

Supporting Information

Peptidic boronic acid *Plasmodium falciparum* SUB1 inhibitors with improved selectivity over human proteasome

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1. Effect of human proteasome inhibitors on activity of recombinant PfSUB1

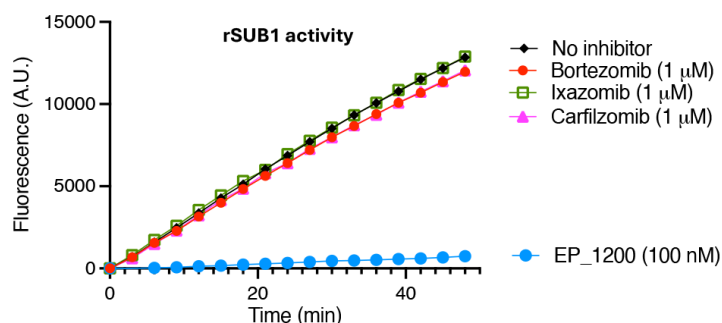
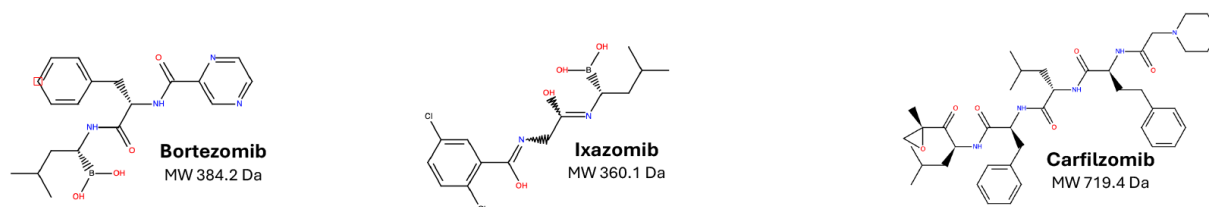


Figure S1. Human proteasome inhibitors do not inhibit recombinant PfSUB1. In vitro PfSUB1 activity assay showing lack of inhibition by bortezomib, ixazomib or carfilzomib (1 μ M final concentration). EP_1200 (used as a positive control) completely inhibited recombinant PfSUB1 activity at 100 nM. The assay shown is typical of duplicate assays.

2. 20S Human Proteasome dose response assay for peptidic boronic acid SUB1 inhibitors

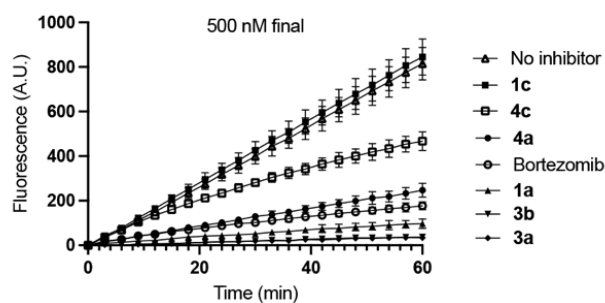
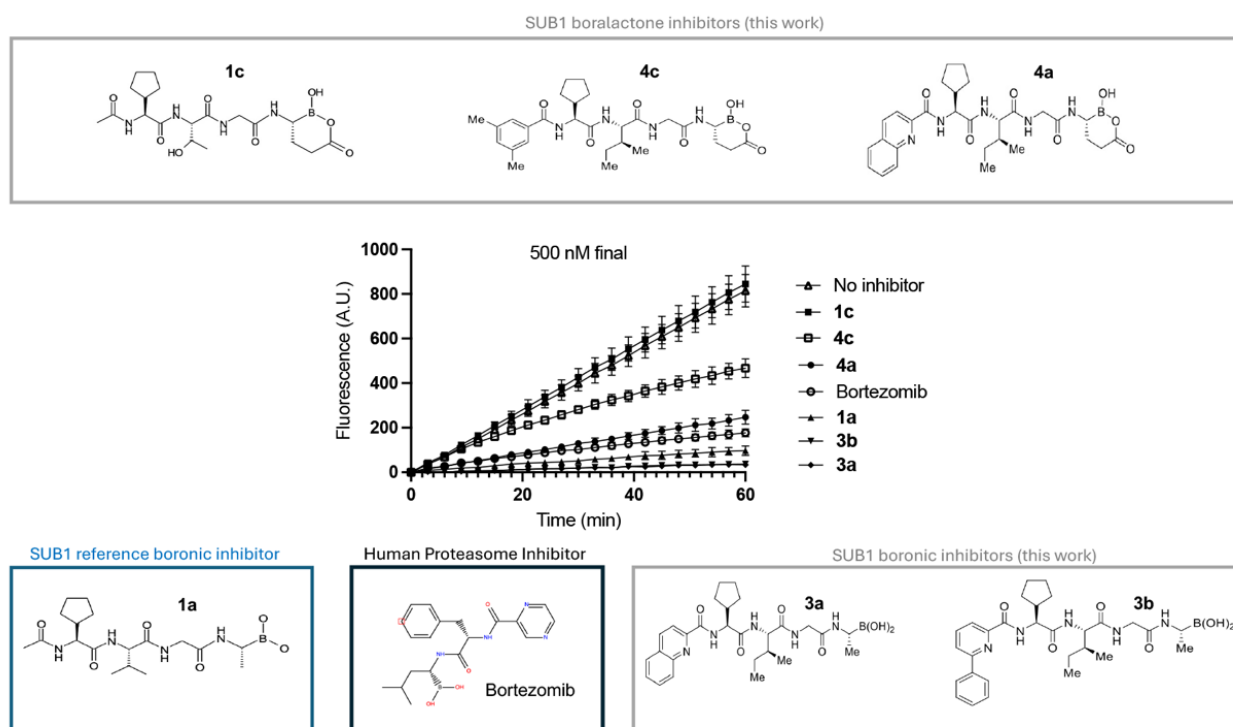


Figure S2. 20S Human proteasome dose response assay. The peptidic boronic acid PfSUB1 inhibitors **1a**, **3a**, **3b**, the borolactone PfSUB1 inhibitors **1c**, **4c**, **4a** and the bortezomib control were used at 500 nM final in a fluorescence-based enzymatic assay measuring the chymotrypsin-like activity (β 5) of the human 20S proteasome. All measurements were performed in duplicate, with points shown as mean values. Error bars, S.D.

3. Docking of bortezomib and inhibitors 1a, 3b, 1c and 4c into PfSUB1

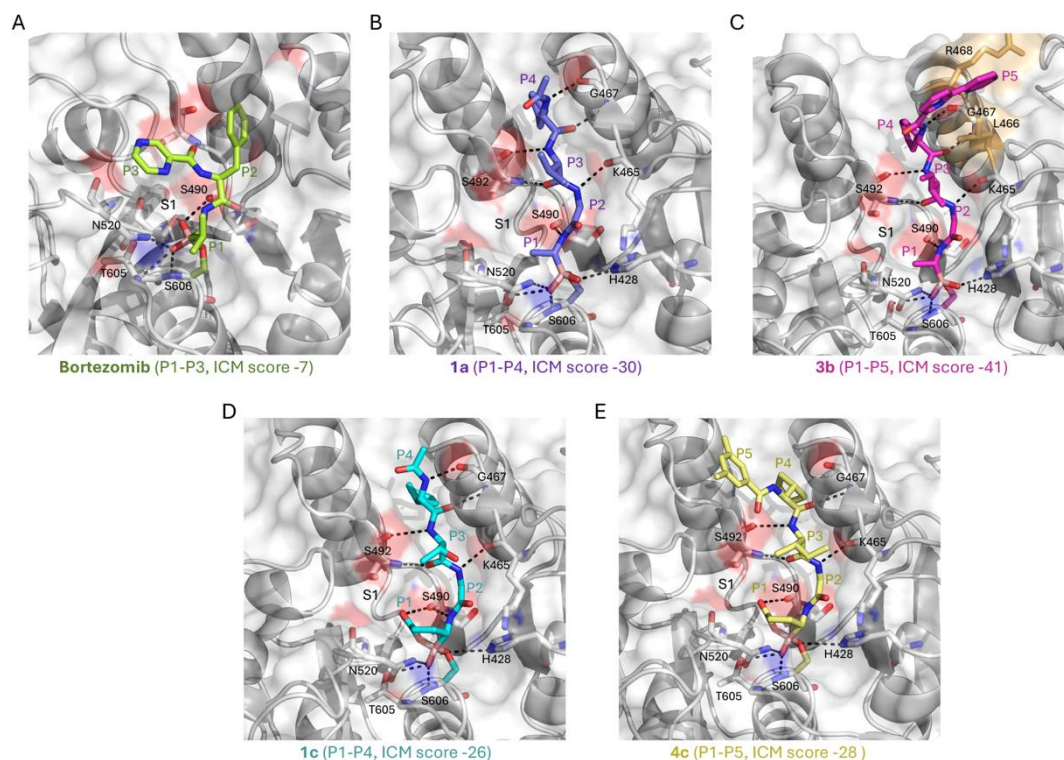
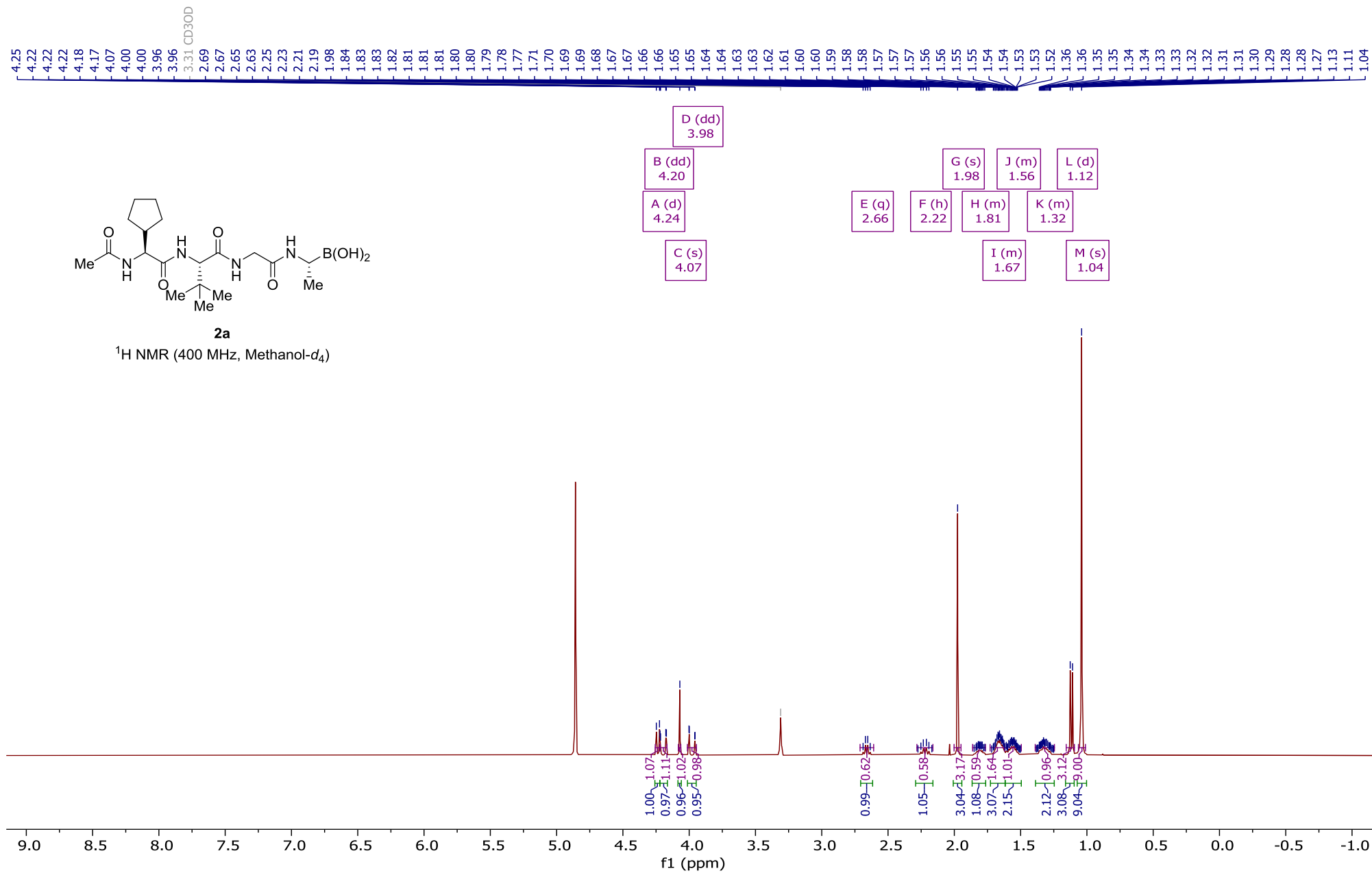
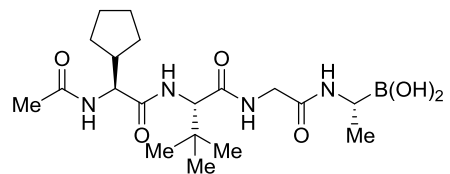


Figure S3. Bortezomib, compound **1a**, **3b**, **1c** and **4c** docked into PfSUB1(PDB: 4lvn). SUB1 is shown as a cartoon with a semi-transparent molecular surface colored by elements (O: red, N: blue). Positions P1 to P5 of inhibitors are indicated, the boron atom is colored in pink. Stabilizing H-bonds are shown as black dashed lines. **A.**: Bortezomib docked into the active site of PfSUB1 (ICM score -7) is shown as green sticks colored by elements. The P1 Leu side chain did not fill the S1 polar pocket and faced outwards the pocket. **B.**: compound **1a** docked into the active site PfSUB1 (ICM score -30) is shown as purple sticks colored by elements. The P1 Ala side chain fitted the S1 pocket. **C.**: compound **3b** docked into the active site PfSUB1(ICM score -41) is shown as magenta sticks colored by elements. The P1 Ala side chain fitted the S1 pocket. The P5 phenyl pyridine capping group was stabilized by Pi-stacking interactions involving Leu466 and Arg468 (in wheat colour). **D.**: compound **1c** docked into the active site of PfSUB1 (ICM score -26) is shown as teal sticks colored by elements. The boralactone group fitted the S1 polar pocket. **E.**: compound **4c** docked into the active site of PfSUB1 (ICM score -28) is shown as yellow sticks colored by elements. The boralactone group fitted the S1 polar pocket

4. Compound 2a–h, 3a–j, 4a–c characterization spectra



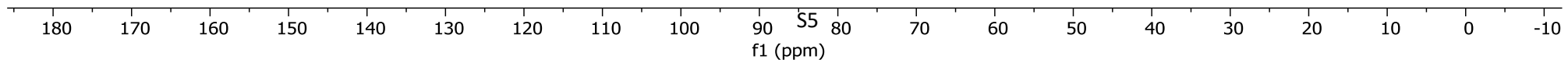
176.2
174.8
173.5
173.4

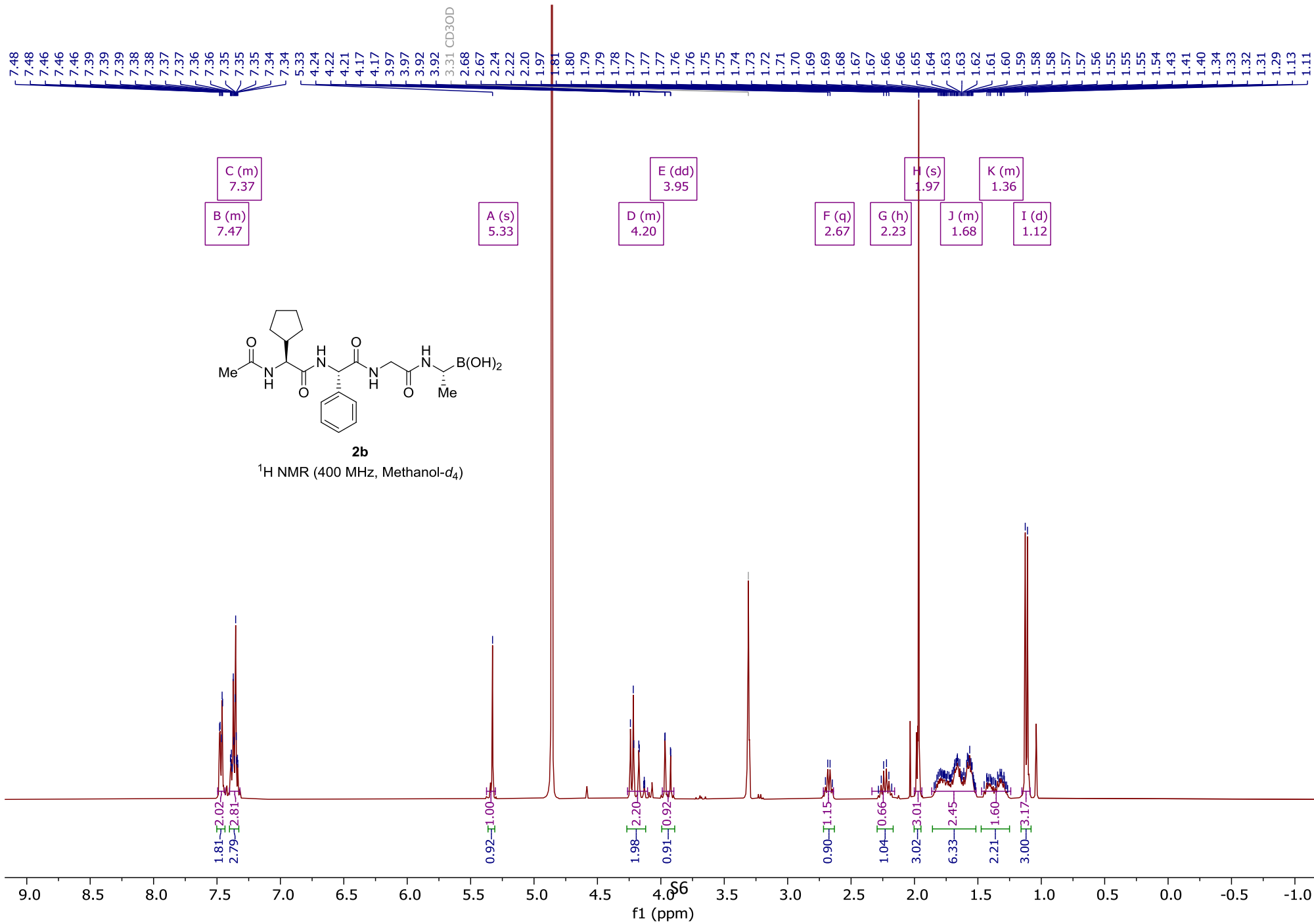


¹³C NMR (101 MHz, Methanol-d₄)

63.5
58.9

42.9
41.9
39.5
34.6
30.4
30.3
27.0
26.3
25.9
22.3
15.9





176.2
174.4
173.4
173.1

137.3

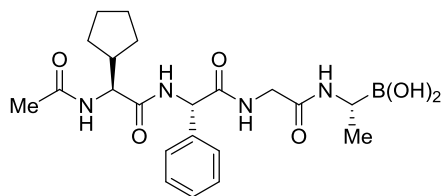
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129.7
129.0

59.4
58.6

43.2
41.9
39.9

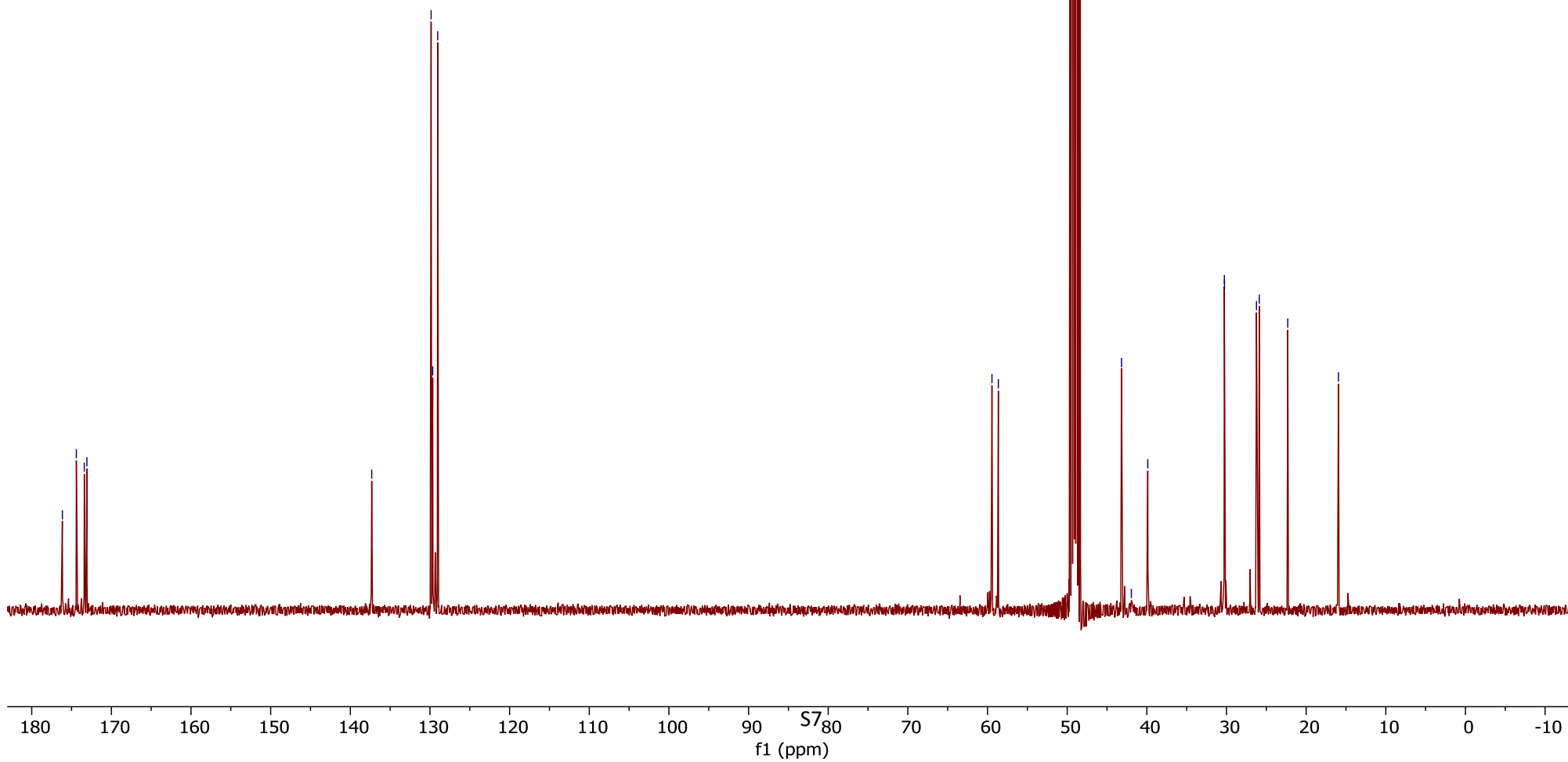
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30.2
26.2
25.9
22.3

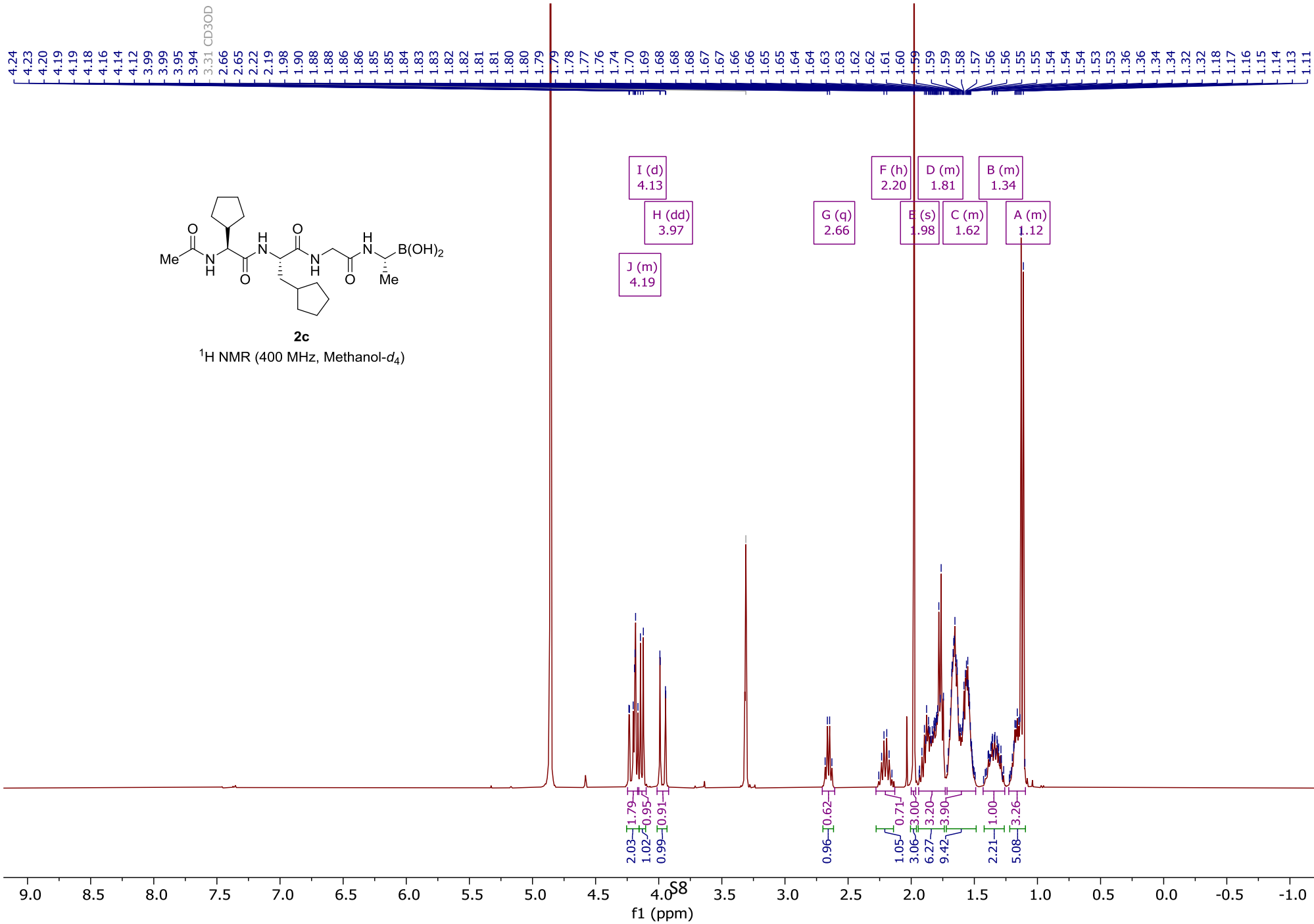
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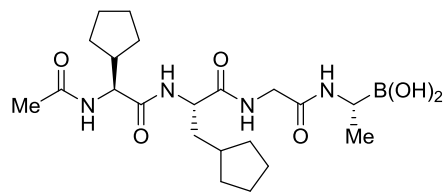
2b

¹³C NMR (101 MHz, Methanol-d₄)





176.3
175.3
175.0
173.5

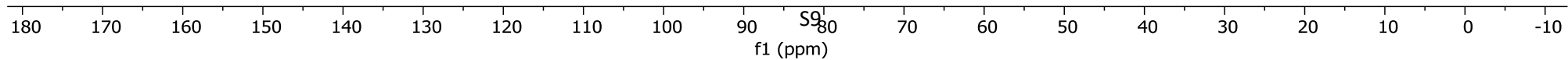


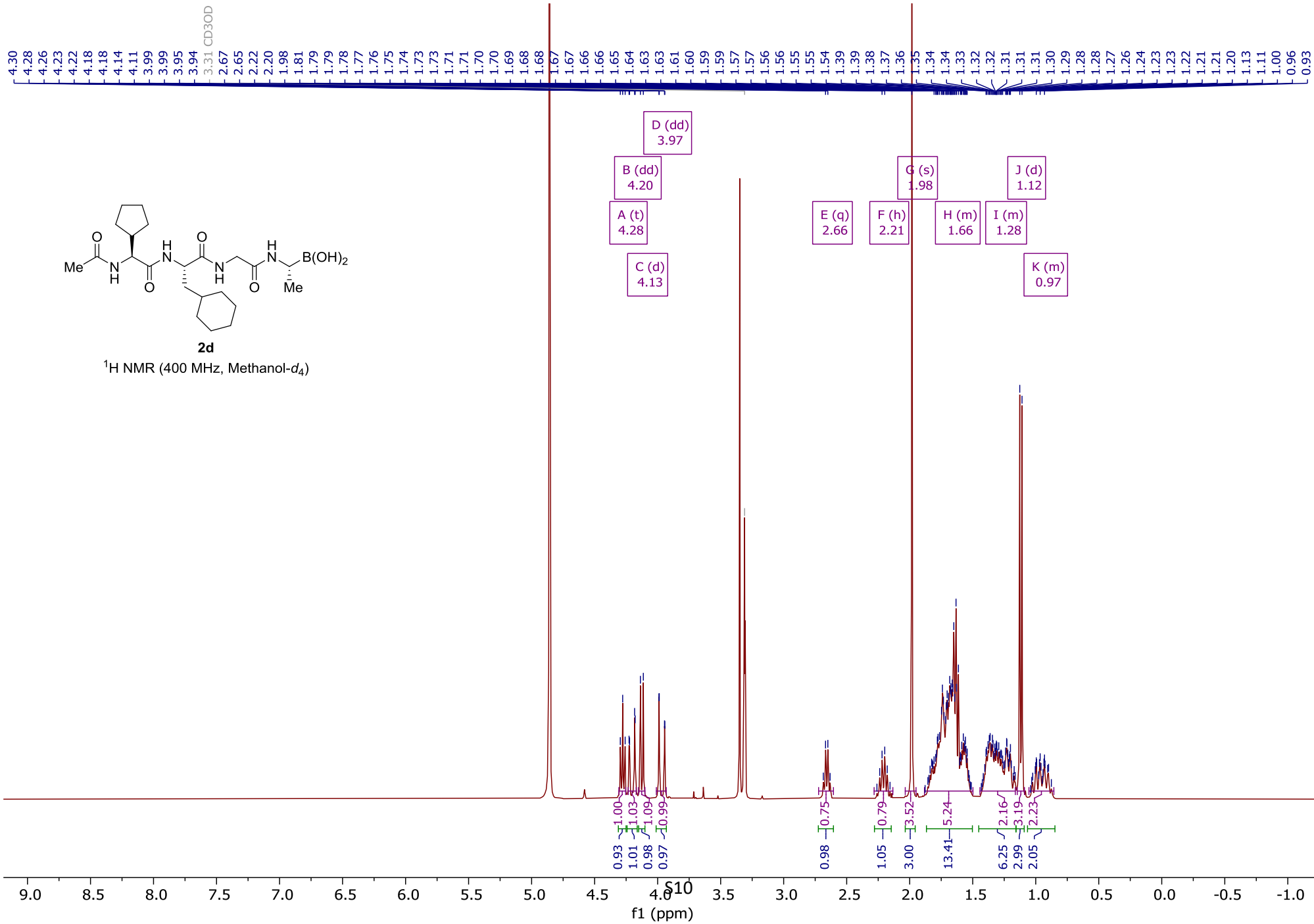
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¹³C NMR (101 MHz, Methanol-d₄)

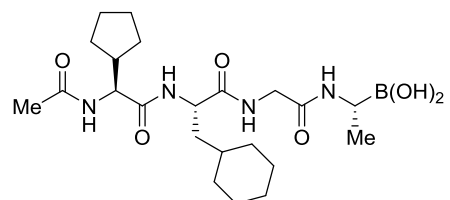
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55.1

43.1
42.0
39.8
38.3
37.7
33.7
33.2
30.4
30.2
26.2
26.1
26.0
25.9
22.3
15.9





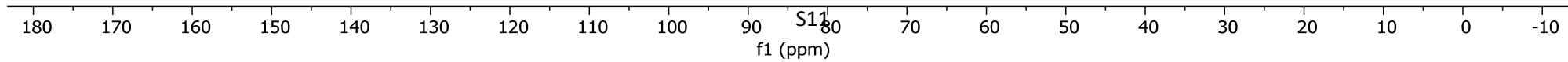
176.3
175.5
175.0
173.5

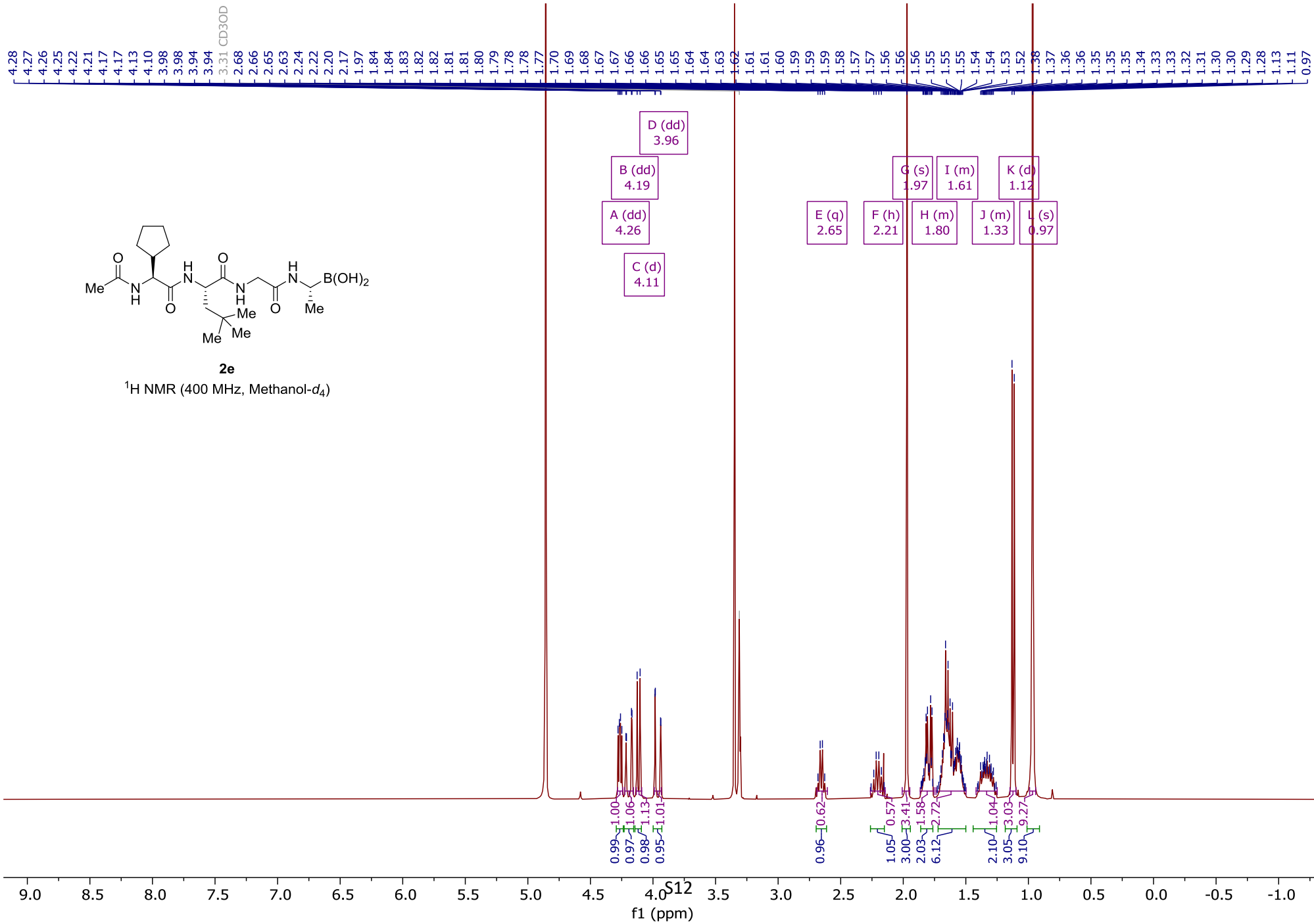


2d

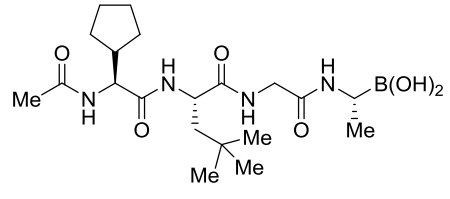
^{13}C NMR (101 MHz, Methanol- d_4)

59.1
53.1
49.0 CD3OD
43.0
41.9
39.8
39.5
35.2
34.8
33.5
30.4
30.2
27.6
27.4
27.2
26.2
25.9
22.4
16.0





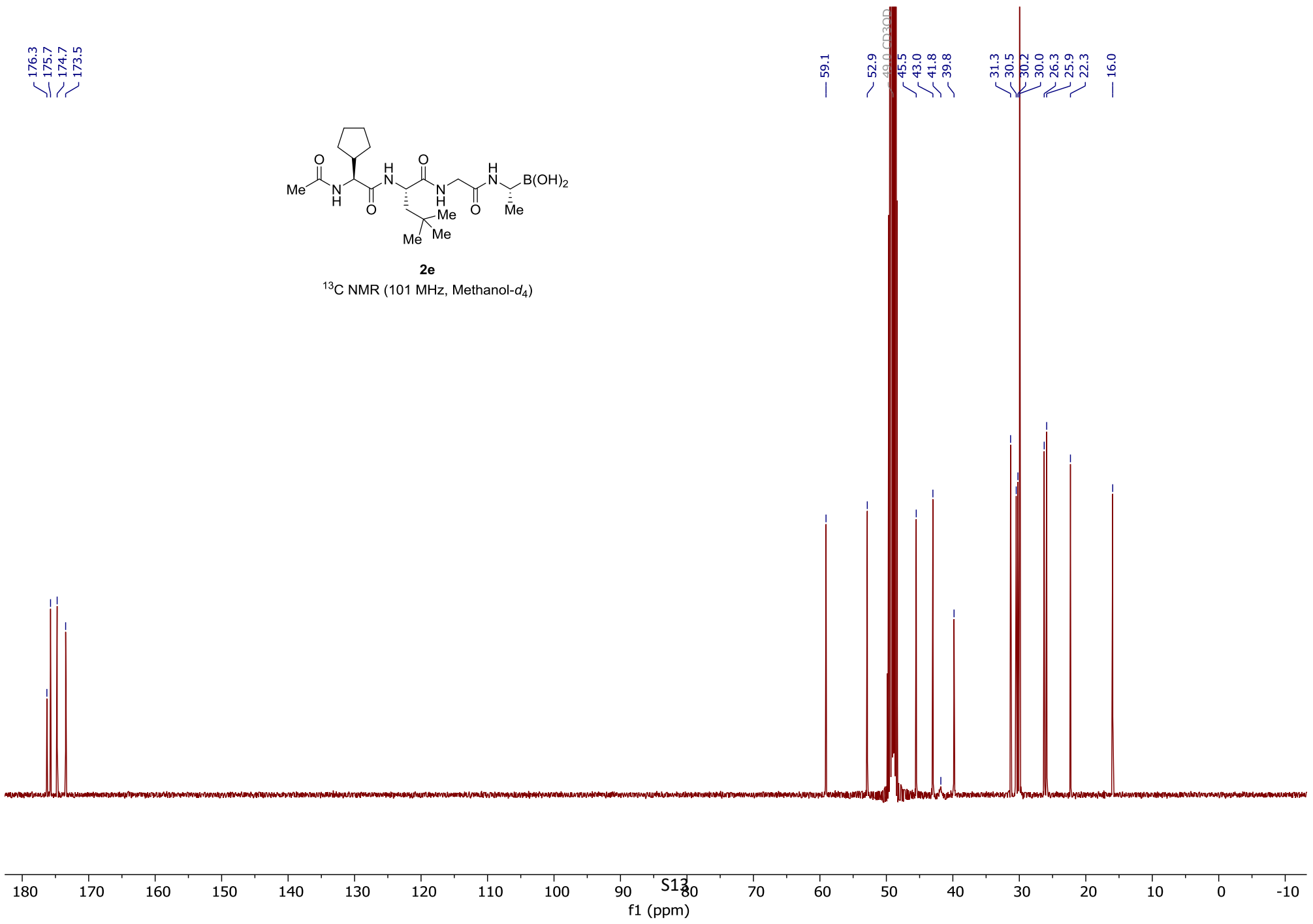
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173.5

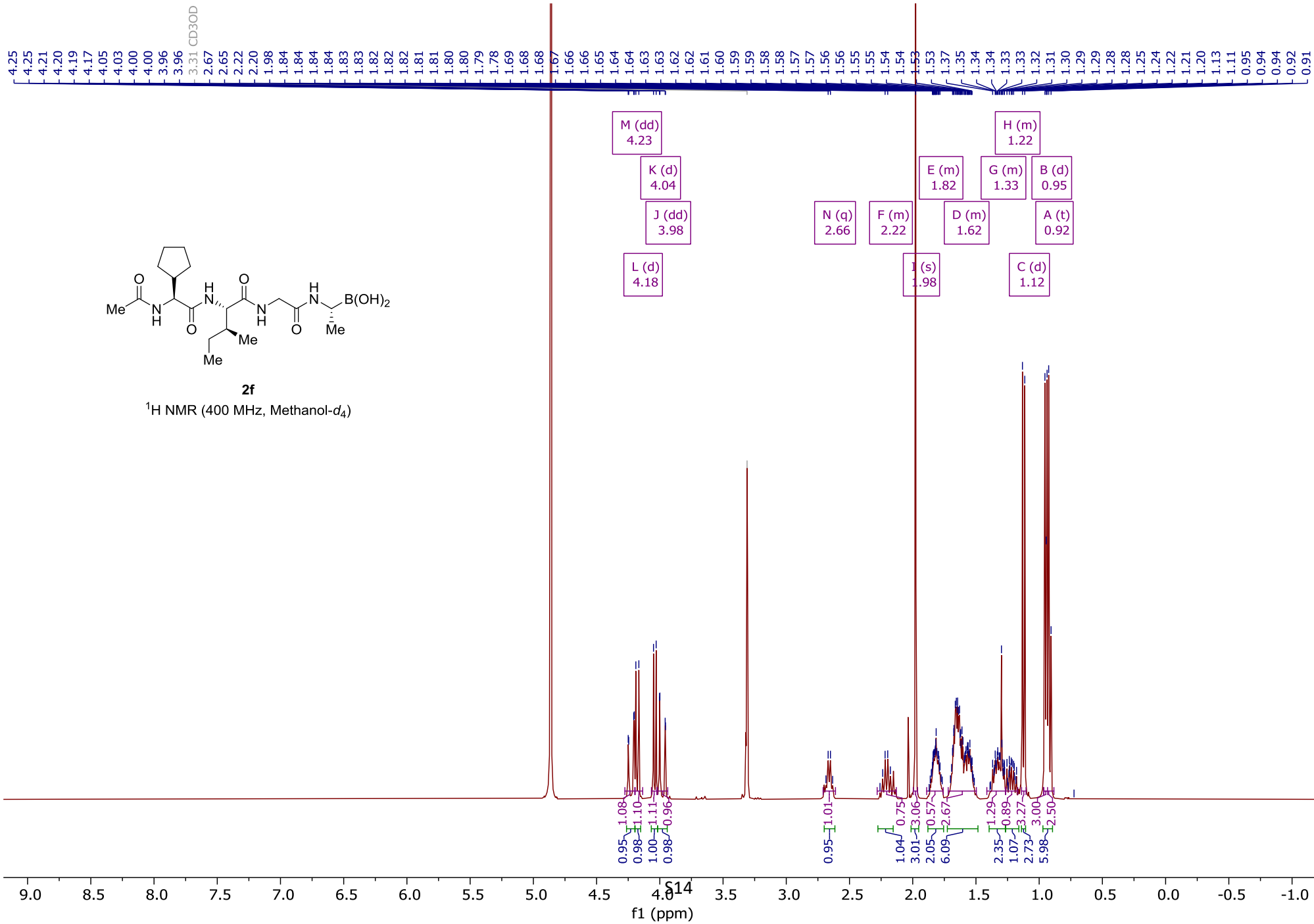


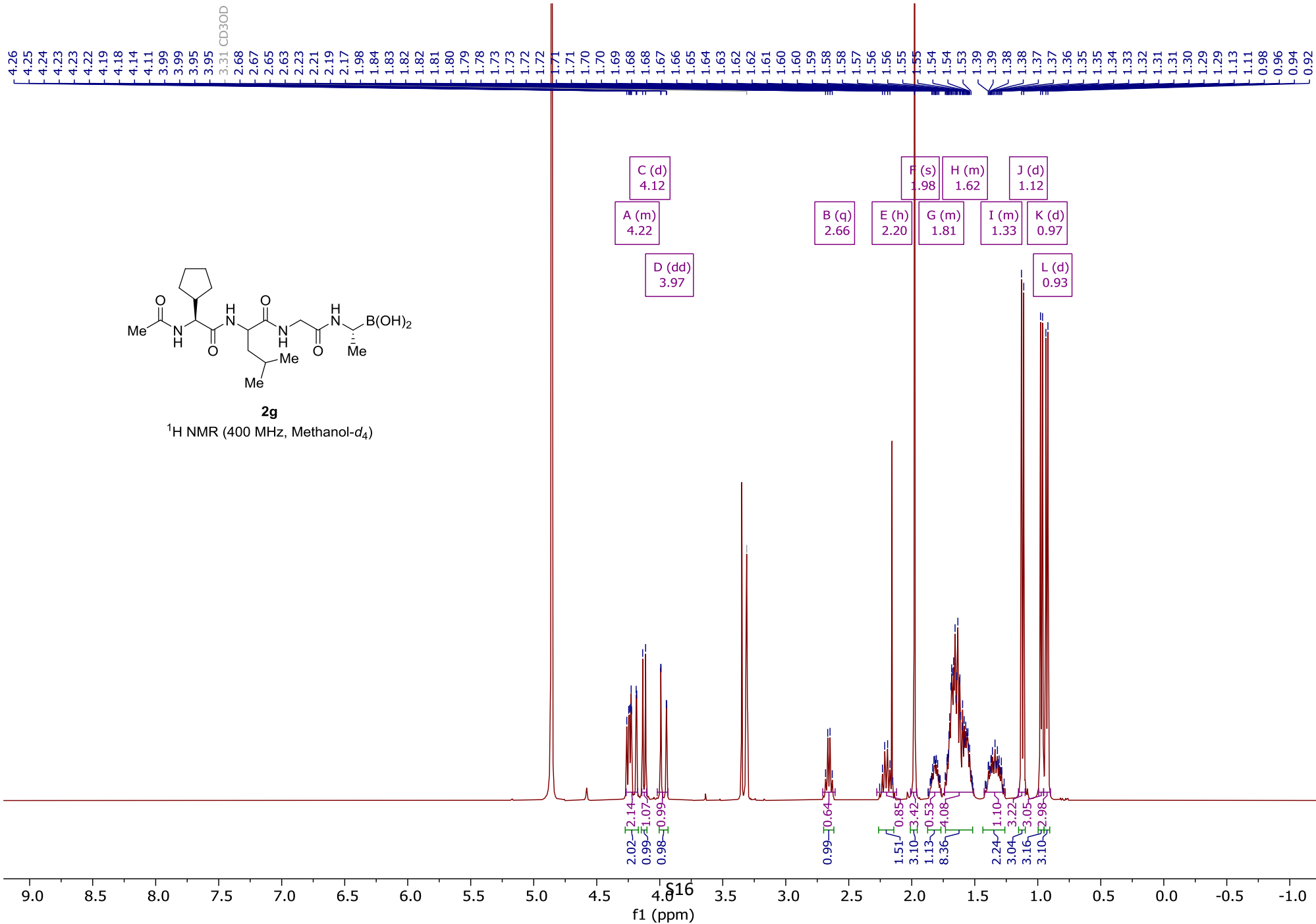
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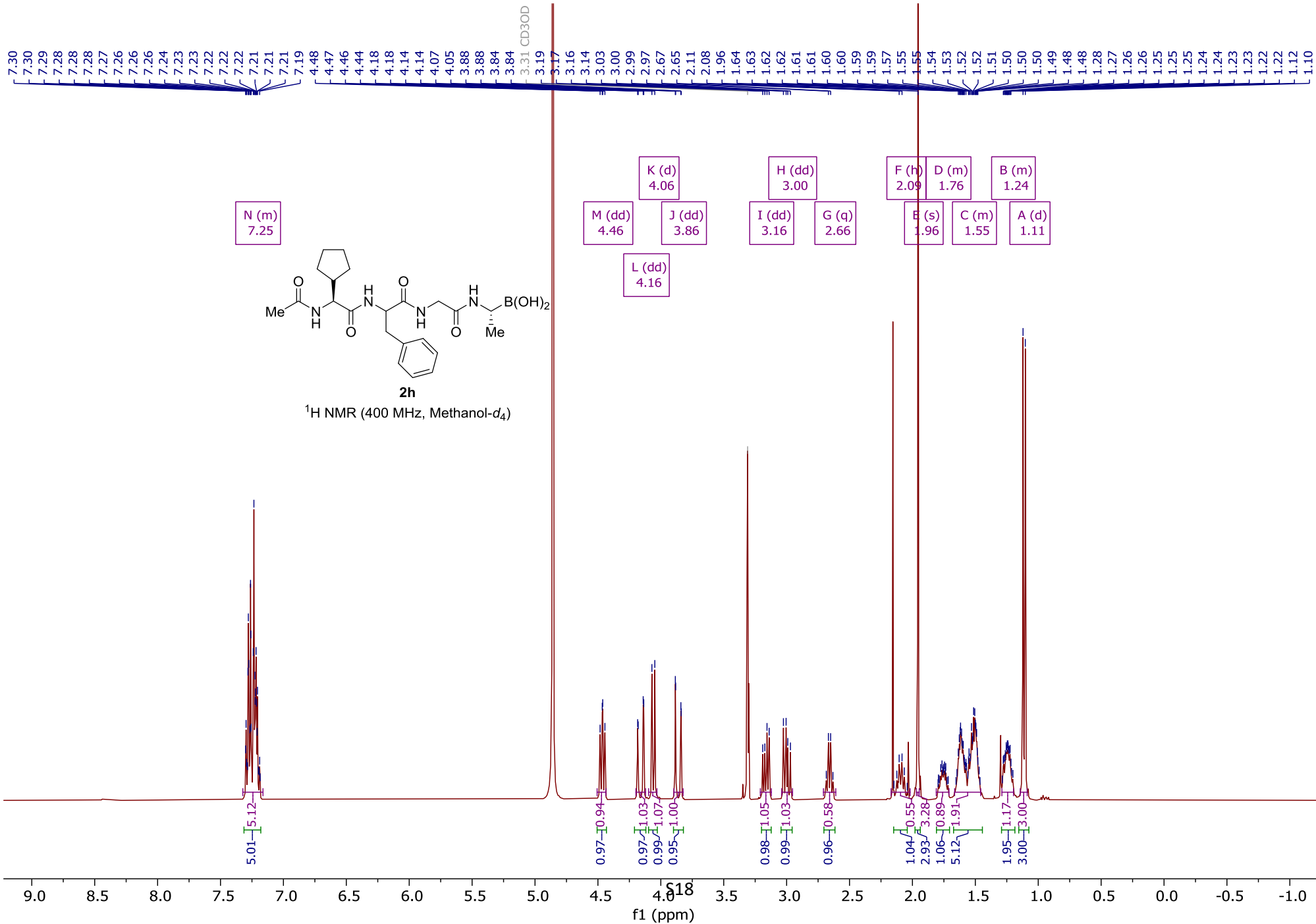
¹³C NMR (101 MHz, Methanol-d₄)

59.1
52.9
49.0 CD3OD
45.5
43.0
41.8
39.8
31.3
30.5
30.2
30.0
26.3
25.9
22.3
16.0









176.2
174.8
174.2
173.6

138.2

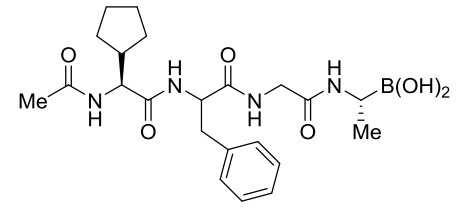
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56.8

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37.8

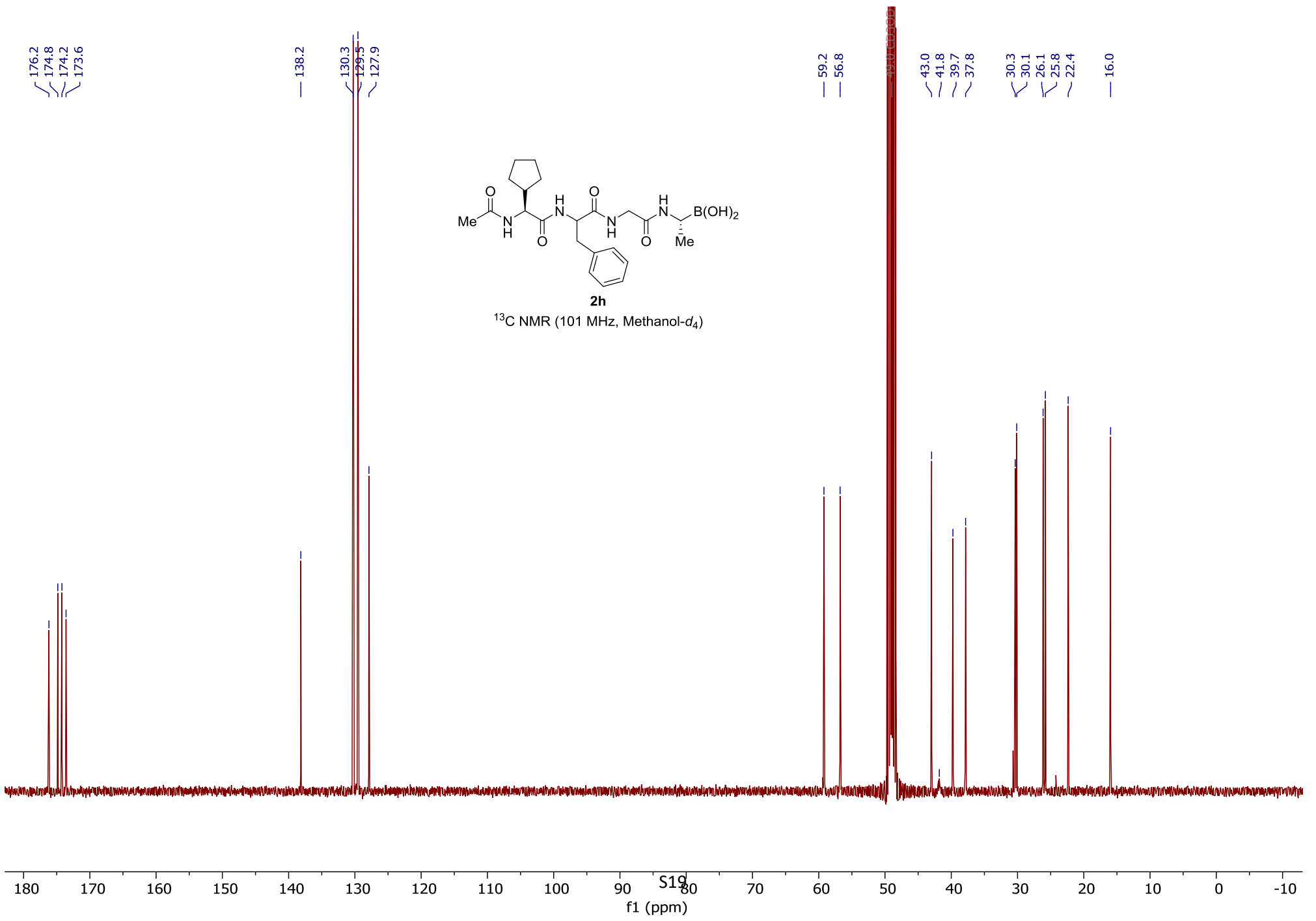
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22.4

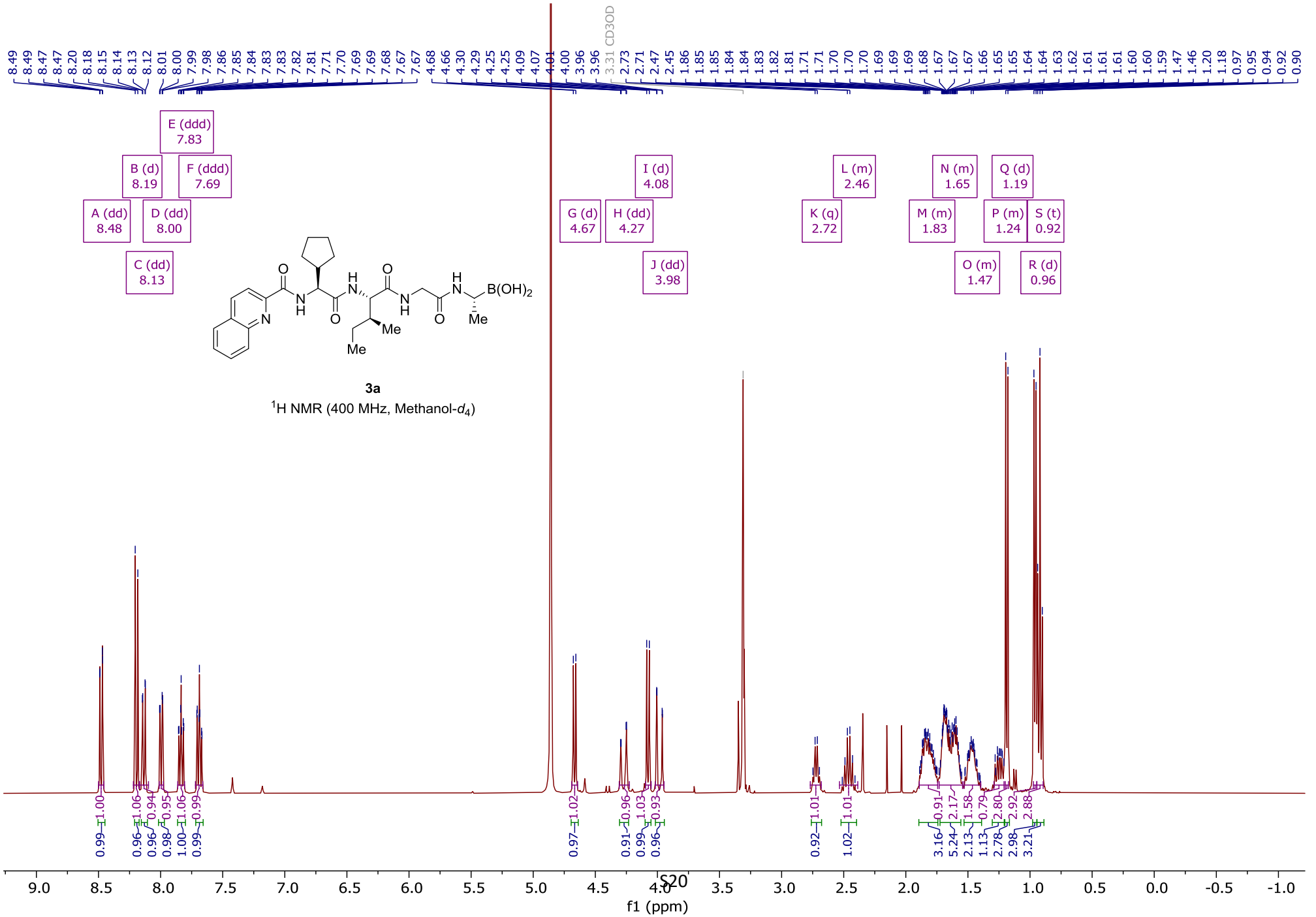
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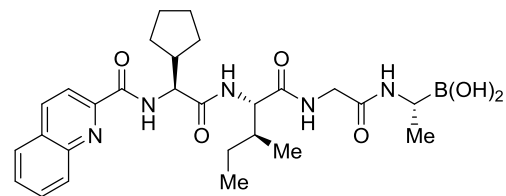


2h

¹³C NMR (101 MHz, Methanol-d₄)

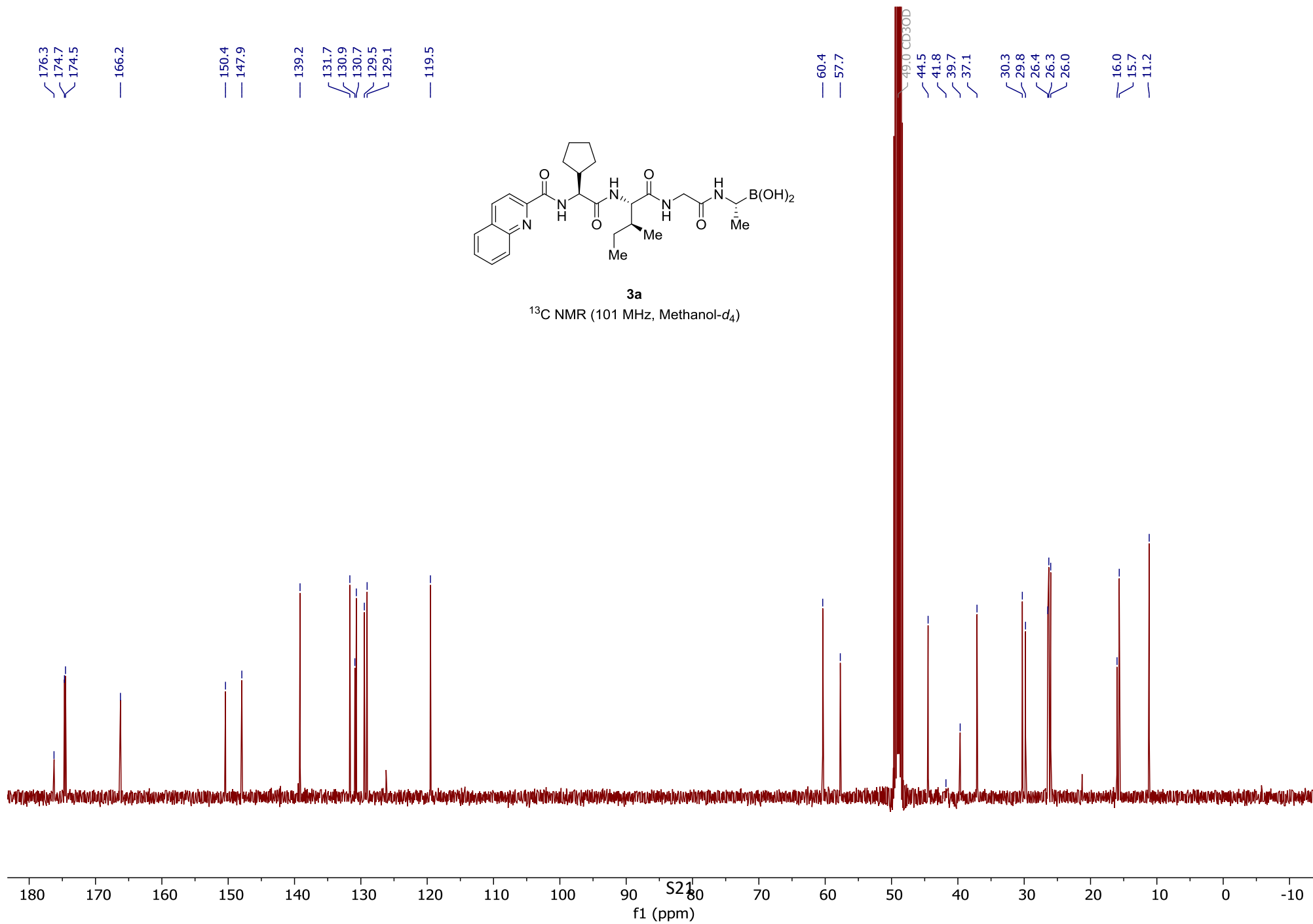


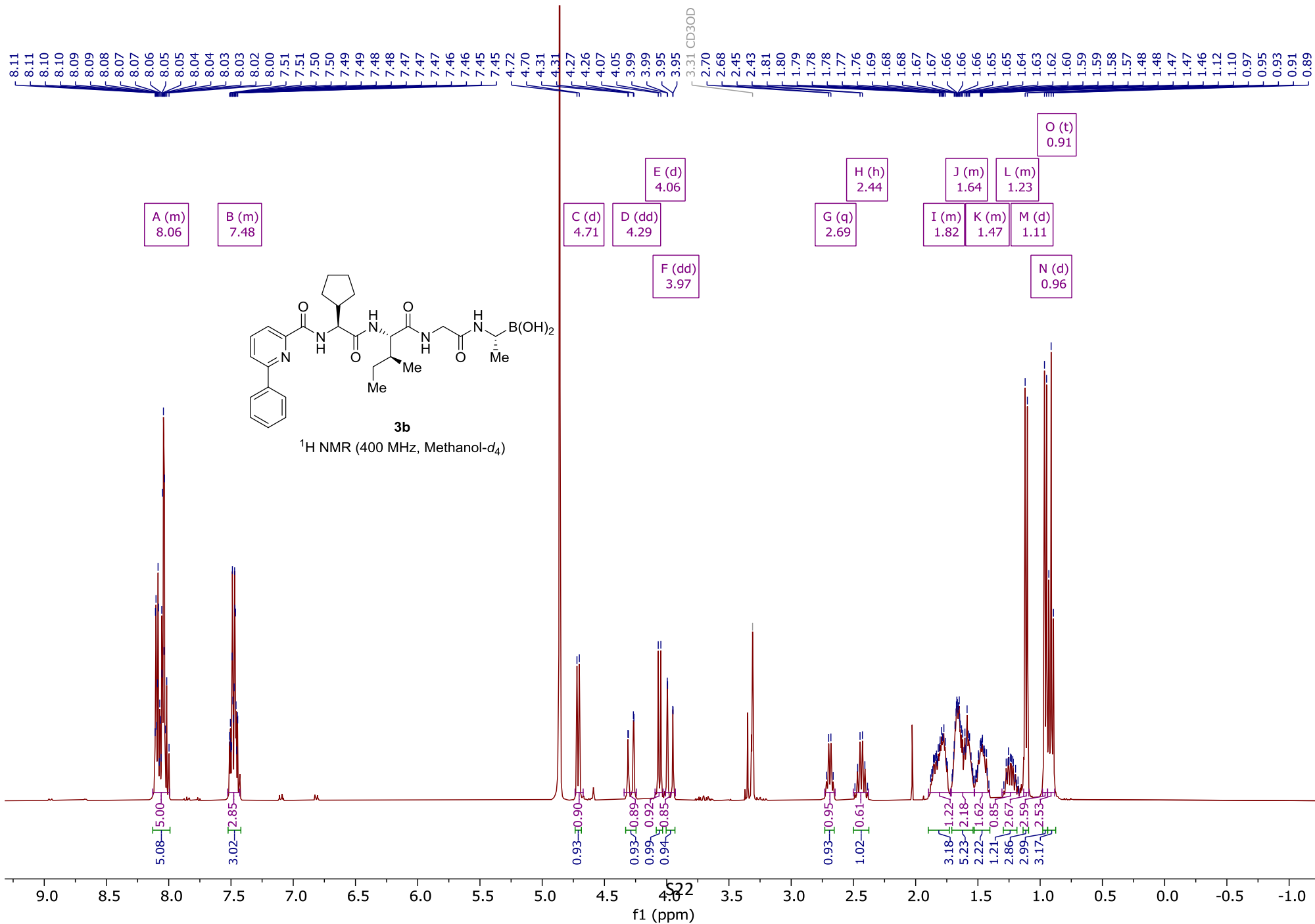


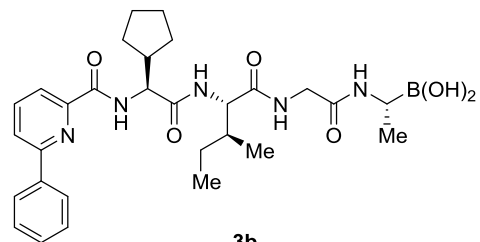


3a

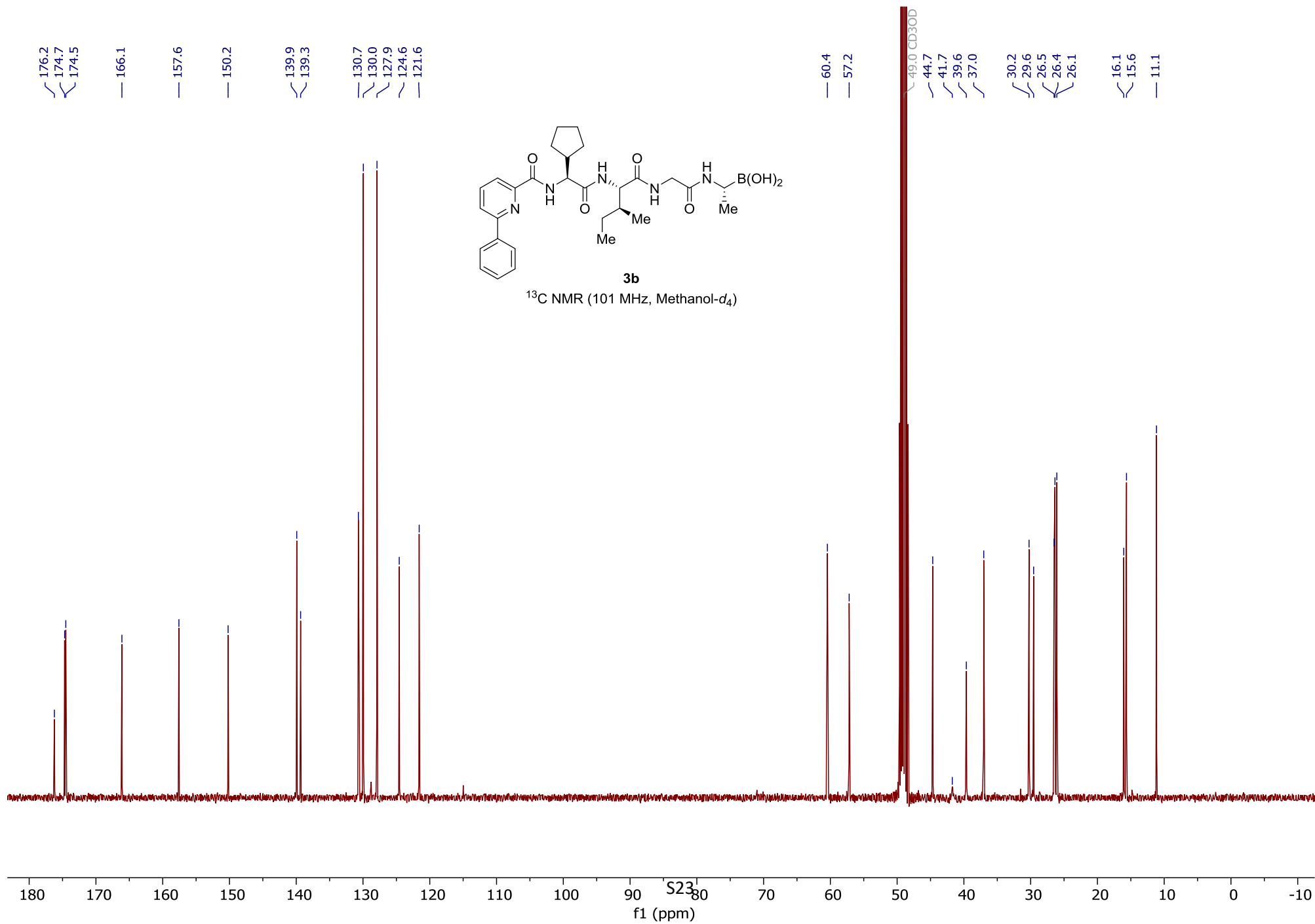
¹³C NMR (101 MHz, Methanol-*d*₄)

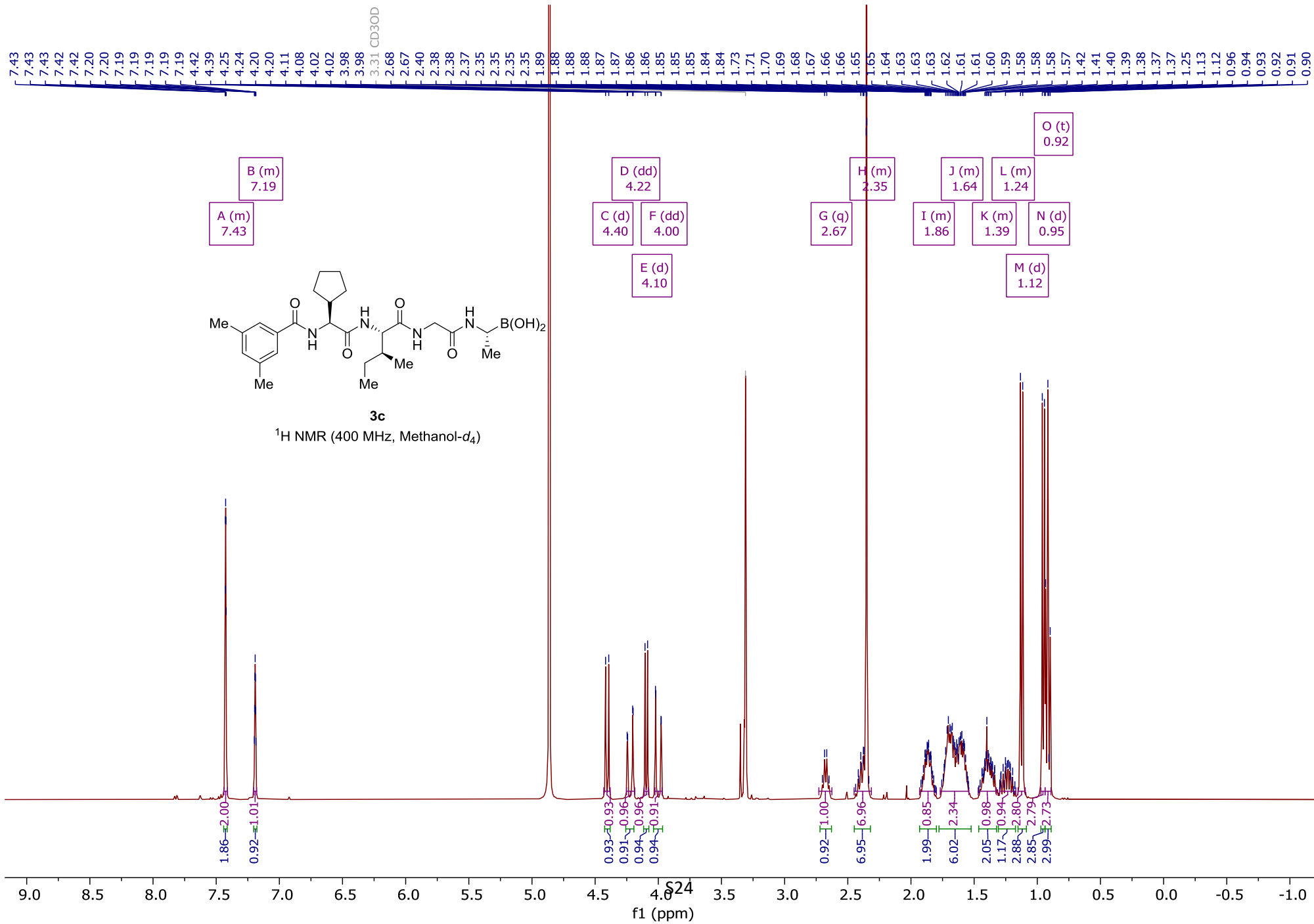






¹³C NMR (101 MHz, Methanol-d₄)





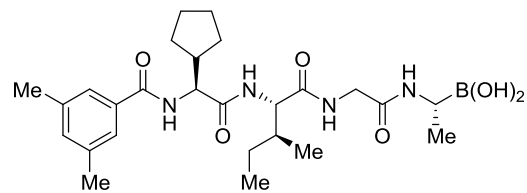
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175.0
174.5
170.8

139.4
135.3
134.3

126.2

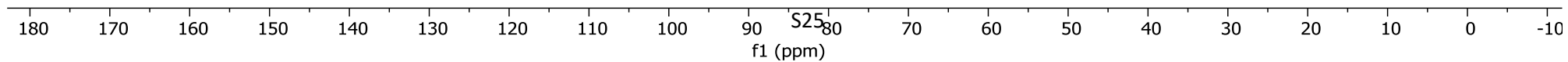
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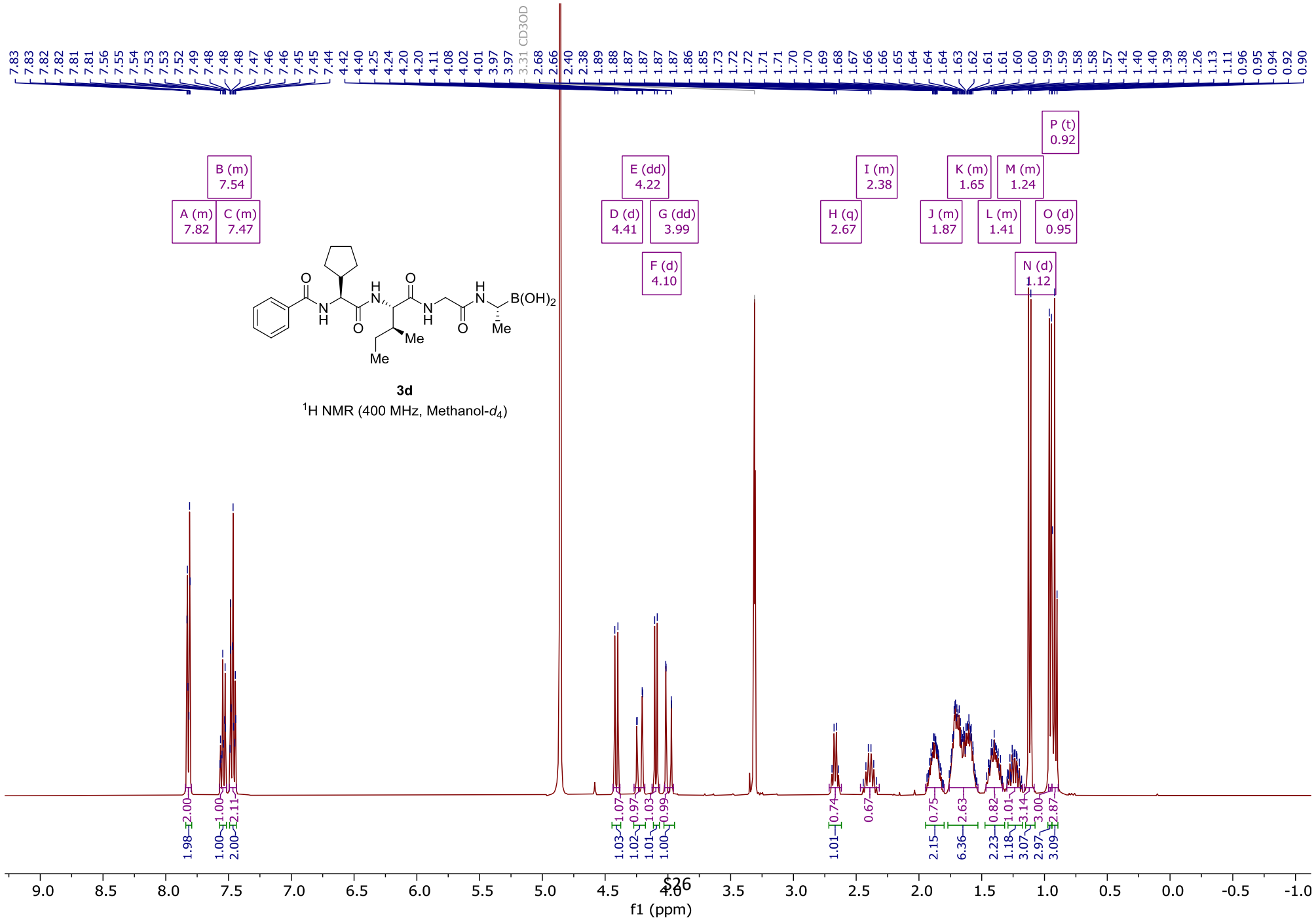
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41.9
39.6
37.4
30.7
30.5
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25.9
21.3
21.3
21.2
15.9
15.7
11.2

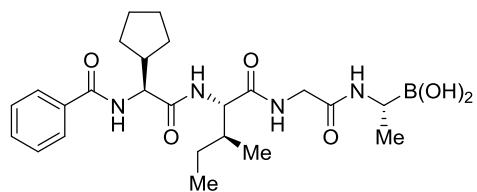


3c

¹³C NMR (101 MHz, Methanol-d₄)

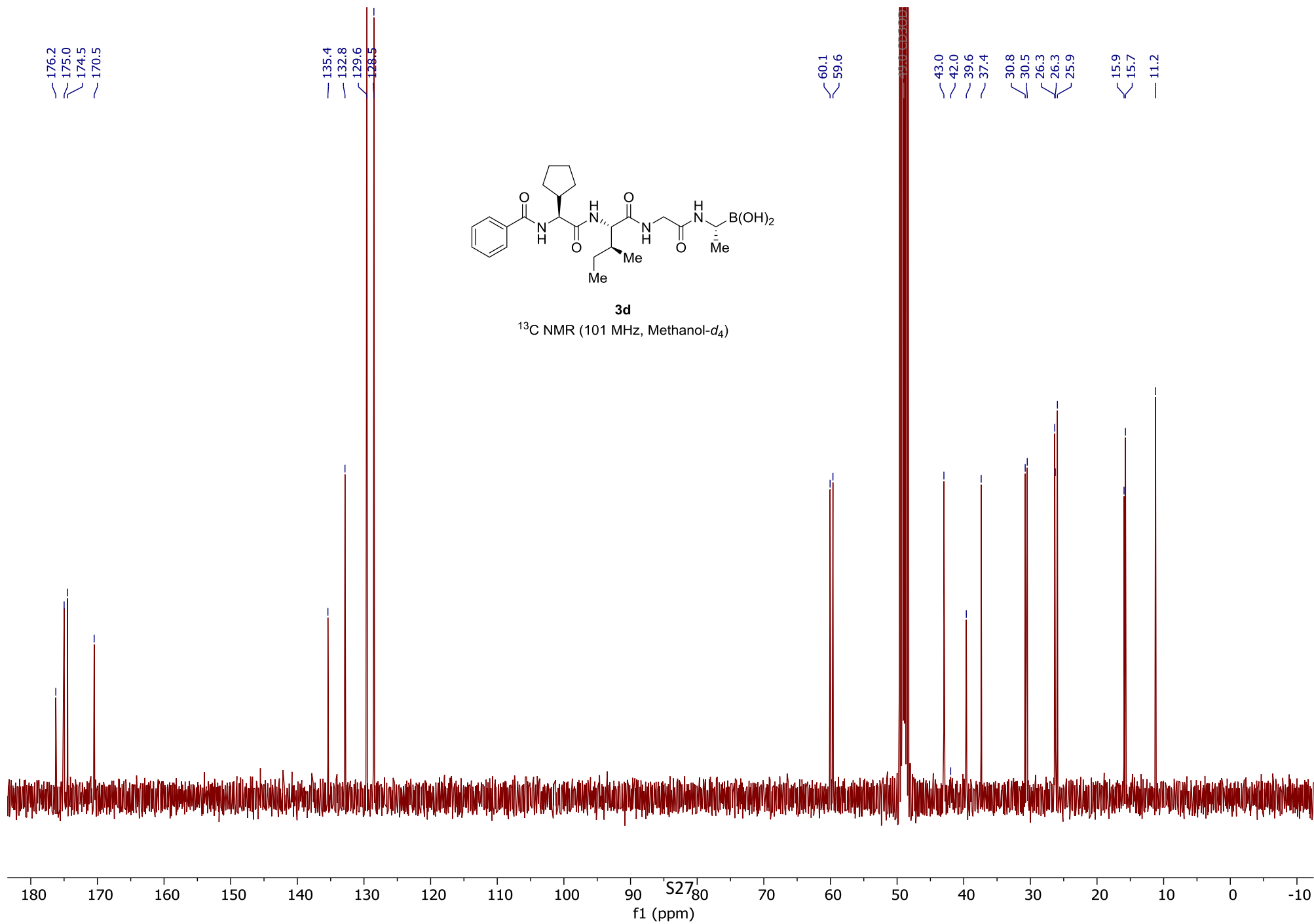


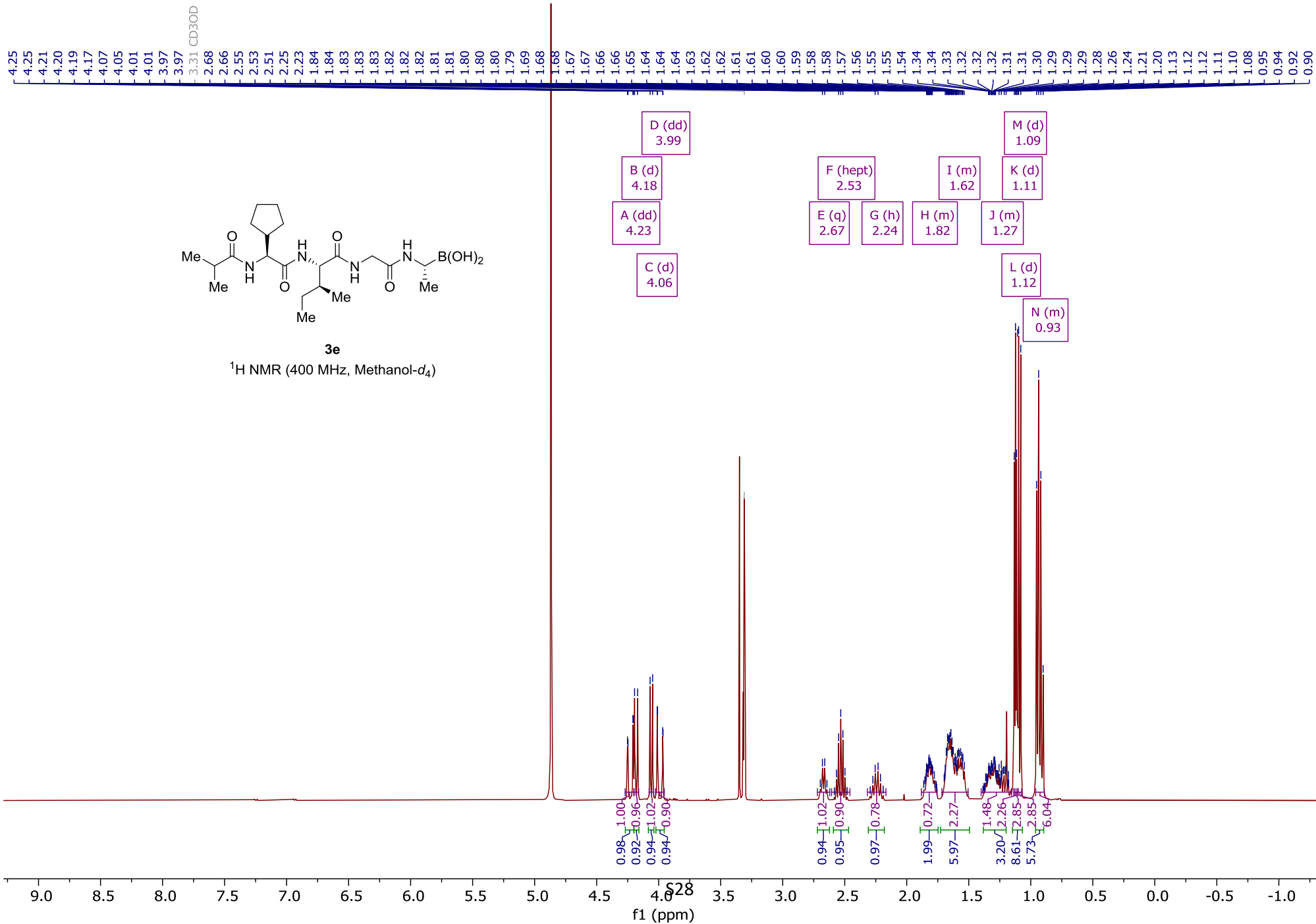




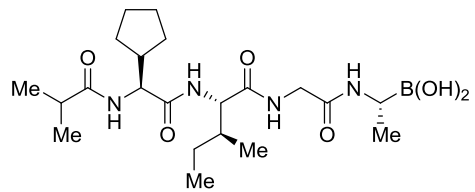
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^{13}C NMR (101 MHz, Methanol- d_4)





180.2
176.4
175.0
174.5

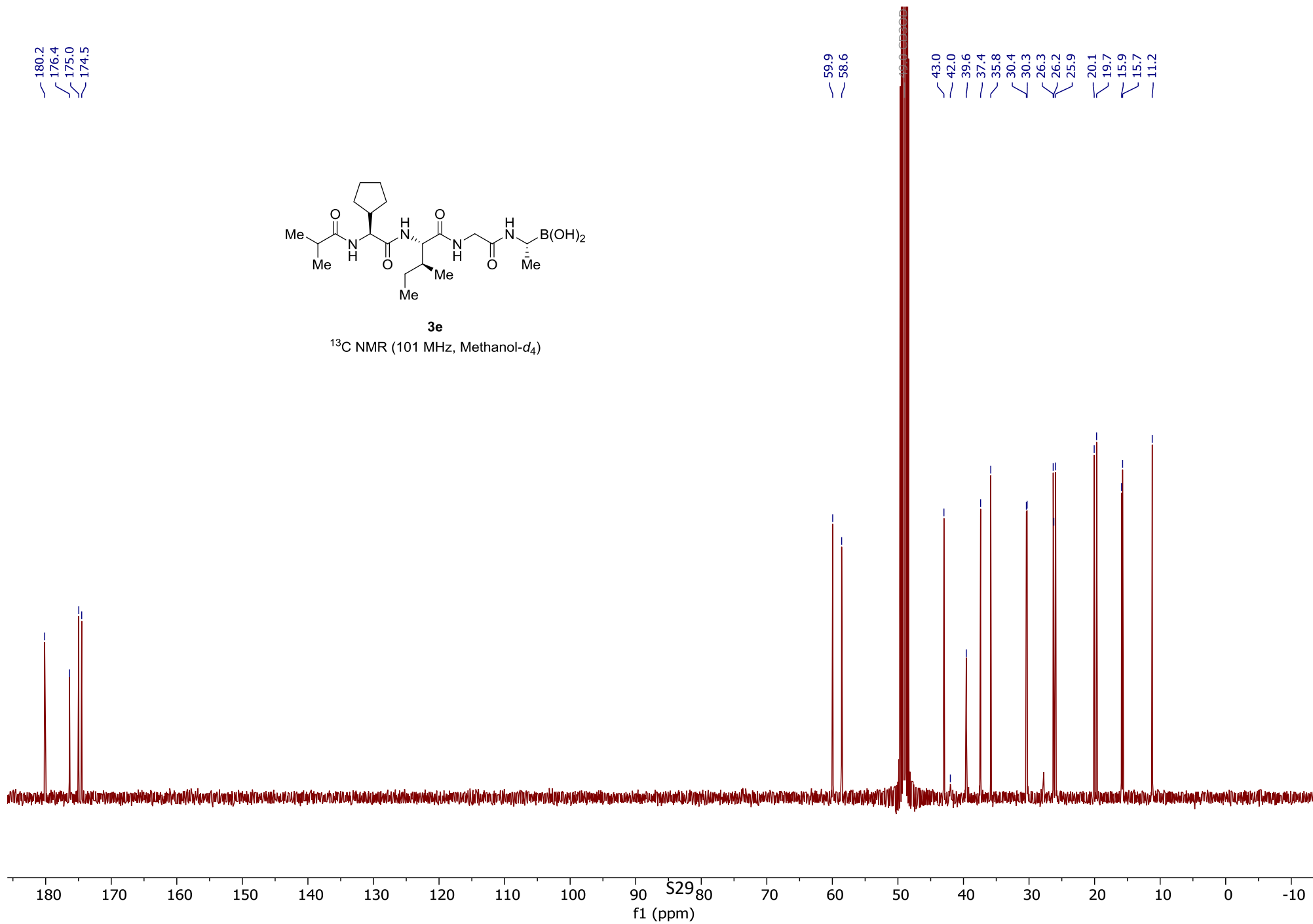


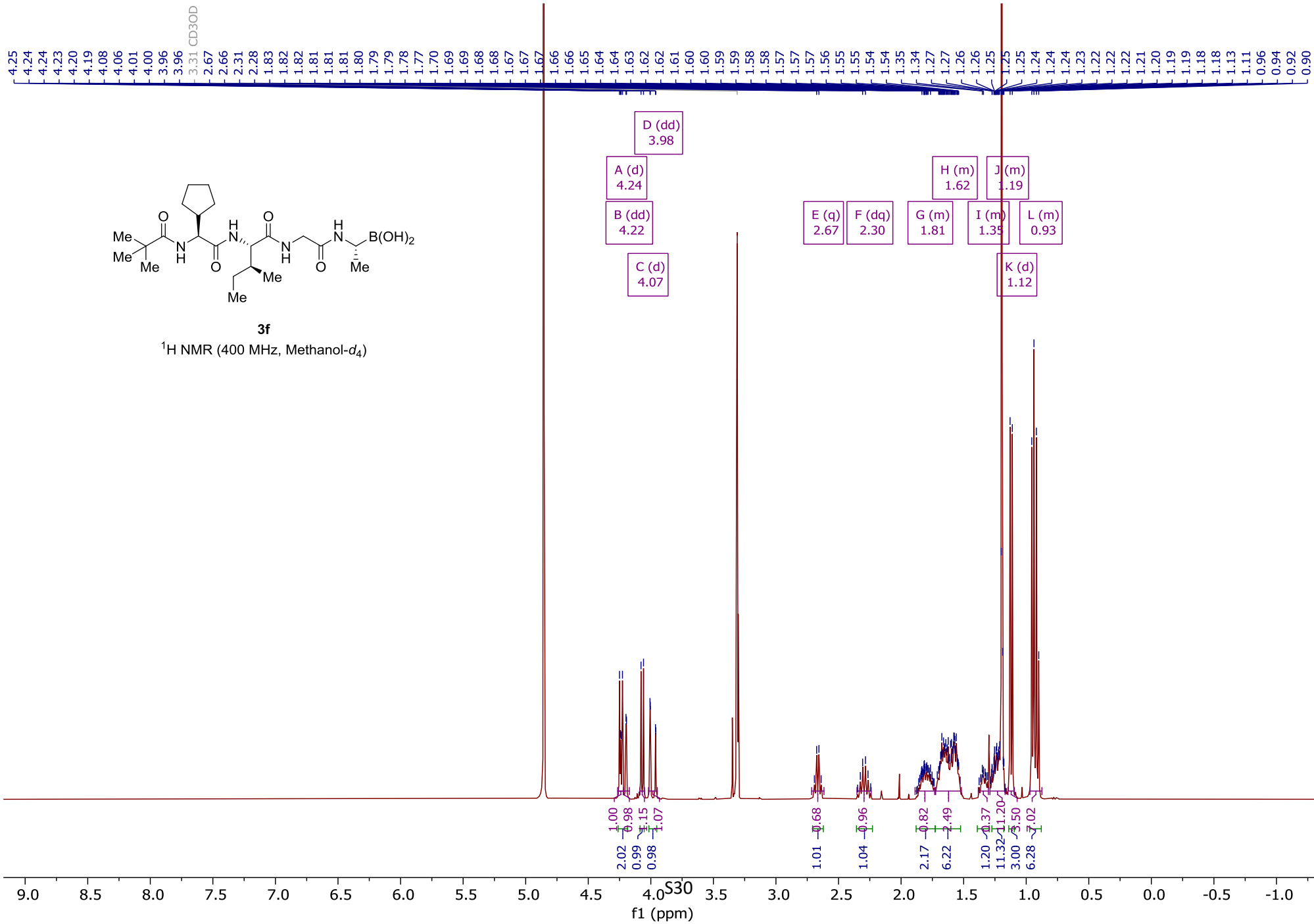
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^{13}C NMR (101 MHz, Methanol- d_4)

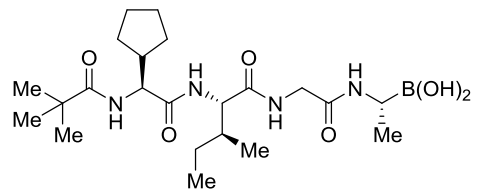
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58.6

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15.7
11.2





181.1
176.3
174.9
174.4



3f

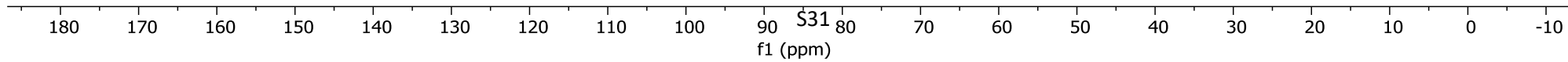
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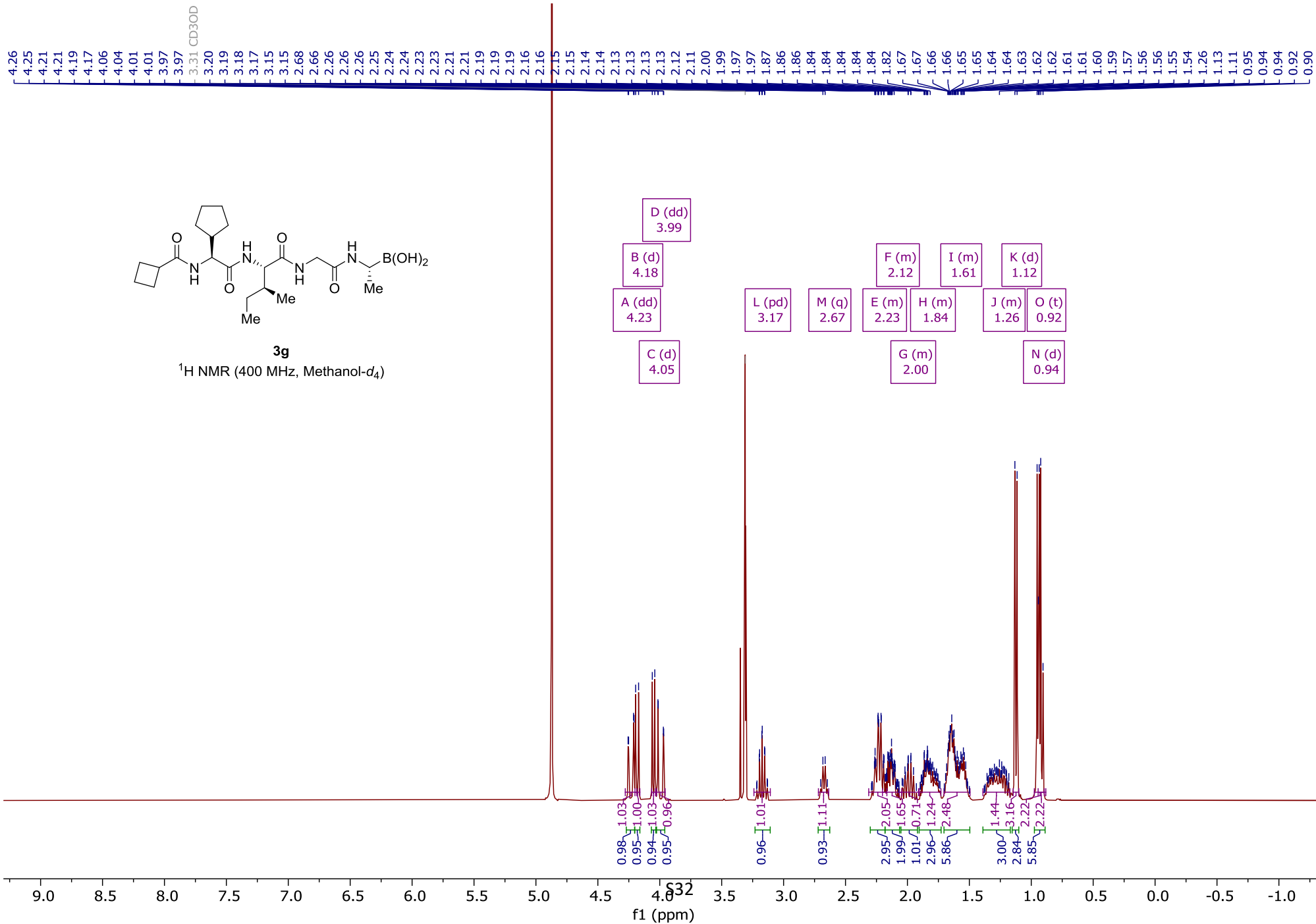
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58.6

49.0
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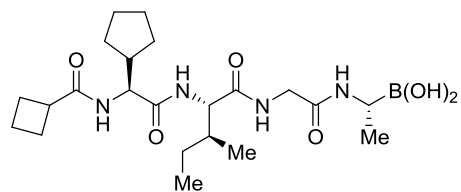
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39.8
39.6
37.5
30.4
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26.2
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15.9
15.7
11.2





177.7
176.4
175.0
174.5

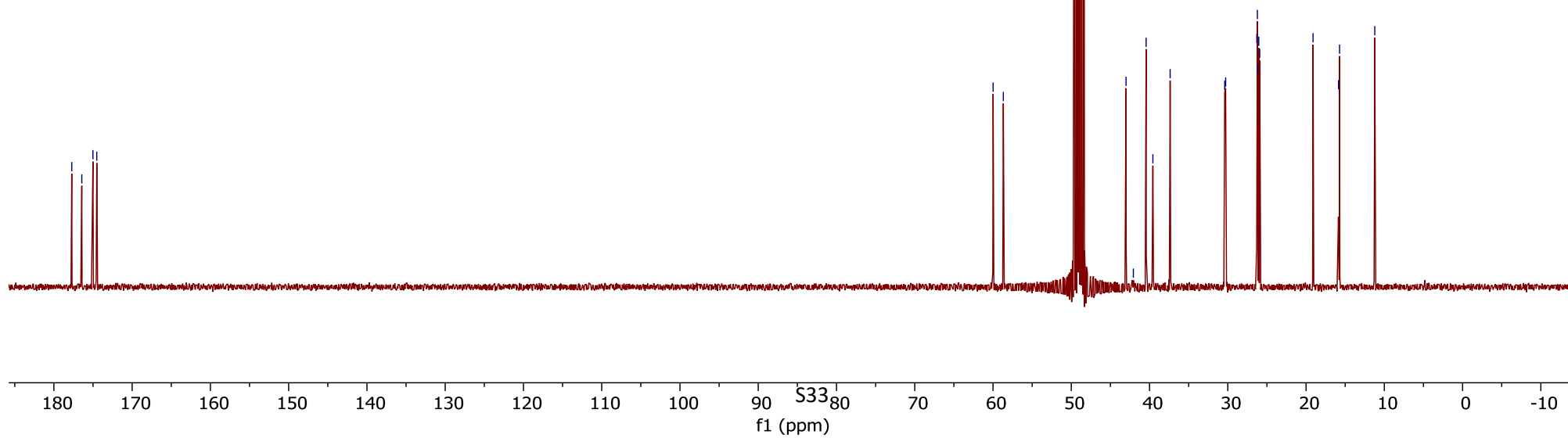


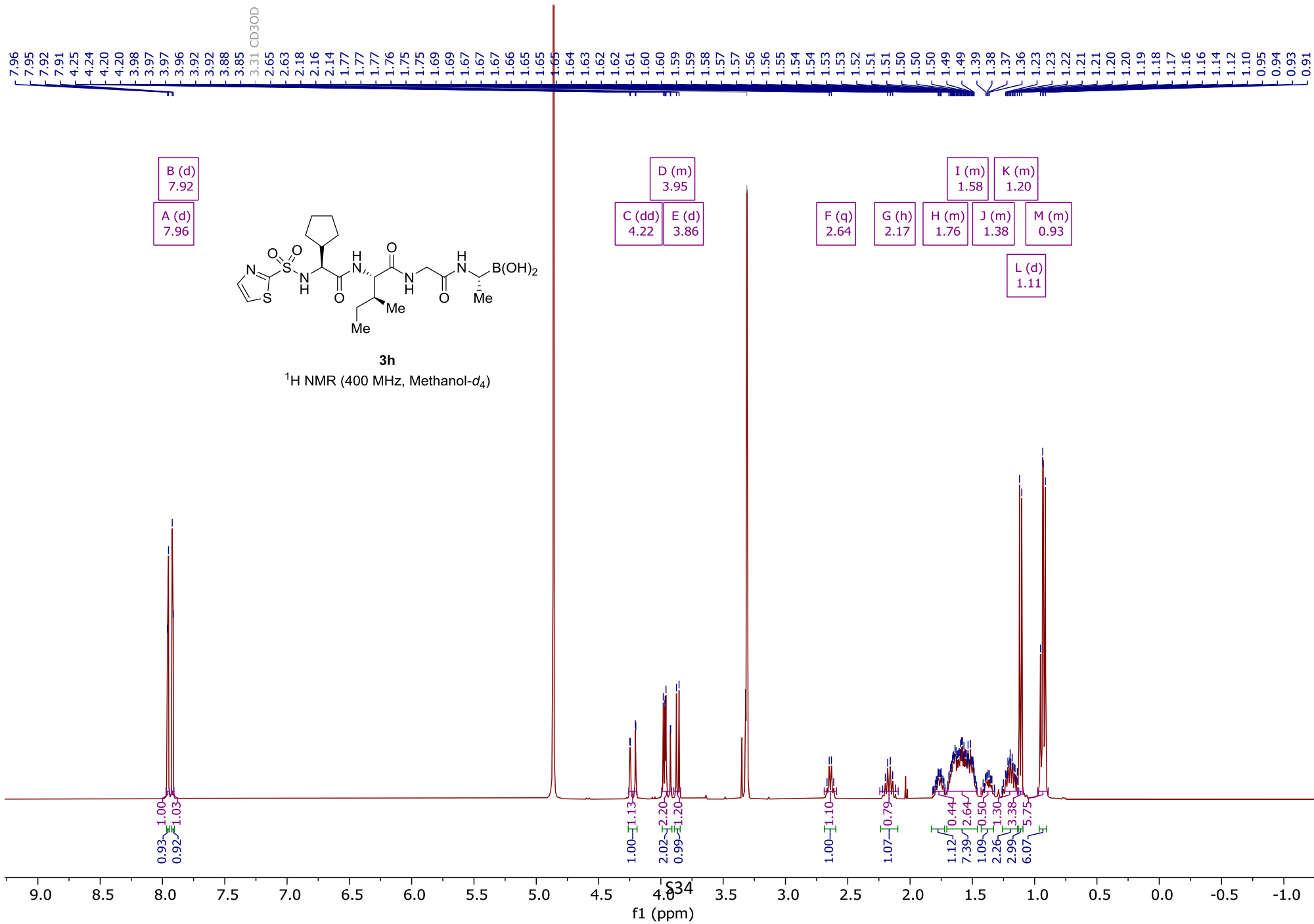
3g

¹³C NMR (101 MHz, Methanol-d₄)

60.0
58.7

43.0
42.1
40.4
39.6
37.4
30.4
30.3
26.3
26.2
26.0
25.9
19.1
15.9
15.7
11.2





176.3
174.4
174.1
168.0

145.1

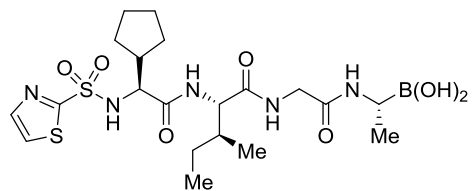
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61.6
60.3

44.1
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39.6
37.1

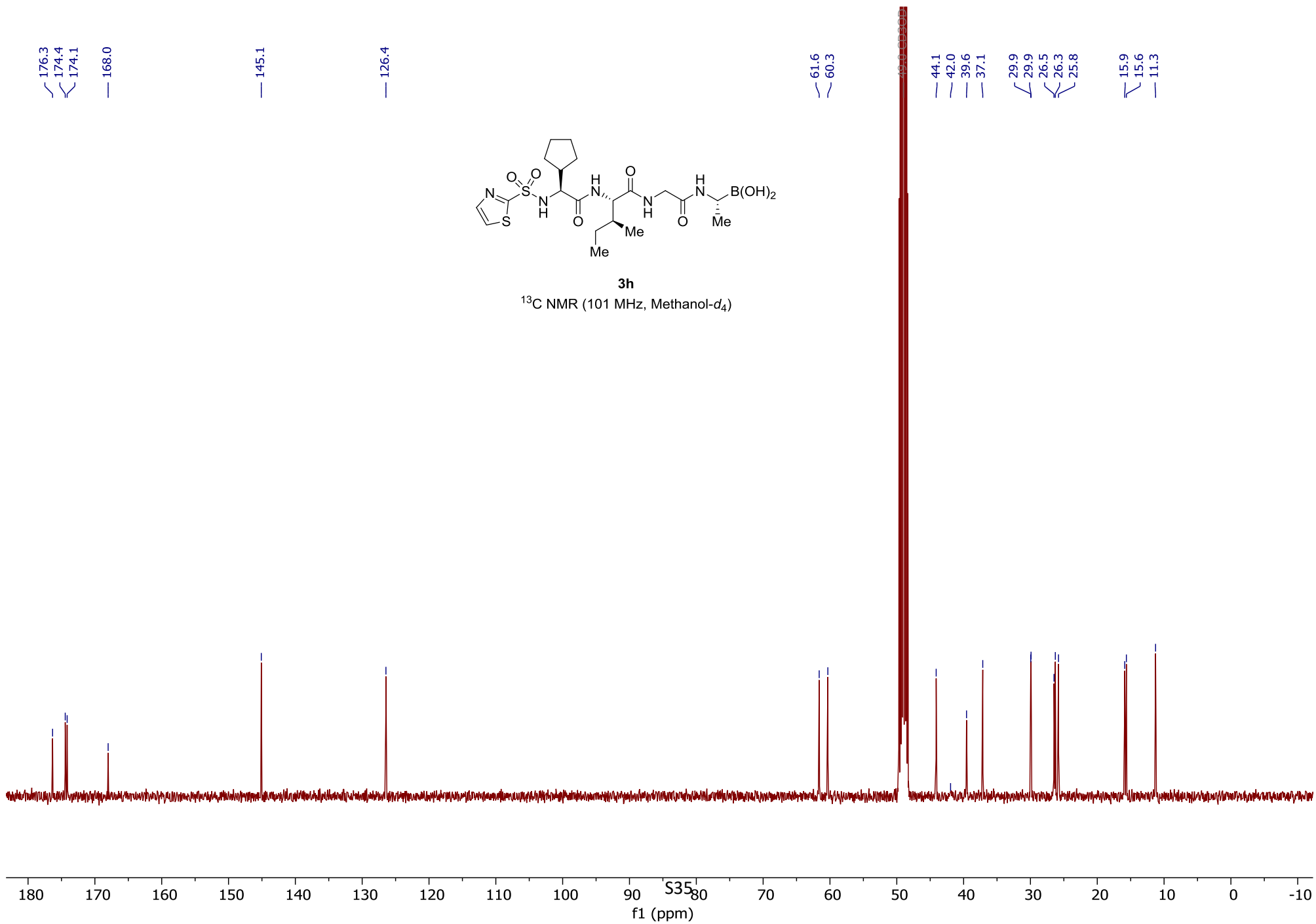
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29.9
26.5
26.3
25.8

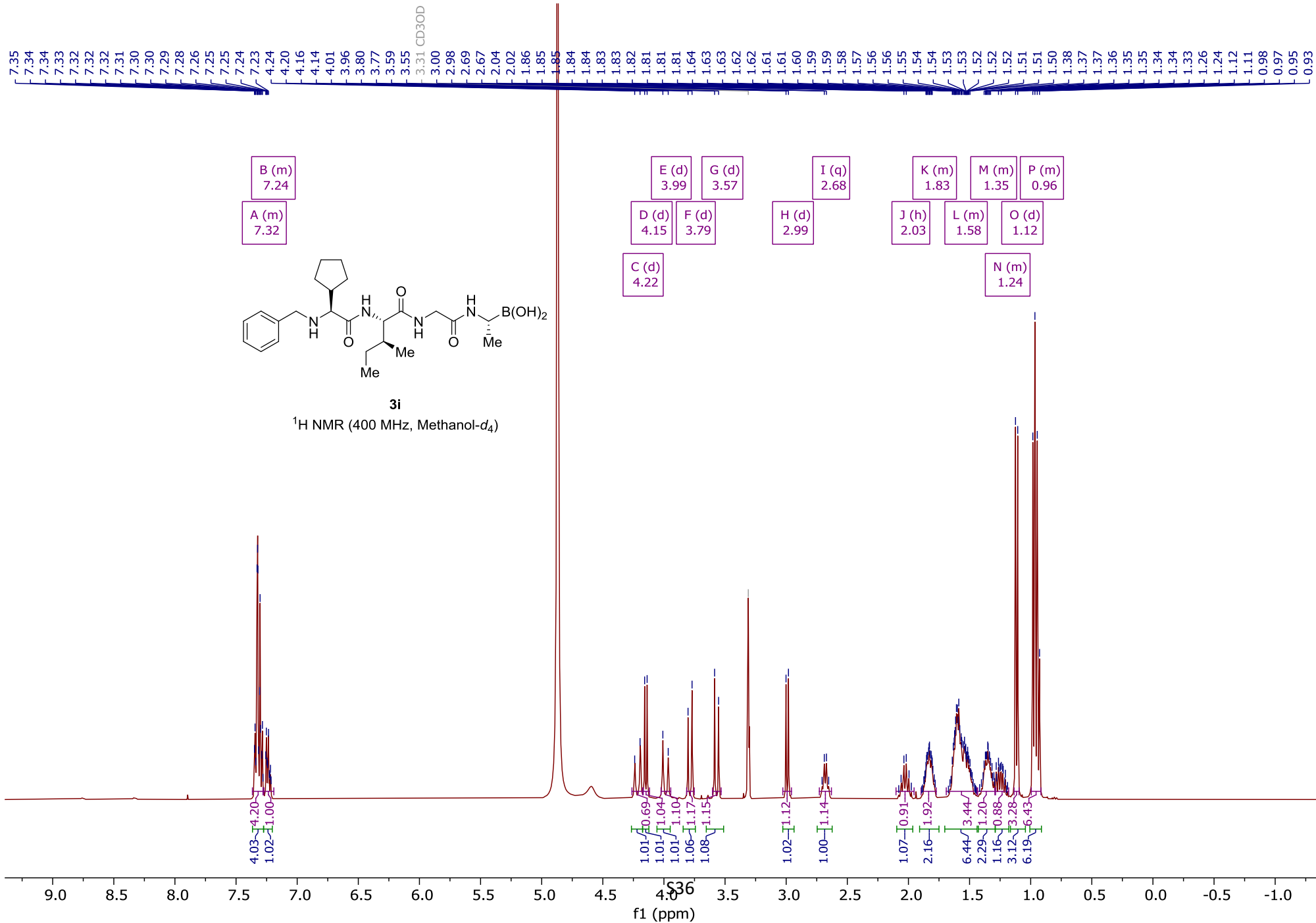
15.9
15.6
11.3



3h

¹³C NMR (101 MHz, Methanol-d₄)





177.6
176.0
174.5

140.9

129.5
129.4
128.2

66.9

59.4

53.0

49.0

45.0

41.7

39.8

37.4

30.6

30.1

26.3

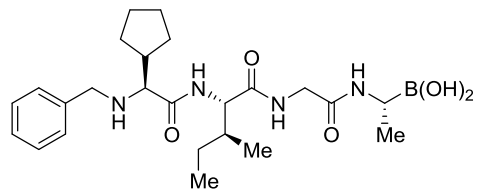
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26.0

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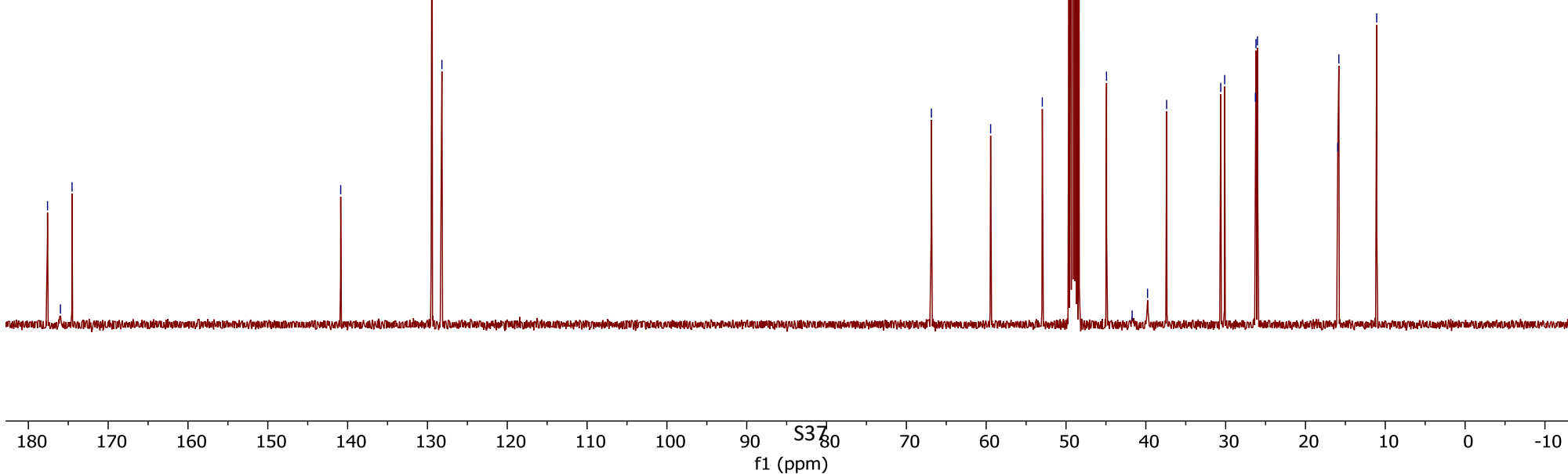
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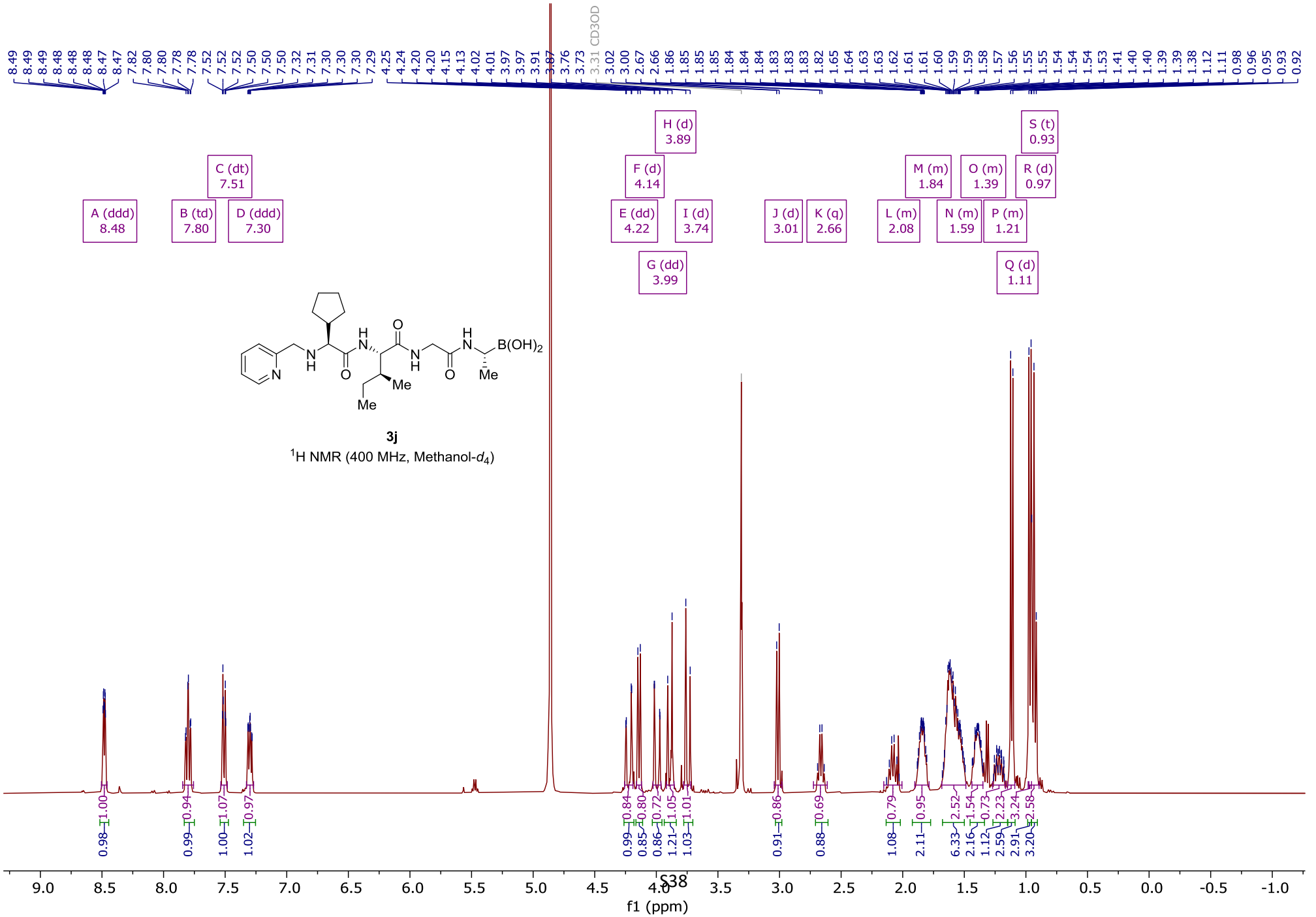
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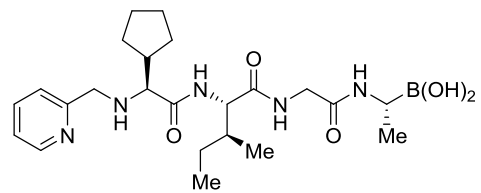


3i

¹³C NMR (101 MHz, Methanol-d₄)

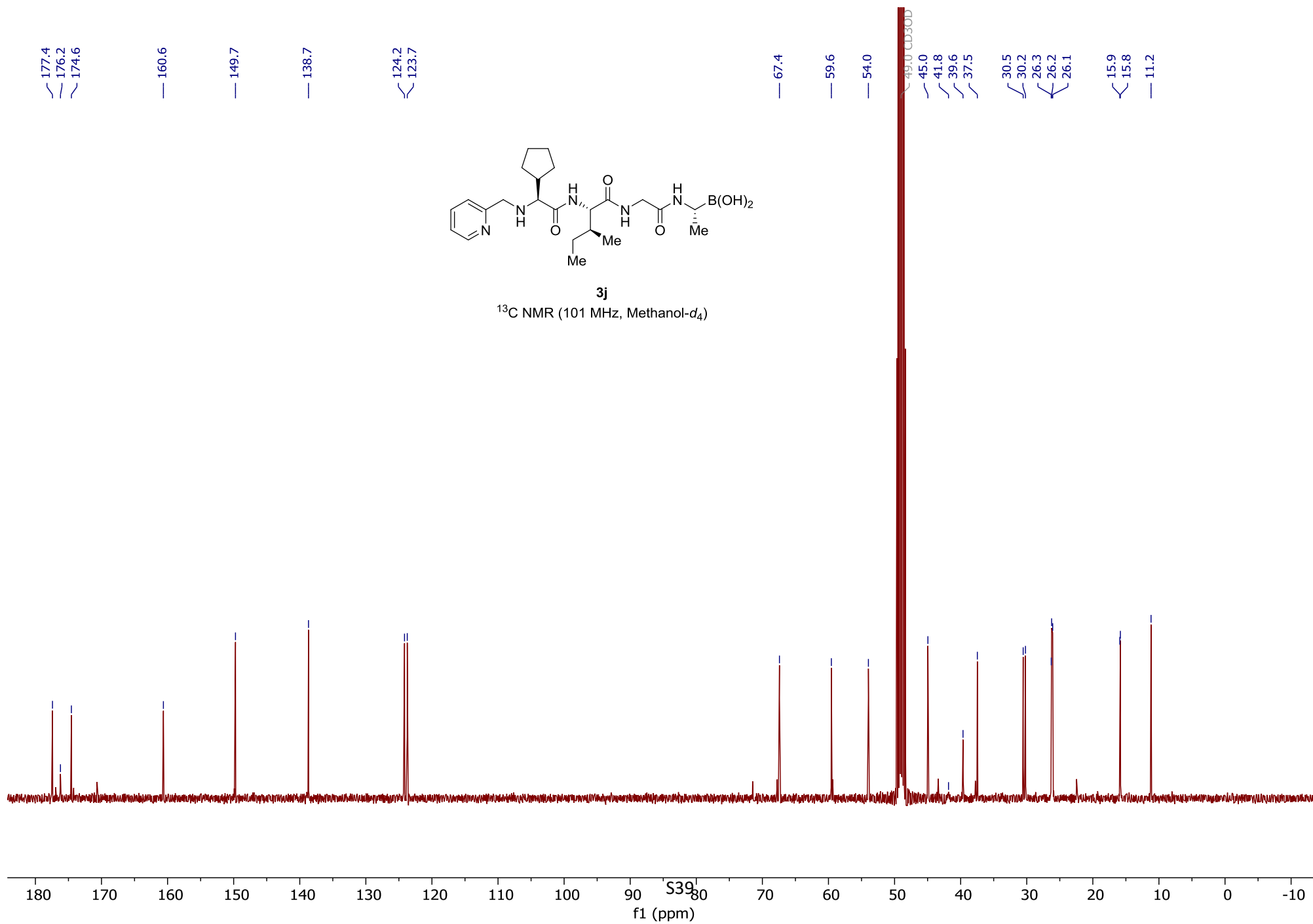


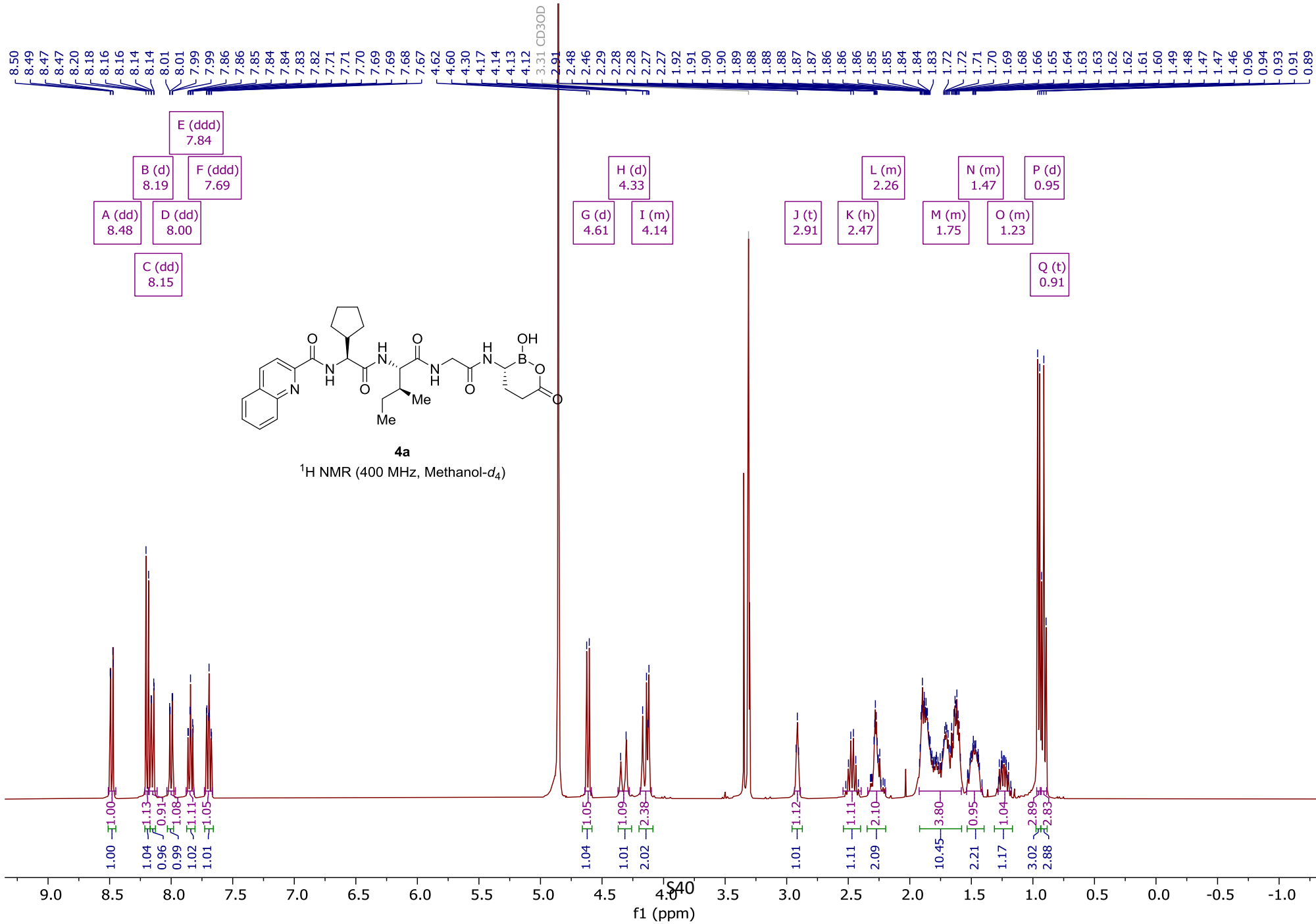




3j

¹³C NMR (101 MHz, Methanol-*d*₄)





179.8
176.7
174.7
174.5

166.4

150.4
147.9

139.2

131.7
130.9
130.7
129.5
129.1

119.5

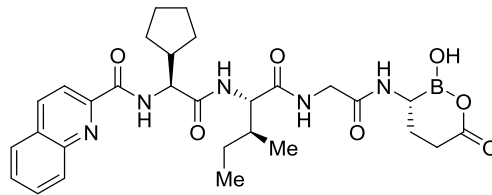
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58.2

44.1
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39.3
37.3

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30.1
29.2
26.3
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25.6

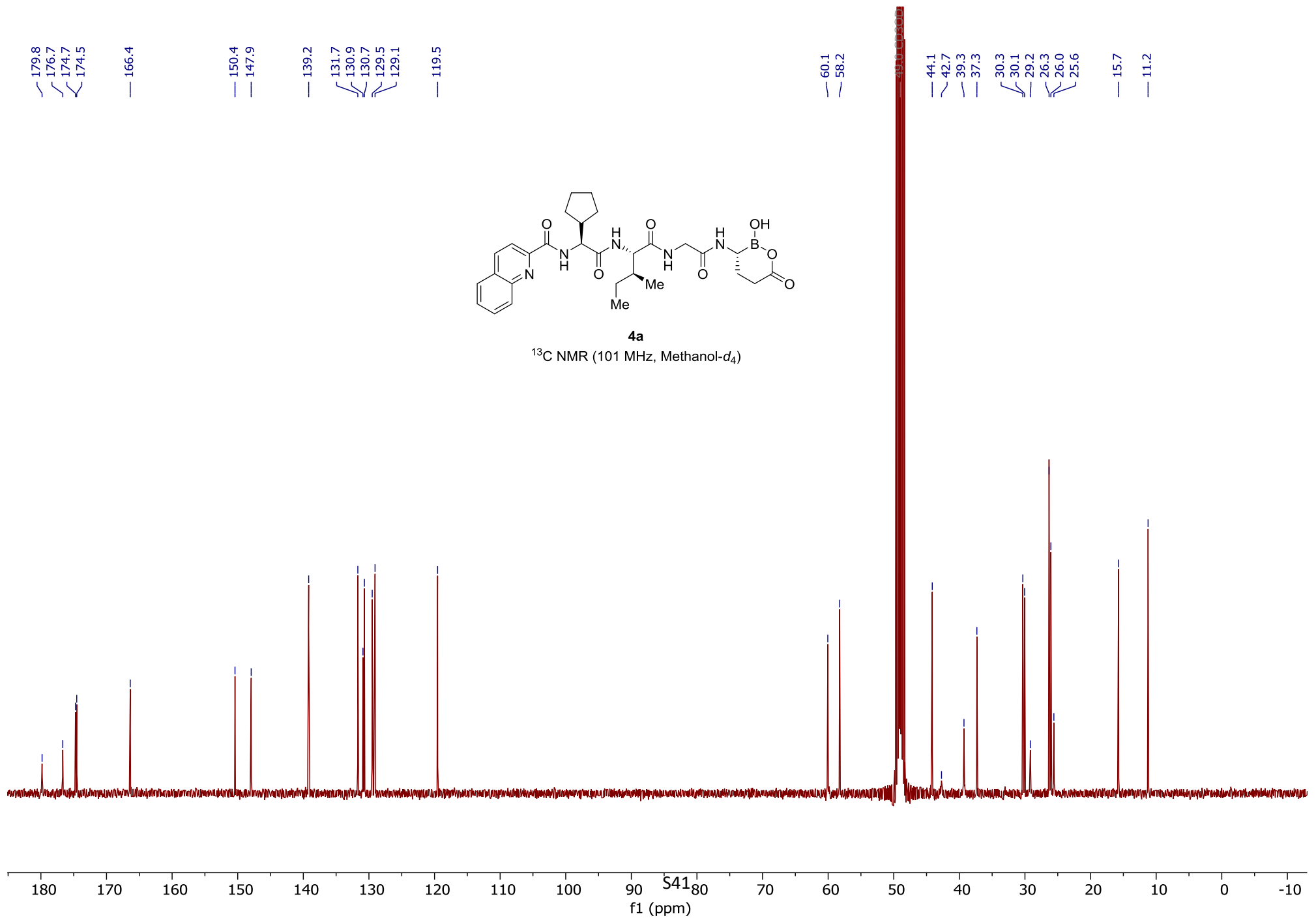
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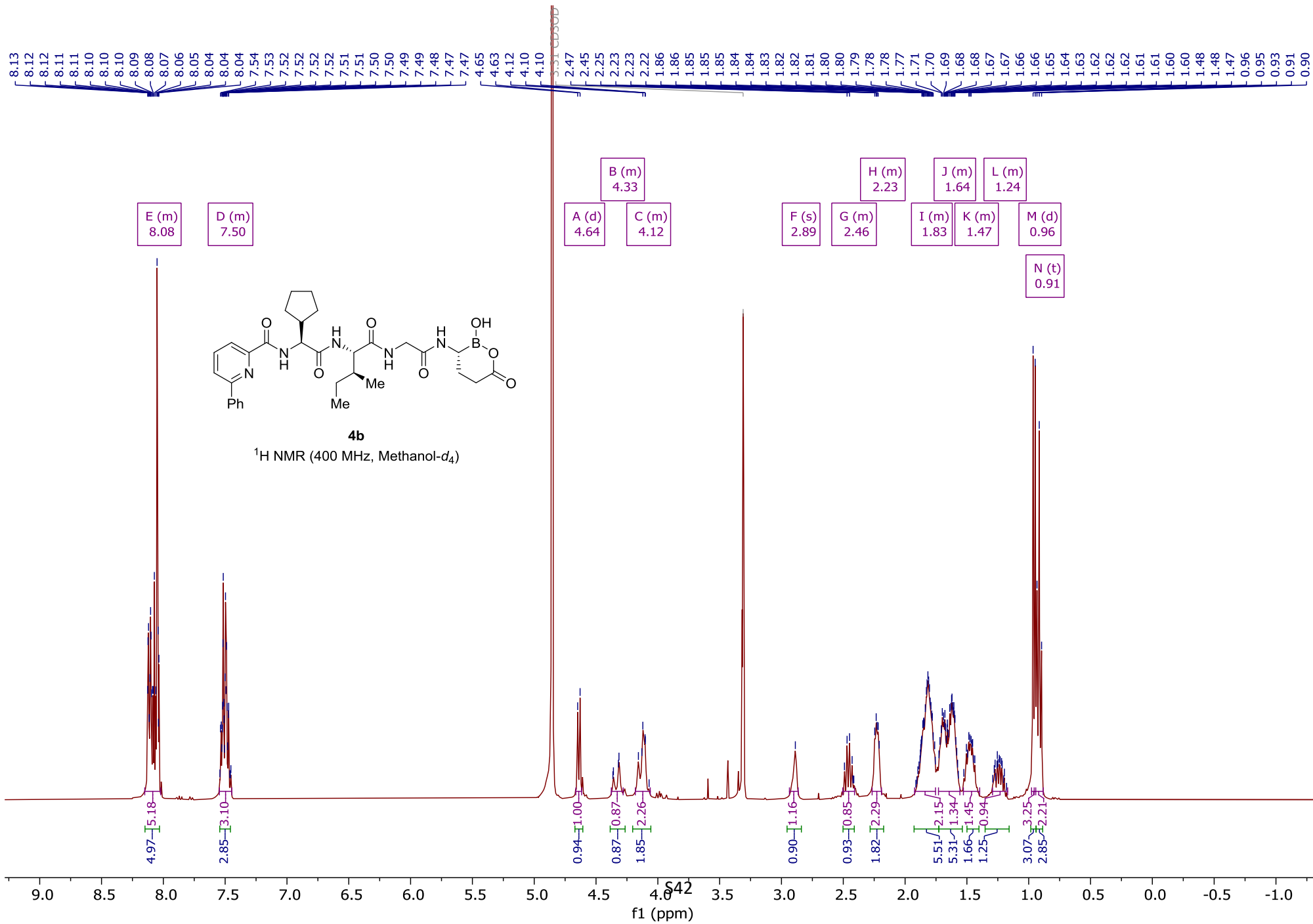
11.2



4a

¹³C NMR (101 MHz, Methanol-d₄)





179.8
176.6
174.8
174.5

166.3

157.7

150.2

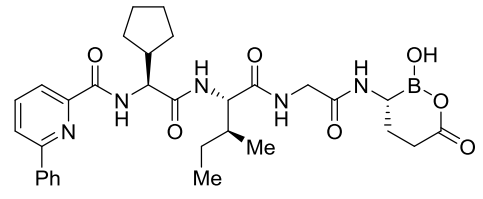
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124.7
121.6

60.1
57.7

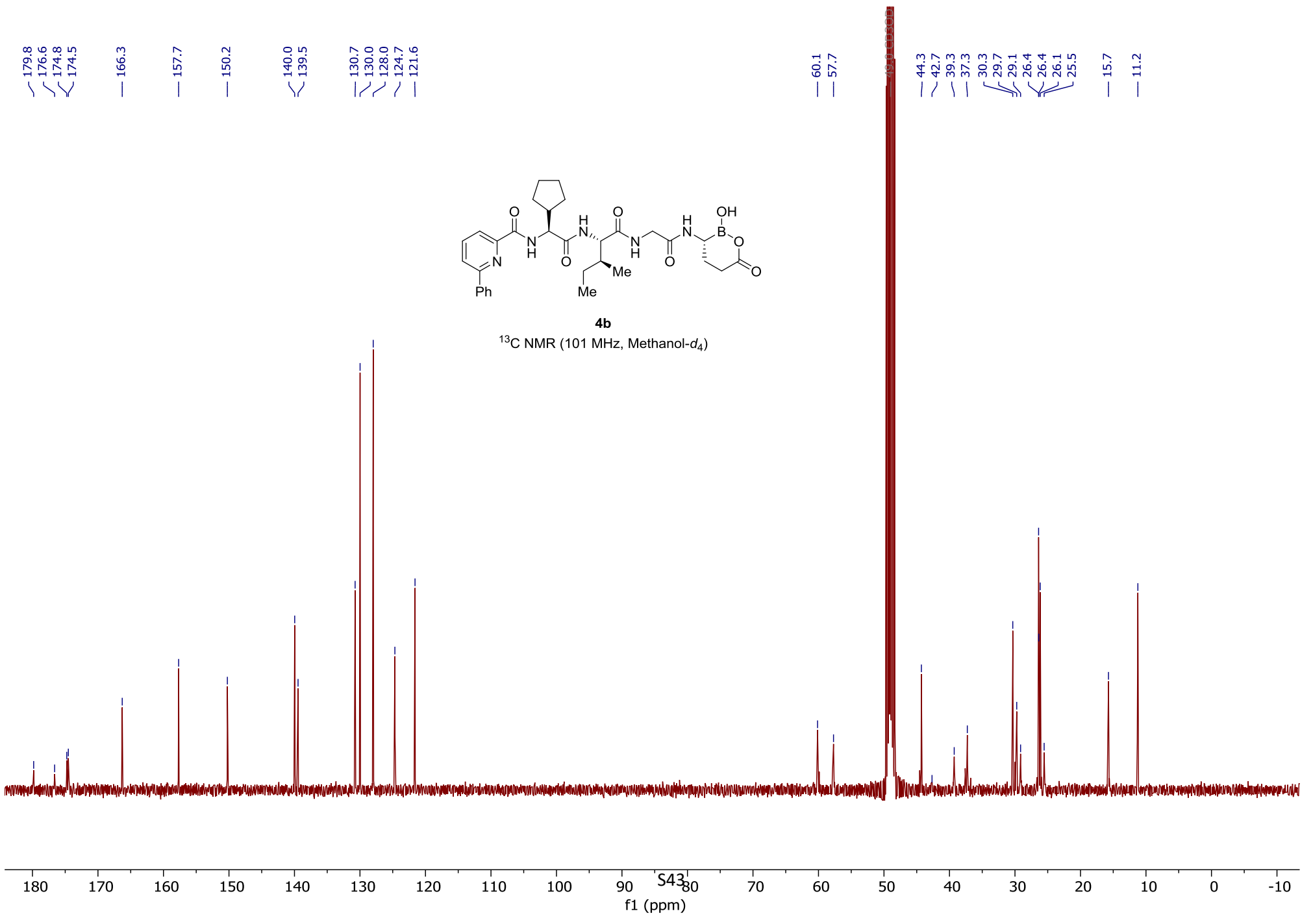
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29.1
26.4
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25.5

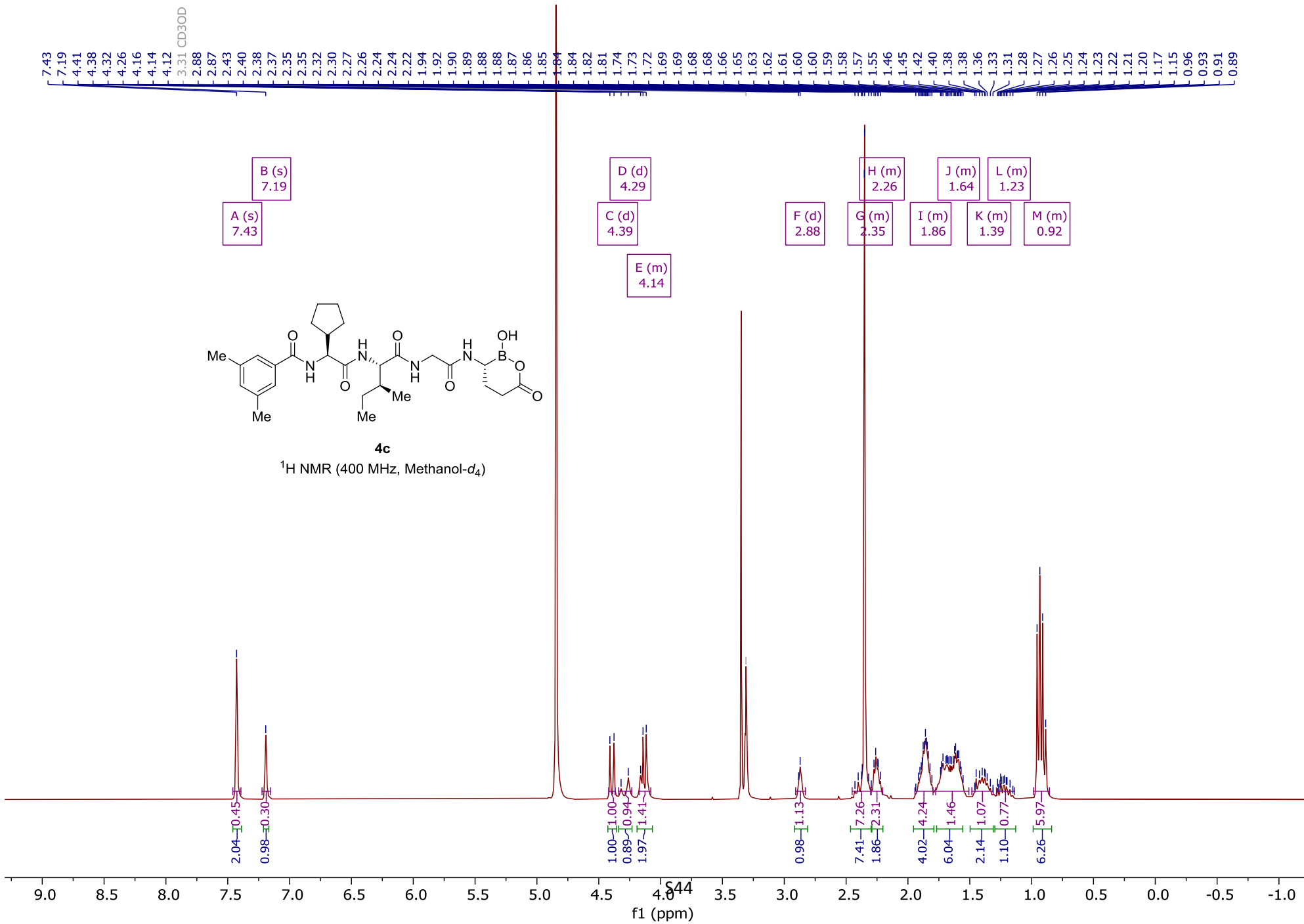
15.7
11.2



4b

¹³C NMR (101 MHz, Methanol-d₄)





179.8
176.7
175.1
174.6
170.9

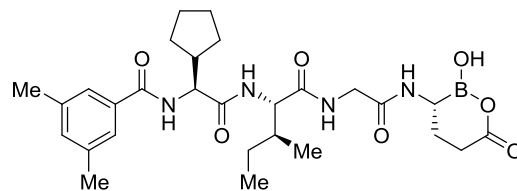
139.5
135.3
134.3

126.2

59.9
59.7

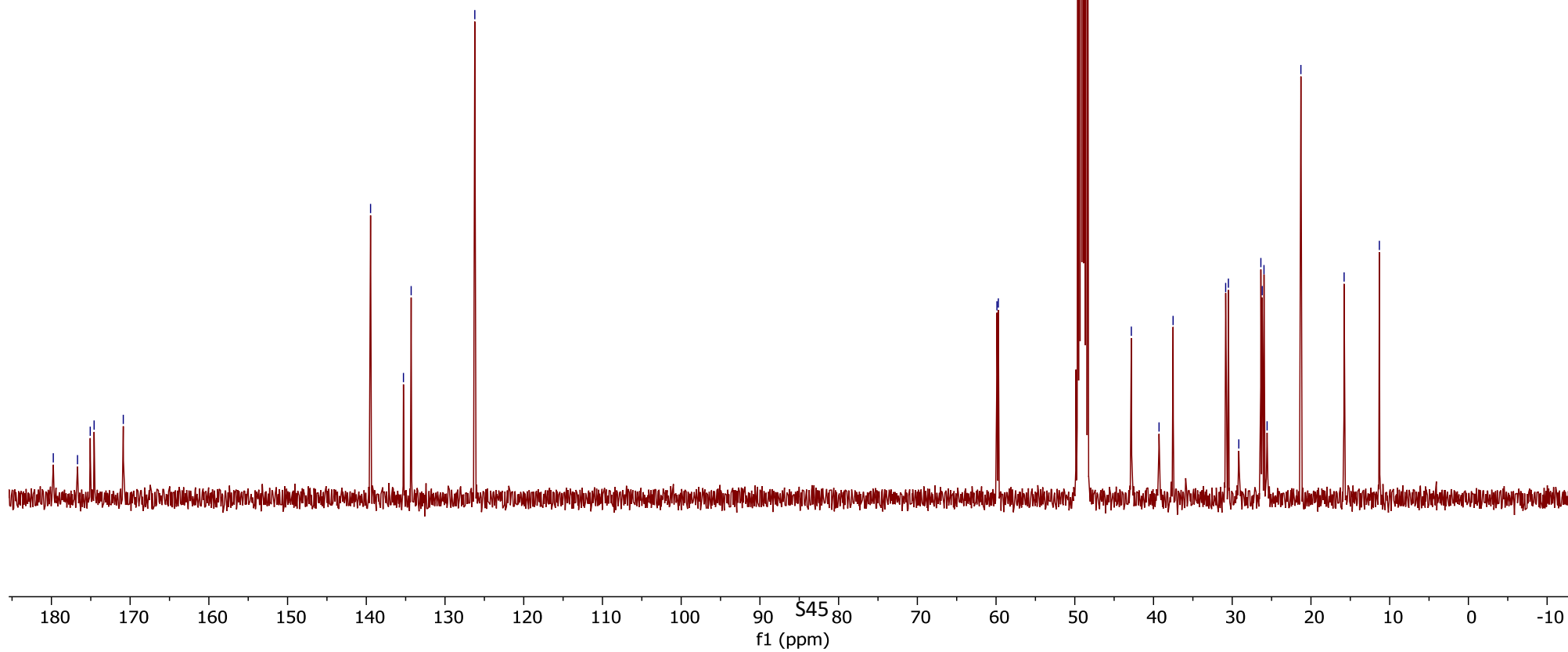
42.8
39.3
37.5

30.8
30.5
29.2
26.4
26.2
26.0
25.6
21.3
15.8
11.3



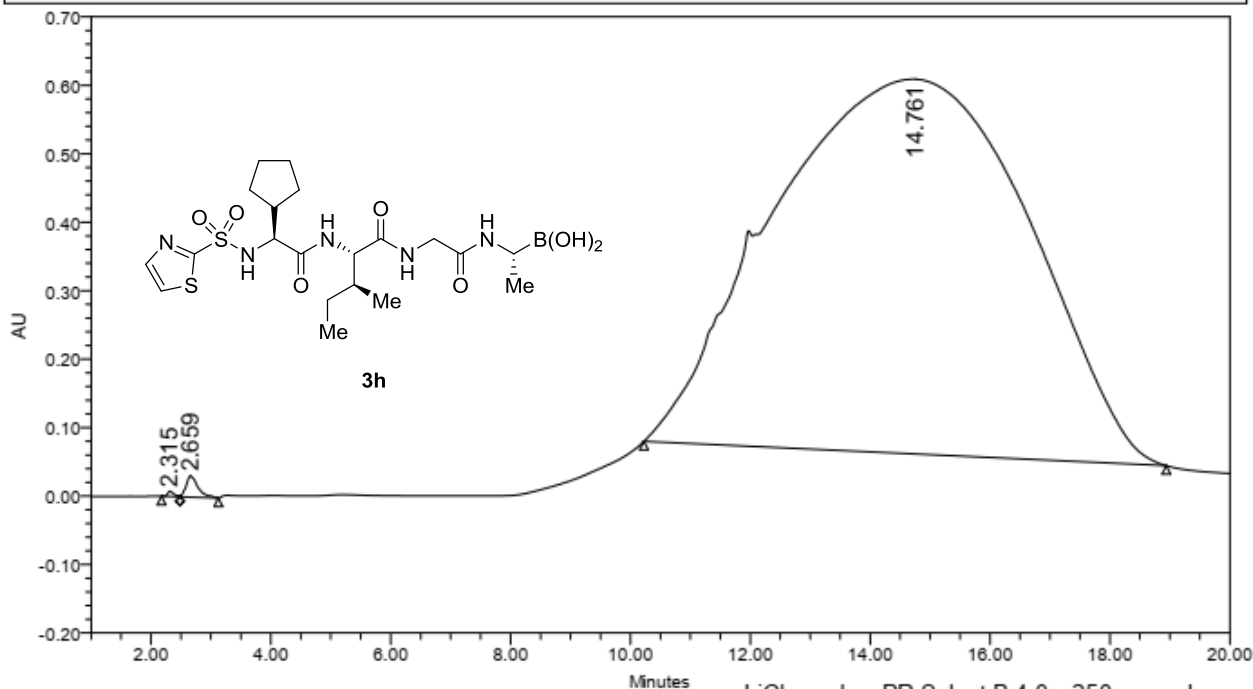
4c

¹³C NMR (101 MHz, Methanol-d₄)



5. Representative examples of HPLC purity

SAMPLE INFORMATION			
Sample Name:	osm-EP_1506	Acquired By:	Olita
Sample Type:	Unknown	Sample Set Name:	26042024p
Vial:	5	Acq. Method Set:	MeCN_0_5min_100%
Injection #:	1	Processing Method:	Processing
Injection Volume:	30.00 ul	Channel Name:	W2489 ChA
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 210nm
Date Acquired:	2024.04.26. 15:31:28 EET		
Date Processed:	2024.04.26. 16:24:34 EET		



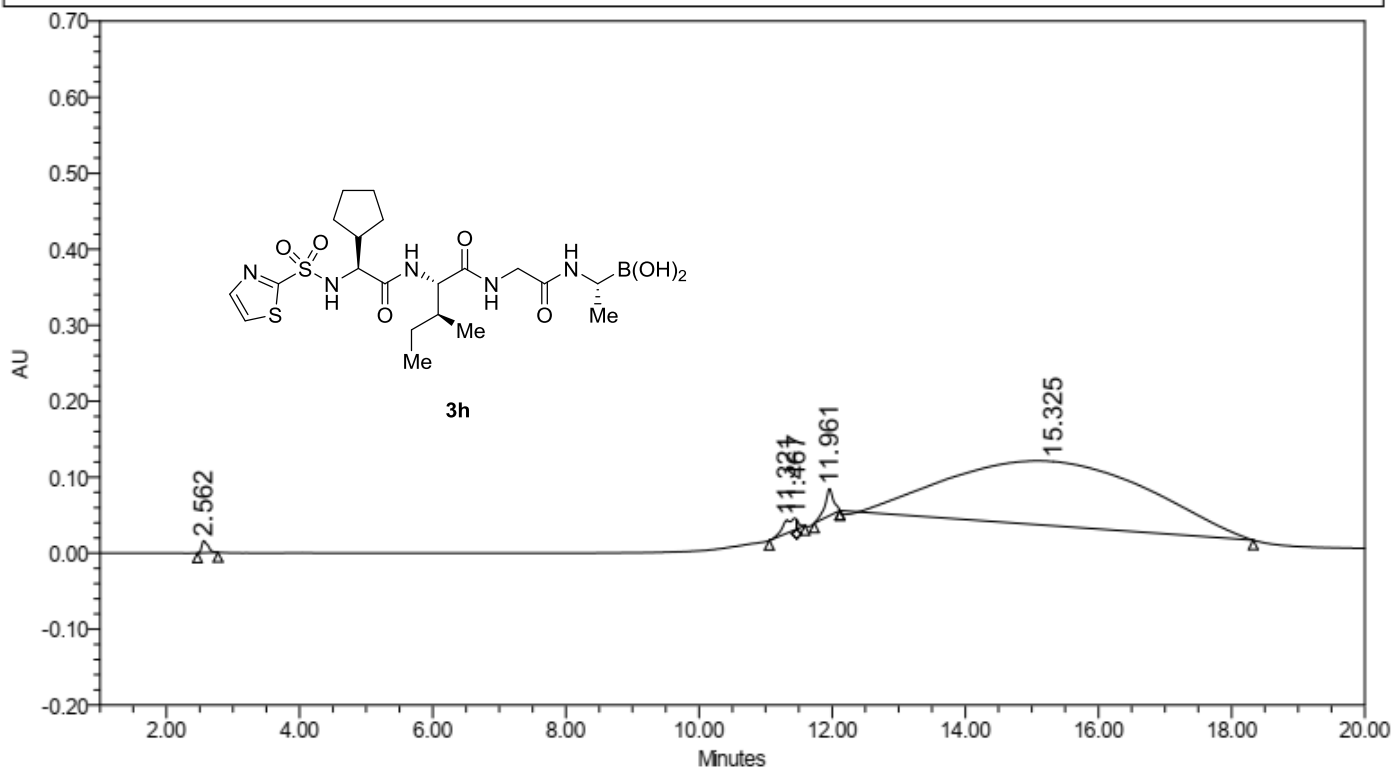
	RT	Area	% Area	Height
1	2.315	56447	0.03	7455
2	2.659	431249	0.27	31180
3	14.761	161940290	99.70	547420

Reported by User: Olita
 Report Method: Default Individual Report
 Report Method ID 12125
 Page: 1 of 1

Project Name: Martins
 Date Printed:
 2024.04.26.
 16:25:36 Europe/Riga

SAMPLE INFORMATION

Sample Name: osm-EP_1506	Acquired By: Olita
Sample Type: Unknown	Sample Set Name: 26042024p
Vial: 5	Acq. Method Set: MeCN_0_5min_100%
Injection #: 1	Processing Method: Processing
Injection Volume: 30.00 ul	Channel Name: W2489 ChB
Run Time: 20.0 Minutes	Proc. Chnl. Descr.: W2489 ChB 254nm
Date Acquired: 2024.04.26. 15:31:28 EET	
Date Processed: 2024.04.26. 16:24:05 EET	



	RT	Area	% Area	Height
1	2.562	103532	0.56	15944
2	11.321	220761	1.19	16922
3	11.467	31279	0.17	12164
4	11.961	314452	1.69	35193
5	15.325	17937149	96.40	84529

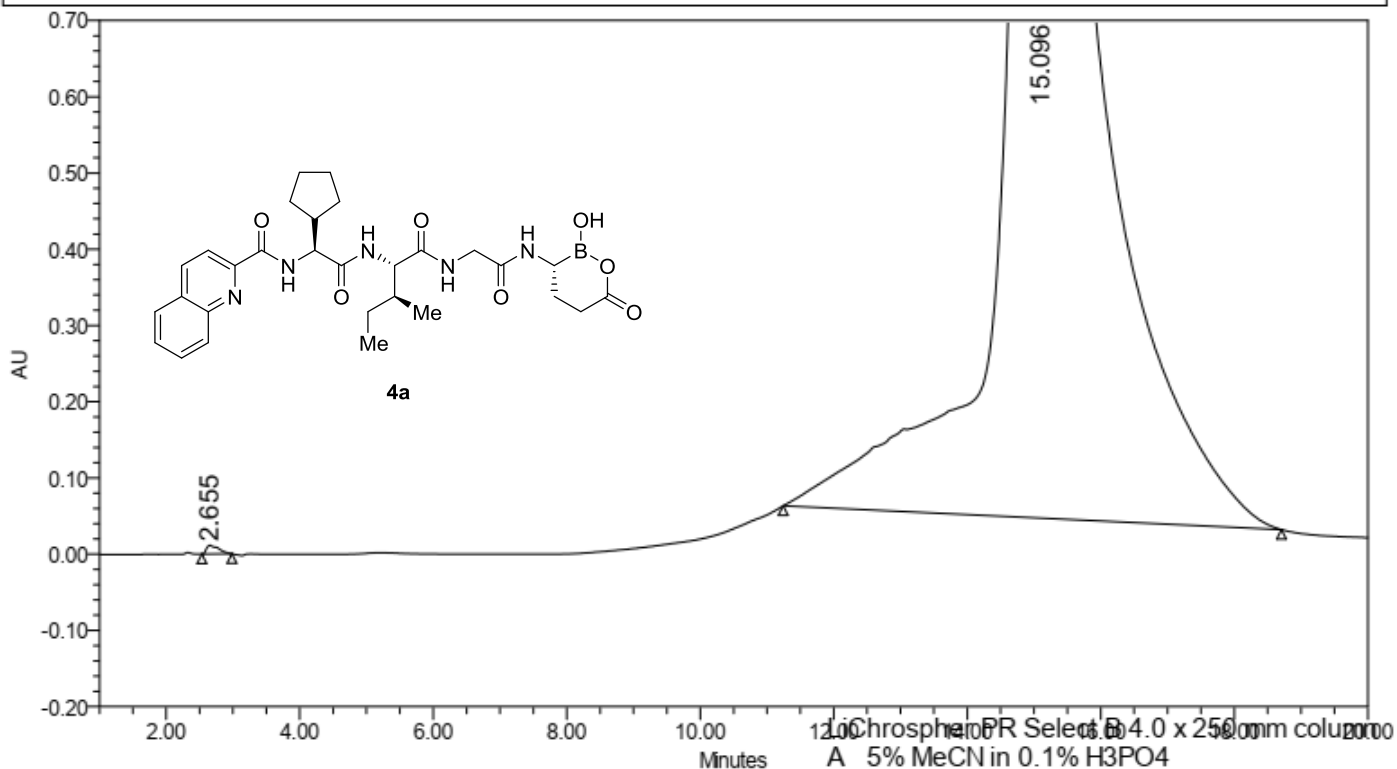
LiChrospher PR Select B 4.0 x 250 mm column
 A 5% MeCN in 0.1% H₃PO₄
 B 95% MeCN in 0.1% H₃PO₄
 Gradient 15 min 0% A to 100% B; 5 min 100% B
 Column Temperature 40°C
 Flow Rate: 1.0 ml/min

Reported by User: Olita
 Report Method: Default Individual Report
 Report Method ID 12125
 Page: 1 of 1

Project Name: Martins
 Date Printed:
 2024.04.26.
 16:25:05 Europe/Riga

SAMPLE INFORMATION

Sample Name: osm-EP_1800 Sample Type: Unknown Vial: 7 Injection #: 1 Injection Volume: 30.00 ul Run Time: 20.0 Minutes Date Acquired: 2024.04.26. 12:53:45 EET Date Processed: 2024.04.26. 13:35:35 EET	Acquired By: Olita Sample Set Name: 26042024 Acq. Method Set: MeCN_0_5min_100% Processing Method: Processing Channel Name: W2489 ChA Proc. Chnl. Descr.: W2489 ChA 210nm
--	---



	RT	Area	% Area	Height
1	2.655	145944	0.10	11451
2	15.096	146271984	99.90	1576171

Reported by User: Olita
 Report Method: Default Individual Report
 Report Method ID 12125
 Page: 1 of 1

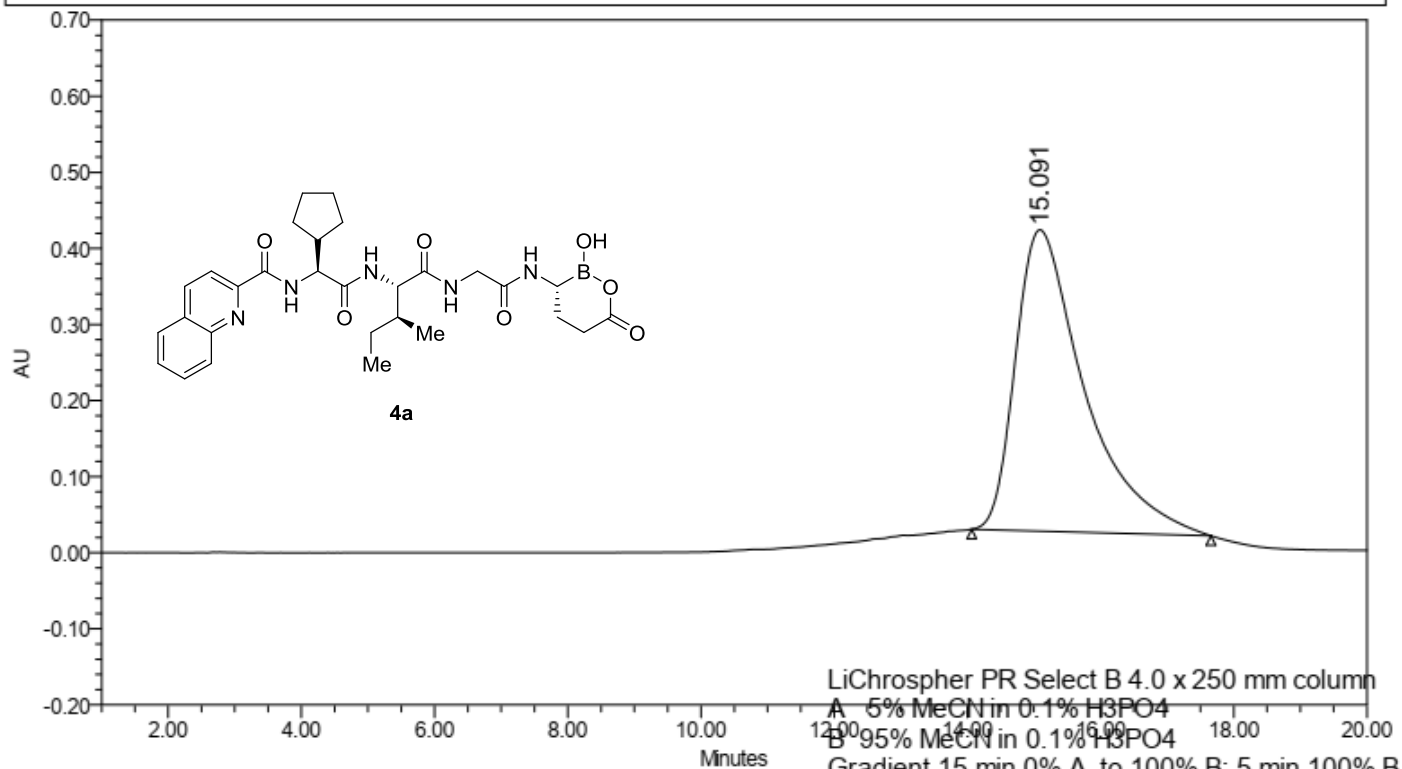
Project Name: Martins
 Date Printed:
 2024.04.26.
 13:37:22 Europe/Riga

SAMPLE INFORMATION

Sample Name: osm-EP_1800
 Sample Type: Unknown
 Vial: 7
 Injection #: 1
 Injection Volume: 30.00 ul
 Run Time: 20.0 Minutes

Acquired By: Olita
 Sample Set Name: 26042024
 Acq. Method Set: MeCN_0_5min_100%
 Processing Method: Processing
 Channel Name: W2489 ChB
 Proc. Chnl. Descr.: W2489 ChB 254nm

Date Acquired: 2024.04.26. 12:53:45 EET
 Date Processed: 2024.04.26. 13:34:37 EET



LiChrospher PR Select B 4.0 x 250 mm column
 A 5% MeCN in 0.1% H₃PO₄
 B 95% MeCN in 0.1% H₃PO₄
 Gradient 15 min 0% A to 100% B; 5 min 100% B
 Column Temperature 40oC
 Flow Rate: 1.0 ml/min

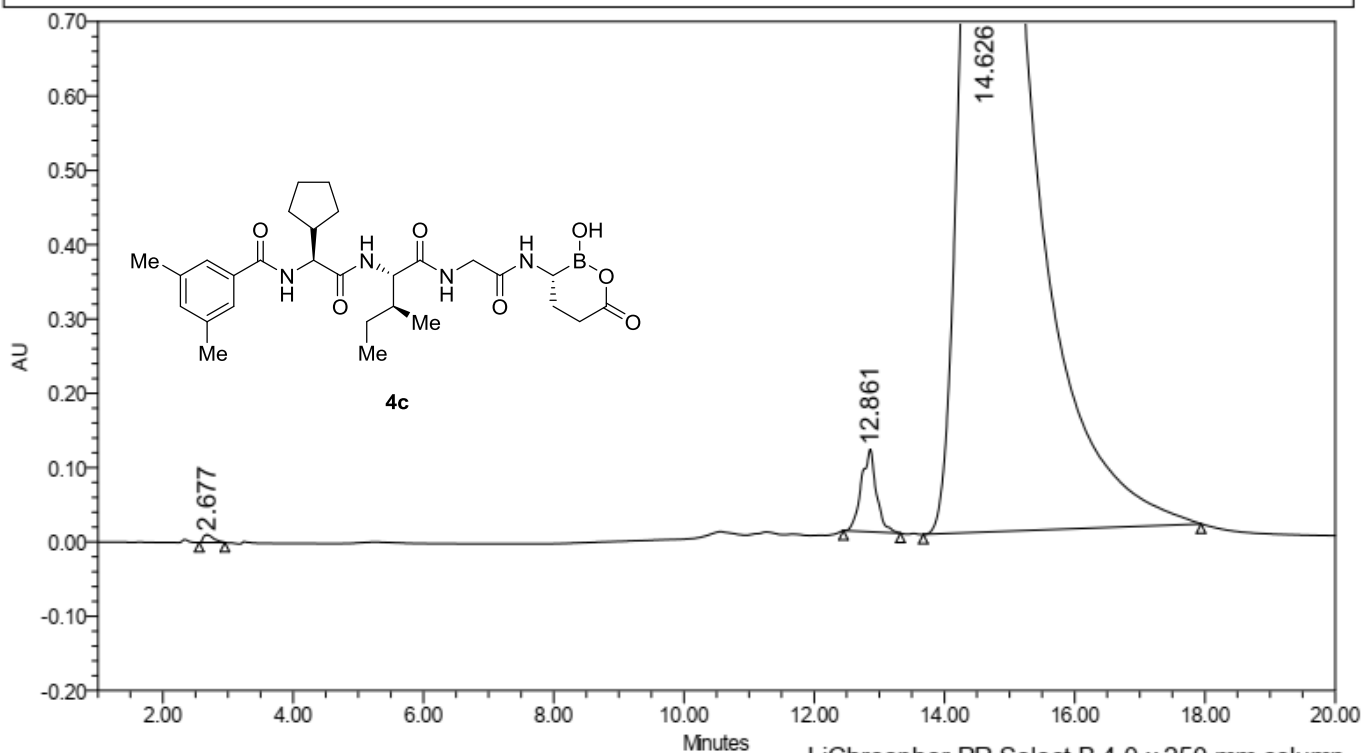
	RT	Area	% Area	Height
1	15.091	28469907	100.00	395971

Reported by User: Olita
 Report Method: Default Individual Report
 Report Method ID 12125
 Page: 1 of 1

Project Name: Martins
 Date Printed: 2024.04.26.
 13:36:48 Europe/Riga

SAMPLE INFORMATION

Sample Name: osm-EP_1913 Sample Type: Unknown Vial: 2 Injection #: 1 Injection Volume: 30.00 ul Run Time: 20.0 Minutes Date Acquired: 2024.04.26. 10:44:42 EET Date Processed: 2024.04.26. 13:32:01 EET	Acquired By: Olita Sample Set Name: 26042024 Acq. Method Set: MeCN_0_5min_100% Processing Method: Processing Channel Name: W2489 ChA Proc. Chnl. Descr.: W2489 ChA 210nm
--	---



LiChrospher PR Select B 4.0 x 250 mm column
 A 5% MeCN in 0.1% H₃PO₄
 B 95% MeCN in 0.1% H₃PO₄
 Gradient 15 min 0% A to 100% B; 5 min 100% B
 Column Temperature 40°C
 Flow Rate: 1.0 ml/min

RT	Area	% Area	Height
1	112442	0.11	10184
2	1778266	1.76	110924
3	99338490	98.13	1527402

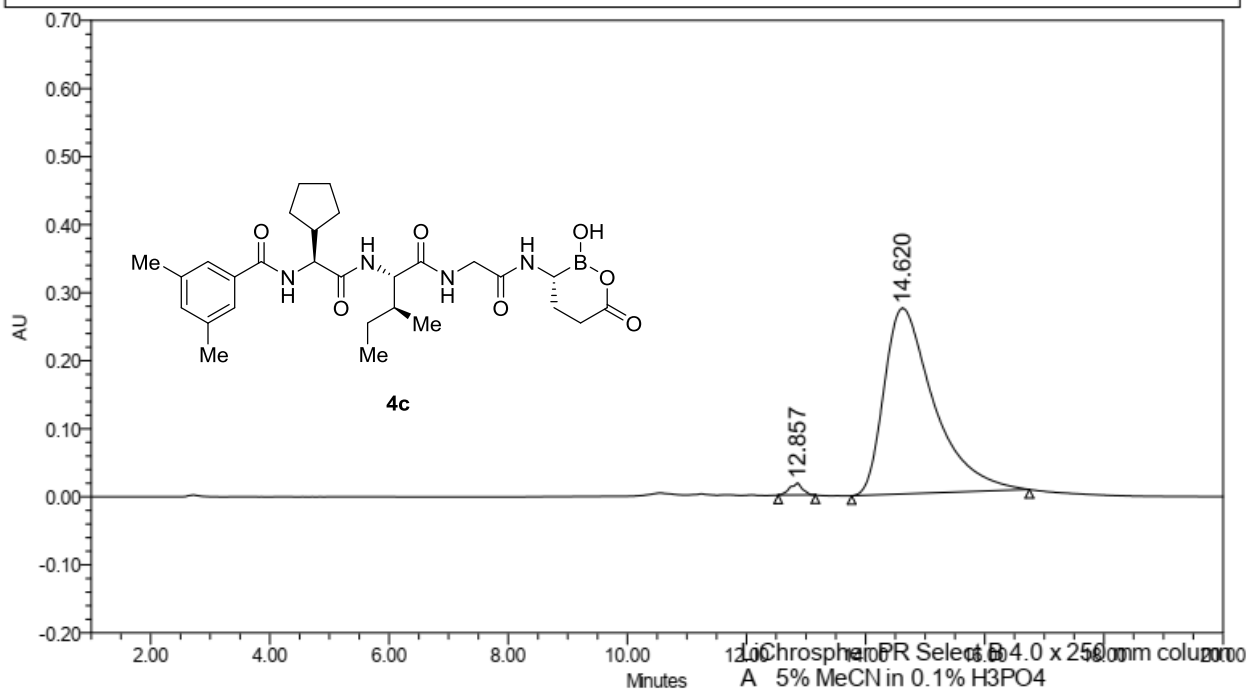
Reported by User: Olita
 Report Method: Default Individual Report
 Report Method ID 12125
 Page: 1 of 1

Project Name: Martins
 Date Printed:
 2024.04.26.
 13:36:11 Europe/Riga

SAMPLE INFORMATION

Sample Name: osm-EP_1913	Acquired By: Olita
Sample Type: Unknown	Sample Set Name: 26042024
Vial: 2	Acq. Method Set: MeCN_0_5min_100%
Injection #: 1	Processing Method: Processing
Injection Volume: 30.00 ul	Channel Name: W2489 ChB
Run Time: 20.0 Minutes	Proc. Chnl. Descr.: W2489 ChB 254nm

Date Acquired: 2024.04.26. 10:44:42 EET
 Date Processed: 2024.04.26. 13:27:48 EET



	RT	Area	% Area	Height
1	12.857	253479	1.57	17289
2	14.620	15915867	98.43	273005

Chromatogram PR Select 64.0 x 250 mm column
 A 5% MeCN in 0.1% H3PO4
 B 95% MeCN in 0.1% H3PO4
 Gradient 15 min 0% A to 100% B; 5 min 100% B
 Column Temperature 40oC
 Flow Rate: 1.0 ml/min

Reported by User: Olita
 Report Method: Default Individual Report
 Report Method ID 12125
 Page: 1 of 1

Project Name: Martins
 Date Printed:
 2024.04.26.
 13:28:39 Europe/Riga