SUPPORTING INFORMATION
FOR THE PAPER ENTITLED

Synthesis of New Sulfenic Acid-Reactive Compounds Based on 1, 3-Cyclohexadione

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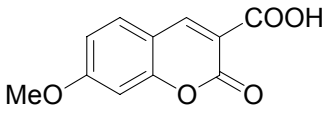
Supporting information

Chemistry

General

Glassware was oven-dried before use and cooled to room temperature under N_2. All reagents and solvents were obtained from a commercial source and used without further purification unless noted otherwise. Analytical thin layer chromatography (TLC) was performed on 250 µm silica gel 60 plates (DC-Fertigplatten Krieselgel 60 F254). Visualization was accomplished with UV light and ethanolic phosphomolybdic acid solution followed by heating. Purification of the reaction products was carried out by flash column chromatography using silica gel 60 (32-63 µm). NMR were recorded on a Bruker DPX 300 spectrometer, operating at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR) respectively, with chemical shifts referenced to the residual solvent peak. ^{1}H NMR data are reported as follows: chemical shift, integration, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet) and coupling constants (Hz). Melting points were determined on a Thomas Hoover uni-melt capillary melting point apparatus and are uncorrected.

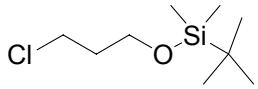
7-methoxy-3-carboxycoumarin



This was synthesized essentially as previously described for 2-methyl-7-methoxy-3-carboxycoumarin:[1] A mixture of 2-hydroxy-4-methoxybenzaldehyde (0.5 g, 3.29 mmol), Meldrum's acid (0.47 g, 3.29 mmol) and piperidine (3 µL, 32.9 µmol) was stirred at rt for 20 min and then at reflux for 2 h. Upon cooling the reaction mixture was filtered and washed with MeOH (20 mL), followed by DCM (30 mL), and the resultant solid recrystallized to purity (from EtOH) to yield an off-white solid (0.52 g, 71.8%). Mp. 197-199°C (lit. 198-199°C)[2]; ^{1}H NMR ($CDCl_3$) δ 12.19 (1H, bs), 8.86 (1H, s), 7.65 (1H, d, *J* = 8.7 Hz), 7.02 (1H, dd, *J* = 8.7 Hz, 2.4 Hz), 6.93 (1H, d, *J* = 2.4 Hz), 3.96 (3H, s); ^{13}C NMR (DMSO-d_6) δ 164.6, 164.1, 157.2, 156.9, 149.1, 131.5, 113.8, 113.3, 111.6, 100.3, 56.2.

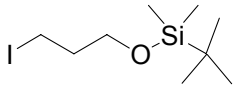
[1] Song, A; Wang, X; Lam, KS. Tet. Letts. (2003) 44:1755-1758.

3-Chloro-1-*tert*-butyldimethylsiloxypropane



This was synthesized as previously described for 3-bromo-1-*tert*-butyldimethylsiloxypropane:[3] To a solution of 3-chloro-propan-1-ol (3.54 mL, 42.3 mmol) in DCM (100 mL) was added imidazole (4.32 g, 63.5 mmol). After 5 min of stirring the solution was cooled to 0^{o}C and *tert*-butyldimethylsilyl chloride (9.57 g, 63.5 mmol) in DCM (60 mL) added dropwise over 15 min. The reaction was allowed to rise to rt and stirred for an additional 16 h before being diluted with diethyl ether (100 mL) and washed with sat. NH_4Cl (50 mL). The aqueous phase was further washed with diethyl ether (3 x 50 mL), the organic phases combined and washed with brine (100 mL), dried over anhydrous $MgSO_4$ and reduced to dryness. The resultant syrup was purified by flash column chromatography with gradient elution (hexanes to hexanes/EtOAc 9/1) to yield a clear liquid (8.64 g, 97.8%). *Rf* 0.19 (hexanes); ^{1}H NMR ($CDCl_3$) δ 3.75 (2H, t, *J* = 5.7 Hz), 3.65 (2H, t, *J* = 6.3 Hz), 1.95 (2H, pentet, *J* = 6.3 Hz), 0.90 (9H, s), 0.06 (6H, s); ^{13}C NMR ($CDCl_3$) δ 59.6, 41.9, 35.6, 26.1, 18.5, -5.3.

3-Iodo-1-*tert*-butyldimethylsiloxypropane



A solution of 3-chloro-1-*tert*-butyldimethylsiloxypropane (4.5 g, 21.6 mmol) and NaI (29 g, 0.19 mol) in acetone (120 mL) was refluxed for 16 h. Upon cooling, the reaction mixture was diluted with diethyl ether (150 mL), washed with 10% $Na_2S_2O_7$ solution (100 mL) and brine (100 mL), dried over anhydrous $MgSO_4$ and reduced to dryness. The resultant syrup was purified by flash column chromatography (hexanes/DCM 9/1) to yield a clear liquid (5.64 g, 87.1%). *Rf* 0.10 (hexanes); ^{1}H NMR ($CDCl_3$) δ 3.67 (2H, t, *J* = 5.7 Hz), 3.28 (2H, t, *J* = 6.6 Hz), 2.03-1.95 (2H, m), 0.90 (9H, s), 0.06 (6H, s); ^{13}C NMR ($CDCl_3$) δ 62.5, 36.3, 26.1, 18.5, 3.8, -5.2.

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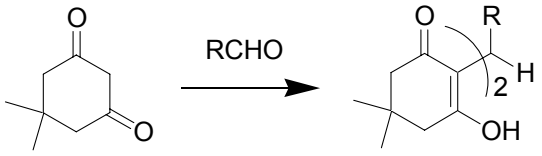
[2] Shirokova, EA. Bioorganicheskaya Kimiya (1998) 14;236-242.
[3] Yotsu-Yamashita, M; Yasumoto, T; Rawal, VH. Heterocycles (1998) 48(1):79-93.

Dimedone reactivity towards aldehydes

The reactivity of dimedone with aldehydes has long been known, and the crystalline dimethone adducts formed have been utilized as a means of identification and characterization of aldehydes.[4] In order to determine whether our fluorescent derivatives of 1,3-cyclohexadione could possibly form similar 2-alkylidene 1,3-diones, we investigated the conditions under which 1,3-diones (dimedone) would react with aldehydes. The ability of dimedone to react with aldehydes in DCM under mildly acidic conditions (silica gel) has previously been reported.[5] Thus our investigations concentrated on the use of neutral or basic conditions and are summarized below.

Dimedone did not appreciably react with acetaldehyde or butyraldehyde in aqueous conditions or in the absence of base. Dimedone reacted with benzaldehyde irrespective of whether or not a base was present in organic solvent, but formation of the dimethone adduct was decreased in aqueous conditions.



dimethone

To a solution of dimedone (280.4 mg, 2 mmol) in DCM (10 mL) or 50% EtOH solution (10 mL) was added the aldehyde (1 mmol) and the mixture stirred under various conditions for 3 h. The solvent was removed under reduced pressure and the resultant dimethone derivatives (if formed) were isolated as white solids from column chromatography (hexanes/EtOAc 3/2), and recrystallized from dilute EtOH to yield analytically pure samples.

[4] Vogel's textbook of practical organic chemistry. Fifth edition. 2005. Publ. Pearson education. Ed. Furniss, BS; Hannaford, AJ; Smith, PWG; Tatchell, AR.
[5] Fuchs, K; Paquette, LA. J. Org. Chem. (1994);59:528-532.

RCHO	Conditions	Piperidine	Yield of dimethone
CH_3CHO	DCM, r.t	None	0
″	DCM, reflux	None	0
″	DCM, reflux	1 mmol	2.6%
″	50% EtOH, r.t	None	0
$CH_3CH_2CH_2CHO$	DCM, r.t	None	1.0%
″	DCM, reflux	None	2.1%
″	DCM, reflux	1 mmol	93.1%
″	50% EtOH, r.t	None	6.6%
PhCHO	DCM, reflux	None	48.8%
″	DCM, reflux	1 mmol	58.3%
″	50% EtOH, r.t	None	28.7%

2,2'-Phenylmethylenebis(3-hydroxy-5,5'-dimethyl-2-cyclohexen-1-one)

Rf 0.61(hexanes/EtOAc 3/2); mp. 194-195°C (lit. 195 °C)[4]; ^{1}H NMR ($CDCl_3$) δ 11.83 (1H, bs), 11.55 (1H, bs), 7.19 (2H, 2d, *J* = 8.6 Hz), 7.10 (1H, t, *J* = 6.4 Hz), 7.02 (2H, d, *J* = 8.0 Hz), 5.47 (1H, s), 2.30 (8H, 4s), 1.16 (6H, s), 1.03 (6H, s); ^{13}C NMR ($CDCl_3$) δ 190.5, 189.5, 138.2, 128.3, 126.9, 125.9, 115.7, 47.1, 46.5, 32.8, 31.5, 29.7, 27.5.

2,2'-Propylmethylenebis(3-hydroxy-5,5'-dimethyl-2-cyclohexen-1-one)

Rf 0.60 (hexanes/EtOAc 3/2); mp. 133-134°C (lit. 142°C)[4]; ^{1}H NMR ($CDCl_3$) δ 12.48 (1H, bs), 11.54 (1H, bs), 3.90 (1H, t, *J* = 8.1 Hz), 2.28 (4H, s), 2.27 (4H, s), 1.97 (2H, q, *J* = 7.8 Hz), 1.25 (1H, t, *J* = 7.8Hz), 1.19 (1H, t, *J* = 7.2 Hz), 1.06 (6H, s), 1.05 (6H, s), 0.87 (3H, t, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 190.1, 189.8, 116.8, 47.2, 46.4, 31.5, 31.3, 30.1, 29.5, 26.8, 22.3, 14.0.

2,2'-Methylmethylenebis(3-hydroxy-5,5'-dimethyl-2-cyclohexen-1-one)

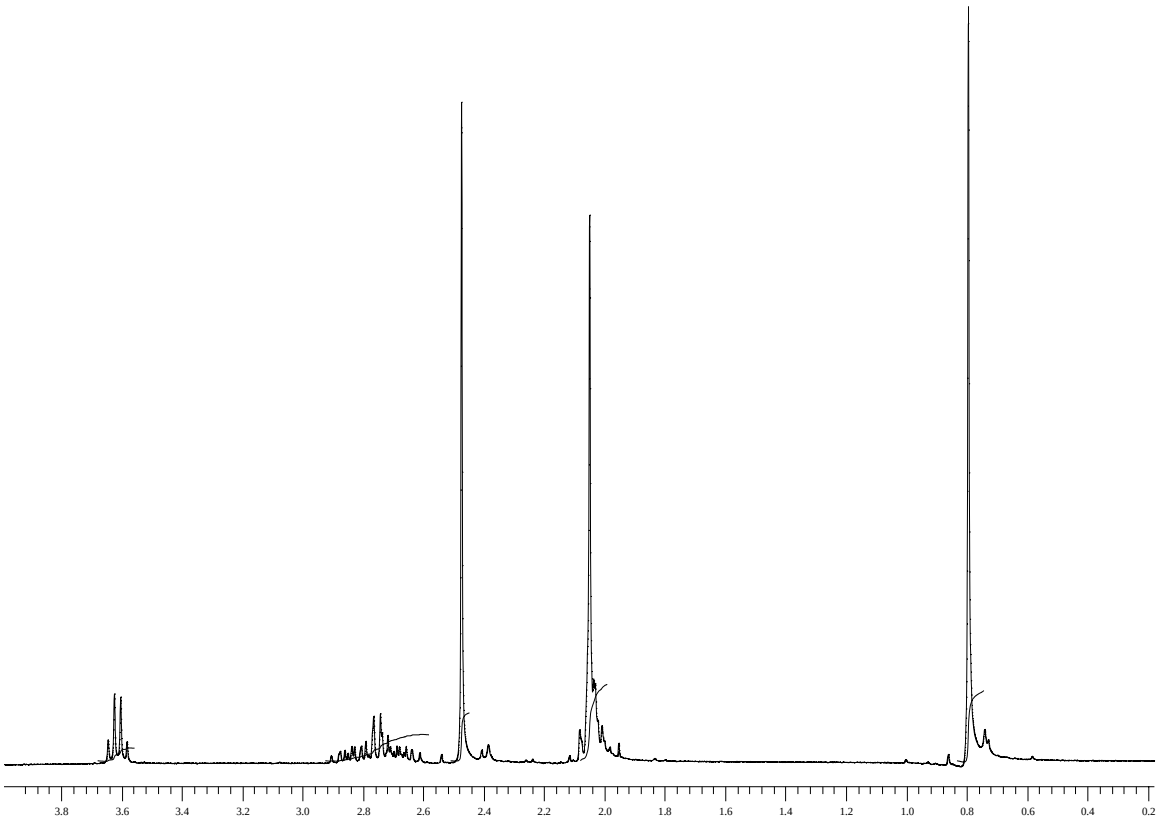
Rf 0.60 (hexanes/EtOAc 3/2); mp. 132-134°C (lit. 141°C)[4]; ^{1}H NMR ($CDCl_3$) δ 12.45 (1H, bs), 11.51 (1H, bs), 4.07(1H, q, *J* = 7.5 Hz), 2.12 (8H, m), 1.42 (3H, d, *J* = 7.5 Hz), 0.98 (12H, s).

Dimedone reactivity towards sulfoxides

The ability of dimedone to react with and trap sulfoxides was investigated, and the results (described below) indicate that dimedone shows little/no reactivity with sulfoxides. Thus, our fluorescent, 1,3-cyclohexadione derivatives should exhibit similar unreactivity towards sulfoxides.

To a saturated solution of dimedone in D_2O (2 mL) was added L-methionine S-oxide (9.0 mg) and the solution stirred at r.t and monitored over time by NMR (aliquots taken and ^1H NMR spectra recorded). The ^1H NMR showed little change over time indicating low/no reactivity of dimedone with L-methionine S-oxide (see example spectra below).

Dimedone (4.0 mg) was solubilised in DMSO-d_6 and the ^1H NMR spectra recorded over various time intervals. The ^1H NMR showed no change over time further confirming low/no reactivity of dimedone towards sulfoxides.



Dimedone reactivity with amines

1,3-Dicarbonyls are known to react with amines to form enamines. Indeed, dimedone has previously been utilized as an alternative N-protecting group for amino acids, forming stable enamines.[6] However, as this reaction was performed in an organic solvent over 24 h and involves the elimination of water, we were interested in whether or not these enamines could form under aqueous conditions. As indicated from our limited investigations, below, these products were not formed under aqueous conditions, suggesting that this potential side-reaction for our fluorescent, 1,3-cyclohexadione derivatives would be negligible.

To a solution of dimedone (280.4 mg, 2 mmol) in DCM (10 mL) or 50% EtOH solution (10 mL) was added the amine (2 mmol) and the mixture stirred at rt for 3 h. The reaction was monitored by TLC and after 3 h the solvent was removed under reduced pressure (lyophilization for aqueous phase) and the resultant products (if formed) were isolated as white solids from column chromatography (gradient elution, 100% EtOAc to EtOAc/MeOH 85/15).

Amine	Conditions	Yield of adduct
$BnNH_2$	DCM, r.t	65.4%
″	50% EtOH, r.t	0
L-methionine S-oxide	50% EtOH, r.t	0

3-Benzylamino-5,5'-dimethyl-2-cyclohexen-1-one

Rf 0.18 (EtOAc); mp. 123-125°C (lit. 124-125°C)[7]; ^{1}H NMR ($CDCl_3$) δ 7.39-7.27(5H, m), 5.58 (1H, bs), 5.14 (1H, s), 4.24 (2H, d, *J* = 5.3 Hz), 2.26 (2H, s), 2.16 (2H, s), 1.08 (6H, s); ^{13}C NMR ($CDCl_3$) δ 197.1, 163.1, 137.0, 128.9, 127.9, 127.6, 96.1, 50.4, 47.2, 43.5, 32.9, 28.4.

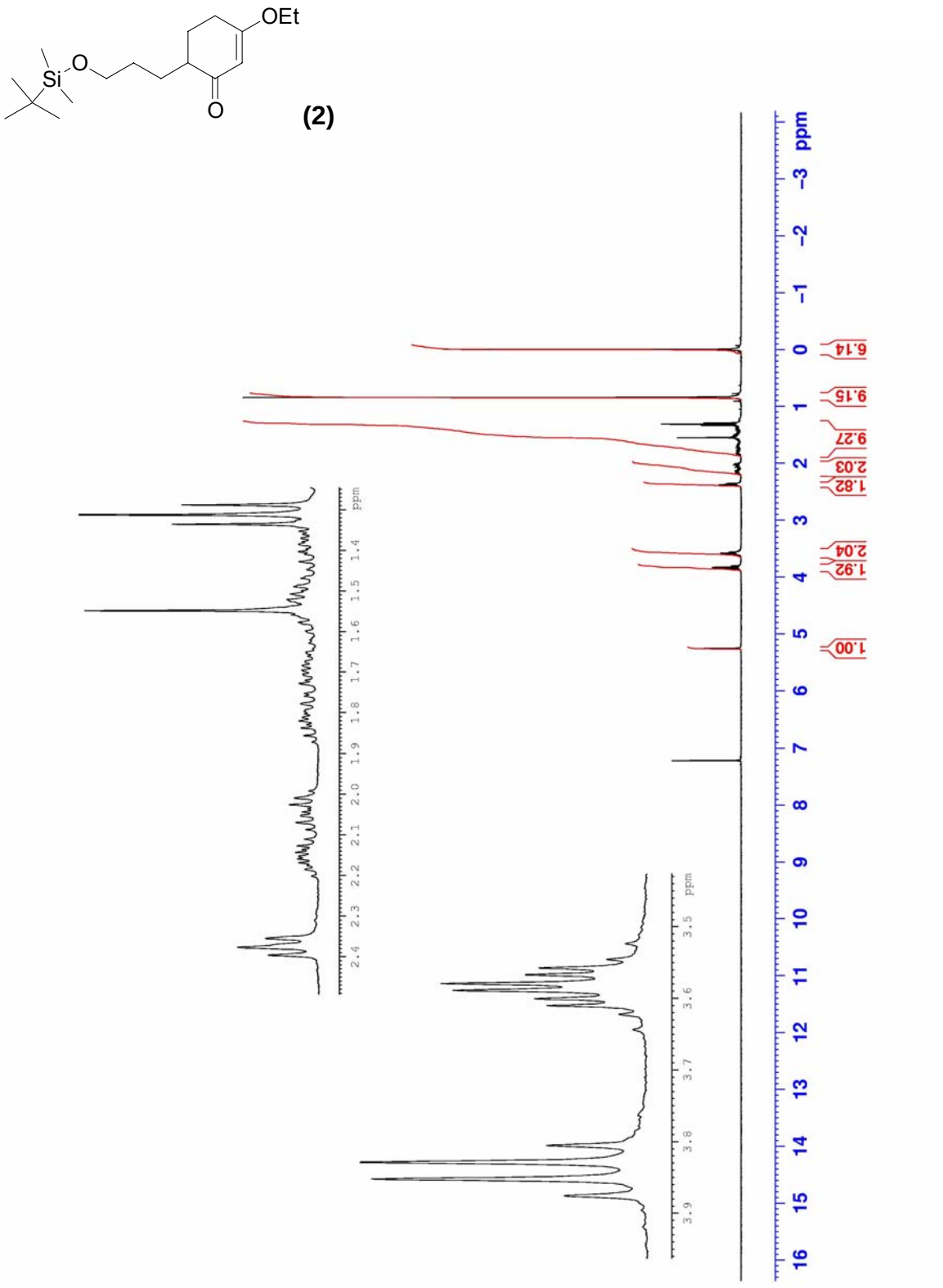
[6] Halpern, B; James, LB. Aust. J. Chem. (1964); 17:1282-1287.
[7] Jirkovsky, I. Can. J. Chem. (1974); 52:55-65.

Dimedone reactivity with S-nitroso thiols

S-Nitroso thiols comprise another group of biologically relevant protein modifications that contain an electrophilic sulfur atom. Control experiments were performed to determine the reaction of dimedone with this functional group.

S-Nitrosothiol Stability Study—S-Nitrosoglutathione was prepared as described.[8] Dimedone (3.5 mg, 0,025 mmol) dissolved in DMSO (100 mL) was added to a solution of S-nitrosoglutathione (7.5 mg, 0.89 mmol, 0.89 mM) in distilled water (25 mL). An aliquot of this mixture was transferred to a UV cuvette and the absorbance at 336 nm was monitored at room temperature for 1 hour.

[8] Hart, T. W. Tetrahedron Lett. (1985); 26:2013-2016.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9.27
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1.82
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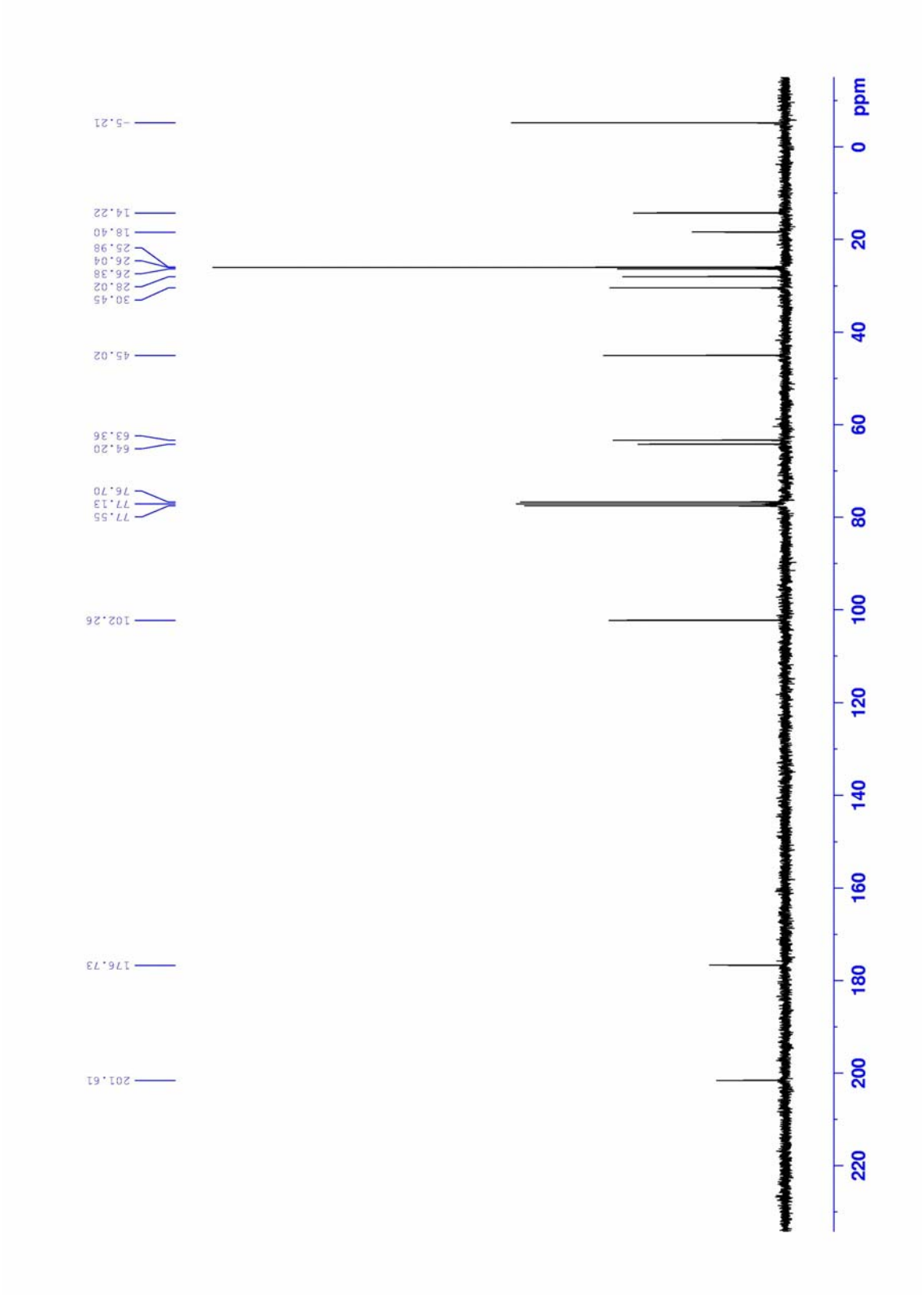
2.04
1.00

(2)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77.13
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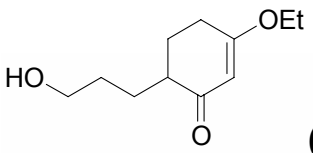
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HO
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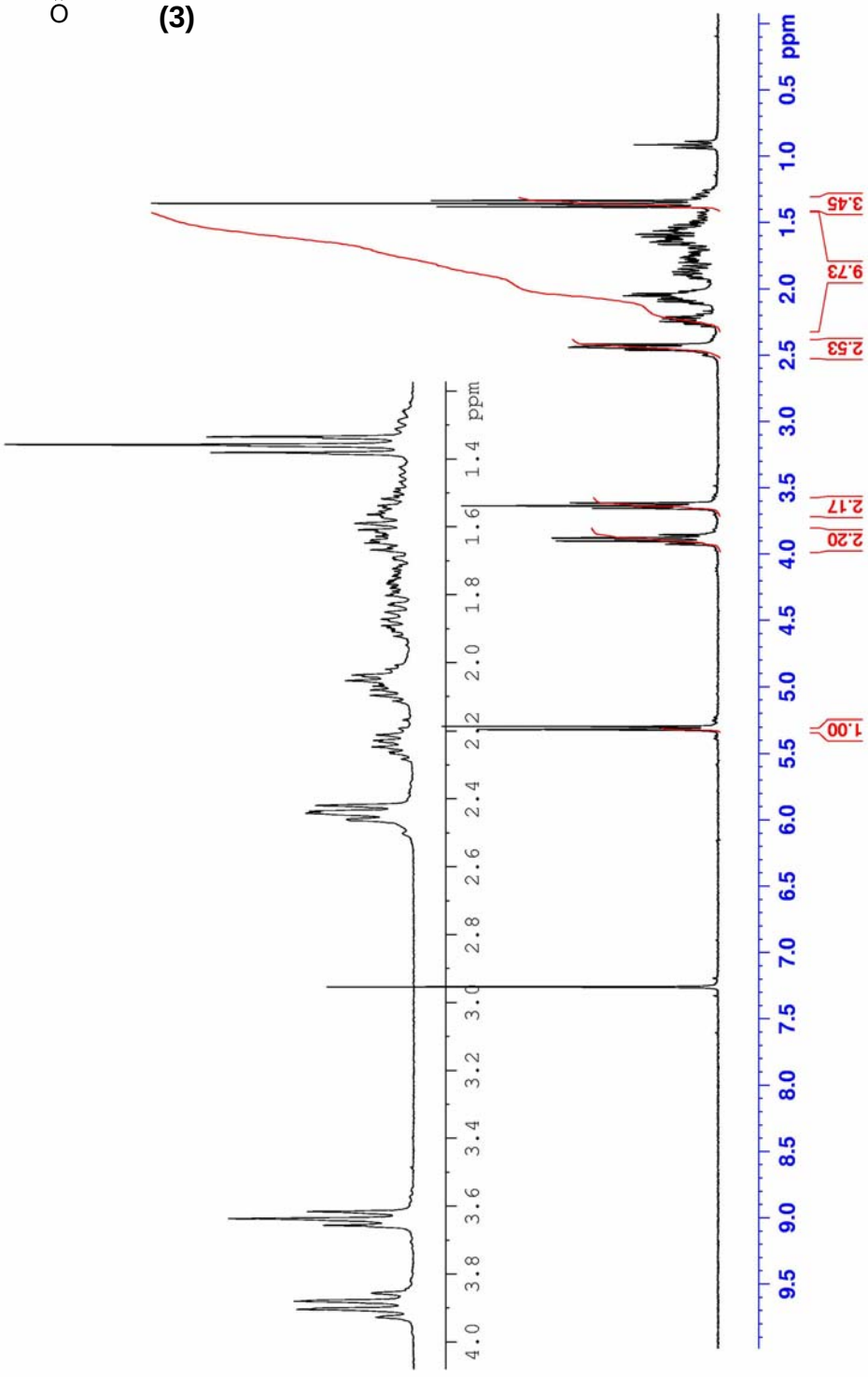
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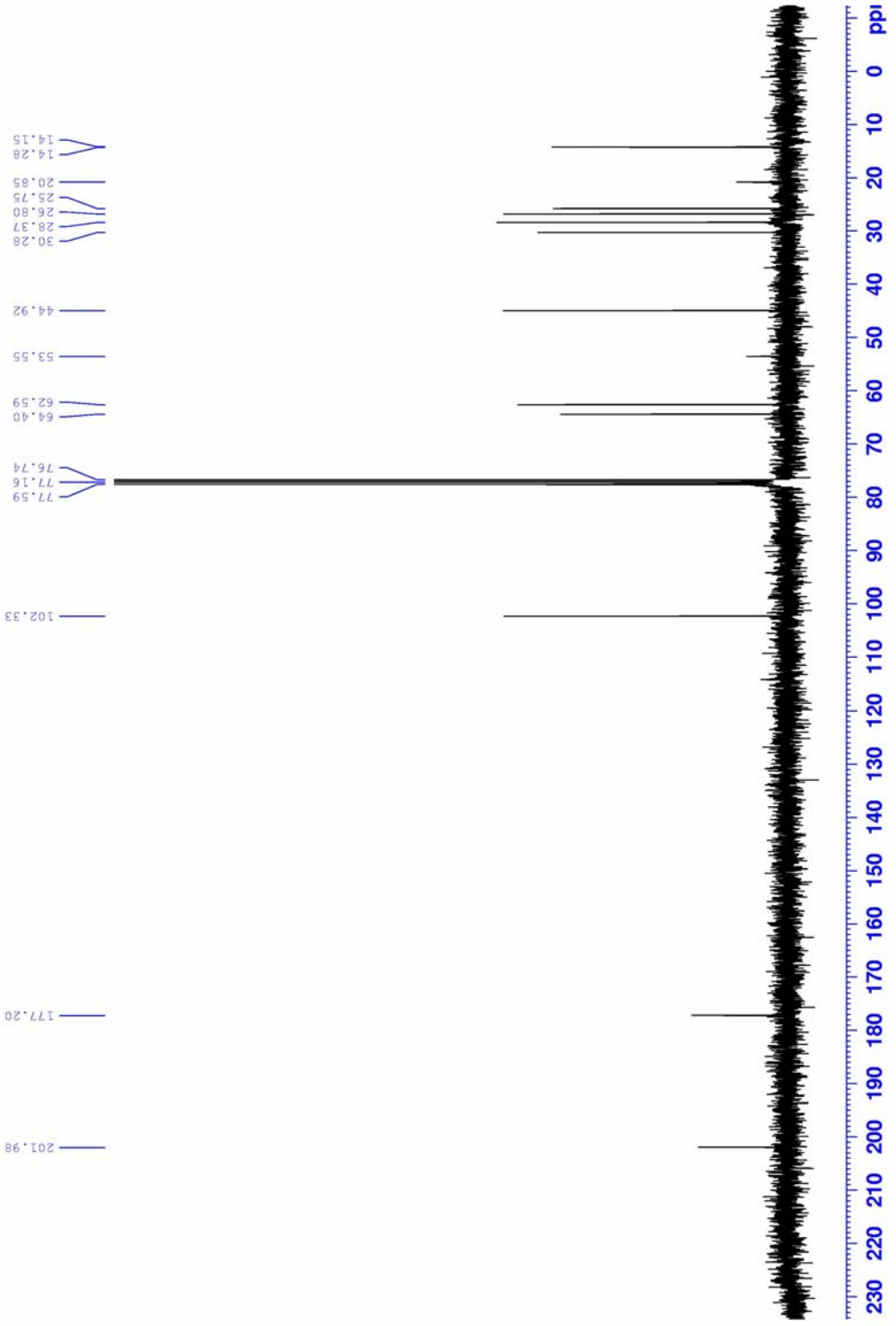
3.6
3.8
4.0

(3)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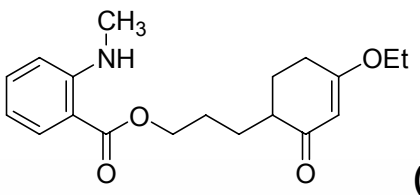
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O
O
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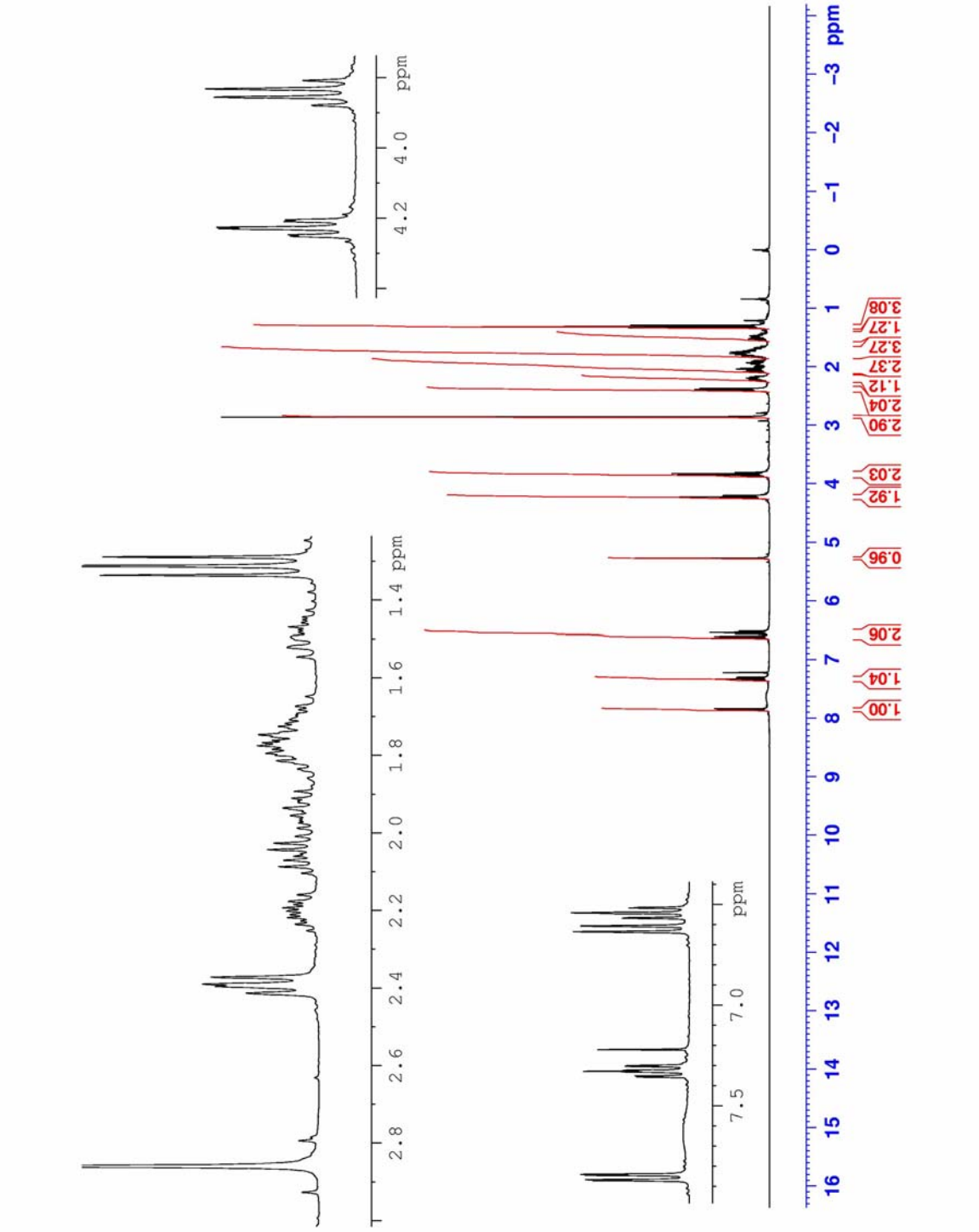
(4)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2.04
1.12
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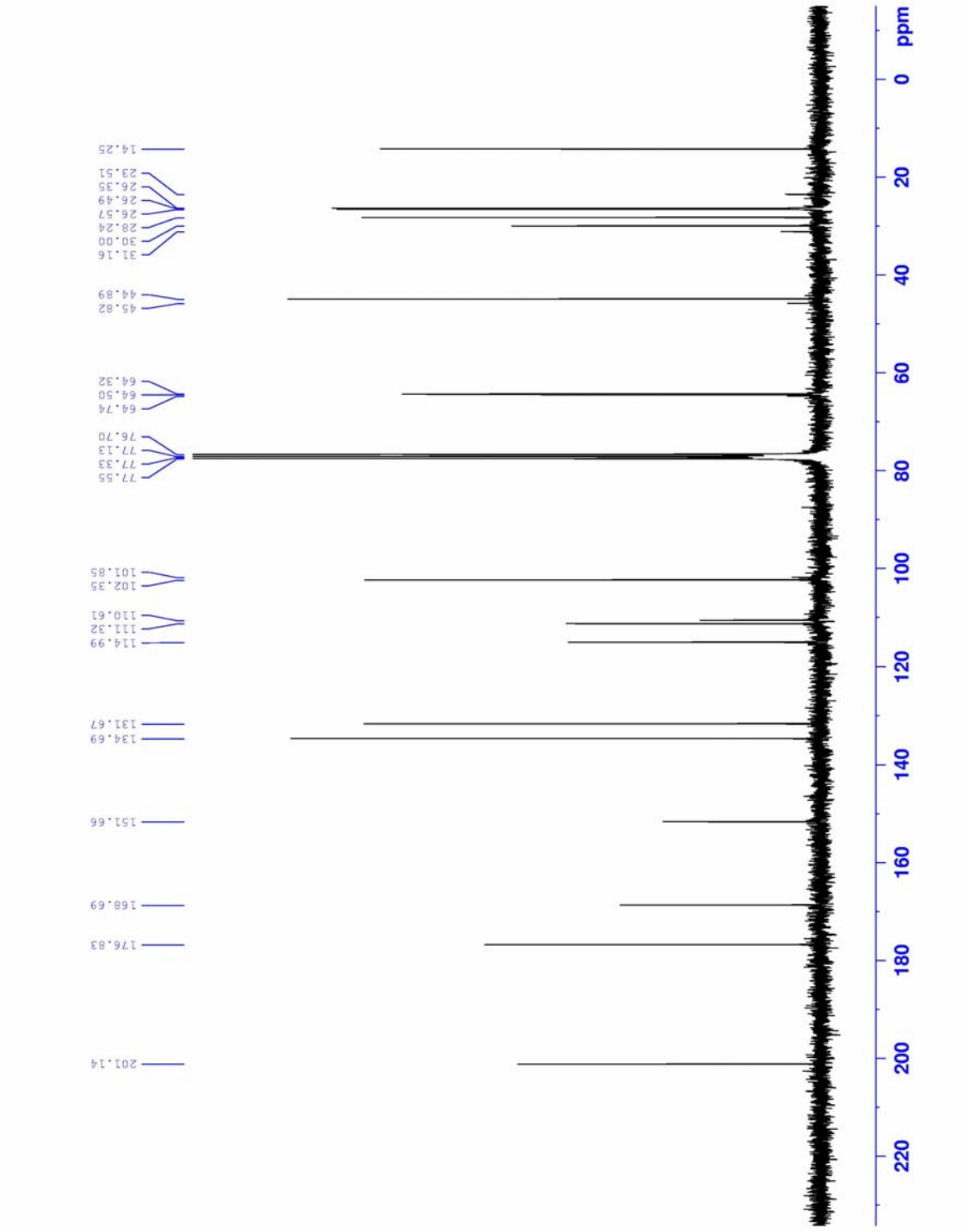
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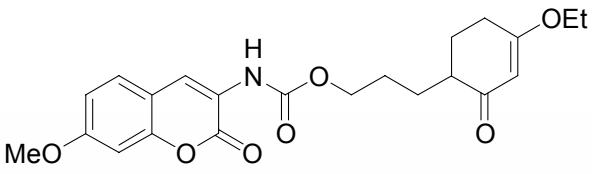

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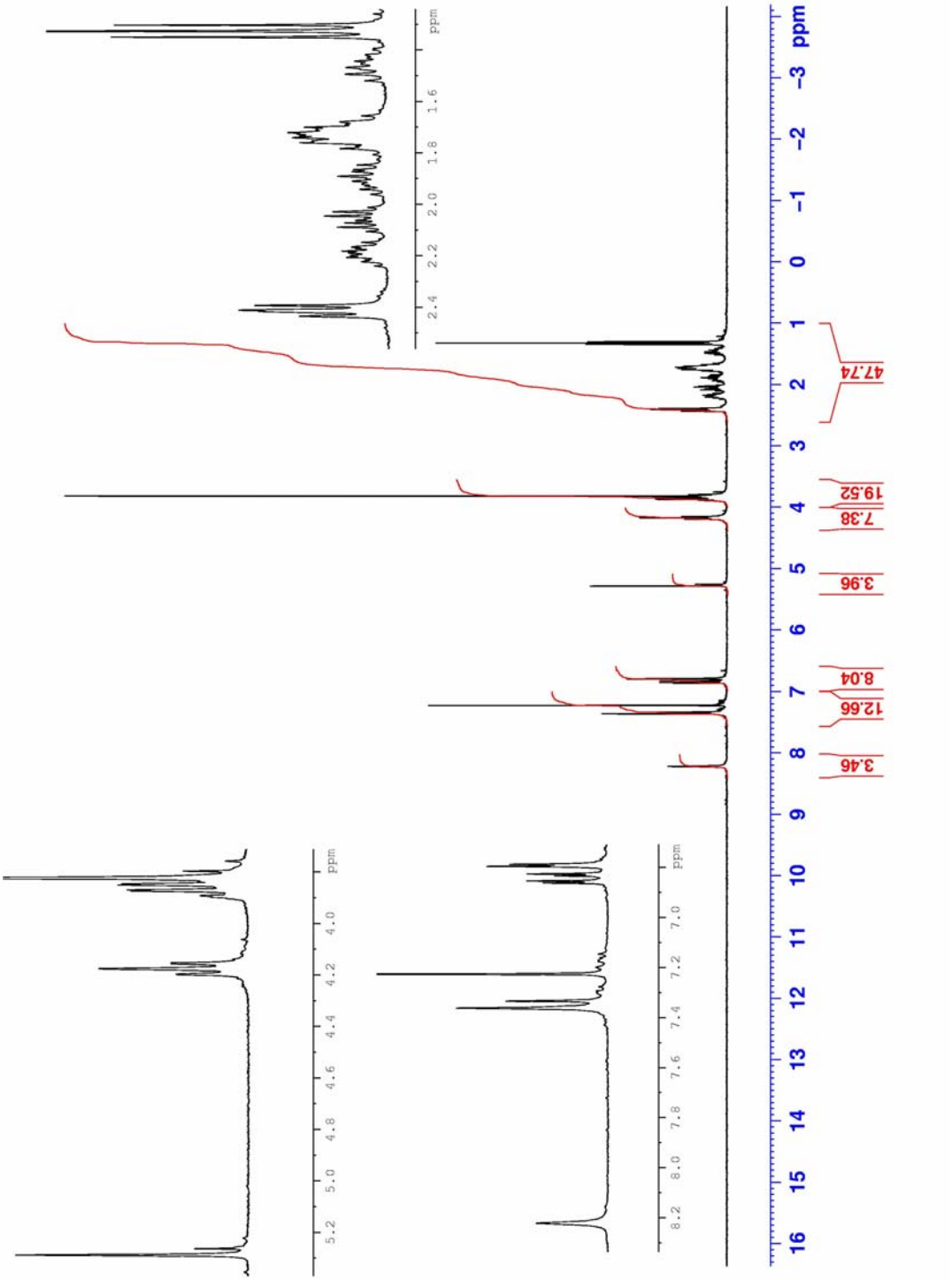

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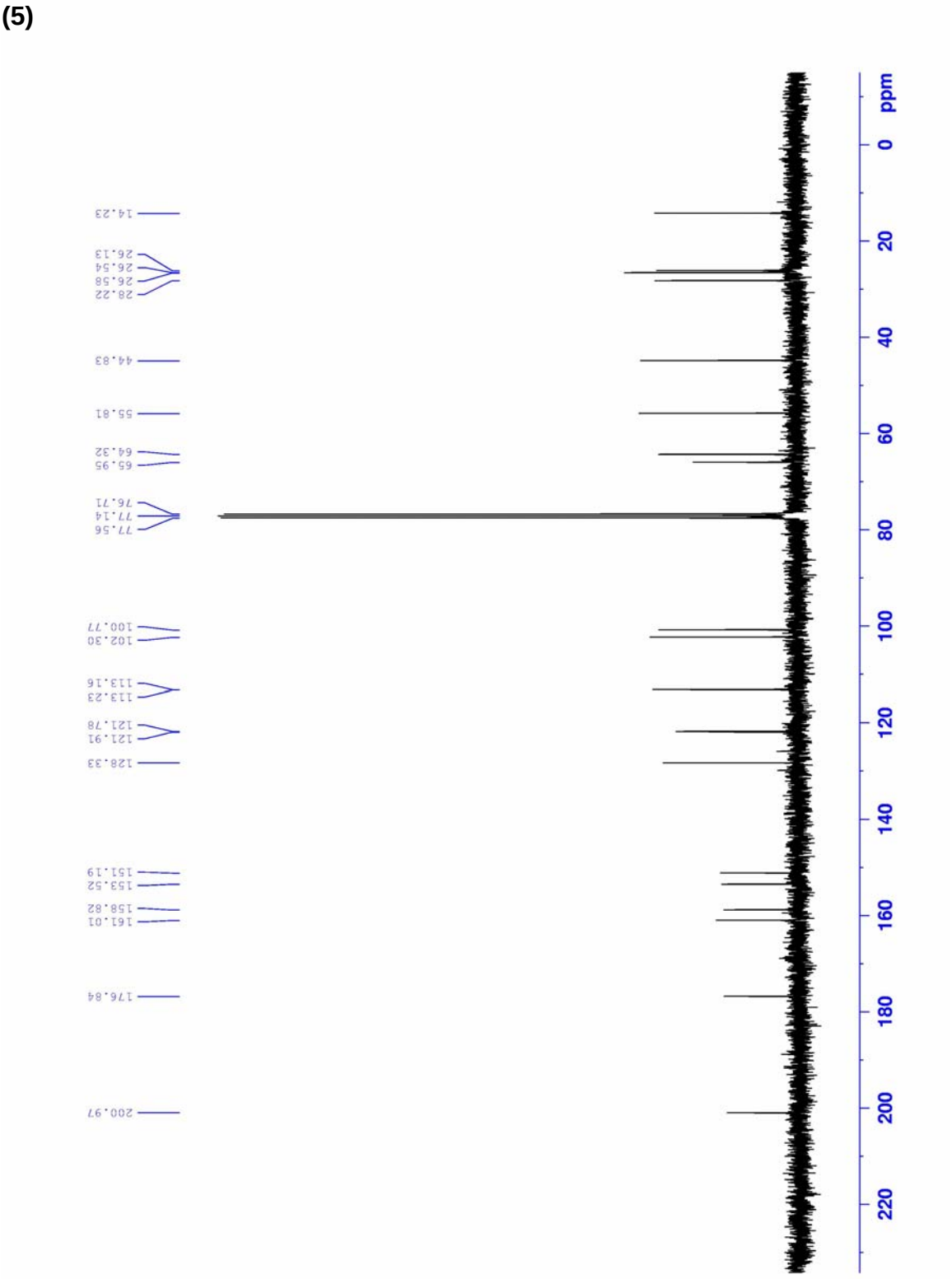
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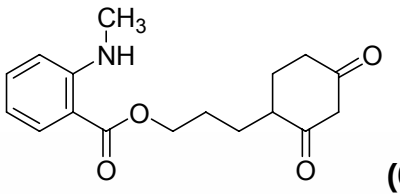
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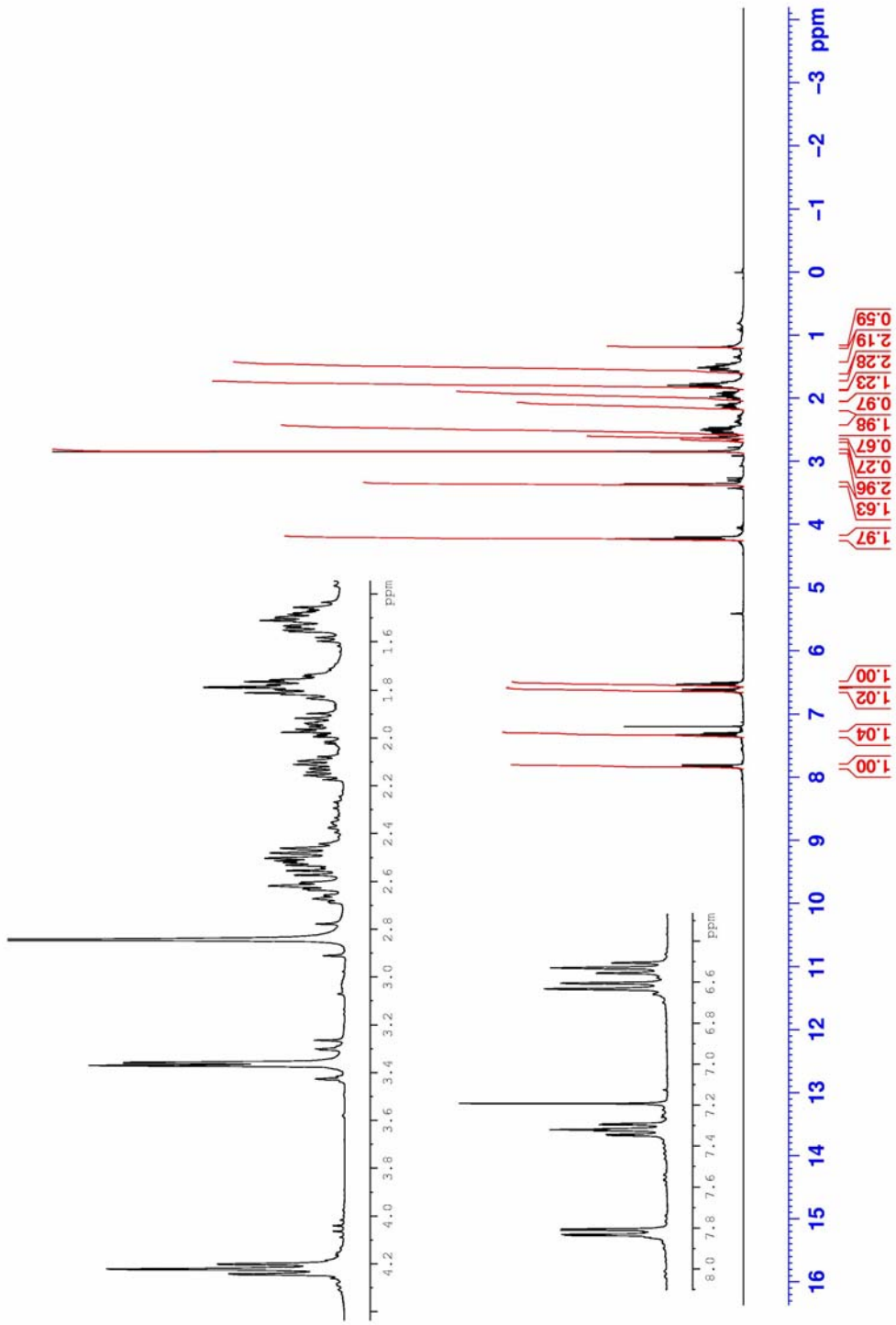
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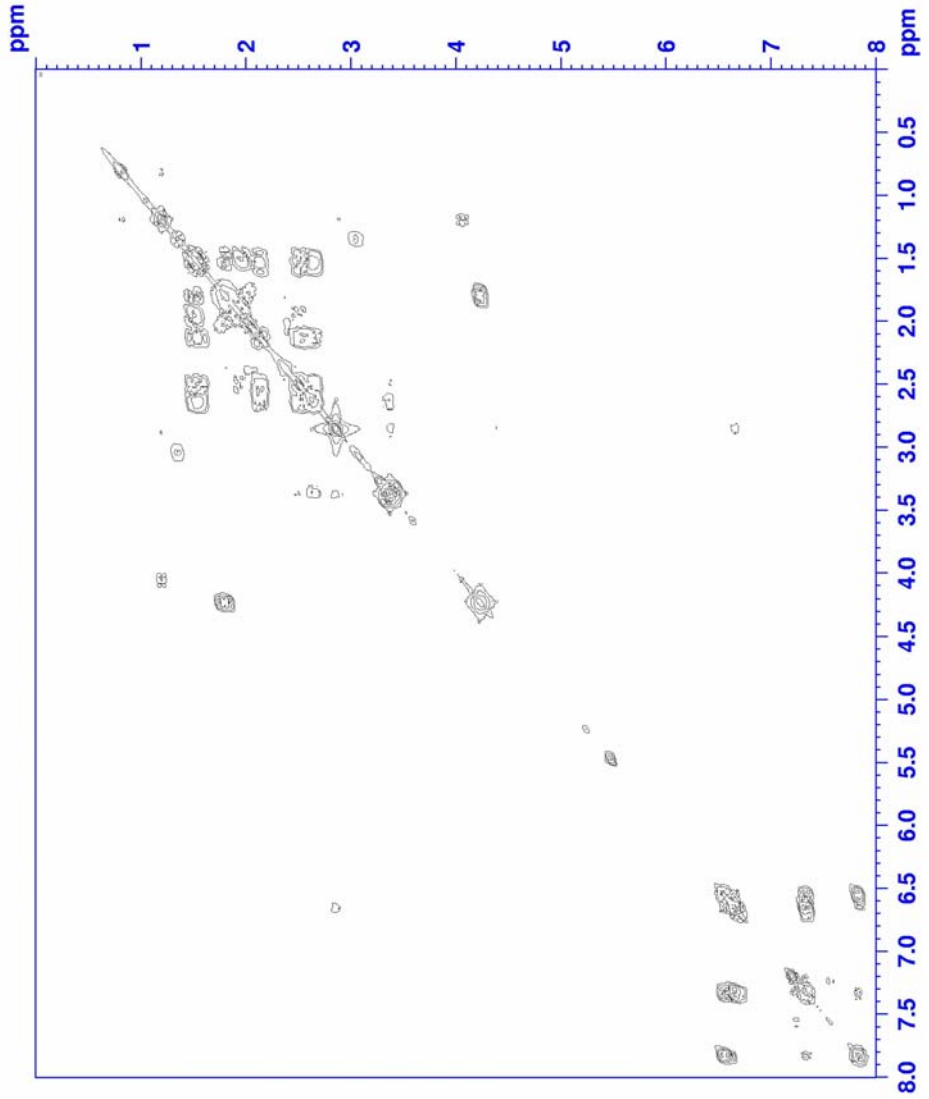

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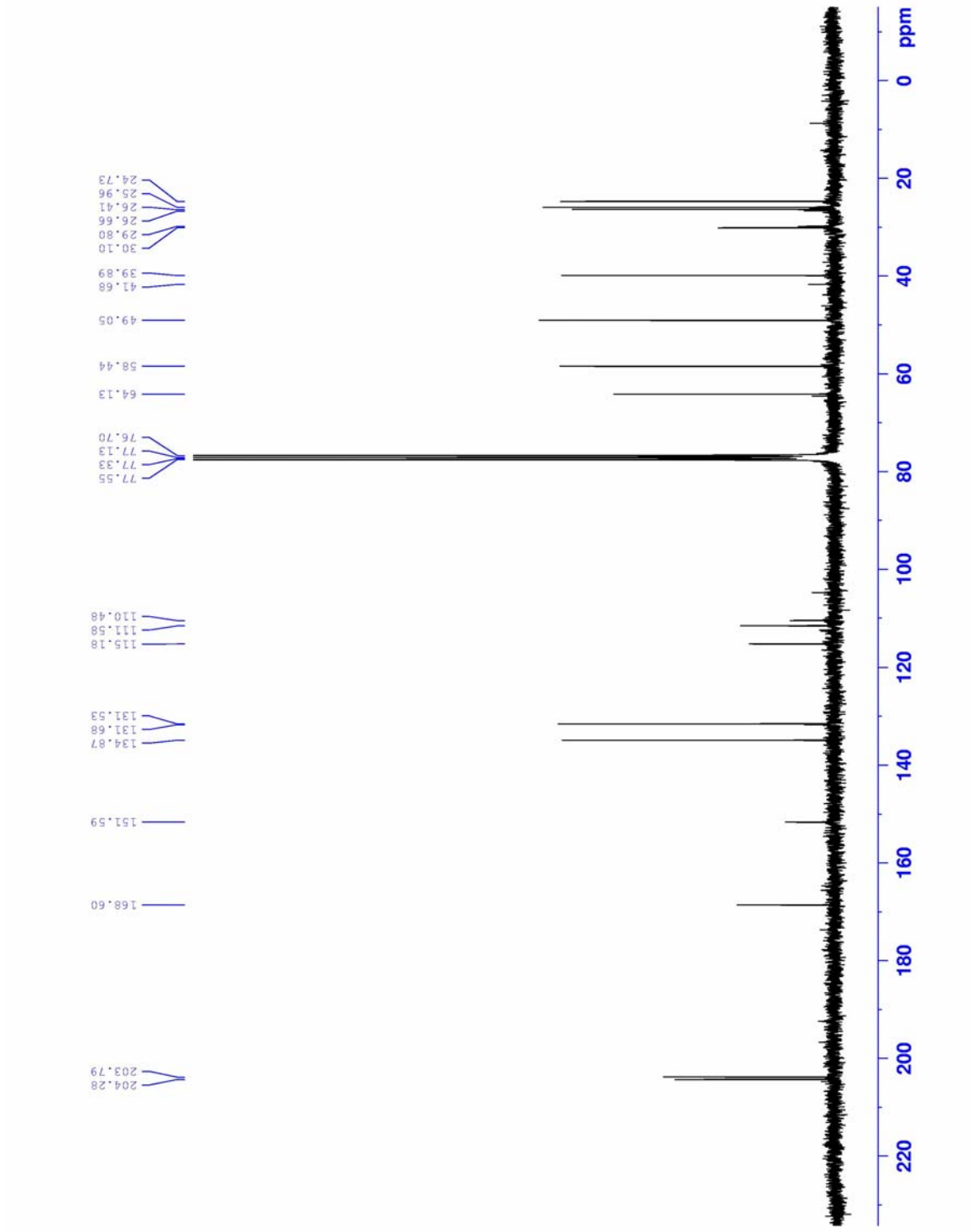
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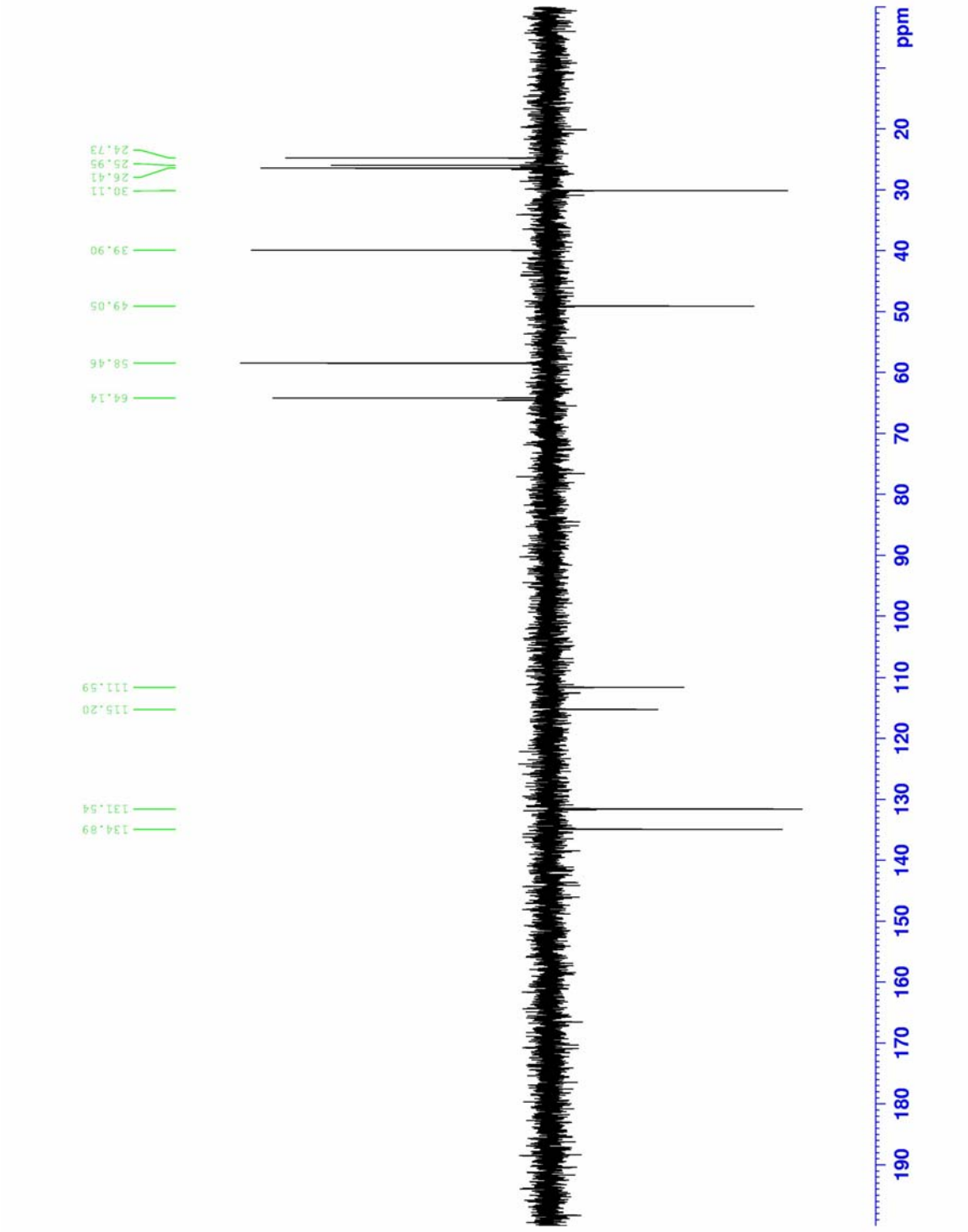
(6)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64.14
111.59
115.20
131.54
134.89
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60
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80
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100
110
120
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180
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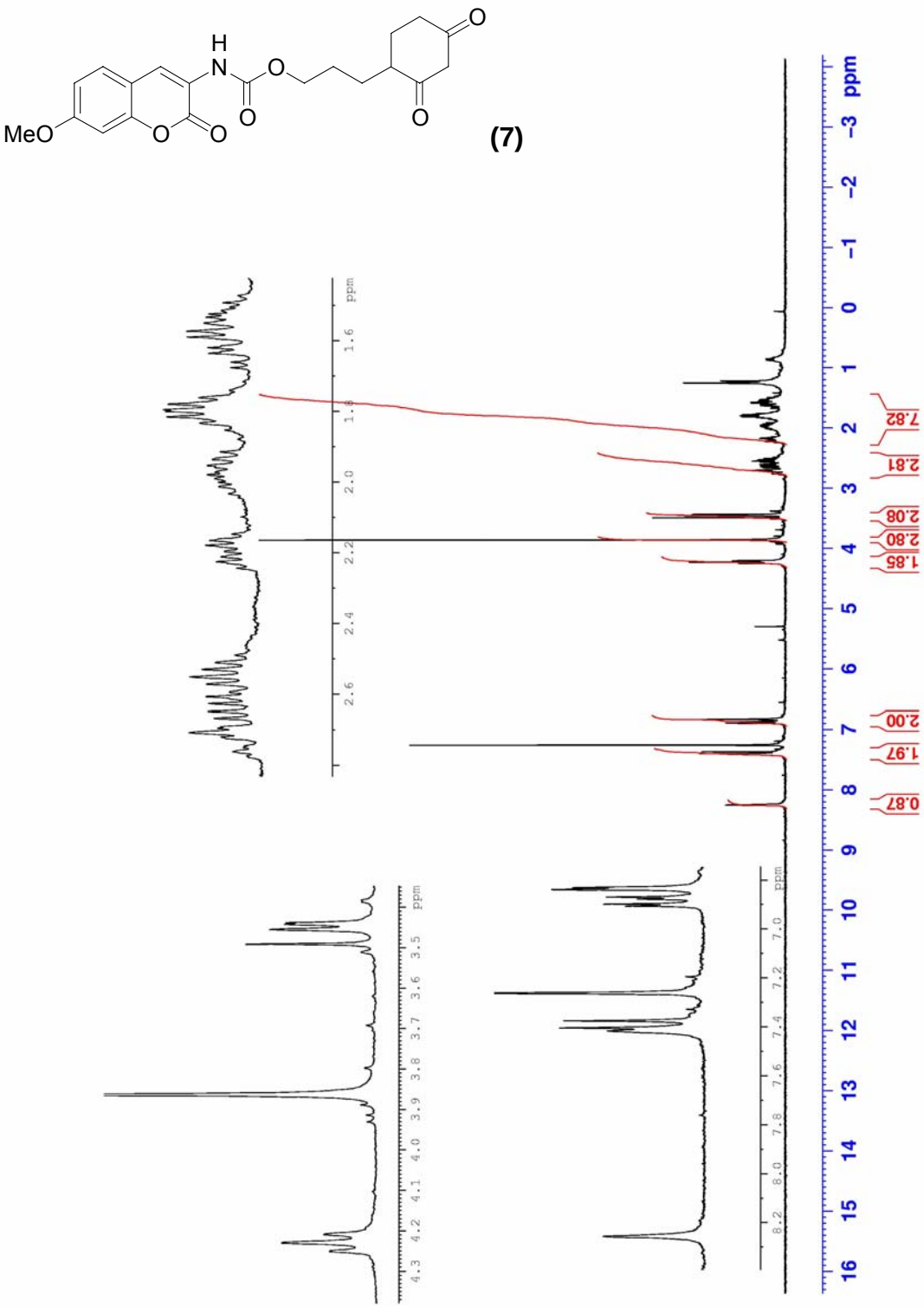
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O
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O
(7)
ppm
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
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−2
−3
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2.00
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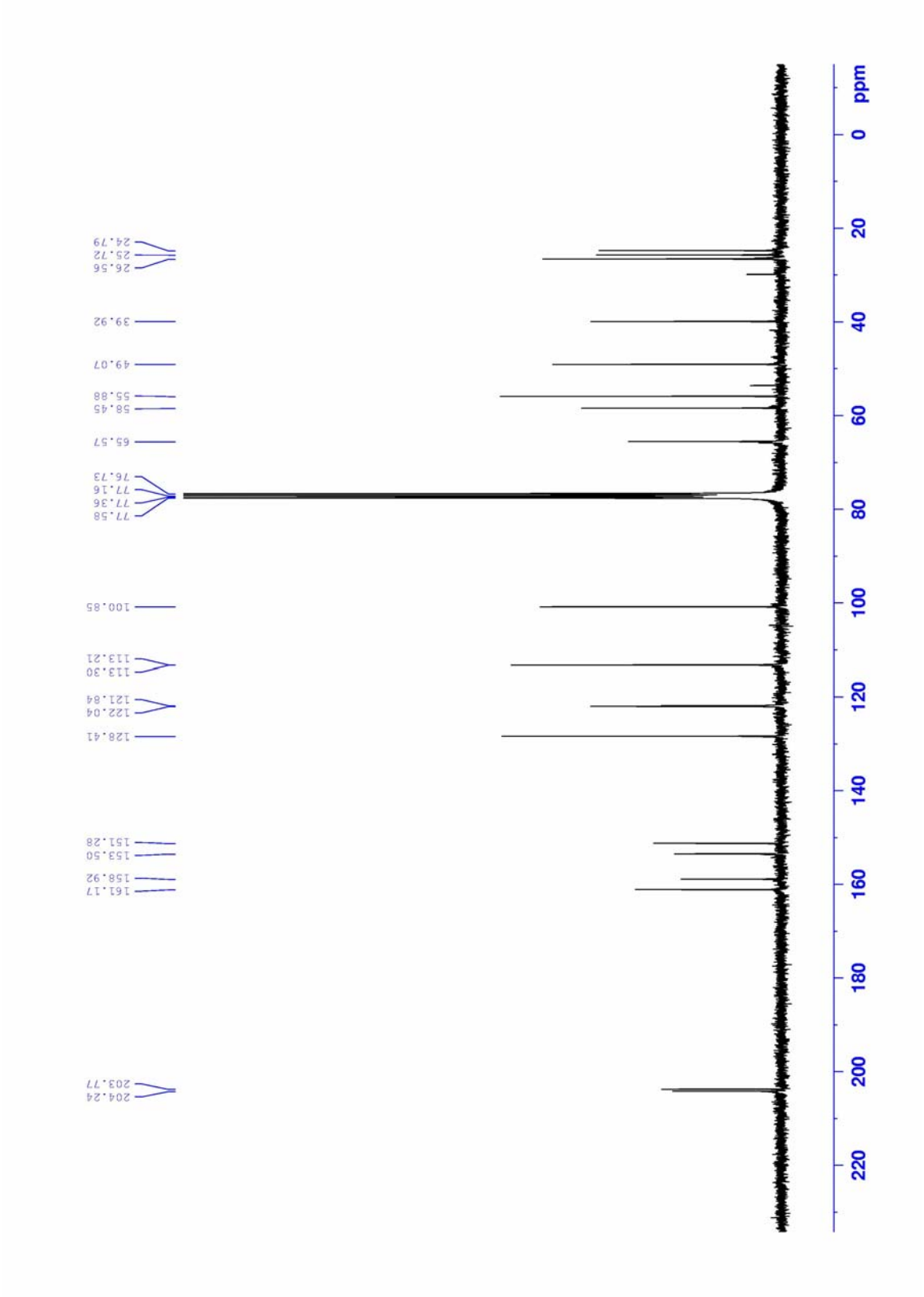
2.80
2.08
2.81
7.82

(7)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65.57
76.73
77.16
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77.58
100.85
113.21
113.30
121.84
122.04
128.41
151.28
153.50
158.92
161.17
203.77
204.24
ppm
0
20
40
60
80
100
120
140
160
180
200
220