Supporting Information

Discovery of a Small-molecule Modulator of the Autophagy-Lysosome Pathway that Targets Lamin A/C and LAMP1, Induces Autophagic Flux, and Affects Lysosome Positioning in Neurons

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001-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.515	0.331	0.568	13.160
	CQ,20 μM	10.764	0.856		7.953

001-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	1.974	0.209	0.562	10.613
	CQ,20 μM	9.002	0.816		9.063

002-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.586	0.246	0.694	9.521
	CQ,20 μM	11.123	0.625		5.616

002-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.566	0.313	0.736	12.211
	CQ,20 μM	11.064	0.435		3.932

003-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.107	0.241	0.509	11.430
	CQ,20 μM	8.958	0.881		9.835

003-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	1.993	0.220	0.461	11.061
	CQ,20 μM	8.723	0.988		11.328

004-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.307	0.250	0.671	10.824
	CQ,20 μM	10.831	0.684		6.314

004-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.542	0.202	0.711	7.932
	CQ,20 μM	10.823	0.596		5.503

005-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.728	0.257	0.670	9.426
	CQ,20 μM	10.627	0.610		5.745

005-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.685	0.282	0.629	10.498
	CQ,20 μM	9.899	0.610		6.157

006-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.856	0.230	0.595	8.070
	CQ,20 μM	10.496	0.801		7.633

006-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.863	0.318	0.595	11.121
	CQ,20 μM	10.768	0.749		6.960

007-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.221	0.387	0.584	17.416
	CQ,20 μM	9.239	0.586		6.338

007-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.584	0.353	0.600	13.673
	CQ,20 μM	10.189	0.661		6.484

008-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.371	0.373	0.632	15.743
	CQ,20 μM	9.886	0.548		5.542

008-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.422	0.282	0.678	11.628
	CQ,20 μM	9.753	0.505		5.180

009-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.273	0.265	0.536	11.652
	CQ,20 μM	9.468	0.849		8.962

009-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.394	0.333	0.592	13.892
	CQ,20 μM	9.962	0.697		6.993

010-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.380	0.263	0.624	11.046
	CQ,20 μM	9.800	0.668		6.812

010-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.464	0.323	0.648	13.106
	CQ,20 μM	9.879	0.548		5.543

011-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.051	0.202	0.690	9.865
	CQ,20 μM	9.967	0.616		6.182

011-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.286	0.201	0.655	8.775
	CQ,20 μM	10.224	0.712		6.968

012-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.397	0.228	0.657	9.498
	CQ,20 μM	10.124	0.656		6.477

012-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.201	0.331	0.734	15.031
	CQ,20 μM	10.331	0.391		3.781

013-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	1.737	0.184	0.598	10.600
	CQ,20 μM	8.383	0.707		8.429

013-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.232	0.235	0.681	10.530
	CQ,20 μM	9.630	0.552		5.730

014-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.370	0.221	0.725	9.337
	CQ,20 μM	10.221	0.498		4.871

014-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.310	0.592	0.464	25.635
	CQ,20 μM	10.547	0.879		8.333

015-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.177	0.224	0.694	10.294
	CQ,20 μM	9.601	0.533		5.553

015-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.571	0.404	0.605	15.720
	CQ,20 μM	9.780	0.545		5.573

016-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	1.868	0.187	0.688	9.991
	CQ,20 μM	8.603	0.515		5.981

016-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.021	0.316	0.616	15.639
	CQ,20 μM	8.681	0.536		6.171

017-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.396	0.299	0.685	12.470
	CQ,20 μM	9.441	0.440		4.658

017-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	1.989	0.246	0.542	12.352
	CQ,20 μM	8.305	0.719		8.655

018-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.850	0.357	0.604	12.515
	CQ,20 μM	10.415	0.641		6.157

018-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	0.272	0.272	0.611	9.430
	CQ,20 μM	10.715	0.743		6.933

019-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.703	0.150	0.587	5.547
	CQ,20 μM	10.115	0.871		8.611

019-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.560	0.215	0.635	8.417
	CQ,20 μM	9.875	0.674		6.822

020-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.343	0.261	0.648	11.127
	CQ,20 μM	9.735	0.606		6.225

020-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	1.897	0.925	0.335	48.782
	CQ,20 μM	10.292	0.935		9.084

021-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.640	0.305	0.711	11.534
	CQ,20 μM	10.607	0.462		4.355

021-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.764	0.237	0.692	8.618
	CQ,20 μM	10.826	0.592		5.467

022-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.589	0.323	0.666	12.494
	CQ,20 μM	10.083	0.511		5.073

022-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.478	0.346	0.654	13.975
	CQ,20 μM	10.552	0.586		5.555

023-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.683	0.189	0.736	7.062
	CQ,20 μM	10.673	0.514		4.818

023-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.697	0.352	0.682	13.057
	CQ,20 μM	10.529	0.477		4.530

024-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.086	0.335	0.436	16.043
	CQ,20 μM	9.668	1.091		11.286

024-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.469	0.256	0.635	10.375
	CQ,20 μM	10.292	0.696		6.767

025-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.214	0.310	0.480	13.979
	CQ,20 μM	9.558	0.940		10.089

025-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.142	0.227	0.581	10.582
	CQ,20 μM	9.399	0.786		8.364

026-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	1.899	0.237	0.499	12.484
	CQ,20 μM	8.929	0.936		10.486

026-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	1.919	0.478	0.490	24.900
	CQ,20 μM	9.208	0.760		8.257

027-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.639	0.176	0.669	6.680
	CQ,20 μM	10.982	0.746		6.789

027-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	1.917	0.236	0.383	12.299
	CQ,20 μM	8.621	1.143		13.259

028-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.729	0.227	0.721	8.319
	CQ,20 μM	10.226	0.469		4.586

028-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.762	0.198	0.657	7.171
	CQ,20 μM	9.974	0.627		6.287

029-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.098	0.213	0.622	10.161
	CQ,20 μM	10.463	0.839		8.023

029-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.244	0.256	0.535	11.385
	CQ,20 μM	10.552	1.032		9.770

030-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.090	0.282	0.455	13.494
	CQ,20 μM	9.889	1.134		11.472

030-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.153	0.256	0.560	11.891
	CQ,20 μM	10.334	0.944		9.133

031-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.372	0.177	0.673	7.467
	CQ,20 μM	9.257	0.573		6.192

031-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.294	0.290	0.424	12.636
	CQ,20 μM	8.696	0.940		10.809

032-3 Dup1	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.366	0.268	0.445	11.347
	CQ,20 μM	8.673	0.898		10.357

032-3 Dup2	Controls:	Avg puncta/cell	Std dev	Z'	%CV
	DMSO	2.512	0.262	0.678	10.437
	CQ,20 μM	9.503	0.488		5.140

Table S1: Reproducibility of high-content screen.
The high-content screen was assessed using Z' and % CV of two biological replicates.

13 elements included exclusively in *Biotin*-RH1115:
Isoform 4 of Tropomyosin alpha-1 chain (TPM1)
Prelamin-A/C (LMNA)
Heterogeneous nuclear ribonucleoprotein K (HNRNPK)
Nucleoside diphosphate kinase A (NME1)
Fructosamine-3-kinase (FN3K)
Ketosamine-3-kinase (FN3KRP)
Hemoglobin subunit alpha (HBA1)
Ubiquitin-60S ribosomal protein L40 (UBA-52)
Calmodulin-like protein 5 (CALML5)[1]
RNA-binding protein Raly (RALY)
Small nuclear ribonucleoprotein E (SNRPE)
Lysosome-associated membrane glycoprotein 1 (LAMP1)
HLA class I histocompatibility antigen, A-24 alpha chain (HLA-A)

	Potential targets
	Different isoform, lack of disease relevance, or no expression in brain cells
	Eluted in presence of competitor

Table S2: Proteins exclusive to ***Biotin*-RH1115** in the pulldown assay.
Thirteen proteins were exclusively pulled down by ***Biotin*-RH1115**, of which three were selected as putative targets. Putative targets were narrowed down based on several criteria: presence of various isoforms in solution, lack of disease relevance in the literature, and expression levels in the brain (CALML5) [1].

Antibody	Dilution	Experiment	Company (Catalog No.)
β-Actin (i^{3}Neurons)	1:30,000	Western Blot	ABClonal (AC026)
β-Actin (HeLa)	1:2000	Western Blot	Cell Signaling (8457L)
hLamp1 (i^{3}Neurons)	1:3000 1:500	Western Blot IF	Cell Signaling (9091)
Tau (i^{3}Neurons)	1:400	IF	Cell Signaling (4019S)
LC3 (i^{3}Neurons)	1: 1000	Western Blot	Cell Signaling (4108)
LC3 (HeLa)	1:1000	Western Blot	Cell Signaling (2775S)
Phospho-S6 (i^{3}Neurons)	1:7500	Western Blot	Cell Signaling (4858)
p70S6K (HeLa)	1:1000	Western Blot	Cell Signaling (9205S)
S6 (i^{3}Neurons)	1:1000	Western Blot	Cell Signaling (2217)
70S6K (HeLa)	1:1000	Western Blot	Cell Signaling (2708S)
LAMP1 (HeLa)	1:1000	Western Blot	Cell Signaling (3243S)
Lamin A/C (HeLa)	1:1000	Western Blot	Cell Signaling (2032S)
UBA52 (HeLa)	1:1000	Western Blot	ABCam (ab109227)

Table S3: Antibodies
Antibodies and specific experiments, dilutions, and companies are listed.

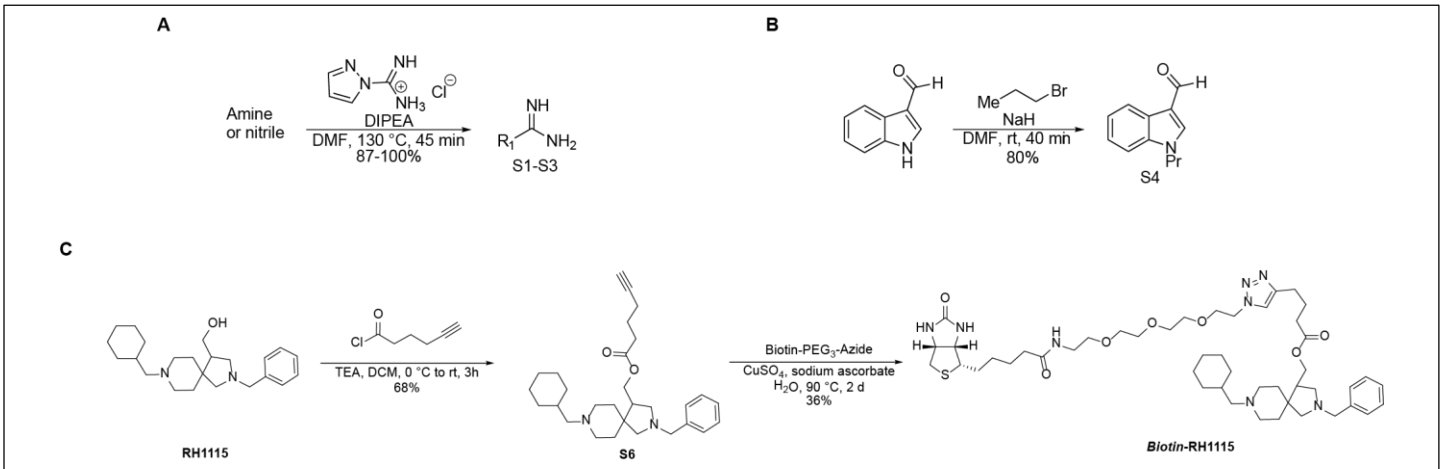


Figure S1: Additional synthetic efforts.
(**A**) Synthetic method to generate substituted guanidine and amidine reagents. (**B**) Synthetic method to generate the propyl substituted aldehyde reagent. (**C**) Synthetic route to access ***Biotin*-RH1115**.

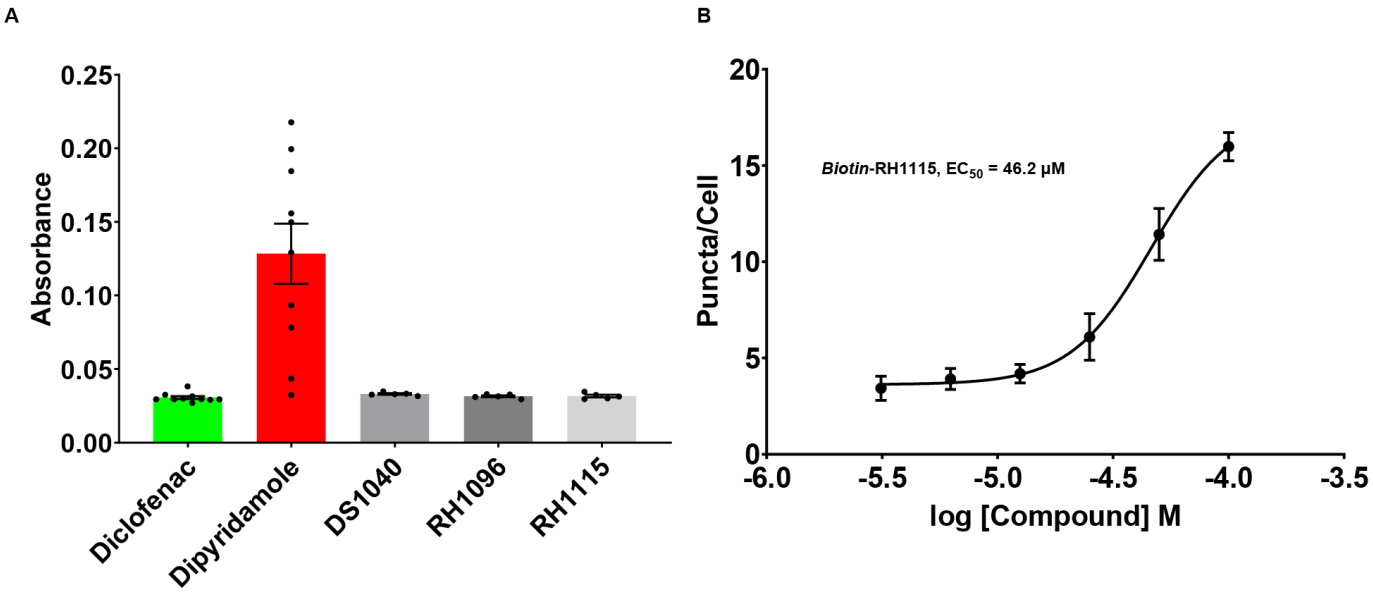


Figure S2: Solubility and confirmation of biotin probe activity.
(**A**) Kinetic aqueous solubility was performed on select compounds (100 µM) as a solution in 1x PBS. Optical density calculations were performed at 620 nm. Data are presented as the mean ± SEM of five independent experiments. (**B**) eGFP-LC3 dose response was performed on ***Biotin*-RH1115** to ensure activity was retained. Data are presented as mean ± SEM from three independent experiments, each with duplicate biological replicates.

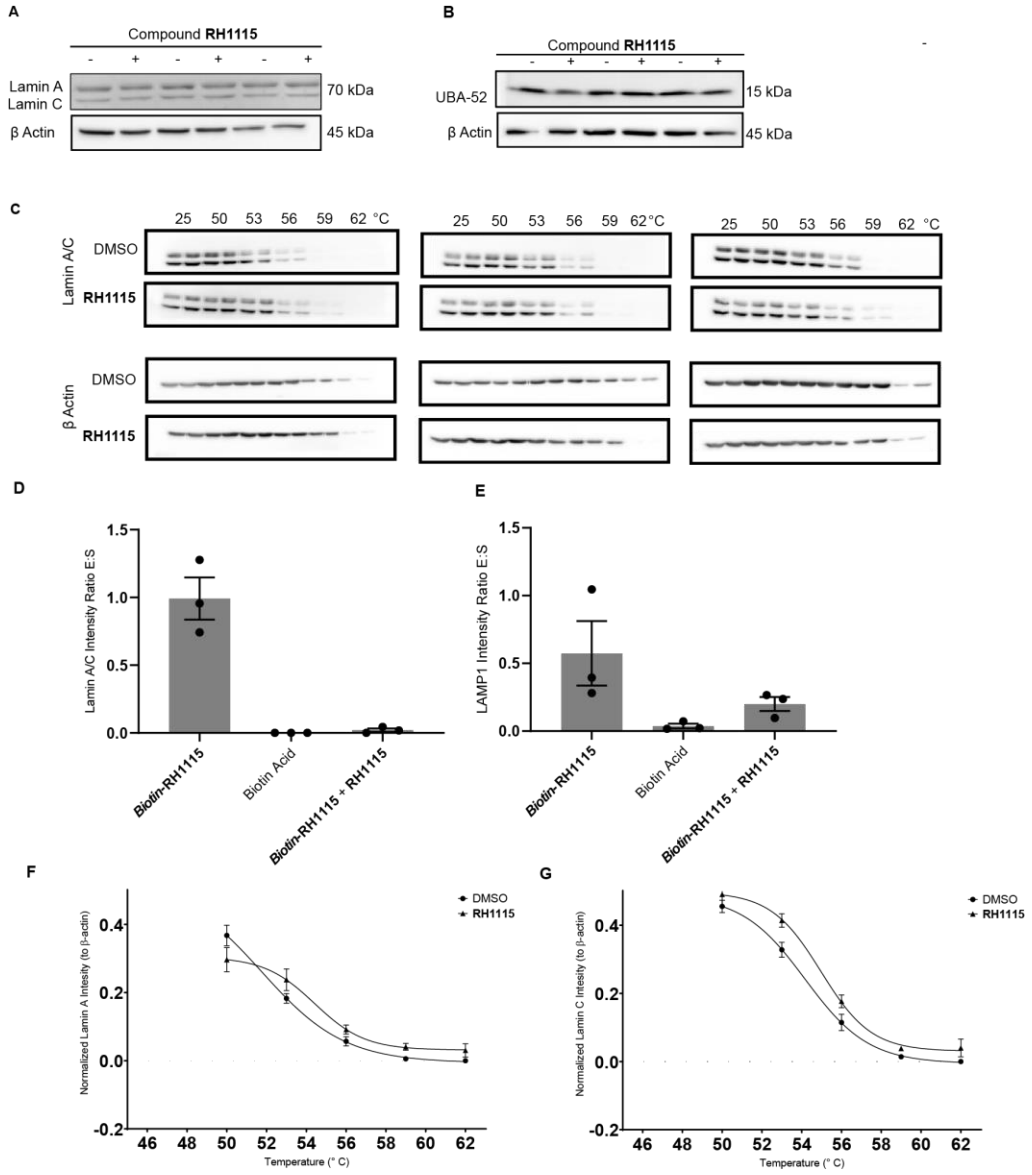


Figure S3: Expression levels of putative targets and validation.
Expression levels of Lamin A/C (**A**) and UBA-52 (**B**) were quantified using immunoblotting. Data are presented from three biological replicates. (**C**) Three independent Cellular Thermal Shift Assays were performed with A549 cells treated with **RH1115** (100 µM) or DMSO for 24 h and heated at each temperature in 3 minutes. Data are presented from two biological replicates at each temperature. (**D**) Ratio of the quantification of Lamin A/C protein levels in the eluent over the supernatant for the pulldown assay. Lysates were treated with ***Biotin*-RH1115** (50 µM), biotin acid (50 µM), and ***Biotin*-RH1115** (50 µM) in addition to **RH1115** in excess (100 µM). Data are presented as the mean ± SEM of three independent experiments. (**E**) Ratio of the quantification of LAMP1 protein levels in the eluent over the supernatant for the pulldown assay. Lysates were treated with ***Biotin*-RH1115** (50 µM), biotin acid (50 µM), and ***Biotin*-RH1115** (50 µM) in addition to **RH1115** in excess (100 µM). Data are presented as the mean ± SEM of three independent experiments. (**F**) Quantification of three independent cellular thermal shift assays in duplicate for Lamin A (ΔT_m = 2.64 °C). Data are presented as mean ± SEM of three independent experiments in duplicate. (**G**) Quantification of three independent cellular thermal shift assays in duplicate for Lamin C (ΔT_m = 0.8 °C). Data are presented as mean ± SEM of three independent experiments in duplicate.

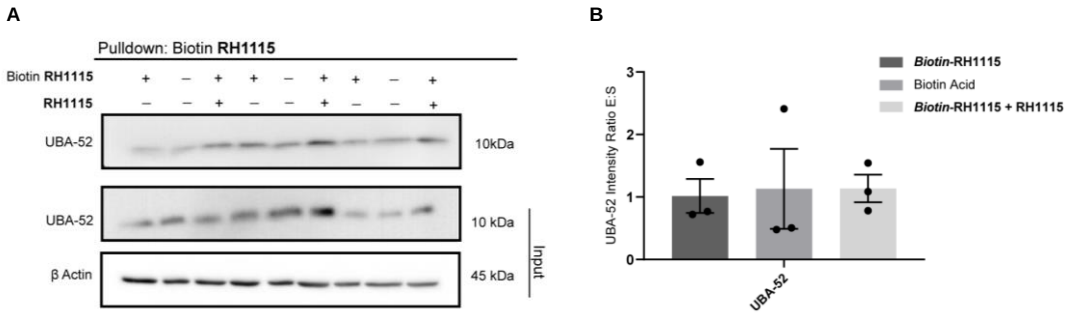


Figure S4: Exclusion of UBA52 as a putative target.
(**A**) Immunoblot analysis of UBA52 capture by streptavidin pulldown protocol in the presence of ***Biotin*-RH1115** (50 µM) (+/-), biotin acid (-/-), ***Biotin*-RH1115** (50 µM) with excess **RH1115** (100 µM) following lysate addition to the beads (+/+). β-actin was used as a loading control. Data are presented from three independent experiments. (**B**) Ratio of the quantification of UBA52 protein levels in the eluent over the supernatant for the pulldown assay shown in (**A**). Data are presented as the mean ± SEM of three independent experiments.

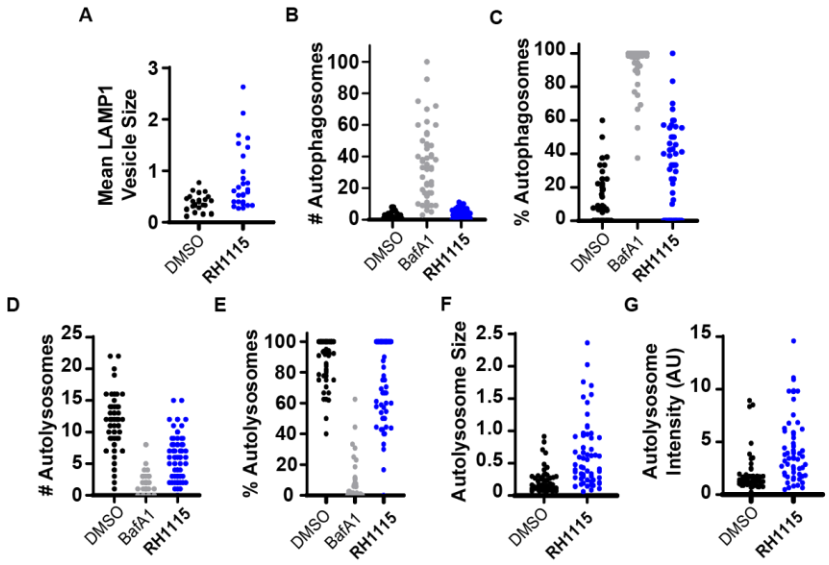


Figure S5: RH1115 increases LAMP1 vesicle size and activates autophagy in neurons.

(**A**) ADIV10 i3Neurons were immunostained for LAMP1. Mean LAMP1 vesicle size in each i3Neuron analyzed across all experiments for DMSO or RH1115 (15 μM). (**B**) DIV10 i^{3}Neurons stably expressing LC3-RFP-GFP were live imaged and the properties of each cell's autophagosomes and autolysosomes were quantified. Neurons were treated with DMSO, BafA1 (100 nM), or **RH1115** (15 μM). Data are presented from three independent experiments, 60-70 neurons per condition. The number of autophagosomes for each individual i^{3}Neuron were analyzed. (**C**) Percent autophagosomes for each individual i^{3}Neuron. (**D**) Number of autolysosomes for each individual i^{3}Neuron. (**E**) Percent autolysosomes in each individual i^{3}Neuron. (**F**) Mean autolysosome size in each individual i^{3}Neuron. (**G**) Autolysosomal intensity in each individual i^{3}Neuron.

Uncropped Western Blots

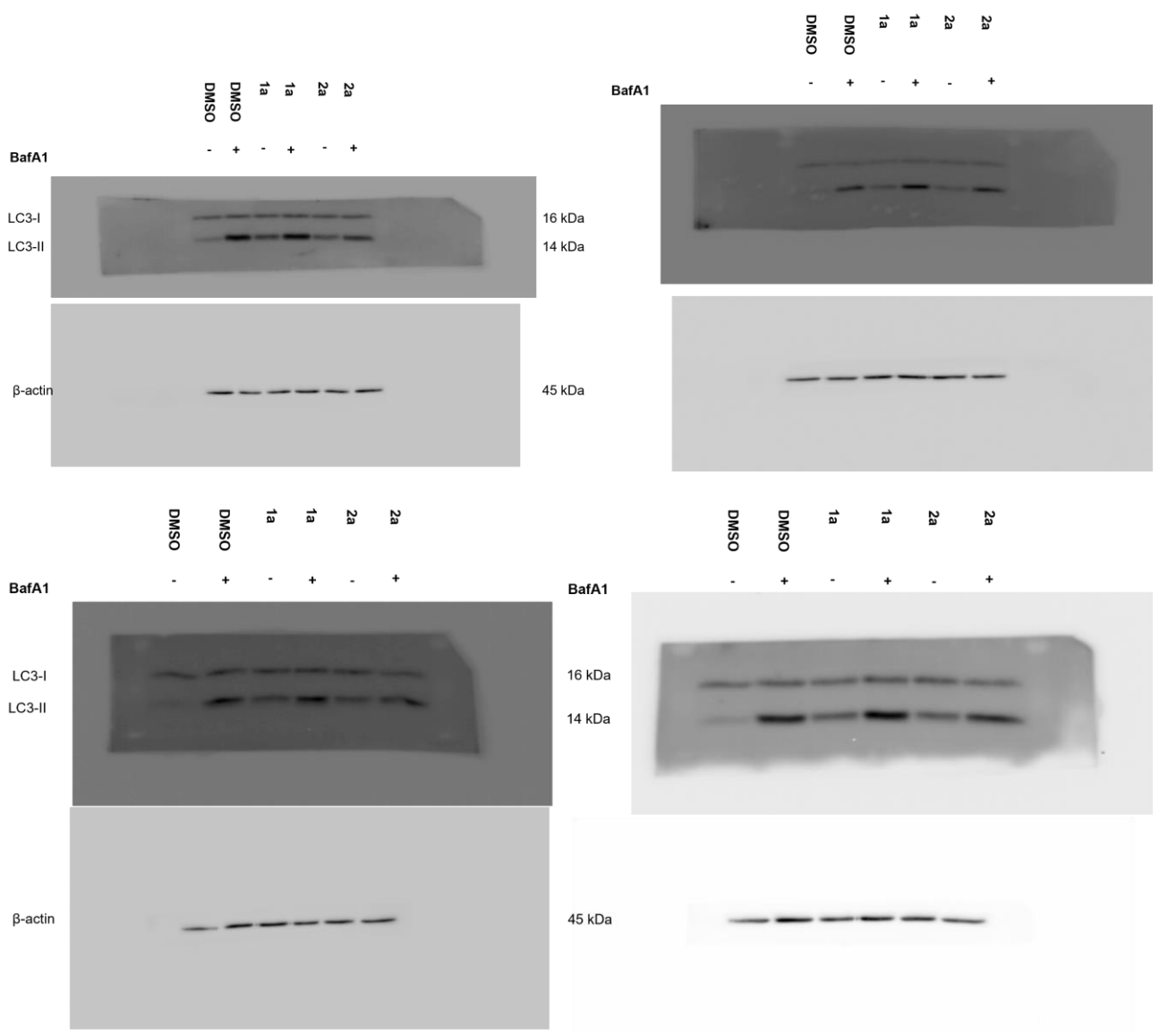


Figure 1E

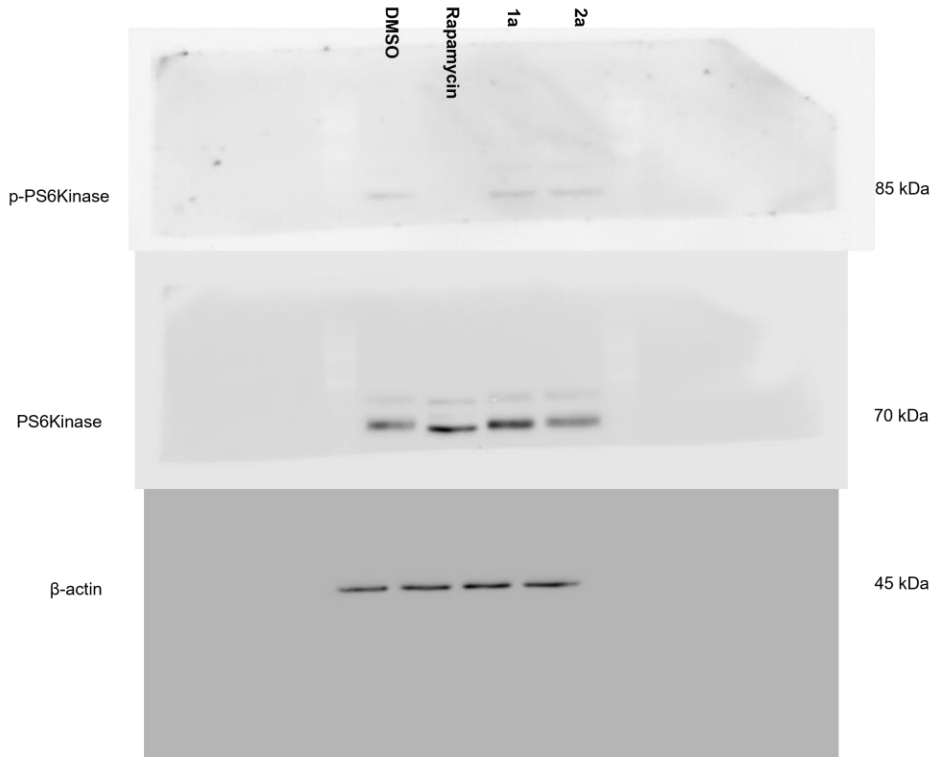


Figure 1G

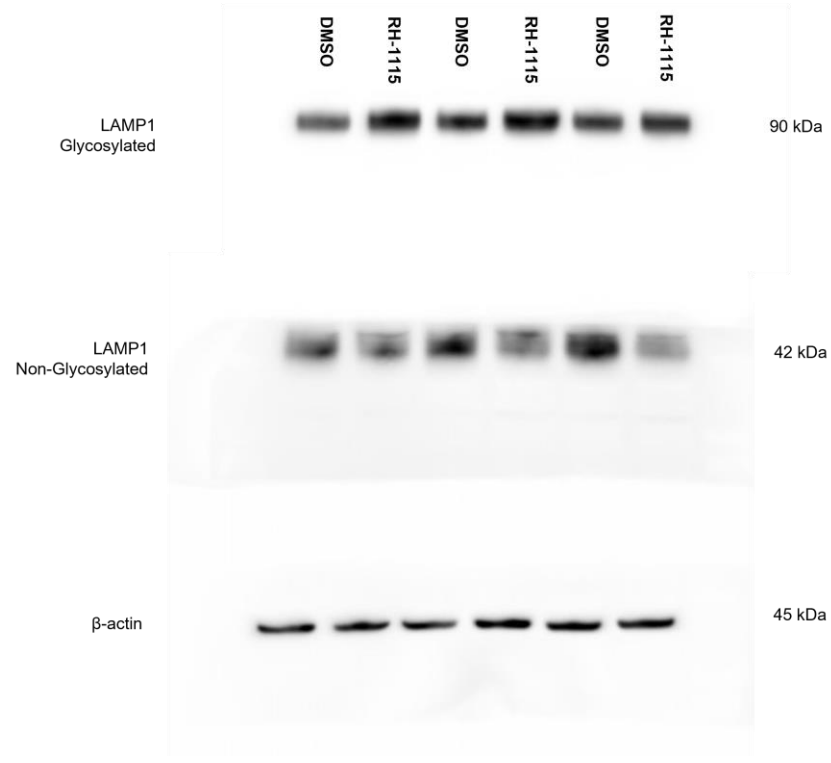


Figure 3C

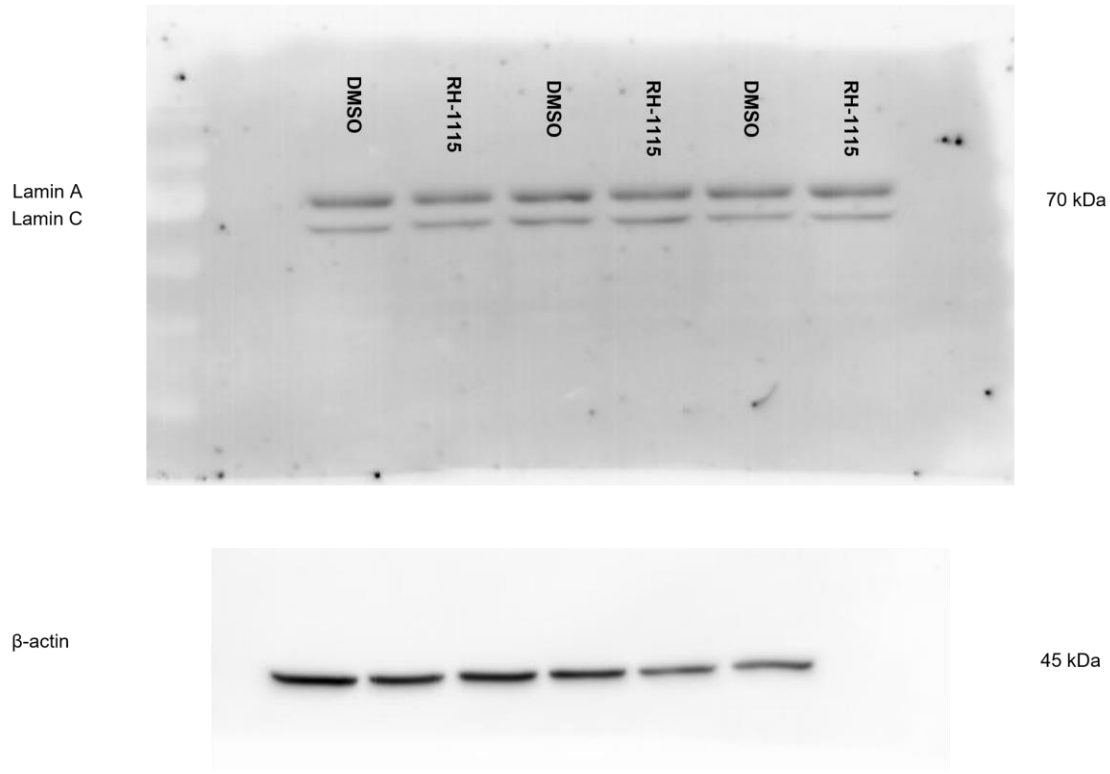


Figure 3D, S3A

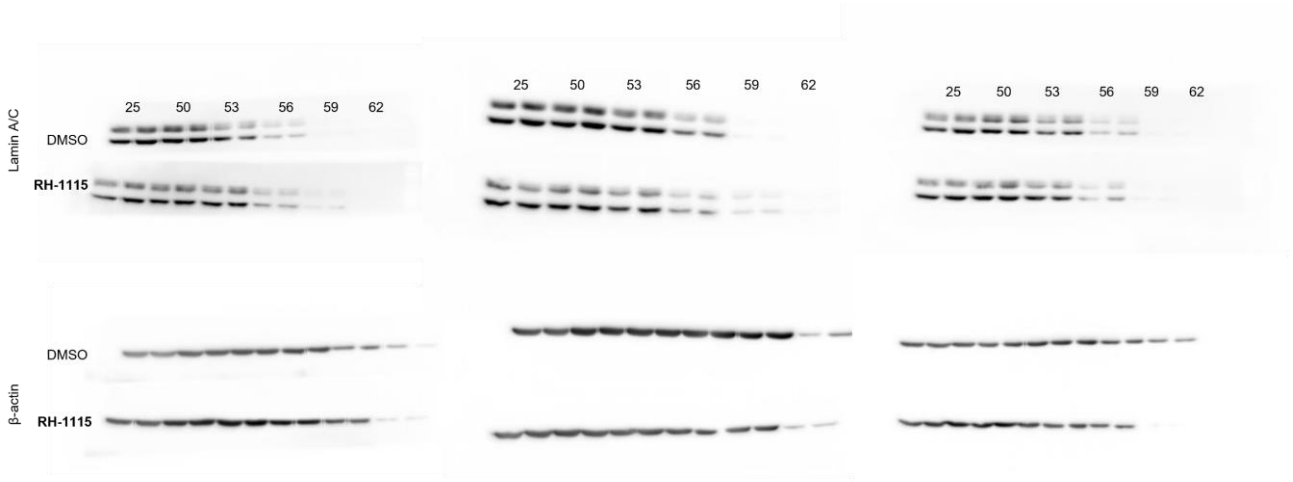


Figure 3E, S3C

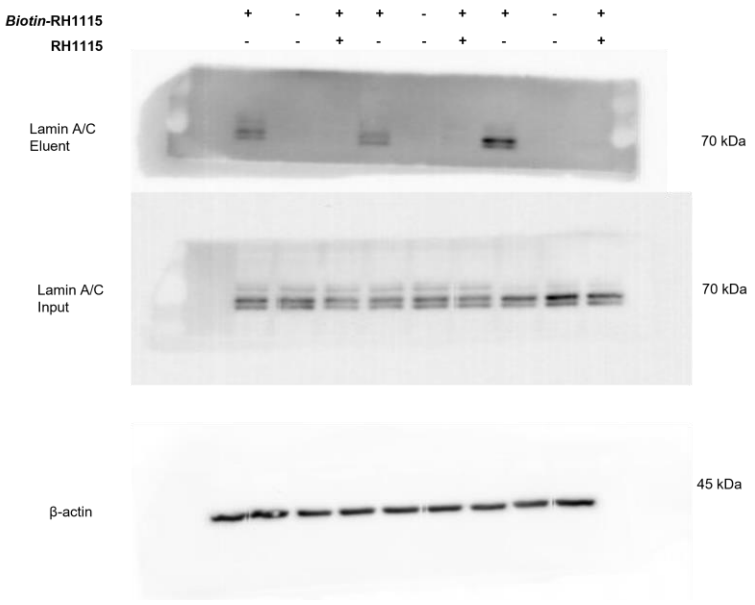


Figure 3F

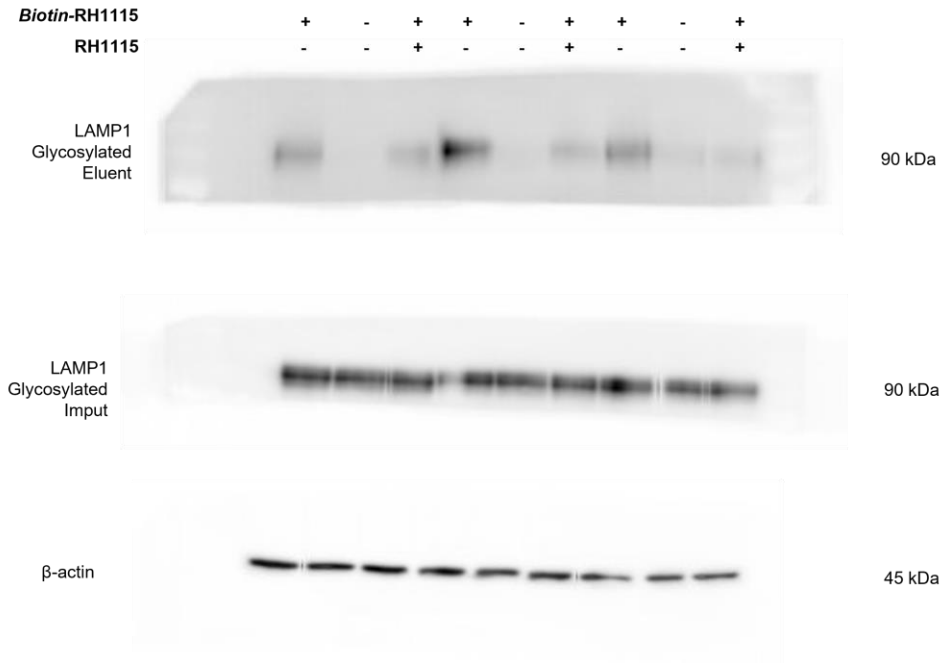


Figure 3G

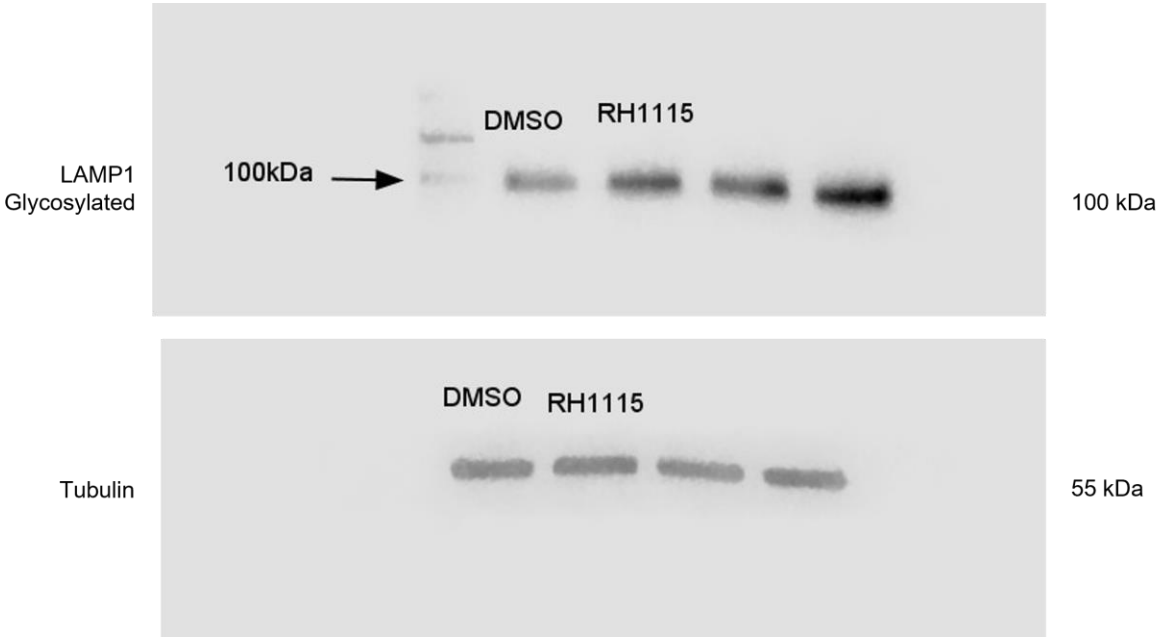


Figure 4F

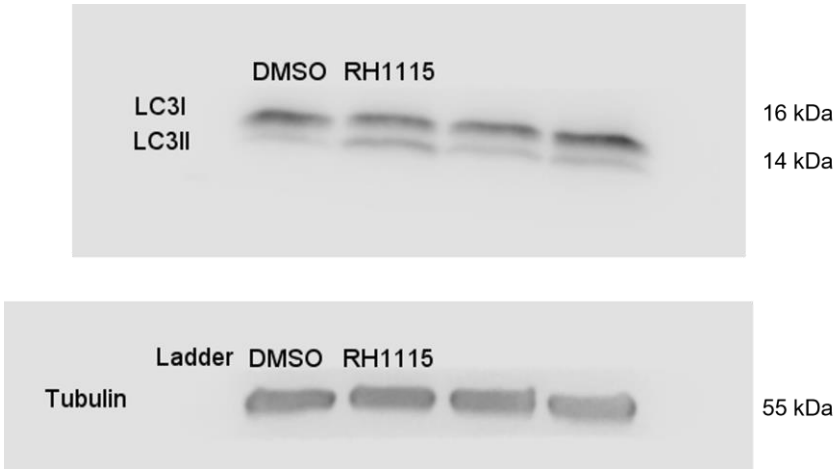


Figure 5a

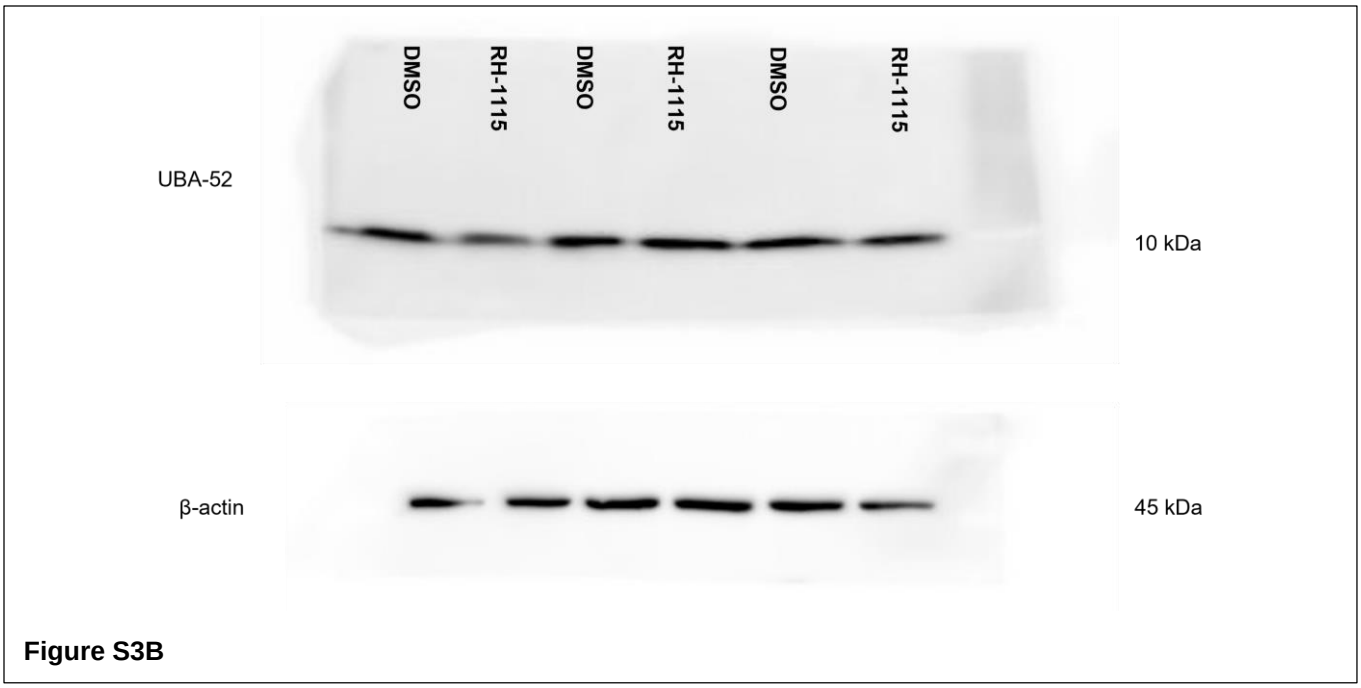


Figure S3B

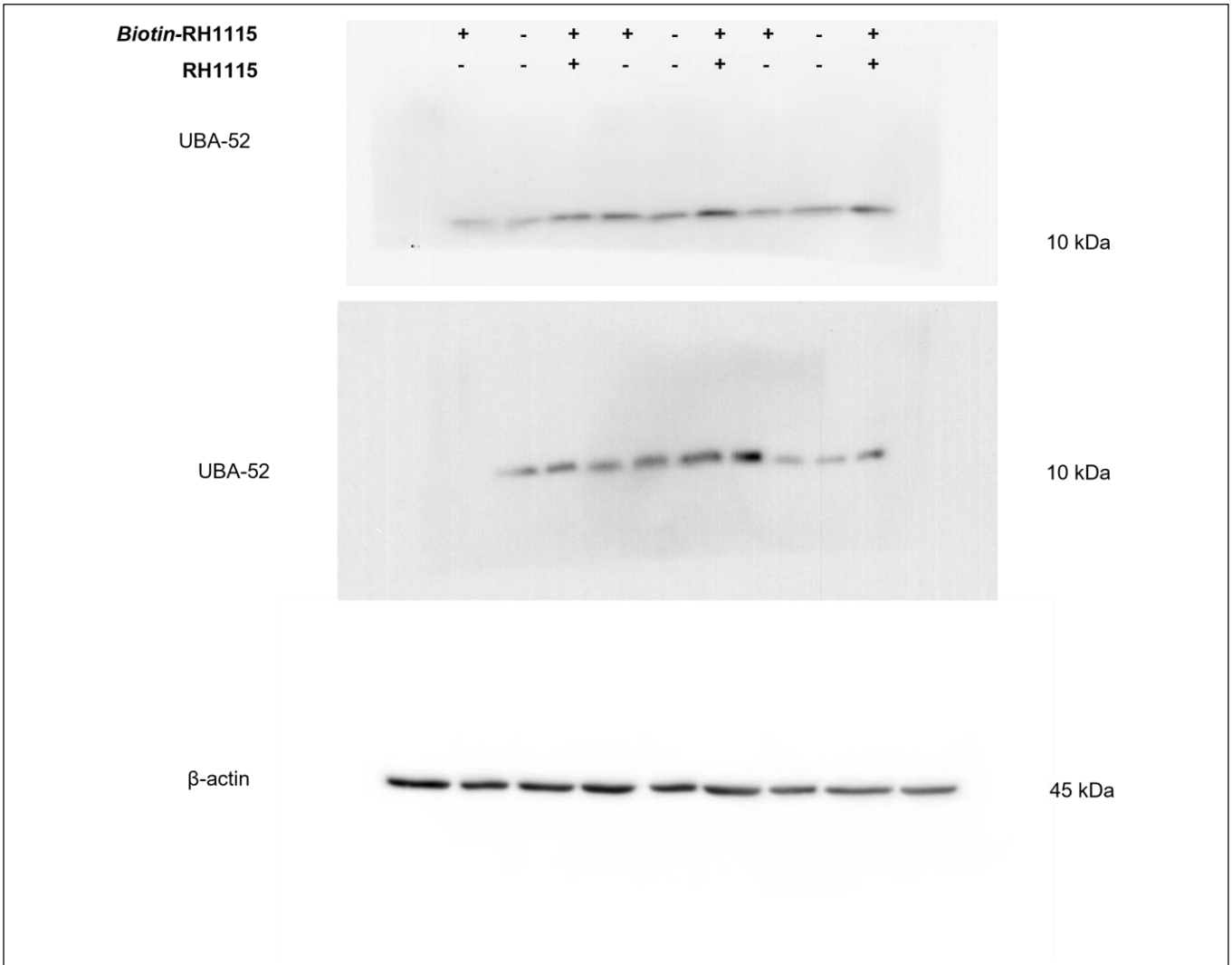
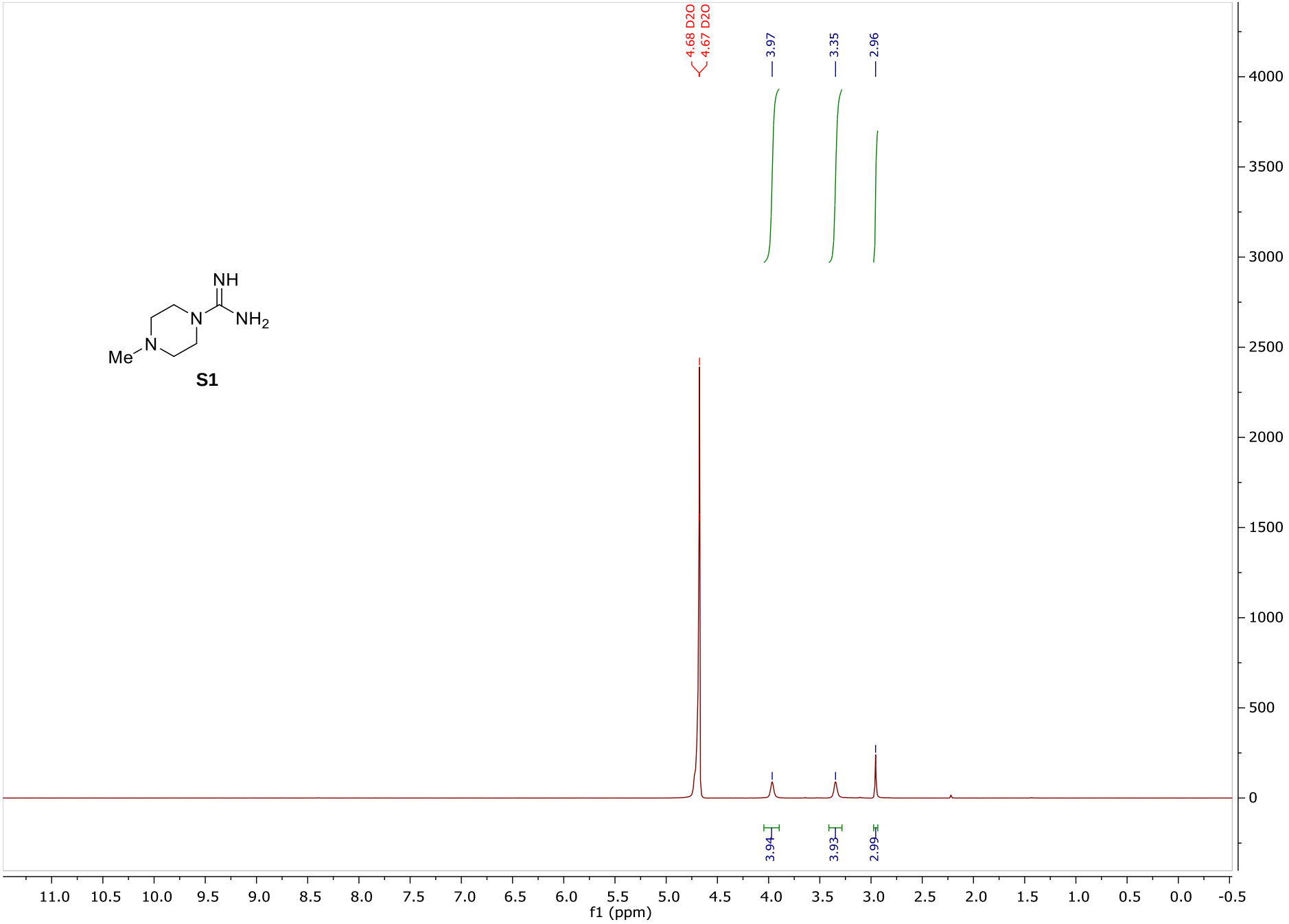
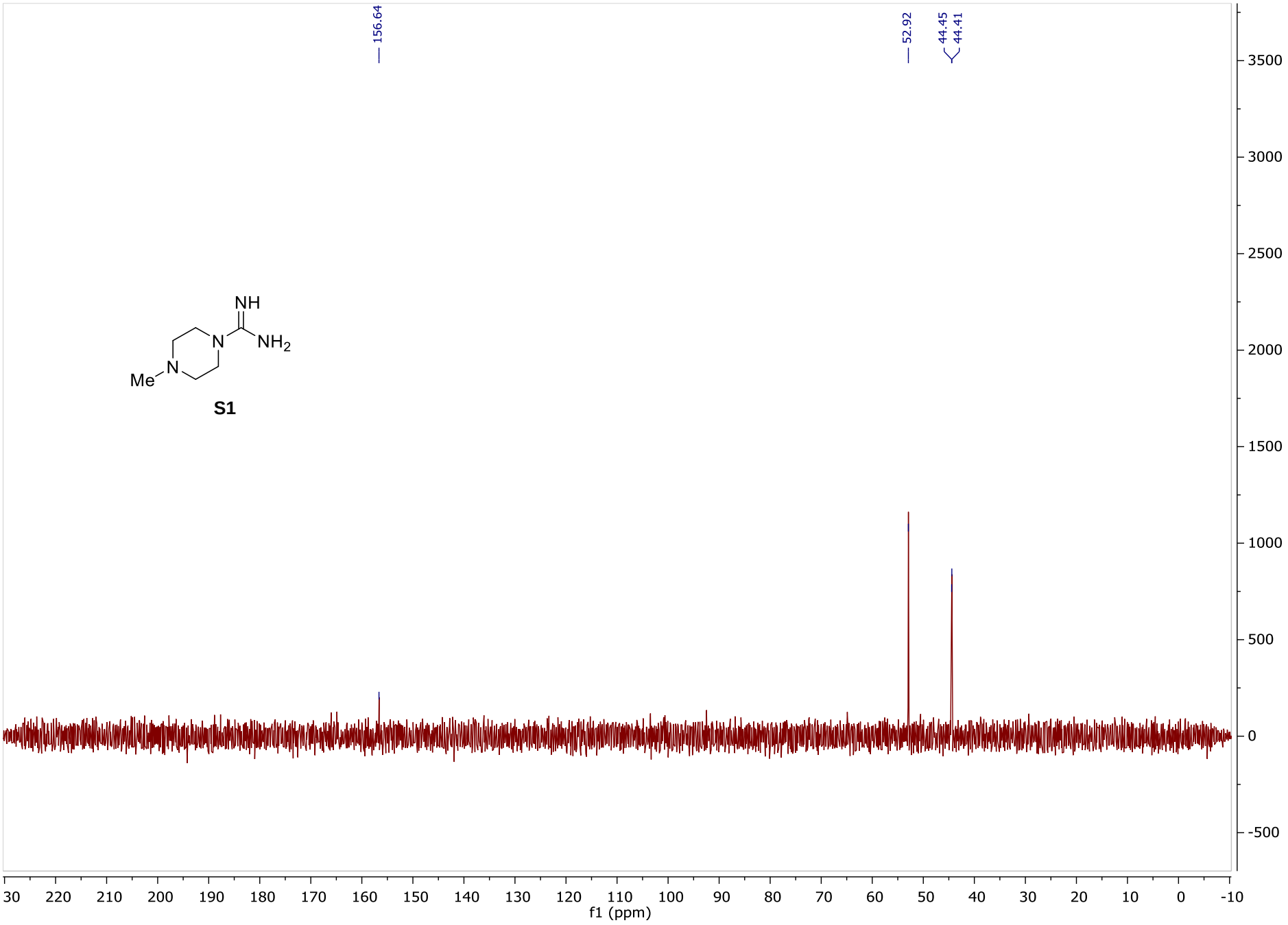


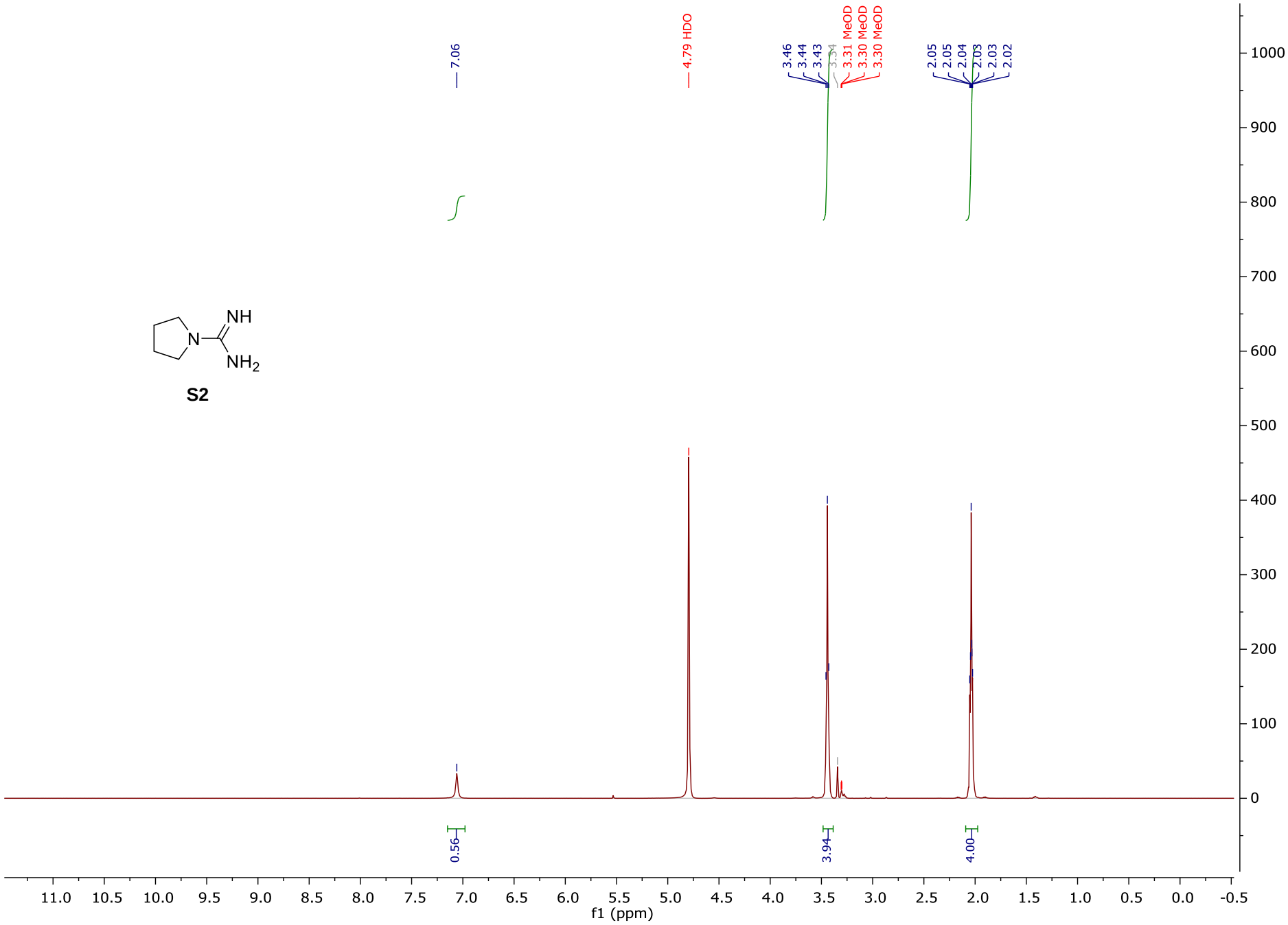
Figure S4A

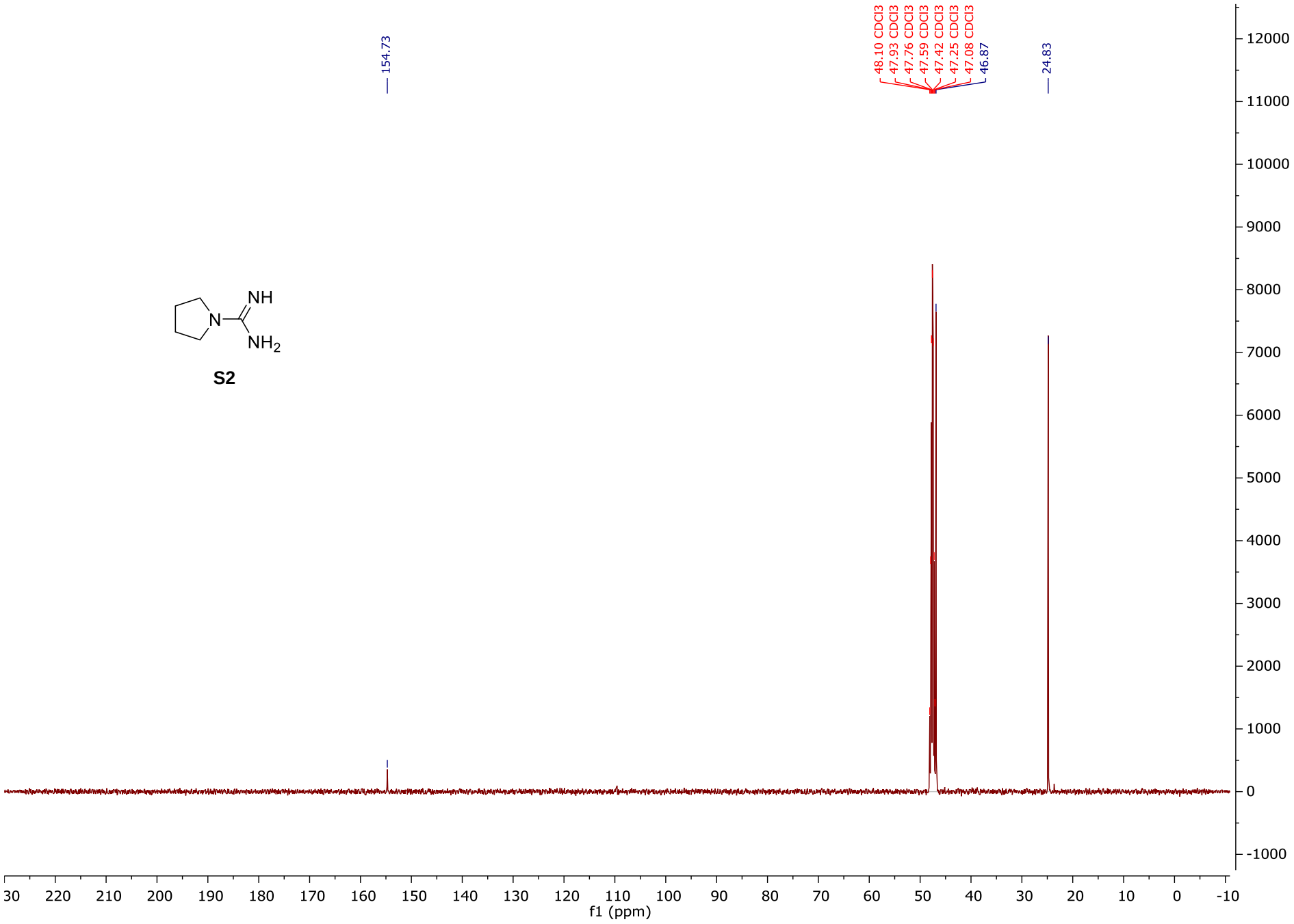
References

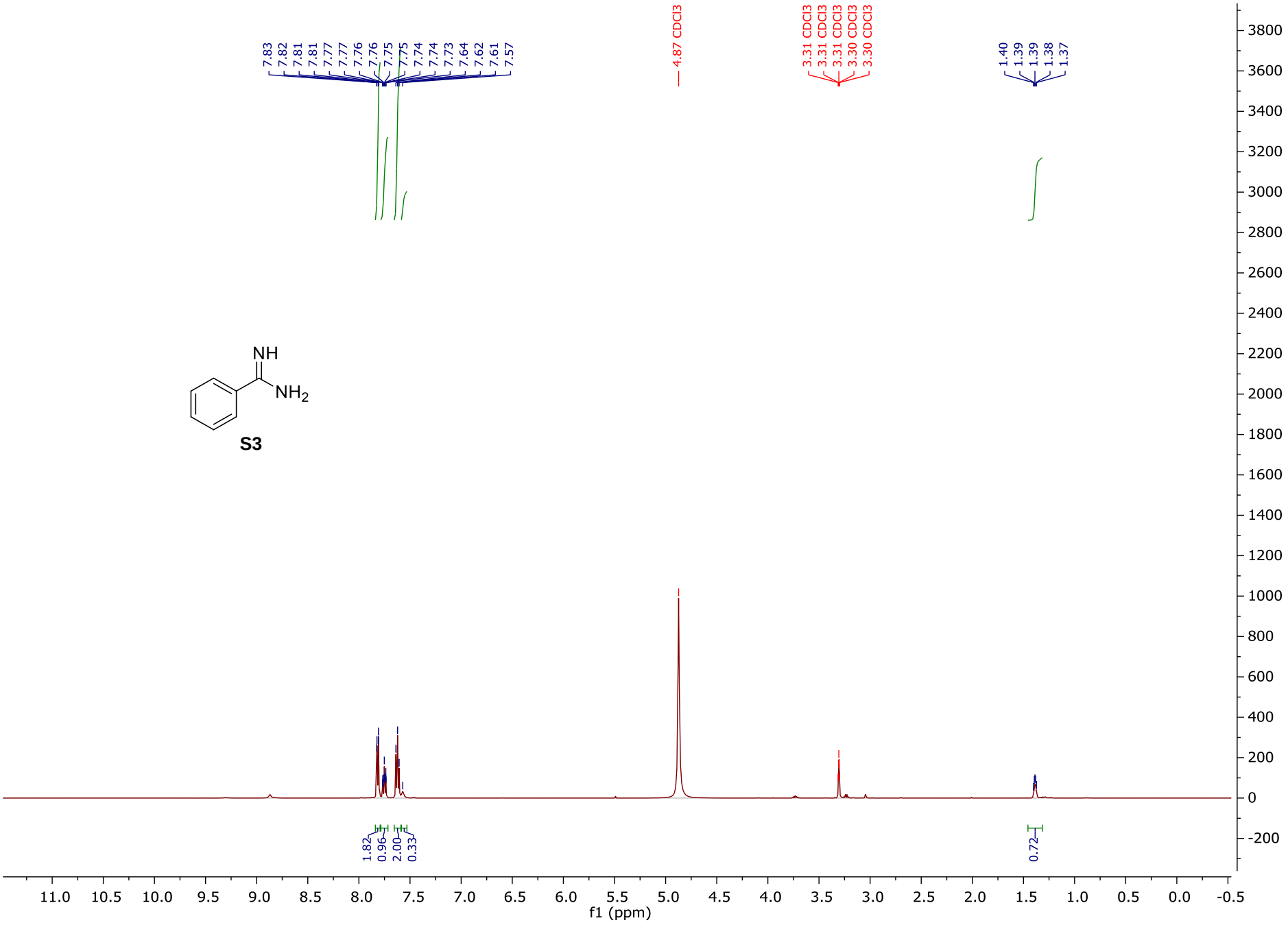
(1) Brain tissue expression of CALML5 Summary, The Human Protein Atlas. https://www.proteinatlas.org/ENSG00000178372-CALML5/brain (accessed 2023-04-11).


4.68 D2O
4.67 D2O
3.97
3.35
2.96
NH
NH2
N
N
Me
S1
3.94
3.93
2.99
f1 (ppm)


156.64
52.92
44.45
44.41
NH
N
NH2
Me
N
S1
3500
3000
2500
2000
1500
1000
500
0
-500
30
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


7.06
4.79 HDO
3.46
3.44
3.43
3.34
3.31 MeOD
3.30 MeOD
3.30 MeOD
2.05
2.05
2.04
2.03
2.03
2.02
NH
N
NH2
S2
0.56
3.94
4.00
f1 (ppm)


154.73
48.10 CDCl3
47.93 CDCl3
47.76 CDCl3
47.59 CDCl3
47.42 CDCl3
47.25 CDCl3
47.08 CDCl3
46.87
24.83
NH
N
NH2
S2
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
30 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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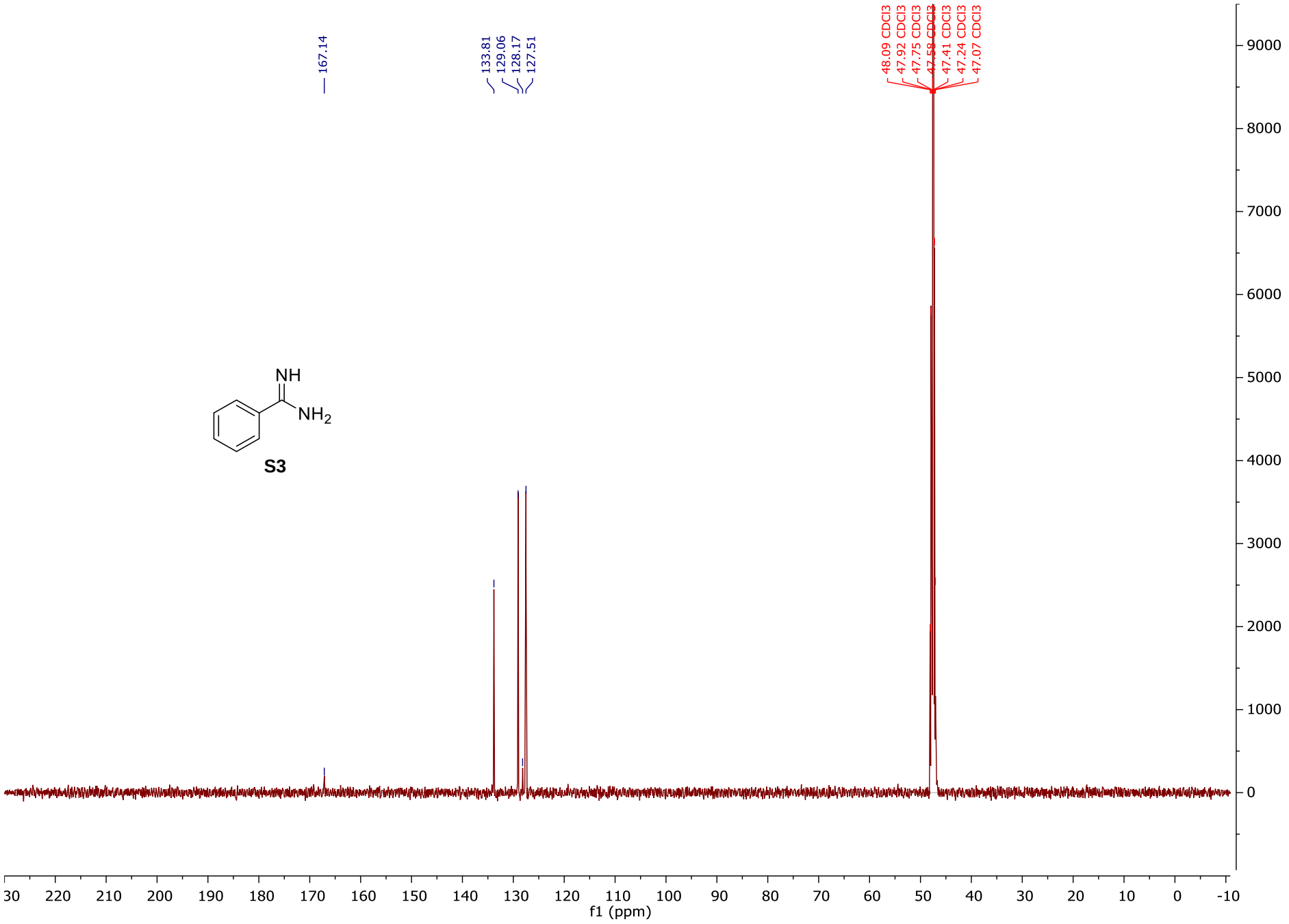
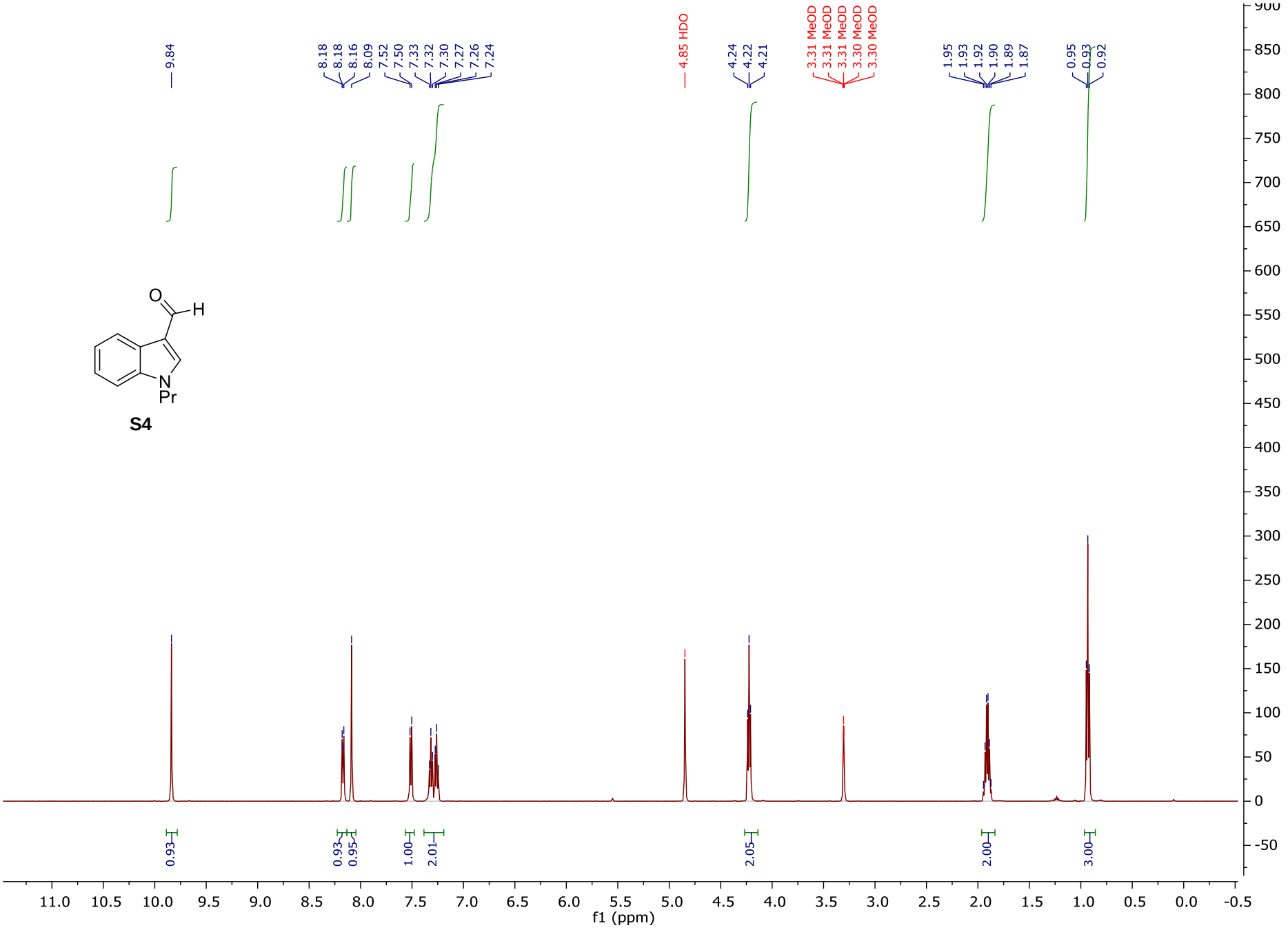
7.82
7.81
7.81
7.77
7.77
7.76
7.76
7.75
7.75
7.74
7.74
7.73
7.64
7.62
7.61
7.57
4.87 CDCl3
3.31 CDCl3
3.31 CDCl3
3.31 CDCl3
3.30 CDCl3
3.30 CDCl3
1.40
1.39
1.39
1.38
1.37
NH
NH2
S3
1.82
0.96
2.00
0.33
0.72
f1 (ppm)


167.14
133.81
129.06
128.17
127.51
48.09 CDCl3
47.92 CDCl3
47.75 CDCl3
47.58 CDCl3
47.41 CDCl3
47.24 CDCl3
47.07 CDCl3
NH
NH2
S3
f1 (ppm)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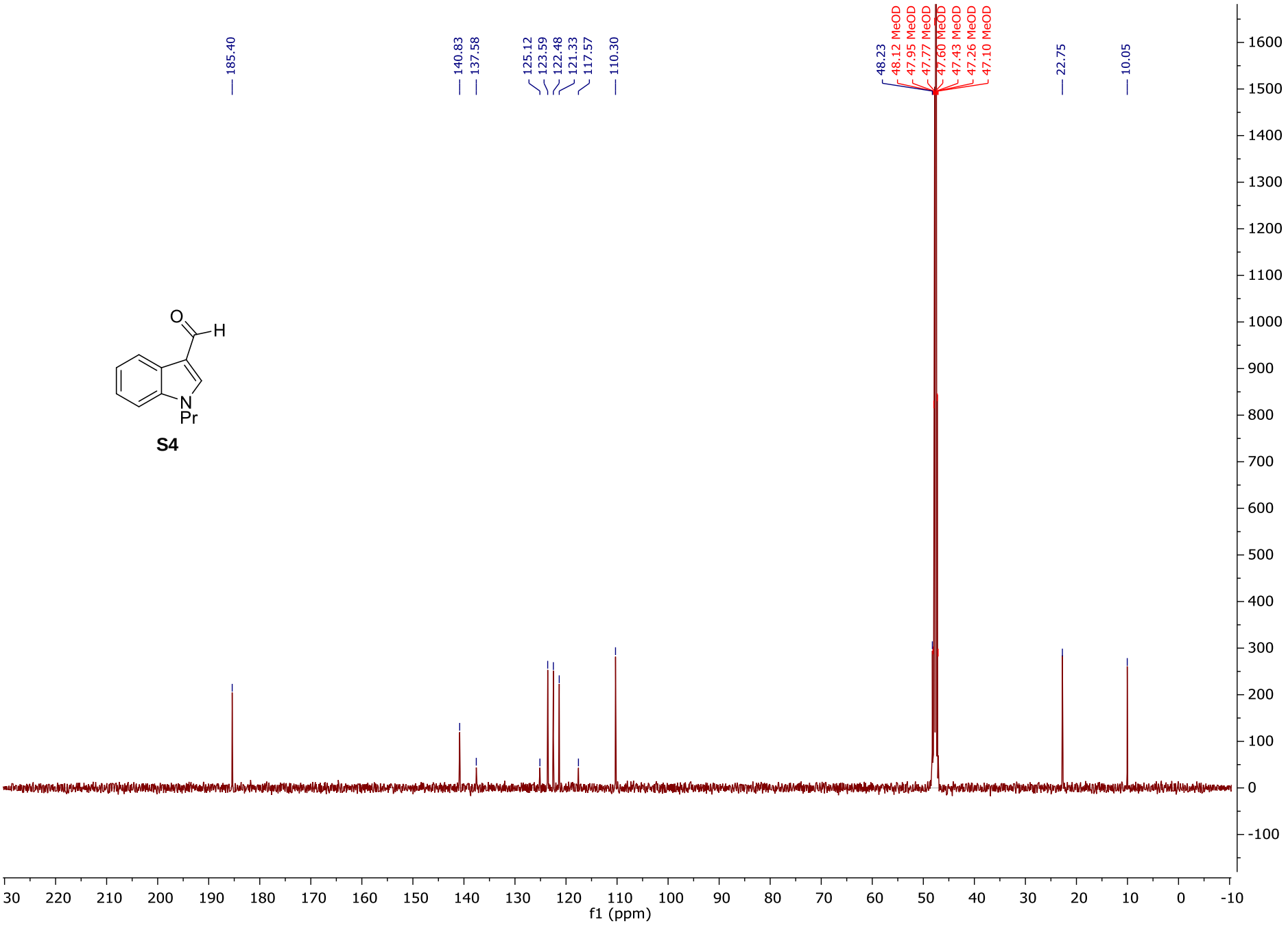
8.18
8.18
8.16
8.09
7.52
7.50
7.33
7.32
7.30
7.27
7.26
7.24
4.85 HDO
4.24
4.22
4.21
3.31 MeOD
3.31 MeOD
3.31 MeOD
3.30 MeOD
3.30 MeOD
1.95
1.93
1.92
1.90
1.89
1.87
0.95
0.93
0.92
O
H
N
Pr
S4
0.93
0.93
0.95
1.00
2.01
2.05
2.00
3.00
f1 (ppm)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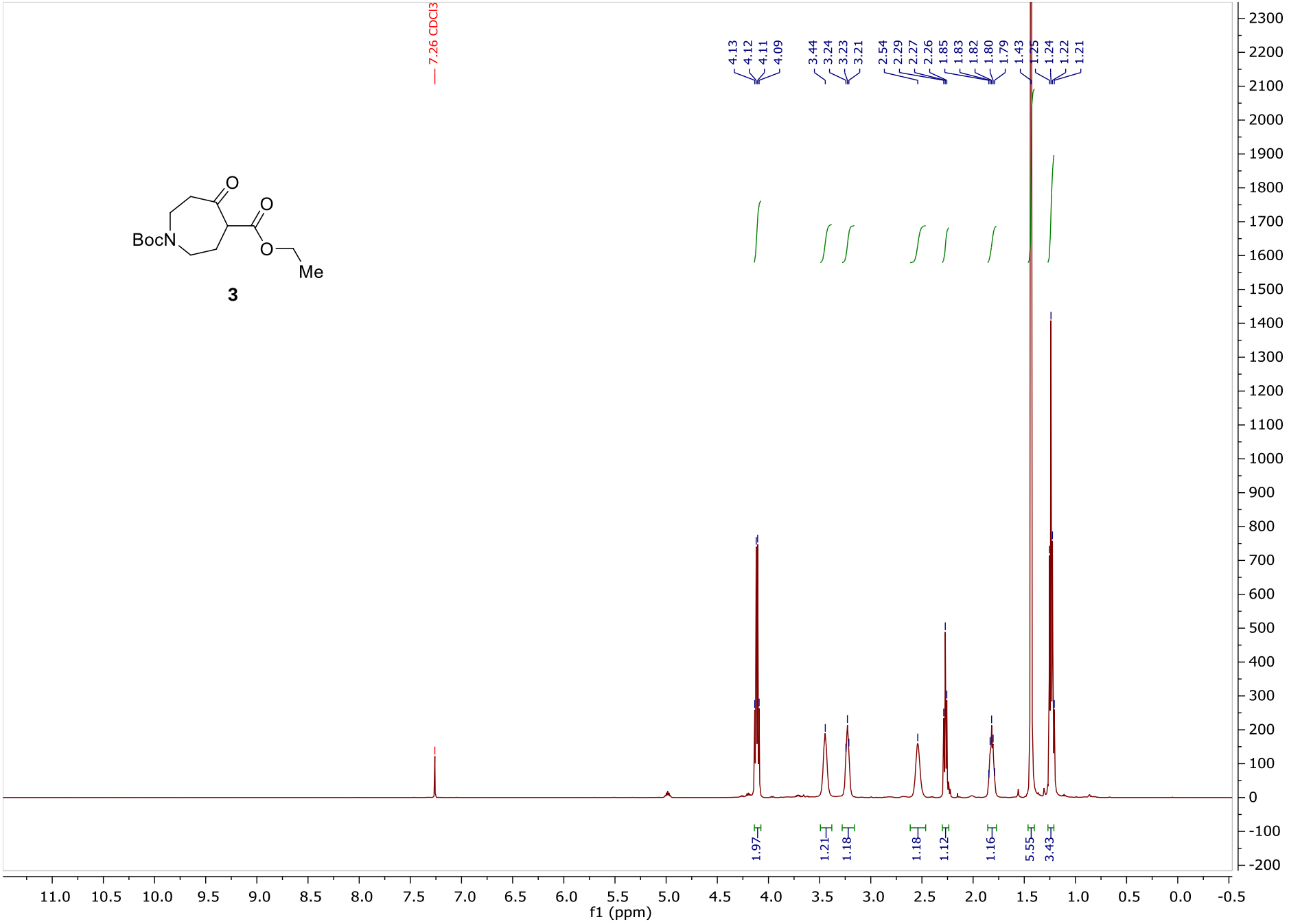
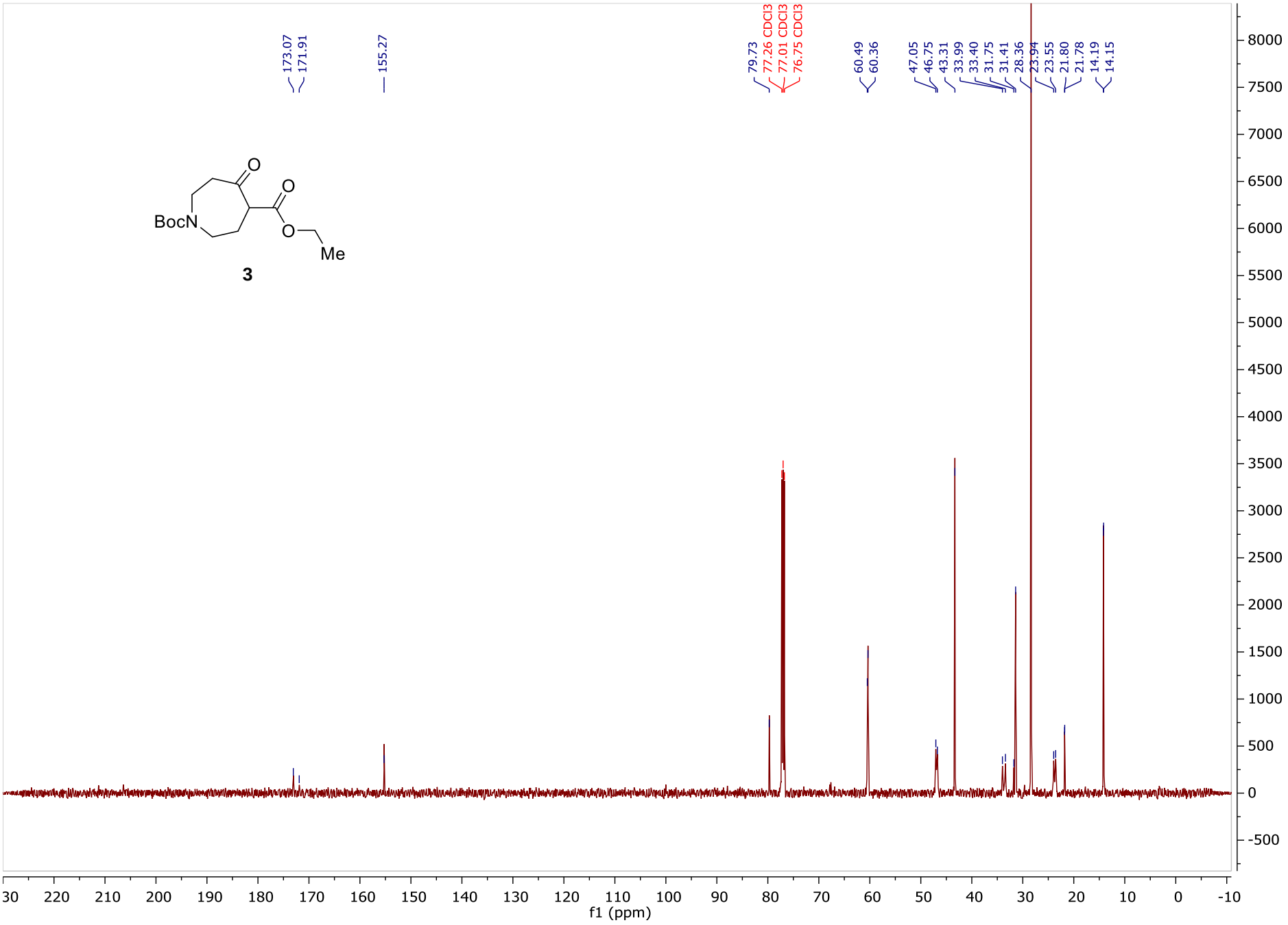
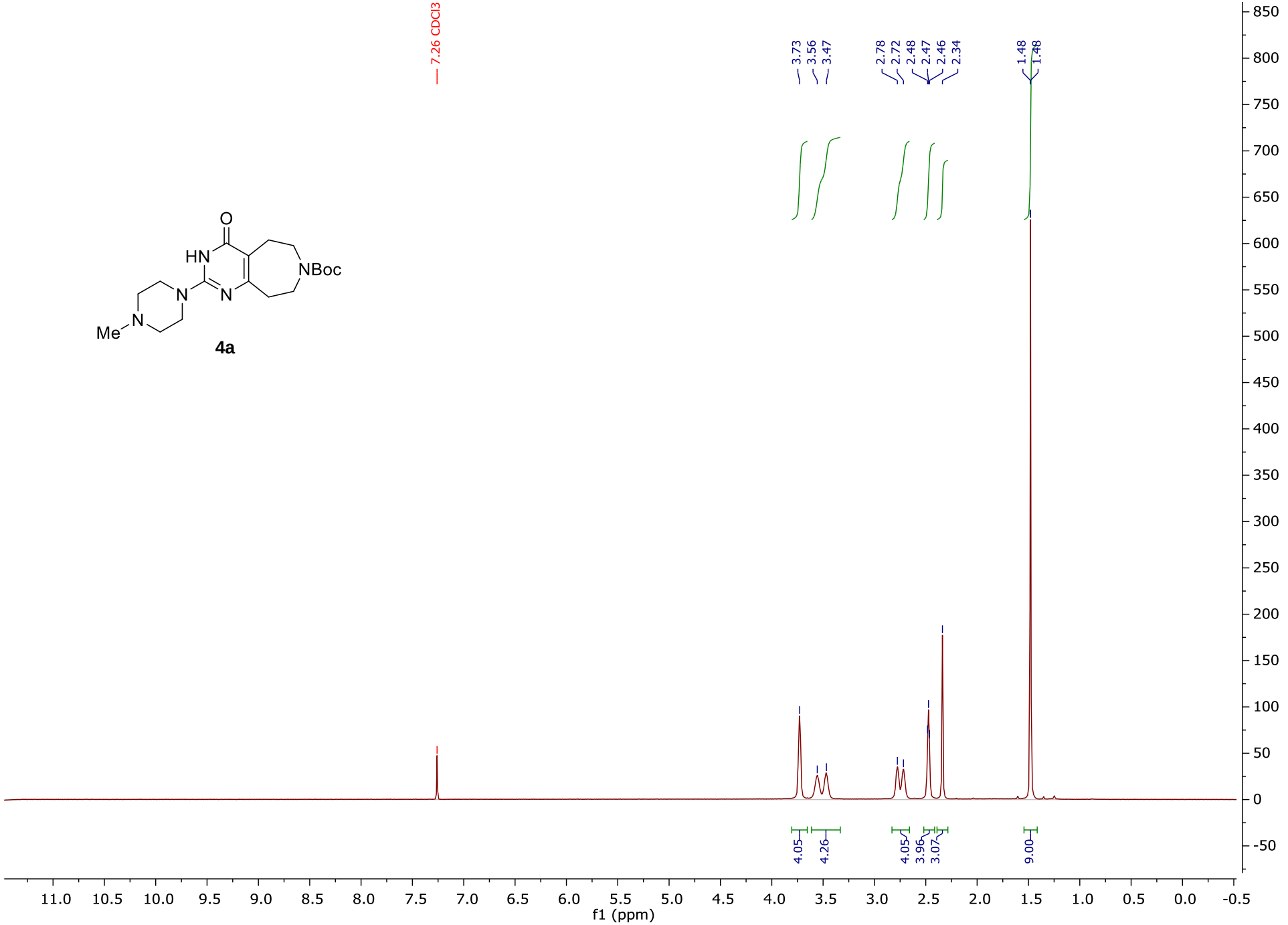
140.83
137.58
125.12
123.59
122.48
121.33
117.57
110.30
48.23
48.12 MeOD
47.95 MeOD
47.77 MeOD
47.60 MeOD
47.43 MeOD
47.26 MeOD
47.10 MeOD
22.75
10.05
O
H
N
Pr
S4
f1 (ppm)


7.26 CDCl3
4.13
4.12
4.11
4.09
3.44
3.24
3.23
3.21
2.54
2.29
2.27
2.26
1.85
1.83
1.82
1.80
1.79
1.43
1.25
1.24
1.22
1.21
BocN
O
O
O
Me
3
1.97
1.21
1.18
1.18
1.12
1.16
5.55
3.43
f1 (ppm)


173.07
171.91
155.27
79.73
77.26 CDCl3
77.01 CDCl3
76.75 CDCl3
60.49
60.36
47.05
46.75
43.31
33.99
33.40
31.75
31.41
28.36
23.94
23.55
21.80
21.78
14.19
14.15
BocN
O
O
O
Me
3
f1 (ppm)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3.56
3.47
2.78
2.72
2.48
2.47
2.46
2.34
1.48
1.48
O
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N
4a
4.05
4.26
4.05
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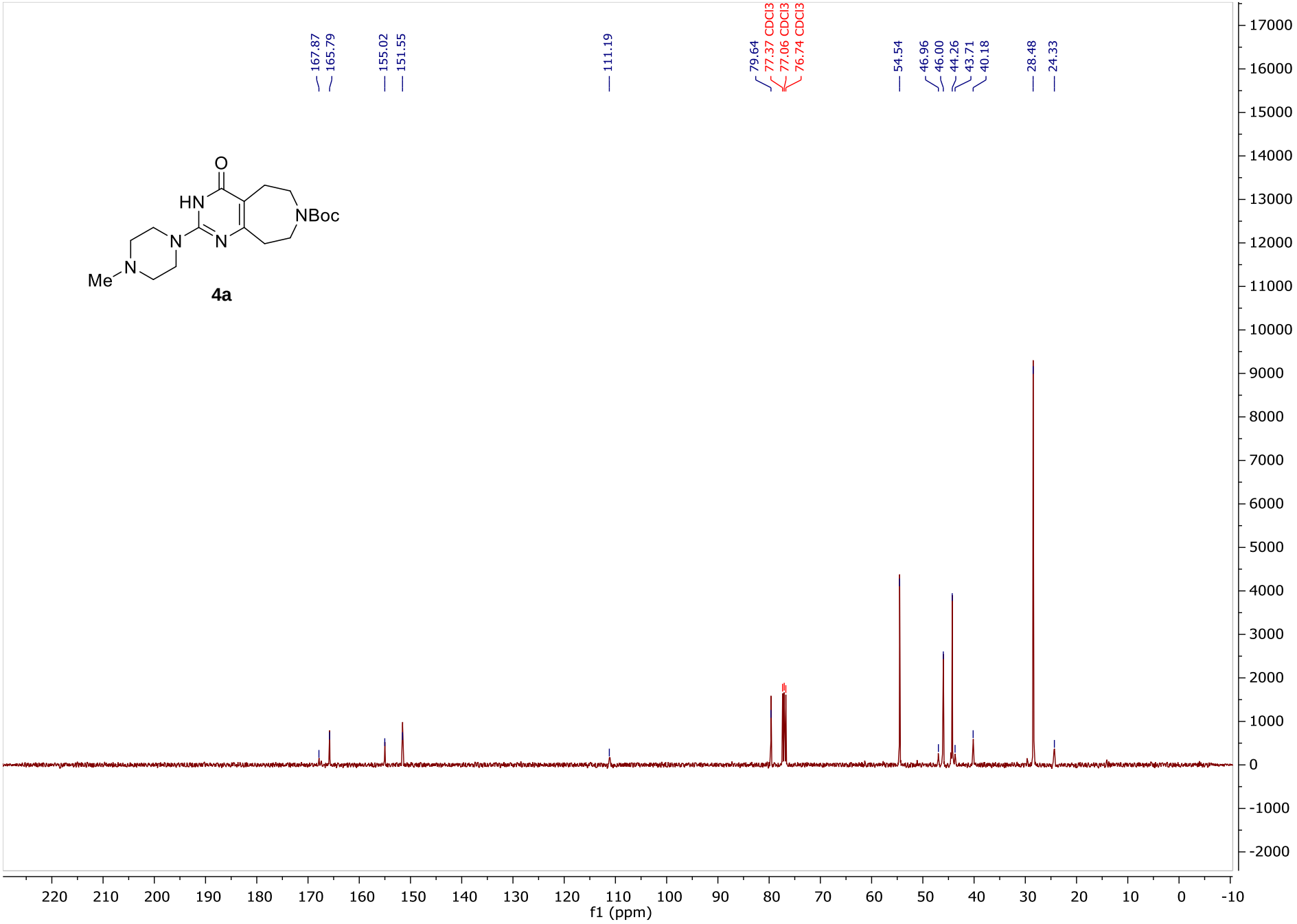
3.07
9.00
f1 (ppm)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151.55
111.19
79.64
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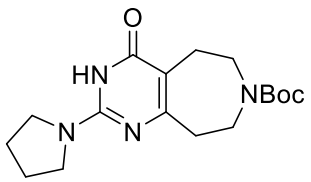
77.06 CDCl3
76.74 CDCl3
54.54
46.96
46.00
44.26
43.71
40.18
28.48
24.33
O
HN
NBoc
N
N
Me
N
4a
f1 (ppm)


O
HN
NBoc
N
N

4b

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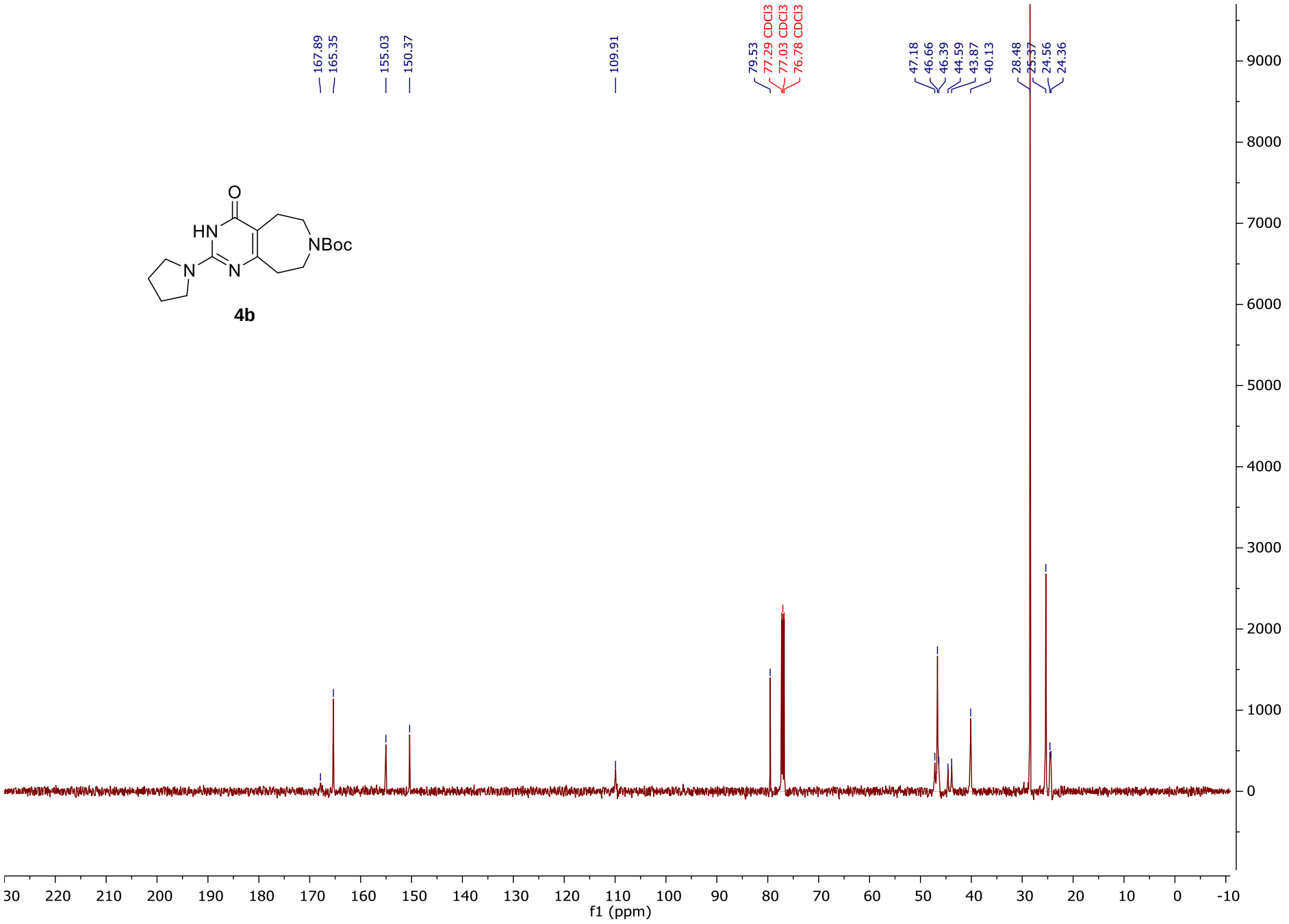
3.54
3.53
3.45
2.77
2.76
2.75
2.72
1.98
1.97
1.95
1.46
6.11
1.94
4.01
4.01
9.00
f1 (ppm)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109.91
79.53
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76.78 CDCl3
47.18
46.66
46.39
44.59
43.87
40.13
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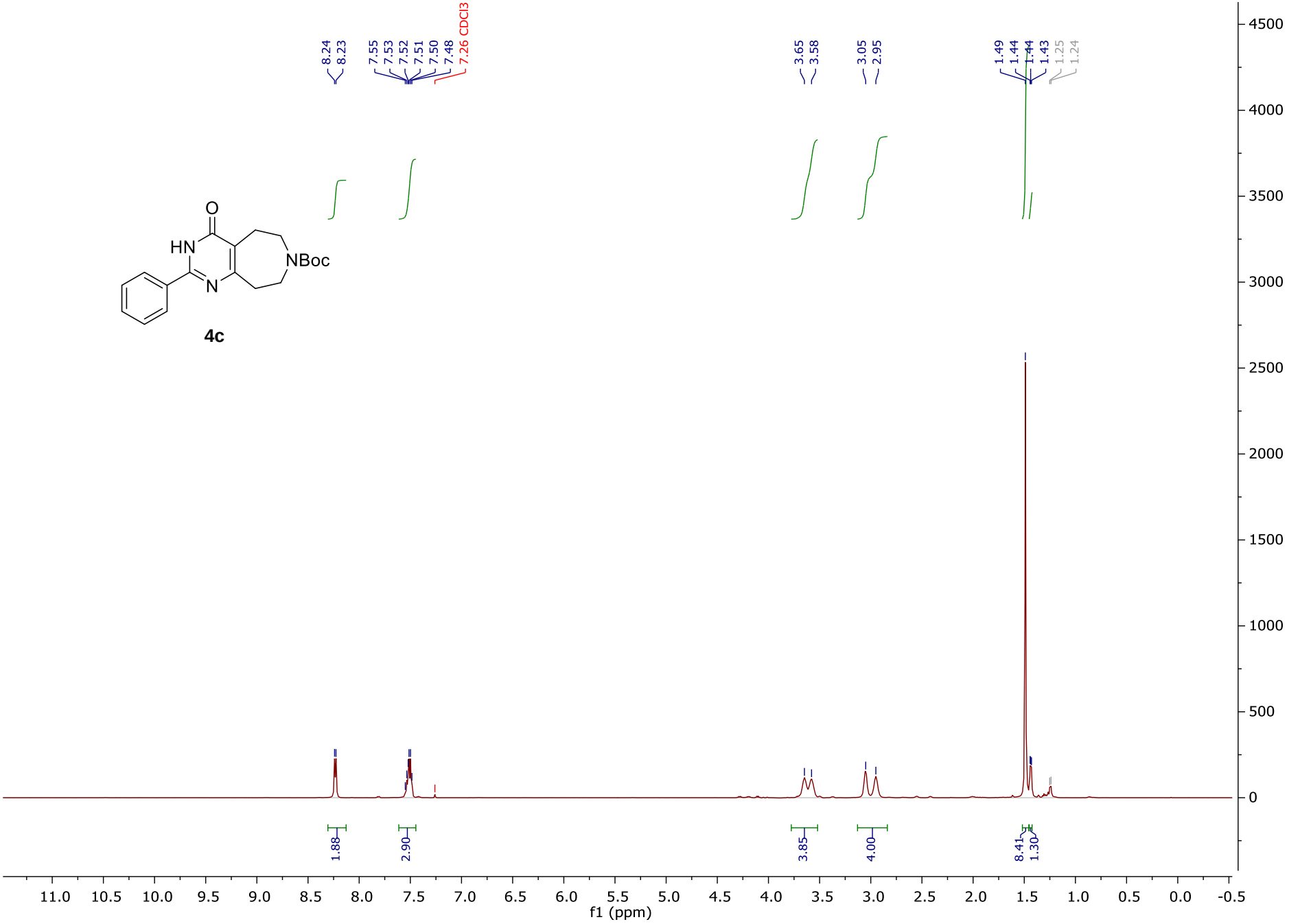
25.37
24.56
24.36
O
HN
N
N
NBoc
4b
f1 (ppm)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8.23
7.55
7.53
7.52
7.51
7.50
7.48
7.26 CDCl3
3.65
3.58
3.05
2.95
1.49
1.44
1.44
1.43
1.25
1.24
O
HN
N
NBoc
4c
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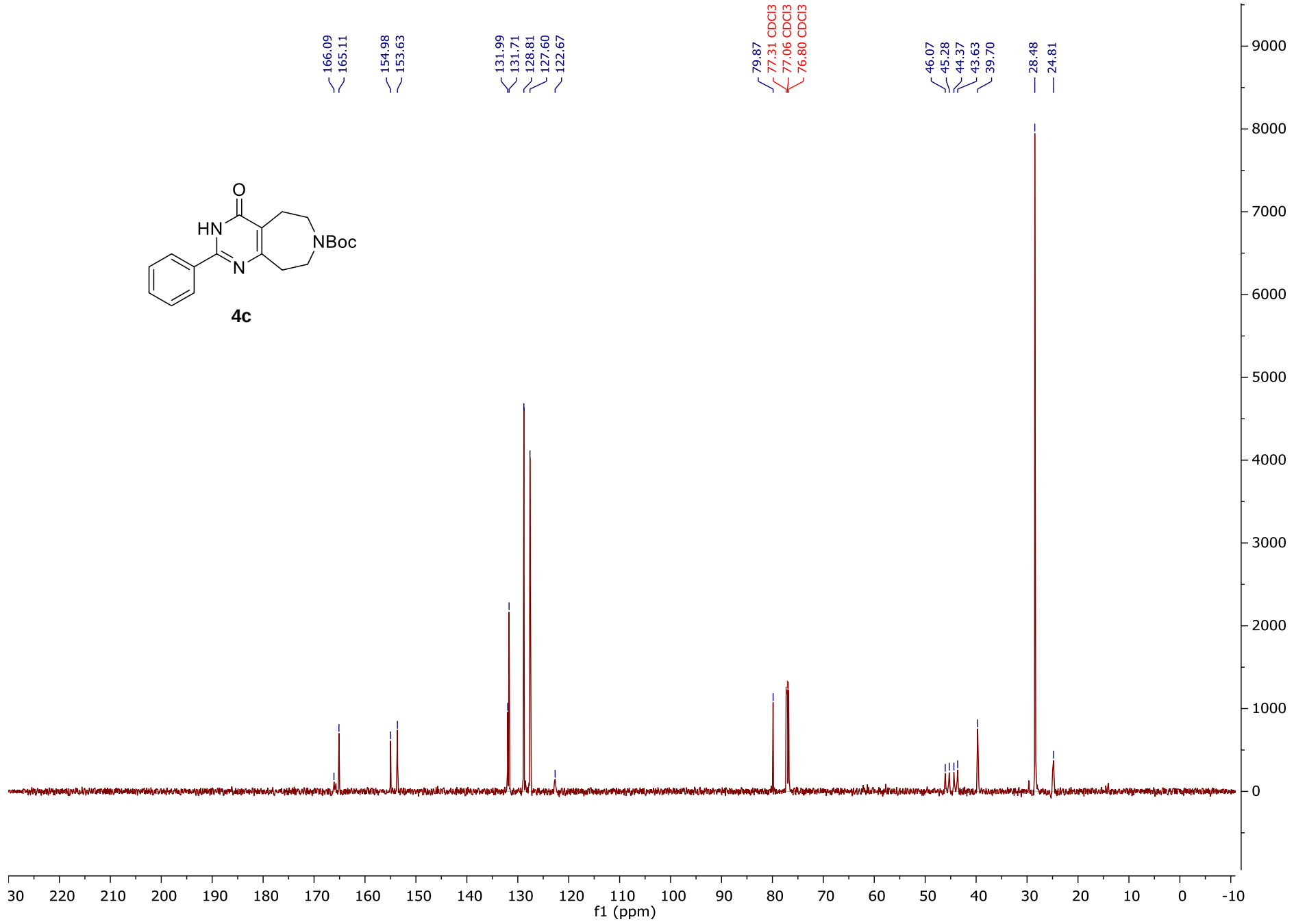
2.90
3.85
4.00
8.41
1.30
f1 (ppm)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153.63
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128.81
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76.80 CDCl3
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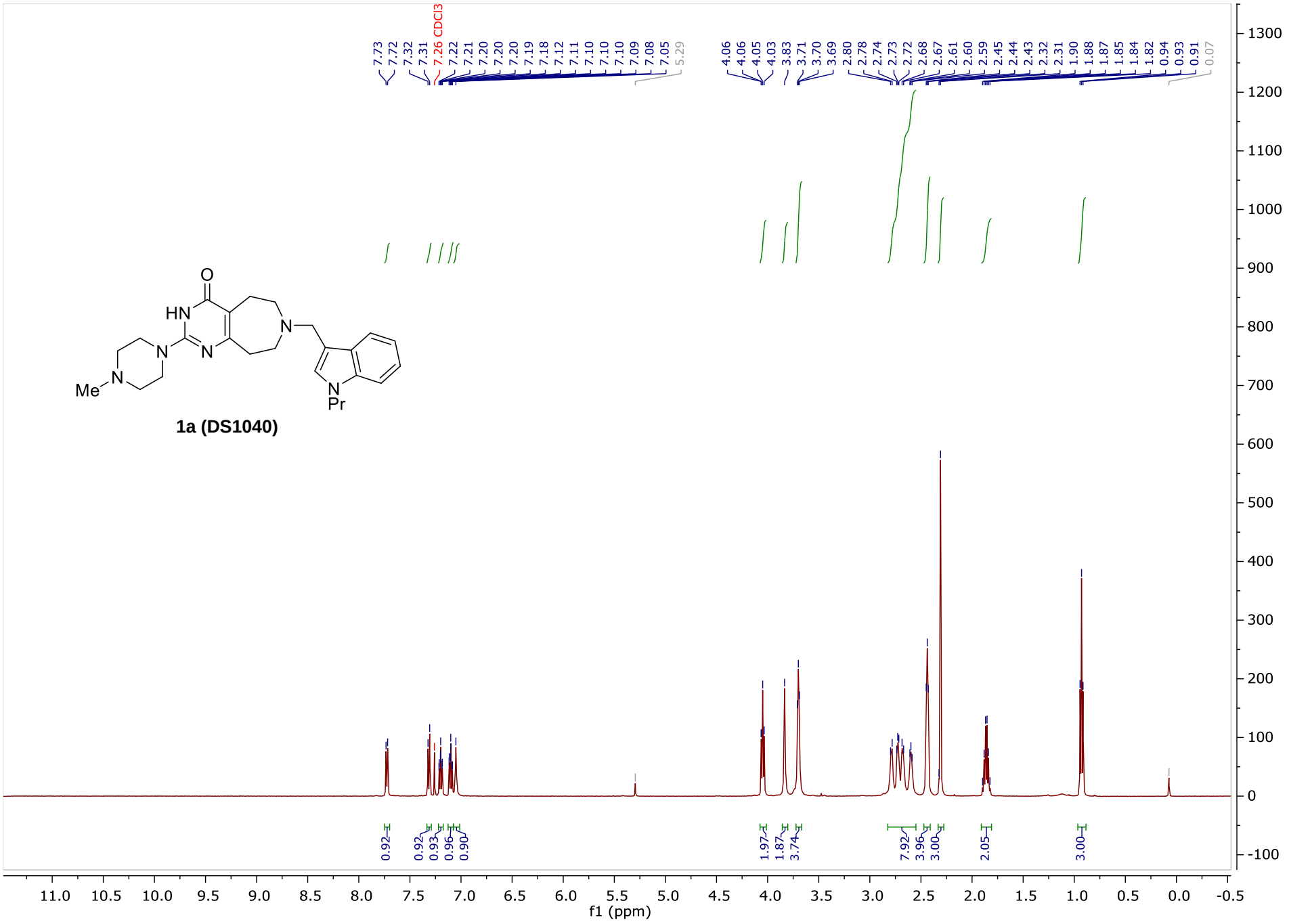
24.81
O
HN
N
NBoc
4c
f1 (ppm)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7.19
7.18
7.12
7.11
7.10
7.10
7.10
7.09
7.08
7.05
5.29
4.06
4.06
4.05
4.03
3.83
3.71
3.70
3.69
2.80
2.78
2.74
2.73
2.72
2.68
2.67
2.61
2.60
2.59
2.45
2.44
2.43
2.32
2.31
1.90
1.88
1.87
1.85
1.84
1.82
0.94
0.93
0.91
0.07
O
HN
N
N
N
Me
N
Pr
1a (DS1040)
0.92
0.92
0.93
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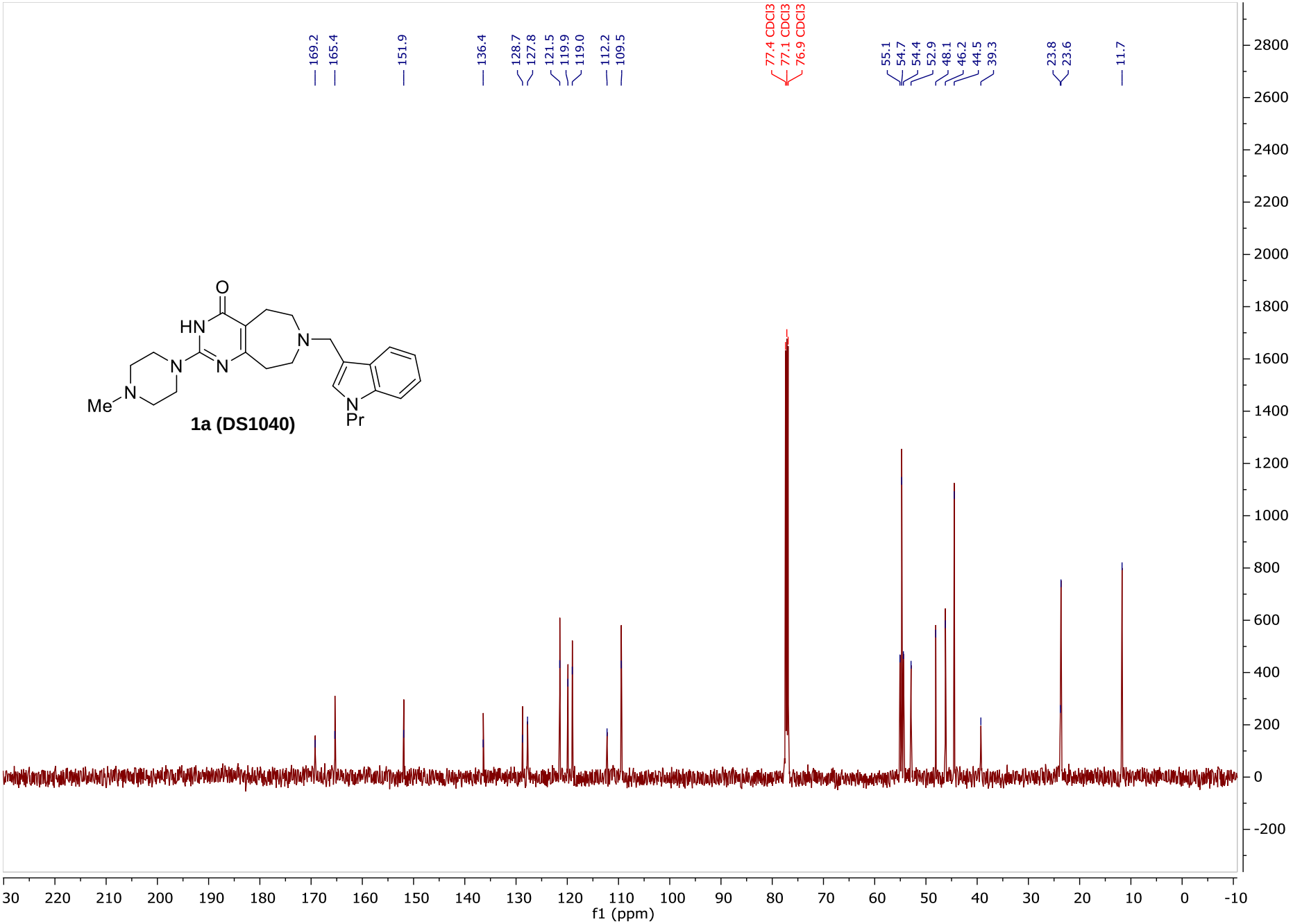
1.97
1.87
3.74
7.92
3.96
3.00
2.05
3.00
f1 (ppm)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136.4
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119.9
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112.2
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77.1 CDCl3
76.9 CDCl3
55.1
54.7
54.4
52.9
48.1
46.2
44.5
39.3
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23.6
11.7
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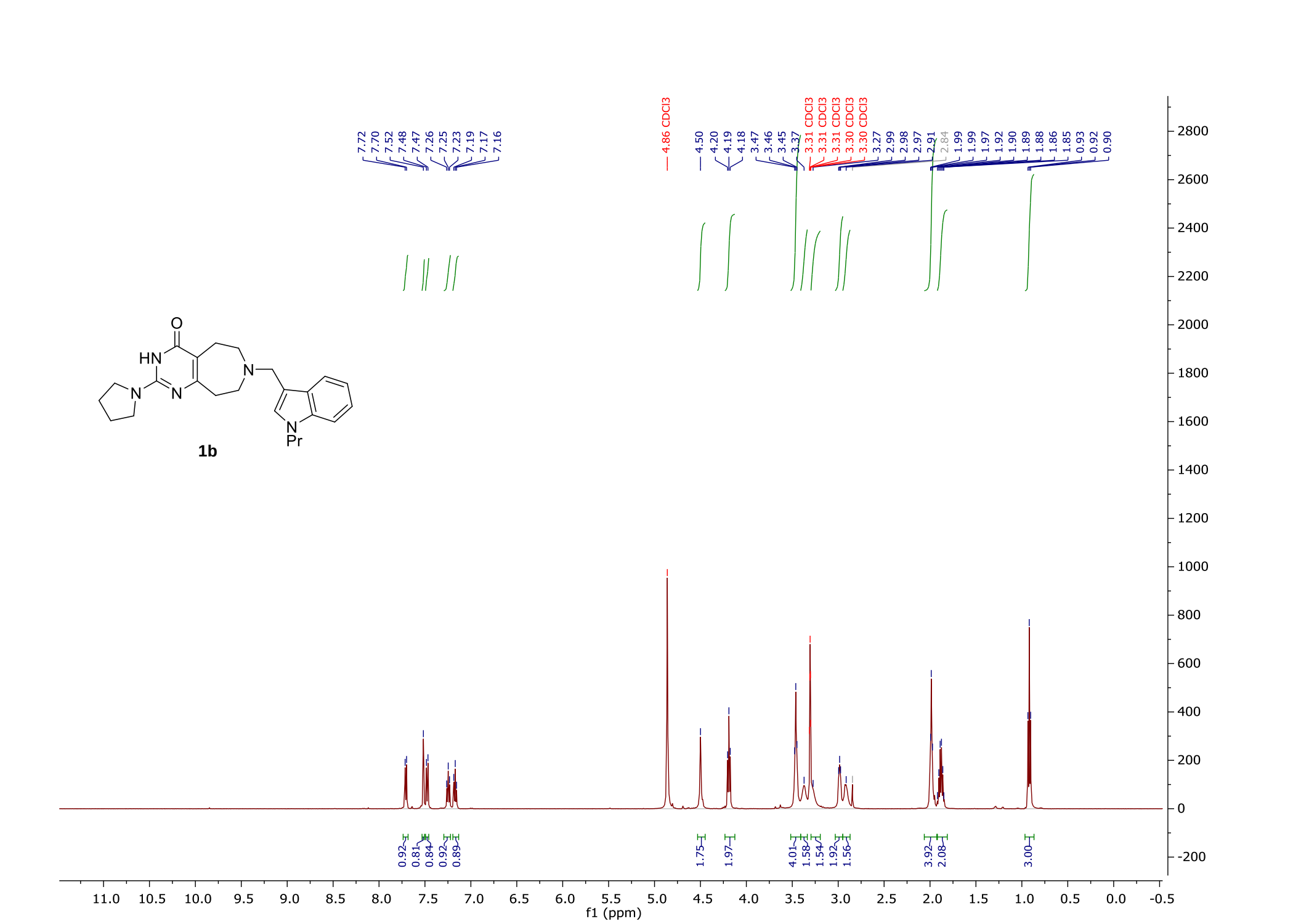
N
N
N
Me
N
Pr
1a (DS1040)
f1 (ppm)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7.23
7.19
7.17
7.16
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4.50
4.20
4.19
4.18
3.47
3.46
3.45
3.37
3.31 CDCl3
3.31 CDCl3
3.31 CDCl3
3.30 CDCl3
3.30 CDCl3
3.27
2.99
2.98
2.97
2.91
2.84
1.99
1.99
1.97
1.92
1.90
1.89
1.88
1.86
1.85
0.93
0.92
0.90
O
HN
N
N
N
N
Pr
1b
0.92
0.81
0.84
0.92
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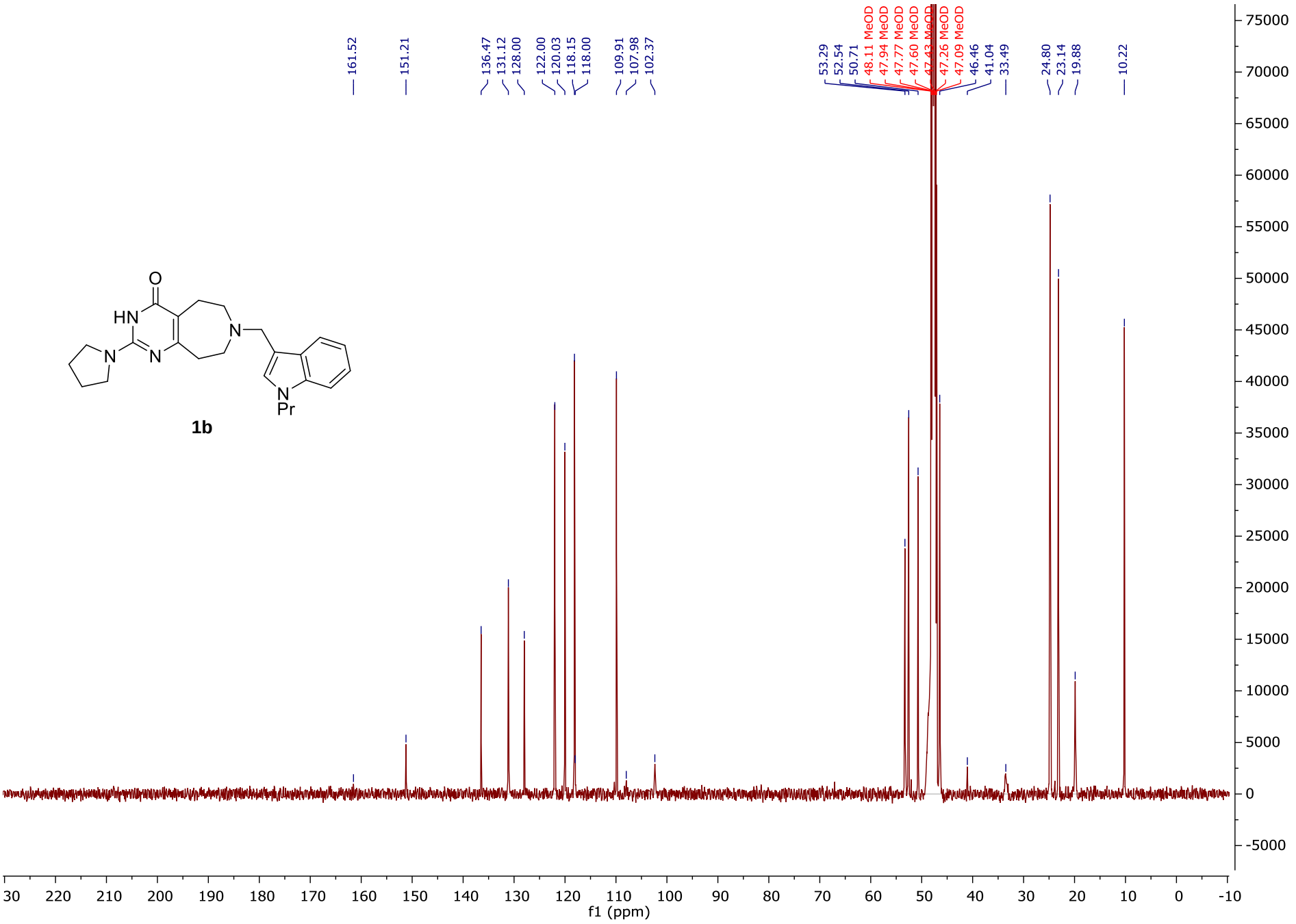
1.54
1.92
1.56
3.92
2.08
3.00
f1 (ppm)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122.00
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107.98
102.37
53.29
52.54
50.71
48.11 MeOD
47.94 MeOD
47.77 MeOD
47.60 MeOD
47.43 MeOD
47.26 MeOD
47.09 MeOD
46.46
41.04
33.49
24.80
23.14
19.88
10.22
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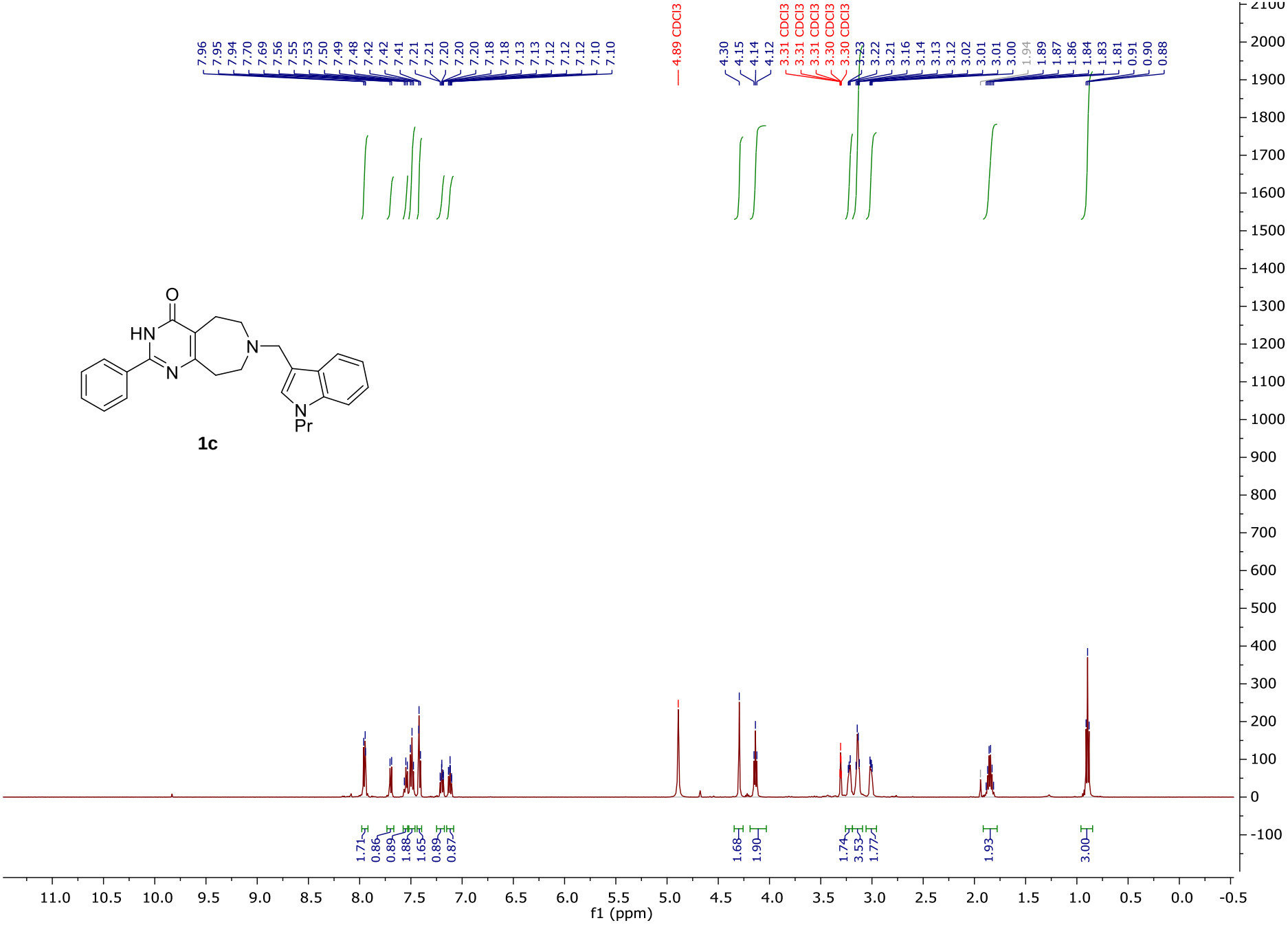
HN
N
N
N
N
Pr
1b
f1 (ppm)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7.94
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7.49
7.48
7.42
7.42
7.41
7.21
7.21
7.20
7.20
7.20
7.18
7.18
7.13
7.13
7.12
7.12
7.12
7.10
7.10
4.89 CDCl3
4.30
4.15
4.14
4.12
3.31 CDCl3
3.31 CDCl3
3.31 CDCl3
3.30 CDCl3
3.30 CDCl3
3.23
3.22
3.21
3.16
3.14
3.13
3.12
3.02
3.01
3.01
3.00
1.94
1.89
1.87
1.86
1.84
1.83
1.81
0.91
0.90
0.88
HN
O
N
N
N
Pr
1c
1.71
0.86
0.89
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1.90
1.74
3.53
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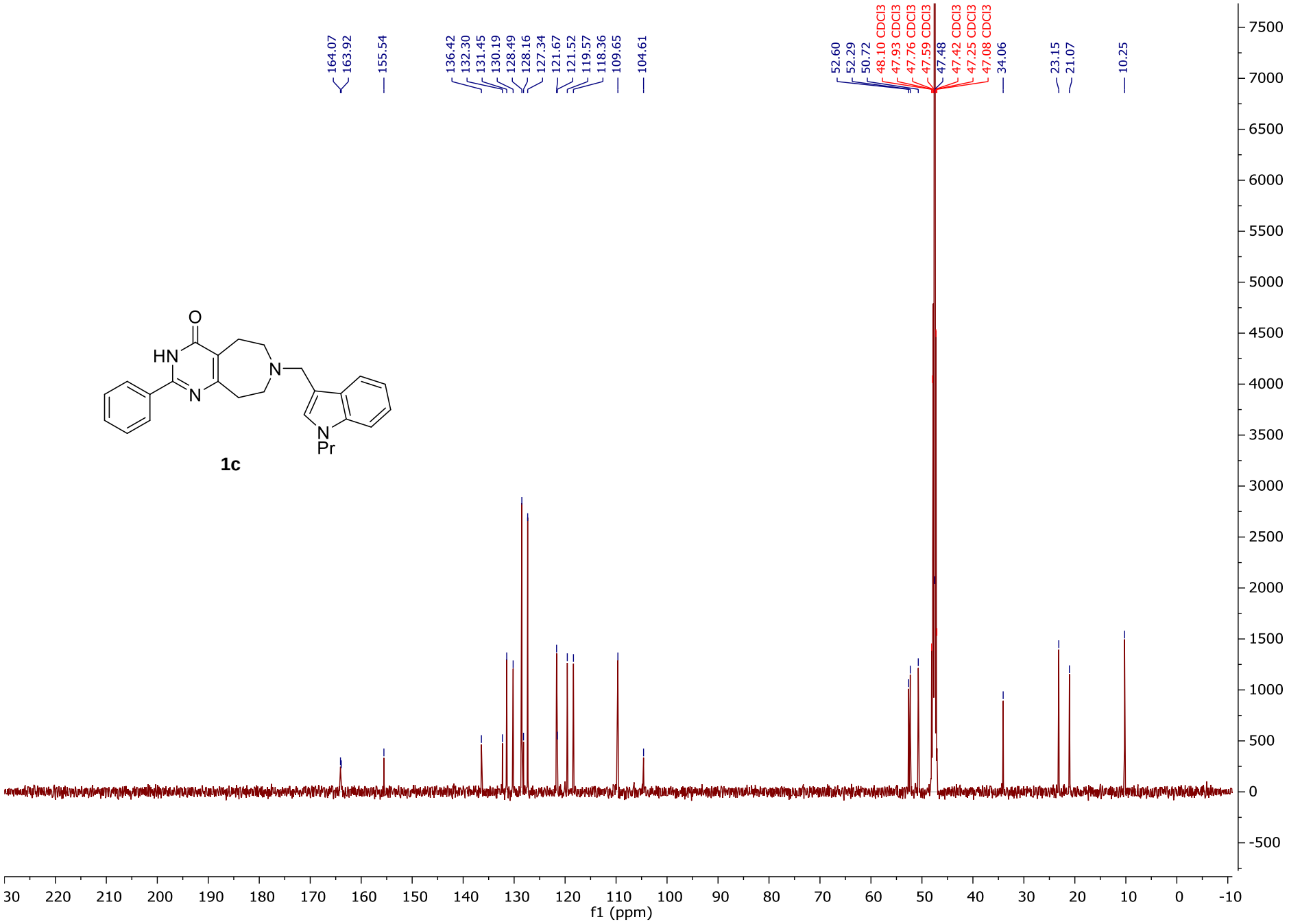
1.93
3.00
f1 (ppm)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118.36
109.65
104.61
52.60
52.29
50.72
48.10 CDCl3
47.93 CDCl3
47.76 CDCl3
47.59 CDCl3
47.48
47.42 CDCl3
47.25 CDCl3
47.08 CDCl3
34.06
23.15
21.07
10.25
O
HN
N
N
N
Pr
1c
7500
7000
6500
6000
5500
5000
4500
4000
3500
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500
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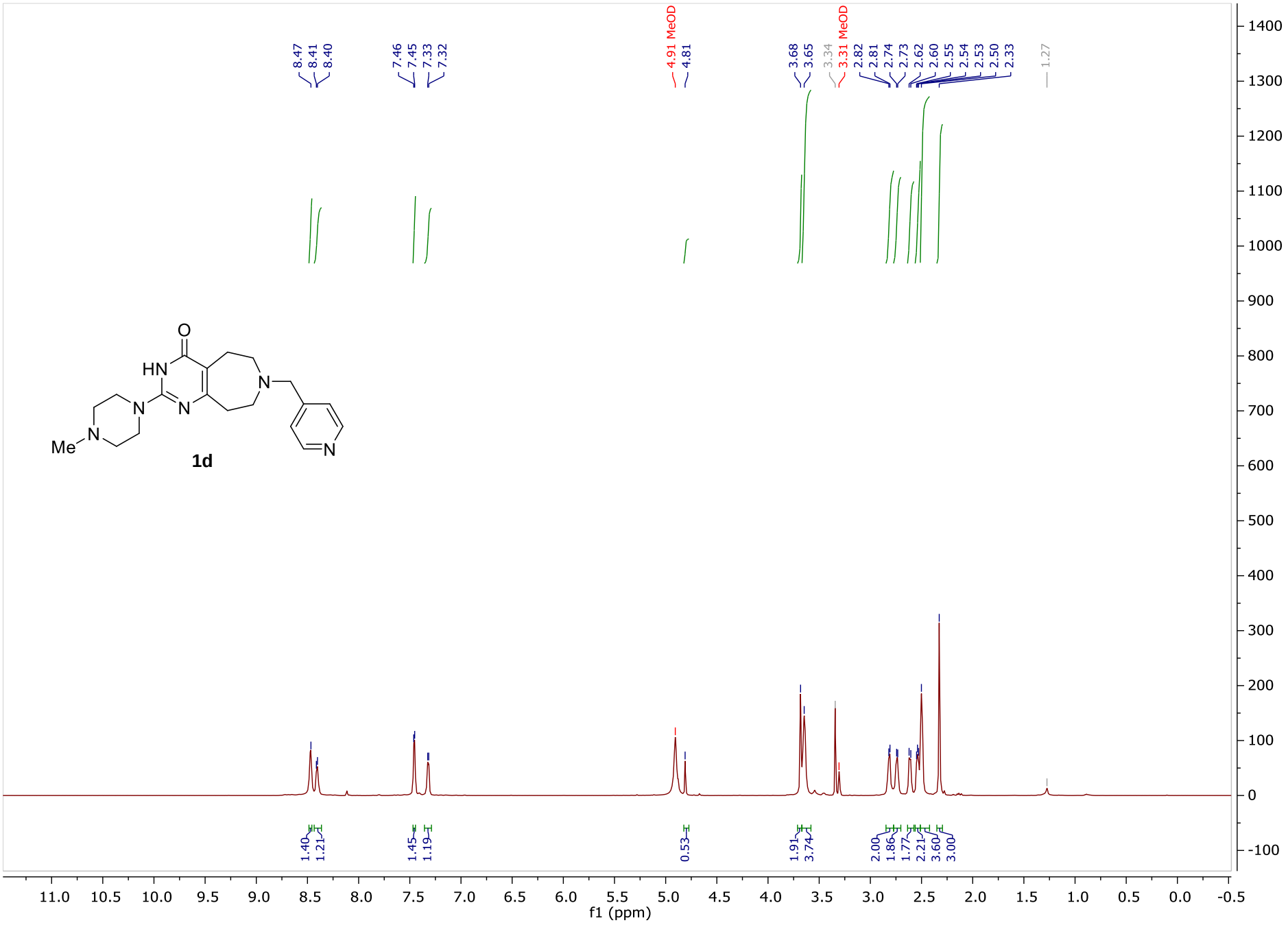
f1 (ppm)


8.47
8.41
8.40
7.46
7.45
7.33
7.32
4.91 MeOD
4.81
3.68
3.65
3.34
3.31 MeOD
2.82
2.81
2.74
2.73
2.62
2.60
2.55
2.54
2.53
2.50
2.33
1.27
1.40
1.21
1.45
1.19
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3.74
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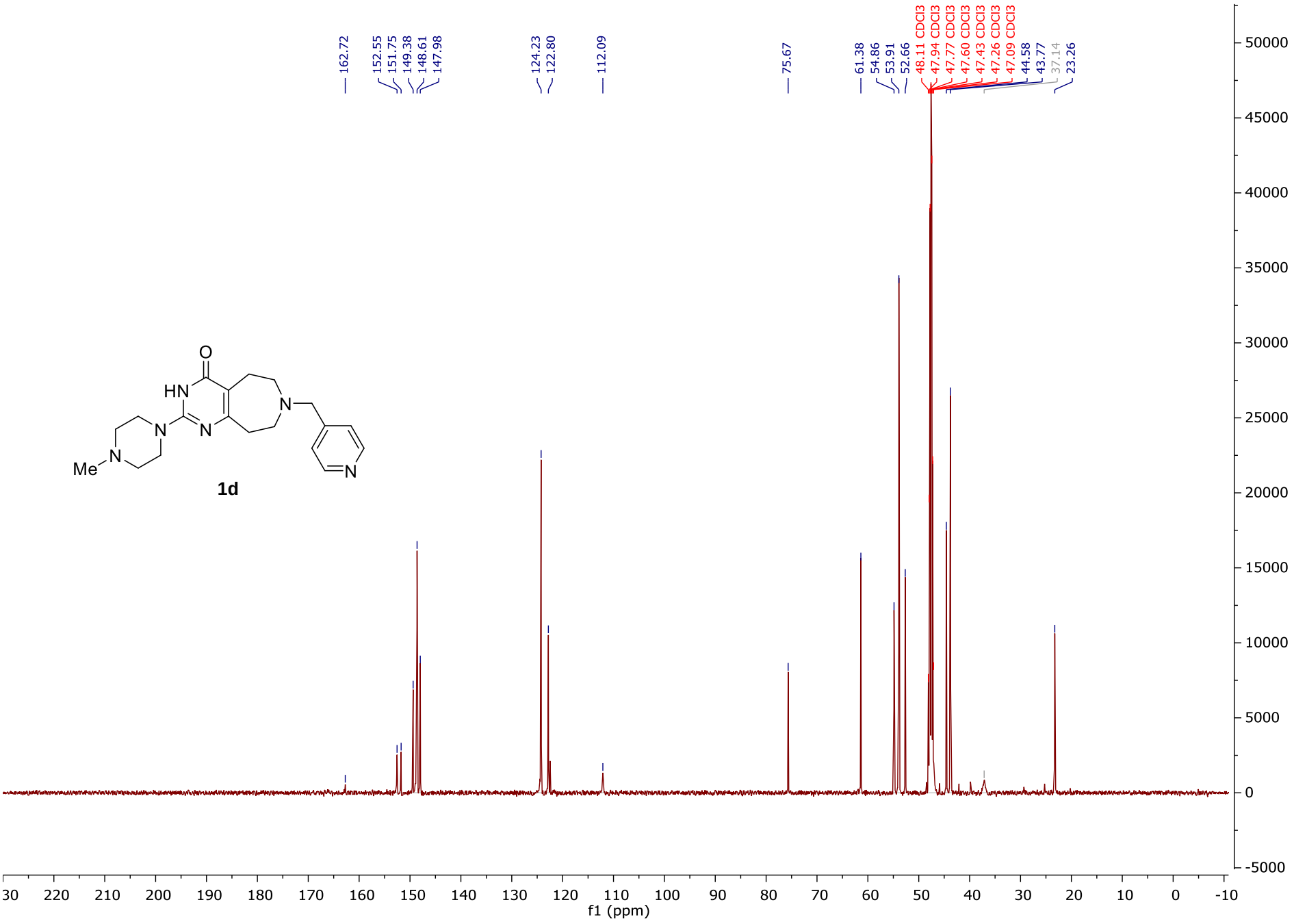
1.86
1.77
2.21
3.60
3.00
f1 (ppm)

1d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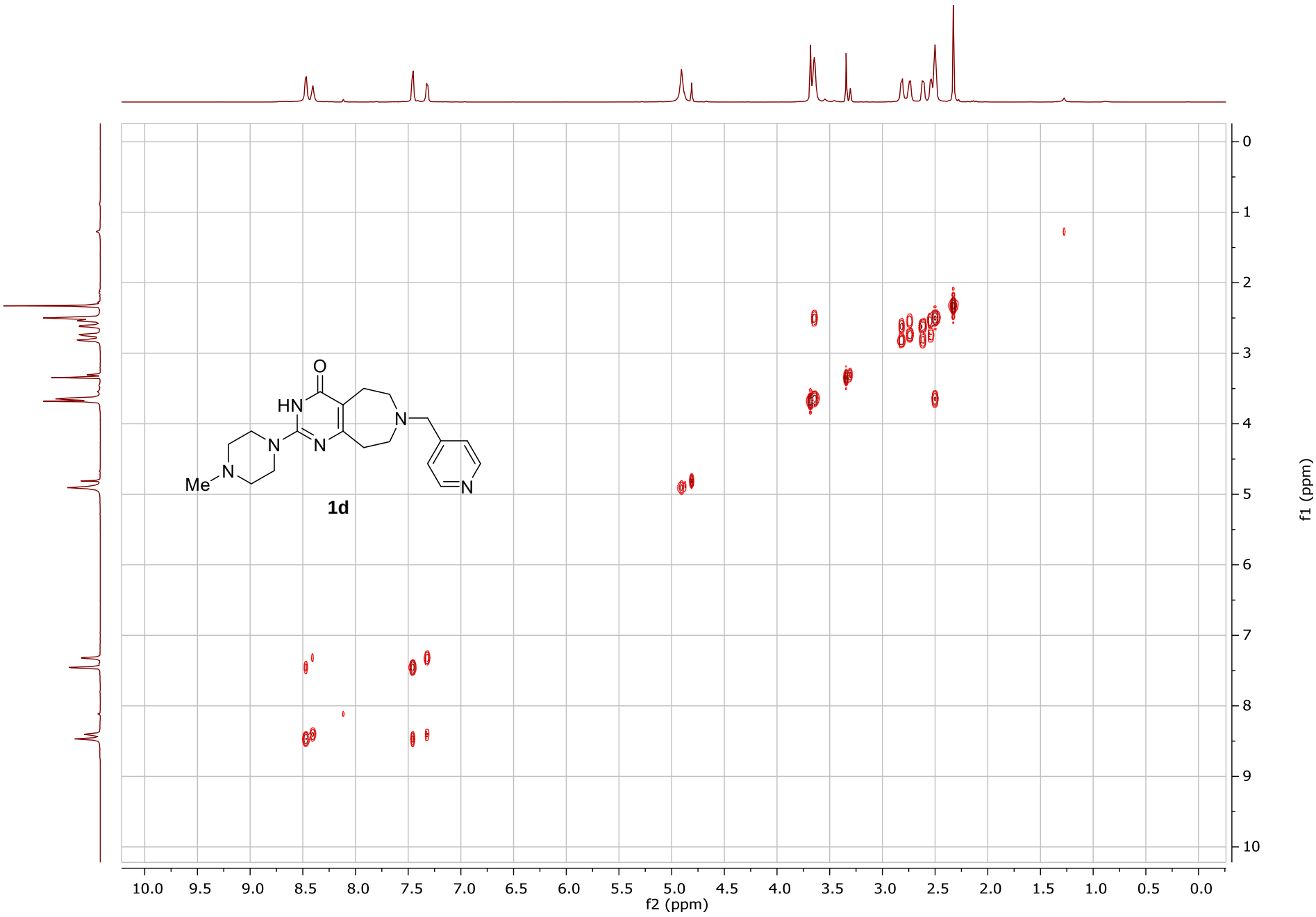
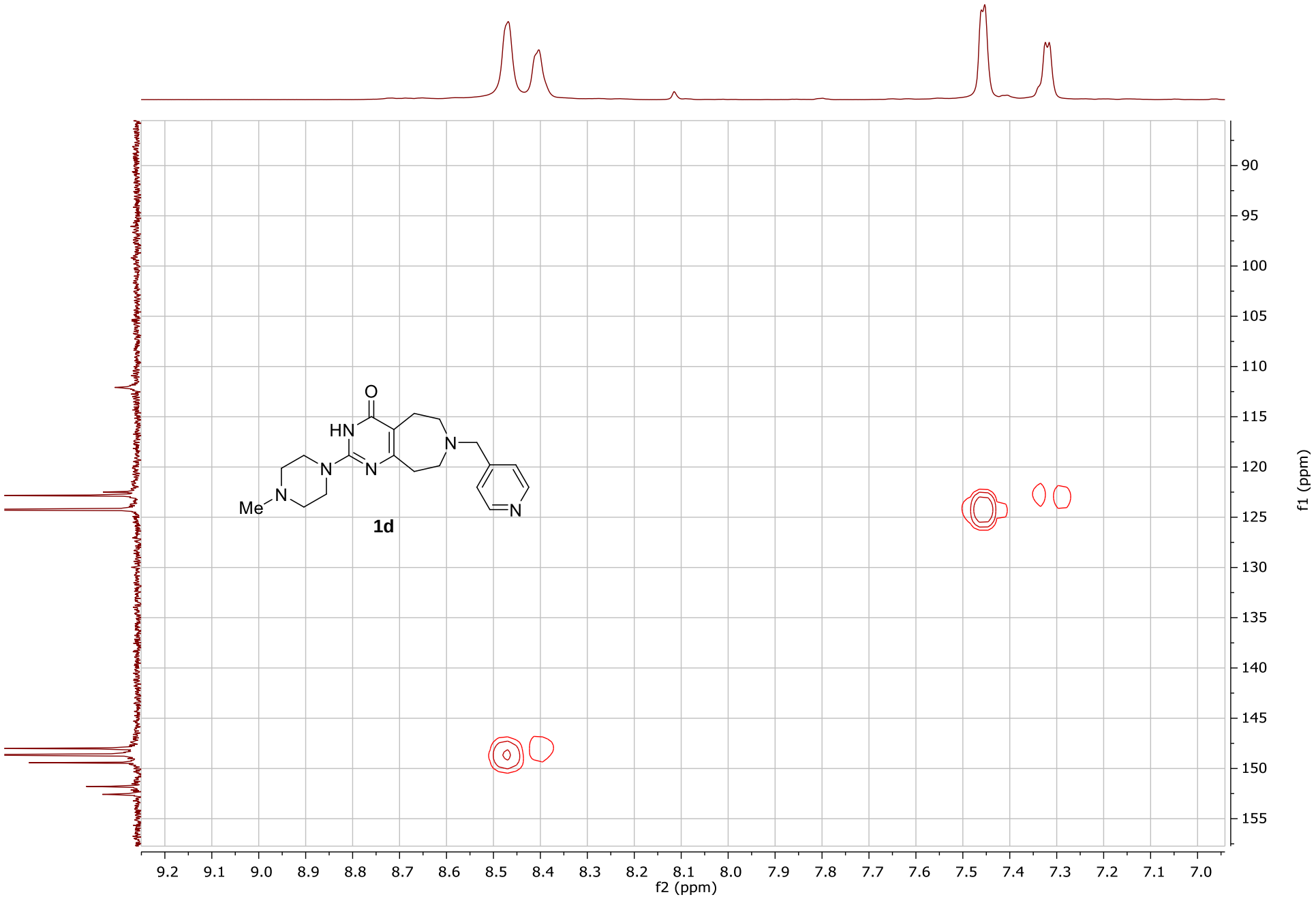
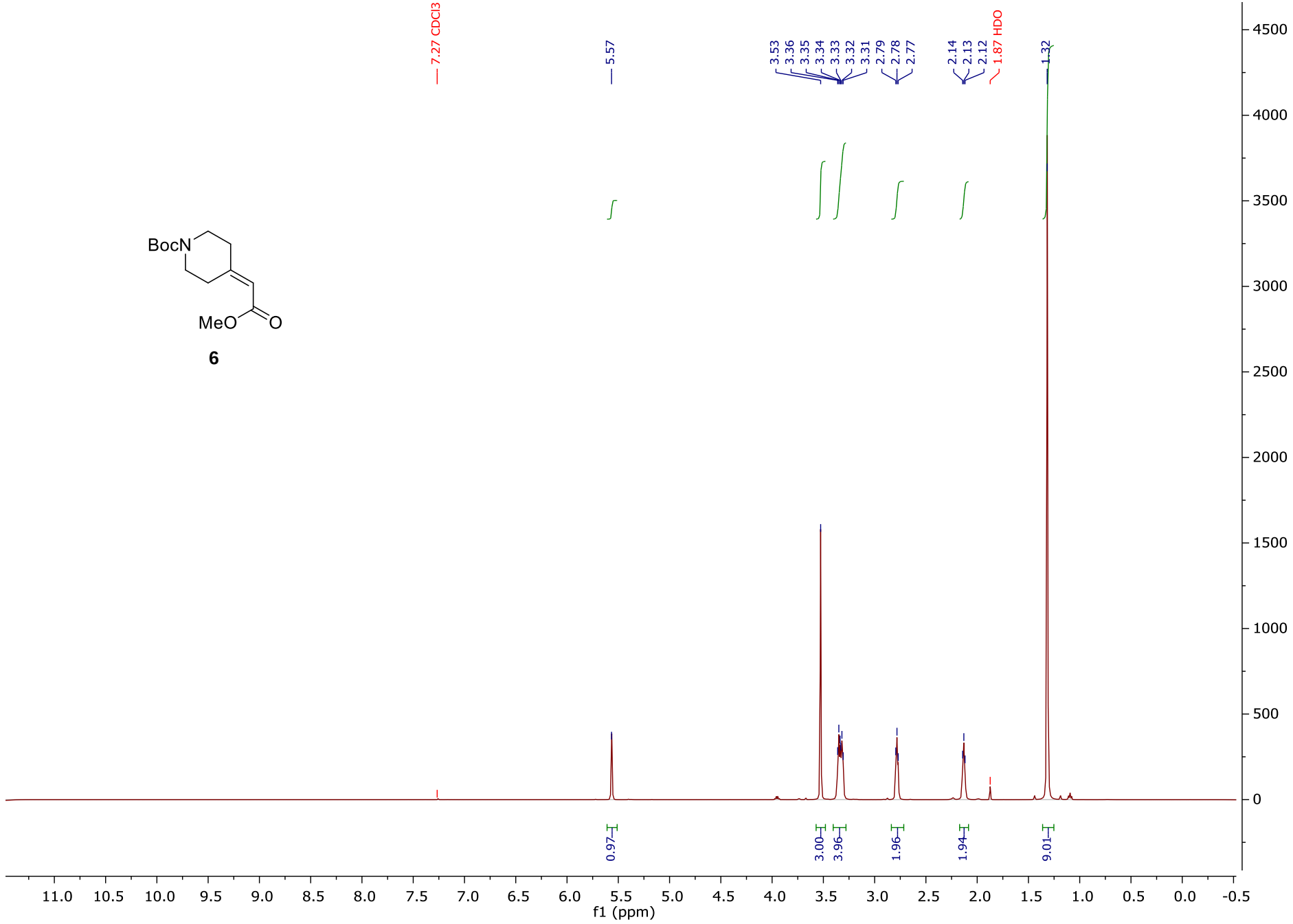
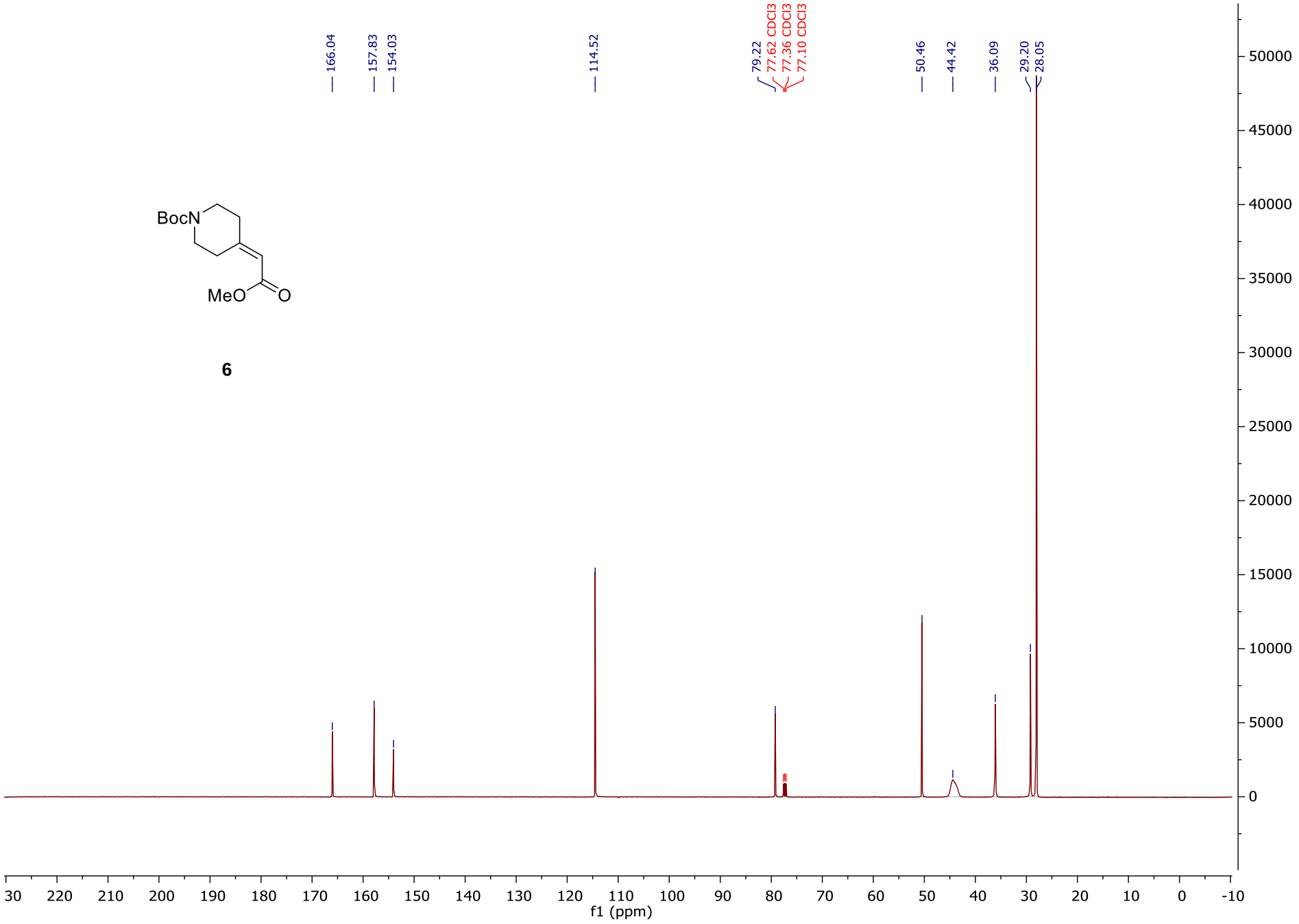
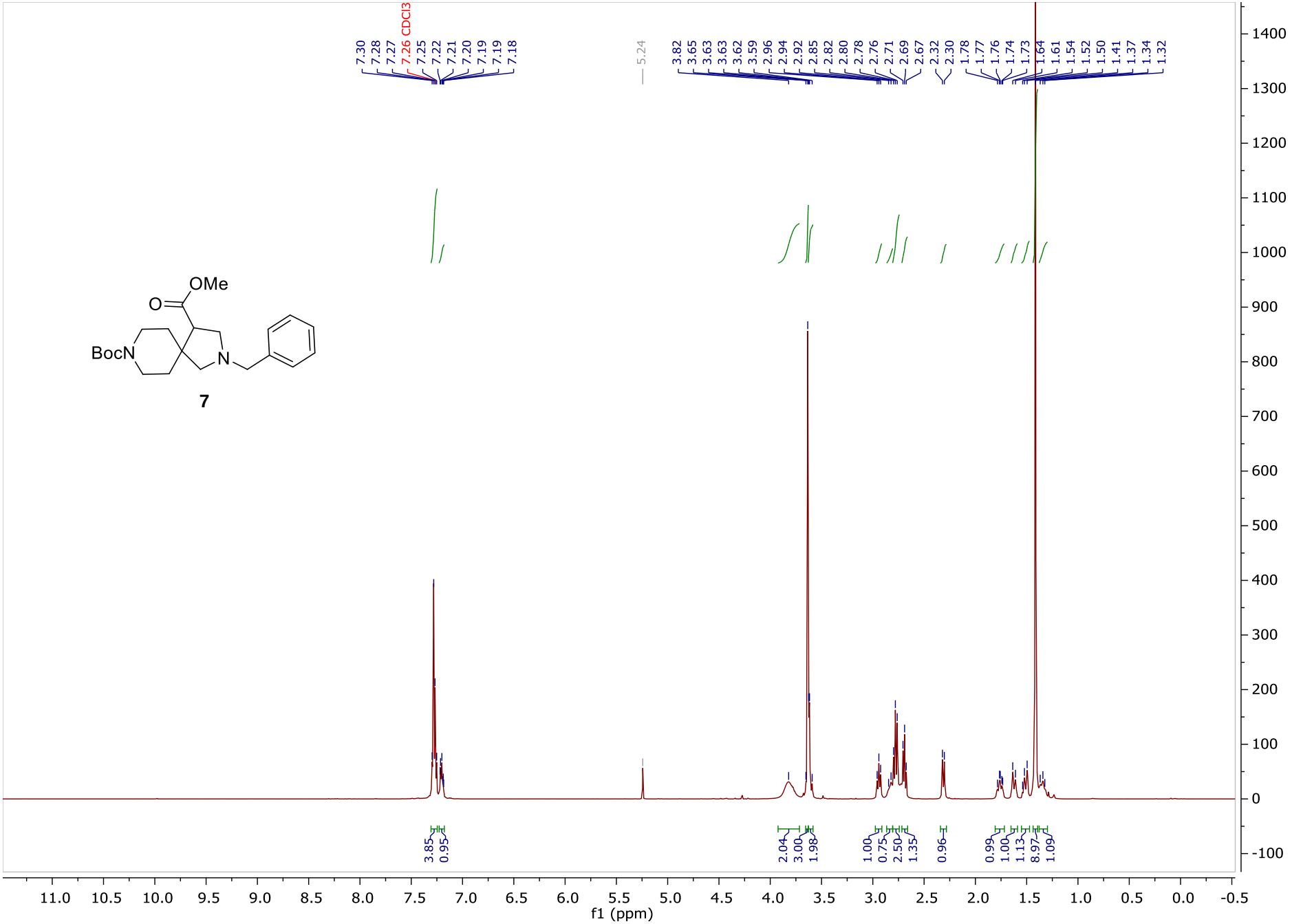
152.55
151.75
149.38
148.61
147.98
124.23
122.80
112.09
75.67
61.38
54.86
53.91
52.66
48.11 CDCl3
47.94 CDCl3
47.77 CDCl3
47.60 CDCl3
47.43 CDCl3
47.26 CDCl3
47.09 CDCl3
44.58
43.77
37.14
23.26
O
HN
N
N
N
Me
N
1d
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000
30 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

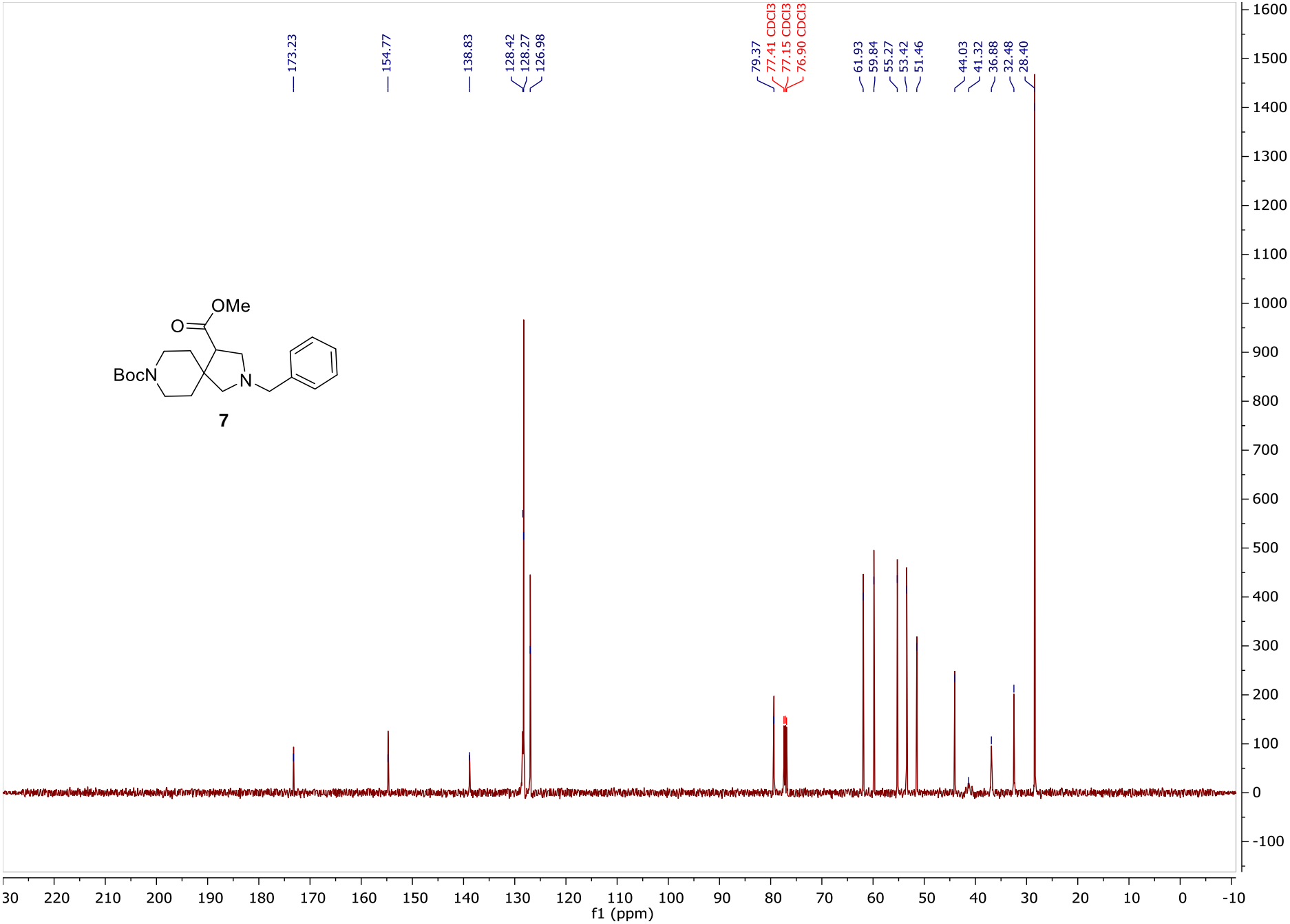

O
HN
N
N
N
Me
N
1d
f2 (ppm)
f1 (ppm)

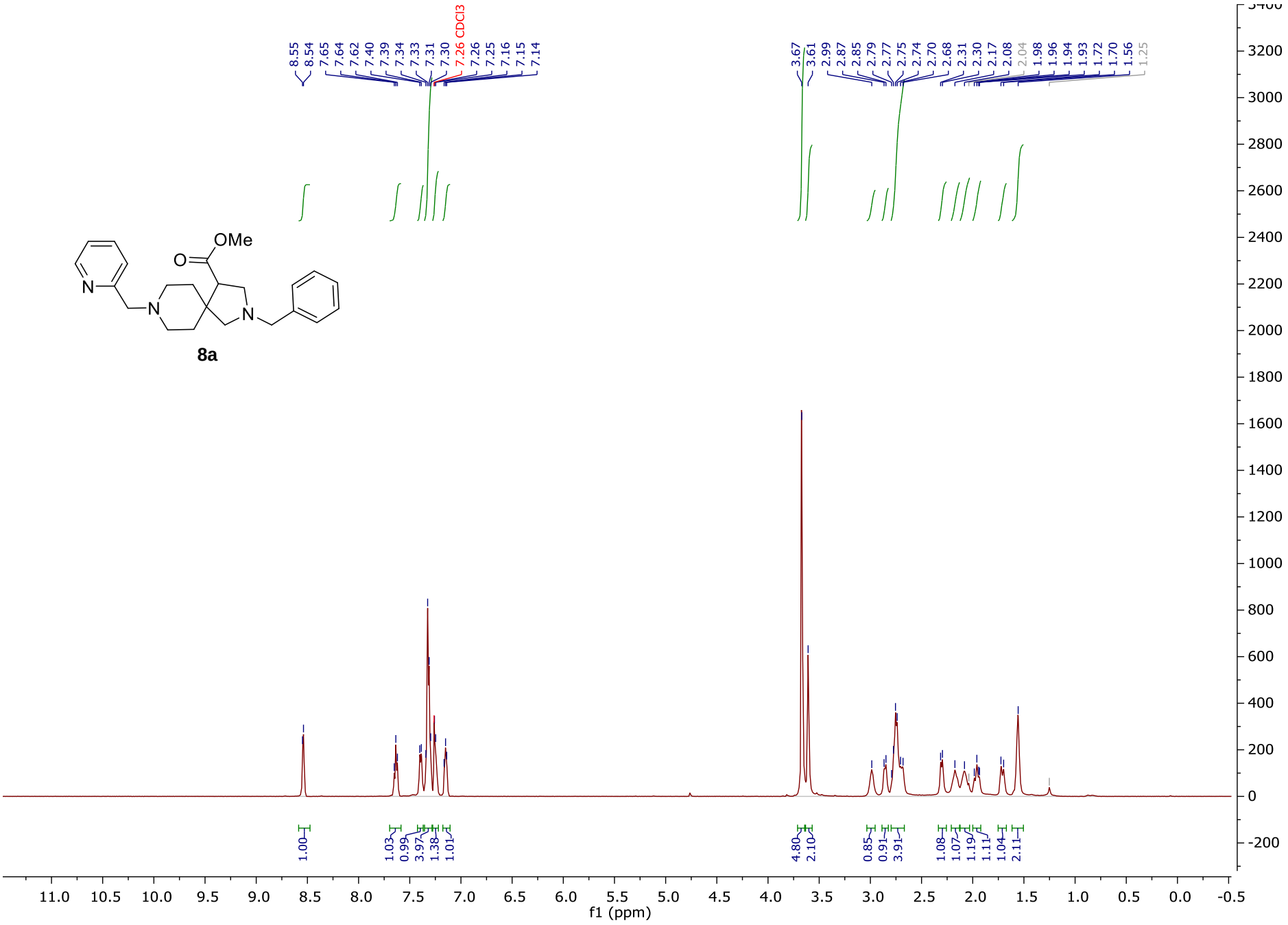

O
HN
N
N
N
Me
N
1d
f2 (ppm)
f1 (ppm)

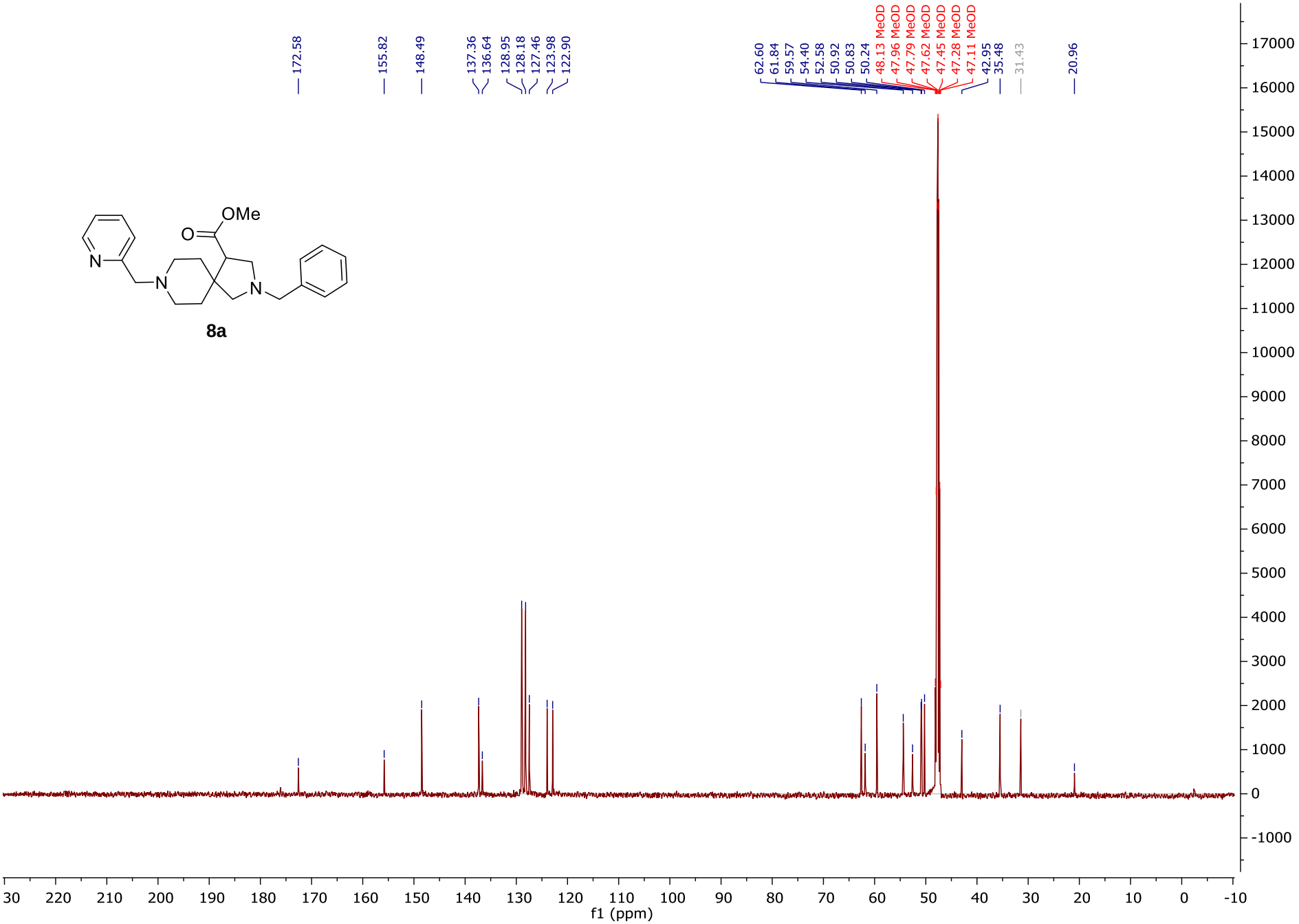

7.27 CDCl3
5.57
3.53
3.36
3.35
3.34
3.33
3.32
3.31
2.79
2.78
2.77
2.14
2.13
2.12
1.87 HDO
1.32
BocN
MeO
O
6
0.97
3.00
3.96
1.96
1.94
9.01
f1 (ppm)

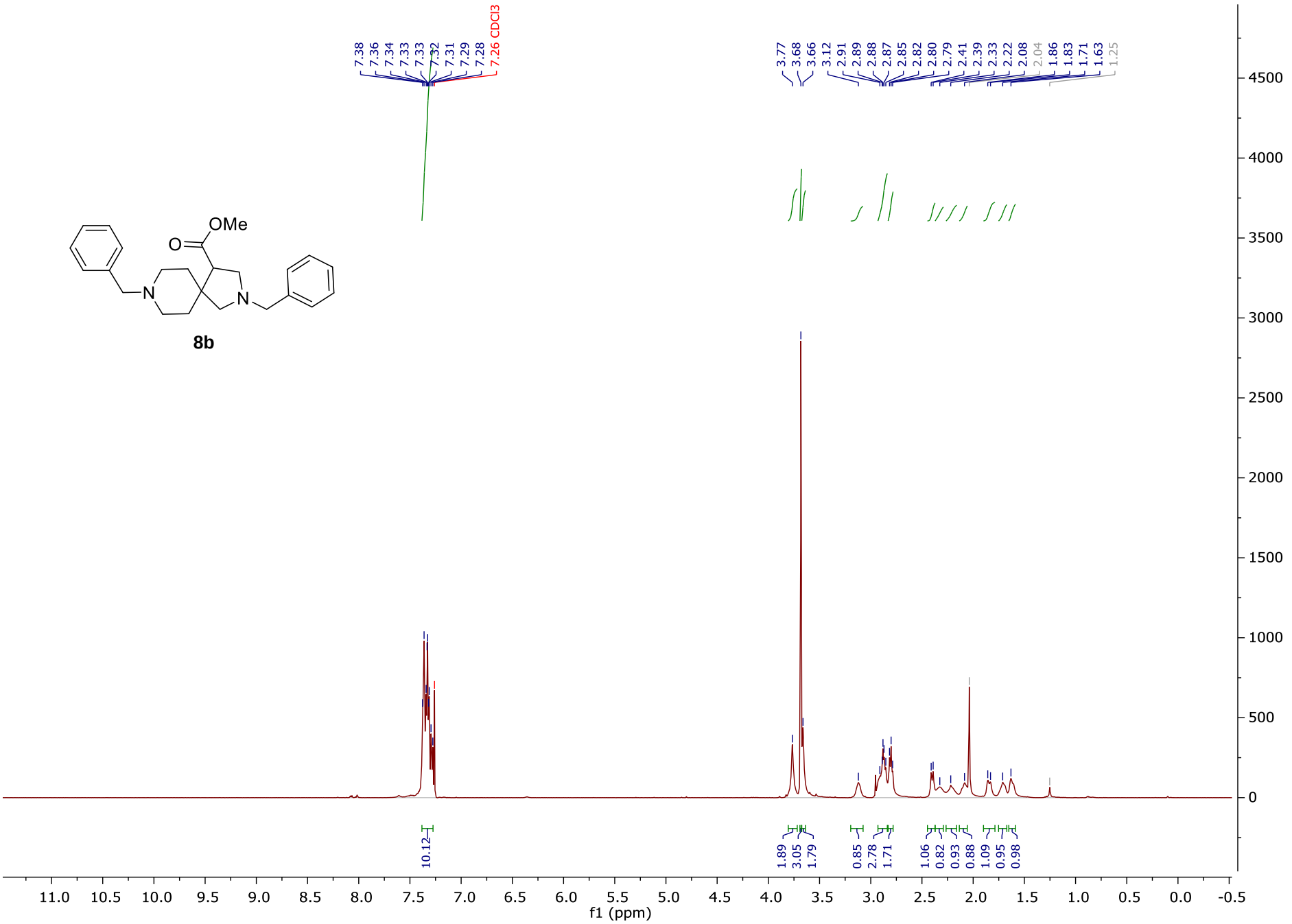

166.04
157.83
154.03
114.52
79.22
77.62 CDCl3
77.36 CDCl3
77.10 CDCl3
50.46
44.42
36.09
29.20
28.05
BocN
MeO
O
6
f1 (ppm)

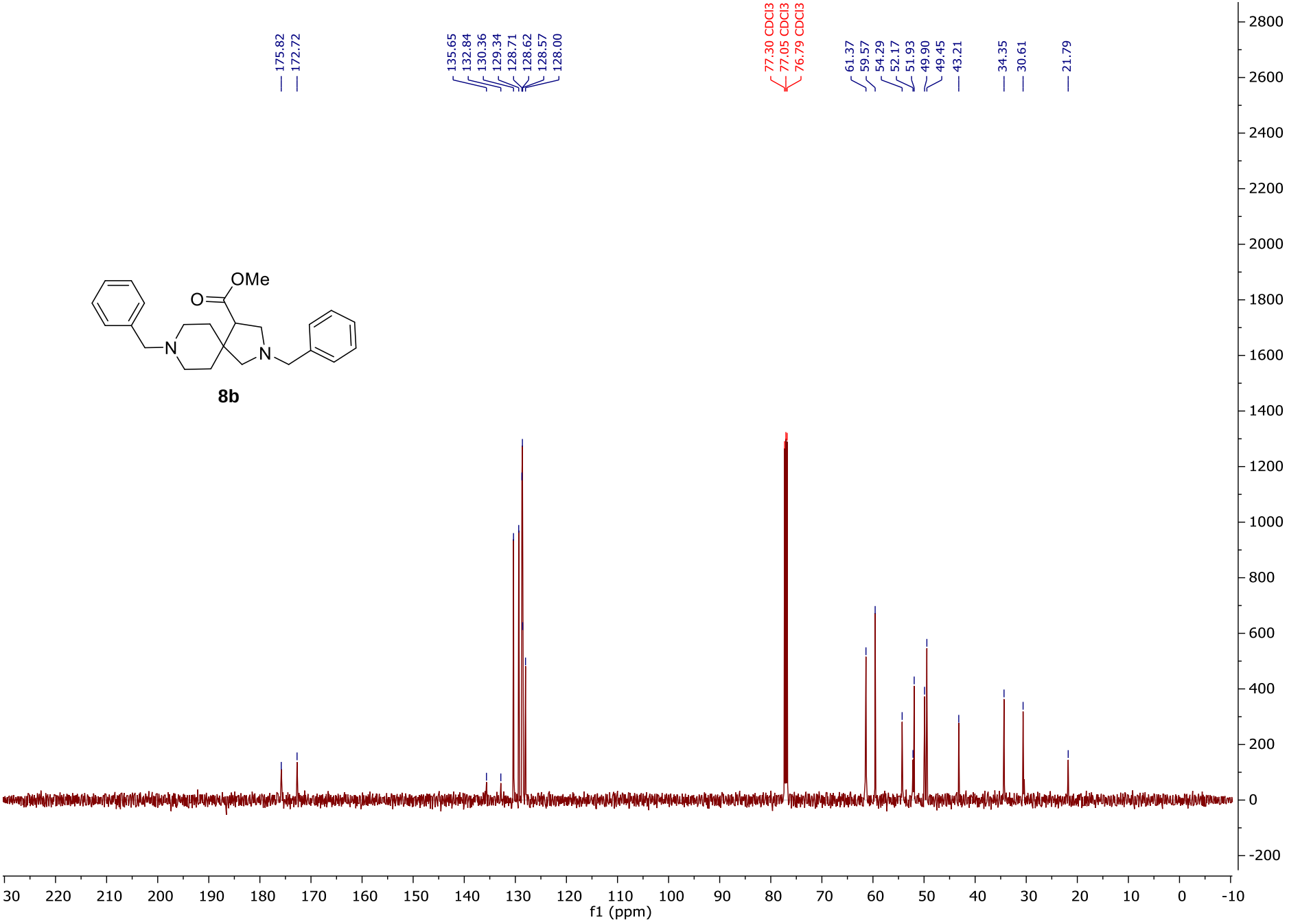

7.30
7.28
7.27
7.26 CDCl3
7.25
7.22
7.21
7.20
7.19
7.19
7.18
5.24
3.82
3.65
3.63
3.63
3.62
3.59
2.96
2.94
2.92
2.85
2.82
2.80
2.78
2.76
2.71
2.69
2.67
2.32
2.30
1.78
1.77
1.76
1.74
1.73
1.64
1.61
1.54
1.52
1.50
1.41
1.37
1.34
1.32
OMe
O
BocN
N
7
3.85
0.95
2.04
3.00
1.98
1.00
0.75
2.50
1.35
0.96
0.99
1.00
1.13
8.97
1.09
f1 (ppm)


173.23
154.77
138.83
128.42
128.27
126.98
79.37
77.41 CDCl3
77.15 CDCl3
76.90 CDCl3
61.93
59.84
55.27
53.42
51.46
44.03
41.32
36.88
32.48
28.40
OMe
O
BocN
N
7
f1 (ppm)


8.55
8.54
7.65
7.64
7.62
7.40
7.39
7.34
7.33
7.31
7.30
7.26 CDCl3
7.26
7.25
7.16
7.15
7.14
3.67
3.61
2.99
2.87
2.85
2.79
2.77
2.75
2.74
2.70
2.68
2.31
2.30
2.17
2.08
2.04
1.98
1.96
1.94
1.93
1.72
1.70
1.56
1.25
OMe
O
N
N
N
8a
1.00
1.03
0.99
3.97
1.38
1.01
4.80
2.10
0.85
0.91
3.91
1.08
1.07
1.19
1.11
1.04
2.11
f1 (ppm)


172.58
155.82
148.49
137.36
136.64
128.95
128.18
127.46
123.98
122.90
62.60
61.84
59.57
54.40
52.58
50.92
50.83
50.24
48.13 MeOD
47.96 MeOD
47.79 MeOD
47.62 MeOD
47.45 MeOD
47.28 MeOD
47.11 MeOD
42.95
35.48
31.43
20.96
OMe
O
N
N
N
8a
f1 (ppm)


7.38
7.36
7.34
7.33
7.33
7.32
7.31
7.29
7.28
7.26 CDCl3
3.77
3.68
3.66
3.12
2.91
2.89
2.88
2.87
2.85
2.82
2.80
2.79
2.41
2.39
2.33
2.22
2.08
2.04
1.86
1.83
1.71
1.63
1.25
OMe
O
N
N
8b
10.12
1.89
3.05
1.79
0.85
2.78
1.71
1.06
0.82
0.93
0.88
1.09
0.95
0.98
f1 (ppm)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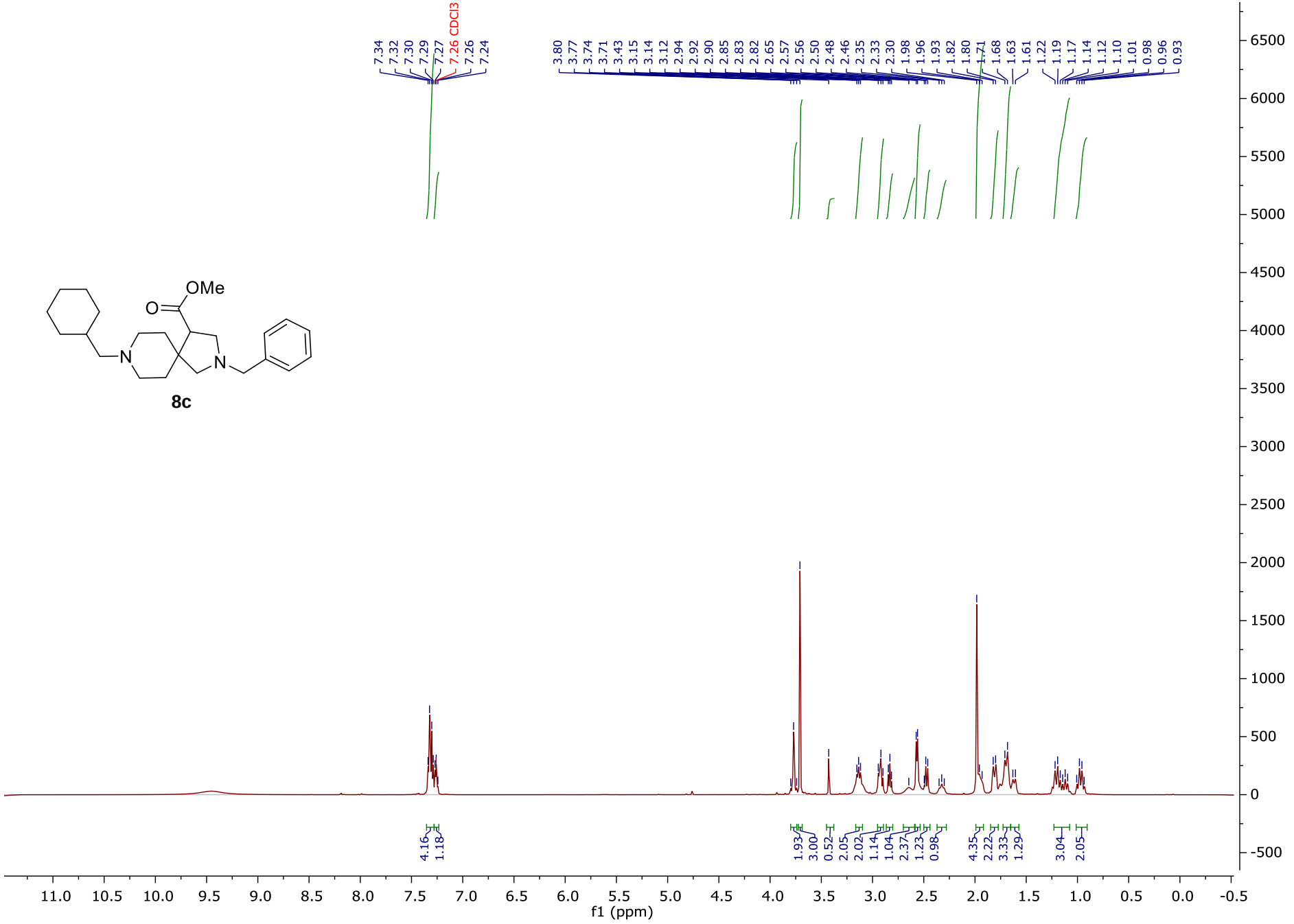
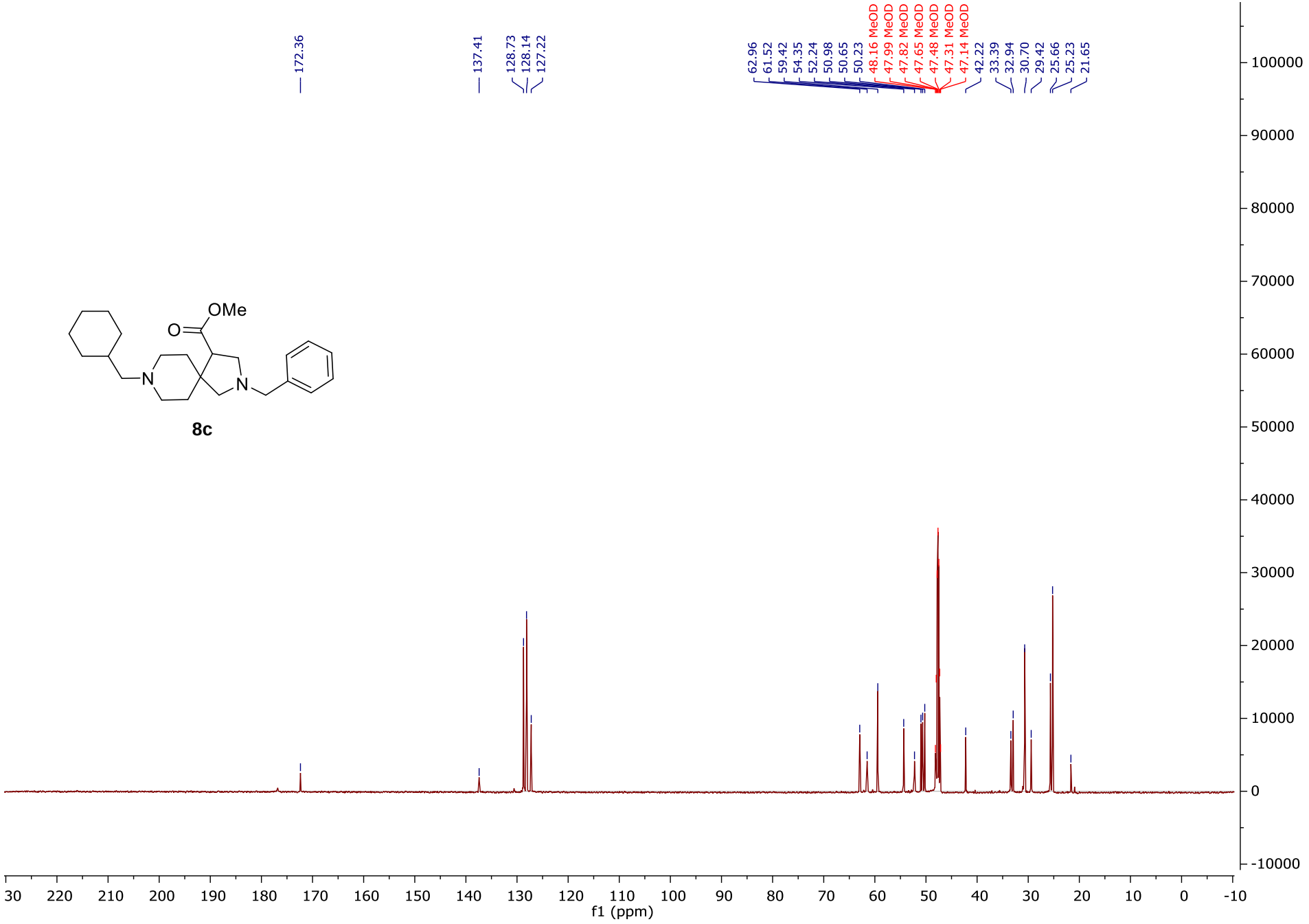
172.72
135.65
132.84
130.36
129.34
128.71
128.62
128.57
128.00
77.30 CDCl3
77.05 CDCl3
76.79 CDCl3
61.37
59.57
54.29
52.17
51.93
49.90
49.45
43.21
34.35
30.61
21.79
OMe
O
N
N
8b
f1 (ppm)


7.34
7.32
7.30
7.29
7.27
7.26 CDCl3
7.26
7.24
3.80
3.77
3.74
3.71
3.43
3.15
3.14
3.12
2.94
2.92
2.90
2.85
2.83
2.82
2.65
2.57
2.56
2.50
2.48
2.46
2.35
2.33
2.30
1.98
1.96
1.93
1.82
1.80
1.71
1.68
1.63
1.61
1.22
1.19
1.17
1.14
1.12
1.10
1.01
0.98
0.96
0.93
OMe
O
N
N
8c
4.16
1.18
1.93
3.00
0.52
2.05
2.02
1.14
1.04
2.37
1.23
0.98
4.35
2.22
3.33
1.29
3.04
2.05
f1 (ppm)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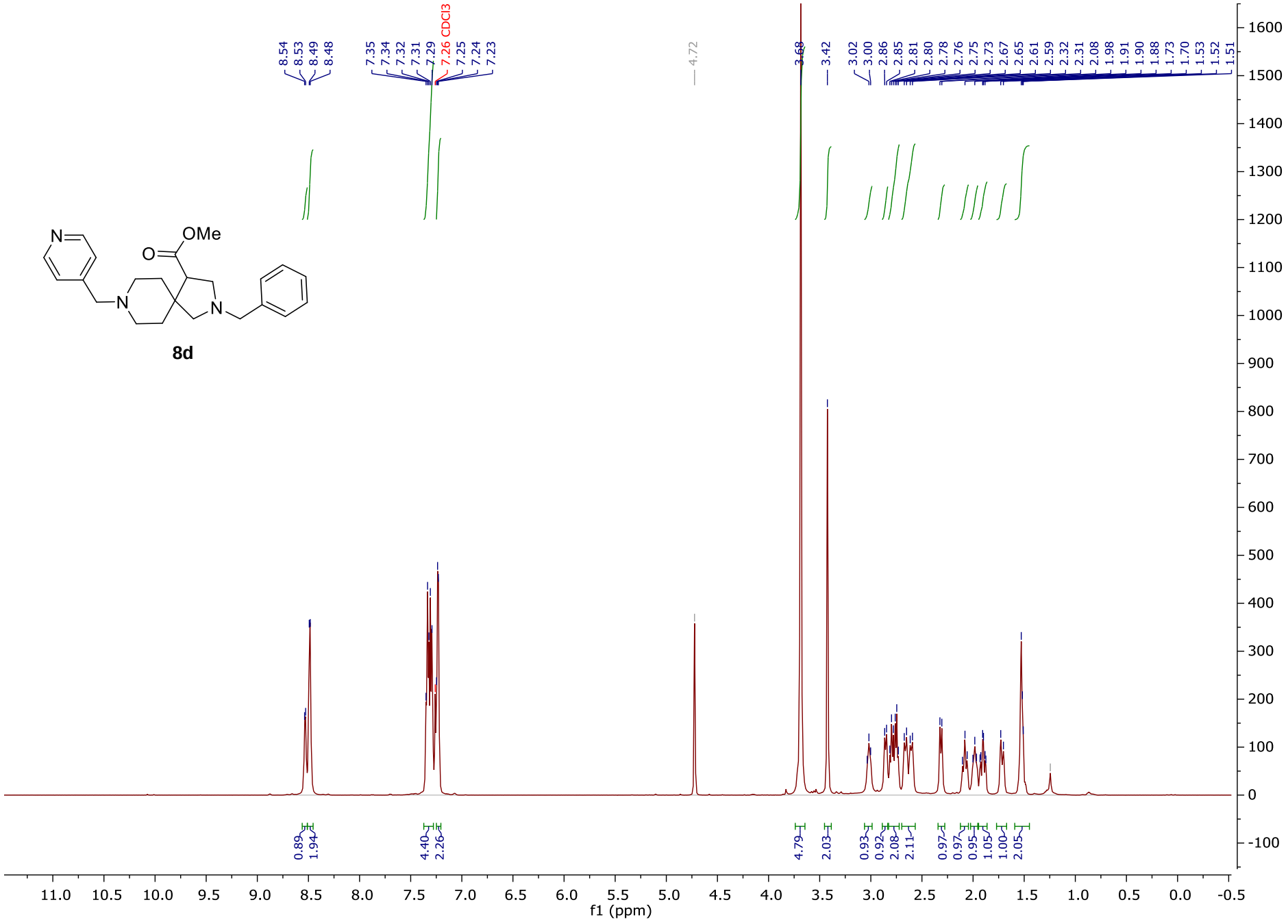
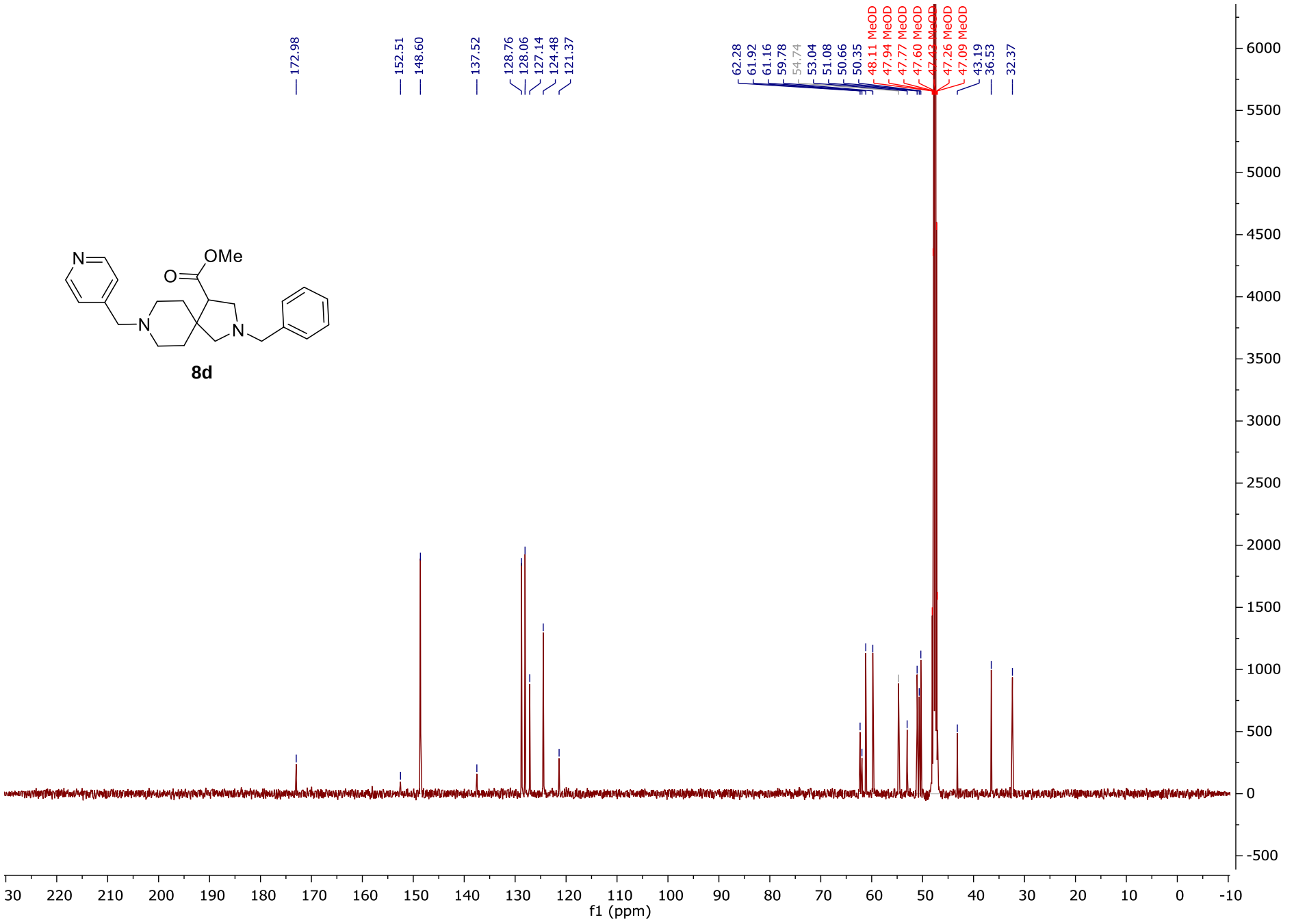
137.41
128.73
128.14
127.22
62.96
61.52
59.42
54.35
52.24
50.98
50.65
50.23
48.16 MeOD
47.99 MeOD
47.82 MeOD
47.65 MeOD
47.48 MeOD
47.31 MeOD
47.14 MeOD
42.22
33.39
32.94
30.70
29.42
25.66
25.23
21.65
OMe
O
N
N
8c
f1 (ppm)


8.54
8.53
8.49
8.48
7.35
7.34
7.32
7.31
7.29
7.26 CDCl3
7.25
7.24
7.23
4.72
3.68
3.42
3.02
3.00
2.86
2.85
2.81
2.80
2.78
2.76
2.75
2.73
2.67
2.65
2.61
2.59
2.32
2.31
2.08
1.98
1.91
1.90
1.88
1.73
1.70
1.53
1.52
1.51
0.89
1.94
4.40
2.26
4.79
2.03
0.93
0.92
2.08
2.11
0.97
0.97
0.95
1.05
1.00
2.05
f1 (ppm)

8d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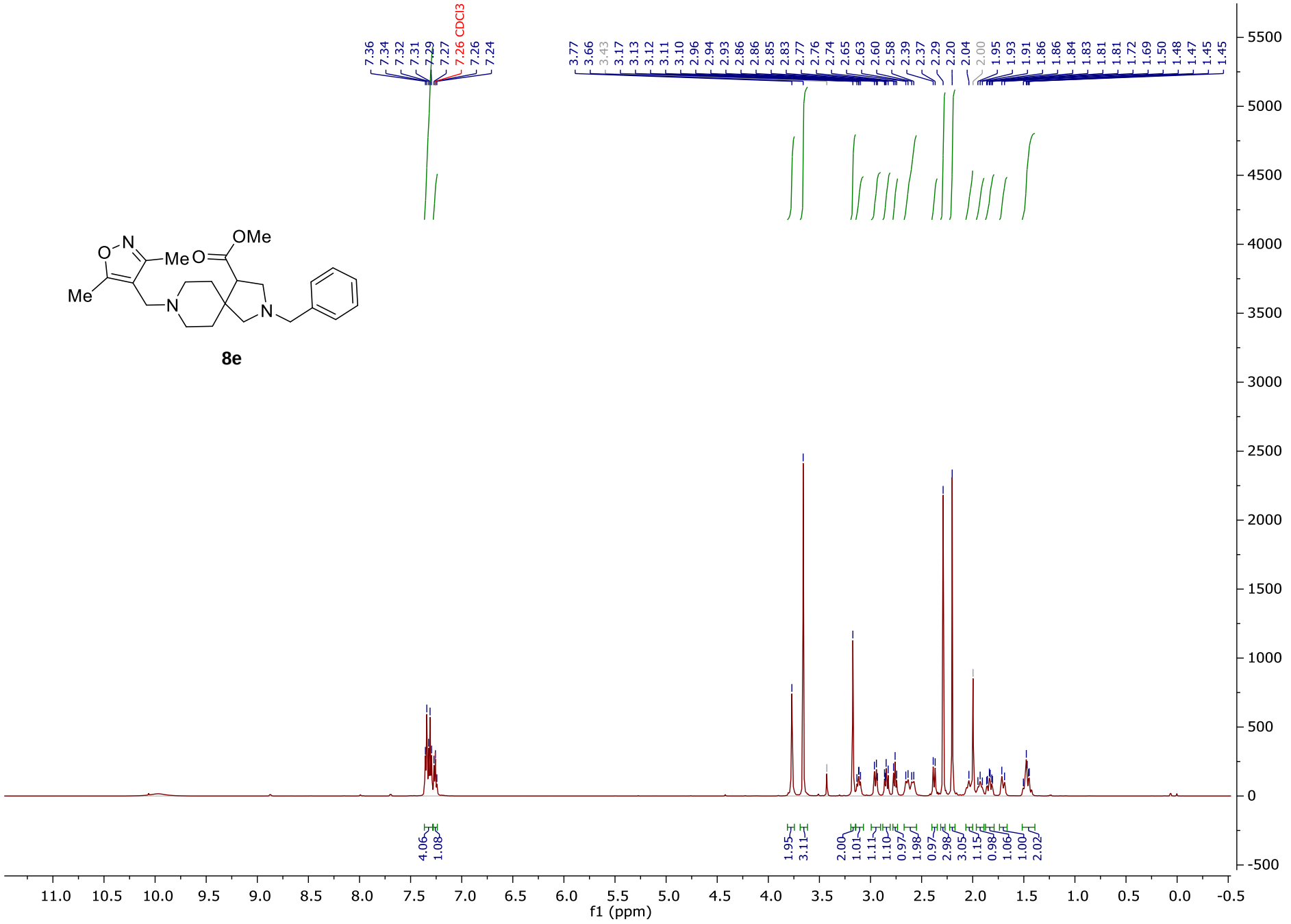
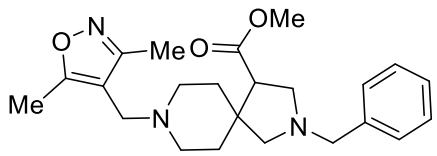
152.51
148.60
137.52
128.76
128.06
127.14
124.48
121.37
62.28
61.92
61.16
59.78
54.74
53.04
51.08
50.66
50.35
48.11 MeOD
47.94 MeOD
47.77 MeOD
47.60 MeOD
47.43 MeOD
47.26 MeOD
47.09 MeOD
43.19
36.53
32.37
N
O
OMe
N
N
8d
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
30 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

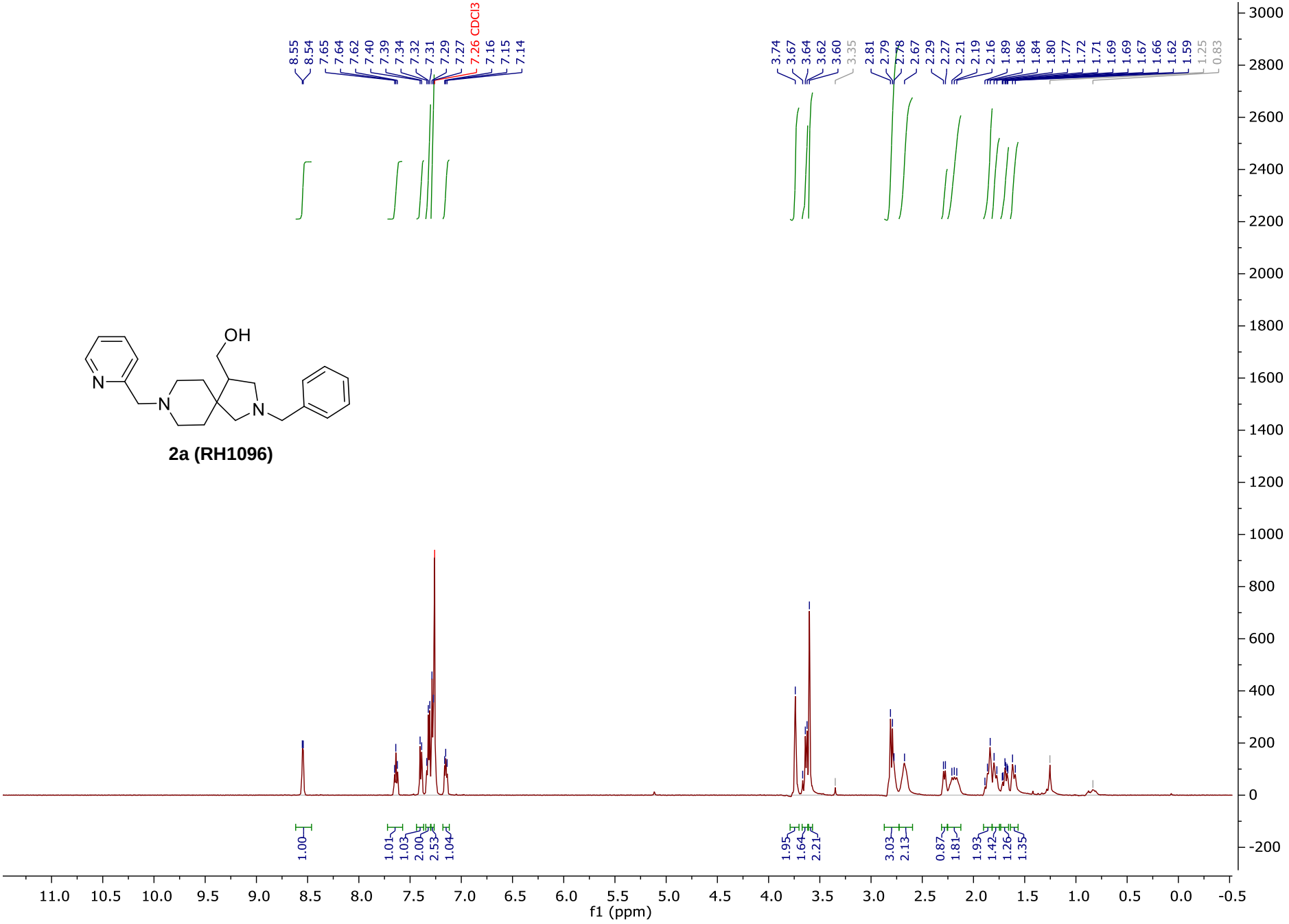

7.36
7.34
7.32
7.31
7.29
7.27
7.26 CDCl3
7.24
3.77
3.66
3.43
3.17
3.13
3.12
3.11
3.10
2.96
2.94
2.93
2.86
2.86
2.85
2.83
2.77
2.76
2.74
2.65
2.63
2.60
2.58
2.39
2.37
2.29
2.20
2.04
2.00
1.95
1.93
1.91
1.86
1.86
1.84
1.83
1.81
1.81
1.72
1.69
1.50
1.48
1.47
1.45
1.45
O
N
Me
O
OMe
Me
N
N
4.06
1.08
1.95
3.11
2.00
1.01
1.11
1.10
0.97
1.98
0.97
2.98
3.05
1.15
0.98
1.06
1.00
2.02
f1 (ppm)

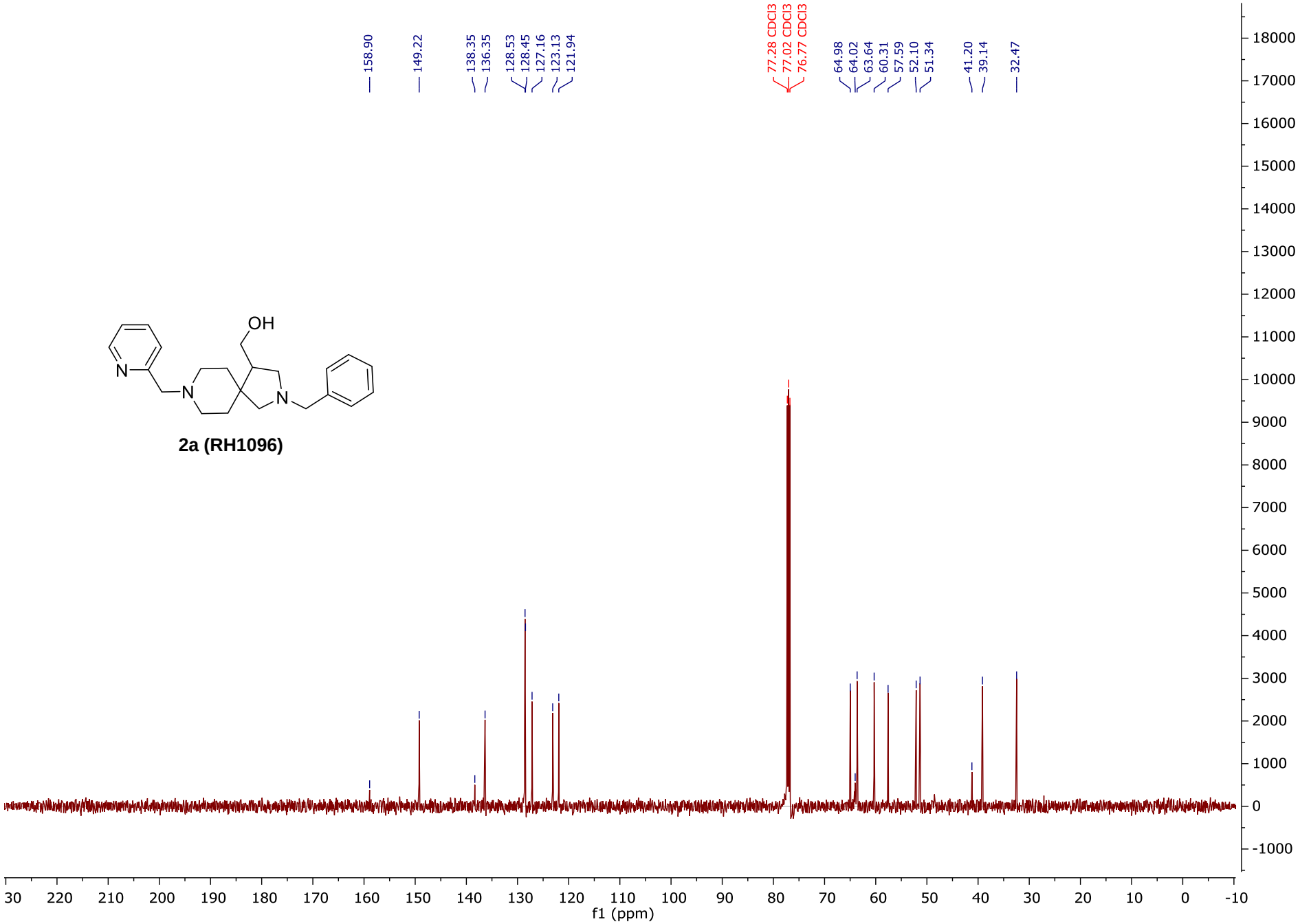
8e

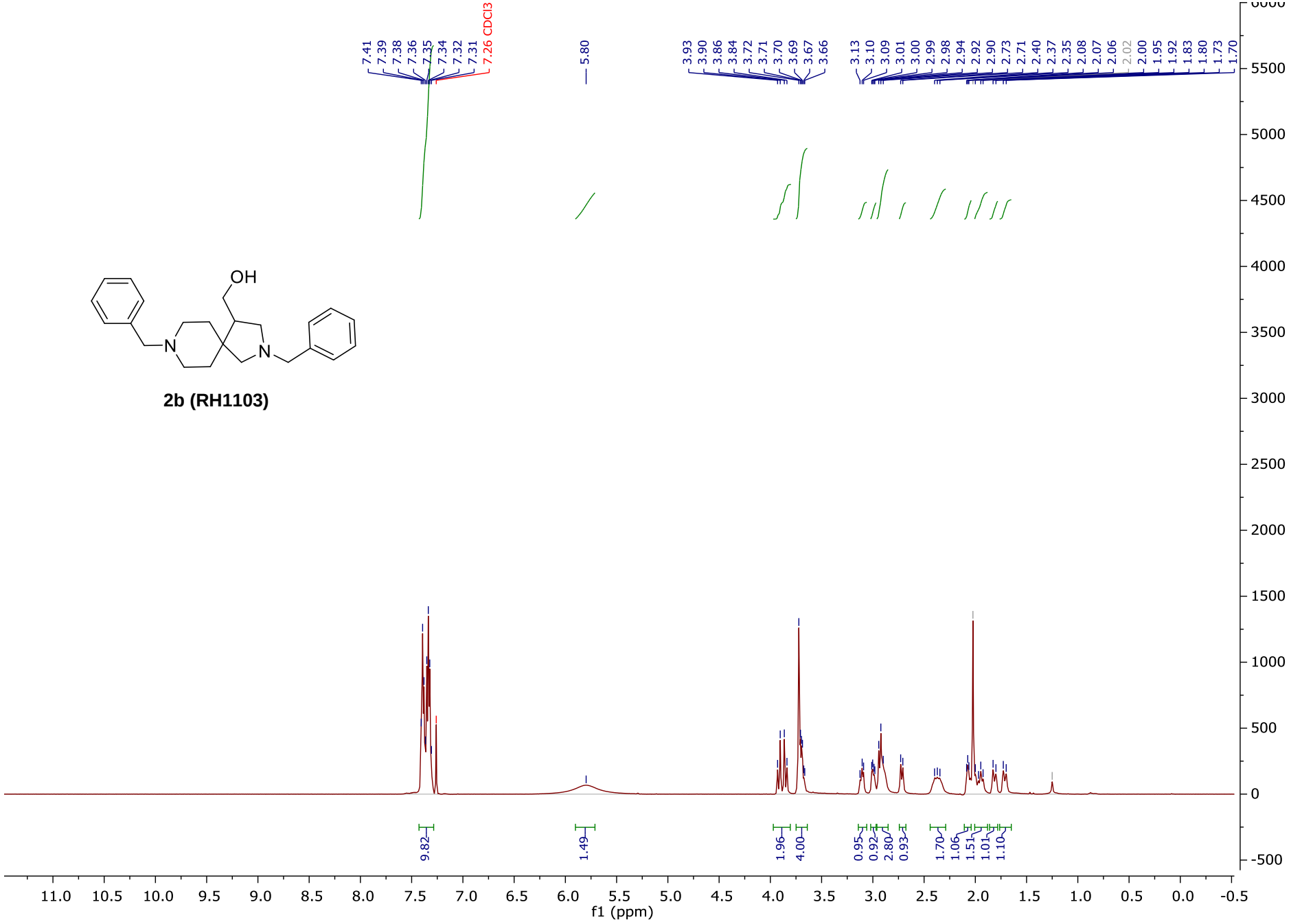

OMe
O
N
O
Me
Me
N
N

8e

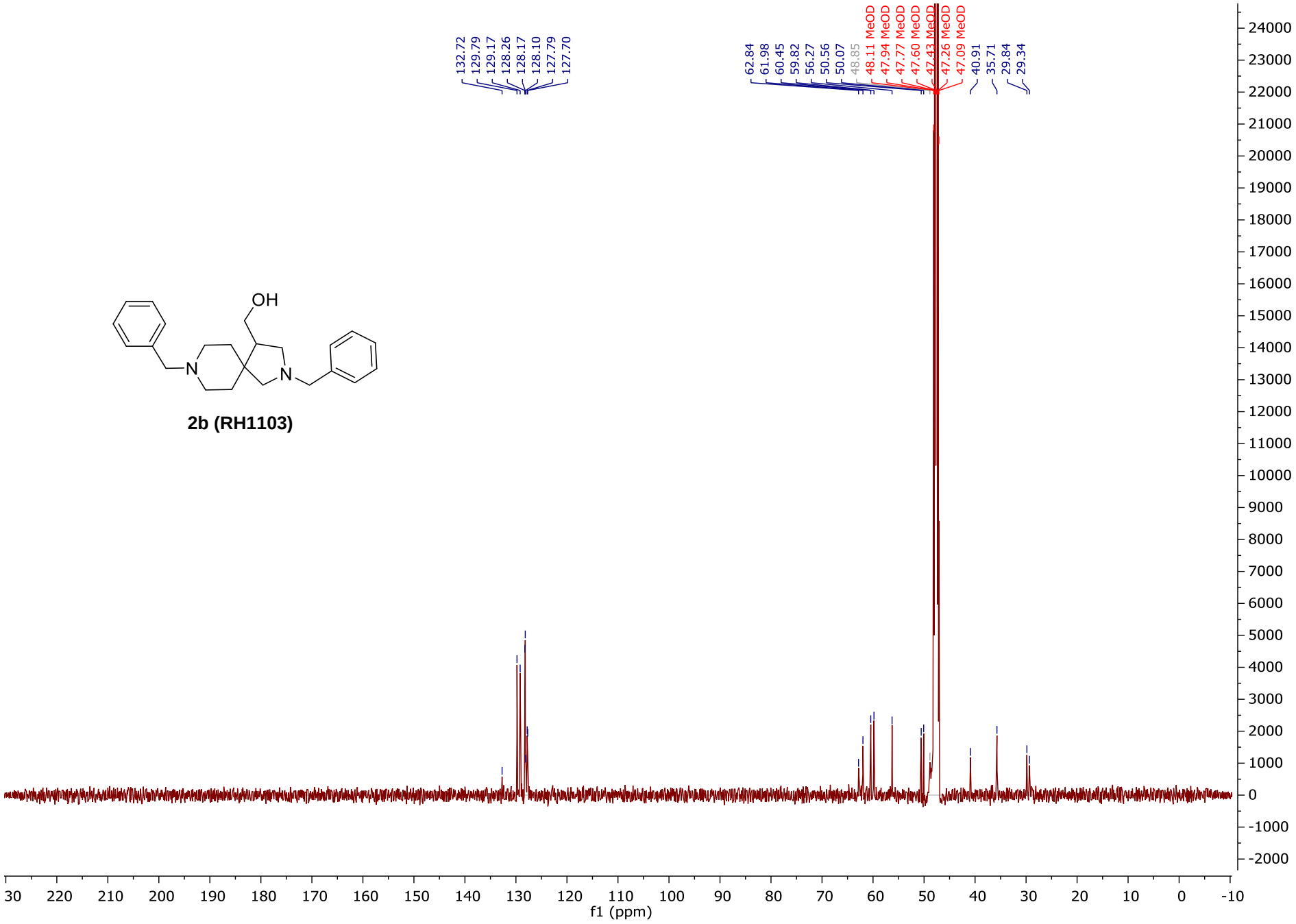
173.43
166.50
160.39
138.55
128.61
128.30
127.06
110.48
77.34 CDCl3
77.09 CDCl3
76.83 CDCl3
62.49
59.91
55.21
53.41
51.44
51.07
50.58
50.40
43.88
37.24
32.89
11.05
10.28
170000
160000
150000
140000
130000
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
-10000
30 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

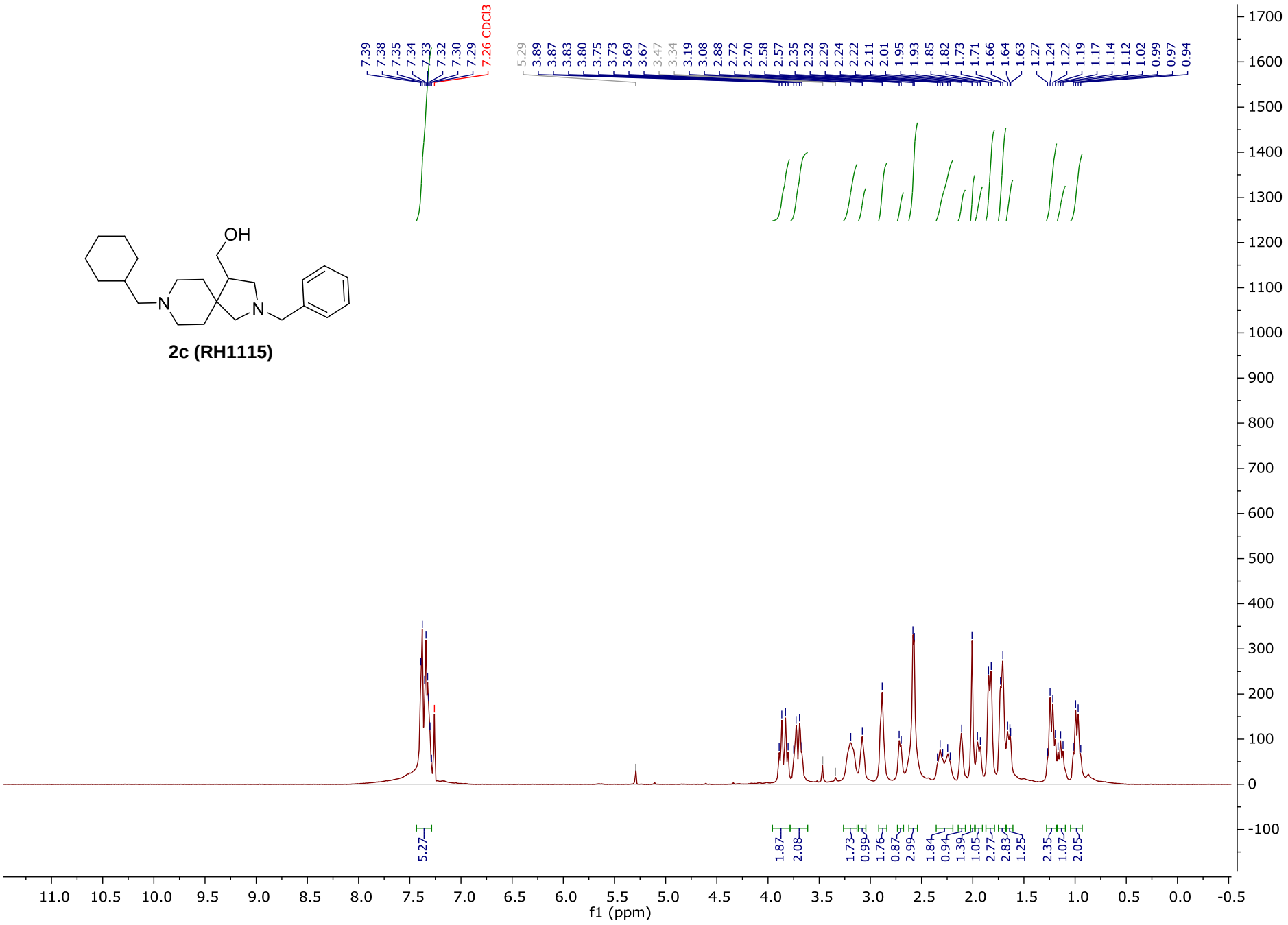

8.55
8.54
7.65
7.64
7.62
7.40
7.39
7.34
7.32
7.31
7.29
7.27
7.26 CDCl3
7.16
7.15
7.14
3.74
3.67
3.64
3.62
3.60
3.35
2.81
2.79
2.78
2.67
2.29
2.27
2.21
2.19
2.16
1.89
1.86
1.84
1.80
1.77
1.72
1.71
1.69
1.69
1.67
1.66
1.62
1.59
1.25
0.83
OH
N
N
N
2a (RH1096)
1.00
1.01
1.03
2.00
2.53
1.04
1.95
1.64
2.21
3.03
2.13
0.87
1.81
1.93
1.42
1.26
1.35
f1 (ppm)

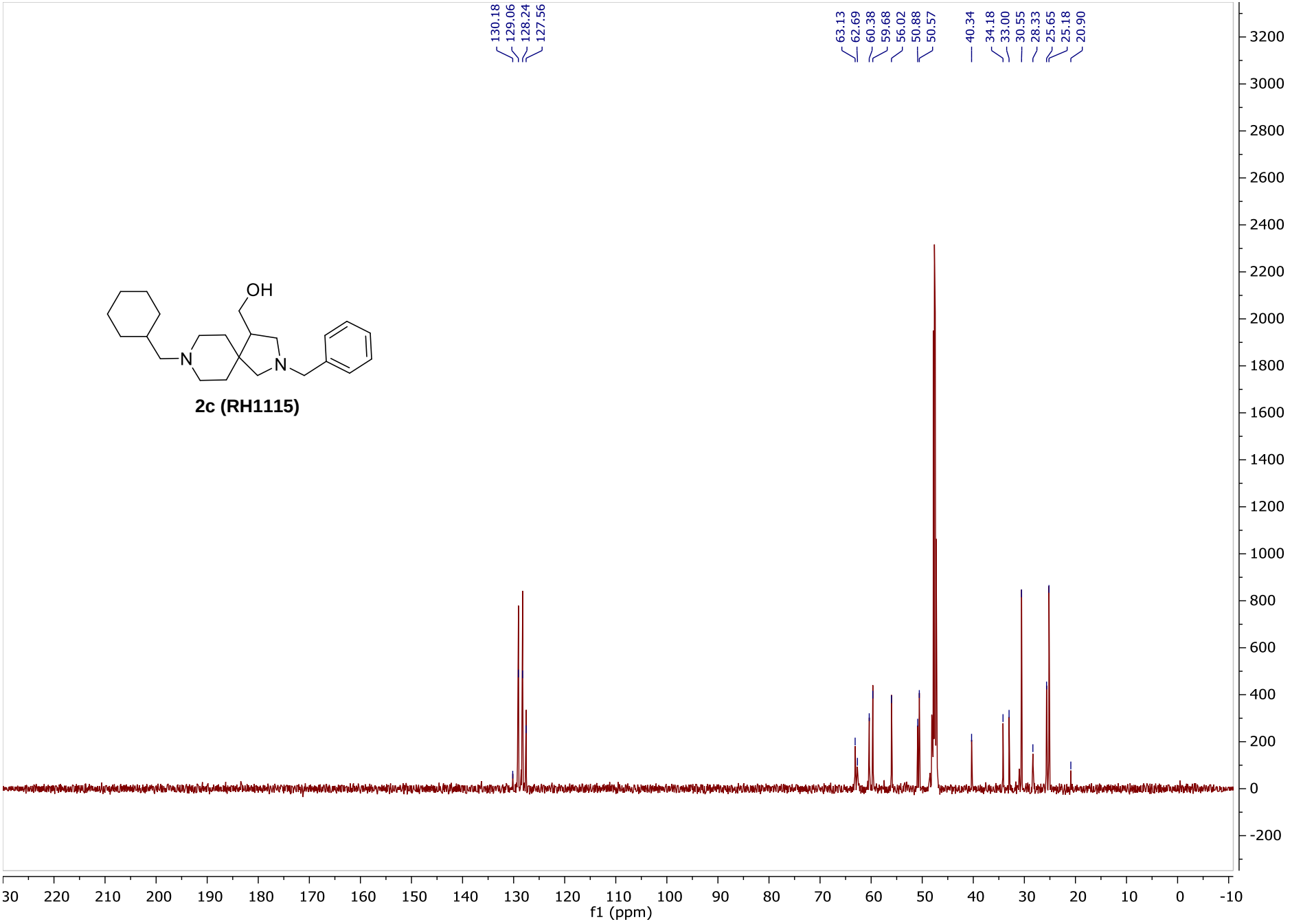

158.90
149.22
138.35
136.35
128.53
128.45
127.16
123.13
121.94
77.28 CDCl3
77.02 CDCl3
76.77 CDCl3
64.98
64.02
63.64
60.31
57.59
52.10
51.34
41.20
39.14
32.47
OH
N
N
N
2a (RH1096)
f1 (ppm)

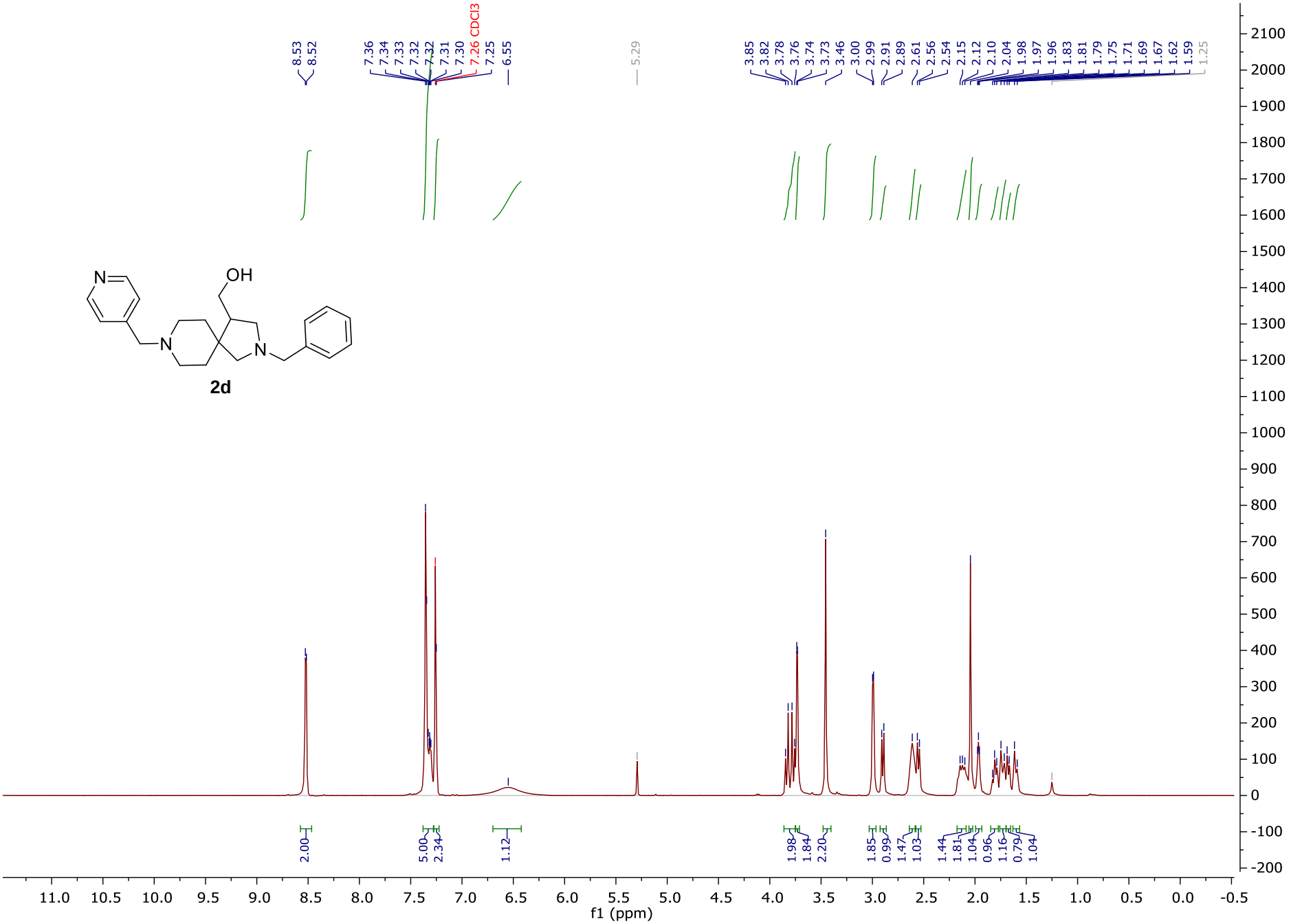

7.41
7.39
7.38
7.36
7.35
7.34
7.32
7.31
7.26 CDCl3
5.80
3.93
3.90
3.86
3.84
3.72
3.71
3.70
3.69
3.67
3.66
3.13
3.10
3.09
3.01
3.00
2.99
2.98
2.94
2.92
2.90
2.73
2.71
2.40
2.37
2.35
2.08
2.07
2.06
2.02
2.00
1.95
1.92
1.83
1.80
1.73
1.70
OH
N
N
9.82
1.49
1.96
4.00
0.95
0.92
2.80
0.93
1.70
1.06
1.51
1.01
1.10
f1 (ppm)

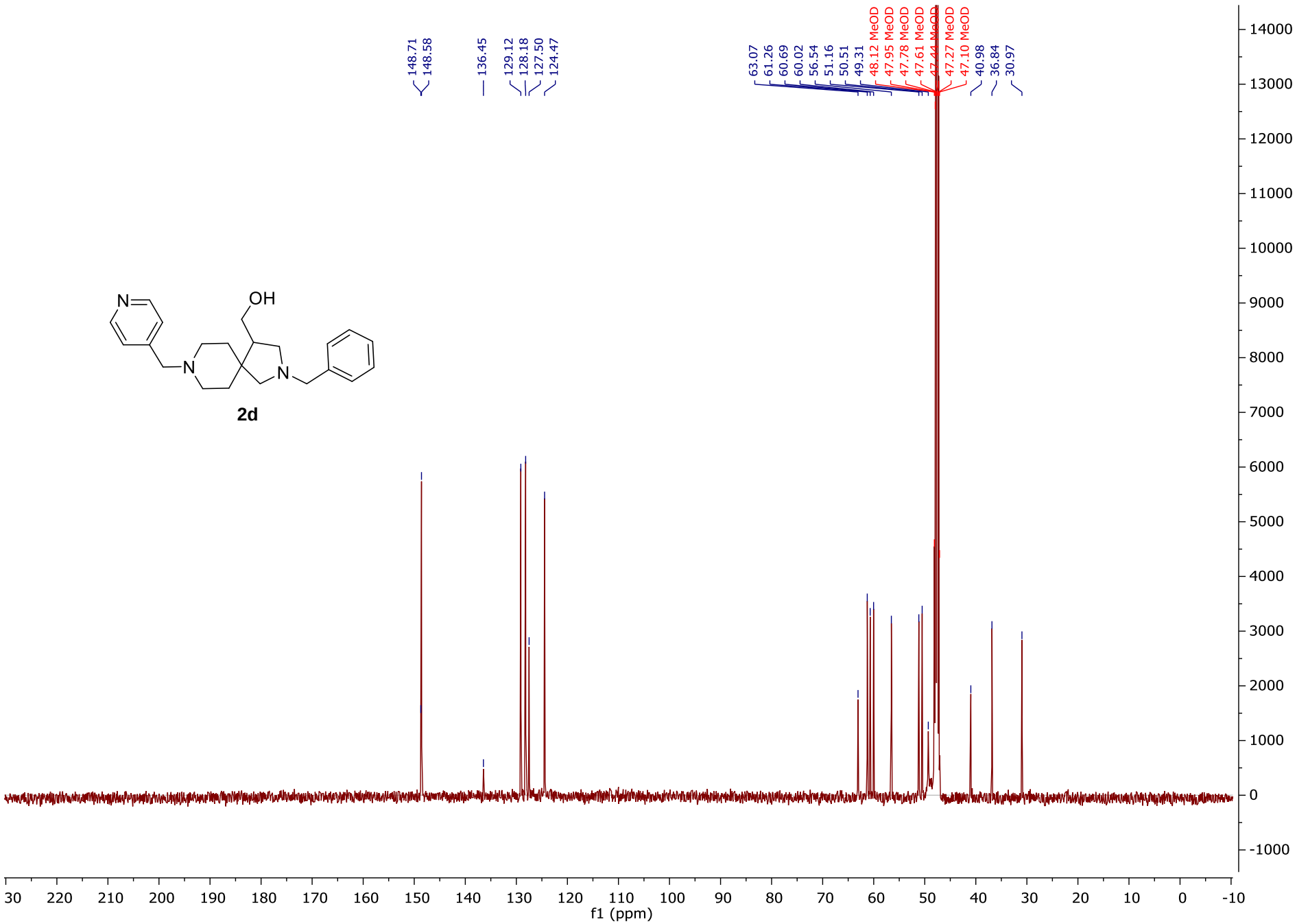
2b (RH1103)

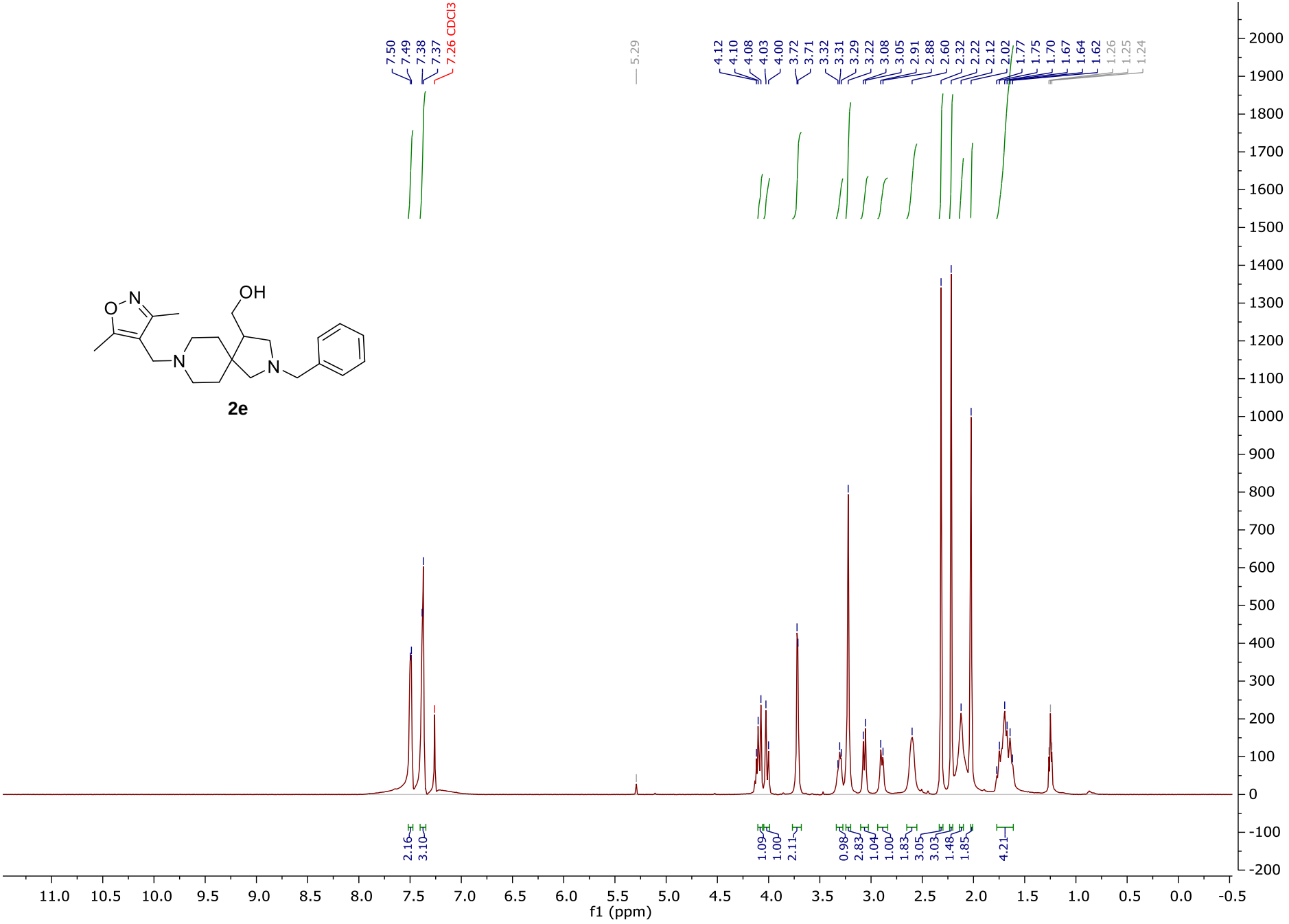

132.72
129.79
129.17
128.26
128.17
128.10
127.79
127.70
62.84
61.98
60.45
59.82
56.27
50.56
50.07
48.85
48.11 MeOD
47.94 MeOD
47.77 MeOD
47.60 MeOD
47.43 MeOD
47.26 MeOD
47.09 MeOD
40.91
35.71
29.84
29.34
OH
N
N
2b (RH1103)
f1 (ppm)


7.39
7.38
7.35
7.34
7.33
7.32
7.30
7.29
7.26 CDCl3
5.29
3.89
3.87
3.83
3.80
3.75
3.73
3.69
3.67
3.47
3.34
3.19
3.08
2.88
2.72
2.70
2.58
2.57
2.35
2.32
2.29
2.24
2.22
2.11
2.01
1.95
1.93
1.85
1.82
1.73
1.71
1.66
1.64
1.63
1.27
1.24
1.22
1.19
1.17
1.14
1.12
1.02
0.99
0.97
0.94
OH
N
N
2c (RH1115)
5.27
1.87
2.08
1.73
0.99
1.76
0.87
2.99
1.84
0.94
1.39
1.05
2.77
2.83
1.25
2.35
1.07
2.05
f1 (ppm)


130.18
129.06
128.24
127.56
63.13
62.69
60.38
59.68
56.02
50.88
50.57
40.34
34.18
33.00
30.55
28.33
25.65
25.18
20.90
OH
N
N
2c (RH1115)
f1 (ppm)


8.53
8.52
7.36
7.34
7.33
7.32
7.32
7.31
7.30
7.26 CDCl3
7.25
6.55
5.29
3.85
3.82
3.78
3.76
3.74
3.73
3.46
3.00
2.99
2.91
2.89
2.61
2.56
2.54
2.15
2.12
2.10
2.04
1.98
1.97
1.96
1.83
1.81
1.79
1.75
1.71
1.69
1.67
1.62
1.59
1.25
N
OH
N
N
2d
2.00
5.00
2.34
1.12
1.98
1.84
2.20
1.85
0.99
1.47
1.03
1.44
1.81
1.04
0.96
1.16
0.79
1.04
f1 (ppm)


148.71
148.58
136.45
129.12
128.18
127.50
124.47
63.07
61.26
60.69
60.02
56.54
51.16
50.51
49.31
48.12 MeOD
47.95 MeOD
47.78 MeOD
47.61 MeOD
47.44 MeOD
47.27 MeOD
47.10 MeOD
40.98
36.84
30.97
N
OH
N
N
2d
f1 (ppm)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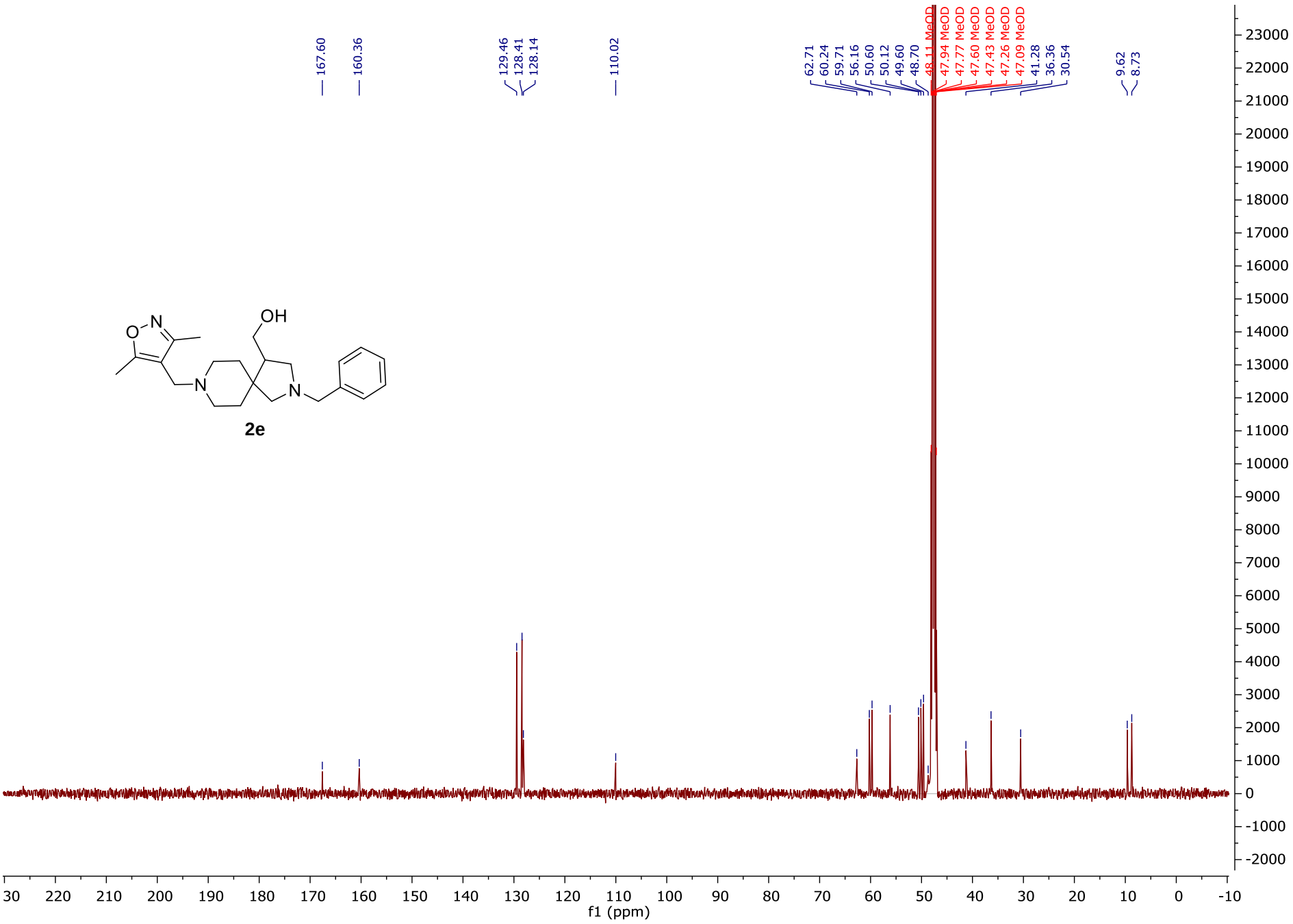
7.49
7.38
7.37
7.26 CDCl3
5.29
4.12
4.10
4.08
4.03
4.00
3.72
3.71
3.32
3.31
3.29
3.22
3.08
3.05
2.91
2.88
2.60
2.32
2.22
2.12
2.02
1.77
1.75
1.70
1.67
1.64
1.62
1.26
1.25
1.24
OH
N
O
N
N
2e
2.16
3.10
1.09
1.00
2.11
0.98
2.83
1.04
1.00
1.83
3.05
3.03
1.48
1.85
4.21
f1 (ppm)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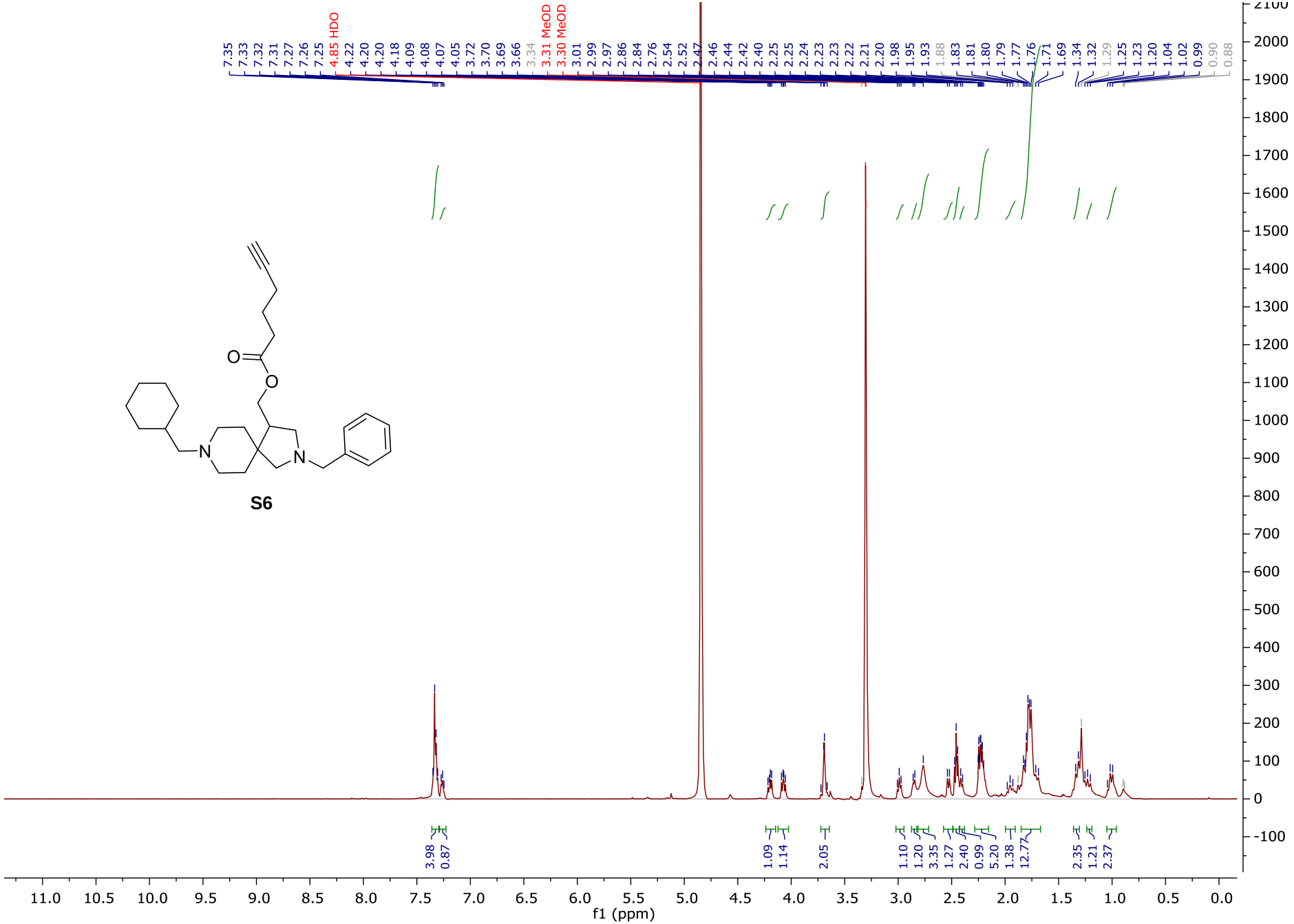
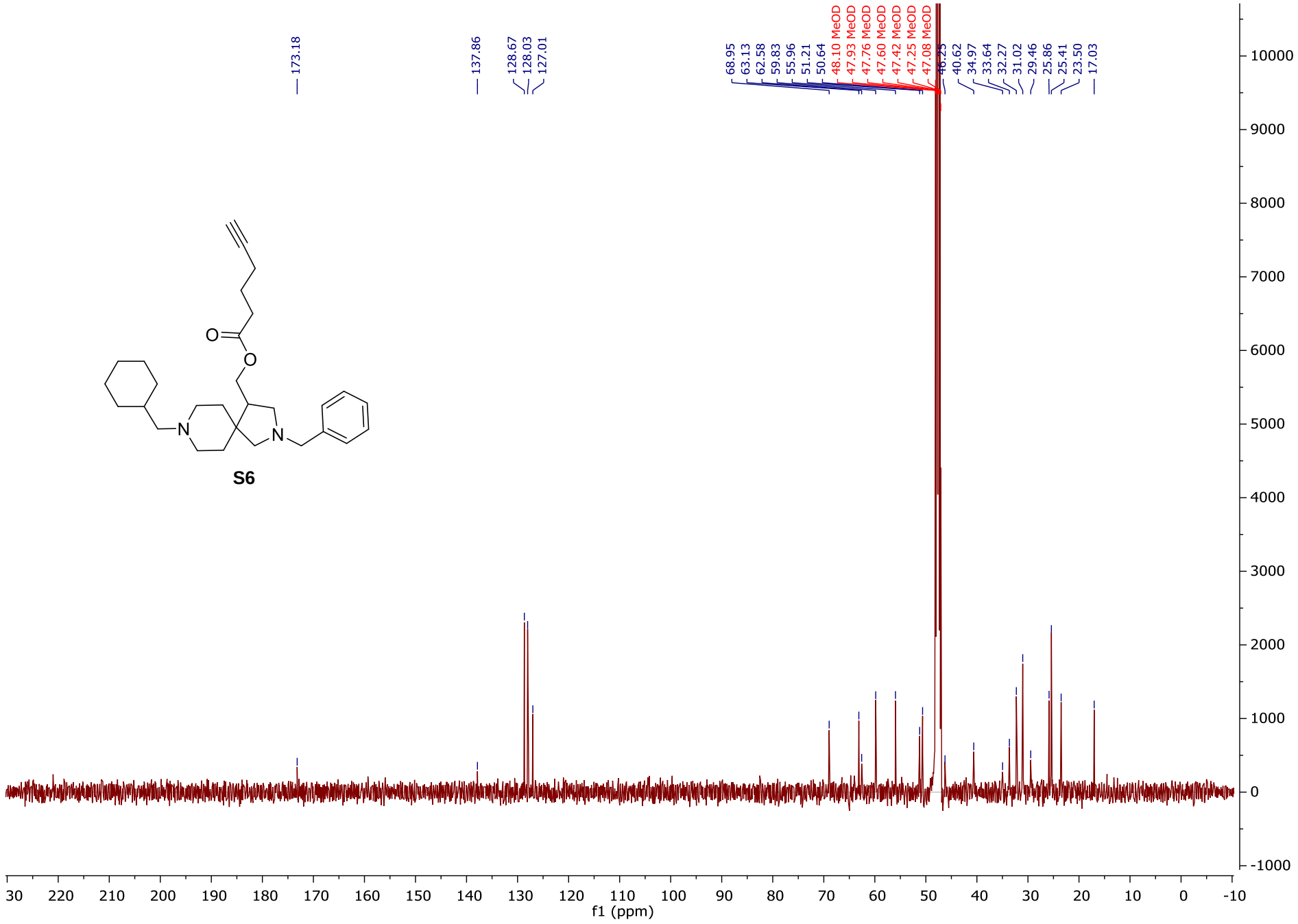
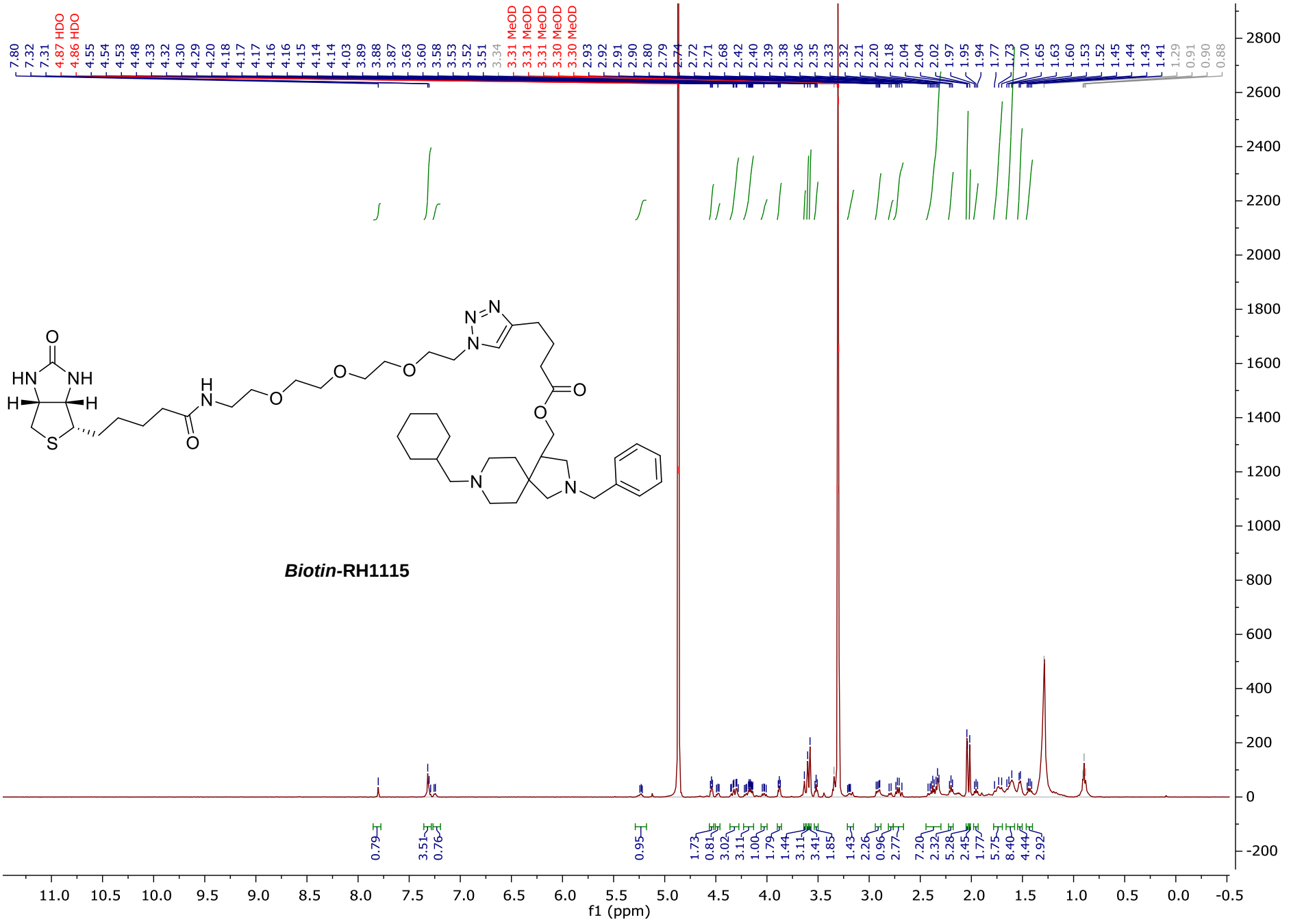
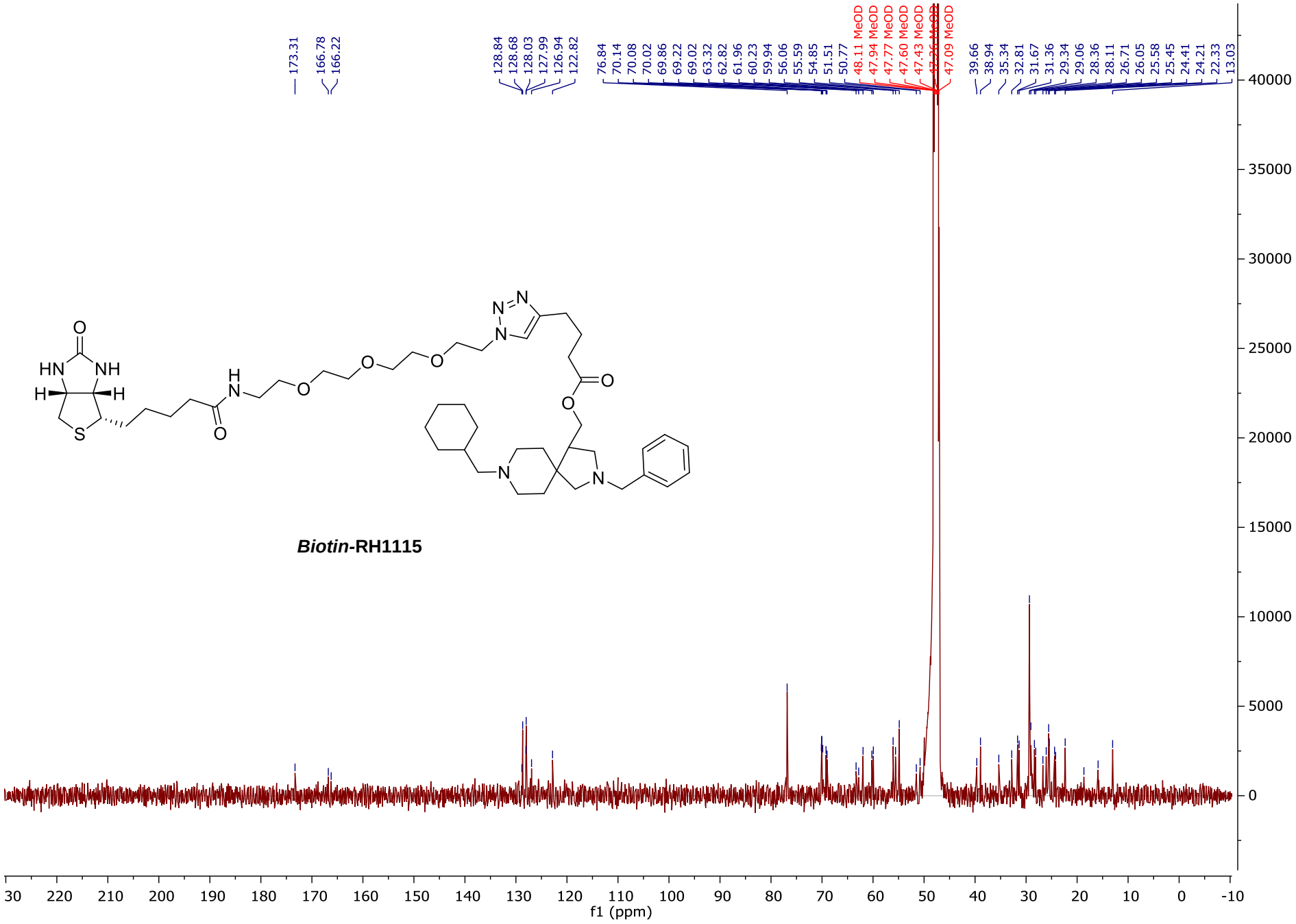
160.36
129.46
128.41
128.14
110.02
62.71
60.24
59.71
56.16
50.60
50.12
49.60
48.70
48.11 MeOD
47.94 MeOD
47.77 MeOD
47.60 MeOD
47.43 MeOD
47.26 MeOD
47.09 MeOD
41.28
36.36
30.54
9.62
8.73
OH
N
O
N
N
2e
f1 (ppm)


7.35 7.33 7.32 7.31 7.27 7.26 7.25 4.85 HDO 4.22 4.20 4.20 4.18 4.09 4.08 4.07 4.05 3.72 3.70 3.69 3.66 3.34 3.31 MeOD 3.30 MeOD 3.01 2.99 2.97 2.86 2.84 2.76 2.54 2.52 2.47 2.46 2.44 2.42 2.40 2.25 2.25 2.24 2.23 2.23 2.22 2.21 2.20 1.98 1.95 1.93 1.88 1.83 1.81 1.80 1.79 1.77 1.76 1.71 1.69 1.34 1.32 1.29 1.25 1.23 1.20 1.04 1.02 0.99 0.90 0.88
O
O
N
N
S6
3.98 0.87 1.09 1.14 2.05 1.10 1.20 3.35 1.27 2.40 0.99 5.20 1.38 12.77 2.35 1.21 2.37
11.0 10.5 10.0 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 0.0
f1 (ppm)


173.18
137.86
128.67
128.03
127.01
68.95
63.13
62.58
59.83
55.96
51.21
50.64
48.10 MeOD
47.93 MeOD
47.76 MeOD
47.60 MeOD
47.42 MeOD
47.25 MeOD
47.08 MeOD
40.62
34.97
33.64
32.27
31.02
29.46
25.86
25.41
23.50
17.03
O
O
N
N
S6
f1 (ppm)


7.80 7.32 7.31 4.87 HDO 4.86 HDO 4.55 4.54 4.53 4.48 4.33 4.32 4.30 4.29 4.20 4.18 4.17 4.17 4.16 4.16 4.15 4.14 4.14 4.03 3.89 3.88 3.87 3.63 3.60 3.58 3.53 3.52 3.51 3.34 3.31 MeOD 3.31 MeOD 3.31 MeOD 3.30 MeOD 3.30 MeOD 2.93 2.92 2.91 2.90 2.80 2.79 2.74 2.72 2.71 2.68 2.42 2.40 2.39 2.38 2.36 2.35 2.33 2.32 2.21 2.20 2.18 2.04 2.04 2.02 1.97 1.95 1.94 1.77 1.73 1.70 1.65 1.63 1.60 1.53 1.52 1.45 1.44 1.43 1.41 1.29 0.91 0.90 0.88
HN NH H H S O O H N O O O O N N N O O N N
Biotin-RH1115
0.79 3.51 0.76 0.95 1.73 0.81 3.02 3.11 1.00 1.79 1.44 3.11 3.41 1.85 1.43 2.26 0.96 2.77 7.20 2.32 5.28 2.45 1.77 5.75 8.40 4.44 2.92
11.0 10.5 10.0 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 0.0 -0.5
f1 (ppm)
2800 2600 2400 2200 2000 1800 1600 1400 1200 1000 800 600 400 200 0 -200



***Biotin*-RH1115**