Synthesis of the Phosphinic Analogue of Thyrotropin Releasing Hormone

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Supporting Information

NMR, MS, HRMS spectra, and HPLC chromatograms

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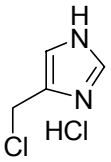
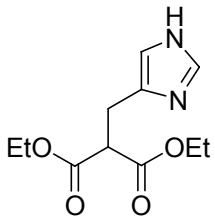
General considerations: All of the compounds, for which analytical and spectroscopic data are quoted, were homogenous by TLC. TLC analyses were performed using silica gel plates (E. Merck silica gel 60 F-254) and components were visualized by the following methods: ultraviolet light absorbance, charring after spraying with a solution of $(NH_4)HSO_4$, and by Pauli test for imidazole (spraying with a fresh mixture of equal volumes of sulfanilic acid 1% in 1M HCl and $NaNO_2$ 5%, and Na_2CO_3 10% solution after 5 minutes). Column chromatography was carried out on silica gel (E.Merck, 70-230 mesh), height 40 cm and diameter 1.5 cm. HPLC analyses were carried out on a MZ-Analytical Column 250x4mm, Kromasil, 100, C18, 5μm, at a flow rate of 0.5 mL/min. Solvent A: 10% CH_3CN, 90% H_2O, 0.1% TFA. Solvent B: 90% CH_3CN, 10% H_2O, 0.09% TFA. The gradient system used was *t*=0 min (0% B), *t*=10 min (25% B), *t*=45 min (75% B), *t*=50 min (100% B), *t*=55 min (100% B), *t*=60 min (40% B) and eluted peaks were detected at 254 nm, except for compound **8**, which was detected at 220 nm, using Solvent C: 3% CH_3CN, 97% H_2O, 0.1% TFA. Given times (where more than one) correspond to two pairs of diastereoisomers and are counted in minutes. All the compounds were characterized by ^{1}H, ^{13}C and ^{31}P-NMR spectroscopy. ^{13}C and ^{31}P-NMR spectra are fully proton decoupled. ^{31}P- chemical shifts are reported on δ scale (in ppm) downfield from 85% H_3PO_4. ESI mass spectral analysis was performed using direct sample injection. Negative or positive ion ESI spectra were acquired by adjusting the needle and cone voltages accordingly, while HRMS mass spectral analysis was performed using an orthogonal geometry hybrid quadrupole – TOF mass spectrometer retrofitted with a nanoelectrospray source. Commercially available reagents, were used without further purification.

Abbreviations: AcOH: acetic acid, AcOEt: ethyl acetate, Bu^t: *tert*-butyl, DIPEA: diisopropylethylamine, HOBt: 1-hydroxybenzotriazole, MeOH: methanol, P.E. 40-60°C: petroleum ether 40-60°C.

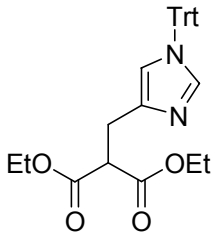
Detailed Experimental Procedures:



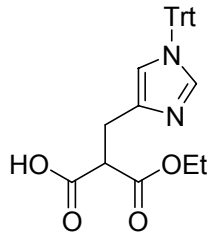
4-Chloromethyl-1*H*-imidazole hydrochloride (1): To a suspension of 4-(hydroxymethyl)imidazole hydrochloride (10 g, 74.3 mmol) in benzene (15 mL), $SOCl_2$ (11.9 g, 100 mmol) in benzene (7.5 mL) are added dropwise. The resulting mixture is refluxed for 6 h. Filtration of the mixture, washings with benzene and Et_2O provides **7** (11.4 g, 99%) as off-white solid. ^{1}H-NMR (200 MHz, d_6-DMSO) δ 4.9, (2H), 7.4-7.5 (m, 1H), 9.1-9.2 (m-1H); ^{13}C-NMR (50 MHz, d_6-DMSO) 34.3, 118.5, 129.4. 135.2.



2-(1*H*-Imidazol-4-ylmethyl)-malonic acid diethyl ester (2): Na (5.13 g, 223 mmol) is dissolved in abs. EtOH (180 mL), and diethyl malonate (35.7 g, 223 mmol) is added to this solution. Upon heating to 50^{o}C, a solution of **7** (11.4 g, 74.3 mmol) in EtOH (70 mL) is added dropwise. The reaction mixture is stirred for 16 h at 50^{o}C. Removal of solvent, dissolution in 1 M HCl, washings with Et_2O, neutralization with 10% Na_2CO_3,, extraction with AcOEt, washing with brine, drying over Na_2SO_4 and solvent removal provides **2** (14.6 g, 82%) as orange oil. TLC R_f 0.14 ($CHCl_3$/MeOH =95:5); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.17 (t, J = 7.1 Hz, 6H), 3.18 (d, J = 7.6 Hz, 2H), 3.71 (t, J = 7.6 Hz, 1H), 4.11 (q, J = 7.1 Hz, 4H), 6.82 (s, 1H), 7.55 (s, 1H); ^{13}C-NMR (50 MHz, $CDCl_3$) 13.9, 26.2, 52.1, 61.4, 117.7, 133.4, 134.9, 169.1; ESMS *m/z* calcd for $C_{11}H_{17}N_2O_4$ $(M + 1)^+$ 241.1, found 241.1.

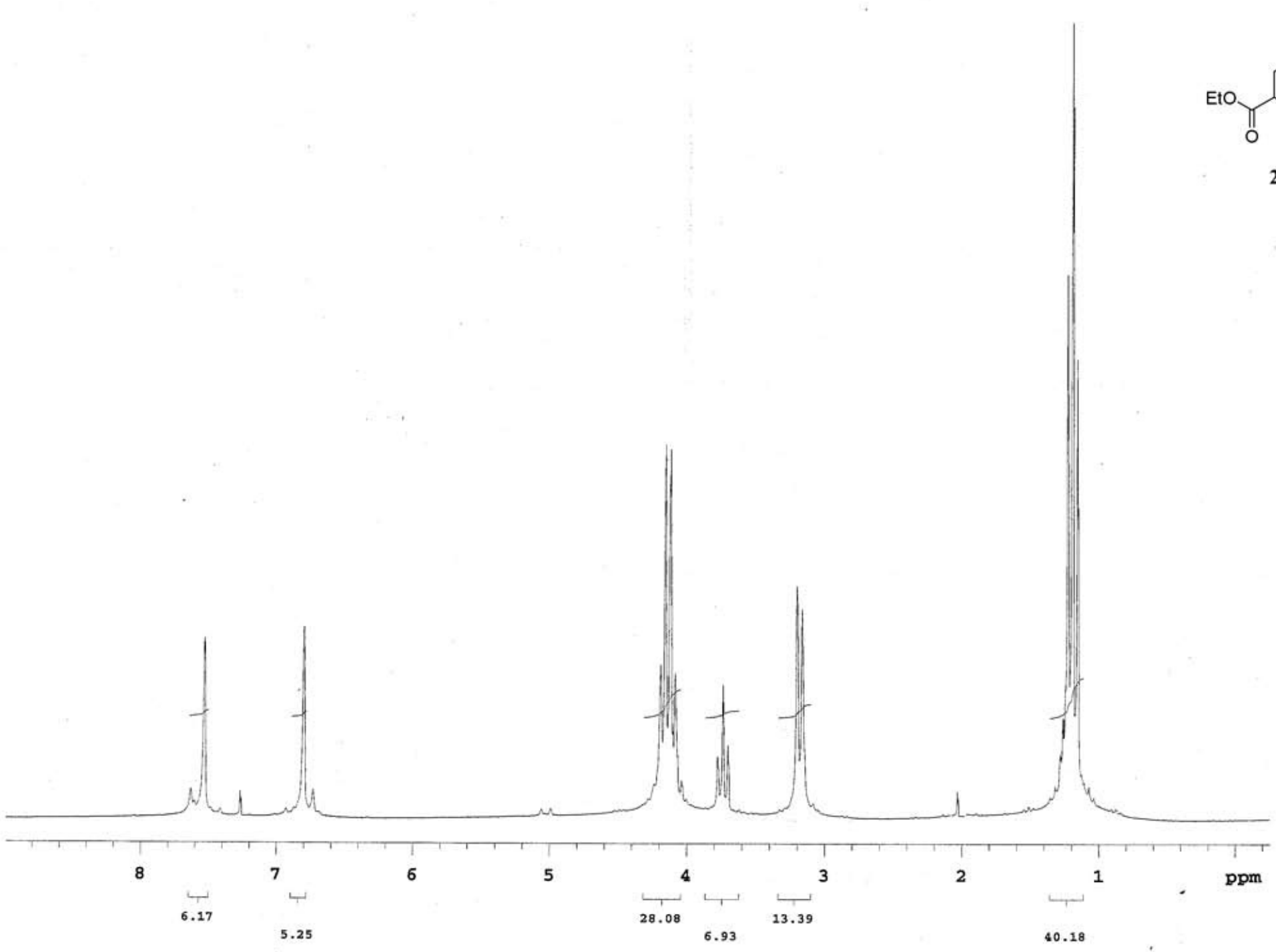


(1-Trityl-1*H*-imidazol-4-ylmethyl)-malonic acid diethyl ester (3): In a solution of **2** (14 g, 58.3 mmol) in anhydrous CH_2Cl_2 (150 mL) and DIPEA (9.05 g, 70 mmol), TrtCl (19.5 g, 70 mmol) in CH_2Cl_2 (60 mL) is added dropwise at 0°C and the reaction mixture is stirred at rt for 16 h. Removal of solvent, dissolution in AcOEt, washings with 0.5 M HCl to pH 1, 5% $NaHCO_3$ and brine, drying over Na_2SO_4, solvent removal and column purification with CH_2Cl_2/MeOH = 97:3 affords **3** (28.1 g, 95%) as yellow oil. TLC R_f 0.68 ($CHCl_3$/MeOH =95:5); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.17 (t, J = 7.1 Hz, 6H), 3.10 (d, J = 8.2 Hz, 2H), 3.78 (t, J = 8.2 Hz, 1H), 4.08 (q, J = 7.1 Hz, 4H), 6.63 (s, 1H), 7.03-7.37 (m, 16H); ^{13}C-NMR (50 MHz, $CDCl_3$) 13.9, 27.6, 52.0, 61.1, 74.9, 118.8, 126.7, 127.5, 127.8, 128.5, 129.5, 137.4, 138.3, 142.2, 168.8; ESMS *m/z* calcd for $C_{30}H_{31}N_2O_4$ $(M + 1)^+$ 483.2, found 483.4.



2-(1-Trityl-1*H*-imidazol-4-ylmethyl)-malonic acid monoethyl ester (4): In a solution of **3** (28 g, 58 mmol) in abs EtOH (65 mL), KOH (3.57 g, 63.8 mmol) in EtOH (65 mL) is added dropwise at 0°C and the reaction mixture is stirred at rt for 1 h. Removal of solvent, dissolution in H_2O, washings with Et_2O, acidification with 1 M HCl to pH 1, extraction with AcOEt, washing with brine, drying over Na_2SO_4 and solvent removal affords **4** (25.8 g, 98%) as pale yellow foam. TLC R_f 0.16 ($CHCl_3$/MeOH =95:5); ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.02 (t, J = 7.2 Hz, 3H), 3.17 (d, J = 6.6 Hz, 2H), 3.77 (t, J = 6.6 Hz, 1H), 3.95 (q, J = 7.2 Hz, 2H), 6.70 (s, 1H), 6.88-7.38 (m, 15H), 8.13 (s, 1H); ^{13}C-NMR (50 MHz, $CDCl_3$) 13.8, 24.2, 51.3,

61.3, 78.1, 121.0, 128.5, 128.9, 129.3, 132.0, 135.3, 139.6, 168.4, 169.9; ESMS *m/z* calcd for $C_{28}H_{27}N_2O_4$ $(M + 1)^+$ 455.2, found 455.3.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OEt
O
O
2
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7
6
5
4
3
2
1
ppm
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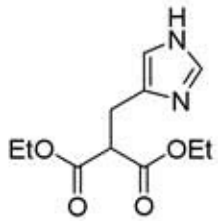
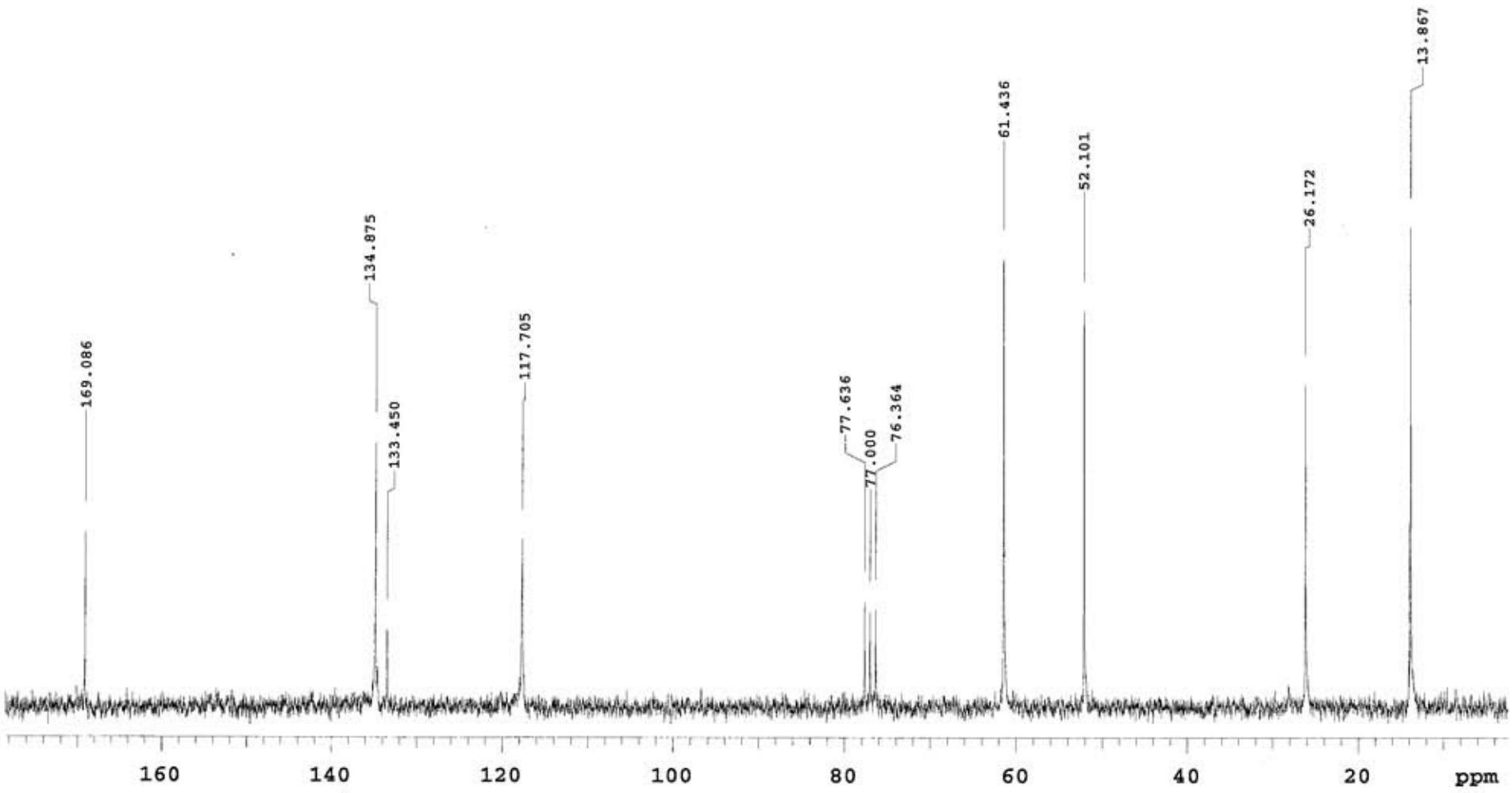
6.93
13.39
40.18


H
N
N
EtO
OEt
O
O

2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117.705
77.636
77.000
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61.436
52.101
26.172
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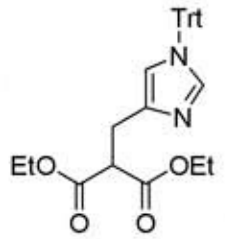
160
140
120
100
80
60
40
20
ppm



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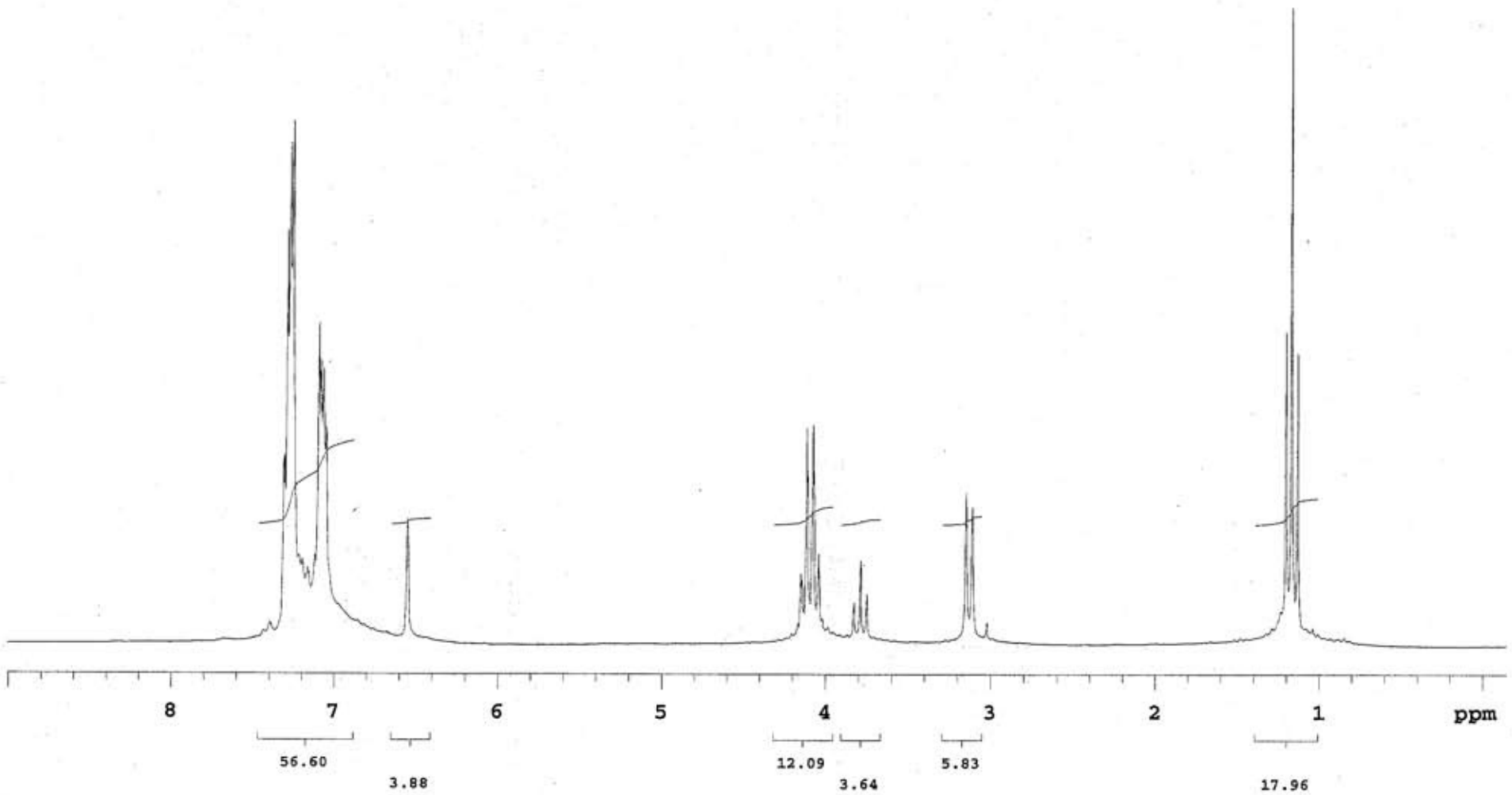

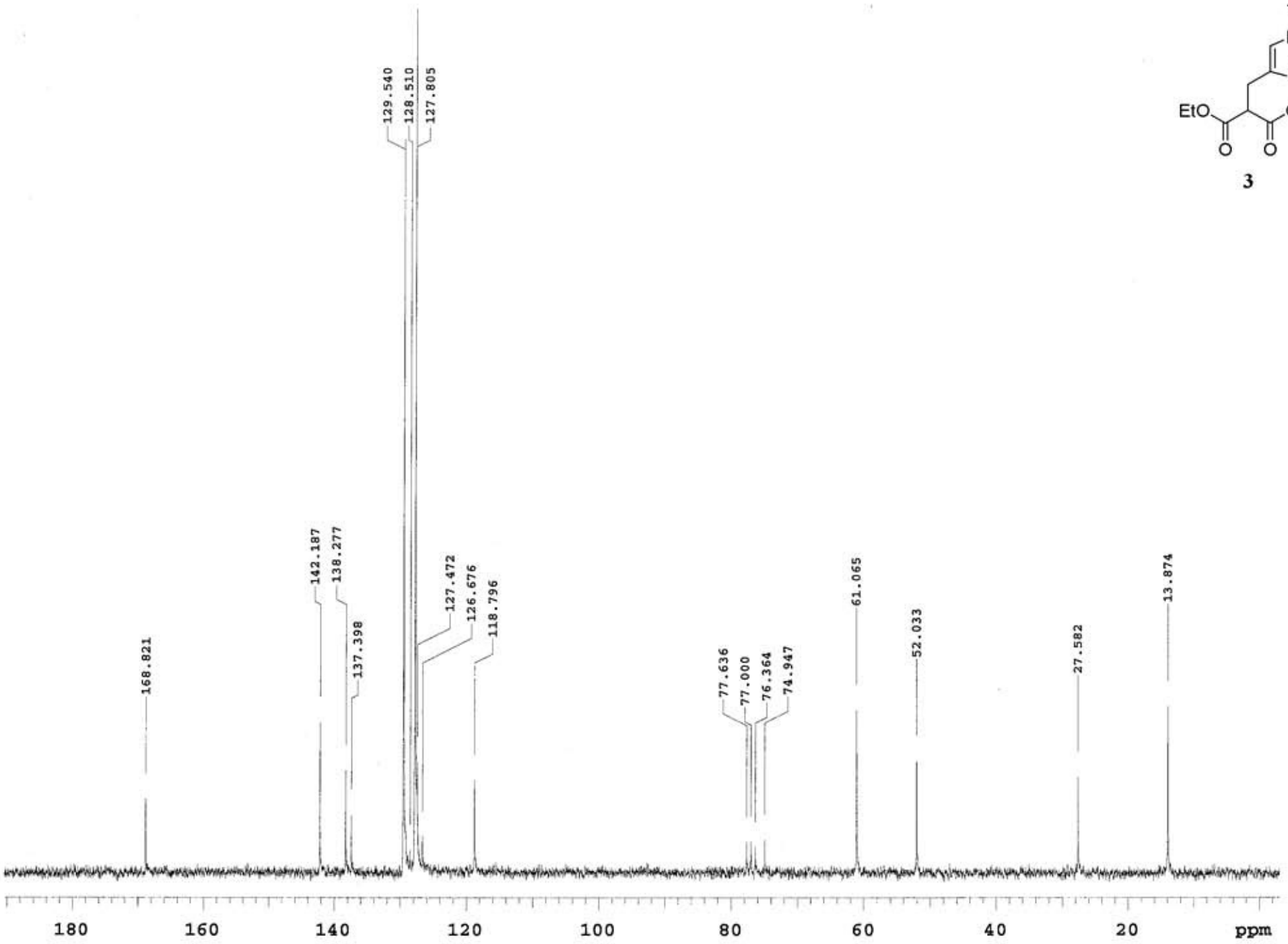
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127.472
126.676
118.796
168.821
77.636
77.000
76.364
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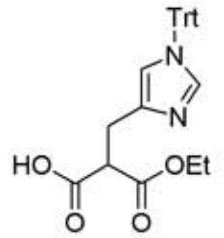
100
80
60
40
20
ppm



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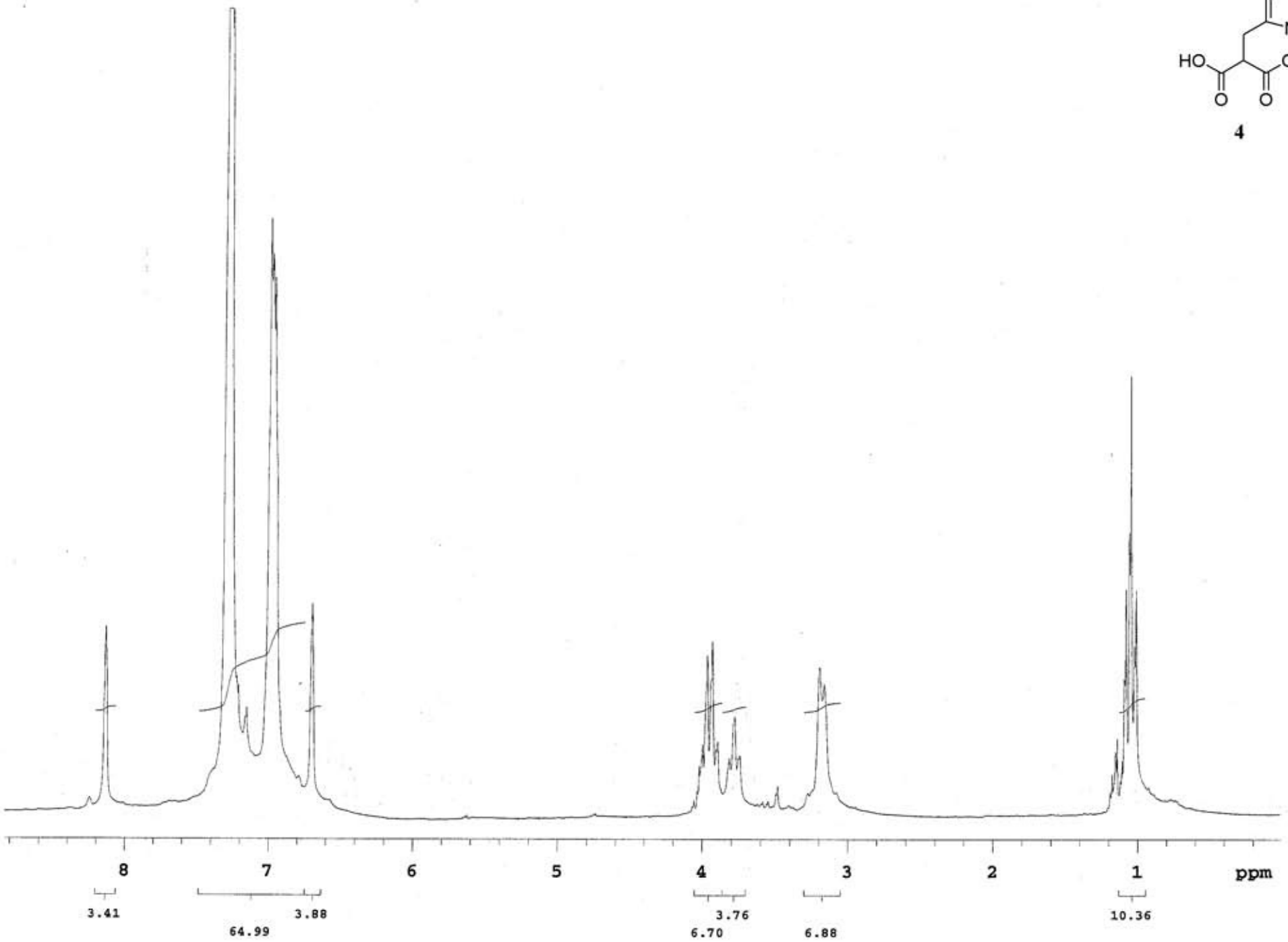

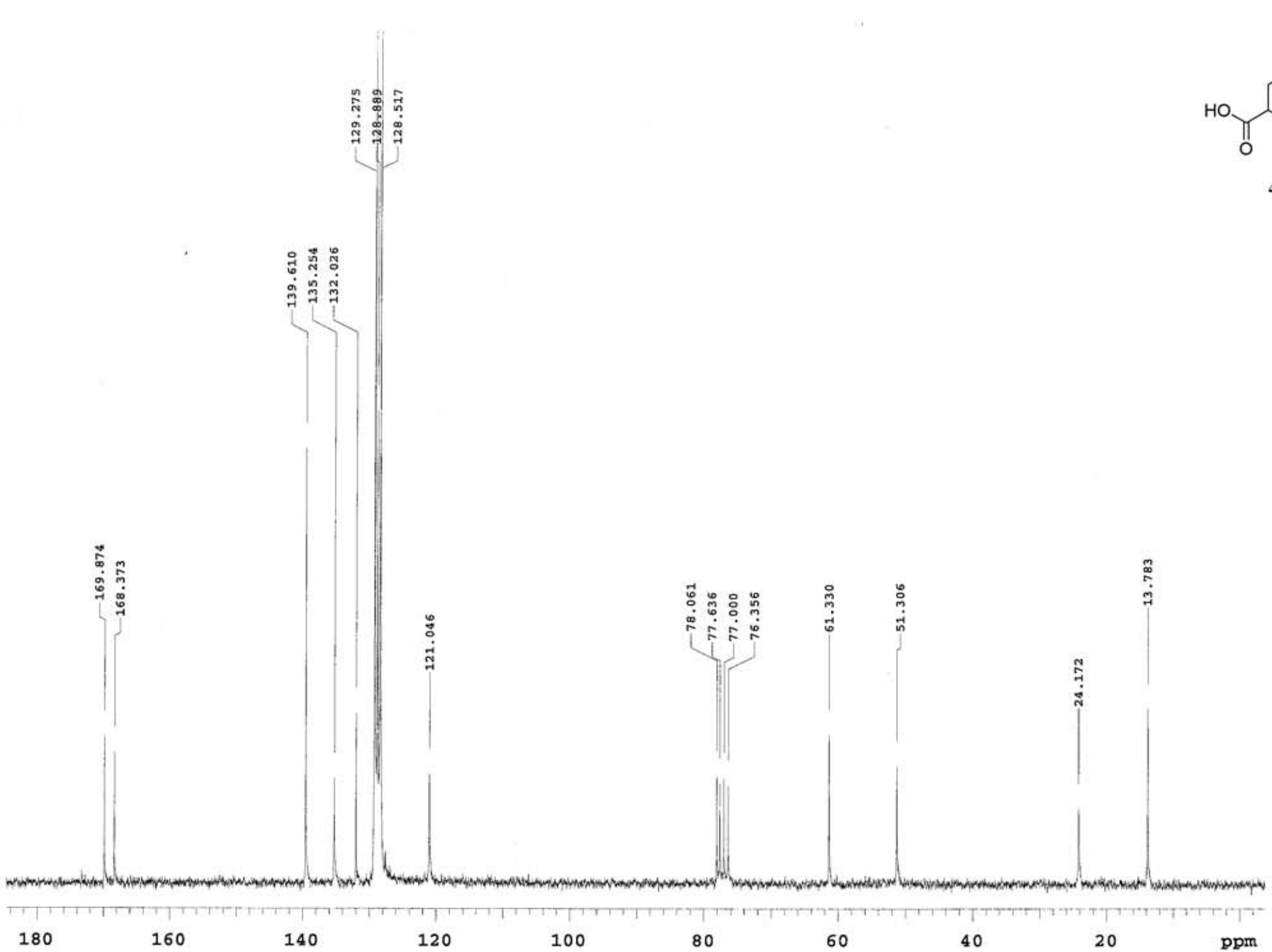
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OEt
O
O
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128.517
121.046
78.061
77.636
77.000
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140
120
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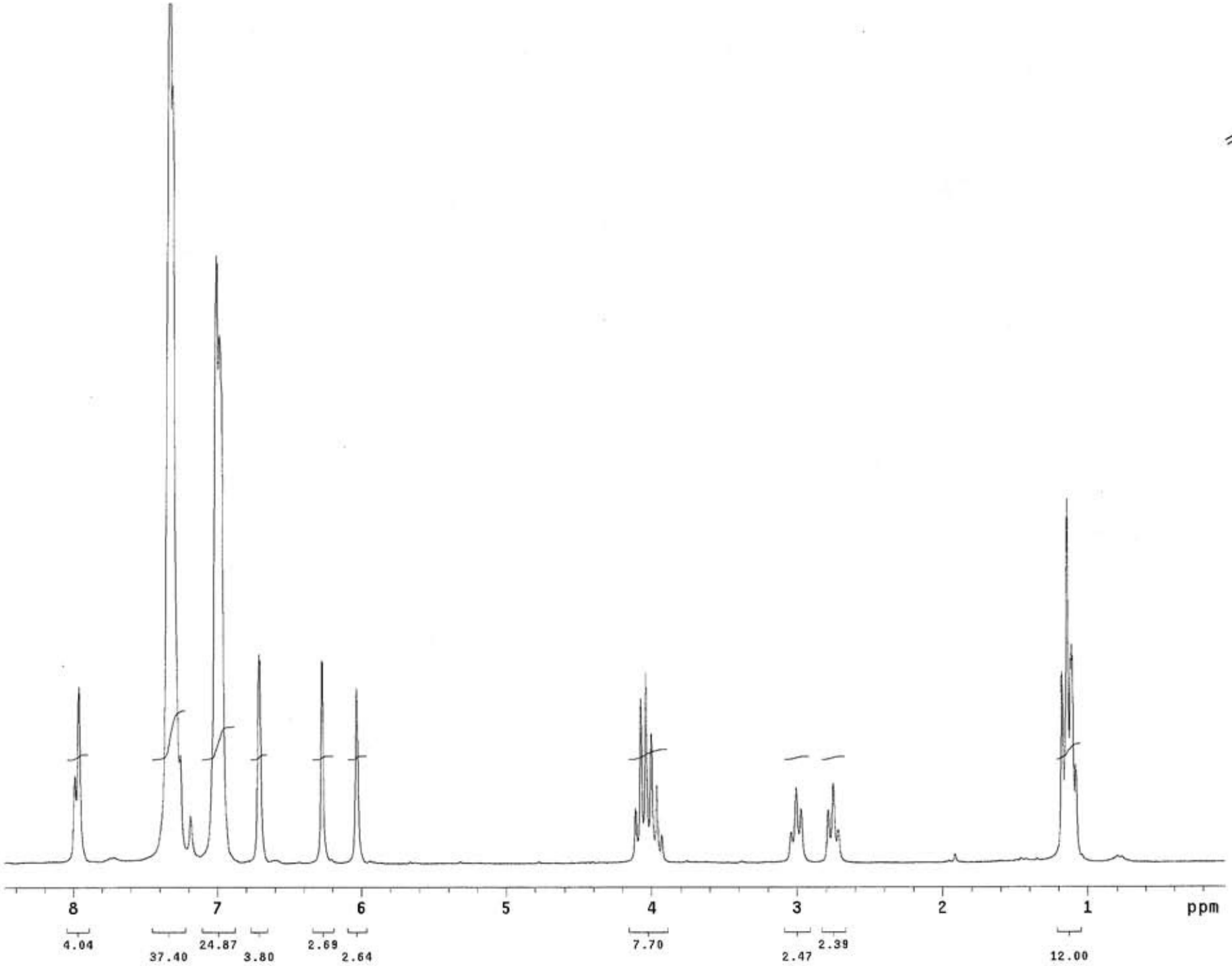
80
60
40
20
ppm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7
6
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4
3
2
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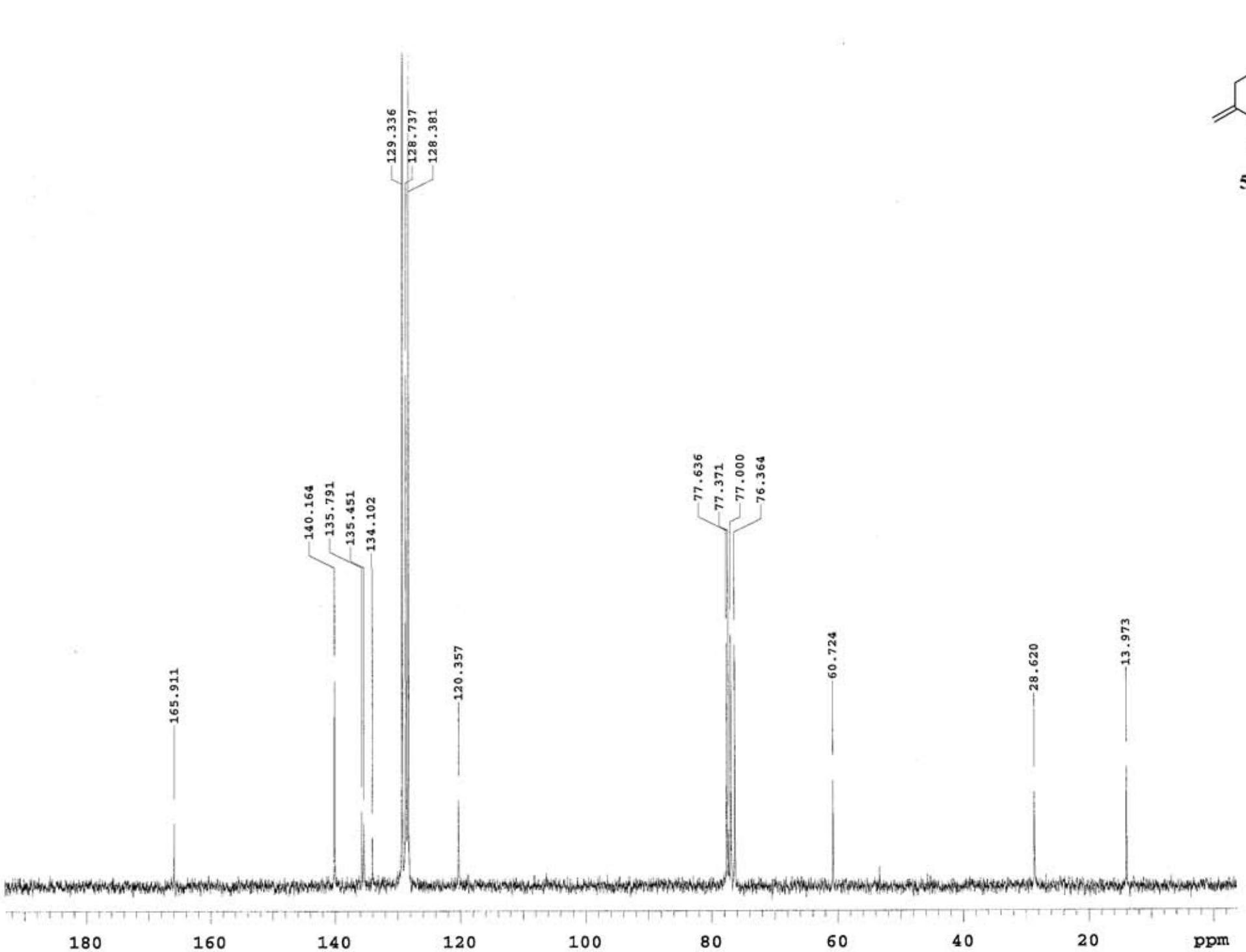
7.70
2.47
2.39
12.00

5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129.336
128.737
128.381
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77.636
77.371
77.000
76.364
60.724
28.620
13.973
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160
140
120
100
80
60
40
20
ppm

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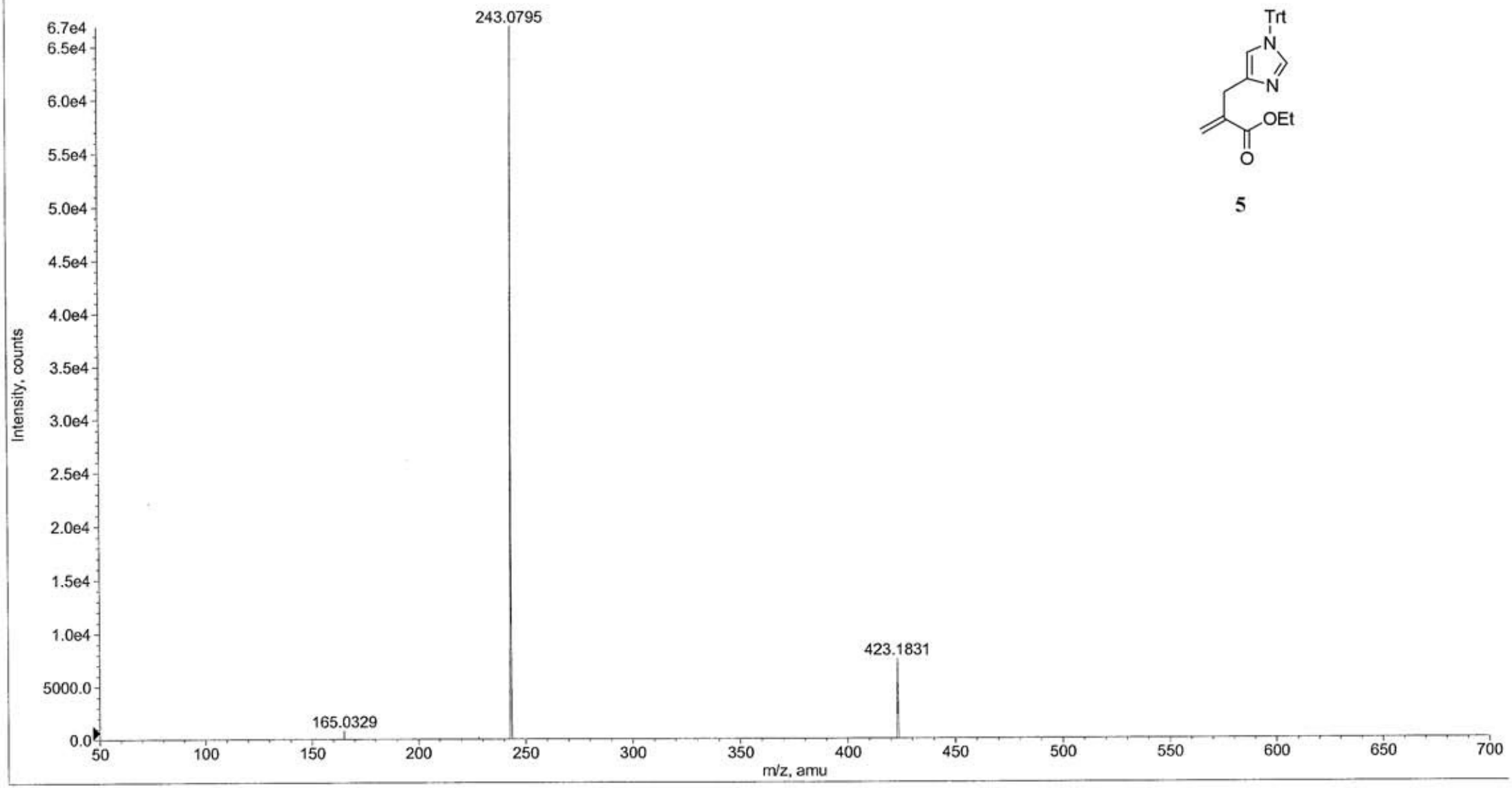

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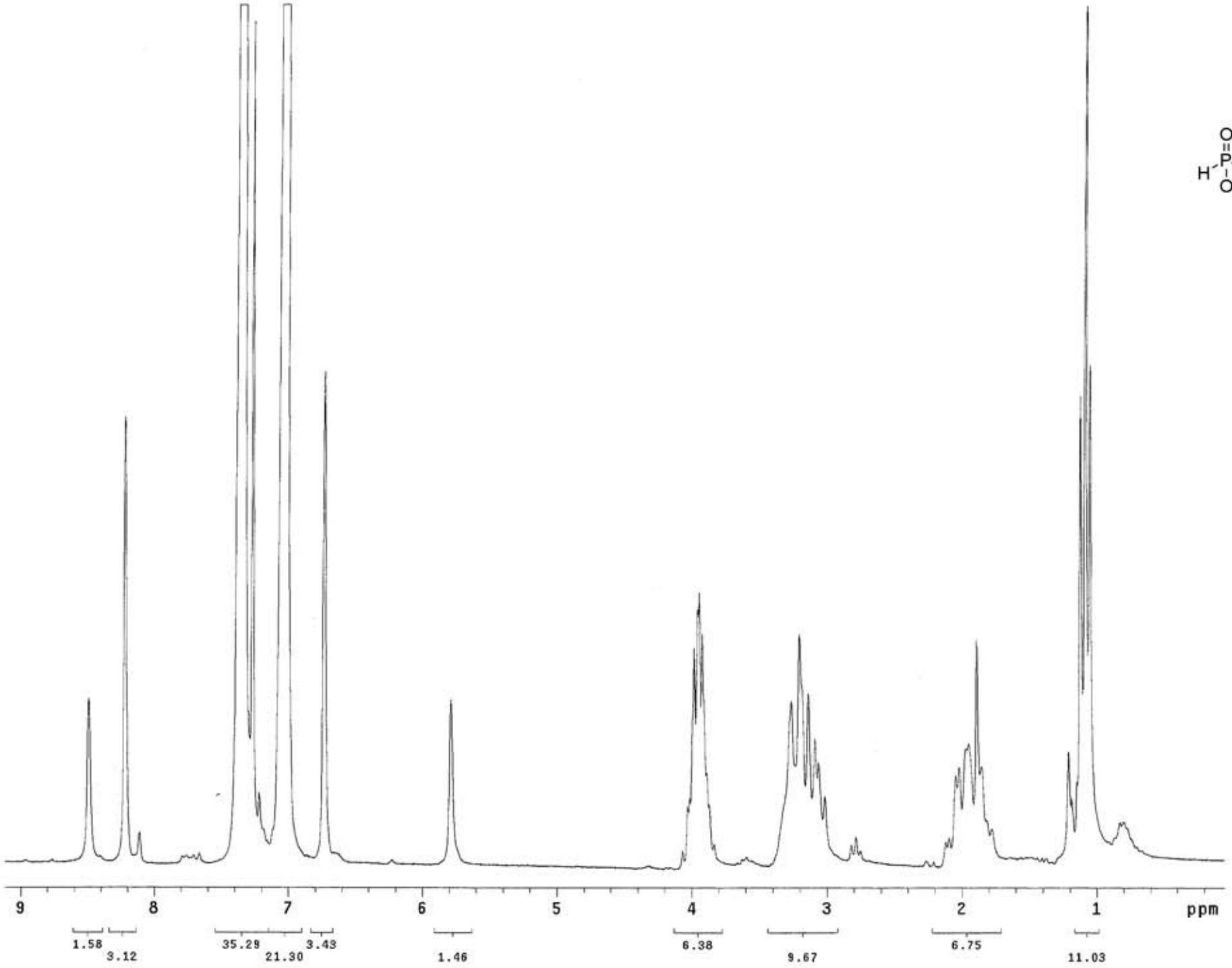
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6
5
4
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1.46
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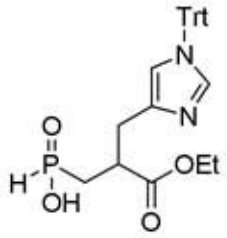
9.67
6.75
11.03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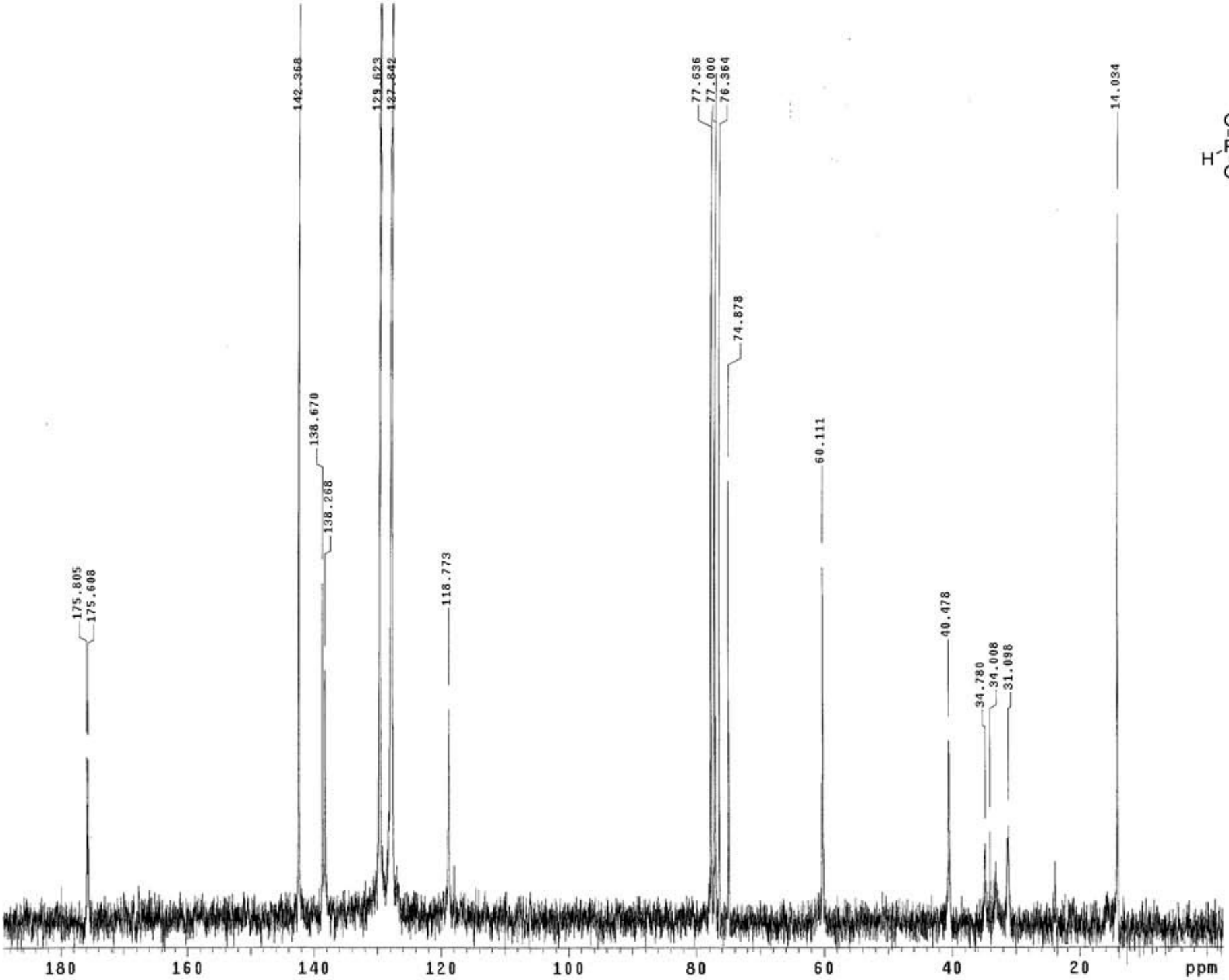
N
N
O
H
P
OH
OEt
O

6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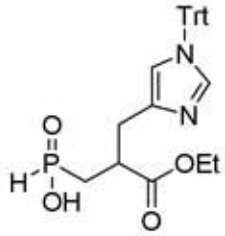
80
60
40
20
ppm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N
O
P
H
OH
OEt
O

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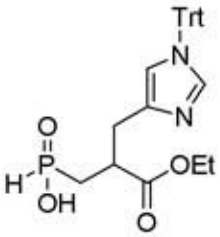

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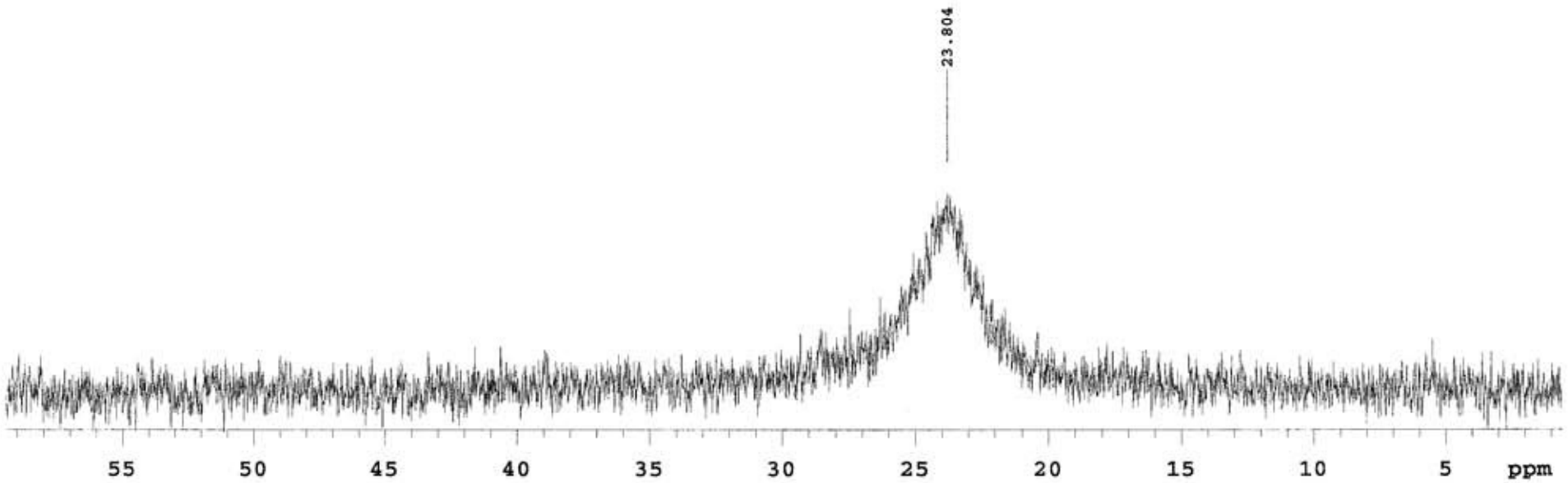

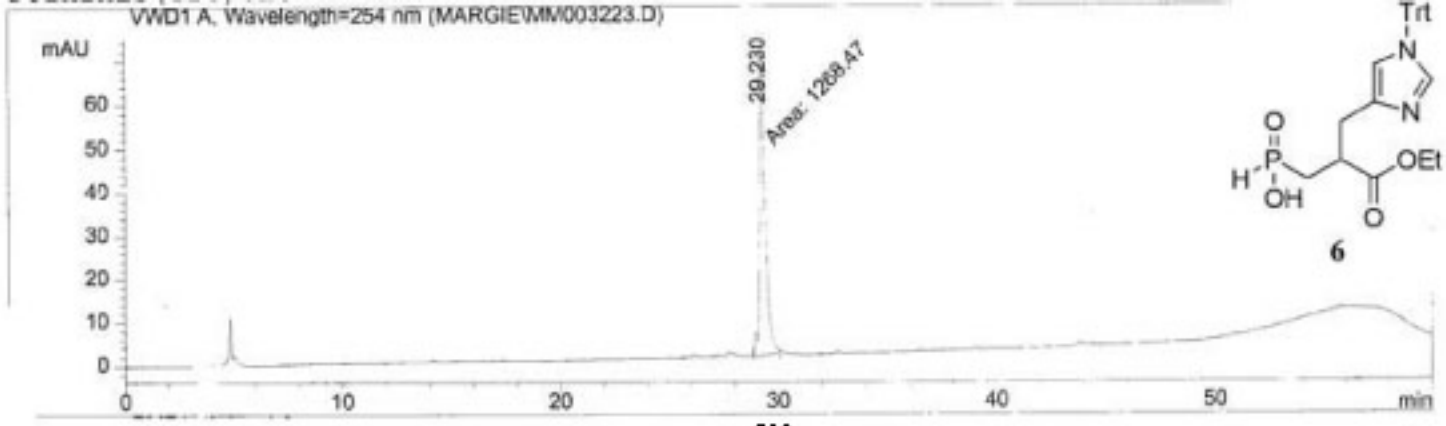
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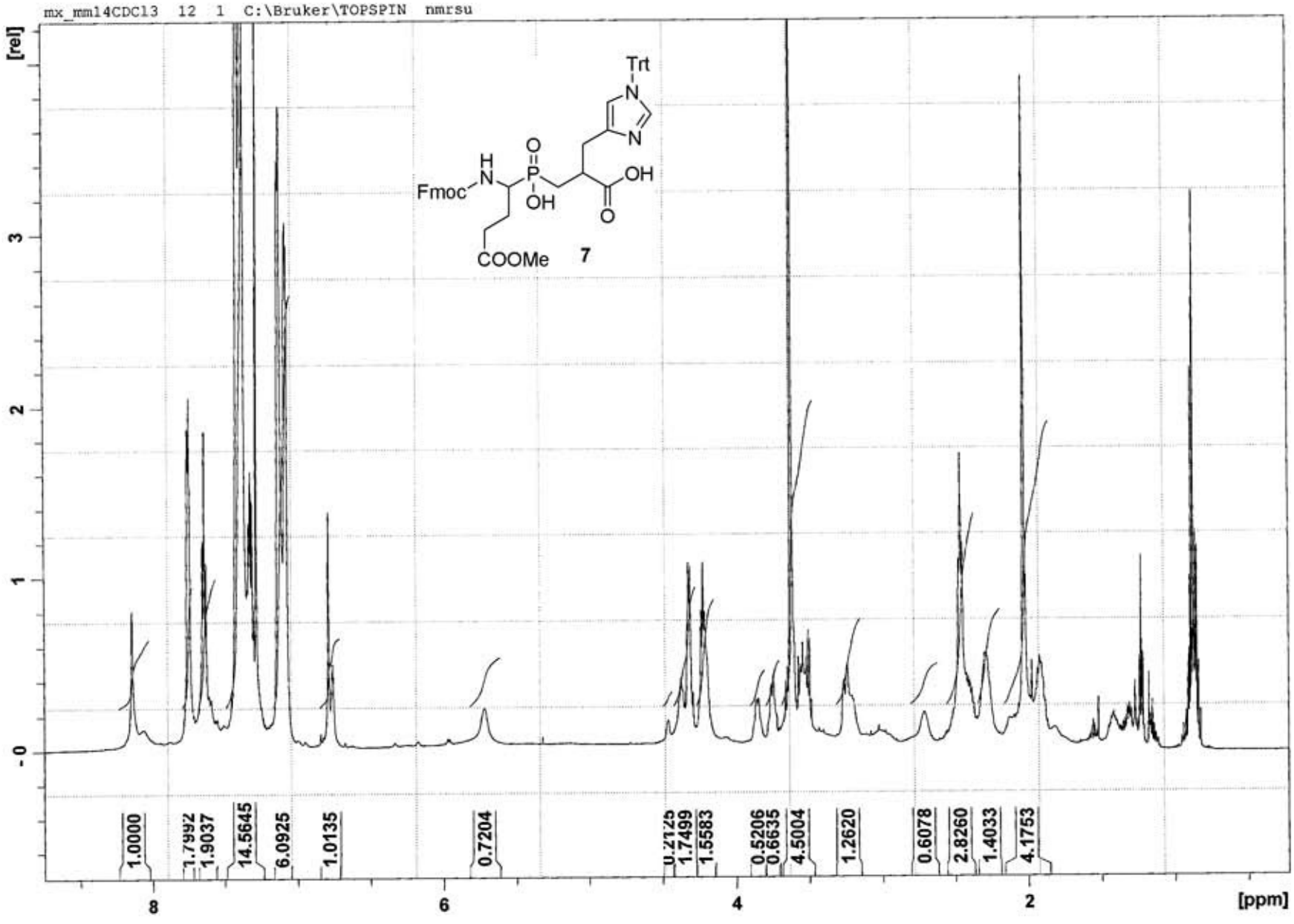
40
30
20
10
0
29.230
Area: 1268.47
0
10
20
30
40
50
min
Trt
N
N
O
P
H
OH
OEt
O
O
6


mx_mm14CDCl3 12 1 C:\Bruker\TOPSPIN nmrsu
[rel]
Trt
Fmoc
COOMe
7
1.0000
1.7992
1.9037
14.5645
6.0925
1.0135
0.7204
1.7499
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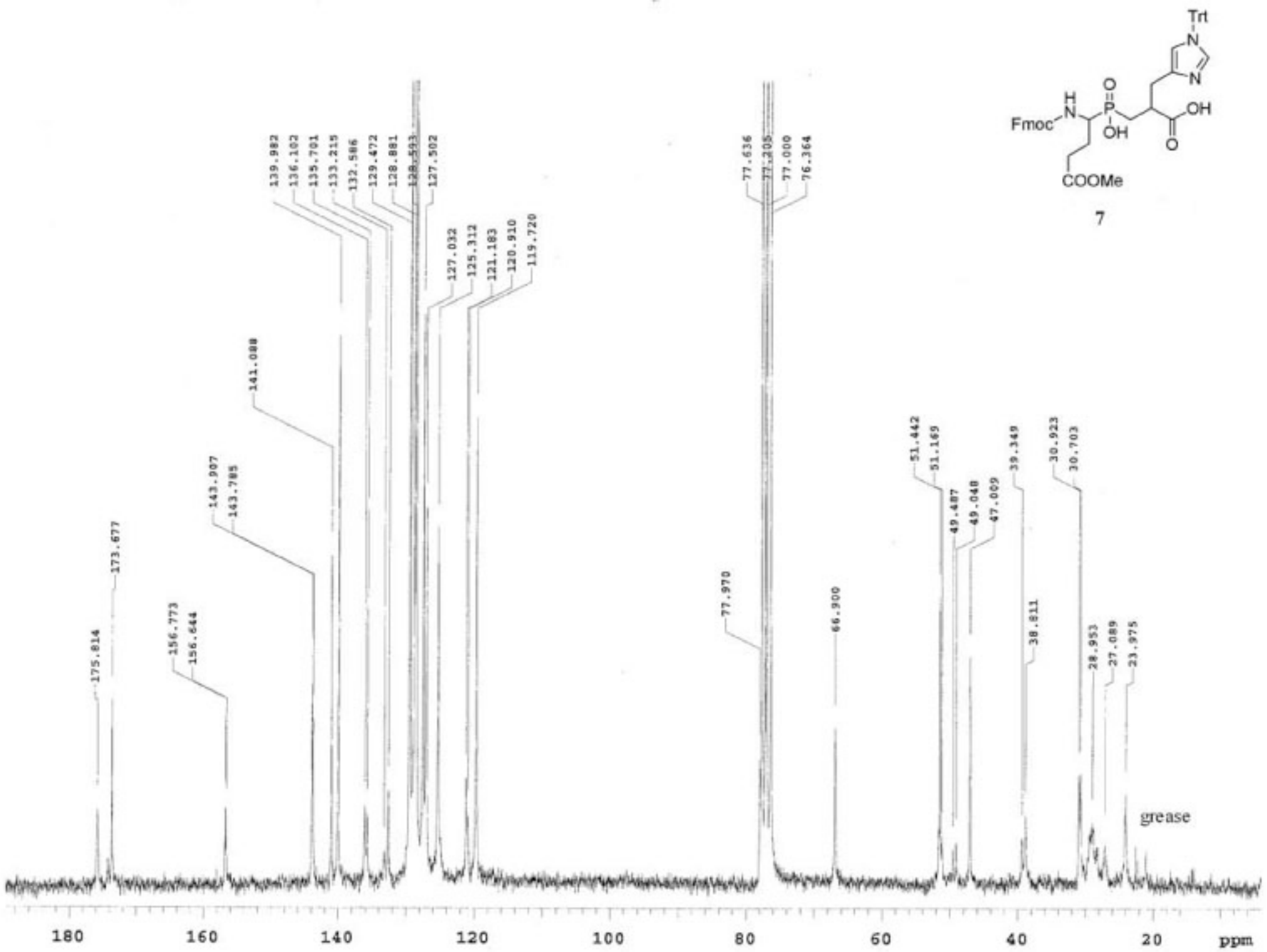
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Fmoc
NH
P
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OH
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COOMe
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136.102
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128.881
128.593
127.502
127.032
125.312
121.183
120.910
119.720
141.088
143.907
143.785
156.773
156.644
175.814
173.677
77.636
77.205
77.000
76.364
77.970
66.900
51.442
51.169
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49.048
47.009
39.349
38.811
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20
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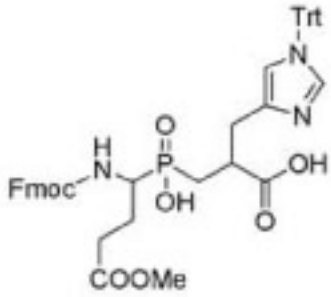

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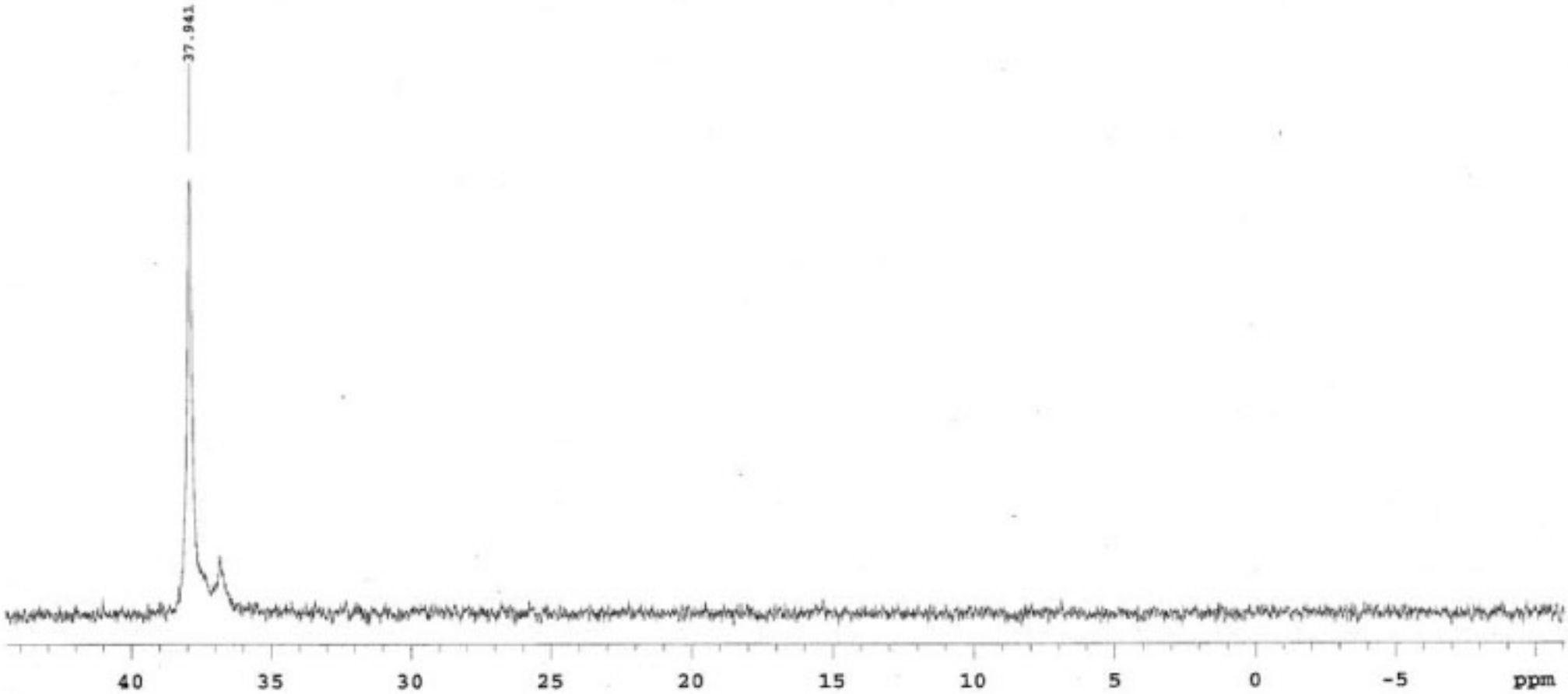

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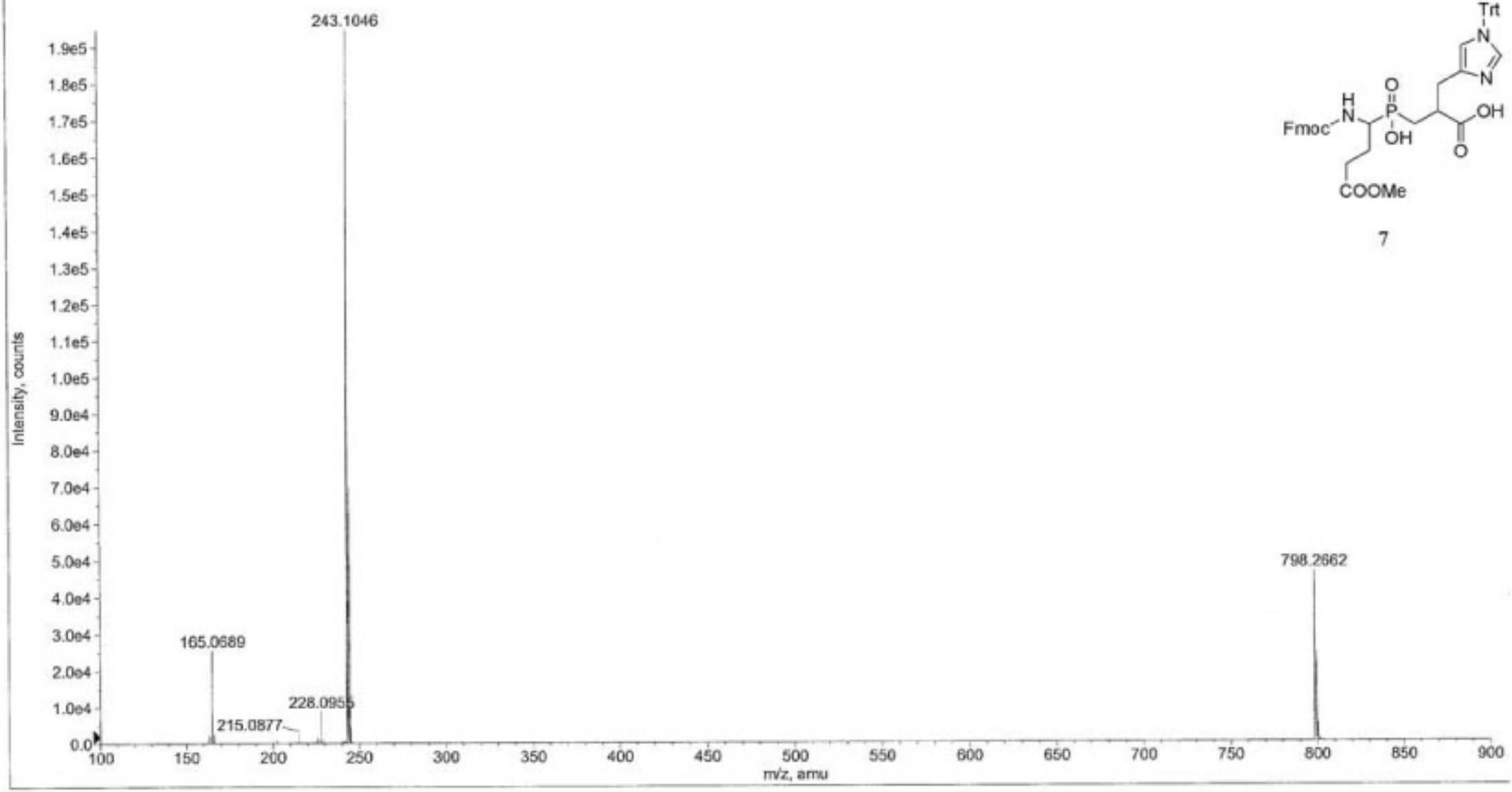
Max. 1.9e5 counts.



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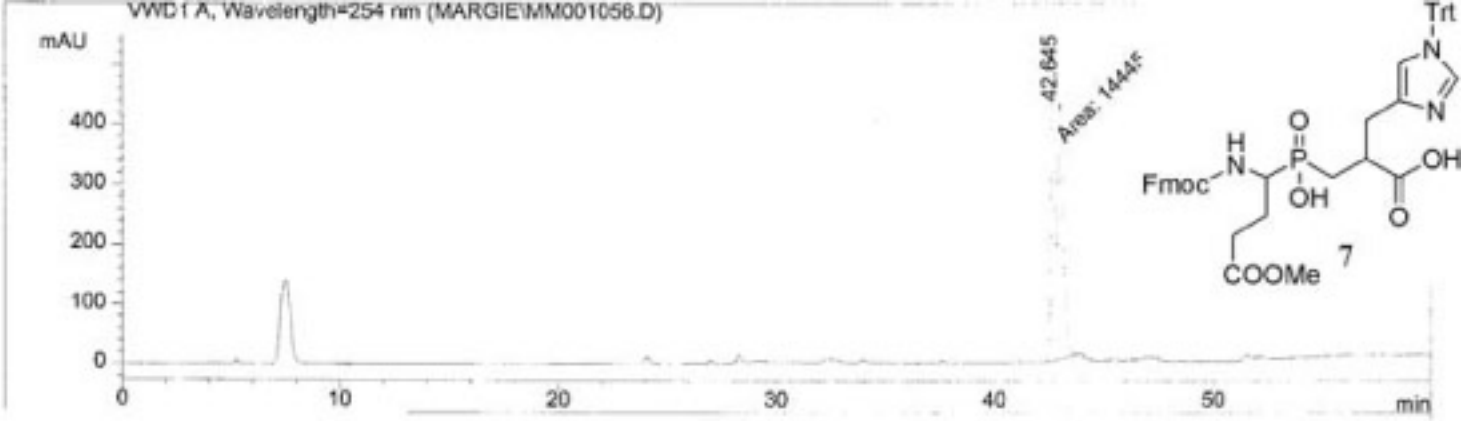
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20
30
40
50
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O
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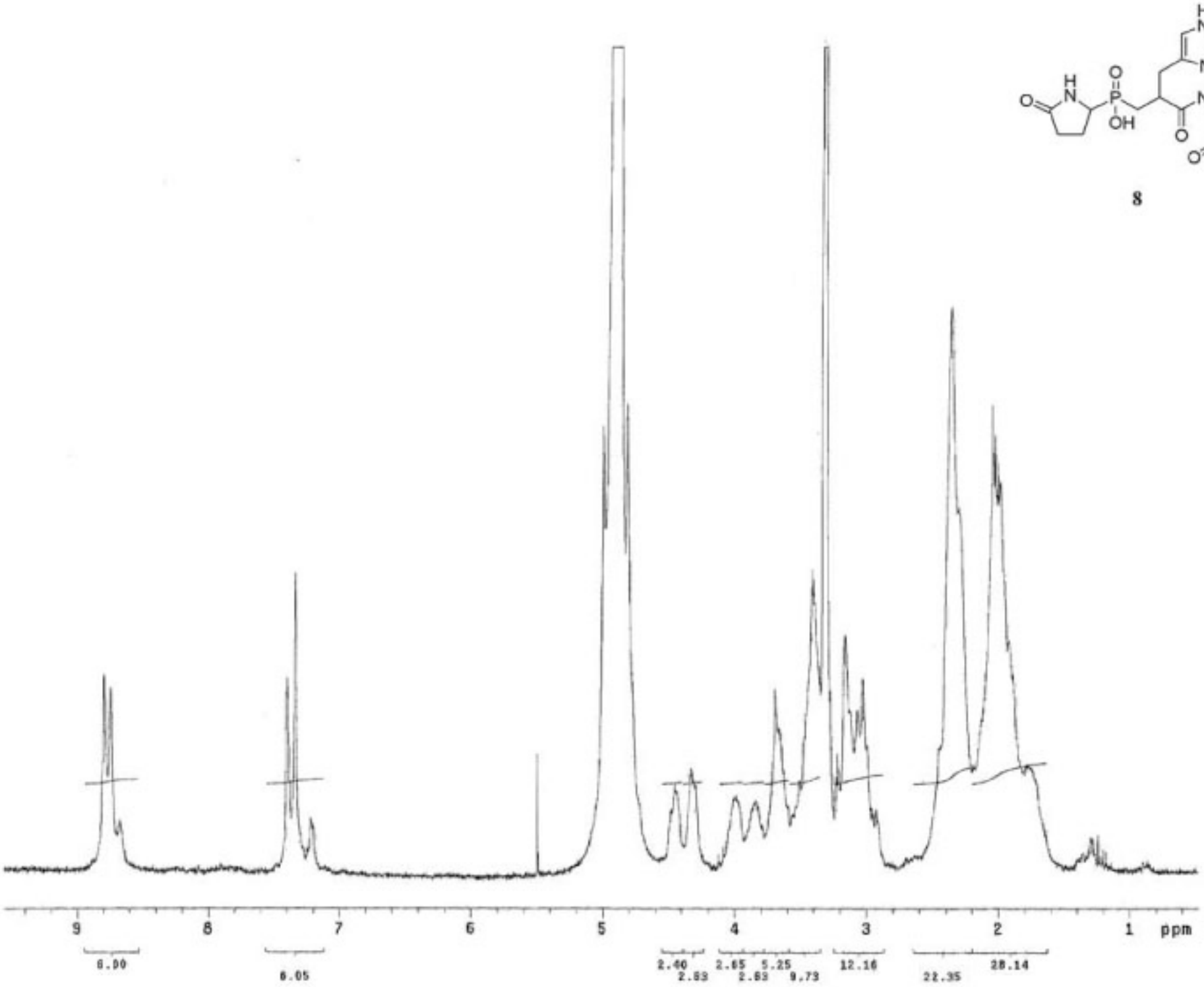
OH
OH
O
COOMe
7



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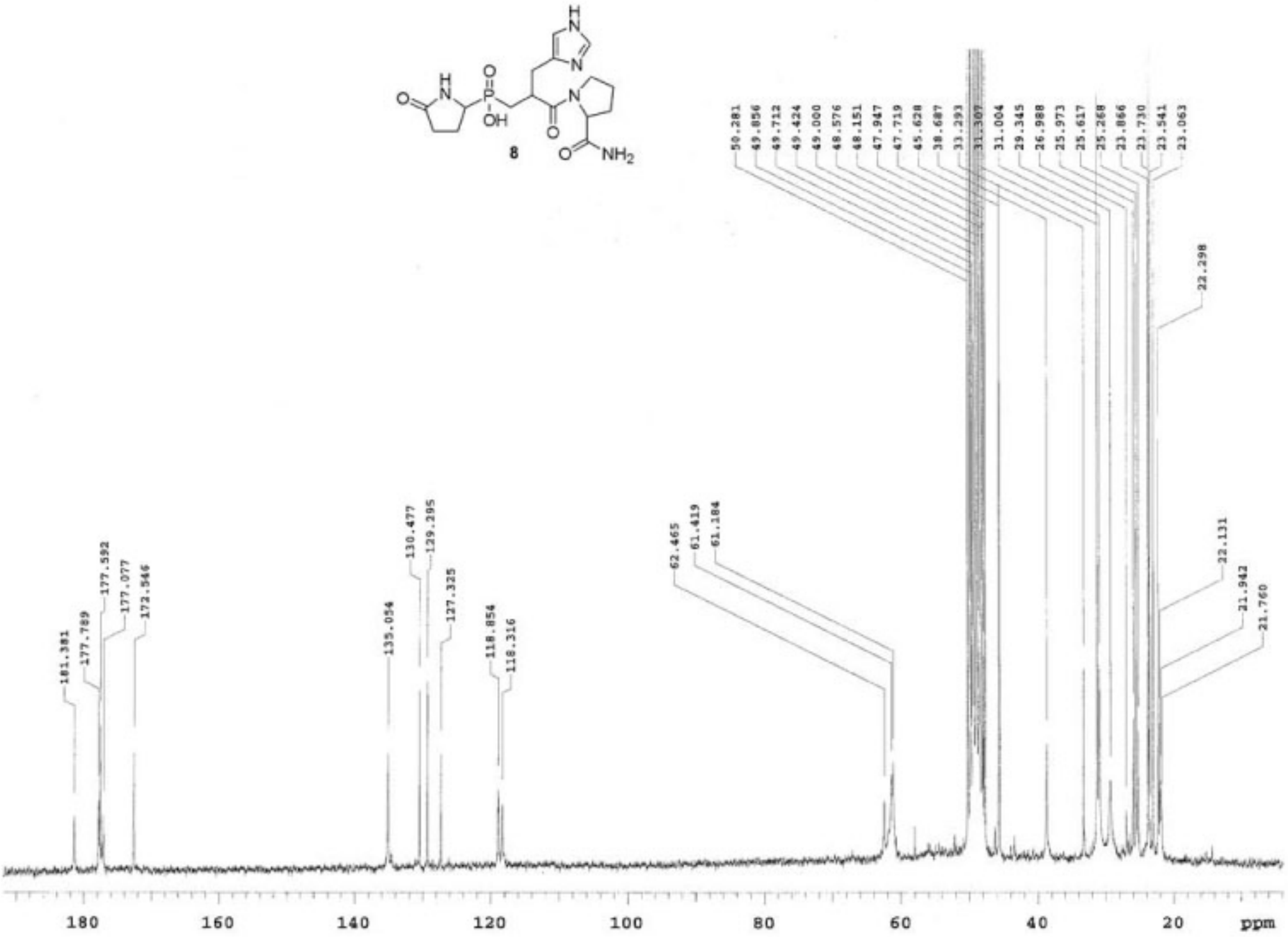
8
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177.789
177.592
177.077
172.546
135.054
130.477
129.295
127.325
118.854
118.316
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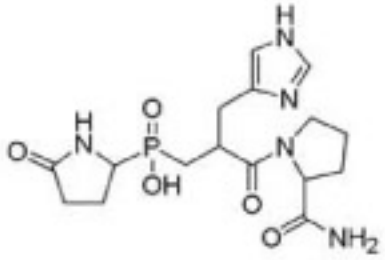
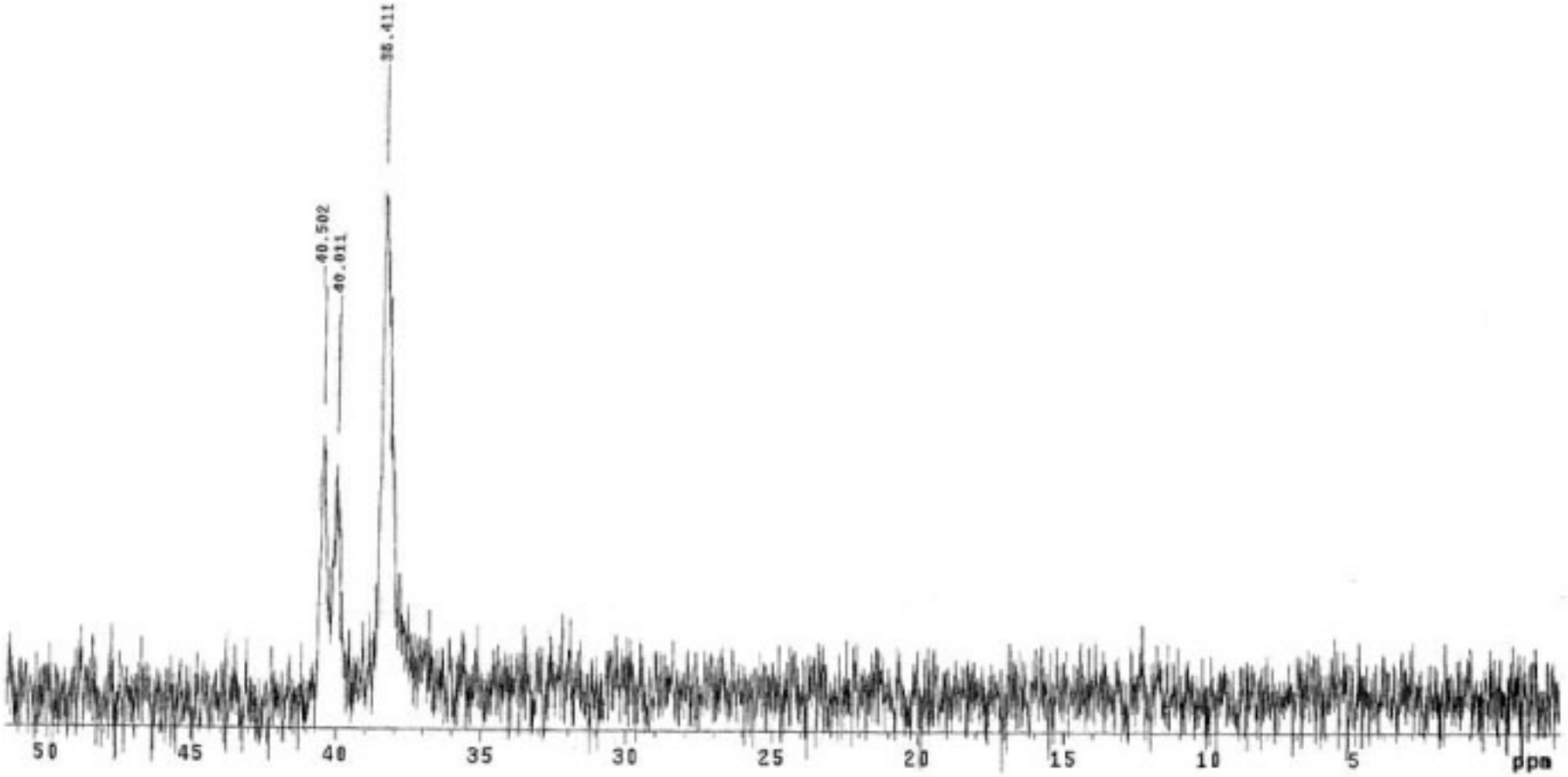
61.419
61.184
50.281
49.856
49.712
49.424
49.000
48.576
48.151
47.947
47.719
45.628
38.687
33.293
31.307
31.004
29.345
26.988
25.973
25.617
25.268
23.866
23.730
23.541
23.063
22.298
22.131
21.942
21.760
180
160
140
120
100
80
60
40
20
ppm

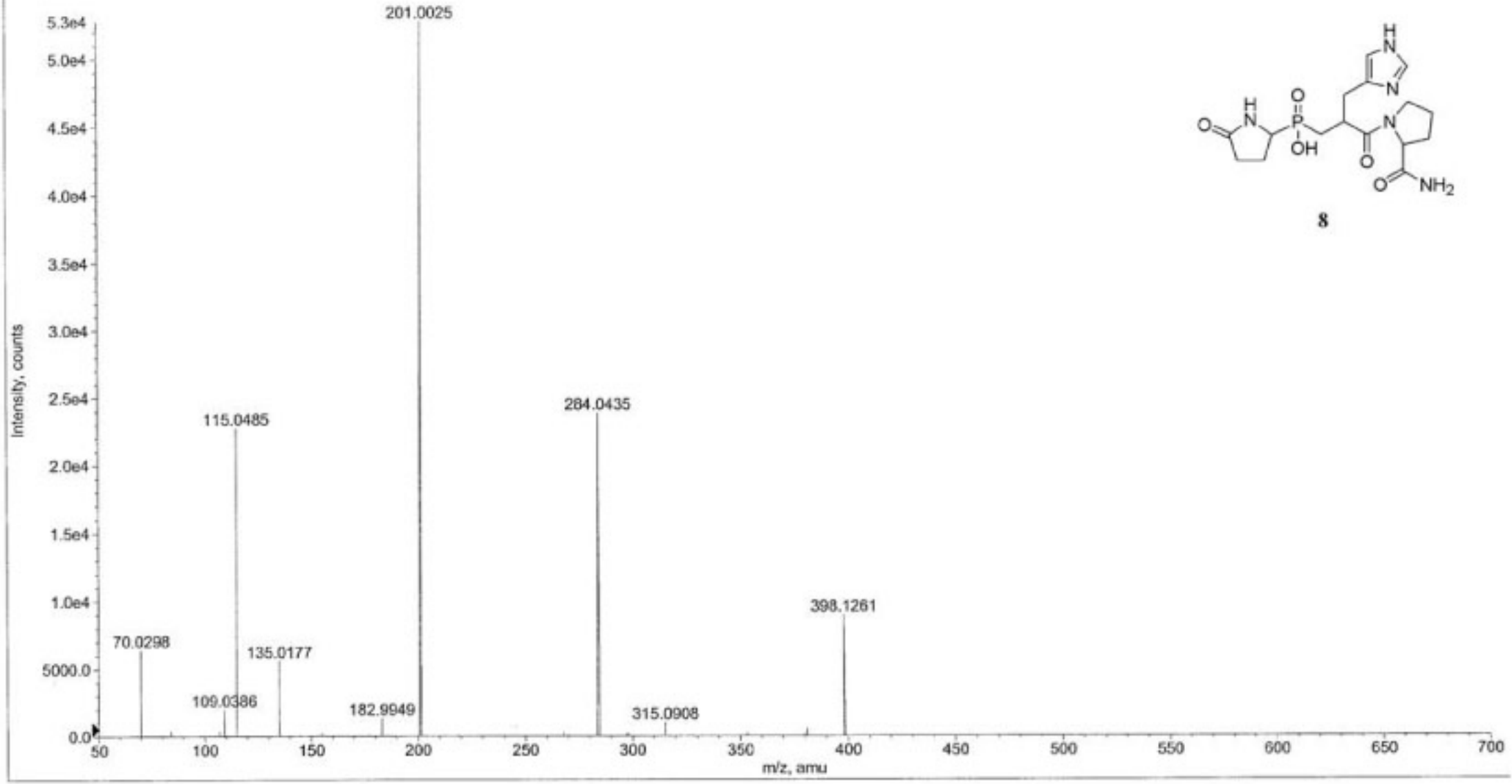


8



+TOF Product (398.0): 51 MCA scans from Sample 2 (398) of 08FEB08_MMX135.wiff
a=3.57067227063213480e-004, t0=2.54448378721826880e+001

Max. 5.3e4 counts.



8


VWD1 A, Wavelength=220 nm (MARGIE\MM001057.D)
mAU
2000
1500
1000
500
0
0
5
10
15
20
25
min

8