Supplemental material for

Mapping peptide thiol accessibility in membranes using a quaternary ammonium isotope-coded mass tag (ICMT)

Chiao-Yung Su,[†] Erwin London, [†,§] and Nicole S. Sampson[†,*]

[†]Department of Chemistry, Stony Brook University, Stony Brook, NY 11794-3400, [§]Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY 11794-5215

RUNNING TITLE: Peptide thiol ICMT maps membrane topology

*Correspondence should be addressed to:

Dr. Nicole S. Sampson

Department of Chemistry

Stony Brook University

Stony Brook, NY 11794-3400

Phone: 631-632-7952

FAX: 631-632-5731

E-mail: nicole.sampson@stonybrook.edu

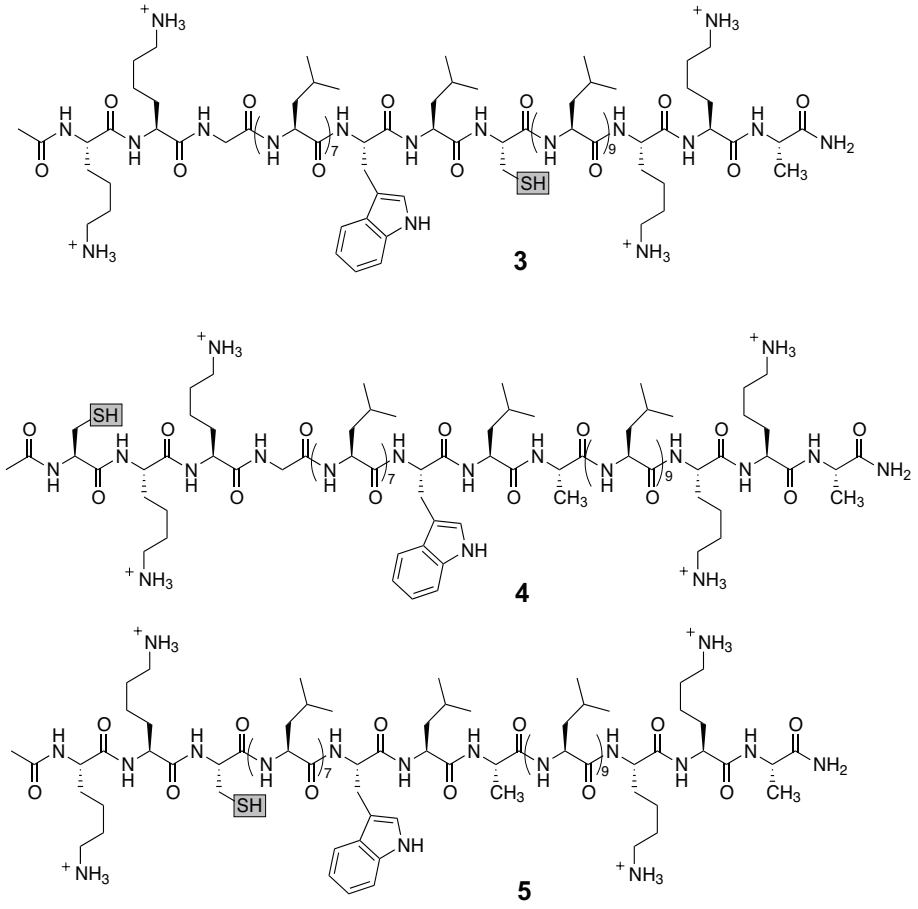


Figure S1. **Structures of model α-helical peptides.** Peptide **3** (cysteine inaccessible), peptide **4** (cysteine accessible), and peptide **5** (cysteine partially accessible).

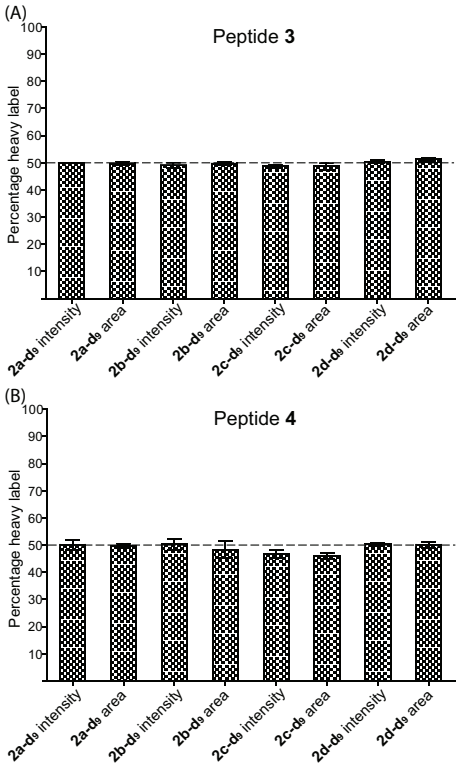


Figure S2. Accuracy and precision of labeling peptide 3 and peptide 4 with ICMT probes. Peptide **3** (panel A) or peptide **4** (50 μM) (panel B) dissolved in 0.5% (w/v) SDS, 50 mM phosphate, pH 7.0 was labeled with a mixture of light (9 mM) and heavy (9 mM) ICMT probe. The percentage of peptide labeled with heavy probe was determined from either the average intensity of the isotopomer in each envelope or the area under the entire isotopic envelope. Average values from three replicate experiments and the standard deviations are shown. The dashed line represents the expected value of 50%.

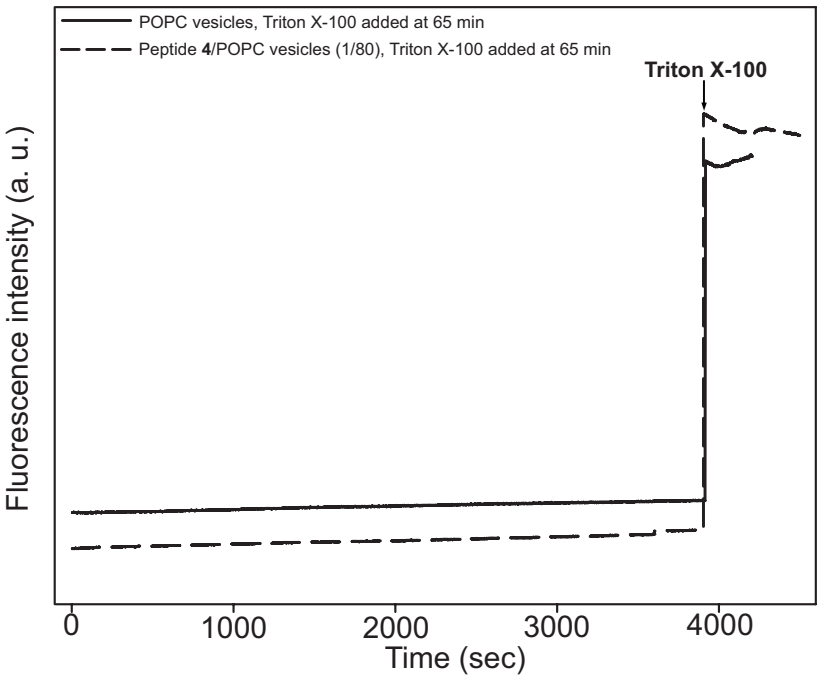


Figure S3. Vesicle permeability assay. Carboxyfluorescein (100 mM) was encapsulated in peptide **4**/POPC (1/80) vesicles or POPC vesicles (285 μM total lipid). Fluorescence emission intensities (λ_{ex} = 492 nm, λ_{em} = 517 nm) were monitored for 70 min, and Triton X-100 was added to completely lyse vesicles at 65 min.

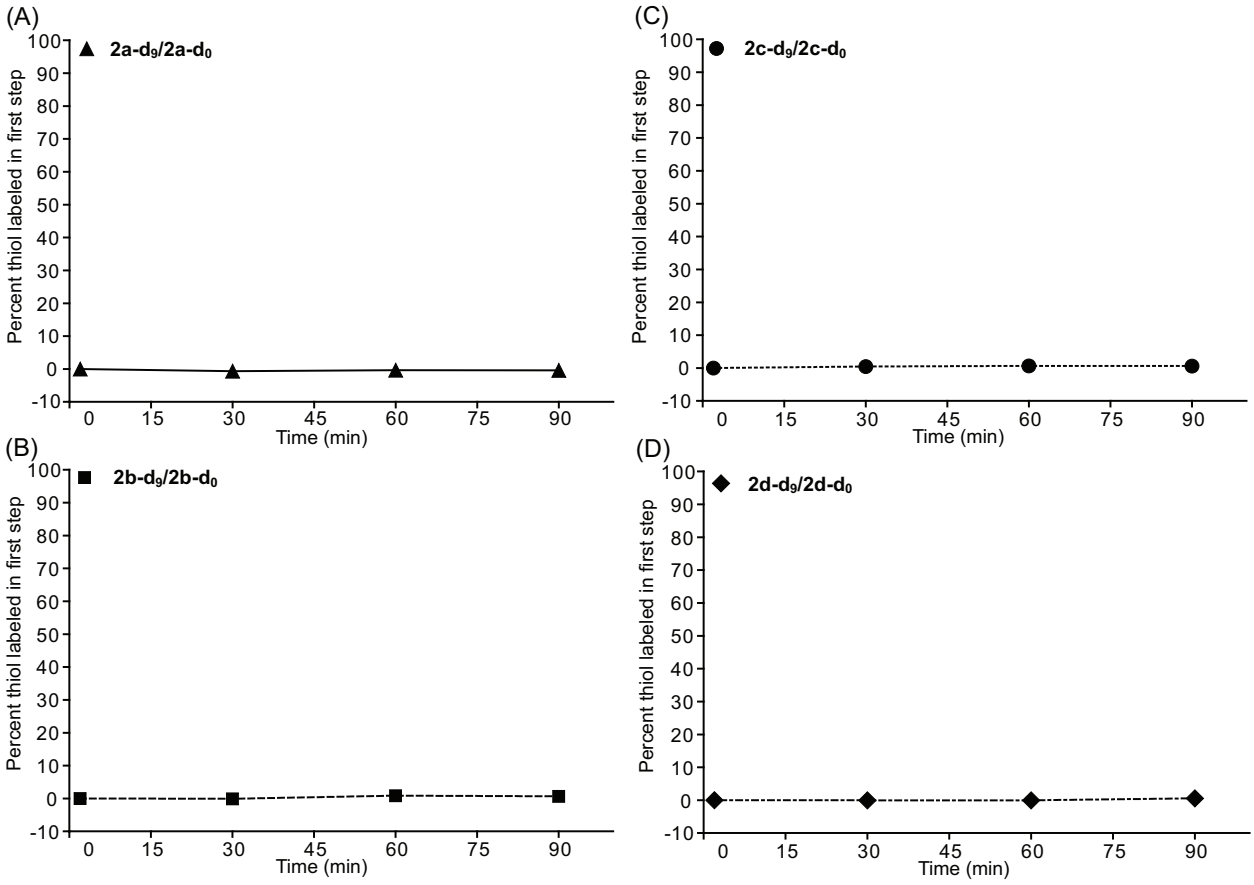


Figure S4. ICMT permeability tests with peptide 4/POPC (1/80) vesicles. Tri-peptide NB-ECD-OMe (3 mM) was encapsulated in peptide **4**/POPC (1/80) vesicles. Intact vesicles were labeled with 6 mM **2a-d$_9$**, **2b-d$_9$**, **2c-d$_9$**, or **2d-d$_9$** heavy probe for 2, 30, 60 or 90 minutes, quenched with 7 mM DTT, 0.03% Triton X-100 to lyse vesicles, and were then labeled with 15 mM **2a-d$_0$**, **2b-d$_0$**, **2c-d$_0$**, or **2d-d$_0$** light probe for 1 hour. The reactions were analyzed by LC/MS and extracted ion chromatograms were integrated. Percentage heavy label indicates the percent of total labeling that occurred with intact vesicles. Average values from three replicate experiments and the standard deviations are shown. Tests with (A) **2a-d$_9$**/**2a-d$_0$** probe. (B) **2b-d$_9$**/**2b-d$_0$** probe. (C) **2c-d$_9$**/**2c-d$_0$** probe. (D) **2d-d$_9$**/**2c-d$_0$** probe.

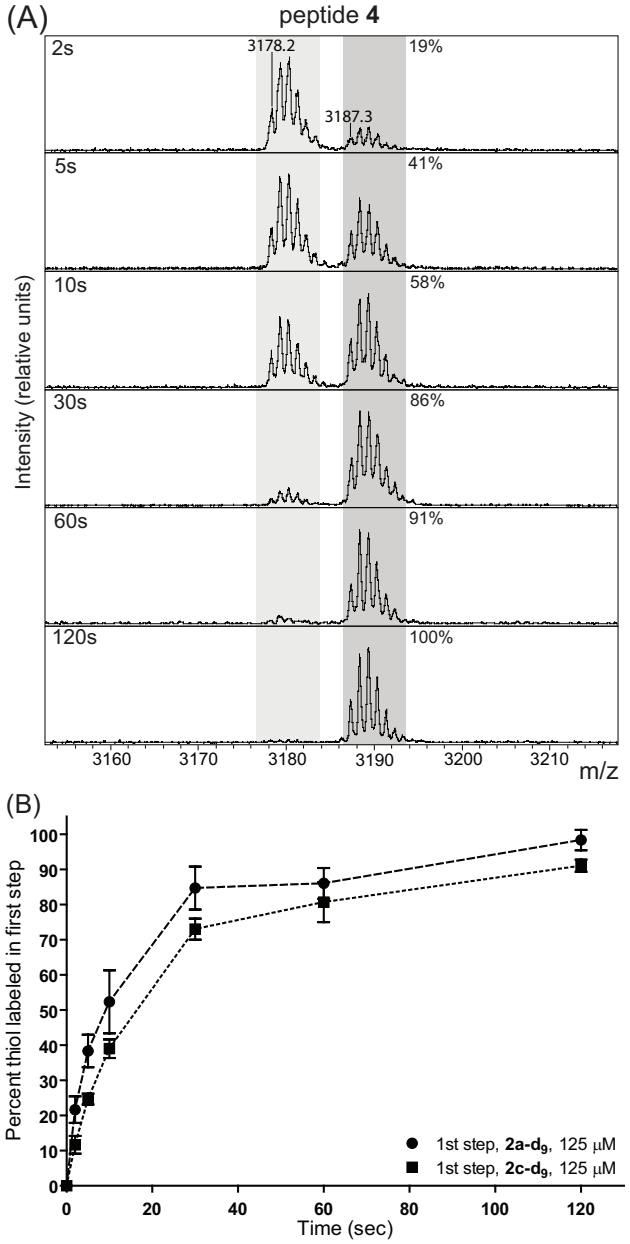
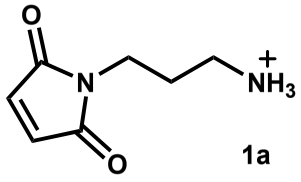


Figure S5. Kinetics of labeling peptide 4/POPC vesicles (1/80). The vesicles were labeled with 125 μM **2a-d$_9$** or **2c-d$_9$**, **after 2, 5, 10, 30, 60, or 120 s** followed by 1.25 mM **2a-d$_0$** or **2c-d$_0$**, respectively. (A) Mass spectra of peptide **4** labeled with **2a-d$_9$/2a-d$_0$** at different time points. (B) Time courses for the two labeling experiments. Integrated areas under the isotopic envelope were used to calculate percent labeling.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N
NH3
O

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2.06
2.00
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9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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4.0
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3.0
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1.5
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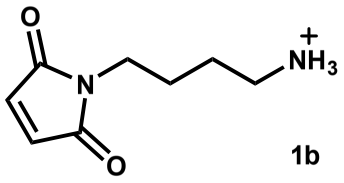
0.5
0.
ppm



1b

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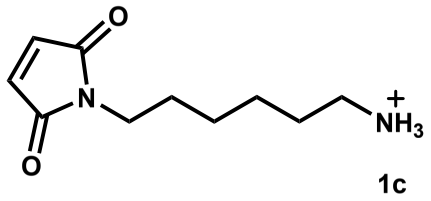
ppm



1c

S9

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2.00
4.06
4.14

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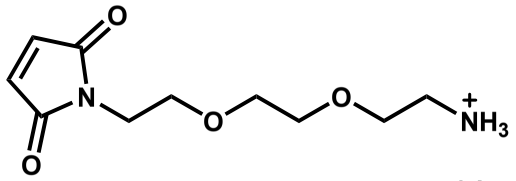

1d

1.61

10.13

2.00

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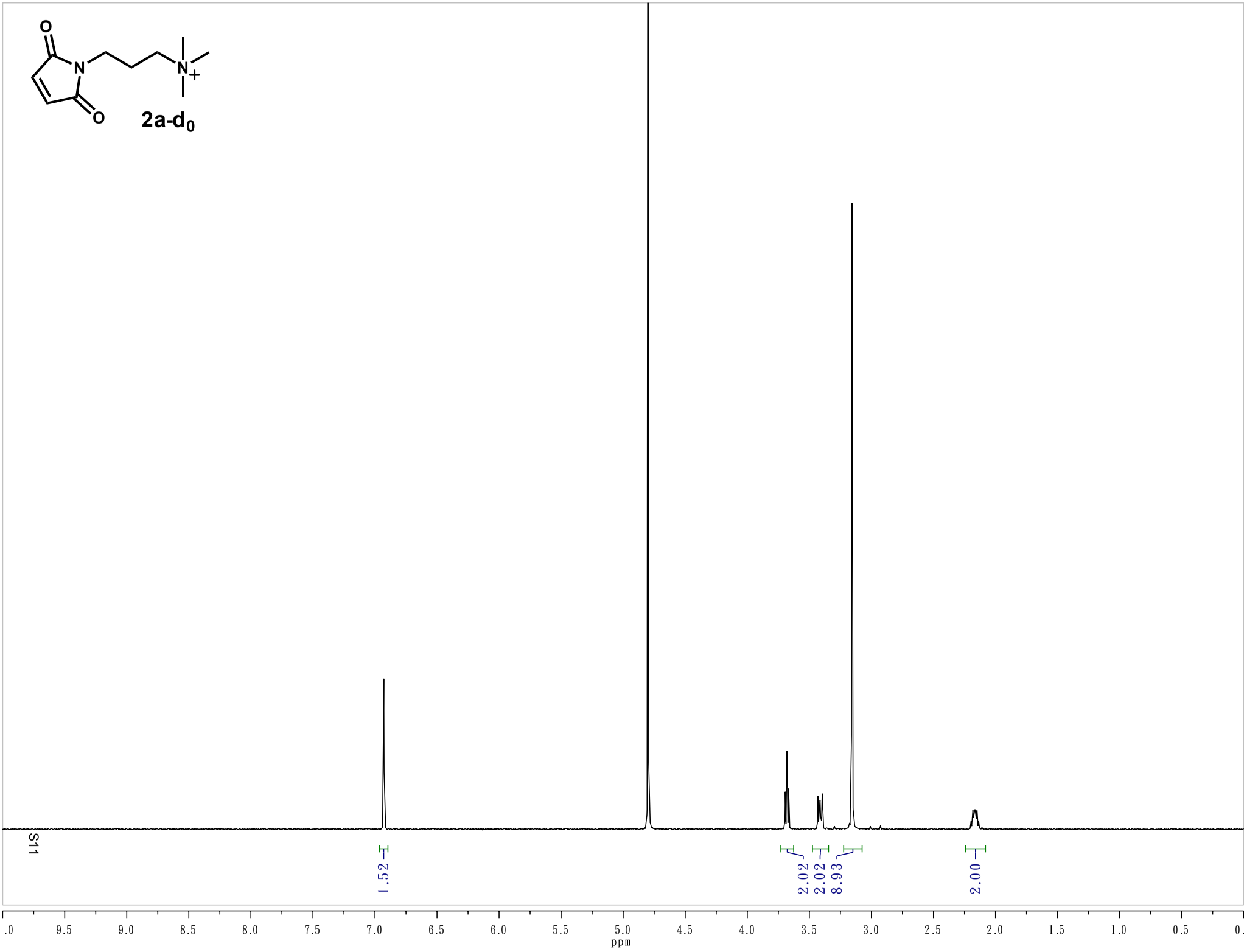
ppm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N+
O
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9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
ppm

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10
5
0
[ppm]
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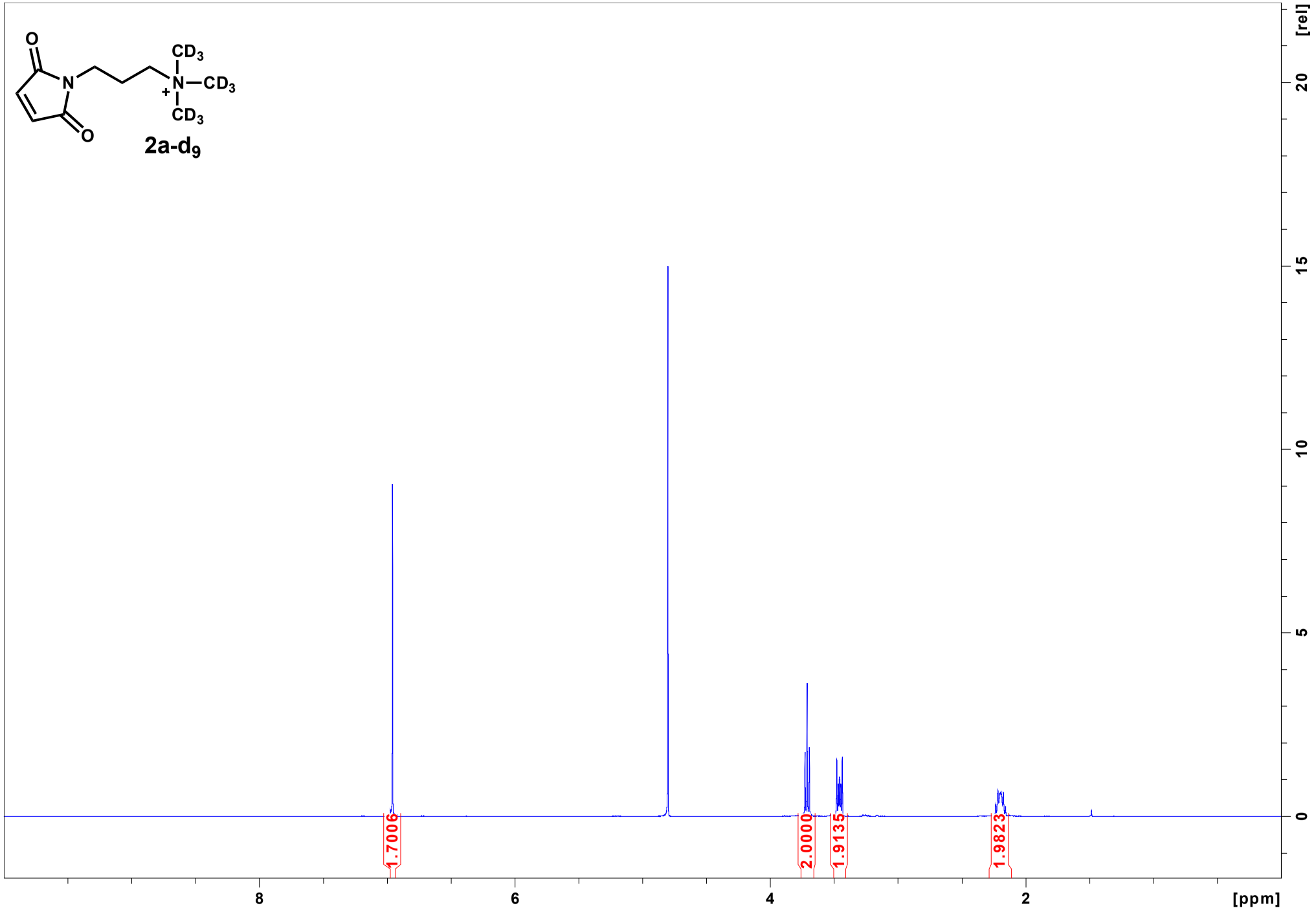
172.9189
2a-d0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O
CD3
N
CD3
CD3
+
2a-d9
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6
4
2
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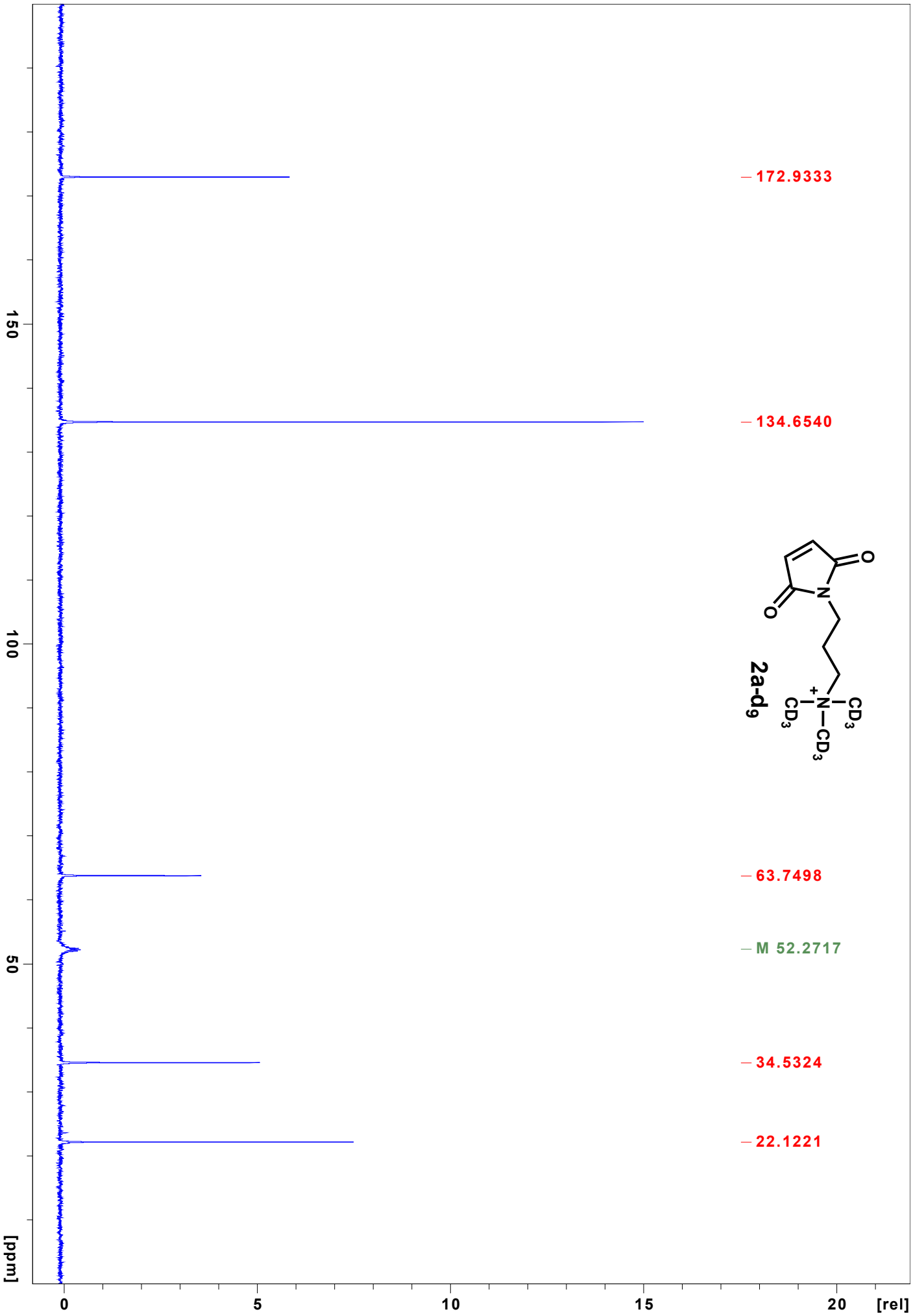
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20
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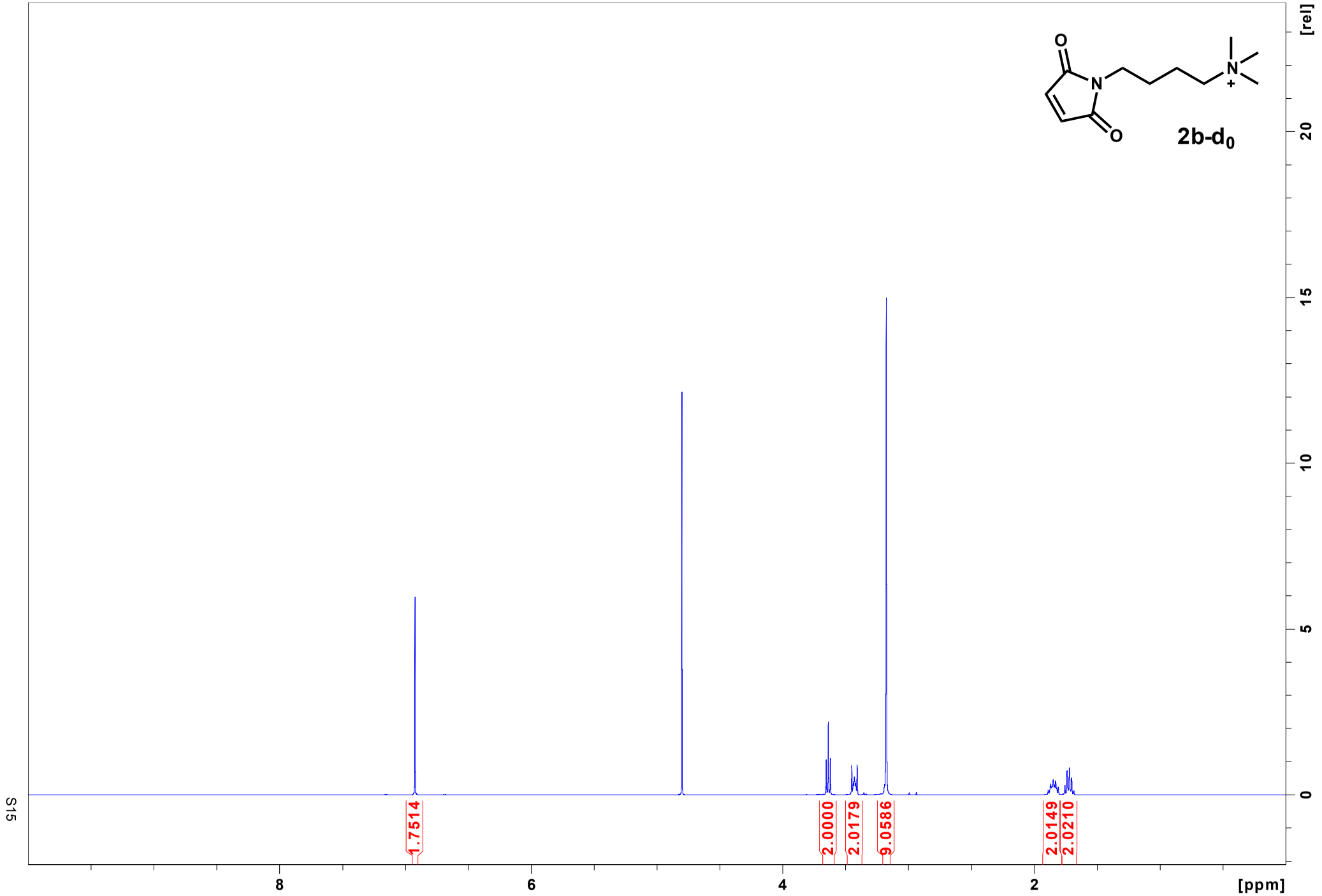
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N
O
N+
2b-d0
[rel]
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4
2
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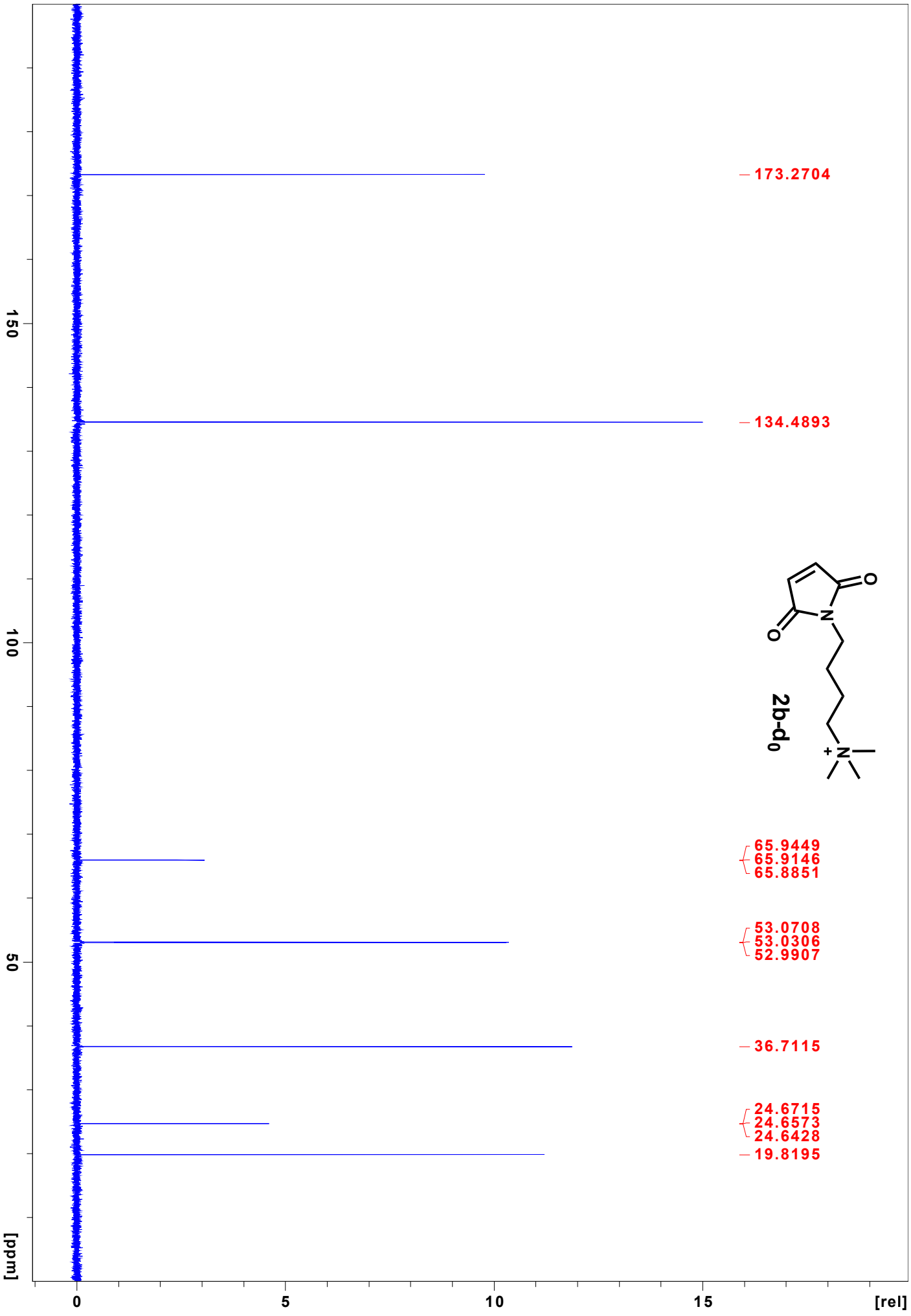
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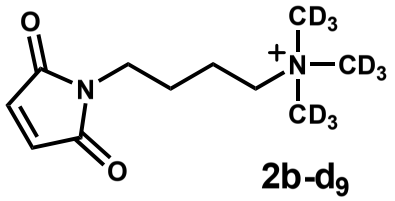

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ppm

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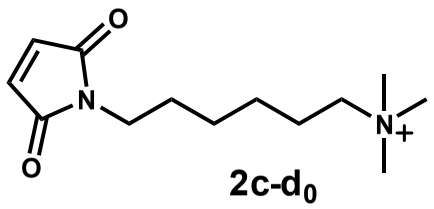
2b-d9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N
O
N+

2c-d_0

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2.00
2.00
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9.0
8.5
8.0
7.5
7.0
6.5
6.0
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3.0
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0.5
0.
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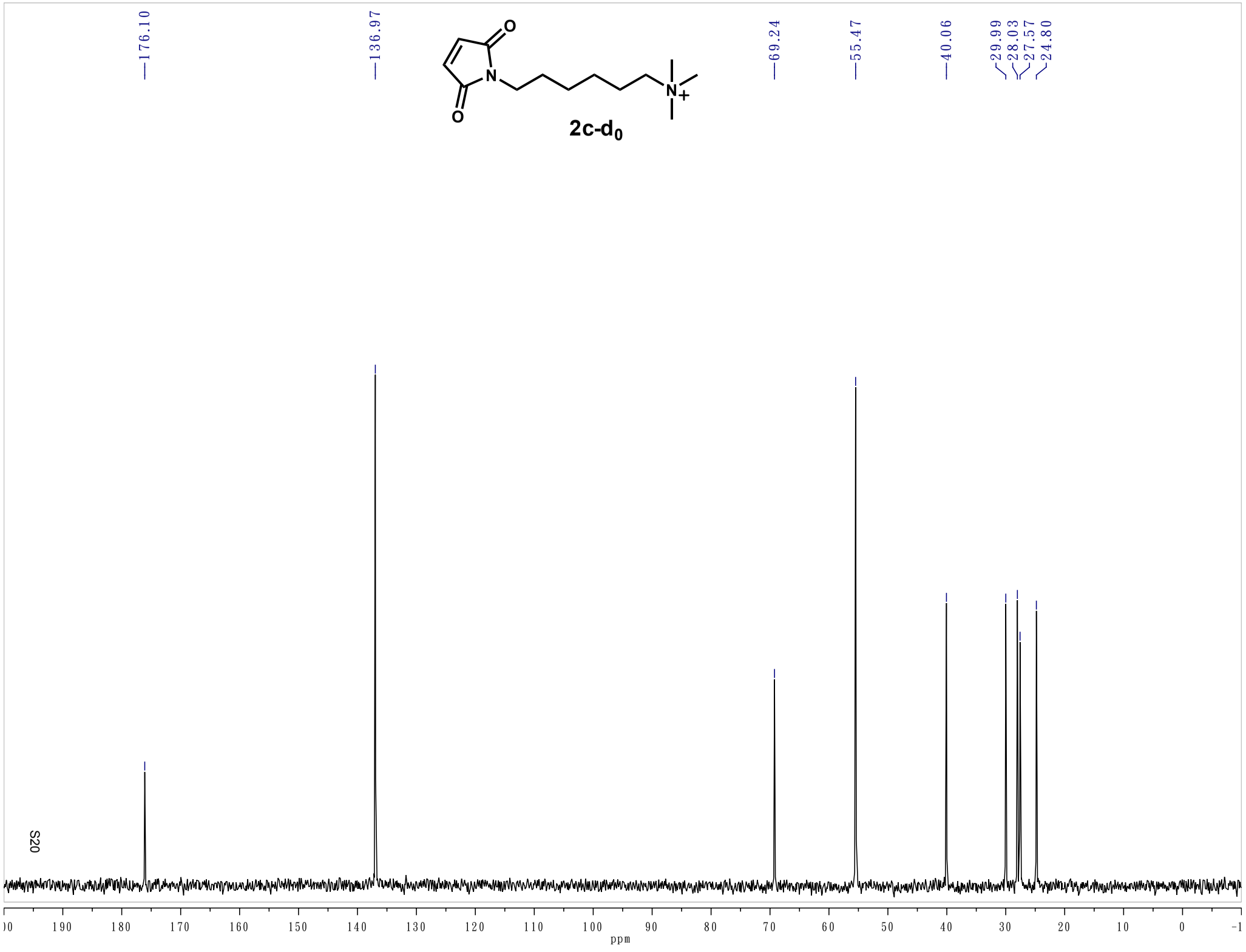
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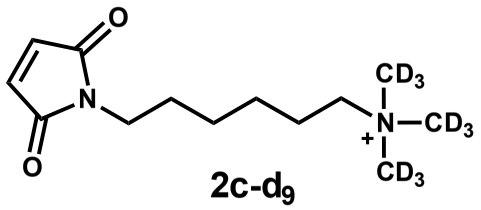
N
O
N+
2c-d0
S20
ppm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CD3
N—CD3
+
CD3

2c-d_9

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2.0000
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4
2
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[rel]
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[ppm]
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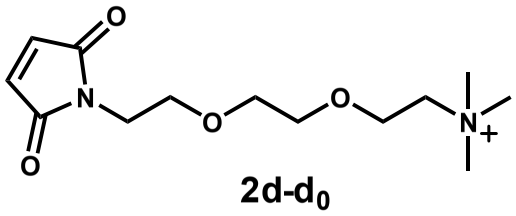

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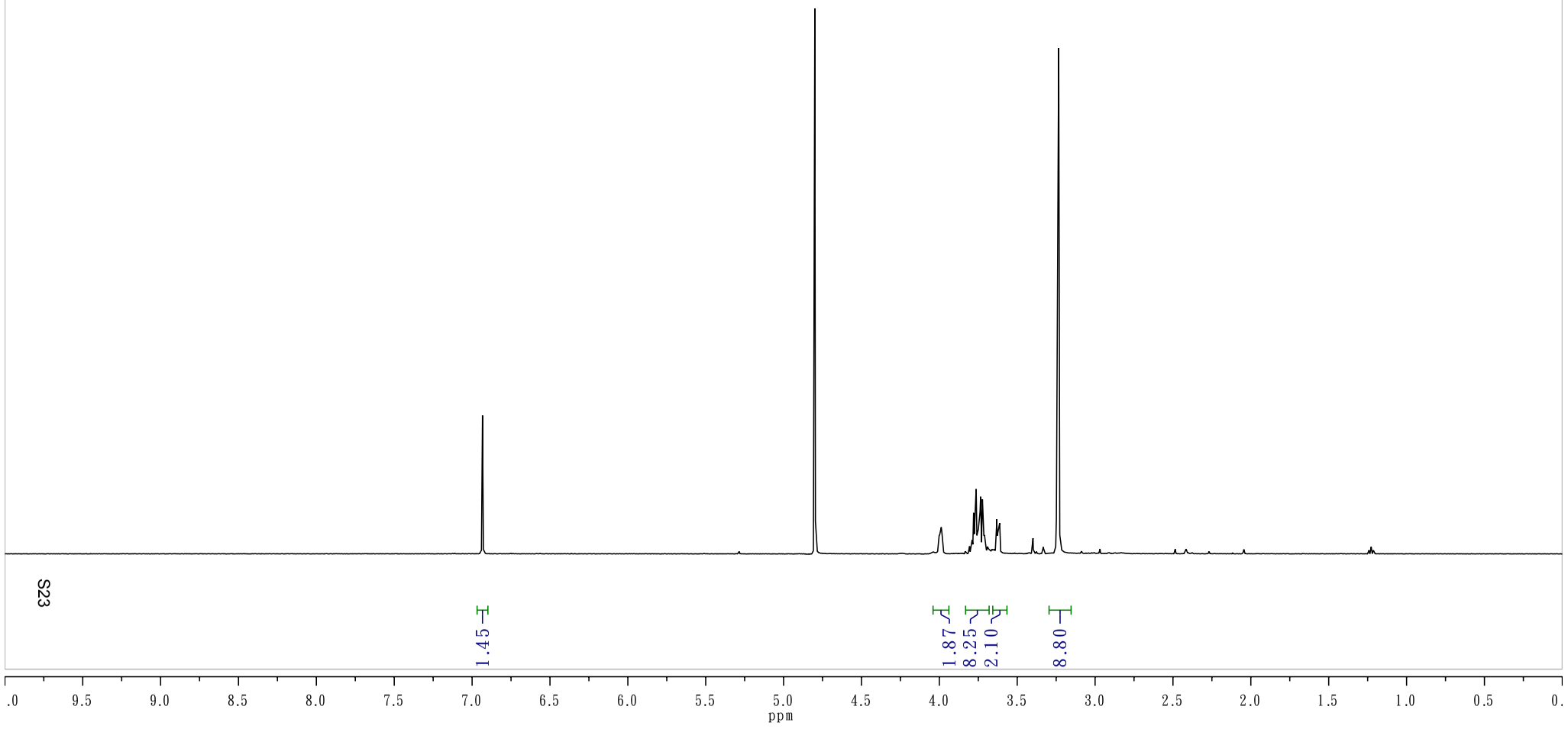
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6.0
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3.0
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2.0
1.5
1.0
0.5
0.
ppm

2d-d0
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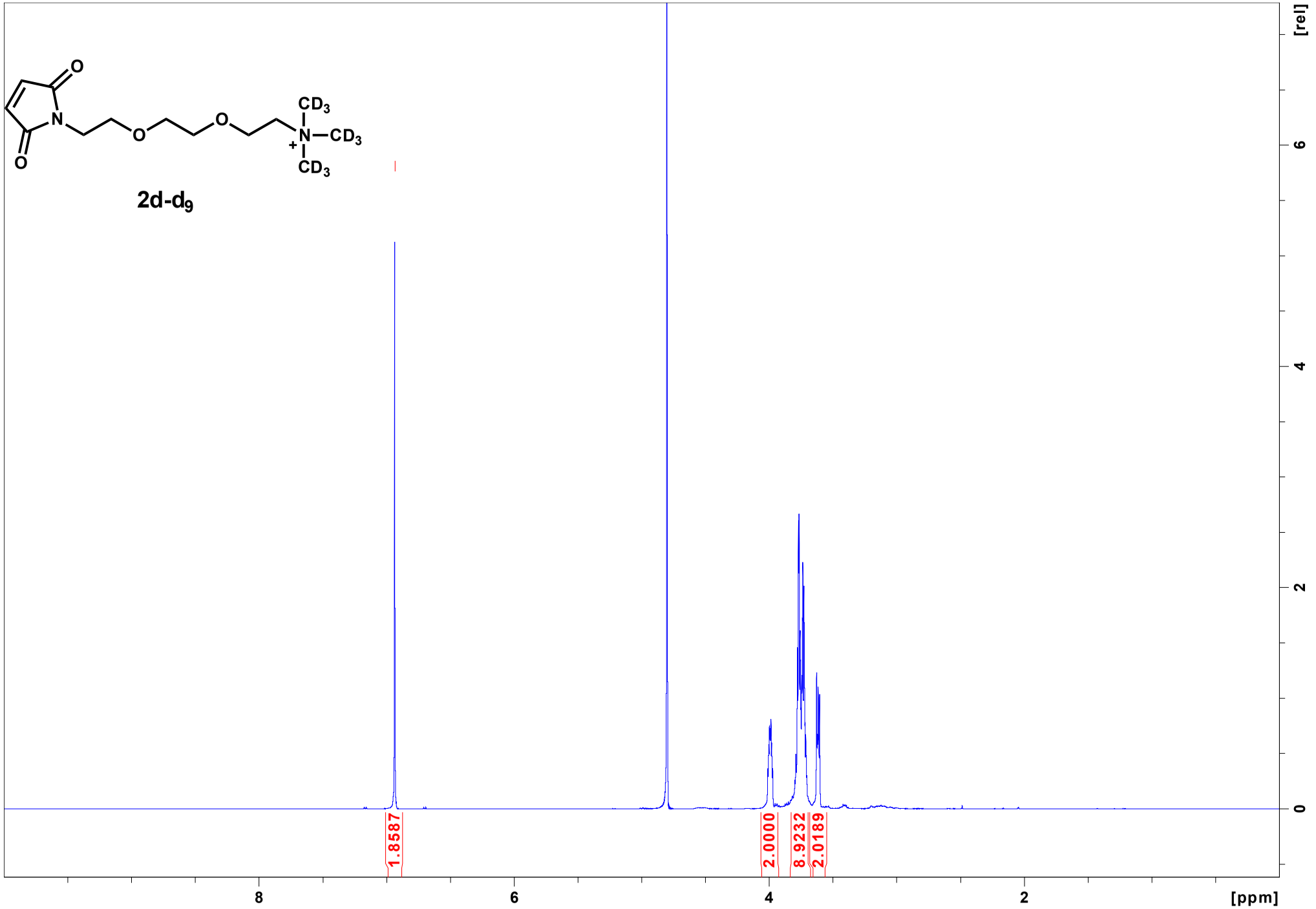
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O
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+
CD3
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2
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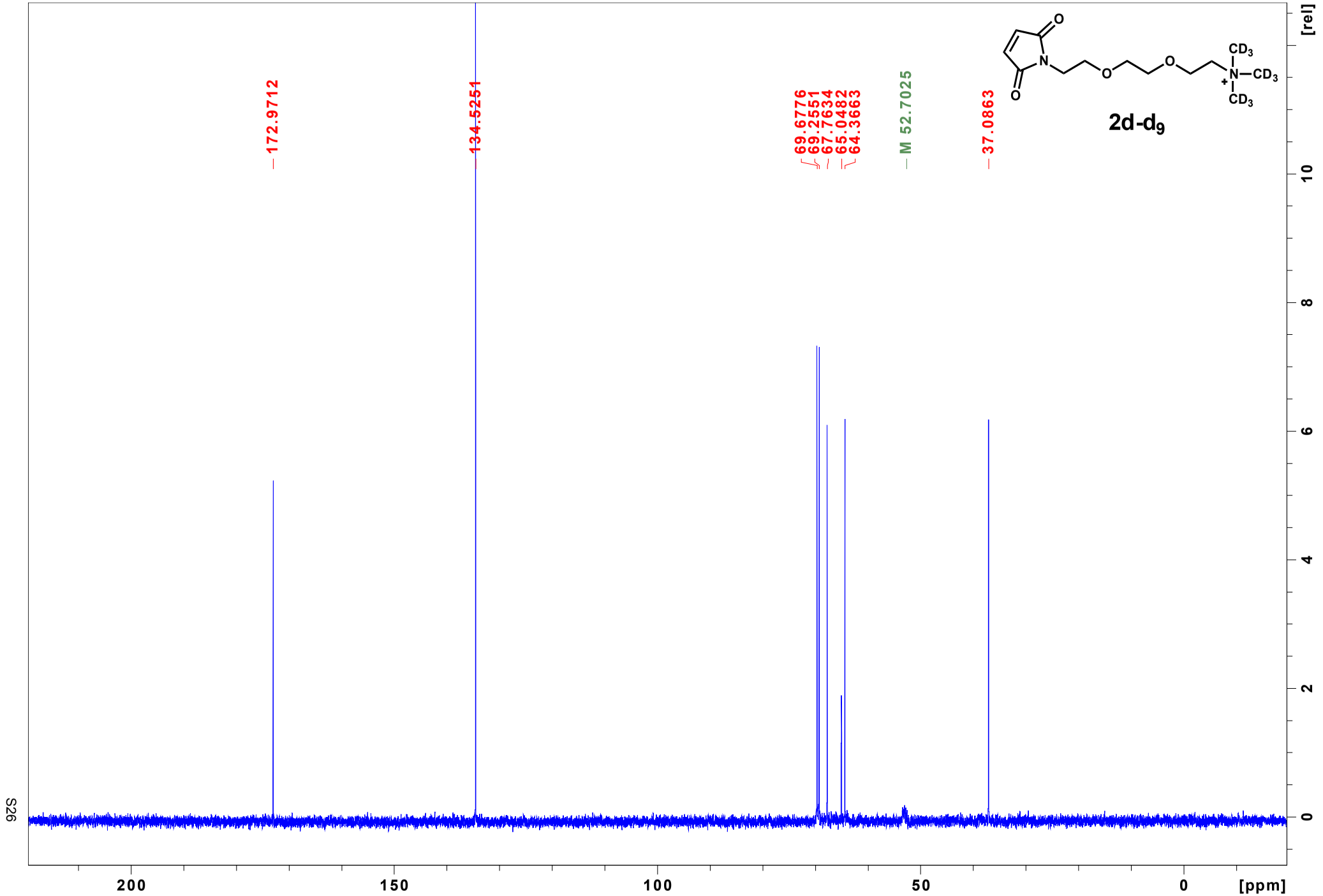
6
[rel]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8
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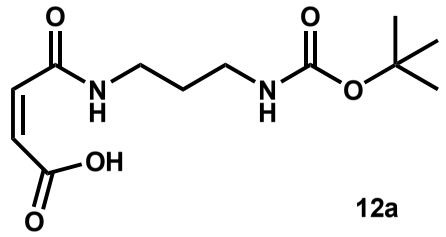

12a

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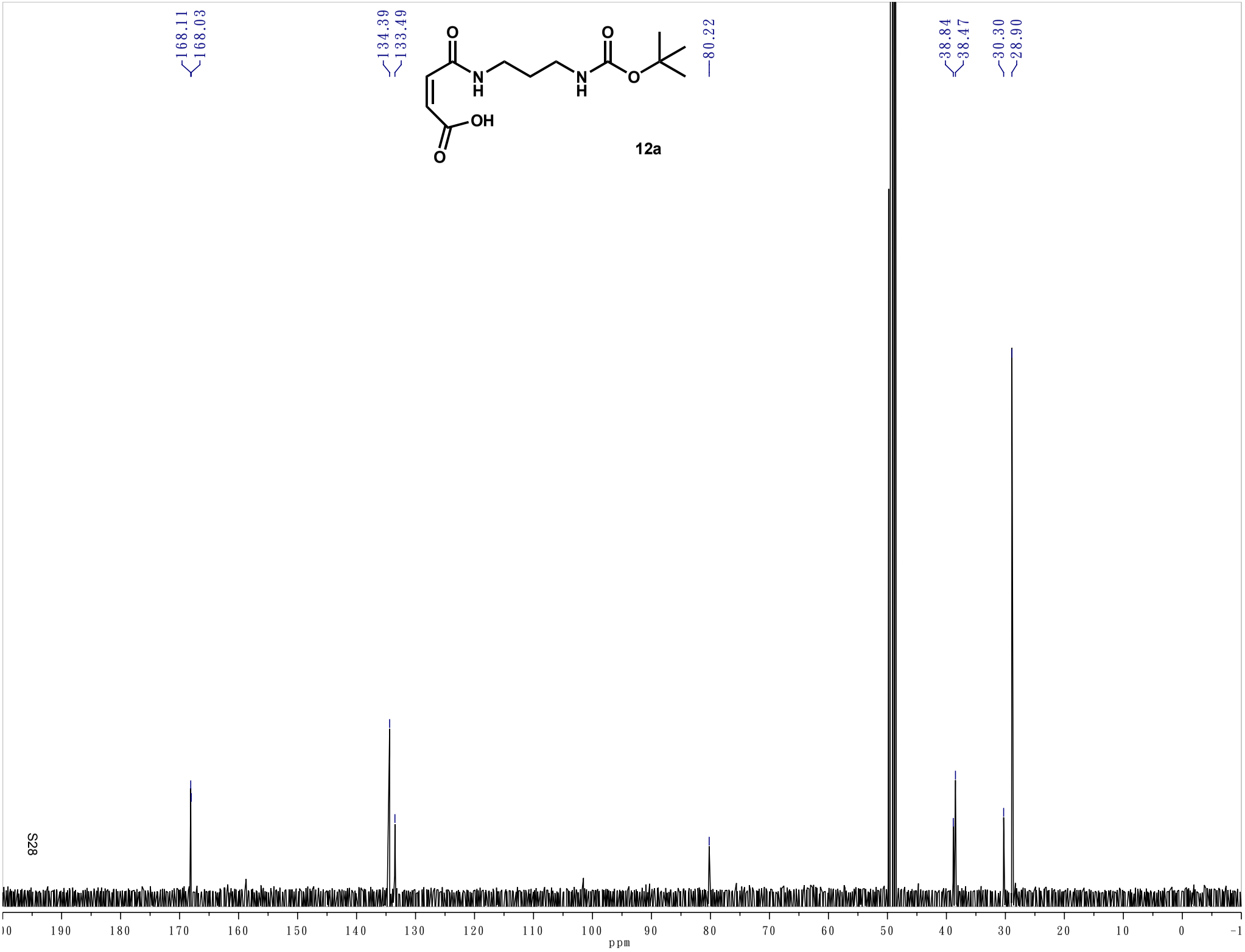
ppm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134.39
133.49
80.22
38.84
38.47
30.30
28.90
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N
H
N
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OH
O
12a
S28
190
180
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160
150
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130
120
110
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90
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50
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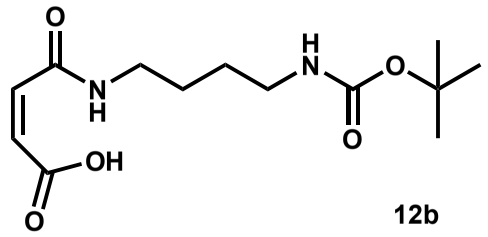
ppm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H
O
O
OH
O

12b

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2.00
4.07
8.88
.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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2.0
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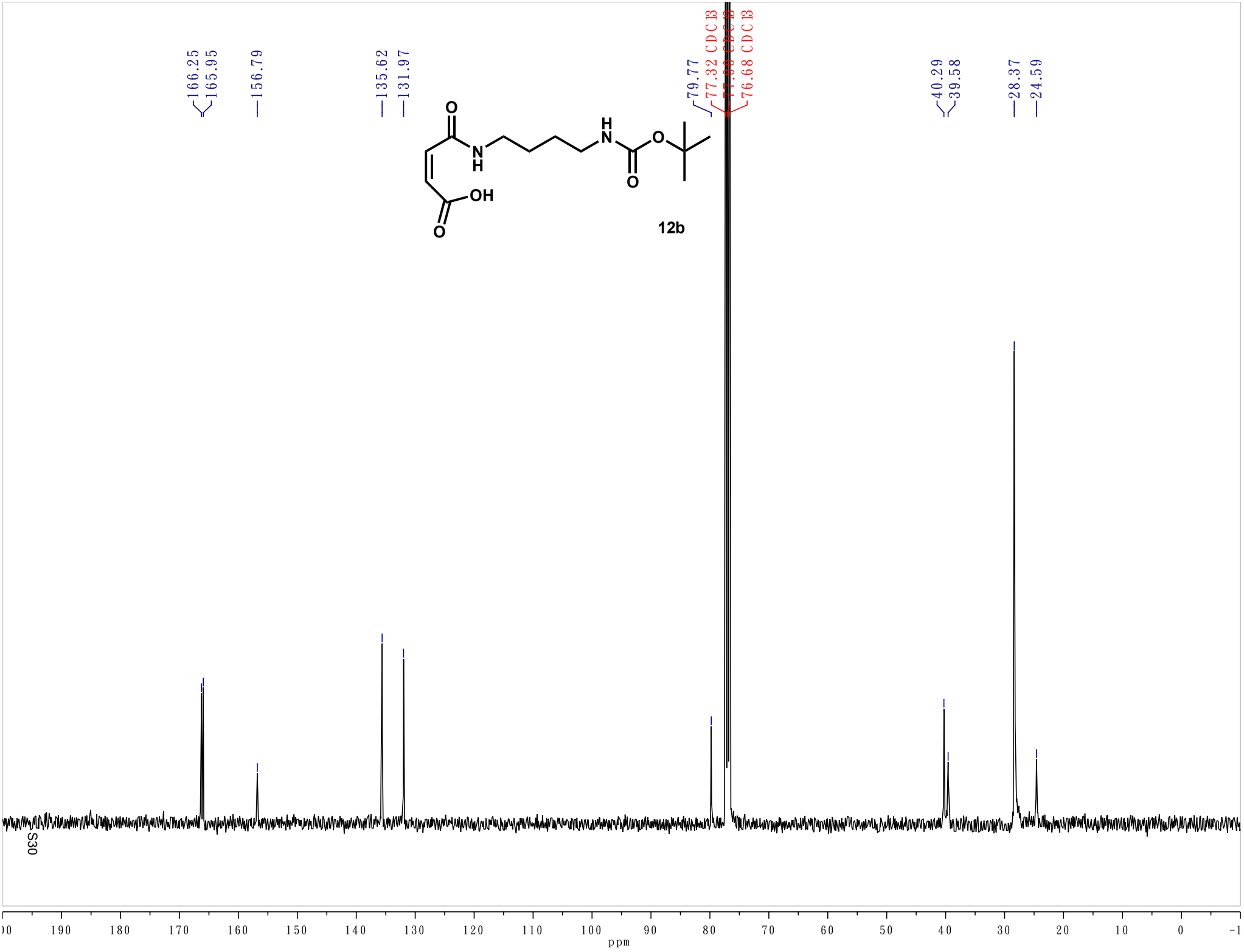
1.0
0.5
ppm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156.79
135.62
131.97
79.77
77.32 CDC13
77.00 CDC13
76.68 CDC13
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28.37
24.59
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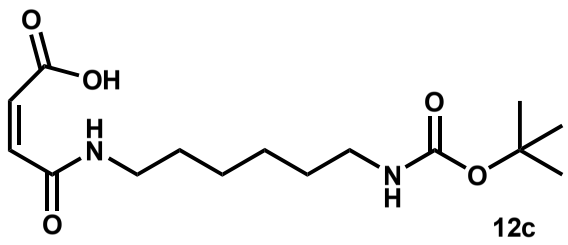
H
H
N
O
O
OH
O
12b
S30
ppm



S31

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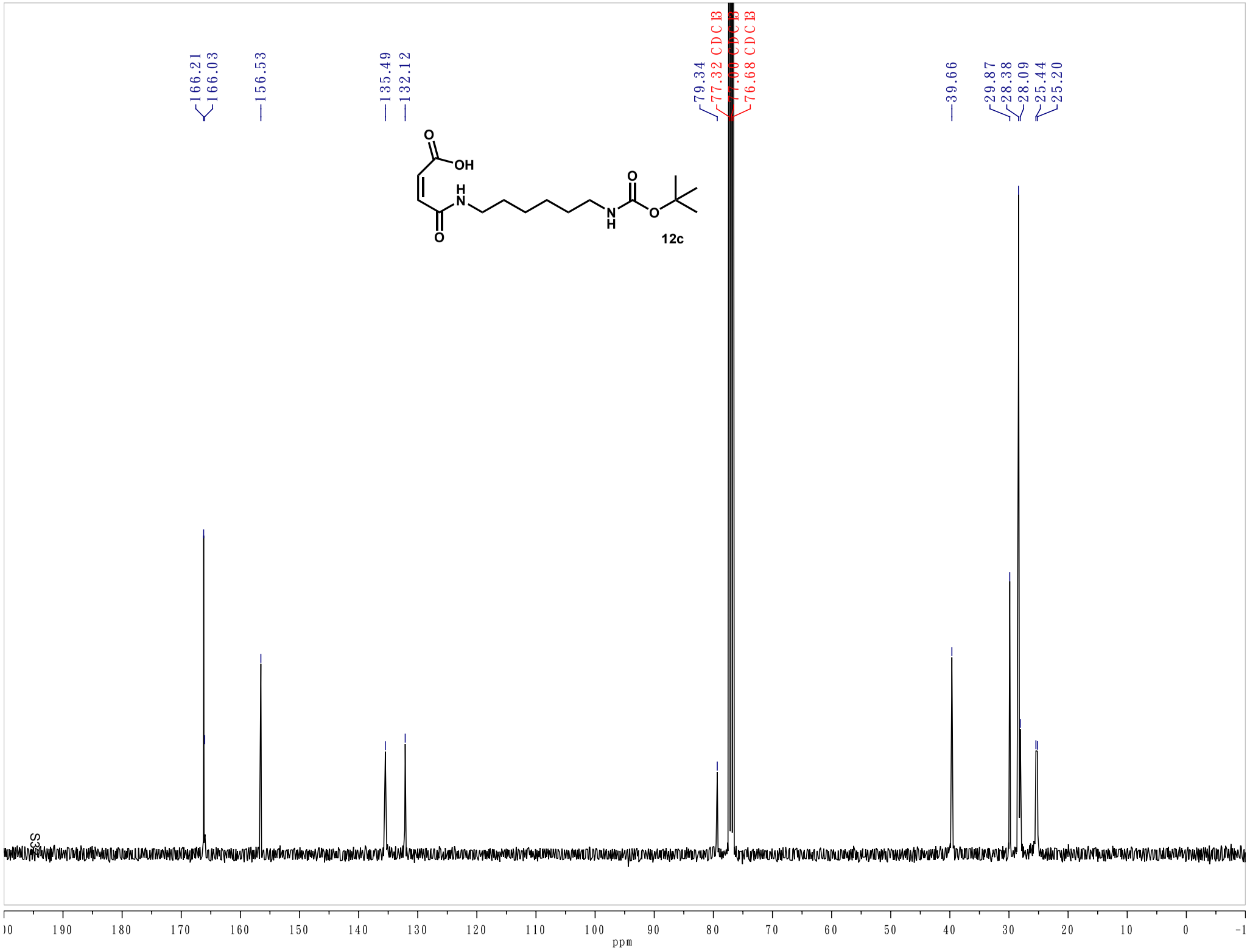
ppm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156.53
135.49
132.12
79.34
77.32 CDCl3
77.00 CDCl3
76.68 CDCl3
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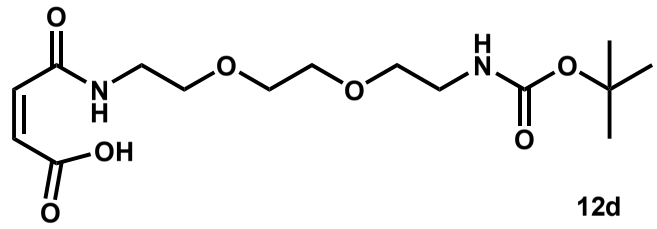
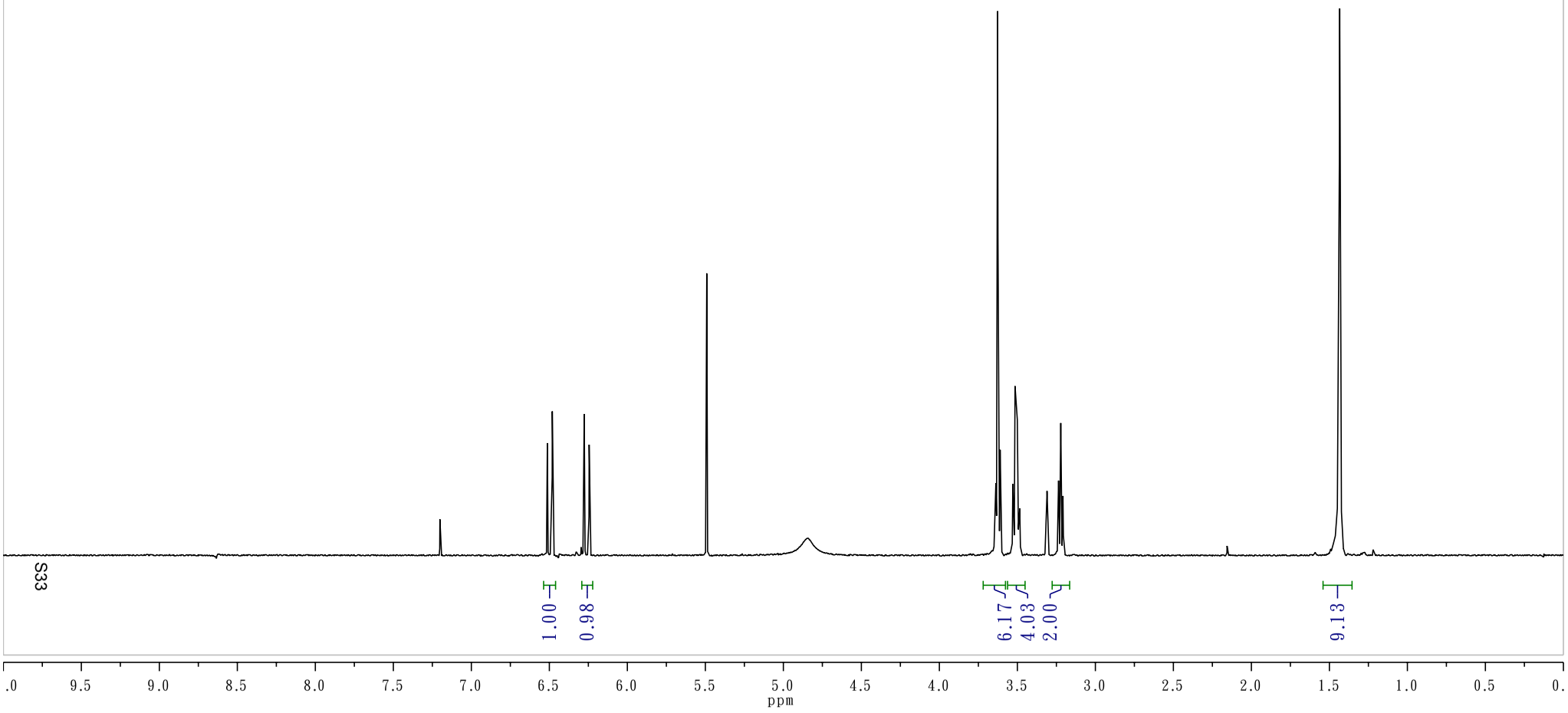
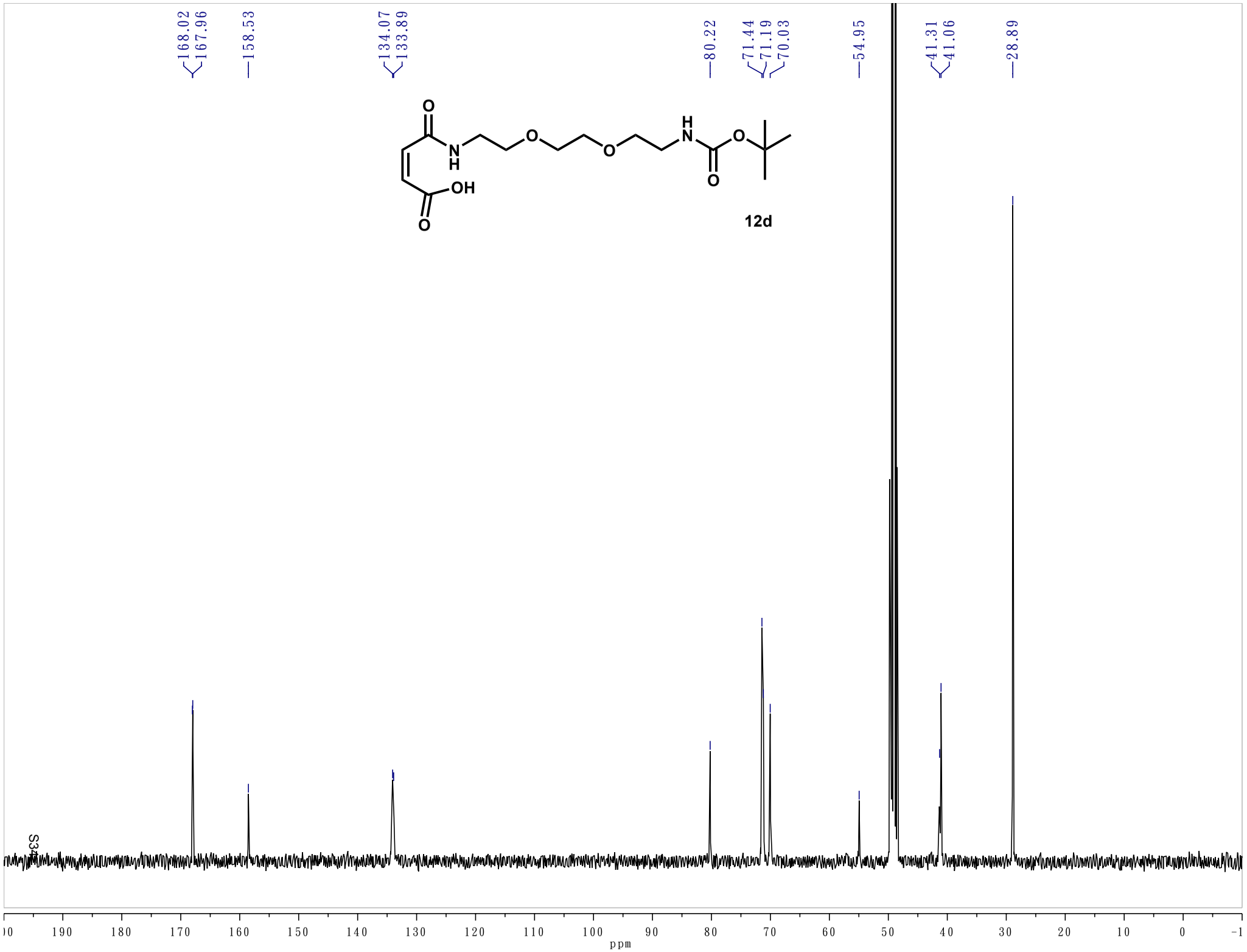


12d

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158.53
134.07
133.89
80.22
71.44
71.19
70.03
54.95
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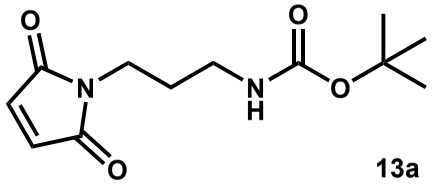
41.06
28.89
O
N
H
O
O
H
N
O
O
OH
O
12d
ppm


O
N
O
H
N
O
O

13a

S35
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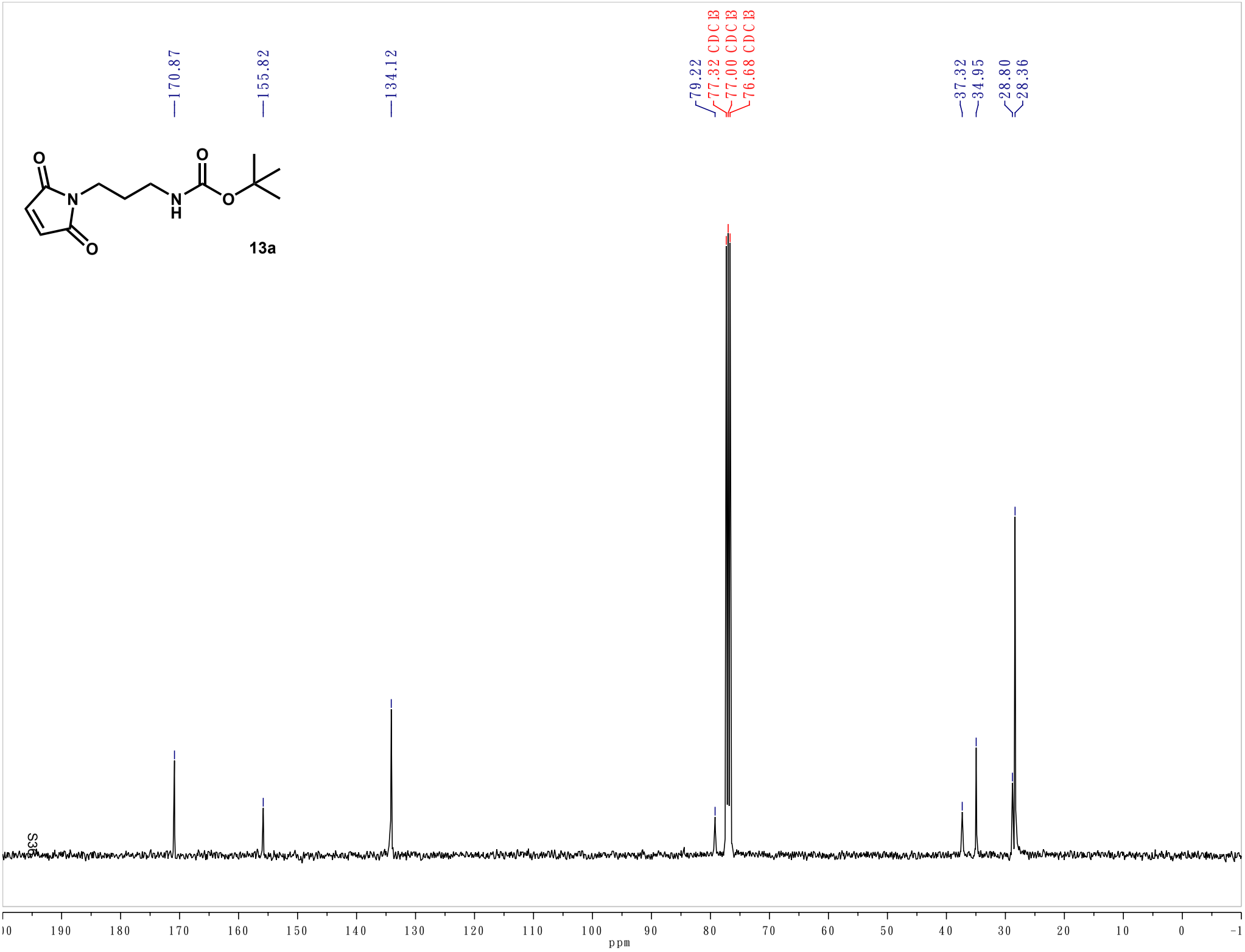
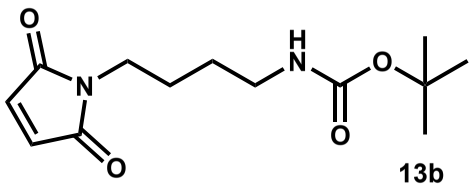
0.90
2.00
2.01
2.20
8.90
.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
ppm


170.87
155.82
134.12
79.22
77.32 CDC13
77.00 CDC13
76.68 CDC13
37.32
34.95
28.80
28.36
13a
S33
ppm

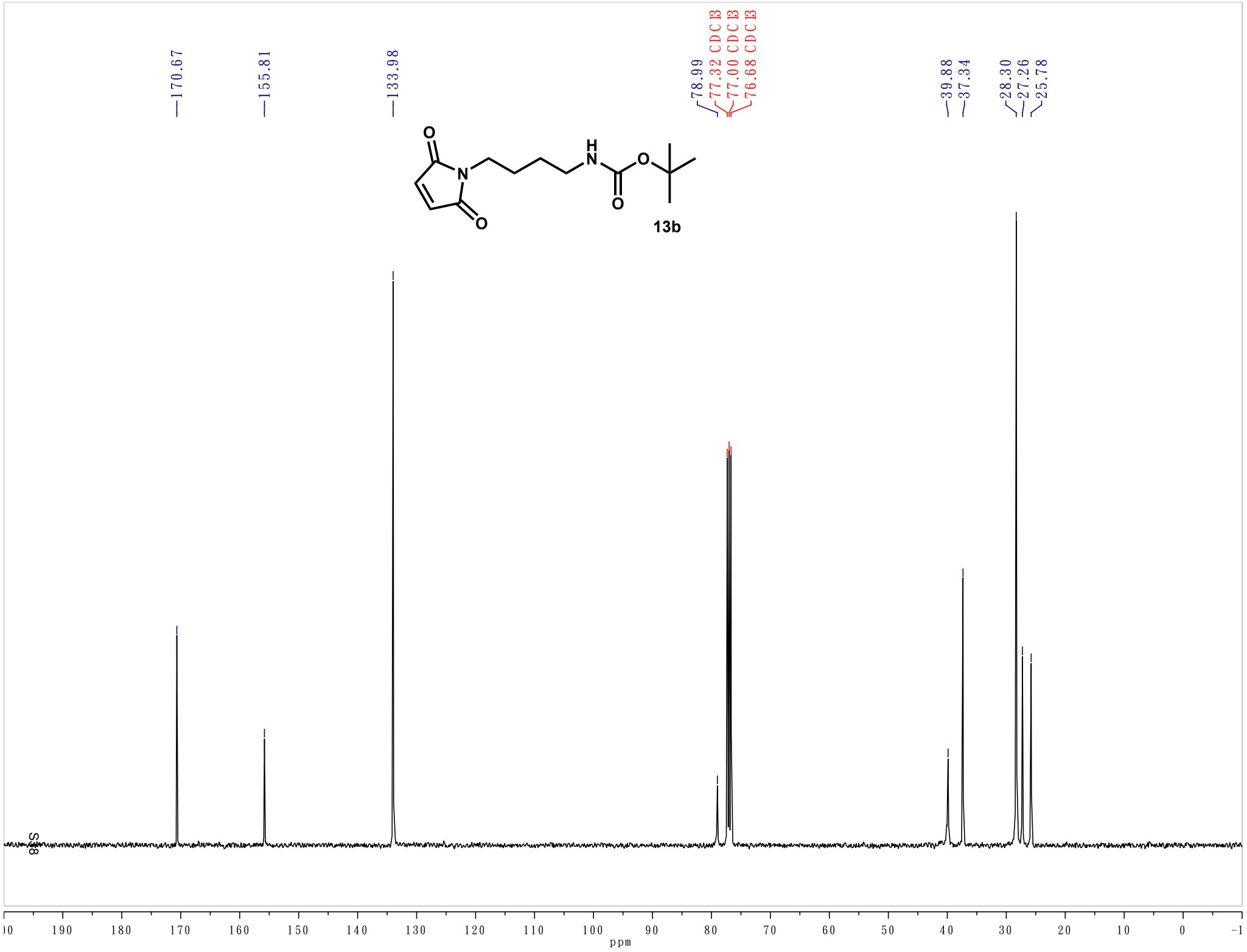


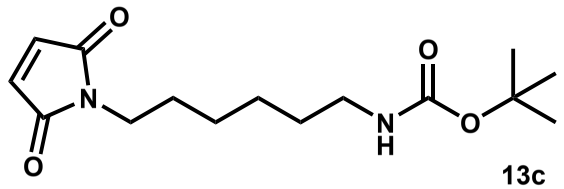
13b

1.74 0.88 1.78 2.00 1.86 11.09

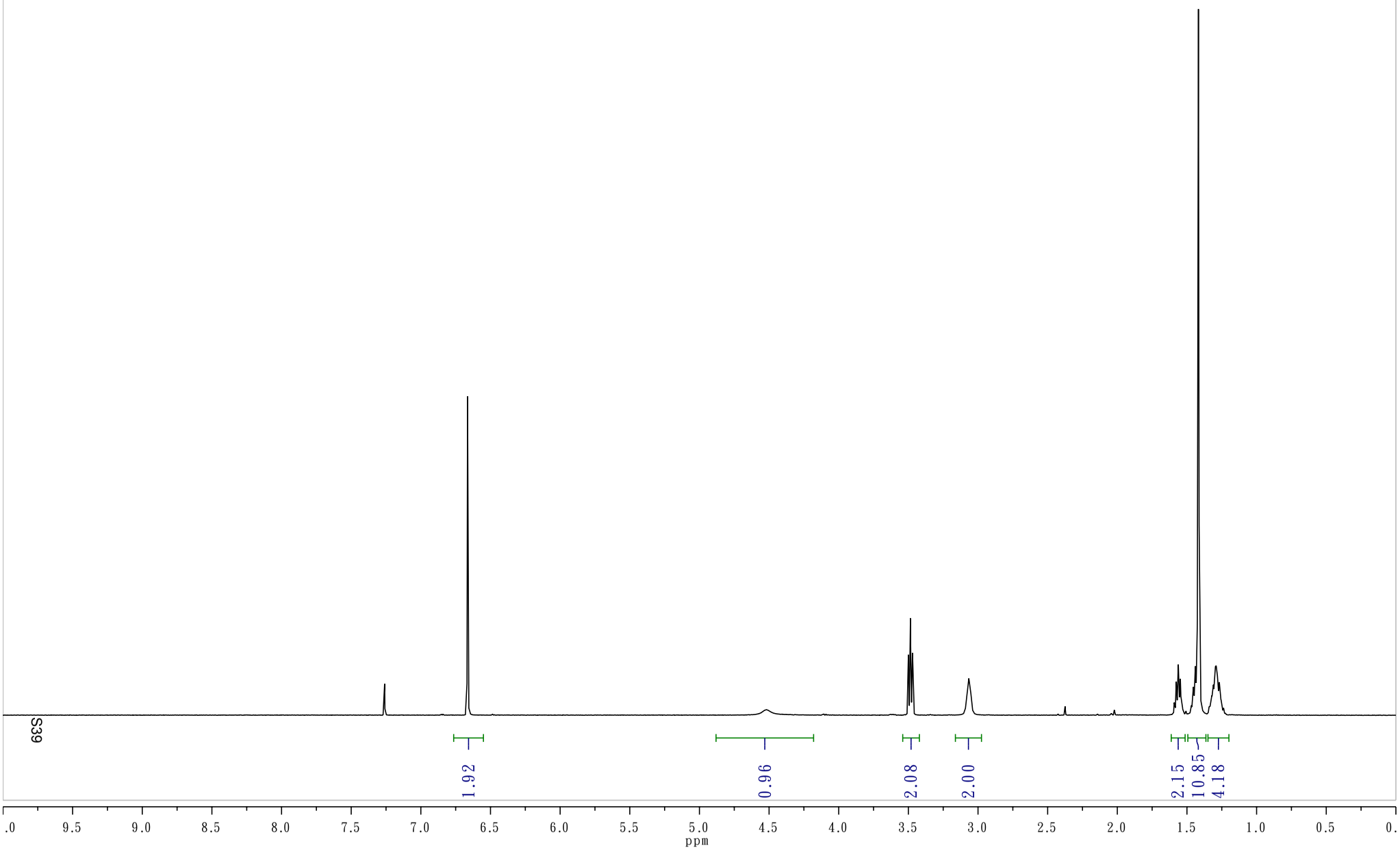
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5

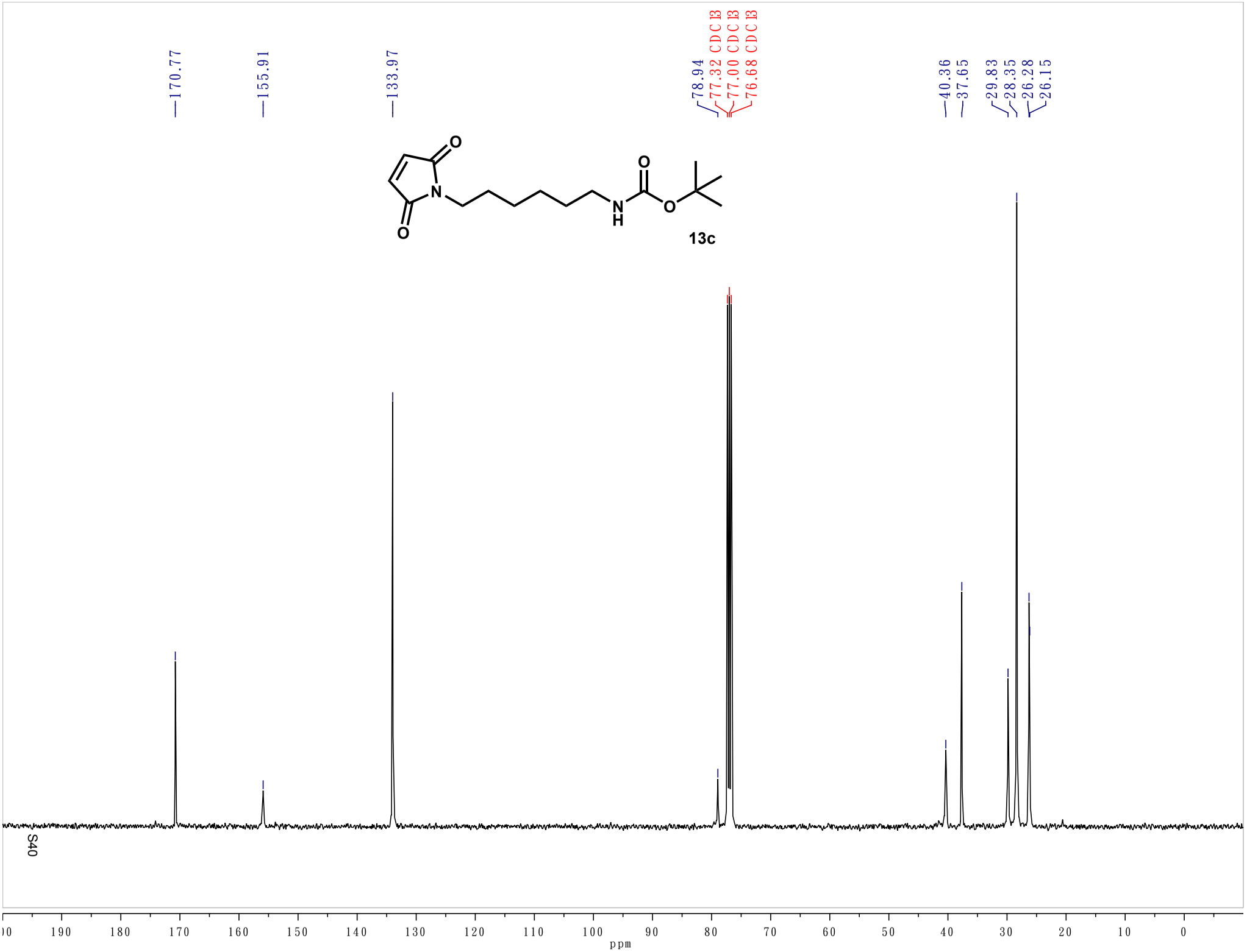
ppnm

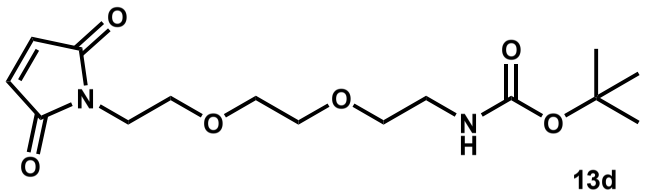

170.67
155.81
133.98
78.99
77.32 CDC13
77.00 CDC13
76.68 CDC13
39.88
37.34
28.30
27.26
25.78
13b
S88
ppm


O
N
O
O
N
H
O

13c


S39
1.92
0.96
2.08
2.00
2.15
10.85
4.18
.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
ppm


170.77
155.91
133.97
78.94
77.32 CDC13
77.00 CDC13
76.68 CDC13
40.36
37.65
29.83
28.35
26.28
26.15
O
O
N
N
H
O
O
13c
S40
ppm

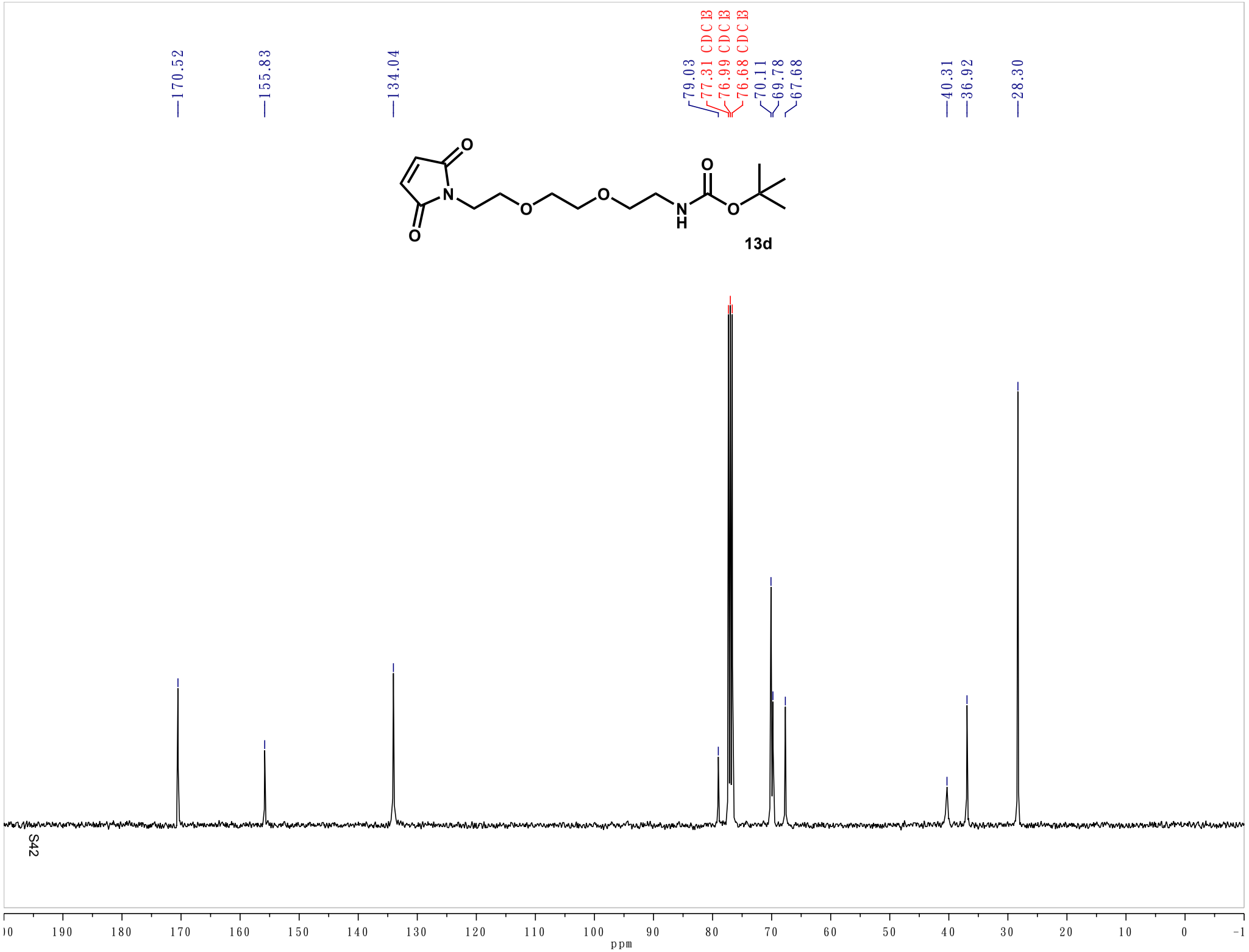


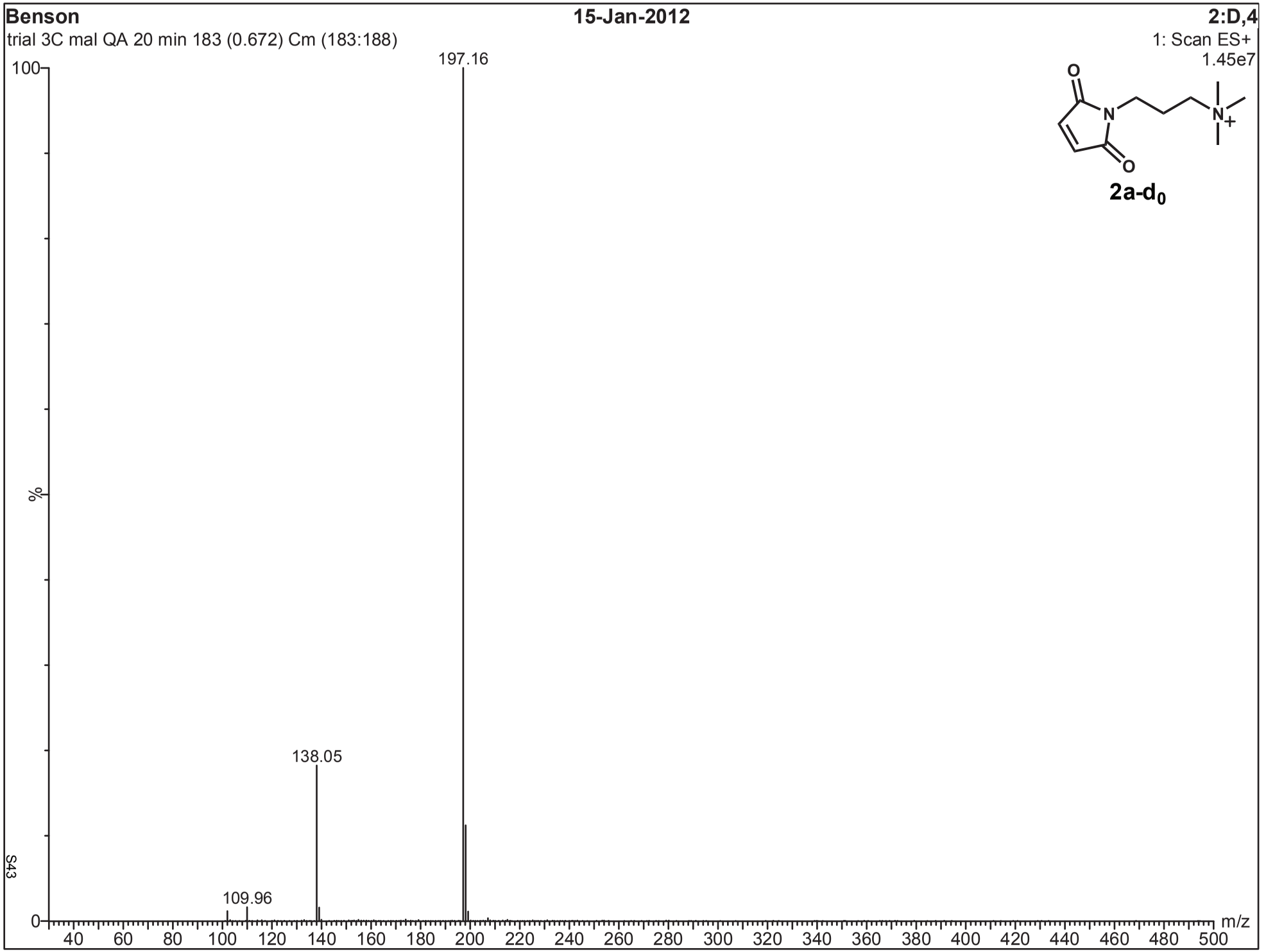
13d

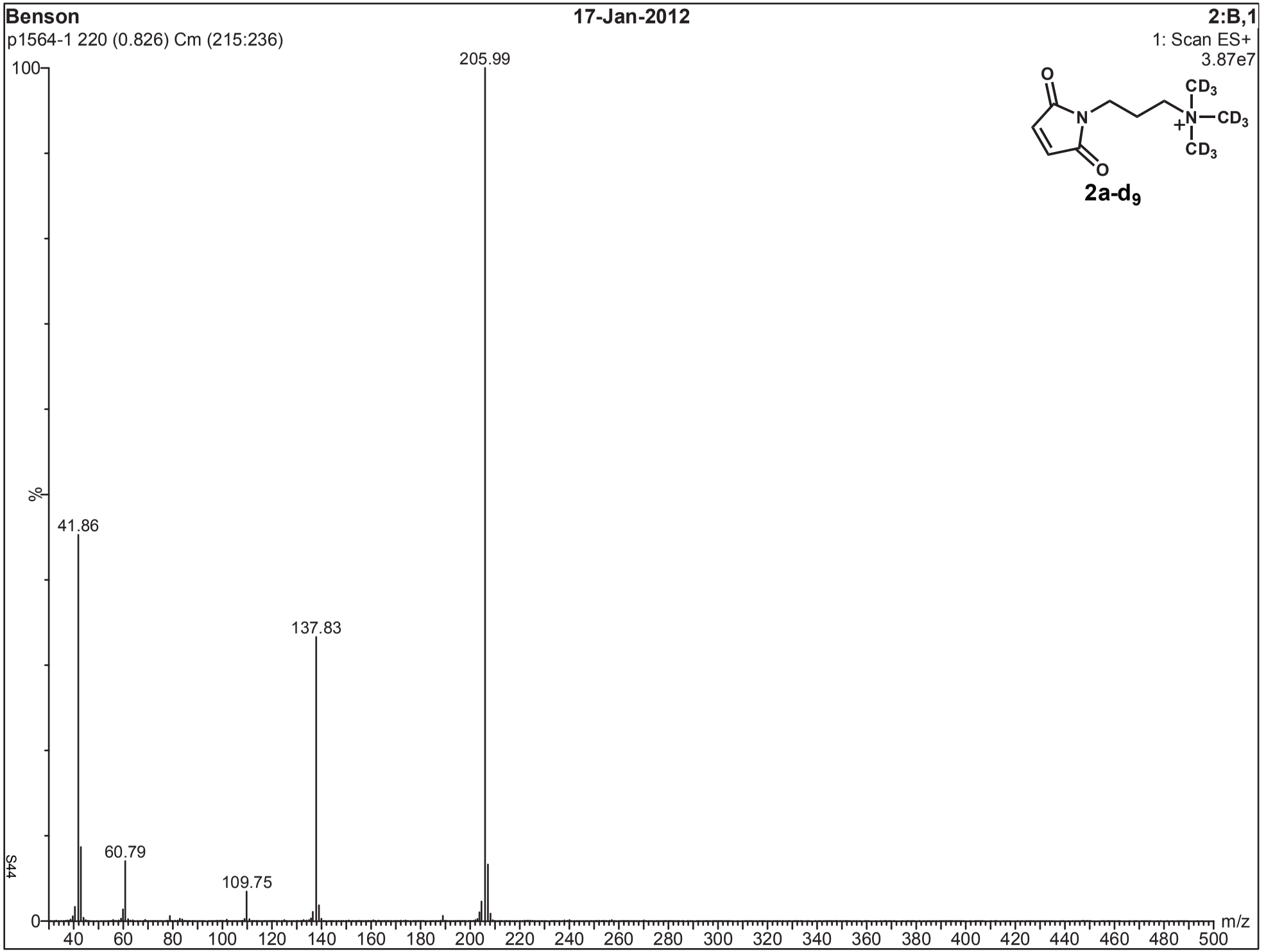
1.76 0.91 2.02 2.04 2.06 2.10 2.03 2.00 9.03

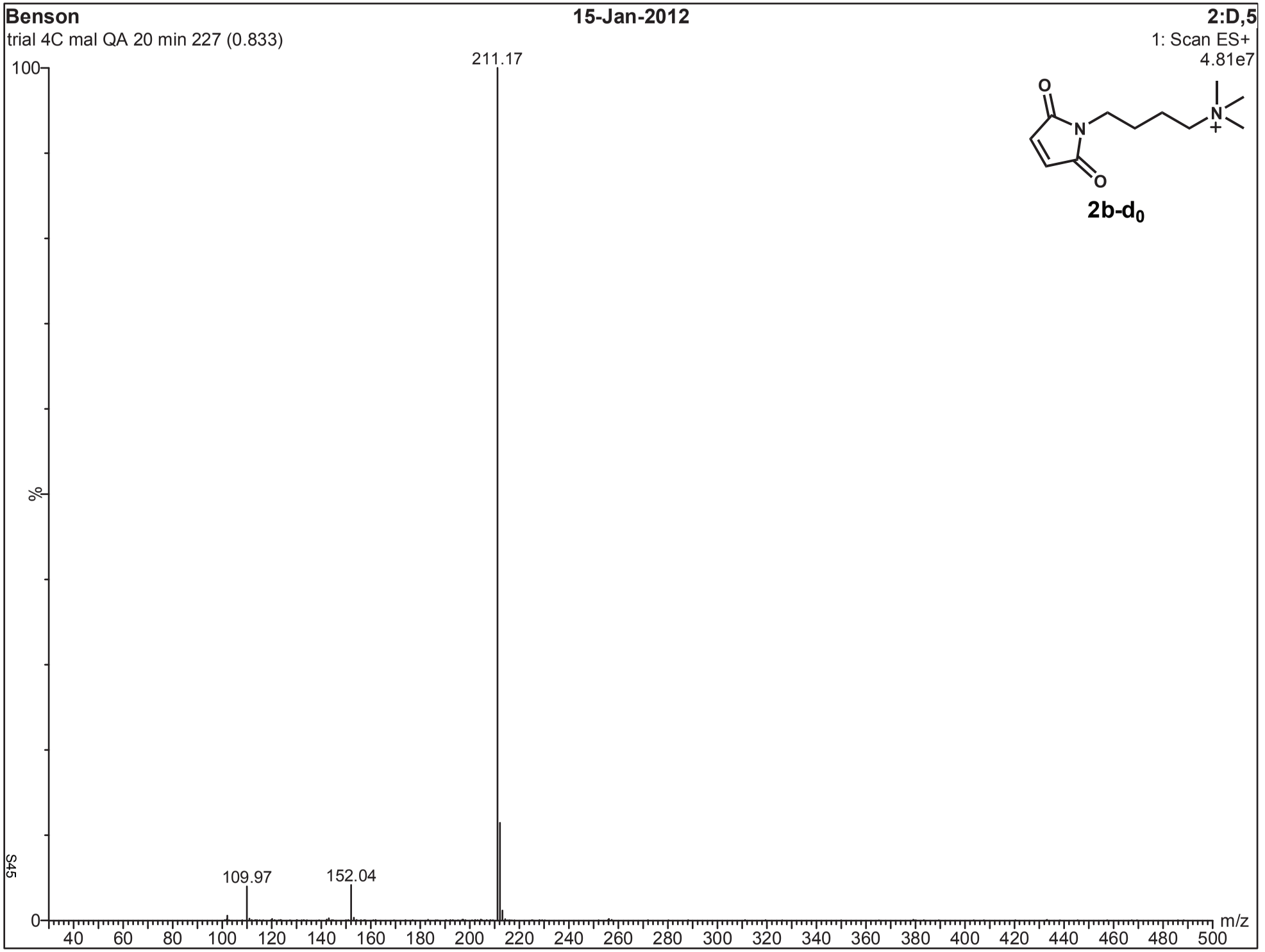
.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.

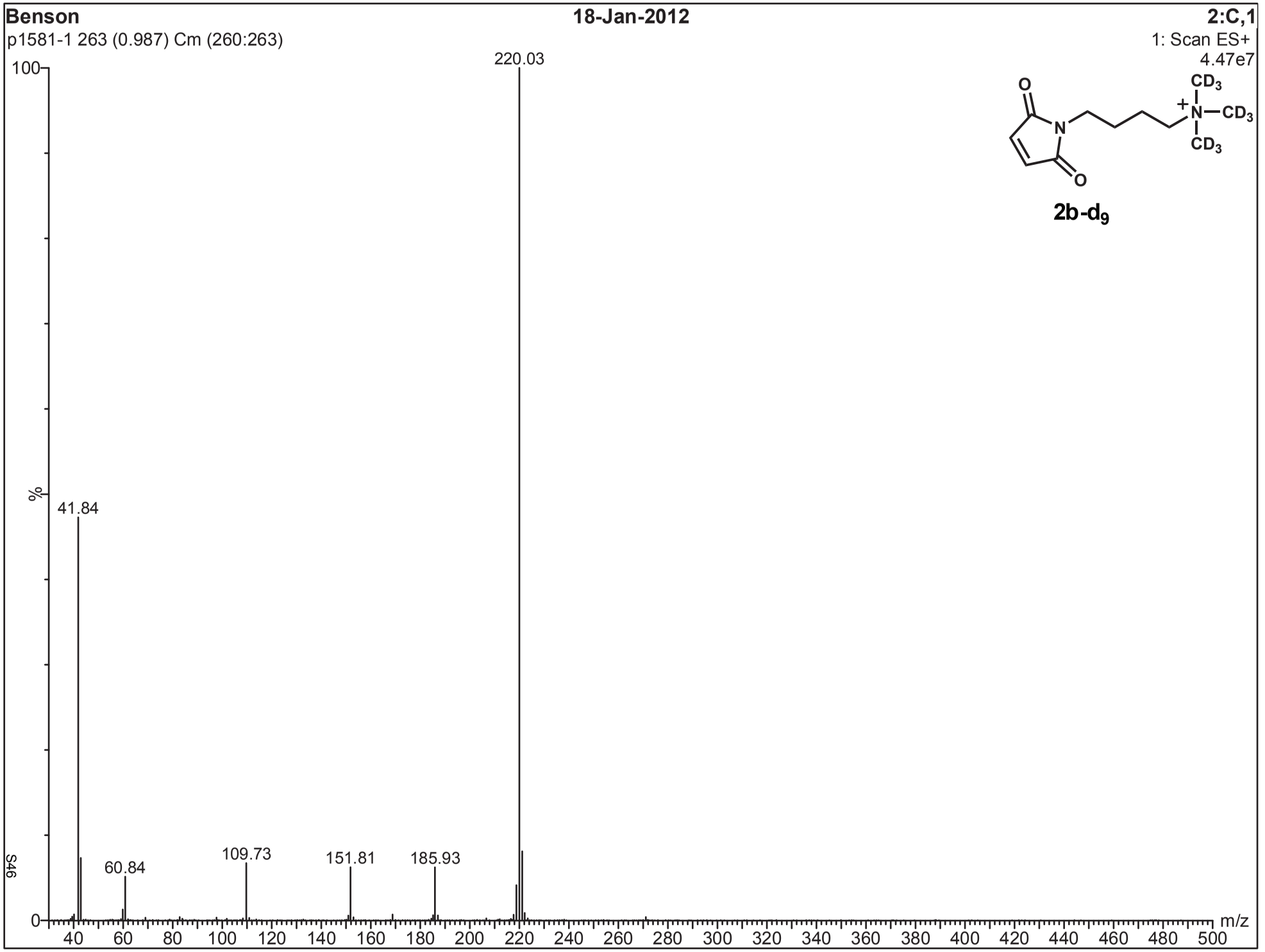
ppm

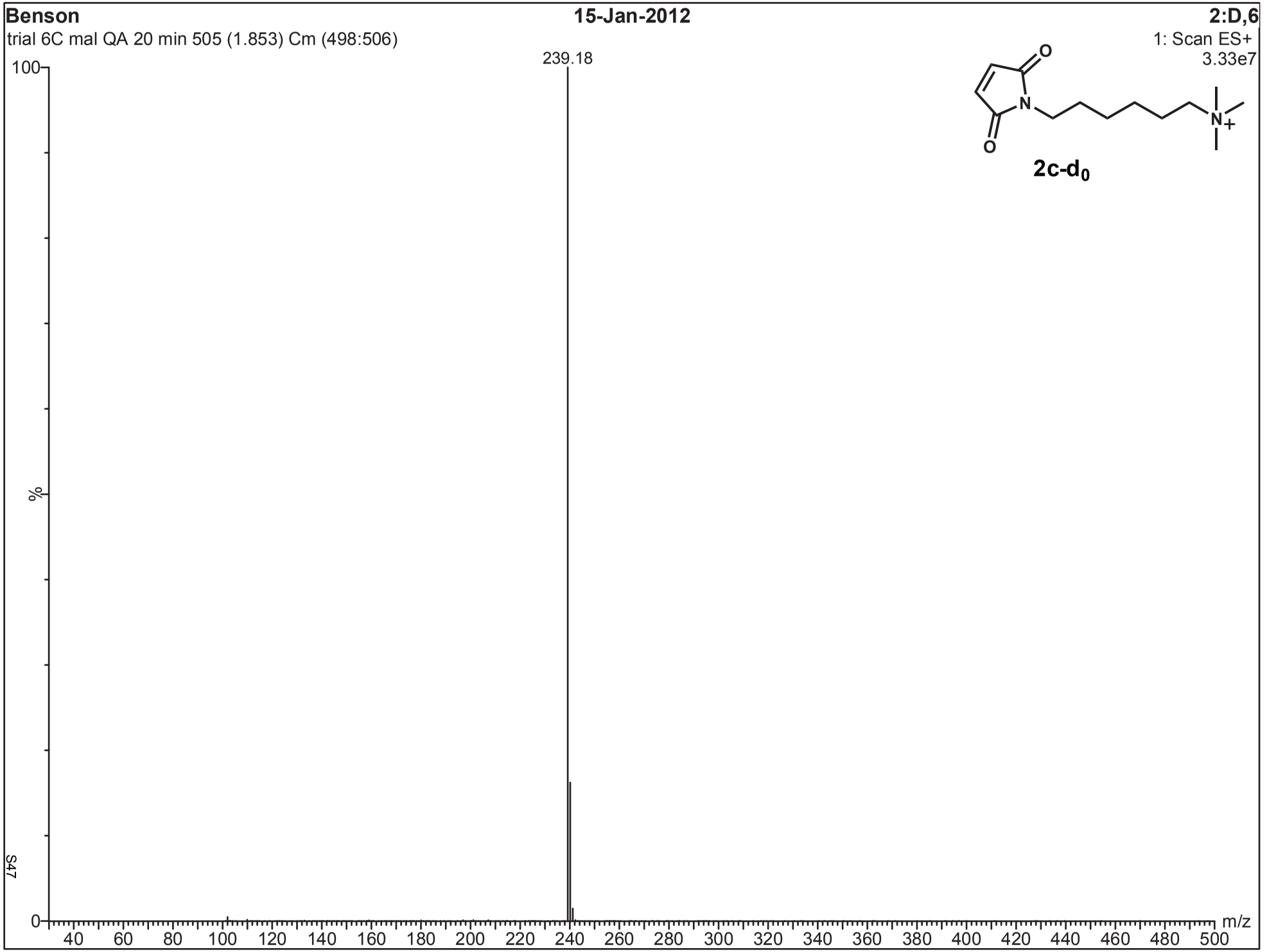

170.52
155.83
134.04
79.03
77.31 CDCl3
76.99 CDCl3
76.68 CDCl3
70.11
69.78
67.68
40.31
36.92
28.30
O
N
O
O
O
N
H
O
O
13d
S42
ppm

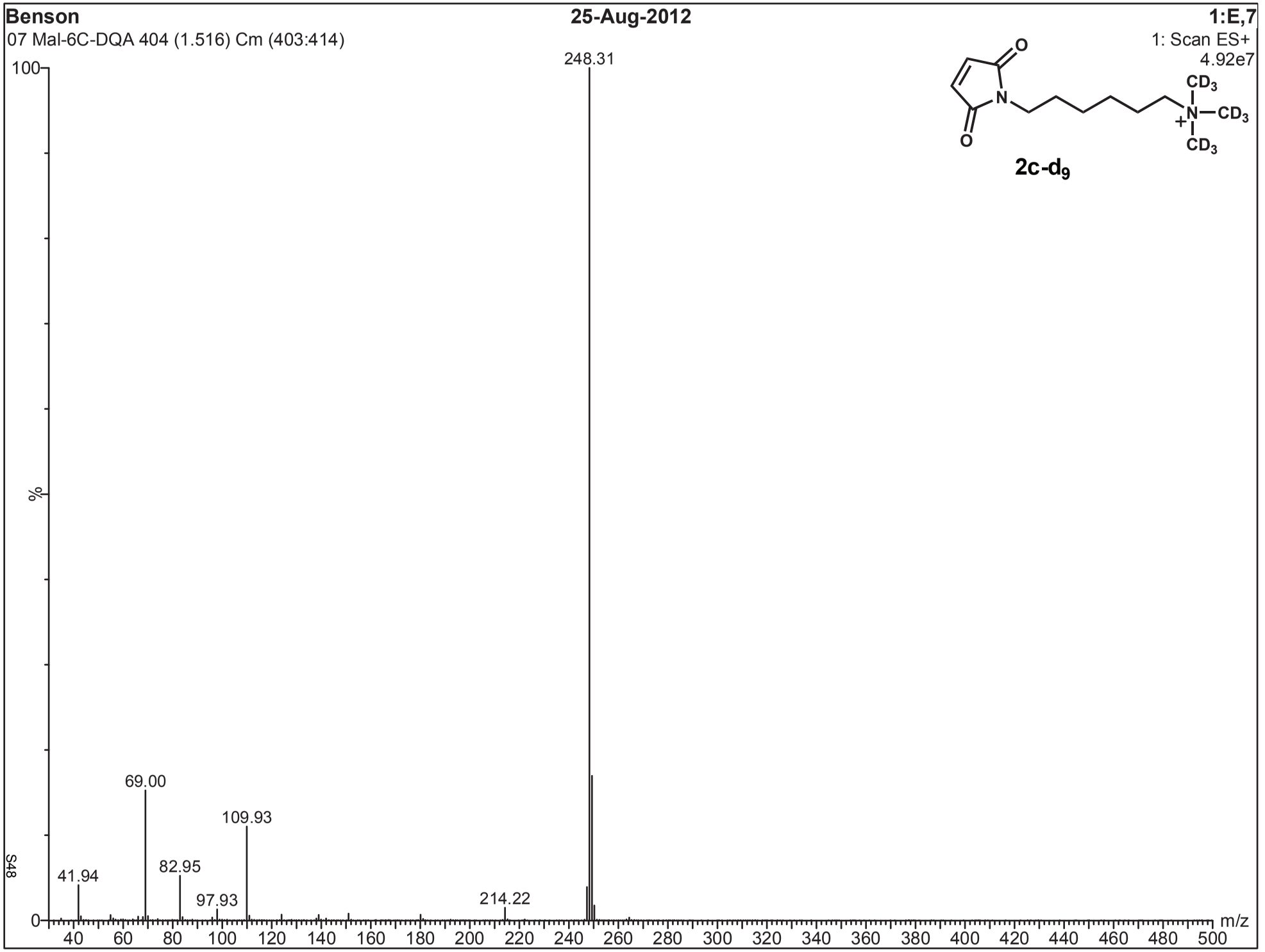

Benson
15-Jan-2012
2:D,4
trial 3C mal QA 20 min 183 (0.672) Cm (183:188)
1: Scan ES+
1.45e7
197.16
138.05
109.96
100
%
0
S43
m/z
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
O
N
N+
O
2a-d0

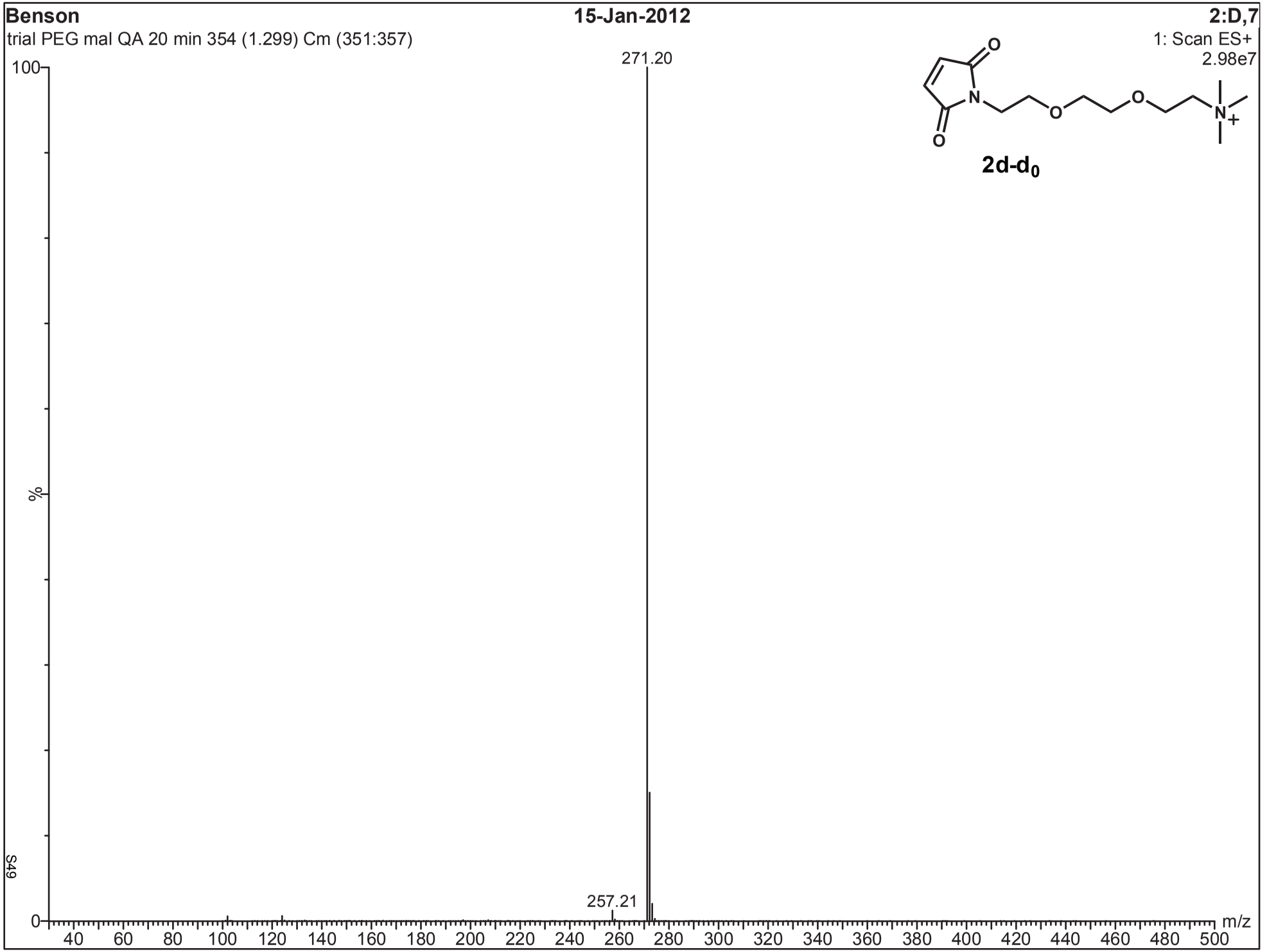

Benson
17-Jan-2012
2:B,1
p1564-1 220 (0.826) Cm (215:236)
1: Scan ES+
3.87e7
205.99
41.86
137.83
60.79
109.75
100
%
0
m/z
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
O
N
O
CD3
N
CD3
CD3
+
2a-d9
S44

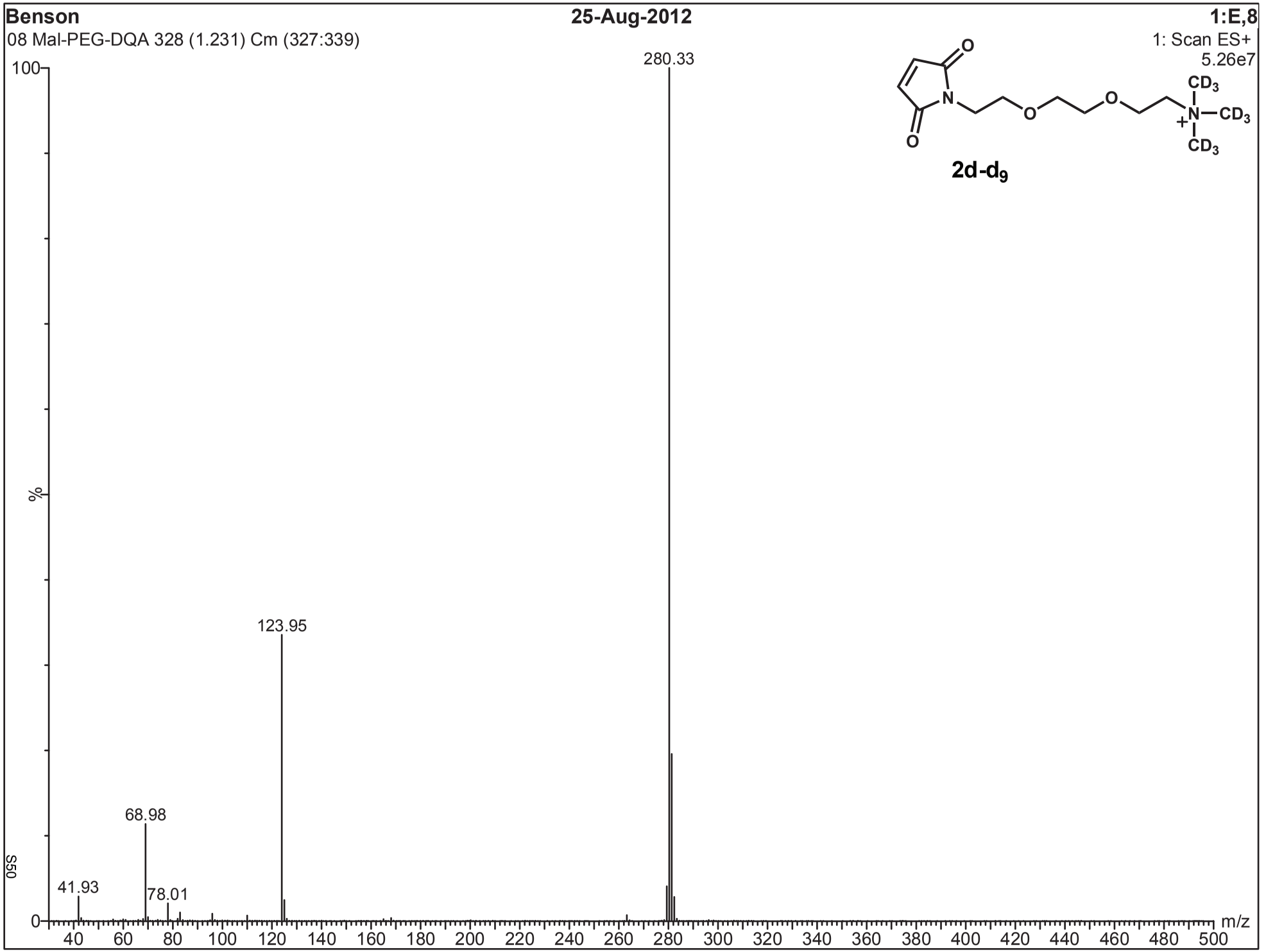

Benson
15-Jan-2012
2:D,5
trial 4C mal QA 20 min 227 (0.833)
1: Scan ES+
4.81e7
211.17
109.97
152.04
100
%
0
S45
m/z
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
O
N
+
O
2b-d0


Benson
18-Jan-2012
2:C,1
p1581-1 263 (0.987) Cm (260:263)
1: Scan ES+
4.47e7
100
%
0
220.03
41.84
60.84
109.73
151.81
185.93
S46
m/z
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
O
N
O
+
N
CD3
CD3
CD3
2b-d9


Benson
15-Jan-2012
2:D,6
trial 6C mal QA 20 min 505 (1.853) Cm (498:506)
1: Scan ES+
3.33e7
239.18
100
%
0
m/z
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
O
N
O
N+
2c-d0
S47


Benson
25-Aug-2012
1:E,7
07 Mal-6C-DQA 404 (1.516) Cm (403:414)
1: Scan ES+
4.92e7
248.31
100
%
0
69.00
109.93
41.94
82.95
97.93
214.22
S48
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
m/z
O
N
O
CD3
N
CD3
+
CD3
2c-d9


Benson
15-Jan-2012
2:D,7
trial PEG mal QA 20 min 354 (1.299) Cm (351:357)
1: Scan ES+
2.98e7
271.20
257.21
100
%
0
m/z
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
O
N
O
O
O
N+
2d-d0
S49


Benson
25-Aug-2012
1:E,8
08 Mal-PEG-DQA 328 (1.231) Cm (327:339)
1: Scan ES+
5.26e7
280.33
123.95
68.98
41.93
78.01
100
%
0
S50
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
m/z
O
N
O
O
O
+
N
CD3
CD3
CD3
2d-d9