

Advanced
**Synthesis &
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Supporting Information

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Supporting Information for

Nitrilase Catalyzed Selective Hydrolysis of Dinitriles and Green Access to the Cyanocarboxylic Acids of Pharmaceutical Importance

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Characterization data for w-cyanocarboxylic acids and dinitriles:

2-Cyano-2-methylpropionic acid,^[1] 224 mg, white solid, m.p. 54-56°C, lit.^[1] 54-55°C; ¹H NMR (CDCl₃) δ = 1.66 (s, 6H), 9.34 (br, 1H); ¹³C NMR (CDCl₃) δ = 25.0, 39.2, 120.4, 175.3.

3-Cyanoacrylic acid,^[2, 3] 208 mg, white solid, m.p. 74-76°C, lit.^[3] 75-77°C; ¹H NMR (CDCl₃) δ = 6.60 (d, J = 16 Hz, 1H), 6.75 (d, J = 16 Hz, 1H), 9.50 (br, 1H); ¹³C NMR (CDCl₃) δ = 115.2, 115.7, 139.0, 168.0.

3-Cyanopropanoic acid,^[4] 240 mg, white solid, m.p. 46-48°C, lit.^[4] 49.5-51°C; ¹H NMR (CDCl₃) δ = 2.68 (m, 2H), 2.78 (m, 2H), 9.61 (br, 1H); ¹³C NMR (CDCl₃) δ = 13.1, 30.1, 118.7, 175.8.

4-Cyanobutanoic acid,^[4] 224 mg, white solid, m.p. 36-38°C, lit.^[4] 39.6-40.2°C; ¹H NMR (CDCl₃) δ = 2.01 (m, 2H), 2.50 (m, 2H), 2.59 (m, 2H), 9.64 (br, 1H); ¹³C NMR (CDCl₃) δ = 16.8, 20.8, 32.5, 119.4, 178.0.

5-Cyanopentanoic acid,^[4, 5] 227 mg, colorless oil; ¹H NMR (CDCl₃) δ = 1.77 (m, 4H), 2.41 (m, 4H), 9.65 (br, 1H); ¹³C NMR (CDCl₃) δ = 17.3, 24.0, 25.0, 33.4, 119.7, 179.1.

6-Cyanohexanoic acid,^[5] 208 mg, colorless oil. ¹H NMR (CDCl₃) δ = 1.55 (m, 2H), 1.70 (m, 4H), 2.39 (m, 4H), 9.74 (br, 1H); ¹³C NMR (CDCl₃) δ = 17.4, 24.2, 25.4, 28.4, 34.0, 120.0, 180.0.

7-Cyanoheptanoic acid,^[5] 200 mg, white solid, m.p. 35-36°C, lit.^[5] 38-39°C. ¹H NMR (CDCl₃) δ = 1.25-1.49 (m, 4H), 1.67 (m, 4H), 2.35 (m, 4H), 9.84 (br, 1H); ¹³C NMR (CDCl₃) δ = 17.5, 224.6, 25.5, 28.5, 28.7, 34.2, 120.1, 180.2.

9-Cyanononanoic acid,^[6] 196 mg, white solid, m.p. 48-50°C, lit.^[6] 49-50°C. ¹H NMR (CDCl₃) δ = 1.34 (m, 6H), 1.46 (m, 2H), 1.66 (m, 4H), 2.35 (m, 4H), 9.64 (br, 1H); ¹³C NMR (CDCl₃) δ = 17.5, 24.9, 25.7, 28.9, 29.0, 29.2, 29.3, 34.4, 120.2, 180.5.

1-Cyanocyclopentaneacetoneitrile,^[7, 8] ¹H NMR (CDCl₃) δ = 1.83 (m, 4H), 1.91 (m, 2H), 2.27 (m, 2H), 2.74 (s, 2H), ¹³C NMR (CDCl₃) δ = 24.6, 27.0, 38.1, 40.6, 116.2, 122.9.

1-cyanocyclohexaneacetoneitrile,^[8, 9] ¹H NMR (CDCl₃) δ = 1.23 (m, 1H), 1.48 (m, 1H), 1.66-1.82 (m, 6H), 2.11 (m, 2H), 2.69 (s, 2H), ¹³C NMR (CDCl₃) δ = 23.1, 24.9, 29.4, 35.3, 37.5, 115.4, 121.2.

1-Cyanocycloheptaneacetoneitrile,^[9] ¹H NMR (CDCl₃) δ = 1.58 (m, 2H), 1.75 (m, 8H), 2.15 (m, 2H), 2.69 (s, 2H), ¹³C NMR (CDCl₃) δ = 23.7, 27.6, 30.3, 38.1, 40.2, 115.9, 122.1.

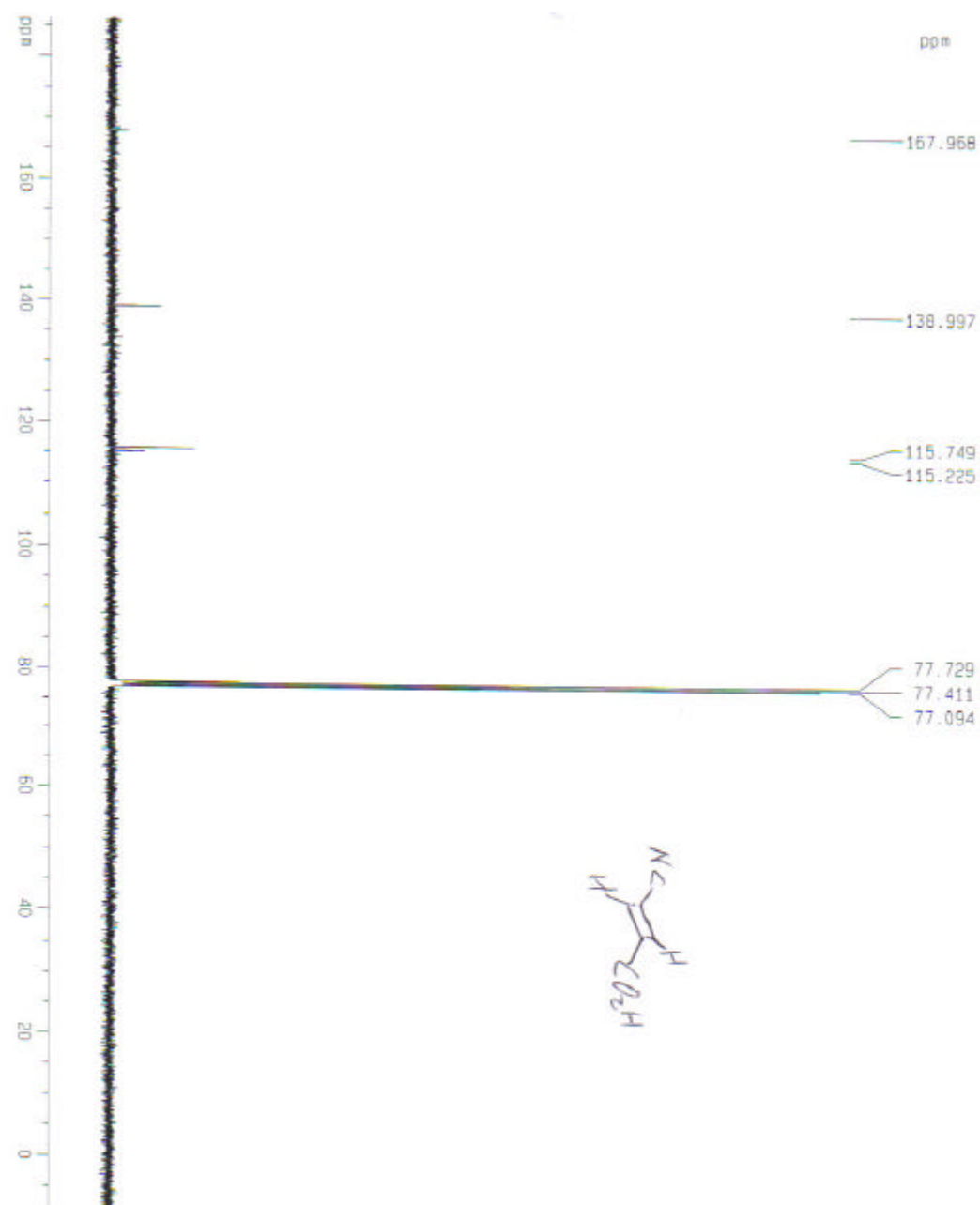
Reaction of w-cyanocarboxylic acids with nitrilase (Using 5-Cyanopentanoic acid as an example). To a solution of 5-cyanopentanoic acid (50 mg, 0.39 mmol) in potassium phosphate buffer (50 ml, 100 mM, pH = 7.2), enzyme (5.0 U) was added and the reaction mixture was incubated at 30°C for 24 hrs. The mixture was acidified with 1 N HCl solution to pH ~ 5, saturated with NaCl and extracted with ethyl acetate. The organic extract was dried over anhydrous sodium sulfate. Removal of solvent provided a residue, the ¹H and ¹³C NMR of which was identical to the starting mono-acid and no di-acid was detected.

References

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J. J. Kinsora, S. M. Lotarski, A. El-Kattan, K. Walters, M. Cherukury, C. P. Taylor, D. J. Wustrow, J. B. Schwarz, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2333-2336.

¹³C NMR spectra of cyanocarboxylic acids.



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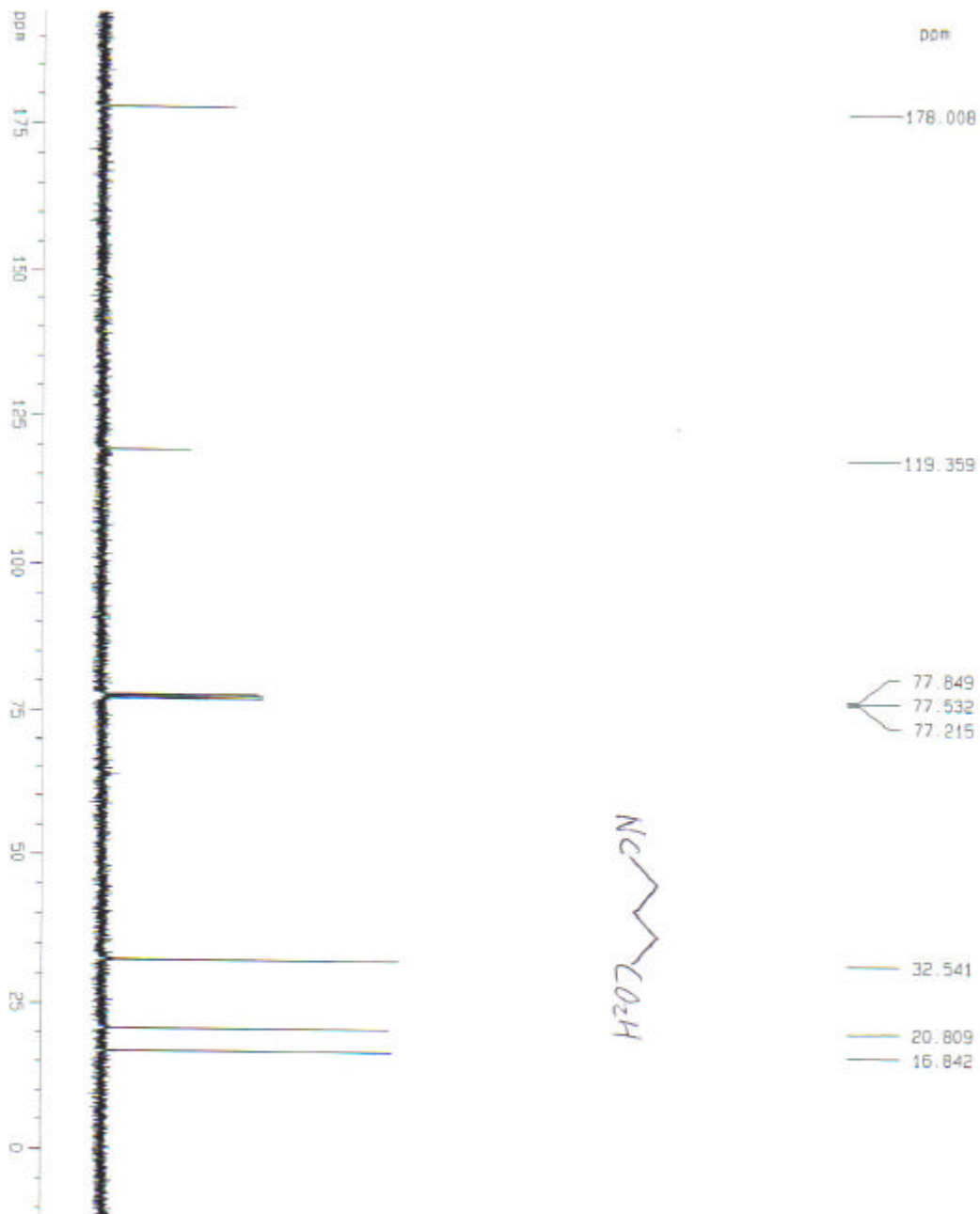
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10 NMR plot parameters

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F1	18735.21 Hz
F2P	-9.148 ppm
FZ	-920.41 Hz
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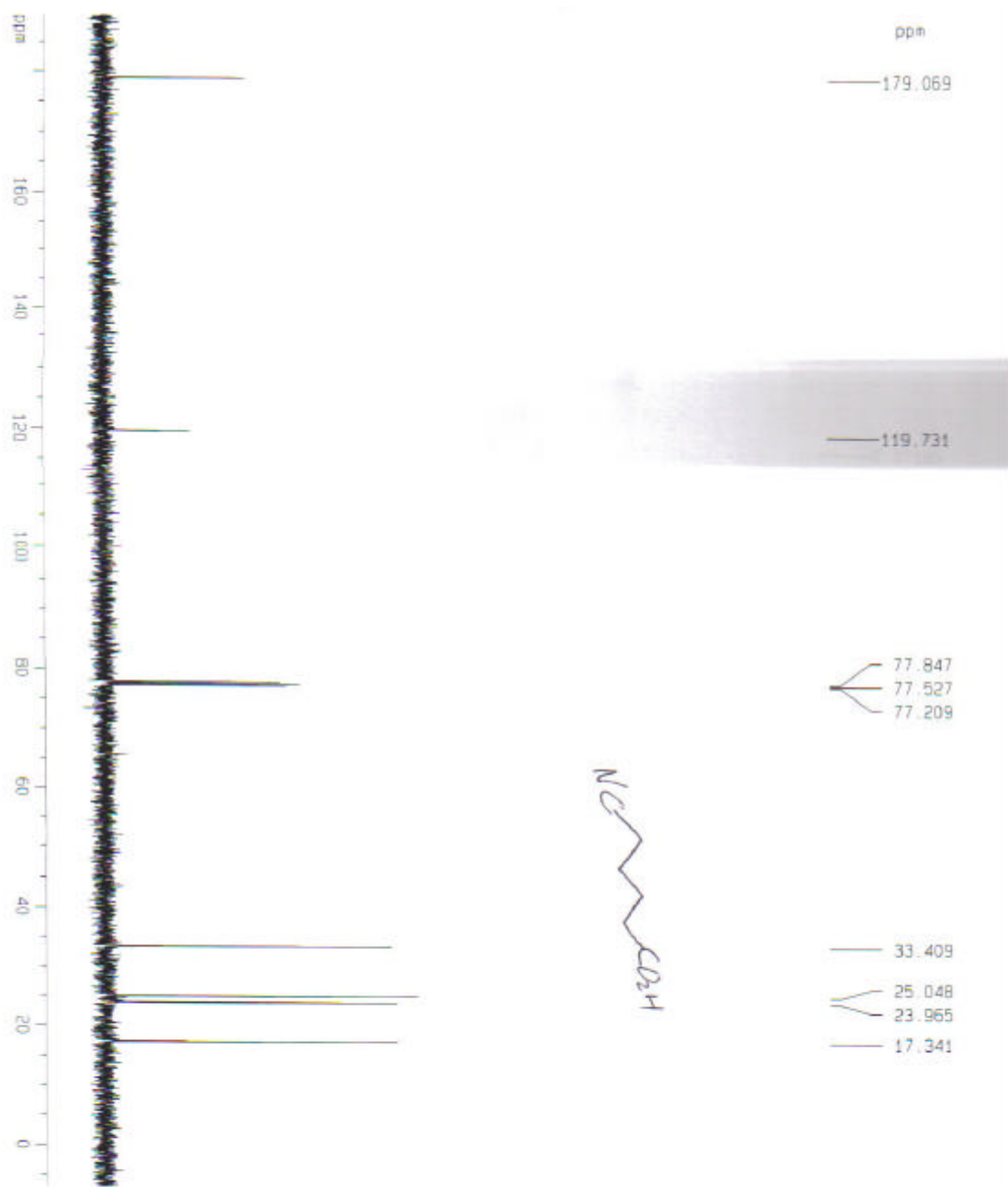
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FIDRES 0.365918 Hz
AQ 1.3864756 sec
RG 4597.0
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LB 1.00 Hz
GB 0
PC 1.40

1D NMR plot parameters
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CY 5.15 cm
F1P 104.092 GHz
F1 19538.16 MHz
F2P -11.775 GHz
F2 -1184.73 MHz
BPMCH 10.20337 GHz/cm
H2CH 1035.84491 Hz/cm



Current Data Parameters

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PROCNO	1

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DS	2
SWH	23980.814 Hz
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RG	4597.6
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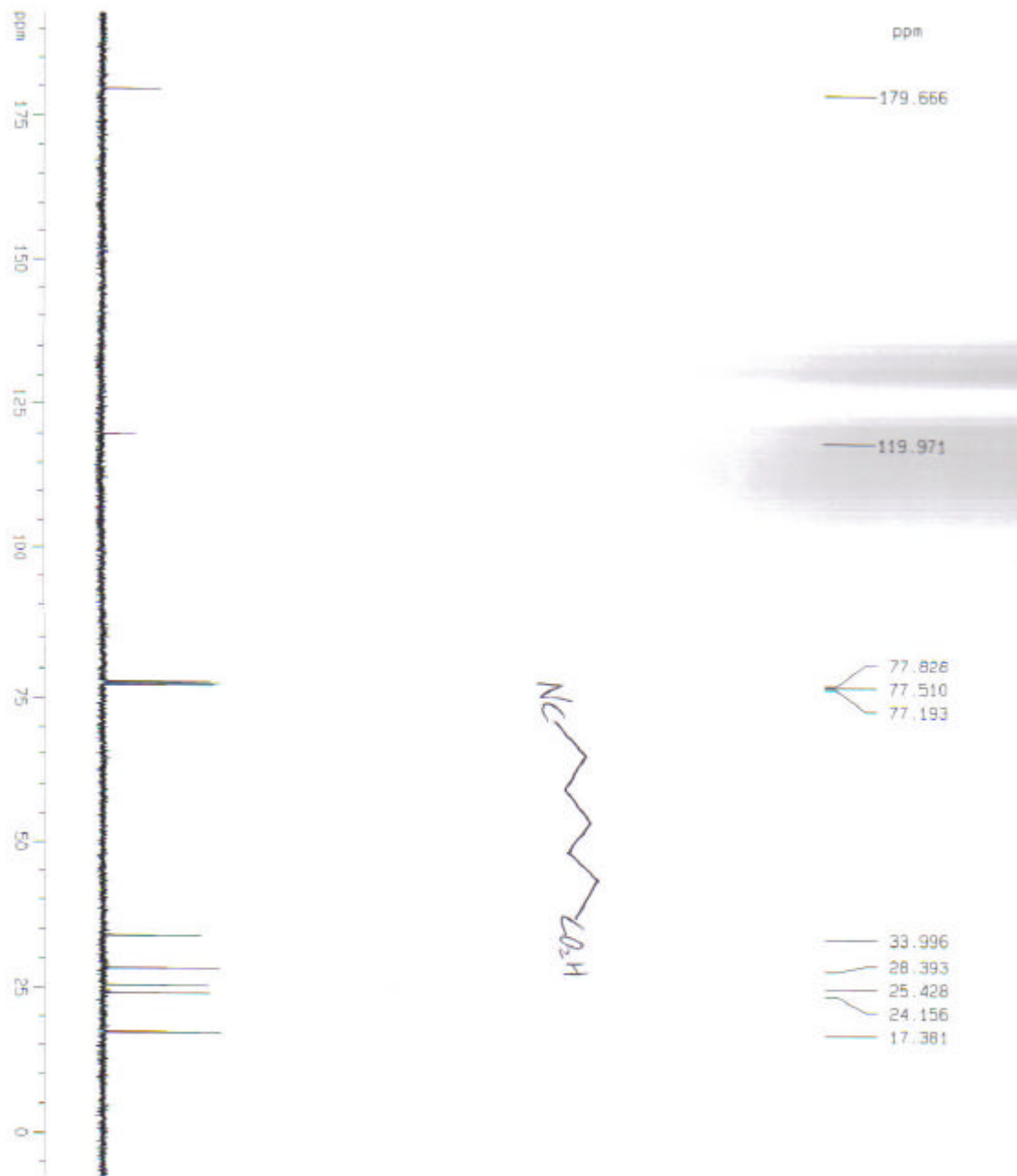
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F2 - Processing Parameters

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ID parameters

CH	20.00 cm
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F1	190.71.51 Hz
F20	-6.999 ppm
F2	-704.15 Hz
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RG	5160.6
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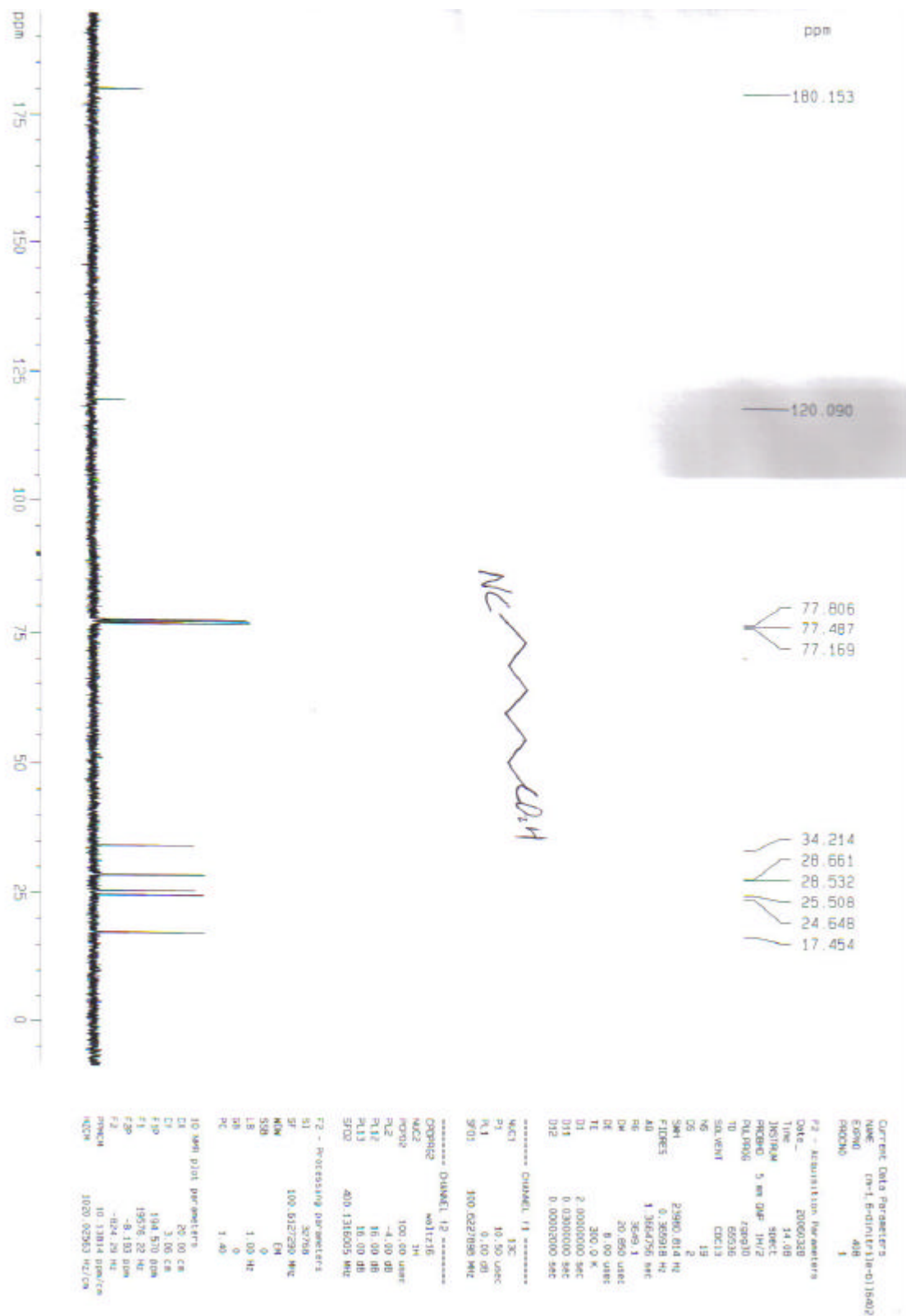
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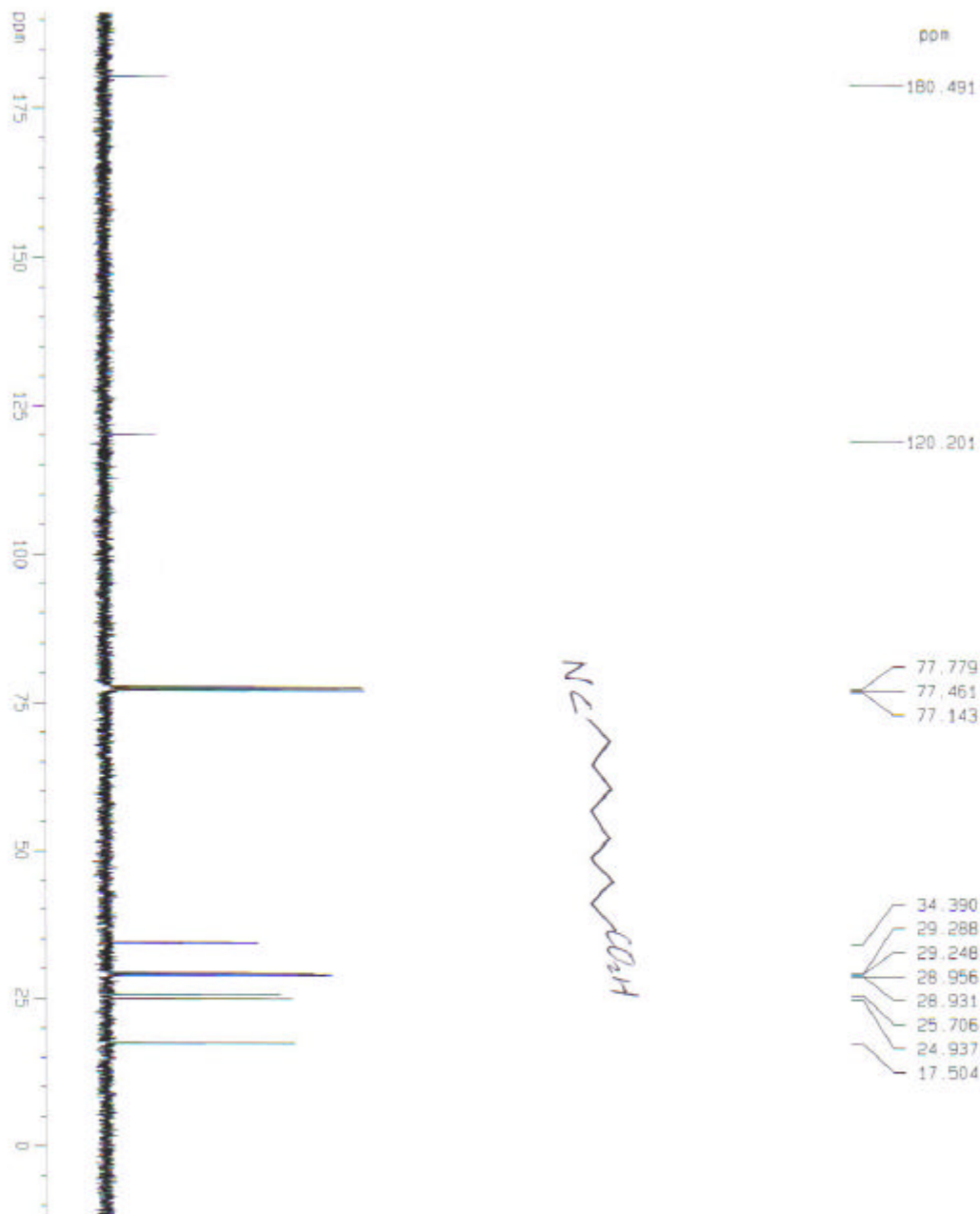
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3D NMR plot parameters

CX	20.00 cm
CY	2.08 cm
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KCM	1008.01123 Hz/cm





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PROCNO    1

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SOLVENT   CDCl3
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DS         2
SWH        23980.814 Hz
FIDRES     0.365918 Hz
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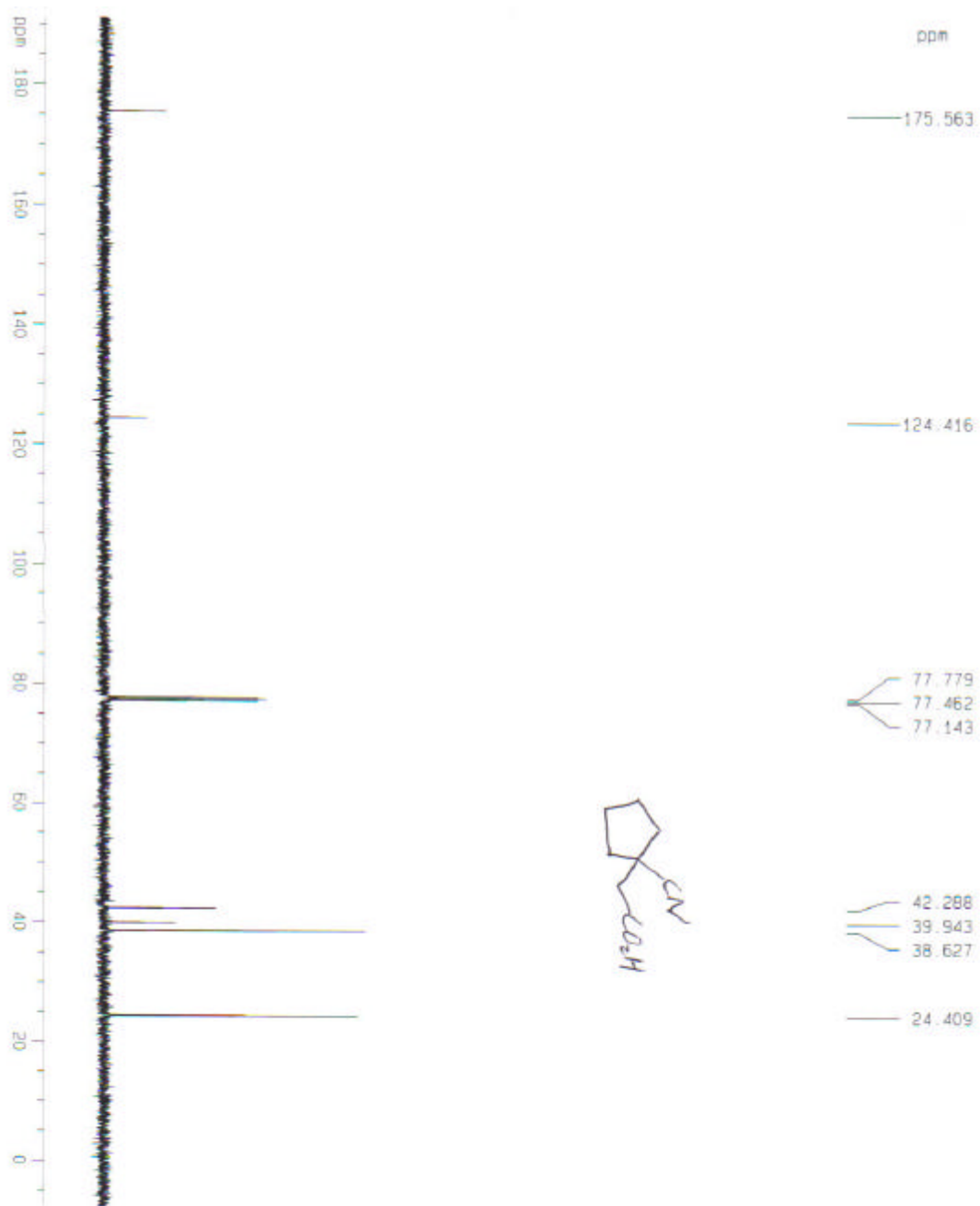
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NUC2       1H
PCPD2      100.00 usec
PL2        -4.00 dB
PL12       15.00 dB
PL13       16.00 dB
SFO2       400.1316005 MHz

F2 - Processing parameters
SI         32768
SF         100.6127250 MHz
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SSB        0
LB         1.00 Hz
GB         0
PC         1.40

1D NMR plot parameters
CX         20.00 cm
CY         4.42 cm
F1P        191.226 MHz
F1         192.35 MHz
F2P        -12.014 MHz
F2         -1208.75 MHz
FREQCH     10.16202 MHz/cm
HZCM       1022.42853 Hz/cm

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Current Data Parameters

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EXPNO	44
PROCNO	1

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DS	4
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***** CHANNEL f2 *****

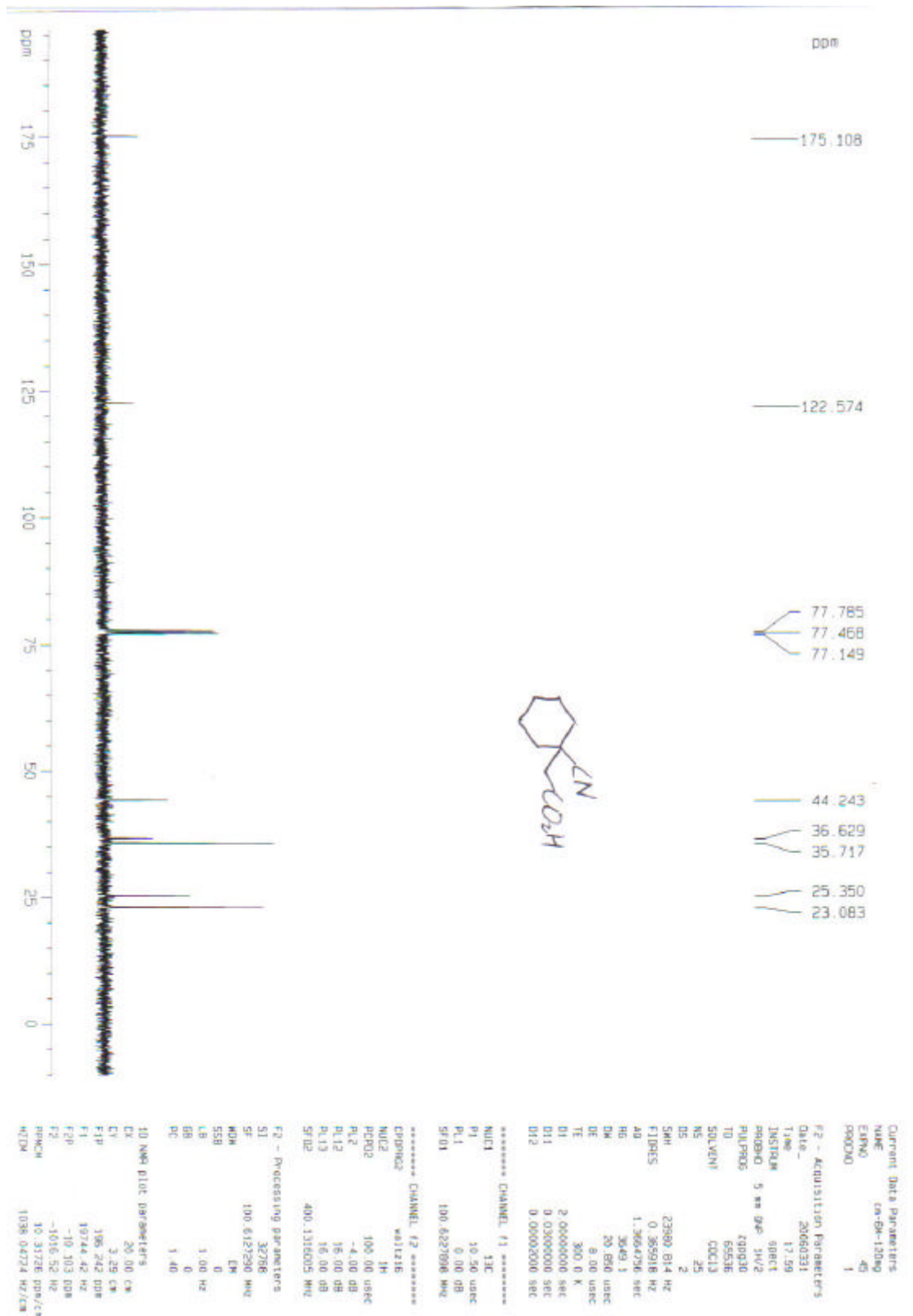
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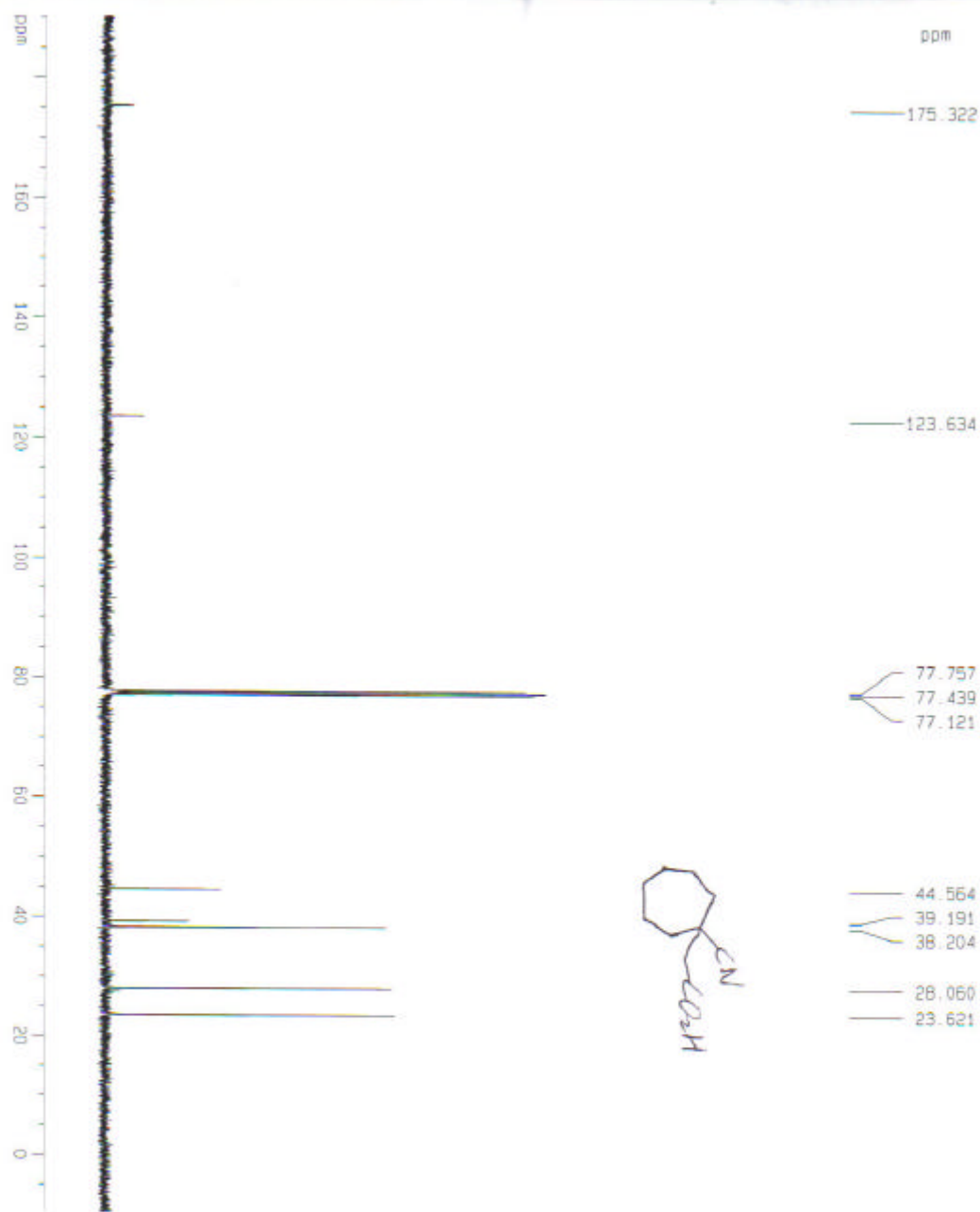
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Current Data Parameters
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PROCNO 1

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D12 0.00020000 sec

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PL13 16.00 dB
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GB 0
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