:05:09 AM Inj Volume : 10 µl

Acq. Method : C:\HPCHEM\1\METHODS\DH.M
Last changed : 5/24/2009 11:34:17 PM by DH
(modified after loading)
Analysis Method : C:\HPCHEM\1\METHODS\DH.M
Last changed : 5/24/2009 7:59:02 PM by TMA



Fraction Information

Fraction collection off

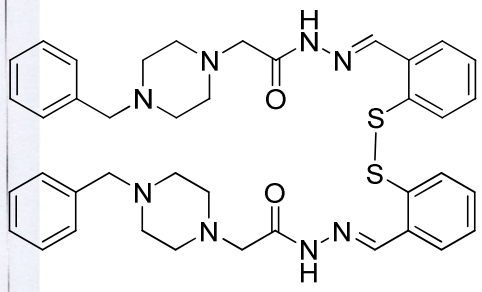
No Fractions found.

Area Percent Report

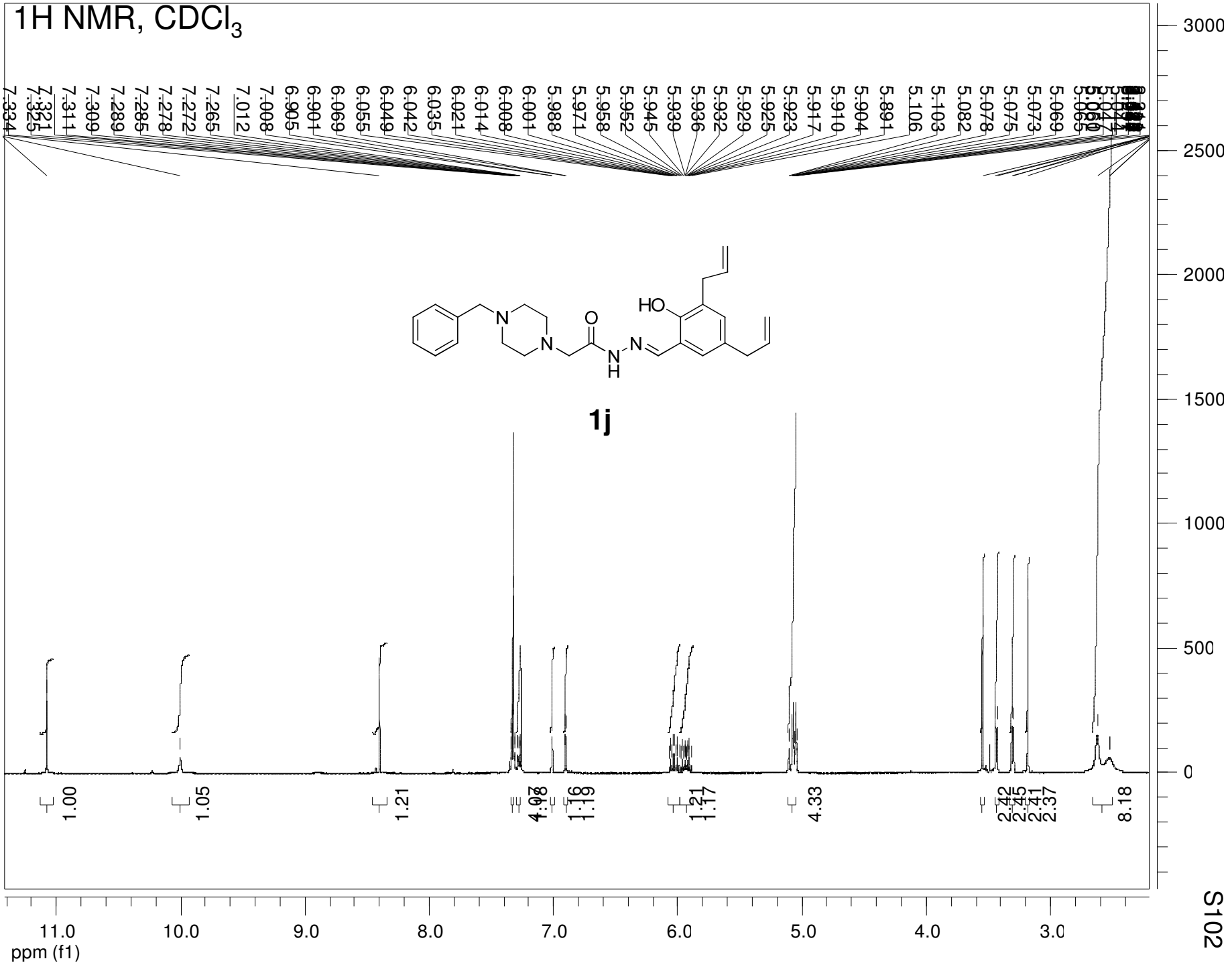
Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

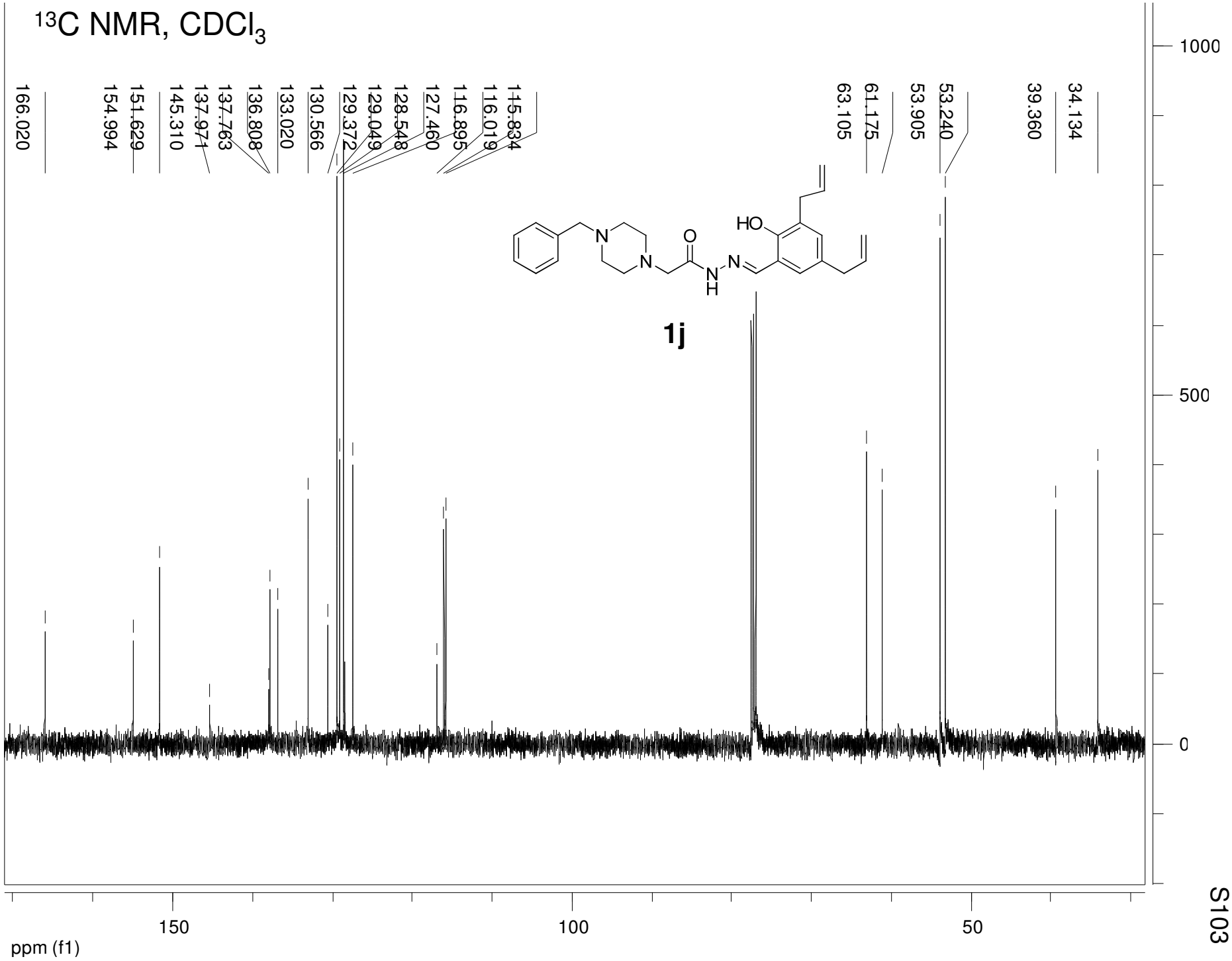
Signal 1: MWD1 A, Sig=280,8 Ref-off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.180	MM	0.2207	120.01580	9.06414	0.5257
2	9.760	MM	0.2389	2.23939e4	1562.06506	98.0908
3	13.090	MM	0.4011	315.85379	13.12333	1.3835
Totals :				2.28298e4	1584.25253	

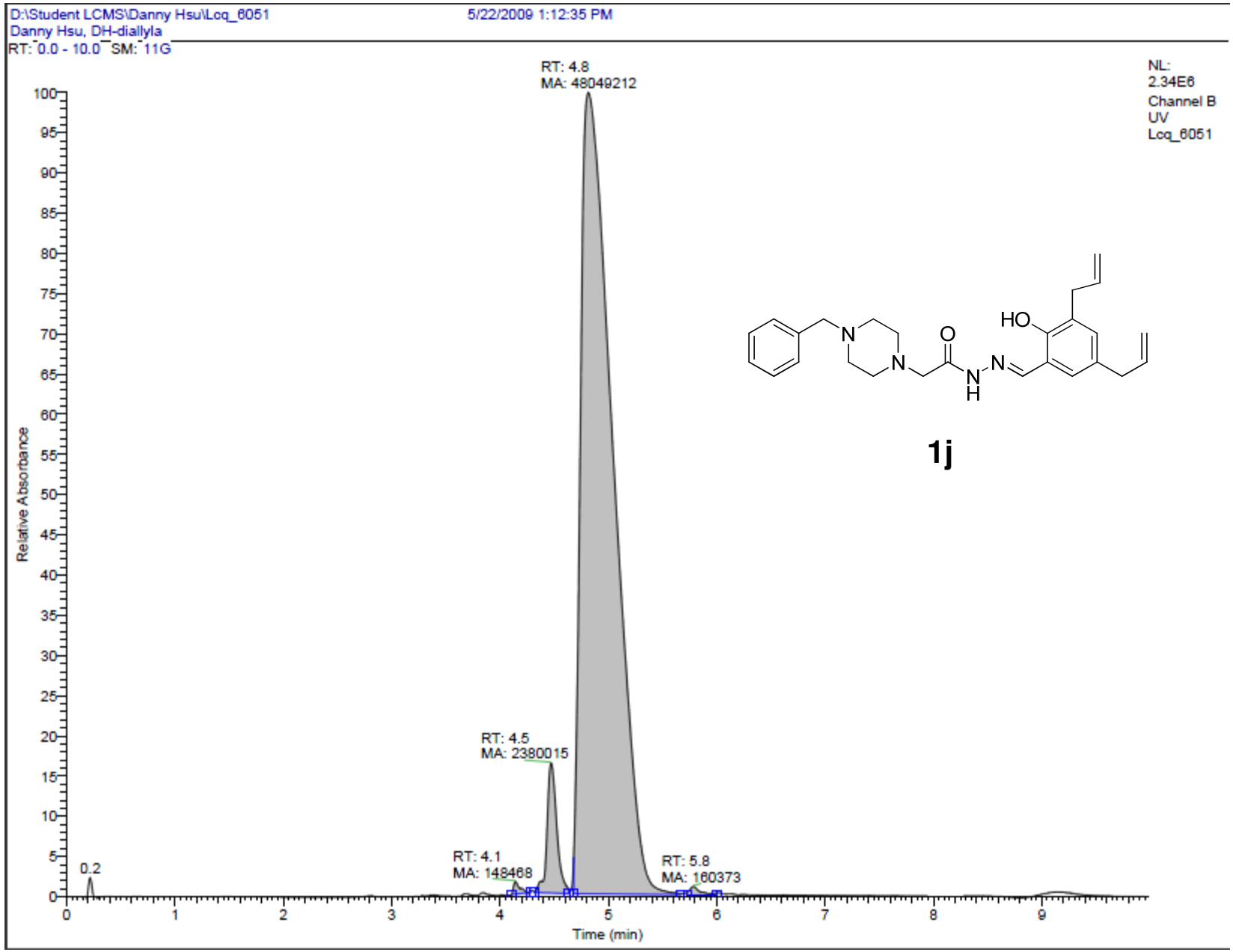


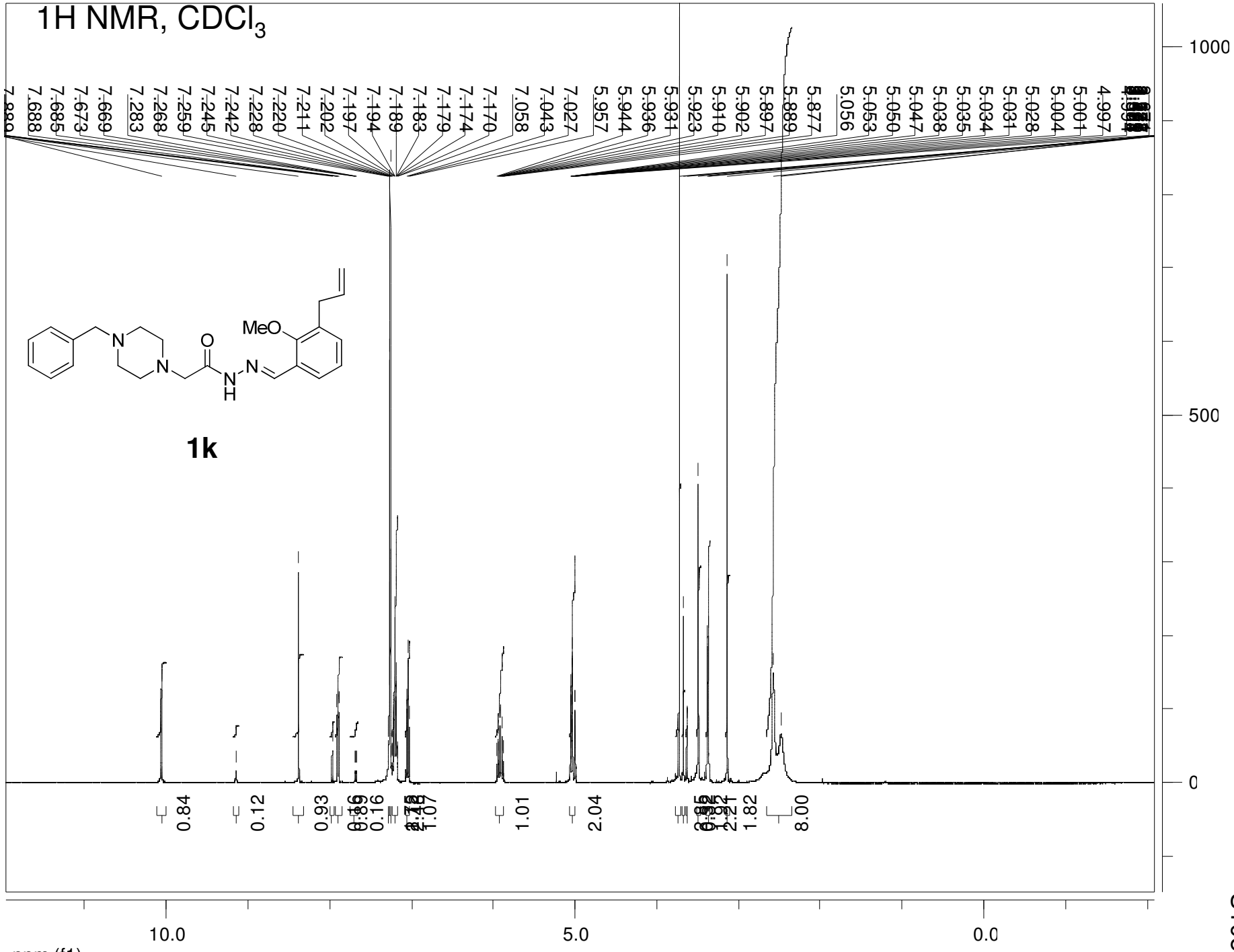
1i


1H NMR, CDCl3
HO
O
N
N
N
N
H
1j
3000
2500
2000
1500
1000
500
0
1.00
1.05
1.21
4.07
1.16
1.19
1.21
1.17
4.33
2.42
2.45
2.41
2.37
8.18
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)

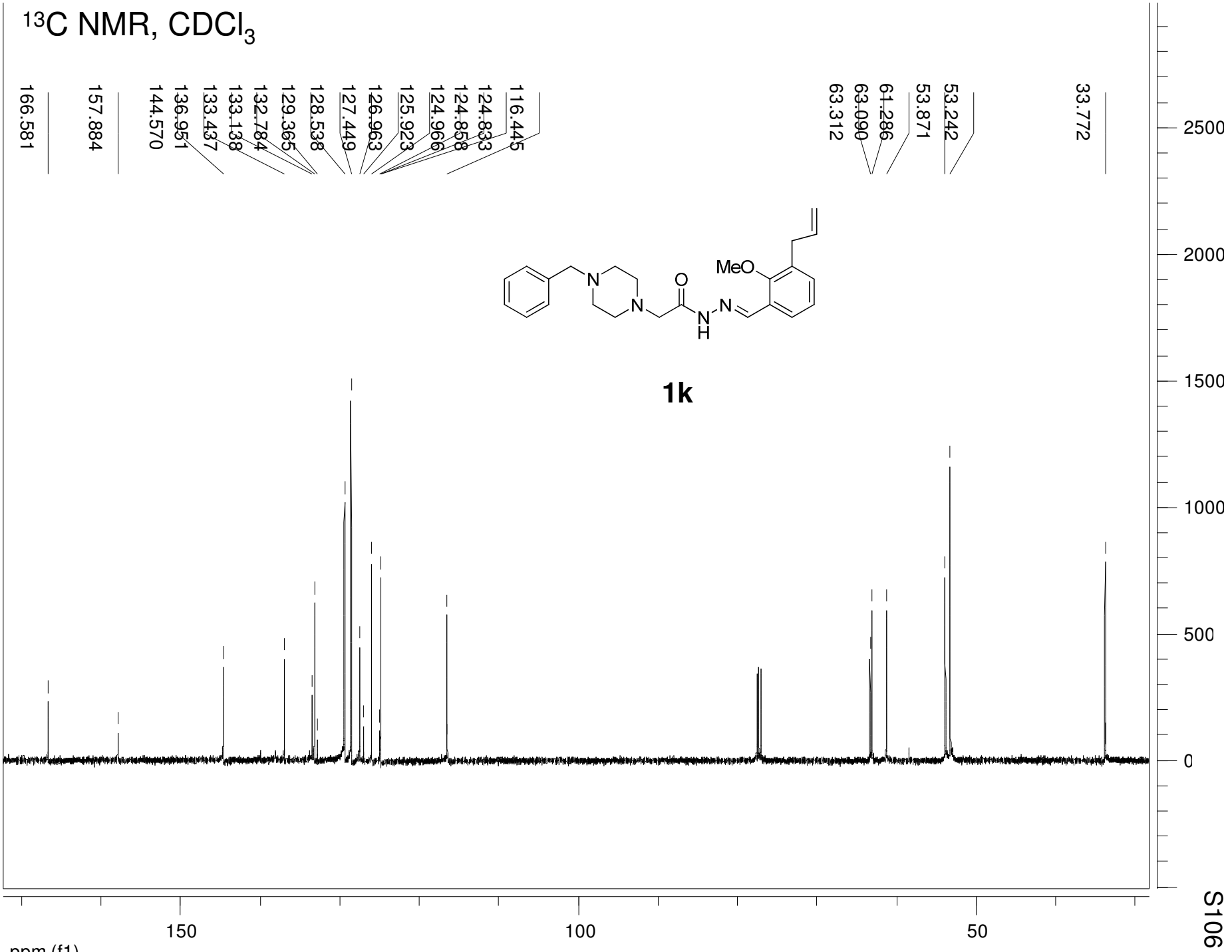

^{13}C NMR, $CDCl_3$
166.020
154.994
151.629
145.310
137.971
137.763
136.808
133.020
130.566
129.372
129.049
128.548
127.460
116.895
116.019
115.834
63.105
61.175
53.905
53.240
39.360
34.134
HO
N
O
N
NH
N
1j
1000
500
0
150
100
50
ppm (f1)

LC-MS
Purity: 95%



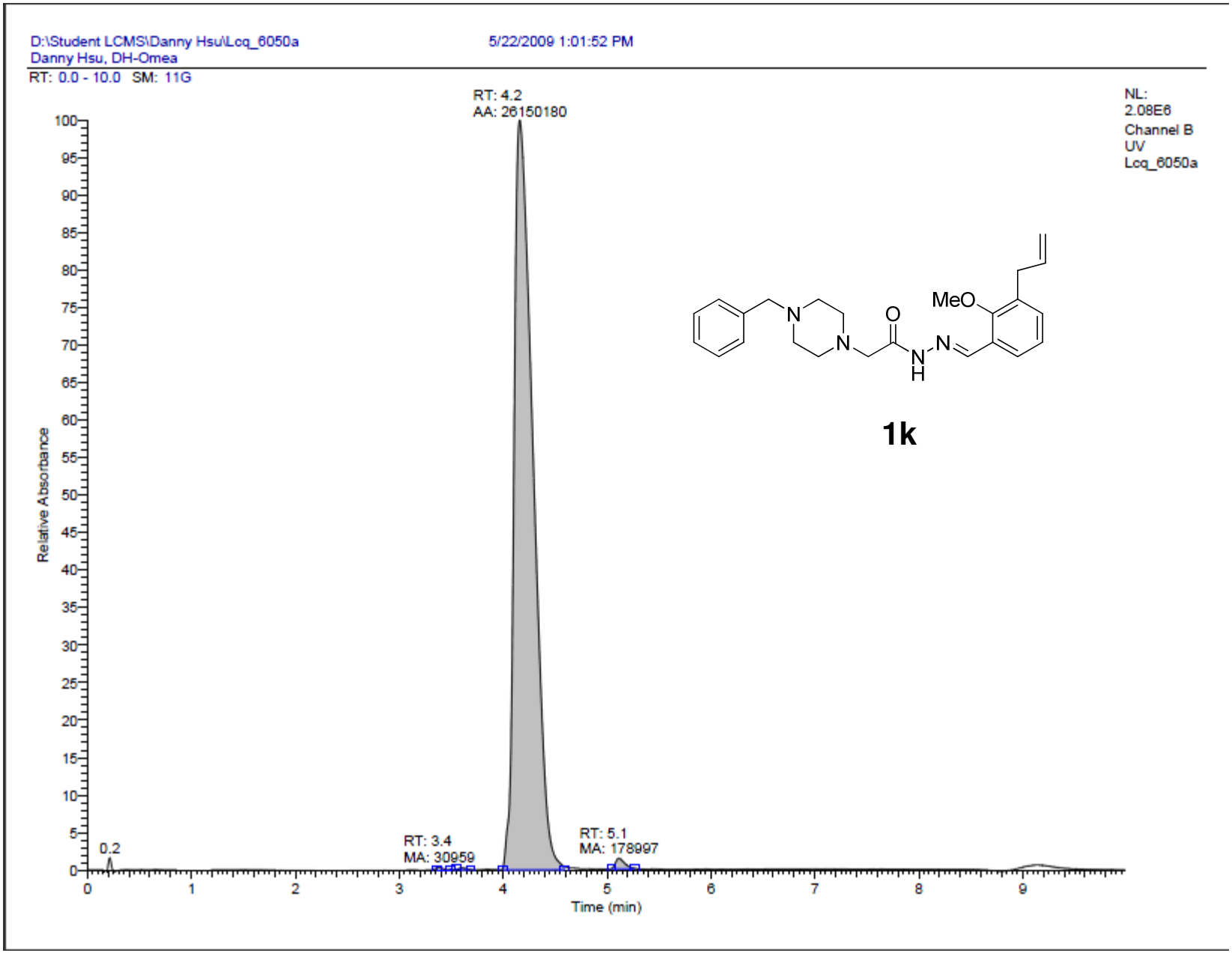

1H NMR, CDCl3
MeO
N
O
N
N
H
1k
1000
500
0
10.0
5.0
0.0
ppm (f1)

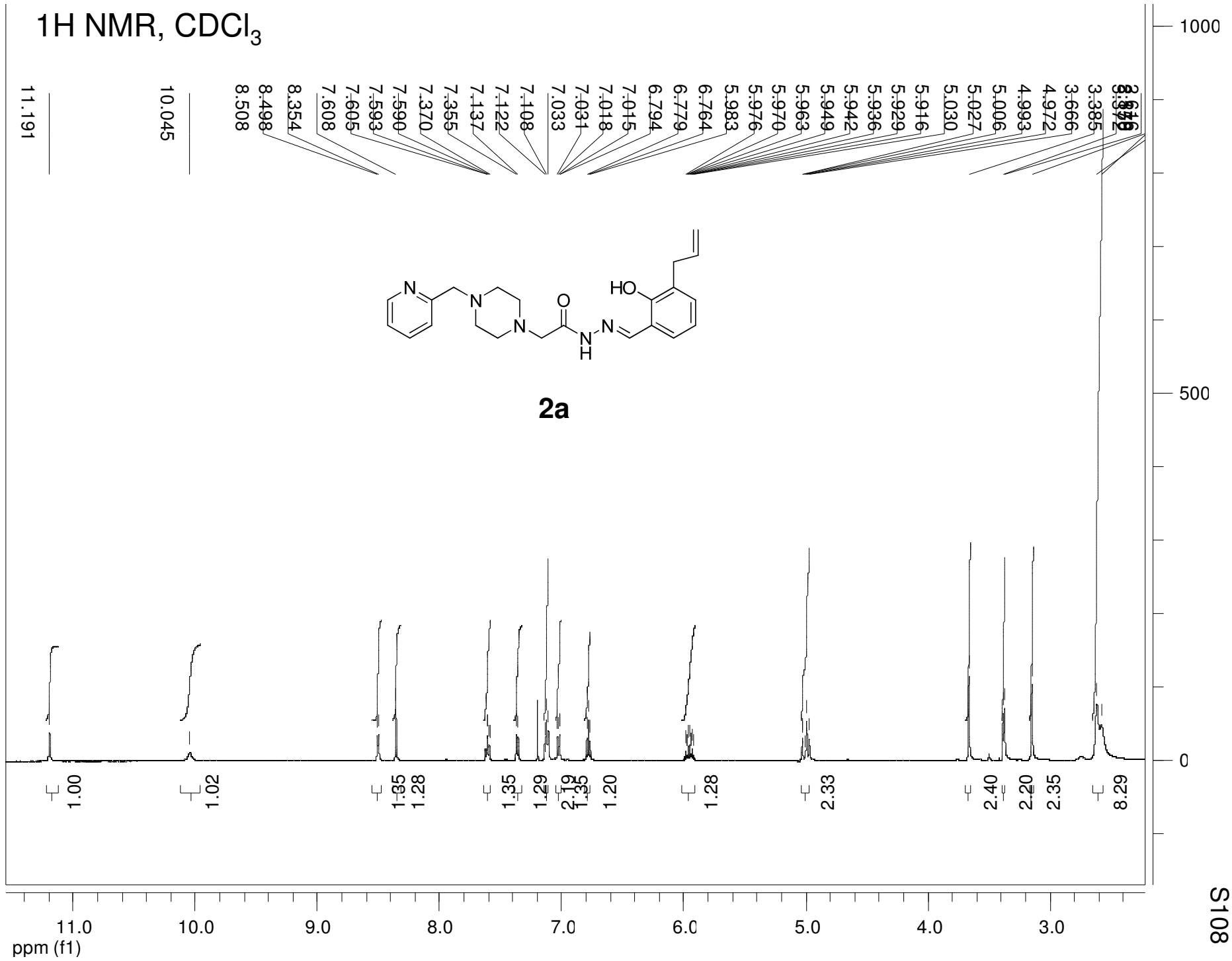
^{13}C NMR, $CDCl_3$



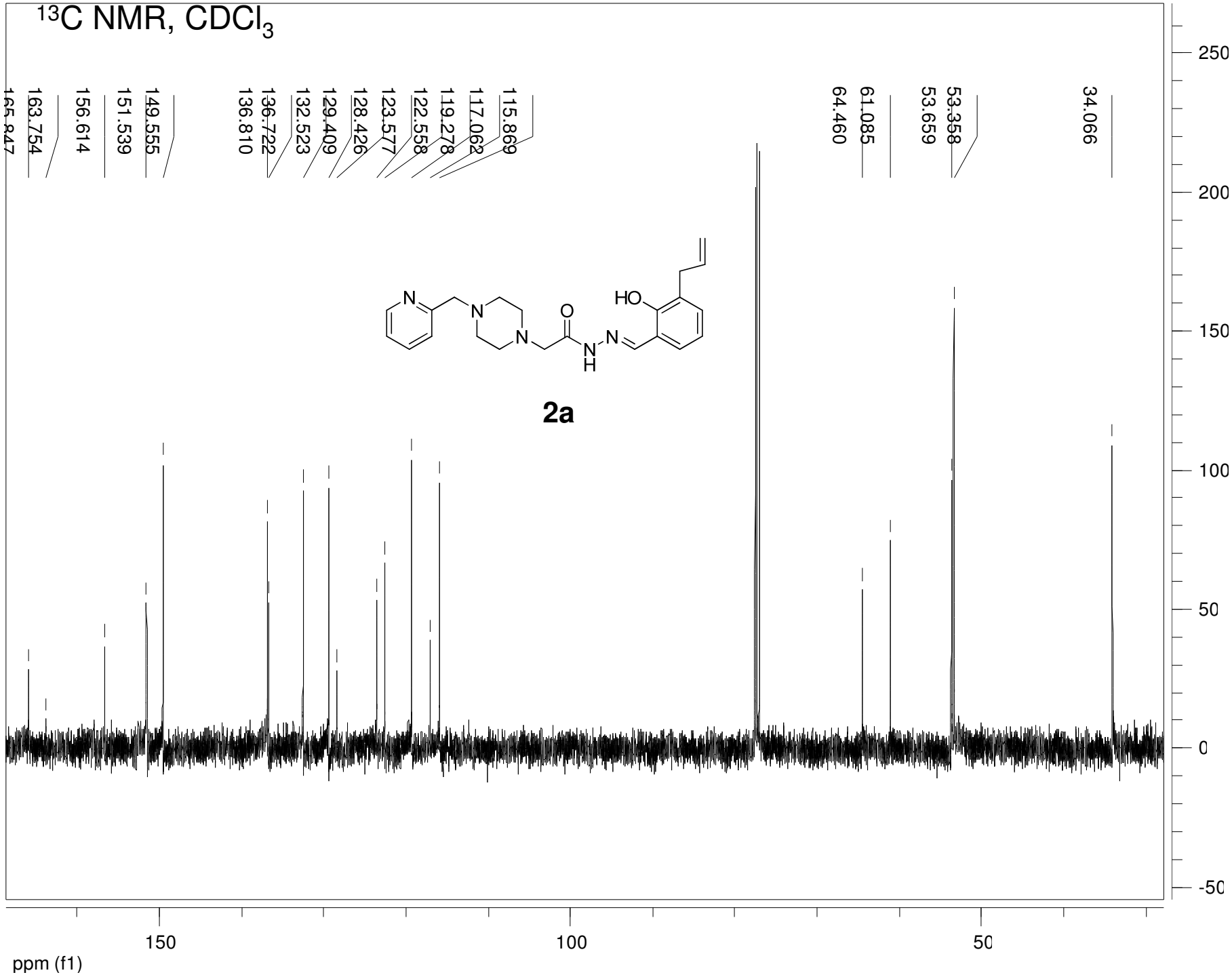
1k

LC-MS
Purity: 98%

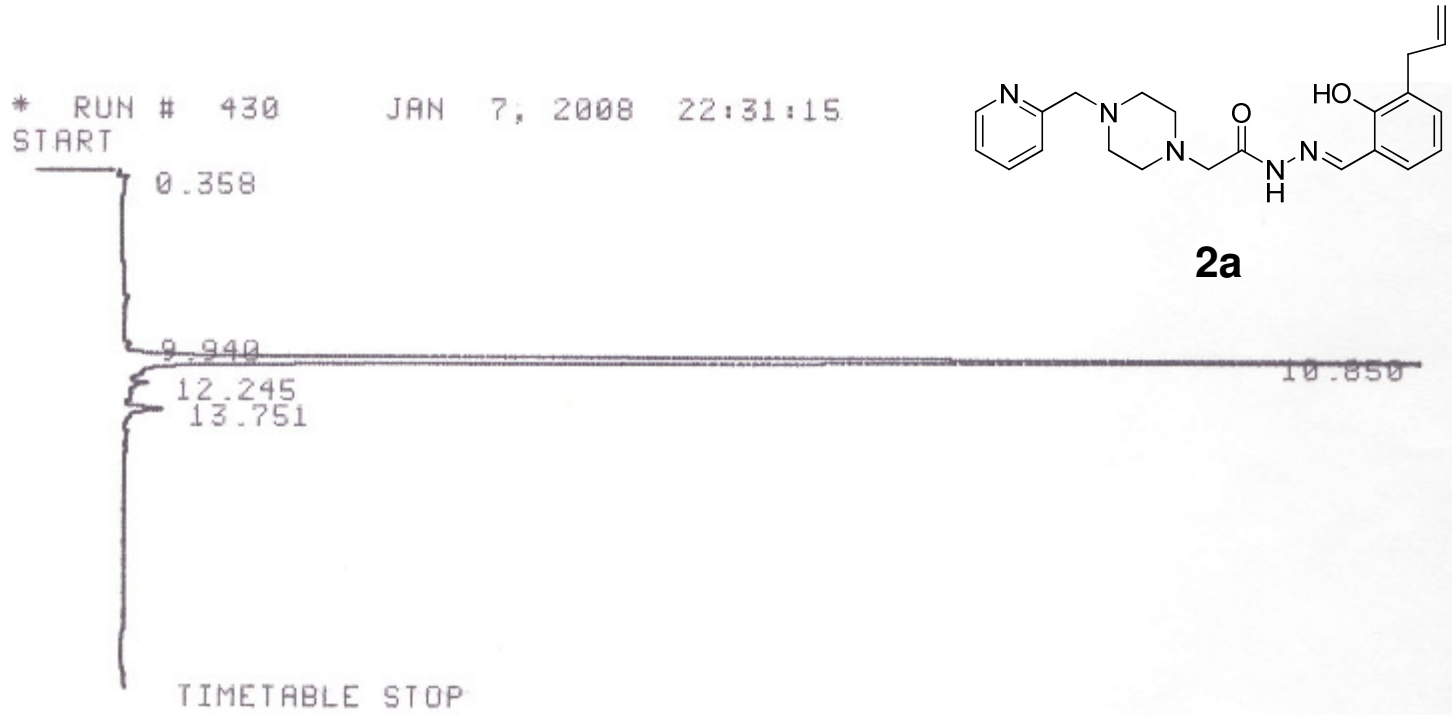



1H NMR, CDCl3
11.191
10.045
8.508
8.498
8.354
7.608
7.605
7.593
7.590
7.370
7.355
7.137
7.122
7.108
7.033
7.031
7.018
7.015
6.794
6.779
6.764
5.983
5.976
5.970
5.963
5.949
5.942
5.936
5.929
5.916
5.030
5.027
5.006
4.993
4.972
3.666
3.385
HO
N
O
1.00
1.02
1.35
1.28
1.35
1.29
2.19
1.35
1.20
1.28
2.33
2.40
2.20
2.35
8.29
1000
500
0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)

2a


^{13}C NMR, $CDCl_3$
165.847
163.754
156.614
151.539
149.555
136.810
136.722
132.523
129.409
128.426
123.577
122.558
119.278
117.062
115.869
64.460
61.085
53.659
53.358
34.066
HO
N
O
NH
2a
250
200
150
100
50
0
-50
150
100
50
ppm (f1)

HPLC



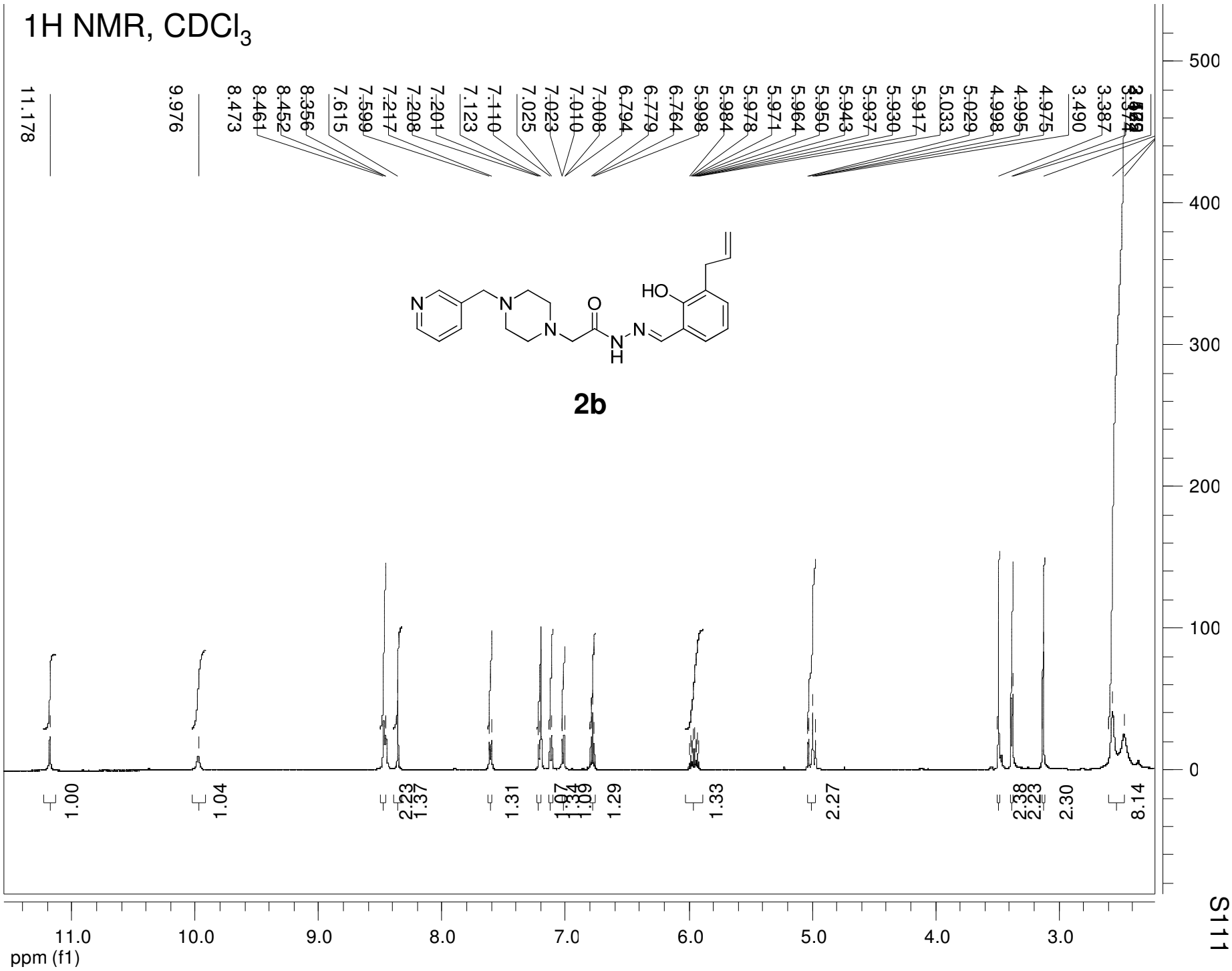
2a

RUN# 430 JAN 7, 2008 22:31:15

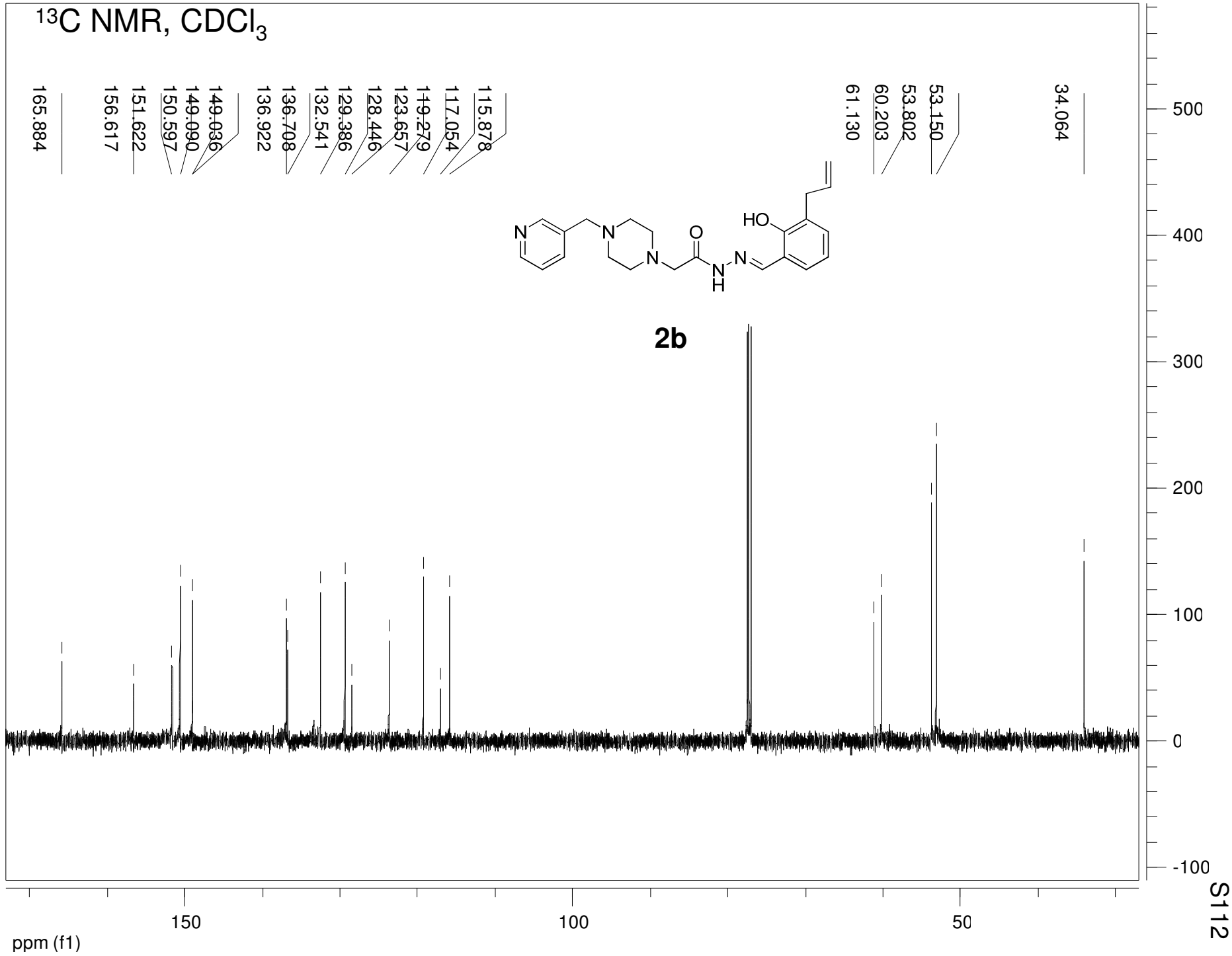
AREA%

RT	AREA	TYPE	WIDTH	AREA%
.358	156343	BB	.167	.35943
9.940	90278	BP	.166	.20755
10.850	41843424	PB	.281	96.19763
12.245	364354	PP	.209	.83765
13.751	1042960	BV	.308	2.39776

TOTAL AREA=4.3497E+07
MUL FACTOR=1.0000E+00

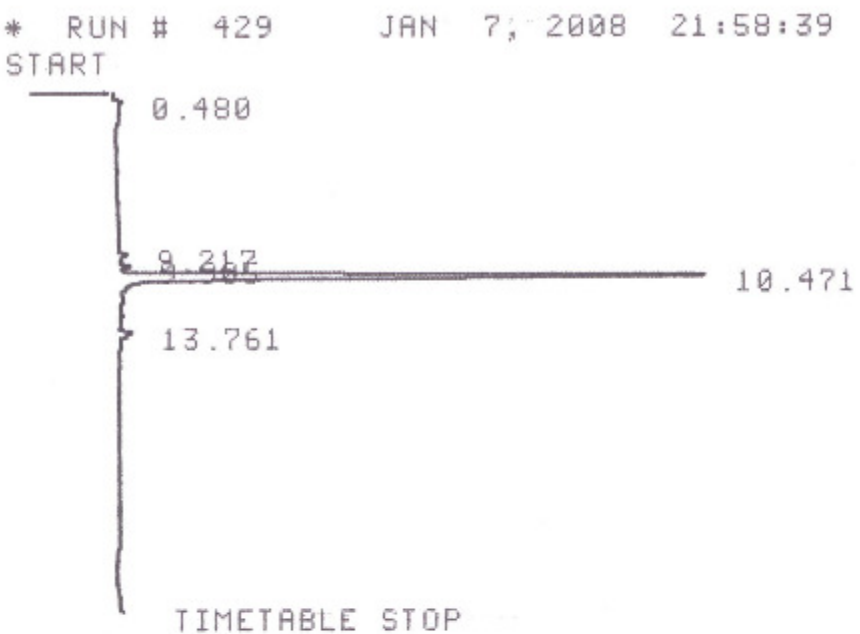

1H NMR, CDCl3
11.178
9.976
8.473
8.461
8.452
8.356
7.615
7.599
7.217
7.208
7.201
7.123
7.110
7.025
7.023
7.010
7.008
6.794
6.779
6.764
5.998
5.984
5.978
5.971
5.964
5.950
5.943
5.937
5.930
5.917
5.033
5.029
4.998
4.995
4.975
3.490
3.387
HO
N
O
NH
2b
1.00
1.04
2.23
1.37
1.31
1.07
1.34
1.09
1.29
1.33
2.27
2.38
2.23
2.30
8.14
500
400
300
200
100
0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)

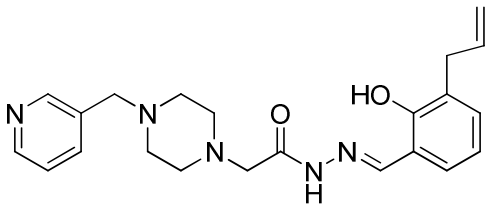
^{13}C NMR, $CDCl_3$



2b

HPLC





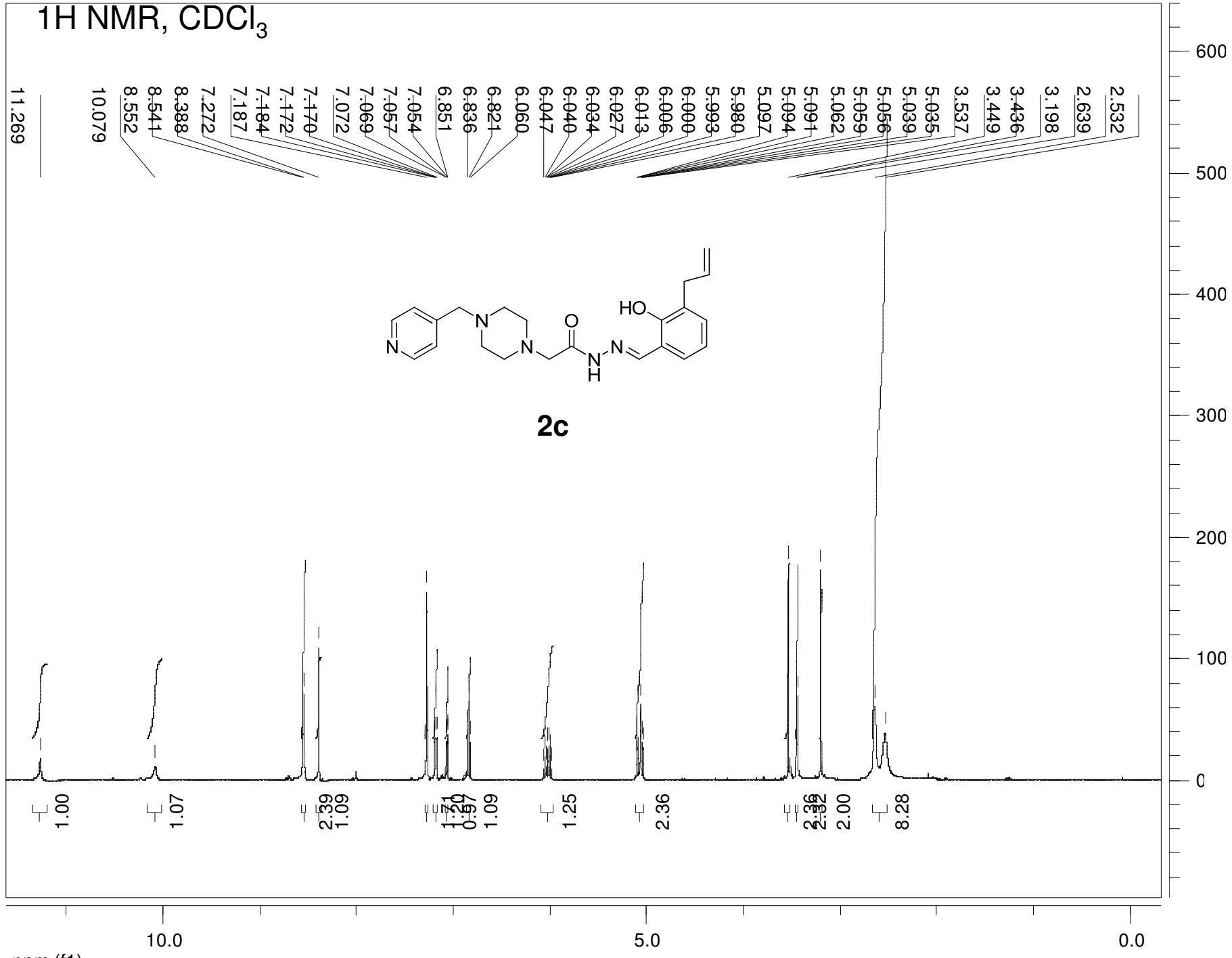
2b

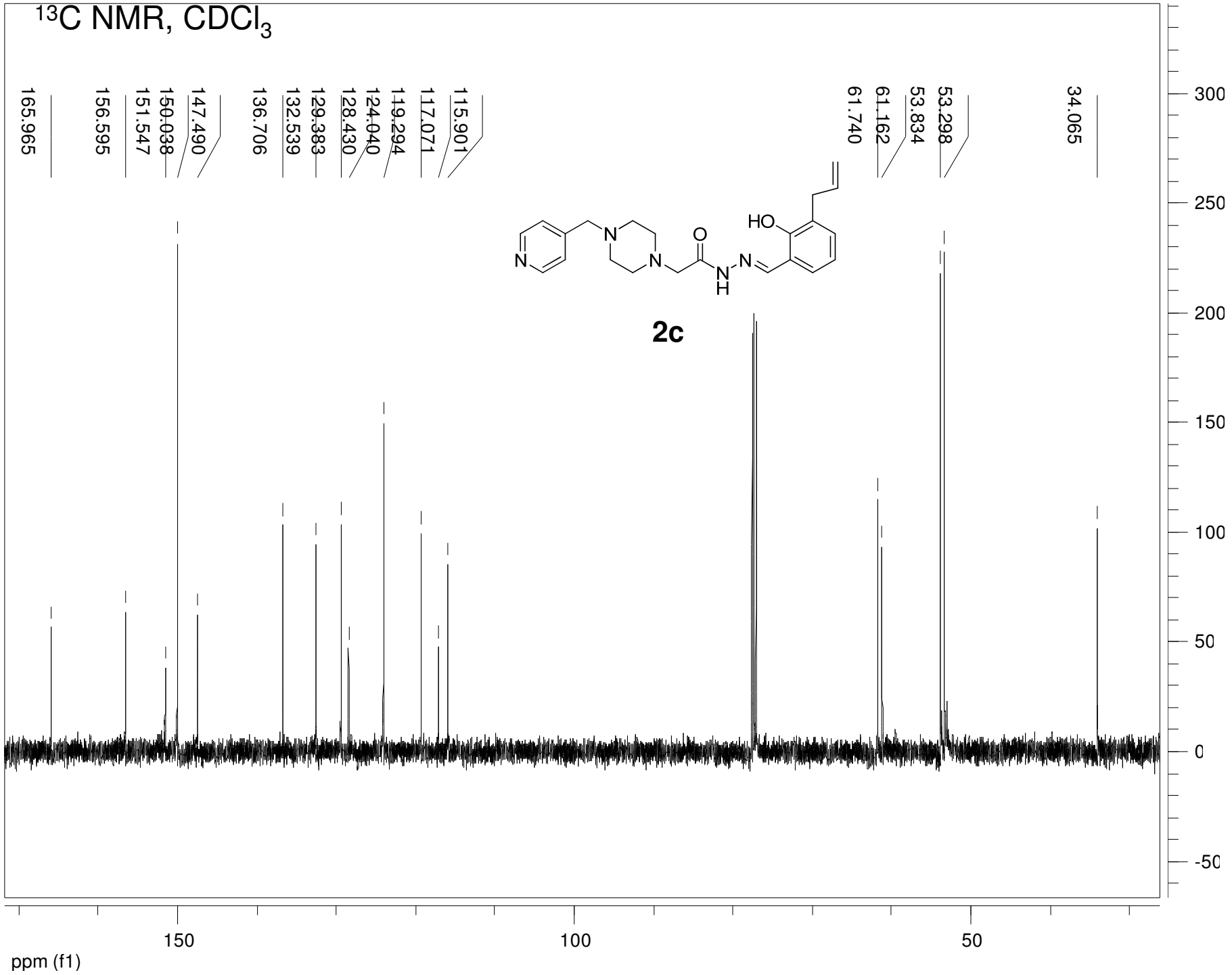
RUN# 429 JAN 7, 2008 21:58:39

AREA%

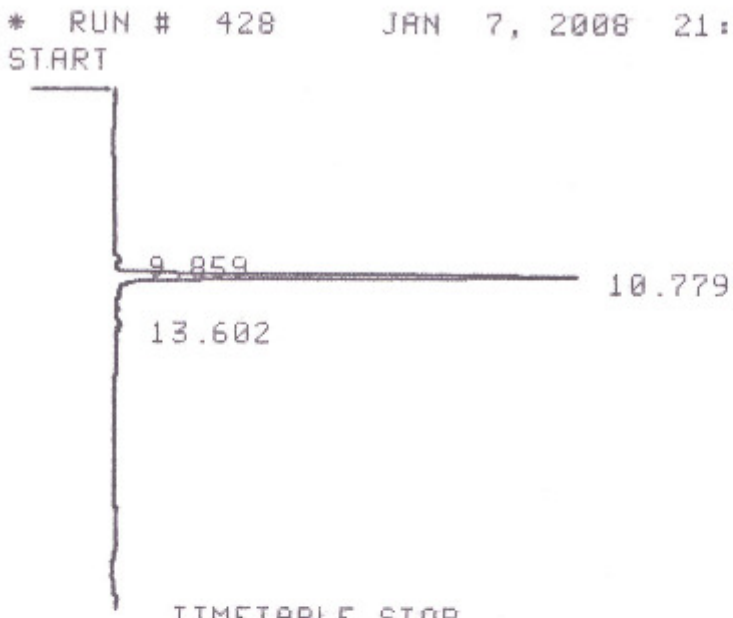
RT	AREA	TYPE	WIDTH	AREA%
.480	155050	BB	.215	.93098
9.217	171835	BV	.190	1.03176
9.905	190472	BV	.162	1.14366
10.471	15849544	VB	.286	95.16627
13.761	287685	BV	.209	1.72736

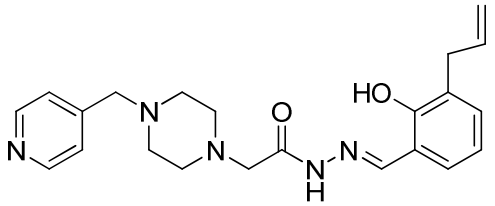
TOTAL AREA=1.6655E+07
MUL FACTOR=1.0000E+00


1H NMR, CDCl3
11.269
10.079
8.552
8.541
8.388
7.272
7.187
7.184
7.172
7.170
7.072
7.069
7.057
7.054
6.851
6.836
6.821
6.060
6.047
6.040
6.034
6.027
6.013
6.006
6.000
5.993
5.980
5.097
5.094
5.091
5.062
5.059
5.056
5.039
5.035
3.537
3.449
3.436
3.198
2.639
2.532
HO
N
O
N
N
N
H
2c
1.00
1.07
2.39
1.09
1.21
1.20
0.97
1.09
1.25
2.36
2.00
8.28
600
500
400
300
200
100
0
10.0
5.0
0.0
ppm (f1)


^{13}C NMR, $CDCl_3$
165.965
156.595
151.547
150.038
147.490
136.706
132.539
129.383
128.430
124.040
119.294
117.071
115.901
61.740
61.162
53.834
53.298
34.065
HO
N
O
N
N
N
H
2c
300
250
200
150
100
50
0
-50
150
100
50
ppm (f1)

HPLC





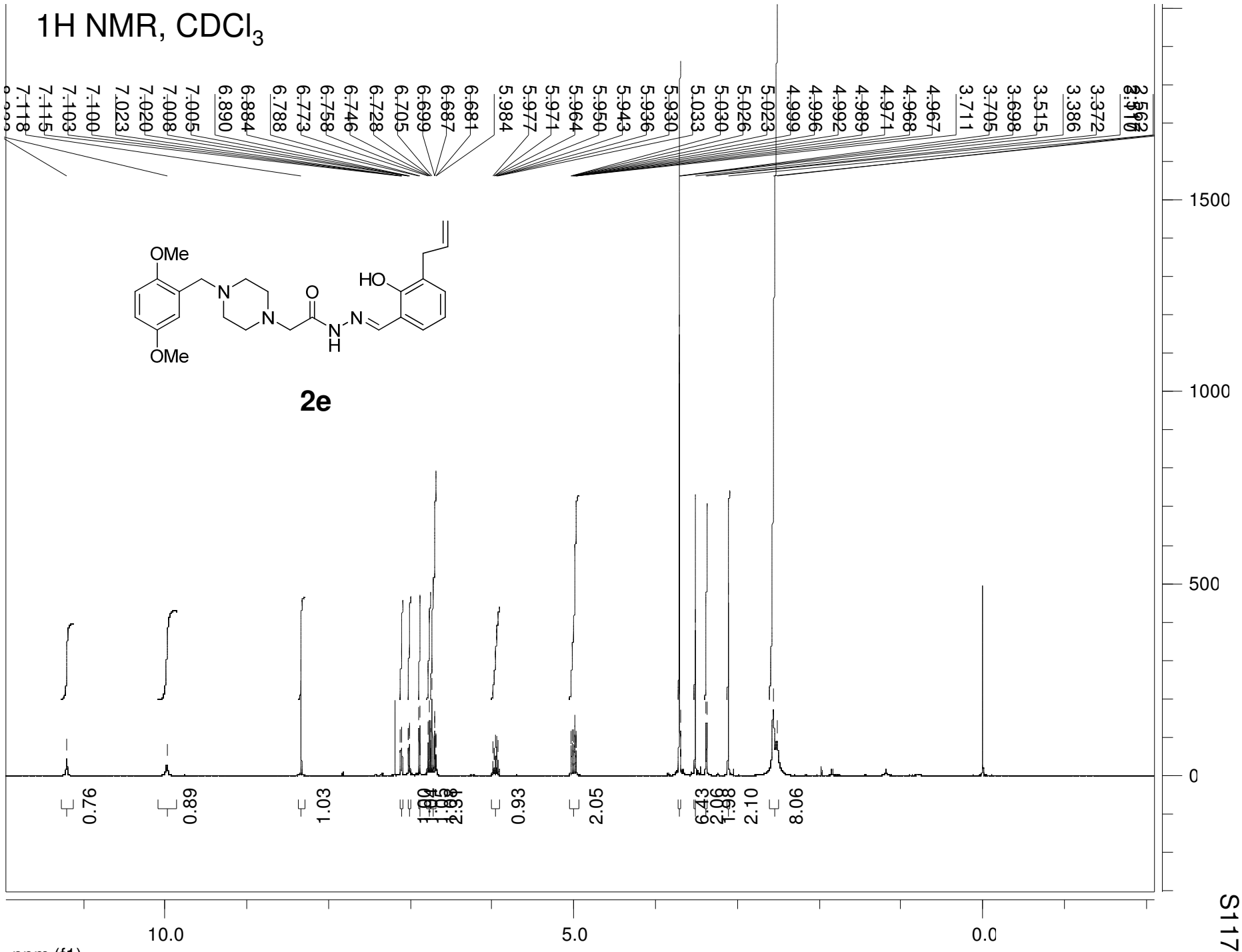
2c

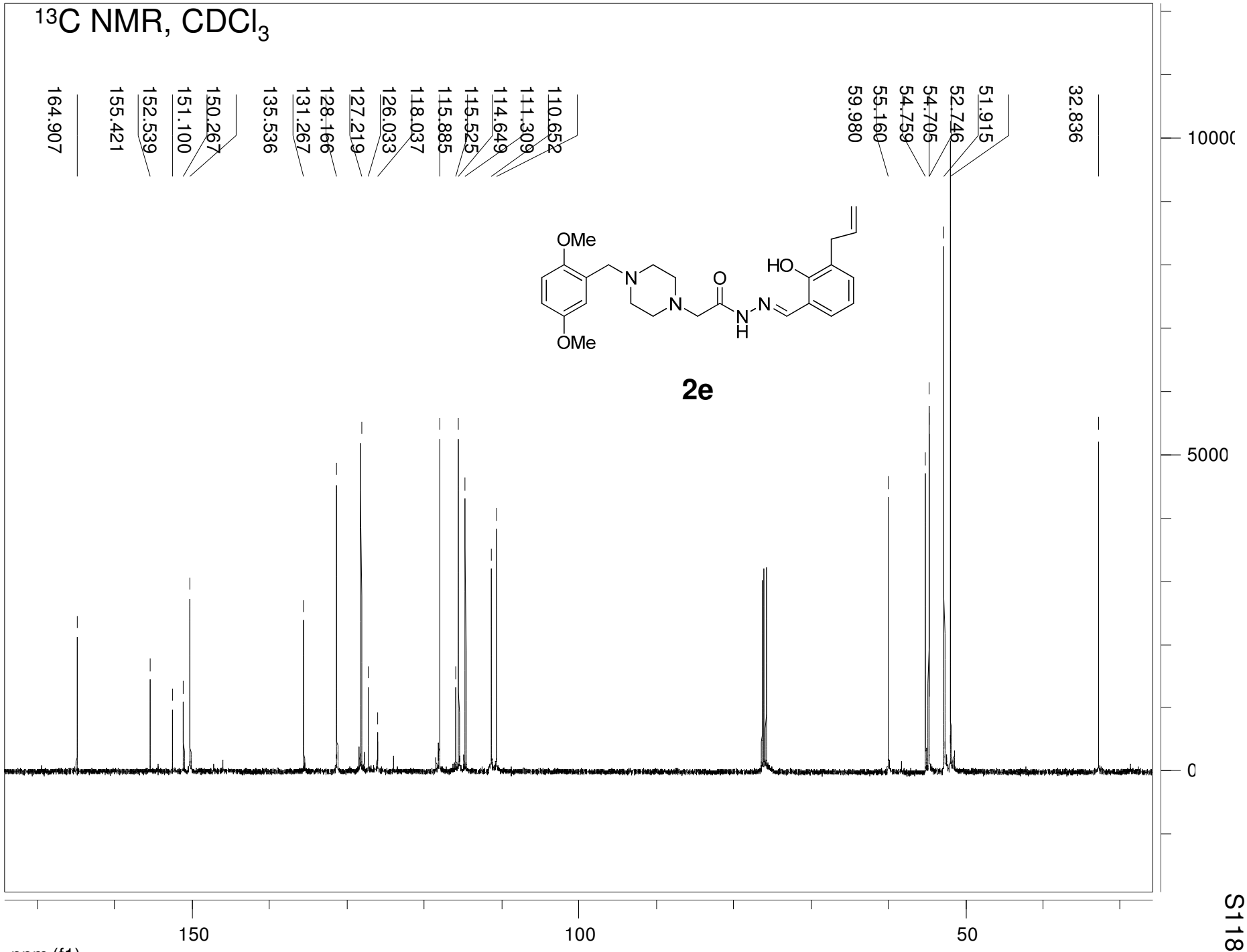
RUN# 428 JAN 7, 2008 21:26:17

AREA%

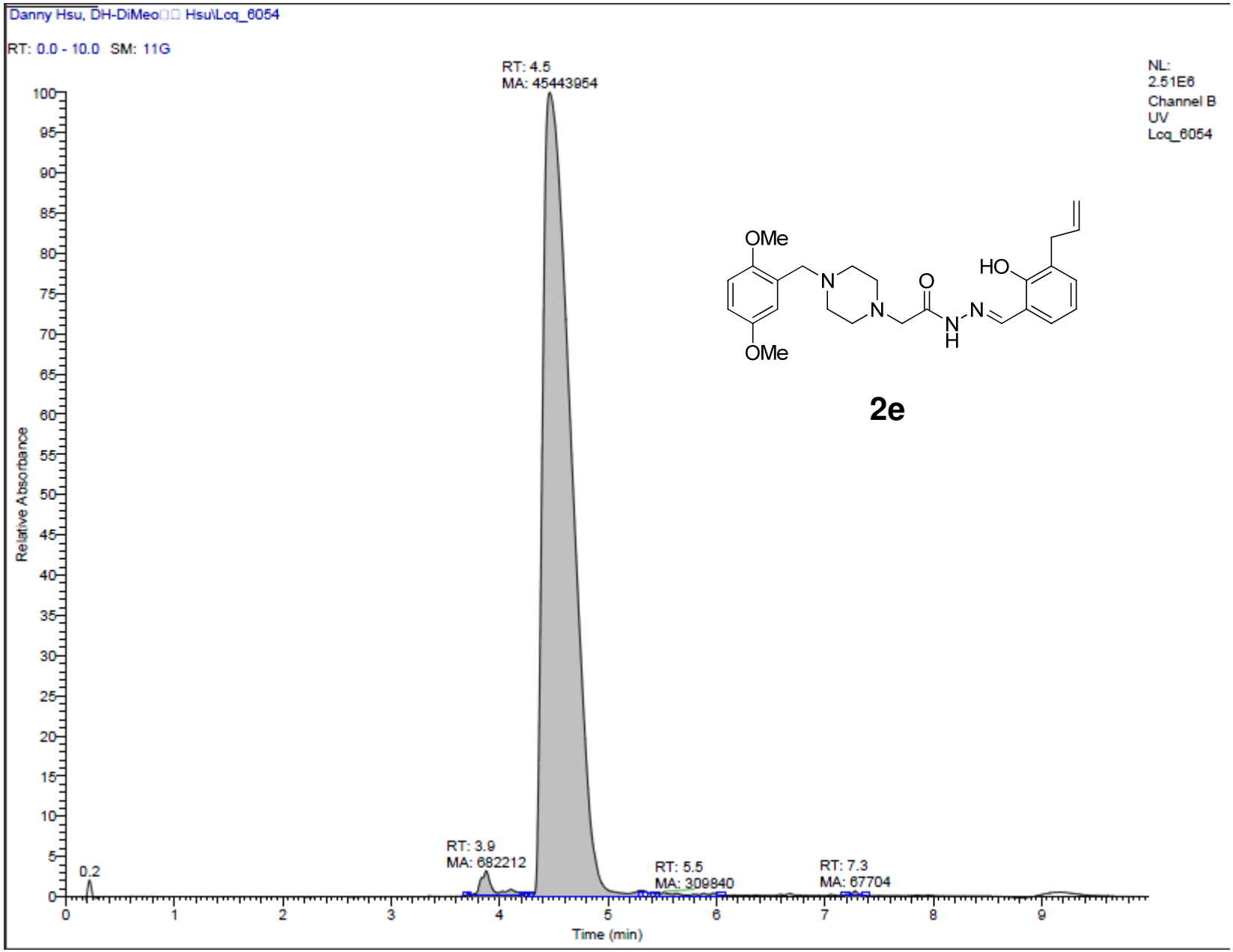
RT	AREA	TYPE	WIDTH	AREA%
9.859	192383	BP	.312	1.62955
10.779	11429840	PB	.261	96.81466
13.602	183675	BV	.299	1.55579

TOTAL AREA=1.1806E+07
MUL FACTOR=1.0000E+00

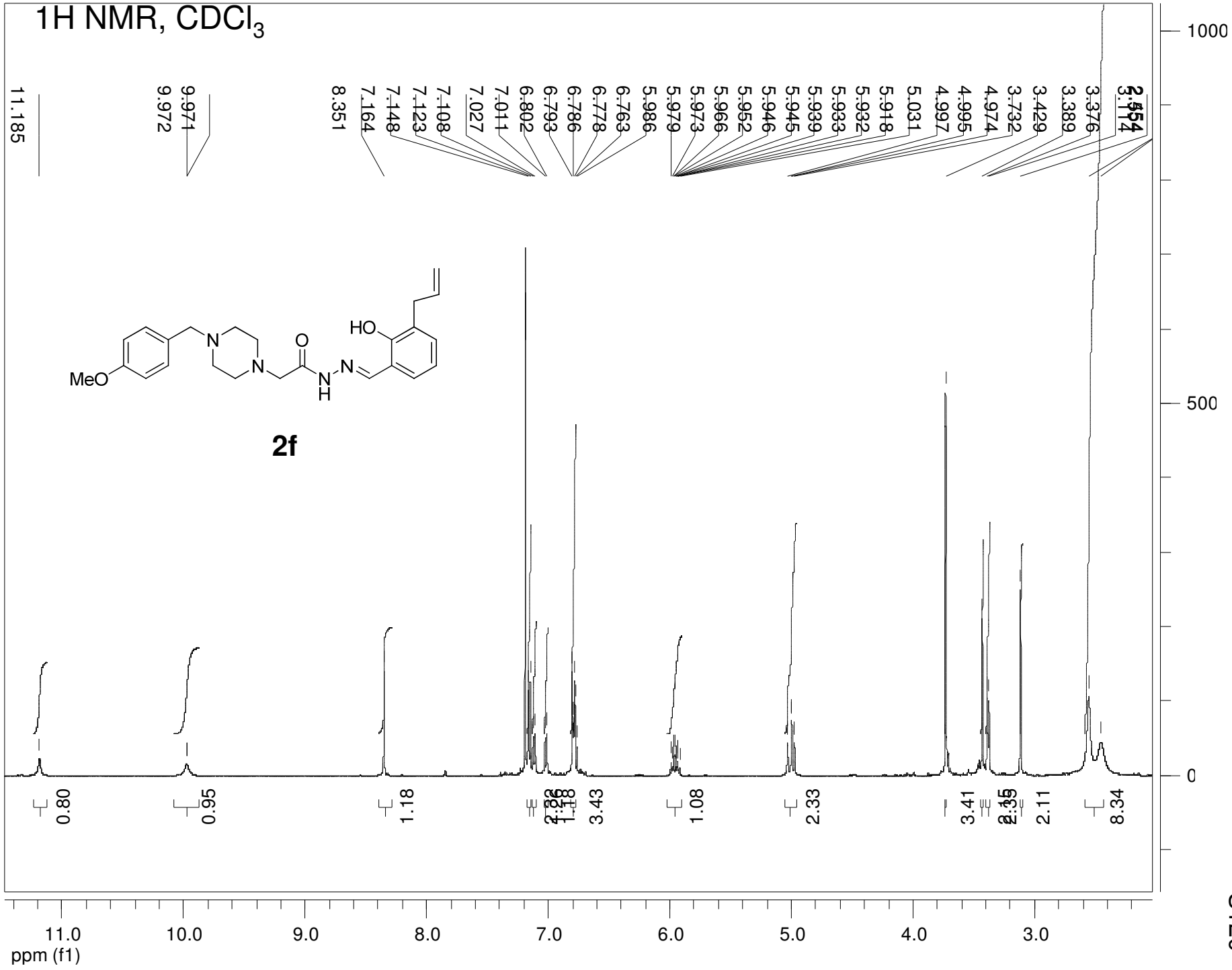

1H NMR, CDCl3
OMe
OMe
N
N
O
N
H
HO
2e
1500
1000
500
0
10.0
5.0
0.0
ppm (f1)

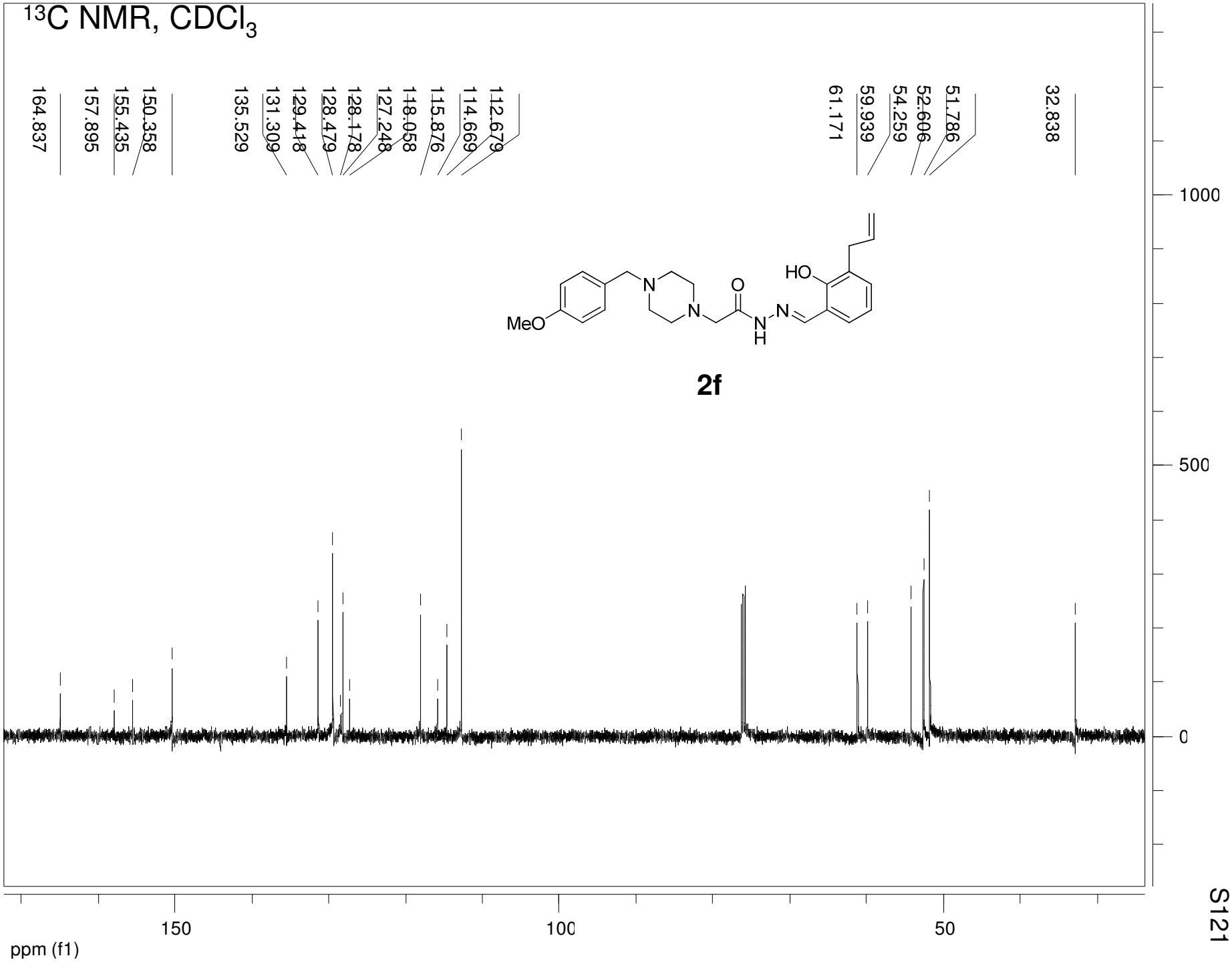

^{13}C NMR, $CDCl_3$
164.907
155.421
152.539
151.100
150.267
135.536
131.267
128.166
127.219
126.033
118.037
115.885
115.525
114.649
111.309
110.652
59.980
55.160
54.759
54.705
52.746
51.915
32.836
OMe
OMe
HO
N
N
O
N
H
N
2e
10000
5000
0
150
100
50
ppm (f1)

LC-MS
Purity: 95%

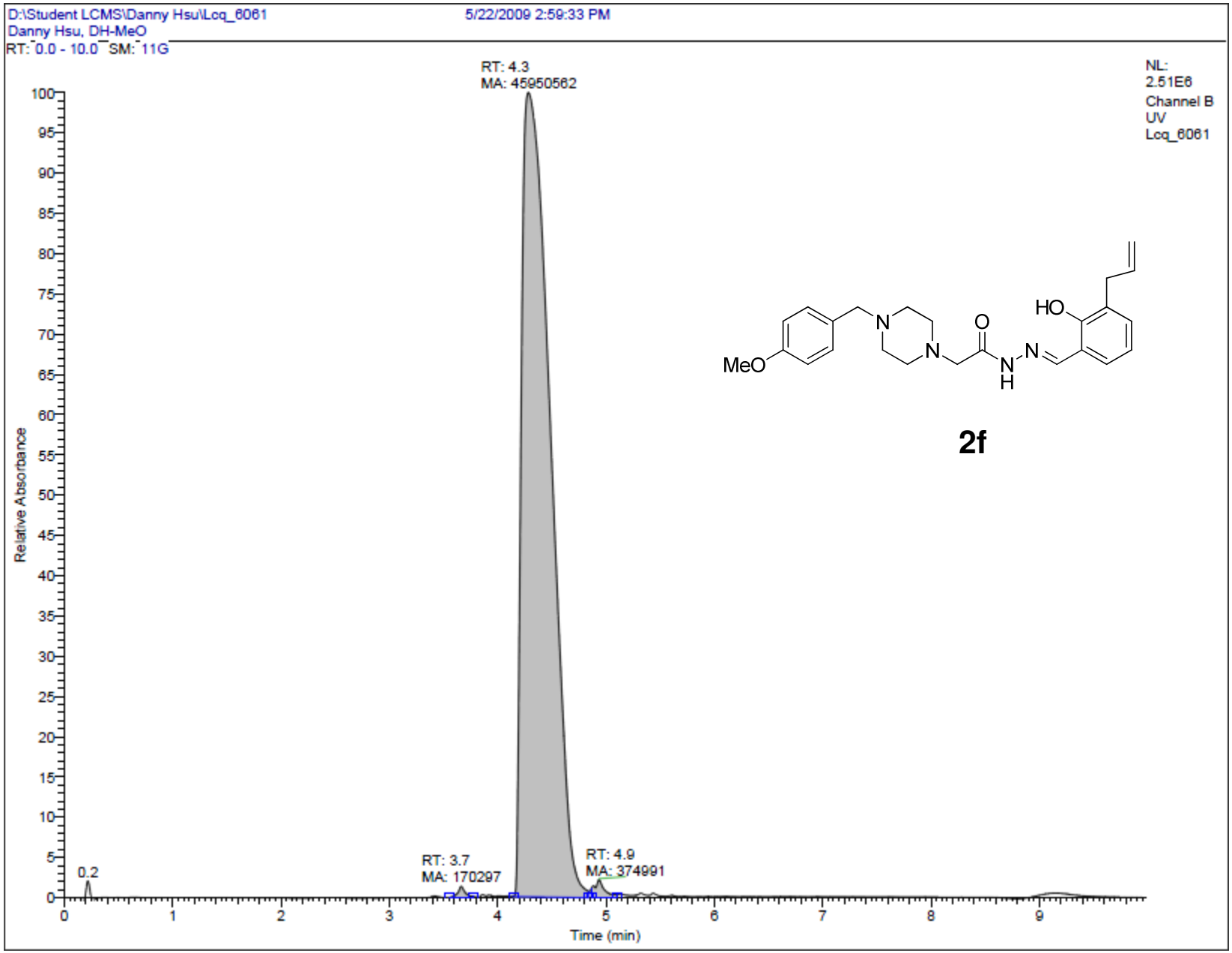


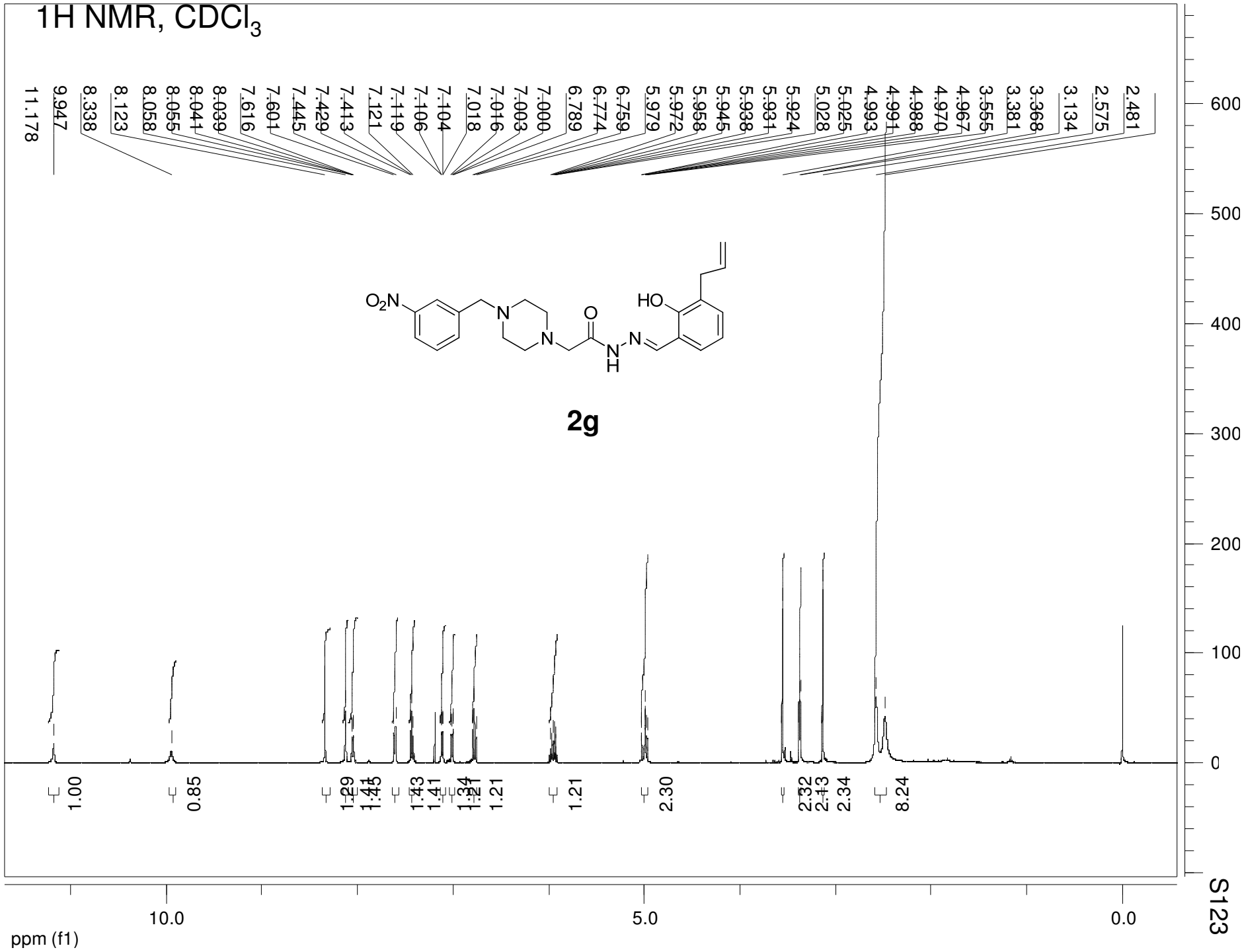
2e

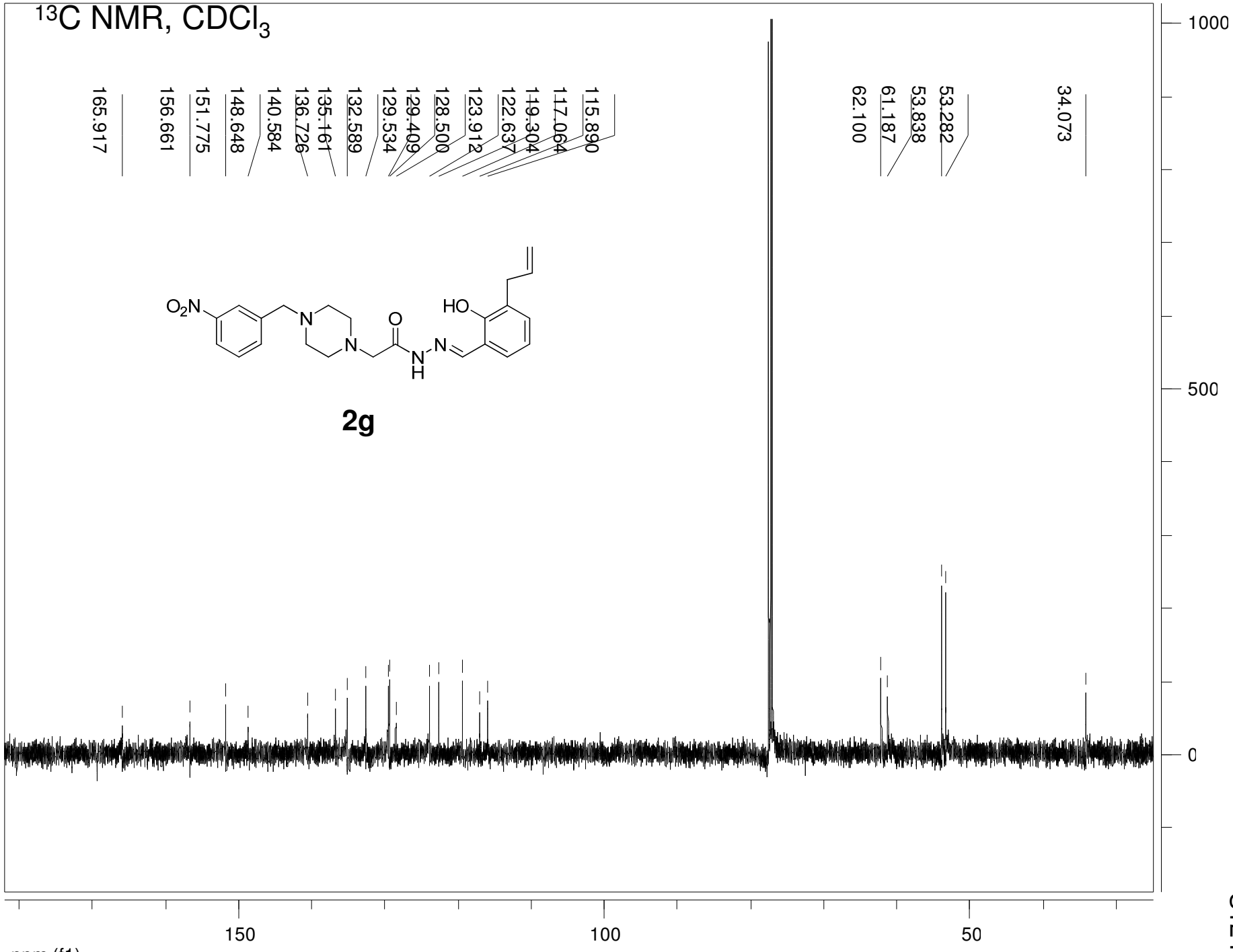

1H NMR, CDCl3
11.185
9.971
9.972
8.351
7.164
7.148
7.123
7.108
7.027
7.011
6.802
6.793
6.786
6.778
6.763
5.986
5.979
5.973
5.966
5.952
5.946
5.945
5.939
5.933
5.932
5.918
5.031
4.997
4.995
4.974
4.732
3.429
3.389
3.376
2.554
3.114
HO
O
N
N
N
H
MeO
2f
0.80
0.95
1.18
3.43
1.08
2.33
3.41
2.11
8.34
1000
500
0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)


^{13}C NMR, $CDCl_3$
164.837
157.895
155.435
150.358
135.529
131.309
129.418
128.479
128.178
127.248
118.058
115.876
114.669
112.679
61.171
59.939
54.259
52.606
51.786
32.838
MeO
N
N
O
N
H
N
HO
2f
1000
500
0
150
100
50
ppm (f1)

LC-MS
Purity: 99%




1H NMR, CDCl3
11.178
9.947
8.338
8.123
8.058
8.055
8.041
8.039
7.616
7.601
7.445
7.429
7.413
7.121
7.119
7.106
7.104
7.018
7.016
7.003
7.000
6.789
6.774
6.759
5.979
5.972
5.958
5.945
5.938
5.931
5.924
5.028
5.025
4.993
4.991
4.988
4.970
4.967
3.555
3.381
3.368
3.134
2.575
2.481
O2N
N
N
O
N
H
N
HO
2g
1.00
0.85
1.29
1.45
1.43
1.41
1.34
1.21
1.21
2.30
2.32
2.13
2.34
8.24
600
500
400
300
200
100
0
10.0
5.0
0.0
ppm (f1)
S123

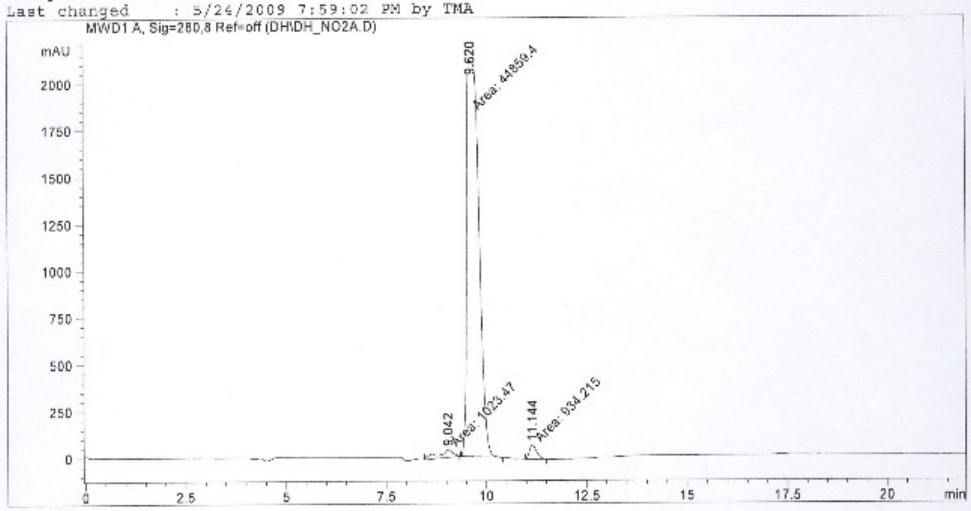

^{13}C NMR, $CDCl_3$
165.917
156.661
151.775
148.648
140.584
136.726
135.161
132.589
129.534
129.409
128.500
123.912
122.637
119.304
117.064
115.890
62.100
61.187
53.838
53.282
34.073
O_2N
N
N
O
N
H
HO
2g
1000
500
0
150
100
50
ppm (f1)

HPLC

Data File D:\HPCHEM\DATA\2009\DH\DH_NO2A.D
Sample Name: DH-NO2a

Acq. Operator : DH
Acq. Instrument : El Chupacabra Location : P1-F-08
Injection Date : 5/25/2009 12:57:01 AM Inj Volume : 10 µl

Acq. Method : C:\HPCHEM\1\METHODS\DH.M
Last changed : 5/24/2009 11:34:17 PM by DH
(modified after loading)
Analysis Method : C:\HPCHEM\1\METHODS\DH.M
Last changed : 5/24/2009 7:59:02 PM by TMA



Fraction Information

Fraction collection off

No Fractions found.

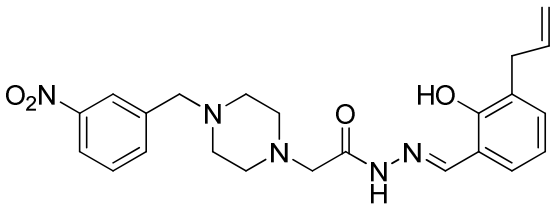
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

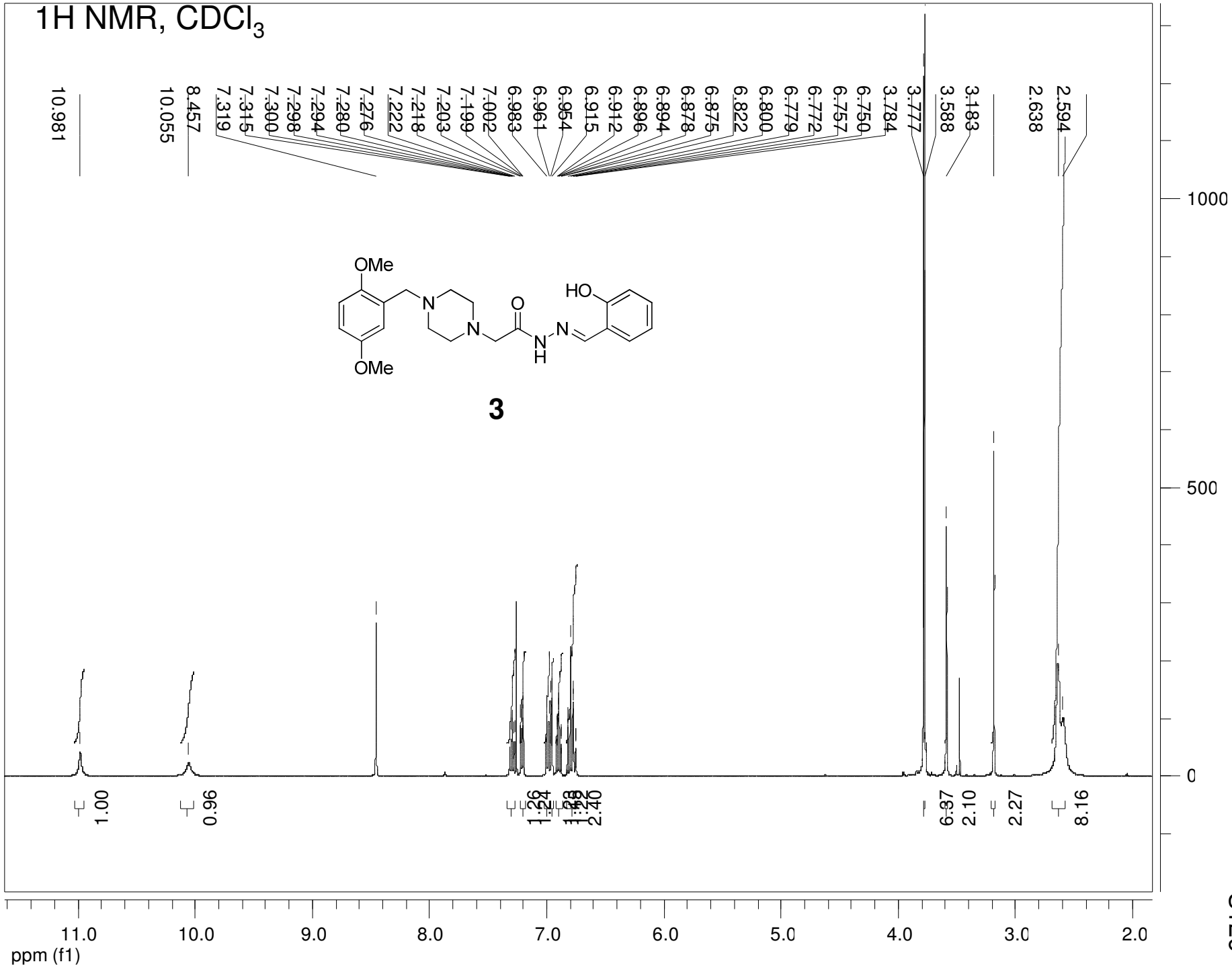
Signal 1: MWD1 A, Sig=280,8 Ref=off

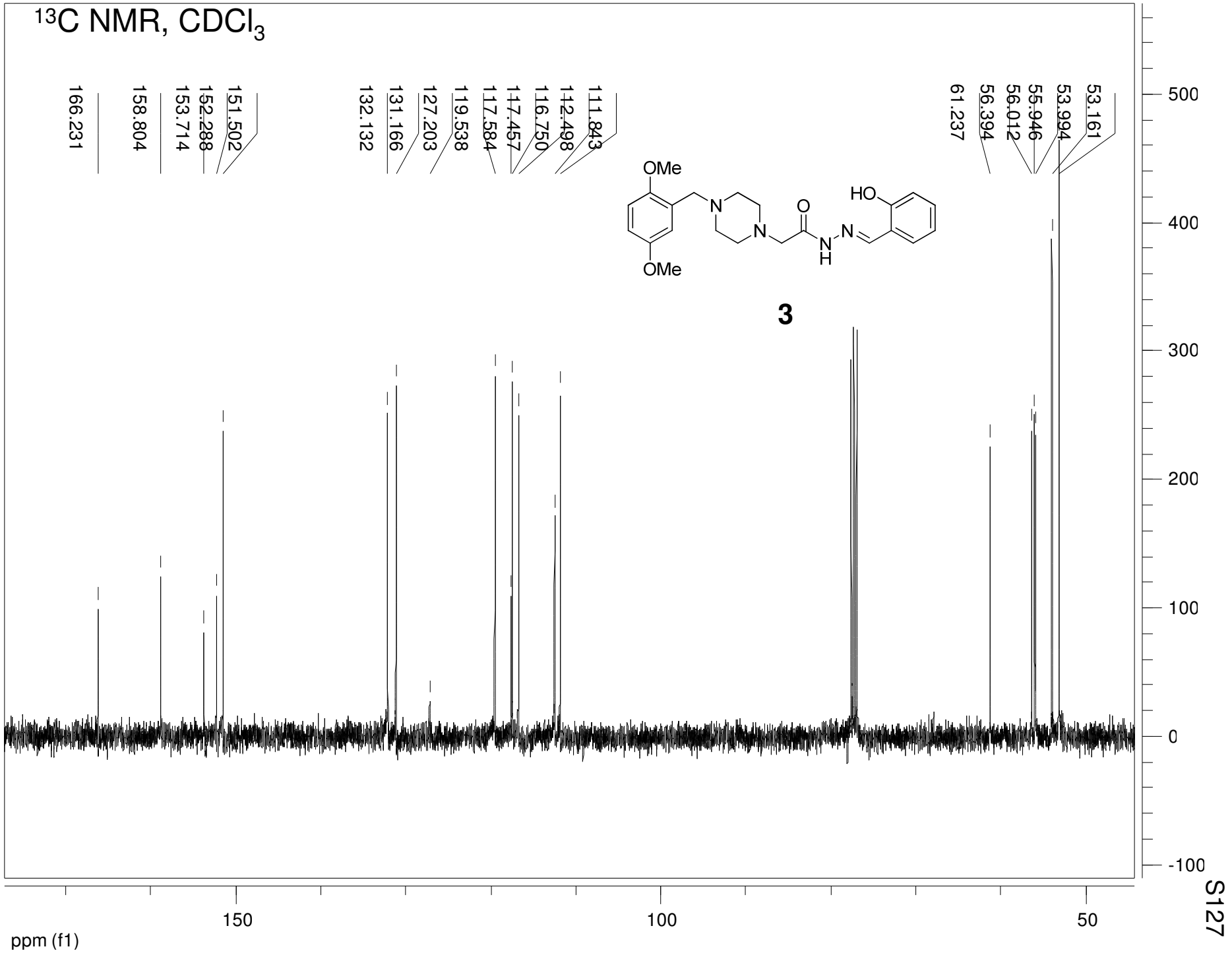
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.042	MM	0.4191	1023.45661	40.69639	2.1861
2	9.620	MM	0.3536	4.48594e4	2114.29907	95.8184
3	11.144	MM	0.2047	934.21515	76.05640	1.9955
Totals :				4.68171e4	2231.05187	

El Chupacabra 5/25/2009 1:21:39 AM DH Page 1 of 2

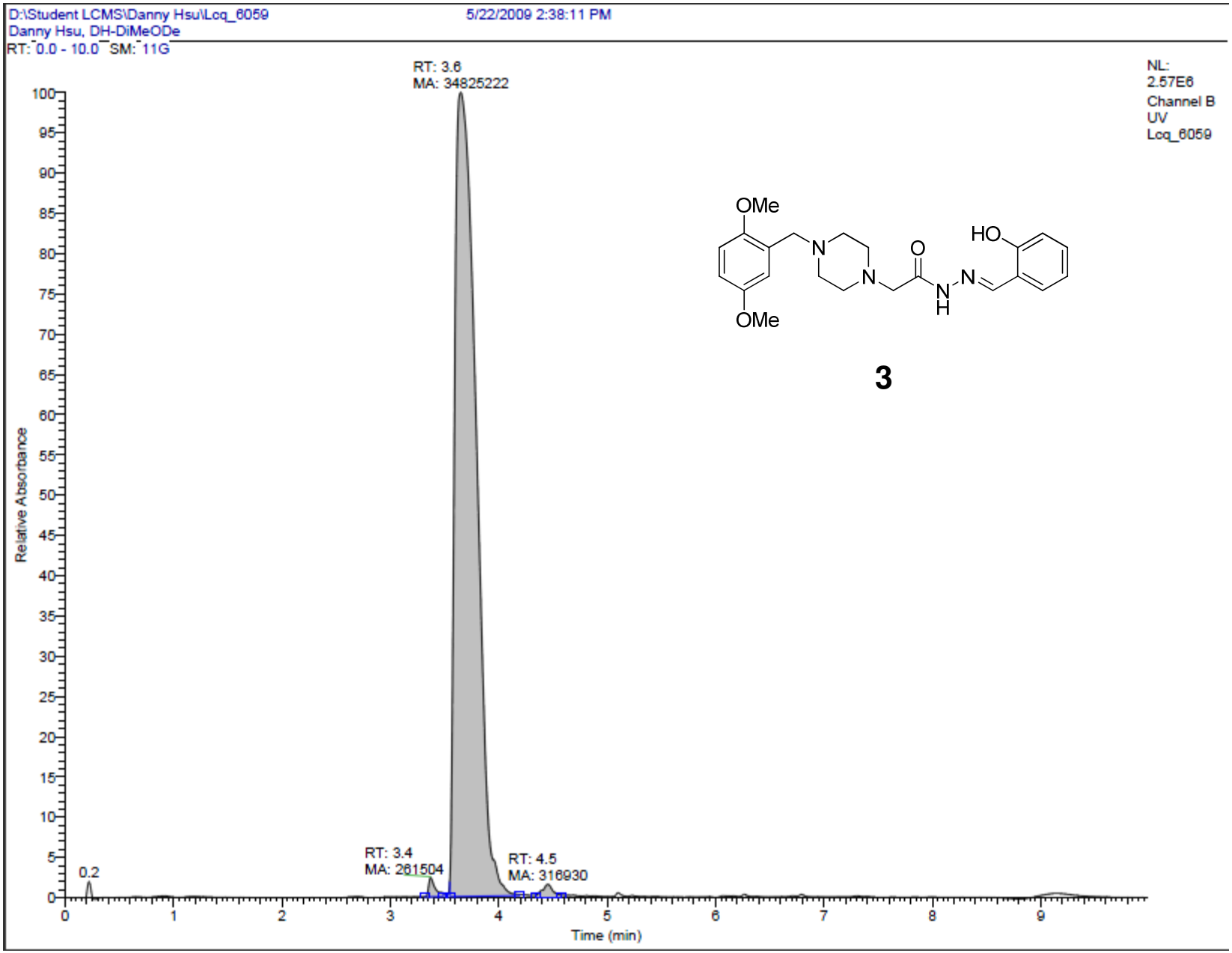


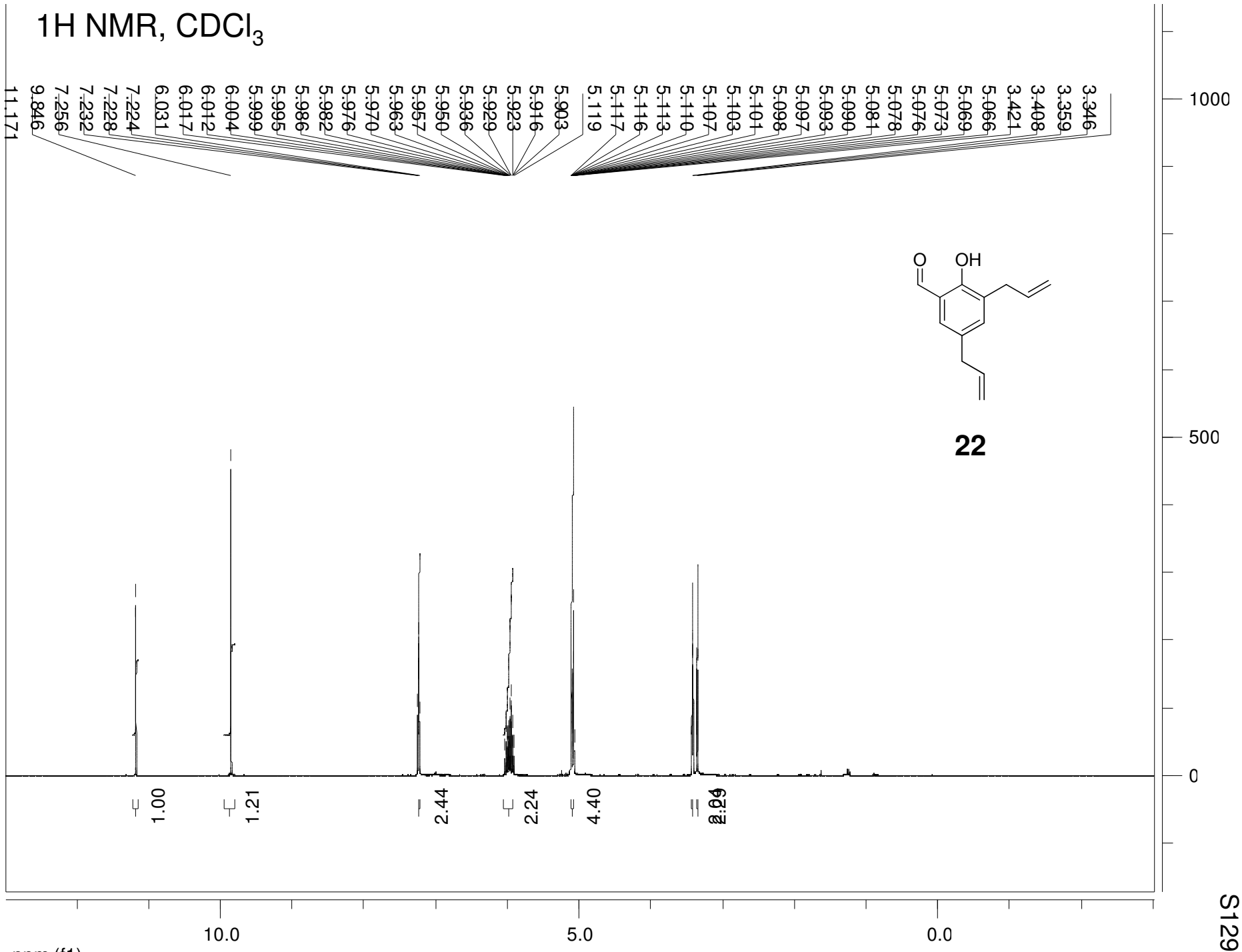
2g


1H NMR, CDCl3
10.981
8.457
10.055
7.319
7.315
7.300
7.298
7.294
7.280
7.276
7.222
7.218
7.203
7.199
7.002
6.983
6.961
6.954
6.915
6.912
6.896
6.894
6.878
6.875
6.822
6.800
6.779
6.772
6.757
6.750
3.784
3.777
3.588
3.183
2.638
2.594
OMe
OMe
HO
N
N
O
N
H
N
3
1000
500
0
1.00
0.96
6.87
2.10
2.27
8.16
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (f1)


^{13}C NMR, $CDCl_3$
166.231
158.804
153.714
152.288
151.502
132.132
131.166
127.203
119.538
117.584
117.457
116.750
112.498
111.843
61.237
56.394
56.012
55.946
53.994
53.161
OMe
OMe
HO
N
N
O
N
H
3
500
400
300
200
100
0
-100
150
100
50
ppm (f1)

LC-MS
Purity: 98%




1H NMR, CDCl3
11.171
9.846
7.256
7.232
7.228
7.224
6.031
6.017
6.012
6.004
5.999
5.995
5.986
5.982
5.976
5.970
5.963
5.957
5.950
5.936
5.929
5.923
5.916
5.903
5.119
5.117
5.116
5.113
5.110
5.107
5.103
5.101
5.098
5.097
5.093
5.090
5.081
5.078
5.076
5.073
5.069
5.066
3.421
3.408
3.359
3.346
O
OH
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1000
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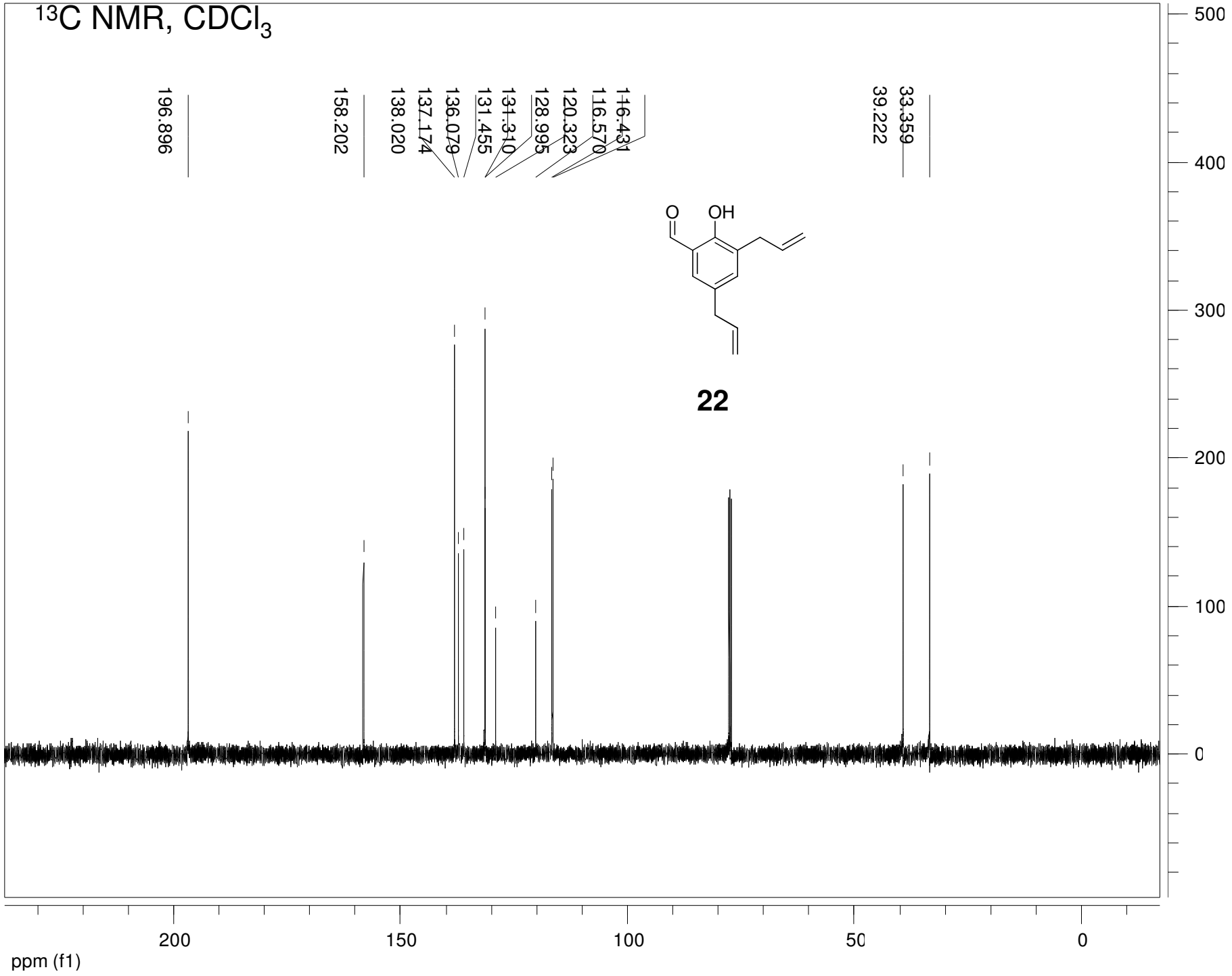
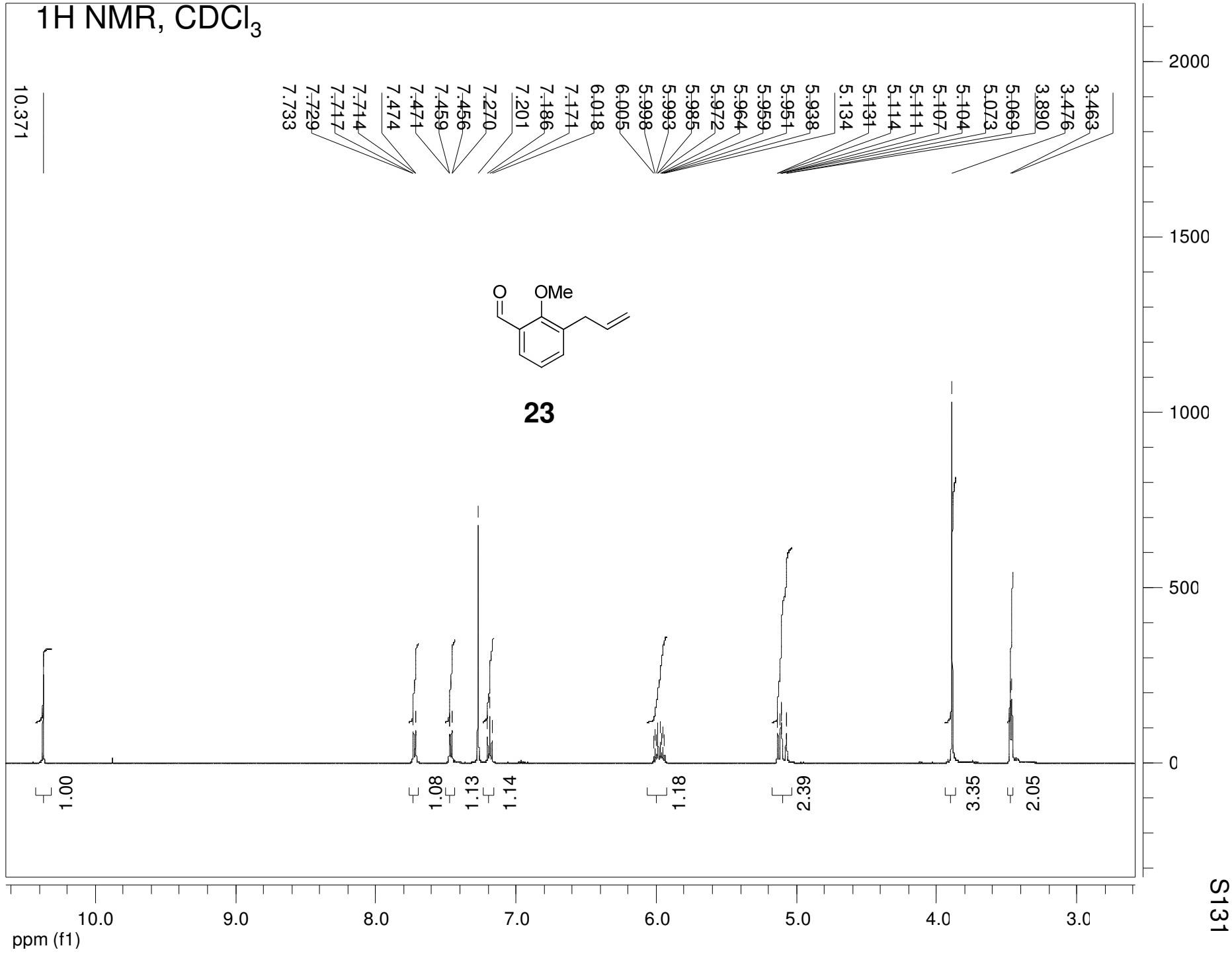
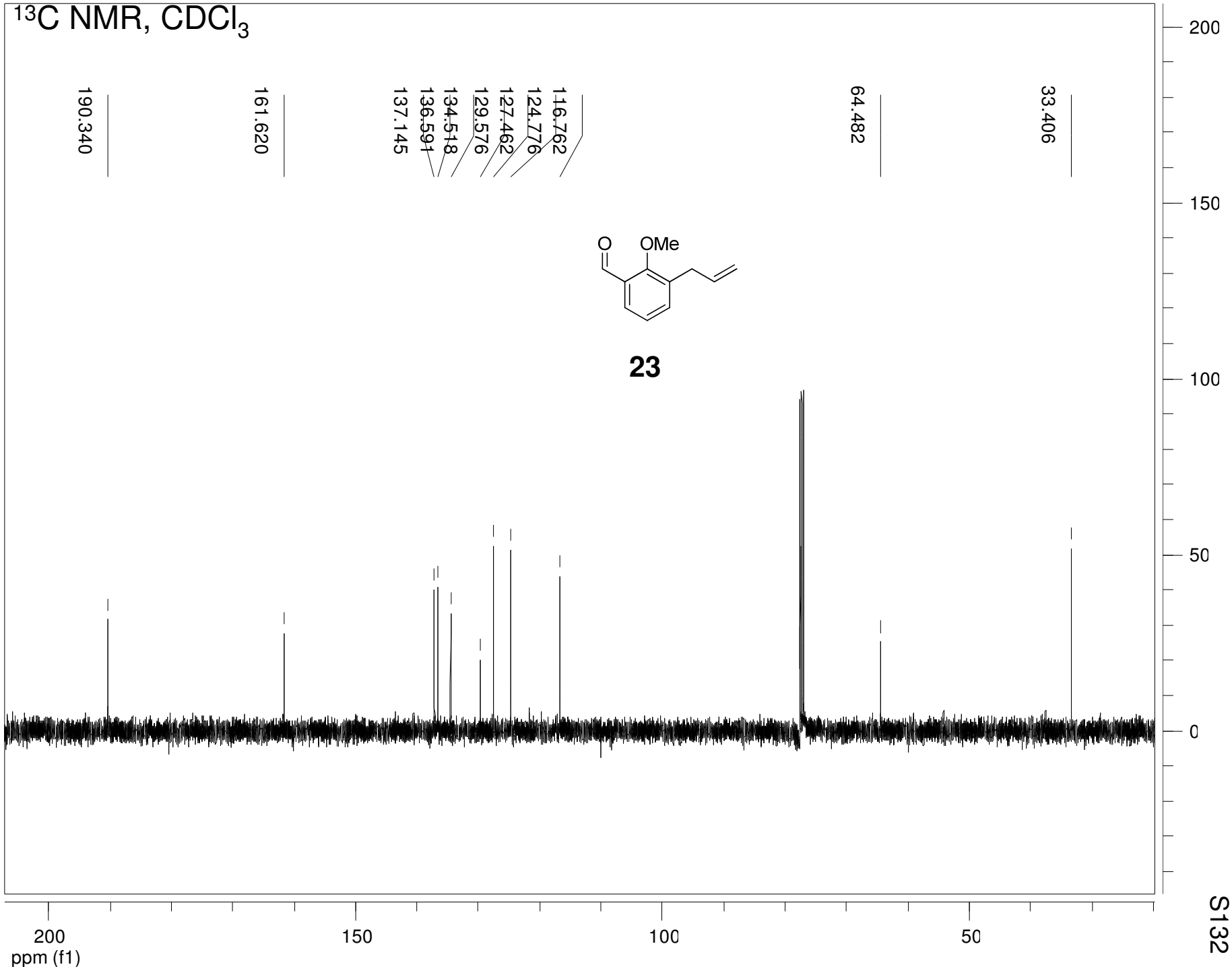
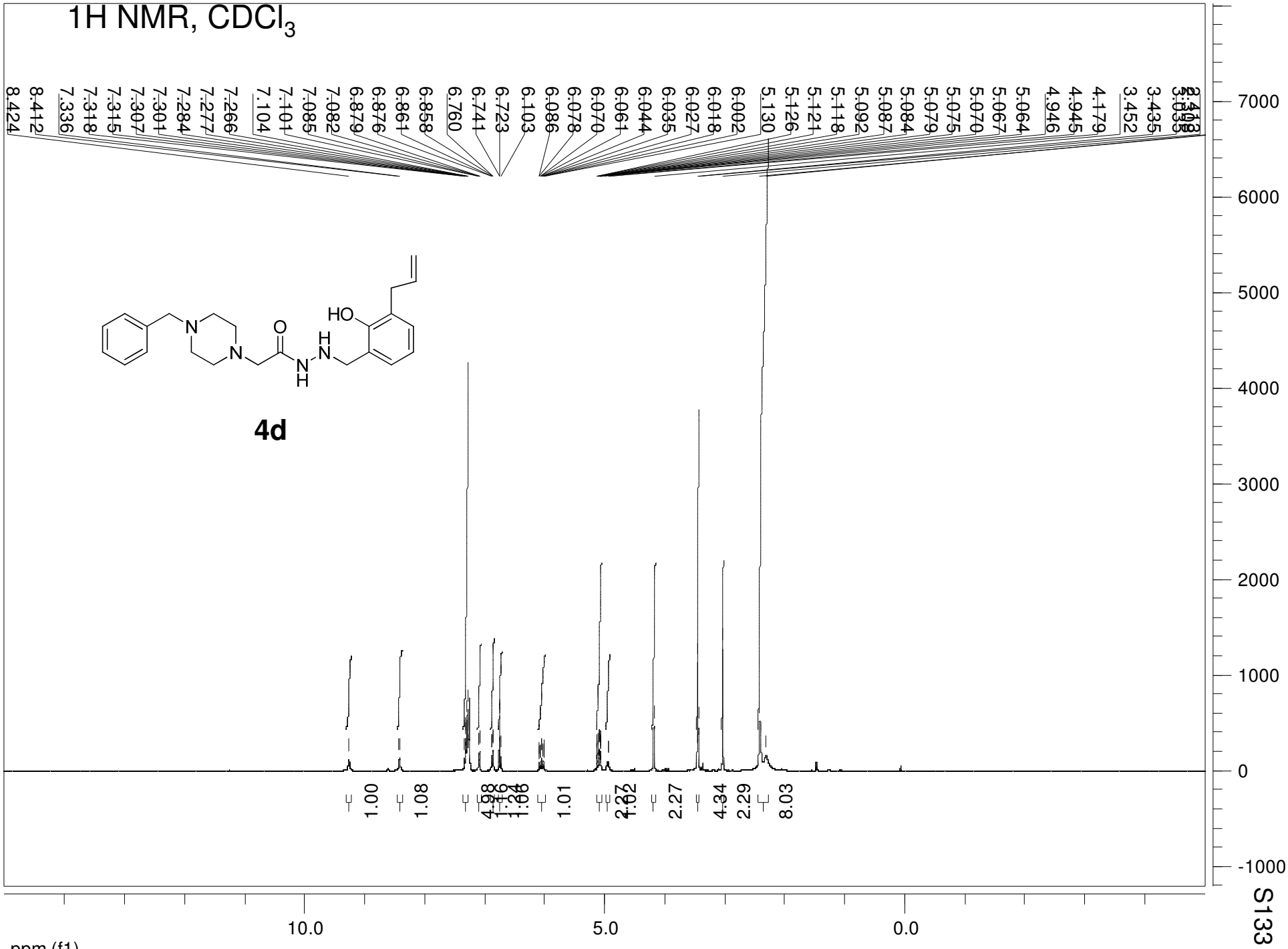
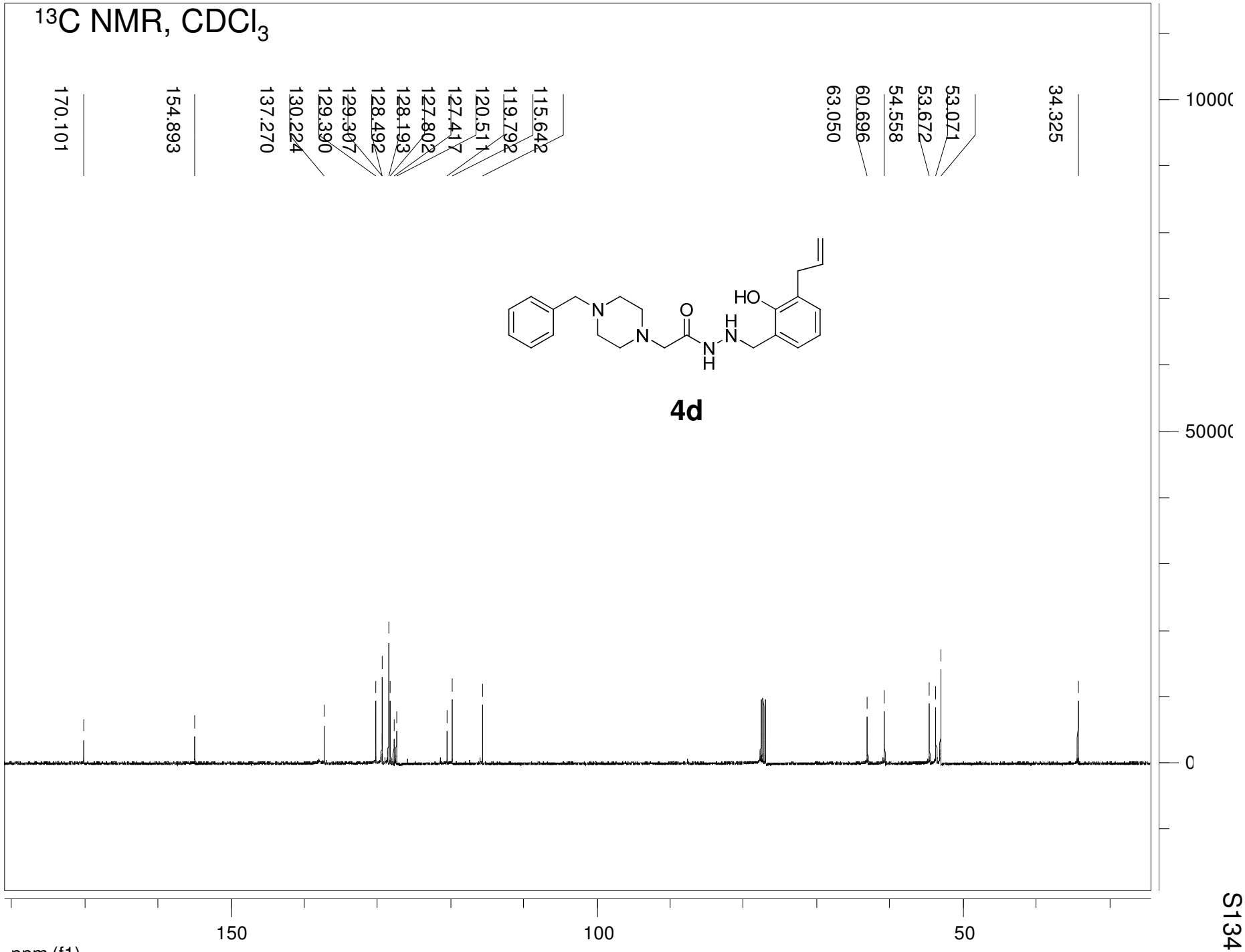
0
1.00
1.21
2.44
2.24
4.40
10.0
5.0
0.0
ppm (f1)


^{13}C NMR, $CDCl_3$
196.896
158.202
138.020
137.174
136.079
131.455
131.310
128.995
120.323
116.570
116.431
39.222
33.359
O
OH
22
500
400
300
200
100
0
200
150
100
50
0
ppm (f1)


1H NMR, CDCl3
10.371
7.733
7.729
7.717
7.714
7.474
7.471
7.459
7.456
7.270
7.201
7.186
7.171
6.018
6.005
5.998
5.993
5.985
5.972
5.964
5.959
5.951
5.938
5.134
5.131
5.114
5.111
5.107
5.104
5.073
5.069
3.890
3.476
3.463
O
OMe
23
2000
1500
1000
500
0
1.00
1.08
1.13
1.14
1.18
2.39
3.35
2.05
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)


13C NMR, CDCl3
190.340
161.620
137.145
136.591
134.518
129.576
127.462
124.776
116.762
64.482
33.406
O
OMe
23
200
150
100
50
0
200
150
100
50
ppm (f1)


1H NMR, CDCl3
8.424
8.412
7.336
7.318
7.315
7.307
7.301
7.284
7.277
7.266
7.104
7.101
7.085
7.082
6.879
6.876
6.861
6.858
6.760
6.741
6.723
6.103
6.086
6.078
6.070
6.061
6.044
6.035
6.027
6.018
6.002
5.130
5.126
5.121
5.118
5.092
5.087
5.084
5.079
5.075
5.070
5.067
5.064
4.946
4.945
4.179
3.452
3.435
HO
N
O
H
N
N
4d
1.00
1.08
4.98
1.16
1.24
1.08
1.01
2.07
2.27
4.34
2.29
8.03
7000
6000
5000
4000
3000
2000
1000
0
-1000
10.0
5.0
0.0
ppm (f1)
S133

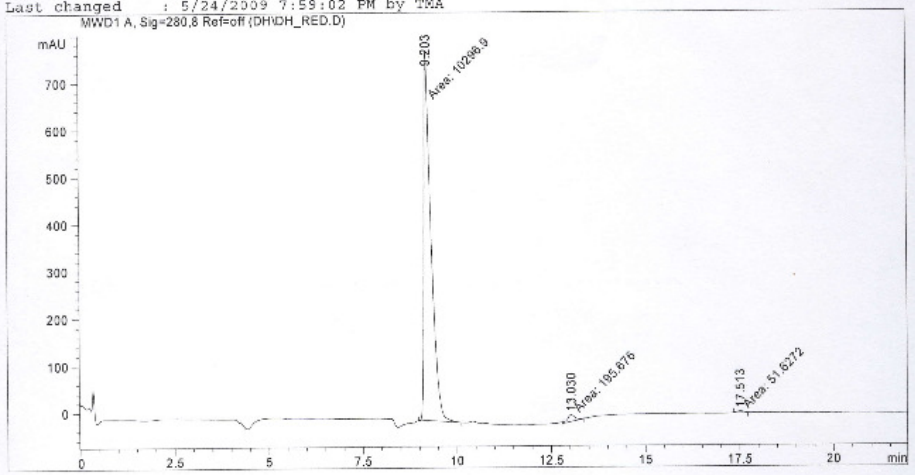

^{13}C NMR, $CDCl_3$
170.101
154.893
137.270
130.224
129.390
129.307
128.492
128.193
127.802
127.417
120.511
119.792
115.642
63.050
60.696
54.558
53.672
53.071
34.325
HO
O
N
N
NH
HN
4d
10000
50000
0
150
100
50
ppm (f1)

HPLC

Data File D:\HPCHEM\DATA\2009\DH\DH_RED.D
Sample Name: DH-RED

Acq. Operator : DH
Acq. Instrument : El Chupacabra Location : P1-F-07
Injection Date : 5/25/2009 12:29:23 AM Inj Volume : 10 µl

Acq. Method : C:\HPCHEM\1\METHODS\DH.M
Last changed : 5/24/2009 11:34:17 PM by DH
(modified after loading)
Analysis Method : C:\HPCHEM\1\METHODS\DH.M
Last changed : 5/24/2009 7:59:02 PM by TMA



Fraction Information

Fraction collection off

No Fractions found.

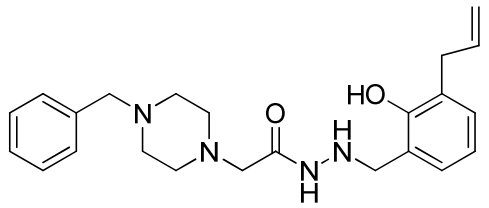
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

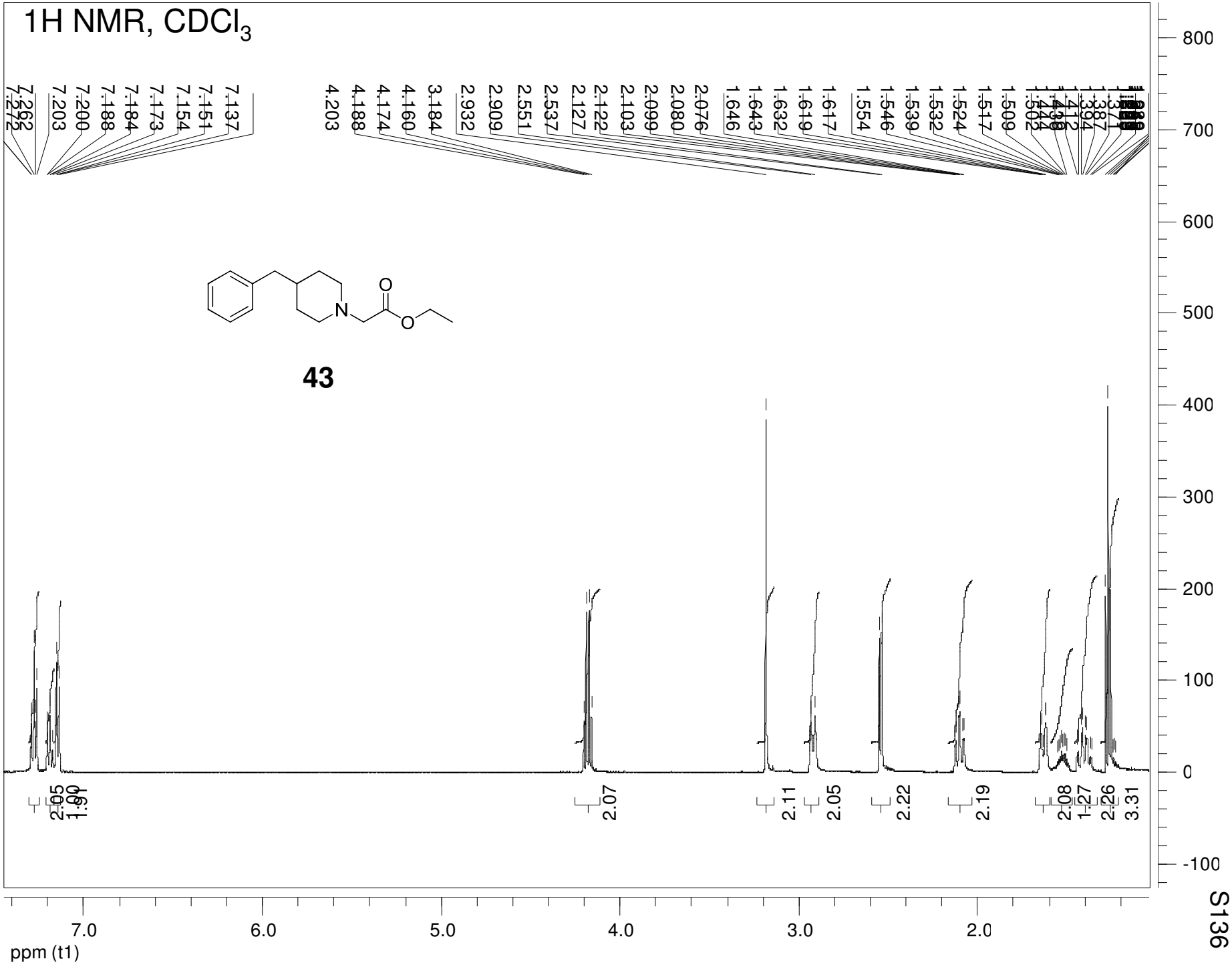
Signal 1: MWD1 A, Sig=280,8 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.203	MM	0.2198	1.02969e4	780.93475	97.6546
2	13.030	MM	0.2233	195.67628	14.60288	1.8558
3	17.513	MM	0.2340	51.62722	3.67708	0.4896
Totals :				1.05442e4	799.21470	

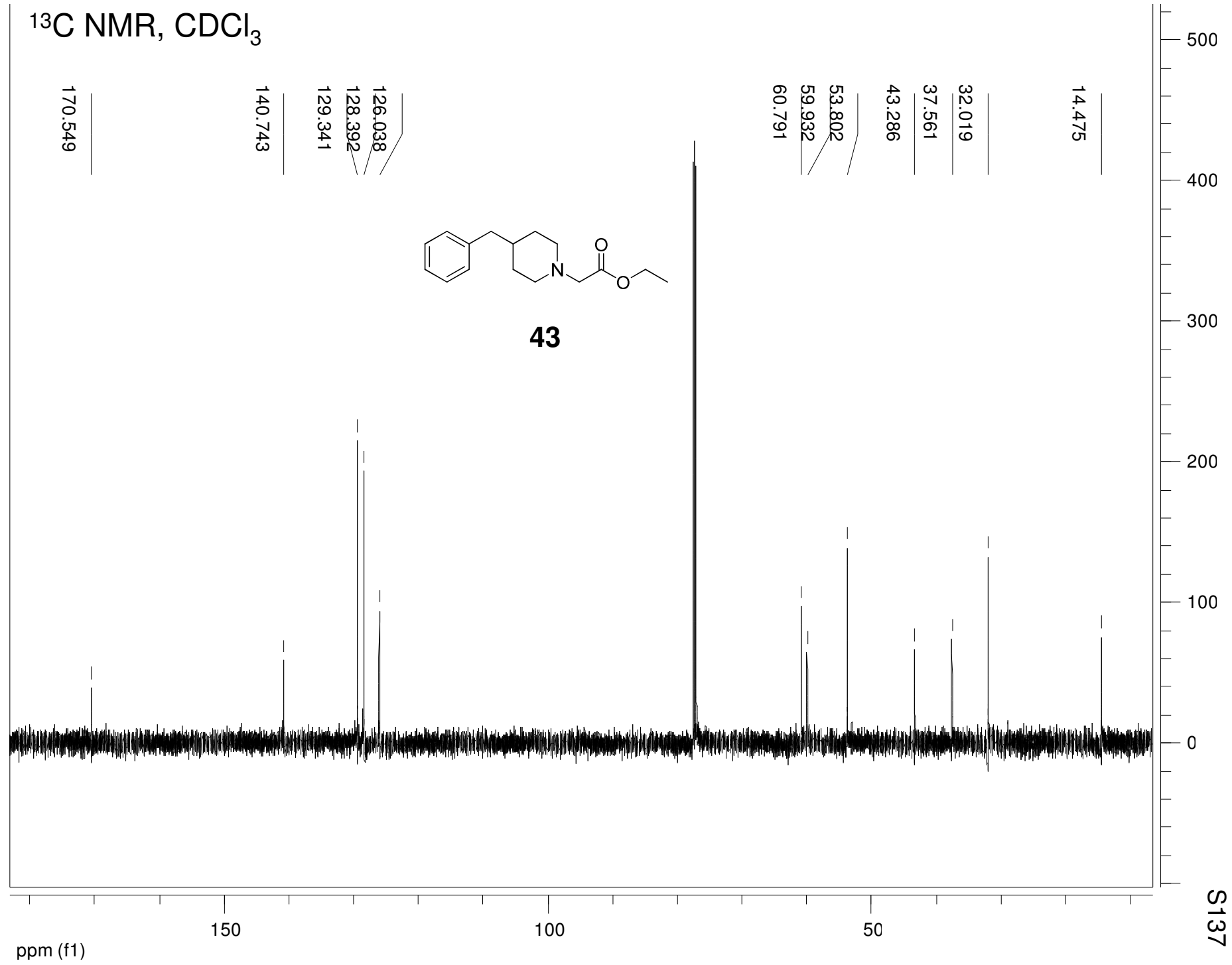
El Chupacabra 5/25/2009 12:55:07 AM DH Page 1 of 2



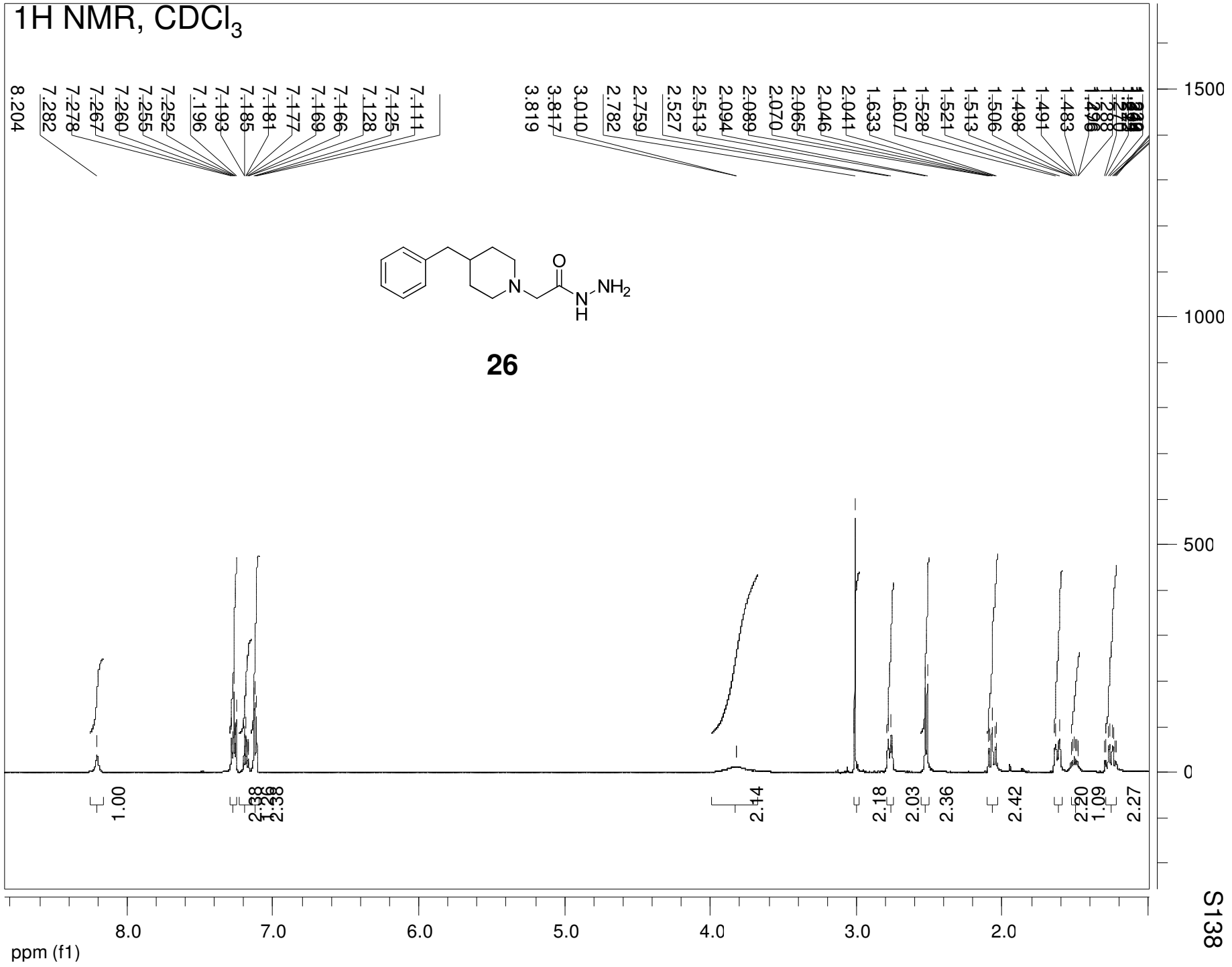
4d

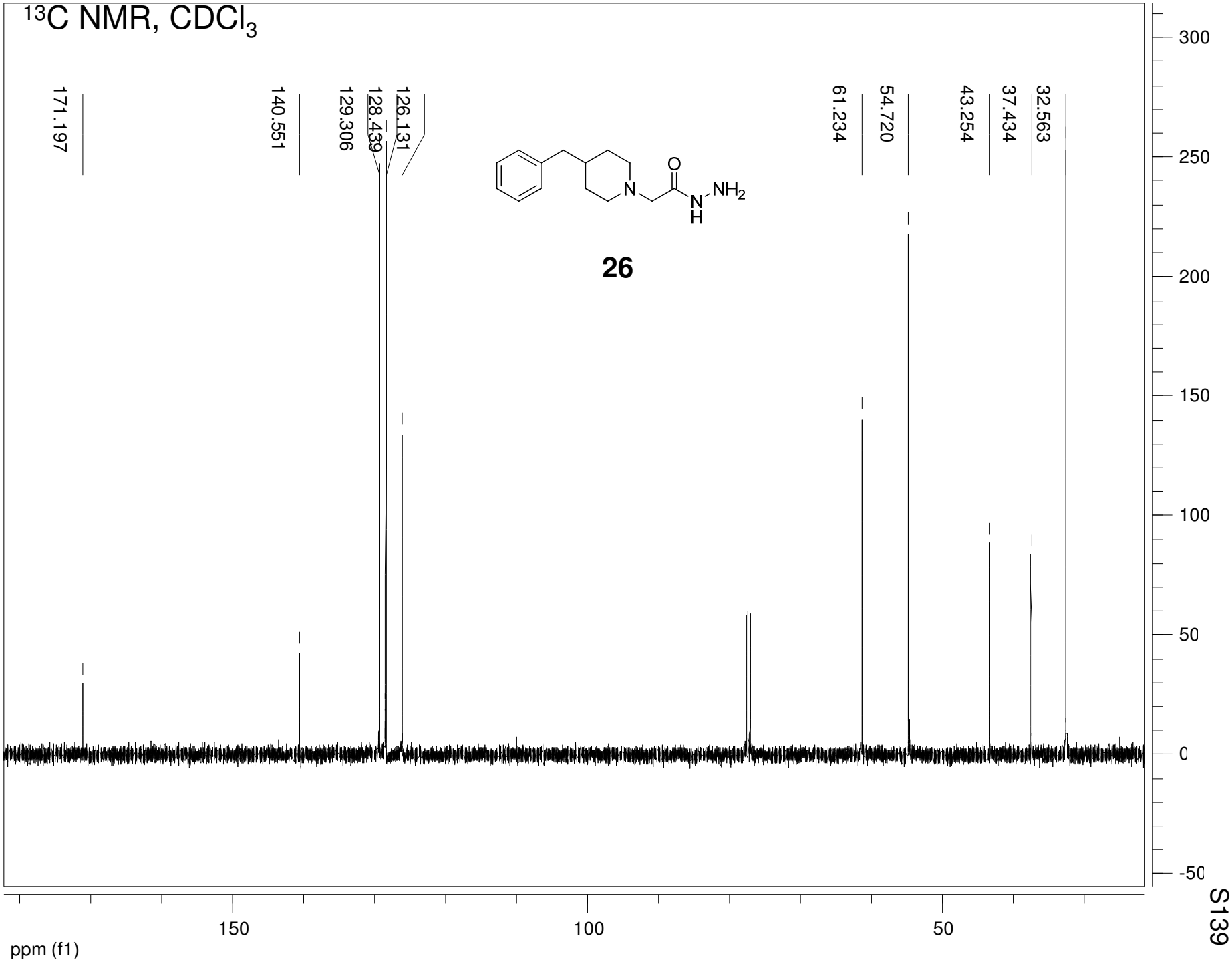

1H NMR, CDCl3
7.272
7.262
7.203
7.200
7.188
7.184
7.173
7.154
7.151
7.137
4.203
4.188
4.174
4.160
3.184
2.932
2.909
2.551
2.537
2.127
2.122
2.103
2.099
2.080
2.076
1.646
1.643
1.632
1.619
1.617
1.554
1.546
1.539
1.532
1.524
1.517
1.509
1.502
1.394
1.387
1.371
O
N
O
43
2.05
1.00
2.07
2.11
2.05
2.22
2.19
2.08
1.27
2.26
3.31
800
700
600
500
400
300
200
100
0
-100
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)

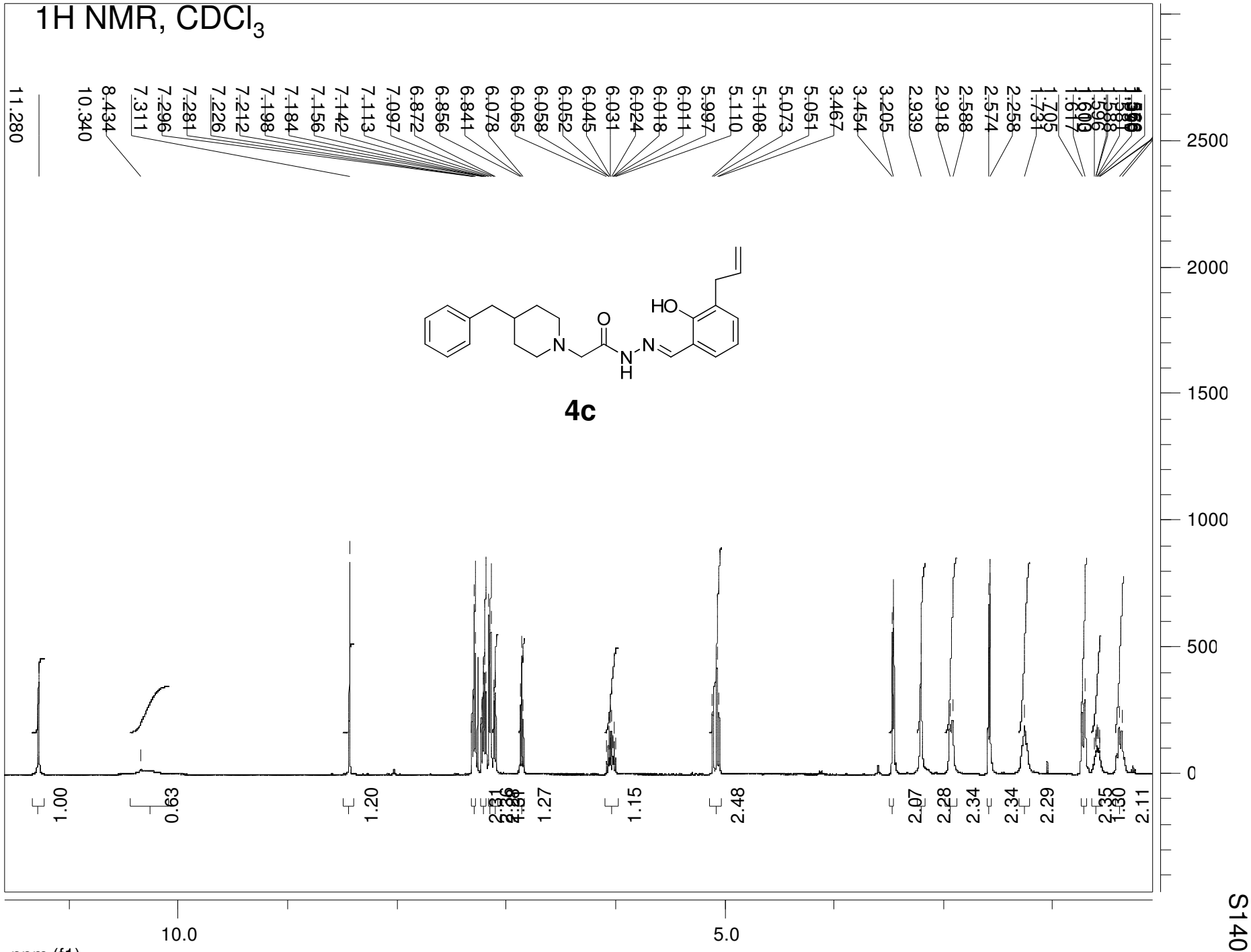
^{13}C NMR, $CDCl_3$

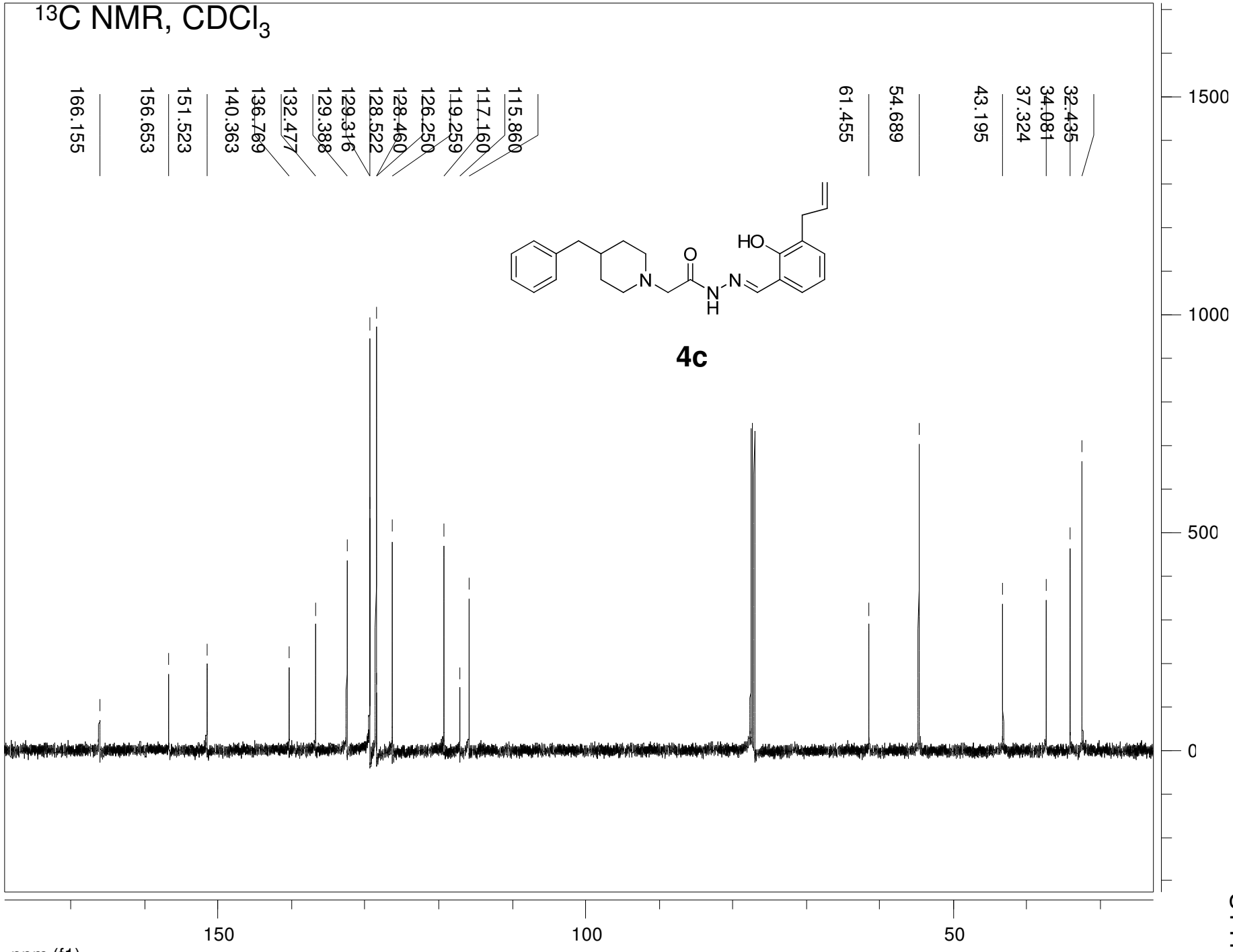


43

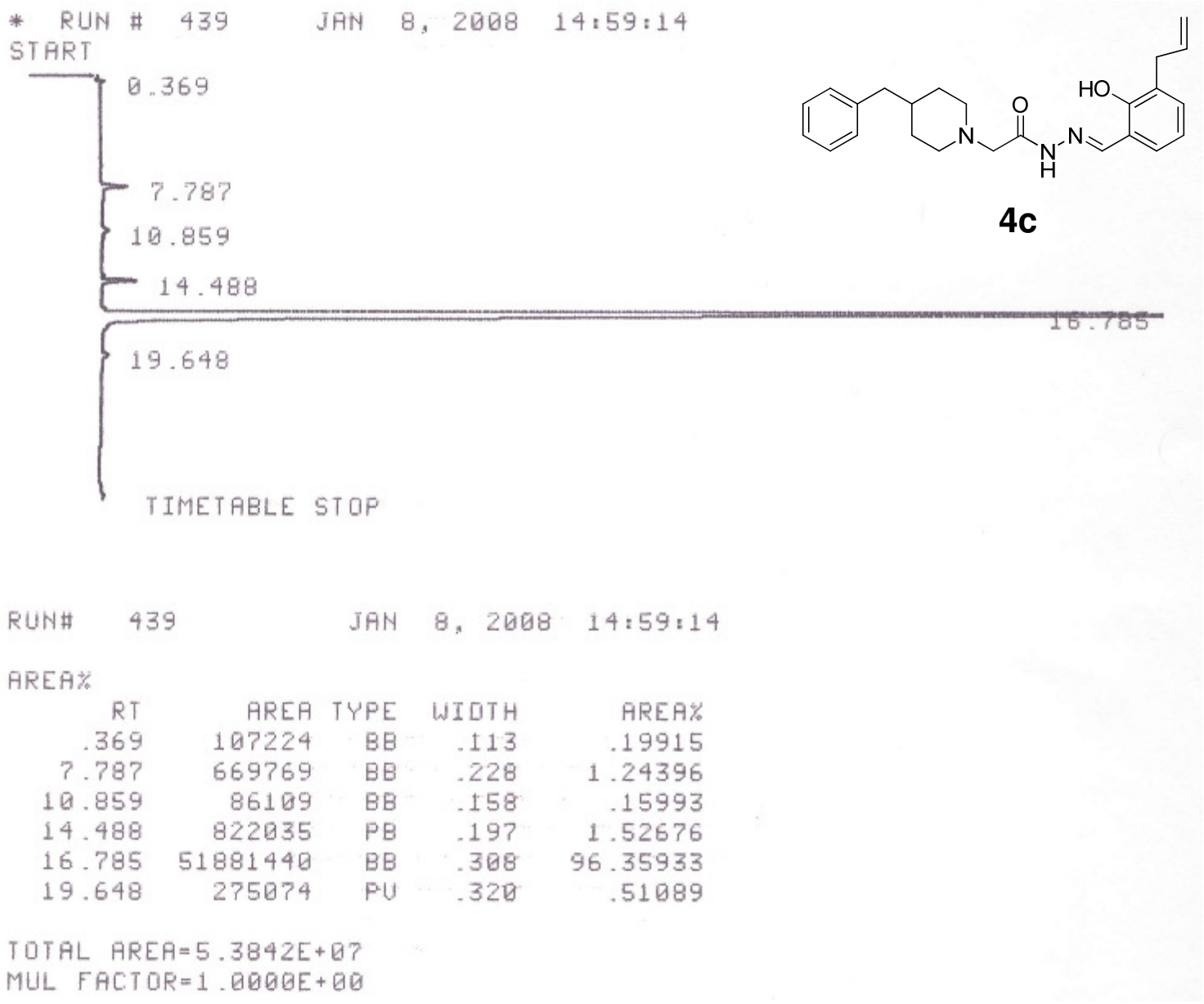

1H NMR, CDCl3
8.204
7.282
7.278
7.267
7.260
7.255
7.252
7.196
7.193
7.185
7.181
7.177
7.169
7.166
7.128
7.125
7.111
3.819
3.817
3.010
2.782
2.759
2.527
2.513
2.094
2.089
2.070
2.065
2.046
2.041
1.633
1.607
1.528
1.521
1.513
1.506
1.498
1.491
1.483
1.496
O
N
NH2
N
H
26
1500
1000
500
0
1.00
2.38
2.38
2.38
2.14
2.18
2.03
2.36
2.42
2.50
1.09
2.27
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (f1)


^{13}C NMR, $CDCl_3$
171.197
140.551
129.306
128.439
126.131
61.234
54.720
43.254
37.434
32.563
O
N
NH$_2$
N
H
26
300
250
200
150
100
50
0
-50
150
100
50
ppm (f1)


1H NMR, CDCl3
11.280
10.340
8.434
7.311
7.296
7.281
7.226
7.212
7.198
7.184
7.156
7.142
7.113
7.097
6.872
6.856
6.841
6.078
6.065
6.058
6.052
6.045
6.031
6.024
6.018
6.011
5.997
5.110
5.108
5.073
5.051
3.467
3.454
3.205
2.939
2.918
2.588
2.574
2.258
1.731
1.705
1.617
1.603
HO
O
N
N
H
N
4c
2500
2000
1500
1000
500
0
1.00
0.93
1.20
2.31
1.27
1.15
2.48
2.07
2.28
2.34
2.34
2.29
2.35
1.30
2.11
10.0
5.0
ppm (f1)


13C NMR, CDCl3
166.155
156.653
151.523
140.363
136.769
132.477
129.388
129.316
128.522
128.460
126.250
119.259
117.160
115.860
61.455
54.689
43.195
37.324
34.081
32.435
HO
O
N
N
H
4c
1500
1000
500
0
150
100
50
ppm (f1)

HPLC



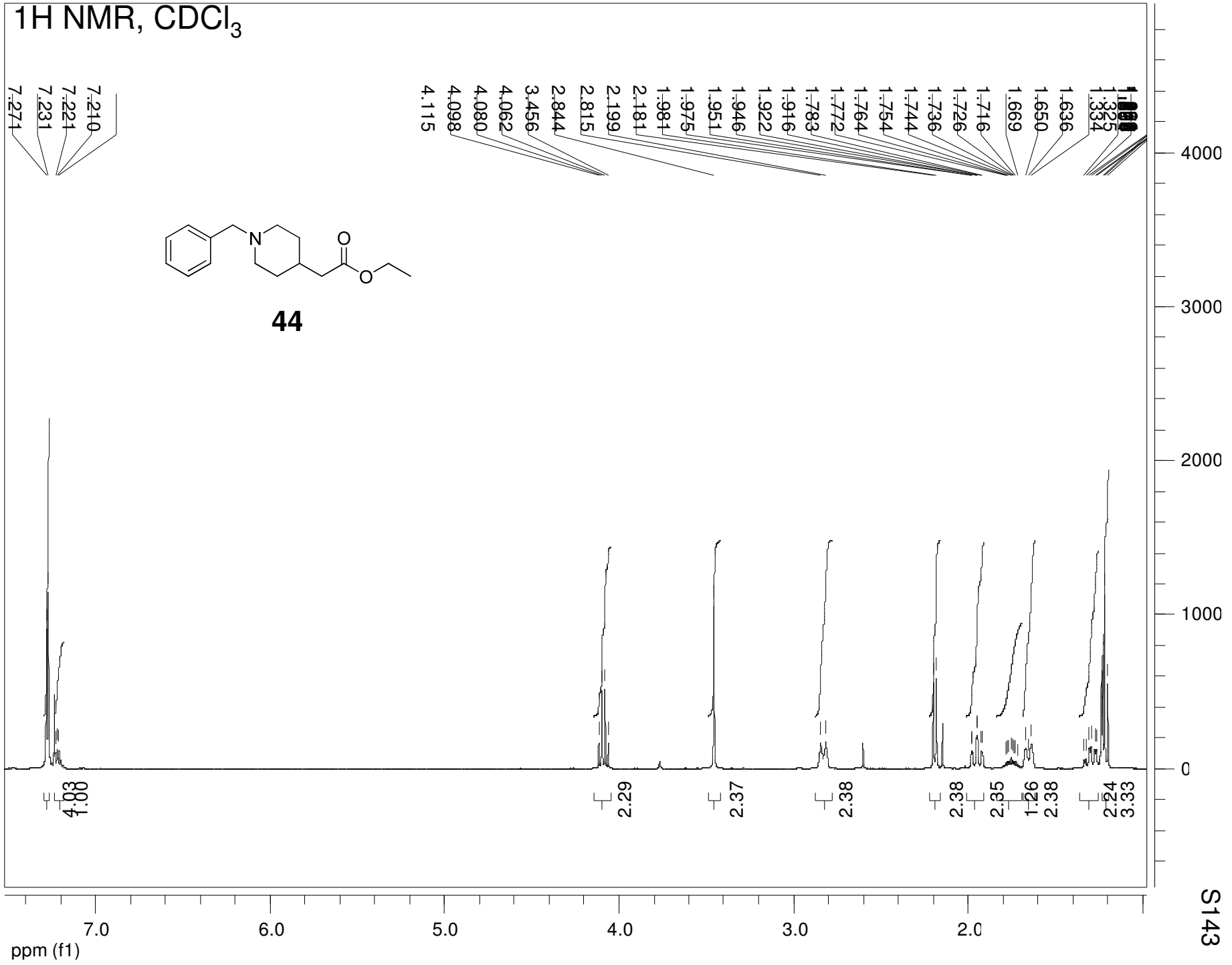
4c

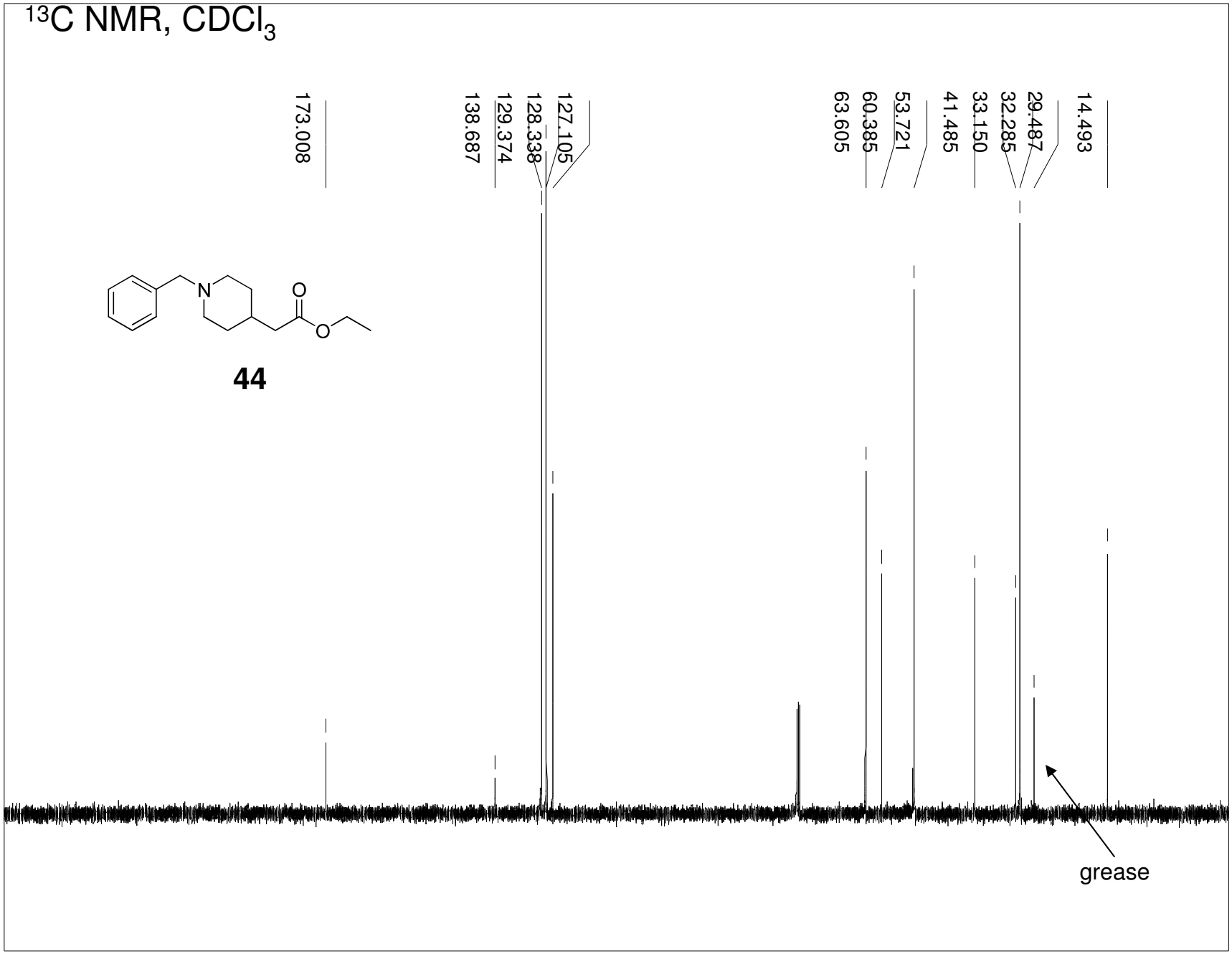
RUN# 439 JAN 8, 2008 14:59:14

AREA%

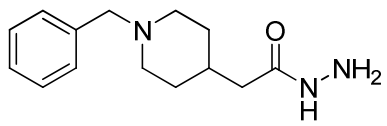
RT	AREA	TYPE	WIDTH	AREA%
.369	107224	BB	.113	.19915
7.787	669769	BB	.228	1.24396
10.859	86109	BB	.158	.15993
14.488	822035	PB	.197	1.52676
16.785	51881440	BB	.308	96.35933
19.648	275074	PU	.320	.51089

TOTAL AREA=5.3842E+07
MUL FACTOR=1.0000E+00

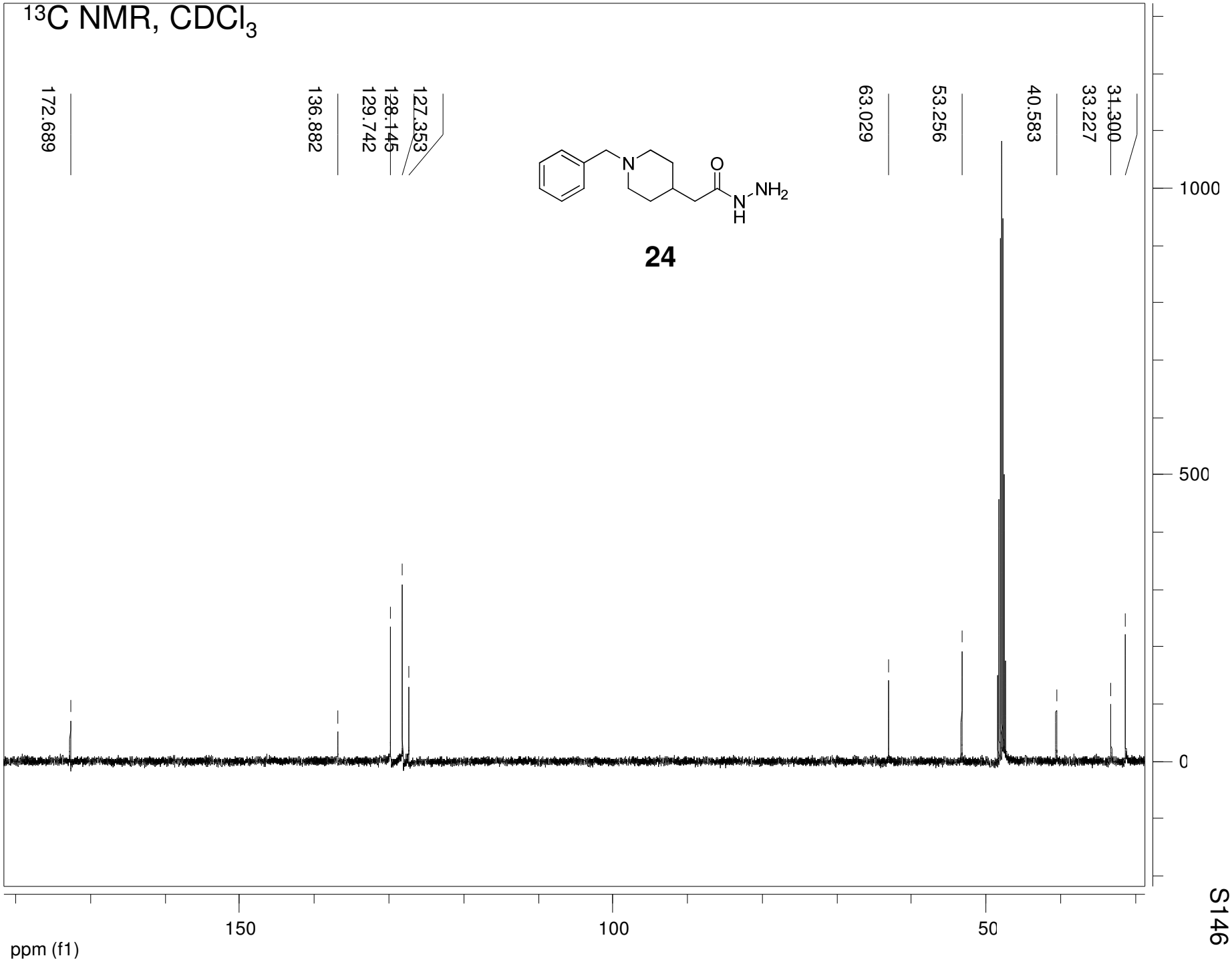

1H NMR, CDCl3
7.271
7.231
7.221
7.210
4.115
4.098
4.080
4.062
3.456
2.844
2.815
2.199
2.181
1.981
1.975
1.951
1.946
1.922
1.916
1.783
1.772
1.764
1.754
1.744
1.736
1.726
1.716
1.669
1.650
1.636
1.334
N
O
O
44
4000
3000
2000
1000
0
4.03
1.00
2.29
2.37
2.38
2.38
2.35
1.26
2.38
2.24
3.33
7.0
6.0
5.0
4.0
3.0
2.0
ppm (f1)


13C NMR, CDCl3
173.008
138.687
129.374
128.338
127.105
63.605
60.385
53.721
41.485
33.150
32.285
29.487
14.493
N
O
O
44
grease
600
500
400
300
200
100
0
200
150
100
50
0
ppm (f1)

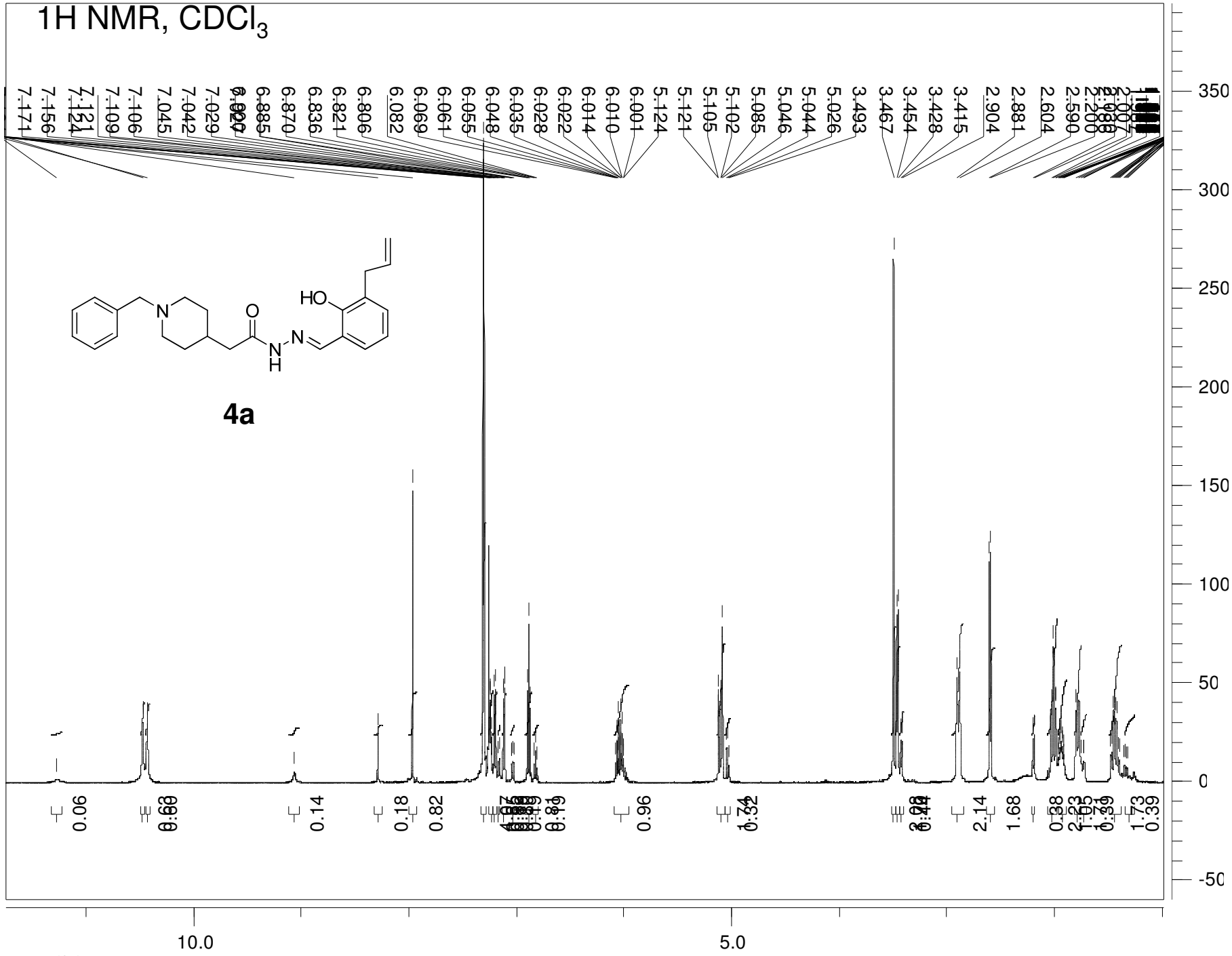
1H NMR, $CDCl_3$

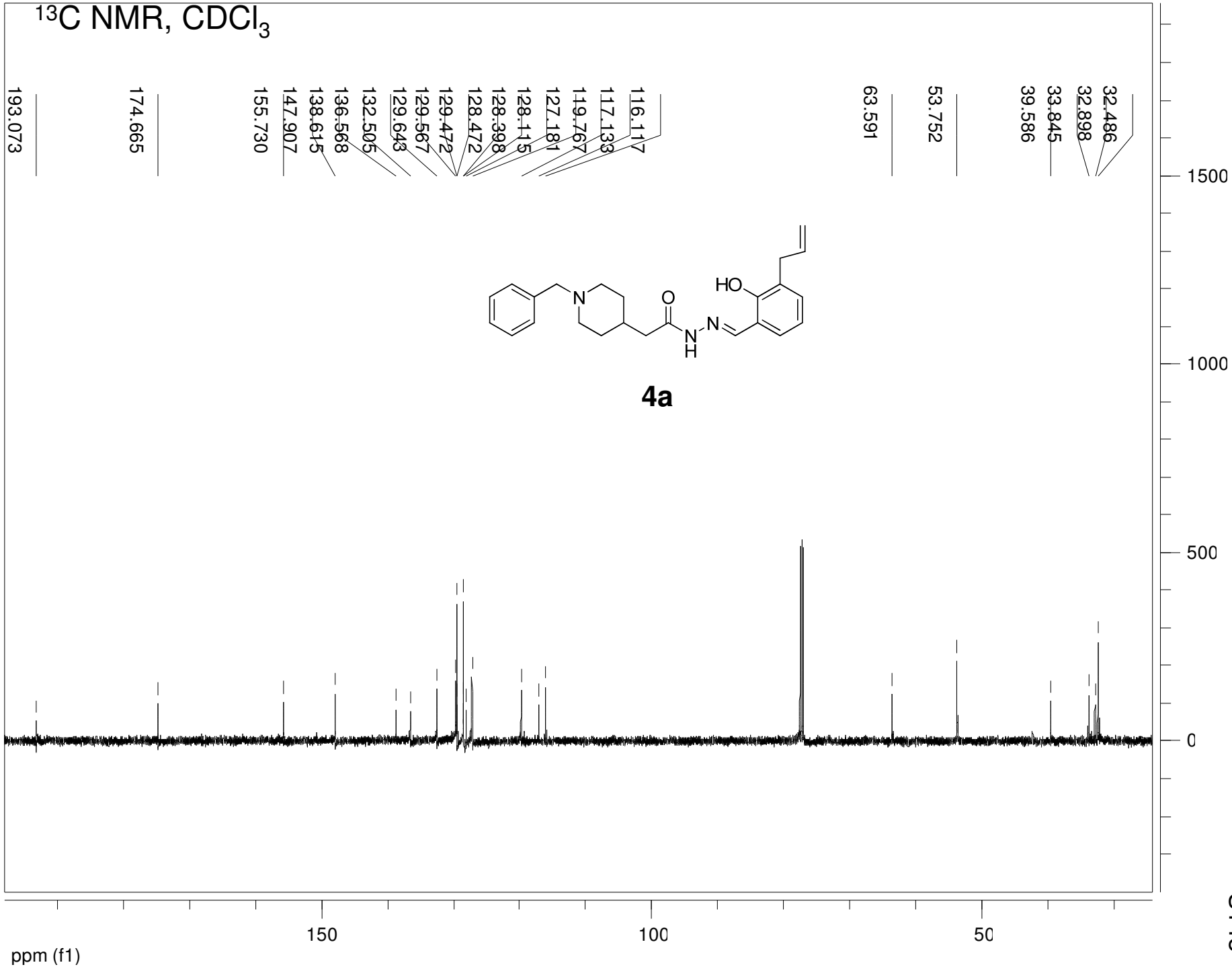


24

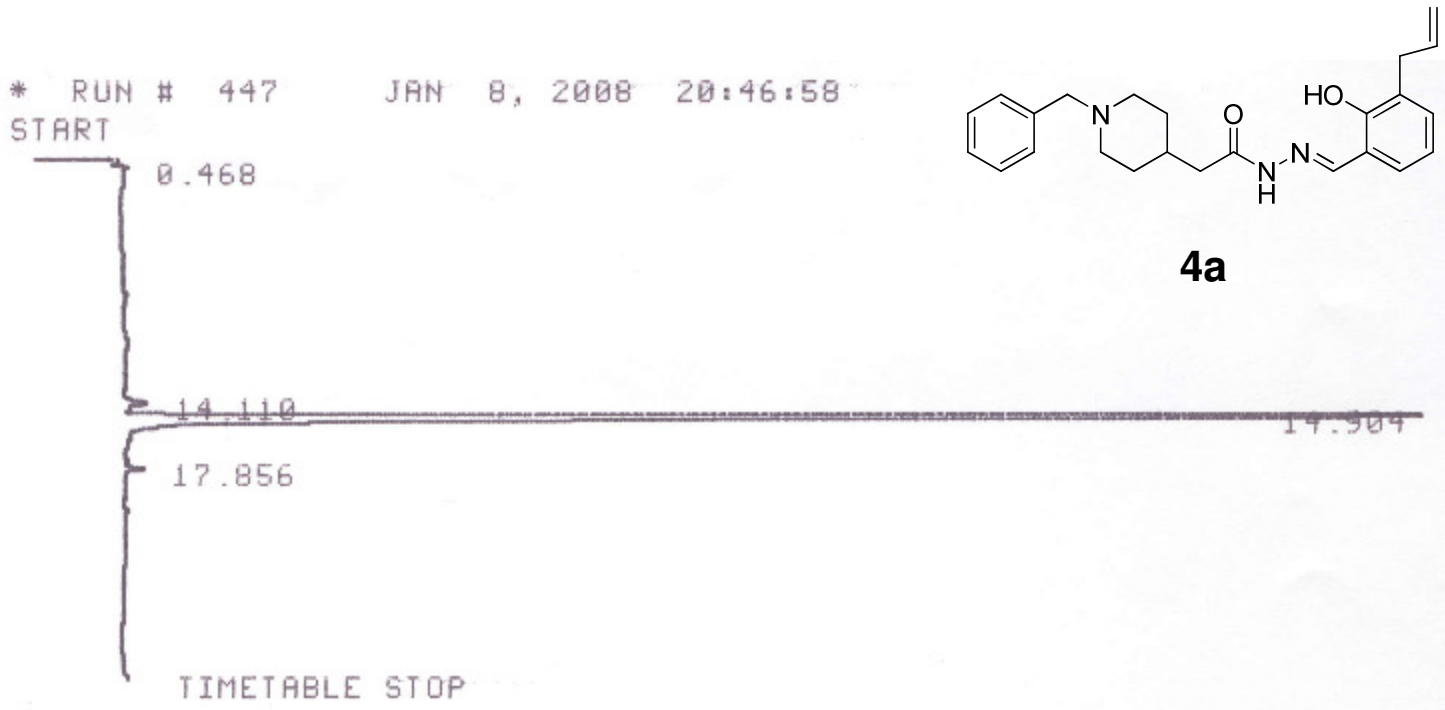

^{13}C NMR, $CDCl_3$
172.689
136.882
129.742
128.145
127.353
63.029
53.256
40.583
33.227
31.300
1000
500
0
150
100
50
ppm (f1)

24


1H NMR, CDCl3
HO
O
N
N
NH
4a
ppm (f1)
10.0
5.0


^{13}C NMR, $CDCl_3$
193.073
174.665
155.730
147.907
138.615
136.568
132.505
129.643
129.567
129.472
128.472
128.398
128.115
127.181
119.767
117.133
116.117
63.591
53.752
39.586
33.845
32.898
32.486
HO
N
O
N
NH
4a
1500
1000
500
0
150
100
50
ppm (f1)

HPLC



4a

RUN# 447 JAN 8, 2008 20:46:58

AREA%

RT	AREA	TYPE	WIDTH	AREA%
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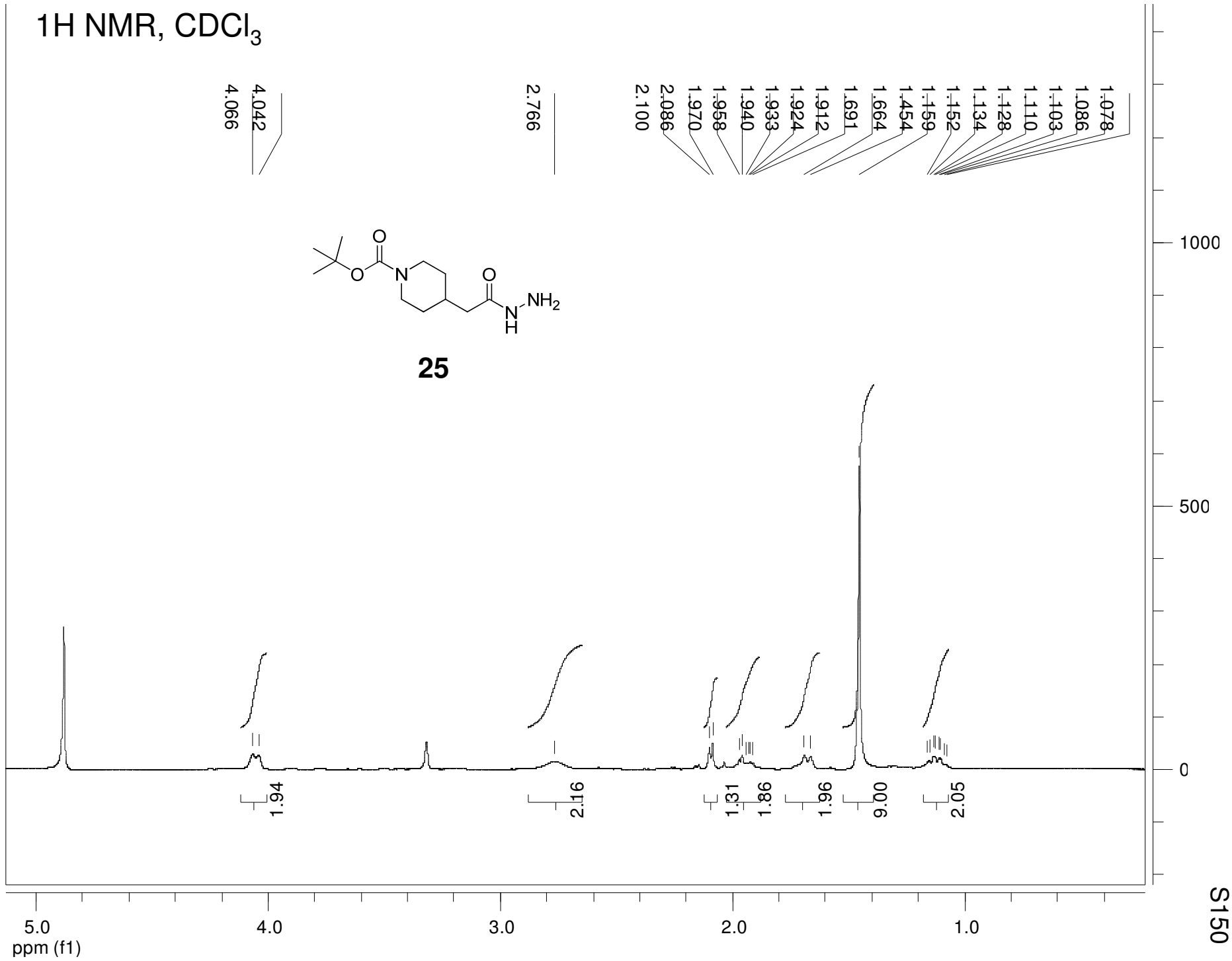
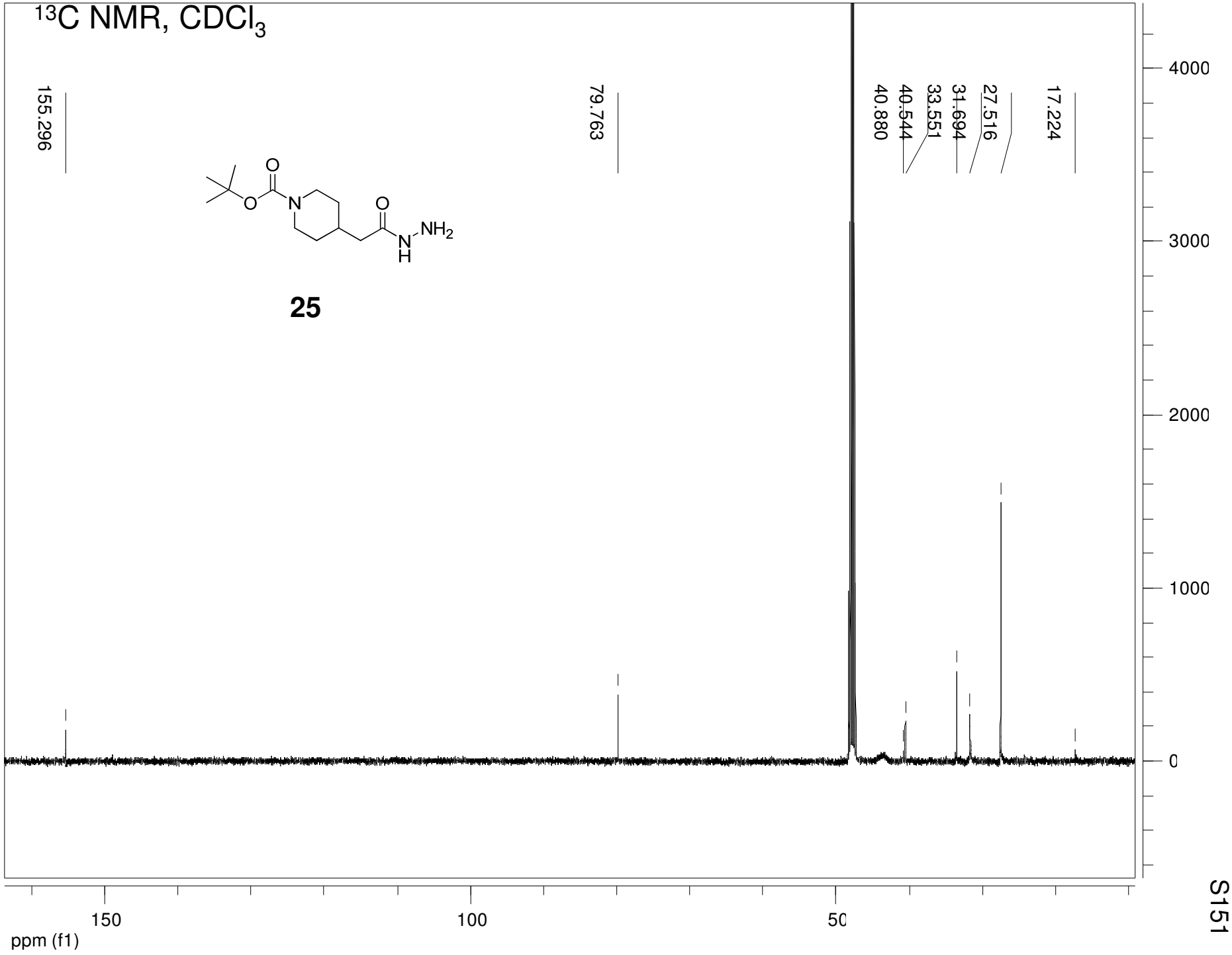
14.110	656174	BP	.319	1.53580
14.904	41376064	PB	.293	96.84227
17.856	339703	BB	.186	.79509

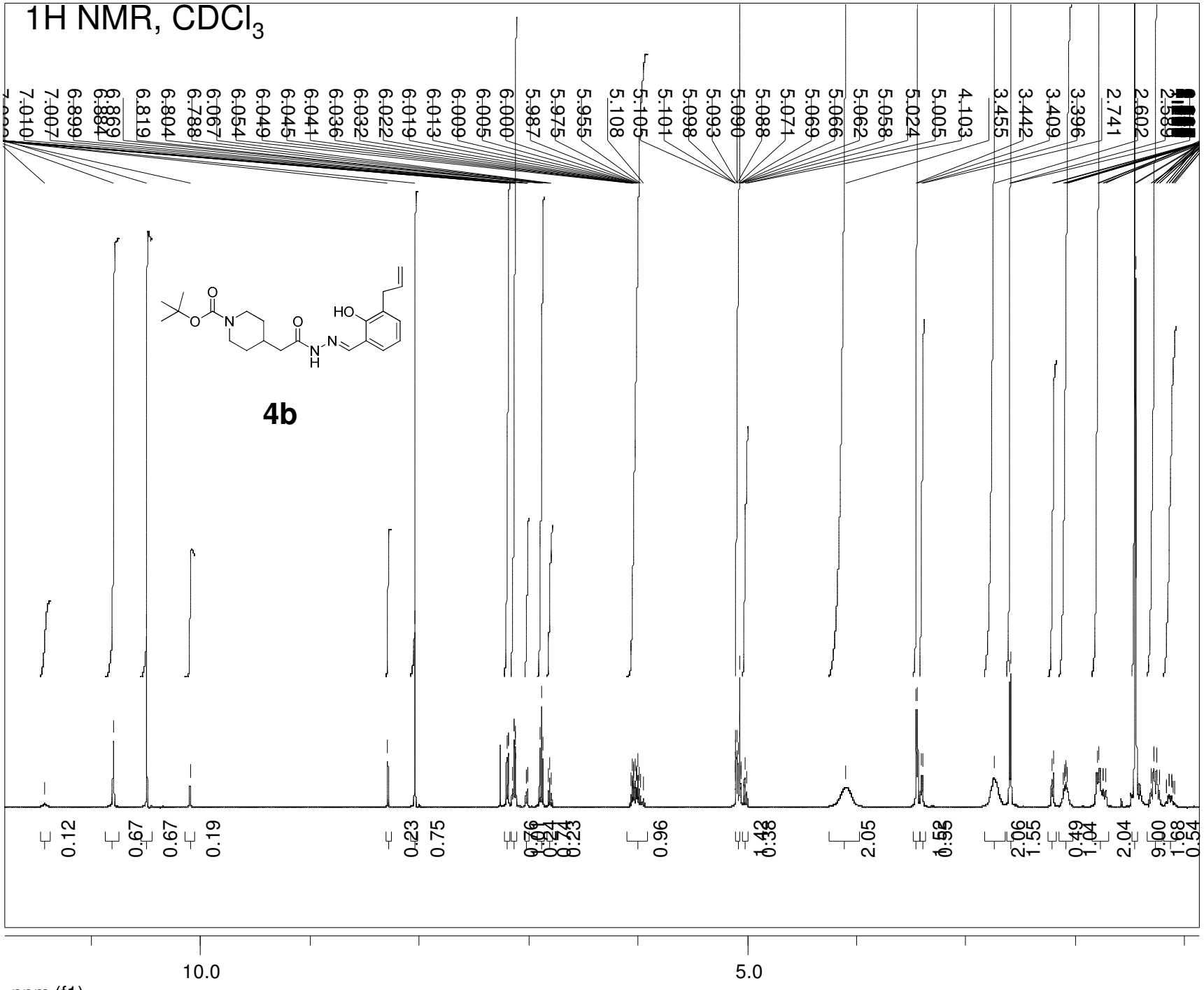
TOTAL AREA=4.2725E+07
MUL FACTOR=1.0000E+00

1H NMR, $CDCl_3$

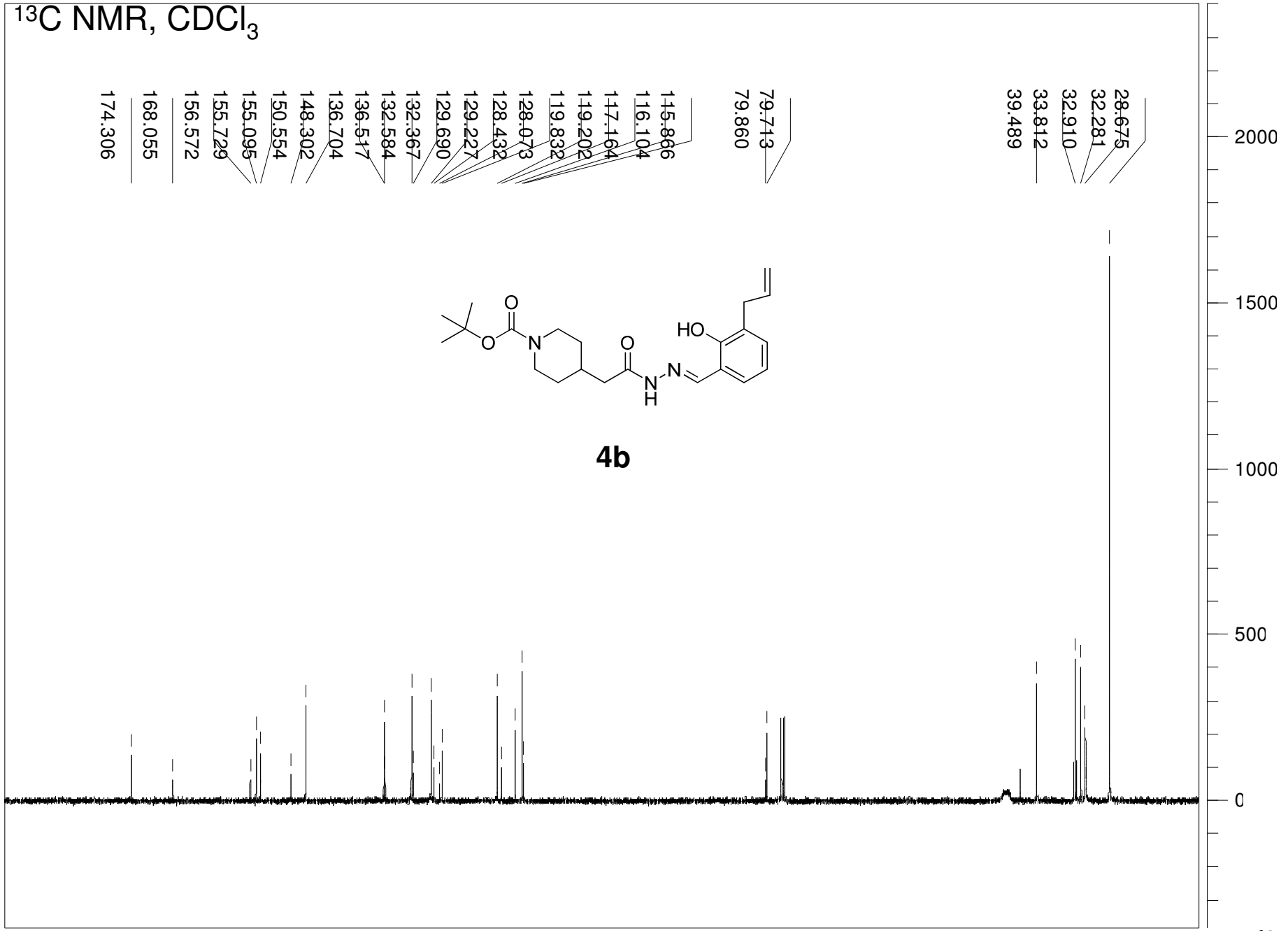


25


13C NMR, CDCl3
155.296
79.763
40.880
40.544
33.551
31.694
27.516
17.224
O
O
N
O
N
H
NH2
25
4000
3000
2000
1000
0
150
100
50
ppm (f1)


1H NMR, CDCl3
4b
HO
O
N
H
ppm (f1)
10.0
5.0

^{13}C NMR, $CDCl_3$



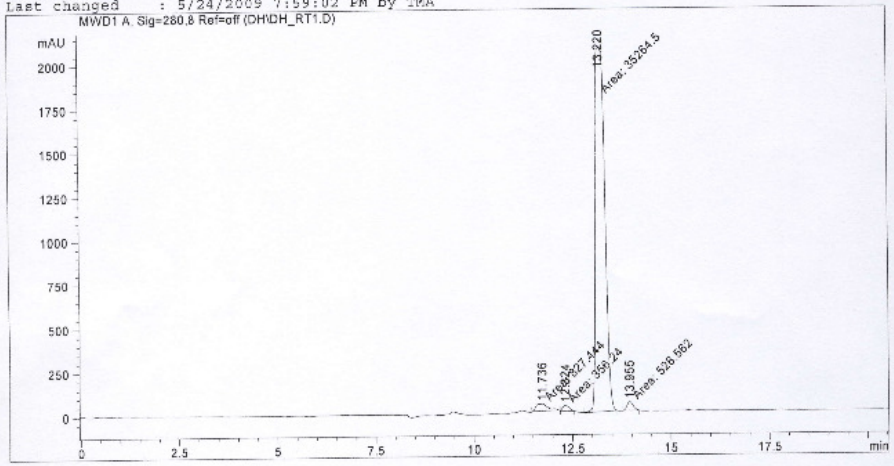
4b

HPLC

Data File D:\HPCHEM\DATA\2009\DH\DH_RT1.D
Sample Name: DH-RT1

Acq. Operator : DH
Acq. Instrument : El Chupacabra Location : P1-F-05
Injection Date : 5/24/2009 11:37:49 PM Inj Volume : 10 µl

Acq. Method : C:\HPCHEM\1\METHODS\DH.M
Last changed : 5/24/2009 11:34:17 PM by DH
(modified after loading)
Analysis Method : C:\HPCHEM\1\METHODS\DH.M
Last changed : 5/24/2009 7:59:02 PM by TMA



Fraction Information

Fraction collection off

No Fractions found.

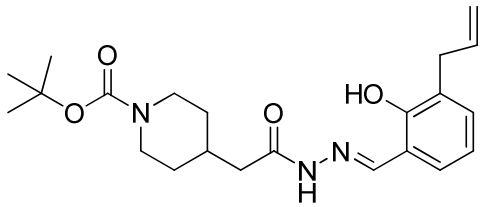
Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

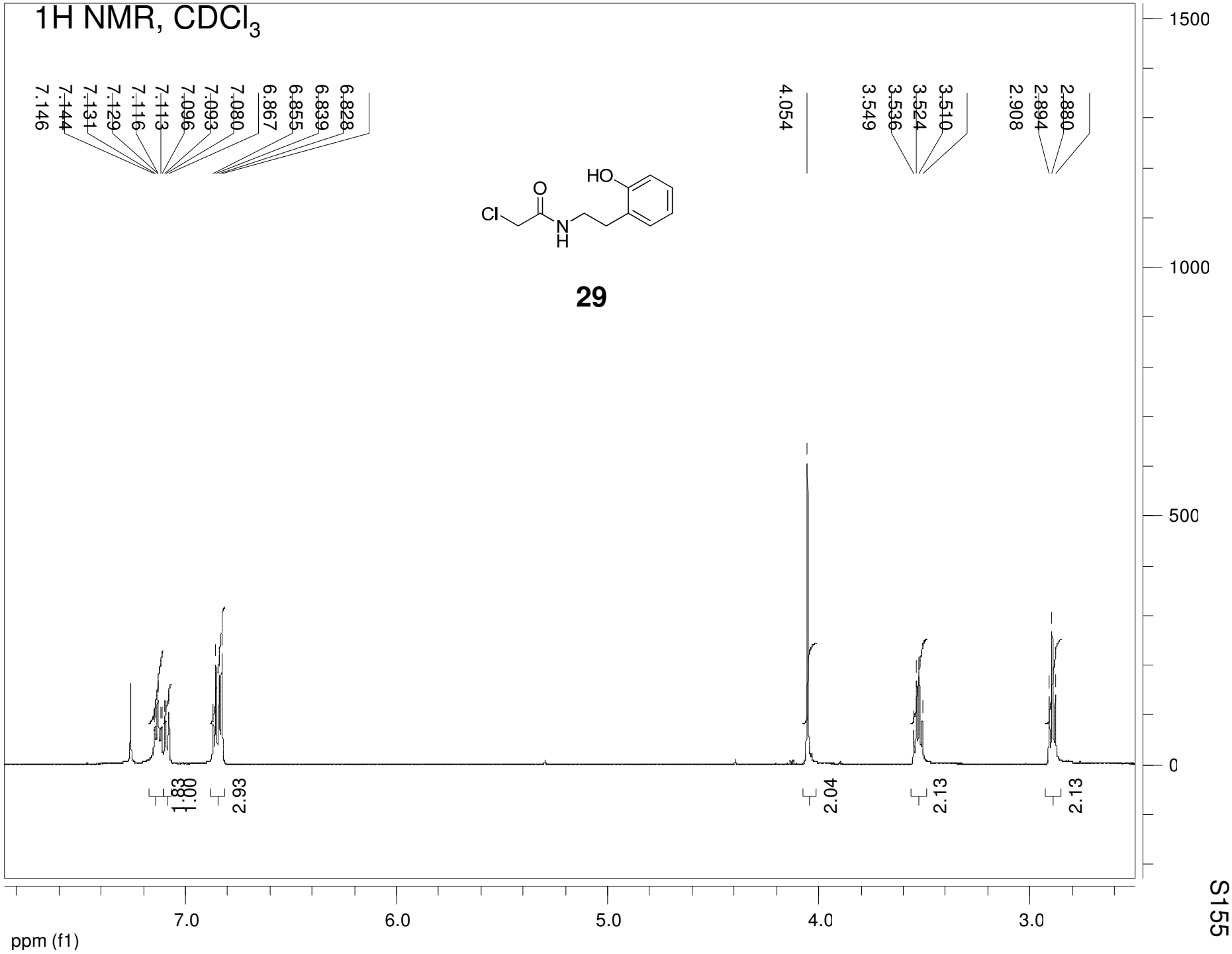
Signal 1: MWD1 A, Sig=280,8 Ref=off

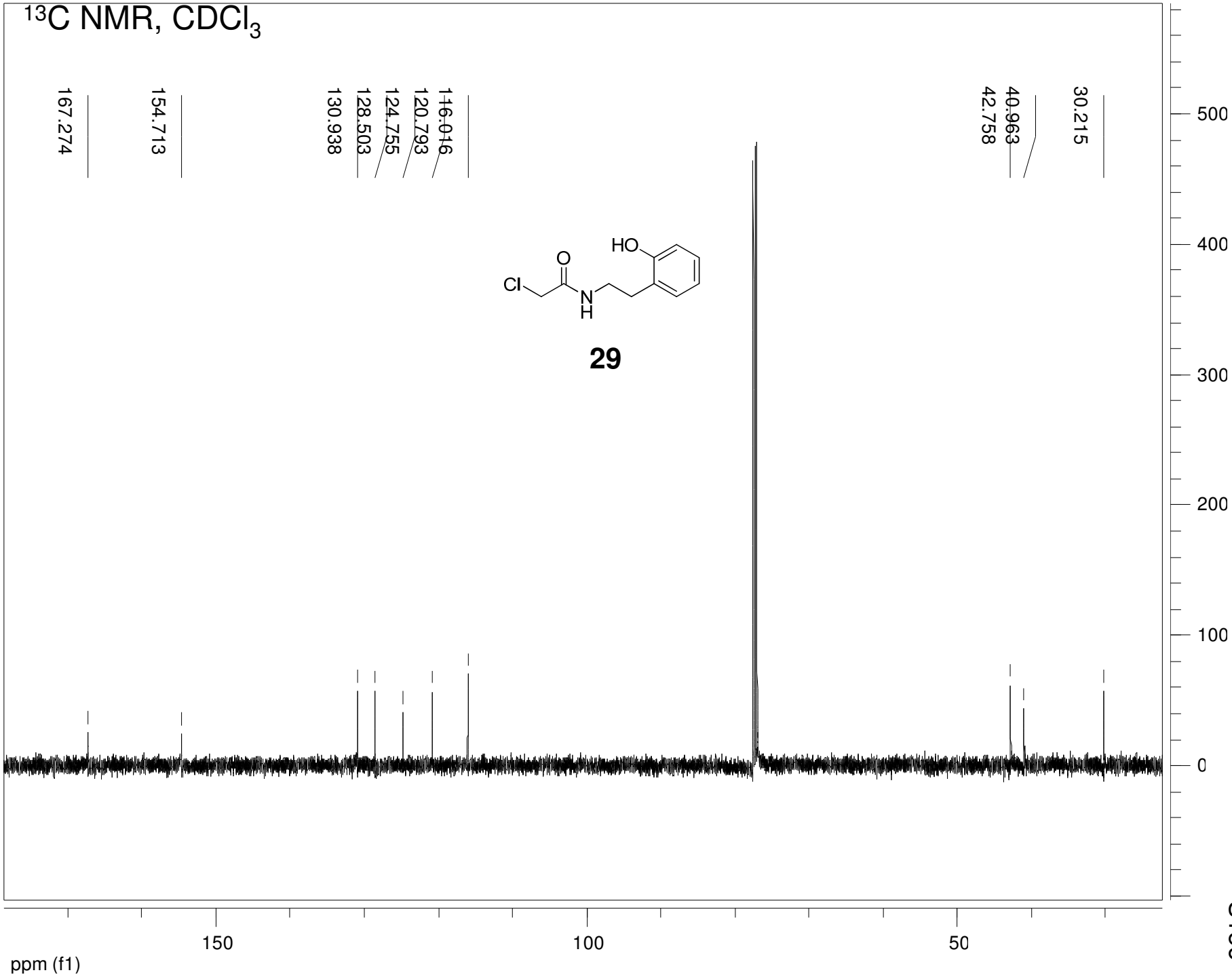
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.736	MM	0.3369	827.44397	40.93148	2.2377
2	12.324	MM	0.1806	356.24042	32.88172	0.9634
3	13.220	MM	0.2834	3.52645e4	2073.87036	95.3694
4	13.955	MM	0.1756	528.56183	50.16971	1.4294
Totals :				3.6976Be4	2197.85326	

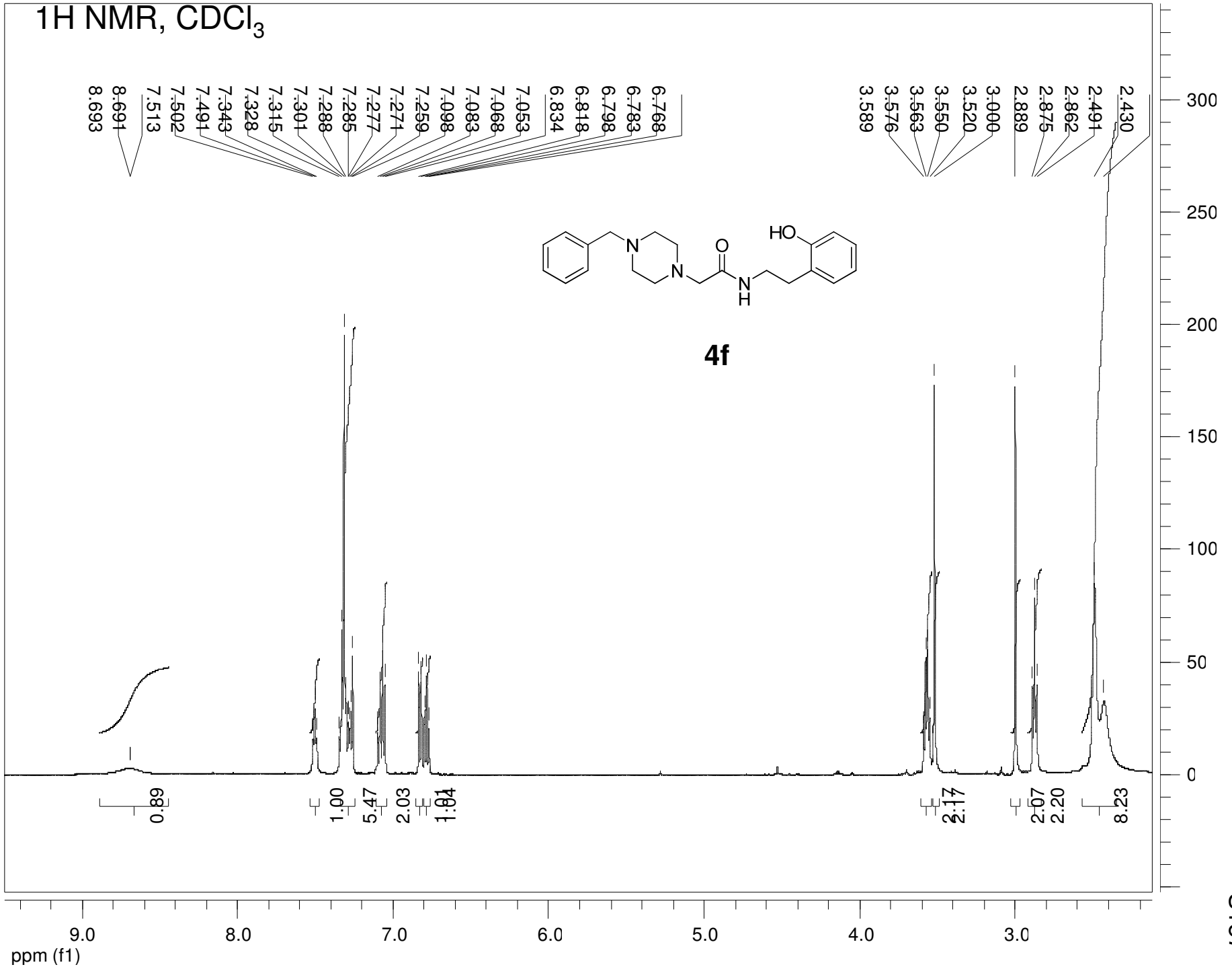
El Chupacabra 5/25/2009 12:01:22 AM DH Page 1 of 2

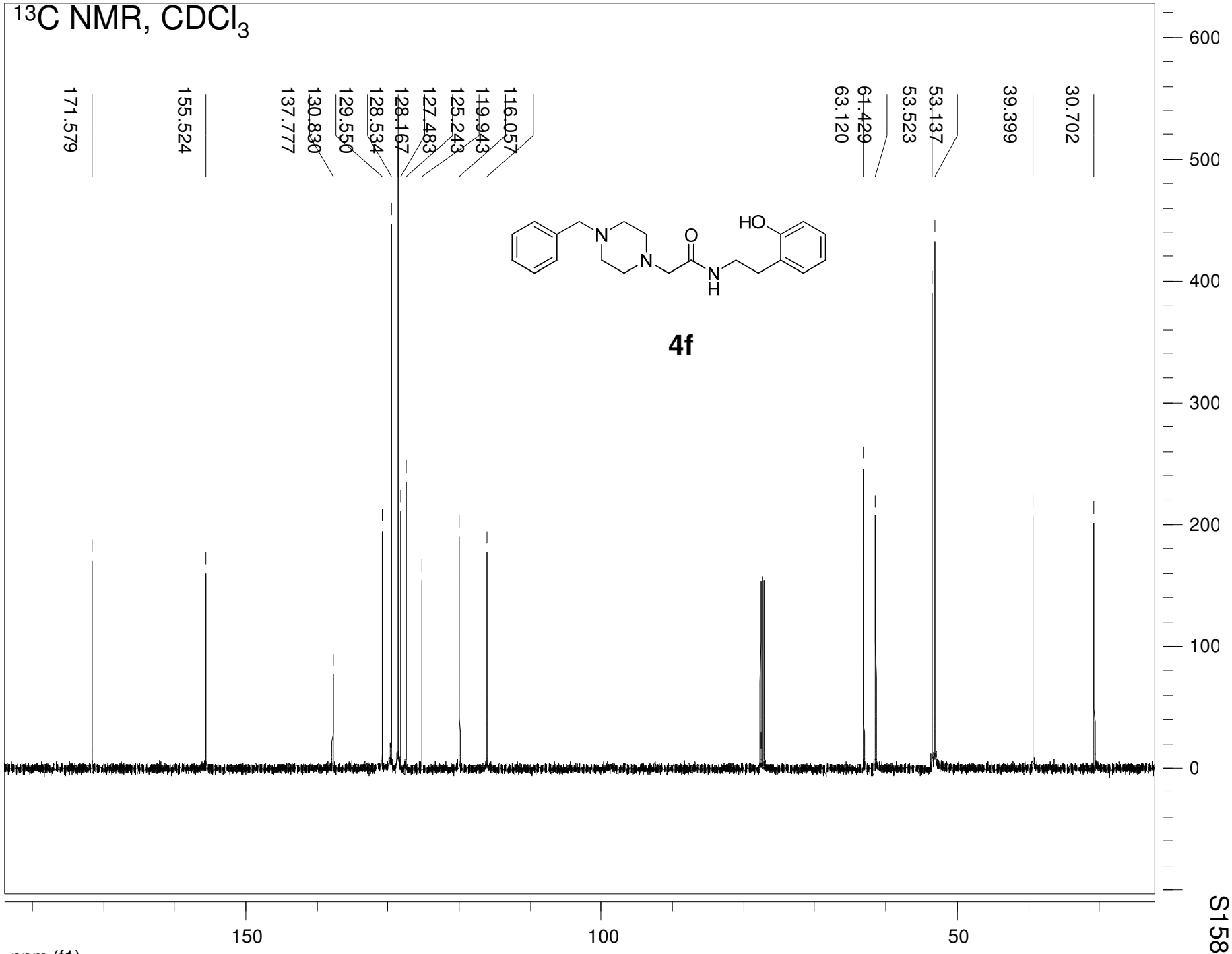


4b

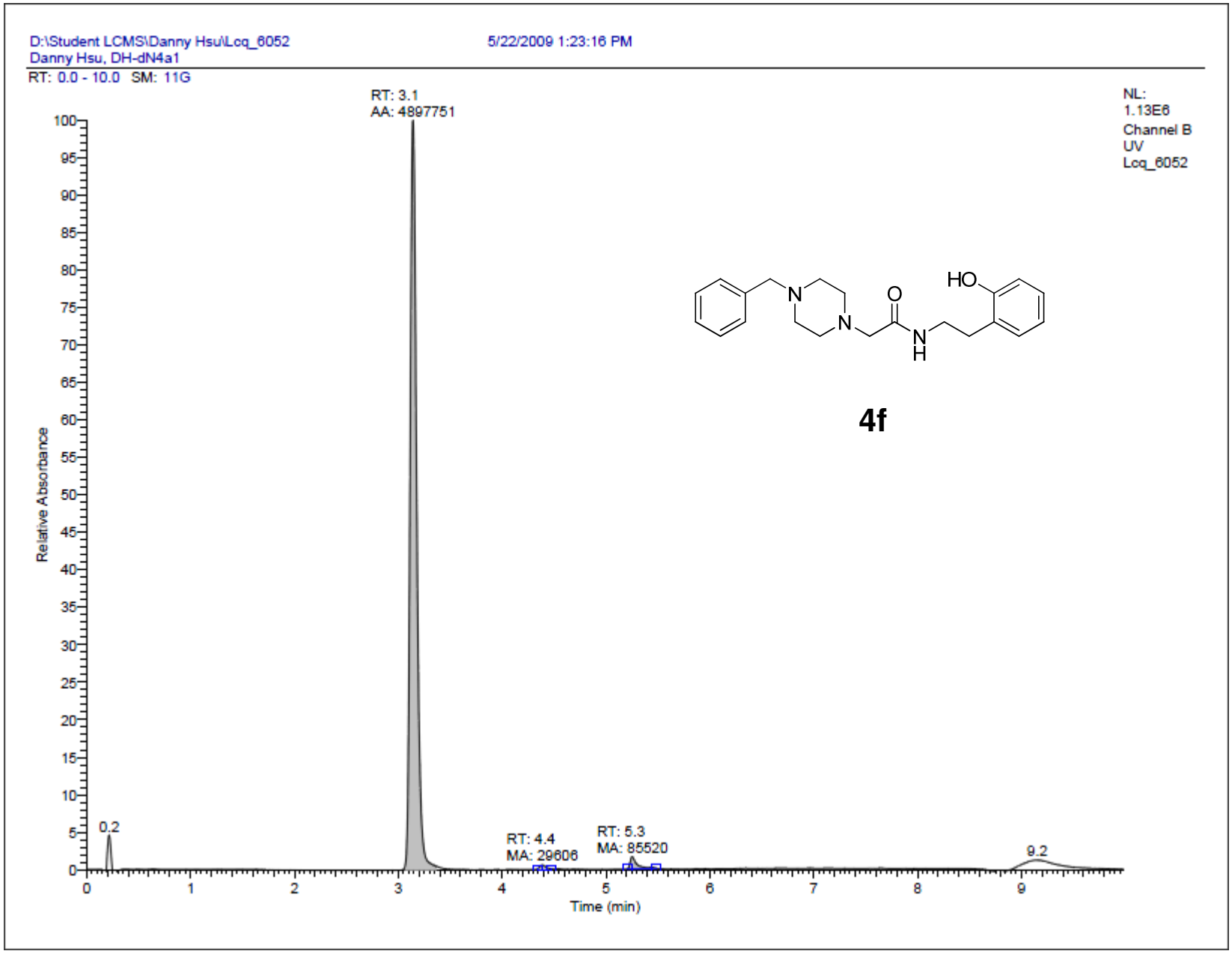

1H NMR, CDCl3
7.146
7.144
7.131
7.129
7.116
7.113
7.096
7.093
7.080
6.867
6.855
6.839
6.828
4.054
3.549
3.536
3.524
3.510
2.908
2.894
2.880
HO
O
Cl
N
H
29
1.83
1.00
2.93
2.04
2.13
2.13
1500
1000
500
0
7.0
6.0
5.0
4.0
3.0
ppm (f1)

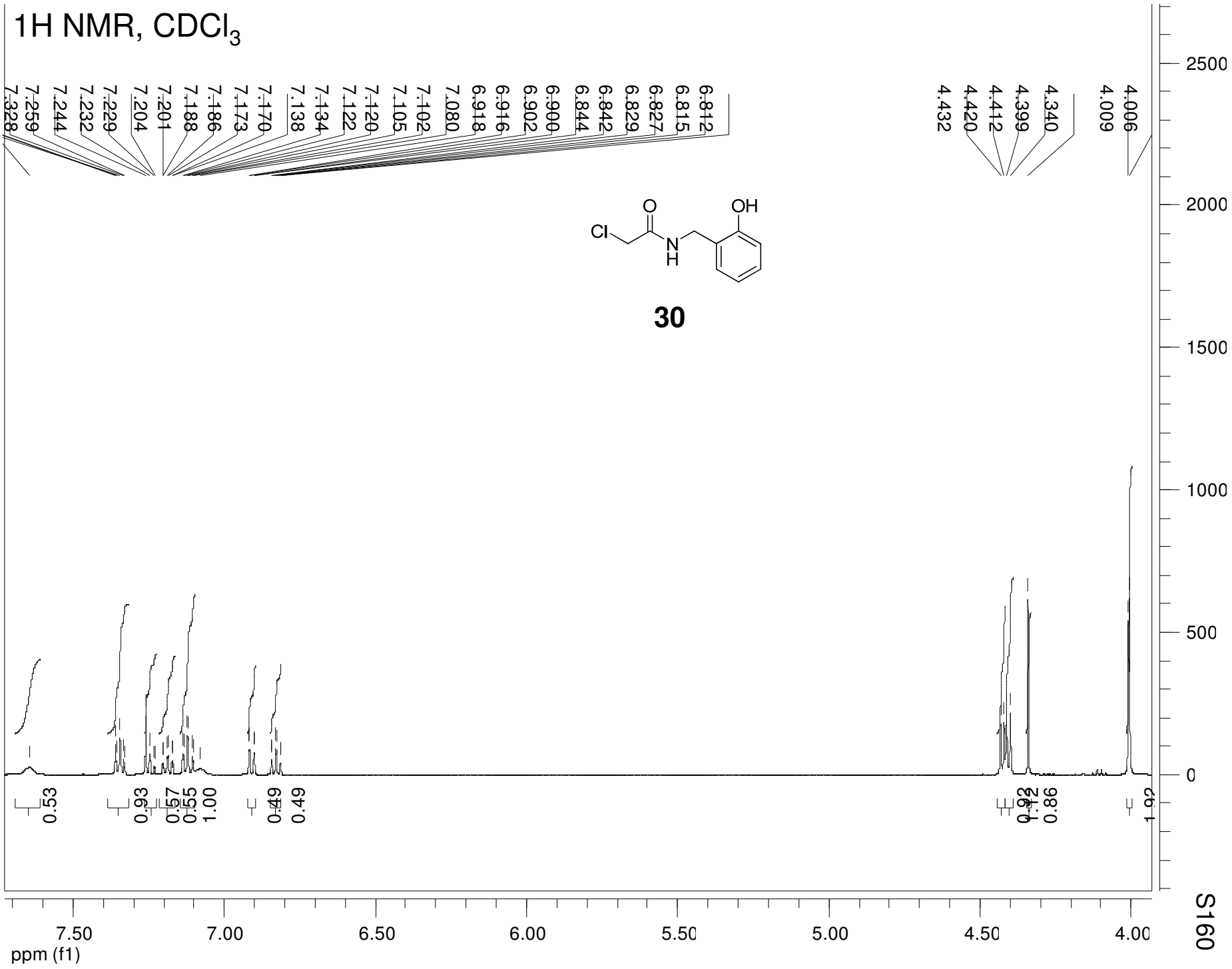

^{13}C NMR, $CDCl_3$
167.274
154.713
130.938
128.503
124.755
120.793
116.016
42.758
40.963
30.215
HO
O
Cl
N
H
29
500
400
300
200
100
0
150
100
50
ppm (f1)


1H NMR, CDCl3
8.693
8.691
7.513
7.502
7.491
7.343
7.328
7.315
7.301
7.288
7.285
7.277
7.271
7.259
7.098
7.083
7.068
7.053
6.834
6.818
6.798
6.783
6.768
3.589
3.576
3.563
3.550
3.520
3.000
2.889
2.875
2.862
2.491
2.430
HO
N
O
N
H
4f
0.89
1.00
5.47
2.03
1.01
2.17
2.07
2.20
8.23
300
250
200
150
100
50
0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)


^{13}C NMR, $CDCl_3$
171.579
155.524
137.777
130.830
129.550
128.534
128.167
127.483
125.243
119.943
116.057
63.120
61.429
53.523
53.137
39.399
30.702
HO
N
N
O
NH
4f
600
500
400
300
200
100
0
150
100
50
ppm (f1)

LC-MS
Purity: 95%




1H NMR, CDCl3
7.328
7.259
7.244
7.232
7.229
7.204
7.201
7.188
7.186
7.173
7.170
7.138
7.134
7.122
7.120
7.105
7.102
7.080
6.918
6.916
6.902
6.900
6.844
6.842
6.829
6.827
6.815
6.812
4.432
4.420
4.412
4.399
4.340
4.009
4.006
Cl
O
N
H
OH
30
0.53
0.63
0.57
0.55
1.00
0.49
0.49
0.92
1.12
0.98
2500
2000
1500
1000
500
0
7.50
7.00
6.50
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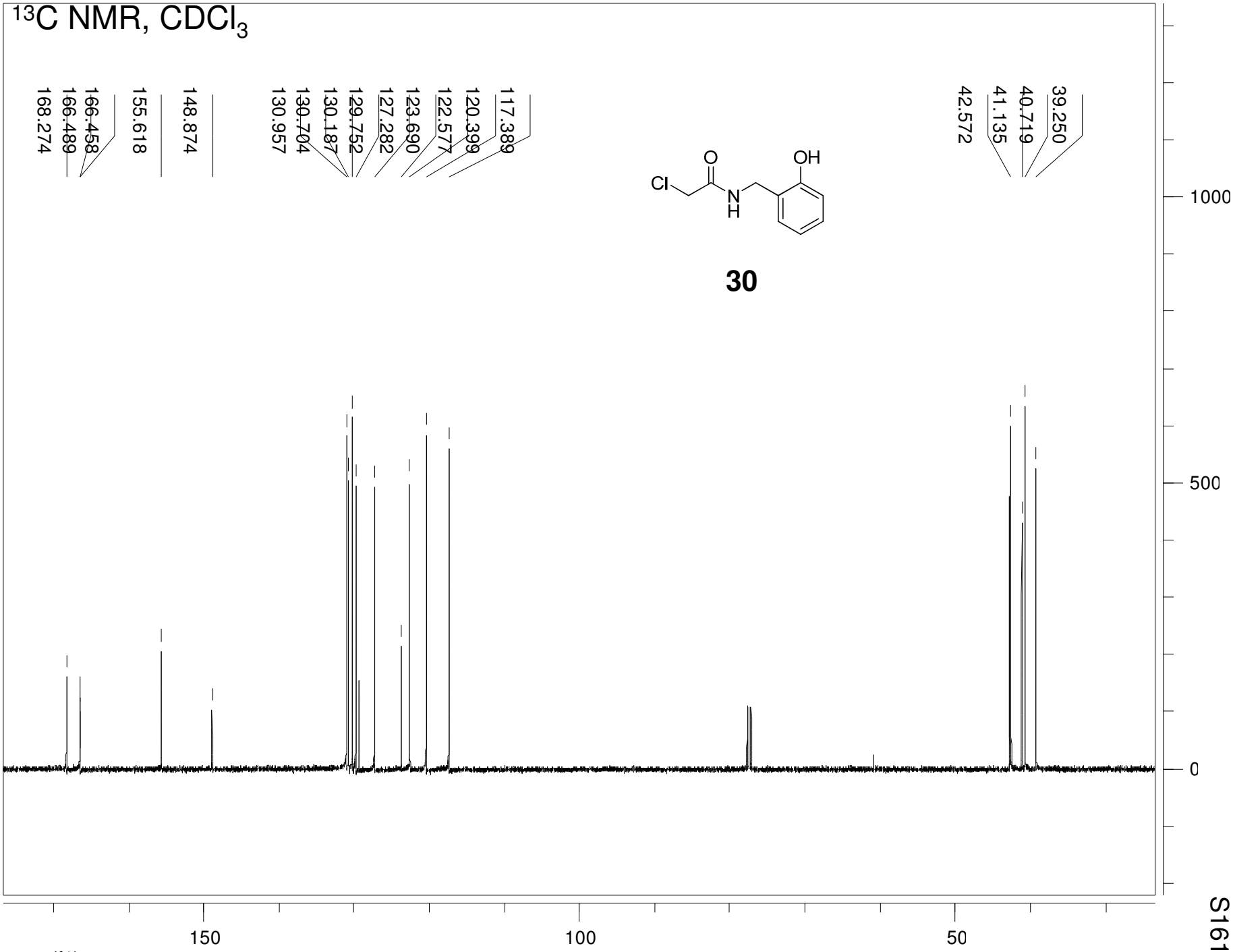
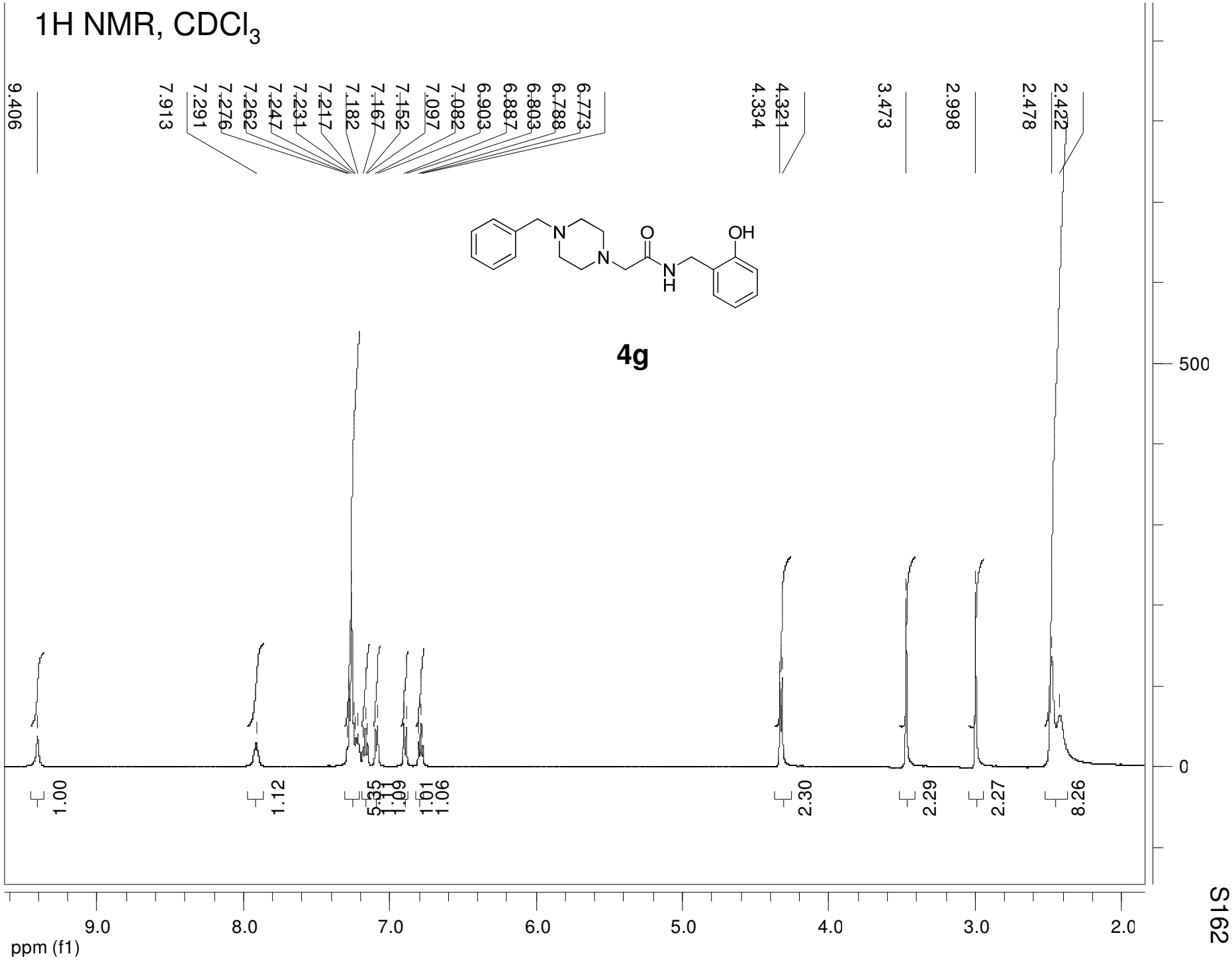
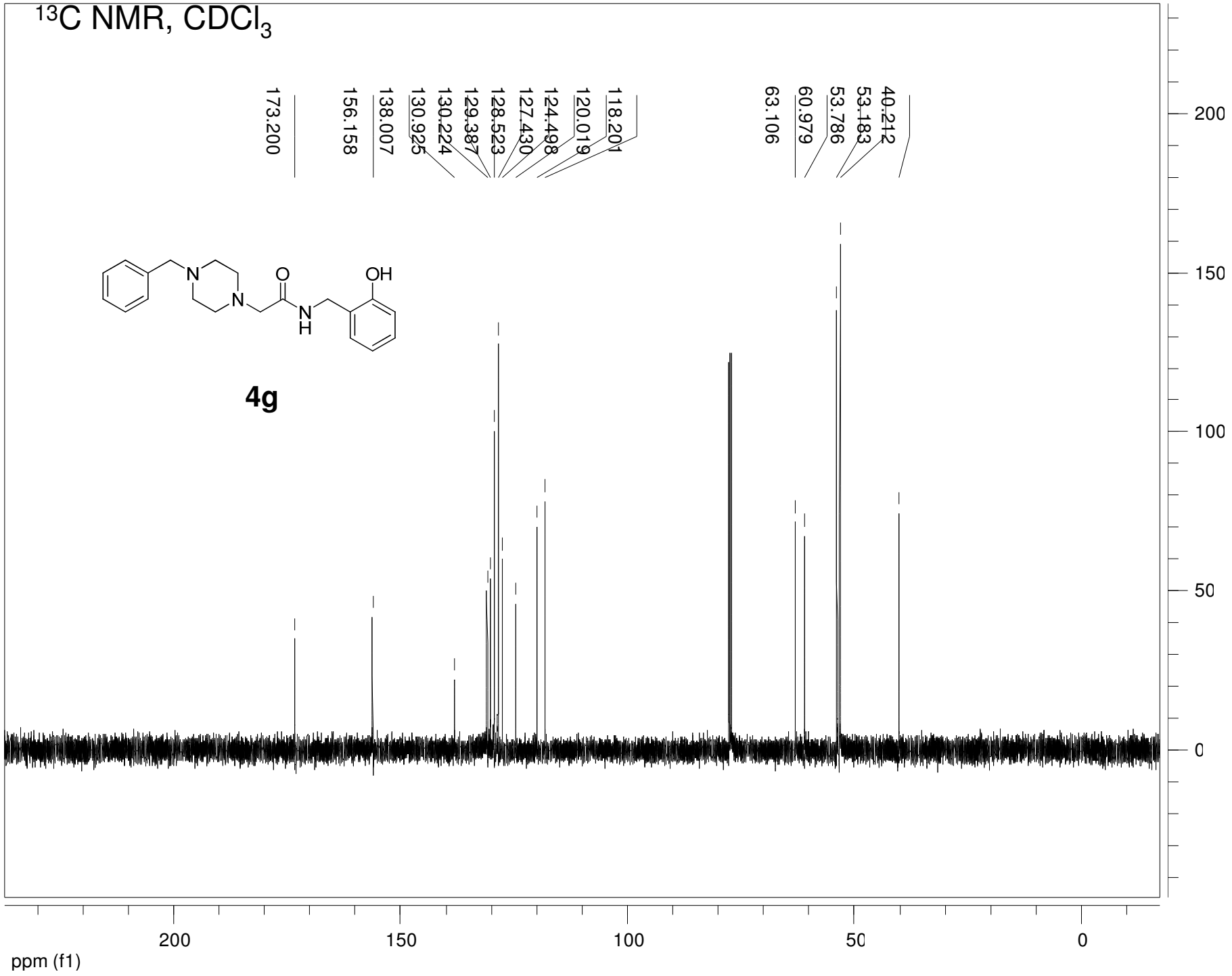
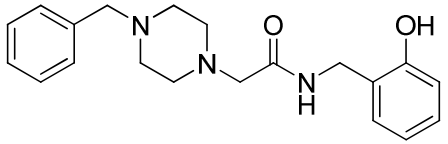
5.50
5.00
4.50
4.00
ppm (f1)


13C NMR, CDCl3
168.274
166.489
166.458
155.618
148.874
130.957
130.704
130.187
129.752
127.282
123.690
122.577
120.399
117.389
42.572
41.135
40.719
39.250
Cl
O
N
H
OH
30
1000
500
0
150
100
50
ppm (f1)

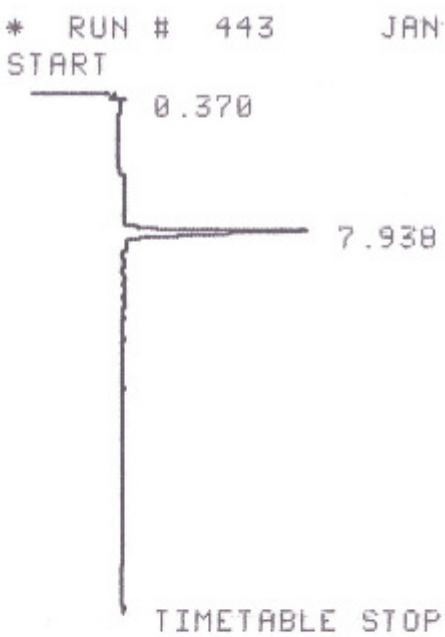

1H NMR, CDCl3
9.406
7.913
7.291
7.276
7.262
7.247
7.231
7.217
7.182
7.167
7.152
7.097
7.082
6.903
6.887
6.803
6.788
6.773
4.334
4.321
3.473
2.998
2.478
2.422
N
N
O
N
H
OH
4g
500
0
1.00
1.12
5.35
1.11
1.09
1.01
1.06
2.30
2.29
2.27
8.26
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (f1)


^{13}C NMR, $CDCl_3$
173.200
156.158
138.007
130.925
130.224
129.387
128.523
127.430
124.498
120.019
118.201
63.106
60.979
53.786
53.183
40.212
N
N
O
N
H
OH
4g
200
150
100
50
0
200
150
100
50
0
ppm (f1)

HPLC



4g



RUN# 443 JAN 8, 2008 17:15:55

AREA%

RT	AREA	TYPE	WIDTH	AREA%
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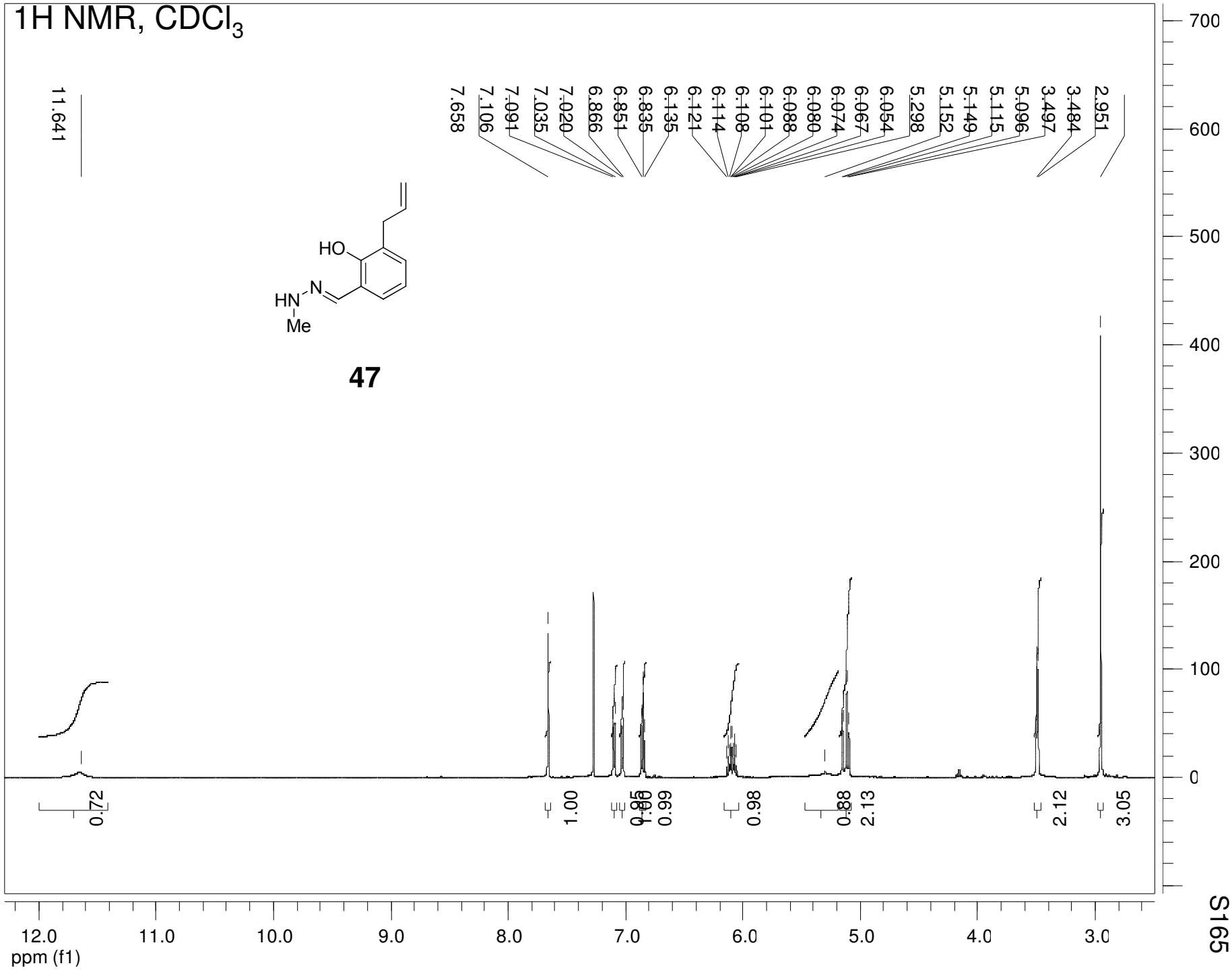
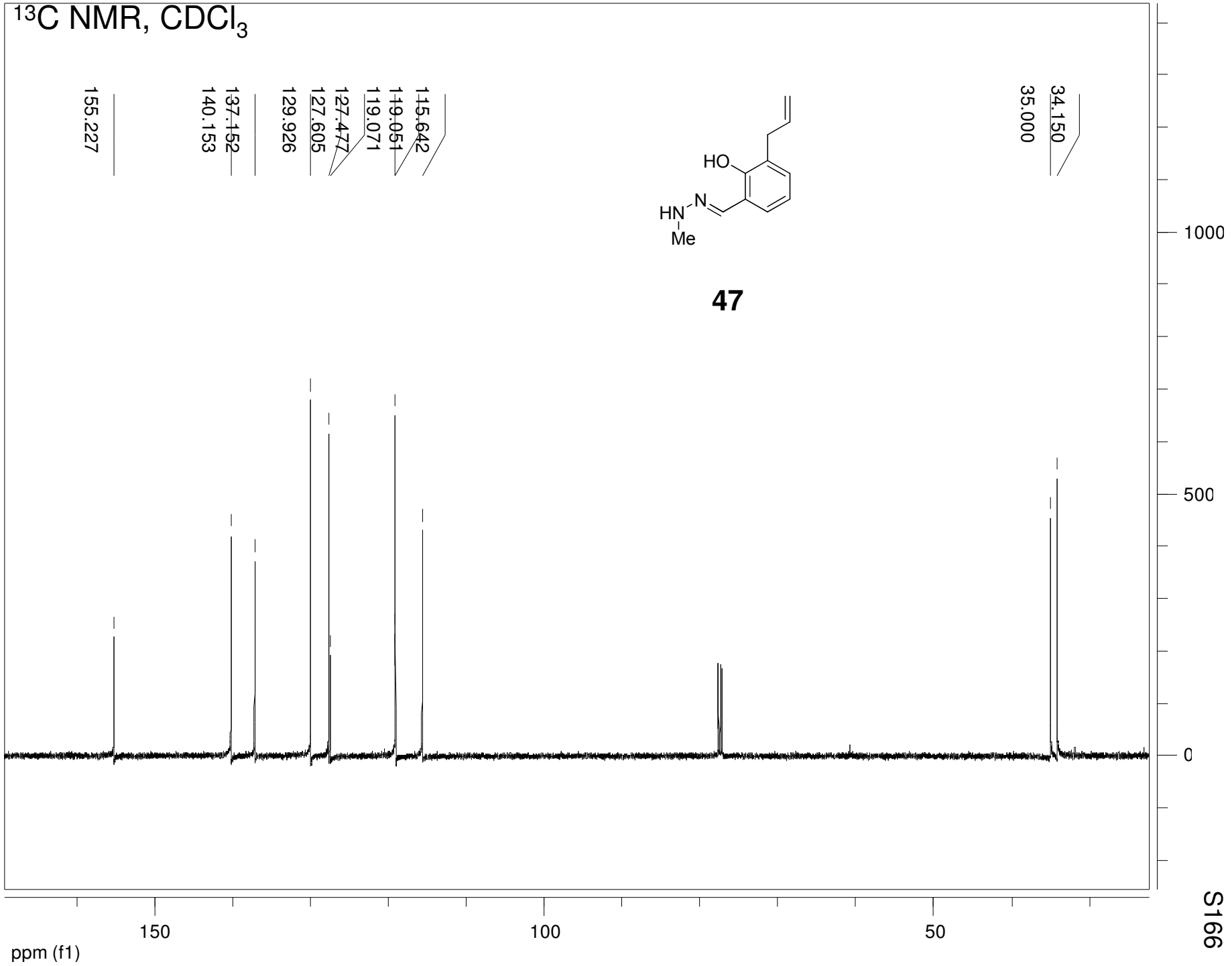
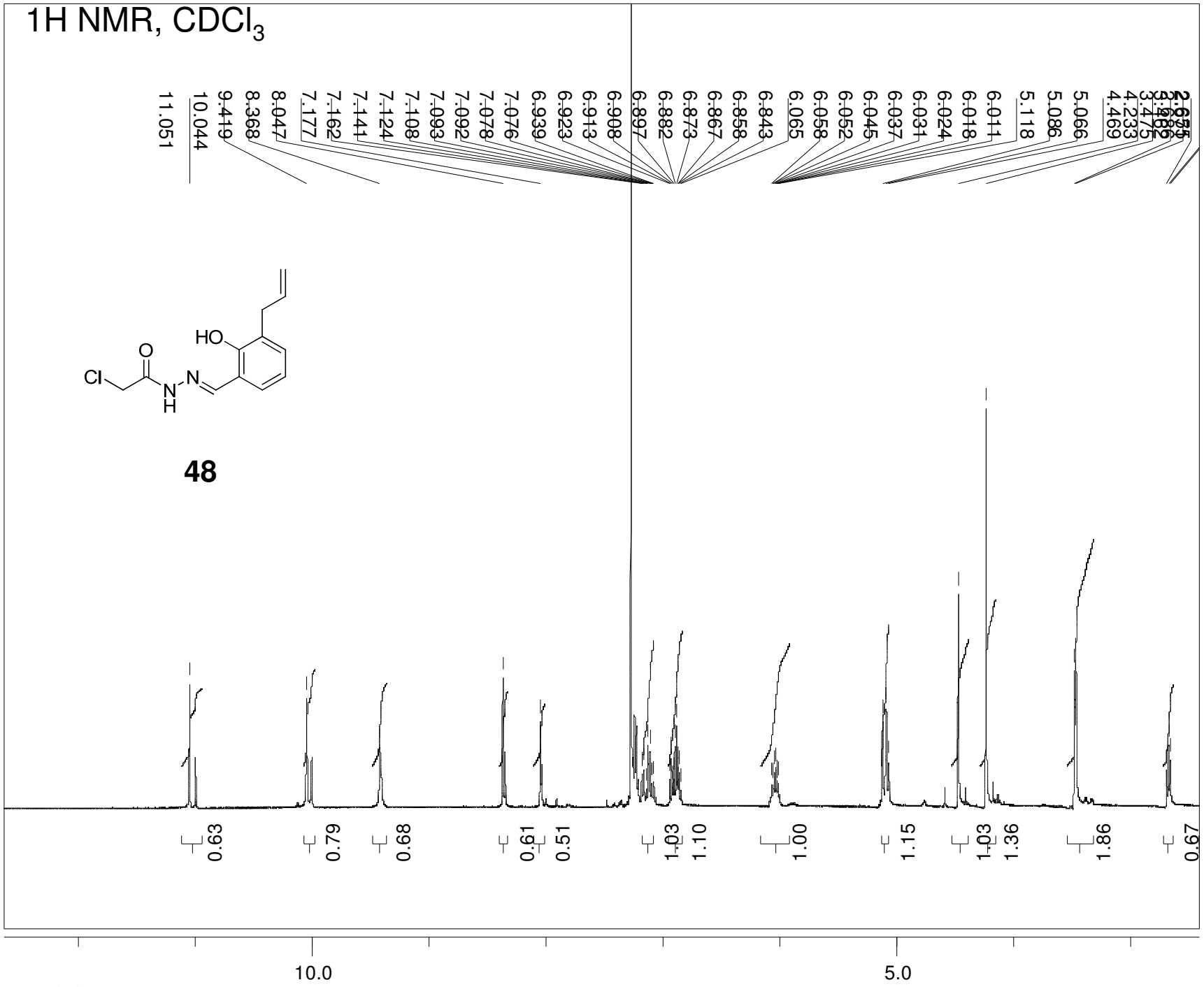
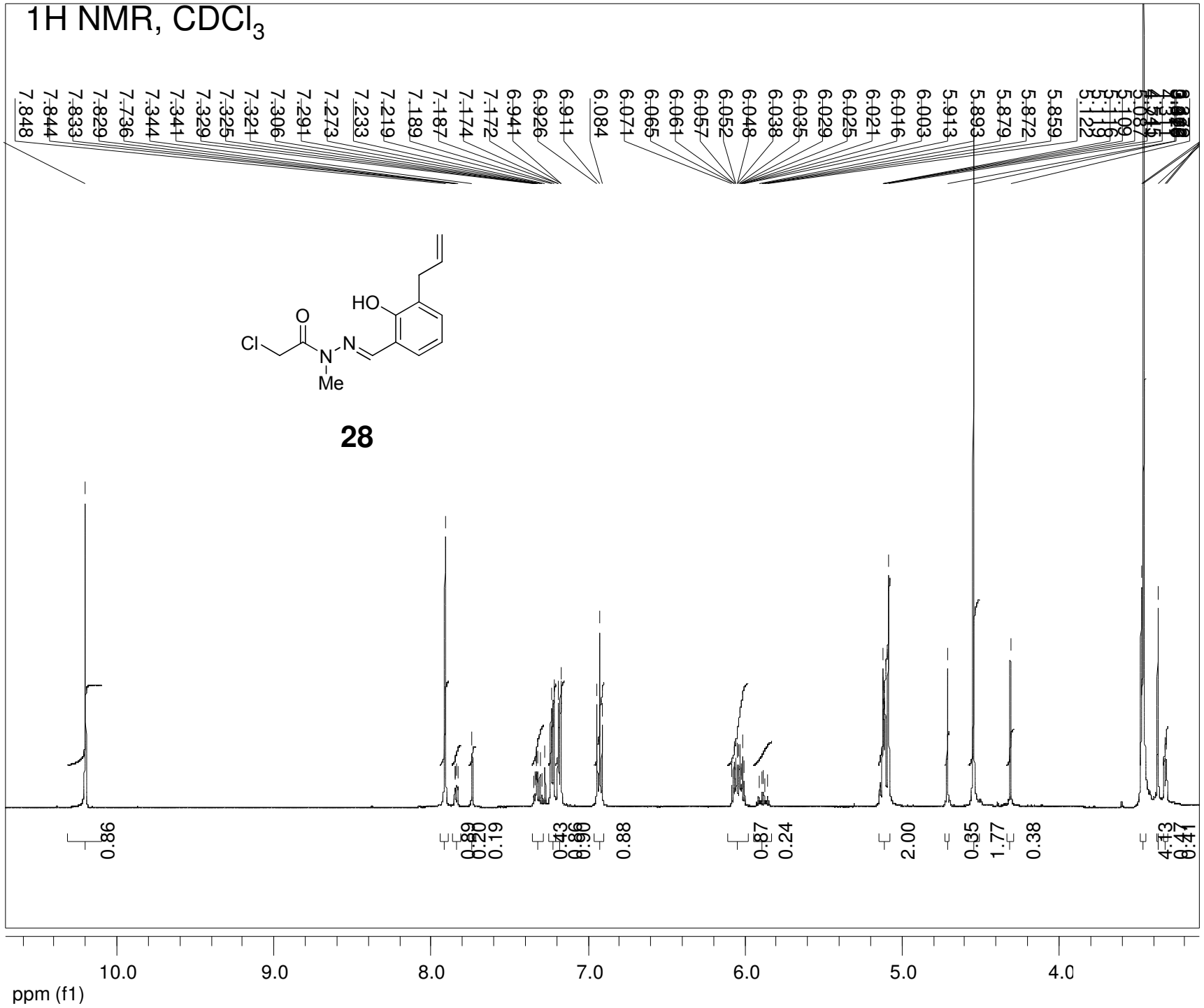
7.938	5225914	BB	.300	96.61555

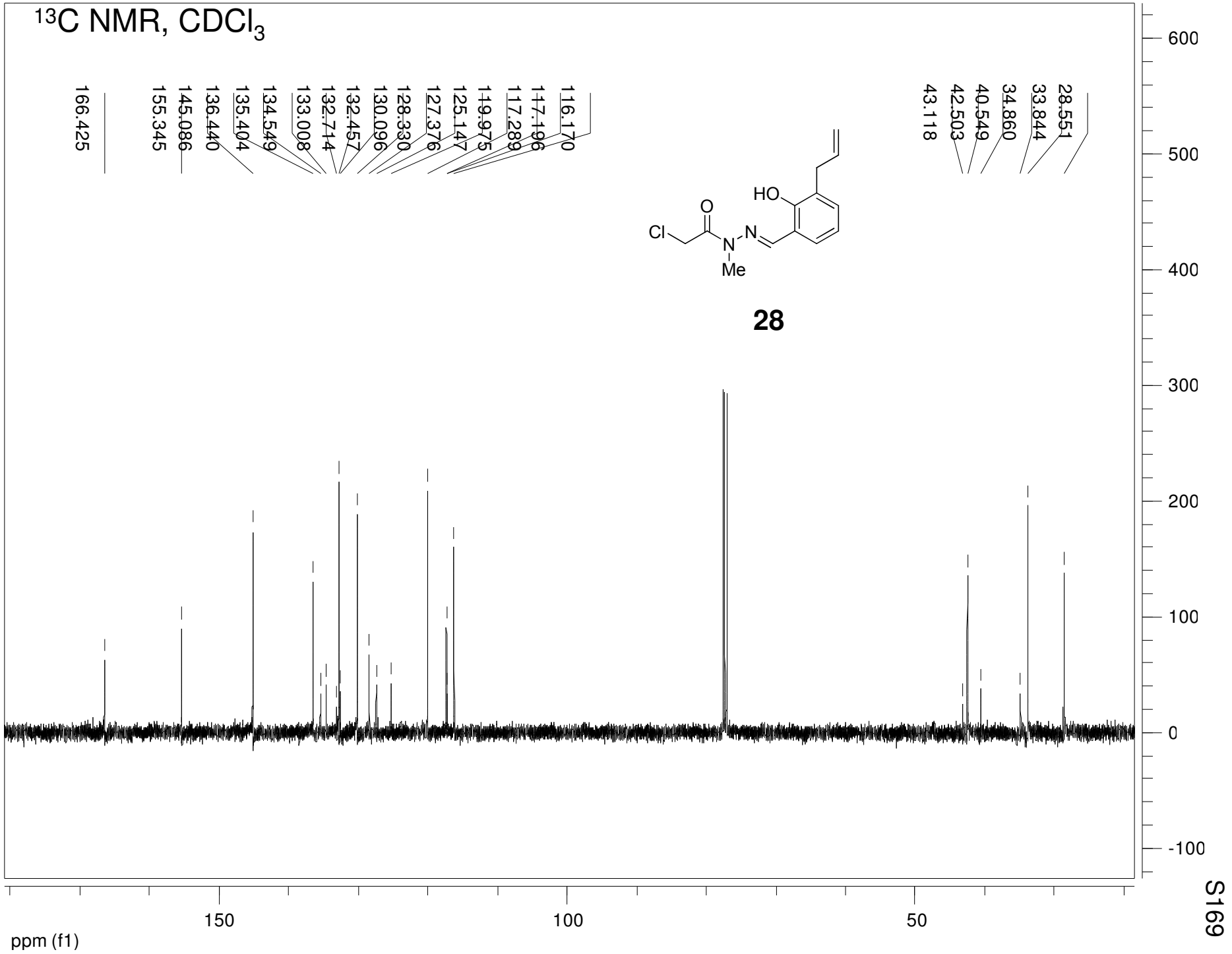
TOTAL AREA=5408976
MUL FACTOR=1.0000E+00

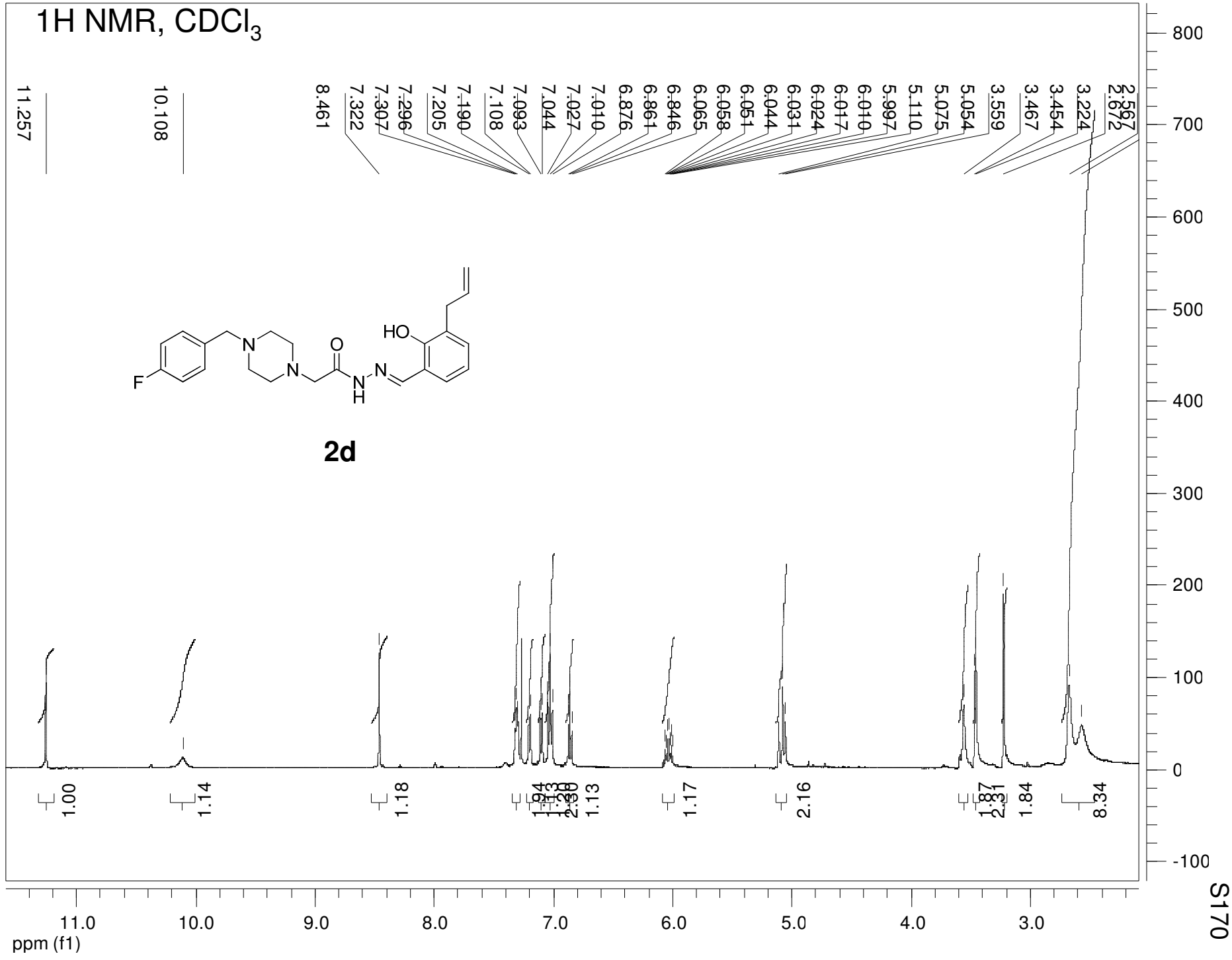

1H NMR, CDCl3
11.641
7.658
7.106
7.091
7.035
7.020
6.866
6.851
6.835
6.135
6.121
6.114
6.108
6.101
6.088
6.080
6.074
6.067
6.054
5.298
5.152
5.149
5.115
5.096
3.497
3.484
2.951
HO
HN
N
Me
47
0.72
1.00
0.85
0.99
0.69
0.98
0.88
2.13
2.12
3.05
700
600
500
400
300
200
100
0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)


^{13}C NMR, $CDCl_3$
155.227
140.153
137.152
129.926
127.605
127.477
119.071
119.051
115.642
35.000
34.150
HO
N
HN
Me
47
1000
500
0
150
100
50
ppm (f1)

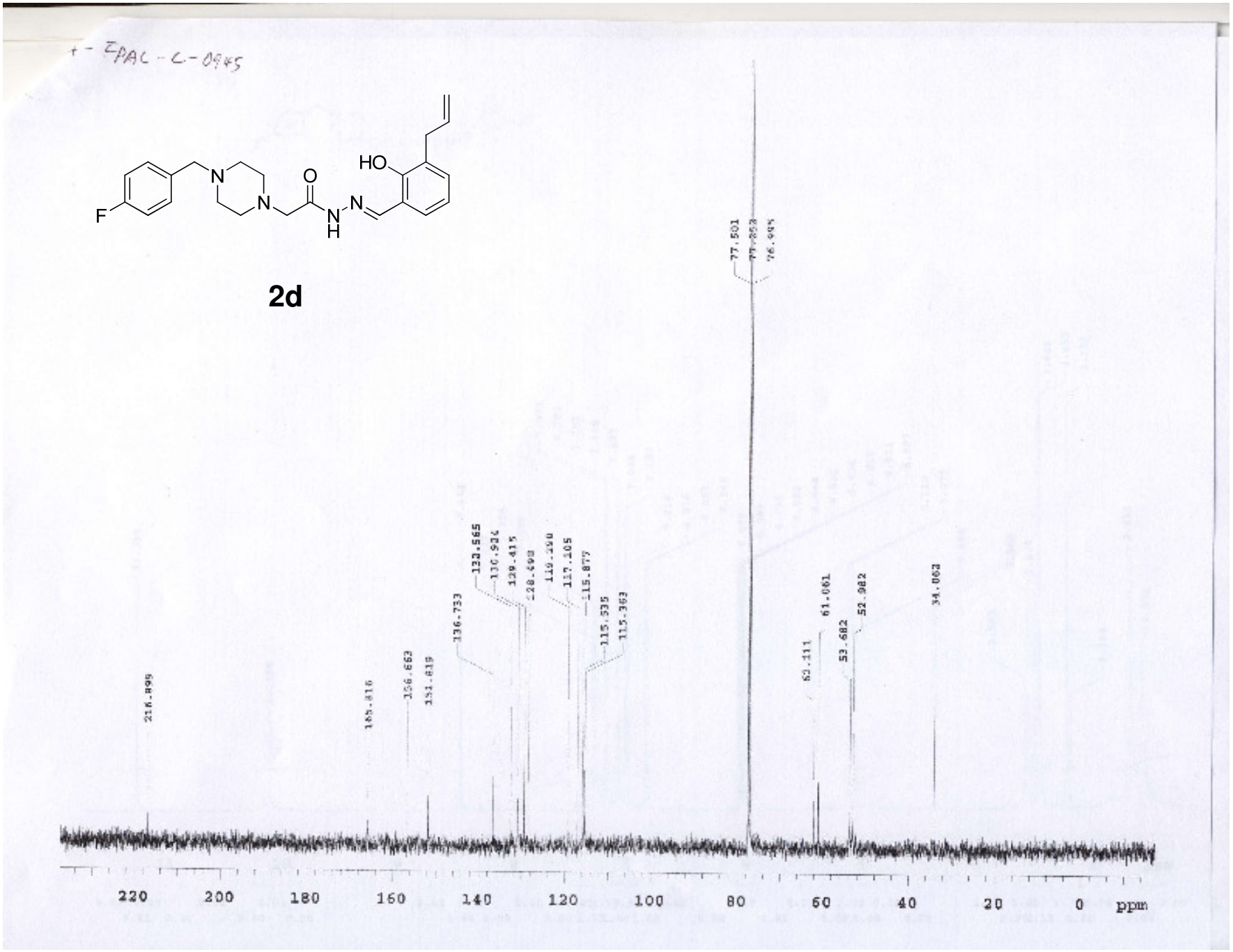

1H NMR, CDCl3
11.051
10.044
9.419
8.368
8.047
7.177
7.162
7.141
7.124
7.108
7.093
7.092
7.078
7.076
6.939
6.923
6.913
6.908
6.897
6.882
6.873
6.867
6.858
6.843
6.065
6.058
6.052
6.045
6.037
6.031
6.024
6.018
6.011
5.118
5.086
5.066
4.469
4.233
3.475
2.655
HO
O
Cl
N
H
48
0.63
0.79
0.68
0.61
0.51
1.03
1.10
1.00
1.15
1.03
1.36
1.86
0.67
200
150
100
50
0
10.0
5.0
ppm (t1)


1H NMR, CDCl3
7.848
7.844
7.833
7.829
7.736
7.344
7.341
7.329
7.325
7.321
7.306
7.291
7.273
7.233
7.219
7.189
7.187
7.174
7.172
6.941
6.926
6.911
6.084
6.071
6.065
6.061
6.057
6.052
6.048
6.038
6.035
6.029
6.025
6.021
6.016
6.003
5.913
5.893
5.879
5.872
5.859
5.122
5.116
5.109
5.087
HO
O
Cl
N
N
Me
28
0.98
0.89
0.20
0.19
0.43
0.86
0.88
0.87
0.24
2.00
0.95
1.77
0.38
4.13
0.47
500
400
300
200
100
0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
ppm (f1)

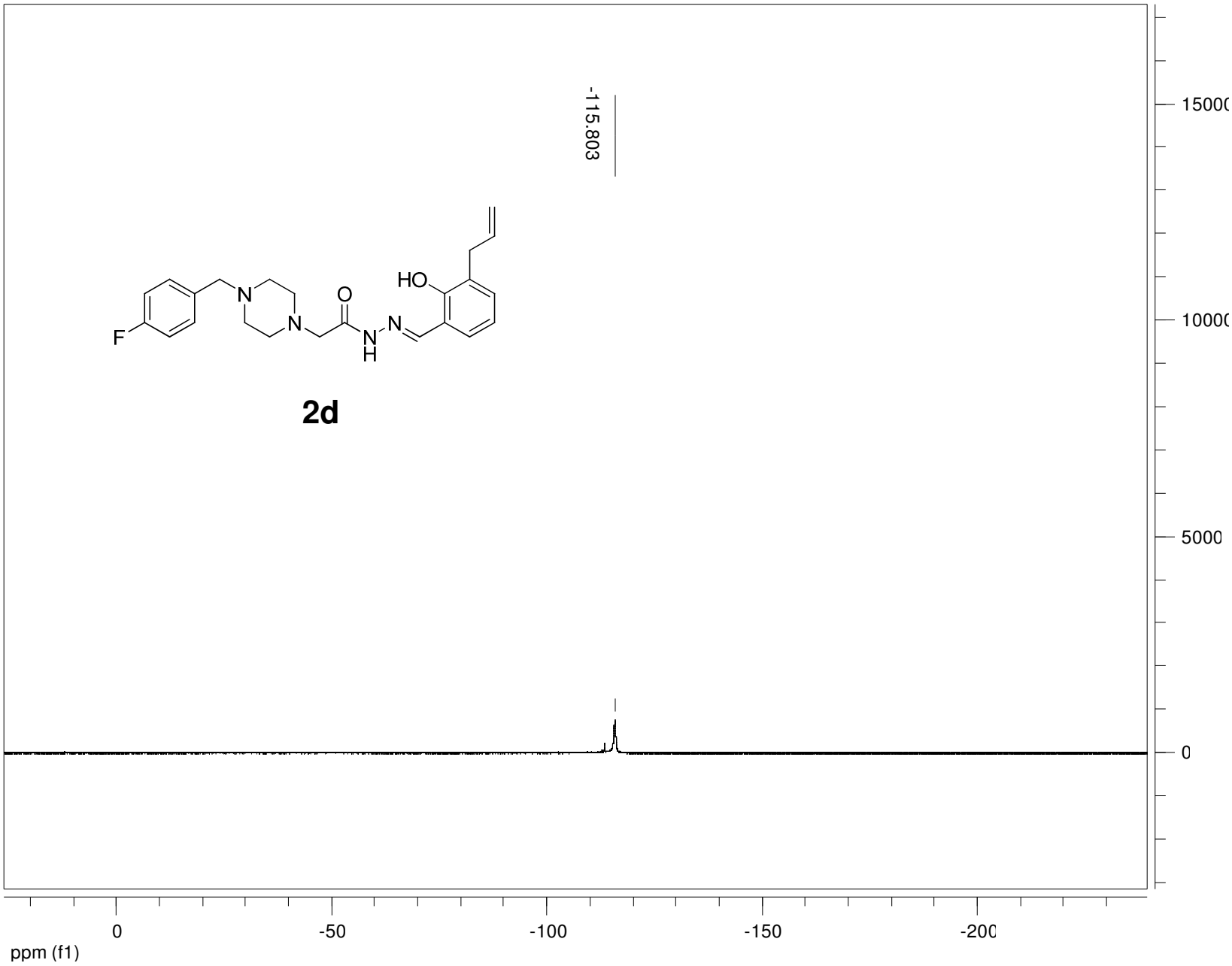

13C NMR, CDCl3
166.425
155.345
145.086
136.440
135.404
134.549
133.008
132.714
132.457
130.096
128.330
127.376
125.147
119.975
117.289
117.196
116.170
43.118
42.503
40.549
34.860
33.844
28.551
HO
O
Cl
N
N
Me
28
600
500
400
300
200
100
0
-100
150
100
50
ppm (f1)


1H NMR, CDCl3
11.257
10.108
8.461
7.322
7.307
7.296
7.205
7.190
7.108
7.093
7.044
7.027
7.010
6.876
6.861
6.846
6.065
6.058
6.051
6.044
6.031
6.024
6.017
6.010
5.997
5.110
5.075
5.054
3.559
3.467
3.454
3.224
2.567
2.672
HO
O
N
N
N
H
F
2d
1.00
1.14
1.18
1.94
1.13
2.30
1.13
1.17
2.16
1.87
2.31
1.84
8.34
800
700
600
500
400
300
200
100
0
-100
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)

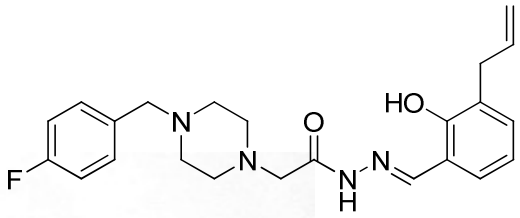
^{13}C NMR, $CDCl_3$



^{19}F NMR, $CDCl_3$



HPLC



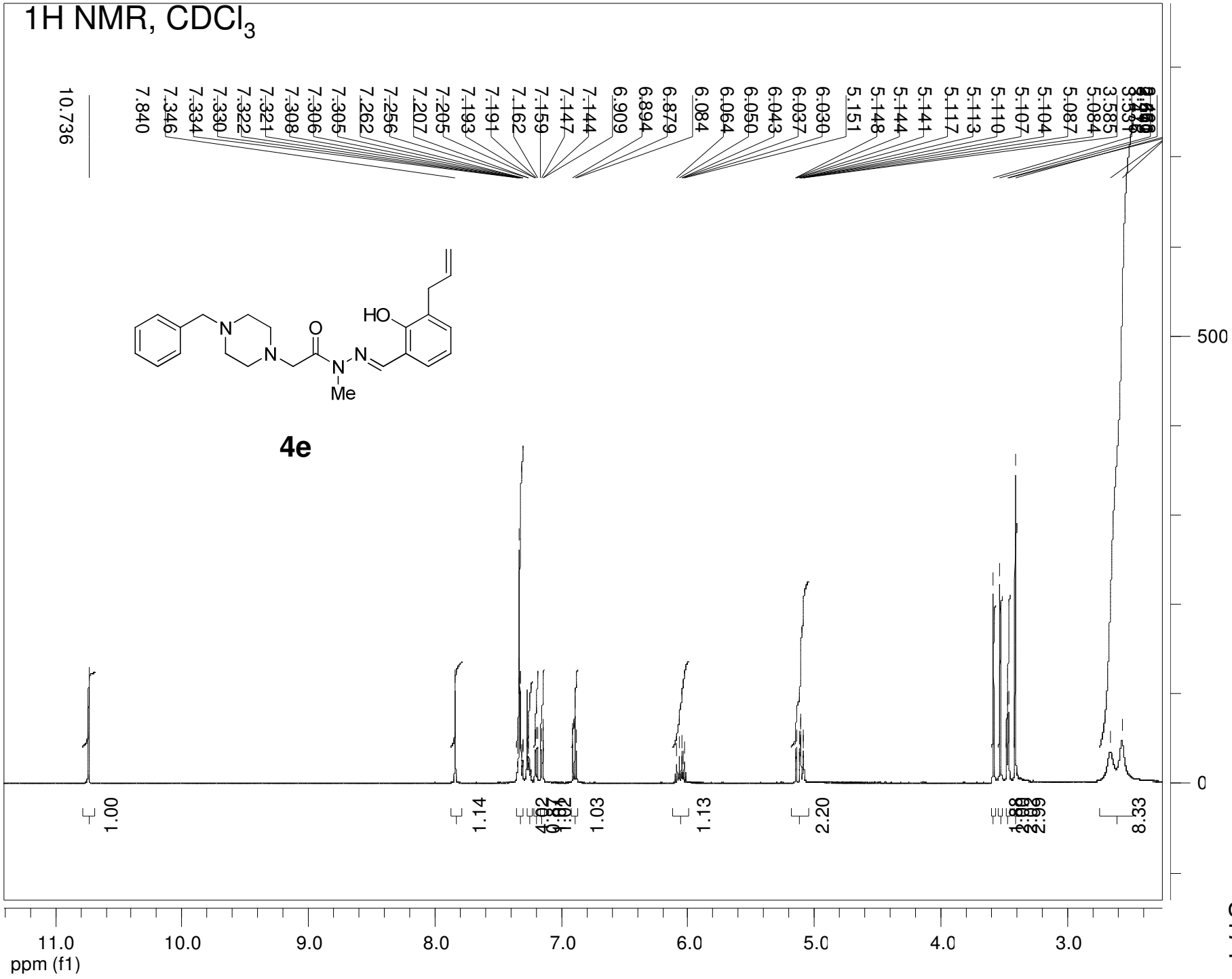
2d

RUN# 436 JAN 8, 2008 13:19:46

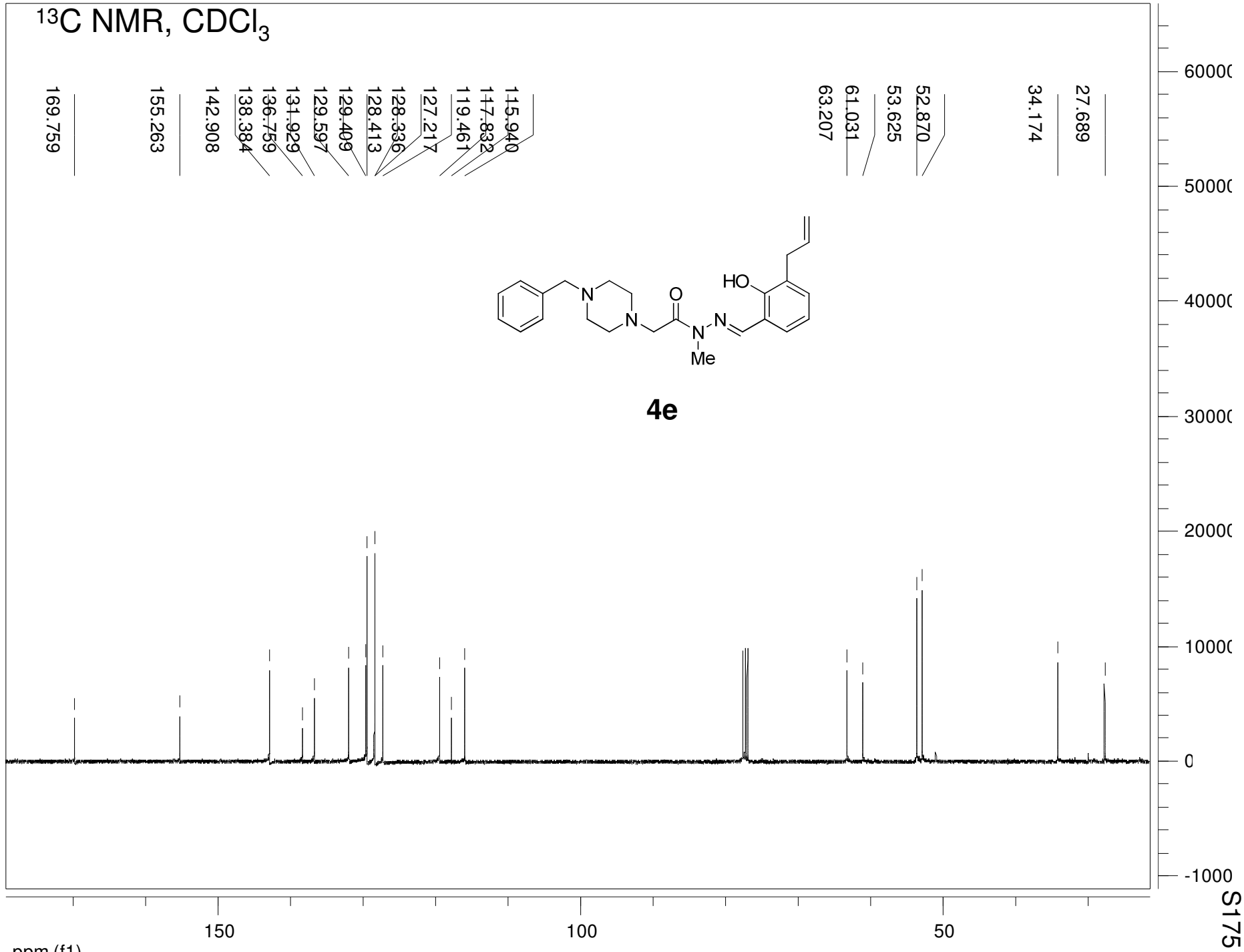
AREA%

RT	AREA	TYPE	WIDTH	AREA%
10.483	238865	BV	.217	.31261
11.131	1223276	VV	.289	1.60094
12.861	110514	PP	.236	.14463
13.886	73565312	SBB	.213	96.27734
14.972	197511	BB	.303	.25849
16.766	1074339	BB	.271	1.40602

TOTAL AREA=7.6410E+07
MUL FACTOR=1.0000E+00

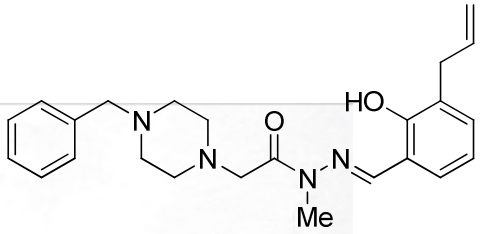

1H NMR, CDCl3
10.736
7.840
7.346
7.334
7.330
7.322
7.321
7.308
7.306
7.305
7.262
7.256
7.207
7.205
7.193
7.191
7.162
7.159
7.147
7.144
6.909
6.894
6.879
6.084
6.064
6.050
6.043
6.037
6.030
5.151
5.148
5.144
5.141
5.117
5.113
5.110
5.107
5.104
5.087
5.084
3.585
HO
O
N
N
N
Me
4e
500
0
1.00
1.14
1.03
1.13
2.20
8.33
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)

^{13}C NMR, $CDCl_3$

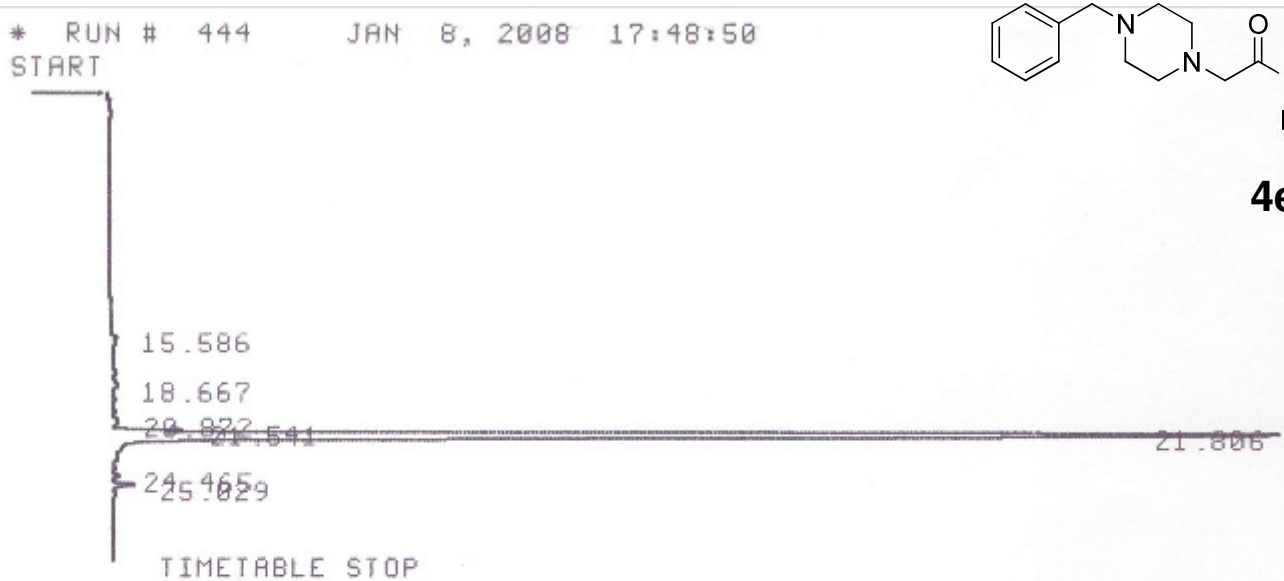


4e

HPLC



4e



RUN# 444 JAN 8, 2008 17:48:50

AREA%

RT	AREA	TYPE	WIDTH	AREA%
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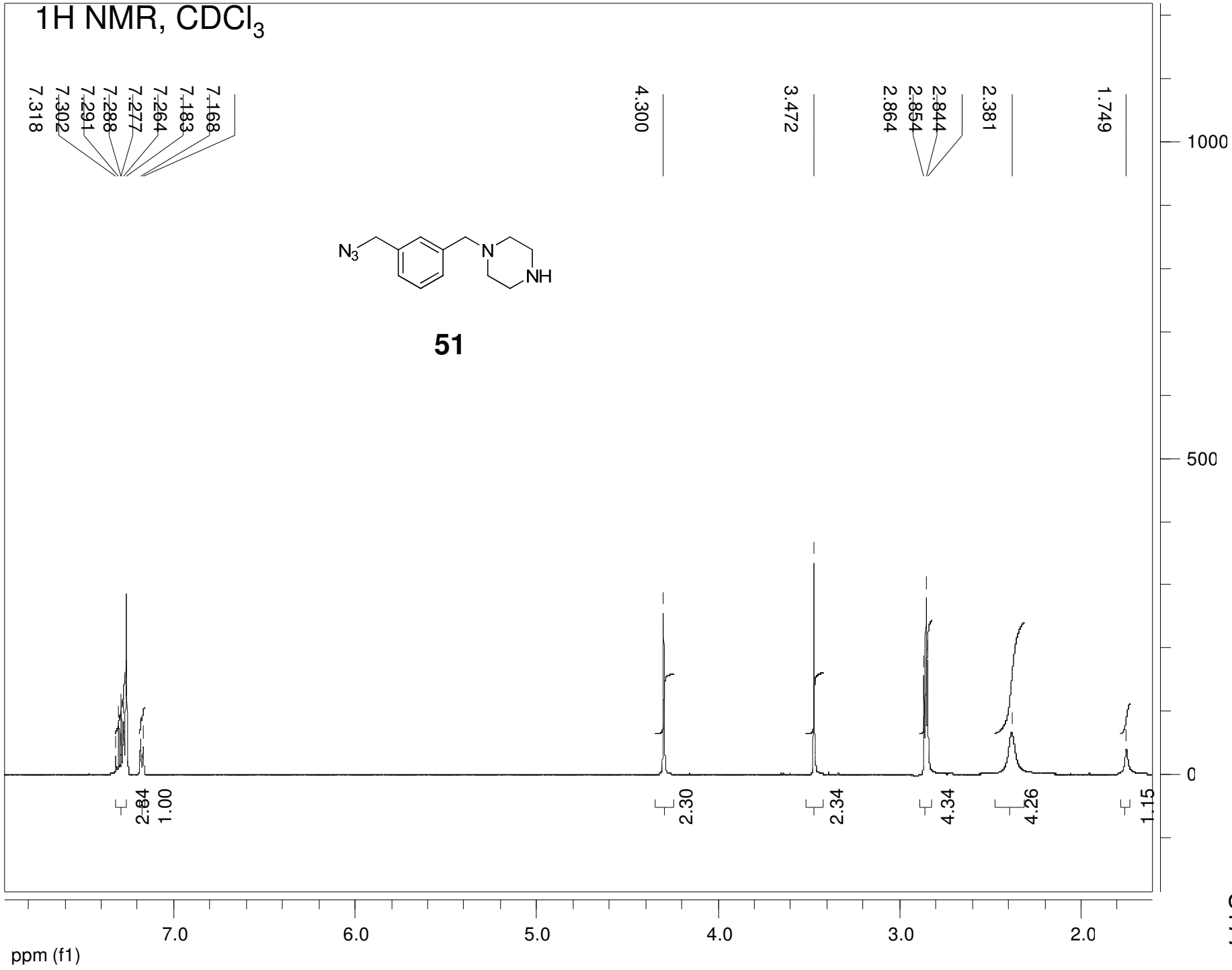
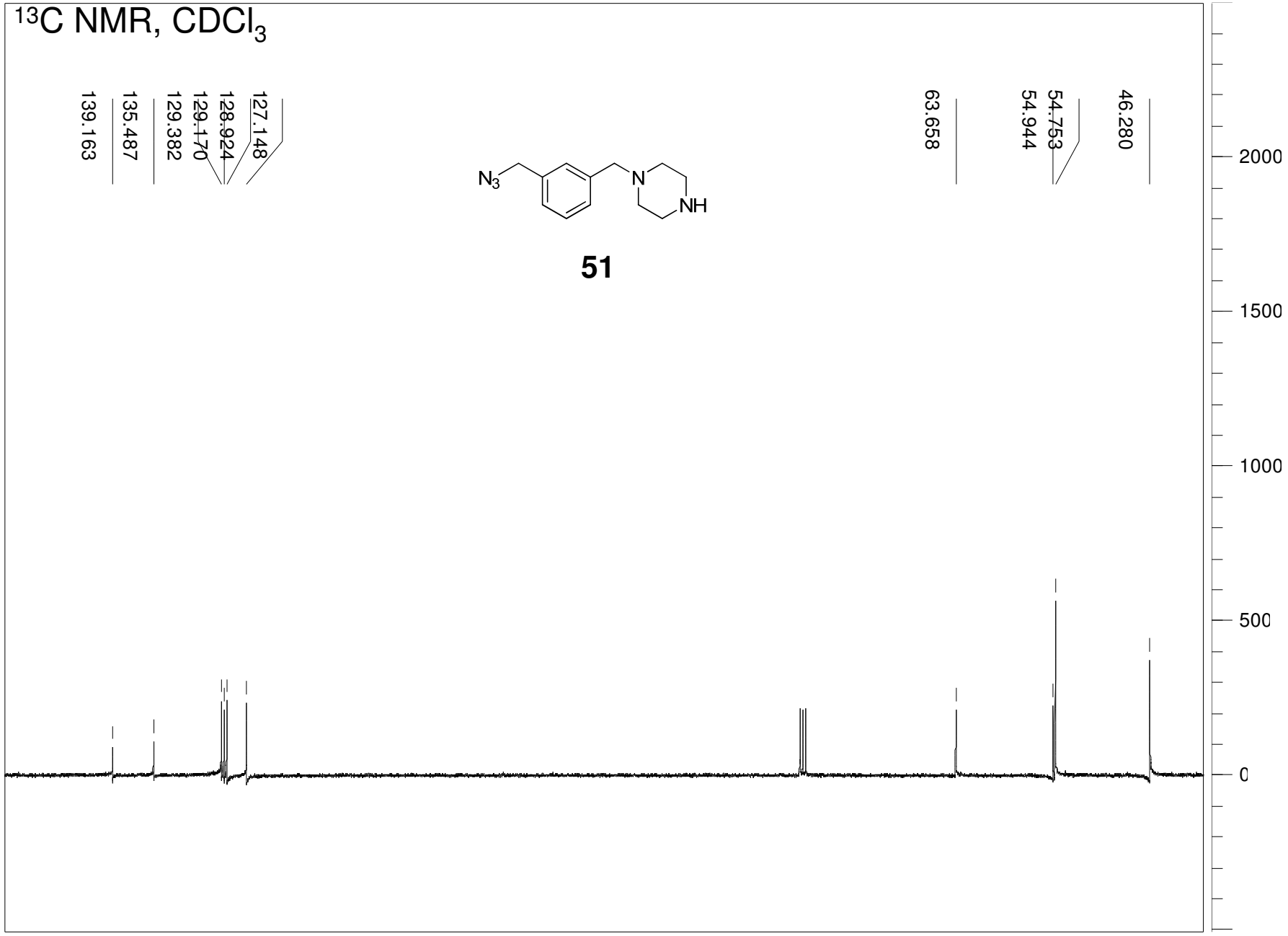
18.667	171570	PB	.301	.35191
20.872	218436	BP	.368	.44804
21.541	778833	PV	.106	1.59749
21.806	46910688	VB	.373	96.22000
24.465	156491	BP	.222	.32098
25.029	461187	PB	.201	.94596

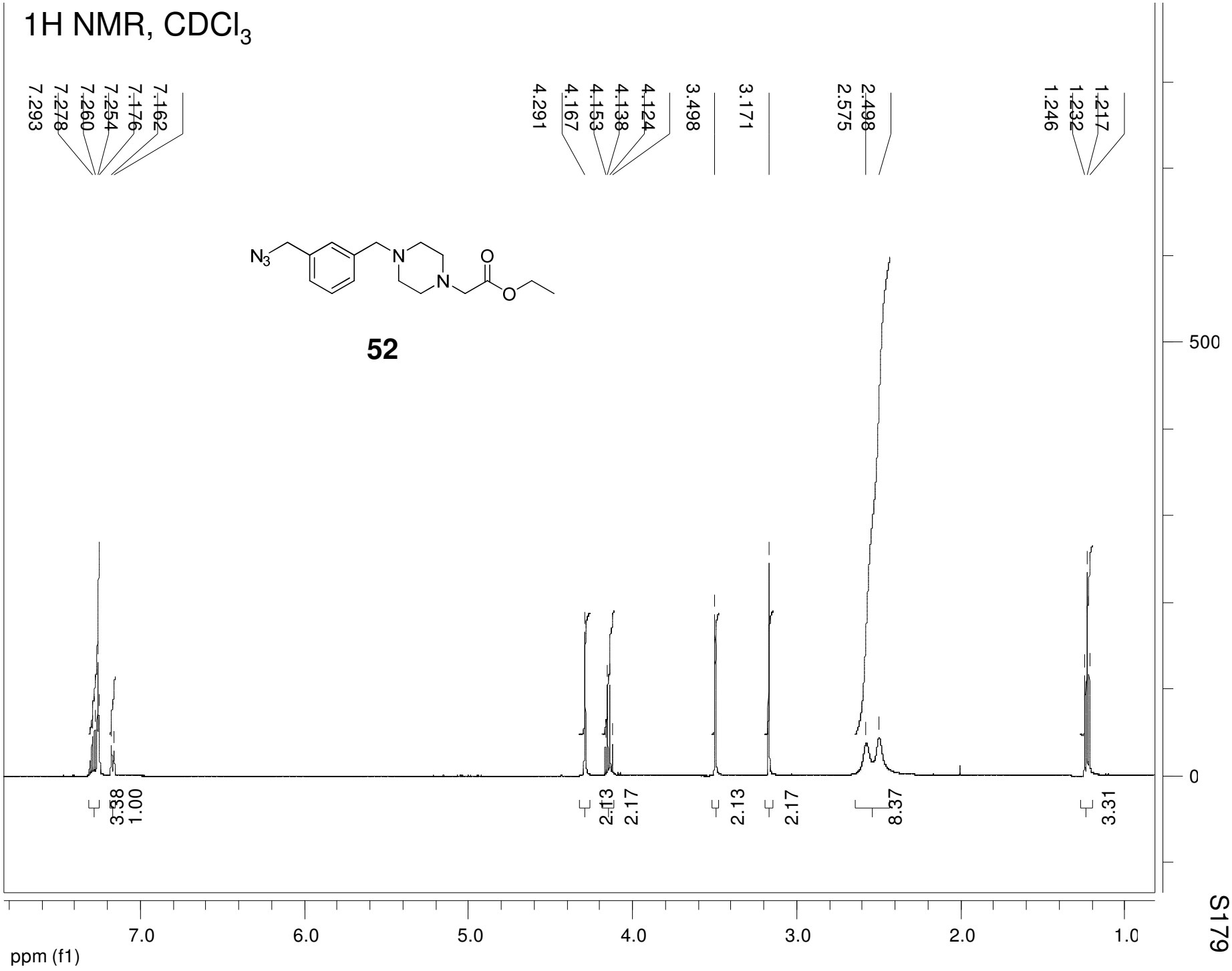
TOTAL AREA=4.8754E+07
MUL FACTOR=1.0000E+00

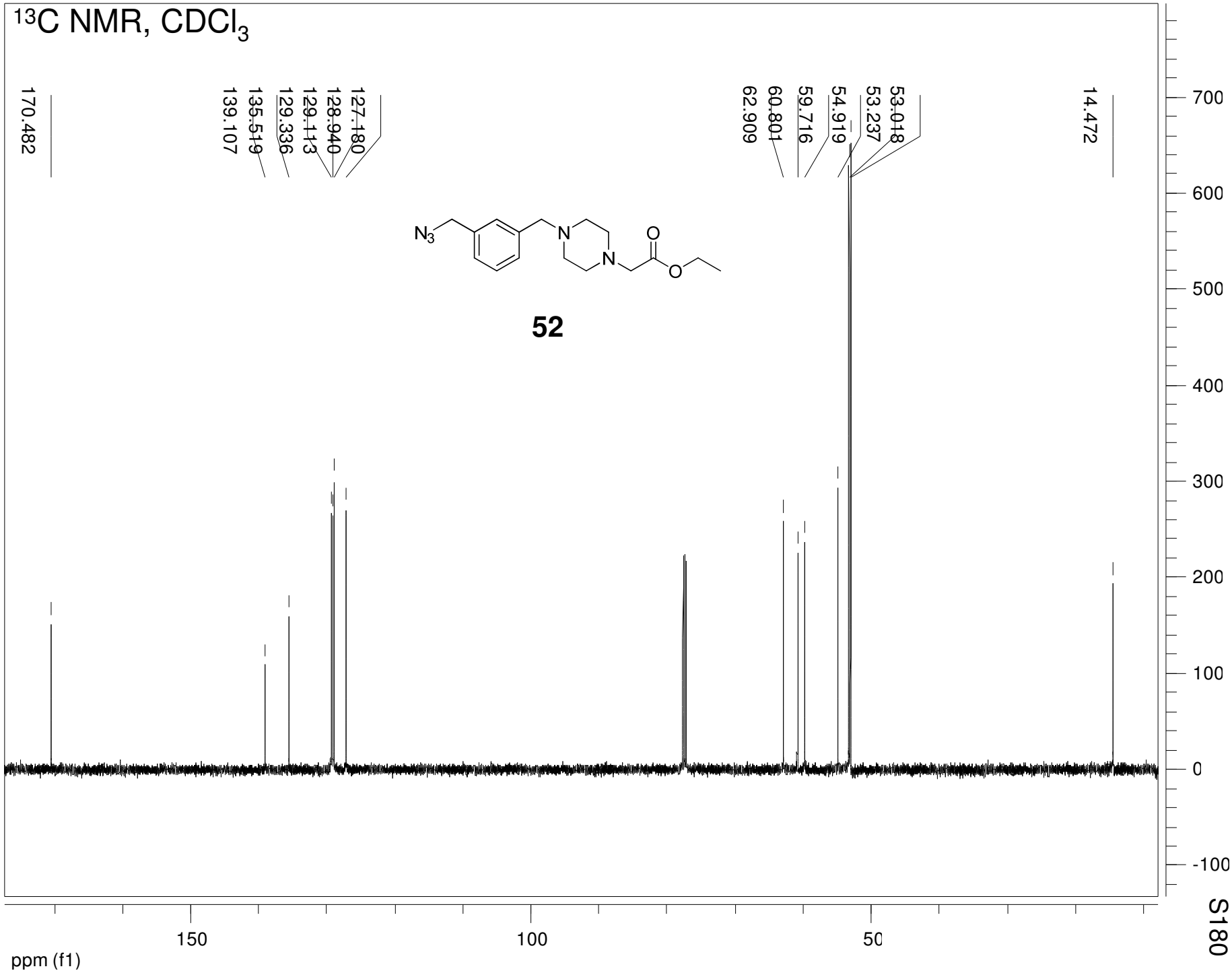

1H NMR, CDCl3
7.318
7.302
7.291
7.288
7.277
7.264
7.183
7.168
4.300
3.472
2.864
2.854
2.844
2.381
1.749
N3
N
NH
51
1000
500
0
2.04
1.00
2.30
2.34
4.34
4.26
1.15
7.0
6.0
5.0
4.0
3.0
2.0
ppm (f1)

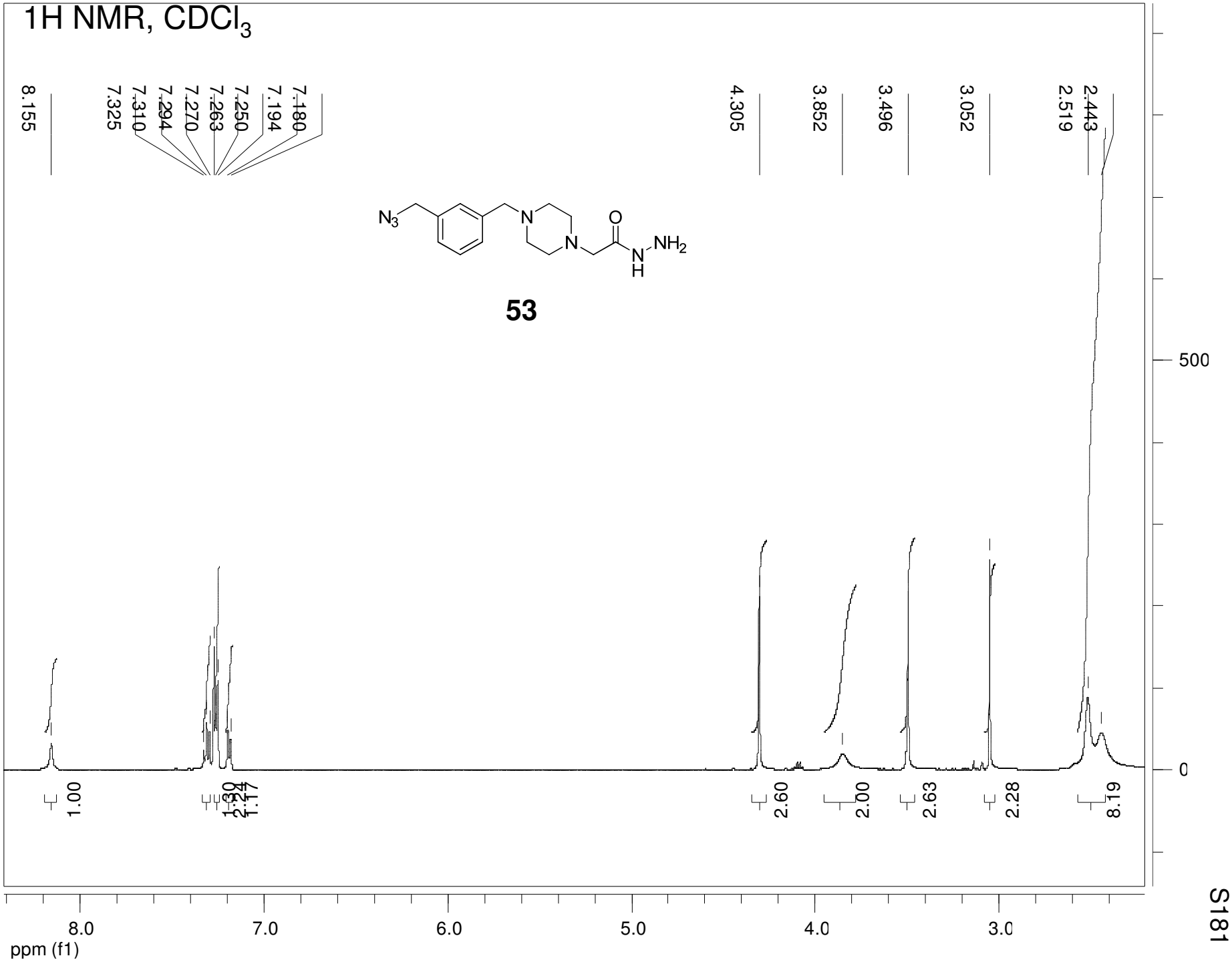
^{13}C NMR, $CDCl_3$



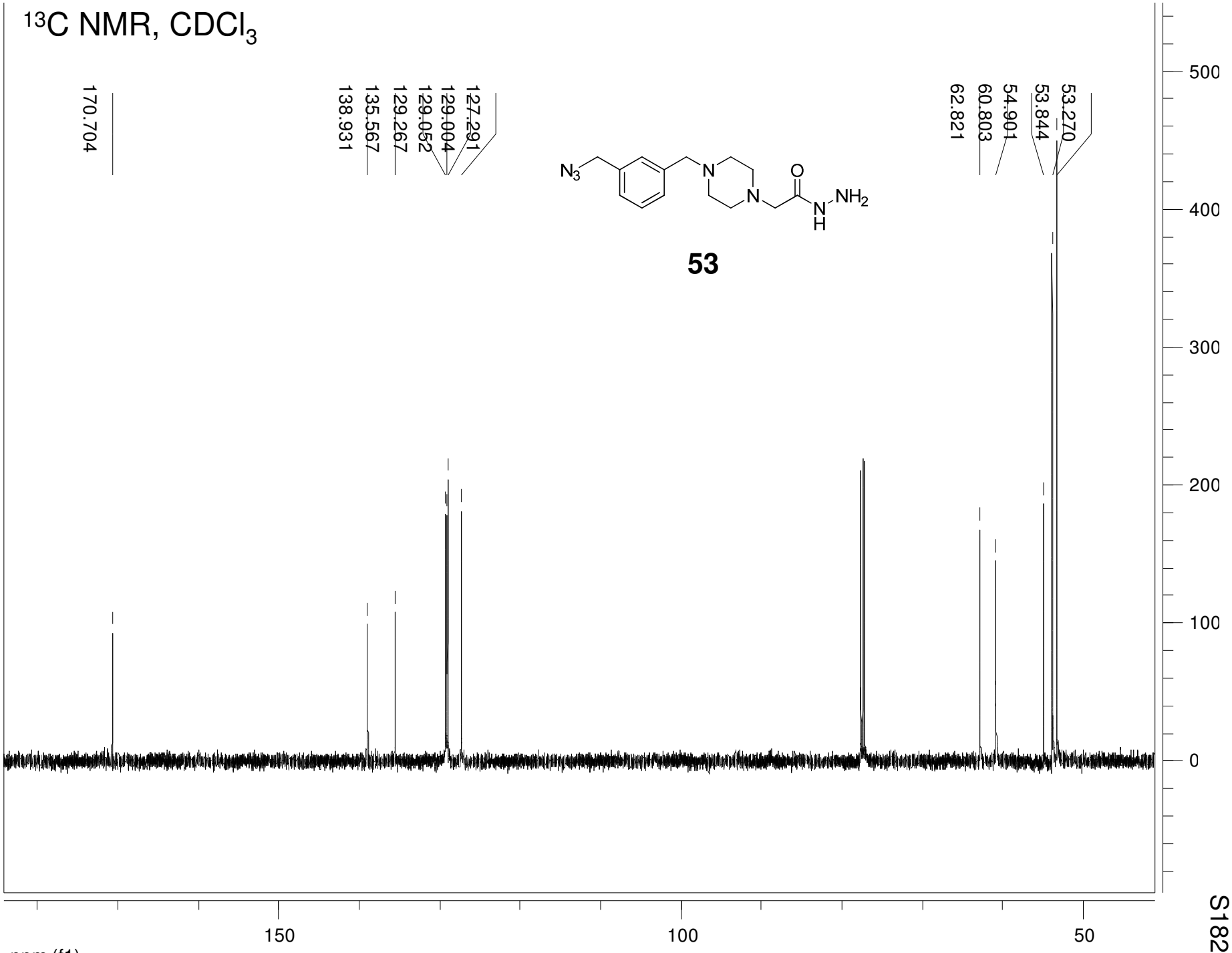
51


1H NMR, CDCl3
7.293
7.278
7.260
7.254
7.176
7.162
4.291
4.167
4.153
4.138
4.124
3.498
3.171
2.575
2.498
1.246
1.232
1.217
N3
N
N
O
O
52
500
0
3.38
1.00
2.13
2.17
2.13
2.17
8.37
3.31
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)

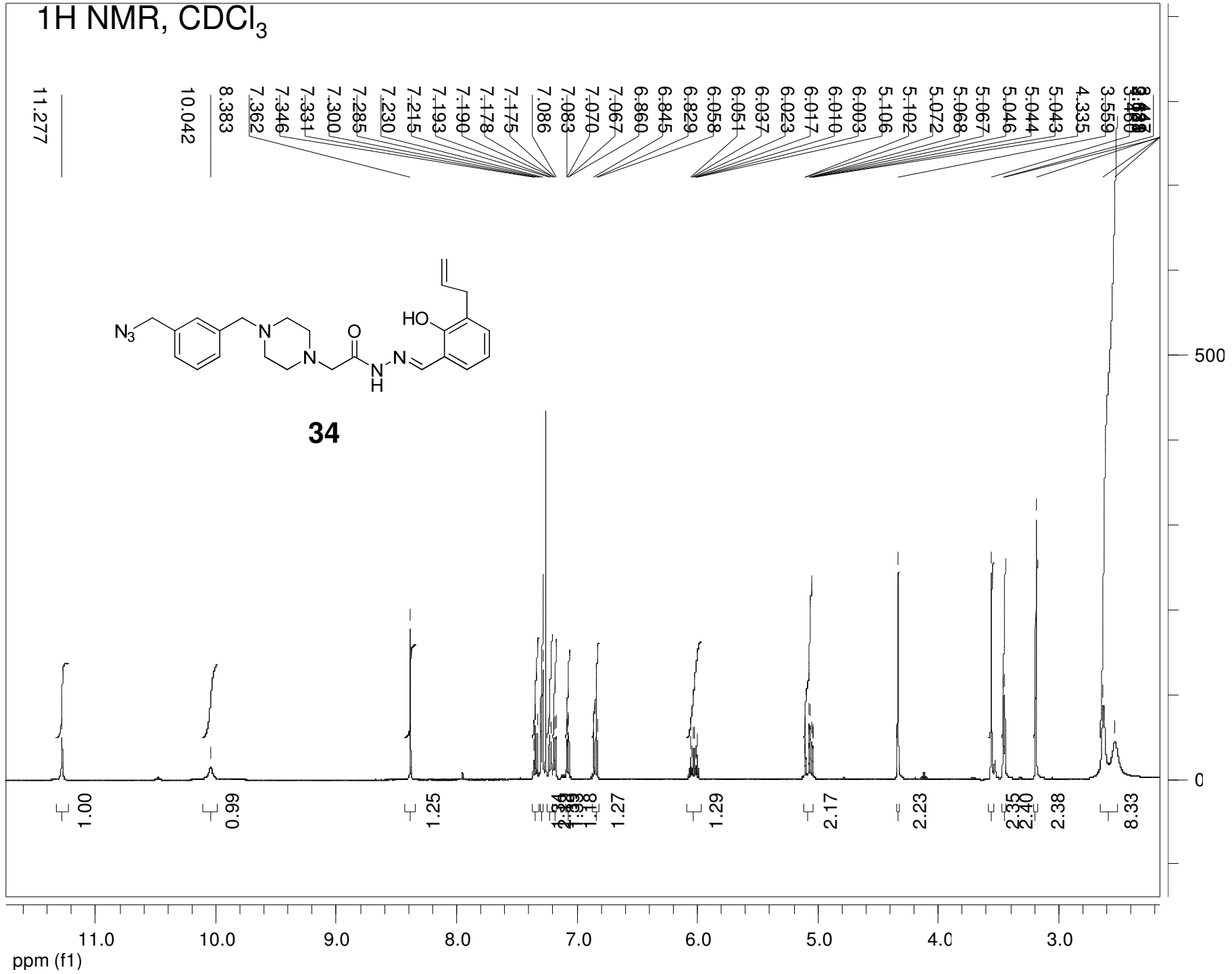

13C NMR, CDCl3
170.482
139.107
135.519
129.336
129.113
128.940
127.180
62.909
60.801
59.716
54.919
53.237
53.018
14.472
N3
N
N
O
O
52
700
600
500
400
300
200
100
0
-100
150
100
50
ppm (f1)

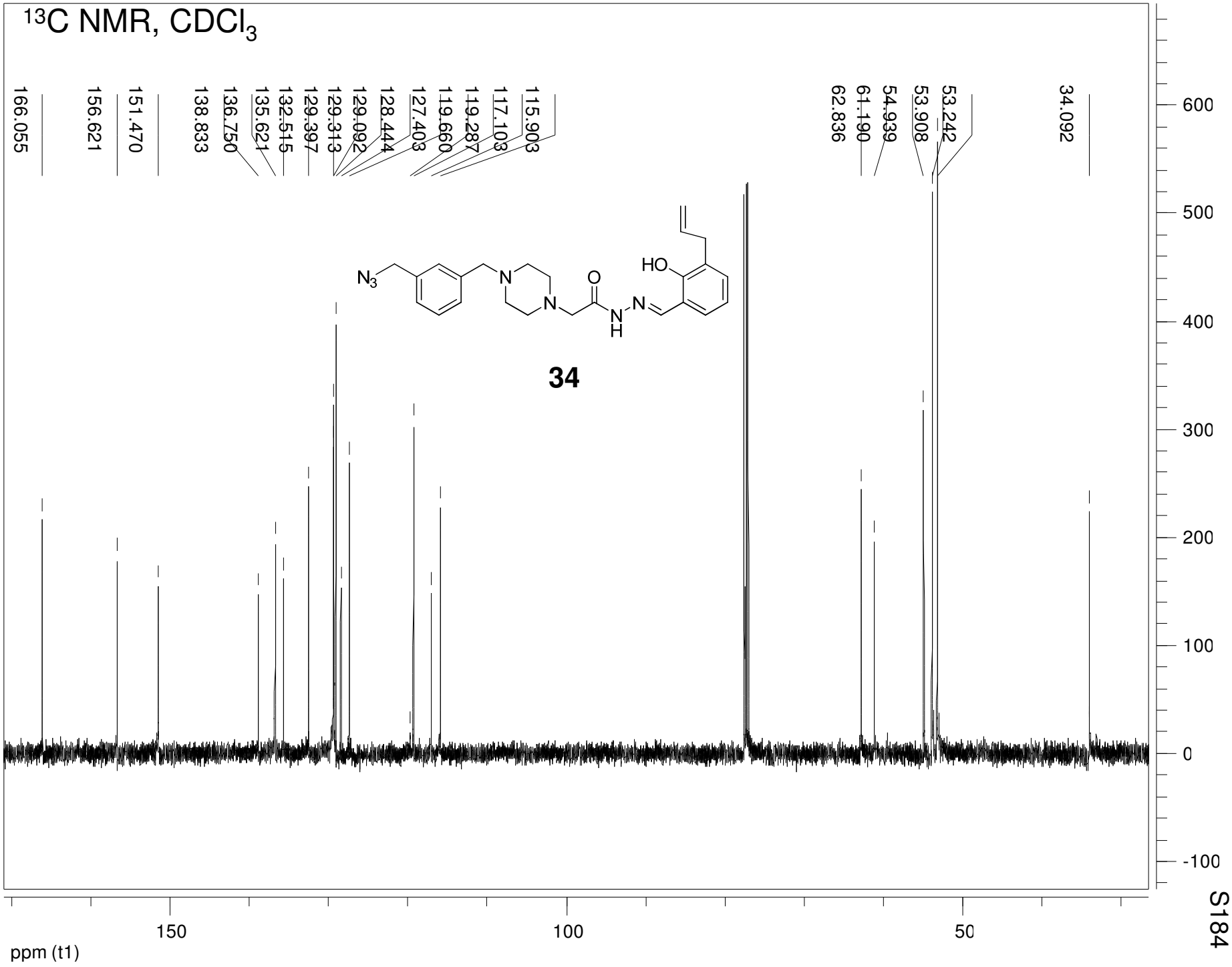

1H NMR, CDCl3
8.155
7.325
7.310
7.294
7.270
7.263
7.250
7.194
7.180
4.305
3.852
3.496
3.052
2.519
2.443
N3
N
N
O
N
H
NH2
53
500
0
1.00
2.90
2.00
2.93
2.28
8.19
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)

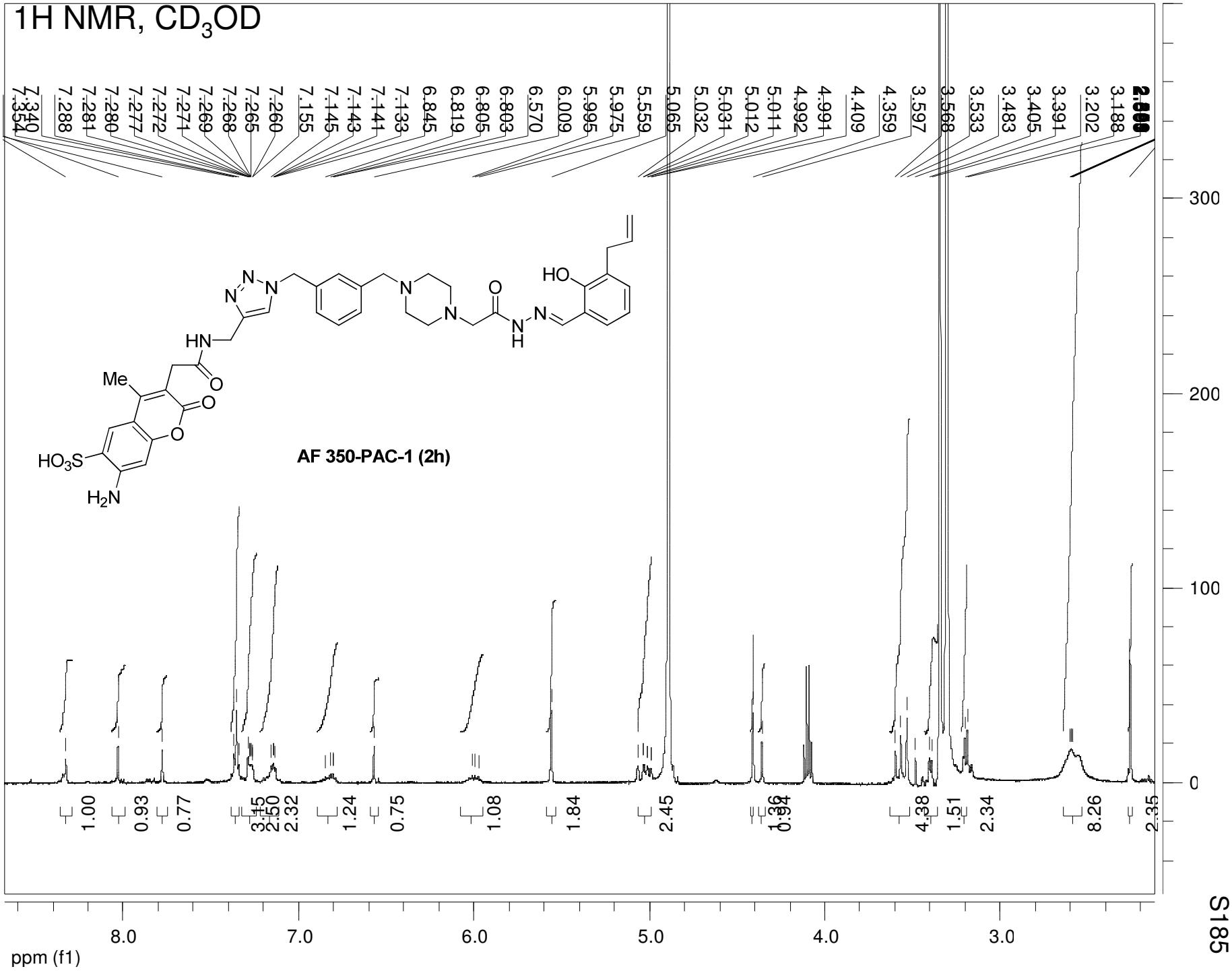
^{13}C NMR, $CDCl_3$



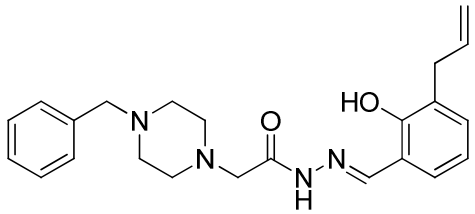
53


1H NMR, CDCl3
11.277
10.042
8.383
7.362
7.346
7.331
7.300
7.285
7.230
7.215
7.193
7.190
7.178
7.175
7.086
7.083
7.070
7.067
6.860
6.845
6.829
6.058
6.051
6.037
6.023
6.017
6.010
6.003
5.106
5.102
5.072
5.068
5.067
5.046
5.044
5.043
4.335
3.559
N3
N
O
HO
N
NH
N
34
500
0
1.00
0.69
1.25
1.27
1.29
2.17
2.23
2.38
8.33
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (f1)

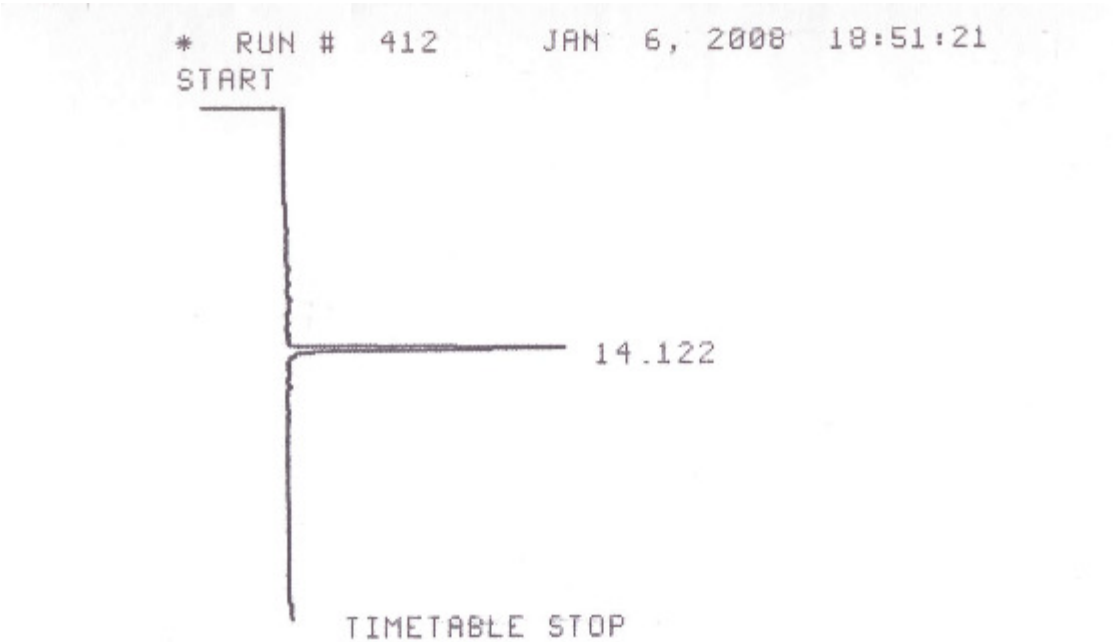

13C NMR, CDCl3
166.055
156.621
151.470
138.833
136.750
135.621
132.515
129.397
129.313
129.092
128.444
127.403
119.660
119.287
117.103
115.903
62.836
61.190
54.939
53.908
53.242
34.092
HO
N3
O
N
N
N
H
34
600
500
400
300
200
100
0
-100
150
100
50
ppm (t1)


1H NMR, CD3OD
AF 350-PAC-1 (2h)
ppm (f1)

HPLC



PAC-1 (1)



RUN# 412 JAN 6, 2008 18:51:21

AREA%

RT	AREA	TYPE	WIDTH	AREA%
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TOTAL AREA=5036704
MUL FACTOR=1.0000E+00