Supporting Information

A High-yielding Solid-Phase Total Synthesis of Daptomycin using a Fmoc SPPS Stable Kynurenine Synthon.

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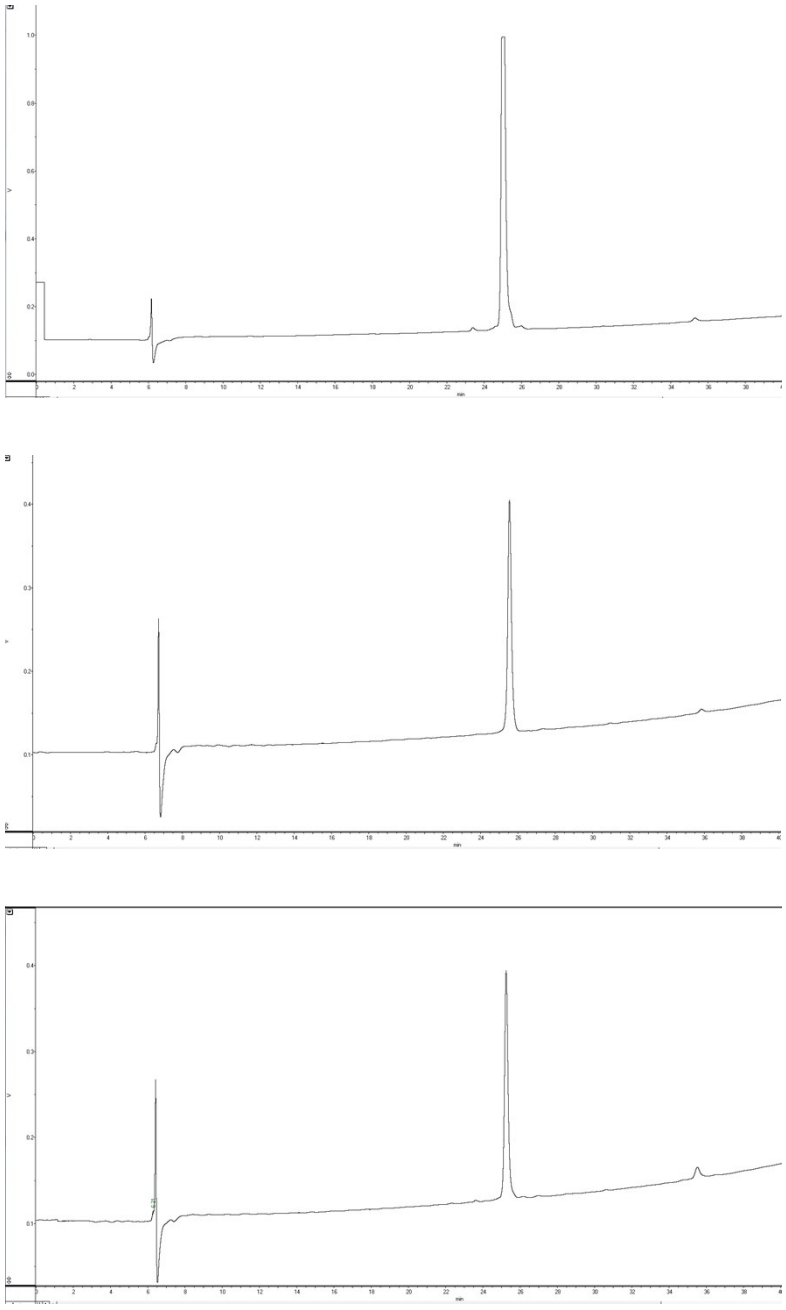


Figure S1: RP-HPLC chromatograms of authentic daptomycin (top), synthetic daptomycin prepared via Scheme 1 (middle) and a mixture of both authentic and synthetic daptomycin prepared via Scheme 1 (bottom). A linear gradient 10:90 MeCN: H_2O (0.1% TFA) to 90:10 MeCN: H_2O (0.1% TFA) over 40 min was used. All chromatograms were obtained using λ = 220 nm.

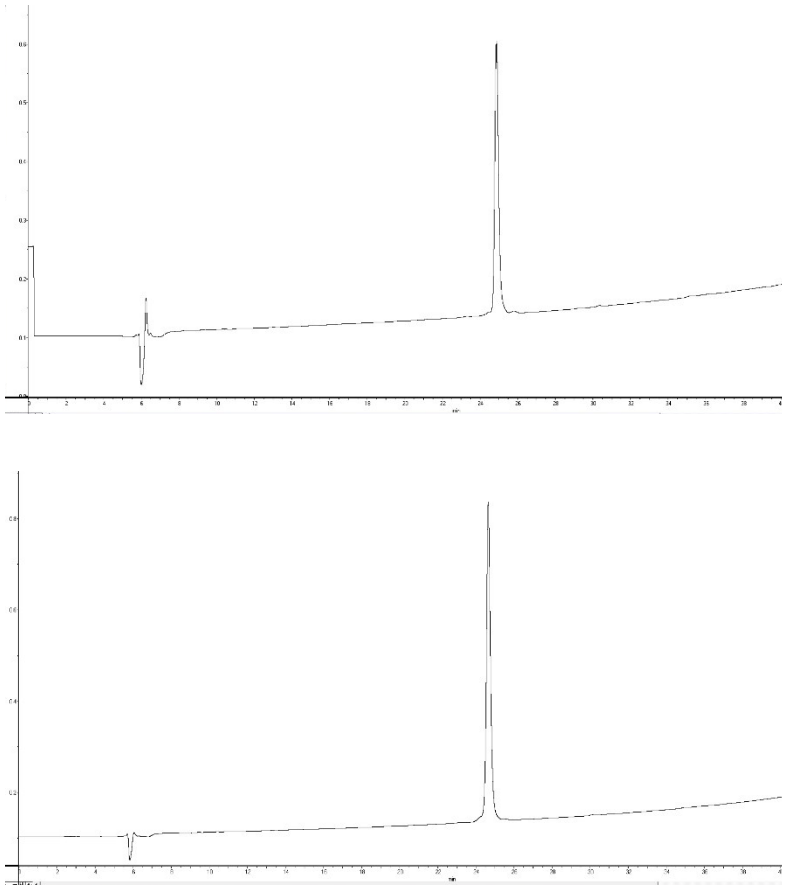


Figure S2: RP-HPLC chromatograms of synthetic daptomycin prepared via Scheme 5 (top) and a mixture of both authentic and synthetic daptomycin prepared via Scheme 5 (bottom). A linear gradient 10:90 MeCN: H_2O (0.1% TFA) to 90:10 MeCN: H_2O (0.1% TFA) over 40 min was used. All chromatograms were obtained using $\lambda = 220$ nm.

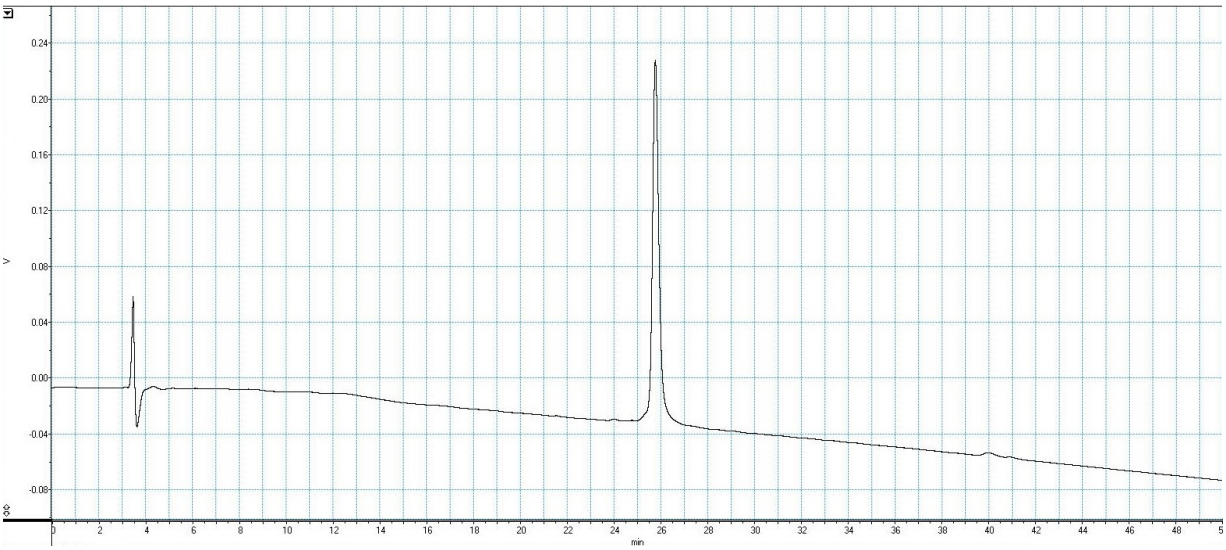


Figure S3: RP-HPLC chromatogram of peptide **7**. A linear gradient of 10:90 MeCN: H_2O (0.1% TFA) to 90:10 MeCN: H_2O (0.1% TFA) over 50 min was used. This chromatogram was obtained using λ = 220 nm.

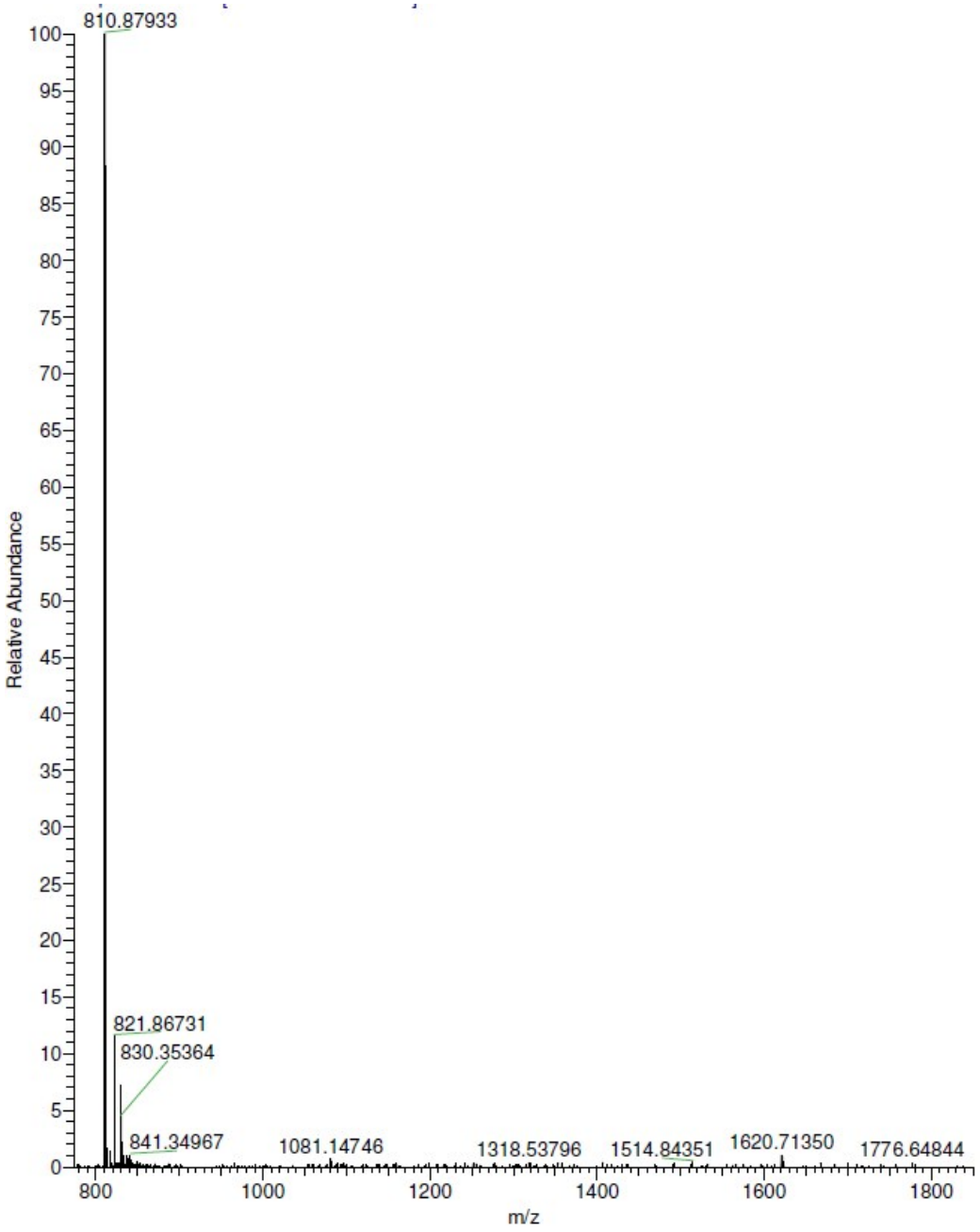


Figure S4: ESI-MS of synthetic dap prepared via Scheme 1 (1:1 MeOH:H_2O + 0.1% formic acid).

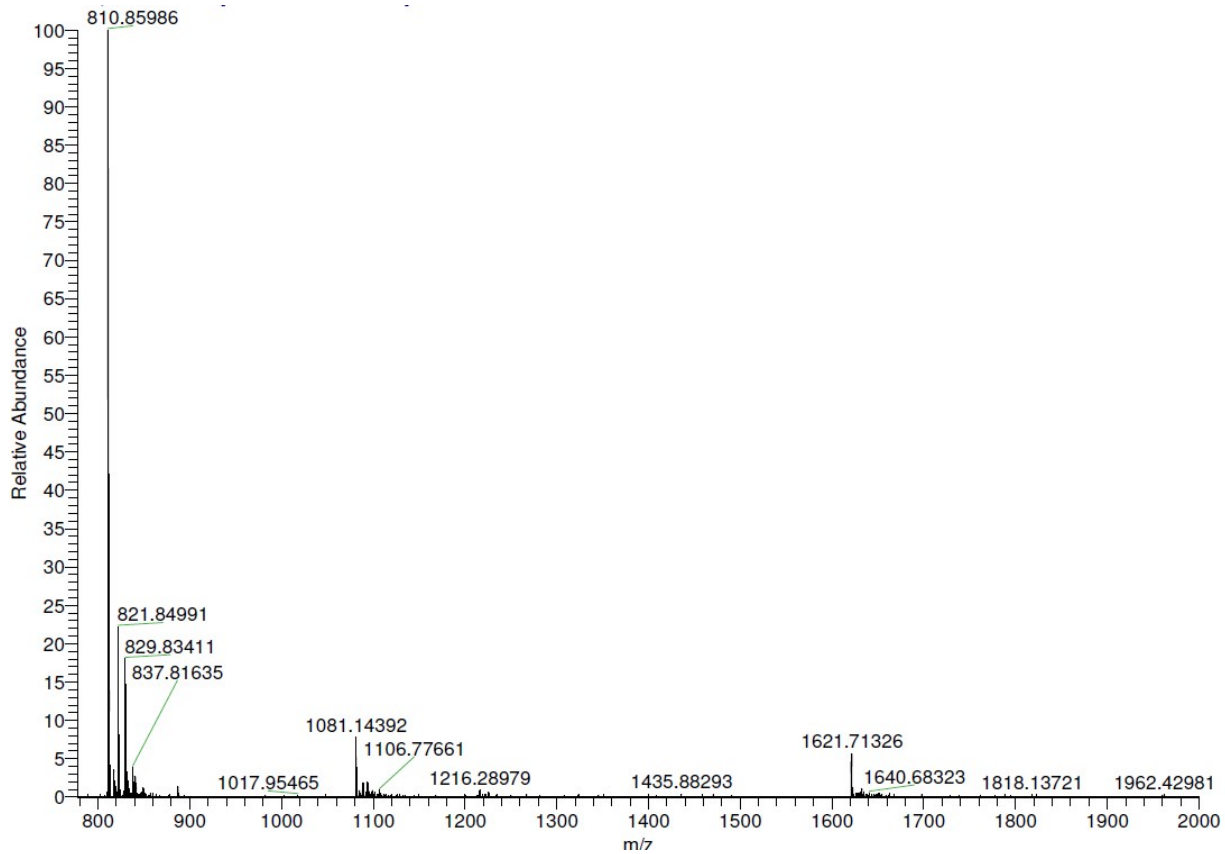


Figure S5: ESI-MS of synthetic dap prepared via Scheme 5 (1:1 MeOH:H_2O + 0.1% formic acid).

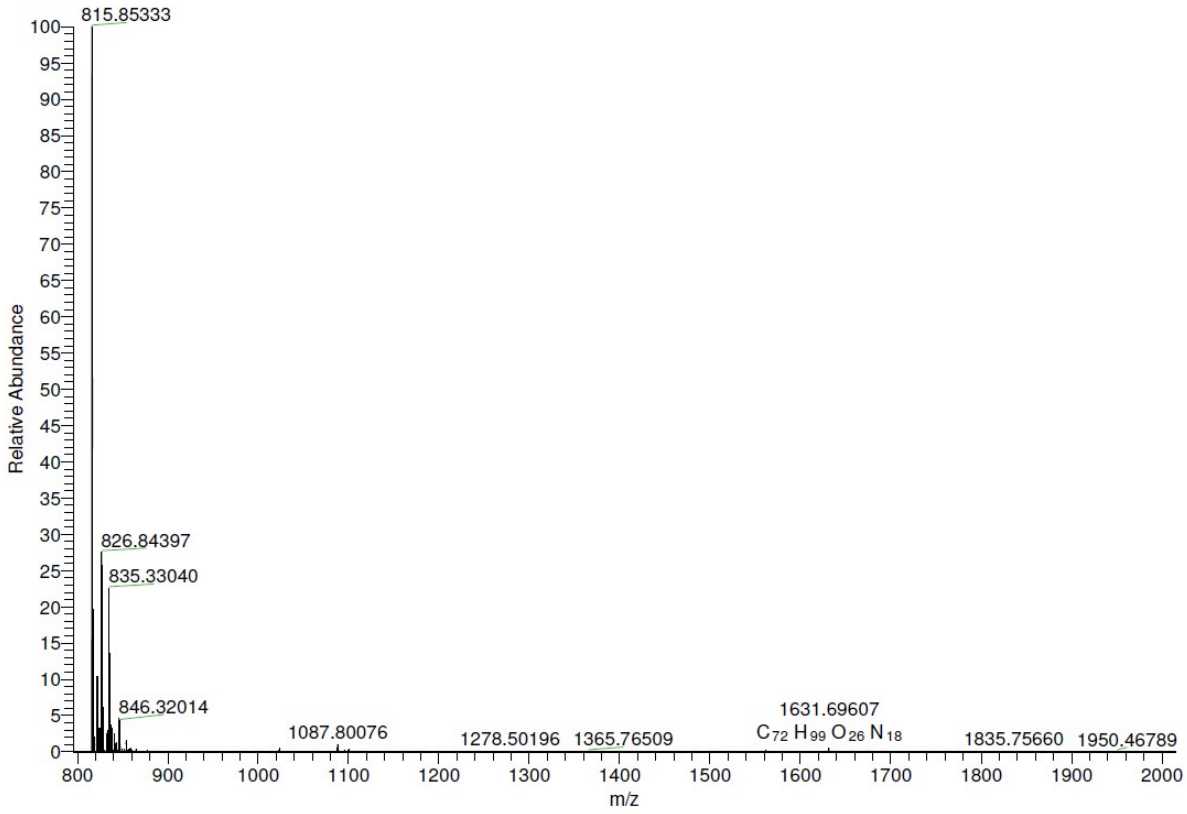
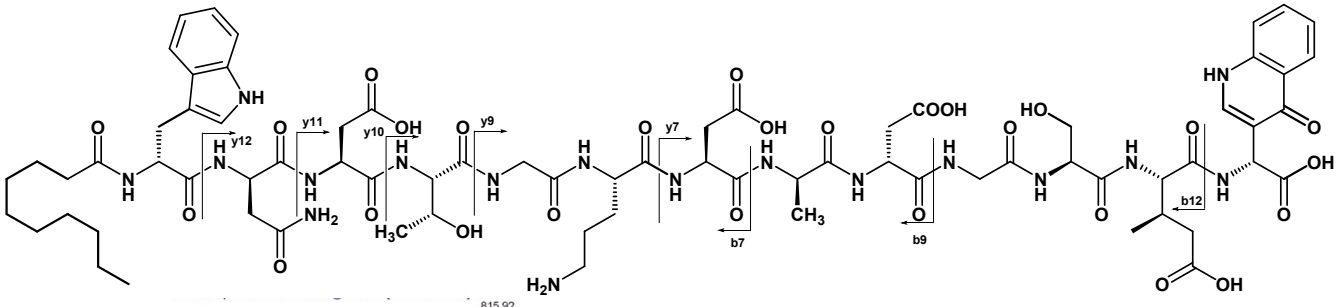
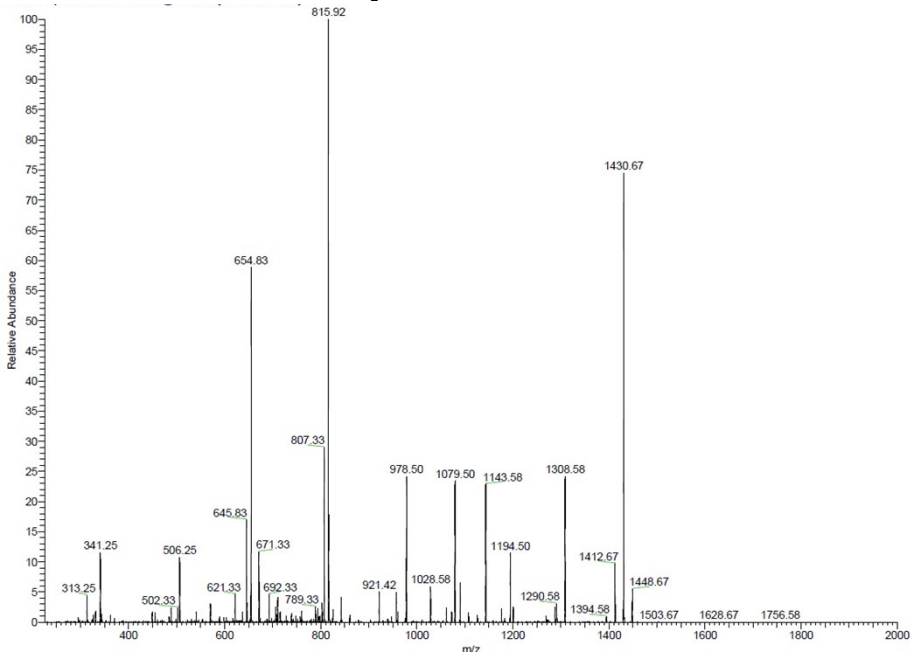


Figure S6: ESI-MS of peptide **7** (1:1 MeOH:H_2O + 0.1% formic acid).

Table S1. MS-MS sequencing of peptide **7** following ester bond hydrolysis in 0.1 N LiOH.





Ion	Molecular formula	Observed fragment (Calculated mass) (Da)	Ion	Molecular formula	Observed fragment (Calculated mass) (Da)
b7	$C_{44}H_{65}N_{10}O_{14}^{+}$	957.6 (957.5)	y9	$C_{40}H_{56}N_{11}O_{18}^{+}$	978.5 (978.4)
b9	$C_{51}H_{75}N_{12}O_{18}^{+}$	1143.6 (1143.5)	y10	$N_{12}O_{20}^{+}$	1079.5 (1079.4)
b12	$C_{62}H_{92}N_{15}O_{24}^{+}$	1430.7 (1430.6)	y11	$C_{48}H_{68}N_{13}O_{23}^{+}$	1194.5 (1194.5)
y7	$C_{33}H_{43}N_{8}O_{16}^{+}$	807.3 (807.3)	y12	$C_{52}H_{74}N_{15}O_{25}^{+}$	1308.6 (1308.5)

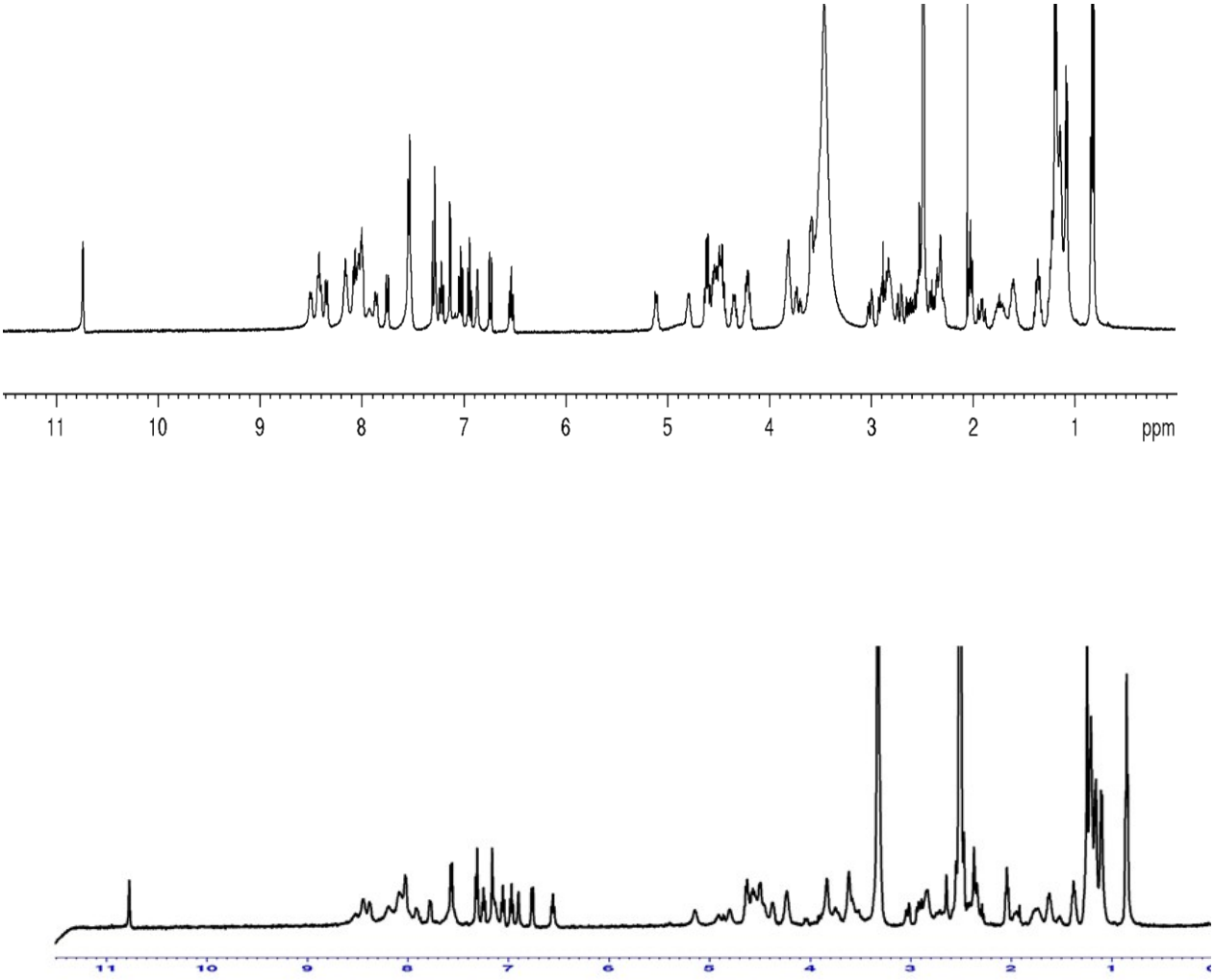


Figure S7: ^{1}H NMR spectra of Brimble and coworkers' authentic sample of dap in DMSO-d_6 (top; 500 MHz NMR, 11.7 mg/mL in DMSO-d_6)[6] and the synthetic dap prepared via Scheme 1 (bottom; 500 MHz NMR, 5 mg/mL in DMSO-d_6).

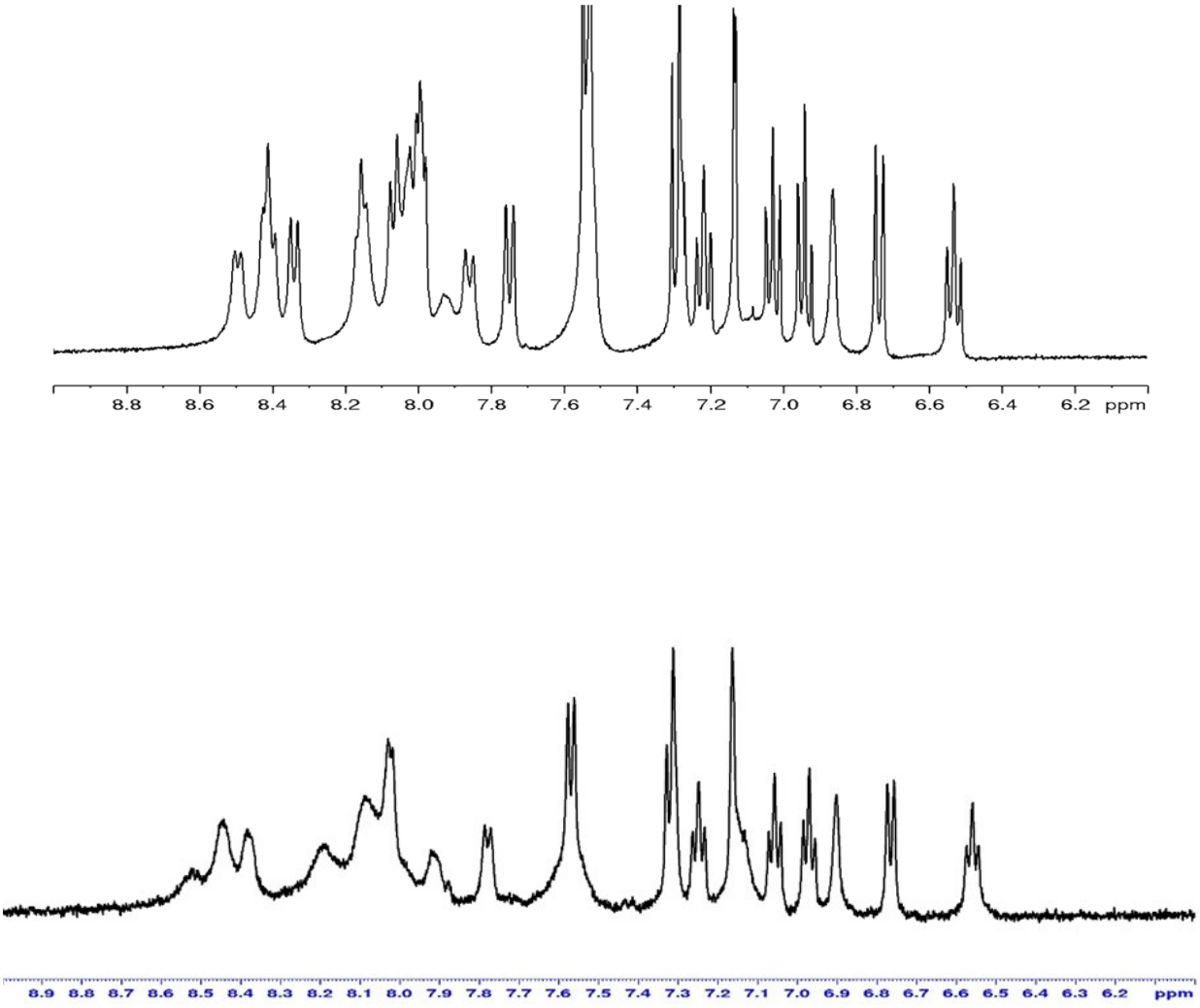


Figure S8: Amide and aromatic region of the ^{1}H NMR spectra of Brimble and coworkers' authentic sample of dap in DMSO-d_6 (top; 500 MHz NMR, 11.7 mg/mL in DMSO-d_6)[6] and the synthetic dap prepared via Scheme 1 (bottom; 500 MHz NMR, 5 mg/mL in DMSO-d_6).

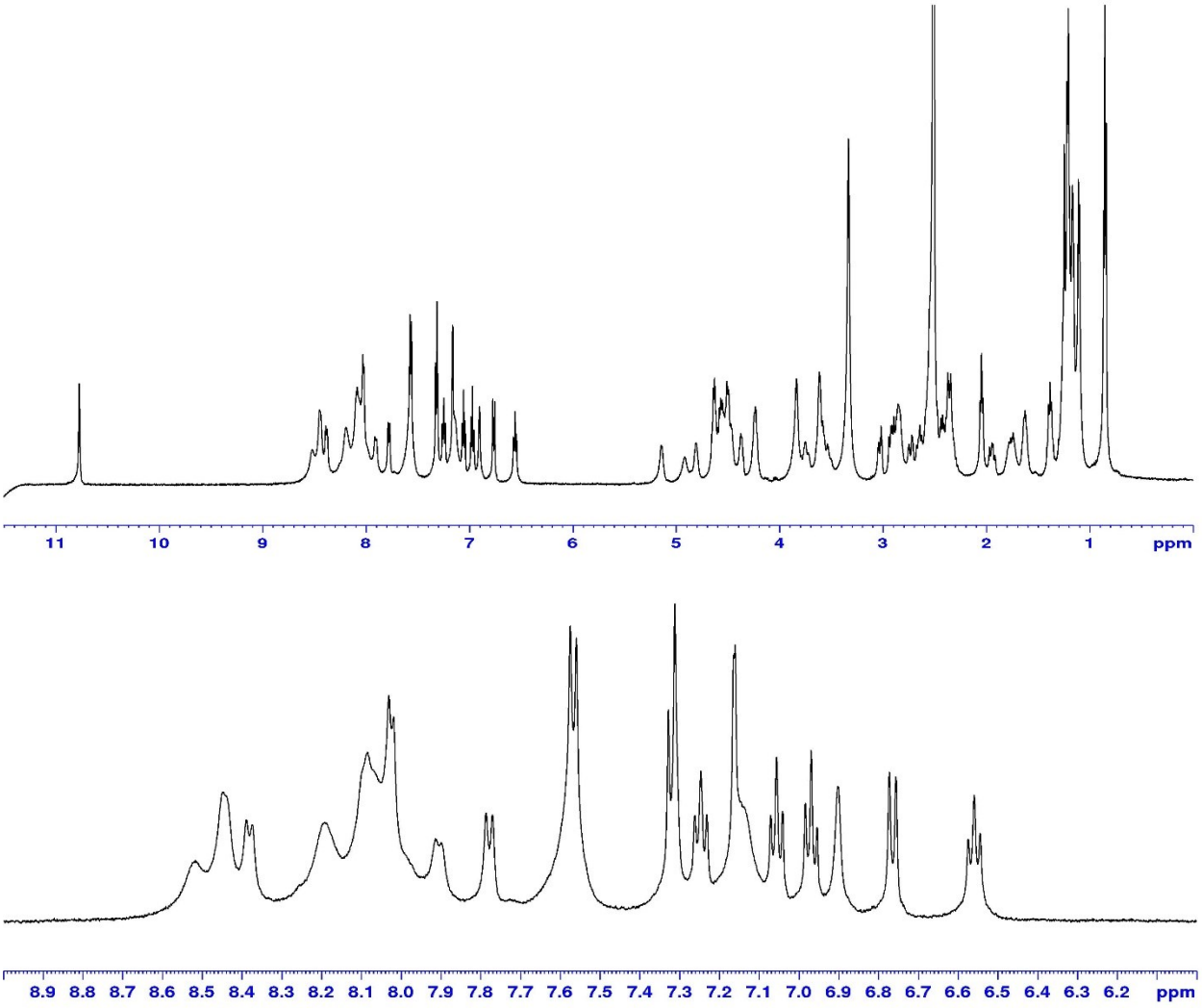


Figure S9: ^{1}H NMR of synthetic dap prepared via Scheme 5 (top; 500 MHz NMR, 8 mg/mL in DMSO-d_6). Amide and aromatic region of the same spectra (bottom).

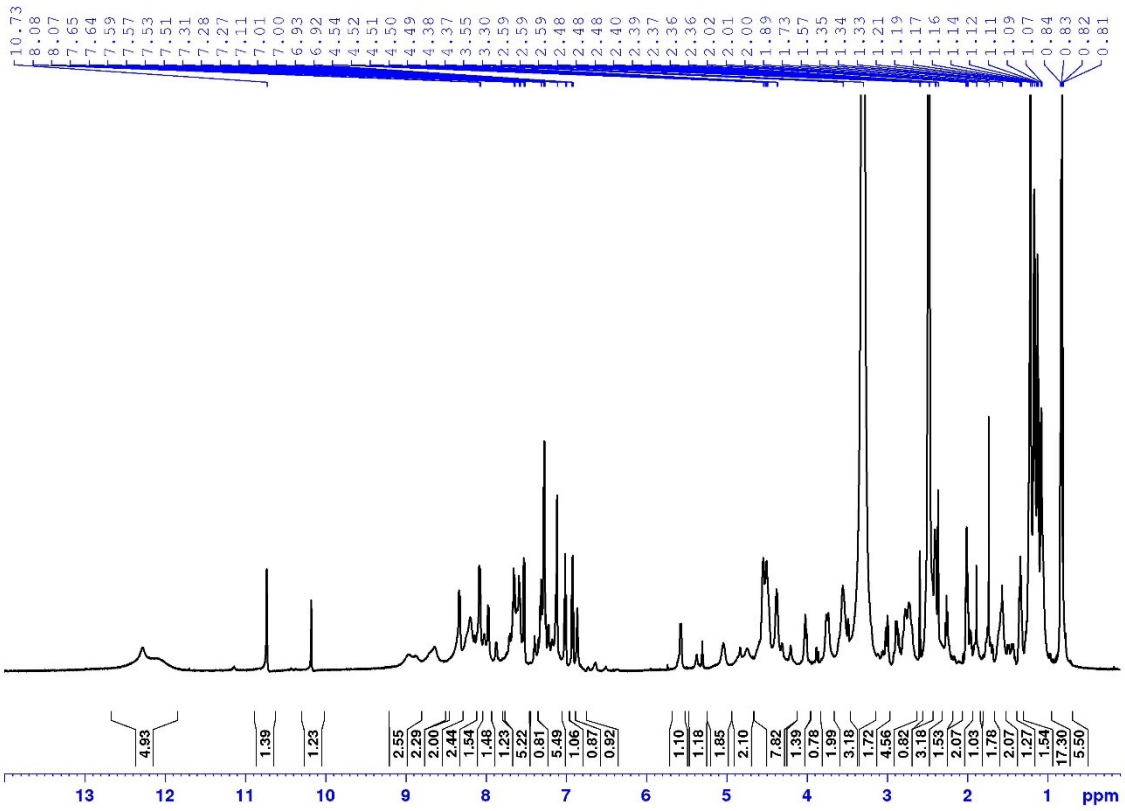


Figure S10: ^{1}H NMR spectrum of peptide **7** in DMSO-d_6 (600 MHz NMR).

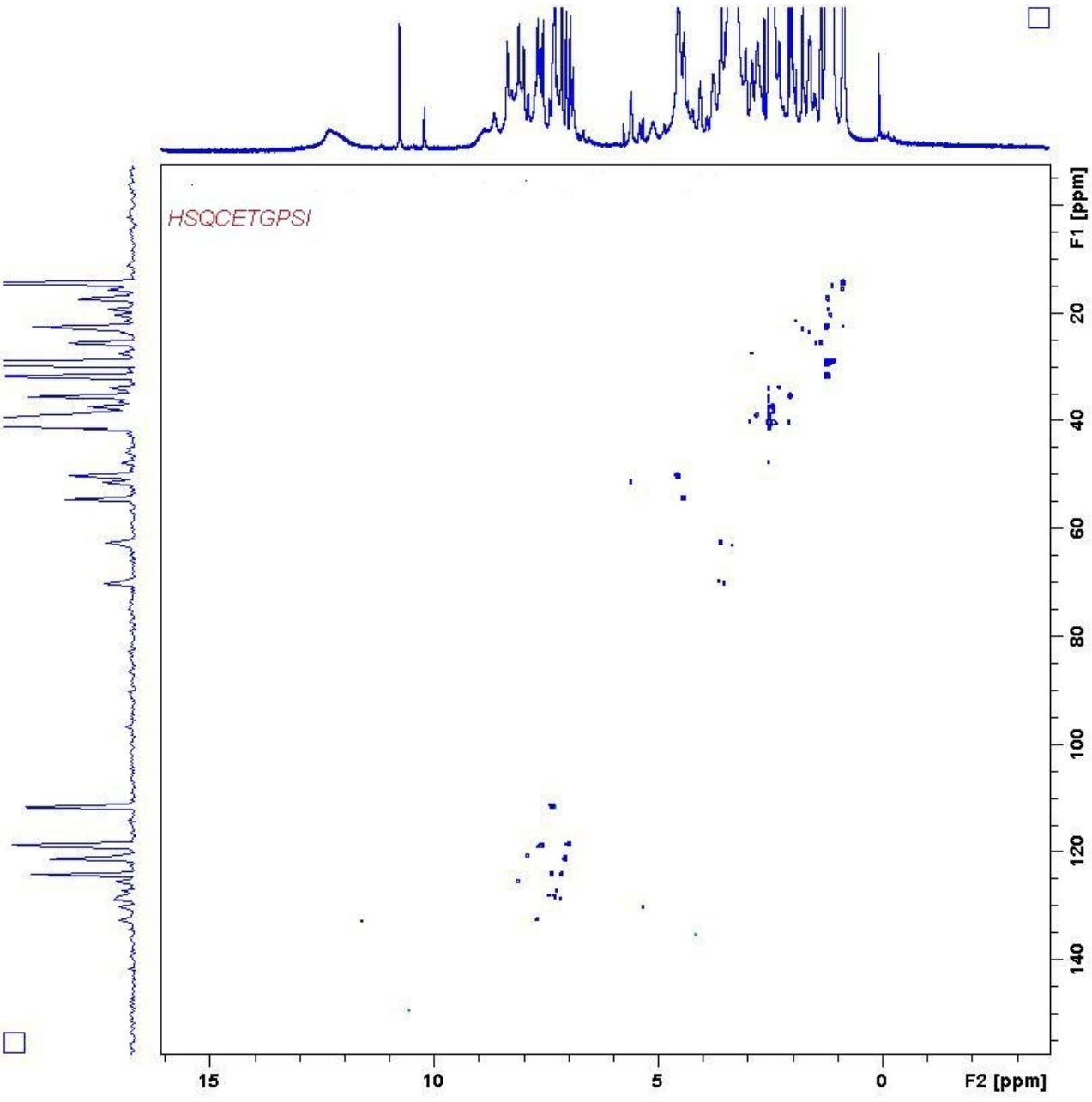


Figure S11: HSQC of **7** in DMSO-d_6 (600 MHz NMR)

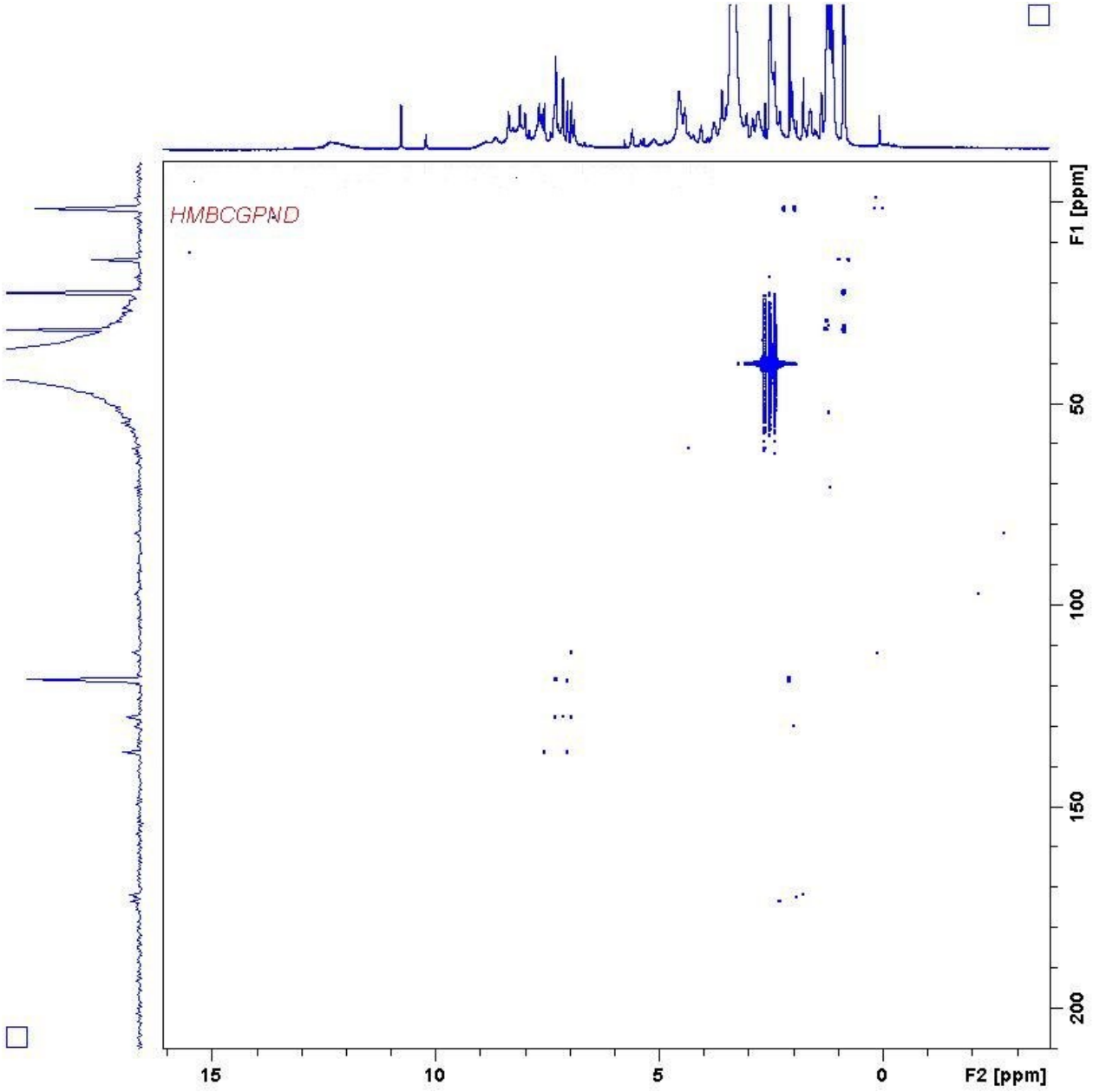


Figure S12: HMBC of **7** in DMSO-d_6 (600 MHz NMR).

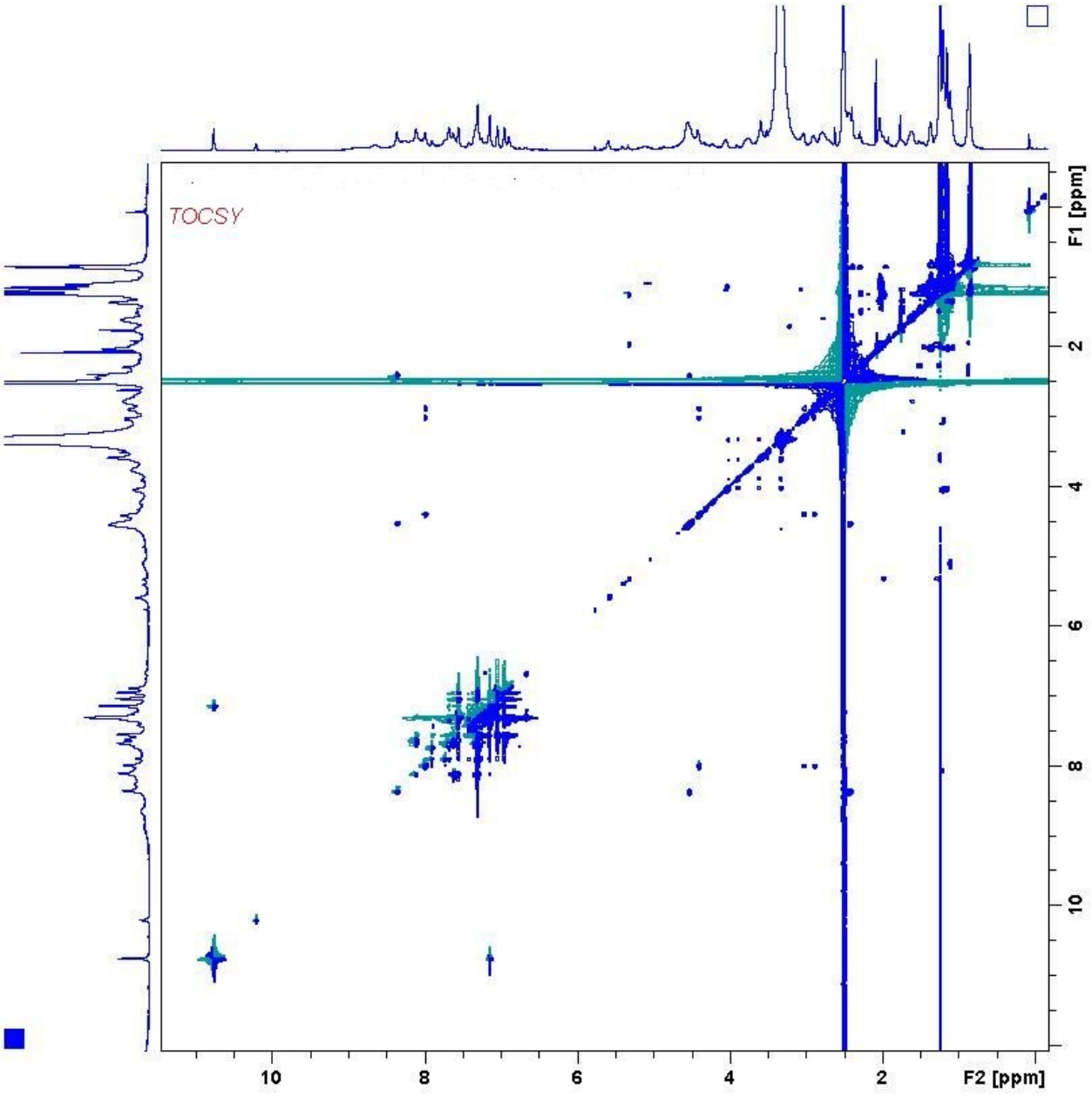


Figure S13: TOCSY of peptide **7** in DMSO-d_6 (600 MHz NMR)

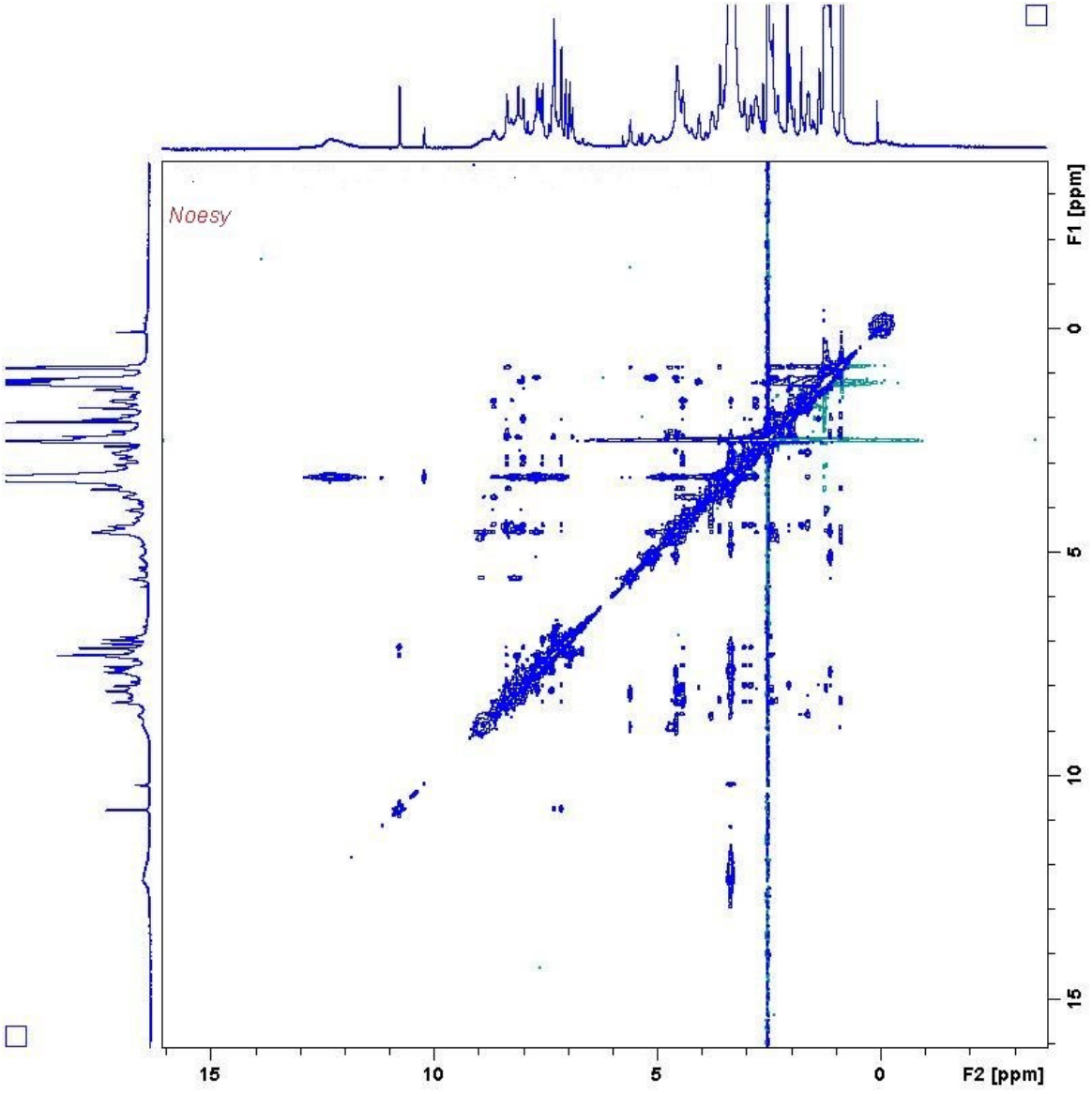


Figure S14. NOESY of peptide **7** in DMSO-d_6 (600 MHz NMR)

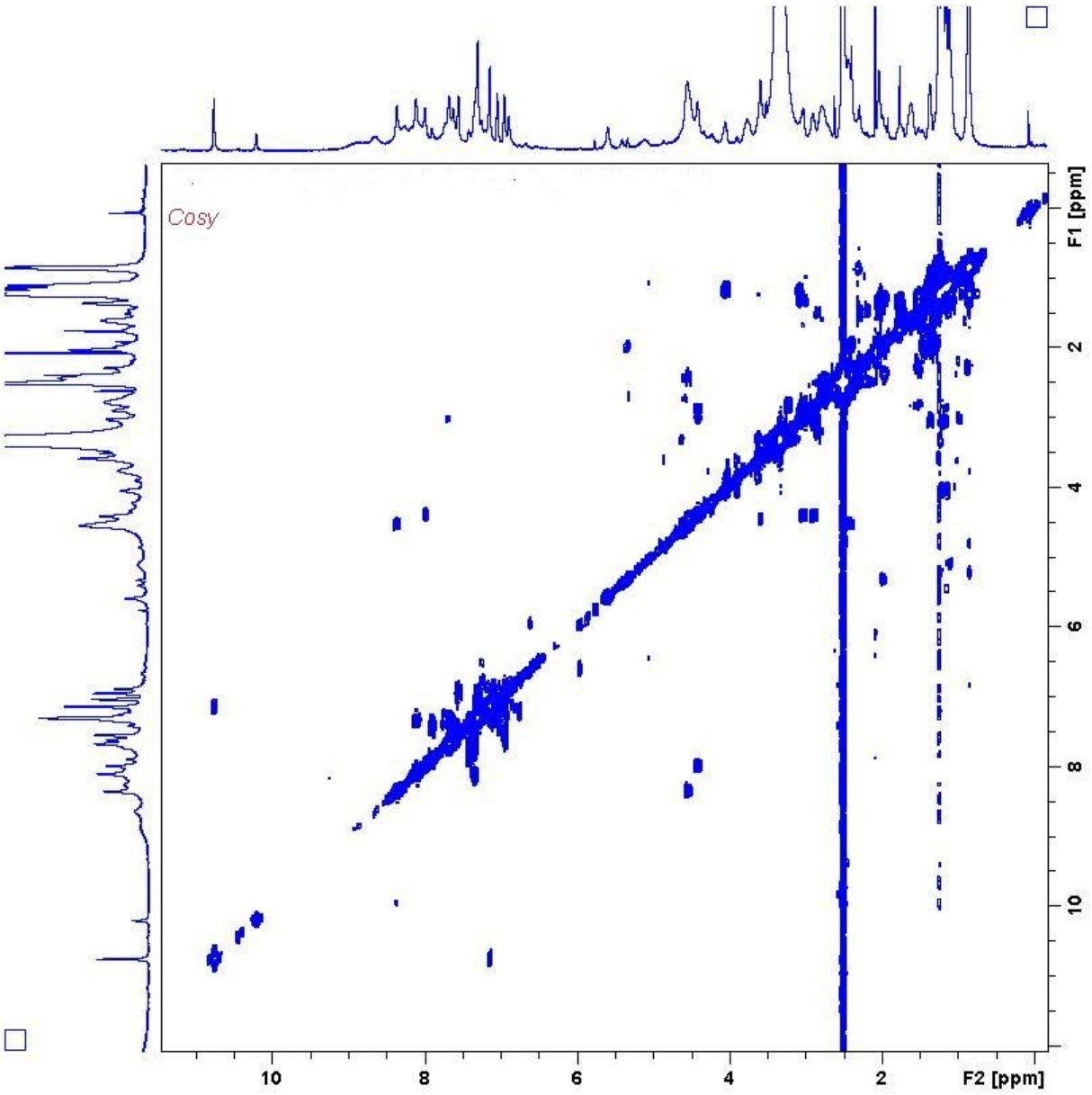
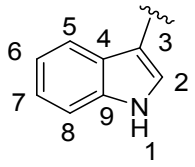
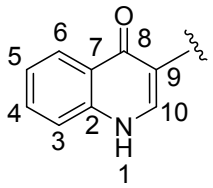


Figure S15. COSY of peptide **7** in DMSO-d_6 (600 MHz NMR)

Table S2. Chemical shift assignment of **7**

Residue	Position	δ_C	δ_H (J value, Hz)
Trp1 	NH	--	7.99
	α	54.6**	4.41
	β	27.6	3.03, 2.89
	1	--	10.77 s
	2	124.2	7.14 s
	3 (quat)	--	--
	4	118.9	7.55 d (7.3)
	4a (quat)	127.8	--
	5	118.6	6.95 dd (7.1, 7.3)
	6	121.3	7.04 dd (7.5, 7.3)
	7	111.7	7.30 d (7.5)
	7a (quat)	136.6	--
D-Asn2	NH	--	8.35
	α	50.2**	4.55
	β	37.5	2.42, 2.29
	C=O	--	--
	$CONH_2$	--	6.89
Asp3	NH	--	8.35
	α	50.5	4.59
	β	39.0**	2.75,2.44
	C=O	--	--
	COOH	--	12.32**
Thr4	NH	--	7.46
	α	50.0	4.56
	β	70.5	5.11
	γ	17.5	1.21
	C=O	--	--
Gly5	NH	--	--
	α	42.9**	3.88/3.63**
	C=O	--	--
Orn6	NH	--	8.32
	α	54.5**	4.37/4.41**
	β	--	1.66
	γ	24.7	1.49
	δ	39.0**	2.79**
	NH_2	--	--
	C=O	--	--
Asp7	NH	--	7.97
	α	54.5**	4.42
	β	--	2.90
	C=O	--	--
	COOH	--	12.32**
D-Ala8	NH	--	--

	α β C=O	49.9 20.4 --	4.06 1.15 --
Asp9	NH α β C=O COOH	-- 50.2** 38.5 -- --	8.36 4.54 2.41 -- 12.32**
Gly10	NH α C=O	-- 42.9** --	-- 3.88/3.63** --
D-Ser11	NH α β OH C=O	-- 63.1 65.9 -- --	10.20 3.34 4.04, 3.90 -- --
3mGlu12	NH α β γ1 γ2 C=O COOH	-- -- 34.0 40.5 15.8 -- --	-- 4.78 2.31 1.52, 2.07 0.85 -- 12.32**
4-QO13 	NH α 1 2 3 4 5 6 7 8 9 10 C=O	-- 51.4 -- -- 119.0 132.5 124.8 125.5 -- -- -- 135.3 --	8.99 5.59 12.32** -- 7.63 7.66 7.34 8.11 -- -- -- 7.65 --
Decanoic tail	--	--	--

'—'indicates that the signal was not assigned. '**' indicates that the assignment is ambiguous due to overlapping peaks.

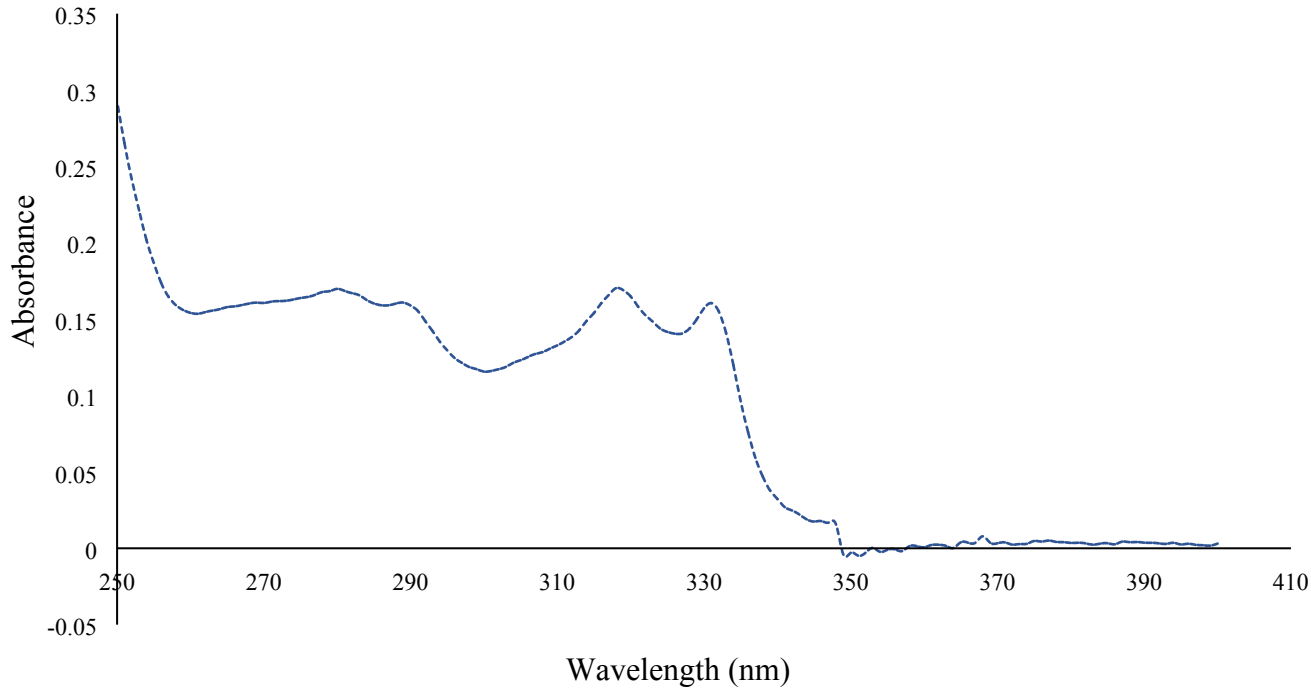
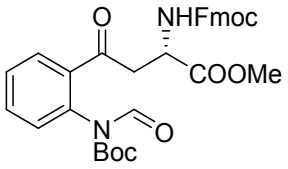
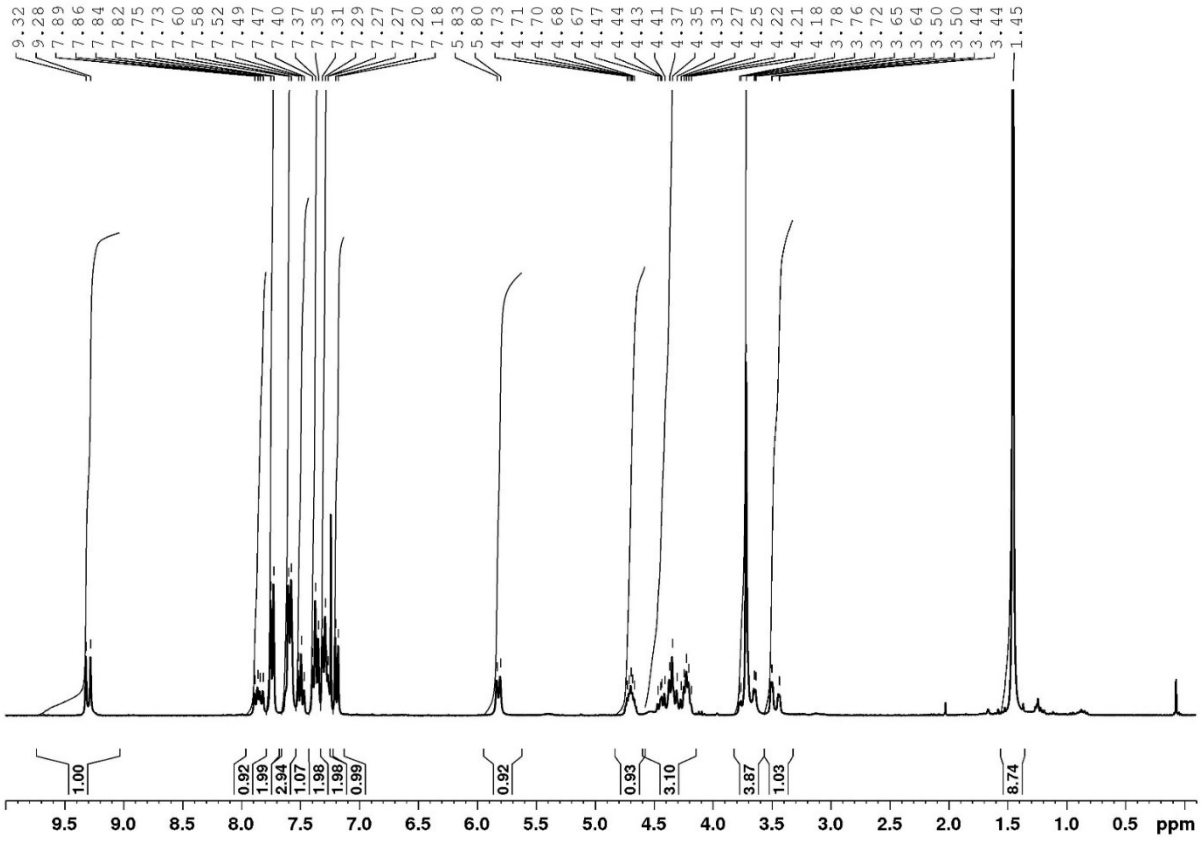


Figure S16. UV-Vis trace of peptide **7** in H_2O at a concentration of 32.5 µg/mL


O
NHFmoc
COOMe
N
O
Boc

^{1}H NMR of **11**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9.28
7.89
7.86
7.84
7.82
7.75
7.73
7.60
7.58
7.52
7.49
7.47
7.40
7.37
7.35
7.31
7.29
7.27
7.27
7.20
7.18
5.83
5.80
4.73
4.71
4.70
4.68
4.67
4.47
4.44
4.43
4.41
4.37
4.35
4.31
4.27
4.25
4.22
4.21
4.18
3.78
3.76
3.72
3.65
3.64
3.50
3.50
3.44
3.44
1.45
1.00
0.92
1.99
2.94
1.07
1.98
1.98
0.99
0.92
0.93
3.10
3.87
1.03
8.74
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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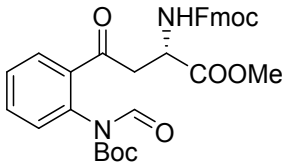
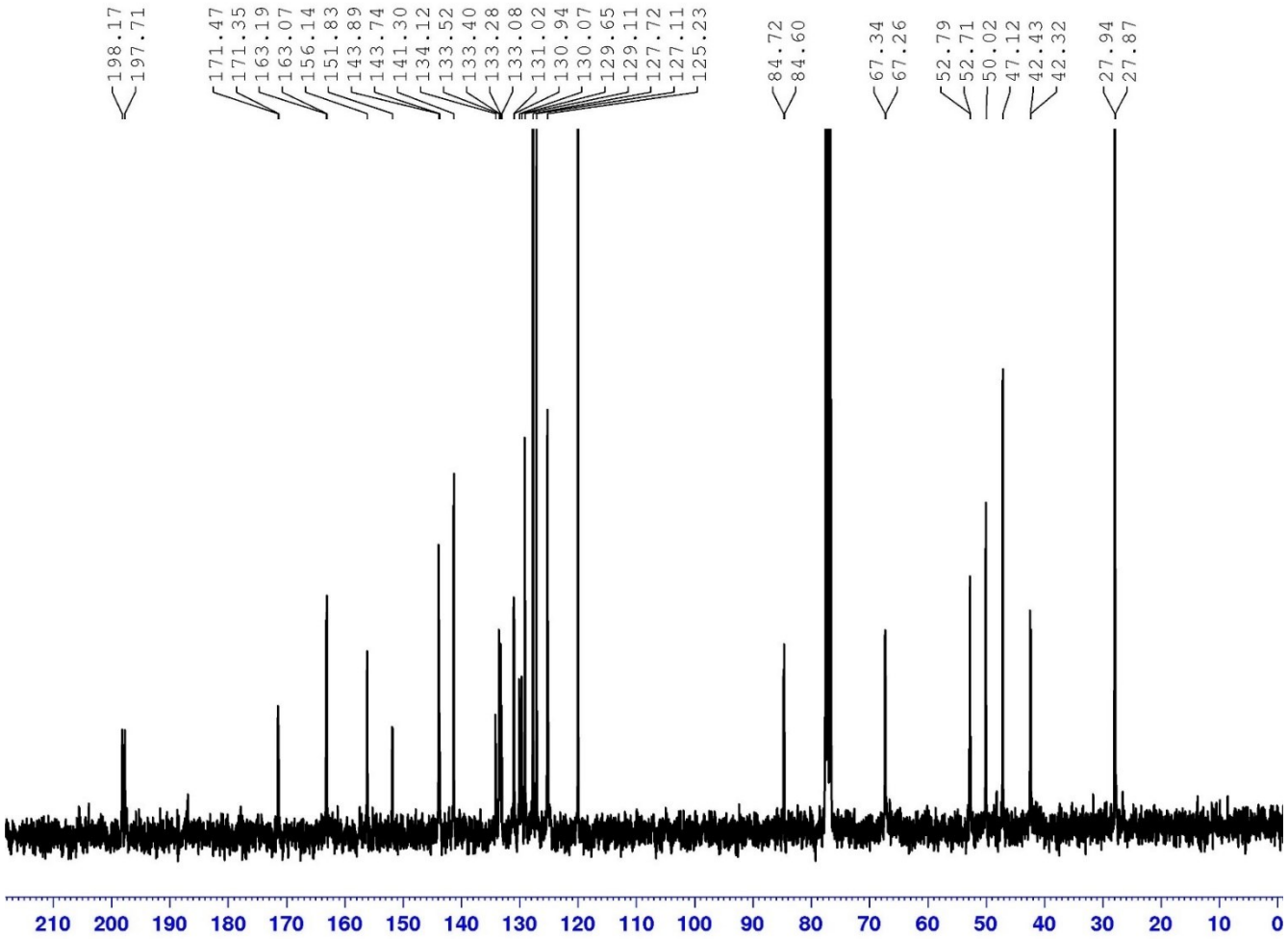
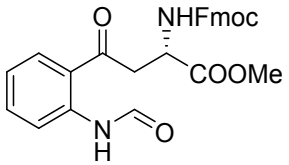
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm


O
NHFmoc
COOMe
N
O
Boc

^{13}C NMR of **11**


198.17
197.71
171.47
171.35
163.19
163.07
156.14
151.83
143.89
143.74
141.30
134.12
133.52
133.40
133.28
133.08
131.02
130.94
130.07
129.65
129.11
127.72
127.11
125.23
84.72
84.60
67.34
67.26
52.79
52.71
50.02
47.12
42.43
42.32
27.94
27.87
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0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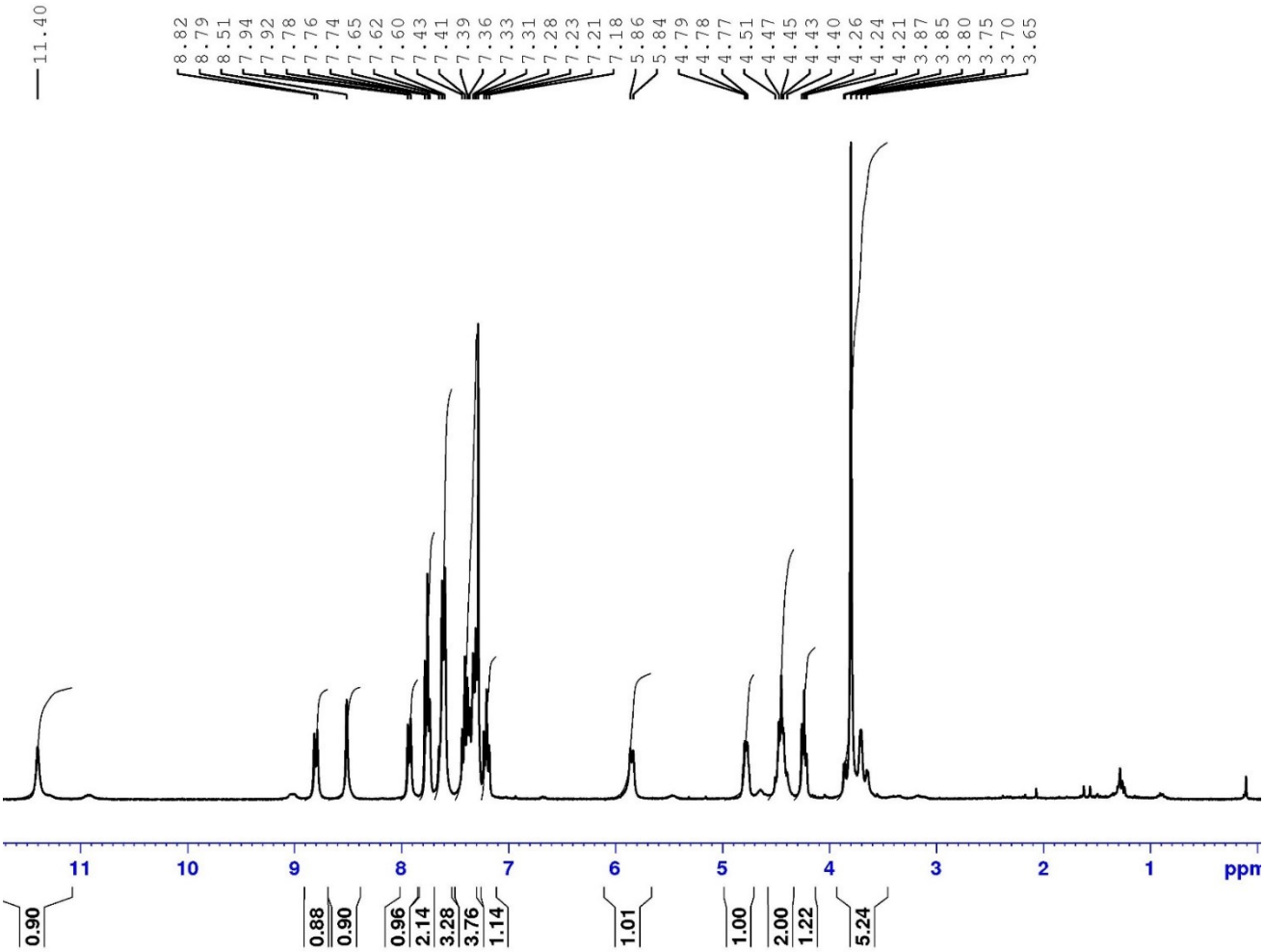
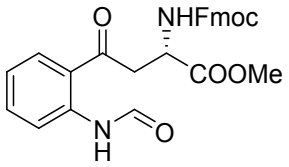
NHFmoc
COOMe
N
H
O

^{1}H NMR of **12**


11.40
8.82
8.79
8.51
7.94
7.92
7.78
7.76
7.74
7.65
7.62
7.60
7.43
7.41
7.39
7.36
7.33
7.31
7.28
7.23
7.21
7.18
5.86
5.84
4.79
4.78
4.77
4.51
4.47
4.45
4.43
4.40
4.26
4.24
4.21
3.87
3.85
3.80
3.75
3.70
3.65
11
10
9
8
7
6
5
4
3
2
1
ppm
0.90
0.88
0.90
0.96
2.14
3.28
3.76
1.14
1.01
1.00
2.00
1.22
5.24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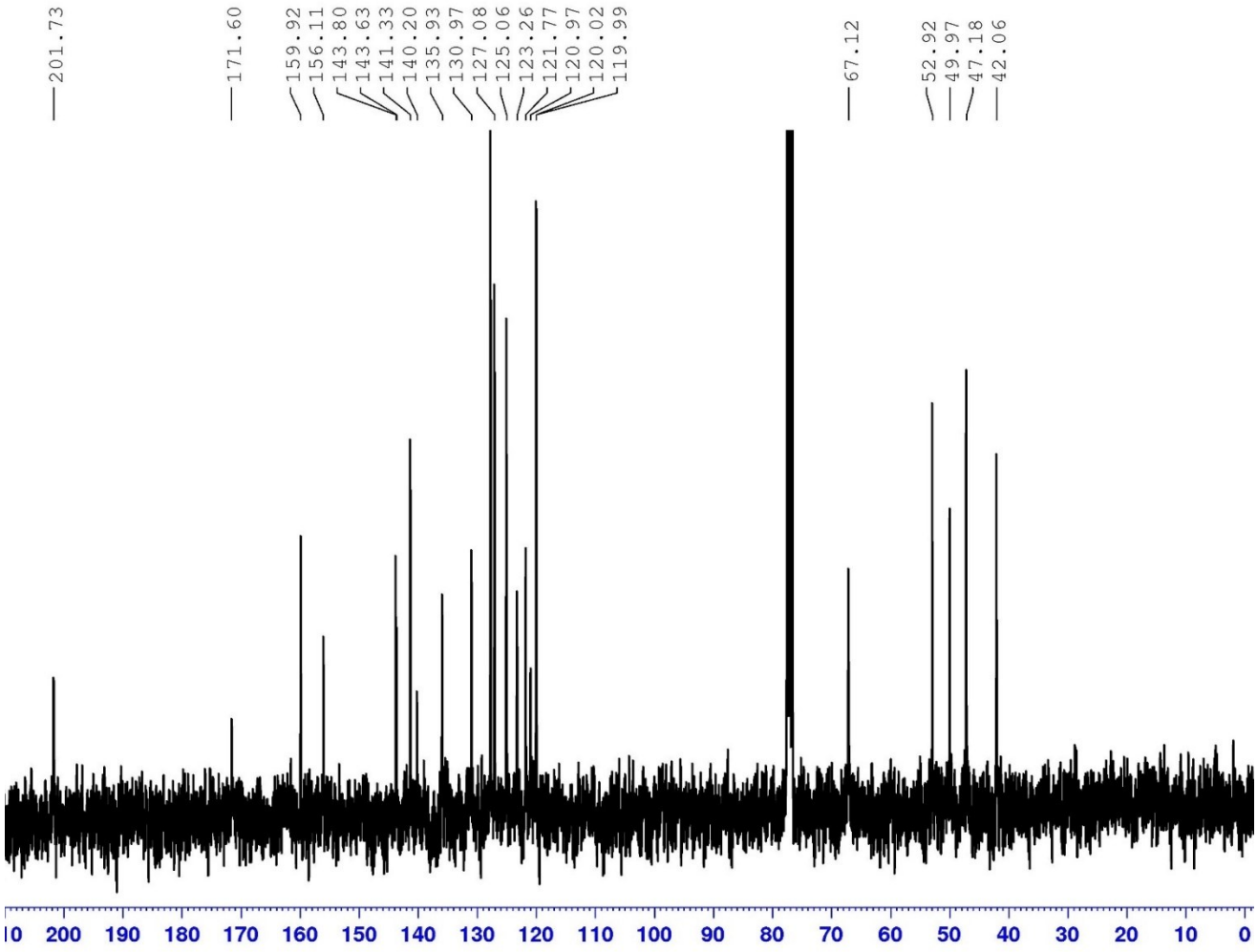
NHFmoc
COOMe
N
H
O

^{13}C NMR of **12**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159.92
156.11
143.80
143.63
141.33
140.20
135.93
130.97
127.08
125.06
123.26
121.77
120.97
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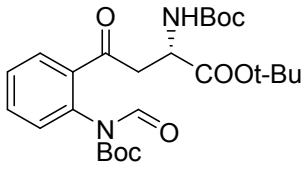
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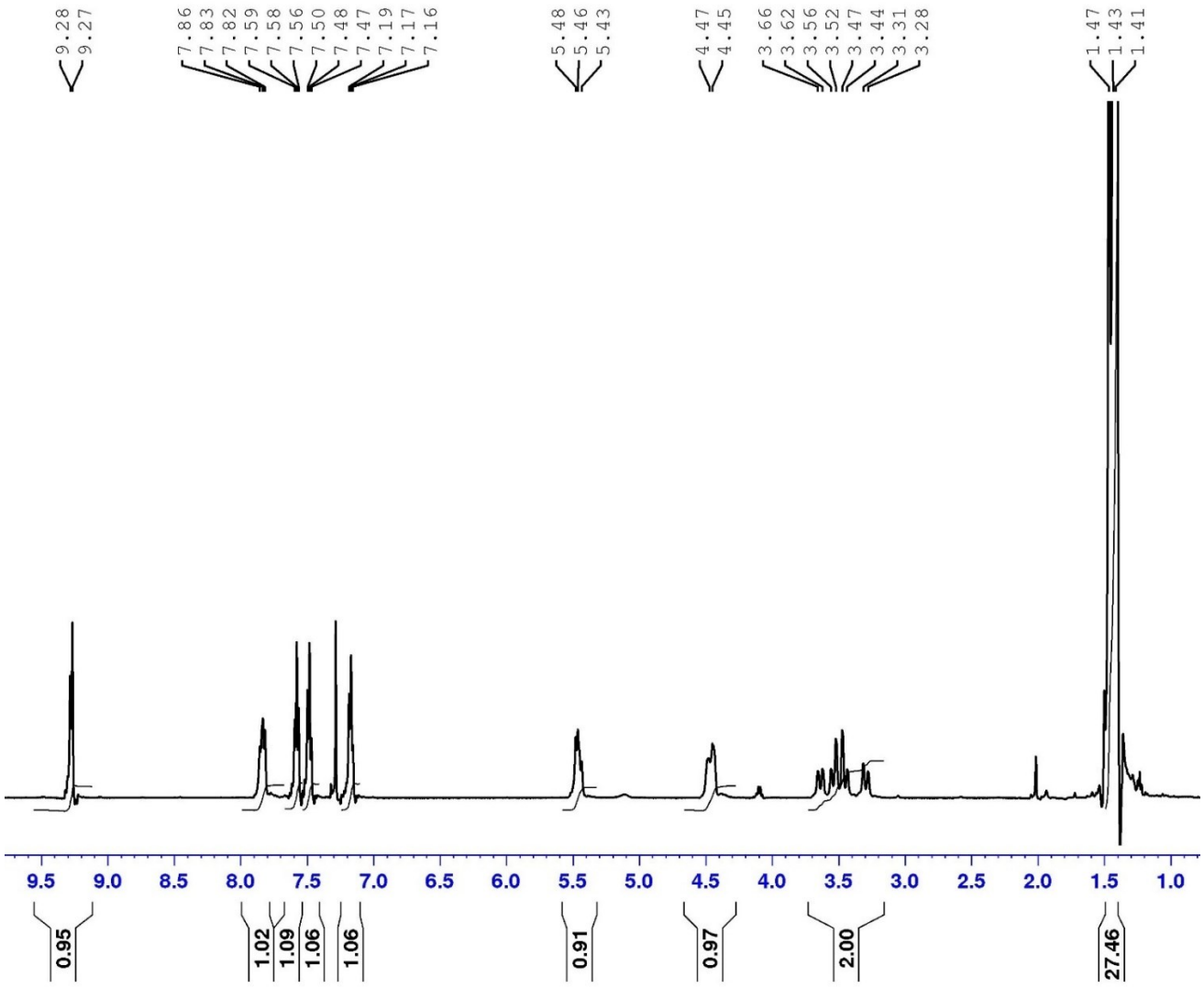
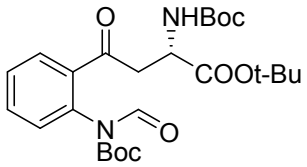
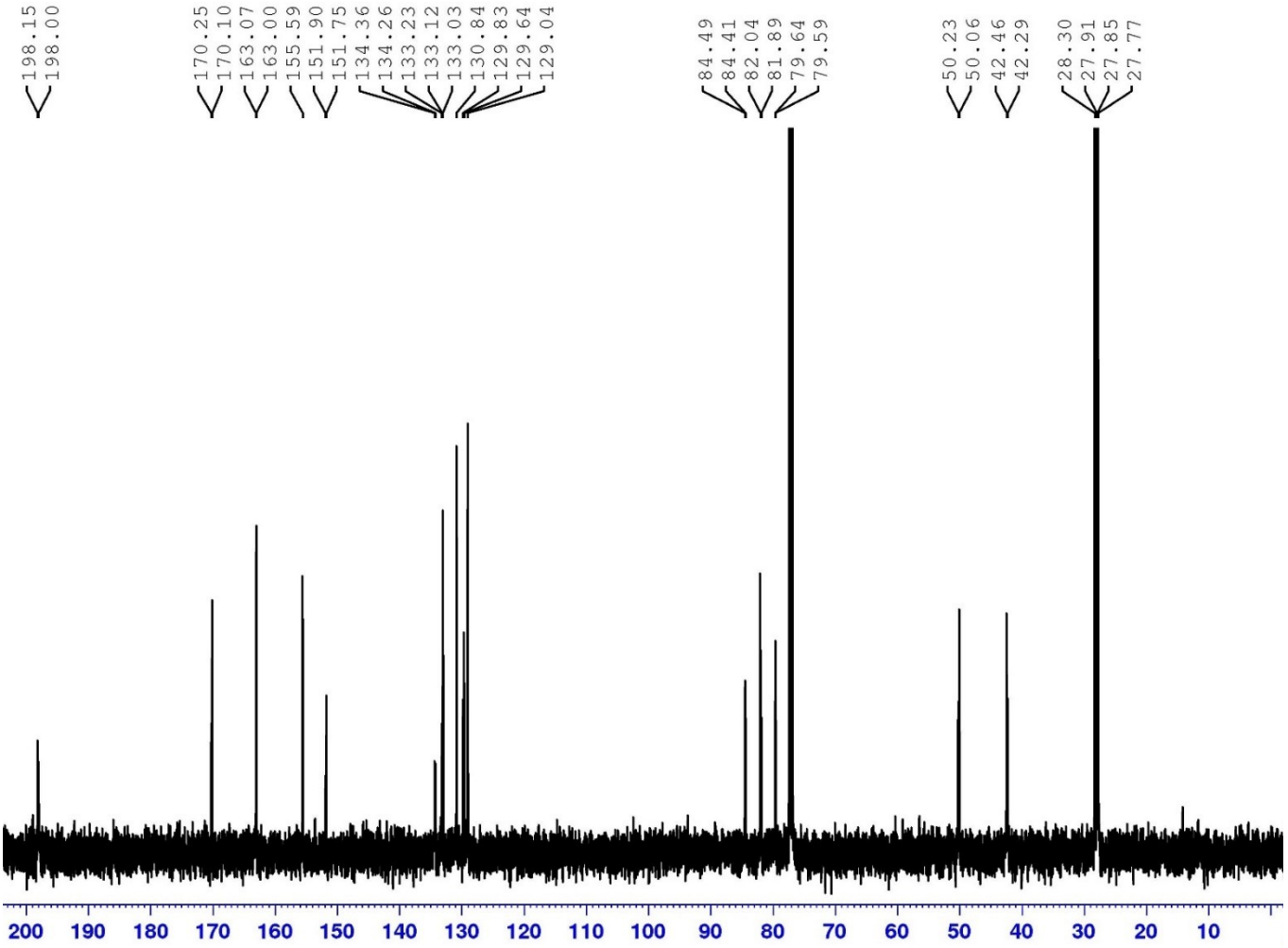
NHBoc
COOt-Bu
N
O
Boc

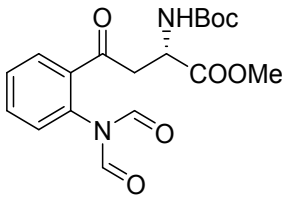
^{1}H NMR of **13**


9.28
9.27
7.86
7.83
7.82
7.59
7.58
7.56
7.50
7.48
7.47
7.19
7.17
7.16
5.48
5.46
5.43
4.47
4.45
3.66
3.62
3.56
3.52
3.47
3.44
3.31
3.28
1.47
1.43
1.41
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
0.95
1.02
1.09
1.06
1.06
0.91
0.97
2.00
27.46

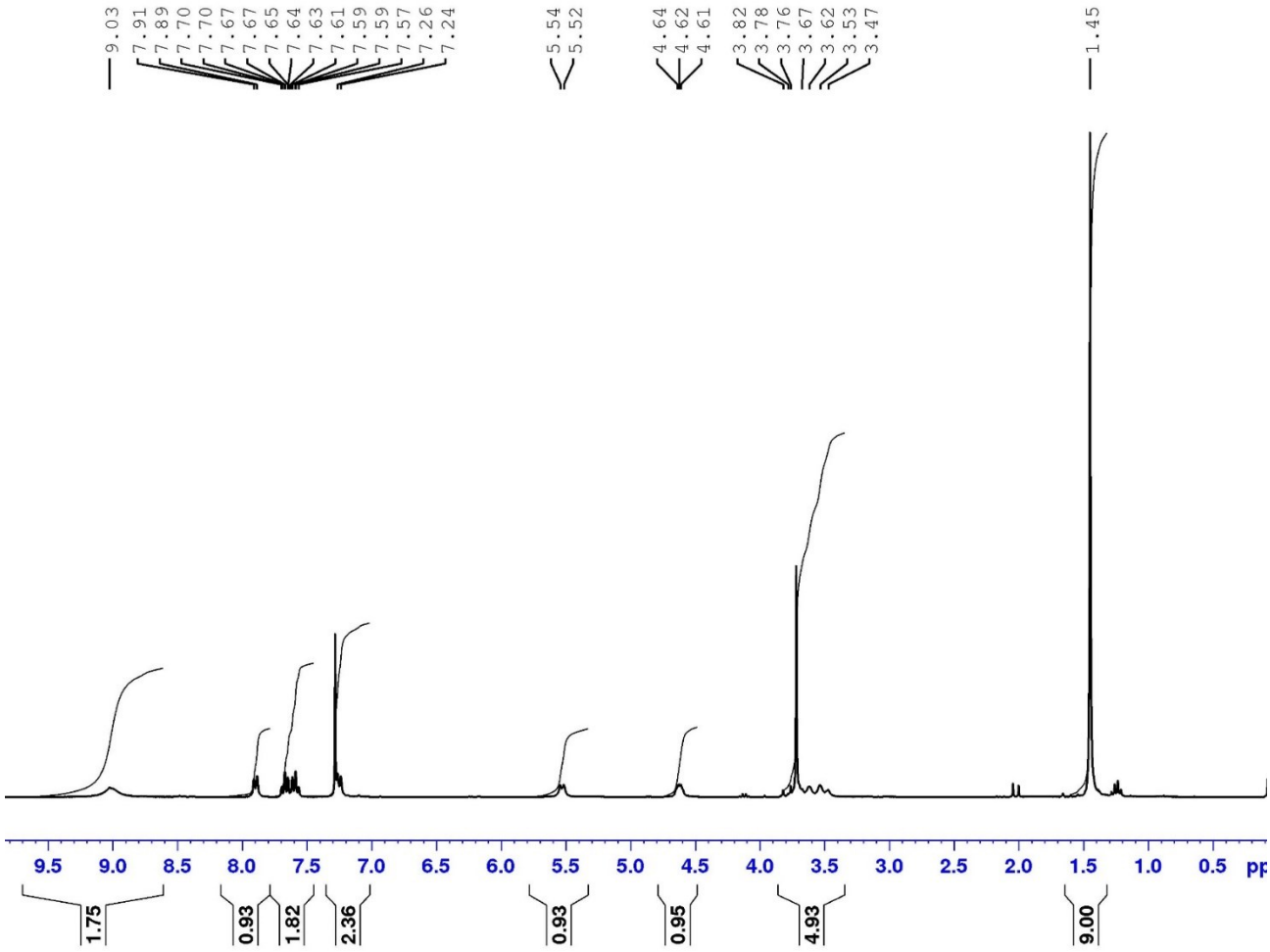

O
NHBoc
COOt-Bu
N
O
Boc

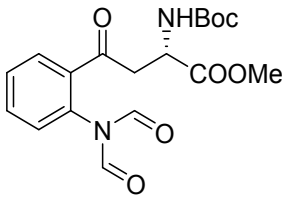
^{13}C NMR of **13**


198.15
198.00
170.25
170.10
163.07
163.00
155.59
151.90
151.75
134.36
134.26
133.23
133.12
133.03
130.84
129.83
129.64
129.04
84.49
84.41
82.04
81.89
79.64
79.59
50.23
50.06
42.46
42.29
28.30
27.91
27.85
27.77
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

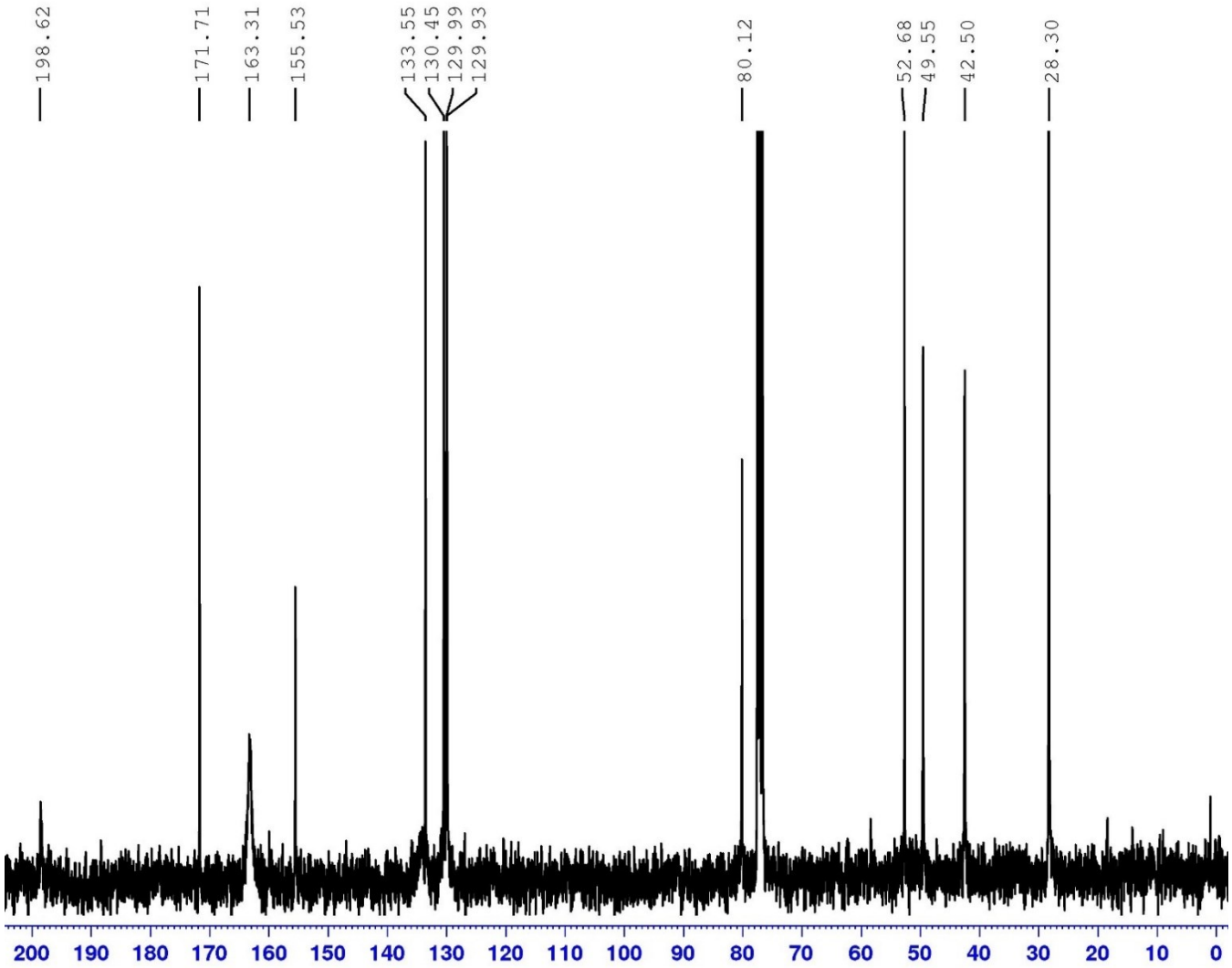

O
NHBoc
COOMe
N
O
O

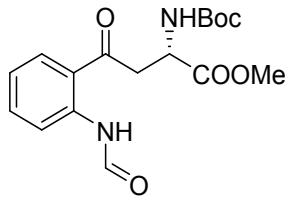
^{1}H NMR of **14**


9.03
7.91
7.89
7.70
7.70
7.67
7.67
7.65
7.64
7.63
7.61
7.59
7.59
7.57
7.26
7.24
5.54
5.52
4.64
4.62
4.61
3.82
3.78
3.76
3.67
3.62
3.53
3.47
1.45
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
1.75
0.93
1.82
2.36
0.93
0.95
4.93
9.00

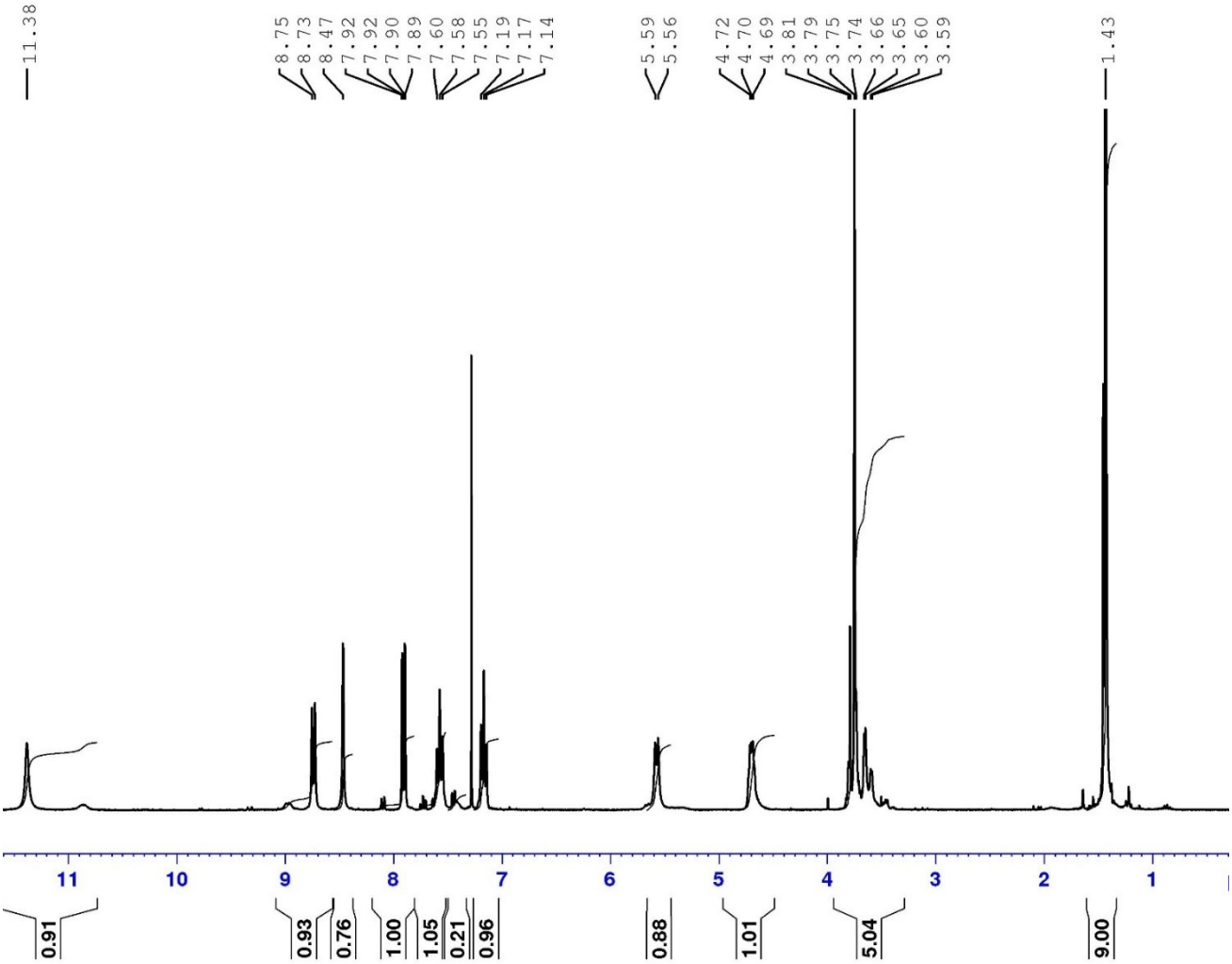

O
NHBoc
COOMe
N
O
O

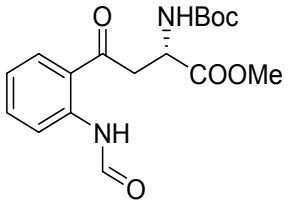
^{13}C NMR of **14**


198.62
171.71
163.31
155.53
133.55
130.45
129.99
129.93
80.12
52.68
49.55
42.50
28.30
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

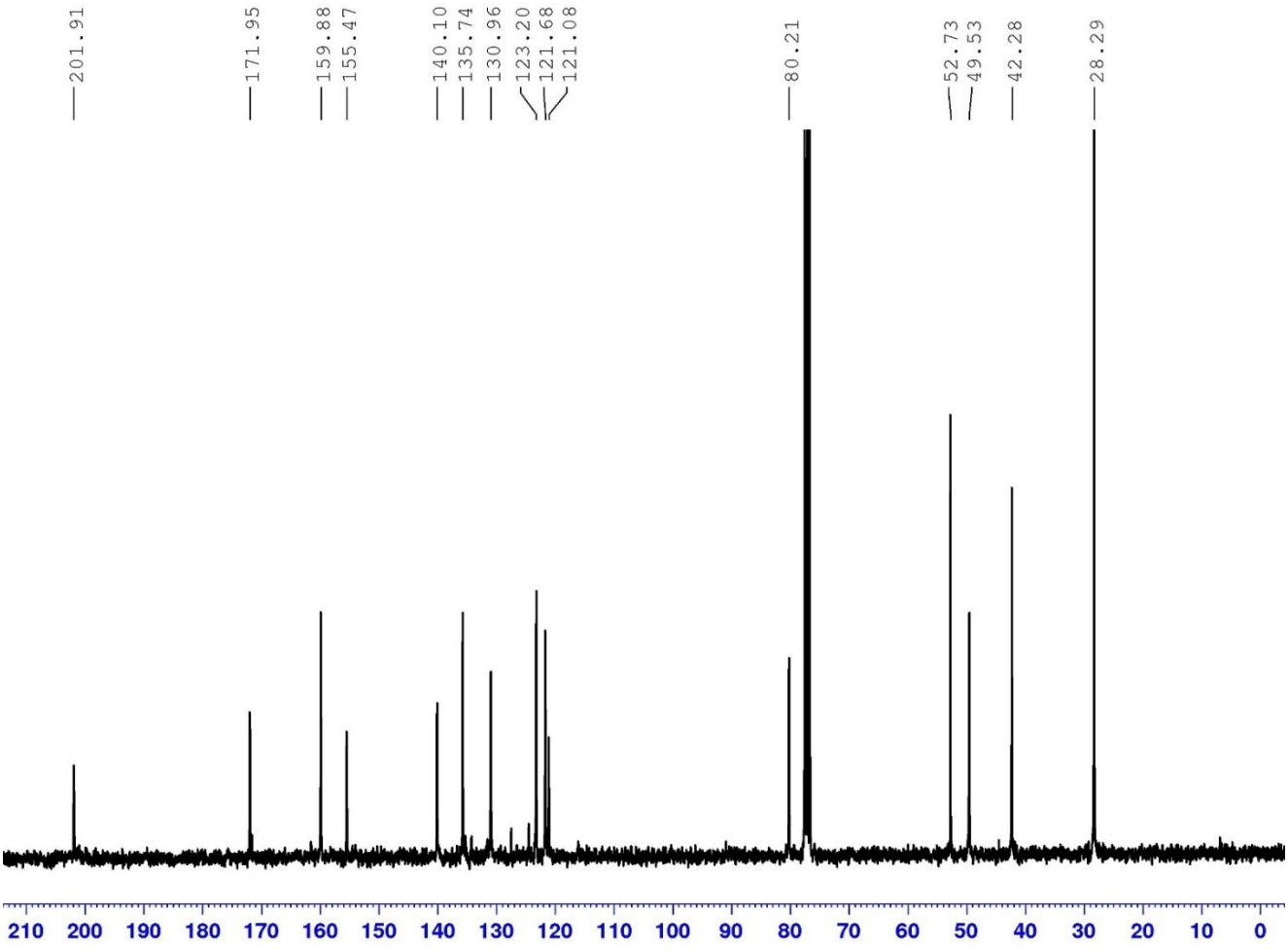

O
NHBoc
COOMe
NH
O

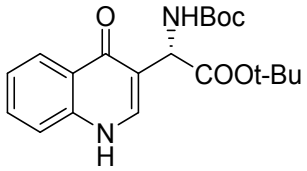
^{1}H NMR of **15**


11.38
8.75
8.73
8.47
7.92
7.92
7.90
7.89
7.60
7.58
7.55
7.19
7.17
7.14
5.59
5.56
4.72
4.70
4.69
3.81
3.79
3.75
3.74
3.66
3.65
3.60
3.59
1.43
11
10
9
8
7
6
5
4
3
2
1
0.91
0.93
0.76
1.00
1.05
0.21
0.96
0.88
1.01
5.04
9.00

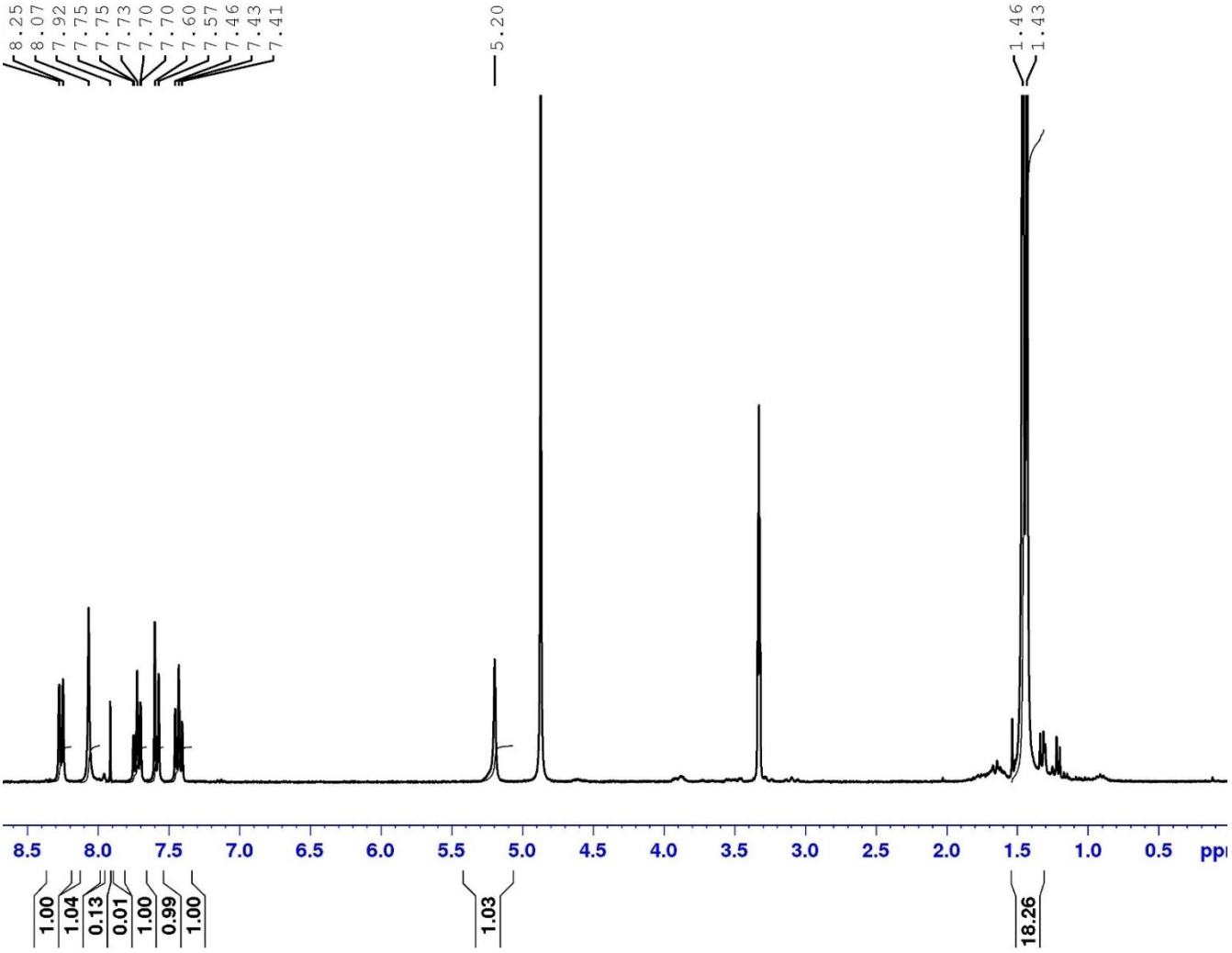

O
NHBoc
COOMe
NH
O

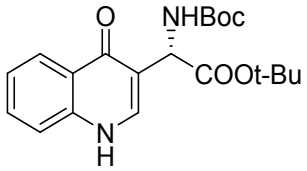
^{13}C NMR of **15**


201.91
171.95
159.88
155.47
140.10
135.74
130.96
123.20
121.68
121.08
80.21
52.73
49.53
42.28
28.29
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

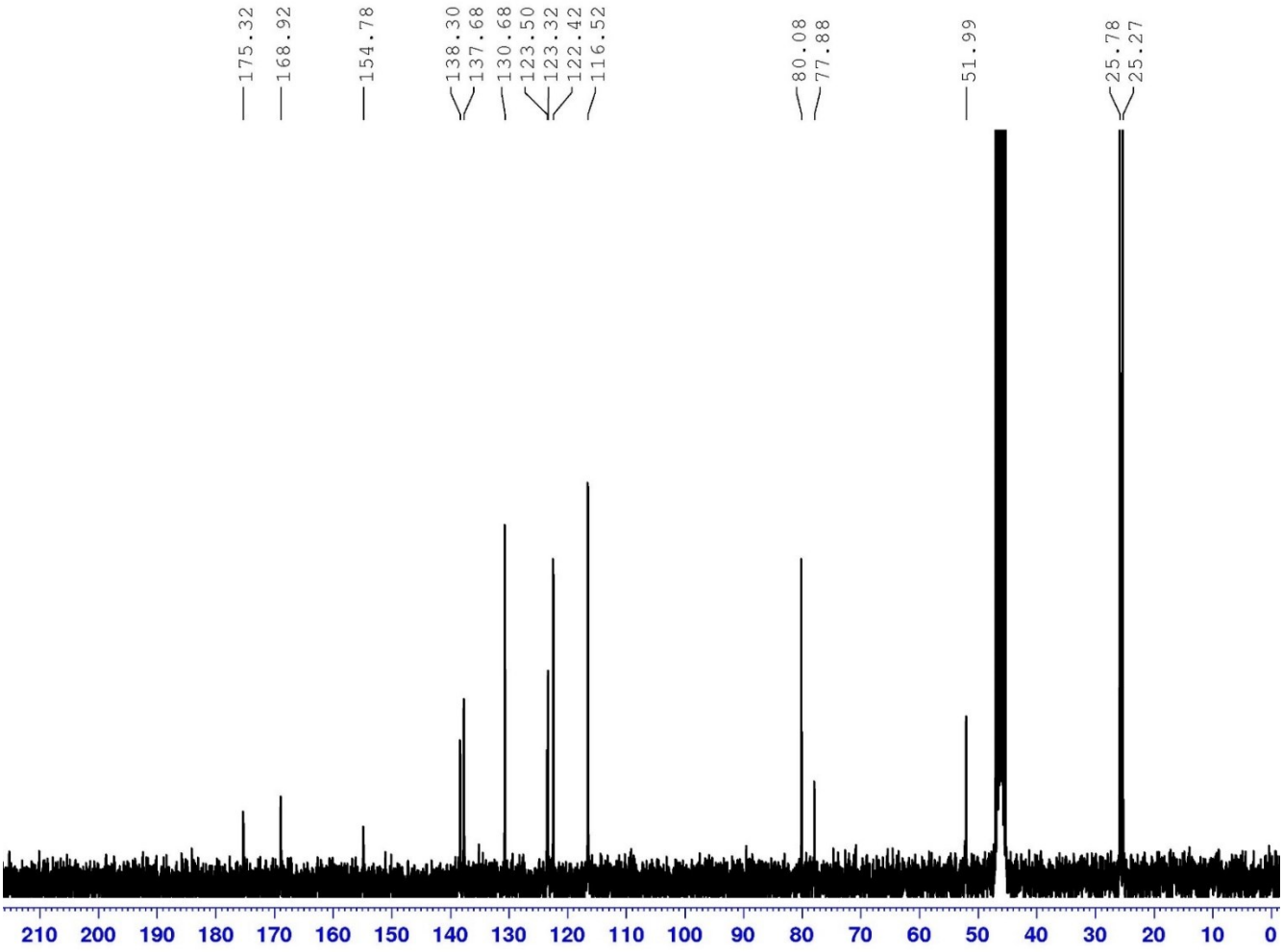

O
NHBoc
COOt-Bu
N
H

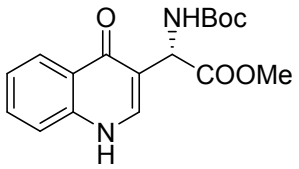
^{1}H NMR of **16**


8.25
8.07
7.92
7.75
7.75
7.73
7.70
7.70
7.60
7.57
7.46
7.43
7.41
5.20
1.46
1.43
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
1.00
1.04
0.13
0.01
1.00
0.99
1.00
1.03
18.26

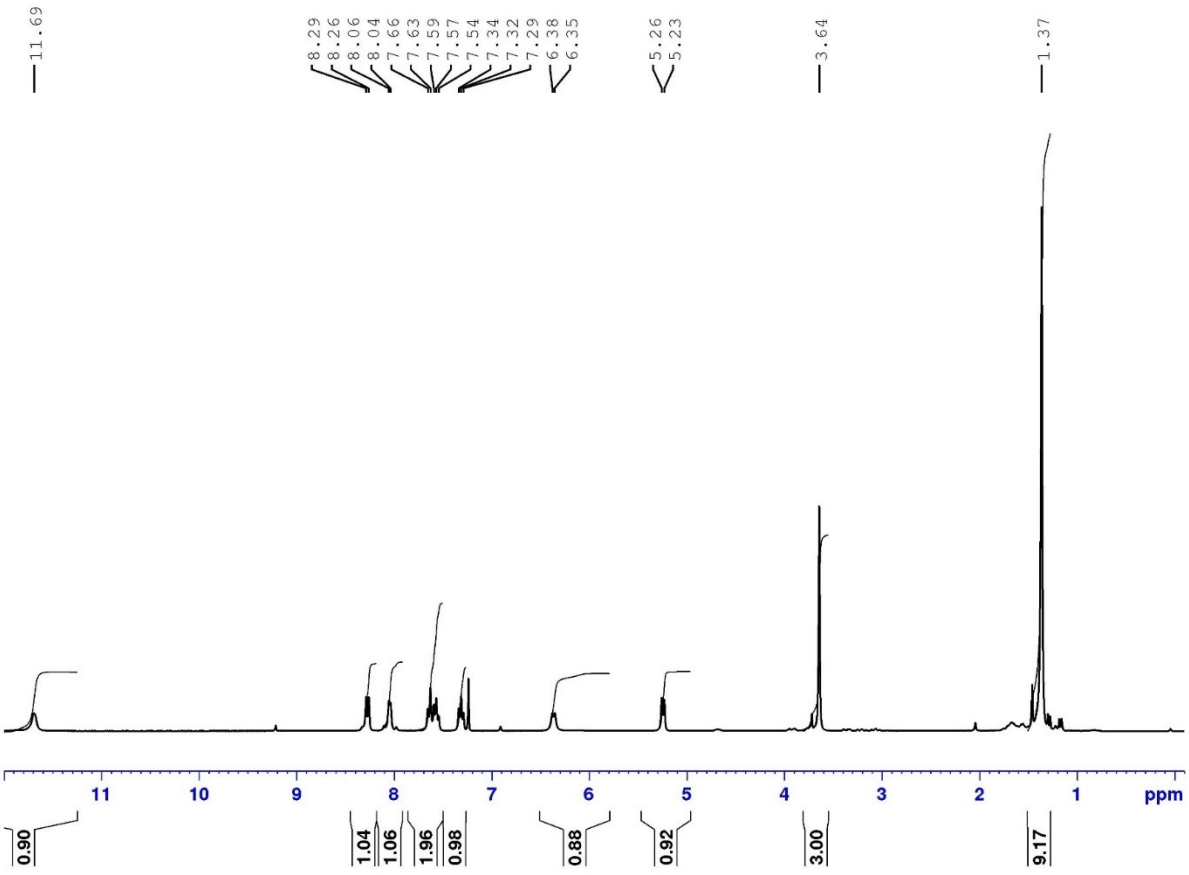

O
NHBoc
COOt-Bu
N
H

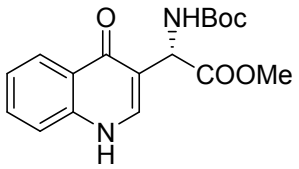
^{13}C NMR of **16**


175.32
168.92
154.78
138.30
137.68
130.68
123.50
123.32
122.42
116.52
80.08
77.88
51.99
25.78
25.27
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

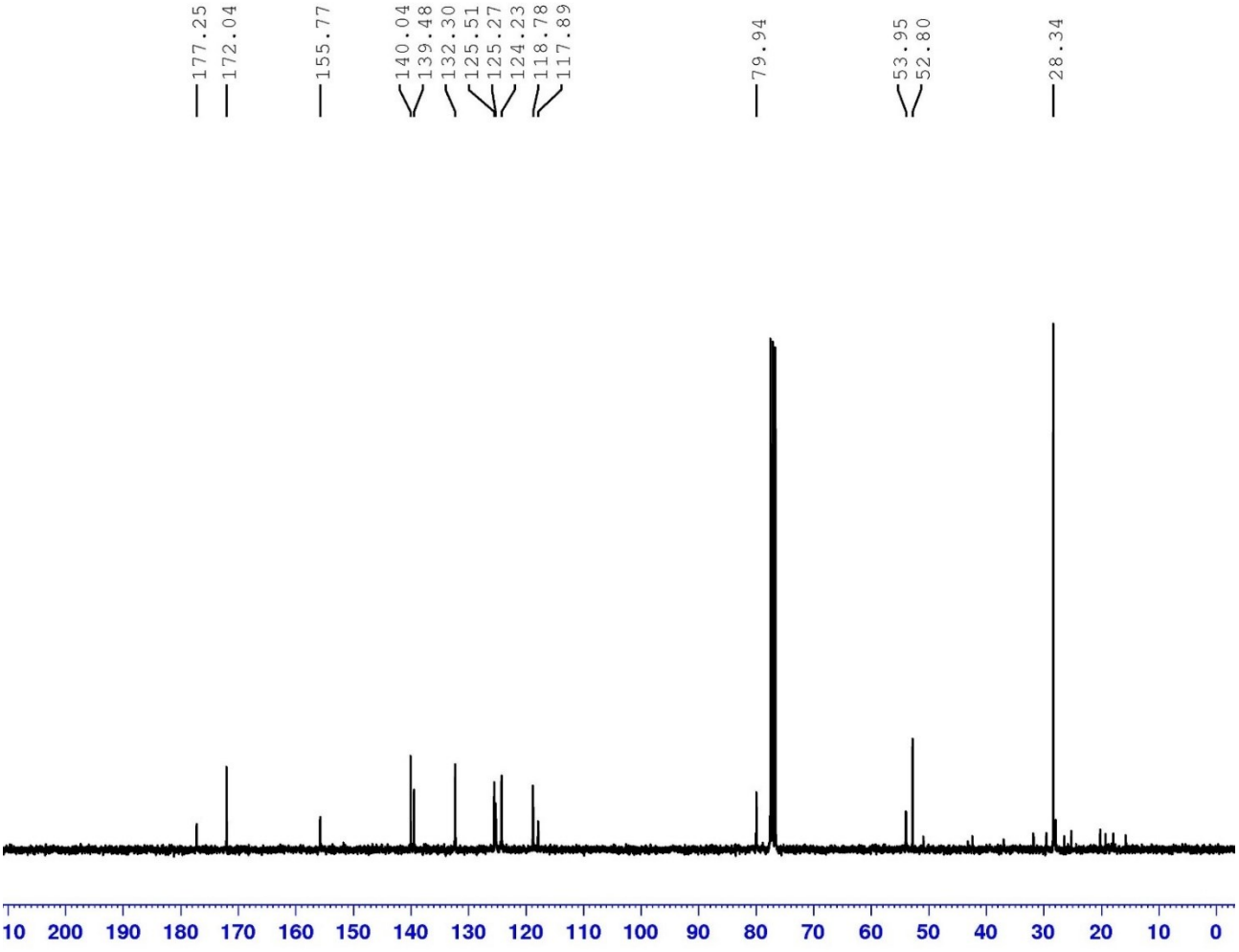

O
NHBoc
COOMe
N
H

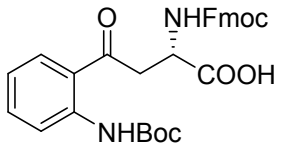
^{1}H NMR of **17**


11.69
8.29
8.26
8.06
8.04
7.66
7.63
7.59
7.57
7.54
7.34
7.32
7.29
6.38
6.35
5.26
5.23
3.64
1.37
11
10
9
8
7
6
5
4
3
2
1
ppm
0.90
1.04
1.06
1.96
0.98
0.88
0.92
3.00
9.17

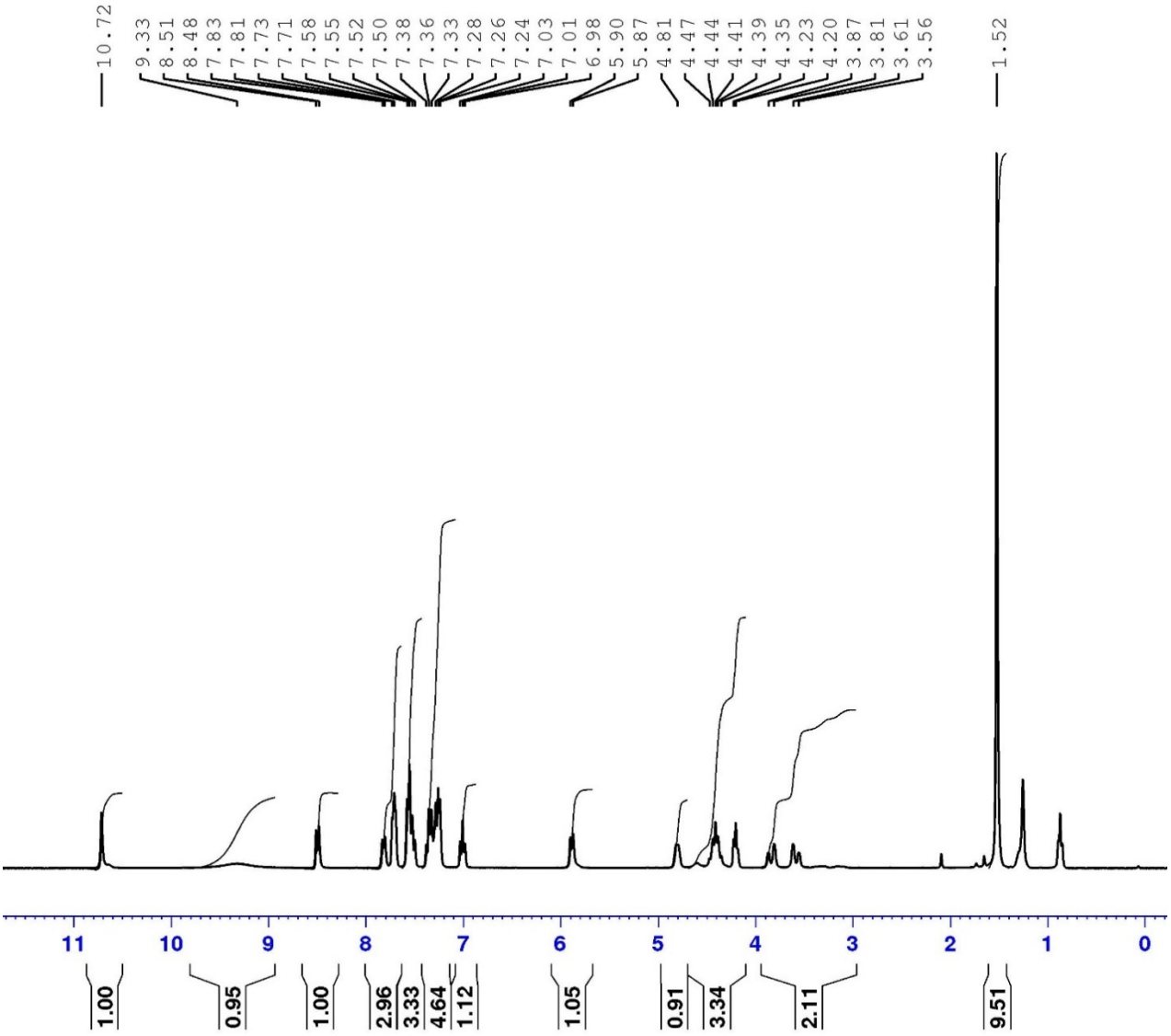

O
NHBoc
COOMe
N
H

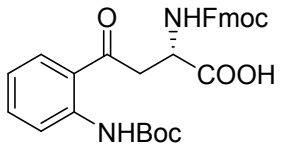
^{13}C NMR of **17**


177.25
172.04
155.77
140.04
139.48
132.30
125.51
125.27
124.23
118.78
117.89
79.94
53.95
52.80
28.34
10 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

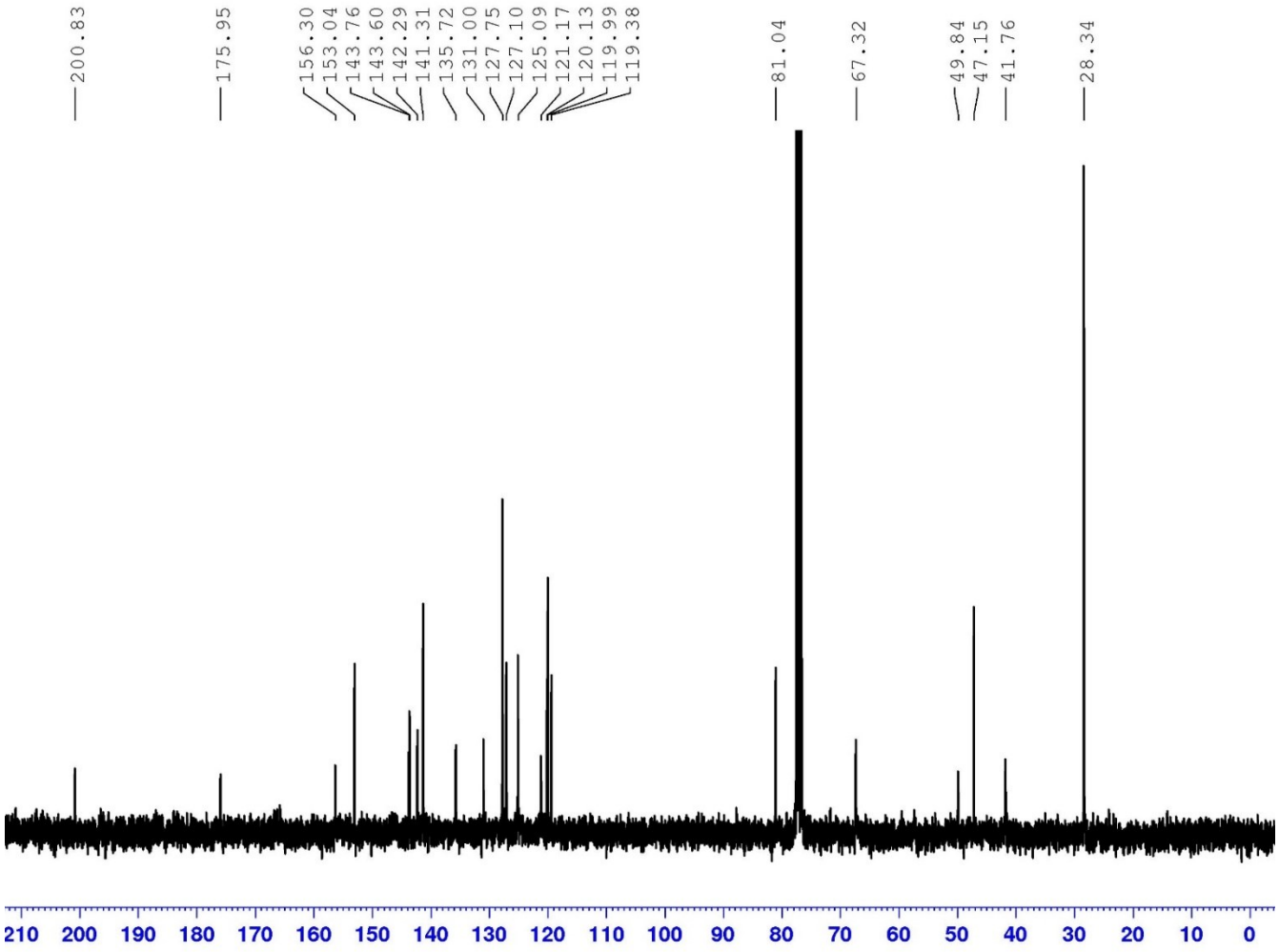

O
NHFmoc
COOH
NHBoc

^{1}H NMR of **18**


10.72
9.33
8.51
8.48
7.83
7.81
7.73
7.71
7.58
7.55
7.52
7.50
7.38
7.36
7.33
7.28
7.26
7.24
7.03
7.01
6.98
5.90
5.87
4.81
4.47
4.44
4.41
4.39
4.35
4.23
4.20
3.87
3.81
3.61
3.56
1.52
11
10
9
8
7
6
5
4
3
2
1
0
1.00
0.95
1.00
2.96
3.33
4.64
1.12
1.05
0.91
3.34
2.11
9.51


O
NHFmoc
COOH
NHBoc

^{13}C NMR of **18**


200.83
175.95
156.30
153.04
143.76
143.60
142.29
141.31
135.72
131.00
127.75
127.10
125.09
121.17
120.13
119.99
119.38
81.04
67.32
49.84
47.15
41.76
28.34
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0