Novel Insights on Human Carbonic Anhydrase Inhibitors Based on Coumalic Acid: Design, Synthesis, Molecular Modeling Investigation, and Biological Studies

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* Correspondence: paolo.guglielmi@uniroma1.it (P.G.); claudiu.supuran@unifi.it (C.T.S.);

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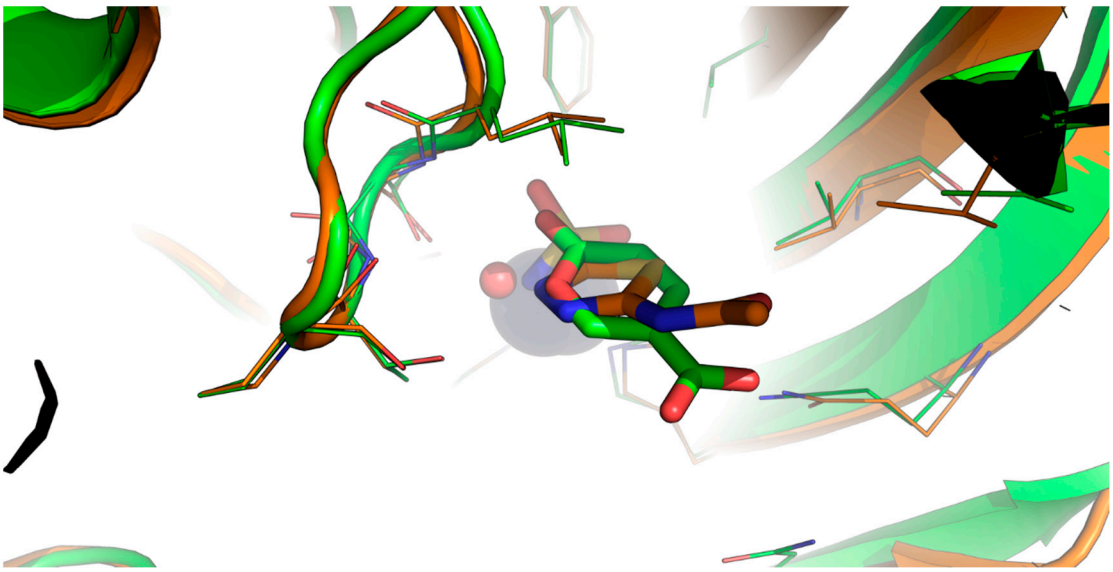
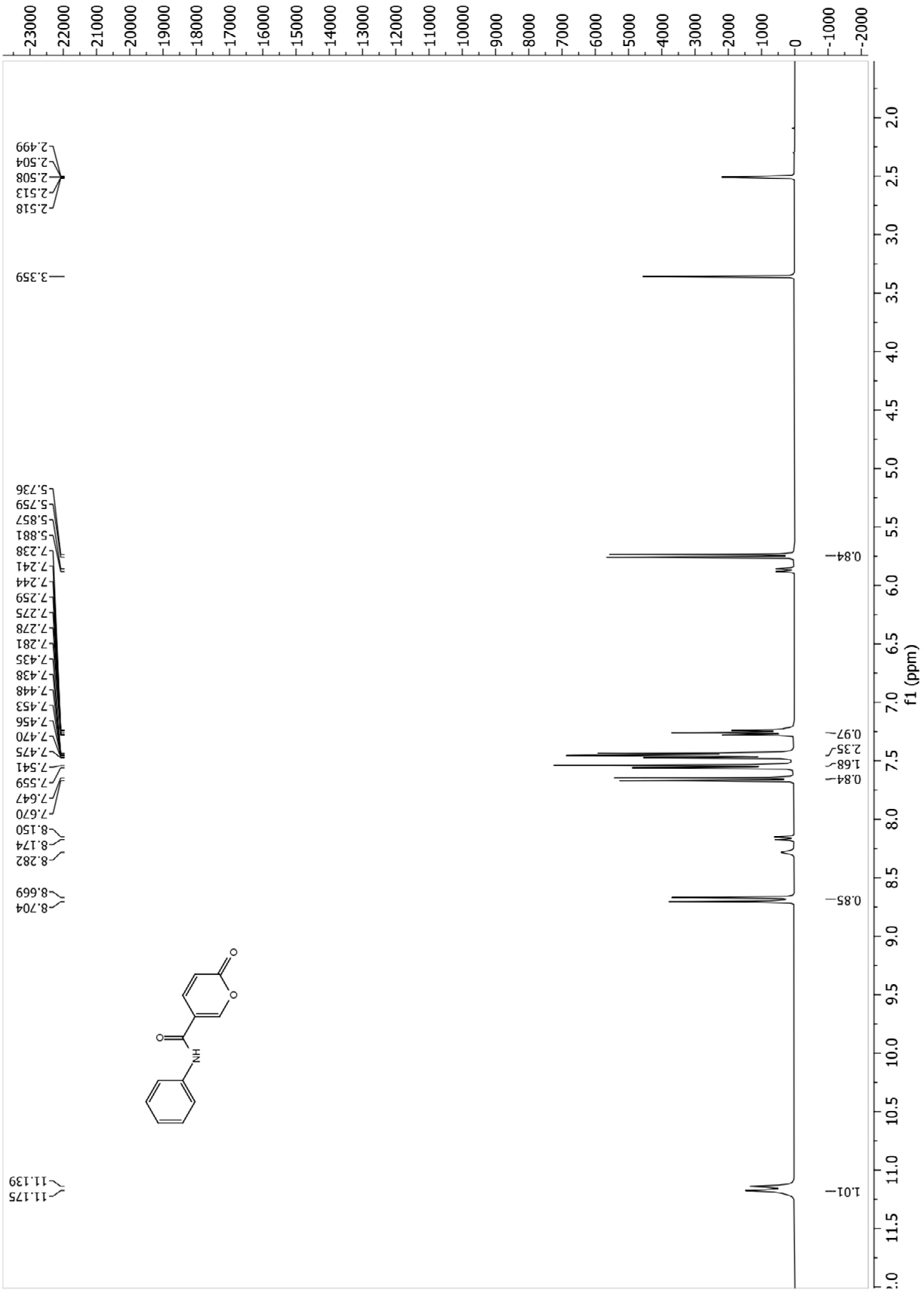
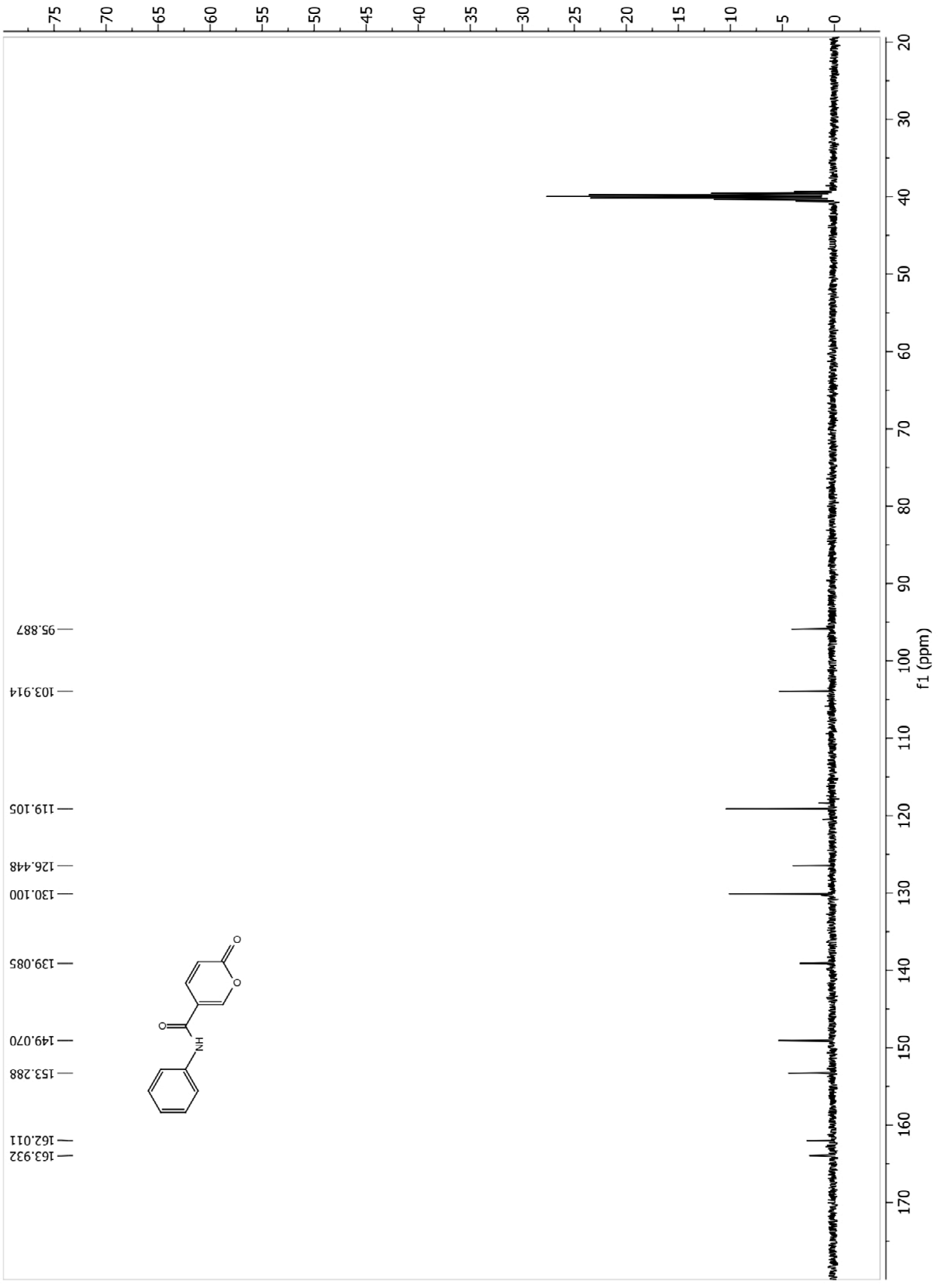


Figure S1. Overimposition between the crystallographic complex between hCA IX and acetazolamide (coloured orange) and the MD-representative structure of the complex between **1** (in its closed form) and hCA IX, which is coloured green. Residues within 4 Å from the ligands are shown as lines. The Zn(II)-bound water molecule in the MD structure is shown as a small red sphere, the catalytic Zn(II) ion is shown as a grey sphere in both structures.

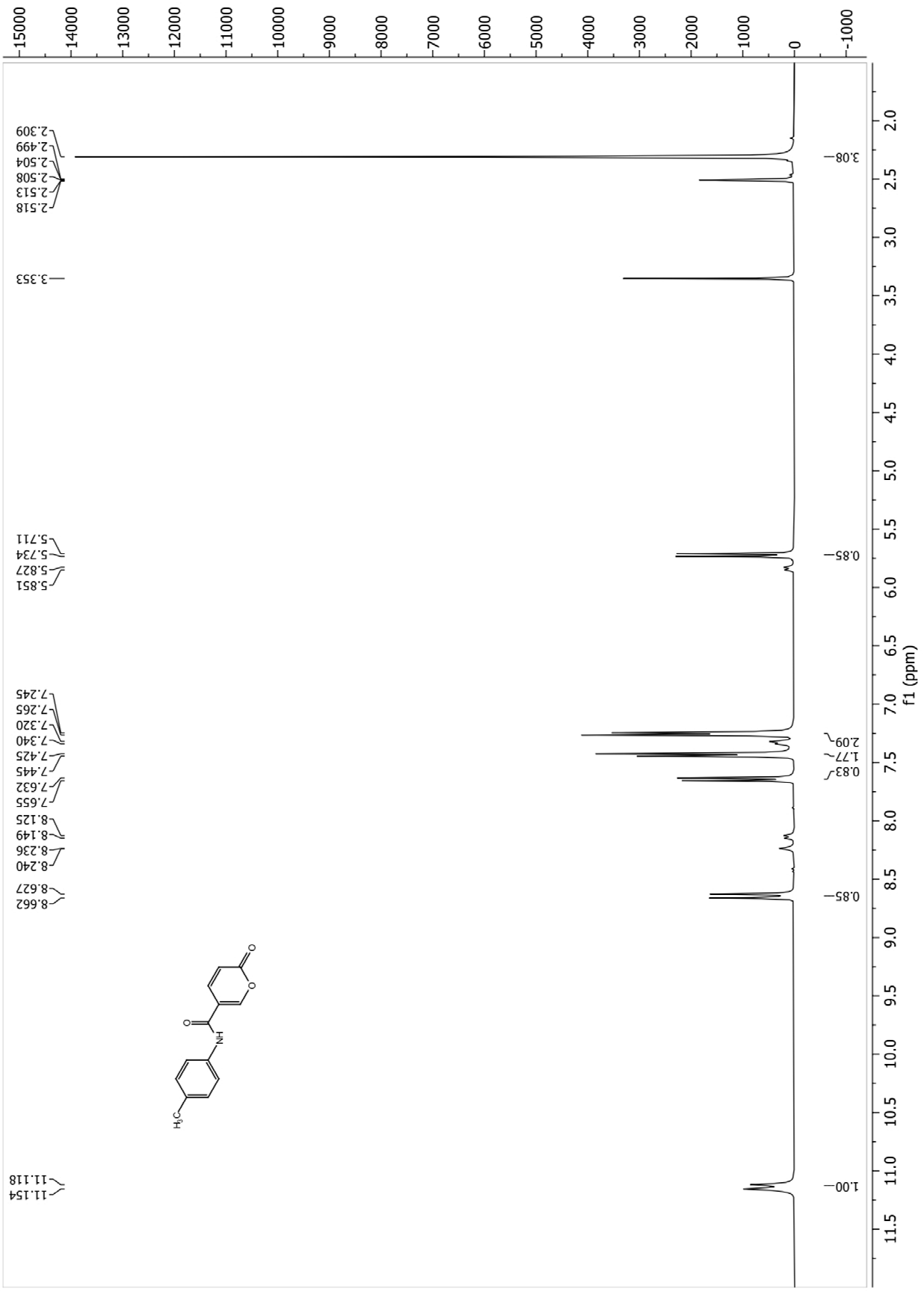
^{1}H NMR spectrum of compound **2**



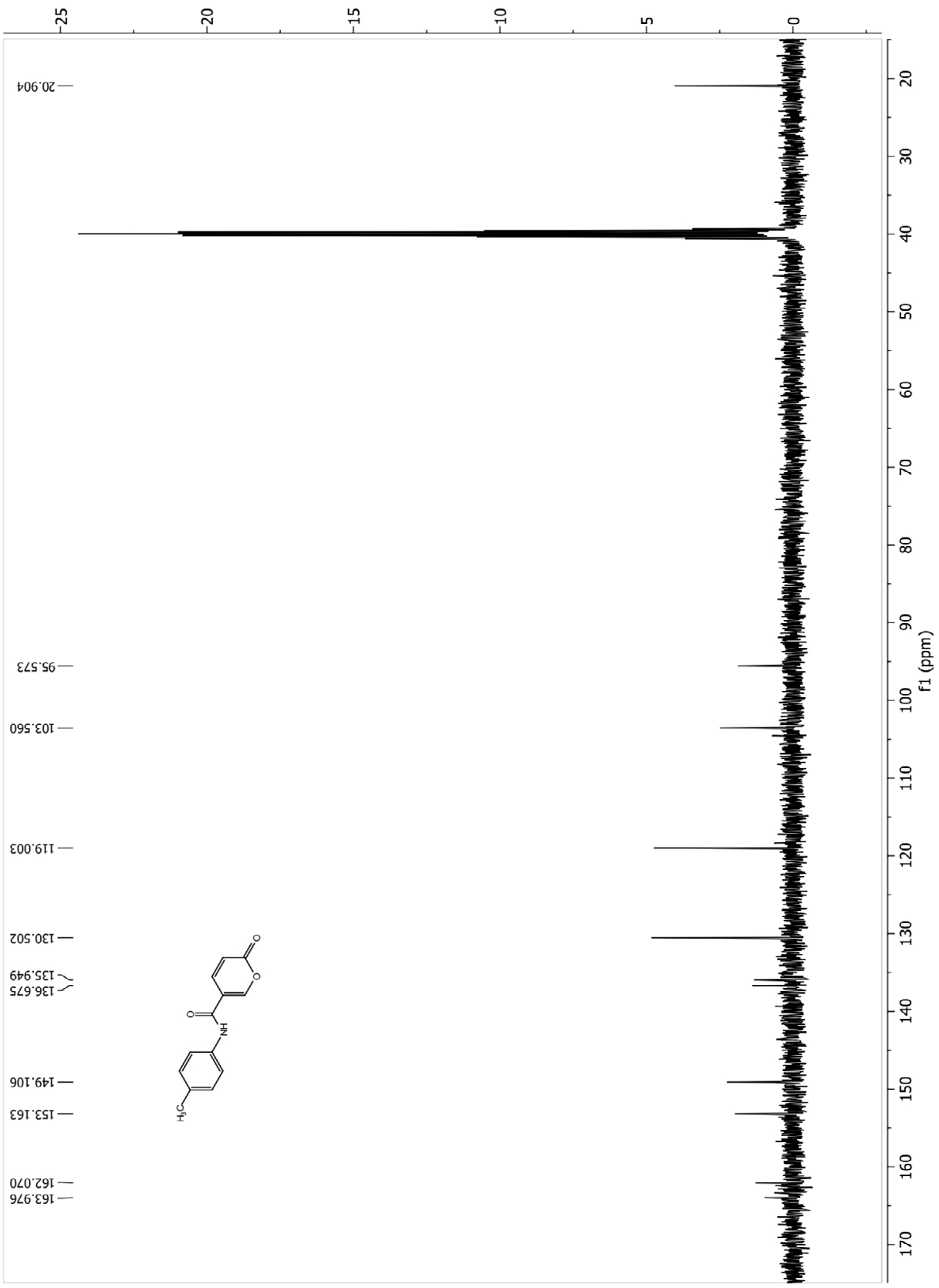
^{13}C NMR spectrum of compound **2**



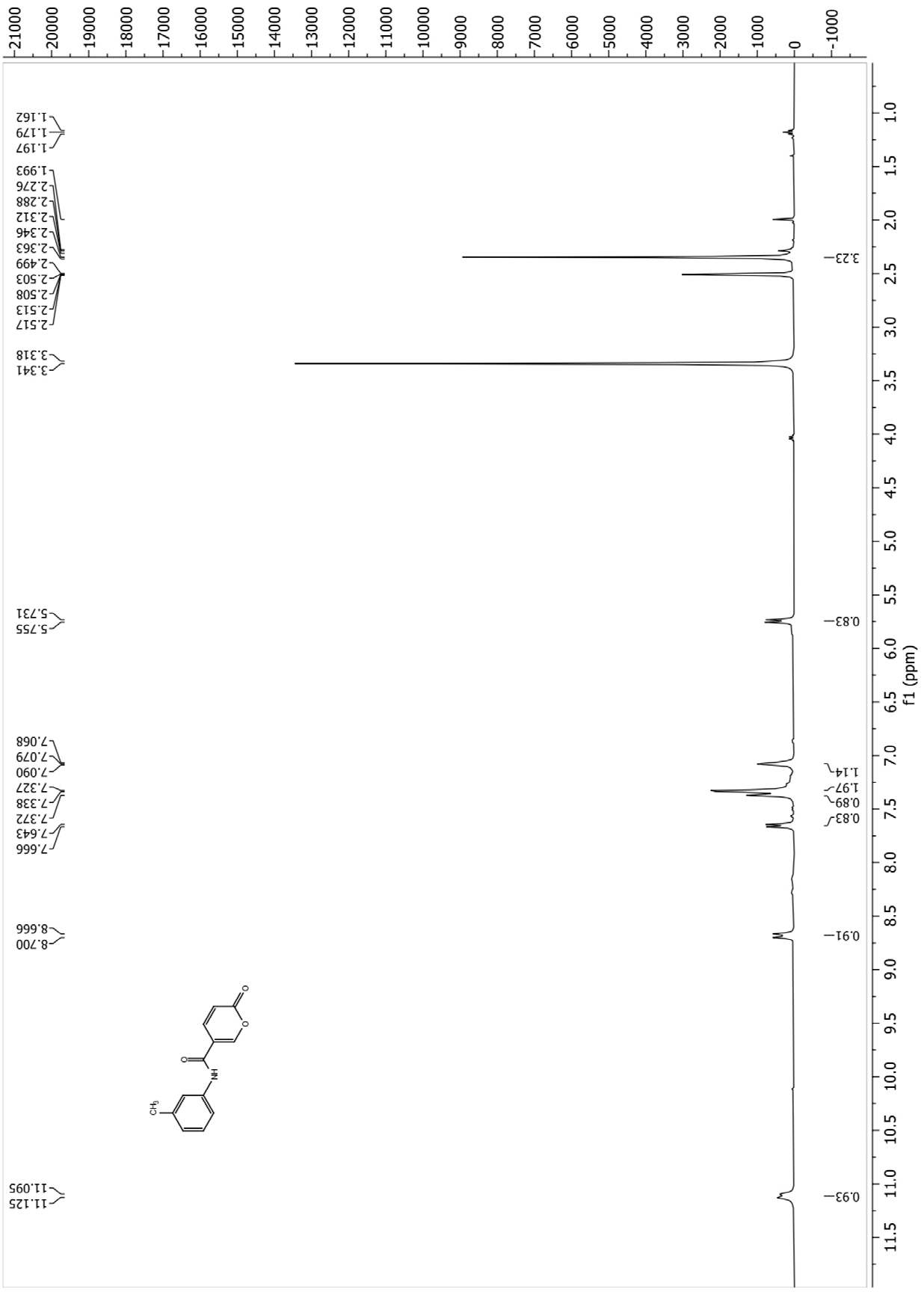
^{1}H NMR spectrum of compound **3**



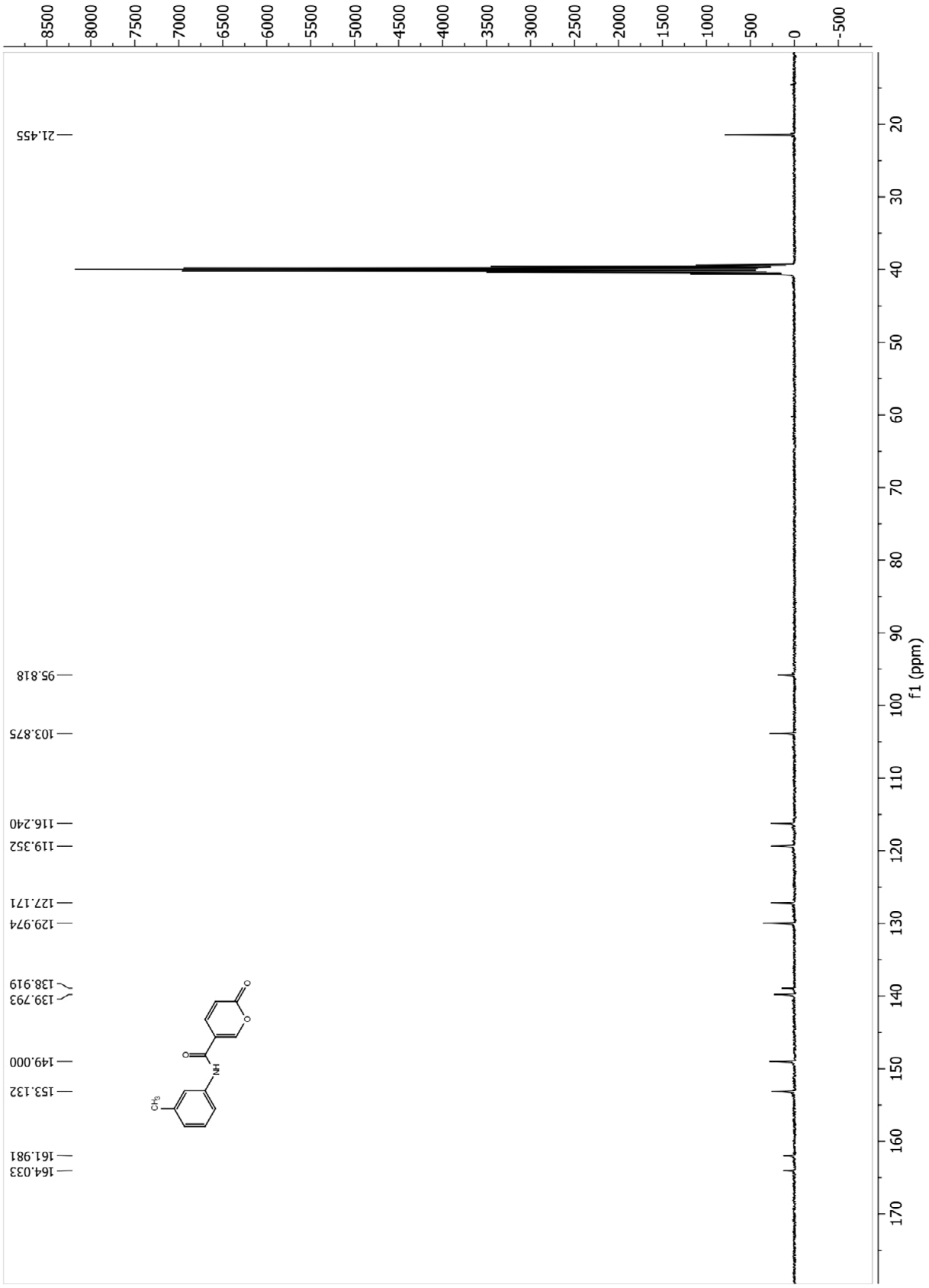
^{13}C NMR spectrum of compound **3**



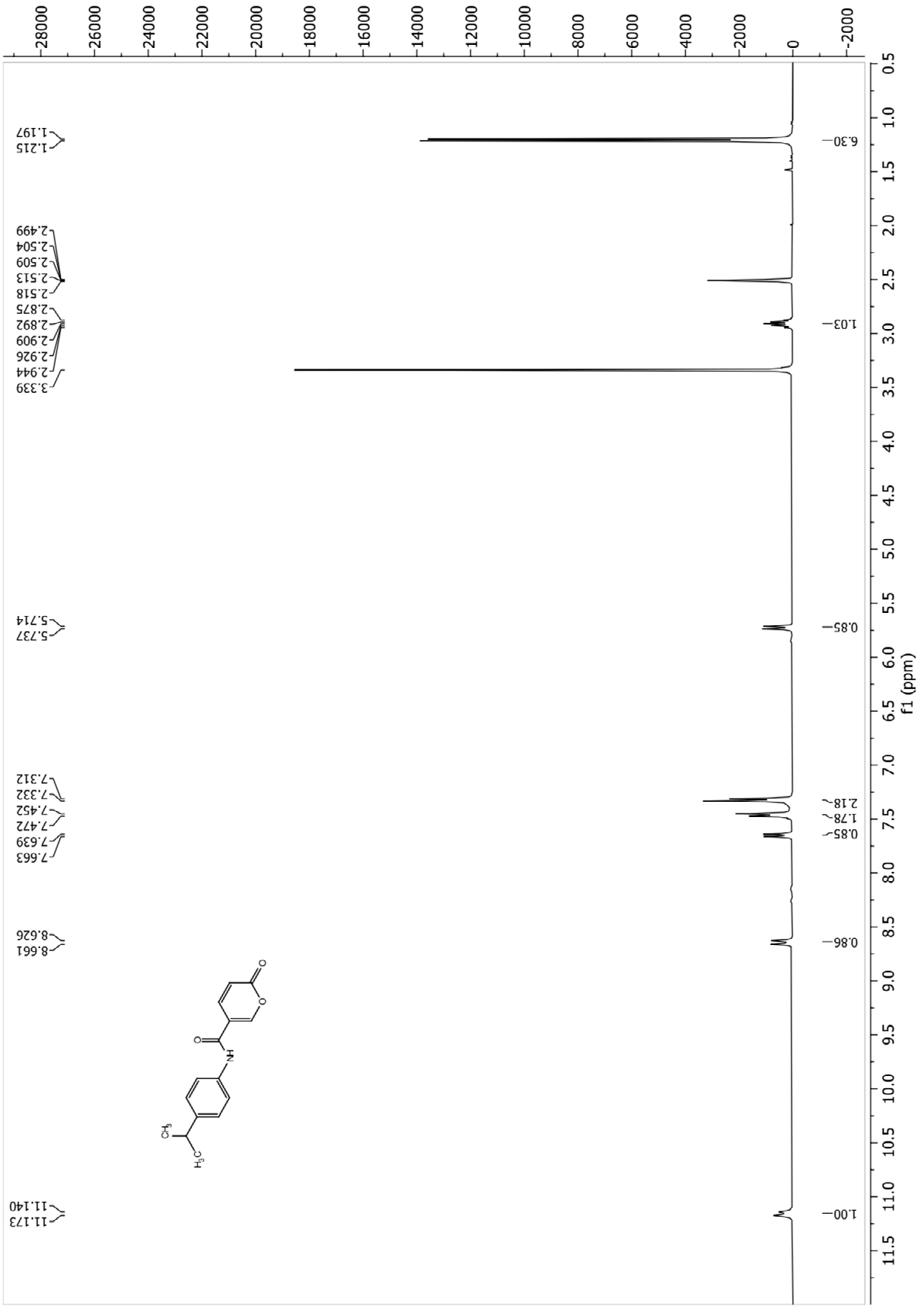
^{1}H NMR spectrum of compound **4**



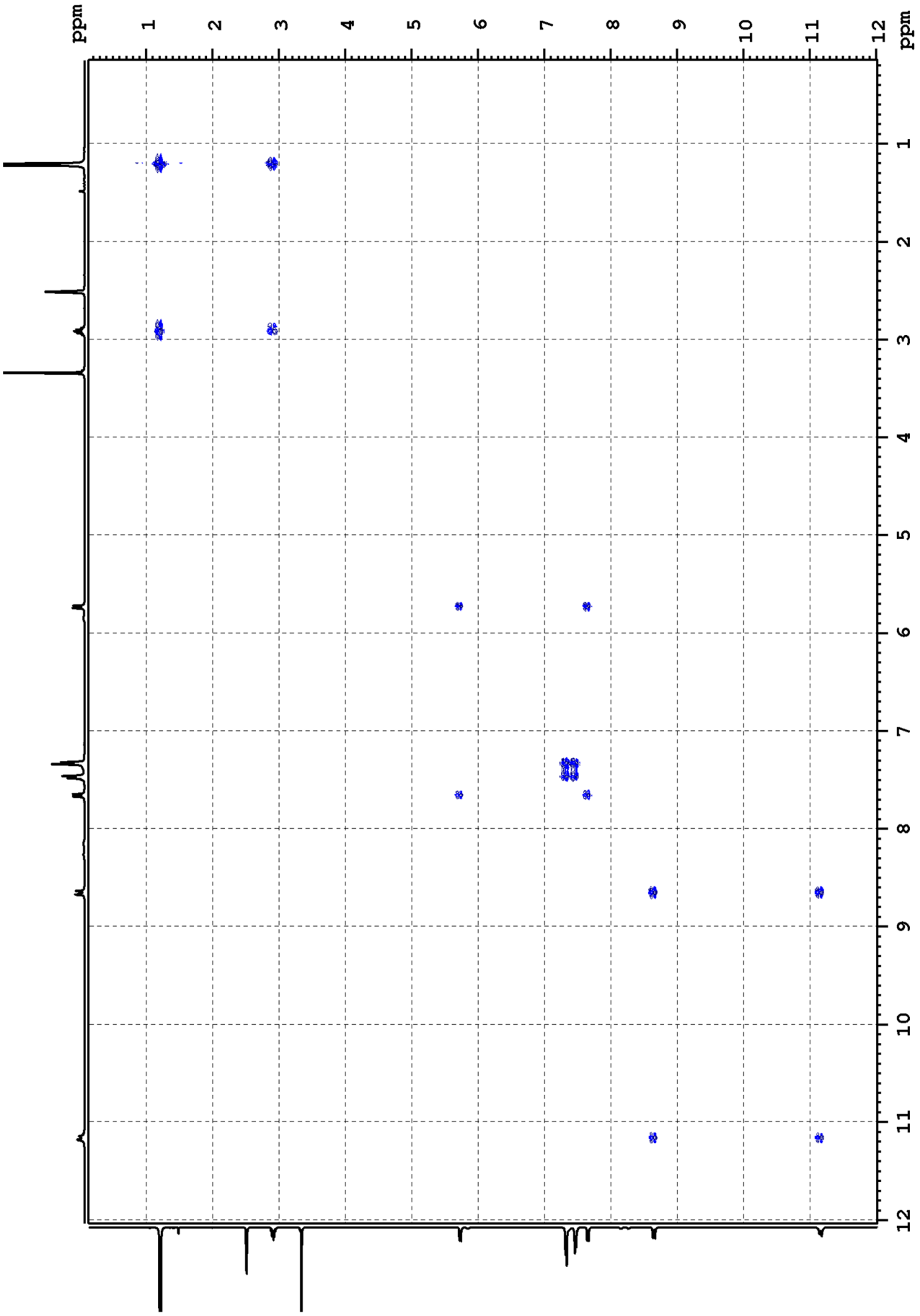
^{13}C NMR spectrum of compound **4**



^{1}H NMR spectrum of compound **5**



^{1}H-^{1}H NMR-COSY spectrum of compound **5**



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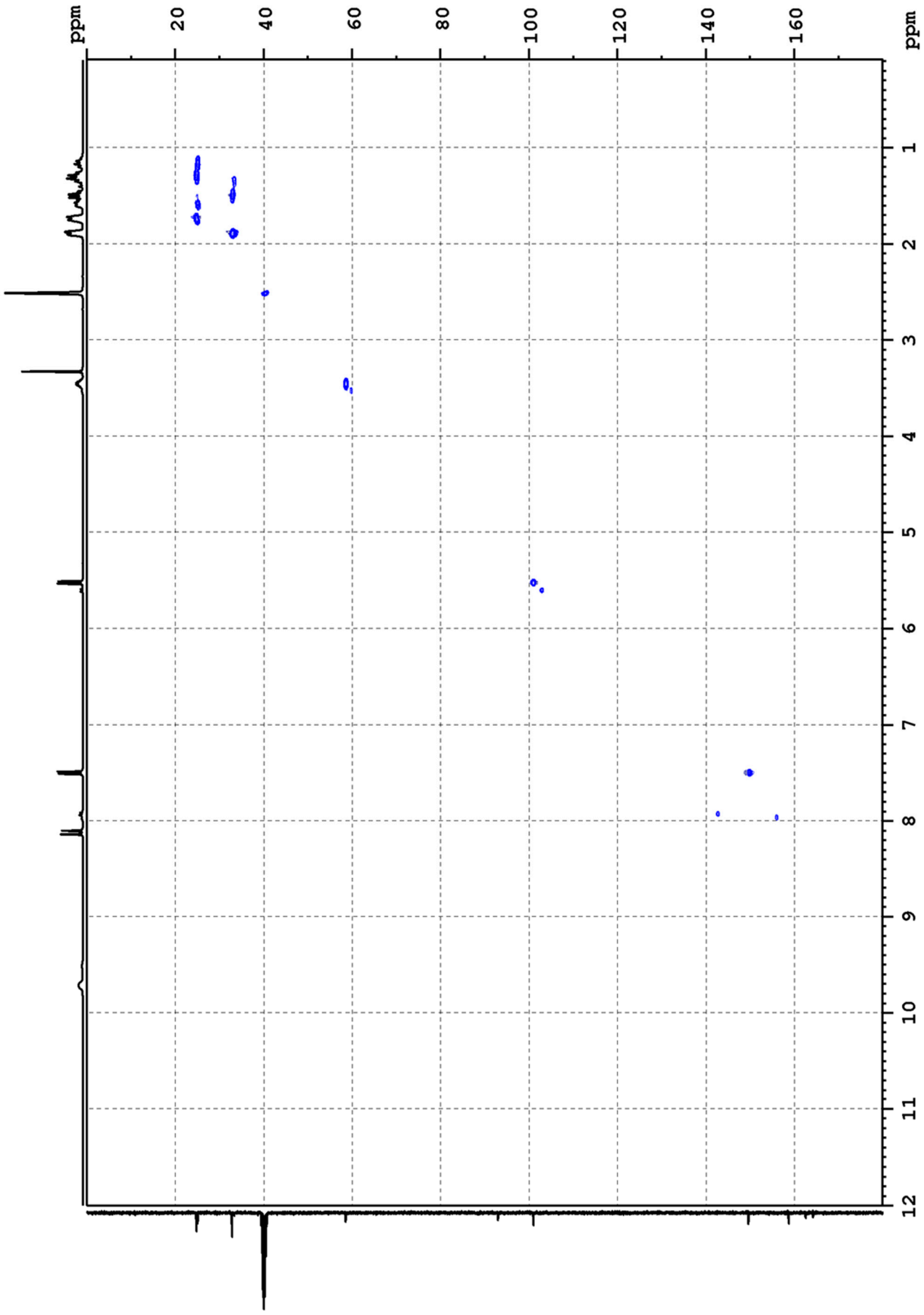
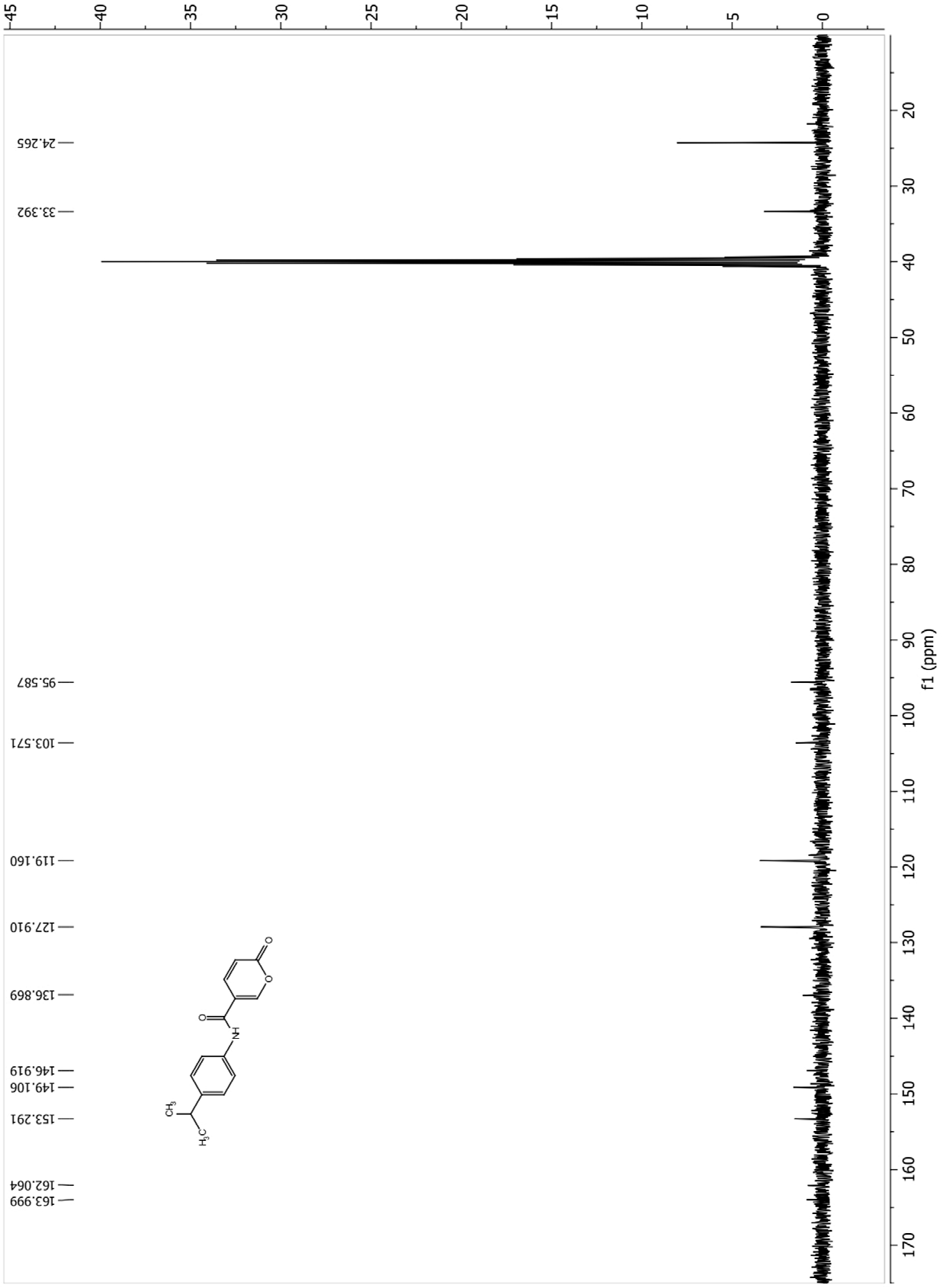


^{13}C NMR spectrum of compound **5**



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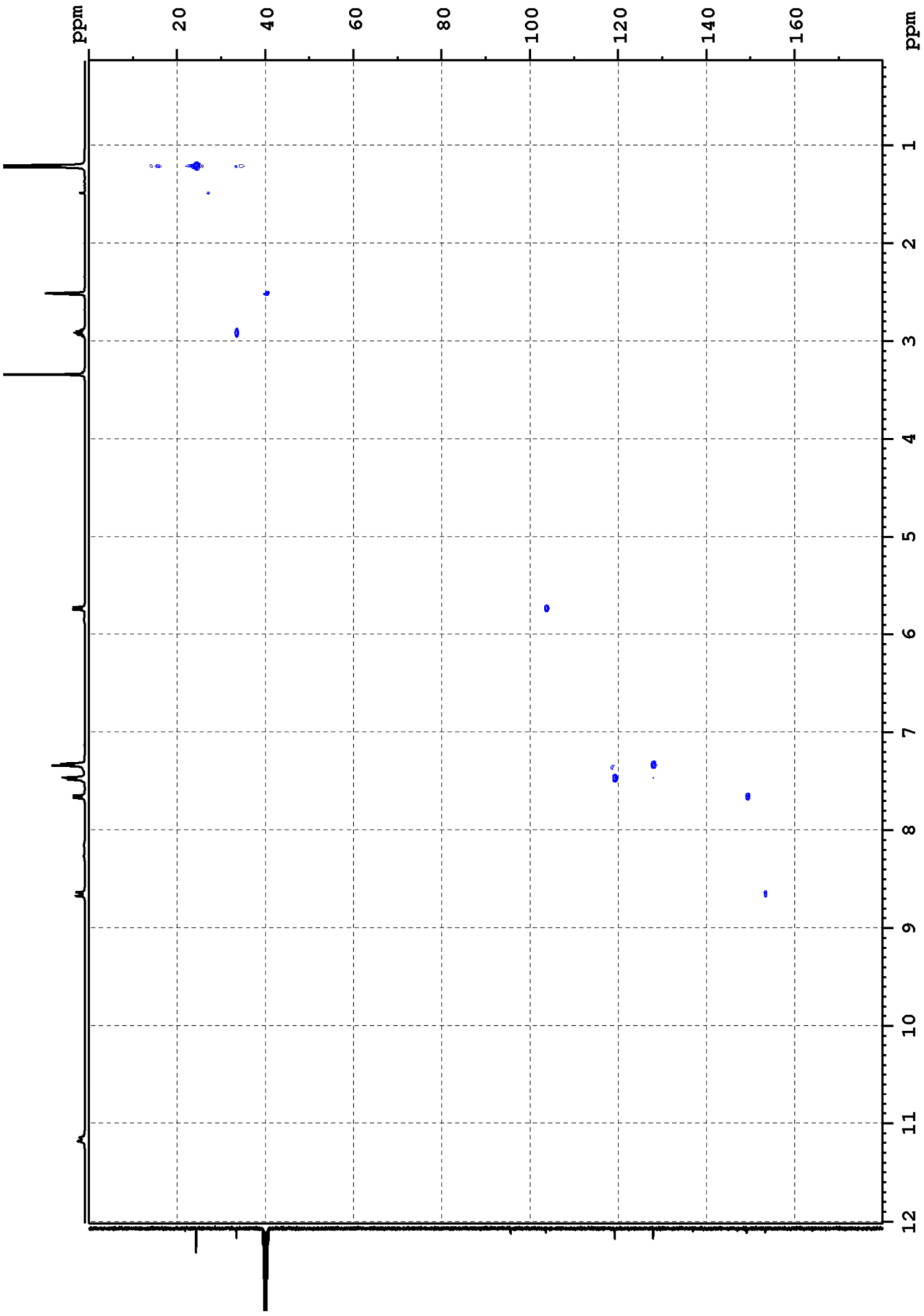

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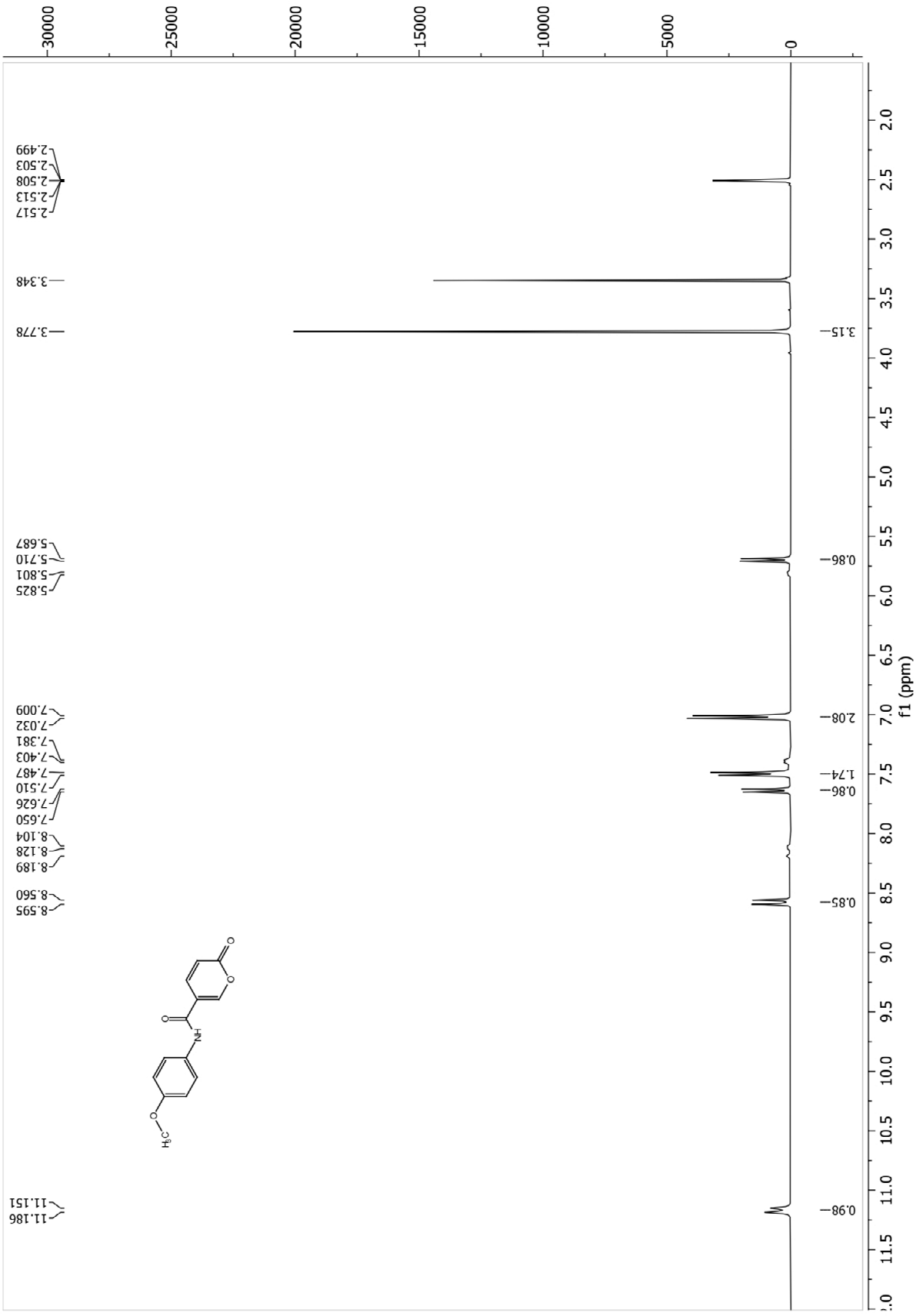
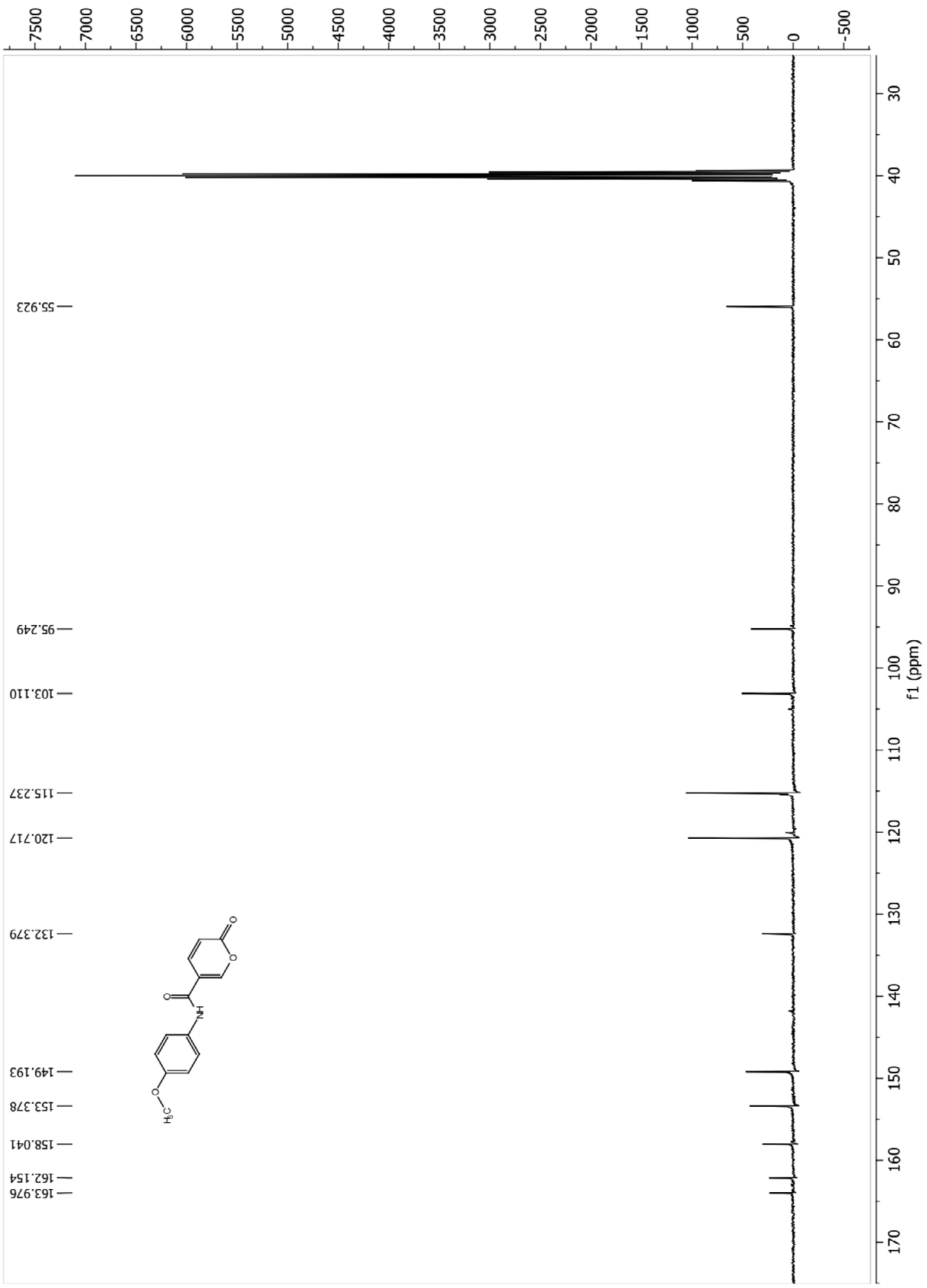
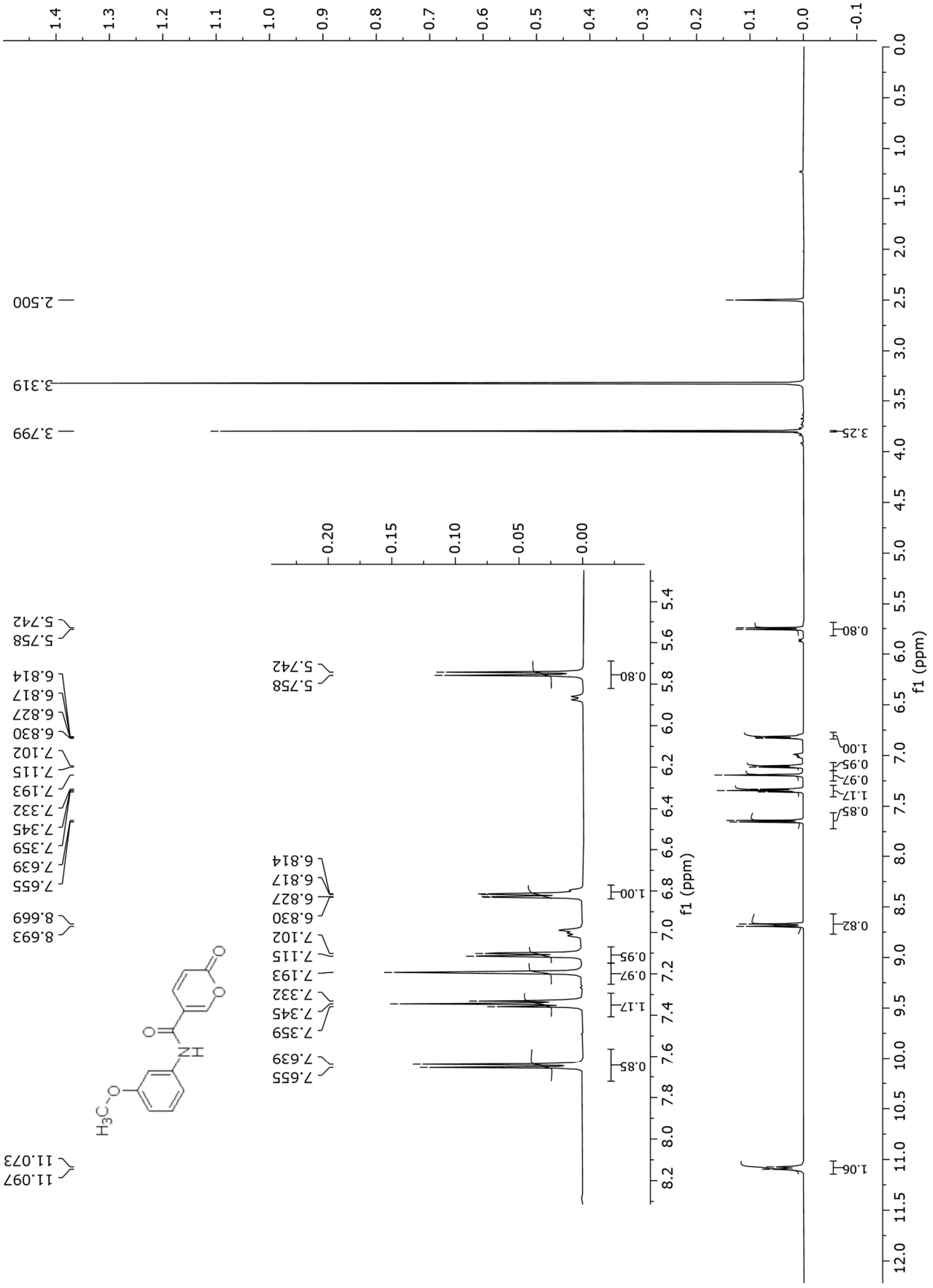


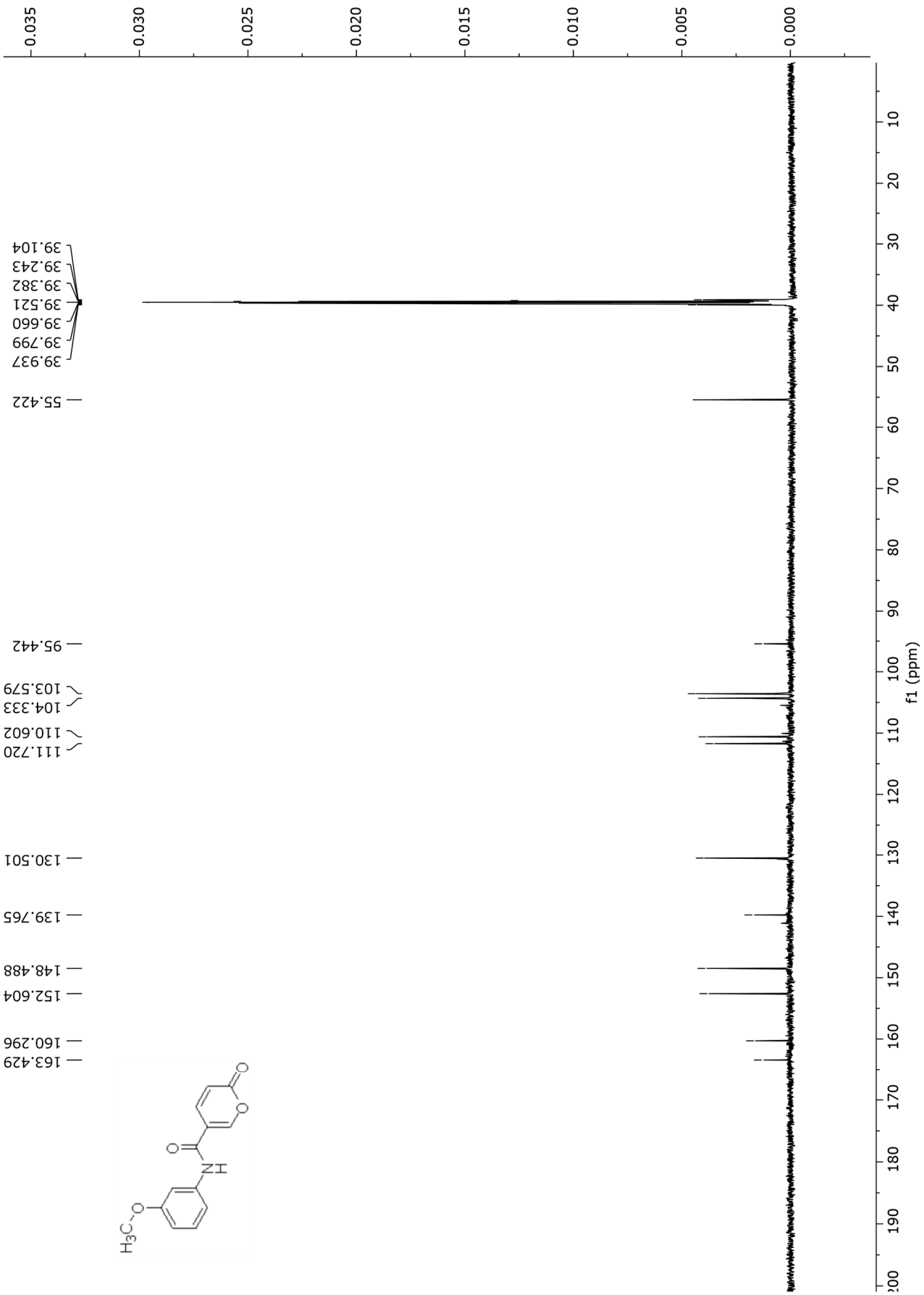
^{13}C NMR spectrum of compound **6**



^{1}H NMR spectrum of compound **7**



^{13}C NMR spectrum of compound **7**



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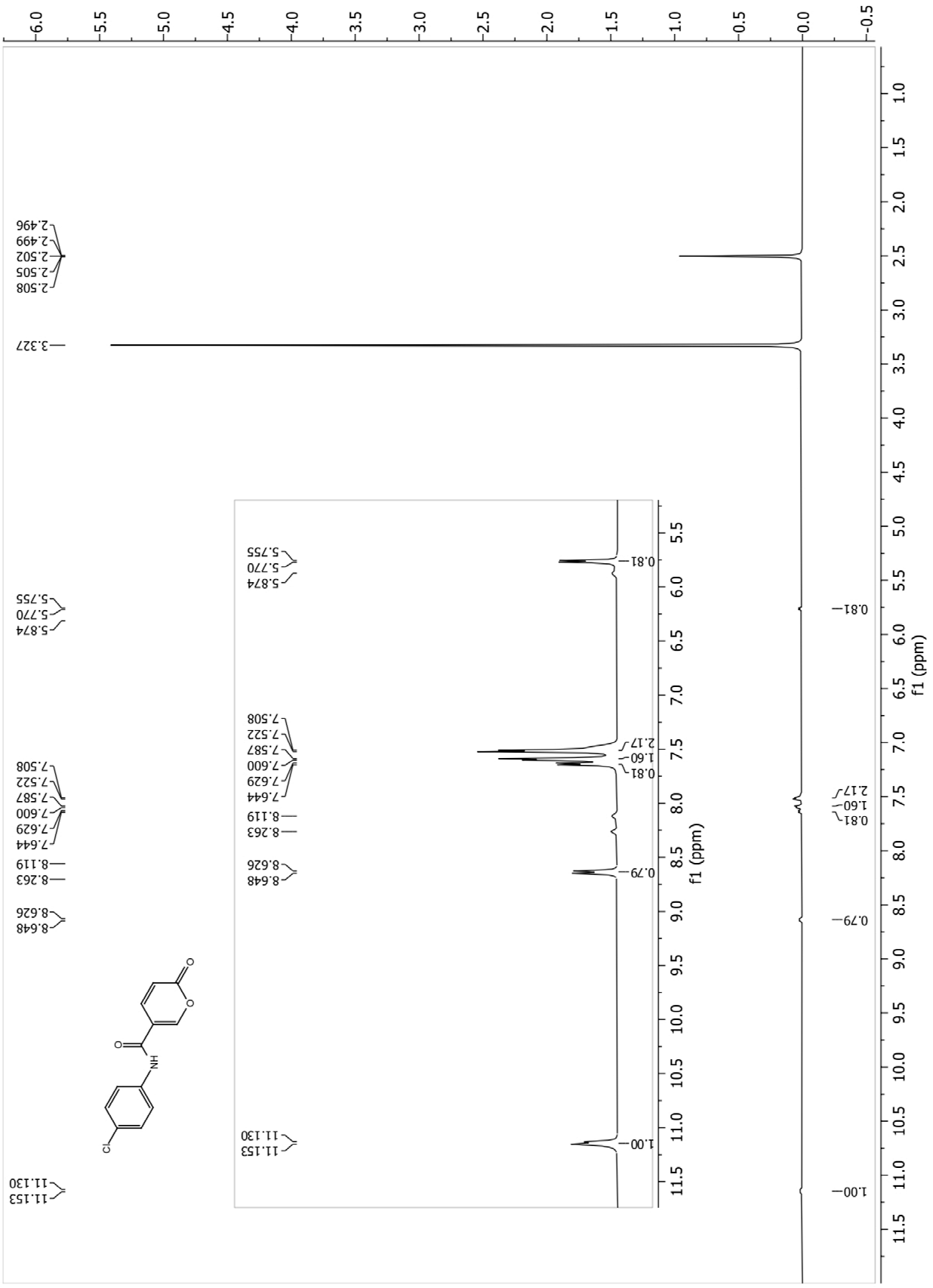

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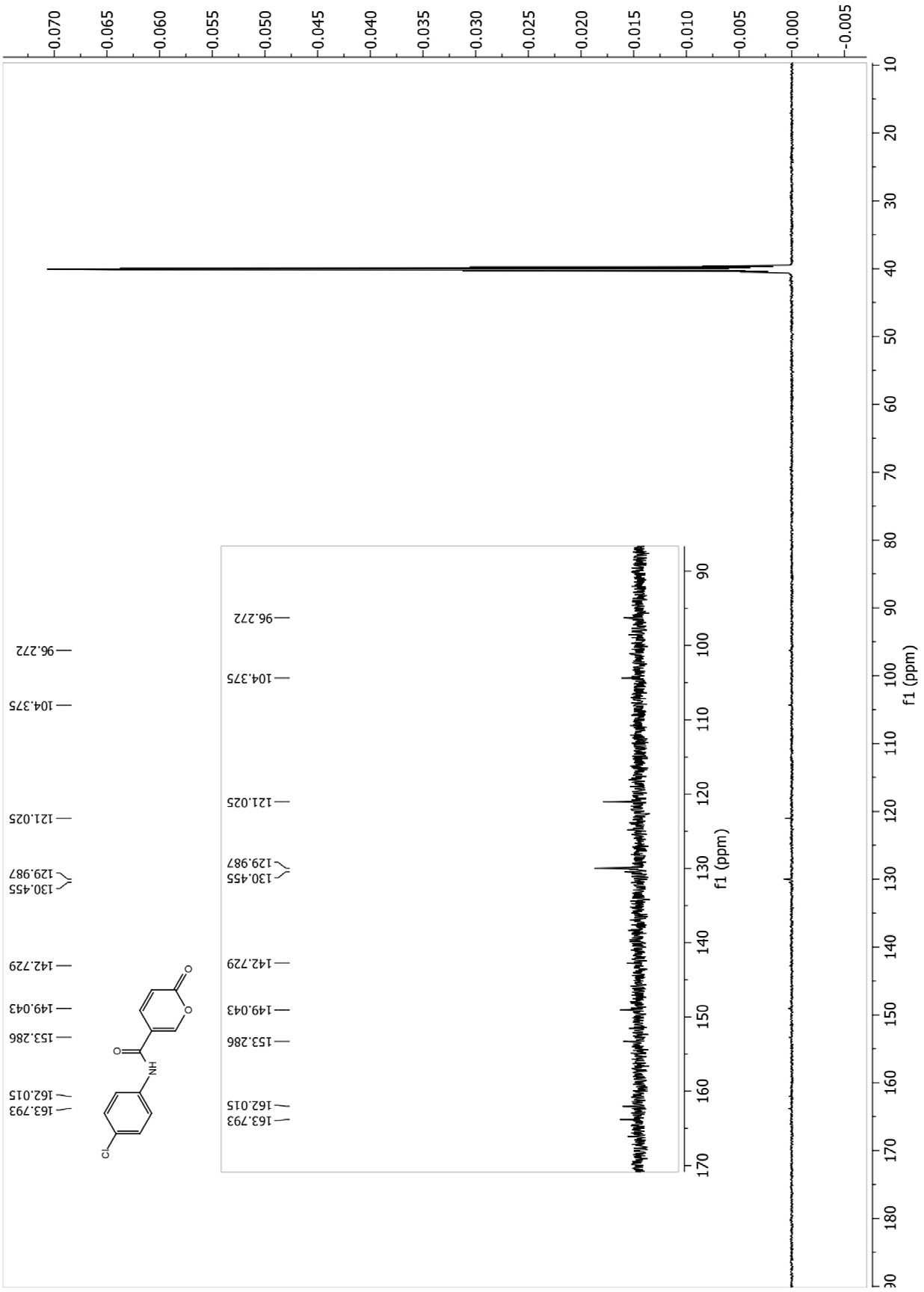

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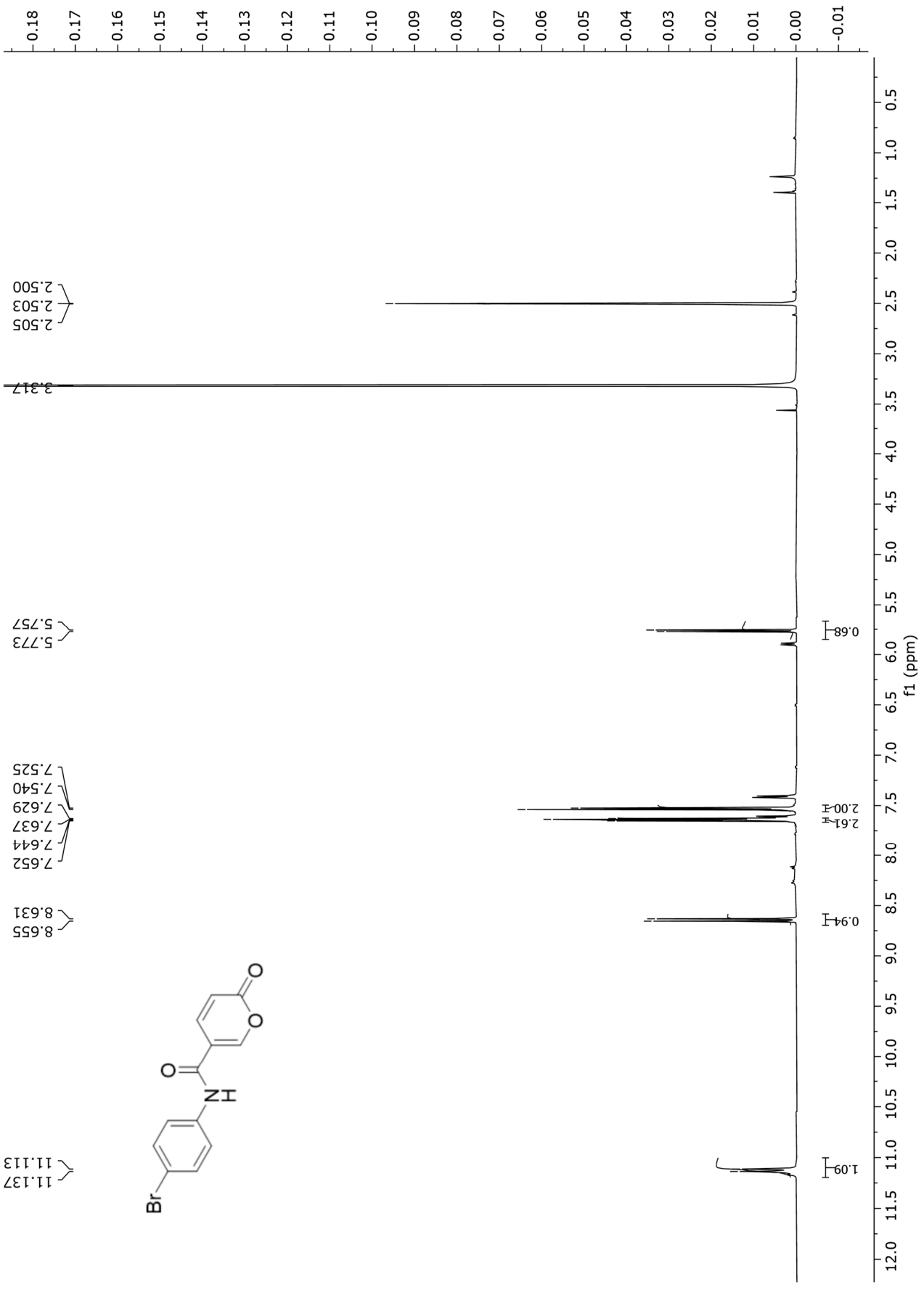

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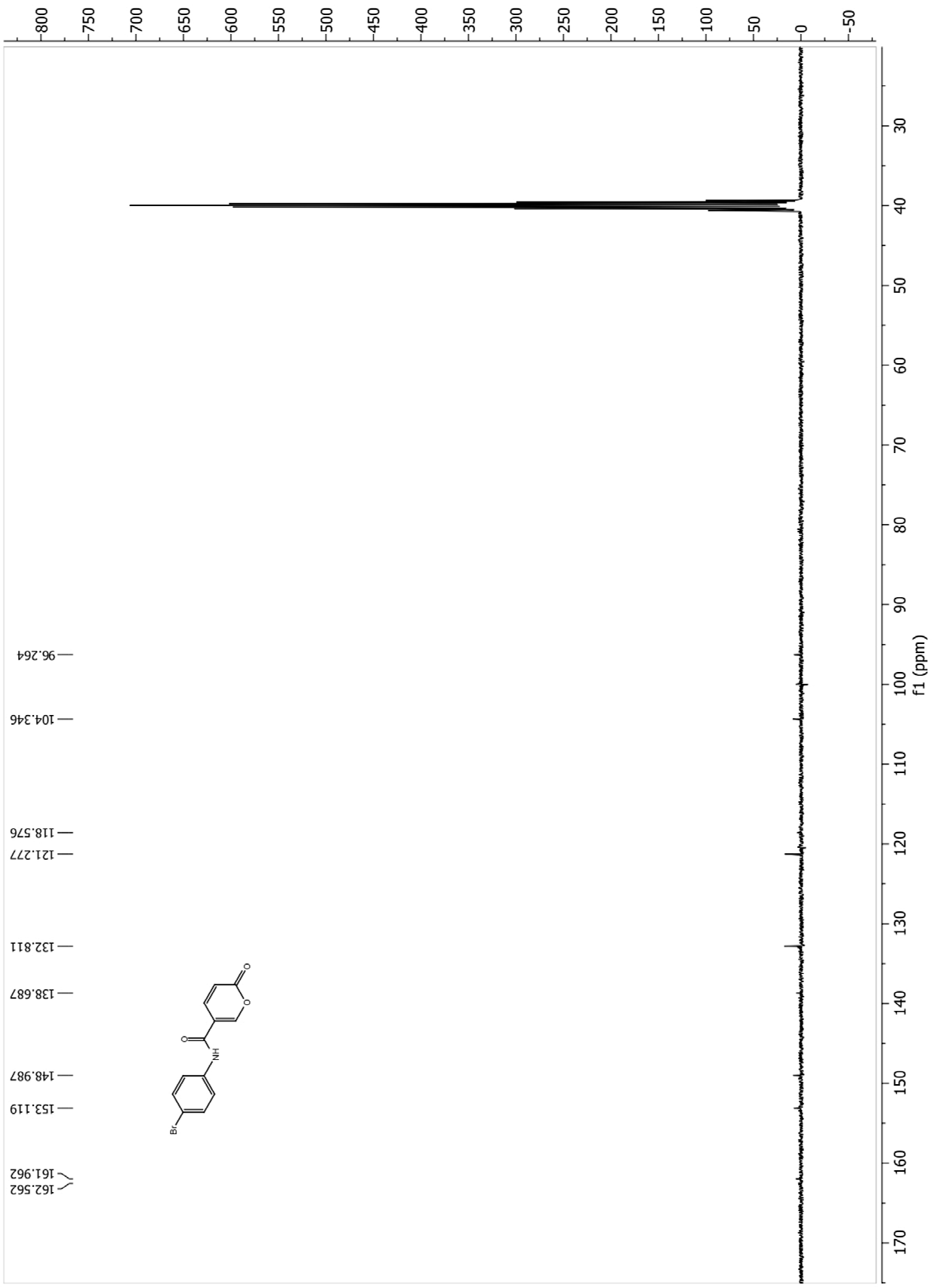

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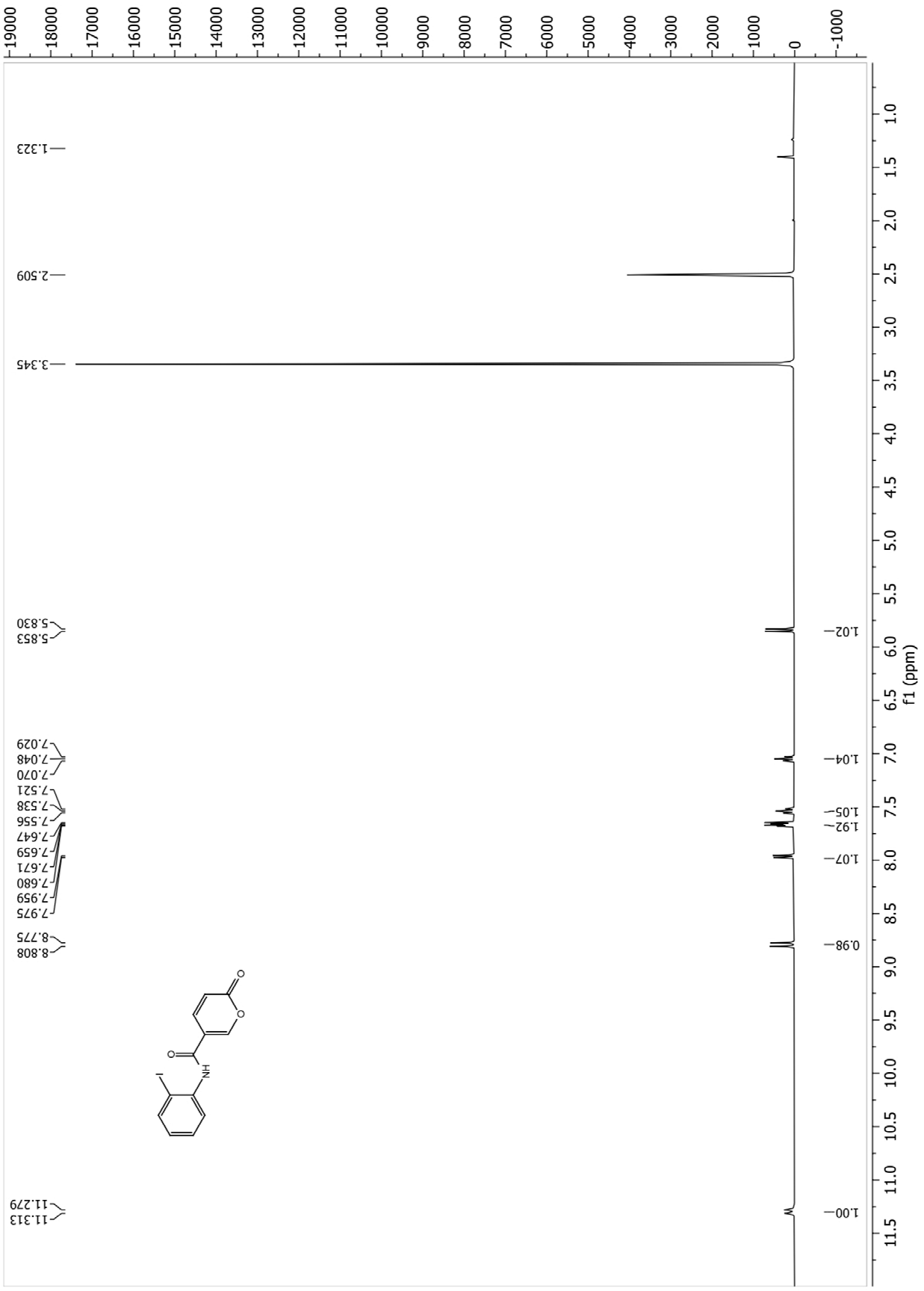

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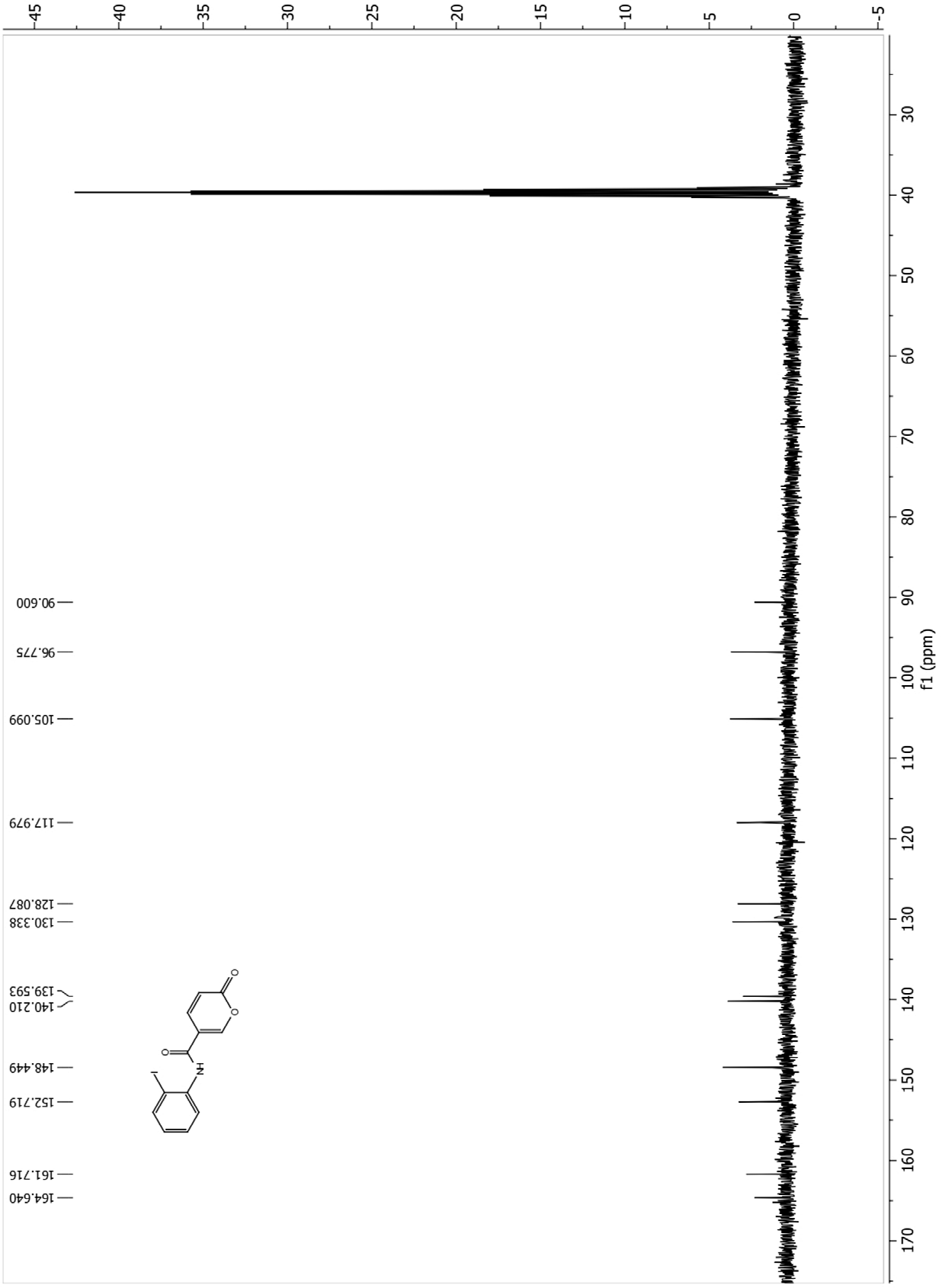
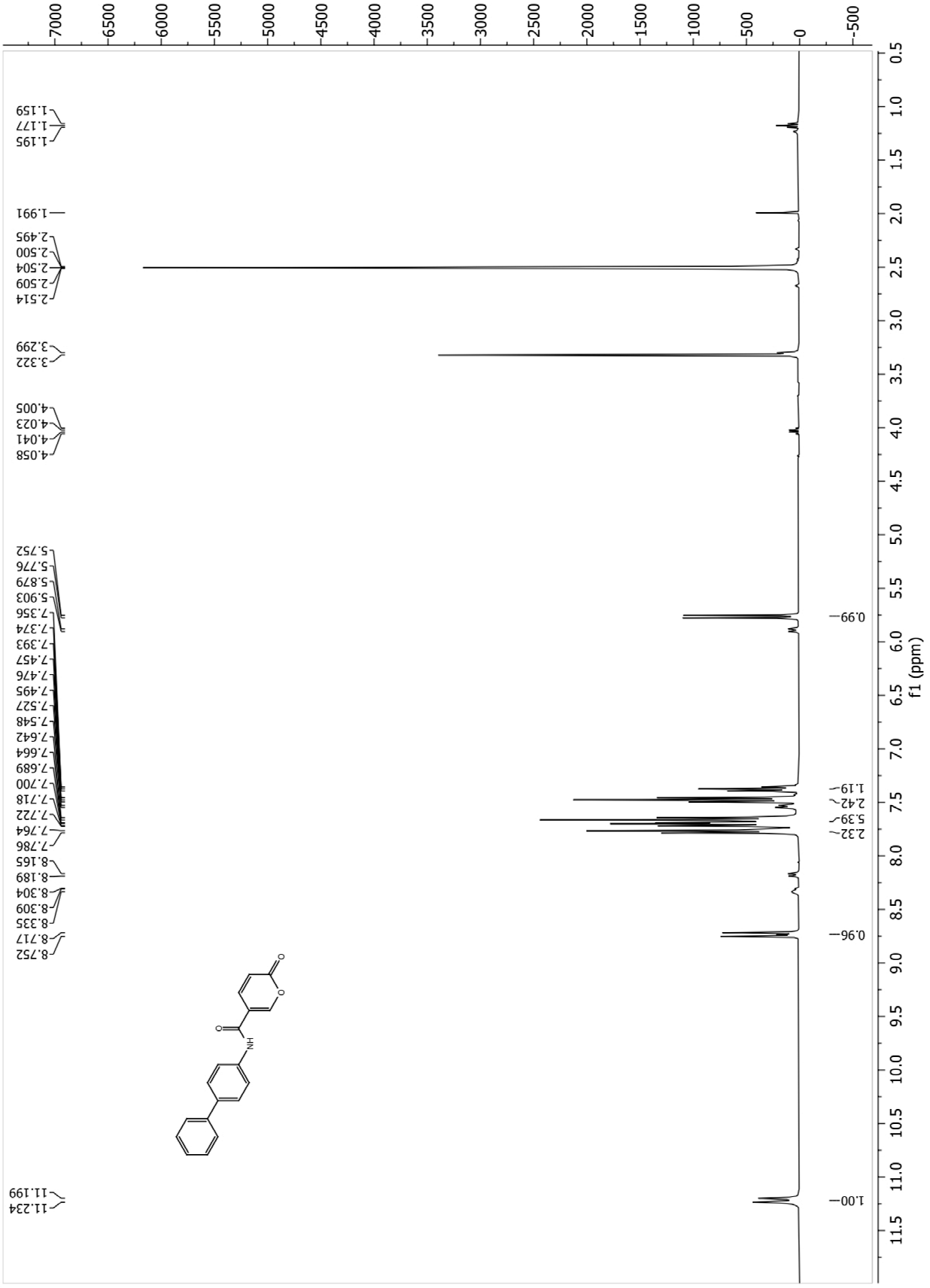
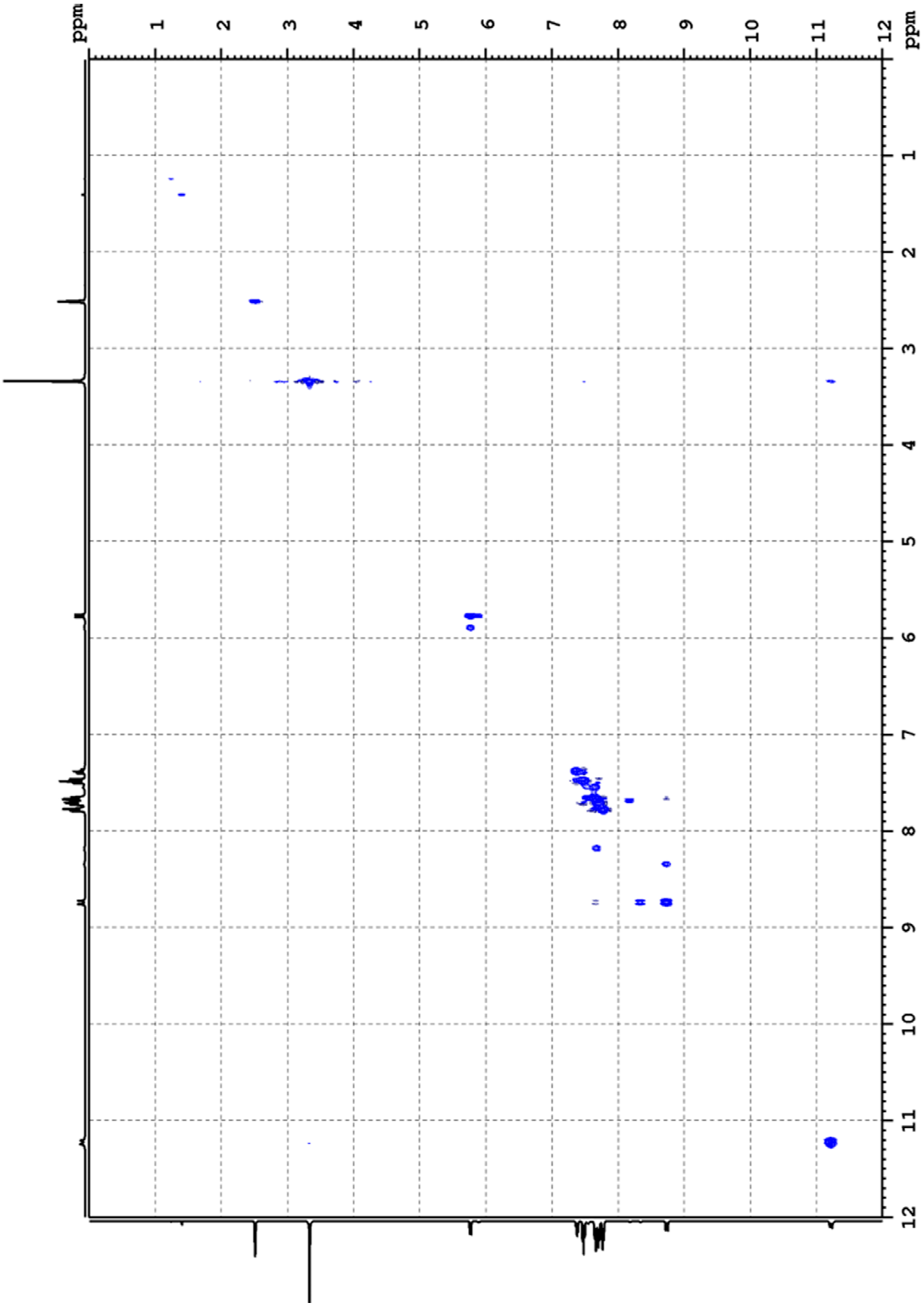


^{1}H NMR spectrum of compound **11**



^{1}H-^{1}H NMR-NOESY spectrum of compound **11**



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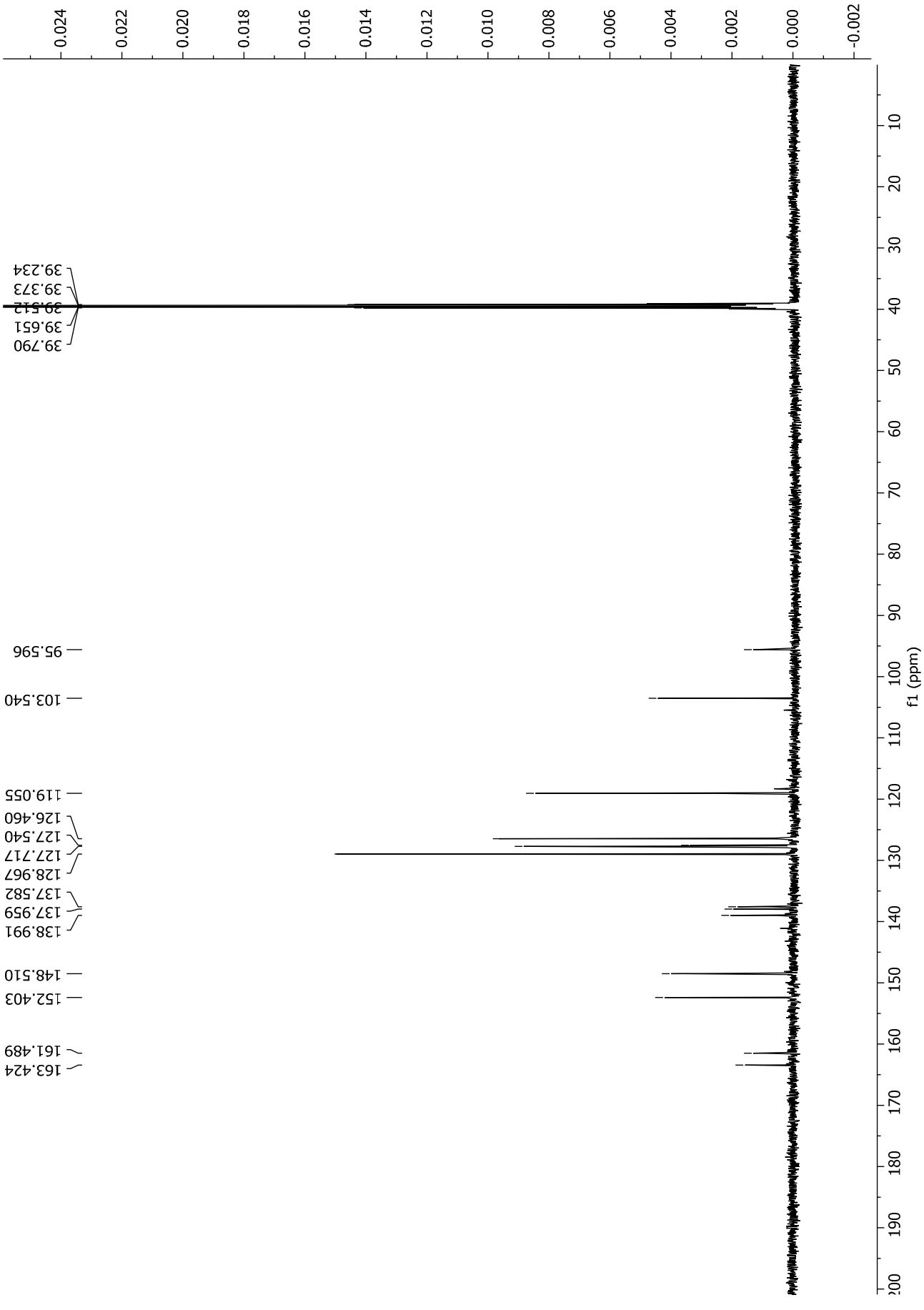

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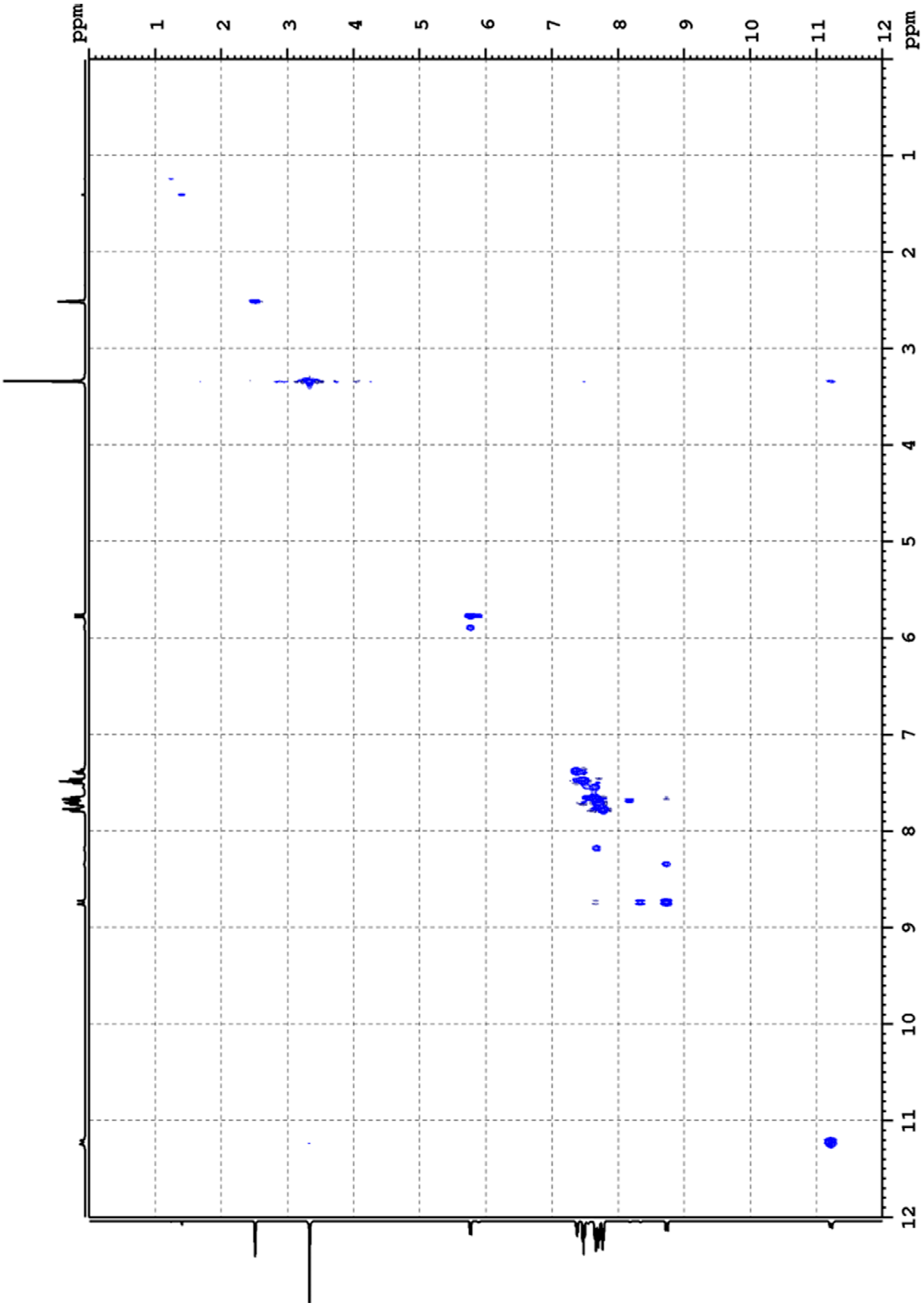

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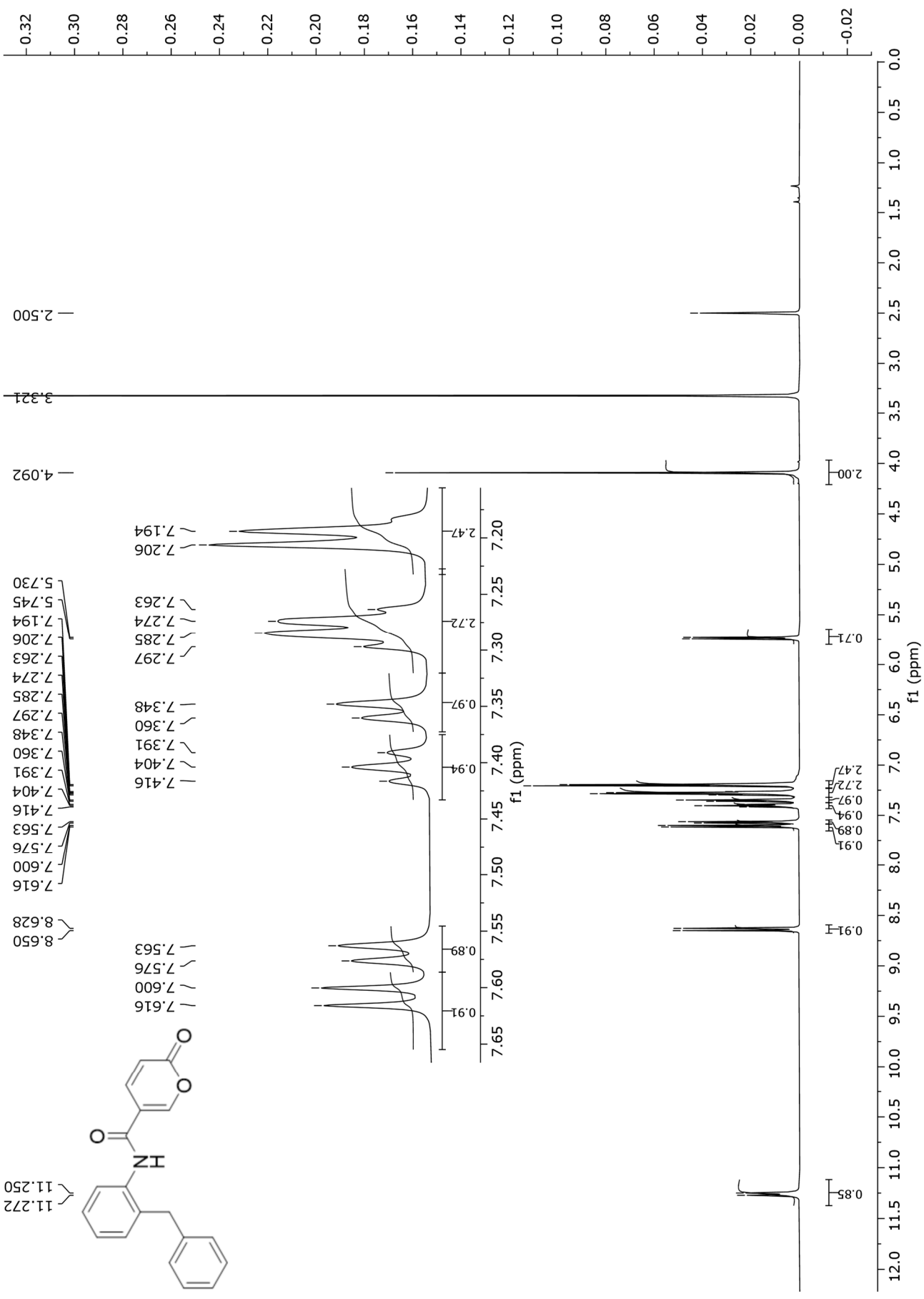

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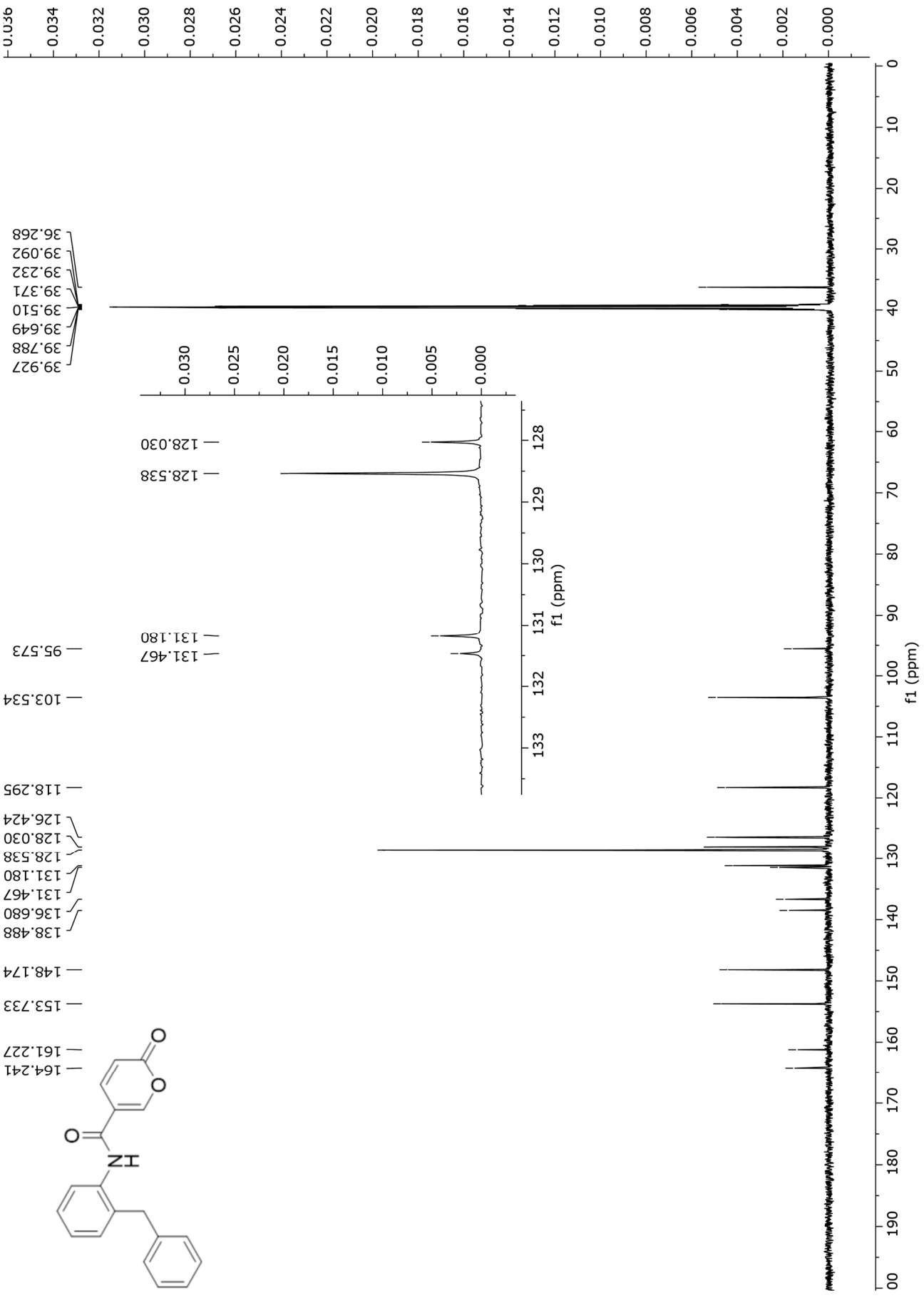

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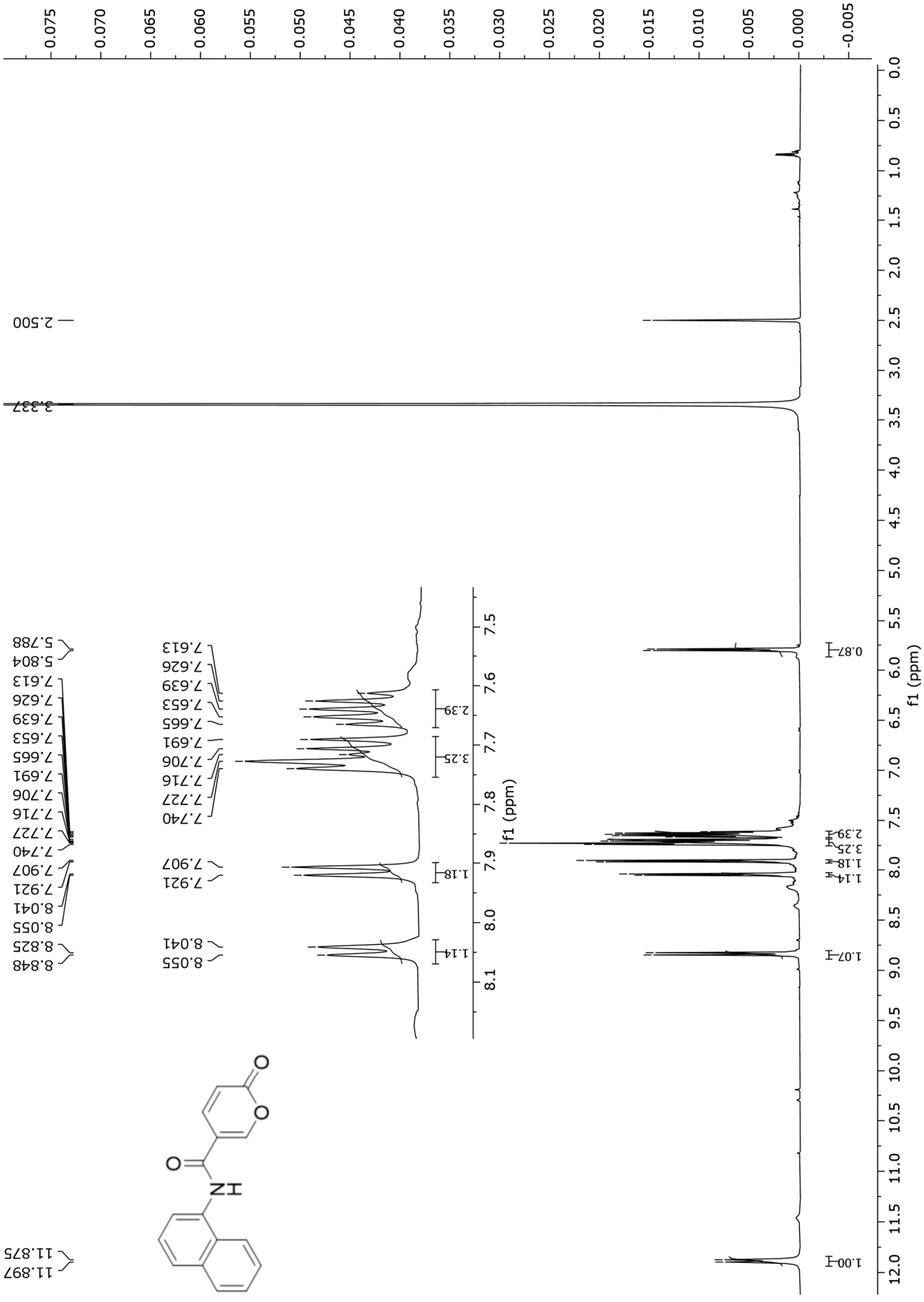

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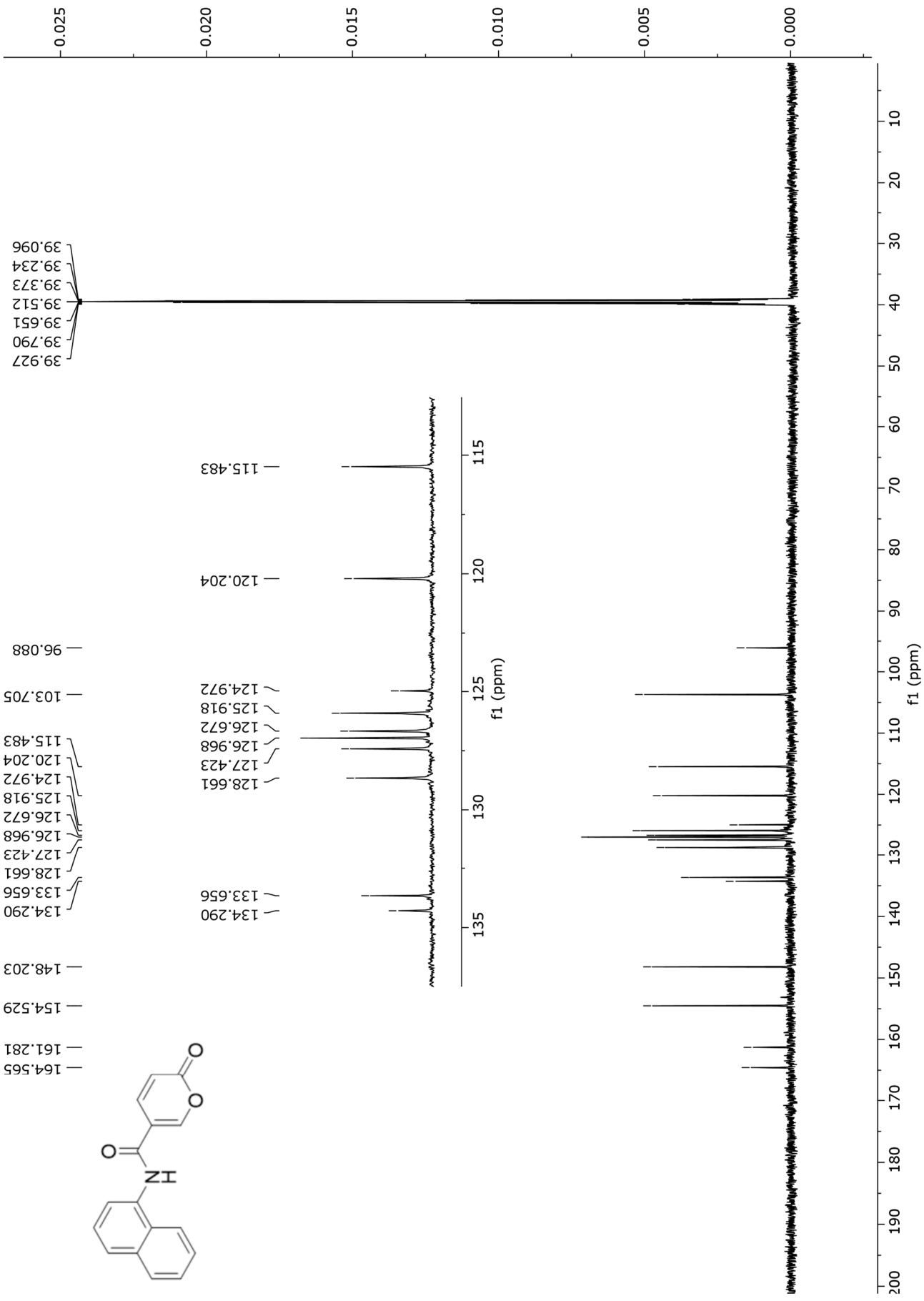
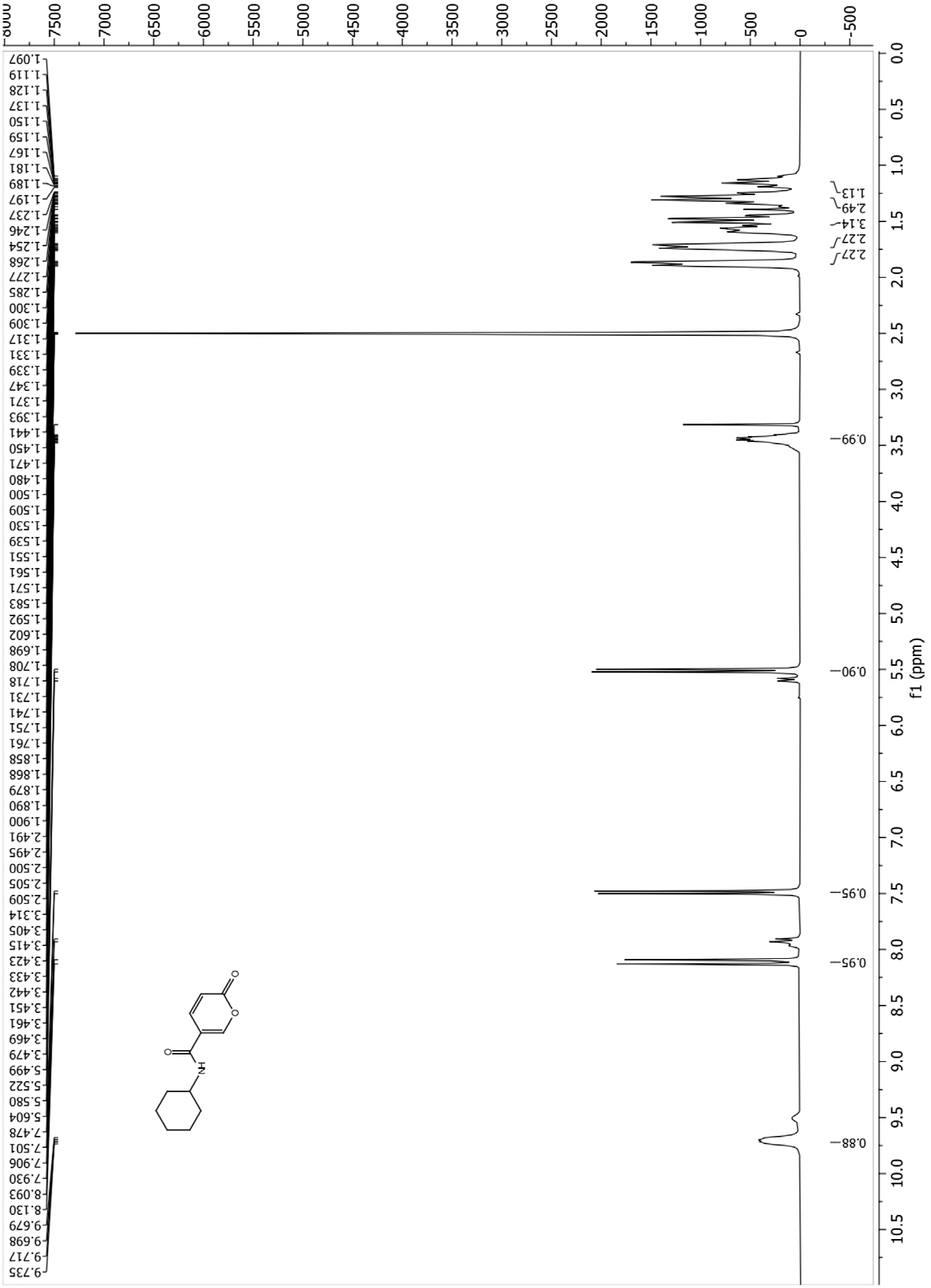
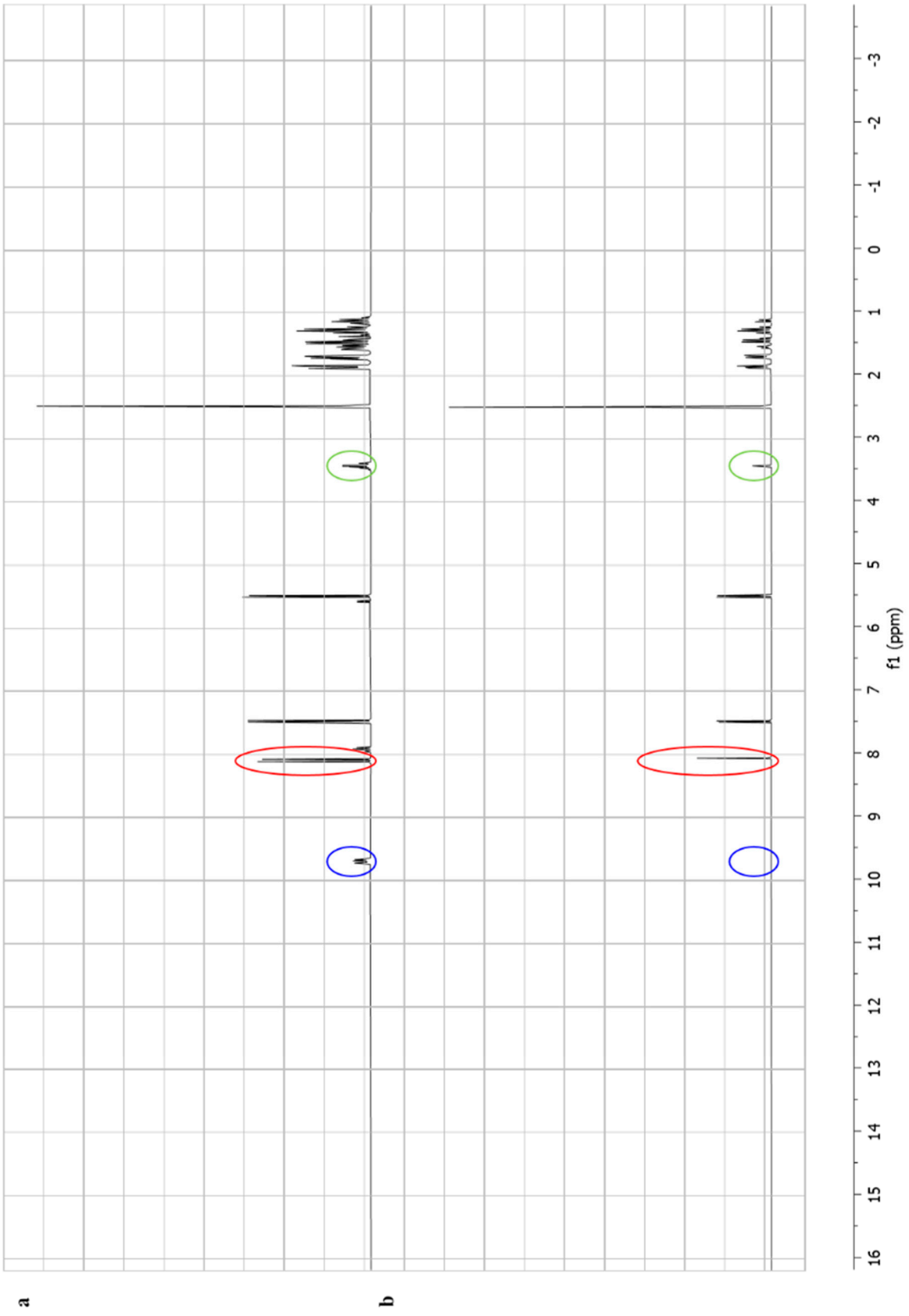


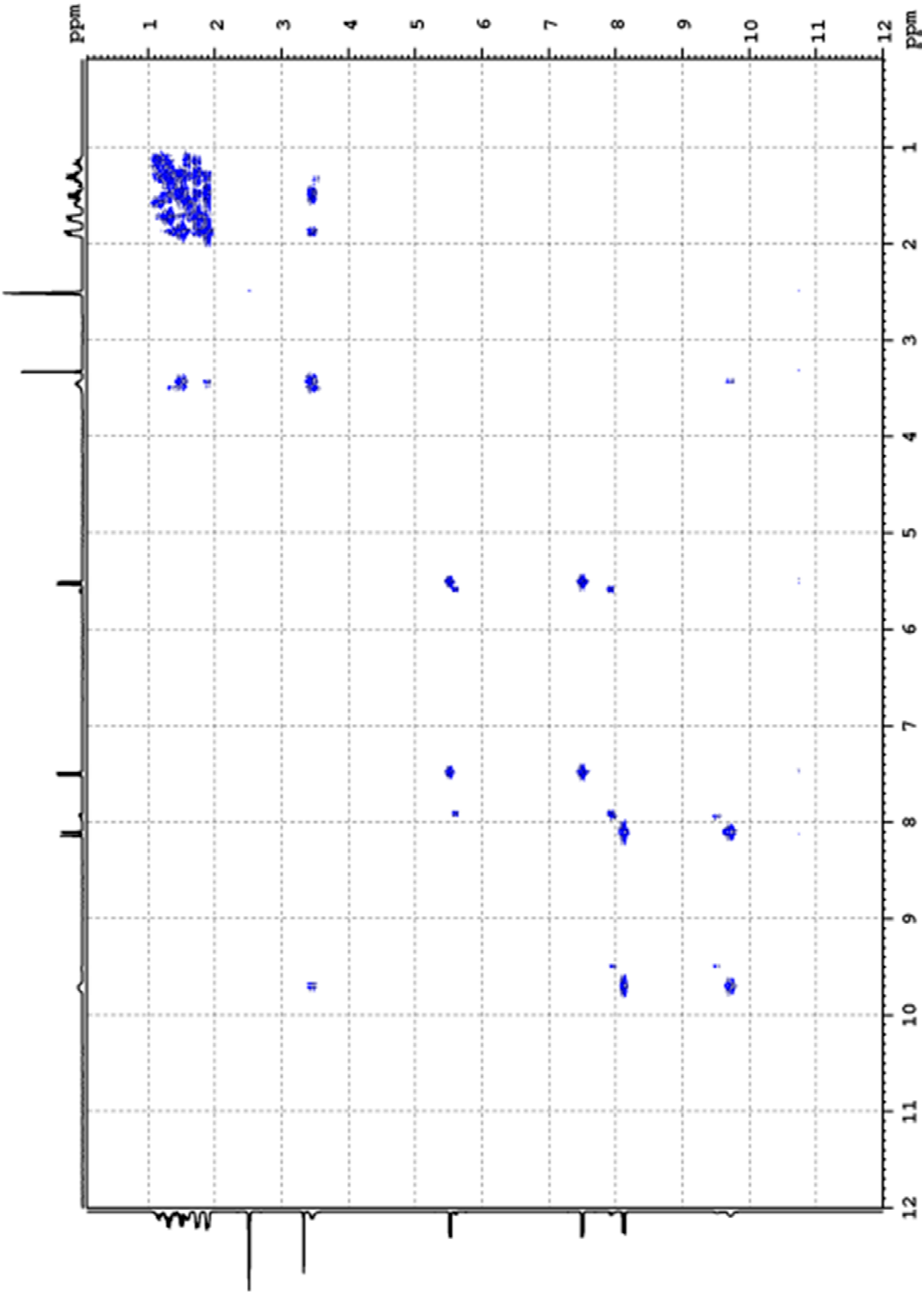
^{1}H NMR spectrum of compound **14**



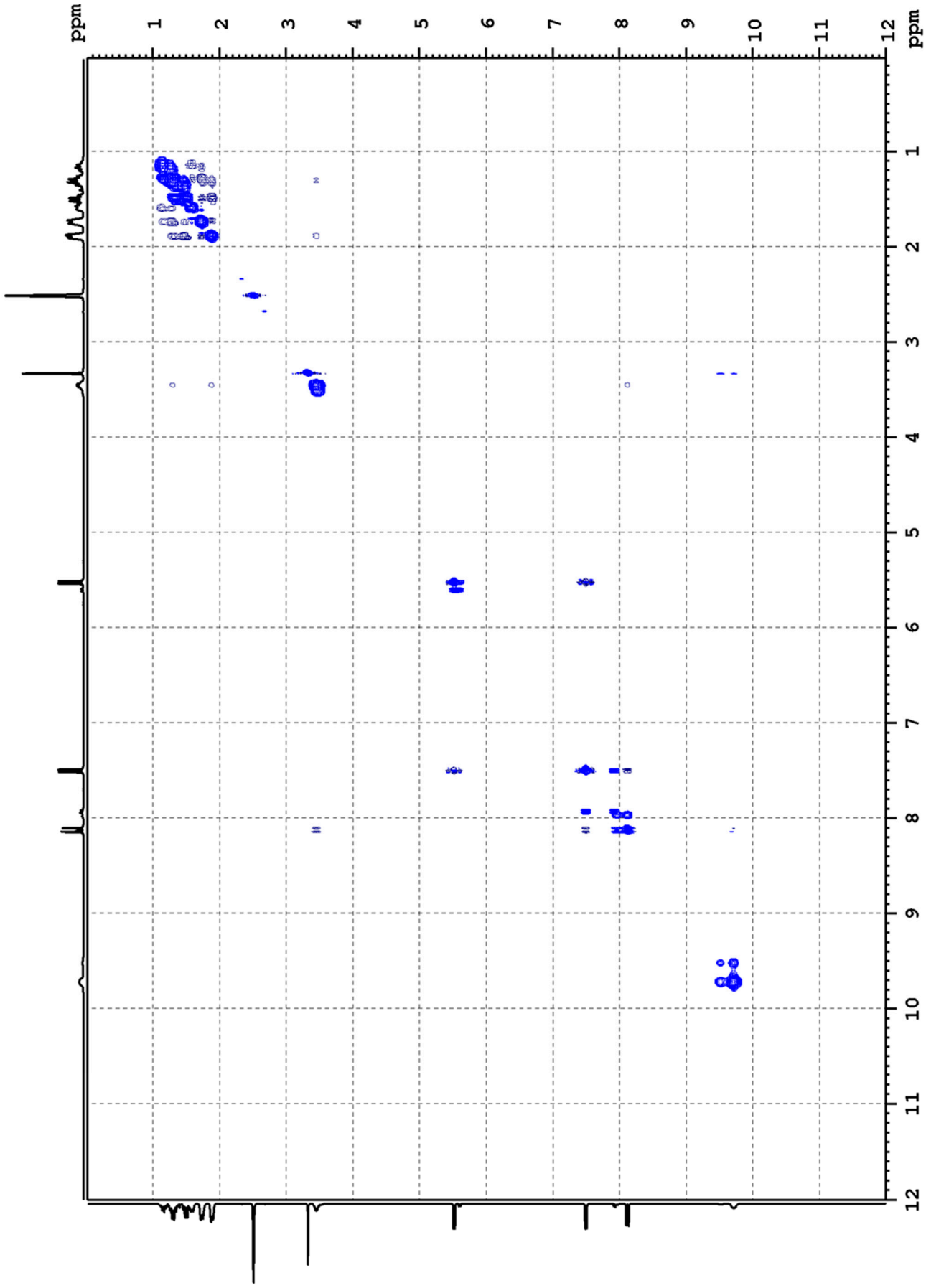
^{1}H NMR spectrum of compound **14** without (a) and in presence (b) of D_2O



^{1}H-^{1}H NMR-COSY spectrum of compound **14**



^{1}H-^{1}H NMR-NOESY spectrum of compound **14**



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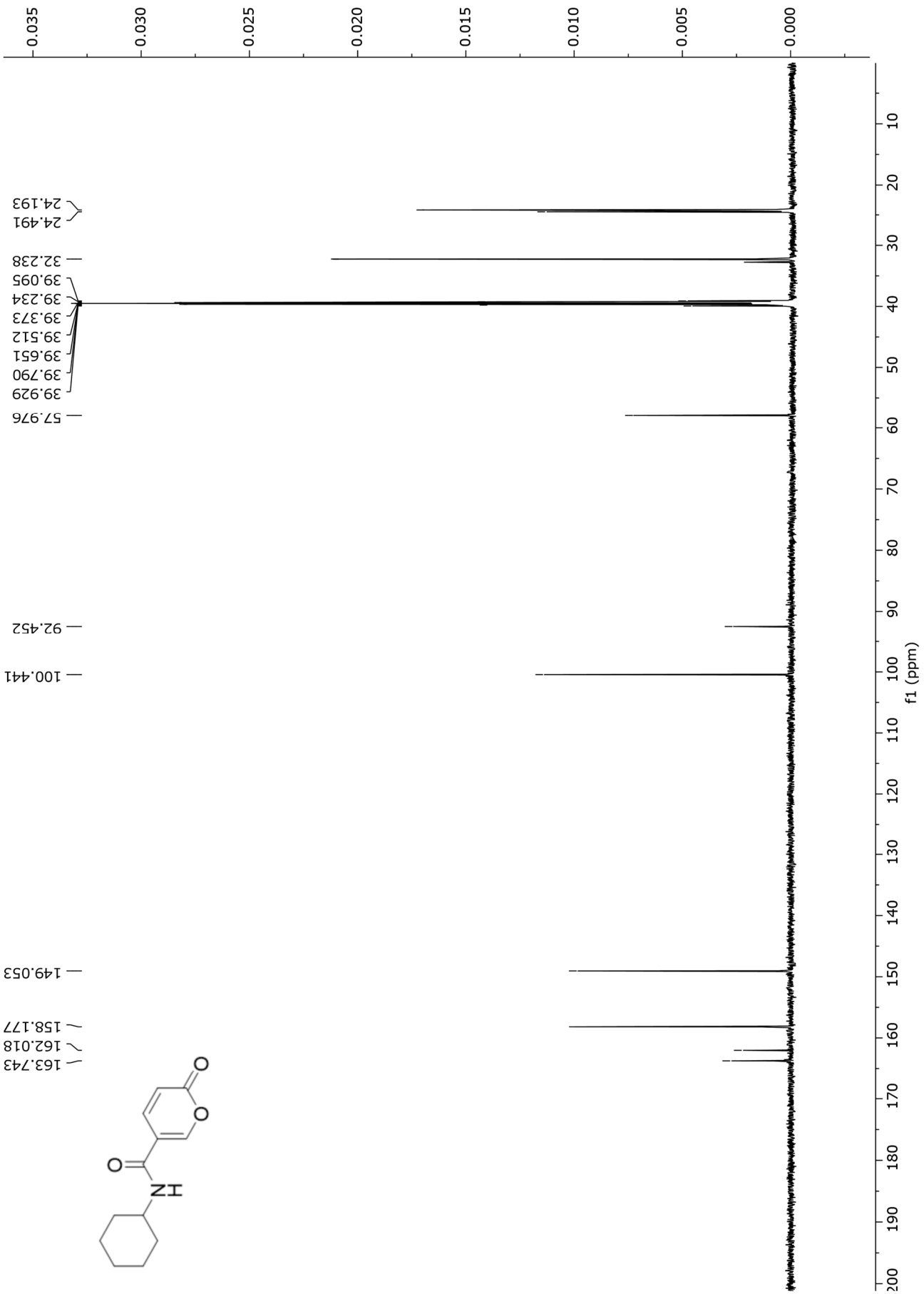

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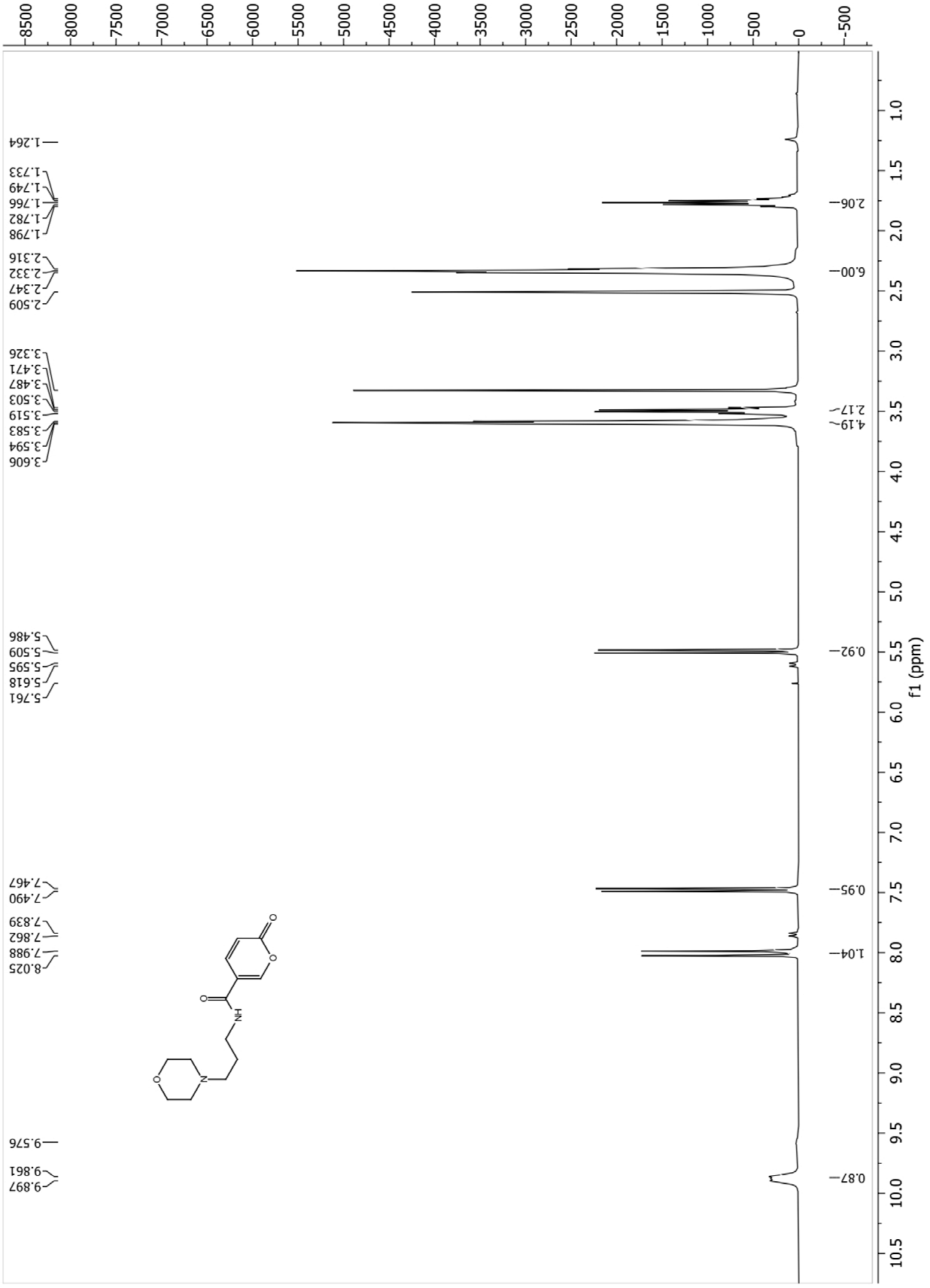

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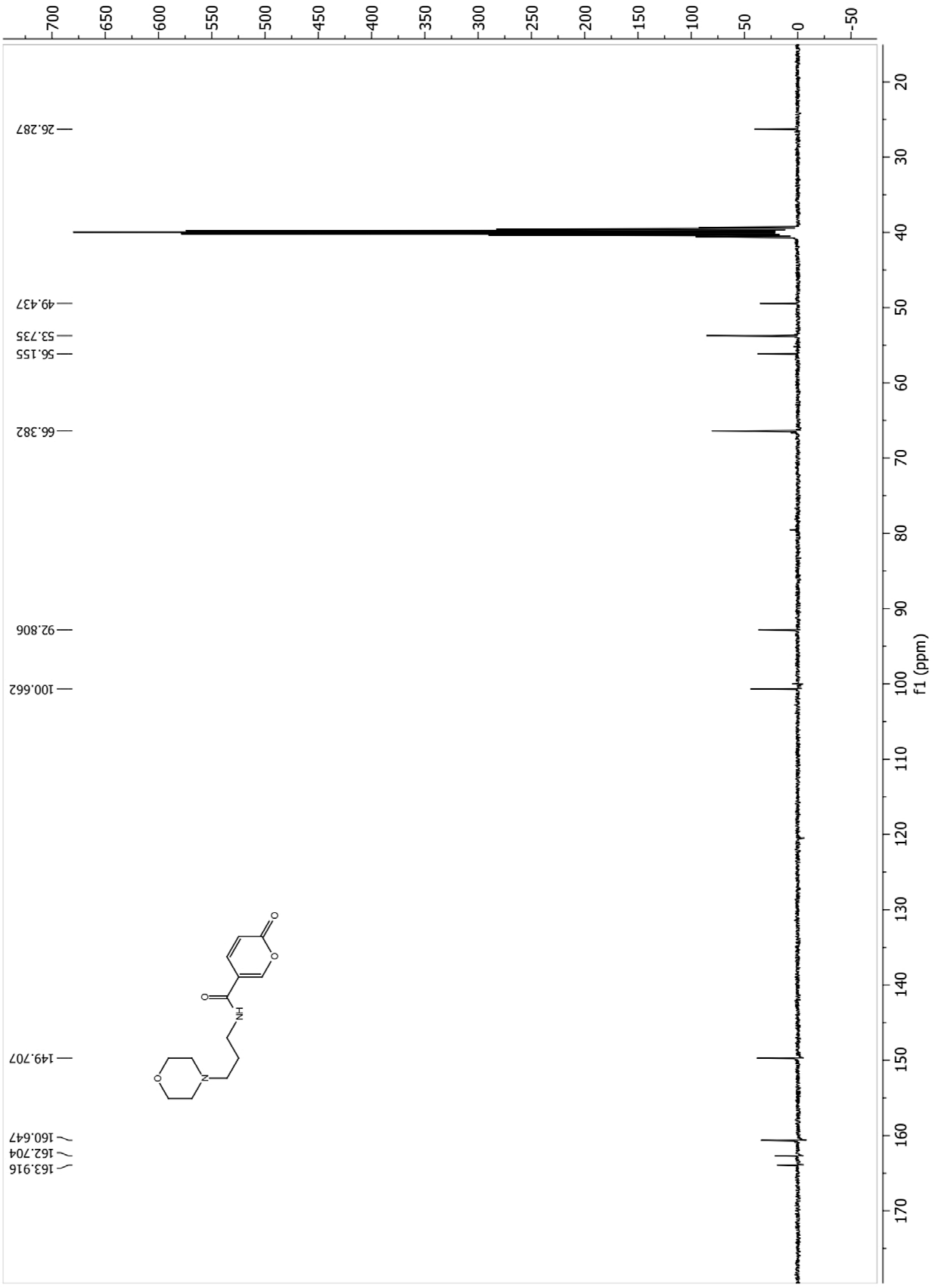
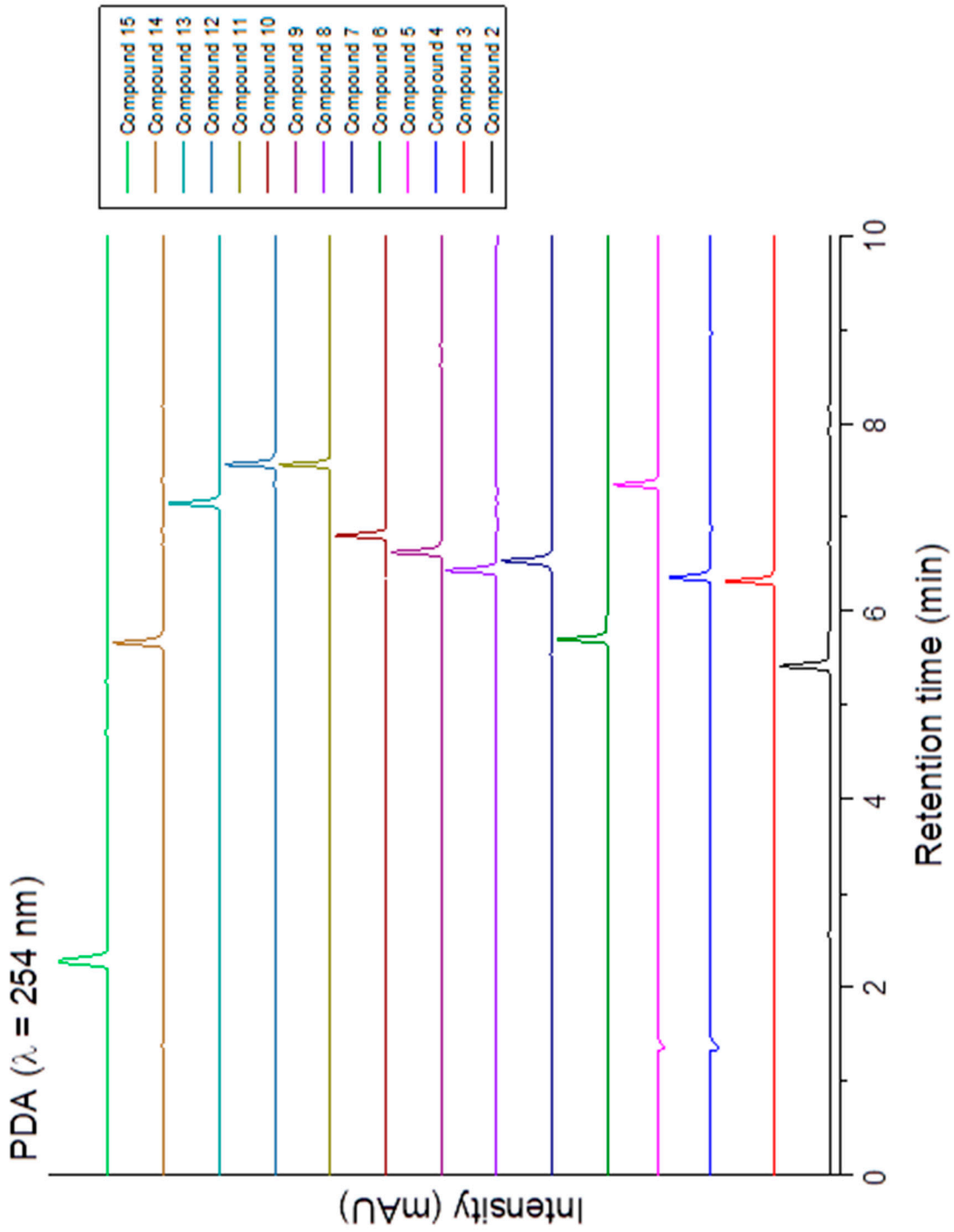
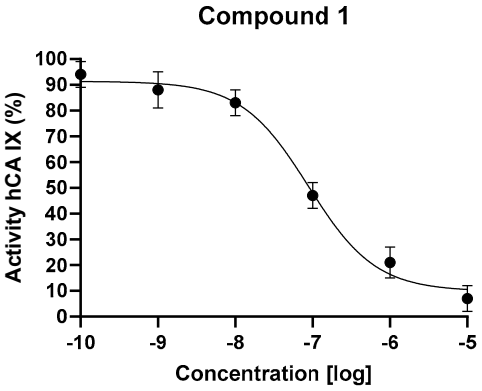
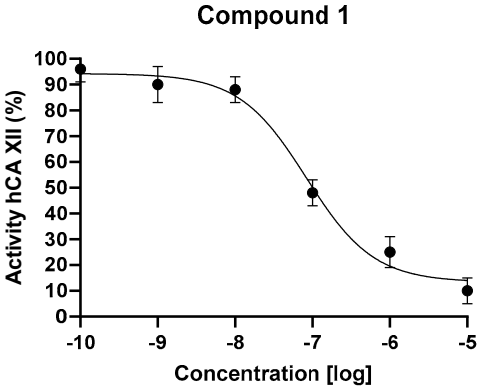


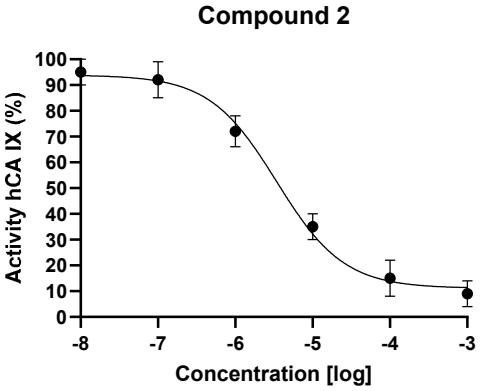
HPLC chromatograms of compounds 2-15

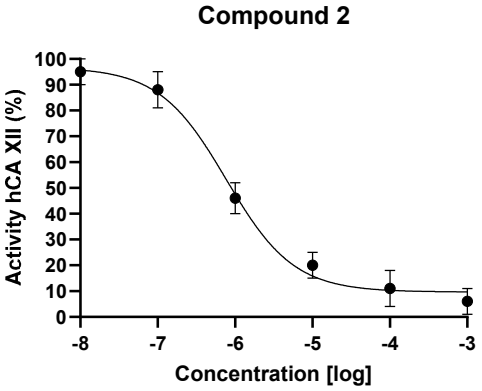


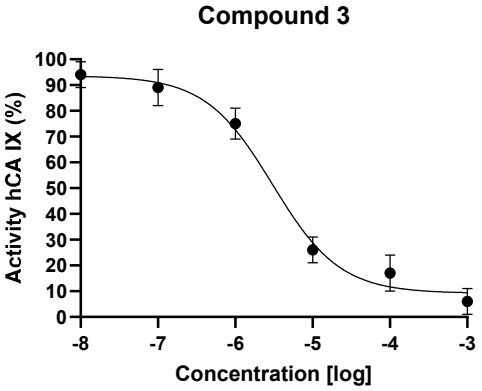
Enzyme inhibition curves of compounds 2-15

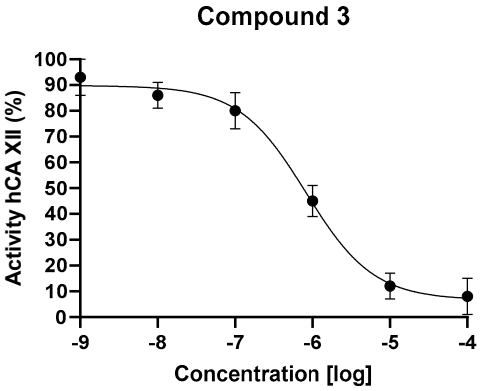


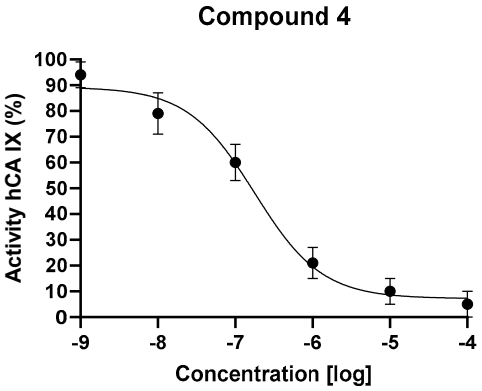


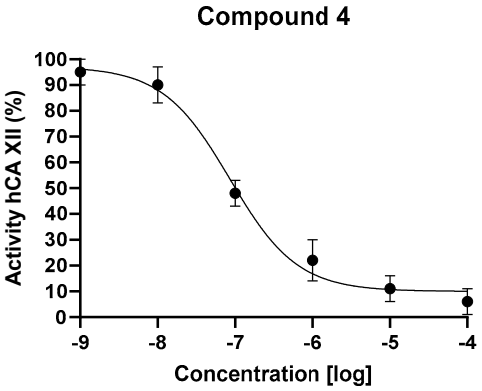


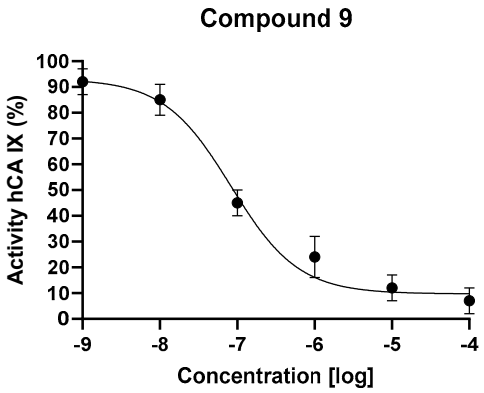


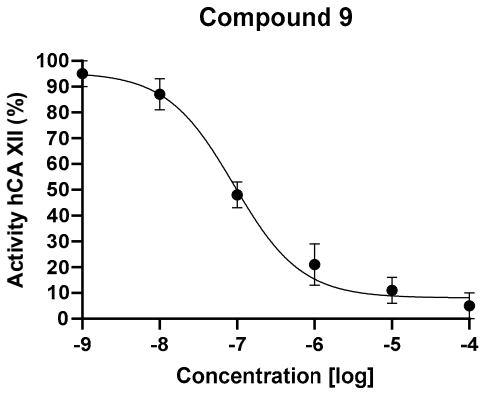


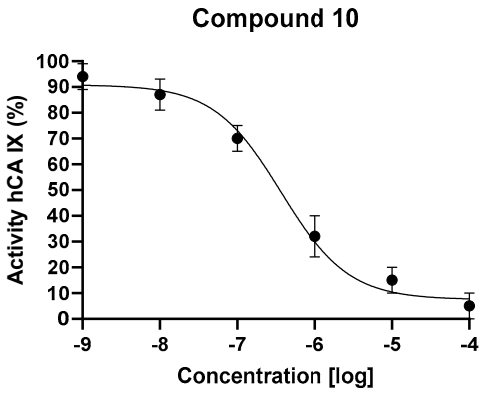


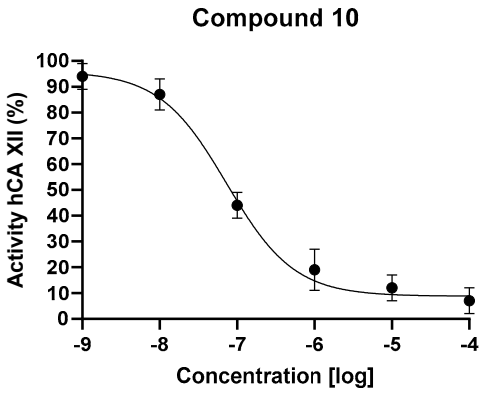


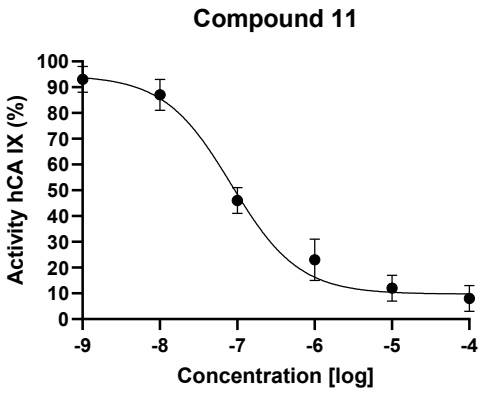


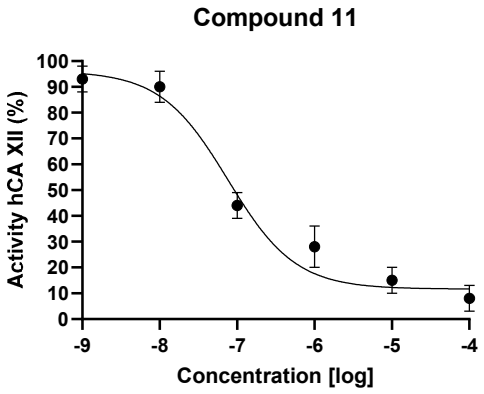

Compound 9
Activity hCA IX (%)
Concentration [log]

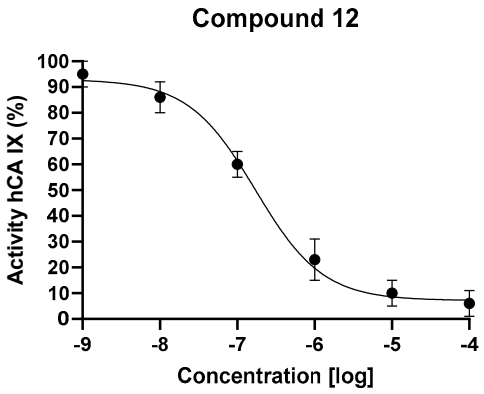

Compound 9
Activity hCA XII (%)
Concentration [log]

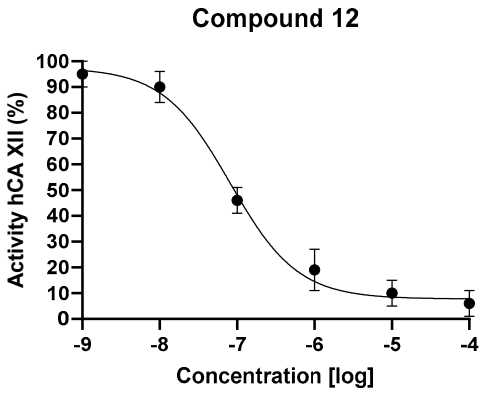

Compound 10
Activity hCA IX (%)
Concentration [log]

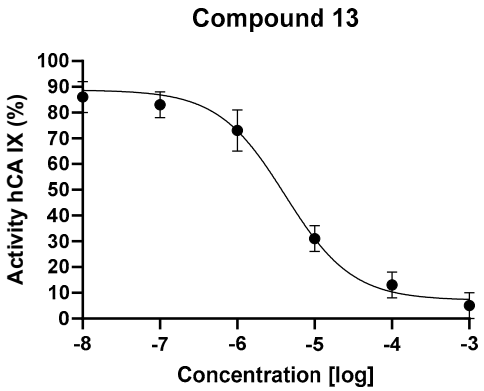

Compound 10
Activity hCA XII (%)
Concentration [log]

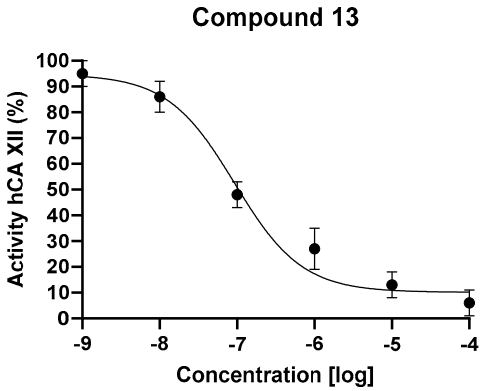

Compound 11
Activity hCA IX (%)
Concentration [log]

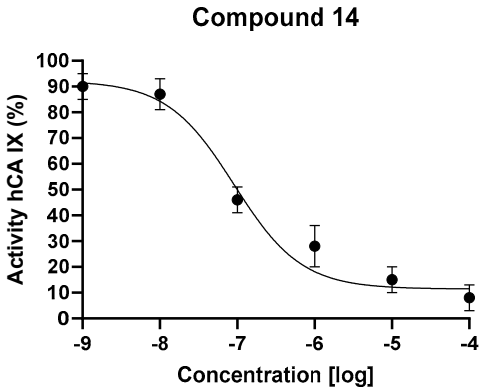

Compound 11
Activity hCA XII (%)
Concentration [log]

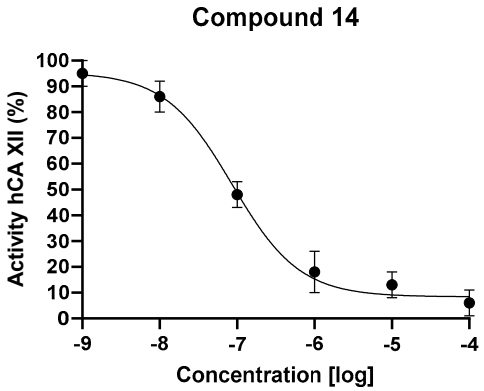

Compound 12
Activity hCA IX (%)
Concentration [log]

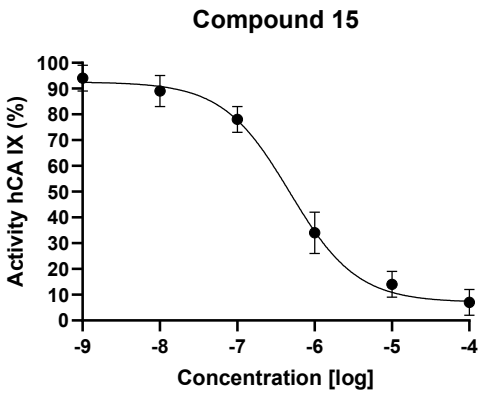

Compound 12
Activity hCA XII (%)
Concentration [log]


Compound 13
Activity hCA IX (%)
100
90
80
70
60
50
40
30
20
10
0
-8
-7
-6
-5
-4
-3
Concentration [log]


Compound 13
Activity hCA XII (%)
100
90
80
70
60
50
40
30
20
10
0
-9
-8
-7
-6
-5
-4
Concentration [log]


Compound 14
Activity hCA IX (%)
100
90
80
70
60
50
40
30
20
10
0
-9
-8
-7
-6
-5
-4
Concentration [log]


Compound 14
Activity hCA XII (%)
100
90
80
70
60
50
40
30
20
10
0
-9
-8
-7
-6
-5
-4
Concentration [log]


Compound 15
Activity hCA IX (%)
100
90
80
70
60
50
40
30
20
10
0
-9
-8
-7
-6
-5
-4
Concentration [log]

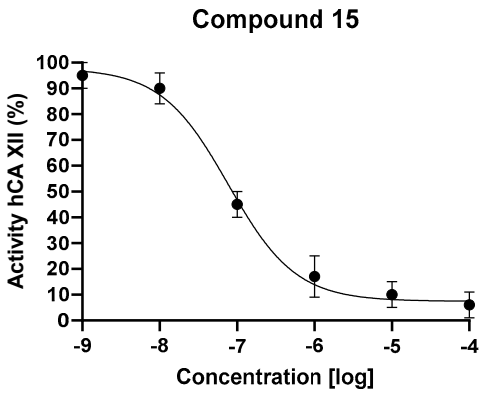
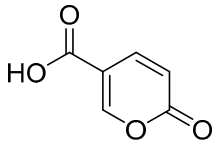
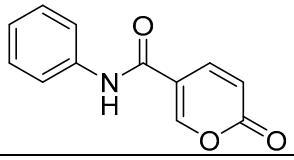
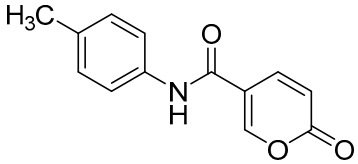
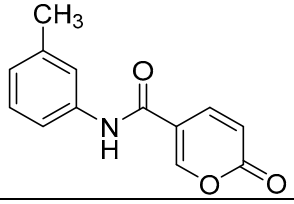
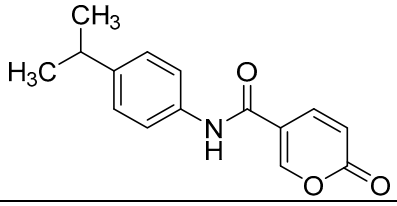
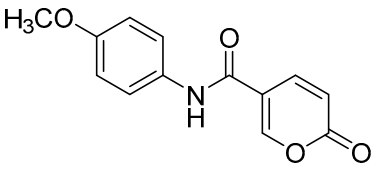
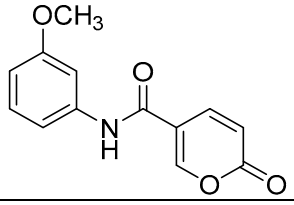
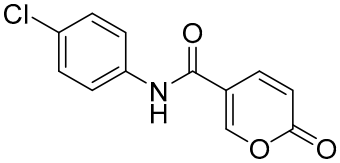
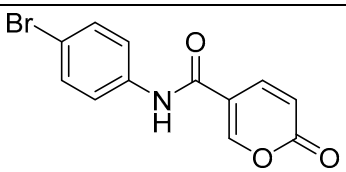
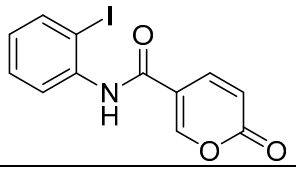
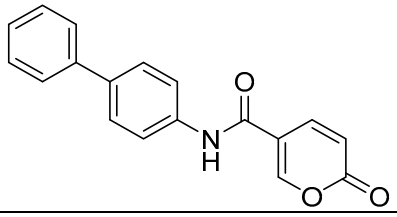
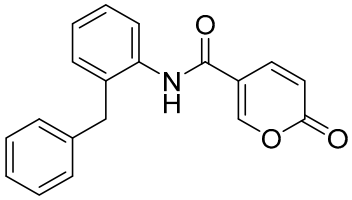
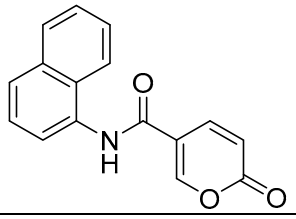
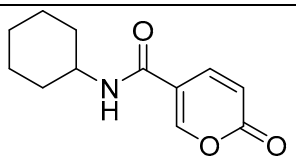
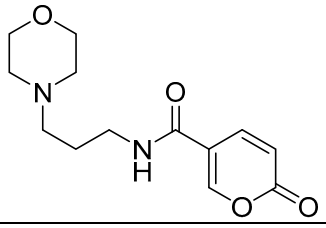

Compound 15
Activity hCA XII (%)
100
90
80
70
60
50
40
30
20
10
0
-9
-8
-7
-6
-5
-4
Concentration [log]

Table S2. Instrument parameters for exact mass determination

Positive Ion Mode	
Compound	**Instrument parameters**
7	Spray Voltage (|kV|): 4.50, Sheath Gas Flow Rate: 7, Aux Gas Flow Rate: 0, Swe Gas Flow Rate: 0. Capillary Voltage (V): 30.00, Capillary Temperature: 270°C, T Lens voltage (V): 50.00, Skimmer Voltage (V): 22.00.
12	Spray Voltage (|kV|): 4.50, Sheath Gas Flow Rate: 7, Aux Gas Flow Rate: 0, Swe Gas Flow Rate: 0. Capillary Voltage (V): 67.50, Capillary Temperature: 270°C, T Lens voltage (V): 95.00, Skimmer Voltage (V): 20.00.
13	Spray Voltage (|kV|): 4.50, Sheath Gas Flow Rate: 7, Aux Gas Flow Rate: 0, Swe Gas Flow Rate: 0. Capillary Voltage (V): 32.50, Capillary Temperature: 270°C, T Lens voltage (V): 85.00, Skimmer Voltage (V): 24.00.
14	Spray Voltage (|kV|): 2.50, Sheath Gas Flow Rate: 7, Aux Gas Flow Rate: 0, Swe Gas Flow Rate: 0. Capillary Voltage (V): 60.00, Capillary Temperature: 270°C, T Lens voltage (V): 100.00, Skimmer Voltage (V): 32.00.
Negative Ion Mode	
Compound	**Instrument parameters**
9	Spray Voltage (|kV|): 2.50, Sheath Gas Flow Rate: 5, Aux Gas Flow Rate: 2, Swe Gas Flow Rate: 0. Capillary Voltage (V): -90.00, Capillary Temperature: 200°C, T Lens voltage (V): -90.00, Skimmer Voltage (V): -26.00.
11	Spray Voltage (|kV|): 2.50, Sheath Gas Flow Rate: 5, Aux Gas Flow Rate: 2, Swe Gas Flow Rate: 0. Capillary Voltage (V): -90.00, Capillary Temperature: 200°C, T Lens voltage (V): -90.00, Skimmer Voltage (V): -26.00.

Table S2. Purification conditions for compounds **2-15**.

Entry	Structure	Purification method	
		Chromatography	**Crystallization**
1		Commercial	/
2		Stationary phase: silica gel Mobile phase: ethyl acetate/cyclohexane (33% *v*/*v*)	
3		Stationary phase: silica gel Mobile phase: ethyl acetate/cyclohexane (33% *v*/*v*).	Mixture of dichloromethane/cyclohexane (4:1 ratio v/v).
4		Stationary phase: silica gel Mobile phase: ethyl acetate/cyclohexane (33% *v*/*v*).	Mixture of dichloromethane/cyclohexane (4:1 ratio *v*/*v*).
5		Stationary phase: silica gel Mobile phase: ethyl acetate/cyclohexane (33% *v*/*v*).	Mixture of dichloromethane/cyclohexane (4:1 ratio *v*/*v*).
6		Stationary phase: silica gel Mobile phase: ethyl acetate/cyclohexane (33% *v*/*v*).	Mixture of dichloromethane/cyclohexane (4:1 ratio *v*/*v*).
7		Stationary phase: silica gel Mobile phase: ethyl acetate/cyclohexane (33% *v*/*v*).	Mixture of dichloromethane/cyclohexane (4:1 ratio *v*/*v*).
8		Stationary phase: silica gel Mobile phase: ethyl acetate/cyclohexane (33% *v*/*v*).	Mixture of dichloromethane/cyclohexane (4:1 ratio *v*/*v*).
9		Stationary phase: silica gel Mobile phase: ethyl acetate/cyclohexane (33% *v*/*v*)	Mixture of dichloromethane/cyclohexane (4:1 ratio *v*/*v*). Performed two-times.

10		Stationary phase: silica gel Mobile phase: ethyl acetate/cyclohexane (33% *v*/*v*)	Mixture of dichloromethane/cyclohexane (4:1 ratio *v*/*v*).
11		Stationary phase: silica gel Mobile phase: ethyl acetate/cyclohexane (33% *v*/*v*).	Mixture of dichloromethane/cyclohexane (4:1 ratio *v*/*v*). Performed two-times.
12		Stationary phase: silica gel Mobile phase: ethyl acetate/cyclohexane (33% *v*/*v*)	Mixture of dichloromethane/cyclohexane (4:1 ratio *v*/*v*). Performed two-times.
13		Stationary phase: silica gel Mobile phase: ethyl acetate/cyclohexane (33% *v*/*v*)	Mixture of dichloromethane/cyclohexane (4:1 ratio *v*/*v*). Performed two-times.
14		Stationary phase: silica gel Mobile phase: ethyl acetate/cyclohexane (33% *v*/*v*)	Mixture of dichloromethane/cyclohexane (4:1 ratio *v*/*v*). Performed two-times.
15		Stationary phase: silica gel Mobile phase: ethyl acetate/cyclohexane (33% *v*/*v*)	Mixture of dichloromethane/cyclohexane (4:1 ratio *v*/*v*). Performed two-times.

High resolution mass analysis of compound 7

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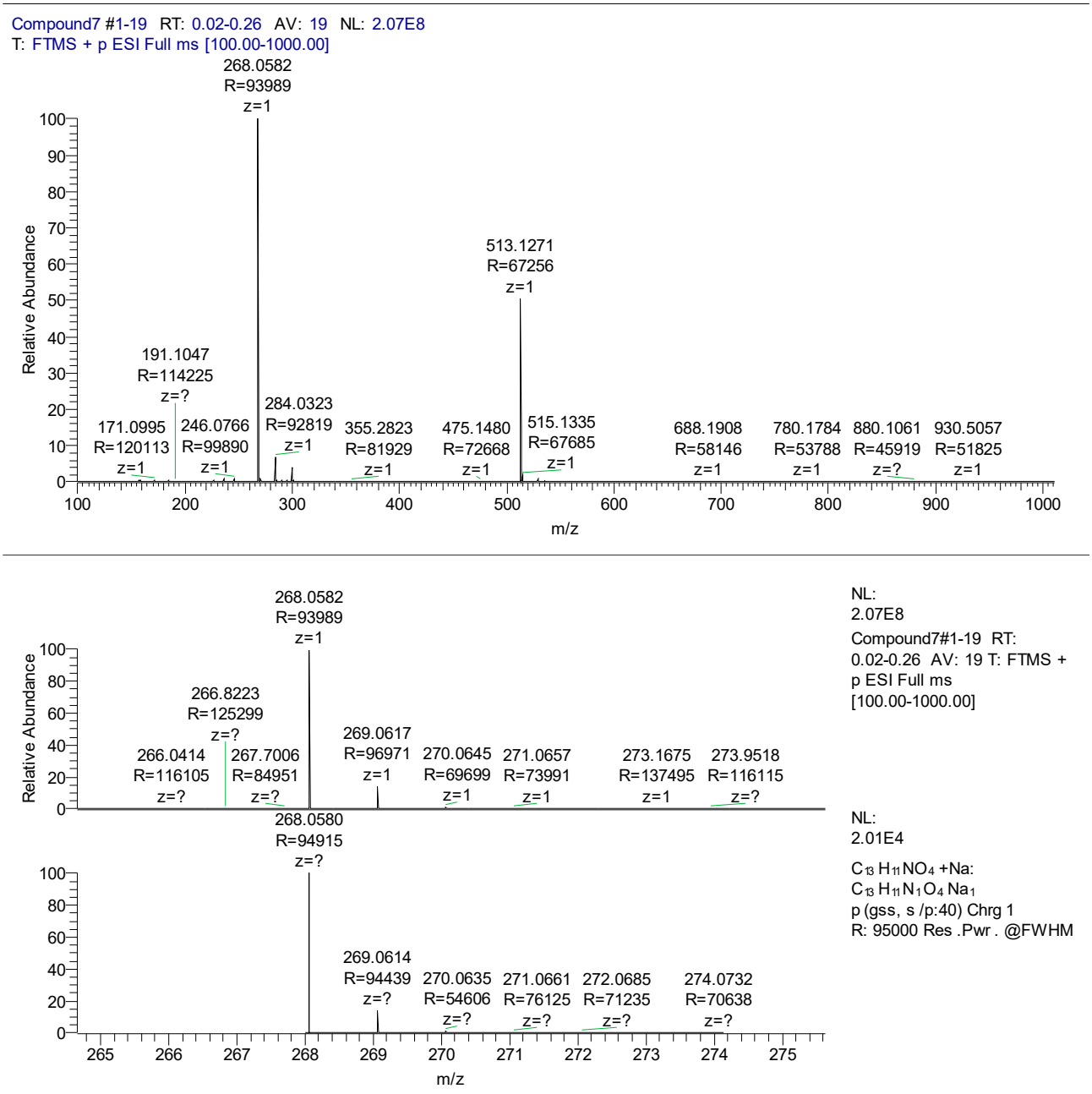

NL:
2.07E8
Compound7#1-19 RT: 0.02-0.26 AV: 19 T: FTMS + p ESI Full ms [100.00-1000.00]

NL:
2.01E4
$C_{13}H_{11}NO_4$ +Na:
$C_{13}H_{11}N_1O_4Na_1$
p (gss, s /p:40) Chrg 1
R: 95000 Res .Pwr . @FWHM

High resolution mass analysis of compound 9

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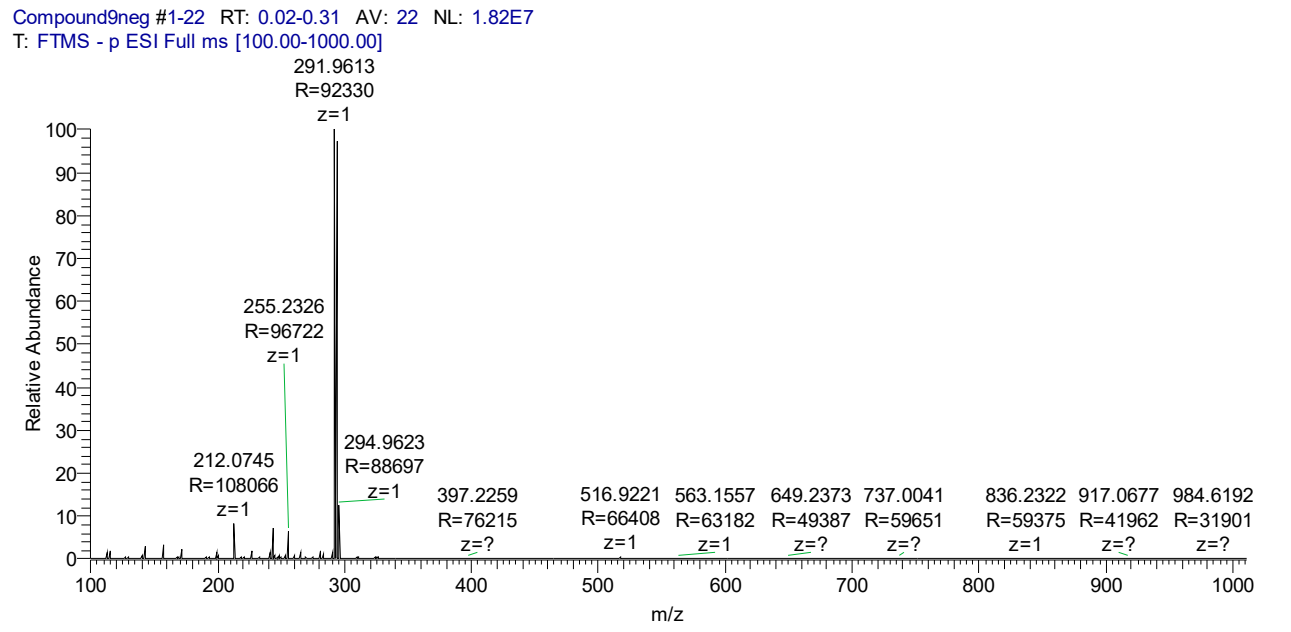

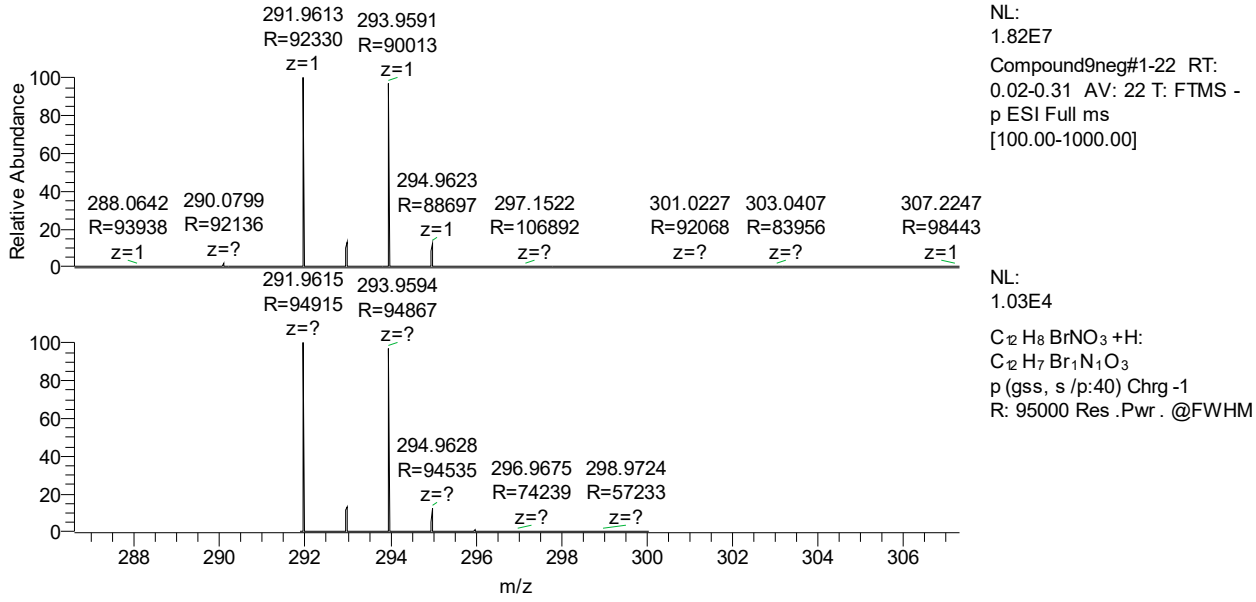

High resolution mass analysis of compound 11

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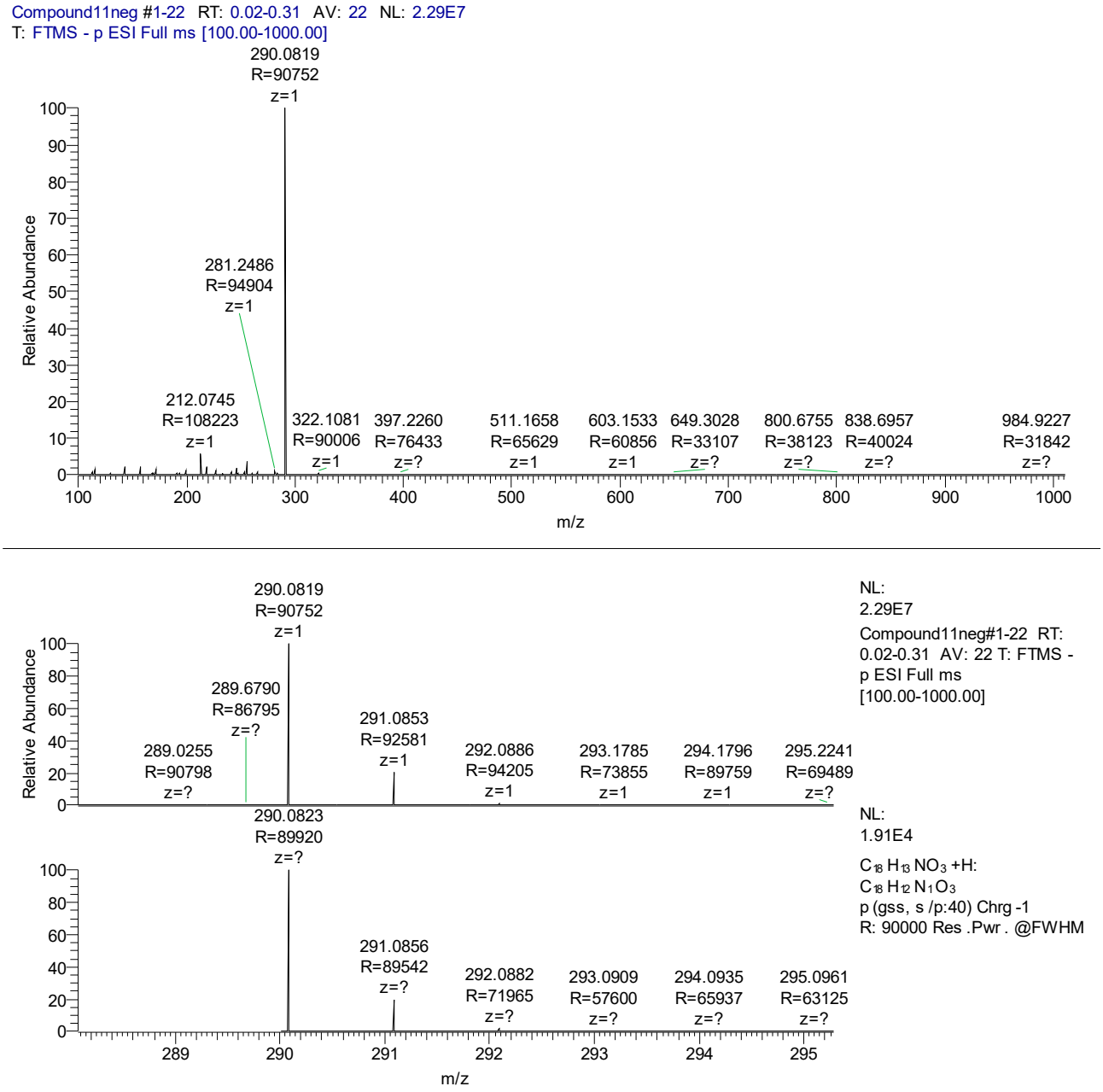

High resolution mass analysis of compound 12

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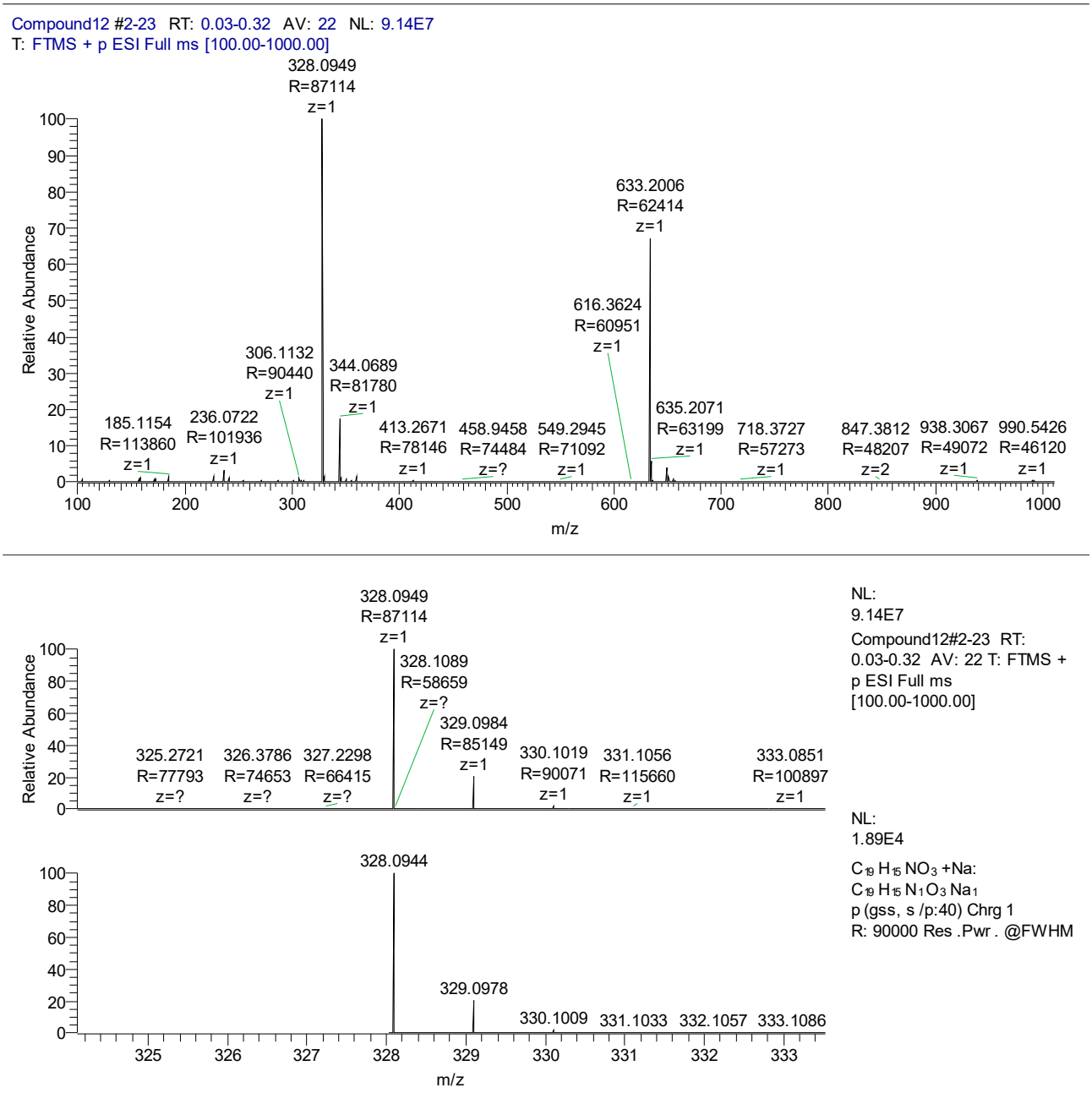
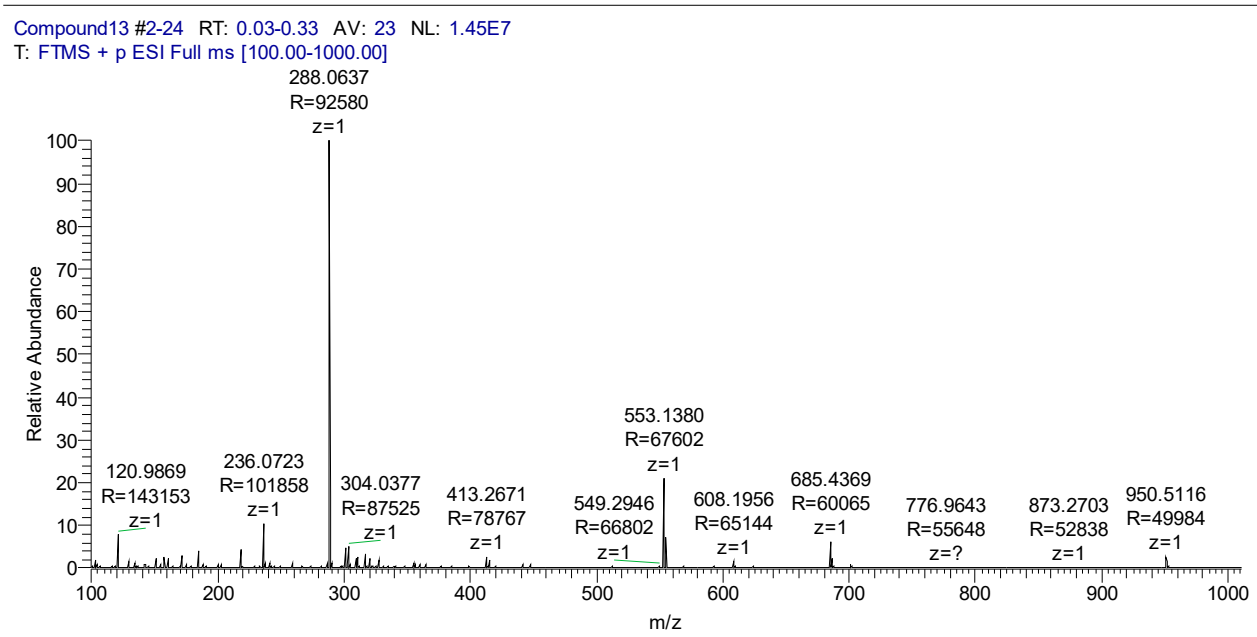
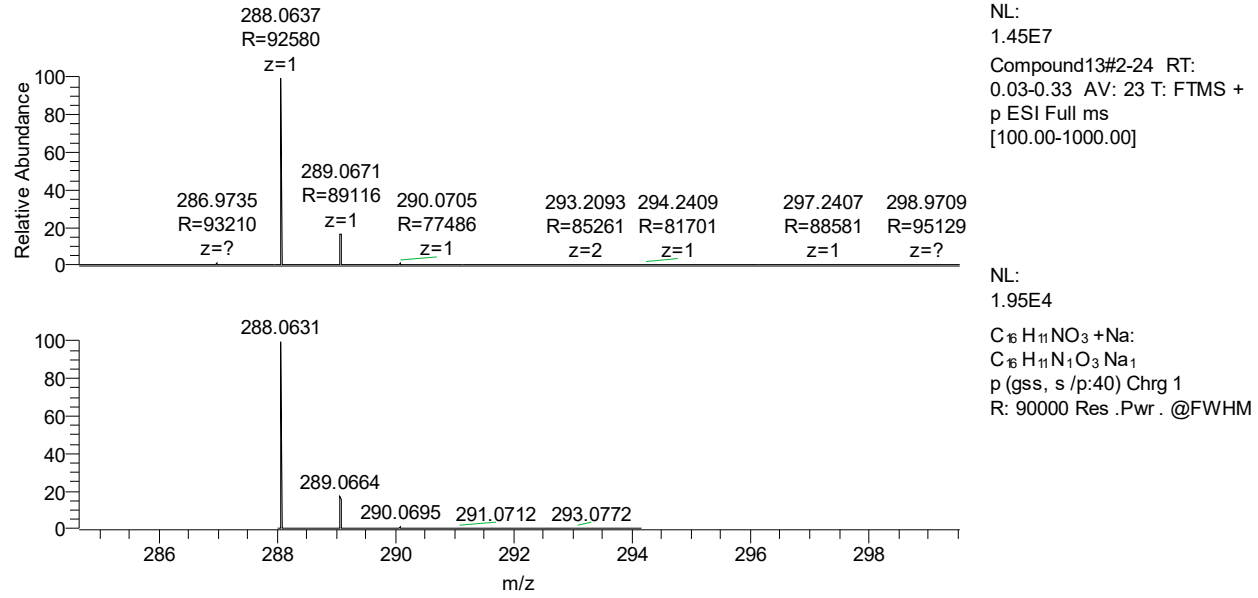


High resolution mass analysis of compound 13

C:\Xcalibur\...\Secci\Compound13 6/23/2022 6:24:33 PM





High resolution mass analysis of compound 14

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